Supporting Information

Magnetic Tweezers Measurement of the Bond Lifetime-Force Behavior of the IgG-Protein A Specific Molecular Interaction

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Materials and Methods

Preparation of superparamagnetic particles

The superparamagnetic particles were prepared by emulsion templated self-assembly of Fe_3O_4 nanoparticles [1]. The Fe_3O_4 nanoparticles were prepared by co-precipitation of a ferric and ferrous salt solution using concentrated ammonia. An aqueous Fe^{2+}/Fe^{3+} solution was prepared by dissolving 12.0 g ferrous chloride tetrahydrate (Sigma Chemical, St Louis, MO) and 24.3g ferric chloride (Mallinckrodt, Phillipsburg, NJ) in 50 ml deoxygenated water. Forty milliliters of ammonium hydroxide (Mallinckrodt) was rapidly added to this solution with vigorously stirring before the solution was heated to 70°C for 5 min. A volume of 5 ml of oleic acid (Sigma Chemical) was then added to the mixture and the solution was heated for an additional 25 min. The resulting black slurry was washed with ethanol, 18% (v/v) perchloric acid (Mallinckrodt), and water. These nanoparticles were resuspended in a 100 μM benzophenone (Alfa Aesar, Ward Hill, MA) hexane (Mallinckrodt) solution at 2.4% (w/v). A crude oil-in-water emulsion was then prepared by vortexing the nanoparticle solution with a 30% dextran and 1% sodium dodecyl sulfate (SDS, Mallinckrodt) solution at 1:4 ratio. This crude emulsion was broken up into uniform size droplets by extrusion through a membrane with 5 micron pores (Isopore, Millipore). Microparticles were then formed by allowing the hexane to evaporate overnight at room temperature. The microparticles were subsequently mixed with 10% acrylic acid and 10% SDS in an aqueous solution. Polymerization was carried out by exposing the microparticles to a 20 mW/cm^2 ultraviolet light source for 10 min. The microparticles were collected with a permanent magnet, rinsed with water three times, and stored at 4 ºC.

The magnetic particles were then covalently functionalized with mouse IgG type 2a (anti-M13, GE Healthcare, Piscataway, NJ) through a MW 3,400 poly(ethylene glycol) (PEG) monolayer [2]. The microparticles were first coated with a monolayer of primary amines by physically adsorbing polyethylene imine (PEI, Polymin SNA, BASF, Rensseler, NY) on the negatively charged microparticles. These surfaces were then functionalized with a vinyl-sulfone PEG monolayer by reacting α-vinyl sulfone, ω-N-hydroxysuccinimidyl ester of poly(ethylene glycol)-propionic acid (NHS-PEG-VS) (Nektar, Huntsville, AL) with the PEI coated particles. The IgG was modified with sulfhydryl groups using *N*-succinimidyl-S-acetylthioacetate (SATA, Pierce Biotechnology, Rockford, IL) in 100 mM phosphate buffer, 150 mM NaCl, and 10 mM EDTA at pH 7.2. The sulfhydryl groups were activated with a deacetylation buffer at pH 8.2. Approximately 1×10^{10} PEG-VS microparticles were reacted with 700 μg of the SATA activated antibody. The antibody coverage on the microparticles was measured as approximately 9,400 antibodies/μm^2 [2].

Preparation of the protein A functionalized polystyrene microreactors.

Polystyrene microreactors of 0.2 mm in height and 8.8 mm in diameter were modified with a mixed PEG monolayer baring methoxy and primary amine groups using a three step surface chemistry[2]. The surfaces were first oxidized with a concentrated solution of $KMnO_4$-H_2SO_4 and then coated with a monolayer of polyethylene imine[3]. Second, the surfaces were reacted with a solution of 10 mg/ml *N*-hydroxysuccinimidyl ester of methoxy-terminated poly(ethylene glycol) (mPEG-NHS) and 10 mg/ml α-tert-butyloxycarbonyl, ω-N-hydroxysuccinimidyl ester of poly(ethylene glycol) BOC-PEG-NHS (Nektar, San Carlos, CA) in a carbonate buffer with 0.6 M K_2SO_4 (Mallinckrodt

Baker, paris, KY), at pH 8.2 and 50ºC, for 2 hrs. These surfaces were stable for several months when stored at -20^{o}C. The surface was then activated by deprotecting the BOC group with trifluoroacetic acid (Acros, Morris Plains, NJ) to release the primary amine [4]. The protein A used in this study was cultured from the NCTC 8325 strain of *Staphlococcus aureus* and purified using affinity chromatography (Sigma)[5]. Protein A was grafted to the PEG monolayers using a heterobifunctional cross-linker. The primary amines on the surface were first converted to sulfhydryl groups with SATA as described above. Protein A was activated with the heterobifunctinal cross-linker succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC, Pierce Biotechnology) at a 1:10 molar ratio in 100 mM phosphate buffer and 150 mM NaCl at pH 7.2 [6]. The activated protein A was reacted with the PEG monolayers at a concentration of 1-50 μg/ml at 4 ºC for 12 hours.

The protein A density on the PEG monolayers was then determined using a colorimetric assay with an IgG-horse radish peroxidase conjugate (IgG-HRP). First, the protein A-PEG modified polystyrene surfaces were reacted with 1 mg/ml of the mouse anti-M13 IgG-HRP conjugate (GE Healthcare, Piscataway, NJ) in 12 mM phosphate buffered with 100 mM NaCl (PBS) at pH 7.2 for 2 hr at room temperature. Second, the colorimetric reaction was carried out by adding 1 ml of the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS, Pierce Biotechnology) substrate to each well at 10 mg/ml and carrying out the reaction for exactly 5 min. Third the relative density of HRP, and thus protein A, was determined by measuring the optical density of each sample at 405 nm using a spectrophotometer (Lambda EZ210, Perkin Elmer, Wellesley, MA). The absolute density of protein A was determined by reacting known quantities of

IgG-HRP with a saturated monolayers of protein A formed on the bare polystyrene reactors. These standard surfaces were prepared by incubating 1 mg/ml of protein A with the polystyrene surfaces in PBS overnight at 4^{o}C and then adding 25.7, 34.3, 51.5, 103, 133 and 200 ng IgG-HRP conjugate to each well.

Magnetic tweezers instrument

Figure 1 presents a schematic of the magnetic tweezers instrument that was constructed around an inverted optical microscope (TE300, Nikon, Melville, NY) equipped with a CCD camera (Sony, DXC-107A, Japan). Individual magnetic microparticles could be identified using this simple CCD camera in bright field illumination due to the strong optical adsorption of the Fe_3O_4 in the particles. Images of the magnetic particles on the surface of the polystyrene microreactors were captured in a 220×165 μm^2 field view at 0.1 sec intervals for 30 sec using a Roper Scientific frame grabber (Duluth, GA). The number of particles was automatically counted using digital acquisition software (Scion Image, Frederick, MD).

A constant force was applied to the magnetic particles with an NdFeB rare earth permanent magnet assembly. The magnet assembly was constructed from four 12.7×12.7×3.175 mm N42 NdFeB magnets (K&J Magnetics, Jamison, PA) with like poles facing each other. The 1-3 mm air gap between the magnet assemblies was adjusted to optimize the transmitted light illumination of the magnetic particles. This optimized design produced a high and uniform field and field gradient over a 0.5x12.5 mm area directly below the gap. This magnet assembly was mounted between the microscope condenser and stage. The position of the assembly was controlled with a three dimensional translation stage with 0.1 mm accuracy (Newport, Irvine, CA). This

allowed a specific force to be held at 1.5, 8 and 12 pN by varying the distance between the magnet assembly and the microreactor. The magnetic field generated by the magnet assembly was measured using a Hall probe (DTM-133 digital teslameter, GMW Associates, San Carlos, CA).

References

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