CY305 Molecular Architecture and Evolution of Functions

Report on

The Role of Biology in Materials Science

By,
R.Vinay CH03B044
Arjun Kannan CH03B007

Introduction

History is testimony to the fact that all the "**best**" designs, till date, have come from the nature. There have been numerous instances of scientists, engineers, architects gaining inspiration from the "Mother Nature". Take for example the invention of aero plane, the Wright brothers took flight only after watching the vultures swoop. As a matter of fact, Gustave Eiffel's tower, one of the eight wonders of the world, rests on elegantly designed curved structure inspired by the bone structure. In addition to this the bone structure has also been the flag bearer for the design of the modern crane. In 1886, C Culman in Zurich was inspired by the trabeculae of the porous material which led him to design of modern crane. Joseph Paxton is said to have paid tribute to the ribbed stem of a lily leaf in his Crystal Palace, which housed the Great Exhibition of 1851.
So to say, the nature has been the "guru" for all the scientists, engineers and artificers. It is interesting to note how the school of thought that was the premise of earlier age in terms of morphology, forces in nature etc, nevertheless present even now, is the guide for discovery of new age materials and devices that are frightfully forward looking and put to shame the best of engineers, scientists in the world.
Biomimetics is the key paradigm that has been hinted here. Now it's well established that the nature's designs and materials are the best and that no one so far has ever been able to come up with better ones, why not just mimic the nature, and create tailor made materials with desired properties using the same principles that the nature has used in making its own fantastic materials. In other words, why not cook food to our taste but use the nature's recipe. Thus, it right to say, "Man has indeed evolved from apes!"

Biology – An indispensable part of the "Material World"

Traditionally material scientists gained tremendous inspiration from nature and focused on emulating the biosystems using synthetic components to create materials with similar properties. For e.g. plastic films having microstructure similar to that of shark skin as drag reducing coating in aeroplanes, manufacture of glass optical fibers having layered structure inspired from sponge spicule, etc. But now focus has shifted to using the self assembling nature of the proteins as a platform to create novel materials in mild conditions expending as little energy as possible.
Biology plays a very important role in materials science. Firstly, it can act as a guide in designing materials with better mechanical, optical, electronic properties and other properties. Secondly, the fabrication of novel nanomaterials can be done by using the self assembling property of biological molecules like proteins.

The former procedure involves a thorough understanding of the structure and mechanics of biomaterials and ***use of synthetic components*** to develop new synthetic materials by mimicking the biomaterials. These new materials can have a variety of applications like in aeronautics & space exploration, biomedicine, and telecommunication to name a few. For example the understanding of structure and properties (and also structure-property relationships) of the sponge spicule can provide the necessary guidance in developing novel optical fibers that have fantastic properties. Also synthetic composite materials can be made, that are structurally similar to mollusk shells. These would be stronger, stiffer,

and more fracture-resistant and would have tremendous potential for use in aircraft and spacecraft industry. Study of the ability of cell membranes and other biological materials to sense and respond to their surroundings could lead to synthetic "smart" materials capable of sensing and repairing material fatigue or damage. And the list goes on. A detailed analysis, of structures of different naturally occurring substances, will be done in the following sections.

The latter procedure, of using the ***self assembling*** property of natural molecules like proteins, involves first creating genetically engineered short polypeptides that have long range order meaning these can assemble over large topographies. These polypeptides are generally 7- 15 amino acids long and they are created by computational biology approaches which shall be discussed later. These polypeptides have an unique property of binding to specific inorganic materials. Once created, these engineered inorganic binding polypeptides can further be genetically engineered to get suitable material properties, material surfaces, morphologies, and crystal chemistries. Thus these have tremendous potential in many applications.
Both the approaches are discussed in detail in the following sections.

Molecular Biology and Materials Science – Strange bed fellows

The idea is to imbibe the features of molecular biology to create materials of required properties using synthetic components. Some of the naturally occurring materials include mollusk shells, dental enamel junction, sponge spicule, etc. These have unusual engineering properties compared to the synthetic materials and serve as guides for synthesis of novel technological materials having practical applications. A detailed investigation of these biologically synthesized materials is attempted in the following sections that helps in establishing the structure-material property relationships which is the first step in realizing the biomimetics paradigm.

The Shark Skin [1]

The shark skin is an excellent example of how the nature can be a guide to show us the enlightened path to sound engineering. The studies of shark skins have elegantly led to creation of the drag reducing coatings. Let's see what makes up the structure of the shark skin and what makes it so special. The shark skin from a microscopic view looks something like this:

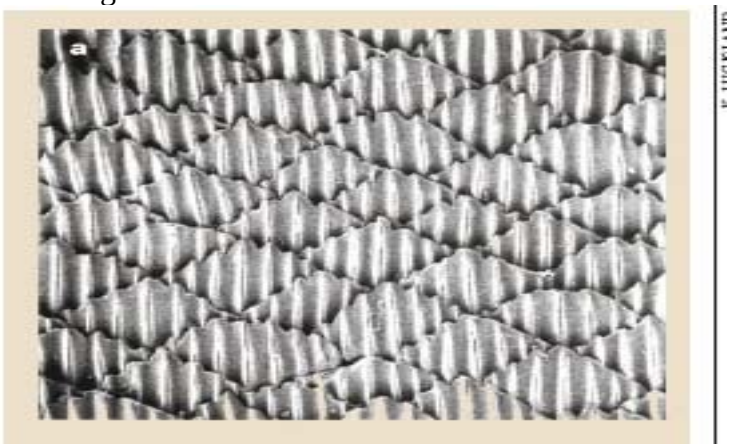

Figure 1. Ribbed structure of shark skin

The figure shows the ribbed structure of the shark skin viewed under microscope. It is observed that the shark skin has many corrugations on its surface. The turbulent boundary layer is affected by such surface irregularities which results in improper boundary layer development resulting drag reduction. Thus such a ribbed structure seems to provide a superior aerodynamic efficiency when compared to the smooth surface. So to mimic this feature, plastic films of same microscopic structure with ribs parallel to the direction of flow have been used to coat aircrafts reducing the drag by 8% and resulting in fuel reduction by 1.5%.

Figure 2 Trials on an aircraft coated with a plastic film with the same microscopic texture.

Not only does the shark skin very efficient in reducing drag but also the texture provides a self – cleaning function. In other words, the rough surface has low wettability. It is also well known that rough surfaces can increase the contact angle by 180 even though they may be hydrophilic ones.

The lesson that we learn from nature is that micro scale protrusions result in low wettability and also low drag. Yet another testimony to this fact is the lotus leaf which has non-wettable surface due to many micro scale protrusions. Thus to surmise to produce materials having low wettability and low drag, they must have microscopic texture having many ribs or corrugations on the surface.

Sponge Spicule [2]

Biological hard tissues namely bone, teeth, shells, skeletal units, and spicules have excellent mechanical properties. These are synthesized by many multicellular organisms.

These are nothing but composites of inorganic materials and organic matrices (such as proteins). These have better properties than most of the synthetic materials having the same phase composition. Sponge spicule is one such biological hard tissue which provides ample opportunities to learn from the nature and build novel materials by mimicking the nature's designs. It is fibrous, glassy silica having concentrically layered structure having optical and mechanical properties. It is hydrated form of glassy silica

and biosynthesized in the most elementary conditions in sea water. Thus, it is evident that nature always goes for best output with minimum input. One such sponge species is the *Rosella racovitzea* (Rosella) lives 120 m under water in Ross Sea near Antarctica.
The Rosella has pentactinal spicules that are 10 cm long and have 200-300 µm diameter. It also has cross-shaped apexes that act as lenses giving excellent optical properties. There is a green filamentous algae present in the interior of the sponge which adapted to live under dim light conditions.
One may wonder as to what can the optical properties of sponge spicule attributed to?
Well the answer lies in the very structure of the sponge spicule which acts as optical waveguides. All in all, the spicules and the algae residing in the interior form a symbiotic system. The spicules are the optical lenses or the fibers which transfer dim light available at the depth and the algae uses the dim light producing nutrients to the whole system to function.
Now lets us look into the very structure of the sponge spicule and phantom out the reasons behind the observed mechanical and optical properties. As far as the optical properties are considered a mere experimentation would reveal that the index of refraction is constant throughout the thickness giving it qualities that are at par with the synthetically manufactured glass fiber. But then what why does the sponge spicule score above the conventional glass fiber? Well, the answer lies in establishing the structure property relationships existing in sponge spicules and comparing it with the conventional glass fiber for durability and of course mechanical integrity. In this regard, we present an SEM image of the sponge spicule.

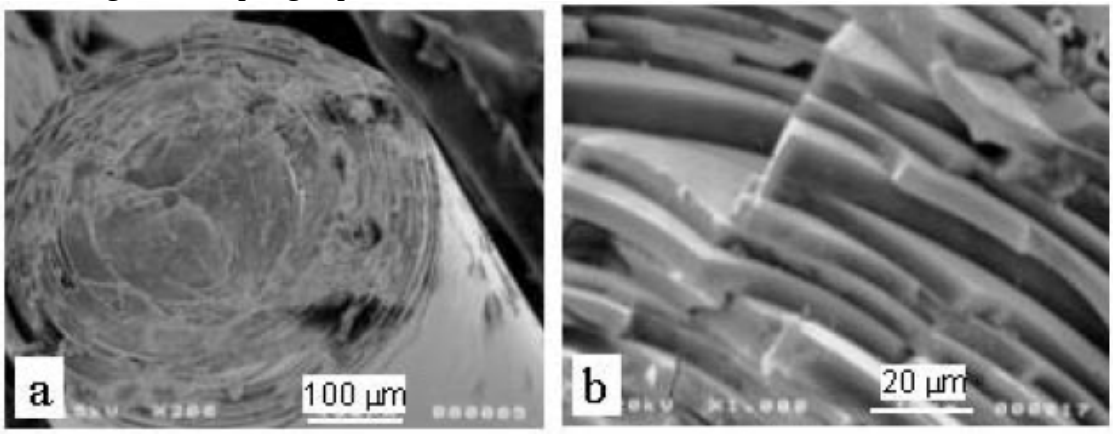

Figure 3. SEM image of a fractured surface of sponge spicule showing concentric layering.

From the figure its clear that the sponge spicule has concentric layered structure. This is surprising as from outside the sponge spicule looks like a solid bar and one would expect smooth fracture surface (Take for example a silica bar, cut it and a smooth fracture surface can be observed). And this is key thing that differentiates the normal silica bar and the sponge spicule in terms of mechanical properties. All the fractured surfaces display a layered structure with layer having a thickness of about 2-15 µm across the diameter. These concentric layers surround an inner proteinaceous central filament. One may be tempted to ask as to what makes the layered structure so special. The point to be

noted here is that the interface between the layers is weak and the layers might slide over one another when subjected to shear. This inhibits the development of cracks.
Thus a weak interface deflects the crack and stops it from propagating from one layer to another when a fracture occurs.
The ideal way to gage the performance of sponge spicule is to test it under standard conditions and compare it with silica glass rods. Three point bend tests would reveal that sponge spicule has fracture strength, stress to strain failure and fracture energy several times that of silica glass rods.
In this regard, it is ideal to give the stress vs. strain curve.

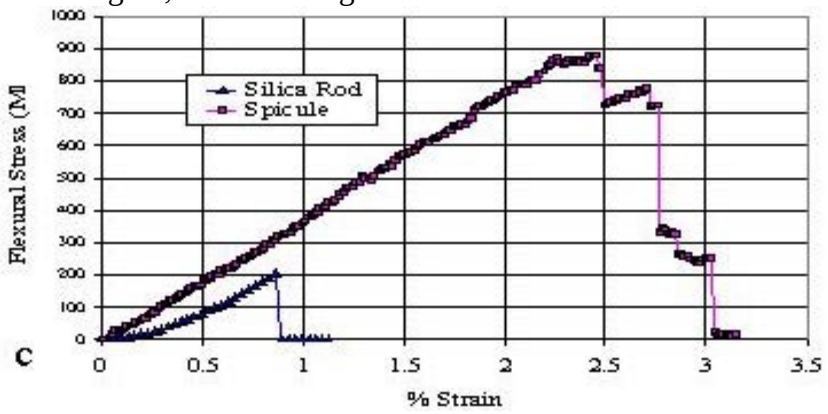

Figure 4. Stress vs. Strain relationship
Also it's interesting to see that the under bending stresses, the sponge spicule undergoes a soft failure indicated from the slow decrease of stress with increase in strain. Whereas for silica glass rods, the stress suddenly jumps to zero at some point indicating that the silica glass rods fail catastrophically under bending stresses.
To dig deeper and gain better understanding of mechanical properties, nanoindentation tests can be performed. Details of such test can be found elsewhere[3]. The results obtained from such tests reveal that elastic modulus and hardness of spicules are half that of commercially available glass rods
To surmise the observed mechanical properties are due to layered microstructure which can be mimicked. However physical layering should ensure that refractive index be constant across the layers to have uniform optical properties.

Mollusk shells [4]

Shells are hard coverings formed by certain organisms to protect them from predators and environmental hazards. They also provide them the necessary rigidity and shape. One group of animals that form shells is called the mollusks. These mollusks are grouped according to the kind of shells they form into eight classes viz. Gastropods, Bivalvia Cephalopoda, Caudofoveata, Aplacophora, Monoplacophora, Polyplacophora, and Scaphopoda. Mollusk shells are made of calcium carbonate derived either from sea water or from the food mollusks eat. There are two common forms of calcium carbonate viz. aragonite and calcite. Both differ in crystal shape but have same chemical formula. The calcite form usually occurs in hard tissues for e.g. found in hard, outer shell of oyster.
Abalones and some oysters produce shells having aragonite microstructure. The substance they produce is called as "mother of pearls" or nacre. The following section

describes in detail structure of nacre and the reason for its unique properties.

Nacre: "Mother of pearl"[3, 4]

Nacre is one of the most widely studied aragonite microstructures. It is found in shells of many families of mollusks viz. Gastropods, bivalves, and cephalopod. It is a composite with a layered structure of brick (aragonite platelets) and mortar (protein–polysaccharide matrix). Take for e.g. in red abalone which belongs to gastropod family, nacre is characterized by 300±10 nms thick hexagonally shaped platelets surrounded by thin film of organic matrix (10±5 nms.). These aragonite platelets (orthorhombic $CaCO_3$) form a layered structure which looks like brick-mortar structure, as the platelets are closely packed in a given layer but are staggered throughout.

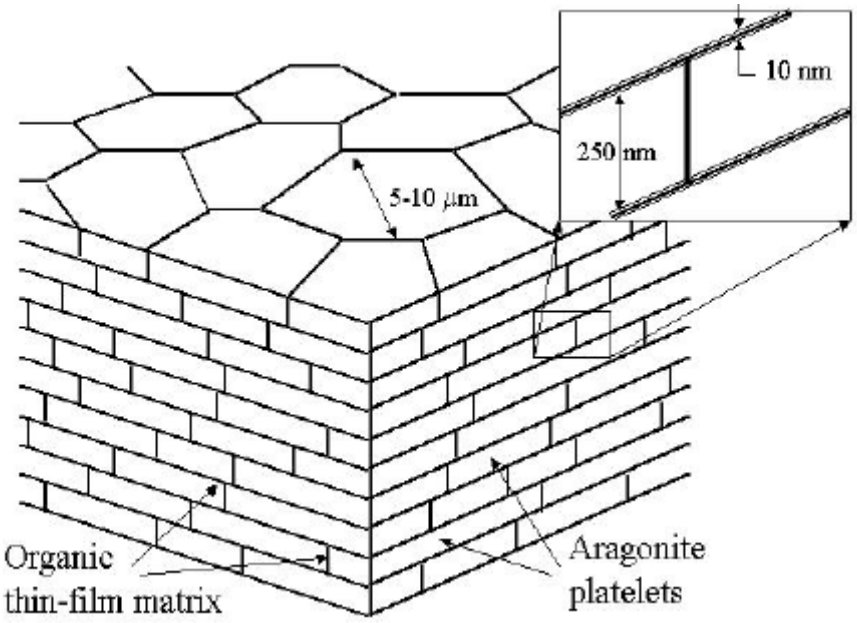

Figure 5a. Schematic representation of aragonite microstructure of nacre.

Gathering insights from the Nacre's microstructure and relating to its excellent properties:

Nacre has high fracture toughness and specific strength. The high fracture toughness is due to the presence of thin films of organic matrix framework. The organic framework has nano columns which are randomly arranged. These act as 'mineral bridges' to connect two aragonite platelets through the matrix. It is observed that when a fracture occurs, the crack propagates through the organic matrix or gaps in the nacre. When the crack is deflected into the matrix, it splits into two opposite directions. Then they are stopped by the mineral bridges. As larger and larger cracks are set up, it is seen that the crack passes through one layer and then is stopped by the mineral bridges.
This is why nacre has high fracture toughness.
The segmented structure to a great extent affects its behavior to stresses. Stresses normal to platelet plane are tackled by the organic matrix that bridges between the platelets, thereby keeping them together and preventing the uncontrolled crack growth. If the

stresses are shear, then the platelets slide over the organic matrix and dissipate the stresses. The behavior of nacre towards is explained by the following diagram

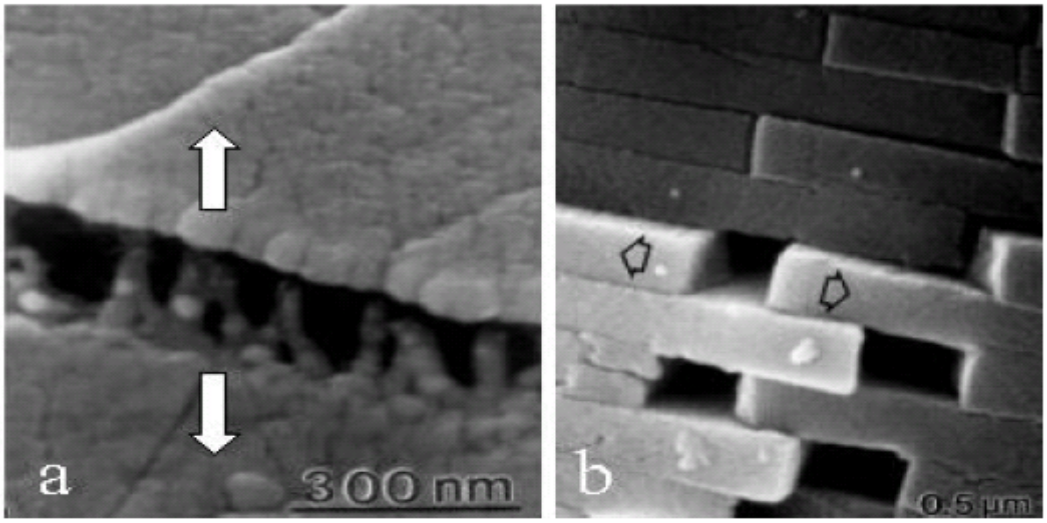

Figure 5b. SEM images demonstrating deformation behavior of nacre under static loading, representing normal and shear resolved stresses

To surmise, the layered structure can be further toughened by using segmented design as seen in nacre. Toughening effects of layered structure of nacre is evident from the highly tortuous fracture surface with exposed aragonite platelets. The dimensional features of the rigid phase and the thickness and composition of the organic matrix are also critical factors.

Dentin Enamel Junction [3, 5]

So what is there in a tooth? Well, for one, it has the hardest tissue in the body, namely the enamel. But that is not the only reason that tooth is studied these days. The transition of mechanical properties as we go from the outer to the inner parts of the tooth is a great source of information on how we can develop similar materials.

A profile of the tooth is shown below.

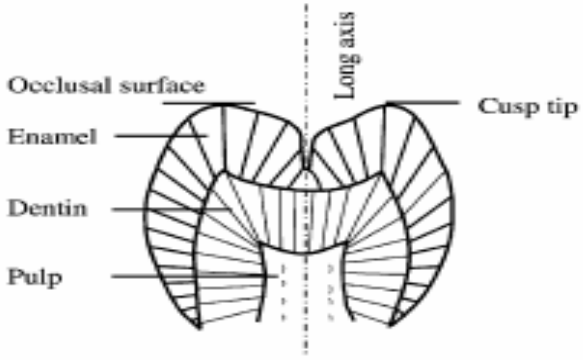

Figure 6. Profile of DEJ.

It consists of the outer hard enamel, which covers the softer dentin. The interface between the two is the dentin-enamel junction (DEJ). The innermost part of the tooth is the pulp.

The enamel is made up of highly organized inorganic hydroxyapatite crystals. These minerals give it the great hardness and wear resistance that enamel is known for. Dentin is made up of HAP crystals arranged randomly in a collagenous matrix. This makes dentin competitively softer than enamel. The DEJ is the bridge which is shaped such that it allows the interpenetration of the two layers. Thus it is usually convex towards the dentin. Collagen fibers from the dentin enter the DEJ and are then joined to the highly mineralized enamel.

The wear and fracture resistance are measured using indentation tests. Usually the tooth is cut and polished either along the occlusal surface (shown in the above figure) or a surface perpendicular to it. The sampled is set in a mould, cleaned with alcohol and roughness is checked to make sure the tooth is polished well. It is then set in an artificial salivary environment. Micro or nano-hardness tests are done using balls of appropriate weight. A triangular or quadrilateral mark is usually made at various points. The diagonal of the mark is measured and correlated to hardness. Wear tests are conducted using a ball which varies the tangential force on the tooth and gets a displacement as the response. Keeping a constant normal force, cycles of different tangential forces are done. In the occlusal section layer upon layer are examined by a optical microscope and the thickness and depth of the mark are measured. Then the next wear test is done. A similar pattern is also done for the axial direction except that the alignment of marks is different. Thus the process gets the wear and hardness values of layers of enamel, DEJ and dentin.

Friction[5]

The tests in the occlusal section show the friction coefficient as a function of the number of cycles. As we go from enamel (a in the graph) to dentin (e in the graph). In most cases there is a slow initial increase in the coefficient which suddenly shoots up and then saturation is reached. However closer to DEJ there is a faster rise in the coefficient. In the dentin the coefficient immediately increases.

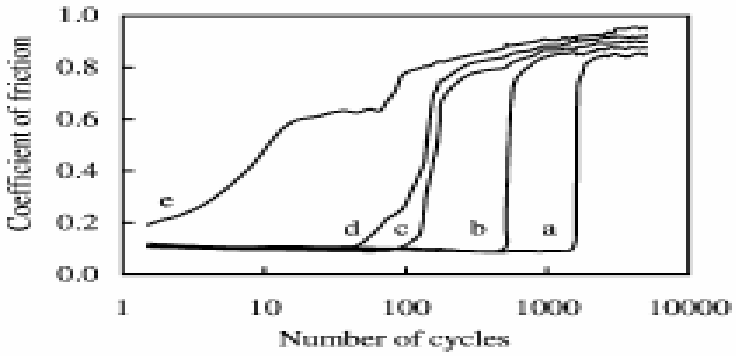

Figure 7. Friction coefficient

In the axial section, the broad results (shown below) were repeated in that in the enamel (a in the graph) and DEJ (b in the graph) increase rapidly and then saturate. However, dentin (c in the graph) has two regions of steady increase which show its collagenous structure. As is evident, there are more fluctuations in this section.

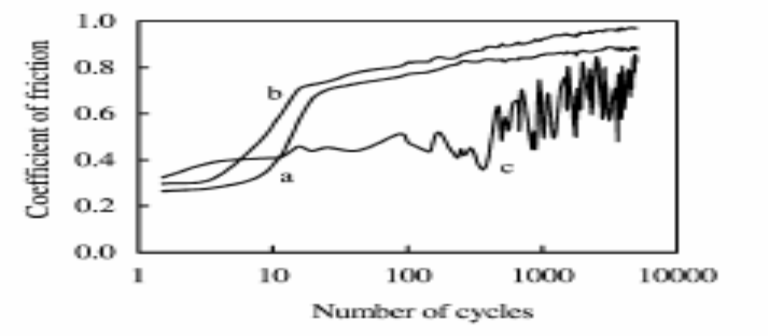

Figure 8. Friction coefficient

Wear behavior[5]

The method of using wear tests was mentioned earlier. When typical wear marks, corresponding to dentin, enamel and DEJ are seen under a microscope the following is observed. Dentin marks are a series of ploughs or troughs in the surface. This confirms the thought that the mechanism of wear is due to plastic deformation. Enamel on the other hand has a layer of particles on it. This is due to the hardness and hence cracking of enamel. The DEJ clearly shows both these patterns as shown below.

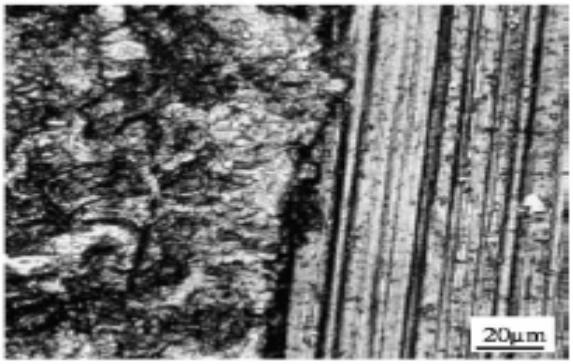

Figure 9. Wear behavior.

The depth and breadth of the marks is more in dentin and hardness is less, in accordance with the earlier discussion. The difference in the tribological properties of the aforementioned sections agrees with findings that in the occlusal section the HAP is more symmetrical than in the axial section. Similarly dentin is more tubular in the axial section.

Crack propagation[5]

Cracks in enamel are caused because of the mastication done while grinding of food. These cracks pass through the gaps in the HAP crystals (it is easier than through the rod). Due to the orientation of the DEJ the cracks will come to it. Now it can be deflected as sometimes happens in many mechanical structures or pass to the dentin where plastic deformation will stop the crack. The former is dangerous as enamel would be easily eroded and superficial dentin would be exposed. Instead crack propagates through the DEJ. This is because the gradual change in properties gives close values of elastic modulus required for crack propagation. Again cracks propagate through normal surfaces. In DEJ the geometry with many shapes causes cracks to orient towards normal surfaces and pass on to the dentin where it is stopped.

The above properties can be used to construct better materials and contacts between hard and soft materials for preventing failure through cracks, wear and friction.

Biomineralization & Biomaterials[6]

Biomaterials are unique from the point of view of their structure. These materials are made up of hierarchical arrangement of molecular units to subunits and then to higher sized units. High degree of organization is seen at all length scales of these biomaterials. Moreover architectures at the nanoscale are intricate and very intriguing and make up variety of soft and hard tissues. Another feature that is unique to biomaterials and absent in traditional synthetic materials is the use of biomacromolecules like proteins, carbohydrates and lipids. This is evident from all the examples seen so far. These biomaterials are created in the mild aqueous environments unlike the synthetic materials which require high temperature and pressure etc. Proteins are cynosure of any biomaterial and they decide the whole structural conformation of the biomaterial. Proteins both collect and transport raw materials required for material synthesis. Moreover, they self-assemble and co- assemble giving rise to nanoconstructs neatly arranged in organized fashion to result in macroscopic dimensions. They act as templates, growth modifiers, nucleators etc. Thus proteins are an indispensable part of the biomaterials synthesis.

Fabricating Materials – The nature's way!

The unique performance of the natural materials like soft and hard tissues (bones, tendons, mollusk shell, etc) can be attributed to the fashion in which their molecular units are arranged into subunits which in turn are combined and intertwined into larger and larger units. This feature is difficult to imbibe in synthetically manufactured materials. These are called as hierarchical structures. From a detailed analysis of biomaterials, one approach that can be thought of in achieving this end is by utilizing the self assembling nature of proteins.

Molecular self assembly[7]

This is a powerful tool and is characterized by weak non-covalent bonds such as hydrogen bonds, ionic bonds (electrostatic interactions), hydrophobic interactions, van-der-Waal's interactions and water mediated hydrogen bonds. Though these interactions are insignificant in isolation, when combined as a whole they govern the whole structural conformation of the biomolecules and also the interaction of these biomolecules with other molecules. Water mediated hydrogen bonds are especially important since all the biological systems interact with water. All the peptides, proteins and other biomolecules self assemble resulting in formation of well defined structures. Thus by seeing how these peptides self assemble, novel materials can be fabricated by exploiting the self assembly. Nature uses the paradigm of self assembly and has created many materials like collagen, keratin, pearl shell, coral & calcite microlenses, optical waveguides etc. This is new "bottom-up" approach in fabrication of novel materials, also called as "molecular biomimetics".

Molecular Biomimetics in Materials Science[7, 8]

Controlling the binding and assembly of engineered proteins or polypeptides is the essence of "modern biological materials science". Engineered polypeptides combined with other synthetic molecules having specific functions leads gives rise to heterofunctional building blocks for fabricating novel materials which are of tremendous use in electronic and photonics industry. The nanoscale materials or nanostructures that can be created by utilizing the self assembly of Genetically Engineered Proteins for Inorganics (GEPI) can revolutionize nanobiotechnolgy and can have tremendous potential for use in drug delivery systems. Also self assembled monolayers of GEPI which was not possible with normal proteins earlier can result in fabrication of novel material surfaces. So, molecular biomimetics has tremendous potential for combining with traditional material science creating a hybrid material science technology that can have far reaching effects in the future. A schematic of such a hybrid technology is presented.

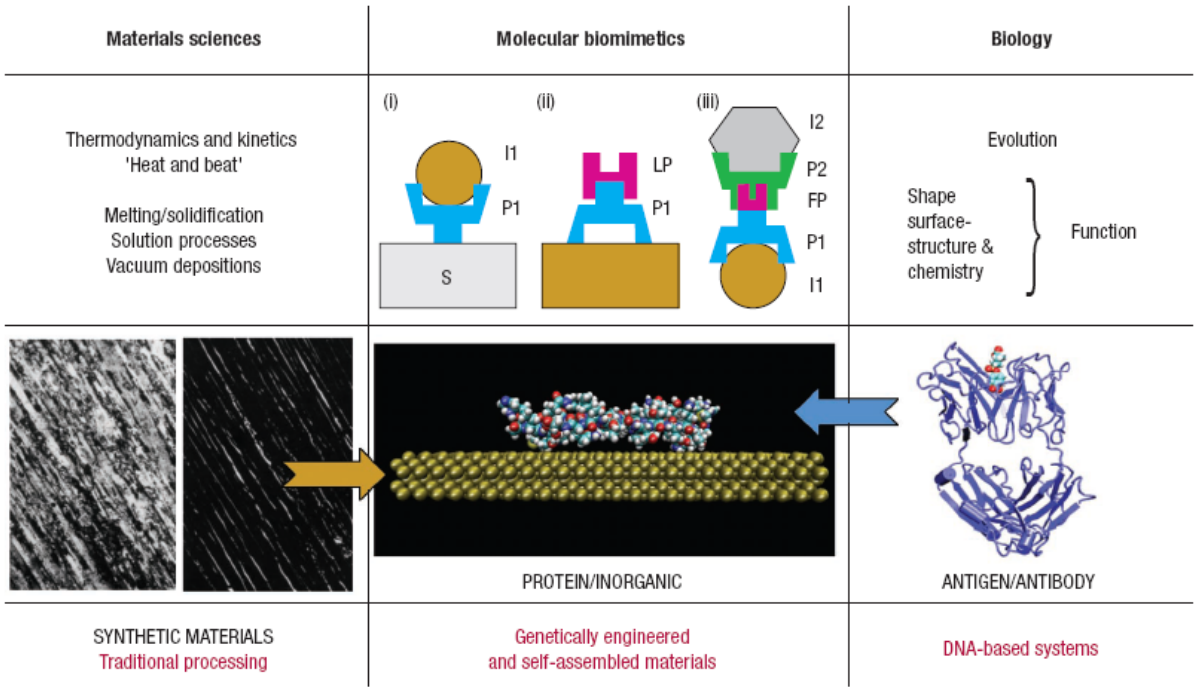

Figure 10. Hybrid technology imbibing the aspects of both traditional materials sciences and biology resulting in what is known as "molecular biomimetics"

In the following section design strategies to produce engineered proteins that bind to inorganics is presented.

Approaches in Designing Inorganic – Binding Proteins[7, 9]

Only certain polypeptides have been identified that specifically bind to inorganics. One such example is the ice – binding proteins. These are synthesized by many fish species, plants and insects. These are also called as antifreeze proteins (AFPs) and have repeating

polypeptide units that bind to ice in the internal fluids to control particle size and morphology.

One of the methods to design such inorganic – binding proteins is to use computational methods or theoretical molecular approach that is usually followed in designing drugs in the pharmaceutical industry. But this method proves to be very expensive and is impractical.

Another approach is to extract these proteins from living organisms. These could be biomineralizing proteins which are extracted from hard tissues, purified and cloned from their genes. However, this approach is time consuming and difficult. Several such proteins have been extracted by this method. For example, amelogenins in mammalian enamel synthesis, silicatein, in sponge spicular formation, calcite- and aragonite-forming polypeptides in mollusk shells etc.

However there is a major drawback with this approach. Hard tissues have different kinds of proteins each of which plays a crucial role in the biomineralization process. Thus extraction of each kind of protein becomes difficult. For e.g., more than 20 known proteins have been implicated in the synthesis of human enamel, and over 10 polypeptides have been identified in mollusk shells. In addition to this, the versatility of the extracted protein is severely restricted if it is extracted from a hard tissue. These proteins would bind to only those inorganics which they were associated with originally in the hard tissue.

This leaves us seeking for alternative sources of obtaining the inorganic – binding proteins. As mentioned earlier, combinatorial biology paradigm is a viable option. This involves searching for protein having a specific sequence that strongly binds to inorganics of interest from a random library of peptides. This library would, of course have peptides having same number of amino acids, but varying compositions. This is called as Display technology. There are three kinds of display technologies used viz. Phage display (PD), Cell Surface Display (CSD), and ribosome display (RD). The display technologies are useful in characterizing the receptor antibody binding sites, for studying protein – ligand interactions, and for the isolation and evolution of proteins or enzymes exhibiting improved or otherwise altered binding characteristics for their ligands. The phage display technology for selecting inorganic-binding protein applied in electronic industry is shown below:

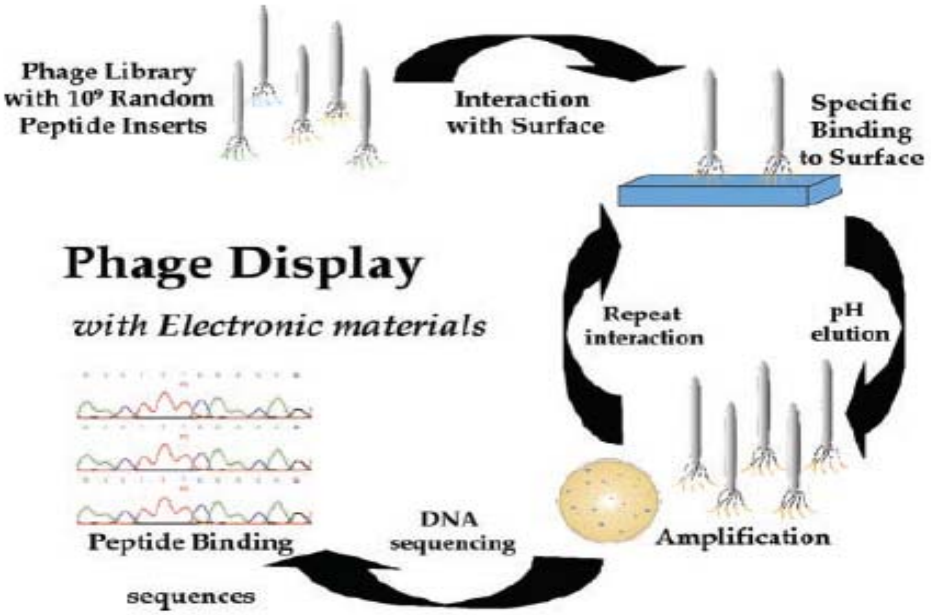

Figure 11. Peptide selection for electronic materials. The $1.9*10^9$ random peptide sequences are exposed to the different crystal substrates; nonspecific peptide interactions are removed with extensive washes. The phages that bind are eluted by lowering the pH and disrupting the surface interaction. The eluted phages are amplified by infecting the *E. coli* ER2537 host, producing enriched populations of phage, displaying peptides that interact with the specific crystal substrate. The amplified phage are isolated, titered, and reexposed to a freshly prepared substrate surface, thereby enriching the phage population with substrate-specific binding phage. This procedure is repeated three to five times to select the phage with the tightest and most specific binding. The DNA of phage that shows specificity is sequenced to determine the peptide-binding sequence.

The details for other display technologies can be found elsewhere[9]

The search for such inorganic – binding proteins is the key to fabricate novel materials as it is the proteins, binding to specific surface/size/morphology of an inorganic compound, that promote the assembly of the intricate, hybrid structures comprising of proteins themselves, inorganic compounds, and in some cases functional polymers. Such short peptides are also called as genetically engineered polypeptides for inorganics (GEPIs).

What makes the protein binding to inorganics so special? - "Understanding the protein-inorganic interactions" [7, 9]

This question can be answered by detailed understanding of the chemical specificity and the physical specificity of the protein – inorganic interactions. The chemical specificity generally refers to the adsorption selectivity. There are two kinds of adsorption possible viz. physisorption and chemisorption. While the former refers to low-to-moderate energy adsorption that is reversible and usually occurs for small molecules, the latter refers to moderate-to-high adsorption energy and posses specific surface selectivity. This kind of

analysis, which works well for describing interactions like thiol-terminated molecules with noble metal surfaces or silanes with metal oxide surfaces, need not necessarily explain the observed specificity of protein binding with inorganics or target substrate.
This is because the protein which has a spatial arrangement of amino acids will surely interact with the surface in a much complex manner. These interactions would depend on the nature of amino acids, various interactions among the amino acids and also side chain interactions. Varied chemical and physical traits of the amino acid sequence would be a governing factor. There are 20 generally occurring amino acids. This makes it difficult to guess as to what kind of right combinations of the amino acid sequence that would give the required selectivity and bonding. Nevertheless, as new inorganic-binding sequences are reported in literature, a better understanding of the characteristics of the inorganic-binding polypeptides could be achieved by statistical analyses.

Characteristics of certain inorganic-binding polypeptides:

In silver binders, the position and the enrichment of proline residues (an amino acids) along with presence of polar, hydrophobic, and hydroxyl-containing small amino acids seems to be the governing factor. Whereas gold binding polypeptides are characterized by the hydrophobicity and polar nature of the small amino acids but the presence of proline is not observed. Also none of the gold polypeptides contain the amino acid cysteine which is characteristic for its binding through thiol linkage seen in the self assembled monolayer formation. The Pt and Pd binders are characterized by hydrophobic and contained aliphatic and hydroxyl side chain amino acids. These are generated by using disulfide heptapeptide constrained library unlike gold and silver binders. These binders are enriched in serine (S), threonine (T), and proline (P) residues.
It is surprising that cysteine (C) and histidine (H) which are usually expected to bind to metals are not observed in noble metal binders.

Thus from the aforementioned examples, it can be concluded that it is difficult to discern as to what kind of amino acid sequence characterizes the observed specificity.

Physical Specificity:
However as far as the protein binding to surface of substrate is concerned, a detailed knowledge of molecular architecture would provide invaluable insights to understand the specificity of polypeptide on the surface. Also atomic structure of the inorganic materials needs to be known. Techniques such as X-ray crystallography, NMR spectroscopy could provide this information.

Now there is one way to circumvent the problem of haziness in understanding of the protein-inorganic binding characteristics. This could be done by molecular modeling.

Molecular Modeling to the Rescue!

As was mentioned in preceding section, the specificity of polypeptide binding to an inorganic is dependant on its molecular structure and also the atomic structure of the inorganic material. Thus structural information of the protein or polypeptide is imperative

from the point of view of molecular recognition process. Now that there are no generalized rules as to why certain amino acid sequence should work well for certain cases and not for others, molecular modeling can be sought as a viable option of circumventing this problem. Molecular dynamics and simulated annealing approaches could help in getting the averaged lowest energy structure of as many GEPIs as possible. These structures could yield information about the orientation and binding energetics at specific interfaces with other materials (inorganics or any other). Thus peptides can be "ranked" depending on the interfacial interaction energies and a general mapping of required specificity to inorganics and the preferred side chains, their alignment etc can be done. This when fed to the display library can complement the experimental observations and result in better understanding of the inorganic-protein interactions.

GEPI – The vanguard of "Modern Material Science"[7, 9]

The potential utility of GEPIs can be categorized as linkers for nanoparticle immobilization, functional molecules that assemble on substrates and heterofunctional linkers joining two or more peptides along with many inorganic nanoparticles. This can be schematically represented as follows:

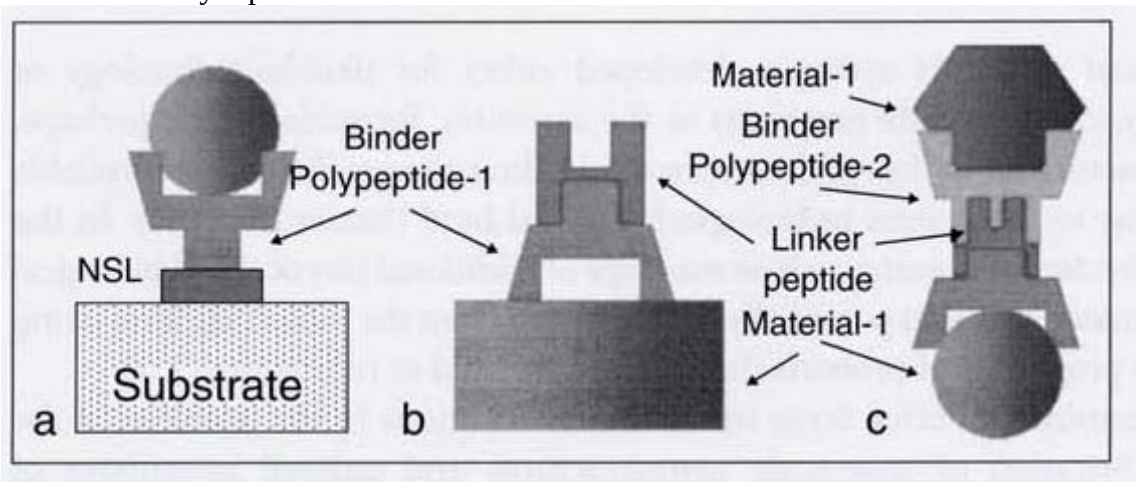

Figure 12. Showing the potential utility of GEPIs a) nanoparticle immobilization b) self assembly over specific substrates c) multiple linking

Use of GEPIs ensures control at the lowest dimension scale possible as these can be selected and engineered at the molecular level. Such proteins can be used as linkers or "molecular erector" (see schematic) linking nanoparticles, functional molecules and other nanostructures on molecular templates. Also the self assembling nature (already mentioned) can be put to use in creating hierarchical structures. Thus these properties make it most useful as heterofunctional building blocks in molecular electronics and photonics. These molecular erector sets have tremendous potential for nanotechnological and nanobiotechnological applications. Apart from materials sciences these find use in Drug delivery systems, tissue engineering etc.

Certain Specific Applications of GEPIs[7, 9]

The potential of GEPI for use in novel materials fabrication has already been mentioned. The following sections elaborate on this aspect detailing the various applications of GEPI and reiterate as to how biology plays a crucial role in fabrication of novel materials.

Creating Monolayers by Self Assembly of Inorganic – binding polypeptides:

Self assembled monolayers are molecular architectures grafted on surfaces. These have specific properties of surface coatings, chemical functionality, corrosion resistance, etc. Monolayers grown on evaporated thin gold films are well known. These are usually alkanethiolate monolayers. The basic idea of creating monolayers is that, if a molecular surface is created then its functions can help in growing other materials over it. Several such monolayers have been reported. Generally, placing of biomolecules directly onto metal surfaces results in protein denaturing. Hence earlier the proteins were placed over traditional self assembled monolayers formed by silane and thiol linkers. Now there have been reports that genetically engineered proteins can chemically recognize a particular surface and assemble over it with a long range order [1].

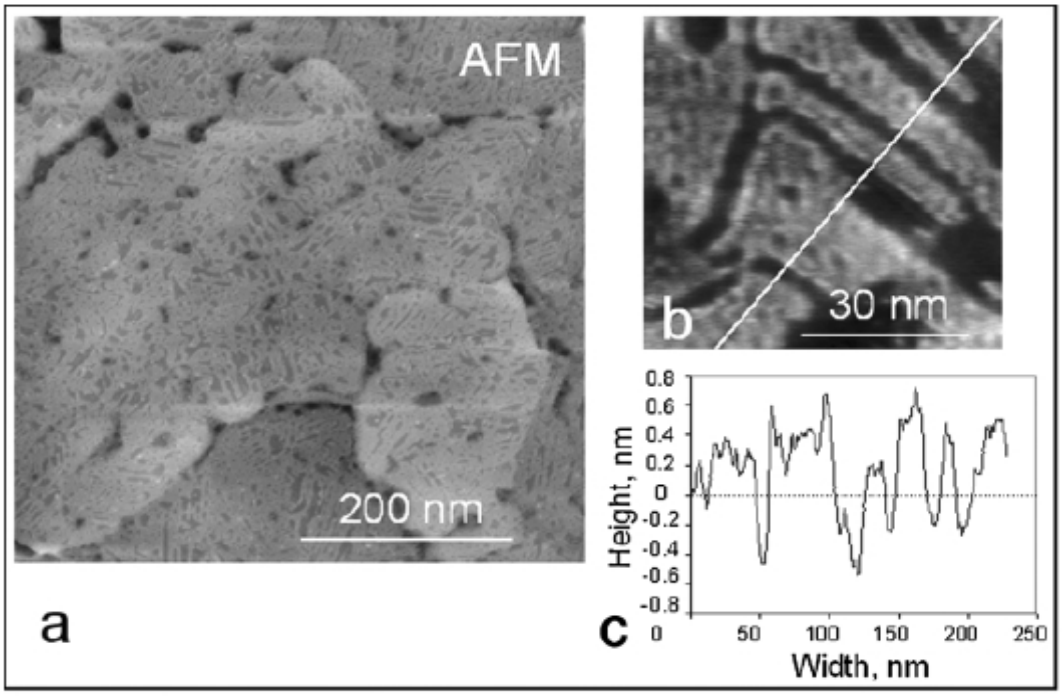

Figure 13. Self-assembled three-repeat GBP-1 on Au(111) (AFM image)

One such example is the monolayer of gold binding protein on Au (111) surface. From the diagram it is observed that the domain boundaries make 60 and 120 angles which would be possible only if six fold lattice symmetry of Au (111) is recognized by the GBP. Thus it is clear from this example that GEPI monolayer can serve as functional linkages over which other specific materials (inorganics, functional polymers etc) could

be added to create novel materials having hierarchical structures giving them fantastic properties.

Nanoparticle assembly governed by Engineered Peptides[7, 9]

Now that GEPI can form SAMs on substrate surface, the resulting molecular structure can be used to immobilize nanoparicles in two and three dimensions. By doing this one can create nanostructures which could be building blocks for future novel materials and devices. Generally speaking creation of nanostructures involves numerous challenges like maintaining uniformity in size, controlling minerology, surface structures, chemistry and also their spatial distribution. These are easily overcome by using GEPI as they specifically bind to materials based on their chemical and physical property and hence control the morphology and uniformity of the materials formed. If GEPIs can bind to different inorganics, then heterostructures can be created.
The best example of GEPI immobilizing a nanoparticle is that of creation of quantum dot. Traditionally quantum dots are created by vacuum technology (like MBE etc) involving high temperature and low pressures in toxic environments.
Synthetic molecules like thiols, lipids, and biological molecules like polypeptides, amino acids etc can be used to couple inorganic particles. Assembly to novel materials is made possible because of the recognition and self assembling properties of these molecules. However there is one major drawback in using the synthetic molecules like thiols etc as these are nonspecific. For e.g. thiols bind to gold particles as well as silver particles, and citrate ions bind to almost all noble metals. Thus, GEPIs which maintain specificity in binding to inorganic particles have a great potential in creating nanostructures by assembly of nanopartilces. Furthermore, these GEPIs can be combined with other functional biomolecules to produce heterobiofunctional molecular units.

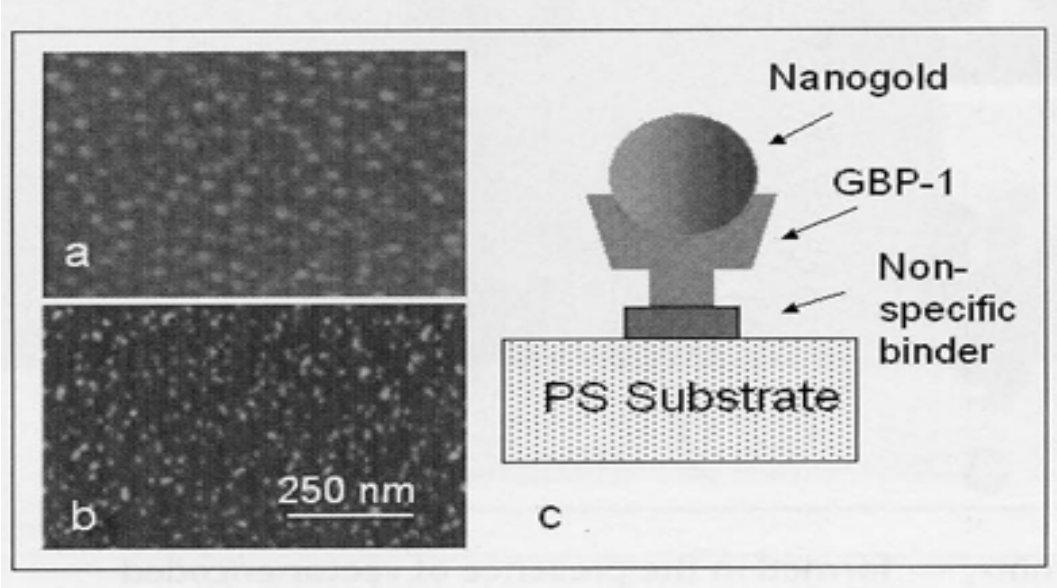

Figure 14. AFM image of GaInAs dots assembled on GaAs substrate a) MBE b) assembly of gold nanoparticles over mica sheets via GBP-1

Altering the material surface by Molecular Self-Assembly[10]

Materials are generally coated to alter their texture, color and their responsiveness to particular environment. For e.g. Zn coatings are put on iron plates to prevent rusting in highly humid atmosphere. Conventional coatings are simply applied by painting or electroplating. However, these coatings suffer from the problem of erosion as these are usually in ten and hundred micron size ranges and this makes poor compatibility of the interface at the molecular level.

In this regard, molecular self assembly of proteins can provide a great help. New technologies that have been developed like micro contact printing technology help in utilizing the self assembly of proteins to create material coatings that are complementary at the molecular scale. The general procedure in such a technology involves imbibing or functionalizing a given surface with different molecules (like peptides etc) using variety of methods like covalent coupling, dip-pen lithography, coordination chemistry etc.

An AFM tip dipped into protein solution can be used to transfer the proteins onto a substrate surface. In other words, the proteins and polypeptides can be printed onto the surface. Then, these polypeptides self assemble into a monolayer giving rise to certain ordered/ hierarchical structures that have specific desired properties. The polypeptides are infact bound to certain inorganic compounds, sometimes to certain functional molecules or functional polymers to obtain a desired property at the surface. Also these peptides can be used as ink for inkjet printers as shown in the diagram:

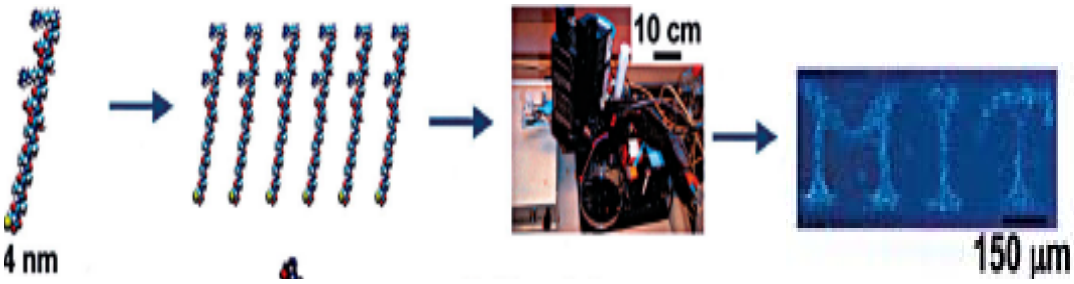

Figure 15. Surface coating nanopeptide. This consists of three segments viz. functional segment, which interacts with other proteins, a linker segment that sets the distance from the surface and an anchor segment for covalent attachment to the surface.

Creating nanowires for electronic industry using peptides[10]

Self assembling peptides can serves as templates for metallization. Metal particles can be introduced onto peptides (that specifically bind to metal particles) and the peptide can be arranged in the form of wires. When the organic framework is removed what is left behind is a conducting wire immobilized on a surface. Such nanowires have been created by coating lipid tubules with gold and silicon nanocrystals[11]. Such nanowires have been used as coatings in a numerous industrial applications too.

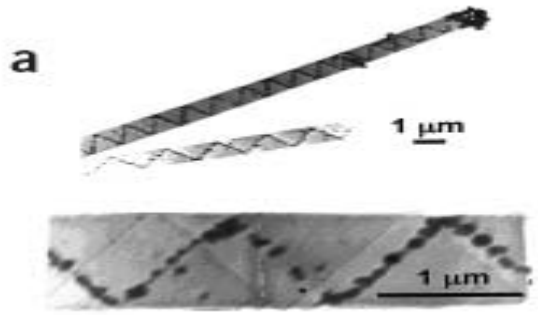

Figure 16. Lipid tubule coated wire.

Conclusions

It worth reiterating the fact that time and again nature has inspired and motivated man to great inventions. Influence of nature in man's endeavors is ubiquitous and renders unimaginable prospects in fabricating novel materials, thereby aiding man's quest to foray into the unknown. As far as materials science is concerned there are numerous examples as to how nature has played a crucial role. Many new materials have come up by mere mimicking of biomaterials. Micro patterned single crystals and photonics inspired by sponge spicules have tremendous potential for use in communication technology[12]. Advent to nanotechnology and subsequent technological advances in electron microscopy, scanning probe microscopy (AFM etc.) have realized the dream of molecular biomimetics which was largely elusive a few years back. The semiconductor and communication industry faces an enormous challenge in creating smaller and smaller features by top-down approach. This problem can be circumvented by bottom-up approach by use of GEPIs which is the cynosure of molecular biomimetics. This way biology can provide invaluable inputs for creation of novel materials.

The future studies will, in fact, focus on development of GEPIs and expanding the library further. However, it is not to be forgotten that the field of molecular biomimetics is still at the stage of infancy. Self assembly of biological molecules can revolutionize materials sciences and can promise materials, devices, and technologies beyond our current imagination.

References

[1] Phillip, B., "Shark Skin and other solutions", News and Views, *Nature* **400**, (1999)

[2] Mehmet, S., Hanson, F., Sopp, Kalpana, S., Katti, and George, M., Biomimetics: Nanomechanical Design of Materials Through Biology, *15th ASCE Engineering Mechanics Conference* June 2-5, 2002, Columbia University, New York, NY

[3] Oliver, W. C. & Pharr, G. M. Pharr "Nanoindentation," *J. Mater. Res*., **4 (6)** 1564 (1992)

[4] Antonio, G. C., Alejandro, B. R, "Self-organisation of nacre in the shells of Pterioida (Bivalvia: Mollusca)", *Biomaterials* **26**, 1071–1079 (2005)
[5] Fong, H., Sarikaya, M., White, S. N. & Snead, M. L. "Nanomechanical Properties Profiles Across DEJ of Human Incisor Tooth," *J. Mater. Sci. & Eng.C*, 7, 119-128 (2000)
[6] Sarikaya, M., Tamerler, C., Schulten, K., and Baneyx, F., "Molecular biomimetics: nanotechnology through biology" *Nature Materials* **2**, 577-586, (2003)
[7] Sarikaya, M., Tamerler, C., Schwartz, Baneyx, F, "Materials Assembly and formation using engineering polypeptides" *Ann. Rev. Mater. Res.*, **34**, 373-408, (2004)
[8] Brad Newsome, "Molecular Substrates for Nanobiotechnology" NNIN REU Site: Center for Nanotechnology, University of Washington, (2004) *NNIN REU Research Accomplishments*
[9] Sarikaya, M., "Nanofabrication towards biomedical Applications", Wiley-VCH Verlag GmbH & Co. KGaA, (2005)
[10] Zhang, S., "Fabrication of novel biomaterials through molecular self assembly", *Nature Biotechnology*, **21**, 1171-1179, (2003)
[11] Lvov ,Y.M,.et al., "Imaging Nanoscale patterns on biologically derived microstructure", *Langmuir*, **16**, 5932-5935 (2000)
[12] Aizenberg, J., Tkachenko, A., Weiner, S., Addiadi, L., & Hendler, G., "Calcitic microlenses as a part of photoreceptor system in brittle stars", *Nature* **412**, 819-822, (2001)

Additional References:

[1] William, L.M., David, J.M., "Molecular scale Biomimicry", *Nature biotechnology, News and Views*, **20**, 30-31, (2002)
[2] Tadashi, M, Toshifumi, S., "Molecular Mechanism of Magnet Formation in Bacteria", *Journal of Biosciences and Bioengineering*, **90**, 1-13, (2000)
[3] www.nasa.com
[4] www.sciam.com