Poster

SYNTHESIS AND CHARACTERIZATION OF FERRITIN DIMERS: A NEW APPROACH TOWARDS MULTIFUNCTIONAL MATERIALS

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Biological moulds, such as apoferritin, can be used to synthesize nanoparticles [1]. This biomimetic approach offers a number of advantages over other synthetic methods. Worth mentioning, among them, are the good homogeneity in the size and shapes of these moulds and the possibility of functionalizing the biological envelope with different chemical groups. Apoferritin robustness against relatively large variations in temperature and pH enables the synthesis of a variety of inorganic materials, from magnetic oxides such as γ-Fe_2O_3 [1] and Gd complexes [2], to metals (Cu [3], Ni, Cr [4], etc) and even semiconductors as CdS [5] or CdSe [6]. For this reason, ferritin-based nanoparticles are seen as promising materials for a vast number of applications. The versatility of ferritin as a mould could also be advantageously employed to obtain multifunctional materials, by e.g. linking together ferritin molecules with different functionalities (e.g. a large magnetic moment in one monomer plus an optically active centre in the other). Although ferritin dimers and higher oligomers are present in solution [7], these molecular associations are mainly composed of reversible dimers with very weak interaction [8]. Here, as a first step, we report the synthesis and purification of dimers of natural ferritin molecules bound covalently by chemical linkers.

Ferritin molecules were covalently bound using the cross-linking agent sulfo-LC-SPDP, a sulfonated and longer analog of SPDP. This heterobifunctional cross-linker contains an amine-reactive N-hydroxysuccinimide (NHS) ester that reacts with lysine residues to form a stable amide bond. The other end of the spacer arm is terminated in the pyridyl disulfide group that reacts with sulfhydryls to form a reversible disulfide bond. Different portions of functionalized ferritin were made to react in 1:1 stoichiometry, favoring the production of dimers. The chromatogram (Figure 1) showed three well defined peaks, the first corresponding hypothetically to dimers, the second to monomers and the third to non-reacted SPDP.

The fractions of the sample containing respectively ferritin monomers (peak 2 in Fig. 1) and dimers (peak 1 in Fig. 1) were further investigated by AFM. Typical images are shown in Fig. 2. The peak attributed to ferritin shows well-separated, 20-40 nm wide, molecules with an apparent height of 10 ± 2 nm. The apparent width is considerably larger than the true dimension due to tip broadening. Dimers show 40-80 nm width and approx the same height as monomers, indicating that the conformation of the protein has not been altered during dimerization. Further evidence of the formation of stable dimers is provided by the magnetic characterization of aqueous solutions of the two samples. As *T* decreases, the susceptibility of the monomers and dimers shows the typical drop or “superparamagnetic blocking” that is also observed in natural ferritin samples [9]. However, the blocking temperature of the dimers is higher, by more than a 50 percent, than the blocking temperature of the monomers. It is well known that the dipolar interactions between magnetic nanoparticles can modify, often

increase, the energy barriers for the magnetization reversal [10] thus leading to an increase in T_b, as observed here. In fact, these artificially engineered ferritin dimers provide model systems to investigate how interparticle interactions modify the magnetic memory of nanoparticles.

In conclusion, we have achieved the synthesis of covalently bound natural ferritin dimers, which are stable in solution. These materials open the door to the design of biocompatible multifunctional nanomaterials, e.g. by the combination of two ferritin monomers encapsulating nanoparticles of two different compounds. This work is currently underway.

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Figures:

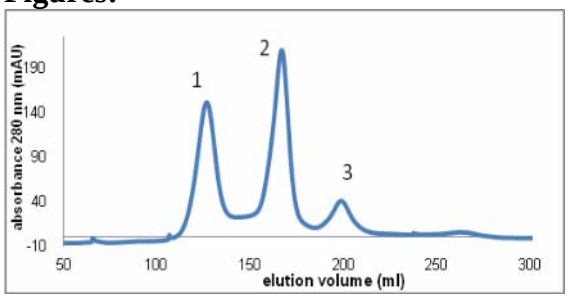


Fig. 1. Chromatogram following absorbance at wavelength 280 nm vs volume corresponding to the separation of dimers from monomers by size exclusion chromatography. From right to left the molar mass grows.

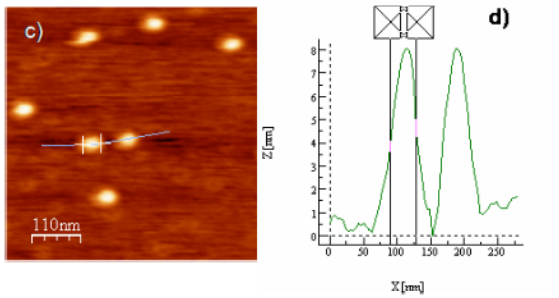
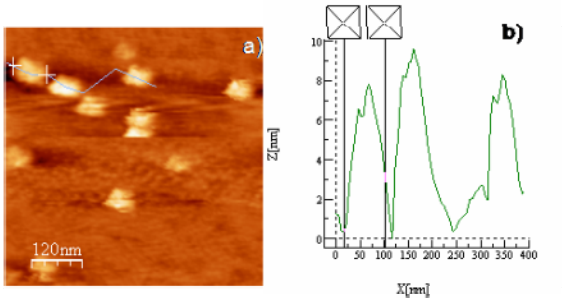


Fig. 2. AFM images in Jumping Mode of a characteristic region of the sample taken in contact conditions in acetate / glycine buffer, pH 4. a) sample from Peak 2; b) profile of the dimerized protein from a); c) sample from Peak 1; d) profile of the monomer protein from c).



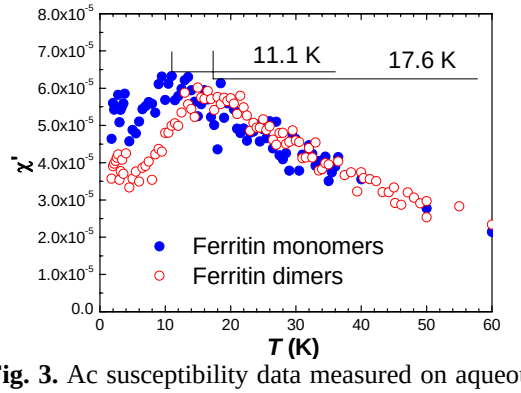


Fig. 3. Ac susceptibility data measured on aqueous solutions of ferritin monomers and dimers.