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REVIEW

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Biomolecular rods and tubes in nanotechnology

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Abstract Biomolecules are vitally important elements in nanoscale science and also in future nanotechnology. Their shape and their chemical and physical functionality can give them a big advantage over inorganic and organic substances. While this becomes most obvious in proteins and peptides, with their complicated, but easily controlled chemistry, other biomolecular substances such as DNA, lipids and carbohydrates can also be important. In this review, the emphasis is on one-dimensional molecules and on molecules that self-assemble into linear structures, and on their potential applications. An important aspect is that biomolecules can act as templates, i.e. their shape and chemical properties can be employed to arrange inorganic substances – such as metals or metal compounds – on the nanometre scale. In particular, rod- and tube-like nanostructures can show physical properties that are different from those of the bulk material, and thus these structures are likely to be a basis for new technology.

Introduction

When one, two and three dimensions in a sample of a material are restricted to the nanometre scale, the resulting object is a nanolayer or nanoslab (two-dimensional, 2D), a nanorod or nanotube (1D), and a nanodot (0D), respectively; and it can play a role as a building block in nanoscale science and nanotechnology. While nanoscale objects such as pigment particles have been produced for many decades, and in some cases have been known for centuries (José-Yacamán et al. 1996), a relatively new aspect is the controlled fabrication and integration into devices – both with nanometre precision – as proposed by Feynman in 1959 (Feynman 1960). An example for such a device would be an artificial nanoscale motor, in analogy to biological motor proteins such as kinesin, myosin and ATPase. Building such nanodevices, i.e. nanoscale structures whose function is based on nanoscale effects, as nature does it, namely from atoms and small molecules, is now becoming possible for relatively simple systems, e.g. mechanical DNA "tweezers" (Yurke et al. 2000) or carbon nanotube transistors (Keren et al. 2003). It is still impossible to create any desired nanostructure (or arrangement of atoms or "molecule"), even if it is stable, since the tools are not yet developed or are too slow. If this goal is reached, the enormous diversity of the structures will allow the building of a nanodevice with various, even multiple, functions and physical properties, which can be integrated into a micro- or macro-scale device.

Over the past decades, new instrumental developments, such as the scanning probe methods, have allowed for synthesis and/or analysis on ever decreasing length scales. Surface science and its analysis methods have played a key role: with the help of atom-by-atom assembly, nano-objects can be created, usually on well-defined, atomically smooth surfaces. It is obvious that functional devices are more complex and thus call for a more elegant strategy, i.e. for "parallel fabrication" such as in organic synthesis and biochemistry. In this way, chemistry is entering the field of nanoscale science, which was originally physics-oriented; a good example is the use of metal nanoclusters, in most cases synthesized by wet chemistry. The devices created in this way should not only be demonstrated merely to work; a future "nanotechnology" in the strict sense requires that they are available in large quantities. This criterion is currently fulfilled for the (mechanical) tip of the atomic force microscope (AFM) and the (magnetic) layers in reading heads, for example.

Today, the driving force to create new and more complex nanostructures is on the one hand technology, e.g. a scaling-down of computing, memory storage and sensor devices; on the other hand, nano-objects exhibit physical properties that are different from those of the bulk material, such as quantization of electrical conductance, (dis)appearance of ferromagnetic coupling, or

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changes in light absorption and emission. In this respect, inorganic structures (e.g. metals, metal compounds and ionic crystals) and organic/inorganic composites are often more attractive than organic molecules. Although biochemical synthesis provides a huge toolbox for molecular assembly, more complex structures such as the composites require new strategies. At this point, biomolecules enter the stage: even very simple life forms are built on a whole range of complex functional nanostructures, of which 1D structures such as microtubuli and actin fibres are of special importance. Hence a future nanotechnology could be built very elegantly on a combination of biology with chemistry and nanoscale physics (Drexler 1981; Martin and Kohli 2002; Niemeyer 2001; Rietman 2001), maybe with the aim of constructing nanodevices from small molecules.

Here 1D (linear) biomolecules (and also some examples of 0D and 2D) are reviewed as building blocks and as templates for (in)organic nanostructures. The importance of this subtopic of nanoscale science and nanotechnology is that these structures can help to tackle some of today's most pressing issues such as placing a nano-object with nanometre precision, and the transition from assembly on surfaces to assembly in three dimensions. Both are required to build the above-mentioned devices, the fundamental test for a "true nanotechnology" (as opposed to mere fabrication of sub-μm structures). A good example for the use of biomolecules is a nanomechanical device for which a biological motor protein was assembled on a surface, and its moving part combined with an artificial structure (Soong et al. 2000). Similar inorganic devices cannot yet be made this small, but electron or ion beams will soon access feature sizes that correspond to a single protein. This should trigger an exciting competition between sequential "top-down" structuring and parallel "bottom-up" self-assembly: the conventional scaling down of devices as known from microelectronics can only work sequentially, i.e. slowly, on the sub-10 nm scale, while molecular recognition and self-assembly build complex molecules and nanostructures very quickly from smaller units, which assemble in a well-defined way.

It is hoped that nanoscale science (inspired by physics and chemistry) will not only be combined with biology (nature), but will also result in a conceptually new "nanobiotechnology" (not covered in this review). In addition to the new physical properties, biological functions and chemical reactions can be also exploited on the nanoscale. For example, an enzymatic reaction or biomolecular recognition can change the physical properties of a single biomolecule (such as size and luminescence), which are measured and yield a signal. On the road to this new technology, the use of different approaches and languages in the various scientific disciplines can be an obstacle. A typical example is the lack of overlap between chemical (molecular) concepts and solid-state physics, such as chemical valency of the atoms vs. physical conductivity of the molecule, or molecular self-assembly vs. biological recognition. In this review, the problem is addressed from a chemical viewpoint, which is anticipated to open up several new perspectives.

Outline

A sample of artificial nanostructures exhibits in most cases a size distribution, i.e. not all objects in a sample are of the same size, while biomolecules (in a purified sample) have identical size, (complex) shape, and chemical groups (see Fig. 1), which will be the focus of **Section 1**. This is especially important for 1D objects, where inhomogeneities in diameter are typical of inorganic structures. The potential for nanotechnology lies in the large variety of their functionality and in the availability of the biomolecules: complex artificial molecules have to be synthesized stepwise, which is often more difficult than the isolation and purification of biomolecules. From a chemist's perspective, the chemistry of proteins appears very attractive: proteins are the prime example of highly complex and extremely variable nanostructures, although they are built on a simple construction principle. In addition, the construction is programmable in DNA, which makes protein synthesis by expression in bacteria or yeasts far simpler than organic synthesis. However, one has to admit that it is still impossible – for chemistry and biochemistry alike – to predict all the properties of a nanosized molecule; despite highly developed modelling procedures, this task is hard for even the chemically simplest nanostructures, clusters with several hundred identical atoms. Note that the functionality and the chemical complexity of biomolecules are ingeniously

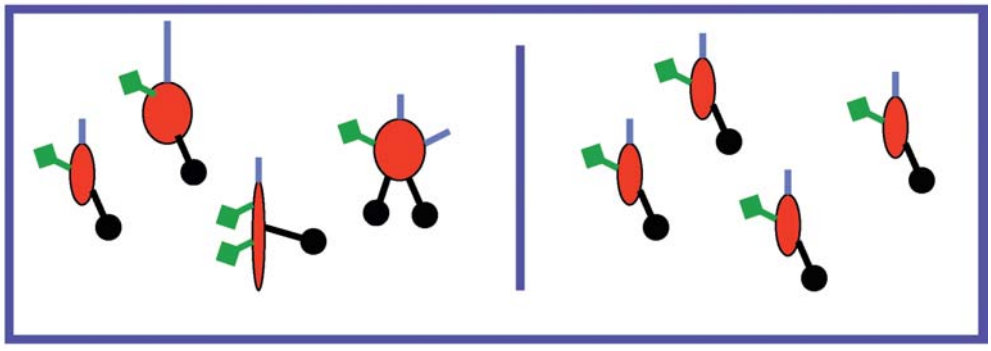

Fig. 1 Typical samples of inorganic or organic nanostructures/particles (*left*) and of biomolecules (*right*). *Each colour* refers to chemical group, and *each shape* to a conformation

Fig. 2 Device with 1D-biostructure (or composite) (*green*). The structure is anchored by antibody linkages (*red*) to contacts (*black*) on a solid surface (*blue*) and could e.g. function as sensor for molecules in a fluid phase or as antenna for electromagnetic waves

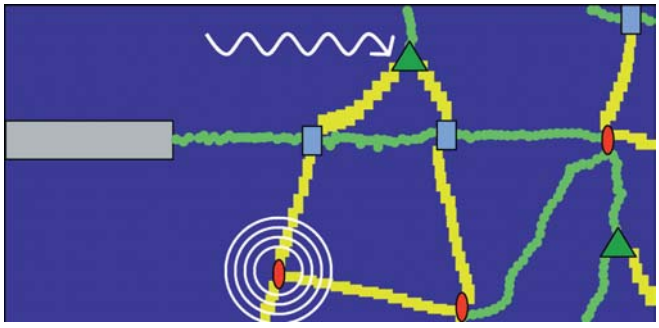

Fig. 3 Highly complex nanodevice, connected at the left to a microstructure. *Each shape* refers to a biomolecule that can either self-assemble or connect to other molecules, *each colour* reflects a special physical property (e.g. electrical conduction) which is cooperative, i.e. depending on the properties of neighbouring molecules. Electromagnetic interactions can reach further; the *wavy arrow* could correspond to excitation by an external light source, the *concentric circles* to a static magnetic field

used by nature in self-assembly to even more complex nano- and microstructures; this method is increasingly used also for building artificial nanostructures. As yet only simple examples for large artificial molecules exist – at least when comparing organic chemistry to nature, which is completely based on self-assembly, as is obvious for viruses. This is of special significance for 1D assemblies, since practically all of them are constructed from a large number of small units. **Section 2** deals with viruses, prime examples for this principle. However, the viruses are in most cases modified with inorganic compounds, for several reasons.

A nano-object can act as a transducer when contacted or addressed in some way, for example, direct chemical sensing by highly selective binding to an analyte, thereby producing a physical signal (see Fig. 2). All physical properties of the object show effects of reduced dimensionality, and all are potentially exploitable: *optical* (e.g. anisotropic and energy-shifted luminescence), *magnetic* (e.g. loss of ferromagnetic coupling), *electronic* (e.g. change of conductivity) and *mechanical* (change of shape) (see Table 1). Note that these properties are rarely unique on their own – they can be found in macroscopic structures or in nonbiological nanostructures – but are nearly always unique in their *combination*. However, properties such as the conduction of electrical current or magnetism are usually associated with inorganic (nano)objects, which is a disadvantage of using biomolecules in nanoscale science. This means that biomolecules are often not interesting as such, but merely as *templates* for other (inorganic and organic) nanostructures. A bio-(in)organic "nanocomposite" is formed, in which the biomolecule determines the arrangement (distribution) of an organic or inorganic substance that is synthesized at the biological surface(s). In this case, mainly the properties of the inorganic and organic nanostructures are searched for. The biomolecules simply provide their shape as a mould or mask for the structures, and they can be removed (and recycled) after the process. **Section 3** provides details on several synthetic methods that take account of these requirements.

The future may also again favour pure organic molecules or biomolecules: a single molecule can – in analogy with composites – exhibit electrical conductivity, an interesting electronic structure, and defined mechanical behaviour. Indeed, recent measurements prove that relatively small molecules (2 nm length), preferably with pi-electron systems, show conductivity and even non-Ohmic behaviour (Reichert et al. 2002). The **Perspectives** section presents some other possibilities to be explored. The ultimate goal can be formulated as the self-assembly-based formation of a 2D (or 3D) network (Eichen et al. 1998) in which each molecule is multifunctional and cooperates with at least its neighbours (Fig. 3). Such a network requires electrical or optical or mechanical contact only at few input and output points. In the case of not only tens, but thousands of molecules, the network can perform virtually any desired task. A first step has been demonstrated recently (nanoscale field effect transistor assembled with recombinant DNA; see Keren et al. 2003).

Table 1 Examples for artificial, biological and composite 1D nanostructures with mechanical, electrical/electronic, magnetic and optical properties

Nanostructures	Biological	Artificial	Composites
Mechanical	Actin, microtubules	Carbon nanotube actuators	DNA "tweezers"
Electrical/electronic	Ion channels	InP nanowires	Peptide nanotubes with Ag nanowires
Magnetic	Magnetosomes	Ni nanowires	Phages with CoPt particles
Optical	Retinal rod cells	CdS nanowires	Microtubules with fluorescent markers

See Lee et al. 2003; Zhou et al. 2001 (biological); Pradhan et al. 1999 (artificial); Yurke et al. 2000; Reches and Gazit 2003; Reiss et al. 2004; Lee et al. 2003 (composites)

Section 1: Biomolecules in nanoscale science

Two big problems in the synthesis of nanostructures are size and shape control. These properties are of utmost importance since they determine to what extent effects of the reduced dimensionality come into play; they also determine presence, intensity and anisotropy of optical, magnetic, mechanical and electronic effects. Size and shape distributions mean that different nano-objects in a sample behave more or less differently – the narrower the distributions, the closer the physical properties of the objects. Near-ideal 2D nanostructures (ultrathin layers) can extend over macroscopic distances; membranes belong to this field of nanoscale science, and especially the bacterial outer membrane proteins (Koebnig et al. 2000) are very valuable for nanoscale science. 1D and 0D shapes are both found for many biomolecules, and they are of special interest: *spheres* (0D structures) since they can exhibit isotropic properties, and highly anisotropic 1D structures such as *rods* ("wires") and *tubes* (Martin and Kohli 2002), since they can provide a connection between nanostructures and the macroscopic world, for instance as electrical contacts (see Fig. 2), and maybe also as antennae for electromagnetic waves. This concept was only recently demonstrated: a carbon nanotube was biochemically coupled to an ssDNA/nucleoprotein filament that was bound to a specific location on a long dsDNA; metallization produced a nanoscale field effect transistor (Keren et al. 2003). For an electrical connection, all 0D nano-objects require a 1D connection. Ideally, one would wish to control the orientation of the 1D object to facilitate making contacts (see Fig. 4; in comparison with Fig. 3 the scenario is more realistic, since errors occur and unwanted byproducts are produced). Note that with current technology such assembly tasks require in nearly all cases a supporting flat substrate and careful control of this surface's chemistry. In this context one has to note that multiple interactions between large molecules (biomolecules of >1 kDa) and flat surfaces are still not well studied on the atomic scale (with respect to which group in which conformation and chemical state interacts with the surface), while interactions between small molecules and flat solid surfaces are a very active field of research (surface science) (Kasemo 1998).

When the template function is invoked, a "nanocomposite" of biomolecule and inorganic (or organic) matter is synthesized. For rods and tubes it becomes possible to arrange the substance in a 1D fashion on the nanoscale (Fig. 5). Here the advantage of biomolecules becomes obvious: Few nanotemplates are available in organic and inorganic chemistry, and their shape, size and chemistry are in most cases neither well defined nor easily varied. Surfaces of biomolecules offer excellent control of arrangement and orientation of covalently linked and adsorbed substances. However, more and better-defined inorganic nanostructures are becoming available, and hence biomolecular templates may not be the sole solution to nano-object synthesis.

Possible uses of nanosized biomolecules or composites are in magnetic recording (when combined with magnetic nanoclusters; Mayes et al. 2003), molecular-scale spintronics (magnetic switches) (Ruben et al. 2003), coupling of nanodevices with optical signals [already shown for virions (Dragnea et al. 2003; Islam et al. 1997)], and molecular-scale electronic switches (Keren et al. 2003). For extreme cases, quantum mechanics plays a huge role, e.g. for single electron transfer, electron tunnelling, and single photon emission. However, quantum effects are not automatically the basis for a nanodevice, for instance, electrical currents can flow without quantum effects in metal wires of down to 2 nm diameter. For possible applications it is important that biomolecules can be very neatly arranged and oriented on a large variety of substrates. Figure 4 shows an as yet not realized scenario where 1D nanostructures bind to microscopic contacts, self-assemble into a long wire, and are chemically modified with extremely high specificity by inorganic and organic substances. This can be the first step towards a multifunctional cross-linked network (see Fig. 3).

Important requirements for nanotechnological applications of biomolecules are that they should be mechanically stable, chemically resistant against solvents or gases, and ideally also stable at elevated temperatures for building up a nanodevice (and in some cases also for operating it). These arguments at first glance prohibit such an application; however, they merely mean that part of the very large family of biomolecules does not qualify. Indeed biomolecules such as DNA, some plant viruses, membrane proteins of archaea, prions and prion-related peptides, and also carbohydrates show a thermal and chemical stability that could be acceptable for building and operating stable devices. Moreover, any potential nanodevice might be prepared under biocompatible conditions; already present-day copper interconnects in computer chips are fabricated electrochemically, i.e. at ambient pressure and temperature. Note that all mentioned molecules, except the carbohydrates, can be modified by recombinant DNA techniques; all of them, including the carbohydrates (Gabius 2000), can carry a code since they contain a sequence of coding elements (base pairs, amino acids or sugars, the basis for the structures in Figs. 1, 2, 3, 4 and 5) – a feature unknown in nonbiological molecules.

In the following, biochemically "active" molecules (e.g. enzymes) will be distinguished from "passive" ones (e.g. membrane-building molecules). This classification is not strict and depends on the desired application: DNA can be addressed as active, especially when coding, replication and recognition are possible; or as passive, when solely the chemical groups are to be used. Artificial nanostructures with passive biomolecules might be applied in a future nanotechnology as functional building blocks (Bashir 2001), while biologically active structures are assumed to be the basis for nanobiotechnology (Niemeyer 2001). On first sight, active molecules appear much more interesting: they can replicate (e.g. for DNA computing; Liu et al. 2000), they can synthesize nanos-

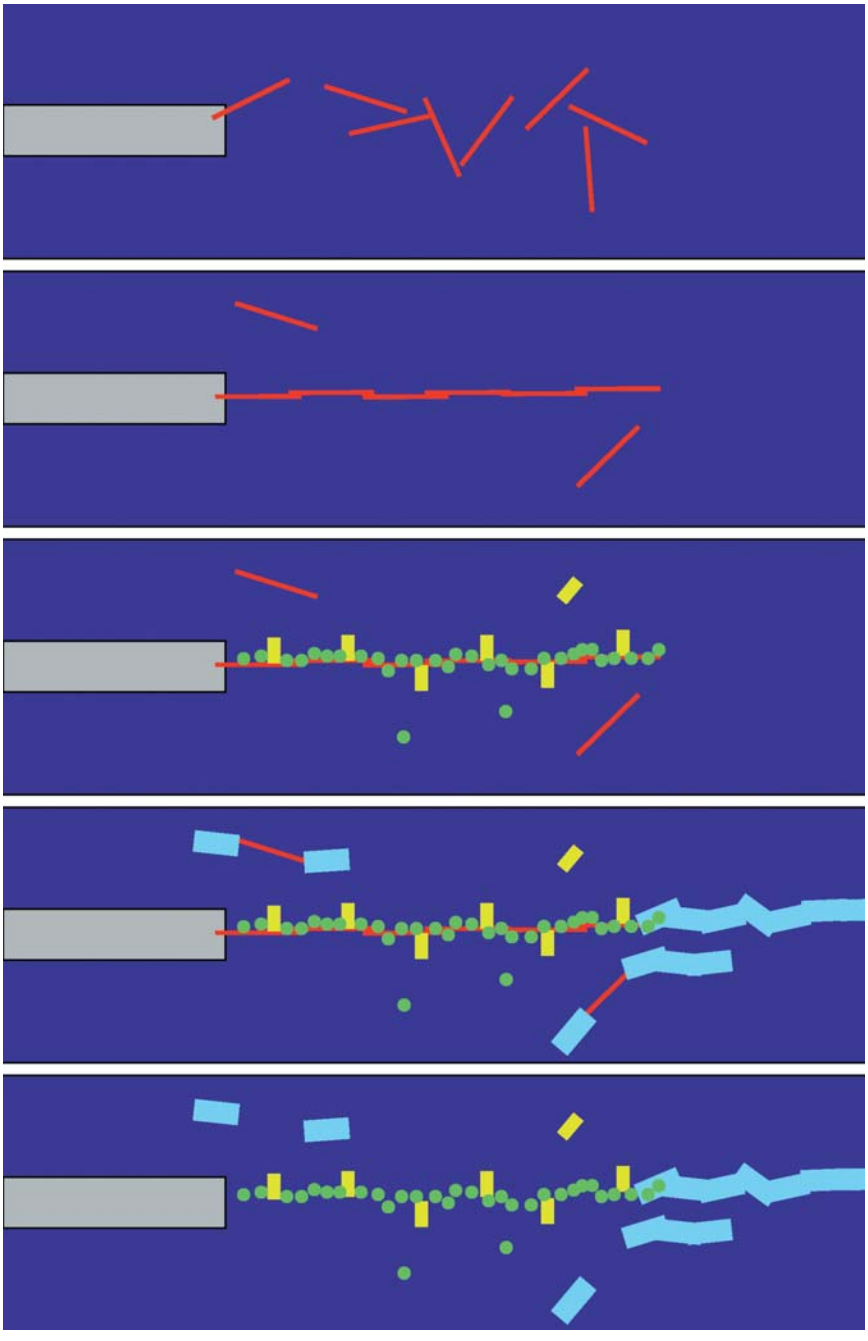

Fig. 4 Assembly of a complex 1D biostructure on a solid substrate; its length can be 1,000 or only 10 nm. Hence the image can be 10 or 0.1 μm wide, referring to assembling a large nanostructure in a given microstructure or a tiny nanostructure in nanoscale connectors. **a** The 1D object (*red*) attaches chemically to a contact (*grey*); **b** self-assembly leads to local ordering (wire); **c** chemical modification with two reagents (organic or inorganic) yields the desired complex functionality; **d** other biomolecules form a second, specifically attached self-assembling wire; **e** disassembly and recycling of the original structure. Some byproducts are immobilized, but are not functional

tructures or molecules and thereby create chemical signals as a basis for sensitive detectors (indirect sensing), and they can work as motors (Lee et al. 2003) for example, ATPase coupled to a nickel nanopropeller (Soong et al. 2000) and DNA-fuelled DNA machines (Yurke et al. 2000). It must be stressed that passive molecules, too, are functional by virtue of their chemical functional groups or their shape, especially when they are (quasi) one-dimensional.

Materials

In the following, several passive biomolecules and biomaterials (Mann 1996) that are used for nanoscale science are presented, especially 1D structures. Note that a complete overview is not attempted; i.e. not all materials and not all publications are cited, and rarely employed structures are not included. By far the most commonly used substance is DNA, for instance as nanowire (Bashir 2001), as template for nanowires (Braun et al. 1998; Harnack et al. 2002; Keren et al. 2003; Park et al. 2002), and for chain-like arrangements of metal (Deng and Mao 2003; Kumar et al. 2001; Nakao et al. 2003b; Warner and

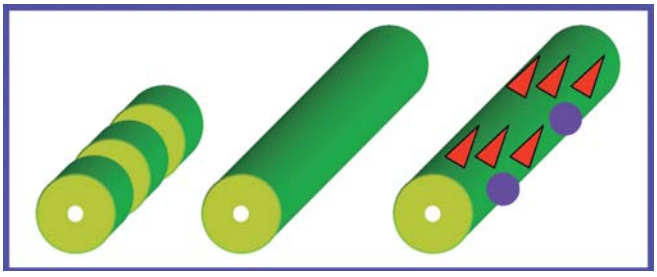

Fig. 5 General 1D biostructure, e.g. a filamentous plant virus. The left tube forms via self-assembly from proteins (here stacks; they may also be helically arranged). The right one is modified (by mutagenesis) to a composite with two different chemical groups or quantum dots (*red triangles* and *purple dots*), offering a unique nanoscale architecture that is only available with biomolecules. The inner channel (*white*) also can be accessible for molecular species or clusters

Hutchison 2003) and semiconductor (Torimoto et al. 1999) clusters. While these clusters were spaced randomly, control of gold cluster spacing on DNA was recently demonstrated (Woehrle et al. 2004). DNA can even be used as template for a wire with the smallest possible diameter, namely linearly arranged ions (Tanaka et al. 2003). Binding fluorescent labels, especially intercalators, is a standard technique in biology – this can be addressed as linear nanoscale arrangement of stacked molecules. More complex geometries are also feasible: DNA plasmids can align semiconductor particles in a circle (Coffer et al. 1996), while "double-crossover" DNA with sticky ends can form 2D crystals (Ding et al. 2004; Winfree et al. 1998). Recombinant techniques ("self-assembly" from a chemical viewpoint) can be used to bind clusters (Park et al. 2002), and maybe even to bind several different clusters in a well-defined sequence – the coding properties of DNA would then be employed to their full extent. Note that RNA, too, can be modified in a similar way, e.g. decorated with gold clusters (Medalia et al. 1999).

Of the proteins, many 0D nanocomposites are known. A heat shock protein can self-assemble into a cage and be filled with iron oxide nanoparticles (Flenniken et al. 2003). The naturally occurring analogue, ferritin, is not only interesting due its cage-like shape, it is also a nanoscale antiferromagnet. It can be demineralized to apoferritin, which in turn can be filled with a whole range of binary compounds, even with ferrimagnetic iron oxide (Mann 1996) and with the technologically relevant CoPt, which shows a high intrinsic magnetic moment (Mayes et al. 2003). The latter example shows that metallization of biotemplates can yield nanoclusters with a narrow size distribution which are otherwise hard to synthesize; moreover, self-assembly into a 2D nanoscale grid was demonstrated, resulting in exactly the structure that is needed for ultimate-density magnetic recording media (highly magnetic bits, combined with the absence of magnetic coupling, translate into bit sizes in the 10 nm range and similar bit-to-bit distances). Other 0D objects, too, can be arranged in 2D. Bacterial S layers are very useful because they form very regular and stable grids; they are used as templates for cluster synthesis and arrangement (Mertig et al. 2001; Shenton et al. 1997; Sleytr et al. 2001).

The natural formation of fibres and tubes from proteins is best known for actin and microtubules (Lee et al. 2003). Short peptides or lipids, too, can assemble into 1D structures, templates for clusters in a wire-like arrangement (Djalali et al. 2002; Scheibel et al. 2003) or for silica layers (Meegan et al. 2004). When tubes form, they may be used as templates for nanowires [peptide tubes (Reches and Gazit 2003), microtubules (Fritzsche et al. 1999) or lipid tubules (Archibald and Mann 1993; Markowitz et al. 1993)]; but usually the exterior surface is coated (e.g. FeOOH on microtubules; Boal et al. 2004). Bolaamphiphiles are not biomolecules, but based on sugars, peptides or nucleobases, they, too, can assemble into 1D nanostructures (Shimizu 2002).

Plant viruses (see Section 2) and phages often exhibit high-aspect ratio 1D structures. They are employed as templates for cluster and wire synthesis (Demir and Stowell 2002; Douglas and Young 1998, 1999; Dujardin et al. 2003; Flynn et al. 2003; Fowler et al. 2001; Fujikawa and Kunitake 2003; Knez et al. 2002, 2003; Mao et al. 2003, 2004; Reiss et al. 2004; Wall 1999). Metal clusters were bound to the tobacco mosaic virus (TMV) as early as 1940 (Kausche 1940; Ruska 1947), and inspired from this and other pioneering work on electron microscopy of biological samples, staining methods with metal clusters were developed. Gold and platinum clusters (Powell et al. 1999) and even gold cluster-tagged antibodies (Bendahmane et al. 1999; Van Regenmortel 1999) are well established as markers – from a chemical viewpoint, the resulting conjugates would be addressed as nanocomposites. Modified phages can form circles (Nam et al. 2004), and modified TMV can bind proteins (Demir and Stowell 2002) and antibodies (Bendahmane et al. 1999; Van Regenmortel 1999). An exciting new concept is to use a living plant as alignment tool for metal clusters (Gardea-Torresdey et al. 2003), comparable to chains of clusters in magnetotatic bacteria (Niemeyer 2001).

Problems and challenges

Biomolecules have a very narrow *size distribution*, but their composites with inorganic materials do not. The template function cannot always yield perfectly defined products. In fact, most reported composites cannot be called "well defined" in a biological or organic chemistry context. However, in view of technological applications, a perfectly defined composite mass might not be as important as the well-defined shape and size; in other words, it often suffices that only *one* property is well controlled (e.g. length *or* diameter *or* magnetism).

The plethora of chemical groups on biomolecules is an enormous advantage over inorganic and even over organic polymer structures, but it can also be a disadvantage. Indeed, chemical reactions with passive biomolecules are only in some cases site-specific, and a spatially

selective binding or synthesis of inorganic materials is usually not achieved, although antibodies can be engineered to bind with extreme specificity (e.g. exclusively to the end of a tubular plant virion; Van Regenmortel 1999).

Spatial selectivity down to the atomic level – as known from organic chemistry – is very difficult to obtain; it was shown for gold clusters that bind selectively to the cysteine groups on an icosahedral plant virus capsid (Wang et al. 2002). The problem of immobilization is closely related: also in this case, a connection to a solid surface is easily achieved, but it is only rarely specific down to the atomic level. Current research in surface science is aimed at this problem by investigating (as yet) relatively small molecules on flat crystalline surfaces under vacuum conditions (Kasemo 1998; Kühnle et al. 2002). Problems such as the synthesis of networks from organic molecules and single metal atoms (Dmitriev et al. 2003) could benefit from introducing more complex biomolecules; on the other hand, large size and charge mean that the usual evaporation strategy in surface science meets its limit.

Connecting nanostructures to microstructures (or larger ones; see Fig. 4) is a very hard task, but possible for nearly all cases. Typical alternatives to self-assembling connections are antibody/antigen and (strept)avidin/biotin linking (Mann et al. 2000). When not self-assembly but structuring is required, microcontact printing (lithography with a surface-structured polymer stamp) is an extremely promising technique that can be employed to build (sub-)micrometre-sized patterns from a large range of – if not all – biomolecules. Even single proteins (Renault et al. 2003) can be arranged in a predefined pattern on a solid surface. Alternatively, lateral forces during adsorption are a simple means to orient 1D molecules or 1D nanocomposites (Deng and Mao 2003; Nakao et al. 2003a). The possibility of orienting the nanocomposites is crucial: only then can defined electrical connections be built (Keren et al. 2002, 2003), only then can photons interact with the structures directionally, and so on. Obviously, in this case 1D structures have advantages over others. A basic question for future applications is whether special properties of single nanostructures are to be exploited (Fig. 5) – here nanostructures or nanodevices must be embedded in microstructures – or whether a not yet constructed *complete network* of nanostructures is required (see Eichen et al. 1998; Figs. 3 and 4); a first step would be crossed DNA-templated wires (Deng and Mao 2003). In the latter case, passive nanostructures must be interconnected with nano-objects; the relationship with (active) biological structures is obvious. However, it is – at least today – obviously impossible to approach the extreme functional complexity of biological systems. The smallest technical devices perform simple tasks on the scale of tens of nanometres (e.g. transistors, wires or scanning probe tips), while biological structures of this size reach such a level of complexity that their function is not known in complete detail (e.g. the ribosome).

Using self-assembly is probably the most elegant and also efficient way to built complex structures. However, self-assembly implies also *self-disassembly*, the rate of which is tightly controlled in biological systems like the cell. The lack of control, especially in passive structures, means that a certain fraction of nanostructures will disintegrate. Remedies could be posttreatments such as covalent linkages after the self-assembly. On the other hand, disassembly could even be beneficial through the recycling of valuable biomolecules (Fig. 4).

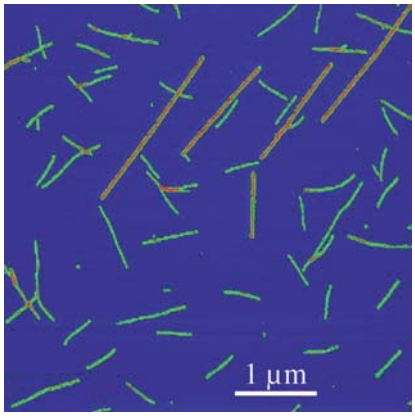

Fig. 6 Potato virus X (*PVX*, *green*, slightly bent) and tobacco mosaic virus (*TMV*, *red*, linear aggregates of more than 1 µm length) adsorbed on mica. The AFM image is height-coloured, and TMV with its larger diameter (18 nm, collapsed to 14 nm due to the adsorption) is clearly distinguishable from PVX. In addition, PVX is more flexible and thus most virions are slightly bent

Flexibility is crucial for 1D structures, as shown by elasticity measurements on single biomolecules (microtubules) (Kis et al. 2002). Usually a stiff structure is needed (see Fig. 5; for an example see also Fig. 6), especially after immobilization. On the other hand, building up nanostructures sometimes requires flexibility, e.g. pH-induced opening of virus capsids (Douglas and Young 1998) and temperature-induced denaturing of DNA. Here a posttreatment is usually not helpful; since 1D nanostructures can contact a surface while they change their conformation, they will be immobilized as curved structures. The same applies when a 1D nanostructure contacts inorganic reagents in solution. To alleviate the problem, highly specific chemical modifications are often impossible, as discussed above. However, for some protein-based structures, mutations can be of help. For example, the Glu50 → Gln50 mutation of the TMV coat protein increases interprotein interactions and results in higher stability of the virion (Lu et al. 1996).

Section 2: Virus nanostructures

Viruses were investigated on the nanoscale already with the first transmission electron microscopes (Kausche et al. 1939; Ruska 1943). Virions consist of a nucleic acid strand and a protein cage or tube that is made up from a large number of identical proteins. The proteins can also be glyco- or lipoproteins; they are most commonly arranged in a near-spherical (e.g. icosahedral) or helically

wound (tubular) exterior shell, the capsid. Viruses are usually specialized for certain hosts – the tobacco mosaic virus does not infect mammals (Schwarz et al. 1966) – hence phages and plant viruses (Fig. 6) can be handled with simple safety measures. Note that an infection with plant viruses requires living organisms or lesions of plant tissues (or direct uptake by the root system), and that the mechanical infection paths usually rely on a certain chemical and mechanical stability of the virions – a great advantage for use in nanoscale science. The simple structure of the virions, too, is very important since it allows us to understand and to explore their chemical behaviour. Virions are (today) used as passive molecules, especially as templates for inorganic nanostructures.

An example is the tobacco mosaic virus (TMV) (Kausche et al. 1939), which is the ideal model substance for self-assembly processes, and at the same time the perfect model for viruses in general, especially for filamentous plant viruses. Its genome is completely characterized for a range of strains: 2,130 protein molecules are helically arranged with 16.3 units building up one turn (cf. Fig. 5). A helical RNA strand (8 nm diameter) is deeply buried inside the protein structure without being exposed to the central channel of 4 nm diameter. The protein loop that forms the wall of the central channel shows less conformational stability than the bulk protein structure, which is based on the rigid barrel type with four alpha helices. The particle length is 300 nm with 18 nm exterior diameter (Henrick and Thornton 1998; Klug 1999; Namba et al. 1989; Pattanayek and Stubbs 1992; Stubbs 1999), but linear head-to-tail alignment is frequently found. Similarly, the pure protein can self-assemble into long, 18-nm-wide tubular structures, which are of course non-infective (Schramm 1943). TMV tolerates ethanol, aqueous dimethyl sulphoxide and temperatures up to 90°C. TMV is not affected by pH values from 3.5 up to about 9 for at least several hours – some virions in a sample remain active even at pH 1.5 (Kausche 1938b) – and it retains its infectivity also in dried leaves in cigarettes (Wetter 1975). Site-directed mutations such as Glu50 → Gln50 can enhance the stability; consequently the infectivity diminishes (Lu et al. 1996).

In addition to the discussed "bottom-up" self-assembly, one can ask whether it is possible to arrange this biomolecule in a defined way with a "top-down" method. Recent research points to a positive answer: many elegant methods have been developed for a spatially selective and orienting immobilization, for example of DNA (Deng and Mao 2003; Nakao et al. 2003b; Winfree et al. 1998). TMV can easily be bound to surfaces, even technically important ones like silicon wafers or glass (see Fig. 7a). For the binding in this case the hydrophilic and charged nature of both virion and substrate is decisive: the exterior TMV surface provides Ser and Thr as well as the C-terminus of the coat protein, and it is negatively charged when the pH in a suspension is higher than the pI value of 3.5. Immobilization is fast and strong when the pH value of a TMV suspension is in the range 3.5–7, and when the substrate contains hydrophilic or positively charged groups. Even covalent linkages to a surface are possible (Knez et al. 2004b). Consequently the binding on hydrophobic, uncharged graphite is slow and weak. One could assume that such conditions allow for surface diffusion, and finally for arrangement towards 2D crystals. However, a typical property of 1D molecules is that they crystallize neither in 3D nor in 2D, but form oriented fibres or liquid crystals. This is well known for TMV (Kausche 1939), and only very small 2D domains are found on a solid substrate (Nedoluzhko and Douglas 2001). Hence ordered adsorption might be impossible on macroscale surfaces. However, forces that arise during normal sample handling, namely suction of a droplet and blowing dry with a gas stream, can orient single 1D macromolecules in parallel, as demonstrated in Fig. 7b. An example for microcontact printing (a "top-down" technique) of virions is presented in Fig. 7c. In combination with the above-mentioned orienting methods ("bottom-up"), the aim is to place biomolecules on a predefined location in a defined orientation, without destruction of their chemical and physical properties. Recently, an AFM lithography approach was demonstrated for plant viruses (Cheung et al. 2004). The interest in dielectrophoretic capture of nano-objects, also of virions (Akin and Bashir 2004), is growing fast.

Of the plant viruses, TMV (Demir and Stowell 2002; Douglas and Young 1999; Dujardin et al. 2003; Fujikawa

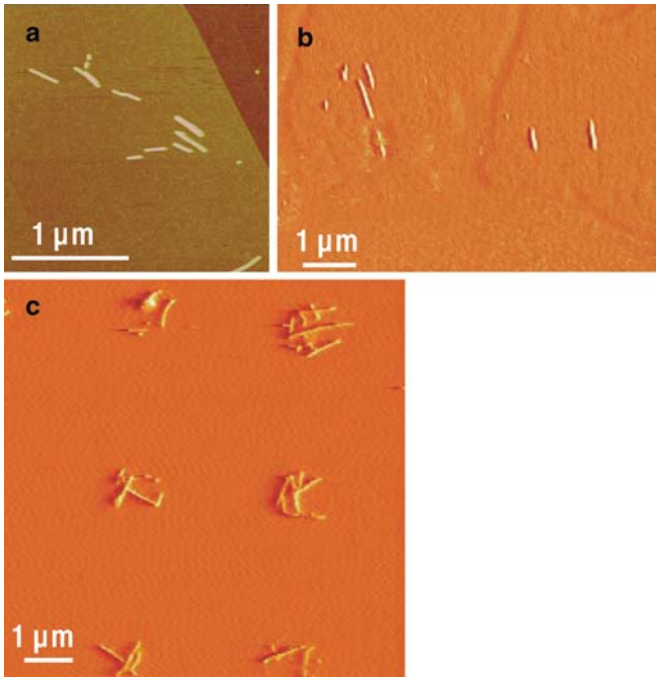

Fig. 7 a Atomic force microscopy (*AFM*, in contact mode) of tobacco mosaic viruses (*TMVs*) adsorbed on glass. **b** AFM image (noncontact mode) of TMVs aligned by a stream of argon during adsorption on an oxide-covered silicon wafer. **c** Microcontact printing of TMVs on mica. TMVs arrange randomly, but only inside the square-shaped contact areas. The random orientation of the mid-right, mid-left and lower left parts might be translated as "change" (hua_4), "must" (bi_4) and "fire" (huo_3) in Chinese

and Kunitake 2003; Knez et al. 2004a, 2004b; Shenton et al. 1999), the cowpea mosaic virus (decorated with gold clusters; Wang et al. 2002) and the brome mosaic virus (Dragnea et al. 2003) have been used in nanoscale science; and several other near-spherical viruses can also form 2D layers (Kuznetsov et al. 2001). In fact, a range of interesting alternatives with nanometre diameter and various chemical behaviour is available for 1D applications, for instance filamentous viruses, such as potato virus X, with its special surface features (Parker et al. 2002). The M13 phage, well known from the phage display technique, can easily be handled and modified, and has been employed in nanoscale science for some years (Flynn et al. 2003).

Section 3: Synthetic methods

Binding of inorganic nanostructure precursors to biomolecular templates

Binding organic molecules, and especially biomolecules, to a biomolecule [here: to the (passive) template] are the best-known chemical modifications of a nano-object and rely on classic biochemistry. (Strept)avidin–biotin and Ni(II)–His interactions are widely used in nanoscale science. Other examples are TMV, where dyes (Kausche 1938a) and avidin (Demir and Stowell 2002) can bind to the exterior surface, and DNA, to which polymers can bind (Eichen et al. 1998). In contrast, binding inorganic structures is often not straightforward, but more and more recipes are currently being developed (Niemeyer 2001). A templating action requires as first step some chemical interaction of the template with the precursor molecules, which later react to produce the nanostructure. In vacuum, the precursors may be atoms, as demonstrated by the standard method of metal shadowing for electron microscopy, which most probably destroys the chemical functionality, and by the creation of metal nanoparticle grids on archaea S layers (Mertig et al. 2001). In order to preserve the chemical structure, binding molecules or metal cations (or complexes) from aqueous solutions is preferable. Examples for all possible interactions between template and metal ions exist: covalent linking by chemical reactions (Au–S binding for cluster immobilization; Wang et al. 2002), complex formation, ion-pair (electrostatic) binding (Au and CdS particles on DNA; Kumar et al. 2001; Torimoto et al. 1999; Warner and Hutchison 2003; Woehrle et al. 2004), CdS and ZnS on phages (Mao et al. 2003), hydrogen bridges on DNA (Nakao et al. 2003b) and Van der Waals interactions. However, a closer look shows that a direct proof for the presence of such an interaction is extremely difficult, due to the complicated chemistry of proteins. Indeed strategies or recipes for site-specific binding of metal ions or complexes exist only in rare cases (Mann 1996), although the basic concepts of inorganic complex chemistry can be applied (Berthon 1995). For future work, theoretical studies of the selective binding to amino acid sites (Rulisek and Havlas 2003) may be helpful. Strategies are better developed for a special case of ion binding, the isomorphic heavy atom substitutions in protein crystals. To improve the situation, mutations can be employed to create a certain number of reactive groups at preselected sites of a protein or of a protein assembly (e.g. a virion; Wang et al. 2002). One of the most advanced methods is the combinatorial synthesis (by mutation) and optimization of random amino acid sequences via phage display (Flynn et al. 2003). From such results, one can derive sequences that bind a certain metal cation strongly. Unfortunately, even in this case a generalization is not possible.

In conclusion, the understanding of biomolecular surfaces is – although highly developed – still insufficient to predict metal ion-binding properties (with the possible exception of the Ni(II)–His interaction which has become a standard linking method). A possibility to circumvent this – at least for relatively large nanostructures – is to employ "biosurface binding" via antibodies (e.g. immunostaining with gold clusters bound to antibodies), antigens or coenzymes. As yet, no strategy exists to place inorganic structures well aligned (this is of special interest for 1D biomolecules) or well ordered on a biomolecule. A first step is the recently reported control of interparticle spacing of gold nanoclusters on DNA (Woehrle et al. 2004).

Electroless deposition: a method for the synthesis of biomolecule/metal nanocomposites

As already discussed, the first step in the nanocomposite synthesis is the binding of metal cations to the biotemplate. In the following step, anions can react with the bound metal ions to yield salts or other binary compounds (followed by more cations and anions to create clusters), which constitutes a "biomineralization on the nanoscale". Alternatively, a metallization can be achieved via reduction to the zerovalent state.

The tubular shape of TMV and also its high aspect ratio makes it an interesting passive nano-object, especially as a template, as demonstrated by the crystallization ("biomineralization") of inorganic materials (Shenton et al. 1999), and by attaching metal clusters to its surfaces (metallization, see Fig. 8a) (Bendahmane et al. 1999; Demir and Stowell 2002; Dujardin et al. 2003; Kausche 1940; Knez et al. 2002; Ruska 1947). The coat protein contains no His and no Met, and only a single non-accessible Cys (in the rigid barrel inside the protein); hence naturally occurring TMV lacks the most effective metal binding sites. A huge drawback of the reduction method for the creation of metal nanostructures is that only those metal ions that are already bound to the biomolecule can be reduced, which results ultimately in small nanoclusters, similar to binding very small prefabricated clusters – no complex structures are attainable, and the clusters remain isolated. Such clusters have been formed on the exterior TMV surface, but also inside its channel (Du-

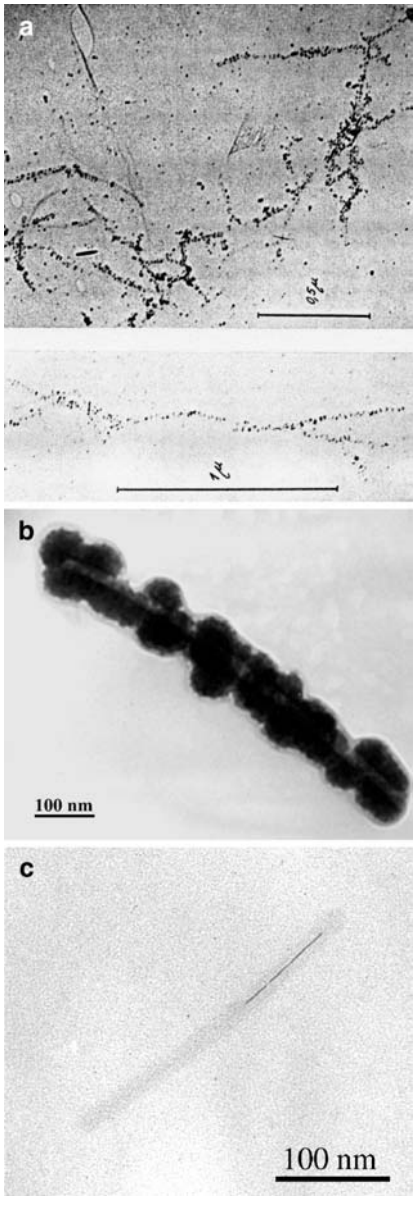

Fig. 8 Transmission electron micrographs of tobacco mosaic viruses (TMVs). **a** Treated with a 3 nm diameter gold sol; the scale bars are 0.5 μm and 1 μm long (from Kausche 1940). **b** TMV coated with clusters of electrolessly deposited nickel that coalesced to a metallic coating. The TMV is still clearly visible; it fills an 18-nm-wide hole inside the metal structure. **c** 3-nm-wide nickel wire deposited inside the interior channel of TMV (see text)

jardin et al. 2003; Knez et al. 2002, 2004a), which automatically means alignment. In particular, the physical properties can be further exploited, when the clusters – in their linear or helical arrangement – are separated by *defined* distances. This aim is very hard to achieve on any biotemplate – the first examples might be gold clusters on the cowpea mosaic virus (Wang et al. 2002) and on DNA (Woehrle et al. 2004) – the reason being the still insufficient understanding of protein chemistry, combined with the complexity of the biochemical manipulations (most unsuccessful experiments are unlikely to be published).

When clusters grow sufficiently fast on a biomolecular surface, they can coalesce, as shown in Fig. 8b. Similar arguments apply also for "biomineralizations", e.g. of TiO_2 on TMV (Fujikawa and Kunitake 2003) or of CdS on a phage (Mao et al. 2004). Hence for wire-like metal structures, a two-step electroless deposition (comparable to the "enhancement" of latent photographic patterns) is useful. A solution with the metal ions, complexants and a reductant (e.g. Ag^+, NH_3 and glucose) is contacted with a biomolecular surface that has been pretreated with noble metal complexes such as Pt(II), Pd(II) or Au(I). It is quite likely that the metal ions are in part already reduced during binding to biomolecules; the pretreatment alters the biomolecular surface. Since only extremely small clusters at a low coverage are formed (Knez et al. 2003), the biomolecule is preserved much better than with other methods. The details of this pretreatment (first step), the so-called "activation" or "sensitation", are not yet known, although some theoretical studies exist (Mertig et al. 2002; Nakai et al. 2001). After exchanging the solution to the electroless deposition bath, the deposition starts (second step). It is catalysed by the noble metal nanoparticles. The crucial point is that the deposited metal also catalyses its own reductive deposition; hence the metal particles can *continue* to grow. Obviously, such an autocatalytic process is hard to control. However, some technical processes like photography or staining of biological samples (including SDS-PAGE gels) show that sufficient control is achievable. The importance in the context of nanotechnology is that electroless deposition does work on the whole length scale that is relevant for materials and biological structures, i.e. from metres down to nanometres. Generally, electroless deposition is advantageous for high-aspect ratio structures (hence for tubes) since they can be filled or decorated without clogging up or closing an orifice.

Recently, true nanowires [as opposed to wire-like assemblies of (aligned) clusters, discussed above] were synthesized in the inner channel of tubular biomolecules with electroless deposition (Knez et al. 2003; Reches and Gazit 2003). In the case of TMV, a suspension was pretreated ("sensitized") with $PtCl_4^{2-}$, washed and contacted with an electroless nickel bath with a borane compound reductant, yielding a nickel wire of only 3 nm diameter (see Fig. 8c). Exciting physical properties such as electrical conduction (already shown for 30-nm-thick wires in microtubules; Reches and Gazit 2003) and magnetism are expected. In the presence of phosphate traces, a preferable adsorption of Pt(II) on the exterior viral surface is induced. The resulting Pt clusters are too small to be detected even with transmission electron microscopy, but after electroless deposition of nickel, large (but still nanoscale) clusters coalesce. The produced structure is a nickel tube with a well-defined tubular hole of 18 nm diameter (Fig. 8b). The coalescence leads to relatively large (>10 nm) structures, a problem encountered with all biological rods and tubes (e.g. for nickel on microtubules; Kirsch et al. 1997), and especially well known for DNA (Braun et al. 1998; Deng and Mao 2003; Harnack et al.

2002; Kumar et al. 2001; Mertig et al. 2002; Nakao et al. 2003b; Warner and Hutchison 2003) and TMV (Demir and Stowell 2002; Dujardin et al. 2003; Kausche 1940; Kausche and Ruska 1939; Knez et al. 2002, 2004a; Ruska 1947). Improvement of the bath should allow to control the deposition much better, and very thin nickel "skins" of the virion should be attainable, as known for TiO_2 (Fujikawa and Kunitake 2003) and SiO_2 (Fowler et al. 2001) crystallizations. The strength of the protein (virion) template is that site-directed mutations allow to express chemical groups selectively on the exterior surface, as shown for Lys on TMV (Demir and Stowell 2002) and for the M13 phage (Mao et al. 2004) and thereby to bind the metal ions with higher rate and coverage.

Confinement templating

As far as uniformity and shape control are concerned, using confinement inside templates is the most successful route towards nanocomposites. When a biomolecule is used as template, its inner surfaces have to be attacked selectively. Again, understanding the chemistry of such a surface has not been achieved, although several impressive experiments have been conducted: for 0D, inorganic nanoparticles inside apoferritin (Mann 1996; Mayes et al. 2003), inside the cowpea chlorotic mottle virions (Douglas and Young 1998), inside the brome mosaic virion (Dragnea et al. 2003), and inside a heat shock protein cage (Flenniken et al. 2003); for 1D, metal and inorganic nanoclusters inside TMV (Dujardin et al. 2003; Knez et al. 2002, 2003, 2004a) and silver inside peptide fibres (Reches and Gazit 2003). Some basic considerations concerning local charges and possible complexation sites have been formulated for TMV (Dujardin et al. 2003; Knez et al. 2004a). The smallest yet obtained 1D structure, with only 3 nm diameter, is shown in Fig. 8c. In this case electroless deposition was able to deliver a rather large flow of ions and reductant inside the central channel. While in other cases a biomolecular shell might be penetrated or cages may open and close again (Douglas and Young 1998), both are impossible for TMV. The example proves the ability of electroless deposition to access extremely small cavities. Evaporation of metal in vacuum, the standard metallization method in nanoscale science, would have closed the channel before filling it.

Now a rather unexpected advantage becomes clear: carbon nanostructures (e.g. nanotubes) feature hydrophobic cavities which cannot be wetted by aqueous solutions, and even molten metals do not enter easily (Ni wires of 4 nm diameter can be grown by vapour deposition of nickelocene; Pradhan et al. 1999). In contrast, many biomolecules have accessible hydrophilic cavities. One can estimate roughly whether ions or complexes can reach the interior surface of a nanotube. The average diffusion distance is $(2Dt)^{1/2}$ where t is the time and D (the diffusion coefficient) is of the order of 10^{-9} m^2/s. The fact that 300 nm distance requires merely tens of microseconds means that the complete inner channel of TMV is fully accessible in typical experimental times (seconds to minutes). These considerations cannot be valid when the pore approaches the size of a single water molecule, ~0.3 nm. Such cases, namely pore proteins and ion pumps in membranes, have been intensively studied from a biochemical and structural biology point of view; experiments on ion movement through water pores (Murata et al. 2000) and ion pores (Zhou et al. 2001) were awarded the 2003 Nobel prize in chemistry. The water movement through the passage, including reorientation, has been modelled (Groot and Grubmüller 2001), and even the translocation of extremely long molecules (DNA) through very narrow pores (hemolysin) is well documented (Meller et al. 2001). An exciting transition from passive structures (discussed above) to active ones like gated pore proteins can thus become feasible in nanoscale science.

The ideal nanostructure, a single atom-wide chain, should be attainable by confinement. The first example is probably a chain of Cu(II) ions inside modified DNA (Tanaka et al. 2003). Such structures show extraordinary physical phenomena: the electrical conduction must be quantized, and ferromagnetic coupling becomes possible in non-magnetic metals. For larger structures, a size control is possible simply by choosing a template with very high symmetry. In the 0D case this is ideally a sphere, and for a virion e.g. an icosahedron as approximation. Fine tuning can be achieved by mutagenesis as shown for the cowpea mosaic virus (Wang et al. 2002) and for a protein cage (Flenniken et al. 2003). Similar arguments apply to 1D structures. However, until now a nanoscale control of the length has not been demonstrated. This problem is very obvious with protein fibres, whose length cannot be selected (the same problem exists for carbon nanotubes). Filamentous plant viruses and linear phages with their well-defined length offer an elegant solution. Moreover, when coat proteins and nucleic acids are reconstituted to a virion, an artificial nucleic acid could be used to tune the length of the template (as done in the plant cell with the natural nucleic acid).

Perspectives

Biomolecules which are not yet common in nanotechnology include carbohydrates (e.g. saccharides), viroids (pure RNA loops which are highly infective for certain plants), cells (which may be too complex and not sufficiently stable) and larger parts of cells (e.g. organelles), while viruses and protein and lipid fibres (or tubules) are already widely used. They all offer chemical and structural functionalities that are as yet largely unexploited for nanoscale science, let alone nanotechnology; this also applies to the widely used single molecules and self-assembled molecular structures. In other cases, biomolecules are already being investigated in detail for possible applications; for example, actin and myosin should be promising elements for artificial linear motors, and hemolysine could form tunable molecular pores or

channels. Another less common topic is a biomolecule/organic composite as "passive" nanostructure: although the attachment of organic molecules to biomolecules is very well studied (e.g. antigen–antibody) and will most likely form the basis for new biosensors, and although fluorescence from attached markers is a standard analytical technique, physical properties that emanate directly from the attachment (e.g. changes in the molecular conductivity) have not yet been investigated.

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References

Akin D, Li H, Bashir R (2004) Real-Time virus trapping and fluorescent imaging in microfluidic devices. Nano Lett 4:257–259

Archibald DD, Mann S (1993) Template mineralization of self-assembled anisotropic lipid microstructures. Nature 364:430–433

Bashir R (2001) DNA-mediated artificial nanobiostructures: state of the art and future directions. Superlattices Microstructures 29:1–16

Bendahmane M, Koo M, Karrer E, Beachy RN (1999) Display of epitopes on the surface of tobacco mosaic virus: impact of charge and isoelectric point of the epitope on virus–host interactions. J Mol Biol 290:9–20

Berthon G (ed) (1995) Handbook of metal–ligand interactions in biological fluids. Dekker, New York

Boal AK, Headley TJ, Tissot RG, Bunker BC (2004) Microtubule-templated biomimetic mineralization of lepidocrocite. Adv Funct Mater 14:19–24

Braun E, Eichen Y, Sivan U, Ben-Yoseph G (1998) DNA-templated assembly and electrode attachment of a conducting silver wire. Nature 391:775–778

Cheung CL, Camarero JA, Woods BW, Lin T, Johnson JE, De Yoreo JJ (2004) Fabrication of assembled virus nanostructures on templates of chemoselective linkers formed by scanning probe nanolithography. J Am Chem Soc 125:6848–6849

Coffer JL, Bigham SR, Li X, Pinizzotto RF, Rho YG, Pirtle RM, Pirtle IL (1996) Dictation of the shape of mesoscale semiconductor nanoparticle assemblies by plasmid DNA. Appl Phys Lett 69:3851–3853

Demir M, Stowell MHB (2002) A chemoselective biomolecular template for assembling diverse nanotubular materials. Nanotechnology 13:541–544

Deng Z, Mao C (2003) DNA-templated fabrication of 1D parallel and 2D crossed metallic nanowire arrays. Nano Lett 3:1545–1548

Ding B, Sha R, Seeman NC (2004) Pseudohexagonal 2D DNA crystals from double crossover cohesion. J Am Chem Soc 126:10230 –10231

Djalali R, Chen YF, Matsui H (2002) Au nanowire fabrication from sequenced histidine-rich peptide. J Am Chem Soc 124:13660–13661

Dmitriev A, Spillmann H, Lin N, Barth JV, Kern K (2003) Modular assembly of two-dimensional metal-organic coordination networks at a metal surface. Angew Chem Int Ed 42:2670–2673

Douglas T, Young M (1998) Host–guest encapsulation of materials by assembled virus protein cages. Nature 393:152–155

Douglas T, Young M (1999) Virus particles as templates for materials synthesis. Adv Mater 11:679–681

Dragnea B, Chen C, Kwak E-S, Stein B, Kao CC (2003) Gold nanoparticles as spectroscopic enhancers for in vitro studies on single viruses. J Am Chem Soc 125:6374–6375

Drexler KE (1981) Molecular engineering: an approach to the development of general capabilities for molecular manipulation. Proc Natl Acad Sci 78:5275–5278

Dujardin E, Peet C, Stubbs G, Culver JN, Mann S (2003) Organization of metallic nanoparticles using tobacco mosaic virus templates. Nano Lett 3:413–417

Eichen Y, Braun E, Sivan U, Ben-Yoseph G (1998) Self-assembly of nanoelectronic components and circuits using biological templates. Acta Polymer 49:663–670

Feynman RP (1960) There's plenty of room at the bottom (at Annual meeting 1959, Am. Phys. Soc.). Engineering and Science (Caltech, Pasadena) February 1960

Flenniken ML, Willits DA, Brumfield S, Young MJ, Douglas T (2003) The small heat shock protein cage from *Methanococcus jannaschii* is a versatile nanoscale platform for genetic and chemical modification. Nano Lett 3:1573–1576

Flynn CE, Lee S-W, Peelle BR, Belcher AM (2003) Viruses as vehicles for growth, organization and assembly of materials. Acta Mater 51:5867–5880

Fowler CE, Shenton W, Stubbs G, Mann S (2001) Tobacco mosaic virus liquid crystals as templates for the interior design of silica mesophases and nanoparticles. Adv Mater 13:1266–1269

Fritzsche W, Böhm KJ, Unger E, Köhler JM (1999) Metallic nanowires created by biopolymer masking. Appl Phys Lett 75:2854–2856

Fujikawa S, Kunitake T (2003) Surface fabrication of hollow nanoarchitectures of ultrathin titania layers from assembled latex particles and tobacco mosaic viruses as templates. Langmuir 19:6545–6552

Gabius HJ (2000) Biological information transfer beyond the genetic code: the sugar code. Naturwissenschaften 87:108–121

Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M (2003) Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir 19:1357–1361

Groot BL de, Grubmüller H (2001) Water permeation across biological membranes. Science 294:2353–2357

Harnack O, Ford WE, Yasuda A, Wessels JM (2002) Tris(hydroxymethyl)phosphine-capped gold particles templated by DNA as nanowire precursors. Nano Lett 2:919–923

Henrick K, Thornton JM (1998) PQS: a protein quaternary structure file server http://pqs.ebi.ac.uk/pqs-bin/macmol.pl?filename=2tmv. Trends Biochem Sci 23:358–361

Islam MN, Zhao XK, Said AA, Mickel SS, Vail CF (1997) High-efficiency and high-resolution fiber-optic probes for near field imaging and spectroscopy. Appl Phys Lett 71:2886–2888

José-Yacamán M, Rendón L, Arenas J, Puche MCS (1996) Maya blue paint: an ancient nanostructured material. Science 273:223–225

Kasemo B (1998) Biological surface science. Curr Opin Solid State Mater Sci 3:451–459

Kausche GA (1938a) Über den färberischen Nachweis des Tabakmosaikvirus. Naturwissenschaften 26:741–742

Kausche GA (1938b) Über eine Trennungsmöglichkeit von Mischviren auf Grund ihrer differenten pH-Stabilität. Naturwissenschaften 26:219–219

Kausche GA (1939) Über die Bildung von hexagonalen Viruskristallen aus Suspensionen des Tabakmosaikvirus in vitro. Naturwissenschaften 27:77–78

Kausche GA (1940) Über den Mechanismus der Goldsolreaktion beim Protein des Tabakmosaik- und Kartoffel-X-Virus. Biol Zbl 60:179–199

Kausche GA, Ruska H (1939) Die Sichtbarmachung der Adsorption von Metallkolloiden an Eiweißkörper. I. Die Reaktion kolloides Gold-Tabakmosaikvirus. Kolloid Z 89:21–26

Kausche GA, Pfankuch E, Ruska H (1939) Die Sichtbarmachung von pflanzlichem Virus im Übermikroskop. Naturwissenschaften 27:292–299

Keren K, Krueger M, Gilad R, Ben-Yoseph G, Sivan U, Braun E (2002) Sequence-specific molecular lithography on single DNA molecules. Science 297:72–75

Keren K, Berman RS, Buchstab E, Sivan U, Braun E (2003) DNA-templated carbon nanotube field-effect transistor. Science 302:1380–1382

Kirsch R, Mertig M, Pompe W, Wahl R, Sadowski G, Böhm KJ, Unger E (1997) Three-dimensional metallization of microtubules. Thin Solid Films 305:248–253

Kis A, Kasas S, Babic B, Kulik AJ, Benoit W, Briggs GAD, Schönenberger C, Catsicas S, Forro L (2002) Nanomechanics of microtubules. Phys Rev Lett 89:2481011–2481014

Klug A (1999) The tobacco mosaic virus particle: structure and assembly. Philos Trans R Soc Lond B 354:531–535

Knez M, Sumser M, Bittner AM, Wege C, Jeske H, Kooi S, Burghard M, Kern K (2002) Electrochemical modification of individual nano-objects. J Electroanal Chem 522:70–74

Knez M, Bittner AM, Boes F, Wege C, Jeske H, Maiss E, Kern K (2003) Biotemplate synthesis of 3-nm nickel and cobalt nanowires. Nano Lett 3:1079–1082

Knez M, Sumser M, Bittner AM, Wege C, Jeske H, Martin TP, Kern K (2004a) Spatially selective nucleation of metal clusters on the tobacco mosaic virus. Adv Funct Mater 14:116–124

Knez M, Sumser MP, Bittner AM, Wege C, Jeske H, Hoffmann DMP, Kuhnke K, Kern K (2004b) Binding the tobacco mosaic virus to inorganic surfaces. Langmuir 20:441–447

Koebnig R, Locher KP, Gelder PV (2000) Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Mol Microbiol 37:239–253

Kühnle A, Linderoth TR, Hammer B, Besenbacher F (2002) Chiral recognition in dimerization of adsorbed cysteine observed by scanning tunnelling microscopy. Nature 415:891–893

Kumar A, Pattarkine M, Bhadbhade M, Mandale AB, Ganesh KN, Datar SS, Dharmadhikari CV, Sastry M (2001) Linear superclusters of colloidal gold particles by electrostatic assembly on DNA templates. Adv Mater 13:341–344

Kuznetsov YG, Malkin AJ, Lucas RW, Plomp M, McPherson A (2001) Imaging of viruses by atomic force microscopy. J Gen Virol 82:2025–2034

Lee BS, Lee SC, Holliday LS (2003) Biochemistry of mechanoenzymes: biological motors for nanotechnology. Biomed Microdevices 5:269–280

Liu Q, Wang L, Frutos AG, Condon AE, Corn RM, Smith LM (2000) DNA computing on surfaces. Nature 403:175–179

Lu B, Stubbs G, Culver JN (1996) Carboxylate interactions involved in the disassembly of tobacco mosaic tobamovirus. Virology 225:11–20

Mann S (ed) (1996) Biomimetic materials chemistry. VCH, New York

Mann S, Shenton W, Li M, Connolly S, Fitzmaurice D (2000) Biologically programmed nanoparticle assembly. Adv Mater 12:147–150

Mao C, Flynn CE, Hayhurst A, Sweeney R, Qi J, Georgiou G, Iverson B, Belcher AM (2003) Viral assembly of oriented quantum dot nanowires. Proc Natl Acad Sci USA 100:6946–6951

Mao C, Solis DJ, Reiss BD, Kottmann ST, Sweeney RY, Hayhurst A, Georgiou G, Iverson B, Belcher AM (2004) Virus-based toolkit for the directed synthesis of magnetic and semiconducting nanowires. Science 303:213–217

Markowitz M, Baral S, Brandow S, Singh A (1993) Palladium ion assisted formation and metallization of lipid tubules. Thin Solid Films 224:242–247

Martin CR, Kohli P (2002) The emerging field of nanotube biotechnology. Nat Rev Drug Discovery 2:29–37

Mayes E, Bewick A, Gleeson D, Hoinville J, Jones R, Kasyutich O, Nartowski A, Warne B, Wiggins J, Wong KKW (2003) Biologically derived nanomagnets in self-organized patterned media. IEEE Trans Magn 39:624–627

Medalia O, Heim M, Guckenberger R, Sperling R, Sperling J (1999) Gold-tagged RNA: a probe for macromolecular assemblies. J Struct Biol 127:113–119

Meegan JE, Aggeli A, Boden N, Brydson R, Brown AP, Carrick L, Brough AR, Hussain A, Ansell RJ (2004) Designed self-assembled beta-sheet peptide fibrils as templates for silica nanotubes. Adv Funct Mater 14:31–37

Meller A, Nivon L, Branton D (2001) Voltage-driven DNA translocations through a nanopore. Phys Rev Lett 86:3435–3438

Mertig M, Wahl R, Lehmann M, Simon P, Pompe W (2001) Formation and manipulation of regular metallic nanoparticle arrays on bacterial surface layers: an advanced TEM study. Eur Phys J D 16:317–320

Mertig M, Ciacchi LC, Seidel R, Pompe W, De Vita A (2002) DNA as selective metallization template. Nano Lett 2:841–844

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y (2000) Structural determinants of water permeation through aquaporin-1. Nature 407:599–605

Nakai H, Homma T, Komatsu I, Osaka T (2001) Ab initio molecular orbital study of the oxidation mechanism of hypophosphite ion as a reductant for an electroless deposition process. J Phys Chem B 105:1701–1704

Nakao H, Gad M, Sugiyama S, Otobe K, Ohtani T (2003a) Transfer-printing of highly aligned DNA nanowires. J Am Chem Soc 125:7162–7163

Nakao H, Shiigi H, Yamamoto Y, Tokonami S, Nagaoka T, Sugiyama S, Ohtani T (2003b) Highly ordered assemblies of Au nanoparticles organized on DNA. Nano Lett 3:1391–1394

Nam KT, Peelle BR, Lee S-W, Belcher AM (2004) Genetically driven assembly of nanorings based on the M13 virus. Nano Lett 4:23–27

Namba K, Pattanayek R, Stubbs G (1989) Visualization of protein–nucleic acid interactions in a virus: refined structure of intact tobacco mosaic virus at 2.9 Å resolution by X-ray fiber diffraction. J Mol Biol 208:307–325

Nedoluzhko A, Douglas T (2001) Ordered association of tobacco mosaic virus in the presence of divalent metal ions. J Inorg Biochem 84:233–240

Niemeyer CM (2001) Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. Angew Chem Int Ed 40:4128–4158

Park SJ, Taton TA, Mirkin CA (2002) Array-based electrical detection of DNA with nanoparticle probes. Science 295:1503–1506

Parker L, Kendall A, Stubbs G (2002) Surface features of potato virus X from fiber diffraction. Virology 300:291–295

Pattanayek R, Stubbs G (1992) Structure of the U2 strain of tobacco mosaic virus refined at 3.5 Å resolution using X-ray fiber diffraction. J Mol Biol 228:516–528

Powell RD, Halsey CMR, Liu W, Joshi VN, Hainfeld JF (1999) Giant platinum clusters: 2-nm covalent metal cluster labels. J Struct Biol 127:177–184

Pradhan BK, Kyotani T, Tomita A (1999) Nickel nanowires of 4 nm diameter in the cavity of carbon nanotubes. Chem Commun 1317–1318

Reches M, Gazit E (2003) Casting metal nanowires within discrete self-assembled peptide nanotubes. Science 300:625–627

Reichert J, Ochs R, Beckmann D, Weber HB, Mayor M, Löhneysen H v (2002) Driving current through single organic molecules. Phys Rev Lett 88:1768041–1768044

Reiss BD, Mao C, Solis DJ, Ryan KS, Thomson T, Belcher AM (2004) Biological routes to metal alloy ferromagnetic nanostructures. Nano Lett 4:1127–1132

Renault JP, Bernard A, Bietsch A, Michel B, Bosshard HR, Delamarche E, Kreiter M, Hecht B, Wild UP (2003) Fabricating arrays of single protein molecules on glass using microcontact printing. J Phys Chem B 107:703–711

Rietman EA (2001) Molecular engineering of nanosystems. Springer, Berlin Heidelberg New York

Ruben M, Breuning E, Lehn J, Ksenofontov V, Renz F, Gutlich P, Vaughan G (2003) Supramolecular spintronic devices: spin transitions and magnetostructural correlations in [Fe4IIL4]8+ [2×2]-grid-type complexes. Chemistry European Journal 9:4422–4429

Rulisek L, Havlas Z (2003) Theoretical studies of metal ion selectivity. 3. A theoretical design of the most specific combinations of functional groups representing amino acid side chains for the selected metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}). J Phys Chem B 107:2376–2385

Ruska H (1943) Versuch zu einer Ordnung der Virusarten. Arch Ges Virusforsch 2:480–498

Ruska H (1947) Über die Bindung des Sublimats an Bakterien und Virus. Arch Exp Pathol Pharmakol 204:576–585

Scheibel T, Parthasarathy R, Sawicki G, Lin XM, Jaeger H, Lindquist SL (2003) Conducting nanowires built by controlled self-assembly of amyloid fibers and selective metal deposition. Proc Natl Acad Sci USA 100:4527–4532

Schramm G (1943) Über die Spaltung des Tabakmosaikvirus in niedermolekulare Proteine und die Rückbildung hochmolekularen Proteins aus den Spaltstücken. Naturwissenschaften 31:94–96

Schwarz T, Schmidt F, Proll E (1966) Versuche zur tierpathogenen Wirksamkeit des Tabakmosaik-Virus. Naturwissenschaften 53:485–486

Shenton W, Pum D, Sleytr UB, Mann, S (1997) Synthesis of cadmium sulphide superlattices using self-assembled bacterial S-layers. Nature 389:585–587

Shenton W, Douglas T, Young M, Stubbs G, Mann S (1999) Inorganic–organic nanotube composites from template mineralization of tobacco mosaic virus. Adv Mater 11:253–256

Shimizu T (2002) Bottom-up synthesis and structural properties of self-assembled high-axial-ratio nanostructures. Macromol Rapid Commun 23:311–331

Sleytr UB, Sára M, Pum D, Schuster B (2001) Characterization and use of crystalline bacterial cell surface layers. Prog Surf Sci 68:231–278

Soong RK, Bachand GD, Neves HP, Olkhovets AG, Craighead HG, Montemagno CD (2000) Powering an inorganic nanodevice with a biomolecular motor. Science 290:1555–1558

Stubbs G (1999) Tobacco mosaic virus particle structure and the initiation of disassembly. Philos Trans R Soc Lond B 354:551–557

Tanaka K, Tengeiji A, Kato T, Toyama N, Shionoya M (2003) A discrete self-assembled metal array in artificial DNA. Science 299:1212–1213

Torimoto T, Yamashita M, Kuwabata S, Sakata T, Mori H, Yoneyama H (1999) Fabrication of CdS nanoparticle chains along DNA double strands. J Phys Chem B 103:8799–8803

Van Regenmortel MHV (1999) The antigenicity of tobacco mosaic virus. Philos Trans R Soc Lond B 354:559

Wall JS (1999) Visualizing "greengold" clusters in the STEM. J Struct Biol 127:161–168

Wang Q, Lin T, Tang L, Johnson JE, Finn MG (2002) Icosahedral virus particles as addressable nanoscale building blocks. Angew Chem Int Ed 41:477–480

Warner MG, Hutchison JE (2003) Linear assemblies of nanoparticles electrostatically organized on DNA. Nat Mater 2:272–277

Wetter C (1975) Tabakmosaikvirus und Para-Tabakmosaikvirus in Zigaretten. Naturwissenschaften 62:533–533

Winfree E, Liu F, Wenzler LA, Seeman NC (1998) Design and self-assembly of two-dimensional DNA crystals. Nature 394:539–544

Woehrle GH, Warner MG, Hutchison JE (2004) Molecular-level control of feature separation in one-dimensional nanostructure assemblies formed by biomolecular nanolithography. Langmuir 20:5982–5988

Yurke B, Turberfield AJ, Mills AP Jr, Simmel FC, Neumann JL (2000) A DNA-fuelled molecular machine made of DNA. Nature 406:605–608

Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001) Chemistry of coordination and hydration revealed by a K+ channel–Fab complex at 2 Å resolution. Nature 414:43–48