Biomimetic Materials and Transport Systems

by Reinhard Lipowsky, MPI of Colloids and Interfaces

Summary

Biological cells have amazing material properties which are based on their macromolecular and supramolecular (or colloidal) building blocks. During the last decade, much progress has been made in order to identify these different components and their interactions. The next step will consist in the integration of these different components into higher level systems. Some examples for such biomimetic systems are: supramolecular architectures containing membranes and polymers; polymer networks as models for the cytosceleton; biomimetic mineralization; transport via molecular motors; biomimetic recognition and signal transduction.

These research activities are interdisciplinary and involve the combined efforts of physicists, chemists, molecular cell biologists and bioengineers. The biomimetic model systems which will arise from these efforts have many potential applications in bioengineering, pharmacology and medicine.

There are various places in Europe which already pursue related research on a local scale. What is still missing, however, is an initiative which combines these efforts on the European scale. Such an initiative is necessary in order to compete with the science community in the US where several 'BioX'–centers are currently being set up.

1 Introduction: The Goals of Biomimetics

Biological cells are built up from macromolecules and supramolecular assemblies (or colloids) in water. Both the intracellular architectures and the interactions between cells are based on soft and flexible nanostructures which are multifunctional and highly intelligent materials. In addition, cells are able to build up hard materials in the form of biominerals and to control their morphology on the nanometer scale.

All cells contain similar macromolecules but different types of cells can survive in very different environments. Indeed, cells can live in boiling water, in strong acids, at the low temperatures of the antarctic, and under the enormous pressures of the deep sea. These different adaptations, which are based upon different supramolecular architectures, demonstrate the wide range of possible material properties of these architectures.

Another intriguing aspect of biological cells is that they contain a large variety of very efficient transport systems. The latter systems are based on

motor proteins or molecular motors, which transform chemical energy into mechanical work. These nanoengines are responsible for the transmembrane transport of ions and macromolecules, for the regulated adhesion and fusion of membranes, for the intracellular transport of vesicles and organelles, for cell division and cell locomotion.

Research on biomimetic materials and transport systems has four goals:

(i) *Understanding* the material and transport properties of biological cells and tissues. Since these latter systems are very complex, such an understanding can only arise if one focusses on certain aspects of these structures. Thus, one is led to the

(ii) *Construction of model systems* to which one can apply the experimental and theoretical methods of physics and chemistry. The coevolution of experiment and theory is a necessary condition in order to transform vague ideas into useful knowledge.

(iii) The knowledge obtained from the biomimetic model systems can then be used in order to develop *new types of designed materials* which are biocompatible and which have defined physical, chemical or biological characteristics.

(iv) *Applications* of these biomimetic materials in bioengineering, pharmacology and medicine.

2 State of the Art

The structural organization within biological cells has many levels. As one goes 'bottom–up', i. e., from small to large structures, the first three levels are:
(1) the level of macromolecules (or copolymers) which have a backbone of monomers connected by covalent bonds;
(2) the level of supramolecular assemblies of many similar molecules, the formation of which is governed by noncovalent forces such as the hydrophilic or hydrophobic interactions with water;
(3) the level of complex architectures which contain different types of building blocks and/or different types of assemblies.
These different levels will be discussed in the following subsections.

2.1 Recent Developments: Macromolecules

The macromolecular components of the cell (proteins, nucleic acids, polysaccharides, lipids) are known for a long time. All of these macromolecules are copolymers which are built up from a certain number of different monomers or building blocks. In addition, the three–dimensional conformation of these

biopolymers is determined, to a large extent, by the water solubility or hydrophilicity of these building blocks. From the viewpoint of material science, one simple and useful property of biopolymers is that all members of the same molecular species have the same length. In contrast, synthetic polymers always exhibit some length distribution or polydispersity.

In the last decade, new synthetic methods have been developed which allow the construction of hybrid molecules consisting of biopolymers coupled to synthetic ones. In this way, one can design new biomimetic polymers which combine the properties of both the natural and the synthetic component. In addition, new experimental procedures, so–called 'single molecule methods', have been established by which one can determine the physical properties of single macromolecules.

On the one hand, one can label these molecules by a fluorescent probe and then track their motion both in solution and bound to a sheet–like membrane or rod–like filament. On the other hand, one can firmly anchor them at a solid surface and then probe individual macromolecules by various experimental methods. Using optical methods, for instance, one can directly observe the thermally–excited transitions between two different conformations of RNA strands. One may also use the tip of an atomic force microscope in order to pull at a single copolymer which is anchored to a solid surface. In this way, one can determine the functional relationship between the force and the linear extension of the molecule. These force–extension curves are rather reproducible and, thus, reflect the forces which determine the three–dimensional conformation of the polymer.

A third area where single molecule methods have led to much insight is the active movement of molecular motors along filaments. For some cytoskeletal motors, see Figure 1, it has been possible to resolve single motor steps which

Figure 1: Cartoon of two molecular motors, in this case two kinesins, bound to a microtubule filament. The microtubule has a thickness of 25 nanometers. Each kinesin walks along the filament by making steps of 8 nanometer.

are of the order of 10 nanometers. In the cell, these motors are responsible for the directed transport of vesicles and organelles over tens of micrometers or even centimeters.

2.2 Recent Developments: Supramolecular Assemblies

If one looks into a typical animal or plant cell, one sees two types of supramolecular assemblies which determine the spatial organization of the cell over a wide range of length scales: (i) Compartments bounded by sheet–like membranes and (ii) Networks of rod–like filaments. These two types of structures are displayed in Figure 2 and Figure 3. Both structures are assembled on the molecular scale, i. e., on the scale of a few nanometers, but are able to organize much larger spatial regions up to tens of micrometers!

Biomembranes are highly flexible and, thus, can easily adapt their shape to external perturbations. In spite of this flexibility, they are rather robust and keep their structural integrity even under strong deformations. This *combination of stability and flexibility* is a consequence of their internal fluidity. This was first realized in the context of lipid bilayers which are the simplest biomimetic membranes.

Fluid membranes have unusual elastic properties which determine their morphology. These properties are now understood in a quantitative way using (i) mesoscopic models which describe the membranes as elastic sheets and

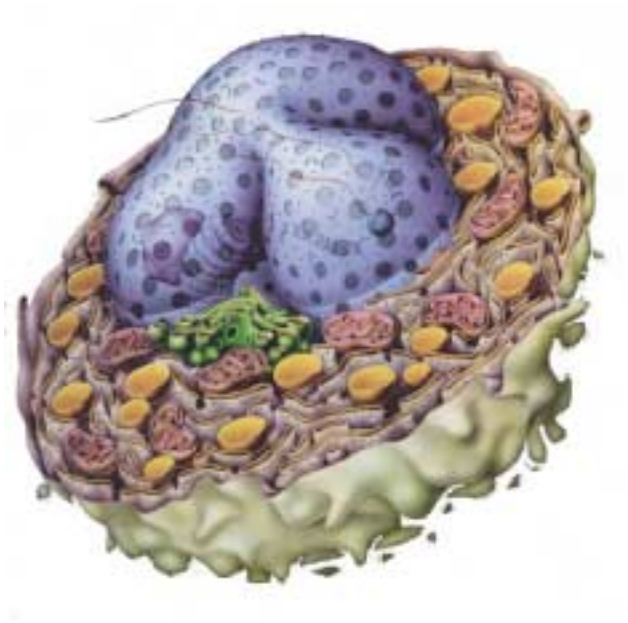

Figure 2: The spatial organization of a typical animal cell is based on membrane–bounded compartments. The diameter of the cell is of the order of 20 micrometers.

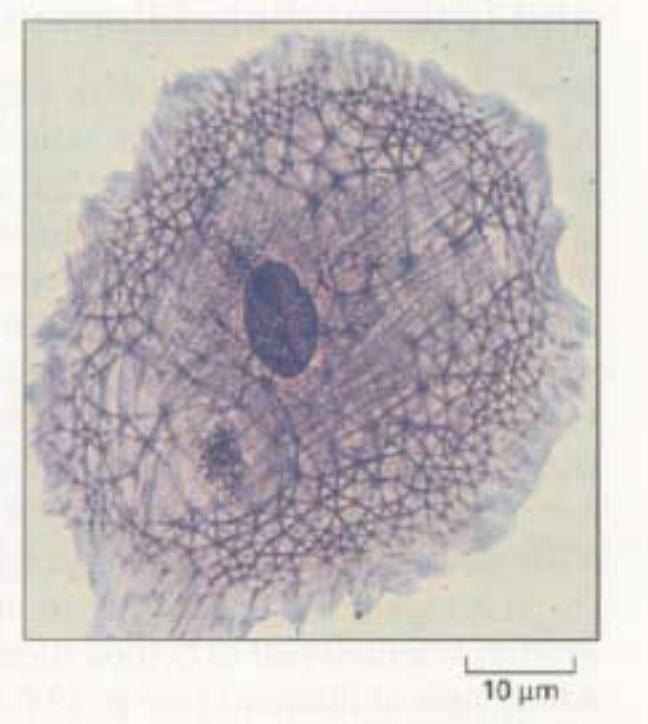

Figure 3: Cytoskeleton of a large animal cell. This network of filaments is primarily built up from microtubules, which emanate from the center, and from actin filaments at the periphery of the cell.

(ii) models with molecular resolution which can be studied by computer simulations and can be used to relate the elastic parameters with the molecular interactions.

The stability of lipid bilayers makes it possible to isolate them and to manipulate them in various ways: one can suck them into micropipettes, attach them to other surfaces, and grap them with optical tweezers generated by focussed laser beams. These membranes are even self–healing: if one pinches small holes into them, these holes close again spontaneously.

In the last couple of years, new types of biomimetic membranes have been constructed. One example is provided by bilayers of amphiphilic diblock copolymers. Both artificial and hybrid copolymers have been found to undergo spontaneous bilayer formation. The underlying mechanism is the same as for lipids. Another type of biomimetic membrane is provided by polyelectrolyte multilayers. These multilayers are constructed in a layer–by–layer fashion where one alternatively adds negatively and positively charged polyelectrolytes onto solid templates. These multilayers form dense polymer networks which are reminiscent of the filament networks close to the outer plasma membrane of cells. These new types of biomimetic membranes have a large potential for applications as drug delivery systems.

In addition to the soft and flexible assemblies discussed so far, biomimetic

research has also produced hard materials in the form of biomimetic minerals. These minerals are typically built up from rather simple building blocks such as hydroxyl apatite or calcium carbonate. However, biological cells are able to control the detailed morphology of these minerals. As a result, the same building block such as hydroxyl apatite leads to rather different materials such as teeth and bone. It has been recently shown that such processes can be mimicked, to a certain extent, by growing the minerals in the presence of organic additives such as synthetic copolymers in aqueous solution.

2.3 Recent Developments: Complex Architectures

The next level of complexity consists in supramolecular architectures which incorporate different types of building blocks and/or which contain different types of supramolecular assemblies.

Several attempts have been made to built up complex architectures consisting of rodlike filaments within membrane compartments. It has been demonstrated that both actin filaments and microtubules can be polymerized within lipid vesicles. This can be directly observed in the light microscope since the growing filaments induce morphological transformations of the vesicles. In the case of actin, two different procedures have been realized. One of these procedures led to shells which are reminiscent of the cytoskeleton cortex, the other to protrusions which resemble microvilli.

The layer–by–layer construction of polyelectrolyte multilayers makes it possible to incorporate layers of different species of polyelectrolytes and/or of other types of colloids. In this way, one can construct complex multilayers which represent multifunctional interfaces.

Complex architectures may also be constructed using chemically structured surfaces. Indeed, the multifunctional interfaces of biomembranes arise from the lateral organization of these membranes into specialized membrane domains. New techniques have been developed which make it possible to chemically structure solid surface on the nanometer scale. These structured surfaces can be used to built up supramolecular architectures with a defined lateral organization.

3 Future Perspectives

There are many challenges for biomimetics on the supramolecular (or colloidal) scale. One important and general goal is to gain *improved control* over the structure formation and over the morphology of the supramolecular assemblies. In particular, one would like to construct biomimetic systems which undergo reversible transformations and, thus, can be switched forward and backward between different types of assemblies or between different types

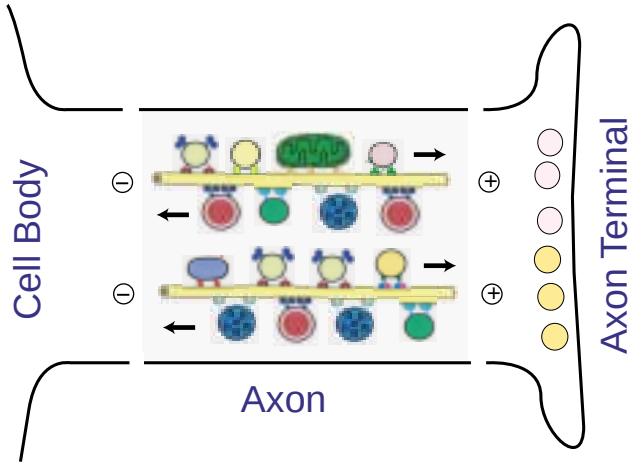

Figure 4: All microtubule filaments within an axon have the same orientation. Cytoskeletal motors (indicated by small 'feet') are responsible for the directed transport of various particles along these filaments

of morphologies. Likewise, one would like to incorporate such reorganizable structures as subsystems into larger architectures.

Future research projects which will lead to such an improved control include:

(i) Construction of spatial patterns of filaments. This could be achieved, e.g., by anchorage to chemically structured substrates;
(ii) Controlled assembly and *disassembly* of filaments;
(iii) Lateral organization of membranes into well–defined domains;
(iv) Biomimetic recognition systems or biosensors based on immobilized membranes;
(iv) Controlled formation of membrane buds mimicking the cellular processes of endocytosis and exocytosis;
(v) Model systems for the fusion of membranes;
(vi) Minerals with defined morphologies on the nanometer scale;
(vii) Biomimetic transport systems based on filaments and molecular motors in open and closed compartments. The latter type of systems is inspired by the directed transport as found in the axons of nerve cells, see Figure 4.

A more ambitious long–term goal would be to combine biomimetic sensors and motors in order to get model systems for biological signal transduction. Thus, one may envisage autonomous nanorobots which receive physical or chemical signals from their environment and respond to this information with some 'action'.

In the very long run, research on biomimetic systems could lead to 'construction kits' by which one can create artificial cells. At present, this vision must still be regarded as science fiction.