**Planned Experiments**

Part 1) Learn how to make TX-TL

Part 2) Check to see if ATP is the limiting factor (no encapsulation)

* Use ATP fluorescence assay to make calibration curve
* Perform experiments with bulk TX-TL - quantify ATP and protein production
  + Add different amounts of ATP, 3PGA, Energy Mix, DNA in the beginning to understand which one affects protein production the most
  + Add different amounts of ATP, 3PGA, Energy Mix, DNA at different times within the process to see if extract can be ‘revived.’
  + Take fluorescence measurements at regular time intervals

Given that data from Part 2 indicates ATP is limiting factor:

Part 3) Characterize ATP and protein production in encapsulated TX-TL

* Learn how to make vesicles and how to encapsulate

Part 4) Bind ATP Synthase to vesicle membrane via gene expression

* Add ATP Synthase F0 and F1 DNA templates, let TX-TL create proteins
* If no spontaneous integration, try detergent method
* If this doesn’t work – Bind ATP Synthase to membrane via purification and reconstitution

Part 5) Bind proton pump to membrane via gene expression

**Lab Equipment Needed**

For TX-TL: Autoclave, Plates, Centrifuge, Echo, French Press, Dialysis (in cold room), Magnetic Stirrer, Pipettes, Cuvettes

For data collection: Biotek plate reader, Computers, Possibly Olympus IX81 microscope

For encapsulation: Fume hood

**Place where I need help**

Making TX-TL, liposomes, encapsulation

Using lab equipment: Echo, French Press, Dialysis

Finding Reagents

**Place where I can help**

Cleaning dishes, taking out plates

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.