**Questions for Manisha**

TxTl vs PURE? (She doesn’t need)

Express or purify and integrate and why?

ATP assay?

How to control actuation direction and membrane integration location? Maybe use an light-activated proton pump?

What are you using the microscope for?

How to detect ph inside a liposome? Or testing extract?

**Questions for Miki**

Can I make liposomes?

Can I make txtl extract batches? – don’t know how to use French press,rosetta, dialysis etc.happy to read protocols

* This updated: <https://www.cds.caltech.edu/biocircuits/index.php/File:TX-TL_Protocol.docx>
* From 2 Nov 2017

How to order atp dye?

* 189$ -20C,6months stable
* Has 5x/2x
* What types of plates are these experiments performed on

How to order ATP synthase genes? What website to use? (IDT)

How to order ph sensitive dye – will do with Manisha?

How to update wiki?

**Current plan**

Basics

Learn how to make vesicles look at wiki protocols

Learn how to make txtl (?) look at protocols

How to encapsulate txtl? Look at wiki protocols

Characterize (practice Basics)

ATP assay/dye- aliquot out a bit every x hours and test atp fluor in biotek

(<https://www.caymanchem.com/pdfs/700410.pdf>)

Know how much /rate of protein (way to practice protocol)

Use Translational Reporters:

Positive Control - pR(pLambda):UTR1:deGFP:T500 on ColE1 (pBEST/IA\_v1-1) backbone

Part 1: is ATP Limiting Factor?

Adjust txtl to have more atp in beginning

Aliquot atp in the middle (might have to add some enzymes to get rid of toxic pi concentration)

Add GFP DNA template – test every x hours

Part 2a: Bound ATP Synthase via Gene Expression

Add ATP Synthase F0 and F1 parts (DNA Template)

Tag with GFP? Does it reconstitute in the membrane?

Part 2b: Bound ATP Synthase via Membrane Reconstitution

Get parts (work with Manisha)

How to get correct membrane integration

Part 3a/b: Bound Proton Pump

Try to express – more research

**Lab Equipment**

Biotek plate reader

Olympus IX81 microscope (inverted microscope)

Ice

Computers

Fume hood (for encapsulation)

Pipettes

French Press, BL21 Rosetta, Dialysis comparison

**Places where I need help**

Making liposomes, txtl, encapsulation

Using echo

**Where I can help**

Cleaning dishes

Taking out plates

Will update as I get more experience