Supplementary Information

Following aptamer-ricin specific binding by single molecule recognition and force spectroscopy measurements

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SI-1. Materials and methods for AFM experiments.

The aptamer sequence was obtained from literature, and the 5’ terminal was modified with a short hydrocarbon linker and amine group (Figure SI-1A). This modified sequence was purchased from Integrated DNA Technologies (Coralville, IA, USA). The ricin sample was provided by Vector Laboratories (Burlingame, CA). The polymer linker thiol-(polyethylene glycol)-acid (HS-PEG-COOH, M.W. 2000) was purchased from Creative PEGWorks (Winston Salem, NC, USA). The N-hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (EDC) was purchased from Flucka Chemicals (Sigma-Aldrich, St. Louis, MO, USA). The lipoic acid-NHS ester (LA-NHS) was synthesized in our lab. Phosphate buffer saline (PBS, pH 7.2) was purchased from Pierce (Thermo Scientific, Waltham, MA, USA). Triplet DI water was provided by a Barnstead Nanopure Diamond Laboratory Water System.

In the AFM molecular recognition experiments, TopMAC mode (Agilent Technologies, Santa Clara, CA, USA) is used to obtain the topography images, and PicoTREC module (Agilent Technologies, Santa Clara, CA, USA) is used to obtain the recognition images. The AFM tip was coated with the magnetic material and gold. The antibody and aptamer are connected to the gold surface of the tip with different functionalized PEG2000 linker molecule. The ricin molecules were immobilized on Au(111) surface by LA-NHS. The reaction conditions have been optimized to make sure the single-molecule interaction happens.


It is very difficult, if not impossible, to obtain the crystal structures of aptamer and ricin-aptamer complex, so we have to use molecular modeling and simulation to help us understand their structures and possible conformations during the AFM recognition process. The ricin protein structure we are using is obtained from protein databank (PDB), with the PDB code of 2AAI. The small sugar ligands and water molecules were removed from the structure. Then this ricin structure was minimized using molecular dynamics software AMBER 10 for 500 steps of steepest descent minimization and followed by 500 steps of conjugate gradient minimization in vacuum. The sequences and secondary structure of aptamers was obtained from the reference. Then a NAB program (included in AMBER 10 package) was used to generate
possible 3D folding structures of this aptamers (Figure SI-1B). The antibody used in the AFM experiment is from goats, but its PDB is not available, so we use one PDB file of human IgG1 as a homological structure. The initial PDB file was obtained from the literature, and then the amino acid residues in complementarity determining regions (CDRs) were replaced according to literatures so that the CDRs are specific to ricin. This replacement is done by Sirius visualization software (version 1.2, University of California San Diego). Then the new model was minimized using AMBER 10 for 500 steps of steepest descent minimization and followed by 500 steps of conjugate gradient minimization in vacuum (Figure SI-1C).

Figure SI-1: The molecular structures of the aptamer and antibody. (A) The amine-modified aptamer sequence. (B) The aptamer folding structure is colored in blue, and the active residues to ricin are colored in red. (C) The antibody structure with two heavy chains in yellow and blue, two light chains in orange and black, and the active residues are colored in purple.

The complex formed by ricin and aptamer was obtained by molecular docking method since the binding conformation of this type of complexes is not available from experimental methods. We uploaded PDB files of the ricin, aptamers, and antibody (only Fab part) generated by ourselves to HADDOCK webserver and obtained the binding conformation of the complexes with the lowest value of HADDOCK score (more negative the HADDOCK score, more favorite
the conformation is)\textsuperscript{11,12}. The HADDOCK webserver need users to provide restraint definitions for the docking sites. These restraints of the binding sites were assigned based on our knowledge of biochemistry and structural biology. Based on the x-ray crystallography and site-directed mutagenesis study of ricin with other antibodies, the possible binding sites for aptamer may include ricin A chain residues Tyr80, Val81, Gly121, Tyr123, Glu177, and Arg180\textsuperscript{13,14}. These residues were assigned as active residues for ricin. For the aptamer, residues A17 to T22 were assigned as active residues since they locate at the top of the hairpin loop. For the ricin-antibody interactions, research has found that the CDR-H3 region at the center of the antibody binding region is the most important fragment for the repertoire diversity\textsuperscript{15}. The CDR-L3 region is in close contact with CDR-H3 so two residues from this fragment were also considered as the active residues. Therefore, in this modified human IgG1 structure, the heavy chain residues Asp104, Glu105, Arg106, Phe107, Asp108, Phe109, and light chain residues Leu93, Thr94 together were assigned as active residues in HADDOCK calculation. The binding sites on ricin were also determined by literatures. The ricin A chain residues Asn97 to Phe108 were found to have strong affinity to a monoclonal IgG antibody “R70” for mice\textsuperscript{9}. Therefore, these residues were assigned to be the active residues of ricin. Here we used intellectual guess (aka educated guess) to search the possible binding sites of the ricin, aptamers, and antibody, and then used molecular docking methods to test our predictions. After we obtained the binding conformations by HADDOCK web server, the binding residues were checked again manually in visualization software VMD. We find that the residues showing close contacts in the docking results were “shifted” from the input active residues. For the ricin-aptamer complex, ricin residues Tyr80, Tyr123, Glu177, and Arg180, and aptamer residues G18, T19, G20, and T22 were in close contacts. For ricin-antibody complex, ricin residues Glu102, Ile104, Thr105, His106, Thr109, Asp110, and Arg114, and antibody heavy chain Asp104, Glu105, Arg106, Phe107, Ala108, light chain Thr33, Lys67, Thr94, and Ser95 were in close contacts. Therefore, they are considered as the active residues in the docking conformations and labeled in Fig. 2 of the paper.

These predictions using simulation methods cannot represent real molecular structures in the experiments, but they are based on the results from other research work using conventional biochemistry methods mentioned above. Therefore, we believe these simulations and predictions can be used as references to our AFM experiments.
SI-3. Surface modification of AFM tips with the aptamer.

Figure SI-2 shows the reaction scheme for the tip modification. AFM tip was first immersed in HS-PEG-COOH solution (0.2 mg/mL in DMSO) at room temperature for 3 hours. After washing three times in DMSO and keeping 30 min in warm DI water (<50 °C) to remove physically adsorption, the tip was put into the EDC/NHS solution (10 mM, 1:1 in PBS, pH7.2) for 30 min. Next, the tip was washed with DI water once and dipped into aptamer solution (0.2 μM in PBS buffer pH 7.2) overnight at 4 °C. Finally, followed by washing with PBS buffer 3 times, and kept in PBS buffer for further use.

![Reaction scheme for the AFM tip modification with the aptamer.](image)

**Figure SI-2**: Reaction scheme for the AFM tip modification with the aptamer.

SI-4. Blocking experiments.

The blocking experiment was conducted in the flow-through liquid cell. The ricin immobilization and AFM tip modification were carried out with the same methods used before. The tip continued the scanning before and after the injection of aptamer solution. It is supposed to be a competition process between the aptamer in solution and the aptamer on the tip surface as both of them can react with ricin. The changes of topography and recognition images were
monitored and the representative result is shown in Figure SI-3. The recognition signals reduced significantly one hour after the injection, which proved the specificity of aptamer-ricin interaction. Here, the aptamer molecules in blocking solution deposited on the gold surface and covered the binding sites of ricin molecules during the scanning, so the aptamer on AFM tip could not interact with ricin anymore. However, the aptamer molecules in the block solution continued to deposit on the gold surface during the scanning, which changed the surface morphology and made the topography image blur. Finally it is very difficult to distinguish individual ricin and aptamer molecule (Figure SI-3, lower left). Besides that, the blocking effect is not consistent to every ricin molecule. Some ricin molecules still show weak recognition signals. It means that the aptamer molecules in solution didn’t block all of the ricin on the substrate during the period of one hour.

**Figure SI-3:** Blocking of the recognition signals using the aptamer as the blocking reagent. Upper parts: topography and recognition images obtained before injection of 1 μM aptamer solution. Lower parts: topography and recognition images obtained one hour after the injection of 1 μM aptamer solution. Left side are the topography image, right side are the recognition images. Scan size 450 × 450 nm² for all four images.
To further prove the specificity of aptamer-ricin binding, we did another blocking experiment using anti-ricin antibody as blocking reagent. After 1.3 μM anti-ricin antibody was injected into the liquid cell, the topography and recognition images were monitored (Figure SI-4). The antibody didn’t block the recognition signal because the antibody binding site on ricin is different from the aptamer binding site on ricin. Therefore, the aptamer on AFM tip can still bind to ricin with the existence of antibody and show recognition signals.

**Figure SI-4:** Blocking of ricin recognition signals using anti-ricin antibody as the blocking reagent. Upper parts: topography and recognition images obtained before injection of 1.3 μM antibody solution. Lower parts: topography and recognition images obtained 40 mins after the injection of 1.3 μM antibody solution. Left side are the topography image, right side are the recognition images. Scan size 300 × 300 nm² for all four images.

**SI-5. Representative force-distance curves obtained for antibody-ricin interactions.**

To compare the unbinding forces of ricin-aptamer complex with the ones of ricin-antibody complex, the force-distance curves of these two complexes were measured at the same five loading rates. At each loading rates, 250 curves were used to obtain the force distribution.
histogram and the most probably unbinding force was obtained from the fitting plot of each histogram. For ricin-aptamer complex, the representative force-distance curves at each loading rates are shown in Fig. 4A. For ricin-antibody complex, the representative force-distance curves at each loading rates are shown in Figure SI-5. At each loading rate, three force-distance curves were selected and overlaid.

![Force-distance curves](image)

**Figure SI-5:** The representative force-distance curves of ricin-antibody interaction at five loading rates.

**SI-6. Calculation of the dissociation constants of aptamer-ricin and antibody-ricin complexes from dynamic force spectroscopy data.**

According to Bell-Evans single-barrier model for the reaction of two biomolecule, the most probable unbinding force ($F^*$) and the natural logarithm of loading rate ($\ln R$) should have a linear relationship$^{16,17}$. For aptamer-ricin complex and antibody-ricin complex, the fitting plot of $F^*$ vs. $\ln R$ showed linear relationship with the R-square values of 0.973 and 0.966, respectively (Fig. 4C). Therefore the dissociation constant $k_{off}$ of these two reactions could be derived from the equation 1 shown below.

$$F^* = \frac{kT}{x_\beta} \ln R - \frac{kT}{x_\beta} \ln \left[ k_{off} \frac{kT}{x_\beta} \right]$$

(Eq. SI-6-1)

Here $k$ is the Boltzmann constant, $T$ is the temperature, $x_\beta$ is the parameter that represent the position of the energy barrier along the reaction coordinate, and $k_{off}$ is the dissociation constant of the binding complex at the equilibrate state$^{18}$. In the fitting linear plot of $F^*$ vs. $\ln R$, the $x_\beta$
and \( k_{\text{off}} \) can be obtained from the values of the slope, which is \((kT)/x_\beta\), and the intercept, which is 
\[-(kT)/x_\beta \times \ln(k_{\text{off}} \times (kT)/x_\beta)\], the second term on the right side of Eq. SI-61. The experiments were 
considered to be conducted at room temperature \((T = 25 \, ^\circ\text{C} = 298 \, \text{K})\), so \( kT \) value was fixed. For 
aptamer-ricin complex (Fig. 4C red line), the slope value was 8.452 pN, the intercept value was 
43.557 pN, so the \( x_\beta(\text{aptamer}) \) was calculated as 0.49 nm and \( k_{\text{off}}(\text{aptamer}) \) was calculated as 
6.8\times10^{-4}\, \text{s}^{-1}. For antibody-ricin complex (Fig. 4C blue line), the slope of the \( F^* \) vs. \( \ln R \) plot was 
8.614 pN, the intercept was 37.522 pN, so the \( x_\beta(\text{antibody}) \) was calculated as 0.48 nm and 
\( k_{\text{off}}(\text{antibody}) \) was calculated as 1.5\times10^{-3}\, \text{s}^{-1}.

**SI-7. Statistics of different ricin conformations in AFM topography images.**

To have a more comprehensive investigation of ricin immobilized on Au(111) surface, we did 
statistical analysis on various conformations of ricin molecules shown in AFM topography 
images. The ricin molecule was immobilized through the reaction of lysine residue on the 
protein surface with the LA-NHS linker molecule on gold surface (Fig. 1 A and B). Therefore, 
the lysine reacted with LA-NHS is the most important factor to determine the ricin conformation 
on the gold surface. According to the sequence of ricin molecule, seven lysine residues locate in 
ricin B chain and two lysine residues locate in ricin A chain. Therefore, nine conformations 
should be observed if each lysine residue can react with LA-NHS (Figure SI-6). We put these 
nine possible conformations in four conformation groups according to their similarities. The first 
column in each group shows the top view of these conformations with their reacting lysine 
residues pointing into the paper. These surface representations were used to identify the ricin 
conformations in AFM topography images. Among these conformations, eight of them have the 
A chain extended in the solution and B chain attached on gold surface, so the binding sites for 
the aptamer and antibody are available for the reactions. The second column shows the predicted 
side view of each conformation with its reacting lysine residue pointing down to gold surface. 
These four conformation groups can be distinguished with each other in the topography images 
but the conformations in each group cannot, due to the limitation of the AFM resolution. We 
found the corresponding topography images of each conformation group in a total amount of 
2291 ricin molecular images. However, some of these conformations cannot be distinguished in 
the topography images under the experimental conditions we used, so they were put into a group
named “others”. Some of them were too close to each other so the individual conformations were not clear. Other topography images were unidentified because of the tip broadening and compression effect. Table SI-1 shows the statistics of these conformation groups based on the comparison of experimental results and molecular modeling. Four AFM topography images, including the Fig. 3 shown in the article, were used to collect the images of different ricin conformations. Another representative topography image is shown in Figure SI-7. If we neglect the group of “others”, the most abundant conformation group was group I, which includes three conformations with their lysine residues in B chain attached to LA-NHS. Although the conformations in group I seemed to be the most popular ricin conformations, it is difficult to draw a solid conclusion because of the large amount of conformations in the group “others’. However, this statistics shows that AFM topography images can provide some helpful information to investigate the protein conformations when they are immobilized on the substrate.
Figure SI-6. The nine possible binding conformations of ricin with its different lysine residue (pink) attached to LA-NHS. In each group, the first column shows the top view of the conformation. The second column shows the reacting lysine residue (labeled in pink) that bind to the LA-NHS on Au (111) surface. Ricin A chain is in green, B chain in blue, binding sites to aptamer is in red, binding sites to antibody in purple.
Table SI-1. The statistical analysis of conformation groups of ricin shown in AFM topography images.

<table>
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<th>Group I</th>
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</tr>
</tbody>
</table>

Figure SI-7. Another representative AFM image showing different ricin conformations on Au(111) surface. Left: topography image, right: recognition image. The scan size is 1 μm × 1 μm.
Bibliography
14. X. Yan, J. D. Robertus, *Proteins: structure, function, and genetics*, 1998, **31**, 33-41