Supporting Information
Mechanically Resolving Noncovalent Bonds Using Acoustic Radiation Force
Lashan De Silva, Li Yao and Shoujun Xu

Table of Contents:
Modification and power calibration of a sonicator, with Table S1 and Fig. S1;
Experimental details;
Using centrifugal force to measure the binding forces of protein A-IgG, with Fig. S2;
Using centrifugal force to measure the binding forces of DNA duplexes, with Fig. S3.

1. Modification and power calibration of a sonicator

A commercial sonicator, Q55 from Qsonica (Newtown, CT, USA), was modified by replacing the power-adjusting potentiometer (1 turn, 10 kΩ) with a more precise potentiometer (10 turns, 10 kΩ). Therefore, the output power can be more precisely tuned. The position of the potentiometer gives the relative output power, based on information from the manufacturer. An ultrasound transducer with a diameter of 6.5 mm was placed underneath the sample well. A rubber spacer was placed between the transducer tip and the sample well to attenuate the ultrasound power. The duration for each power was 30 s.

The ultrasound power was quantified by a thermal method (T. Kikuchi and Y. Uchida, J. Phys.: Conf. Ser., 2011 279, 012012.), which measures the power as heat absorbed by a fixed volume of liquid. Here, a sample well was filled with 20 μL of buffer. The buffer temperature was measured by a thermal controller (CN9000A, Omega) with ±0.1 °C accuracy. The average power was calculated as \( mC\Delta T/t \), where \( m \) is the mass of the buffer, \( C \) is the heat capacity of water which is considered equal to that of the buffer, \( \Delta T \) is the temperature change, and \( t \) is the ultrasound duration of 30 s. The error for the power measurements was calculated to be ±0.3 mW from the temperature uncertainty. The results are shown in Table S1, and the power density results are plotted in Fig. S1.

Table S1. Power calibration of the attenuated ultrasound radiation

<table>
<thead>
<tr>
<th>Relative Output Power (%)</th>
<th>( \Delta T ) (°C)</th>
<th>Measured Power (mW)</th>
<th>Power Density (mW/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.9</td>
<td>2.0</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>1.3</td>
<td>2.9</td>
<td>36</td>
</tr>
<tr>
<td>40</td>
<td>1.7</td>
<td>3.8</td>
<td>48</td>
</tr>
<tr>
<td>50</td>
<td>2.2</td>
<td>4.9</td>
<td>61</td>
</tr>
<tr>
<td>60</td>
<td>2.8</td>
<td>6.3</td>
<td>79</td>
</tr>
</tbody>
</table>
2. Experimental details

For each protein A-IgG experiment, a 2×4×3 mm3 sample well was prepared, with the bottom surface coated with streptavidin via streptavidin-biotin interaction. Then, a 0.1 mg/mL solution of biotinylated antibodies (mouse IgG1, mouse IgG2a, and mouse IgG2b, from Thermo Scientific) was pipetted into the sample well and incubated for 1 hr. After rinsing with a PBS solution (0.05% Tween-20), the sample was immersed in a 1% (w/w) solution of the blocking agent bovine serum albumin (BSA) for 1 hr. Protein A-coated magnetic dynabeads (Invitrogen) were then added and incubated for 2 hr.

For the DNA experiments, the common strand of the sequences, 3’-GGG TTT TTT TTT TTT GGG-5’, was immobilized on the gold-coated surface via thiol-gold bonds. The respective second strands, which were biotinylated, were then added to the sample well for incubation. Streptavidin-coated magnetic particles (M280, Invitrogen) were then added to bind to the second strands. The remaining experimental parameters are the same as previously described (L. De Silva, L. Yao, Y. Wang and S.-J. Xu, J. Phys. Chem. B, 2013, 117, 7554-7558.).

The samples were magnetized by a permanent magnet for 2 min before measurement. The initial magnetic signal, $B_0$, was then obtained by an atomic magnetometer. The atomic magnetometer had a sensitivity of approximately 100 fT/(Hz)$^{1/2}$. After applying either ARF or centrifugal force with various amplitudes, the magnetic signal of the sample, $B$, was measured and normalized to the corresponding $B_0$. 

---

**Fig. S1** Power density calibration of the attenuated ultrasound radiation.
3. Using centrifugal force to measure the binding forces of protein A-IgG

The binding force $F$ of each bond equals the centrifugal force at which the bonds undergo complete dissociation. Therefore, $F = m\omega^2r$, where $m$ is the buoyant mass of the magnetic particles, $\omega$ is the centrifugal speed in rad/s, and $r$ is the distance between the sample and the center of the centrifuge. The diameter of the protein A-coated magnetic particles was measured as 2.58±0.05 μm by scanning electron microscopy. The particle density was determined to be 1.44±0.02 ×10$^3$ kg/m$^3$ by suspending the particles in a series of Cs$_2$SO$_4$ solutions with known densities. The density of the buffer was 1.0 kg/m$^3$. Therefore, $m = 4.0\times10^{-15}$ kg. For the centrifuge used in this work (Eppendorf 5417R), $r = 8$ cm. Figure S2 shows that the dissociation speeds are 1600 rpm (revolutions per minute), 2600 rpm, and 3000 rpm for IgG$_1$, IgG$_{2b}$, and IgG$_{2a}$, respectively. Therefore, the binding forces were calculated to be 9, 24, and 32 pN for IgG$_1$, IgG$_{2b}$, and IgG$_{2a}$, respectively.

![Fig. S2](image)

**Fig. S2** Magnetic field profiles of the dissociation of protein A-IgG bonds induced by centrifugal force.

4. Using centrifugal force to measure the binding forces of DNA duplexes

The binding forces of DNA duplexes were calibrated as described in the previous section. The dissociation speed was 5800 rpm for duplex 1 and 6000 rpm for duplex 2 (Figure S3). The streptavidin-coated magnetic particles used here have a buoyant mass of 4.6×10$^{-15}$ kg, as measured in a previous work (L. De Silva, L. Yao, Y. Wang and S.-J. Xu, *J. Phys. Chem. B*, 2013, 117, 7554-7558.), and $r = 8$ cm. Therefore, the binding forces are 136 pN and 146 pN for duplex 1 and duplex 2, respectively. The 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’

Fig. S3 Magnetic field profiles of the dissociation of DNA duplexes induced by centrifugal force.