Supporting Information

1 - Experimental Section

1.1 - Materials and instruments

Bovine serum albumin (BSA), fluorescein-modified bovine serum albumin (Fluorescein-BSA), acrylamide (AM), N,N-methylene-bis-acrylamide (MBAM), ammonium persulfate (APS), N,N,N',N'-tertramethyl-ethylene-diamine (TEMED), and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid (iniferter agent) were provided by Sigma-Aldrich. Technic ethanol was provided by VWR Chemicals. All reagents were used as received.

Fourier Transform Infrared (FTIR) spectra were recorded on a Tensor 27, BRUKER spectrophotometer in KBr medium. UV-Vis adsorption spectra were recorded on an UVIKON XL, SECOMAM spectrophotometer. Thermogravimetric analysis (TGA) were carried out for bare maghemite nanoparticles and polymer coated nanoparticles using a SDT-Q600, TA INSTRUMENTS under a nitrogen atmosphere with a heating rate of 10°C/min up to 600°C. Relaxation times were recorded on a Minispec mq20, BRUKER at 20MHz and 25°C. Hydrodynamic diameters were measured using a Zetasizer Nano series, Malvern Instruments.

1.2 - Functionalization of γ-Fe_2O_3 nanoparticles

Maghemite nanoparticles were synthetized as previously described by Massart[1], using a co-precipitation method. Surface of those nanoparticles was functionalized by an iniferter agent using a protocol slightly modified from the one Gonzato et al.[2] used. Briefly, 60 mg of the iniferter agent, 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, were dissolved in 4 mL of technic ethanol, followed by the addition of 26 mL of distilled water and 500 mg of γ-Fe_2O_3 nanoparticles. The reaction was allowed to proceed at room temperature for 18 hours under continuous orbital stirring. Then, functionalized nanoparticles were collected by the application of an external magnetic field and thoroughly washed with technical ethanol and distilled water to remove all remaining unreacted iniferter agent.

1.3 - Synthesis of imprinted and nonimprinted polymers

10 µmol of BSA and 30 mmol of acrylamide were dissolved in 150 mL of distilled water. The mixture was allowed to react and form a pre-polymerization complex for 1 hour at room temperature under constant magnetic stirring. Then, two different synthesis protocols were carried out.

First, 3 mmol of N,N-methylene-bis-acrylamide, 300 mg of functionalized nanoparticles and 25 mg of APS were added and the mixture was nitrogen purged for 15 minutes under magnetic stirring. Lastly, 75 µL of TEMED were added to the mixture and the reaction was allowed to proceed for various amount of time, ranging from 4 hours to 18 hours at room temperature under magnetic stirring.

Second, 3 mmol of N,N-methylene-bis-acrylamide, 300 mg of functionalized nanoparticles and 40 mg of aso-bis-iso-butyronitrile (AIBN) were added and the mixture was nitrogen purged for 15 minutes under magnetic stirring, before being heated at 60°C. The reaction was allowed to proceed for 18 hours.

In both cases, the final product was collected by the application of an external magnetic field and washed thoroughly with deionised water to remove unreacted monomer or remaining protein.

Nonimprinted polymers were synthetized using the same way but without BSA and were allowed to react for 18 hours.

1.4 – Protein extraction

After the synthesis of PIP, proteins have to be extracted from the polymer matrix. A widely described method is the use of acetic acid combined with sodium dodecyl sulfate[3,4]. However, these surfactants can remain adsorbed on the surface of the imprinted polymer, and possibly be at the origin of higher non-specific adsorption, modifying the adsorption capacity of the hybrids nanoparticles[5]. Thus, we chose another extraction method, also previously described[6]. γ-Fe_2O_3@PIP nanoparticles were alternatively washed with a 9/1 methanol/acetic acid mixture and distilled water to remove the template proteins.

1.5 - Protein adsorption experiments

The adsorption capacity (Q) of γ-Fe_2O_3@PIP and γ-Fe_2O_3@NIP nanoparticles was determined as follows: 20 mg of γ-Fe_2O_3@PIP or γ-Fe_2O_3@NIP were dispersed in 3 mL of protein solutions at different initial concentrations in water. The resulting mixtures were shaken at room temperature for 24 hours. Particles were collected by an external magnetic field and supernatants were analysed. The adsorption capacity was determined as follows:

$$Q = \frac{(C_i - C_f)V}{m}$$

where C_i (mg/mL) and C_f (mg/mL) are respectively the initial and final concentrations of the protein samples, determined using UV-Vis spectrophotometry, V (mL) is the volume of the protein solution and m (mg) is the dry mass of γ-Fe_2O_3@PIP or γ-Fe_2O_3@NIP nanoparticles initially dispersed.

1.6 – Selectivity determination and competitive binding assay

Human serum albumin (HAS) and ovalbumin (OVA) were chosen to investigate the selectivity of γ-Fe_2O_3@PIP toward BSA. Experiments were carried out as previously described to determine the adsorption capacity of the imprinted polymers toward these proteins.

Bovine serum albumin modified with fluorescein groups was chosen to investigate the selectivity of γ-Fe_2O_3@PIP toward BSA. Experiments were carried out as previously described to determine the adsorption capacity of the imprinted polymers. Protein solutions contained an equimolar mixture of native BSA and fluorescein-modified BSA.

2 - PIP Characterizations

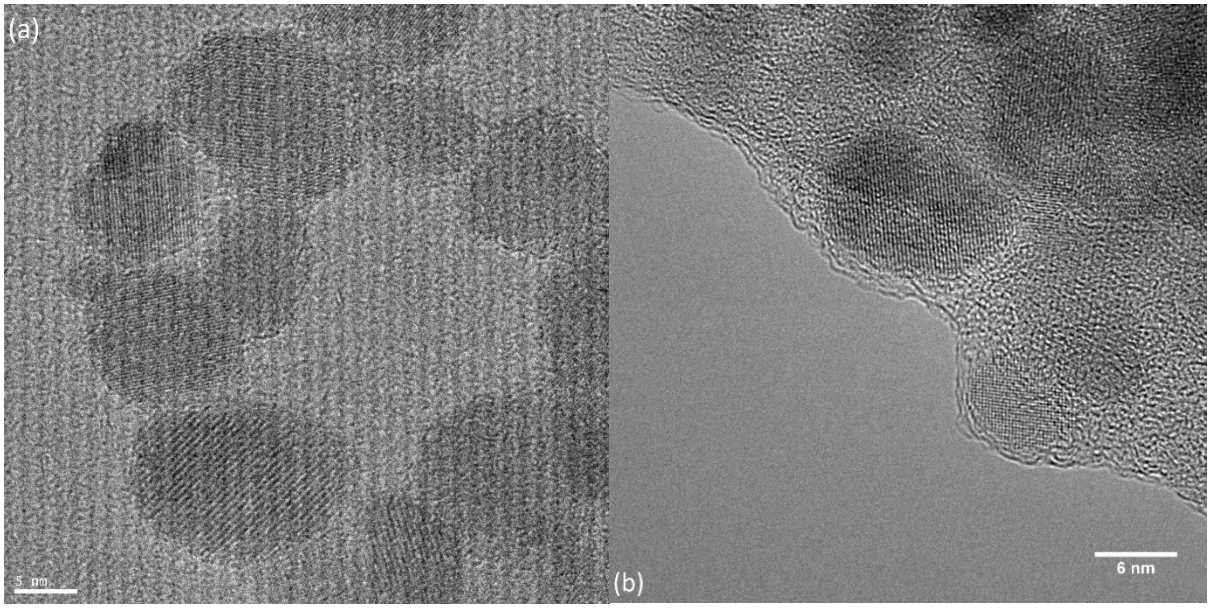

Fig. S1: High-resolution TEM images of bare maghemite nanoparticles (a) and PIP coated maghemite nanoparticles (b).

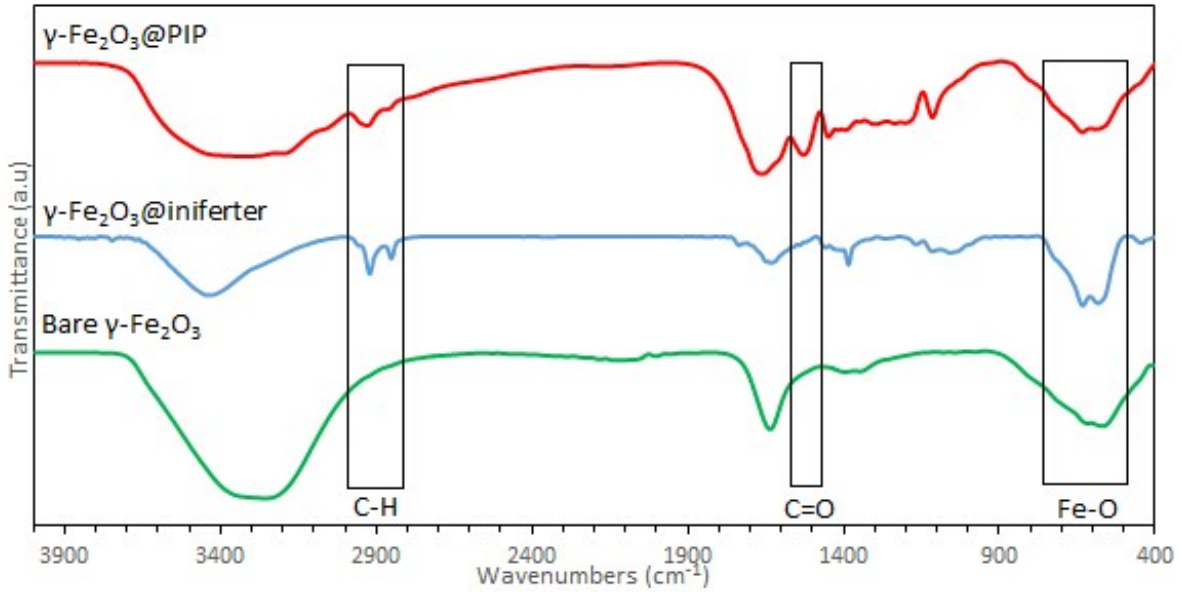

Fig. S2: FTIR spectra of bare γ-Fe_2O_3 nanoparticles, γ-Fe_2O_3@iniferter and γ-Fe_2O_3@PIP nanoparticles

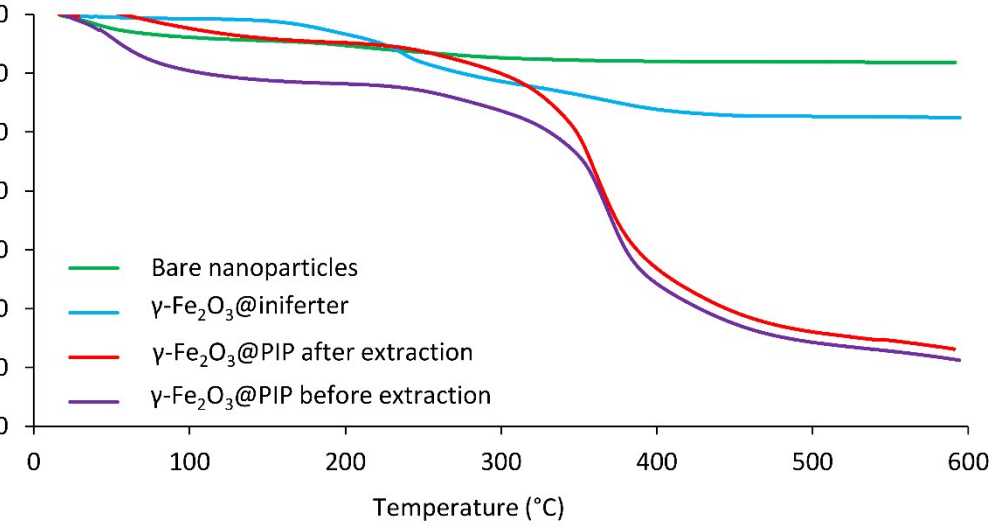

Fig. S3: TGA spectra of bare γ-Fe_2O_3 nanoparticles, γ-Fe_2O_3@iniferter and γ-Fe_2O_3@PIP nanoparticles, before and after protein extraction

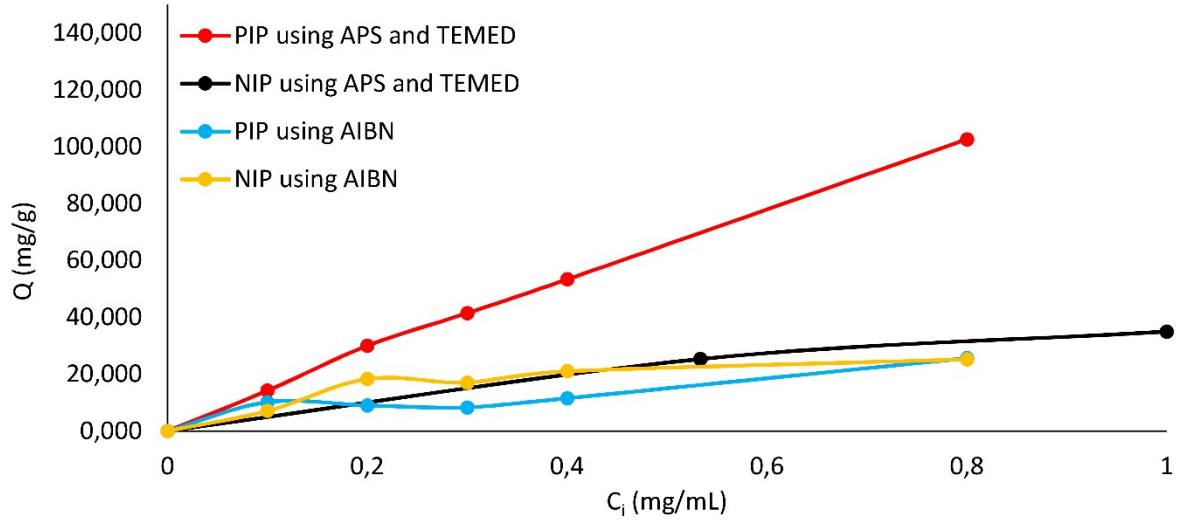

Fig. S4a: Adsorption capacities for γ-Fe2O3@PIP and NIP synthesized with various initiators, V=3mL, m=20mg, room temperature, 24h stirring

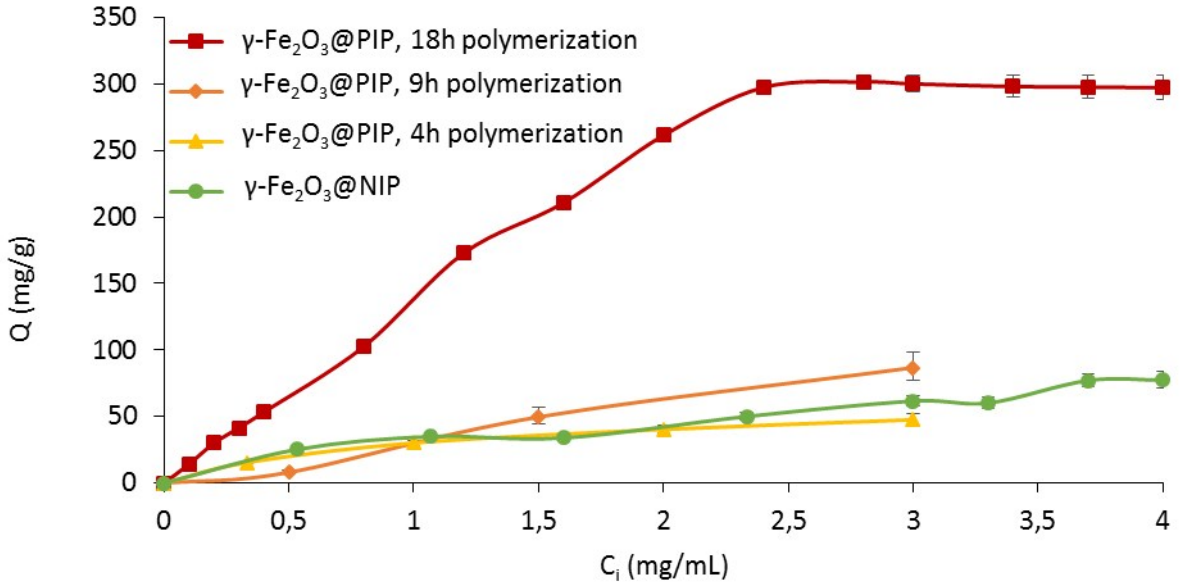

Fig. S4b: Adsorption capacities for γ-Fe_2O_3@PIP synthesized with various polymerization times, V=3mL, m=20mg, room temperature, 24h stirring.

Sample	Hydrodynamic diameter (nm)
Bare nanoparticles	13.9 ± 1.9
γ-Fe_2O_3@PIP, 4 hours polymerization	94.5 ± 3.4
γ-Fe_2O_3@PIP, 9 hours polymerization	157.8 ± 4.4
γ-Fe_2O_3@PIP, 18 hours polymerization	278 ± 7.1

Table S1: Hydrodynamic diameters of PIP magnetic nanoparticles with different polymerization times

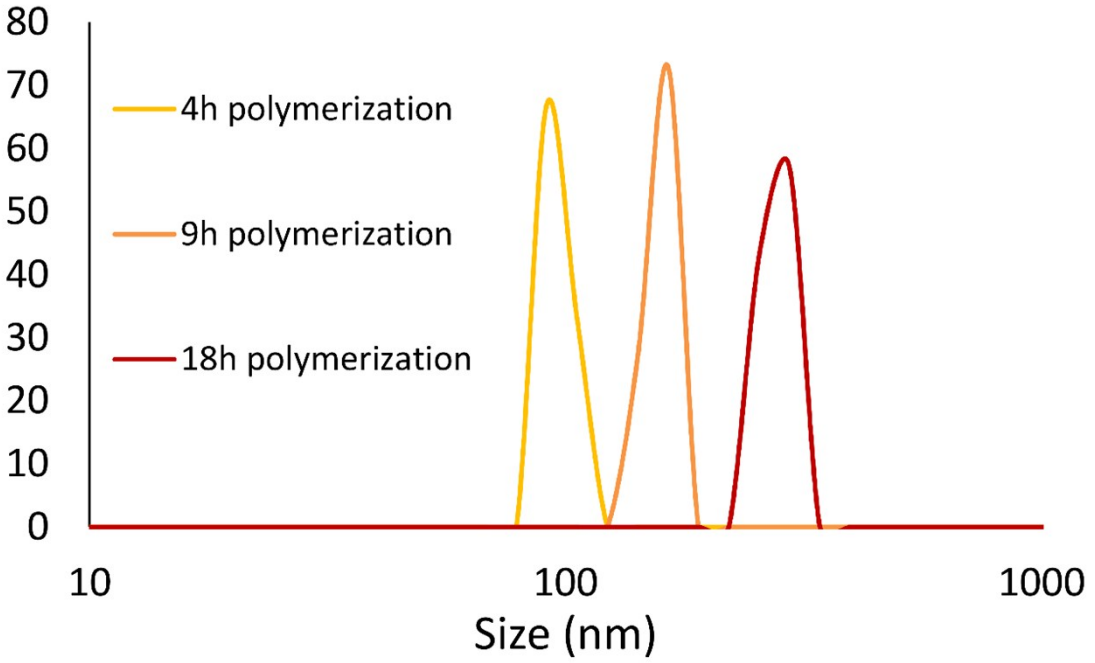

Fig. S5: Light scattering profiles of particles size distribution (intensity profiles)

Sample	R	Q_{max} (mg/g)	K (L/mol)
γ-Fe_2O_3@PIP	0.998	294	$5.6x10^6$
γ-Fe_2O_3@NIP	0.980	80	$6.5x10^4$

Table S2: Langmuir isotherms parameters for adsorption of BSA

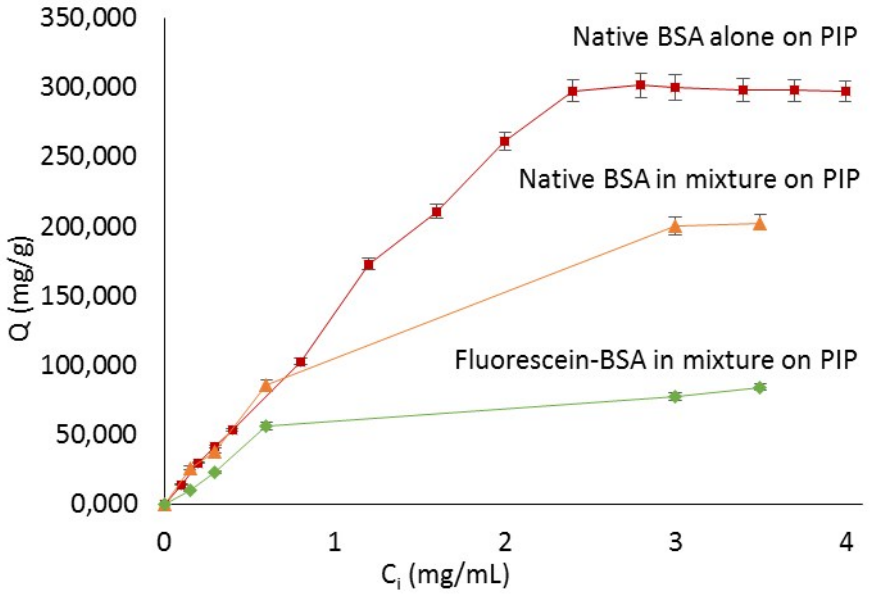

Fig. S6: Competitive adsorption on γ-Fe2O3@PIP, V=3mL, m=20mg, room temperature, 24h stirring. Mixture corresponds to an equimolar solution of native BSA and fluorescein-modified BSA.

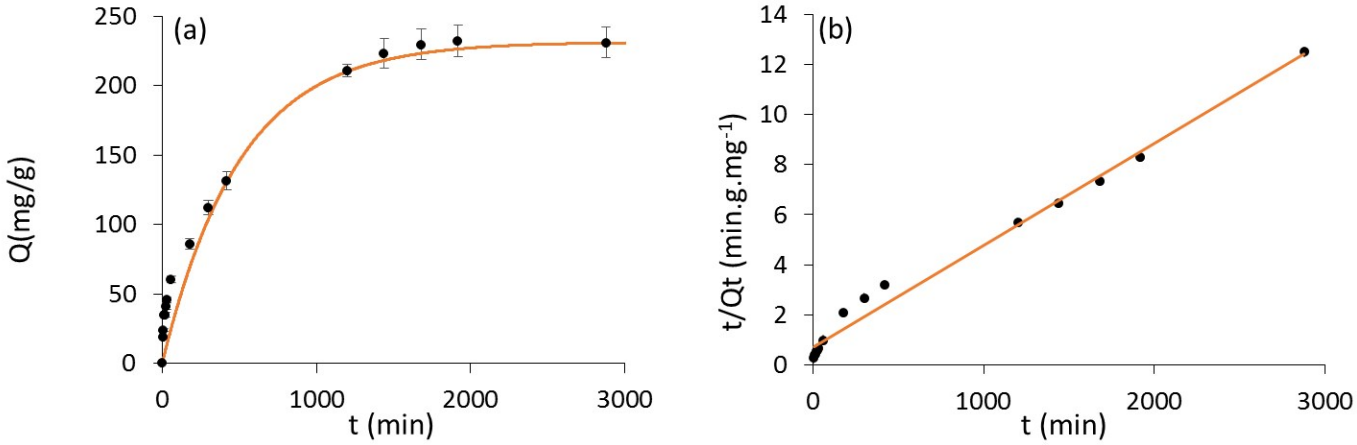

Fig. S7: Adsorption kinetic curves of BSA on PIP with pseudo-first order fit (a) and pseudo-second order fit (b). V=3mL, m=20mg and C_i=0.1mg/mL at room temperature.

Model	R^2	$Q_{e,theoretical}$ (mg/g)	$Q_{e,experimental}$ (mg/g)	k
Pseudo-first order	0.990	231	230	2.32×10^{-5} g.mg^{-1}.min^{-1}
Pseudo-second order	0.988	244	230	1.99×10^{-3} min^{-1}

Table S3: Adsorption kinetic parameters for BSA on PIP

Notes and references

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3 X. Li, B. Zhang, L. Tian, W. Li, T. Xin, H. Zhang and Q. Zhang, *Sens. Actuators B Chem.*, 2014, **196**, 265–271.
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