Published in final edited form as:

Chem Commun (Camb). 2016 February 23; 52(18): 3705–3708. doi:10.1039/c5cc10535h.

Quantitatively Resolving Multivalent Interactions on Macroscopic Scale Using Force Spectroscopy

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Abstract

Multivalent interactions remain difficult to be characterized and consequently controlled, particularly on a macroscopic scale. Using force-induced remnant magnetization spectroscopy (FIRMS), we have resolved the single-, double-, and triple- biotin—streptavidin interactions, multivalent DNA interactions and CXCL12-CXCR4 interactions, on millimetre-scale surfaces. Our results establish FIRMS as a viable method for systematic resolution and controlled formation of multivalent interactions.

Multivalent interactions, which refer to the multiple noncovalent interactions formed between two entities decorated with appropriate molecular pairs, represent the major binding mechanism in many natural and synthetic systems.^{1–3} Examples range from bacteria and cell adhesion,^{1,4,5} drug targeting with synthetic carriers,^{6–9} novel DNA-based materials,^{10–13} to supramolecular devices.^{2,14} The resulting interactions often govern the associated biological functions and the materials' stability. However, resolving the order of multivalent interactions remains a challenge. Atomic force microscopy (AFM) requires one of the binding molecules tethered on the cantilever and is thus inapplicable to study particle-surface interaction.^{15,16} It also has a small field-of-view on the order of only hundreds of micrometers. Optical tweezers^{17–19} and magnetic tweezers²⁰ have small force ranges and also microscopic fields-of-view. Surface plasmon resonance (SPR) usually cannot directly resolve different interaction orders.¹¹

Recently, we developed the force-induced remnant magnetization spectroscopy (FIRMS) technique, which is based on detecting the remnant surface-bound magnetic particles after applying external mechanical forces.²¹ The magnetic particles are tethered to the surface via the noncovalent interactions of interest and are magnetized to generate a magnetic signal. The noncovalent interactions will dissociate when the external mechanical force exceeds their binding force, leading to the randomization of the magnetic dipoles of the magnetic particles or the removal of the particles. Thus a decrease in the magnetic signal will be observed, which is detected by an atomic magnetometer.²² FIRMS enables measurement of

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Electronic Supplementary Information (ESI) available: Experimental procedure, technical details, and supplementary figures S1–S6. See DOI: 10.1039/x0xx00000x

binding forces with high resolution.²³ In addition, it simultaneously measures $\sim 10^5$ interactions within a macroscopic field-of-view of several mm^2 .

Here, we report the use of binding forces obtained by FIRMS as the characteristic parameter to distinguish multivalent interactions and subsequently reveal the well-controlled formation of single-, double-, and triple- interactions. Using the biotin—streptavidin platform, the binding force of the triple interaction reaches 830 pN, far exceeding that of typical noncovalent interactions.²⁴ In addition, multivalent interactions were observed directly on the cell surfaces. Therefore, the force spectra provided by FIRMS can be a general method for studying noncovalent interactions of different orders and types. Furthermore, we show the heterogeneous decay of surface functionalization derived from the change of the binding orders.

Fig. 1a schematically shows the formation of multivalent interactions between the ligand-conjugated particles and receptor-containing surface. In this work, the ligand-receptor pairs are either biotin—streptavidin, complementary DNA strands, or ligand CXCL12 and its corresponding chemokine receptor CXCR4 on cell surfaces. The magnetic particles serve as both the signal producer and force carrier. Mechanical force was exerted onto the noncovalent interactions using a centrifuge (Supplementary Information, Fig. S1).

The experimental results in Fig. 1b show well-resolved streptavidin-biotin interactions between the magnetic particles and the substrate functionalized by biotin-PEG (PEG: polyethylene glycol) at various coating concentrations (Supplementary Information). The biotin—streptavidin interaction is one of the strongest noncovalent interactions²⁵ and has been extensively used to study surface-immobilized biological systems.²⁶ The long flexible PEG linker allows for rapid and free reorientation of the biotin molecules on the surface. When the ratio of biotin-PEG: PEG equals to 0.8% (wt/wt), only one type of interaction with 220 pN binding force was formed (Fig. 1b). Control experiments were performed to confirm that the 220 pN binding force belongs to the single biotin—streptavidin interactions (Supplementary Fig. S2). The force value was calculated from the buoyant mass of the particles²⁷ (Supplementary Fig. S3) and was in excellent agreement with multiple previous reports using AFM.^{15,28}

When the coating density of biotin-PEG was raised to 1.2%, a different form of interaction was revealed, indicated by the dissociation force in the range of 400-500 pN. Because this force was approximately twice the dissociation force of single interaction and there was no binding type in between, we assigned the new one to be the double biotin—streptavidin interaction. The double interaction remained the major product when the biotin-PEG concentration was elevated to 1.6% and 2.4%. Further increasing the biotin-PEG concentration to 3.2% led to the dissociation force of 700-900 pN, which we assigned as the triple interaction because of its immediate appearance after the double interaction and the broad distribution of its magnetic profiles. It is possible that a small portion of quadruple interaction was mixed with the triple interaction, but could not be resolved because of the broad force distribution. When the coating density was increased to 4.0%, only a very small decrease in the magnetic signal was observed, which was 10 pT out of the 69 pT total signal, or 14%. This result shows that most of the interactions have a higher order than three. This

trend continued when the coating density was further increased to 4.8%, in which nearly no signal decrease was observed within 1140 pN. The current upper limit of the mechanical force was determined by the centrifuge speed limit and the buoyant mass of the magnetic particles (Supplementary Information).

To precisely determine the binding forces of the noncovalent interactions of different orders, we took the derivatives of several representative magnetic field profiles in Fig. 1b. The results are shown in Fig. 1c. Gaussian fitting yielded binding forces of 220 ± 45 , 490 ± 120 , and 830 ± 120 pN for the single, double, and triple bonds, respectively. These values clearly show that binding force can be used to resolve noncovalent interactions with different orders. The relatively large uncertainties for the double- and triple- interactions reflect the broadness of their force spectra. The broadness could possibly be attributed to the binding heterogeneity: each streptavidin has four binding sites for biotin, but we cannot distinguish whether the multivalent interactions involve a single or multiple streptavidin molecules.

The number of each order of the interactions can be derived from the signal calibration curve (Supplementary Fig. S4). The typical initial signal of 70 pT in Fig. 1b corresponds to 1.5×10^5 tethered magnetic particles, or the same number of noncovalent interactions of any order. The typical error for magnetic signals is ± 2 pT throughout this work, or $\pm 4 \times 10^3$ interactions.

The correlation between the interaction order and molecular density on the surface is illustrated in Fig. 2a. In general, a higher biotin-PEG density will favor the formation of higher-order interactions between the particles and the surface. The upper limit of coating densities for the single, double, and triple interactions under our experimental conditions are 1.2%, 3.2%, and 4.8%, respectively.

The sensitive dependence of interaction order on receptor density led us to investigate the effect of coating stability to the binding. In Fig. 2b, the magnetic field profiles show that the interaction order decreases as a function of time. The freshly prepared biotin-surface coated at a density of 4.8% yielded bonds mostly with an order higher than three. After 10 days, a decrease of 32 pT in the magnetic signal in between 600 and 1200 pN was observed. This indicates the formation of triple interactions and the remaining being higher-ordered interactions. The decay ratio is 44%, obtained by dividing 32 pT by the total signal of 73 pT. A different experiment was performed after 20 days, a decrease of 19 pT (30% of the overall signal of 64 pT) was detected between 400 and 700 pN, indicating the formation of double interactions. In addition, the 15 pT decrease from 700 and 1200 pN shows the existence of 23% triple interaction. The results show the heterogeneous decay of the surface because interactions of different orders were formed on a single sample surface.

Formation of multivalent interactions can also take place in systems functionalized with DNA strands to produce multiple duplexes. To demonstrate, we conjugated DNA oligomer 5'-CCC AAT CGA CCC-3' onto the magnetic particles and coated its complementary sequence on the surface. In Fig. 3a, the magnetic field profile shows two decreases: one of 17 pT at a lower force value and the other of 6 pT at a higher force. The corresponding force spectrum is shown in Fig. 3b, which contains two well resolved peaks. The first one has a

dissociation force of 44 ± 5 pN, which is assigned to be single DNA duplex. The force value is similar to the previous measurement of 42 pN for another 12-basepair DNA duplex.²³ The second peak corresponds to a dissociation force of 92 ± 10 pN, which belongs to the dissociation of particles tethered through two DNA duplexes onto the surface, i.e., double DNA interactions. The formation of both single and double interactions confirms the heterogeneity of noncovalent binding. The effect of nonspecific binding was removed by applying a force of 23 pN, which is substantially higher than the dissociation force of the nonspecific interactions but weaker than that of the single DNA duplex.²³

The biological significance of using FIRMS to resolve multivalent interactions is demonstrated by studying CXCL12-conjugated magnetic particles binding with CXCR4 receptors on cell surfaces. CXCR4 is an important human chemokine receptor of the family of G protein-coupled receptors (GPCRs).²⁹ Although various features including crystal structures of several GPCRs have been revealed,²⁹⁻³¹ the binding forces of GPCRs with their corresponding ligands have been rarely studied, which may be valuable towards understanding their biological functions. In this study, the CXCR4 receptors were overexpressed on Ghost(3) cells,³² and the CXCL12 ligands were conjugated with magnetic particles (Supplementary Information). The magnetic field profile and its corresponding force spectrum are shown in Fig. 3c and 3d, respectively. The result was similar to that of DNA binding, in which two decreases were observed that represented the single and double bonds, respectively. Dissociation forces of 30 ± 4 pN and 57 ± 5 pN were obtained (Fig. 3d), for the single and double CXCL12-CXCR4 interactions, respectively. No magnetic signal was detected for the control experiment (Supplementary Fig. S5). The cells were presumably dead after fixation, which, however, did not prevent the formation of multivalent interactions. We are currently expanding our capability to study living cells.

The above results allow us to establish a FIRMS diagram to systematically resolve multivalent interactions of different types and orders (Fig. 4). The values from this work include 44 and 92 pN for the 12-basepair DNA single- and double- interactions, respectively; 30 and 57 pN for the CXCL12-CXCR4 single- and double- interactions, respectively; and 220, 490, and 830 pN for the single-, double-, and triple biotin—streptavidin interactions, respectively. Also included in the diagram are results for a series of DNA duplexes with 13-18 basepairs³³ and results for an IgG— α -IgG interaction.³⁴ In addition, the binding force of nonspecific interaction was revealed to be less than 2 ± 1 pN (Supplementary Fig. S6). Therefore, the force diagram consists of three distinct regions. The first is 0-20 pN, for nonspecific interactions. The second is 20-200 pN, representing relatively weak single- and double- noncovalent interactions. Most of our FIRMS results have been in this region. To show the force values clearly, a vertical offset was implemented in this region. The third region is 200-1000 pN, containing stronger noncovalent bonds, such as biotin—streptavidin interaction, and their multivalent derivatives. Clearly, this diagram shows that FIRMS has the capability of distinguishing noncovalent interactions between different molecular pairs and with different binding orders.

In conclusion, the binding forces obtained by FIRMS can be the discriminating parameter for multivalent interactions formed on macroscopic sizes of surfaces, which makes it valuable for surface-based biological research as well as designing supramolecular

materials. The formation of different orders of multivalent interactions can be precisely tuned by varying the receptor density. The effect of receptor density is further demonstrated by the heterogeneity of binding orders after various decaying times. We note that the binding heterogeneity is revealed for a macroscopic field-of-view, which is an advantage of FIRMS over other force-based techniques. In addition, FIRMS provides potential mechanical control of the binding between multivalent particles and the substrate by selectively removing the weaker noncovalent bonds, for example via ultrasound,³⁵ a feat that is difficult for other techniques.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by the National Institutes of Health (R01GM111452) and the Texas Center of Superconductivity at University of Houston (TcSUH). Y.W. acknowledges the Welch Foundation (E-1721) and the NHARP of Texas (003652-0010-2011). The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Ghost(3)X4/R5 from Drs. Vineet N. KewalRamani and Dan R. Littman.

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Fig. 1. Resolving multivalent biotin–streptavidin interactions. **a)** Schematic to show the formation of single-, double-, and triple- multivalent interactions. **b)** Magnetic field profiles at various coating densities. **c.** Representative force spectra of the single- (at 0.8% coating density, red trace), double- (1.2%, blue trace), and triple- (3.2%, green trace) interactions.

Fig. 2. Interaction order controlled by surface coating density. **a)** Correlation between interaction order and coating density. **b)** Magnetic field profiles for surfaces undergoing different decaying times.

Fig. 3. Formation of single- and double- DNA and protein interactions. **a)** Magnetic field profile of DNA binding with two decreases, one for single- and the other for double- interactions. **b)** The corresponding force spectrum of **a**, with Gaussian fittings to extract binding forces. **c)** Magnetic field profile of magnetically labelled CXCL12 ligands binding with CXCR4 receptors on cell surface. The two decreases represent single- and double- interactions, respectively. **d)** The corresponding force spectrum of **c**, with Gaussian fittings.

Fig. 4.

FIRMS diagram for distinguishing noncovalent interactions of various types and orders. I: nonspecific interaction; II: Relatively weak noncovalent interactions; III: Strong biotin—streptavidin interactions. Single-, double-, and triple- interactions are indicated by red, blue, and green, respectively.