Supporting Information

Solventless mechanochemical preparation of novel magnetic bioconjugates

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The X-ray diffraction patterns of both Fe_2O_3 and BSA-DA-Fe_2O_3 nanomaterials were collected using the D8 Advanced Diffractometer (Bruker AXS) with the Lynxeye detector, at room temperature. TEM images were recorded in a FEI Tecnai G^2 system, equipped with a CCD ("charge coupling device") camera. Previously, the sample was dispersed in ethanol and transferred to a copper grid. UV-visible spectra were collected in a Jasco UV-visible-NIR (model V-570) spectrometer.

Particle size distributions and zeta potential of DA functionalized Fe_2O_3 MNPs and BSA-DA-Fe_2O_3 nanobioconjugate were analyzed using Zetasizer Nano ZSP (Malvern Instruments Ltd.,UK) instrument. All samples were diluted in 10 mM of KNO_3 and measurements were recorded in triplicate (n = 3) at 25 °C.

FTIR measurements were performed on the ABB MB3000 infrared spectrophotometer, equipped with an ATR PIKE MIRacle™ sampler, a window of ZnSe, and 256 scans at a resolution of 8 cm^{-1}. The Horizon MBTM software was employed for the spectra acquisition. During the measurements, the sample was purged with a dehydrated and deoxygenated nitrogen flow (20 mL min^{-1}).

Steady-state fluorescence measurements were carried out on the FLS920 Fluorimeter (Edinburgh Instrument Ltd, Livingston, UK).

XPS analysis was conducted using an ultrahigh vacuum (UHV) multipurpose surface analysis system SpecsTM. The analysis was carried out using a conventional X-ray source (XR-50, Specs, Mg-Kα, hv=1253.6 eV, 1 eV = 1.603 x 10^{-19} J) at pressures <10^{-10} mbar and in a "stop and go" mode. The sample was left overnight under vacuum (<10^{-6} Torr). Finally, spectra were acquired at room temperature (pass energy: 25 and 10 eV, step size: 1 and 0.1 eV, respectively) with the Phoibos 150-MCD energy detector. The deconvolution of the obtained curves was accomplished with XPS CASA program. The magnetic susceptibility measurements were performed in a Bartington MS-2 instrument. Nitrogen adsorption measurements were carried out in the Micromeritics ASAP 2000 instrument. Moreover, thermogravimetric analysis was accomplished using the System Setaram Setsys 12 TGA instrument.

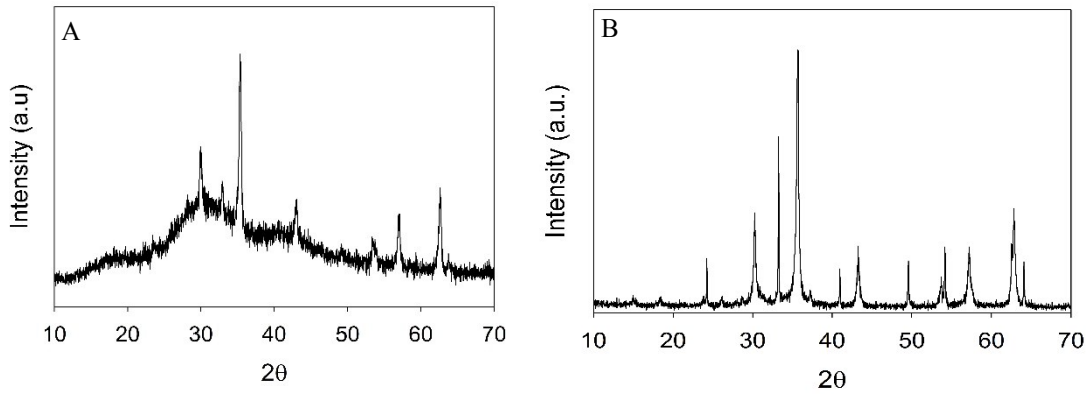

Figure S1. X-ray diffraction patterns of A: BSA-DA-Fe_2O_3 and B: Fe_2O_3.

The XRD pattern of Fe_2O_3 nanoparticles shows a mixture of two phases: maghemite and hematite. In the case of maghemite and magnetite (Fe_3O_4) phases, it is very difficult to clearly distinguish by XRD analysis since these two phases possess a similar XRD pattern. However, the absence of Fe^{2+} species (see XPS analysis) and the reddish-like colour are consistent with a maghemite magnetic phase.

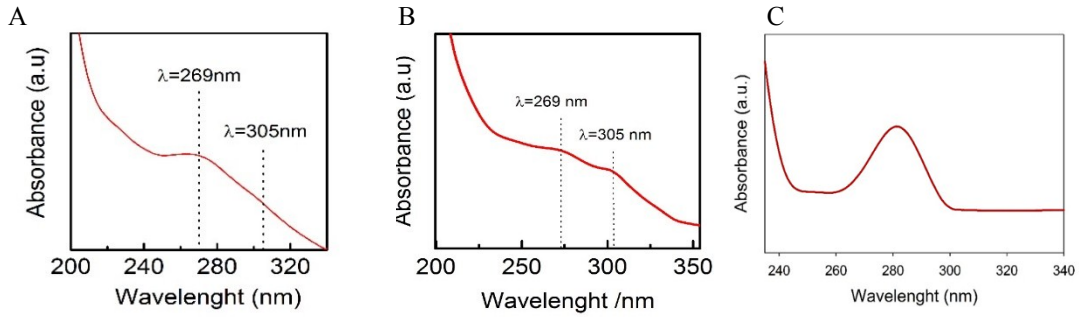

Figure S2. UV-vis spectra of A: DA, B: DA-Fe_2O_3, C: BSA-DA-Fe_2O_3 (1 mg/ml in 0.05 M PBS pH=7)

The UV-vis spectra of DA and DA-Fe_2O_3 was obtained. The main peak around 269 nm, (Q. Wei, F. L. Zhang, J. Li, B. J. Li and C. S. Zhao, Polymer Chemistry, 2010, 1, 1430-1433) in both cases, together with the not appearance of a band at 350 nm corroborate the successful immobilization of the protein and that the attachment of the BSA to DA-Fe_2O_3 nanoconjugate through non-covalent interactions.

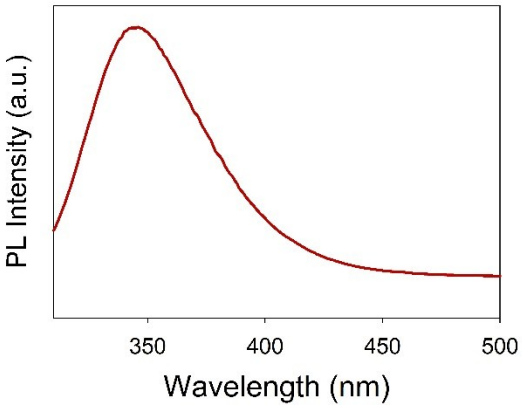

Figure S3. Fluorescence emission spectra of BSA-DA-Fe_2O_3 nanobioconjugates. (Excitation wavelength = 295 nm)

The significant band centered at 345 nm can be associated to native BSA, which is consistent with the fact that there are no changes in the environment of the tryptophan residues and therefore that BSA retain its structural features after the immobilization step. [E. L. Gelamo, M. Tabak, (2000). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2000**, *56*, 2255-2271.]

Table S1. Conformational Analysis of BSA-DA-Fe_2O_3. Structural Percent values are given.

Secondary structure	**Native BSA pH=7.4***	**BSA-DA-Fe_2O_3**
α-helix	68%	67%
β-sheet	20%	18%
β-turn	5%	13%
unordered	4%	2%
Side chain moeties	3%	-

*Values reported in literature [Journal of Colloid and Interface Science 493 (2017) 334–341]

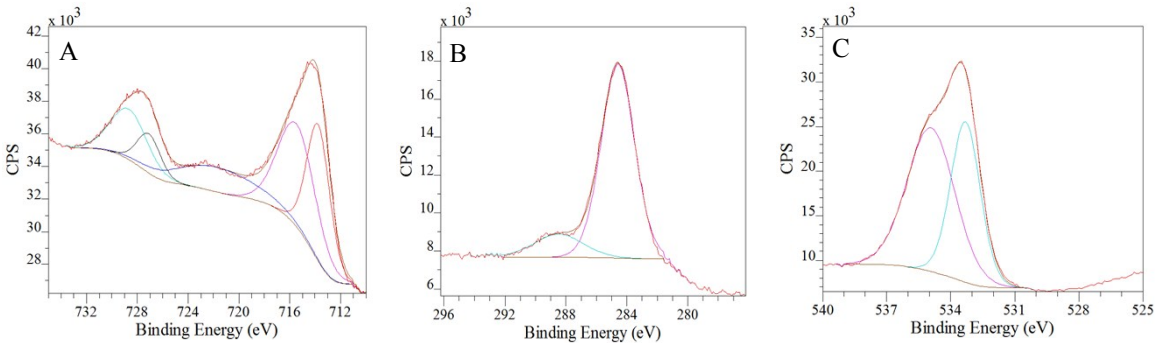

Figure S4. Deconvoluted high-resolution XPS spectra of Fe_2O_3 for a) Fe 2p, b) C 1s and c) O 1s.

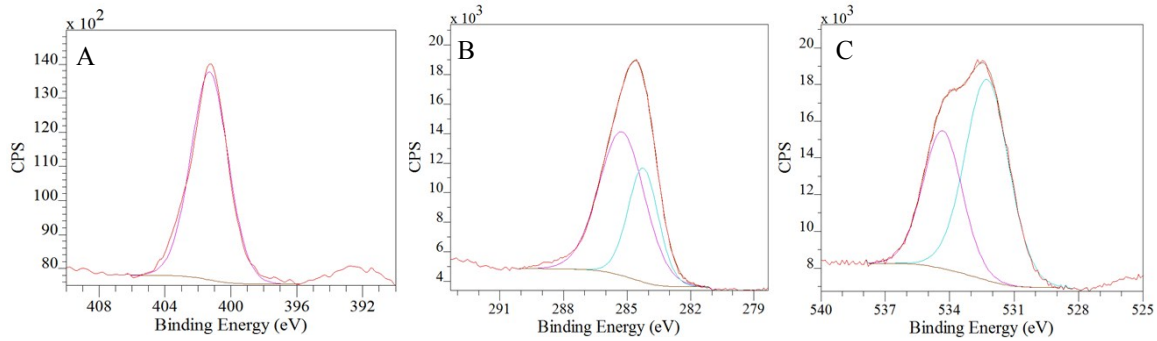

Figure S5. Deconvoluted high-resolution XPS spectra of DA-Fe_2O_3 for a) N 1s, b) C 1s and c) O 1s.

The peak at 401.1 eV can be attributed to the N from amino groups of the dopamine. These results are an evidence of the effective functionalization of the Fe_2O_3 nanoparticles with dopamine.

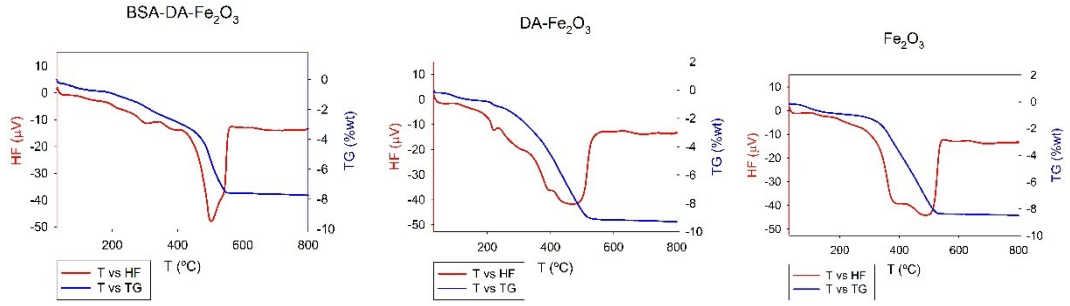

Figure S6. ATG of the BSA-DA-Fe_2O_3, DA-Fe_2O_3 and Fe_2O_3 nanostructures.

The first weight loss at 100 °C in the three materials: BSA-DA-Fe_2O_3, DA-Fe_2O_3, Fe_2O_3 can be correlated to the presence of water in the samples. From 220 °C and 350 °C, a progressive weight loss was observed, that is more marked for the BSA-DA-Fe_2O_3 nanoconjugate and can be associated to the non-oxidative decomposition of organic species in the materials.

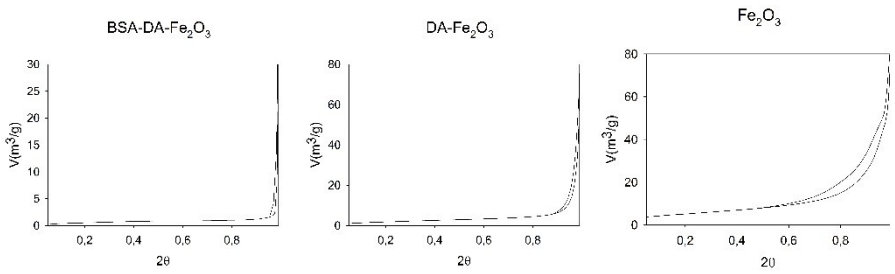

Figure S7. Adsorption-desorption isotherm of the BSA-DA-Fe_2O_3, DA-Fe_2O_3 and Fe_2O_3 nanostructures.

As was expected, the surface area values of the Fe_2O_3, DA-Fe_2O_3 and BSA-DA-Fe_2O_3 nanomaterials were found around 20, 7, and 2 m^2 / g, respectively, lower after the functionalization. The obtained surface area of the MNPs is satisfying, taking into account its preparation methodology.

Table S2. Magnetic susceptibility of the Fe_2O_3 and BSA-DA-Fe_2O_3.

Material	**Magnetic susceptibility (10^{-6} m^3Kg^{-1})**
Fe_2O_3	282
BSA-DA-Fe_2O_3	254