ACCOUNTS
of chemical research

Synthesis of Polymer–Biohybrids: From Small to Giant Surfactants

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RECEIVED ON AUGUST 13, 2008

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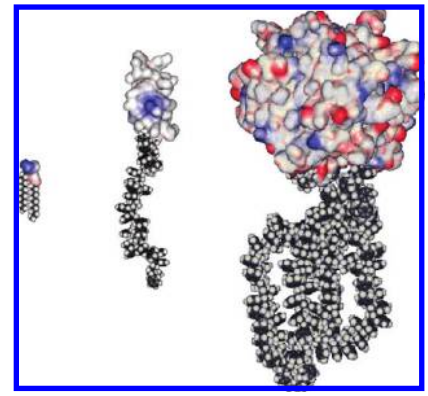

Amphiphiles or surfactants, more popularly known as soaps, are among the oldest known chemical compounds used by man. Written text on a clay tablet dated to 2200 B.C. indicates that the Babylonians were familiar with soap-like substances. According to the Ebers papyrus (1550 B.C.), the ancient Egyptians bathed regularly in a mixture of animal oils, vegetable extracts, and alkaline salts, and a soap factory with bars of scented soap was found in the ruins of Pompeii (79 A.D.). In modern times, the use of soap has become universal, and we now understand reasonably well what happens when soap molecules are dispersed in aqueous solution and how the cleaning properties of soap work. The latter is related to the surface-active behavior of soap molecules, which is a result of their amphiphilic, also called amphipathic, character. Although the cleaning aspect is still an important issue, scientists are increasingly focusing on other properties of soaps, for example, self-assembling behavior and how this can be used in the design and non-covalent synthesis of new (macro)molecular architectures. These new molecules can be employed in nanotechnology and drug delivery, among other applications.

This Account will focus on three different classes of amphiphiles. The first is the low molecular weight amphiphiles, also called classical amphiphiles in this context. A short overview will be given on the research carried out by our group and others on the self-assembly behavior and properties of these compounds; in particular, we focus on the ones that can be stabilized by polymerization (polymerized vesicles). Next, we will introduce the still relatively young field of superamphiphiles, macromolecules consisting of a hydrophobic and a hydrophilic polymeric block. Finally, and this constitutes the main part of this Account, we will provide an overview of a new class of amphiphiles, the so-called giant amphiphiles. These macromolecules have an enzyme or protein as the polar head group and a hydrophobic polymer as a tail. We will finish the Account with conclusions and an outlook to the future.

Introduction: Classical Surfactants

Classic surfactants or amphiphiles are compounds that consist of a hydrophobic (usually a long hydrocarbon chain) and a hydrophilic (ionic or polar) group. Examples of classic amphiphiles are sodium oleate, hexadecyl- (or cetyl-)trimethylammonium bromide (CTAB), and sodium dodecylsulfate (SDS). Extensive studies have revealed that these compounds self-assemble in aqueous solutions forming aggregates such as rod- and sphere-like micelles, vesicles, and multilayer structures with very interesting properties.[1,2] According to the theory of Isrealachvilli,[3] the type of the formed aggregate is related to the shape of the amphiphilic molecule. Most experimental results for phospholipids agree reasonably well with this model, although deviations have been observed for surfactants that contain rigid segments, for example, diphenylazomethine moieties or multiple hydrogen bonding units like urea and acylurea.[4] One of the disadvantages of these assemblies is that they lack long-term stability,

Published on the Web 04/22/2009 www.pubs.acs.org/acr
10.1021/ar800143a CCC: $71.50

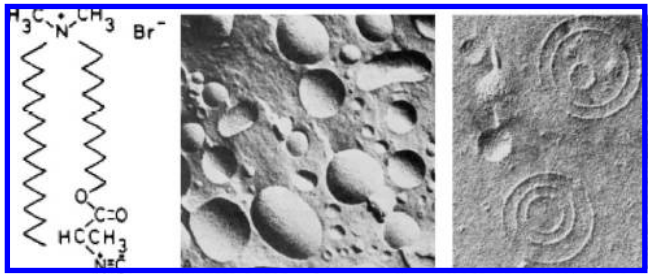

FIGURE 1. Quaternary ammonium surfactant containing a polymerizable isocyano-alanine function (left). Vesicles (middle) and polymerized vesicles (right) formed by this surfactant after dispersion in water. Reproduced from ref 8 with permission. Copyright 1983 American Chemical Society.

which is needed when one intends to use them for certain applications, such as drug delivery.[5] In 1982, Regen and Ringsdorf reported a solution to this problem, that is, polymerization of the core or the corona of the vesicle.[6,7] To this end, the amphiphiles were provided with polymerizable groups such as diacetylene, butadiene, or vinyl moieties, and after polymerization, the resulting assemblies were found to be stable over a long period of time.

Inspired by this work, we synthesized a double chain quaternary ammonium surfactant and provided one of the surfactant tails with a polymerizable isocyanoamino acid residue.[8] This surfactant formed well-defined vesicles, which could be polymerized with a small amount of nickel capronate, yielding structures that were extremely stable and that kept their form even in organic solvents. Interestingly, electron microscopy studies using the so-called freeze−fracture technique provided direct visible proof that polymerized aggregates had been obtained: instead of the usual convex and concave half-balls, circles were now visible, which was the result of the fact that during the applied technique the polymerized aggregates were cross-fractured instead of cleaved along the middle of the bilayer, as is normally the case (Figure 1).

In subsequent studies, the aggregates of the polymerized and unpolymerized amino acid amphiphiles were used as stable, synthetic mimics of cells. It was possible to incorporate synthetic ion channels in the bilayers of the vesicles and to perform ion transport studies.[9] Van Esch et al. used the polymerized isocyanosurfactant vesicles as nanoreactors, that is, small nanosized reaction vessels. They succeeded in incorporating colloidal platinum particles and an electron carrier (methylene blue) in the inner aqueous compartments of the vesicles and an amphiphilic manganese porphyrin in their bilayers. By bubbling molecular hydrogen and molecular oxygen through the solution of the modified vesicles, they were able to convert alkenes into their oxidized products, for example, styrene into styrene oxide, mimicking in this way the action of the membrane-bound enzyme cytochrome P-450.[10] Later Schenning et al. further improved this catalytic system (Figure 2).[11] Since the 1:1 combination of molecular hydrogen and oxygen is dangerous and also gives rise to a side reaction (formation of water), the colloidal platinum was replaced by a rhodium complex, which in the presence of formate ions delivers the electrons that are required for the activation of molecular oxygen and the subsequent oxidation reaction. This modified self-assembled catalytic system behaved beautifully and under precisely set conditions even displayed oscillating behavior, making it the first supramolecular oscillating system to date.[12] Its catalytic activity turned out to be relatively low (ca. 100−400 turnovers per hour) but of the same order of magnitude as the natural cytochrome P-450 (60 turnovers per hour).

The use of polymerizable vesicles has opened a whole new area with applications in the fields of biotechnology and nanotechnology. However, in the mean time a new way of stabilizing the aggregates was explored, namely by using amphiphilic block copolymers.

Supersurfactants

Synthetic Block Copolymers. Block copolymer amphiphiles, also called supersurfactants or superamphiphiles are polymers consisting of a hydrophobic and a hydrophilic block. Similar to their low molecular weight counterparts, they form various aggregates in aqueous solution, such as micelles and vesicles. Although there are structural similarities between the assemblies formed by superamphiphiles and classic amphiphiles, there are also some major differences. The critical micellar concentration (CMC) of the superamphiphiles is usually much lower, the assemblies formed by them are more stable and the exchange dynamics are substantially slower compared with classical amphiphiles. This is a result of a number of factors, such as the higher molecular weight of the compounds, the presence of chain entanglements, and the decreased mobility of the polymer chains in the core of the micelle.[13] The most studied class of amphiphilic diblock copolymers consists of macromolecules having two flexible segments, that is, the coil−coil block copolymers. The formation of micelles by such macromolecules was first reported by Halperin and co-workers[14] and later extensively studied by Zhang and Eisenberg using different polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) copolymers.[15,16] Depending on factors, such as the hydrophilic/hydrophobic ratio, the molecular weight, the presence of homopolymers, and the solvent composition, a large variety of aggregate morphologies was observed.

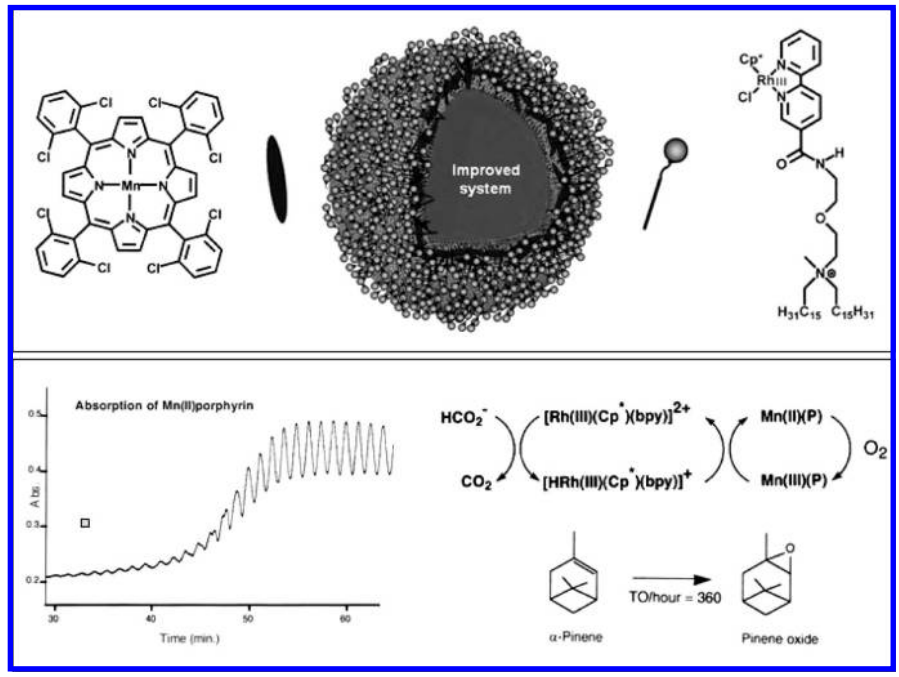

FIGURE 2. Nanoreactor composed of vesicles, which contain in their bilayers a manganese porphyrin and a rhodium(III)–bipyridyl–cyclopentadiene complex (top panel). In the presence of formate ions and molecular oxygen, a catalytic reaction takes place in which the manganese(III) centers are reduced by electrons from the rhodium complex and subsequently reoxidized by molecular oxygen in an oscillating fashion. When substrate is added (e.g., α-pinene), the substrate is catalytically oxidized with a rate of 360 turnovers per hour (lower panel).

In line with the Isrealachvilli rules, Meijer et al. have studied the self-assembling properties of block copolymers containing a hydrophobic polystyrene block and a hydrophilic dendritic segment of varying size. With increasing generation of the dendritic part, the aggregate structures changed from micelles, via micellar rods, to vesicles.[17]

Not only the classical self-assembled structures but also other interesting architectures trapped near equilibrium and metastable morphologies have been observed for superamphiphiles. They are the result of the low mobility of the polymer chains in the aggregates.[18] Trapping of these structures gives detailed insight into the mechanisms of the transitions between the different aggregate morphologies.[19] Because of the larger molecular weight, amphiphilic block copolymers can also exhibit microemulsion character without the need for an oil as is the case for low molecular weight amphiphiles. A nice example has been reported by Jain and Bates, who showed that by varying the weight fraction and length of a poly(ethylene oxide)-*b*-polybutadiene (PEO-*b*-PB) block copolymer in dilute solution, networks and Y-junctions could be obtained.[20] These findings offer new opportunities for designing soft materials with properties that cannot be easily obtained with low molecular weight amphiphiles.

Along this line are also the studies by the groups of Wooley and Pochan on amphiphilic triblock copolymers of the type poly(acrylic acid)-*b*-poly(methyl acrylate)-*b*-polystyrene (PAA_{99}-*b*-PMA_{73}-*b*-PS_{66}). When dispersed in a mixture of water/THF and 2,2′-(ethylenedioxy)diethylamine (EDEA), toroids are formed (Figure 3).[21] The presence of all four components is critical otherwise the toroidal structures, which are very stable, are not observed. In a recent paper, the authors demonstrate that this type of triblock copolymer in combination with EDEA can also generate cylindrical micelles that undergo local, intramicelllar phase separation. These one-dimensional nanostructures are kinetically trapped but stable architectures because they are unable to thermodynamically equilibrate.[22]

Biohybrid Block Copolymers. The introduction of polypeptides as the hydrophilic component in block copolymers is of interest, because the resulting assemblies may combine the properties of the biomolecule, for example, high degree of organization and biocompatibility, with the properties of the synthetic polymer, for example, solubility in various solvents and stability.

Following up on work by Gallot and co-workers,[23] the amine initiated ring-opening polymerization of α-amino acid-*N*-carboxyanhydrides was used by Klok and co-workers to prepare polybutadiene-*b*-poly(L-glutamatic acid) copolymers, which were found to form vesicular aggregates in water.[24,25] The vesicle morphology, however, is independent of the

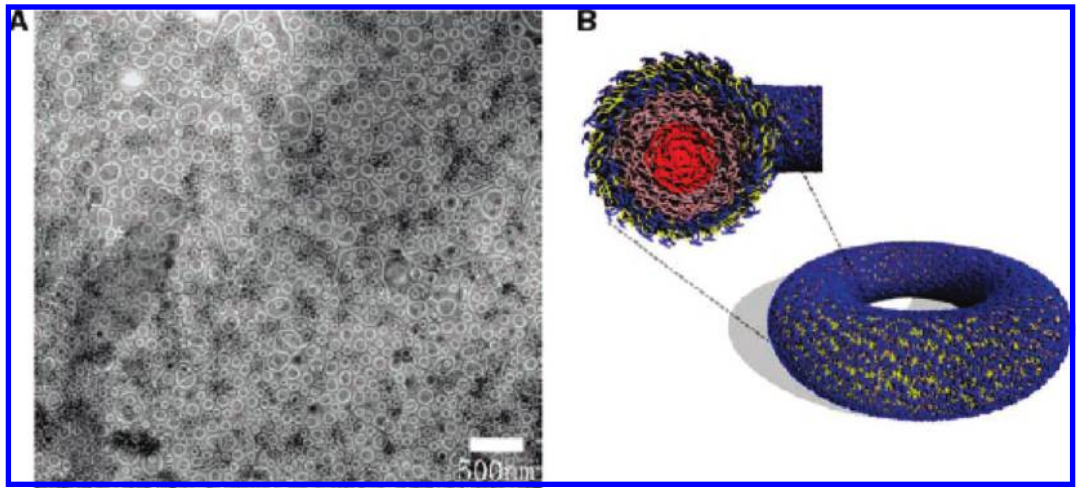

FIGURE 3. (A) Toroidal structures formed by PAA-*b*-PMA-*b*-PS and (B) schematic representation of the aggregates. Reproduced from ref 21 with permission. Copyright 2004 American Association for the Advancement of Science.

polypeptide chain conformation as was shown in an independent study by Kukula et al.[26]

Block copolymers containing a poly(lactic acid) (PLA) and a PEO segment also display amphiphilic behavior; because the PLA block is sparingly soluble in water and has a strong tendency to aggregate in a semicrystalline phase for these materials applications as drug delivery vehicles are suggested.[27]

Assemblies of amphiphilic block copolymers can be further stabilized by cross-linking the unsaturated bonds present in the hydrophobic domains.[28] Also the corona of the aggregates can be stabilized since the side groups of the hydrophilic blocks usually contain amine or carboxylic acid groups, which easily react with other functional groups.[29] Wooley et al. prepared biohybrid block copolymers containing, for example, poly(glutamic acid) segments, and micellar aggregates of these amphiphiles were cross-linked with 2,2′(ethylenedioxy)-diethylamine.[30]

Besides *N*-carboxyanhydride polymerization, biohybrid peptide-containing superamphiphiles can also be prepared by solution phase synthesis. Using this method, Sogah et al.[31] prepared block copolymers containing repetitive amino acid sequences, which form silk fiber-like architectures with crystalline and amorphous domains. Either poly(alanine) or the tetrapeptide GlyAlaGlyAla was used as the crystalline domain (to give the material strength), and poly(ethylene glycol) (PEG) formed the amorphous matrix (allowing orientation under strain). The mechanical properties of fibers and films made from these block copolymers could be modulated by manipulating the length and nature of the constituent blocks. Similar work has been described by Shao et al.[32]

A disadvantage of the solution phase method is that large peptides are very difficult to synthesize. Larger peptide-containing block copolymers, therefore, are better accessible via solid-phase peptide chemistry. Syntheses of polymer−peptide block copolymers using solid phase procedures have been reported employing either nitroxide-mediated radical polymerization (NMRP) or atom transfer radical polymerization (ATRP) for the preparation of the polymer segment.[33,34]

We have used solid-phase peptide synthesis to synthesize amphiphilic tri- and diblock copolymers (Figure 4).[35] The amphiphiles consisted of a β-turn peptide (from the circumsporozoite (CS) protein of the malaria parasite *Plasmodium falciparum*) and one or two polystyrene blocks. In this approach, amine end-capped polystyrene was coupled to an aldehyde functionalized resin by reductive amination. The resulting secondary amine was subsequently used as starting point for the construction of the peptide segment and finally the product was cleaved from the resin to yield the amphiphilic diblock copolymer, which was found to form micellar aggregates in aqueous solution. When, before cleavage from the resin, a carboxylic acid terminated polystyrene was coupled to the N-terminus of the peptide fragment, the triblock copolymer PS-*b*-GANPNAAG-*b*-PS was obtained after cleavage from the resin.

Opsteen and van Hest have recently shown that well-defined block copolymers can be prepared by a modular strategy involving the copper-catalyzed Huisgen [3 + 2] dipolar cycloaddition also called click chemistry to connect the two different polymer segments[36] Click chemistry is particularly efficient in water and therefore can be expected to be very suitable for the synthesis of new biohybrid macromolecular architectures.[37−40] This approach was followed by Dirks et al. by clicking a coumarin-functionalized tripeptide to an azide-terminated polystyrene.[41] The coumarin compound, which is highly fluorescent, facilitates the characterization of the product. This reaction turned out to be very successful, and the method opens many possibilities for the synthesis of biohy-

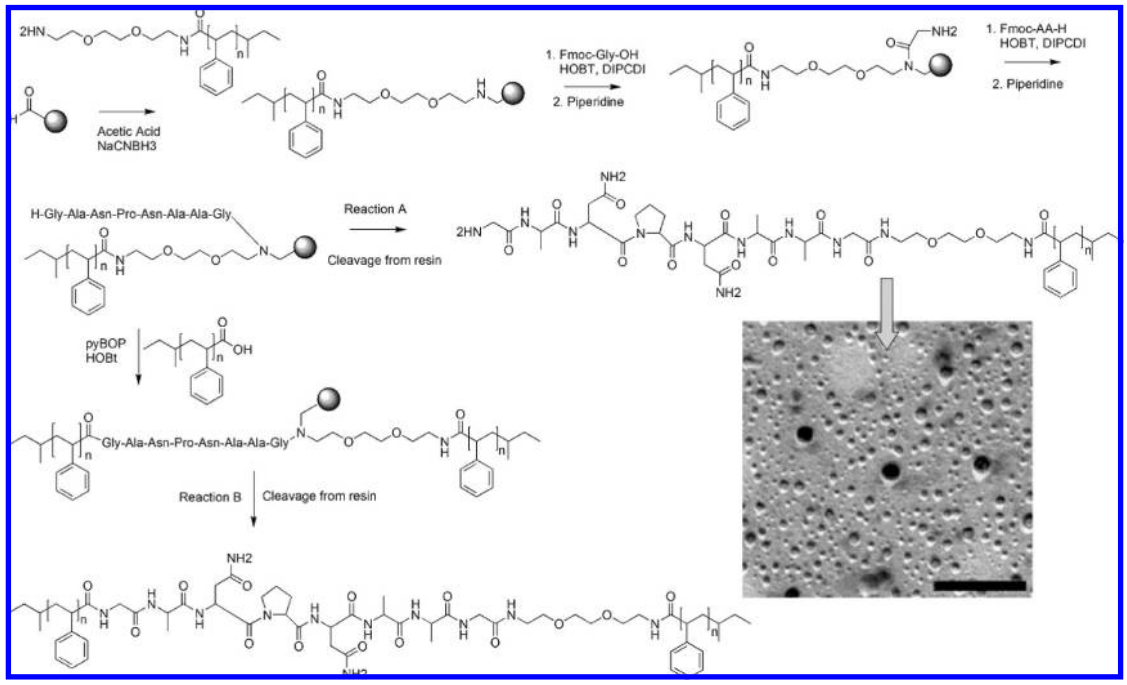

FIGURE 4. Synthesis route of PS_{40}-*b*-GANPNAAG and PS_{40}-*b*-GANPNAAG-*b*-PS_{40}. Inset shows the aggregation behavior of PS_{40}-*b*-GANPNAAG in water.

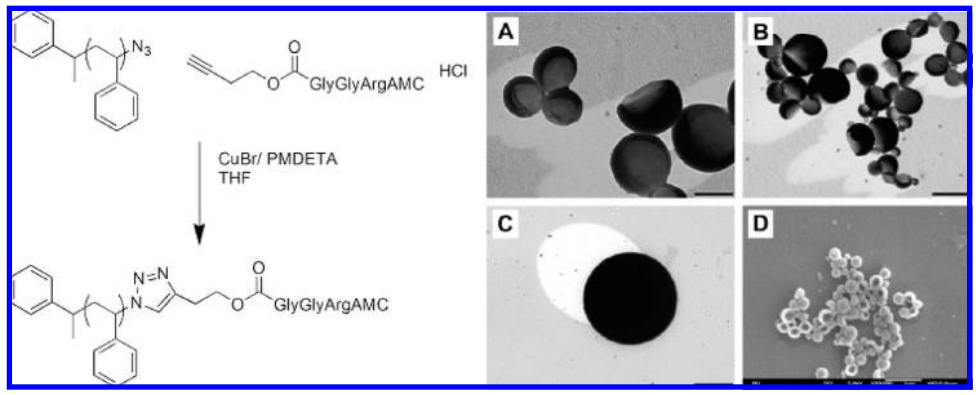

FIGURE 5. Synthetic route toward peptide containing superamphiphiles using click chemistry:[41] right, self-assembly behavior of PS-*b*-GlyGlyArgAMC in aqueous solutions; (A, B) TEM image taken directly after injection (Pt shadowing; scale bars represent 200 and 500 nm for panels A and B, respectively); (C) TEM image taken 18 h after injection (Pt shadowing; scale bar represents 1 μm); (D) SEM image directly taken after injection. Reproduced from ref 48 with permission. Copyright 2005 Royal Society of Chemistry.

brid compounds. The prepared biohybrid amphiphiles formed stable vesicular structures in water (Figure 5) and can be used in biological assays such as thrombin activity tests. The construction of biohybrid materials using click chemistry is an emerging field, and several overviews have recently appeared.[42]

Related to the peptide-containing block copolymers are the polystyrene–polyisocyanopeptide superamphiphiles reported by Nolte, Cornelissen, and others.[43,44] The macromolecules have amino acid based segments that fold into a β-sheet helix, which is also known to occur in Nature, for example, in amyloid fibers that are responsible for diseases like Alzheimer's and Creutzfeldt–Jakob.[45] The block copolymers of styrene and isocyanopeptides self-assembled in water and formed superhelical structures, in which the chiral information was transferred from the amino acid building blocks to the macromolecular polyisocyanide segment and subsequently to the supramolecular superhelices.[43]

A very interesting method to synthesize peptide-containing amphiphiles involves the use of protein engineering, which is a very powerful technique, especially when large peptide sequences are needed.[46] Protein engineering allows one to change structures at the genetic level and opens the possibility to replace specific amino acids and even to introduce nonnatural amino acids. A nice example of a biohybrid block copolymer synthesized in this way was described by Smeenk

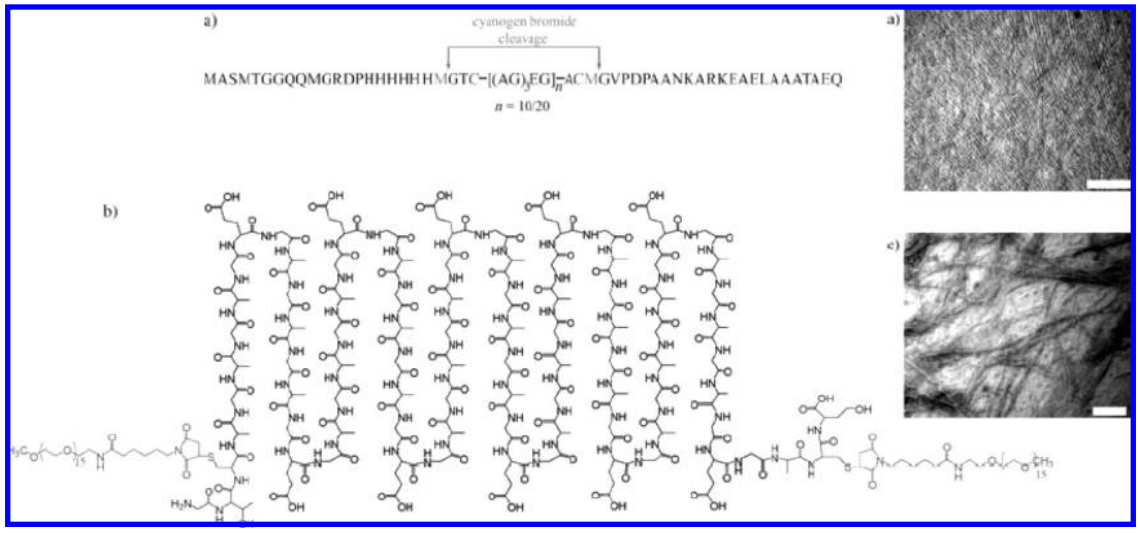

FIGURE 6. (a) Peptide sequence obtained after protein engineering, (b) structure of the triblock copolymer PEG-*b*-$[(AG)_3EG]_n$-*b*-PEG; (c, d) TEM images of fibrils formed from the triblock copolymer. Reproduced from ref 47 with permission. Copyright 2005 Wiley Publishers.

et al.[47] They synthesized, using protein engineering, a triblock copolymer consisting of the repetitive sequence $[(AG)_3EG]_n$ and two PEG blocks (Figure 6). The rationale behind the conjugation of the synthetic polymer to the N and C termini of the polypeptide was to prevent macroscopic crystallization and to allow the formation of self-assembled architectures containing β-sheets of specific width, height, and surface functionality. It was observed that indeed well-defined fibrils in the direction of the β-sheet stacking were formed.

Not only peptide- but also sugar- and DNA-containing amphiphilic block copolymers have been described in the literature. Loos et al.[48] have synthesized hybrid block copolymers containing amylose and polystyrene by covalent attachment of maltoheptose derivatives to end-functionalized polystyrene followed by enzymatic grafting from the heptose primer. Herrmann has prepared DNA-containing multiblock copolymers, which formed micellar structures upon dispersion in aqueous media.[49]

Giant Surfactants

We have described above how simple natural building blocks such as peptides or carbohydrates can be combined with synthetic polymers to form supersurfactants. These compounds display aggregation behavior similar to that of the classic low molecular weight surfactants with the advantage that their assemblies are more stable and can be easily varied by changing the size of the polymeric blocks and the assembling conditions, for example, solvent or pH. In this section, we will discuss a relatively new class of biohybrid amphiphiles, i.e. the giant amphiphiles or surfactants. These very large amphiphilic compounds possess a polymeric tail and an enzyme or protein as headgroup. It should be noted that a wide variety of polymer–enzyme adducts synthesized by more or less randomly coupling an enzyme to a polymeric matrix have been described in the literature.[69,70] The attachment of a polymer chain to a preselected location on the surface of a protein with the objective of constructing a giant amphiphile, however, is new.

An important class of biohybrid conjugates are proteins of which the side chains are modified with PEG. Several pegylated proteins are in clinically use as therapeutics.[50] However, coupling PEG to proteins will not result in amphiphiles, and this subject, therefore, is beyond the scope of this Account.

The synthesis of giant amphiphiles was first reported by the group of Nolte, who used the well-established biotin–streptavidin (Sav) couple to attach a polymer chain to a protein.[51] Sav is a homotetrameric protein with a 2-fold symmetry. It is capable of binding four biotin molecules in a noncooperative way on sites that are arranged in pairs at opposed faces of the molecule. [52,53] The resulting complex with biotin has the highest affinity known in nature between a protein and a ligand ($K_a \approx 10^{13}$ M^{-1}, corresponding to a binding energy of approximately 21 kcal·mol^{-1}).[54] Since the valeric acid carboxyl group of biotin does not play a significant role in the binding process, it can be easily modified and used in numerous applications.[55]

The biohybrid amphiphiles were prepared by binding two molecules of monobiotinylated polystyrene (an amine-termined polystyrene was coupled to the valeric acid carboxyl group of biotin) to streptavidin using monolayer techniques. To this end the biotinylated polymer was spread on the air/water interface in a Langmuir trough followed by the addition of streptavidin to the subphase. Surface pressure–surface

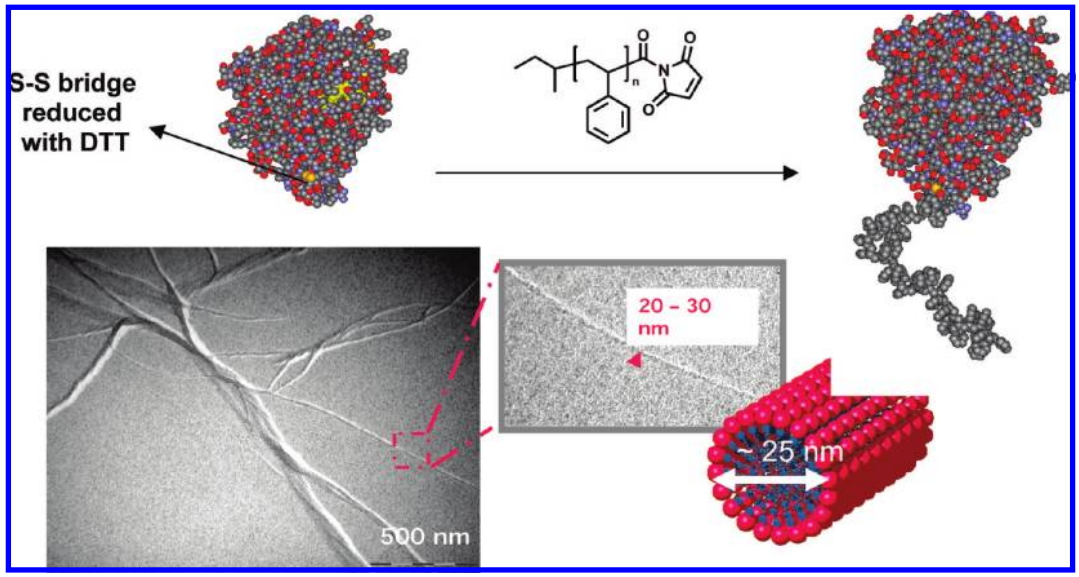

FIGURE 7. Schematic drawing of the synthesis of CALB-*b*-PS, which forms micellar rods when dispersed in water. Adapted from ref 62 with permission. Copyright 2002 American Chemical Society.

area diagrams combined with Brewster angle microscopy (BAM) and atomic force microscopy (AFM) revealed binding of two biotinylated polymers on one side of the streptavidin molecule. The ability of the protein–polymer complex to bind other biotinylated compounds to the unoccupied Sav face was proven with the iron-containing protein ferritin and the enzyme horseradish peroxidase (HRP). The ferritin-containing giant amphiphiles could be visualized by transmission electron microscopy. The HRP containing ones were shown to be catalytically active.

Other examples in which Sav is used as the building block in polymer–protein hybrids have been published in the literature. In these cases, the objective was not to construct giant amphiphiles, but the obtained architectures can be classified as such. Hoffman, Stayton, and co-workers have coupled stimuli-responsive polymers to SAV and other proteins for the reversible precipitation of the latter biomacromolecules[56,57] or to reversibly block the active site of the proteins.[58,59] In the latter case, the thermally responsive polymer poly(*N*,*N*-diethylacrylamide) (PDEAAm) was attached to streptavidin at a short distance from one of the binding sites. Below the lower critical solution temperature (LCST) of PDEAAm, the polymer is in its extended state, allowing binding of biotin to the modified binding site and the neighboring site but not of biotinylated proteins, due to steric hindrance. Above the LCST, the polymer collapses upon the modified binding site preventing access of both small and larger biotin derivatives to this site. In contrast, the crowding around the neighboring site was sharply reduced allowing the binding of both biotin and sterically demanding biotinylated proteins. Kulkarni et al. have synthesized a biotinylated poly(*N*-isopropylacrylamide) (polyNIPAAm) via a reversible addition–fragmentation chain transfer (RAFT) polymerization reaction.[60] Hydrolysis of the thioester end group and subsequent coupling of the thiol group with a maleimide-functionalized biotin formed the biotinylated polyNIPAAm. The particles that were formed after binding with Sav were used in microfluidic devices for the capture and release of biomacromolecules.

The biotin–streptavidin couple has also been extensively used by Niemeyer and co-workers for the construction and manipulation of supramolecular assemblies of DNA, including unusual ones such as nanorings.[61]

Another approach to construct giant amphiphiles introduced by the group of Nolte is the covalent coupling of a polymer to the surface of a protein.[62] In order to achieve this coupling on a predefined position, a disulfide bridge positioned on the outer shell of the lipase B from *Candida antarctica* (CALB) was specifically reduced to provide two free thiol groups (Figure 7). One of these groups was connected to a maleimide end-capped polystyrene of 40 repeat units, yielding a lipase–polystyrene giant amphiphile. Disperson of this giant amphiphile in water led to the formation of micrometer long fibers consisting of bundles of micellar rods. The individual rods possessed a diameter between 25 and 30 nm, closely corresponding to the diameter predicted for micellar architectures built up from these macromolecules. The micellar assemblies exhibited 6–7% of the activity of a free CALB enzyme. This loss of activity can be partially explained by destabilization of the protein molecules and partly by the

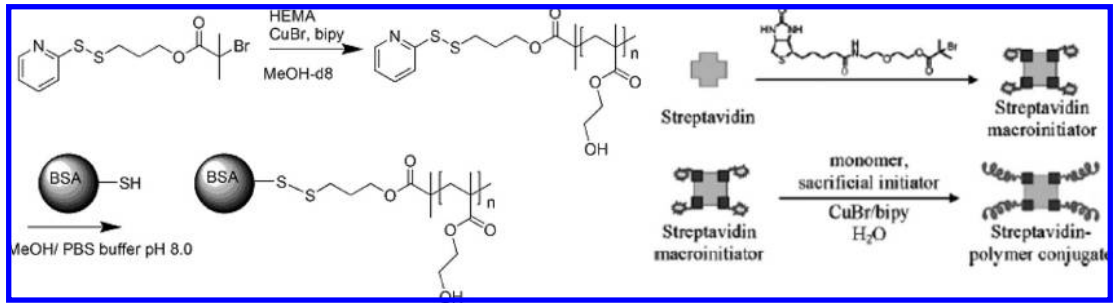

FIGURE 8. Two different routes toward giant amphiphiles: (left) synthesis of a functional polymer with a protected SH group using ATRP followed by coupling to an enzyme; (right) biotin-containing initiator, which after complexation with SAV was used to synthesize a straptavidin–polymer conjugate.

effect of bundling, which makes most of the head groups inaccessible to the substrate molecules.

Besides the thiol group of cysteine residues, also amine side chains of lysine have been targeted with the objective to synthesize biohybrid macromolecules. The excellent reactivity of amines toward a wide range of nucleophiles and the fact that each protein contains at least one amine makes this an attractive method for bioconjugation. Reaction with amines, however, has also disadvantages, one being the fact that often more than one amine is present on the surface of the protein, resulting in nonspecific and random coupling. This heterogeneous conjugation can lead to a significant decrease in bioactivity. Site-specific modification of amines has been attempted by exploiting the lower pK_a of the N-terminal α-amine of proteins; however, even when the coupling reaction is conducted under slightly acidic conditions, heterogeneity is often still observed.[53,63] Detailed reviews on this method have already been written.[53,54]

Also click chemistry (*vide supra*) can be employed to synthesize protein–polymer conjugates by a covalent connection. Dirks et al. have functionalized an enzyme with an azide or acetylene and subsequently coupled an azide or acetylene functionalized polymer to it using standard click reaction conditions. This procedure is very versatile because it can be applied to different enzymes and (poly)peptides and the conversions are very high.[41] It was observed in this particular case that after the click reaction, the giant amphiphiles formed spherical structures. Another example in which click chemistry was used to prepare enzyme dimers has been reported by Hatzakis et al.[64]

Maynard and co-workers have synthesized thioether- and disulfide-containing initiators and employed these compounds in ATRP.[65] Treatment of the resulting polymers (e.g., poly(hydroxyethylmethacrylate) with a reducing agent such as dithiothreitol (DTT) yielded mercapto-terminated polymers, which were coupled to a thiol group of bovine serum albumin (BSA) (see Figure 8). This strategy is applicable to a wide variety of polymer–protein conjugates without the need for postpolymerization modifications of the polymer, and therefore, this is another suitable method for preparing giant amphiphiles. In another example, they used a temperature-responsive polymer for the coupling to BSA.[66] Above the LCST, the synthetic polymer becomes water-insoluble, and a giant amphiphile is obtained.

In the previous examples, a functional polymer was first synthesized by ATRP and then coupled to an enzyme. In an elegant modification of this procedure, Maynard and co-workers have grown polymers directly from a protein initiator.[67] To this end, biotin was functionalized with an initiator, and after complexing with Sav, an ATRP was carried out to obtain the Sav–polymer conjugate (Figure 8). The authors only used the temperature-responsive polymer polyNIPAAm, but this procedure opens the possibility to synthesize all kinds of amphiphilic polymer–protein conjugates, avoiding the tedious coupling of the usually incompatible protein and polymer segments. Further advantages are the easier purification of the product and the fact that the exact number of polymer chains attached to the enzyme is known.[68–70] Protein macroinitiators are also being employed by other groups for the construction of protein–polymer conjugates, however, without aiming for amphiphilic properties.[71]

A different way of attaching the polymer chain to the protein is by using metal–ligand coordination.[72] Following this approach, BSA or CALB were functionalized with a terpyridine ligand using standard protein coupling procedures. Protein–polymer giant amphiphiles were subsequently obtained by adding a polystyrene end-capped with a mono(terpyridine)ruthenium(II) complex. The thus prepared amphiphiles displayed different types of aggregate morphologies, depending on the length of the polystyrene segment.

An interesting approach to synthesize giant amphiphiles is via cofactor reconstitution, that is, the polymer is coupled to the cofactor of an enzyme and subsequently a reconstitution with the apoenzyme is carried out (Figure 9). This method was

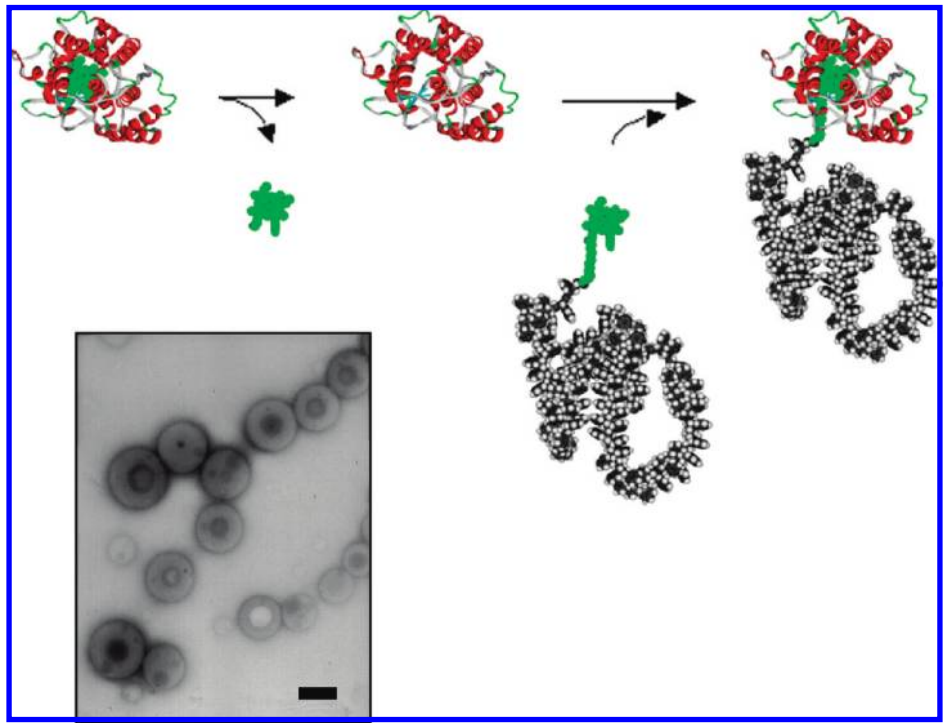

FIGURE 9. Reconstitution method to prepare PS-*b*-Mb and PS-*b*-HRP giant amphiphiles. Inset shows the self-assembly behavior of PS-*b*-HRP forming vesicle structures (scale bar = 100 nm).

employed by Nolte and co-workers to prepare polystyrene–HRP and –myoglobin (Mb) biohybrids.[73,74] The desired biohybrid amphiphiles were obtained by adding a THF solution of the heme cofactor-appended polymer to an aqueous solution of the apoenzyme. The formation of the giant surfactants was confirmed by UV–vis spectroscopy and gel electrophoresis. Electron microscopy (TEM and cryo-TEM) revealed the formation of vesicular aggregates with diameters of 80–400 nm. In most cases, these aggregates enclosed spherical objects, often located away from the center of the aggregates. To explain these structures, it was assumed that the heme-functionalized polymer first forms aggregates on the surface of which the reconstitution reaction with the apo-HRP takes place to form the biohybrid. The latter compound then forms vesicles enclosing the initial aggregates. Enzyme activity measurements on these bioassemblies revealed no activity when HRP was reconstituted at 4 °C. However, when the reconstitution of apo-HRP was carried out at 22 °C, the enzyme–polymer hybrid surprisingly retained much of its activity. Similar experiments were carried out with the oxygen-carrying protein myoglobin. It was found that giant amphiphiles prepared from this protein also self-assembled to generate vesicular structures, which fused in time to form larger vesicles. Again the activity of the protein was retained but turned out to be reduced when compared with the native species.

The cofactor reconstitution method was also used to synthesize biohybrid triblock copolymers, that is, triblock copolymers in which one block is a protein or an enzyme. To this end, the cofactor was functionalized with a diblock copolymer, that is, polystyrene-*b*-poly(ethylene oxide), with the help of click chemistry.[75] Instead of iron protoporphyrin IX, the zinc derivative was chosen because it is fluorescent properties allowing fluorescence spectroscopy to be used as a complementary characterization technique to prove that the desired copolymers are formed. Giant amphiphiles containing either Mb or HRP as the protein block were synthesized and dispersed in an aqueous solution. A large variety of aggregate morphologies were observed, see Figure 10.

Conclusions and Outlook

Over a period of more than 4000 years, surfactants have been used by man, and their unusual behavior has been the subject of numerous studies. Despite this fact, these compounds continue to attract the interest of scientists and have inspired them to design new ones with different and often unexpected self-assembling properties. As shown in this Account, surfactants that are much larger than the classical ones, that is supersurfactants and giant surfactants, can now be easily synthesized starting from synthetic polymers and biopolymers. Like their low molecular weight counterparts, they display intriguing self-assembling properties, which have led to the

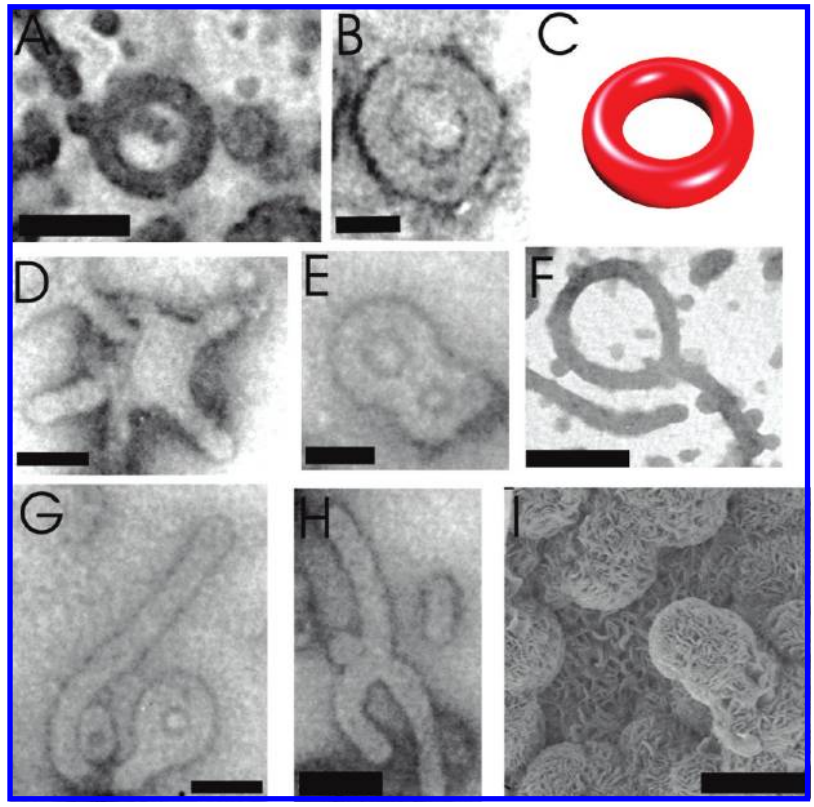

FIGURE 10. Aggregate morphologies observed after dispersing triblock copolymers of Mb-*b*-PS-*b*-PEG and HRP-*b*-PS-*b*-PEG in water: (A, B) toroids; (C) schematic figure of a toroid; (D) octopi; (E) figure eights; (F–H) micellar aggregates; (I) scanning EM (SEM) images of aggregates of MbZn-*b*-PS_{48}-*b*-PEG_{113}, which form spherical aggregates consisting of lamellae. Bars represent 100 nm for panels A–H and 500 nm for panel I. Reproduced from ref 75 with permission. Copyright 2007 American Chemical Society.

discovery of new structures such as toroids and other complex architectures. Since these polymer-based amphiphiles are very stable, they are potentially interesting materials for application in the fields of nanotechnology and life sciences (e.g., as drug delivery systems and as nanoreactors).[76]

The successful synthesis of amphiphiles containing a protein or enzyme as head group opens unique possibilities to construct functional nanosized architectures of a complexity approaching those found in Nature. Although enzymes and proteins have been extensively functionalized in the past, the fact that the simple attachment of a polymer tail can provide them at will with self-assembling properties, similar to those of, for example, phospholipids, opens new avenues for the construction of functional biomimetic assemblages. The construction of these well-defined amphiphiles might, furthermore, provide new insights into the way the (macro)molecular structure dictates the eventual size and shape of the formed assemblies. Within this context, the balance between thermodynamic and kinetic control in the association of amphiphilic molecules will be one of the coming challenges to concur. In particular, predicting and controlling aggregate formation under kinetic conditions, that is, away from equilibrium, we feel is highly interesting.

The next synthetic challenge will be the construction of amphiphiles that are even larger than the ones discussed in this Account. These may be conceived to consist of a long hydrophobic polymer tail and a virus capsid as the hydrophilic headgroup. We have coined the name megasurfactants for such nanosized biohybrid block copolymers and are currently trying to synthesize them starting from the cowpea chlorotic mottle virus.

The Netherlands' Organization for Scientific Research (NWO), the European Science Foundation (ESF), and the Royal Academy for Arts and Sciences (KNAW) are acknowledged for financial support.

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FOOTNOTES

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