

Nanotube Preparation

1. Sonication

- A. 30000 rpm, 4 hour, 4 C
 - i. HiPco looks more diluted than CoMoCAT
 - ii.

2. Dialysis

- A. Prepare 2 L of 1x Sodium Tris-HCl/EDTA Buffed solution (10x solution can be diluted for the use, 10x solution preparing method in the buffer preparation session)
- B. Put X ml of the CNT solution in the dialysis tube. (The tube clipped with clipper(?) and be held with sponge to float it)
- C. Dialysis first solution for 2 hour. Repeat it 3 times
- D. More Dialysis until 24 hour gone
- E. Make a mixture of final concentration of 4 % PEG and 500 mM NaCl in dialyzed DNA-SWCNT solution.
- F. Place the solution in 4 C for 6 hours. Centrifuge it at 10000 g for 15 min. Redisperse the pellets in 1x TBS-EDTA solution

3. CNT length fractionation: Reverse

- A. Make 20% mass fraction of PEG stock solution in DI water.
- B. 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.
- C. Centrifuge at 18000g for 15min. Remove the supernatant and redisperse the pellet in 405 ul 1X Tris buffer. The supernatant has short (~100nm) CNT.
- D. 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.
- E. Centrifuge at 18000g for 15min. Remove the supernatant and redisperse the pellet in 345 ul 1X Tris buffer. The supernatant has medium (~300nm) CNT.
- F. Add 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.

- G. 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 hundreds 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

