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FEATURE ARTICLE

Bionanoparticles and hybrid materials: tailored structural properties, self-assembly, materials and developments in the field

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We discuss new developments with respect to bionanoparticles as well as bionanoparticle hybrid systems and their use in composite materials. The recognition of the particle character and behavior of proteins and viral particles has a major impact on the development of novel nanoparticle systems and adds new functions and possibilities. The types of particles discussed in this review will give access to new functional materials, not only in medical- and biotechnological applications but also in electronic devices and possibly membrane-technology. Though, many systems have been developed, still more is to be discovered and significant advances are expected if more (sub-) disciplines are combined.

1. Introduction

The use of nanoparticles and nanostructures for the formation of new materials is a highly active field of research.¹ Properties of nanoparticles like size, shape and composition can be varied in accordance to their function in devices and self-assembling systems. Though most research using nanoparticles concerns

inorganic particles composed of gold, silver, silicon-oxide, iron, semi-conductors, *etc.*,² more and more the particle character of proteins and viral particles is being recognized.³ These bionanoparticles (BNPs) have very different properties and functions than their inorganic counterparts. Therefore, they can be applied in very different settings and are used for the development of new materials.⁴ The native BNPs by themselves already display interesting properties, not only with respect to catalytic activity, storage capabilities and a wide variety in shapes and sizes but also when introduced into systems which do not comprise a natural setting for these particles *e.g.* behavior at interfaces, serving as a template for the deposition of inorganic materials,

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wagenStiftung. The main research interests of his group include guided self-assembly of block copolymer systems, hierarchical (bio)nanoparticle assemblies and the control of self-assembly processes via external fields.

modifications with synthetic moieties or combining them with other biological structures normally not associated with each other.

Here we discuss recent developments in the use of BNPs and the investigation of their properties in the native form but also as new hybrid materials. By altering the protein structure, addition of new organic or inorganic elements and their combination with different materials, new hybrid BNP structures and materials are formed. This combination allows for the introduction of new properties with respect to self-assembling behavior, electronic properties and sensing capabilities. Recent discoveries are presented in the following, highlighting interesting materials and hybrid materials but also addressing the limitations and possibilities of the developed materials. We end with a discussion on how we feel the field should be developed and where great progress can still be made in order to generate tomorrow's hybrid materials with the same type of particle at its foundation as for that of life—protein structures.

2. Advantages of bionanoparticles

Bionanoparticles have their advantages and disadvantages just like any other system. Though, some of the disadvantages are not necessarily undesirable. Since the BNPs discussed here are proteins, there is always the chance that the specifically folded amino acid sequence is not stable towards certain conditions *e.g.* high temperatures, large pH deviations with respect to physiological conditions, harsh chemicals and hydrolyzing conditions. With respect to these properties, inorganic nanoparticles are vastly superior. However, as will be clear later, the drawbacks named here can also be exploited to their advantage and are in fact key features that produce new and interesting materials.

The main advantages are obvious, recognized by all and indisputable. Most of the advantages of these structures are the reason why life on Earth has developed nicely over the last billion years. The first one is the impeccable ability to have a near perfect reproducibility in formation of 3-dimensional structures. While in some cases, imperfections will lead to a self-regulating sequence in which structures are broken down again or cause the whole biological system to uncontrollably crash which is in a way also a self-regulating sequence, every other production of structures is perfect. Even though the manufacturing of inorganic nanoparticles has also seen major improvements over the years in reproducibility and monodispersity in size and shape, it cannot compete with the synthesis of biological ones.

The second and third advantages are closely related to each other as well as directly connected to the first one and that is the variations in size and shape and their biological function.

The size and shape are of course regulated by the precise sequence of amino acids, the length of the sequence and its final folding. Any variation in amino acid sequence and length will change the overall structure, the precise folding and the function of the protein. While inorganic nanoparticles also possess catalytic functions for organic reactions, proteins are a desired class of catalysts mainly because of their high efficiency as well as their ability to operate under physiological conditions.

As for their size and shape, there are many variations ranging from spherical/globular (*e.g.* CCMV, Ferritin, CPMV), wedges (subunits of TMV, PVX), disks/rings (1st assembled structure of

TMV, PVX), rods (full structure TMV, PVX) and even banana-shaped (Bin/Amphiphysin/Rvs (BAR) domain containing proteins). In addition to the shapes there are even more variations possible when considering that some structures are “massive”, hollow, with or without pores which in some structures are responsive and they vary from just a nanometre to several hundred nanometres either build from one single structure or formed by self-assembled protein capsids (Fig. 1).^{3,5}

As some of the protein structures are composed of several non-covalently connected subunits, they are able to dis-assemble/re-assemble. This dynamic behavior is useful for several features⁶ like when it is combined with *e.g.* inorganic nanoparticles which will be discussed in more detail in one of the next sections. Another appealing feature about this dynamic behavior is that while normally structures like viral particles assemble in the presence of their viral genome, RNA (RNA-virus) or DNA (DNA-virus), into a very specific structure, without this information, other self-assembled structures can be obtained. In many cases the overall structure is the same with or without its genome and only affects one dimension of its morphology as is the case for the length of TMV (Tobacco Mosaic Virus, Fig. 1). However, using different conditions for the aqueous medium, very different morphologies are obtained in addition to its conventional structure.⁷ In the case of TMV single filaments, disks and stacks can be achieved. The usually icosahedral structure of the CCMV (Cowpea Chlorotic Mottle Virus, Fig. 1) can be turned into multi-walled shells and tubular structures.⁸ Proteins which do not rely on the presence of RNA or DNA to guide the structure formation can be dis-assembled/assembled to regain their original structure. For some cage-proteins this property is used to remove some encapsulates as *e.g.* the iron-core in ferritin (Fig. 1) but it can also be used in analogy to the disassembled viral capsids to address other morphologies or again in combination with other structures to form interesting hybrid structures like protein containers of which the structure depends on the type of synthetic nanoparticle introduced.⁹

At the beginning of this section, the relatively easy degradation of the BNP was referred to as a disadvantage. Sometimes it is also utilized to obtain a specific type of material in which the particle is used as a self-assembling template for inorganic materials. Then the ease of removal is an advantage since inorganic nanoparticles are in general more difficult to remove without using extremely harsh conditions, the same also applies for the biodegradation when structures are used *in vivo* and need to be disposed in a natural fashion. More about the use of BNPs as template materials will be discussed in the more material related section.

We have briefly discussed the organic character of the BNP in the sense that they are built from amino acids. Since the amino acids differ in structure, polarity and charge, it is relatively simple to change the charges on the exterior; the interior is often too shielded. By simply changing the pH, the groups in the side-chains composed of carboxylic acids, amines and different hydroxyl-groups, including a phenolic group, can be changed from charged to neutral or *vice versa*. This change will affect its self-assembling behavior. The chargeable groups in the side-chain are in general also accessible for common organic reactions. This gives the possibility to easily add non-natural components with specific properties like fluorescence or specific

Fig. 1 Structures of protein assemblies varying in size and shape. Most frequently used by chemists for the development of new bio-based materials are virus particles but the protein cage ferritin is also often employed as a model structure (the different structures are obtained from ref. 3b, reproduced with permission of AAAS, Copyright 2006; ref. 3c, reproduced with permission of Wiley-VCH, Copyright 2011; ref. 5c, reproduced with permission of American Chemical Society, Copyright 2009).

binding moieties for other proteins, DNA or ions. In the worst case there are no functional groups present on the exterior and chemical modifications by organic synthesis are difficult. Then there is always the possibility to exchange certain non-vital amino acid residues in the sequence for others which do have certain functionality. These days this form of synthesis is common practice for biotechnologists who are able to produce custom made mutants of enzymes and viral protein capsids. The next section will briefly discuss some of the possibilities with respect to modifications either as mutants to yield certain additional properties or combine the protein structures with synthetic materials either inorganic or organic to produce novel hybrid materials. Especially the hybrid materials are a valuable addition to the self-organizing properties of BNPs and will most likely be the foundation of a new generation of materials.

3. Tailored design of bionanoparticles and their modifications

As mentioned in the previous section, BNPs can be modified to improve or alter their function, stability, reactivity towards synthetic additions or combine them with inorganic materials. A few of these modifications are given below to highlight most recent advances.

Mutations in amino acid sequences

Biotechnologists are able to make alterations to proteins in such a way that the proteins are still able to be produced in a biological fashion like being formed inside the cell—usually bacterial cells or yeasts are being used for their biotechnological production. They are able to incorporate alterations in the RNA which is then transfected in the cell and during the formation of the peptide strand; the changes are incorporated into the native strand. This allows for the introduction of amino acids with

specific properties at defined positions. The alterations are often used to stabilize proteins, add functional groups needed for synthetic adjustments,¹⁰ incorporate specific binding sites or change the polarity of a certain part of the protein.¹¹

Additions of inorganic components

Some protein structures naturally contain inorganic material *e.g.* ferritin has an iron-core in the form of crystal ferrihydrite ($5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$) when it originates from mammals, while bacterial ferritins contain also a form of phosphate in some cases referred to as iron-phosphate-oxyhydroxide.¹² Other proteins that can contain inorganic elements are: dps (DNA-binding proteins from starved cells), transferrin and hemoglobin. These mostly contain iron either in the oxide- or in the ionic-form, but other proteins that bind different metals are also known like hemocyanins and blue copper proteins (Cu) and nitrogenases (Mo and V).¹²

In the case of ferritin, alterations to its core structure date back to 1992 when the iron-oxide core of ferritin was exchanged for a ferrimagnetic iron-oxide one (Fe_3O_4) thus introducing magnetism to the structure.¹³ Since then many different core structures have been added¹⁴ each with properties potentially useful for application as contrast agents (ferric iron or molybdenum),¹⁵ anti-bacterial agents (silver),¹⁴ biosensors and imaging (addition of quantum dots for fluorescence tracking and imaging)¹⁶ or as novel biocatalysts (photoactivation by quantum dot followed by oxidation by attached cytochrome P450)¹⁷ (Fig. 2).

Not only replacements have been made in existing inorganic materials containing proteins like exchanging one inorganic core for another but also because of the specific shape of other proteins, they have also been used as templates on which inorganic material is condensed. By attaching inorganic material to the outside or the inside of proteins, the shape is maintained and

Fig. 2 (A) The hollow structure of apoferritin can be filled with a non-natural inorganic core like silver. This occurs through the uptake of silver-ions into the cavity followed by reduction. (B) A Janus-type particle in which enzymes (P450_{B5H}) are attached to a quantum dot (QD) and photo-induced $\text{O}_2^{\cdot-}$ and/or $\cdot\text{OH}$ by irradiation of the QD induces P450 to catalyze a monooxygenation of a fatty acid (images are obtained from ref. 14d, reproduced with permission of American Chemical Society, Copyright 2010; ref. 17, reproduced with permission of Wiley-VCH, Copyright 2006).

inorganic structures can be formed with a relatively high monodispersity in size and shape usually not accessible with conventional synthetic procedures. Especially the TMV is a very popular structure used for the fabrication of gold¹⁸ and silicon oxide nanorods.¹⁹ The combination of inorganic components with protein structures not only provides special inorganic structures but the hybrid materials are also able to complement each other providing new self-assembling properties and functions. These new properties will be more elaborately discussed later.

Functionalization with synthetic molecular components

Instead of altering the basic amino acid sequence or adding inorganic components, new synthetic molecular moieties can also be added to the BNPs. Since there are various amino acids with reactive groups on the side chain, it is not particularly difficult to

add functional molecular components. In many cases one can rely on simple peptide coupling reactions or nucleophilic additions selective to primary amines as well as formation of disulfide bonds (Fig. 3). These reactions already give many possibilities to introduce small molecular components,²⁰ polymers,^{21,66,67} or other reactive groups which are able to perform specific coupling reactions like the often used “click” chemistry between an alkyne and an azide (see ref. 22 for a detailed overview of possible reactions for chemical modification of proteins and viral particles).²² Sometimes the protein structure is interesting in its architecture or function but lacks the common reactive groups needed for certain couplings. In that case unconventional reactions need to be used or the tools of biotechnology are required to implement the alterations in the primary amino acid sequence and to provide the chemist and materials scientist with the proper possibilities for synthetic modifications.

In addition to introducing new molecular properties by the addition of synthetic moieties, the stability of proteins can be enhanced, especially with respect to the tolerance of organic solvents²³ but also the surface polarity can be influenced providing some means to address the self-assembling properties. The next sections will discuss the latest discoveries in BNP hybrid materials, meaning that protein structures are specifically combined with inorganic structures, synthetic molecular structures or even BNPs with other BNPs to obtain new materials with interesting self-assembling properties as well as functions. Though, the hybrid materials will be the driving force for the development of the field, the self-assembling properties of the

Fig. 3 Frequently used reactions for chemical modification of proteins with synthetic components. Most convenient is the modification of either lysines (reactions 1–3) or cysteines (reactions 4–6).²² The reactions add functional groups that can be further transformed by *e.g.* “click-reactions” or Atom Transfer Radical Polymerization (ATRP).

native protein structures in all their diversity cannot be neglected since it is often these properties that are combined with the synthetic structures to create hybrid materials.

4. Self-assembling properties of bionanoparticles and their hybrids

In nature, proteins/bionanoparticles are often not confined to one place inside the cell but are able to travel to various locations, usually guided by the surface charge or distribution of hydrophobic regions, just as a different set of those properties confines them to membranes. Without a larger regulating system, the bionanoparticles still have these properties but do not have the same overall system to guide them and therefore different types of aggregation can be induced. Here we distinguish two levels of self-assembly. On the particle level they can assemble/disassemble reversibly into a discrete structure with a fixed number of assembling units and with specific dimensions. Secondly, the self-assembling properties of the protein structures or the sub-units influenced by changing the environment, by the addition of addressable interfaces either liquid/liquid, liquid/solid or liquid/gas, acting as templates (polar/apolar, oppositely charged) or by the addition of synthetic structures (*e.g.* coordinating or covalently attached polymers and protein conjugation with small molecular components).

Intra-bionanoparticle self-assembling properties in solution

The intra-bionanoparticle self-assembly in solution occurs through their dynamic behavior when composed of several subunits like in the case of cage-proteins. Cage-proteins like ferritin (Fig. 4A), Hsp (heat shock protein) and dps (DNA-binding protein of starved cells) and the well-known viral particles like CCMV and TMV (Fig. 4B) are composed of individual subunits which are able to disassemble/reassemble with high accuracy, even without the genomic information, and in the case of TMV, different self-assembled states can be addressed depending on the choice of the medium (pH and ionic strength) (Fig. 4). This rearranging can also be done with other structures to achieve a variation in the self-assembled structure with respect to the size. This was shown for CCMV which after formation of a dimer could be assembled in spherical structures consisting of 30, 60 or 90 dimer units.²⁴ The advantage is that when there are natural components present *e.g.* RNA or inorganic cores, this can easily be removed. While normally, the structures reassemble into their native structure again, choosing the correct reactive additives which can act as a template, the introduced materials, inorganic, organic or biological, can be bio-functionalized. *Via* coordination by non-covalent interactions or due to chemical reactions, the subunits are transferred to the templates instead of re-assembling into their native structure and newly functionalized nanoparticles are formed or new supra-molecular structures evolve from the coordination.²⁵ These structures will be discussed more elaborately in one of the next sections.

Inter-bionanoparticle self-assembling properties in solution

Proteins and protein sub-units are also able to interact with each other due to non-covalent interactions like hydrophobic

Fig. 4 Dynamic structures of: (A) ferritin and (B) TMV. The formation of the different structures is reversible and depends on the medium with respect to pH and ionic strength (figures obtained from ref. 5c, reproduced with permission of American Chemical Society, Copyright 2006 and ref. 5d, reproduced with permission of The Royal Society, Copyright 1999).

interactions, complementary surface charges or hydrogen bonding motifs to form larger non-native structures. It was found that certain combinations of small peptide structures and proteins are also able to form other structures as well, namely, peptidic macrocycles or "proteinaceous nanostructures" as they are called by Wagner *et al.* Designing different peptide structures increases the scope of materials that are able to be formed.²⁶ Taking a dimeric protein species in combination with a flexible peptide-linker, different self-assembled macro-cycles in solution were obtained. The size of the cycles was found to be dependent on the length and composition of the flexible spacer and the specific structure and interactions of the dimerizer. Another variation also produced nano-rings with catalytic properties which were found to be dependent on the ring size.²⁷

A ring structure triggers immediately the notion of self-assembling units forming tubular structures when stacked on top of each other. This structure is observed in the honeycomb crystal structure of stacked ring-shaped peptides, the

homohexameric Hcp1 from *Pseudomonas aeruginosa*. This observation inspired Mougous and coworkers to introduce specific cysteine mutations in order to allow for the formation of disulfide bonds between macro-cycles in a head-to-tail fashion, creating stable tubular structures in solution²⁸ (Fig. 5A).

Protein–fiber structures are an important feature in biological systems *e.g.* the cytoskeletal build from actin, which is probably why scientists are attempting to recreate these structures and additionally also preparing tubes since an empty inner space would allow for additional functions like storage or perhaps even transport. As in the previous example, it is most convenient to take a promising natural structure and modify it synthetically. There are two ways of addressing this. One approach is to take disk-like protein structures which are not prone to assemble into tubes but after the introduction of a mutation are, as we have seen previously and in other examples (Fig. 5B).²⁹ A second approach is taking a structure from nature that already forms a fiber-like morphology and attempt to introduce more control over its dimensions which was done by introducing a capping protein to the fiber-system. Clark *et al.* showed that by introducing a compatible protein of a similar design which was able to associate into the fiber but prevented further growth.³⁰ One can imagine that control over the dimensions of such structures potentially offer different materials with various properties when different dimensions or hierarchies of self-assembly can be addressed. In some cases one does not need to rely on synthetic modification and the native system already exhibits this control. This form of hierarchically building of structures was found for ADP-ribosyl cyclase which assembles from the monomeric state into defined dimer and subsequently hexamer, finally forming fibers which are crystalline displaying nanorods and

microtubes.³¹ Though not every naturally occurring protein has the properties for the formation of these hierarchically self-assembled structures, it is more and more recognized that other properties can also be exploited. This can range from the use of applying potential differences in order to induce polymerization of actin filaments and orient the fibers they produce.³² But also proteins already prone to form networks as fibrin does in blood clots and thrombi can be used. By isolating the proteins from other interacting species, interesting structures were discovered depending on the concentration as was found for fibrin which forms thin nano-sheets at low concentrations.³³

When proteins are considered as nanoparticulate building blocks, one of the earlier mentioned drawbacks is stability towards conditions which can denature the protein's primary structure. This instability, however, can also be used as a tool to form supra-molecular structures as a response to the denaturing conditions. It was found that hen egg white lysozyme is able to form thermo reversible gels with the use of a denaturing agent like DTT (dithiothreitol).³⁴ That proteins by themselves are able to form gels due to denaturing is trivial, since this is exactly the structure of a boiled egg. However, with the knowledge that this occurs and the realization that this can be induced by different stimuli like heat, reducing agents or even ultra-sound and the fact that proteins behave like particles, interesting structures and morphologies have been discovered,³⁵ especially, in combination with interfacial self-assembly as will be shown in the following section.

Self-assembling properties of bionanoparticles at interfaces

Self-assembly of BNPs is not limited to solute state aggregation. Because of their particle character, it was found that proteins behave in a similar fashion towards polar/apolar interfaces like colloidal particles. The interface between a polar phase (*e.g.* water) and an apolar phase (*e.g.* oil) is then stabilized by micron- to nanometre-sized inorganic particles and the effectiveness of the stabilization depends on the particle-size, particle–particle interactions and the particle interactions with both phases.³⁶ Such an emulsion is called a Pickering emulsion and is very different compared to an emulsion stabilized by conventional surfactants which rely on their amphiphilic character. Recently, Pickering emulsions stabilized by proteins and viral particles were found to exhibit similar properties as they are formed by comparable driving forces^{36,37} which in some case are enhanced by an additional amphiphilic character of the particles.³⁸ Moreover, the interfacial activity can also be influenced changing the surface charges on the protein³⁹ *e.g.* by changing the pH of the aqueous phase to either protonate or deprotonate side groups on the surface of the BNP.

Interfacial self-assembly is not restricted to spherical particles and also anisotropic particles can be used to stabilize the interface. This was found when in addition to CPMV (Cowpea Mosaic Virus)⁴⁰ and TYMV (Turnip Yellow Mosaic Virus),³⁷ which are both icosahedral viral particles, also TMV (Tobacco Mosaic Virus) was introduced to polar–apolar interfaces.³⁷ While both spherical particles were clearly present at the water–oil interface, a more striking effect was observed for the anisotropic rod-like TMV particle. Initially at low concentrations, it was observed that the particles orient parallel to the interface,

Fig. 5 Formation of nanotubes in solution of different length scales; (A) is composed of Hcp1_{CC} protein, a mutant of the Hcp1 *Stacked Pseudomonas aeruginosa* with two cysteine incorporations and displays short non-helical tubes of various lengths up to about 90 nm (scale bar 15 nm); (B) shows single structures of the wild type protein TRAP (trp RNA-binding attenuation protein) from *Bacillus stearothermophilus* on the left and the TRAP–HCCAH mutant in the presence of DTT which induces polymerization, forming long tubular structures (scale bar left: 50 nm; right: 100 nm) (images adapted from ref. 28, reproduced with permission of National Academy of Sciences, Copyright 2008 and ref. 29, reproduced with permission of Wiley-VCH, Copyright 2009, respectively).

which is highly plausible since this orientation will cover the most surface area. However, while with spherical symmetric particles, a fully covered surface remains unchanged and any additional particles stay in solution and do not adsorb to the fluid interface, anisotropic particles apparently behave very differently. Increasing the concentration induced a reorientation of the rod-like particles resulting in rods oriented perpendicular to the interface in order to maintain a high interface coverage while neutralizing inter-rod electrostatic repulsions (Fig. 6A and B).³⁷ This phenomenon not only provides the possibility to control the surface structure of the interface to a certain extent but also allows to introduce a potential channel-like function if one remembers the hollow structure of the TMV, shown in the previous section about BNP structures. That it can act as a channel was in fact shown by incorporation of the TMV in lipid membranes leading to a higher mass transport.⁴¹ Interfacial properties of BNPs have also been used to produce well defined shells around polymer droplets in water, displaying the distinct features of the BNPs on the surface (Fig. 6C).⁴²

As mentioned above, proteins are able to stabilize interfaces.⁴³ There, they can be co-complexed or exchanged by conventional non-ionic and ionic surfactants⁴⁴ or they can denature. During the denaturation process the unfolded peptide-chains stick together and form gel-type and agglomerates. Such a behavior either happens naturally or can be triggered, which is already used in preparations of emulsion foods.⁴⁵ In addition to denaturing proteins like lysozyme with reducing agents, as was shown in the previous section, ultra-sound is also able to induce the denaturation process.⁴⁶ Emulsion preparation with hen egg white lysozyme and crude hen egg white either by introducing air or with the use of oil yields hollow stabilized air capsules⁴⁷ as well as

interesting soft capsule in capsule structures where aqueous stable capsules reside inside a stabilized oil capsule inside an aqueous medium.⁴⁸ A similar approach was also used for the preparation of protein-silica hybrid materials where a silica precursor (tetraethoxyorthosilicate) was employed in combination with lysozyme and silica nanoparticles for the creation of hollow silica capsules.⁴⁹

Self-assembling properties of BNPs are not restricted to liquid-liquid and liquid-gas interfaces. The assembly on liquid-solid interfaces has also been utilized to create ordered structures from BNPs. The additional value of using a liquid-solid interface is that after removal of the liquid phase, the remaining particles on the solid interface can be transferred to other substrates *via* contact printing. The structure of the printed pattern is then the same as the self-assembled structure and by using a preformed solid surface different patterns can be obtained. These different structures are prepared by using PDMS molds. *Via* the molds, square patterns and lines of a few microns in size could be achieved.⁵⁰ By using nano-wrinkled PDMS substrates, smaller print features and a larger number of printed structures could be produced in a single step. Here the TMV particles were used as a model system. They reside at the bottom of the wrinkle grooves and upon printing onto a solid substrate, long lines of TMV structures are formed of just tens of nm in width (Fig. 6D).⁵¹

Self-assembling properties of bionanoparticle-inorganic hybrid structures

We have established that BNPs (proteins and viral particles) are able to self-assemble in solution and at interfaces. However, as mentioned in the previous section addressing chemical modifications of the BNPs, it is the hybrid material that will enable the generation of new materials with multiple functions. Self-assembly is a powerful tool to create a well ordered system based on the properties of the particles. The self-assembly can be used to form a BNP hybrid system or the hybrid is synthesized and able to self-assemble, creating new functional materials.

In the first case, an inorganic structure (particle or flat interface) is usually used as a template on which the BNP is able to adhere. When there is a strong preference for an interface due to electrostatic, protein/protein or chemical interactions (thiol-gold linkage), it is possible to produce a bio-functionalized inorganic structure. This will provide a system that has the properties of inorganic materials *e.g.* fluorescence, magnetism and catalytic properties but because of the adsorption of proteins or protein fragments is also biological compatible, exhibits enhanced water solubility and would be considered as potential biosensor. In order to have particles covalently linked, there should be complementary reactive groups on both particles. One approach is using a thiol-linkage between *e.g.* a protein with freely accessible cysteine groups or a protein modified with a synthetically added thiol-linker. Both approaches have successfully been used to modify either flat surfaces⁵² or nanoparticles.⁵³ Recently, Horse Spleen Ferritin was modified with such thiol-linker tethering it to gold nanoparticles onto surfaces to produce long range ordered arrays.⁵⁴ When looking at the inorganic nanoparticle protein structure as a single species instead of condensed to a surface, the hybrid structure can be seen as a Janus-type nano-assembly which by itself already yields interesting

Fig. 6 (A) Scanning force microscopy analysis of a dried water-oil interphase stabilized by TMV at low concentration displaying parallel orientation with respect to the interface; (B) at high TMV concentration displaying a perpendicular orientation; (C) FESEM image of a polymer droplet (P4PV) stabilized by TMV, the structures are embedded in the surface; (D) self-assembled structures of TMV on a wrinkled PDMS solid-liquid interface which can be transferred to another solid substrate by contact printing (images adapted from ref. 37b, reproduced with permission of American Chemical Society, Copyright 2009; ref. 42, reproduced with permission of RSC, Copyright 2009; ref. 51a, reproduced with permission of RSC, Copyright 2009).

possibilities, especially when the protein has catalytic functions as was mentioned earlier.¹⁷ There are of course other ways to attach particles together and one very elegant example has been shown by Park and coworkers. Instead of relying on a group which is able to interact with the inorganic material, both the inorganic nanoparticle and the protein structure (here an organic viral-like particle, Q β -phage capsid) were modified with complementary DNA-fragments. The particles formed a NaCl-type crystalline structure by self-assembly in solution.⁵⁵ While this example shows the combination between two specific particles, this approach has a universal character. In principle, any two particles are able to be bound in this way and depending on the positions of attachment which can be varied depending on the protein used as well as the number of attachments, a whole range of different materials can be formed with different combinations of inorganic nanoparticles and BNPs.

While covalent systems may provide stable materials, they do provide a certain risk. Once a chemical bond is formed it will not break that easily without destroying the materials itself. Materials formed by self-assembly using non-covalent interactions are able to self-correct and therefore offer a more versatile approach. Interactions range from hydrophobic, coulombic and specific protein–protein interactions, though the last one can in some cases be as strong as covalent interactions concerning streptavidin/biotin and barstar/barnase which have extremely high association constants of 10^{15} and 10^{14} M⁻¹, respectively. One such system that has a similar universal approach to as with the DNA-system was shown by Deyev *et al.* They made use of the barstar/barnase couple to link particles of various types together in solution.⁵⁶ They have constructed a trivalent particle in such a way that a central magnetic particle holding covalently connected barnase was mixed with quantum dots holding two moieties of barstar. This produced a layer of quantum dots around a magnetic particle. Subsequently, an outer layer of a barnase/scFv–antibody complex which is able to recognize certain antigens on cancer cells was attached to the barstar covered surface (Fig. 7A). This sort of particle combines a recognition system together with a biomarker and cytotoxic component.

Other recent approaches are based solely on electrostatic interactions. The surface of an inorganic structure can be negatively charged to allow viral capsid proteins assemble onto the surface providing interesting inorganic core–protein shell structures.⁵⁷ In addition, direct complexation to nickel-ions bound to the surface of inorganic gold nanoparticles in combination with specifically histidine-tagged proteins is also possible. These are able to form various combinations of a single protein connected to a single inorganic nanoparticle and two or three proteins connected to the inorganic nanoparticle as well as core–shell structures. The different complexes were isolated after purification of the protein/nanoparticle mixture.⁵⁸

The structures formed are not always confined to small nanostructures. When several binding sites of the protein are distributed evenly, several larger structures also form depending on the size of the protein.⁵⁹ It was found that a protein like GFP (Green Fluorescent Protein, 27 kDa) that is small compared to an inorganic positively charged gold nanoparticle assembles around the gold nanoparticle. When the protein is larger than the inorganic particle like acid phosphatase (110 kDa) then

Fig. 7 (A) A schematic representation of the build-up of complex nanostructures of a magnetic particle surrounded by quantum dots and with an outer shell of catalysts, held together by non-covalent forces of two complementary proteins. It stains cancer cells which are shown on the left; the fluorescent pattern is made by selective magnetization of the surface where only the cells with the particles adhere. (B) A soft capsule of which the membrane is composed of protein-cages surrounded by UV cross-linkable polymers. The formation occurs by interfacial self-assembly at an oil–water interface with subsequent UV-irradiation (images obtained from ref. 56, reproduced with permission of National Academy of Sciences, Copyright 2010, ref. 66, reproduced with permission of Wiley-VCH, Copyright 2011).

the inorganic particles assemble around the protein structure. The larger extended assemblies occur when the protein and the gold nanoparticle are of similar size as was found for BSA (Bovine Serum Albumin, 66.3 kDa). Not only the size affects the formation of extended aggregates between inorganic nanoparticles and proteins but also the conformation of the protein structure. When Cytochrome c was mixed with negatively charged nanoparticles, only a minor change in size of the composite material was observed in contrast to ApoCytochrome c which has a more random coil structure. ApoCytochrome c is obtained upon removal of the heme-group on Cytochrome c and has a lower defined overall structure. This difference in secondary and tertiary structure induces the formation of larger self-assembled structures depending on the ratio of the ApoCytochrome c/nanoparticle used.⁶⁰

Self-assembling properties of bionanoparticle–polymer hybrid structures

Now we turn from inorganic nanoparticle–protein hybrid structures to the conjugation of polymers to proteins which has gained increasing interest over the last few years. Polymers are available with various properties, *e.g.* with respect to polarity,

charge, responsiveness, cross-linking capabilities and are in general easily accessible with various lengths in organic solvents as well as in water. As with the combination of the particles, polymers and proteins can be used in non-covalent as well as in covalent systems. Non-covalent systems have been used with viral particles like CPMV and TMV in combination with polyelectrolyte polymers and polyaniline. It was found that layer-by-layer assembly of CPMV and TMV onto a surface behaved very differently.⁶¹ Even though the surface of both particles is very similar, the behavior is very different which was attributed to the shape of the particles. CPMV could be incorporated in between thin layers of poly(ethyleneimine) and poly(acrylic acid) while TMV needed much thicker layers of the polyelectrolytes before a second layer could be added and the anisotropic larger particles were not strongly confined to the intermediate phase and were able to float towards the top of the layers. Very recently, similar experiments were performed in solution with the overall negatively charged cowpea chlorotic mottle virus (CCMV) and different cationic linear, Newkome-type dendrons and dendrimers where it was shown that complexation between the species yields large aggregates and that the interactions between the electrolytes can be tuned with the ionic strength of the solution.⁶²

The formation of different morphologies with the use of TMV was also observed in combination with the polymerization of aniline.⁶³ Using different initial TMV concentrations elongated one-dimensional nano-wires were obtained upon polymerization of aniline on the surface of the TMV or when higher concentrations were used a more lateral assembled initial structure was obtained. Both structures resulted in bundled fiber composites of polyaniline and TMV. The overall morphology of the bundled fibers was also adjusted using different pH-values during the polymerization process.

When polymers are conjugated to a protein structure, new properties can be implemented inherent to the properties of the polymer. These polymers may be attached in a single step (grafting to approach) or a polymerization reaction can be performed from the surface of the protein (grafting from approach). This type of hybrid materials provides the possibility to increase interfacial activity, decrease the amount of denaturation of the protein on an interface or render the proteins more compatible with polymer structures. Ferritin is a frequently used protein for these types of modification. It was found that ferritin-PEG (polyethyleneglycol) hybrids take longer to completely saturate the surface of solid silica substrates than unmodified ferritin and that the hybrid is more easily removed from the surface. Also, proteins adhered to interfaces in general denature to some extent which can affect the properties and functions of proteins like enzymatic activity; with the polymer attached it was found that no denaturation takes place.⁶⁴ Similar structures were also used for the integration into polystyrene/polyethyleneoxide diblock copolymer thin films.⁶⁵ In this approach the ferritin protein cage can be directed towards one phase in the thin film. The directionality would be especially interesting in combination with non-natural inorganic ferritin cores like quantum dots and conducting polymer phases.

Additional properties can be incorporated into the ferritin by using random co-polymers attached to the exterior of the ferritin. In our group we recently developed a ferritin-polymer hybrid

composed of the thermo-responsive polymer PNIPAAm (poly (*N*-isopropyl acrylamide)), a UV-responsive chemical cross-linker DMIAAm (2-(dimethyl maleinimido)-*N*-ethylacrylamide) and a fluorescent co-monomer.⁶⁶ It was shown that the polymer composite particles had high interfacial activity and that after the self-assembly of the particles around oil droplets in water they could be additionally stabilized by chemical cross-linking, producing stable soft capsules (Fig. 7B).

Nolte and Cornelissen used a different approach to synthesize polymer-protein conjugates. They attached a single polymer chain on one specific location on the protein. It was found that a giant amphiphile could be created when a polystyrene chain was attached to a single protein acting as a hydrophilic head-group and forming a fiber network in water/THF mixtures.⁶⁷ When a thermo-responsive polymer was attached, a hybrid structure was formed which at low temperatures is sufficiently hydrophilic not to induce aggregation but as soon as the temperature is raised above the lower critical solution temperature of the polymer, the chain becomes more hydrophobic and an amphiphile is created which starts to aggregate.⁶⁸

The systems discussed above are all recently developed and display certain properties with a high potential for possible applications. In the next and final section some recent hybrid materials are highlighted based on the different chemistries discussed in the previous sections.

5. Promising materials, applications and future prospects

The most obvious functions of BNP hybrid systems are usually thought to be for drug/gene-delivery, bio-sensing and bio-imaging. Even though these functions are most likely first to be developed commercially, other applications for composite materials *e.g.* in electronic devices and functional membranes also hold a high potential for introducing BNP composites.⁶⁹

Promising materials and applications

For drug-delivery, bio-sensing, bio-imaging and catalytic bio-reactors, the viral capsids are very promising candidates especially for holding single species in the nanometre regime.⁷⁰ Since these capsids can reversibly open and close upon stimuli or even completely disassemble/reassemble, it provides an easy route for loading and release.⁷¹ In accordance, viral particles have been used to store artificial DNA,⁷² DNA micelles with hydrophobic components inside the hydrophobic domain of the micelle,⁷³ single enzymes⁷⁴ as well as multiple proteins.⁷⁵ This encapsulation offers possibilities to introduce DNA as a gene delivery system or bio-catalysts in a protected environment into the body. When the catalyst would produce molecular oxygen or have specific reducing or oxidizing properties important for cellular functions, it could be used *in vivo* to either restore cellular functions or destroy malignant cells. Also quantum dots packaged inside viral capsids were used to investigate viral behavior inside mammalian cells, mimicking initial behavior of the wild-type variant and showing very different localization inside the cell (the non-encapsulated has non-specific localization).⁷⁶ Not only viral nanoparticles are used but also with ferritin very sophisticated structures are being formed of which many were

produced by Douglas and Young. One promising system was a combination of genetic engineering in order to modify the end terminus of ferritin, a ferrimagnetic iron core and fluorescence labeling.⁷⁷ The particles were able to target cancer cells and it shows that multi-functional BNP hybrid structures can be synthesized with specific functions to be used as a diagnostic imaging agent.

Such small hybrids for bio-imaging do not necessarily need to be formed by protein encapsulation of a fluorescent core. A protein and quantum dot can also be linked together in a Janus-type fashion. Medintz and coworker synthesized a Janus particle in which the protein mCherry was attached to a quantum dot by a peptide linker which can be cleaved by Caspase 3 of which the activity is thought to be related to different kinds of cancer (Fig. 8A).⁷⁸ The mCherry and quantum dot have overlapping spectral properties enabling Förster Resonance Energy Transfer (FRET) which can be measured. If the Caspase 3 is active, the linker will be broken and FRET will not occur. This system is a sensitive tool to measure protein activity and would be a possible diagnostic tool.

When a delivery system for active components is needed that is able to hold larger quantities, then other types of materials are preferably used. For this purpose, protein- or protein-polymer conjugate-stabilized emulsions are used and *via* a secondary stabilization factor, mechanically robust capsules can be formed. The additional stability can be given by *e.g.* gelation of the oil-phase or *via* surface induced bio-mineralization. In the first case an organo-gelator was added to the apolar phase and after the initial stabilization by the protein in a Pickering emulsion, the gelation of the oil-phase was induced by ultra-sound.⁷⁹ As

mentioned earlier, similar stabilized capsules were also prepared by denaturation induced gelation of the protein shell.^{48–51} While the stabilization gives soft capsules, bio-mineralization provides a very strong and tough capsule. Deposition of natural minerals like calcium phosphate onto the interface stabilized by proteins produces capsules with a hard shell of hydroxyapatite which are compatible with biological structures like bone.⁸⁰ In addition to the non-modified proteins, also the protein-polymer hybrids display promising applications. The cross-linkable ferritin-polymer conjugate as mentioned before forms stable o/w capsules.⁶⁶ However, by adjusting the preparation method, not only o/w capsules were obtained but also w/o capsules with the possibility of transferring both capsule types into the same solvent from which the inner compartment is composed of creating w/w and o/o capsules (Fig. 9A).⁸¹

Small structures for sensing or larger spherical capsules are not useful for every type of application and in many materials larger structures like fibers would be preferred. These can be used as wires in electronic devices, larger materials for surface coverage or even modification of actual macroscopic materials as a whole. As mentioned earlier, protein cages and viral particles are able to disassemble and reassemble with high accuracy and return to their original form unless a template is being offered with which the disassembled small protein fragments interact. This behavior was used in a multi-molecular system composed of modified single stranded DNA, cowpea chlorotic mottle virus and chromophores with hydrogen bonding moieties able to coordinate to the DNA.⁸² By combining these components an electronically viable DNA wire was produced and due to the additional assembly of the viral capsid proteins on the surface of that composite, even longer wires in the micrometre range were

Fig. 8 (A) A quantum dot/fluorescent protein biosensor based on a digestible peptide-linker and Förster Resonance Energy Transfer; (B) microcapsules formed and stabilized by ferritin-polymer conjugates which are cross-linked and therefore are stable enough to be transferred to any medium, polar as well as apolar (images obtained from: ref. 78, reproduced with permission of American Chemical Society, Copyright 2009; ref. 81, reproduced with permission of RSC, Copyright 2011).

Fig. 9 (A) A hybrid structure of three proteins connected, each performing a different task to form bio-sensing fibers in solution; (D) a PVX used as a nano-template for multiple biocatalysts mounted on the exterior of the tubular virus particle (images obtained from: ref. 85e, reproduced with permission of Wiley-VCH, Copyright 2010; ref. 92, reproduced with permission of Nature Publishing Group, Copyright 2007).

produced and only about 20 nm in diameter. The material produced could be used in electronic devices as a conducting wire but also other structures useful in nano-electronic devices have been developed also using BNPs as a template.⁸³ Genetically engineered TMV was used to produce nano-structures on gold surfaces with potential as battery electrodes. The TMV was deposited onto a gold surface in an upright position due to specific cysteine binding groups and subsequently an electroless deposition of nickel or cobalt was performed. The uniformly coated TMV structures were found to be still attached to the surface and the overall shape unaffected.⁸⁴ The introduction of conjugated molecules, metals, semiconductors and other electronically active materials in, on or connected to biological species shows great promise⁸⁵ (Fig. 9B), though still a lot of work has to be done to produce working devices.

Future prospects

More sophisticated systems are being prepared with BNPs and slowly in some areas, replacing inorganic nanoparticles or are joined together to induce new properties. It is likely that in the near future, actual products will emerge. However, in order to drive the field of BNP based materials and systems, cross-overs between and the combining of disciplines needs to be enhanced. From the examples discussed above it becomes clear that biotechnology is combined with inorganic chemistry, organic/polymer chemistry, physical chemistry, material science and biology. Though the interdisciplinary aspect is strongly present, it is mostly confined to two or three disciplines. It can be stated that this is not sufficient to really reach the full potential of the materials shown here. A higher degree of interdisciplinary interactions will give a better understanding about different aspects of a system *e.g.* what is synthetically feasible, how does it function *in vivo*, what are the physical and mechanical properties, *etc.*⁸⁶ We feel a strong need to combine more of the specific knowledge provided by the different forms of chemistry in order to exploit every aspect and property of a developed system. Some systems already display some of the “omni-disciplinary” character *e.g.* the ferrite magnetic (inorganic) fluorescently labeled (organic) genetically engineered ferritin cage (biotechnology) which is able to target cancer cells (biology);⁷⁷ the cross-linkable polymer coated (polymer) ferritin (biotechnology) producing macro-structural capsule–fiber network (material science) driven by interfacial activity (physical) with controlled release of active substances⁸¹ and some of the systems where inorganic or organic compounds are combined by encapsulation which are able to enter cells and either sense or image cellular events or perform other duties.^{74,78} Although an increase in the use of BNPs is seen, relatively inactive structures are being used. The inactive protein structures often just serve as templates⁸⁷ and if they do possess certain properties which can be transferred to bulk materials,⁸⁸ the properties are usually synthetically added. By itself this is not a negative aspect, especially the latter mentioned, otherwise the “omni-disciplinary” statement is an empty one but the native biological functions of proteins are often neglected. Instead of a protein-cage with its sole biological function being storage, why not take a protein which is able to perform catalytic reactions. Systems have recently been reported in which the catalytic reaction of an enzyme is able to induce aggregation processes,⁸⁹

perform enzymatically catalyzed hydrolysis at interfaces⁹⁰ or show mass transfer using selective gating proteins which can be pressure-activated.⁹¹ With respect to hybrid structures, even a viral-enzyme hybrid system has been developed in which a viral particle has been used as a scaffold to mount bio-catalysts which still showed activity.⁹²

Of course when proteins are being used for their catalytic activity extra care needs to be taken not to disturb its structural features, accessibility of the reactive center and space for rearrangements during the catalytic cycle. Using the native catalytic functions of proteins more frequently will aid in the development of new and interesting materials.

6. Conclusions

In conclusion bio-inspired materials with additional or altered functions based on the recognition of the particle character is vastly being explored. Though, new materials and systems have been developed based on protein structures in combination with inorganic particles, polymers, and other proteins which show tremendous possibilities for being used in pharmaceuticals, bio-sensing and bio-imaging, still the full potential of the systems has not been reached. In order to get more functionality of a system and to speed-up the development towards actual commercial product, more cross-over between scientific disciplines is needed than has been shown so far. Like the sum of the individual components in the cell is greater and more sophisticated than the individual components, also a vast cross-talk between many disciplines will yield an added value to research on BNP hybrid systems.

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Notes and references

- 1 S. Mann, *Nat. Mater.*, 2009, **8**, 781–792.
- 2 (a) P. V. Kamat, *J. Phys. Chem. B*, 2002, **106**, 7729–7744; (b) Y. Sun and J. A. Rogers, *Adv. Mater.*, 2007, **19**, 1897–1916; (c) Z. Niu, J. He, T. P. Russell and Q. Wang, *Angew. Chem., Int. Ed.*, 2010, **49**, 10052–10066.
- 3 (a) M. Uchida, M. T. Klem, M. Allen, P. Suci, M. Flenniken, E. Gillitzer, Z. Varpness, L. O. Liepold, M. Young and T. Douglas, *Adv. Mater.*, 2007, **19**, 1025–1042; (b) T. Douglas and M. Young, *Science*, 2006, **312**, 873–875; (c) E. M. Plummer and M. Manchester, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2011, **3**, 174–196.
- 4 M. Fischlechner and E. Donath, *Angew. Chem., Int. Ed.*, 2007, **46**, 3184–3193.
- 5 (a) S. E. Anlagyei, C. DuFort, C. C. Kao and B. Dragnea, *J. Mater. Chem.*, 2008, **18**, 3763–3774; (b) D. J. Kushner, *Bacteriol. Rev.*, 1969, 302–345; (c) J. Swift, C. A. Butts, J. Cheung-Lau, V. Yerubandi and I. J. Dmochowski, *Langmuir*, 2009, **25**(9), 5219–5225; (d) A. Klug, *Philos. Trans. R. Soc. London, Ser. B*, 1999, **354**, 531–535.
- 6 T. Ueno, *J. Mater. Chem.*, 2008, **18**, 3741–3745.
- 7 (a) H. D. Nguyen and C. L. Brooks, III, *Nano Lett.*, 2008, **8**, 4574–4581; (b) H. D. Nguyen, V. S. Reddy and C. L. Brooks, III, *J. Am. Chem. Soc.*, 2009, **131**, 2606–2614.
- 8 L. Lavelle, M. Gingery, M. Phillips, W. M. Gelbart, C. M. Knobler, R. D. Cadena-Nava, J. R. Vega-Acosta, L. A. Pinedo-Torres and J. Ruiz-Garcia, *J. Phys. Chem. B*, 2009, **113**, 3813–3819.

- 9 J. Sun, C. DuFort, M.-C. Daniel, A. Murali, C. Chen, K. Gopinath, B. Stein, M. De, V. M. Rotello, A. Holzenburg, C. C. Kao and B. Dragnea, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 1354–1359.
- 10 M. T. Dedeo, K. E. Duderstadt, J. M. Berger and M. B. Francis, *Nano Lett.*, 2010, **10**, 181–186.
- 11 (a) T. S. Wong and U. Schwaneberg, *Curr. Opin. Biotechnol.*, 2003, **14**, 590–5962; (b) M. Sarikaya, C. Tamerler, A. K.-Y. Jen, K. Schulten and F. Baneyx, *Nat. Mater.*, 2003, **2**, 577–585.
- 12 A. G. Sykes, *Advances in Inorganic Chemistry*, Academic press, 1991, vol. 36.
- 13 F. C. Meldrum, B. R. Heywood and S. Mann, *Science*, 1992, **257**, 522–523.
- 14 (a) M. Ceolín, N. Gálvez, P. Sánchez, B. Fernández and J. M. Domínguez-Vera, *Eur. J. Inorg. Chem.*, 2008, 795–801; (b) M. Suzuki, M. Abe, T. Ueno, S. Abe, T. Goto, Y. Toda, T. Akita, Y. Yamadae and Y. Watanabe, *Chem. Commun.*, 2009, 4871–4873; (c) Y. Shin, A. Dohnalkova and Y. Lin, *J. Phys. Chem. C*, 2010, **114**, 5985–5989; (d) O. Kasyutich, A. Ilari, A. Fiorillo, D. Tatchev, A. Hoell and P. Ceci, *J. Am. Chem. Soc.*, 2010, **132**, 3621–3627.
- 15 K. M. Bennett, E. M. Shapiro, C. H. Sotak and A. P. Koretsky, *Biophys. J.*, 2008, **95**, 342–351.
- 16 B. Hennequin, L. Turyanska, T. Ben, A. M. Beltrán, S. I. Molina, M. Li, S. Mann, A. Patané and N. R. Thomas, *Adv. Mater.*, 2008, **20**, 3592–3596.
- 17 B. I. Ipe and C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2006, **45**, 504–507.
- 18 K. M. Bromley, A. J. Patil, A. W. Perriman, G. Stubbs and S. Mann, *J. Mater. Chem.*, 2008, **18**, 4796–4801.
- 19 E. S. Royston, A. D. Browna, M. T. Harris and J. N. Culver, *J. Colloid Interface Sci.*, 2009, **332**, 402–407.
- 20 (a) R. A. Miller, A. D. Presley and M. B. Francis, *J. Am. Chem. Soc.*, 2007, **129**, 3104–3109; (b) M. Endo, M. Fujitsuka and T. Majima, *Chem.-Eur. J.*, 2007, **13**, 8660–8666; (c) N. F. Steinmetz, A. Bize, K. C. Findlay, G. P. Lomonosoff, M. Manchester, D. J. Evans and D. Prangishvili, *Adv. Funct. Mater.*, 2008, **18**, 3478–3486.
- 21 K. L. Heredia and H. D. Maynard, *Org. Biomol. Chem.*, 2007, **5**, 45–53.
- 22 (a) M. A. Gauthier and H.-A. Klok, *Chem. Commun.*, 2008, 2591–2611; (b) L. A. Lee, Z. Niu and Q. Wang, *Nano Res.*, 2009, **2**, 349–364.
- 23 P. G. Holder, D. T. Finley, N. Stephanopoulos, R. Walton, D. S. Clark and M. B. Francis, *Langmuir*, 2010, **26**, 17383–17388.
- 24 J. M. Johnson, J. Tang, Y. Nyame, D. Willits, M. J. Young and A. Zlotnick, *Nano Lett.*, 2005, **5**(4), 765–770.
- 25 C. B. Chang, C. M. Knobler, W. M. Gelbart and T. G. Mason, *ACS Nano*, 2008, **2**, 281–286.
- 26 J. C. T. Carlson, S. S. Jena, M. Flenniken, T.-F. Chou, R. A. Siegel and C. R. Wagner, *J. Am. Chem. Soc.*, 2006, **128**, 7630–7638.
- 27 T. F. Chou, C. So, B. R. White, J. C. T. Carlson, M. Sarikaya and C. R. Wagner, *ACS Nano*, 2008, **2**, 2519–2525.
- 28 E. R. Ballister, A. H. Lai, R. N. Zuckermann, Y. Cheng and J. D. Mougous, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3733–3738.
- 29 F. F. Miranda, K. Iwasaki, S. Akashi, K. Sumitomo, M. Kobayashi, I. Yamashita, J. R. H. Tame and J. G. Hedde, *Small*, 2009, **5**, 2077–2084.
- 30 T. A. Whitehead, A. L. Meadows and D. S. Clark, *Small*, 2008, **4**(7), 956–960.
- 31 Q. Liu, I. A. Kriksunov, Z. Wang, R. Graeff, H. C. Lee and Q. Hao, *J. Phys. Chem. B*, 2008, **112**, 14682–14686.
- 32 I. Y. Wong, M. J. Footer and N. A. Melosh, *J. Am. Chem. Soc.*, 2008, **130**, 7908–7915.
- 33 E. T. O'Brien, III, M. R. Falvo, D. Millard, B. Eastwood, R. M. Taylor, II and R. Superfine, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 19438–19443.
- 34 H. Yan, H. Frielinghaus, A. Nykanen, J. Ruokolainen, A. Saianid and A. F. Miller, *Soft Matter*, 2008, **4**, 1313–1325.
- 35 T. P. J. Knowles, T. W. Oppenheim, A. K. Buell, D. Y. Chirgadze and M. E. Welland, *Nat. Nanotechnol.*, 2010, **5**, 204–207.
- 36 A. Böker, J. He, T. Emrick and T. P. Russell, *Soft Matter*, 2007, **3**, 1231–1248.
- 37 (a) G. Kaur, J. He, J. Xu, S. V. Pingali, G. Jutz, A. Böker, Z. Niu, T. Li, D. Rawlinson, T. Emrick, B. Lee, P. Thiagarajan, T. P. Russell and Q. Wang, *Langmuir*, 2009, **25**, 5168–5176; (b) J. He, Z. Niu, R. Tangirala, J.-Y. Wang, X. Wei, G. Kaur, Q. Wang, G. Jutz, A. Böker, B. Lee, S. V. Pingali, P. Thiagarajan, T. Emrick and T. P. Russell, *Langmuir*, 2009, **25**, 4979–4987.
- 38 (a) M. B. Linder, *Curr. Opin. Colloid Interface Sci.*, 2009, **14**, 356–363; (b) W. Wohlleben, T. Subkowski, C. Bollschweiler, B. von Vacano, Y. Liu, W. Schrepp and U. Baus, *Eur. Biophys. J.*, 2010, **39**, 457–468.
- 39 Z. Zhang, J. Buitenhuis, A. Cukkemane, M. Brocker, M. Bott and J. K. G. Dhont, *Langmuir*, 2010, **26**, 10593–10599.
- 40 J. T. Russell, Y. Lin, A. Böker, L. Su, P. Carl, H. Zettl, J. He, K. Sill, R. Tangirala, T. Emrick, K. Littrell, P. Thiagarajan, D. Cookson, A. Fery, Q. Wang and T. P. Russell, *Angew. Chem., Int. Ed.*, 2005, **44**, 2420–2426.
- 41 L. D. Zorova, B. F. Krasnikov, A. E. Kuzminova, I. A. Polyakova, E. N. Dobrov and D. B. Zorov, *FEBS Lett.*, 2000, **466**, 305–309.
- 42 T. Li, L. Wu, N. Suthiwangcharoen, M. A. Bruckman, D. Cash, J. S. Hudson, S. Ghoshroy and Q. Wang, *Chem. Commun.*, 2009, 2869–2871.
- 43 (a) G. Muschiolik, *Curr. Opin. Colloid Interface Sci.*, 2007, **12**, 213–220; (b) V. S. Alahverdijeva, D. O. Grigoriev, J. K. Ferri, V. B. Fainerman, E. V. Aksenenko, M. E. Leser, M. Michel and R. Miller, *Colloids Surf., A*, 2008, **323**, 167–174.
- 44 V. S. Alahverdijeva, D. O. Grigoriev, V. B. Fainerman, E. V. Aksenenko, R. Miller and H. Möhwald, *J. Phys. Chem. B*, 2008, **112**, 2136–2143.
- 45 (a) E. Silletti, M. H. Vingerhoeds, W. Norde and G. A. van Aken, *J. Colloid Interface Sci.*, 2007, **313**, 485–493; (b) A. Drakos and V. Kiosseoglou, *J. Agric. Food Chem.*, 2006, **54**, 10164–10169.
- 46 (a) B. Lei, K. Majumder, S. Shen and J. Wu, *Food Chem.*, 2011, **124**, 808–815; (b) P. B. Stathopoulos, G. A. Scholz, Y.-M. Hwang, J. A. O. Rumfeldt, J. R. Lepock and E. M. Meiering, *Protein Sci.*, 2004, **13**, 3017–3027; (c) X. Wang, J. A. Klugea, G. G. Leiskb and D. L. Kaplan, *Biomaterials*, 2008, **29**, 1054–1064.
- 47 M. Zhou, F. Cavalieri and M. Ashokkumar, *Soft Matter*, 2011, **7**, 623–630.
- 48 P. van Rijn, H. Wang and A. Böker, *Soft Matter*, 2011, **7**, 5274–5280.
- 49 (a) T. Shiomi, T. Tsunoda, A. Kawai, H. Chiku, F. Mizukami and K. Sakaguchi, *Chem. Commun.*, 2005, 5325–5327; (b) T. Shiomi, T. Tsunoda, A. Kawai, F. Mizukami and K. Sakaguchi, *Chem. Mater.*, 2007, **19**, 4486–4493.
- 50 S. Balci, D. M. Leinberger, M. Knez, A. M. Bittner, F. Boes, A. Kadri, C. Wege, H. Jeske and K. Kern, *Adv. Mater.*, 2008, **20**, 2195–2200.
- 51 (a) A. Horn, H. G. Schoberth, S. Hiltl, A. Chiche, Q. Wang, A. Schweikart, A. Fery and A. Böker, *Faraday Discuss.*, 2009, **143**, 143–150; (b) A. Horn, S. Hiltl, A. Fery and A. Böker, *Small*, 2010, **6**, 2122–2125.
- 52 J. M. Lee, H. K. Park, Y. Jung, J. K. Kim, S. O. Jung and B. H. Chung, *Anal. Chem.*, 2007, **79**, 2680–2687.
- 53 (a) R. K. Dani, M. Kang, M. Kalita, P. E. Smith, S. H. Bossmann and V. Chikan, *Nano Lett.*, 2008, **8**, 1229–1236; (b) C. Staii, D. W. Wood and G. Scoles, *J. Am. Chem. Soc.*, 2008, **130**, 640–646.
- 54 Y. Hu, D. Chen, S. Park, T. Emrick and T. P. Russell, *Adv. Mater.*, 2010, **22**, 2583–2587.
- 55 P. Cigler, A. K. R. Lytton-Jean, D. G. Anderson, M. G. Finn and S. Y. Park, *Nat. Mater.*, 2010, **9**, 918–922.
- 56 M. P. Nikitina, T. A. Zdobnova, S. V. Lukash, O. A. Stremovskiy and S. M. Deyev, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 5827–5832.
- 57 C. Chen, M.-C. Daniel, Z. T. Quinkert, M. De, B. Stein, V. D. Bowman, P. R. Chipman, V. M. Rotello, C. C. Kao and B. Dragnea, *Nano Lett.*, 2006, **6**, 611–615.
- 58 M. Hu, L. Qian, R. P. Briñas, E. S. Lymar and J. F. Hainfeld, *Angew. Chem., Int. Ed.*, 2007, **46**, 5111–5114.
- 59 M. De, O. R. Miranda, S. Rana and V. M. Rotello, *Chem. Commun.*, 2009, 2157–2159.
- 60 H. Bayraktar, S. Srivastava, C.-C. You, V. M. Rotello and M. J. Knapp, *Soft Matter*, 2008, **4**, 751–756.
- 61 N. F. Steinmetz, K. C. Findlay, T. R. Noel, R. Parker, G. P. Lomonosoff and D. J. Evans, *ChemBioChem*, 2008, **9**, 1662–1670.
- 62 M. A. Kostianen, P. Hiekkataipale, J. A. de la Torre, R. J. M. Nolte and J. J. L. M. Cornelissen, *J. Mater. Chem.*, 2011, **21**, 2112–2117.
- 63 Z. Niu, M. A. Bruckman, S. Li, L. A. Lee, B. Lee, S. V. Pingali, P. Thiagarajan and Q. Wang, *Langmuir*, 2007, **23**, 6719–6724.
- 64 Y. Kumashiro, Y. Ikezoe, K. Tamada and M. Hara, *J. Phys. Chem. B*, 2008, **112**, 8291–8297.
- 65 Y. Hu, D. Samanta, S. S. Parelkar, S. W. Hong, Q. Wang, T. P. Russell and T. Emrick, *Adv. Funct. Mater.*, 2010, **20**, 3603–3612.

- 66 N. C. Mougín, P. van Rijn, H. Park, A. H. E. Müller and A. Böker, *Adv. Funct. Mater.*, 2011, **21**, 2470–2476.
- 67 K. Velonia, A. E. Rowan and R. J. M. Nolte, *J. Am. Chem. Soc.*, 2002, **124**, 4224–4225.
- 68 C. Lavigueur, J. González García, L. Hendriks, R. Hoogenboom, J. J. L. M. Cornelissen and R. J. M. Nolte, *Polym. Chem.*, 2011, **2**, 333–340.
- 69 E. Katz and I. Willner, *Angew. Chem., Int. Ed.*, 2004, **43**, 6042–6108.
- 70 Z. Su and Q. Wang, *Angew. Chem., Int. Ed.*, 2010, **49**, 10048–10050.
- 71 A. de la Escosura, R. J. M. Nolte and J. J. L. M. Cornelissen, *J. Mater. Chem.*, 2009, **19**, 2274–2278.
- 72 S. Mukherjee, C. M. Pfeifer, J. M. Johnson, J. Liu and A. Zlotnick, *J. Am. Chem. Soc.*, 2006, **128**, 2538–2539.
- 73 M. Kwak, I. J. Minten, D.-M. Anaya, A. J. Musser, M. Brasch, R. J. M. Nolte, K. Müllen, J. J. L. M. Cornelissen and A. Herrmann, *J. Am. Chem. Soc.*, 2010, **132**, 7834–7835.
- 74 M. Comellas-Aragonès, H. Engelkamp, V. I. Claessen, N. A. J. M. Sommerdijk, A. E. Rowan, P. C. M. Christianen, J. C. Maan, B. J. M. Verduin, J. J. L. M. Cornelissen and R. J. M. Nolte, *Nat. Nanotechnol.*, 2007, **2**, 635–639.
- 75 I. J. Minten, L. J. A. Hendriks, R. J. M. Nolte and J. J. L. M. Cornelissen, *J. Am. Chem. Soc.*, 2009, **131**, 17771–17773.
- 76 F. Li, Z.-P. Zhang, J. Peng, Z.-Q. Cui, D.-W. Pang, K. Li, H.-P. Wei, Y.-F. Zhou, J.-K. Wen and X.-E. Zhang, *Small*, 2009, **5**, 718–726.
- 77 M. Uchida, M. L. Flenniken, M. Allen, D. A. Willits, B. E. Croley, S. Brumfield, A. F. Willis, L. Jackiw, M. Jutila, M. J. Young and T. Douglas, *J. Am. Chem. Soc.*, 2006, **128**, 16626–16633.
- 78 K. Boeneman, B. C. Mei, A. M. Dennis, G. Bao, J. R. Deschamps, H. Mattoussi and I. L. Medintz, *J. Am. Chem. Soc.*, 2009, **131**, 3828–3829.
- 79 Y. Han, D. Shchukin, J. Yang, C. R. Simon, H. Fuchs and H. Möhwald, *ACS Nano*, 2010, **4**, 2838–2844.
- 80 G. Jutz and A. Böker, *J. Mater. Chem.*, 2010, **20**, 4299–4304.
- 81 P. van Rijn, N. C. Mougín, D. Franke, H. Park and A. Böker, *Chem. Commun.*, 2011, **47**, 8376–8378.
- 82 A. de la Escosura, P. G. A. Janssen, A. P. H. J. Schenning, R. J. M. Nolte and J. J. L. M. Cornelissen, *Angew. Chem.*, 2010, **122**, 5463–5466.
- 83 I. Yamashita, *J. Mater. Chem.*, 2008, **18**, 3813–3820.
- 84 E. Royston, A. Ghosh, P. Kofinas, M. T. Harris and J. N. Culver, *Langmuir*, 2008, **24**, 906–912.
- 85 (a) K. Gerasopoulos, M. McCarthy, P. Banerjee, X. Fan, J. N. Culver and R. Ghodssi, *Nanotechnology*, 2010, **21**, 055304, 11pp; (b) M. Kobayashi, M. Seki, H. Tabata, Y. Watanabe and I. Yamashita, *Nano Lett.*, 2010, **10**, 773–776; (c) A. F. Loftus, K. P. Reighard, S. A. Kapourales and M. C. Leopold, *J. Am. Chem. Soc.*, 2008, **130**, 1649–1661; (d) V. K. Kodali, J. Scrimgeour, S. Kim, J. H. Hankinson, K. M. Carroll, W. A. de Heer, C. Berger and J. E. Curtis, *Langmuir*, 2011, **27**, 863–865; (e) Y. Leng, H.-P. Wei, Z.-P. Zhang, Y.-F. Zhou, J.-Y. Deng, Z.-Q. Cui, D. Men, X.-Y. You, Z.-N. Yu, M. Luo and X.-E. Zhang, *Angew. Chem., Int. Ed.*, 2010, **49**, 7243–7246.
- 86 A. E. Nel, L. Mädler, D. Velegol, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova and M. Thompson, *Nat. Mater.*, 2009, **8**, 543–557.
- 87 D. M. Hess, R. R. Naik, C. Rinaldi, M. M. Tomczak and J. J. Watkins, *Chem. Mater.*, 2009, **21**, 2125–2129.
- 88 Y. Gu, X. Liu, T. Niu and J. Huang, *Chem. Commun.*, 2010, **46**, 6096–6098.
- 89 A. R. Hirst, S. Roy, M. Arora, A. K. Das, N. Hodson, P. Murray, S. Marshall, N. Javid, J. Sefcik, J. Boekhoven, J. H. van Esch, S. Santabarbara, N. T. Hunt and R. V. Uljin, *Nat. Chem.*, 2010, **2**, 1089–1094.
- 90 K. Mircheva, T. z. Ivanova, I. Panaiotov, V. Duce and F. Boury, *J. Colloid Interface Sci.*, 2010, **347**, 69–73.
- 91 W. K. Zhang, D. Wang, Y. Duan, M. M. T. Loy, H. C. Chan and P. Huang, *Nat. Cell Biol.*, 2010, **12**, 507–513.
- 92 N. Carette, H. Engelkamp, E. Akpa, S. J. Pierre, N. R. Cameron, P. C. M. Christianen, J. C. Maan, J. C. Thies, R. Weberskirch, A. E. Rowan, R. J. M. Nolte, T. Michon and J. C. M. van Hest, *Nat. Nanotechnol.*, 2007, **2**, 226–229.