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Force-Induced Selective Dissociation of Noncovalent Antibody−Antigen Bonds

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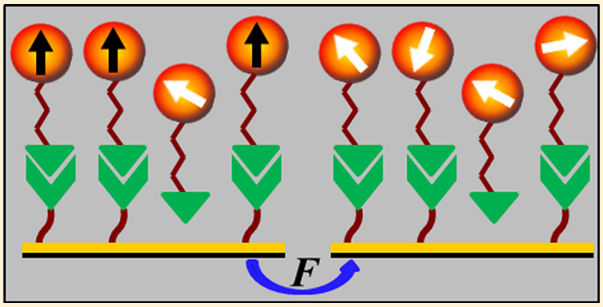
Supporting Information

ABSTRACT: Specific noncovalent binding between antibody and antigen molecules is the basis for molecular recognition in biochemical processes. Quantitative investigation of the binding forces could lead to molecular specific analysis and potentially mechanical manipulation of these processes. Using our force-induced remnant magnetization spectroscopy, we revealed a well-defined binding force for the bonds between mouse immunoglobulin G and magnetically labeled α-mouse immunoglobulin G. The force was calibrated to be 120 ± 15 pN. In comparison, the binding force was only 17 ± 3 pN for physisorption and much higher than 120 pN for biotin−streptavidin bonds. A unique rebinding method was used to confirm the dissociation of the antibody−antigen bonds. A well-defined and molecule-specific binding force opens a new avenue for distinguishing different noncovalent bonds in biochemical processes.



INTRODUCTION

Reversible and molecule-specific noncovalent bonds are the foundation for molecular recognition in many processes in chemistry and biology, such as enzyme catalysis, cancer diagnosis, and drug delivery.[1−3] The noncovalent bonds possess a broad range of binding strengths, which are often measured as dissociation forces in piconewtons.[4] A characteristic dissociation force will be suitable as a discriminating parameter for identifying specific receptor molecules in molecular diagnosis and selective manipulation of biochemical processes.[5] Current techniques for measuring the forces include atomic force microscopy (AFM), dynamic force microscopy, optical tweezers, and magnetic tweezers.[6−9] While these techniques provide detailed information on dynamic dissociation forces and energy landscape, they are sometimes inconvenient for practical molecular and cellular diagnosis.[10−12] For example, because AFM detects a single dissociation event at a time, many repeated measurements are necessary to ensure the statistical significance of the results.

Recently, we reported a force-induced remnant magnetization spectroscopy (FIRMS) technique, which uses a shaking force to distinguish the physisorbed ligand molecules from those bound via molecule-specific noncovalent bonds.[13] This technique is based on the following concept: the magnetically labeled ligand molecules undergo Brownian motion once they dissociate from the receptor molecules or surface, which consequently randomizes the magnetic dipoles of the particles and results in a magnetization decrease. Therefore, the measured magnetization represents only the quantity of the bound particles, hence the noncovalent bonds. So far, it remains challenging to quantify the dissociation force for noncovalent antibody−antigen bonds and to confirm the dissociation of the desired bonds, both of which are critical for molecular recognition and sensing.

Here we show the noncovalent bonds between antibody and antigen molecules can be dissociated by an external force but only when the force exceeds a well-defined value. We have chosen three types of interactions with significantly different binding strengths: nonspecific physisorption which is the weakest, specific binding between mouse immunoglobulin G (IgG) and goat α-mouse IgG (simplified as IgG−α-IgG hereafter) with a dissociation force between 50 and 150 pN, and biotin−streptavidin which is one of the strongest noncovalent bonds, with greater than 250 pN dissociation force, as reported in the majority of literature based on single-molecule methods.[4,14] Using a unique rebinding approach, we are able to confirm the dissociation of the specific noncovalent bonds. The number of the bonds, hence the quantity of the targeted receptor molecules, is also revealed.

EXPERIMENTAL METHODS

Figure 1 shows the schematic of the experimental setup. The receptor molecules are immobilized on a substrate surface. Their corresponding ligand molecules, which are conjugated with magnetic particles, are incubated with the receptor molecules. Specific bonds are formed between the receptor and ligand molecules as well as physisorption between the substrate surface and the ligand molecules. Forces with increasing amplitudes, provided by either a shaker (VWR, 12620-942) or centrifuge (Eppendorf MiniSpin), are then

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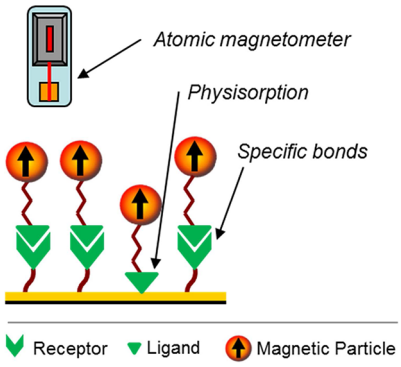

Figure 1. Schematic of the experimental setup. The targeted receptor molecules are immobilized on the surface of a substrate, while the ligand molecules are conjugated with magnetic particles. The magnetization of the magnetic particles is measured by an atomic magnetometer as a function of an external force to distinguish different molecular interactions.

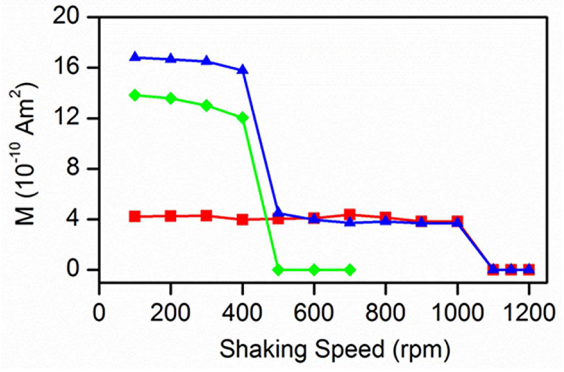

Figure 2. Plots of magnetization M vs shaking force for magnetically labeled α-mouse IgG binding to a bare gold surface (green trace) and IgG-coated gold surface (red and blue traces). M is the total magnetic moment of the magnetic particles. The error bar for the magnetization values is approximately 5×10^{-12} Am^2.

applied to induce the dissociation of interactions. After each force, the magnetization of the magnetic particles is measured by an atomic magnetometer with a sensitivity of ~150 fT/(Hz)$^{1/2}$.[15]

The sample cells were 4 × 1 × 1 mm^3. The bottom glass surface of the sample cells was coated with either bare gold, gold conjugated with mouse IgG, or biotin. After incubation with the appropriate magnetic particles, the samples were magnetized for 2 min using a permanent magnet. Forces with varying amplitudes were applied on the samples by gradually increasing the speed of the shaker or the centrifuge. The duration for each force was 5 min. Durations of 10 and 15 min were also attempted and showed the same results as 5 min. Magnetic particles conjugated with goat α-mouse IgG and streptavidin were 1−4 μm in size for both conjugates (Pierce Biotechnology). The 2.8 μm particles were purchased from Invitrogen.

For IgG binding, a sample cell with a gold-coated (30 nm thick) bottom was immersed in a 0.2 mM ethanolic solution of dithiobis(succinimidyl undecanoate) for ~24 h. After rinsing the cell with ethanol and drying it with a nitrogen stream, the cell was incubated with 4 μL of mouse IgG in borate buffer (containing 0.02 wt % sodium azide and 1% Tween 80) for 24 h in a humidity chamber. The cell was then immersed in a 0.5% (w/w) bovine serum albumin (BSA) solution in borate buffer for 1 h, followed by incubation with 4 μL magnetic particles (~8 × 10^4 particles) overnight.

For biotin−streptavidin binding, a biotin-coated glass slide (MicroSurfaces) formed the bottom surface of the sample cell. A 4 μL suspension of streptavidin particles (containing 0.5% BSA) was incubated for 1 h.

RESULTS AND DISCUSSION

Figure 2 shows the results for physisorption and IgG−α-IgG bonds under different conditions. Here, α-mouse IgG molecules were labeled with magnetic particles. The surface was either bare gold or gold coated with mouse IgG.[16] A digital shaker was used for force-induced bond dissociation. The sample cell is mounted on the motor of the shaker. The shaking motion creates a vortex in the buffer fluid that applies a mechanical force on the magnetic particles. The magnetization (M) value is measured by an atomic magnetometer, which is the total magnetic moment of the magnetic particles. For binding α-mouse IgG to the bare gold surface (green trace), an abrupt transition in magnetization (M) decrease occurred from 400 to 500 rpm (revolutions per minute). In addition, the magnetization decreased to zero, indicating no bound particles remained afterward. This feature was assigned to the dissociation of physisorbed α-mouse IgG by the gold surface, as the receptor IgG molecules were absent on the surface. For α-mouse IgG binding to an IgG-coated gold surface (red trace), a sharp magnetization decrease occurred from 1000 to 1100 rpm; the magnetization value also became zero after the decrease. We assigned this transition as the dissociation of IgG−α-IgG bonds. When loading excessive α-mouse IgG-conjugated magnetic particles on another IgG-coated surface (blue trace), both transitions were observed, which confirms our assignments. The uncertainty of the magnetization values in this work is approximately 5×10^{-12} Am^2, which is estimated from the sample distance to the atomic magnetometer and the sensitivity of the instrument.[15]

To calibrate the force, we used centrifugal force to dissociate the same IgG−α-IgG bonds with uniform 2.8 μm magnetic particles. The magnetization profile is shown in Figure 3a. The inset shows the corresponding force-induced remnant magnetization (FIRM) spectrum, which is obtained by taking the derivative of the magnetization profile. The broad character of the force spectrum is due to the mass distribution of the particles. The centrifugal force is given by $f = m\omega^2 r$, where m is the buoyant mass of the particles, ω is the centrifuge angular velocity, and r is the distance of the sample from the rotation center (4 cm in this work).[17] From this spectrum and the mass distribution of the particles, we obtained a dissociation force of 120 ± 15 pN for the IgG−α-IgG bond. The dissociation force for physisorption was consequently obtained to be 17 ± 3 pN based on the ω values in the inset in Figure 3a. These values are consistent with the results using single-molecule techniques.[4,18,19] The magnetization values are lower than the values in Figure 2 because the particles used here have weaker magnetic moments than the particles used in the shaking experiment in Figure 2.

After determining the force, we varied the speed of the shaker at its finest step to improve the force resolution. Two similar transitions to Figure 2 were observed (Figure 3b), and the corresponding FIRM spectrum was derived (inset in Figure 3b). The two peaks in the spectrum correspond to the two dissociation transitions, at 120 ± 15 pN for IgG−α-IgG bonds

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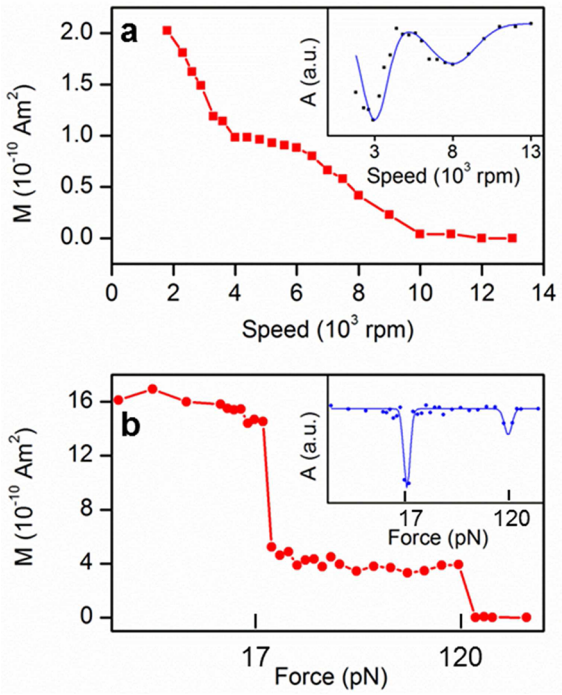

Figure 3. (a) Magnetization profile vs centrifuge speed for IgG–α-IgG binding. (b) Magnetization profile vs calibrated shaking force for the same interaction. The insets show the corresponding FIRM spectra. *A*: amplitude.

and 17 ± 3 pN for physisorption, based on the force calibration in Figure 3a. The decrease of magnetization to zero at 120 pN also suggests that the binding between the magnetic particles and the surface is mostly single IgG–α-IgG bonds.

We verified the dissociation force of the IgG–α-IgG bond by rebinding experiments (Figure 4) and kinetics study (Figure 5). As shown in Figure 4a, there are three possible positions for the dissociation to occur: between the magnetic particle and the ligand molecule (position A), the desired position between the

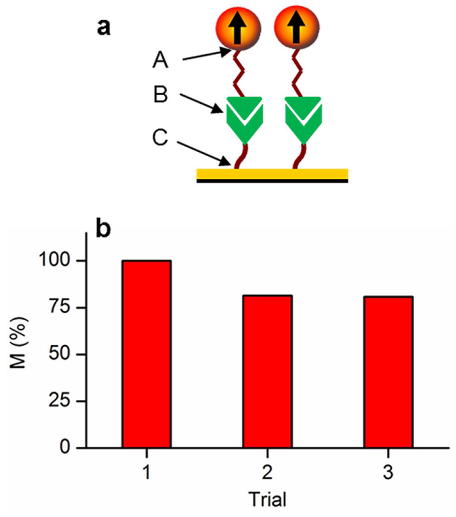

Figure 4. (a) Schematic of three possible positions, A, B, and C, for the dissociation between magnetically labeled ligand molecules and immobilized receptor molecules. (b) Magnetization comparison between the first binding experiment (trial 1) and two rebinding experiments (trials 2 and 3).

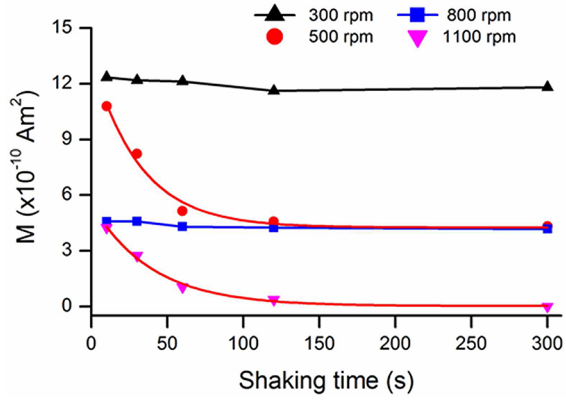

Figure 5. Dissociation kinetics at four different forces. The 300 and 500 rpm are smaller and greater than the dissociation force of 437 rpm for the physisorption, respectively. The 800 and 1100 rpm are smaller and greater than the dissociation force of 1025 rpm for the IgG–α-IgG bonds, respectively.

ligand and receptor molecules (position B), and between the receptor molecule and the surface (position C). For rebinding, the same IgG-coated surface was used for binding three times. The bound α-IgG-conjugated particles were measured each time and then removed entirely by shaking and rinsing with buffer. Then, the washed surface was incubated with a new batch of α-IgG-conjugated magnetic particles and was shaken at 800 rpm for 5 min to remove physisorption. The magnetization of the remaining specifically bound magnetic particles was measured (trial 2). The whole process was repeated for trial 3. The magnetizations for bound particles in the rebinding experiments were each 82% of that of the first binding experiment (Figure 4b). The high percentages of rebinding particles eliminated the possibility of IgG dissociation from the gold surface and α-IgG dissociation from the magnetic particles. If either had occurred, the surface would not be able to specifically rebind α-IgG-conjugated particles. Only when the dissociation takes place at position B will the surface be able to form noncovalent bonds with a new batch of receptors. Rebinding is a unique advantage of our approach for verifying the dissociation of specific bonds.

The dissociation kinetics shown in Figure 5 confirmed that the dissociation force was well-defined. Magnetization vs duration of force was studied for 300 and 500 rpm (before and after dissociation of physisorption, respectively) and 800 and 1100 rpm (before and after IgG–α-IgG dissociation, respectively). No significant decrease was observed for 300 and 800 rpm, indicating no dissociation of physisorption and IgG–α-IgG, respectively. Dissociation at 500 rpm (for physisorption) and 100 rpm (for IgG–α-IgG bonds) took less than 2 min, verifying that 5 min of force duration was sufficient for completing the dissociation processes.

Another consideration is the effect of magnetization relaxation of the particles in our experiments. We measured the magnetization of an ensemble of the particles versus time and observed a decrease of only 7% after 8 h. Therefore, relaxation is not a significant factor for our experiments. The magnetization decreases were due to the randomization of the magnetic dipoles following the dissociation of the corresponding molecular interactions.

The magnetization decrease is a direct measurement for the quantity of its corresponding interaction. One advantage of using atomic magnetometers for detection is that they measure

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the absolute magnetic fields and hence the magnetization values.[15] We measured the magnetization of various amounts of the magnetic particles used in labeling the α-mouse IgG molecules. A linear correlation was obtained (Figure 6), which

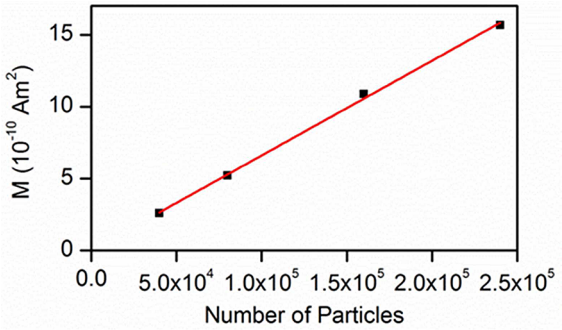

Figure 6. Calibration for the number of magnetically labeled molecules.

was the basis for obtaining the quantity of magnetic particles from the measured magnetization. For physisorption, the decrease is 1.2×10^{-9} Am^2, corresponding to 1.8×10^5 magnetic particles; for specific binding, the magnetization decrease is 4×10^{-10} Am^2, equivalent to 6×10^4 magnetic particles.

To demonstrate that different bonds possess distinctive dissociation forces, we investigated biotin–streptavidin bonds, which are widely used in biochemical research.[20] We incubated streptavidin-coated particles with IgG- and biotin-coated surfaces (Figure 7). At the IgG-coated surface, only

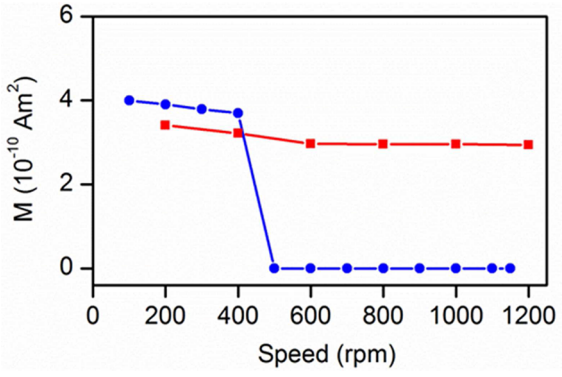

Figure 7. Plots of magnetization vs shaking force for biotin–streptavidin bonds (red trace) and control experiment without biotin (blue trace).

physisorption should take place, as IgG is not the matching receptor for streptavidin. Consistently, magnetization decreased to zero at a shaking speed of 500 rpm, the same force noted in the previous physisorption. Using a biotin-coated surface, however, the magnetization underwent no change even at 1200 rpm, which represents the current force limit. This result is consistent with the observation that the dissociation force for biotin–streptavidin bonds is 257 pN,[14,21,22] substantially stronger than IgG–α-IgG bonds. The force limit can be extended by using different forms of force. For example, the fluid dragging force can reach several nN, which should be sufficient to dissociate most noncovalent bonds.[23] Ultrasound has been used to dissociate covalent bonds,[24] which are in general stronger than noncovalent bonds investigated in here. Related work is currently under investigation.

CONCLUSIONS

We demonstrate a well-defined binding force for noncovalent antibody–antigen bonds. The force is potentially capable of serving as an indicator for a particular biomarker. Using our FIRMS technique, the dissociation forces of the bonds are obtained without the need of sample purification. Furthermore, the experimental setting is suitable for analyzing opaque samples because the detection technique is magnetic-based. Therefore, our work will open a new avenue for molecular recognition and biomedical diagnostics.

ASSOCIATED CONTENT

Supporting Information

Force calibration details and consideration of magnetization relaxation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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