Adjustable Multi-Enzyme to Cell Surface Anchoring Protein

Missouri Miners iGEM Team

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Abstract

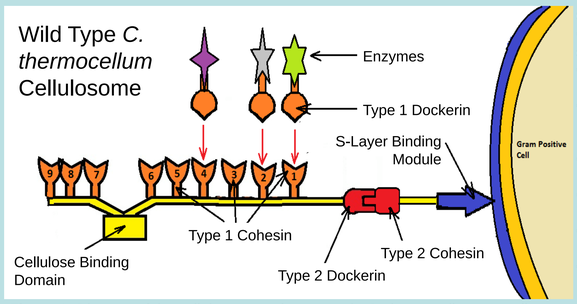
There are a plethora of enzymes that occur in the natural world which perform reactions that could be immensely useful to humans. Unfortunately, the efficiency of some of these reactions render their applications impractical. The cellulosome scaffolding protein produced by *Clostridium thermocellum* has been shown to significantly increase the efficiency of cellulose degradation. This scaffolding protein can be reduced in size and adapted for the cell surface of *Escherichia coli*. Different cohesion sites on the new cell surface display protein can be introduced to allow for attachment of desired enzymes. Future applications would include producing a collection of distinct versions of the scaffolding protein for unique arrangements and concentrations of enzymes, enabling the construction of extra-cellular assembly lines for a variety of multi-enzymatic reactions. This would lay the foundation for making previously infeasible applications of multi-enzymatic reactions possible through increased efficiency.

1 Background

Inspiration:
- Increase in cases of drug resistant *Mycobacterium tuberculosis*[4]
- Mycolic acids aid in drug resistance[2]
  - Protects the bacteria from host's immune system
  - Makes antibiotic treatment difficult[3]

Challenge:
- Mycolic acids are hydrophobic, complex fatty acids
- Degrade the mycolic acids extracellularly
- Maintain efficiency of the required multi-enzyme process

Solution:
- Engineer *E. coli* with an extracellular enzyme scaffold to break down mycolic acids
- Use cellulosome structure from *Clostridium thermocellum*
  - Contains binding sites for cellulose degrading enzymes
  - Increases efficiency of extracellular cellulose degradation[1]



Components:
- Cellulose-degrading enzymes
- Type 1 dockerin regions
  - bound to enzymes
- Type 1 cohesin regions
  - bound to scaffoldin
  - binds to type 1 dockerin
- Cellulose binding domain
  - bound to cellulose
- Type 2 dockerin region
  - bound to scaffoldin
- Type 2 cohesin region
  - binds to type 2 dockerin
- S-layer binding module
  - anchors to cell surface

Figure 1: Diagram of the cellulosome as it appears in *C. thermocellum* naturally

Broader Impact:
- Scaffolds can be used in a variety of applications
  - Plastic degradation
  - Bioremediation
  - Biofuels from waste products

2 Project Description

Purpose:
Create a customizable BioBrick tool kit that would allow teams to anchor multiple enzymes to the external surface of *E. coli*.

Goals:
- Replace the Gram-positive binding module of *C. thermocellum* with a protein compatible with Gram-negative bacteria
- Reduce the size of the cellulosome scaffoldin gene
- Compile and standardize a variety of cohesin and dockerin regions from other organisms

Steps:
1. Combine type 2 cohesin module (coh2) with the *E. coli* trans-membrane protein LPP-OmpA, BBa_K103006 to create Bba_K877001 (Figure 2)

2. Isolate both miniA1 and miniA2 (Figure 3) fragments from *C. thermocellum*'s scaffoldin CipA gene

3. Combine miniA1 and miniA2 to make miniA (BBa_K877000, Figure 4)

4. Link type 1 dockerin gene fragments to fluorescent protein (Figure 5)

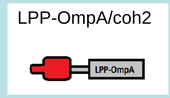


Figure 2: A diagram of our LPP-OmpA/coh2 part (BBa_K877001)

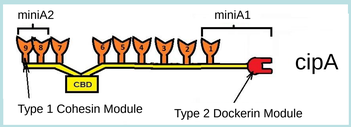


Figure 3: Source of miniA1 and miniA2 fragments from cipA

Figure 4: Diagram of miniA part (BBa_K877000)

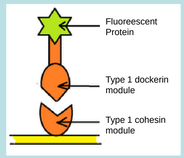


Figure 5: Diagram of fluorescent protein attachment

References

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3 Making Our Parts

BBa_K877001: LPP-OmpA and CtCoh2
Stage: Planning
Description: This part is composed of the cellulosome's type 2 cohesin module and the Warsaw iGEM team's LPP-OmpA trans-membrane anchoring module.

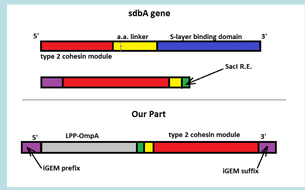

Figure 6: Replacement of the native s-layer binding module with the LPP-OmpA part to create our LPP-OmpA/CtCoh2 part

BBa_K877000: MiniA coding region
Stage: Submitted
Description: This part is composed of the miniA1 and miniA2 fragments. miniA1 codes for the type 2 dockerin and the first type 1 cohesin module of the cellulosome cipA gene. miniA2 includes the 8th and 9th cohesin modules of the cellulosome cipA gene.

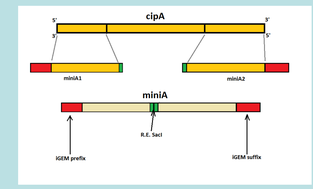

Figure 7: Assembly of our miniA part from portions of the cipA gene

4 Modeling

This model predicts the increase in reaction efficiency when all steps in a multi-enzyme process are localized.

$$\dot{n} = R_{in} - R_{out} = \beta\rho(N-n) - \frac{n}{\tau}$$

N = total number of enzymes present
n = number of enzymes with substrate bound
τ = average time an enzyme stays bound to substrate
ρ = density of enzymes present (effectively increased by scaffoldin)
β = enzyme affinity for substrate

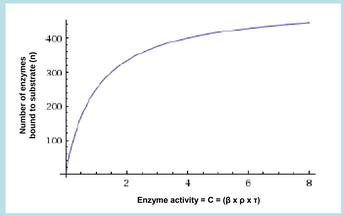

Figure 8: The effect of enzyme activity on number of substrate-bound enzymes

5 Future Steps

- Determine the effects of the scaffoldin on fatty acid oxidizers
- Create a library of standardized cohesin and dockerin modules from a variety of species for simplified customization of scaffodin

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