

Colorimetric recognition of the coralyne–poly(dA) interaction using unmodified gold nanoparticle probes, and further detection of coralyne based upon this recognition system†

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Among the functional nucleic acids studied, adenine-rich nucleic acids have attracted attention due to their critical roles in many biological processes and self-assembly-based nanomaterials, especially deoxyribonucleic acids (abbreviated as poly(dA)). Therefore the ligands binding to poly(dA) might serve as potential therapeutic agents. Coralyne, a kind of planar alkaloid, has been firstly found that it could bind strongly to poly(dA). This work herein reports an approach for visual sensing of the coralyne–poly(dA) interaction. This method was based on the coralyne inducing poly(dA) into the homo-adenine DNA duplex and the difference in electrostatic affinity between single-stranded DNA and double-stranded DNA with gold nanoparticles (GNPs). Furthermore, we applied the recognition process of the interaction between coralyne and poly(dA) into specific coralyne detection with the assistance of certain software (such as Photoshop). A linear response from 0 to 728 nM was obtained for coralyne, and a detection limit of 91 nM was achieved.

Introduction

Beyond the Watson–Crick DNA duplex,¹ a lot of other kinds of functional nucleic acids (such as aptamers, catalytic nucleic acids, i-motifs, G-quadruplexes, triplex and Holliday junctions) have been extensively studied over the past decades due to their importance to sensors, the assembly of nanomaterials, nanomachines, diagnostics, and therapies.^{2–8} For example, a lot of aptasensors have been fabricated to detect varieties of targets ranging from small molecules to proteins or cells.⁹ A light-induced conformational switch of i-motif DNA was designed by coupling with a light-induced hydroxide ion emitter.^{8c} An anti-VEGF (vascular endothelial growth factor) aptamer as therapeutic drug has been *in vitro* selected and approved by the FDA.¹⁰

Among the functional nucleic acids studied, much attention has been focused on adenine-rich nucleic acids, especially deoxyribonucleic acids (abbreviated as poly(dA)), due to their critical roles in many biological processes and self-assembly-based nanomaterials.¹¹ For example, many poly(dA) tracts in most eukaryotes may affect chromatin structure and thus may serve as a major DNA marker system.^{11a} Therefore some recent researches have been devoted to discover the ligands that could bind poly(dA) and thus might serve as potential therapeutic agents. In fact, few molecules seem to bind to single-stranded

nucleic acids.¹² Coralyne, a kind of planar alkaloid, has been particularly noticed for its high propensity to stabilize triplex DNA, anti-leukemic activity in animal models and as a dual poison of topoisomerase I and II.¹³ It has been firstly found by Ren and Chaires that coralyne could intercalate strongly to poly(dA) with an apparent association constant of $(1.05 \pm 0.1) \times 10^5 \text{ M}^{-1}$.¹⁴ Furthermore, Hud *et al.* have found that coralyne promoted the formation of an anti-parallel homo-adenine duplex with a melting temperature around 50 °C, with a stoichiometry of one coralyne per four adenine bases.^{11b–d}

Despite such progress, the study of molecular recognition between ligands and poly(dA) and the screening of potential therapeutic drugs are still great challenge. Up to now, most techniques used for the investigation of ligands binding to the homo-adenine secondary structures are electrophoresis, UV-vis and circular dichroism spectroscopy.^{11a–d} For further study, some novel techniques are needed to investigate the interaction between poly(dA) and its potential ligands, among which simple colorimetry, especially GNP-based colorimetry, reveals increasing potential for its simplicity, high sensitivity, and low cost.¹⁵

The GNP-based colorimetry relies on the fact the dispersed GNP solution is a wine-red color whereas the aggregated one is purple.¹⁵ Song *et al.* reported the coralyne-assisted assembly of GNPs and its potential in screening homo-adenine DNA duplex binders.^{11e} Though GNP-based colorimetry was employed to monitor the assembly, Song's method needed the steps of modifying HS-poly(dA) onto the GNPs and separating the modified GNPs from the unmodified GNPs. Such steps might bring relatively complex operation and high cost. This kind of labeled colorimetry could be further simplified and improved via a label-free approach. This label-free colorimetry used unmodified GNPs as sensing probes and was based on the different adsorption abilities of single-stranded DNA and double-stranded

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DNA onto the GNPs.¹⁶ Since Li and Rothberg's pioneering work in label-free GNP-based colorimetry, substantial progress has been made in this area.¹⁷ For example, Hg²⁺-promoted formation of thymine–thymine base pairs has been investigated by using this kind of label-free GNP probe.¹⁸ To the best of our knowledge, no one has so far studied the ligand-induced formation of the homo-adenine DNA duplex *via* label-free GNP-based colorimetry. Herein, we firstly report a simple colorimetric recognition of ligand inducing poly(dA) into homo-adenine DNA duplex using unmodified GNPs as probes, taking coralyne as the model ligand (Scheme 1). Also, this process was further used for coralyne detection.

Experimental

Chemical and reagents

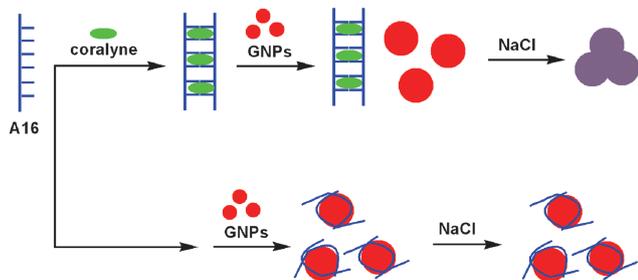
Coralyne was purchased from Acros (NJ, USA). Rhodamine B, methylene blue, sodium citrate and sodium chloride were purchased from Beijing Chemical Reagent Company (Beijing, China). Chloroauric acid (HAuCl₄) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Other reagents and chemicals were of at least analytical reagent grade. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA). The poly(dA) oligonucleotide sequence (5' AAAA AAAA AAAA AAAA 3', abbreviated as A16) and control random oligonucleotide sequence (5' CAAG TGTC GGCA TCAT 3', abbreviated as R16) were synthesized by the TaKaRa Biotechnology Co., Ltd (Dalian, China).

Apparatus

Absorption spectra were recorded on a Cary 500 Scan UV-Vis-NIR Spectrophotometer (Varian, Harbor City, CA, USA) at room temperature.

Preparation of gold nanoparticles (GNPs)

13 nm GNPs were prepared according to a literature method.¹⁹ Briefly, a sodium citrate solution (0.1 M, 1.94 mL) was rapidly added to a boiled HAuCl₄ solution (50 mL H₂O, 0.167 mL 10% HAuCl₄) under vigorous stirring. The mixed solution was boiled for 10 min and further stirred for 15 min. The resulting wine-red-colored solution was cooled to room temperature, and was stored in the 4 °C refrigerator before use.



Scheme 1 Colorimetric recognition of coralyne-induced poly(dA) into the homo-adenine DNA duplex using unmodified GNPs.

Colorimetric recognition of coralyne-induced formation of homo-adenine DNA duplex

A typical colorimetric recognition was realized as the following procedure (see Scheme 1). First, 4 μM A16 and 16 μM coralyne were mixed and incubated in 10 mM phosphate buffer (pH 7.0, containing 200 mM NaCl) for 20 minutes. Second, 5 μL of the A16/coralyne solution was added into 100 μL of 13 nm GNP solution and was sonicated for 40 seconds. Third, 5 μL of 680 mM NaCl was added into the A16/coralyne/GNP solution and was sonicated for 40 seconds to produce a color change. The final concentrations of coralyne, A16 and NaCl in GNP solution were 728 nM, 182 nM and 40 mM, respectively.

In control experiments, R16 was investigated instead of A16 (or rhodamine B and methylene blue were investigated instead of coralyne) under other conditions identical to those used for A16 and coralyne.

Coralyne detection and quantitative calculation of the relative intensity of the dose-dependent formation reaction with Photoshop7.0

Concentration-dependent experiments for coralyne detection was carried out by mixing different amount of the coralyne and A16, and the mixtures were added to the GNPs. The specific processes were followed by above-mentioned experiment details. The coralyne concentration-dependent response was analysed quantitatively with the Photoshop7.0. The relative intensity could be measured as follows: (1) select an area you want to measure, (2) choose Image>Histogram, (3) select Chanel:Red, and read the value of Mean. The Mean value is the intensity we used in our experiment. The dose-dependent response could be quantitatively calculated as: relative intensity = [intensity of coralyne–A16–GNPs]/[intensity of GNPs alone] × 100%.

Results and discussion

Mechanism for the sensing system

The mechanism of colorimetric recognition of the coralyne–poly(dA) interaction using the unmodified GNP probes could be explained as follows. The 13 nm GNPs used were coated with citrate, which stabilized the GNPs *via* their electrostatic repulsion against van der Waals attraction.¹⁹ However, this electrostatic repulsion was so weak that it could be screened by NaCl added and thus the GNPs could be aggregated easily by NaCl. As with previous reports,^{16,17a} the unfolded single-stranded DNA could be adsorbed onto the GNPs surface *via* coordination interaction, which could enhance the GNPs stability remarkably against NaCl-induced aggregation. Here, A16 (or R16 as discussed later) alone was in an unfolded state without any self-structure pair. Therefore, it could protect GNPs and ensure they remained a red color after the addition of NaCl. As for double-stranded DNA, the outer phosphate groups prevented its interaction with negatively charged GNPs, so it could not protect GNPs against NaCl-induced aggregation.^{16,17a} Therefore, after coralyne induced unfolded A16 to form the homo-adenine DNA duplex, the duplex could not stabilize the GNPs any more, which led to their aggregation after NaCl addition and the development of a corresponding purple color.

Optimizing of the concentration of coralyne

Coralyne is a kind of planar alkaloid with a positive charge (see ESI†), so a high concentration of coralyne itself could effectively aggregate the GNPs. In order to exclude the impact of coralyne, 5 μL of different concentrations of the coralyne were added to 95 μL of 13 nm GNP solution before incubation for 40 minutes. Fig. 1 shows photographs of 100 μL GNPs containing various concentrations of coralyne only. It could be clearly seen that coralyne could aggregate the GNPs when the final concentration of coralyne was greater than or equal to 1 μM . Thus to guarantee that the color change of the GNPs originated from the coralyne-induced formation of the homo-adenine DNA duplex but not from coralyne itself, the final concentration of coralyne in the GNP solution was optimized below 1 μM in our work.

Optimizing of the concentration of DNA

Polak and Hud have reported that coralyne promoted the formation of the homo-adenine duplex with a stoichiometry of one coralyne per four adenine bases.^{11b} The A16 used in our experiments had 16 adenine bases. Therefore, in order to make sure that coralyne could promote A16 to form the homo-adenine duplex completely, the mole ratio between coralyne and A16 was chosen as 4 : 1, the same mole ratio between coralyne and R16 was chosen. In order to optimize the concentration of A16, we should study the ability of single-stranded DNA protecting GNPs against both NaCl- and coralyne-induced aggregation. We chose R16 as an example of single-stranded DNA because R16 could not be introduced into the duplex chain by coralyne. We incubated R16 at various primal concentrations of 1 μM , 2 μM , 3 μM , 4 μM and 5 μM , coralyne at various primal concentrations of 4 μM , 8 μM , 12 μM , 16 μM and 20 μM . As shown in Fig. 2, R16 at lower primal concentrations of 1 μM , 2 μM and 3 μM , for which the final concentrations of were 45 nM, 91 nM and 136 nM in GNP solutions, could not protect GNPs effectively against NaCl-induced aggregation. R16 at primal concentrations of 4 μM and 5 μM , for which the final concentrations of were 182 nM and 227 nM in GNP solutions, could protect the GNPs and ensure that they remained a red color after the addition of NaCl.

Furthermore, R16 at the final concentration of 182 nM could protect GNPs containing various concentrations of coralyne against NaCl-induced aggregation as shown in Fig. 3. Therefore, the final concentration of A16 (or R16) in GNP solution was chosen as 182 nM.

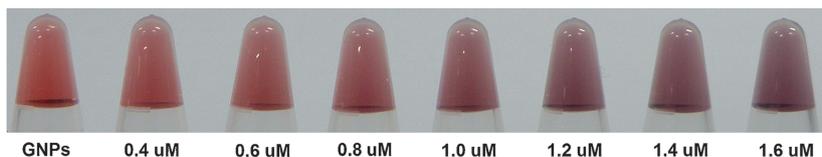


Fig. 1 Photographs of 100 μL of 13 nm GNPs containing various concentrations of coralyne. The first photograph (from left) was GNPs alone without coralyne. The second to eighth photographs (from left to right) contained coralyne of a final concentration of 0.4 μM , 0.6 μM , 0.8 μM , 1.0 μM , 1.2 μM , 1.4 μM , 1.6 μM , respectively.

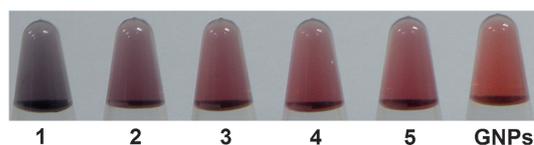


Fig. 2 Photographs of 110 μL of 13 nm GNPs containing 40 mM NaCl in the presence of: (1) 45 nM R16 and 182 nM coralyne; (2) 91 nM R16 and 364 nM coralyne; (3) 136 nM R16 and 546 nM coralyne; (4) 182 nM R16 and 728 nM coralyne; (5) 227 nM R16 and 910 nM coralyne. The last photograph (from left) was for GNPs alone.

Colorimetric recognition of coralyne inducing poly(dA) into the homo-adenine DNA duplex

Shown in Fig. 4 is a typical colorimetric recognition of the coralyne-induced formation of the homo-adenine DNA duplex. In the absence of coralyne (Fig. 4b), the single-stranded poly(dA) was adsorbed on the GNP probes and stabilized the GNP probes against a given high concentration of salt. In this case, the solution appeared red. However, in the presence of coralyne (Fig. 4a), it induced the formation of the homo-adenine DNA duplex *via* intercalation. Such a formed poly(dA) duplex could not be adsorbed on the GNP probes and could not stabilize the GNP probes against the high salt. Accordingly, aggregation of GNP probes was induced with a purple solution appearing. By the color change of the unmodified GNP probes from red to purple, the coralyne-induced formation of the homo-adenine DNA duplex could be directly monitored with the naked eye.

Detection of coralyne

Because coralyne was a very important alkaloid, we further applied the recognition process of the interaction between coralyne and poly(dA) to the specific detection of coralyne. The coralyne concentration-dependent response is shown in Fig. 5. It was found in the coralyne concentration range from 0 to 728 nM that the GNP probe color gradually turned from red to purple (Fig. 5a). And as low as 91 nM coralyne could be recognized directly with the naked eye. Meanwhile, this trend of color change further confirmed that coralyne could sensitively induce the formation of the A16 duplex, and the more coralyne added the more duplex formed. Usually, the quantitative data analysis of label-free colorimetry was performed with UV-vis spectroscopy. However, with the assistance of certain software (such as Photoshop), quantitative analysis could be readily obtained just with the photographs. Here, with Photoshop7.0, the dose-dependent response could be quantitatively calculated as: relative

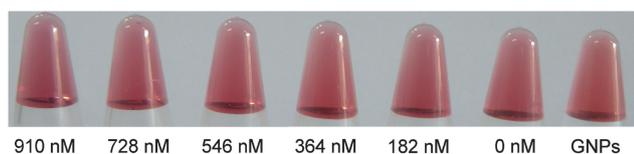


Fig. 3 Photographs of 110 μL of 13 nm GNPs containing 182 nM R16 and 40 mM NaCl in the presence of various concentrations of coralyne. The last photograph (from left) was for GNPs alone without R16 and coralyne. The first to sixth photographs (from left to right) contained coralyne with a final concentration of 910 nM, 728 nM, 546 nM, 364 nM, 182 nM and 0 nM, respectively.

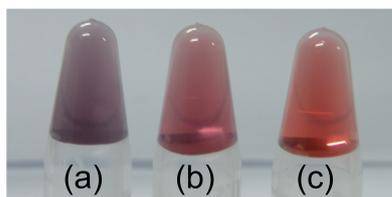


Fig. 4 Colorimetric recognition of coralyne inducing poly(dA) into homo-adenine DNA duplex. Photographs of 110 μL of 13 nm GNPs containing 182 nM A16 and 40 mM NaCl in the presence (a) and absence (b) of 728 nM coralyne. (c) GNPs alone as control.

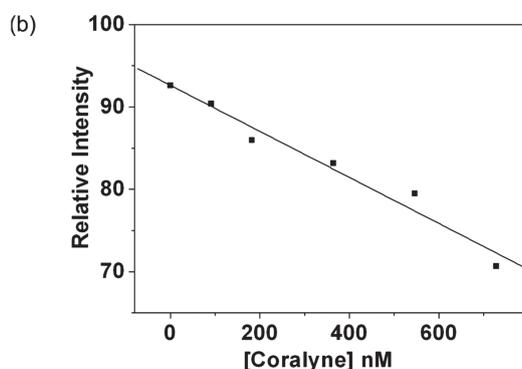
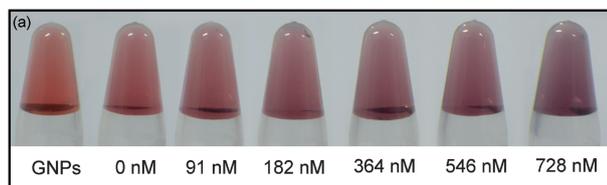


Fig. 5 (a) Photographs of 110 μL of 13 nm GNPs containing 182 nM A16 and 40 mM NaCl in the presence of various concentrations of coralyne. The first photograph (from left) was GNPs alone without A16 and coralyne. The second to seventh photographs (from left to right) contained coralyne with a final concentration of 0 nM, 91 nM, 182 nM, 364 nM, 546 nM, and 728 nM, respectively. (b) Plots of intensity versus coralyne concentration.

intensity = [intensity of coralyne–A16–GNPs]/[intensity of GNPs alone] \times 100%. As shown in Fig. 5b, a linear response from 0 nM to 728 nM was obtained for coralyne, and a detection limit of 91 nM was achieved.

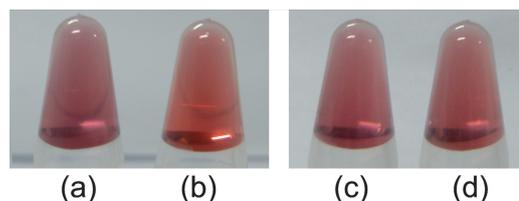


Fig. 6 Photographs of 110 μL of 13 nm GNPs containing 182 nM R16 and 40 mM NaCl in the presence (a) and absence (b) of 728 nM coralyne; and photographs of 110 μL of 13 nm GNPs containing 182 nM A16 and 40 mM NaCl in the presence of (c) 728 nM methylene blue and (d) 728 nM rhodamine B.

Selectivity of the sensing system

To testify that the recognition procedure is specific, control experiments were done using random single-stranded DNA (here R16) and other ligands (here rhodamine B and methylene blue). As shown in Fig. 6, R16 used here could also protect GNP probes from NaCl-induced aggregation but exhibited no significant response to coralyne under the same experimental conditions. In addition, when rhodamine B and methylene blue substituted coralyne, still no color change of the GNP probes took place under the same amount of NaCl. All of these control experiments further proved the specific interaction between A16 and coralyne.

Conclusions

In summary, a simple label-free colorimetric recognition of coralyne inducing poly(dA) to form the homo-adenine DNA duplex was realized using GNP probes, which may offer a useful method to screen potential therapeutic molecules in the future. Further, the sensitive detection of coralyne was accomplished in this way. Through this method, commonly used steps such as labeling, modification and separation were effectively avoided. So it provided a much simpler, faster, and more economical alternative to traditional methods.^{11e} Furthermore, this work extended the application of label-free GNP colorimetry from sensors to recognition interaction between ligands and functional nucleic acids.

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References

- J. D. Watson and F. H. C. Crick, *Nature*, 1953, **171**, 737.
- (a) A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818; (b) C. Tuerk and L. Gold, *Science*, 1990, **249**, 505; (c) T. Hermann and D. J. Patel, *Science*, 2000, **287**, 820.
- (a) D. L. Robertson and G. F. Joyce, *Nature*, 1990, **344**, 467; (b) S. W. Santoro and G. F. Joyce, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 4262; (c) R. R. Breaker, *Nat. Biotechnol.*, 1997, **15**, 427.
- J. L. Leroy, M. Gueron, J. L. Mergny and C. Helene, *Nucleic Acids Res.*, 1994, **22**, 1600.
- J. T. Davis, *Angew. Chem., Int. Ed.*, 2004, **43**, 668.
- G. Felsenfeld and A. Rich, *Biochim. Biophys. Acta*, 1957, **26**, 457.

- 7 P. R. Bianco, R. B. Tracy and S. C. Kowalczykowski, *Front. Biosci.*, 1998, **3**, 570.
- 8 (a) D. Li, B. Shlyahovsky, J. Elbaz and I. Willner, *J. Am. Chem. Soc.*, 2007, **129**, 5804; (b) J. Liu and Y. Lu, *Nat. Protocols*, 2006, **1**, 246; (c) H. J. Liu, Y. Xu, F. Y. Li, Y. Yang, W. X. Wang, Y. L. Song and D. S. Liu, *Angew. Chem., Int. Ed.*, 2007, **46**, 2515; (d) S. D. Jayasena, *Clin. Chem. (Washington, D. C.)*, 1999, **45**, 1628; (e) M. Famulok, G. Mayer and M. Blind, *Acc. Chem. Res.*, 2000, **33**, 591.
- 9 (a) C. -C. Huang, Y. -F. Huang, Z. H. Cao, W. H. Tan and H. -T. Chang, *Anal. Chem.*, 2005, **77**, 5735; (b) J. Wang, L. H. Wang, X. F. Liu, Z. Q. Liang, S. P. Song, W. X. Li, G. X. Li and C. H. Fan, *Adv. Mater.*, 2007, **19**, 3943; (c) M. Mascini, G. G. Guilbault, S. J. Lebrun and D. Compagnone, *Anal. Lett.*, 2007, **40**, 1386; (d) V. Pavlov, Y. Xiao, B. Shlyahovsky and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 11768; (e) D. H. Shangguan, Y. Li, Z. W. Tang, Z. H. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. Y. J. Yang and W. H. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 11838.
- 10 C. Pestourie, B. Tavitian and F. Duconge, *Biochimie*, 2005, **87**, 921.
- 11 (a) E. P. Economou, A. W. Bergen, A. C. Warren and S. E. Antonarakis, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 2951; (b) M. Polak and N. V. Hud, *Nucleic Acids Res.*, 2002, **30**, 983; (c) S. S. Jain, M. Polak and N. V. Hud, *Nucleic Acids Res.*, 2003, **31**, 4608; (d) O. Persil, C. T. Santai, S. S. Jain and N. V. Hud, *J. Am. Chem. Soc.*, 2004, **126**, 8644; (e) G. T. Song, C. Chen, X. G. Qu, D. Miyoshi, J. S. Ren and N. Sugimoto, *Adv. Mater.*, 2008, **20**, 706.
- 12 J. B. Chaires, *Top. Curr. Chem.*, 2005, **253**, 33.
- 13 (a) J. S. Lee, L. J. P. Latimer and K. J. Hampel, *Biochemistry*, 1993, **32**, 5591; (b) A. A. Moraru-Allen, S. Cassidy, J.-L. Asensio Avarez, K. R. Fox, T. Brown and A. N. Lane, *Nucleic Acids Res.*, 1997, **25**, 1890; (c) M. S. Keppler, S. Neidle and K. R. Fox, *Nucleic Acids Res.*, 2001, **29**, 1935; (d) K. Zee-Cheng, K. D. Paull and C. C. Cheng, *J. Med. Chem.*, 1974, **17**, 347; (e) D. W. Wilson, A. N. Gough and J. J. Doyle, *J. Med. Chem.*, 1976, **19**, 1261; (f) D. Makhey, B. Gatto, C. Yu, A. Liu, L. F. Liu and E. J. LaVoie, *Med. Chem. Res.*, 1995, **5**, 1; (g) D. Makhey, B. Gatto, C. Yu, A. Liu, L. F. Liu and E. J. LaVoie, *Bioorg. Med. Chem. Lett.*, 1996, **4**, 781; (h) B. Gatto, M. M. Sanders, C. Yu, H. Wu, D. Makhey, E. J. LaVoie and L. F. Liu, *Cancer Res.*, 1996, **56**, 2795.
- 14 J. S. Ren and J. B. Chaires, *Biochemistry*, 1999, **38**, 16067.
- 15 (a) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, *Science*, 1997, **277**, 1078; (b) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959.
- 16 H. Li and L. Rothberg, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 14036.
- 17 (a) L. H. Wang, X. F. Liu, X. F. Hu, S. P. Song and C. H. Fan, *Chem. Commun.*, 2006, 3780; (b) Z. Wang, J. H. Lee and Y. Lu, *Adv. Mater.*, 2008, **20**, 3263; (c) H. Wei, B. L. Li, J. Li, E. K. Wang and S. J. Dong, *Chem. Commun.*, 2007, 3735; (d) C. Chen, G. T. Song, J. S. Ren and X. G. Qu, *Chem. Commun.*, 2008, 6149.
- 18 (a) L. H. Wang, J. Zhang, X. Wang, Q. Huang, D. Pan, S. P. Song and C. H. Fan, *Gold Bull.*, 2008, **41**, 37; (b) D. Li, A. Wiecekowska and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 3927.
- 19 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.