Protein Corona Affects Relaxivity and MRI Contrast Efficiency of Magnetic Nanoparticles

Houshang Amiri[1*], Lorenzo Bordonali[5], Alessandro Lascialfari[5,6], Sha Wan[2], Marco P. Monopoli[2], Iseult Lynch[2,∫*], Sophie Laurent[7], and Morteza Mahmoudi[3,4*]

[1]*Radiology Department, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*
[2]*Centre for BioNano Interactions, School of Chemistry and Chemical Biology and UCD Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland*
[3]*Department of Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[5]*Physics Department, University of Milan, Milan, Italy*
[6]*Physics Department "A. Volta", Univerity of Pavia, Pavia, Italy*
[7]*Department of General, Organic, and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Avenue Maistriau 19, B-7000 Mons, Belgium*

The first three authors contributed equally as "first author" to this work.

***Corresponding Authors:**

(HA) Web: runmc-radiology.nl; email: H.Amiri@ncmls.ru.nl

(M.M.) Web: www.biospion.com; email: Mahmoudi-M@TUMS.ac.ir

(I.L.) email: i.lynch@bham.ac.uk

∫ Current address: School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston,
Birmingham B15 2TT, United Kingdom

Differential Centrifugal Sedimentation:

Differential centrifugal sedimentation experiments wereperformed with a CPS Disc Centrifuge DC24000. The analyzer measures particle size distributions using centrifugal sedimentation within an optically clear spinning disc that is filled with fluid. Sedimentation is stabilized by a density gradient within the fluid, and accuracy of the measured sizes is insured through the use of a known size calibration standard run immediately before each measurement. The use of a biological sample with a large amount of proteins requires a new sucrose gradient to be prepared for each measurement. The concentration of the particles at each size is determined by continuously measuring the turbidity of the fluid near the outside edge of the rotating disc. The turbidity measurements are converted to a weight distribution by Mie theory light scattering calculations. The choice of the experimental parameters, namely the fluid gradient, the speed of rotation, etc. is based on the type of material being analyzed and the range of sizes being measured. The primary information from the analytical disk centrifuge is the time taken for the particles to travel from the centre of the disk through a defined viscous sucrose gradient to a detector placed at the outer rim of the disk under a strong centrifugal force. For materials with homogenous density and simple shape (for example spherical particles) one can directly relate this time to a particle size.

Where objects are inhomogeneous, or irregular in shape, the different arrival times still allows one to distinguish between them. In the simplest approximation one still approximates them to an equivalent uniform sphere, and this is the meaning of the size cited on the x-axis of all figures presented and hence it should be considered as an ‘apparent’ size. Moreover, for the sake of clarity in the comparison of different samples, we chose to show data as relative weight particle size distribution. The tallest peak (highest weight value) in the distribution is called the ‘base’ peak (has a value of 100%) and all other particle size (multimer) peaks are then normalized against this base peak to give a relative weight distribution. It is important to

emphasize that the conversion from absorption raw data to molecular weight data is correct as long as the optical parameters and the density for particles and fluid are correct and the particles are spherical. In this regard, the information we get about the large NP-protein clusters at about 500 nm are merely indicative of their existence but do not provide insight regarding their real size or their absolute amount.

In order to define the protein thicknesses at the surface of various SPIONs, a simple core-shell method was used, with dextran-coated SPIONs as the core and protein biomolecules as the shell, to calculate 2 densities using the following equation[1]:

$$\frac{(\rho_c - \rho_s)}{(\rho_c - \rho_f)}\frac{D_c{}^3}{D_s} + \frac{(\rho_s - \rho_f)}{(\rho_c - \rho_f)}D_s{}^2 = D^2$$

where ρ_c is the density of the inner core, ρ_s is the density of the shell, ρ_f is the density of the fluid, D_c is the diameter of the core, $D_s(D_c+2\delta)$ is the total diameter of the core-shell particles, and D is a diameter measured by the DCS method. The protein thicknesses thus obtained are presented in Table 2 of the manuscript. It is notable to mention that the density of core@shell nanoparticles were calculated as follows: density core*volume core + density shell*volume shell (the diameters of core and shell were obtained from TEM images)

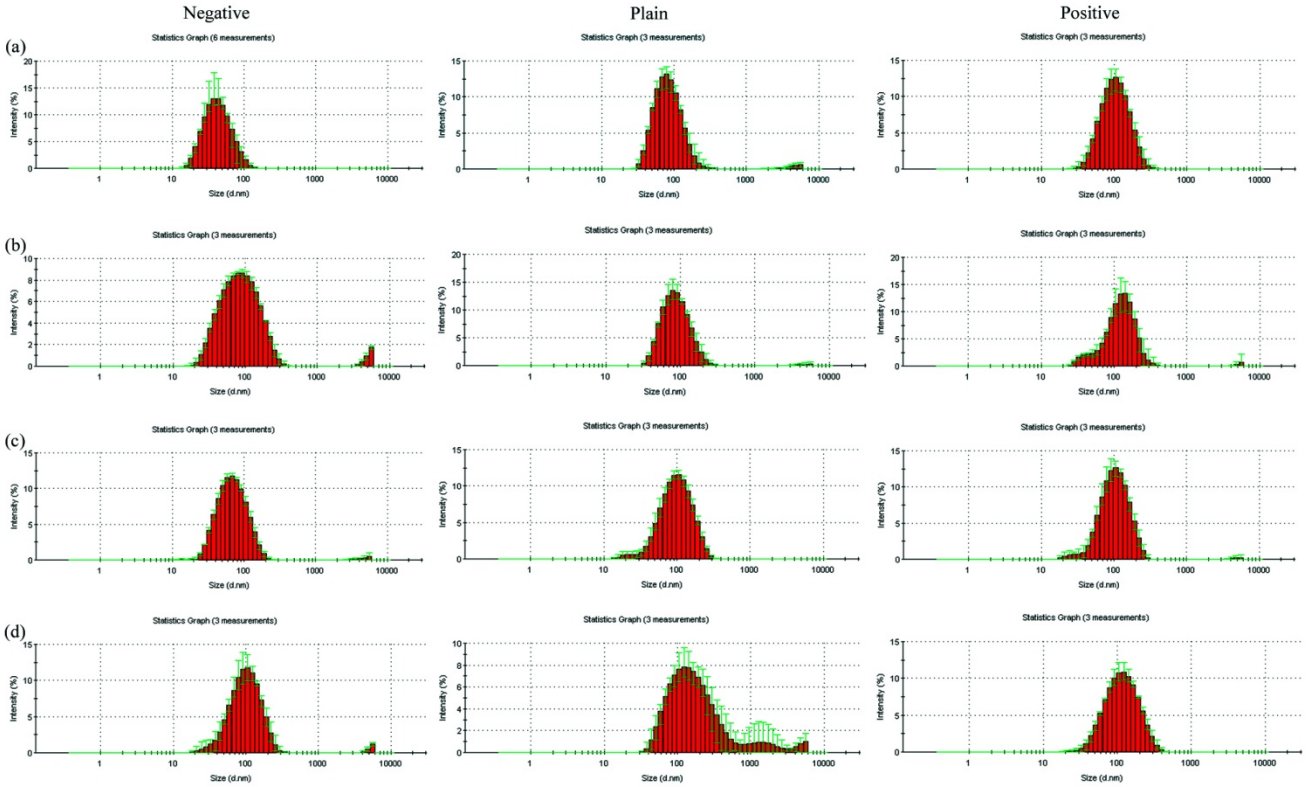

Figure S1: CONTIN distribution curves for the single coated-SPIONs dispersed in (a) PBS and (b) FBS. (c) and (d) CONTIN distribution curves for the double coated-SPIONs dispersed in PBS and FBS, respectively.

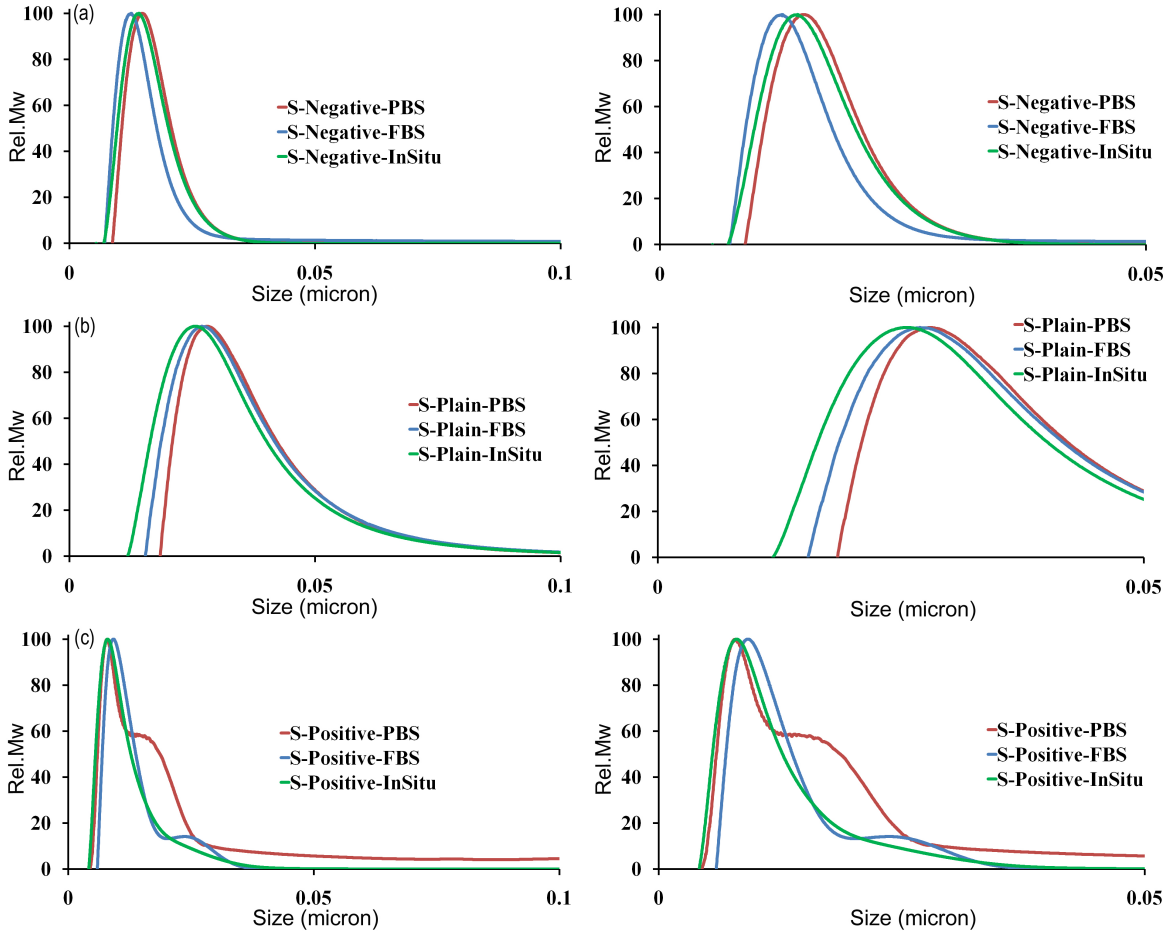

Figure S2: **DCS size distribution data for various single-layer dextran-coated SPIONs.** (a) Negative, (b) Plain, and (c) positive single-layer dextran coated SPIONs (Left) and enlargement of the main peak areas (Right) to enhance the shift to larger size before, and after interactions with FBS in the absence (i.e. S-Surface Charge-FBS) and presence (i.e. S-Surface Charge-InSitu) of excess proteins. Note the presence of a second peak in the Positive-dextran coated SPIONs, as a result of particle-agglomeration, which was less prominant in the protein solutions than in PBS.

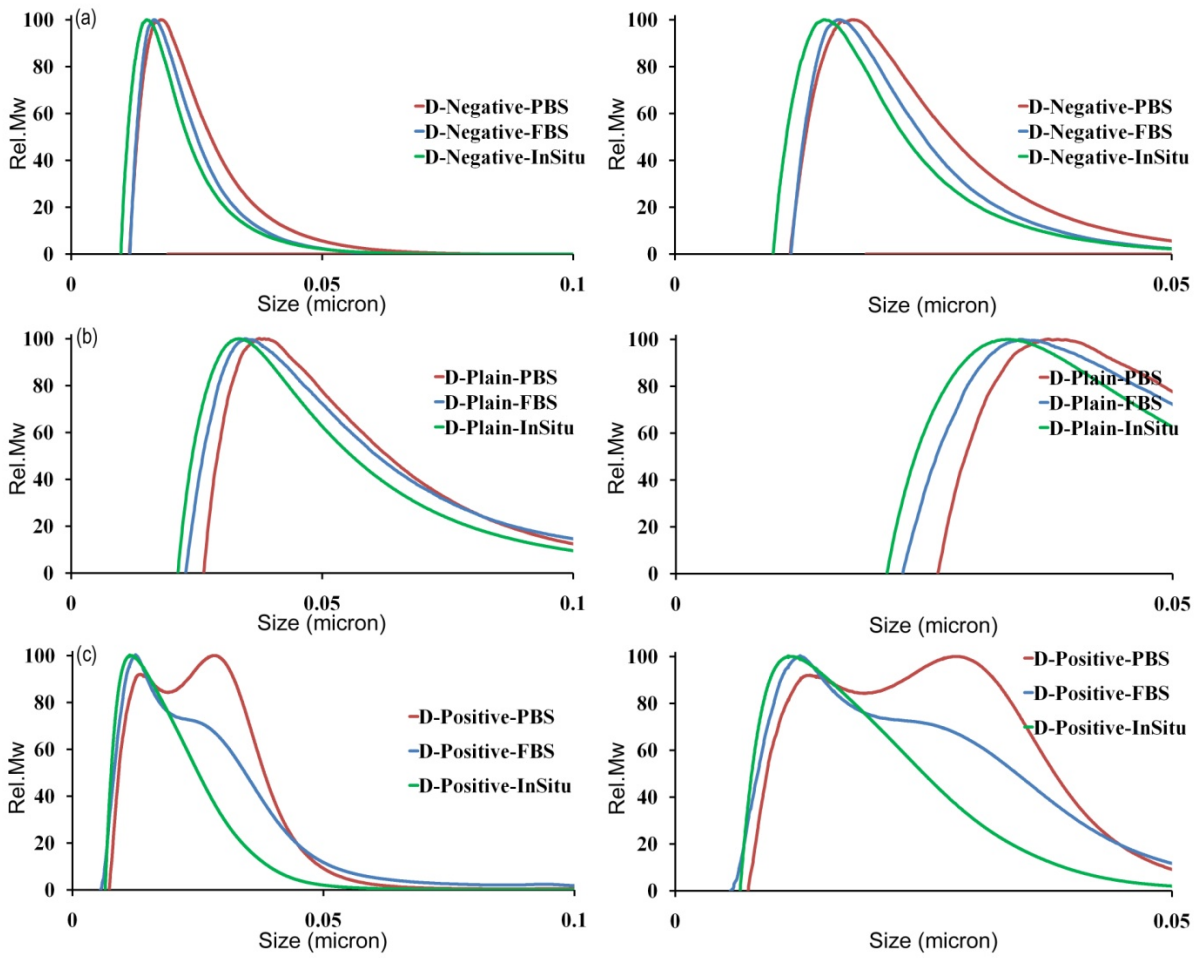

Figure S3: **DCS size distribution data for various dextran double-coated SPIONs** (a) Negative, (b) Plain, and (c) positive double-layer dextran coated SPIONs (Left) and enlargement of the main peak areas (Right) to enhance the shift to larger size before, and after interactions with FBS in the absence (i.e. D-Surface Charge-FBS) and presence (i.e. D-Surface Charge-InSitu) of excess proteins. Note that it appears that the second layer did not form effectively for the Plain SPIONs.

References

[1] D. Walczyk, F. B. Bombelli, M. P. Monopoli, I. Lynch, K. A. Dawson, *Journal of the American Chemical Society* **2010**, *132*, 5761–5768.