SPR imaging analysis of protein-receptor binding in supported membrane arrays on gold substrates with calcinated silicate films

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Supporting information

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Materials:
NBD-PC, or 1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)-amino]hexanoyl]-sn-glycerol-3-phosphocholine, was obtained from Avanti Polar Lipids.

Vesicle Preparation:
Stock solutions of lipids in chloroform were evaporated in a nitrogen stream, followed by resolubilization in Tris buffer (10 mM with 150 mM NaCl, pH 7.4) and probe sonication for 15 min. The resulting vesicles were purified by centrifugation and extrusion as necessary and incubated at 4° for 1 h before use.

Silicate layer preparation:
Clean gold SPR substrates were immersed in 3-MPA solution (10 mM) overnight, then rinsed well with ethanol and water and placed in a vertical position. Poly(allylamine) hydrochloride (PAH, 1 mg/mL in D.I. water, adjusted to pH 8.0) and sodium silicate solution (22 mg/mL, adjusted to pH 9.5) were sequentially sprayed on the substrates using Nalgene hand-pumped bottles from Fisher. For each layer, 30 s was allowed before rinsing with water. The chips were dried with nitrogen and calcinated in a furnace with an initial heating rate of 15° per min and a final temp of 450° C for 4 h.

Photolithographic patterning:
The calcinated surfaces were cleaned in an equal volume mixture of acetone and detergent, followed by copious rinsing with D.I. water and drying in a N_2 stream. The surface was covered with a thin layer of photoresist and spun on at 3500 rpm, soft-baked on a hot plate for 1 min, covered by a photomask, and UV exposed for 14 s. The pattern was developed for 70 s with mild agitation and post-baked for 3 min, followed by etching in 1:360 dilution of concentrated HF with gentle agitation. The chip was then rinsed with water and the remaining photoresist was removed with acetone and rinsed with water. Photomasks were made using either a laser-printed transparency (600 dpi) or with TEM grids to demonstrate higher resolution pattern fidelity and dense arrays.

Atomic Force Microscopy (AFM):
Clean and dry SPR slides (2 cm x 2 cm) were placed on an AFM (Novascan) sample holder. The AFM tip was positioned over the region of interest with the aid of a microscope. The samples were scanned via contact mode at a scan rate of 1 Hz, and a scan resolution of 400 x 400. The scanned area was an 80 µm x 80 µm region. Best images were obtained when the set point was set at -0.1 V.

SPR measurement:
The SPR measurement of CT/GM1 interaction was carried out at a near equilibrium state. During CT sample injection, the flow in the chamber was stopped when the concentration of CT reached its maximum value. A 12 min incubation period without flow followed. The flow was then resumed, followed by rinsing with a Tris buffer solution.

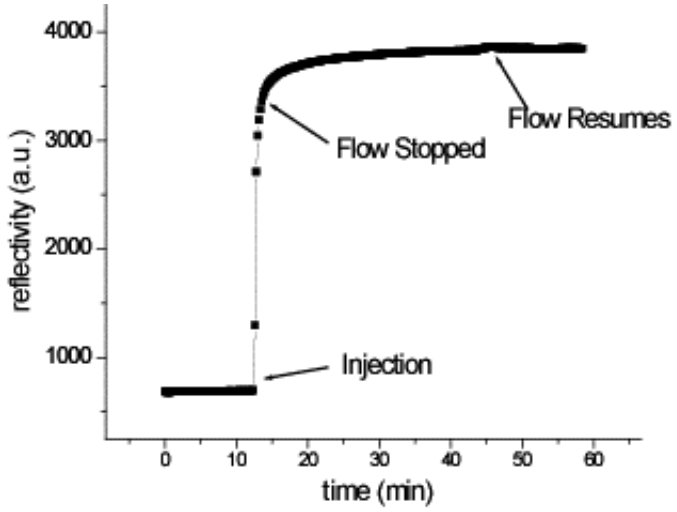

Figure S1. Vesicle fusion in the nanowells tracked by SPRi.

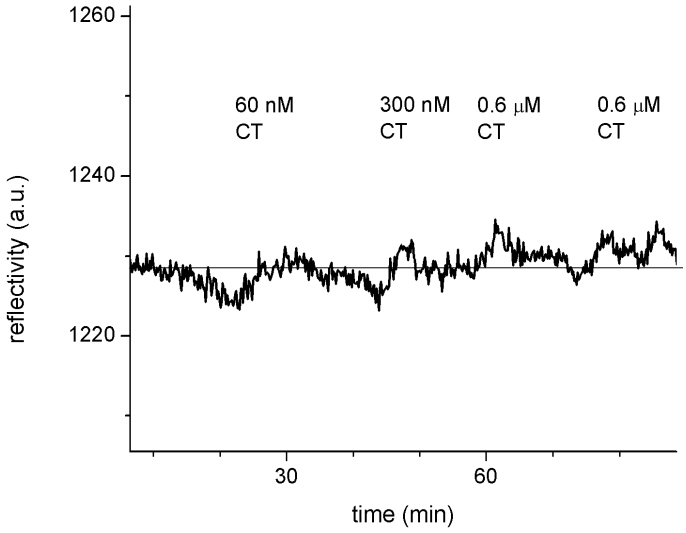

Figure S2. SPR reflectivity for control experiment in which CT was injected over an etched silicate substrate with egg PC supported membranes.