Supplementary Information

Stable polymersomes based on ionic-zwitterionic block copolymers modified with superparamagnetic iron oxide nanoparticles for biomedical applications

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1. AFM measurements

AFM measurements in dry state were conducted to confirm the ability of polymersomes to enclose SPION/CCh. To see the differences between free and enclosed nanoparticles, this experiment was performed using the sample that was not purified from the free SPION/CCh. Topography images were obtained using a Quantitative Nanomechanical Mapping (QNM) mode with lower and higher deflection setpoints of 1 and 25 nN, respectively. As shown in Fig. S1, the images of the same SPION/CCh-polymersome recorded for different deflection setpoints show different structural topographies. The image of the SPION/CCh-polymersome recorded at the lower deflection setpoint (Fig. S1A) shows that the polymersomes are significantly flattened both due to adsorption on the surface and pressure applied by the tip. The surface of the SPION/CCh-polymersome was imaged and SPION/CCh enclosed in the polymersome are not visible as clearly as free ones, which are present in the corners of the image. On the other hand, a stiff and hard AFM tip and higher deflection setpoint forced thin polymersome membrane to stick to the enclosed SPION/CCh surface (Fig. 1B), which are now clearly visible. Hard SPION/CCh, which were not enclosed inside the polymersomes, did not change their shapes.

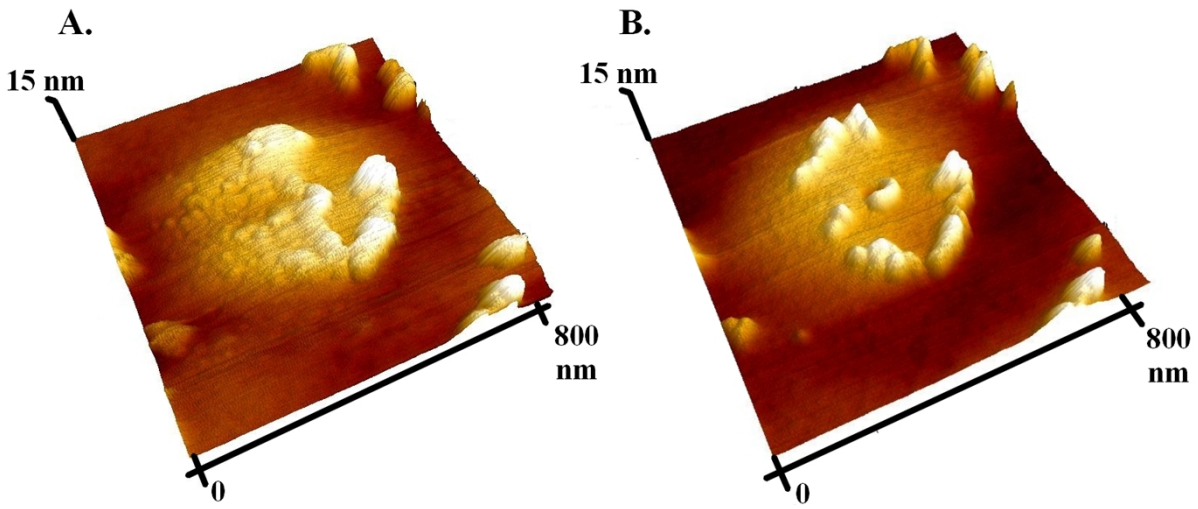

Fig. S1. AFM topography image of SPION/CCh-polymersome: (A) lower deflection setpoint (1 nN), (B) higher deflection setpoint (25 nN). Free SPION/CCh are visible in the corners.

Using QNM mode adhesion, tip deformation and DMT modulus images were obtained as well. As shown in Fig. S2, the difference between enclosed (blue arrows) and free (red arrows) SPION/CCh is clearly visible. Enclosed SPION/CCh indicate adhesion values similar to the polymersome surroundings. Moreover, tip deflection change for them is almost insignificant like for the polymer. For free SPION/CCh, both adhesion contrast and tip deflection value are high. Finally, DMT modulus signal exists only for hard free SPION/CCh. This very sensitive method does not detect differences in the signal for enclosed SPION/CCh and polymersomes without SPION/CCh. That admittedly confirms that SPION/CCh (except those added in purpose as free) are enclosed inside polymeric structures.

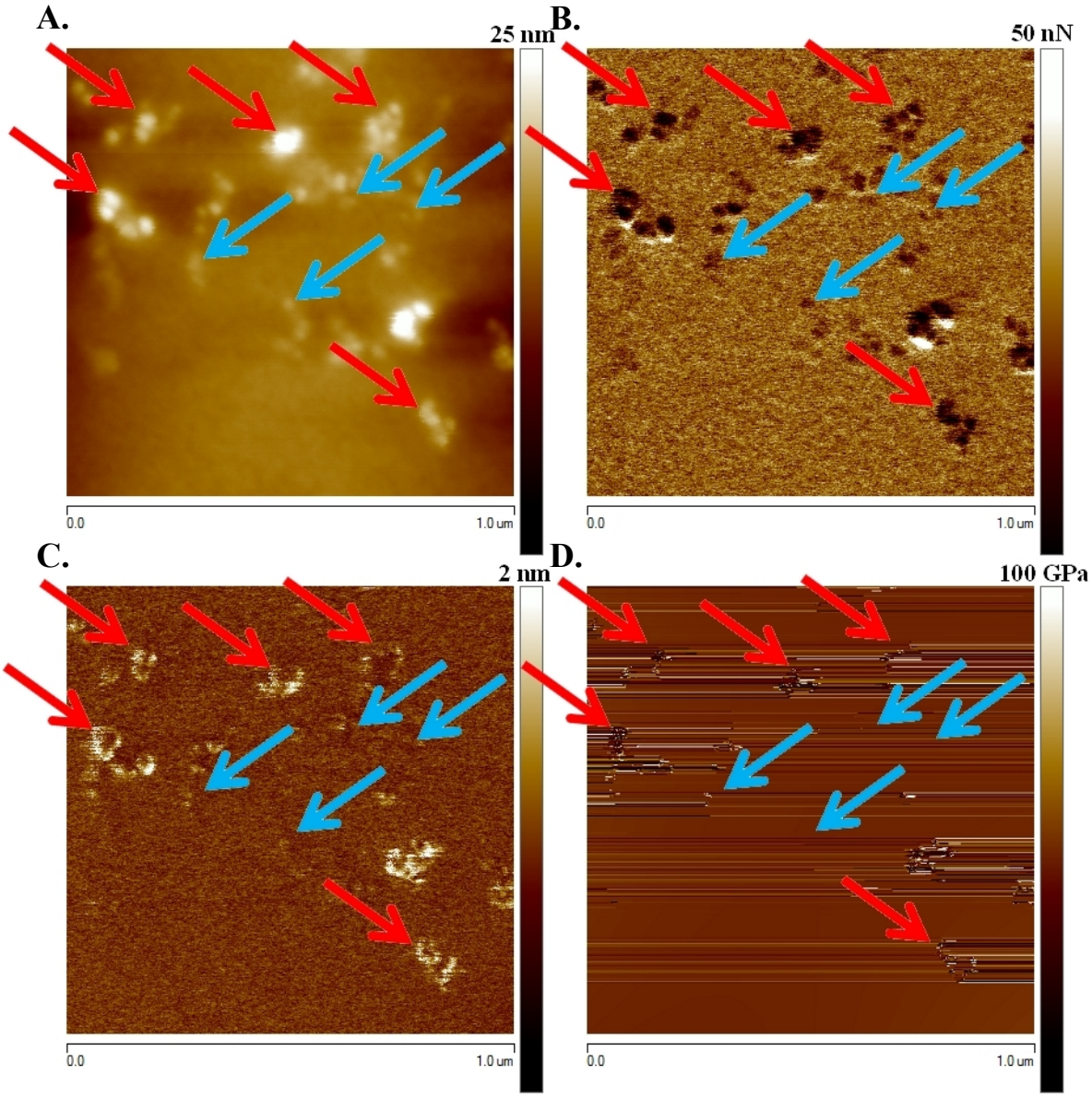

Fig. S2. AFM images of SPION/CCh-polymersomes with free (red arrows) and enclosed SPION/CCh (blue arrows): A. topography, B. adhesion, C. tip deformation, D. DMT modulus.

2. Relaxivity measurements

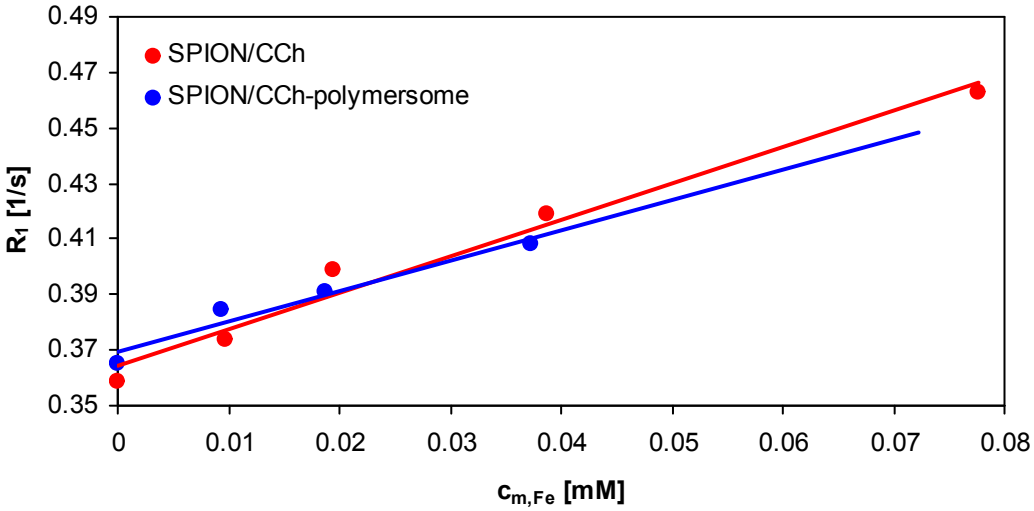

Fig. S3. Dependence of longitudinal relaxation rate on iron concentration in SPION/CCh and SPION/CCh-polymersome at 9.4 T.

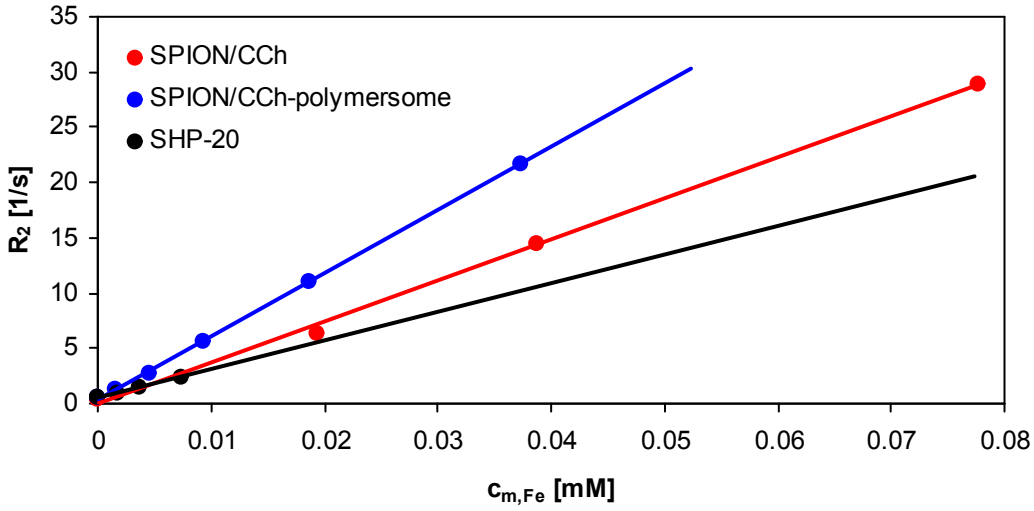

Fig. S4. Dependence of transverse relaxation rate on iron concentration in SPION/CCh, SPION/CCh-polymersome and the commercial MRI contrast agent (SHP-20, Ocean NanoTech) at 9.4 T.

3. Confocal measurements

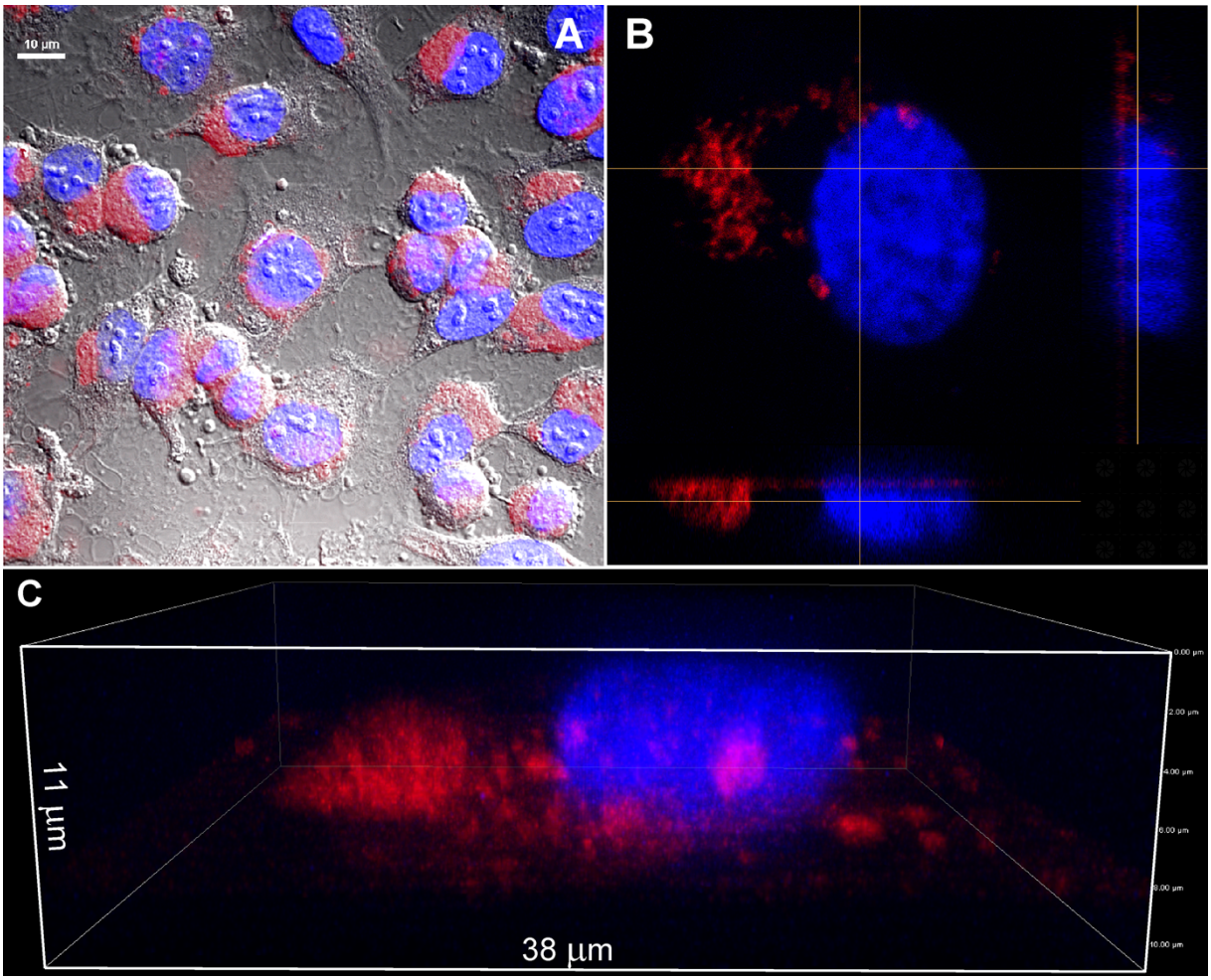

Fig. S5. (A) Confocal micrograph showing fluorescence of fluorescently modified SPION/CCh-polymersomes (red) superimposed on the transmitted light image of EA.hy926 cells. The nuclei were stained with Hoechst 33342 (blue). (B) Cross sections of the EA.hy926 cell incubated with fluorescently modified SPION/CCh-polymersomes and (C) three-dimensional image of the cell constructed from a series of fluorescence micrographs.