3/1/2020

Maybe consider a rheostat to monitor both ATP and NADPH levels

ATP needed for most things – including translation, transcription

The Adenosine triphosphate (**ATP**) molecule is the nucleotide known in biochemistry as the "molecular currency" of intracellular energy transfer; that is, **ATP** is able to store and transport chemical energy within cells. **ATP** also plays an important role in the synthesis of nucleic acids.

**NADPH** is **used in** the biosynthesis (production) of lipids (fatty acids and cholesterols), neurotransmitters, nucleotides and amino acids. It also plays a major role in plant photosynthesis as an electron acceptor in the light reaction and donor in the light-independent reactions.

3/2/2020 – William Meeting

My goals:

* Extend the lifetime of TX/TL function in liposomes (from 4-6 to 10-12 hrs)
  + Do by ATP regeneration
* Show that we can generate greater amounts of protein when Tx/TL system is in the presence of a molecular rheostat that maintains ATP levels
* Molecular rheostat was generated in Bowie Lab
  + 2 possible pathways
    - ATP generating pathway when there are high free phosphate concentrations
    - no ATP generated pathway when low free phosphate concentration
  + They were able to show prolonged ATP production

With modeling and simulation:

* Recreate COPASI modeling done in Bowie paper with the molecular rheostat (the current model)
  + See if it can truly extend lifetime of transcription and translation function
* Explore and understand reasons why ATP regeneration stops – what are the main inhibitors (ADP accumulation etc.)
  + Try different types of perturbations and mitigating approaches related to energy usage to understand how different concepts affect the energy lifetime

Forward it to Opgenorth

ATP regeneration limited by gunk,

Bioscrape

4/5/2020

**Ideal Figures**

Ideally, we would consider that the ATP production remained at a constant steady state level for at least 12 hours

We would also hope to see increased amount of absolute protein production with consistent ATP production. We would also hope the rate of protein production would be high.

Currently: Can get about 6-10 hours of gene expression

Overarching goal: extend lifetime of anabolic (atp using, protein producing) processes in synthetic cells

First, let’s make a diagram that depicts our overarching goal  
Metabolic process: anything that converts food to energy, things that develop

Anabolic: synthesis of compounds (tx/tl) (require energy)

Catabolic: break down of compounds (release energy)

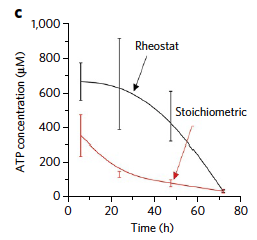
ADP + Pi

ATP

Anabolic Process

Notes:

* Specifically, study tx-tl process
* ATP is limited – overcome by ATP regeneration pathway
  + Proposing: molecular rheostat (Bowie Lab)
  + Produces isobutanol from glucose (has nonATP and ATP producing pathway)
  + Can make ATP up to 72 hrs
  + \*\*SHOW how much excess ATP is made that can contribute to anabolic process\*\*
  + Apply that with TxTL system to see if we can get prolonged protein production
    - Greater amounts of absolute protein production
    - Rate of protein production at medium-high level steady state for extended period of time
    - More protein production and consistent rate
* First half
  + Extending metabolic process
  + Diagram with metabolic processes with constant supply of ATP
* Next half – described goals and how to attack
  + Molecular rheostat diagram (how u propose ATP regeneration)
  + Have some sort of ideal figure
    - ATP production 72 hr diagram from opgsenorth paper
    - X axis – time, y-axis – protein production rate
      * Show that it plateaus to some point for x amount of time and then it may decay but u know absolute amount of protein is higher



4/7/2020

Incoherent type 1 FFL (I1-FFL): pulse generator and response accelerator. The two signal pathways of the I1-FFL act in opposite directions where one pathway activates Z and the other represses it. When the repression is complete, this leads to pulse-like dynamics. Can serve as a response accelerator, can speed-up response of any gene. Can generate non-monotonic input function in synthetic and naïve systems. Provide adaptation to the amount of DNA template and can be superior to simple combinations of constitutive promoters.

* Need an input of some type of DNA that the TX-TL system can process and output protein
* Cognate – has restriction sites for enzymes to cut
* For protein-mediated networks, must add protease ClpXP for protein degradation
* Do you want to do Rna-mediated network of protein-mediated?
  + Both – I was initially interested in protein-mediated networks but it might be simpler to start with RNA-mediated network so I’ll be sure to consider that when modeling
  + Initially, I was thinking of just having a system that activates GFP and see for how long I can get GFP expression
* Fixed concentrations of nutrient resources which limit the lifetime of reactions
* ATP conc in ecoli change during growth transitions and control the rate of rRNA transcription initiation at those times.
* 2 ATP and 2GTP are consumed per peptide bond, need more GTP as well?
* In modern cell-free txtl systems, batch mode reactions terminate because of **chemical energy limitations** and accumulation of byproducts
* Let’s try to maintain ATP and GTP concentrations at initial conc, recycle inorganic phosphate (byproduct which can be an inhibitor of protein synthesis)
* Currently, an ATP regeneration system composed of a phosphate donor (like creatinine phosphate) and a kinase is also added to the energy mixture. Such a system extends gene expression up to 3-4 hours
* **Can add a carbon source (maltose, maltodextrin) to extend protein synthesis up to 10 hours by activating glycolytic pathway**
  + Acts as a substrate for ATP regeneration and recycling of inorganic phosphate
  + Hopefully I can get 12-14 hrs
* Adding ATP won’t work because u get a buildup of ADP and AMP which is toxic. And you also need to add magnesium because ATP is negatively charged
* There are hypotheses that involve doing a constant buffer exchange through some dialysis membrane or using microfluidics (based on diffusion exchange or continuous dilution)
  + Might be something to explore in the future
* There are other limiting resources – such as amino acids being unstable, pH decrease, and finite conc of core txtl machinery
* Transcription rate limits protein production rate in vitro
* **ATP is about 1-2 mM (or 1000 µm) but they are ate about 700-500µm for the steady state conc’s**