

Supporting Information for

**Protein-Protected Au Clusters as a New Class of Nanoscale Biosensors for
Label-Free Fluorescence Detection of Proteases with High Sensitivity****

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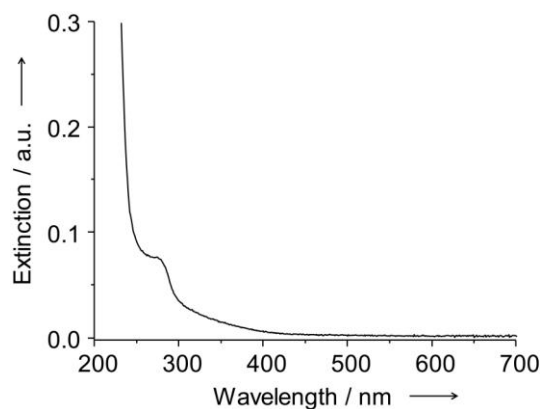


Figure S1. UV-vis extinction spectrum of an aqueous suspension of BSA-AuCs. The aqueous suspension of AuCs had an extinction peak around 280 nm due to the absorption of aromatic amino acids in BSA. No extinction peak was observed at 520 nm, indicating the absence of large Au nanoparticles in the sample.

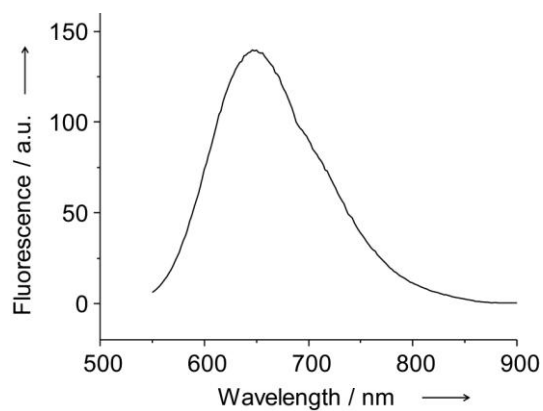


Figure S2. Fluorescence emission spectrum ($\lambda_{\text{ex}} = 500$ nm) of an aqueous suspension of BSA-AuCs.

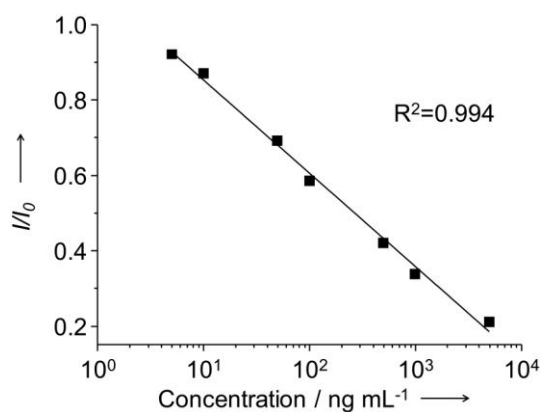


Figure S3. Linear fit for a plot of I/I_0 as a function of proteinase K concentration after the mixtures of BSA-AuCs and protease had been incubated for 4 h. I and I_0 represent fluorescence intensities of BSA-AuCs in the absence and presence of proteases, respectively

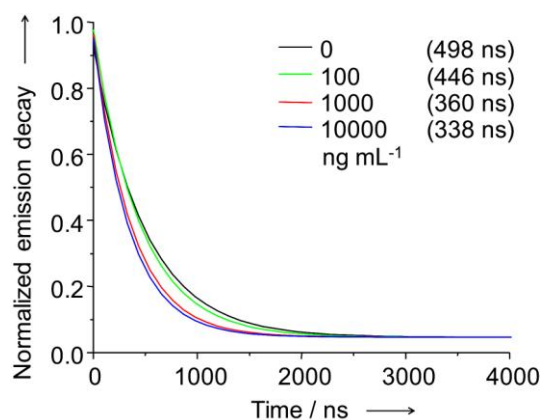


Figure S4. Fluorescence decay curves for aqueous suspensions of BSA-AuCs after incubation with proteinase K at different concentrations for 4 h. Numbers in the parenthesis represent the corresponding calculated fluorescence lifetimes.

Table S1. Summary of protein substrates and proteases that have been examined for the fluorescence-based detection using protein-protected AuCs.

Protein substrate	Protease	Linear range (ng mL ⁻¹)	$I/I_0 = A \log(C) + B^a$		
			A	B	R ²
BSA	α -Chymotrypin	10 – 1 × 10 ⁶	-0.14	1.06	0.993
BSA	Trypsin	10 – 1 × 10 ⁶	-0.11	1.03	0.987
BSA	Papain	1 × 10 ³ – 5 × 10 ⁵	-0.21	1.65	0.991
BSA	Protease XIII	N.A. ^b	N.A.	N.A.	N.A.
BSA	Proteinase K	5 – 5 × 10 ³	-0.20	1.08	0.994
Insulin	Proteinase K	1 × 10 ⁴ – 1 × 10 ⁶	-0.19	1.83	0.991
Lysozyme	Proteinase K	1 × 10 ³ – 1 × 10 ⁶	-0.22	1.59	0.993
Transferrin	Proteinase K	10 – 1 × 10 ⁵	-0.21	1.25	0.984

^a I_0 and I represent intensities of fluorescence from BSA-AuCs in the absence and presence of proteases, respectively. C represents the concentration of protease in ng mL⁻¹.

^b Not available.