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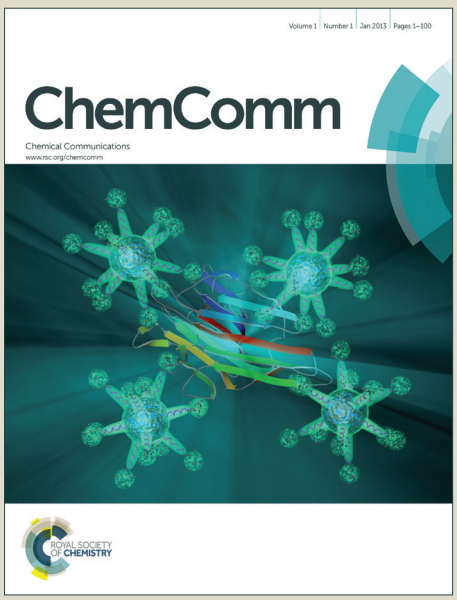

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COMMUNICATION

Mechanically resolving noncovalent bonds using acoustic radiation force

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The resolution of molecular bonds and subsequent selective control of their binding are of great significance in chemistry and biology. We have developed a method based on the use of acoustic radiation force to precisely dissociate noncovalent molecular bonds. The acoustic radiation force is produced by extremely low-power ultrasound waves and is mediated by magnetic particles. We successfully distinguished the binding of antibodies of different subclasses and the binding of DNA duplexes with a single-base-pair difference. In contrast to most ultrasound applications in chemistry, the sonication probe is noninvasive and requires a sample volume of only a few microliters. Our method is thus viable for noninvasive and accurate control of molecular bonds that are widely encountered in biochemistry.

Noncovalent molecular binding is a major pathway for molecular recognition in chemical and biological processes.[1,2] The resulting bonds are usually specific to molecular structures, including antibody subclasses and DNA sequences, with characteristic binding strengths. Current research has primarily focused on characterizing these molecular bonds. Representative techniques include atomic force microscopy (AFM),[3-5] optical tweezers (OT),[6,7] and the recently developed force-induced remnant magnetization spectroscopy (FIRMS).[8,9] However, noninvasive control of molecular binding remains a challenge: AFM is a single-molecule technique and requires the molecular system to be directly attached to the force medium; the OT technique is also based on single molecules and has a relatively small force range; and FIRMS currently uses shaking or centrifugal forces that are difficult to implement for direct bond manipulation.[10]

A new form of mechanical force is thus needed to couple with FIRMS. One possible candidate is acoustic radiation force (ARF) generated by ultrasound radiation.[11] Ultrasound radiation has been commonly used for cleaning, extracting biological entities from cells, and medical imaging.[12,13] Recently, its application scope has been extended to organic chemistry to promote the synthesis of target products.[14,15] Polymers containing a weak bond in the central portion can be selectively dissociated.[16,17] Despite these wide-range applications, there have been no reports on the use of precisely controlled ARF for mechanical manipulation of noncovalent bonds.

Here, we report that ARF, produced by extremely low-power ultrasound radiation and mediated by magnetic particles, can selectively dissociate noncovalent bonds according to their different binding strengths. The transducer is not immersed in the sample, which paves the way for noninvasive control of molecular binding. The principle is illustrated in Fig. 1. The sample well contains multiple types of noncovalent bonds with various abundances. Two types of bonds are shown as an example. One bond occurs between a magnetically labelled ligand and receptor 1. The other bond occurs between the ligand and receptor 2, which is assumed to have a weaker binding force than the former. When low-power ultrasound radiation is applied, the resulting ARF exerted on the magnetic particles will only dissociate the weaker bonds between the ligand and receptor 2. The dissociated magnetic particles will yield a decrease in the magnetic signal because of the randomization of their magnetic dipoles. This is the basis of the FIRMS technique.[8] Then, a stronger ARF produced by a slightly higher-power ultrasound can dissociate the stronger bonds between the ligand and receptor 1. The process can be repeated until all noncovalent bonds are resolved based on their binding forces, which will be indicated by a zero magnetic field.

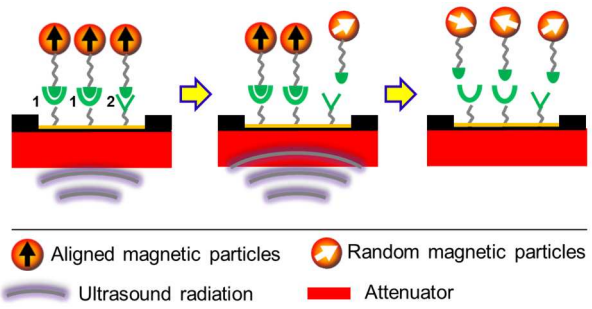

Fig. 1 Principle of the ARF-based FIRMS technique for the selective dissociation of noncovalent molecular bonds. 1 and 2 indicate two different types of receptors on the surface.

We have chosen a molecular system of protein A binding with three mouse IgG subclasses: IgG_1, IgG_{2b}, and IgG_{2a}. The order of the IgG antibodies represents their increasing binding strengths for protein A.[18] The antibodies were immobilized on the surface, while protein A was conjugated to the magnetic particles. The ultrasound power of a modified sonicator was calibrated using a thermal method (Supporting Information, Fig. S1).[19] The radiation power was in the range of mW/cm^2, with duration of 30 s. Figure 2 shows the results of the ARF-induced dissociation of each type of bond and their respective FIRM spectra, which were obtained by taking the derivative of the corresponding magnetic signal profile. The magnetic signals were detected by an atomic magnetometer (Supporting Information). For an incremental

power step of 3 mW/cm^2, the dissociation ultrasound powers for the three IgG-protein A bonds were found to be 22, 34, and 47 mW/cm^2, respectively. The different dissociation power values are consistent with the order of the binding strengths of the three IgG antibodies interacting with protein A. The results also indicate the capability of this technique to resolve different noncovalent bonds by adjusting the ultrasound power and hence the resulting ARF.

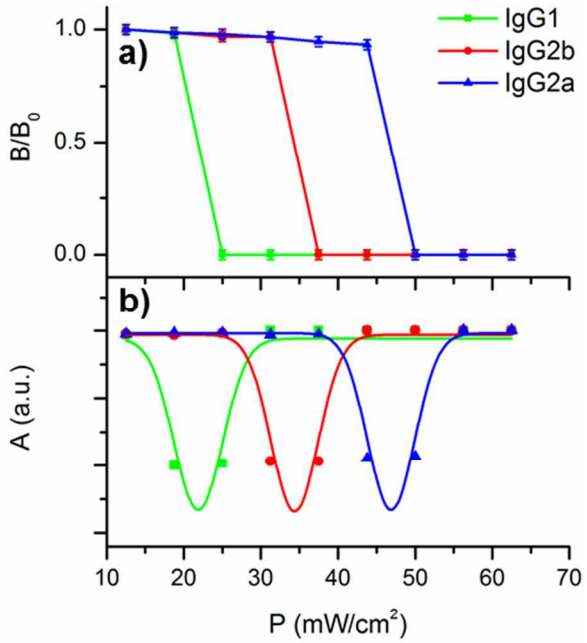

Fig. 2 ARF-induced selective dissociation of protein A−mouse IgG bonds. a) Relative magnetic signal as a function of ultrasound power for three different bonds. b) Corresponding FIRM spectra for the profiles in a).

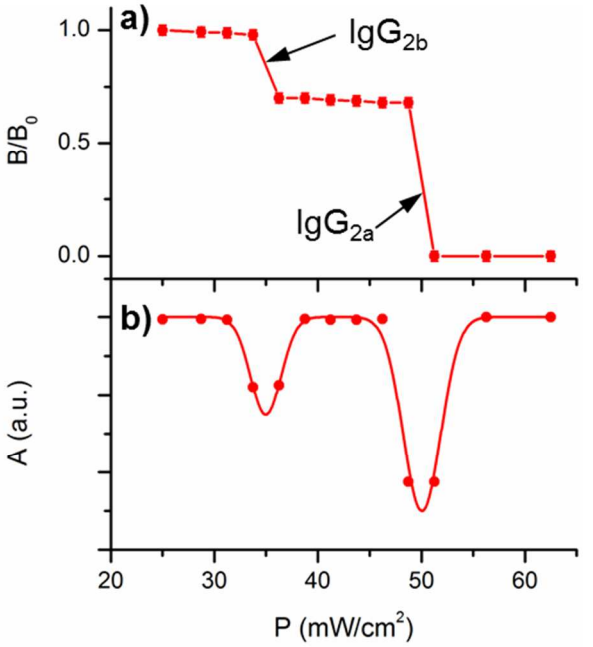

Fig. 3 Resolving noncovalent bonds using ARF. a) Magnetic signal profile of ARF-induced dissociation of protein A-IgG$_{2b}$ and protein A-IgG$_{2a}$ in a single sample. b) Corresponding FIRM spectra of a).

To demonstrate the resolving capability of ARF for different bonds, we applied more precisely adjusted ultrasound radiation to a sample well containing both IgG$_{2a}$ and IgG$_{2b}$ (Fig. 3). The incremental power step was reduced to 1.5 mW/cm^2. Two dissociations were observed, one at 35 mW/cm^2 and one at 50 mW/cm^2. Based on the individual studies presented in Fig. 2, we attributed the former dissociation to the protein A-IgG$_{2b}$ bonds and the latter to the protein A-IgG$_{2a}$ bonds. Differentiation of the profile yielded a FIRM spectrum consisting of two well-resolved peaks (Fig. 3b). The peak positions represent the respective binding strengths, and the peak heights correspond to the respective abundances.

The binding forces of the noncovalent bonds were obtained with FIRMS by employing a centrifugal force (Supporting Information, Fig. S2).[9,20] The dissociation speeds were 1600, 2600, and 3000 rpm (revolutions per minute) for IgG$_1$, IgG$_{2b}$, and IgG$_{2a}$, respectively. These values correspond to 9±2, 24±2, and 32±3 pN for protein A binding to IgG$_1$, IgG$_{2b}$, and IgG$_{2a}$, respectively. The force errors were based on the minimum increment of 100 rpm in the centrifugal speed. The correlation between the binding force and the ultrasound power is plotted in Fig. 4. Because an exact calculation of the ARF is challenging,[21] the use of bonds with well-characterized binding forces offers a viable scale for ARF calibration.

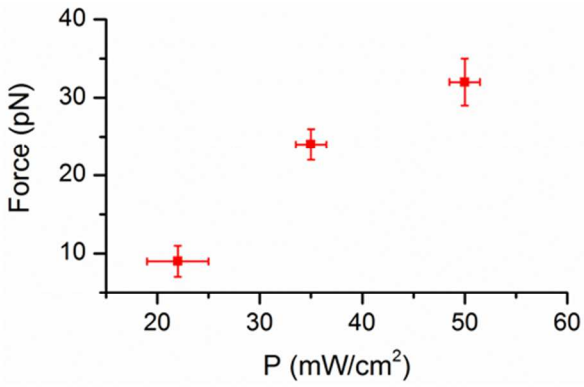

Fig. 4 Correspondence of ultrasound power with the binding forces of noncovalent bonds.

We compare our application of ARF with other ultrasound applications in chemistry. First, the ultrasound power in this work is much lower than that of other methods.[14-17] The attenuation factor resulting from a 6.5-mm-thick rubber layer placed between the sample and the transducer is estimated to be 5600, by comparing the manufacturer-specified power and the attenuated power. In contrast, ultrasound-induced dissociation of covalent bonds typically requires two orders of magnitude higher power. Second, the duration of this application is only 30 s, compared to the several hours required in organic synthesis assisted by ultrasound. Third, the ARF was precisely tuned to selectively dissociate different molecular bonds, which has not been achieved in previous works. Coupled with a noninvasive scheme in which the transducer does not contact the sample, this work paves the way for mechanical control of molecular bonding.

The power used in this work is nearly an order of magnitude below the cavitation threshold for 20-kHz ultrasound radiation.[22] Therefore, the effects associated with cavitation can be excluded. In addition, due to the low power and short duration of our

approach, the thermal effect in our experiments was minimal. For example, the dissociation power for IgG_{2a}, the highest of the three, was 50 mW/cm^2. This power corresponds to a mere 2.2 °C increase in the sample temperature.

To further illustrate the resolving capability of ARF for noncovalent bonds, we designed two DNA duplexes with only a 3 °C difference in melting temperatures using mfold software.[23] The two sequences are as follows:

Duplex 1: 3′-GGG TTT TTT TTT TTT GGG-5′
5′-CCC GGG AAA AAA AAA CCC-3′
Duplex 2: 3′-GGG TTT TTT TTT TTT GGG-5′
5′-CCC GGA AAA AAA AAA CCC-3′

The duplexes were designed such that the dissociation power remained low to avoid substantial thermal effects but was higher than that of protein A-IgG_{2a} to expand the force range for studying more molecular binding systems. One of the strands in the duplexes was immobilized on the gold-coated bottom surface of the sample well, and the other was labeled with magnetic particles (Supporting Information).

Figure 5 shows the results of ARF-induced dissociation of the DNA duplexes. The increment of the ultrasound power was 1.5 mW/cm^2. With such fine tuning, the dissociation power of the two DNA duplexes was well characterized. For duplex 1, the dissociation power was 67 mW/cm^2, and for duplex 2, it was 72 mW/cm^2. The difference of 5 mW/cm^2 is substantially greater than the 1.5-mW/cm^2 uncertainty. Therefore, ARF can dissociate stronger noncovalent bonds and can still distinguish between them with high resolution. The difference of 3 °C in melting temperatures is significantly smaller than the difference of 7 °C between the two DNA duplexes that we previously reported for centrifugal force.[10]

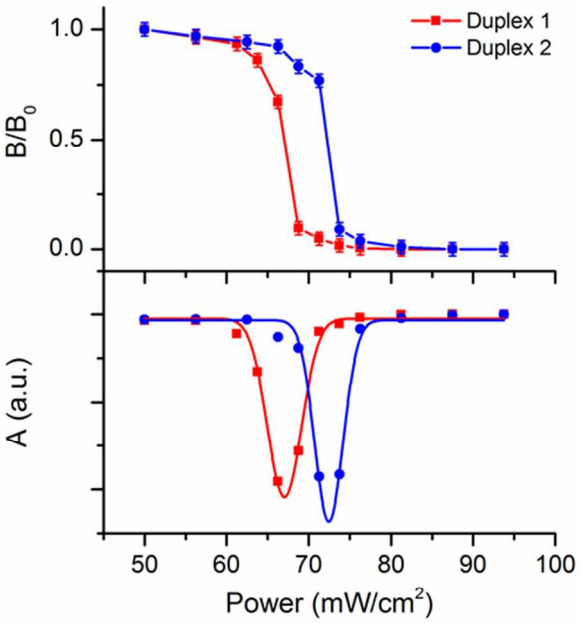

Fig. 5 ARF-induced selective dissociation of two DNA duplexes. a) Relative magnetic signal profiles as a function of ultrasound power. b) Corresponding FIRM spectra of the profiles in a).

The binding forces of the duplexes were also measured using centrifugal force. The dissociation speeds were 5800 and 6000 rpm for duplex 1 and duplex 2, respectively. Consequently, the binding forces are 136 and 146 pN for duplex 1 and duplex 2, respectively (Supporting Information, Figure S3). The higher binding forces demonstrate the wide application range of ARF-based FIRMS technique in resolving noncovalent bonds, which have typical binding forces between 10 and 150 pN.[1]

The use of ARF for highly selective bond dissociation represents a new branch of mechanochemistry. Compared to the previously reported shaking and centrifugal forces, ARF is advantageous in that the force generator can be integrated with the atomic magnetometer. The ultrasound probe is much smaller than either a shaker or a centrifuge, allowing it to be potentially placed inside the magnetic shield of the magnetometer. This implementation will eliminate the need for a manual sample transfer between the force application and signal measurement. The development of ARF-based bond dissociation also allows for the study of molecular interactions under conditions, for example in vivo, that cannot be employed in a shaker or centrifuge.

One unknown aspect of ARF is how the ultrasound frequency will affect the dissociation of noncovalent bonds. Frequency plays a role in both power reduction due to the attenuation and penetration of the ultrasound into the medium.[24,25] Research related to this issue is currently being performed.

In conclusion, we have shown that selective dissociation of noncovalent bonds can be achieved by ARF. The force produced by precisely adjusted ultrasound radiation is capable of resolving different antibodies and DNA duplexes. Due to their small size, ultrasound probes can be integrated with an atomic magnetometer. Consequently, ARF-based FIRMS will be capable of noninvasive mechanical manipulation of molecular interactions.

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Notes and references

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† Electronic Supplementary Information (ESI) available: experimental details, signal of DNA exchange using two different types of magnetic particles. See DOI: 10.1039/b000000x/

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