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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I - Nanobiotechnology – SBTA1503

NANOTECHNOLOGY

Introduction

The prefix nano in the word nanotechnology means a billionth ($1x10^{-9}$). Nanotechnology deals with various structures of matter having dimensions of the order of billionth of a meter. While the word nanotechnology is relatively new, the existence of functional devices and structures of nanometer dimensions is not new, and in fact such structures have existed on Earth as long as life itself. The abalone, a mollusk, constructs very strong shells having irrridescent inner surface by organizing calcium carbonate into strong nanostrucutred bricks held together by a glue made of a carbohydrate-protein mix. Cracks initiated on the outside are unable to move through the shell because of the nanostructured bricks. The shells represent a natural demonstration that a structure fabricated from nanoparticle can be much stronger.

Historical Developments

- In the fourth-century A.D Roman glassmakers were fabricating glasses containing nanosized metals. An artifact from this period called the Lycurgus cup resides in the British Museum in London. The cup, which depicts the death of King Lycurgus, is made from soda lime glass containing silver and gold nanoparticles. The color of the cup changes from green to deep red when a light source is placed inside it. The great varieties of beautiful colors of the windows of medieval cathedrals are due to the presence of metal nanoparticles in the glass.
- Photography is an advanced and mature technology, developed in the eighteenth and nineteenth centuries, which depends on production of silver nanoparticles sensitive to light. Photographic films is an emulsion, a thin layer of gelatin containing silver halides, such as silver bromide, and a base of transparent cellulose acetate. The light decomposes the silver halides, producing nanoparticles of silver, which are the pixels of the image.
- In 1857, Michael Faraday published a paper in the Philosophical Transactions of the Royal Society, which attempted to explain how metal particles affect the color of church windows. Gustav Mie was the first to provide an explanation of the dependence of the color of the glasses on metal size and kind. His paper was published in the German Journal Annalen der Physik in 1908.

- Richard Feynman was awarded the Nobel Prize in physics in 1965 for his contributions to quantum electrodynamics. In 1960 he presented a visionary and prophetic lecture at a meeting of the American Physical Society, entitled “There is Plenty of Room at the Bottom”, where he speculated on the possibility and potential of nanosized materials. He envisioned etching lines a few atoms wide with beams of electrons, effectively predicting the existence of electron-beam lithography, which is used today to make silicon chips. He proposed manipulating individual atoms to make new small structures having very different properties. He envisioned building circuits on the scale of nanometers that can be used as elements in more powerful computers. He also recognized the existence of nanostructures in biological systems. Many of Feynman’s speculations have become reality. However, his thinking did not resonate with scientists at the time.
- There were other visionaries. Ralph Landauer, a theoretical physicist working or IBM in 1957, had idea on nanoscale electronics and realized the importance that quantum-mechanical effects would play in such devices. Uhlir reported the first observation of porous silicon in 1956, but it was not until 1990 when room temperature fluorescence was observed in this material that interest grew. Other work in this era involved making alkali metal nanoparticles by vaporizing sodium or potassium metal and then condensing them on cooler materials called substrates. Magnetic fluids called ferrofluids were developed in the 1960s. They consist of nanosized magnetic particles dispersed in liquids. The particles were made by ballmilling in the presence of a surface-active agent and liquid carrier. Another area of activity in the 1960s involved electron paramagnetic resonance (EPR) of conduction electrons in metal particles of nanodimensions referred to as colloids. Structural features o metal nanoparticles such as existence of magic numbers were revealed in the 1970s using mass spectroscopic studies of sodium metal beams. Group at Bell Laboratories and IBM fabricated the first two-dimensional quantum wells in the early 1970s. It was not until the 1980s with the emergence of appropriate methods of fabrication of nanostructures that a notable increase in research activity occurred, and a number of significant developments resulted.
- In 1981, a method was developed to make metal clusters using a high-powered focused laser to vaporize metals into a hot plasma. In 1985, this method was used to synthesize the fullerene (C60). In 1982, two Russian scientists, Ekimov and Omushchenko,

reported the first observation of quantum confinement. The scanning tunneling microscope was developed during this decade by G.K. Bining and H. Roher of the IBM Research Laboratory in Zurich, and they were awarded Nobel Prize in 1986 for this. The invention of the scanning tunneling microscope (STM) and the atomic force microscope (AFM), provided new important tools for viewing, characterizing and atomic manipulation of nanostructures. This period was marked by development of methods of fabrication such as electron-beam lithography, which are capable of producing 10-nm structures. Also in this decade layered alternating metal magnetic and nonmagnetic materials, which displayed the fascinating property of giant magnetoresistance, were fabricated. The layers were a nanometer thick, and the materials have an important application in magnetic storage device in computers.

- In the 1990, Iijima made carbon nanotubes, and superconductivity and ferromagnetism were found in C60 structures. Efforts also began to make molecular switches and measure the electrical conductivity of molecules. A field-effect transistor based on carbon nanotubes was demonstrated. The study of self-assembly of molecules on metal surfaces intensified. Self-assembly refers to the spontaneous bonding of molecules to metal surfaces, forming an organized array of molecules on the surface. Self-assembly of thiol and disulfide compounds on gold has been most widely studied.
- In 1996, a number of government agencies led by National Science Foundation commissioned a study to assess the current worldwide status of trends, research and development in nanoscience and nanotechnology. Two general findings emerged from the study. The first observation was that materials have been and can be nanostructured for new properties and novel performance. The second observation of the U.S government study was a recognition of the broad range of disciplines that are contributing to developments in the field. These disciplines include physics, chemistry, biology and engineering (electrical, mechanical and chemical engineering). The interdisciplinary nature of the field makes it somewhat difficult for researchers in one field to understand and draw on developments in another area. To explore the potential of nanotechnology it is essential to know what are nanomaterials, how and why do they differ from other materials, how to synthesize/analyze the nanomaterials and organize them to apply in different areas.

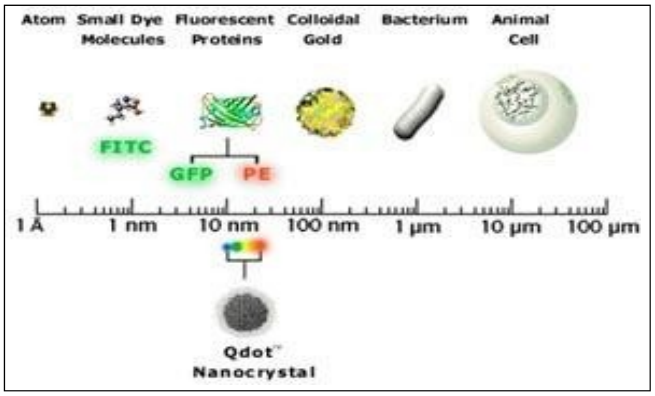
- ***What is Nanotechnology?*** Broadly speaking however, nanotechnology is the act of purposefully manipulating matter at the atomic scale, otherwise known as the "nanoscale." Coined as "nano-technology" in a 1974 paper by Norio Taniguchi at the University of Tokyo, and encompassing a multitude of rapidly emerging technologies, based upon the scaling down of existing technologies to the next level of precision and miniaturization. Taniguchi approached nanotechnology from the 'top-down' standpoint, from the viewpoint of a precision engineer.

  K. Eric Drexler introduced the term "nanotechnology" to the world in 1986, using it to describe a 'bottom-up' approach. Drexler approaches nanotechnology from the point-of-view of a physicist, and defines the term as "large-scale mechanosynthesis based on positional control of chemically reactive molecules."

  At the nanoscale, the physical, chemical, and biological properties of materials differ in fundamental and valuable ways from the properties of individual atoms and molecules or bulk matter. Nanotechnology R&D is directed toward understanding and creating improved materials, devices, and systems that exploit these new properties.

- **SIZE :** A *meter* is about the distance from the tip of your nose to the end of your hand (1 meter = 3.28 feet). One *thousandth* of that is a *millimeter*. Now take *one thousandth* of that, and you have a *micron*: a thousandth of a thousandth of a meter. Put another way: a *micron is a millionth of a meter*, which is the scale that is relevant to - for instance - building computers, computer memory, and logic devices. Now, let's go smaller, to the *nanometer*: A nanometer is one thousandth of a micron, and a thousandth of a millionth of a meter (a billionth of a meter). Imagine: *one billion nanometers in a meter*.

Figure 1.1 : Nanoscale features



- **Nanoscale features:** Nanomaterials are characterised at the nanometre scale in one, two or three dimensions, leading to quantum wells (e.g., thin films, layers, surface coatings), quantum wires (e.g., nanotubes, nanowires) or quantum dots (qdots), respectively (Figure 1.1). Nanoparticles with a diameter of less <100 nm are for example fullerenes,dendrimers and semiconductor quantum dots. The word quantum is associated withthese three structures because profound changes in material properties emanate fromthe quantum mechanical nature of physics that rules the world in the ultra-small andwhere material properties no longer obey the classical macroscopic laws of physics.Materials can be scaled down many orders of magnitude from macroscopic tomicroscopic without any or little change in expected properties occurring. However,when the nanoworld is entered, characteristic changes are observed. For the time beingno strict dimensional limits can be defined for this phenomenon. At the nanoscale,physics, chemistry, biology, material science, and engineering converge toward thesame principles and tools. As a result, progress in nanoscience will have very far-reaching impact. The nanoscale is not just another step toward miniaturisation, but aqualitatively new scale. The change in behaviour is dominated in the first place byquantum mechanics, as mentioned above and is additionally attributable to materialconfinement in small structures, and the increase in surface area per volume (or massunit). At the larger end of the nanometre scale other phenomena are crucial, such assurface tension and Brownian motion. Nanoscience is concerned with understandingthese effects and their influence on material properties. Nanotechnology aims to exploitthese effects to create structures, devices, and systems with novel properties andfunctions due to their size (The Royal Society & The Royal Academy of Engineering,2004). In contrast to other key technologies, such as biotechnology, information andcommunication technology, nanotechnology is much less well-defined and well-structured. In fact, nanotechnology is immensely complex and covers multipledisciplines ranging from physics, chemistry, and biology to engineering disciplines. The Royal Society & The Royal Academy of Engineering (2004) definitions were givenfor nanoscience and nanotechnology:

  Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at larger scale.

Nanotechnology is the design, characterisation, production and applications of structures, devices and systems by controlling shape and size at the nanometre scale.

- Despite the apparent simplicity of definition, nanotechnology actually encompasses diverse lines of inquiry. Nanotechnology cuts across many disciplines, including colloidal science, chemistry, applied physics, materials science, and even mechanical and electrical engineering. It could variously be seen as an extension of existing sciences into the nanoscale, or as a recasting of existing sciences using a newer, more modern term.

  Related and interwoven fields include, but are not limited to: Nanomaterials, Nanomedicine, Nanobiotechnology, Nanolithography, Nanoelectronics, Nanomagnetics, Nanorobots, Biodevices (biomolecular machinery), AI, MEMS (MicroElectroMechanical Systems), NEMS (NanoElectroMechanical Systems), Biomimetic Materials, Microencapsulation, and many others.

- Two main approaches are used in nanotechnology: one is a "bottom-up" approach where materials and devices are built from molecular components which assemble themselves chemically using principles of molecular recognition; the other being a "top-down" approach where nano-objects are constructed from larger entities without atomic-level control. (i) Bottom-up approaches: These seek to arrange smaller components into more complex assemblies. DNA Nanotechnology utilises the specificity of Watson-Crick basepairing to construct well-defined structures out of DNA and other nucleic acids. More generally, molecular self-assembly seeks to use concepts of supramolecular chemistry, and molecular recognition in particular, to cause single-molecule components to automatically arrange themselves into some useful conformation. (ii)Top-down approaches: These seek to create smaller devices by using larger ones to direct their assembly. Many technologies descended from conventional **solid-state silicon methods** for fabricating microprocessors are now capable of creating features smaller than 100 nm, falling under the definition of nanotechnology. Giant magnetoresistance-based hard drives already on the market fit this description, as do atomic layer deposition (ALD) techniques. Solid-state techniques can also be used to create devices known as **nanoelectromechanical systems** or NEMS, which are related to microelectromechanical systems or MEMS. Atomic force microscope tips

can be used as a nanoscale "write head" to deposit a chemical on a surface in a desired pattern in a process called **dip pen nanolithography**. This fits into the larger subfield of nanolithography. (iii) Functional approaches: These seek to develop components of a desired functionality without regard to how they might be assembled. **Molecular electronics** seeks to develop molecules with useful electronic properties. These could then be used as single-molecule components in a nanoelectronic device. For example rotaxane. Synthetic chemical methods can also be used to create **synthetic molecular motors**, such as in a so-called nanocar.

- **Four Generations:** Mihail (Mike) Roco of the U.S. National Nanotechnology Initiative has described *four generations* of nanotechnology development (Figure1.2). The current era, as Roco depicts it, is that of passive nanostructures, materialsdesigned to perform one task. The second phase, which we are just entering, introducesactive nanostructures for multitasking; for example, actuators, drug delivery devices, and sensors. The third generation is expected to begin emerging around 2010 and will feature nanosystems with thousands of interacting components. A few years after that, the first integrated nanosystems, functioning (according to Roco) much like a mammalian cell with hierarchical systems within systems, are expected to be developed

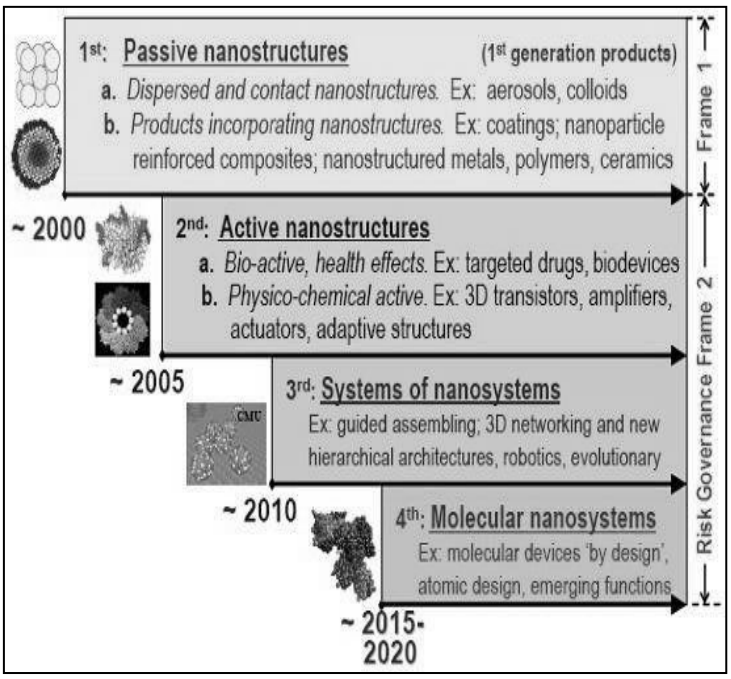


Figure 1.2: Generations of Nanotechnology

- Many scientists and technologists believe that nanoscience will provide the basis for an industrial revolution in the 21st century that will have an impact on the health, wealth, and security of the world's people as significant as the combined influence of antibiotics, integrated circuits, and human made polymers. Already, impressive examples demonstrate the potential impact of nanotechnology:
    - Carbon nanotubes have been shown to be ten times as strong as steel with one sixth of the weight and to exhibit semiconducting properties similar to silicon on the nanometer scale.
    - Nanoparticle-reinforced polymers, with lightweight and strong mechanical strength, improve fuel efficiencies and increase safety for transportation vehicles.
    - Molecular switches that could potentially improve computer storage capacity by a million times have been demonstrated.
    - Nanostructured silicates and polymers are used as effective contaminant scavengers for a cleaner environment.
    - New drugs made of nanoparticle powder have nearly ten times the bioavailability and faster response times compared with conventional drugs.
    - Patterning of nanoporous surface texturing at the interface between medical implants and their biological substrates has provided a powerful new way to encourage tissue integration.

Examples of the impact on key industries include the following:

- Medical device industry with 300-500 large companies and small start-ups: to enable extreme miniaturization and the development of new types of products;
- High-tech materials and manufacturing industry: to enable the development of consumer and defense products based on new materials;
- Biotechnology industry: to enable the development of pharmaceutical products with highly controlled effects and the production of superior agricultural products;
- Data storage, information processing, and telecommunications industries: to produce highly advanced systems based on radically new technologies;
- Instrument and sensor industry: to enable the development of ultra-small sensors for process control and health diagnostics

PROPERTIES OF NANOMATERIALS

Nanomaterials: Generally, nanomaterials are defined as materials with grain sizes below 100 nm. More stringent: Nanomaterials are materials with special properties depending on their small grain size**.** In many cases, the latter definition restricts nanomaterials to grain sizes below 10 nm.

The second definition is the more useful one, because nanomaterials are expensive. An expensive material without very special properties is senseless. Some nanocrystalline ceramic materials or nanoglasses with particle sizes below 10 nm exhibit interesting physical properties. Except for properties related to grain boundaries, these are properties of single isolated particles. These special properties may be lost in the case that the particles are interacting. This phenomenon leads to the necessity of nanocomposites

Materials behave differently at this scale: Nanomaterials have the structural features in between of those of atoms and the bulk materials. While most microstructured materials have similar properties to the corresponding bulk materials, the properties of materials with nanometer dimensions are significantly different from those of atoms and bulks materials.

Why do materials behave differently at the nanoscale? Materials behave differently at this scale for two reasons: Firstly, very small particles have a larger surface area compared to the same amount of material in a larger lump (for example, grains of sand would cover a bigger surface than the same amount of sand compressed into a stone). As the surface of the particle is involved in chemical reactions, the larger surface area can make materials more reactive – grains of salt dissolve in water much more quickly than a rock of salt for example. In fact, some materials that are generally inactive in their larger form can be more reactive in nanoscale. Secondly, when we look at materials on a nanoscale level, the relative importance of the different laws of physics shift and effects that we normally do not notice (such as quantum effects) become more significant, especially for sizes less than 20nm.

This is mainly due to the nanometer size of the materials which render them:

(i) large fraction of surface atoms;
(ii) high surface energy;
(iii) spatial confinement;
(iv) reduced imperfections, which do not exist in the corresponding bulk materials.

Due to their small dimensions, nanomaterials have extremely large surface area to volume ratio, which makes a large fraction of atoms of the materials to be the surface or interfacial atoms, resulting in more “surface” dependent material properties. Especially when the sizes of nanomaterials are comparable to Debye length, the entire material will be affected by the surface properties of nanomaterials. This in turn may enhance or modify the properties of the bulk materials. For example, metallic nanoparticles can be used as very active catalysts. Chemical sensors from nanoparticles and nanowires enhanced the sensitivity and sensor selectivity.

The nanometer feature sizes of nanomaterials also have spatial confinement effect on the materials, which bring the quantum effects. Nanoparticles can be viewed as a zero dimension quantum dot while various nanowires and nanotubes can be viewed as quantum wires. The quantum confinement of nanomaterials has profound effects on the properties of nanomaterials. The energy band structure and charge carrier density in the materials can be modified quite differently form their bulk count part and in turn will modify the electronic and optical properties of the materials. For example, lasers and light emitting diodes (LED) from both of the quantum dots and quantum wires are very promising in the future optoelections. High density information storage using quantum dot devices is also a fast developing area. Reduced imperfections are also an important factor in determination of the properties of the nanomaterials.

Nanosturctures and nanomaterials favors of a self-purification process in that the impurities and intrinsic material defects will move to near the surface upon thermal annealing. This increased materials perfection affects the properties of nanomaterials. For example, the chemical stability for certain nanomaterials may be enhanced, the mechanical properties of nanomaterials will be better than the bulk materials. The superior mechanical properties of carbon nanotubes are well known.

Due to their nanometer size, nanomaterials are already known to have many novel properties. Many novel applications of the nanomaterials rose from these novel properties have also been proposed. In this chapter, the properties of nanomaterials including the mechanical, thermal, biological, optical and chemical properties of nanomaterials will be addressed.

General Properties

(a)Mechanical Properties

Mechanical properties of materials depend upon the composition on bonds between the atoms viz. covalent, metallic, ionic etc., As a result , purest materials may be inherently weak or strong or brittle. Presence of impurities affects all the properties.

When the size of materials is reduced to nanoscale, materials tend to be single crystals. It has been shown in case of metallic nanocrystalline materials that elastic modulii reduce dramatically. For example in case of magnesium nanocrystalline materials (grains ~12nm size) Young's modulus was observed to be 3900 N/mm2 as against 4100 N/mm2 for polycrystalline (grain size>1um) magnesium. Palladium nanocrystallites of ~8nm size had Young's modulus 8800 N/mm2 as against 1230 N/mm2 for polycrystalline palladium.

Plastic deformation in nanocrystallline materials strongly differs from that of polycrystalline bulk counter part. In nickel stress removal results in more effective recovery of the materials as compared to corresponding polycrystalline material.

Hardness of materials is also related to grain size. For copper in micrometer grain size range there is a linear dependence of hardness on particle size. It increase with increase of grain size. However in nanometer size range the hardness increases with decrease of particle size linearly. Similar results are found in case of palladium nanoparticles and microparticles.

Applications of Mechanical Properties of Nanomaterials

Tougher and harder cutting tools: Cutting tools made of nanomaterials, such as tungsten carbide, tantalum carbide, and titanium carbide, are much harder, much more wear-resistant, erosion-resistant, and last longer than their conventional (large-grained) counterparts. Also, for the miniaturization of microelectronic circuits, the industry needs micro drills (drill bits with diameter less than the thickness of an average human hair or 100 μm) with enhanced edge retention and far better wear resistance. Since nanocrystalline carbides are much stronger, harder, and wear-resistant, they are currently being used in these micro drills.

Automobiles with greater fuel efficiency: In automobiles, since nanomaterials are stronger, harder, and much more wear-resistant and erosion-resistant, they are envisioned to be used in spark plugs. Also, automobiles waste significant amounts of energy by losing the thermal energy generated by the engine. So, the engine cylinders are envisioned to be coated with

nanocrystalline ceramics, such as zirconia and alumina, which retain heat much more efficiently that result in complete and efficient combustion of the fuel.

Aerospace components with enhanced performance characteristics: One of the key properties required of the aircraft components is the fatigue strength, which decreases with the component's age. The fatigue strength increases with a reduction in the grain size of the material. Nanomaterials provide such a significant reduction in the grain size over conventional materials that the fatigue life is increased by an average of 200-300%. In spacecrafts, elevated-temperature strength of the material is crucial because the components (such as rocket engines, thrusters, and vectoring nozzles) operate at much higher temperatures than aircrafts and higher speeds. Nanomaterials are perfect candidates for spacecraft applications, as well.

Ductile ceramics: Ceramics are very hard, brittle, and hard to machine even at high temperatures. However, with a reduction in grain size, their properties change drastically. Nanocrystalline ceramics can be pressed and sintered into various shapes at significantly lower temperatures. Zirconia, for example, is a hard, brittle ceramic, has even been rendered superplastic, i. e., it can deformed to great lengths (up to 300% of its original length). However, these ceramics must possess nanocrystalline grains to be superplastic. Ceramics based on silicon nitride (Si3N4) and silicon carbide (SiC), have been used in automotive applications as high-strength springs, ball bearings, and valve lifters, and because they possess good formability and machinabilty combined with excellent physical, chemical, and mechanical properties. They are also used as components in high-temperature furnaces.

Better insulation materials: Aerogels are nanocrystalline porous and extremely lightweight materials and can withstand 100 times their weight. They are currently being used for insulation in offices, homes, etc. They are also being used as materials for "smart" windows, which darken when the sun is too bright and they lighten themselves otherwise.

(b) Structural Properties

Small clusters or nanoparticles are not just the fragments of bulk materials. There can entirely different structure as well as bonds and bond strengths in nanomaterials. As an example consider silicon crystal. Bulk silicon crystallizes in diamond structure. Small clusters of silicon atoms can be considered as fragments of the unit cell.

Even though some nanomaterials with slightly large number of atoms (>50-60 atoms) may acquire bulk crystalline materials, it is found that the lattice parameters may not be the same as in the bulk materials. For example, X-ray diffraction patterns of ZnS of 1.4 nm particles had liquid like disorder. However, larger nanocystals of ZnS indeed show same sphalerite (cubic) structure as in the bulk. It has been observed that there is a lattice contraction of ~1 for 1.4 nm ZnS nanoparticles. Other small particles also show upto ~2.3% lattice constant deviations compared to bulk crystalline materials.

Temperature and pressure also have profound effect on crystal structure. With increase in temperature the disordered structure of small particles of ZnS were found to transform to wurtzite (hexagonal) structure. Further, chemical capping, often used in the synthesis of nanoparticles, gets removed and particles tend to agglomerate or coalesce forming large particles.

Effect of pressure on structural properties (using x-ray diffraction) has also been well investigated for some nanoparticles. It has been found that indeed the structural transformations do take place in case of nanoparticles with applied pressure. However, the pressures required for this are larger for nanoparticles than for corresponding bulk material and depend upon the particle size for CdSe nanoparticles. Thus CdSe nanoparticles of 2 to 4 nm size required 4.9 GPa to 3 GPa pressure to transform them from wurtzite to rock salt structure. Bulk CdSe needs just 2.0 GPa for the same transformation.

(c) Melting

A variety of nanoparticles like Au, Ag, CdS etc., have been investigated for their thermal stability and melting. Melting begins at the surface. As the particle size decreases, surface t bulk atom ratio increases dramatically. In small particles or cluster the central atom may be considered as surrounded by first, second, third, … compact shell of atoms. First shell would have 12 atoms, second shell would have 42 atoms and so on. The number of surface atoms is quite large in nanoparticles and surface to bulk atoms ratio goes on increasing with decreasing particle size (or shells). Large surface is related to large surface energy. This energy can be lowered by melting. Melting temperature of gold nanoparticles of 3-4 nm size is reduced by ~500 C compared to bulk melting point.Melting of nanoparticles is determined either by X-ray diffraction or electron diffraction. Heating increases the lattice parameter and at melting long range order is lost.

(d) Electrical Conductivity

Materials are often classified according to their ability to let current flow through them. Conductivity is defined in terms of the properties of electrons in the solids. Resistivity is the inverse of conductivity. Metals are characterized by very low resistivity (~10-6 ohms.cm). Semiconductors have medium resistivity (few ohmcm) and insulators have larger resistivity (> 10^3 ohm.cm). The resistivity (or conductivity) in solids can be measured in principle by connecting electrically conducting wires to solid material of known geometry, applying a voltage difference across it and measuring the current flowing through it. Current flowing through it is given by Ohm's law. For a metal, current-voltage is a linear graph.

If we reduce the dimensions of metal piece (or introduce a semiconductor nanoparticle or quantum dot) to ~100 nm or less and wish to measure its conductivity, then it is useful to put capacitors on either side so that direct contact between electrodes and metal particle is avoided. There appears then a region around zero voltage for which there is no current flow. This phenomenon is known as Coulomb blockade. Repeated tunneling of single electrons produces what is known as Coulomb Staricase.

Resistivity in nanomaterials is in general larger than that in polycrystalline materials. The electrons get scattered at grain boundaries resulting into increase of resistance. Therefore, electrical resistance of polycrystalline materials is larger than that of corresponding single crystal materials. In materials having nanocrystalline grains have larger number of boundaries exist, compared to polycrystalline materials having micrometer sized grains. Therefore, resistivity of materials having nano sized grains is generally quite large.

Applications of Electrical Properties of Nanomaterials: High energy density batteries. Conventional and rechargeable batteries are used in almost all applications that require electric power. The energy density (storage capacity) of these batteries is quite low requiring frequent recharging. Nanocrystalline materials are good candidates for separator plates in batteries because they can hold considerably more energy than conventional ones. Nickel-metal hydride batteries made of nanocrystalline nickel and metal hydrides are envisioned to require far less frequent recharging and to last much longer.

Large electrochromic display devices; An electrochromic device consists of materials in which an optical absorption band can be introduced, or an existing band can be altered by the passage of current through the materials, or by the application of an electric field. They are

similar to liquid-crystal displays (LCD) commonly used in calculators and watches and are primarily used in public billboards and ticker boards to convey information. The resolution, brightness, and contrast of these devices depend on the tungstic acid gel's grain size. Hence, nanomaterials, such as tungstic oxide gel, are being explored for this purpose.

(e) Optical Properties

Nanocrystalline systems have attracted much interest for their novel optical properties, which differ remarkably from bulk crystals. Key contributory factors include quantum confinement of electrical carriers within nanoparticles, efficient energy and charge transfer over nanoscale distances and in many systems a highly enhanced role of interfaces. With the growing technology of these materials, it is increasingly necessary to understand the detailed basis for nanophotonic properties.The linear and nonlinear optical properties of such materials can be finely tailored by controlling the crystal dimensions, and the chemistry of their surfaces, fabrication technology becomes a key factor for the applications.

Surface Plasmons (SP) are the origin of the color of nanomaterials. An SP is a natural oscillation of the electron gas inside a given nanosphere. If the sphere is small compared to a wavelength of light, and the light has a frequency close to that of the SP, then the SP will absorb energy. The frequency of the SP depends on the dielectric function of the nanomaterial, and the shape of the nanoparticle. For a gold spherical particle, the frequency is about 0.58 of the bulk plasma frequency. Thus, although the bulk plasma frequency is in the UV, the SP frequency is in the visible (close to 520 nm)

Suppose we have a suspension of nanoparticles in a host. If a wave of light is applied, the local electric field may be hugely enhanced near an SP resonance. If so, one expects various nonlinear susceptibilities, which depend on higher powers of the electric field, to be enhanced even more.

Luminescence can be excited in some molecules or solids using an external stimulus like electrons, photons or electric field. Semiconductor nanoparticles – doped or undoped- exhibit enchanced luminescence compared to their bulk counterparts.

Applications of Optical Properties of Nanomaterials: Glues containing nanoparticles have optical properties that give rise to uses in optoelectronics. Casings, containing nanoparticles

used in electronic devices, such as computers, offer improved shielding against electromagnetic interference. Electrochromic, devices are similar to liquid-crystal displays (LCD), are been developed with nanomaterials. The incorporation of nanomaterials in surface coatings can provide long-term abrasion resistance without significantly effecting optical clarity, gloss, color or physical properties.

(f) Magnetic properties

Magnetism is a very important property of materials as it has diverse applications like information storage, electron circuits, transformers, motors, actuators, sensors and medical field. Magnetic nanoparticles, assemblies of nanoparticles, magnetic nanowires, magnetic thin films or multilayers films and metal oxide films show interesting magnetoresistive or magneto optical properties.

Ferromagnetic materials like Fe, Co, Ni have very interesting behavior below a critical size, characteristic of each material. Bulk ferromagnetic materials have spontaneously magnetized domains. However, below the critical size domain formation is not energetically favoured and materials prefers to be single domain. In such a situation all the spins of atoms are oriented in one direction. Typically, the particles with a size below 100 nm are likely to be single domain. Single domain particles of extremely small size which do not show coercivity hysterisis are known as superparamagnetic materials. In superparamagenetic particles, spins are oriented in one direction and switch coherently in the opposite direction.

Small particles are characterized by large surface to volume ratio. Therefore surfaces and interfaces play an important role their magnetic properties of nanostructures. At surface there is not only the symmetry breaking of the bulk crystal structure but there is a change in the coordination number as well as change in the lattice constant. Such effects can give rise to observation of ferromagnetic behavior of materials which are not ferromagnetic in the bulk form.

Deposition of one kind of material over the other, of a few nanometer thick, and repeating it several times gives rise to a multilayer. The multilayers are characterized by the presence of a large number of interfaces. The properties of multilayers are therefore governed not only by the parent materials but also by their surface and interface properties. Magnetic multilayers can be ferromagentically or antiferromagnetically coupled. This gives rise to magnetoresistivitly which depends upon the orientation of the magnetic layers. Mangetoresistance (MR) is the

relative change in electric resistance of a material on the application of magnetic field. The change in the resistivity can be quite large and is known as Giant Magneto Resistance (GMR).

Based on GMR effect multilayer structures have been designed for various applications some of which are magnetic tunnel junction (MTJ) and spin valve. A spin valve is a thin film made up of essentially magnetic tri-layers. One layer is magnetically very soft material, meaning it is very sensitive to small magnetic fields. The other is made of a magnetically 'hard' meaning insensitive to fields of moderate size. The central part consists of two magnetic layers, separated by a Cu spacer layer. Spin valves are commercially used in computer read heads.

MTJ material is made of at least two magnetic layers separated by an insulating tunnel barrier.The current flows perpendicular to the film plane. The 3-d transition metal oxides, particularly the manganites which have pervoskite structure have improved deviceperformance as compared to the GMR materials. These oxides display a diverse nature of properties such as paramagnetic to ferromagnetic transition accompanied by insulator to metal transition and realization of high magnetoresistance on application of low magnetic field.

Applications of Magnetic Properties of Nanomaterials: High-power magnets Magnets made of nanocrystalline yttrium-samarium-cobalt grains possess very unusual magnetic properties due to their extremely large surface area. Typical applications for these high-power rare-earth magnets include quieter submarines, automobile alternators, land-based power generators, and motors for ships, ultra-sensitive analytical instruments, and magnetic resonance imaging (MRI) in medical diagnostics.

(g) Chemical Properties

One of the important factors for the chemical applications of nanomaterials is the increment of their surface area which increases the chemical activity of the material.

Applications of Chemical Properties of Nanomaterials: Due to their enhanced chemical activity, nanostructural materials can be used as catalysts to react with such noxious and toxic gases as carbon monoxide and nitrogen oxide in automobile catalytic converters and power generation equipment to prevent environmental pollution arising from burning gasoline and coal. Fuel cell technology is another important application of the noble metal nanoparticles relating the catalysis of the reactions. In the present, the fuel cell catalysts are based on platinum group metals (PGM). Pt and Pt-Ru alloys are some of the most frequently used catalysts from

this group. In fact, the use of these metals is one major factor for cell costs, which has been one of the major drawbacks preventing it from growing into a more important technology. One possibility to produce economical catalysts is the use of bimetallic nanoparticles.

Metal Nanoparticles – properties

(a) Magic numbers: A high intensity laser bean is incident on a metal rod, causing evaporation of atoms from the surface of the metal. The atoms are then swept away by a burst of helium and passed through an orifice into a vacuum where the expansion of the gas causes cooling and formation of clusters of the metal atoms. These clusters are then ionized by UV radiation and passed into a mass spectrometer that measures their mass:charge ratio. The mass spectrum shows that clusters of 7 and 10 atoms are more likely than other clusters, which means that these clusters are more stable than clusters of other sizes. The ionization potential is the energy necessary to remove the outer electron from the atom. The mamimum ionization potential occurs for the rare-gas atoms. More energy is required to remove electron from filled orbitals than from unfilled orbitals. Peaks are observed at clusters having two and eight atoms. These numbers are referred to as electron magic numbers.

(b) Jellium model: The jellium model envisions a cluster of atoms as a large atom. The positive nuclear charge of each atom of the cluster is assumed to be uniformly distributed over the sphere of the cluster.

(c) Geometric Structure: Generally the crystal structure of large nanoparticles is the same as the bulk structure with somewhat different lattice parameters. X-ray diffraction studies of 80-nm aluminium particles have shown that it has the face-centered cubic (FCC) unit cell, which is the structure of the unit cell of bulk aluminium. However, in some instances it has been shown that small particles having diameters of <5nm may have different structures. For example, it has been shown that 3-5 nm gold particles have an icosahedrdal structure rather than the bulk FCC structure.

(d) Electronic structure: When atoms form a lattice, the discrete energy levels of the atoms are smudged out into energy bands. The term density of states refers to the number of energy levels in a given interval of energy. For a metal, the top band is not totally filled. In the case of a semiconductor the top occupied band, called the valence band, is filled, and there is a small energy separation referred to as the band gap between it and the next higher unfilled band.

When a metal particle having bulk properties is reduced in size to a few hundered atoms, the density of states in the conduction band, the top band containing electrons, changes dramatically. The continuous density of states in the band is replaced by a set of discrete energy levels, which may have energy level spacings larger than the thermal energy and gap opens up. The small cluster is analogous to a molecule having discrete energy levels with bonding and antibonding orbitals. Eventually a size is reached where the surface of the particles are separated by distances which are in the order of the wavelengths of the electrons. In this situation the energy levels can be modeled by the quantum-mechanical treatment of a particle in box. This is referred to as the quantum size effect.

The color of material is determined by the wavelength of light that is absorbed by it. The absorbtion occurs because electrons are induced by the photons of the incident light to make transitions between the lower-lying occupied levels and higher unoccupied energy levels of the materials. Clusters of different sizes will have different electronic structures, and different energy-level separations. Light induced transitions between these levels determines the color of materials. This means that clusters of different sizes can have different colors, and the size of cluster can be used to engineer the color of material.

(e) Reactivity: Since the electronic structure of nanoparticles depends on the size of the particle, the ability of the cluster to react with other species should depend on cluster size. This has important implications for the design of catalytic agents. High catalytic activity is observed for gold nanoparticles smaller than 3-5 nm, where the structure is icosahedrdal instead of the bulk FCC arrangement. This work has led to the development of odor eaters for bathrooms based on gold nanoparticles on a Fe_2O_3 substrate.

Semiconducting nanoparticles- properties

(a) Optical properties: Nanoparticles made of cadmium, germanium, or silicon are not themselves semiconductors. A nanoparticles of Si can be made by laser evaporation of a Si substrate in the region of helium gas pulse. The beam of neutral clusters is photolyzed by a UV laser producing ionized clusters whose mass to charge ratio is then measured in a mass spectrophotometer.

The most striking property of nanoparticles made of semiconducting elements is the pronounced changes in their optical properties compared to those of the bulk material. There is significant shift in the optical absorption spectra toward the blue (shorter wavelength) as the particle size is reduced.

In a bulk semiconductor a bound electron-hole pair, called an exciton, can be produced by a photon having an energy greater than that of the band gap of the material. The band gap is the energy separation between the top filled energy level of the valence band and the nearest unfilled level in the conduction band above it. The photon excites and electron from the filled band to the unfilled band above. The result is a hole in the otherwise filled valence band, which corresponds to an electron with an effective positive charge. Because of the Columb attraction between the positive hole and the negative electron, a bound pair, called an exciton, is formed that can move through the lattice. The separation between the hole and the electron is many lattice parameters. The existence of the exciton has a strong influence on the electronic properties of the semiconductor and its optical absorption.

An exciton can move in the crystal whose center of mass motion is quantized. Different kinds of excitons are identified in a variety of materials. When the electron-hole pair is tightly bound with distance between electron and hole comparable to lattice constant then it is called Frenkel exciton. At the other extreme, one may have an exciton with electron-hole separation much larger compared to lattice constant. Such a weakly bound electron-hole pair is called Mott-Wannier exciton.

Cd_2P_2 is a dark brown semiconductor with energy gap of approximately 0.5 eV. When its particles are made, it progressively passes through a series of colours like brown, red, yellow and white with particle size changing from ~30A to ~15A. For ~15 A particles the band gap increases to 4 eV. The same is true for CdS. The bulk semiconductor with energy gap of 2.42 eV is orange in colour. As the particles become smaller and energy gap increases it becomes yellowish and ultimately white.

(b) Luminescence:

Luminescence can be excited in some molecules or solids using an external stimulus like electrons, photons or electric field. Semiconductor nanoparticles- doped or undoped- have been widely investigated as they exhibit enhanced luminescence compared to their bulk counterparts.

(i) photoluminescence: When the external source is photons, the luminescence is known as photoluminescence. An electron from a valence band can be excited to a level in the conduction band if photon of sufficient energy to make a transition is available. This process

leaves a hole in the valence band. The excited electron can loose energy by emission of photon in a relatively shorter time before it can relax and make a radiative transition.

(ii) Electroluminescence: Luminescence observed by the application of an electric field to a material is known as electrolumnisence. It can be observed by applying either low or high field; accordingly it is classified as 'injection luminescence' and 'high field electroluminescence' respectively. Light emitting diodes are based on the principle of minority carrier injection in a diode. High field electroluminescence is used in 'display panel'. Emission of electron by application of very high electric field is known as field emission.

(iii) Cathodoluminescence: Electrons of very high energy striking a semiconductor material produce luminescence known as 'cathodoluminescence'. The incident electrons here are from some filament or field emission cathode. Phenomenon of cathodoluminescence is used in oscilloscope, TV etc.,

(iv) Thermoluminescence: In semiconductors with large band gaps it is found that if they are excited at very low temperatures with photons in the UV range, on heating to some temperature which depends upon the dopant ions, light is emitted even in the absence of any other stimulus. The phenomenon is known as thermoluminescence or after glow. Thermoluminescence is quite strong in nanomaterials. Thermoluminescence has been reported for ZnS nanoparticles doped with copper.

(c) Photofragmentation

It has been observed that nanoparticles of silicon and germanium can undergo fragmentation when subjected to laser light from a Q-switched Nd:YAG laser. The products depend on the size of the cluster, the intensity of laser light, and the wavelength.

(d) Coulombic explosion

Multiple ionization of clusters causes them to become unstable, resulting in very rapid high-energy dissociation or explosion. The fragment velocities from this process are very high. The phenomenon is called Coulombic explosion.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – II - Nanobiotechnology –SBTA1503

CHARACTERIZATION OF NANOMATERIALS

Introduction

The current revolution in nanoscience was brought about by concomitant development of several advances in technology. One of factor responsible for the nanotechnology revolution has been the improvement of old and the introduction of the new instrumentation systems for evaluating and characterizing nanostructures. Although the techniques to be used would depend upon the type of material and information one needs to know, usually one is interestedin first knowing the size, crystalline type, composition and then chemical state, optical, magnetic and other properties. Some of the commonly used techniques are:

- **Microscopy:** Microscopy is useful to investigate morphology, size, structure and even composition of solids depending upon the type of microscope. Some of the microscopes are able to resolve structures up to atomic resolution. Combined with some other techniques, microscopes can give information about optical, magnetic and other properties of nanomaterials.

  Optical microscope, Confocal Microscope, Scanning Electron Microscope(SEM), Transmission Electron Microscope (TEM) , Scanning Tunnelling Microscope (STM), Atomic Force Microscope (AFM), Scanning Near-Field Optical Microscope (SNOM).

- **Spectroscopy:** Spectroscopies are useful for chemical state analysis (bonding or charge transfer amongst the atoms), electronic structure (energy gaps, impurity levels, band formation, transition probabilities etc.,) and other properties of materials.

  UV-VIS-IR spectroscopy, Fourier Transform Infra Red (FTIR), Atomic absorption Spectroscopy, Electron Spin Resonance (ESR), Nuclear Magnetic Resonance (NMR), Raman Spectroscopy, Auger Electron Spectroscopy.

- **Diffraction:** Diffraction techniques are often used in average particle size analysis as well as structural determination.

  X-ray Diffraction, Electron Diffraction, Neutron Diffraction, Small Angle X-ray scattering (SAXS), Small Angle Neutron Scattering (SANS).

Atomic structure

To understand a nanomaterial we must, first, learn about its structure, meaning that we must determine the type of atoms that constitute its building blocks and how these atoms are arranged relative to each other. Most nanostructures are crystalline, meaning that their thousands of atoms have a regular arrangement in space on what is called a crystal lattice. This lattice can be described by assigning the positions of atoms in a unit cell, so the overall lattice arises from the continual replication of this unit cell throughout space. There are 17 possible types of crystal structures called space groups, meaning 17 possible arrangements of atoms in unit cells in two dimensions. The characteristics of the parameters are a, b, and . In three dimensions the situation is much more complicated. There are now three lattice constants a, b, c, for the three dimensions x, y, z with the respective angles between them. There are seven crystal systems in three dimensions with a total of 230 space groups The objective of a crystal structure analysis is to distinguish the symmetry and space group, to determine the values of lattice constants and angles, and to identify the positions of the atoms in the unit cell.

Certain special cases of crystal structures are important for nanocrystals, such as those involving simple cubic (SC), body-centered cubic(BCC), and face-centered cubic(FC) unit cells. Another important structural arrangement is formed by stacking planar hexagonal layers, which for a monoatomic crystal provides the highest density of closest-packed arrangement of identical spheres. If the third layer is placed directly above the first layer, the fourth directly above the second, and so on, in an A-B-A-B… type sequence, the hexagonal close-packed (HCP) structure results. If, on the other hand, this stacking is carried out by placing the third layer in a third position and the fourth layer above the first, and so forth, the result is an A-B-C-A-B-C-A….. sequence, and the structure is FCC. The later arrangement is more commonly found in nanocrystals.Some properties of nanostructures depend on their crystal structure, while other properties such as catalytic reactivity and adsorption energies depend on the type of exposed surface.

Microscopy

(a) Electron Microscopes: In electron microscopes electrons and electrostatic or magnetic lenses are used. According to wave-particle duality, electrons have both particle and wave nature. Therefore, just like electromagnetic radiation, which can be used to image the objects, electrons can be used to image the objects. Advantage of using electrons is that their wavelength can be tuned to a very small value, just by changing their energies so that the

resolution can be increased. Although the wavelengths appear to be very small and one would expect extremely high resolution, in general the interactions between electrons and solids are quite complicated due to charge on electrons and subsequent interaction with electrons and ions in solids. This interaction results into back scattering of electrons, production of Auger electrons, visible light, UV light, X-rays etc., depending upon the energy of electrons, type of sample and thickness of sample. There are two types of electron microscopes viz., scanning electron microscope (SEM) and Transmission electron microscope (TEM). Scanning electron microscope uses backscattered electron from a sample for imaging and transmission electron microscope utilizes electrons transmitted through a sample. SEM can be used to image the surface of thick sample but TEM needs to have think sample so that high energy electron which need to reach the sample without getting scattered by air. Therefore the electron microscope need vacuum for their operation.

(i) Scanning electron microscope: In an electron microscope electrons emitted from a hot filament is usually used. However, sometimes cold cathode (a cathode which emits electrons without heating it) is also used. A cold cathode emits electrons under the application of a very high electric field. It is also known as field emitter. Such SEMs are known as FE-SEM and are able to give better images than hot filament SEM. In scanning electron microscope backscattered electrons or secondary electrons are detected. Due to interaction of focused beam with solid, the backscattered electrons are defocused resulting into lowered resolution than one would expect. In an electron microscope, the electron beam can be focused to a very small spot size using electrostatic or magnetic lenses. Usually the electrostatic lenses are used for an SEM. The fine beam is scanned or rastered on the sample surface using a scan generator and back scattered electrons are collected by an appropriate detector.

Signal from scan generator along with amplified signal from the electron collector generates the image of sample surface. In order to avoid the oxidation and contamination of filament as well as reduce the collision between air molecules and electrons, filament and sample have to be housed in a vacuum chamber. Usually vacuum ~10-5 torr is necessary for a normal operation of scanning of electron microscope. Electrons are accelerated as usual in a vacuum system but they enter the sample chamber through a thin foil or aperture so that a large pressure difference can be maintained.

One disadvantage of SEM is that insulating samples cannot be analyzed directly as they get charged due to incident electrons and images become blurred/faulty. Therefore, insulating

solids are coated with a very thin metal film like gold or platinum making them conducting without altering any essential details of the sample. The metal film is usually sputter coated on the sample to be investigated prior to the introduction into SEM.

(ii) Transmission electron microscope: TEM is ideally suitable for investigating the nanomaterials as very high resolution is possible using it. As the name suggests the electrons are transmitted through the specimen in this microscope. Electrons of very high energy are used which pass through a series of magnetic lenses, as in SEM. The various components of TEM are electron source, condenser lens, specimen, objective lens, and a fluorescent screen inthe given order. There may be additional lenses in different microscope in order to improve the image quality and resolution. The lenses are electromagnetic whose focal lengths are varied to obtain optimized images. Similar to SEM, the components of a TEM also have to be housed in a chamber having vacuum for its proper functioning.

(b) Scanning probe microscopes (SPM): SPM is a generic name given to a family of microscopes in which a sharp tip of a metal is scanned across a sample surface in a raster mode to produce the images of samples even at subatomic resolution. The first SPM known as Scanning Tunneling Microscope (STM) was developed in 1982 by G. Binnig and H. Rohrer for which they received Nobel Prize in 1986 along with Ernst Ruska. Subsequently many other SPMs like Atomic Force Microscope (AFM), Scanning Near Optical Field Microscope (SNOM) were developed to overcome some of the limitations as well as carry out 'spectromicroscopies' i.e., microscopy as well as spectroscopy combined in the same instrument so that spectroscopy of same sample area is performed. With spectromicroscopy, one is able to get not only the details of morphology and structure of a material but also know the chemical nature or electronic structure of the material and study mechanical, thermal, optical, magnetic and many other physical properties too. Besides these benefits, great advantage is that unlike in electron microscopy, no special sample preparation is necessary nor vacuum is necessary. One can use even liquid environment for these microscopes. In STM, AFM, and SNOM microscopes for nanotechnology work, scanning probe and raster principle is common. Probe is a fine metal tip of ~10 nm diameter. Tip materials are Si, Pt-Ir, Pt-Rh etc. Even diamond film coated tips are used. Tips are obtained by etching a fine metal wire in some suitable chemicals or lithography. In case of STM, tip is directly mounted on specially designed piezo drive (or piezo tube). For AFM, investigation tip is mounted on a cantilever which is then mounted on a piezo drive. Function of a piezo drive is to scan the sample surface to be imaged. Materials like lead zirconium titanate (PZT) are known to be piezo crystals. Using dopants

properties of piezo crystals can be changed. SPM scans are made over few nm to 100 um in the horizontal plane and about few nm to 10 um in vertical plane. Scanning is usually fast in one line going slowly to the other line. An image of the predetermined surface area is usually acquired in few minutes. Piezo drives along with the tip are very crucial in determining the resolution of the acquired images. In order to achieve very high resolutions, it is essential that the microscopes be shielded from mechanical vibrations. As the forces involved are very small, influence of external magnetic field as well as electrical noise need to be avoided.

(i) Scanning Tunneling Microscope (STM): It is based on tunneling principle. When two metals say M1 and M2 are brought at small distance (but larger than about 10 nm) even though their Fermi level do not coincide, transfer of electrons from one metal to the other is not possible. To transfer electrons from one metal to the other, it is necessary for the electrons in the vicinity of the Fermi level to overcome the potential barrier known as the work function of the material. Typically the work functions of metals are few electron volts (2-5 eV) and transfer of electrons at room temperature is forbidden. However, the metals brought in extremely close distance of the order of a few nanometers (usually less than 10 nm) behave differently. Electrons can be transferred from one metal to the other to establish a common Fermi level without going over the potential barrier, set by the work function. At short distance of few nanometers, the wave functions of electrons from either side decay into the other metal. In other words, electrons can 'tunnel' from one metal to the other to occupy state of lower energy. This causes Fermi level of the two metals to coincide with a small 'contact potential'.

An STM can be operated in two different modes:

- Constant current mode: Probe in the form of a sharp metal tip is moved slowly on the sample surface such the current between the tip and sample remains constant. In order to maintain the constant current between the tip and the sample, distance between the tip and the atomic corrugations also need to be kept constant. Thus the tip will have to follow the atom contours. By successively scanning the desired sample area in a raster mode, profile of surface atoms can be generated as an image. This is known as constant current mode.
- Constant height mode: Alternatively, the tip can be moved on the sample surface at a constant height (typically >0.5 nm). As there is relation between current and the distance, a surface profile can be constructed from the variations observed in the tunnel current. Thus the image is constructed from the variations of current as the tip scans the

desired area of the sample surface. Advantage of the constant height mode as compared to the constant current mode is that the tip can be moved faster on the sample surface as there is no necessity of the feed back circuit.

Major limitation of STM is that tunneling current has to flow between the sample and the probe. Although, the current is very small, it can be detected. However, in case of insulating samples, current is not obtained. Therefore, realizing this problem other scanning probe microscopes were developed. Limitation of an STM is that it requires the sample to be a conductor or at least a semiconductor.

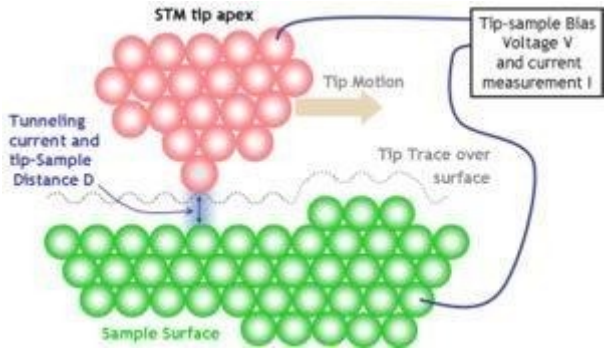


Figure 2.1 Basic overview of the scanning tunneling microscope tip-sample interaction. When the tip is within atomic distance of the sample surface and a small bias voltage about a volt or so is applied, tunneling current can be measured. Adjusting the height of the tip while scanning the tip over the surface with a fixed bias voltage to always maintain a constant tunnel current will map out the sample topography.

(ii) *Atomic Force Microscope:* AFM has a flexible cantilever ~100 um long, 10 um width and 1um in height attached to a piezo drive. A tip is mounted on cantilever, which can be brought close to sample surface. The tip experiences a repulsive force which results into minute amount of bending of the cantilever. A laser beam is directed on back of the cantilever which after reflection passes to a position sensitive detector. Small deflections caused by the tip-sample interaction are recorded by a position sensitive photodiode. By rastering the probe on the sample surface and measuring the cantilever deflections, surface image is obtained. An AFM can be operated in three different modes:

- Contact mode: In this case the tip is contact with the sample surface and is almost forced into it. However, due to repulsive interaction between electron charge cloud of the tip atom and that of the surface atom, the tip is repelled back which bends the cantilever and deviates the direction of the laser beam. The main disadvantage of this mode is that the tip or sample can get damaged due to forcing of the tip into sample.
- Non-contact mode: In this mode the tip or the probe moves at some small distance away from the sample surface. Therefore it cannot damage the sample.
- Tapping mode: It is the combination of contact and non-contact modes. The resolution if contact mode is higher than that due to non-contact mode, because in contact mode the interaction between tip and surface atoms is much more sensitive to the distance as compared to that in non-contact mode. With tapping mode, high resolution advantage of contact mode and non-destructiveness of non-contact mode are achieved.

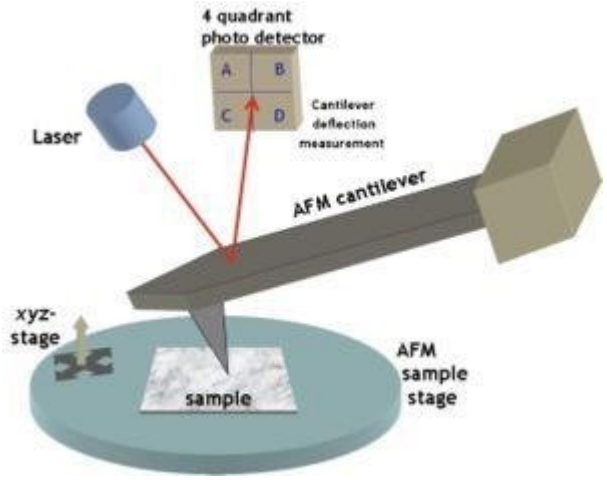
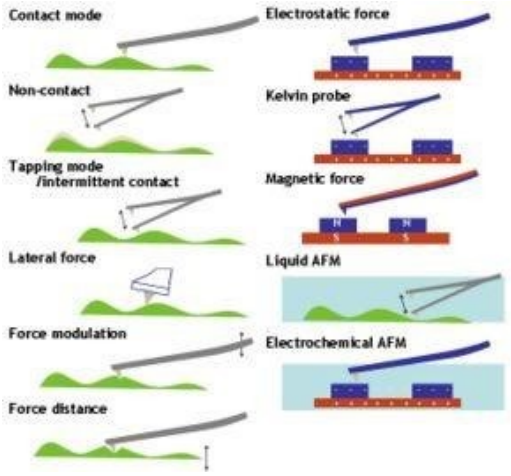


Figure 2.2. Typical AFM setup. The deflection of a microfabricated cantilever with a sharp tip is measuredbe reflecting a laser beam off the backside of the cantilever while it is scanning over the surfaceof the sample.

Figure 2.3. Methods in AFM:



A wealth of techniques are used in AFM to measure the topography and investigate the surface forces on the nanoscale:

For imaging sample topography:

- Contact mode, where the tip is in contact with the substrate. Gives high resolution but can damage fragile surfaces.
- Tapping / intermittent contact mode (ICM), where the tip is oscillating and taps the surface.
- Non-contact mode (NCM), where the tip is oscillating and not touching the sample.

For measuring surface properties (and imaging them):

- Lateral force microscopy (LFM), when the tip is scanned sideways it will tilt and this can be measured by the photodetector. This method is used to measure friction forces on the nanoscale.
- Force Modulation Microscopy. Rapidly moving the tip up and down while pressing it into the sample makes it possible to measure the hardness of the surface and characterize it mechanically.
- Electrical force microscopy. If there are varying amount of charges present on the surface, the cantilever will deflect as it is attracted and repelled. kelvin probe microscopy will normally be more sensitive than measuring s static deflection.

    - Kelvin probe microscopy. By applying an oscillating voltage to an oscillating cantilever in non-contact mode and measuring the charge induced oscillations, a map can be made of the surface charge distribution.
    - Dual scan method - an other kelvin probe method described below.
- Magnetic Force Microscopy. If the cantilever has been magnetized it will defelct depending on the magnetization of the sample.
- Force-spectroscopy or force-distance curves. Moving the cantilever up and down to make contact and press into the sample, one can measure the force as function of distance.
- Nanoindentation. When pressing the cantilever hard into a sample it can leave an imprint and in the force distance curve while doing indentation can tell about the yield stress, elastic plastic deformation dynamics.
- Liquid sample AFM. By immersing the cantilever in a liquid one can also image wet samples. It can be difficult to achieve good laser alignment the first time.
- Electrochemical AFM.
- Scanning gate AFM
- Nanolithography
- Dip-pen lithography

(iii) *Scanning Near-Field Optical Microscope (SNOM):* The imaging in conventional optical microscope is based on the principle of interference of light waves. It is known that the reflected light or the light leaving from the luminous object has two components viz., evanescent beam and propagating beam. Evanescent beam is related to what is known as near field and propagating beam is related to far field. Near field extends to very small distance and evanescent beam does not have a propagating wave nature. However photons cannot be trapped and must escape as propagating waves. This in fact is quite advantageous. If the near field zone is disturbed it also affects the far field and propagating waves. This idea is used to overcome the diffraction limit and obtain a high resolution using scanning near-field microscope.

A special probe with very small aperture of few nm diameter is brought very close to the sample surface. The diameter of the aperture as well as distance between aperture and sample has be smaller than the wavelength of light. Under such conditions light leaves the sample before diffracting. The resolution obtained in a SNOM will depend upon the size of the aperture and the distance at which the probe can be placed. Very fine optical fibers are tapered to the

diameter of less than 100 nm and coated with some metal like aluminium. Metal coating of the fiber aperture is necessary because narrower the aperture greater are the chances that the light can escape from the sides of the aperture wall. SNOM is integrated with other techniques like STM, AFM, Raman spectroscopy etc. so that on the same sample different analysis can be performed.

An intense beam from a laser source is passed through an optical fiber which is tapered at one end forming an aperture few nm in size. This form a probe of SNOM. Using the piezo drives similar to STM or AFM, the probe is brought in the vicinity of the sample to be analyzed. Keeping some very small fixed distance from the sample, the probe is scanned over the sample surface. Intense laser light illuminates the surface in such a way that the beam reaches the sample before diffraction, satisfying the condition necessary to observe the near-field variations. The reflected or transmitted light carries, therefore, the signature of the near-field changes of the sample. Measurement in 'far-field' mode of the propagating waves, just as in conventional optical microscope still carries the near-field information. Observation in both reflection and transmission geometries are possible.

Spectroscopy

The spectroscopic techniques described below do not provide a three-dimensional picture of a molecule, but instead yield information about certain characteristic features. A brief summary of this information follows:

- **Mass Spectrometry**: Sample molecules are ionized by high energy electrons. The mass to charge ratio of these ions is measured very accurately by electrostatic acceleration and magnetic field perturbation, providing a precise molecular weight. Ion fragmentation patterns may be related to the structure of the molecular ion.
- **Ultraviolet-Visible Spectroscopy**: Absorption of this relatively high-energy light causes electronic excitation. The easily accessible part of this region (wavelengths of 200 to 800 nm) shows absorption only if conjugated pi-electron systems are present.
- **Infrared Spectroscopy**: Absorption of this lower energy radiation causes vibrational and rotational excitation of groups of atoms. within the molecule. Because of their characteristic absorptions identification of functional groups is easily accomplished.
- **Nuclear Magnetic Resonance Spectroscopy**: Absorption in the low-energy radio-frequency part of the spectrum causes excitation of nuclear spin states. NMR

spectrometers are tuned to certain nuclei (e.g. ^{1}H, ^{13}C, ^{19}F & ^{31}P). For a given type of nucleus, high-resolution spectroscopy distinguishes and counts atoms in different locations in the molecule.

(a) Laser Scanning Confocal Microscopy (LSCM): Confocal laser scanning microscopy (CLSM or **LSCM)** is a technique for obtaining high-resolution optical images. The key feature of confocal microscopy is its ability to produce in-focus images of thick specimens, a process known as *optical sectioning*. Images are acquired point-by-point and reconstructed with a computer, allowing three-dimensional reconstructions of topologically-complex objects. Confocal laser scanning microscopy is a technique that allows a much better resolution from optical microscopes and three dimensional imaging.

Using a high NA objective also gives a very shallow depth of focus and hence the image will be blurred by structures above or below the focus point in a classical microscope. A way to circumvent this problem is the confocal microscope, or even better the Laser Scanning Confocal Microscope (LSCM). Using a laser as the light source gives better control of the illumintaion, especially when using fluorescent markers in the sample. The theoretical resolution using a 1.4 NA objective can reach 140nm laterally and 230nm vertically [1] while the resolution quoted in ref [2] is 0.5×0.5×1μm. The image in the LSCM is made by scanning the sample in 2D or 3D and recordning the signal for each point in space on a PC which then generates the image.

(b) Photoemission and X-ray spectroscopy: Photoemission spectroscopy (PES) measures the energy distribution of electrons emitted by atoms and molecules in various charge and energy states. A material irradiated with ultraviolet light (UPS) or X-rays (XPS) can emit electrons called photoelectrons from atomic energy levels with a kinetic energy.

X-ray microscopy uses X-rays to image with much shorter wavelength than optical light, and hence can provide much higher spatial resolution and use different contrast mechanisms. X-ray microscopy allows the characterization of materials with submicron resolution approaching the 10's of nanometers. X-ray microscopes can use both laboratory x-ray sources and synchrotron radiation from electron accelerators. X-ray microscopes using synchrotron radiation provide the greatest sensitivity and power, but are unfortunately rather large and expensive. X-ray microscopy is usually divided into two overlapping ranges, referred to as soft x-ray microscopy (100eV - 2keV) and hard x-ray microscopy (1keV-40keV). All x-rays

penetrate materials, more for higher energy x-rays. Hence, soft x-ray microscopy provides the best contrast for small samples. Hard x-rays do have the ability to pass nearly unhindered through objects like your body, and hence also give rather poor contrast in many of the biological samples you would like to observe with the x-ray microscope. Nevertheless, hard x-ray microscopy allows imaging by phase contrast, or using scanning probe x-ray microscopy, by using detection of fluorescent or scattered x-rays. Despite its limitations, X-ray microscopy is a powerful technique and in some cases can provide characterization of materials or samples that cannot be done by any other means.

An **X-ray microscope** uses electromagnetic radiation in the soft X-ray band to produce images of very small objects.Unlike visible light microscopes, X-rays do not reflect or refract easily, and they are invisible to the human eye. Therefore the basic process of an X-ray microscope is to expose film or use a charge-coupled device (CCD) detector to detect X-rays that pass through the specimen, rather than light which bounces off the specimen. It is a contrast imaging technology using the difference in absorption of soft x-ray in the water window region (wavelength region: 2.3 - 4.4 nm, photon energy region: 0.28 - 0.53 keV) by the carbon atom (main element composing the living cell) and the oxygen atom (main element for water).

Sources of soft X-rays suitable for microscopy, such as synchrotron radiation sources, have fairly low brightness of the required wavelengths, so an alternative method of image formation is scanning transmission soft X-ray microscopy. Here the X-rays are focused to a point and the sample is mechanically scanned through the produced focal spot. At each point the transmitted X-rays are recorded with a detector such as a proportional counter or an avalanche photodiode.

The resolution of X-ray microscopy lies between that of the optical microscope and the electron microscope. It has an advantage over conventional electron microscopy in that it can view biological samples in their natural state. Electron microscopy is widely used to obtain images with nanometer level resolution but the relatively thick living cell cannot be observed as the sample has to be sliced thinly and then dried to get the image.

Additionally, X-rays cause fluorescence in most materials, and these emissions can be analyzed to determine the chemical elements of an imaged object. Another use is to generate diffraction patterns, a process used in X-ray crystallography. By analyzing the internal reflections of a diffraction pattern (usually with a computer program), the three-dimensional structure of a crystal can be determined down to the placement of individual atoms within its molecules. X-

ray microscopes are sometimes used for these analyses because the samples are too small to be analyzed in any other way.

(c) Infra red and Raman Spectroscopy: Vibrational spectroscopy involves photons that induce transitions between vibrational states in molecules and solids, typically in infrared (IR) frequency range from 2 to 12x1013 Hz. The energy gaps of many semiconductors are in this same frequency region, and can be studied by infrared techniques.

Infrared spectroscopy (IR spectroscopy) is the subset of spectroscopy that deals with the infrared region of the electromagnetic spectrum. It covers a range of techniques, the most common being a form of absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify compounds or investigate sample composition. Infrared spectroscopy correlation tables are tabulated in the literature.

The infrared spectra of a sample is collected by passing a beam of infrared light through the sample. Examination of the transmitted light reveals how much energy was absorbed at each wavelength. This can be done with a monochromatic beam, which changes in wavelength over time, or by using a Fourier transform instrument to measure all wavelengths at once. From this, a transmittance or absorbance spectrum can be produced, showing at which IR wavelengths the sample absorbs. Analysis of these absorption characteristics reveals details about the molecular structure of the sample.

This technique works almost exclusively on samples with covalent bonds. Simple spectra are obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra. The technique has been used for the characterization of very complex mixtures

A beam of infrared light is produced and split into two separate beams. One is passed through the sample, the other passed through a reference which is often the substance the sample is dissolved in. The beams are both reflected back towards a detector, however first they pass through a splitter which quickly alternates which of the two beams enters the detector. The two signals are then compared and a printout is obtained.

A reference is used for two reasons:

- This prevents fluctuations in the output of the source affecting the data

- This allows the effects of the solvent to be cancelled out (the reference is usually a pure form of the solvent the sample is in

Raman spectroscopy is a spectroscopic technique used in condensed matter physics and chemistry to study vibrational, rotational, and other low-frequency modes in a system. It relies on inelastic scattering, or Raman scattering(**Raman scattering** or the **Raman effect** is the inelastic scattering of a photon. When light is scattered from an atom or molecule, most photons are elastically scattered (Rayleigh scattering). The scattered photons have the same energy (frequency) and wavelength as the incident photons. However, a small fraction of the scattered light (approximately 1 in 1 million photons) is scattered by an excitation, with the scattered photons having a frequency different from, and usually lower than, the frequency of the incident photons. In a gas, Raman scattering can occur with a change in vibrational, rotational or electronic energy of a molecule. Chemists are concerned primarily with the vibrational Raman effect) of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the phonon modes in the system. Infrared spectroscopy yields similar, but complementary information.

Typically, a sample is illuminated with a laser beam. Light from the illuminated spot is collected with a lens and sent through a monochromator. Wavelengths close to the laser line, due to elastic Rayleigh scattering, are filtered out while the rest of the collected light is dispersed onto a detector.

Spontaneous Raman scattering is typically very weak, and as a result the main difficulty of Raman spectroscopy is separating the weak inelastically scattered light from the intense Rayleigh scattered laser light. Raman spectrometers typically use holographic diffraction gratings and multiple dispersion stages to achieve a high degree of laser rejection. In the past, PMTs were the detectors of choice for dispersive Raman setups, which resulted in long acquisition times. However, the recent uses of CCD detectors have made dispersive Raman spectral acquisition much more rapid.

Raman spectroscopy has a stimulated version, analogous to stimulated emission, called stimulated Raman scattering.

Resonance Raman (RR) spectroscopy is a specialized implementation of the more general Raman spectroscopy. As in Raman spectroscopy, RR spectroscopy provides information about the vibrations of molecules, and can also be used for identifying unknown substances. RR spectroscopy has found wide application to the analysis of *bioinorganic* molecules. Although the technique uses a different part of the electromagnetic spectrum than infrared (IR) spectroscopy, the two methods are actually complementary. Both are used to measure the energy required to change the vibrational state of a chemical compound.

IR spectroscopy involves measuring the direct absorption of photons with the appropriate energy to excite molecular bond vibrations. The wavelengths of these photons lie in the infrared region of the spectrum, hence the name of the technique. Raman spectroscopy measures the excitation of bond vibrations in an indirect manner. The two methods are complementary because some vibrational transitions that are observed in IR spectroscopy are not observed in Raman spectroscopy, and vice versa. RR spectroscopy is an improvement of traditional Raman spectroscopy that has increased sensitivity and is better suited for the study of complicated systems.

X-Ray Raman Scattering

In the x-ray region, enough energy is available for making electronic transitions possible. At core level resonances, X-Ray Raman Scattering can become the dominating part of the x-ray fluorescence spectrum. This is due to the resonant behavior of the Kramers-Heisenberg formula in which the denominator is minimized for incident energies that equal a core level. This type of scattering is also known as resonant inelastic x-ray scattering (RIXS). In the soft x-ray range, RIXS has been shown to reflect crystal field excitations, which are often hard to observe with any other technique. Application of RIXS to strongly correlated materials is of particular value for gaining knowledge about their electronic structure. For certain wide band materials such as graphite, RIXS has been shown to (nearly) conserve crystal momentum and thus has found use as a complementary band mapping technique.

Conventional Raman spectroscopy is limited to a spatial resolution on the micron scale. By using novel techniques and materials, information can be gained from structures on a sub-micron or nanometre scale e.g. Raman may be used to classify the diameter of carbon nanotubes, given that the frequency of the radial breathing mode (RBM) is related to the tube diameter. Pioneering products such as the award winning Nanonics NSOM/AFM 100

Confocal™/Renishaw Raman microscope system have demonstrated superior spatial resolution than is possible with the normal far-field diffraction limit.

Surface Enhanced Raman Spectroscopy, often abbreviated **SERS**, is a surface sensitive technique that results in the enhancement of Raman scattering by molecules adsorbed on rough metal surfaces. The enhancement factor can be as much as 10^{14}-10^{15}, which allows the technique to be sensitive enough to detect single molecules.

Raman scattering or the **Raman effect** is the inelastic scattering of a photon.

When light is scattered from an atom or molecule, most photons are elastically scattered (Rayleigh scattering). The scattered photons have the same energy (frequency) and wavelength as the incident photons. However, a small fraction of the scattered light (approximately 1 in 1 million photons) is scattered by an excitation, with the scattered photons having a frequency different from, and usually lower than, the frequency of the incident photons. In a gas, Raman scattering can occur with a change in vibrational, rotational or electronic energy of a molecule. Chemists are concerned primarily with the vibrational Raman effect.

(d) Magnetic Resonance: Another branch of spectroscopy that has provided information on nanostructures is magnetic resonance. It involves the study of microwave (radar frequency) and radiofrequency transitions. Most magnetic resonance measurements are made in fairly strong magnetic fields, typically B~0.33 T for electron spin resonance (ESR), and B~10T for nuclear magnetic resonance (NMR).

Diffraction

Diffraction techniques using electrons, X-rays or neutrons produce information about crystal structure and are used to understand structure (Bravais lattice) of bulk materials and can be extended to investigate nanomaterials. The diffraction analysis relies on the long range periodic arrangement of atoms/molecules.

X-ray diffraction: There are different types of X-ray diffractometers available for crystal structure analysis. The most commonly used diffractometer is known as Powder Diffractometer or Debye- Scherrer diffractometer. This diffractometer allows determination of crystal structure of polycrystalline samples, thin films and nanoparticles. The diffractometer consists of a monochromatic source of X-rays (usually from a copper target), sample holder and an X-ray detector. Both sample and detector move around an axis passing through sample centre and

normal to the plane of the paper. Samples in the form of powder, thin films etc. can be used. The diffracted rays make angle 2 at the detector with respect to incident beam direction. A plot of intensity (counts), as a function of angle 2 (20 to 60), is a diffraction pattern. Detector is a suitable photon counter like Geiger Muller tube, scintillation counter etc. Due to finite size of X-ray beam ~1-2 mm2, smaller angles (<20) are not accessible using these diffractometers.

X-ray scattered by atoms enable us to understand about arrangement of atoms in solids.

Nanomanipulation

AFM manipulation: With AFM nanostructures such as nanotubes and nanowires lying on surfaces can be manipulated to make electrical circuits and measure their mechanical properties and the forces involved in manipulating them.

STM manipulation: Using an STM individual atoms can be manipulated on surfaces to create quantum corals, structuring the wavefunction of the surface electrons between individual atoms added to the surface. The probably demonstrates the higest resolution nanomanipulation

In-situ SEM manipulation: To monitor a three-dimensional nanomanipulation process, in-situ SEM or TEM manipulation seems preferable. AFM (or STM) does have the resolution to image nanoscale objects, even down to the sub-atomic scale, but the imaging frame rate is usually slow compared to SEM or TEM and the structures will normally have to be planar. SEM offers the possibility of high frame rates; almost nanometer resolution imaging of three-dimensional objects; imaging over a large range of working distances; and ample surrounding volume in the sample chamber for the manipulation setup. TEM has a much more limited space available for the sample and manipulation systems but can on the other hand provide atomic resolution. For detailed studies of the nanowires' structure, TEM is a useful tool, but for the assembly of nanoscale components of a well defined structure, such as batch fabricated nanowires and nanotubes, the SEM resolution should be sufficient to complete the assembly task.

To date the tools used for in-situ SEM nanomanipulation have almost exclusively been individual tips (AFM cantilever tips or etched tungsten tips), sometimes tips used together with electron beam deposition have been used to create nanowire devices. Despite the availability of commercial microfabricated grippers in the last couple of years, little has been reported on the use of such devices for handling nanostructures. Some electrical measurements and

manipulation tasks have been performed in ambient conditions with carbon nanotube nanotweezers

In-situ TEM manipulation: TEM offers atomic 3D resolution but the extreme requirements on stability combined with very limited sample space makes the contruction of in-situ TEM manipulation equipment quite a task. With such systems, people have observed freely suspended wires of individual atoms between a gold tip and a gold surface; carbon nanotubes working as nanoscale pipettes for metals and a wealth of other exotic phenomena

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – III - Nanobiotechnology – SBTA1503

Synthesis of Nanomaterials

Introduction

There are a large number of techniques available to synthesize different types of nanomaterials in the form of colloids, clusters, powders, tubes, rods, wires, thin films etc. There are various physical, chemical, biological and hybrid techniques available to synthesize nanomaterials. The technique to be used depends upon the material of interest, type of nanostructure viz., zero dimensional, one dimensional, or two dimensional material size, quantity etc.

- **Physical methods:** *(a) mechanical:* ball milling, melt mixing
  (*b)Vapor:* physical vapor deposition, laser ablation, sputter deposition, electric arc deposition, ion implantation
- **Chemical methods:** colloids, sol-gel, L-B films, inverse micelles.
- **Biological methods:** biomembranes, DNA, enzymes, microorganisms.

Physical methods

(a) Ball milling: It is used in making of nanoparticles of some metals and alloys in the form of powder. Usually the mill contains one or more containers are used at a time to make fine particles. Size of container depends upon the quantity of interest. Hardened steel or tungsten carbide balls are put in containers along with powder or flakes (<50 um) of a material of interest. Initial material can be of arbitrary size and shape. Container is closed with tight lids. The containers are rotated at high speed (a few hundreds of rpm) around their own axis. Additionally they may rotate around some central axis and are therefore called as 'planetary ball mill'. When the containers are rotating around the central axis, the material is forced to the walls and is pressed against the walls. But due to the motion of the containers around their own axis, the material is forced to other region of the container. By controlling the speed of rotation of the central axis and container as well as duration of milling, it is possible to ground the material to fine powder whose size can be quite uniform. Some of the materials like Co, Cr, W, Ni-Ti, Al-Fe, Ag-Fe etc. are made nanocrystalline using ball mill.

Large balls, used for milling, produce smaller grain size and larger defects in the particles. The process may add some impurities from balls. The container may be filled with air or inert gas. However, this can be an additional source of impurity. A temperature rise in the range of 100

to 1100 C is expected to take place during the collisions. Cryo-cooling is used to dissipate the generated heat.

(b) Melt Mixing: It is possible to form or arrest the nanoparticles in glass. Structurally, glass is an amorphous solid, lacking long range periodic arrangement as well as symmetry arrangement of atoms/molecules. When a liquid is cooled below certain temperature, it forms either a crystalline or amorphous solid (glass). Nuclei are formed spontaneously with homogenous (in the melt) or inhomogeneous (on the surface of other materials) nucleation, which can grow to form ordered, crystalline solid. Usually, metals form crystalline solids but, if cooled at very high cooling rate, they can form amorphous solids. Such solids are known as metallic glasses. Even in such cases the atoms try to reorganize themselves into crystalline solids. Addition of elements like B, P, Si etc. helps to keep the metallic glasses in amorphous state. It is possible to form nanocrystals within metallic glasses. It is also possible to form some nanoparticles by mixing the molten streams of metals at high velocity with turbulence. On mixing thoroughly, nanoparticles are formed.

(c) Physical Vapor Deposition: It involves material for evaporation, an inert gas or reactive gas for collosion of material vapor, a cold finger on which clusters or nanoparticles can condense, a scraper to scrape the nanoparticles and piston- anvil (an arrangement in which nanoparticle powder can be compacted). All the processes are carried out in a vacuum chamber so that the desired purity of the end product can be obtained.

Metals or high vapor pressure metal oxides are evaporated or sublimated from filaments or boats of refractory metals like W, Ta, Mo in which materials to be evaporated are held. Size, shape and even the phase of evaporated material can depend upon the gas pressure in deposition chamber. Clusters or nanoparticles condensed on the cold finger (water or liquid nitrogen cooled) can be scraped off inside the vacuum system. The process of evaporation and condensation can be repeated several times until enough quantity of material falls through a funnel in which a piston-anvil arrangement has been provided.

(d) Ionized Cluster Beam Deposition: It is useful to obtain adherent and high quality single crystalline thin films. The set up consists of a source of evaporation, a nozzle through which material can expand into the chamber, an electron beam to ionize the clusters, an arrangement to accelerate the clusters and a substrate on which nanoparticle film can be deposited, all housed in a suitable vacuum chamber. Small clusters from molten material are expanded through the fine nozzle. The vapor pressure, ~10 torr to 10-2 torr needs to be created in the source and the

nozzle needs to have a diameter larger than the mean free path of atoms or molecules in vapor form in the source to form the clusters. On collision with electron beam clusters get ionized. Due to applied accelerating voltage, the clusters are directed towards the substrate. By controlling the accelerating voltage, it is possible to control the energy with which the clusters hit the substrate. Thus it is possible to obtain the films of nanocrystalline material using ionized cluster beam.

(e) Laser Vaporization: In this method, vaporization of the material is effected using pulses of laser beam of high power. The set up is a ultra high vacuum or high vacuum system equipped with inert or reactive gas introduction facility, laser beam, solid target and cooled substrate. Clusters of any material of which solid target can be made are possible to synthesize. Usually laser giving UV wavelength such as excimer laser is necessary because other wavelengths like IR or visible are often reflected by some of the metal surface. A powerful beam of laser evaporates the atoms from a solid source, atoms collide with inert gas atoms (or reactive gases) and cool on them forming clusters. They condense on the cooled substrate. The method is often known as laser ablation. Gas pressure is very critical in determining the particle size and distribution. Simultaneous evaporation of another material and mixing the two evaporated materials in inert gas leads to the formation of alloys or compounds.

(f) Laser Pyrolysis or Laser Assisted Depositon: Here a mixture of reactant gases is decomposed using a powerful laser beam in presence of some inert gas like helium or argon. Atoms or molecules of decomposed reactant gases collide with inert gas atoms and interact with each other, grow and are then get deposited on cooled substrate. Many materials like Al_2O_3, WC, Si_3Ni_4 etc. are synthesized in nanocrystalline form by this method. Here too, gas pressure plays an important role in deciding the particle size and their distribution.

(g) Sputter Deposition: In sputter deposition, some inert gas ions like Ar are incident on a target at a high energy. The ions become neutral at the surface but due to their energy, incident ions may get implanted, get bounded back, create collision cascades in target atoms, displace some of the atoms in the target creating vacancies, interstitials and other defects, desorb some adsorbents, create photons while loosing energy to target atoms or even sputter out some target atoms/molecules, clusters, ions and secondary electrons. Sputter deposition is a widely used thin film deposition technique, specially to obtain stoichiometric thin films from target material. Target material may be some alloy, ceramic or compound. It is a very good technique to deposit multilayer films for mirrors or magnetic films for spintronic applications. Sputter

deposition can be carried out using Direct Current (DC) sputtering, Radio Frequency (RF) sputtering or magnetron sputtering. In all these methods, one uses discharge or plasma of some inert gas atoms or reactive gases. The deposition is carried out in a required gas pressurized high vacuum or ultra high vacuum system equipped with electrodes, one of which is a sputter target and the other is a substrate, gas introduction facility etc.

(h) Chemical Vapour Deposition (CVD): It is a hybrid method using chemicals in vapour phase. Basic CVD process can be considered as a transport of reactant vapour or reactant gas towards the substrate kept at some high temperature where the reactant cracks into different products which diffuse on the surface, undergo some chemical reaction at appropriate site, nucleate and grow to form the desired material film. The by-products created on the substrate have to be transported back to the gaseous phase removing them from the substrate. Vapours of desired material may be often pumped into reaction chamber using some carrier gas. In some cases the reactions may occur through aerosol formation in gas phase. There are various processes such as reduction of gas, chemical reaction between different source gases, oxidation or some disproportionate reaction by which CVD can proceed. However, it is preferable that the reaction occurs at the substrate rather than in the gas phase. Usually temperature ~ 300 to 1200 C is used at the substrate. There are two ways viz., hot wall and cold wall by which substrates are heated. In hot wall set up the deposition can take place even on reactor walls. This is avoided in cold wall design. Besides this, the reaction can take place in gas phase with hot wall design,which is suppressed in cold wall set up. Further, coupling of plasma with chemical reaction in cold wall set up is feasible. Usually gas pressures in the range of 0.1 torr to 1.0 torr are used. Growth rate and film quality depend upon the gas pressure and the substrate temperature. When the growth takes place at low temperature, it is limited by the kinetics of surface tension.

(i) Electric Arc Deposition: This is one of the simplest and useful methods, which leads to mass scale production of fullerenes and carbon nanotubes. It requires water cooled vacuum chamber and electrodes to strike an arc between them. The positive electrode itself acts as the source of material. If some catalyst are to be used, there can be some additional thermal source of evaporation. Inert gas or reactive gas introduction is necessary. Usually the gap between the electrodes is ~1mm and high current ~50 to 100 amperes is passed from a low voltage power supply (~12-15 volts). Inert gas pressure is maintained in the vacuum system. When an arc is set up, anode material evaporates. This is possible as along as the discharge can be maintained. By striking the arc between the two graphite electrodes, it is possible to get fullerenes in large

quantity. In case of fullerenes, the formation occurs at low helium pressure as compared to that used for nanotube formation. Also, fullerenes are obtained by purification of soot collected from inner walls of vacuum chamber, whereas nanotubes are found to be formed only at high He gas pressure and in the central portion of the cathode. No carbon nanotubes are found on the chamber walls

(j) Ion Implantation: In this method high energy (few keV to hundereds of keV) or low energy (<200 eV) ions are used to obtain nanoparticles. Ions of interest are usually formed using an ion gun specially designed to produce metal ions, which are accelerated to high or low energy towards the substrate heated to few hundered of C. Depending upon the energy f the incident ions, various other processes like sputtering and generation of electromagnetic radiation may take place. It is possible to obtain single element nanoparticles or compounds and alloys of more than one element. In some experiments it has been possible to even obtain doped nanoparticles using ion implantation. There is possibility of making nanoparticles using swift heavy ions (few MeV energy) employing ion accelerators like a pelletron.

(k) Molecular beam epitaxy (MBE): This technique of deposition can be used to deposit elemental or compound quantum dots, quantum wells, quantum wires in a very controlled manner. High degree of purity in materials is achievable using ultra high vacuum (better than torr). Special sources of deposition known as Kundsen cell (K-cell) or effusion cell are employed to obtain molecular beams of the constituent elements. The rate of deposition is kept very low and substrate temperature is rather high in order to achieve sufficient mobility of the elements on the substrate and layer by layer growth to obtain nanostructures.

(l) Thermolysis: Nanoparticles can be made by decomposing solids at high temperature having metal cations, and molecular anions or metal organic compounds. The process is called thermolysis. For example, small lithium particles can be made by decomposing lithium azide, LiN3. The material is placed in an evacuated quartz tube and heated to 400 C. At but 370 C LiN3 decomposes, releasing N2 gas, which is observed by an increase in the pressure on the vacuum gauge. In a few minutes the pressure drops back to its original low value, indicating that all the N2 has been removed. The remaining lithium atoms coalesce to form small colloidal metal particles. Particles less than 5nm can be made by this method. Passivation can be achieved by introducing an appropriate gas.

(m) Pulsed laser method: Pulsed lasers have been used in the synthesis of nanoparticles of silver. Silver nitrate solution and a reducing agent are flowed through a blenderlike device. In the blender there is a solid disk, which rotates in the solution. The solid disk is subjected to pulses from a laser beam creating hot spots on the surface of the disk. Silver nitrate and the reducing agent react at these hot spots, resulting in the formation of small silver particles, which can be separated from the solution using a centrifuge. The size of particles is controlled by the energy of the laser and rotation speed of the disk. This method is capable of a high rate of production.

Chemical Methods (Wet Chemical route)

There are numerous advantages of using chemical methods, which are –

- Inexpensive, less instrumentation compared to many physical methods
- Low temperature (< 350 C) synthesis
- Doping of foreign atoms (ions) possible during synthesis
- Variety of size and shapes are possible
- Self assembly or patterning is possible

(a) Colloids and Colloids in solutions: A class of materials in which two or more phases (solid, liquid, gas) of same or different materials co-exist with at least one dimension less than a micrometer is known as colloids. Colloids may be particles, plates, or fibers. Nanomaterials are a sub-class of colloids, in which one of the dimensions of colloids is in about 1 to 100 nm range. Colloids are the particles suspended in some host matrix.

Syntheis: Chemical reactions in which colloidal particles are obtained are carried out in glass reactor of suitable size. Glass reactor usually has a provision to introduce some precursors, gases as well as measure temperature, pH etc. during the reaction. It is usually possible to remove the products at suitable time intervals. Reaction is usually carried out under inert atmosphere like argon or nitrogen gas so as to avoid any uncontrolled oxidation of the products. There is also provision made to stir the reactants during the reaction by using Teflon coated magnetic needle.

Colloidal metal nanoparticles are often synthesized by reduction of some metal salt or acid. For example highly stable gold particles can be obtained by reducing choloroauric acid (HAuCl4) with tri sodium citrate ($Na_3C_6H_5O_7$). The reaction takes place as follows –

HAuCl4 + Na3C6H5O7 ⟶ Au+ +C6H5O- + HCl + 3NaCl.

Au atoms are formed by nucleation and condensation. They grow bigger in size by reduction of more Au+ ions on the surface. These atoms are stabilized by oppositely charged citrate ions. Metal gold nanoparticles exhibit intense red, magenta etc., colours, depending upon the particle size. Gold nanoparticles are stabilized by repulsive Coulomb interaction. It is also possible to stabilize gold nanoparticles using thiol or some other capping molecules. In a similar manner, silver, palladium, copper and other metal nanoparticles can be synthesized using appropriate precursors, temperature, pH, duration of synthesis etc., Particle size, size distribution and shape strongly depend on the reaction parameters and can be controlled to achieve desired results. It is also possible to synthesize alloy nanoparticles using appropriate precursors.

(b) Langmuir-Blodgett (L-B) method: This technique to transfer organic layers at air-liquid interface onto solid substrates is known for nearly 70 years. The technique was developed by the two scientists Langmuir and Blodgett. In this technique one uses amphiphilic long chain molecules like that in fatty acids. An amphiphilic molecule has a hydrophilic group (water loving) at one end and a hydrophobic group (water hating) at the other end. As an example consider the molecule of arachidic acid, which ahs a chemical formula [CH3(CH2)16 COOH]. There are many such long chain organic chains with general chemical formula [CH3(CH2)nCOOH], where n is a positive integer. In this case, -CH3 is hydrophobic and –COOH is hydrophilic in nature.

Usually molecules with n>14 are candidates to form L-B films. This is necessary in order to keep hydrophobic and hydrophilic ends well separated from each other. When such molecules are put in water, the molecules spread themselves on surface of water in such a way that their hydrophilic ends, often called as heads, are immersed in water, whereas the hydrophobic ends called as tails remain in air. They are also surface active agents or surfactants. Surfactants are amphiphilic molecules i.e. an organic chain molecule in which at one end there is polar, hydrophilic (water loving) and at the other a nonpolar, hydrophobic (water hating) group of atoms. Using a movable barrier, it is possible to compress these molecules to come close together to form a monolayer and align the tails. It is however necessary that hydrophilic and hydrophobic ends are well separated. Such a monolayer is two dimensionally ordered and can be transferred on some suitable solid substrates like glass, silicon etc. This is done by dipping the solid substrate in the liquid, in which ordered organic molecular monolayer is already formed.

Deposition of L-B films is done by following steps: (1) A monolayer of amphiphilic molecules is formed (2) A substrate is dipped in the liquid (3) The substrate is pulled out, during which ordered molecules get attached to the substrate (4) When the substrate is again dipped, molecules again get deposited as the substrate forming a second layer on the substrate (5) As the substrate is again pulled out a thin layer gets deposited. By repeating the procedure large number of ordered layers can be transformed on a substrate.

(c) Sol-Gel Method: As the name implies sol-gel involves two types of materials or components 'sol' and 'gel'. There are several advantages of sol-gel: All sol-gel formation process is usually a low temperature process. This means less energy consumption and less pollution too. Some of the benefits like getting unique materials such as aerogels, zeolites, ordered porous solids by organic-inorganic hybridization are unique to sol-gel process. It is also possible to synthesize nanoparticles, nanorods, nanotubes etc., using sol-gel technique.

Sols are solid particles in a liquid. They are thus a subclass of colloids. Gels are nothing but a continuous network of particles with pores filled with liquid (or polymers containing liquid). A sol-gel process involves formation of 'sols'in a liquid and then connecting the sol particles (or some subunit capable of forming a porous network) to form a network. By drying the liquid, it is possible to obtain powders, thin films or even monolithic solid.

Synthesis of sol-gel in general involves hydrolysis of precursors, condensation followed by polycondensation to form particles, gelation and drying process by various routes. Precursors (starting chemicals) are to be chosen so that they have a tendency to form gels. Both alkoxides or metal salts can be used. Alkoxides have a general formula M(ROH)n, where M is a cation, R an alcohol group, and n is the number of (ROH) groups with each cation. Salts are denoted as MX, in which M is a cation and X is an anion. Although it is not mandatory that only oxides be formed by a sol-gel process, often oxide ceramics are best synthesized by a sol-gel route. For example in silica, SiO4 group with Si at the centre and four oxygen atoms at the apexes of tetrahedron are very ideal for forming sols with interconnectivity through the corners of tetrahedrons, creating some cavities or pores. By polycodensation process (i.e., many hydrolyzed units coming together by removal of some atoms from small molecules like OH) sols are nucleated and ultimately solgel is formed. Sol-gel method is particularly useful to synthesize ceramics or metal oxides although sulphides, borides and nitrides also are possible.

(d) Microemulsion: Synthesis of nanoparticles in the cavities produced in microemulsion is a widely used method. Advantage of this method is the biocompatibility and biodegradability of synthesized materials. Biocompatability is useful in drug delivery of nanomaterials and biodegradability is environmentally useful. Whenever two immiscible liquids are mechanically agitated or stirred together, they are known to form what is called 'emulsion'. The tendency of the liquids is such that the liquid is smaller quantity tries to form small droplets, coagulated droplets or layers so that they are all separated from the rest of the liquid (for example droplets of fat in milk). The droplet sizes in emulsion are usually larger than 100 nm upto even few millimeters. Emulsions are usually turbid in appearance. On the other hand, there is another class of immiscible liquids, known as microemulsions which are transparent and the droplets are in the range of ~1 to 100 nm. This is size needed for the synthesis of nanomaterials. Microemulsions are stabilized using surfactants (surface stabilized active agents). When an organic liquid or oil (O), water (W) and surfactant (T) are mixed together, under some critical concentration, 'micelles' or inverse micelles are formed, depending upon the concentration of water and organic liquid. Micelles are formed with excess water and inverse micelles are formed in excess of organic liquid or oil. The ratio of water, oil and surfactant is important to decide which type of micelle will be formed and can be represented in a ternary phase diagram, using a triangle. Composition can be determined by drawing lines parallel to all three sides of the triangle. A modified phase diagram known as 'Winsor Diagram' also can be constructed for finer details. The critical micelle concentration (CMC) depends upon all W, O and T concentrations. Effect of T is to reduce the surface tension of water dramatically below CMC and remain constant above it, as the organic solvent concentration is kept on increasing. There are four types of surfactants in general:

Cationic: eg. CTAB

Anionic: eg. R-SO3-Na+

Nonionic: R-(CH2-CH2-O)20-H

Amphoeric: eg. betaines.

A large number of nanoparticles of (metals, semiconductors and insulators) cobalt, copper, CaCO3, BaSO4, CdS, ZnS etc, have been synthesized using microemulsions or inverse micelles. Eg. synthesis of cobalt nanoparticles – A reverse miceller solution of water and oil can be stabilized using a monlayer of surfactant like sodium bis (2-ethylhexyl) sulfosuccinate or Na(AOT). The droplet diameter is controlled simply by controlling the amount of water.

Two micellar solutions having same diameter of droplets can be formed. Thus one solution shuld have Co(AOT)2 i.e., cobalt bis (2-ethylhexyl) sulfosuccinate and the other should have sodium tetrahydroborate (NaBH4). When two solutions are mixed together the solution appears clear but the color changes from pink to black. One can find by electron microscopy analysis that cobalt nanoparticles are formed.

Biological Methods

Synthesis of nanomaterials using biological ingredients can be roughly divided into following three types:

- use of microorganisms
- use of enzymes or plant extracts
- use of templates like DNA, membranes, viruses

(a) Synthesis using microorganisms: Microorganisms are capable of interacting with metals coming in contact with them through their cells and form nanoparticles. Different processes of metal-microorganism interactions are: (i) Some microorganisms produce hydrogen sulfide (H2S). It can oxidize organic matter forming sulphate, which in turn acts like an electron acceptor for metabolism. This H2S can, in presence of metal salt, convert metal ions into metal sulphide, which deposits extracellulary. (ii) In some cases, metal ions from a metal salt enter the cell. The metal ions are then converted into a nontoxic form and covered with proteins in order to protect the remainder of cell from toxic environment. (iii) certain microorganisms are capable of secreting some polymeric materials like polysaccharides. They have some phosphate, hydroxyl and carboxyl anionic groups which complex with metal ions and bind extracellularly (iv) cells are also capable of reacting with metals or ions by processes like oxidation, reduction, methylation, demethylation etc.

Examples:

- Pseudomonas stutzeri Ag259 bateria are found in silver mines and are capable of accumulating silver inside or outside of their cells walls. Using this fact these bacterial strains can be challenged with high concentration of silver salt like AgNO3. Numerous silver nanoparticles of different shapes can be produced having size <200 nm intracellularly.
- Low concentrations of metal ions (Au+, Ag+ etc.) can be converted to metal nanoparticles by Lactobacillus strain present in butter milk. By exposing the mixture of

two different metal salts to bacteria, it is indeed possible to obtain alloys under certain conditions.

- Fusarium oxysporum challenged with gold or silver salt for approximately three days produces gold or silver particles extracellularly.. Extremophilic actinomycete Thermomonospora sp. produces gold nanoparticles extracellularly.
- When silver metal salt is treated with fungus Verticillium sp. the nanoparticles can be produced intracellularly. Changes in biomass colour from initial yellow to final brown, after exposure to silver salt, is a visual indication of silver nanoparticles formation. Particles can be recovered by washing with some suitable detergent or ultrasonication. In a similar way, gold nanoparticles can be produced using Verticillium sp. However, the colour of biomass is from pink to blue depending upon the particle size.
- Semiconductor nanoparticles like CdS, ZnS, PbS etc. can be produced using different microbial routes. Desulfobacteriaceae can form 2-5 nm ZnS nanoparticles. Bacteria Klebsilla pneumoniae can be used to synthesize CdS nanoparticles. When Cd(NO3)2 is mixed in a solution containing bacteria and solution is shaked for about one day at ~38 C, then the CdS nanoparticles in the size range ~5-200 nm can be formed. CdS nanoparticles with narrow size distribution can be synthesized using the yeasts like Candida glabrata and Schizosaccharomyces pombe. Similarly it is possible to synthesize PbS by challenging Torulopsis sp. with lead salt like PbNO3.

(b) Synthesis using plant extracts: It has been reported that live alfalfa plants are found to produce gold nanoparticles from solids. Leaves from geranium plant (pelargonium graveolens) have also been used to synthesize nanoparticles of gold. Nanoparticles obtained using Collectotrichum sp. Fungus related to geranium plant has a wide distribution of sizes and particles are mostly spherical. On the other hand, geranium leaves produce rod and disk shaped nanoparticles. Synthesis procedure to obtain gold nanoparticles form geranium plant extract is as follows: Finely crushed leaves are put in Erlenmeyer flask and boiled in water just for a minute. Leaves get ruptured and cells release intracellular material. Solution is cooled and decanted. This solution is added to HAuCl4 aqueous solution and nanoparticles of gold start forming within a minute.

(c) Use of templates: DNA, S-layers or some membranes have long range periodic order in terms of some molecular groups of their constituents. Therefore on some periodic active sites preformed nanoparticles can be anchored. Alternatively, using certain protocols nanoparticles

can be synthesized using DNA, membranes etc., as templates. Such ordered arrays are formed as a result of various interactions that take place between the templates and the particles.

Ferritin is a colloidal protein of nanosize. It stores iron in metabolic process and is abundant in animals. It is also capable of forming uniform three dimensional hierarchical architechture. There are 24 protein (peptides) subunits in a ferritin, which are arranged in such a way that they create a central cavity of ~6nm. Diameter of polypeptide shell is 12 nm. Ferritin can accommodate 4500 Fe atoms. They are in Fe^{3+} state as hydrated iron oxide mineral, ferrihydrite. The protein subunits are composed of light as well as heavy chains having dinuclear ferroxide centres. These centres are catalysts for in vitro oxidation of Fe^{2+} ions. The ferritin without inorganic matter in its cavity is known as apoferritin and can be used to entrap desired nanomaterial inside the protein cage. Therefore, first step is to remove iron from ferritin to form apoferritin and then introduce metal ions to form metal nanoparticles inside the cavity or carry out some controlled reaction with metal ions to make a compound inside the cavity. In any case, ions can be removed or introduced inside the ferritin, through some available channels.

Horse spleen ferritin , diluted with sodium acetate buffer, should be placed in dialysis bag. A solution of sodium acetate and thioglycolic acid is made in which dialysis bag is kept under nitrogen gas flow for 2-3hours. Solution needs to be replaced from time to time for total 4-5 hours. Further dialysis of apoferritin solution should be done against saline for one hour and in refreshed saline for ~15-20 hours. Apoferriting should then be mixed with solution having sodium chloride (NaCl) and N-tris (hydroxymethyl) methyl-2-aminoethanosuphonic acid (TES). Aqueous cadmium acetate is added to this solution and stirred continuously with constant N_2 gas purging. Process of CdS formation is stepwise with Cd loading of 55 atoms per apoferritin colloid taking place in each step. Higher loading like 110, 165, 220 are possible. Due to remarkably constant size of ferritin colloids and apoferritin derived from them, it is possible to obtain nanoparticles of very uniform size. Besides CdS there ae several other examples like controlled iron oxide, manganese, uranyl oxide, cobalt, cobalt-platinum alloy etc., being synthesized inside ferritin. It is possible to fabricate ordered arrays of ferritin as well as of nanoparticles inside them.

DNA can be used for preformed charged nanoparticles can get bonded with phosphate group of DNA and even form organized arrays of nanoparticles. CdS (or other sulfide) nanoparticles can be synthesized using DNA. Organic molecules can cap the surfaces of nanoparticles

growing in solutions. Similarly one can use DNA to bind with surface of growing nanoparticles. For example, double stranded Salmon sperm DNA can be sheared to an average size of 500 bp. Cadmium acetate can be added to desired medium like water, dimethylformamide, ethanol, propanol etc., and reaction carried out in a glass flask with facility to purge the solution and flow with an inert gas like nitrogen. Addition of DNA shouldbe made and then Na2S can be added dropwise. Depending upon the concentrations of cadmium acetate, sodium chloride and DNA nanoparticles of CdS with size less than ~10 nm can be obtained. DNA probably bonds through its negatively charged phosphate group to positively charged (Cd+) nanoparticle surface.

FABRICATION

Introduction

One of the widely used method for the fabrication of nanostructures is lithography, which makes use of radiation-sensitive layer to form well-defined patterns on a surface. Current technologies for the fabrication of nanoscale structure are limited in terms of the minimum feature size that can be achieved. Natural macromolecules and the processes through which their highly controlled assembly is carried out have become a source of interest to create novel devices and materials.

Strategies for nanoarchitecture

One approach to the preparation of nanostructure, called the *bottom-up approach*, is to collect, consolidate, and fashion individual atoms and molecules into the structure. This is carried out by a sequence of chemical reactions controlled by catalysts. It is a process that is widespread in biology where catalysts called enzymes assemble amino acids to construct living tissue that forms and supports the organs of the body. It is the process of self-assembly.

The opposite approach to the preparation of nanostructure is called the *top-down method*, which starts with large-scale object or pattern and gradually reduces its dimension or dimensions.

Lithography

Conventional ‘Lithography’ is a top down approach. The word lithography has its origin in the Greek work ‘litho’ which means stone. Lithography therefore literally means carving a stone or writing on a stone. It is now used now to mean a process in which a sample is patterned by removing some part of it or sometimes even organizing some material on a suitable substrate.

Lithography is extensively used in electronics industry so as to obtain integrated circuits (IC) or very large scale integration (VLSI) on small piece of semiconductor substrate often called a 'chip'.

Different lithography techniques like optical lithography, x-ray lithography, electron beam lithography and some other have been developed. They depend upon using photons or particle radiations for carving the materials. The lithography technique involve transfer of some pre-designed geometrical pattern (called master or mask) on a semiconductor (silicon) or directly patterning (often known as writing) using suitable radiation. Mask is usually prepared by creating radiation opaque and transparent regions on glass or some other material. Pre-designed patterns can be transferred on a substrate much faster as compared to direct writing. Direct writing being a slower process is overall expensive.

Common principle in most of the lithographic technique is to expose a material sensitive to either electromagnetic radiation or to particles at some regions. Such a radiation sensitive material is known as resist. The selection of area is made using a mask, which is transparent is some regions and opaque in the other regions. This causes selective exposure of the resist, making it weaker or stronger compared to unexposed material depending upon the type of the resist being used. By removing the exposed or unexposed material in suitable chemicals or plasma, desired pattern is obtained.

Various steps involved in photolithography to transfer a pattern on some semiconductor surface: A thin film coating of a metal (like chromium) is deposited on a suitable substrate (for example glass or silicon). A positive or a negative photoresist, usually some polymer, is coated on metal thin film. Positive photoresist material has the property that when exposed to the appropriate radiation it degrades or some chemical bonds are broken. Negative resist on the other hand is a material, which hardens (crosslinks) on exposure to a radiation. A mask is placed between the resist coated substrate and the source of light. By using a suitable chemical (developer) the weakened portion is removed (or image is developed). Remaining unexposed part also can be removed by appropriate chemical treatment. The remaining material can be dissolved in one step and the hardened material in another step.

Depending upon the radiation used like visible light, X-rays, electrons, ions etc., the lithography name is tagged with it.

Nanolithography is the art and science of etching, writing, or printing at the microscopic level, where the dimensions of characters are on the order of nanometers (units of 10^{-9} meter, or millionths of a millimeter). This includes various methods of modifying semiconductor chips at the atomic level for the purpose of fabricating integrated circuits (ICs). Instruments used in nanolithography include the scanning probe microscope (SPM) and the atomic force microscope (ATM). The SPM allows surface viewing in fine detail without necessarily modifying it. Either the SPM or the ATM can be used to etch, write, or print on a surface in single-atom dimensions.

Lithography using photons

It is possible to use visible, ultraviolet, extreme ultraviolet (EUV) or X-rays to perform lithography. Highest resolution of the generated features ultimately depend upon the wavelength of radiation used and interaction of radiation with matter as well as mask and optical elements used. Smaller the wavelength used smaller can be feature size. Depth of focus depends upon the penetration of incident radiation. In the visible range glass lenses and masks can be used. In the UV range fused silica or calcium fluoride lenses are used.

There are three methods used to pattern a substrate viz., proximity, contact and projection. In proximity method, mask is held close to the photoresist coated metallized substrate, whereas in contact method the mask is in contact with photoresist. In both proximity and contact methods a parallel beam of light falls on the mask, which transmits the radiation through some windows but blocks through opaque parts. Although better resolution is achieved with contact method as compared to proximity method, in contact method the mask gets damaged faster. In case of projection method a focused beam is scanned through the mask, which allows good resolution to be achieved along with the reduced damage of the mask. However, scanning is a slow process and also requires scanning mechanism adding to the cost.

(a) UVlight and Laser Beams: Using monochromatic light in the visible to UV range, features as small as 1 to 1.5 um size can be routinely obtained. Often g-line (436 nm) from mercury lamp is used. Laser beam of KrF (248 nm) or ArF (193 nm) also are employed reaching ~150 nm as the smallest feature size.

(b) X-ray lithography: Smaller features are possible to obtain by employing X-rays also. However, it is difficult to make suitable masks for X-ray lithography. X-rays in the 0.1-5nm range are used with appropriate metal masks in proximite geometry. Absorption of X-rays in materials not only depends upon the thickness of the material but is also complicated by the

presence of adsorption edges. Metal masks are fabricated in such a way that through thin portions they are transmitted and absorbed in thicker regions. Gold masks are often used.

X-ray lithography is a next generation lithography that has been developed for the semiconductor industry. Batches of microprocessors have already been produced. The short wavelengths of 0.8 nm X-rays overcome diffraction limits in the resolution of otherwise competitive optical lithography. The X-rays illuminate a mask placed in proximity to a resist-coated wafer. No lenses are used, and only rudimentary collimating mirrors. The X-rays are broadband, typically from a compact synchrotron radiation source, allowing rapid exposure. Deep X-ray lithography uses yet shorter wavelengths, about 0.1 nm with modified procedures, to fabricate deeper structures, sometimes three dimensional, with reduced resolution. The mask consists of an X-ray absorber, typically of gold or compounds of tantalum or tungsten, on a membrane that is transparent to X-rays, typically of silicon carbide or diamond. The pattern on the mask is written by direct write electron beam lithography onto a resist that is developed by conventional semiconductor processes. The membrane can be stretched for overlay accuracy.

Most X-ray lithography demonstrations have been performed by copying with image fidelity, i.e. without magnification, 1x, on the line of fuzzy contrast as illustrated in the figure. But with the increasing need for high resolution, X-ray lithography is now performed on the Sweet Spot, using local “demagnification by bias.” Dense structures are developed by multiple exposures with translation. Many advantages accrue from the application of 3x “demagnification": the mask is more easily fabricated; the mask to wafer gap is increased; and the contrast is higher. The technique is extensible to dense 15 nm prints. The resulting printing has high contrast. X-rays generate secondary electrons as in the cases of extreme ultraviolet lithography and electron beam lithography. While the fine pattern definition is due principally to secondaries from Auger electrons with a short path length, the primary electrons will sensitize the resist over a larger region than the X-ray exposure. While this does not affect the pattern pitch resolution (determined by wavelength and gap), the image exposure contrast $(max - min) / (max + min)$ is reduced since the pitch is on the order of the primary photo-electron range. Several prints at about 20 nm have been published. Another manifestation of the photoelectron effect is exposure to X-ray generated electrons from thick gold films used for making daughter masks. Simulations suggest that photoelectron generation from the gold substrate may affect dissolution rates.

Lithography using particle beams

Optical lithography is an important manufacturing tool in the semiconductor industry. However, to fabricate semiconductor devices smaller than 100nm, ultraviolet light of short wavelengths (193 nm) is required, but this will not work because the materials are not transparent at these wavelengths. Electron beam and X-ray lithography can be used to make nanostructures, but these processes are not amenable to the high rate of production that is necessary for large-scale manufacturing. Electron-beam lithography uses a finely focused beam of electrons, which is scanned in a specific pattern over the surface of a material. It can produce a patterned structure on a surface having 10-nm resolution. Because it requires the beam to hit the surface point by point in a serial manner, it cannot produce structures at sufficiently high rates to be used in assembly-line manufacturing processes. X-ray lithography can produce pattern on surfaces having 20nm resolution, but its mask technology and exposuresystems are complex and expensive for practical applications.

All the moving particles are associated with wavelength known as de Broglie wavelength. All kinds of particles can in principle be used. But to achieve high resolution wavelength should be as small as possible. Thus large mass and large velocity of particle makes is possible to get adequate resolution. In fact it is possible using neutral atoms, ions or electrons to bring down the particle associated wavelength to any desired value, even as small as 0.1 nm.

(a) Electron Beam Lithography: It is very similar to a scanning electron microscope and requires vacuum. Sometimes SEM is modified in order to use it as a lithography set up. Electron beam lithography is a direct writing method i.e., no mask is required to generate a pattern. Rather, pattern or masters required for other lithography processes like optical lithography and soft lithography can be generated using electron beam lithography. Electrons with high energy (~5KeV) are incident on the photoresist. Here also positive or negative photoresists can be used. Common positive resists are polymethylmethacrylate (PMMA) and polybutane-1-sulphone (PBS). Negative resist often used in electron beam lithography is polyglycidylmethacrylate coehylacrylate (COP). Developers used are methylisobutylkeone (MIBK) and isopropylalcohal (IPA) in 1:1 ratio. A focused electron beam in electron beam lithography is used in two modes viz., vector scan and raster scan. In vector scan the electron beam writes on some specified region. After one region is completed the X-Y scanning stage on which substrate to be patterned is mounted, moves. During its movement electron beam is put off. Then a new region is selected and written with the beam. This is continued until whole

pattern is generated. In raster scan the beam is rastered or moved continuously over a small area, line by line. The X-Y stage of sample moves at right angles to the beam. The beam is turned off or turned on depending on the pattern. Although very high resolution (~50 nm) is routinely possible using this lithography, due to scanning mode it is rather slow.

The practice of using a beam of electrons to generate patterns on a surface is known as Electron beam lithography. The primary advantage of this technique is that it is one of the ways to beat the diffraction limit of light and make features in the sub-micrometre regime. Beam widths may be on the order of nanometers. This form of lithography has found wide usage in research,but has yet to become a standard technique in industry. The main reason for this is speed. Thebeam must be scanned across the surface to be patterned -- pattern generation is serial. This makes for very slow pattern generation compared with a parallel technique like photolithography (the current standard) in which the entire surface is patterned at once. As an example, to pattern a single layer of semiconductor containing 60 devices (each device consistsof many layers) it would take an electron beam system approximately two hours; compared with less than two minutes for an optical system.

One caveat: While electron beam lithography is used directly in industry for writing features, the process is used mainly to generate exposure masks to be used with conventional photolithography. However, when it is more cost-effective to avoid the use of masks, e.g., low volume production or prototyping, electron-beam direct writing is also used.

For commercial applications, electron beam lithography is usually produced using dedicated beam writing systems that are very expensive. For research applications, it is very common to produce electron beam lithography using an electron microscope with a home-made or relatively low cost lithography accessory. Such systems have produced linewidths of ~20 nm since at least 1990, while current systems have produced linewidths on the order of 10 nm or smaller. These smallest features have generally been isolated features, as nested features exacerbate the proximity effect, whereby electrons from exposure of an adjacent feature spill over into the exposure of the currently written feature, effectively enlarging its image, and reducing its contrast, i.e., difference between maximum and minimum intensity. Hence, nested feature resolution is harder to control. For most resists, it is difficult to go below 25 nm lines and spaces, and a limit of 20 nm lines and spaces has been found.

With today's electron optics, electron beam widths can routinely go down to a few nm. This is limited mainly by aberrations and space charge. However, the practical resolution limit is determined not by the beam size but by forward scattering in the photoresist and secondary electron travel in the photoresist. The forward scattering can be decreased by using higher energy electrons or thinner photoresist, but the generation of secondary electrons is inevitable. The travel distance of secondary electrons is not a fundamentally derived physical value, but a statistical parameter often determined from many experiments or Monte Carlo simulations down to < 1 eV. This is necessary since the energy distribution of secondary electrons peaks well below 10 eV. Hence, the resolution limit is not usually cited as a well-fixed number as with an optical diffraction-limited system. Repeatability and control at the practical resolution limit often require considerations not related to image formation, e.g., photoresist development and intermolecular forces. In addition to secondary electrons, primary electrons from the incident beam with sufficient energy to penetrate the photoresist can be multiply scattered over large distances from underlying films and/or the substrate. This leads to exposure of areas at a significant distance from the desired exposure location. These electrons are called backscattered electrons and have the same effect as long-range flare in optical projection systems. A large enough dose of backscattered electrons can lead to complete removal of photoresist in the desired pattern area.

(b) Ion Beam Lithography: Very small features (~5-10 nm) can be written using high-energy ion beams. Major advantage of using ion beams is that resists are more sensitive to ions as compared to electron and have low scattering in the resist as well as from the substrate. Commonly used ions are He+, Ga+ etc., with energy in the 100-300 KeV range.

(c) Neutral Beam Lithography: Neutral atoms like argon or cesium have been allowed to impinge on substrates to be patterned through the mask. Such beams cause less damage to the masks. Self assembled monolayers on gold substrate have been often patterned using neutral beams.

Scanning Probe Lithography

STM and AFM microscopes using sharp tips or probes for imaging can be used for lithography purpose. This has evolved scanning probe lithography (SPL). One major advantage is that like optical lithography it is also carried out in air. There are different ways in which SPL can be carried out viz., mechanical scratching or movement, optical, thermomechanical and electrical.

(a) Mechanical Lithography: In mechanical lithography there are different modes like scratching, pick up and pick down or dip pen lithography.

(i) Scratching: Pits or lines can be produced using either STM tip or AFM tip on surface of bulk material or surface of a thin film. Formation of pits or lines by scratching is like ploughing, in which scratched material is piled up around the indented region. Variety of materials like nickel, gold, copper, polymers, Langmuir Blogett films, high temperature superconductors are possible to scratch. Often diamond tips can be used. Pits as small as 30 nm diameter and 10 nm depth are possible to make.

(ii) Pick-up and Pick-down: Atoms are picked up one by one and arranged in desired pattern on a substrate. STM/AFM tips can be used to move atoms or molecules. Systematic work by IBM scientists made it possible for them to pile up xenon atoms on a metal substrate and write a letter patter IBM for the first time. Some scientists moved 30 nm GaAs particles on a GaAs substrate. Letter patterns as high as 50 nm in height were made using AFM tip. Now the technique is used to fabricate some circuits.

(iii) Dip-pen lithography: The method bears a similarity to writing on a piece of paper with ink. That is why the name dip pen lithography is given. An AFM tip is used as a pen and molecules are used as ink. Appropriate molecules picked up by the tip from the source of molecules can be transported and transferred at desired place on the substrate. Letters with line thickness as small as 15nm have been written. Overwriting and erasing capability of dip pen lithography is quite a unique feature.

Dip Pen Nanolithography (DPN) is a scanning probe lithography technique where an atomic force microscope tip is used to transfer molecules to a surface via a solvent meniscus. This technique allows surface patterning on scales of under 100 nanometres. DPN is the nanotechnology analog of the dip pen (also called the quill pen), where the tip of an atomic force microscope cantilever acts as a "pen," which is coated with a chemical compound or mixture acting as an "ink," and put in contact with a substrate, the "paper." DPN enables direct deposition of nanoscale materials onto a substrate in a flexible manner. The vehicle for deposition can include pyramidal scanning probe microscope tips, hollow tips, and even tips on thermally actuated cantilevers. Applications of this technology currently range through chemistry, materials science, and the life sciences, and include such work as ultra high density

biological nanoarrays, additive photomask repair, and brand protection for pharmaceuticals. The technique was discovered in 1999 by a research group at Northwestern University led by Chad Mirkin. The company NanoInk, Inc. holds a patent on Dip Pen Nanolithography, and "DPN" and "Dip Pen Nanolithography" are trademarks or registered trademarks of NanoInk.

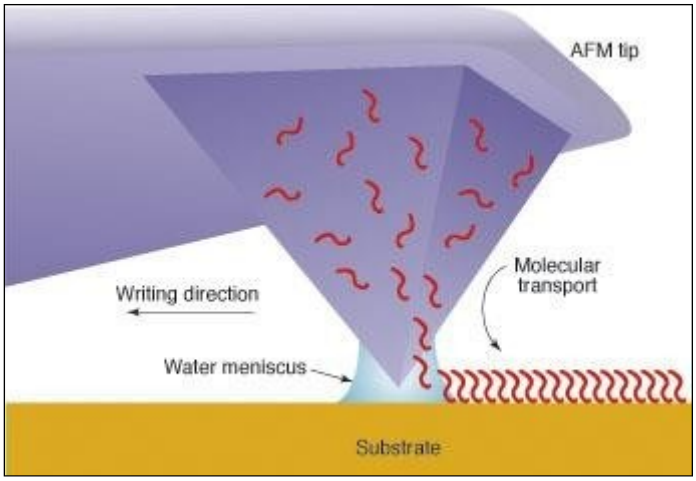


Figure 3.1. Dip pen lithography

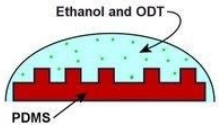
(b) Optical scanning probe lithography: Very high resolution ~20-50 nm is possible, overcoming the diffraction limit, with visible light using Scanning Near-Field Optical Microscope (SNOM). This is attributed to near-field component of electromagnetic radiation. In SNOM, a fine spot of light emerging through an aperture, scans on the surface within a distance of less than wavelength of light used for scanning. By placing the aperture close to the photoresist coated substrate, it is possible to obtain as small as ~50nm size features routinely.

(c) Thermo-mechanical lithography: It is also possible to use an AFM tip along with a laser beam and carry out nanolithography. While the AFM tip is in contact with a coating like polymethylmethaacrylate (PMMA), laser beam strikes the same point of the coating. This heats the film locally enabling the tip to penetrate in the material and make a pit. This thermo-mechanical method is capable of producing resolution as high as ~30nm.

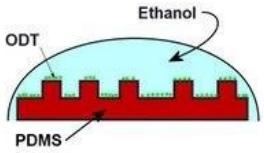
(d) Electrical Scanning Probe Lithography: In this method, a voltage is applied between the SPM tip and the sample. Above some critical voltage if large current flows between the tip and the sample, an irreversible change can occur in sample surface. Variety of bulk solid and think films surfaces has been patterned using this method. In silicon or modified silicon surface ~30 -60 nm wide and ~5-10 nm deep lines have been engraved.

Soft Lithography

The name soft lithography is used to mean the techniques using materials like polymers, organic materials or self assembled films. It is a useful alternative to obtain resolution better than ~100nm at low cost. Moreover, the method is applicable from few nm to few um size features. In general, soft-lithography technique involves fabrication of a patterned master, molding of master and making replicas. A master is usually made using X-ray or electron beam lithography. It is supposed to be quite rigid. A mold is usually made using a polymer like polydimethylsiloxane (PDMS), epoxide, polyeurethane etc., PDMS is most common amongst the polymers used for molding due to its attractive properties like thermal stability (~150 C), optical transparency, flexibility (~160% elongation), capability of cross-linking using IR or UV radiation etc. However, during molding, some distortions can take place and adequate control has to be practiced to achieve reproducible and required results.



"Inking" a stamp. PDMS stamp with pattern is placed in Ethanol and ODT solution



ODT from the solution settles down onto the PDMS stamp. Stamp now has ODT attached to it which acts as the ink.

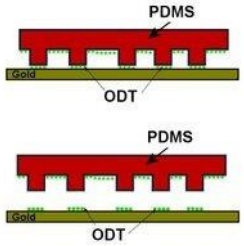


Figure 3.2 Steps in Soft lithography

The PDMS stamp with the ODT is placed on the gold substrate. When the stamp is removed, the ODT in contact with the gold stays stuck to the gold. Thus the pattern from the stamp is transferred to the gold via the ODT "ink."

In technology, ***soft lithography*** refers to a set of methods for fabricating or replicating structures using "elastomeric stamps, molds, and conformable photomasks. It is called "soft" because it uses elastomeric materials. Soft lithography is generally used to construct features measured on the nanometer scale. ***Soft lithography*** includes the technologies of Micro Contact Printing (μCP), replica molding (REM), microtransfer molding (μTM), micromolding in capillaries (MIMIC) and solvent-assisted micromolding (SAMIM). One of the soft lithography procedures is as follows:

1. The steps of any of your favorite micro- or nano- scale lithography procedures (photolithography, EBL, etc.) are followed to etch a desired pattern onto a substrate (usually silicon)
2. Next, the stamp is created by pouring a degassed resin overtop of the etched wafer. Common resins include PDMS and Fluorosilicone.
3. Removing the cured resin from the substrate, a stamp contoured to your pattern is acquired.
4. The stamp is then "inked" by placing it, pattern-up, in a bath of inking solution (for example, ODT in ethanol) for a short period of time. The ink molecules will fall and adhere to the surface of the stamp creating a single-molecule layer of the ink on the stamp.
5. The inked stamp is then pressed on the substrate and removed, leaving the desired single-molecule thick pattern on the substrate
6. Steps 4 and 5 are repeated for each substrate on which the pattern is desired

Advantages: Soft lithography has some unique advantages over other forms of lithography (such as photolithography and electron beam lithography). They include the following:

- Lower cost than traditional photolithography in mass production
- Well-suited for applications in biotechnology
- Well-suited for applications in plastic electronics
- Well-suited for applications involving large or nonplanar (nonflat) surfaces
- More pattern-transferring methods than traditional lithography techniques (more "ink" options)
- Does not need a photo-reactive surface to create a nanostructure
- Smaller details than photolithography in laboratory settings (~30nm vs ~100nm)

(a) Nanoimprint lithography: It has been developed that may provide a low-cost, high-production rate manufacturing technology. Nanoimprint lithography patterns a resist by physically deforming the resist shape with a mold having a nanostructure pattern on it, rather than by modifying the resist surface by radiation, as in conventional lithography. A resist is a coating material that is sufficiently soft that an impression can be made on it by a harder material. A mold having a nanoscale structured pattern on it is pressed into a thin resist coating on a substrate, creating a contrast pattern in the resist. After the mold is lifted off, an etching process is used to remove the remaining resist material in the compressed regions. The resist is a thermoplastic polymer, which is a material that softens on heating. It is heated during the molding process to soften the polymer relative to the mold. The polymer is generally heated above its glass transition temperature, thereby allowing it to flow and conform to the mold pattern. The mold can be a metal, insulator or semiconductor fabricated by conventional lithography methods. Nanoimprint lithography can produce patterns on a surface having 10 nm resolution at low cost and high rates because it does not require the use of sophisticated radiation beam generating pattern for the production of each structure.

(b) Microcontact Printing (uCP): A PDMS stamp is dipped in an alanethiol solution and pressed against the metallized (Au, Ag, Cu) substrate. Those parts of substrate, which come in contact with the PDMS receive layers of alkanethiol. The monolayers do not spread on the substrate. Further, these self assembled monolayers can be used as resists for selectiveetchingor deposition. The printing being simulateous, it is a fast method.

(c) Relica Molding: In this method, a PDMS master or stamp is used to replicate a number o copies. For example a solution of polyurathene is poured in PDMS and cured using UV light or thermal treatment so that polyurathene becomes solid. PDMS can be easily removed so that a pattern opposite to that is produced in polyurathene. By applying small pressure on PDMS, it is possible to further reduce the size o the features smaller than in the original pattern. Nanostructure ~30 nm have been achieved using this method.

Self Assembly

The ‘bottom up’ approach of nanofabrication proposes to overcome the limitations of traditional ‘top down’ lithographic techniques by relying on the self-organization of molecular building blocks into higher order assemblies having a desired configuration. A variety of molecular building blocks with programmed non-covalent recognition sites have been designed and produced by organic synthesis. Nature has been working on nanoscale self-assembly.

Consequently, today biological materials are used as building blocks for self-assembly applications. These biological materials include DNA nanostructures, small peptides, S-layer glycoproteins, viral proteins and even whole bacteriophages.

(a) Process of self-assembly: The spontaneous organization of small molecules into larger well-defined, stable, ordered molecular complexes or aggregates is known as self assembly. Self assembly takes place spontaneously by adsorption of atoms or molecules onto a substrate in a systematic, ordered manner. This process involves the use of weak, reversible interactions between parts of molecules without any central control, and the result is a configuration that is in equilibrium. The procedure is automatically error-checking, so faulty or improperly attached subunits can be replaced during the growth.

The traditional organic synthesis of very large molecules called macromolecules comprises a number of time-consuming steps that involve breaking and remaking strong covalent bonds, and these steps are carried out under kinetic control. They yields are small, and errors are not readily recognized or corrected. In contrast to this, self-assembly variety of synthesis makes use of weak, noncovalent bonding interactions such as those involving hydrogen bonds and van der Waals forces, which permit the reactions to proceed under thermodynamic control, with continual correction of errors. The initial individual molecules or subunits are usually small in size and number and easy to synthesize, and the final product is produced in a thermodynamically equilibrium state.

On one hand, fabrication methods in micro- and nanoscience allow for batch processing. That is, we have the ability to make many copies of the same device simultaneously. How do we design these devices so that they spontaneously assemble themselves into a useful working structure? On the other hand, traditional fabrication methods are limited in resolution. To make smaller structures, i.e., true nanoscale structures, requires the development of new methods. Taking their cue from nature, coaxing nanostructures into self assembling is an avenue many scientists are exploring. Ultimately, a deeper understanding of self assembly may shed light on the nature of life itself.

Assembly by capillary forces: G.M. Whitesides and his group at Harvard University have designed and studied various self assembling systems. Many of these are based on the so-called "capillary bond." This "bond" exploits two properties of objects in water. First, small objects resting on the surface of water attract one another. In this way, interacting particles feel a force of attraction. Second, when two hydrophobic surfaces come into contact they remain in contact.

In other words, they bond. In their experiments Whitesides et. al. have used the polymer polydimethylsiloxane (PDMS) to fabricate their self assembling shapes. PDMS is naturally hydrophobic and its surface properties can be easily changed from hydrophobic to hydrophilic by treating with an oxygen plasma. In this way interacting particles with varying surface properties can be fabricated. Whitesides et. al. have designed planar systems that self assemble to tile the plane, tile the plane with gaps, and form chain-like structures.

Assembly by electrostatic forces: Assembly by capillary forces relies upon particle-particle interaction and interaction between particles and their enviornment. The particles have surface properties (hydrophobic or hydrophillic) and the enviornment is water.

Assembly by magnetic forces: simple to construct self assembling system involves magnets. In its simplest incarnation, the system is no more than a collection of disk shaped magnets randomly strewn about a inside a container. Here, simple shaking is enough to cause formation of a more highly ordered state

(b) Interactions governing self-assembly: Self organization and self-assembly are ubiquitous in nature, ranging from the simplest chemical reactions to the formation of living organisms. Self-assembly is synonymous for the spontaneous occurrence of order in a given open system. Any spontaneous process is irreversible and is accompanied by an increase in the combined entropy for both the system and its environment. The basic thermodynamic quantity, which dictates these processes at constant pressure, is system's Gibbs free energy change.

$\Delta G=\Delta H-\Delta TS$, where ΔH and ΔS are the changes of enthalpy and entropy, respectively, after the reaction or self-assembly processes. For spontaneous process, ΔG must to be <0. One major contribution to ΔH comes from the potential attractive interactions. The forces that determine ΔH include: van der Waals, hydrogen bonding, electrostatic, hydrophobic, and other non-bonding interactions. Entropy is related to quality of energy and order in any given system and is measured by the statistical probability. In the process of self-organization and self-assembly of the inorganic materials, major contributions to ΔG come from the system's enthalpy change, because entropy change is generally of less importance due to the limited freedom of inorganic compounds in condensed phase.

Self assembly is the organization of molecules or materials into order from disorder. Quantum mechanics determines the forces that organize the system on the nanometer scale. There are only four forces known in nature, these are the strong and weak forces existing in the nuclei,

gravitational forces, and electromagnetic forces. Of these only the electromagnetic force will be relevant for the self-assembly. The chemical bonding interaction is one the strongest among all electromagnetic forces. Since self-assembly does not involve the breaking or the formation of chemical bonds, only non-bonding interactions are important. The non-bonding electromagnetic force is expressed in various forms e.g van der Waals forces, hydrogen bonding, electrostatic forces, magnetic interactions and others such as cation- and metal complexation interactions. In some cases, one type of interaction dominates, in others, several interactions may be of equal importance and these interactions may be cooperative instead of additive.

Van der Waals interaction is a weak short-range attractive force due to the temporary dipole-dipole moment interaction that results from electron movement surrounding the nuclei. It is non-discriminate and contributes to less than 2kcal/mol for each pair of interacting dipole moments. It is a high order interaction. Electrostatic interaction is Columbic in nature. Its interacting energy is inversely proportional to the distance between the charged particles. Electrostatic interaction is strong and constitutes one of the major forces. Temperature does not have a strong effect on electrostatic interactions. The hydrogen bonding interaction very unique interaction between hydrogen bond donors (e.g N-H, O-H) and acceptors (eg. N, O). Hydrogen bonding plays an important role in hydrophobic interactions and in molecular recognition of proteins because of its directionality. It was originally thought that hydrogen bonding interaction is mostly electrostatic in nature, but it is now generally accepted that the hydrogen bond consists of many different interactions, including Coulombic attraction, exchange repulsion, polarization and charge transfer and dispersion. The hydrogen bonding interaction contributes to about 2-20 kcal/mol to the total energy. If one or both of the donor and acceptor are charged, the interaction becomes much stronger. Hydrophobic interactions between the non-polar solutes in aqueous solution arises from the stronger attraction between the water-water molecules than between the solute-water molecules. Metal complexation is duemostly to a combination of electronic, electrostatic, charge transfer, van der Waals interactionsand the polarization effect. In the case of complexation between transition metal ions and ligands, four or six coordinates is generally found.

(c) Examples of self-assembly: (i) Semicondutor islands: One type of self-assembly involves the preparation of semiconductor islands. It can be carried out by a technique called heteroepitaxy, which involves the placement or deposition of the material that forms the islands on a supporting substrate called a substrate made of a different material with a closely matched

interface between them. It involves bringing atoms or molecules to the surface of the substrate where they do one of three things. They either are adsorbed and diffuse about on the surface until they join or nucleate with another adatom to form an island, attach themselves to or aggregate into an existing island, or desorb and thereby leave the surface. Small islands can continue to grow, migrate to other positions, or evaporate. There is critical size at which they become stable, and no longer experience much evaporation. Thus there is an initial nucleation stage when the number of islands increases with the coverage. This is followed by an aggregation stage when the number of islands levels off and the existing ones grow in size. Finally there is the coalescence stage when the main events that take place involve the merge of exisiting islands with each other to form larger clusters.

It is possible to spontaneously create quantum dots of Ge on Si, InAs on GaAs etc. The origin of self assembly is strain induced. For example germanium and silicon have only 4% lattice mismatch. Therefore Ge can be deposited epitaxially on Si single crystal upto 3-4 monolayers. This results into spontaneous formation of nanosized islands or quantum dots. However, temperature of substrate has to be >350 C during deposition or post deposition annealing is required. Size of the islands depends upon growth temperature as well as substrate plane on which it grows.

(ii) Monolayers: A model system that well illustrates the principles and advantages of the self-assembly process is a self-assembled monolayer. The Langmuir-Blodgett technique, had been widely used for the preparation and study of optical coatings, biosensors, ligand-stabilized Au clusters, antibodies and enzymes. It involves starting with clusters, forming them into monolayer at an air-water interface, and then transferring the monolayer to a substrate in the form of what is called a Langmuir-Blodgett film. Self assembled monolayers, are stronger, are easier to make, and make use of a wide variety of available starting materials.

Self-assembled monolayers and multilayers have been prepared on various metallic and inorganic substrates such as Ag, Au, Cu, Ge, Pt, Si, GaAs, SiO2, and other materials. This has been done with aid of bonding molecules or ligands such as alkanethiols RSH, sulfides RSR', disulfides RSSR', acids RCOOH, and siloxanes RSiOR3, where the symbols R and R' designate organic molecule groups that bond to a thiol radical –SH or an acid radical –COOH. The binding to the surface for the thiols, sulfides, and disulfides is via the sulfur atom; that is, the entity RS-Au is formed on a gold substrate, and the binding for the acid is RCO2-(MO)n where MO denotes a metal oxide substrate ion, and the hydrogen atom H of the acid is released

at the formation of the bond. The alkanethiols RSH are the most widely used ligands because of their greater solubility, their compatibility with many organic functional groups, and their speed of reaction. They spontaneously adsorb on the surface; hence the term self-assemble is applicable.

For self-assembled monolayers to be useful in commercial microstructures, they can be arranged in structured regions or pattern on the surface. An alkaenthio ‘ink’ can systematically form or write pattern on a gold surface with alkanethiolate. The monolayer-forming ‘ink’ can be applied to the surface by a process called microcontact printing, which utilizes an elastomer, which is a material with rubber like properties, as a ‘stamp’ to transfer the pattern. The process can be employed to produce think radiation-sensitive layers called resists for nanoscale lithography. The monolayers themselves can serve for a process called passivation by protecting the underlying surface from corrosion.

Self-assembled monolayer: Self assembled monolayers (SAMs) are surfaces consisting of a single layer of molecules on a substrate. Rather than having to use a technique such as chemical vapor deposition or molecular beam epitaxy to add molecules to a surface (often with poor control over the thickness of the molecular layer), self assembled monolayers can be prepared simply by adding a solution of the desired molecule onto the substrate surface and washing off the excess.

A common example is an alkane thiol on gold. Sulfur has particular affinity for gold, with a binding energy in the range of 20–35 kcal/mol (85–145 kJ/mol). An alkane with a thiol head group will stick to the gold surface and form an ordered assembly with the alkyl chains packing together due to van der Waals forces. For alkyl thiols on gold, the extended alkyl chains typically orient with an angle of ~30 degrees from the perpendicular of the substrate, and are assumed to be in a fully extended linear arrangement. There has been a great deal of work done determining the process by which alkyl thiol on gold assemblies are produced. It is generally thought that alkyl thiol molecules first bind to the gold surface in a 'lying down' position, where the alkyl chain tails of the molecules lie flat on the gold surface. The thiol interaction provides about 20–30 kcal/mol (85–130 kJ/mol) of driving force for the initial binding, which is modeled as a Langmuir binding isotherm. These binding events continue until the lying down molecules are dense enough on the surface to interact with each other. At some point the alkyl chains lift off the substrate and point outwards, tethered by the thiol anchor to the surface. There is a shift to a mixture of lying down molecules and island domains of upright alkyl chains, tilted at 30

degrees to the normal. At this stage binding kinetics become more complex and can no longer be modeled with a simple Langmuir binding isotherm. Over time the island domains merge and cover the bulk of the substrate, and the process can be compared to a 2-D crystallization process on a surface. Alkyl thiol SAMs exhibit grain boundaries and defects even after long periods of assembly. The initial stage of SAM formation usually takes minutes or less under the normal conditions of 0.1-10 mmol/L thiol concentration in a solvent. More ordering of the assembly can take place over days or months, depending on the molecules involved.

A variety of other self-assembled monolayers can be formed, although there is always debate about the degree to which systems self-assemble. Alkyl thiols are known to assemble on many metals, including silver, copper, palladium, and platinum. Alkyl silane molecules (e.g. octadecyltrichlorosilane) are another well-known example of self-assembly on silicon oxide surfaces and potentially be of greater technical relevance than alkyl thiol assembly on metals. Alkyl carboxylates are known to assemble on a variety of surfaces, such as aluminium and mica. Silicon has been used through the reaction of silicon hydride surface and a radical generator, such as heat, UV or radical initiator molecule, or with reagents such as Grignard and chlorosilanes. Once assembly has been accomplished, chemistry can be performed on the layer, especially if self-assembly places a reactive functional group on the outside of the monolayer.

SAMs have several applications in scientific research; they tend to have quite different chemical kinetics than the same molecules in another form, because of their exposed, 2-dimensional distribution, and as such are useful for some chemical and biochemical experiments. They can also be used for simulation of biological membranes and as substrates for cell culture. As technology develops to control the functional groups present in SAMS, either by direct deposition of molecules with those groups or by chemical modification of the layer, many other applications are also developing, for example in nanoscale fabrication of electronics.

(iii) Protein folding and aggregation: Protein folding is a typical example of self-assembly regulated by many subtle interacting forces. Protein folding is the process by which the polypeptide is folded into a functional three-dimensional protein structure based on the amino-acid sequence. When polypeptides are newly produced under physiological conditions, their folding is based on the interacting forces and the laws of thermodynamics. However, in some cases, molecular chaperones have to be employed to assist protein folding to prevent incorrect interactions within and between non-native polypeptides.

Molecular recognition means that there is specific interaction between protein and ligand, or between antibody and antigen. As was observed experimentally, topography (conformational match seems to play an essential role in protein-ligand recognition. Because hydrophobic and van der Waals interactions are not specific; they cannot alone be responsible for molecular recognition. Directional hydrogen bonding and electrostatic interactions play an important role.

Hydrophobic and hydrophilic interactions are important because many molecules in biology such as proteins and the molecules that make up the cell membrane have hydrophilic and hydrophobic regions on the same molecule. When put in water these molecules automatically organize themselves into more complex and biologically useful structures. This process is termed self assembly. It is illustrated in the diagram for a molecule with a polar head and a non polar tail.

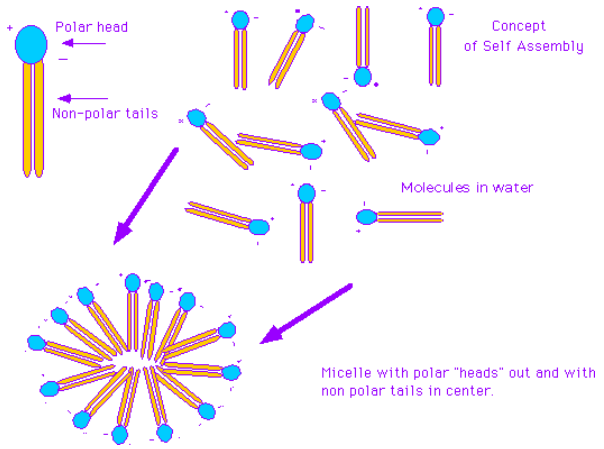


Figure 3.3 Self assembly for a molecule with a polar head and a non polar tail.

(d) Applications: (i) Self assembly using biological templates: There are many examples of self assembly at biological templates like S-layers, proteins, DNA, lipids etc. When organized arrays of inorganic crystals are embedded in biological systems they are often referred to as biomineralized systems.

Mangetotactic bacteria are small bacteria, ~35 to 120 nm sized permanent magnets are present inside them. The magnets are of either iron sulphide (Fe3S4-gregite) or iron oxide (Fe3O4 – magnetite). Such magnetic particles make a chain of nanomagnets. It is useful for navigation of bacteria. Earth's magnetic field has a dip in the north and south hemisphere which helps bacteria to seek direction.

Another example of self-assembly in biological systems is S-layers. They are part of cell envelope of prokaryotic organisms. They are two dimension, crystalline single proteins or glycoprotein monomers organized in hexagonal, oblique or square lattices. These lattices have ordered pores. The periodicity of pores can vary, depending upon the protein, from 3-35 nm. Such S-layers after extraction from bacterial cells have been transferred on some metallic substrates (or grids). When treated with cadmium salt and subsequently with Na2S, ordered arrays of CdS nanoparticles could be formed. S-layers have been used to assemble Au, Pt, Fe, Ni etc., metal nanoparticles. In general, S-layers extracted from the biological cells can be directly used to deposit nanoparticles from liquid phase.

Ferritins are protein colloids of 12 nm size found in all animals. Ferritins have a cavities ~6-8 nm in size filled with iron oxide. It is possible to remove iron oxide and replace it with metal or other nanoparticle. Further it is possible to make a two dimensional array of ferritins in solution. For example, ferritin solution in NaCl and phosphate at ~5.8 pH can be filled in a trough. Chloroform containing dichloroacetic acid can be used to dissolve poly-1-benzal-L-histidine (PBLH) and spread over ferritin solution in trough. After about two hours the solution can be heated at 38 C for one hour and cooled back to room temperature. This produces ordered layer of ferritin at liquid-air interface. The layer can be transferred on silicon substrate by dipping in the solution.

DNA is a long helical molecule. It has large aspect ratio and acts like a long one dimensional template in its simplest form. Its four nucleotide bases viz., guanine, cytosine, adenine, and thymine can form a rich variety of sequences and structures. Thus, cirucular, square, branched etc. long or short DNA templates are possible. Besides planar geometry, they can adopt even three dimensional structures. As DNA has alternate sugar and phosphate groups on its strands, it is possible to anchor metal, semiconductor or oxide particles by different bonding on DNA to have assembly of particles.

(ii) Self assembly using organic molecules: Preformed inorganic nanoparticles can be assembled on solid substrates through some organic molecules adsorbed on their surfaces. CdS nanoparticles functionalized with carboxylic group can be transferred to aluminium thin films. Dithiols adsorbed on metals surface also could adsorb CdS nanoparticles to form layers of them. Silver particles have been adsorbed on oxidized aluminium layers using bifunctional molecule such as 4-carboxythiophenol. These molecules bind to aluminium oxide layer by carboxylic group and thiol attaches to silver particles.

Using a two phase reaction alkanethiol or alkylamine capped gold, silver and palladium nanoparticles have been self assembled. Here chemical reaction takes place in an aqueous medium. The particles are then transferred into an organic solvent. Solvent is allowed to evaporate which leaves self-assembled layer.

Using Langmuir Blodgett technique it is possible to transfer organic layers along with the attached nanoparticles in liquid subphase to form ordered monolayer or multiple layers on solid substrate. It is possible to order different bilayers of nanoparticles by simply dipping the same substrate alternately in different baths.

Others

(a) Sputtering (a bottom-up approach): One method used to make thin layers of material that are only a few atoms thick is called "sputtering." Sputtering involves transferring atoms from a block of source metal over to a surface waiting to be coated. The atoms are knocked loose from the source metal by bombarding them with other high-energy particles. The common aproach taken when explaining sputtering is to imagine billiard balls being struck by the cue ball. The cue ball is rather like the hihg-energy incident particle. As it strikes a bunch of billiard balls (atoms in a block of source metal) they scatter from one another. This is where the analogy breaks down, though, as there is no second surface that the billiard balls attach to besides the pool table. In sputtering, however, the loose atoms are free to deposit on some material that needs to be coated.

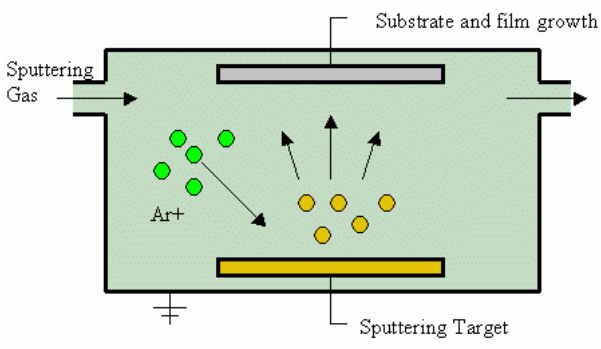


Figure 3.4 Sputtering

(b) Nanoscale Crystal Growth (a bottom-up approach): Just like it sounds, this method involves rather tricky selection of seed crystals and growing conditions with the hopes of creating crystals that have unusual shapes. Nanowires, which happen to exhibit tremendous conductivity, are typically created in this way.

(c) Focused ion beam: **Focused ion beam, also known as FIB, is a scientific instrument that resembles a scanning electron microscope. However, whereas the SEM uses a focused beam of electrons to image the sample in the chamber, a FIB instead uses a focused beam of gallium ions. Gallium is chosen because it is easy to build a gallium liquid metal ion source (LMIS). In a Gallium LMIS, gallium metal is placed in contact with a tungsten needle and heated. Gallium wets the tungsten, and a huge electric field (greater than 10^8 volts per centimeter) causes ionization and field emission of the gallium atoms. These ions are then accelerated to an energy of 5-50 keV (kiloelectronvolts), and then focused onto the sample by electrostatic lenses. A modern FIB can deliver tens of nanoamps of current to a sample, or can image the sample with a spot size on the order of a few nanometers.**

Unlike an electron microscope, the FIB is inherently destructive to the specimen. When the high-energy gallium ions strike the sample, they will sputter atoms from the surface. Gallium atoms will also be implanted into the top few nanometers of the surface, and the surface will be made amorphous. Because of the sputtering capability, the FIB is used as a micro-machining tool, to modify or machine materials at the micro- and nanoscale. A FIB can also be used to deposit material via ion beam induced deposition. FIB-assisted chemical vapor deposition occurs when a gas, such as tungsten carbonyl ($W(CO)_6$) is introduced to the vacuum chamber and allowed to chemisorb onto the sample. By scanning an area with the beam, the precursor gas will be decomposed into volatile and non-volatile components; the non-volatile component, such as tungsten, remains on the surface as a deposition. This is useful, as the deposited metal can be used as a sacrificial layer, to protect the underlying sample from the destructive sputtering of the beam. Other materials such as platinum can also be deposited.

FIB is often used in the semiconductor industry to patch or modify an existing semiconductor device. For example, in an integrated circuit, the gallium beam could be used to cut unwanted electrical connections, or to deposit conductive material in order to make a connection. The FIB is also commonly used to prepare samples for the transmission electron microscope. The TEM requires very thin samples, typically ~100 nanometers. Other techniques, such as ion milling or electropolishing can be used to prepare such thin samples. However, the nanometer-scale resolution of the FIB allows the exact thin region to be chosen. This is vital, for example, in integrated circuit failure analysis. If a particular transistor out of several million on a chip is bad, the only tool capable of preparing an electron microscope sample of that single transistor

is the FIB. The drawback to FIB sample preparation is the above-mentioned surface damage and implantation. However, this is usually only noticeable in high-resolution "lattice imaging" TEM. By lightly ion-milling the sample after completing the FIB preparation, much of this damage can be removed. In short, the FIB is a useful and versatile tool in the materials sciences and semiconductor fields.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV - Nanobiotechnology – SBTA1503

PROTEINS AS COMPONENTS FOR NANODEVICES

Introduction

The goal of nanobiotechnology is to build tiny devices that respond to the environment, perform computations and carry out tasks. Considerable progress has been made in building protein components for such devices, and here we describe examples, including self-assembling protein arrays, pores with triggers and switches, and motor proteins harnessed for specific tasks.

Proteins represent fertile territory for nanobiotechnology because they have properties ideal for engineering purposes. They possess sophisticated architectures at nanoscale dimensions, rich chemistry and versatile enzymatic activities. Proteins are capable of carrying out complex tasks in cells. We need think only of examples such as the flagellar motors of bacteria, the linear motors of muscle and the cytoskeleton, voltage-gated ion channels, DNA replication complexes, or the photosynthetic reaction centers. By genetic engineering and/or chemical modification or by using proteins in ways not found in nature, nanobiotechnology can harness the power of proteins to create new components for materials and devices.

What properties might proteins bring to nanodevices ? Nanodevices might use motor proteins to move linearly, by rotation, or in a more complex three dimensional manner. Nanodevices might respond to the environment through proteins with built-in switches that operate in a simple on-off way or through more finely tuned and complex logic gates with graded or multiple inputs. In this way, nanodevices will sense their environment. More advanced functions might include transport (uptake, movement and delivery of cargoes utilizing protein transporters and pores) and chemical transformation, by enzymatic catalysis, for example. To perform these functions, the nanodevice must use energy and might even transduce and storeit by using, for example, the biological energy currency of ATP. The nanodevice might deal with data, by storing it or performing computations with protein switches, combined perhaps with DNA-based components. Like many protein complexes, the nanodevice is likely to be capable of self-assembly, and perhaps repair and even replication.

Three classes of protein components for nanodevices are presented in order of complexity: planar crystalline arrays, engineered protein pores, and molecular motors.

Ordered protein arrays

Two-dimensional protein crystals might provide useful scaffolds for nanobiotechnology. While several proteins form non-natural planar arrays, the premier examples of planar protein assemblies are naturally occurring bacterial surface-layers. S-layers are composed of identical protein or glycoprotein subunits and self-assemble into lattices that form the outermost component of the cell envelopes of many species of bacteria and most archaea. S-layer lattices exhibit either oblique, square or hexagonal symmetry with morphological unit cells ranging in size from 3 nm to 30 nm. The pores in the protein lattice can vary in diameter from 2 nm to 6 nm. The lattice thickness is between 5 nm and 10 nm. Several applications have been suggested for S-layers, such as their use as templates for the nanoscale patterning of inorganic materials or as immobilization matrices for biomedical applications. Indeed, lattices of cadmium sulfide quantum dots have been synthesized by using self-assembled bacterial S-layers as templates. The nucleation of nanoparticle growth was confined to the pores of the S-layer lattice. Au and CdSe nanoparticles have also been directly deposited onto the protein lattice. Given that the macroscopic electronic or magnetic properties of nanoparticle arrays are influenced by the interparticle distance and geometry, it should be possible to use various natural or engineered S-layer lattices as a ‘tuneable’ system to obtain nanoparticle assemblies with designed properties for materials science. In a second example, S-layer–streptavidin fusion proteins were assembled into crystalline sheets to generate a molecular affinity matrix. First, an S-layer–streptavidin fusion protein was mixed with streptavidin and refolded to obtain heterotetramers of 1:3 stoichiometry. Chimeric S-layer lattices were then formed on various substrates. When biotinylated ferritin was allowed to bind to the lattice and visualized by TEM, the ferritin molecules could be seen to reflect the organization of the underlying lattice, albeit not perfectly. By using a similar genetic engineering approach, S-layer fusion proteins including an IgG-binding domain were constructed for the development of high-density adsorbents for extracorporeal blood purification . Two copies of the protein A related Z-domain, capable of binding the Fc portion of IgG, were fused to a C-terminal truncation mutant of an S-layer protein. The self-assembled S-layers were coated onto microbeads, which thereby attained an IgG-binding capacity at least 20 times higher than commercial immunoadsorbents used to remove autoantibodies from the sera of patients suffering from autoimmune disease.

In the future, engineered S-layer proteins might be used as toolkits for the positioning of proteins or nanoparticles in nanopatterned arrays. By using dip-pen nanolithography, patterns might be created, for example, by the direct deposition of biotinylated particles from AFM tips onto a streptavidin–S-layer lattice. An alternative and faster route to nanopatterns might be another a top-down approach wherein S-layer proteins are assembled on nanolithographically structured substrates. Metallic or semiconductor nanoparticle assemblies generated in this way will form the basis of materials with tailored electronic or magnetic properties.

Proteins that would not naturally form supramolecular assemblies have also been engineered to self-assemble into designed networks. A C4-symmetric tetrameric aldolase was used to forma 'quadratic network'. The aldolase, which has the form of a 7 _ 7 _ 5 nm flattened cube, was engineered to form a rigid four-way connector by covalently tethering two biotins to cysteines placed on each 7 _ 5 face. The tetrameric biotin-binding protein streptavidin was used to connect the aldolase cubes in a controlled, stepwise assembly procedure. Streptavidin has D2 symmetry and two of the four binding sites were used to connect with each face of the aldolase. The length of the bivalent streptavidin connectors was varied by forming stiff streptavidin rods held together with bis-biotin linkers. More extensive networks were formed when the aldolase was confined through His tags on the protein to a monolayer containing Ni-NTA lipids. However, at this stage of development, the largest networks extend over only a few hundred nanometers. The biotin–streptavidin interaction is extremely strong, but it might be possible to further extend and repair the constructs by forming annealable networks from modified biotins and engineered streptavidins with weakened affinities.

Protein nanopores

Protein nanopores have beenengineered for applications in nanobiotechnology. Thea-hemolysin(aHL)pore has been especially well explored and exemplifieswhat can be done. Modified aHL pores have been developed into sensors to detect environmental pollutants, chemical or biological weapons, and medical analytes at the single molecule level by stochastic sensing. These analytes range from small ions and organic molecules to nucleic acids and proteins, and recently reactive molecules. Importantly, unusual approaches to protein engineering have been developed in conjunction with these applications. For example, it was shown that adapter molecules can lodge within the lumen of the aHL pore and thereby change its properties. Based on this observation, more complex constructs such as pores that carry two different cyclodextrin adapters have been assembled. In another approach, a polymer was

attached within the lumen of the aHL pore and the biotinylated free end was observed to move back and forth from one side of a lipidbilayer to the other, a distance ofat least 10 nm. Recently, step-by-step polymer growth within the aHL pore has been observed, and it may be possible to use this approach to construct, in situ, capped polymers of precisely defined length. It has also been shown that up to 175 exogenous amino acids can be packed within the lumen of the aHLpore as an internal loop in one of the seven subunits. This work sets the stage for the placement of functional polymers or even small folded proteins within a protein nanopore. New developments include the demonstration that aHL and other b-barrel transmembrane pores can operate at temperatures approaching the boiling point of water and that individual pores can be placed in bilayers by using amechanical probe, which opens up new possibilities for device fabrication by pick-and-place techniques. Here, we focus briefly on recent developments with light modulatable pores because light, from either an external or internal source, represents an excellent way of triggering or switching components of nanodevices, and of pumping energy into a device.

aHL pores have been made that open in response to chemical derivatization, light and protease digestion. Switchable aHL pores that respond to metal ions have also been prepared. Recently, two new efforts in this area have appeared. Inspired by the pioneering work of Henry Lester on the acetylcholine receptor, Kramer and colleagues have made a light-switchable (as opposedto triggered) K channel. A channel blocker was anchored to a specific site on the protein through a photoisomerizable arm containing an azobenzene group. In the trans configuration, the azobenzene allows the appended blocker, a tetraalkyl ammonium group, access to its binding site at the external mouth of the channel. After irradiation at 380 nm, causing trans–cis isomerization, the blocker can no longer reach its binding site and the channel is activated causing a 10-fold increase in current flow. Irradiation at 500 nm causes cis– trans isomerization and the channel is blocked again. Presumably, the tetrameric channel carries four azobenzene blockers and therefore efficient conversion to the cis formis required for switching. Koc¸er and colleagues have examined photomodulatable derivatives of the E. coli mechanosensitive channel MscL. MscL is a pentameric channel that is normally opened by membrane tension to form a 3 nm-diameter pore. The presence of polar groups at position 22 in the M1 transmembrane helix causes MscL to open at zero applied pressure. Therefore, a light-removable 2-nitro- 4,5-dimethoxybenzyl-containing group was placed on a cysteine residue that had been introduced at position 22, such that a charged carboxymethylcysteine would remain after photolysis. As predicted, after photolysis, the engineered protein exhibited

transient openings in patch clamp recordings. A spiropyran was then placed at the same position. When irradiated, spiropyrans ring-open to the polar merocyanine form. In this case, a reversible response was demonstrated in patch clamp experiments. Pores controlled by light might be used to allow molecules to move into or out of devices, or to travel from one compartment to another within a device.

Other applications of light-activated membrane proteins in nanobiotechnology include components for memory storage and energy transduction. For example, assemblies of bacteriorhodopsin, which in nature is a light-driven proton pump containing a photoisomerizable retinylidene chromophore, have been used as photorewriteable information storage devices or as photochromic ink for security card applications. In 1974, Racker and colleagues reported that the photogenerated proton gradient produced by bacteriorhodopsin across liposomal membranes can be used to power ATP synthesis by ATP synthase (F1FOATPase). Recently, Montemagno and colleagues have shown that such liposomes can be incorporated into silica sol-gels, a prelude to their use in nanodevices provided the long-term stability of sol-gels can be addressed.

Molecular motors

Cells contain a variety of motor proteins that move in a linear fashion (e.g. the kinesin or myosin motors) or rotate (e.g. ATP synthase or bacterial flagellar motors). One element of the ATP synthase is an ATP-driven rotary motor, the F1-ATPase. Two properties of the F1-ATPase that would be useful in nanodevices have been demonstrated: an interface with abiotic materials and an on-off switch. Normally, the ATP synthase uses the energy available from the downhill transport of protons across a membrane to convert ADP and Pi to ATP. Proton movement through the membrane-embedded FO domain drives the rotation of a central stalk inside the trimeric [(ab)3] F1 domain. Successive conformational changes of the F1 subunits force the release of ATP, generated from bound ADP and Pi. Conversely, the hydrolysis of ATP can drive the rotation of the central stalk in the opposite direction. This was dramatically demonstrated in 1997, when Noji and colleagues coupled a fluorescently tagged actin molecule, 1 mm to 4 mm in length, to the stalk and observed its rotation by fluorescence microscopy.

Subsequently, in a step towards incorporation into nanodevices, the F1-ATPase motor has been interfaced with inorganic materials. The ‘bottom’ of the protein was attached to a Ni-capped post 80 nm in diameter and 200 nm high. The stalk, which protrudes at the top of the protein,

was attached to a Ni propeller 750 nm to 1400 nm in length and 150 nm in width. In both cases, attachment was through the side-chains of histidine residues, which appear to bond effectively to Ni metal. Upon Protein components for nanodevices the addition of ATP, the propellers rotated, the short ones at _8 rps and the long ones at _1 rps. Although the success rate was poor (_1% rotated), when rotation did occur it lasted for more than 2.5 h.

In another study, the F1-ATPase was equipped with an engineered switch to control the rotation of the motor by an external chemical stimulus. The switch consisted of three histidine residues strategically positioned at the interface between two of the subunits in the F1- ATPase (a and b). When Zn(II) is coordinated by the three histidines (and a water molecule), the conformational change involved in catalysis is blocked and rotation ceases as observed at the single molecule level. The addition of a chelating agent restores rotation. If the F1-ATPase rotary motor can be incorporated into nanodevices, for example to provide propulsion, engineered switches will allow sensing of the environment and feedback control.

Other molecular motors move along linear tracks. The ATP-powered motor activity of myosin, kinesin or dynein is usually visualized by tracking the movement of fluorescently labeled protein fibers relative to immobilized motor proteins. Several groups have investigated the use of taxol-stabilized microtubules as shuttles to move cargo along engineered kinesin tracks. For example, the feasibility of using complex track networks to transport microtubule shuttles along micropatterned surfaces has been tested. In one example, Vogel and colleagues examined kinesin-coated figure-of-eight circuits on glass with crossing points and dead-ends in which microtubules turned around. To carry out transport, cargo must be loaded and unloaded, and the speed of the system must be controlled. Control over microtubule velocity has already been achieved by the photolytic cleavage of caged ATP. Loading and unloading might similarly be controlled through the use of photoisomerizable ligands as ‘hooks’. Vogel and colleagues also envisage laboratory-on-a-chip devices with picoliter volumes powered by the motorized transport of reagents instead of pressure-driven or electroosmotic bulk flow. They also suggest that motors might be used to produce unusual materials trapped in non-equilibrium states. Indeed, they have recently demonstrated the ATP-driven assembly of ‘nanowires’ and ‘nanospools’ from microtubules, made sticky through biotinylation and the presence of a sub-saturating concentration of streptavidin. These structures would not be formed in normal thermally activated processes. Microtubules are very hard to bend (persistence length >5 mm) and require the ATPdriven process to form the _2 mm diameter circles that are observed.

In another example, immobilized myosin has been used to transport actin-based conductive metallic nanowires. Like the study of the F1-ATPase, this work indicates that biological activity is compatible with inorganic/ organic hybrid structures. To form nanowires, G-actin derivatized with 1.4 nm Au nanoparticles was polymerized. This was followed by the catalyticenlargement of the nanoparticles to yield gold wires (1–4 mm long and 80–200 nm high), whichexhibited high electrical conductivity. Polymerization of the Au nanoparticle/G-actin monomerfollowed by the polymerization of free G-actin, or alternatively polymerization of the Au- nanoparticlelabelled G-actin on polymerized F-actin, followed by the catalytic enlargement ofthe particles, gave patterned actin-Au wire-actin or Au wire-actin-Au wire nanostructures suitable for transport by the myosin motor.

DNA as a Construction Material

There are several advantages to using DNA for nanotechnological constructions. First, the ability to get sticky ends to associate makes DNA the molecule whose intermolecular interactions are the most readily programmed and reliably predicted: Sophisticated docking experiments needed for other systems reduce in DNA to the simple rules that A pairs with T and G pairs with C. In addition to the specificity of interaction, the local structure of the complex at the interface is also known: Sticky ends associate to form B-DNA. A second advantage of DNA is the availability of arbitrary sequences, due to convenient solid support synthesis. The needs of the biotechnology industry have also led to straightforward chemistry to produce modifications, such as biotin groups, fluorescent labels, and linking functions. The recent advent of parallel synthesis is likely to increase the availability of DNA molecules for nanotechnological purposes. DNA-based computing is another area driving the demand for DNA synthetic capabilities. Third, DNA can be manipulated and modified by a large battery of enzymes, including DNA ligase, restriction endonucleases, kinases and exonucleases. In addition, double helical DNA is a stiff polymer in 1-3 turn lengths, it is a stable molecule, and it has an external code that can be read by proteins and nucleic acids.

There are two properties of branched DNA that one cannot ignore: First, the angles between the arms of branched junctions are variable. In contrast, to the trigonal or tetrahedral carbon atom, ligation-closure experiments, have demonstrated branched junctions are not well-defined geometrically. Thus, the cube and the truncated octahedron discussed above are molecules

whose graphs correspond to the graphs of those ideal objects, but only their branching connectivity has been (or probably can be) demonstrated. Simple branched junctions apparently do not lead to geometrical control. This places a greater burden on specificity: The construction illustrated in Figure would not lead exclusively to the quadrilateral depicted there unless the inter-arm angles were fixed to be right angles. Nevertheless, it is possible to generate a quadrilateral by using four different sticky end pairs to make each of the four edges.

Second, it is imperative to recognize that DNA is a helical molecule. For many purposes, the double helical half-turn is the quantum of single-stranded DNA topology. Figure illustrates two variants of Figure 1, one with an even number of half-turns between vertices, and the other with an odd number. With an even number of half-turns, the underlying substructure is a series of catenated single-stranded cycles, much like chain-mail, but an odd number leads to an interweaving of long strands. If the edges flanking a face of a polyhedron contain an exact number of helical turns, then that face contains a cyclic strand as one of its components; this strand will be linked (in the topological sense) to the strands of the adjacent faces, once for every turn in their shared edges. We used this design motif with both the cube and the truncated octahedron, so they are really a hexacatenane and a 14-catenane. In general, the level control over linking topology available from DNA is almost equal to the level of control over branching topology. Consequently, a number of topological species have been constructed relatively easily from DNA, even though they represented extremely difficult syntheses using the standard tools of organic and inorganic chemistry.

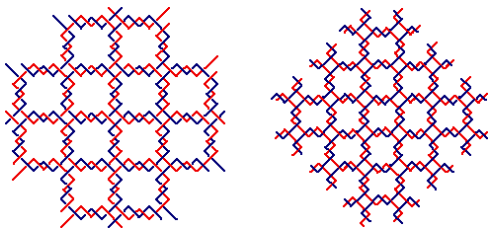

Figure 4.1 DNA origami

Topological Consequences of Ligating DNA Molecules Containing Even and Odd Numbers of DNA Half-Turns in Each Edge. These diagrams represent the same ligation shown in Figure However, they indicate the plectonemic winding of the DNA, and its consequences. The DNA is drawn as a series of right-angled turns. In the left panel, each edge of each square contains

two turns of double helix. Therefore, each square contains a cyclic molecule linked to four others. In the right panel, each edge of each square contains 1.5 turns of DNA. Therefore, the strands do not form cycles, but extend infinitely in a warp and weft meshwork.

The Construction and Analysis of DNA Polyhedra

The combination of branched DNA and sticky-ended ligation results in the ability to form stick figures whose edges consist of double helical DNA, and whose vertices are the branch points of the junctions. The flexibility of the angles that flank the branch points of junctions results in the need to specify connectivity explicitly. This may be done either by a set of unique sticky end pairs, one for each edge, or by utilizing a protection-deprotection strategy so that only a given pair is available for ligation at a particular moment. The first strategy was used in the construction of the DNA cube, which was done in solution.

We found that we had too little control over the synthesis when it was done in solution, so we developed a solid-support-based methodology. This approach allows convenient removal of reagents and catalysts from the growing product. Each ligation cycle creates a robust intermediate object that is covalently closed and topologically bonded together. The method permits one to build a single edge of an object at a time, and to perform intermolecular ligations under conditions different from intramolecular ligations. Control derives from the restriction of hairpin loops forming each side of the new edge, thus incorporating the technique of successive deprotection. Intermolecular reactions are done best with asymmetric sticky ends, to generate specificity. Sequences are chosen in such a way that restriction sites are destroyed when the edge forms. One of the major advantages of using the solid support is that the growing objects are isolated from each other. This permits the use of symmetric sticky ends, without intermolecular ligation occurring. More generally, the solid support methodology permits one to plan a construction as though there were only a single object to consider. Many of the differences between a single molecule and a solution containing 10^{12} molecules disappear if the molecules are isolated on a solid support. We utilized the solid-support methodology to construct the DNA truncated octahedron.

The polyhedra we made were objects that were topologically specified, rather than geometrically specified; consequently, our proofs of synthesis were also proofs of topology. In each case, we incorporated restriction sites in appropriate edges of the objects, and then broke them down to target catenanes, whose electrophoretic properties could be characterized against

standards. For example, the first step of synthesizing the cube resulted in the linear triple catenane corresponding to the ultimate left-front-right sides of the target. When the target was achieved, one of the most robust proofs of synthesis came from the restriction of the two edges in the starting linear triple catenane, to yield the linear triple catenane corresponding to the top-back-bottom of the cube, as shown in Figure 4.1. A similar approach was taken with the proof of the truncated octahedron synthesis: The presence of the six square strands was demonstrated first. Then the octacatenane corresponding to the eight hexagonal faces was shown by restricting it down to the tetracatenane flanking each square, for which we were able to make a marker.

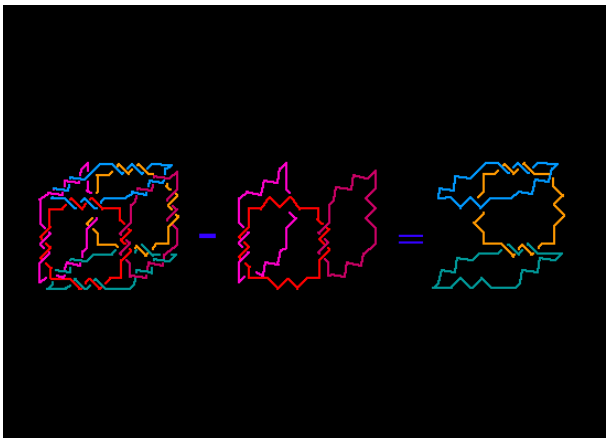

Figure 4.2 DNA nanostructure

The Linear Triple Catenanes that Link to Form the Cube. The target cube is shown at the left of the figure. The starting material for its synthesis was the linear triple catenane shown at the center of the drawing. This catenane corresponds to the left, front and right faces of the cube. When the cube is restricted on its two front edges, the starting linear triple catenane is destroyed. However, when the cube is successfully synthesized, a linear triple catenane results. This catenane corresponds to the top, back and bottom faces of the cube.

The solid-support based methodology appears to be quite powerful. We feel that we could probably construct most Platonic, Archimedean, Catalan, or irregular polyhedra by using it. The cube is a 3-connected object, as is the truncated octahedron. The cube was constructed from 3-arm branched junctions, but the truncated octahedron was constructed from 4-arm branched junctions, because we had originally planned to link the truncated octahedra together. The connectivity, of an object or a network determines the minimum number of arms that can flank the junctions that act as its vertices. Thus, one must have at least 5-arm branched junctions to construct an icosahedron, and one must have 12-arm branched junctions to build a cubic-

close-packed (face-centered cubic) lattice. We have built junctions with up to 6 arms, but there seem to be no impediments to making junctions containing arbitrary numbers of arms. The one *caveat* to observe is that the lengths of the arms necessary for stabilization tend to increase with the number of arms.

Topological Construction

The key requisite for constructing topological targets is the ability to produce at will a chemical version of a node or a crossing (sometimes called a unit tangle) in the target. The strength of DNA in this regard derives from the fact that a half-turn of DNA corresponds exactly to this necessary component. It is easy to understand this relationship by looking at Figure 7. Here, a trefoil knot has been drawn, with an arbitrary polarity. Squares have been placed about each of the crossings, so that the portions of the knot contained within each square act as its diagonals. These diagonals divide the square into four regions, two between parallel strands, and two between antiparallel strands. Whereas the strands of double helical DNA are antiparallel, one should design the sequence of the DNA strand so that pairing occurs over a half-turn segment (ca. 6 nucleotide pairs) in the regions between antiparallel strands. Thus, it is possible to make the transition from topology to nucleic acid chemistry by specifying complementary sequences to form desired nodes. Linker regions between the nodes usually consist of oligo-dT.

Nodes as Half-Turns of Double Helical DNA

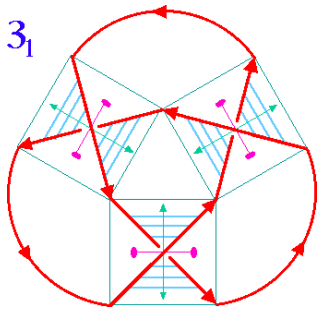

Figure 4.3 *The Relationship Between Nodes and Antiparallel B-DNA Illustrated on a Trefoil Knot.*A trefoil knot is drawn with negative nodes. Nodes are also known as crossings or unit tangles.The path is indicated by the arrows and the very thick curved lines connecting them. The nodesformed by the individual arrows are drawn at right angles to each other. Each pair of arrows forming a node defines a quadrilateral (a square in this figure), which is drawn in dotted lines.

Each square is divided by the arrows into four domains, two between parallel arrows and two between antiparallel arrows. The domains between antiparallel arrows contain lines that correspond to base pairing between antiparallel DNA (or RNA) strands. Dotted double-arrowheaded helix axes are shown perpendicular to these lines. The twofold axis that relates the two strands is perpendicular to the helix axis; its ends are indicated by lens-shaped figures. The twofold axis intersects the helix axis and lies halfway between the upper and lower strands. The amount of DNA shown corresponds to about half a helical turn. It can be seen that three helical segments of this length could assemble to form a trefoil knot. The DNA shown could be in the form of a 3-arm DNA branched junction. A trefoil of the opposite sense would need to be made from Z-DNA, in order to generate positive nodes.

There are two kinds of nodes found in topological species, positive nodes and negative nodes. As illustrated at the top of Figure 8, these nodes are mirror images of each other. B-DNA is a right-handed helical molecule. Its crossings generate nodes that are designated to have negative signs, as illustrated at the bottom-left side of the drawing. Fortunately, there is another form of DNA, Z-DNA, shown at the bottom-right, whose helix is left-handed. Z-DNA is not the geometrical mirror image of B-DNA, because it still contains D-deoxyribose sugar residues, and, in addition, its structure is qualitatively different. However, from a topological standpoint, it is the mirror image of B-DNA, and it can be used to supply positive nodes when they are needed.

Fig 4.4. Node Chirality

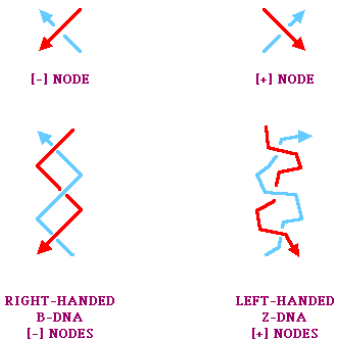

Figure. *Nodes and DNA Handedness.* The upper part of this drawing shows positive and negative nodes, with their signs indicated. It is useful to think of the arrows as indicating the 5'-->3' directions of the DNA backbone. Below the negative node is a representation of about

one and a half turns of a right-handed B-DNA molecule. Note that the nodes are all negative. Below the positive node is a left-handed DNA molecule, termed Z-DNA. The Z-DNA molecule has a zig-zag backbone, which we have tried to indicate here. However, the zig-zag nature of the backbone does not affect the fact that all the nodes are positive.

The Z-forming propensity of a segment of DNA is a function of two variables, the sequence, and the conditions. Not all sequences undergo the B-->Z transition under the mild conditions compatible with enzymatic ligation. The sequence of conventional nucleotides that undergoes the transition most readily contains the repeating dinucleotide sequence dCdG. Furthermore, the ease with which a segment undergoes the B-->Z transition can be made a function of base modification; DNA in which a methyl group has been added to the 5-position of cytosine undergoes the transition under milder conditions. However, in the absence of Z-promoting conditions, the sequence will remain in the B-form.

Fig.4.5 A DNA Strand in Four Topological States

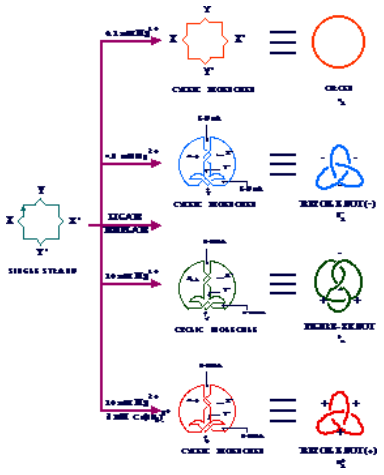

Figure *A DNA Strand is Ligated into Four Topological States by Variation of Ligation Conditions.* The left side of this synthetic scheme indicates the molecule from which the target products are produced. The four pairing regions, X and its complement X', Y and its complement Y' are indicated by the bulges from the square. The 3' end of the molecule is denoted by the arrowhead. The four independent solution conditions used to generate the target products are shown to the right of the basic structure. The pairing and helical handedness expected in each case is shown to the right of these conditions, and the molecular topology of

the products is shown on the far right of the figure. The species are, from the top, the circle, the trefoil knot with negative nodes, the figure-8 knot, and the trefoil knot with positive nodes.

The favored topology of each of the species in figure is a function of solution conditions. If one of these molecules is placed in solution conditions that favor one of the other knots, it cannot convert to the new favored structure without breaking and rejoining its backbone. However, type I DNA topoisomerases can catalyze this interconversion. Figure 10 illustrates the stepwise interconversion of the different species, under solution conditions that promote the B-->Z or Z-->B transitions.

Interconversion of DNA Knots

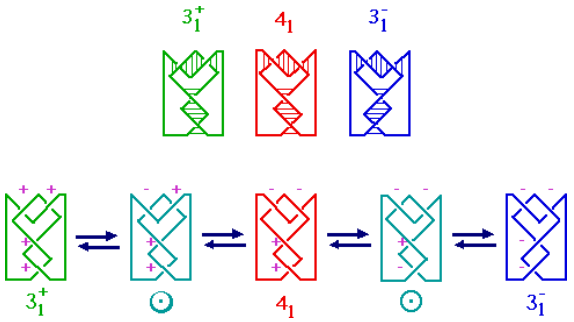

Figure 4.6 *DNA Knots Interconverted by Type I DNA topoisomerases.* On the top of this figure are the three knots that are interconverted, the trefoil knot with positive nodes, The figure-8 knot, and the trefoil knot with negative nodes. The nucleotide pairs that give rise to the nodes are indicated between strands. The same knots are shown in the bottom portion of the figure, interspersed by circles drawn with the node structures of dumbbells. The lines indicating the base pairs have been removed for clarity. The '+' and '-' signs near the nodes indicate their topological signs. The equilibria indicated between structures are catalyzed by the *E. coli* DNA Topoisomerases I and III. The trefoil knot on the left has all positive signs, and the signs of a single node at a time are switched from positive to negative in each of the structures as one proceeds towards the right of the figure. Changing the sign of a single node in the positive trefoil knot produces a circle (dumbbell), and changing a second node in the same domain produces a figure-8 knot. Changing the sign of another positive node in the figure-8 knot produces the circle (dumbbell) on the right, and changing the sign of the last node generates

the negative trefoil knot. It is important to realize that the two circles shown may interconvert without the catalytic activity of a topoisomerase.

This ability of topoisomerases to interconvert synthetic DNA knots suggested to us that it would be possible to use an RNA knot to assay the presence of an RNA topoisomerase, a species unknown previously. By preparing both an RNA knot and an RNA circle, we found that it was possible to catalyze the interconversion of these cyclic molecules by the presence of *E. coli* DNA topoisomerase III. This experiment is illustrated in figure

Fig. Discovery of an RNA Topoisomerase

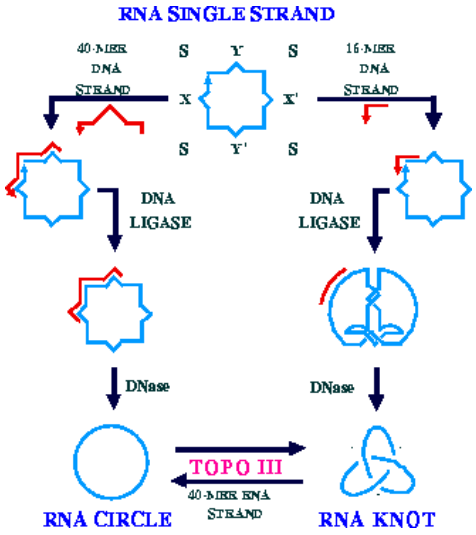

Figure 4.7 *The Discovery of an RNA Topoisomerase* An RNA single strand is shown at the top ofthis diagram. Its Watson-Crick pairing regions, X, Y, X' and Y' are illustrated at bumps on thesquare, and the spacers, denoted by S are shown as the corners of the square. The arrowhead denotes the 3' end of the strand. The pathway to the left illustrates formation of the RNA circle: A 40 nucleotide DNA linker (incompatible with knot formation) is annealed to the molecule, and it is ligated together to form an RNA circle, which survives treatment with DNase. In the other pathway, a 16 nucleotide DNA linker is used in the same protocol to produce the RNA trefoil knot, whose three negative nodes are indicated. The interconversion of the two species by *E. coli* DNA Topoisomerase III (Topo III) is shown at the bottom of the figure. The 40-mer RNA strand promotes somewhat the formation of the circle from the knot. *E. coli* DNA Topoisomerase I does not catalyze this reaction.

NANOMOTORS AND CELLULAR NAVIGATION

Introduction

Nanotechnology can best be defined as a description of activities at the level of atoms and molecules that have applications in the real world. A nanometer is a billionth of a meter, that is, about 1/80,000 of the diameter of a human hair, or 10 times the diameter of a hydrogen atom. The size-related challenge is the ability to measure, manipulate, and assemble matter with features on the scale of 1-100nm.

Nature deploys proteins to perform various cellular tasks – from moving cargo, to catalyzing reactions, while it has kept DNA as an information carrier. It is hence understandable that most of the natural machinery is built from proteins. With the powerful crystallographic techniques available in the modern world, the protein structures are clearer than ever. The ever increasing computing power makes it possible to dynamically model protein folding processes and predict the conformations and structure of lesser known proteins. All this helps unravel the mysteries associated with the molecular machinery and paves the way for the production and application of these miniature machines in various fields including medicine, space exploration, electronics and military.

This section focuses on the study of the following main protein based molecular machines:

i. ATP Synthase

ii. The Kinesin, Myosin, Dynein and

iii. Flagella Molecular Motors

A **nanomotor** is a nanotechnology-based device, operating at a molecular level, and which is capable of effecting forces of the order of piconewtons. Energy acquired by a nanomotor can thus be converted into motion at the molecular level.

It has been proposed by nanotechnology-based scientists to integrate molecular motor proteins that occur in living cells into molecular motors for other purposes. These molecular motors, or ‘nanomotors’ as they are dubbed, can be implanted in artificial devices to perform much the same functions that their living-cell counterparts perform. Such a motor protein developed can be able to move required ‘objects’ within the device.

If successfully applied, nanomotors could become an important component of the now-nascent field of nanotechnology.

An important way of controlling particles and to measure the fundamental properties of individual nanoparticles is by the production and use of nanomotors. They are a powerful means by which rheological nanoenvironments and biomolecular motors can be investigated. Factors that affect movement speeds of nanomotors include the torque magnitude involved and the viscosity of the surrounding medium. Biotechnologists have observed that every unique organism can have specific nanomotors. Each of these nanomotors can be evolved through a set of stages to perform transporting functions at the molecular level, across cell membranes in living tissue.

ATP Synthase – a true nano rotary motor

Synthesis of ATP is carried out by an enzyme, known as ATP Synthase. The inner mitochondrial membrane contains the ATP Synthase. The ATP Synthase is actually a combination of two motors functioning together. This enzyme consists of a proton-conducting F_0 unit and a catalytic F_1 unit. The subunits in side the two motor components. F_1 constitutes of 33αβγδε subunits. F_0 has three different protein molecules, namely, subunit a, b and c. The γ-subunit of F_1 is attached to the c subunit of F_0 and is hence rotated along with it. The 33αβsubunits are fixed to the b-subunit of F_0 and hence do not move. Further the b-subunit is held inside the membrane by a subunit of F_0.

ATP Synthase 'nano' Properties

1. Reversibility of the ATP Synthase: There are two directions in ATP Synthase system and these two directions correspond to two different functionalities and behavior. This two-way behavior is because of the reversible nature of the ATP-ADP cycle and the structure of the ATP Synthase. Let us term the forward direction as when the F_0 drives the γ-subunit (because of Proton Motive force) of F_1 and hence ATP synthesis takes place. And the backward direction is when hydrolysis of ATP counter-rotated the γ-subunit and hence the F_0 motor and leads to pumping back the protons. Therefore the forward direction is powered by the proton motive force and the backward direction is powered by the ATP hydrolysis. Which particular direction

is being followed depends upon the situation and the environmental factors around the ATP Synthase.

2. Coupling of Proton Flow (F_0) and the ATP synthesis and hydrolysis (in F_1): Boyer proposed a model which predicted that the F_0 and F_1 motors are connected through the γ subunit. Further he proposed that this connection was mechanical in nature.

3. Boyer's binding Change Mechanism: Boyer isolated the F_1 part of the ATP Synthase complex. It was found that the alpha and beta subunits alternate in this cylindrical part of the F_1 structure. As per this model each α and β pair forms a catalytic site. The rotation of the γ subunit induces structural conformation in the $\alpha_3\beta_3$ subunits. Although the three catalytic units are identical in their chemistry but they are functionally very different at any given point in time. These conformal changes induce a change in the binding affinities of all the three catalytic sites towards the ATPase reactants (ADP, P_i, ATP etc.).

4. F_1-ATPase a true nano rotary motor: Till today the exact mechanism of the molecular motor characterized by F_1-ATpase has not been fully determined. Research by Kinosita's lab is a step towards this goal and proposes some very conclusive models for the same. The results obtained show not only the various methods through which we can analyze these nano devices, but also predicts many characteristics for these.

What is known till now is that γ subunit rotates inside the alpha-beta hexamer, but whether the rotation is continuous or is random was not known. Kinosita's lab solved this problem by imaging the F_1-ATPase molecule. The objective of their experiment was to determine the uniqueness of the rotary motion and its characteristics. They attached a micrometer long actin filament to the γ subunit. This actin filament was fluorescently label, so that its fluorescence could be measured under a microscope. Hydrolysis of the ATP (when introduced in the experiment) led to the rotation of the γ subunit and in effect the rotation of the actin filament. As reported by the authors, not all the actin filaments were observed to have rotation. But some percentage of them did rotate and that too in a unique direction and without having much reversibility in the direction. This direct imaging proved that the structure solved by Walker and group was indeed correct and there exists rotary motion between γ subunit and the alpha and beta hexamer.

Electric magnets were used to rotate this bead attached to the γ subunit. The rotation resulted in appearance of ATP in the medium (which was initially immersed in ADP). Thus the connection between the syntheses of ATP as a result of the mechanical energy input is established.

The exact mechanism of the F_1-ATPase rotation is still an active area of research today and many groups are working towards finding it. The key to solving the mechanism is solving the transient conformation of the catalytic sites and the γ subunit when rotation is taking place. What is not clear is the correspondence between the chemical reactions at the catalytic sites and their influence on the rotation of the γ subunit. Which event triggers the rotation and which not has still to be exactly determined? Many models have been predicted, but they all still elude the reality of the rotational mechanism.

The Kinesin, Myosin, and Dynein - linear motors

With modern microscopic tools, we view a cell as a set of many different moving components powered by molecular machines rather than a static environment. Molecular motors that move unidirectionally along protein polymers (actin or microtubules) drive the motions of muscles as well as much smaller intracellular cargoes. In addition to the F_0-F_1-ATPase motors inside the cell, there are linear transport motors present as tiny vehicles known as motor proteins that transport molecular cargoes that also require ATP for functioning. These minute cellular machines exist in three families - the kinesins, the myosins and the dyneins. The cargoes can be organelles, lipids or proteins etc. They play an important role in cell division and motility. There are over 250 kinesin-like proteins, and they are involved in processes as diverse as the movement of chromosomes and the dynamics of cell membranes.

The only part they have in common is the catalytic portion known as the motor domain. They have significant differences in their location within cells, their structural organization, and the movement they generate. Muscle myosin, whose study dates back to 1864, has served as a model system for understanding motility for decades. Kinesin however was discovered rather recently using in vitro motility assays in 1985. Conventional Kinesin is a highly processive motor that can take several hundred steps on a microtubule without detaching whereas muscle myosin executes a single "stroke" and then dissociates . A detailed analysis and modeling of these motors has been done.

Kinesin and myosin make up for an interesting comparison. Kinesin is microtubule-based; it binds to and carries cargoes along microtubules whereas myosin is actin-based. The motor domain of kinesin weighs one third the size of that of myosin and one tenth of that of dynein. Before the advent of modern microscopic and analytic techniques, it was believed that these two have little in common. However, the crystal structures available today indicate that they probably originated from a common ancestor.

The Myosin Linear Motor

Myosin is a diverse superfamily of motor proteins. Myosin-based molecular machines transport cargoes along actin filaments - the two stranded helical polymers of the protein actin, about 5-9 nm in diameter. They do this by hydrolyzing ATP and utilizing the energy released. In addition to transport, they are also involved in the process of force generation during muscle contraction, wherein thin actin filaments and thick myosin filaments slide past each other. Not all members of the myosin superfamily have been characterized as of now. However, much is known about the structure and function. Myosin molecules were first sighted through electron microscope protruding out from thick filaments and interacting with the thin actin filaments in late 1950s. Since then it was known that ATP plays a role in myosin related muscle movement along actin. However, the exact mechanism was unknown, which was explained later in 1971 by Lymn and Taylor.

(a) Structure of Myosin Molecular Motor: Myosin molecule has a size of about 520 kilodaltons (kD) including two 220 kD heavy chains and light chains of sizes between 15-22 kD. They can be visualized as two identical globular 'motor heads', also known as motor domains, each comprising of a catalytic domain (actin, nucleotide as well as light chain binding sites) and about 8 nm long lever arms. The heads, also sometimes referred to as S1 regions (subfragment 1) are shown in blue, while the lever arms or the light chains, in yellow. Both these heads are connected via a coiled coil made of two α-helical coils (grey) to the thick base filament. The light chains have considerable sequence similarity with the protein 'calmodulin' and troponin C, and are sometimes referred to as calmodulin-like chains. They act as links to the motor domains and do not play any role in their ATP binding activity but for some exceptions. The motor domain in itself is sufficient for moving actin filaments. Three-dimensional structures of myosin head revealed that it is a pear-shaped domain, about 19 nm long and 5 nm in maximum diameter.

(b) Function of Myosin Molecular Motor: A crossbridge-cycle model for the action of myosin on actin has been widely accepted since 1957. Since the atomic structures of actin monomer and myosin were resolved this model has been refined into a 'lever-arm model' which is now acceptable. Only one motor head is able to connect to the actin filament at a time, the other head remains passive. Initially the catalytic domain in the head has ADP and P_i bound to it and as a result, its binding with actin is weak. With the active motor head docking properly to the actin-binding site, the P_i has to be released. As soon as this happens, the lever arm swings counterclockwise due to a conformational change. This pushes the actin filament down by about 10 nm along its longitudinal axis. The active motor head now releases its bound ADP and another ATP molecule by way of Brownian motion quickly replaces it, making the binding of the head to the actin filament weak again. The myosin motor then dissociates from the actin filament, and a new cycle starts. However, nano-manipulation of single S1 molecules (motor domains) show that myosin can take multiple steps per ATP molecule hydrolyzed, moving in 5.3 nm steps and resulting in displacements of 11 to 30 nm.

The Kinesin Linear Motor

Kinesin and Dynein family of proteins are involved in cellular cargo transport along microtubules as opposed to actin in the case of myosin. Microtubules are 25 nm diameter tubes made of protein tubulin and are present in the cells in an organized manner. Microtubules have polarity; one end being the plus (fast growing) end while the other end is the minus (slow growing) end. Kinesins move from minus end to plus end, while dyneins move from plus end to the minus end of the microtubules. Microtubule arrangement varies in different cell systems. In nerve axons, they are arranged longitudinally in such a manner that their plus ends point away from the cell body and into the axon. In epithelial cells, their plus end points towards the basement membrane. They deviate radially out of the cell center in fibroblasts and macrophages with the plus end protruding outwards. Like myosin, kinesin is also an ATP-driven motor. One unique characteristic of kinesin family of proteins is their processivity – they bind to microtubules and literally 'walk' on it for many enzymatic cycles before detaching. Also, each of the globular heads/motor domains of kinesin is made of one single polypeptide unlike myosin (heavy and light chains and dynein heavy, intermediate and light chains).

(a) Structure of Kinesin Molecular Motor: A lot of structural information about kinesin is now available through the crystal structures. The motor domain contains a folding motif similar to that of myosin and G proteins. The two heads or the motor domains of kinesin are linked via

'neck linkers' to a long coiled coil, which extends up to the cargo. They interact with the α and β-subunits of the tubulin hetrodimer along the microtubule protofilament. The heads have the nucleotide and the microtubule binding domains in them.

(b) Function of Kinesin Molecular Motor: While kinesin is also a two-headed linear motor, its modus operandi is different from myosin in the sense that both its head work together in a coordinated manner rather than one was being left out. Fig. 8b shows the kinesin walk. Each of the motor heads is near the microtubule in the initial state with each motor head carrying an ADP molecule. When one of the heads loosely binds to the microtubule, it looses its ADP molecule to facilitate a stronger binding. Another ATP molecule replaces the ADP which facilitates a conformational change such that the neck region of the bound head snaps forward and zips on to the head. In the process it pulls the other ADP carrying motor head forward by about 16 nm so that it can bind to the next microtubule-binding site. This results in the net movement of the cargo by about 8 nm. The second head now binds to the microtubule by losing its ADP, which is promptly replaced by another ATP molecule due to Brownian motion. The first head meanwhile hydrolyses the ATP and loses the resulting P_i. It is then snapped forward by the second head while it carries its ADP forward. Hence coordinated hydrolysis of ATP in the two motor heads is the key to the kinesin processivity. Kinesin is able to take about 100 steps before detaching from the microtubule while moving at 1000 nm/sec and exerting forces of the order of 5-6 pN at stall.

The Dynein Motor

Dynein superfamily of proteins was introduced in 1965. Dyneins exist in two isoforms, the cytoplasmic and the axonemal. Cytoplasmic dyneins are involved in cargo movement, while axonemal dyneins are involved in producing bending motions of cilia and flagella.

(a) Structure of Dynein Molecular Motor: The structure consists of two heavy chains in the form of globular heads, three intermediate chains and four light intermediate chains. Recent studies have exposed a linker domain connecting the 'stem' region below the heads to the head itself. Also from the top of the heads the microtubule binding domains protrude out. The ends of these stalks have smaller ATP sensitive globular domains which bind to the microtubules. Cytoplasmic dynein is associated with a protein complex known as dynactin, which contains ten subunits. Some of them are shown in the figure as p150, p135, actin related protein 1 (Arp1), actin, dynamitin, capping protein and p62 subunit. These play an important regulatory

role in the binding ability of dynein to the microtubules. The heavy chains forming the two globular heads contain the ATPase and microtubule motor domains.

One striking difference that dynein exhibits compared to kinesins and myosins is that dynein has AAA (ATPases Associated with a variety of cellular Activities) modules, which indicate that its mode of working will be entirely different from kinesins and myosins. This puts dyneins into the AAA superfamily of mechanoenzymes. The dynein heavy chains contain six tandemly linked AAA modules with the head having a ring-like domain organization, typical of AAA superfamily. Four of these are nucleotide binding motifs, named P1-P4, but only P1 (AAA1) is able to hydrolyse ATP.

(b) Function of Dynein Molecular Motor: Because dynein is larger and more complex structure as compared to other motor proteins, its mode of operation is not as well known. However, very recently, using electron microscopy and image processing it has been shown the structure of a flagellar dynein at the start and end of its power stroke; giving some insight into its possible mode of force generation. When the dynein contains bound ADP and V_i (vandate), it is in the pre-power stroke conformation. The state when it has lost the two, known as the apo-state is the more compact post power stroke state. There is a distinct conformational change involving the stem, linker, head and the stalk that produces about 15 nm of translation onto the microtubule bound to the stalk.

The Flagella Motors

Unicellular organisms, such as, E. coli have an interesting mode of motility. They have a number of molecular motors, about 45 nm in diameter, that drive their 'feet' or the flagella that help the cell to swim. Motility is critical for cells, as they often have to travel from a less favorable to a more favorable environment. The flagella are helical filaments that extend out of the cell into the medium and perform a function analogous to what the oars perform to a boat. The flagella and the motor assembly are called a flagellum. The flagella motors impart a rotary motion into the flagella. In addition to a rotary mechanism, the flagella machines consist of components such as rate meters, particle counters, and gearboxes. These are necessary to help the cell decide which way to go, depending on the change of concentration of nutrients in the surroundings. The rotary motion imparted to the flagella needs to be modulated to ensure the cell is moving in the proper direction as well as all flagella of the given cell are providing a concerted effort towards it . When the motors rotate the flagella in a counterclockwise direction as viewed along the flagella filament from outside, the helical flagella create a wave

away from the cell body. Adjacent flagella subsequently intertwine in a propulsive corkscrew manner and propel the bacteria. When the motors rotate clockwise, the flagella fly apart, causing the bacteria to tumble, or change its direction. These reversals occur randomly, giving the bacterium a 'random walk', unless of course, there is a preferential direction of motility due to reasons mentioned earlier. The flagella motors allow the bacteria to move at speeds of as much as 25 μm/s with directional reversals occurring approximately 1 per second. A number of bacterial species in addition to E. coli., depend on flagella motors for motility. Some of these are Salmonella enterica serovar Typhimurium (Salmonella), Streptococcus, Vibrio spp., Caulobacter, Leptospira, Aquaspirrilum serpens and Bacillus. The rotation of flagella motors is stimulated by a flow of ions through them which is a result of a build-up of a transmembrane ion gradient. There is no direct ATP-involvement; however the proton gradient needed for the functioning of flagella motors can be produced by ATPase.

(a) Structure of the Flagella Motors: A complete part list of the flagella motors may not be available as of now. Continued efforts dating back to early 1970s have however revealed much of their structure, composition, genetics and function. Newer models of the motor function are still being proposed with an aim to explain observed experimental phenomena. That means that we do not fully understand the functioning of this motor. A typical flagella motor from E. coli. consists of about 20 different proteins, while there are yet more that are involved in the assembly and functioning. There are 14 Flg-type proteins named FlgA to FlgN; 5 Flh-type proteins called FlhA to FlhE; 19 Fli-type proteins named FliA to FliT; MotA and MotB making a total of 40 related proteins. The name groups Flg, Flh, Fli and Flg originate from the names of the corresponding genes. Out of these the main structural proteins are FliC or the filament; FliD (filament cap); FliF or the MS-ring; FliG; FliM and FliN (C-ring); FlgB, FlgC and FlgF (proximal rod); FlgG (distal rod); FlgH (L-ring); FlgI (P-ring); FlgK and FlgL (hook-filament junction); and MotA-MotB (torque generating units). Earlier it was believed that the M and S are two separate rings and M was named after membrane and S after supramembranous. Now they are jointly called the MS-ring as it has been found that they are two domains of the same protein FliF. The C-ring is named after cytoplasmic, while the names of the P and L-rings come from 'peptidoglycan' and 'lipopolysaccharide' respectively. The FlhA,B, FliH,I,O,P,Q,R constitute the 'transport apparatus'.

The hook and filament part of the flagellum is located outside the cell body, while the motor portion is embedded in the cell membrane with parts (the C-ring and the transport apparatus) that are inside the inner membrane in the cytoplasmic region. MotA and MotB are arranged in

a circular array embedded in the inner membrane, with the MS-ring at the center. Connected to the MS-ring is the proximal end of a shaft, to which the P-ring, which is embedded in the peptidoglycan layer, is attached. Moving further outwards, there is the L-ring embedded in the outer cell membrane followed by the distal shaft end that protrudes out of the cell. To this end there is an attachment of the hook and the filament, both of which are polymers of hook-protein and flagellin respectively.

(b) Function of the Flagella Motors: The flagellar motors in most cases are powered by protons flowing through the cell membrane (protonmotive force, defined earlier) barring exceptions such as certain marine bacteria, for example, the Vibrio spp., which are driven by Na+ ions. There are about 1200 protons required to rotate the motor by one rotation. A complete explanation of how this proton flow is able to generate torque is not available as of today. From what is known, the stator units of MotA and MotB play an important role in torque generation. They form a MotA/MotB complex which when oriented properly binds to the peptidoglycan and opens proton channels through which protons can flow. It is believed that there are eight such channels per motor. The protonmotive force is a result of the difference of pH in the outside and the inside of the cell. The E. coli cells like to maintain a pH of 7.6-7.8 on their inside, so depending on the pH of the surroundings, the protonmotive force will vary, and hence the speed of rotation of their motors. To test how the speed of rotation depends on the protonmotive force, the motors were powered by external voltage with markers acting as heavy loads attached to them. It was found that the rotation indeed depends directly on the protonmotive force. According to the most widely accepted model, MotA/MotB complex interacts with the rotor via binding sites. The passage of protons through a MotA/MotB complex (stator or torque generator) moves it so that they bind to the next available binding site on the rotor, thereby stretching their linkage. When the linkage recoils, the rotor assembly has to rotate by one step. Hence whichever complex receives protons from the flux will rotate the rotor, generating torque. The torque-speed dependence of the motor has been studied in detail and indicates the torque range of about 2700 pN-nm to 4600 pN-nm.

Synthetic molecular motors are molecular machines capable of rotation under energy input. Although the term "molecular motor" has traditionally referred to a naturally occurring protein that induces motion, some groups also use the term when referring to non-biological, non-peptide synthetic motors. Many chemists are pursuing the synthesis of such molecular motors

e.g Triptycene motors

Helicene motors

RNA nanomotor: One particular virus that invades bacteria gets the bacteria to synthesize an unus powerful phi29 encoded **RNA** to build little **motors** so the virus can drive *its* DN

protective protein shells that are then inserted into the bacteria and take over the

cells' programming.

Cellular navigation

In order to achieve cost-effectiveness in nanotechnology it will be necessary to automate molecular manufacturing. The engineering of molecular products needs to be carried out by robotic devices, which have been termed nanorobots. A nanorobot is essentially a controllable machine at the nano meter or molecular scale that is composed of nano-scale components. The field of nanorobotics studies the design, manufacturing, programming and control of the nano-scale robots.

Nanorobots would constitute any passive or active structure (nano scale) capable of actuation, sensing, signaling, information processing, intelligence, swarm behavior at nano scale. These functionalities could be illustrated individually or in combinations by a nano robot (swarm intelligence and co-operative behavior). So, there could be a whole genre of actuation and sensing or information processing nano robots having ability to interact and influence matter at the nano scale. Some of the characteristic abilities that are desirable for a nanorobot to function are:

i. Swarm Intelligence – decentralization and distributive intelligence

ii. Cooperative behavior – emergent and evolutionary behavior

iii. Self assembly and replication – assemblage at nano scale and ‘nano maintenance’

iv. Nano Information processing and programmability – for programming and controlling nanorobots (autonomous nanorobots)

v. Nano to macro world interface architecture – an architecture enabling instant access to the nanorobots and its control and maintenance.

There are many differences between macro and nano-scale robots. However, they occur mainly in the basic laws that govern their dynamics. Macro scaled robots are essentially in the Newtonian mechanics domain whereas the laws governing nanorobots are in the molecular quantum mechanics domain. Furthermore, uncertainty plays a crucial role in nanorobotic systems. The fundamental barrier for dealing with uncertainty at the nano scale is imposed by the quantum and the statistical mechanics and thermal excitations. For a certain nano system at some particular temperature, there are positional uncertainties, which can not be modified or further reduced.

The nanorobots are invisible to naked eye, which makes them hard to manipulate and work with. Techniques like Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) are being employed to establish a visual and haptic interface to enable us to sense the molecular structure of these nano scaled devices. Virtual Reality (VR) techniques are currently being explored in nano-science and bio-technology research as a way to enhance the operator's perception (vision and haptics) by approaching more or less a state of 'full immersion' or 'telepresence'. The development of nanorobots or nano machine components presents difficult fabrication and control challenges. Such devices will operate in microenvironments whose physical properties differ from those encountered by conventional parts.

Mother Nature has her own set of molecular machines that have been working for centuries, and have been optimized for performance and design over the ages. As our knowledge and understanding of these numerous machines continues to increase, we now see a possibility of using the natural machines, or creating synthetic ones from scratch, using nature's components. The main goal in the field of molecular machines is to use various biological elements — whose function at the cellular level creates motion, force or a signal — as machine components. These components perform their preprogrammed biological function in response to the specific physiochemical stimuli but in an artificial setting. In this way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these different components were assembled together in the proper proportion and orientation they would form nano devices with multiple degrees of freedom, able to apply forces and manipulate objects in the nanoscale world. The advantage of using nature's machine components is that they are highly efficient and reliable.

Nanomotors inside the body: Biological nanotechnology, or nanobiotechnology, is the incorporation of nano-scale machines into biological organisms for the ultimate purpose of

improving the organism's quality-of-life. To date there are a few methods for synthesizing nano-devices that have the potential for being used in an organism without risk of being rejected as antigens. Any nano-device needs a motor to power the device and a power supply for that motor. A motor is any object or device that can contain moving or fixed parts that converts one form of energy to another. The entire process that the energy undergoes during conversion from one form to another inside the motor is considered a mechanism. Currently, research is being done into using the ATPase rotor-pump inside cells and mitochondria to power nanobiosystems. Other research involves the identification of pathogens in the human body and the destruction of these detrimental cells, drug delivery to sick cells, and the treatment and prevention of cancer using nanodevices.

"NEMS, or nano-electro-mechanical systems" are biologically-based nanosystems that are powered by biological motors and chemical energy sources. F1 ATPase is a biological pump that exists in mitochondria which phosphorylates ADT into ATP, providing an energy supply for the cell The force generated by this motor protein is >100 pico-Newtons, which is among the greatest of any known molecular motor" . Thus, this protein has the potential to be an almost perfect nano-motor to power nano-devices. For the nano-devices to be powered by the ATPase, modifications have to be made to the ATPase so it will provide energy for the mitochondria and the cell as well as the nano-devices, including "[M]utations in the g initiation codon from GTG (Valine) to ATG (Methionine), and Stop Codons from TAG to TAA" Still further modification is needed for the attachment and motion of the specific biomolecular motors. This is accomplished by attaching a Histidine containing synthetic peptide, a Histidine tag, to the g subunit, which creates unique Cysteine residues. These special residues can allow for the attachment of both inorganic and organic compounds using thiol chemistry. This modification is accomplished using thermophilic bacterium in vitro. This setup using ATPase, a naturally occurring proton pump, accomplishes three goals: First, it provides easy manipulation of the codon sequence to supply power for nano-devices. Secondly, it allows for the possible production of large quantities of proteins. Finally, the setup imparts the ability to attach "handles" such as the Cysteine residues for the attachment of nano-devices and nano-motors. Still the question remains: how does one build a nano-device, now that we know how to power it?

One answer is structural DNA nanotechnology. This term describes the "construction of nano-sized molecules from the basic DNA components". Molecules are assembled via 'sticky end'

interaction and bonding among strands of DNA by use of RFLPs. ***Figure*** shows the interaction of sticky ends after the DNA has been cut via RFLP, EcoR1. In structural DNA nanotechnology, instead of just working with double helix shaped DNA, the RFLPs are made to interact with the DNA to produce three-dimensional structures and molecules that interact with each other and cell membranes based on polarity and topological structure.

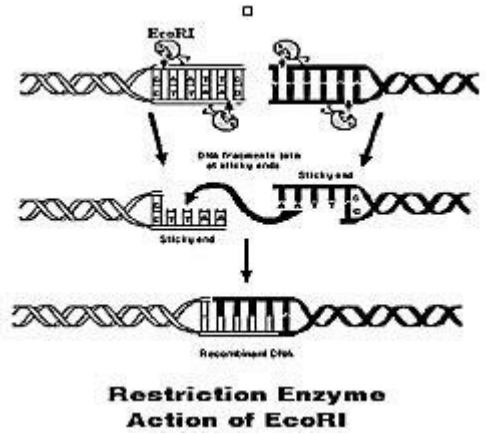

Figure 4.8

Constructing Biological Motor Powered Nanomechanical Devices

A confluence in scientific advancements associated with molecular biology and nanofabrication technology now offer, for the first time, the potential of engineering functional hybrid organic/inorganic nanomechanical systems. Our long-term goal is the integration of the biological motor, F_1-ATPase with nano-electro-mechanical systems (NEMS) to create a new class of hybrid nanomechanical devices. The F_1-ATPase biomolecular motor is capable of generating a force >100 pN, has a calculated no-load rotational velocity of 17 r.p.s., and a diameter of less than 12 nm. These characteristics are consistent with the engineering features associated with currently producable nanomechanical structures. Thus, the potential to seamlessly integrate the motive power of life with engineered nanofabricated devices now exists. This paper will address current research on the integration of the F_1-ATPase motor protein with NEMS specifically designed to evaluate motor performance.

Scientific advancements in both molecular biology and nanofabrication technology now provide the potential of engineering functional hybrid organic/inorganic nanomechanical systems. Scientists have been studying a wide range of organic molecular motors for some

time. Concurrently, inorganic, primarily silicon based, micromechanical devices have been pursued as useful devices. Only very recently has the size scale of nanofabricated inorganic mechanical devices approached a size scale that could conceivably be compatible with the force production and dimensions of molecular motors. Our long-term objective is to utilize the best attributes associated with both the organic and inorganic worlds for the examination and creation of nano-electro-mechanical systems (NEMS) that are powered by biological motors and chemical energy sources. We envision that F_1-ATPase motors will pump fluids, and open and close valves in microfluidic devices, and provide mechanical drives for a new class of nanomechanical devices.

The rotary motion of the γ subunit of F_1-ATPase in response to the synthesis/hydrolysis of ATP has been previously demonstrated. The force generated by this motor protein was >100 pN, which is among the greatest of any known molecular motor. With a calculated no-load rotational velocity of 17 r.p.s. and a diameter of less than 12 nm, the F_1-ATPase protein is a tailor made nano-motor. These properties, coupled with the fact F_1-ATPase is automatically synthesized using the machinery of life, opens the door to the potential for creating chemically powered nanomechanical devices. Integration of nanoscale biocompatible lithographic processes with biological molecular motors may provide the means for creating a transparent interface between the organic/inorganic world. Insertion and self-assembly of hybrid ATPase-powered NEMS in host cells may be possible by taking advantage of cell physiological processes. In addition, host cells may provide power for the device in the form of ATP, as well as maintain a system for replacing the molecular motors when function ceases.

ATPase is a ubiquitous enzyme that is found in virtually every living organism. It consists of two separate portions: (1) F_0, the hydrophobic, membrane-bound portion that is responsible for proton translocation, and (2) F_1, the hydrophilic portion that is responsible for ATP synthesis and hydrolysis. As protons flow through the F_0, the subunit of the F_1-ATPase rotates clockwise and ATP is synthesized. Hydrolysis of ATP results in counterclockwise rotation of the γ subunit, and drives the reverse flow of protons. The a, b, and c subunits of the F_0-ATPase form the channel which allows protons to flow through the membrane. The nucleotide binding and catalytic sites are located on the three a and three b subunits of the F_1-ATPase, respectively. The γ subunit is centrally located within the $_3$ $_3$ hexamer, and rotates as a function of ATP synthesis/hydrolysis.

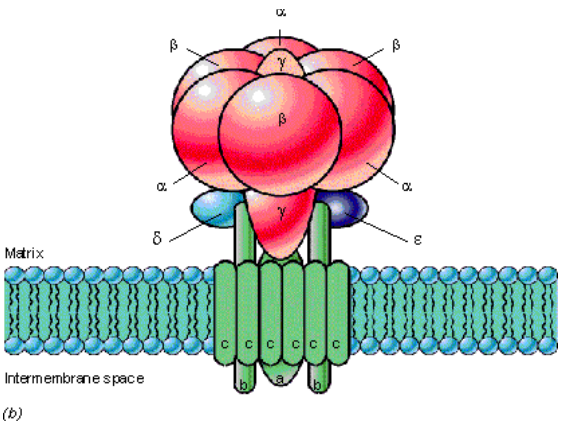

Fig 4.9 Structure FoF1 ATPase

During hydrolysis, counterclockwise rotation of the subunit provides interaction with all three forms of the subunits in the order: AMP-PNP > ADP > empty form. The exact mechanism of interaction has yet to be determined. Crystallization of of the F_1-ATPase has revealed that all three sites must contain bound nucleotides in order for rotation of the subunitto occur. Further, the subunit is displaced from its central axis during rotation.

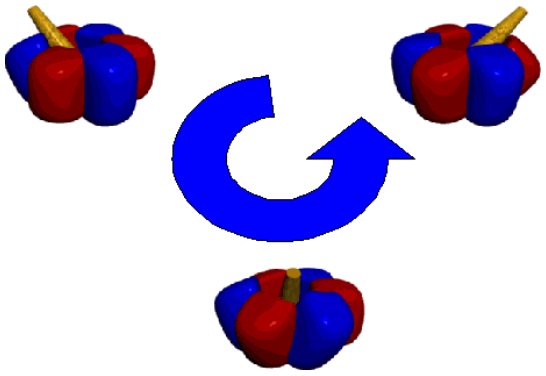

Fig 4.10 F_1-ATPase motor protein

Despite the superb performance of the F_1-ATPase motor protein, little is truly known about how this enzyme generates rotary motion. Neither the useful life of the motor nor the impact of local environmental variables such as pH and temperature on enzyme activity has been determined. The impact of motor generated waste products (i.e., protons and heat), as well as the effects of load on the performance and life of the motor need to be identified. A rigorous evaluation of the engineering properties of the F_1-ATPase motor protein necessitates the development of assays that will provide consistent measurements of the performance of the F_1-ATPase motor protein under different operating conditions. Our current research effort has

focused on integrating the F_1-ATPase with NEMS specifically designed to evaluate motor performance. We will present the results of this effort to construct a hybrid organic/inorganic nanoscale system that both provides insight into the basic mechanics of motor protein motion and establishes a technological foundation for functionally integrating these molecules with manufactured devices.

Integration of biological motors and NEMS: Platforms for the production of both biomolecular motors and NEMS must be established in order to integrate these technologies and produce hybrid systems. Initial research efforts, therefore, have focused on the development of these platforms. In addition, we also have begun evaluating the engineering properties of F_1-ATPase. Expression of the recombinant F_1-ATPase was induced by the addition of 1 mM IPTG approximately 3 hours after inoculation of M9 minimal media. Native protein was extracted using lysozyme/sonication, and purified using Ni^{2+}-NTA affinity chromatography. Approximately 50 mg of F_1-ATPase was purified per liter of cell extract, and analyzed using SDS-polyacrylamide gel electrophoresis. The activity of the purified protein was measured using an ATP regeneration assay

Attachment of biological molecules to nanofabricated substrates: In order to integrate biomolecular motors into NEMS, procedures for the specific attachment and positioning of these motors is essential. Therefore, the objective of this experiment was to evaluate the binding of biological molecules to nanofabricated substrates. Electron beam lithography was utilized to etch an array pattern on a 25 mm coverslip that had been coated with a resist bilayer.Coverslips then were patterned with metal substrates using evaporative deposition of gold, copper, or nickel. Subsequently, the bilayer was removed to expose the array.

A six His-tag peptide was covalently coupled to carboxylate-modified 1 mm fluorescent microspheres using a water-soluble carbodiimide. The His-tagged microspheres were allowed to attach to gold-, copper-, and nickel-coated coverslips for 15 minutes at room temperature. Unattached microspheres were removed through a series of washes, and coverslips were observed using fluorescence microscopy. His-tagged microspheres attached to all three substrates; however, attachment was most frequently observed with nickel-coated coverslips.

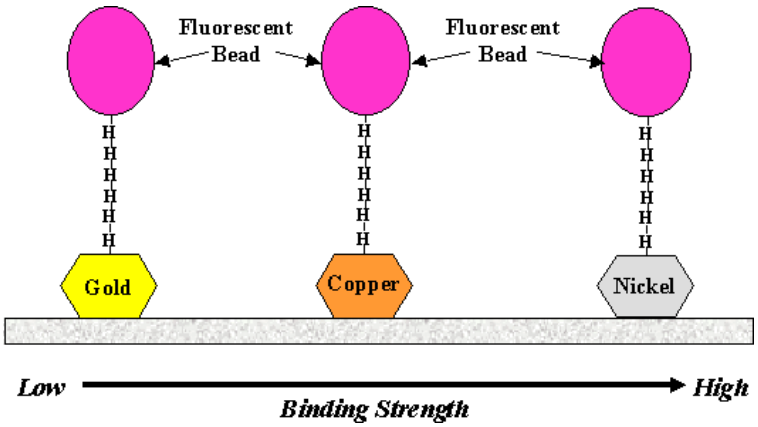

Fig 4.11 chemical mechanism for protein binding and positioning to engineered structures

To test the strength of attachment, laser tweezers were used to remove the microspheres from the substrate. The laser tweezers, however, were unable to remove microspheres from any of the three substrates suggesting that the bonding strength was greater than 600 pN. Further attempts to remove the microspheres with high velocity flow suggest that the bonding strength increases from gold to unoxidized copper to nickel. Oxidized copper does not serve as a suitable surface for binding of His-tagged microspheres. These experiments demonstrate a chemical mechanism for protein binding and positioning to engineered structures that are compatible with current nanofabrication technologies. Using this knowledge in conjunction with standard e-beam lithographic methods we can now attach individual motor protein molecules with a precision greater than 30 nm

Attachment and movement of individual biomolecular motors: Although the biological and chemical aspects of F1-ATPase have been studied, relatively little is know about the engineering properties of this enzyme. The objectives of this experiment were to: (i) attach F_1-ATPase to a nanofabricated substrate, and (ii) measure the rotational velocity and angle of deformation of the g subunit. Analysis of crystallized F_1-ATPase suggests that the g subunit is displaced from the central axis during rotation a distance >20 Å. By attaching a 1 mm microsphere to the g subunit, the displacement and angle of deformation of the g subunit can be determined by measuring the radial displacement of the microsphere. The angle of deformation will provide valuable insight on the mechanism behind rotation of the g subunit**.**

The g subunit of the recombinant F_1-ATPase was specifically biotinylated through disulfide linkage to the gCys. The biotinylated protein then was attached to an array of 30 nm gold dots deposited on a coverslip. Fluorescent 1 mm microspheres coated with streptavidin were

allowed to bind to the biotinylated g subunits. Subsequently, unattached microspheres were

removed through a series of washes. Rotation of the g subunit was initiated by the addition of 2 mM Na_2ATP in presence of 4 mM $MgCl_2$. Movement of the microsphere was measured using a differential interferometer. Images of microsphere movement were also captured at 1 msec intervals using the CCD kinetics camera.

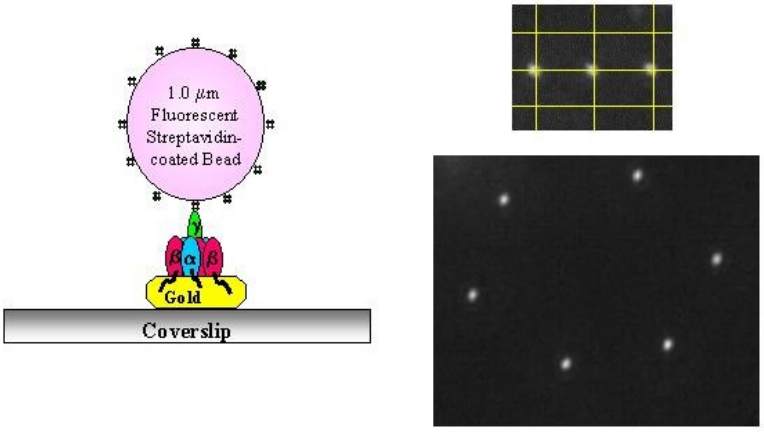

Figure 4.12.Image analysis demonstrated that microsphere movement occurred in three discrete steps following a counterclockwise pattern at a rate of approximately 3-4 r.p.s. Both the interferomenter and image data confirm a counterclockwise, three step rotational mechanism of hydrolysis. Microsphere movement ceased approximately 40 minutes following the initial addition of ATP. Prior to stopping, microspheres would remain at rest for periods up to approximately 600 msec, followed by 500 msec of continuous movement. We attributed this pattern of movement to low concentration of ATP in solution. Continuous movement was reinitiated following the addition of fresh ATP to the flow cell, suggesting that movement of the microsphere (rotation of the subunit) is dependent upon the presence of ATP.

We have established biological and nanofabrication platforms for the production of organic/inorganic hybrid NEMS. Because of its size and force generation, F_1-ATPase serves as an excellent model system for evaluating the use of biomolecular motors in these hybrid devices. The biological platform that has been established allows us to: (1) easily manipulate the coding sequence, (2) produce large quantities of protein, and (3) place specific "handles" on the enzyme for the precise attachment to nanofabricated devices and substrates. Moreover, the presented nanofabrication platform permits both the construction of chemically active sites that are consistent with the size of the protein and the development of devices that are capable of translating the energy of a biomolecular motor into useful work. These results represent the enabling technologies necessary for integrating NEMS into living organisms.

Further investigation of the engineering properties and motor performance are necessary for the production of functional nanomechanical devices powered by F_1-ATPase. For example, the impact of waste products such as heat and protons on motor performance must be evaluated. Motor performance must be evaluated as a function of heat, pH, load, and local environmental conditions. Moreover, dissecting the interaction between the subunits of the a_3b_3g complex may allow us to specifically engineer the protein for increased performance as a biomolecularmotor. These efforts will provide a significant step toward the seamless integration of nanoscale technologies into living system, and are central to the creation of organic/inorganicintelligent systems.

Nano-biosensors / biodetection

A biosensor is generally defined as a measurement system that consists of a probe with a sensitive biological recognition element, or bioreceptor, a physicochemical detector component, and a transducer in between. A nanobiosensor or nanosensor is a biosensor that has dimensions on the nanometre size scale. Nanosensors could provide the tools to investigate important biological processes at the cellular level *in vivo*. Two types of nanosensors with medical application possibilities are cantilever array sensors and nanotube/nanowire sensors.

Cantilever array sensors

Microfabricated cantilever array sensors are used as ultra-sensitive mechanical sensors converting (bio)chemical or physical processes into a recordable (electrical) signal in microelectromechanical systems (MEMS) or nanoelectromechanical systems (NEMS). Cantilevers are typically rectangular-shaped silicon bars. The unique feature of microcantilevers is their ability to undergo bending due to molecular adsorption or binding induced changes in surface tension. The major advantages of such miniaturised sensors are their small size, fast response times, high sensitivity, and direct transduction without the need for any labels.

(a) Medical applications of cantilever-based sensors have been proposed for early diagnosis of diabetes mellitus and can improve blood glucose monitoring using small and ultra-sensitive analytical platforms. In patients with diabetes mellitus, ketones are produced due to the deterioration of blood insulin concentrations. Acetone is one of these ketones which is excreted in urine or expired as vapour in exhaled air. Disposable test kits are used to detect acetone in urine. Acetone in exhaled air can only be detected by the physician as a putrid smell without

any quantification. Small amounts of acetone in a patient's breath can be detected by cantilever array sensor technique which may attribute to early diagnosis of diabetes mellitus.

(b) Devices have also been developed to detect bacteria, fungal spores, and viruses. The interaction between specific antibodies, for instance antibodies to *Escherichia coli*, immobilised on the surface of the cantilever, and antigens on cell membrane surface results in additional mass loading detected by the device. The detection sensitivity is in the order of a single bacterium corresponding to a mass of ~1 pg (pico (p) = 10-12), single fungal spore, and single vaccinia virus particles corresponding to a mass of ~10 fg (femto (f) = 10-15). Cantilever arrays allow detection of vital functionalised fungal spores *in situ* within ~4 hours, which is more than ten times faster than current applied procedures for fungal detection. Recently, a NEMS device with molecular recognition for virus particle detection has been developed, allowing improvement of the detection sensitivity up to 6 bound baculovirus particles. Once these devices with on-chip antibody-based recognition are integrated with sample concentrators, nanomechanical oscillators may prove to present a viable strategy for ultra-sensitive detection of airborne bacteria, fungi, and virus particles.

(c) Cantilever arrays can aid cancer diagnosis and can be engineered to bind to molecules associated with cancer, such as DNA sequences, singlenucleotide polymorphisms, and proteins. When the cancer-associated molecules bind to the cantilevers, changes in surface tension cause the cantilever to bend. By monitoring whether or not the cantilevers are bending, the presence of cancer-associated molecules can be demonstrated. Significant bending should be evident when the molecules are present in very low DNA concentrations (femtomoles detection). Recently, the mass detection limitation of NEMS cantilevers has been improved to the enumeration of a single DNA molecule consisting of ~1600 base pairs and weighing ~1000 kD, which is ~1 ag (atto (a) = 10-18). The cantilever technology could be useful in high-throughput nanomechanical genomic analysis and proteomics detecting early molecular events in the development of cancer. Microcantilever-based, multiplexed DNA assays to detect mutations have recently been introduced. The specificity and sensitivity of these arrays do not yet offer substantial advantages over conventional detection methods, although the use of nanoparticle probes might allow for individual single-pair mismatch discrimination. Rather, the breakthrough potential of micro- and nanomechanical cantilevers resides in their extraordinary multiplexing capabilities. It is realistic to envision arrays of thousands of cantilevers constructed on individual centimetre-sized chips, enabling the simultaneous reading of proteomic profiles or, ultimately, the entire proteome

Nanotube-based sensors

(a) Carbon nanotubes are promising sensing candidates to monitor glucose in blood and urine. MWCNTs as well as SWCNTs have been used to develop enzymatic amperometric biosensors or fluorimetric biosensors. The enzyme glucose oxidase is either immobilised inside MWCNTs or non-covalently attached to the surface of SWCNTs enabling the catalysis of glucose with hydrogen peroxide as co-product. For the amperometric biosensor the enzyme immobilisation allows for the direct electron transfer from the enzyme to a gold or platinum transducer producing the response current. The fluorescence biosensor could be used in a new type of implantable biological sensor such as near-infrared nanoscale sensor. This sensor could be inserted into tissue, excited with a laser pointer, and provide real-time, continuous monitoring of blood glucose levels. It consists of protein-encapsulated SWCNTs functionalised with potassium ferrocyanide, a substance that is sensitive to hydrogen peroxide. The ferrocyanide ion adsorbs on the surface through the porous monolayer. When present, hydrogen peroxide will form a complex with the ion, which changes the electron density of the carbon nanotube and consequently its optical properties. The more glucose that is present, the brighter the carbon nanotube will fluoresce. The sensor can be loaded into a porous capillary and inserted into tissue. As carbon nanotubes do not degrade like organic molecules that fluoresce, these nanoparticle optical sensors would be suitable for long-term monitoring applications. Proof-of-concept studies to detect glucose levels have been performed *in vitro*, i.e. in blood samples. Practical use is five to ten years ahead, according to the researchers. Self-assembled peptide nanotubes can be used in an electrochemical biosensor. The presence of the peptide nanotubes improves the sensitivity of the device several fold. Peptide nanotubes offer several advantages over carbon nanotubes, since they are biocompatible, water-soluble, inexpensive, easy to manufacture, and can be chemically modified by targeting their amino or carboxyl groups. The sensing technique can be used as a platform for ultra-sensitive detection of biological and chemical agents.

(b) MWCNT-based nanoelectrode arrays embedded in SiO2 matrix have been integrated into a electrochemical system for ultra-sensitive and rapid DNA detection. A bottom-up approach is used for the fabrication of individually addressed nanoelectrode arrays, that results in precisely positioned and well aligned MWCNT arrays on a silicon wafer. Subsequently, the open ends of MWCNTs are functionalised with oligonucleotide probes. Combining the

nanoelectrode arrays with redoxactive molecule-mediated (e.g., Ru(bpy)3 2+) guanine oxidation, the hybridisation of less than a few attomoles of oligonucleotide targets (~3.5×106 DNA molecules) can be easily detected by voltametric measurement. The proof-of-concept has been demonstrated for clinical relevant DNA molecules related to wild-type alleles associated with cancer genes.

(c) Carbon nanotube-based chemical gas sensors have great potential in medical applications. Currently, Nanomix Inc. (Emeryville, California, USA) is developing a medical capnography sensor using polyethylene-imine-coated carbon nanotubes. Capnography is the measurement of carbon dioxide concentration in human respiration and is a indicator of patient status during administration of anaesthesia.

(d) Various applications have been reported illustrating the broad potential of carbon nanotube based biosensors, such as biosensing platforms for the simultaneous detection of dopamine and ascorbic acid for the diagnosis of Parkinson's disease, and dopamine and serotonin, and a nitric oxide radical biosensor.

Nanowire-based sensors

(a) Semiconducting silicon nanowires can be configured as field-effect transistors for the electrical detection of viruses in solutions. When a single charged virus binds to receptors (e.g., antibodies) linked to the nanodevice the conductance of a semiconducting nanowire changes from the baseline value, and when the virus unbinds, the conductance returns to the baseline value. The conductance of a second nanowire device without receptors should show no change during the same time period and can serve as an internal control. Nanowires are confined to a central region that is coupled to a microfluidic channel for sample delivery and the conductance response can be recorded while solutions with viruses flow at a constant rate. Modification of different nanowires within an array with receptors specific for different viruses provides a means for simultaneous detection of multiple viruses at the single particle level. The potential of nanowire-based electrical detection of viruses exceeds the capabilities of other methods such as polymerase chain reaction-based assays and micromechanical devices.

(b) Silicon nanowire field-effect transistor devices have been used for detection of small molecule inhibitors of ATP binding to AbI, which is a protein kinase whose activity is responsible for chronic myelogenous leukaemia. In addition, real-time, label free detection of DNA and DNA mismatches is also feasible. Silicon nanowire sensors functionalised with peptide nucleic acid receptors can distinguish wild-type from the mutation type in the cystic

fibrosis transmembrane receptor. Cystic fibrosis is one of the most common fatal genetic diseases among populations of European origin.

Optical-based sensors

Normal Raman spectrometry detects physiological concentrations of glucose *in vitro* from a simulated aqueous humour solution, in serum and in blood, though high laser power and long acquisition time render normal Raman spectrometry clinically not practicable. However, surface-enhanced Raman spectrometry possesses many advantages allowing chemical analysis of in vivo molecular substances including high specificity, micromolar to picomolar concentration sensitivity, and interfacial generality. For the first time the concept-of-proof toward the development of a glucose sensor using surface-enhanced Raman spectroscopy has recently been demonstrated. Glucose is partitioned into an alkanethiol monolayer (~2 nm thick) adsorbed on a silver film (200 nm thick) over nanosphere surface (polystyrene latex spheres ~390 nm in diameter). Spectra are measured from backscattered laser light indicating the glucose concentration. On the long term, the surface-enhanced Raman spectrometry substrate can be miniaturised to a microscale of even nanoscale device that can be implanted subcutaneously or can be incorporated as a component of a prosthetic lens in the eye with little or no discomfort to diabetic individuals.

Nanoarray-based biodetection

Viruses in human blood samples, such as HIV-1, can be detected using nanoscale antibody array-based devices. Dip-pen nanolithography was used to pattern 16-mercaptohexadecanoic acid into an array of 60 nm dots on a gold thin film. Monoclonal antibodies to the HIV-1 p24 antigen were immobilised on the dots. The analysis consists of immersing the array for one hour in a blood plasma sample. Subsequently, the signal from the antigen-array binding was amplified using gold nanoparticles probes functionalised with polyclonal antibodies in a solution for one more hour. A measurable amount of HIV-1 p24 antigen in blood plasma from humans with less than 50 copies of RNA/ml is feasible demonstrating that nano-based assays can far exceed the 5 pg/ml (pico (p) = 10-12) detection limit of conventional enzyme-linked immunosorbent assays and provide sensitivity comparable to a polymerase chain reaction-based assay, without target amplification. Nanobased array biodetection could enable HIV-1 diagnosis in mother-to-child transmission.

Nanoparticle-based biodetection

One of the major drawbacks of conventional protein or antigen detection methods (e.g., enzyme-linked immunoassays, blotting assays) is the relative insensitivity for the target. Ultra-sensitive tests are needed for patient screening and diagnosis in the early stage of diseases enabling detection of very low concentrations of pathogenic biomarkers and conclusive confirmation of the disease in living patients. Recently, an ultra-sensitive bio-bar code assay has been developed for the detection of protein/antigen analytes at clinically relevant attomolar (atto = 10-18) concentrations which is five to six orders of magnitude less compared to conventional clinical assays. The bio-bar code assay uses two types of probes: gold nanoparticle (13-30 nm in diameter) probes heavily functionalised with hundreds of identical hybridized oligonucleotides (DNA strands or "bar-code DNA" acting as an identification label) and polyclonal antibodies, and magnetic microparticle (1-μm diameter polyamine particle with magnetic iron oxide core) probes functionalised with monoclonal antibodies. The polyclonal and monoclonal antibodies recognize and bind to the same target protein, sandwiching the protein between the nano- and microparticle (Figure 13). After the "sandwich" is removed magnetically from the solution, the bar-code DNA strands are released and read using standard DNA detection methodologies. The increased sensitivity of the assay derives mainly from the very effective sequestration of the protein/antigen and the amplification process that occurs as a result of the large number of barcode DNA strands (for 13 nm nanoparticles, each nanoparticle can support up to 100 strands of DNA) released for each recognition and binding event.

The bio-bar code assay technology has been tested to detect very low concentrations free of prostate-specific antigens. Prostate-specific antigens are associated with prostate and breast cancer. In women with breast cancer, free prostate-specific antigen is found in serum at much lower concentration than in men and it is being explored as a breast cancer screening target. Recently, the bio-bar code assay technology has successfully been applied for the first time to detect amyloid-β-derived diffusible ligands in cerebrospinal fluid of living patients with Alzheimer's disease.

Recently, dye-doped silica nanoparticles have been used to develop an assay tool for *in situ* pathogen quantification in water samples enabling the detection of one bacterium cell. This ultra-sensitive detection method uses fluorescent-bioconjugated silica nanoparticles (~60 nm in diameter). Within each silica nanoparticle thousands of fluorescent dye molecules are

trapped. The silica matrix not only provides high photostability of the dye molecules inside the nanoparticle, but it also enables easy modification of the surface by conjugation of various biomolecules to the nanoparticles. Monoclonal antibodies against antigens of bacteria are covalently immobilised onto the nanoparticles, which are then used in an immunoassay. High fluorescent signal amplification is achieved when the antibody bioconjugated nanoparticles bind to antigens on the surface of the bacteria enabling detection of bacteria using a spectrofluorometer. The single-bacterium assay can be adapted for multiple-sample determination (>300 samples at one time) and is rapid, taking <20 minutes to complete sample preparation, instrumentation preparation, and sample determination. In addition, the bioassay can be used for multiple-pathogen quantification *in situ* with high specificity.

Drug delivery

Introduction

Rapid advances in proteomics & genomics coupled with rational drug design and rapid screening techniques have led to revolution in the drug discovery process resulting in introduction of large number of novel therapeutics at proliferative rate. However, the use of these novel therapeutics in medicine is frequently opposed by the lack of efficiency in delivery of these therapeutic agents to the target organs. Consequently, in the last three decades, there has been a great focus on the development of drug delivery systems (DDSs) for the treatment of diseases. In very simple terms, drug delivery can be defined as the process of releasing a carried bioactive agent at a specific site, at a specific rate. The drug candidates often present a multiplicity of delivery challenges, including issues of solubility, in vivo stability, poor pharmacokinetics, and undesirable toxicity and side effect profiles, all of which must be dealt with simultaneously in order for the candidate to become a successful therapeutic. Formulation scientists have always struggled to overcome these problems but with the advent of nanotechnology the conventional challenges can now be looked upon as new opportunities

Nanotechnology deals with phenomena whose physics or chemistry differs from that of bulk materials of the same composition. Extending this interpretation, nanoparticles are particles in which the small size influences the intrinsic properties or behavior of the particle. Properties of interest may be: surface properties, quantum mechanical properties, chemical or biological reactivity, etc. The term “nanoparticles” varies greatly based on the specific definition that is used. National Science Foundation and the National Nanotechnology Initiative define

nanoparticles as particles having dimensions of 1-100nm. Interestingly, much of what we know about the bulk properties of materials breaks down at these scales. For example, nanomaterials such as carbon nanotubes and gold nanoparticles have physical properties that are different from their bulk counterparts. Therefore, such technologies generate new opportunities and applications. However, in case of drug delivery, nanoparticles are no longer confined to strict size range of 1-100nm In case of drug delivery, the properties that hold premier interest are: surface properties (i.e. particle size, surface area, surface free energy and surface-to-volume ratio) and biological reactivity (circumventing opsonization). These properties can be modulated at sub micron size ranges and there's no stringent requirement to hold on to the sizes of below 100nm. Formulators however, have there own way of defining nanoparticles, where the boundaries of size ranges dissolves away and anything submicron is considered to be a part of nanotechnology.

Nanoparticles and nanoformulations have already been applied as drug delivery systems with great success; and nanoparticulate drug delivery systems have still greater potential for many applications, including anti-tumour therapy, gene therapy, AIDS therapy, radiotherapy, in the delivery of proteins, antibiotics, virostatics, vaccines and as vesicles to pass the blood-brain barrier.

Nanoparticles provide massive advantages regarding drug targeting, delivery and release and, with their additional potential to combine diagnosis and therapy, emerge as one of the major tools in nanomedicine. The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, improve drug loading, targeting, transport, release, and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remains a major problem, and improvements in biocompatibility obviously are a main concern of future research.

Nanotechnology and Drug Delivery- Fundamentals

Some of the fundamentals on which nanotechnology based drug delivery systems are designed:

(a) Particle Size, Surface area, Surface Free Energy: Around 40% of drugs developed today are poor candidates for drug delivery formulations owing to their limited water solubility. Nano-sizing drug or formulating drug as a nano particulate system results in better dissolutionand solubilization of drug. The "top-down" technique used for fabricating nano-structured

materials results in increasing the effective surface area (surface area available for medium interaction) and imparting high free surface energy to the particles which in-turn helps in entropically driven effective solubilization.

(b) Surface-to-Volume Ratio: The earliest concept for targeting therapeutic to specific site included attachment of targeting moieties to the drug molecule (Immunoconjugates). This concept was hardly considered convincing as it required attachment of one targeting moiety per drug molecule; also the attachment of immunoglobulin (targeting moiety) to naked drug molecule posed a big risk of affecting the biological activity of drug. Nanoparticles could be advantageously used to overcome these problems in targeted drug delivery. Nanoparticles act as a carrier for drug delivery with number of drug molecules encapsulated in single nanoparticle. Moreover, the enhanced surface–to-volume ratio further allows effective attachment of targeting moieties onto the surface of nanoparticles. Thus, the drug molecules are safely carried to the target site without undergoing any chemical modifications.

(c) Particle Size & Biological System: Living organisms are built of cells that are typically 10 μm across. However, the cell parts are much smaller and are in the sub-micron size domain. Even smaller are the proteins with a typical size of just 5 nm, which is comparable with the dimensions of smallest manmade nanoparticles. This simple size comparison gives an idea of using nanoparticles as an effective tool in delivering drug to the target sites. Infact, nanoparticles are the only colloids that can be given intravenous (IV route) because they don't settle or aggregate in the blood and thus cause no embolism. The smaller size also ensures easy and effective penetration not only through the biological membrane but also through the cellular pores achieving greater transfection and enabling manipulation at molecular level.

(d) Biological Reactivity: The trek of a "therapeutic" from the point of administration to the intended target is full of perils, biological barriers might arise in form of tight junctions between epithelial cells, Immunological hurdles are created by opsonization mediated by macrophages of RES (Reticulo Endothelial System) and biophysical obstacles include the charge related agglomeration and bio-distribution. Nanotechnology based systems presents themselves as well equipped delivery agents by overcoming various barriers and other related hurdles by the virtue of their modified properties. The small particle size and uniform particle distribution helps nanoparticles to overcome the biological barriers by effective and efficient transfer across biological membranes and tight junctions. Nanoparticles can be sized down below the cut-off range for easy penetration across the barriers and because of the hydrophobicity of the particles

their journey across the membranes is not that difficult as the membranes themselves are made up of lipophilic moieties.

(e) Opsonization: Opsonization which is thought to be the greatest threat to any injectable xenobiotics, leads to engulfment of foreign particles injected into the blood stream by specific macrophages cells of RES, resulting in removal of therapeutics from the circulation and ultimately decreasing efficacy and potency of the therapy. The entire process of opsonization depends on the interaction of opsonin (endogenous proteins) with the foreign object; this interaction in turn depends on the surface physiochemical properties i.e. size, shape, charge, density and surface hydrophobicity. All of these can be modulated based on the techniques used for fabrication and post fabrication modification of nanostructured particles for e.g PEGylation, which includes hydrophilic coating of Poly Ethylene Glycol on nanoparticle surface. Other non-covalent approaches include layer by layer deposition of ionic polymers, such as quantum dots. Layer-by layer methods alter the surface charge of nanoparticles resulting in prevention of particle agglomeration and regulated nanoparticle biodistribution.

Drug Delivery Systems

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-

sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Controlled drug release and subsequent biodegradation are important for developing successful formulations. Potential release mechanisms involve: (i) desorption of surface-bound /adsorbed drugs; (ii) diffusion through the carrier matrix; (iii) diffusion (in the case of nanocapsules) through the carrier wall; (iv) carrier matrix erosion; and (v) a combined erosion /diffusion process. The mode of delivery can be the difference between a drug's success and failure, as the choice of a drug is often influenced by the way the medicine is administered. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature).

Drug Carriers

A successful drug carrier system needs to demonstrate optimal drug loading and release properties, long shelf-life and low toxicity. Colloidal systems, such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10 - 400 nm diameter show great promise as carriers in drug delivery systems.

(a) Micelles: Drugs can be trapped in the core of a micelle and transported at concentrations even greater than their intrinsic water solubility. A hydrophilic shell can form around the micelle, effectively protecting the contents. In addition, the outer chemistry of the shell may prevent recognition by the reticuloendothelial system, and therefore early elimination from the bloodstream. A further feature that makes micelles attractive is that their size and shape can be

changed. Chemical techniques using crosslinking molecules can improve the stability of the micelles and their temporal control. Micelles may also be chemically altered to selectively target a broad range of disease sites.

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as drug delivery systems. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity. The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possesses amphiphilic and/or mesogenic properties.

Micelles formed by self-assembly of amphiphilic block copolymers (5-50 nm) in aqueous solutions are of great interest for drug delivery applications. The drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water- solubility. Moreover, the hydrophilic blocks can form hydrogen bonds with the aqueous surroundings and form a tight shell around the micellar core. As a result, the contents of the hydrophobic core are effectively protected against hydrolysis and enzymatic degradation. In addition, the corona may prevent recognition by the reticuloendothelial system and therefore preliminary elimination of the micelles from the bloodstream. A final feature that makes amphiphilic block copolymers attractive for drug delivery applications is the fact that their chemical composition, total molecular weight and block length ratios can be easily changed, which allows control of the size and morphology of the micelles. Functionalization of block copolymers with crosslinkable groups can increase the stability of the corresponding micelles and improve their temporal control. Substitution of block copolymer micelles with specific ligands is a very promising strategy to a broader range of sites of activity with a much higher selectivity.

(b) Liposomes: Liposomes are vesicles that consist of one to several, chemically-active lipid bilayers. Drug molecules can be encapsulated and solubilised within the bilayers. Certain (channel) proteins can be incorporated in the membrane of the liposome, which act as size-selective filters only allowing the diffusion of small solutes such as ions, nutrients and antibiotics. Thus, drugs encapsulated within a liposome ‘nanocage’ that has been functionalized with channel proteins, are effectively protected from premature degradation.

The drug molecule, however, is able to diffuse through the channel, driven by the concentration difference between the interior and the exterior of the ‘nanocage’.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes. Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients and antibiotics.

(c) Dendrimers: Dendrimers are nanometre-sized, polymer macromolecules. They consist of a central core, branching units and terminal functional groups. The core chemistry determines the solubilizing properties of the cavity within the core, whereas external chemical groups determine the solubility and chemical behavior of the dendrimer itself. Targeting is achieved by attaching specific linkers to the external surface of the dendrimer which enable it to bind to a disease site, while its stability and protection from phagocytes is achieved by ‘decorating’ the dendrimers with polyethylene glycol chains.

A dendrimer is an artificially manufactured or synthesized large molecule comprised of many smaller ones linked together - built up from branched units called monomers. Technically, dendrimers are a unique class of a polymer, about the size of an average protein, with a compact, tree-like molecular structure, which provides a high degree of surface functionality and versatility. Their shape gives them vast amounts of surface area, making them useful building blocks and carrier molecules at the nanoscale and they come in a variety of forms, with different physical (including optical, electrical and chemical) properties.

Dendrimer As A Biologically Active Carrier: Dendrimers can act as biologically active carrier molecules in drug delivery to which can be attached therapeutic agents and as scavengers of metal ions, offering the potential for environmental clean-up operations because their size allows them to be filtered out with ultra-filtration techniques.

(d) Liquid Crystals: Liquid Crystals combine the properties of both liquid and solid states. Liquid crystals can be made to form different geometries, with alternate polar and non-polar layers (i.e., lamellar phases), within which aqueous drug solutions can be incorporated.

(e) Nanoparticles: Nanoparticles, including nanospheres and nanocapsules, can be amorphous or crystalline. They are able to adsorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. In nanocapsules, the drug is confined to a cavity surrounded by a polymer membrane, while nanospheres are matrix systems within which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles have attracted considerable attention in the controlled release of drugs in targeting particular organs/tissues, as carriers of DNA in gene therapy and in their ability to deliver proteins, peptides and genes by the oral route.

(f) Hydrogels: Hydrogels are three-dimensional polymer networks that swell but do not dissolve in aqueous media. They are used to regulate drug release in reservoir-based systems or as carriers in swelling-controlled release devices. On the forefront of controlled drug delivery, hydrogels, as enviro-intelligent and stimuli-sensitive gel systems, can modulate drug release in response to pH, temperature, ionic strength, electric field, or specific analyte concentration differences. Release can be designed to occur within specific areas of the body. Hydrogels as drug delivery systems are very promising if combined with the technique of molecular imprinting.

(g) Molecularly Imprinted Polymers: Molecularly imprinted polymers have an enormous potential for drug delivery systems. Examples include: rate-programmed drug delivery, where drug diffusion from the system has to follow a specific rate profile; activation-modulated drug delivery, where the release is activated by some physical, chemical or biochemical processes; and feedback-regulated drug delivery, where the rate of drug release is regulated by the concentration of a triggering agent, which is activated by the drug concentration in the body.

Despite already-developed applications, the incorporation of the molecular imprinting approach for the development of drug delivery systems is at an early stage. It can be expected that in the next few years significant progress will occur, taking advantage of the improvements in this technology in other areas.

(f) Conjugation of Biological Molecules and Synthetic Polymers: The conjugation of biological molecules (peptides/proteins) and synthetic polymers is an efficient means of improving control over the nanoscale structure formation of synthetic polymers that can be used as drug delivery systems. The conjugation of suitable synthetic polymers to peptides or proteins can reduce toxicity, prevent immunogenic or antigenic side reactions, enhance blood circulation times and improve drug solubility. Modification of synthetic polymers with peptide sequences, which can act as antibodies to specific epitopes, can also prevent random distribution of drugs throughout a patient's body by active targeting. The functionalisation of synthetic polymers with peptide sequences derived from extracellular matrix proteins is an efficient way to mediate cell adhesion, for example. In addition the ability of cationic peptide sequences to complex DNA and oligonucleotides offers prospects for the development of non-viral vectors for gene delivery, based on synthetic polymeric hybrid materials.

(g) In-Situ Forming Implants: The field of in-situ forming implants has grown exponentially in recent years. Liquid formulations generating a (semi-) solid depot after subcutaneous injection are attractive delivery systems for parenteral (non-oral) application because they are less invasive and painful compared to implants. They enable drugs to be delivered locally or systemically over prolonged periods of time, typically up to several months. These depot systems could minimize side effects by achieving constant, 'infusion-like' drug profiles, especially important for delivering proteins with narrow therapeutic indices. They also offer the advantage of being relatively simple and cost effective to manufacture.

(h) Microelectromechanical Systems (MEMS): The ultimate goal in controlled release is the development of a microfabricated device with the ability to store and release multiple chemical substances on demand. Recent advancement in microelectromechanical systems (MEMS) have enabled the fabrication of controlled-release microchips, which have the following advantages:

- Multiple chemicals in any form (e.g. solid, liquid or gel) can be stored and released
- Chemical release is initiated by the disintegration of the barrier membrane by applying an electric potential
- A variety of highly potent drugs can potentially be delivered accurately and safely

 Complex release patterns (e.g. simultaneous constant and pulsatile release) can be achieved

 Local delivery is possible, achieving high concentrations of drug where needed, while keeping the systemic concentration of the drug at a low level

 Water penetration into the reservoirs is avoided by a barrier membrane and thus the stability of protein based drugs with limited shelf-life is enhanced.

(i) Gold Nanoparticles Form Basis of Intelligent Drug Delivery System: The technology, which involves miniscule gold particles only nanometres in size, has been used to create intelligent delivery systems that may have potential as drug carriers. To develop the intelligent delivery systems, the researchers lined the walls of microscopic polymer 'delivery-vehicle' particles with gold nanoparticles. Because laser light is absorbed by the gold nanoparticles, they found that by simply shining a laser on loaded delivery vehicles (i.e. particles filled with various contents, such as an enzyme or drug), the walls could be opened and the contents released. By encasing biologically significant substances, such as drugs, within the gold nanoparticle-shelled delivery vehicles, release of the active materials can be remotely controlled by shining a laser on the gold nanoparticles, which then opens the particle walls.

Inducing release of the delivery vehicle contents is so fast, it is feasible that large areas of interest could be scanned quickly even with a relatively low-power, low-cost laser. Also, there is no risk that the laser energy will be significantly absorbed by biological structures such as bodily organs because the absorption of the gold-coated delivery vehicles in the near infrared light region is intentionally engineered in the wavelength regime for which light has a maximum penetration depth in tissue. In addition to drugs, these gold-coated vehicles could be used for the controlled delivery of a wide range of other substances including genes, pesticides, cosmetics and food stuffs.

A technique was devised to suspend high concentrations of gold nanoparticles in water without them settling to the bottom or sticking together (called high colloidal stability). This gold nanoparticle technology forms the basis for the technique used in the delivery vehicles.

(j) Uniform, Self-Assembled Nanocells for Drug Delivery: A new method for producing uniform, self-assembled nanocells has been developed by researchers at the National Institute

of Standards and Technology (NIST). The method may have applications as an improved method for encapsulating drug therapies. Current bulk methods for producing nanocells called liposomes—a type of artificial cell—produce particles in a wide range of sizes. The sizes must be sorted and filtered before being used for drug delivery, since dosage depends critically on size.

The new NIST method uses micrometer-size channels etched into a device to produce self-assembled liposomes of specific sizes from as large as about 240 nanometers (nm) to as small as about 100 nm. A stream of natural fats (lipids) dissolved in alcohol—is directed at an intersection of two channels that looks like a micro version of a four-way stop. A water-based liquid containing medicines or other substances is sent toward the lipid stream from two opposing directions. Rather than mixing with the water, the lipids surround it, forming self-assembled nanocells.

Controlling flow rates in the microchannels produces nanocells of specific sizes. Faster flows produce smaller cells. Medicine-filled liposomes made in nanosizes should allow for more accurate drug delivery. In particular, liposomes have been studied for years as a way to concentrate the effectiveness of cancer chemotherapy while minimizing harmful side effects.

(j) Nanocapsules: A nanocapsule is any nanoparticle that consists of a shell and a space, in which desired substances may be placed. Technologies for microencapsulating materials have been around for several years, primarily for applications involving minimisation of hygroscopy and chemical interactions, elimination of oxidation, and controlled release of nutraceuticals

The Use Of Man-Made Liposomes:Man-made liposome’s have been used in cosmetics for some years to control the release of substances or protect them from the environment. Recently many other materials, such as polymers, have been used to make nanocapsules.

The Properties Of Polymeric Nanocapsules: Polymeric nanocapsules can be made in specific sizes, shapes, and in reasonable quantities. Nanocapsules can be made to function in various ways. They can be produced as monodisperse particles with exactly defined biochemical, electrical, optical, and magnetic properties. They can be tailored to suit the complexity of whatever application they are intended for, such causing the release of the contents in response to a particular bimolecular triggering mechanism in targeted drug-delivery systems.

The Use Of Nanocapsules As Smart Drugs: Nanocapsules can be used as smart drugs that have specific chemical receptors and only bind to specific cells. It is this receptor that makes the drug ‘smart,’ allowing it to target cancer or disease. The advantages of nano-encapsulation technologies for pharmaceutical applications include:

- Higher dose loading with smaller dose volumes
- Longer site-specific dose retention
- More rapid absorption of active drug substances
- Increased bioavailability of the drug
- Higher safety and efficacy
- Improved patient compliance

The Future Benefits Of Nanocapsules In Drugs: Beyond the ability to deliver existing drugs to their target A nanocapsule is any nanoparticle that consists of a shell and a space, in which desired substances may be placed. Technologies for microencapsulating materials have been around for several years, primarily for applications involving minimisation of hygroscopy and chemical interactions, elimination of oxidation, and controlled release of nutraceuticals.

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The Future Benefits Of Nanocapsules In Drugs: Beyond the ability to deliver existing drugs to their target, nanocapsules would allow for as much as a 10,000-fold decrease in drug dosages, reducing the harmful side effects of drugs used in chemotherapy. Quite often, drugs don’t make

it to market is because they have too many unwanted side effects. However, placing the same drug inside a nanocapsule and delivering it directly to its intended target in a reduced dosage, eliminates some of those side effects, or at least reduces them to an acceptable level.

Further Applications Of Nanocapsules: Nanocapsules also have potential applications in agrochemicals, cosmetics, genetic engineering, wastewater treatments, cleaning products, and adhesive component applications. They can be used to encapsulate enzymes, catalysts, oils, adhesives, polymers, inorganic micro- and nanoparticles, latex particles, or even biological cells.

Drug Encapsulation

What is Encapsulation and What Can It Be Used For? Nanotechnology enables companies to manipulate the properties of the outer shell of a capsule in order to control the release of the substance to be delivered. 'Controlled release' strategies are highly prized in medicine since they can allow drugs to be absorbed more slowly, at a specific location in the body or at the say-so of an external trigger. With potential applications across the food chain (in pesticides, vaccines, veterinary medicine and nutritionally-enhanced food), these nano- and micro-formulations are being developed and patented by agribusiness and food corporations such as Monsanto, Syngenta and Kraft.

Different Types of Encapsulation at the Nanoscale Examples of nano and microcapsule designs:

- Slow release - the capsule releases its payload slowly over a longer period of time (e.g., for slow delivery of a substance in the body);

- Quick-release - the capsule shell breaks upon contact with a surface (e.g. when pesticide hits a leaf);

- Specific release - the shell is designed to break open when a molecular receptor binds to a specific chemical (e.g., upon encountering a tumour or protein in the body);

- Moisture release - the shell breaks down and releases contents in the presence of water (e.g. in soil);

- Heat-release - the shell releases ingredients only when the environment warms above a certain temperature;

- pH release - nanocapsule breaks up only in specific acid or alkaline environment (e.g., in the stomach or inside a cell);

- Ultrasound release - the capsule is ruptured by an external ultrasound frequency;

Magnetic release - a magnetic particle in the capsule ruptures the shell when exposed to a magnetic field;

- DNA nanocapsule - the capsule smuggles a short strand of foreign DNA into a living cell which, once released, hijacks cell machinery to express a specific protein (used for DNA vaccines).

Background: Nanoencapsulation is the coating of various substances within another material at sizes on the nano scale. This technique is already commonplace within a range of industries but it is accepted that only around 10% of potential applications are being exploited.

Microencapsulation: Microencapsulation is similar to nanoencapsulation aside from it involving larger particles and having been done for a greater period of time than nanoencapsulation. Nanoencapsulation has evolved from and can be considered to be the miniaturisation of microencapsulation.

Encapsulation Terms: The encapsulated material is commonly referred to as the internal phase, the core material, the filler or the fill. The encapsulation material is known as the external phase, the shell, coating or membrane.

Nanocapsule Appearance: Common macro sized capsules used for off the shelf pharmaceuticals and vitamins are smooth uniformly sized object but they are vastly different to micro and nanoencapsulated materials. As the core material for different nanocapsules may vary greatly in size, shape and composition, the encapsulated particle can be have anappearance that ranges from having regular, uniform shape through to being jagged and irregular.

Nanoencapsulation Techniques: A multitude of techniques are used in nanoencapsulation and as the field is an emerging one, new techniques are constantly being developed. The more popular techniques include:

(a) Fluid bed coating Wax and lipid coating (b) Spray drying (c) Spray congealing (d) Hydrogel encapsulation

(e) Melt extrusion

Application: The basic reason for nanoencapsulation is to protect the core material and to then release it when it is required. Applications for this include:

- Targeted drug delivery systems that release the drug only when the drug has arrived at the site in the body where it is required.
- Timed release drug delivery where the nanoencapsulation material slowly allows the drug to be released into the body – such as nasal delivery of insulin. The coating material can be customised to determine the rate of delivery
- Embedded fragrances for branded perfumed clothing
- Food additions and food enhancements such as Omega-3 fatty acid additions to bread that do not alter taste
- Increasing shelf life and stability of products like vitamins

Targeted Drug Delivery Systems

The long-term objective of drug delivery systems is the ability to target selected cells and/or receptors within the body. At present, the development of new drug delivery techniques is driven by the need on the one hand to more effectively target drugs to the site of disease, to increase patient acceptability and reduce healthcare costs; and on the other hand, to identify novel ways to deliver new classes of pharmaceuticals that cannot be effectively delivered by conventional means. Nanotechnology is critical in reaching these goals. Already now nanoparticle formulations make use of the fact that an enlarged surface/volume ratio results in enhanced activity. Nanoparticles are also useful as drug carriers for the effective transport of poorly soluble therapeutics. When a drug is suitably encapsulated, in nanoparticulate form, it can be delivered to the appropriate site, released in a controlled way and protected from undergoing premature degradation. This results in higher efficacy and dramatically minimises undesirable side effects. Such nanoparticulate delivery systems can be used to more effectively treat cancer and a wide range of other diseases, which call for drugs of high potency.

Drug-delivering microchip technology, resulting from the convergence of controlled release and fabrication technologies evolved for the electronics industry, is also benefiting from the application of nanotechnology. urther miniaturization and the ability to store and release chemicals on demand offer new treatment options in the fight against disease. A future vision is that nanoparticles will carry therapeutic payloads or genetic content into diseased cells, minimising side effects as the nanoparticles will only become active upon reaching their ultimate destination. They may even check for overdosage before becoming active, thus preventing drug-related poisoning. In the past three decades, the number and variety of controlled release systems for drug delivery applications has increased dramatically. Many utilize polymers that have particular physical or chemical characteristics, such as biodegradability, biocompatibility or responsiveness to pH or temperature changes. In spite of many successful examples, the notion of combining polymer science with concepts from structural biology to provide new strategies and opportunities in the design of novel drug delivery systems adapted to today's demands, has not been fully embraced. In part progress has been slowed by regulatory submissions.

The very slow progress in the treatment of severe diseases has led to the adoption of a multidisciplinary approach to the targeted delivery and release of drugs, underpinned by nanoscience and nanotechnology. New drug delivery systems (DDS) combine polymer science, pharmaceutics, bioconjugate chemistry and molecular biology. The aim is to better control drug pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity and biorecognition of systems in the quest for improved efficacy.

Drug delivery and targeting systems under development aim to minimize drug degradation and loss, prevent harmful side effects and increase the availability of the drug at the disease site. Drug carriers include micro and nanoparticles, micro and nanocapsules, lipoproteins, liposomes, and micelles, which can be engineered to slowly degrade, react to stimuli and be site-specific. Targeting mechanisms can also be either passive or active. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the differences in the vascularization of the tumor tissue compared with healthy tissue. Active targeting involves the chemical 'decorating' of the surface of drug carriers with molecules enabling them to be selectively attached to diseased cells.

The controlled release of drugs is also important for therapeutic success. Controlled release can be sustained or pulsatile. Sustained (or continuous) release of a drug involves polymers that

release the drug at a controlled rate, by diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often preferred, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g. exposure to light, changes in pH or temperature).

Other nano-based approaches to drug delivery are focused on crossing a particular physical barrier, such as the blood-brain barrier; or on finding alternative and acceptable routes for the delivery of a new generation of protein-based drugs other than via the gastro-intestinal tract, where degradation can occur. Nanoscience and nanotechnology are thus the basis of innovative delivery techniques that offer great potential benefits to patients and new markets to pharmaceutical and drug delivery companies.

Controlled and Targeted Drug Delivery Systems: In medical therapy, a substantial application field for nanotechnology is the controlled and targeted transport of drugs ("drug delivery"). The use of nanoscale transportation vehicles should make it possible to achieve, that the active drugs affect selectively the targeted regions of the human body only, minimizing unwanted side effects.

How Nanotechnology Can Improve Targeted Drug Delivery Methods: Targeted drug delivery systems can convey drugs more effectively and/or more conveniently, increase patient compliance, extend the product life cycle, provide product differentiation, and reduce health care costs. Drug delivery systems that rely on nanomaterials also allow for targeted delivery of compounds characterized by low oral bioavailability due to poor water solubility, permeability and/or instability and provide for longer sustained and controlled release profiles. These technologies can increase the potency of traditional small molecule drugs in addition to potentially providing a mechanism for treating previously incurable diseases.

Benefits of Coupling Drugs with Nanoparticles: By the coupling of drugs with nanoparticles, less burdening application procedures can be realized like inhalation instead of infusions, for example. By functionalised nanostructured coating of the drug particles, the deposition speed can be controlled and smaller doses can be applied reducing unwanted side effects.

Using Magnetic Nanoparticles Targeted Drug Delivery - What This ‘Tag and Drag’ Process Involves

The use of magnetic nanoparticles in targeted drug delivery systems is under investigation by several research groups. Therapeutic drug molecules have been immobilized on the surface of magnetic nanoparticles or nanocrystals and directed to a specific target tissue using a magnetic field gradient. The drug is released by applying a radio frequency (RF) pulse. Gold coated iron, nickel and cobalt ferromagnetic nanoparticles have been employed in this "tag and drag" approach. In hypothermal treatment, magnetic nanoparticles are directed to diseased tissue containing heat sensitive tumors. An AC magnetic field is applied such that the nanoparticles become heated, causing destruction of the cancerous cells. More effective radiation therapy for tumor treatment can also be expected using metallic nanoparticles instead of, for example, magnetite. The nanoparticles allow the application of higher dosages of radiation at the tumor while sparing normal tissue.

Drug Transportation Systems Based on Nanoscale Cage Molecules, Nanoparticles and Nanoscale Suspensions

Such transportation systems could be realized, in principle, from nanoscale cage molecules (e.g. liposomes, fullerenes or other cage molecules such as dendrimers) or by coupling with nanoparticles. The goal here is to carry the active drugs selectively to the targeted cells, by means of nanoparticles with specific surface functionalization. Nanoparticles are small enough to penetrate cell membranes and overcome physiological barriers (e.g. blood-brain barrier) in the organism. Furthermore, nanoparticles and nanoscale suspensions improve the solubility and bio-availability of drugs and allow the application of drugs which are, so far, not applicable.

'Smart Drugs' - How Nanocapsules Reach Their Targets

As with pesticide delivery, the big interest is in 'controlled release.' Many of the big pharma and animal pharma companies working on nano-drugs are using encapsulation technologies such as nanocapsules to smuggle active compounds into and around the body. The capsules can be functionalised to bind at specific places in the body, or be activated by an external trigger, such as a magnetic pulse or ultrasound. The USDA compares these functionalised drug nanocapsules, called "Smart Delivery Systems," to the postal system, where molecular-coded "address labels" ensure that the packaged pharmaceutical reaches its intended destination.

Other Types of Nanomaterials Used in Drug Delivery Systems: Besides capsules, other nanomaterials being used to deliver drugs are listed below:

BioSilicon: BioSilicon is a highly porous silicon-based nanomaterial product, which can release a medicine slowly over a period of time. Developed by Australian company pSivida, the company uses its BioSilicon technology to fashion tiny capsules (to be swallowed) and also tiny needles that can be built into a patch to invisibly pierce the skin and deliver drugs.

Fullerenes: Fullerenes, the so called “miracle molecules” of nanotechnology (buckyballs and carbon nanotubes are included in this class of carbon molecules), are hollow cages of sixty carbon atoms less than a couple of nanometers wide. Because they are hollow, pharma companies are exploring filling the fullerenes with drug compounds and then functionalising them to bind in different parts of the body.

Dendrimers: Dendrimers are branching molecules that have a tree-like structure and are becoming one of the most popular tools in nanotechnology. Because of their shape and nano-size, dendrimers have three advantages in drug delivery:

- First, they can hold a drug’s molecules in their structure and serve as a delivery vehicle;
- Second, they can enter cells easily and release drugs on target;
- Third, and most importantly, dendrimers don’t trigger immune system responses.

Dendrimers can also be used for chemical analysis and diagnosis – raising the future possibility of synthetic molecules that can locate, diagnose and then treat tumours or other sick cells.

DNA Nanocapsules: DNA nanocapsules smuggle strands of viral DNA into cells. Once the capsule breaks down, the DNA hijacks the cells’ machinery to produce compounds that would be expected in a virus attack, thus alerting and training the immune system to recognise them. DNA nanocapsule technology could also be used to hijack living cells to produce other compounds such as new proteins or toxins. As a result, they must be carefully monitored as a potential biowarfare technology.

Administration Routes

The choice of drug is often influenced by the way it is administered, as this can make the difference between a drug’s success and failure. So the choice of a delivery route can be driven

by patient acceptability, important properties of the drug (e.g. solubility), the ability to target the disease location, or effectiveness in dealing with the specific disease.

Oral route : The most important drug delivery route is the peroral route. An increasing number of drugs are protein and peptide-based. They offer the greatest potential for more effective therapeutics, but they do not easily cross mucosal surfaces and biological membranes, they are easily denatured or degraded, they are prone to rapid clearance in the liver and other body tissues and they require precise dosing. At present, protein drugs are usually administered by injection, but this route is less accepted by patients and also poses problems of oscillating blood drug concentrations. So, despite the barriers to successful drug delivery that exist in the gastrointestinal tract (e.g. acid-induced hydrolysis in the stomach, enzymatic degradation throughout the gastrointestinal tract, bacterial fermentation in the colon), the peroral route is still the most intensively investigated as it offers advantages of convenience, cheapness of administration and manufacturing cost savings.

Parenteral Routes: Parenteral routes (e.g. intravenous, intramuscular or subcutaneous) are very important. The only nanosystems presently on the market, liposomes, are administered intravenously. Nanoscale drug carriers have a great potential for improving the delivery of drugs through nasal and sublingual routes, both of which avoid first-pass metabolism; and for difficult access ocular, brain and intra-articular cavities.

It has been possible to deliver peptides and vaccines systemically using the nasal route through the association of active drug macromolecules with nanoparticles. In addition, there is the possibility of improving the ocular bioavailability of drugs if administered in a colloidal drug carrier.

Pulmonary Delivery: Pulmonary delivery is also important and is effected in a variety of ways - via aerosols, metered dose inhaler systems, powders (dry powder inhalers) and solutions (nebulizers), which may contain nanostructures such as liposomes, micelles, nanoparticles and dendrimers. Aerosol products for pulmonary delivery comprise more than 30% of the global drug delivery market. Research into lung delivery is driven by the potential for successful protein and peptide drug delivery by this route and by the promise of an effective delivery mechanism for gene therapy (e.g. in the treatment of cystic fibrosis), as well as the need to replace chlorofluorocarbon propellants in metered dose inhaler systems. Pulmonary drug delivery offers local targeting for the treatment of respiratory diseases and increasingly appears

to be a viable option for the delivery of drugs systemically. However, the success of pulmonary delivery of protein drugs is diminished by proteases in the lung, which reduce their overall bioavailability, and by the barrier between capillary blood and alveolar air (the air-blood barrier).

Transdermal Drug Delivery: Transdermal drug delivery avoids problems such as gastrointestinal irritation, metabolism, variations in delivery rates and interference due to the presence of food. It is also suitable for unconscious patients. The technique is generally non-invasive, well accepted by patients and can be used to provide local delivery over several days. Limitations include slow penetration rates, lack of dosage flexibility and/or precision, and a restriction to relatively low dosage drugs.

Trans-Tissue and Local Delivery Systems: Trans-tissue and local delivery systems are systems that require to be tightly fixed to resected tissue during surgery. The aim is to produce an elevated pharmacological effect, while minimizing systemic, administration-associated toxicity. Trans-tissue systems include: drug-loaded gelatinous gels, which are formed in-situ and adhere to resected tissues releasing drugs, proteins or gene-encoding adenoviruses; antibody-fixed gelatinous gels (cytokine barrier) that form a barrier that on a target tissue could prevent the permeation of cytokines into that tissue; cell-based delivery, which involves a gene-transduced oral mucosal epithelial cell-implanted sheet; device directed delivery - a rechargeable drug infusion device that can be attached to the resected site.

Gene Delivery :Gene delivery is a challenging task in the treatment of genetic disorders. Plasmid DNA has to be introduced into the target cells. It then needs to be transcribed, and the genetic information ultimately translated into the corresponding protein. To achieve this, a number of hurdles have to be overcome. The gene delivery system has to be targeted to the target cell, transported through the cell membrane, taken up and degraded in the endolysosomes, and the plasmid DNA trafficked intracellularly to the nucleus.

Table 1: Summary of application areas for nanoscale pharmaceuticals and medicine in drug delivery.

Material/technique	Property	Applications
Drug delivery		
Nanoparticles in the range of 50–100 nm.	Larger particles cannot enter tumour pores while nanoparticles can easily move into a tumour.	Cancer treatment.
Nanosizing in the range of 100–200 nm.	Low solubility.	More effective treatment with existing drugs.
Polymers.	These molecules can be engineered to a high degree of accuracy.	Nanobiological drug carrying devices.
Ligands on a nanoparticle surface.	Table These molecules can be engineered to a high degree of accuracy.	The ligand target receptors can recognise damaged tissue, attach to it and release a therapeutic drug.
Nanocapsules.	Evading body's immune system whilst directing a therapeutic agent to the desired site.	A Buckyball-based AIDS treatment is just about to enter clinical trials.
Increased particle adhesion.	Degree of localised drug retention increased.	Slow drug release.
Nanoporous materials.	Evading body's immune system whilst directing a therapeutic agent to the desired site.	When coupled to sensors, drug-delivering implants could be developed.
'Pharmacy-on-a-chip'	Monitor conditions and act as an artificial means of regulating and maintaining the body's own hormonal balance.	E.g. diabetes treatment.
Sorting biomolecules.	Nanopores and membranes are capable of sorting, for example, left- and right-handed versions of molecules.	Gene analysis and sequencing.

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – V- Nanobiotechnology – SBTA1503

Nanotoxicity

Nanomaterials have at least one primary dimension of less than 100 nanometers, and often have properties different from those of their bulk components that are technologically useful. Because nanotechnology is a recent development, the health and safety effects of exposures to nanomaterials, and what levels of exposure may be acceptable, is not yet fully understood.[2] Nanoparticles can be divided into combustion-derived nanoparticles (like diesel soot), manufactured nanoparticles like carbon nanotubes and naturally occurring nanoparticlesfrom volcanic eruptions, atmospheric chemistry etc. Typical nanoparticles that have been studied are titanium dioxide, alumina, zinc oxide, carbon black, carbon nanotubes, and buckminsterfullerene.

Nanotoxicology is a sub-specialty of particle toxicology. Nanomaterials appear to have toxicity effects that are unusual and not seen with larger particles. For example, even inert elements like gold become highly active at nanometer dimensions. Nanotoxicological studies are intended to determine whether and to what extent these properties may pose a threat to the environment and to human beings. Nanoparticles have much larger surface area to unit mass ratios which in some cases may lead to greater pro-inflammatory effects in, for example, lung tissue. In addition, some nanoparticles seem to be able to translocate from their site of deposition to distant sites such as the blood and the brain.

Nanoparticles can be inhaled, swallowed, absorbed through skin and deliberately or accidentally injected during medical procedures. They might be accidentally or inadvertently released from materials implanted into living tissue. One study considers release of airborne engineered nanoparticles at workplaces, and associated worker exposure from various production and handling activities, to be very probable.

Factors affecting nanotoxicity

size is a key factor in determining the potential toxicity of a particle. However it is not the only important factor. Other properties of nanomaterials that influence toxicity include: chemical composition, shape, surface structure, surface charge, aggregation and solubility,] and the presence or absence of other chemicals. The large number of variables influencing toxicity means that it is difficult to generalise about health risks associated with exposure to nanomaterials – each new nanomaterial must be assessed individually and all material properties must be taken into account.

Composition

Metal-based

Metal based nanoparticles (NPs) are a prominent class of NPs synthesized for their functions as semiconductors, electroluminescents, and thermoelectric materials. Biomedically, these antibacterial NPs have been utilized in drug delivery systems to access areas previously inaccessible to conventional medicine. With the recent increase in interest and development of nanotechnology, many studies have been performed to assess whether the unique characteristics of these NPs, namely their large surface area to volume ratio, might negatively impact the environment upon which they were introduced. Researchers have since found that many metal and metal oxide NPs have detrimental effects on the cells with which they come into contact including but not limited to DNA breakage and oxidation, mutations, reduced cell viability, warped morphology, induced apoptosis and necrosis, and decreased proliferation.

Carbon-based

The latest toxicology studies on mice as of 2013 involving exposure to carbon nanotubes (CNT) showed a limited pulmonary inflammatory potential of MWCNT at levels corresponding to the average inhalable elemental carbon concentrations observed in U.S.-based CNT facilities. The study estimated that considerable years of exposure are necessary for significant pathology to occur

One review concludes that the evidence gathered since the discovery of fullerenes overwhelmingly points to C_{60} being non-toxic. As is the case for toxicity profile with any chemical modification of a structural moiety, the authors suggest that individual molecules be assessed individually

Dispersion state

Many nanoparticles agglomerate or aggregate when they are placed in environmental or biological fluids. The terms agglomeration and aggregation have distinct definitions according to the standards organizations ISO and ASTM, where agglomeration signifies more loosely bound particles and aggregation signifies very tightly bound or fused particles (typically occurring during synthesis or drying). Nanoparticles frequently agglomerate due to the high ionic strength of environmental and biological fluids, which shields the repulsion due to charges on the nanoparticles. Unfortunately, agglomeration has frequently been ignored in nanotoxicity studies, even though agglomeration would be expected to affect nanotoxicity since it changes the size, surface area, and sedimentation properties of the nanoparticles. In

addition, many nanoparticles will agglomerate to some extent in the environment or in the body before they reach their target, so it is desirable to study how toxicity is affected by agglomeration.

The agglomeration/deagglomeration (mechanical stability) potentials of airborne engineered nanoparticle clusters also have significant influences on their size distribution profiles at the end-point of their environmental transport routes. Different aerosolization and deagglomeration systems have been established to test stability of nanoparticle agglomerates.

Surface chemistry and charge

NPs, in their implementation, are covered with coatings and sometimes given positive or negative charges depending upon the intended function. Studies have found that these external factors affect the degree of toxicity of NPs.

Administration methods

Respiratory

Inhalation exposure is the most common route of exposure to airborne particles in the workplace. The deposition of nanoparticles in the respiratory tract is determined by the shape and size of particles or their agglomerates, and they are deposited in the lungs to a greater extent than larger respirable particles. Based on animal studies, nanoparticles may enter the bloodstream from the lungs and translocate to other organs, including the brain.] The inhalation risk is affected by the dustiness of the material, the tendency of particles to become airborne in response to a stimulus. Dust generation is affected by the particle shape, size, bulk density, and inherent electrostatic forces, and whether the nanomaterial is a dry powder or incorporated into a slurry or liquid suspension

Animal studies indicate that carbon nanotubes and carbon nanofibers can cause pulmonary effects including inflammation, granulomas, and pulmonary fibrosis, which were of similar or greater potency when compared with other known fibrogenic materials such as silica, asbestos, and ultrafine carbon black. Some studies in cells or animals have shown genotoxic or carcinogenic effects, or systemic cardiovascular effects from pulmonary exposure. Although the extent to which animal data may predict clinically significant lung effects in workers is not known, the toxicity seen in the short-term animal studies indicate a need for protective action for workers exposed to these nanomaterials. As of 2013, further research was needed in long-term animal studies and epidemiologic studies in workers. No reports of actual adverse health effects in workers using or producing these nanomaterials were

known as of 2013. Titanium dioxide (TiO_2) dust is considered a lung tumor risk, with ultrafine (nanoscale) particles having an increased mass-based potency relative to fine TiO_2, through a secondary genotoxicity mechanism that is not specific to TiO_2 but primarily related to particle size and surface area

Dermal

Some studies suggest that nanomaterials could potentially enter the body through intact skin during occupational exposure. Studies have shown that particles smaller than 1 μm in diameter may penetrate into mechanically flexed skin samples, and that nanoparticles with varying physicochemical properties were able to penetrate the intact skin of pigs. Factors such as size, shape, water solubility, and surface coating directly affect a nanoparticle's potential to penetrate the skin. At this time, it is not fully known whether skin penetration of nanoparticles would result in adverse effects in animal models, although topical application of raw SWCNT to nude mice has been shown to cause dermal irritation, and *in vitro* studies using primary or cultured human skin cells have shown that carbon nanotubes can enter cells and cause release of pro-inflammatory cytokines, oxidative stress, and decreased viability. It remains unclear, however, how these findings may be extrapolated to a potential occupational risk. In addition, nanoparticles may enter the body through wounds, with particles migrating into the blood and lymph nodes

Gastrointestinal

Ingestion can occur from unintentional hand-to-mouth transfer of materials; this has been found to happen with traditional materials, and it is scientifically reasonable to assume that it also could happen during handling of nanomaterials. Ingestion may also accompany inhalation exposure because particles that are cleared from the respiratory tract via the mucociliary escalator may be swallowed

The extremely small size of nanomaterials also means that they much more readily gain entry into the human body than larger sized particles. How these nanoparticles behave inside the body is still a major question that needs to be resolved. The behavior of nanoparticles is a function of their size, shape and surface reactivity with the surrounding tissue. In principle, a large number of particles could overload the body's phagocytes, cells that ingest and destroy foreign matter, thereby triggering stress reactions that lead to inflammation and weaken the body's defense against other pathogens. In addition to questions about what happens if non-degradable or slowly degradable nanoparticles accumulate in bodily organs, another concern

is their potential interaction or interference with biological processes inside the body. Because of their large surface area, nanoparticles will, on exposure to tissue and fluids, immediately adsorb onto their surface some of the macromolecules they encounter. This may,for instance, affect the regulatory mechanisms of enzymes and other proteins.

Nanomaterials are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot. Nanomaterials can gain access to the blood stream via inhalation] or ingestion.] Broken skin is an ineffective particle barrier, suggesting that acne, eczema, shaving wounds or severe sunburn may accelerate skin uptake of nanomaterials. Then, once in the blood stream, nanomaterials can be transported around the body and be taken up by organs and tissues, including the brain, heart, liver, kidneys, spleen, bone marrow and nervous system. Nanomaterials have proved toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production and cell death.

Oxidative stress

For some types of particles, the smaller they are, the greater their surface area to volume ratio and the higher their chemical reactivity and biological activity. The greater chemical reactivity of nanomaterials can result in increased production of reactive oxygen species (ROS), including free radicals. ROS production has been found in a diverse range of nanomaterials including carbon fullerenes, carbon nanotubes and nanoparticle metal oxides. ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity; it may result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA

Cytotoxicity

A primary marker for the damaging effects of NPs has been cell viability as determined by state and exposed surface area of the cell membrane. Cells exposed to metallic NPs have, in the case of copper oxide, had up to 60% of their cells rendered unviable. When diluted, the positively charged metal ions often experience an electrostatic attraction to the cell membrane of nearby cells, covering the membrane and preventing it from permeating the necessary fuels and wastes.[9] With less exposed membrane for transportation and communication, the cells are often rendered inactive.

NPs have been found to induce apoptosis in certain cells primarily due to the mitochondrial damage and oxidative stress brought on by the foreign NPs electrostatic reactions

Genotoxicity

Metal Oxides such as copper oxide, uraninite, and cobalt oxide have also been found to exert significant stress on exposed DNA.[9] The damage done to the DNA will often result in mutated cells and colonies as found with the HPRT gene test.

Standards

Characterization of a nanomaterial's physical and chemical properties is important for ensuring the reproducibility of toxicology studies, and is also vital for studying how the properties of nanomaterials determine their biological effects. The properties of a nanomaterial such as size distribution and agglomeration state can change as a material is prepared and used in toxicology studies, making it important to measure them at different points in the experiment

With comparison to more conventional toxicology studies, in nanotoxicology, characterisation of the potential contaminants is challenging. The biological systems are themselves still not completely known at this scale. Visualisation methods such as electron microscopy (SEM and TEM) and atomic force microscopy (AFM) analysis allow visualisation of the nano world. Further nanotoxicology studies will require precise characterisation of the specificities of a given nano-element: size, chemical composition, detailed shape, level of aggregation, combination with other vectors, etc. Above all, these properties would have to be determined not only on the nanocomponent before its introduction in the living environment but also in the (mostly aqueous) biological environment.

There is a need for new methodologies to quickly assess the presence and reactivity of nanoparticles in commercial, environmental, and biological samples since current detection techniques require expensive and complex analytical instrumentation.

Regulation.

The Royal Society identifies the potential for nanoparticles to penetrate the skin, and recommends that the use of nanoparticles in cosmetics be conditional upon a favorable assessment by the relevant European Commission safety advisory committee.

The Woodrow Wilson Centre's Project on Emerging Technologies conclude that there is insufficient funding for human health and safety research, and as a result there is currently limited understanding of the human health and safety risks associated with nanotechnology. While the US National Nanotechnology Initiative reports that around four percent (about $40 million) is dedicated to risk related research and development, the Woodrow Wilson Centre

estimate that only around $11 million is actually directed towards risk related research. They argued in 2007 that it would be necessary to increase funding to a minimum of $50 million in the following two years so as to fill the gaps in knowledge in these areas

The potential for workplace exposure was highlighted by the 2004 Royal Society report which recommended a review of existing regulations to assess and control workplace exposure to nanoparticles and nanotubes. The report expressed particular concern for the inhalation of large quantities of nanoparticles by workers involved in the manufacturing process

Stakeholders concerned by the lack of a regulatory framework to assess and control risks associated with the release of nanoparticles and nanotubes have drawn parallels with bovine spongiform encephalopathy (‘mad cow's disease'), thalidomide, genetically modified food, nuclear energy, reproductive technologies, biotechnology, and asbestosis. In light of such concerns, the Canadian-based ETC Group have called for a moratorium on nano-related research until comprehensive regulatory frameworks are developed that will ensure workplace safety