Supporting Information

for

Antifouling Lipid Membranes over Protein A for Orientation Controlled Immunosensing in Undiluted Serum and Plasma

Kristy S. McKeating,[†,∥] Samuel S. Hinman,[‡,#] Nor Akmaliza Rais,[§] Zhiguo Zhou[¶] and Quan Cheng[*,†,‡]

[†]Department of Chemistry, [‡]Environmental Toxicology, and [§]Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, CA 92521, USA
[¶]Luna Innovations Inc., Danville, VA 24541, USA

[*]E-mail: quan.cheng@ucr.edu

Supplementary Experimental Methods

Materials and Reagents. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-ethylphosphocholine (EPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPG), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) were obtained from Avanti Polar Lipids (Alabaster, AL). Silane-PEG-Maleimide was obtained from Nanocs (New York, NY). Thiolated recombinant protein A was obtained from Protein Mods (Madison, WI). Functionalized oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). Anti-Mouse IgG (rabbit polyclonal purified IgG) was obtained from EMD Millipore (Temecula, CA). Biotinylated antibodies were conjugated in house using the EZ-Link^{TM} Sulfo-NHS-Biotin labeling kit obtained from Thermo Fischer Scientific (Waltham, MA). All other reagents were obtained from Millipore-Sigma (St. Louis, MO).

Preparation of Gold Surrogate Surface for Fluorescence Microscopy. Glass coverslips were cleaned with boiling Piranha solution (3:1 H_2SO_4 / 30% H_2O_2) for 2 h then thoroughly cleaned with water and ethanol before drying with compressed air. The coverslips were then submerged in a 1 mg / mL solution of Silane-PEG-Maleimide in ethanol overnight at room temperature. After cleaning with ethanol and drying with compressed air, a 10 μg / mL solution of thiolated protein A in 10 mM PBS was added to the surface for 2 h at room temperature. Coverslips were then washed with distilled water and dried under a stream of N_2 before being coated with a 1 mM solution of 3-mercapto-1-propanol (MPO) in 10 mM PBS for 1 h at room temperature. After washing with distilled water and drying with N_2, lipid vesicles at 1 mg mL^{-1} were added to the surface in PDMS wells for 1 h and protected from ambient lighting. Lipids were prepared according to a standard protocol and consisted of either EPC, POPC, or a

1:1 mixture of EPC / POPC with 2% (w/w) NBD-PE, as specified in the main text. The coverslips were then rinsed thoroughly with distilled water, taking care not to expose the lipid surfaces to air, and then mounted onto glass slides. For fluorescent measurements of lipids not formed over a protein A surface, the lipids were directly added to the piranha treated glass coverslips.

Gold Nanoparticle Conjugation. Gold nanoparticles were prepared using a standard citrate reduction. Briefly, a 12.5 mM solution of $HAuCl_4$ was dissolved in 500 mL of distilled water in glassware previously cleaned with aqua regia (3:1 HCl to HNO_3). This solution was heated until boiling with vigorous stirring, then a 30 mM solution of sodium citrate tribasic dihydrate was added, and the solution boiled for another 15 min before being allowed to cool to room temperature. The size of gold nanoparticles was determined from UV-Vis method and is the core diameter of the gold nanoparticles prior to functionalization[3]. These nanoparticles were functionalized with thiolated oligonucleotides consisting of poly-thymine and biotinylated poly-thymine in a 1:1 ratio according to a protocol previously reported in our group.[4] The as-synthesized gold nanoparticles were concentrated by a factor of 3 before the addition of 2.5 nmol DNA (100 μM, H_2O) to 1 mL of concentrated gold nanoparticles. Immediately, 20 μL of citrate-HCL (500 mM, pH 3.0) was added and the solution vortexed before the addition of 10 μL of 10% (v/v) Tween 20. Finally, the NaCl concentration was increased to 1 M and the nanoparticles were incubated overnight at 4 °C. Excess reagents were removed from the nanoparticle solution by centrifugal filtration (Amicon, MWCO 50 kDa), and the resulting conjugates were stored at 4 °C.

MUA vs. Protein A Comparison. This was carried out following a basic protocol previously reported by our group,[5] with necessary changes made for the detection of the

antibodies within this paper. Gold chips were fabricated with a 2 nm layer of chromium and 50 nm layer of gold via electrodepostion, washed with ethanol, and dried under compressed air. Next the chips were soaked in 1 mM of 1-mercaptoundecanoic acid (MUA) (in ethanol) solution overnight to form a self-assembled monolayer with carboxyl functional groups on the surface. The chips were washed with deionized water and dried under a stream of N_2. In the SPR instrument, a mixture of 0.4 M EDC and 0.1 M NHS was injected into the flow cell and incubated for 30 min. After a 15 min wash with PBS, 100 μg / mL anti-cholera toxin (anti-CT) was injected and incubated for 30 min to allow formation of covalent amide linkages. Following this, passivation of the unused activated carboxyl groups was carried out by injecting a 4 mM PEG-amine and incubated for 20 min. Next 25 μg / mL of CT was injected and incubated for 30 min to allow binding with the capture anti-CT. Finally, 100 μg / mL of anti-CT was injected to amplify the detection signal, then incubated for 30 min before a 30 min washing step. All injection steps were separated by a 15 min washing with PBS unless different washing time is specified.

Supplementary Figures

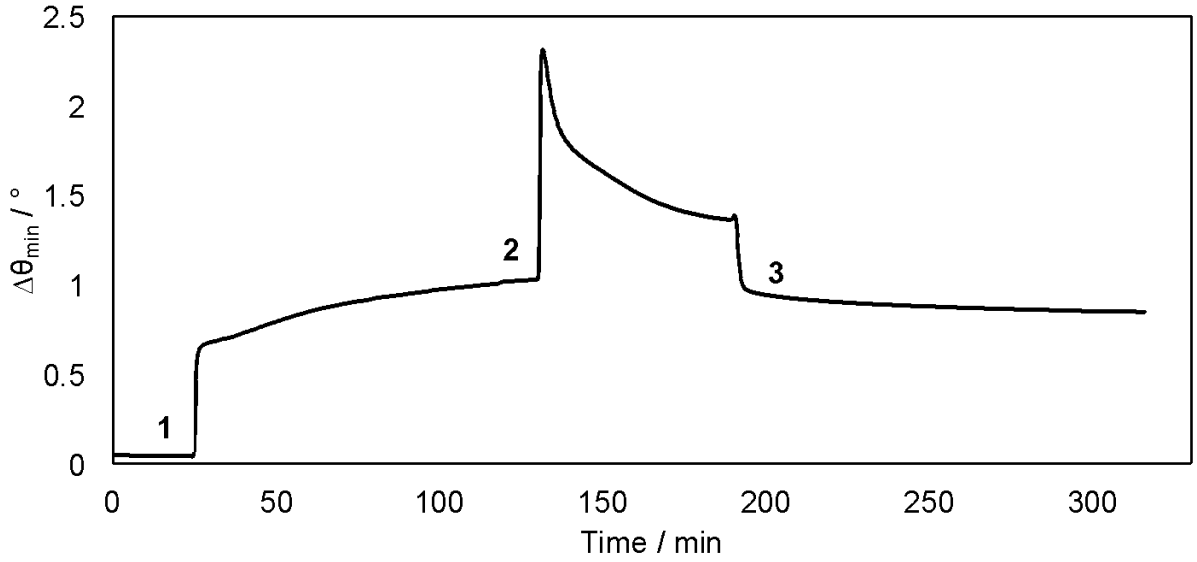

Figure S1. Sensorgram depicting an extended wash step on an antifouling lipid bilayer formed over the protein A/MPO gold surface. EPC was injected over the surface (1) and incubated for 1 h, followed by the injection of undiluted human serum (2) for 30 min, and finally a 2 h PBS wash (3) was carried out to assess stability of the membrane over time.

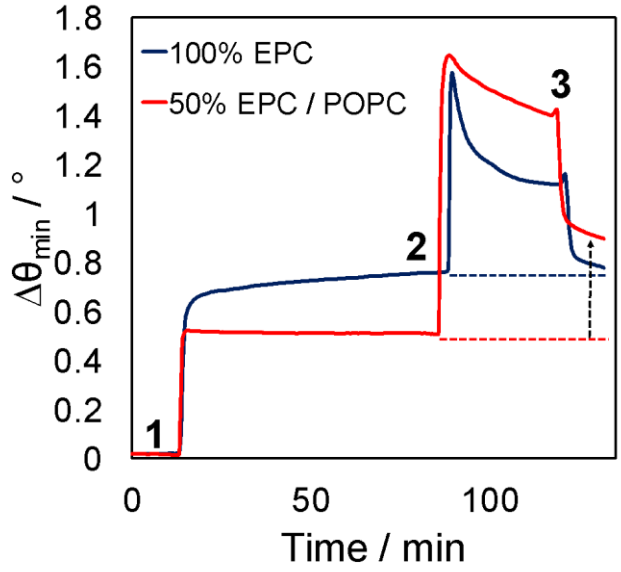

Figure S2. Sensorgram depicting the extent of non-specific binding from human serum components onto lipid bilayers when the overall charge is reduced. Gold chips were coated with protein A and MPO according to the standard protocol. A solution of EPC lipid vesicles (black) or a 50% mixture of EPC and POPC were injected for 1 h (1), followed by undiluted human serum for 30 min (2), and a PBS wash (3).

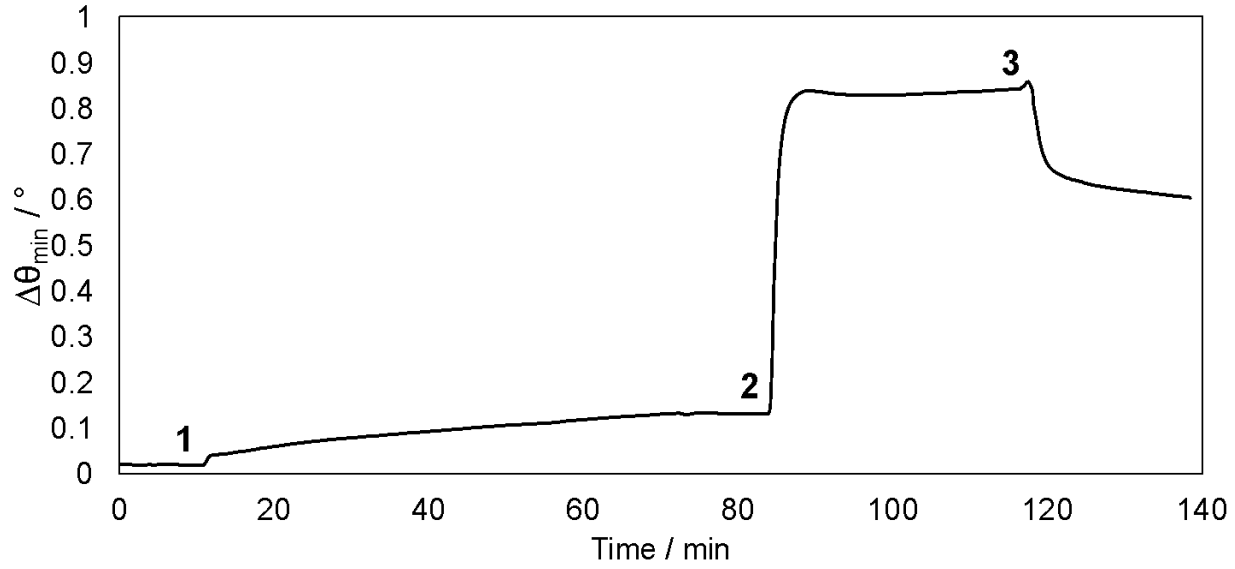

Figure S3. Sensorgram depicting the extent of non-specific binding from human serum onto a gold chip modified with protein A, MPO and POPG. POPG was injected over the surface (**1**) and incubated for 1 h, followed by the injection of undiluted human serum (**2**) for 30 min, and finally a PBS wash (**3**) was used to remove unbound serum components.

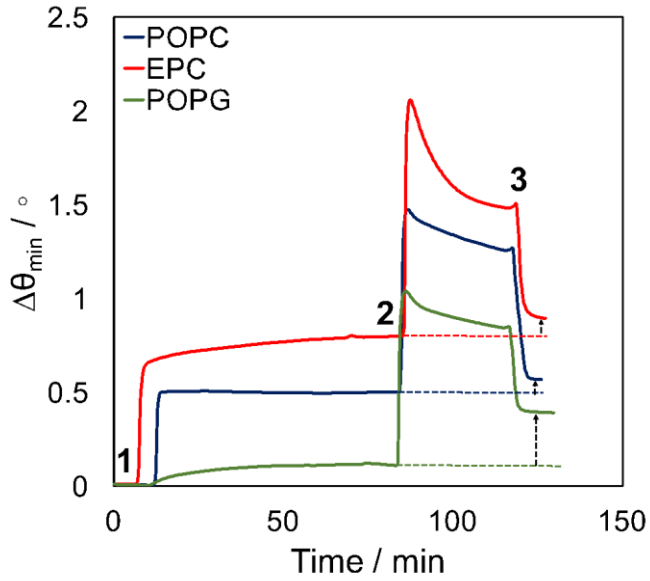

Figure S4. Sensorgram depicting the extent of non-specific binding from human serum onto various lipids formed over silica coated gold chips. POPC (blue), EPC (red), and POPG (green) were injected onto the surface (1) and incubated for 1 h, followed by the injection of undiluted human serum (2) for 30 min, and finally a PBS wash (3) was used to remove unbound serum components.

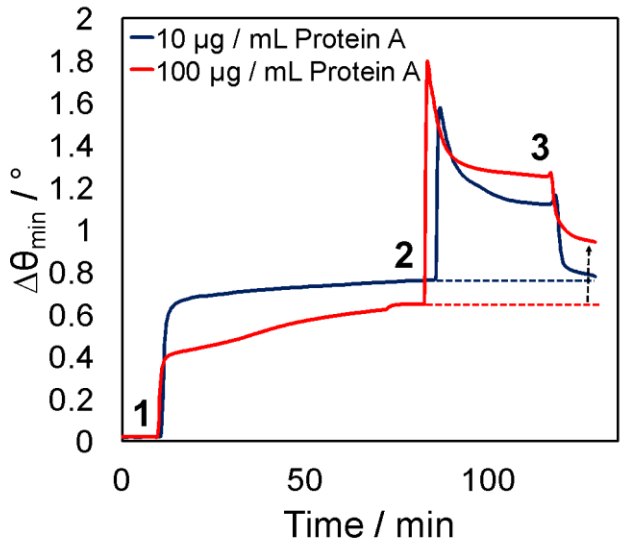

Figure S5. Sensorgram depicting the formation of EPC lipid vesicles over an increased concentration of protein A. Gold chips were coated with protein A at a concentration of either 10 µg / mL (black) or 100 µg / mL (red), and 1 mM MPO. A 1 mg / mL solution of EPC lipid vesicles were injected for 1 h (1), followed by undiluted human serum for 30 min (2), and a PBS wash (3).

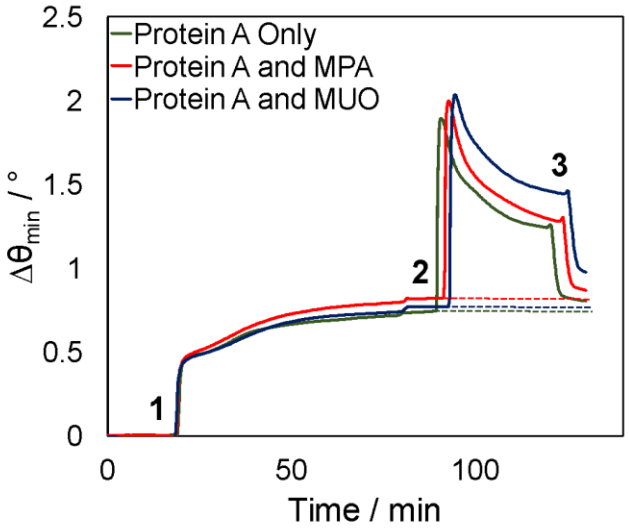

Figure S6. Sensorgram depicting the extent of non-specific binding from human serum components onto a protein A surface functionalized surface with varying spacers. Gold chips were coated with 10 µg / mL protein A only (green), protein A and 1 mM mercaptopropionic acid (blue), or protein A and 1 mM mercapto-un-decanol. EPC lipid vesicles were injected for 1 h (1), followed by undiluted human serum for 30 min (2), and a PBS wash (3).

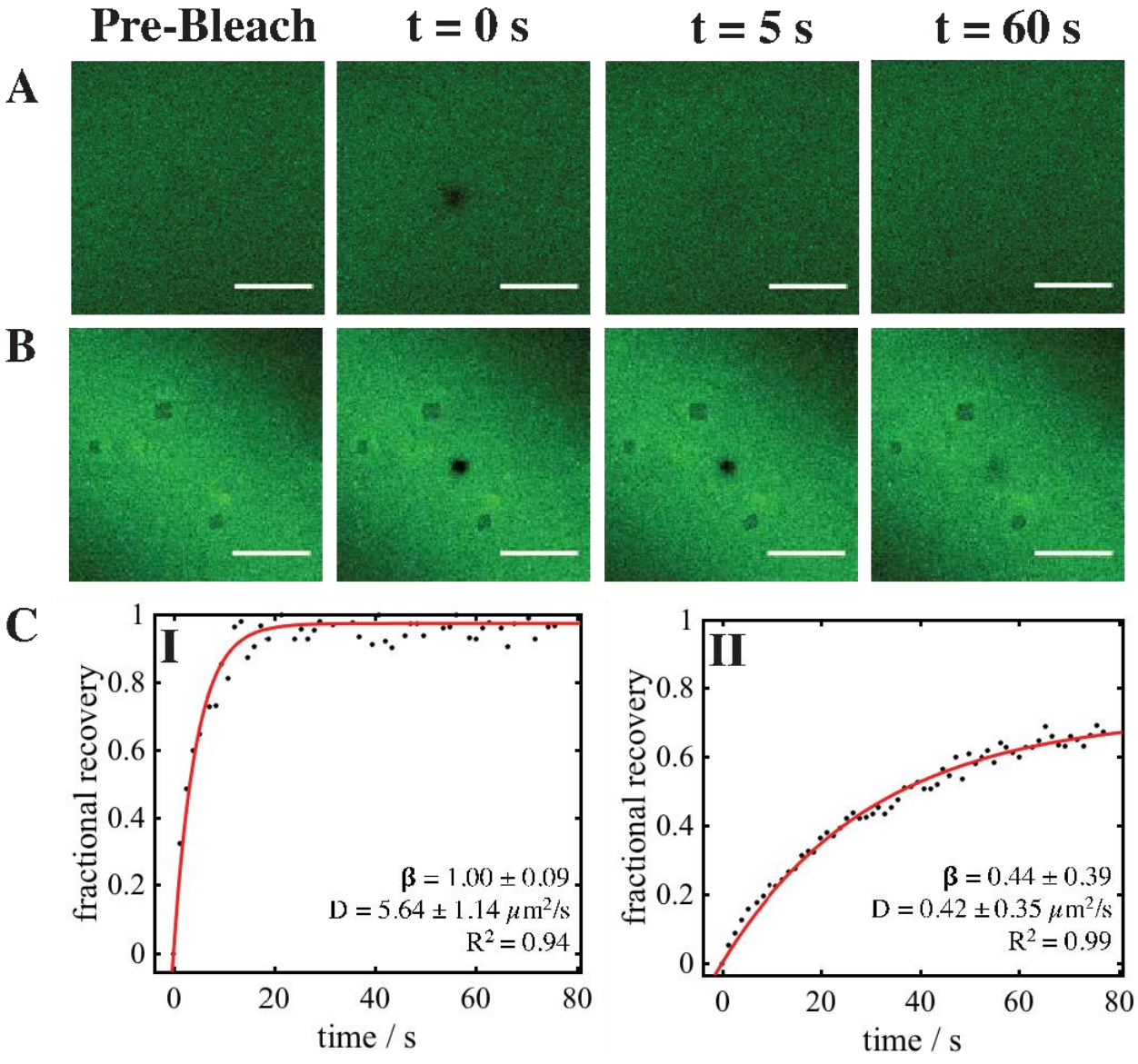

Figure S7. Images depicting FRAP analysis of a 50% EPC / POPC lipid mixture formed over (A) a glass coverslip and (B) a protein A coated glass chip, and the corresponding recovery data (C) for the glass (I) and protein A surface (II). Standard deviations represent 3 replicate experiments, and scale bars represent 30 μm.

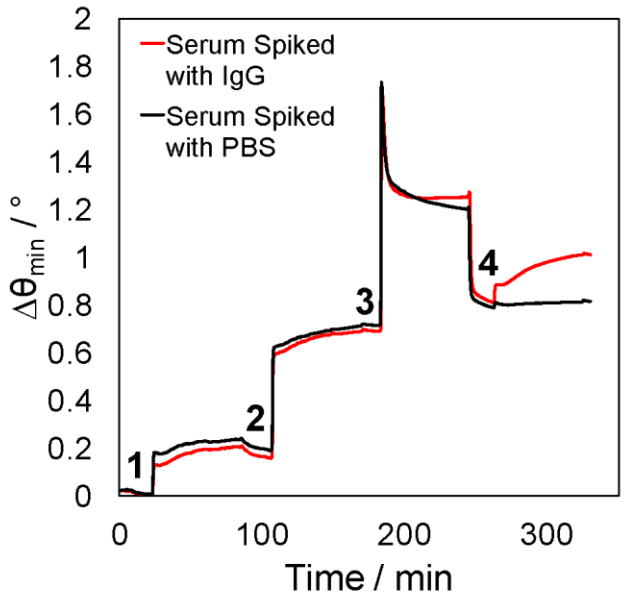

Figure S8. Sensorgrams representing the detection of mouse IgG (red) in undiluted human serum where the capture antibody is injected (1), followed by the EPC lipid vesicles (2), the plasma spiked with mouse IgG (3), and finally a detection antibody for enhancement (4). A 10 min PBS wash was included in between each step and a control sample was carried out by spiking the plasma with PBS only (black).

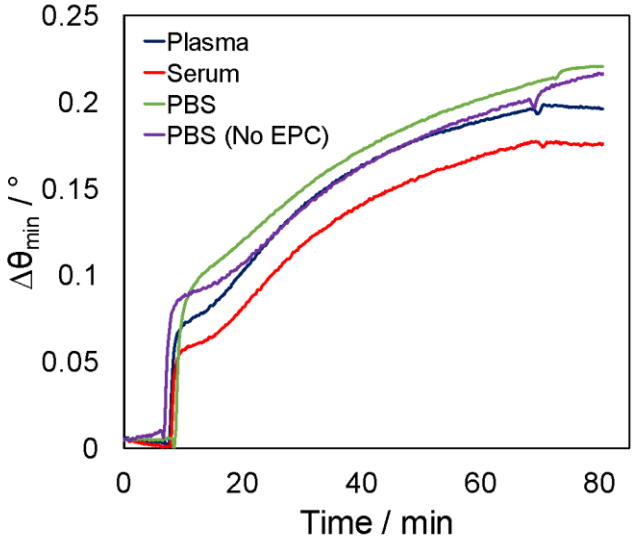

Figure S9. Comparison of the antibody enhancement step for IgG detection in various matrices including plasma (blue), serum (red), and PBS (green), along with a no EPC analysis in PBS (purple).

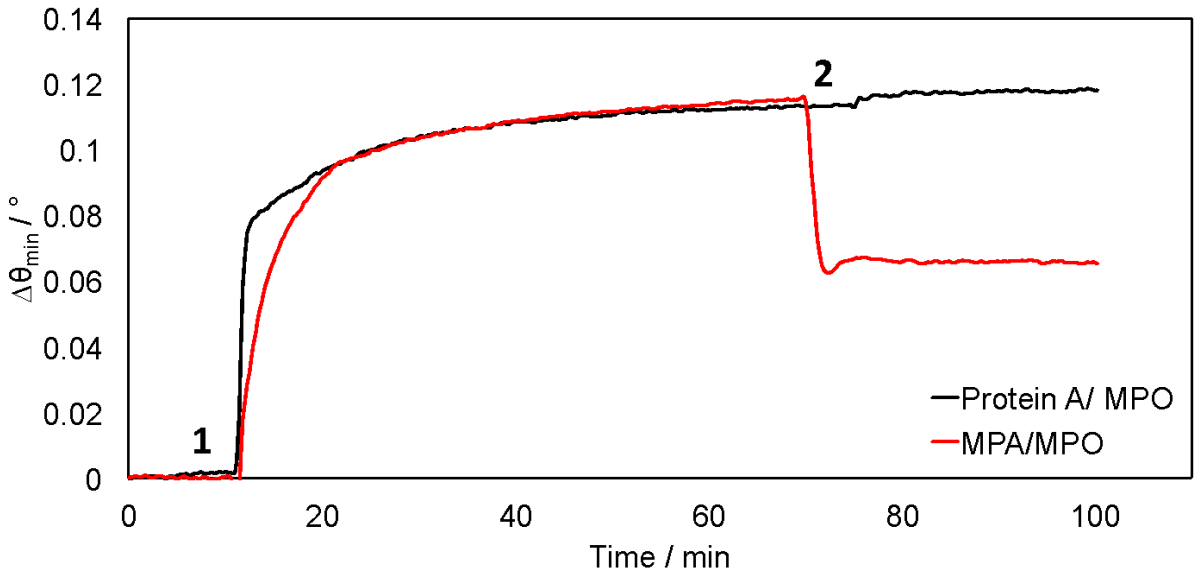

Figure S10. Comparison of the capture antibody attachment method, where anti-CT was conjugated to the surface via protein A (black) or via EDC/NHS coupling chemistry to 11-mercaptoundecanoic acid (red). Sensorgrams depict the addition of a secondary antibody after the detection of CT (1) and a PBS wash (2).

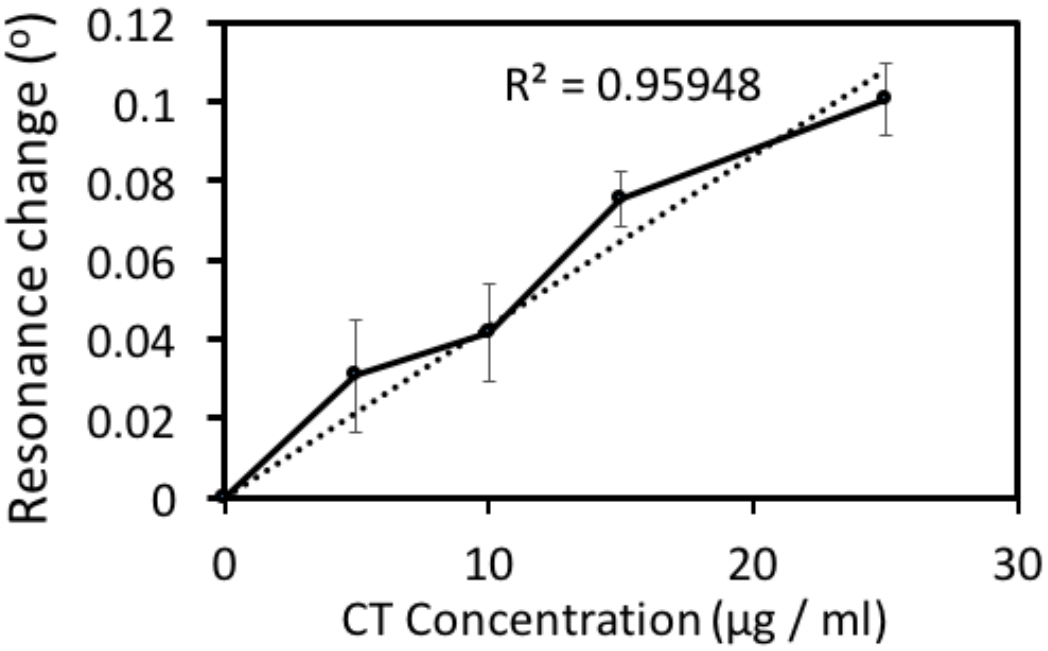

Figure S11. Calibration curve for detection of Cholera Toxin (CT) using an anti-CT enhancement step

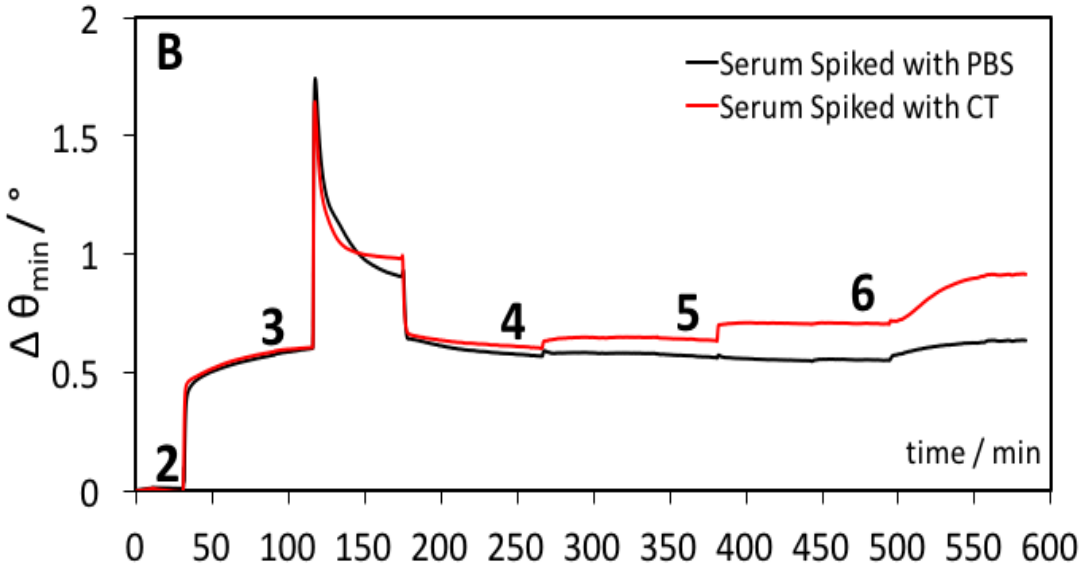

Figure S12. Sensorgram representing the detection of CT (red) in undiluted human serum where the capture anti-CT is injected offline and the EPC lipid vesicles injected online (2), followed by the serum spiked with CT (3), a biotinylated detection anti-CT (4), streptavidin bridge (5) and biotin labelled gold nanoparticles (6) for enhancement. A 15 minute PBS wash was included in between each step and a control sample was carried out by spiking the serum with PBS only (black).

Table S1

Summary of SPR data presented in the manuscript for both IgG and CT in different test medium, detection mode (e.g., via detection antibody, AuNPs) and their angle shifts

Medium	IgG (μg mL^{-1})	IgG (No. of molecules)	Δθ (anti-IgG)	Δθ (AuNPs)	Figure
Blood plasma	10	4.4 x 10^{12}	0.25°		5A
Human Serum	10	4.4 x 10^{12}	0.25 °		S8
Human Serum	10	4.4 x 10^{12}		0.20 °	5C

Medium	CT (μg mL^{-1})	CT (No. of molecules)	Δθ (anti-CT)	Δθ (AuNPs)	Figure
Human Serum	10	7.9 x 10^{12}	0.05°		5B
Human Serum	0.05	4.0 x 10^{10}		0.13 °	S12

The number of molecules was calculated using injection volume (110μL) and molecular weight of IgG and CT of 150 kDa and 83 kDa, respectively.

References

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