Anionic Deep Cavitands Control the Adhesion of Unmodified Proteins at a Membrane Bilayer

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Electronic Supplementary Information

1. SPR Binding Analysis (sensorgrams not shown in the text)

1) Bovine Serum Albumin (BSA)

*a. POPC:cavitand **1** bilayer*

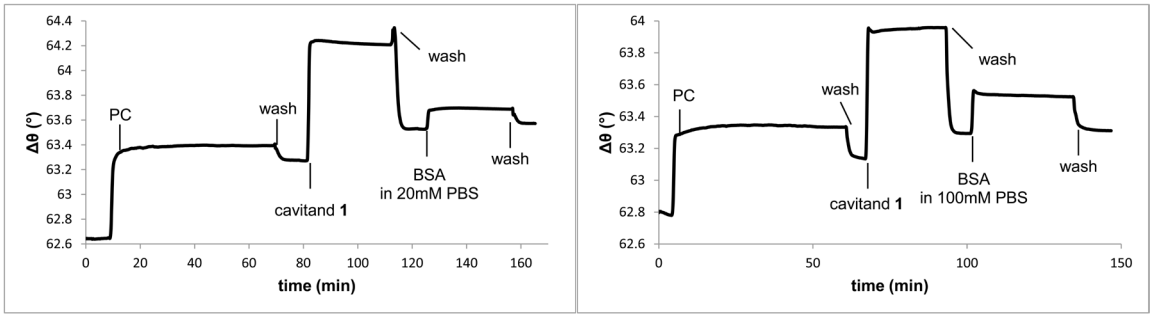


Figure *S*-1. SPR sensorgrams of the binding event between POPC:cavitand **1** and BSA in 20 mM PBS (left) or 100 mM PBS (right).

*b. POPC:cavitand **2** bilayer*

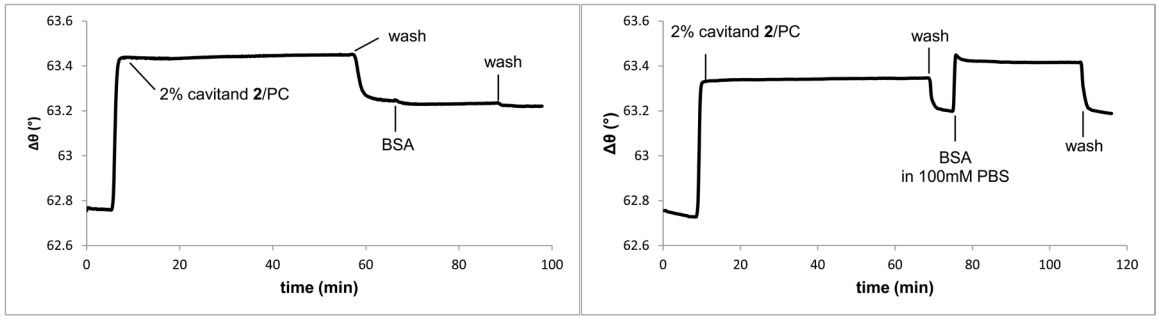


Figure *S*-2. SPR sensorgrams of the binding event between POPC:2% cavitand **2** and BSA in water (left) or 100mM PBS (right).

c. POPC:sodium palmitate bilayer

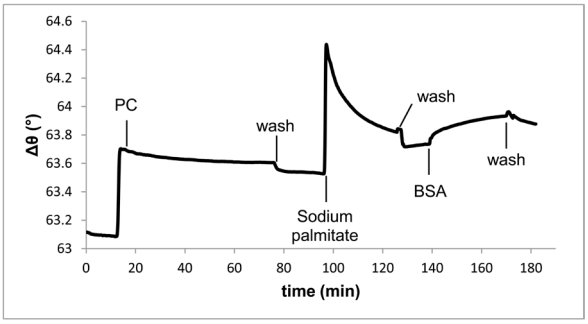


Figure *S*-3. SPR sensorgram of the binding event between POPC:sodium palmitate and BSA in water.

2) Cytochrome c (cyt *c*)

*a. POPC:cavitand **1** bilayer*

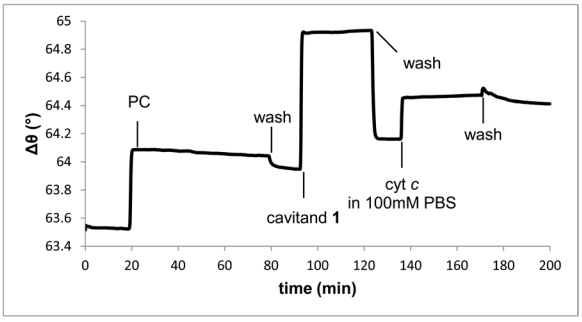


Figure *S*-4. SPR sensorgram of the binding event between POPC:cavitand **1** and cyt *c* in 100 mM PBS.

*b. POPC:cavitand **2** bilayer*

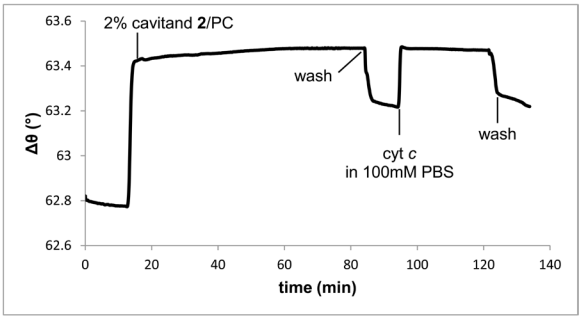


Figure *S*-5. SPR sensorgram of the binding event between POPC:2% cavitand **2** and cyt *c* in 100 mM PBS.

3) Myoglobin

*a. POPC:cavitand **1** bilayer*

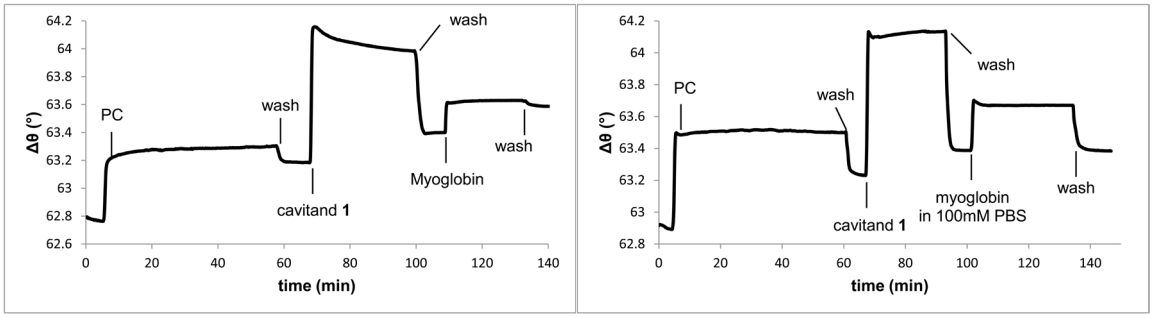


Figure *S*-6. SPR sensorgrams of the binding event between POPC:cavitand **1** and myoglobin in water (left) or 100 mM PBS (right).

b. Control experiment (POPC bilayer alone)

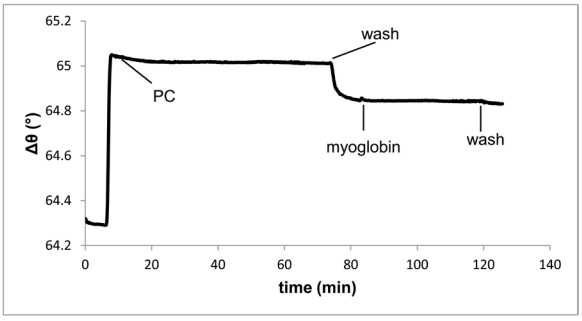


Figure *S*-7. SPR sensorgram of the binding event between POPC membrane and myoglobin in water.

*c. POPC:cavitand **2** bilayer*

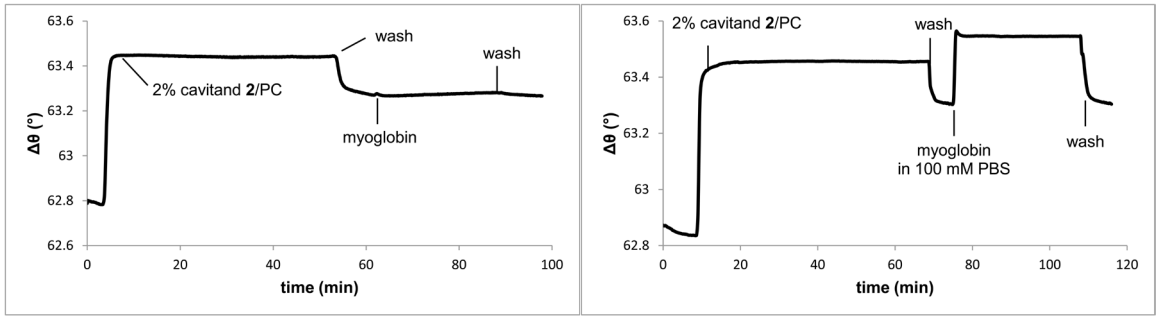


Figure *S*-8. SPR sensorgrams of the binding event between POPC:2% cavitand **2** and myoglobin in water (left) or 100 mM PBS (right).

4) Trypsin

*a. POPC:cavitand **1** bilayer*

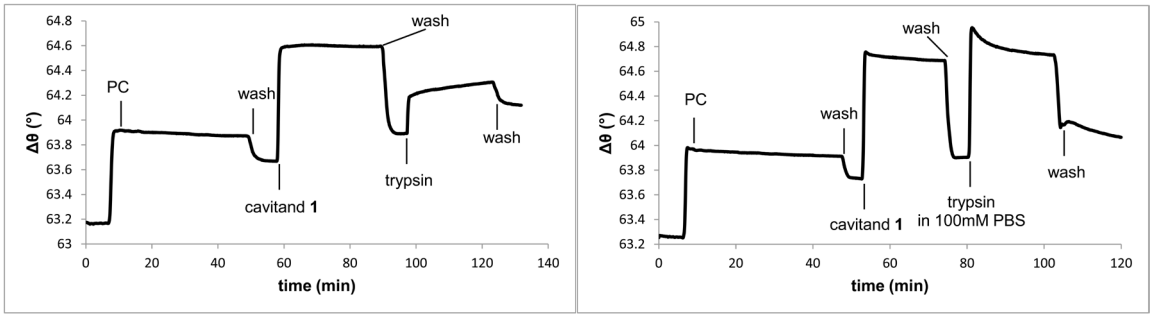


Figure *S*-9. SPR sensorgrams of the binding event between POPC:cavitand **1** and trypsin in water (left) or 100 mM PBS (right).

b. Control experiment (POPC bilayer alone)

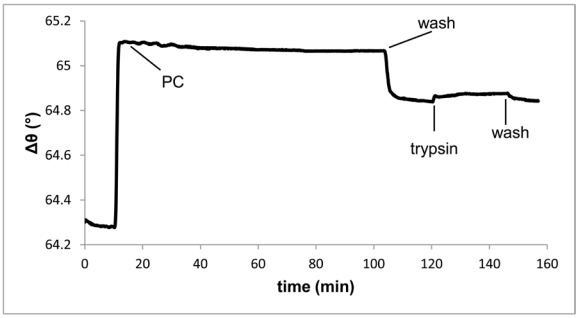


Figure *S*-10. SPR sensorgram of the binding event between POPC membrane and trypsin in water.

5) TPCK-trypsin

*a. POPC:cavitand **1** bilayer*

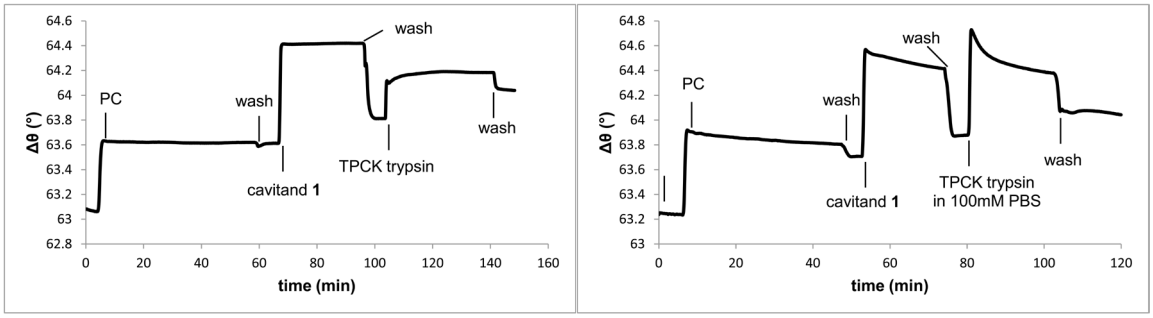


Figure *S*-11. SPR sensorgrams of the binding event between POPC:cavitand **1** and TPCK-trypsin in water (left) or 100 mM PBS (right).

b. Control experiment (POPC bilayer alone)

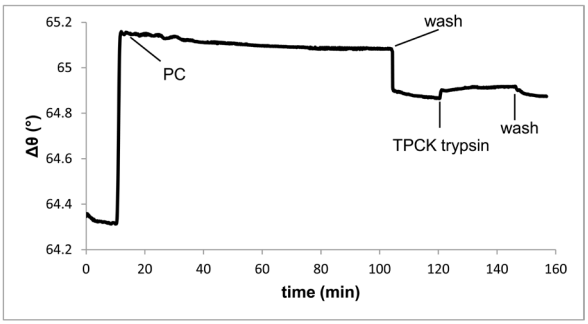


Figure *S*-12. SPR sensorgram of the binding event between POPC membrane and trypsin in water.

2. Capillary Electrophoresis Binding Analysis

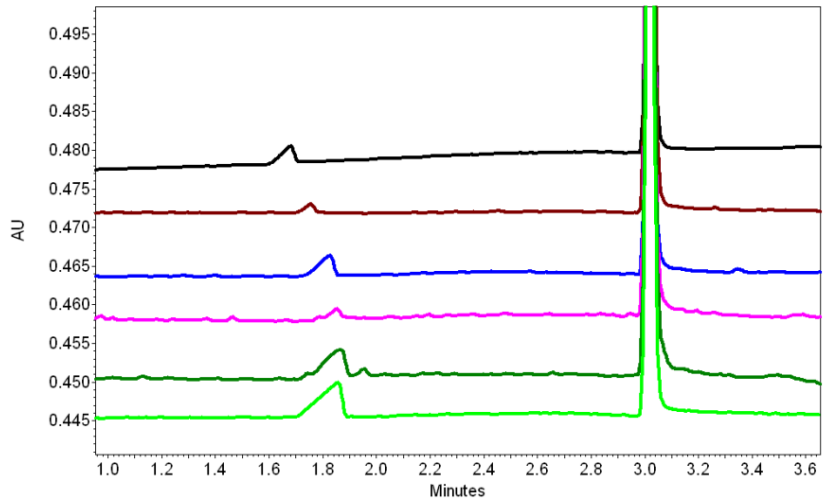
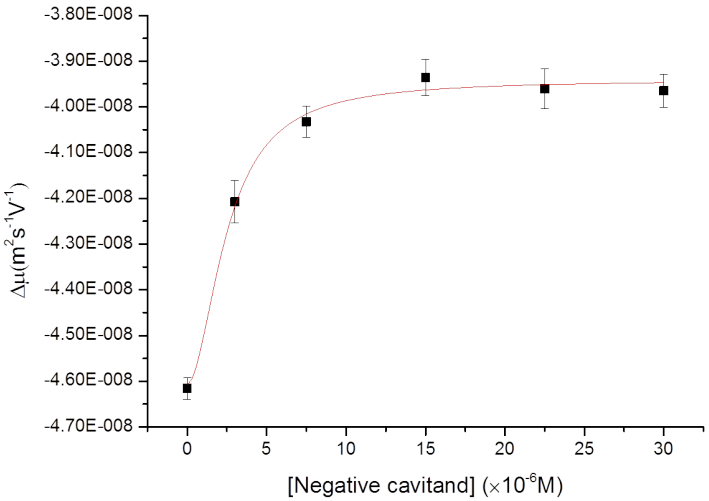


Figure *S*-13. Electropherograms for cyt *c* incubated with cavitand **1** as running buffer at 191 nm**,** [**1**] = 3-30 µM, [Cyt *c*] = 3 µM)**.**



Sample ID (Cavitand: cyt C molar ratio)	Mobility 1	Mobility 2	Mobility 3	Average Mobility	Stdev Mobility
Cyt C control	-4.64E-08	-4.59E-08	-4.62E-08	-4.62E-08	2.43E-10
3uM:3uM	-4.26E-08	-4.20E-08	-4.16E-08	-4.21E-08	4.60E-10
7.5uM:3uM	-4.03E-08	-4.00E-08	-4.07E-08	-4.03E-08	3.42E-10
15uM:3uM	-3.92E-08	-3.98E-08	-3.90E-08	-3.94E-08	3.98E-10
22.5uM:3uM	-3.98E-08	-3.99E-08	-3.91E-08	-3.96E-08	4.36E-10
30uM:3uM	-3.92E-08	-3.98E-08	-3.99E-08	-3.97E-08	3.64E-10

Figure *S*-14. Mobility shift of cyt *c* vs. [**1**] and binding constant calculation: K_d = 2.5 x 10^{-6} M.

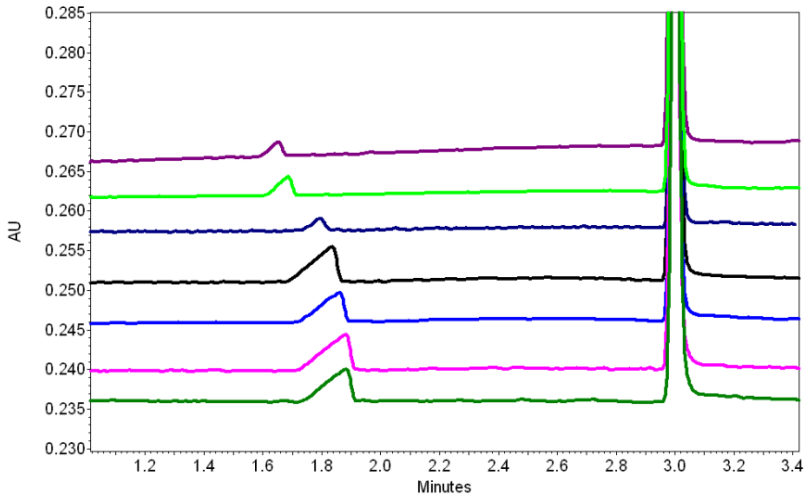
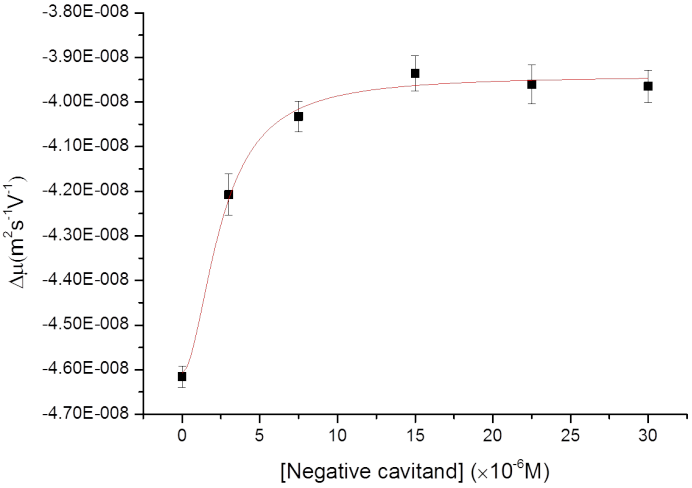


Figure *S*-15. Electropherograms for cyt *c* incubated with cavitand **1** and POPC lipids as running buffer at 191 nm, [**1**] = 3-30 μM, [Cyt *c*] = 3 μM, [POPC] = 15.8 μM)**.**



Sample ID (Cavitand: cyt C molar ratio)	Mobility 1	Mobility 2	Mobility 3	Average Mobility	Stdev Mobility
Cyt C control	-4.67E-08	-4.60E-08	-4.60E-08	-4.62E-08	4.22E-10
3uM:3uM	-4.51E-08	-4.41E-08	-4.51E-08	-4.48E-08	5.77E-10
7.5uM:3uM	-4.10E-08	-4.07E-08	-4.16E-08	-4.11E-08	4.66E-10
15uM:3uM	-3.75E-08	-3.72E-08	-3.80E-08	-3.75E-08	4.04E-10
19uM:3uM	-3.71E-08	-3.76E-08	-3.66E-08	-3.71E-08	5.00E-10
22.5uM:3uM	-3.55E-08	-3.63E-08	-3.63E-08	-3.60E-08	4.62E-10
30uM:3uM	-3.61E-08	-3.70E-08	-3.68E-08	-3.66E-08	4.73E-10

Figure *S*-16. Mobility shift of cyt *c* vs. [**1**] in the presence of POPC lipid vesicles and binding constant calculation: K_d = 7.59 x 10^{-6} M.

3. Trypsin digestion on bioreactive surface

a) Unreacted Oxidized Insulin chain B

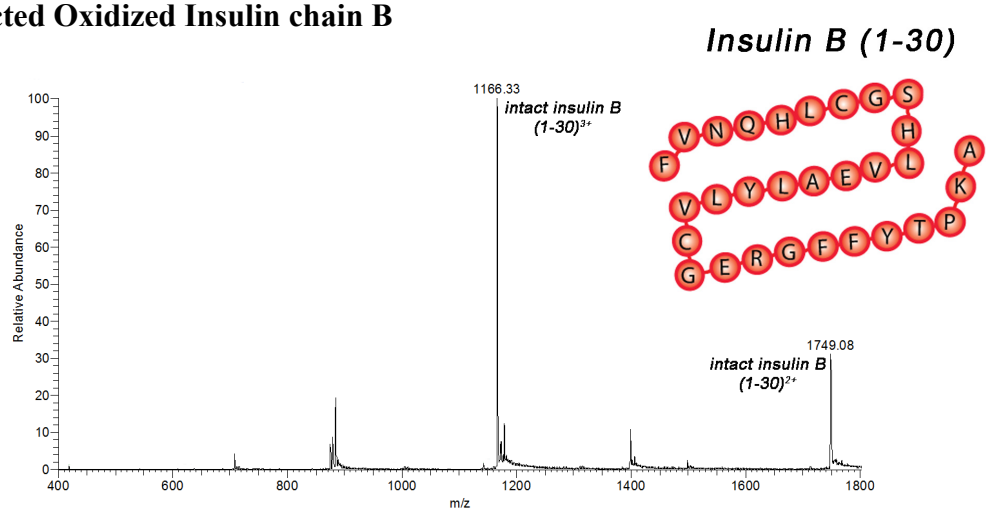


Figure *S*-17. ESI-MS analysis of oxidized insulin chain B.

b) Trypsin digestion of Insulin B in solution

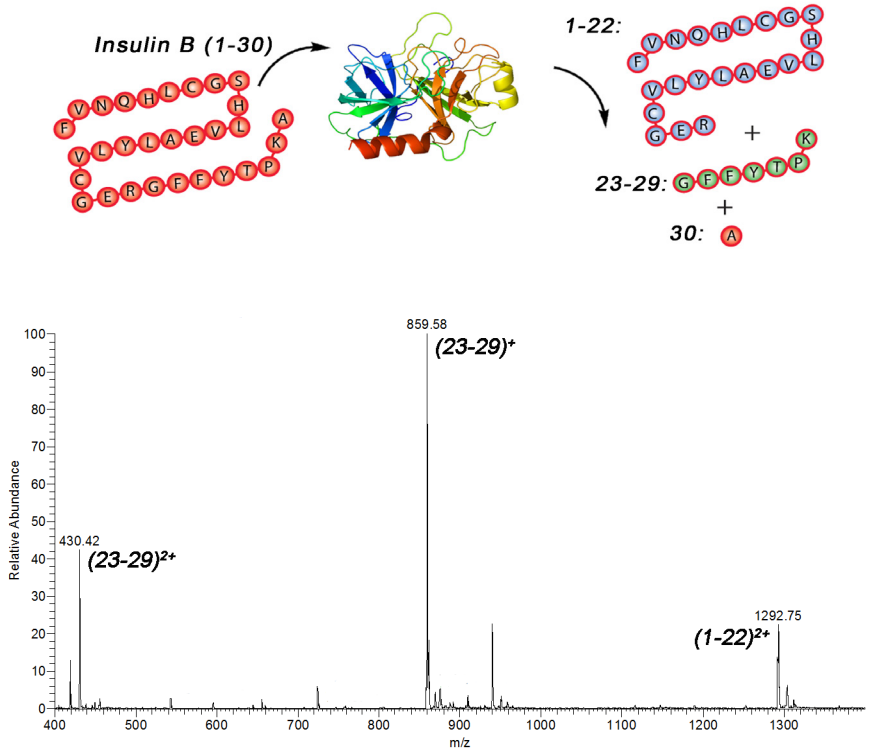


Figure *S*-18. ESI-MS analysis of trypsin (7.5 μM) digestion of insulin chain B (150 μM) for 1 h at 298 K in aqueous solution.

c) Trypsin digestion of Insulin B at the Bioreactive surface

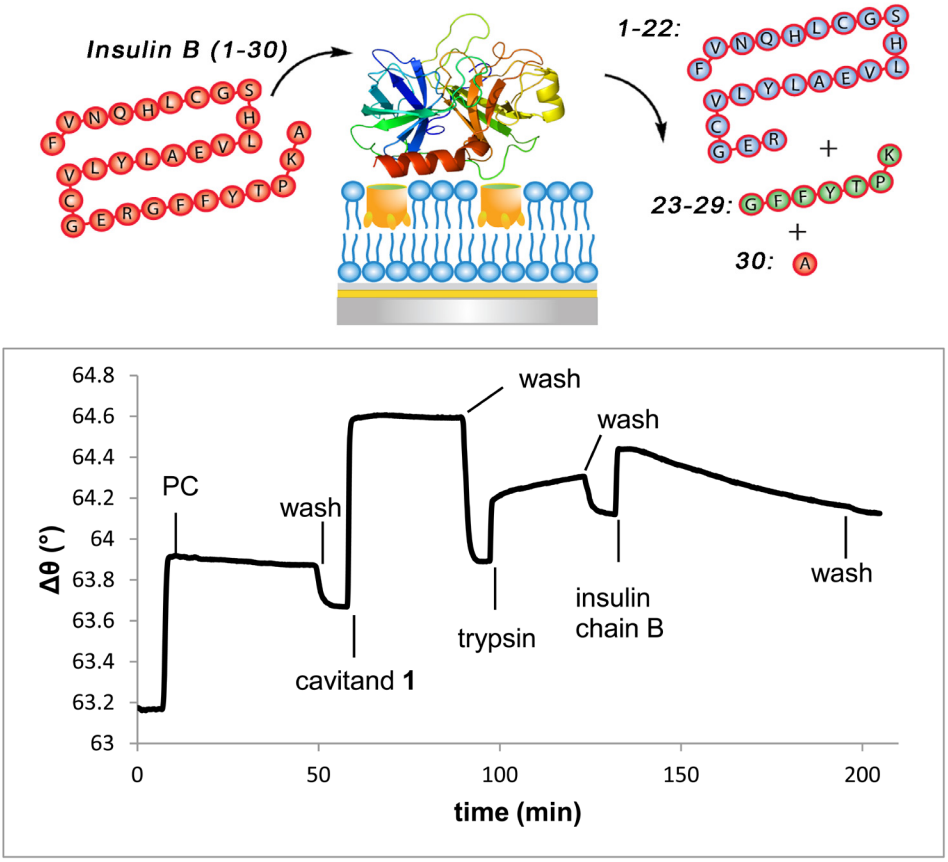


Figure *S*-19. SPR sensorgram of trypsin digestion of insulin chain B for 1 h at 298 K at the POPC:**1**:trypsin surface.

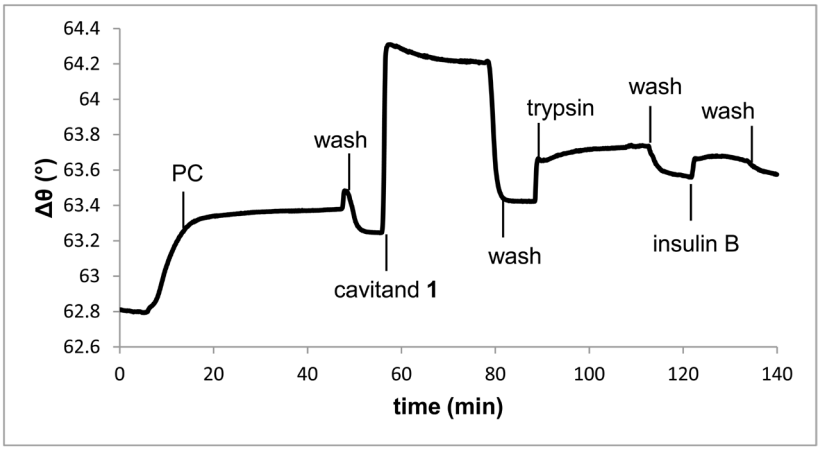


Figure *S*-20. SPR sensorgram of trypsin digestion of insulin chain B for 10 min at 298 K in SPR.

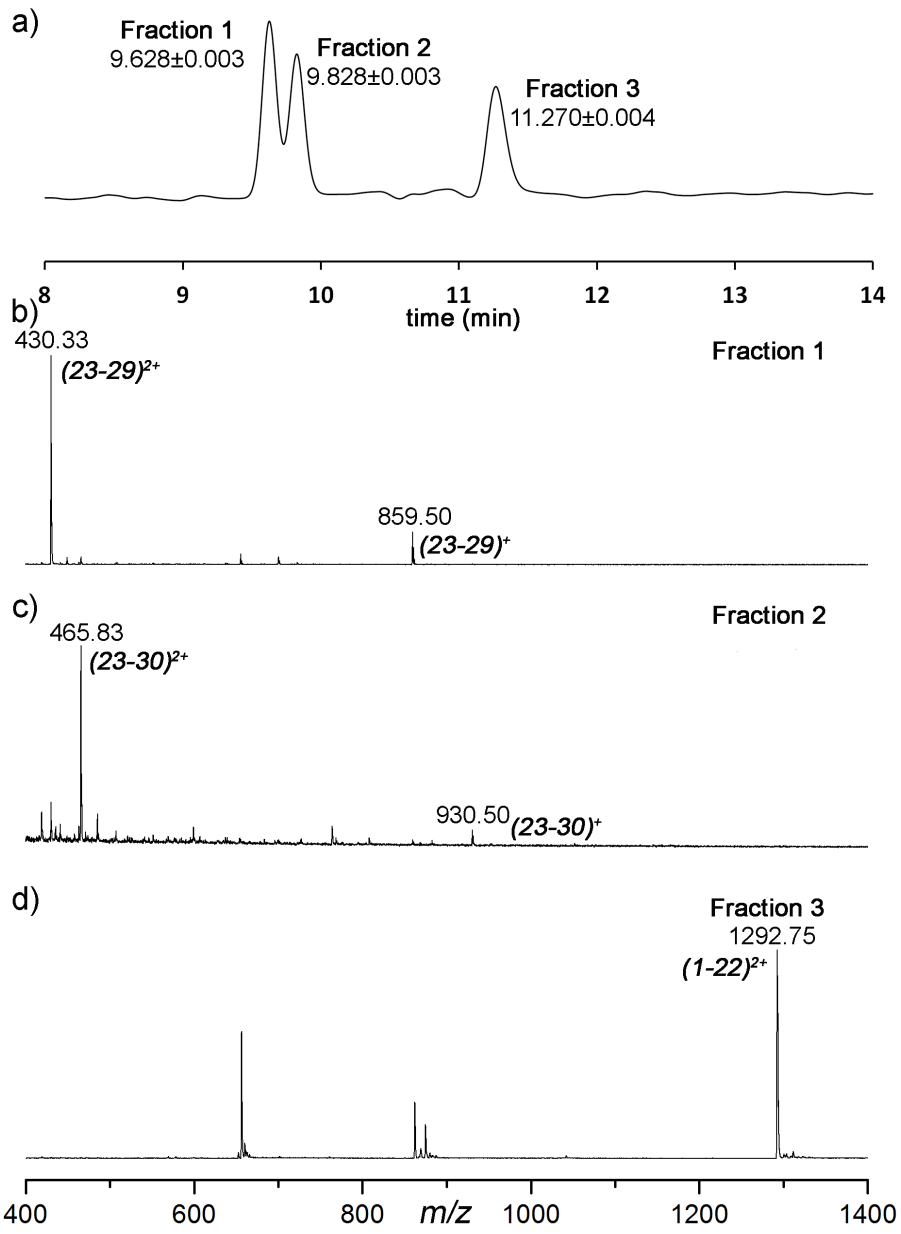


Figure *S*-21. HPLC and ESI-MS analysis of trypsin digestion of insulin chain B for 1 h at 298 K in SPR. a) HPLC-ESI mass spectra of collected fractions from HPLC: b) fraction 1; c) fraction 2; and d) fraction 3.

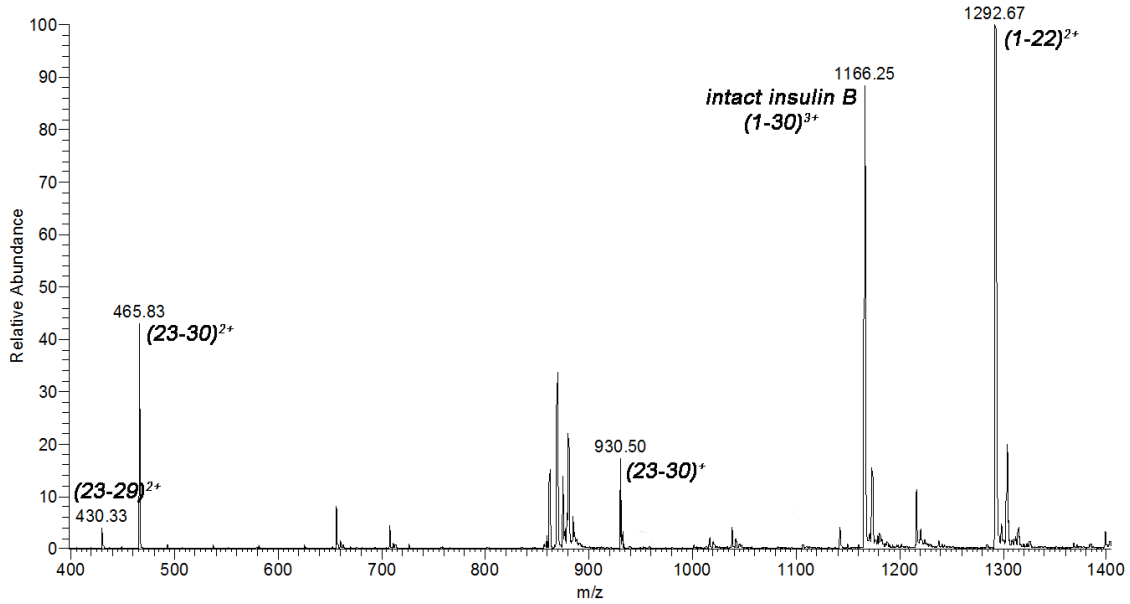


Figure *S*-22. ESI-MS analysis of trypsin digestion of insulin chain B for 10 min in SPR.

d) Surface Reusability: multiple trypsin digestions at the same surface

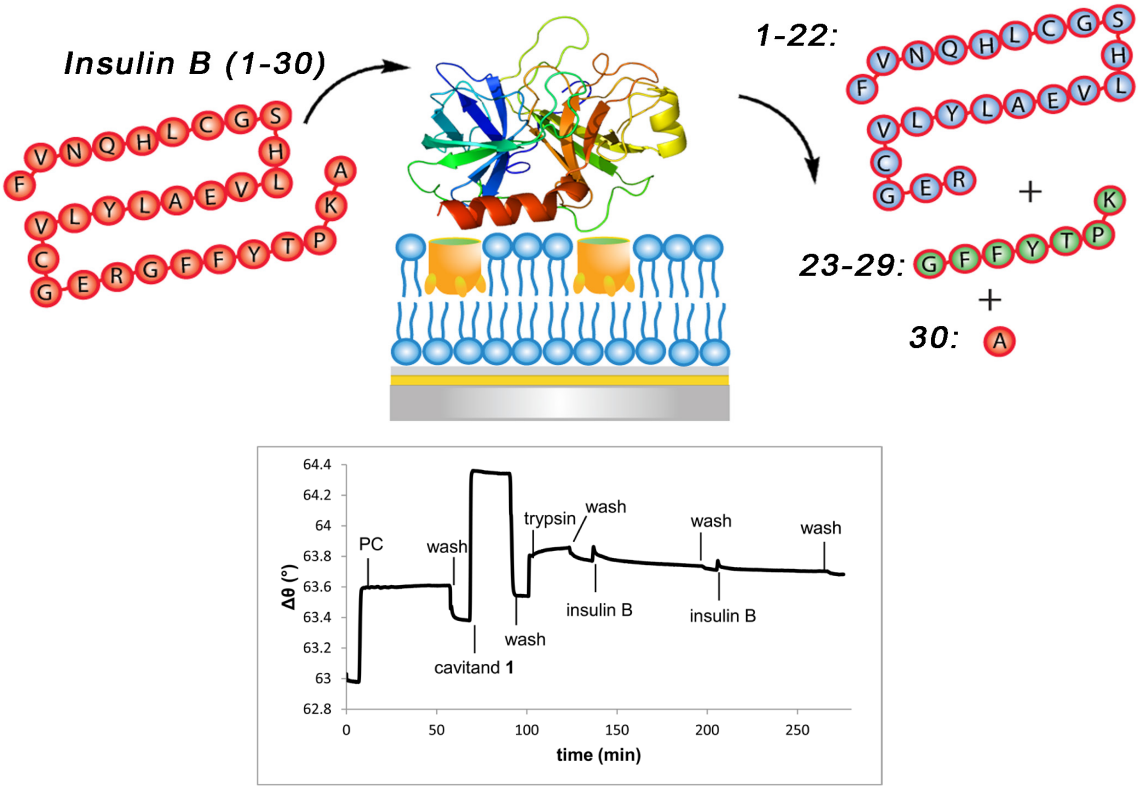
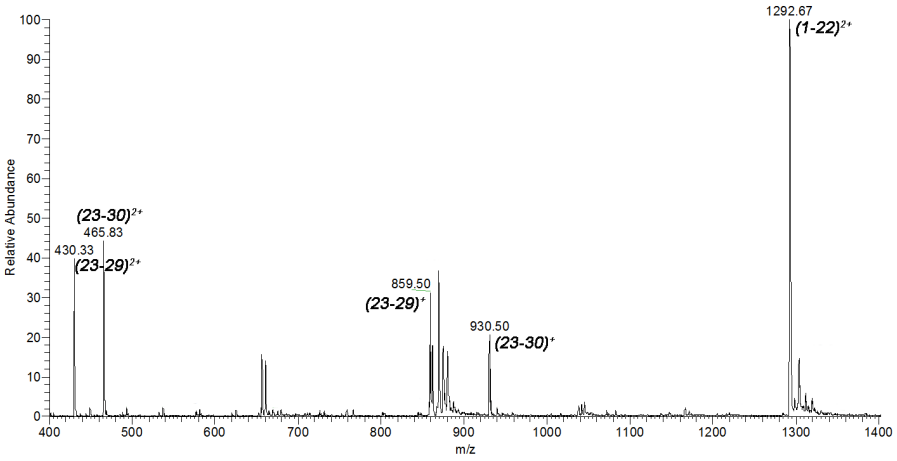


Figure *S*-23. SPR sensorgram of multiple trypsin digestions of insulin chain B for 1 h at 298 K in SPR.

a)



b)

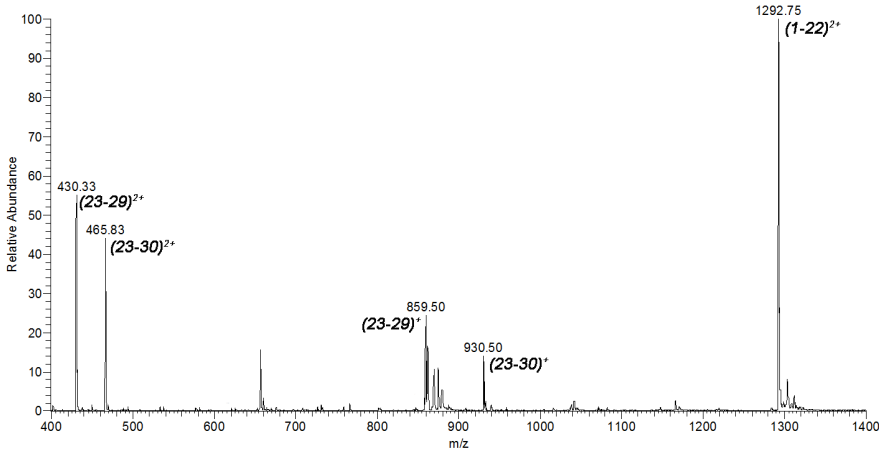


Figure *S*-24. ESI-MS analysis of multiple trypsin digestions of insulin chain B for 1 h at 298 K in SPR: a) first digestion, b) second digestion.

e) Inhibition of trypsin digestion by addition of benzamidine hydrochloride

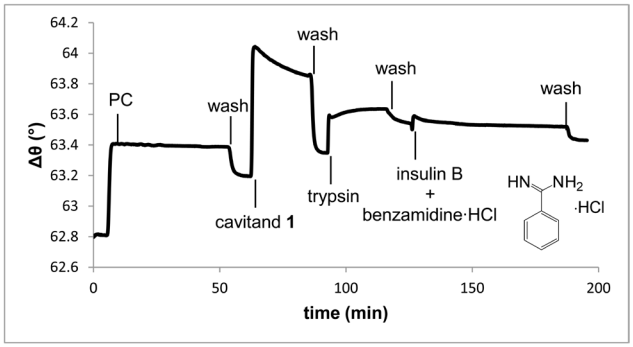


Figure *S*-25. SPR sensorgram of trypsin digestion of insulin chain B with 100 mM benzamidine hydrochloride for 1 h at 298 K in SPR.

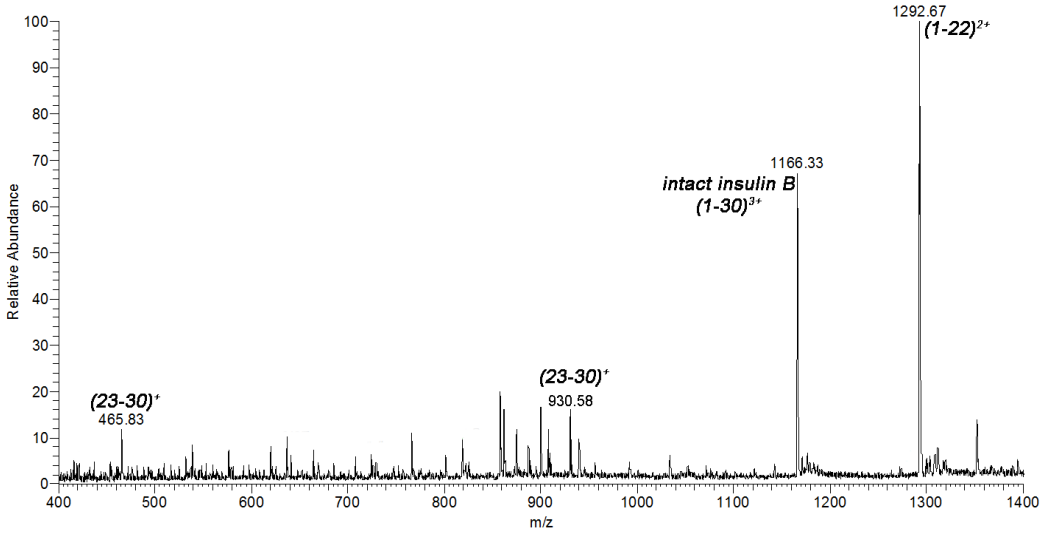


Figure *S*-26. ESI-MS analysis of trypsin digestion of insulin chain B with 100 mM benzamidine hydrochloride for 1 h at 298 K in SPR.

f) TPCK-trypsin digestion

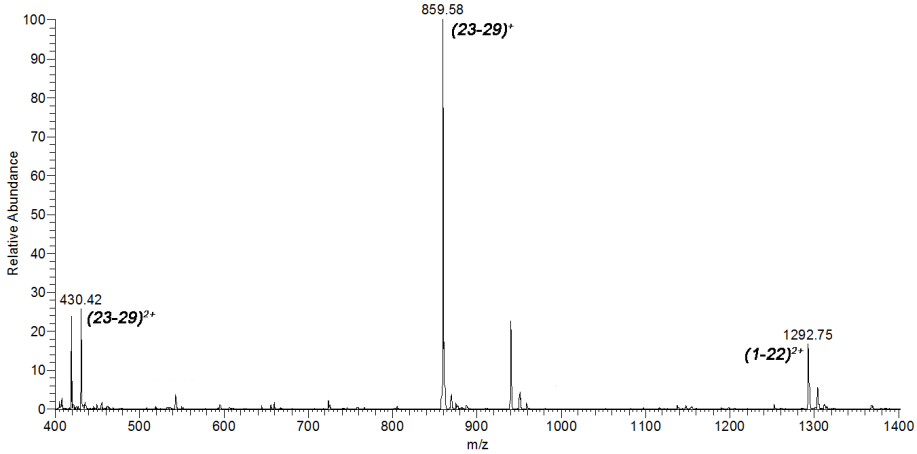


Figure *S*-27. ESI-MS analysis of TPCK-trypsin (7.5 μM) digestion of insulin chain B (150 μM) for 1 h at 298 K in aqueous solution.

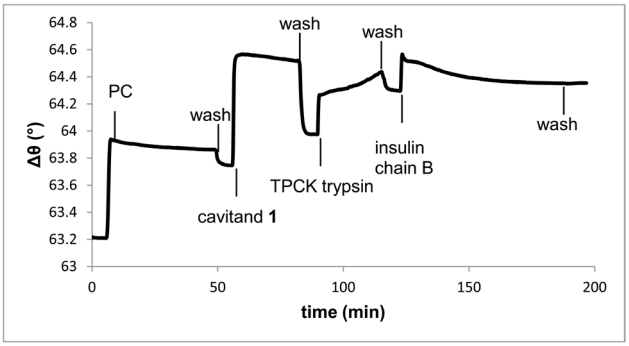


Figure *S*-28. SPR sensorgram of TPCK-trypsin digestion of insulin chain B for 1 h at 298 K in SPR.

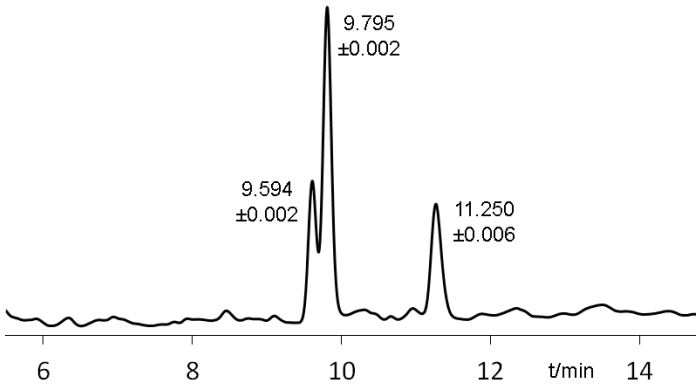


Figure *S*-29. HPLC analysis of TPCK-trypsin digestion of insulin chain B for 1 h at 298 K in SPR.

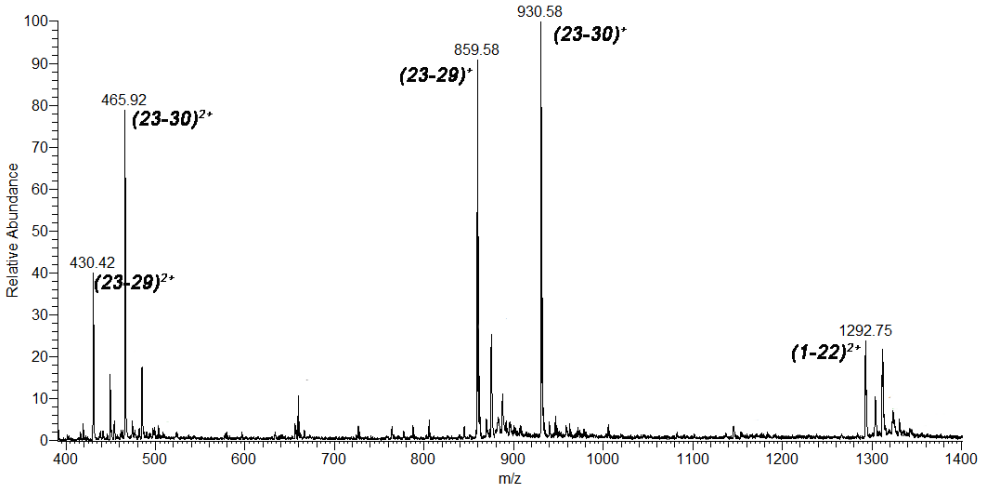


Figure *S*-30. ESI-MS analysis of TPCK-trypsin digestion of insulin chain B for 1 h at 298 K in SPR.

4. CD Analysis of injected protein structure

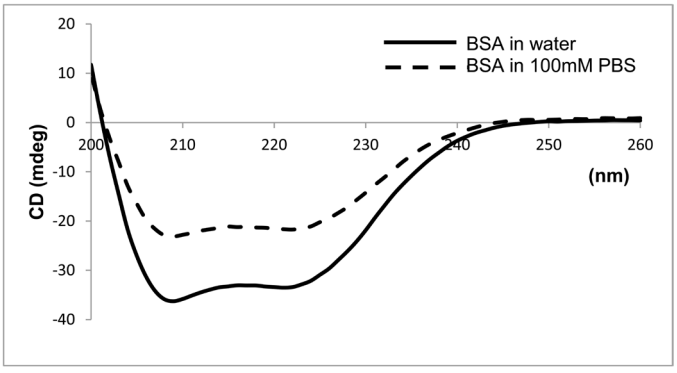


Figure *S*-31: CD spectrum of 2 µM BSA in water (solid line) or 100 mM PBS (dotted line).

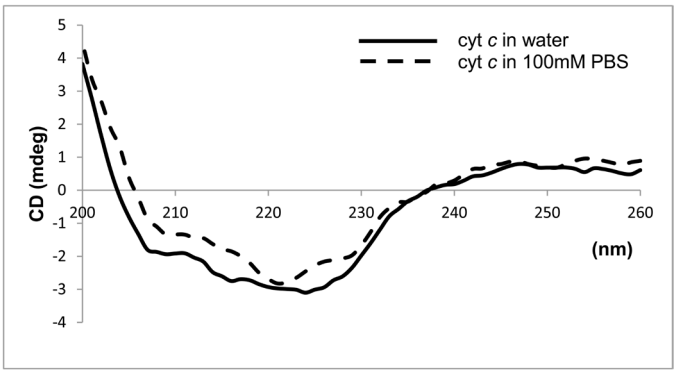


Figure *S*-32: CD spectrum of 2 μM cyt *c* in water (solid line) or 100 mM PBS (dotted line).

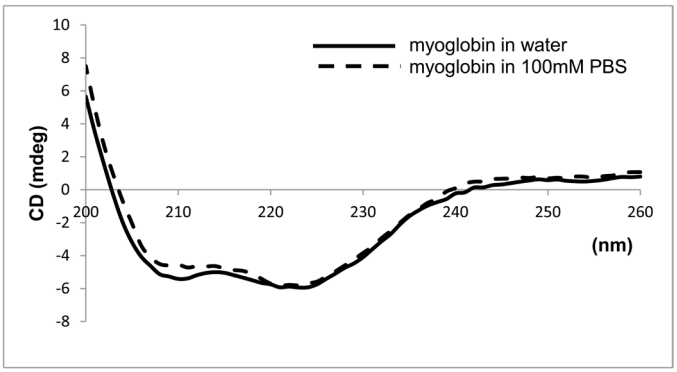


Figure *S*-33: CD spectrum of 2 μM myoglobin in water (solid line) or 100 mM PBS (dotted line).