

*Supplementary Information*

**Super-Resolution Force Spectroscopy Reveals Ribosomal Motion at  
Sub-Nucleotide Step**

Haina Jia,<sup>a</sup> Yuhong Wang,<sup>\*b</sup> Shoujun Xu<sup>\*a</sup>

<sup>a</sup>*Department of Chemistry,* <sup>b</sup>*Department of Biology and Biochemistry, University of Houston,*  
*Houston, TX 77204, USA*

**Table of Contents:**

1. Experimental procedures.
2. Technical details of SURFS.
3. Repeated SURFS results of the 12-bp DNA and 11-bp DNA with daunomycin, with Fig. S1.
4. FIRMS results of the 12-bp DNA and 11-bp DNA with daunomycin, with Fig. S2.
5. Force-voltage correlation by comparing SURFS with FIRMS results, with Fig. S3.
6. Repeated SURFS results of ribosome complexes Pre and Post using P12 DNA probe, with Fig. S4.
7. FIRMS results for the Post-P11 complex, with Fig. S5.
8. Comparing dissociation forces of mRNA-DNA duplex with and without spectinomycin, with Fig. S6.

## 1. Experimental procedures

*Materials* All DNAs and the mRNA were purchased from IDT (Integrated DNA Technologies). The magnetic particles were purchased from Invitrogen under the commercial name M280. All other chemicals were obtained from Sigma-Aldrich unless otherwise specified.

The DNA sequences were as follows:

DNA<sub>1</sub>: 5'-Bio-CCC AAT CGA CCC-3', Bio: biotin functionalized;

DNA<sub>2</sub>: 3'-GGG TTA GCT GGG-Bio-5', forming 12-bp (bp: basepair) duplex with DNA<sub>1</sub>;

DNA<sub>3</sub>: 3'-GG TTA GCT GGG-Bio-5', forming 11-bp duplex with DNA<sub>1</sub>;

DNA<sub>4</sub>: 3'-GG TTA CCT GGG-Bio-5', forming 10-bp duplex with DNA<sub>1</sub> due to the mismatching C (underlined).

The mRNA in the ribosome complexes and the two probing DNAs, P12 and P11, were:

mRNA: 5'-Bio-CAA UGU UAA UUA AAU UAA AUU AAA AAG GAA AUA AAA AUG UUU GAA AAA CGC UAC GUA **AAU CUA CUG CUG** AAC UC-3';

P12: 3'-**TTT AGA TGA CGA** GAA CTC-Bio-5', which forms 12-bp duplex with the mRNA using the complementary bases in bold;

P11: 3'-**ATT AGA TGA CGA** GAA CTC-Bio-5', which forms 11-bp duplex with the mRNA using the complementary bases in bold. Note P11 differs from P12 only by the 3'-end A.

*Preparation of the DNA samples* A plastic sample well of 4×3×2 mm<sup>3</sup> (L×W×D) was glued with biotin-coated glass as the bottom surface. Then 20 μL solution of 0.25 mg/mL streptavidin was loaded into the sample well and incubated for 1 hr. Then the sample well was rinsed twice with TE buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, and 0.05% Tween-20, pH = 7.5). Meanwhile, mix equal amount of DNA<sub>1</sub> and one other DNA (DNA<sub>2</sub>, DNA<sub>3</sub>, or DNA<sub>4</sub>) in a microtube, and incubate the microtube at 95 °C for 5 min. Allow the microtube to naturally cool down to room temperature. Next, 20 μL of 1 μM the formed DNA duplex was immobilized on the streptavidin-decorated surface and incubated for 1 hr. Subsequently, the sample well was rinsed with buffer solution twice. The streptavidin-coated magnetic particle were pre-washed three times with TE buffer and incubated with the DNA duplex for 2 hr. The physically absorbed magnetic particles were removed from the surface by centrifuging for 20 min at 2000 rpm (revolution per minute). Then the sample well was magnetized for 2 min by using a permanent

magnet and sealed with tape. When needed, daunomycin was introduced to the sample well and incubated for 1 hr.

*Preparation of the ribosome samples* All experiments were carried out in TAM<sub>10</sub> buffer (20 mM tris-HCl, 30 mM NH<sub>4</sub>Cl, 10 mM MgAc<sub>2</sub>, 70 mM KCl, 5 mM EDTA, 7 mM BME (2-mercaptoethanol), pH 7.6). Five mixtures, ribosome mix, TuWG mix, Tu0G mix, A mix, A-Lys mix, and A-Arg mix, were prepared. The ribosome mix contained 1 μM ribosome, 1.5 μM of IF 1, 2, 3, 2 μM of mRNA, 4 μM of charged fMet-tRNA<sup>fMet</sup>, and 4 mM of GTP. The TuWG mix contained 6 μM EFTu, 3 μM EF-G, 4 mM GTP, 4 mM PEP, and 0.02 mg/mL Pyruvate Kinase. The Tu0G mix contained no EF-G but all the rest components in TuWG. The A mix contained 100 mM Tris (pH 7.8), 20 mM MgAc<sub>2</sub>, 1 mM EDTA, 4 mM ATP, 0.1 mg/mL total synthetase, 50 A<sub>260</sub>/ml total tRNA, and 0.25 mM of phenylalanine, glutamic acid. The A-Lys mix contained 100 mM Tris (pH 7.8), 20 mM MgAc<sub>2</sub>, 1 mM EDTA, 4 mM ATP, 0.1 mg/mL total synthetase, 2 A<sub>260</sub>/ml tRNA<sup>Lys</sup>, and 0.25 mM of lysine. The A-Arg mix was similar to A-Lys mix except that tRNA<sup>Arg</sup> and arginine had replaced the Lysine counterparts.

To form the ribosome Pre and Post complexes, the five mixes were incubated at 37 °C for 25 min. Then, the resulted ribosome mixture, TuWG and A mixes were mixed with 1:2:2 ratio and then incubated at 37 °C for 15 min. The resulting ribosome complex was purified via 1.1 M sucrose cushion ultracentrifugation. The ribosome complex was then incubated with the A-Lys and Tu0G mixes at 37 °C for 2 min, followed by incubating with the EF-G solution that contained 2 μM EF-G, 4 mM GTP, 4 mM PEP, and 0.02 mg/ml Pyruvate Kinase at 37 °C for 30 min. The obtained MFEK Post (denoted as Post) was purified via 1.1M sucrose cushion ultracentrifugation. The MFEKR Pre (denoted as Pre) was formed by incubating MFEK Post (1 μM) with A-Arg mix in the ratio of 1:2 at 37 °C for 30 min. MFEKR Pre was purified via 1.1 M sucrose cushion ultracentrifugation.

The mRNA positions in the Post and Pre complexes were measured with probing DNAs P12 and P11 using SURFS. First, 20 μL of 0.04 μM biotinylated Post or Pre solution was immobilized on the streptavidin-decorated surface and incubated for 1 hr, followed by rinsing with buffer twice. Then 20 μL of 2 μM probe DNA P12 or P11 was loaded in the well and incubated for 1.5 hr. The streptavidin-coated magnetic particles were pre-washed three times using TAM<sub>10</sub> buffer and incubated with DNA duplex for 2 hr. The sample well was magnetized

for 2 min. The physically absorbed magnetic particles were removed from the surface by applying centrifuge with the speed of 2000 rpm for 20 min. Then the well was sealed with tape before SURFS measurements

For Post interacting with puromycin, first Post was incubated with 1mM puromycin for 10 min at 37 °C. Then, 20  $\mu\text{L}$  of 0.04  $\mu\text{M}$  the Post solution was immobilized on the streptavidin-decorated surface and incubated for 1 hr. Subsequently, the sample well was rinsed with buffer solution twice. Then 20  $\mu\text{L}$  of 2  $\mu\text{M}$  probe DNA P12 or P11 was loaded in the well and incubated for 1.5 hr, followed by SURFS measurement.

For Pre interacting with spectinomycin, a similar procedure as above was followed. The only difference was 1mM spectinomycin contained in the TAM<sub>10</sub> buffer.

*FIRMS measurements of the samples* The sample containing magnetically labeled noncovalent bonds were subject to centrifugal speed of 2000 rpm to remove the nonspecifically bound particles. Then a permanent magnet was used for magnetizing the particles. The particles were allowed to relax for 1 hr before the first magnetic field measurement. Centrifugal forces of increasing amplitudes were sequentially applied on the sample, and the magnetic signal was measured after each force application. The centrifugal force was calculated according to  $F = m\omega^2r$ , in which  $m$  is the buoyant mass of the magnetic particles,  $\omega$  is the centrifugal speed, and  $r$  is the distance between the sample and the center of the centrifuge motor (L. De Silva, L. Yao, Y. Wang and S. Xu, *J. Phys. Chem. B*, 2013, **117**, 7554.). The value of  $r$  is 8 cm for Eppendorf 5417R centrifuge. A magnetic field profile was obtained by plotting the maxima of the scanning profiles vs. the centrifugal forces. Derivative of the curve gives its corresponding force spectrum.

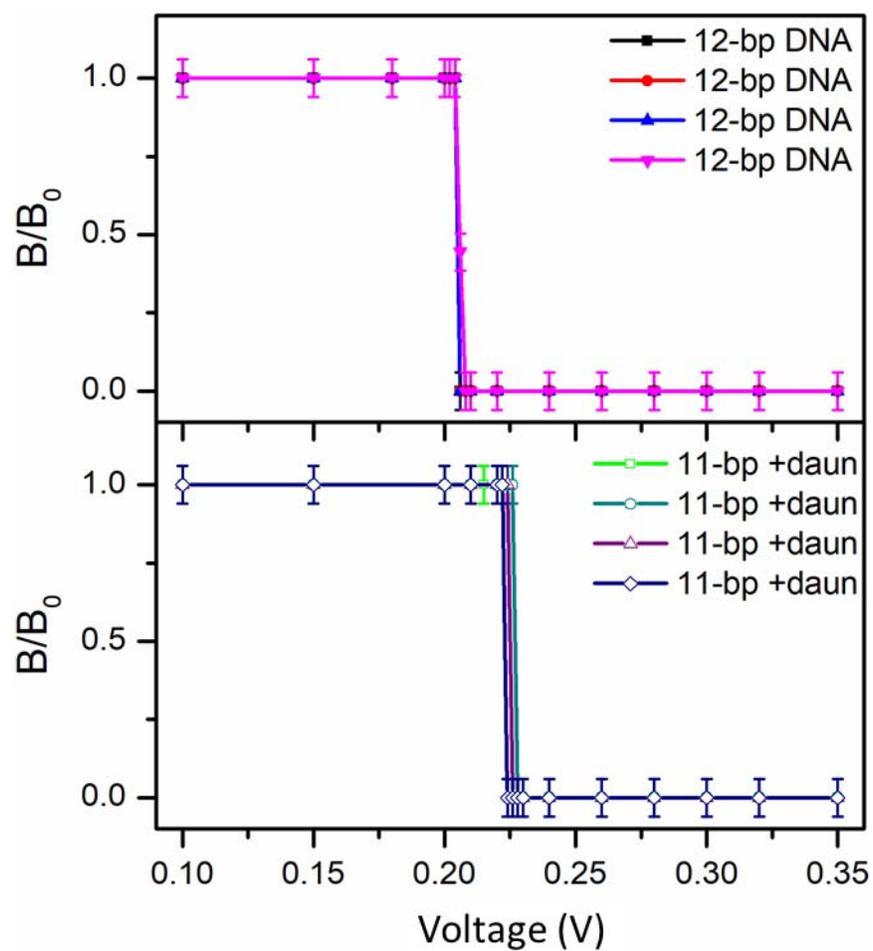
Detection of the magnetically labeled noncovalent bonds was achieved by using an atomic magnetometer. The sample well was mounted on a linear actuator and scanned in the vicinity of the atomic sensor that was located at the center of the magnetic shield. A magnetic field profile was obtained, which reached maximum when the sample well was the closest to the atomic sensor (L. Yao and S. Xu, *Angew. Chem. Int. Ed.*, 2009, **48**, 5679.).

## **2. Technical details of SURFS**

A function generator (DS345, Stanford Research Systems) was used to generate 1.0 MHz ac signal with various amplitudes. The generated function was amplified by a RF amplifier (75A250A from AR). The amplified ultrasound drove a piezo disk that had resonance frequency of 1.0 MHz. The piezo disk was mounted at motorized stage for position adjustments. The sample was initially centrifuged at 2000 rpm for 20 min to remove nonspecific absorption. Then, it was placed on top of a spacer containing water. Beneath the plastic spacer located the piezo disk. A lower voltage of the function was first applied, typically 0.1 V, which was gradually increased to up to 0.3 V. Each time the radiation was applied for 30 sec.

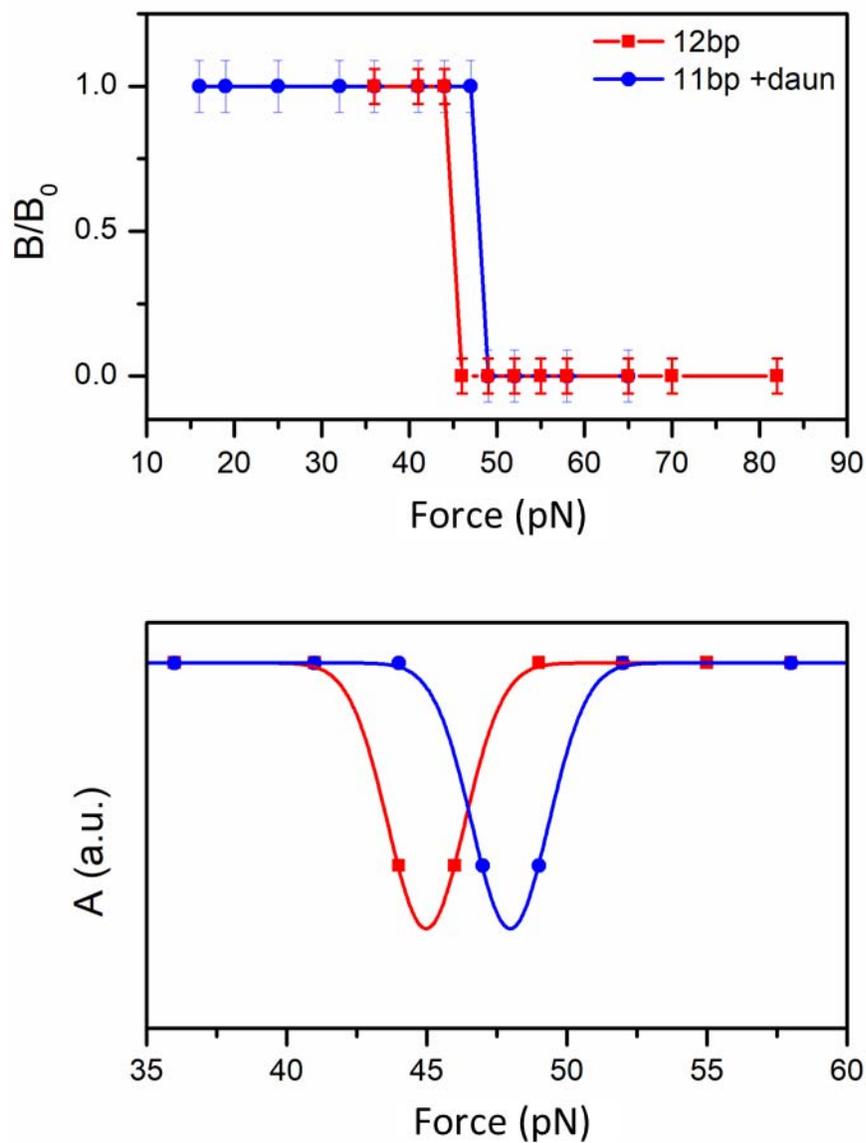
The piezo disk was purchased from Steiner & Martins, Inc. ([www.steminc.com](http://www.steminc.com)). The diameter was 15 mm and thickness 2.1 mm.

### 3. Repeated SURFS results of the 12-bp DNA and 11-bp DNA with daunomycin



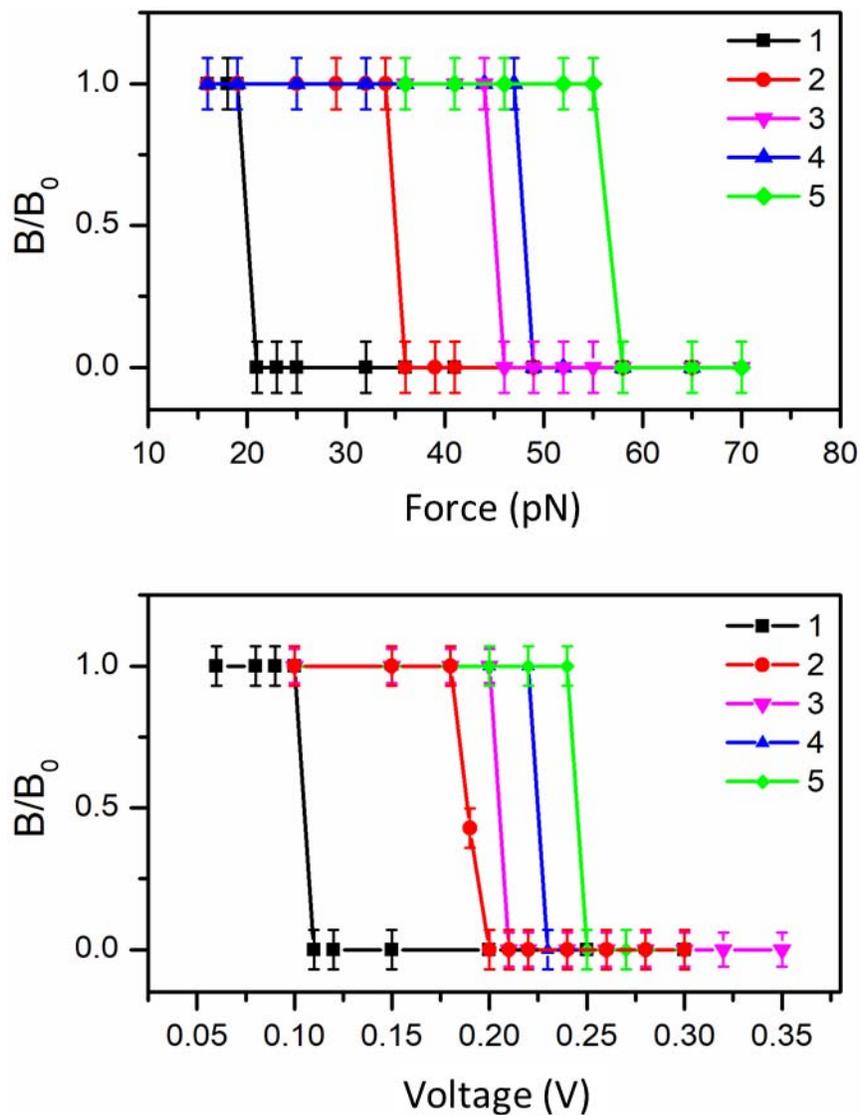
*Fig. S1. Repeated SURFS measurements for the 12-bp DNA duplex (top) and 11-bp DNA intercalated with daunomycin (bottom). Daun: daunomycin.*

#### 4. FIRMS results of the 12-bp DNA and 11-bp DNA with daunomycin



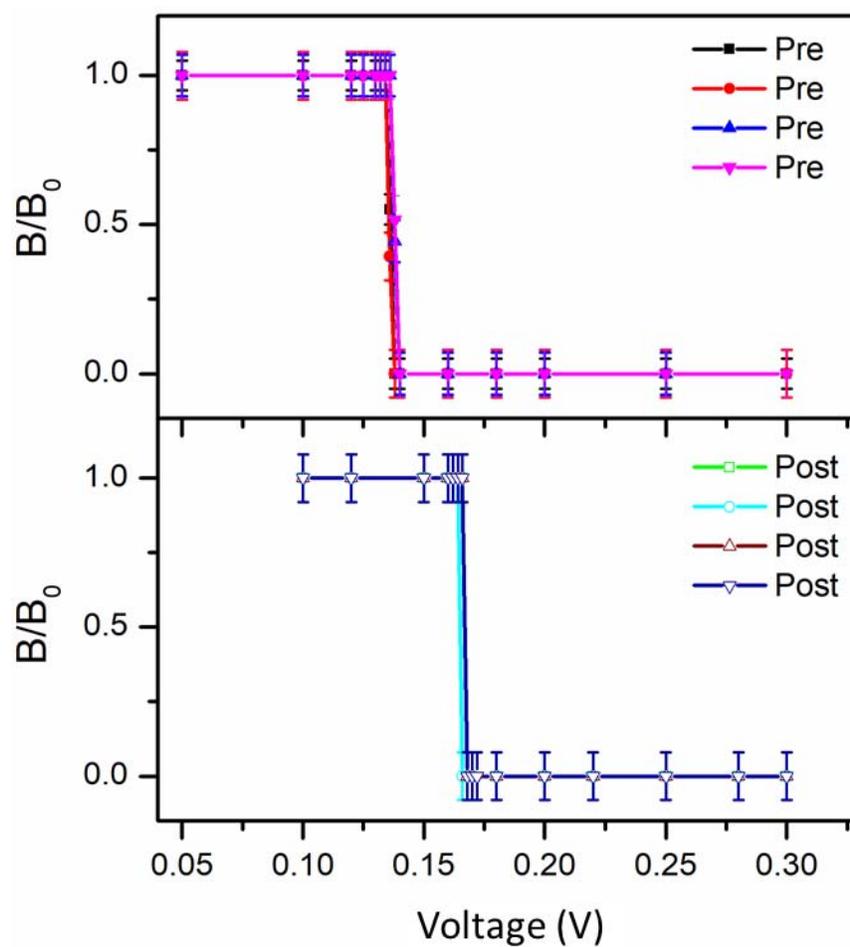
*Fig. S2. FIRMS results of the 12-bp DNA and 11-bp DNA with daunomycin. Top: magnetic field profiles vs. Centrifugal force. Bottom: derivatives of the above profiles in an expanded range to show incomplete peak separation. Daun: daunomycin.*

## 5. Force-voltage correlation by comparing SURFS with FIRMS results



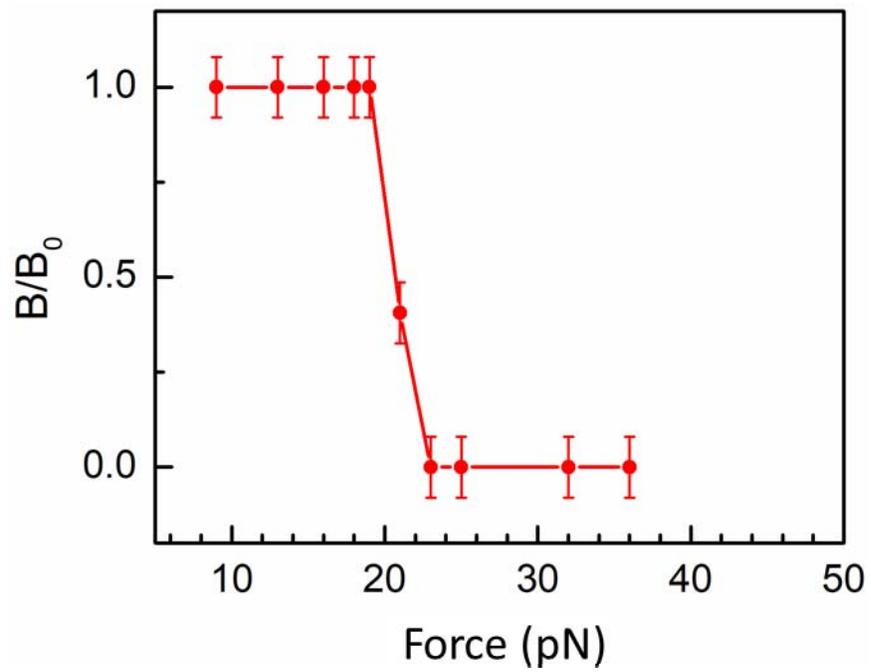
**Fig. S3.** Dissociation forces measured by FIRMS (top) and voltages by SURFS (bottom) for five different DNA systems. 1: 10-bp duplex; 2: 11-bp duplex; 3: 12-bp duplex; 4: 11-bp duplex intercalated with daunomycin; 5: 12-bp duplex intercalated with daunomycin.

6. Repeated SURFS results of ribosome complexes Pre and Post using P12 DNA probe



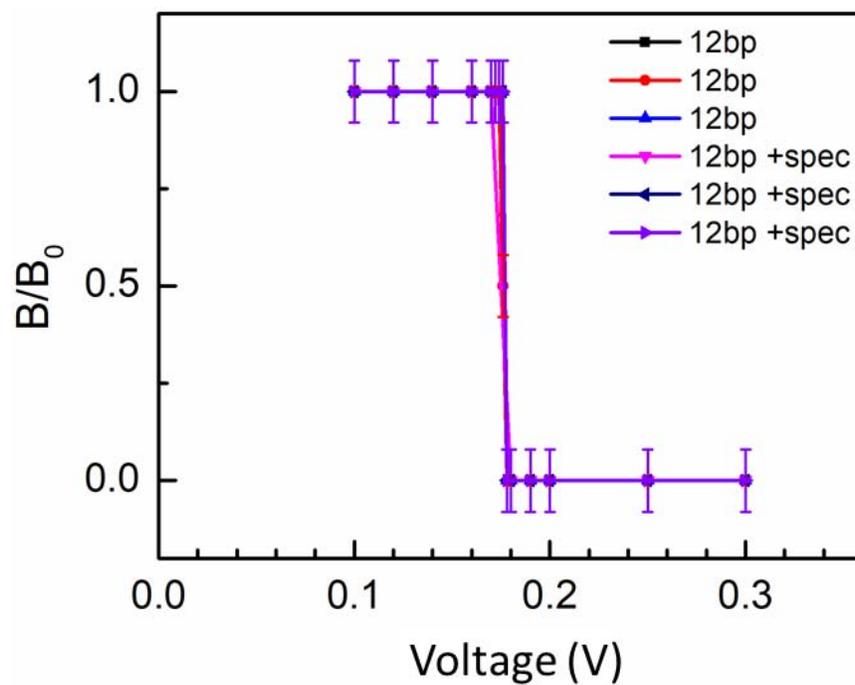
*Fig. S4. Repeated SURFS results for the Pre and Post complexes using P12 DNA probe.*

## 7. FIRMS results for the Post-P11 complex



*Fig. S5. The dissociation force of the Post-P11 complex using FIRMS.*

### 8. Comparing dissociation forces of mRNA-DNA duplex with and without spectinomycin



**Fig. S6.** SURFS results of the mRNA-P12 duplex in the absence and presence of spectinomycin.