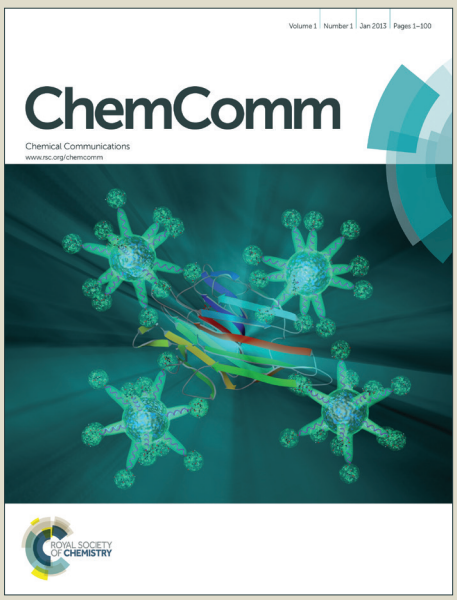
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm

RSCPublishing

COMMUNICATION

Fluorescence Turn-On Detection of Glucose via the Ag Nanoparticle Mediated Release of a Perylene Probe

Cite this: DOI: 10.1039/x0xx00000x

Juanmin Li,[ab] Yongxin Li,[a] Sohail Anjum Shahzad,[ac] Jian Chen,[a] Yang Chen,[ab] Yan Wang,[ab] Meiding Yang,[ab] and Cong Yu*[ab]

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

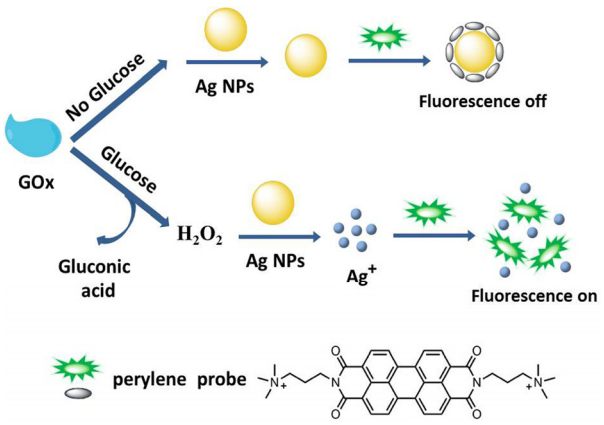
A novel fluorescence turn-on strategy for glucose sensing is demonstrated. The fluorescence of a perylene probe could be quenched by the silver nanoparticles (Ag NPs). The Ag NPs could be etched by H_2O_2 generated from the enzymatic oxidation of glucose. And efficient probe fluorescence recovery was detected.

Diabetes mellitus is a disease of glucose metabolic disorder which results from insulin deficiency or resistance. It has become one of the most significant health concerns worldwide. The fluctuation of blood glucose level beyond normal range could result in serious complications such as high risks of heart disease, kidney failure, nerve damage and blindness.[1] Medical treatment of diabetes requires frequent monitoring of daily blood glucose level. Hence it is of great importance to develop a glucose sensor with excellent selectivity and sensitivity.

Various glucose sensing approaches have been proposed such as the chemiluminescence,[2] surface-enhanced Raman scattering,[3] mass spectrometry,[4] colorimetric,[5] electrochemical[6] and fluorometric[7] methods. Among these, fluorescence sensors have attracted special interests because of their simplicity, rapid response and high sensitivity. In addition, fluorescence sensors usually do not require expensive or sophisticated instrumentation, complicated operation and detection procedures. Many glucose fluorescence sensors have been fabricated. However, in many cases probe molecules covalently linked with boronic acid functionalities are employed, which considerably increase the complexity of probe synthesis, and usually with less selectivity. In addition, some methods employ turn-off detection mode or have less satisfactory detection limit. Therefore, it is of great value to explore new fluorescence turn-on strategy for the determination of glucose level in complicated biological sample mixtures (for example, in human serum samples).

Noble metal nanoparticles (e.g., Au and Ag NPs) possess many unique optical properties, such as characteristic surface plasmon resonance absorption, high extinction coefficient and the size-dependent quantum confinement effects. Noble metal nanoparticles have been explored to construct novel sensors. For example, some glucose sensors have been developed based on tuning surface plasmon resonance absorption by etching.[8] It has been demonstrated that Ag NPs can be used as excellent quencher for various fluorescence probes, such as organic dyes[9] and QDs.[10] A number of strongly fluorescent perylene probes with high fluorescence quantum yield and good photostability in an aqueous buffer solution have recently been synthesized by our research group. The perylene probes have been used for the development of a number of novel sensing techniques.[11]

Herein we present a new protocol for glucose sensing utilizing a perylene probe for the first time. As depicted in Scheme 1, the perylene probe could be closely attached to the surface of the Ag NPs due to strong electrostatic attractive and hydrophobic interactions.[11] As a result, the fluorescence of the perylene probe is effectively quenched. However, enzymatic oxidation of glucose by glucose oxidase (GOx) takes place in the presence of dissolved oxygen after the addition of glucose. The in situ generated H_2O_2 leads to etching of the Ag NPs. The perylene probe could not be quenched and a turn-on fluorescence signal is detected, which could



Scheme 1 Strategy for fluorescence turn-on glucose selective sensing.

be used for the quantification of glucose. This newly developed fluorescence turn-on sensor is simple, highly sensitive and selective toward glucose. Accurate determination of glucose level in human serum samples is also demonstrated.

The perylene probe emits strong fluorescence at 545 nm upon excitation at 495 nm as shown in Fig. 1-A. When mixed with the Ag NPs, it was observed that the fluorescence of the probe was efficiently quenched. TEM images clearly show that the Ag NPs were well dispersed in the absence of the perylene probe. After the addition of probe, Ag NPs formed aggregates (Fig. S1, ESI†). The changes in surface plasmon absorption of the Ag NPs also suggest strong aggregation of the NPs (Fig. S2). Zeta potential analysis shows that the Ag NPs were negatively charged (-7.9 mV) (Fig. S3). The positively charged perylene probe could be adsorbed onto the surface of the Ag NPs as a result of the strong attractive electrostatic and hydrophobic interactions. Consequently, the negative charge on the surface of the Ag NPs was neutralized, which resulted in strong NPs aggregation. And the perylene probe monomer fluorescence was efficiently quenched. The fluorescence quenching of the perylene probe at different concentrations was investigated with the amount of the Ag NPs kept constant (Fig. S4-A). Only slight increase in fluorescence intensity was observed when the probe concentration remained no more than 160 nM. Quenching efficiency reached about 90.9% at 160 nM probe concentration (Fig. S4-B). Further increase in probe concentration caused steeper increase in emission intensity (and decrease in quenching efficiency). Therefore, 160 nM probe concentration was selected in the following experiments.

The fluorescence intensity of the perylene probe could recover almost 100% in the presence of glucose and GOx (Fig. 1-B). A similar recovery in probe fluorescence intensity was also observed in the presence of H_2O_2 (Fig. S5). On the contrary, various concentrations of GOx in the absence of glucose did not induce noticeable fluorescence recovery and the intensity of fluorescence remained unaltered (Fig. S6). These results clearly suggest that GOx itself is not involved in probe fluorescence recovery. The in situ generated H_2O_2, which was originated from the enzymatic oxidation of glucose, is responsible for the fluorescence recovery.

TEM images also provide convincing evidence to support the assay rationale. Fig. 2 shows that the synthesized Ag NPs are mostly

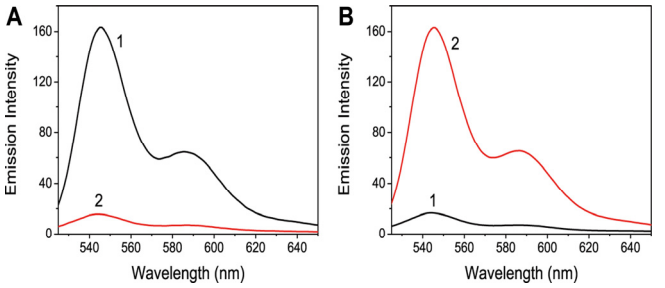


Fig. 1 (A) Fluorescence spectra of the perylene probe (1), and probe mixed with the Ag NPs (2). (B) Fluorescence spectra of the perylene probe mixed with Ag NPs + GOx (1), and with Ag NPs + glucose + GOx (2). Final concentrations: citrate buffer 5 mM (pH 5.5), Ag (0) 50.5 μM, perylene probe 160 nM, glucose 500 μM, GOx 0.5 U/mL, respectively.

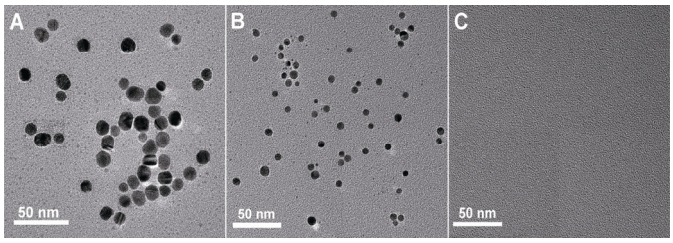


Fig. 2 TEM images of the Ag NPs (A), and the NPs mixed with glucose of different concentrations [100 μM (B), 500 μM (C)]. Final concentrations: citrate buffer 5 mM (pH 5.5), Ag (0) 50.5 μM, GOx 0.5 U/mL, respectively.

spherical with a narrow size variation and exhibit an average diameter of around 10.1 nm. After treatment with GOx and glucose, the Ag NPs were etched to smaller NPs with an average size of about 6.5 nm. With further increase of the glucose concentration, no Ag NPs could be observed. The results clearly indicate that the Ag NPs were etched and transformed to Ag^+. Fig. S7 shows that the Ag NPs exhibit a characteristic surface plasmon absorption band at around 390 nm.[12] In the presence of 100 μM glucose and 0.5 U/mL GOx, the surface plasmon absorption band decreased significantly. The 390 nm band disappeared completely upon further increase of the glucose concentration to 500 μM. The changes in surface plasmon absorption spectrum of the Ag NPs are in consistent with the TEM results.

Some crucial assay conditions were optimized to get the best sensing performance. Fig. S8 shows that in the presence of 500 μM glucose, the emission intensity of the perylene probe at 545 nm reached maximum when 0.5 U/mL GOx was employed. Insufficient amount of GOx would obviously generate not enough H_2O_2. We also studied the kinetics of the reaction as shown in Fig. S9. In the presence of 500 μM glucose and 0.5 U/mL GOx, the emission intensity of the perylene probe increased gradually with prolonged reaction time and reached its maximum at 90 min. Further increase of the reaction time caused no further increase in probe emission intensity. Therefore, 0.5 U/mL was thus selected as the optimal GOx concentration, and 90 min was selected as the optimal reaction time.

The glucose detection was performed under the optimal conditions. The fluorescence emission intensity increased with increasing concentrations of glucose (0 – 500 μM). And a linear response (R^2 = 0.997) was obtained over a wide glucose concentration range of 0 – 200 μM (Fig. 3). Fig. S10 shows the linear curve at a lower glucose concentration range of 0 – 50 μM. The linear regression equation is F = 0.61C + 17.70, in which 'F' is the emission intensity of the perylene probe at 545 nm and 'C' is the glucose concentration in μM. Our assay is highly sensitive and 2.5 μM glucose could be easily detected (Figure S10).[3,4,7]

The selectivity of the assay toward glucose over some potential interfering substances was tested, including fructose, maltose, lactose and sucrose. Remarkably, only glucose induced a significant increase in fluorescence emission intensity of the perylene probe while other carbohydrates could not induce noticeable increase in emission intensity (Fig. 4). The assay thus shows excellent selectivity for glucose.

To validate the practical application of the assay in biological

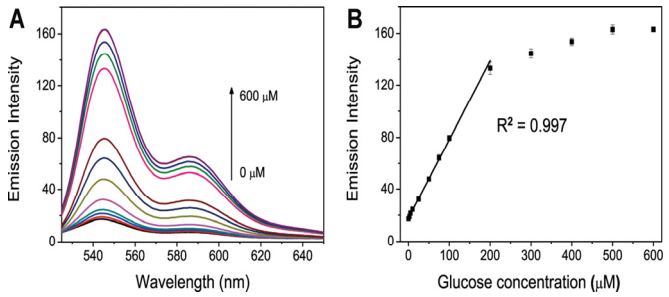


Fig. 3 (A) Changes in fluorescence spectrum of the perylene probe with glucose concentration. (B) Plot of the changes in emission intensity of the perylene probe at 545 nm against glucose concentration. Final concentrations: citrate buffer 5 mM (pH 5.5), Ag (0) 50.5 μM, perylene probe 160 nM, GOx 0.5 U/mL, glucose 0, 2.5, 5, 10, 25, 50, 75, 100, 200, 300, 400, 500 and 600 μM, respectively.

samples, the concentrations of glucose in human serum samples (S1 and S2) were determined. The glucose concentration was obtained by employing a standard addition method.[13] Samples were diluted and spiked with the standard glucose solutions of different concentrations. The concentrations of glucose spiked were plotted against fluorescence response as shown in Fig. S11. The intercept of the linear curve on the X axis gave the serum sample glucose concentration. The determined glucose concentrations were 13.38 mM and 6.18 mM for samples S1 and S2, respectively. The glucose concentrations determined by a commercial glucose meter were 13 mM and 5.9 mM, respectively. These results clearly demonstrate that our method could be used for accurate glucose quantification in human serum samples.

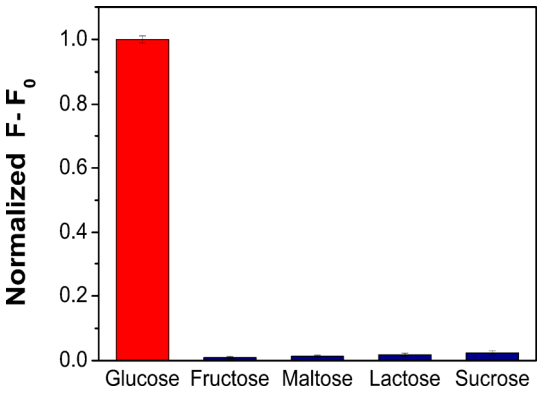


Fig. 4 Selectivity of the assay. Final concentrations: citrate buffer 5 mM (pH 5.5), Ag (0) 50.5 μM, perylene probe 160 nM, GOx 0.5 U/mL, respectively. Glucose and the other carbohydrates: 500 μM each.

Conclusions

In conclusion, a highly sensitive and selective fluorescence turn-on approach for glucose detection has been developed. It was observed that the fluorescence of a perylene probe could be efficiently quenched by the Ag NPs. The Ag NPs could be etched by H_2O_2 generated from the enzymatic oxidation of glucose. As a consequence, the perylene probe could not be quenched and a fluorescence turn on signal was detected. The recovered fluorescence emission of the perylene probe could be directly related to the glucose concentration. And accurate quantification of glucose in serum samples demonstrates the practicality of our assay.

This work was supported by the National Basic Research Program of China (973 Program, 2011CB911002), the National Natural Science Foundation of China (21275139 and 21405151), and the Pillar Program of Changchun Municipal Bureau of Science and Technology (14KG062). S. A. Shahzad acknowledges the receipt of a Chinese Academy of Sciences fellowship for postdoctoral and visiting scholars from developing countries (2014FFGB0009).

Notes and references

[a] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China*
E-mail: congyu@ciac.ac.cn; Fax: +86 431-8526-2710
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China*
[c] *Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan*
† Electronic supplementary information (ESI) available: Experimental details, supporting figures and table. See DOI: 10.1039/c000000x/

1 (a) J. Wang, *Chem. Rev.*, 2008, **108**, 814-825; (b) S. P. Nichols, A. Koh, W. L. Storm, J. H. Shin and M. H. Schoenfisch, *Chem. Rev.*, 2013, **113**, 2528-2549.
2 (a) D. Lan, B. X. Li and Z. J. Zhang, *Biosens. Bioelectron.*, 2008, **24**, 934-938; (b) C. S. Zhou, Y. L. Shi, X. D. Ding, M. Li, J. J. Luo, Z. Y. Lu and D. Xiao, *Anal. Chem.*, 2013, **85**, 1171-1176; (c) P. Yang, S. Y. Jin, Q. Z. Xu and S. H. Yu, *Small*, 2013, **9**, 199-204; (d) C. K. Lim, Y. D. Lee, J. Na, J. M. Oh, S. Her, K. Kim, K. Choi, S. Kim and I. C. Kwon, *Adv. Funct. Mater.*, 2010, **20**, 2644-2648.
3 (a) K. E. Shafer-Peltier, C. L. Haynes, M. R. Glucksberg and R. P. Van Duyne, *J. Am. Chem. Soc.*, 2003, **125**, 588-593; (b) K. V. Kong, Z. Y. Lam, W. K. O. Lau, W. K. Leong and M. Oivo, *J. Am. Chem. Soc.*, 2013, **135**, 18028-18031; (c) K. V. Kong, C. J. H. Ho, T. X. Gong, W. K. Lau and M. Olivo, *Biosens. Bioelectron.*, 2014, **56**, 186-191.
4 (a) R. Chen, W. J. Xu, C. Q. Xiong, X. Y. Zhou, S. X. Xiong, Z. X. Nie, L. Q. Mao, Y. Chen and H. C. Chang, *Anal. Chem.*, 2012, **84**, 465-469; (b) S. M. Chen, H. Z. Zheng, J. N. Wang, J. Hou, Q. He, H. H. Liu, C. Q. Xiong, X. L. Kong and Z. X. Nie, *Anal. Chem.*, 2013, **85**, 6646-6652.
5 (a) F. Wang, X. Q. Liu, C. H. Lu and I. Willner, *ACS Nano*, 2013, **7**, 7278-7286; (b) B. Malvi, C. Panda, B. B. Dhar and S. Sen Gupta, *Chem. Commun.*, 2012, **48**, 5289-5291; (c) P. Roy, Z. H. Lin, C. T. Liang and H. T. Chang, *Chem. Commun.*, 2012, **48**, 4079-4081; (d) S. Chen, X. Hai, X. W. Chen and J. H. Wang, *Anal. Chem.*, 2014, **86**, 6689-6694.
6 (a) Y. C. Fu, P. H. Li, Q. J. Xie, X. H. Xu, L. H. Lei, C. Chen, C. Zou, W. F. Deng and S. Z. Yao, *Adv. Funct. Mater.*, 2009, **19**, 1784-1791; (b) L. N. Cella, W. Chen, N. V. Myung and A. Mulchandani, *J. Am. Chem. Soc.*, 2010, **132**, 5024-5026; (c) C. C. Mayorga-Martinez, M. Guix, R. E. Madrid and A. Merkoci, *Chem. Commun.*, 2012, **48**, 1686-1688; (d) A. Koh, Y. Lu and M. H. Schoenfisch, *Anal. Chem.*, 2013, **85**, 10488-10494. (e) K. H. Cha, G. C. Jensen, A. S. Balijepalli, B. E. Cohan and M. E. Meyerhoff, *Anal. Chem.*, 2014, **86**, 1902-1908.
7 (a) X. R. Wang, J. M. Hu, G. Y. Zhang and S. Y. Liu, *J. Am. Chem. Soc.*, 2014, **136**, 9890-9893; (b) Y. Y. Li and S. Q. Zhou, *Chem. Commun.*, 2013, **49**, 5553-5555; (c) Y. J. Huang, W. J. Ouyang, X. Wu, Z. Li, J. S. Fossey, T. D. James and Y. B. Jiang, *J. Am. Chem. Soc.*, 2013, **135**, 1700-1703; (d) F. He, Y. L. Tang, M. H. Yu, S. Wang, Y. L. Li and D. B. Zhu, *Adv. Funct. Mater.*, 2006, **16**, 91-94; (e) X. D. Hong, Y. Peng, J. L. Bai, B. A. Ning, Y. Y. Liu, Z. J. Zhou and Z. X. Gao, *Small*, 2014, **10**, 1308-1313; (f) Y. N. Zhao, B. G. Trewyn, I. I. Slowing and V. S. Y. Lin, *J. Am. Chem. Soc.*, 2009, **131**, 8398-8400; (g) Y. C. Shiang, C. C. Huang and H. T. Chang, *Chem. Commun.*, 2009, 3437-3439; (h) Y. H. Yi, J. H.

Deng, Y. Y. Zhang, H. T. Li and S. Z. Yao, *Chem. Commun.*, 2013, **49**, 612-614.
8 (a) H. L. He, X. L. Xu, H. X. Wu and Y. D. Jin, *Adv. Mater.*, 2012, **24**, 1736-17401; (b) Y. S. Xia, J. J. Ye, K. H. Tan, J. J. Wang and G. Yang, *Anal. Chem.*, 2013, **85**, 6241-6247; (c) L. Saa, M. Coronado-Puchau, V. Pavlov and L. M. Liz-Marzan, *Nanoscale*, 2014, **6**, 7405-7409; (d) X. Liu, S. Y. Zhang, P. L. Tan, J. Zhou, Y. Huang, Z. Nie and S. Z. Yao, *Chem. Commun.*, 2013, **49**, 1856-1858.
9 (a) L. Deng, X. Y. Ouyang, J. Y. Jin, C. Ma, Y. Jiang, J. Zheng, J. S. Li, Y. H. Li, W. H. Tan and R. H. Yang, *Anal. Chem.*, 2013, **85**, 8594-8600; (b) Z. P. Zhou, H. D. Huang, Y. Chen, F. Liu, C. Z. Huang and N. Li, *Biosens. Bioelectron.*, 2014, **52**, 367-373.
10 X. Y. Cao, F. Shen, M. W. Zhang and C. Y. Sun, *Sens. Actuators B.*, 2014, **202**, 1175-1182.
11 (a) B. Wang and C. Yu, *Angew. Chem. Int. Ed*, 2010, **49**, 1485-1488; (b) B. Wang, H. P. Jiao, W. Y. Li, D. L. Liao, F. Y. Wang and C. Yu, *Chem. Commun.*, 2011, **47**, 10269-10271; (c) W. Y. Li, J. Chen, H. P. Jiao, Q. F. Zhang, H. P. Zhou and C. Yu, *Chem. Commun.*, 2012, **48**, 10123-10125; (d) D. L. Liao, J. Chen, H. P. Zhou, Y. Wang, Y. X. Li and C. Yu, *Anal. Chem.*, 2013, **85**, 2667-2672. (e) B. Wang, Q. K. Zhu, D. L. Liao and C. Yu, *J. Mater. Chem.*, 2011, **21**, 4821-4826.
12 Y. Wan, Z. R. Guo, X. L. Jiang, K. Fang, X. Lu, Y. Zhang and N. Gu, *J. Colloid. Interf. Sci.*, 2013, **394**, 263-268.
13 L. Saa and V. Pavlov, *Small*, 2012, **8**, 3449-3455.

