Last Update: March 22, 2023

Ref: 1) Jing Pan, Yancheng Du, Hengming Qiu, Luke R. Upton, Feiran Li, and Jong Hyun Choi, *Nano Letters* **2019** *19* (12), 9138-9144, DOI: 10.1021/acs.nanolett.9b04428

2) Hengming Qiu, Feiran Li, Yancheng Du, Ruixin Li, Ji Yeon Hyun, Sei Young Lee, and Jong Hyun Choi, *ACS Synthetic Biology* **2021** *10* (6), 1268-1276, DOI: 10.1021/acssynbio.0c00550

Azide-DNA Synthesis (for small unilamellar vesicle synthesis)

1. Mix approximately 20 µl 1 mM amine-DNA (dissolved in deionized or DI water, stored in -20 °C), 39 µl 60 mM azide-$(PEG)_4$-NHS (dissolved in dimethylformamide or DMF, stored in -20 °C), 40 µl DMF and 1 µl TEA (Triethylamine), mix with pipette and react for two hours.

- Leave it at room temperature in the dark.
- A large container (preferably thermocol) with a lid can be used to block light from entering.


Thermocol Box with lid

2. Add approximately 200 µl ethanol and 7.5 µl 4 M NaCl solution to the mixed solution, mix with pipette and put in a refrigerator at -20°C for 30 minutes.

3. Centrifuge the solution at 20,000 g for 30 minutes.

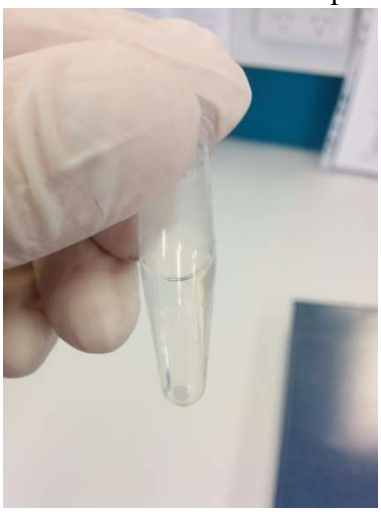
- Centrifugation can be done at the highest RPM (15000 RPM) configuration.
- Use Eppendorf tubes for this centrifugation. **Do not use filter tubes**.



Eppendorf tube (√) Filter tube (filter separated) (X) Eppendorf tube and filter tube

4. Remove the supernatant and add 200 µl ethanol to resuspend the precipitant.

- Be sure to do this immediately after the centrifugation is over. Do not let the sample sit as it may start dissolving again.
- After centrifugation, a precipitate should be observed. This is the sample we desire for this step.
- While removing the supernatant, do not disturb the precipitate. Leaving a small amount of supernatant is okay.
- After adding ethanol, pipette it for some time so that the precipitate resuspends in ethanol.



Example of DNA pellet in solution

5. Centrifuge the solution (precipitate resuspended in ethanol) at 20,000 g for 10 minutes.

*Step 4-5 may be repeated for 2-3 times for better purification.

6. Remove the supernatant and dry the precipitant with a vacuum desiccator.

- For using the vacuum desiccator, use a needle to punch a hole in the cap of the Eppendorf tube and then put this tube on the tube holder and put into the vacuum desiccator for 15-20 minutes.
- The machine has a pipe connected to a pump. Push the power button near the pump after aligning the lid with the base.
- After a few seconds, you can try to gently lift the lid. The lid gets lifted along with the base. This confirms that a vacuum has been created.
- Once the vacuum process is completed, turn off the power button and very gently remove the pipe connecting the setup to the pump. Be careful not to disturb the sample holder or the sample.
- After removing the pipe, lift the lid and take the sample out.
- The sample should be completely dry now. If not, this process can be repeated.

Vacuum Desiccator

7. Resuspend the dried azide-DNA in 100 µl phosphate-buffered saline (PBS).

- Pipette again after each resuspension step to dissolve the precipitate.

8. Determine the final concentration of the azide-DNA solution from optical absorption of DNA at 260 nm using a Perkin-Elmer Lambda-950 spectrophotometer.

- For baseline, use PBS buffer. Simply measure the sample having PBS buffer only and do the baseline correction.
- The absorbance peak at 260 nm is our focus. Use A = ε.c.l where ε is the extinction coefficient (266877 $M^{-1} \cdot cm^{-1}$), c is the concentration, l is the length (usually 1 cm).
- For different sequences of DNA, the extinction coefficient (molar concentration) can be different.

DNA-lipid Conjugate (for small unilamellar vesicle synthesis)

1. Dissolve DSPE-PEG(2000)-DBCO (powder, stored in -20 °C) in ethanol to form a 1 mM solution.

2. Mix the azide-DNA solution (in PBS solution, prepared according to **Azide-DNA Synthesis)** and DSPE-PEG(2000)-DBCO solution at a 1:5 molar ratio.

- The concentration of azide-DNA was found previously using its absorbance.
- **Note**: This is NOT the final DNA concentration.

3. Store the mixed solution in dark (thermocol box) at room temperature overnight.

4. Add approximately 200 µL ethanol and 7.5 µL of 4M NaCl to the solution. Incubate solution at -20°C for 30 minutes.

5. Centrifuge the solution at 20,000 g for 30 minutes.

- Centrifugation at higher RPM (15000 RPM) can also be used.

6. Remove the supernatant and add 200 µl ethanol to resuspend the precipitant.

7. Centrifuge the solution at 20,000 g for 10 minutes.

8. Repeat steps 6-7 for 3 times to thoroughly remove the unreacted DSPE-PEG(2000)-DBCO.

9. After washing, dry the precipitant with a vacuum chamber for 15 minutes.

- Same as the vacuum desiccation step described in **Azide-DNA Synthesis**.

10. Resuspend the dried DNA-lipid conjugate was resuspended in 100 µl PBS.

11. Determine the final concentration of the DNA-lipid solution from optical absorption of DNA at 260 nm using a Perkin-Elmer Lambda-950 spectrophotometer.

- This is used to calculate the final DNA concentration. Using this, number of DNA strands per vesicle can be calculated.
- The DNA concentration in this step should be lesser than the value calculated previously.

*Step 4-11 may refer to step 2-8 in **Azide-DNA Synthesis**

DBCO-RNA synthesis (for RNA surface preparation)

1. Mix approximately 20 µl 1 mM amine modified RNA (stored in -20 °C, dissolved in DI water), 39 µl 60 mM DBCO-NHS (stored in -20°C, dissolved in DMF), 40 µl DMF and 1 µl TEA and incubate for two hours.

- Incubate at room temperature in thermocol box in dark.

2. Add 200 µl ethanol and 7.5 µl 4 M NaCl solution to the mixture.

3. Incubate the solution at -20°C for 30 minutes and then centrifuge at 20,000 g for 30 minutes.

4. Remove the supernatant and add 200 µl ethanol was added to resuspend the precipitant.

5. Centrifuge the solution at 20,000 g for 10 minutes.

- Centrifugation at maximum RPM (15000 RPM) can be used.

*Step 4-5 may be repeated for 2-3 times for better purification.

6. Remove the supernatant and dry the precipitant with a vacuum chamber.

*Step 2-6 may refer to step 2-6 in **Azide-DNA Synthesis.**

7. Dissolve the dried DBCO-RNA in 100 µl 0.1% PBST (v/v, 0.1 % Tween 20 in 1× PBS).

- Prepare fresh PBST every week as needed. Avoid using PBST older than 1 week.
- Tween 20 should be kept inside cabinets and must not be left outside in light.

- After preparing PBST, it can be stored at room temperature in dark (inside a container that can block light).

Tween 20