Glutathione-bound gold nanoclusters for selective-binding and detection of glutathione S-transferase-fusion proteins from cell lysates†

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A straightforward method for the rapid detection of the presence of glutathione S-transferase (GST)-tagged proteins from sample solutions using glutathione (GSH)-bound gold nanoclusters (Au@GSH NCs) with luminescence properties as the detection probes by simple observation with the naked eye was proposed in this study.

Recombinant proteins are generally designed to be tagged by glutathione S-transferase (GST), which can then be easily purified from complex cell lysates by using glutathione (GSH)-bound affinity chromatography *via* GSH–GST interactions.[1] For quantitative analysis, sandwich enzyme-linked immunosorbent assay (ELISA) has been developed by using an antifusion protein antibody for recognition of GST fusion protein, followed by detection with a labelled second antibody.[2] Antibodies are expensive, so developing alternative approaches for reducing cost and analysis time should be desirable. Because of their high surface-to-volume ratio, nanoparticles (NPs) have been used as effective affinity probes to trap specific targets such as histidine-tagged proteins,[3] phosphorylated peptides/proteins,[4] and GST-tagged proteins.[5] The surfaces of the NPs are functionalized to have the capability to interact with target species. Lately, there have been a number of studies done on NP-based affinity chromatography to enrich target species such as histidine-tagged proteins[3] and phosphorylated proteins/peptides;[4] however, only a few reports[5] have addressed the use of NPs in the binding of GST fusion proteins from complex samples.

GSH, a tripepetide consisting of *N*-γ-glutamyl-cysteinyl-glycine, has been used as a linker to bind with gold NPs through its thiol group.[6] Gold NPs with sizes below 2 nm with luminescence are especially denoted as gold nanoclusters (NCs).[7] A feature unique to gold NCs, which distinguishes them from NPs is their photoluminescence property. Thus, fluorescent gold NCs are suitable for use in chemical sensing and biosensing.[8] In addition, gold NCs have other remarkable features such as good water solubility, excellent stability, and low-toxicity, which also make them potentially applicable to bioanalytical and biomedical research. Luminescent GSH-bound gold NCs (Au@GSH NCs) were generated by one step synthesis,[7] which was conducted by stirring GSH with $HAuCl_4$ for 72 h under room lighting. This method was quite straightforward. We reduced the reaction time by slightly raising the reaction temperature. The Au@GSH NCs used were generated by stirring GSH (2.5 mM, 1 mL) and $HAuCl_4$ (2.5 mM, 1 mL) at 32 °C for 36 h under room lighting. The generated Au@GSH NCs had good water solubility and stability, as well as good luminescence. Since the reaction was mainly based on Zheng's study,[7] the characteristics of the as-prepared Au@GSH NCs are similar to the properties reported previously. Nevertheless, the absorption spectrum (Fig. S1a), fluorescence spectrum (Fig. S1b), XPS result (Fig. S2) of the as-prepared NCs, and additional discussion are provided in the ESI.†

The generated NCs from the aqueous solution did not readily settle down when subjected to centrifugation at ~15 682 *g*. We suspected that when the Au@GSH NCs bound with GST/GST-tagged proteins *via* GSH–GST interactions, the NC-target species conjugates could be readily spun down with centrifugation at ~15 682 *g* as a result of the attachment of the proteins on the NCs. Based on these premises, we performed the experiment by simply mixing the Au@GSH NCs with cell lysates containing GST and GST-tagged proteins to demonstrate the feasibility of using the Au@GSH NCs as the detection probes for GST-tagged proteins from complex samples. To further confirm the presence of GST-tagged proteins, matrix-assisted laser desorption/ionization (MALDI) mass spectrometric analysis was employed for protein identification.

Figs. 1a–f display the photographs obtained after reacting GSH with $HAuCl_4$ for 30 min, 2 h, 4 h, 16 h, 24 h, and 36 h, respectively. The solution gradually became yellowish. Panels g–l in Fig. 1 display the same samples as shown in panels a–f in Fig. 1, but these were observed under the illumination of an ultraviolet (UV) lamp (λ_{max} = 365 nm). The intensity of the fluorescence of the solution was noted to be gradually increased, and a bright reddish orange fluorescence was observed at the end of the reaction. After removing large particles by centrifugation, the generated Au@GSH NCs were rinsed several times by deionized water before use. The quantum yield (QY) was estimated *ca.* 1.4%, which was determined by using riboflavin 5′-mono-phosphate as the standard (QY = 26%) ($\lambda_{excitation}$ = 395 nm; $\lambda_{emission}$ = 610 nm) for estimation. The size of the NCs was *ca.* 2.2 ± 0.3 nm (see Fig. 2).

Five standard proteins were used as the samples to examine the binding affinity of the Au@GSH NCs for their target species. Fig. 3a–h present the photographs obtained after incubation of the Au@GSH NCs with phosphate buffer solution (PBS), cytochrome C, myoglobin, carbonic

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† Electronic supplementary information (ESI) available: Characterization data of the Au NCs and identifications of the proteins trapped by the NCs are provided. See DOI: 10.1039/b916919a

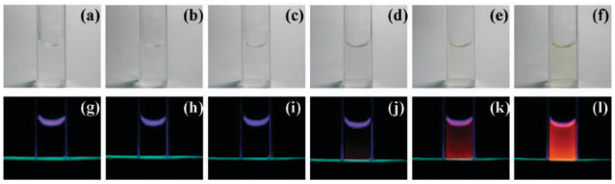

Fig. 1 Photographs obtained during one-pot-synthesis of Au@GSH NCs. Panels (a)–(f) were obtained at the reaction time of 30 min, 2 h, 4 h, 16 h, 24 h, and 36 h, respectively. Panels (g)–(l) were obtained at the reaction time of 30 min, 2 h, 4 h, 16 h, 24 h, and 36 h, respectively, under illumination of a UV lamp (365 nm).

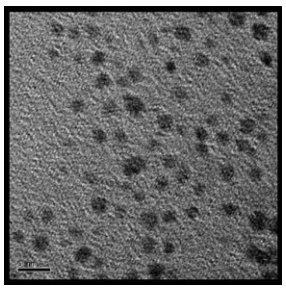

Fig. 2 TEM image of the Au@GSH NCs.

anhydrase, GST, and bovine serum album (BSA), the mixture containing four standard proteins (cytochrome C, myoglobin, carbonic anhydrase, and BSA) (10^{-5} M each), and the mixture containing the five standard proteins (cytochrome C, myoglobin, carbonic anhydrase, BSA, and GST) (10^{-5} M each) respectively, followed by centrifugation at ~15 682 *g*. Results show that conjugates at the bottom of the sample vials visible under UV light illumination were seen only in the vials containing GST (tubes e and h).

Clumping factor A (ClfA) is a surface protein of *Staphylococcus aureus* that can bind fibrinogen γ chain C-terminus with a high affinity and destroy thrombus formation.[9] The fibrinogen-binding segment of ClfA located at residues 221–550 with GST tag (GST-ClfA$_{221–550}$) has been produced by recombinant technology.[9,10] To demonstrate the current approach's applicability in real cases, we employed the cell lysates containing GST and GST-ClfA$_{221–550}$ as the samples to examine the feasibility of the trapping capacity of the Au@GSH NCs with proteins with the GST tag. The cell

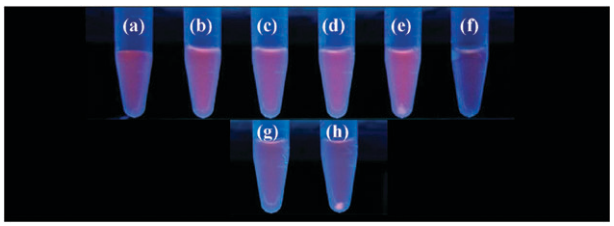

Fig. 3 Photographs obtained after incubation of the Au@GSH NCs with (a) PBS buffer only; (b) cytochrome C (10^{-5} M); (c) myoglobin (10^{-5} M); (d) carbonic anhydrase (10^{-5} M); (e) GST (10^{-5} M); (f) BSA (10^{-5} M); (g) the protein mixtures including cytochrome C, myoglobin, carbonic anhydrase, and BSA (10^{-5} M each); (h) the protein mixtures including cytochrome C, myoglobin, carbonic anhydrase, BSA, and GST (10^{-5} M each) followed by centrifugation at 15 682 *g*. All the samples were prepared in PBS buffer.

lysate resulting from *E. coli* DH5α-competent cells not containing GST/GST-ClfA$_{221–550}$ plasmid were used as the control samples for comparison. Fig. 4 presents the photographs of the samples obtained by incubating the Au@GSH NCs with the cell lysate samples for 15 min refrigerated at 4 °C for 1 h followed by centrifugation at 15 682 *g*. The Eppendorf tubes marked a and b are the samples of the cell lysates containing GST and GST-ClfA$_{221–550}$, respectively. Tube c contains the cell lysate of *E. coli*-competent cells (DH5α) as the control sample. Bright spots are observed to be aggregated on the tubes a and b, while no such aggregations are visible at the bottom of tube c. We initially suspected that the aggregated spots resulted from the formation of the Au@GSH–GST/GST-ClfA$_{221–550}$ conjugates, which were heavier than the free Au@GSH NCs. As a result, the heavy conjugates were easily spun down after centrifugation. On the other hand, in the absence of GST and GST-ClfA$_{221–550}$ proteins, Au@GSH NCs remain suspended in the solution after the same treatment. The results imply that Au@GSH NCs can selectively bind with GST and GST-ClfA$_{221–550}$ from the complex cell lysates. Furthermore, the presence of GST/GST-tagged proteins in the sample solution can be easily observed by the naked eye by using this approach, as supported by the observation of the bright conjugates on the bottom of the sample tubes after centrifugation under illumination of UV light. To further characterize the species trapped by the Au@GSH NCs, we isolated the conjugates from tubes a and b and rinsed the conjugates by phosphate buffer solution (PBS, 0.1 mL × 3) containing Triton X-100 (1%) to remove non-specific binding species. The conjugates were then re-dissolved in deionized water (10 μL) and mixed with sinapinic acid (15 mg mL^{-1}) prepared in acetonitrile/deionized water (2 : 1, v/v) containing 0.1% trifluoroacetic acid. It appears that the peaks at *m*/*z* 27 k and 63 k, which match to the molecular weights of GST and GST-ClfA$_{221–550}$, dominate the mass spectra (see Fig. S3 in ESI†). Protein identifications using proteomics strategies was also conducted (see ESI†). The detection limit of this approach by simple observation with the naked eye was also examined. Fig. 5a displays the photograph obtained by incubating our NCs with GST-ClfA$_{221–550}$ at the concentrations of 0–10^{-5} M followed by centrifugation at 15 682 *g*. It appears that the Au@GSH NC-GST conjugates are still visible at the concentration of 7.5×10^{-7} M. However, as the concentration of GST was further decreased to 5×10^{-7} M, the conjugates became less visible to the naked eye. Therefore, the detection limit of this approach by simple observation with the naked eye is *ca.* 7.5×10^{-7} M. We also subjected the supernatant of these samples to fluorescent spectroscopy. Fig. 5b displays the fluorescence spectra of the supernatant obtained after incubation of the Au@GSH NCs with the aqueous samples containing GST-ClfA$_{221–550}$ at 0–10^{-5} M. The results show that there is a slight decrease in the band at the wavelength of 610 nm of the supernatant obtained from the GST-ClfA$_{221–550}$ sample at the concentration of 2.5×10^{-7} M incubated with the Au@GSH NCs. The results indicate that the detection limit with fluorescent spectroscopy is *ca.* 2.5×10^{-7} M, which is slightly lower than that observed simply with the naked eye.

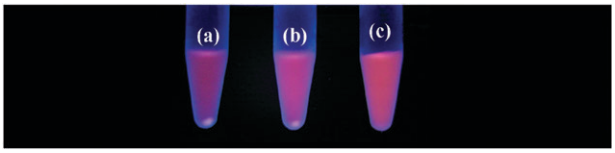

Fig. 4 Photographs obtained after incubation of the Au@GSH NCs with the cell lysates containing (a) GST and (b) GST-ClfA$_{221–550}$ followed by centrifugation at 15 682 *g*. Panel (c) was obtained by incubating the same NCs with the cell lysate of *E. coli* DH5α in the absence of GST-related plasmids.

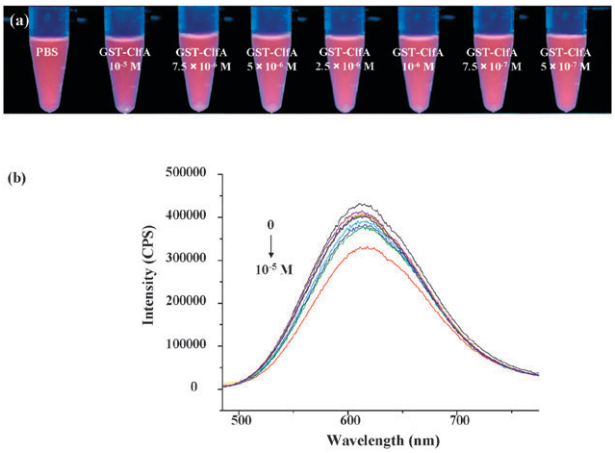

Fig. 5 (a) Photographs obtained after incubation of the Au@GSH NCs with the aqueous samples containing GST-ClfA$_{221–550}$ at 0 M, 10^{-5} M, 7.5×10^{-6} M, 5×10^{-6} M, 2.5×10^{-6} M, 10^{-6} M, 7.5×10^{-7} M and 5×10^{-7} M (from left to right) followed by centrifugation at 15 682 *g*, under illumination of a UV lamp (λ_{max} = 365 nm). (b) Fluorescence spectra of the supernatants obtained after incubation of the Au@GSH NCs with the aqueous samples containing GST-ClfA$_{221–550}$ at 0 M, 2.5×10^{-7} M, 5×10^{-7} M , 7.5×10^{-7} M, 10^{-6} M, 2.5×10^{-6} M, 5×10^{-6} M, 7.5×10^{-6} M, and 10^{-5} M ($\lambda_{excitation}$ = 395 nm) (from top to bottom).

In conclusion, a straightforward method for targeting GST-fusion proteins by using Au@GSH NCs as detection probes was demonstrated in this study. Under UV light illumination, fluorescent Au@GSH NC-GST-tagged protein conjugates were visible at the bottom of the sample vial after centrifugation. To the best of our knowledge, this is the first report on using fluorescent NCs as the visible probes for direct examination of the presence of GST/GST-tagged proteins in complex cell lysate samples. This approach is uncomplicated and only requires a short period of time for analysis and examination. Furthermore, expensive antibodies are not required in this approach. The main advantages of using this approach for the detection of GST/GST fusion proteins include short analysis time and low cost. On the basis of the results demonstrated in this study, we believe this approach could be very suitable for use in the rapid examination and characterization of the presence of GST-fusion proteins from complex cell lysates.

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Notes and references

1 (*a*) C. H. Hsieh, L. F. Liu, S. P. Tsai and M. F. Tam, *Biochem. J.*, 1999, **343**, 87; (*b*) C. Di Ilio, A. Aceto, R. Piccolomini, N. Allocati, A. Faraone, T. Bucciarelli, D. Barra and G. Federici, *Biochim. Biophys. Acta*, 1991, **1077**, 141; (*c*) J. M. Harris, D. J. Meyer, B. Coles and B. Ketterer, *Biochem. J.*, 1991, **278**, 137; (*d*) C. Z. Liu, H. J. Jeng and L. Y. Chang, *Protein Expression Purif.*, 2008, **61**, 31.
2 (*a*) D. U. Rabin, R. Palmer-Crocker, D. V. Mierz and K. K. Yeung, *J. Immunol. Methods*, 1992, **156**, 101; (*b*) T. P. J. Mulder, W. H. M. Petrers, D. A. Cxourt and J. B. M. J. Jansen, *Clin. Chem.*, 1996, **42**, 416.
3 (*a*) K. S. Lee and I. S. Lee, *Chem. Commun.*, 2008, 709; (*b*) Y. C. Li, Y. S. Lin, P. J. Tsai, C. T. Chen, W. Y. Chen and Y. C. Chen, *Anal. Chem.*, 2007, **79**, 7519; (*c*) H. W. Gu, K. M. Xu, C. J. Xu and B. Xu, *Chem. Commun.*, 2006, 941; (*d*) I. S. Lee, N. Lee, J. Park, B. H. Kim, Y. W. Yi, T. Kim, T. K. Kim, I. H. Lee, S. R. Paik and T. Hyeon, *J. Am. Chem. Soc.*, 2006, **128**, 10658.
4 (*a*) M. Rainer, H. Sonderegger, R. Bakry, C. W. Huck, S. Morandell, L. A. Huber, D. T. Gjerde and G. K. Bonn, *Proteomics*, 2008, **8**, 4593; (*b*) C. T. Chen and Y. C. Chen, *J. Biomed. Nanotechnol.*, 2008, **4**, 73; (*c*) C. Y. Lo, W. Y. Chen, C. T. Chen and Y. C. Chen, *J. Proteome Res.*, 2007, **6**, 887; (*d*) C. T. Chen, W. Y. Chen, P. J. Tsai, K. Y. Chien, J. S. Yu and Y. C. Chen, *J. Proteome Res.*, 2007, **6**, 316; (*e*) C. T. Chen and Y. C. Chen, *Anal. Chem.*, 2005, **77**, 5912.
5 (*a*) M. Zheng and X. Y. Huang, *J. Am. Chem. Soc.*, 2004, **126**, 12047; (*b*) T. H. Ha, S. O. Jung, J. M. Lee, K. Y. Lee, Y. Lee, J. S. Park and B. H. Chung, *Anal. Chem.*, 2007, **79**, 546; (*c*) Y. B. Shin, J. M. Lee, M. R. Park, M. G. Kim, B. H. Chung, H. B. Pyo and S. Maeng, *Biosens. Bioelectron.*, 2007, **22**, 2301.
6 (*a*) J. P. Wilcoxon, J. E. Martin, F. Parsapour, B. Wiedenman and D. F. Kelley, *J. Chem. Phys.*, 1998, **108**, 9137; (*b*) T. G. Schaaff, G. Knight, M. N. Shafigullin, R. F. Borkman and R. L. Whetten, *J. Phys. Chem. B*, 1998, **102**, 10643; (*c*) M. M. Alvarez, J. T. Khoury, T. G. Schaaff, M. N. Shafigullin, I. Vezmar and R. L. Whetten, *J. Phys. Chem. B*, 1997, **101**, 3706; (*d*) T. G. Schaaff, M. N. Shafigullin, J. T. Khoury, I. Vezmar, R. L. Whetten, W. G. Cullen and P. N. First, *J. Phys. Chem. B*, 1997, **101**, 7885; (*e*) M. J. Hostetler, J. E. Wingate, C. J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans and R. W. Murray, *Langmuir*, 1998, **14**, 17; (*f*) T. G. Schaaff and R. L. Whetten, *J. Phys. Chem. B*, 2000, **104**, 2630.
7 J. Zheng, *PhD Dissertation*, Georgia Institute of Technology, 2005.
8 (*a*) C. C. Huang, C. T. Chen, Y. C. Shiang, Z. H. Lin and H. T. Chang, *Anal. Chem.*, 2009, **81**, 875; (*b*) C. C. Huang, C. K. Chiang, Z. H. Lin, K. H. Lee and H. T. Chang, *Anal. Chem.*, 2008, **80**, 1497.
9 (*a*) C. Z. Liu, M. H. Shih and P. J. Tsai, *Thromb. Haemost.*, 2005, **94**, 286; (*b*) C. Z. Liu, M. H. Shih, P. J. Tsai, P. J. Tsai, L. Y. Chang and M. C. Chang, *Thromb. Res.*, 2007, **121**, 183.
10 (*a*) D. H. Smith and K. S. Johnson, *Gene*, 1988, **67**, 31; (*b*) J. S. Hawiger, S. Timmons, D. D. Strong, B. A. Cottrell, M. Riley and R. F. Doolittle, *Biochemistry*, 1982, **21**, 1407.