Supporting Information for:

A method to measure the denatured proteins in the corona of nanoparticle based on the specific adsorption of Hsp90ab1

*Shang Liu[1], Xinbang Jiang[1], Xuejiao Tian[1], Zhenzhen Wang[1,2], Zhen Xing[1], Jiahui Chen[1], Junfeng Zhang[1], Chunming Wang[2], Lei Dong[1,3] **

1. State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences & Medical School, Nanjing University, 163 Xianlin Avenue, Nanjing 210093, China.
2. State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau SAR.
3. Chemistry and Biomedicine Innovative Center, Nanjing University, 163 Xianlin Avenue, Nanjing 210093, China

* Corresponding Authors: L.D., Email: leidong@nju.edu.cn.

Table S1. Characterization of experimental particles: To determine their particle size, the particles were imaged with transmission electron microscopy (TEM) and were suspended in deionized water for nanoparticle tracking analysis (NTA) using NanoSight NS300 instrument. The zeta potential was determined by Zetasizer Nano-Z Zeta Potential Analyzer. Surface Area was determined by Brunauer, Emmett and Teller (BET) analysis. SD (standard deviations).

Name	**TEM diameters**	**Hydrodynamic diameter +/- SD (nm)**		**Zeta Potential +/- SD (mV)**		**Elemental Analysis (%)**			**Surface Aera**
	In dry state	In ddH_2O	+Albumin	In ddH_2O	+Albumin	C	H	N	(m^2/g)
FeCoNiNPs	99.1+/-10.9	234.3+/-22.8	277.1+/-16.6	-15.67+/-0.58	-4.03+/-0.42		NI		8.14
Fe_3O_4NPs	102.2+/-10.5	163.0+/-15.3	151.0+/-15.2	-12.90+/-0.82	-9.22+/-0.62	0.39	0.31	0	40.0
CNPs	146.0+/-7.9	163.3+/-10.5	231.6+/-10.6	-24.77+/-0.78	-24.87+/-0.47		NI		280.0
SiO_2NPs	78.9+/-5.7	310.9+/-2.9	238.5+/-16.3	-18.20+/-0.62	-18.03+/-0.25		NI		400.0
AuNPs	75.4+/-8.5	170.9+/-4.3	282.1+/-5.5	-23.13+/-0.72	-24.27+/-0.38		NI		15.2
APTES-Fe_3O_4NPs	NI	316.8+/-8.7	199.3+/-16.8	-7.37+/-1.06	-7.66+/-0.56	0.78	0.34	0	NI
20 nm Fe_3O_4NPs	NI	191.6+/-9.7	196.3+/-10.3	-1.66+/-0.42	8.17+/-0.65		NI		NI
200 nm Fe_3O_4NPs	NI	292.0+/-14.8	296.3+/-10.8	-15.07+/-0.50	-10.53+/-0.21		NI		NI
800 nm Fe_3O_4NPs	NI	734.8+/-7.8	729.4+/-6.6	-9.15+/-1.64	-14.20+/-0.46		NI		NI
20 nm PEG-Fe_3O_4NPs	NI	42.8+/-4.4	44.6+/-7.5	2.20+/-0.36	-1.05+/-0.25	1.46	0.98	0	NI

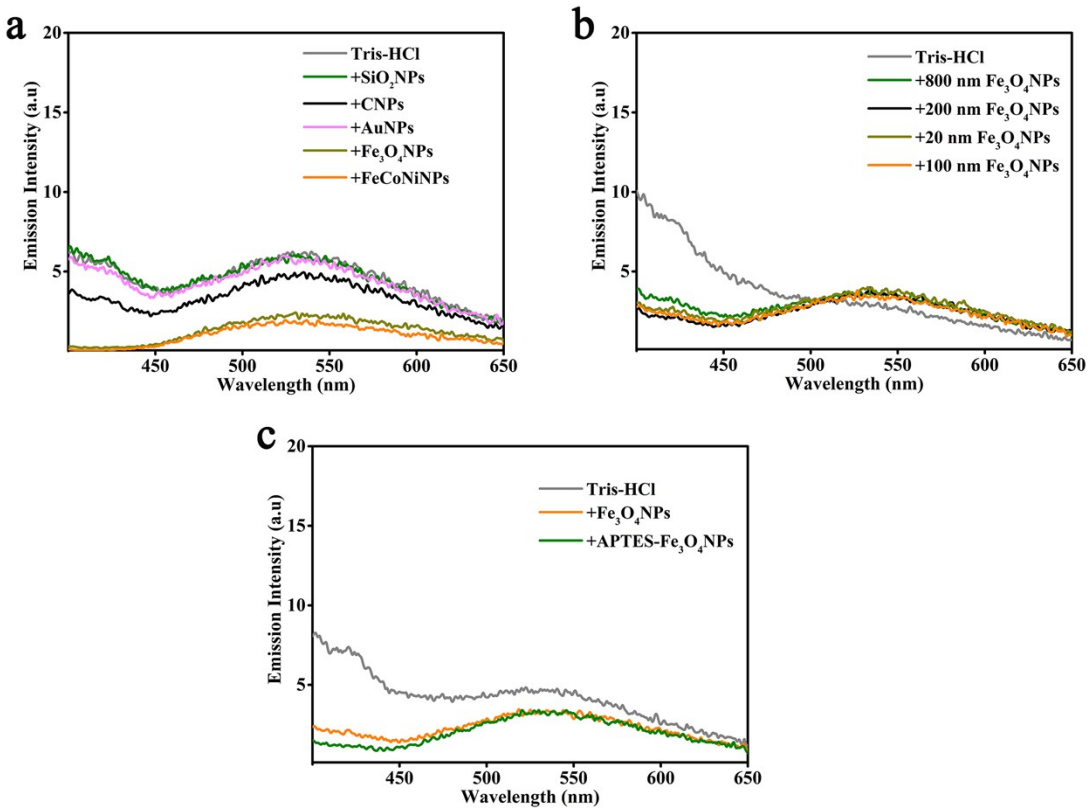

Fig. S1 Emission spectra of 1-anilinonaphthalene-8-sulfonate (ANS) in Tris-HCl solution in presence of (**a**) different kinds of NPs (FeCoNi NPs, Fe_3O_4 NPs, C NPs, SiO_2 NPs, and Au NPs), (**b**) Fe_3O_4 NPs with different sizes and (**c**) Fe_3O_4 NPs modified with or without aminopropyltriethoxysilane (APTES), with the same surface area.

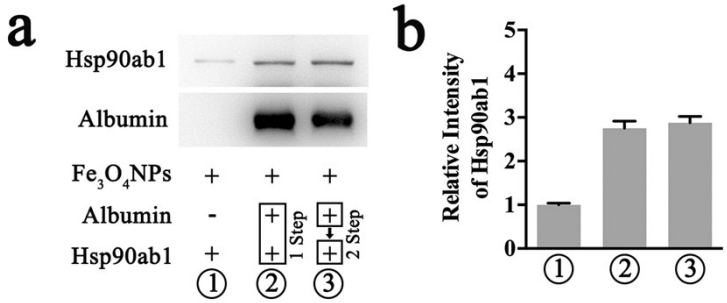

Fig. S2 (**a**) WB analysis and (**b**) the relative intensity of Hsp90ab1 in the corona of Fe_3O_4 NPs, incubated with Hsp90abl exclusively (group 1), or incubated albumin and Hsp90ab1 simultaneously (group 2), or incubated with albumin followed by Hsp90ab1 after washing (group 3). All of intensities of Hsp90ab1 were normalized to averaged intensity of Hsp90ab1 in group 1. Representative results from three independent experiments are presented as the means ± SD.

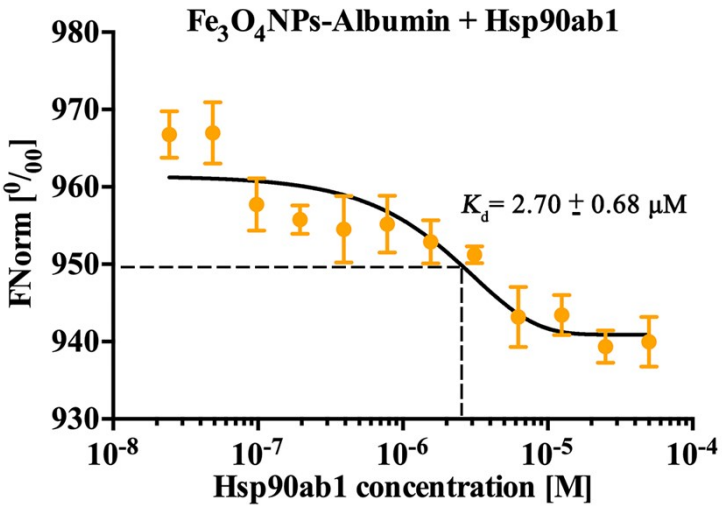

Fig. S3 Determination of the dissociation constants for the interaction between Hsp90ab1 with Cy5-Fe_3O_4 NPs-albumin corona complex by microscale thermophoresis (MST) analysis. K_d value was calculated by fitting data with Hill equation, and samples were excited at 650 nm with an excitation power of 40%, and thermophoresis analyses were conducted at room temperature (RT) with a 40% MST power. Representative results from three independent MST experiments are presented as the means ± SD.