Continue with ATP regeneration

Implement ATP synthase in membrane

Test / ATP data collection

Implement proton pump in membrane

Test / ATP data collection

**Questions for** **John**

Where do you see the future of dna messaging going? What are necessary next steps?

**Dna messaging not really used or an immediate thing. Will be used later to create more complex circuits probably.**

What are possible applications? Mentioned quorum sensing

**Not yet**

Anyone else in lab who works on integrases/excisionases?

**Andrey**

When are you done? Who can teach me techniques?

**Have two more years**

Are you publishing a paper with this work and when?

**In two more years**

What is DNA brush?

**dsDNA attached to a surface**

Weizmann institute has spatially distributed ‘artificial cells’ that allow for programmable interactions.

From proposal my understanding is what I would look into developing a set of fundamental operations that can be connected together to implement more complex computationsl systems. AND OR NOT gates, pulse generators (feedforward loops), timers and counters (oscillation circuits), memory and event ordering (DNA itnegratino sites), and comparison functions (A>B with molecular sequestration).

Which of these do you think is feasible to do…what has community already done…what may take a longer time?

**All the low hanging fruit has already been done. Would have to be more complex things. Perhaps combinations. But I think there are limits that come with trying to do these kinds of experiments.**

***Integrase Notes / Reading***

Serine Integrases: Advancing Synthetic Biology

<https://pubs.acs.org/doi/pdf/10.1021/acssynbio.7b00308>

* Precise rearrangement of DNA through site-specific recombination of small sequence of DNA called attachment (att) sites
* Serine integrases are highly directional, can only be reversed in presence of RDF (recombination directionality factor)
  + so they can be used for controlled rearrangement and modification of DNA
* site-specific recombination requires no extensive DNA sequence homology, no synthesis or degradation of DNA, and has no reliance on endogenous repair pathways or cofactors
* serine integrases subfamily of serine recombinases
  + recombinases make dsDNA breaks
  + tyrosine recombine make ss break and rejoin strands via holliday junction
  + serine integrases only requires the integrase protein and small att sites ( < 50 bp)
* serine integrases can catalyze recombination between att sites on linear or circulat DNA substrates and, depending on position and orientation, integrase, excise, or invert sections of DNA
* serine integrases are encoded by temperate bacteriophages and catalyze their integration into bacterial genomes through recombination of *attP* (phage) and attB (bacteria) attachment sites, generating attL (left) and attR (right) sites
* integrases preferentially form complexes with attP and attB sites, and prohibit attL and attR recombinations
* serine residues cleave the att sites, leaves 3-2 overhang, switch and bind
* RDFs are phage-encoded proteins that bind integrase proteins, stimulate recombination of attL and attR sites, and inhibit recombination of attP and attB sites
* Integration can be targeted to a specific locus known to have minimal positional effects on transgene expression
* Most commonly used: load DNA into pre-existing enomic loci (pseudosites), those that use a ‘landing pad’ (single att site integrated in genome), and recombinase-mediate cassette exchange (RMCE)
* Increasing information storage capacity of integrase-based logic and memory devices
* Use CRISPR-cas9 to record presence of biological signals

A single-input binary counting module based on serine integrase site-specific recombination

<https://academic.oup.com/nar/article/47/9/4896/5430839>

Logic Synthesis of Recombinase-Based Genetic Circuits (2017)

<https://www.nature.com/articles/s41598-017-07386-3.pdf>

* Synthetic two-input logic gates in E coli cells with long-term memory based on DNA inversion induced by recombinases
* DNA inversion mediated by genome editing tools is possible
* CRISPR-cas9 can be exploited to implement large-scale recombinase-based circuits
* How to construct arbitrary Boolean functions based on emerging technologies?
* Use existing electronic design automation (EDA) tools to automate the synthesis of complex recombinase-based genetic circuits with respect to area and delay optimization
* *In silico* experimental results demonstrate applicability of proposed methods as a useful tool for recombinase-based genetic circuit synthesis and optimization
* Transgenerational memory – store the computational results in separate synthetic memory devices which can be duplicated in cell divisions
* Computational result automatically stored in computing circuit configuration and changes of configuration can be propagated to its descendant cells
  + For 90 generations
* AHL acitavtes expression recombinase Bxb1 and aTc for phiC31
* GFP when both AHL and aTc in high concentration
* Try to construct complex logic gates with more inputs – 4 input logic circuits
* Shorter DNA sequence is more likely to succeed in vector insertion to deploy genetic circuit into host cell to conduct intended computation – Gibson assembly

An Automated Design Framework for Multicellular Recombinase Logic 2018

<https://pubs.acs.org/doi/pdf/10.1021/acssynbio.8b00016>

* Issue – current designs are performed on a case-by-case basis, limiting their scalability and requiring time consuming optimization
* Automated workflow for designing recombinase logic devices executing Boolean functions
* CALIN – Composable asynchronous logic using integrase networks
* When greater than 4 inputs – can cause metabolic burden and affect cellular viability
* Distributed multicellular computation (DMC)
  + Based on the decomposition of a Boolean function into various subfunctions, each performed by a particular subpopulation of cells
* While highly scalable, the need for spatial separation between each subpopulation prevents these systems from operating autonomously
* Their design for Boolean logic based on a reduced library of cellular computing units responding to one or multiple inputs
* Logic system is single layer, doesn’t need cell-cell communication or spatial separation
* CALIN takes truth tables as inputs and providing corresponding DNA designs and sequences as outputs
* If one cellular subpopulation is ON (expression of the output gene), then the global output of the system is ON

Permanent genetic memory with >1-byte capacity 2014

<https://www.rle.mit.edu/sbg/wp-content/uploads/2014/10/Permanent-genetic-memory-with-1-byte-capacity.pdf>