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Using Nature´s concepts to synthesize materials – an example of biomimetic chemistry

by

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Introduction

Everyone with a qualified training in chemistry is aware of the fact that Nature is a master of preparative chemistry. This applies to chemistry both on the molecular level and on the supramolecular level. Organic chemists, who usually work on the molecular level, know that Nature has a lot to teach them. As early as 1917 the British chemist Sir Robert Robinson introduced the term biomimetic synthesis for biologically inspired preparative organic chemistry[1]. Also more recent organic chemists like E.J. Corey and Ronald Breslow have used the terms biomimetic synthesis and biomimetic chemistry in their work[2]. These terms are also appropriate within biochemistry. An obvious example is the design, synthesis and use of artificial enzymes. These may even have certain technical advantages, such as better thermal stability or broader pH window than the natural enzyme which they are supposed to mimic[2].

The term biomimetic chemistry is highly relevant also to supramolecular chemistry. Then we are moving away from pure organic chemistry or biochemistry into what is normally referred to as materials chemistry. Everybody knows that Nature has an amazing talent to build complicated structures with a high degree of precision and with a remarkable reproducibility. Materials chemists have a lot to learn from Nature in this respect, a fact that is today widely recognized in the scientific community. The interest in mimicking Nature has come much later in the supramolecular field than in the molecular field and the delay is mainly due to the analytical challenges involved in deciphering the biological structures and synthesis routes involved in many of the interesting materials that we have around us. Analysis of molecular events may not always be easy but is still much more straight-forward that analysis of supramol-

ecular events. It is only during the last decades than there are tools available that can give information about many of the interesting structures detailed enough to be of practical value for the chemist involved in supramolecular synthesis of biomimetic nature.

In this paper the concept *supramolecular biomimetic chemistry* will be illustrated by three examples, which are all important and subject to large current research activities. The examples are very different in character and in practical use, however, which has been the author's intention. The choice of topics has been made with the aim to illustrate the broad scope of the topic and also the versatility of Nature when it comes to preparative supramolecular chemistry. However, before discussing the first example, a short background to the field will be given. What do we expect from supramolecular biomimetic chemistry? Why is there today such a strong scientific interest in this field?

Background

What is the main incentive for trying to copy how Nature builds materials, i.e. supramolecular biomimetic chemistry? There are a number of options:

1. *Environmental aspects.* Environmental considerations are currently a very important driving force for research and development in chemistry and this relates to both academic and industrial activities[3]. Yet, environmental aspects are not a very important driving force for biomimetic chemistry. With the term 'biomimetic synthesis' we mean that the synthesis procedure is bioinspired. Still, it does not necessarily mean that we use natural materials in the synthesis. We may do so but we can also use synthetic building blocks to make materials in a bioinspired way. Thus, the sustainability aspect need not be important for the biomimetic approach.
2. *Toxicological aspects*. Will materials made according to Nature's approach be less toxic? One may intuitively think so because it is generally perceived as gentle to living organisms. However, Nature can also produce extremely toxic compounds. The Botulinum toxin, which is a protein produced by the bacterium *Clostridium botulinum* is the most acutely lethal toxin known,

orders of magnitude more toxic that the most potent of the war gases produced in the 20th century[4]. It has been estimated that 4 kg of the toxin would be enough to kill the entire population on earth. There is a plethora of other toxic compounds in Nature, although fortunately without the extreme potency that the toxin from *Clostridium botulinum* exhibits. Toxins in different kinds of mushrooms are well-known examples. Thus, one may conclude that doing the synthesis according to Nature's principles does not automatically lead to materials and synthesis byproducts of lower toxicity than what the non-bioinspired methods do.

3. *Economic aspects*. Is the bioinspired approach likely to be less expensive than the normal synthetic route? Will supramolecular biomimetic chemistry result in cheaper materials in the future? The answer is: probably not. Nature frequently uses complicated approaches and biomimetic routes are often quite advanced from a preparative point of view. Complicated synthesis procedures tend to result in relatively expensive materials.
4. *Performance*. Yes, that is the key point! The way Nature makes materials usually leads to products with outstanding properties. This will be illustrated by the three examples following this section. It is in the search of very high quality materials that scientists believe that following Nature's path is beneficial.

Example 1: Ordered mesoporous materials

Mesoporous materials are solids with pores in the range 2–50 nm. Materials with pores having a diameter below 2 nm are classified as 'microporous', and the term 'macroporous' is used for materials with pore diameters above 50 nm. There is a considerable current interest in mesoporous materials and in particular in materials with sizes in the lower part of the interval, typically 2–15 nm. Scientists see a broad range of different uses for such materials, ranging from very technical ones such as catalysis to life science related applications such as delivery systems for drugs and other active substances. (Silica, the most common mesoporous material, is basically the same as sand and regarded as totally safe to humans and has an established use as carrier for orally administrated drugs.)

Nature makes a variety of porous inorganic materials with pore size, pore shape and distance between the pores controlled in a very strict manner, so-called ordered porous materials. A marvelous example – among many – is the shell of diatoms. Diatoms are a group of algae sharing the unique feature to be enclosed in a cell wall made of silica. This inorganic wall can take many different forms, as the four images shown in Figure 1 indicate, but characteristic for them all is that the wall is porous and the pores are highly ordered. It is likely that the pores are there to let nutrients in and degradation products out from the cell.

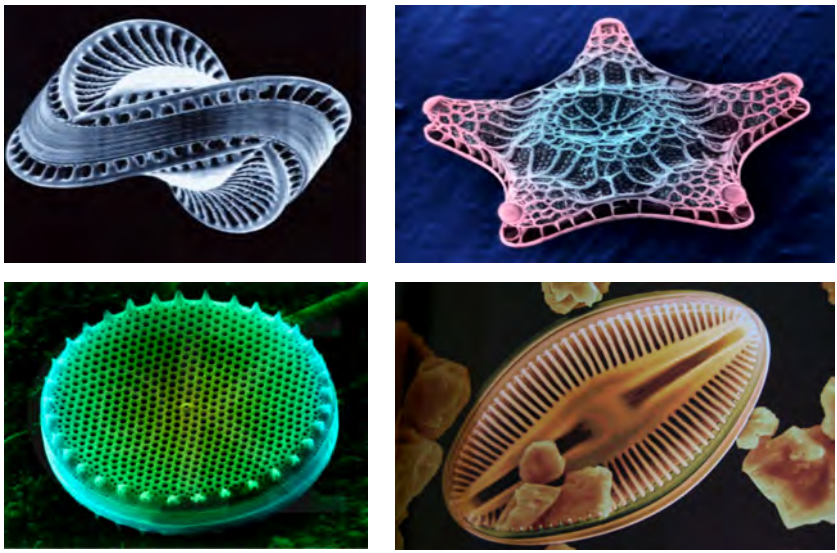

Figure 1. Scanning electron microscopy images of four different diatom shells. The colours are added in order to help the eye distinguish the object from the background but the shapes are the real ones. The objects are typically 20–100 μm in size.

The porous diatom shells are created by an advanced multistep procedure. A somewhat simplified description is the following. First, a well-controlled network is created by self-assembly of water soluble biomolecules. Soluble silicates naturally present in both see water and fresh water will be adsorbed to the network where they polymerize and eventually form three-dimensional silica. This results in a composite material

composed of an organic template made of thin threads with silica as filler material in between. In a subsequent step the organic material is spontaneously removed either by leaching or by some kind of chemical degradation. The pores that appear then originate from the previous template threads.

Around 1990 scientists in Japan and in the US started to use a bioinspired route for making ordered mesoporous materials, first with pores in the size range 2–5 nm, later somewhat larger [5–7]. An organic template was first created, but synthetic molecules with a strong tendency to self-assemble were used instead of the biomolecules that diatoms (and many other water-living organisms) use. The self-assembling molecules are amphiphilic and are usually referred to as surfactants in the technical literature. Figure 2 shows a schematic of a surfactant molecule. It also depicts some of the self-assembled structures that surfactants spontaneously form in water if the conditions are right.

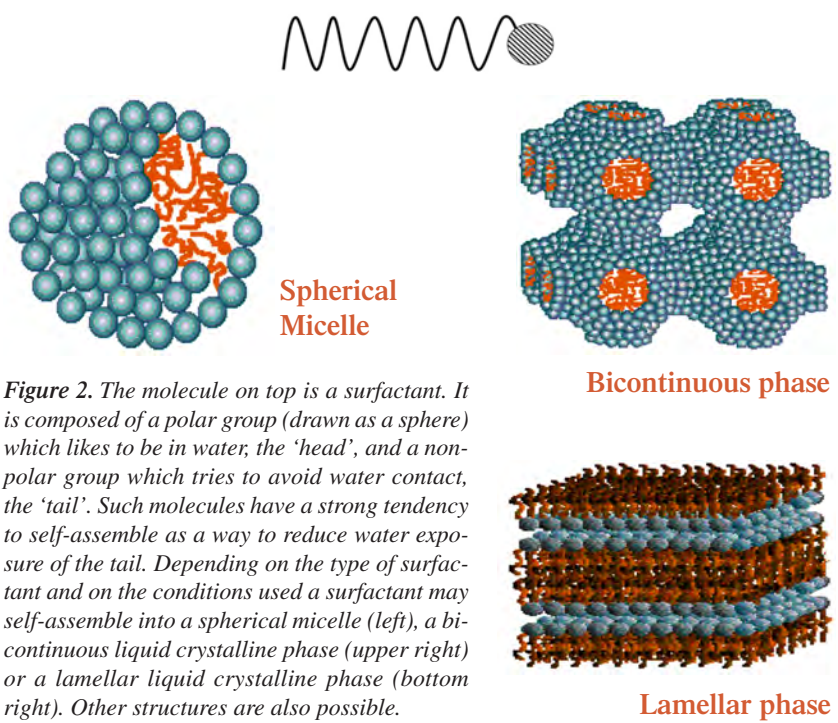

Figure 2. *The molecule on top is a surfactant. It is composed of a polar group (drawn as a sphere) which likes to be in water, the 'head', and a non-polar group which tries to avoid water contact, the 'tail'. Such molecules have a strong tendency to self-assemble as a way to reduce water exposure of the tail. Depending on the type of surfactant and on the conditions used a surfactant may self-assemble into a spherical micelle (left), a bicontinuous liquid crystalline phase (upper right) or a lamellar liquid crystalline phase (bottom right). Other structures are also possible.*

The set of sequences used in the artificial route is in principle the same as Nature uses when preparing porous materials with a high degree of order; the step to remove the template, though, is made differently. Instead of leaching or allowing chemical degradation, the template is simply burned off, a process called calcination. Depending on the choice of surfactant used, mesoporous materials with different arrangement of the pores can be obtained. Figure 3 illustrates the process for preparing mesoporous silica with hexagonal structure.

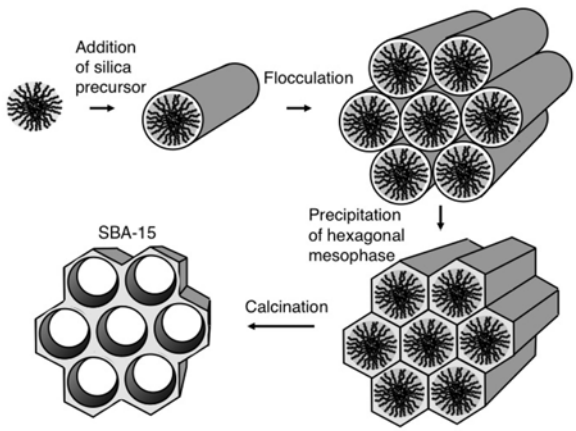

Figure 3.
Formation of mesoporous silica with hexagonal packing of the pores.
A surfactant is used as template and self-assembles into micelles. On addition of the silica precursor, usually tetraethylorthosilicate, the micelles become elongated. The cylindrical micelles form a liquid crystalline phase with hexagonal geometry. Reduction of pH from highly alkaline towards neutral leads to formation of a silica layer around the micelles; each cylindrical micelle is now surrounded by a solid shell. Calcination leads to the mesoporous material. (From Ref. 8, with permission.)

The process illustrated in Figure 3 can lead to mesoporous materials with very good control of pore size and of the arrangement of the pores. Many other materials than silica can be prepared by this bioinspired principle. Mesoporous alumina and titania are examples of other common materials that have been produced by the same route. Figure 4 shows a transmission electron microscopy image of mesoporous silica with hexagonal packing. As can be seen, the pores are ordered in a way that resembles the ordering of some of the diatom shells shown in Figure 1, although the dimensions are different.

As mentioned above, there are many potential uses for the man-made mesoporous materials. One interesting application is the use of the pores as hosts for enzymes. Since the pore dimension can be tailored with a great deal of precision by the choice of surfactant, materials with pores that fit a specific enzyme can be prepared. An enzyme that is inserted into the pores will remain active, yet be protected for instance from protease-catalyzed degradation. After use the enzyme loaded materials can be removed from a reaction batch by filtration or centrifugation and then reused. Figure 5 illustrates loading of a mesoporous material with an enzyme.

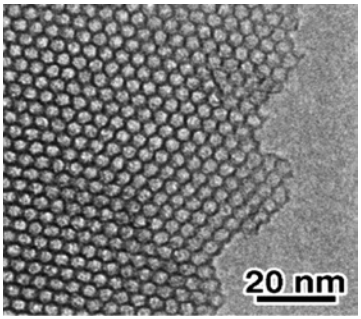

Figure 4. Transmission electron microscopy image of mesoporous silica with hexagonal packing of the pores. As the scale bar indicates, each pore is around 4 nm in diameter.

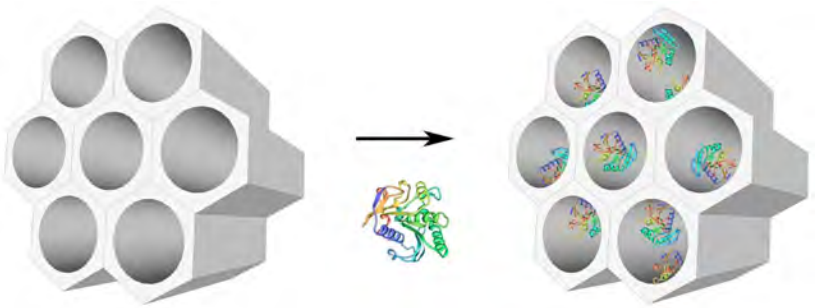

Figure 5. Loading of a mesoporous material with hexagonal packing of the pores with an enzyme. (From Ref. 8, with permission.)

Example 2: The spider thread

The thin threads that a spider produces so rapidly are truly amazing. The best spider silks are claimed to have a tensile strength higher than steel, counted on a weight basis. The threads, which can be as thin as 2–3 nm, are made of protein chains which are produced in the silk gland. This has been known for quite some time but what has remained a mystery

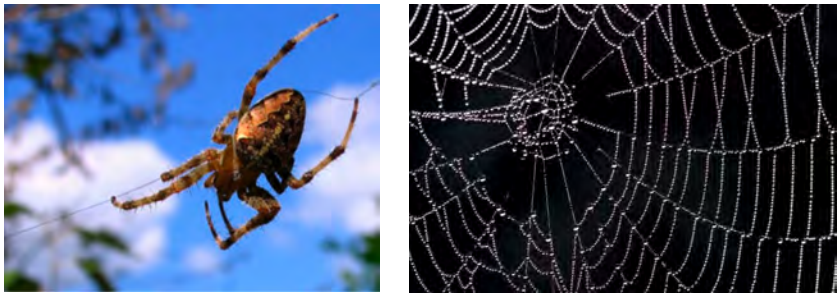

Figure 6. A spider thread (left) and the ready-made web (right).

until now is how the long protein molecules that eventually make up a thread can be housed so close together in the gland without clumping. It now seems that scientists in Germany have unlocked this secret. The explanation combines advanced physical chemistry and biochemistry. Plans are that this knowledge will lay the foundation for a synthetic production of fiber based on the same principle – a truly bioinspired development.

Polymer chemists have known since long back that the packing of the individual polymer chains that make up a fiber is crucial. The chains must be aligned properly so that when a load is applied all the chains will contribute in preventing the fiber to crack. If the polymer molecules are not properly aligned there is a risk that one molecule breaks first, then a second one, then a third one, and so on. The tensile strength of such a fiber is very low because of the lack of cooperativity.

The importance of having all polymer chains aligned in parallel is well established and the remarkable strength of polyamide fibers is believed to be due to this phenomenon. In polyamide fibers the polymer molecules are held together by intermolecular hydrogen bonds. This principle was recognized already by Wallace Carothers, the inventor of the Nylon fiber in the 1930's. It was later found that aromatic polyamide fibers had even higher tensile strength than Nylon, which is based on aliphatic polyamides, because the aromatic moieties in adjacent chains will attract each other, a so-called π–π interaction. Thus, aromatic polyamide fibers, out of which Kevlar is the best known example, have superior strength because of very good alignment of the individual

polyamide chains, which are hold together by a combination of hydrogen bonds between amide linkages and π–π interactions between aromatic rings. This packing is illustrated in Figure 7. Fibers based on polymers that do not possess amide bonds or aromatic rings have much lower tensile strength.

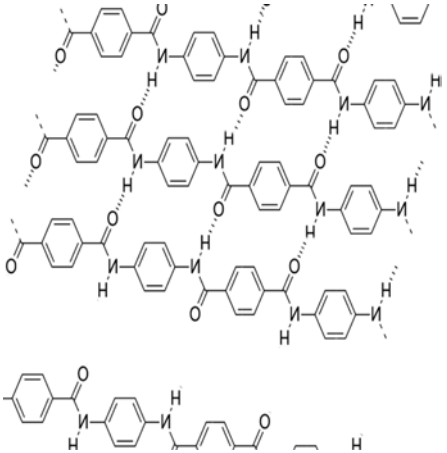

Figure 7. The aromatic polyamide fiber called Kevlar has very high tensile strength because the chains are aligned in parallel and held together by a combination of hydrogen bonds between amide linkages and π–π interactions between aromatic rings.

Against this background it was anticipated that also the protein chains that build up the spider threads were arranged in parallel so that the individual protein molecules would act in concert when a load is applied on the thin thread. But how is this possible considering that the spider must be able to eject the thread from the gland with very limited mechanical force? Thousands of protein molecules held together with hydrogen bonds and maybe also other attractive interaction would give a highly viscous solution which would be very difficult for the spider to spit out. The answer to this intriguing question seems to be that within the gland there is no or little attraction between the individual protein chains. It is only at the outlet of the gland, when the protein solution is mixed with a second stream of water that the proteins become 'sticky'. There seem to be two parameters that are vital in this respect: a change of pH from neutral to acidic and a dilution of the electrolyte concentration, i.e. a transition from high to low salt concentration.

A protein has two ends. On one side the protein ends with a carboxyl group, on the other with an amino group. These two ends are called C-terminal and N-terminal, respectively. The proteins present in the spider gland are built up in such a way that at neutral pH and high electrolyte concentration there is very little attraction between the end groups. The

ends are not 'sticky'. However, things will change drastically when pH is reduced and the solution is diluted so that the electrolyte concentration becomes low. At low pH the free amino group in the N-terminal end starts to become protonated. At some pH some of the N-terminal groups are cationic and some are non-charged. This leads to hydrogen bond interactions, i.e. the N-terminals become sticky. The net effect is that an N-terminal group from one protein ties to an N-terminal group from another protein. This is a mechanism for spontaneous elongation of the protein molecule. This process continues as long as the spider secrets the protein solution from its gland, which means that there is almost no upper limit to the length of the chain produced.

Also the C-terminal end changes character when it meets the acidic water at the outlet. Inside the gland the electrolyte concentration is very high, which from a physical chemical perspective means that all electrostatic interactions – attractive and repulsive – are virtually eliminated. On dilution with water the electrolyte concentration goes down and electrostatic forces come into play. Also the C-end of the protein now becomes sticky. A C-end from one protein associates with a C-end from a neighboring protein, inducing close packing of the chains. This close packing of parallel chains is augmented by hydrogen bonds between amide linkages, as described above for the synthetic polyamide fibers Nylon and Kevlar. The series of events, involving both the N-terminal end and the C-terminal end of the proteins that are present in the silk gland, is schematically shown in Figure 8 [9, 10].

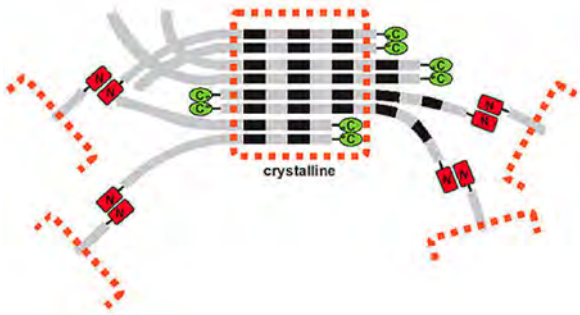

Figure 8. Organization of individual spider gland proteins into bundles. Attraction between C-terminal ends induces tight packing of chains. The packing can be so tight that sections become crystalline. Attraction between N-terminal ends leads to elongation into infinitely long threads. (From Ref. 11.)

In the language of polymer chemists the proteins in the gland are 'pre-polymers' and the mechanism for generation of the thread is that of a 'two-component reactive system'. The first component is the neutral solution of the protein chains in the gland at high electrolyte concentration and the second component is the acidic water of low electrolyte concentration. When the two components meet at the exit of the gland an extremely well ordered and almost infinitely long bundle of proteins will be formed. This is the spider thread and from a materials chemist's point of view its synthesis route is fascinating.

There is some resemblance between how the spider thread is produced and the technical process for making viscose (rayon) fiber. In that process cellulose xanthate is kept in solution in strong alkali and then extruded into a bath of concentrated sulfuric acid. The acid removes the xanthate groups and regenerates cellulose which without the xanthate substituents becomes insoluble in water. This process is a crude one and lacks the sophistication of the thread production by the spider. Materials chemists have much to learn from Nature in making strong fibers, and large research programs today are directed towards this type of bioinspired polymer chemistry.

Example 3: The blue mussel adhesive

Everyone who owns a small boat is aware of the amazing ability of many see-living organisms to adhere to the hull of the boat. After a summer in the water the bottom of the boat may be so covered by various objects that the underlying plastic, or wood, is no longer visible. There are a number of fouling organisms, and they can be of either animal or plant origin. Some of the more common species are barnacles, algae, mussels, clams, hydroids and tube worms. They all adhere to the hull of the boat by different mechanisms. In recent years scientists have been particularly interested in the mechanism behind the way the blue mussel adheres to surfaces, and there are currently great expectations that if the way the blue mussel binds to surfaces can be revealed, we will be able to produce a glue, and maybe also a paint, that is water based and still useful on wet surfaces.

The blue mussel's ability to stick to all kinds of surfaces – stone, iron, wood, plastics, etc. – is astounding. As shown in Figure 9, the mussel

Figure 9. *A mussel connected to a stone by a number of thin threads.*

connects itself to the surface via long threads that are secreted from the foot of the mussel. The threads are made of proteins, just as the spider thread discussed in the previous section. What is fascinating about the mussel adhesive is that it works on such a broad range of surfaces, that a strong joint is obtained without any mechanical help and that it is efficient under wet conditions.

The ability of the blue mussel to stick to wet surfaces in spite of the fact that the glue itself is water-based opens fascinating perspectives. As will be discussed below, the adhesive is in reality a two component system, just as was the case for the spider thread. One of the target applications for a water-based glue to be used on wet surfaces is the so-called surgical glue, i.e. a glue that can be used instead of stiches to repair the skin. Surgical glues are actually starting to appear on the market, but present state technology is not up to the standard of the mussel's glue. A true blue mussel glue, obtained from the natural source, would probably not be an option for such an application, however, because of the risk of immune defense stimulation by foreign proteins. It is the principle that the mussels use that is of interest. Figure 10 shows a cartoon of the use of a two-component surgical glue.

Scientists have known for some time that the blue mussel glue contains one specific structure normally not present in glues which may be the explanation to the performance of the mussel adhesive. That struc-

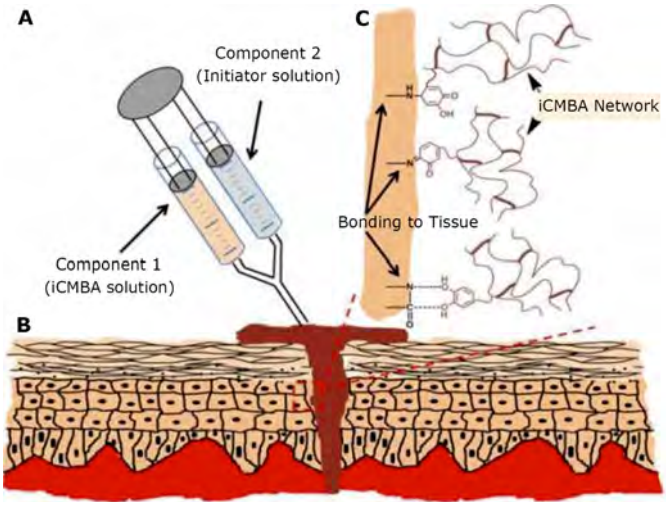

Figure 10. *A two-component surgical glue. (From Ref. 12.)*

tural element is the amino acid L-di-*ortho*-hydroxyphenylalanine. This is a fascinating observation because that specific amino acid is of considerable medical interest. L-di-*ortho*-hydroxyphenylalanine, or L-DOPA for short, is a precursor of the neurotransmitter serotonin. Patients suffering from Parkinson's disease have too low serotonin levels and the discovery that administration of L-DOPA to such patients is a way to combat the disease rendered Arvid Carlsson the Nobel Prize in Physiology or Medicine in 1990. Figure 11 shows the structure of L-DOPA.

It must be a pure coincidence that the characteristic structure of the blue mussel adhesive is the same as the active agent against Parkinson's disease. In fact, it is not the entire amino acid that is believed to be vital for the adhesion. It seems that the binding structure is the catechol part of the molecule, i.e. the aromatic ring with two neighboring hydroxyl groups, which constitutes the left part of the molecule in Figure 11.

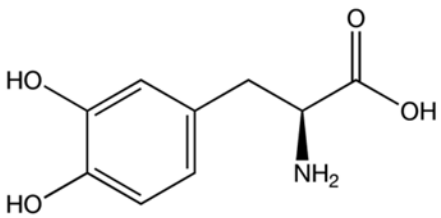

Figure 11. *The structure of L-DOPA, a medicine against Parkinson's disease.*

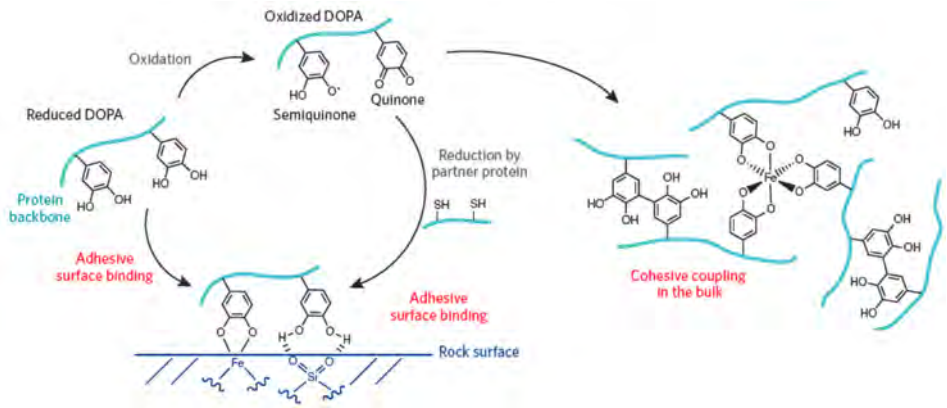

Figure 12. Proposed mechanism for the adhesion of the blue mussel. (From Ref. 14, with permission.)

Figure 12 illustrates the current view on how the blue mussel adhesive works and the arrows indicate that it is a stepwise process. First a protein rich in DOPA is excreted from the mussel foot onto the surface that the mussel wants to attach to. Some segments from the large protein molecule bind to the surface. In a second step oxidation of other catechol units into o-quinones occurs. This oxidation is known to occur as a free radical process. It may be catalyzed by an enzyme that the mussel also excretes and it may also be catalyzed by trivalent ferric ions, Fe^{3+}, which are abundant in sea water (and which are, of course, very abundant at the surface of iron objects). The oxidized structures are reactive. A carbon-carbon bond is rapidly formed between two aromatic rings and this process leads to crosslinking when the aromatic rings are situated on different protein strands. The crosslinking generates a three-dimensional network, which, in turn, is responsible for the high tensile strength of the threads[13, 14].

There are obvious similarities between how the spider thread and the blue mussel thread (the byssus thread) are formed. Both are based on proteins excreted from the living organism. Both proteins contain specific moieties that are responsible for cohesion and adhesion: the spider protein carries C- and N-terminal ends, which, when the conditions are

right, give rise to strong attraction with other protein chains; the blue mussel protein contains catechol groups, some of which attach to surfaces and some of which give rise to crosslinking.

The knowledge about the principle by which the blue mussel adhesive works has emerged during the last decades and has triggered a lot of interest in the materials science community. Water-based glues and paints that give strong adhesion to wet surfaces is a very attractive concept from an industrial perspective. It is generally agreed that some kind of two-component system will be needed to achieve a workable solution and the route that the blue mussel uses with catechol moieties that both bind to surfaces and undergo oxidation and subsequent crosslinking is very attractive. Polymer chemists are currently pursuing this approach, attaching catechol groups to different types of polymers. This is indeed a very good example of bioinspired materials design.

Summary

Bioinspired materials science is today attracting a lot of attention. The routes that Nature uses for preparation of materials are often ingenious and the chemistry involved can be quite advanced. They can teach us synthesis strategies that can be useful for a variety of applications. In this brief account of biomimetic chemistry applied to materials the following three areas have – somewhat arbitrarily – been chosen as examples:

- Ordered mesoporous materials
- The spider thread
- The blue mussel adhesive

Together these areas illustrate the broad scope of this type of supramolecular biomimetic chemistry.

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Krister Holmberg, professor in Surface Chemistry, was acting chairman of the academy in year 2014.