**Questions for Manisha**

~~TxTl vs PURE? (She doesn’t need)~~

ATP Synthase Protein Questions

I know that you plan to purify and integrate the protein - What is the benefit of that vs express genes with tx-tl extract?

~~You’re choosing to purify and integrate the proteins – this is just done by purifying and adding the subunits to the mixture where the liposomes lie?~~

* ~~I’ve heard it’s difficult to purify proteins so why not express the genes maybe with tx-tl extract on the outside of the liposomes~~

To validate my models I’ll be worried about how much atp synthase/proton pump are actually binding to the membrane. How do you plan on quantifying how much and where ATP synthase attaches to the membrane? Tag GFP to ATPase or try to visualize under a microscope?

One of my issues will be whether or not just expressing the proteins within the vesicle will lead to spontaneous membrane integration. Do you plan on using the detergent method that Zoila is using to control or aid membrane integration?

Energy

Are you worried about having enough energy? Will you be supplying a source of ATP or ADP in the surrounding environment? Is there one particular direction of rotation that you prefer – whether that’s atp hydrolysis or synthesis or is just the consistent rotation of the f1 subunit what matters?

Assay/Experimental

I want to use an ATP assay to detect how much ATP is within a liposome but I’m confused how I actually access the contents inside the liposome. Along the similar lines, are you planning to detect ph inside a liposome? How do you do that? Do you extract items out of vesicles or do you not plan on using vesicles?

Other

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

**Questions for Miki**

I will want to make liposomes and different types of txtl extract – some with atp assays and extra sources of atp…is this doable? I understand that txtl extract takes a very specific timeline to make so I’m not sure how that will work.

I don’t know how to use French press, rosetta, dialysis etc – are there protocols/manuals I can find somewhere on the wiki? How will I learn these?

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

How do I encapsulate txtl in liposomes?

* Emulsion transfer protocol’

Can I and how to order atp dye? Is there a spectrophotometer in the lab?

* (<https://www.caymanchem.com/pdfs/700410.pdf>)
  + 189$ -20C,6months stable
  + Has 5x/2x
* What types of plates are these experiments performed on
* Option 2: https://www.abcam.com/luminescent-atp-detection-assay-kit-ab113849.html
  + 295$, 300 tests

Need to order GFP protein detection gene: Positive Control - pR(pLambda):UTR1:deGFP:T500 on ColE1 (pBEST/IA\_v1-1) backbone

How to order ATP synthase genes? What website to use? (IDT) Probably won’t need to do soon?

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

Do I need to order ATP? What’s in the lab?

* If need ATP: <https://www.sigmaaldrich.com/catalog/product/sigma/a3377?lang=en&region=US>

I don’t have a wiki page…

Any slack channels I should be a part of?

**Current plan**

Part 1: 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

* Emulsion transfer protocol?

Part 2: 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, or to allow atp to go inside)

Add GFP DNA template – test every x hours

If need ATP: <https://www.sigmaaldrich.com/catalog/product/sigma/a3377?lang=en&region=US>

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

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

bulk with no encapsulation

1. Base Extract = Extract+DNA+Energy Mix. Possible Additives = [Water (Control), ATP (is ATP) the limiting factor? 3PGA (is the main energy source the limited factor?), and Energy Mix (maybe the limiting factor is in the energy mix but is not ATP or 3PGA?).
2. Base Extract = Extract +/- Energy Mix. Additives = Same as 1 + but mixed with DNA. Does extract die without DNA? Can protein expression be started in extract later?
3. Base Extract = Extract + DNA (no Energy Mix). Additives = Energy Mix (at different times) does extract die before it is active?

 I would tentatively suggest 0, 3, 6, and 12 hours or something along those lines (0, 2, 4, and 8 would probably be just as good and would make your life easier). For your initial experiments, however, I would just try these experiments at one timepoint - probably 2 or 3 hours. Make sure that the volume of the additives you use are always the same so concentrations of extract & non-added ingredients are consistent across experiments.

Part 3: 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 4a: 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 4b: Bound ATP Synthase via Membrane Reconstitution

Get parts (work with Manisha)

How to get correct membrane integration

Part 5a/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

If things go online, I will either model more mechanisms for atp regeneration OR start to study/model ways that integrases can be of unique interest in synthetic cells.