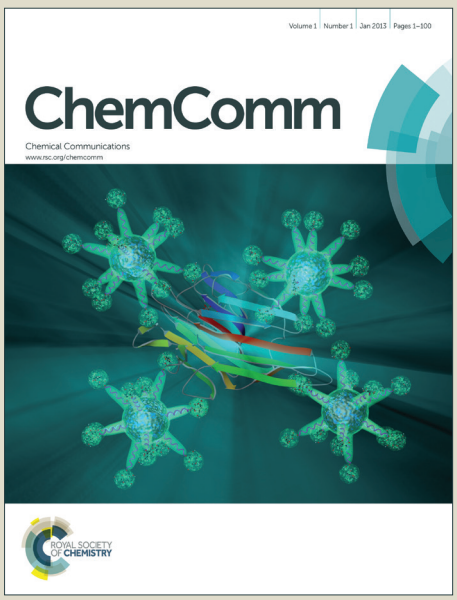
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

Highly Sensitive and Selective Detection of miRNA: DNase I-Assisted Target Recycling using DNA Probes Protected by Polydopamine Nanospheres

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Yi Xie, Xiaoyan Lin, Yishun Huang, Rujun Pan, Zhi Zhu, Leiji Zhou, and Chaoyong James Yang*

Based on the protective property of polydopamine nanospheres for DNA probes against nuclease digestion, we have developed a DNase I-assisted target recycling signal amplification method for highly sensitive and selective detection of miRNA.

MicroRNAs (miRNAs) are single-stranded, endogenously expressed, small noncoding RNAs of ~22 nucleotides. They play important roles as endogenous gene regulators by mediating translation of target mRNAs. It has been estimated that miRNAs can target more than 30% of the human genome. With the great progress of miRNA-related biological studies, miRNAs are being considered as novel biomarkers and potential therapeutic targets for various diseases.[1] As a consequence, sensitive and selective miRNA detection is very significant for miRNA discovery, study and clinical diagnosis.[2] However, miRNA detection is challenged by the characteristics of low cellular abundance and short length. Currently, many methods have been developed for the detection of miRNA, including northern-blotting with LNA probes,[3] nanoparticle amplification methods,[4] quantitative RT-PCR,[5] and so on.[6] Although these methods can detect miRNA with good sensitivity and selectivity, they have several limitations. First, many current techniques require specialized and expensive reagents and time-consuming processes, which are costly and complicated. Second, the ability to distinguish highly related miRNAs is still in great demand to improve selectivity. Third, many methods require relatively clean samples, which preclude *in situ* analysis of miRNAs in complicated biological samples. Therefore, a rapid, simple and cost-effective method for miRNA detection with high sensitivity and selectivity is still in great demand.

Owing to their unique structural, optical and catalytic properties, nanomaterials have been used as novel biosensing platforms.[7] Over the past years, many fluorescent biosensors have been exploited by using nanomaterials as quenchers. Gold nanoparticles (AuNPs),[8] graphene oxide (GO),[9] carbon nanoparticles,[10] and MoS_2 nanosheets[11] have been used as highly efficient nanoquenchers to develop novel fluorescent sensors. More recently, the fluorescence quenching ability of polydopamine nanospheres (PDANSs) has been reported by Xu *et al*, showing that 6-Carboxyfluorescein (FAM) labelled ssDNA can be adsorbed on PDANSs by π-π stacking and resulted in quenching by fluorescence resonance energy transfer.[12] Subsequently, combined with fluorophore-labelled DNA probes, a sensing method for the detection of DNA and proteins was developed based on target recognition-induced desorption and subsequent fluorescence recovery of the DNA probe.[12] Single-stranded DNA has been reported to tightly adsorb on several nanomaterials including grapheme oxide (GO),[9] carbon nanotubes,[13] carbon nanoparticles,[10] MoS_2,[11] and gold nanoparticles.[8] This tight binding also results in the quenching of fluorophores labelled on the ssDNA sequence. Another important property resulting from the adsorption of ssDNA on the surface of these nanomaterials is protection of the DNA probe against nuclease digestion.[9-10] However, up to now, there is no report on the protective properties of PDANSs against enzymatic cleavage of ssDNA. Because it has properties similar to those of the above nanomaterials, we speculated that PDANS may be able to protect ssDNA adsorbed on its surface from nuclease digestion. In this work, we investigated and found that a single-stranded fluorescent DNA probe adsorbed on PDANSs can be effectively protected from enzymatic cleavage. Based on this finding, we have successfully developed a DNase I-assisted target recycling signal amplification method for the analysis of miRNA.

Polydopamine nanospheres were synthesized by self-polymerization of dopamine (Figure S1, ESI) in a mixed solvent of Tris-buffer and isopropyl alcohol, according to a previously reported method.[12] Monodisperse polydopamine nanospheres with a diameter of approximately 250 nm were obtained (Figure S2, ESI). The ssDNA binding and fluorescence quenching ability of PDANSs was confirmed

by adding different concentrations of the nanoparticles to a solution containing 50 nM fluorophore labelled ssDNA probe (5'- AAC TAT ACA ACC TAC TAC CTC A FAM-3'). The sequence of the probe is complimentary to miRNA let-7a (see Table S1). As shown in Figure S3, a dramatic reduction in fluorescence intensity was observed when different concentrations of PDANSs were added, and a quenching efficiency of 99.1% was obtained with 0.33 mg/mL PDANSs.

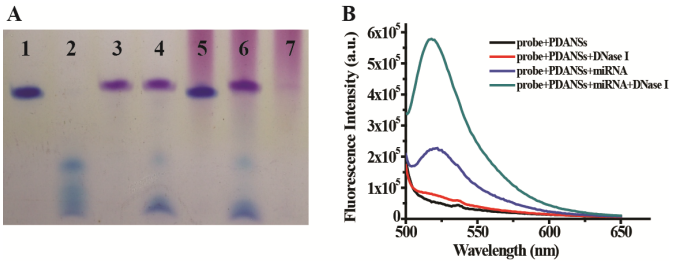
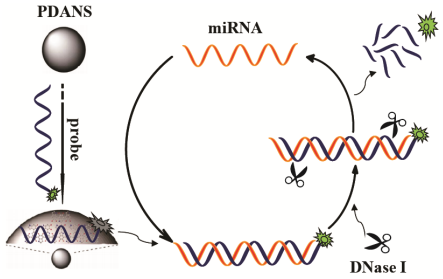


Figure 1. (A) Gel electrophoresis results of probe/miRNA and probe/miRNA-PDANSs with and without DNase I treatment. Lane1: probe, lane2: probe after DNase I digestion, lane3: miRNA after DNase I digestion, lane4: probe + miRNA after DNase I digestion, lane5: probe/ PDANSs complex after DNase I digestion, lane6: probe adsorbed on PDANS + miRNA after DNase I digestion, lane7: PDANSs. (B) Fluorescence emission spectra of probe (50nM) at different conditions.

The protective property of PDANSs for ssDNA probe was then investigated. Free probe, its target miRNA, and mixture of the probe with its target miRNA were each treated with DNase I both without and with PDANSs for 1 hr before being analyzed by gel electrophoresis. As shown in Figure 1A, probe without (lane 2) or with (lane 4) target miRNA was completely digested by DNase I. Because DNase I can only catalyze the hydrolysis of DNA, miRNA remained intact in the samples, regardless the presence of the DNase I (lane 3, lane 4). When mixed with PDANS, however, no clear hydrolysis of the DNA probe was observed (lane 5). In contrast, when the probe was mixed with its complimentary miRNA, the probe was completely digested even in the presence of PDANSs (lane 6), indicating that the miRNA/probe complex had desorbed from the PDANSs surface, followed by digestion of the probe by DNase I. The above experiments established that the ssDNA probe could be protected from enzymatic digestion when adsorbed on the surface of PDANSs.

Based on this new finding that ssDNA can be protected from DNase I digestion, together with the previously reported preferential binding property of PDANS for ssDNA over dsDNA,[12] we proposed a DNase I-assisted target recycling signal amplification method for the analysis of miRNA based on PDANSs. The working principle of the method is illustrated in Scheme1. In the absence of miRNA, FAM–labelled DNA probes are in the flexible single-stranded state and adsorb on the PDANSs and, as a result, the fluorescence is quenched by PDANSs. Upon the addition of miRNA, the probes bind to the miRNA and the DNA probe/miRNA complexes desorb from the PDANSs surface, and probe fluorescence is restored. Furthermore, the probe immediately becomes the substrate for DNase I digestion, subsequently releasing the miRNA to bind to another probe on PDANSs to initiate t he next round of cleavage. This cyclic reaction repeats until all the related probes are consumed and all fluorophores emit, resulting in significant fluorescence signal amplification.



Scheme 1. Working principle of DNase I-assisted target recycling signal amplification for miRNA based on polydopamine nanosphere protection and quenching of ssDNA probes.

Fluorescence measurements were first carried out to verify the DNase I-assisted target recycling process and fluorescence signal amplification. As shown in Figure 1B, the probes (black line) showed low fluorescence intensity after incubation with PDANSs, demonstrating the efficient adsorption and quenching ability of PDANSs towards fluorescent ssDNA probes. After the addition of miRNA, there was an increase in the fluorescence intensity (blue line). Without miRNA, the presence of DNase I led to a slight fluorescence enhancement, suggesting that some of the probes absorbed on PDANSs were hydrolyzed by DNase I (red line). By contrast, in the presence of both DNase I and miRNA, the probes hybridized with miRNA and desorbed from the PDANS surface (green line).The probes were then digested by DNase I, followed by the release of miRNA and to bind to another probe and repeat the cyclic cleavage reaction, resulting in a remarkable fluorescence enhancement. The result verified that DNase I-assisted digestion of probes leads to a target recycling reaction, thus resulting in amplified fluorescence intensity. By comparing the signal-to-background ratio (Figure S4, ESI), the optimal PNANS concentration was found to be 0.09 mg/mL, which was used in the following experiment.

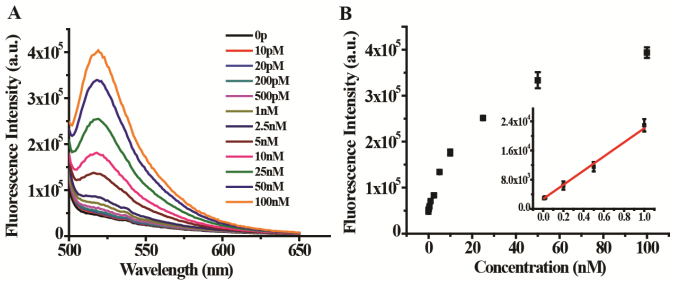


Figure 2. (A) Fluorescence emission spectra of DNA probes with different concentrations of let-7a. (B) Plot of fluorescence intensity as a function of the concentration of let-7a.

The DNase I-assisted target recycling signal amplification leads to highly sensitive detection of miRNA. After the addition of 20 units of DNase I and different concentrations of miRNA, the solution was incubated at 37°C for 90 min and the fluorescence intensities were detected afterwards. As shown in Figure 2A, a dramatic increase in fluorescence intensity was observed with increasing concentrations of let-7a. Figure 2B shows the relationship between the fluorescence intensity and the concentration of let-7a. The fluorescence intensity exhibited a good linear positive correlation with the concentration of let-7a within the range from 10 pM to 1 nM. The detection limit of the method was calculated to be 2.3 pM based on the 3σ method. In order to confirm that it is DNase I-assisted target recycling signal

amplification that contributes to the high sensitivity, control experiments with let-7a at various concentrations reacting in the absence of DNase I were also carried out. However, the detection limit was only 2.5 nM, which is 3 orders of magnitude less sensitive than DNase I-assisted target recycling signal amplification method (Figure S5, ESI).

We further tested the selectivity of the let-7a detection method. The miRNA family often comprises highly homogeneous sequences with only one-base variation, posing a great challenge for distinguishing different miRNA members. Because of the inherent specificity of DNA probes, the binding of probe and let-7a was found to be highly selective. Let-7f, let-7i and mir122 were chosen to evaluate the selectivity of our strategy (see Table S1). As shown in Figure 3A, the fluorescence signal of let-7f, which differs from let-7a only one base, is only 18% of that produced by let-7a, with a negligible signal in the presence of let-7i and mir122, which differ from let-7a by more than 4 bases. The results established that single-base mismatch could be distinguished with high selectivity among the closely related miRNA sequences by the DNase I-assisted target recycling signal amplification method.

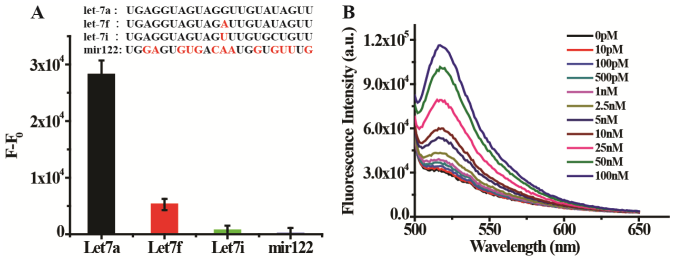


Figure 3. (A)Selectivity of DNase I-assisted target recycling signal amplification method for let-7a over let-7f, let-7i and mir122. The bases differing from those in let-7a are marked in red. (B)Fluorescence emission spectra of DNase I-assisted signal amplification strategy with different concentrations of miRNA in cell lysate.

Considering the significance of miRNA analysis in complex biological samples, we challenged our new method to detect miRNA in the lysate of the breast cancer cell MDA-MB-231, which has been reported with very low express ion levels of let-7a.[14] As demonstrated in Figure 3B, with the addition of increasing concentrations of miRNA, the fluorescence intensity shows a proportionate increase. The minimum detectable concentration was found to be 10 pM, which is consistent with that obtained in pure buffer solution. The result strongly confirmed the adaptability of our amplification approach for miRNA detection complicated biological matrices.

In conclusion, for the first time we demonstrated that ssDNA probes absorbed on PDANSs can be effectively protected from digestion by DNase I. Based on PDANSs-protected ssDNA probes, we have developed a DNase I-assisted target recycling signal amplification strategy for highly sensitive and selective analysis of miRNA. The proposed approach shows advantages in high sensitivity, excellent selectivity and simplicity. Our method can achieve a detection limit as low as 2.3 pM for miRNA, 3 orders of magnitude lower than that of non-amplified methods. Furthermore, the high binding selectivity between probes and miRNA leads to high selectivity that can distinguish a single-base-mismatched miRNA from miRNA target. In addition, our method allows direct miRNA analysis in complicated biological samples without any sample pre-treatment, thereby greatly simplifying the procedure for rapid detection. When used in nuclease-assisted target recycling signal amplification methods for miRNA detection, compared to other materials, PDANS shows its advantages of higher sensitivity and better biocompatibility (Table S2). Therefore, the reported DNase I-assisted target recycling signal amplification strategy based on PDANS-protected ssDNA probes has great potential for miRNA discovery, functional studies, and clinical diagnoses relying on miRNA biomarkers.

We thank the National Science Foundation for Distinguished Young Scholars of China (21325522), the National Science Foundation of China (91313302, 21205100, 21275122), and the National Basic Research Program of China (2013CB933703) for financial support.

Notes and references

[a] MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, Collaborative Innovation Center of Chemistry for Energy Materials, Key Laboratory of Chemical Biology of Fujian Province, State Key Laboratory of Physical Chemistry of Solid Surfaces, and Department of Chemistry, College of Chemistry & Chemical Engineering, Xiamen University, Xiamen 361005, P. R. China; E-mail: cyyang@xmu.edu.cn, ljzhou@xmu.edu.cn.

Electronic Supplementary Information (ESI) available: Experimental Section, Sequences used in this work, and Figures. See DOI: 10.1039/c000000x/See DOI: 10.1039/c000000x/

1. H. Dong, J. Lei, L. Ding, Y. Wen, H. Ju and X. Zhang, *Chem. Rev.*, 2013, **113**, 6207-6233.
2. C. Arenz, *Angew. Chem. Int. Ed.*, 2006, **45**, 5048-5050.
3. A. Valoczi, C. Hornyik, N. Varga, J. Burgyan, S. Kauppinen and Z. Havelda, *Nucleic Acids Res.*, 2004, **32**.
4. S. P. Fang, H. J. Lee, A. W. Wark and R. M. Corn, *J. Am. Chem. Soc.*, 2006, **128**, 14044-14046.
5. (a) Y. Q. Cheng, X. Zhang, Z. P. Li, X. X. Jiao, Y. C. Wang and Y. L. Zhang, *Angew. Chem. Int. Ed.*, 2009, **48**, 3268-3272; (b) Y. F. Peng, G. S. Yi and Z. Q. Gao, *Chem. Commun.*, 2010, **46**, 9131-9133.
6. (a) H. X. Jia, Z. P. Li, C. H. Liu and Y. Q. Cheng, *Angew. Chem. Int. Ed.*, 2010, **49**, 5498-5501; (b) D. Li, S. P. Song and C. H. Fan, *Acc. Chem. Res.*, 2010, **43**, 631-641.
7. (a) G. Aragay, F. Pino and A. Merkoci, *Chem. Rev.*, 2012, **112**, 5317-5338; (b) J. P. Lei and H. X. Ju, *Chem. Soc. Rev.*, 2012, **41**, 2122-2134; (c) M. Swierczewska, G. Liu, S. Lee and X. Y. Chen, *Chem. Soc. Rev.*, 2012, **41**, 2641-2655.
8. D. J. Maxwell, J. R. Taylor and S. M. Nie, *J. Am. Chem. Soc.*, 2002, **124**, 9606-9612.
9. L. Cui, Y. L. Song, G. L. Ke, Z. C. Guan, H. M. Zhang, Y. Lin, Y. S. Huang, Z. Zhu and C. J. Yang, *Chem. Eur. J.*, 2013, **19**, 10442-10451.
10. X. Y. Lin, L. Cui, Y. S. Huang, Y. Lin, Y. Xie, Z. Zhu, B. C. Yin, X. Chen and C. J. Yang, *Chem. Commun.*, 2014, **50**, 7646-7648.
11. C. F. Zhu, Z. Y. Zeng, H. Li, F. Li, C. H. Fan and H. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 5998-6001.
12. W. Qiang, W. Li, X. Li, X. Chen and D. Xu, *Chem. Sci.*, 2014, **5**, 3018.
13. Z. Zhu, R. H. Yang, M. X. You, X. L. Zhang, Y. R. Wu and W. H. Tan, *Anal. Bioanal. Chem.*, 2010, **396**, 73-83.
14. F. Yu, H. Yao, P. C. Zhu, X. Q. Zhang, Q. H. Pan, C. Gong, Y. J. Huang, X. Q. Hu, F. X. Su, J. Lieberman and E. Song, *Cell*, 2007, **131**, 1109-1123.