

Label-free microRNA detection based on exchange-induced remnant magnetization†

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We report a technique that is based on exchange-induced remnant magnetization for microRNA (miRNA) detection. In sequence-specific exchange reactions between label-free miRNA and magnetically labelled RNA with one mismatched base, the decrease in magnetization quantitatively represents the target miRNA. The detection limit reaches the zeptomole regime, with no amplification or washing procedures. Therefore, our technique will be suitable for precise miRNA profiling to aid in early diagnosis of cancers.

MiRNAs, which are short RNA strands containing 18–25 nucleotides, play important roles in gene expression, cell differentiation, and disease development.^{1–3} The mature miRNAs are incorporated into RNA-induced silencing complexes that bind with messenger RNA based on partial sequence complementarity and consequently cause inhibition of protein translation. The regulation by miRNA depends on its sequence, expression level, and cooperation with other miRNAs. Therefore, sensitive and specific detection of miRNAs is an essential step towards understanding their functions in gene regulation and their expression as biomarkers for cancer diagnostics.

A variety of techniques have been used for miRNA sequencing and profiling. Conventional methods include northern blotting,⁴ reverse transcriptase polymerase chain reaction,⁵ *in situ* hybridization,⁶ and microarray.⁷ Many 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 methods.¹³ Each of these techniques offers certain specific advantages. However, it remains difficult to simultaneously achieve high sensitivity, single-base specificity, and a broad dynamic range, because miRNAs have very short strands and diverse expression levels. In addition, reproducibility is a significant issue when

comparing results from different techniques, due to the many steps and various protocols involved in the measurements.¹⁴

Here, we show a technique, exchange-induced remnant magnetization (EXIRM), that uses the exchange reaction between the target miRNA and magnetically labelled RNA with one mismatched base during competitive binding with the complementary sequence of the target miRNA. The principle is demonstrated in Fig. 1. A sample containing RNA duplexes is initially prepared: the strand complementary to the target miRNA is immobilized on the surface of a sample well (strand 1) and the other strand with one mismatched base is labelled with a magnetic particle (strand 2). Then the target miRNA (strand 3) is introduced and incubated with the prepared RNA duplexes. Exchange reactions take place, in which the strand 3 miRNA replaces the mismatched strand 2 in the duplexes. This is because the former has thermodynamically stronger binding with the immobilized strand 1, due to sequence complementarity, than the latter. The dissociation of strand 2 leads to randomization of the magnetic dipoles of the magnetic particles due to Brownian motion induced by a weak mechanical force.¹⁵ Therefore, the exchange reaction will produce a decrease in the magnetic signal that can be measured using an atomic magnetometer, which is arguably the most sensitive device for magnetic sensing.^{16,17}

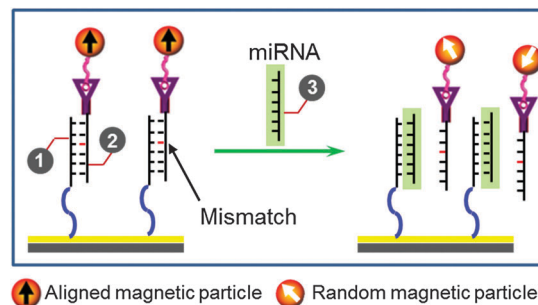


Fig. 1 Principle of the EXIRM technique. Three RNA strands are involved: immobilized strand 1, strand 2 with one mismatched base, and strand 3 of the target miRNA. Strand 2 is hybridized with strand 1 and is labelled by a magnetic particle. The particles are magnetically aligned before exchange but randomized after exchange-induced dissociation.

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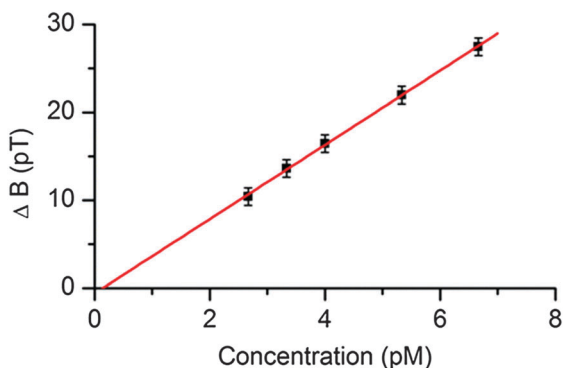


Fig. 5 Magnetic signal decreases, ΔB , for various concentrations of the target DNA, using 1 μm diameter magnetic particles.

because let-7a complements strand 1 and should only replace strand 2 in the left sample well. In contrast, let-7c and strand 2 are both one-base mismatched to strand 1, so no exchange reaction should occur in the right sample well (at 182 nm). Similarly, when let-7c was added to both sample wells, only the right sample well produced a magnetic signal decrease (Fig. 4c). In a control experiment in which no miRNA was added to the sample wells, no magnetic signal change was observed in either sample well (Fig. 4d). This confirms that the responses were due to the presence of sequence-specific miRNA. This single-base specificity is required for miRNA profiling because miRNAs in the same family often differ by one or a few nucleotides.

In addition to the detection limit and specificity, the dynamic range is another important parameter for a detection technique. A broad dynamic range is desired for miRNA profiling because it is well known that the expression levels may be drastically different among miRNAs.²² For EXIRM, the dynamic range can be adjusted by varying the size of the magnetic labels. Fig. 5 shows the results of using 1 μm diameter magnetic particles instead of the 2.8 μm particles in the exchange reactions involving the 12-base DNA (ESL,† Fig. S2). The detection concentration is one order of magnitude higher than the values in Fig. 3. This is because the fixed surface area of the sample well can accommodate more of smaller particles. Ultimately, the upper detection limit is determined by our atomic magnetometer, which is ~ 15 nT;¹⁹ the lower limit is determined by the sensitivity, which is 150 fT. Therefore, the dynamic range can reach five orders of magnitude by adjusting the size of the magnetic particles. In addition, detection of a single DNA or miRNA molecule is potentially possible if a strong magnetic particle is used.²³

Other significant features of our technique include no need for amplification, no multiple washing or transfer procedures, and no sequencing in a base-by-base fashion. These characteristics substantially simplify the analysis process and improve the reliability of the measurements.

There are two main issues concerning the use of the EXIRM technique in the present stage. One is that the size of the sensor is not convenient for high throughput analysis. Miniaturized atomic sensors have been demonstrated with high sensitivity, but have not been used for the detection of miRNA.²⁴ Atomic sensors of 900 nm thickness have also been studied.²⁵ The other is that the reaction time remains fairly long. While the

biological samples under our current *in vitro* conditions are usually stable, a long reaction time can lead to less efficient analysis. To shorten the reaction time, one approach may be careful control of the temperature, so that it is high enough to accelerate the exchange reactions but not too high to induce nonspecific dissociation. Detailed investigation is ongoing.

In conclusion, we have shown an EXIRM technique that represents a new avenue for miRNA sequencing. The high sensitivity of atomic magnetometers enables detection of as few as 10^4 molecules. Single-base resolution has been achieved from sequence-specific exchange reactions. Cross-talking is not observed between miRNAs with one base difference. With future developments in control and miniaturization, EXIRM will be capable of sensitive, quantitative, and reliable miRNA profiling and will have broader applications in cancer diagnostics.

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