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).](image)
b. POPC:cavitand 2 bilayer

![Figure S-2](image)

**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](image)

**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](image)

**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.](image)

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).](image)

b. **Control experiment (POPC bilayer alone)**

![Figure S-7. SPR sensorgram of the binding event between POPC membrane and myoglobin in water.](image)
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 sensorogram 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.

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

Figure S-16. Mobility shift of cyt c vs. [1] in the presence of POPC lipid vesicles and binding constant calculation: $K_d = 7.59 \times 10^{-6}$ M.
3. Trypsin digestion on bioreactive surface

a) Unreacted Oxidized Insulin chain B

![Figure S-17. ESI-MS analysis of oxidized insulin chain B.](image)

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.](image)
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.
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.
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).