Designing starch – harnessing carbohydrate polymer synthesis in plants

Oliver Ebenhöh, HHU Düsseldorf, Germany
Sam Zeeman, ETH Zurich, Switzerland
Rob Field, JIC Norwich, UK

ERA-CAPS Grant Holders Workshop, Lisbon, May 8, 2015

ERA-CAPS

ERA-NET for Coordinating
Action in Plant Sciences

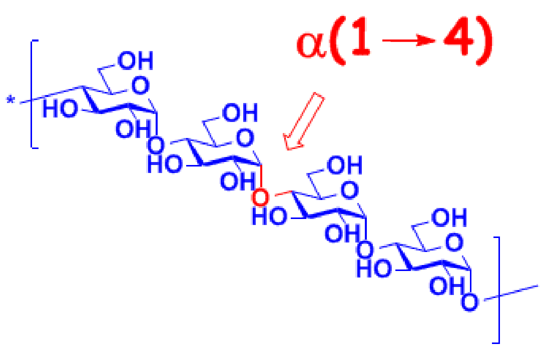
Starch – half of the calories in the human diet

pictures from:

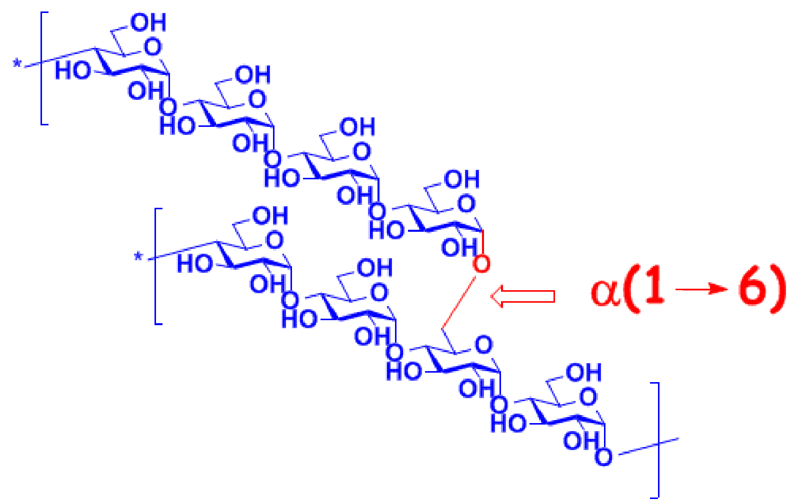
1 – cropsforthefuture.org / commons.wikimedia.org (Author: NusHub)
2 – nutr130.wikispaces.com
3 – nutr130.wikispaces.com
4 – newworldencyclopedia.org
5 – freefoodfotos.com
6 – commons.wikimedia.org (Author: KATORISI)
7 – mappingignorace.org (Sanjeev Gupta / EPA)
8 – commons.wikimedia.org (Author: P. Brundel)

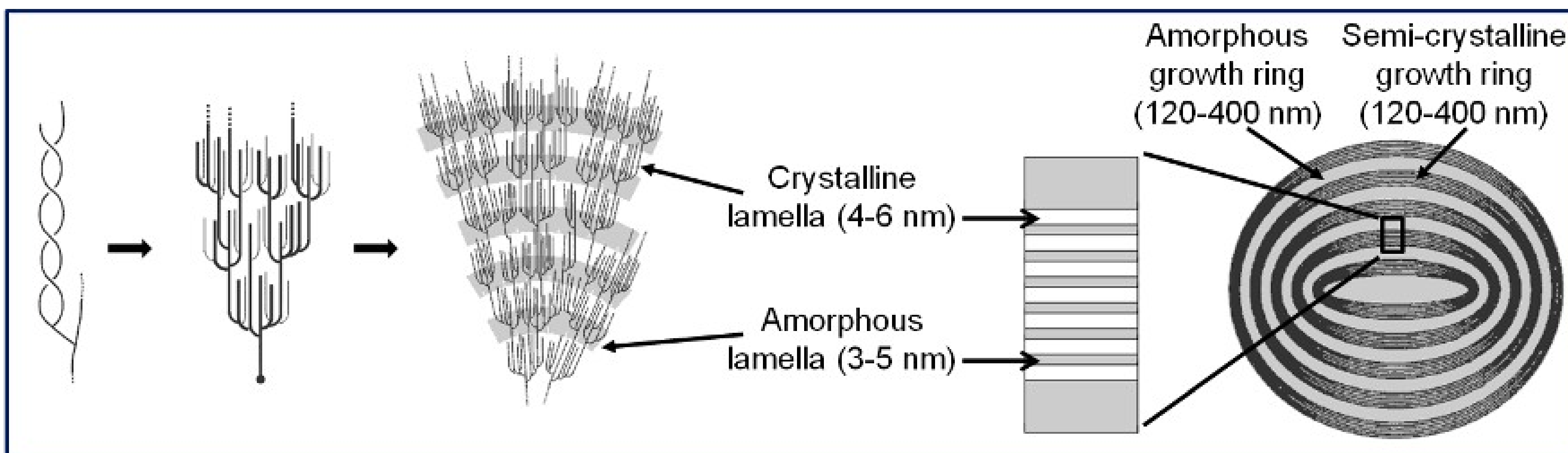
The structure of a starch granule

Amylose
(MW 32,000-113,000)



Amylopectin
(MW 10^7-10^9)





Starch as a bulk commodity

Food	Confectionery	Pharmacy	Plastic and Textiles	Various
Mayonnaise	Jelly beans	Tablets	Biodegradable plastic	Water treatment
Baby food	Boiled sweets	Dusting powder	Warp	Detergent
Bread	Jellies	**Agriculture**	Fabrics	Oil drilling
Buns	Fruit fillings Marshmallows	Seed coating	Yarns	Stain remover
Meat sausages	Marmalade	Fertiliser	Hygienic diapers	
Meat rolls and loaves	Jam	Feed pellets	Baby diapers	
Ketchup	Ice cream	**Building**	Sanitary napkins	
Soups	Dairy cream	Mineral fibre tiles	Corrugated board	
Snacks	**Beverages**	Gypsum board	Printing paper	
Sauces	Soft drinks	Concrete	Packaging	
Low fat foods	Beer	Gypsum plaster	Glue	
Noodles	Alcohol			

Annual global starch trade projected to exceed 5Bn tonnes by 2015

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Move the generation of starch functional diversity from the chemical plant to the crop plant

Wouldn't it be great...

...if we could design starch with desired properties *in vivo*?

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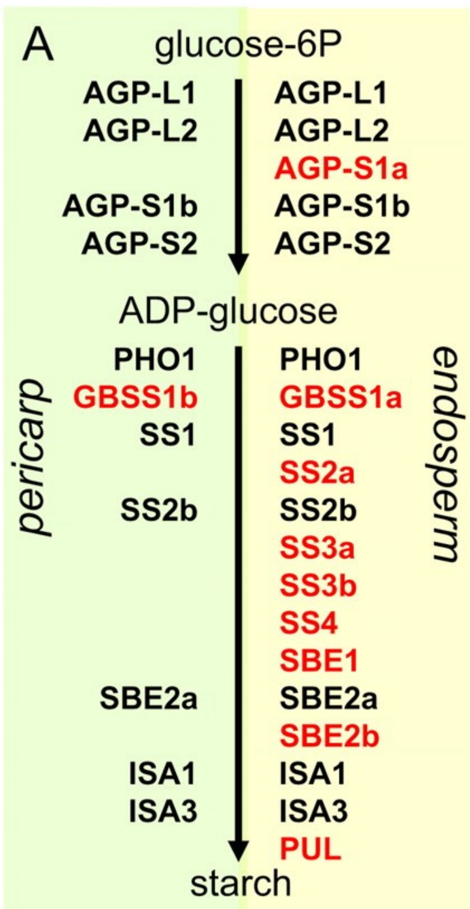
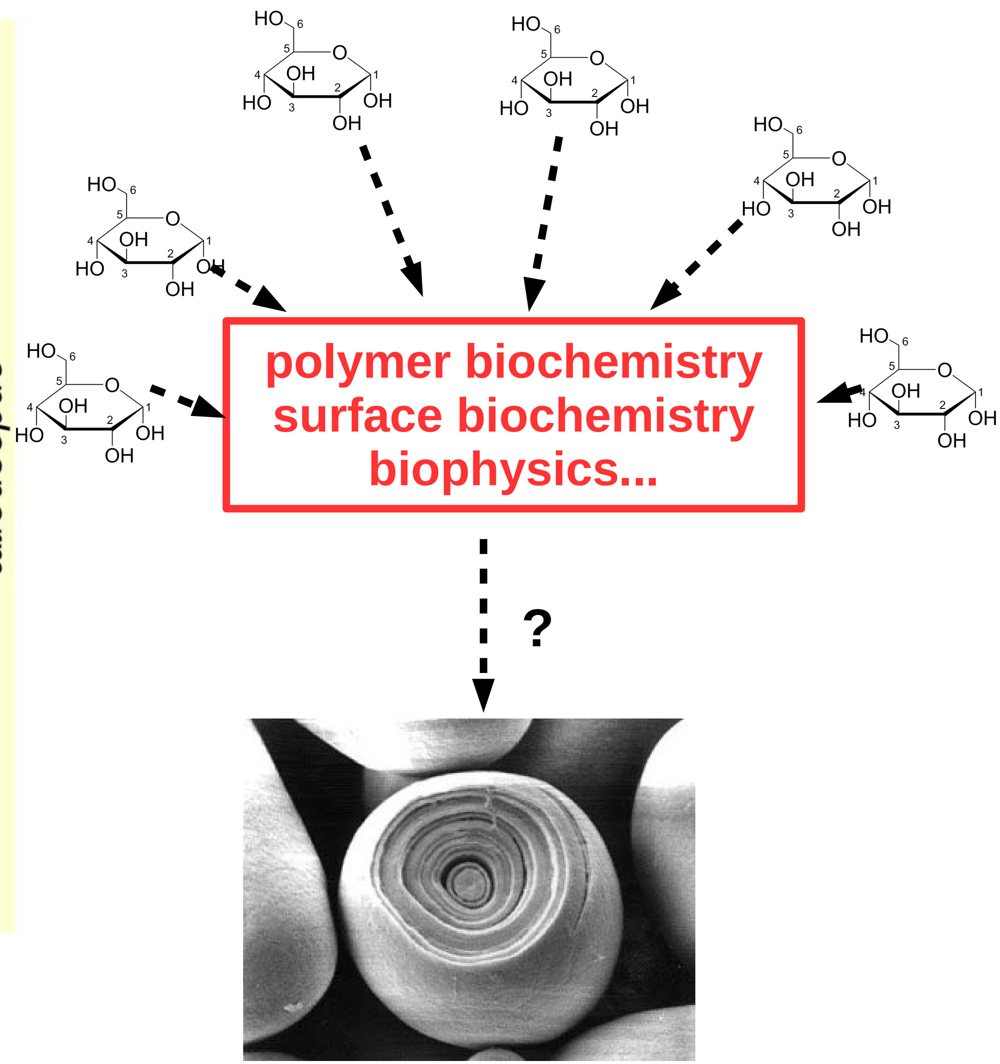
...if we could design starch with desired properties *in vivo*?

But how do all these factors actually play together?



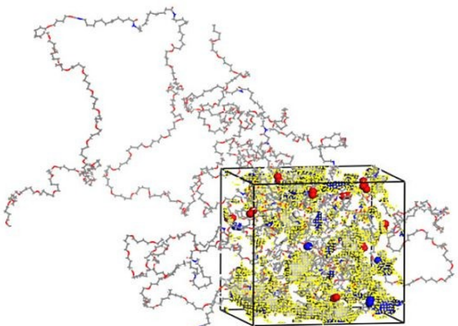
(Radchuk et al, 2009)



A classical physics problem

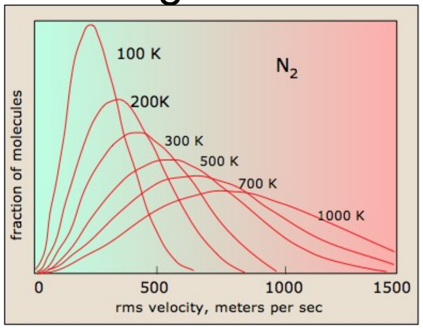
PHYSICS

molecular interactions



(from: De Lorenzo et al, 2012)

gas



(from: chemwiki.udavies.edu)

<u>collective behaviour</u>

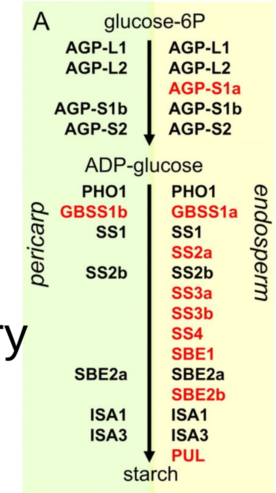
- pressure
- temperature

microscopic

macroscopic scale

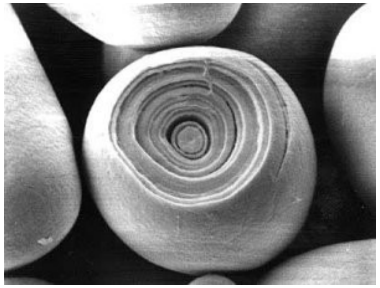
BIOLOGY

enzymes
surface physics
polymer chemistry



(from: Radchuk et al, 2009)

starch granule



(from: braukaiser.com)

- chain length distr.
- branching pattern
- glycaemic index

www.nobelprize.org

Richard Feynman:

“What I cannot create, I do not understand!”

www.nobelprize.org

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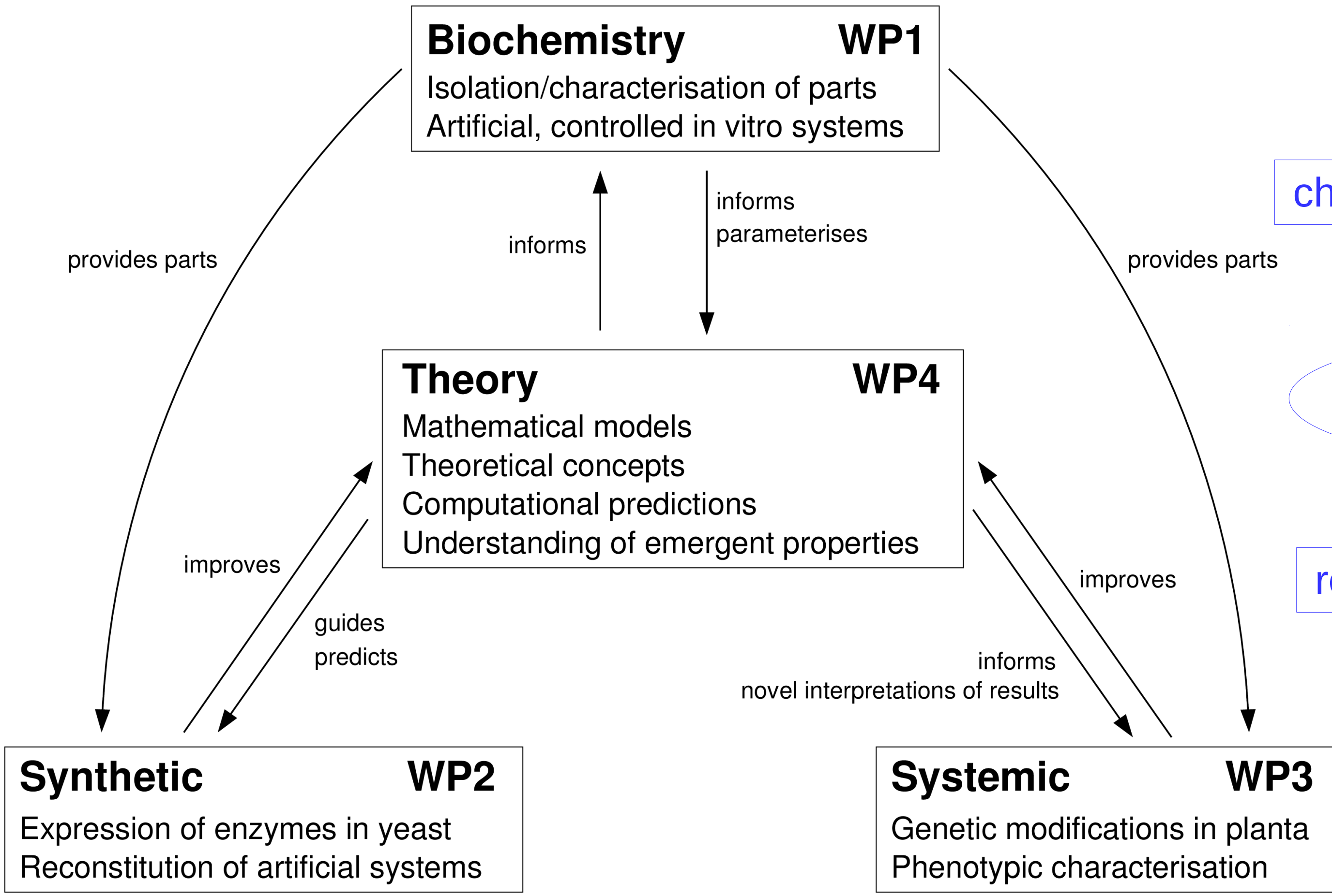
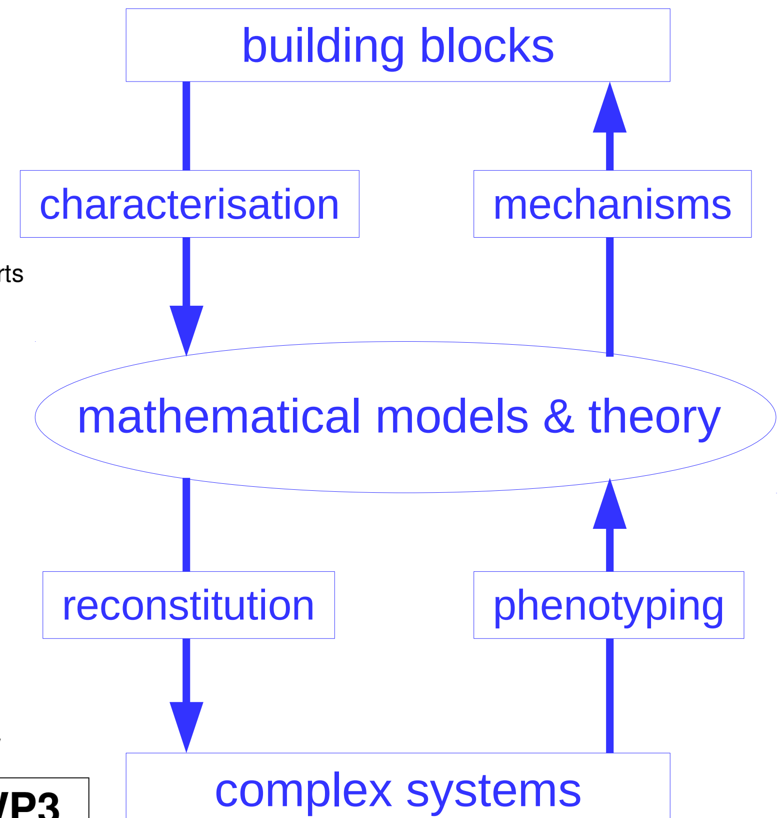
⟹ Let's build starch!

DesignStarch: the project

TOP-DOWN AND BOTTOM-UP



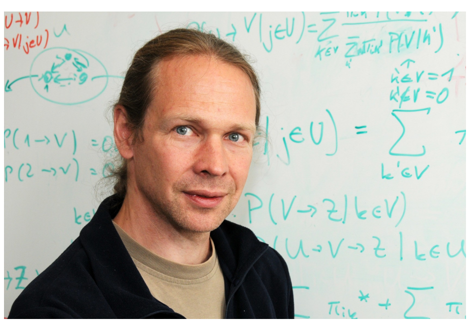


The team

Sam Zeeman

- expression of enzymes in yeast
- targeted modification of plants

Oliver Ebenhöh



- mathematical models
- data management
- project coordination

Rob Field

- in vitro surface biochemistry
- in vitro polymer biochemistry

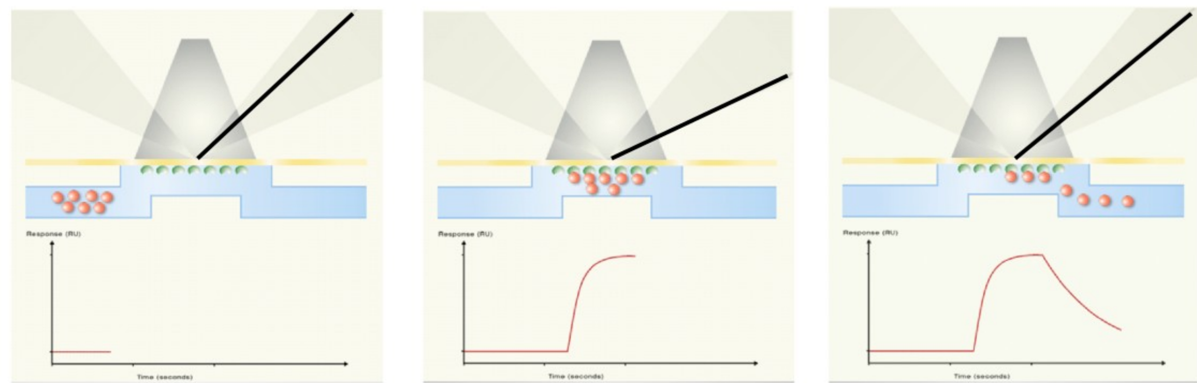
Preliminary work:
in vitro surface & polymer biochemistry

<u>How can you measure *in vitro* kinetics for surface-active enzymes?</u>

Surface Plasmon Resonance (SPR)

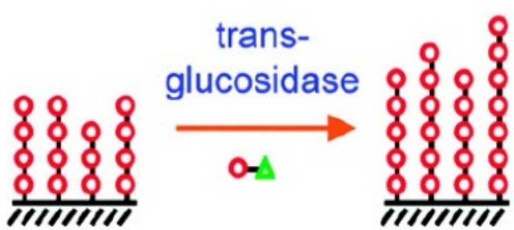
Changes on the surface are detected by changes in resonance wavelength

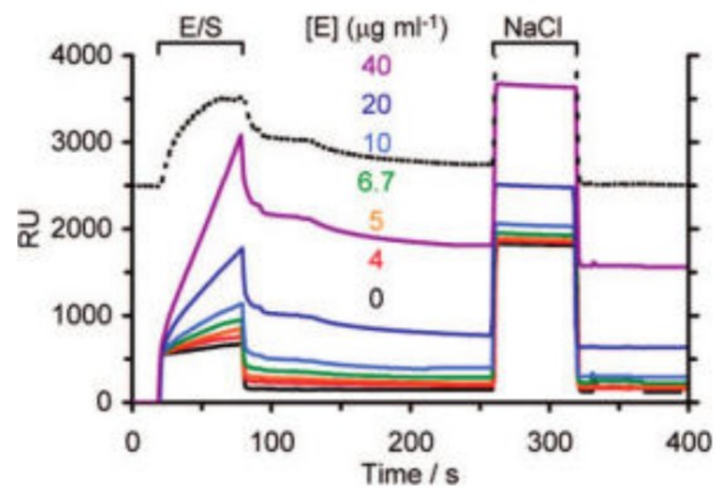
Chem. Commun, 2005, 3334; Glycoconj. J., 2008, 25, 69; ChemBioChem, 2008, 9, 1568; Chem. Sci., 2011, 2, 1952



Baseline | Association | Dissociation

Application to surface-active carbohydrate-active enzymes:

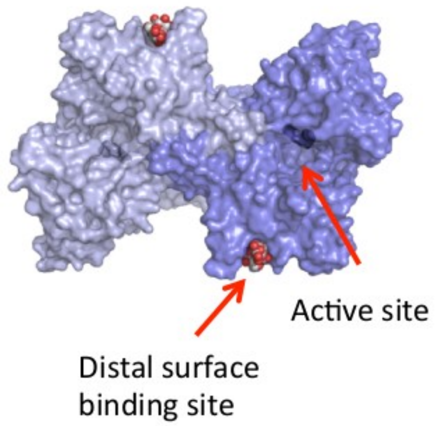


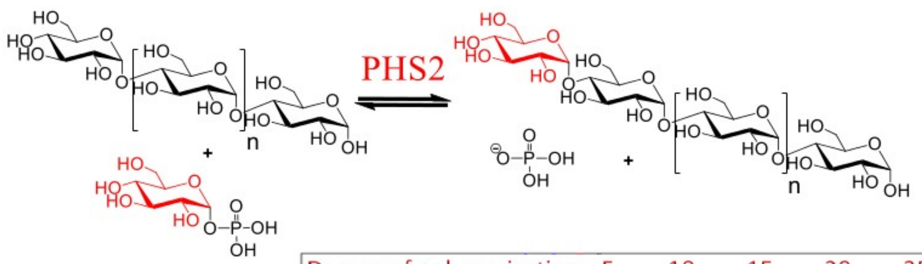


JACS, 2008, 130, 15234

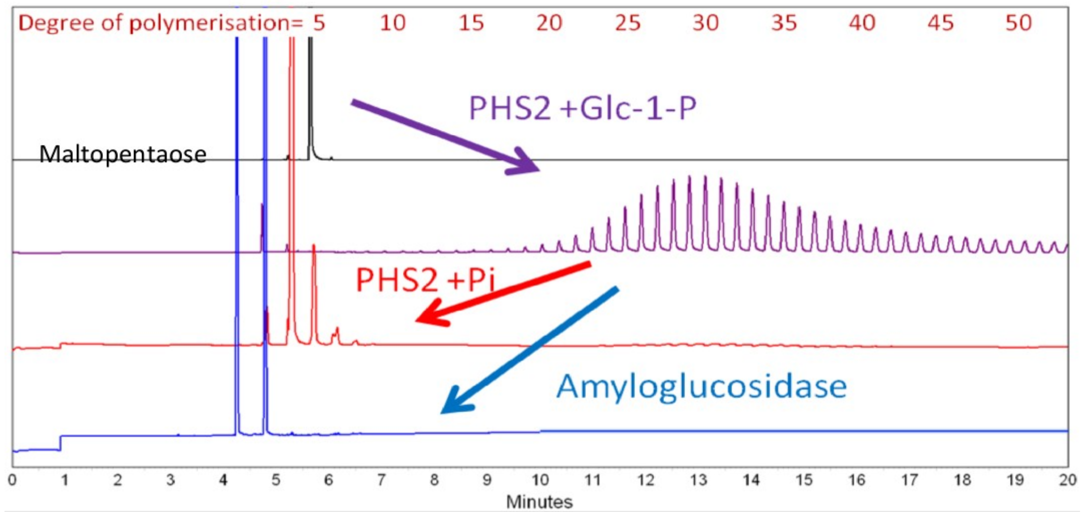
Preliminary work:
in vitro surface & polymer biochemistry

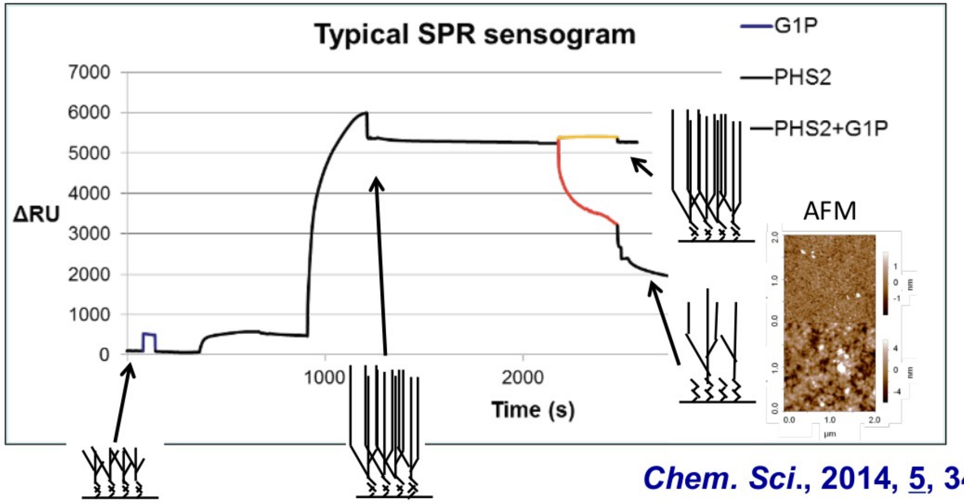
Phosphorylase (PHS2) is involved in glucan synthesis





Reversible formation of glucan chains



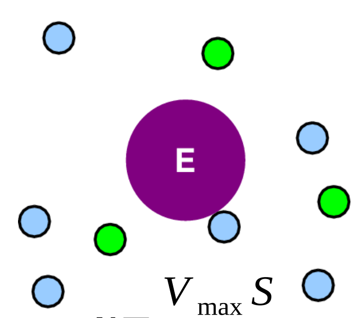


Generation of artificial 'starch-like' surfaces

Chem. Sci., 2014, 5, 341

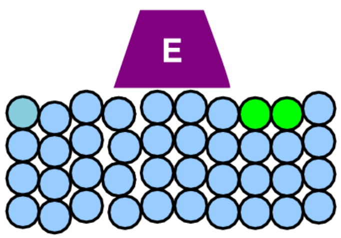
Preliminary work:
in silico surface & polymer biochemistry

dissolved substrate



$$v = \frac{V_{max} S}{K_M + S}$$

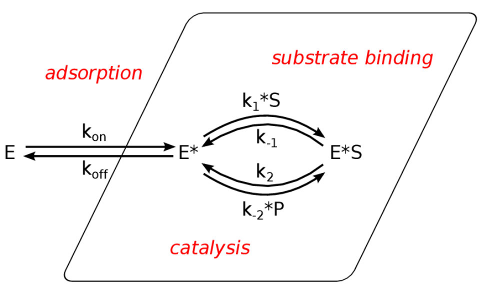
aggregated substrate (with interfacial reaction space)

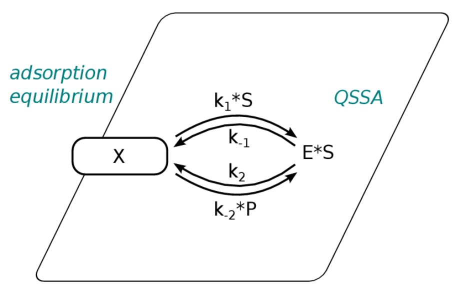


$$v = f(?)$$

<u>Fundamental question:</u>
What is an appropriate rate law for surface-active enzymes?

<u>Derivation of a generic surfactive rate-law</u>





$$v = \frac{k_A a_s \Phi_{eq}[M][E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + k_A a_s \Phi_{eq}[M]\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)} = \frac{V_M^{app}\frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}$$

Kartal and Ebenhöh (2013) FEBS Letters – centenary issue commemorating Michaelis-Menten 'Kinetik der Invertinwirkung'

Preliminary work:
in silico surface & polymer biochemistry

<u>Fundamental question:</u>
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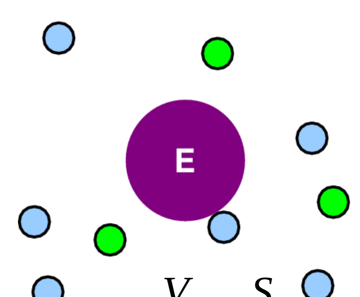

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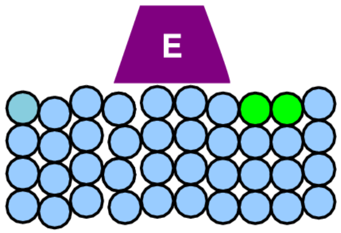
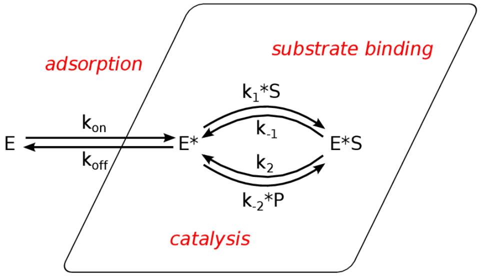
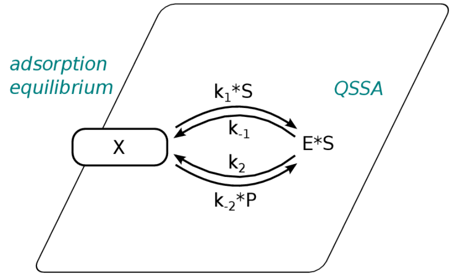


$$v = f(?)$$

<u>Derivation of a generic surfactive rate-law</u>





$$v = \frac{k_A \boxed{a_s}\boxed{\Phi_{eq}}[M][E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + k_A \boxed{a_s}\boxed{\Phi_{eq}}[M]\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)} = \frac{V_M^{app}\frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}$$

specific surface area

“few big objects behave different to many small objects”

available area function

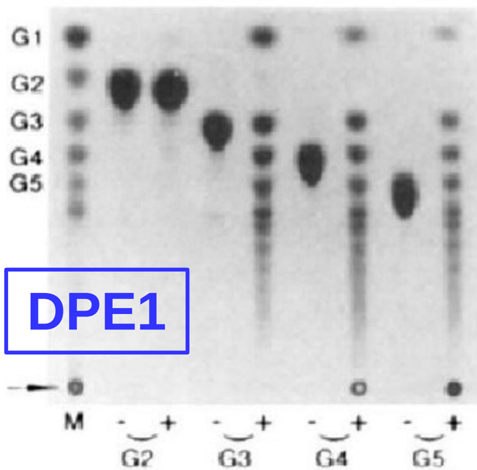
“many enzymes (also others) jam the surface”

⇨ Important implication for the interpretation of *in vitro* kinetics

Kartal and Ebenhöh (2013) FEBS Letters – centenary issue commemorating Michaelis-Menten 'Kinetik der Invertinwirkung'

Preliminary work: *in silico* surface & polymer biochemistry

<u>Problem</u>: polymer-active enzymes catalyse an ***infinite*** number of individual reactions



(from: Takaha et al., JBC 1993)

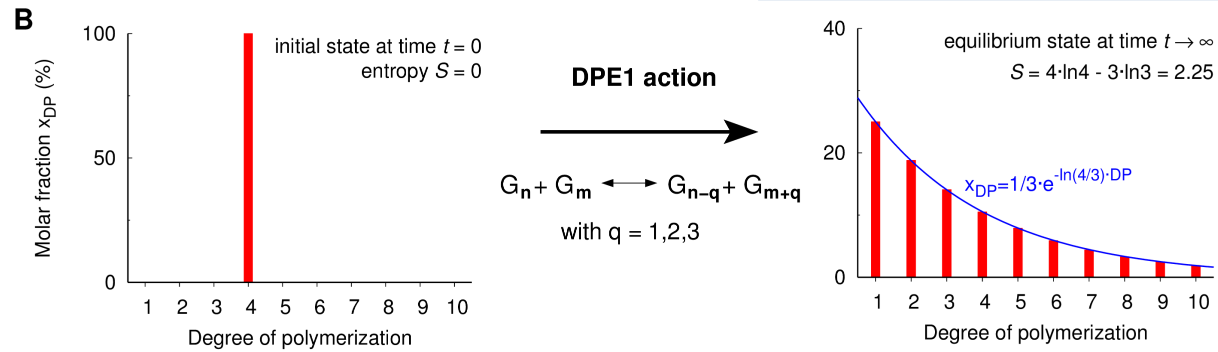
What determines the equilibrium of

$$G_n + G_m \longleftrightarrow G_{n-q} + G_{m+q} \quad ?$$

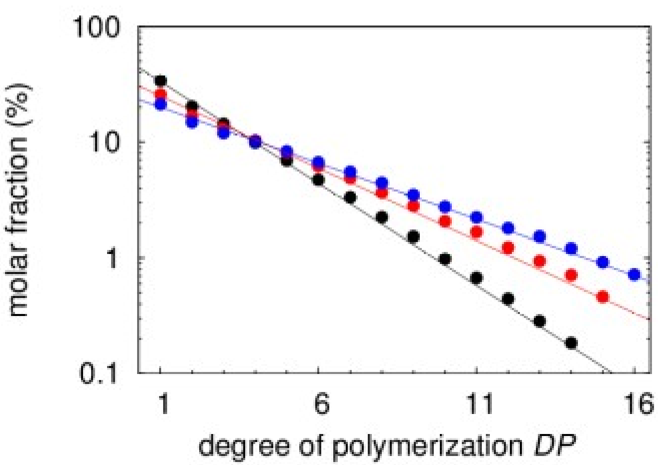
<u>max. entropy principle:</u>

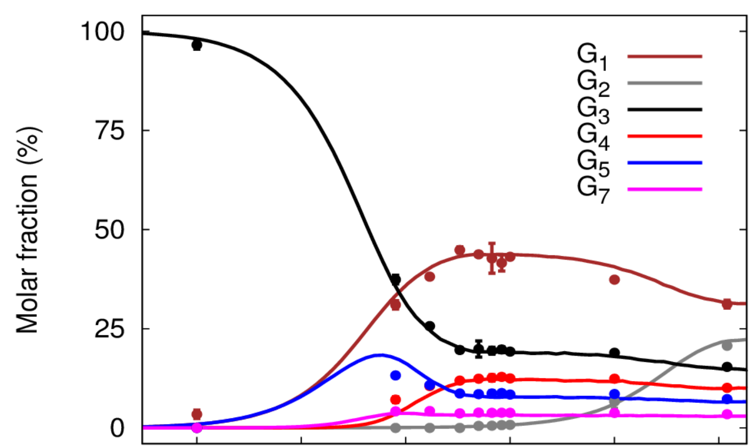
$$S = -\sum x_k \ln x_k \rightarrow \max!$$

$$\boxed{x_i = \frac{1}{Z} e^{-\beta E_i}, \quad \beta = \ln \frac{\mathrm{DP}_{\mathrm{ini}}}{\mathrm{DP}_{\mathrm{ini}} - 1}}$$



Theory can explain equilibria and time courses:

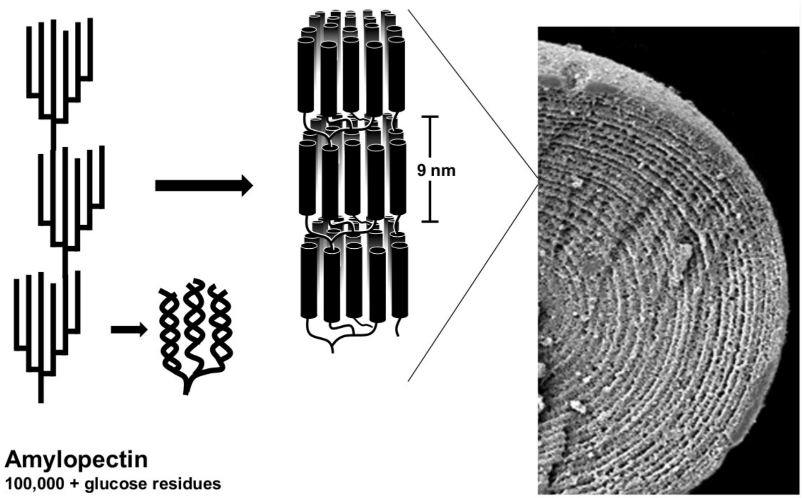




(Kartal et al, 2011, Mol Syst Biol)

Preliminary work:
expressing starch-like polymers in yeast

The branching pattern matters!



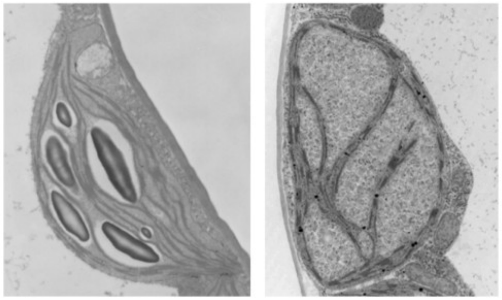
ADPglucose → Chain elongation → Branching → **Debranching !**

5 iso-enzymes	2+ iso-enzymes	3 iso-enzymes

Plants

SS1 Synthesis of short chains
SS2 Elongation of intermediate chains
SS3 Synthesis of long cluster-spanning chains
SS4 Granule initiation and shape

GBSS Amylose synthesis within the granule



Debranching enzymes are critical for making branched glucans!

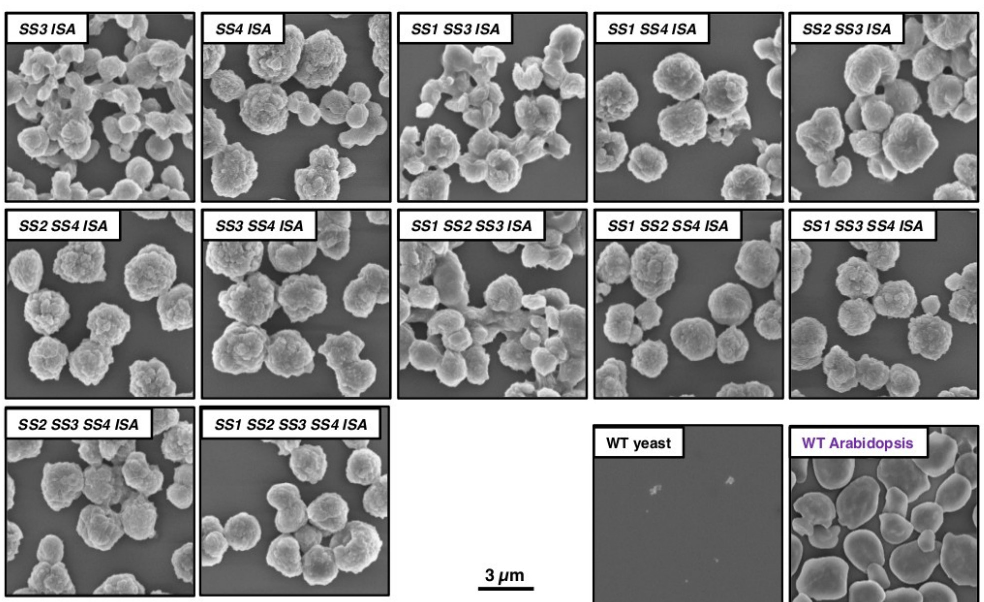
Preliminary work: expressing starch-like polymers in yeast

STARCH IN YEAST?

Barbara Pfister

- Delete all 7 glycogen biosynthesis genes
- Progressively add Arabidopsis genes
- All lines express AGPase and both BE isoforms
- Variable combinations of starch synthases with the presence/absence of ISA

Iodine-stained galactose plate



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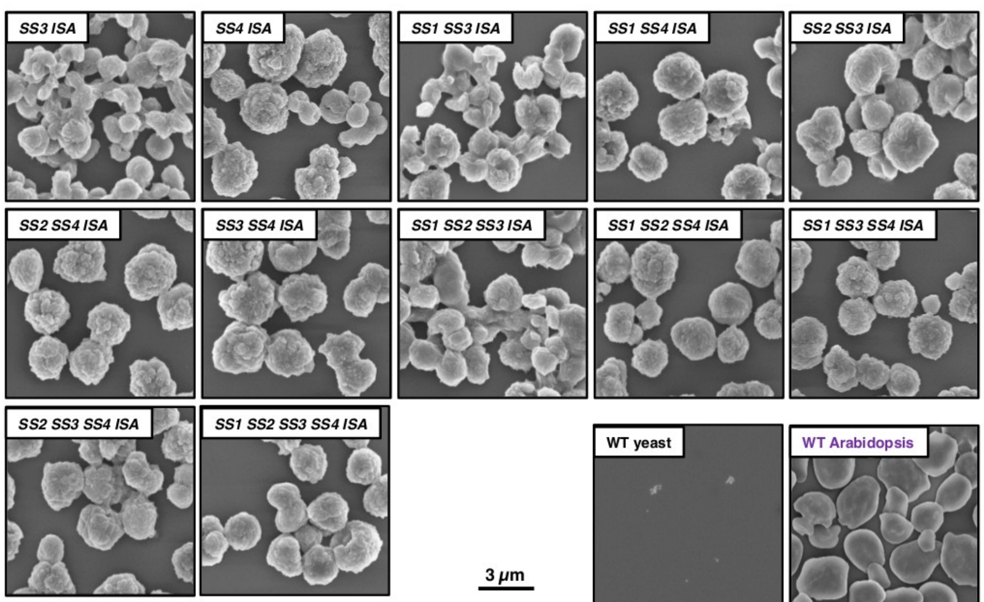

Conclusion & Outlook:

- We are only beginning to understand...
- We get something that looks like starch, but is not!
- How does this actually work?
- How can we control the properties of the insoluble glucans?

Thanks...

Reviewers

“This is a very bold project indeed.”

“...it is very much worth the gamble: this should be funded.”

“I have to cast strong doubt on the feasibility of the proposed work plan.”

Funding agencies

DFG Deutsche Forschungsgemeinschaft

FNSNF
Fonds national suisse
Schweizerischer Nationalfonds
Fondo nazionale svizzero
Swiss National Science Foundation

BBSRC
bioscience for the future

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Action in Plant Sciences