12 Helix Bundle DNA Origami Folding Protocol

This protocol describes the folding reaction for creating DNA origami from a long ssDNA scaffold strand and the core stables. The handles and antihandle conjugated to fluorophores or biotin are also folded into the structure at this point. Any chemical moiety conjugated to oligonucleotides can be added at this point provided it can withstand the temperature ramp. For more sensitive components like proteins, it is recommended to add them afterward. See Derr, Goodman et al. 2012. Science 338: 662-665 for additional information.

Protocol

1) Mix 50 ul of folding reactions at room temperature with the following items:
   - 2.5 ul of 20X folding buffer.
   - 5 ul of 1 uM p8064 scaffold (final scaffold concentration is 100 nM, see "Purification of p8064 Scaffold ssDNA").
   - Core staples to a final concentration of 600 nM (Table S1, Derr et al 2012).
   - Negative handle staples (Table S2, Derr et al 2012) to a final concentration of 600 nM
   - Positive handle staples (Table S3, Derr et al 2012) to a final concentration of 10 uM
   - If using fluorophore-oligos that bind to positive handles, they should be added to a final concentration of ~3X the concentration of the complementary handles to which they bind.
   - Add supplemental $MgCl_2$ to bring final concentration to 16 mM. (Note that the folding buffer already has some $MgCl_2$ in it.)
   - Add water to final volume of 50 ul.
2) Using a thermal cycler, run the following protocol:
   - 80°C for 5 minutes.
   - -1°C per cycle for 14 additional cycles.
   - 65°C for 30 minutes.
   - -1°C per cycle for 35 additional cycles.
   - Hold at 4°C.
3) Store at 4C for a few days or proceed directly to the purification of the 12hb structure from excess staples (recommended).

Additional notes:

- Make sure you use either a positive or negative handle staple (BUT NOT BOTH) at each handle site.
- Core staples should all have a final concentration of 6X the scaffold.
- All positive handle staples should have a final concentration of 100X the scaffold.
- Folding and purification can be assayed using 2% agarose gel electrophoresis. Gels and running buffers should be supplemented with 11

mM MgCl2. Gels may need to be run in water baths to prevent hot gels from melting origami structures. This will appear as a smear rather than tight bands.

20X Folding Buffer

- 100mM Tris pH 8.0
- 20mM EDTA
- 200mM $MgCl_2$

Gradient Purification of 12hb Origami DNA

This protocol purifies the folded 12 hb origami away from excess staple strands by using gradient centrifugation. Depending on the concentrations of origami used for the downstream protocols, buffer exchange can be performed to remove the glycerol and/or exchange buffers.

Protocol

1) Prepare a gradient the night before by the following protocol:
   - Lay glycerol gradient layers. I usually do 7 layers, 80 µL per layer, from 45% (bottom) to 15% (top) with -5% increment per layer.
   - Put the tube with gradient layers in a cold room and incubate o/n.
2) Using 50 µl volume of a folding reaction for origami purification add 5.5ul of 100% glycerol to make final glycerol concentration 10%, mix well.
3) Load the sample containing 10% glycerol to the top of the gradient carefully attempting not to disturb the gradient.
4) Centrifuge the gradient in a SW55 rotor for 45,000 rpm for 2:10 hrs at 4C.
5) Collect fractions of 50 µL from the top down, which leads to about 12-14 fractions.
6) Run ~5ul per fraction on a 2% agarose TBE gel to determine the content in each fraction. Run with constant voltage at 70V for 90min.
7) Buffer exchange/Concentrate using Amicon Ultra 0.5mL 30K or 100K MWCO filters.

Buffers:

10X TBE Buffer

- 450mM Tris pH 8.1
- 450mM Boric Acid
- 10mM EDTA

Agarose Gel

- 2% Agarose
- 1X TBE buffer
- Boil to dissolve agarose
- Add $MgCl_2$ to 11mM

Gradient Buffers:

- 1X TBE buffer
- 11mM $MgCl_2$
- 15%-45% Glycerol

Agarose Gel Running Buffer

- 1X TBE buffer
- 11mM $MgCl_2$

Additional Notes:

- When making the agarose gel, add the $MgCl_2$ **AFTER** boiling the agarose.
- When running the gel, make sure to run no higher than 70V. If the gel overheats, the origami will denature and run as a smear. If heat is a concern, run the gel apparatus in an icebox to dissipate heat.
- If running longer than 2hrs, exchange the running buffer. After about 2hrs, the salts will be depleted (white precipitant) and the origami will denature.