Preparation of Lipidic Cubic Phase (LCP) using LCP Mixer

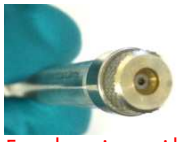
Additional Materials required

- Protein sample volume ratio approximately 2:3 (protein:lipid)
- Lipid for hosting protein (normally monoolein)
- Pipettes and tips for range 5 – 50 µL

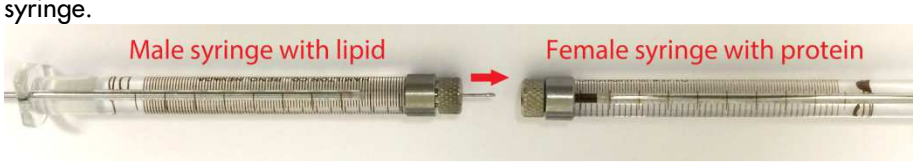
Preparation of the Lipidic Cubic Phase

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 (see References for the JOVE video link).
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 the two PTFE ferrules are compressed. Prime the female syringe so that the protein is just coming out of the PTFE ferule (shown in image above).
5. Connect the male and female syringes by inserting the 7mm needle into the PTFE ferules of the female syringe.



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 then waiting 2 minutes. Then repeat until mixture is transparent and uniform.

8. Ensure all the LCP is in the male syringe then separate the syringes. The LCP is now ready for use.

References:

- Caffrey & Cherezov (2009) Crystallizing membrane proteins using lipidic mesophases. *Nature Protocols* **4**:706.
- Caffrey & Porter (2010) Crystallizing membrane proteins for structure determination using lipidic mesophases. *J Vis Exp.* **45**:1712.
- https://www.jove.com/video/1712/crystallizing-membrane-proteins-for-structure-determination-using

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