

FIRMS: A Novel Technique for Magnetic Molecular Imaging

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ABSTRACT

Molecular imaging usually employs a labeling technique, mainly optical labeling or magnetic labeling, to highlight the target molecules. While optical labeling uses a wavelength parameter to achieve molecular specificity, there is no analogous parameter in magnetic labeling to achieve the same goal. To solve this problem, we developed a magnetic-based technique that uses the binding force between the target molecules and the receptor molecules, one of which is labeled with magnetic particles. This force-induced remnant magnetization spectroscopy (FIRMS) technique measures the magnetization of the magnetic particles as a function of an external mechanical force that is used to induce dissociation of the noncovalent bonds between the probe target molecules. The high force resolution of 1 pN allows us to distinguish antibodies of different subclasses and DNA duplexes with a single-basepair difference.

Keywords: force spectroscopy, noncovalent bond, magnetic particles, atomic magnetometer, bond dissociation

1 INTRODUCTION

Molecular recognition usually relies on the specific noncovalent bonding between the ligand and receptor molecules, which is often used to detect the molecule of interest.¹ A common method for detecting the target molecules is using fluorophores to label the molecules and detect the optical signal, wherein the wavelength is a key parameter for distinguishing different molecules.^{2,3} One of the major limitations of this approach is the incapability for opaque conditions. In addition, autofluorescence and quenching may interfere with the desired signal. Another approach is magnetic labeling. However, the magnetic signal usually lacks of a spectroscopic parameter based on which different molecular signatures can be resolved.

One potential parameter for molecular magnetic imaging is the binding force of the noncovalent bonds, which ranges in tens to hundreds of pN (10^{-12} N).⁴ Current techniques that measure noncovalent binding forces include atomic force microscopy and optical tweezers.⁵ These two techniques measure a single bond at a time, which limits their application in practical diagnostics. In addition, the broad force range that they provide makes it difficult to

distinguish different molecular bonds. Some experimental parameters involved in the techniques, such as the stiffness of the cantilever in atomic force microscopy and force application history, significantly affect the experimental measurements.

Here, we demonstrate the force-induced remnant magnetization spectroscopy (FIRMS) technique that measures the dissociation of tens of thousands of noncovalent bonds simultaneously and provides the binding force with ultra-narrow force distribution.⁶⁻⁸ By using an atomic magnetometer to detect the magnetic signal, the measurement has minimum invasiveness and hence provides highly reliable results.⁹ Molecular bonds with similar strength can be completely resolved; the number of each type of bonds can be precisely determined. The FIRMS technique has the potential of wide applications in molecule-specific imaging and manipulation, for both fundamental biochemical research and medical diagnosis.

2 METHOD

FIRMS is based on the randomization of the magnetic particles once they dissociate from the noncovalent bonds under the influence of an external mechanical force.⁶ The magnetic signal is measured as a function of the amplitude of the external force. A signal decrease quantitatively represents the number of a particular type of bonds.

2.1 Experimental Scheme

As shown in Fig. 1, one of the binding pairs, generally termed as the receptor, is immobilized on the surface. The other, the ligand, which is labeled with a magnetic particle, forms specific noncovalent bonds with the immobilized receptor. In addition, there is nonspecific physisorption between the ligand and the surface or other species, which is normally much weaker. The magnetized particles give a total magnetic signal. When a weak external force of f_1 is applied, the physisorption will dissociate, leading to the randomization of the magnetic dipoles of the magnetic particles. A decrease in magnetic signal is thus produced. When the force matches the binding force of the specific bonds between the ligand and the receptor, shown as f_2 , the dissociation of the molecular bonds yields another decrease in the magnetic signal. Therefore, by ramping the force amplitude, different bindings can be resolved.

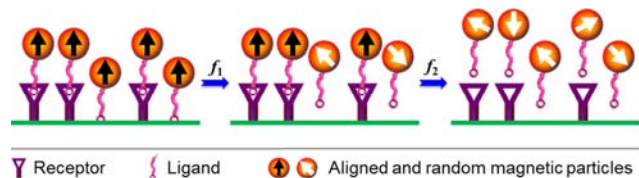


Figure 1: The schematic of FIRMS for distinguishing noncovalent bonds based on their binding strengths.

2.2 Sources for External Force

We have shown two different types of mechanical forces to induce bond dissociation. The first is shaking force by a commercial shaker, for example VWR 12620-942. Specific binding of antibody-antigen has been resolved from the much weaker nonspecific physisorption. The second is centrifugal force by a commercial centrifuge, for example Eppendorf MiniSpin and 5417R.

2.3 Detection with an Atomic Magnetometer

Sensitive detection is required to quantify the number of the magnetically labeled noncovalent bonds. We use an atomic magnetometer to measure the magnetic signal vs. the external force, with a sensitivity of approximately $80 \text{ fT}/(\text{Hz})^{1/2}$.¹⁰ The magnetic particles is initially magnetized by a permanent magnet before applying the external force.

To decouple the distance dependence of magnetic signal, a scanning magnetic imaging scheme has been adopted.¹¹ Instead of a single point measurement, a magnetic field profile is obtained by scanning the sample along the atomic sensor. Fitting the profile provides both the sample distance and the magnetization. The latter represents the number of magnetic particles that are associated with noncovalent bonds. Typically 10^4 molecular bonds can be detected using magnetic microparticles such as M280 (Invitrogen). Magnetically stronger particles will improve the detection limit.

3 RESULTS AND DISCUSSION

Figure 2 shows the FIRMS result of the binding between mouse IgG and its corresponding antigen α -mouse IgG. The top panel shows the relative magnetization as a function of the shaking speed. The first decrease, at 450 rpm (resolution per minute) indicates the weaker nonspecific physisorption, while the second decrease at 1050 rpm belongs to the specific antibody-antigen bonds. The two features are clearly resolved, validating the FIRMS technique for molecular magnetic detection. A re-binding experiment showed a second batch of magnetically-labeled antigen was able to bind specifically with the washed surface coated with the antibody, confirmed the 1050 rpm transition being the dissociation of the antibody-antigen

bonds. Otherwise, either the antibody would have been washed away or saturated with antigen.

The derivative of the magnetization profile produces a FIRMS spectrum (bottom panel, Fig. 2). The peak height represents the quantity of the corresponding bonds and the peak position indicates the binding force. The force spectrum is analogous to an optical spectrum, which resolves molecules by their characteristic absorption bands. Therefore, FIRMS implements molecular signature into magnetic sensing for the first time.

Force calibration was obtained by using a centrifuge, which can be calculated from $f = m\omega^2 r$.⁷ Here, m is the buoyance mass of the magnetic particles, ω is the speed of the centrifuge, and r is the distance between the sample and the center of the motor. The buoyance mass of M280 was measured to be $4.6 \times 10^{-15} \text{ kg}$; r is 4 cm for the MiniSpin and 8 cm for 5417R.^{7,8} Using this method, we have obtained the binding force between the mouse IgG and α -mouse IgG to be 120 pN, and the physisorption binding force to be 17 pN.

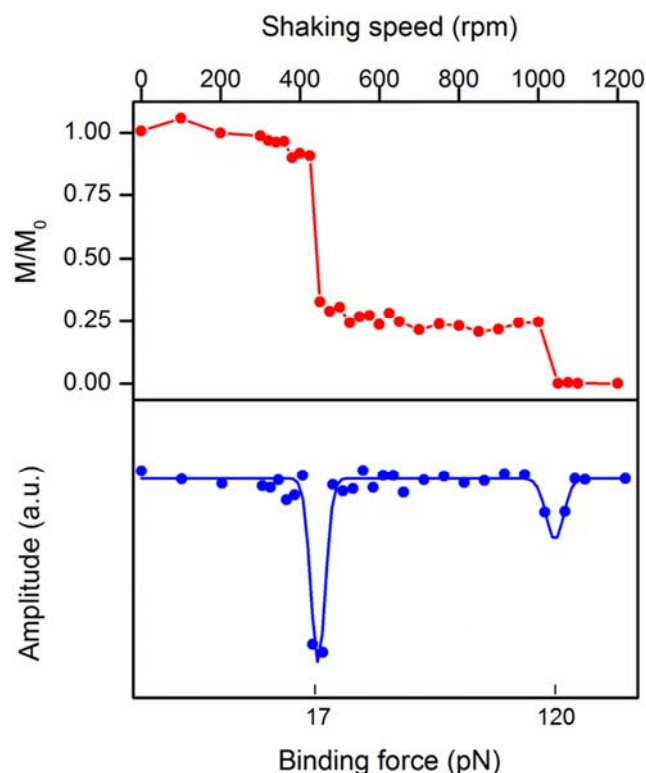
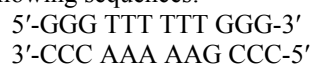


Figure 2. FIRMS of antibody-antigen binding. (Modified with permission from *J. Phys. Chem.* **116**, 9944, 2012. Copyright 2012 American Chemical Society.)

Ultra-high force resolution FIRMS was achieved by using centrifugal force to distinguish DNA duplexes with different numbers and positions of basepairs. Figure 3 shows the results of an 11-basepair DNA duplex with the following sequences:



The first strand was immobilized on the surface and the second was labeled with magnetic particles. The top panel shows the magnetization vs. the centrifugal speed after a weak centrifugal force to remove the physisorbed magnetic particles. The sharp transition at 2700 rpm indicates the well-defined binding force of the DNA duplex. The FIRMS spectrum on the bottom panel gives a half-width half-maximum of 80 rpm, which corresponds to 1.8 pN force resolution. The distribution of binding force is much narrower than that of single-molecule-based techniques.¹²

The narrow force distribution enables distinguishing different DNA duplexes based on their binding forces. The binding force of a 12-basepair DNA duplex, in which the second strand was 3'-CCC AAA AAA CCC-5', was measured to be 42 pN. More importantly, the two duplexes within the same sample can be completely resolved from the FIRMS spectrum. In addition, the different positions of mismatched base can lead to different binding forces, which can be possibly resolved.⁸

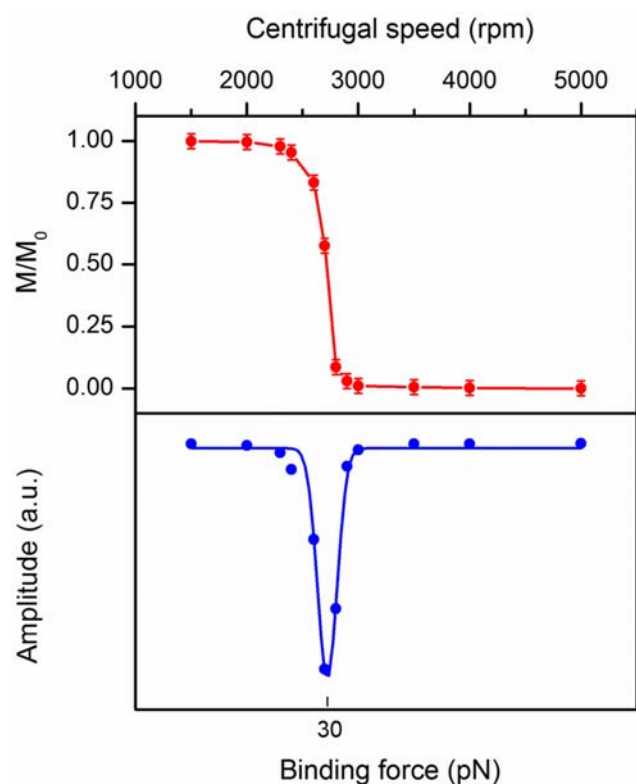


Figure 3. FIRMS of a DNA duplex. (Modified with permission from *J. Phys. Chem.* **117**, 7554, 2013. Copyright 2013 American Chemical Society.)

The number of the noncovalent bonds being detected can be obtained from the magnetization value and the calibration curve of the magnetic particles being used. We have experimentally determined the magnetization of the M280 under our magnetizing condition follows a linear progression.⁷ The detection limit of the atomic magnetometer is 80 fT for 1 s integration time. Given the sample to the detector distance of approximately 8 mm, the

minimum magnetization will be $2 \times 10^{-13} \text{ Am}^2$, equivalent to 4×10^3 molecular bonds labeled with M280. Using magnetically stronger particles will lead to even better detection limit. For example, we have demonstrated several tens of magnetic particles of 4.5 μm diameter can be detected.¹⁰

The force resolution may also be improved. At present, the limiting factor seems to be the smallest incremental step of the centrifugal speed, which is 100 rpm. Implementing a more precise centrifuge in the speed range of 2000-8000 rpm will probably improve the force resolution.

We compare our new FIRMS technique with the widely used AFM and optical tweezers. First, the force distribution of FIRMS is only 2 pN, nearly an order of magnitude narrower than that of the other two techniques. The highly precise binding force enables resolution of different molecular bonds in a single sample. Such capability facilitates practical diagnostics. Second, FIRMS typically measures 10^3 - 10^6 bonds at a time; the results do not need to be repeated many times, in contrary to the other two techniques that are based on single-molecule detection. Third, the atomic magnetometer used for FIRMS is mechanically uncoupled from the molecular system, different from the AFM in which the cantilever needs to be connected with the molecular bonds. This noninvasive detection eliminates possible interferences such as the force constant of the cantilever.

4 CONCLUSION

The FIRMS technique implements molecular specificity into magnetic sensing for the first time. The binding force serves as the discriminating parameter, which is analogous to the wavelength parameter in optical-based detection. Because of its advantage of opaque-condition applicability, magnetic sensing with FIRMS can find broad applications in diagnosis.

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