small NANO MICRO

EcoRI-Modified Gold Nanoparticles for Dual-Mode Colorimetric Detection of Magnesium and Pyrophosphate Ions

Hongbo Wang, Wei Xu, Hao Zhang, Dawei Li, Zhaoqi Yang, Xiaoji Xie, Tianhu Li, and Xiaogang Liu**

Magnesium in its ionic form (Mg^{2+}) is essential for many physiological processes, including much of metabolism, enzyme activation and catalysis, photosynthesis development, signal transduction, and protection against hypertension and blood vessel spasm.[1] Therefore, it has been long recognized that for suitable diagnosis of various ailments, the accurate and rapid measurement of Mg^{2+} is important. Additionally, the ability to detect Mg^{2+} is also important in the area of environmental monitoring for effective pollution control.[2] Colorimetric methods are known for the detection of Mg^{2+} in various fluids.[3] These methods usually involve the use of a metal-complexing reagent, such as thymolphthalein or arsenazo III, which forms a colored complex with Mg^{2+} present in fluids.[3] However, these methods in general require sample pretreatment, and lack detection sensitivity and selectivity. For example, proteins and calcium ions present in fluids can also complex with magnesium complexing dyes, thereby causing interference in magnesium detection.[4] Thus, a rapid, inexpensive, selective, and sensitive method that permits real-time detection of Mg^{2+} still remains elusive.

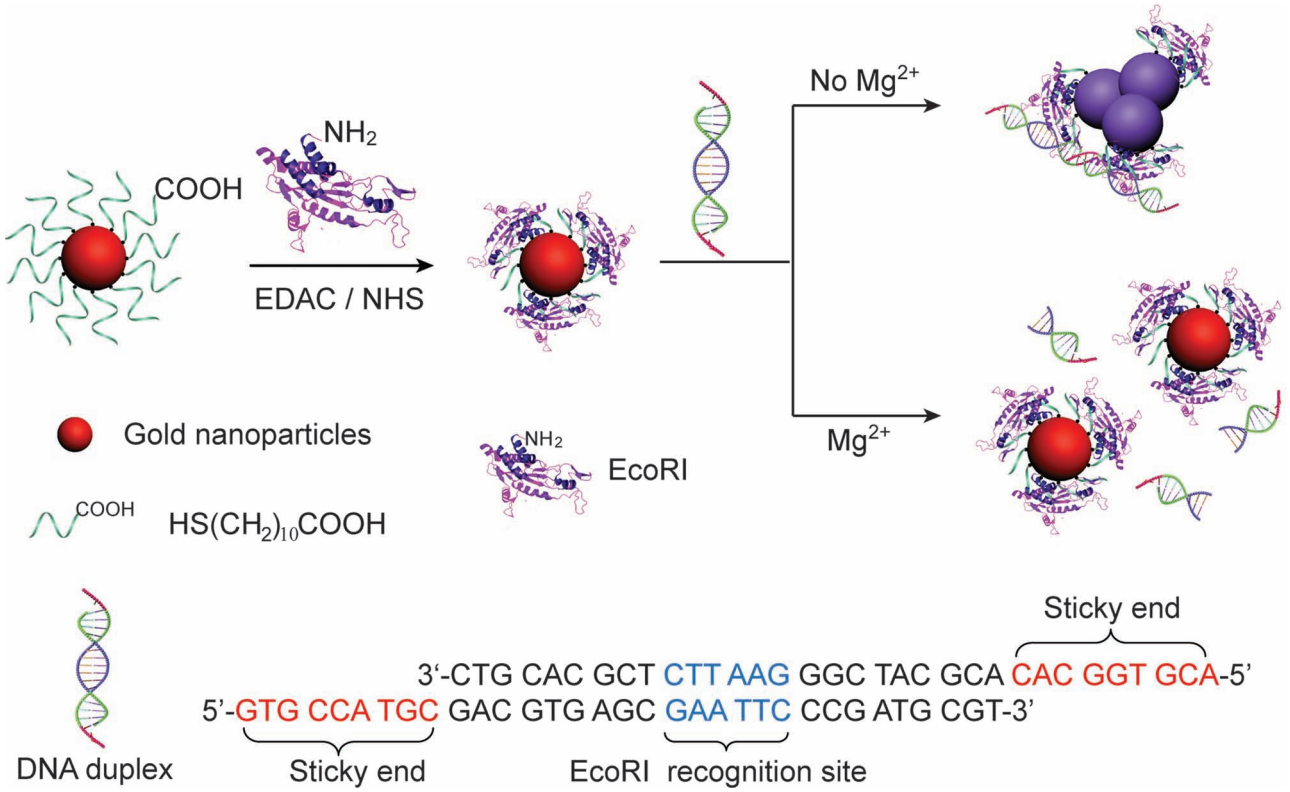
Recent advances in gold nanoparticles functionalized with thiolated DNA have enabled colorimetric detection of biological species and heavy metal ions.[5] For detection or screening of heavy metal ions, the colorimetric sensing technique offers several distinct advantages over other methods such as spectrophotometric analysis and ion chromatography.[6] This technique requires minimal sample preparation and offers a simple, convenient method for detection.[7] Good detection limits and wide dynamic ranges have been demonstrated in real time and in complex media. It is also capable of providing a multiplex assay for the simultaneous detection of trace heavy metal ions.[8] However, the method for rapid identification of divalent alkaline earth metal ions is significantly less exploited. Here, we address this issue by developing a platform based on a combination of an EcoRI-modified nanoparticle and a double-stranded DNA with sticky ends; EcoRI is an enzyme obtained from *Escherichia coli*. Divalent magnesium ions in the sub-micromolar concentration range can be readily detected using our approach. We also demonstrate the utilization of this nanoparticle system for rapid screening of physiologically important pyrophosphate ions.

The basic design for detecting Mg^{2+} is composed of EcoRI-modified nanoparticles and a specifically designed double-stranded DNA (**Scheme 1**). The DNA duplex contains an EcoRI-recognition site and complementary sticky ends that can pair with each other when mixed.[9] Upon addition of the nanoparticles to the DNA duplex, we anticipate that particle aggregation occurs due to the EcoRI–DNA binding and spontaneous pairing of DNA sticky ends. A visual, colorimetric readout, as a result, would be possible. However, in the case of a sufficient amount of magnesium ions present in solution, the solution color may remain intact because the divalent Mg^{2+} can serve as a cofactor for activating enzymatic cleavage of the double-stranded DNA.[10] This high-specificity nature of Mg^{2+}-mediated DNA cleavage would enable selective detection of Mg^{2+} among other metal ions.

As a proof-of-concept experiment, the EcoRI conjugation was first carried out by coupling 1-mercaptoundecanoic-acid-stabilized nanoparticles (~14 nm) with EcoRI (~0.1 μM) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) and *N*-hydroxysuccinimide (NHS). The bioconjugated nanoparticles resulted in no color change and no noticeable shift in the surface plasmon band in the absorbance spectrum (Supporting Information, Figure S1). The EcoRI-modified nanoparticles were then incubated with an aliquot of magnesium chloride (250 pmol) for 5 min, at which time a solution of the DNA duplex (15 pmol) was then added to the mixture. As anticipated, no particle aggregation was observed for the sample containing Mg^{2+} (**Figure 1**a). In contrast, solution-based control investigation showed

H. Wang, W. Xu, X. Xie, Prof. X. Liu
Department of Chemistry
National University of Singapore
3 Science Drive 3, Singapore 117543
E-mail: chmlx@nus.edu.sg
H. Zhang, D. Li, Z. Yang, Prof. T. Li
Division of Chemistry & Biological Chemistry
School of Physical & Mathematical Sciences
Nanyang Technological University
Singapore 637371
Email: thli@ntu.edu.sg
Prof. X. Liu
Institute of Materials Research and Engineering
3, Research Link, Singapore 117602

DOI: 10.1002/smll.201100470



Scheme 1. Rational design of gold-nanoparticle-based colorimetric detection of magnesium ions.

that nanoparticles immediately aggregated in the absence of Mg^{2+}. The particle aggregation suggests that the sticky end-paired DNA duplex is bound to the EcoRI-modified nanoparticles. In the UV–vis spectrum, the plasmon absorption shifted from 520 to 570 nm and broadened in comparison with the dispersed nanoparticle solution (Figure 1b). Transmission electron microscopy (TEM) images showed these aggregated nanoparticles and confirmed that the nanoparticle aggregation is a consequence of simultaneous EcoRI–DNA binding and sticky end pairing under these conditions (Figure 1c).

To determine if the detection system would provide selectivity for Mg^{2+} over other metal ions, we tested the response of a variety of metal ions including monovalent ions (K^+ and Na^+), divalent ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Mn^{2+}, and Ni^{2+}), and trivalent ion (Fe^{3+}). Results from trials showed that particle aggregation was consistently observed when other metal ions were introduced to the nanoparticle solution (Figure 1d). In the absence of magnesium ions, the nanoparticles efficiently bound to DNA, but enzymatic cleavage did not occur. The remarkable degree of detection specificity can be attributed to the strong catalytic activity dependence of EcoRI on the presence of magnesium ions.[9] The replacement of Mg^{2+} with other metal ions leads to reduced catalytic activity for DNA cleavage.

To improve the detection limit and probe the dynamic range of the assay, a chip-based scanometric system was developed by using a flatbed scanner (Supporting Information, Figure S2). In a typical experiment, a glass microscope slide was first modified with a capture DNA strand. Aqueous solutions containing EcoRI-modified gold nanoparticles, a linkerDNA strand, and different concentrations of magnesium ions were then spotted onto the glass slide. After incubation for 4 h in a DNA hybridization chamber, the glass slide was rinsed in buffer and subsequently subjected to silver amplification. As expected, without added Mg^{2+}, significant darkening of the spotted surface was observed (**Figure 2**a). This indicates the specific binding of EcoRI-modified nanoparticles to the DNA duplex that contains an EcoRI recognition site. In contrast, in the presence of Mg^{2+}, enzymatic cleavage of the DNA duplex was observed and the cleavage efficiency increased with increasing Mg^{2+} concentration (0.1–100 μM) as measured by the grayscale intensities of the spotted areas (Figure 2a).

Importantly, EcoRI-modified nanoparticles have remained dispersed in solution after storage at 4° C for over one week with no indication of enzyme deactivation or marked particle aggregation. The bioactivity of EcoRI-modified nanoparticles after several days of storage was confirmed by [^{32}P]-labeled denaturing polyacrylamide gel electrophoresis (PAGE, 20%). As shown in Figure 2b, even after 8 days of storage, the EcoRI-modified nanoparticles efficiently cleaved the DNA duplex into fragments under the optimum reaction conditions (Figure 2b). Another intriguing property of the EcoRI-modified nanoparticles is their ability to be recycled. After the presence of Mg^{2+} was confirmed, ethylenediaminetetraacetic acid (EDTA) was added to the solution to uptake the Mg^{2+} occupied at the EcoRI-binding site. The noncomplexed EcoRI-modified nanoparticles were isolated by centrifugation and redispersed for repeated use. The recycled nanoparticles showed consistent catalytic

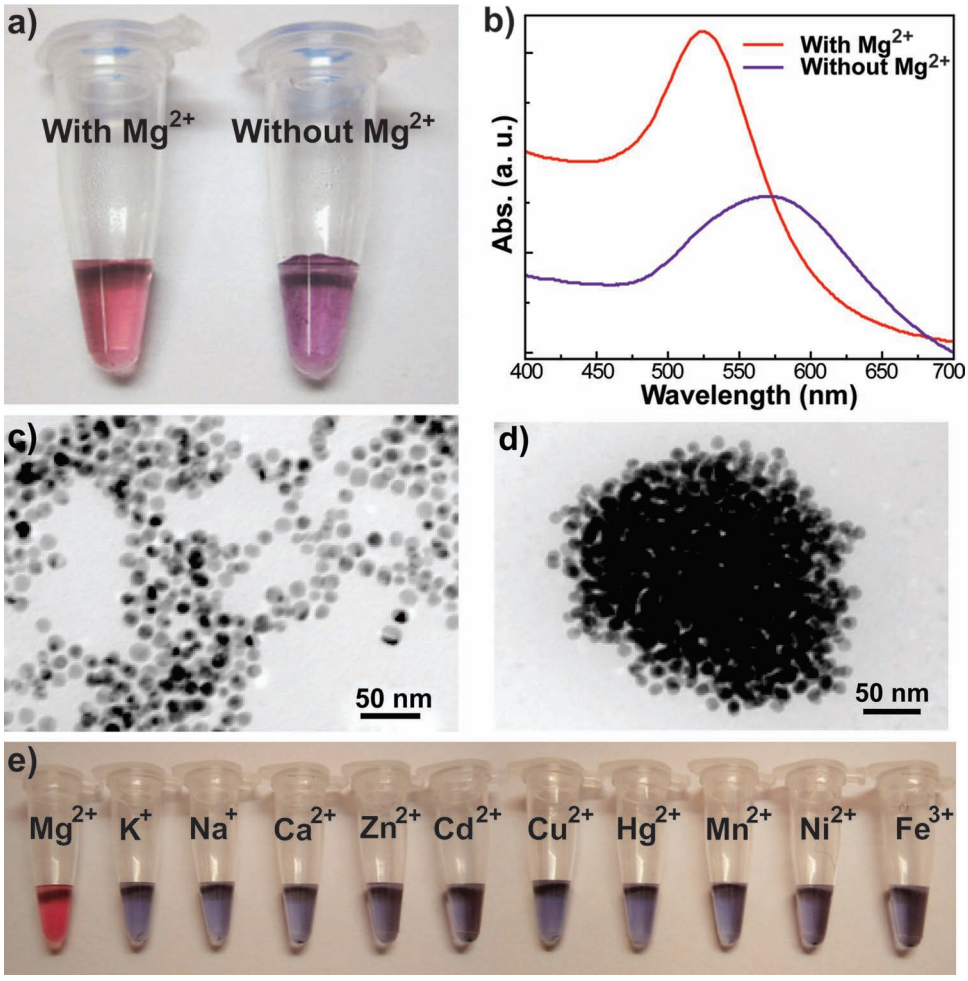


Figure 1. Colorimetric detection of Mg^{2+}. a) Color response of a 14-nm nanoparticle detection system (~4 nM particles; 0.3 μM DNA duplex) in the presence (5 μM) or absence of Mg^{2+}. b) The corresponding UV–vis spectra of the particle solutions with or without Mg^{2+}. c,d) The corresponding TEM images taken for the samples with and without Mg^{2+}. e) Colorimetric response of the detection system (~4 nM particles; 0.3 μM DNA duplex) in the presence of a selection of metal ions (10 μM each).

activity for three consecutive rounds, as evidenced by the absorbance spectra recorded for each particle aggregation step in comparison with that of the redispersed particle solution (Figure 2c).

Apart from providing a convenient detection method for Mg^{2+}, the EcoRI-modified particle system is useful for pyrophosphate ion (PPi: $P_2O_7^{4-}$) screening. PPi plays an important role in a wide range of chemical and biological process, and is well known to inhibit enzyme activity, primarily due to its complexation with Mg^{2+}.[11] Thus, the design of enzyme deactivation through Mg^{2+} complexation with PPi, coupled with gold nanoparticles, can be developed for colorimetric PPi detection. As a demonstration of this concept, we first added different amounts of PPi (0–1.5 nmol) to a solution of Mg^{2+} (250 pmol). The resulting mixture was then transferred to a solution of gold nanoparticles followed by the addition of the EcoRI-recognition DNA duplex. We observed a gradual, visible color change from red to blue with increased PPi content, indicating that enzyme cleavage was disrupted by Mg–PPi complexation (**Figure 3**a). On the basis of this approach, the colorimetric detection of PPi at concentration down to 10 μM could be achieved. The addition of PPi to the particles led to the gradual shift in the absorbance band corresponding to DNA-duplex-bound nanoparticles (Figure 3b). To validate the anion selectivity of the sensor system, we tested a variety of anions including monovalent anions (F^-, Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, HCO_3^-, $H_2PO_4^-$, CH_3COO^-), divalent anions (SO_4^{2-}, HPO_4^{2-}), and tetravalent PPi as the control. Upon addition of each anion (50 μM) to the particles, a noticeable color change from red to blue was observed only for the solution with the PPi content (Figure 3c).

In conclusion, we have presented a rapid colorimetric method, based on EcoRI-modified nanoparticles and DNA sticky end pairing, for magnesium ion detection. When combined with the scanometric technique, this method can detect the presence of Mg^{2+} at concentrations as low as 0.1 μM. The EcoRI-modified nanoparticles have also shown utility for rapid PPi sensing over a variety of potentially interfering anions. The high selectivity and excellent stability of the particle system should enable a broad spectrum of potential applications in the monitoring and detection

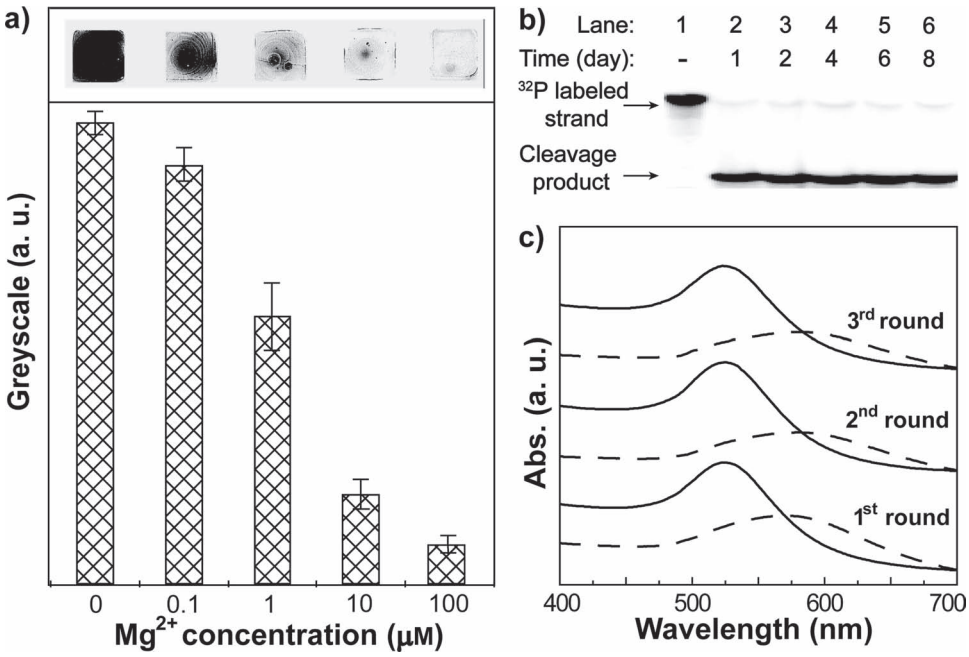


Figure 2. a) Top: scanometric images taken after silver enhancement of the DNA-modified glass slide incubated with EcoRI-modified nanoparticles in the presence of various concentrations of Mg^{2+} (0, 0.1, 1, 10, and 100 μM). Bottom: the corresponding grayscale values of darkened areas obtained as a function of Mg^{2+} concentration. b) Autoradiogram of polyacrylamide-gel-separated products obtained from DNA cleavage experiments using EcoRI-modified nanoparticles stored for different periods of time (Lane 1: control experiment without the addition of EcoRI-modified nanoparticles). c) UV–vis spectra of the recycled particle solutions for the redispersed EcoRI-modified nanoparticles (solid lines) in comparison to particle aggregation (dashed lines).

for magnesium and pyrophosphate ions in complex settings. We also envision that the design of the detection system can be extended to other types of functional nanomaterials such as luminescent quantum dots and upconversion nanoparticles.[12]

Experimental Section

EcoRI-Modified Gold Nanoparticles for Mg^{2+} Detection: In a typical experiment, a solution-based detection system containing EcoRI-modified particles and a specific DNA duplex with sticky ends

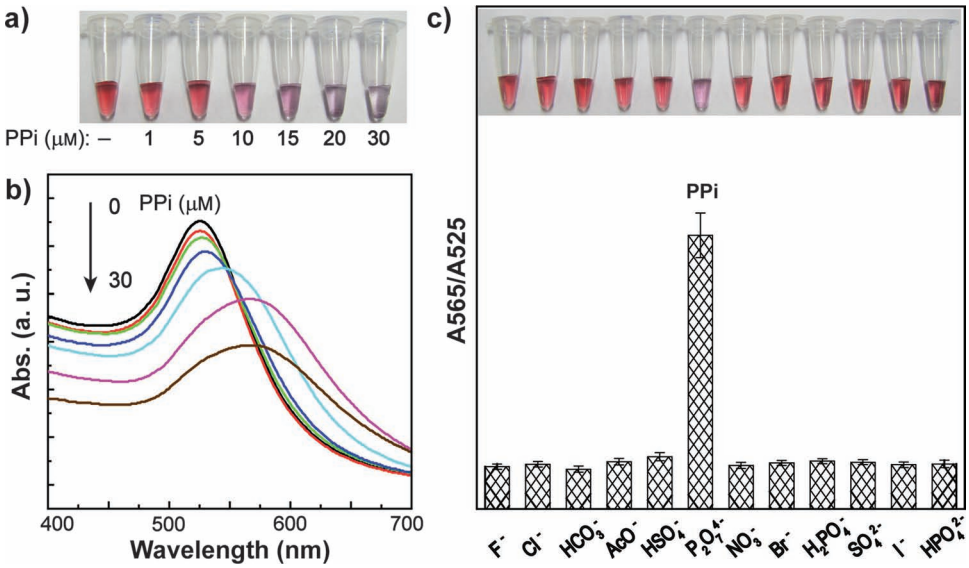


Figure 3. a) Colorimetric response of the detection system (~4 nm particles; 0.3 μM DNA duplex) in the presence of various PPi contents (0–30 μM). b) The corresponding UV–vis spectra of the particle solutions in the presence of different PPi concentrations. c) Top: colorimetric response of the particle solutions containing different anions. Bottom: the corresponding UV–vis absorption ratio of the particle solution at 565 to 525 nm as a function of different anion (50 μM each).

NANO MICRO small

was used for colorimetric detection of magnesium ions. The DNA duplex was prepared by mixing and annealing two single-stranded sequences (5′-GTG CCA TGC GAC GTG AGC GAA TTC CCG ATG CGT-3′ and 5′-GCA GGT CAC ACG CAT CGG GAA TTC GCT CAC GTC-3′, 10 μM each in 10 mM phosphate buffer (pH 7.0), and 0.3 M NaCl) at 90 °C for 5 min, followed by slow cooling to room temperature. The EcoRI-modified nanoparticles (~4 nM, 48 μL) were then incubated with a 0.5-μL solution of magnesium chloride (0.5 mM) for 5 min, at which time a 1.5-μL solution of the double-stranded DNA (10 μM) was then added to the mixture. The colorimetric response of the solution was recorded after 5 min.

PAGE Analysis:[13] The strand scission process for the EcoRI-recognition DNA was monitored by denaturing PAGE with autoradiography. In a typical experiment, an oligonucleotide strand (5′-GTG CCA TGC GAC GTG AGC GAA TTC CCG ATG CGT-3′; 100 pmol) was first labeled with [γ-^{32}P]-ATP at the 5′ end in the presence of T4 polynucleotide kinase (T4 PNK), followed by purification by electrophoresis through a denaturing 20% polyacrylamide gel. The slice of band containing the [^{32}P]-labeled DNA strand was cut out of the gel and eluted by soaking in tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl; 10 mM, pH 7) for 2 h, followed by purification by gel filtration chromatography (NAP-25, GE Healthcare) eluting with ultrapure water. The purified [^{32}P]-labeled strand (12.5 pmol) was mixed with a solution of EcoRI-modified nanoparticles (4 nM) in the presence of another single-stranded DNA (5′-GCA GGT CAC ACG CAT CGG GAA TTC GCT CAC GTC-3′; 12.5 pmol). Upon incubation at 37 °C for 1 h, the reaction mixture was centrifuged and the particles were separated. An aliquot of 6 μL of denaturing loading buffer (95% v/v formamide, 18 mM EDTA, 0.025% w/w sodium dodecyl sulfate, 10% w/w xylene cyanole FF, and bromophenol blue) was then added to the resulting solution. The mixture was stored at −20 °C for gel electrophoresis. The reaction products were analyzed by electrophoresis in a 20% polyacrylamide gel. Autoradiographic images were analyzed and quantified using the ImageQuant software.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work is supported by the National University of Singapore (R-143-000-427), the Ministry of Education (R-143-000-453, M45110014, and M58110042), and the Singapore-MIT Alliance.

This Communication is part of the Special Issue dedicated to Chad Mirkin in celebration of 20 years of influential research at Northwestern University.

[1] a) C. A. Smith, I. Rayment, *Biochemistry* **1996**, *35*, 5404; b) J. Durlach, P. Bac, V. Durlach, M. Bara, A. GuietBara, *Magnesium. Res.* **1997**, *10*, 169; c) B. Biteau, J. Labarre, M. B. Toledano, *Nature* **2003**, *425*, 980; d) E. G. Vescovi, F. C. Soncini, E. A. Groisman, *Cell* **1996**, *84*, 165; e) N. Mochizuki, R. Tanaka, A. Tanaka, T. Masuda, A. Nagatani, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 15184.

[2] a) H. Siren, S. Vantsi, *J. Chromatogr. A.* **2002**, *957*, 17; b) Y. Izumi, F. Kiyotaki, T. Minato, Y. Seida, *Anal. Chem.* **2002**, *74*, 3819; c) D. Yan, L. Yang, Q. Wang, *Anal. Chem.* **2008**, *80*, 6104.

[3] a) Z. O. Tesfaldet, J. F. van Staden, R. I. Stefan, *Talanta* **2004**, *64*, 981; b) R. Shen, X. Pan, H. Wang, L. Yao, J. Wu, N. Tang, *Dalton. Trans.* **2008**, *27*, 3574; c) F. B. Gomez, F. B. Reig, P. C. Falco, *Talanta* **1999**, *49*, 155; d) E. G. Lamkin, M. B. Williams, *Anal. Chem.* **1965**, *37*, 1029.

[4] a) A. Feofanov, A. Lanoul, V. Oleinikov, S. Gromov, O. Fedorova, M. Alfimov, I. Nabiev, *J. Phys. Chem.* **1996**, *100*, 2154; b) K. M. Hirshfield, D. Toptygin, G. Grandhige, H. Kim, B. Z. Packard, L. Brand, *Biophys. Chem.* **1996**, *62*, 25; c) K. Ohta, K. Tanaka, *Anal. Chim. Acta.* **1998**, *373*, 189; d) E. Nemutlu, N. Ozaltin, *Anal. Bioanal. Chem.* **2005**, *383*, 833.

[5] a) C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607; b) D. J. Maxwell, J. R. Taylor, S. Nie, *J. Am. Chem. Soc.* **2002**, *124*, 9606; c) N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. R. Lytton-Jean, M. S. Han, C. A. Mirkin, *Science* **2006**, *312*, 1027; d) X. Liu, N. Wu, B. H. Wunsch, R. J. Barsotti, F. Stellacci, *Small* **2006**, *2*, 1046; e) J. Liu, Y. Lu, *J. Am. Chem. Soc.* **2007**, *129*, 8634; f) J. Zhang, L. Wang, D. Pan, S. Song, F. Y. C. Boey, H. Zhang, C. Fan, *Small* **2008**, *4*, 1196; g) W. Zhao, M. M. Ali, M. A. Brook, Y. Li, *Angew. Chem.* **2008**, *120*, 6428; *Angew. Chem. Int. Ed.* **2008**, *47*, 6330; h) X. Qian, X. Zhou, S. Nie, *J. Am. Chem. Soc.* **2008**, *130*, 14934; i) X. Xue, F. Wang, X. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 3244; j) W. Xu, X. Xue, T. Li, H. Zeng, X. Liu, *Angew. Chem.* **2009**, *121*, 6870; *Angew. Chem. Int. Ed.* **2009**, *48*, 6849; k) T. Jiang, R. Liu, X. Huang, H. Feng, W. Teo, B. Xing, *Chem. Commun.* **2009**, *1972*; l) K. J. Jang, H. Lee, H. L. Jin, Y. Park, J. M. Nam, *Small* **2009**, *5*, 2665; m) H. Zhang, R. J. Barsotti, C. Wong, X. Xue, X. Liu, F. Stellacci, J. T. L. Thong, *Small* **2009**, *5*, 2797; n) C. Ananthanawat, T. Vilaivan, V. P. Hoven, X. Su, *Biosens. Bioelectron.* **2010**, *25*, 1064; o) Z. Zhu, C. Wu, H. Liu, Y. Zou, X. Zhang, H. Kang, C. J. Yang, W. Tan, *Angew. Chem.* **2010**, *122*, 1070; *Angew. Chem. Int. Ed.* **2010**, *49*, 1052; p) J. H. Oh, J. S. Lee, *Chem. Commun.* **2010**, *46*, 6382; q) X. Zhou, P. Cao, Y. Tian, J. Zhu, *J. Am. Chem. Soc.* **2010**, *132*, 4161; r) D. Zhu, X. Jiang, C. Zhao, X. Sun, J. Zhang, J. Zhu, *Chem. Commun.* **2010**, *46*, 5226; s) L. Li, H. Liu, Y. Shen, J. Zhang, J. Zhu, *Anal. Chem.* **2011**, *83*, 661; t) X. Xie, W. Xu, T. Li, X. Liu, *Small* **2011**, *7*, 1393; u) X. Xue, F. Wang, X. Liu, *J. Mater. Chem.* **2011**, doi: 10.1039/C1JM11401H.

[6] a) Z. Wang, J. Zhang, J. M. Ekman, Y. Lu, *Nano Lett.* **2010**, *10*, 1886; b) X. Xue, W. Xu, F. Wang, X. Liu, *J. Am. Chem. Soc.* **2009**, *131*, 11668; c) N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**, *105*, 1547; d) R. Kanjanawarut, X. Su, *Anal. Chem.* **2009**, *81*, 6122; e) J. Y. Kim, J. S. Lee, *Chem. Mater.* **2010**, *22*, 6684; f) Y. P. Kim, L. D. Weston, Z. Xia, H. Xie, C. A. Mirkin, J. Rao, *Chem. Commun.* **2010**, *46*, 76; g) D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, C. A. Mirkin, *Angew. Chem.* **2010**, *122*, 3352; *Angew. Chem. Int. Ed.* **2010**, *49*, 3280; h) F. Wang, D. Banerjee, Y. Liu, X. Chen, X. Liu, *Analyst* **2010**, *135*, 1839; i) F. Wang, X. Liu, *Chem. Soc. Rev.* **2009**, *38*, 976.

[7] a) J. Liu, Z. Cao, Y. Lu, *Chem. Rev.* **2009**, *109*, 1948; b) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078; c) J. M. Nam, C. S. Thaxton, C. A. Mirkin, *Science* **2003**, *301*, 1884; d) R. Liu, R. S. Liew, H. Zhou, B. Xing, *Angew. Chem.* **2007**, *119*, 8955; *Angew. Chem. Int. Ed.* **2007**, *46*, 8799; e) X. Qian, S. Nie, *Chem. Soc. Rev.* **2008**, *37*, 912; f) J. H. Lee, Z. Wang, J. Liu, Y. Lu, *J. Am. Chem. Soc.* **2008**, *130*, 14217; g) X. Su, R. Kanjanawarut, *ACS Nano* **2009**, *3*, 2751; h) M. Yang, T. Chen, W. S. Lau, Y. Wang, Q. Tang, Y. Yang, H. Chen, *Small* **2009**, *5*, 198; i) K. Wang, Z. Tang, C. J. Yang, Y. Kim, X. Fang,

W. Li, Y. Wu, C. D. Medley, Z. Cao, J. Li, P. Colon, H. Lin, W. Tan, *Angew. Chem.* **2009**, *121*, 870; *Angew. Chem. Int. Ed.* **2009**, *48*, 856; j) S. Kim, J. W. Park, D. Kim, D. Kim, I. Lee, S. Jon, *Angew. Chem.* **2009**, *121*, 4202; *Angew. Chem. Int. Ed.* **2009**, *48*, 4138; k) M. M. Ali, Y. Li, *Angew. Chem.* **2009**, *121*, 3564; *Angew. Chem. Int. Ed.* **2009**, *48*, 3512; l) D. Li, S. Song, C. Fan, *Acc. Chem. Res.* **2010**, *43*, 631.

[8] a) C. Zhao, K. Qu, Y. Song, C. Xu, J. Ren, X. Qu, *Chem. Eur. J.* **2010**, *16*, 8147; b) D. Zhang, M. Deng, L. Xu, Y. Zhou, J. Yuwen, X. Zhou, *Chem. Eur. J.* **2009**, *15*, 8117; c) Y. Chen, H. Chang, Y. Shiang, Y. Hung, C. Chiang, C. Huang, *Anal. Chem.* **2009**, *81*, 9433; d) X. Xu, W. L. Daniel, W. Wei, C. A. Mirkin, *Small* **2010**, *6*, 623; e) Z. Hu, C. Lin, X. Wang, L. Ding, C. Cui, S. Liu, H. Lu, *Chem. Commn.* **2010**, *46*, 3765; f) C. Lin, C. Yu, Y. Lin, W. Tseng, *Anal. Chem.* **2010**, *82*, 6830.

[9] a) C. Mao, W. Sun, N. C. Seeman, *J. Am. Chem. Soc.* **1999**, *121*, 5437; b) H. Yu, H. Liu, Y. Chen, Y. Tian, Z. Deng, S. H. Ko, T. Ye, C. Mao, *Microsc. Res. Techniq.* **2007**, *70*, 522; c) Y. Ke, Y. Liu, J. Zhang, H. Yan, *J. Am. Chem. Soc.* **2006**, *128*, 4414.

[10] J. R. Taylor, M. M. Fang, S. Nie, *Anal. Chem.* **2000**, *72*, 1979.

[11] a) R. K. Airas, *Eur. J. Biochem.* **1996**, *240*, 223; b) R. K. Airas, *Biophys. Chem.* **2007**, *131*, 29.

[12] a) W. C. W. Chan, S. Nie, *Science* **1998**, *281*, 2016; b) F. Wang, Y. Han, C. S. Lim, Y. Lu, J. Wang, J. Xu, H. Chen, C. Zhang, M. Hong, X. Liu, *Nature* **2010**, *463*, 1061; c) X. Ye, J. E. Collins, Y. Kang, J. Chen, D. T. N. Chen, A. G. Yodh, C. B. Murray, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 22430; d) C. Wang, L. Cheng, Z. Liu, *Biomaterials* **2011**, *32*, 1110; e) F. Wang, X. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 5642; f) F. Wang, J. Wang, X. Liu, *Angew. Chem.* **2010**, *122*, 7618; *Angew. Chem. Int. Ed.* **2010**, *49*, 7456; g) J. Wang, F. Wang, J. Xu, Y. Wang, Y. Liu, X. Chen, H. Chen, X. Liu, *C. R. Chim.* **2010**, *13*, 731; h) F. Wang, J. Wang, J. Xu, X. Xue, H. Chen, X. Liu, *Spectrosc. Lett.* **2010**, *43*, 400; i) C. Li, J. Lin, *J. Mater. Chem.* **2010**, *20*, 6831; j) J. Zhou, M. Yu, Y. Sun, X. Zhang, X. Zhu, Z. Wu, D. Wu, F. Li, *Biomaterials* **2011**, *32*, 1148; k) D. Chen, Y. Yu, F. Huang, P. Huang, A. Yang, Y. Wang, *J. Am. Chem. Soc.* **2010**, *132*, 9976; l) C. Li, J. Lin, *J. Mater. Chem.* **2010**, *20*, 6831; m) W. Feng, L. Sun, C. Yan, *Chem. Commun.* **2009**, 4393; n) H. S. Mader, P. Kele, S. M. Saleh, O. S. Wofbeis, *Curr. Opin. Chem. Biol.* **2010**, *14*, 582.

[13] T. Li, D. S. Weinstein, K. C. Nicolaou, *Chem. Biol.* **1997**, *4*, 209.

Received: March 11, 2011
Revised: April 11, 2011
Published online: June 14, 2011