Electronic Supplementary Information *for*

Cholesterol determination using protein-templated fluorescent gold nanocluster probes

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Experimental

Chemicals and reagents. Phosphate buffered saline (PBS) tablets (catalog no. P4417; 10 mM phosphate buffer, 2.7 mM KCl, and 137 mM NaCl, pH 7.4 at 25 °C), tetrachloroauric(III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$; 520918; ≥99.9% trace metals basis), sodium hydroxide (306576; pellets, semiconductor grade, 99.99% trace metals basis), bovine serum albumin (BSA; A7030; essentially fatty acid free, essentially globulin free, ≥98% by agarose gel electrophoresis), Triton™ X-100 (TX-100; X100; laboratory grade), cholesterol (C8667; ≥99%), and cholesterol oxidase (ChOx) from *Brevibacterium* sp. (C8868; ≥50 units/mg protein, recombinant, expressed in *E. coli*) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Aqueous solutions were prepared from freshly drawn ultrapure water from a Direct-Q 3 UV system polished to a resistivity of 18.2 MΩ cm.

Preparation of AuNCs@BSA. 15 mL of 10 mM $HAuCl_4$ (*aq*) was mixed with 15 mL of 50 mg mL^{-1} BSA (*aq*). After stirring for a few minutes, 1.5 mL of 1.0 N NaOH (*aq*) was added under constant stirring. The reaction mixture was incubated at 38 °C for 24 h to generate AuNCs@BSA.

Preparation of TX-100 stock solution. 15.0 g of TX-100 was dissolved in water, adjusted to a total volume of 100 mL, and warmed to 65 °C overnight to assist solubilization, yielding a final stock 0.24 M in TX-100.

Preparation of cholesterol stock solution. 500 mg of cholesterol were dissolved in 100 mL of 0.24 M TX-100 at 65 °C to achieve a cholesterol standard stock solution at a concentration of 12.8 mM.

Preparation of ChOx stock solution. 3.0 mg of ChOx from *Brevibacterium* sp. was dissolved in 3.00 mL of PBS solution to yield a 1.0 mg mL^{-1} ChOx enzyme stock.

Preparation of sample and control groups. Sample and controls groups were prepared following Table S2. Appropriate volumes of AuNCs@BSA and PBS stocks were combined with cholesterol and TX-100 stocks and then aged at 40 °C for 3 h. To initiate the reaction, 40 µL of as-prepared ChOx stock was injected at time zero.

Preparation of samples with different cholesterol levels. Samples were prepared following Table S3. A 0.2 mL volume of AuNCs@BSA and 0.8 mL of PBS were combined with cholesterol and TX-100 stocks and then aged at 40 °C for 3 h. To initiate the reaction, 40 µL of as-prepared ChOx stock was injected at time zero.

Table S1. Comparison of a few illustrative cholesterol detection methods.

Method	Detection Range	LOD	Response Time	Ref
Amperometric biosensor	0.5–15 μM	0.5 μM	5 s	2
HPLC	2.7–8.6 mM	0.12 mM	0.5 h	4
Enzymatic methods	0–12 mM	1.35 mM	40 s	8
Nanocluster probes	0–300 μM	12 μM	> 1 h	current

Table S2. Composition of sample and controls presented in Fig. 3.

Sample	AuNCs@BSA	PBS	TX-100	ChOx	cholesterol
a)	0.2 mL	0.8 mL	20 μL	40 μL	20 μL
b)	0.2 mL	0.8 mL	40 μL	40 μL	0 μL
c)	0.2 mL	0.8 mL	20 μL	0 μL	20 μL

[a] Stock descriptions are provided in the above Experimental. Cells shaded aqua denote aqueous stocks; pink cells indicate a solvent system containing 0.24 M aqueous TX-100.

Table S3. Composition of samples containing different cholesterol concentrations for construction of a calibration curve (see Fig. 4).[a]

Sample	AuNCs@BSA	PBS	TX-100	ChOx	cholesterol
1	0.2 mL	0.8 mL	40 μL	40 μL	0 μL
2	0.2 mL	0.8 mL	35 μL	40 μL	5 μL
3	0.2 mL	0.8 mL	30 μL	40 μL	10 μL
4	0.2 mL	0.8 mL	25 μL	40 μL	15 μL
5	0.2 mL	0.8 mL	20 μL	40 μL	20 μL
6	0.2 mL	0.8 mL	15 μL	40 μL	25 μL

[a] Stock descriptions are provided in the Experimental. Cells shaded aqua denote aqueous stocks; the pink cells indicate a solvent system composed of 0.24 M TX-100 (*aq*).

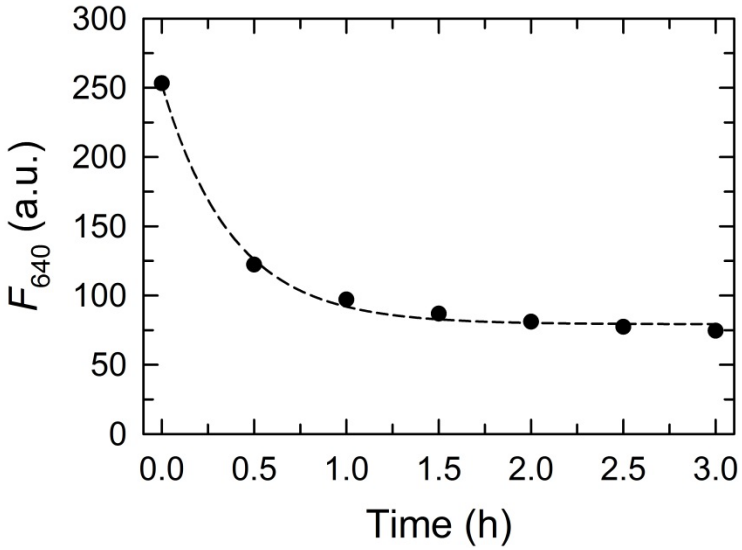

Fig. S1 Time-dependent fluorescence intensity of AuNCs@BSA following injection into a solvent system containing 0.8 mL of PBS and 0.2 mL of 0.24 M TX-100 (*aq*). As is clearly evident, after about an hour the sample has adjusted to the new solvent composition and reached a stable signal. The dashed curve is an exponential-decay fit to the data corresponding to $F = 79 + 173*\exp(-2.63t)$. Accordingly, the fluorescence decreased *ca.* three-fold (*i.e.*, (79 + 173)/79) as a result of the presence of surfactant.

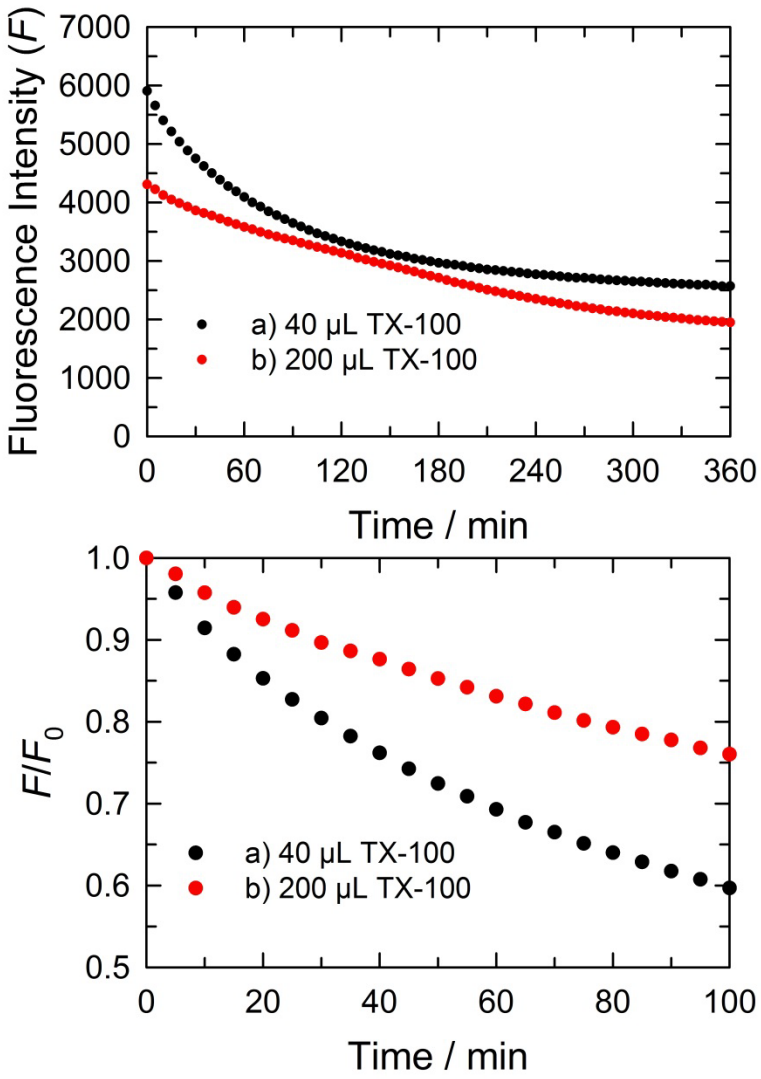

Fig. S2 Integrated fluorescence intensity (630–640 nm) of AuNCs@BSA solutions containing different amounts of TX-100 as a function of time after ChOx injection at 40 °C. Both samples contain identical ChOx and cholesterol concentrations. Sample a) containing 40 µL of TX-100 (black symbols) shows faster initial kinetics than sample b) (red), which contains 200 µL of TX-100. Conditions are the same as for sample 6 in Table S3 with the exception that b) contains an additional 160 µL of 0.24 M TX-100 and only 0.64 mL of PBS.

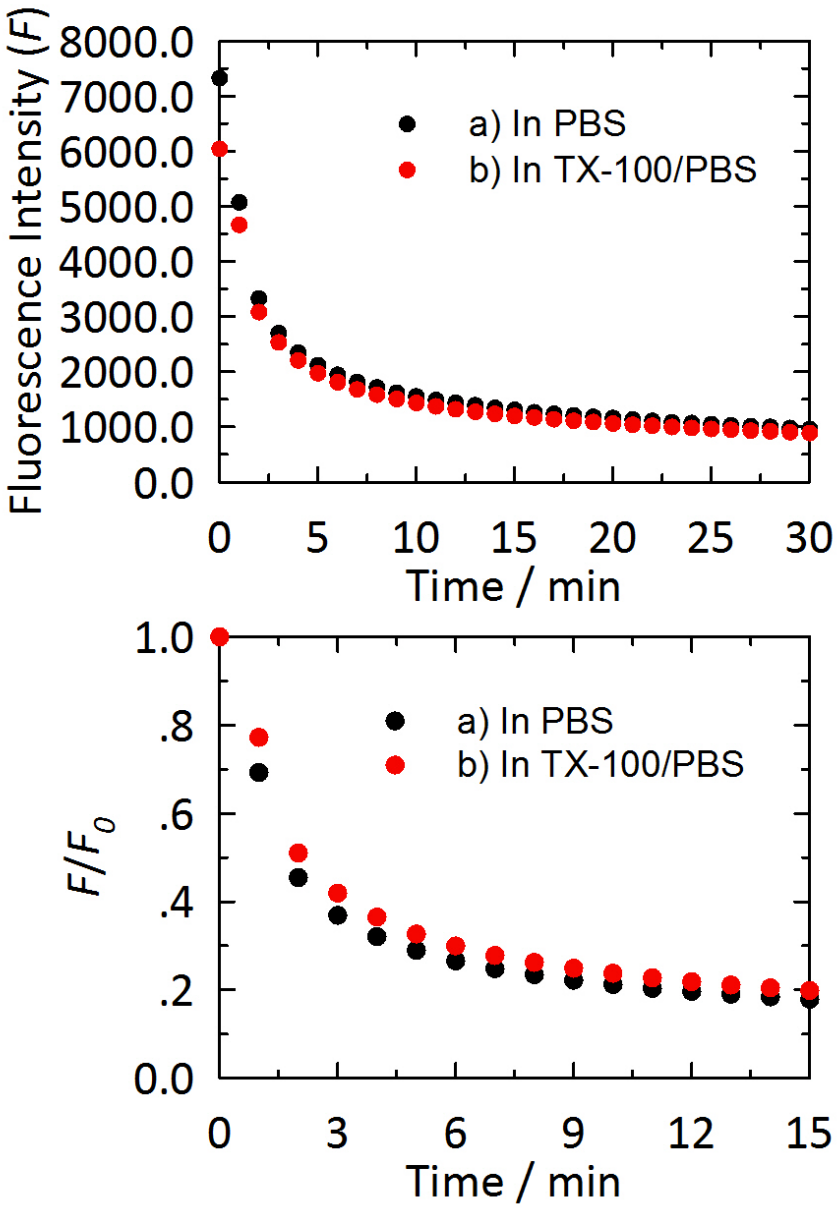

Fig. S3 Fluorescence intensity integrated between 630 and 640 nm for AuNC@BSA solution as a function of time after H_2O_2 injection at 40 °C. In these examples, 5 µL of 30 wt% H_2O_2 (*aq*) were injected at time zero. Sample a) containing no TX-100 (black symbols) shows comparable kinetics to sample b) (red), which contains TX-100. Both samples contain 0.2 mL of AuNCs@BSA and 0.8 mL of PBS whereas b) contains an additional 40 µL of TX-100. These samples contain neither ChOx nor cholesterol, eliminating the enzymatic production of H_2O_2 as a possibility.

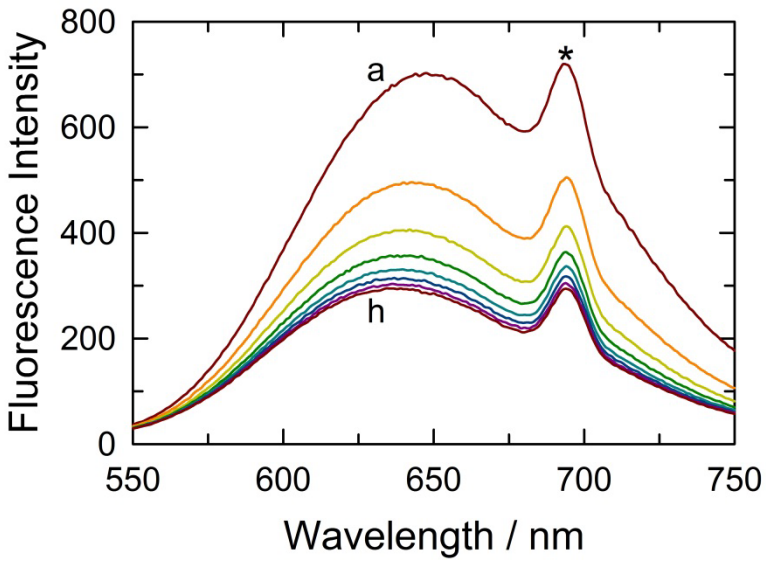

Fig. S4 Emission spectra of AuNCs@BSA after ChOx injection. From top (a) to bottom (h), the spectra were collected 0, 50, 100, 150, 200, 250, 300, and 350 min post ChOx injection. Conditions are as per sample 6 in Table S3. Note that the peak labeled * arises from doubled Rayleigh scatter. Excitation was at 350 nm.