# Exploring luminescence-based temperature sensing using protein-passivated gold nanoclusters

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## Experimental Section

**Chemicals and Reagents.** 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); tetramethyl orthosilicate (TMOS; 341436; ⩾99%); aluminum isopropoxide ($Al(O\text{-}i\text{-}Pr)_3$); and acetic acid (ACS reagent, ⩾99.7%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris base (catalog no. BP152-500) was purchased from Fisher Scientific. Diglycerylsilane (DGS) was prepared following a published procedure.[1] 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 freshly prepared 50 mg $mL^{-1}$ BSA (*aq*). After stirring for a few minutes, 1.5 mL of 1.0 M NaOH (*aq*) was added under constant stirring. The reaction mixture was incubated at 38 °C for 24 h to generate AuNCs@BSA. As-prepared AuNCs@BSA were diluted 9-fold with ultrapure $H_2O$ and incubated at room temperature for 24 h before studying them.

**Thermally Annealed AuNCs@BSA.** Samples were iteratively heated and cooled using a temperature-controlled cell holder connected to a circulation bath and mounted within a fluorimeter. During annealing, samples were heated from 10 to 60 °C at a rate of ~1 °C $min^{-1}$ and held at 60 °C for 15 min followed by cooling down to 10 °C and another 15 min hold. This constitutes one cycle. Two additional cycles were applied. Samples treated in this way are referred to as thermally "annealed" AuNCs@BSA.

## Sol–Gel-Coated AuNCs@BSA

**Preparation of TMOS Silica Sol–gel-modified AuNCs@BSA.** 0.5 mL of as-prepared AuNCs@BSA was combined with 1.0 mL of 10 mM Tris base (*aq*). After stirring for a few minutes, 5 μL of TMOS was added to the reaction mixture followed by another 5 μL addition 12 h later. The mixture was diluted 3-fold with water and incubated at room temperature for another 24 h prior to measurement.

**Preparation of DGS Silica Sol–gel-modified AuNCs@BSA.** 0.15 g of diglycerylsilane (DGS) was dissolved in 10 mL of ultrapure $H_2O$. After stirring for 30 min, 0.5 mL of as-prepared DGS (*aq*) was added to 0.5 mL of as-prepared AuNCs@BSA twice at an interval of 12 h. The mixture was diluted 3-fold in water and incubated at room temperature for 24 h before testing.

**Preparation of Alumina Sol–gel-modified AuNCs@BSA.** 1.51 g of aluminum isopropoxide ($Al(O\text{-}i\text{-}Pr)_3$) was dissolved into 25 mL of ultrapure $H_2O$ with vigorous stirring to form a uniform milky solution. 1.5 mL of as-prepared AuNCs@BSA was added to the solution followed by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was incubated at 90 °C for 2 h and then cooled to room temperature naturally. The sample was dried in the air for a week to generate $Al_2O_3$-stablized AuNCs@BSA.

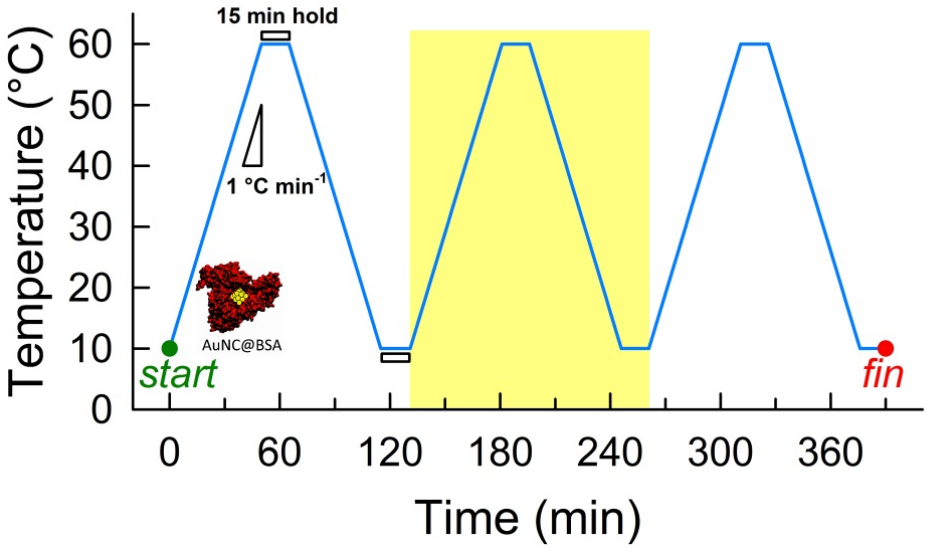
**Preparation of Heat-Denatured AuNCs@BSA (abbreviated as AuNCs@hBSA).** AuNCs@hBSA were prepared following the same strategy for normal AuNCs@BSA, except that BSA was incubated at 80 °C for 30 min prior to addition of $HAuCl_4$. Before measurements were made, the as-prepared AuNCs@hBSA were diluted 9-fold in water and incubated at room temperature for 24 h.

**Preparation of $NaBH_4$-reduced AuNCs@BSA (AuNCs@rBSA).** AuNCs@rBSA was prepared followed a similar approach reported by Chen's group with slight modification.[2] Typically, 0.5 g of BSA was dissolved in 12.5 mL of ultrapure water. Next, 1.0 mL of 15 mg $mL^{-1}$ freshly prepared $NaBH_4$ (*aq*) was added under constant stirring. The mixture was held at room temperature for 30 min until frothing could no longer be observed. The mixture was then incubated at 70 °C until the generation of $H_2$(g) subsided. After the solution was naturally cooled to room temperature, 4.5 mL of 24.3 mM $HAuCl_4$ was added under stirring, and 2 min later, 2.0 mL of 1.0 M NaOH added. The reaction continued overnight (12 h) at room temperature. Following the reaction, the solution was dialyzed against ultrapure $H_2O$ using a 2,000 MWCO membrane (Slide-A-Lyzer dialysis cassette, Thermo Scientific). Before conducting tests, as-prepared AuNCs@rBSA was 9-fold diluted by water and followed by holding at room temperature for 24 h.

**Preparation of Halide-Treated AuNCs@BSA.** 1.0 mL of as-prepared AuNCs@BSA was mixed with 1.0 mL of 1.0 M aqueous salt (NaCl, KBr, or KI) followed by the addition of 1.0 mL of $H_2O$. These halide-treated AuNCs@BSA samples were then 3-fold diluted and incubated at room temperature for 24 h before study.

**References**

1. B. Satishkumar, S. K. Doorn, G. A. Baker and A. M. Dattelbaum, *ACS nano*, 2008, **2**, 2283-2290.
2. H. Li, Y. Guo, L. Xiao and B. Chen, *Analyst*, 2013, **139**, 285-289.




**Scheme S1.** Thermal program used to "anneal" AuNCs@BSA.

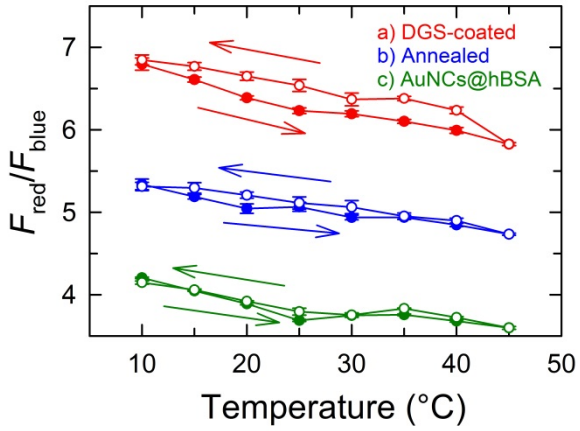



**Fig. S1 Ratio of red-to-blue integrated fluorescence intensities during heating (filled symbols) and cooling (empty symbols) for** DGS-coated AuNCs@BSA (red profiles), **annealed AuNCs@BSA (blue),** and AuNCs@hBSA (green)**. The "red" and "blue" band intensities were determined by integration from 640–650 nm and 330–340, respectively. $\lambda_{exc}$ = 250 nm.**

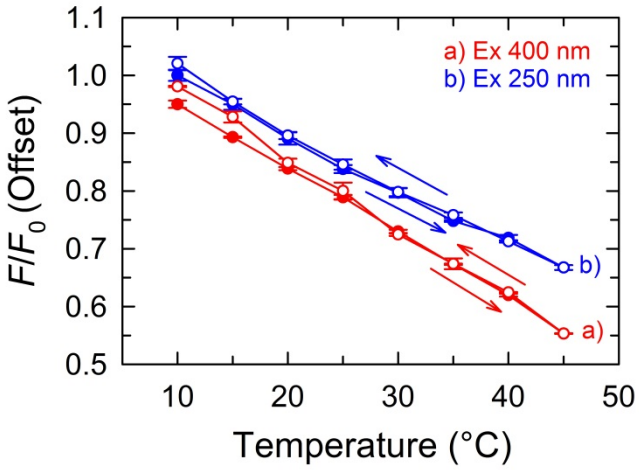



**Fig. S2** Comparison of thermal cycles constructed from the integrated fluorescence intensity (640–650 nm) for annealed AuNCs@BSA during heating (filled symbols) and cooling (empty symbols) for excitation at 400 nm (red symbols) and 250 nm (blue symbols). $F/F_0$ denotes the fraction of fluorescence remaining, as normalized to the initial integrated fluorescence intensity at 10 °C. Data for 400 nm-excitation are shifted downward by 0.05 to avoid graphical congestion. These results demonstrate that the overall response to temperature is not strongly affected by excitation wavelength for the red emission band arising from $Au_{25}$.

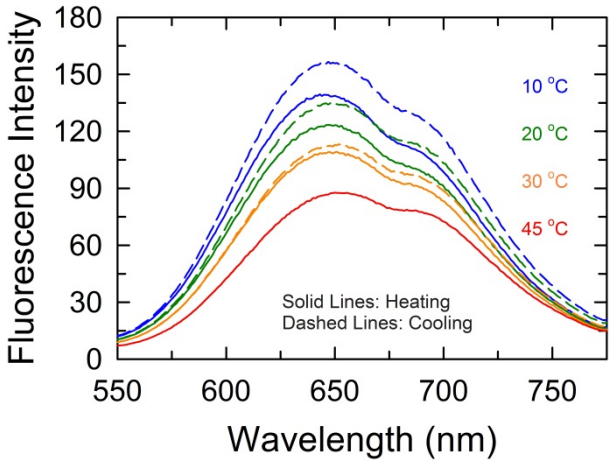



**Fig. S3 Steady-state fluorescence emission spectra of untreated AuNCs@BSA during controlled heating (solid lines) and cooling (dashed lines), starting at 10 °C. The hysteresis after a single heating–cooling cycle is abundantly clear. $\lambda_{exc}$ = 400 nm.**

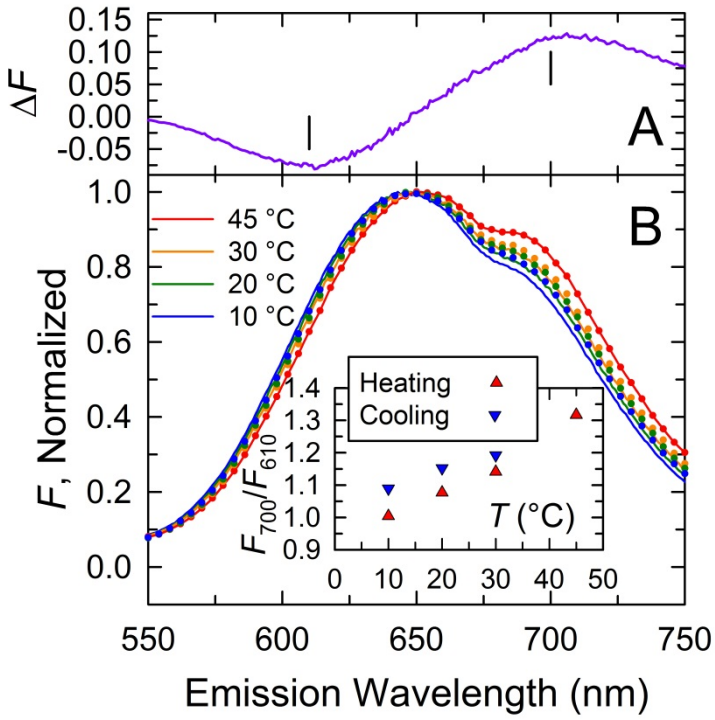



**Fig. S4 (A) The spectrum constructed as the difference in the normalized emission spectra for AuNCs@BSA measured at 45 °C and 10 °C. The vertical marks denote the peak positions at 610 nm and 700 nm. (B) Normalized steady-state fluorescence emission spectra taken of virgin (untreated) AuNCs@BSA during controlled heating (shown as solid curves) and cooling (symbols) segments of a single thermal cycle. $\lambda_{exc}$ = 400 nm.**

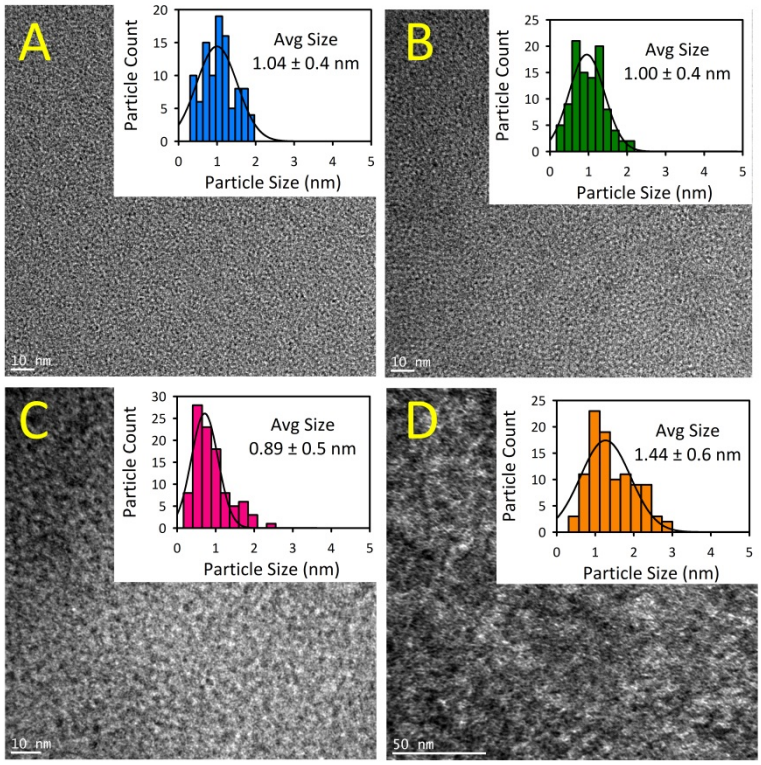



**Fig. S5 Representive TEM images of (A) AuNCs@BSA, (B) TMOS-coated AuNCs@BSA, (C) DGS-coated AuNCs@BSA, and (D) $Al_2O_3$-coated AuNCs@BSA with average particle sizes of 1.04 ± 0.4 nm, 1.00 ± 0.4 nm, 0.89 ± 0.5 nm, and 1.44 ± 0.6 nm, respectively.**

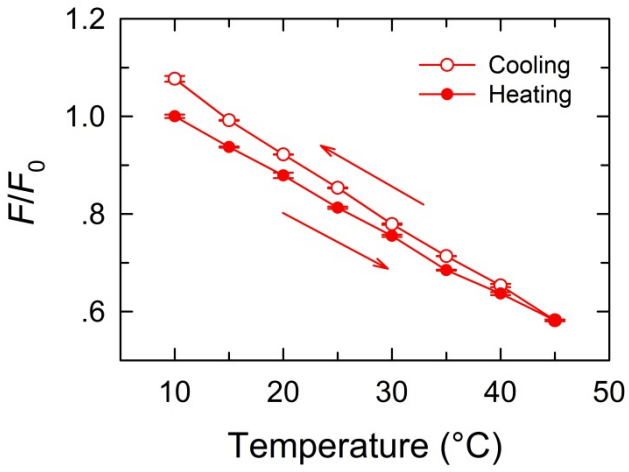



**Fig. S6 Ratio of fluorescence intensity of red peak (integrated from 640–650 nm) to blue peak (integrated from 330-340 nm) during heating (filled symbols) and cooling (empty symbols) processes in thermal cycling on $NaBH_4$-reduced AuNCs@rBSA. $\lambda_{exc}$ = 250 nm.**

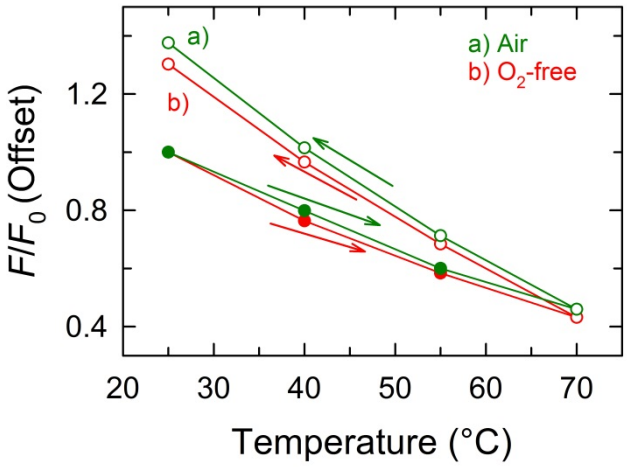



**Fig. S7 Thermal cycling curve constructed from integrated fluorescence intensity (640-650 nm) of original AuNCs@BSA (red curves) and $O_2$ excluded AuNCs@BSA (green curves) during heating (filled symbols) and cooling (open symbols). $F/F_0$ denotes the fraction of fluorescence remaining, normalized to the initial fluorescence at 10 °C. $\lambda_{exc}$ = 400 nm. We note that similar insensitivity to $O_2$ was also observed for AuNCs@hBSA.**

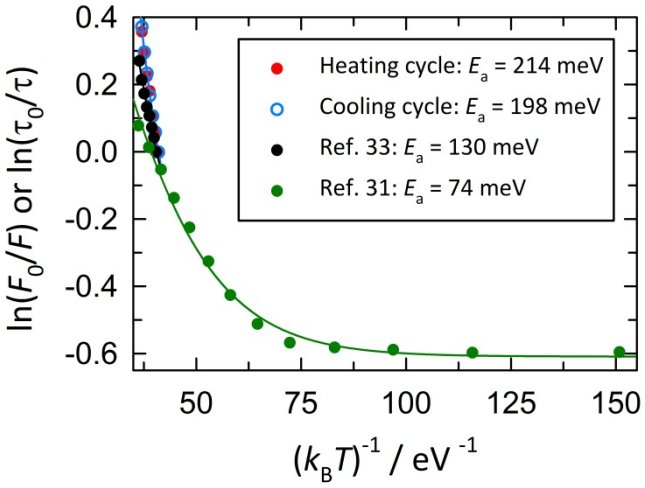



**Fig. S8 Expanded-range analog of the Arrhenius plot presented in Fig. 5. The references quoted in the inset refer to those in the main body of the paper.**

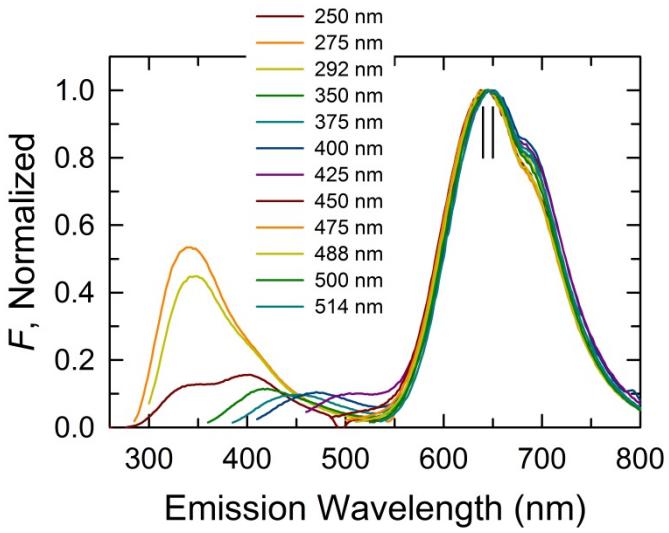



**Fig. S9 Excitation wavelength dependent normalized emission spectra of AuNCs@hBSA. The two vertical lines denote the range for integration of the red band.**