EXIRM: A Novel Technique for Label-Free Detection of MicroRNA

Yuhong Wang* and Shoujun Xu**

*Department of Biology and Biochemistry, University of Houston
Houston, Texas 77204, USA, ywang60@uh.edu
**Department of Chemistry, University of Houston
Houston, TX 77204, USA, sxu7@uh.edu

ABSTRACT

We report a sensitive and label-free detection method for microRNAs. The detection limit reaches zeptomole regime and the detection is a one-step process. MicroRNAs with a single-nucleotide difference can be distinguished. This recently invented technique, exchange-induced remnant magnetization (EXIRM) quantitatively reveals target microRNA molecules that exchanges with one strand of a surface immoblilized duplex. One of the duplex strand is tethered directly to the surface, and the other one is labeled with a magnetic bead. The disruption of the duplex causes the detachment of the magnetic beads from the surface that can be quantified with an atomic magnetometer. Potential applications and important parameters for future optimization are discussed.

Keywords: microRNA, magnetic particles, exchange reaction, atomic magnetometer, label-free

1 INTRODUCTION

MicroRNAs (miRNAs) play important roles in gene expression and have emerged as potential biomarkers for various cancers.[1-3] The regulation by miRNA depends on the sequence, expression level, and in cooperation with other miRNAs. Therefore, sensitive and sequence-specific detection is an essential step towards understanding their roles in protein synthesis, cellular proliferation and differentiation, and disease development.

Many conventional biological techniques, such as northern blotting[4], real time polymerase chain reaction,[5] in situ hybridization,[6] and microarray,[7] have been used for miRNA detection. However, mature miRNAs consist of only 19-25 nucleotides, making them difficult for conventional methods to detect specifically. A range of new techniques have also been developed, including bioluminescence,[8] surface plasmon resonance,[9] surface-enhanced Raman spectroscopy,[10] electrochemical detection,[11] fluorescence,[12] and photonic[13] methods. However, it remains difficult for a single technique to achieve high sensitivity and specificity, broad dynamic range, and reproducibility. Therefore, research effort needs to be devoted into developing new approaches.

2 METHOD

We present a novel EXIRM technique for sensitive miRNA detection.[14] This technique is based on the exchange reaction between the label-free miRNA and a magnetically labeled RNA that has one base difference from the miRNA. The exchange reaction is because of the more thermodynamically stable binding of the miRNA onto its complimentary sequence than the mismatched RNA. Details are as follows.

2.1 Exchange Scheme

The EXIRM method is schematically shown in Fig. 1. Three nucleic acid sequences are involved. First, the RNA (or DNA, denoted as Strand 1) complimentary to the target miRNA is incubated with a RNA strand (Strand 2) that differs from the target miRNA by one base. Strand 1 is immobilized on the surface of the sample well and Strand 2 is labeled with a magnetic bead. Then, the target miRNA (Strand 3) is added into the sample well. It will replace Strand 2 because of the stronger binding between Strand 3 and Strand 1 due to the complete complementarity. After detachement from the surface, Strand 2 undergoes Brownian motion, which results in randomization of the magnetic dipoles of the magnetic particles. Therefore, the exchange reaction will produce a decrease in the magnetic signal. The decrease amplitude linearly correlates with the quantity of the target miRNA molecules. Because of the sensitivity of atomic magnetometer, the absolute number of miRNAs are directly measured without PCR amplifications. In addition, no other enzymatic or fluorescence labeling reactions is needed.

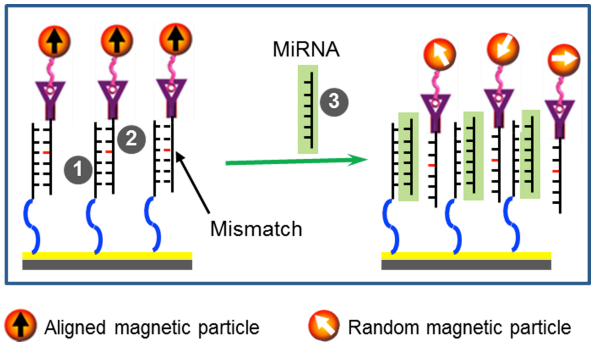

Figure 1: The schematic of EXIRM for detecting miRNA.

2.2 Sample Preparation

Experimentally, the immobilization of Strand 1 can be achieved by either thiolated nucleic acid binding onto a gold surface or biotinylated nucleic acid binding onto streptavidin-coated surface. The magnetic bead being used include Dynabeads and M280 (Invitrogen), which have diameters of 1 μm and 2.8 μm, respectively. The magnetic beads are decorated with streptavidin so that they will bind with biotinylated Strand 2. The temperature for the exchange reactions has been tested for room temperature and 37 ºC. The latter gives a much faster reaction rate.

2.3 Detection with an Atomic Magnetometer

The detection of the magnetic signal before and after the exchange reaction is achieved by using an atomic magnetometer, with a typical sensitivity of 80-100 $fT/(Hz)^{1/2}$.[15] Atomic magnetometers are based on the magneto-optical resonance of polarized alkali atoms interacting with a near-resonant laser beam. The laser beam first polarizes the alkali atoms in the ground state. Then the precession of the atomic alignment rotates the polarization of the laser, with a frequency determined by the type of the alkali atom and the magnetic field to be measured. Therefore, measuring the optical rotation will yield the absolute amplitude of the magnetic field produced by the magnetic beads in the experiments here. With a sensitivity of tens of $fT/(Hz)^{1/2}$ for dc signal and below 1 $fT(Hz)^{1/2}$ for ac signal, atomic magnetometers are arguably the most sensitive devices for magnetic sensing.[16,17]

A scanning magnetic imaging scheme is used for measurement, in which the sample well is scanned across the magnetometer to obtain a magnetic field profile.[18] Fitting this profile yields the magnetization of the magnetic beads, hence the number of replaced RNA molecules, and the distance between the sample scanning trajectory and the magnetometer.

3 RESULTS AND DISCUSSION

The EXIRM method has been demonstrated by detecting miRNAs let-7a and let-7c, which differ by only one base. The sequences are the following:

Let-7a: UUGAUAUGUUGGAUGAUGGAUG

Let-7c: UUGGUAUGUUGGAUGAUGGAUG

Their respective complementary sequences were used as Strand 1 for their detection. A common Stand 2 was used that had one mismatching base from both miRNAs, with sequence

Strand 2: UUGUUAUGUUGGAUGAUGGAUG

The scheme and results are shown in Fig. 2. Two sample wells were located on different positions (indicated by x alue in Fig. 2a) on the sample inlet system. The left one was designed for detecting let-7a via implementing Strand 1 that is complementary to let-7a. Similarly, the right sample well implemented the duplex to detect let-7c. When let-7a was added into both sample wells, only the left one gave a difference in the magnetic signal (Fig. 2b); Addition of let-7c to both samples caused a magnetic signal change only in the right sample well (Fig. 2c). A random miRNA was used as a control experiment, which caused no change in either sample well (Fig. 2d). Therefore, selective detection was observed in which no cross talking occurred between the two miRNAs.

The concentration of the miRNAs was 300 fM, which generated aprroximately 12 pT signal that corresponded to approximately 10^6 molecules. Given the sensitivity of 80 fT for 1 s integration time, the detection limit of approximately 10^4 can be achieved. This value may be further reduced by using larger magnetic beads. For example, we have shown that tens of 4.5 μm-diameter magnetic beads can be detected.[19]

In addition to single-base specificity and high sensitivity, another related parameter is the detection dynamic range. For the atomic magnetometer used in this work, the upper limit is approximately 20 nT.[19] Therefore, the dynamic range can be obtained as 5 orders of magnetitude, given the lower limit being 80 fT. In addition, by adjusting the size of the magnetic beads, this range can be expanded. For instance, use of smaller magnetic beads will lead to a higher detection upper limit, because of both the weaker magnetic signal of the beads and the increased number of beads covering the surface of the sample well.

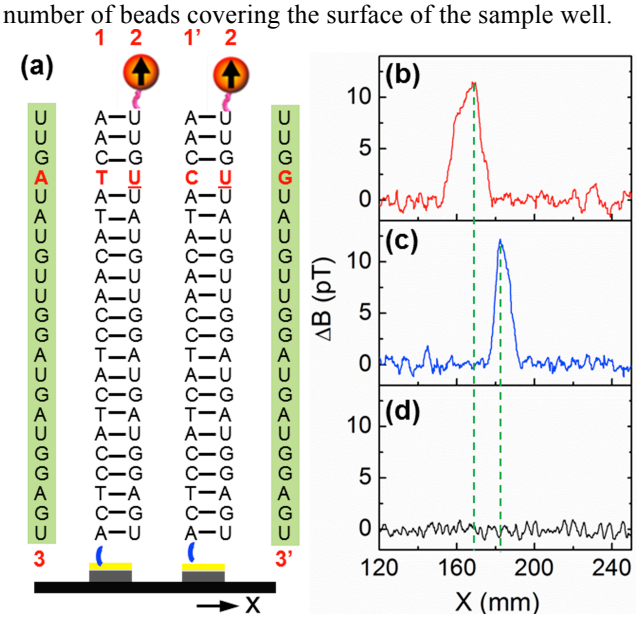

Figure 2: Results of miRNA detection using EXIRM. a) Experimental scheme showing the sequences of the two sample wells. b-d) Magnetic field measurements of let-7a, let-7c, and a random sequence miRNA, respectively. (First published by RSC)

In the following table, we compare our EXIRM technique with other well-established techniques including real time polymerase chain reaction (RT-PCR) and surface plasmon resonance (SPR).[5,7,20.21]

Method	Sensitivity[a)]	Dynamic Range[b)]
North Blotting	10^{14}	3
Microarray	10^{8}	5
RT-PCR	10^{8}	7
SPR	10^{5}	>3
Fluorescence	10^{6}	4
Electrochemical	10^{6}	3
EXIRM	10^{4}	>5

Table 1: Comparison of various miRNA detection methods. [a)]number of molecules; [b)]orders of magnitudes.

The comparison shows that EXIRM has the potential of offering high sensitivity and a broad dynamic range. Furthermore, a significant advantage of our technique is that there is no amplification step. The detection of the target miRNA is achieved in a one-step process. The change in magnetic field correlates linearly with the amount of the sample.

A critical parameter for future optimization is the reaction temperature. It appears higher temperature accelarates the exchange reaction. Because the melting temperature of a typical miRNA duplex is above 60 ºC, we can try to carry out the measurement under a higher temperature than the current 37 ºC. We expect the exchange time will be reduced accordingly.

In addition to miRNA detection, the exchange principle of EXIRM is also valuable to detect other noncovalent binding molecules, such as antibodies and viruses.[22] For these applications, a weakly bound protein or DNA can be designed to bind with the complimentary binding receptor of the target ligand. The target ligand can then be detected using the EXIRM approach.

4 CONCLUSION

We conclude that the EXIRM technique offers the following several advantages. First, the signal is several orders more senstive than flourescence, therefore no PCR amplification is needed. Second, it is suitable for both opaque or translucent conditions, in contrast to optical techniques. Third, it is one-step and label-free for the target miRNAs, unlike fluorescence-based detections, in which cDNA library and primer tagging are inevitable. Fourth, it relies on the exchange reaction driven by thermodynamic stability. Thus no scanning external force is needed. These advantages will promote EXIRM to be an innovative avenue for miRNA profiling in cancer diagnostics.

REFERENCES

[1] Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281-297 (2004).

[2] Di leva, G. & Croce, C. M. miRNA profiling of cancer. *Curr. Opin. Genet. Dev.* **23**, 3-11 (2013).

[3] Lee, Y. S. & Dutta, A. MicroRNAs in cancer. *Annu. Rev. Pathol.* **4**, 199-227 (2009).

[4] Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. Identification of novel genes coding for small expressed RNAs. *Science* **294**, 853-858 (2001).

[5] Benes, V. & Castoldi, M. Expression profiling of microRNA using real-time quantitative PCR, how to use it and what is available. *Methods* **50**, 244-249 (2010).

[6] Obernosterer, G., Martinez, J. & Alenius, M. Locked nucleic acid-based in situ detection of microRNAs in mouse tissue sections. *Nat. Protoc.* **2**, 1508-1514 (2007).

[7] Sudo, H., Mizoguchi,A., Kawauchi, J., Akiyama, H. & Takizawa, S. Use of non-amplified RNA samples for microarray analysis of gene expression. *Plos One* **7**, 1-6 (2012).

[8] Cissell, K. A., Rahimi, Y., Shrestha, S., Hunt, E. A. & Deo, S. K. Bioluminescence-based detection of MicroRNA, miR21 in breast cancer cells. *Anal. Chem.* **80**, 2319-2325 (2008).

[9] Fang, S. P., Lee, H. J., Wark, A. W. & Corn, R. M. Attomole microarray detection of MicroRNAs by nanoparticle-amplified SPR imaging measurements of surface polyadenylation reaction. *J. Am. Chem. Soc.* **128**, 14044-14046 (2006).

[10] Driskell, J. D., Seto, A. G., Jones, L. P., Jokela, S., Dluhy, R. A., Zhao, Y.-P. & Tripp, R. A. Rapid microRNA (miRNA) detection and classification via surface-enhanced Raman spectroscopy (SERS). *Biosens. Bioelectron.* **24**, 917-922 (2008).

[11] Yang, H., Hui, A., Pampalakis, G., Soleymani, L., Liu, F. F., Sargent, E. H. & Kelley, S. O. Direct, electronic MicroRNA detection for the rapid determination of differential expression profiles. *Angew. Chem. Int. Ed.* **48**, 8461-8464 (2009).

[12] Jiang, L., Duan, D. M., Shen, Y. & Li, J. Direct microRNA detection with universal tagged probe and time-resolved fluorescence technology. *Biosens. Bioelectron.* **34**, 291-295 (2012).

[13] Qavi, A. J., Kindt, J. T., Gleeson, M. A. & Bailey, R. C. Anti-DNA:RNA antibodies and silicon photonic microring resonators: increased sensitivity for multiplexed microRNA detection. *Anal. Chem.* **83**, 5949-5956 (2011).

[14] Yao, L., Wang, Y. & Xu, S.-J. Ultrasensitive microRNA sequencing using exchange-induced remnant magnetization. *Chem. Commun.* **49**, 5183-5185 (2013).

[15] Garcia, N. C., Yu, D., Yao, L. & Xu, S.-J. Optical atomic magnetometer at body temperature for magnetic particle imaging and nuclear magnetic resonance. *Opt. Lett*. **35**, 661-663 (2010).

[16] Dang, H. B., Maloof, A. C. & Romalis, M. V. Ultrahigh sensitivity magnetic field and magnetization measurements with an atomic

magnetometer. *Appl. Phys. Lett.* **97**, 151110 (2010).
[17] Budker, D. & Romalis, M. V. Optical magnetometry. *Nat. Phys.* **3**, 227-234 (2007).
[18] Yao, L., Jamison, A. C. & Xu, S.-J. Scanning imaging of magnetic nanoparticles for quantitative molecular imaging. *Angew. Chem. Int. Ed.* **49**, 7493-7496 (2010).
[19] Yu, D. S., Ruangchaithaweesuk, S., Yao, L. & Xu, S.-J. Detecting molecules and cells labeled with magnetic particles using an atomic magnetometer. *J. Nanoparticle Res.* **14**, 1135 (2012).
[20] Qavi, A. J., Kindt, J. & Bailey, R. C. Sizing up the future of microRNA analysis. *Anal. Bioanal. Chem.* **398**, 2535-2549 (2010).
[21] de Planell-Saguer, M. & Rodicio, M. C. Analytical aspects of microRNA in diagnostics: A review. *Anal. Chim. Acta.* **699**, 134-152 (2011).
[22] Yao, L. & Xu, S.-J. Force-induced selective dissociation of noncovalent antibody-antigen bonds. *J. Phys. Chem. B* **116**, 9944-9948 (2012).