

Preparing LCP with LCP Mixer Kit

Method adapted from:

- Caffrey, M., Cherezov, V. Crystallising membrane proteins in lipidic mesophases. *Nat. Protoc.* 4, 706-731 (2009).
- Caffrey, M., Porter, C. Crystallizing Membrane Proteins for Structure Determination using Lipidic Mesophases. *J. Vis. Exp.* (45), e1712, doi:10.3791/1712 (2010).

<https://www.jove.com/video/1712/crystallizing-membrane-proteins-for-structure-determination-using>
www.douglas.co.uk/videos.htm Video of LCP mixer

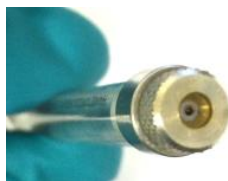
Materials required:

- 2 x Hamilton syringes. One with a 6.5 mm needle for the lipid and one (**labelled female**) for the protein with an enlarged hole on its knurled syringe nose fitting and with two ferrules instead of one.
- Protein sample volume ratio approximately 2:3 (protein:lipid).
- Lipid for hosting protein (normally monoolein).
- LCP mixer device.
- Pipettes and tips for range 5 – 50 μ L.

1. Heat the lipid to its melting temperature approximately 45° C. Ensure the syringes and pipette tips are slightly warm so that the lipid remains liquid during the procedure.
2. Remove the plunger of the lipid syringe and pipette the liquid lipid into the syringe. Prime the syringe so that the lipid is just coming out of needle. Air bubbles should rise to the top of lipid when priming (shown in JOVE video, website link above).
3. Load the protein into the female syringe. We recommend removing the plunger of the protein (female) syringe and pipetting the protein into the back end of the syringe. Ensure that there are no air bubbles are in protein or lipid.

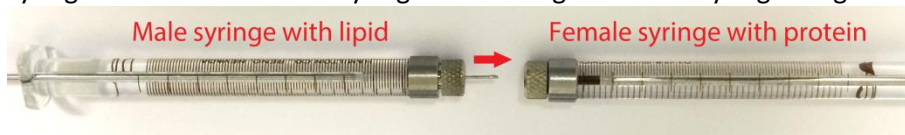


Male syringe with lipid



Female syringe with protein

4. Tighten the knurled syringe nose fitting on female syringe, such that PTFE ferrules are compressed. Prime the female syringe so that the protein is just coming out of the PTFE ferrule (shown in image above).
5. Connect the male and female syringes by inserting the 7mm needle into the PTFE ferrules of the female syringe. Ensure the knurled syringe nose fitting on female syringe is tightened.



6. Place the syringes in the mixer. Adjust the position of the moveable end stop using the thumb screw, to take up the slack.
7. Move the mixer back and forth to mix the LCP. We recommend mixing ~25 times, waiting 2 minutes and then repeat until mixture is transparent and homogenous.



8. Ensure all of the LCP is in the male syringe (un-labelled) then separate the syringes. The LCP is now ready for use.
9. **Important:** After use Loosen the female syringe fitting to avoid compression and deformation of PTFE ferrules. Non deformed PTFE ferrules are required to make a seal between the syringes