HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

CEPLAS
Cluster of Excellence on Plant Sciences

1495
UNIVERSITY OF ABERDEEN

Mathematical Models of Plant Energy Metabolism

Towards synthetic starch

Oliver Ebenhöh

AcclіPhot
www.accliphot.eu

aSSB Strasbourg, 25.3.2015

Why do we need mathematical models?

- Simplified representation of reality
- Reduction to the essentials

“Simplicity is the ultimate sophistication”

(Leonardo da Vinci)

Why do we need mathematical models?

- Simplified representation of reality
- Reduction to the essentials

“Simplicity is the ultimate sophistication”

(Leonardo da Vinci)

⇨ Models help to discover general principles!

Why do we need mathematical models?

- Simplified representation of reality
- Reduction to the essentials

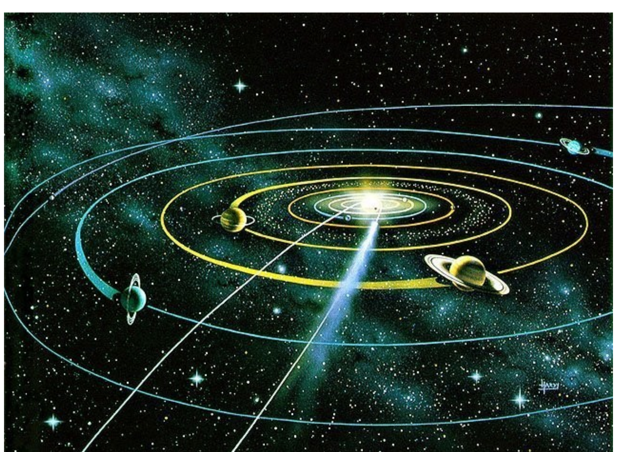
"Simplicity is the ultimate sophistication"

(Leonardo da Vinci)

⇨ Models help to discover general principles!

Example from physics:

$$\vec{F} = m \cdot \vec{a}$$

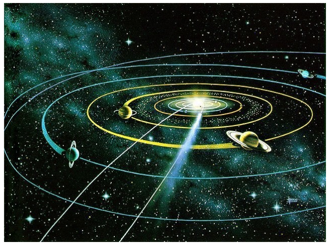


www.thehungryandfoolish.com

www.hh.schule.de

www.welt.de

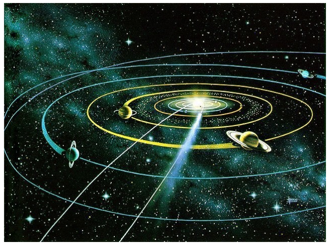
How does one find principles (theory building)?



???

$$\vec{F} = m \cdot \vec{a}$$

How does one find principles (theory building)?



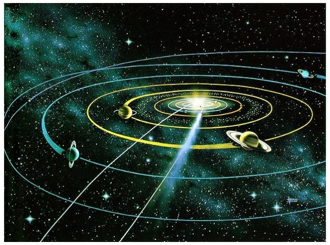
???

$$\vec{F} = m \cdot \vec{a}$$

Intuition

Every model is a small step on this path

How does one find principles (theory building)?

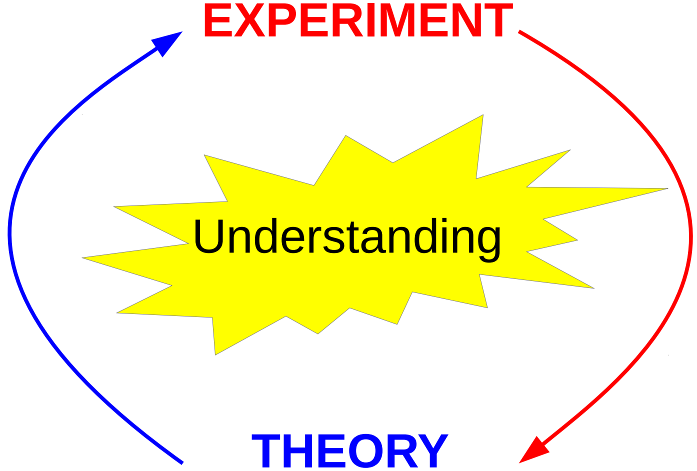


??? → $\vec{F} = m \cdot \vec{a}$

Intuition

Every model is a small step on this path

- Model predictions / new hypotheses
- Suggestions for new experiments
- Improvement of experimental design



- Initial model formulation
- Confirmation / falsification of predictions
- New model assumptions

The Systems biology principle

What's special about plants?

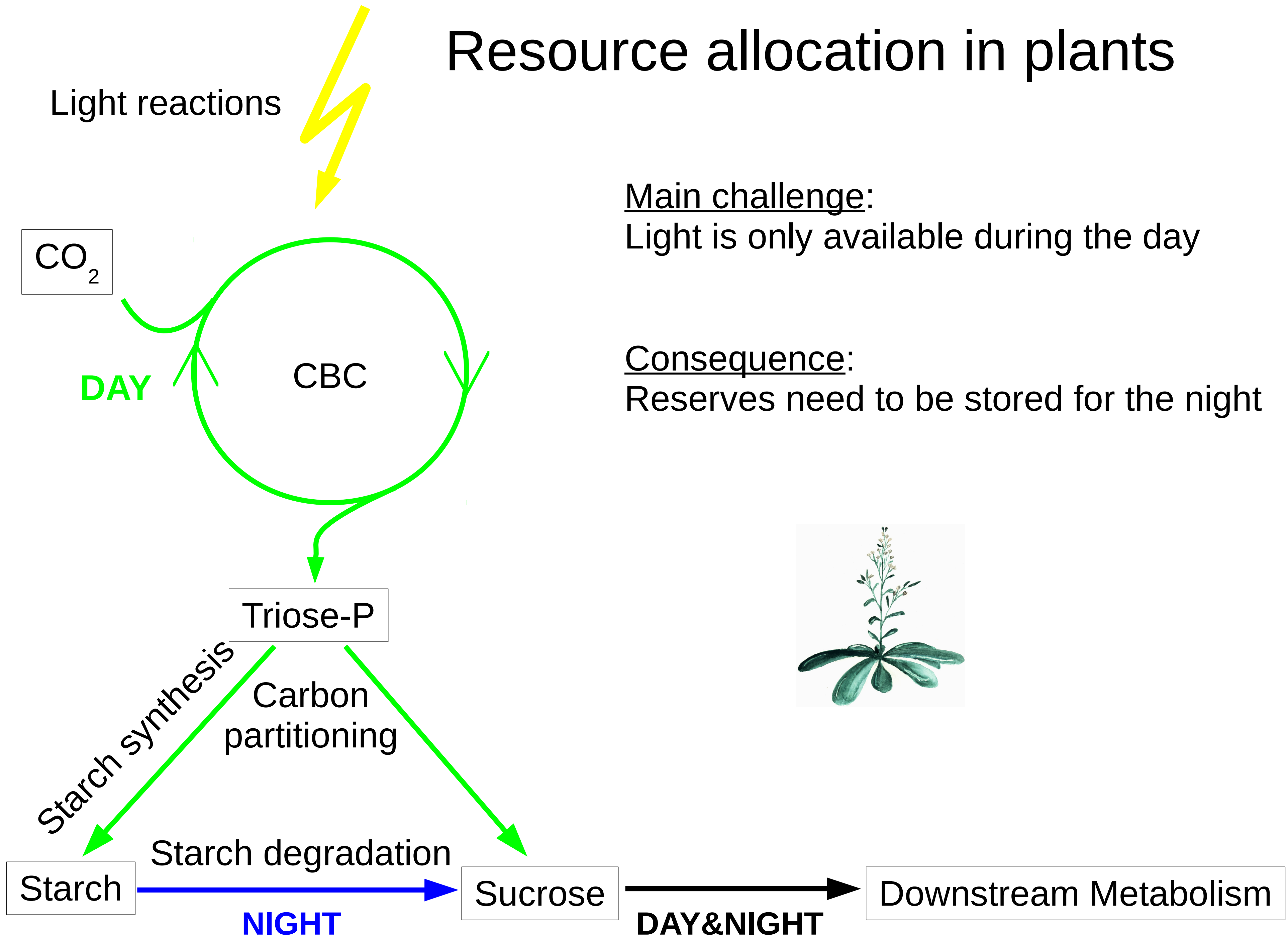
1. Photosynthesis
2. Can't run away!

⇨ Experts in chemical warfare!

Estimated > 200,000 secondary metabolites!

(commons.wikimedia.org)

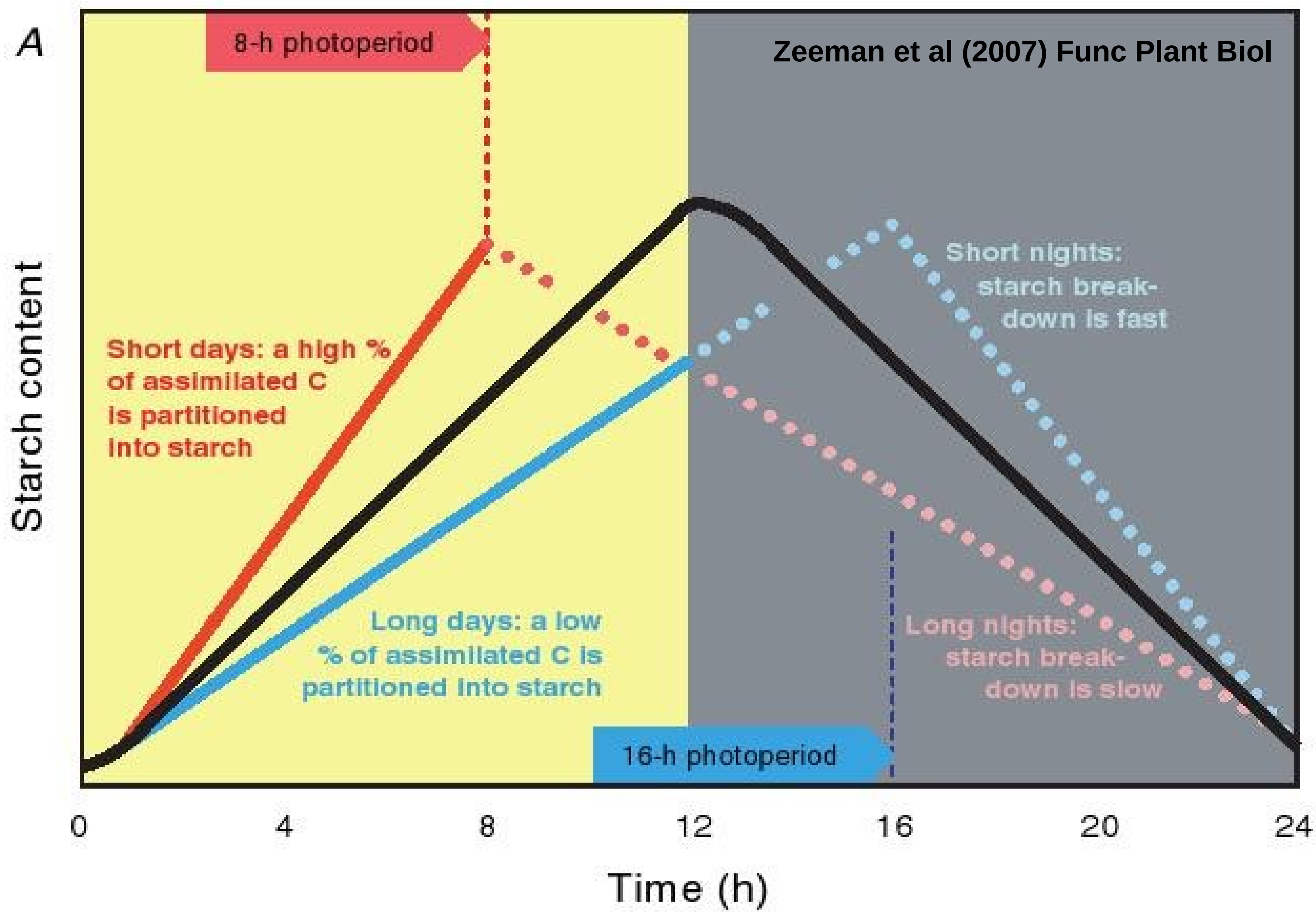
Resource allocation in plants



<u>Main challenge</u>:
Light is only available during the day

<u>Consequence</u>:
Reserves need to be stored for the night

The diurnal turnover of starch



Open questions

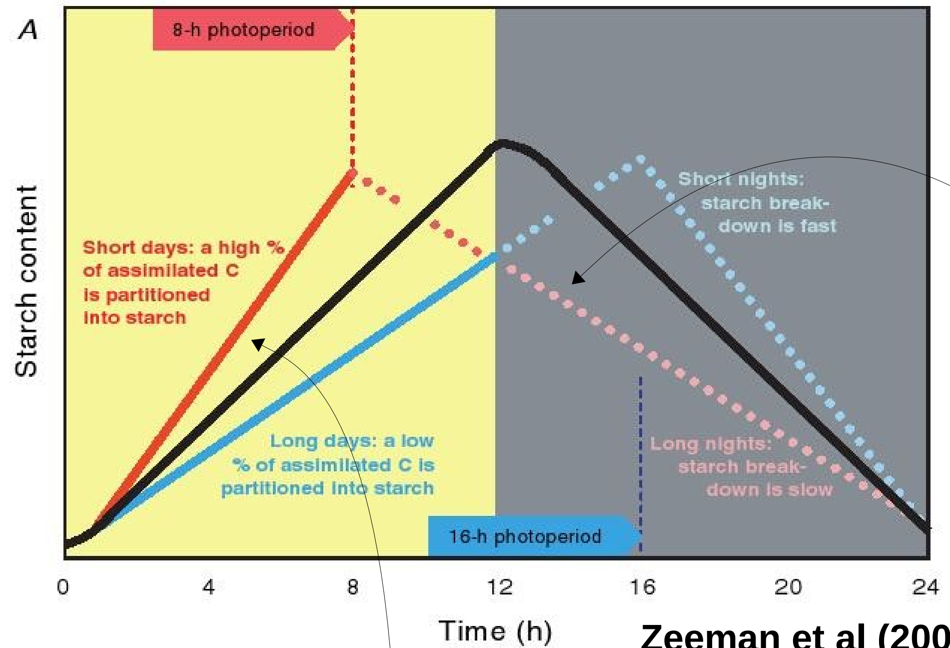
?

?

How does the clock 'tell' expected length of day/night?

What measures the starch content?

?



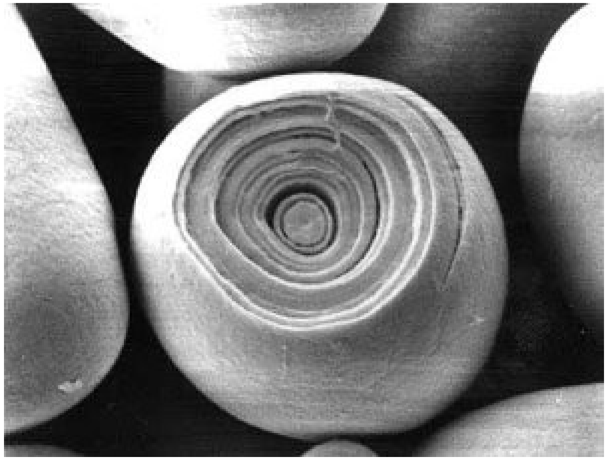
How is the correct breakdown rate 'calculated'?

Zeeman et al (2007) Func Plant Biol

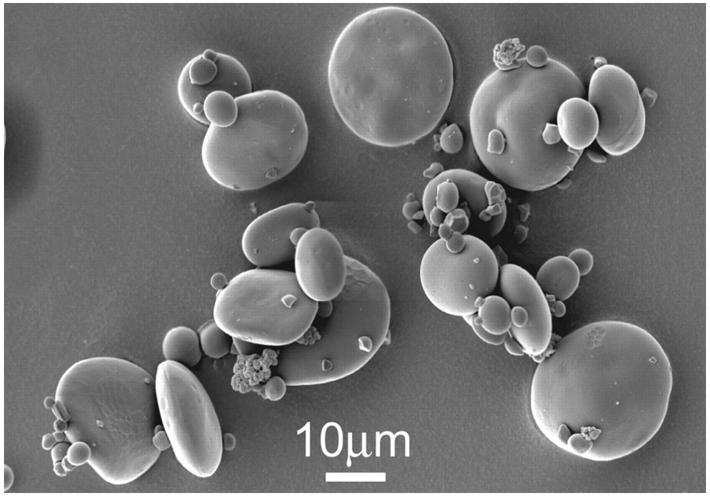
TiMet

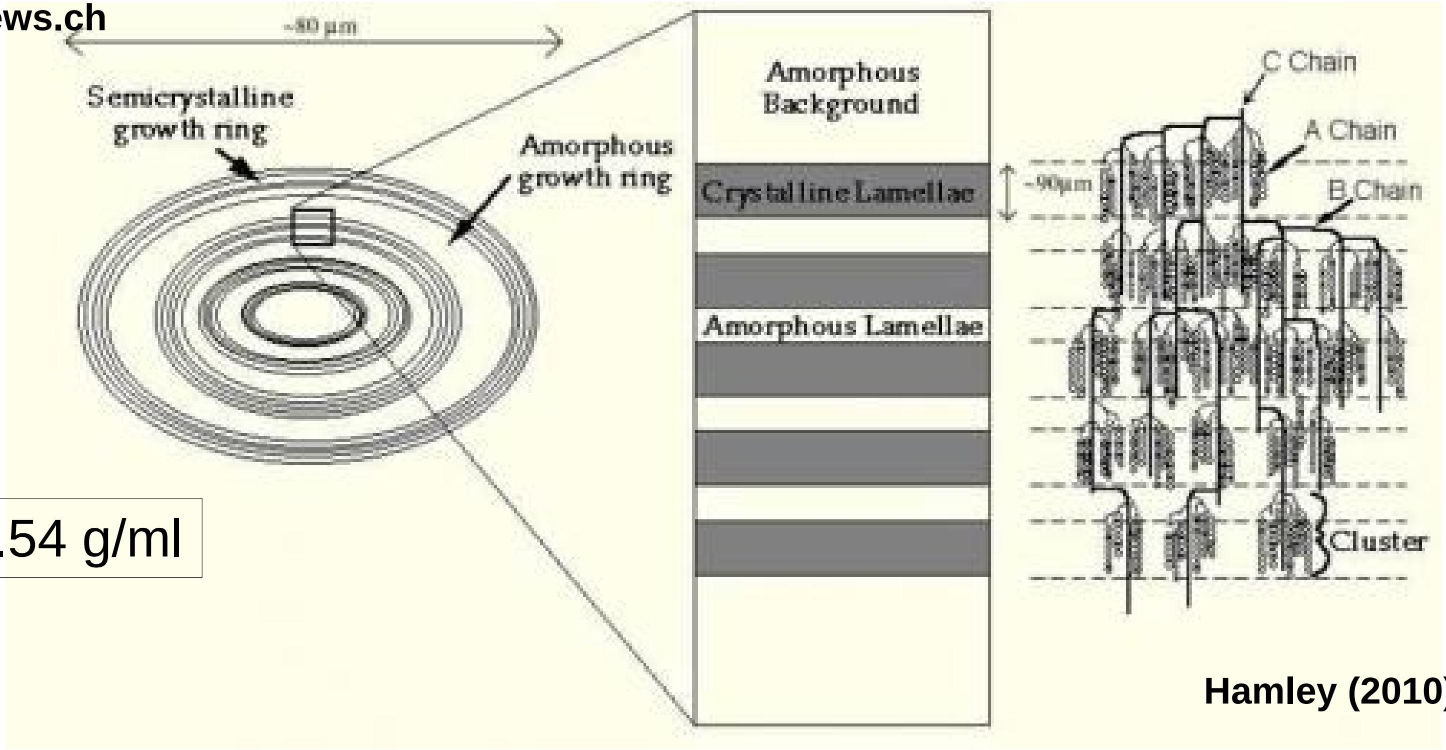
How is carbon partitioning controlled?

Why starch?



http://foodnews.ch





Density: 1.54 g/ml

Hamley (2010) Soft Matter

The structure of starch allows for an extremely high energy storage density

Alternatives

	energy content (kJ/g)
Carbohydrates	17
Lipids	38
Proteins	17
Alcohol	30

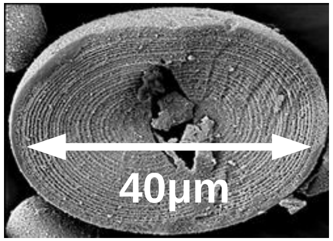
We (animals and fungi) predominantly use glycogen

big molecule (up to 10 MDa)

Possible advantages of starch

- low osmolarity
- large size
- high density

still small compared to starch



http://swissplantscienceweb.ch

→ $3 \cdot 10^{10}$ Da!!!

Alternatives

	energy content (kJ/g)
Carbohydrates	17
Lipids	38
Proteins	17
Alcohol	30

We (animals and fungi) predominantly use glycogen

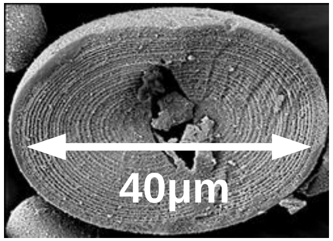
trade-off between storage density and rapid mobilization

big molecule (up to 10 MDa)

Possible advantages of starch

- low osmolarity
- large size
- high density

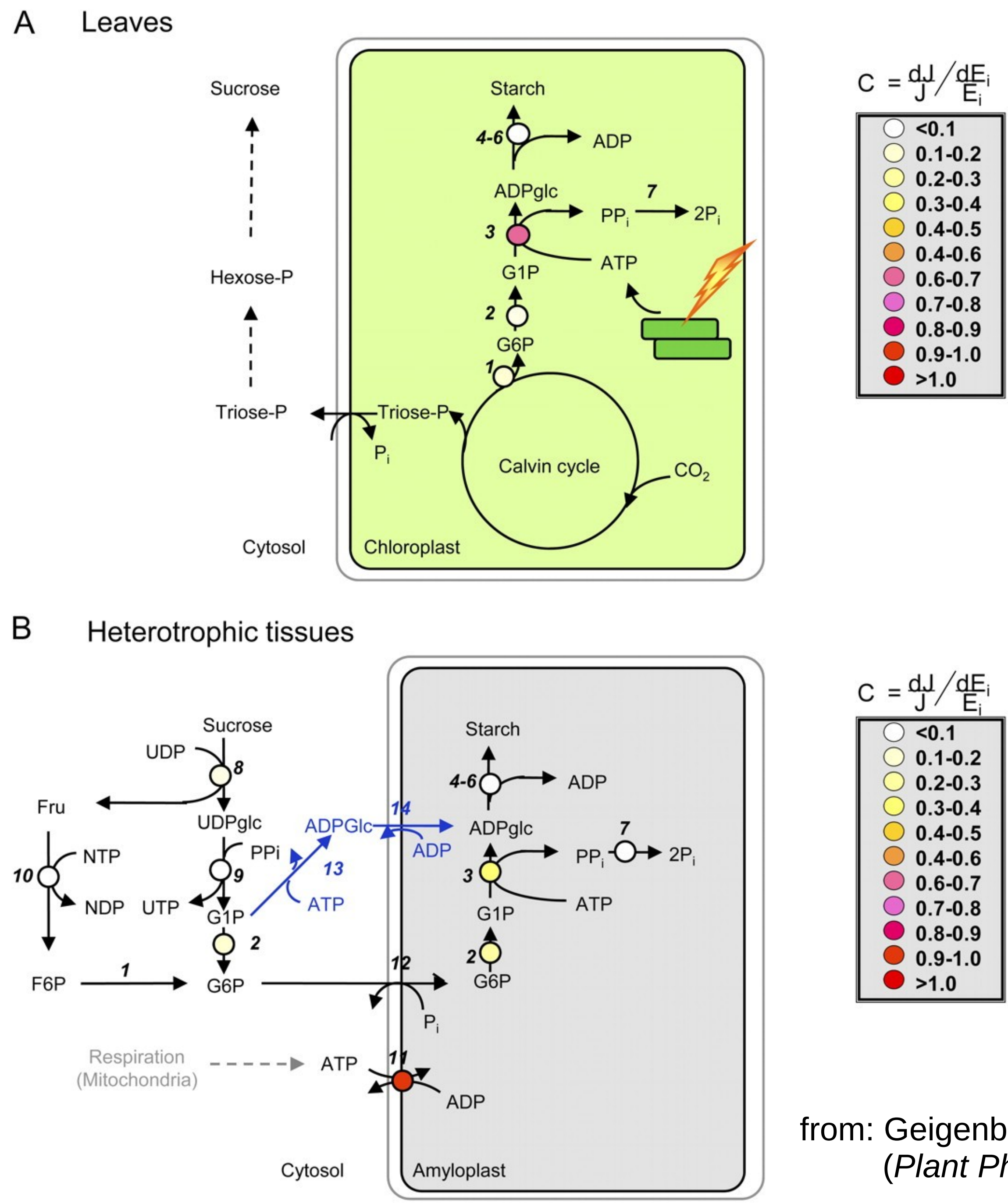
still small compared to starch



$3 \cdot 10^{10}$ Da!!!

optimised for storage density, slower deployment

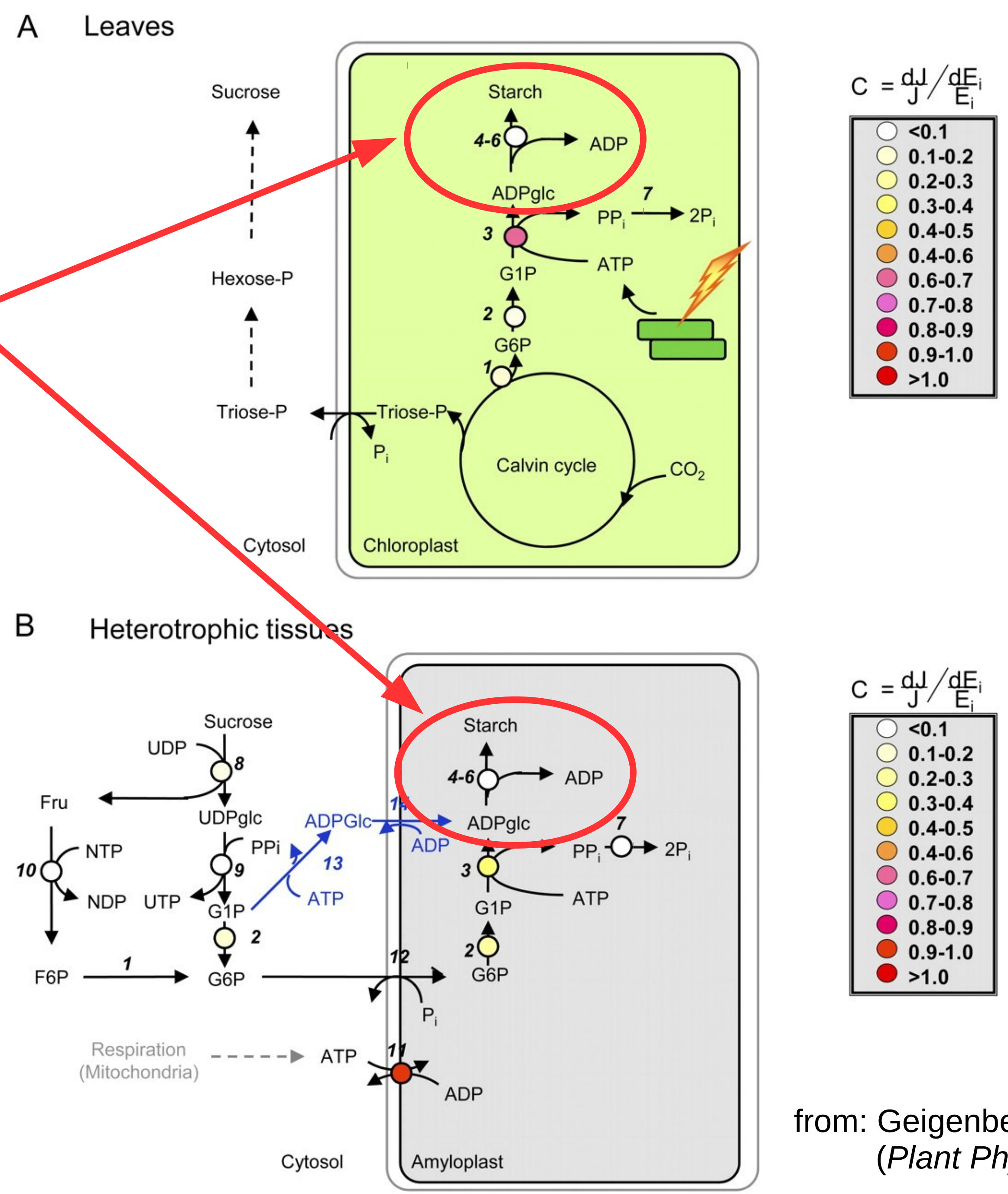
How is starch made?



from: Geigenberger 2011 (*Plant Phys*)

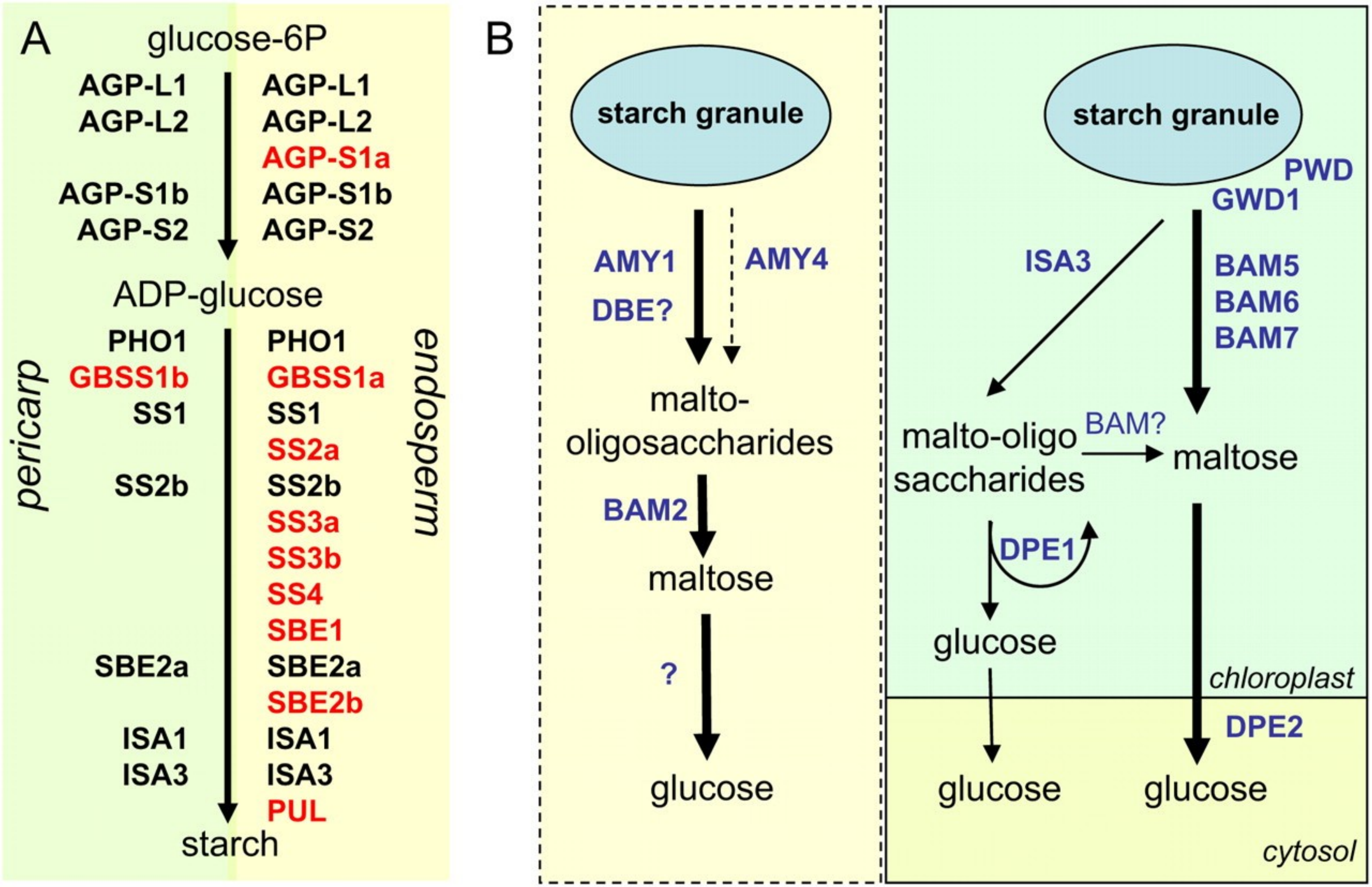
How is starch made?

What's behind these?



from: Geigenberger 2011 (*Plant Phys*)

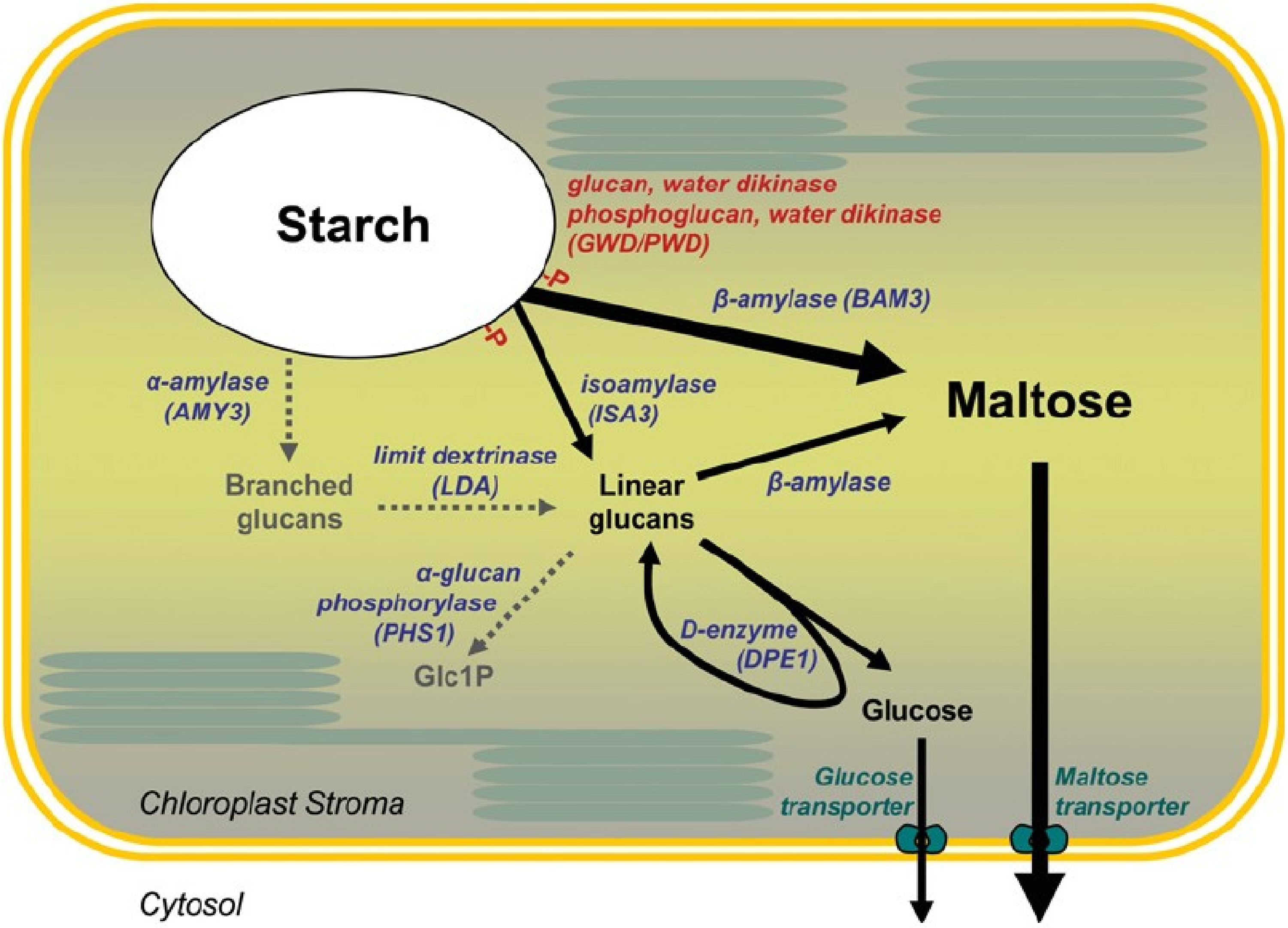
Many enzymes are involved in starch synthesis



- starch synthases
- branching enzymes
- phosphorylases
- isoamylases

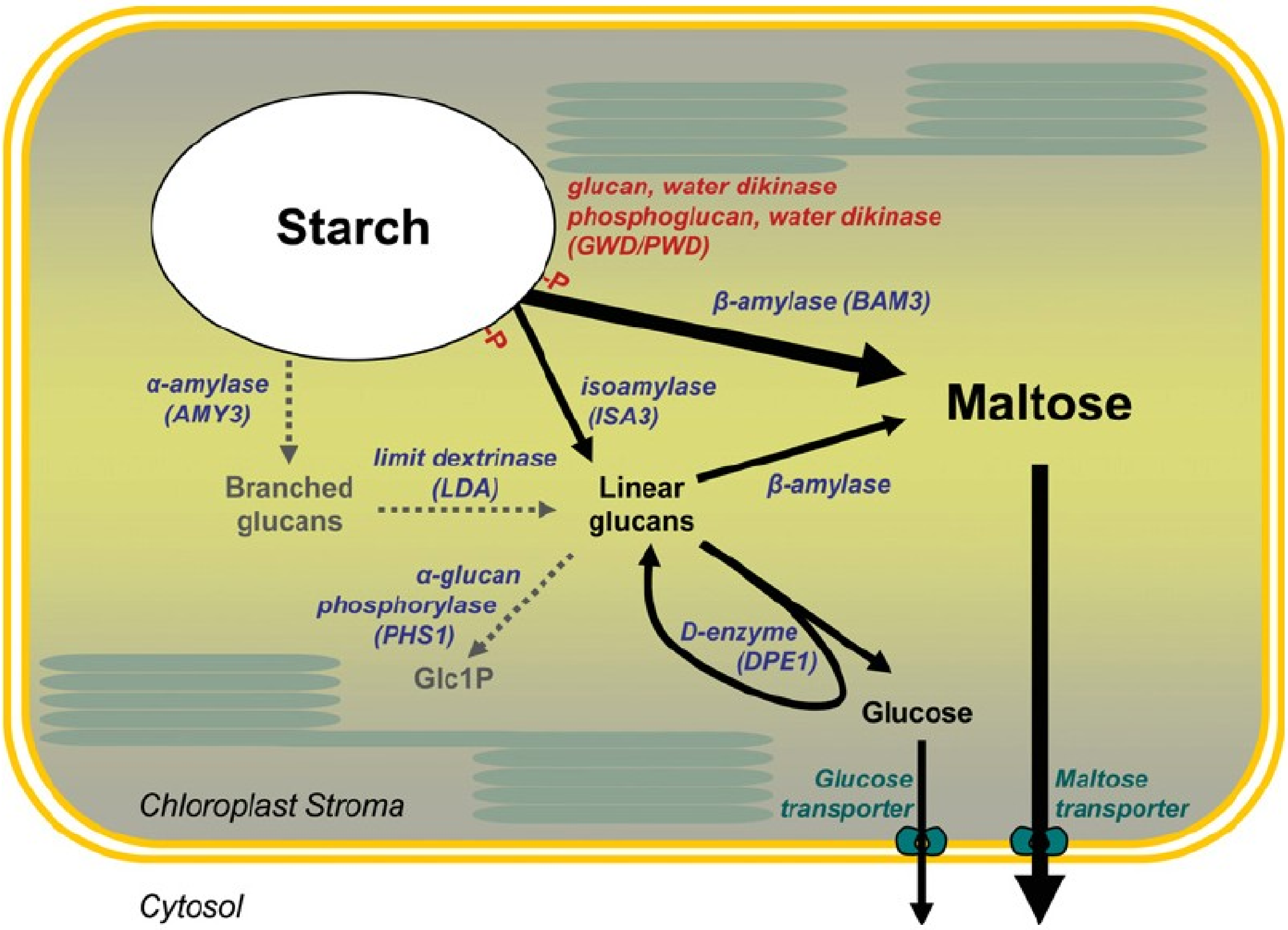
from: Radchuk et al 2009 (*Plant Phys*)

...and starch breakdown



from: Zeeman et al, 2007, *Biochem J*

...and starch breakdown



Many enzymes

- **are surface-active**

or

- **act on polymers**

⇩

hard to describe with traditional modelling approaches

from: Zeeman et al, 2007, *Biochem J*

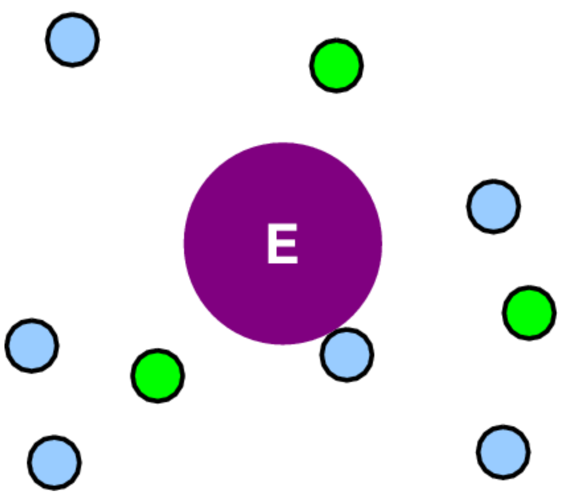
Challenges / Topics of lecture

1. Surface-active enzymes
2. Polymer-active enzymes
3. Timing of starch metabolism

1. Surface-active enzymes

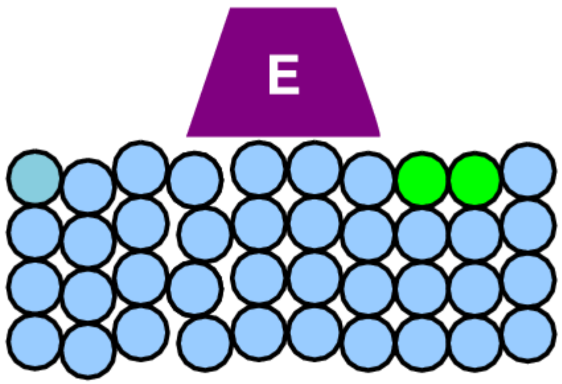
Rate laws for surfactive enzymes

dissolved substrate



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

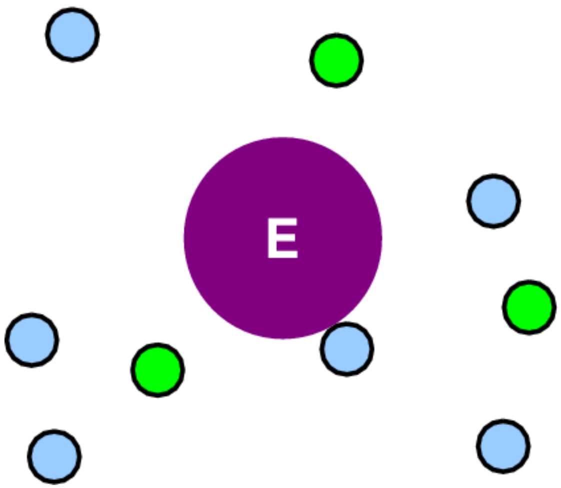
aggregated substrate (with interfacial reaction space)



$$v = f(?)$$

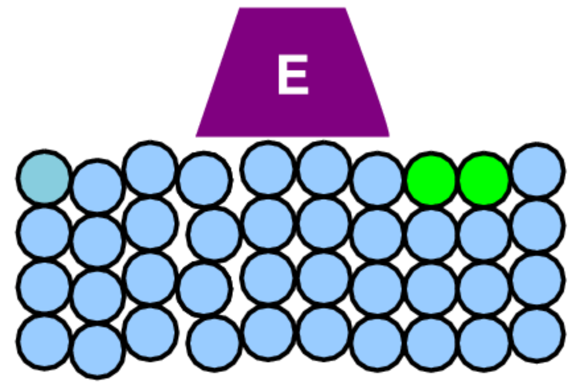
Rate laws for surfactive enzymes

dissolved substrate



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

aggregated substrate
(with interfacial reaction space)

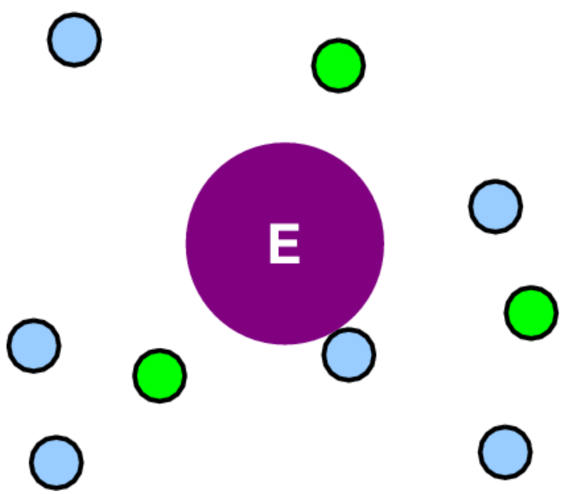


$$v = f(?)$$

Reaction space confined to 2D!

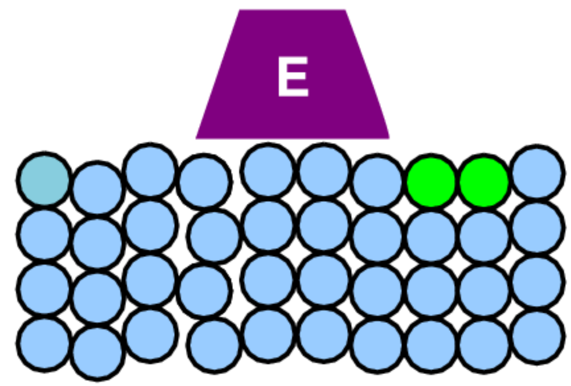
Rate laws for surfactive enzymes

dissolved substrate



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

aggregated substrate (with interfacial reaction space)



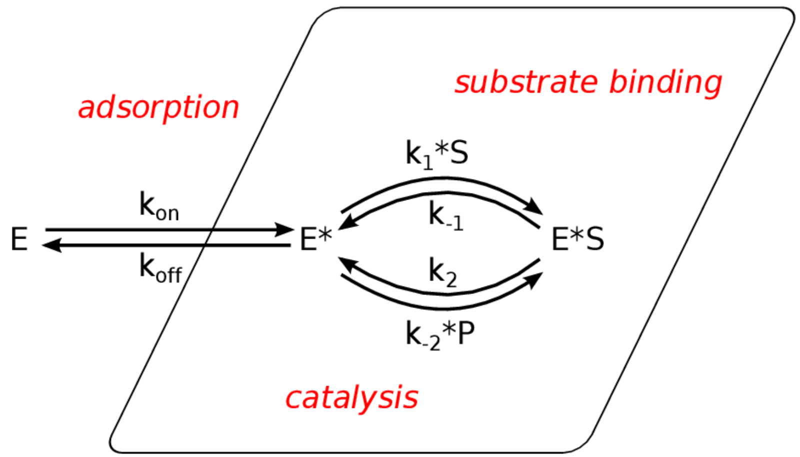
$$v=f(?)$$

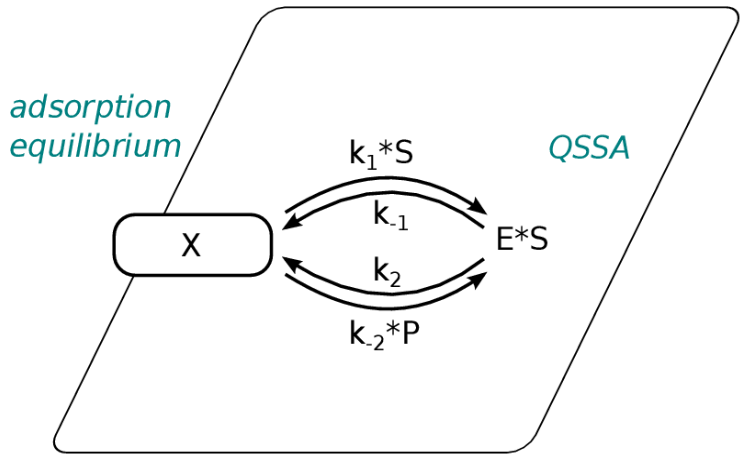
Reaction space confined to 2D!

Implications! - Fundamental differences to the classical case in solution:

- Relative activity dependent on enzyme concentration (jamming)
- Rate not independent on presence of other enzyme species! (competition)

Derivation of a generic surfactive rate-law

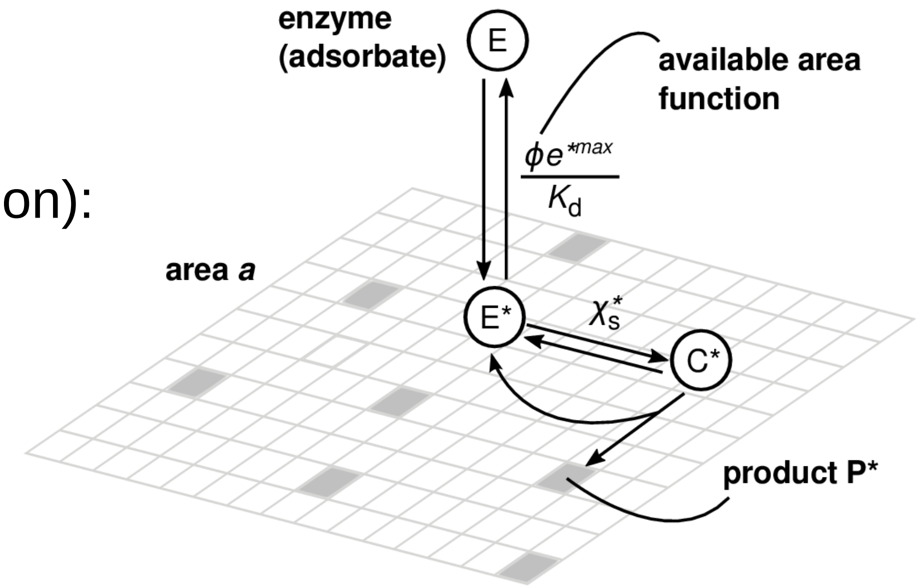




Kartal and Ebenhöh (2013) FEBS Letters – centenary issue commemorating Michaelis-Menten 'Kinetik der Invertinwirkung'

The adsorption equilibrium

<u>The Langmuir isotherm</u>
(a concept from surface physics)



Adsorption coverage (surface concentration):

$$\theta_E = \frac{n(E)}{n(E)_{\max}} = \frac{n(E)}{E_{\max} \cdot S}$$

Adsorption rate: $r_a \propto c(E) \cdot (1 - \theta_E)$

Desorption rate: $r_d \propto \theta_E$

The adsorption equilibrium

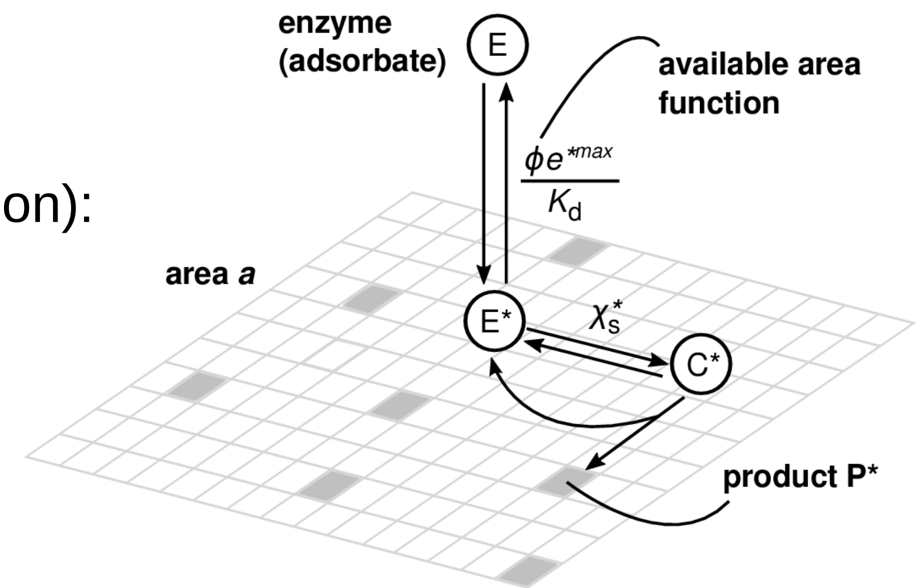
<u>The Langmuir isotherm</u>
(a concept from surface physics)

Adsorption coverage (surface concentration):

$$\theta_E = \frac{n(E)}{n(E)_{\max}} = \frac{n(E)}{E_{\max} \cdot S}$$

Adsorption rate: $r_a \propto c(E) \cdot (1-\theta_E)$

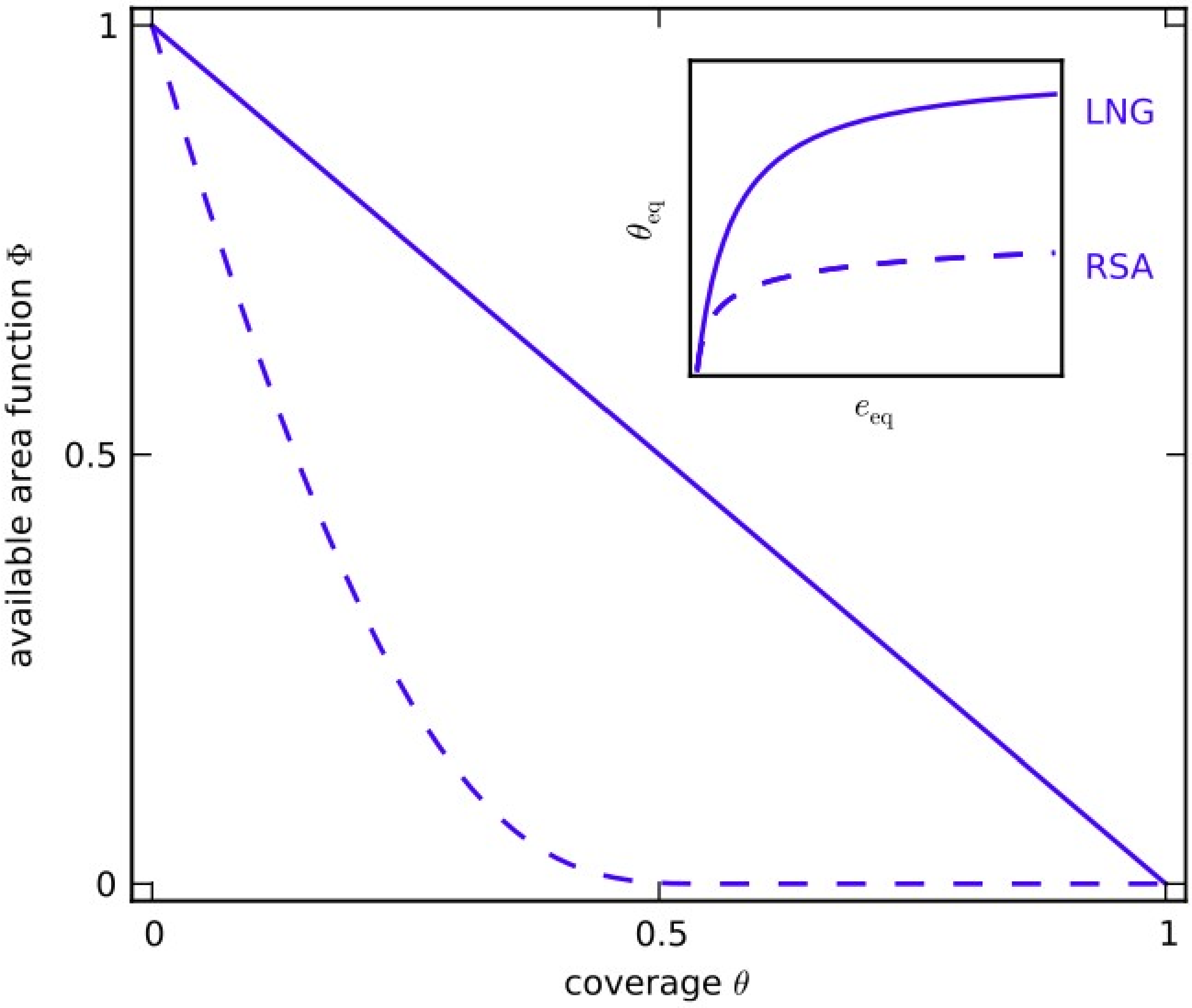
Desorption rate: $r_d \propto \theta_E$



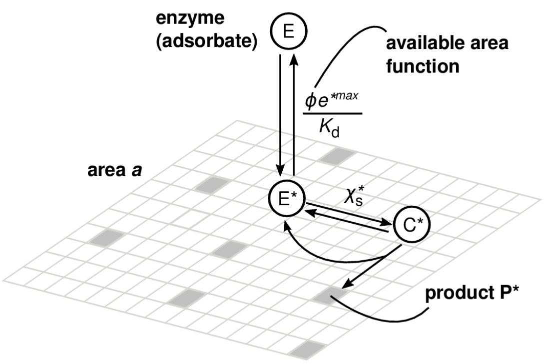
Available area function

The adsorption equilibrium

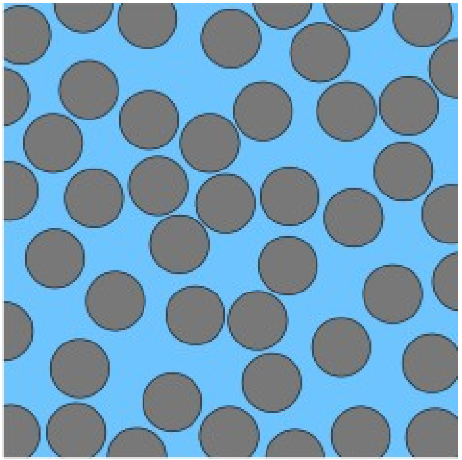
Other adsorption models can give quite different results:



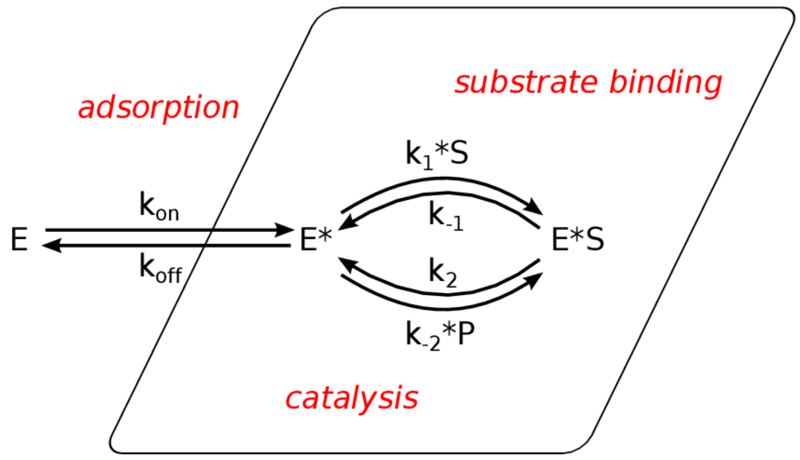
LNG

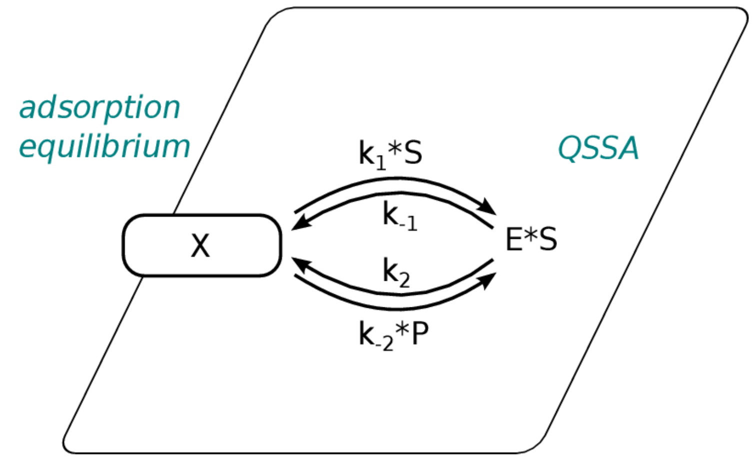


RSA



Derivation of a generic surfactive rate-law

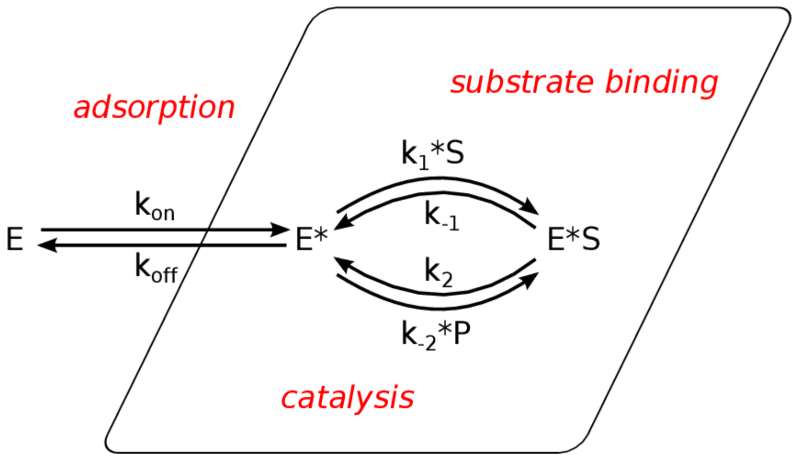


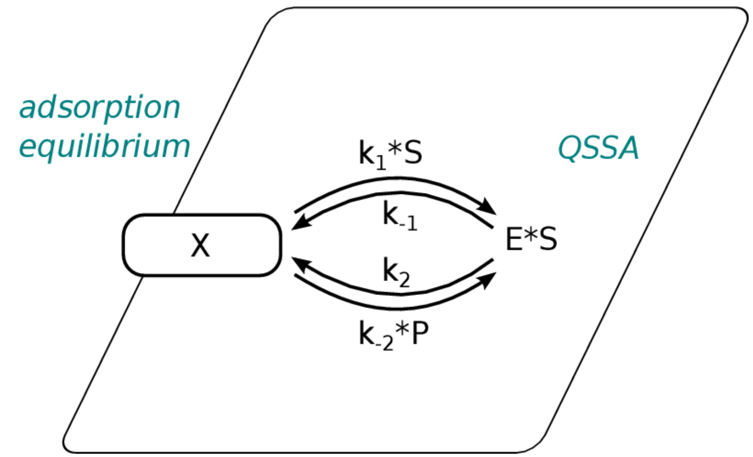


$$v = \frac{k_A a_s \Phi_{eq}[M][E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + k_A a_s \Phi_{eq}[M]\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)} = \frac{V_M^{app}\frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}$$

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

Derivation of a generic surfactive rate-law





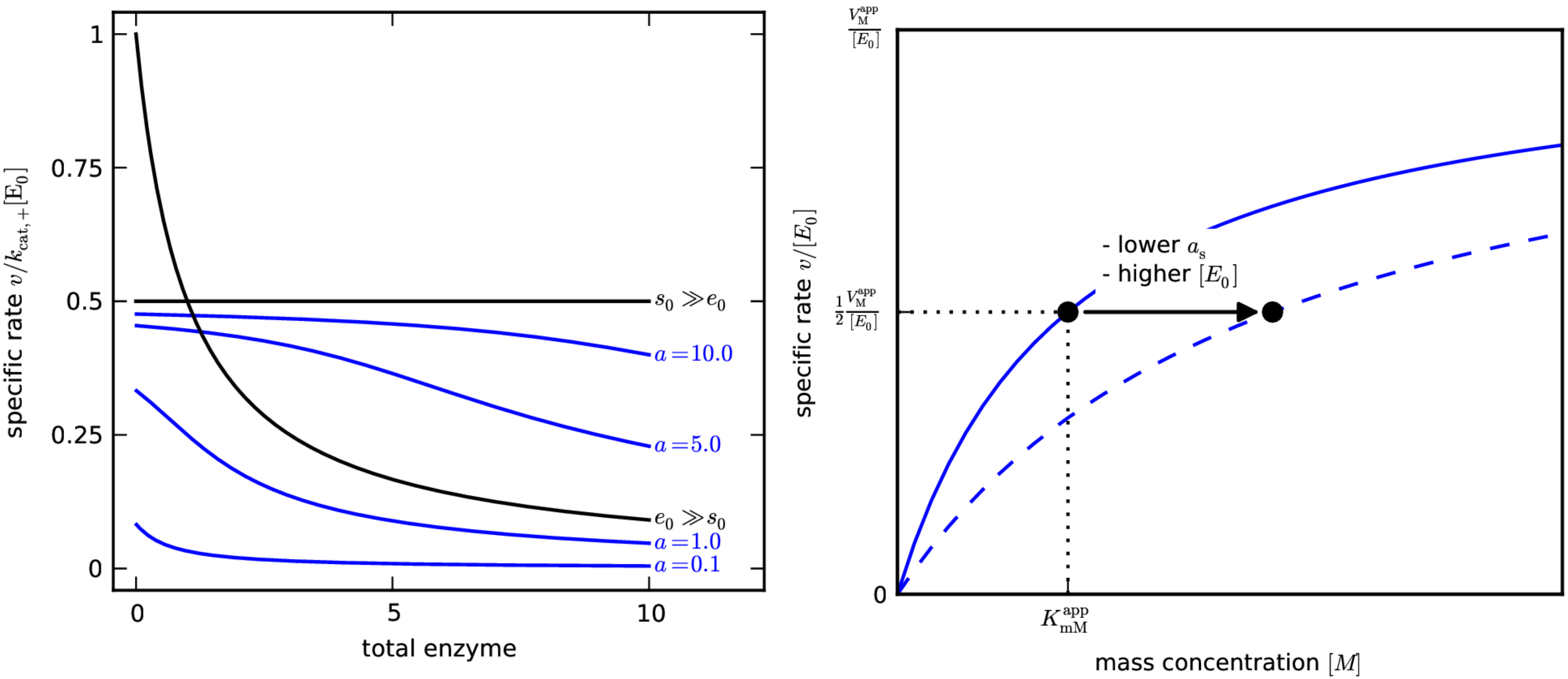
$$v = \frac{k_A a_s \Phi_{eq} [M][E_0](k_S \langle *S \rangle - k_P \langle *P \rangle)}{1 + k_A a_s \Phi_{eq} [M] \left(1 + \frac{\langle *S \rangle}{K_{mS}} + \frac{\langle *P \rangle}{K_{mP}}\right)} = \frac{V_M^{app} \frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}$$

specific surface area — "few big objects behave different to many small objects"

available area function — "many enzymes (also others) jam the surface"

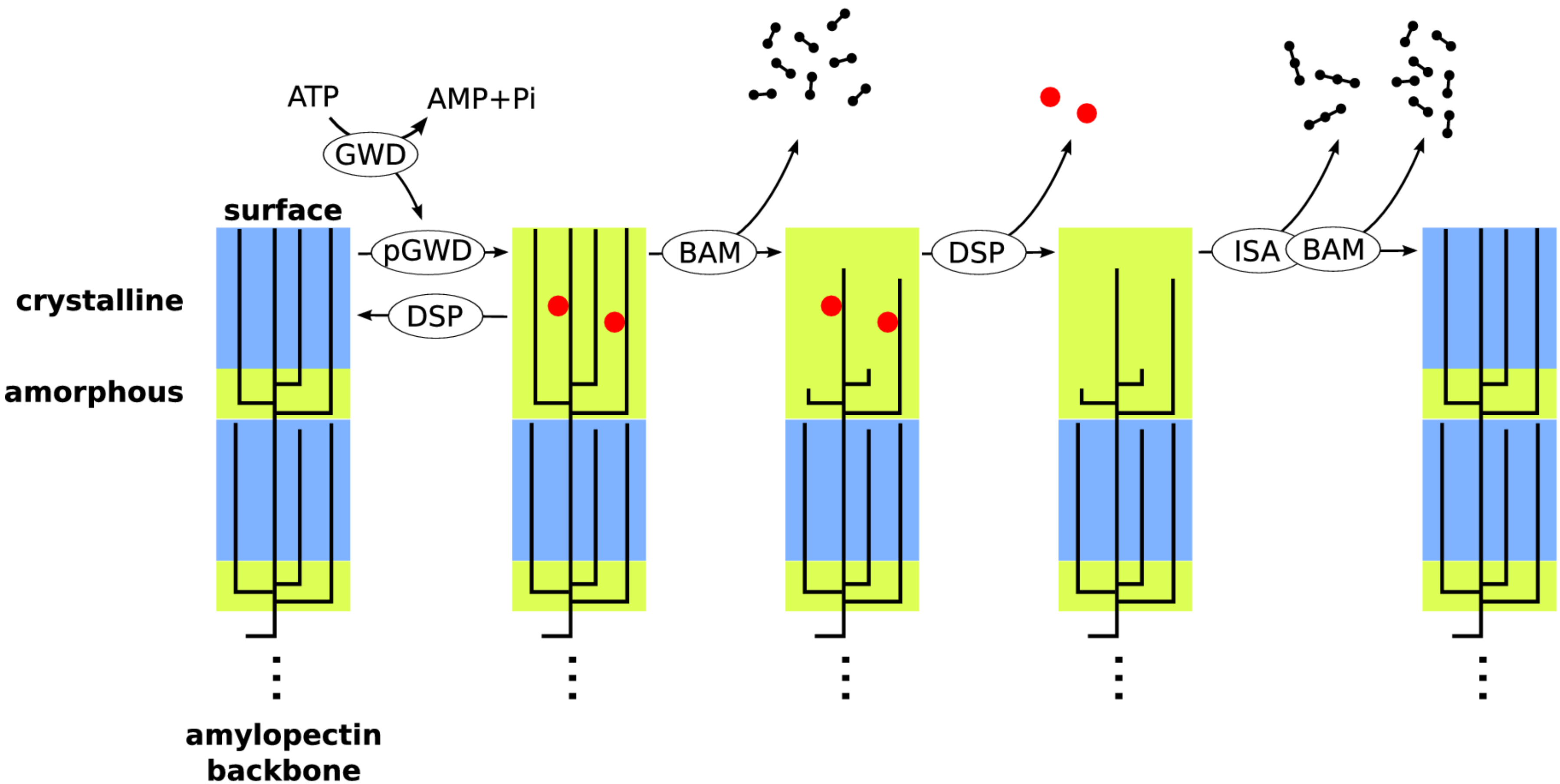
Kartal and Ebenhöh (2013) FEBS Letters – centenary issue commemorating Michaelis-Menten 'Kinetik der Invertinwirkung'

Consequences for experimental design



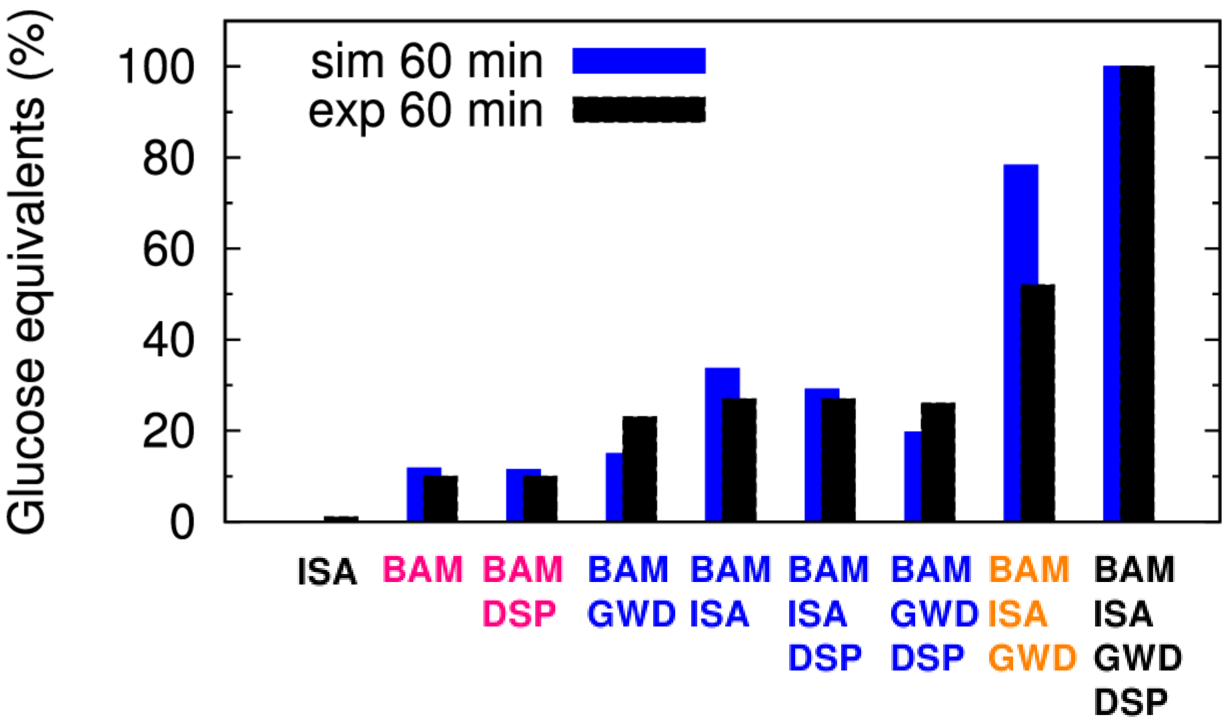
mass alone is insufficient!

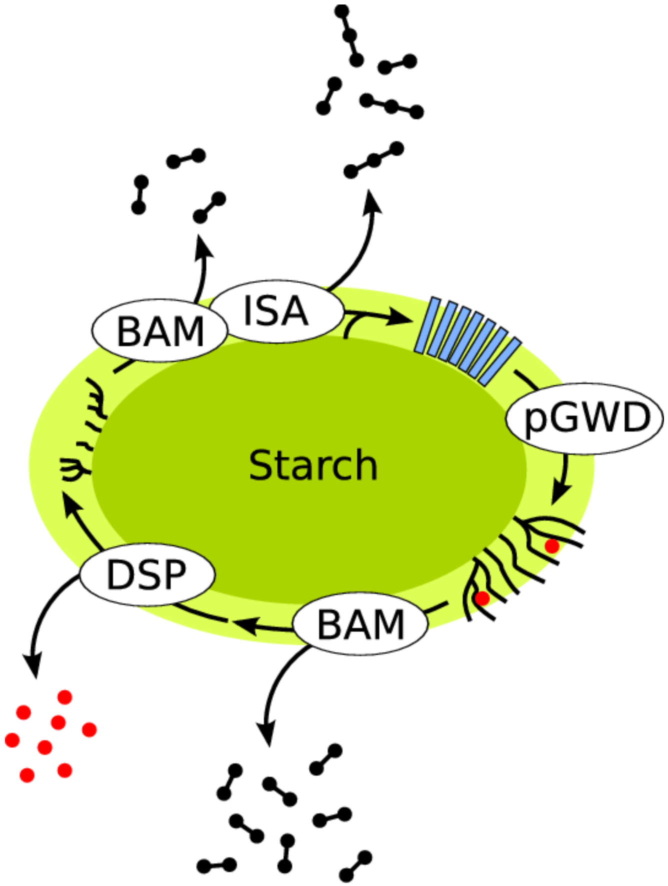
A kinetic model of starch surface attack



- Disruption of crystalline surface by phosphorylation allows access for BAM and ISA
- Dephosphorylation by DSP enables further degradation

Simulations compared to experiment



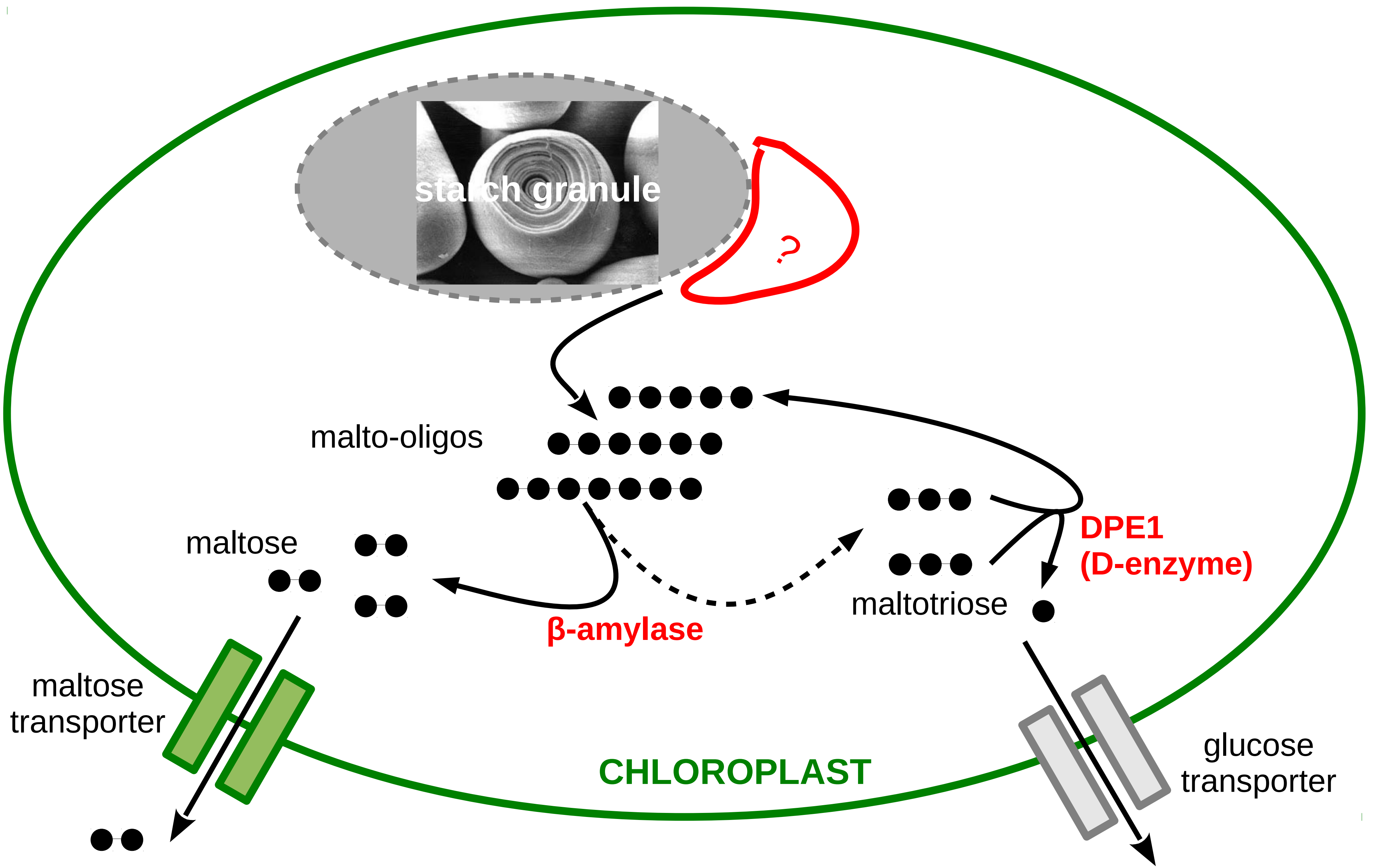


Good agreement with data from Kötting et al (2009) *Plant Cell*

But: only one time point!

2. Polymer Biochemistry

Starch degradation - disproportionation



Disproportionating enzymes (D-enzymes)

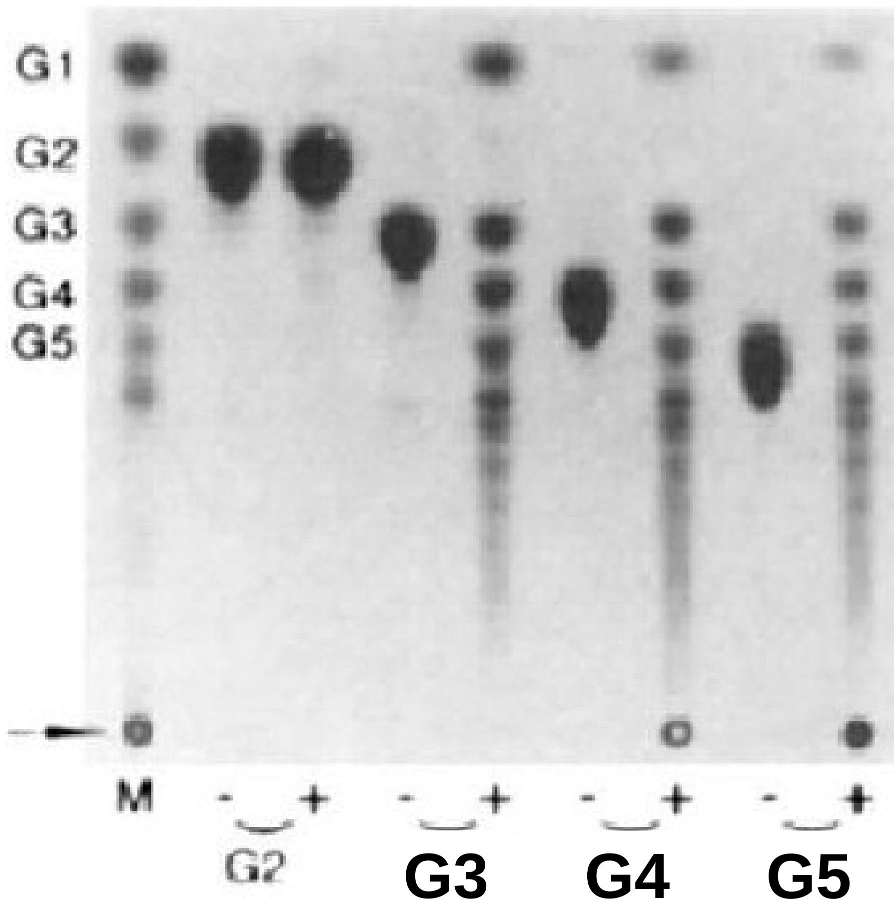
DPE1

EC: 2.4.1.25

catalyses $2\,\text{maltotriose} \leftrightarrow \text{maltopentaose} + \text{glucose}$

$$G3 + G3 \leftrightarrow G5 + G1$$

but not only!



DPE1 produces a set of glucans of different length in *in vitro* assays.

(Takaha et al., JBC 1993)

Disproportionating enzymes (D-enzymes)

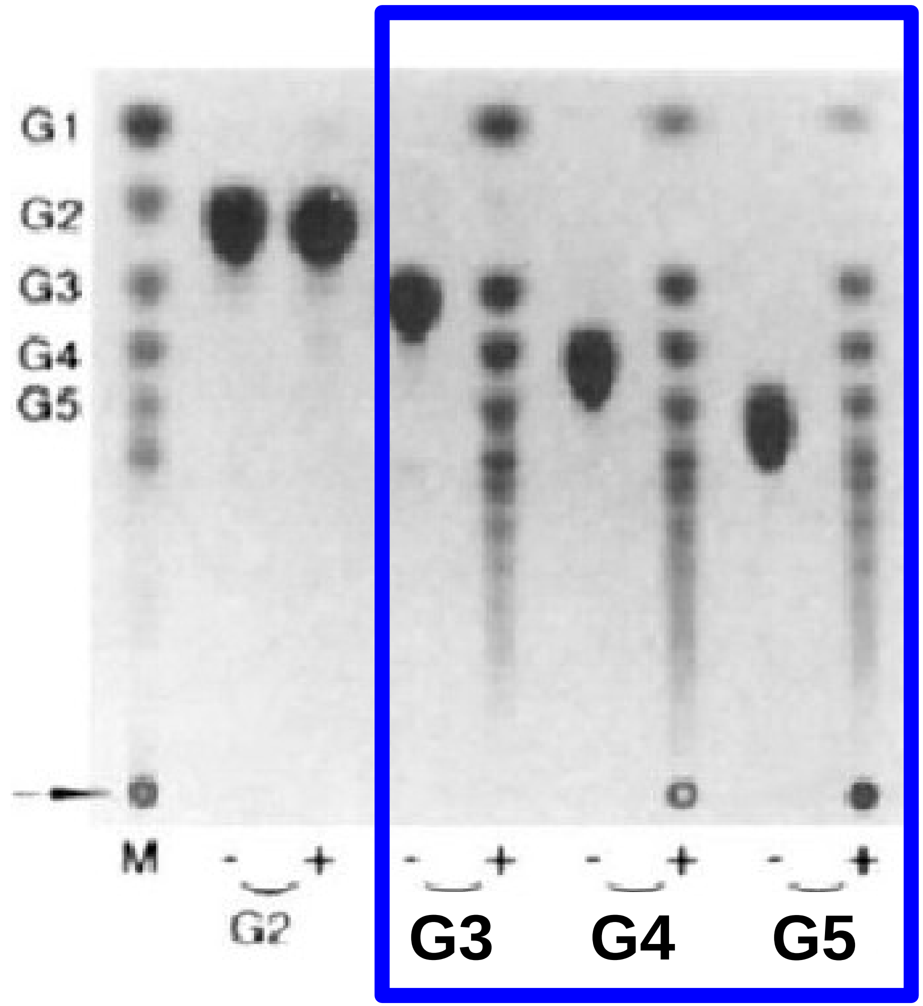
DPE1

EC: 2.4.1.25

but not only!

catalyses $$2\,\text{maltotriose} \leftrightarrow \text{maltopentaose} + \text{glucose}$$

$$G3 + G3 \leftrightarrow G5 + G1$$



(*Takaha et al., JBC 1993*)

DPE1 produces a set of glucans of different length in *in vitro* assays.

Equilibrium distribution depends on initial conditions!

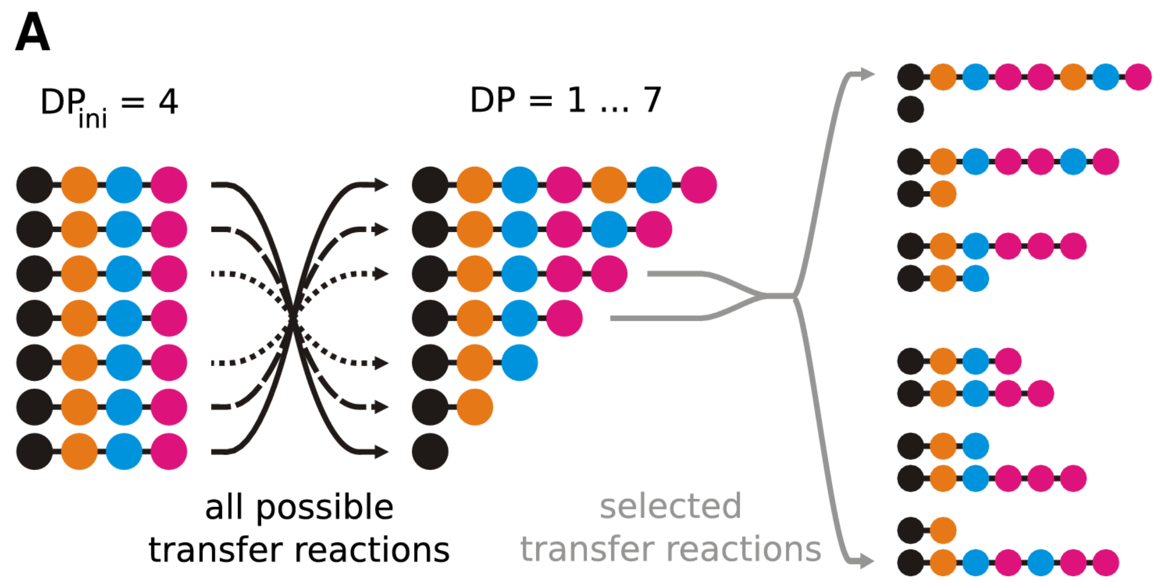
K_{eq}???

Disproportionating enzymes (D-enzymes)

DPE1

EC: 2.4.1.25

Disproportionating Enzyme randomises DPs



transfers glucosyl residues from one glucan to another: $G_n + G_m \longleftrightarrow G_{n-q} + G_{m+q}$

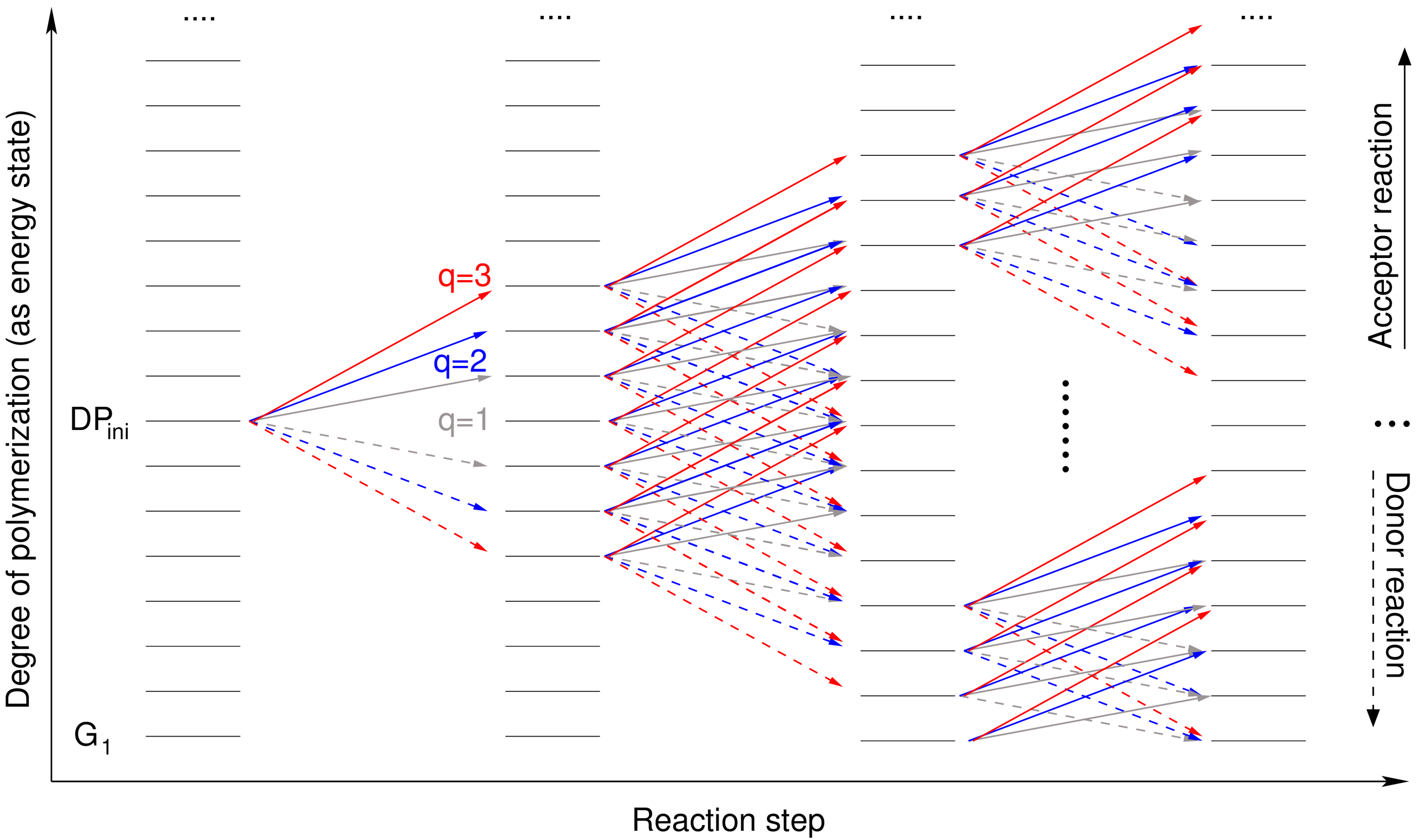
reaction must proceed towards a smaller Gibbs free energy : $\Delta G = \Delta H - T \Delta S < 0$

energy neutral (enthalpy of α-1,4-bond hydrolysis independent on position): $\Delta H = 0$
(Goldberg et al, 1992)

⇨ DPE1 maximises the entropy of the polydisperse reactant mixture

The thermodynamic picture

- Different DPs are interpreted as different energy states (energy of formation)
- Enzymes mediate transitions between these states



Polydisperse mixtures as statistical ensembles

x_i : molar fraction of glucans with length *i*
corresponds to occupation number of state *i*

The distribution $\{x_i\}$ fully characterises the polydisperse reactant mixture

The entropy of the statistical ensemble is $S = -\sum x_k \ln x_k$

<u>Equilibrium is determined by maximal entropy:</u>

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

Maximum entropy principle under constraint that #bonds and #molecules is conserved!

conservation of #molecules: $\sum x_k = 1$

conservation of #bonds: $\sum k \cdot x_k = b$ ← **determined by initially applied mixture of maltodextrins**

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

$$\frac{\partial L}{\partial x_k} = 0 \quad \text{with} \quad L(x_k;\alpha,\beta) = \sum_k x_k \ln(x_k) + \alpha\left(\sum_k x_k - 1\right) + \beta\left(\sum_k k \cdot x_k - b\right)$$

$$\Leftrightarrow \quad \ln(x_k) + 1 + \alpha + k\beta = 0 \quad \text{for all} \quad k$$

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

$$\frac{\partial L}{\partial x_k} = 0 \quad \text{with} \quad L(x_k; \alpha, \beta) = \sum_k x_k \ln(x_k) + \alpha\left(\sum_k x_k - 1\right) + \beta\left(\sum_k k \cdot x_k - b\right)$$

$$\Leftrightarrow \quad \ln(x_k) + 1 + \alpha + k\beta = 0 \quad \text{for all} \quad k$$

$$\Rightarrow \quad \boxed{x_k = \frac{1}{Z} e^{-k\beta}} \quad \text{with} \quad Z = \sum_k e^{-k\beta}$$

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

$$\frac{\partial L}{\partial x_k} = 0 \quad \text{with} \quad L(x_k; \alpha, \beta) = \sum_k x_k \ln(x_k) + \alpha\left(\sum_k x_k - 1\right) + \beta\left(\sum_k k \cdot x_k - b\right)$$

$$\Leftrightarrow \quad \ln(x_k) + 1 + \alpha + k\beta = 0 \quad \text{for all} \quad k$$

$$\Rightarrow \quad \boxed{x_k = \frac{1}{Z} e^{-k\beta}} \quad \text{with} \quad Z = \sum_k e^{-k\beta}$$

Analogy to statistical physics!

$$\left(\text{There}, \beta = \frac{1}{k_B \cdot T}\right)$$

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

$$\frac{\partial L}{\partial x_k} = 0 \quad \text{with} \quad L(x_k;\alpha,\beta) = \sum_k x_k \ln(x_k) + \alpha\left(\sum_k x_k - 1\right) + \beta\left(\sum_k k \cdot x_k - b\right)$$

$$\Leftrightarrow \quad \ln(x_k) + 1 + \alpha + k\beta = 0 \quad \text{for all} \quad k$$

$$\Rightarrow \quad \boxed{x_k = \frac{1}{Z} e^{-k\beta}} \quad \text{with} \quad Z = \sum_k e^{-k\beta}$$

Analogy to statistical physics! $\left(\text{There}, \beta = \frac{1}{k_B \cdot T}\right)$

Calculation of β: $-\frac{1}{Z}\frac{\partial Z}{\partial \beta} = b \Leftrightarrow \beta = \ln\frac{b+1}{b}$

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

$$\frac{\partial L}{\partial x_k}=0 \quad \text{with} \quad L(x_k;\alpha,\beta)=\sum_k x_k \ln(x_k)+\alpha\left(\sum_k x_k-1\right)+\beta\left(\sum_k k\cdot x_k-b\right)$$

$$\Leftrightarrow \quad \ln(x_k)+1+\alpha+k\beta=0 \quad \text{for all} \quad k$$

$$\Rightarrow \quad \boxed{x_k=\frac{1}{Z}e^{-k\beta}} \quad \text{with} \quad Z=\sum_k e^{-k\beta}$$

Analogy to statistical physics! $\left(\text{There}, \beta = \frac{1}{k_B \cdot T}\right)$

Calculation of β: $-\frac{1}{Z}\frac{\partial Z}{\partial \beta} = b \Leftrightarrow \beta = \ln\frac{b+1}{b}$

Maximal entropy in equilibrium: $S_{max} = (b+1)\ln(b+1)-b\ln b$

Entropic approach

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

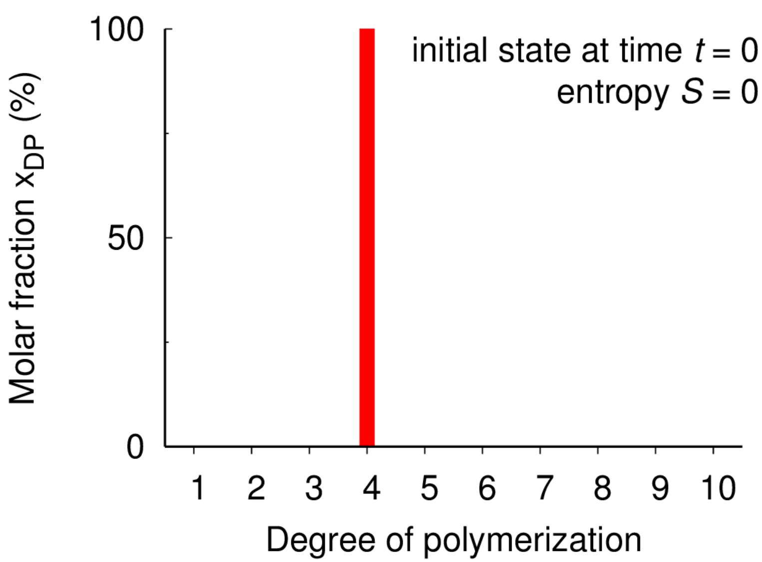
conservation of #molecules: $\sum x_k = 1$

conservation of #bonds: $\sum k \cdot x_k = \mathrm{DP}_{\mathrm{ini}} - 1$

implies

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

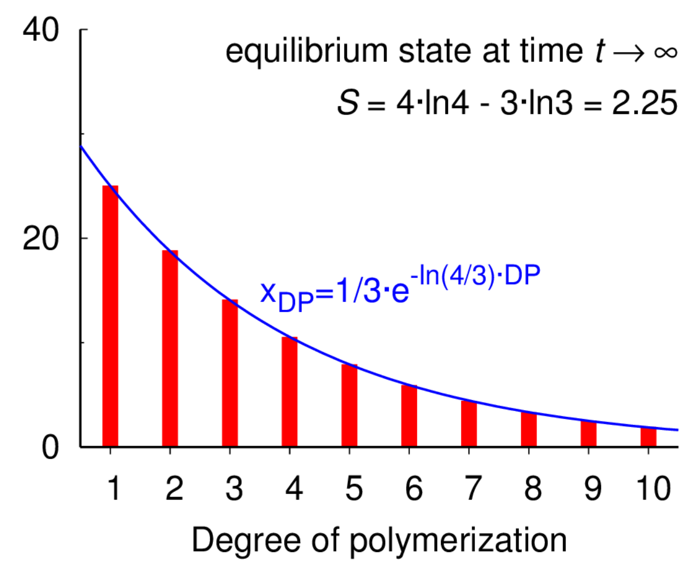
predicts



DPE1 action

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

with q = 1,2,3

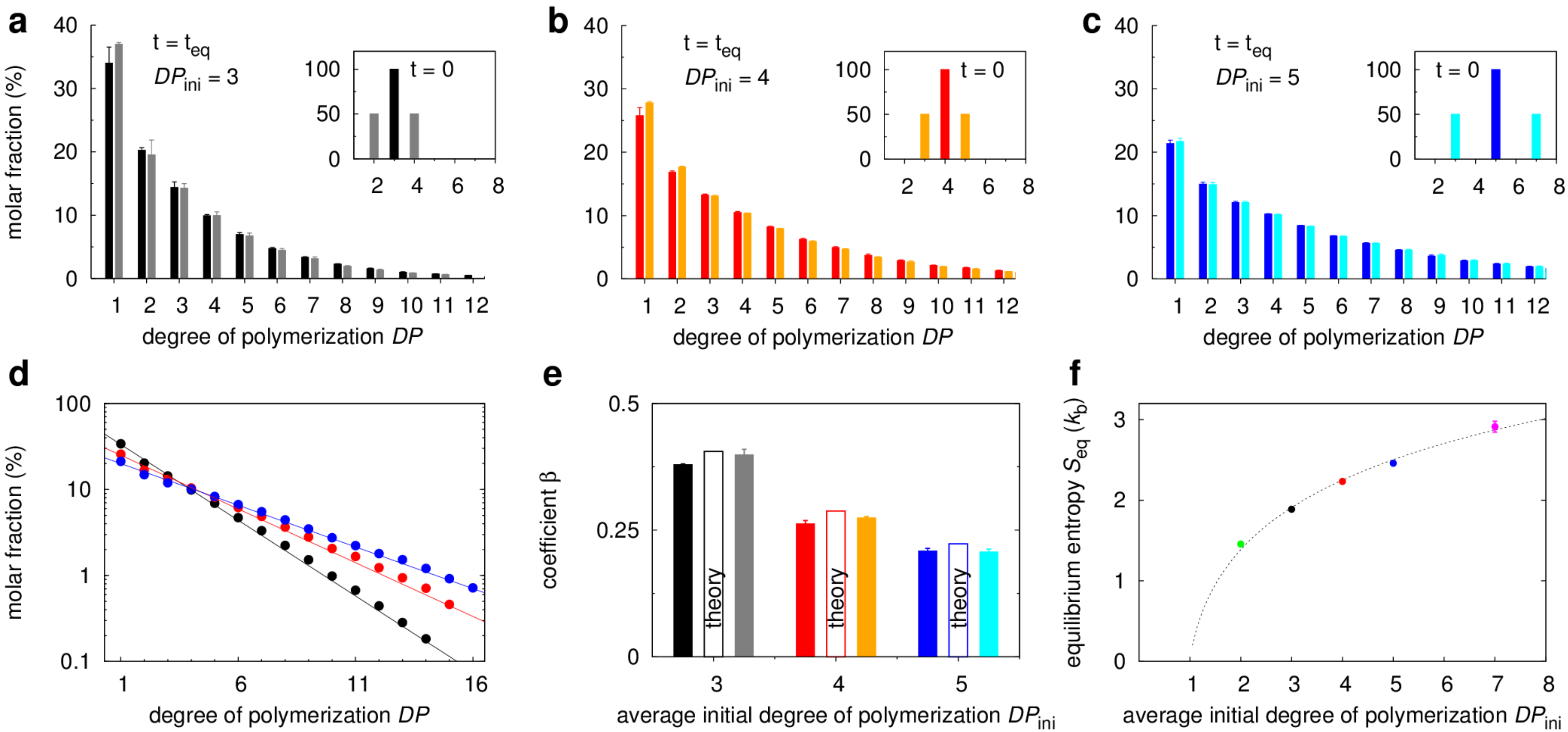


An instance of the 2^{nd} law of TD!

DPE1 is entropy driven

Experiments with Martin Steup, University of Potsdam

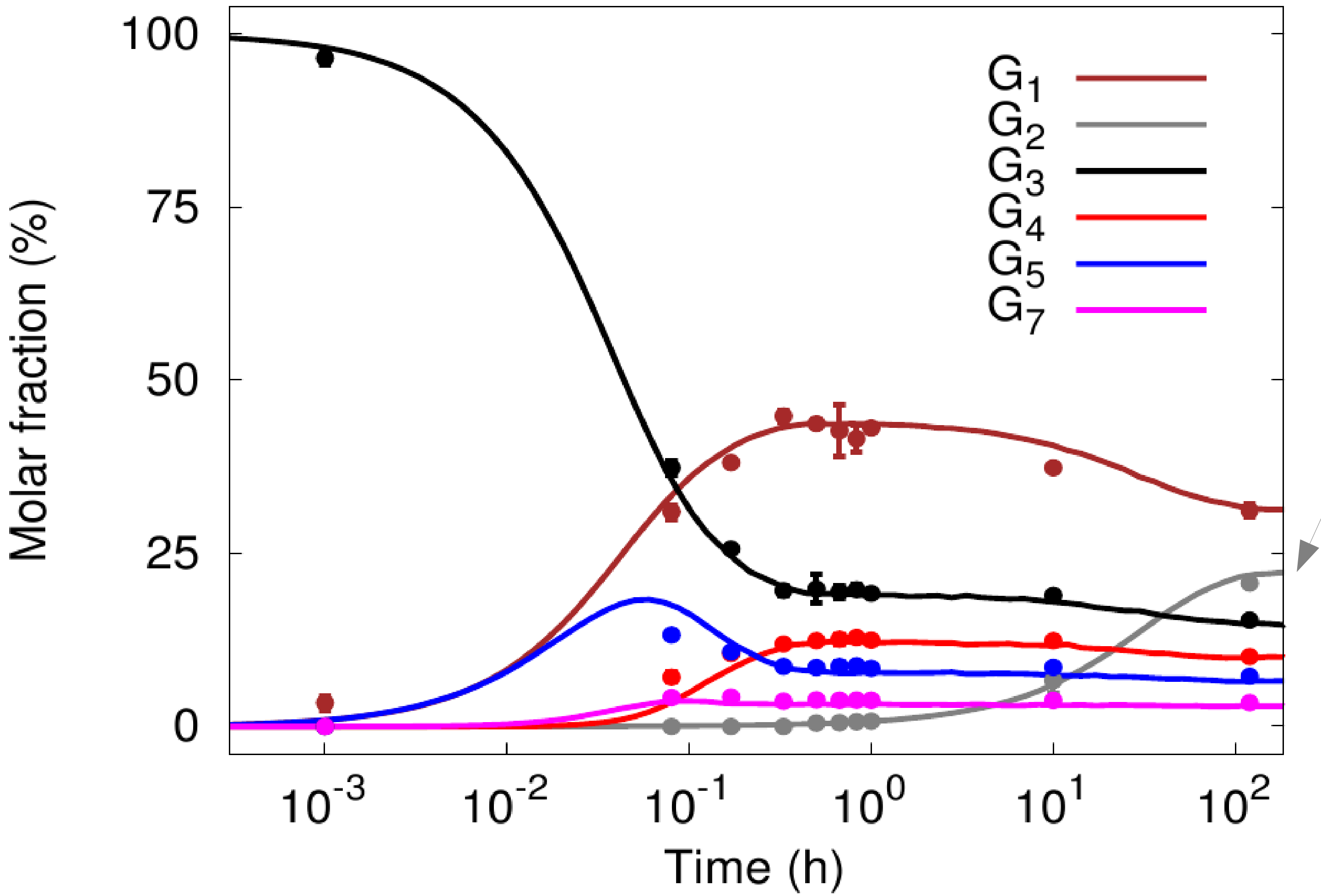
method: capillary electrophoresis



β is a generalisation of the equilibrium constant for polydisperse mixtures

(Kartal et al, 2011, Mol Syst Biol)

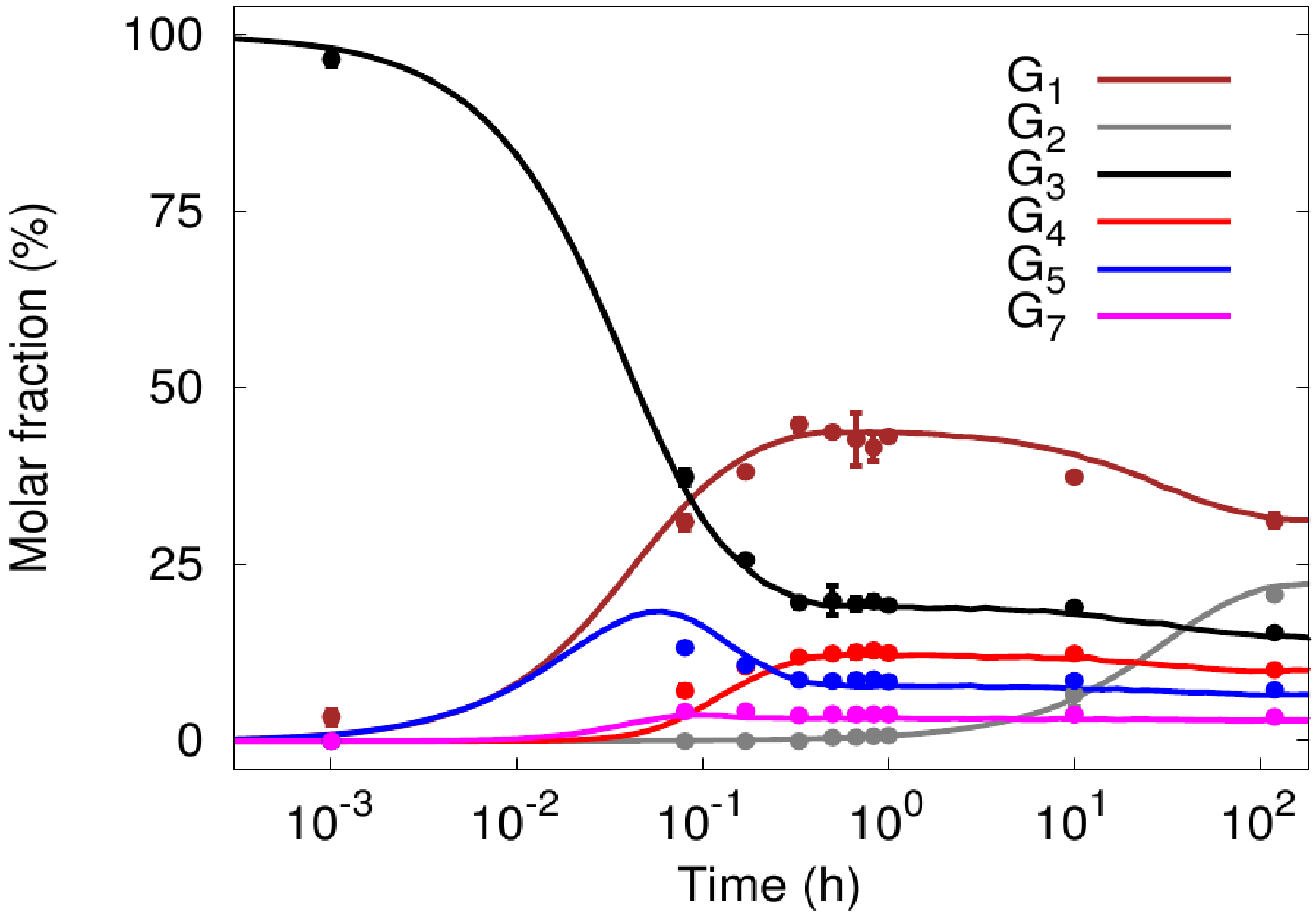
The dynamics of DPE1



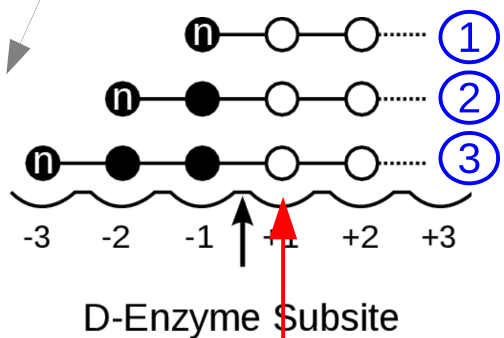
maltose is formed late

Two time scales!

The dynamics of DPE1



maltose is formed late



subsite +1 repellent (binding of G2 unlikely)

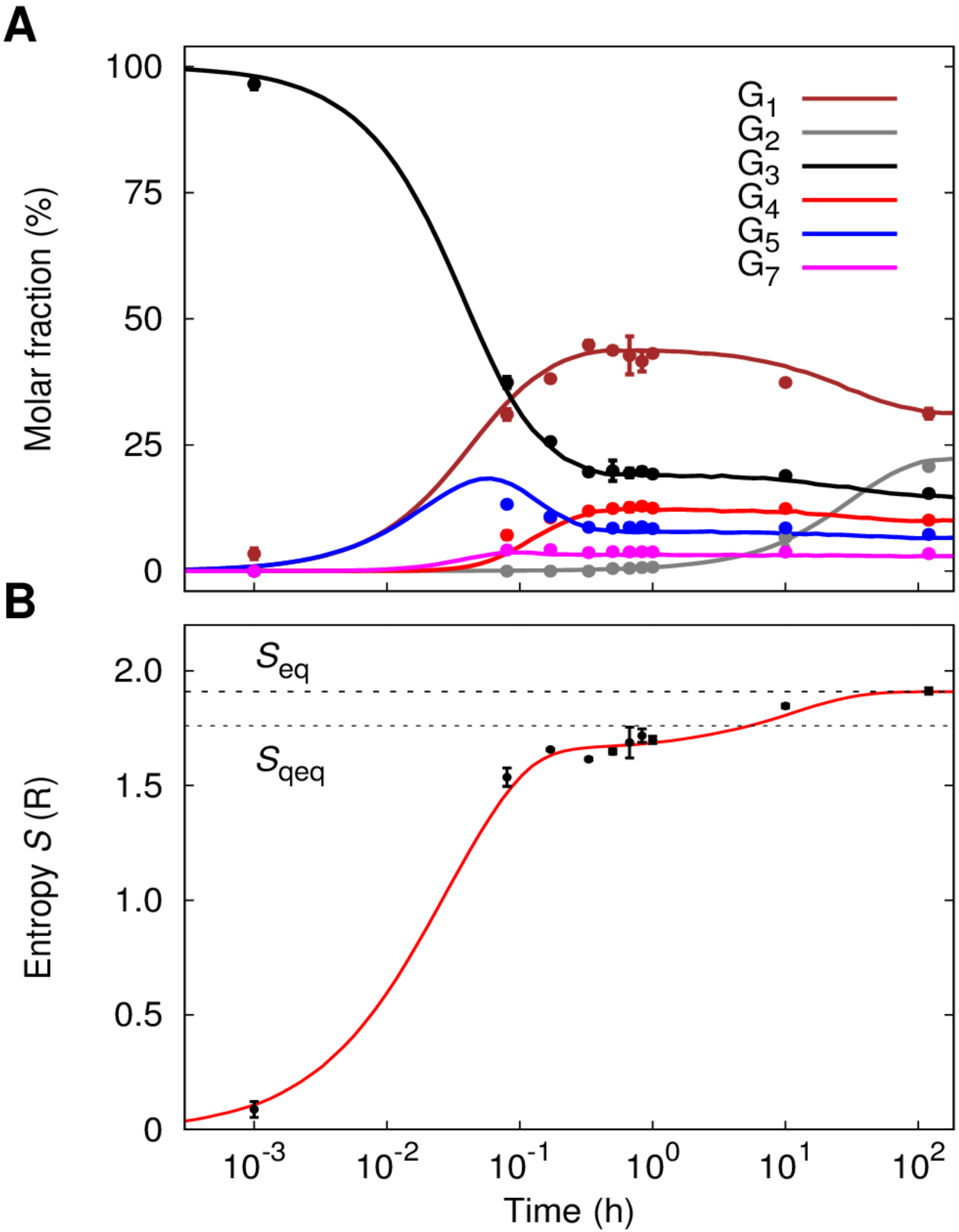
Two time scales!

The simulations used 3 parameters:

- maximal turnover
- affinity for positional isomer 1
- affinities for positional isomers 2 and 3

ratio 1:800

This system allows to follow the entropy *experimentally*!



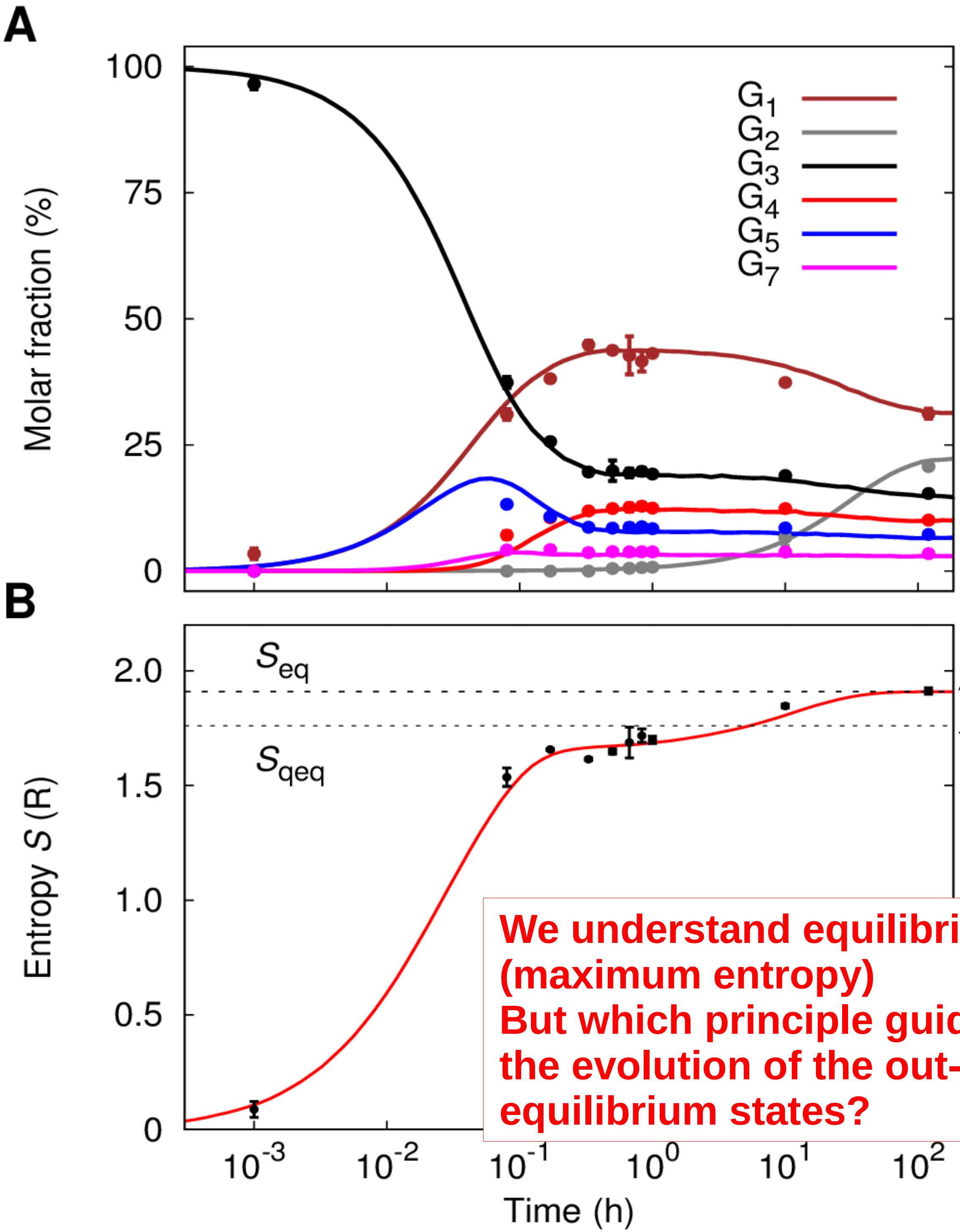
"true" equilibrium

(calculated as previously)

"quasi" equilibrium

(calculated with the same approach but omitting maltose from the statistical ensemble)

This system allows to follow the entropy *experimentally*!



“true” equilibrium

(calculated as previously)

“quasi” equilibrium

(calculated with the same approach but omitting maltose from the statistical ensemble)

We understand equilibrium (maximum entropy)
But which principle guides the evolution of the out-of-equilibrium states?

Theory is also confirmed by DPE2

DPE2 vs DPE1

- transfers single glucosyl residues
- G2 only used as donor
- G3 only used as acceptor

<u>Generic reaction catalysed:</u>

$$G_n + G_1 \longleftrightarrow G_{n-1} + G_2$$

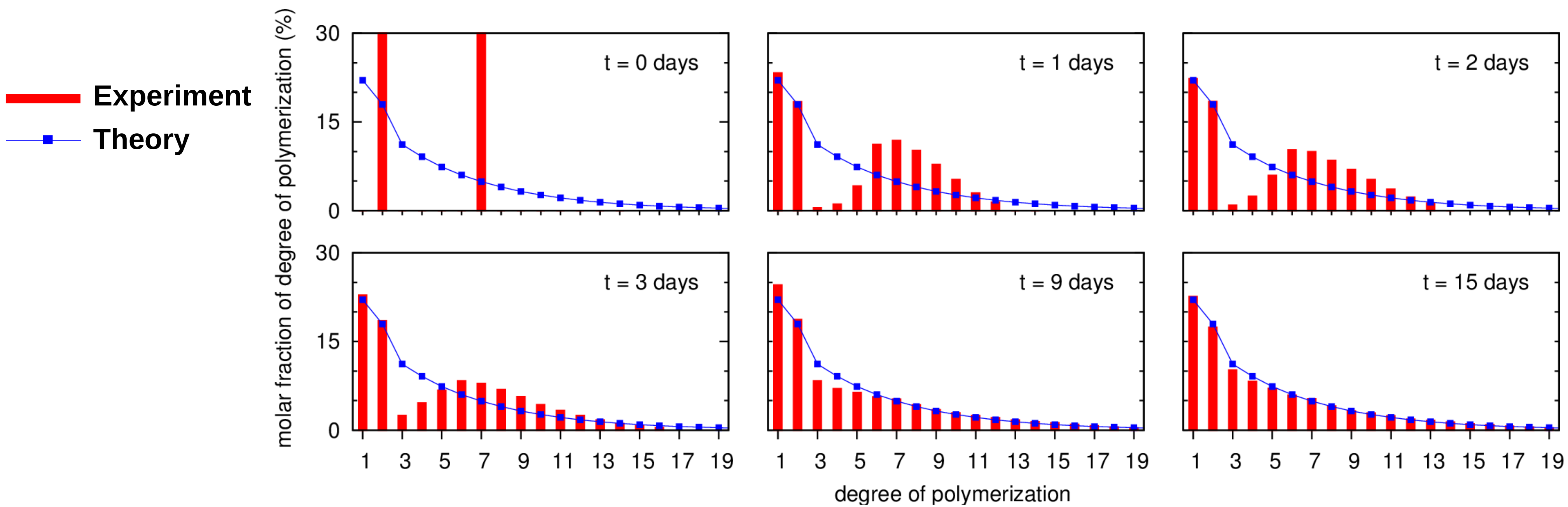
<u>Entropic principle:</u>

$$S = -\sum_k x_k \ln x_k \rightarrow \max$$

with one additional side constraint

$$x_1 + x_2 = m = \text{const.} \quad \left(\text{and} \sum x_k = 1 ; \sum k \cdot x_k = b\right)$$

$$\Rightarrow \quad x_i = \frac{1}{Z} e^{-\beta E_i} \quad \text{for} \quad i \geqslant 3 \qquad \text{where } \beta \text{ fulfils} \qquad b - 2(1-m) = m \cdot \frac{e^{-\beta}}{1+e^{-\beta}} + (1-m) \cdot \frac{e^{-\beta}}{1-e^{-\beta}}$$



Theory is also confirmed by DPE2

DPE2 vs DPE1

- transfers single glucosyl residues
- G2 only used as donor
- G3 only used as acceptor

Generic reaction catalysed:

$$G_n + G_1 \longleftrightarrow G_{n-1} + G_2$$

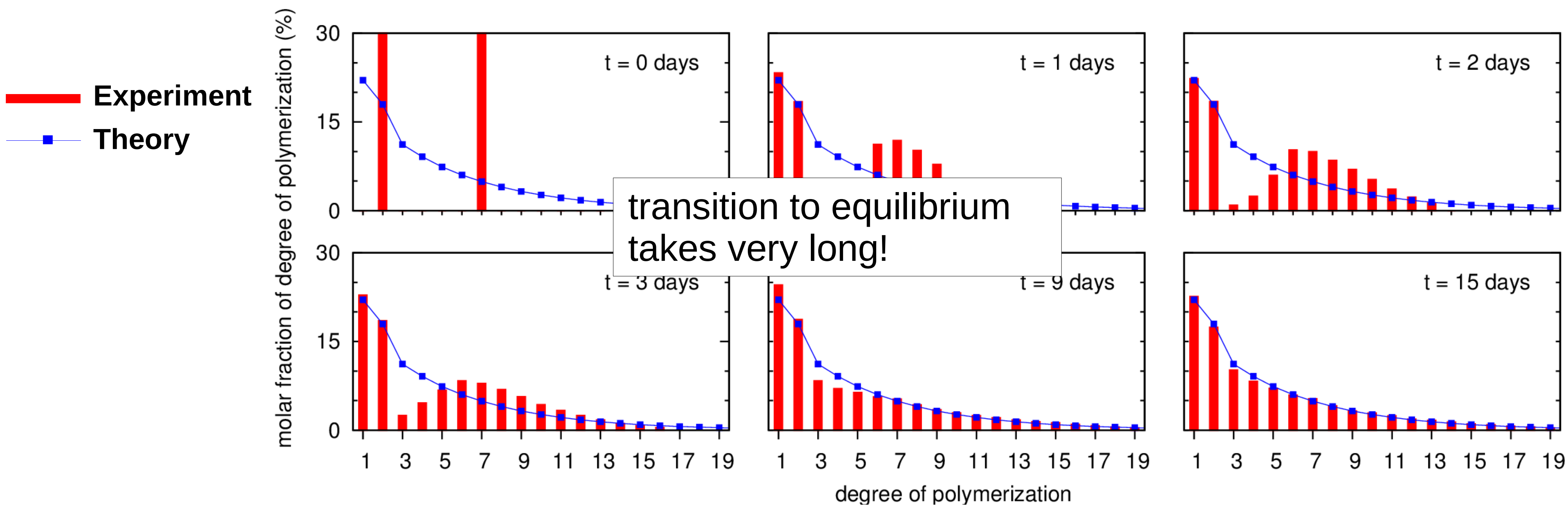
Entropic principle:

$$S = -\sum_k x_k \ln x_k \rightarrow \max$$

with one additional side constraint

$$x_1 + x_2 = m = \text{const.} \quad \left(\text{and} \sum x_k = 1 ; \sum k \cdot x_k = b\right)$$

$$\Rightarrow \quad x_i = \frac{1}{Z} e^{-\beta E_i} \quad \text{for} \quad i \geqslant 3 \qquad \text{where } \beta \text{ fulfils} \qquad b - 2(1-m) = m \cdot \frac{e^{-\beta}}{1+e^{-\beta}} + (1-m) \cdot \frac{e^{-\beta}}{1-e^{-\beta}}$$



transition to equilibrium takes very long!

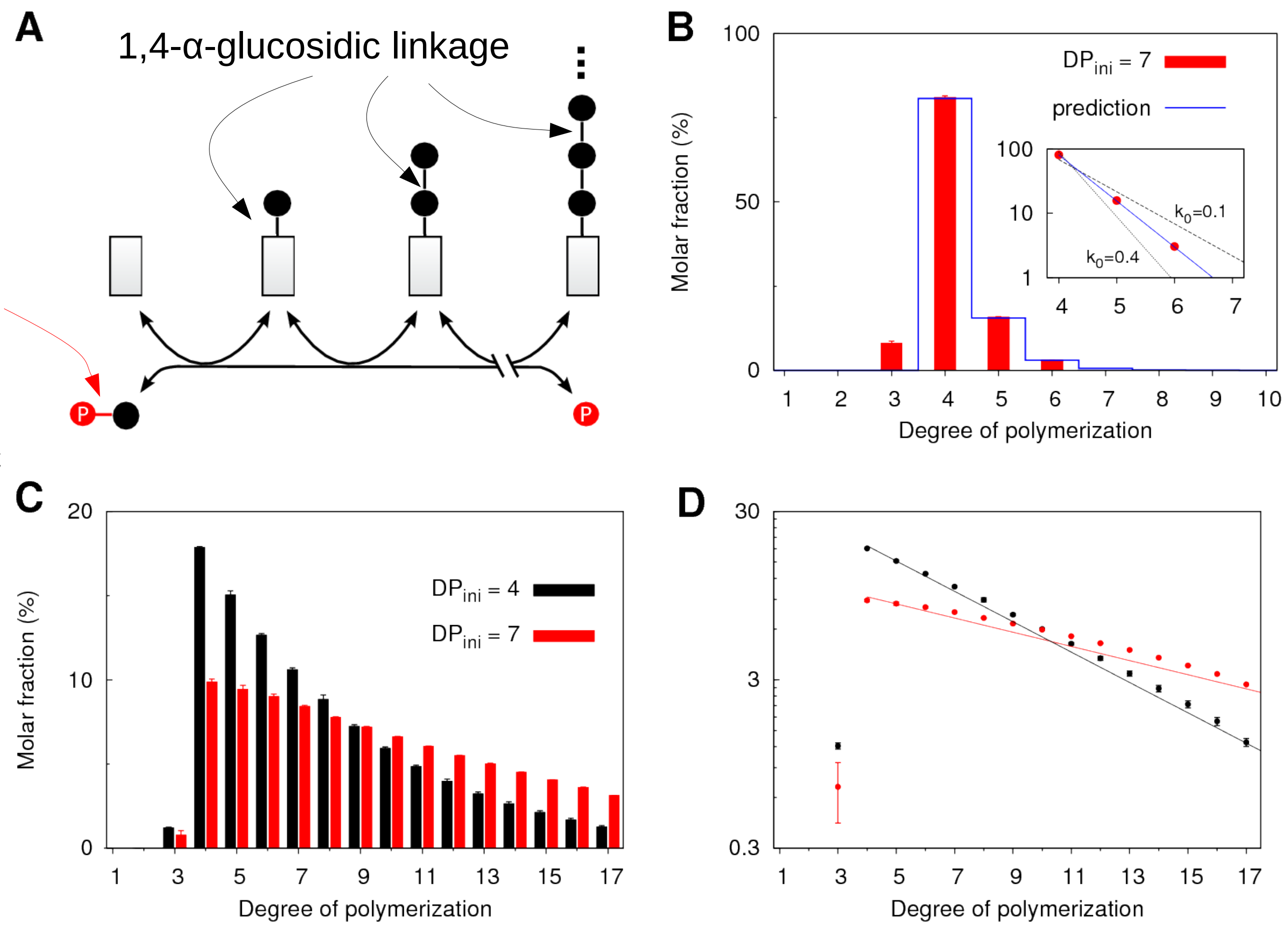
Generalisation to non-zero enthalpy changes

Phosphorylase (cPho):

$$P_i + G_n \longleftrightarrow G1P + G_{n-1}$$

$$\Delta H \neq 0!$$

phosphoester bond



Generalisation by including energetic and entropic contributions:

$$G \;=\; G^f - T \cdot S_{mix} \rightarrow \min!$$

Gibbs energy of formation

mixing entropy:

$$S_{mix} = -R \sum x_k \ln x_k$$

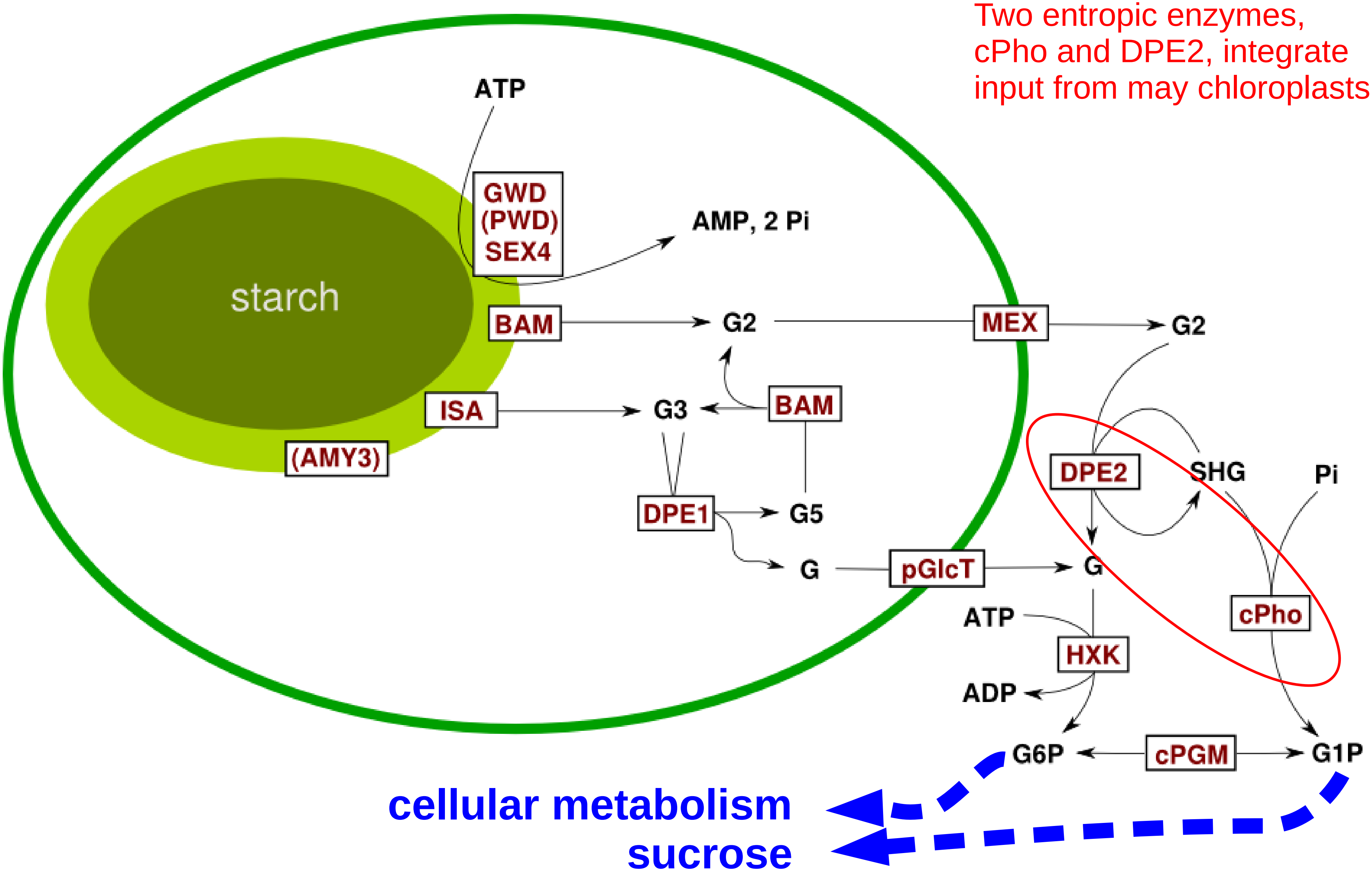
<u>Prediction</u>: Similar pattern as for DPE2

Experimentally confirmed.

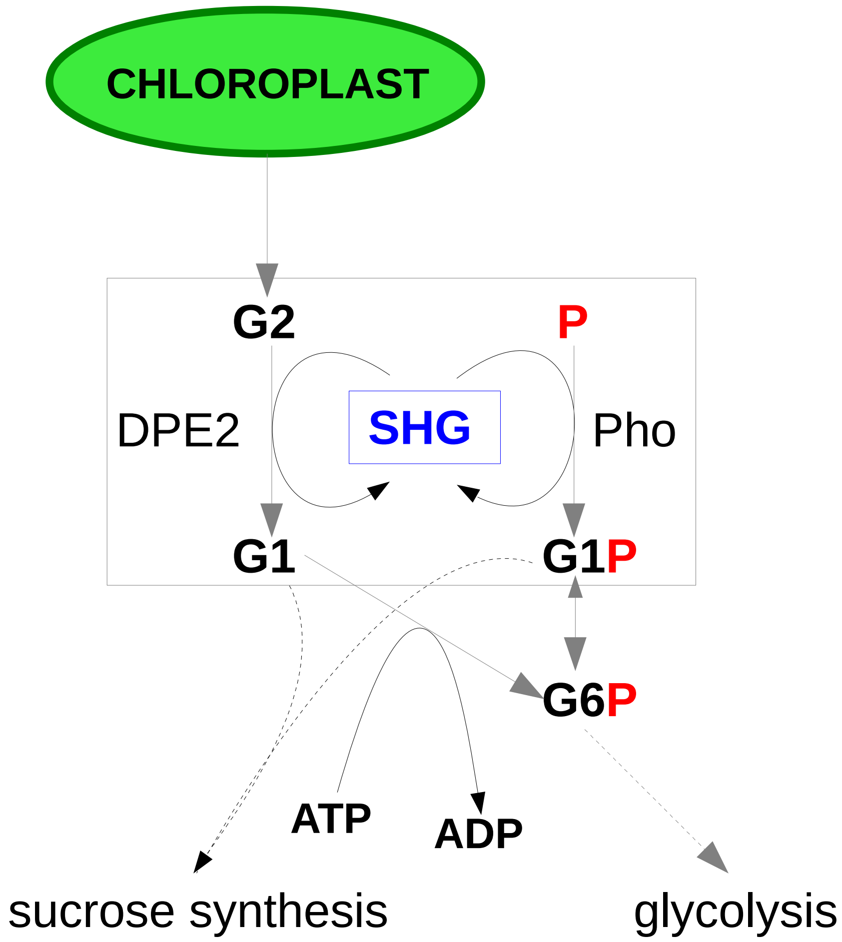
(Kartal et al, Supp to MSB 2011; Ebenhöh et al, Proc 5th ESCEC 2013)

An entropy-driven buffer

Two entropic enzymes, cPho and DPE2, integrate input from may chloroplasts



What is the role of the SHG pool?

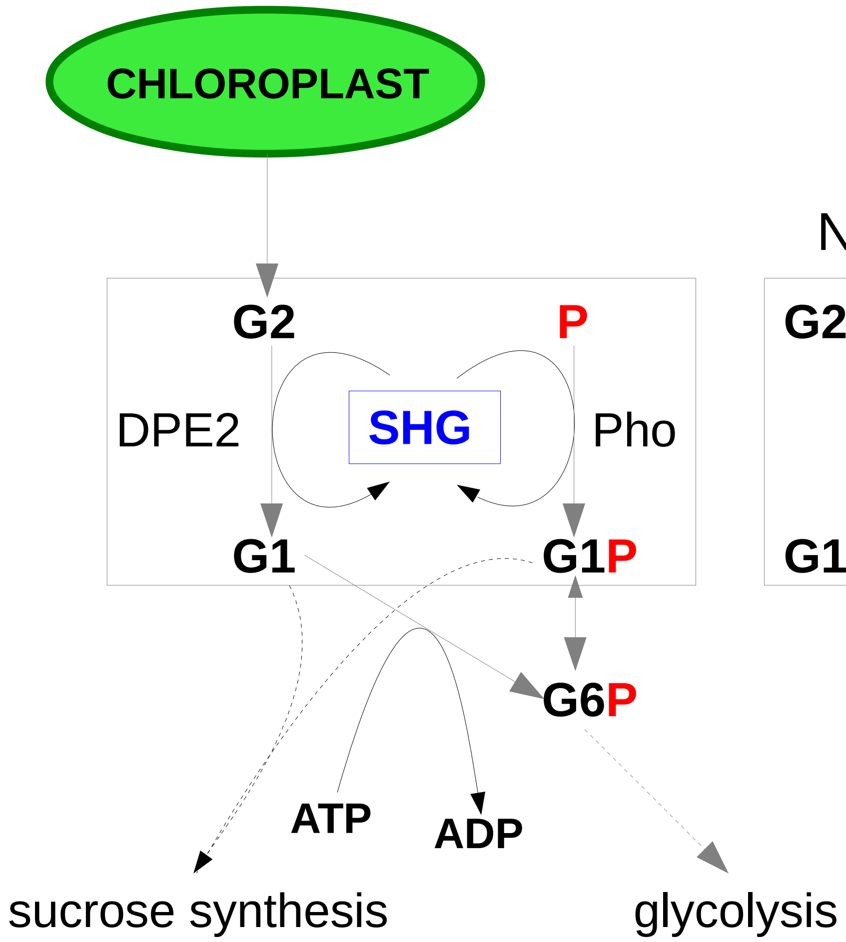


Two 'entropic' enzymes mediate the turnover of a polydisperse pool

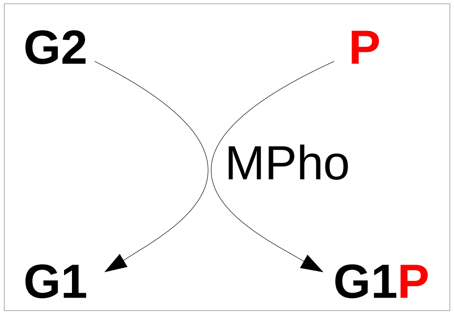
What is the advantage over other hypothetical systems?

What is the role of the SHG pool?

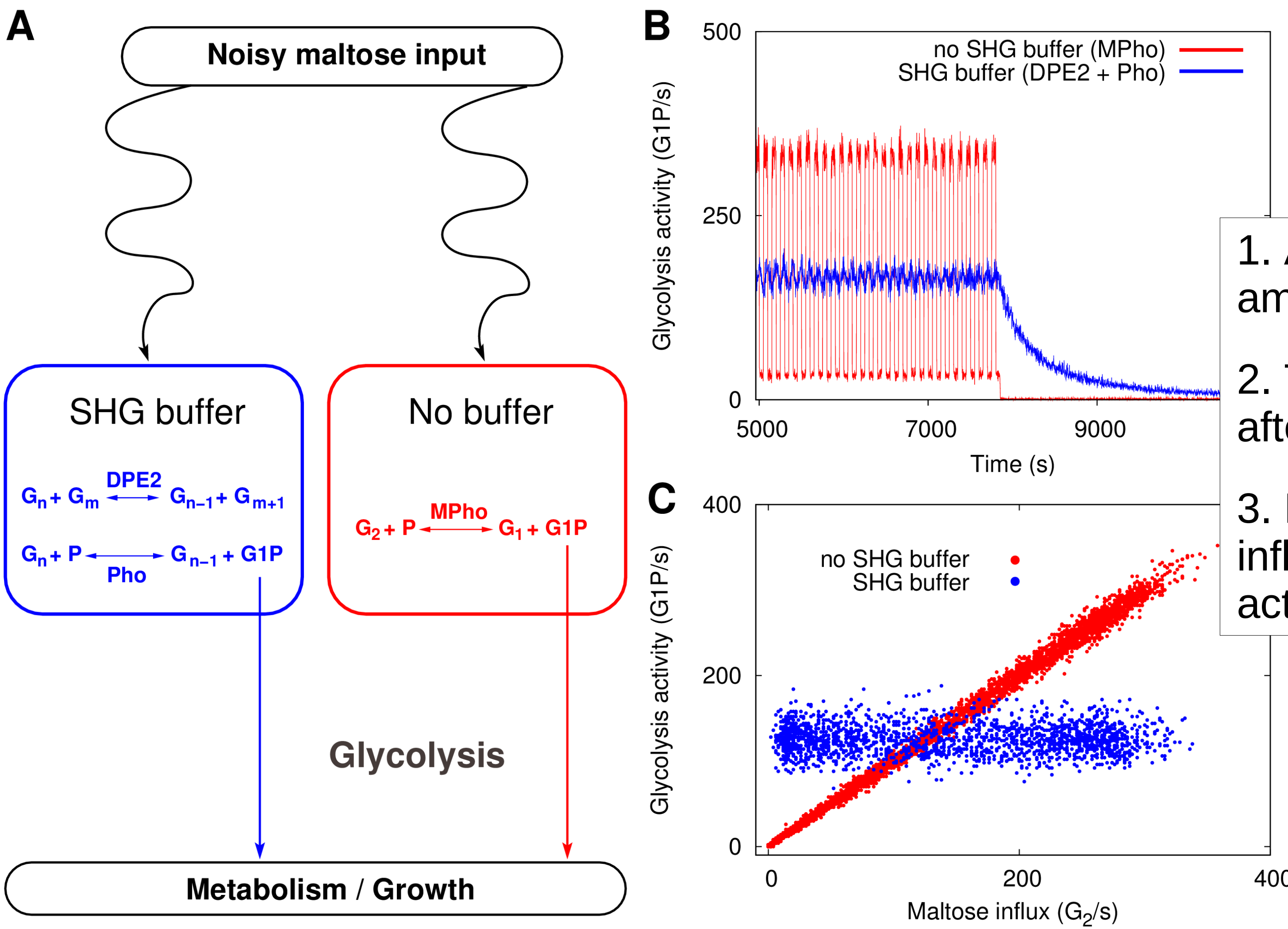
Comparison with alternative



No buffer



Polydisperse SHG pools increases robustness *in vivo*



1. Attenuation of fluctuation amplitude (low-pass filter)

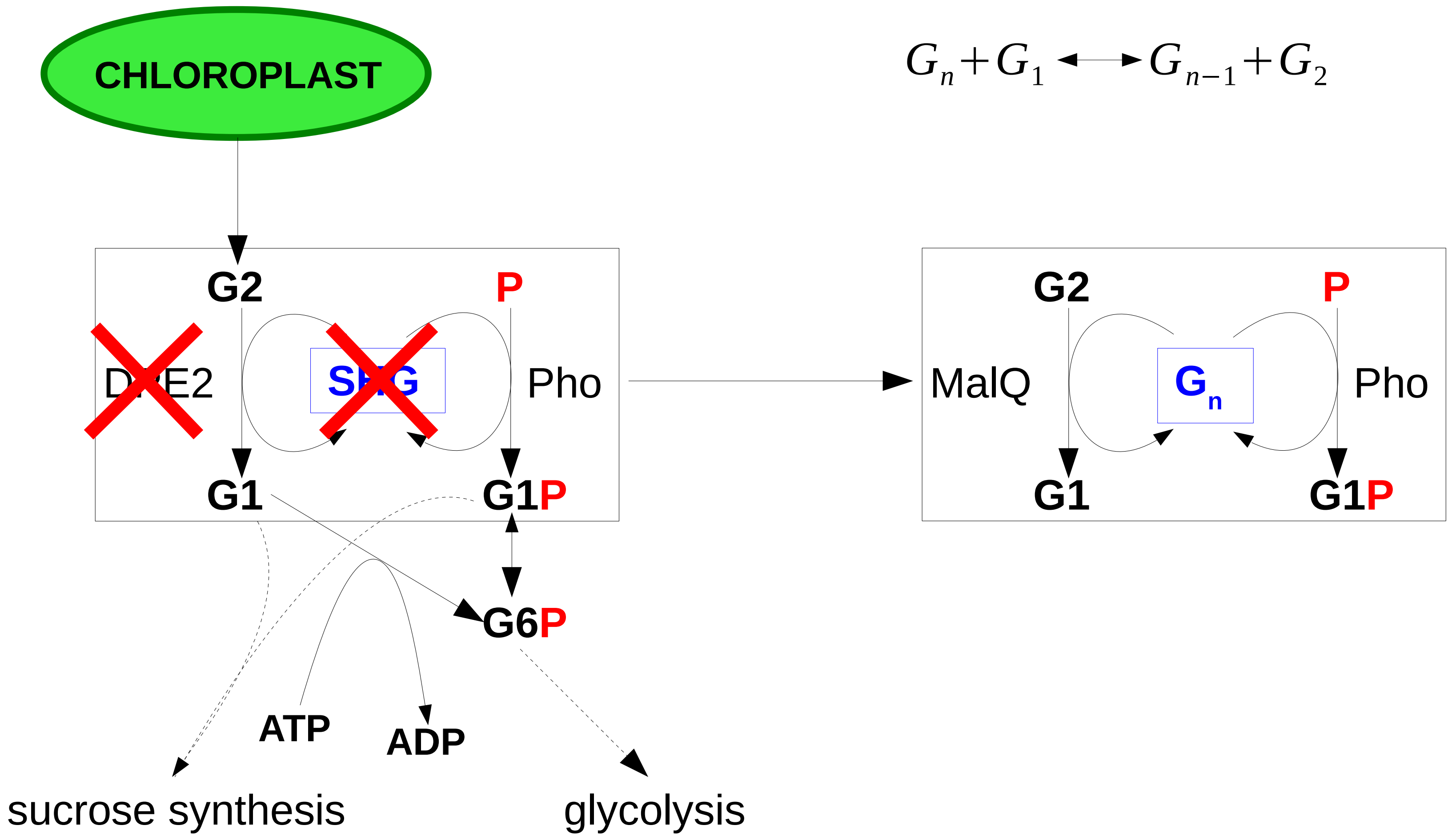
2. Transient support of activity after drop of maltose influx

3. Buffering large variations in influx to provide robust output activity

Replacing DPE2 by MalQ

MalQ does the same as DPE2, but does not use SHG

$$G_n + G_1 \leftrightarrow G_{n-1} + G_2$$



Simulating MalQ in vitro kinetics

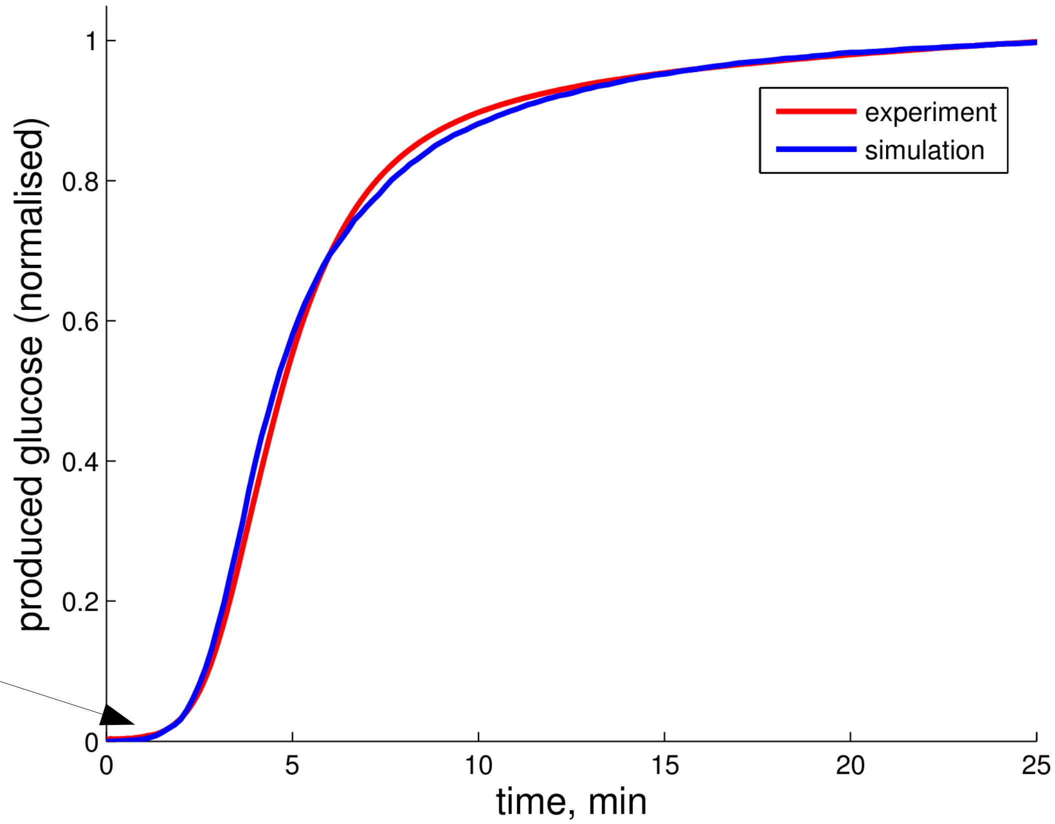
In vitro system: DPE1 + HXK

$$G_n + G_1 \longleftrightarrow G_{n-1} + G_2 \quad n \neq 3$$

$$G_1 \longrightarrow \emptyset$$

Incubation with G_2 only!

delayed start presumably due to enzyme-bound glucose residues

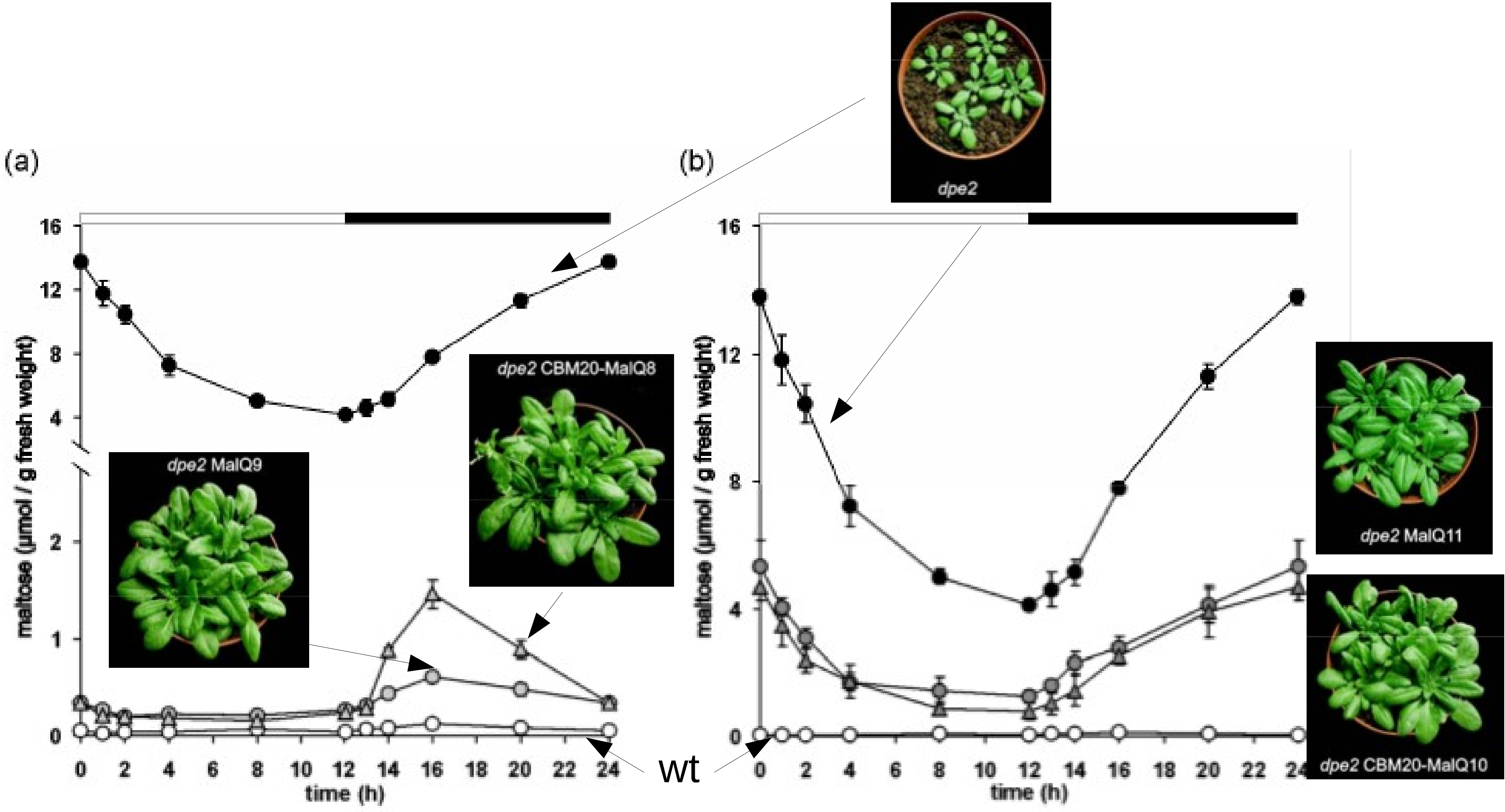


Moderate growth phenotype

(Julia Smirnova, PhD thesis; Ruzanski et al, JBC 2013)

complemented plants grow OK!

Maltose turnover

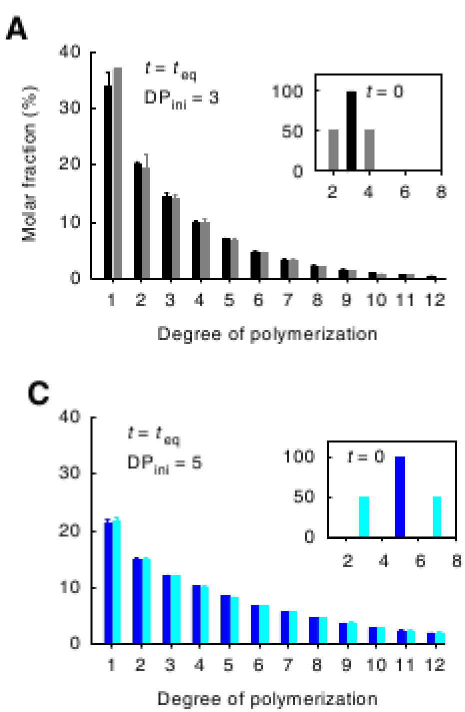


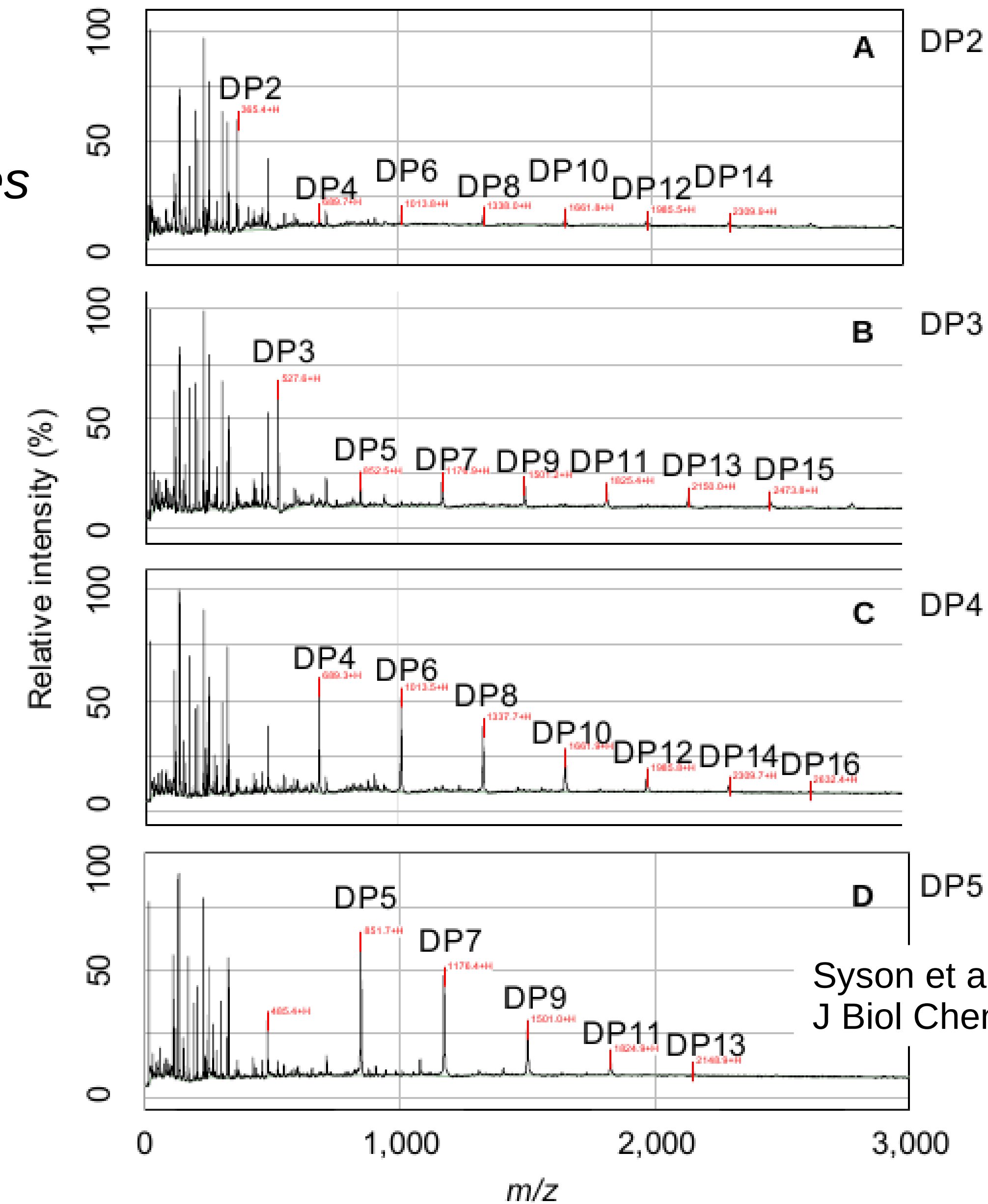
Where else do find entropic enzymes?

...for example

Maltosyltransferases in *Streptomyces*

"Acceptor specificity" can be explained by entropic principles





Syson et al, 2011, J Biol Chem

3. Timing of Metabolism

Open questions

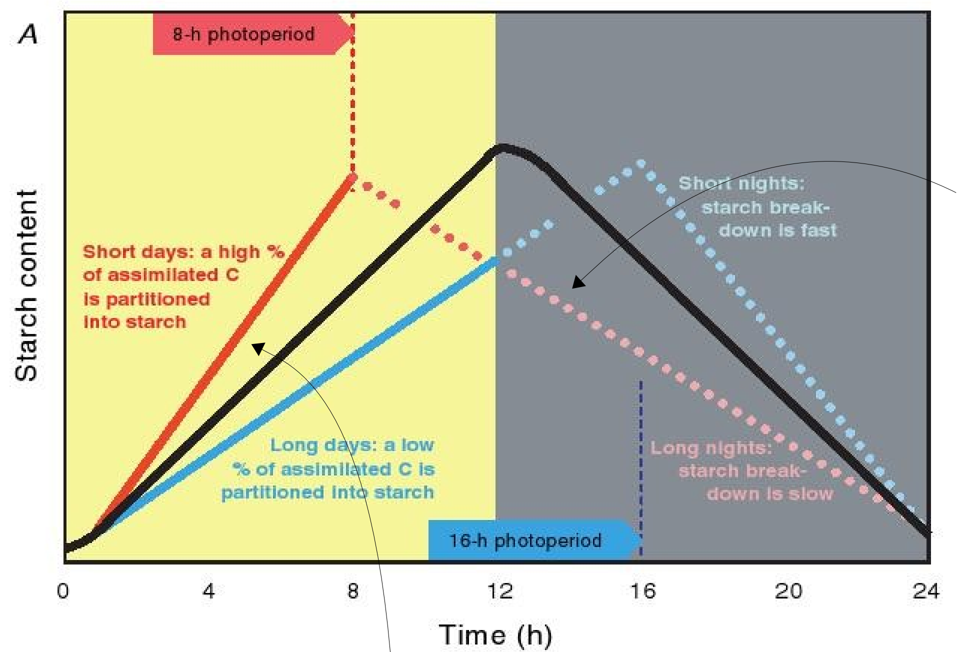
?

?

How does the clock 'tell' expected length of day/night?

What measures the starch content?

?

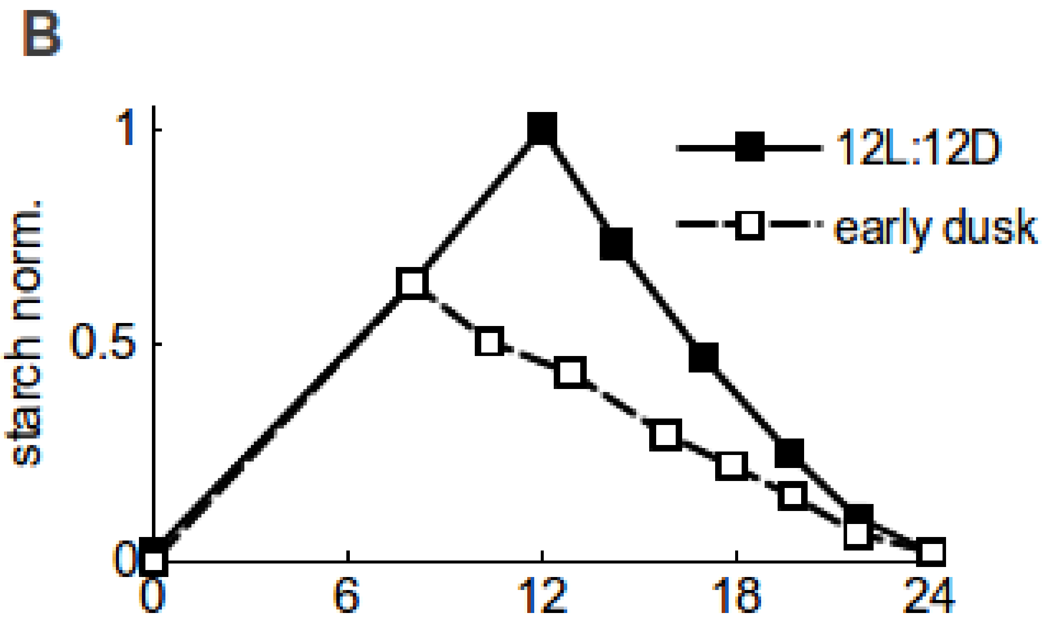


How is the correct breakdown rate 'calculated'?

How is carbon partitioning controlled?

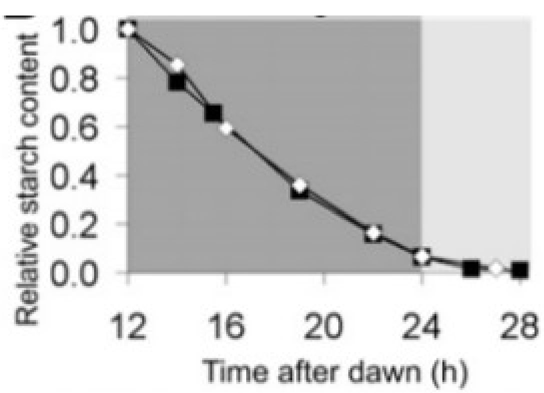
...even more mysteries...

The 'early dusk' experiment by
Alexander Graf,
(Graf et al 2010, PNAS)



Even when 'surprised' by a 4 hour shorter day, plants 'know' what to do!

The circadian clock is apparently important, because:



Plants cannot adapt to T-cycles different than 24h!

Building a mathematical model

Alexandra Pokhilko

Known:
- Metabolism
- Circadian clock

Unknown:
- Regulation of starch synthesis
- Regulation of starch breakdown
- How is starch content measured?

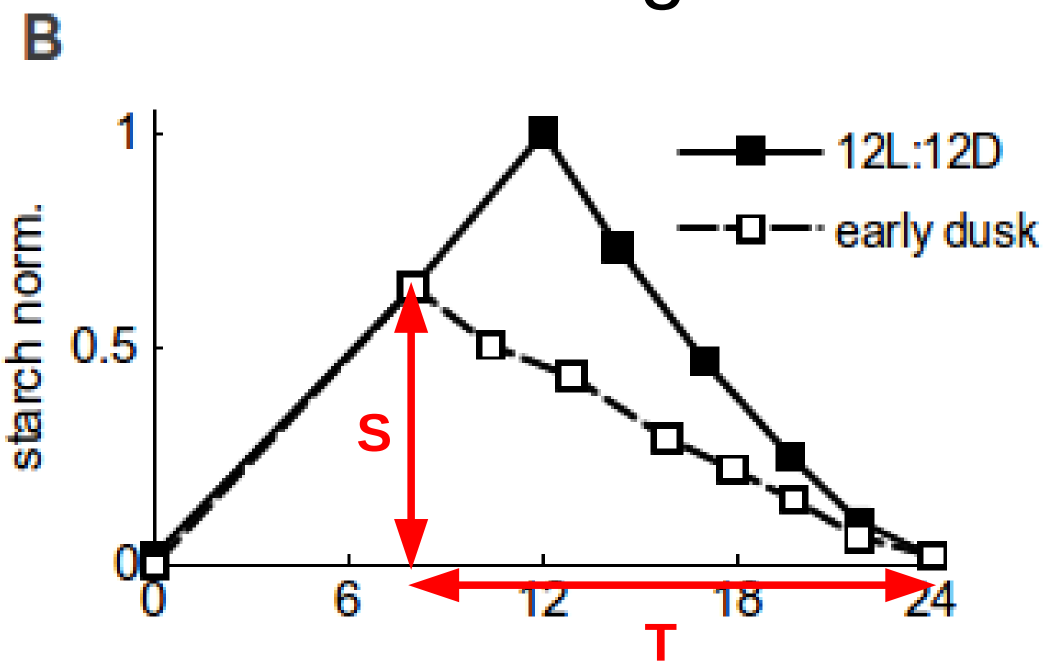
Challenges:

1. The model must combine known systems with plausible, but hypothesised regulatory mechanisms

2. To keep the model tractable, we need to find a compromise between detailedness and simplification

Seaton et al, 2013, *Roy Soc Interface*;
Pokhilko et al, 2014, *Mol BioSyst*;
Pokhilko et al, 2015, *Roy Soc Interface*

How to regulate starch degradation?



<u>Arithmetic division</u>

$$v = \frac{S}{T}$$

<u>Simplest solution:</u>

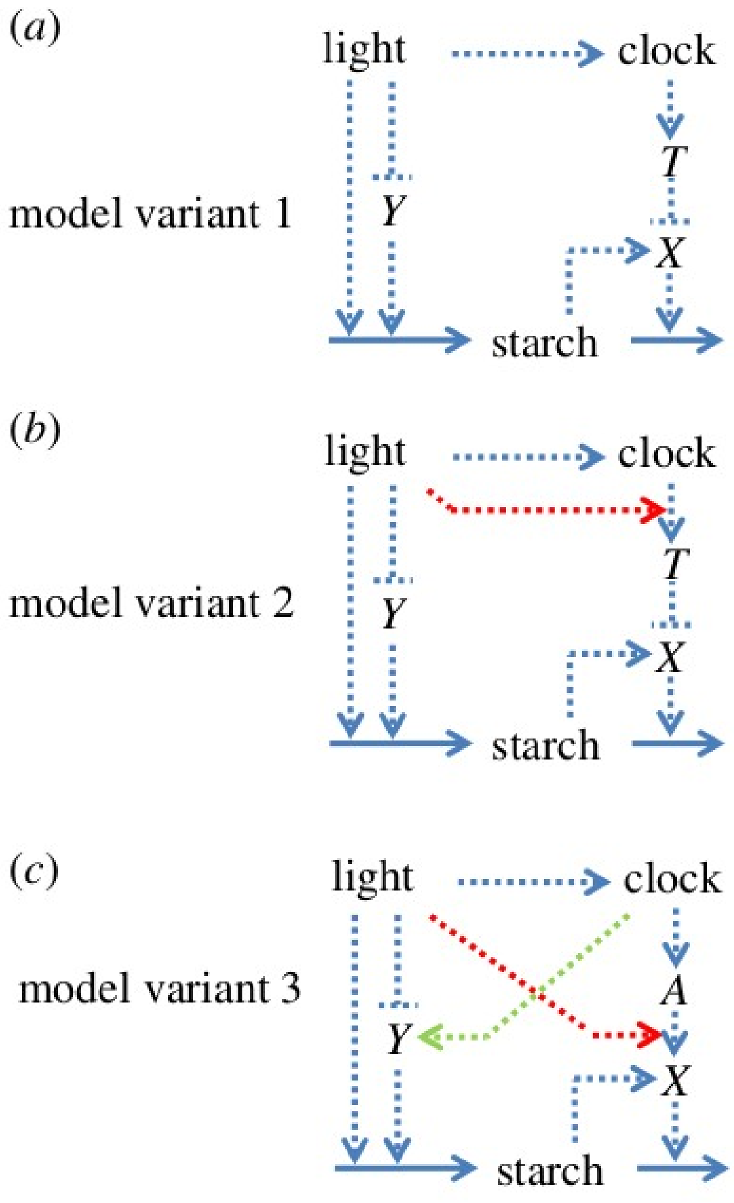
Auxiliary compound *X* (e.g. active form of starch degrading enzyme):

$$\frac{dX}{dt} = k_1 S - k_2 X\, T$$

Rapid activation/deactivation: $\frac{dX}{dt} = 0 \Leftrightarrow X = \frac{k_1}{k_2} \cdot \frac{S}{T}$

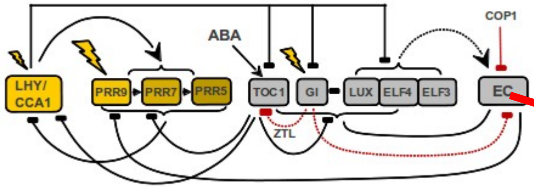
Scaldione et al (2013), eLife: **Arabidopsis plants perform arithmetic division to prevent starvation at night**

The evolution of a model



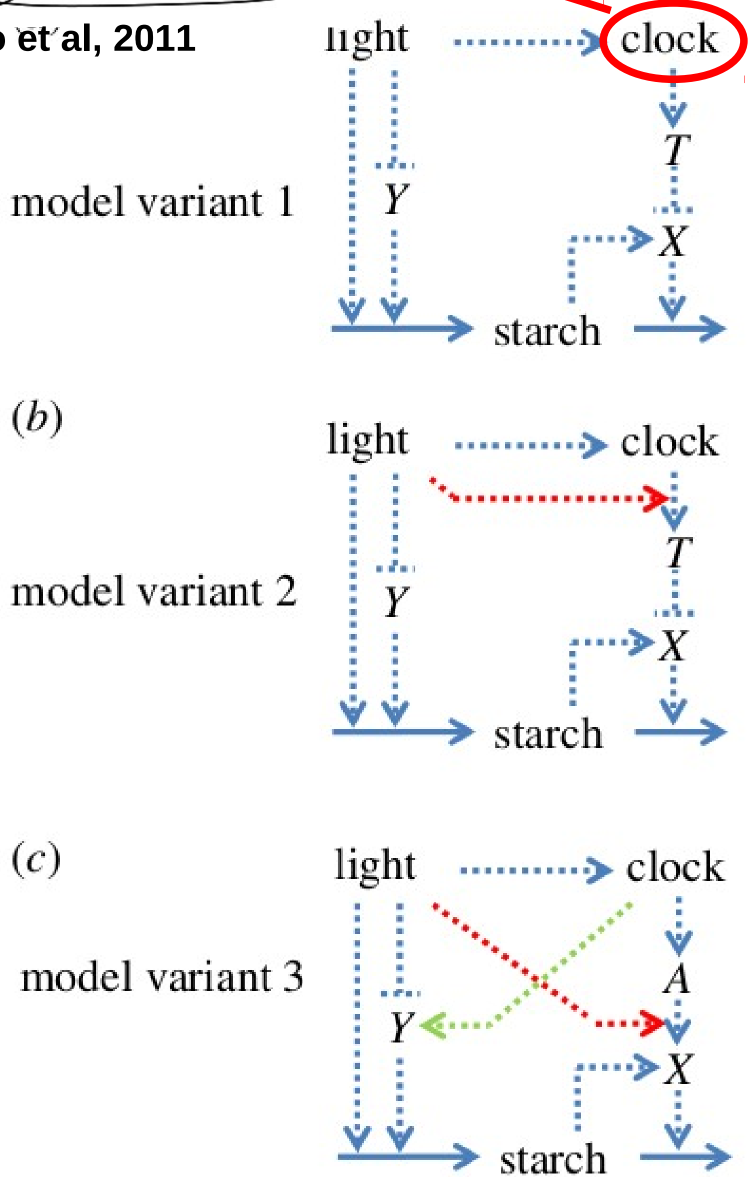
In Seaton et al, 2013:

- Testing basic regulatory mechanisms



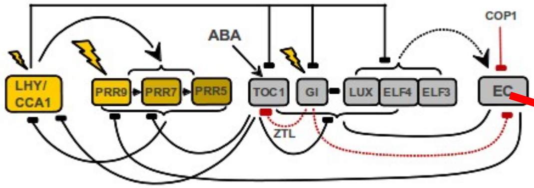
Pokhilko et al, 2011

The evolution of a model



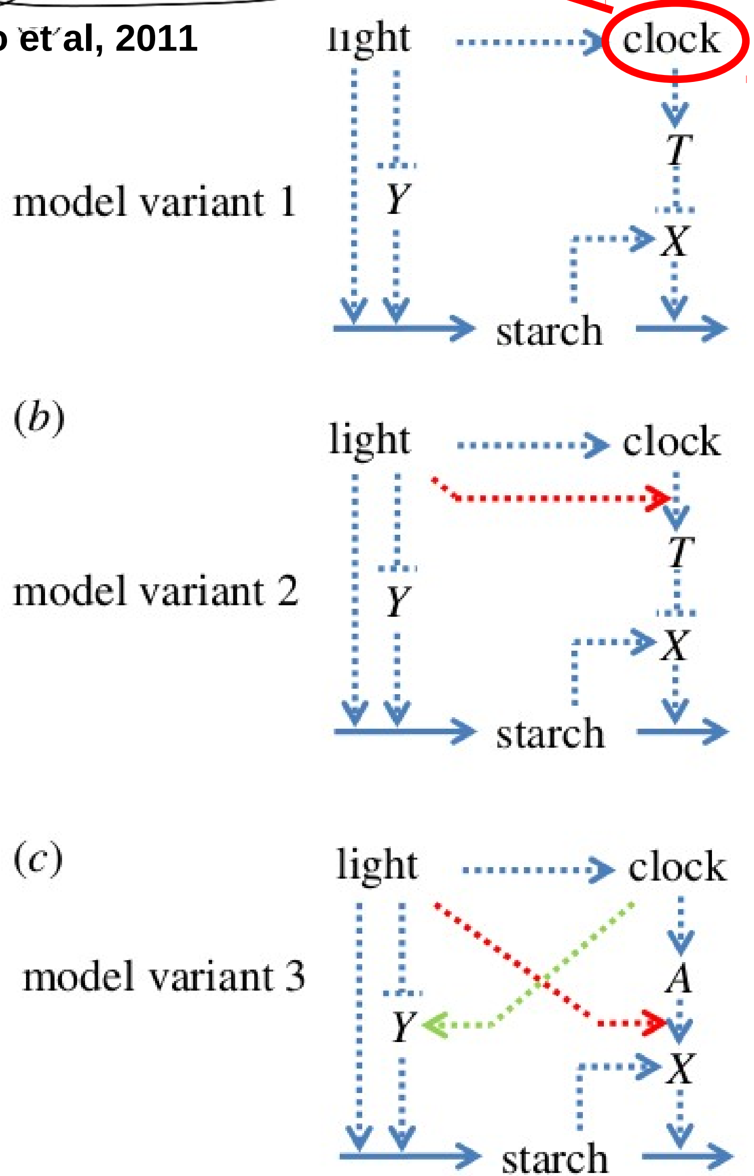
In Seaton et al, 2013:

- Testing basic regulatory mechanisms
- Replacing 'clock' by a detailed model



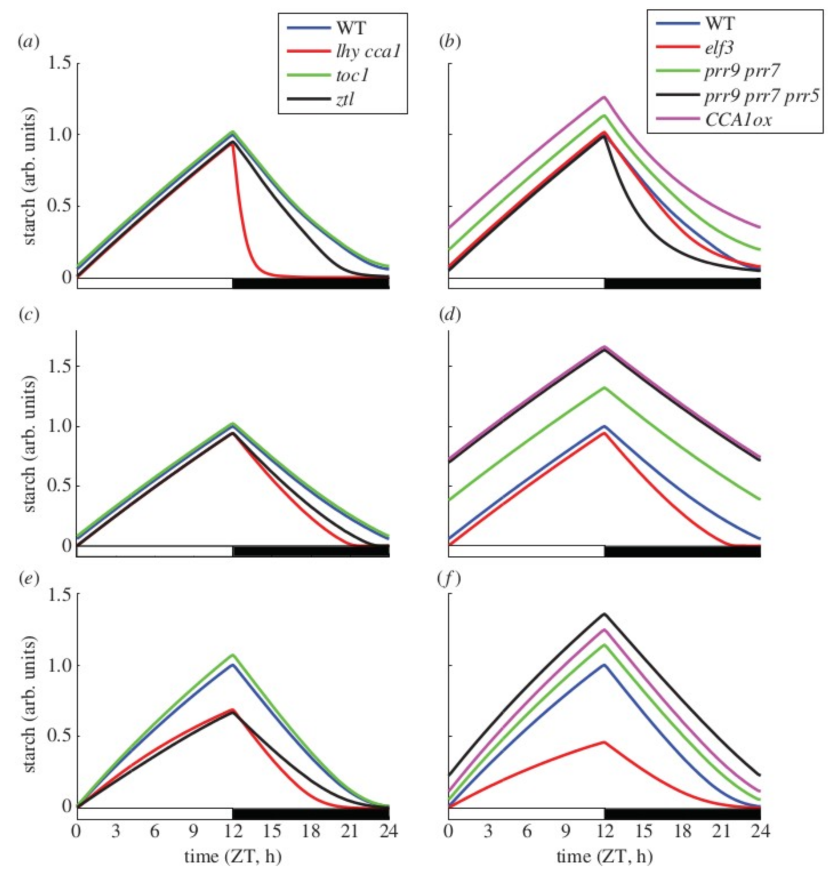
Pokhilko et al, 2011

The evolution of a model

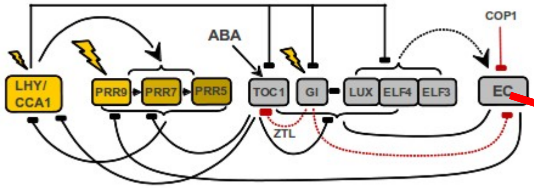


In Seaton et al, 2013:

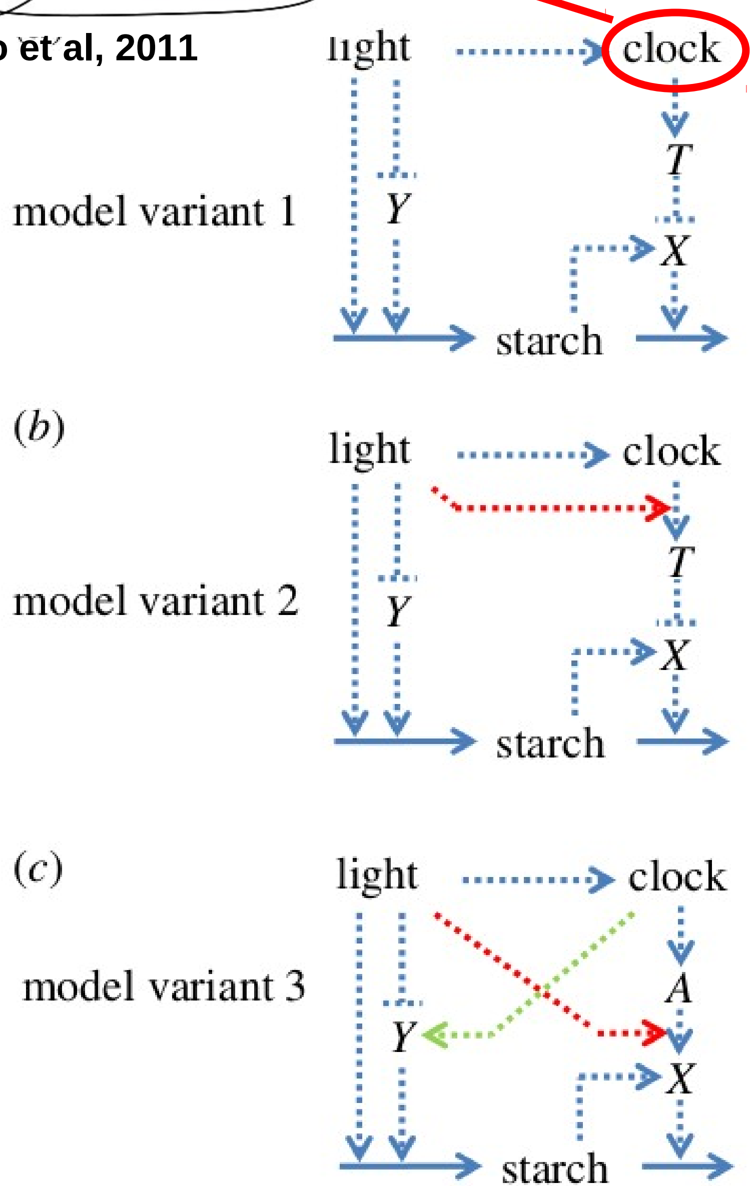
- Testing basic regulatory mechanisms
- Replacing 'clock' by a detailed model
- Simulate clock mutants



The evolution of a model

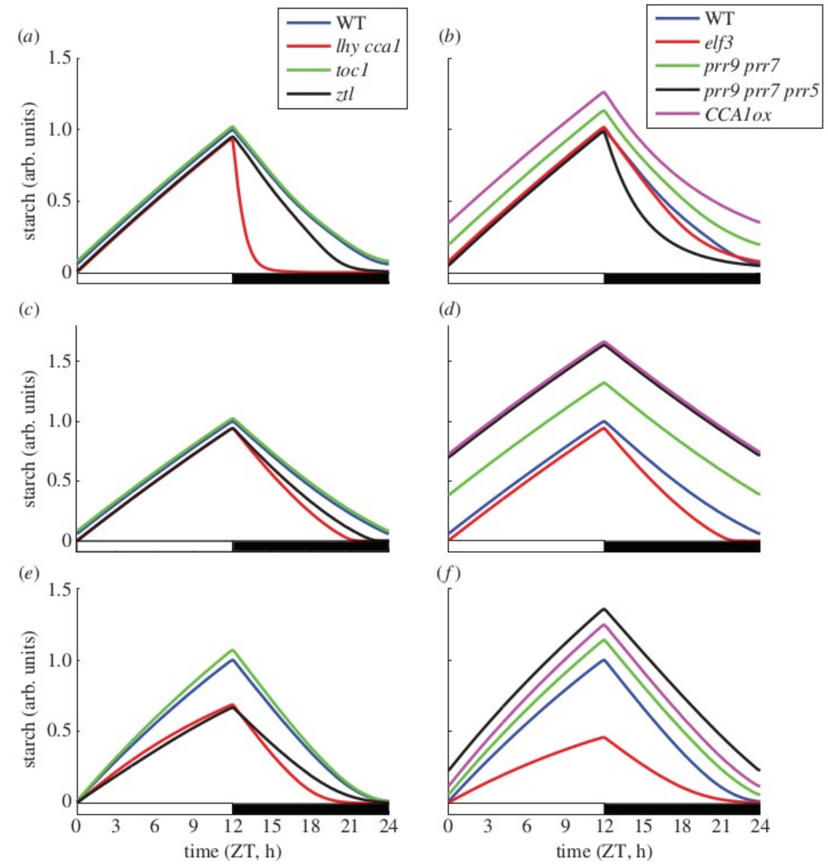


Pokhilko et al, 2011



In Seaton et al, 2013:

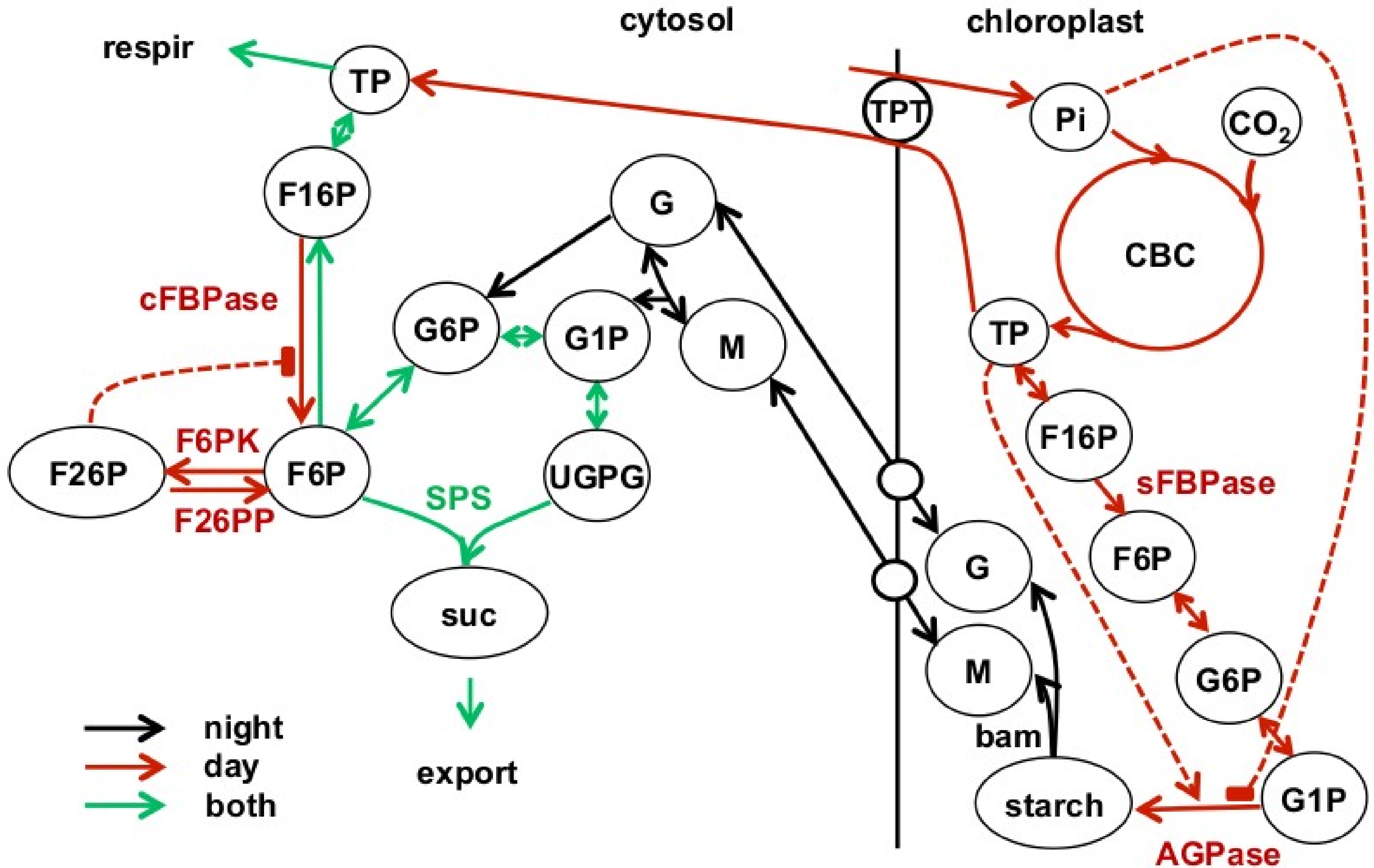
- Testing basic regulatory mechanisms
- Replacing 'clock' by a detailed model
- Simulate clock mutants



<u>Conclusions:</u>

- Variants 2 & 3 ok, more tests needed
- Components A,X,Y remain hypothetical

Adding more details of metabolism



- Carbon fixation
- Starch synthesis
- Starch breakdown
- Sucrose synthesis
- Sucrose export

Pokhilko et al, 2014, Mol Biosystems

Include key steps but simplify pathways!

Model assumptions (postulates)

1. Key sensors:

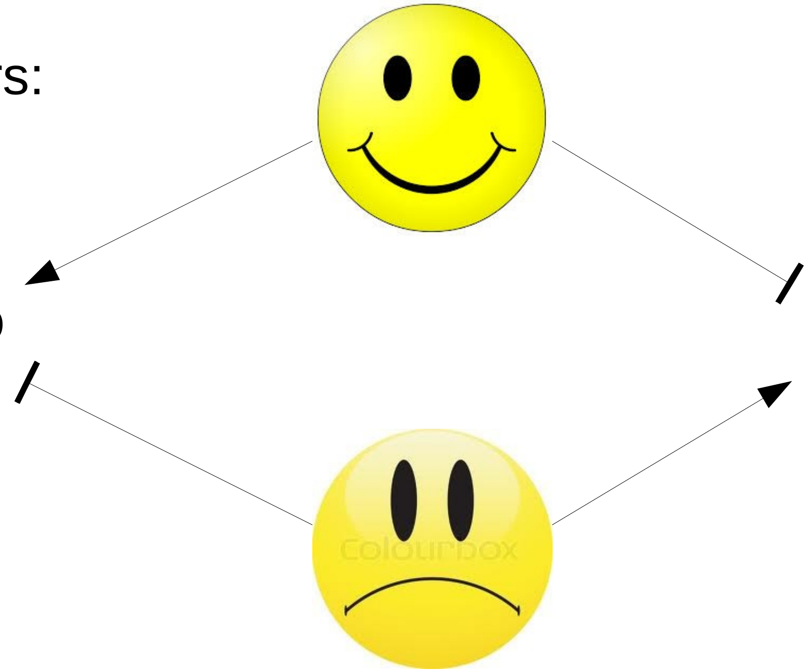
Timer α

time-to-dawn

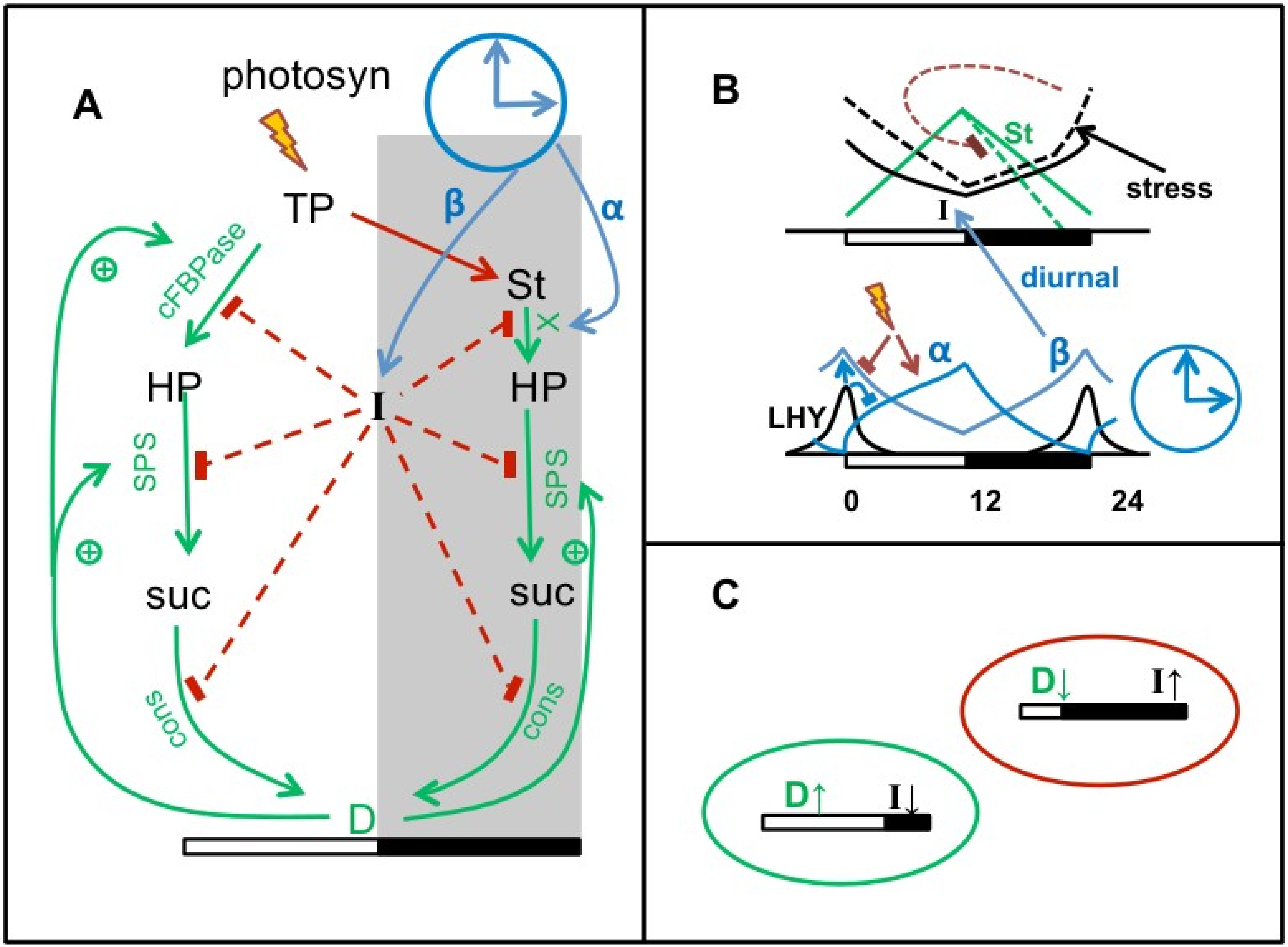
dark sensor β

carbon limitation

2. Global regulators:

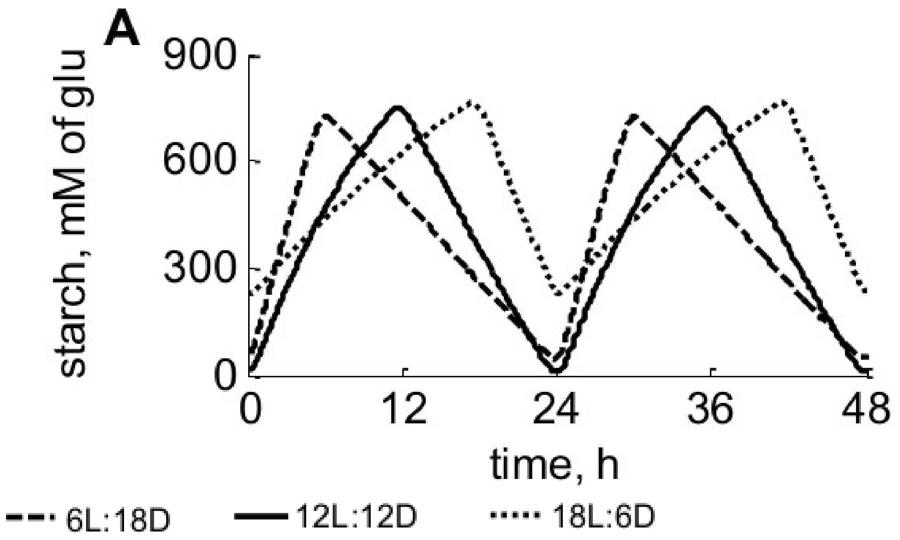


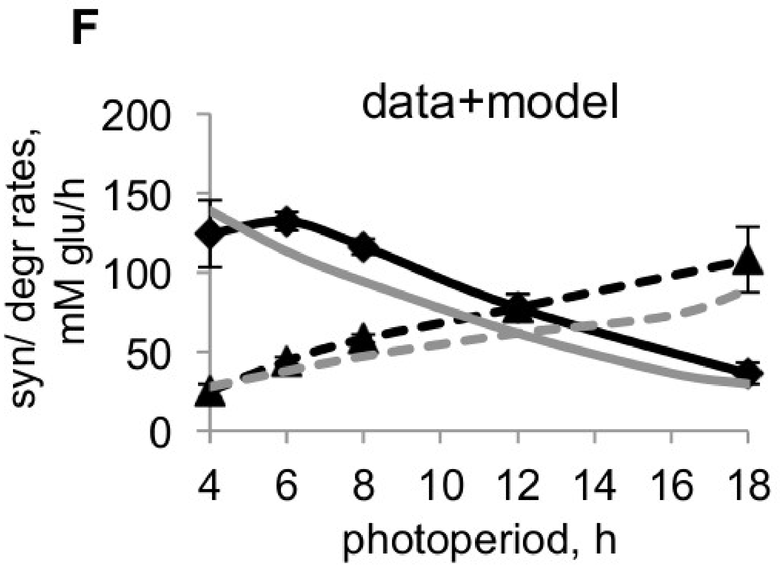
Regulatory principles



Simulations wild-type

Regulatory principles allow to explain wild-type starch turnover under various photoperiods





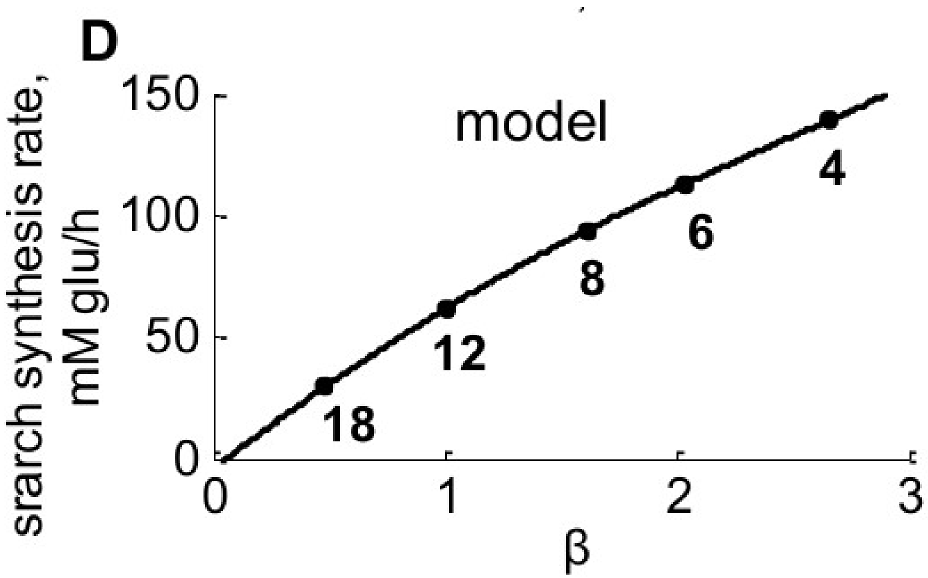
What are the unknown components?

Model allows to make predictions of their behaviour

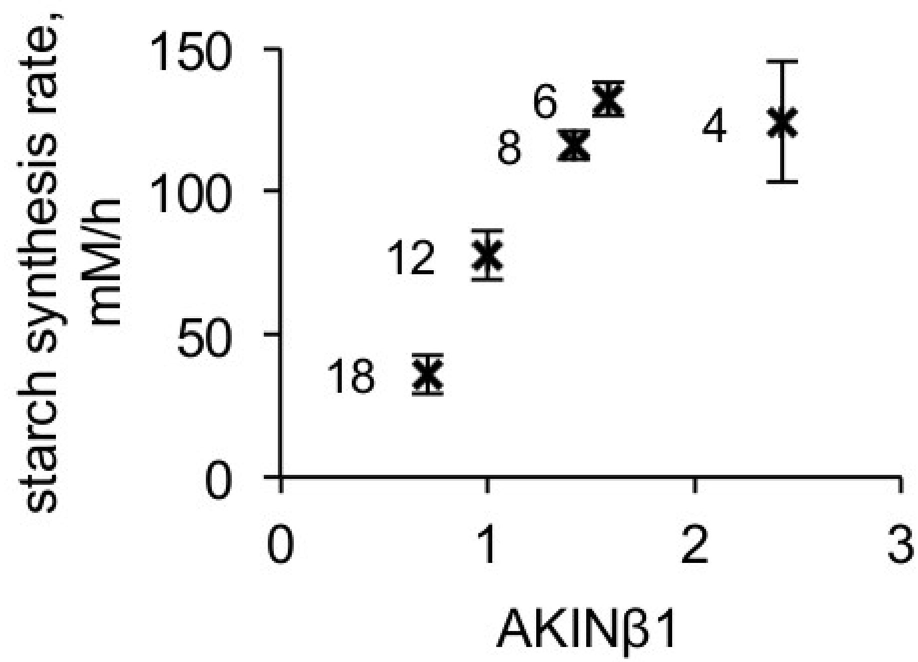
⇨ Helps to identify candidates from expression / proteomics data

For example, the component β:

Predicted peak-levels at dawn



Microarray data for β-subunit of SNRK1

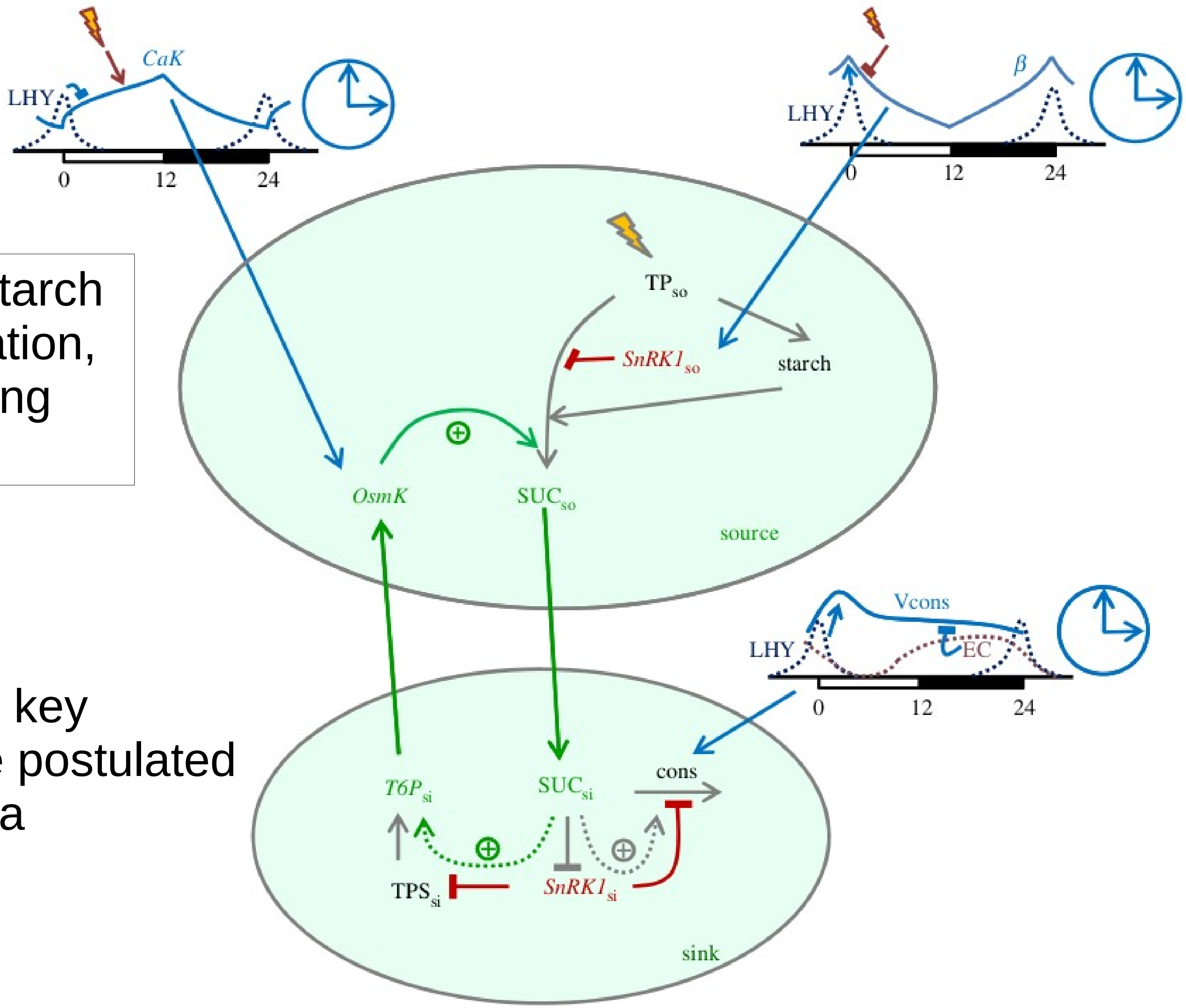


Promotor structure also supports AKINβ1 as good candidate for β

Other regulatory components still unknown!

The third generation

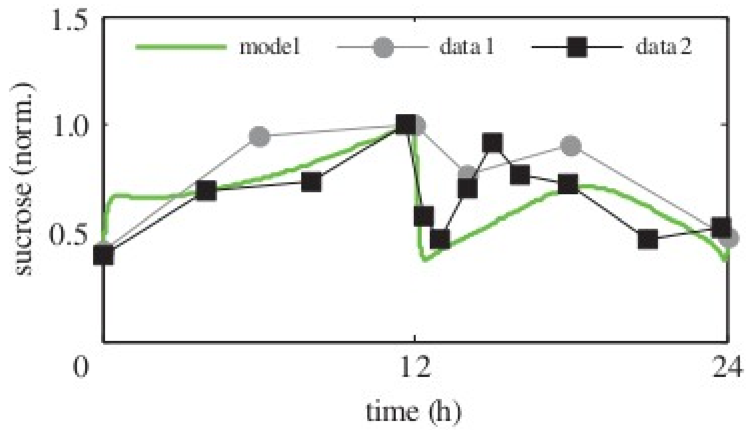
A combined regulation of starch turnover by demand regulation, carbon sensing, light sensing and timing (clock)

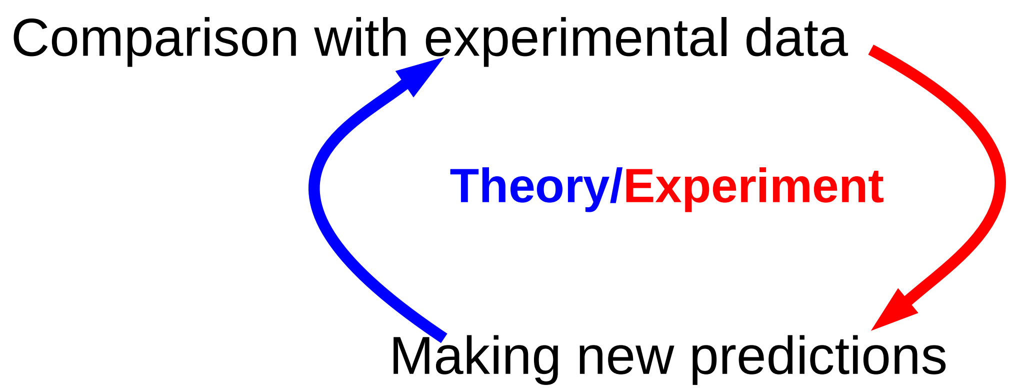


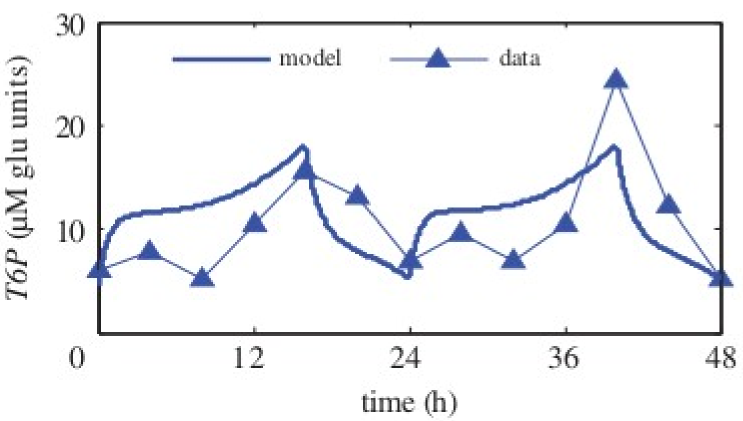
The molecular nature of all key regulatory components are postulated based on experimental data

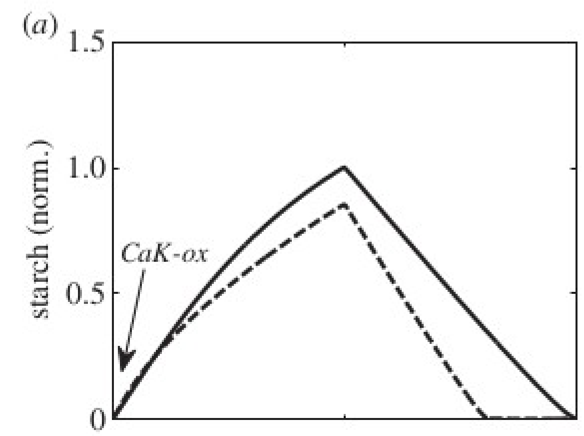
Pokhilko et al, 2015, Roy Soc Interface

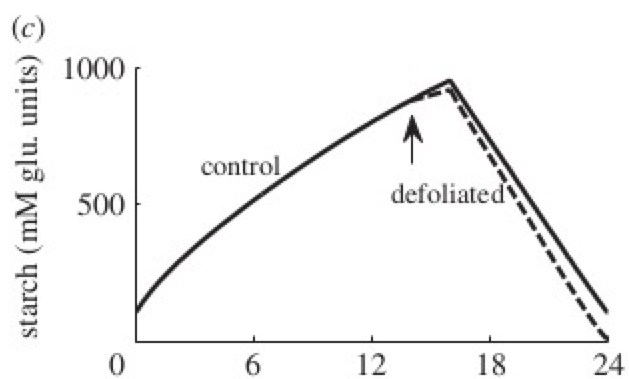
Improved results and new predictions

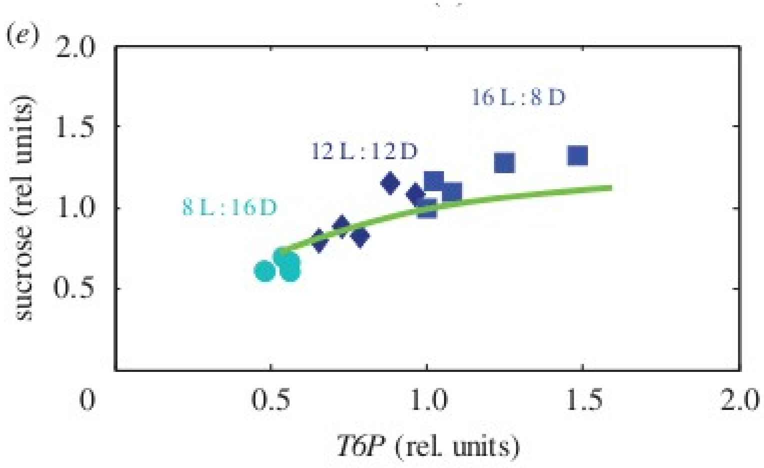


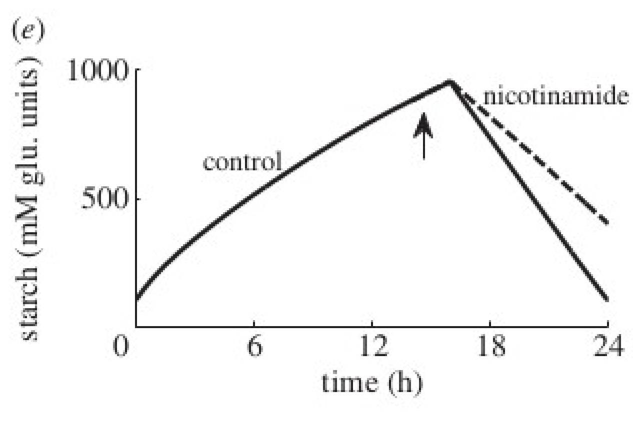


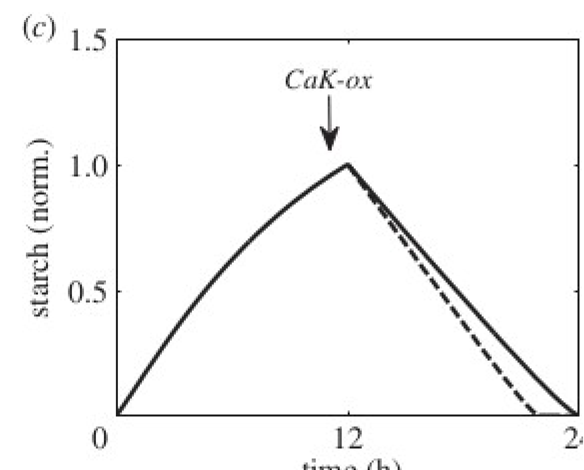












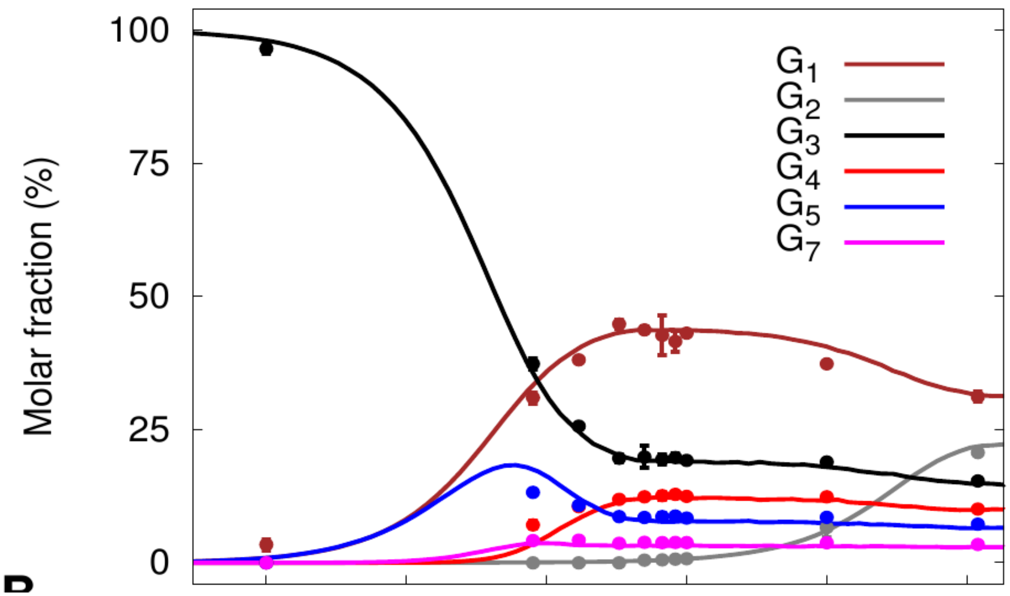
Outlook – towards designing starch

What do we need to model & design starch?

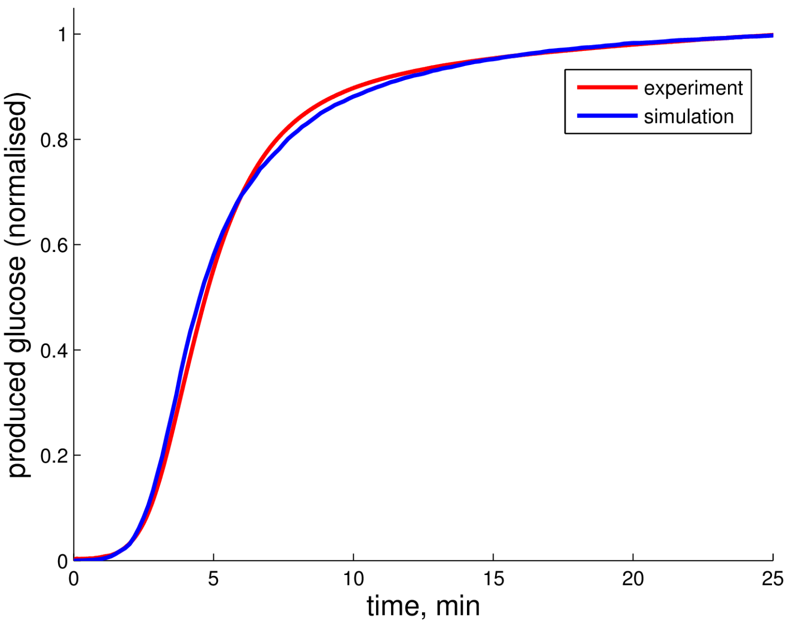
1. Understand and describe polymer-active enzymes

What do we need to model & design starch?

1. Understand and describe polymer-active enzymes



DPE1



MalQ

What do we need to model & design starch?

1. Understand and describe polymer-active enzymes

OK

Require more data:
- in vitro kinetics of enzymes
- chain-length distributions for knockouts / synthetic in vitro-systems

2. Understand and describe surface-active enzymes

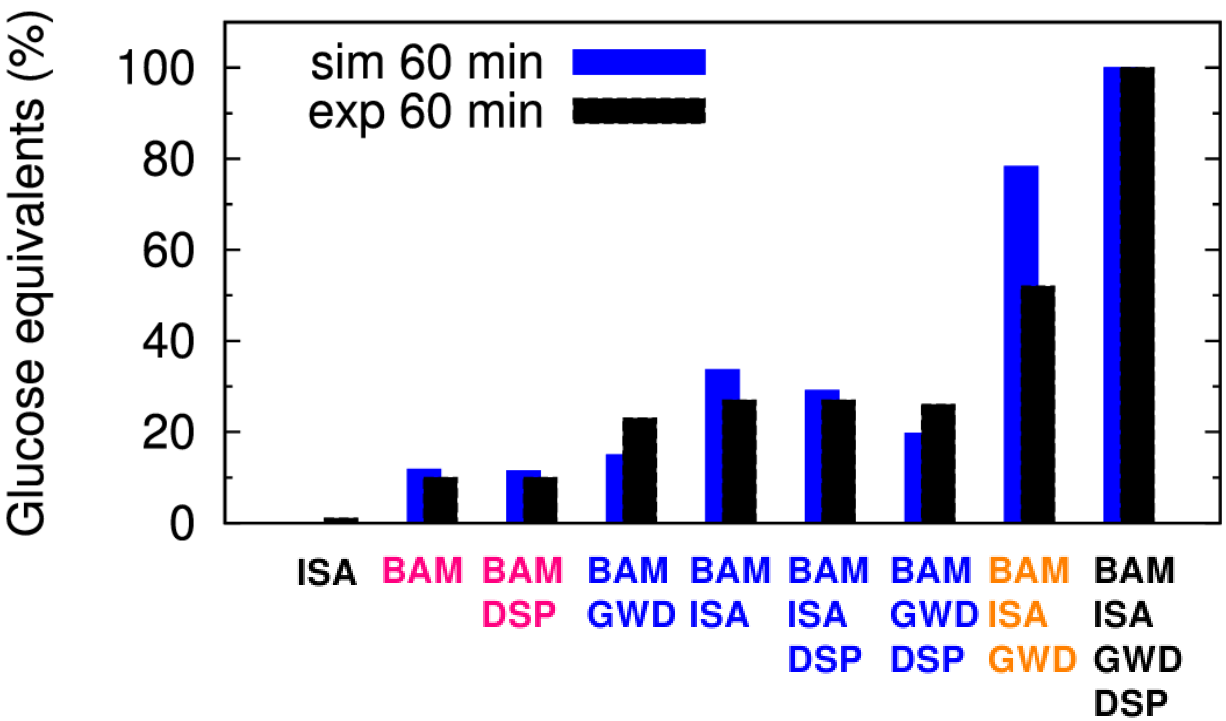
What do we need to model & design starch?

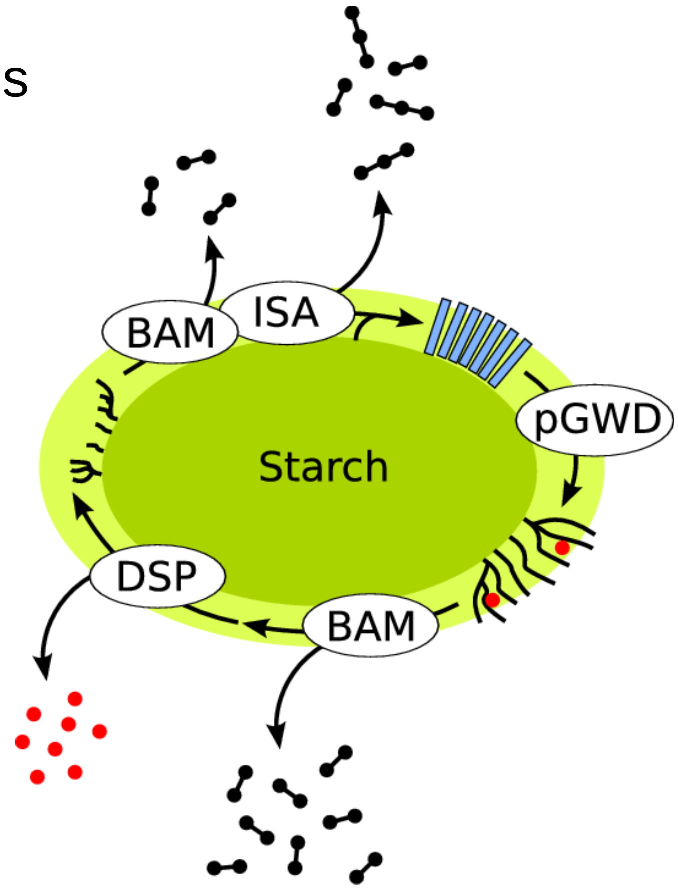
1. Understand and describe polymer-active enzymes

OK

Require more data:
- in vitro kinetics of enzymes
- chain-length distributions for knockouts / synthetic in vitro-systems

2. Understand and describe surface-active enzymes





What do we need to model & design starch?

1. Understand and describe polymer-active enzymes

   OK

   Require more data:
   - in vitro kinetics of enzymes
   - chain-length distributions for knockouts / synthetic in vitro-systems

2. Understand and describe surface-active enzymes

   OK

   Require more data:
   - in vitro kinetics of enzymes (difficult!)
   - synthetic in-vitro systems with crystallised (ideal) starch
   - time-resolved data!

3. Find the missing links!

What do we need to model & design starch?

1. Understand and describe polymer-active enzymes

OK

Require more data:
- in vitro kinetics of enzymes
- chain-length distributions for knockouts / synthetic in vitro-systems

2. Understand and describe surface-active enzymes

OK

Require more data:
- in vitro kinetics of enzymes (difficult!)
- synthetic in-vitro systems with crystallised (ideal) starch
- time-resolved data!

3. Find the missing links!

For example:
- formation of double helices (α-1,4-glucans)
- cooperation of biochemical and biophysical processes

Modelling 3D structure of polysaccharides

POLYS 2.0: An Open Source Software Package for Building Three-Dimensional Structures of Polysaccharides

Søren B. Engelsen,[1] Peter I. Hansen,[1] Serge Pérez[2]

[1] *Spectroscopy & Chemometrics, Faculty of Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Copenhagen, Denmark*

[2] *Centre de Recherches sur les Macromolécules Végétales, CNRS, BP 53 X, 380451 Grenoble, Cedex, France*

Received 23 June 2013; revised 18 November 2013; accepted 19 November 2013
Published online 30 November 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/bip.22449

The next steps...

- Systematic *in vitro* characterisation of surface-active and polymer-active enzymes (Rob Field, JIC Norwich)
- Systematic experiments in yeast and combination of enzymes *in vitro* (Sam Zeeman, ETH Zurich)
- Combine existing modelling approaches (Oliver Ebenhöh, HHU Düsseldorf)

⇨

ERA-CAPS Project ***DesignStarch***

Postdoc needed!

- Envisaged start: June 2015
- Goals:
  - synthesise starch *in vitro* and in yeast
  - model these processes
  - predict physico-chemical properties from biochemistry/biophysics
  - design starch!

Acknowledgements

Collaborators

Potsdam
Martin Steup
Sebastian Mahlow
Julia Smirnova
Mark Stitt
Anna Flis

Universität Potsdam
Max-Planck-Institut für Molekulare Pflanzenphysiologie

Aberdeen
Alexandra Pokhilko

1495 UNIVERSITY OF ABERDEEN

Edinburgh
Andrew Millar
Daniel Seaton

THE UNIVERSITY OF EDINBURGH

Zurich
Önder Kartal
Sam Zeeman

University of Zurich UZH
ETH Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Financial Support

DFG
CEPLAS Cluster of Excellence on Plant Sciences
AcciPhot