Last Update: 22 March 2023

CNT Length Sorting

- **CNT length fractionation: Reverse**

1. Make 20% mass fraction of PEG stock solution in DI water.
2. Add 125 ul of PEG stock solution into 450 ul of SWCNTs solution (4.2% mass fraction of PEG). Add 25 ul 6M NaCl solution (0.25M NaCl). Incubate for 6hrs at 4C.
3. Centrifuge at 18000g for 15min. Remove the supernatant and redisperse the pellet in 405 ul 1X Tris buffer. The supernatant has short (~100nm) CNT.
4. Add 75ul of PEG stock solution into redispersed SWCNTs solution (3.06% mass fraction PEG). Add 20ul 6M NaCl solution (0.25M NaCl). Incubate for 6hrs at 4C.
5. Centrifuge at 18000g for 15min. Remove the supernatant and redisperse the pellet in 345 ul 1X Tris buffer. The supernatant has medium (~300nm) CNT.
6. Avs bnwsadd 42ul of PEG stock solution into redispersed SWCNTs solution (2.08% mass fraction PEG). Add 13ul 6M NaCl solution (0.25M NaCl). Incubate for 6hrs at 4C.
7. Centrifuge at 18000g for 15min. Remove the supernatant and redisperse the pellet in 100 ul 1X Tris buffer. The supernatant has medium-long (~500nm) CNT. Add 100ug/ml DNA to the redispersed CNTs for long-term storage. Use column filtration to remove free DNA upon usage.

- **Hongjie Dai Protocol**

1. Preparation of PL-PEG
   a) SWNTs are suspended with 1 % wt/wt sodium deoxycholate aqueous solution by 1 h sonication
   b) Ultracentrifuged at 300,000g to remove the bundles and other large aggregates
   c) The supernatant was retained and 0.75 mg ml−1 of DSPE-mPEG (5 kDa) along with 0.25 mg ml−1 of DSPE-PEG (5 kDa)-NH2 was added.
   d) Sonicated briefly for 5 min dialyzed at pH 7.4 in a 3,500-Da membrane (Fisher) with a minimum of six water changes and a minimum of 2 h between water changes
   e) The suspension was ultracentrifuged again for 1 h at 300,000g

2. Preparation of PL-PEG-Alpha
   a. PL-PEG-FA (FA, folic acid – used for FA depleted Cell medium experiment, easily internalized by folate receptor)
      i. FA (3.5 mM) and EDC (5 mM, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide) added to a solution of 0.35 mM PL-PEG-NH2 in 10 mM PBS at pH 7.5

ii. Dialysis under 10 mM PBS at pH 7.5 using a membrane (MW cutoff = 1,000) to remove unreacted FA and EDC

iii. Dialysis for 3 days.

b. PL-PEG-FITC (FITC, Fluorescein isothiocyanate)

i. 3 mg of PL-PEG-NH2 was dissolved in 1.5 ml of 0.1 M carbonate buffer solution (pH 8.0).

ii. 100 µl of 13mM solution of FITC in DMSO adds to the i. solution. (Overnight reaction, needs light protection)

iii. Gel chromatography purification

1. loading 1 ml of the solution to a Sephadex G-25 column
2. An elution solvent (DI) was flown through the column
3. Fractions are collected

- **Pu Chun Ke Protocol**

1. PL-NT

A. SWNTs of 1 mg a few hundred ug in 40 mg in 1 mL PBS (pH 7.4)

B. Bath Sonication for 1 hour

- **Our Protocol - PEG-NHS/amine-DOPE-CNT synthesis**

1. Weigh 5 mg of PEG-NHS and dissolve in 1ml of NaHCO3 buffer.
2. Add 10ul DOPE stock solution (10mg/ml) to 90 ul of PEG-NHS solution. Wait for 2hr in room temperature.
3. Mix 1ml SC-CNT solution and 100ul DOPE-PEG solution. Add the mixed solution into 3500 MWCO dialysis cassette. Dialyze against 1x tris buffer for 2+2+8 hrs.
4. Remove the solution from dialysis cassette. Use column filtration (100k Da MWCO) to remove unbound PEG/DOPE/DSPE-PEG.

LPC 18:0, LPA 16:0, and LPG 16:0 and surfactant SDS. The numbers “18” and “0” in LPC 18:0 denote the total number of carbon atoms and the total number of double bonds contained in the sum of the fatty acyl chains