Electronic Supplementary Material (ESI) for Analyst.

β-lactoglobulin amyloid fibrils templated gold nanoclusters for cellular multicolor fluorescent imaging and colorimetric blood glucose assay

Zhiguang Suo[a], Xialing Hou[a], Yu Liu[b], Feifei Xing[c], Yingying Chen[a], Lingyan Feng*[a]

[a] *Materials Genome Institute, Shanghai University, 99 Shangda Road, Shanghai 200444, People's Republic of China*

[b] *College of Qianweichang, Shanghai University, 99 Shangda Road, Shanghai 200444, People's Republic of China*

[c] *College of Science, Department of Chemistry, Shanghai University, 99 Shangda Road, Shanghai 200444, People's Republic of China*

*Corresponding authors.
E-mail address: lingyanfeng@t.shu.edu.cn
Tel/Fax: +86-21-66136189

1. Experimental Details

1.1 *Reagents*

β-Lactoglobulin (from bovine milk) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich. $HAuCl_4 \cdot 4H_2O$, H_2O_2 solution (30%), Ascorbic acid, 3,3,5,5-tetramethylbenzidine (TMB), D-fructose, α-lactose monohydrate, D-maltose monohydrate, and D-glucose (GO) were obtained from Sinopharm. Glucose oxidase (GOx, from Aspergillus Niger, Specific activity ≥100 U/mg) and DMSO were supplied by BBI (Ontario, Canada). Thiazolyl blue tetrazolium bromide (MTT) was obtained from Yeasen Biotech Co., Ltd. Human serum sample was obtained from Ruijin Hospital. A549 cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai). Fetal bovine serum (FBS) was bought from PAA laboratories. Streptomycin, penicillin, 0.25% Trypsin-EDTA and DMEM medium were purchased from Gibco. All other chemicals, such as HCl, H_2SO_4, Na_2HPO_4 and NaH_2PO_4 were of analytical grade. Milli-Q water (18.2 MΩ□cm; Millpore Co., USA) was used in all experiments. Filters (0.22 μm and 0.45 μm) and centrifugal flter devices (0.5 mL 30 K) were supplied from Millipore.

1.2 *Production of β-lactoglobulin amyloid fibrils*

Before the experiment, all glassware and magneton were thoroughly washed with freshly prepared aqua regia (HCl: HNO_3 = 3:1, v/v), rinsed with ethanol and ultrapure water and dried in an oven at 60 °C for 3-4 h. First, the BLG powder was dissolved in ultrapure water at 10 wt% and adjusted to pH 4.6 using 1 M HCl. The protein solution was centrifuged 15000 rmp over 15 min at 20℃, and then the supernatant was collected and adjusted to pH 2. The supernatant was filtered by a 0.22 μm filter to remove insoluble proteins. In order to reduce the effect of ions on protein aggregation, the filtered BLG solution was purified by an ultra-filter device (0.5 mL, 3 K). The filtered solution was freeze-dried and stored for further use. Purified β-lactoglobulin powder was dissolved and centrifuged 10800 g over a period of 1 h at 20℃. The filtered solution adjusted to pH 2 and through a 0.45 μm filter before heat-treatment. Then, the BLG solution was heated with vigorous stirring at 90 ^{o}C for 5 h. After heat treatment, the heated BLG solution was quickly cooled in ice-water mixtures to quench the protein aggregation process.[1]

1.3 *Preparation of BLGF-AuNCs*

The multicolor BLGF-Au NCs were prepared by one-pot method. Briefly, 2.0 mL of $HAuCl_4$ solution (4 mM) was added into 2 mL BLGF solution (20 $mg{\cdot}mL^{-1}$) under vigorous stirring for 2 min. Then, 1.0 M NaOH solution was introduced into the mixture for the reaction at pH 12, and the reaction was allowed to proceed for 24 h. Then the red fluorescence BLGF-Au NCs (R-BLGF-Au NCs) were obtained. The green fluorescence BLGF-Au NCs (G-BLGF-Au NCs) were prepared by adjusting the pH values of 2.0 mL of $HAuCl_4$ solution (4 mM) and 2 mL BLGF solution (20 $mg{\cdot}mL^{-1}$) mixture to ~1 by 1M HCl. The reaction was proceed under 37 ℃ over 100 h.[2] The BLGF-Au NCs with a blue fluorescence (B-BLGF-Au NCs) were prepared by adding 2.0 mL $HAuCl_4$ (10 mM) to 2 mL BLGF solution (20 $mg{\cdot}mL^{-1}$) under vigorous stirring for 2 min. 20 μL of ascorbic acid (0.35 $mg{\cdot}mL^{-1}$) was added above mixture under pH 8 environment for 5 h at 37 ℃.[3] The final BLGF-Au NCs compounds were freeze-dried after purification. A 10 $mg{\cdot}mL^{-1}$ BLGF-Au NCs solution was obtained for future use by dissolving the powder into ultrapure water.

1.4 *Characterization of the BLGF-Au NCs*

β-lactoglobulin amyloid fibrils were primarily characterized by AFM in tapping mode using a MicroNano D-5A AFM system. Far-UV circular dichroism(CD) spectra were recorded with a path length of 1 mm on a Jasco J-1500 spectropolarimeter under nitrogen atmosphere. An Edinburgh FS-5 UK fluorescence spectrometer was used for fluorescence spectroscopy measurements. The spectrum correction of back ground was selected for both excitation and emission at the slits of 5/5nm. The fluorescence lifetimes were performed using an Edinburgh Photonics Mini-Tau time-resolved fluorometer (excited at 365 nm TCSPC). The decay datas were performed following two-exponential model in Fluoracle program from Edinburgh instruments Inc. Quantum yield (QY) measurement of Au NCs was carried out using an Edinburgh FS-5 UK fluorescence spectrometer by an absolute method. The QY was measured by direct excitation in the integrating sphere. And, the final calculation of the quantum yield is supplied with the Fluoracle software. The absolute fluorescence quantum yield is calculated as follows:

$$QY = \frac{N^{em}}{N^{abs}} = \frac{E_b - E_a}{S_a - S_b}$$

where N^{em} and N^{abs} are the number of photons emitted and absorbed respectively. E_a and E_b are the number of photons emitted of solvent and sample. S_a and S_b are the number of photons absorbed of solvent and sample. All UV-vis absorption spectra were performed using a cuvette of 1 cm path length in Jasco J-750 spectrophotometer. The sizes and morphologies of Au NCs were obtained using Hitachi HT7700 high-resolution transmission electron microscope. The diluted Au NCs sample was placed on a carbon support copper grid. X-ray photoelectron spectroscopy(XPS) measurements were performed on an Escalab 250xi spectrophotometer. Fourier transform infrared (FT-IR) spectra were taken on a Thermo Nicolet 6700 FT-IR spectrometer. The cell imaging was obtained on Olympus FV3000 confocal microscope. All measurements were carried out at least three times to obtain reproducible results.

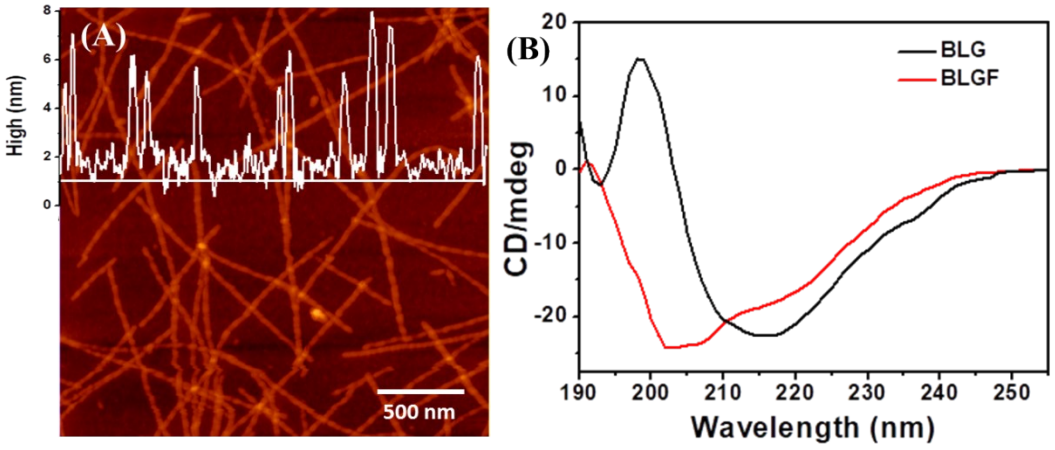

Fig. S1 (A) AFM height image of BLGF deposited on mica substrates. (B) Far-UV circular dichroism (CD) spectra of BLG (black) and BLGF (red). (n=3)

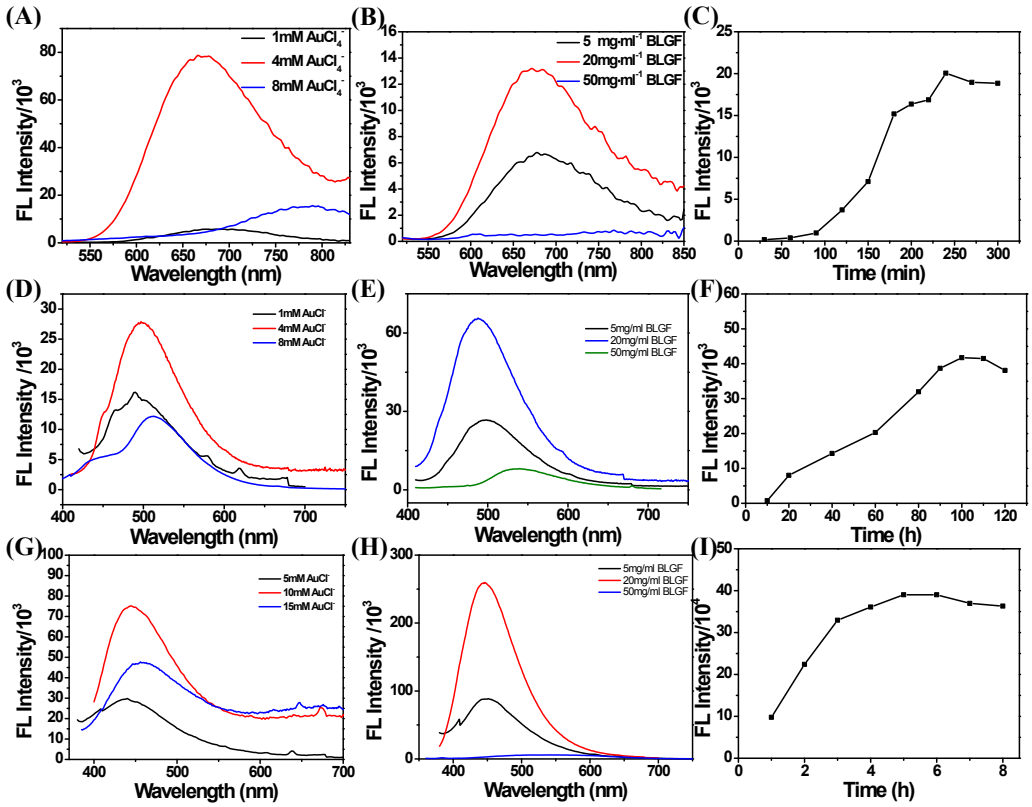

Fig. S2 Emission spectra of red (A,B,C), green (D,E,F), blue (G,H,I) fluorescence BLGF-Au NCs at different concentration of (A,D,G) AuCl4- , (B,E,H) BLGF and reaction time (C,F,I).

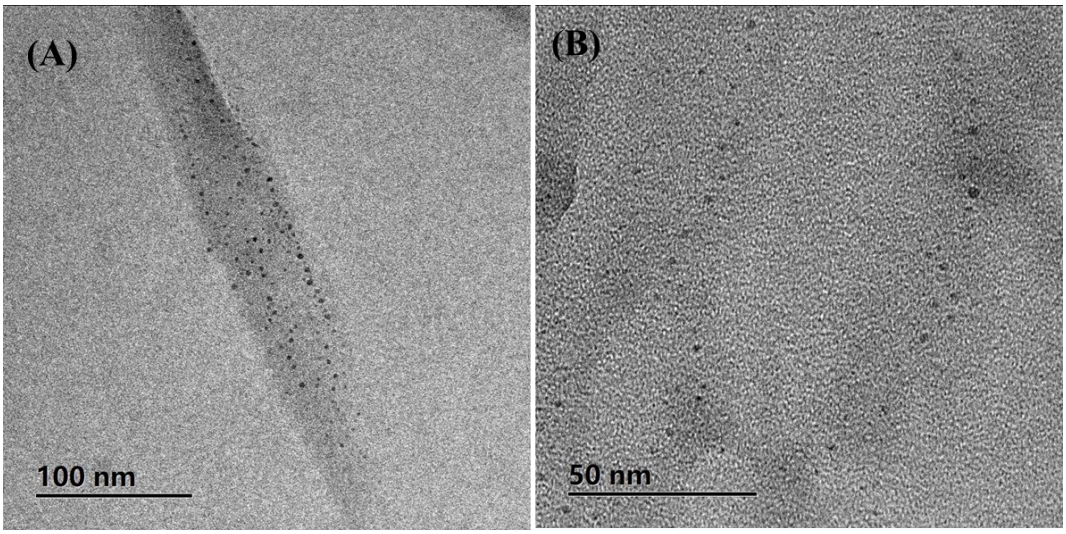

Fig. S3 TEM images of aqueous solutions of BLGF-Au NCs.

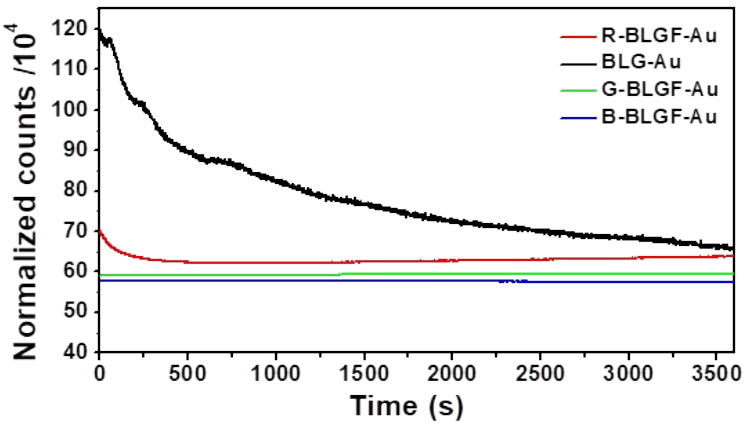

Fig. S4 Time response of the fluorescence intensity of BLG-Au NCs (black) and BLGF-Au NCs (red, green, blue) within 1h.

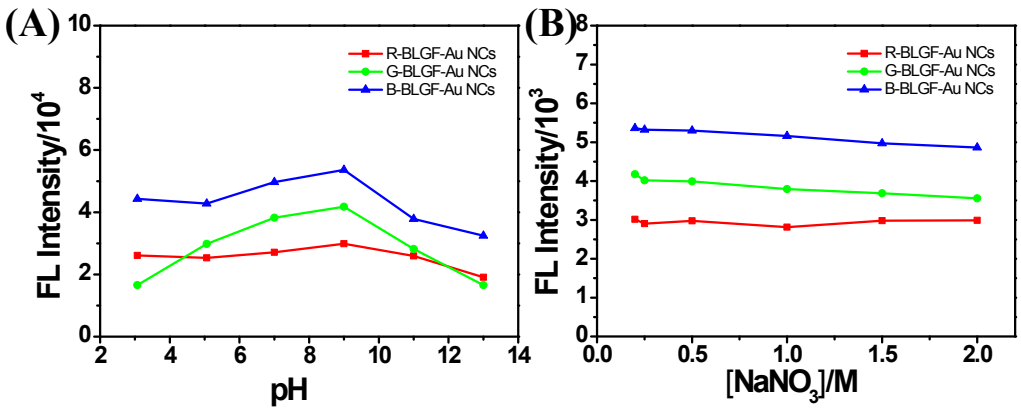

Fig. S5 (A) Fluorescence stability of R-BLGF-Au NCs at different pH. (B) Fluorescence intensity of R-BLGF-Au NCs over a salt concentration of 0.2 M to 2 M $NaNO_3$.

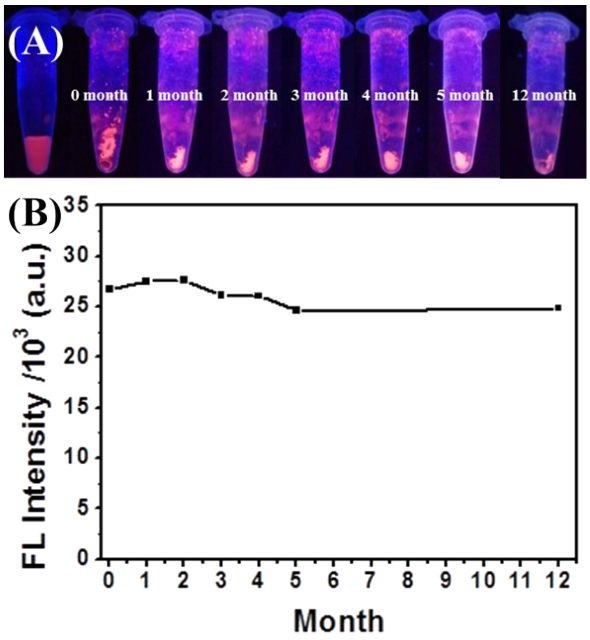

Fig. S6 Fluorescence stability of R-BLGF-Au NCs in solution and powder form within 12 month, (A) Images of R-BLGF-Au NCs powder under UV light (365 nm). (B) Emission spectra changes of aqueous solutions over 12 month.

Table S1. Comparison of different fluorescent probes for cell imaging.

Materials	Cell line	Colour	Dose	Incubation time (h)	ref
R-PEG-20	A549	Red	10 μg·mL^{-1}	3	4
N-CQDs	MCF-7	Blue	200 μg·mL^{-1}	24	5
N-CDs	786–0	Green, bule	300 μg·mL^{-1}	20	6
PEI-CuNCs	293T	Blue	20 μg·mL^{-1}	12	7
Au NCs	SH-SY5Y	Red	50 μg·mL^{-1}	24	8
BLGF-Au NCs	A549	Red, green, bule	200 μg·mL^{-1}	14	This work

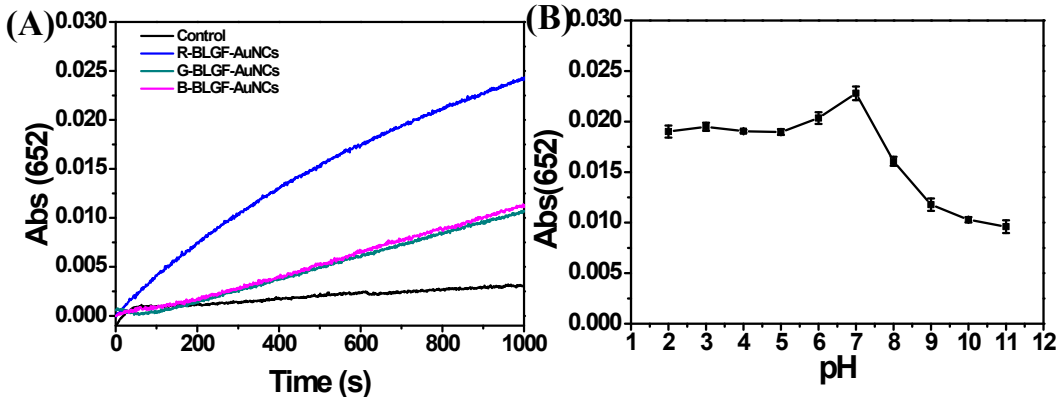

Fig. S7 (A) The time-dependent absorbance (652 nm) changes using AuNCs with red, green and blue. (B) pH effect on the peroxidase-like activity of R-BLGF-Au NCs. The error bar represents standard deviation of three replicated measurements. Reaction conditions: R-, G-, B-BLGF-AuNCs, 10 μg· mL^{-1}; H_2O_2, 30 μmol· L^{-1}, TMB, 35 mM in 2 mL buffer.

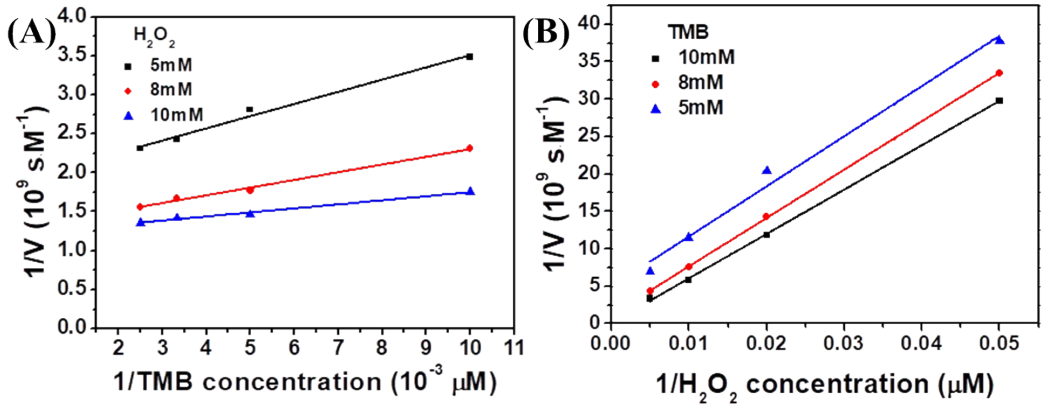

Fig. S8 Double-reciprocal plots for catalytic activity of R-BLGF-Au NCs versus different concentration TMB (A) or H_2O_2 (B).

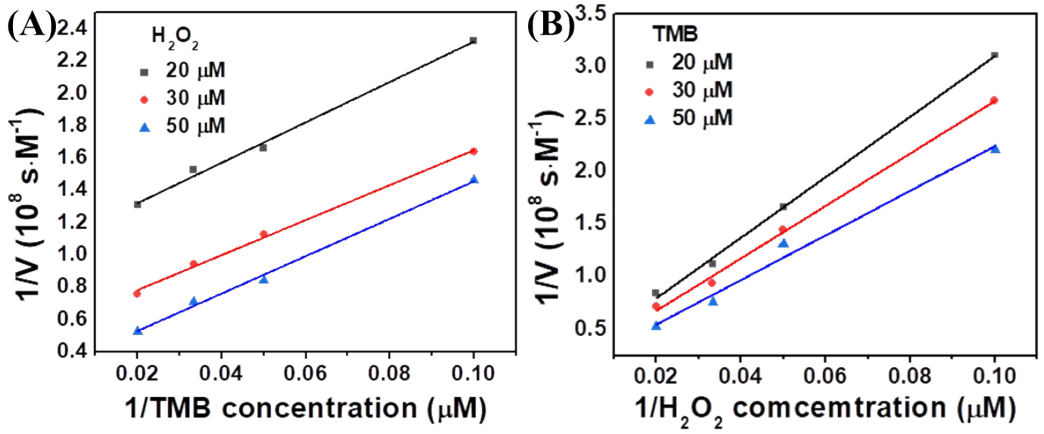

Fig. S9 Double-reciprocal plots for catalytic activity of natural peroxidase HRP versus different concentration TMB (A) or H_2O_2 (B).

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