Abstract

Currently no tools are available to the synthetic biology community that allow for the separation of engineered biochemical processes in order to improve characterization and the stability of these systems. To achieve this we developed microcompartments that will mimic the compartmentalization which occurs in eukaryotes, allowing the ability to selectively target proteins into this compartment. The system we are creating is based on an engineered lumazine synthase protein containing point mutations to generate a highly negative interior. This protein can attract highly positive 10 arginines (R$_{10}$) tags, enabling a selective targeting system. We have successfully created the cloning vectors to introduce these signal peptides to either the N- or C-terminus of any protein of interest. We have modeled the lumazine synthase microcompartment and shown that it has a volume of 299 and 369 nm$^3$ which is able to accommodate proteins of up 80 kDa in size. The pore size however will not allow for the escape of these proteins from the compartment. In order to test co-localization of two proteins into the microcompartment we will test this system using the fluorescence resonance energy transfer (FRET) between cyan and yellow fluorescent proteins. An alternative approach to compartmentalization is the creation of nanoparticles which we are going to optimize using the nanoparticle producing protein Mms6 within Escherichia coli.

Microcompartments

Lumazine synthase (LS) from Aquifex aeolicus was mutated at four positions by introducing glutamates. The system was modeled and the mutations were found to result in a more negative formal charge of -480 in comparison to the wild type +180 for a 60 subunit capsid. The nanoparticle producing protein (Mms6) has been constructed based on the protein found in Magnetospirillum magneticum.

Further modeling demonstrated that the capsid interior diameter is between 83 and 89 nm. The fluorescent protein (FP) volume was calculated to be approximately 55.49 nm$^3$ showing that between 5 or 6 proteins can fit into the capsid unable to exit through the 1.93 nm pores.

Figure 1. Electrostatic surface potential map of two subunits of the pentamer mutant LS of the a) inside face and b) outside face. Areas of positive and negative charge are shown in blue and red, respectively.

Figure 2. Electrostatic surface potential map of 11 subunits map with 1 subunit cap removed of the a) wild type and b) mutant LS. Areas of positive and negative charge are shown in blue and red, respectively.

Figure 3. A representation of cyan and yellow fluorescent proteins (FP) within the LS microcompartment.

Figure 4. Schematic representation of the construction strategy of R$_{10}$ fusion proteins (Biofusion, Silver Lab) which can be targeted to the LS microcompartment by the electrostatic forces.

Figure 5. Fluorescence of cell extracts containing R$_{10}$ YFP constructs.

Figure 6. Schematic representation of FRET occurring between the donor CFP (excitation 439 nm, emission 476 nm) and acceptor YFP (excitation 514 nm, emission 527 nm).

Figure 7. The final LS microcompartment co-localization construct.

Figure 8. The final Mms6 construct.

Figure 9. Electron microscope images of magnetite nanoparticles obtained by co-precipitation of FeCl$_3$ and FeCl$_2$ in solution a) without protein and b) with Mms6 (Prozorov et al., 2007).

Nanoparticles

The nanoparticle producing protein (Mms6) has been constructed based on the protein found in Magnetospirillum magneticum.

Future Directions

- Complete the assembly of the lumazine synthase microcompartment co-localization construct.
- Characterize the lumazine synthase capsids
- Size of assembled capsids
- Characterize the method of co-localization
- Independently control lumazine synthase and fluorescent protein expression
- Localization photosynthetic protein(s) within the lumazine synthase microcompartment
- Characterization and optimization nanoparticle production based on localization of the Mms6 protein.
- Assembly and optimization of the photosynthetic fuel cell.

Judging Criteria

- Had a fun summer, now we are here!!!
- Complete and submit the iGEM 2009 Judging form
- Create and share a description of the team’s project
- Present a Poster and Talk at the iGEM Jamboree
- Enter information detailing new standard BioBrick Parts
- DNA submitted for new BioBrick Parts
- Demonstrate function new BioBrick Parts
- Characterize or improve an existing BioBrick Part or Device
- Help another iGEM team
- Detail a new approach to an issue of Human Practice
- Answered the four iGEM safety questions

References


Tsujiura et al. (2001). Photosynthetic bioelectrochemical cell utilizing cyanobacteria and water-generating oxidase. Enzyme and Microbial Technology, 29, 225-231

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A Synthetic Future: Microcompartments, Nanoparticles and the BioBattery

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