Protein – Polymer Hybrid Amphiphiles**

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Low molecular weight amphiphilic molecules are known to form a large variety of self-assembled structures, for example monolayers, micelles, vesicles, and rod- and sheetlike structures, in water.[1] Well-defined diblock copolymers—so-called superamphiphiles—such as poly(ethyleneglycol) – poly(benzyl ether) dendrimers, polystyrene – poly(propyleneimine) dendrimers, polystyrene-*block*-poly(2-vinylpyridine)s, and block copolymers of styrene and isocyanopeptides, have been shown to generate these highly ordered structures as well.[2] Herein we describe a new class of amphiphiles, namely "giant amphiphiles", in which a protein or enzyme acts as a polar head group and polymers act as the apolar tails (Figure 1). These biohybrids differ from other protein – polymer systems described in the literature, in the sense that the protein to polymer ratio is predefined and the position of the conjugation site is precisely known.

The hybrid amphiphiles were prepared by the association of two molecules of monobiotinylated polystyrene (**1**; $M_n = 9147$, $M_w/M_n = 1.03/1$, Figure 1a) with streptavidin. This 60-kD protein consists of four identical subunits, each of which can bind one biotin molecule.[3, 4] The affinity between streptavidin and biotin is so high ($K_a \approx 10^{15}\,\text{M}^{-1}$; 21 kcal mol^{-1}) that the complex formation can be regarded as irreversible. The valeric acid carboxyl group of biotin can be used for modification because it does not play a significant role in the binding process.[3, 5] The binding sites are positioned in pairs on opposite faces of the protein, which allows the use of the protein as a versatile modular building block in the construction of supramolecular systems (Figure 1b).[6]

The ability of biotinylated polystyrene **1** to specifically bind streptavidin was ascertained using the HABA – streptavidin assay.[7] In this test the dye HABA (4′-hydroxyazobenzene-2-carboxylic acid) is expelled from the protein binding pocket by the biotin end group of the polymer, which results in changes in the relative intensities of the UV/Vis absorption bands of the dye.

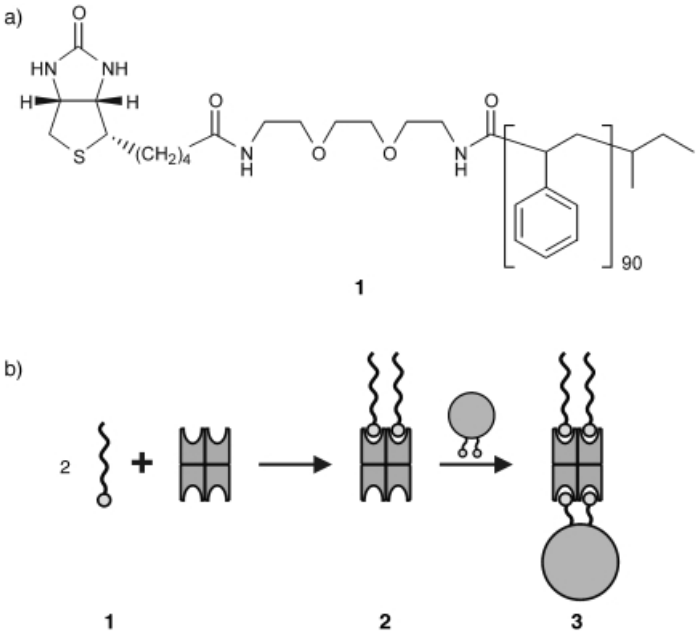

Figure 1. a) Structure of biotinylated polystyrene **1**. b) Schematic representation of giant amphiphile **2** and the modular construction of the functional protein – polymer hybrids **3**.

The protein – polymer hybrids were constructed by making use of a Langmuir trough, which allowed us to monitor the formation of the giant amphiphiles. The biotinylated polymer **1** was spread at the air/water interface, and subsequent compression of the monolayer resulted in the formation of a rigid film. Brewster angle microscopy (BAM) and atomic force microscopy (AFM) revealed that this monolayer was the result of merging separate microscopic domains of biotinylated polymer (Figure 2a). The surface pressure/surface area isotherm of **1** in the absence of streptavidin (Figure 3) showed a lift-off area of 5.2 nm^2 and a molecular area of 3.9 nm^2. A dramatic increase in these two values per biotinylated macromolecule was observed (to 14.8 and 12.0 nm^2, respectively) in the presence of active streptavidin (0.5 equiv). A molecular area of 24 nm^2 was deduced[8] for the amphiphilic complex from the obtained area per polymer chain. This value agrees well with those of 23.5 – 35.7 nm^2 reported for streptavidin in the literature.[9] To confirm that the increase in area is largely a consequence of the specific binding of the protein to the biotinylated polymer, the isotherm of **1** was recorded on a subphase containing deactivated streptavidin (Figure 3). This isotherm showed only slight differences from that of **1** on a buffer solution as a result of unspecific binding of the protein.[10]

Monolayers of the giant amphiphiles, prepared with rhodamine Red-X labeled streptavidin, were transferred to solid substrates by the Langmuir – Schaefer (LS) technique.[11] Confocal fluorescence microscopy ($\lambda_{ex} = 543$ nm) in combination with AFM revealed fluorescent domains with a thickness of 7 nm (Figure 2c,d). Investigation of these do-

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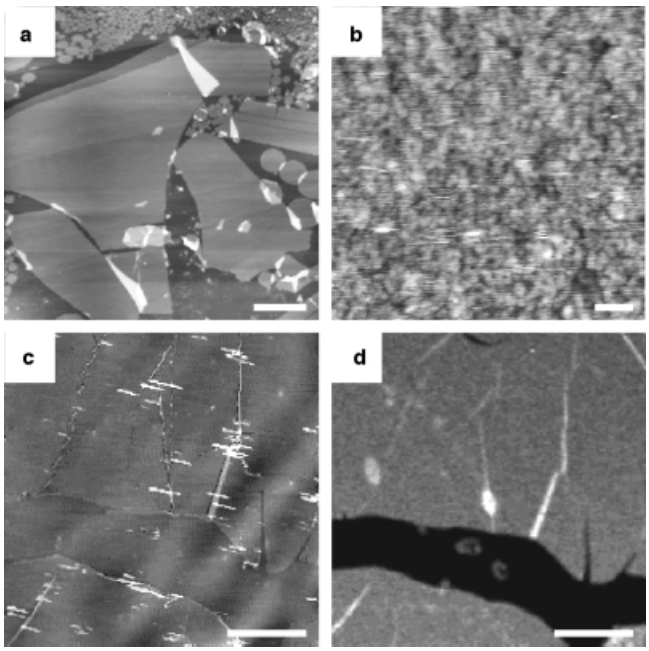

Figure 2. Topographic images of a monolayer of polymer **1** measured with confocal-AFM in water: a) pure polymer (scale bar = 1 μm); b) polymer with bound streptavidin (scale bar = 50 nm). Monolayer of rhodamine Red-X labeled protein–polymer hybrids: c) AFM topographic image (scale bar = 10 μm); d) confocal fluorescence image (λ_{ex} = 543nm, scale bar = 10 μm).

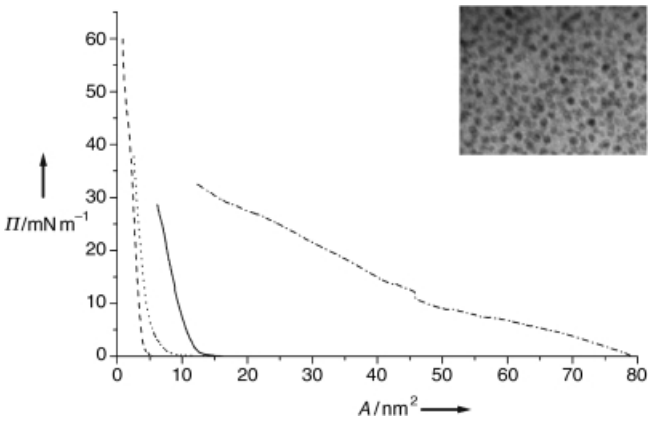

Figure 3. Surface pressure/surface area isotherms of biotinylated polystyrene **1** recorded on a subphase without protein (– – –); a subphase containing streptavidin (——); a subphase containing deactivated streptavidin (····); and a subphase containing streptavidin and biotinylated ferritin (·–·–). The inset shows a transmission electron microscope image of a compressed ferritin-streptavidin–polystyrene monolayer (Π = 35 mN m^{-1}) transferred to a formvar coated electron microscope grid.

mains in a separate experiment demonstrated that the surfaces of these layers consisted of spherical particles. The dimensions of these particles agreed well with those expected for streptavidin (Figure 2b), when AFM tip-broadening effects and the mobile nature of the ethyleneglycol moiety of polymer **1** under the process of liquid imaging are taken into account.[12] To confirm that the pair of binding sites of streptavidin opposite to that occupied by the polymer chains was still available, the sample was incubated in a solution of fluorescein-labeled biotin. Fluorescence imaging (λ_{ex} = 488 nm) suggested that the geographic location of the bound fluorescein indeed corresponded with that of the rhodamine Red-X label of streptavidin.

The ability of the protein–polymer hybrid to complex other biotinylated molecules was tested in the case of the 460-kD iron storage protein ferritin. Biotinylated ferritin was added to the subphase underneath a noncompressed monolayer of streptavidin–polystyrene conjugates. The resulting system could be compressed to a uniformly dense film in which the ferritin molecules are closely packed (Figure 3). The molecular area of the conjugate was estimated from the recorded isotherm to be 126 nm^2 (63 nm^2 per polymer chain), which agrees well with values reported for the cross-sectional area of ferritin (133nm^2).[13]

Enzyme functionalized protein–polymer hybrids could be prepared from a covalent horse radish peroxidase(HRP)–streptavidin (1/1) conjugate by using the approach described above for ferritin. Extensive washing of the subphase was performed to exclude the presence of unbound HRP–streptavidin molecules. The catalytic activity of the resulting polymer–enzyme hybrid system was assessed by using the standard ABTS/H_2O_2 assay.[14] To this end hydrogen peroxide and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were injected into the subphase, which was circulated through a UV flowcell. This procedure meant that the reaction could be monitored in real time. The slope of the activity plot proved to be independent of the applied surface pressure, which indicates that the catalytic activity of the enzyme is not influenced by lateral pressure in the range measured (Π = 7–33 mN m^{-1}, Figure 4). Confirmation that

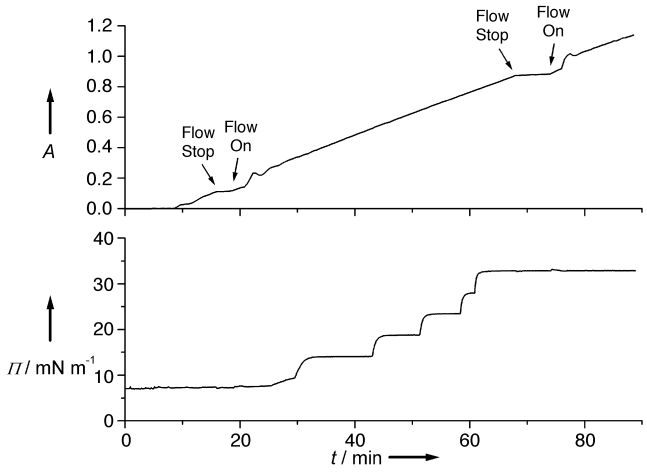

Figure 4. UV absorption at 420 nm of the subphase (top) and the surface pressure of the monolayer (bottom) as a function of time for the HRP–streptavidin–polystyrene hybrid system. The arrows indicate when the circulation of the aqueous subphase through the UV cell was switched on or off.

the oxidation reaction occurred at the monolayer of HRP–streptavidin–polystyrene hybrids and not in the subphase was obtained from the observation that the slope of the curve immediately leveled off when the circulation of the aqueous subphase through the UV cell was stopped.

In summary, we have constructed amphiphilic protein–polymer hybrids by the association of two biotinylated polystyrene molecules **1** and streptavidin. The pair of free

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binding sites opposite to that occupied by the polymer chains can be used to bind other biotinylated molecules, as was demonstrated for the iron storage protein ferritin. The giant amphiphiles can also be functionalized with enzymes as was shown with horse radish peroxidase. The resulting hybrids retained their catalytic activity. The precise control over the structure of the giant amphiphiles in principle allows for tuning of their aggregate morphologies. In this way well-defined structures analogous to those reported for low molecular weight surfactants and surfactants derived from block copolymers will become attainable.

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A Tetrameric Nickel(II) "Chair" with both Antiferromagnetic Internal Coupling and Ferromagnetic Spin Alignment**

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Interest in polynuclear complexes of the 3d metals has been stimulated by the search for new magnetic materials[1, 2] and by demonstration of the occurrence of oligonuclear metal centers in proteins such as urease.[3] Of the relatively small number of reported tetranuclear complexes of $S = 1$ nickel(II) of known structure, the majority have a hemicubane- or "butterfly-" rather than a squarelike core.[4] Of these molecules, just one is entirely antiferromagnetic,[5] while the remainder entail purely ferromagnetic interactions amongst the nickel(II) ions.[6–10] Oximes have shown promise as bridging ligands for the preparation of polynuclear complexes.[11, 12] The reaction of 1,4,7-triazaheptane (diethylenetriamine, Dien) with the monooxime of 2,3-butanedione (ModaH) in the presence of Ni^{II} ions, instead of yielding the anticipated Schiff base derivative, gave the tetranuclear Ni^{II} compound **1** (dark brown crystals; $C_4H_8O_2$ = 1,4-dioxane), containing uncondensed but coordinated ketone and amine groups. Figure 1a shows the structure of the the cation of **1**, while Figure 1b highlights its Ni^{II} core.

$[\{Ni(Dien)\}_2(\mu_3\text{-}OH)_2\{Ni_2(Moda)_4\}](ClO_4)_2 \cdot 2\,C_4H_8O_2 \cdot 2\,H_2O$ **1**

The centrosymmetric $[\{Ni(Dien)\}_2(\mu_3\text{-}OH)_2\{Ni_2(Moda)_4\}]^{2+}$ ion possesses an Ni_4O_2 core based on a "chair" topology.[13] Two central Ni atoms (Ni_c) with pseudo-octahedral N_2O_4 donor ligand sets are bridged by hydroxyl ions to form a central Ni_2O_2 parallelogram (Figure 1b) in which the Ni_c–O(μ) distances are 2.038 Å, the Ni-O-Ni angles are 97.73°, and the Ni_c–Ni_c separation is 3.070 Å. The $Moda^-$ ligands are coordinated to the Ni_c atoms through their ketone-O and oximate-N atoms. The Ni_c–O(ketone) bonds in **1** (2.113 and 2.114 Å) are akin to another recently described[14]

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