Selective Protein Recognition in Supported Lipid Bilayer Arrays by Tailored, Dual-Mode Deep Cavitand Hosts

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Electronic Supplementary Information

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1. General Information

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz NMR spectrometer and processed using MestReNova by Mestrelab Research S.L. Proton (^{1}H) chemical shifts are reported in parts per million (δ) with respect to tetramethylsilane (TMS, δ=0), and referenced internally with respect to the protio solvent impurity. Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories, Inc., Andover, MA, and used without further purification. All other materials were obtained from Aldrich Chemical Company (St. Louis, MO), Fisher Scientific (Fairlawn, NJ), or TCI (Tokyo, Japan) and were used as received. Solvents were dried through a commercial solvent purification system (Pure Process Technologies, Inc.). Mass spectra were recorded by electrospray ionization with a LTQ-XL linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA). Surface Plasmon Resonance spectroscopic measurements were performed with a dual-channel SPR spectrometer, NanoSPR6-321 (NanoSPR, Chicago, IL), with a GaAs semiconductor laser light source (λ = 650 nm). The device was equipped with a manufacturer-supplied high-refractive index prism (n = 1.61) and a 30 μL flow cell. Surface interactions at the gold interface were monitored using the resonance angle tracking mode.

2. Synthesis of New Compounds

2-Biotinamido-N,N,N-trimethylethanaminium Iodide 4: Biotin N-hydroxysuccinimidyl ester (NHS Biotin, 100 mg, 0.293 mmol) was added to a 10 mL round bottom flask with a stir bar. The system was purged and placed under nitrogen followed by addition of dry THF (3 mL). Unsym-N,N dimethylethylene-diamine was added (0.296 mmol) and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated via rotary evaporation and

triturated with ether and hexanes before drying under vacuum. The product was then placed in a 10 mL round bottom flask with a stir bar under nitrogen. Dry THF (2 mL) was added followed by iodomethane (41.6 mg, 18.2 µL, 0.293 mmol). The reaction mixture was stirred at room temperature for 4 h then filtered and triturated with dry dichloromethane and hexanes. The product (48 mg, 36 % yield) was collected as a white solid. ^{1}H NMR (400 MHz, D_2O): δ 4.64 (dt, *J* = 8, 4 Hz, 1H), 4.47 (dd, *J* = 8, 4 Hz, 1H), 3.72 (t, *J* = 6 Hz, 2H), 3.52 (t, *J* = 7, 6, 2H), 3.36 (dd, *J* = 13, 5 Hz, 1H) 3.31 (dd, *J* = 13, 5Hz, 1H), 3.21 (s, 9H), 3.03 (dd, *J* = 13, 5, Hz, 1H), 2.61 (m, 2H), 2.34 (t, *J* = 7 Hz, 2H), 1.67 (m, 2H), 1.44 (m, 2H) ^{13}C NMR (100 MHz, DMSO): δ 172.8, 162.8, 63.8, 61.1, 59.2, 55.4, 52.6, 44.3, 35.0, 33.0, 30.4, 28.0, 25.0. ESI *m/z* expected: 329.48, found $[MH^+]$ = 329.22

N^1-(3-acetamidopropyl)-N^3,N^3,N^3-trimethylpropane-1,3-diaminium Iodide 5: NHS Biotin (100 mg, 0.293 mmol) was added to a 10 mL round bottom flask with a stir bar. The system was purged and placed under nitrogen followed by addition of dry THF (3 mL). N,N-dimethylpropylenediamine was added (0.296 mmol) and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated via rotary evaporation and triturated with ether and hexanes before drying under vacuum. The product was then placed in a 10 mL round bottom flask with a stir bar under nitrogen. Dry THF (2 mL) were added followed by iodomethane (41.6 mg, 18.2 µL, 0.293 mmol). The reaction mixture was stirred at room temperature for 4 h then filtered and triturated with dry dichloromethane and hexanes. The product (85 mg, 55% yield) was collected as a white solid. ^{1}H NMR (400 MHz, D_2O): 4.63 (td, *J* = 8, 4 Hz, 1H), 4.46 (td, *J* = 8, 4 Hz, 1H), 3.47 (m, 2H), 3.35 (m, 2H), 3.32 (t, J= 7 Hz, 2H), 3.20 (s, 10H), 3.12, (t, J= 7 Hz, 2H), 3.06 (m, 4H), 2.31 (t, *J* = 7 Hz, 2H), 1.89 (m, 2H), 1.66 (m, 4H), 1.44

(m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9, 162.7, 63.7, 61.0, 59.2, 55.4, 54.9, 52.3, 45.9, 45.2, 36.2, 35.2, 28.4, 28.2, 27.8, 25.2, 21.6. ESI-MS *m/z* expected: 400.60, found [MH^+] = 400.30.

2-((2-biotinamidoethyl)thio)-4-oxo-4-((2-(trimethylammonio)-ethyl)amino)butanoate 6: NHS Biotin (100 mg, 0.293 mmol) was added to a 10 mL round bottom flask with a stir bar. The system was purged and placed under nitrogen followed by addition of dry THF (3 mL). Cystamine dihydrochloride was added (0.147 mmol) and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated via rotary evaporation and triturated with ether and hexanes before drying under vacuum to afford a white solid (60 mg, 68% yield) as product. ^{1}H NMR (400 MHz, D_2O): δ 4.64 (dt, *J* = 8,4 Hz, 2H), 4.47 (dd, *J* = 8,4 Hz, 2H), 3.42 (t, *J* = 6 Hz, 6H), 3.04 (t, *J* = 8 Hz, 6H), 2.79 (m, 6H), 1.82 (m, 4H), 1.66 (m, 4H), 1.55 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.2, 162.6, 61.0, 59.2, 55.2, 37.8, 33.9, 30.0, 27.8, 27.6, 25.4, 24.3. ESI-MS *m/z* expected: 604.20, found [MH^+] = 605.21. The disulfide product (1 mg, 1.65 μmol) was then placed in a glass vial equipped with a stir bar under. Nanopure water (500 μL) and excess tris(2-carboxyethyl)phosphine (TCEP) were added (8.3 mg, 33 μmol) and the solution was stirred at room temperature for 1 h. An aliquot of a solution of **7** (1.65 μmol) was added to the solution and stirred at room temperature for 1 h. This solution was then directly used for SPR studies.

(Z)-4-oxo-4-((2-(trimethylammonio)ethyl)amino)but-2-enoate 7: Maleic anhydride (500 mg, 5.10 mmol) was added to a 100 mL round bottomed flask with a stir bar followed by ether (50 mL). N,N-dimethylethylenediamine (5.10 mmol, 450 mg, 511μL) was then added dropwise while stirring and the solution as allowed to stir at room temperature for 10 min (when precipitate was formed). The solid was then filtered, washed with ether and dried. 100 mg (0.540 mmol) of solid was then placed in a round bottom flask followed by DMF (3 mL) and methyl iodide (76.6 mg,

33.6 µL, 0.54 mmol). The reaction mixture was then stirred at room temperature for 4 h. The solid was then filtered and washed with ether and hexanes to afford a yellow-white solid (125 mg, 10 % yield) as product. ^{1}H NMR (400 MHz, D_2O): δ 6.51 (d, *J* = 12 Hz, 1H), 6.36 (t, *J* = 11 Hz, 1H), 3.80 (t, *J* = 7 Hz, 2H), 3.57 (t, *J* = 7 Hz, 2H), 3.22 (s, 9H). ^{13}C NMR (100 MHz, D_2O): δ 221.3, 220.1, 132.5, 129.8, 64.0, 53.5, 33.7. ESI-MS: *m/z* expected: 201.24, found: [MH^+] = 201.14

(Z)-4-oxo-4-((2-(trimethylammonio)hexyl)amino)but-2-enoate 8: Maleic anhydride (170 mg, 1.73 mmol) was added to a 50 mL round bottomed flask with a stir bar followed by ether (10 mL). 6-(dimethylamino)hexylamine (300 µL, 1.73 mmol) was then added dropwise while stirring and the solution as allowed to stir at room temperature for 10 min (when precipitate was formed). The solid was then filtered, washed with ether and dried. 84 mg (0.347 mmol) of the resulting solid was then placed in a round bottomed flask followed by DMF (1 mL) and methyl iodide (49 mg, 22.0 µL, 0.35 mmol). The reaction mixture was then stirred at room temperature for 4 h. The solid was then filtered and washed with ether and hexanes to afford a thick orange oil (90 mg, 68 % yield) as product. ^{1}H NMR (400 MHz, D_2O): δ 6.48 (d, *J* = 12.4 Hz, 1H), 6.30 (d, *J* = 12.2 Hz, 1H), 3.32 (m, 4H), 3.12 (s, 9H), 1.82 (q, *J* = 7 Hz, 2H), 1.59 (q, *J* = 7, 2 Hz), 1.42 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ 169.2, 167.3, 133.5, 130.3, 66.8, 53.1, 39.7, 27.8, 25.7, 25.2, 22.4. ESI-MS: *m/z* expected: 257.35, found: [MH^+] = 257.15.

3. SPR Data

Native Protein Immobilization:

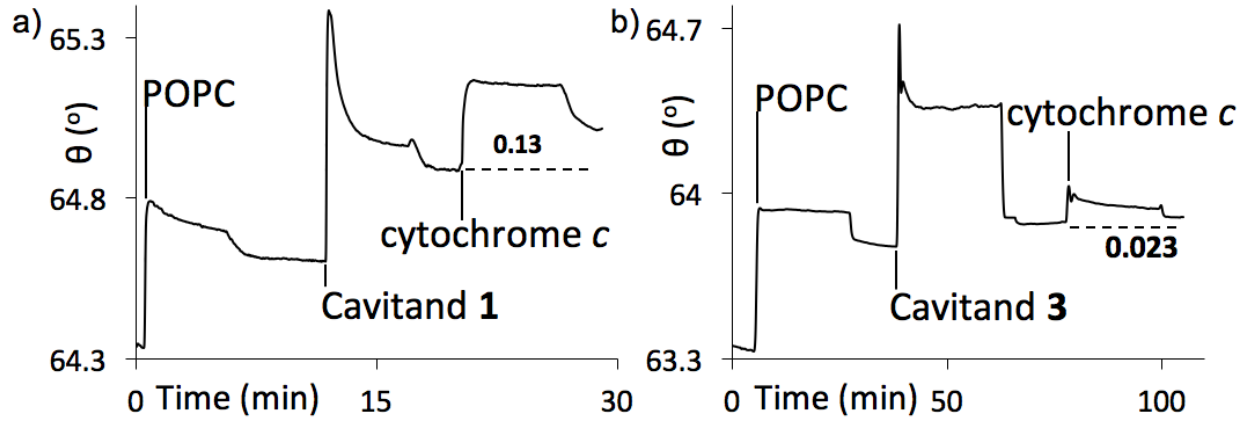

Figure S-1. SPR sensorgrams showing immobilization of cytochrome *c* at the a) cavitand **1**:POPC interface and b) cavitand **3**:POPC interface.

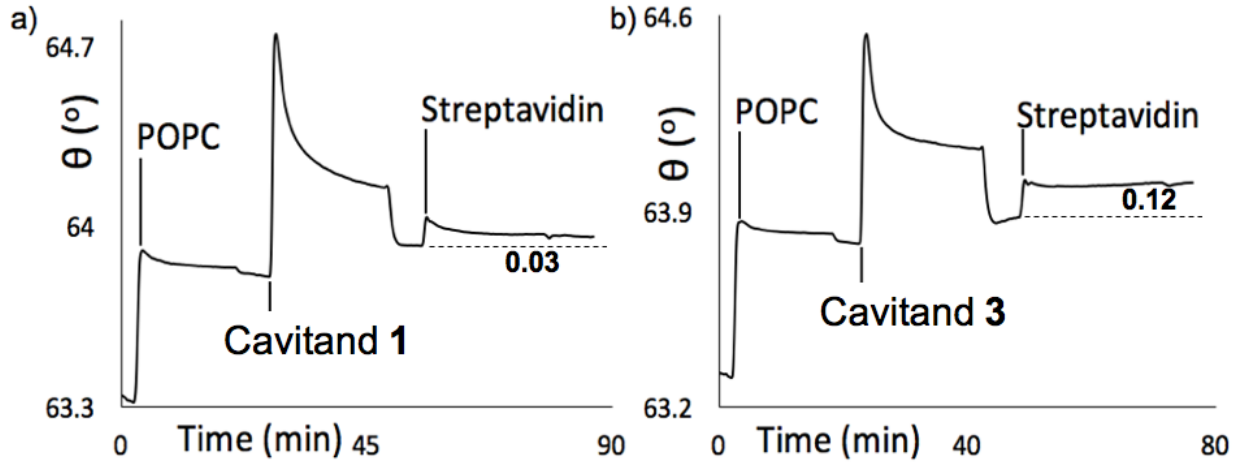

Figure S-2. SPR sensorgrams showing immobilization of streptavidin at the a) cavitand **1**:POPC interface and b) cavitand **3**:POPC interface.

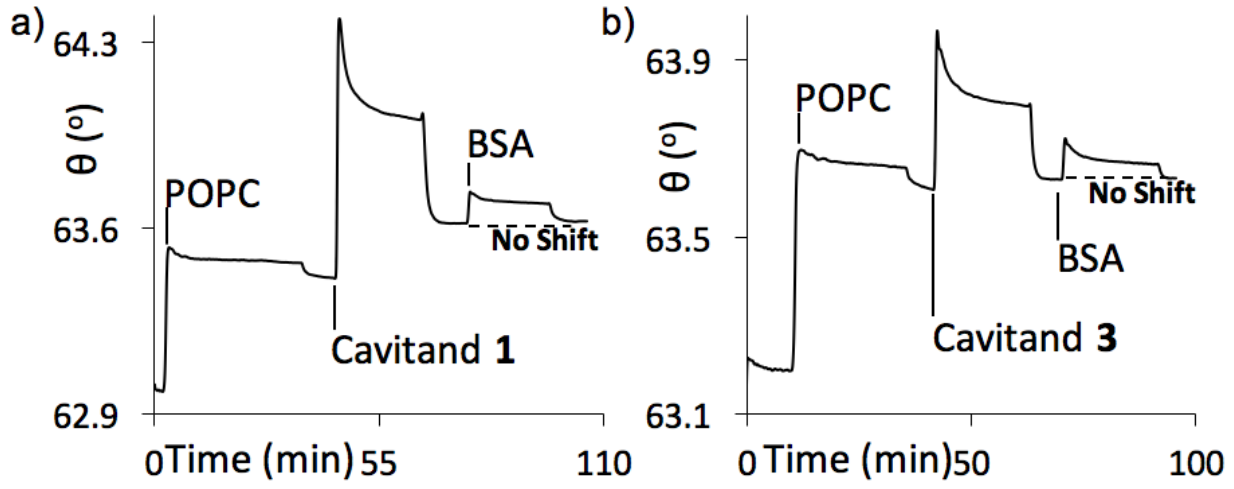

Figure S-3. SPR sensorgrams showing immobilization of BSA at the a) cavitand **1**:POPC interface and b) cavitand **3**:POPC interface.

Avidin Selectivity of Cavitand 1:

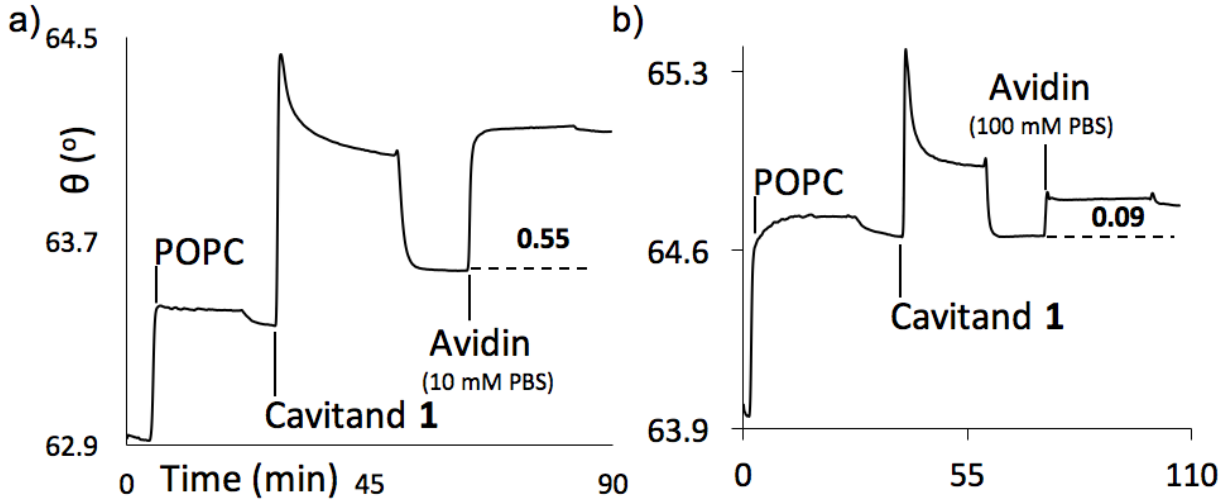

Figure S-4. SPR sensorgrams showing immobilization of avidin by the cavitand **1**:POPC interface. Avidin injection medium: a) 10 mM PBS; b) 100 mM PBS.

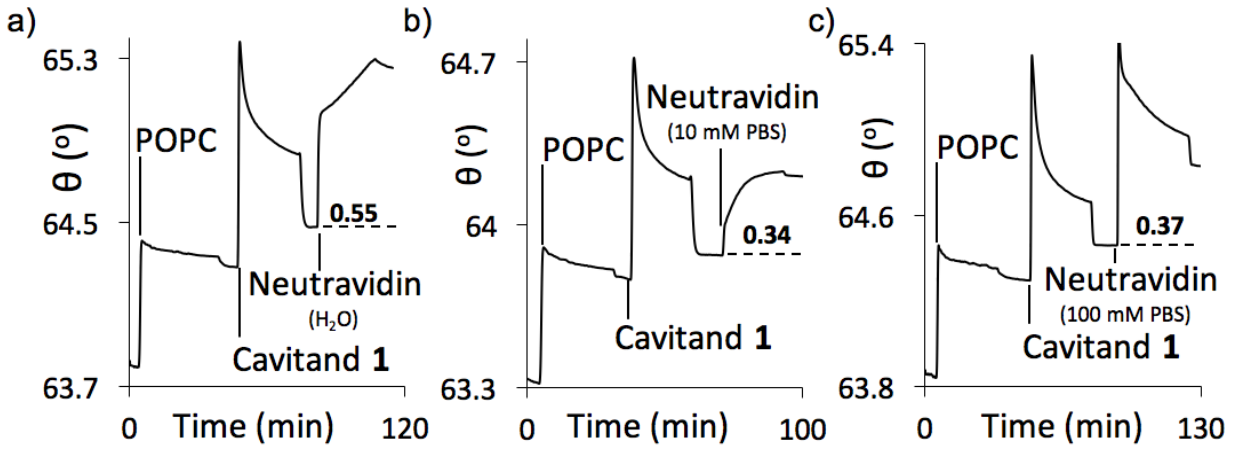

Figure S-5. SPR sensorgrams showing immobilization of neutravidin by the cavitand **1**:POPC interface. Neutravidin injection medium: a) Nanopure H_2O; b) 10 mM PBS; c) 100 mM PBS.

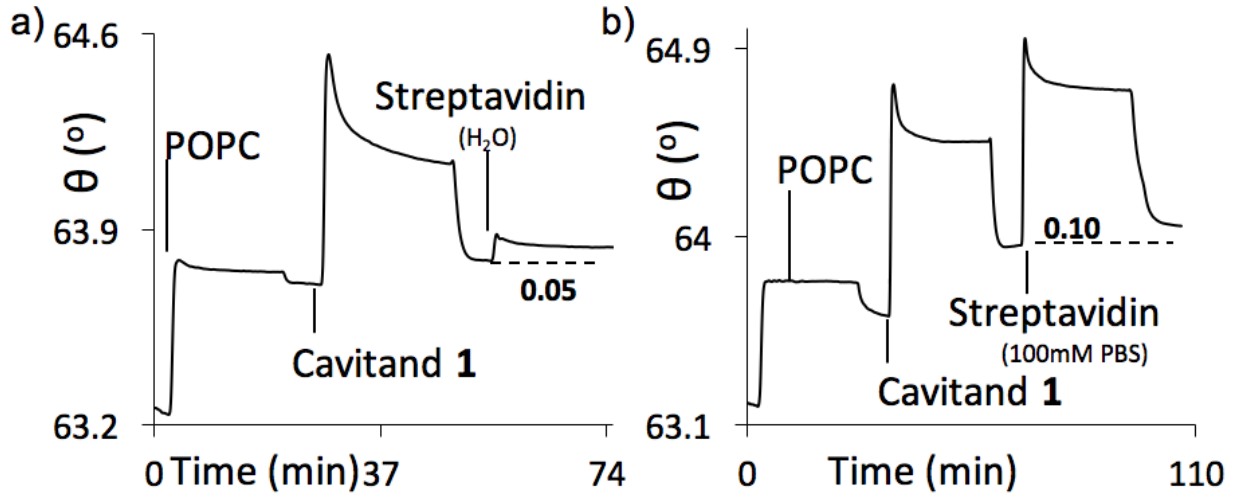

Figure S-6. SPR sensorgrams showing immobilization of streptavidin by the cavitand **1**:POPC interface. Streptavidin injection medium: a) Nanopure H_2O; b) 100 mM PBS.

Dual Mode Binding Guests:

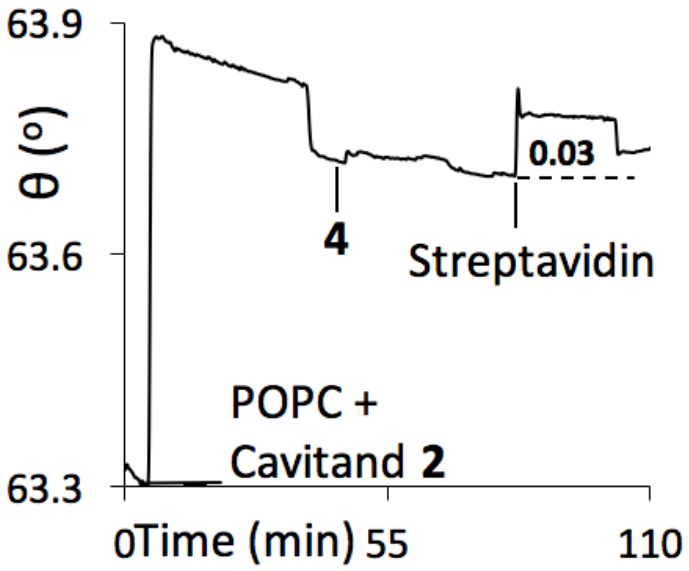

Figure S-7. SPR sensorgrams showing immobilization of streptavidin in 10 mM PBS by the **4**:cavitand **2**:POPC interface.

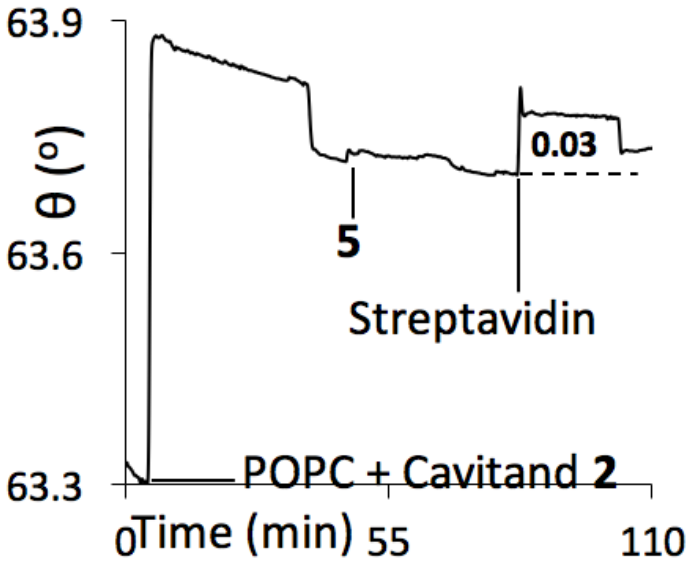

Figure S-8. SPR sensorgrams showing immobilization of streptavidin in 10 mM PBS by the **5**:cavitand **2**:POPC interface.

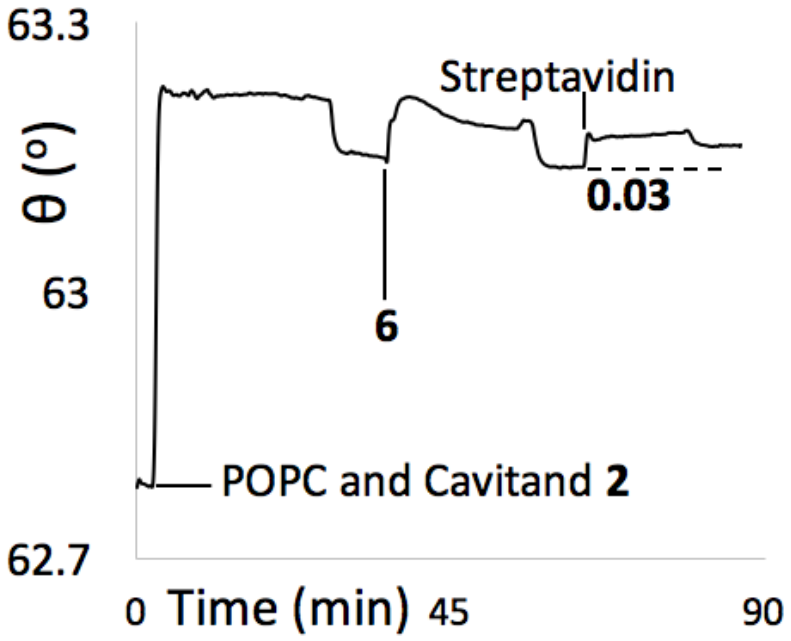

Figure S-9. SPR sensorgrams showing immobilization of streptavidin in 10 mM PBS by the **6**:cavitand **2**:POPC interface.

Streptavidin Binding Optimization

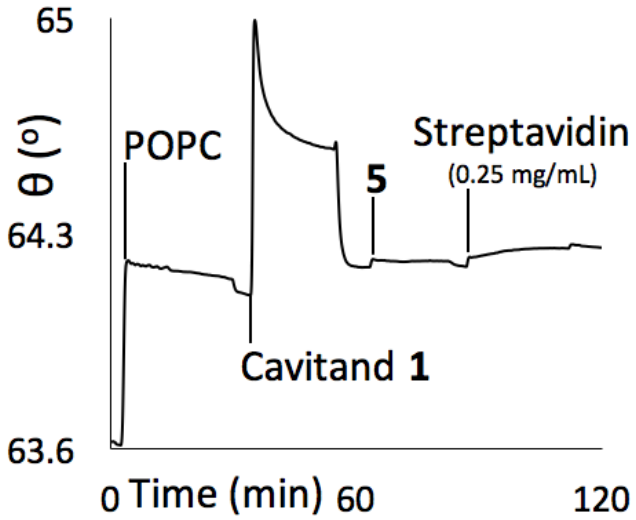

Figure S-10. SPR sensorgrams showing immobilization of streptavidin in 10 mM PBS (0.25 mg/mL) by the **5**:cavitand **1**:POPC interface.

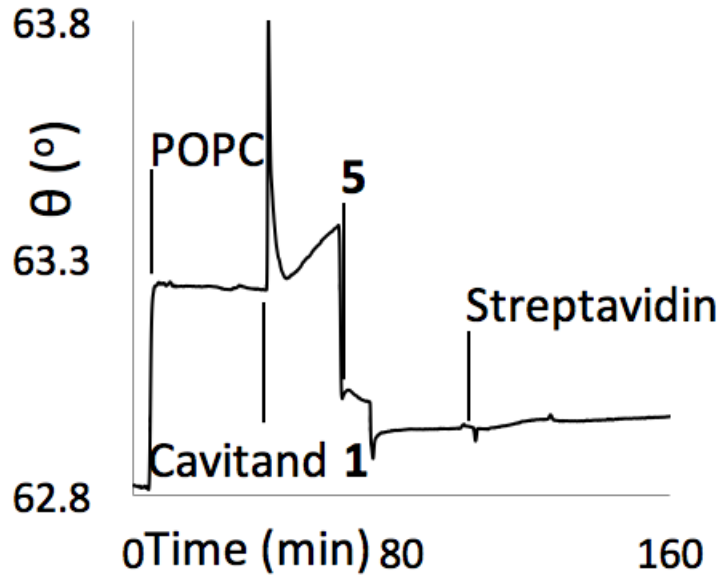

Figure S-11. SPR sensorgrams showing immobilization of streptavidin in 10 mM PBS by the **5**:cavitand **1**:POPC interface using 10 mM PBS as running buffer.

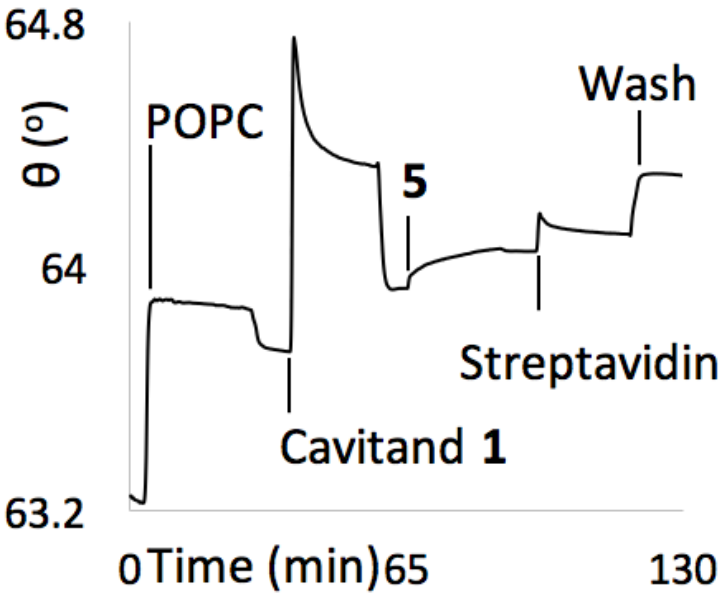

Figure S-12. SPR sensorgrams showing immobilization of streptavidin in 10 mM PBS by the **5**:cavitand **1**:POPC interface by increasing the injection time from 2 minutes and 35 seconds to 3 minutes and 30 seconds.

Affinity Determination

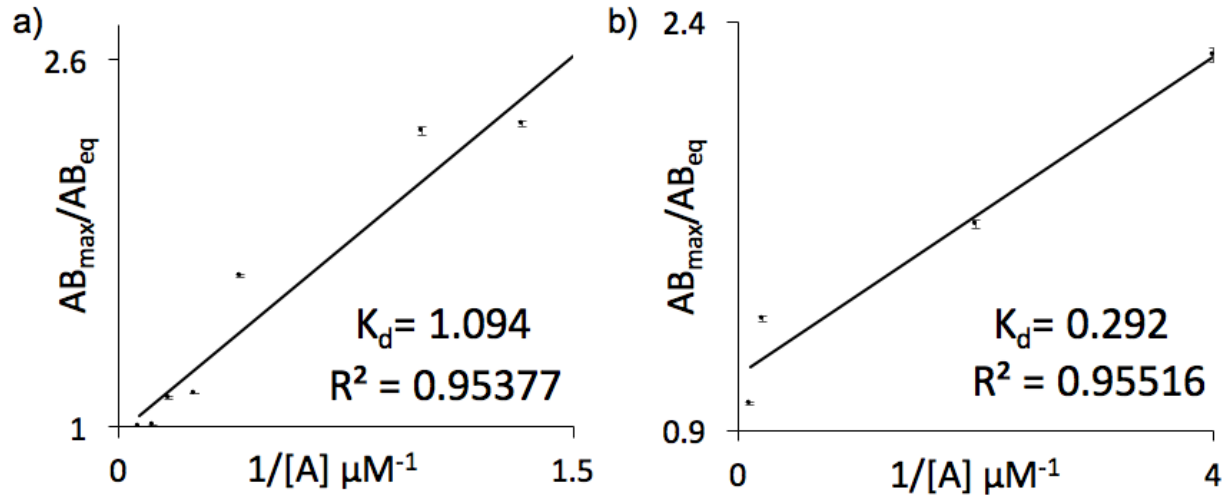

Figure S-13. Equilibrium dissociation constant (K_d) determination for cavitand **3** binding with a) **8**•BSA and b) **7**•BSA.

Control Sensorgrams

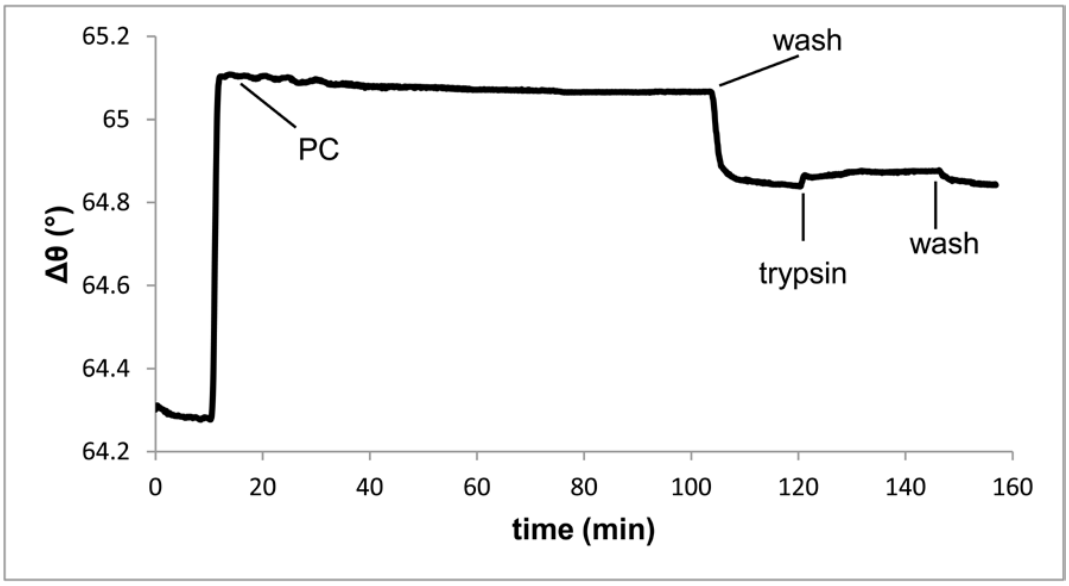

Figure S-14. Control SPR sensorgram of the addition of trypsin to a pristine POPC membrane in the absence of cavitand, showing no immobilization.

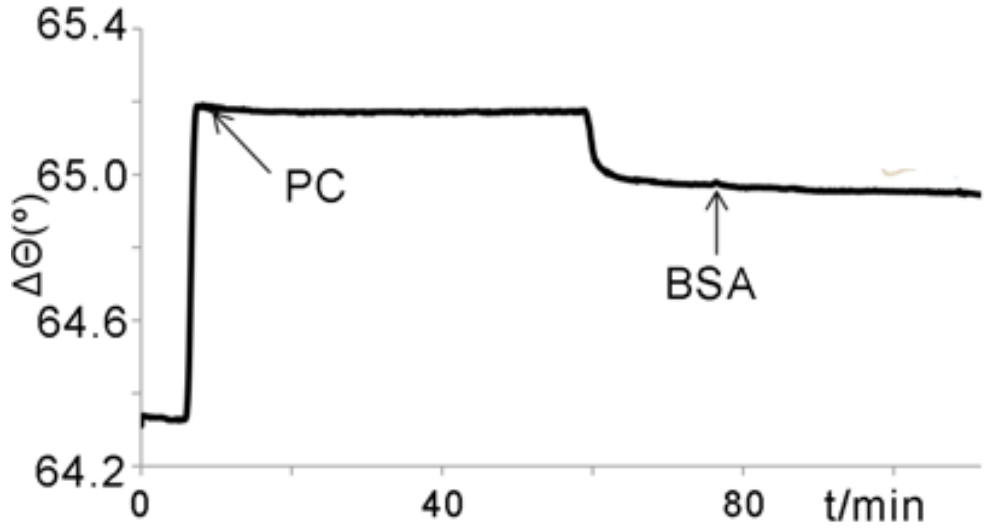

Figure S-15. Control SPR sensorgram of the addition of BSA to a pristine POPC membrane in the absence of cavitand, showing no immobilization.

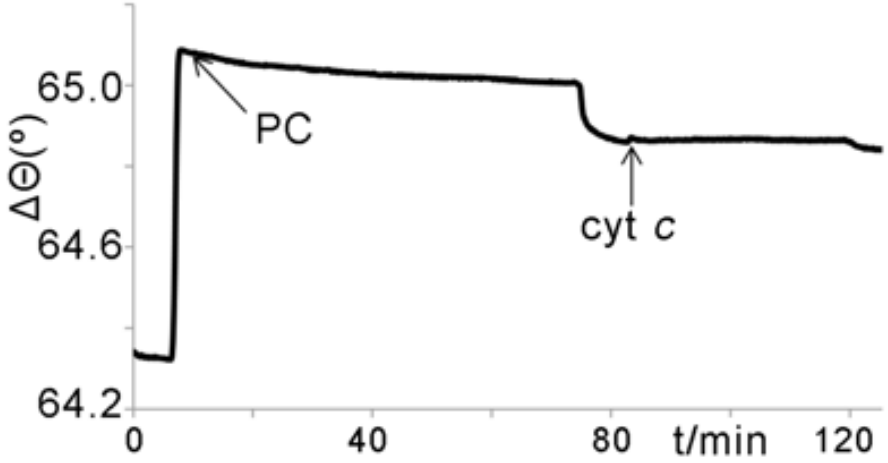

Figure S-16. Control SPR sensorgram of the addition of cytochrome *c* to a pristine POPC membrane in the absence of cavitand, showing no immobilization.

4. SPRi Data

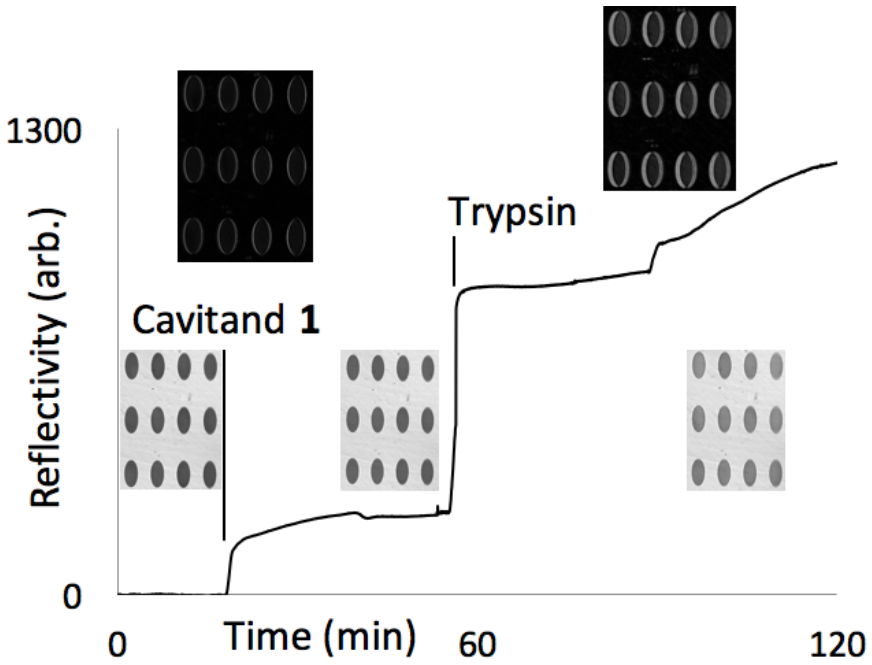

Figure S-17. Mean SPRi sensorgram of the array spots showing immobilization of trypsin in 10 mM PBS at the cavitand **1**:POPC interface.

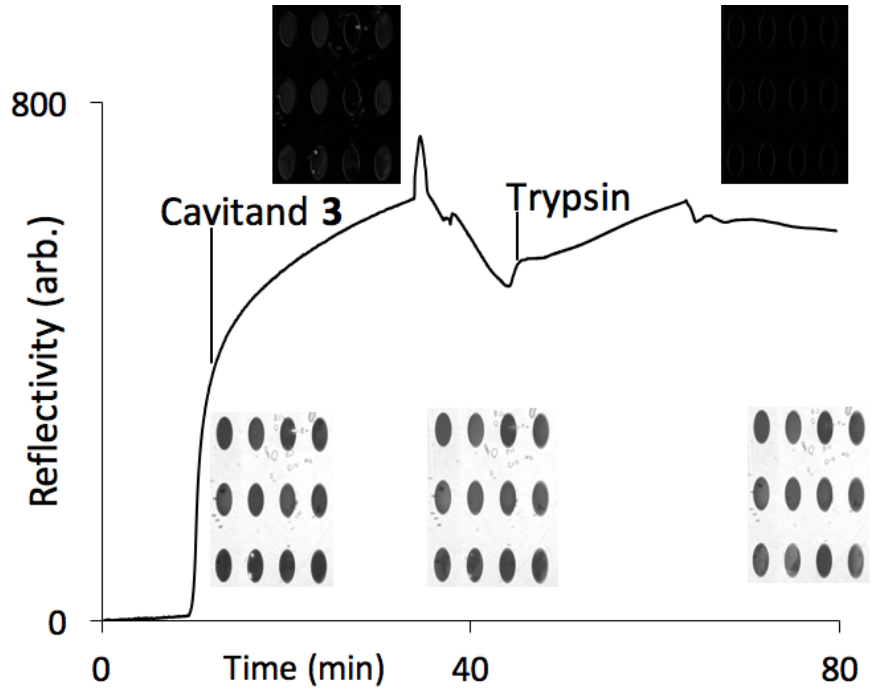

Figure S-18. Mean SPRi sensorgram of the array spots showing immobilization of trypsin in 10 mM PBS at the cavitand **3**:POPC interface.

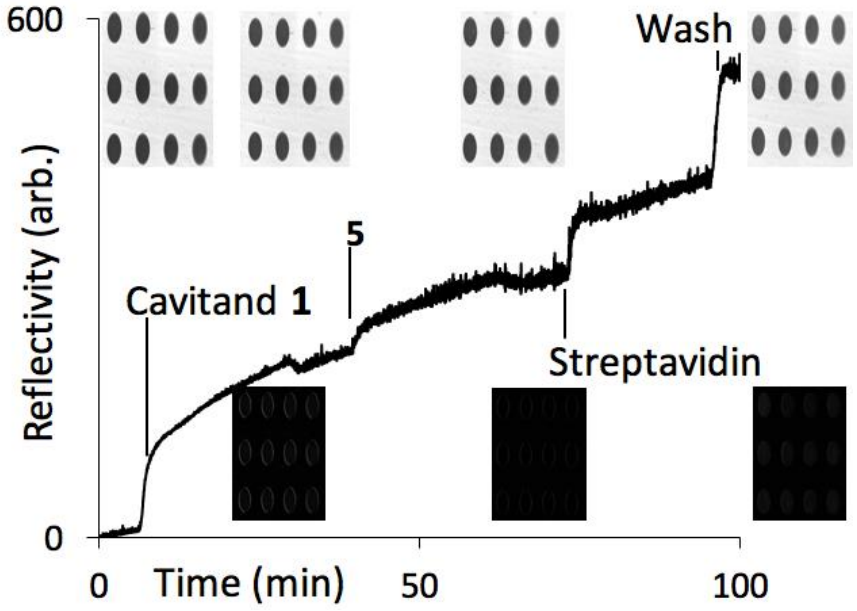

Figure S-19. Mean SPRi sensorgram of the array spots showing immobilization of streptavidin in 10 mM PBS by the **5**:cavitand **1**:POPC interface.

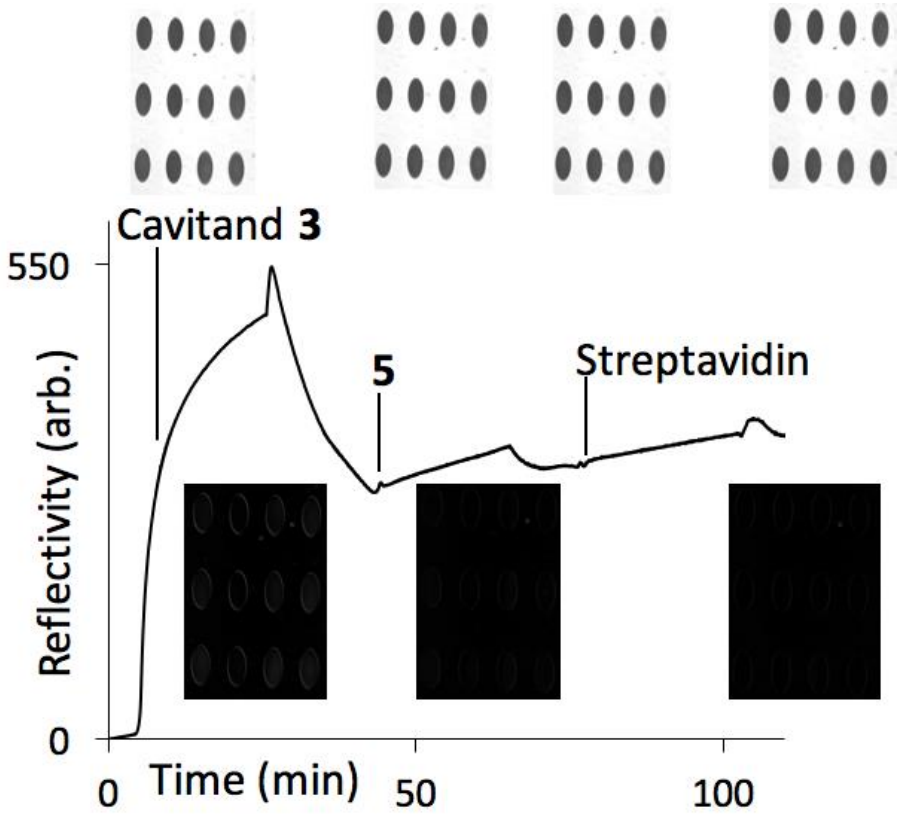

Figure S-20. Mean SPRi sensorgram of the array spots showing immobilization of streptavidin by the **5**:cavitand **3**:POPC interface.