

**Question:** What is the difference if the lipid film was used immediately or stored for use later?

### Step 1: Preparation of the oil containing lipids

1. Mix mineral oil by gentle inversion before use

20~1 sec inversions

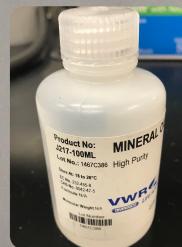
2. Place 0.5 mL of mineral oil into each of the vials.

3. Incubate at ~~55~~ degC for 10 min. (no 60°C heater)  
-dry bath (buried)

4. Vortex for 10 mins

5. Incubate vials for 3 hrs at 60 degC.

6. Wrap up the top of the vials with aluminum foil and seal with parafilm.



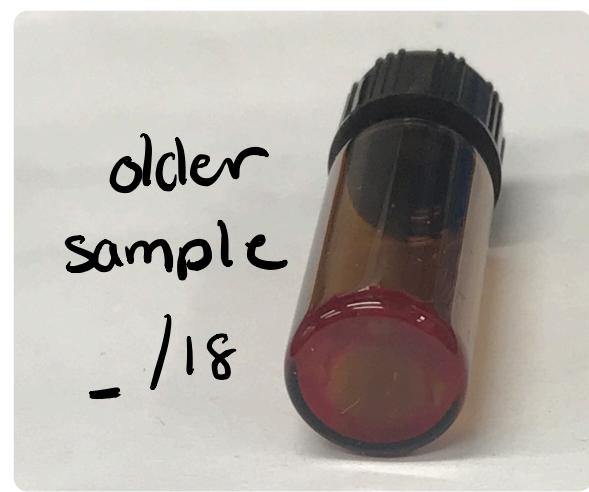
liposome-kit/txt-liposome\_water-in-oil.md at master · BuildACell/liposome-kit  
7. Sonicate in an heated water bath for 30 minutes, at 60 degC.  
a. Note: If a sonicator is unavailable then repeat 60 degC incubation and vortexing until the lipid is completely dissolved.

\* Immediate use test on a single vial test... along with older vial for comparison using Github method.

## Samples used

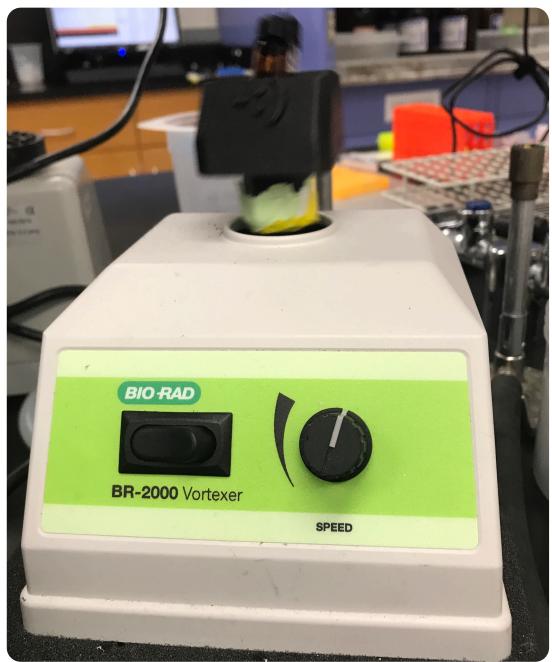


- odorless
- no change after first heating



- fishy odor (common in the past)
- no change after initial heating

5:33pm, 5:40pm



- Using Vortex holder, made by Andrey. (some wobbling)
- Keeps vials at 45°
- Vials position switched after 5min
  - no change, vials 'cold' to touch
  - difficult to remove
- not a perfect vortex, more of a mixer

\* Left in heated water bath + 1 hr

→ the older sample after vortex suspended

the lipids

→ the new sample lipids were not suspended  
(maybe a little bit)

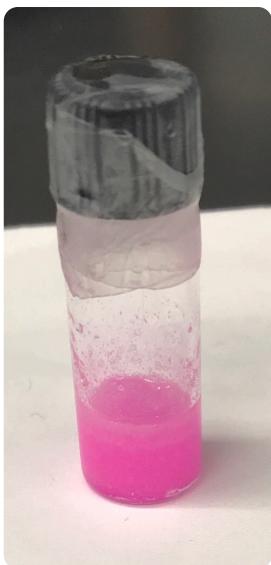
\* Left in 55°C bath overnight

- in order to deal with this in the morning "

September 13, 2018

Removed at ~1pm

Vortexed for 30s, lipids seem to be settled on bottom



• consistency looked a little grainy, not sure why



- Consistency as expected
- Lipids settled again after initial vortex last night

Question: Left in heat for <12 hours, what effect may this have?

#### Step 2: Self-assembly of the Liposomes by centrifugation

1. Place 225 uL of centrifuge buffer (100 mM HEPES + 200 mM glucose, pH 8) into a labeled eppendorf tube.

a. Note: When TX-TL systems are used, the outer solution should also contain the small molecular weight components of the TX-TL system.

2. Add in 30 uL of inner solution (100 mM HEPES, 200 mM sucrose pH 8, and, if needed, 2 uM of a water-soluble fluorescent dye like HPTS or calcein.) to 500 uL of suspended lipid in oil from Step

i. Vortex for 30 s. Equilibrate for 10 min at 4 degC.

o Note: When TX-TL systems are used, buffer is omitted. TX-TL systems come with their own buffers.

3. Add the emulsion on top of the 225 uL centrifuge buffer by pipetting against the wall of the tube, wait at least 1 minute for the interface to stabilize and flatten between the emulsion and buffer

4. Centrifuge at 18000 rcf at 4 degC for 15 min *< seems too long*

o Note: If the top phase (oil) is clear, that suggests that droplets have passed into the bottom buffer, becoming vesicles.

5. Carefully remove as much mineral oil as possible with a gel-loading-pipette-tip from the top.

o \*\*Note: \*\*The goal is to remove as much oil up to the interface without disturbing the buffer below.

6. Place 225 uL of wash buffer (100 mM HEPES + 250 mM glucose, pH 8) into a labeled eppendorf tube.

7. Add the centrifuge buffer (where the vesicles should have formed) to eppendorf tubes with wash buffer. Make sure to use a new tip to avoid contamination.

o Note: An alternative method is to open the eppendorf tube and use a 21-gauge needle to punch a hole at the bottom of the eppendorf tube. Remove the needle and close the lid to allow the buffer solution to drip out.

8. Centrifuge at 12000 rcf at 4 degC for 5 min

9. Transfer 225 uL from the bottom of the eppendorf tubes with wash buffer into a new labeled eppendorf tube.

## Step 2:



The new sample looked chunky, not smooth in texture.

After waiting 10 min

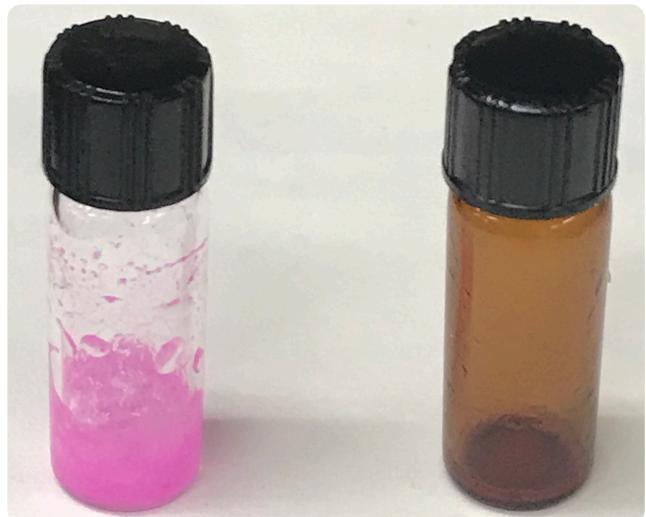
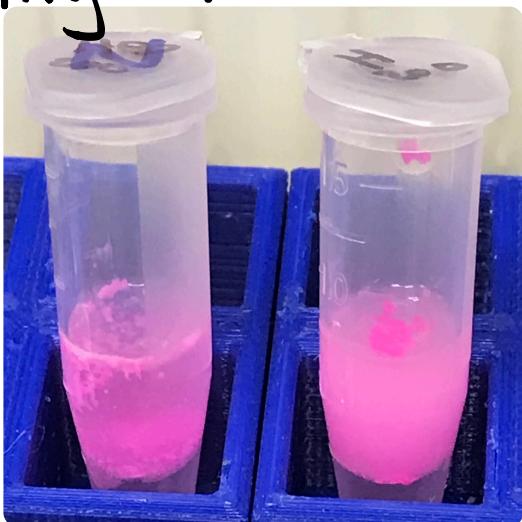


- gel looking  
Cholesterol  
effect?

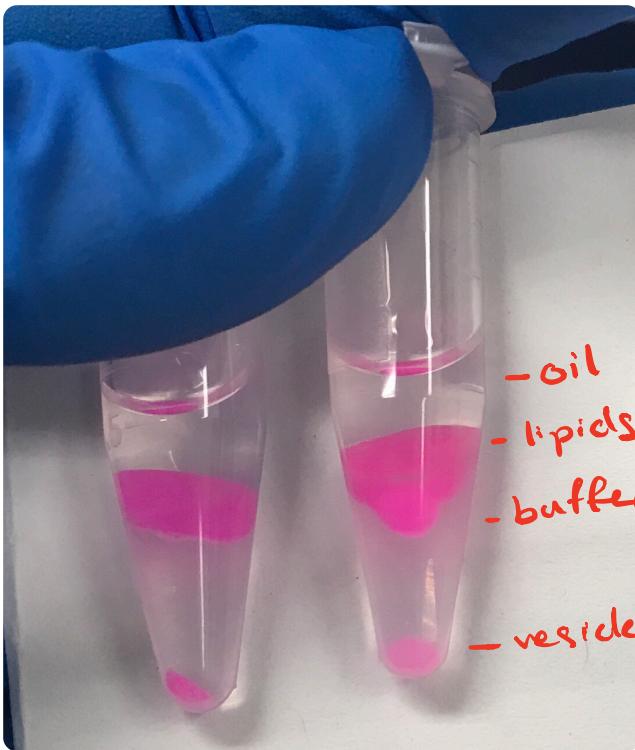


-as before

Moving Lipid + internal solution to tubes



# After centrifuge spin



- looks like 4 different layers
- oil was removed from top
- using  $250\mu\text{L}$  tip was plunge past lipid layer and air was bubbled out
  - all buffer and lower pink section transferred to wash buffer

• Lipids on bottom look like a raft, a little clumpy, not sure what they are

# After Wash



$225\mu\text{L}$  removed from bottom of tube,  $10\mu\text{L}$  was used to take images with

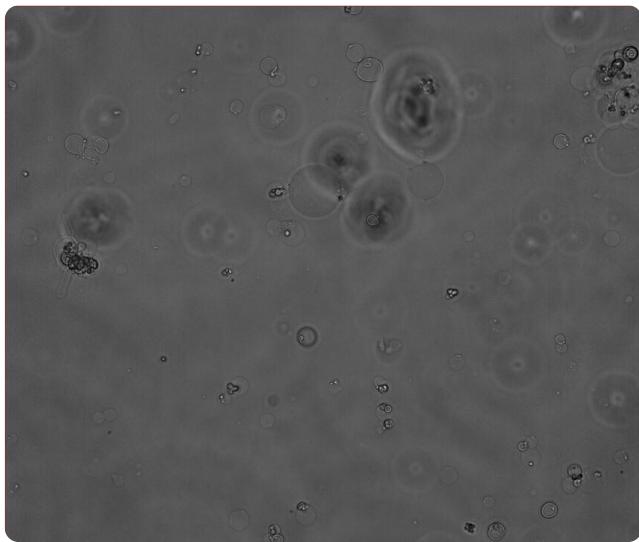
### Step 3: Microscopy Visualization

1. Use Frameseal or Spacer (20 mm D x 0.12 mm depth) to make a small chamber on a microscope slide.
2. Add 10  $\mu$ L of the final solution to the chamber and seal with cover slip
3. Wait 5 minutes before imaging (since the liposomes will float everywhere and can be tricky to catch them).
4. Observe on an inverted microscope. [Sample images] of what you should see:

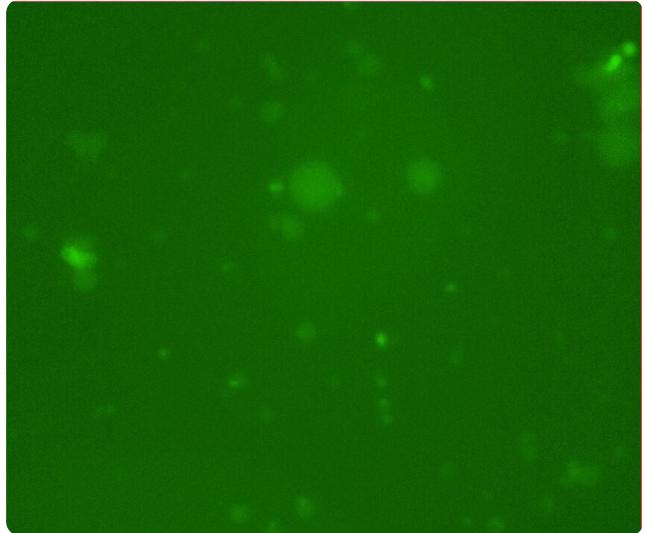
#### Alternative method (neha):

- Use a glass bottom chamber: <https://www.thermofisher.com/order/catalog/product/154453>
- Block glass by adding 0.5 mL of a 1mg/mL BSA in PBS solution for 10 minutes (alternatively block by adding 0.5 mL of SuperBlock for 5 min). The blocking step is important because phospholipid vesicles will rupture to some degree on glass.
- Rinse with PBS 3X. Add 0.5 mL of final buffer from above to chamber. Add 10  $\mu$ L of vesicle sample to chamber, let settle over a few minutes. Image on scope with green and red channels.

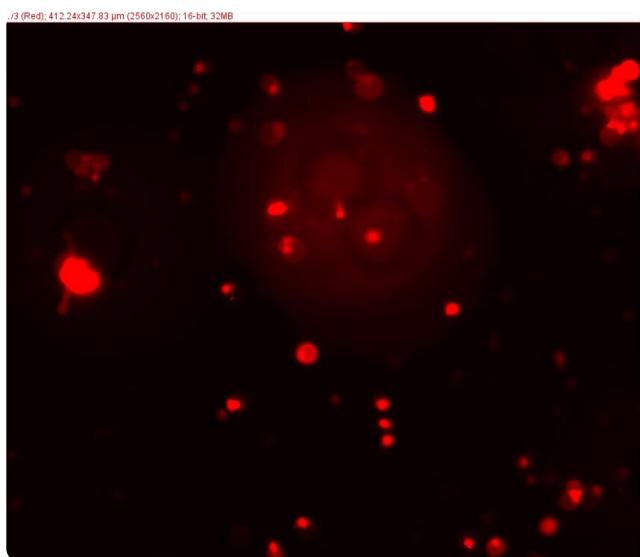
Mag: 40x



BF



GFP



RFP