



Designer cellulosome technology for conversion of cellulosic biomass into biofuels

1st M.O.B.I Workshop

Marine Offshore Biorefinery in Israel

Porter School of Environmental Studies, Tel Aviv

24 May 2017

Ed Bayer

Department of Biomolecular Science

The Weizmann Institute of Science

Rehovot, Israel

Lignocellulosic Biomass

The Fate of Cellulose in Our Environment

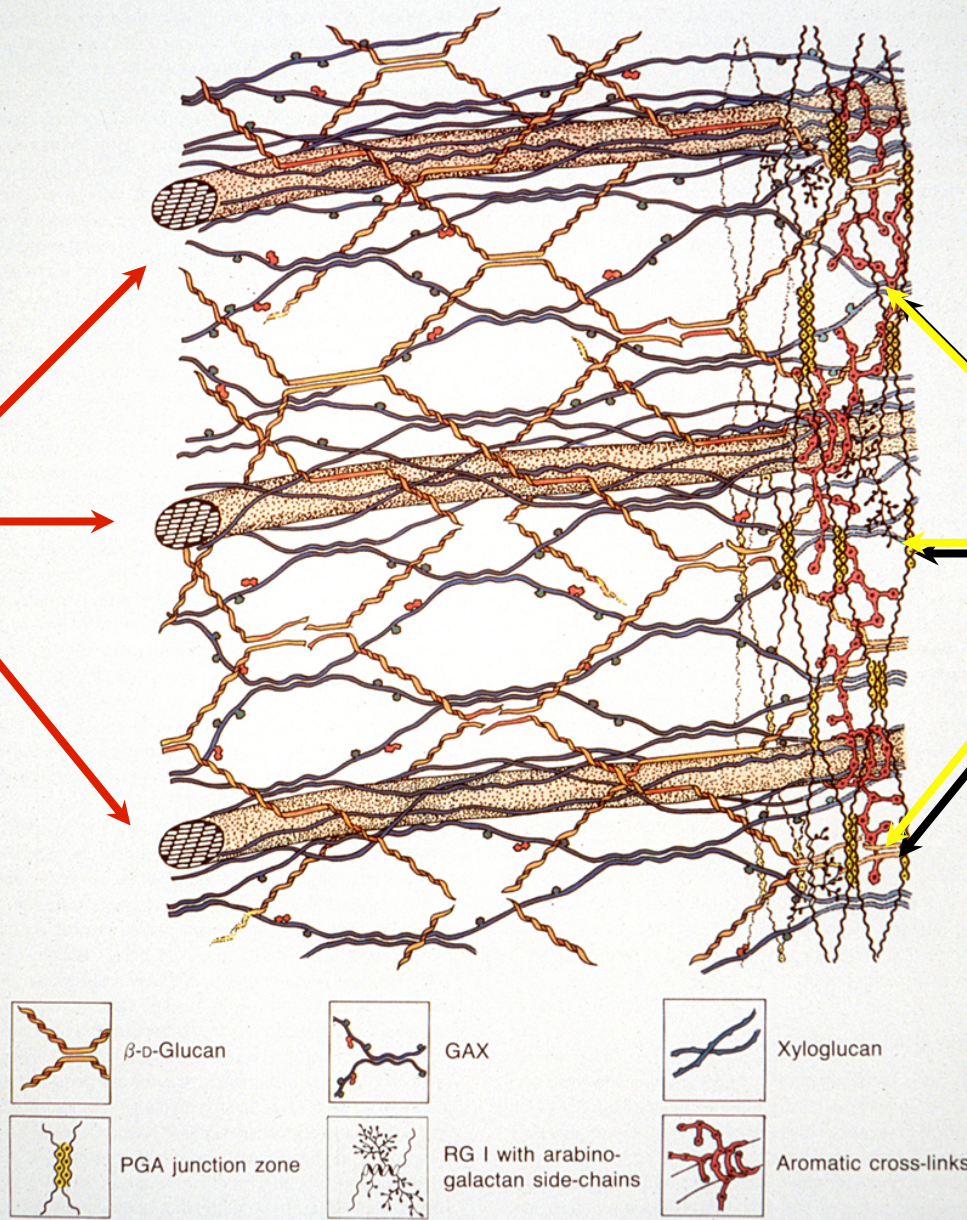


The Plant Cell Wall

Nicholas C. Carpita and David M. Gibeaut

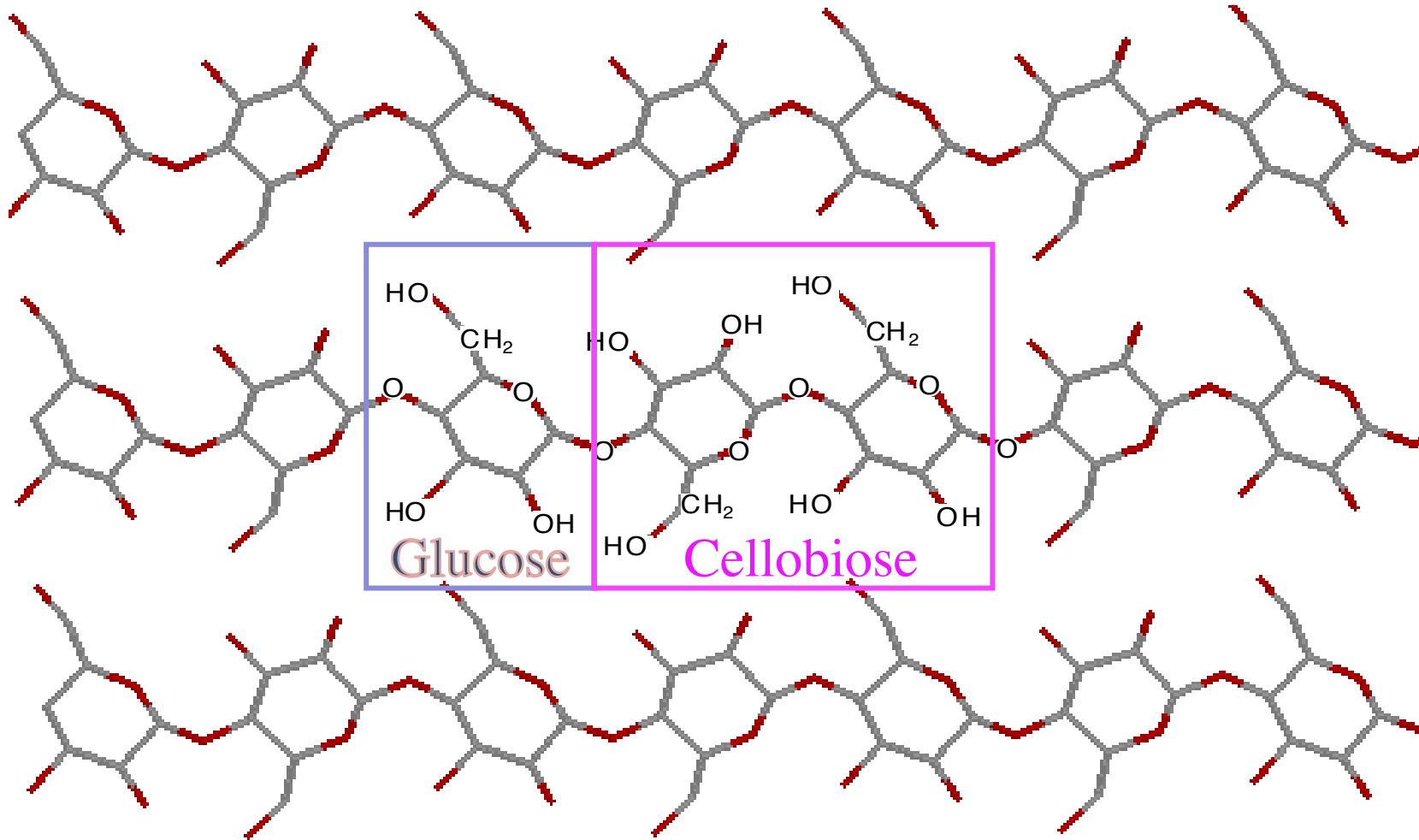
Cellulose

Other stuff

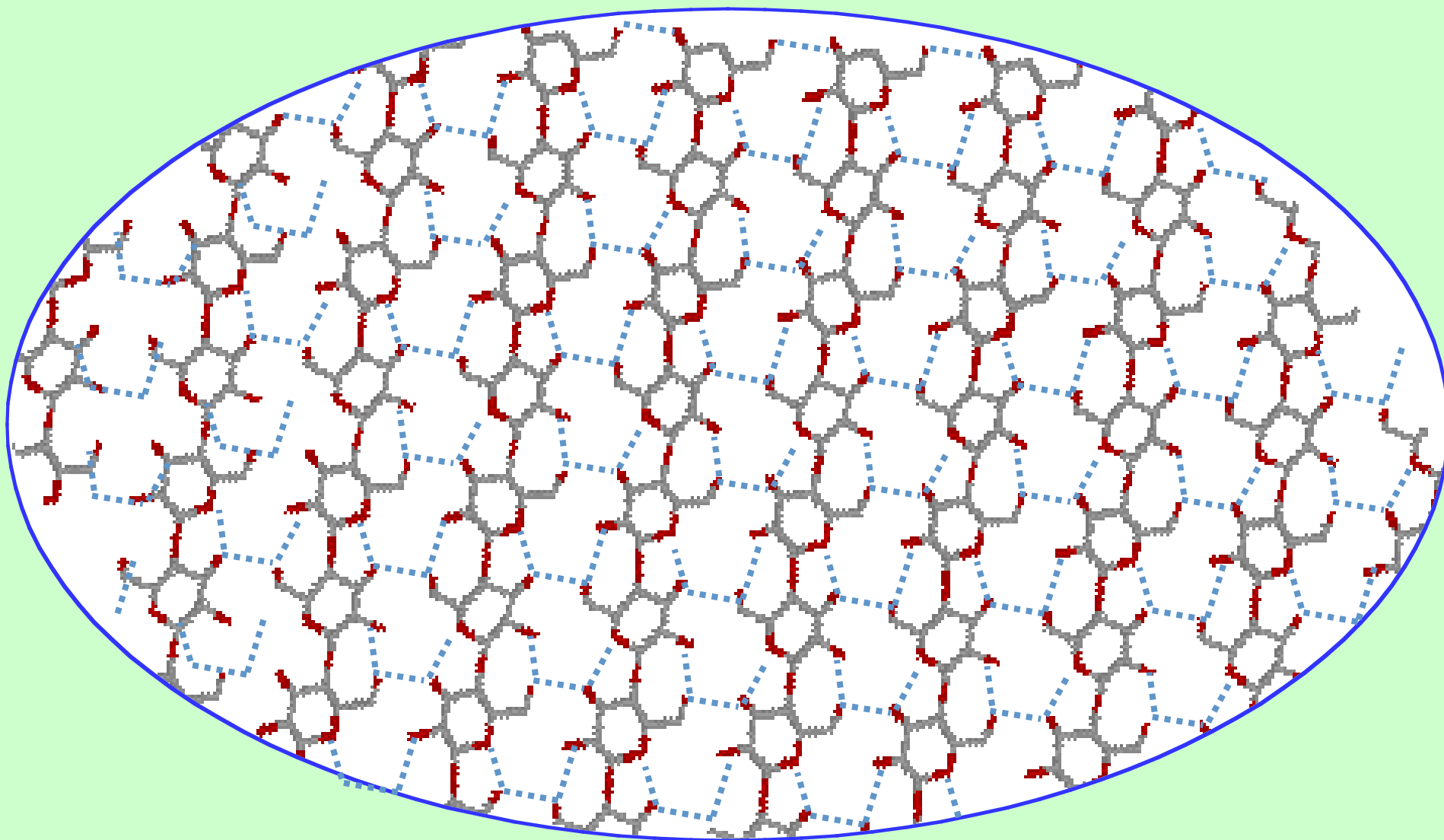


Carpita NC, Gibeaut DM (1993)

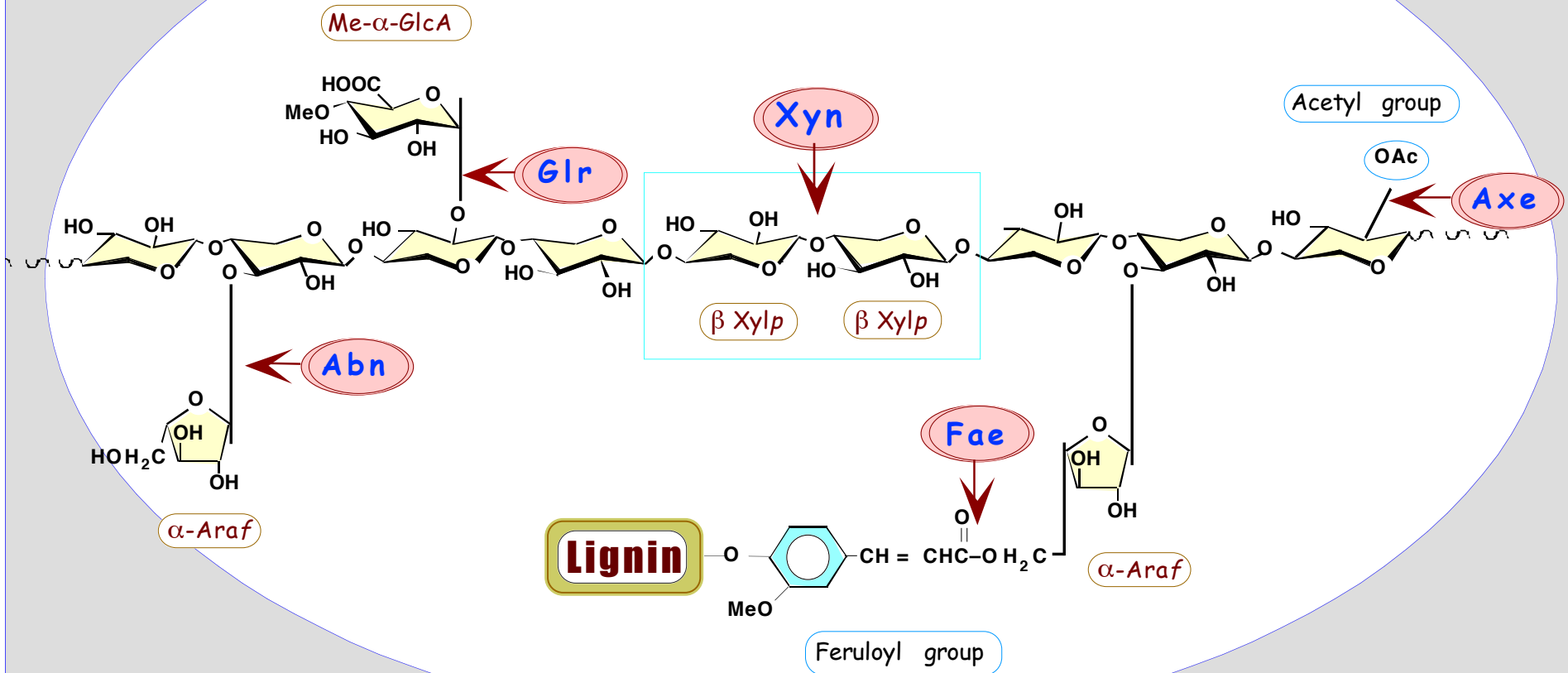
The structure of cellulose



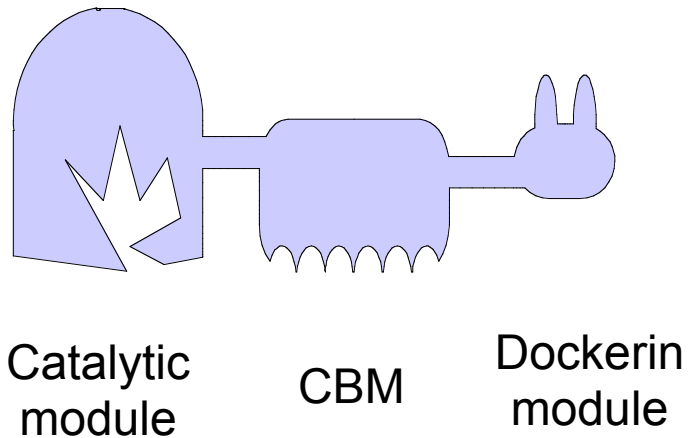
Intra- and Interchain Hydrogen Bonding



Xylan

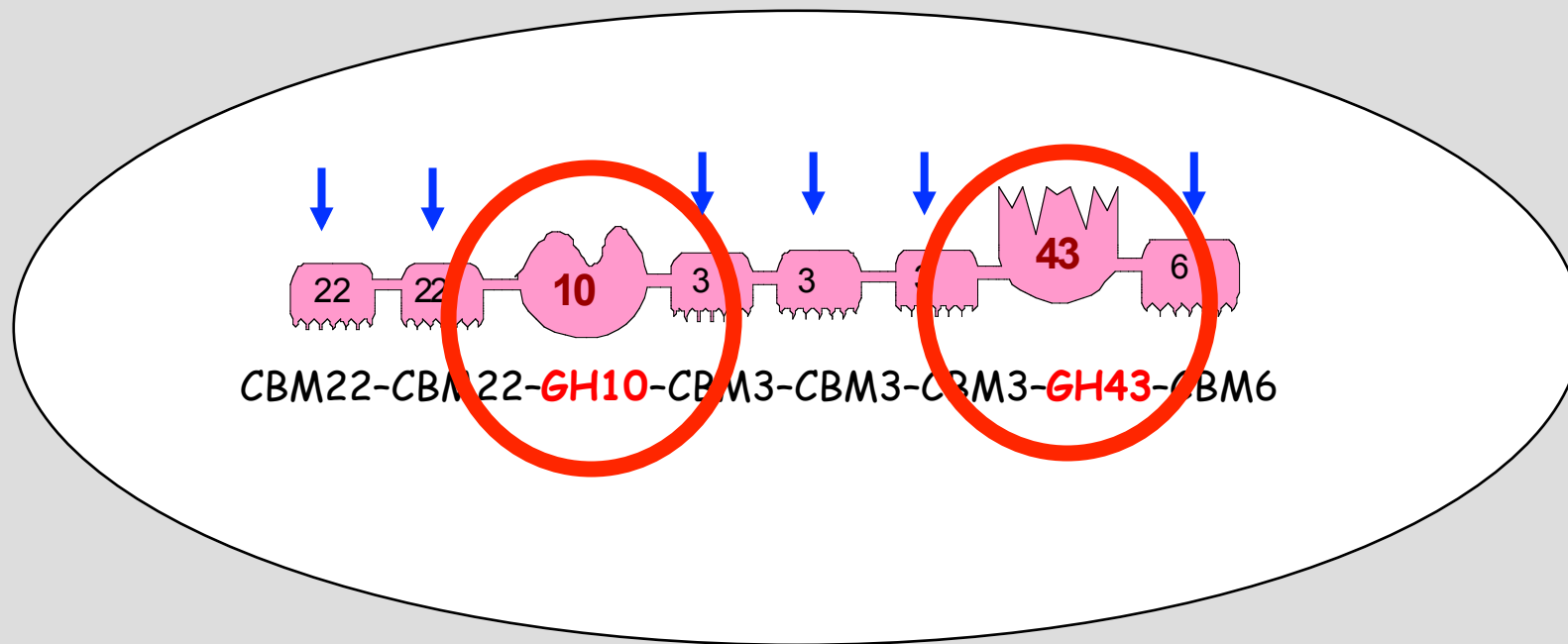


Cellulases (and friends) are Multi-modular Enzymes

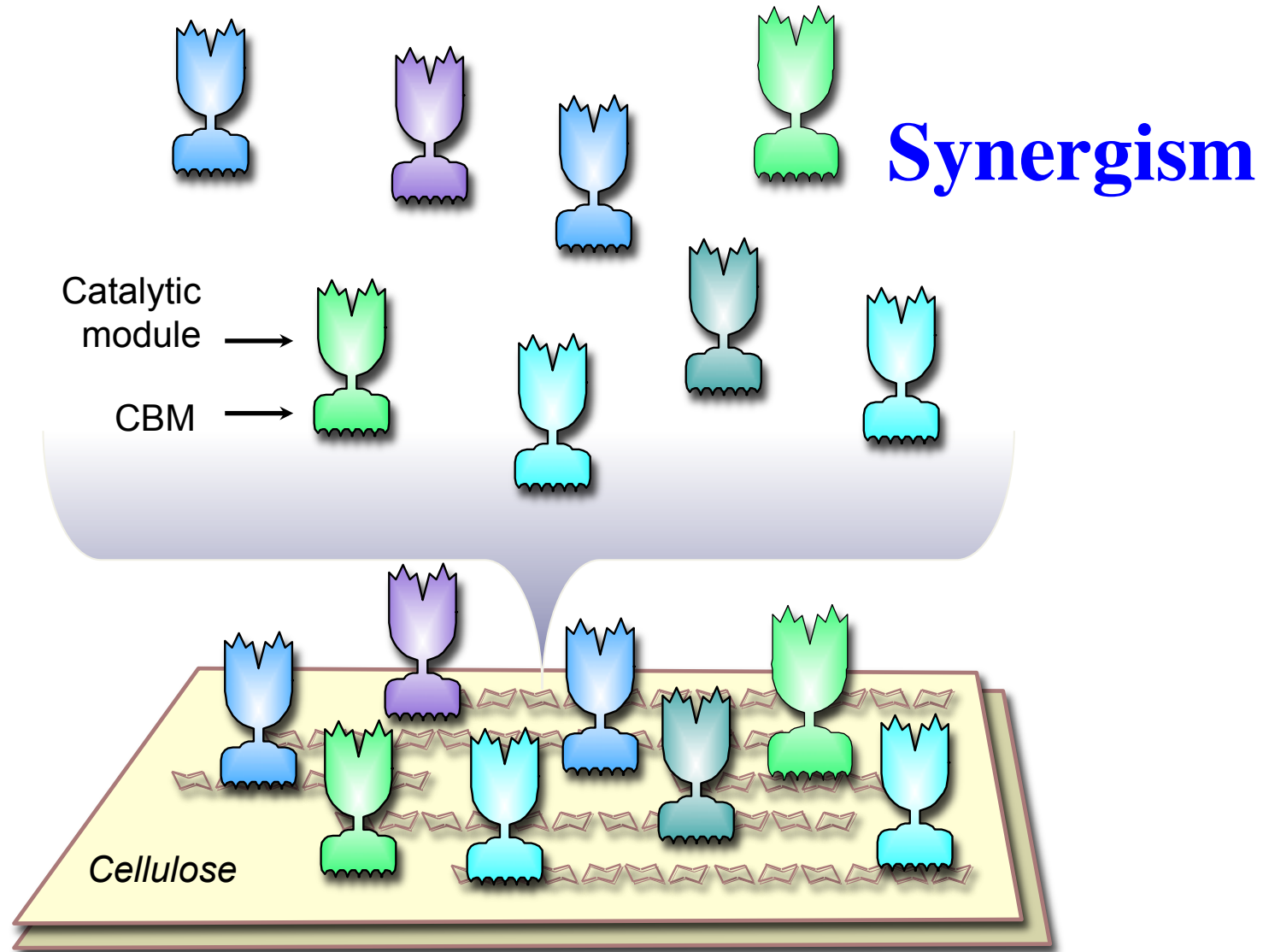


*Cellulases are not “normal” enzymes
Cellulases are sophisticated enzymes*

Multi-modular Xylanase from *Caldicellulosiruptor*



Cellulases: Free Enzyme Paradigm



**Cellulosome
Discovery
1983**

35 years later!!!

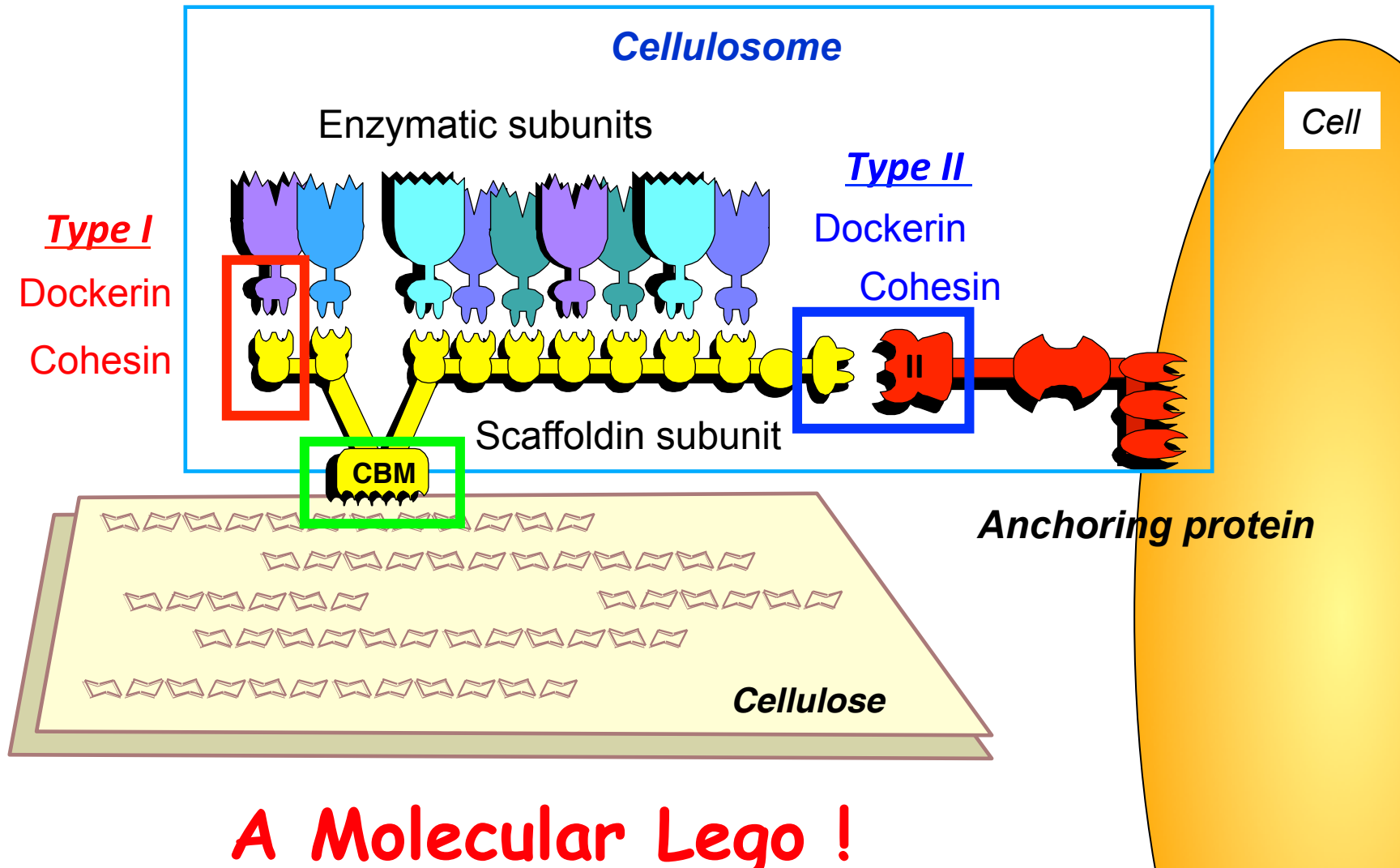


The Cellulosome

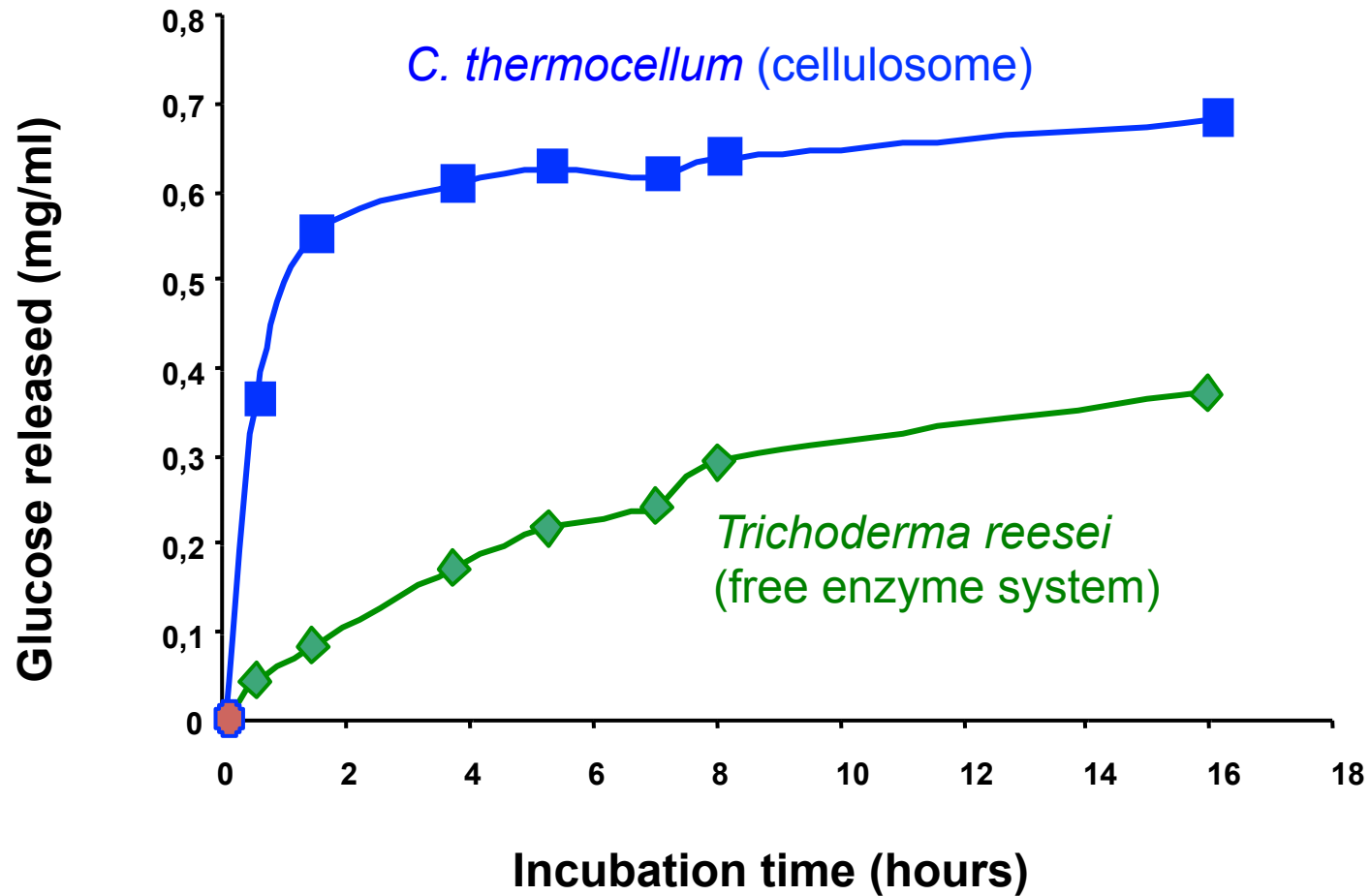
**A discrete, cellulose-binding, multi-enzyme complex
for degradation of plant cell wall polysaccharides**

Bayer & Lamed (1983)

The *Clostridium thermocellum* Cellulosome



C. thermocellum Cellulosome vs. Fungal Enzymes



Cellulosome: Possible Advantages

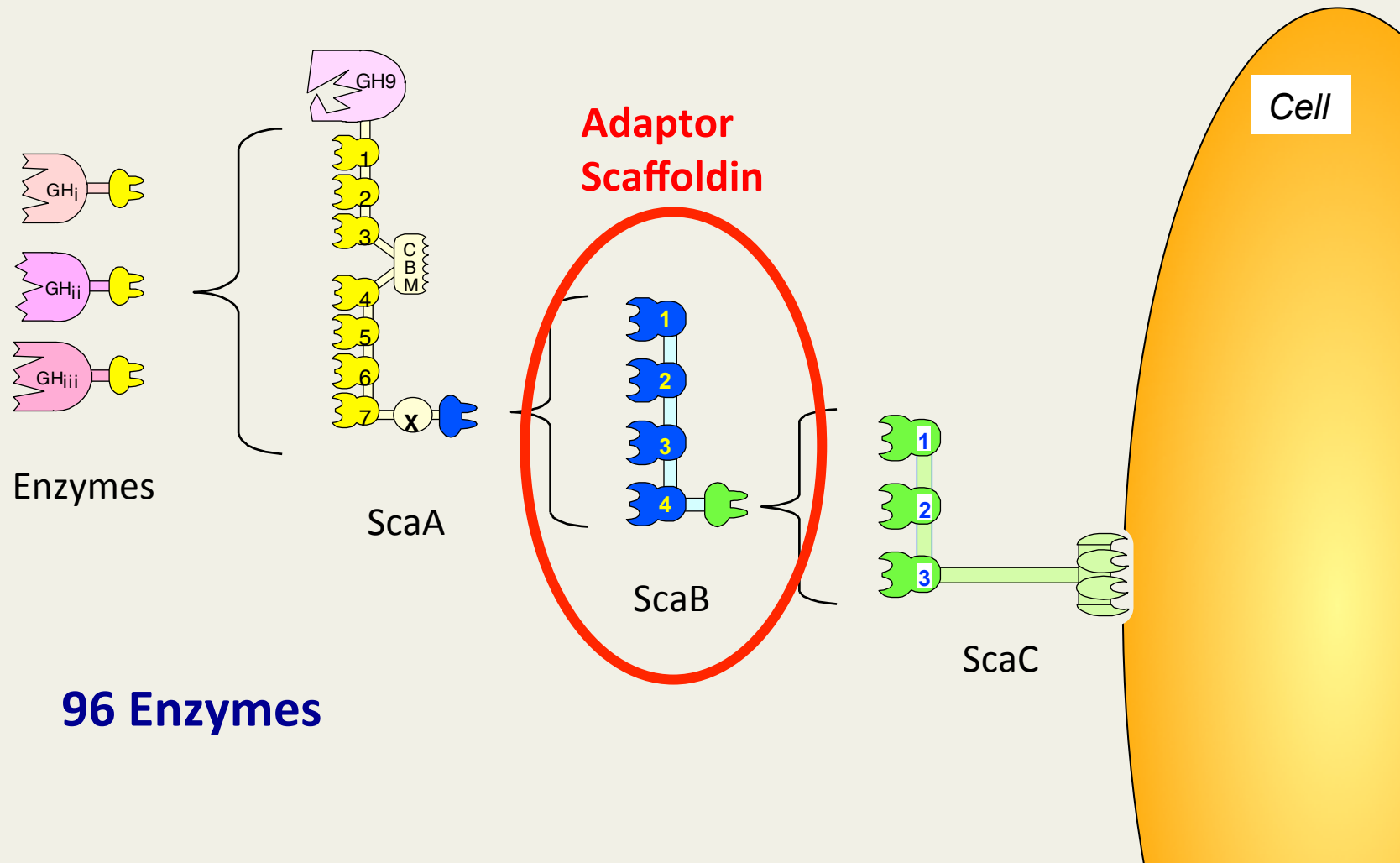
- More efficient synergism due to **enzyme proximity**
- **Common targeting** of enzymes to the substrate
- The enzymes are **attached to the cell**
- The whole cell is **attached to the substrate**

➤ **Minimal diffusion loss of enzymes and hydrolytic products**

Cellulosome Systems...
Not so simple

Theme and Variations: Cellulosome Diversity

The *Acetivibrio cellulolyticus* cellulosome

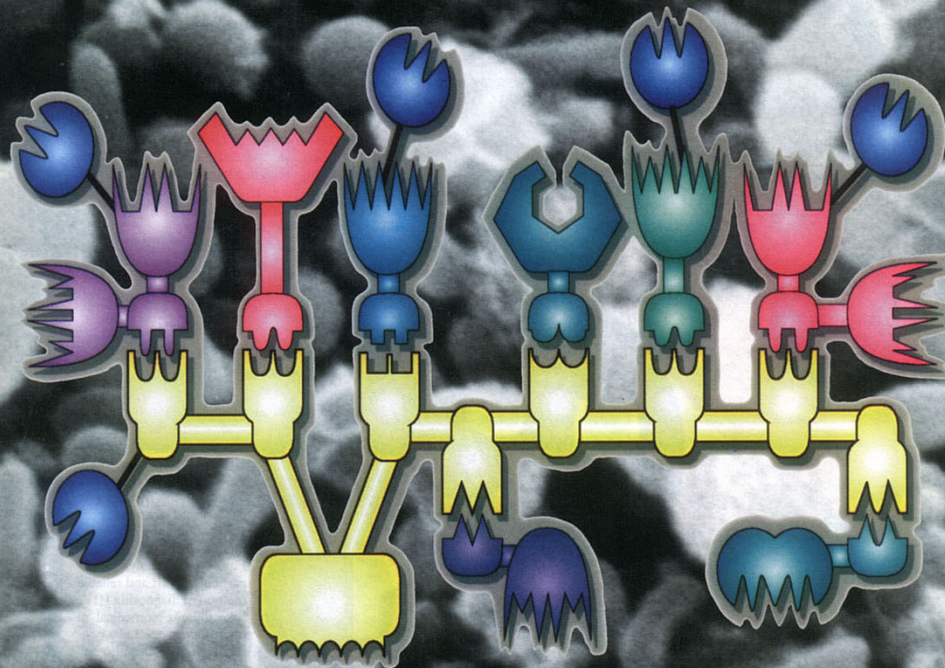


**Designer
Cellulosomes
1994**



trends
in
BIOTECHNOLOGY

TIBTECH September 1994, Volume 12 No. 9 (128)



'Designer' cellulosomes for exploitation and management of cellulotics



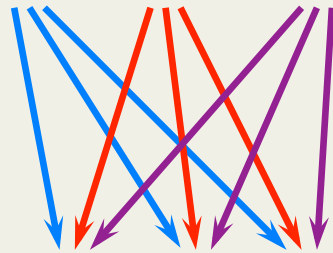
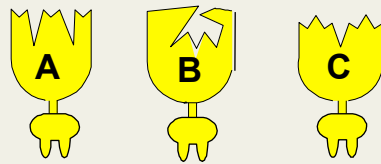
- Selectins as therapeutic targets
- EMS and tryptophan production
- Pharmacological leads from antibodies
- Designing GMMs for release

Bayer et al (1994) Trends Biotechnol. 12, 378

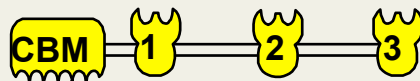
Designer Cellulosomes

Native Cellulosome

Native
dockerin-
containing
enzymes



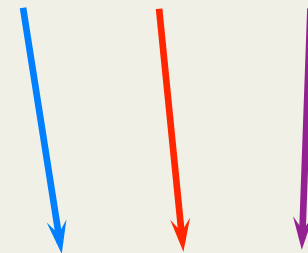
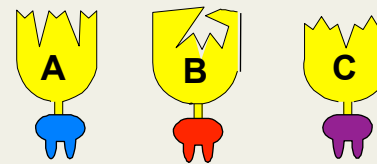
Native
scaffoldin



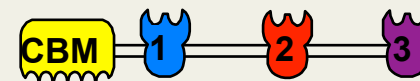
Random incorporation

Designer Cellulosome

Chimaeric
dockerin-
containing
enzymes



Chimaeric
scaffoldin



Controlled incorporation



Designer Cellulosomes



Jonathan Caspi



Sarah Morais



Yael Vazana



Yonit Ben David



Lital Davidi



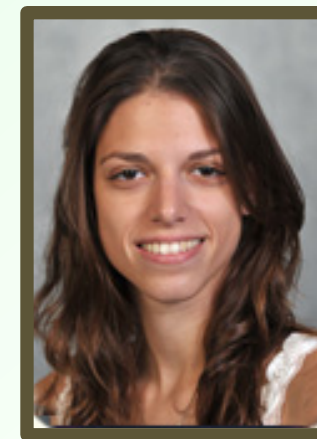
Johanna Stern



Amaranta Kahn



Yonathan Arfi



Lior Artzi

Progressive Enlargement of Designer Cellulosomes



Sarah Morais



Johanna Stern



Sarah Moraïs

Extended Cellulosome Assemblies

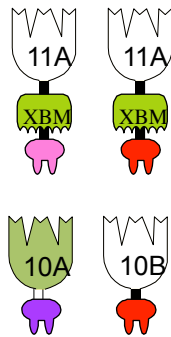
Thermobifida fusca enzymes



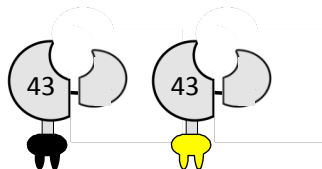
Wheat Straw

Xylanases

Endoxylanases

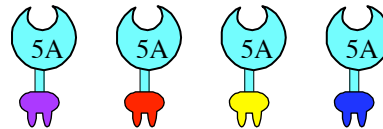


β -Xylosidases

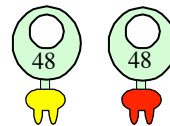


Cellulases

Endoglucanases



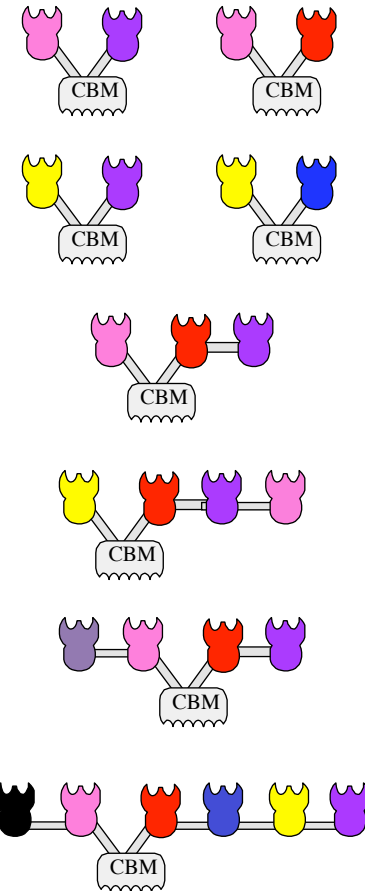
Exoglucanases



β -Glucosidase



Scaffoldins



■ *C. cellulolyticum* ■ *A. cellulolyticus* ■ *C. thermocellum* ■ *A. fulgidus* ■ *B. cellulosolvens* ■ *R. flavefaciens*

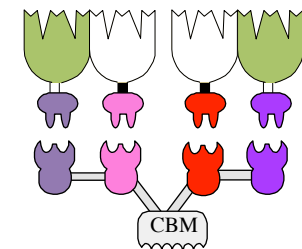
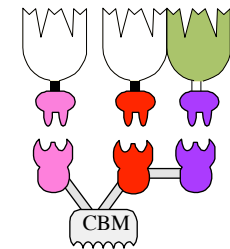
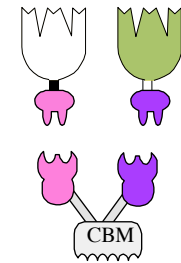


Sarah Moraïs

Incremental Approach



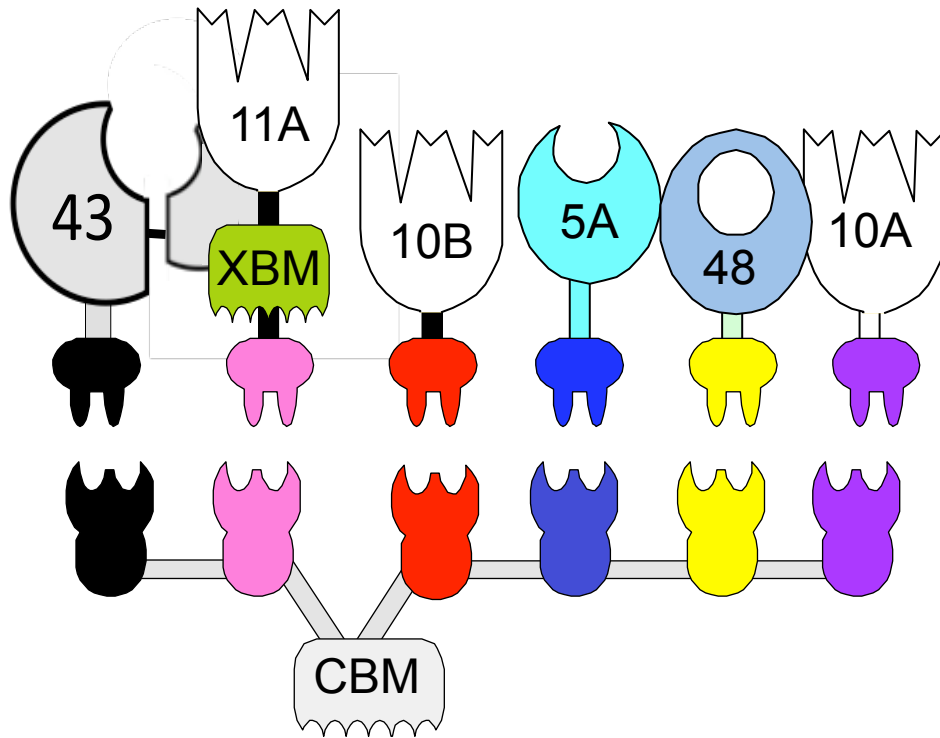
- Enhanced synergy between 2 enzymes in a **divalent** designer cellulosome
- Add another type of enzyme in a **trivalent** designer cellulosome
- Add yet another type of enzyme in a **tetravalent** designer cellulosome
- etc





Sarah Moraïs

Hexavalent Designer Cellulosome



2 Cellulases
4 Xylanases

Hexavalent Scaffoldin

■ *C. cellulolyticum* ■ *A. cellulolyticus* ■ *C. thermocellum* ■ *A. fulgidus* ■ *B. cellulosolvens* ■ *R. flavefaciens*



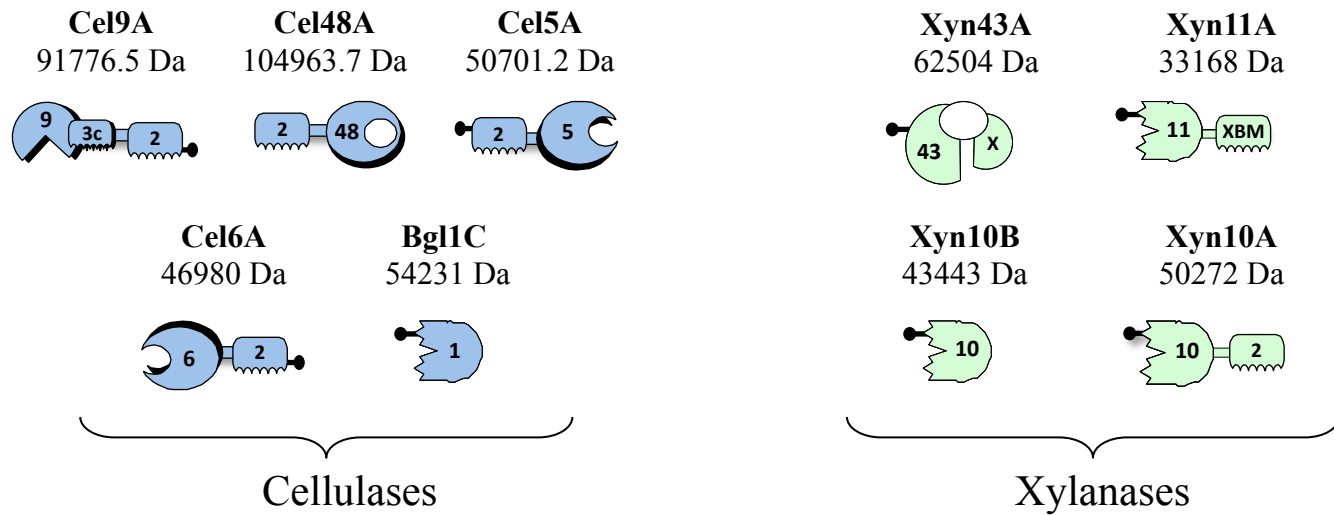
Even Larger Cellulosome Assemblies



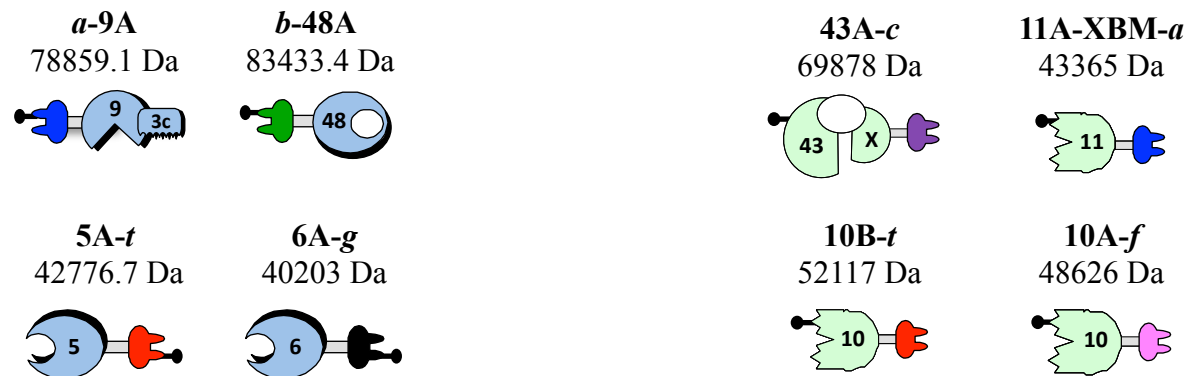
Thermobifida fusca enzymes

Wild-type Enzymes

Johanna Stern



Chimaeric Enzymes





Adaptor Scaffoldins

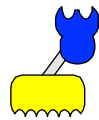


Chimaeric Scaffoldins

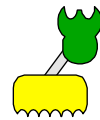
Adaptors

Johanna Stern

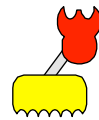
Scaf·cA
36783 Da



Scaf·cB
38572 Da



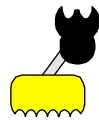
Scaf·cT
36982 Da



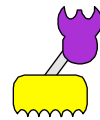
Scaf·T₂c
39628 Da



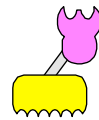
Scaf·cG
38427 Da



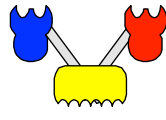
Scaf·cC
36248 Da



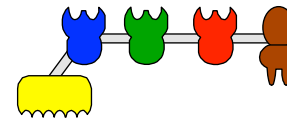
Scaf·cF
35710 Da



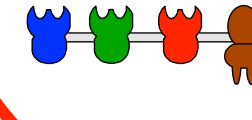
Scaf·AcT
53893 Da



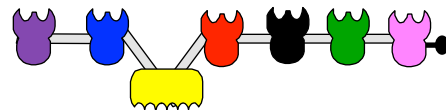
ScAd·cABT₂
92640 Da



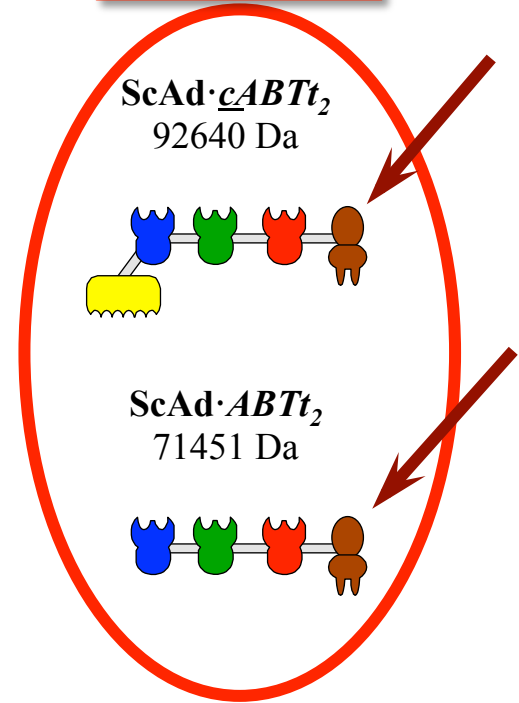
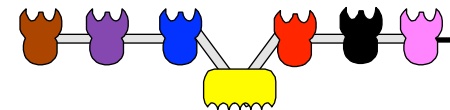
ScAd·ABT₂
71451 Da



Scaf·CAcTGBF
124600 Da



Scaf·T₂CAcTGF
126134 Da



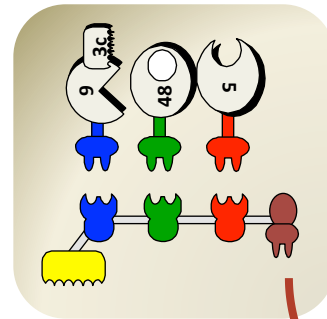


Johanna Stern

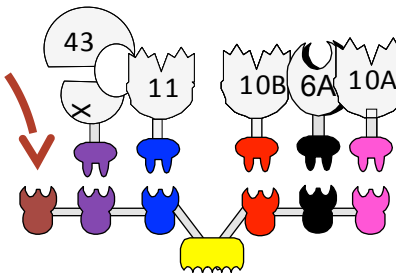
Adaptor Scaffoldins



Adaptor



4 Cellulases
4 Xylanases

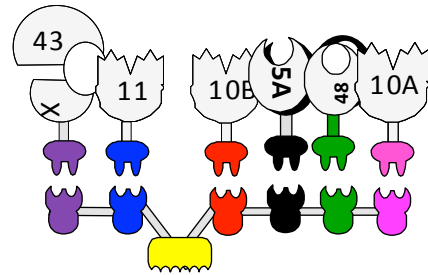


Scaffoldin

Designer Cellulosomes: Where do we stand?



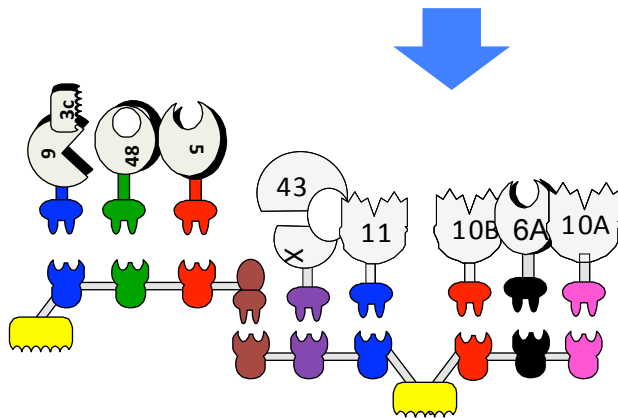
Sarah



6 ENZYMES



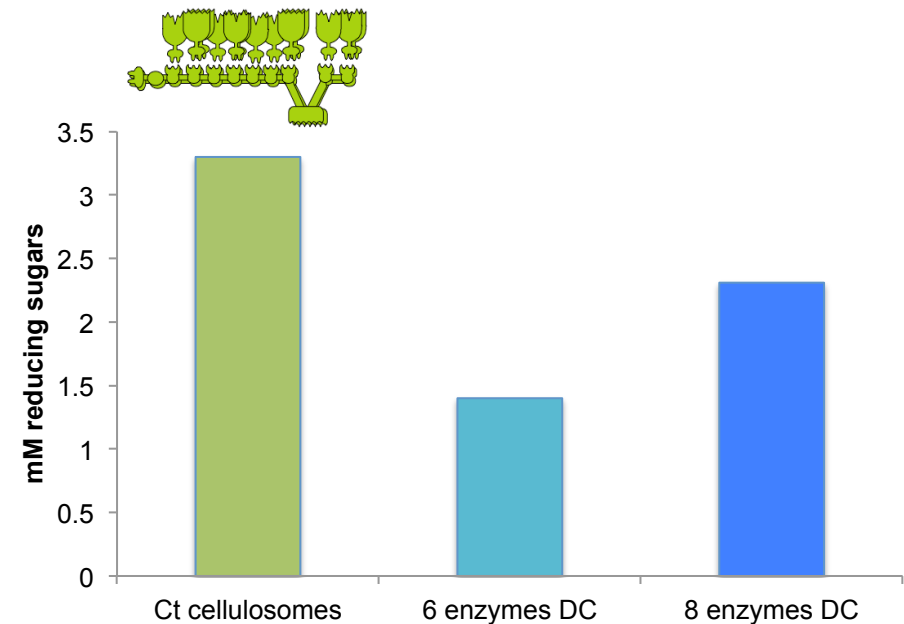
Johanna



8 ENZYMES

40% degradation compared to native cellulosomes

70% degradation compared to native cellulosomes



“Foreign” Enzymes in Designer Cellulosome



Yonathan Arfi

LPMOs



Lital Davidi

Laccase



Gilad Gefen

β -Glucosidase

Offshore Biorefinery

Macroalgae



Seaweeds



Red alga



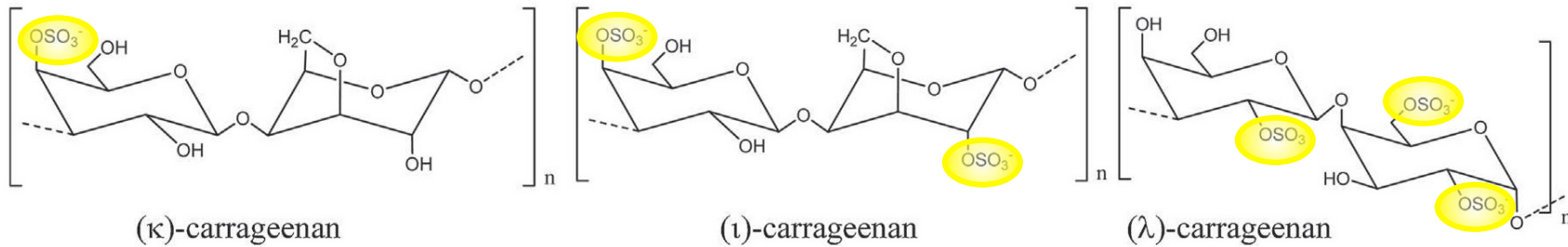
Brown alga



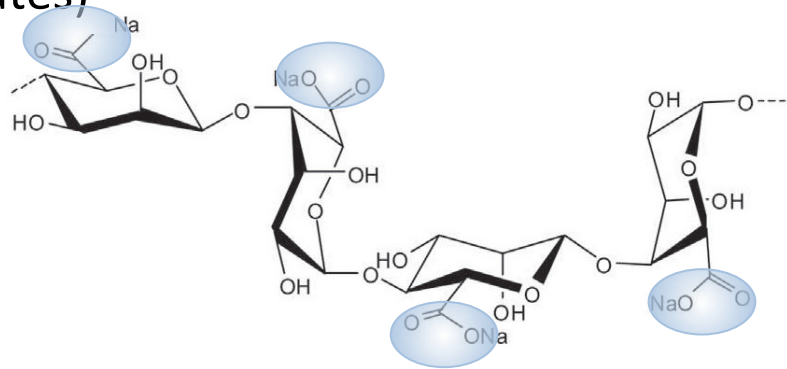
Green alga

Chemical Structures of Macroalgae

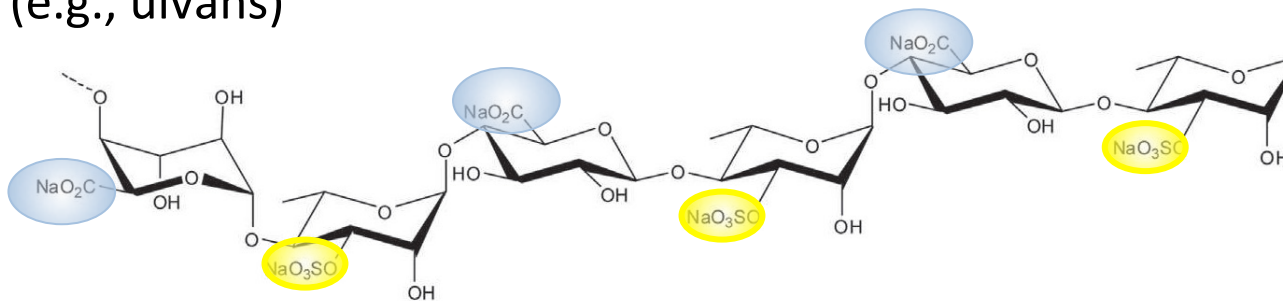
Red (e.g., carrageenans)



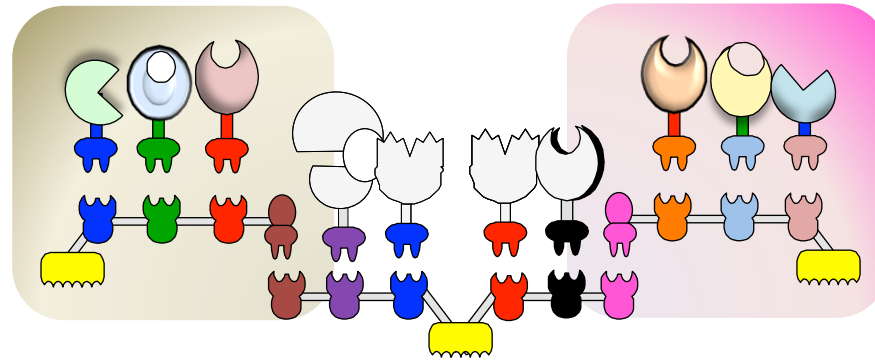
Brown (e.g., alginates)



Green (e.g., ulvans)



Advanced Algae-degrading Cellulosomes



Cellulases

Xylanases

Carrageenan -

Alginate -

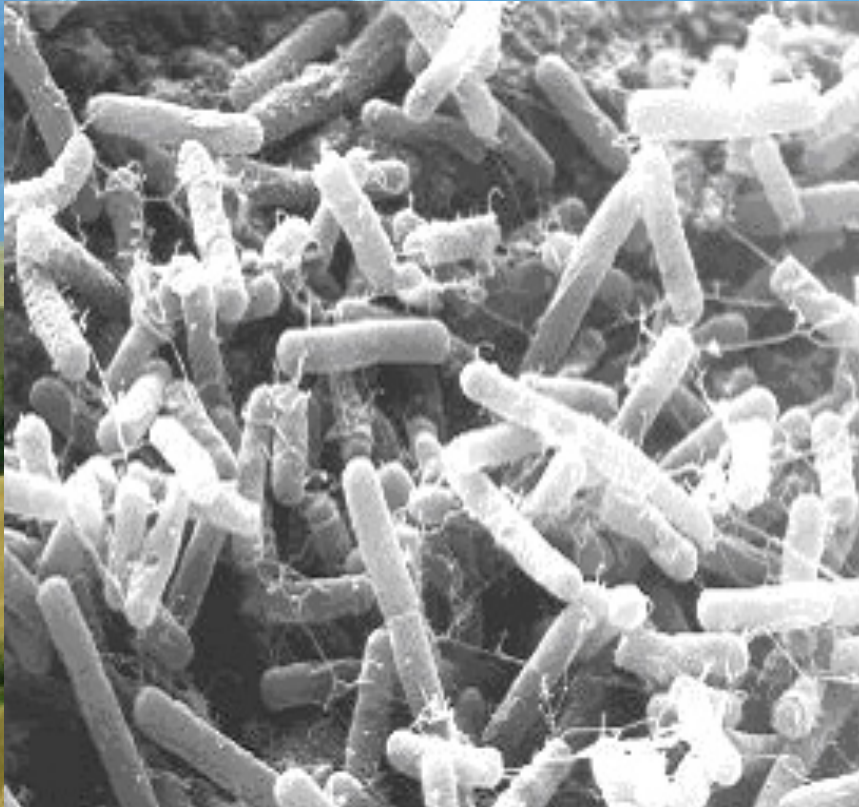
Ulvan -

} degrading Enzymes

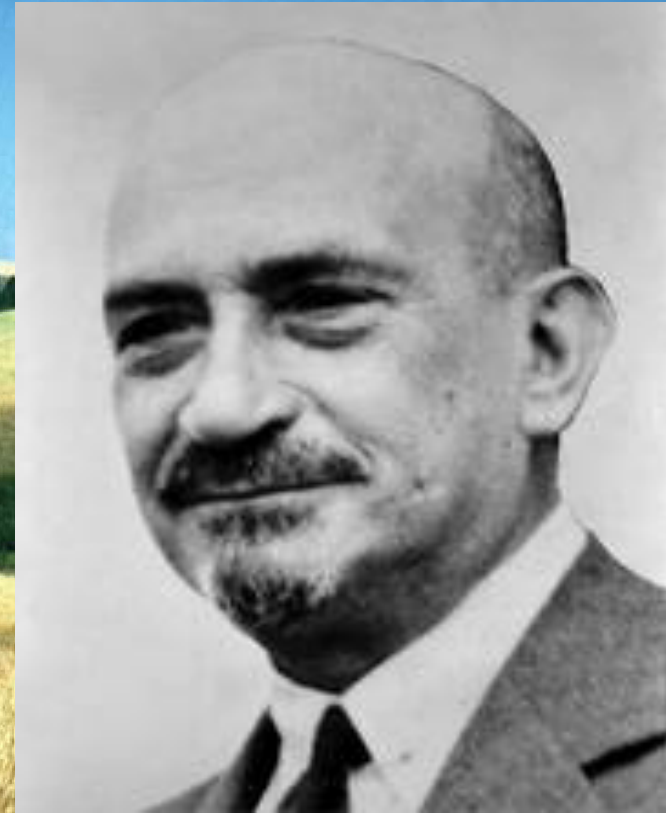
Historical Perspectives

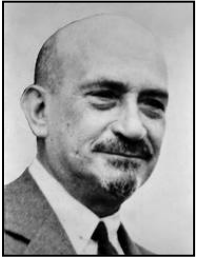
Weizmann's Bug Strikes Again!

Clostridium acetobutylicum



Chaim Weimann

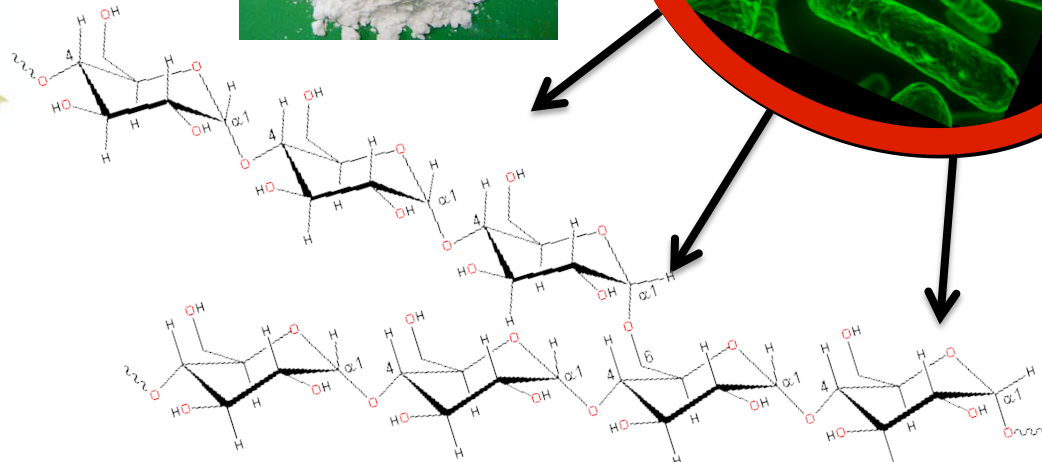




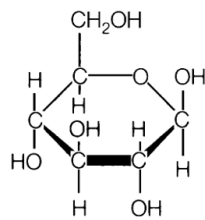
Weizmann's Bug — from Starch to Biofuels/Solvents



Corn (Starch)



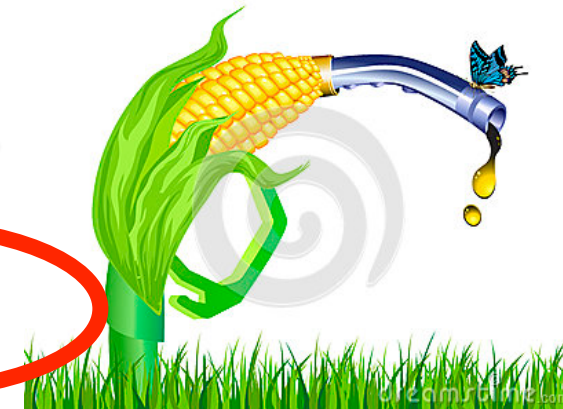
Starch



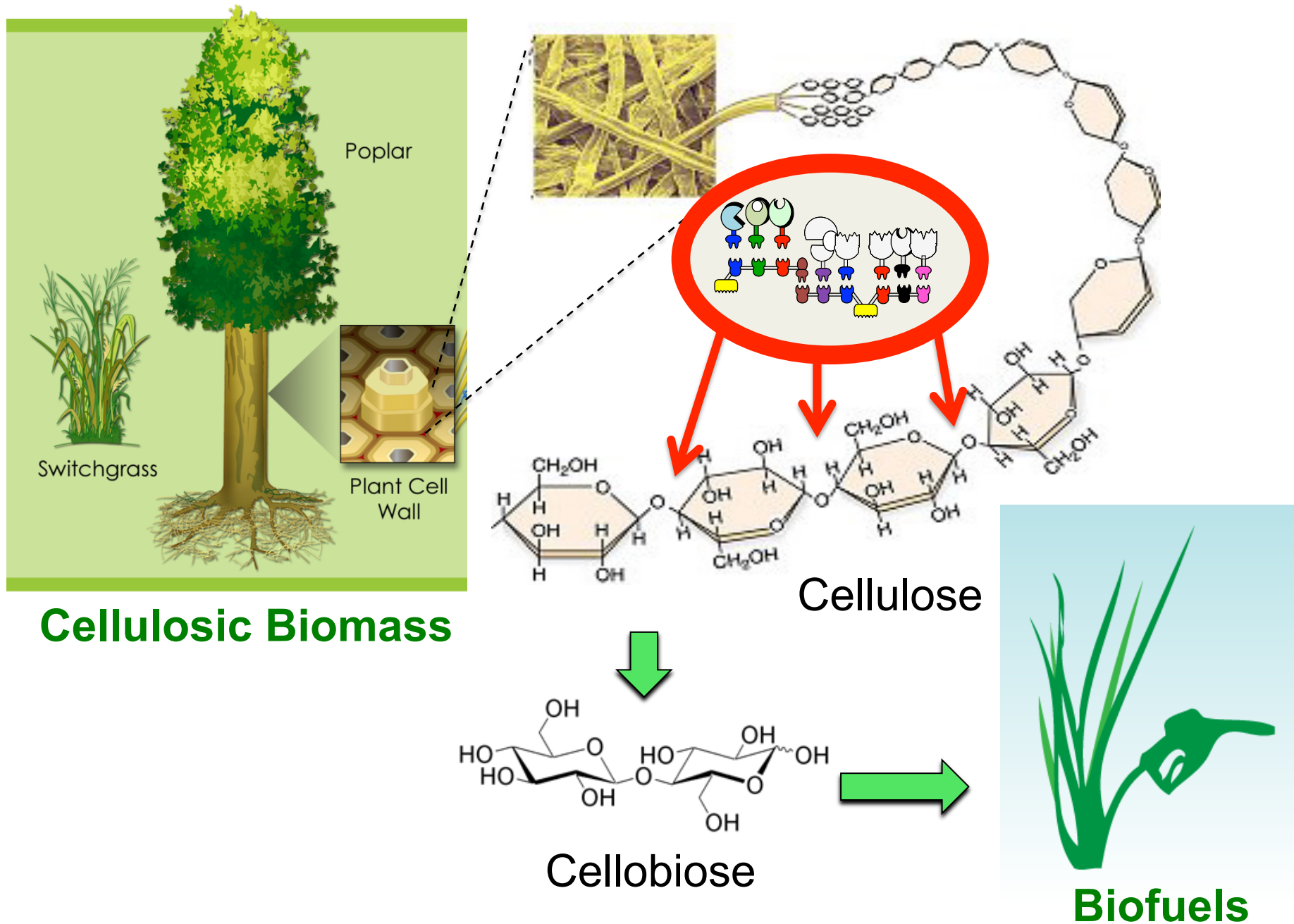
Glucose



- Acetone
- Butanol
- Ethanol



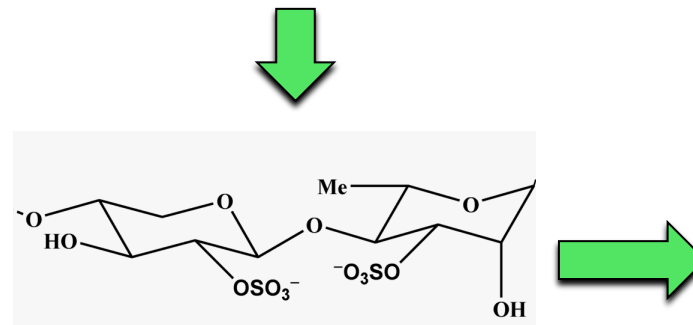
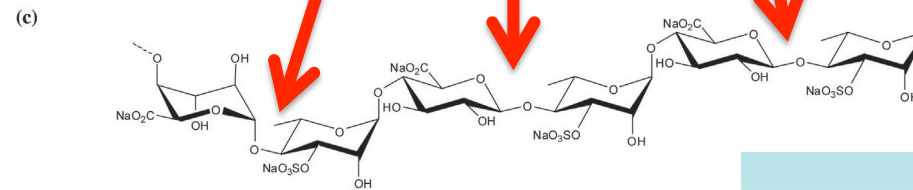
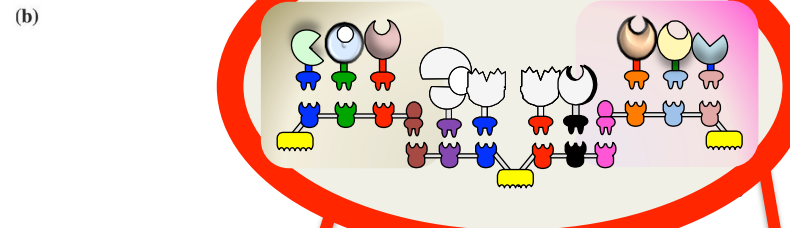
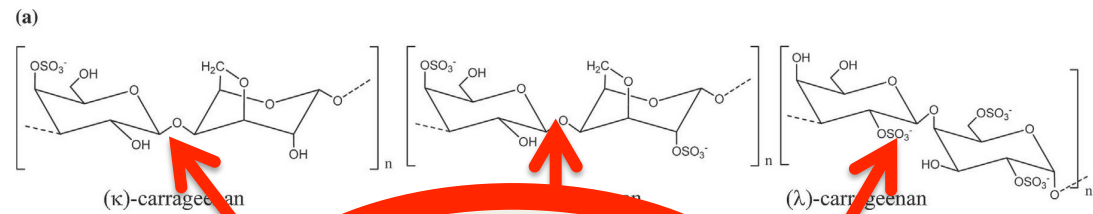
Designer cellulosomes for Cellulosic Biomass to Biofuels



Designer cellulosomes for Algal Biomass to Biofuels



Algal Biomass



Algal Breakdown Products



Biofuels

With thanks....

Group and Collaborators

Israel



Yoav Barak

Rachel Haimovitz

Alon Karpol

Jonathan Caspi

Michael Anbar

Bareket Dassa

Liat Bahari

Dan Fried

Ilit Noach

Orly Alber

Michal Slutzki

Sarah Morais

Yael Vazana

Johanna Stern

Hadar Gilary

Vered Roimy

Yuval Hamberg

Shachar Yoav

Gilad Gefen

Alik Demishtein

Adva Mechaly

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Ely Morag

Yuval Shoham

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Yitzhak Hadar

Itzik Mizrahi

Aharon Gedanken

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Alexander Golberg

Ehud Banin

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Mike Himmel (NREL)

Shi-You Ding (MSU)

Qi Xu (NREL)

Bryan White (U of Ill.)

Steve Brown (ORNL)

Jamie Cate

Klaus Schulten

Raphael Bernardi

Canada



Steve Smith

Jay Adams

Zongchao Jia

Mark Currie

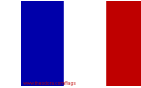
Al Boraston

Japan



Sadanari Jindou

France



Jean-Pierre Belaich

Anne Belaich

Henri-Pierre Fierobe

Florence Mingardon

Frédéric Monot

Antoine Margeot

Chantal Tardiff

Bernard Henrissat

Pedro Coutinho

Veronique Receveur-Brechot

Michal Hammel

Mirjam Czjzek

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Hermann Gaub

Michael Nash

Don C. Lamb

Spain



Victor de Lorenzo

Mariano Carrión

Greece



Dimitris Hatzinikolaou

Australia



Mark Morrison

Thailand



Khanok Ratanakhanokchai

Paripok Phitsuwan

Turkey



Osman Ugur Sezerman

Ozgur Gul

Portugal



Carlos Fontes

Ireland



Damien Thompson

UK



Harry Flint

Harry Gilbert

China



Jian Xu

Qiu Cui

徐健

崔球

With my Great Group – Rehovot 2016

Shahar Alon Jacob Bareket Ronit
Paripok Lior Ama Lizi Sarah Anastasia
Olga Yonit Lital Ed Yuli Melina Vered



THANKS!

