

Impact of Serum Proteins on MRI Contrast Agents: Cellular

Binding and T₂ Relaxation

Supporting Information

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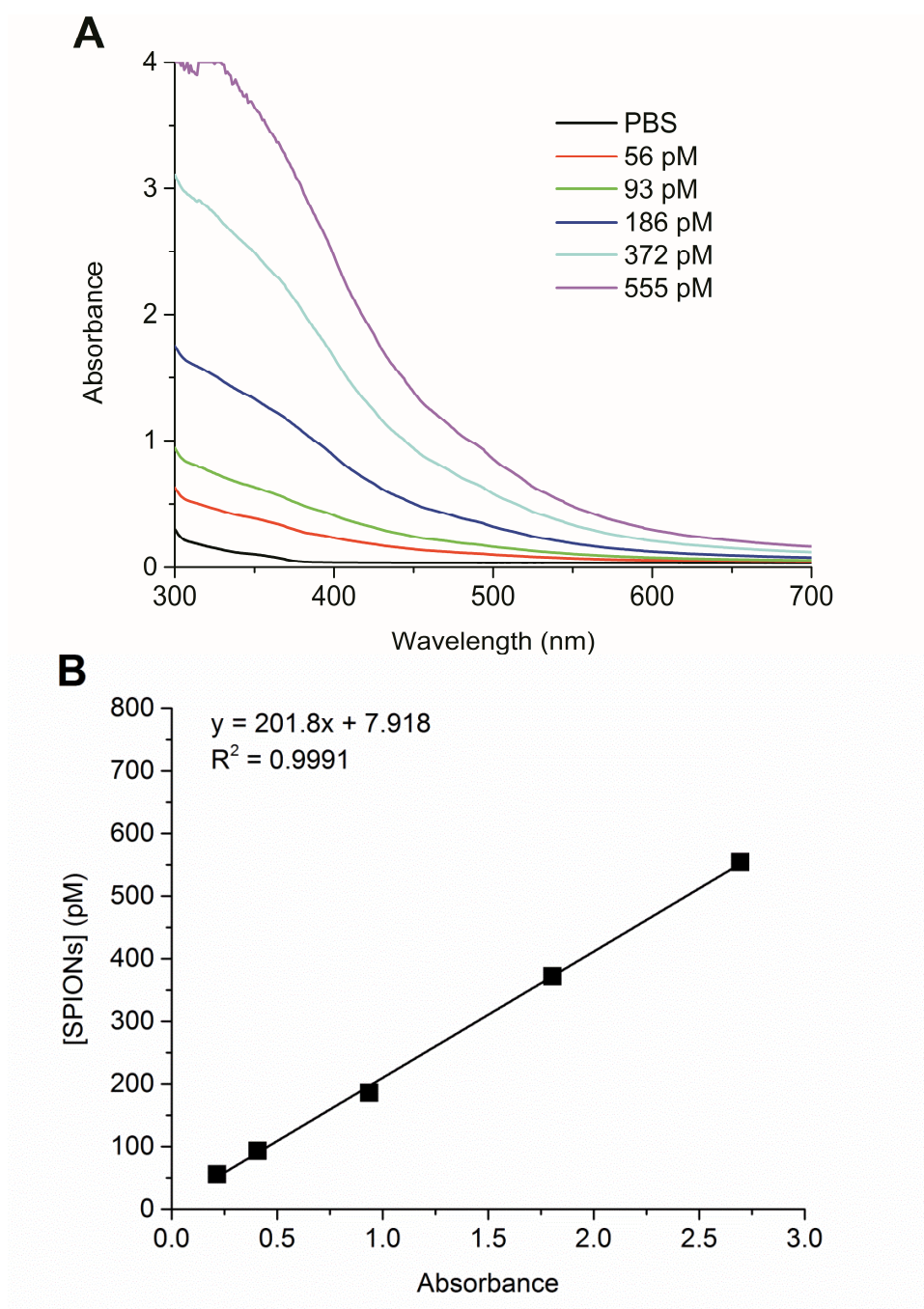


Figure S1 UV-Vis absorption spectroscopy was used to determine the concentration of SPIONs. Initial particle number in a given volume of a SPIONs suspension was calculated using the particle number provided by the supplier ($1.8 \cdot 10^{15}$ nanoparticles/g). (A) Absorbance spectra of SPIONs (0 -555 pM, in PBS). (B) Absorption at 390 nm as a function of SPION concentration. Despite the lack of a distinctive absorption maximum, the absorption values at 390 nm (black squares) showed a linear fit (line). This calibration curve was used to determine the concentration of SPIONs following washing via centrifugation and resuspension.

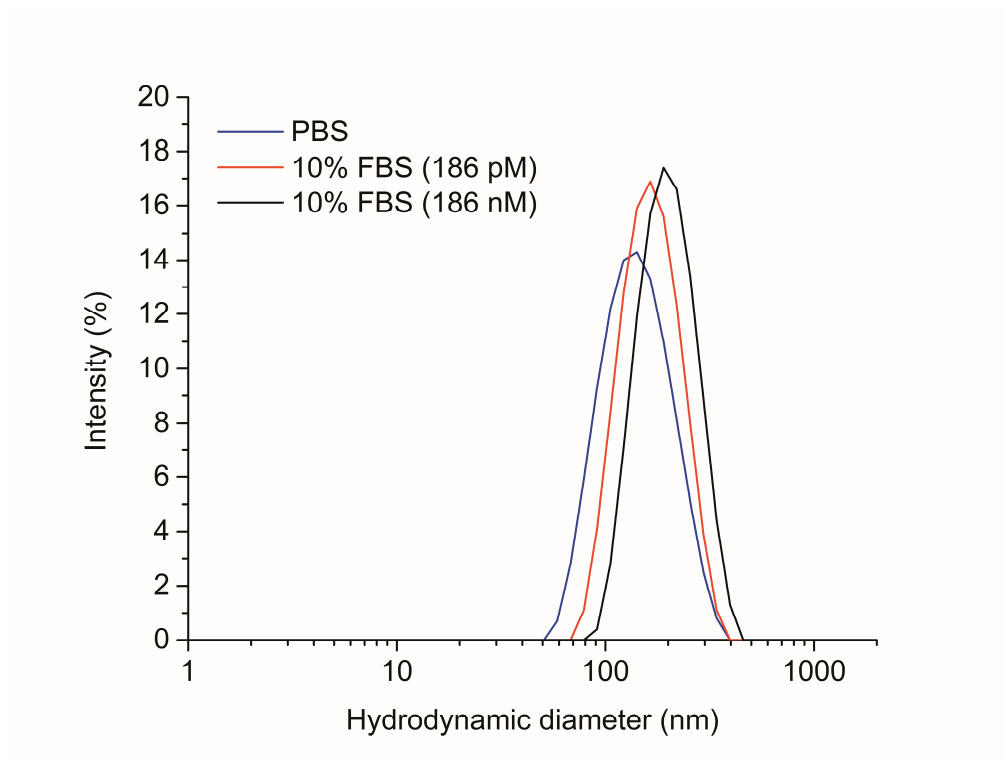


Figure S2 Stability of SPIONs in biological media. SPIONs were diluted in water, PBS lacking Ca^{2+} and Mg^{2+} , and PBS lacking Ca^{2+} and Mg^{2+} supplemented with 10% FBS. The hydrodynamic diameter was measured with dynamic light scattering. The narrow distribution around 110 nm shows that the SPIONs did not aggregate in the media used in the course of experiments. Table S1 includes a complete description of hydrodynamic diameter and polydispersity that includes media in which aggregation occurred.

Table S1 Hydrodynamic diameter and polydispersity index (PDI) of SPIONs in common biological media. SPIONs were characterized in PBS, MEM, DMEM/F12 buffer, and Leibovitz's L15 medium. Divalent cations, Ca^{2+} and Mg^{2+} , led to aggregation. The PBS buffer used for experiments did not contain Ca^{2+} and Mg^{2+} . Although divalent cations are also present in FBS, the proteins in FBS likely stabilize the SPIONs. Measurements were carried out in triplicate and error bars show standard deviation.

Medium	Hydrodynamic diameter (nm)	PDI
PBS ^a	129 ± 2	0.114 ± 0.022
MEM ^b	348 ± 49	0.033 ± 0.024
DMEM/F12 ^c	212 ± 23	0.119 ± 0.026
Leibovitz's L15	176 ± 8	0.096 ± 0.009
PBS + 10% FBS (186 pM)	147 ± 2	0.168 ± 0.017
PBS + 10% FBS (186 nM)	184 ± 7	0.099 ± 0.03
^a Phosphate-buffered saline		
^b Minimal Essential Medium		
^c Dulbecco's Modified Eagle Medium/ Nutrient Mixture F12		

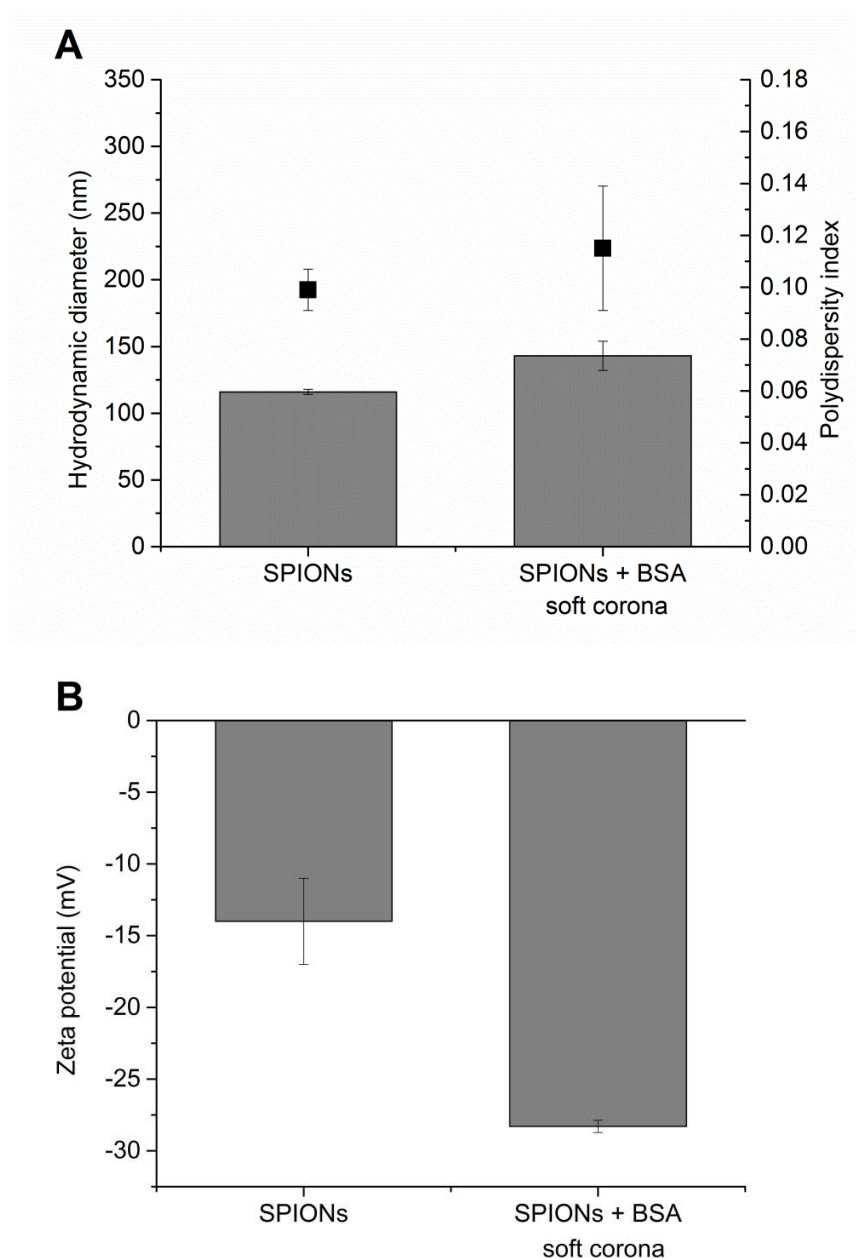


Figure S3 BSA forms a corona on SPIONs. (A) Dynamic light scattering shows an increase in the diameter (gray bars) of SPIONs following incubation of SPIONs (93 pM) in 2.5 mg/mL BSA and one centrifugation and resuspension wash step (soft corona). The polydispersity index indicates that the size increase is not due to aggregation. (B) The decreased zeta potential indicates that the effective charge of the SPIONs decreases following incubation with BSA. Measurements were carried out in triplicate. Error bars show standard deviation (n=3). Isolating SPIONs with a BSA hard corona was not possible, as SPIONs aggregated upon further centrifugation and resuspension.

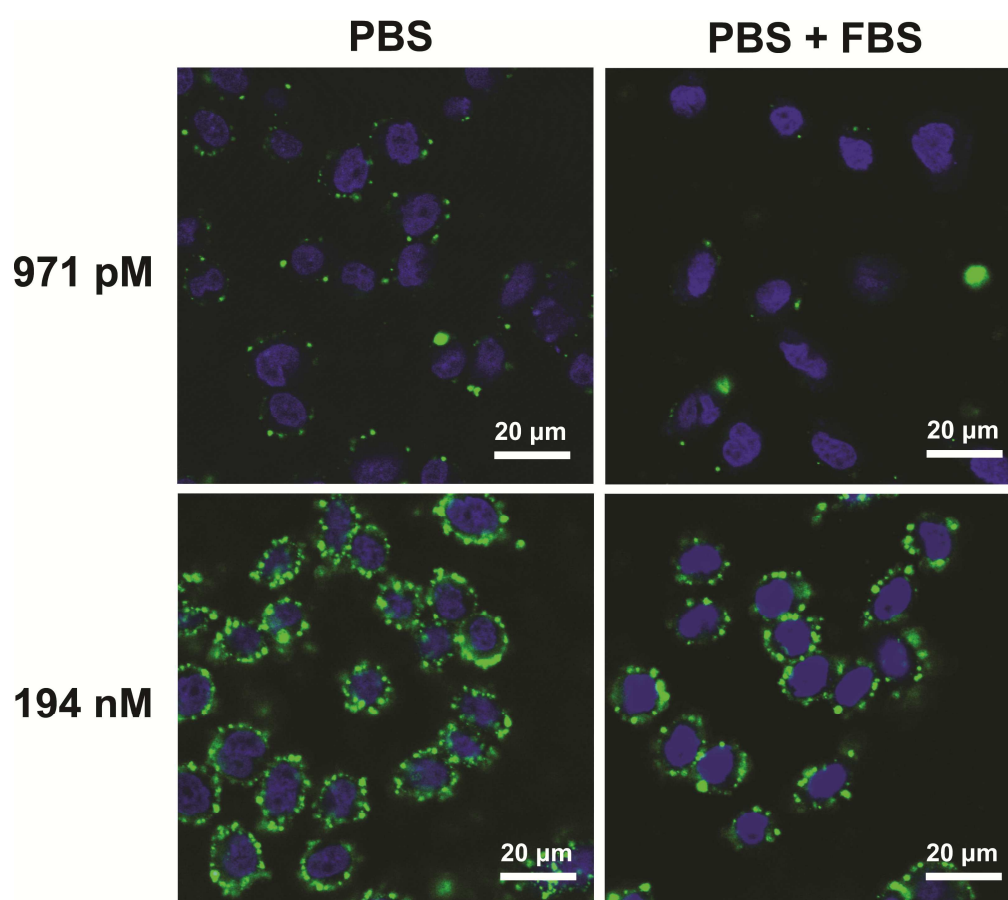


Figure S4 Confocal fluorescence microscopy images show that the decreased cellular binding of SPIONs (green) to CHO cells in the presence of serum proteins also occurs at higher SPION concentrations (971 pM and 194 nM, compared to 186 pM in Fig. 2A). Nuclei were labeled with DAPI (blue).

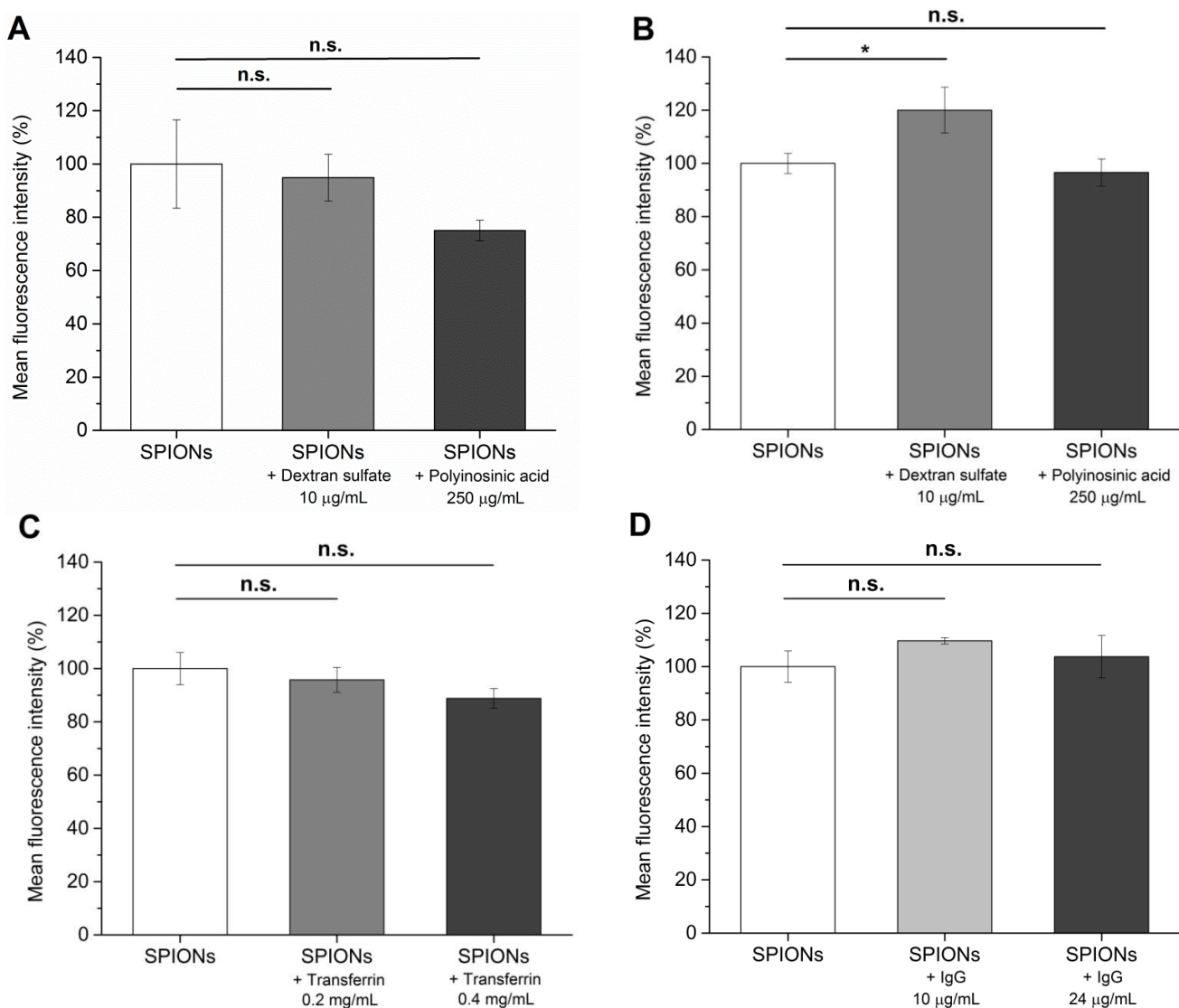


Figure S5 SPIONs do not bind to scavenger, transferrin, or IgG receptors on CHO cells. (A) Flow cytometry shows that the cellular binding of bare SPIONs is not inhibited by dextran sulfate (500 kDa) or polyinosinic acid, standard competitors for scavenger receptors.¹ The concentrations of dextran sulfate (10 μ g/mL) and polyinosinic acid (250 μ g/mL) were chosen based on previous work from Chao and coworkers and Patel and coworkers^{2, 3} and previous work from our group with polystyrene NPs and BS-C-1 cells.⁴ (B) Similarly, dextran sulfate and polyinosinic acid do not inhibit the cellular binding of FBS-coated SPIONs (soft corona). (C) Cellular binding of SPIONs to CHO cells in the presence of transferrin (holo-transferrin, bovine, #T1283, Sigma-Aldrich). The transferrin concentration in 10% FBS varies from 0.18 to 0.22 mg/mL.⁵ (D) Cellular binding in presence of IgG. Concentration of IgG in 10% FBS is approximately 20 μ g/mL.⁶ * indicate p-values < 0.05 determined by ANOVA analysis in combination with the Tukey's range test. Error bars show standard deviation (n=3). SPIONs concentration was 186 pM for all experiments. n.s. indicates not significant.

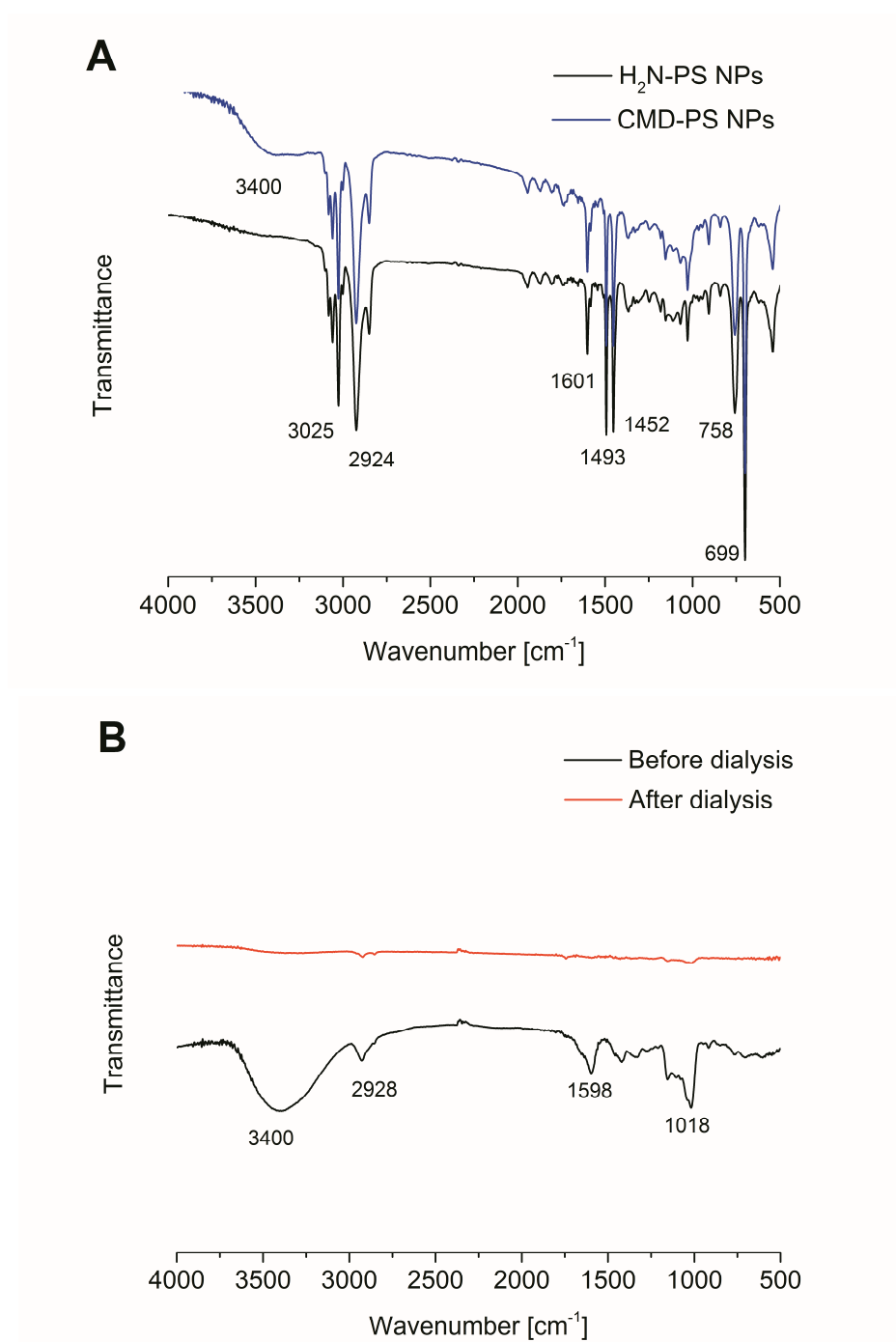


Figure S6 FT-IR characterization of CMD-PS NPs. (A) Spectra of H₂N-PS NPs and CMD-PS NPs. The broad peak at 3400 cm⁻¹ (O-H stretch of dextran) indicates successful functionalization of the PS NPs. (B) Dialysis successfully removes unbound carboxymethyl dextran from the nanoparticle solution. Spectra of carboxymethyl dextran before (black line) and after (red line) dialysis (46 hours, 4 changes of 4 L volume). Pure carboxymethyl dextran solution without nanoparticles (8 mg/mL) was dialyzed under the same conditions as the carboxymethyl dextran-modified polystyrene nanoparticles and served as a control.

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