# NavAb Sodium Flux Assay Protocol

## Making the Liposome

1. Dissolve POPE/POPG (3:1, w/w) in a buffer containing 150 Nacl/20 Hepes, 1% LMNG, NMDG/Hepes adjusted pH 7.5, with final concentration of 5 mg/ml (0.8 ml tube with 4mg of total lipids). Store at -80 Celsius.
2. Thaw the lipid stock in RT, add 3.2 ul TCEP (0.5M=500mM stock) to reach final concentration of 2 mM. Mix with WT NavAb protein at a ratio of 1:8000 (w/w) equivalent to 1ul of NavAb for 800ul of total lipids.
    a. Incubate at 4 Celsius for 20 minutes.
3. Add 0.3g of Bio-beads SM2 to remove detergent overnight.
4. Next day use a 1 ml syringe and 23g needle to suck the liposome out and discard the Bio-beads SM2. Ensure the flat end of the syringe is placed against the wall of the collection tube to avoid sucking in Bio-beads.
5. Store the formed liposomes at -80 Celsius for later use in the liposome flux Assay (LFA).

## Na+ Liposome Flux Assay Version 1

1. Obtain assay plates. Wells that have not been used have no tape on them and can be used for the LFA.
2. Solutions used:
    a. ACMA stock (1mM in DMSO), working conc of 3.5uM (286x)
    b. Valinomycin stock (4.5uM in ethanol), working conc of 0.045uM (100x)-Actual Molarity is about 0.9mM but the dilution factor is still 100x-**NOT USED IN THE NALFA**
    c. CCCP stock (0.1 mM stock in DMSO), working concentration of 1 uM (100x)
    d. 150Kcl/20 Hepes
    e. Hcl
    f. Lidocaine-**LATER**
3. **Compounds are added in this format:**
    a. Make Liposome. Inside liposome = 150 Nacl/20 Hepes (Refer to making liposome above)
    b. Add 150 Kcl/20Hepes to plate (outside) (same pH as inside)
    c. Add liposome/ACMA to plate
    d. Get baseline reading > Pause
    e. For test:
        i. Add Hcl after pause
    f. For Controls (Follow below)
        i. Negative Control:
            1. add CCCP after pause (Note: No Hcl)-Curve could be upward pointing due to K+ influx if not in Eq
            2. add Lidocaine before pause > then Hcl after pause (No signal = Eq)
        ii. Positive Control: Add CCCP **before** the pause and Hcl **after** pause
4. Follow the Excel sheet for calculations (Working on this).

## Na+ Liposome Flux Assay Version 2

1. Obtain assay plates. Wells that have not been used have no tape on them and can be used for the LFA.
2. Solutions used:
    a. ACMA stock (1mM in DMSO), working conc of 3.5uM (286x)
    b. Valinomycin stock (4.5uM in ethanol), working conc of 0.045uM (100x)-Actual Molarity is about 0.9mM but the dilution factor is still 100x-**NOT USED IN THE NALFA**
    c. CCCP stock (0.1 mM stock in DMSO), working concentration of 1 uM (100x)
    d. 150Kcl/20 Hepes
    e. Hcl
    f. Lidocaine-**LATER**
3. **Compounds are added in this format:**
    a. Make Liposome. Inside liposome = 150 Kcl/20 Hepes (Refer to making liposome above)
    b. Add 150 Nacl/20 Hepes to plate (outside) (same pH as inside) + NMDG/Hepes
    c. Add liposome/ACMA to plate
    d. Get baseline reading > Pause
    e. For test:
        i. Add Valinomycin after pause (Expectation really unknown-Josh has data so we now know what to expect)
    f. For Controls (Follow below)
        i. Negative Control:
            1. No Valinomycin add CCCP after pause
            2. OR
            3. add Lidocaine before pause > Add Valinomycin after pause
            4. OR
            5. Add nothing else except buffer+lipo/acma
        ii. Positive Control: Add CCCP **before** the pause and Valinomycin **after** pause
4. Follow the Excel sheet for calculations (Working on this).