## Protocol for Liposome Preparation Through Thin-film Hydration Aqueous Solution Freze Day Organic Solvent Lipid Cake Hydration Agitation LMV Downsized SUV

## **Procedure:**

- 1. The lipids must first be dissolved and mixed in an organic solvent to assure a clear and homogeneous, mixture of lipids.
- 2. Once the lipids are thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film.
- 3. The lipid film is thoroughly dried to remove residual organic solvent by placing the vial or flask on a vacuum pump overnight.
- 4. Add an aqueous medium to the container of dry lipid and agitating. (The temperature of the hydrating medium should be above the gel-liquid crystal transition temperature (Tc or Tm) of the lipid with the highest Tc before adding to the dry lipid. Vigorous shaking, mixing, or stirring is highly recommended)
- 5. The hydrated lipid suspension then was downsized by a variety of techniques, including sonication or extrusion.