Conjugate PCDA to aptamer

"The aptamers were conjugated to the pure PCDA vesicles by using the carbodiimide method in the presence of the EDC and the NHS (Figure 1). Briefly, 6.25 µl of NHS (4 mM), 6.25 µl of EDC (4 mM) and 50 µl of pure PCDA (1 mM) vesicle were mixed in 187.5 µl of deionized water at room temperature. After stirring the mixtures for 2 h to activate carboxy, 10 µl (0.5 mM) DNA (E17F-37, E18R-42 or random DNA) were added into the mixed solution. After incubating the mixtures at room temperature overnight, the aptamers or random DNA were conjugated to the pure PCDA vesicles. Unbound aptamers were removed by dialysis with excess deionized water. The final volume was adjusted to 0.5 ml by adding deionized water and PCDA concentration was approximately 0.1 mM."

1. Mix at room temperature (V_{tot} = 250 µL)
   - 6,25 µL of 4 mM NHS
   - 6,25 µL of 4 mM EDC
   - 50 µL 1 mM PCDA
   - 187,5 µL dH2O
2. Stir mixture for 2h
3. Add 10 µL of 0,5 mM DNA (Aptamer)
4. Incubate overnight at room temperature

Alternative protocol: (Tested on 26-27.07.21)

1. Mix at room temperature (Vtot = 250 µL):
   - 6,25 µL of 4 mM NHS
   - 6,25 µL of 4 mM EDC
   - 237.5 µL 1 mM PCDA
2. Stir mixture for 2h
3. Add 10 µL of 0,5 mM DNA (Aptamer)
4. Incubate overnight at room temperature

Other alternative protocol: (Tested on 10-11.09.21)

1. Mix at room temperature (V_{tot} = 250 µL)
   - 25 µL f 4 mM NHS
   - 25 µL of 4 mM EDC
   - 200 µL 1 mM PCDA
2. Stir mixture for 2h
3. Add 10 µL of 0,5 mM DNA (Aptamer)
4. Incubate overnight at room temperature

Removing unbound aptamers with dialysis [1], [2]

This step depends on the size of the PDAs: if they are in vesicle form, then dialysis can be used as the PDA-conjugated aptamers are significantly larger than the non-conjugated apramers.

0,5 mL Dialysis 20K MW Cut-Off cassette

G2 Dialysis Cassette (Manual)

Use a 200 µL gel loading pipette tip to load the sample, or a syringe.

The total sample volume can be up to 0.5 mL since the sample density is well under the threshold of 1.150 g/mL that allows this.
Our sample density is 4.24 mg/mL (Taking into account NHS, EDC, PCDA and DNA).
The minimum sample volume is 0.350 mL.

A. Hydrate Membrane

1. Remove the cassette from its protective pouch. To prevent membrane contamination, handle the cassette by the plastic frame only. Do not touch the membrane with ungloved hands. The cassette may be placed upright on its bottom end on a flat surface.
2. Immerse the cassette in dialysis buffer for 2 minutes (Figure 1). It may be necessary to hold the cassette under the surface for the hydration step as the air inside the cassette may cause it to float sideways.
   Note: Hydration increases membrane flexibility and allows it to adjust more readily to the sample loading and removal of excess air.
3. Remove cassette from buffer. To remove excess buffer, gently tap the cassette on a paper towel. Turn the cassette upside down and tap again. Do not blot the membrane.

B. Add Sample

Note: When removing excess air by hand, the minimum sample volume required for the 3, 15, 30 and 70mL cassette is approximately ½ of the cassette's maximum volume. For the 0.5mL cassette, the minimum sample volume is > 350µL.

1. Open the cassette by gently twisting the cap counter-clockwise until it stops (~45º angle) and then gently pulling out the cap (Figure 2).
2. Using appropriate device (refer to the Important Product Information Section) add the sample to the cassette, slowly withdrawing the pipette while dispensing. Do not overload the cassette (Figure 3).
   Note: To load the cassette, insert pipette fully into the device and slowly remove pipette while filling. Repeat as needed.
3. Remove the excess air in the cassette by simultaneously pressing the membrane gently on both sides using your gloved thumb and forefinger and inserting the cap (Figure 4).
4. Insert cap and lock by gently twisting it clockwise (Figure 5).

C. Dialyze Sample

1. Float cassette vertically in the dialysis buffer and stir gently to avoid creating a vortex that might pull the cassette down in contact with the stir bar.
2. Dialyze for an amount of time sufficient to remove low molecular weight compounds for the specific downstream application. A typical dialysis procedure is as follows: dialyze for 2 hours at room temperature or 4ºC; change the dialysis buffer and dialyze for another 2 hours; change the dialysis buffer and dialyze overnight. Use the dialysis buffer at a total of at least 300 times the volume of the sample during the course of the dialysis procedure.

D. Remove Sample

Note: If the cassette is swollen recover the sample using a syringe and as described in the Procedure for using a Syringe with the G2 Dialysis Cassette, Section D. Opening a swollen cassette will cause the sample to rise into the loading port and a portion may be lost.

1. Remove cassette from buffer. To remove excess buffer, gently tap the cassette on a paper towel. Turn the cassette upside-down and tap again. Do not blot the membrane.
2. Open the cassette by gently turning the cap counter-clockwise until it stops (~45º angle) and then gently pulling out the cap (Figure 2).
3. Using an appropriate sized serological pipette, retrieve the sample by slowly aspirating while inserting pipette toward the bottom of the cassette (Figure 6).