Towards a self-assembled membrane made of bionanoparticle-polymer conjugates

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INTRODUCTION

Proteins are well characterized nano-objects, which can introduce functionality to polymer films and membranes. Here, we plan to use protein-containing polymer films as scaffolds for nanoporous membranes after denaturation of the protein moieties.

As a model protein particle, horse spleen ferritin is used.[1,2] It is an iron storage protein of 12 nm diameter containing a ferric oxide-phosphate core within a 6 nm central cavity. The protein shell is stable in a broad range of pH and temperature in water. 24 chemically addressable primary amino end groups allow its bioconjugation with an initiator or short polymers.[3-5]

POLYMER CONJUGATION VIA GRAFTING-FROM

Ferritin is transformed into a macroinitiator for Atom Transfer Radical Polymerization (ATRP) by active ester chemistry. Thus a controlled growth of polymer chains from the protein shell may be achieved. SEC in buffer is used to collect monomeric ferritin. Excess 2-bromoisobutyric acid (BIBA) is reacted with monomeric ferritin in presence of *N*-(3-dimethylaminopropyl)-*N´*-ethylcarbodiimide hypochloride (EDC) and sulfo-N-hydroxysuccinimide. The macroinitiator is purified by dialysis against a phosphate buffer (0.1 M, pH 8,4).

N-isopropyl acrylamide (NIPAAm) and oligo(ethylene glycol) methacrylate (OEGMA) are polymerized directly in pure water via ATRP at low temperature from the ferritin macroinitiator.[6, 7] PNIPAAm is a thermo-responsive polymer with a lower critical solution temperature (LCST) of 32°C.[8] PEGMA has a tunable LCST from 26 to 90°C depending on the variation of co-monomer composition, in particular the number of ethylene glycol units in the side (DP from 2 to 9) [9]. The polymerization is conducted in presence of BIBA as a sacrificial initiator at 4 °C with $CuCl/CuCl_2/Me_6TREN$ as catalyst at a ratio [Initiator]:[CuCl]:[$CuCl_2$]:[Me_6TREN] = 1:1.6:0.4:2. Finally, the polymer-conjugate solution is purified by dialysis against a phosphate buffer.

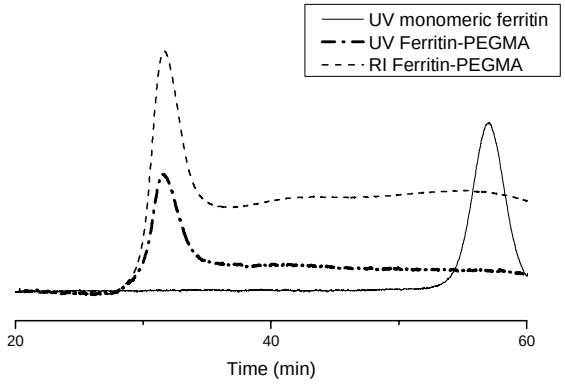

Figure 1. SEC measurement in water of monomeric ferritin before and after polymerization of $O(EG)_9MA$.

SEC measurements (Figure 1) show a shift of the main peak (at 55 min for monomeric ferritin), indicating that the particles become larger (due to the polymer grafted from the protein shell) and electron microscopy reveals that the particles were not crosslinked during the polymerization (Figure 2).

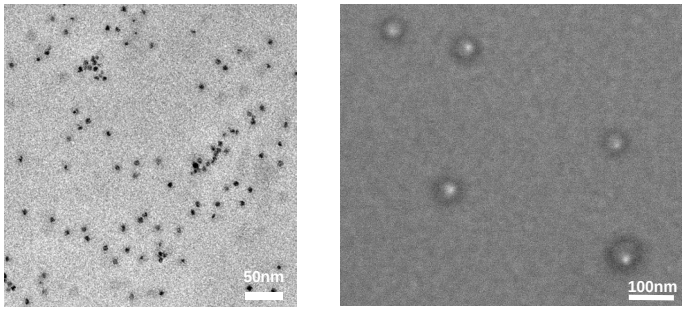

Figure 2. Cryo-TEM (left) and SEM (right) images of ferritin-PEGMA conjugates.

SOLUTION CHARACTERIZATION OF THERMORESPONSIVE CONJUGATES

In the following, we characterized the PNIPAAm-modified particles in solution and observed the effect of temperature. At the LCST, the polymer molecular dimensions change abruptly, followed by an aggregation of individual polymer chains into globular particles yielding an optically detectable phase transition.

UV-Vis turbidity measurements and dynamic light scattering (DLS) allow studying the behavior of the particles in solution as a function of temperature.

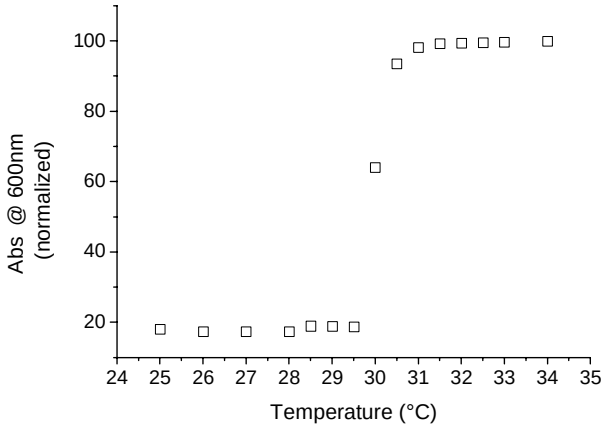

Figure 3. Turbidity measurement of PNIPAAm grafted from ferritin.

For the turbidity measurement (Figure 3), the absorbance at 600 nm was chosen because of the absence of specific absorptions at this wavelength. By increasing the temperature, PNIPAAm undergoes phase separation, resulting in a turbid solution. The cloud point, T_c, defined as the temperature at 50% turbidity, is T_c = 30 ± 0.5 °C. It depends on the hydrophilicity of the end groups and thus on the molecular weight of the polymer.[10,11] Here, we determined the molecular weight of the grafted chains to be M_n = 100 kDa, PDI = 1.2 (Free PNIPAAm chains resulting from the sacrificial initiator used for a better control of the polymerization, are characterized by SEC in NMP with PS as a standard). The observed transition is fast and reversible.

In order to understand the behaviour of the particles in solution as a function of temperature, dynamic light scattering was performed.

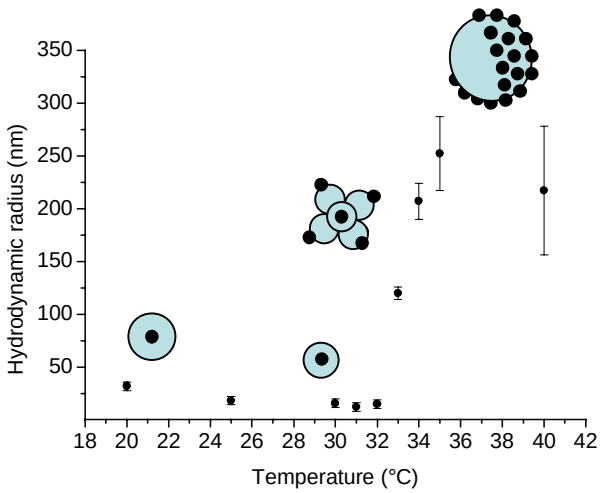

Figure 4. DLS measurements of the ferritin-PNIPAAm conjugate particles.

In Figure 4, it can be seen that the particles start to contract when the temperature is increased from 20 to 32 °C as the cloud point is approached. As a consequence, the hydrodynamic radius of the single particles decreases from 30 nm to 12 nm. After further increase in temperature, the particles slowly start to aggregate. This may be explained by the fact that the PNIPAAm is becoming hydrophobic and tends to reduce its interface with water. Thus, the ferritin particles are orientated towards the water interface while they aggregate via the contracted PNIPAAm. At around 36 °C, there are already large agglomerates of ~250 nm. This temperature-induced aggregation is also observed by TEM as shown in Figure 5.

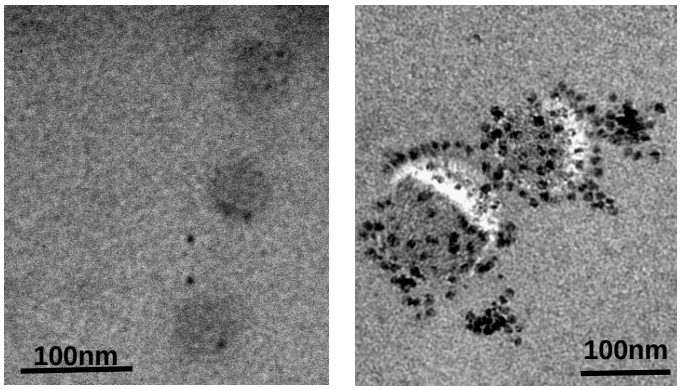

Figure 5. TEM images drop-coated of ferritin-polymer conjugates at room temperature and after heating to 40°C.

SELF-ASSEMBLY AT THE LIQUID-AIR INTERFACE

In a second step, the polymer-modified protein particles were mixed with a high molecular weight PNIPAAm and self-assembled at a liquid-air interface, aiming at the creation of thin films as precursors for nanoporous membranes.

Spin-, dip- and drop-coating procedures were performed to find a suitable method to obtain thin films as homogeneous as possible with good thickness control. Figure 6 shows a representative AFM image of such a polymer-bionanoparticle film.

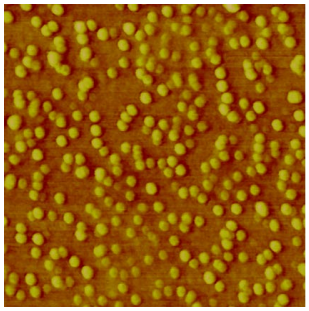

Figure 6. AFM phase image of ferritin-PNIPAAm conjugate particles mixed with PNIPAAm over 1 µm², z-range: 60°.

CONCLUSIONS

In this paper, we have demonstrated the controlled grafting of PNIPAAm and P(OEGMA) from ferritin via ATRP. The resulting bioconjugates are thermoresponsive, as demonstrated by turbidimetry, DLS and electron microscopy. In addition, the self-assembly behavior of these bionanoparticle-polymer conjugates within a polymer matrix was demonstrated. Thus, these particles may serve as precursors for the generation of functional polymeric membranes with controlled pore sizes on the nanometer scale.

ACKNOWLEDGEMENTS

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