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

A new method combining single molecule recognition imaging, dynamic force spectroscopy (DFS) analysis and molecular simulations revealed the details of the binding events between aptamer and ricin. The simulated different ricin binding conformations with aptamer were consistent with AFM images.

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

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Single molecule recognition imaging and dynamic force spectroscopy (DFS) analysis showed strong binding affinity between an aptamer and ricin, which was comparable with antibody–ricin interaction. Molecular simulation showed a ricin binding conformation with aptamers and gave different ricin conformations immobilizing on substrates that were consistent with AFM images.

Single-molecular study can be used to investigate thermodynamic and kinetic properties of biophysical systems, such as protein–ligand or DNA–ligand complexes.1 Among these biomolecular interactions, aptamers with known nucleic acid sequences show specific interactions with certain target molecules, which draw increasing attention in both fundamental and applied research fields, especially protein–aptamer interactions.2,4 The aptamers provide versatile designs and structures in wide applications, such as genetic engineering and biosensors, for different purposes.3,5,6 Compared with antibody, aptamers can be reversibly denatured and easily modified by chemicals without losing activity, which also showed lower cost and less biological immunogenicity.2 However, because of aptamers’ flexible structures, it is difficult to predict their properties and functions, especially the specificity for single-molecule interactions, which limit the application.8,9 So the orientations and conformations of biomolecules during the modifications and interactions need more detailed investigation. Therefore, single-molecule studies of aptamers have been increasingly reported.10–12 Here, taking ricin and its aptamer as an example, we provide a method that combines the atomic force microscopy (AFM) single-molecule experiments with molecular modelling to investigate the binding conformations and affinities of biomolecules.

AFM with a sharp tip and a fine cantilever can provide detailed morphology and mechanical measurement for an aptamer–ricin complex at a single-molecule level. In our AFM molecular recognition and dynamic force spectroscopy (DFS) experiments, an aptamer was attached on an AFM tip as probe molecule to bind the target molecule of ricin on the Au(111) surface (Fig. 1). The detailed procedures are shown in ESI S3.† Briefly, the aptamer used in this work is a single-stranded DNA molecule and its sequence is obtained from ref. 13. The target molecule ricin is one of the most cytotoxic proteins and needs to be monitored strictly.14,15 We used the same methods as described in our previous published papers to synthesize the reaction reagents and immobilize ricin molecules on the Au(111) surface with the linker molecule lipoic acid–N-hydroxysuccinimide ester (LA-NHS).16,17

The schematics of the AFM experiments can be seen in Fig. 1. The first step was to modify the Au(111) surface with LA-NHS (Fig. 1A). Then ricin molecules were put on the surface so that the primary amine on lysine residues reacted with the LA-NHS to form a stable amide bond. As can be seen in Fig. 1B, most of the lysine residues are located on the ricin B chain, so the most probable ricin conformation is like the one shown in Fig. 1B, of which the ricin B chain is in close contact with the gold surface, while the A chain is on the top of the B chain and available to contact to the probe molecules. Therefore, the lysine-LA-NHS
reaction, which effectively constrained the ricin conformation, led to the always available binding sites in the ricin A chain to its probe molecule (Fig. 1B and C).

In order to design aptamers with high affinities to the target molecules, we need better understanding of the mechanism of the aptamer binding. The function of the DNA aptamer is directly related to its sequence and folding structure. Here we use molecular dynamics (MD) and molecular docking methods to predict the folding structure of the aptamer and the possible binding sites of the binding complexes. The details of the MD and docking simulation were shown in ESI S2. Fig. 2 shows the predicted binding conformation of the ricin–aptamer complex and the ricin–antibody complex using AMBER and HADDOCK web server.18–20 The ricin A chain, aptamer, antibody heavy chain, and antibody light chain are colored in green, blue, yellow, and orange, respectively. The predicted binding residues of these two complexes are highlighted in the backbone structures. For the ricin–aptamer complex (Fig. 2A), the ricin residues Tyr80, Tyr123, Glu177, and Arg180, and aptamer residues G18, T19, G20, and T22 were the probable binding residues. For the ricin–antibody complex (Fig. 2B), ricin residues Glu102, Ile104, Thr105, His106, Thr109, Asp110, and Arg114, antibody heavy chain residues Asp104, Glu105, Arg106, Phe107, and Ala108, and light chain residues Thr33, Lys67, Thr94, and Ser95 were the probable binding residues. These simulations predicted that aptamer and antibody have different binding sites on ricin and both of them are located on the ricin A chain. Furthermore, blocking experiments with two different blocking reagents were performed to verify the simulation result (Fig. S3 and S4, ESI†). When using aptamers as blocking reagent, the AFM recognition signal reduced significantly. While using anti-ricin antibody as blocking reagent no significant changes on the recognition signal were observed, indicating that the aptamer on the AFM tip can still bind to the corresponding binding sites on ricin.

Fig. 3 shows the topography and recognition images of ricin molecules on the Au(111) surface. The diameter of the ricin molecule in the topography images (Fig. 3A) is around 20 nm, which is greater than the size of ricin obtained by X-ray crystallography.22 It is mainly due to the tip broadening effect and the compression of the molecules on the surface by the AFM tip during the scanning.17 The recognition image (Fig. 3B) shows 97% recognition efficiency (159 out of 164 ricin molecules showed the corresponding recognition signals). This result is comparable with the AFM recognition using antibody.17 When checking the topography images of single ricin molecules (2291 ricin molecules), we observed different conformations of ricin on the Au(111) surface, which agrees well with ricin structures obtained from MD simulation and can be generally divided into four groups (Fig. 3C, ESI S7†). However, about 30% (718 ricin molecules) of the topographic images cannot fit any simulated structure. This is because the structure we used here was a rigid protein structure and the AFM scanning may cause possible distortion of some of the ricin molecules. Generally, most simulation structures fitted the topography images with certain orientations that keep the ricin B chain at the bottom. Therefore, these topography images of ricin molecules show that the ricin B chain contacted to the gold surface with LA-NHS during the AFM scanning, because most lysine residues are located in the B chain (Fig. 1B), and therefore the ricin A chain was available to interact with probe molecules attached on the AFM tip. This constraint conformation of ricin on the gold surface explained the high efficiency of recognition events.

Next, the affinity of the binding reaction between ricin and aptamer was estimated using the AFM-DFS technique. Based on AFM-DFS experimental data, we compared the interaction...
Fig. 4  The representative data obtained from DFS. (A) Representative force–distance curves under five loading rates. At each loading rate, three force–distance curves were overlaid and colored with red, blue, and black, respectively. (B) The distributions of unbinding force values at five loading rates (0.24 nN s\(^{-1}\)). In these measurements, five relatively slow force–distance curves under five loading rates were collected under each loading rate. The representative force–distance curves of aptamer–ricin binding and antibody–ricin binding were shown in Fig. 4B, and the ones of antibody–ricin unbinding forces were reported in previously published paper. 17

1646/C0 and 51.64 nN s\(^{-1}\) described in ESI S6.

This ricin–aptamer interaction system is assumed to follow the rules of the Bell–Evans single-barrier model, so that the dissociation constant (\(k_{\text{off}}\)) can be derived from the force distribution.11,24–26 In these measurements, five relatively slow loading rates (0.24 nN s\(^{-1}\), 2.72 nN s\(^{-1}\), 15.37 nN s\(^{-1}\), 38.02 nN s\(^{-1}\), and 51.64 nN s\(^{-1}\)) were used to measure the unbinding forces and 250 force–distance curves were collected under each loading rate. The representative force–distance curves of aptamer–ricin binding and antibody–ricin binding were shown under the corresponding loading rates in Fig. 4A and Fig. SI-5 in ESI S5\(^{+}\), respectively. The most probable unbinding force \(F^*\) under each loading rate \(R\) was obtained from the fitting of the histograms of these force distributions. The distributions of aptamer–ricin unbinding forces at these five loading rates were shown in Fig. 4B, and the ones of antibody–ricin unbinding forces were reported in previously published paper.17 The most probable unbinding force \(F^*\) under each loading rate \(R\) was obtained by the Gaussian fitting of the histogram of each force distribution (the inset table in Fig. 4B). Fig. 4C shows the linear fitting plots of \(F^*\) vs. \(R\) (ln \(R\)) for the aptamer–ricin interaction (colored in red) and antibody–ricin interaction (colored in blue). In Fig. 4C, the \(F^*\) values of aptamers were slightly larger than the ones of antibody under all five loading rates. The values of \(k_{\text{off}}\) for aptamer–ricin and antibody–ricin reactions were derived by the same method described in ESI S6.17 \(k_{\text{off}}\) (aptamer) was calculated as 6.8 \(\times\) 10\(^{-3}\) s\(^{-1}\), while \(k_{\text{off}}\) (antibody) was 1.5 \(\times\) 10\(^{-3}\) s\(^{-1}\). Therefore, \(k_{\text{off}}\) (aptamer) obtained from our AFM-DFS experiments is around half of the \(k_{\text{off}}\) (antibody), although it is not significantly different when it comes to the affinities of biomolecules. The comparison of \(k_{\text{off}}\) values demonstrates that the aptamer has slightly higher affinity to ricin.

In conclusion, the AFM recognition and DFS experiments provide both morphology and interaction information of the aptamer and protein, which can be used for the future study on the thermodynamics and kinetics properties of ricin–aptamer/antibody interactions. The immobilization method is proved to be able to constrain the ricin conformations on the modified Au(111) surface. The molecular simulations provide detailed structural information of the aptamer, antibody, and ricin, which are critical when we try to understand the functions of these biomolecules and the thermodynamic processes of the binding events. The combination of AFM techniques and molecular simulations provides a new approach for the single-molecule detection and mechanism study of aptamers.

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Notes and references