Highly efficient binding of paramagnetic beads bioconjugated with 100,000 or more antibodies to protein-coated surfaces

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Supporting Information

Chemicals and materials. Ethanolamine-reagent plus, albumin from bovine serum-lyophilized powder, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. Micro BCA^{TM} protein assay kit from Thermoscientific.

Spectrometer cleaning. Prior to the start of experiments, SPR spectrometer was cleaned in order to ensure no adsorbed substances in the tubing due to previous experiments. A used sensor chip was placed in the SPR spectrometer, and D. I water was used as running buffer with a flow rate of 200 μL min^{-1}. We then injected in the order of absolute ethanol, 0.5 % (w/v) SDS and 50 mM glycine, pH 9.5 for 5 times with an intermittent D.I water washing step. These steps ensured complete desorption of any adsorbed proteins.

Steps in protein binding to immobilized primary antibodies (Ab_1)

Antibody immobilization. Anti-PSA or anti-IL-6 antibody (Ab_1) was immobilized onto activated mSAM coated gold surface using EDC/NHS coupling chemistry. Carboxyl groups on mSAM sensor surface was first activated using 0.4 M/0.1 M EDC/NHS in deionized water at a flow rate of 20 μL min^{-1}. This was followed by the injection of 100 μg mL^{-1} of anti-PSA or anti-IL-6 antibody (Ab_1 in 10 mM sodium acetate pH 5.0 buffer) at a flow rate of 10 μL min^{-1} for 25 min. During the immobilization of antibody the reference channel was disconnected. After the immobilization, the reference channel was reconnected and excess unreacted activated carboxyl groups were capped using 1 M ethanolamine pH 8.5 at a flow rate of 20 μL min^{-1} for a period of 12.5 min. Reference channel subtraction enabled eliminating any effects due to nonspecific binding and any artifacts. Post conditioning was done using 3 times 6 sec pulse of 100 mM HCl at a flow rate of 100 μL min^{-1} in order to remove non-covalently bound antibodies. At this point PBS-T buffer was injected as a blank buffer (2 times) for a period of time similar to association and dissociation of proteins, which was later used for double referencing of obtained curves.

Protein binding to immobilized Ab_1. A flow rate of 50 μL min^{-1} for PSA and 100 μL min^{-1} for IL-6 was set using PBS-T as running buffer during the association and dissociation of proteins to immobilized antibody surface. PSA and IL-6 as obtained were diluted in 0.2 mg mL^{-1} of bovine serum albumin (BSA) in PBS-T buffer at concentrations of 200 nM, 66.6 nM, 22.2 nM, 7.4 nM and 2.5 nM from a 600 nM stock proteins in the order of 3 fold dilution. BSA buffer was used as sample buffer in order to prevent any non-specific binding of protein onto chips or tubing.

Protein binding to Ab_1 surface was allowed for 180s for PSA and 90 s for IL-6. After binding step, 100 mM HCl in D.I water was used to regenerate the surface with 3sec pulse for 3 times at 100 µL min^{-1}. A dissociation rate constant for high affinity antigen-antibody pairs was measured by utilizing longer dissociation times.[1] Extended dissociation of the formed complex for the highest concentration (200 nM) was carried out for 3600 s for protein-Ab_1 complex.

Binding of protein antigens. Figures S1 and S2 shows the association and dissociation of PSA or IL-6 onto Ab_1 surface with surface density of 7.7×10^9 molecules/mm^2 (anti-PSA) and 1.1×10^{10} molecules/mm^2 (anti-IL-6). GraphPad Prism software was used to fit the SPR curves with association and dissociation model as described in main manuscript with eqs 1 and 2 separately. The excellent agreement of the fitted curves (red) and the actual data (black) provides a high degree of confidence. The values of association rate constant $k_a = 3.70 \pm 0.03 \times 10^5$ $M^{-1}s^{-1}$, dissociation rate constant $k_d = 1.11 \pm 0.02 \times 10^{-3}$ s^{-1} and equilibrium dissociation constant K_D= 3.0 ± 0.2 nM was obtained for PSA binding to anti-PSA Ab_1.

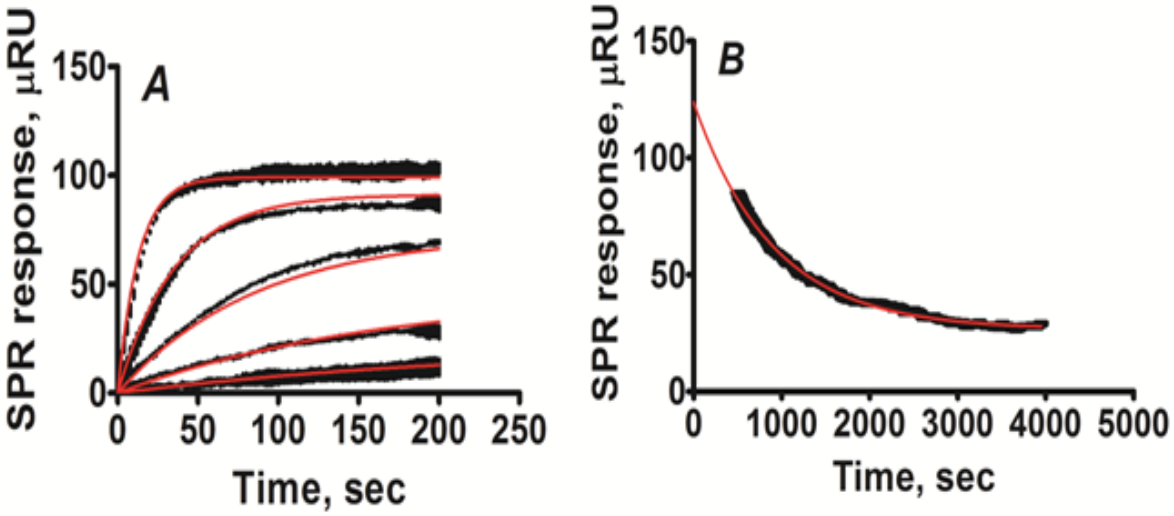

Figure S1. A) Association kinetics of series of PSA concentrations (From top-bottom, 200 nM, 66.7 nM, 22.2 nM, 7.4 nM and 2.5 nM) binding to anti- PSA Ab_1 immobilized on a SPR sensor chip at a flow rate of 50 µL min^{-1} (— SPR association response; mean and error, — simulated fit). B) Extended dissociation of formed PSA-Ab_1 complex over a period of 1 hr for 200 nM PSA. (— SPR response, — simulated fit).

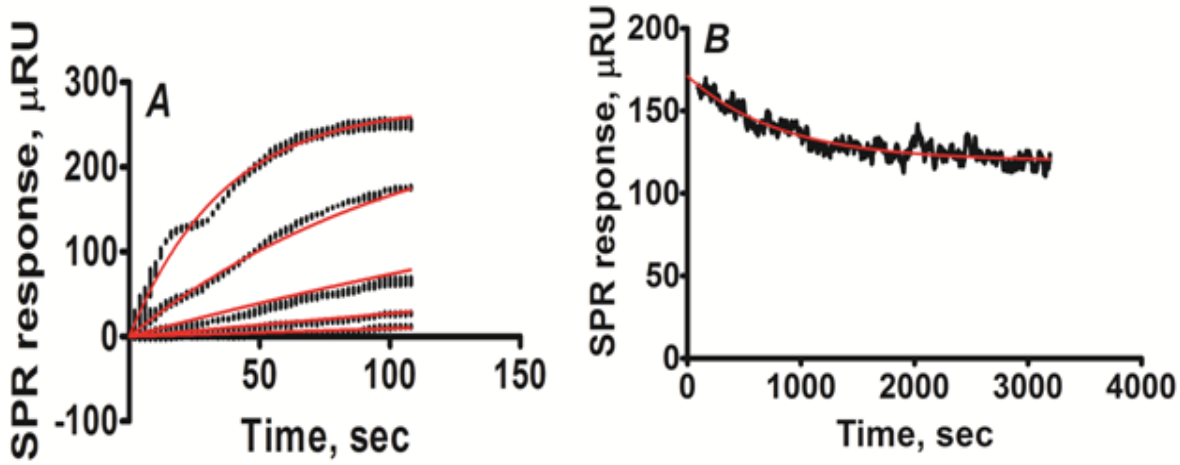

Figure S2. A) Association kinetics of series of interleukin-6 concentrations (from top-bottom, 200 nM, 66.6 nM, 22.2 nM, 7.4 nM and 2.5 nM) binding to human IL-6 mAb (primary antibody) immobilized on a SPR sensor chip at a flow rate of 100 µL min^{-1} (— SPR association response; mean and error, — simulated fit). B) Extended dissociation of formed IL-6-primary antibody complex over a period of 1 hr for 200 nM IL-6. (— SPR response, — simulated fit). Double referenced

The values of $k_a = 1.50 \pm 0.02 \times 10^5$ $M^{-1}s^{-1}$, $k_d = 1.20 \pm 0.03 \times 10^{-3}$ s^{-1} and $K_D = 8.0 \pm 0.2$ nM were obtained for the IL-6 binding to anti-IL-6 Ab_1.

Preparation of MP-Ab_2 conjugates. MP-Ab_2 was prepared according to our previous study. In the first step, 20 µL of tosylated magnetic particles (100 mg mL^{-1}) were magnetically washed with 1 mL of 100 mM pH 9.5 sodium borate buffer for two times. The magnetically separated particles were incubated with mixture of 207 µL 3 M ammonium sulphate in 100 mM pH 9.5 sodium borate buffer, 340 µL of pH 9.5 sodium borate buffer and 80 µL of 1 mg mL^{-1} of either anti-PSA antibody (Ab_2) or anti-IL-6 antibody (Ab_2) in PBS-T buffer in a 1 mL eppendorf vial. This mixture was rotated at 37 ºC in a mixer with slow rotation for a period of 24 hr. Magnetic particle conjugates thus formed were separated using magnet and 625 µL of 0.5 % BSA in PBS-T pH 7.4 was used to passivate and block any free functional tosyl groups for overnight. BSA blocked magnetic particle antibody conjugates (MP-Ab_2) were washed using 0.1% BSA in PBS-T for 4 times and 625 µL of 0.1% BSA buffer was added, which was considered as stock solution for the SPR assay, and stored at 4 ºC before use.

Micro bicinchoninic acid (µBCA)[2] protein assay kit. The amount of Ab_2s coupled to magnetic particles (MP) in each concentrations of MP-Ab_2 conjugates were estimated using protein µBCA assay kit. MP-Ab_2 prepared without BSA blocking step were used for antibody estimation in order to avoid BSA protein in the µBCA protein assay kit. MP-Ab_2 stock was first diluted 8.2 times, which was followed by series of 2-fold dilutions to obtain different concentrations. Similarly MP without secondary antibodies of same stock concentration was diluted to obtain different concentrations which acted as a control. The 450 µL of MP-Ab_2 solution was mixed with 450 µL of BCA reagent and reacted for 1 h at 60 ºC in a water bath. Similar reaction condition was used for MP without Ab_2 as a control. In this reaction Cu^{+2} present in BCA

reagent undergoes reduction to form Cu^{+1} in the presence of protein. Cu^{+1} thus formed chelates between 2 BCA molecules to give a characteristic absorbance at 562 nm corresponding to protein concentration. The supernatant solution obtained after applying magnetic field was used to determine absorbance at 562 nm for MP-Ab_2 and MP (Figure S3). The measured absorbance from each concentration of MP was subtracted from MP-Ab_2 to estimate concentration of Ab_2 present in MP-Ab_2 solution. The subtracted absorbance values at 562 nm of Ab_2 on MP were then correlated to linear calibration of standard antibody Ab_2 concentrations estimated using μBCA protein assay kit (Figure S4). From this, the concentration of Ab_2 in each MP-Ab_2 was accurately determined by this method for anti-PSA Ab_2 and anti-IL-6 Ab_2. The no. of antibodies bound per magnetic particle was determined to be $1.23 \pm 0.12 \times 10^5$ for anti-PSA Ab_2 per MP and $1.22 \pm 0.08 \times 10^5$ for anti-IL-6 Ab_2 per MP.

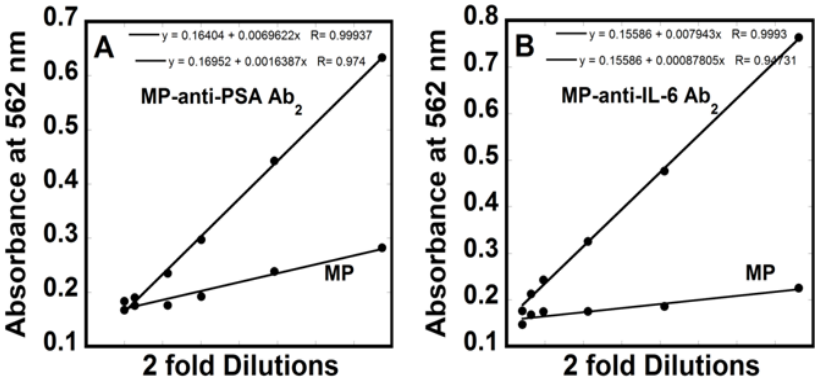

Figure S3. μBCA absorbance response for increasing concentration of MP-Ab_2 and MP at 2-fold dilutions in PBS for A) anti-PSA Ab_2 B) anti-IL-6 Ab_2

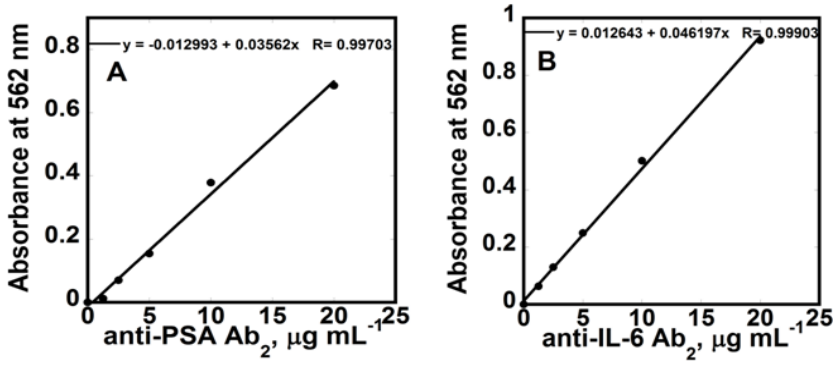

Figure S4. μBCA protein assay for A) standard pure anti-PSA Ab_2 B) standard pure anti-IL-6 Ab_2

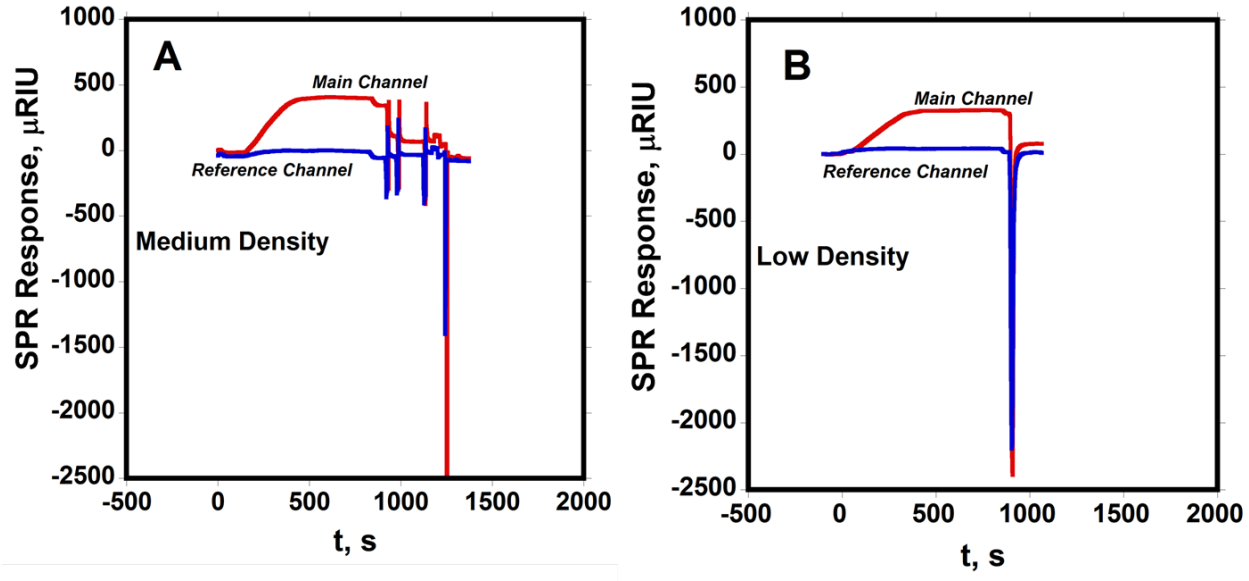

Figure S5. SPR response of detachment of bound particles from A) immobilized PSA (medium density); with total antibody concentration of 67.4 nM B) immobilized IL-6 (low density); with total antibody concentration of 76.1 nM using repeated pulses of 100 mM HCl at a flow rate of 100 µL min^{-1}.

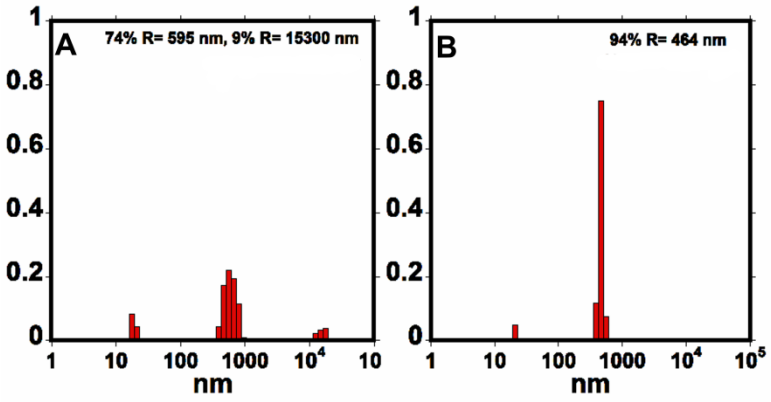

Figure S6. Dynamic light scattering showing the size distribution of offline captured PSA (5 pg/mL) A) by a MP-Ab_2 B) Silica Particles-Ab_2 in PBS-T buffer pH 7.0.

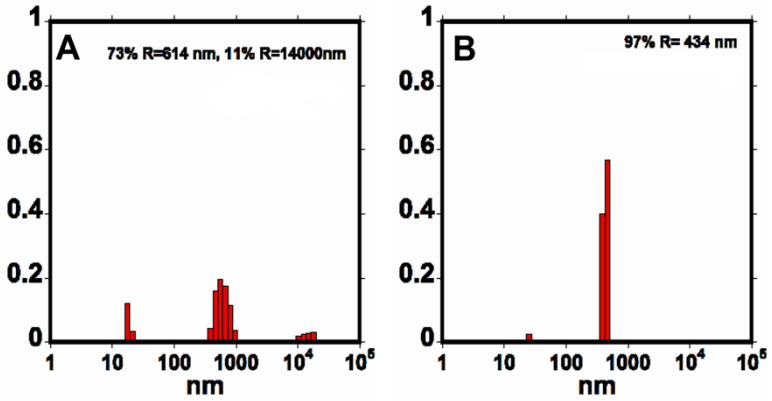

Figure S7. Dynamic light scattering showing the size distribution of A) MP-Ab_2 and B) Silica Particles-Ab_2 with no bound PSA antigen.

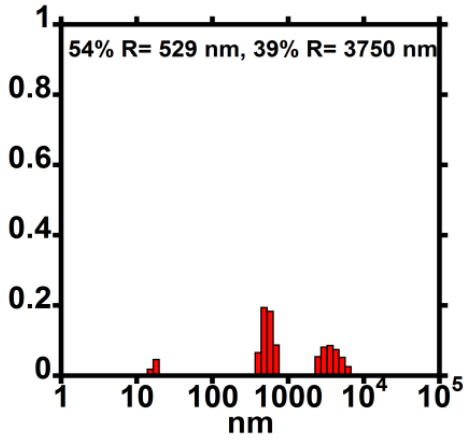

Figure S8. Dynamic light scattering showing the size distribution of tosyl MP without conjugated Ab_2 in 100 mM borate buffer pH 9.5.

References:

(1) Drake, A. W.; Myszka, D. G.; Klakamp, S. L. *Anal. Biochem.* **2004**, *328*, 35-43.
(2) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D.C.; *Anal. Biochem.* **1985**, *150*, 76-85.