Synthetic Cellulosome for Cellulosic Biofuel Synthesis

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Introduction

Cellulosome is a complex of enzymes that degrade plant cell wall polysaccharides. It consists of a central noncatalytic scaffoldin protein bearing up to nine catalytic subunits (Fig. 1). Assembly of cellulosome occurs by a specific high-affinity interaction between cohesin domains of scaffoldin protein and dockerin domains of catalytic subunits. As a multi-enzyme machinery, cellulosome promotes synergistic action among different resident enzymes and enables highly efficient hydrolysis of intractable cellulosic and hemicellulosic materials of plant cell wall (Fig. 1).

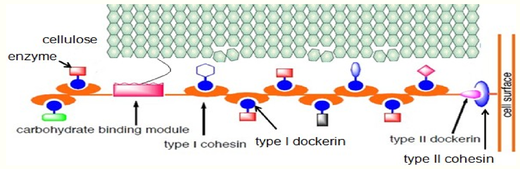


Figure 1. Schematic drawing of cellulosome from *Clostridium thermocellum*.

Our research goal is to expand the repertoire the repertoire of orthogonal cohesin-dockerin pairs, and assemble the synthetic cellulosome which incorporates the cellulases in a controlled way. The designer cellulosome will be tested on degrading various type of plant cellulosic materials for biofuel production.

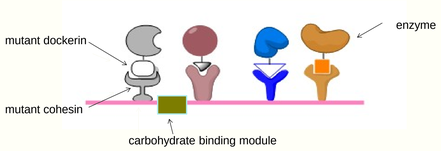
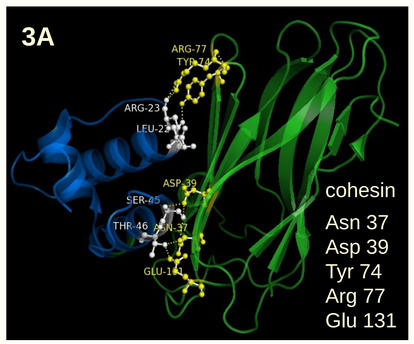


Figure 2. Schematic drawing of designer cellulosome.

Generation of Cohesin Mutant Library



Five residues (Asn37, Asp39, Tyr74, Arg77, and Glu131), which involve in cohesin-dockerin interaction, were randomized using overlapping polymerase chain reaction (PCR).

Library diversity (DNA) = $32^5 = 3.35 \times 10^7$

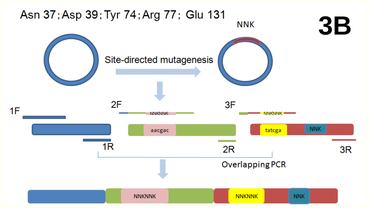


Figure 3. Generation of cohesin mutant library.
3A: Crystal structure of cohesin-dockerin complex; 3B: Flow chart of library construction.

Protein-protein Interaction: Two-hybrid System

In the positive selection, cohesin mutant library was selected against the dockerin mutant. If the cohesin mutants interact with the dockerin mutant, the expression of *His3* gene (Fig. 6), which allows host strain to survive on plates containing 3-amino-1,2,4-triazole (3-AT), is turned on.

In the negative selection, the reporter is URA3 (orotidine 5-phosphate decarboxylase). If cohesin mutants retain effective interaction with wild type dockerin, the expression of URA3 leads to the conversion of 5-FOA into a toxic compound (potent inhibitor to thymidylate synthase) and results in cell death (fails to produce pyrimidines).

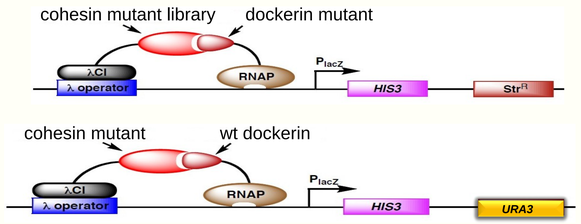
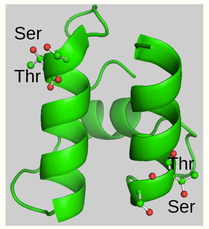


Figure 4. Positive and negative selection system

Generation of the First Coh-doc Pair



Dockerin domain has a internal two-fold symmetry (Fig. 5), both "halves" of the dockerin can interact with cohesins in a similar manner. The dockerin domain from *C. thermocellum* contains a highly conserved serine-threonine motif (Fig. 5), which interacts with cohesin domain. Mutations in this motif caused a 1000-fold reduction in affinity towards wild type cohesin. We constructed a x6b-doc full mutant, which doesn't have interaction with cohesin wt and use it to titrate a partner cohsin mu from cohesin library.

Figure 5. Crystal structure of dockerin domain.

Coh wt	Asn37	Asp39	Tyr74	Arg77	Glu131
Coh 7.5b3	Tyr	Phe	Pro	Arg	Glu

Doc wt	Ser11	Thr12	Ser45	Thr46
X6b-doc full mu	Leu	Leu	Met	Gln

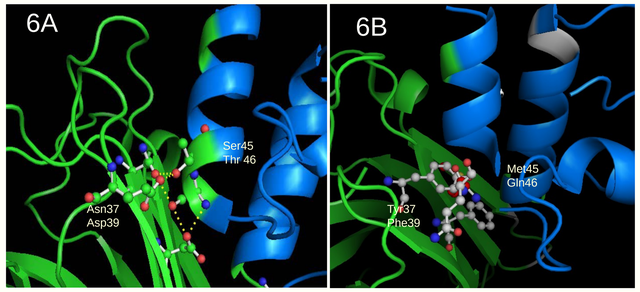
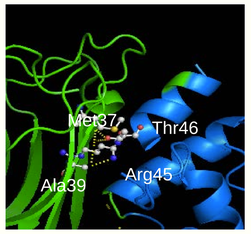


Figure 6A . Crystal structure of wt coh-doc; 6B Modeling of mutant residues contact

Generation of the Second Coh-doc Pair



We use x6b-doc full mu as template, randomized Met45/Gln46 to generate the dockerin mutant library. From the selection between cohesin and dockerin mutant library, a second pair of coh-doc was abtained.

Coh 8	Met37	Ala39	Val74	Arg77	Glu131
Doc 8	Leu11	Leu12	Arg45	Thr462	

Figure 7. Modeling of mutant residue contact

Verification of Orthogonality

	Non-selective	2.5 mM 3-AT
Cohesin8 + x6b-dockerin wt	>100	0
cohesin8 + x6b-dockerin mu	>200	0
Dockerin8 + cohesin wt	>100	0
Dockerin8+ cohesin 7.5b3	>500	0
Cohsin 7.5b3+doc wt	>1000	0
Dockerin full mu +cohesin wt	>1000	0
Cohesin wt+ dockerin wt	>5000	>5000

	Non-sel	5 mM 3-AT	7.5 mM 3-AT	10 mM 3-AT
coh8+doc8	800	>1000	800	>200
7.5b3+doc mu	>5000	>>1000	>1000	500(big, small)
coh wt+doc wt	>5000	>>1000	>>1000	>1000

Future Work

The synthetic scaffoldin is being assembled. To further confirm the interaction and the orthogonality of each coh-doc pair, isothermol titration calorimety will be used to get the dissociation constant of the protein pairs. After incorporation of a series of cellulases, highly efficient designer cellulosomes will be evolved and applied to the production of biofuel from abundant and renewable plant lignocellulosic biomass.

Acknowledgement

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