SUPPORTING INFORMATION

Exploration of possible binding sites of nanoparticles on protein by crosslinking chemistry coupled with mass spectrometry

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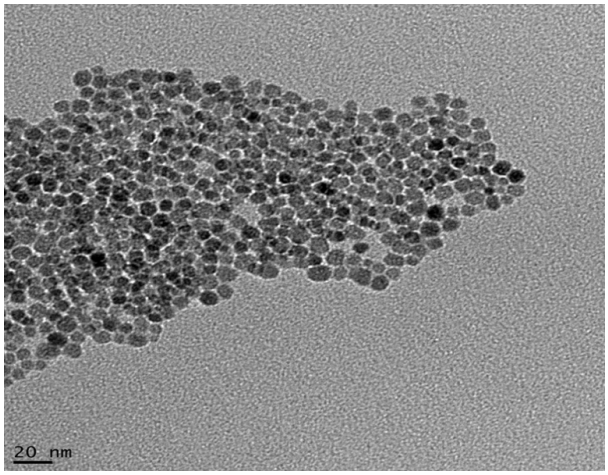
Figure S1a. TEM image of the PAA-Fe_3O_4 NP (average diameter = 8.02 ± 1.26 nm (n = 100)).



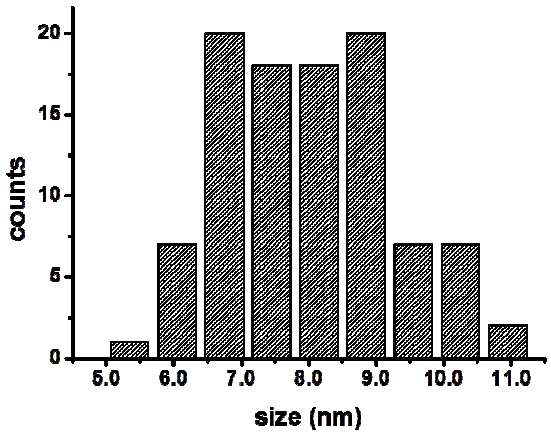
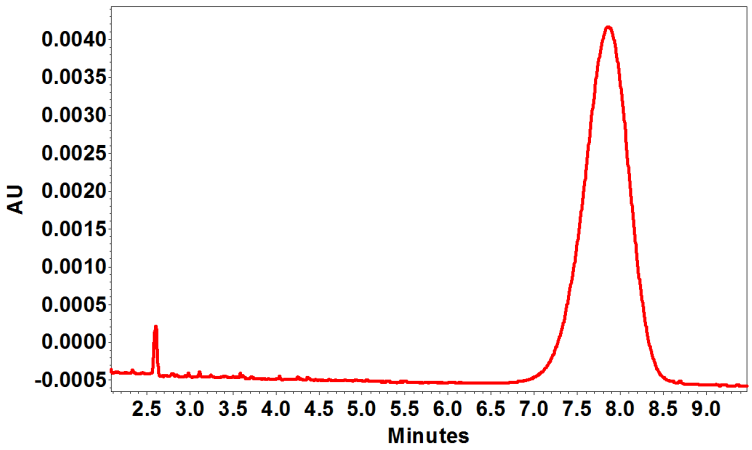


Figure S1b. Zeta potential calculation from CE data. Top: Electropherogram of the 8.02-nm NPs; Bottom: Zeta potential calculated by the electrophoretic mobility of NPs from the equation[1] shown below the plot.



Fe_3O_4 NPs	Electrophoretic mobility ($m^2\ V^{-1}\ S^{-1}$)	Zeta potential (mV)
8.02 nm	$-3.44 \times 10^{-8} \pm 4.01 \times 10^{-11}$	-44.03 ± 0.08

$$\xi = \frac{\mu_e \bullet \eta}{\varepsilon} = \frac{\mu_e \bullet \eta}{\varepsilon_0 \bullet \varepsilon_r} = \frac{\mu_e \bullet 8.90E-4}{8.854187817E-12 \bullet 78.4}$$

Reference:

1. Schnabel, U.; Fischer, C.-H.; Kenndler, E. *J. Microcolumn Sep.* **1997**, 9, 529-534.

Figure S1c. K_D values for interaction of the PAA-Fe_3O_4 NPs with various proteins.

Protein	Mw (kDa)	Dimension (nm)	n	K_D (M)
apo-calmodulin	17	2.1 × 2.1 × 5.8	2.1	1.51×10^{-8}
beta-casein	24		1.3	4.60×10^{-6}
bovine serum albumin	66	2.7 × 2.7 × 11.6	1.1	1.37 ×10-^{5}
gamma-globulin	60-70		1.0	3.36×10^{-7}
Superoxide dismutase	32.5	7.2 × 4.0 × 3.8	Too weak to measure	
Insulin	5.8	2.0 × 2.5 × 2.0	Too weak to measure	

Figure S2. MALDI-Q-TOF MS/MS results for peptide peak at m/z 1640 KVPQVSTPTLVEVSR. Search result was obtained by MASCOT with an ions score of 44. There are 22 matches out of 84 fragment ions using 64 most intense peaks.

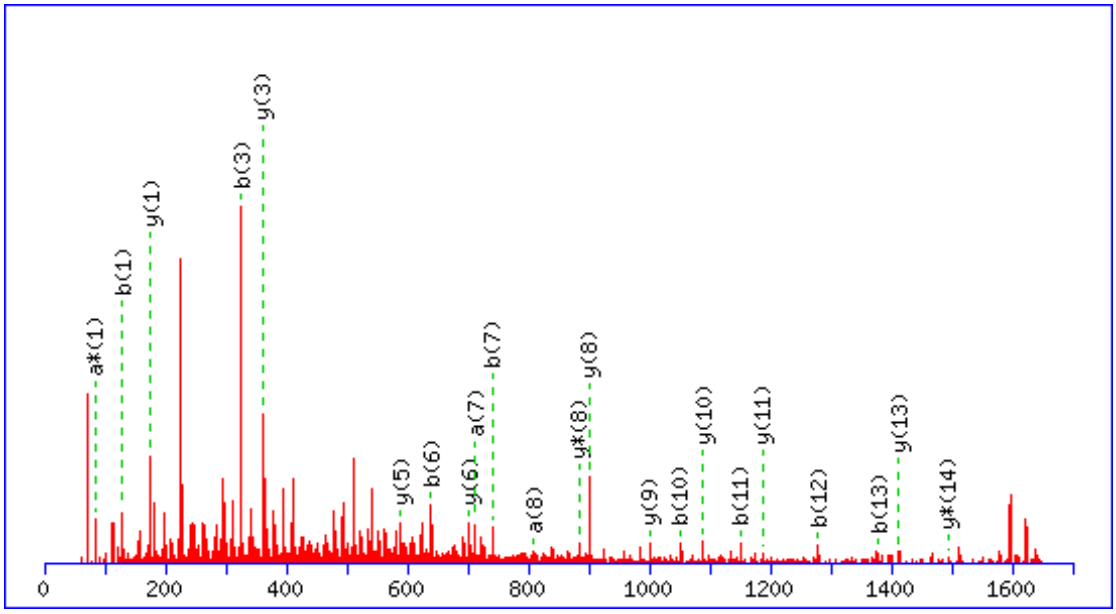
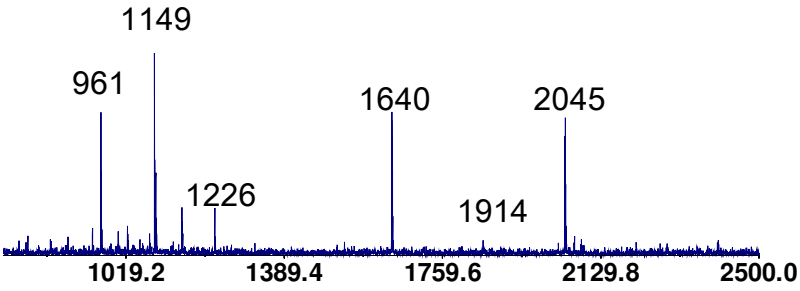


Figure S3. MALDI-TOF-MS spectrum for the tryptic digestion of HSA. A total of 13 peptides were consistently identified and they are listed in the table below.



List of typical HSA peptides found in our samples:

m/z	Sequence
649	CASLQK
674	TPVSDR
715	AACLLPK
789	LVTDLTK
927	YLYEIAR
940	DDNPNLPR
961	FQNALLVR
1074	LDELRDEGK
1149	LVNEVTEFAK
1226	FKDLGEENFK
1386	YICENQDSISSK
1640	KVPQVSTPTLVEVSR
2045	VFDEFKPLVEEPQNLIK

Figure S4. Circular dichroism spectra of 2.2×10^{-7} mol/L HSA (black) and 2.2×10^{-7} mol/L HSA with 2.4×10^{-8} mol/L PAA-Fe_3O_4 NPs (red).

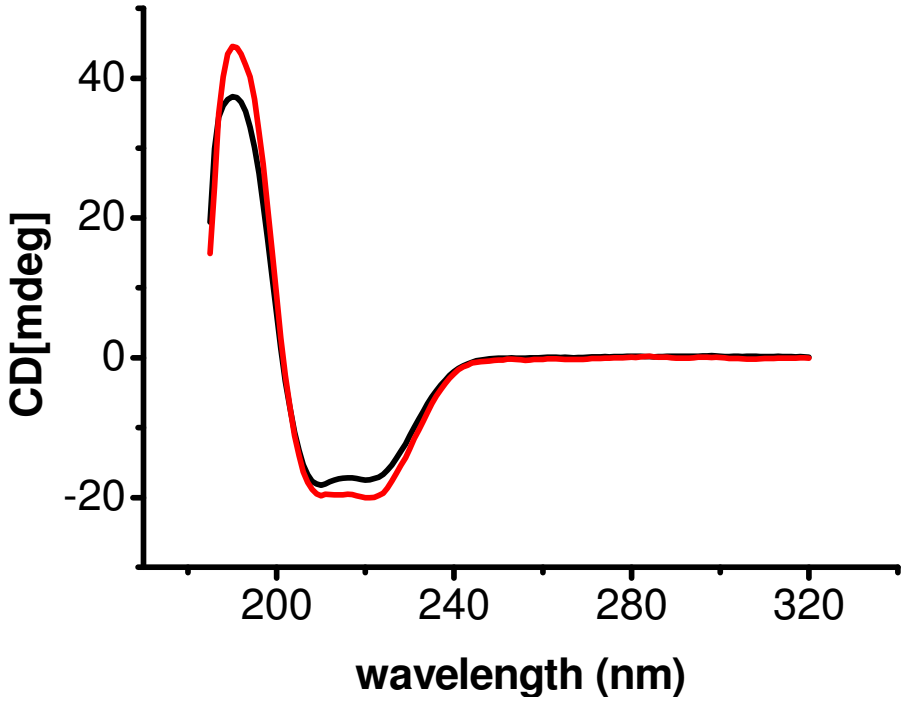


Figure S5a. Electropherograms showing no interaction between ibuprofen and the PAA-coated Fe_3O_4 NPs. In this test, the ibuprofen was dissolved in 50% ethanol (HPLC grade, Fisher Scientific) to make a series of stock solutions with concentration from 4.84×10^{-4} M to 9.70×10^{-3} M. Two micro liters of the drug stock solution at different concentration was mixed with 9.4×10^{-12} mol NPs in a total volume of 20 µL which contained 5% ethanol. After overnight incubation, these samples were analyzed with capillary electrophoresis. No shift was observed in either the peak of NPs or that of ibuprofen, indicating no interaction occurring between these two. All traces were measured at 200 nm.

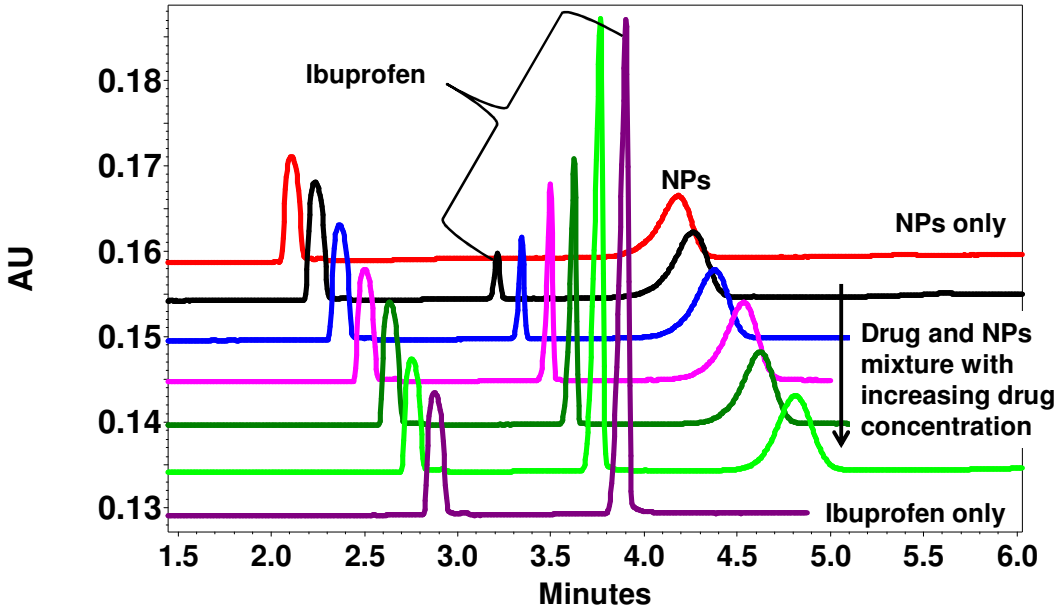


Figure S5b. Electropherograms of incubation of HSA with NPs and ibuprofen at ibuprofen concentration equal to or larger than 9.70×10^{-4} M. Traces were measured at 288 nm.

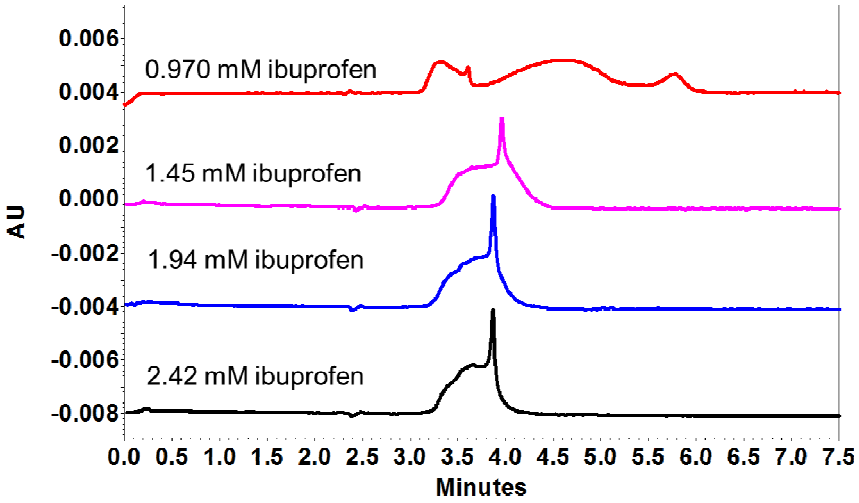


Figure S5c. Crystal structure of HSA bound with fusidic acid (PDB ID: 2VUF)

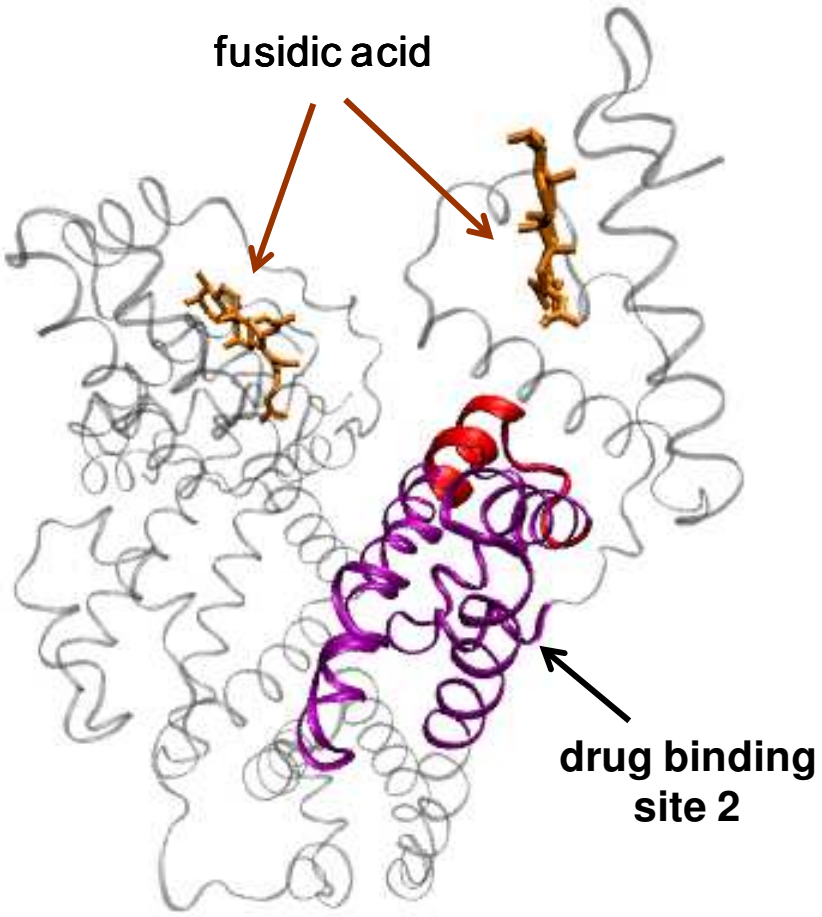


Figure S5d. Effect of fusidic acid on binding affinity between HSA and PAA-Fe_3O_4 NPs. The θ obtained with no fusidic acid was set as 1.0 for normalization.

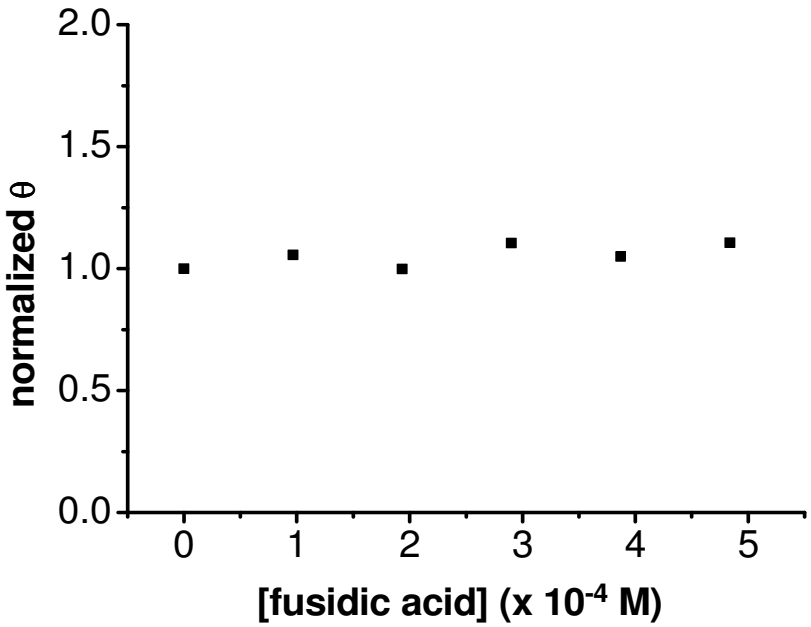
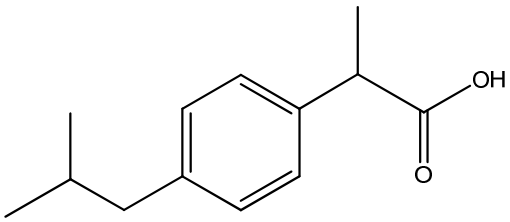
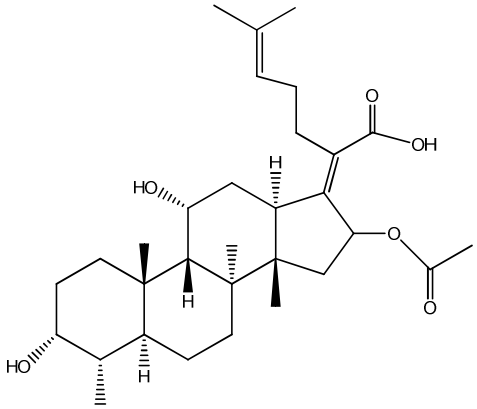


Figure S6. Molecular structures of ibuprofen and fusidic acid.



Ibuprofen



Fusidic Acid