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## CNT Synthesis Protocol

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description: DNA-CNT, Lipid-CNT, PBASE-CNT

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### A. Surfactant separation of SWCNTs

- SDS (or SC) – HiPco SWNT Preparation
  1. Add 4 g SDS into 150 mL H<sub>2</sub>O.
  2. Add 60 mg SWNT (2-3 big scoops).
  3. Add 50 mL H<sub>2</sub>O.
  4. Homogenize at setting 1 for 1 hour. (2 wt.% = 4g / 200 mL (water is 1 g / mL))
  5. Cup-horn sonicate for 10 minutes at amplitude 90%.
  6. Thoroughly weigh out an equal amount of solution in each Beckman centrifuge tube.
  7. Ultracentrifuge for 4 hours at 30,000 RPM.
- SDS (or SC) – CoMoCAT SWNT Dispersion
  1. Disperse 15 mg SWNT and 0.6 g SC in 30 mL H<sub>2</sub>O. (2 wt.% = 0.6 g SC / 30 g H<sub>2</sub>O)
  2. Probe-tip sonicate for 1 hour at 20 W input.
  3. Ultracentrifuge for 2 hours at 30,000 RPM.

### B. DNA-SWCNT synthesis

Note: DNA/CNT stoichiometry Length/bp on CNT ~ 0.5 nm.[1]

- Direct Sonication Method
  1. Weigh 4 mg d(GT)<sub>15</sub> DNA and 1 mg CoMoCAT SWNT (4:1 wt. ratio of DNA:SWNT). Add 1 mL of 0.1 M NaCl into an Eppendorf tube and then shake hard.
  2. Probe-tip sonicate (medium-sized) for 10 minutes at 10 W (60-80 % amplitude).
  3. Benchtop centrifuge for 100 – 150 minutes.
  4. Absorbance measurement at 632 nm (ext. coeff.): SWNT solution should be ~125 mg/L.
- Biotin/DNA CNT synthesis

1. Dilute 100ul SC-CNT in 900ul 1X Tris buffer. Add 100ul 1023 RNA and 25ul (GT)10-Biotin DNA.
2. Transfer the solution obtained in step 1 into a 3000MWCO dialysis cassette using a syringe. Remove excess air from the cassette.
3. Dialyze in 2000ml 1X Tris for 2hrs. Change the dialysis buffer and dialyze for another 2hrs. Change buffer again and dialyze overnight.
4. Recover the solution from the dialysis cassette using a syringe. Pump air into the cassette to make the air/liquid ratio roughly 1:1 before getting the sample.
5. Make a mixture of final concentration of 4% PEG and 500 mM NaCl in dialyzed DNA-SWCNT solution. Place in 4°C for 6 hours. Centrifuge at 10000g for 15 min. Redisperse the pellets in 1X TBS-EDTA solution with desired concentration.

### C. DNA-PBASE-CNT synthesis

- NHS activation (PBA has low solubility in water)
  1. Weigh x mg PBA. Dissolve in a 1.5ml vial with DMSO to make 100mM PBA solution. Brief vortex. [MW: PBA 288.34; ]
  2. Make 100mM EDC solution in 0.1M MES buffer. <pH 6.0> [MW: EDC 191.7]
  3. Add 10ul PBA into 90ul EDC solution. The EDC:PBA molar ratio is 9:1.
  4. Make 225mM Sulfo-NHS solution in 0.1M MES buffer to <pH 6.0> (NHS has a longer half-life under low pH)
  5. Add 90ul Sulfo-NHS solution into the PBA/EDC solution. The NHS:EDC molar ratio is 5:2. [MW: Sulfo-NHS 217.13]
  6. Bathsonicate for 15 mins at room temperature. (The solution color should be yellow/white and turbid)
- Amine reaction synthesis
  1. Add concentrated (1M) PBS or NaHCO<sub>3</sub> and make the solution pH 7~8.
  2. Add 1:10 molar ratio amine-DNA to PBA (100ul maximum). Place on stirrer with 400rpm and wait for 2 hrs at room temperature and in dark. (The solution color should turn red/brown gradually)
- washing (start from 1 if the aqueous solution is clear. start from 3 if the aqueous solution is turbid)
  1. Add 0.1V 3M NaCl and 2~3V Isopropanol
  2. Put in freezer and wait for 10mins.
  3. Centrifuge at 15000g for 10 mins. Remove the supernatant and leave the red/brown pellet.
  4. Redisperse with 200ul 0.1M PBS buffer using bath-sonication for 1 min
- SWCNT functionalization
  1. Mix 1ml of SWCNTs of 100ul of 1mM Pyr-DNA and 25ul of 1mM Pyr-PEG-Biotin.

2. Dialysis using 3400 Da MWCO membrane against 1X Tris pH 7.4 for 24 hrs.
3. Second stage dialysis: using 100kDa MWCO filter in 2000g centrifugation for 4.5 mins. Repeat for 6 times.

### **E. DNA-Lipids-CNT synthesis**

Note: Lipids/CNT stoichiometry c.a.  $10^3 \sim 10^4$  lipids:CNT, weight ratio is 10:1 lipids:CNT.[2]

- DSPE-COOH/amine-DNA Crosslinking
  1. Dilute lipid to make a 10 mM solution. [DOPE stock 10mg/ml in chloroform, MW:866.088 g/mol]
  2. Make 10 mM EDC in 0.1 M MES buffer. <pH 6.0> [MW: EDC 191.7]
  3. Add 10  $\mu$ L lipid solution to 90  $\mu$ L EDC. [Mole ratio of EDC:lipid = 9:1].
  4. Make 25 mM solution of Sulfo-NHS in 0.1 M MES buffer. Add 90  $\mu$ L Sulfo-NHS to the lipid/EDC mixture. Mole ratio of NHS:EDC = 5:2.
  5. Bathsonicate for 15 minutes.
  6. Make the solution slightly basic by adding an equal volume of 1 M PBS (or NaHCO<sub>3</sub>).
  7. Add amine-DNA in a 1:1 mole ratio (DNA:lipid). Add 100  $\mu$ L amine-DNA to mixture.
  8. Place solution on vortex for 30 minutes.
  9. Let the contents react for a total of 2 hours at room temperature.
- DSPE-NHS/amine-DNA Crosslinking
  1. Weigh 1mg of DSPE-NHS and dissolve in 500ul of 1x PBS buffer at pH7.4.
  2. Add 100ul of 1mM DNA.
  3. Let the contents react for a total of 2 hours at room temperature.

### **F. DNA-PEG-Lipids-CNT synthesis**

- PEG-NHS/amine-DOPE-CNT synthesis
  1. weigh 5 mg of PEG-NHS and dissolve in 1ml of NaHCO<sub>3</sub> 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.
- DNA-azide/DBCO-PEG-DSPE-CNT synthesis
  1. Weigh 1mg of DSPE-DBCO and dissolve in 1x PBS buffer at pH 7.4. [Use glass vial for DSPE-DBCO, plastic centrifuge tubes will cause powders to attach to the surface].
  2. Make sonicated SC-SWCNT sample. Adjust the concentration to 20 ug/ml.
  3. Mix X ml DSPE-DBCO and X ml SWCNT. Dialyze for 24 hrs.

4. Make 100uM Azide-DNA solution.
  5. Measure DBCO concentration. Mix Xml DSPE-DBCO-CNT with Xml Azide-DNA and react for 2 hours in PBS buffer and pH 7.4. [The DBCO:Azide ratio should be 1:1~3 ]
  6. Purify the sample using 100kDa Amicon filters in 2000cgf centrifugation for 4.5 mins. Repeat for 6 times.
  7. Characterization of click conjugation: a. kinetics: abs 260 [DNA260-DBCO260] vs abs 309[DBCO309] as a function of time. [extinction coef of DBCO is 12000 M-1Lcm-1 @ 309nm.]
- DNA-amine/COOH(or NHS)-PEG-DSPE-CNT synthesis
    1. Determine the COOH-PEG-DSPE-CNT concentration.
    2. Add EDC into 10ul COOH-PEG-DSPE-CNT solution to make EDC:DSPE = 9:1 ratio.<pH 6.0 MES buffer>
    3. Add sulfo-NHS to make NHS:EDC=5:2 solution.<pH 6 MES buffer>
    4. Bathsonicate for 15 minutes.
    5. Make the solution slightly basic by adding an equal volume of 1 M PBS (or NaHCO<sub>3</sub>).
    6. Add amine-DNA in a 1:1 mole ratio (DNA:lipid).
    7. Place solution on bathsonication for 30 minutes.
    8. Let the contents react for a total of 2 hours at room temperature.

## REF

1. JPCC 2014.
2. Wu, Y., Hudson, J. S., Lu, Q., Moore, J. M., Mount, A. S., Rao, A. M., ... & Ke, P. C. (2006). Coating single-walled carbon nanotubes with phospholipids. The Journal of Physical Chemistry B, 110(6), 2475-2478.