SUPPORTING INFORMATION

DNA Linkers and Diluents for Ultrastable Gold Nanoparticle Bioconjugates in Multiplexed Assay Development

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■ SUPPLEMENTARY METHODS

Buffer Compositions. *Phosphate buffered saline (1×PBS)*: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4. *Phosphate buffered Tween 20 solution (PBT)*: 10 mM Na_2HPO_4, 0.1% (v/v) Tween 20, pH 7.0. *Phosphate citrate buffer (PCB)*: 200 mM Na_2HPO_4, 100 mM trisodium citrate dihydrate, pH 5.0. *2-(N-morpholino)ethanesulfonic acid (MES)*: 10 mM MES, pH 5.5.

SPR Chip Fabrication. BK-7 glass microscope slides were first cleaned using a boiling piranha solution (3:1 v/v H_2SO_4 and 30% H_2O_2) for 30 min, followed by rinsing with DI water and drying under compressed air. 2 nm of chromium (0.5 Å s^{-1}), followed by 46 nm of gold (1.0 Å s^{-1}) were then deposited using electron-beam evaporation (Temescal, Berkeley, CA) at 5×10^{-6} Torr in a Class 1000 cleanroom facility (UCR Center for Nanoscale Science & Engineering). To obtain a hydrophilic surface for lipid bilayer formation, *ca.* 4 nm of SiO_2 was deposited on top of the gold layer using plasma enhanced chemical vapor deposition (PECVD) with a Unaxis Plasmatherm 790 system (Santa Clara, CA).[1,2]

Lipid Vesicle Preparation. An appropriate amount of lipid stock solution consisting of POPC and 5% (n/n) of biotin-PE or 5% (n/n) GM_1 in chloroform was dried in a glass vial under nitrogen to form a thin lipid film. The vial containing dried lipids was placed in a vacuum desiccator for at least 2 h to remove any residual solvent. The lipids were then resuspended in 1×PBS to a concentration of 1 mg mL^{-1}. After vigorous vortexing to remove all lipid remnants from the vial wall, the suspension was bath sonicated for 30 min. Thereafter, the solution was extruded through a polycarbonate filter (Whatman, 100 nm) to produce small, unilamellar vesicles (SUVs) of uniform size. All vesicle suspensions were used within one week and stored at 4 °C. Arraying of SUVs for multiplexed supported bilayer analysis was performed using trehalose-assisted deposition.[2,3]

Gold Nanoparticle Synthesis. Citrate stabilized gold nanoparticles were fabricated by a modified Turkevitch method.[4] For 13 nm spheres, a round bottom flask containing 500 mL of 1 mM gold (III) chloride trihydrate was heated until boiling, after which, 50 mL of 38.8 mM trisodium citrate dihydrate was added. For 30 nm spheres, 510 mL of 0.25 mM gold (III) chloride trihydrate was heated until boiling, and 10 mL of 17 mM trisodium citrate dihydrate was utilized. The mixed solution was boiled and stirred until a deep red color was obtained. After cooling overnight, the nanoparticles were filtered through a cellulose acetate membrane

(Whatman, 0.45 μm) and stored in amber bottles at room temperature. Diameter and concentration were determined according to the methods of Haiss et al.,[5] and verified by transmission electron microscopy (Figure S7).

MHDA/Biotin Modification. Biotin functionalization via 16-MHDA monolayer coupling was performed according the procedure outlined by Aslan et al.[6] 1.5 mL of as-prepared 13 nm gold nanoparticles were first centrifuged and resuspended in 1 mL PBT. Following this, 0.5 mL of 0.5 mM 16-MHDA in ethanol was stirred into the nanoparticle solution and incubated overnight. The nanoparticles were cleaned up from excess 16-MHDA by centrifugation and resuspension in PBT at least three times. Thereafter, the terminal carboxyl groups were activated with 50 mM NHS and 200 mM EDC for 15 min. Excess reagent was cleaned up by centrifugation/resuspension two times, during which, the second resuspension incorporated 22 mM AEE and 2.4 mM BA in PBT. This mixture was incubated for 2 h for the attachment of PEG and biotin to the carboxyl terminals, and the final product was cleaned up through centrifugation/resuspension at least three times.

Ionic Strength Stability Assays. NaCl induced aggregation of bare AuNPs, biotin/MHDA/AuNPs, and **bT$_{20}$**/AuNPs was monitored and compared according to a modification of previously established methods.[7-10] All AuNP concentrations were set to 1.5 nM by adding an appropriate amount of AuNPs (suspended in nanopure water), nanopure water, and 5 M NaCl. The NaCl was mixed immediately before measurement in the Cary 50 spectrophotometer, which monitored the absorbance at 620 nm over the course of 5 min using the "Kinetics" program. Thereafter, a post-salting absorbance spectrum was obtained for each nanoparticle solution using the "Scan" program (Figure S2A, S2C, S2E). The ΔA_{620} at t = 5 min (Figure S2B, S2D, S2F) were quantified in replicate (n = 5) for each nanoparticle solution and salt concentration, and the final values with error bars (± SEM) were plotted together (Figure 2B).

Horseradish Peroxidase Assay. HRP/**cT$_{20}$**/AuNPs, TMB, and hydrogen peroxide were utilized in a colorimetric plate assay format to demonstrate feasibility of the AuNPs for ELISA. 200 μL of 0.1 mg/mL TMB in PCB with 0 – 1 mM H_2O_2 was added to each microplate well, followed by the addition of 100 μL of 1 nM HRP/**cT$_{20}$**/AuNPs (d_a ~13 nm) in PCB. After incubating for 20 min (fresh AuNPs) or 1 h (lyophilized AuNPs), the enzymatic reaction was stopped through the addition of 50 μL of 2 M H_2SO_4. Absorbance at 450 nm was read within five minutes of stop

solution addition on the Biotek microplate reader. For control experiments in which AuNPs were absent (free HRP), a $1{:}1{\times}10^6$ working dilution of the enzyme in PCB was added to the TMB/H_2O_2 wells and analyzed in the same manner as described above.

■ SUPPLEMENTARY TABLE

Table S1. Conjugated AuNP Zeta Potentials

Nanoparticle	ζ-potential (mV)	mobility ($cm^2/V{\bullet}s$)
bare AuNPs	-32.03	$-1.666{\times}10^{-4}$
13 nm $\mathbf{T_{20}}$/AuNPs	-53.21	$-2.766{\times}10^{-4}$
30 nm $\mathbf{T_{20}}$/AuNPs	-45.37	$-2.359{\times}10^{-4}$
13 nm MHDA/AuNPs	-48.62	$-2.528{\times}10^{-4}$

■ SUPPLEMENTARY FIGURES

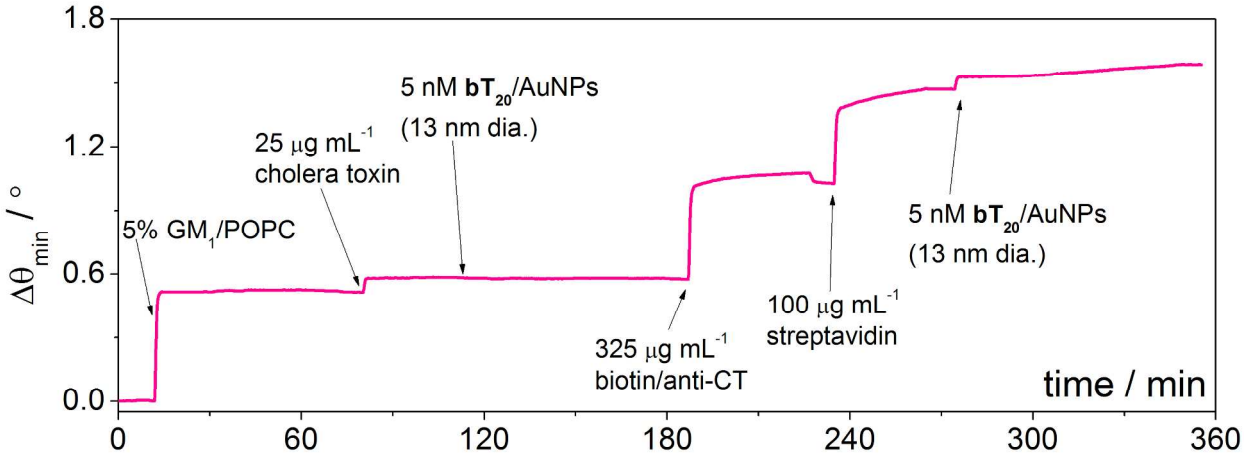

Figure S1. Surface plasmon resonance sensorgram depicting formation of a ganglioside-impregnated supported lipid bilayer, followed by cholera toxin (CT), attempted AuNP recognition, then a biotinylated antibody specific for CT, streptavidin, and another attempt at AuNP recognition. The **bT_{20}**/AuNPs only attach to the surface when streptavidin is present.

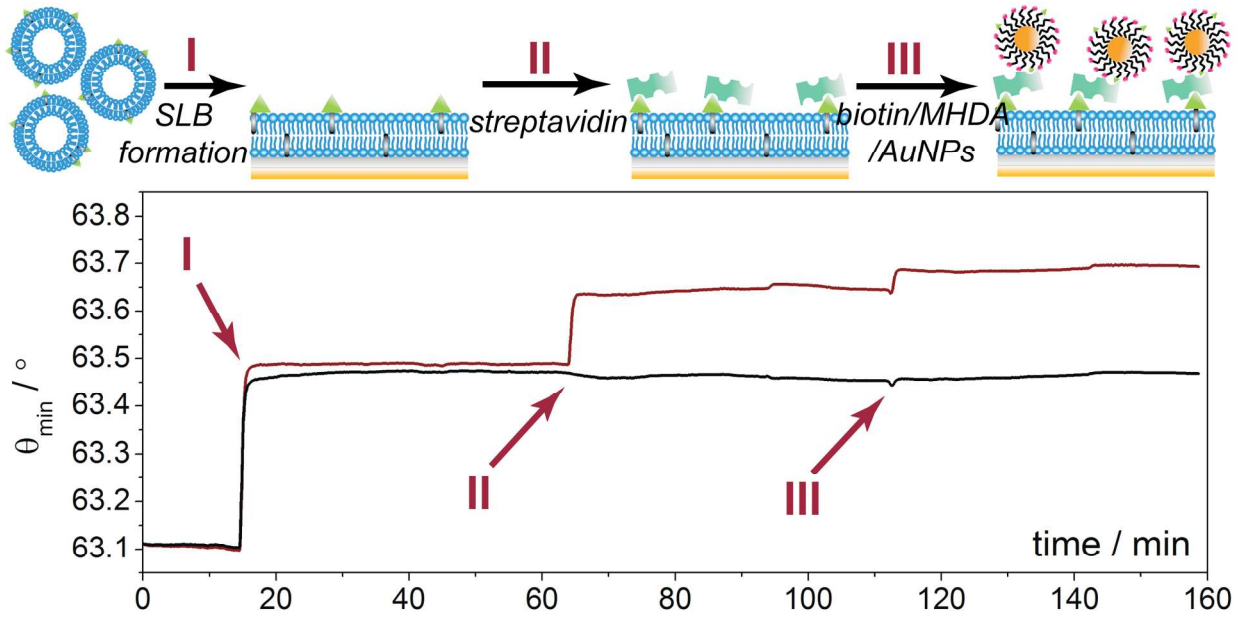

Figure S2. Surface plasmon resonance sensorgram depicting formation of a biotinylated supported lipid bilayer, followed by streptavidin and biotin/MHDA/AuNP recognition. A 100% POPC bilayer is formed in the reference channel and exposed to streptavidin and AuNPs for control purposes.

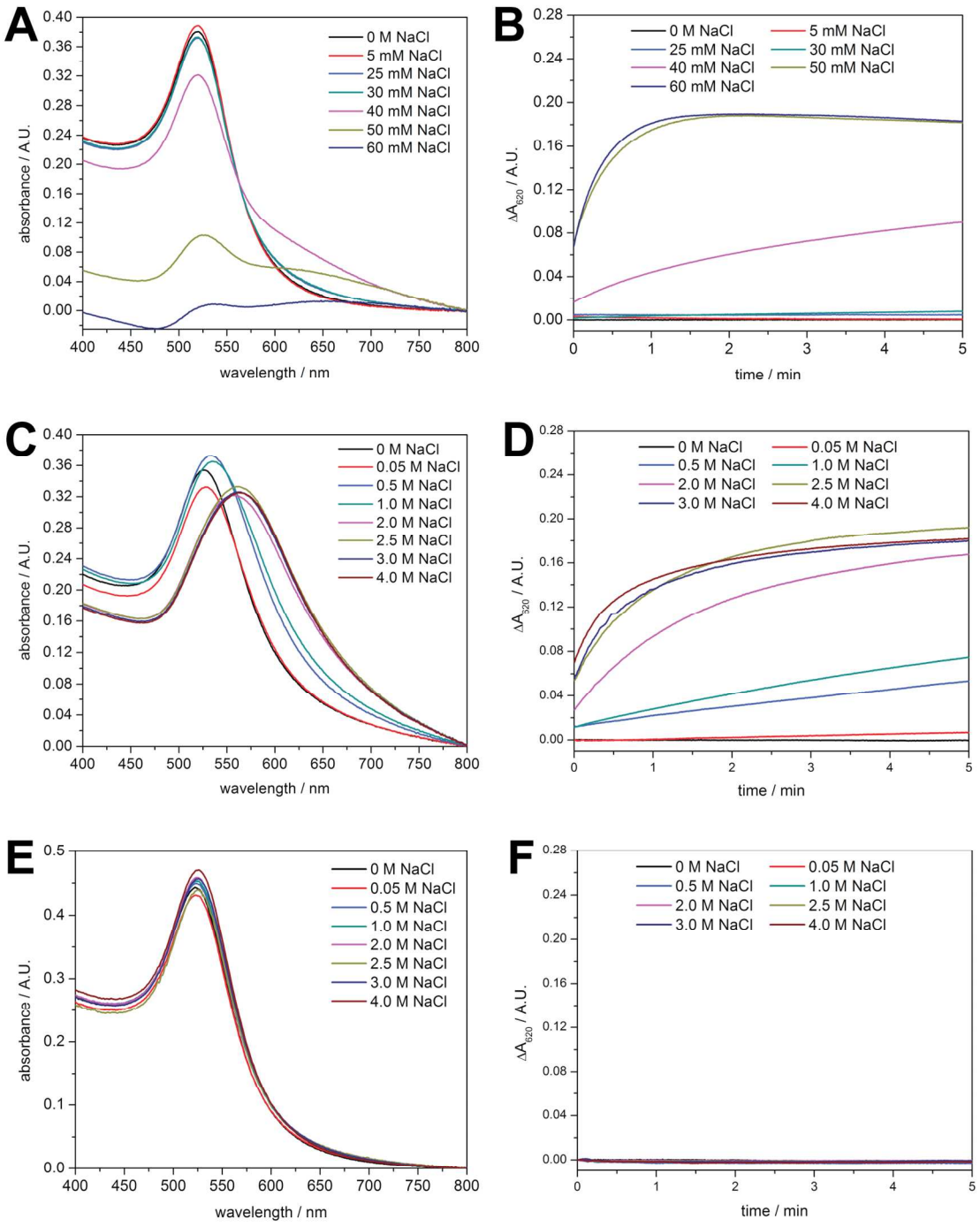

Figure S3. Ionic strength stability assays of bare AuNP (citrate capped), biotin/MHDA/AuNP, and **bT$_{20}$**/AuNP exposures to varying concentrations of NaCl. (A) Bare AuNP absorbance spectra. (B) Bare AuNP ΔA_{620} over 5 min. (C) Biotin/MHDA/AuNP absorbance spectra. (D) Biotin/MHDA/AuNP ΔA_{620} over 5 min. (E) **bT$_{20}$**/AuNP absorbance spectra. (F) **bT$_{20}$**/AuNP ΔA_{620} over 5 min.

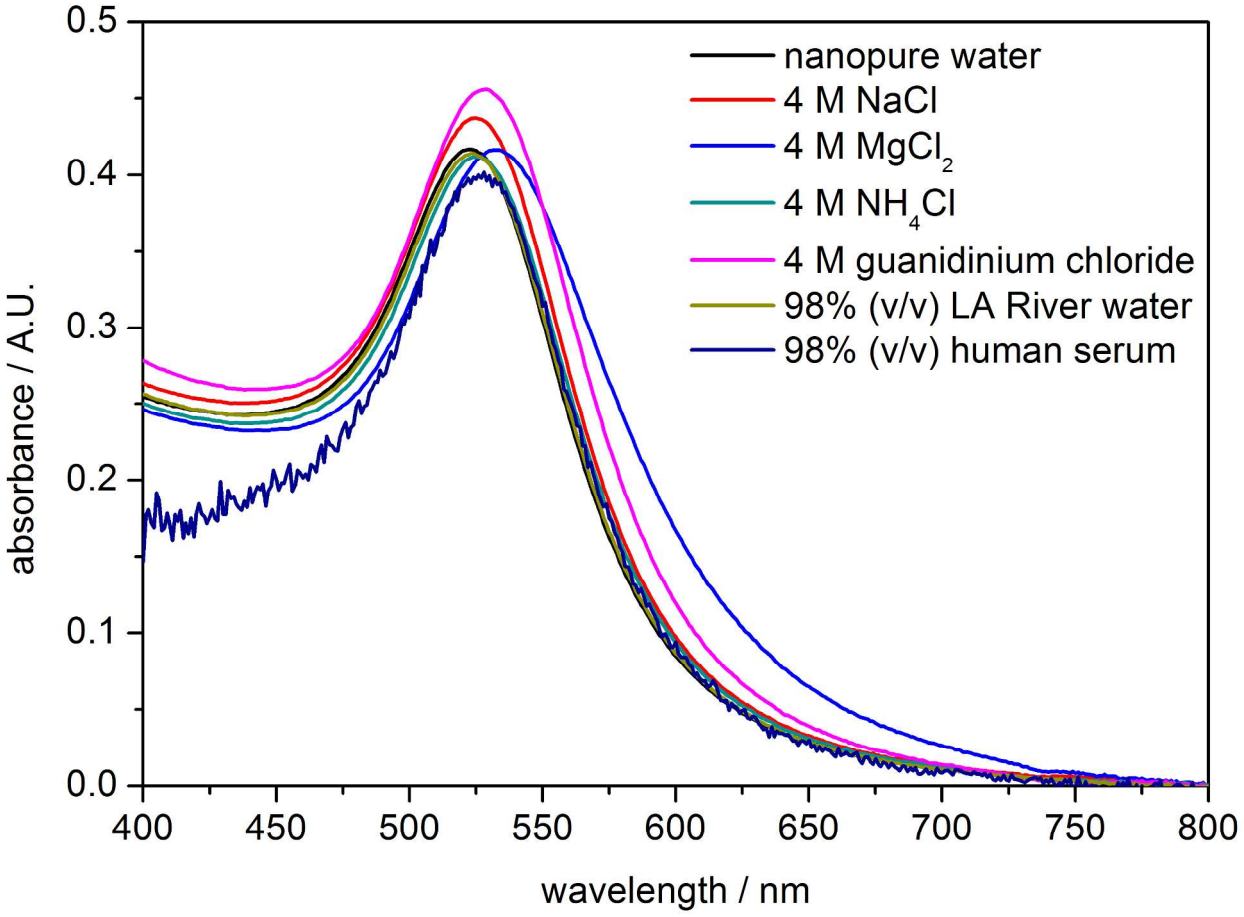

Figure S4. Absorbance spectra of **bT$_{20}$**/AuNPs in nanopure water, 4 M NaCl, 4 M $MgCl_2$, 4 M NH_4Cl, 4 M guanidinium chloride, 98% Los Angeles River water, and 98% human serum.

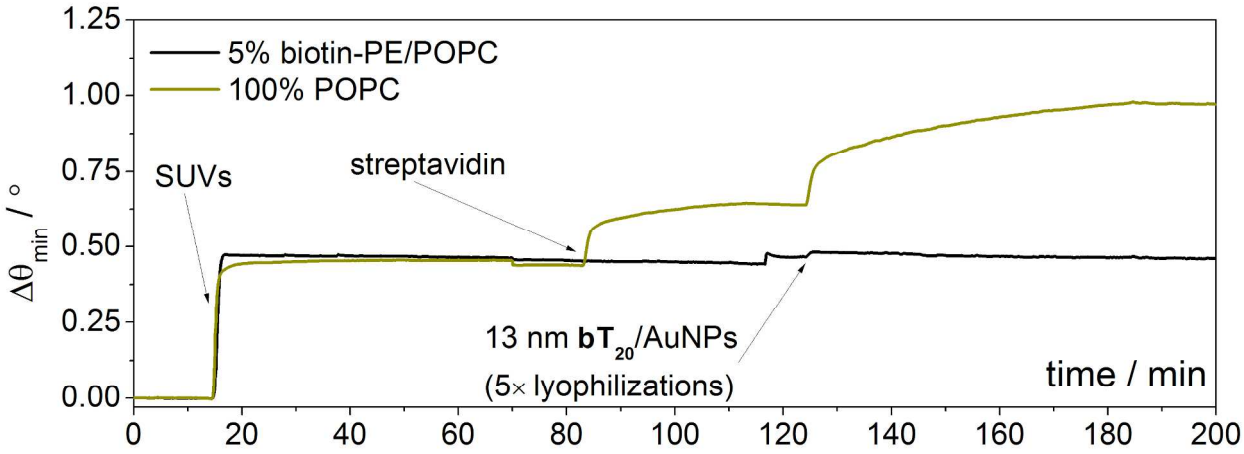

Figure S5. Surface plasmon resonance sensorgram depicting formation of a biotinylated supported lipid bilayer, followed by streptavidin and 13 nm **bT$_{20}$**/AuNP recognition (lyophilized and resuspended five times). A 100% POPC bilayer is formed in the reference channel and exposed to streptavidin and AuNPs for control purposes.

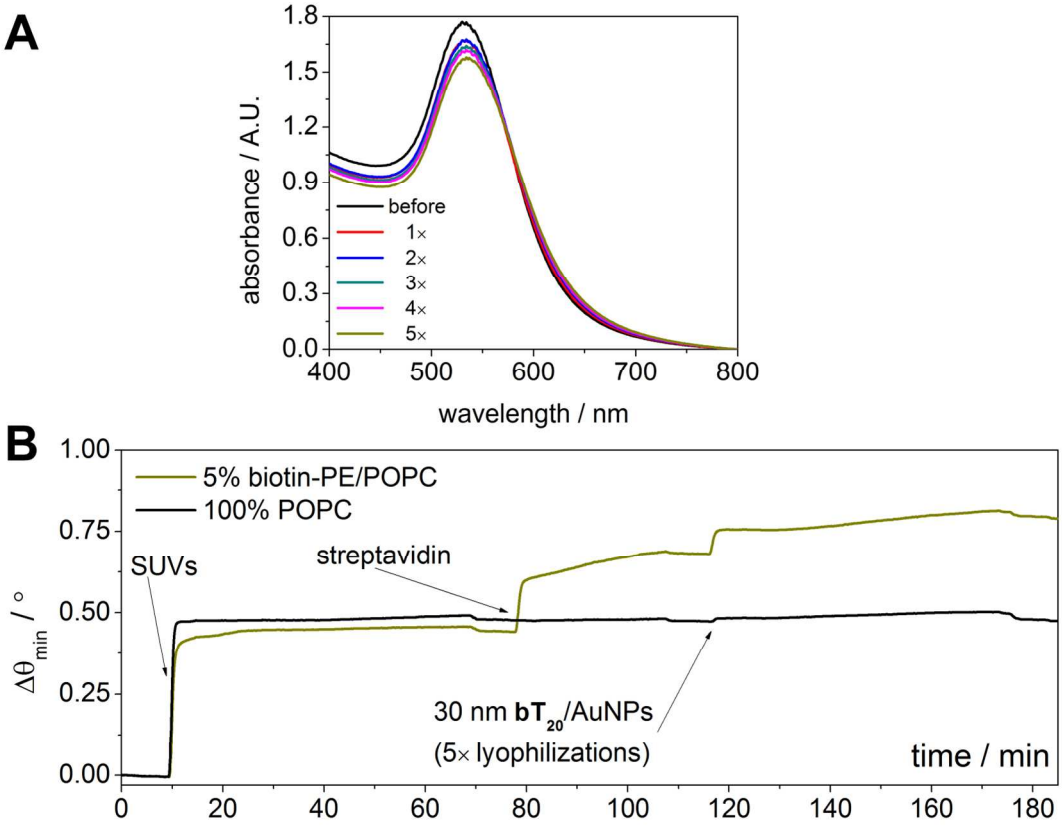

Figure S6. 30 nm **bT$_{20}$**/AuNP lyophilization studies. (A) Absorbance spectra of 30 nm **bT$_{20}$**/AuNPs before and after five lyophilization cycles. (B) Surface plasmon resonance sensorgram depicting formation of a biotinylated supported lipid bilayer, followed by streptavidin and 30 nm **bT$_{20}$**/AuNP recognition (lyophilized and resuspended five times).

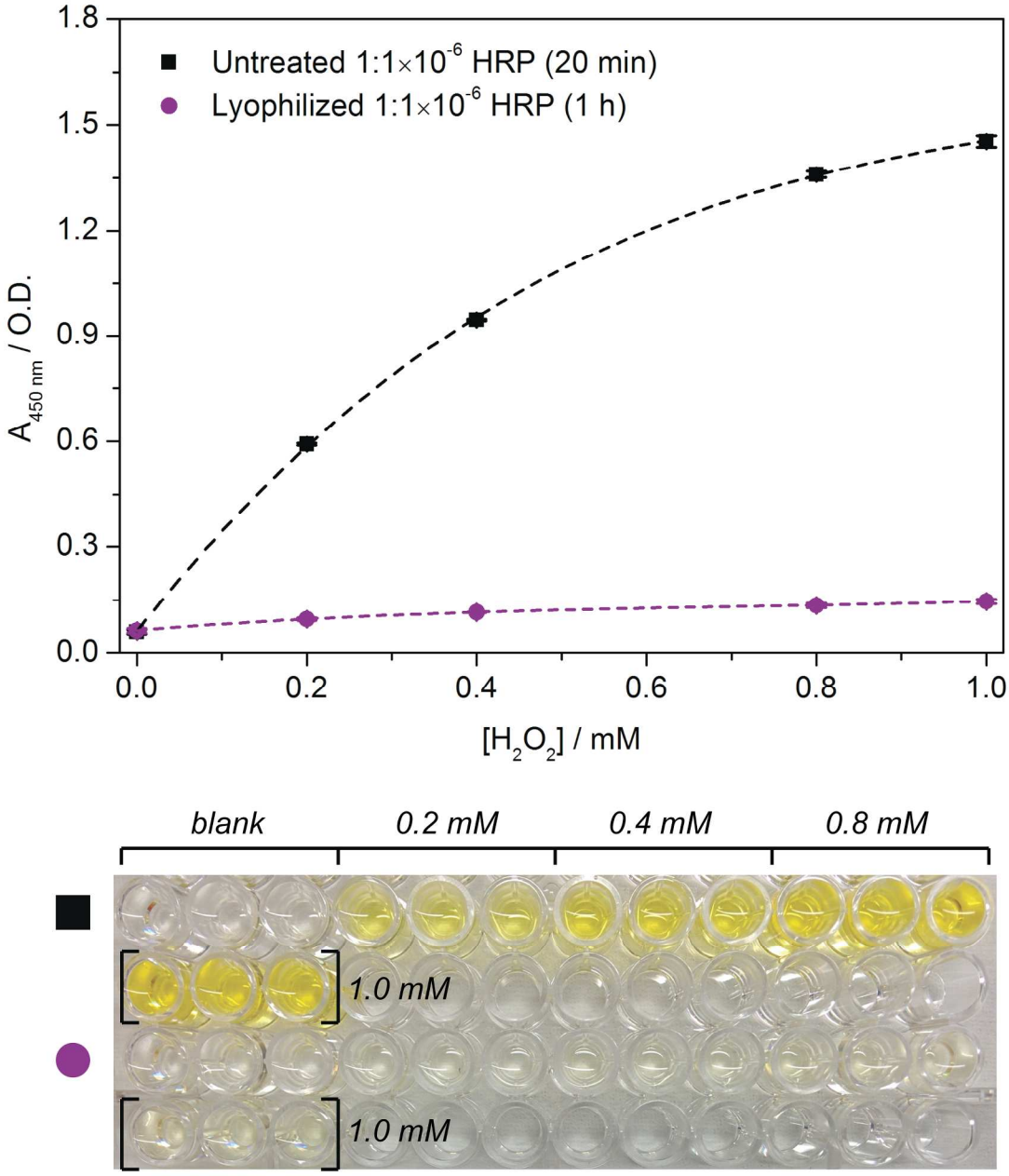

Figure S7. Lyophilization of free horseradish peroxidase (HRP). Results are from hydrogen peroxide assay utilizing TMB and free HRP diluted $1{:}1\times10^{6}$ times.

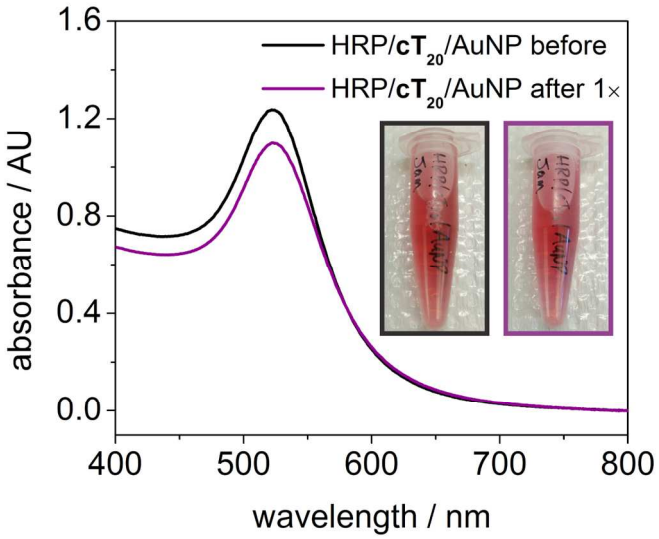

Figure S8. Lyophilization of HRP/$\mathbf{cT_{20}}$/AuNPs: absorbance spectra of the HRP/$\mathbf{cT_{20}}$/AuNPs before and after lyophilization. Inset photographs correlate with samples used for each absorbance spectrum.

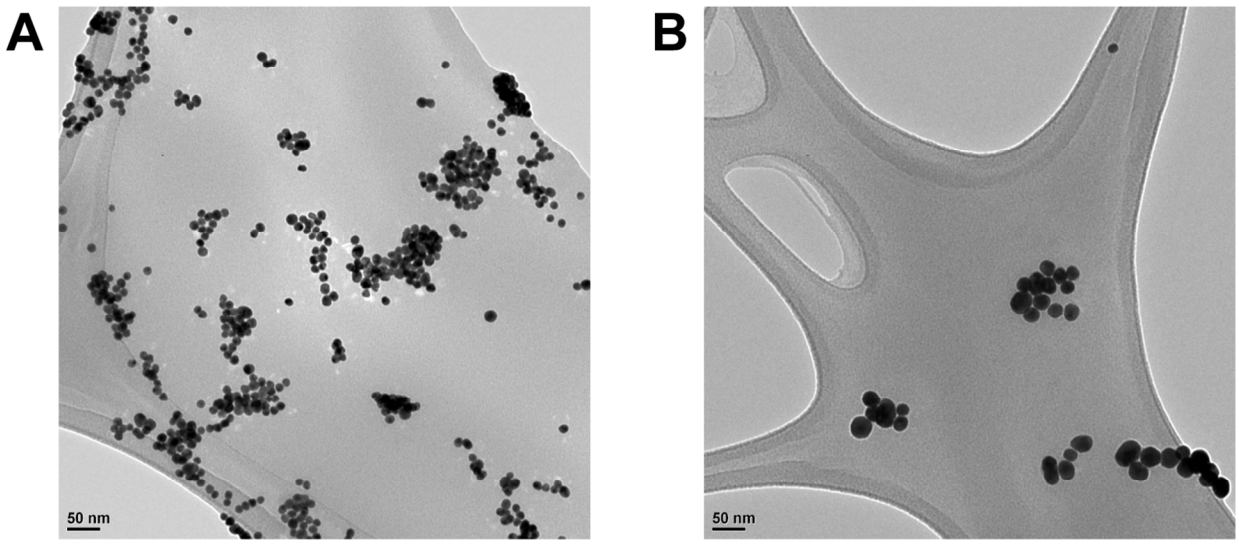

Figure S9. Representative transmission electron micrographs of as-prepared citrate capped (A) 13 nm gold nanoparticles, and (B) 30 nm gold nanoparticles.

■ REFERENCES

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