Electronic Supporting Information

Superparamagnetic Iron Oxide Nanoparticles Functionalized by Peptide Nucleic Acids

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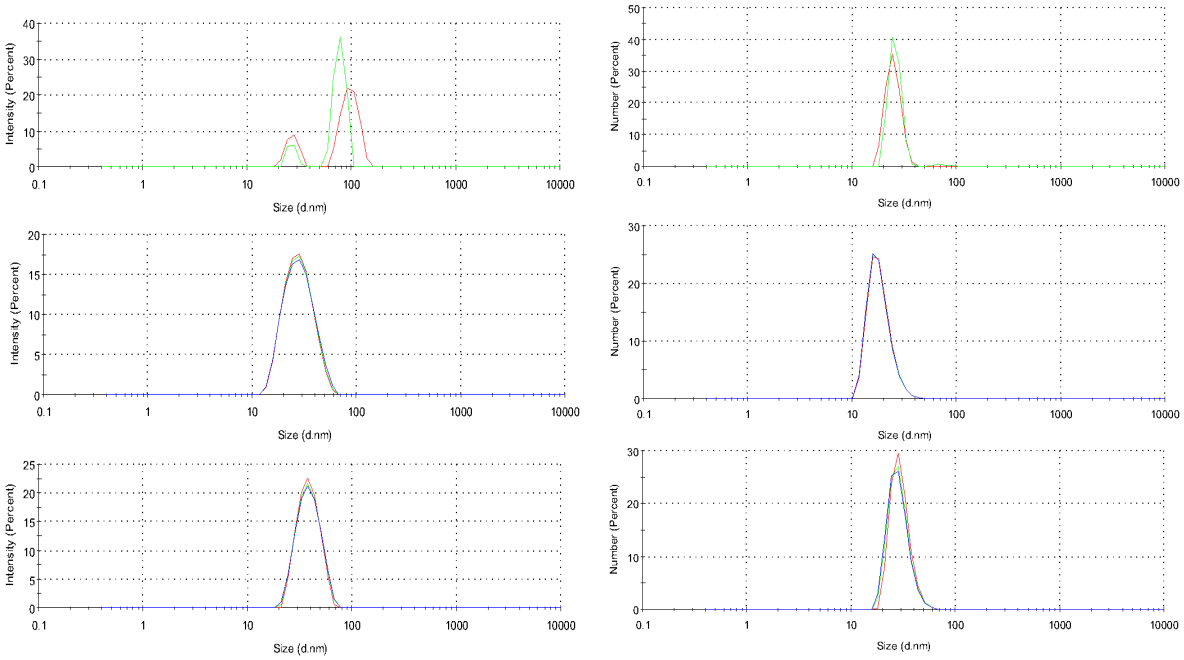
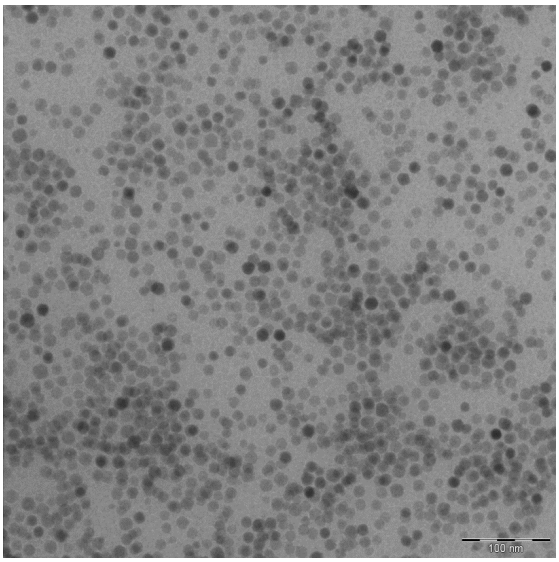
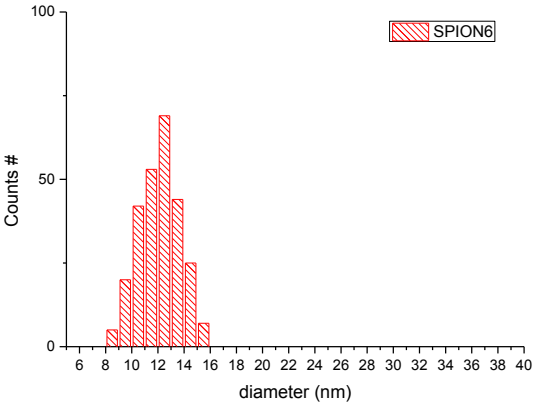
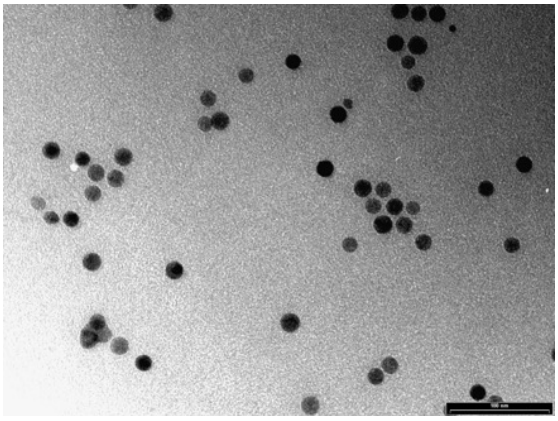


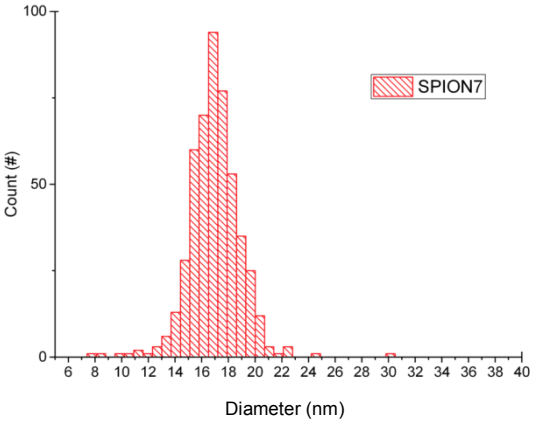
Figure S1. DLS size distribution by intensities (left) and numbers (right) of SPION6@OA, SPION7@OA and SPION8@OA (from top to bottom, respectively) suspended in hexane.



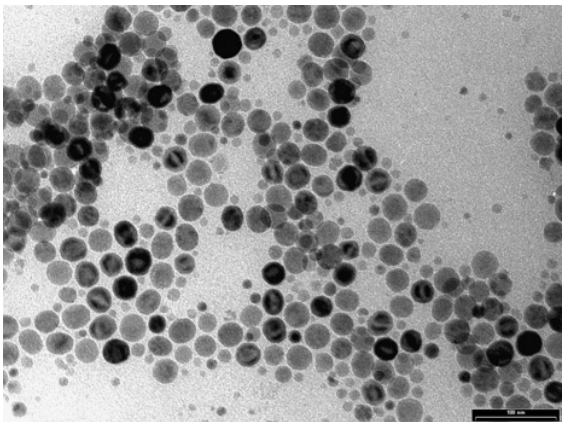


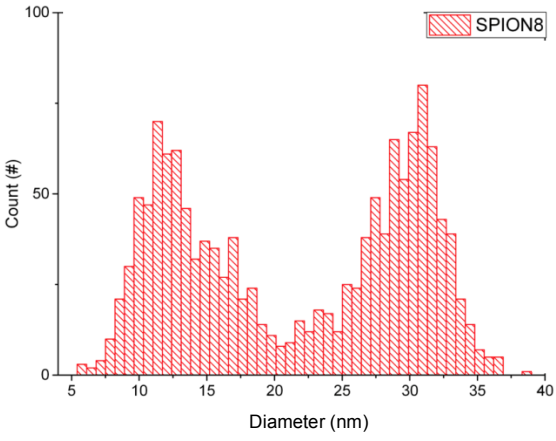
OA:Fe = 6:1; mean diameter = 11.8 ±2 nm





OA:Fe = 7:1; mean diameter = 17.0 ±1.9 nm





OA:Fe = 8:1; mean diameters = 13.5 ± 3.4nm and 29.9 ± 2.6 nm

Figure S2. TEM images of (a) SPION6, (b) SPION7, (c) SPION8, and corresponding size distribution histograms.

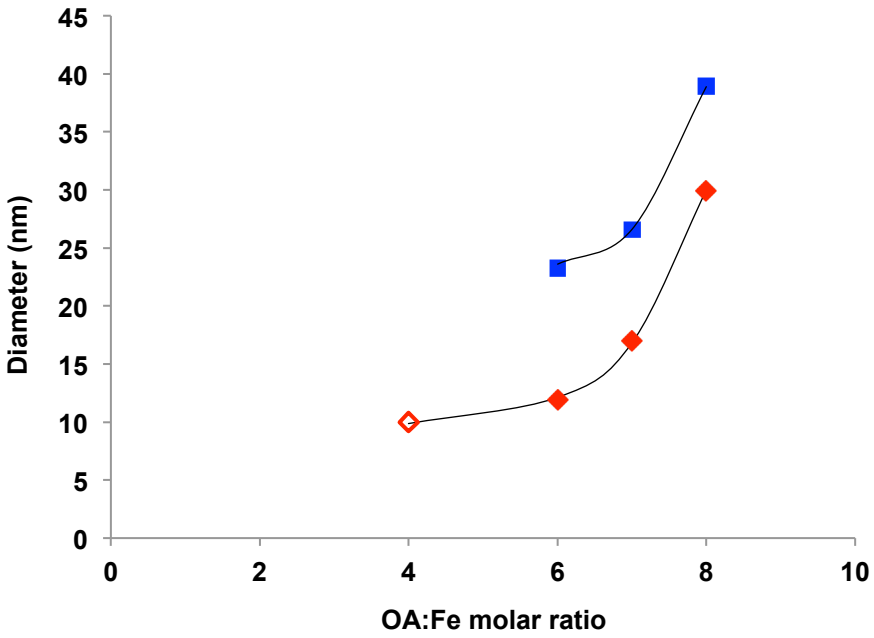


Figure S3. Mean size of the SPION obtained with different OA:Fe molar ratios, as measured by DLS (intensities distribution, blue squares) and TEM (red diamonds). See footnotes of Table 1 for the samples showing two populations. The value at OA:Fe = 4 is from the literature.[1]

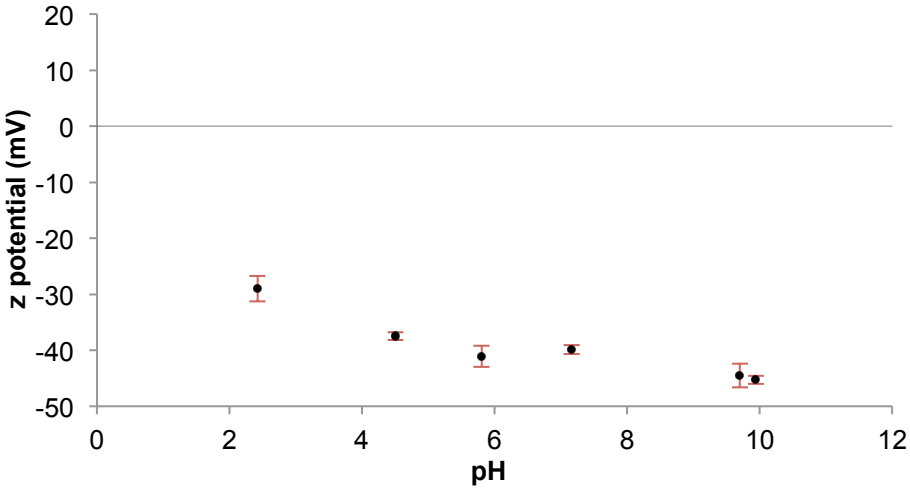


Figure S4. Variation with pH of ζ-potential of SPION@DMSA.

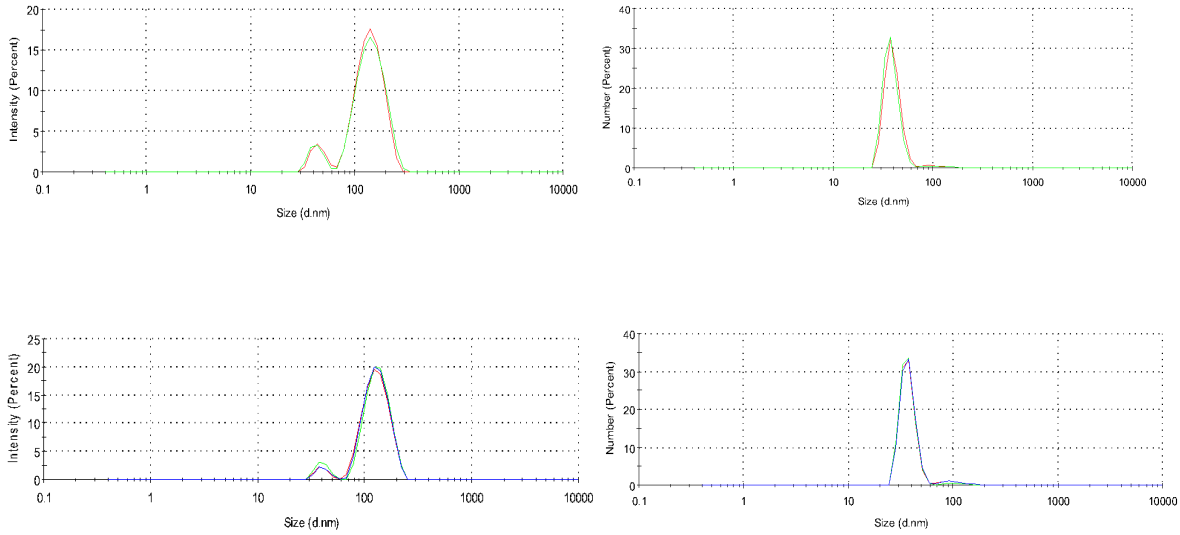
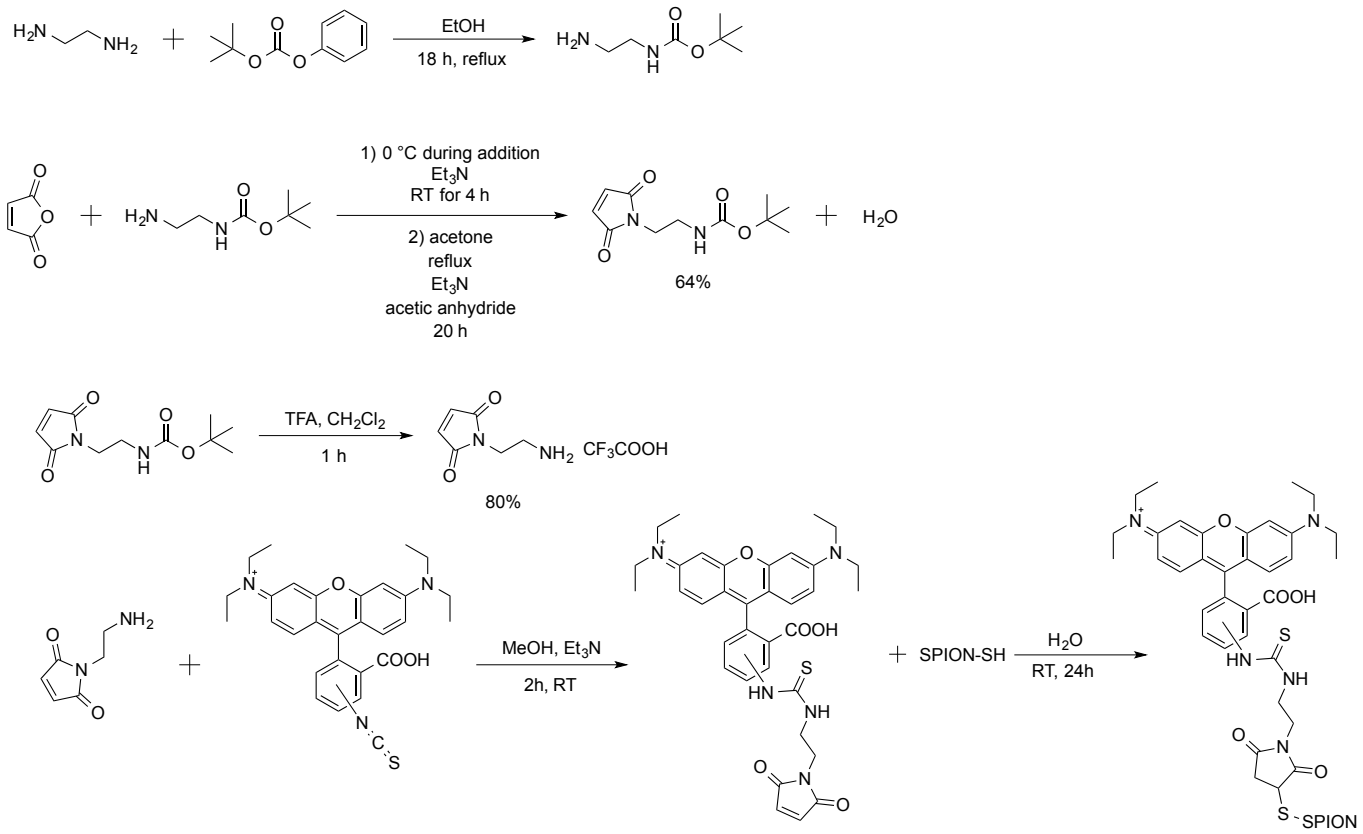


Figure S5. DLS size distribution of SPION@DMSA (top, water at pH = 8.9) and of SPION@DMSA-Mal-PNA (bottom, water at neutral pH) by intensities (left) and numbers (right).



Scheme S1. Synthesis of the maleimide-rhodamine B adduct prepared for testing the feasibility of the maleimide addition to the SH groups on the SPION surface.

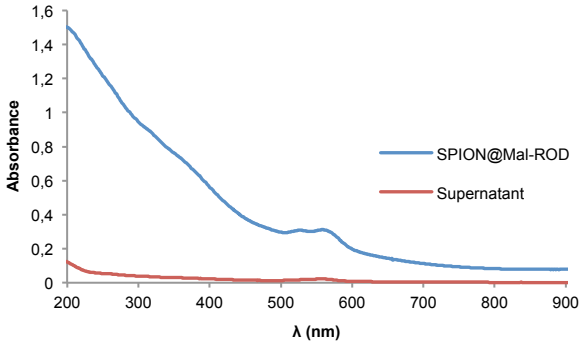


Figure S6. UV-Vis spectra of the suspension of SPION@DMSA-Mal-ROD (Mal-ROD = maleimide-rhodamine adduct) before centrifugation (blue trace) and of the supernatant solution after centrifugation (red trace).

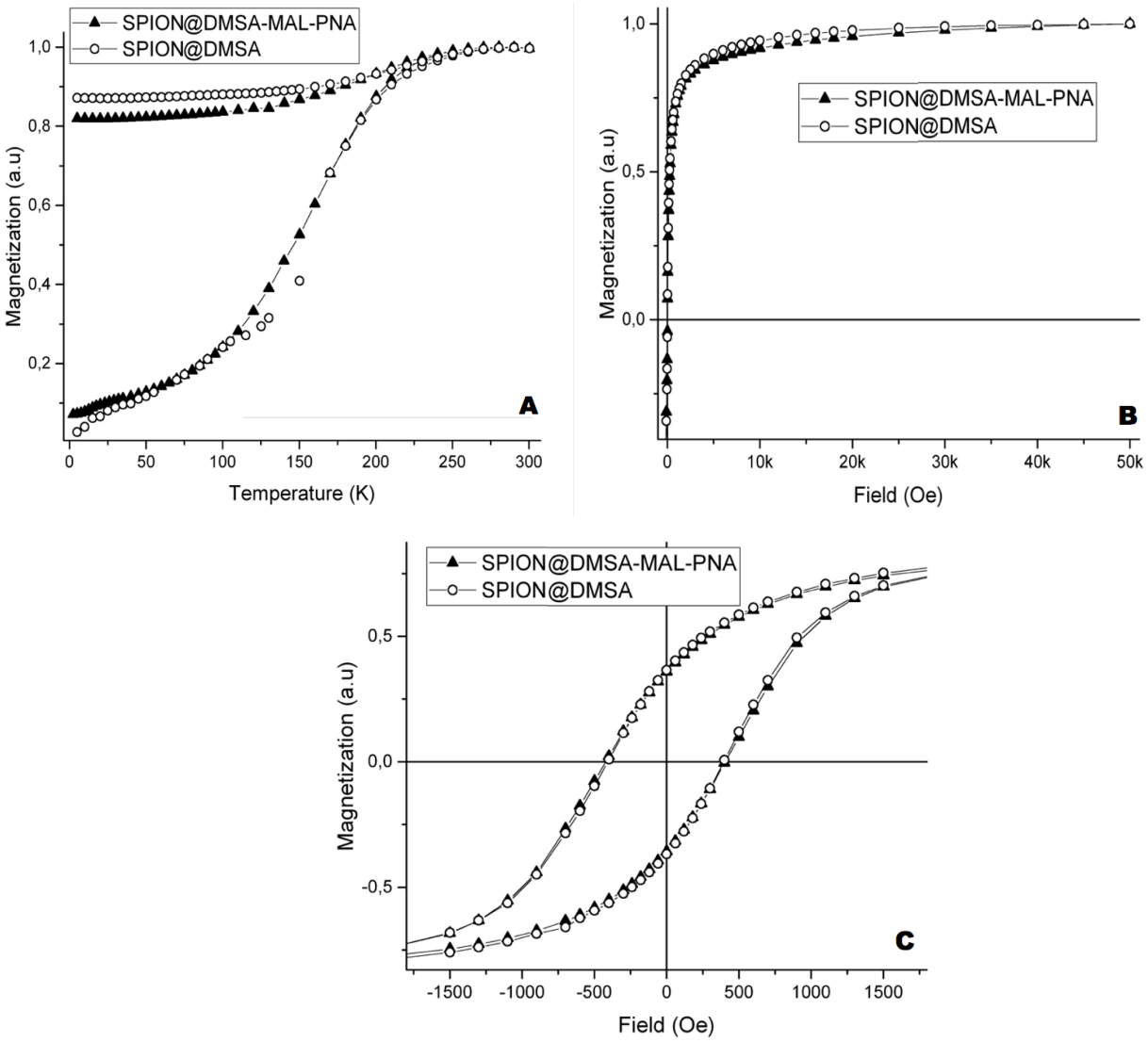


Figure S7. Magnetic properties of SPION@DMSA-Mal-PNA (solid triangles) and SPION@DMSA (empthy circles): a) ZFC-FC magnetization curves; b) Magnetizations at 300 K; c) Enlargement of the low field region of the hysteresis loops at 2.5 K.

The Ellman's Assay. The Ellman's reagent 5,5'-Dithiobis-(2-nitrobenzoic acid), DTNB,[2] is commonly used for the quantification of thiol groups on small molecules. It promptly reacts with thiolate groups in a thiol-disulfide exchange reaction that affords 2-nitro-5-thiobenzoic acid (NTB). The deprotonated form of NTB absorbs in the blue region (maximum at 412 nm, ε = 14150 M^{-1} cm^{-1} at pH = 7.3).[3] For this reason the reaction medium must be at pH > 6, to ensure complete deprotonation of NTB.[4] In the presence of other species absorbing in the same region the quantification is obviously affected by high uncertainty.

Here the reaction of DNTB with SPION@DMSA was performed at room temperature, for 90 min in the dark, and at pH = 8.3 (by using a phosphate buffer). An excess of DTNB had to be used, to allow DNTB to reach all the SH groups on the crowded surface of the SPION@DMSA. The DTNB reactant is responsible for an intense peak at 318 nm (blue trace in Figure S7), whose tail overlaps with the peak of interest of the NTB anion. Due to such DTNB excess, as well as to the absorption of the SPION remaining in solution even after a centrifugation cycle (see red trace in Figure S7), a reliable quantification of the SH groups per SPION was unfeasible. However, the NTB peak was clearly recognizable (see the inset of Figure S7), confirming that accessible SH groups were present on the SPION@DMSA surface.

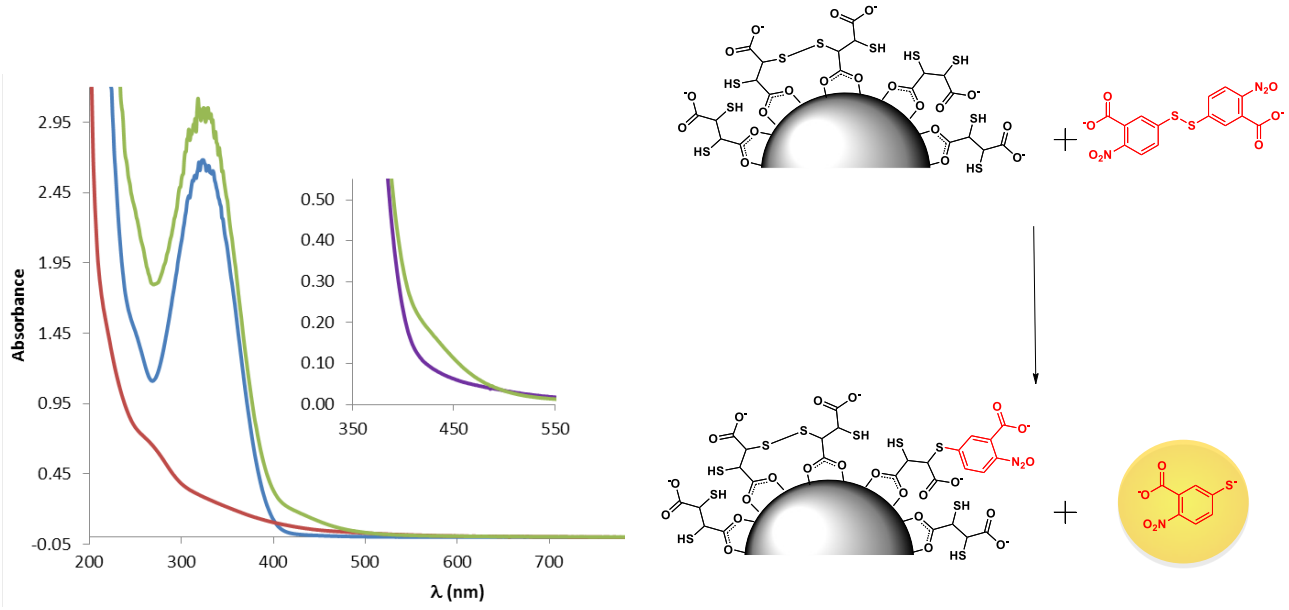


Figure S8. UV-Vis spectra of i) supernatant of the reaction between SPION@DMSA and Ellman's reagent (for 90 min in the dark) after centrifugation (green trace); ii) an aliquot of the same SPION@DMSA suspension used for the Elman assay, subject to the same centrifugation procedure (red trace), iii) the starting buffered solution of the Ellman's reagent used for the assay (blue trace). The inset shows the comparison at longer wavelengths between the spectrum of the reaction mixture (green trace) and the spectrum addition of the red and blue traces (violet trace).

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[1] P. Calcagnile, D. Fragouli, I. S. Bayer, G. C. Anyfantis, L. Martiradonna, P. D. Cozzoli, R. Cingolani, A. Athanassiou, *ACS Nano*, **2012**, *6*, 5413–5419.

[2] G.L. Ellman *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.

[3] P.W. Riddles, R.L. Blakeley, B. Zerner *Methods Enzymol.* **1983**, *91*, 49–60.

[4] R.E. Hansen, H. Ostergaard, P. Norgaard, J.R. Winther *Anal. Biochem.* **2007**, *363*, 77–82.