Supporting Information

Quantitatively Resolving Multivalent Interactions on Macroscopic Scale Using Force Spectroscopy

Qiongzheng Hu[1], Haopeng Yang[2], Yuhong Wang[2,], Shoujun Xu[1,]**

Department of [1]Chemistry, and [2]Biology and Biochemistry, University of Houston, Houston, Texas, 77204, USA

Table of Contents

1. Experimental procedure and instrument

PEGylation of the glass slides. The glass slides, cleaned by KOH solution and dried under nitrogen, were treated with 3-aminopropropyltriethoxysilane (AMEO) (UCT, Inc.) for 30 min, then rinsed and dried. The PEGylation solution was prepared by dissolving 1 mg biotinylated PEG (biotin-PEG, molecular weight 5000, Laysan Bio, Inc.) and 112.5 mg PEG (molecular weight 5,000, Laysan Bio, Inc.) in 450 μL $NaHCO_3$ (8.4 mg/mL). The percentage of biotin-PEG is thus 0.8%. Higher biotin coating densities were obtained by increasing the concentration of biotin-PEG. For each coating density, 20 μL of PEGylation solution was placed onto the glass slides. The glass slides were placed in a wet chamber for 3 h, rinsed with copious water, dried under nitrogen, and stored under vacuum.

Magnetically labelled streptavidin-biotin bonds. The coated glass slides were used as the bottom of the sample wells of 4×2×1 mm^3. After treating the sample well with bovine serum albumin (BSA) (Sigma) for 1 hr, 10 μL of the streptavidin-coated magnetic particles (M280, Invitrogen) was introduced to replace the BSA solution in the sample well and incubated for 2 h.

Control experiments were performed by blocking the streptavidin on the particles surface with free biotin before adding the magnetic particles into the sample well.

Magnetically labelled DNA duplexes. The sample well with biotinylated glass bottom was filled with 10 μL of 0.625 mg/mL streptavidin, incubated for 1 hr and then rinsed. The biotinylated target DNA (10 μL of 20 nM) was transferred onto the streptavidin-decorated surface and incubated for 1 hr. The biotinylated probe DNA was incubated with the magnetic particles for 2 hrs. Next, the DNA-conjugated magnetic particles were added into the sample well to form DNA duplexes. As two control experiments, the bottom surface of the sample well were decorated without the target DNA (denoted as blank) and a random non-complementary DNA (denoted as non-complementary), respectively. The DNA sequences were: target, 5′-GGG TCG ATT GGG-3′; probe, 5′-CCC AAT CGA CCC-3′; non-complementary, 5′-GGG CAA CCC GGG-3′ (all from IDT, Inc.).

Magnetically labelled CXCL12-CXCR4 bonds. Cleaned glass slides were first in contact with 0.01% poly-L-lysine (PLL) (Sigma) at room temperature for 15 min. Then they were drained and dried at 60 ºC for 1 h. Ghost (3) cells were grown on the PLL-coated glass slides in a 37 ºC incubator with 5% CO_2. The cells overexpressing the CXCR4 receptors were received from the NIH AIDS Reagent Program. The cell culture medium (Invitrogen) was 90% high glucose DMEM and 10% fetal bovine serum, supplemented with 500 μg/ml G418, 100 μg/ml hygromycin, pen/strep, and 1 μg/ml puromycin. Cells grown on the glass slides were fixed with 4% paraformaldehyde (PFA) (Sigma) for 30 min. Then the glass slides were glued to assemble the sample wells. Next, the cell-coated glass slides were blocked with an aqueous mixture of 0.3 M glycine (Sigma) and 1% BSA for 2 h. Biotinylated CXCL12 (Chemotactics, Inc.) ligand was conjugated with the streptavidin-coated magnetic particles The resulting magnetically labelled CXCL12 was then added into the sample well to form the CXCL12-CXCR4 bonds on cell surfaces for 2 hr.

Instrument. Detection of the magnetically labeled noncovalent interactions was achieved by using an atomic magnetometer. As shown in Fig. S1, the sample well is mounted on a linear actuator and scanned in the vicinity of the atomic sensor that is located at the center of the magnetic shield. A magnetic field profile is then obtained, which reaches maximum when the sample well is the closest to the atomic sensor (Yao, L.; Xu, S. J. *Angew. Chem. Int. Ed.* **2009**, *48*, 5679). Because the distance between the sample and the atomic sensor along the axis

perpendicular to the scanning direction is fixed, the maximum magnetic signal represents the amount of the magnetically labeled noncovalent bonds. The sample was subject to centrifugal speed of 1500 rpm (revolution per minute) to remove the nonspecifically bound particles. Then a permanent magnet was used to magnetize the particles. 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 $F = m\omega^2 r$, in which m is the buoyant mass of the magnetic particles, ω is the centrifugal speed, and r is the distance between the sample and the center of the centrifuge motor (Halvorsen, K.; Wong, W. P. *Biophys. J.* **2010**, *98*, L53). The maximum centrifuge speed was 14000 rpm. The buoyant mass of the magnetic particles was obtained by measuring their diameter using scanning electron microscope and their density using Cs_2SO_4 solutions. The value of r was 8 cm for Eppendorf 5417R centrifuge.

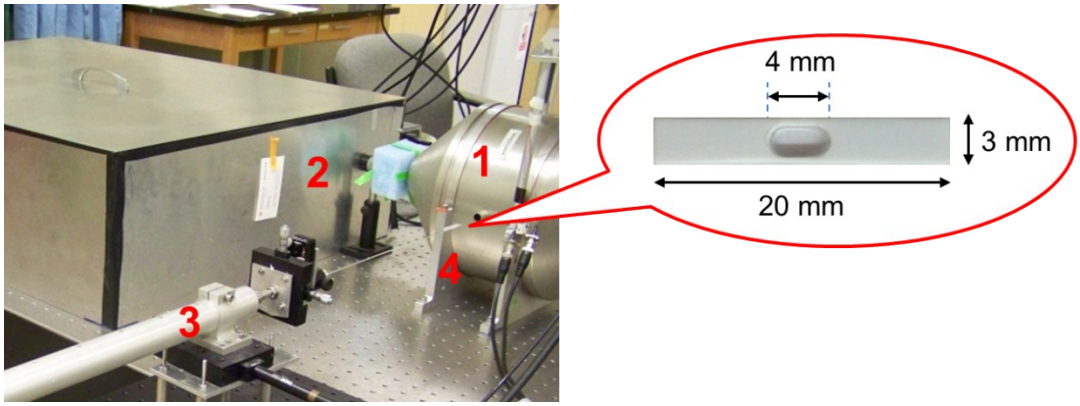

Fig. S1. *Experimental setup. 1: Magnetic shield that houses the atomic sensor; 2. Enclosure for the optical components of the atomic magnetometer; 3. Linear actuator for sample scanning; 4. Sample well, with dimensions shown in the callout box.*

2. Magnetic field profiles of the streptavidin-biotin experiments

A representative magnetic field profile for streptavidin-biotin interactions is shown in Fig. S2 (red trace). Streptavidin was decorated on the magnetic particles, while biotin was coated on the substrate surface. Also shown is a typical magnetic field profile for the control experiment, where the streptavidin molecules on the particles were blocked by free biotin (black trace). The

comparison confirms that the magnetic signal in the red trace only came from the magnetic particles containing streptavidin-biotin interactions.

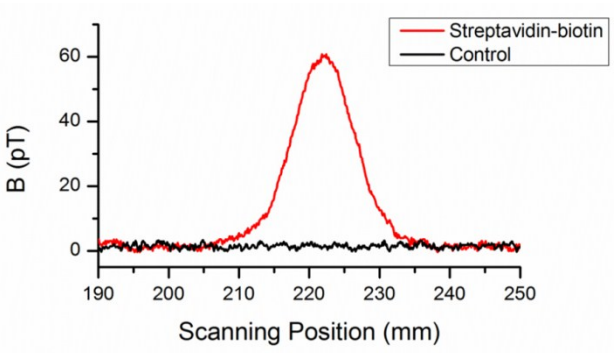

Fig. S2. *Magnetic field profiles of biotin-coated surface bonding with streptavidin-conjugated magnetic particles (red trace) and streptavidin-blocked magnetic particles (black trace, denoted as control).*

3. Characterization of the magnetic particles

The average size of the magnetic particles was obtained by scanning electron microscopy (SEM), with a representative image shown in Fig. S3. The average size was determined to be 2.76±0.1 μm, consistent with the manufacturer's specification of ~2.8 μm.

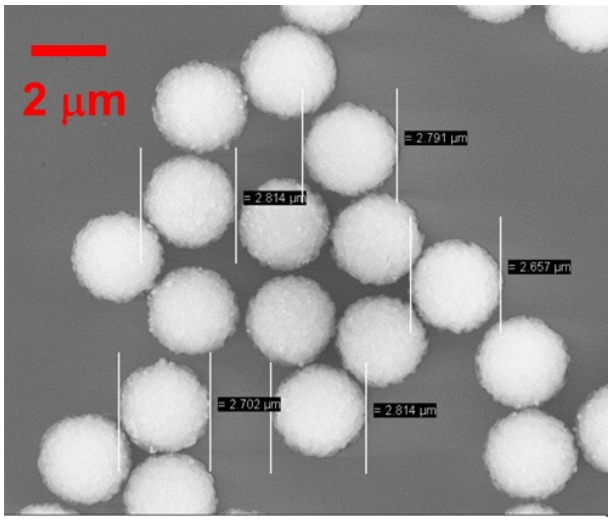

Fig. S3. *A representative SEM image of the magnetic particles.*

To calculate the buoyant mass of the particles, the density of the magnetic particles was determined by dispersing the magnetic particles in Cs_2SO_4 solutions of various concentrations.

The density of the magnetic particles equaled to the solution density when the magnetic particles suspended in the middle of the solution. This method gave a density of 1.61±0.02 mg/mL. The buoyant mass of the magnetic particles was thus calculated to be $(6.63\pm0.05)\times10^{-15}$ kg.

4. Quantification of the noncovalent interactions

The number of the dissociated interactions was obtained by comparing the reduced magnetic signal at the corresponding force value with the calibration curve below. For each data point on the calibration curve, a known number of magnetic particles, calculated from the particle density and sample volume, were dispensed onto a sample well and its magnetic signal was then obtained using the method described in Section 1 of the Supporting Information.

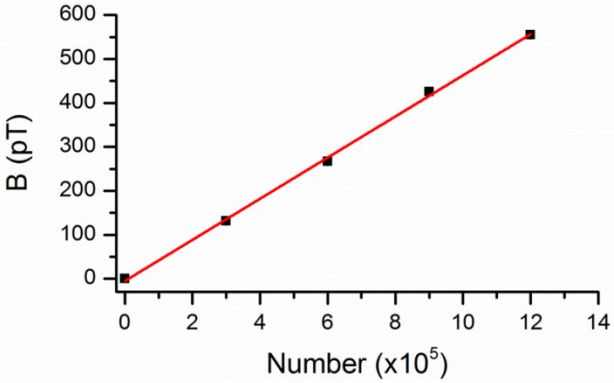

Fig. S4. *The calibration curve for quantifying magnetically labelled noncovalent interactions.*

5. Magnetic field profiles of the CXCL12-CXCR4 experiments

A representative magnetic field profile for magnetic particles containing CXCL12-CXCR4 interactions is shown in Fig. S5 (red trace). Also shown is a magnetic field profile for the control experiment, in which CXCL12 was absent (black trace). The comparison confirmed that the magnetic signal in the red trace belonged to the specific CXCL12-CXCR4 interactions.

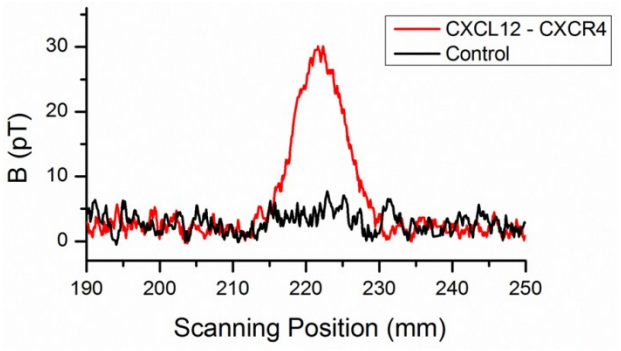

Fig. S5. *Magnetic field profiles of CXCR4 cells binding magnetic particles decorated with CXCL12 (red trace) and magnetic particles without CXCL12 conjugation (black trace, denoted as control).*

6. Nonspecific binding of streptavidin-conjugated particles on biotin surface

The magnetic signals of streptavidin-conjugated magnetic particles on biotin-coated surface were measured in the low force range. The result is shown in Fig. S7. Because the magnetic signal remained constant in between 2 and 20 pN, we determined the nonspecific binding force to be less than 2 pN, with the error bar of 1 pN.

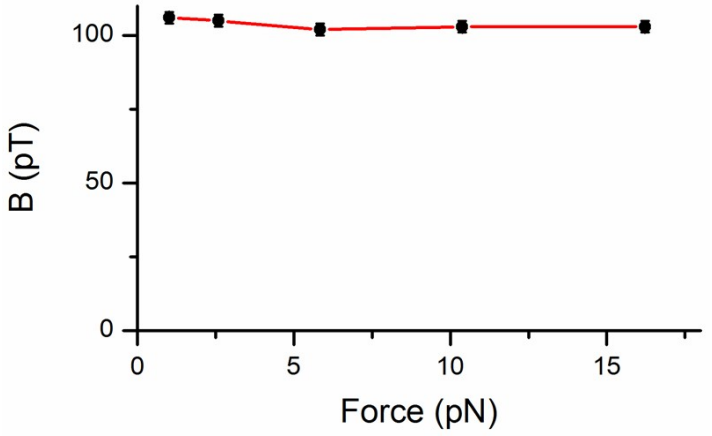

Fig. S6. *Magnetic field profile in the low force range for streptavidin-conjugated magnetic particles on biotin-coated surface.*