Analyst

COMMUNICATION

View Article Online
View Journal | View Issue

Published on 05 September 2014. Downloaded by Queens University - Kingston on 20/10/2014 10:25:57.

Cite this: *Analyst*, 2014, **139**, 5669

Received 5th August 2014
Accepted 5th September 2014

DOI: 10.1039/c4an01438c

www.rsc.org/analyst

Quantification of DNA through a fluorescence biosensor based on click chemistry

Guiyin Yue,[a] Huazhen Ye,[c] Xijing Huang,[a] Wenmei Ye,[a] Suyan Qiu,[b] Bin Qiu,*[a] Zhenyu Lin[a] and Guonan Chen[a]

A simple, sensitive and selective fluorescence biosensor for determination of DNA using CuS particles based on click chemistry is reported. Biotin-modified capture DNA was modified on Streptavidin MagneSphere Paramagnetic Particles (PMPs) and hybridized with target DNA (hepatitis B virus DNA had been chosen as an example), then bound target DNA was hybridized with DNA-CuS particles and formed a sandwich like structure. CuS particles on the sandwich structures can be destroyed by acid to form Cu(II), and Cu(II) can be reduced to Cu(I) by sodium ascorbate, which in turn catalyzes the reaction between a weak-fluorescent 3-azido-7-hydroxycoumarin and propargyl alcohol to form a fluorescent 1,2,3-triazole compound. Using this method, target DNA concentration can be determined by a change in the fluorescence intensity of the system. It is found that the fluorescence increase factor has a direct linear relationship to the logarithm of target DNA concentrations in the range of 0.1 to 100 nM, and the detection limit is 0.04 nM ($S/N = 3$). The proposed sensor not only allows high sensitivity and good reproducibility, but also has a good selectivity to single-nucleotide mismatches.

Introduction

The development of detection methods for various analytes related to health and environment is important in analytical chemistry. DNA detection has attracted increasing attention because of its important roles in pathogen analysis, genetic disorder diagnosis and forensic tests.[1,2] Traditional methods for DNA detection are usually based on DNA microarrays and polymerase chain reaction (PCR).[3] These methods are characterized by high sensitivity and efficiency, but sophisticated instruments and well trained operators are required. Some simple methods, such as fluorescence,[4] nanomaterial-based amplification,[5] and colorimetric,[6] electrochemistry,[7] have been successfully developed and applied for DNA detection. These methods are characterized by high sensitivity, simple equipment and easy operation.

Click chemistry possesses many significant advantages, such as excellent selectivity, high purity, high efficiency and mild reaction conditions.[8] Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) is one of the most mature click reactions, in which azide can rapidly react with terminal alkyne to form 1,2,3-triazole species under mild conditions in the presence of Cu(I) catalyst, which has been applied in diverse areas, such as surface modification, dendrimer design and drug discovery.[9] An early report showed that nonfluorescent 3-azidocoumarins can react with terminal alkynes to yield strong-fluorescent 1,2,3-triazole products through CuAAC reaction.[10] Many sensitive biosensors have been developed based on this reaction for different targets, such as histidine,[11] copper in serum sample and pesticide residues.[12,13] However, to the best of our knowledge, no report on DNA detection based on this reaction had been reported to date.

In this study, by introducing DNA-modified CuS particle to a DNA sandwich assay, we have proposed a highly sensitive and selective fluorescence sensor for DNA (hepatitis B virus (HBV) DNA fragment was selected as an example) detection. This sensor combines the advantages of high selectivity of the CuAAC reaction and high sensitivity of the fluorescence detection method. Specificity and reproducibility of the proposed sensor was also checked.

Experimental

Chemicals

Sodium ascorbate, propargyl alcohol, imidazole and other reagents were obtained from Alfa Aesar China Co. Ltd. (Tianjin). Synthesis of 3-azido-7-hydroxycoumarin has been described

[a]*MOE Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350108, China. E-mail: summer328cn@163.com; Fax: +86-591-22866135; Tel: +86-591-22866135*

[b]*Institute for Quality & Safety and Standards of Agricultural Products Research, Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi, 330200, China*

[c]*Fujian Health College, Fuzhou, Fujian, 350025, China*

Published on 05 September 2014. Downloaded by Queens University - Kingston on 20/10/2014 10:25:57.

elsewhere.[14] Streptavidin MagneSphere Paramagnetic Particles (PMPs) was purchased from Promega Corporation (Madison, Wisconsin, USA). The other chemicals were purchased from Shanghai Chemical Reagent Company (Shanghai, China) and used directly without further purification. 1-Ethyl-3-(3-dimethylammoniapropyl)-carbodiimide (EDC) and bovine serum albumin (BSA) were purchased from Shanghai Sangon Biotech Co. Ltd. (Shanghai, China), and the following oligonucleotides DNA (left to right: 5′ to 3′) were synthesized by Shanghai Sangon Biotech Co. Ltd. (Shanghai, China): 5′-biotin-modified capture DNA for HBV (capt-DNA); biotin-AAAAAAAAAAAAACCTT TAACCTAA 3′-amino group-modified DNA for CuS particle conjugation (NH_2–DNA); TCCTCCCCCAACTCCTCC CAAAAAAAAAAAAA-NH_2 HBV DNA sequence (target DNA); TGGGAGGAGTTGGGGGAGGAGATTAGGTTAAAGGT mismatch sequence of adenine in target DNA (A-mis DNA); TGGGAG GAGTGGGGGGAGGAGATTAGGTAAAAGGT mismatch sequence of guanine in target DNA (G-mis DNA); TGGGAG GAGTGGGGGGAGGAGATTAGGTGAAAGGT mismatch sequence of cytosine in target DNA (C-mis DNA); TGGGAGGAGTGGGGGGAGGAGATTAGGTCAAAGGT.

The target DNA was designed to be considerably longer than the capt-DNA used for detection. In order to maximize its potential to recognize the nucleotide's base at the mismatch site, the locations of the single mismatch site were designed around the middle of the binding arms with capt-DNA. NH_2–DNA was designed to hybridize with the rest of the target DNA, which hybridized with capt-DNA. In addition amino group-modified DNA reacted with carboxyl group-modified CuS particles under catalysis of EDC to form CuS particle-modified DNA.

Buffer solution used in this study: 0.25 M NaCl, 0.15 M sodium phosphate buffer solution, pH 7.3, 0.05% Tween-20.

Synthesis of CuS particles

CuS particles with carboxyl groups were prepared according to the published method.[15] Briefly, 3.0 μL of mercaptoacetic acid as stabilizer was added to 25 mL of 0.4 M $Cu(NO_3)_2$ solution, and the pH of the mixture was adjusted to 9.0 with 0.5 M NaOH solution. After the mixture was bubbled with nitrogen for 30 min, 25 mL of 1.34×10^{-3} M Na_2S solution was added to the solution dropwise. The reaction was carried out for 24 h under nitrogen protection and a brown colloid gradually appeared. After reaction, CuS particles with carboxyl groups were formed.

Modification of NH_2–DNA on CuS particles

200 μL of 0.1 M imidazole solution (pH 6.8) was added into NH_2–DNA (1.24 μM). After gentle shaking for 30 min, 100 μL of 0.1 M EDC solution (cross-linking agent) and 2.0 mL of CuS colloid were added to the mixture and reacted at room temperature for 24 h. Under these conditions, condensation between amino groups and carboxy groups was performed to form NH2–DNA tagged with CuS particles, which was then separated from other reagents by centrifugation at a rotation speed of 10 000 rpm for 30 min. The precipitate was washed 3 times with water and then re-suspended in water. The solution of CuS particles modified with DNA (DNA-CuS particles) was stored at 4 °C for later hybridizations.

Procedures for DNA quantification

A portion of 0.6 mL of 1 mg mL^{-1} PMPs was washed by buffer solution once and then dispersed in 0.6 mL of buffer solution. Capt-DNA was added into the solution to achieve a final concentration of 0.5 μM, and the mixture was mixed on a shaker for 30 min at room temperature. The specific combination of streptavidin with biotin contributed to the combination of PMPs with capt-DNA.[16] After that, PMPs with capt-DNA were separated from the mixture by a magnet because PMPs with paramagnetism could be attracted by a magnet. Then, they were further washed using buffer solution once and dispersed in 0.6 mL of buffer solution. Consequently, PMPs with capt-DNA (PMPs-DNA) were formed.

PMPs-DNA solution (0.5 μM) and various concentrations of target DNA were prepared in buffer solution. In order to ensure the formation of DNA double strand, the mixtures were heated to 37 °C for 2 h. After that, PMP residue was washed with a buffer solution containing 2.0 mg mL^{-1} BSA to remove unbound target DNA and block nonspecific binding sites, and then dispersed in 0.2 mL of buffer solution. DNA-CuS particles (0.465 μM) in buffer solution were added to the above DNA double strand solution, and this mixture was also heated to 37 °C for 2 h. Therefore, DNA-CuS particles were hybridized with target DNA, which had previously formed DNA double strands with PMPs-DNA to generate the sandwich-like structure; we then washed the sandwich-like DNA residue again using buffer solution and dispersed in 0.2 mL of buffer solution. Then, HNO_3 (50 μM) was added to the above mixture to dissolve CuS to produce Cu(II). 2.0 min later, propargyl alcohol (25 μM), 3-azido-7-hydroxycoumarin (25 μM) and sodium ascorbate (3 mM) were added into the above mixed solution. Cu(II) can react with sodium ascorbate to produce Cu(I). The reaction mixture was held for 3.0 h to allow efficient CuAAC reaction between the azide and alkyne groups in the presence of the Cu(I) catalyst at room temperature. Fluorescence spectra of the mixtures were recorded on a Varian Cary Eclipse at the excitation wavelength of 395 nm.

Results and discussion

Design and characterization of the sensor

The principle of a fluorescence sensor is shown in Fig. 1(A). The biotin-modified capt-DNA with PMPs was hybridized to the target DNA, then bound target DNA was hybridized to CuS particle-modified DNA and formed a sandwich-like structure. The sandwich-like DNA could be separated easily by a magnet; it was then redispersed into the buffer solution, and the CuS particles in the sandwich-like structure could be destroyed by an acid to produce Cu(II). In the presence of sodium ascorbate, Cu(II) could be reduced to Cu(I), which in turn initiated the CuAAC reaction between weak-fluorescent 3-azido-7-hydroxycoumarin and propargyl alcohol to form fluorescent 1,2,3-triazole compound. Thus, an obvious fluorescence enhancement

 This journal is © The Royal Society of Chemistry 2014

Published on 05 September 2014. Downloaded by Queens University - Kingston on 20/10/2014 10:25:57.

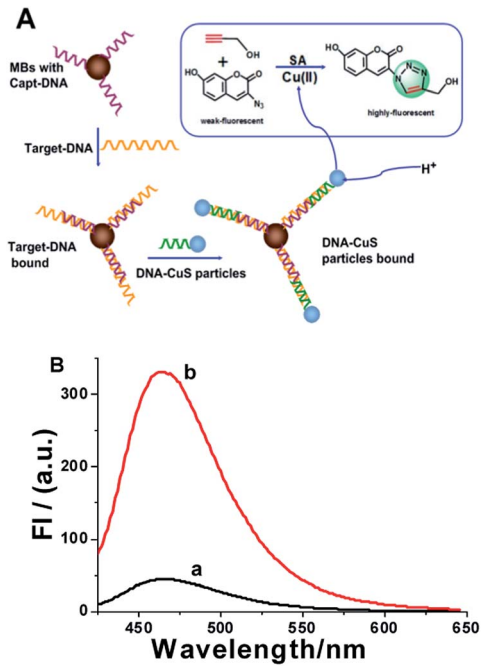

Fig. 1 (A) Schematic representation of fluorescent sensor based on CuAAC reaction. (B) Fluorescence of system in absence (a) and presence (b) of target DNA. Excitation wavelength: 395 nm, $C_{azido} = 2.5 \times 10^{-5}$ M, $C_{alkyne} = 2.5 \times 10^{-5}$ M, $C_{SA} = 3 \times 10^{-3}$ M.

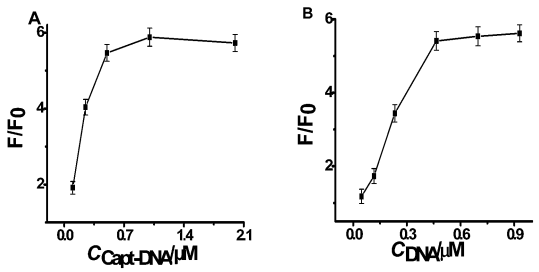

Fig. 2 (A) Relationship between fluorescent increase factor and capt-DNA concentration. (B) Relationship between fluorescence increase factor and concentration of DNA used to modify CuS particles.

could be identified, whereas weak fluorescence intensity could be observed in the absence of the target DNA because the lack of target DNA hindered the formation of the DNA sandwich structure.

Simple experiments were performed to verify our principle. It was found that fluorescence intensity in the absence of the target DNA (curve a in Fig. 1(B)) was considerably lower than that in the presence of target DNA (curve b in Fig. 1(B)). The reason for this is in the fact that the lack of target DNA hindered DNA sandwich hybridization; thus, a structure of a bound DNA-CuS particle sandwich could not be formed; consequently, there was no Cu(II) generated by acid, so Cu(I) could not exist in the reaction system, and the CuAAC reaction did not occur in the absence of Cu(I). Thus, the reaction system showed weak fluorescence in the absence of the target DNA. On the contrary, if the target DNA was added into the reaction system, a strong fluorescence signal could be observed. Moreover, a wavelength shift from 461 nm to 471 nm can be observed in Fig. 1(B). The reason for this lies in the fact that the CuAAC reaction between weak-fluorescent 3-azidocoumarin and propargyl alcohol occurred to yield 1,2,3-triazole compound, which showed a strong fluorescence at 471 nm in the presence of the target DNA.

Optimization of the DNA sensor

In order to understand how to make the system perform best, we studied some experimental conditions that affect the fluorescence enhancement. First, the relationship between fluorescence increase factor (defined as F/F_0, F and F_0 are defined as the fluorescent intensity of the sensor with and without target DNA, respectively) and capt-DNA concentration was studied (Fig. 2(A)). It was found that fluorescence increase factor first increased with the enhancement of capt-DNA concentration, and then it reached a plateau when capt-DNA concentration was over 0.50 μM. Thus, 0.50 μM of capt-DNA was selected in the following study.

The effect of concentration of DNA modified on CuS particles was also investigated (Fig. 2(B)). The system's fluorescence increase factor increased with the extension of DNA concentration in the range of 0.046 to 0.93 μM, and then reached saturation at over 0.46 μM. Therefore, 0.46 μM of DNA was selected as the optimal concentration for the later experiments.

Quantification of DNA

To study the feasibility of this method for the quantitative detection of target DNA, various concentrations of target DNA were added and the fluorescence of the mixed solutions was monitored. Fig. 3(A) shows fluorescence spectroscopy at different DNA concentrations. It was found that fluorescence intensity increased with the extension of the target DNA concentration in the range of 0.03 to 300 nM, and then reached a plateau at over 300 nM. The reason may lie in the fact that a higher target DNA concentration will cause more CuS particle-modified DNA to couple with PMPs, with the result that more Cu(II) can be reduced to Cu(I) by sodium ascorbate, which in turn initiates the CuAAC reaction between weak-fluorescent 3-azido-7-hydroxycoumarin and propargyl alcohol to form more 1,2,3-triazole compound, leading to the increase in fluorescence intensity. However, the CuAAC reaction may have been complete when the target DNA concentration was over 300 nM, and the amount of 1,2,3-triazole compound had no further increase, resulting in the fluorescence intensity showing no obvious change.

Fig. 3(B) shows the relationship between fluorescence increase factor and target DNA concentration. It is seen that fluorescence increase factor increases with an extension of the target DNA concentration. In addition, there is a good linear relationship between the fluorescence increase factor (Y) and the logarithm of target DNA concentration (X) in the range of 0.1 to 100 nM. The equation is:

$$Y = 4.27 + 0.87 \log X \; R^2 = 0.9927$$

Published on 05 September 2014. Downloaded by Queens University - Kingston on 20/10/2014 10:25:57.

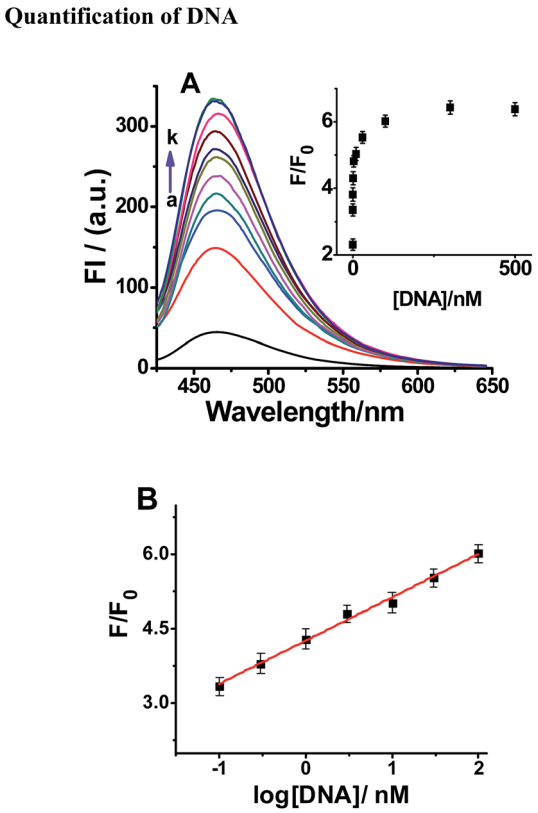

Fig. 3 (A) Fluorescence spectra at different concentrations of target DNA, from a to k: 0 nM, 0.03 nM, 0.1 nM, 0.3 nM, 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM and 500 nM; inset: relationship between values of F/F_0 and different DNA concentrations. (B) Target DNA concentration-dependent change in fluorescence increase factor. Inset shows calibration curve between fluorescence increase factor and logarithm of target DNA concentration.

Detection limit (LOD) is estimated to be 0.04 nM according to the definition of $3\sigma_b$/slope, where σ_b is defined as the standard deviation of the blank samples, and slope is obtained from the calibration curve. The low LOD of the proposed method may lie in the fact that CuS particles are released and function as a catalyst for CuAAC reaction, which amplifies the variation of fluorescence responses at different target DNA concentrations. LOD is better than previously reported fluorescence methods, such as fluorescence turn-on detection (0.41 nM) and fluorescence resonance energy transfer amplification (0.077 nM).[17,18]

Reproducibility and selectivity

In order to investigate the reproducibility of the proposed sensor, five parallel prepared sensors were used to detect target DNA (10 nM). The relative standard deviation (RSD) was calculated to be 4.1%, indicating that the proposed method has good reproducibility. The prepared sandwich-like structure was stored in a refrigerator for six weeks at 4 °C, and then was used to test the fluorescence increase factor; the results showed there was no significant change compared with the freshly prepared one, which suggests the prepared sandwich structure has good stability over a long time.

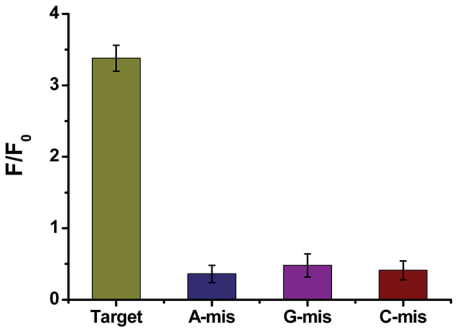

Fig. 4 Selectivity of sensor towards target DNA. DNA concentration is 0.1 nM. $C_{azido} = 2.5 \times 10^{-5}$ M, $C_{alkyne} = 2.5 \times 10^{-5}$ M, $C_{SA} = 3 \times 10^{-3}$ M.

The selectivity of the present sensor was investigated using NH_2-DNA-labeled CuS particles to hybridize with the same concentration of target DNA or with different single-nucleotide mismatch target DNAs. As shown in Fig. 4, the single-nucleotide mismatch caused a little change in the fluorescence increase factor. This is because the single-nucleotide DNA may cause inefficient hybridization, but they cannot stop hybridization completely, and hybridization is enhanced with cooling.[19] Thus, a few of the particles still hybridized with capt-DNA and DNA with CuS particles to form the sandwich-like structure at room temperature, finally induces the CuAAC reaction between 3-azido-7-hydroxycoumarin and propargyl alcohol to form a small amount of fluorescent 1,2,3-triazole compounds. This means mismatch DNA sequences cause no obvious interference to target DNA detection, indicating that the proposed sensor has excellent selectivity for target DNA.

Conclusions

In summary, a novel fluorescence sensor for DNA has been proposed based on CuAAC reaction. Cu(II) comes from target-dependent binding of DNA-CuS particles and can be reduced to Cu(I) by ascorbate, which in turn induces the CuAAC reaction between weak-fluorescent 3-azido-7-hydroxycoumarin and propargyl alcohol to form a fluorescent 1,2,3-triazole compound. It is found that the quantification of DNA is relevant to the fluorescence increase factors. In addition, the proposed sensor shows high sensitivity and good selectivity even in the presence of single-nucleotide mismatches. Moreover, this method may be helpful to expand the utility of click chemistry in fluorescence detection for bioassays.

Acknowledgements

This project was financially supported by the National Basic Research Program of China (no. 2010CB732403), Program for New Century Excellent Talents in University (no. NCET-12-0619). The project was also supported by the National Science Foundation for Fostering Talents in Basic Research of the

 This journal is © The Royal Society of Chemistry 2014

National Natural Science Foundation of China (no. J1103303), Nature Sciences Funding of Fujian Province (no. 2013J01032, no. 2014J06005) and Scientific Research Funding of Fujian Health College (no. 2013-1-4).

Notes and references

1 Y. Huang, Y. Zhang, X. Xu, J. Jiang, G. Shen and R. Yu, *J. Am. Chem. Soc.*, 2009, **131**, 2478–2480.
2 W. Xu, X. Xue, T. Li, H. Zeng and X. Liu, *Angew. Chem., Int. Ed.*, 2009, **48**, 6849–6852.
3 G. M. Makrigiorgos, S. Chakrabarti, Y. Zhang, M. Kaur and B. D. Price, *Nat. Biotechnol.*, 2002, **20**, 936–939.
4 S. Zhu, Z. Liu, W. Zhang, S. Han, L. Hu and G. Xu, *Chem. Commun.*, 2001, 6099–6101.
5 J. M. Nam, S. I. Stoeva and C. A. Mirkin, *J. Am. Chem. Soc.*, 2004, **26**, 5932–5933.
6 H. Zhou, J. Liu, J. Xu and H. Chen, *Chem. Commun.*, 2001, 8358–8360.
7 T. G. Drummond, M. G. Hill and J. K. Barton, *Nat. Biotechnol.*, 2003, **21**, 1192–1199.
8 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
9 Y. Zhou, S. X. Wang, K. Zhang and X. Y. Jiang, *Angew. Chem., Int. Ed.*, 2008, **47**, 7454–7456.
10 R. F. H. Viguier and A. N. Hulme, *J. Am. Chem. Soc.*, 2006, **128**, 11370–11371.
11 S. Y. Qiu, X. H. Li, W. M. Xiong, L. D. Xie, L. H. Guo and B. Qiu, *Biosens. Bioelectron.*, 2013, **41**, 403–408.
12 C. M. Wang, L. J. Lu, W. M. Ye, O. Zheng, B. Qiu, Z. Y. Lin, L. H. Guo and G. N. Chen, *Analyst*, 2014, **139**, 656–659.
13 L. J. Lu, L. L. Yang, H. J. Cai, L. Zhang, Z. Y. Lin, L. H. Guo, B. Qiu and G. N. Chen, *Food Chem.*, 2014, **162**, 242–246.
14 J. P. Collman, N. K. Devaraj and C. E. D. Chidsey, *Langmuir*, 2004, **20**, 1051–1053.
15 T. N. Milica, I. C. Mirjana, V. Veana and I. M. Olga, *J. Phys. Chem.*, 1990, **94**, 6390–6396.
16 P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski and F. R. Salemme, *Science*, 1989, **243**, 85–88.
17 M. Wang, D. Q. Zhang, G. X. Zhang, Y. L. Tang, S. Wang and D. B. Zhu, *Anal. Chem.*, 2008, **80**, 6443–6448.
18 L. G. Xu, Y. Y. Zhu, W. Ma, H. Kuang, L. Q. Liu, L. B. Wang and C. L. Xu, *J. Phys. Chem. C*, 2011, **115**, 16315–16321.
19 C. H. Wohlgamuth, M. A. McWilliams and J. D. Slinker, *Anal. Chem.*, 2013, **85**, 1462–1467.

Published on 05 September 2014. Downloaded by Queens University - Kingston on 20/10/2014 10:25:57.