2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Engineering yeast consortia for surface-display of complex cellulosome structures: A consolidated bioprocessing approach from cellulosic biomass to ethanol

Date: May 21, 2013
Technology Area Review: Biochemical Conversion

Principal Investigator: Wilfred Chen
Organization: University of Delaware

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Goal Statement

- The goal of the project is to develop a synthetic yeast consortium for direct fermentation of cellulose to ethanol, a key program goal for the Biochemical platform. The strategy proposed here emphasizes the efficiency of hydrolysis and synergy among multi-cellulases, rather than focusing on the amount of enzymes produced or used.

Quad Chart Overview

Timeline

- Project start date – 10/1/09
- Project end date – 6/30/13
- Percent complete – 80%

Budget

- Total project funding
  - DOE share – $599,966
  - Contractor share -$152,870

Barriers

- Barriers addressed
  - Feedstocks and Biochemcal.

Partners

- Wilfred Chen is the overall project manager

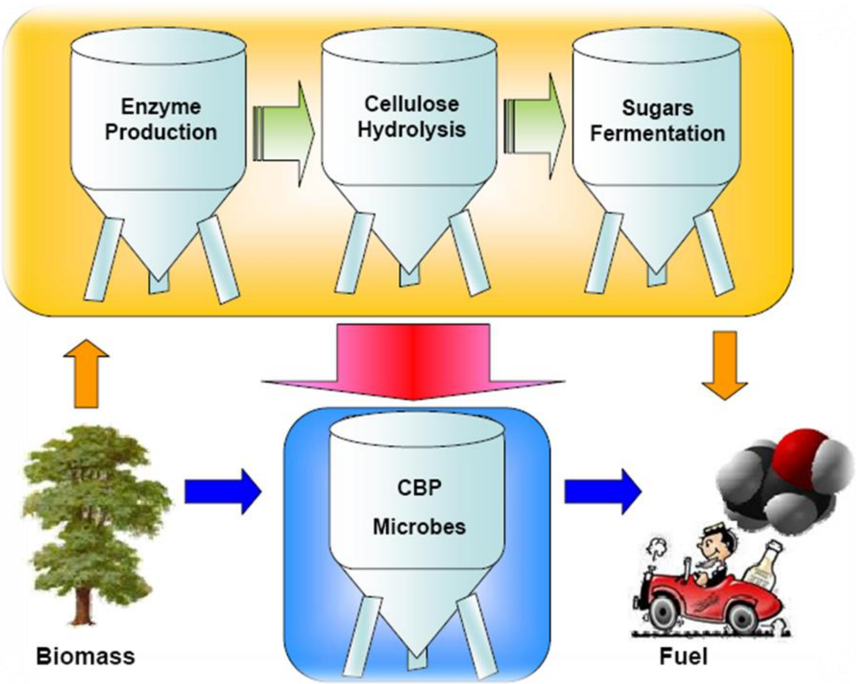
Project Overview

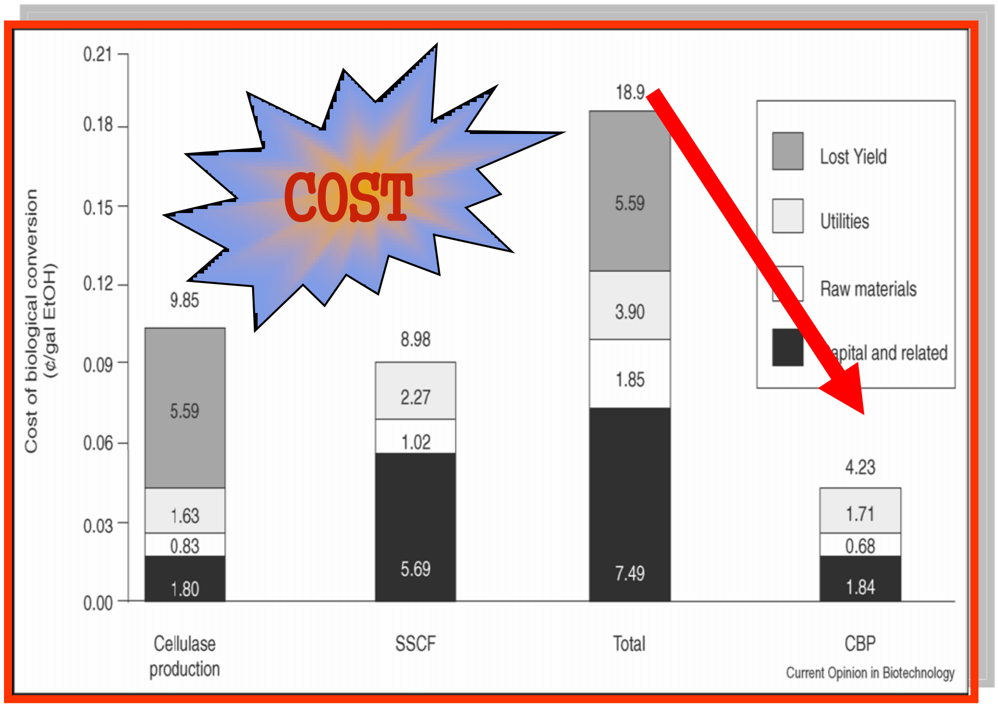
- The overall objective is to emulate the success of a natural cellulose hydrolysis mechanism. A complex cellulosome structure will be assembled onto the yeast cell surface using a synthetic consortium, which will enable the ethanol-producing strains to utilize cellulose and concomitantly ferment the sugars to ethanol.

Approach

- ***The engineering strategy proposed emphasizes the efficiency of hydrolysis and synergy among cellulases, rather than focusing on the amount of enzymes produced or used. To emulate the natural cellulosomes for efficient cellulose hydrolysis, complex cellulosomes will be assembled on the yeast cell surface, enabling the efficient production of ethanol from cellulose. More importantly, by organizing these cellulases in an ordered structure, the enhanced synergy will increase the efficiency in hydrolysis, and thereby enhance ethanol production. The use of a single yeast strain for surface anchoring and cellulase secretion is unlikely to be successful again based on bioenergetic limitations. To solve this problem, a synthetic yeast consortium will be developed for the functional presentation of the complex cellulosome structures.***

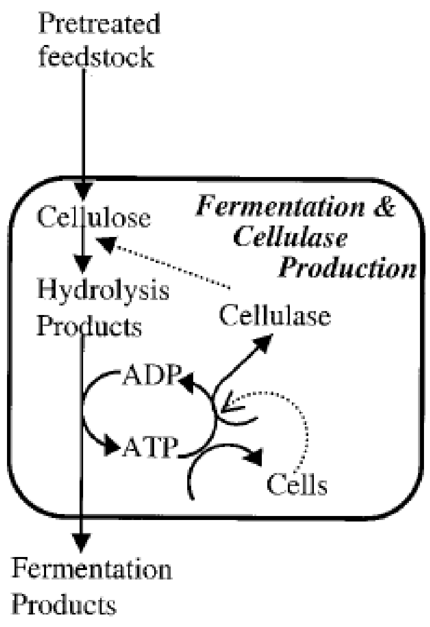
Problems with current systems





Lynd et al., Curr Opin Biotechnol, 2005

Two Possible Approaches



Good cellulolytic microorganisms

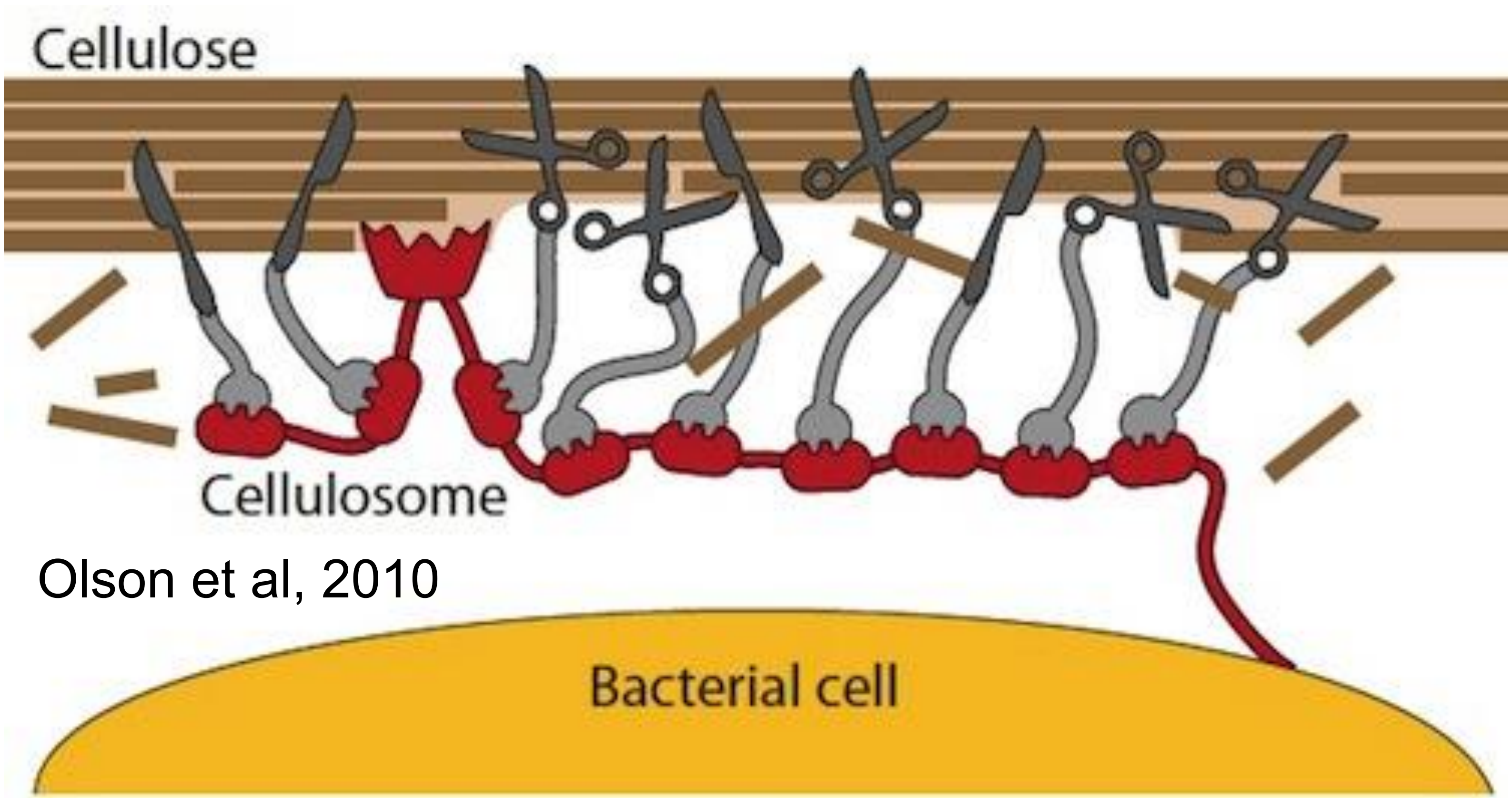
≠

Good ethanol producers

Slow hydrolysis rate of cellulose as high-level secretion of cellulase in a good ethanol producer is **energetically unfavorable under anaerobic condition**

- **Mimic natural anaerobic mechanism - Cellulosome**

Cellulosome



Olson et al, 2010

docke

0 5 10 15 20 25

Time (h)

Haimovitz et al., Proteomics, 2008 er et al., 2006

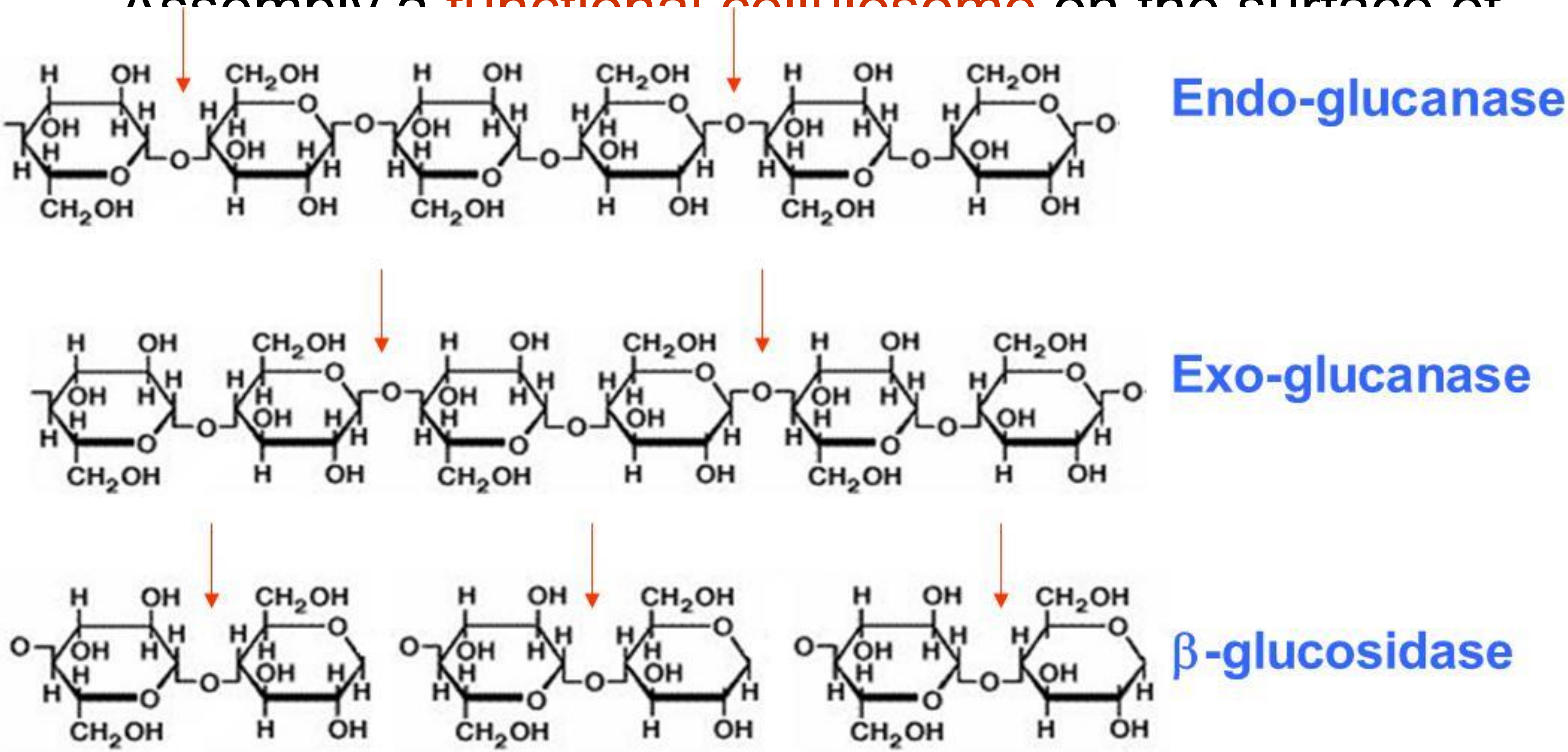
Ding et al., Curr Opin Biotechnol, 2008

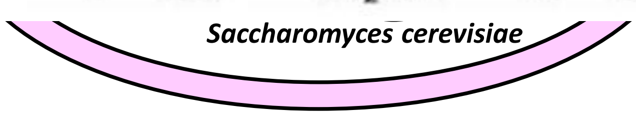
1. A surface-bound enzyme complex found in anaerobic microbes

2. Hydrolyze cellulose up to 50-fold faster due to synergy

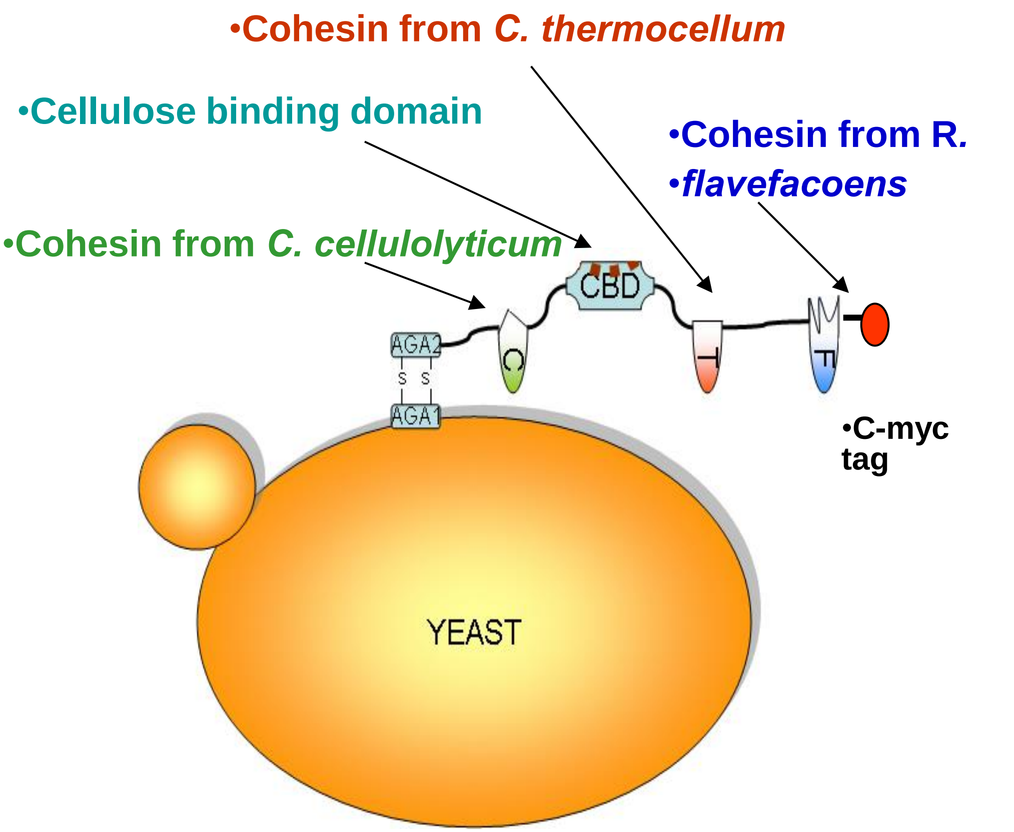
Display of artificial cellulosomes

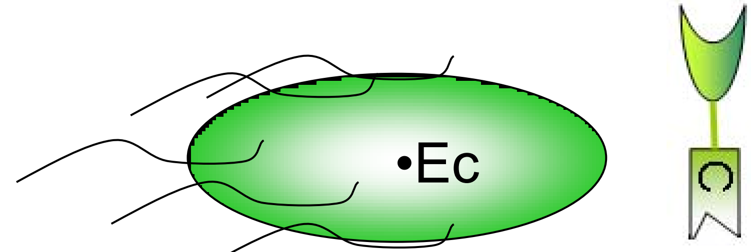
Assembly a functional cellulosome on the surface of



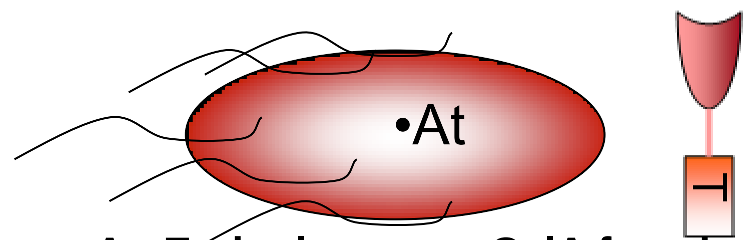


Scaffoldin Displaying Yeast and Cellulase Overexpressing *E. coli*

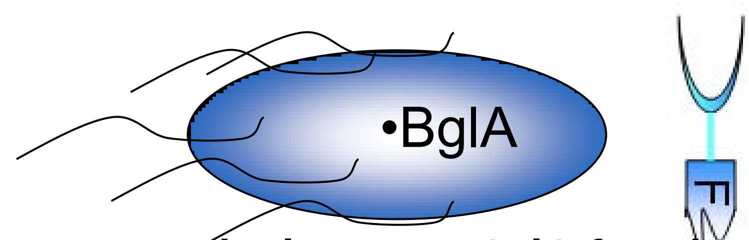




•An Exoglucanase CelE fused with a Dockerin from *C. cellulolyticum*



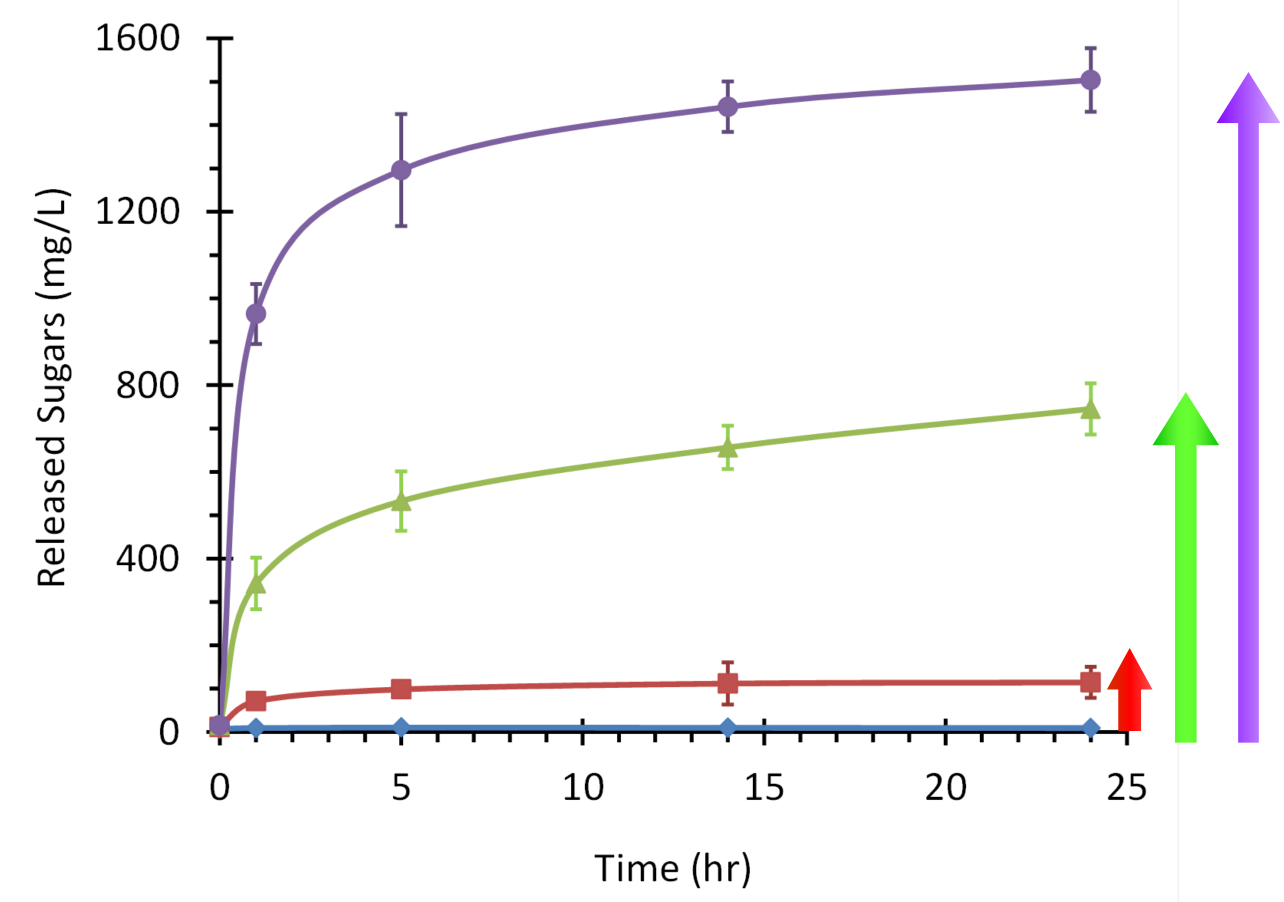
•An Endoglucanase CelA fused with a Dockerin from *C. thermocellum*

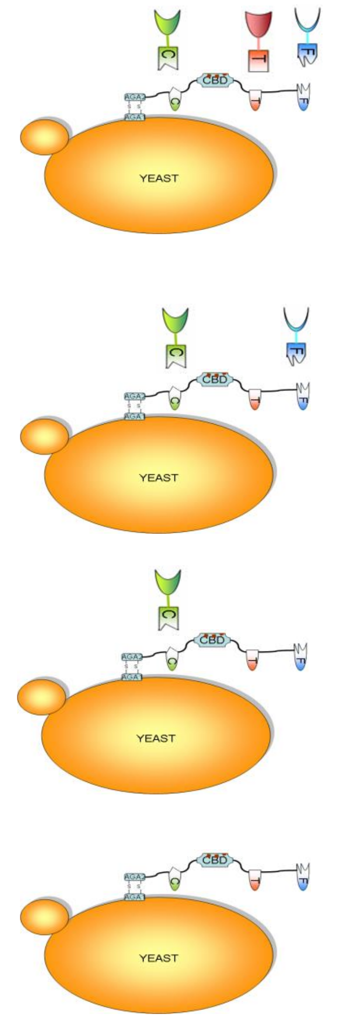


•An Endoglucanase CelG fused with a Dockerin from *R. flavefacoens*

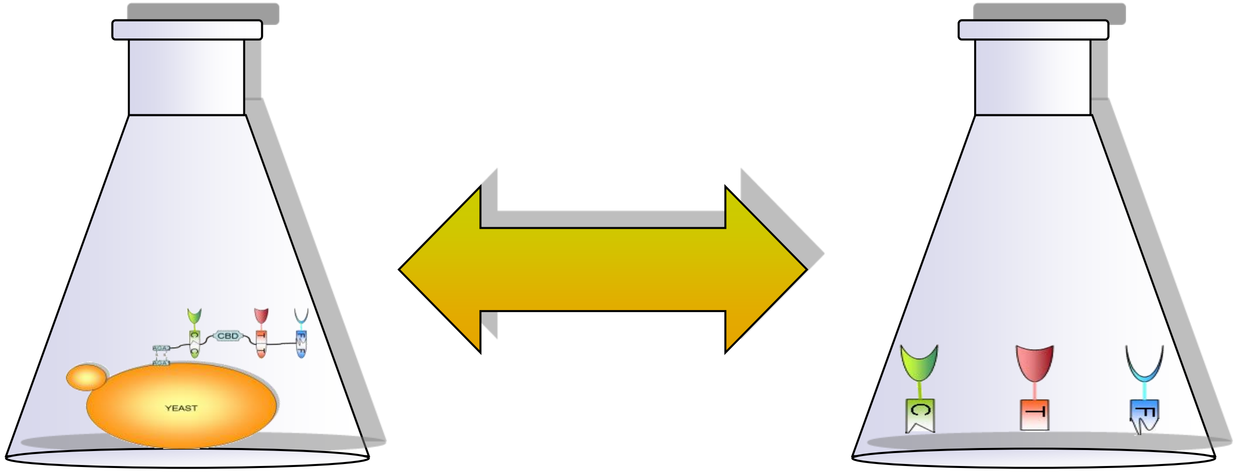
•Tsai et al., Applied and Environmental Microbiology, 2009

Functionality of Mini-cellulosomes





Synergistic Effects of Mini-cellulosomes

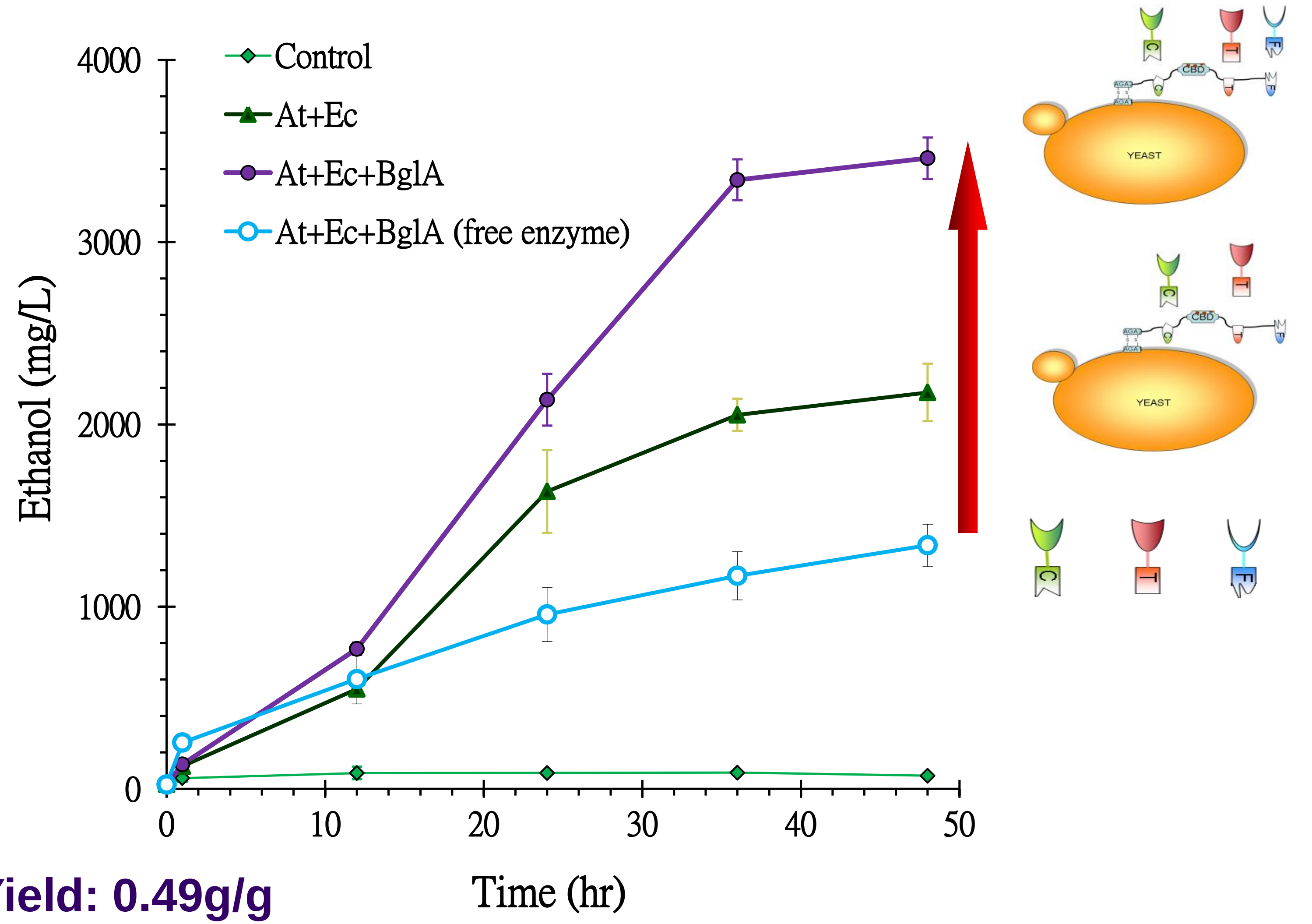


Maximum Synergy [a]		
One	Two	Three
1.70	2.06	2.64

a. $$Synergy = \frac{Reducing \quad sugar \quad from \quad cellulosome}{Reducing \quad sugar \quad from \quad free \quad enzymes}$$

b. The same combination of cellulases but without scaffoldin.

Resting cell ethanol production



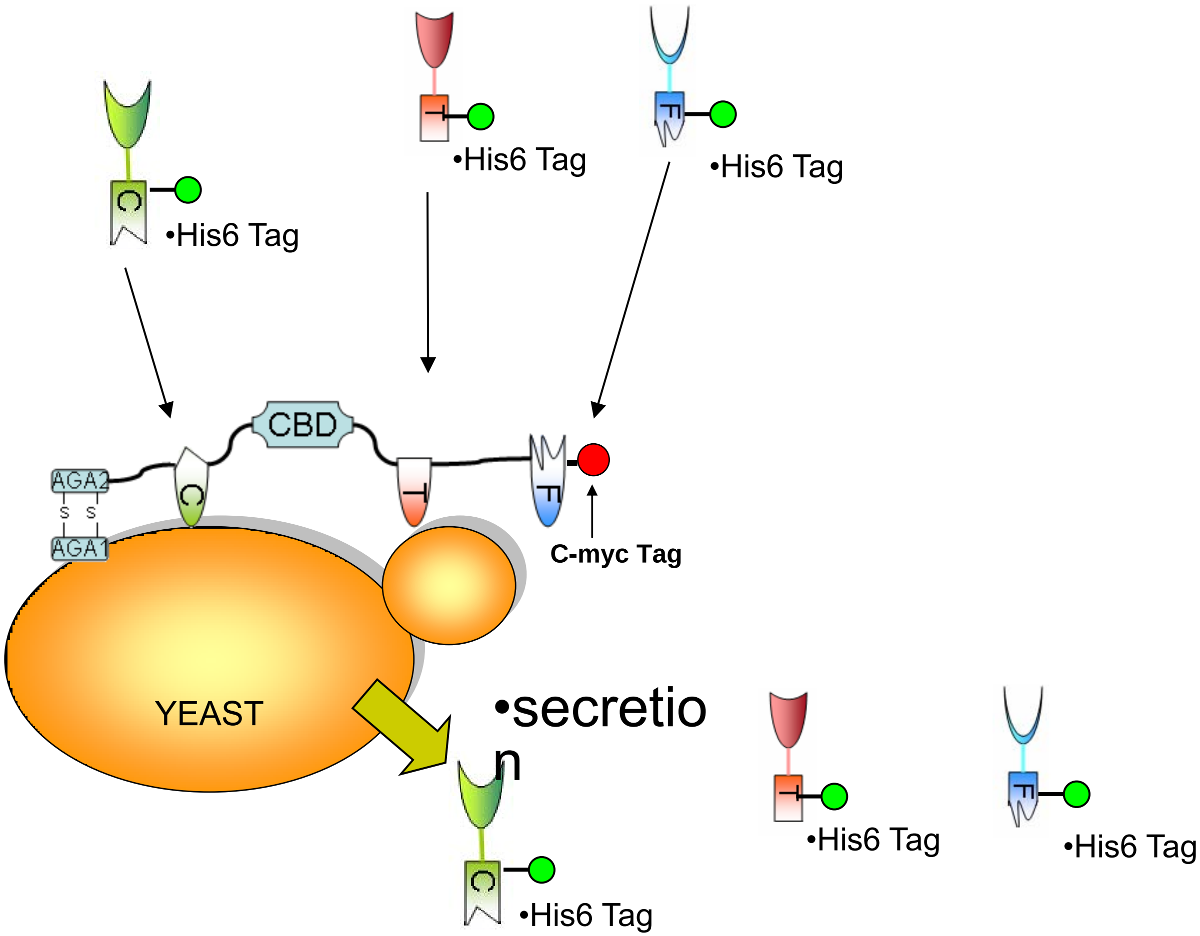
Ethanol Yield: 0.49g/g cellulose

Engineer a super yeast

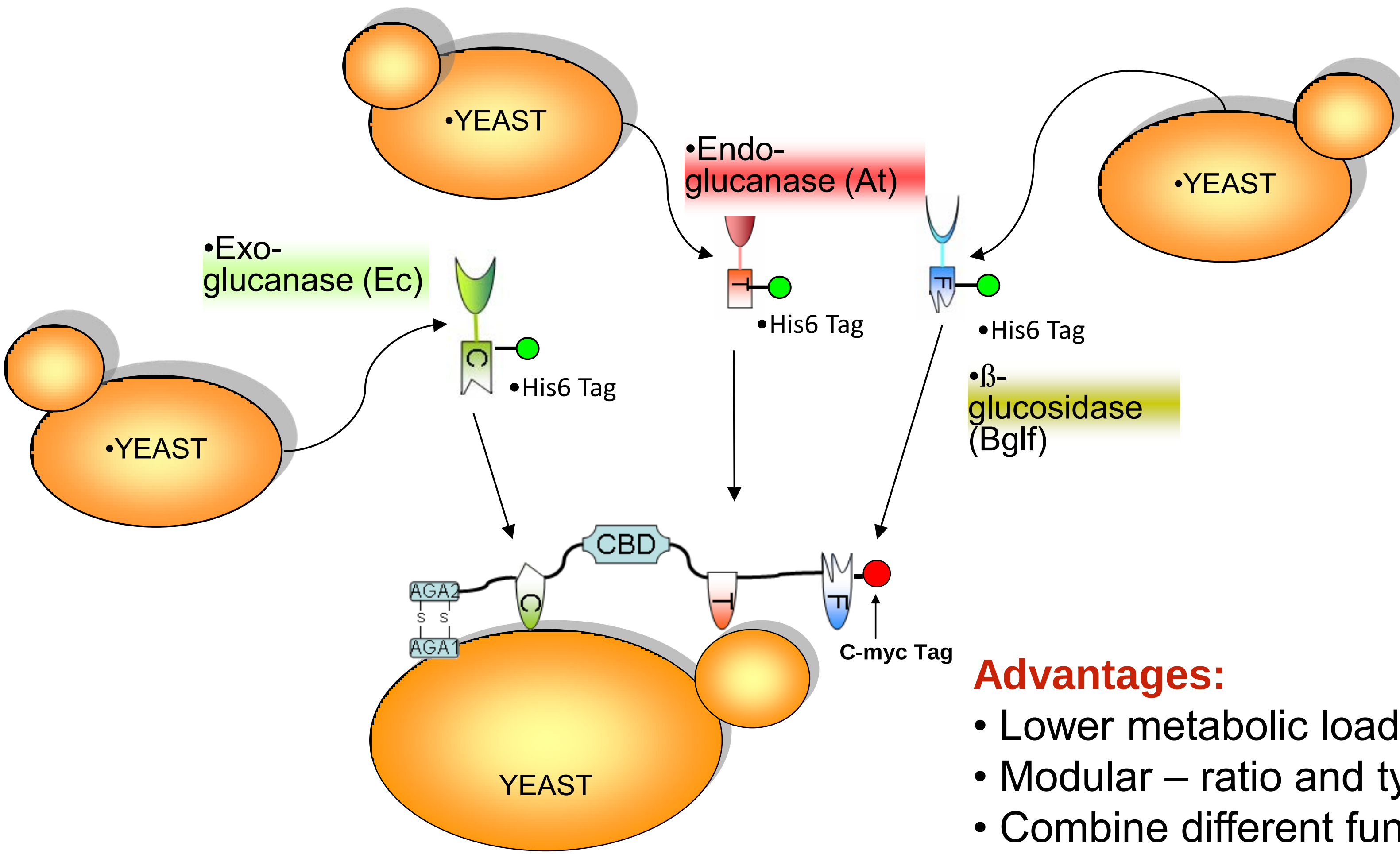
•Disadvantages:

• jamming of the translocation machinery

• energy intensive particularly under anaerobic growth



A Consortium Displaying At, Ec and Bglf

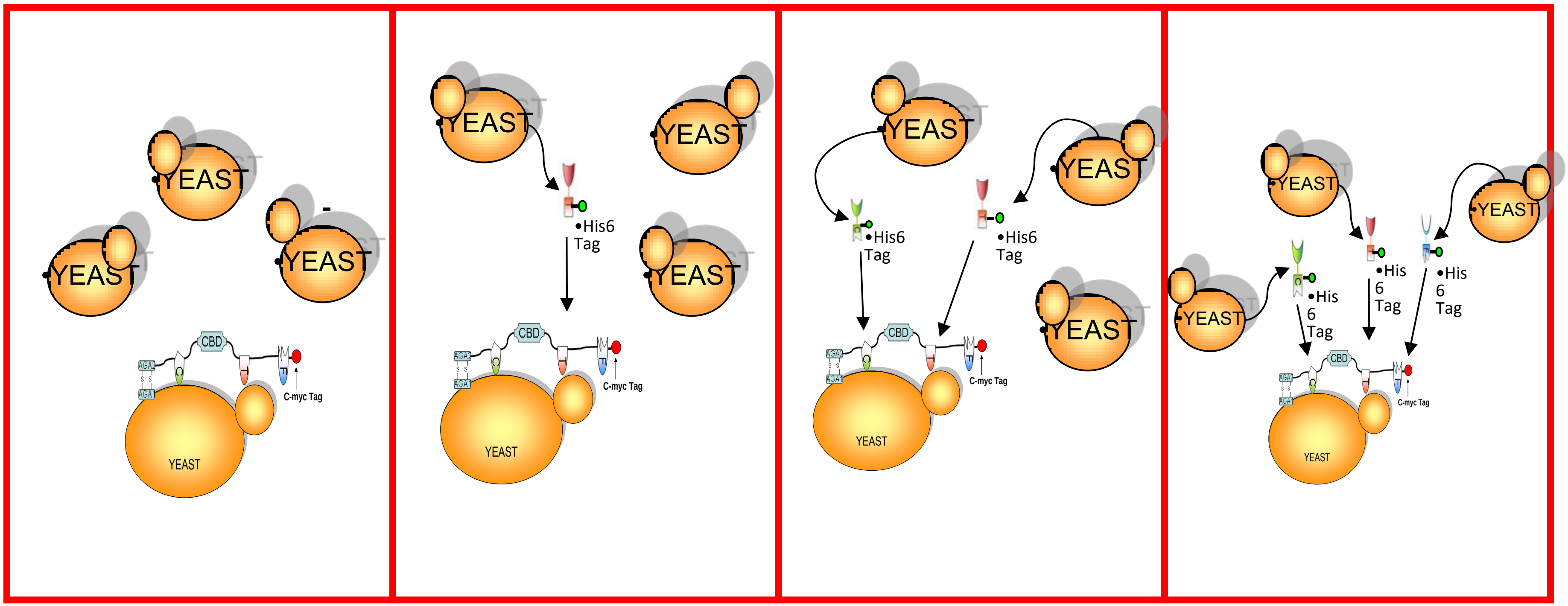


Advantages:

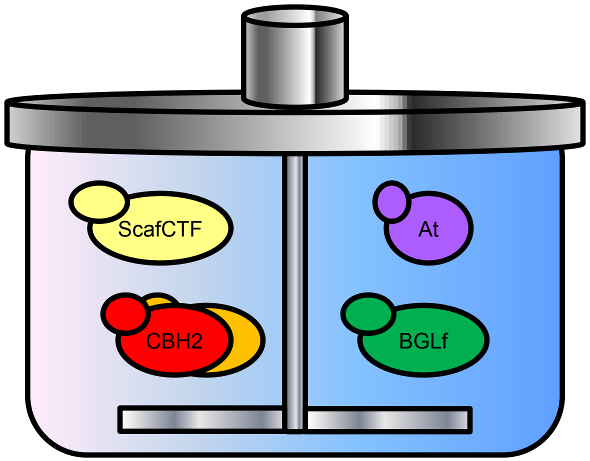
- Lower metabolic load
- Modular – ratio and type
- Combine different functions

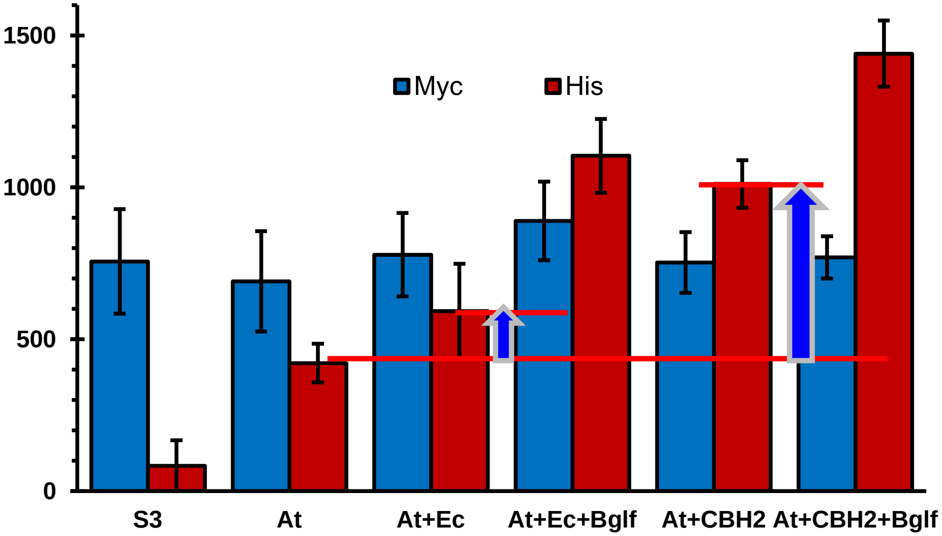
Synthetic Consortia

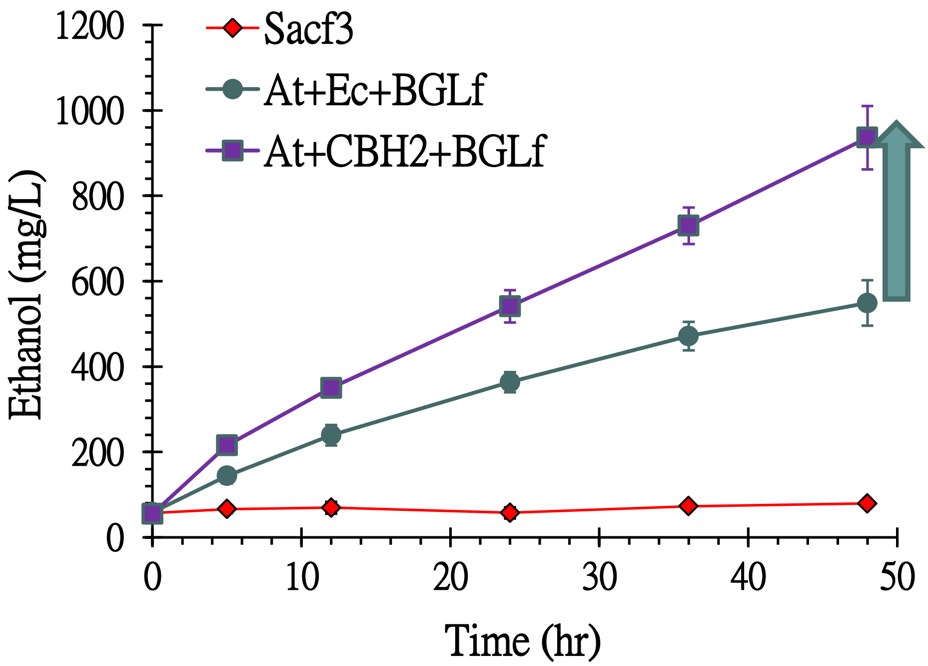
S3	S3, At	S3, At, Ec	S3, At, Ec, BGLf



Interchangeable- easily exchange any of the populations in the system



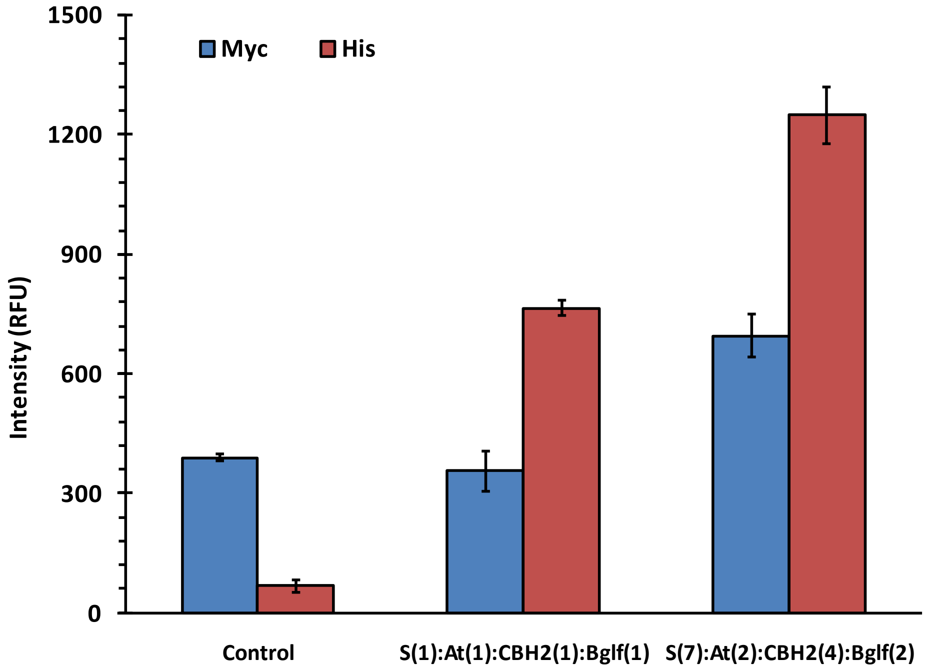


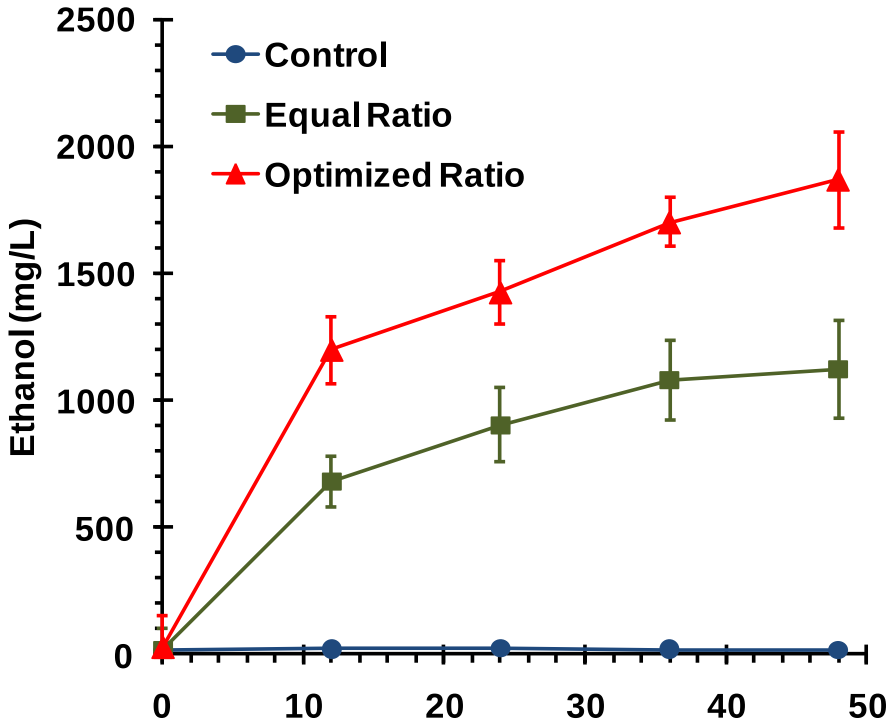


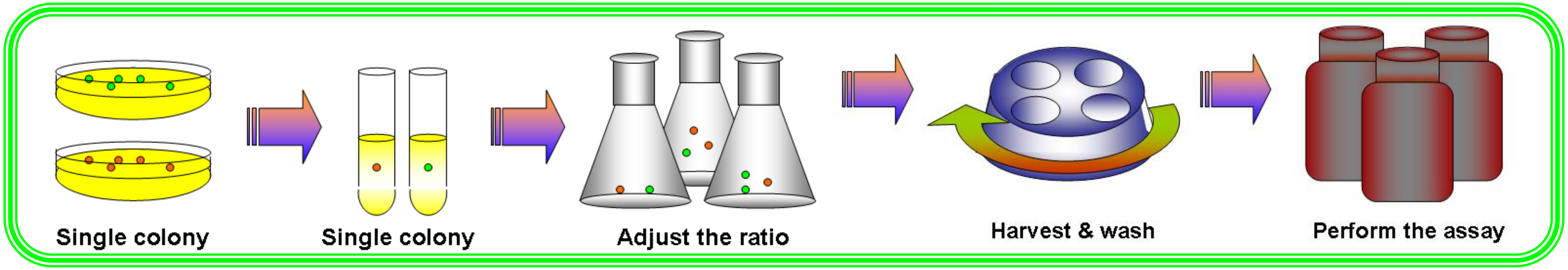
CBH2: a Cellobiohydrolase from *Trichoderma*.

Tsai et al., Applied and Environmental Microbiology, 2010

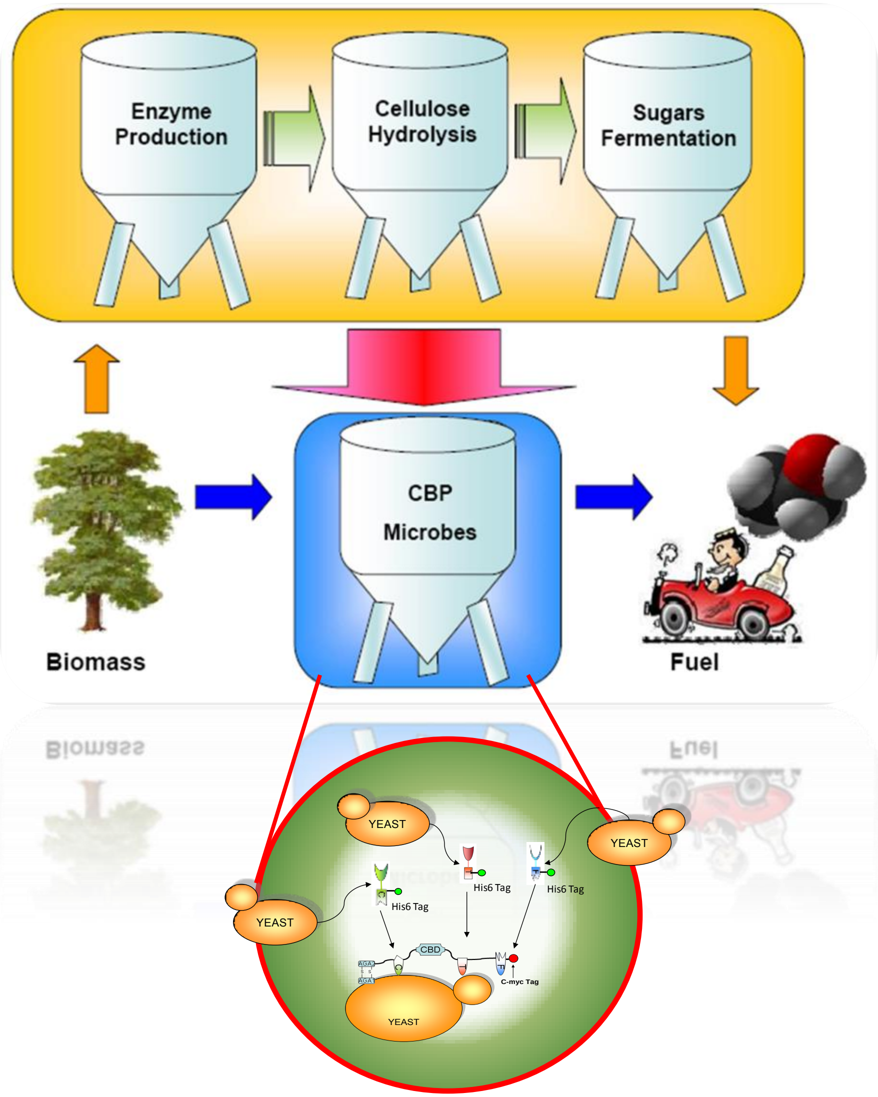
Improved Ratio for the Consortium





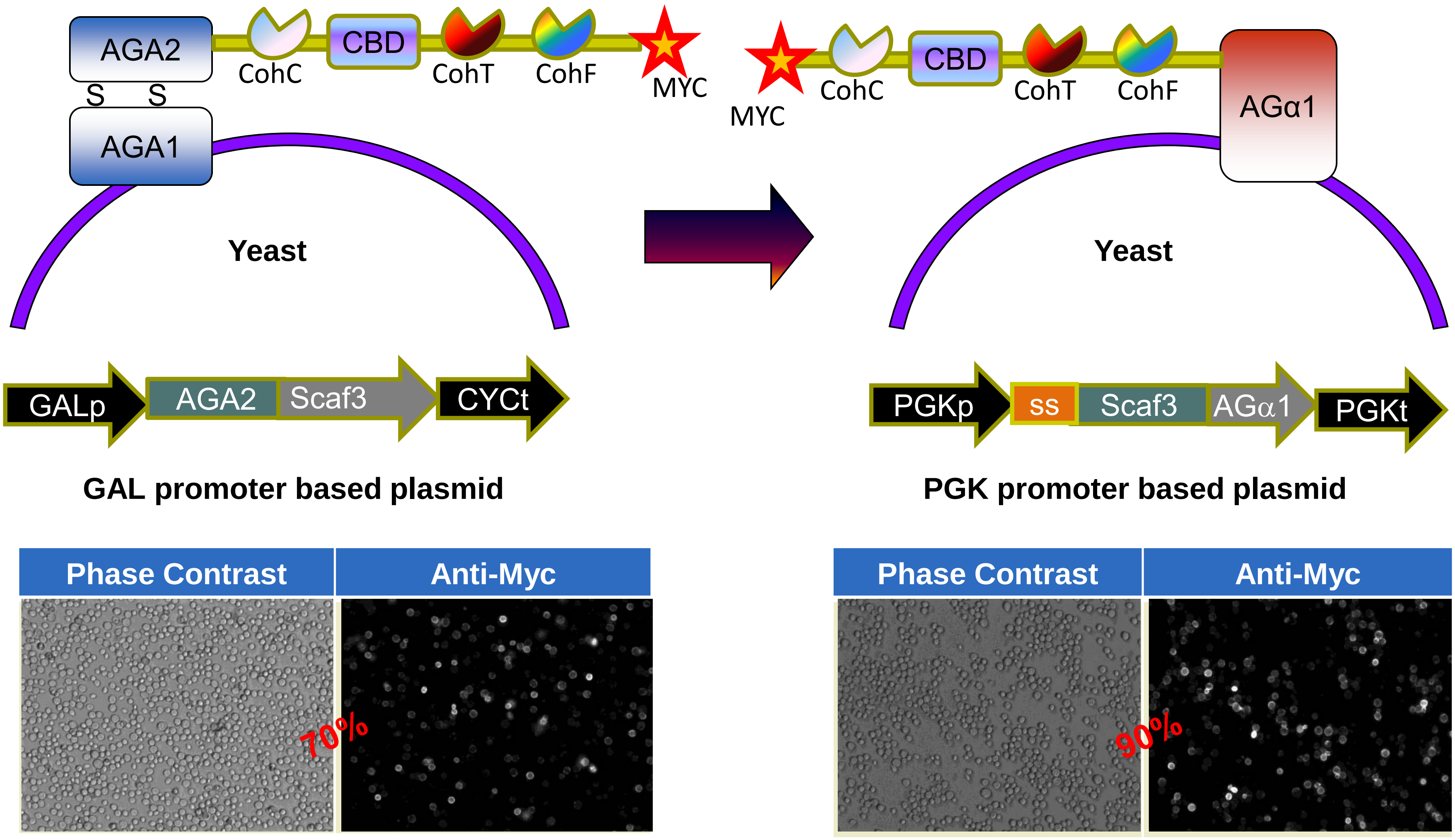


Can we use the consortium for CBP?

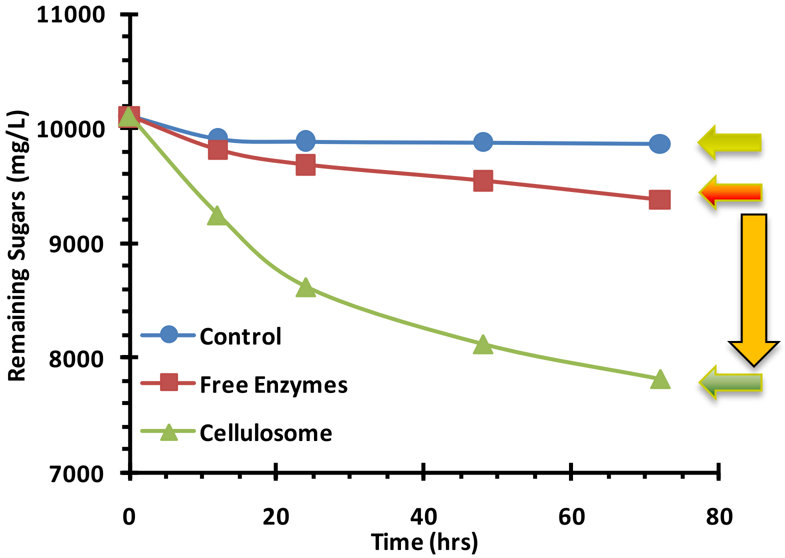


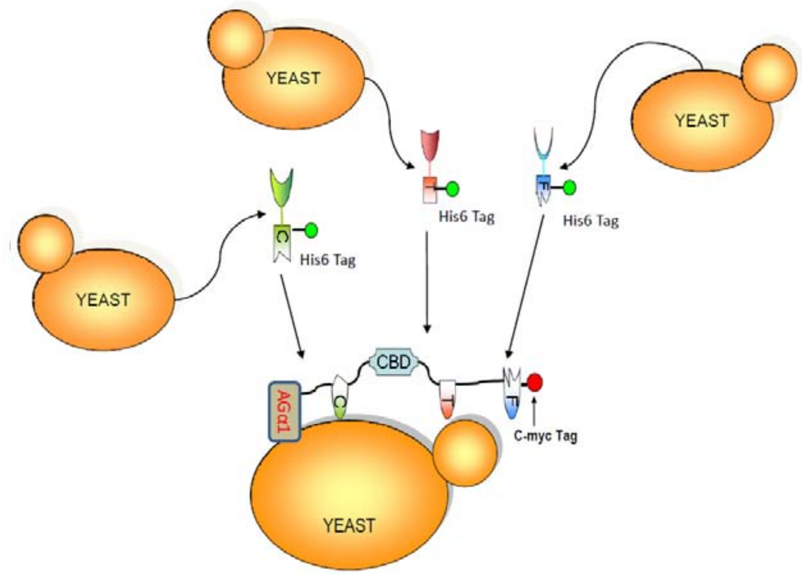
Goyal et al., Microbial Cell Factories, 2011

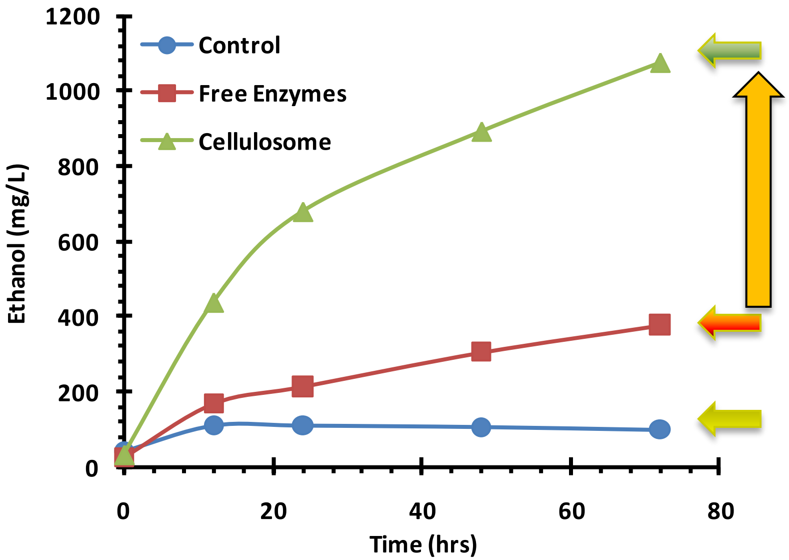
Display of scaffoldin using Agα1

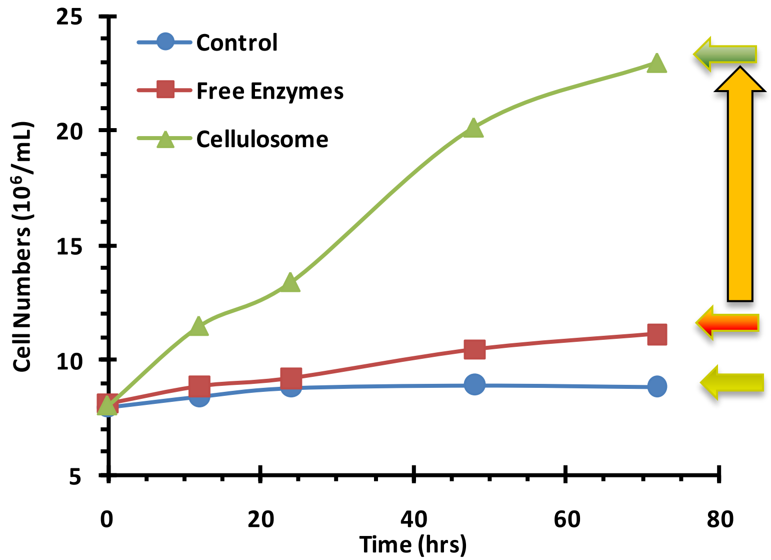


Growth and ethanol productio[illegible]

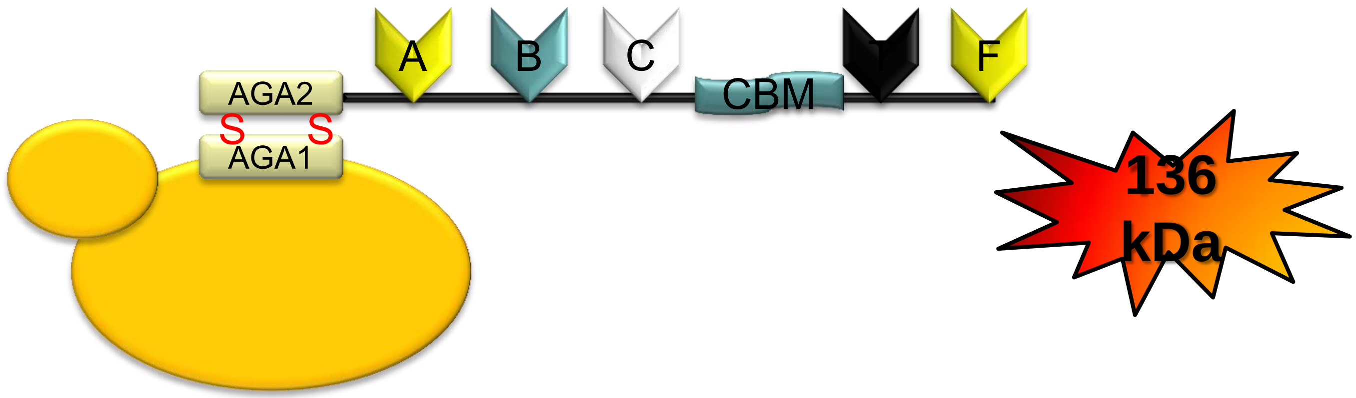


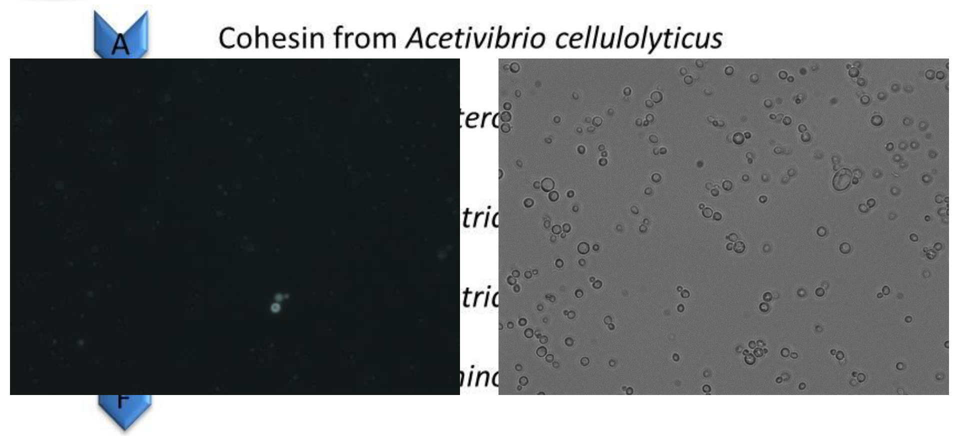






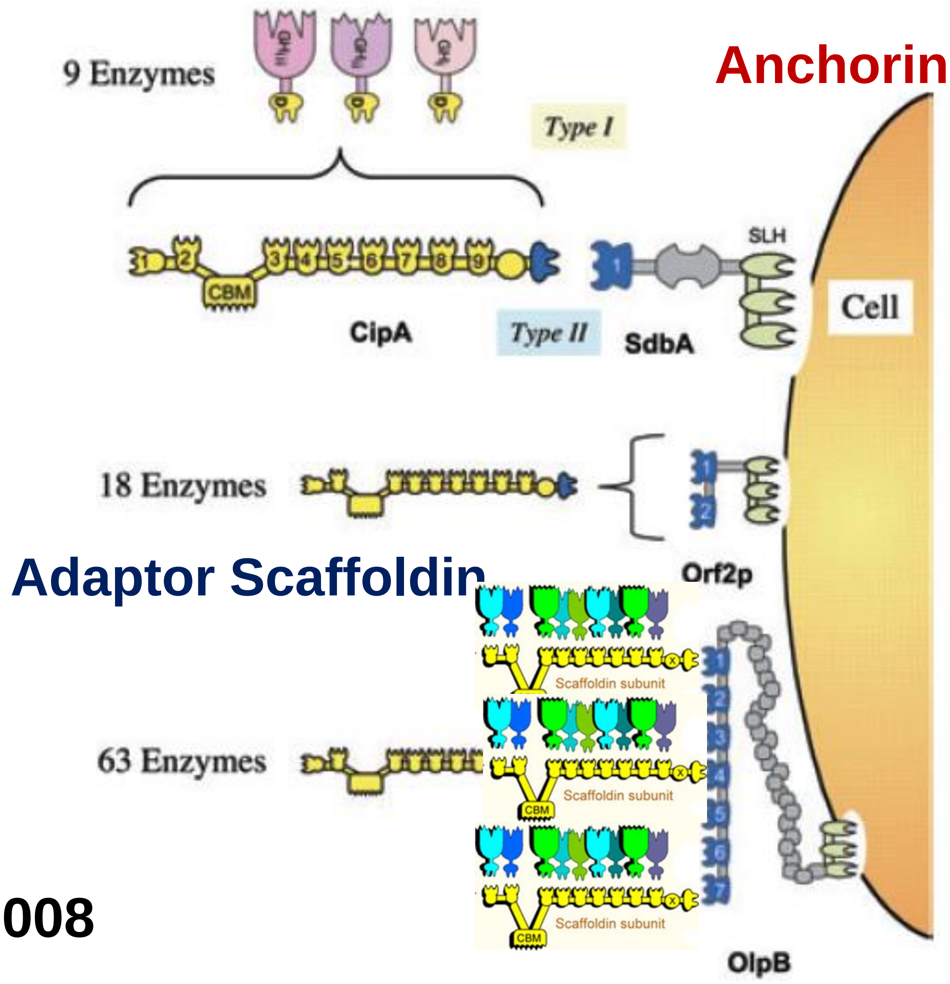
Display of a pentavalent scaffoldin





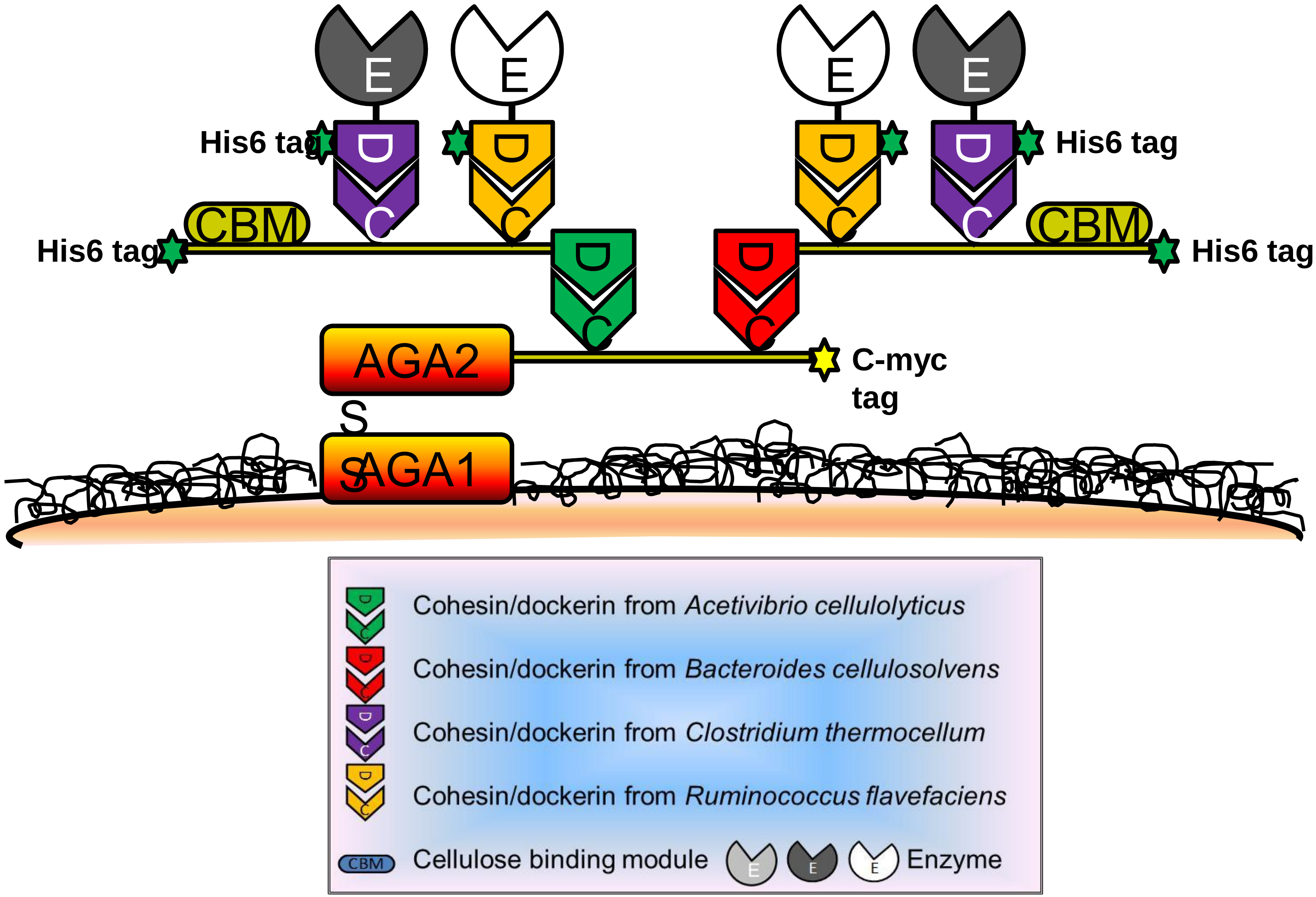
Translocation and folding problems

Improving enzyme density by Adaptive Assembly



Bayer et al, 2008

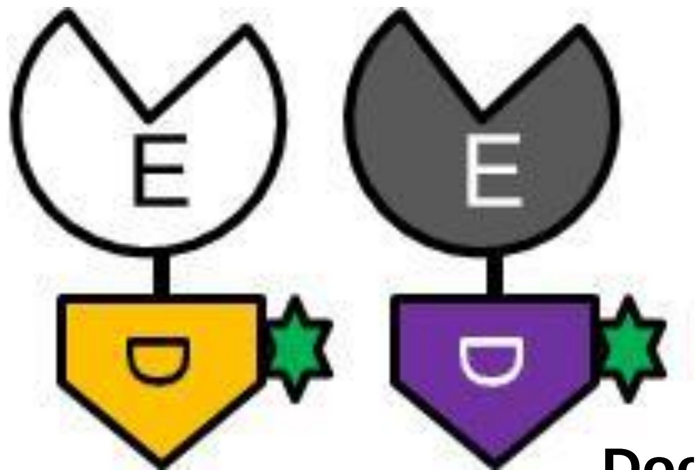
Adaptive assembly

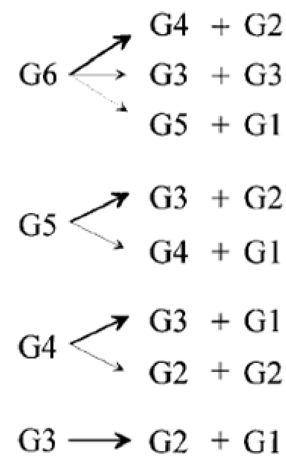


Tsai et al., ACS Synthetic Biology, 2013.

Dockerin-tagged enzymes

β-glucosidase BglA **Endoglucanase CelG**





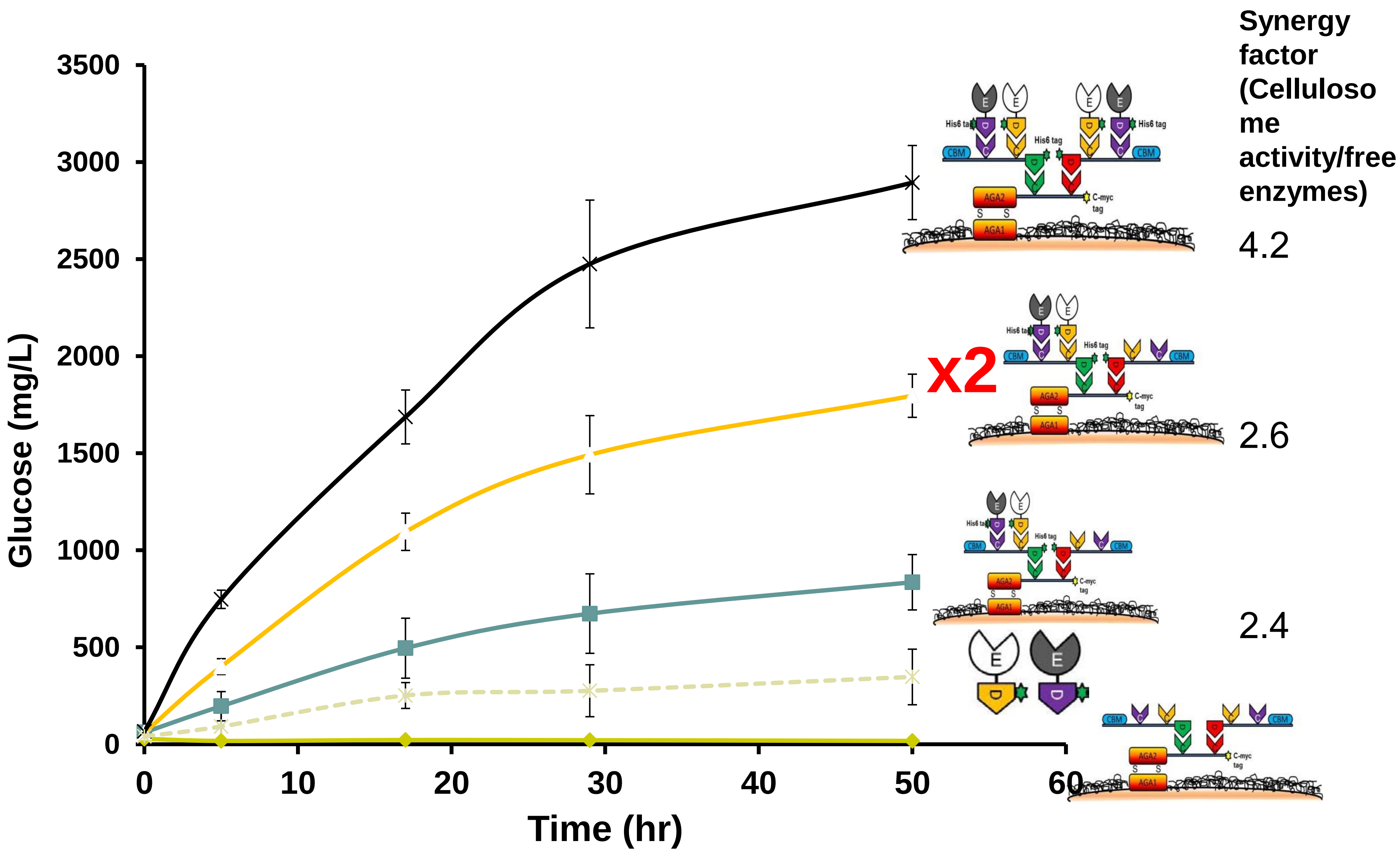
Dockerin from *R. flavefaciens*

Doc *thermocellum*

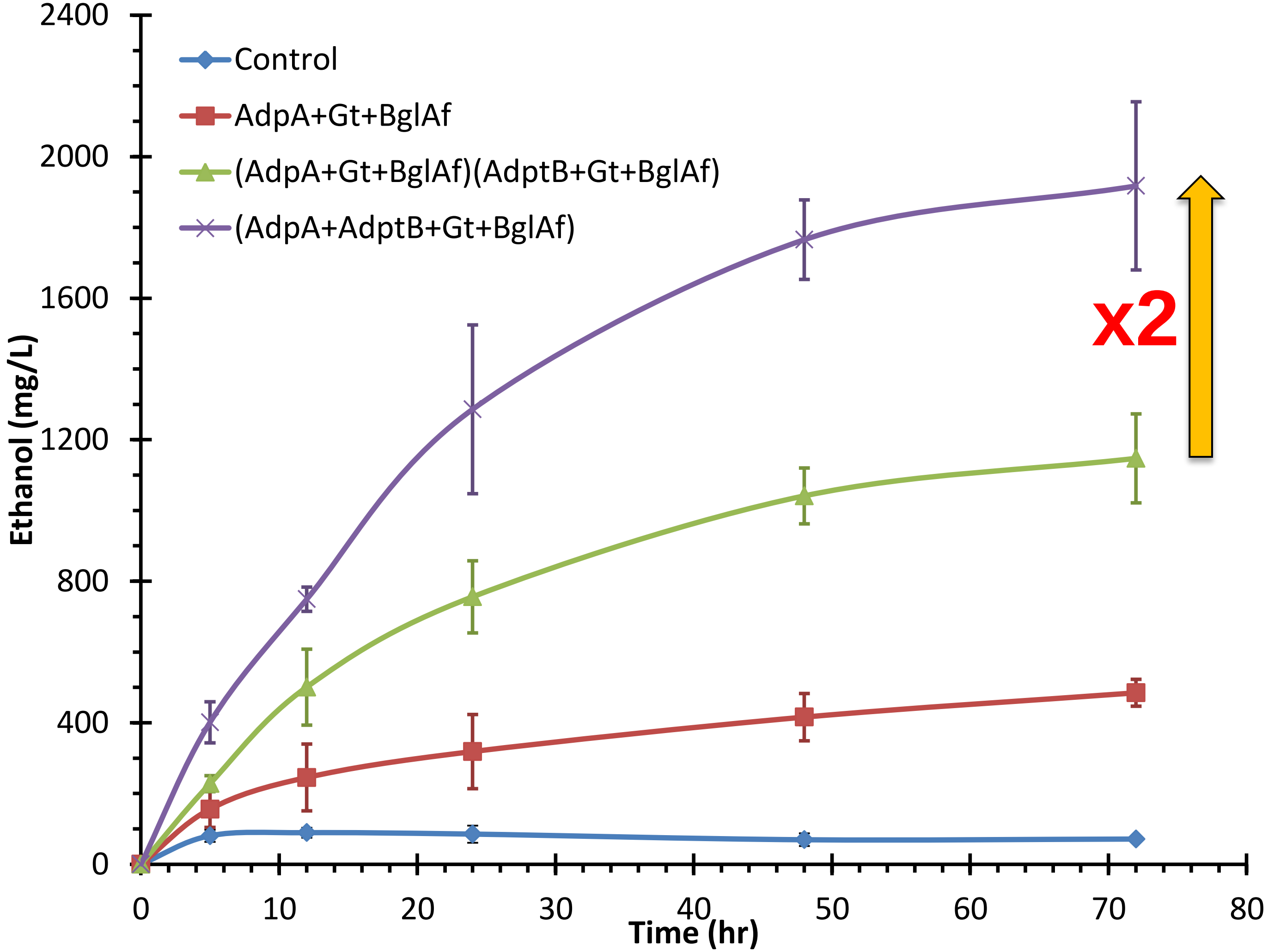
Bglf **Gt**

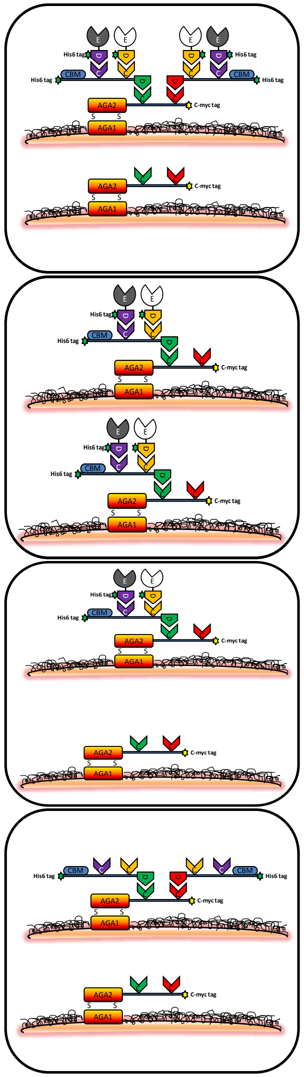
Gal et al, J Bacteriol, 1997

Hydrolysis of cellulose to glucose



Ethanol Production





Tsai et al. ACS Synthetic Biology, In Press.

Xylanosome: Enzymes

Hemicellulase system: Fungal thermostable enzymes, recombinantly expressed in *S. cerevisiae*

= **Endo-β-1,4 xylanase (XynA) from *T. lanuginosus* (literature: *P. pastoris* host)**

= **Acetylxylan esterase (AwAXE) from *A. awamori* (literature: *P. pastoris* host)**

= **β- xylosidase (XlnD) from *A. niger* (literature: *E.coli*; *A. awamori* hosts)**

Substrate for hydrolysis = Birchwood Xylan (Maximal acetic acid substitutions)

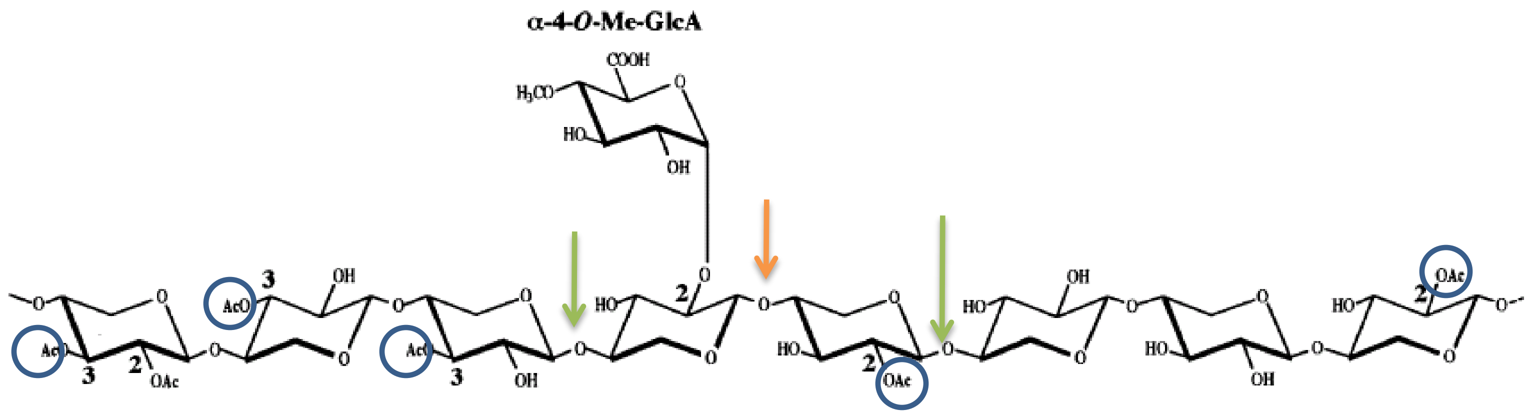
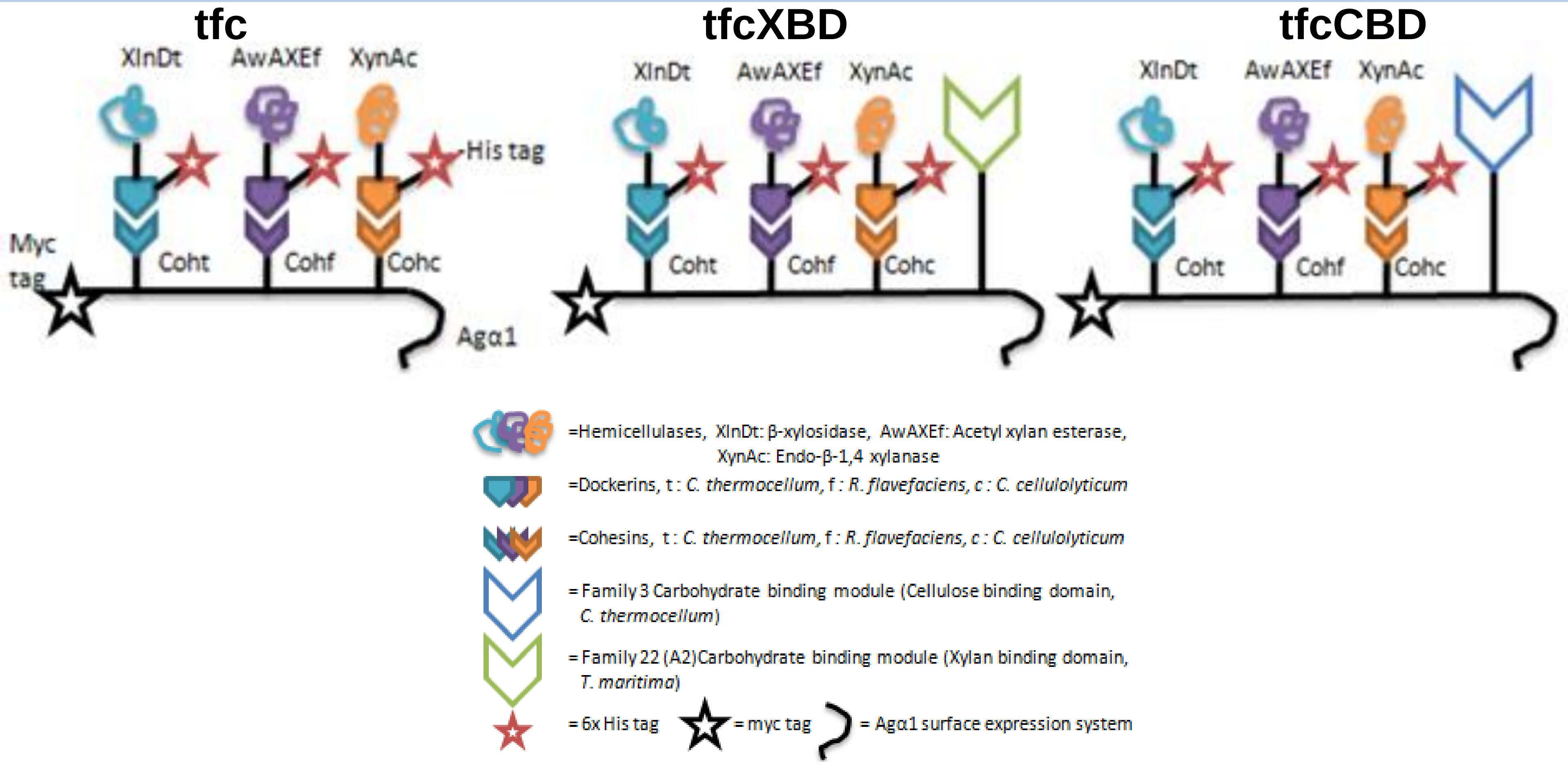


FIGURE 1. Composition of *O*-acetyl-4-*O*-methylglucuronoxylan (hardwood xylan). Numbers indicate the carbon atoms at which substitutions take place. Ac: Acetyl group; α-4-*O*-Me-GlcA: α-4-*O*-methylglucuronic acid.

Ref: "Xylanolytic Enzymes from Fungi and Bacteria", A. Sunna and G. Antranikien, *Critical Reviews in Biotechnology*, 17(1):39-67 (1997)

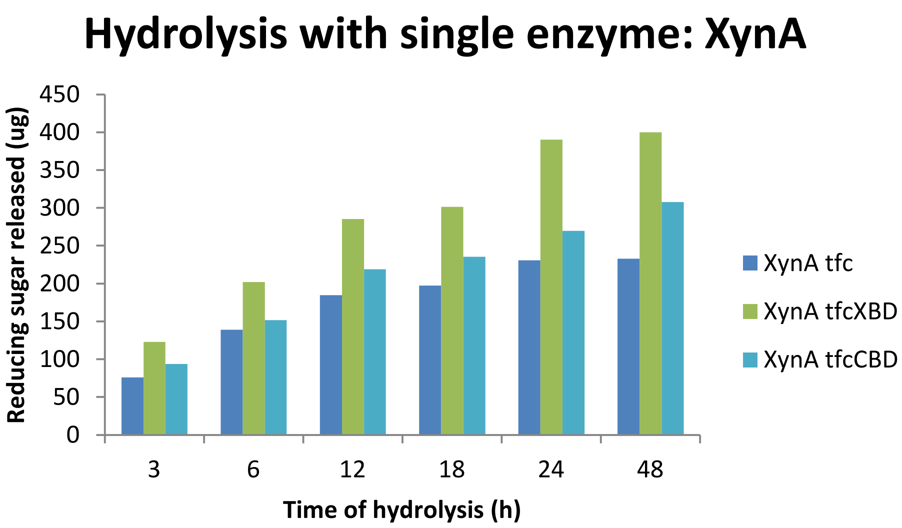
Comparison between binding domains

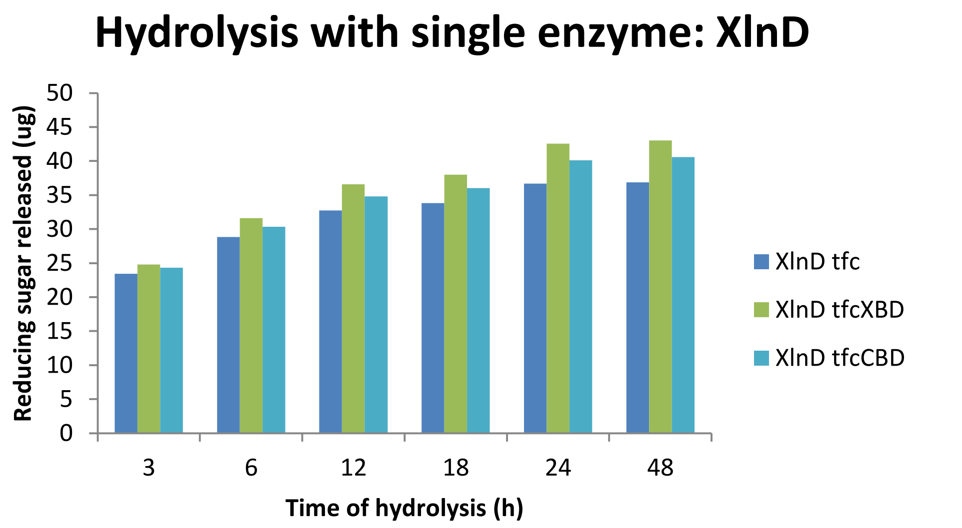


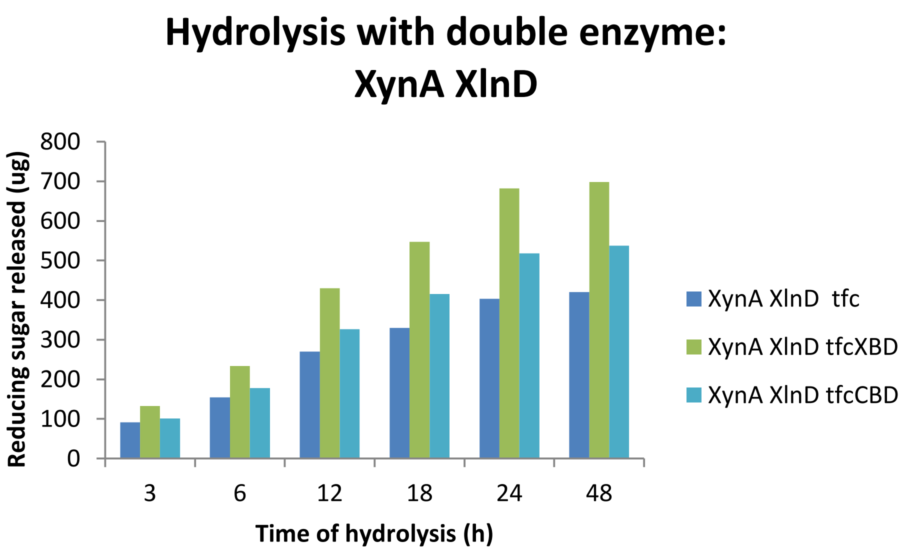
- To compare the effect of **A2 (xylan-binding)** domain with *C. thermocellum* **cellulose binding domain.**

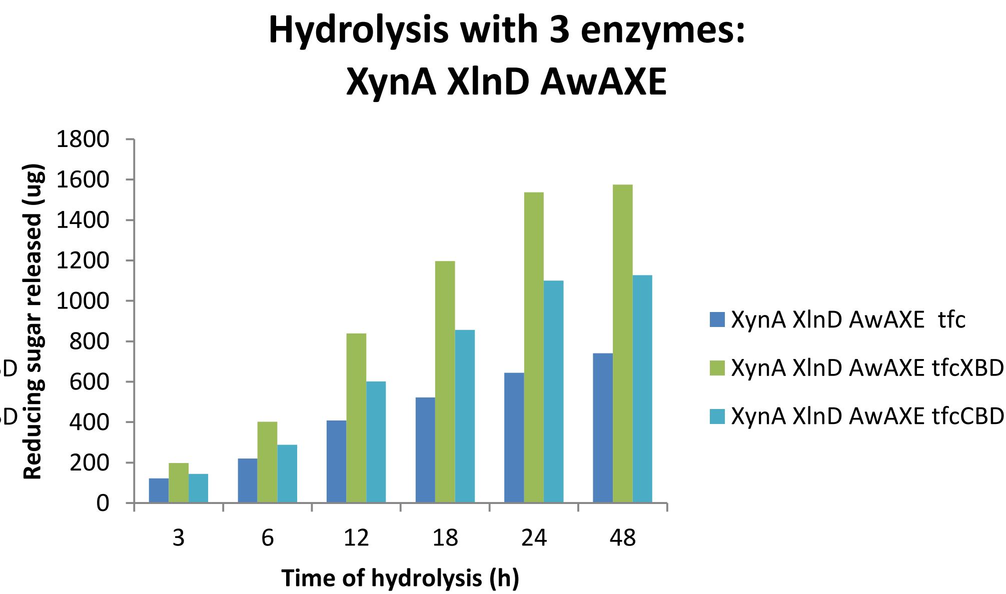
Srikrishnan et al. *Biotechnol. Bioeng.*, **110**, 275–285, 2013

Confirmed enzyme activity on scaffoldins: Study between binding domains, XBD and CBD



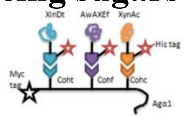
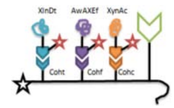
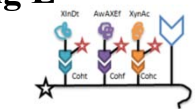






Srikrishnan, **et al**, *Biotechnol.Bioeng.*, **110**, 275–285, 2013

Conclusions from hydrolysis study
"tfcXBD" system shows improved hydrolysis over free enzymes

Enzymes loaded	Reducing sugars released in mg L^{-1}				Fold synergy compared to free enzyme		
	Free enzyme	 tfc(a)	 tfcXBD(b)	 tfcCBD(c)	(a)	(b)	(c)
XynA	214 ± 18	233 ± 16	389 ± 11	308 ± 18	1.1 x	1.8 x	1.4 x
XynA XlnD	324 ± 23	420 ± 10	699 ± 14	537 ± 13	1.3 x	2.2 x	1.7 x
XynA XlnD AwAXE	476 ± 17	740 ± 12	1575 ± 17	1127 ± 19	1.6 x	3.3 x	2.4 x
Fold synergy due to binding module		*1 x*	*2.1 x*	*1.5 x*			

- XynA shows 1.4- 1.8x increased activity with binding module (CBD or XBD)
- tfc XBD shows ~ 3.3x improvement in hydrolysis over free enzymes (tri-enzyme)
- tfc XBD shows ~ 1.4x improvement in hydrolysis over tfc CBD (tri-enzyme)
- Synergy observed due to enzyme proximity for tfc is ~1.6 x (tri-enzyme)
- Synergy observed due to binding module for tfcXBD is ~2.1x (tri-enzyme)

Technical Accomplishments/ Progress/Results

- ***Task 1: Functional assembly of mini-cellulosomes on the yeast surface for cellulose hydrolysis – A paper describing this has been published in AEM***
- ***Task 2: Develop a yeast consortium displaying mini-cellulosomes for cellulose hydrolysis by intracellular complementation – A paper describing this has been published in AEM***
- ***Task 3: Construction of synthetic yeast consortium displaying the mini-cellulosome for the simultaneous cellulose hydrolysis and ethanol production – A paper describing this has been published in Microbial Cell Factories***
- ***Task 4: A yeast strain displaying a complex cellulosome was developed using adaptive assembly – A paper describing this has been published in ACS Synthetic Biology***

Technical Accomplishments/ Progress/Results

- ***For the first time, a functional mini-cellulosome was successfully assembled on the yeast surface***

- ***The engineered yeast strains were able to retain the synergistic effect on cellulose hydrolysis, to produce simple sugars, and to produce ethanol***

- ***A yeast consortium was engineered to display a functional mini-cellulosome***

- ***The yeast consortium can grow and produce ethanol directly from cellulose more efficiently than cells secreting only enzymes***

Relevance

- ***A potentially useful way for ethanol production from cellulosic biomass using the consolidated bioprocessing approach***
- ***Successful display of a complex cellulosome could greatly enhance the overall efficiency and cost of ethanol production***

Success Factors and Challenges

- ***The ability of the surface displayed cellulosomes to retain the synergistic effect on cellulose hydrolysis is the most critical factor for the successful implementation of the approach***
- ***The ability to secrete multiple cellulases and adaptor scaffoldins is the key to the assembly of the complex cellulosome***
- ***This strategy may be a logical first step toward a CBP approach for ethanol production from cellulosic biomass***

Future Work

- ***Construction of yeast strains secreting the the adaptor scaffoldins and /or dockerin-tagged cellulases***

- ***Display of more complex cellulosomes via sortase A-mediated ligation***

Summary

- **Relevance:** ***Successful display of a complex cellulosome could greatly enhance the overall efficiency and cost of ethanol production***
- **Approach: Design a yeast consortium for the display of the complex cellulosome**
- **Technical accomplishments: For the first, a functional mini-cellulosome was successfully assembled on the yeast surface**
- **Success factors and challenges:** ***The ability of the surface displayed cellulosomes to retain the synergistic effect on cellulose hydrolysis is the most critical factor for the successful implementation of the approach***
- **Technology transfer and future work:** ***Display of complex cellulosomes***

Additional Slides

Publications and Presentations

1. Shen-Long Tsai, Garima Goyal, and Wilfred Chen, Surface display of a functional mini-cellulosome by intracellular complementation using a synthetic yeast consortium: Application for cellulose hydrolysis and ethanol production, *Appl. Environ. Microbiol*., **76**, 7514-7520, 2010.
2. Shen-Long Tsai, Jeongseok Oh, Shailendra Singh, Ruizhen Chen**,** and Wilfred Chen, Functional assembly of mini-cellulosomes on the yeast surface for cellulose hydrolysis and ethanol production, *Appl. Environ. Microbiol*., **75**, 6087-6093, 2009.
3. Garima Goyal, Shen Long Tsai, Bhawna Madan, Nancy A. DaSilva, and Wilfred Chen, Simultaneous cell growth and ethanol production from cellulose by an engineered yeast consortium displaying a functional mini-cellulosome, *Microb. Cell Factories*, 10, 89, 2011.
4. Shen-Long Tsai, Nancy A. DaSilva, and Wilfred Chen, Functional display of complex cellulosomes on the yeast surface via adaptive assembly, *ACS Synthetic Biology*, **2**, 14-21, 2013.

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