Analyst

View Online
Dynamic Article Links

Cite this: *Analyst*, 2011, **136**, 764

www.rsc.org/analyst

PAPER

Combing DNAzyme with single-walled carbon nanotubes for detection of Pb(II) in water†

Jingjing Yao,[a] Jishan Li,[ab] Jeremy Owens[c] and Wenwan Zhong*[a]

Received 11th September 2010, Accepted 8th November 2010
DOI: 10.1039/c0an00709a

A sensitive and simple assay for the detection of Pb^{2+} in aqueous solutions is reported. It takes advantage of the high affinity between single-stranded DNA (ssDNA) and single-walled carbon nanotubes (SWCNT) as well as the capability of SWCNT in fluorescence quenching. Lead(II) catalyzes the cleavage of a fluorescently labeled DNA substrate by a DNAzyme, which releases the single-stranded product to be adsorbed onto a SWCNT. The decrease in fluorescence is proportional to the Pb^{2+} concentration. Concentrations as low as 1 nM Pb^{2+} in water could be detected and the detection range spans over 5 orders of magnitude. The unique combination of Pb-specific DNAzyme with SWCNT produces a universal, facile and cost-effective sensing platform for lead ions. The concept can be applied to the design of detection assays for other metal ions or small molecules.

Introduction

Lead-containing cores, paints, petroleum industrial wastes, and alloys are common sources of lead contamination. Due to its detrimental effects on the brain and central nervous system, the level of Pb^{2+} in drinking water and the environment should be monitored very closely to prevent lead-poisoning in humans, especially children.[1] A great number of analytical methods have been developed over the past decades, using techniques like atomic absorption spectrometry,[2] inductively coupled plasma (ICP) mass spectrometry (MS),[3] and electrochemistry.[4] The involvement of sophisticated instruments and complicated sample pretreatment in these methods makes them not suitable for at-the-field detection to promptly recognize the onset of contamination.

Lead(II)-sensors capable of providing immediate optical read-outs with the least instrumentation requirement have drawn great attention. Various Pb^{2+}-responsive molecular probes made of peptides,[5] proteins,[6] polymers,[7] nucleic acids,[8,9] and small ligands[10] have been employed for the specific identification of Pb^{2+}, using fluorescent dyes,[11] gold nanoparticles,[12,13] and chromophores[14] as the signaling labels. Among these Pb-responsive probes, deoxyribozymes, or DNAzymes, have been extensively studied. DNAzymes can catalyze reactions including ligation,[15–17] RNA cleavage,[18,19] DNA phosphorylation,[20] and thymine dimer photoreversion.[21] They are cost effective in production, and have high durability and stability compared to their antibody counterparts. Most DNAzymes rely on divalent metal ions as the cofactors for catalytic reactions; and some exhibit high affinity and specificity to cations such as Pb^{2+} [8,9] Zn^{2+},[22,23] Cu^{2+},[24] Co^{2+},[25] Hg^{2+},[26,27] and UO_2^{2+},[28,29] One of the most effective lead-dependent DNAzymes is the "8–17 DNAzyme" reported by Li and Lu.[8] Lead ion could fit in the highly conserved catalytic core of this DNAzyme and initiate the hydrolytic cleavage of the substrate at its phosphodiester bond between the ribonucleic adenine (rA) and the deoxyribonucleic guanine (G). One way to convert the cleavage events to detectable signals for Pb^{2+} sensing relies on the electrochemically active species being confined onto the electrode surface through interaction with the DNAzyme-substrate structure. Lead-induced cutting of the substrate strand would then release the species and turn off the electrochemical sensor.[30] More commonly, optical detection platforms are used. The 5′-end of the 8–17 DNAzyme is labeled with a quencher and the 3′-end of the substrate strand with a fluorophor.[8] Hybridization of the enzyme and substrate strands quenches the fluorophore, while the cleavage destroys the hybridization and recovers the fluorescence. The quenching efficiency could be further enhanced by labeling both the substrate and enzyme with quenchers,[11] leading to higher sensitivity in Pb^{2+} detection.

It has been reported that single-walled carbon nanotubes (SWCNT) could act as superior quenchers for fluorescent dyes because of their large surface area to volume ratios (200–900 m^2 g^{-1}), great adsorptivity for organic molecules with aromatic structures,[31] and broad UV-Vis-NIR absorption spectra which overlap well with those of several fluorophores.[32–34] On the other hand, SWCNT has been found to strongly interact with single-stranded DNA (ssDNA) *via* π–π stacking.[35,36] The stiffer structure and more exposed negatively charged phosphate backbone

[a]*Department of Chemistry, University of California, Riverside, Riverside, CA, 92507, USA. E-mail: wenwan.zhong@ucr.edu; Fax: +1-951-827-4713; Tel: +1-951-827-4925*
[b]*College of Chemistry and Chemical Engineering, State Key Laboratory for Chemo/Biosensing and Chemometrics, Hunan University, Changsha, 410082, P.R. China*
[c]*Department of Earth Sciences, University of California, Riverside, Riverside, CA, 92507, USA*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c0an00709a

of double-stranded DNA (dsDNA) significantly reduce their affinity with SWCNT.[32] The capability of SWCNT in fluorescence quenching and discriminative binding with ssDNA have been proved to be attractive signalling tools for chemical and biological sensing.[35,37] Herein, SWCNT, together with the 8–17 DNAzyme, was employed to produce a simple but sensitive turn-off assay for Pb^{2+} detection. The 8–17 DNAzyme cleaves the fluorescently labeled substrate in the presence of Pb^{2+} and the single-stranded product binds to SWCNT more strongly than the enzyme-substrate hybridized structure, quenching the fluorophore. The reduced fluorescence is proportional to Pb concentration. SWCNT with different surface modifications were examined, and assay selectivity over interfering divalent cations were tested. The assay was used to dertermine Pb^{2+} concentrations in water samples and the results were verified by conventional instrumental techniques.

Materials and methods

Unmodified SWCNT and SWCNT functionalized with carboxyl group (SWCNT-COOH) or poly(ethylene glycol) (SWCNT-PEG) were obtained from Sigma-Aldrich (St. Louis, MO). The average size (in diameter × length) for SWCNT, SWCNT-COOH and SWCNT-PEG was 1.1 nm × 0.5–100 μm, 4–5 nm × 0.1–1.5 μm and 4–5 nm × 0.5–0.6 μm, respectively. Oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). The sequences are listed in Table 1. Metal salts and reagents for buffer preparation were purchased from Fisher Scientific Inc. (Waltham, MA) and used without further purification. Heavy metal ions were handled and disposed properly to avoid health hazards and environmental pollution.

Cleavage reaction catalyzed by the 8–17 DNAzyme

Typically, 50 nM 17E was mixed with an equimolar amount of Cy3–17S in the reaction buffer (50 mM Tris-acetate and 50 mM $NaNO_3$, pH 7.8). This mixture was denatured at 98 °C for 2 min and slowly cooled to 4 °C over 20 min. Then 10 μl portions of metal solutions were added to 100 μl of the hybridized mixture (17SE) to initiate the cleavage reaction. The reaction lasted for 10 min on ice. Afterwards, 2 μl of SWCNT solution was introduced and the fluorescence was measured. The same reaction procedure was applied to measure the concentration of Pb^{2+} in water samples, except that a 10× concentrated 17E and Cy3–17S hybrid solution was added to a 100 μl portion of water sample.

SWCNT preparation and fluorescence measurement

SWCNT and SWCNT-COOH solutions were prepared in 0.1% dimethylformamide (DMF) at a concentration of 0.25 mg mL^{-1}. To ensure good dispersion, the SWCNT samples were sonicated for 2 h. SWCNT-PEG was dissolved in Milli-Q water (18 MΩ cm) at the same concentration. The quenching efficiency of SWCNT was measured at room temperature with a Spex Fluorolog Tau-3® spectrofluorometer (HORIBA Jobin Yvon Inc., NJ, USA). The excitation wavelength used was 535 nm, and the emission spectrum was measured from 550 to 650 nm using a slit width of 10 nm. The assays for Pb^{2+} detection were carried out in microtiter plates, and the signals were recorded using a Wallac Victor (II) 1420 plate reader with an excitation wavelength filter of 530/30 nm and a long-pass emission filter of 602 nm.

Table 1 Sequence of DNA molecules used in this study

Name	Sequence
Pb^{2+}-dependent enzyme (17E)	5′-CAT CTC TTC TCC GAG CCG GTC GAA ATA GTG AGT -3′
Cy3 labelled substrate (17S)	5′-/cy3/-ACT CAC TAT rA GG AAG AGA TG -3′
Complementary strand to 17S (17D)	5′-CA TCT CTT CCT ATA GTG AGT-3′

Gel electrophoresis

To verify the cleavage, gel electrophoresis was conducted. After a 10 min incubation period with the DNAzyme and metal ions, the reaction was quenched by the addition of an equal volume of the stop buffer (50 mM EDTA, 8 M urea, 90 mM Tris, 90 mM boric acid, 0.05% xylene cyanol and 0.05% bromophenol blue). The mixture was denatured in boiling water for 1 min and cooled on ice before loading. The gel image was scanned with an Amersham Typhoon 9410 (GE Healthcare, WI) and the fluorescence intensity of Cy3 was calculated using Image Quant 5.2. The cleavage yield was defined as the grey density ratio of the cleaved substrate band to the sum of cleaved and uncleaved substrate bands.

Measurement of metal content of water samples

Metals in water samples were screened and quantified either with a PerkinElmer OPTIMA 2000™ inductively coupled plasma atomic emission spectrometer (ICP AES) (Norwalk, CT), or an Agilent 7500c ICP MS. For quantification, standard solutions were prepared by gradually diluting Pb^{2+} solution with 4% (v/v) nitric acid. The water samples were also acidified with 4% HNO_3 before analysis. The instruments were rinsed thoroughly with 4% HNO_3 between measurements to prevent memory effects.

Results and discussions

Adsorption of ss- and dsDNA on SWCNT with different surface modification

The bases of dsDNA are imbeded in the highly hydrophobic helix structure, forming hydrophobic domains only available through major and minor grooves,[38] while the negatively charged phosphate groups face outward and are readily available for electrostatic interaction. In the case of ssDNA, hydrophobic bases are exposed and readily available for the π–π interaction with the side wall of SWCNT. Such structural differences between ss- and dsDNA result in distinct adsorption patterns on SWCNT, with ssDNA being adsorbed more strongly. The smaller size and greater structural flexibility of ssDNA further enhance its affinity to SWCNT. Because fluorophores located close to the SWCNT surface could be quenched, the interaction of ssDNA with SWCNT may be visualized and used to signal DNA cleavage catalyzed by DNAzyme with metal cation as the cofactor. To prove this hypothesis, we studied the fluorescence of Cy3 that was labeled on the substrate of the 8–17 DNAzyme, 17S, when mixed with the unmodified SWCNT, SWCNT-PEG,

and SWCNT-COOH. The Cy3-labeled 17S was either present as ssDNA or hybridized with its complementary strand 17D, forming the dsDNA of 17DS. The fluorescence spectra recorded with 4.2 μg mL^{-1} SWCNT are shown in Figure S1 (ESI).†The quenching effects were highly dependent on the types of SWCNT. The SWCNT-PEG could barely quench the fluorescence of ss-17S or the ds-17DS; the unmodified SWCNT provided certain levels of quenching to both types of DNA, and the SWCNT-COOH completely quenched the ss-17S but left out significant fluorescence from the ds-17SD. The quenching yield $P\%$ was calculated using the following equation:

$$P\% = (F_{17SD} - F_{17S})/F_{17SD} \times 100\%,$$

with F_{17S} and F_{17SD} being the fluorescence of 17S and 17SD in the presence of SWCNT. The quenching yields are presented in Fig. 1. We can clearly see that SWCNT-COOH generated the largest fluorescence difference between the ss- and dsDNA, and obtained a quenching yield of about 97%, which was much higher than those obtained using the unmodified SWCNT and SWCNT-PEG.

Both the aqueous solubility and surface coverage of SWCNT could attribute to the large differences in their quenching yield. The unmodified SWCNT had a lack of hydrophilic groups on their surface and showed very poor solubility in aqueous solutions even after 2 h of sonification. The high van der Waals attraction between the individual SWCNT[39,40] caused them to stack in a bundle or rope formation thereby, limiting their surface availability for DNA adsorption. Both SWCNT-PEG and SWCNT-COOH had superior solubility in water and freely dispersed as individual tubes, but SWCNT-PEG contained 30 wt% PEG on the surface which may block the π–π stacking with the nucleobases. The minimum adsorption of DNA yielded low quenching efficiency. On the other hand, SWCNT-COOH only contains 3–6 atom% carboxylic acid, leaving most of the surface available for DNA adsorption thereby producing a large quenching yield. Therefore, SWCNT-COOH could be utilized to construct a turn-off sensing platform for Pb^{2+} with the 8–17 DNAzyme.

Pb^{2+} sensing with SWCNT-COOH and DNAzyme

A schematic illustration of the SWCNT-based Pb^{2+} sensing method is presented in Fig. 2. The substrate (blue) is labeled with Cy3 and hybridizes to the 8–17 DNAzyme (green) through a complementary region containing a total of 18 nucleotides (nt).

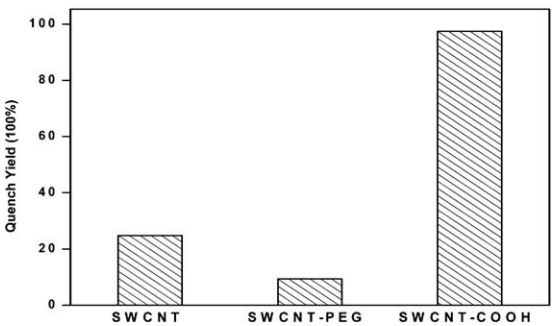

Fig. 1 Comparison of quenching yields obtained with different types of SWCNT at a concentration of 4.2 μg mL^{-1}.

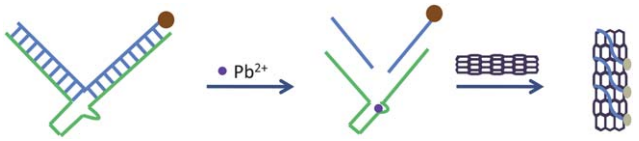

Fig. 2 Schematic illustration using the Cy3 (red dot)-labeled substrate (blue), 8–17 DNAzyme (green), and SWCNT-COOH for Pb^{2+} detection.

The resulting dsDNA is not readily adsorbed onto the SWCNT-COOH thus emitting high fluorescence. In the presence of Pb^{2+}, the enzyme cuts the substrate into half and the released single-stranded product is adsorbed onto the SWCNT-COOH. The fluorescence is completely quenched, and the signal reduction is proportional to the lead concentration. To optimize the quenching effect, we investigated the amounts of SWCNT-COOH and DNA used in our assay. The enzyme-substrate hybrid (17SE) and the substrate 17S were mixed with SWCNT-COOH at varying ratios. The higher the DNA/SWCNT-COOH ratio (nmol mg^{-1}), the higher the fluoresence intensity because the surface area available for adsorption and quenching was smaller (insert of Fig. 3). The quenching yield, $P\% = (F_{17SE} - F_{17S})/F_{17SE} \times 100\%$, reached a maximum at a DNA/SWCNT-COOH ratio of 6 : 1, and dropped substantially when the ratio was higher than 10 : 1 (Fig. 3). Since the 17E contained a loop structure, the catalytic core, which was not complementary to the substrate, the not-perfect match between the 17E and 17S may have left more residue of ssDNA after hybridization and thus the maximum quenching yield was only ~71%, lower than the 97% yield obtained in the above study with the perfectly matched 17S and 17D strands. In the following study, we used the DNA/SWCNT-COOH ratio of 10 : 1 to achieve a relatively high quenching yield with less consumption of SWCNT-COOH.

Sensing performance

The sensing performance of our assay was then tested with microtiter plates. Solutions with different concentrations of Pb^{2+}

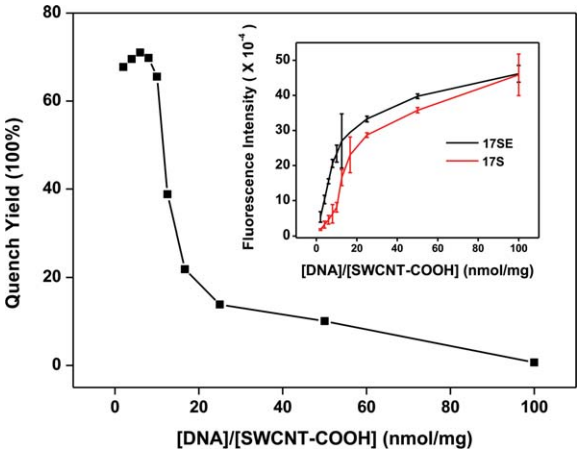

Fig. 3 Quenching yield obtained from different ss- and dsDNA to SWCNT ratio. The concentration of SWCNT-COOH and DNA varied between 1.0–10 μg mL^{-1} and 20–100 nM. The ratios of Cy3–17S or Cy3–17SE (in nmol) to SWCNT-COOH (in mg) were 2, 4, 6, 8, 10, 12.5, 25, 50, and 100. Inset: Plot of fluorescence intensity. The average values and standard deviations (STD, presented as error bars) were obtained from triplicate measurements.

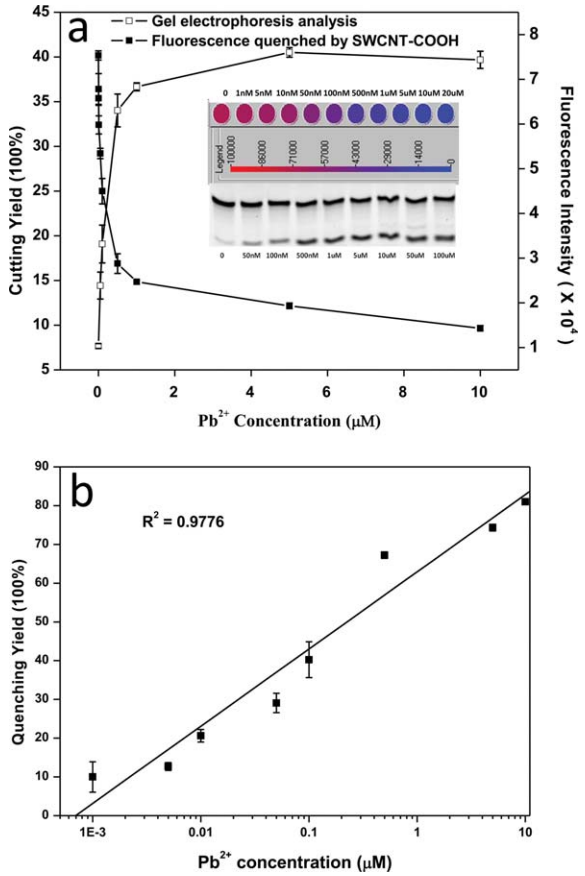

Fig. 4 (a) Cutting yield of Pb^{2+} at a concentration range of 1 nM to 10 μM was obtained by gel electrophoresis (open square), and fluorescence intensity (black square) was measured using a plate reader. The concentration of 17E and Cy3–17S was kept at 50 nM for both tests. Inset: Fluorescence image of the 96-well plate (top) and gel image (bottom). (b) Calibration curve obtained using the quenching yield from the SWCNT-based assay for Pb^{2+} quantification. Each point represents the average and STD of 5 measurements.

were added to the mixture of SWCNT-COOH and 17SE. To further confirm that the quenching was due to substrate cleavage catalyzed by Pb^{2+}, the reaction mixtures were analyzed with 20% denaturing polyacrylamide gel electrophoresis (PAGE) and the cleavage yields were measured. The fluorescence intensity image was obtained by the plate reader and the gel picture was shown in Fig. 4a. The fluorescence reading and cleavage yield were plotted against Pb^{2+} concentration in Fig. 4b. The gel analysis verified that the decrease in fluorescence was due to the cleavage of substrate. We calculated the quenching yield ($(F_{blank} - F_{signal})/F_{blank} \times 100\%$), and found that it increased with the Log [Pb^{2+}] at a concentration range of over 5 orders of magnitude (Fig. 4b). The linear dynamic range was from 5 nM to 1 μM ($R^2 = 0.99$ for the five concentration points within this range). The lowest detectible Pb^{2+} concentration using our assay was 1 nM, which resulted in a fluorescence quenching yield of 10%.

We also evaluated the selectivity of our method. The decrease in fluorescence resulting from the presence of six divalent cations, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+}, were compared with that from Pb^{2+} (Fig. 5a). Only Zn^{2+} at 5 μM led to a quenching yield a little bit higher than 5%. All other interfering cations had negligible quenching effect. Moreover, no interference was observed to the quenching yield measurement when 5 μM of interfering cation was added together with 100 nM Pb^{2+} to the Cy3–17SE hybrid solution. The interfering metal ions could all be the cofactors for the 8–17 DNAzyme, but they react much slower than Pb^{2+}, as discovered by other research groups.[41,42]

Our assay has demonstrated excellent sensitivity and selectivity for Pb^{2+} detection. To verify its applicability, we measured the Pb^{2+} content of tap water samples collected in a chemistry laboratory and at a Riverside household, and compared our results with those obtained by the gold standards in metal quantification, ICP-MS and ICP-AES. The water taps were unused for at least 6 h prior to collection to allow water to reside in the pipes to ensure sufficient metal diffusion for a long enough period. No further purification treatment was applied prior to measurement. The household tap water contained much lower levels of metal than the tap water sample tested, and was therefore analysed by the more sensitive technique of ICP-MS instead of ICP-AES, which was applied to screen the tap water collected in our lab. Cations of Mg^{2+}, Zn^{2+}, Ni^{2+}, and Mn^{2+} were present in the samples (Table S1, ESI)† at levels ranging from 4 to 170 μM, but did not affect the Pb quantification with our assay. The Pb concentrations were calculated using standard curves prepared within the same day. The results are listed in Table 2. We compared the mean values obtained with different methods using the Student's *t* test, and concluded that results of our assay agreed with those of the ICP tests within experimental error at a confidence level of 95% (ICP-MS) and 99% (ICP-AES).

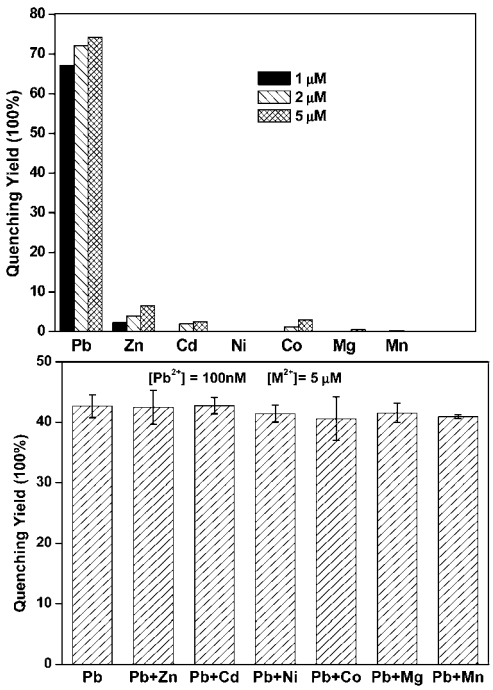

Fig. 5 Selectivity of our assay towards Pb^{2+}. (a) Quenching yields obtained with single cation at concentrations of 1, 2, and 5 μM. (b) Quenching yields obtained with a mixture of 5 μM interfering cation and 100 nM Pb^{2+}. Triplicate tests were performed.

Table 2 Quantification of Pb content in tap water by SWCNT-based assay and by ICP-AES or ICP-MS

	Current assay test (nM) ($n = 3$)	ICP test (nM) ($n = 3$)
UCR CS	226.0 ± 8.8	192.9 ± 5.0
Riverside household	13.2 ± 0.1	14.9 ± 0.0

Conclusions

A simple, selective, and sensitive assay for the detection of Pb^{2+} was developed utilizing the superior fluorescence quenching and DNA-binding abilities of SWCNT-COOH. Our assay can determine Pb^{2+} contents at levels lower than the toxic level defined by the US Environmental Protection Agency (72 nM), with only one fluorophore labeling. The good selectivity towards Pb^{2+} requires minimum sample preparation, and along with the optical signaling format, permits on-site sample detection.

Acknowledgements

The work was supported by the start-up fund provided by the University of California, Riverside to W. Zhong. The authors would like to thank Dr Steven Bates and Professor Timothy W. Lyons from the Department of Earth Sciences, UCR for their assistance in ICP-MS measurement. The Riverside household water was kindly provided by Ms. Leanna Rucker, a high school student from John W. North High, Riverside, CA.

Notes and references

1 V. N. Houk, *J. Pediatr.*, 1978, **93**, 709–720.
2 Y. Shibata, *Jpn. J. Tox. Env. Health.*, 1996, **42**, 385–401.
3 H. F. Hsieh, W. S. Chang, Y. K. Hsieh and C. F. Wang, *Talanta*, 2009, **79**, 183–188.
4 A. I. Stoica, P. Capota and G. E. Baiulescu, *Anal. Lett.*, 2000, **33**, 3025–3035.
5 S. Deo and H. A. Godwin, *J. Am. Chem. Soc.*, 2000, **122**, 174–175.
6 P. Chen, B. Greenberg, S. Taghavi, C. Romano, D. Van der Lelie and C. A. He, *Angew. Chem., Int. Ed.*, 2005, **44**, 2715–2719.
7 I. B. Kim, A. Dunkhorst, J. Gilbert and U. H. F. Bunz, *Macromolecules*, 2005, **38**, 4560–4562.
8 J. Li and Y. Lu, *J. Am. Chem. Soc.*, 2000, **122**, 10466–10467.
9 A. K. Brown, J. Li, C. M. B. Pavot and Y. Lu, *Biochemistry*, 2003, **42**, 7152–7161.
10 R. Metivier, I. Leray and B. Valeur, *Chem. Commun.*, 2003, (8), 996–997.
11 J. W. Liu and Y. Lu, *Anal. Chem.*, 2003, **75**, 6666–6672.
12 J. W. Liu and Y. Lu, *J. Fluoresc.*, 2004, **14**, 343–354.
13 J. W. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2004, **126**, 12298–12305.
14 T. Li, E. Wang and S. Dong, *Anal. Chem.*, 2010, **82**, 1515–1520.
15 W. E. Purtha, R. L. Coppins, M. K. Smalley and S. K. Silverman, *J. Am. Chem. Soc.*, 2005, **127**, 13124–13125.
16 Y. M. Wang and S. K. Silverman, *Biochemistry*, 2005, **44**, 3017–3023.
17 Y. F. Li, Y. Liu and R. R. Breaker, *Biochemistry*, 2000, **39**, 3106–3114.
18 G. M. Emilsson and R. R. Breaker, *Cell. Mol. Life Sci.*, 2002, **59**, 596–607.
19 A. V. Sidorov, J. A. Grasby and D. M. Williams, *Nucleic Acids Res.*, 2004, **32**, 1591–1601.
20 B. Cuenoud and J. W. Szostak, *Nature*, 1995, **375**, 611–614.
21 D. J. F. Chinnapen and D. Sen, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **101**, 65–69.
22 K. E. Nelson, P. J. Bruesehoff and Y. Lu, *J. Mol. Evol.*, 2005, **61**, 216–225.
23 J. Li, W. C. Zheng, A. H. Kwon and Y. Lu, *Nucleic Acids Res.*, 2000, **28**, 481–488.
24 J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 9838–9839.
25 S. Seetharaman, M. Zivarts, N. Sudarsan and R. R. Breaker, *Nat. Biotechnol.*, 2001, **19**, 336–341.
26 D. Zhang, M. G. Deng, L. Xu, Y. Y. Zhou, J. Yuwen and X. Zhou, *Chem.–Eur. J.*, 2009, **15**, 8117–8120.
27 T. Li, S. J. Dong and E. Wang, *Anal. Chem.*, 2009, **81**, 2144–2149.
28 J. W. Liu, A. K. Brown, X. L. Meng, D. M. Cropek, D. J. Istok, D. B. Watson and Y. Lu, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 2056–2061.
29 A. K. Brown, J. W. Liu, Y. He and Y. Lu, *ChemBioChem*, 2009, **10**, 486–492.
30 L. Shen, Z. Chen, Y. Li, S. He, S. Xie, X. Xu, Z. Liang, X. Meng, Q. Li, Z. Zhu, M. Li, X. C. Le and Y. Shao, *Anal. Chem.*, 2008, **80**, 6323–6328.
31 D. A. Britz and A. N. Khlobystov, *Chem. Soc. Rev.*, 2006, **35**, 637–659.
32 C. F. Cao, J. H. Kim, D. Yoon, E. S. Hwang, Y. J. Kim and S. Baik, *Mater. Chem. Phys.*, 2008, **112**, 738–741.
33 G. I. Dovbeshko, O. P. Repnytska, E. D. Obraztsova and Y. V. Shtogun, *Chem. Phys. Lett.*, 2003, **372**, 432–437.
34 M. J. O'Connell, S. M. Bachilo, C. B. Huffman, V. C. Moore, M. S. Strano, E. H. Haroz, K. L. Rialon, P. J. Boul, W. H. Noon, C. Kittrell, J. Ma, R. H. Hauge, R. B. Weisman and R. E. Smalley, *Science*, 2002, **297**, 593–596.
35 R. H. Yang, Z. W. Tang, J. L. Yan, H. Kang, Y. Kim, Z. Zhu and W. Tan, *Anal. Chem.*, 2008, **80**, 7408–7413.
36 A. A. Talin, P. M. Dentinger and F. E. Jones, *J. Vac. Sci. Technol., B*, 2004, **22**, 3107–3111.
37 R. H. Yang, J. Y. Jin, Y. Chen, N. Shao, H. Kang, Z. Xiao, Z. Tang, Y. Wu, Z. Zhu and W. Tan, *J. Am. Chem. Soc.*, 2008, **130**, 8351–8358.
38 M. I. Pividori and S. Alegret, *Top. Curr. Chem.*, 2005, **260**, 1–36.
39 F. Wang, G. Dukovic, L. E. Brus and T. F. Heinz, *Science*, 2005, **308**, 838–841.
40 L. A. Girifalco and M. Hodak, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2002, **65**, 125404–125408.
41 H. K. Kim, J. Liu, J. Li, J. Li, N. Nagraj, M. Li, C. M. B. Pavot and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 6896–6902.
42 H. K. Kim, I. Rasnik, J. Liu, T. Ha and Y. Lu, *Nat. Chem. Biol.*, 2007, **3**, 763–768.

