Supporting Information

Biomimetic sensing based on chemically induced assembly of a signaling DNA aptamer on a fluid bilayer membrane

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Structure of lipids used in this study:

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide] (sodium salt) (MPB-PE)

Experimental Section

Chemicals: All of the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA). Phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). Chloroform was purchased from VWR. Tris(2-carboxyethyl)phosphine (TCEP) and maleimidobutyryloxy-sulfosuccinimide ester (Sulfo-GMBS) were purchased from Sigma, and 100 nm silica nanoparticles modified with NH₂ were purchased from Kisker Biotech GmbH & Co. KG. Sodium chloride, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and adenosine and other nucleotides were purchased from Mandel Scientific (Guelph, Ontario, Canada). Milli-Q water was used for all of the experiments.

Preparation of liposomes: Liposomes were prepared by the standard extrusion method. DOPC and MPB-PE (or DPPC and MPB-PE) were mixed in chloroform at a weight ratio of 20:1 with a total lipid mass of 2.5 mg. The chloroform was removed under a gentle N₂ flow in the fume hood and trace amounts of residual chloroform was then removed by storing the samples in a vacuum oven overnight at room temperature. The lipids were protected under a N₂ environment when taken out of the vacuum oven, and were stored at -20 °C prior to use. To prepare the liposomes, the lipids were rehydrated with 0.5 mL of 150 mM NaCl, 25 mM HEPES, pH 7.6 at room temperature with occasional sonication.
Therefore, the lipid concentration was 5 mg/mL. After the lipids were fully hydrated for ~1 hr, a cloudy lipid suspension was obtained. This lipid was extruded through a polycarbonate membrane (100 nm pore size) with two syringes 21 times. To prepare DPPC liposome, the hydration and extrusion steps were performed at ~50 °C.

**DNA conjugation to liposome:** Each thiol modified DNA (10 µL, 100 µM) was activated by 1 µL TCEP (10 mM) and 1 µL of acetate buffer (400 mM, pH 5) for 1 hr at room temperature. In a typical reaction, 40 µL of the freshly extruded liposome, 2.5 µL of activated FAM DNA and 5 µL of activated TMR DNA were added and incubated at 4 °C overnight. The next day 20 mM NaCl, 10 mM HEPES, pH 7.6 (buffer A) was added to achieve a final volume of 200 µL and the sample was centrifuged at 120000 rpm for 4 hrs at 5 °C. The pellet was dissolved in 200 µL of buffer A.

**DNA conjugation to silica nanoparticles and analysis:** 3.82 mg of Sulfo-GMBS was dissolved in 1 mL of 5 mM HEPES buffer and to this 100 µL of 25 mg/mL of amino-modified silica nanoparticles were added. After 30 min, the sample was washed three times using 5 mM HEPES and dispersed in a final volume of 32.9 µL (silica nanoparticle concentration = ~100 nM). After incubating with activated DNAs at 4 °C overnight, this sample was washed several times to remove free DNA with 5 mM HEPES (8000 rpm for 8 min for each washing). For the fluorescence study, 30 µL of the washed sample was diluted with buffer A to a final volume of 600 µL.

**Steady-state fluorescence studies:** The steady state fluorescence studies were performed using a Varian Carey Eclipse fluorescence spectrophotometer in a quartz cuvette. 20 µL of the above prepared liposome sample was diluted to a final volume of 600 µL in buffer A in the cuvette. The sample was then excited at 485 nm and the emission from 500 nm to 600 nm were recorded.

**Dynamic light scattering**

1 mL of the freshly extruded liposome sample was dispersed in buffer A (concentration = ~0.2 mg/mL DOPC). The hydrodynamic liposome size was determined to be 142.2 nm using dynamic light scattering (Zetasizer Nano, Malvern). Therefore the physical size of liposome was ~140 nm. As shown in Figure S1, the size distribution was relatively narrow.
Figure S1. DOPC liposome size measured using dynamic light scattering. The average hydrodynamic size was determined to be 142.2 nm.

Figure S2. Determination of the coupling efficiency. The liposome samples were purified by ultracentrifugation and the supernatant and liposomes were dissolved in the same volume of buffer. The left panel was the FAM DNA (excited at 485 nm) and the middle panel was the TMR DNA (excited at 540 nm). In the right panel, 10× more liposomes were used.

**Coupling efficiency**

With a concentration of 5 mg/mL, DOPC liposome has a concentration of 31 nM at 140 nm. If the supernatant fluorescence (after centrifugation) and liposome fluorescence was compared (Figure S2), the coupling efficiency can be determined. We determined the coupling efficiency to be 38% for the FAM-labeled DNA and 39% for the TMR-labeled DNA. The initial FAM DNA concentration was 1.86 µM and the TMR DNA concentration was twice of that the FAM DNA. Therefore each liposome has
~60 FAM-labeled DNA and 120 TMR-labeled DNA. In one sample, ten times more liposome was used but the concentration of the DNA was the same. In this case, the coupling efficiency was also increased to ~76% and therefore, the DNA density was diluted by five times.

**Effect of the TMR-labeled DNA concentration.**

In FRET-based signaling, the amount of signal change can be tuned by using different donor to acceptor ratios. In this work, we chose to use a FAM-DNA : TMR-DNA = 1:2 to achieve effective quenching. This ratio is important in sensor performance and its effect has been systematically studied. We fixed the concentration of the FAM-DNA, and varied the TMR-DNA to achieve four ratios. The response of these liposomes to adenosine is shown in Figure S3A. Adenosine could be detected under all the conditions and the performance appears to be better with increasing concentrations of the TMR-DNA. While the signal increased, the initial background also increased (Figure S3B). We chose to use a 2:1 ratio since it has an optimal signal change in terms of FAM fluorescence quenching (Figure S3C). Further increase of TMR had a relatively small effect on the sensor performance.

**Figure S3:** Several liposome systems were made with 40 µL of liposome with addition of 4.63 µM FAM-DNA and the different amounts of TMR-DNA. (A) Detection of adenosine using these liposomes. (B) The initial fluorescence ratio as a function of TMR/FAM ratio. (C) The quenching of the FAM 520 nm emission intensity with addition of 2 mM adenosine.

**Fluorescence lifetime.**

To understand the dynamics of DNA on the bilayer membrane, we carried out fluorescence lifetime studies. We prepared FAM-DNA labeled and also dual-labeled liposomes in the presence or absence of 2 mM adenosine. All of the samples showed very similar fluorescence lifetime decay (Figure S4),
indicating little dynamic quenching or FRET occurred in the whole process. For example, the FAM DNA alone labeled liposome showed a lifetime of 4.05 ns; this lifetime dropped by ~5% to 3.85 ns for the dual labeled liposome. This is consistent with our previous steady-state results that little FRET occurred and there was little interaction between the DNAs. After adding 2 mM adenosine, the lifetime barely changed. Therefore, the ~40% quenching in the steady state can only be attributed to static quenching. The lifetime result is also consistent with the fact that little acceptor fluorescence increase was observed in Figure 2A. The lifetime data indicated that the aptamer/adenosine complex was stable in the excitation lifetime of the FAM fluorophore (e.g. at least for several nanoseconds).

![Fluorescence lifetime traces of the split aptamer probe on DOPC liposome.](image)

**Figure S4.** Fluorescence lifetime traces of the split aptamer probe on DOPC liposome. Three types of conditions were tested.

**Signaling kinetics**

To the quartz cuvette 20 μL of the sample was added to 580 μL of buffer A. The instrument was used in the kinetic mode with excitation wavelength set at 485 nm and emission wavelength set at 520 nm. Fluorescence intensity at 520 nm was measured every 5 sec. Adenosine was added after the 5 min of ever cycle up to 2 mM adenosine. Addition of adenosine was accomplished by pressing pause and then resuming the scan. The results are shown in Figure S5. The signal change was instantaneous upon adenosine addition and the system can reach equilibrium very quickly.
Figure S5: A sample made-up of 40 µL of liposome was treated with 2.5 µL of activated FAM DNA and 5 µL of activated TMR DNA. Initially in the first 5 min no adenosine was added. In the 5 min cycles 0 µM, 100 µM, 500 µM, 1 mM and 2 mM of adenosine was added successively, respectively.

Other control experiments

Figure S6A shows the fluorescence spectra of the split aptamer sensor attached to DOPC liposome upon addition of cytidine. Only a slight decrease due to dilution was observed. Figure S6B is a FAM-labeled DNA functionalized DOPC liposome (no TMR-labeled DNA), showing little fluorescence drop after adding adenosine. Therefore, the adenosine induced fluorescence quenching for the split aptamer sensor was due to aptamer assembly instead of artefacts.

Figure S6. (A) Split aptamer sensor titrated with cytidine. (B) A control experiment using FAM-DNA singly labeled DOPC liposome.
Effect of using lower liposome concentrations.

After reducing the liposome concentration four times, the adenosine titration experiments were performed. As shown in Figure S7, we obtained very similar detection limit (70 μM) and apparent $K_d$ (1.6 mM). Therefore, in our liposome system, the concentration of aptamer was required only for detection. In theory, even a single liposome should also work the same way as long as it can be detected.

**Figure S7.** Fluorescence spectra (A) and fluorescence ratio (B) of split aptamer functionalized liposome titrated with adenosine at low liposome concentration. Inset: the low adenosine region.

**Figure S8.** Fluorescence ratio of split aptamer functionalized liposome with a 5x diluted aptamer density titration with adenosine.
Split aptamer sensor with reduced probe concentration

If the liposomes were incubated with just 20% of normal DNA (i.e. the DNA density was reduced on liposome), an adverse effect on binding was observed (Figure S8). Its $K_d$ was $\sim$9 mM, which was six times higher than the higher DNA density sample.