Supporting Information

Protein-Based Fluorescent Metal Nanoclusters for Small Molecular Drug Screening

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1. Experimental section

Materials and instrumentation. Gold (III) chloride solution ($HAuCl_4$, 30 wt.% in dilute HCl), human serum albumin (HSA, 96 wt.%), bovine serum albumin (BSA, 96 wt.%), ibuprofen (ibp), warfarin (war), phenytoin (phe), sulphanilamide (sul), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. All reagents were used as received and without further purification. All glassware were washed with *Aqua Regia* ($HCl:HNO_3$ volume ratio = 3:1) and rinsed with ethanol and ultrapure water. (*Caution: Aqua Regia is a very corrosive oxidizing agent, which should be handled with great care.*) Ultrapure water with a specific resistance of 18.2 MΩ was used throughout the experiment.

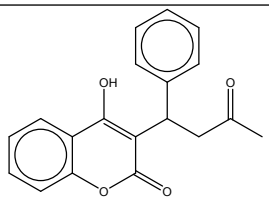
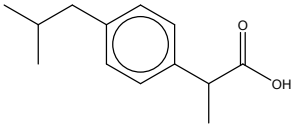
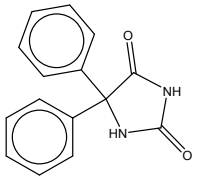
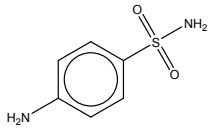
UV-vis absorption and photoluminescence spectra were recorded on a TECAN infinite M200 plate reader. The matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were carried out on a Bruker Daltonics Autoflex II TOF/TOF system. The MALDI-TOF samples were prepared by mixing the samples (2 μL) with the matrix solution [2 μL, saturated 2, 5-dihydroxybenzoic acid (DHB) solution in 50% acetonitrile], followed by a recrystallization in air prior to the measurement. Far-UV circular dichroism (CD) spectra were measured on a Jasco Model J-800 spectropolarimeter with a protein concentration of ~0.007 mM. The helical content was estimated from the mean residue ellipticity (MRE) θ at 220 nm using the equation $\%helix = (MRE_{222} - 4000)/(33000 - 4000) \times 100$. MRE was determined by $MRE = \theta/10nlC_p$, where θ is the ellipticity directly obtained from the spectropolarimeter, n is the number of amino acid residues in HSA (585), l is the optical path length (cm), and Cp is the molar fraction of the proteins (mol/L).

Drug screening based on formation of fluorescent protein-templated Au NCs.

The drug screening process was carried out by synthesizing fluorescent Au NCs templated in drug-loaded proteins. Firstly, the drug-loaded protein was prepared by mixing HSA or BSA (200 μL, 74 mg/mL in water) with a certain type of drug ligand as listed in Table S1 (1 μL, 0.45 M in DMSO) and incubated at room temperature for 1 h. After that, it was heated at 60 °C for 10 min, followed by addition of $HAuCl_4$ (400 μL, 7.5 mM in water). 5 min later, NaOH (20 μL, 1.5 M) were added to above mixture. The fluorescence intensity of resultant sample was measured at different time intervals. The binding strengths of different drugs with a variety of K_D were differentiated by comparing the fluorescence intensity of resultant Au NCs to that of the resultant Au NCs formed in pristine protein. To calculate K_D of a certain drug, the albumin protein was incubated with different amount of the specified drug prior to the synthesis of Au NCs while other reaction conditions were the same. The K_D value was calculated by fitting with the Michaelis-Menten equation using the OriginPro8 (OriginLab) software.

2. Supporting Table and Figures

Table S1. List of several HSA-binding ligands of different binding sites and binding affinity used in this study.

Name	Molecular structure	MW	Site selectivity	Reported K_D (μM)[1]
Warfarin (War)		308.33	I	4.0 ± 2.8
Ibuprofen (Ibp)		206.29	II	0.5 ± 1.0
Phenytoin (Phe)		252.27	I	131.6 ± 12.5
Sulfanilamide (Sul)		172.20	I	N.A.

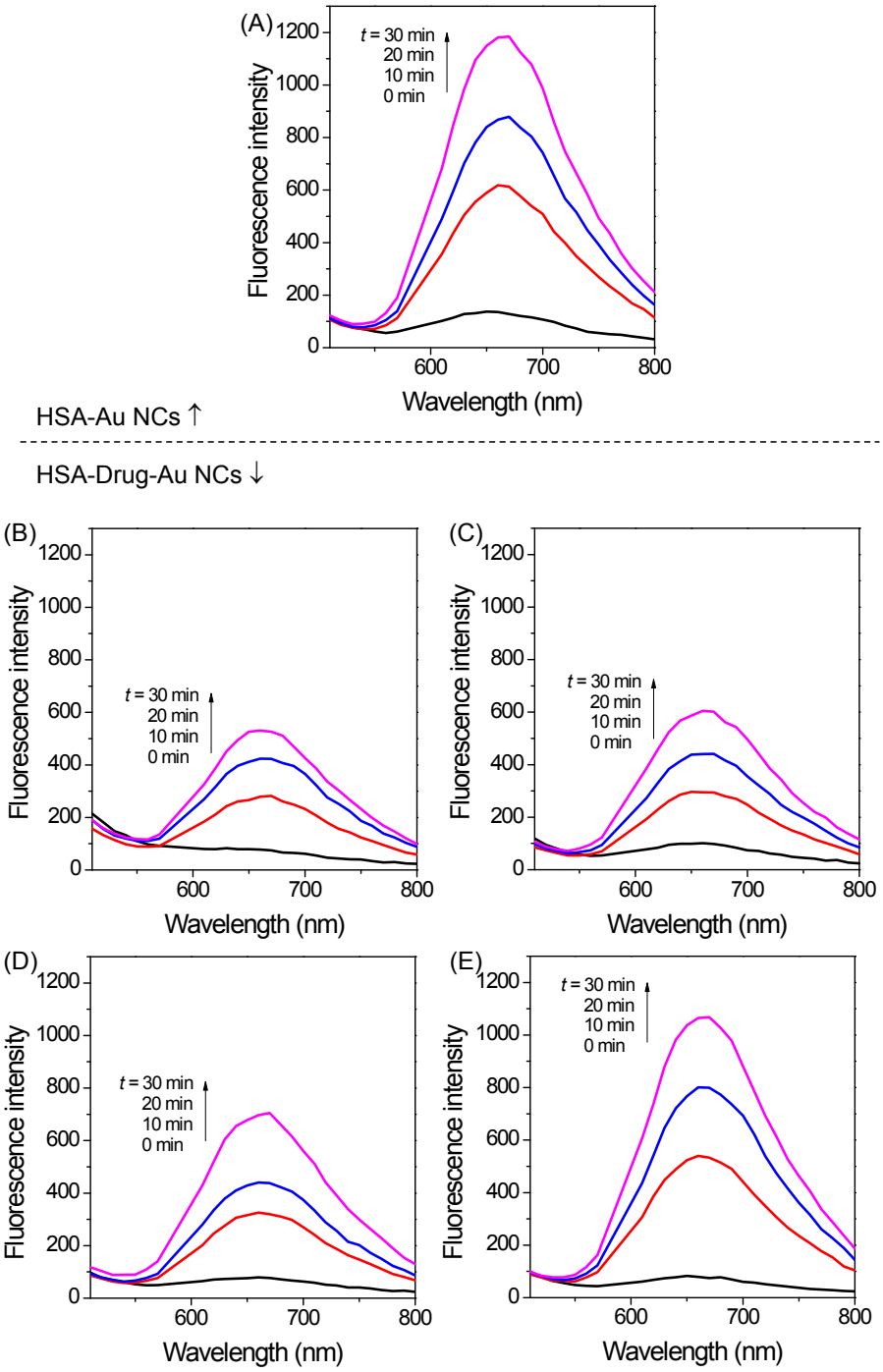


Figure S1. Time resolved photoemission spectra of (A) HSA-Au, (B) HSA-ibuprofen-Au, (C) HSA-warfarin-Au, (D) HSA-phenytoin-Au, and (E) HSA-sulfanilamide-Au NCs.

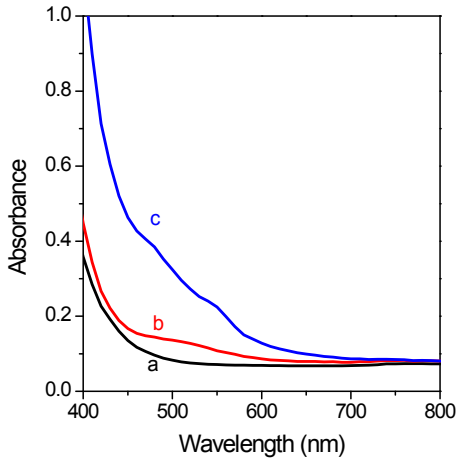


Figure S2. Photoabsorption spectra of HSA-Au NCs prepared at (a) room temperature, (b) 60 °C, and (c) HSA-warfarin-Au NCs prepared at 60 °C. All samples were measured after 10 min of reaction.

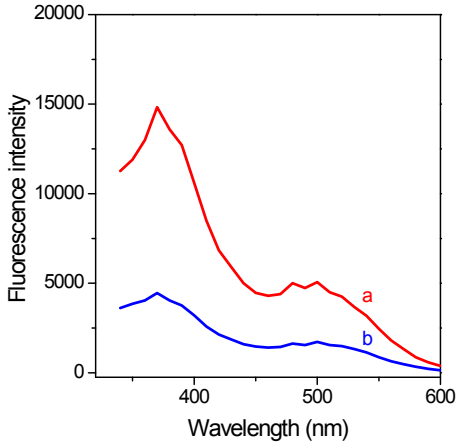


Figure S3. Photoexcitation spectra of (a) HSA-Au NCs and (b) HSA-warfarin-Au NCs prepared after 10 min of reaction at 60 °C.

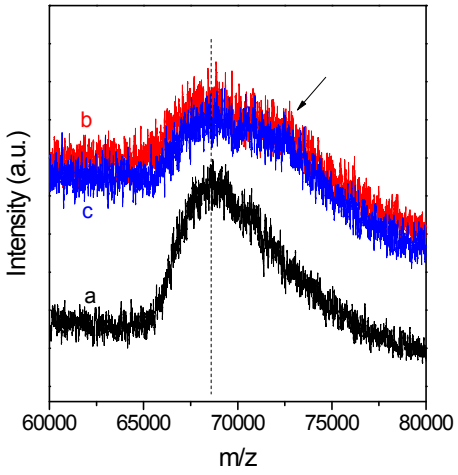


Figure S4. MALDI-TOF spectra of (a) HSA, (b) HSA-Au NCs and (c) HSA-warfarin-Au NCs. The arrow shows m/z peak due to formation of Au NCs inside protein or protein-drug template.

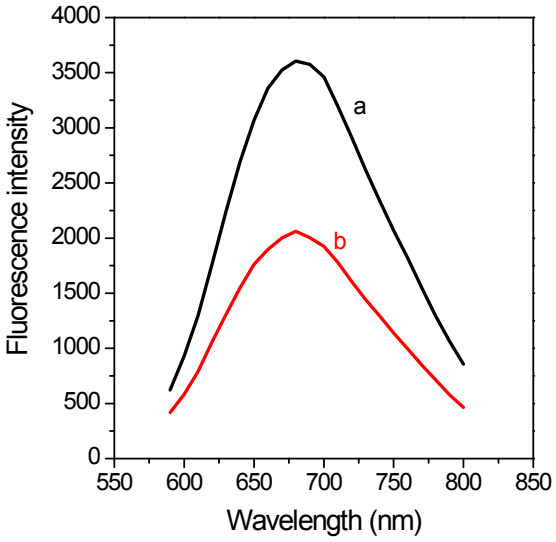


Figure S5. Photoemission spectra of (a) BSA-Au and (b) BSA-warfarin-Au NCs (λ_{ex} = 370 nm).

References

(1) Shortridge, M. D. Nuclear magnetic resonance affinity screening methods for functional annotation of proteins and drug discovery. Dissertation/Thesis, The University of Nebraska - Lincoln, Ann Arbor, 2010.