Spontaneous Talk on Entropy in Carbohydrate Metabolism

Rigi 20-Jan-2015

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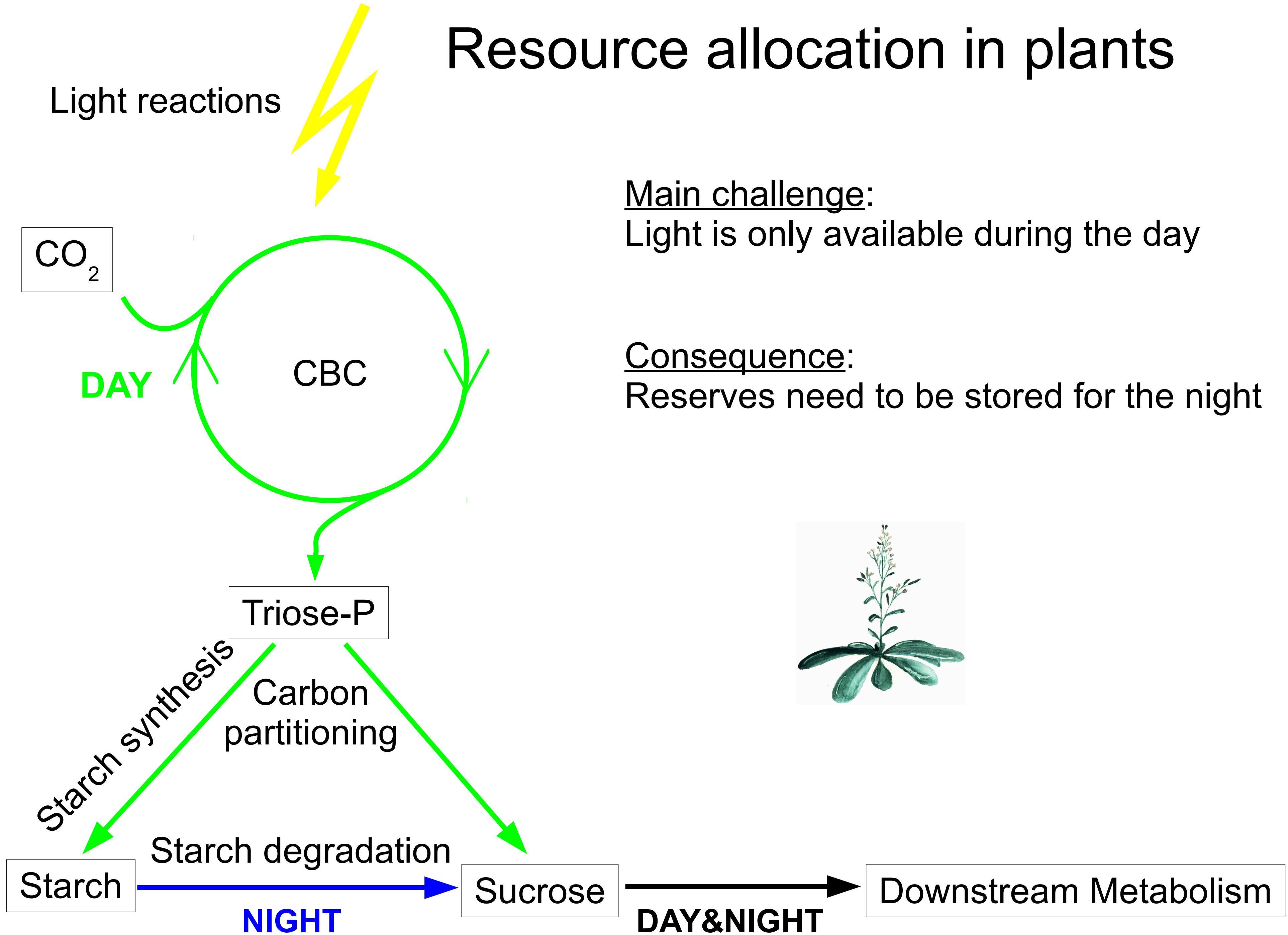
What's special about plants?

1. Photosynthesis
2. Can't run away!

⇨ Experts in chemical warfare!

Estimated > 200,000 secondary metabolites!

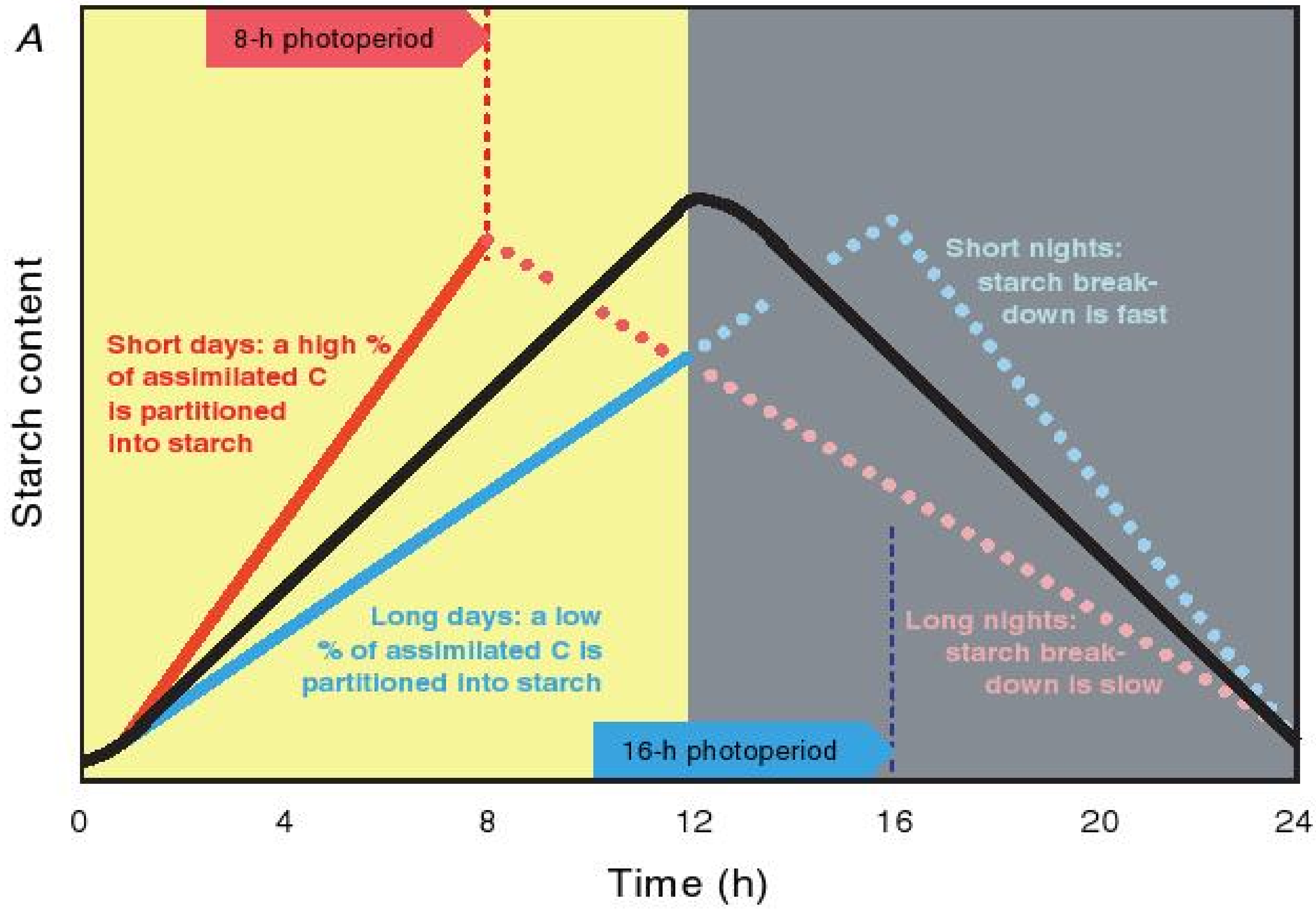
Resource allocation in plants



Main challenge:
Light is only available during the day

Consequence:
Reserves need to be stored for the night

The diurnal turnover of starch



Open questions

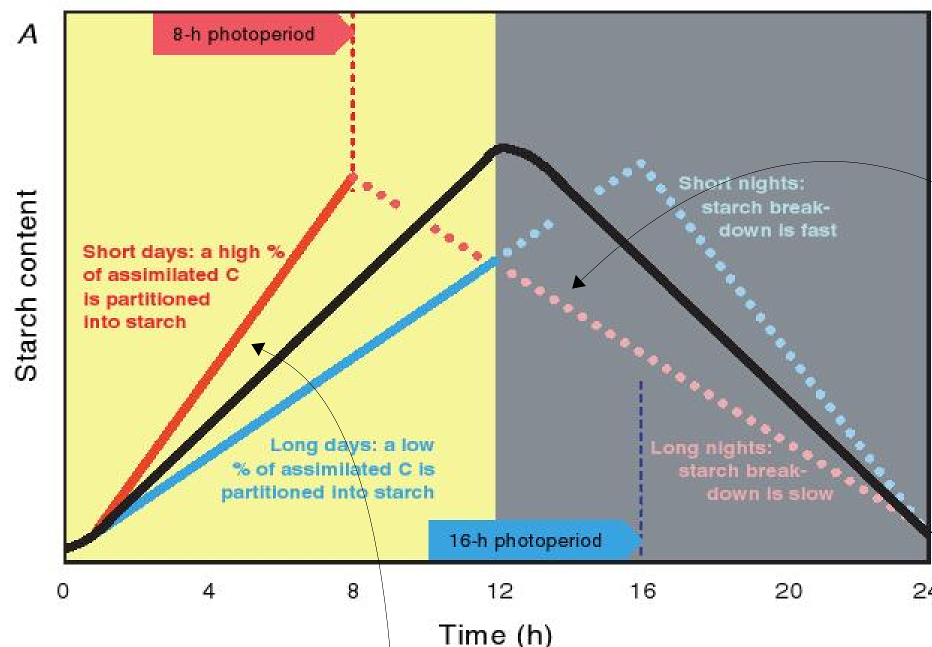
?

?

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

What measures the starch content?

?

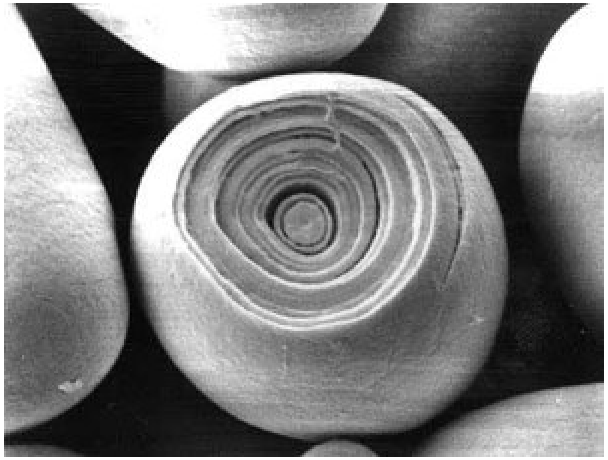


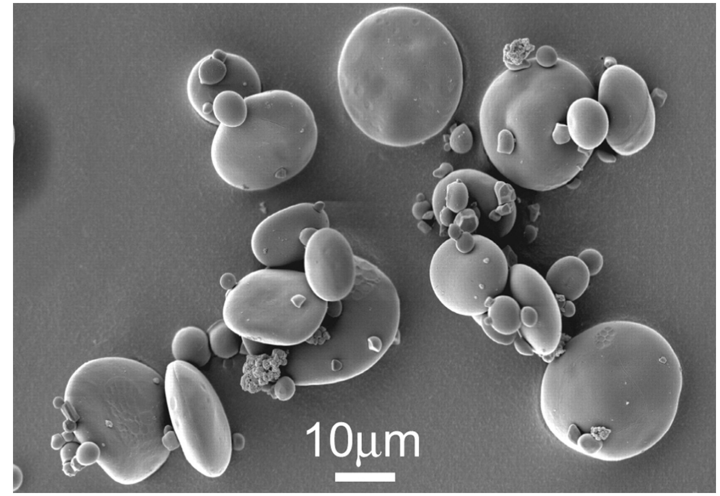
How is the correct breakdown rate 'calculated'?

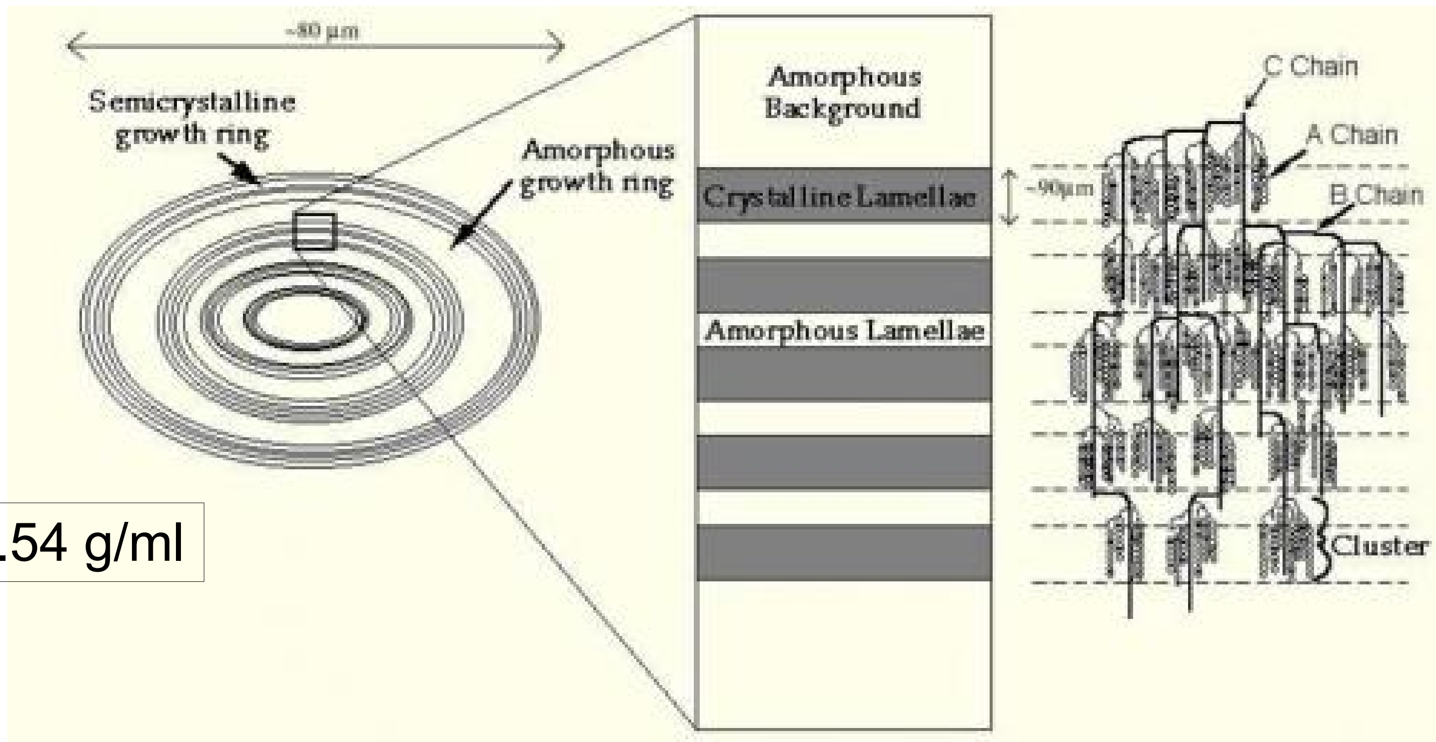
How is carbon partitioning controlled?

TiMet

Why starch?







Density: 1.54 g/ml

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

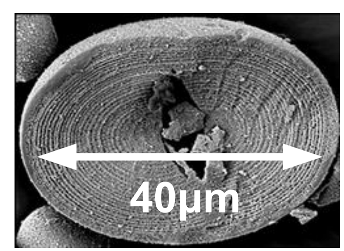
We (animals and fungi) predominantly use glycogen

big molecule (up to 10 MDa)

Possible advantages of starch

- low osmolarity
- large size
- high density

still small compared to starch



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

Alternatives

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We (animals and fungi) predominantly use glycogen

trade-off between storage density and rapid mobilization

big molecule (up to 10 MDa)

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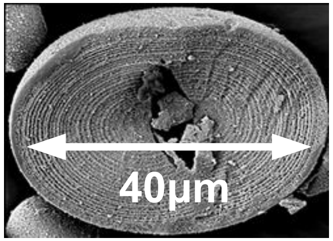
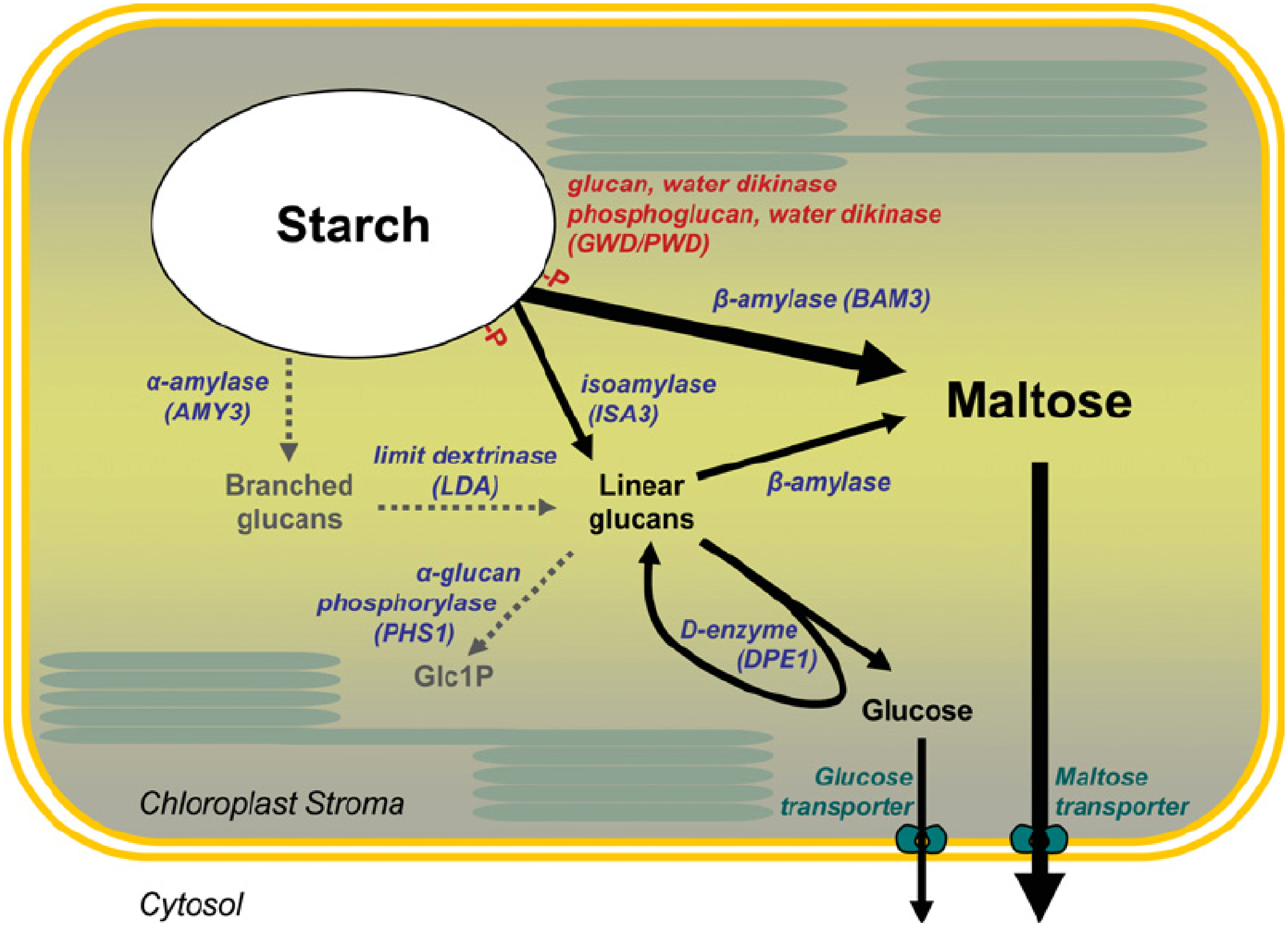


$\Rightarrow$ $3 \cdot 10^{10}$ Da!!!

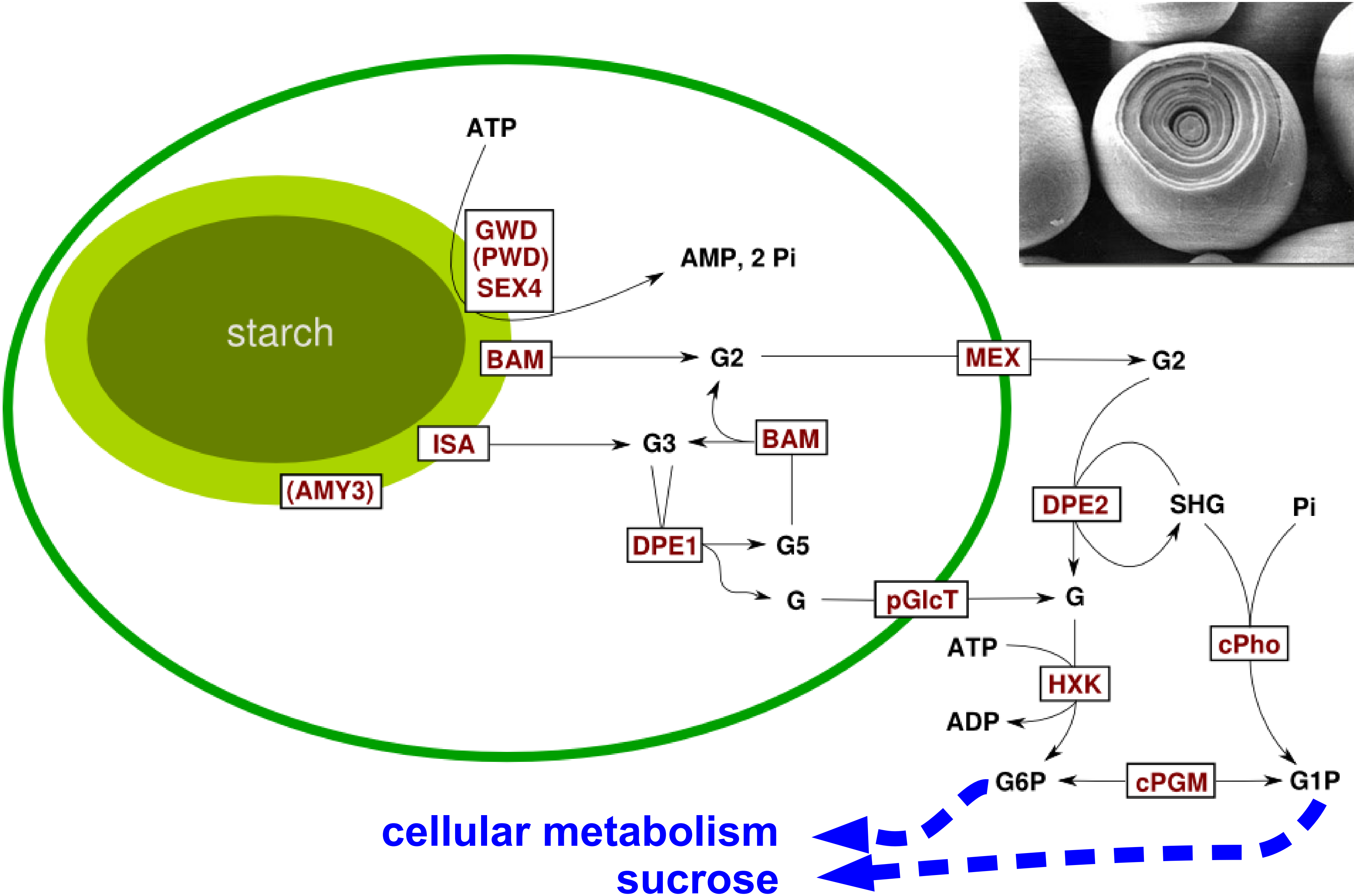
optimised for storage density, slower deployment

The starch breakdown pathway

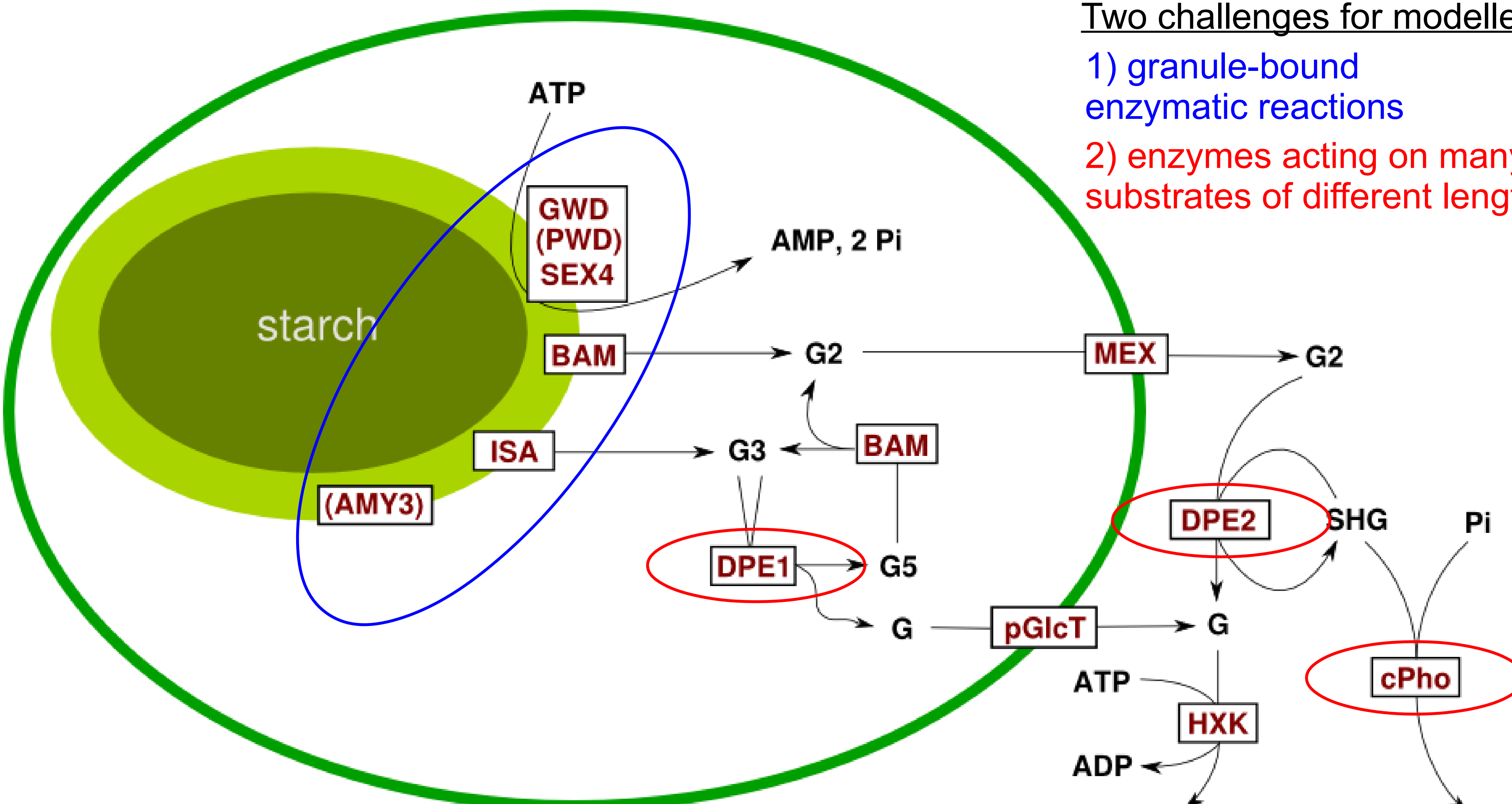


Zeeman et al, 2007

The starch breakdown pathway



Conceptual challenges



Two challenges for modellers:

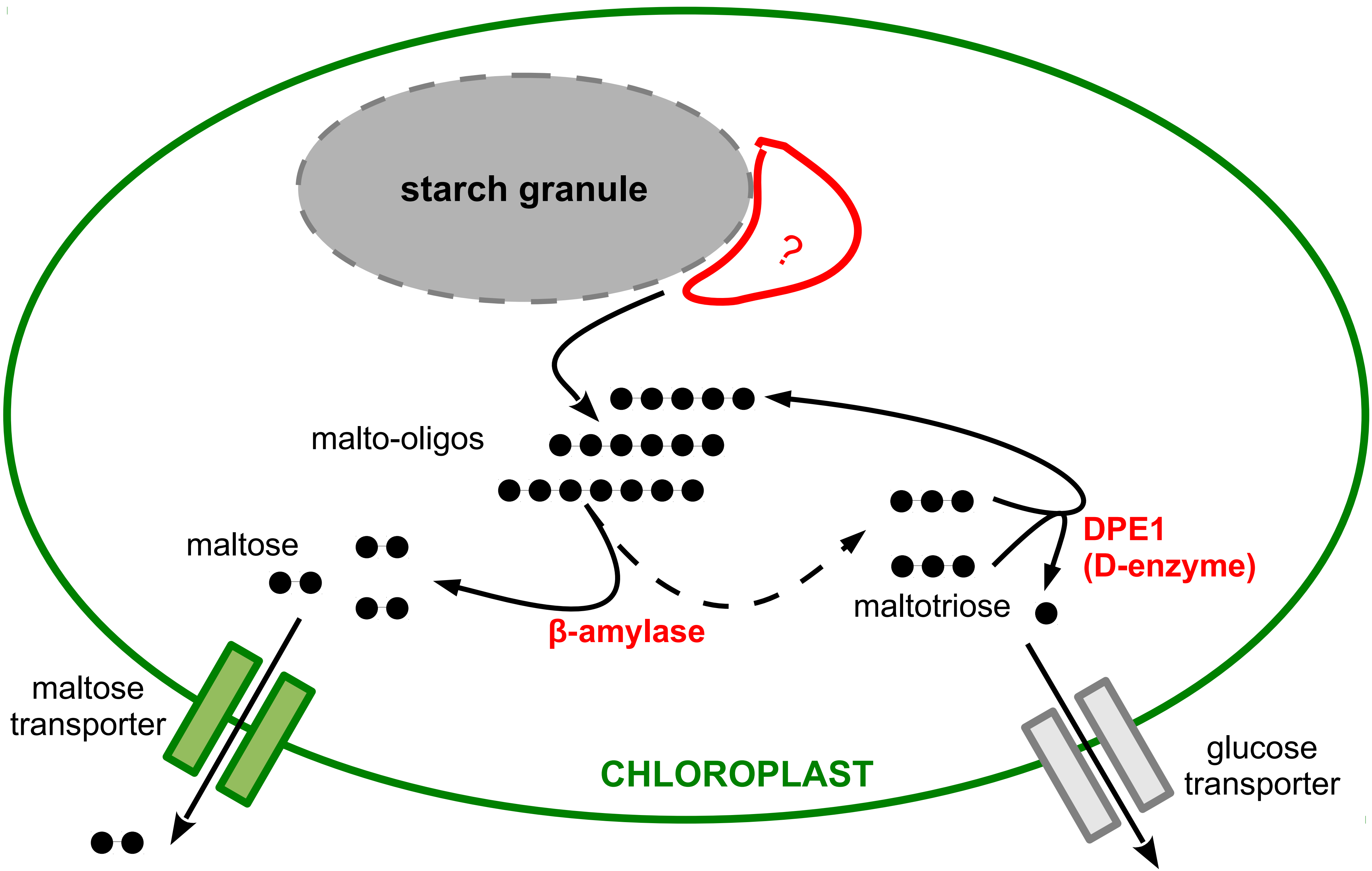
1) granule-bound enzymatic reactions

2) enzymes acting on many substrates of different lengths

cellular metabolism

sucrose

Starch degradation - disproportionation



Disproportionating enzymes (D-enzymes)

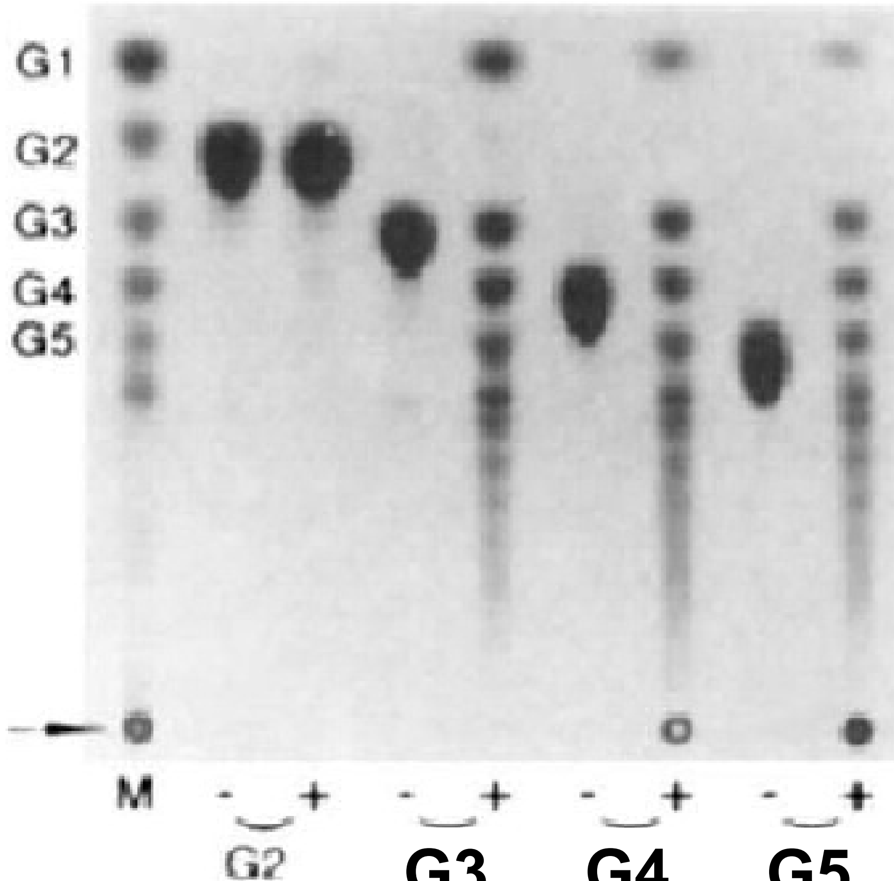
DPE1

EC: 2.4.1.25

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

$$G3 + G3 \longleftrightarrow G5 + G1$$

but not only!



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

(Takaha et al., JBC 1993)

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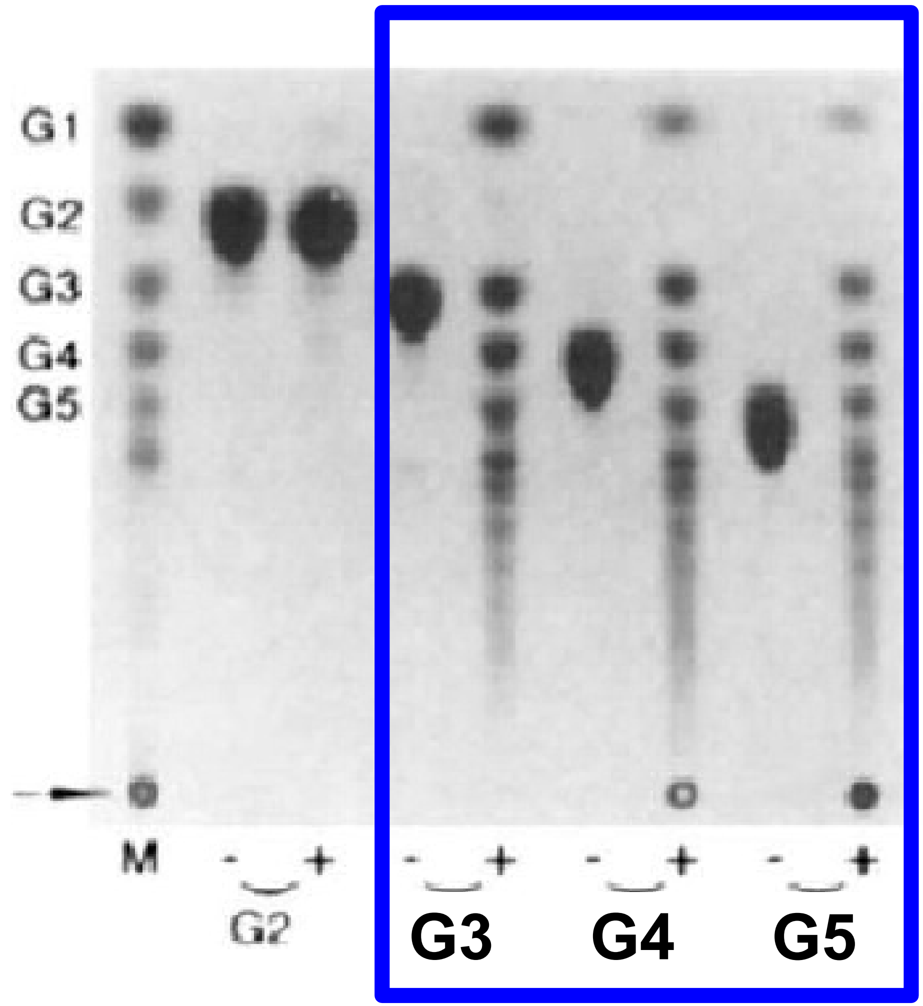


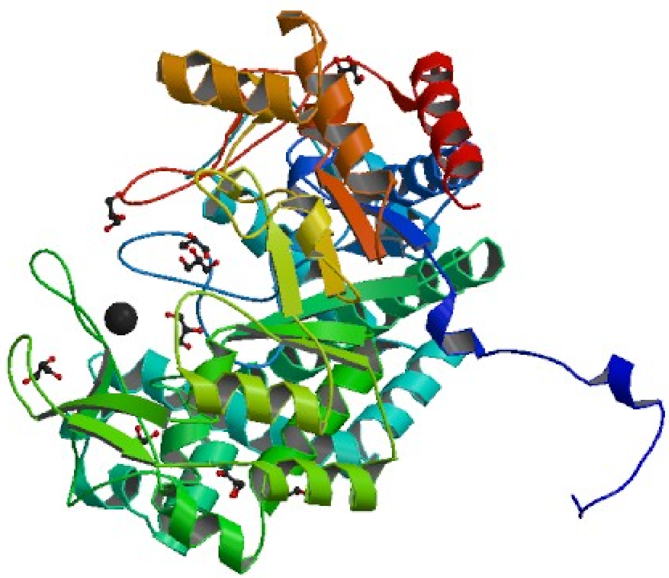
DPE1 produces a set of glucans of different length in *in vitro* assays.

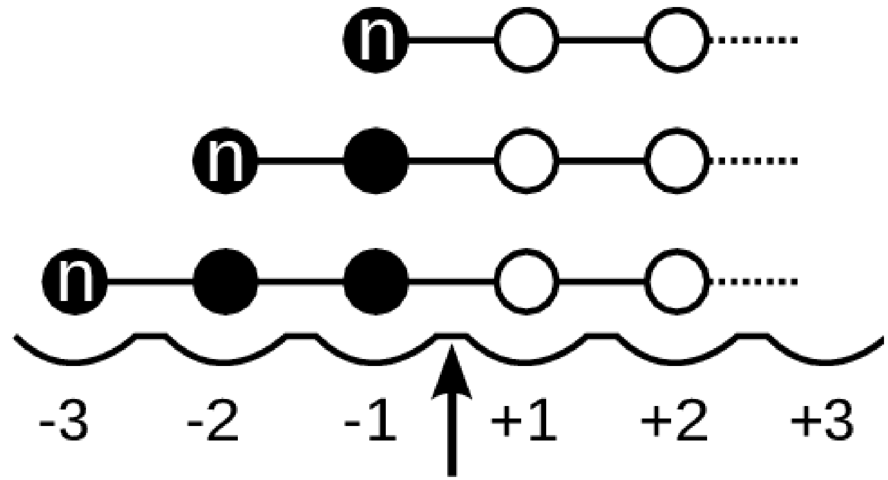
Equilibrium distribution depends on initial conditions!

(*Takaha et al., JBC 1993*)

K_{eq}???

Positional Isomers





D-Enzyme Subsite

Different binding modes of the donor substrate exists

- 1, 2 or 3 glucose residues can be transferred
- The general reaction equation is $G_n + G_m \longleftrightarrow G_{n-q} + G_{m+q}$ with q=1,2,3

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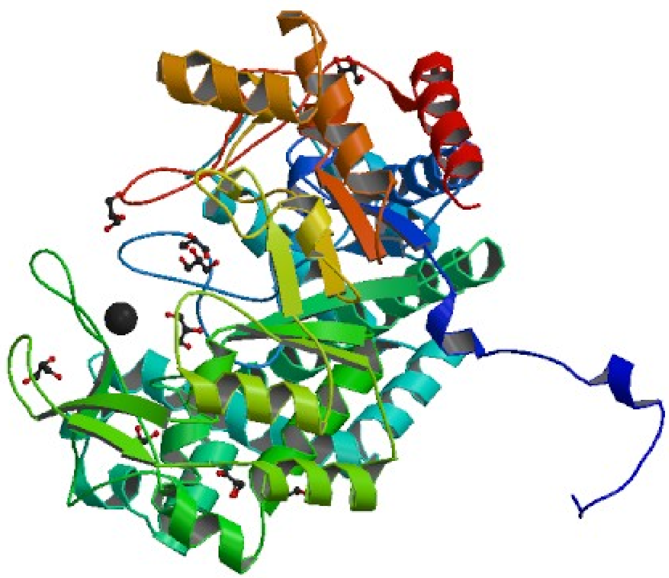

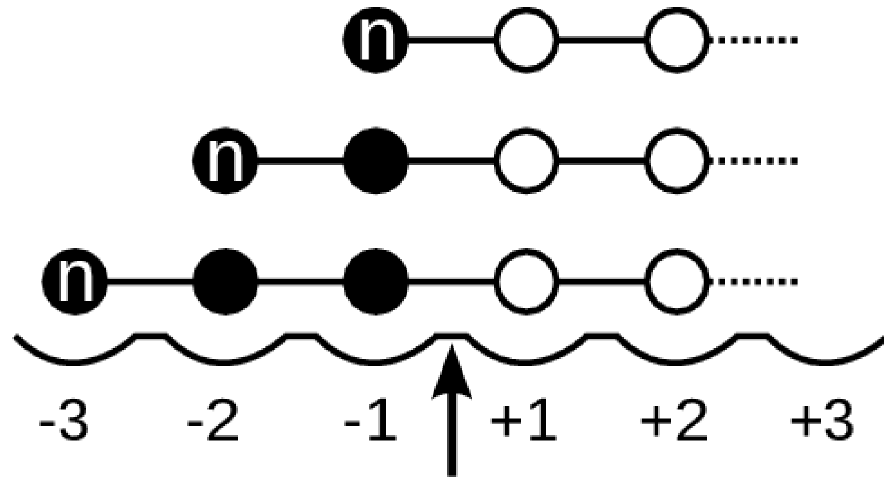

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For such a reaction, what is the meaning of K_M???

Disproportionating enzymes (D-enzymes)

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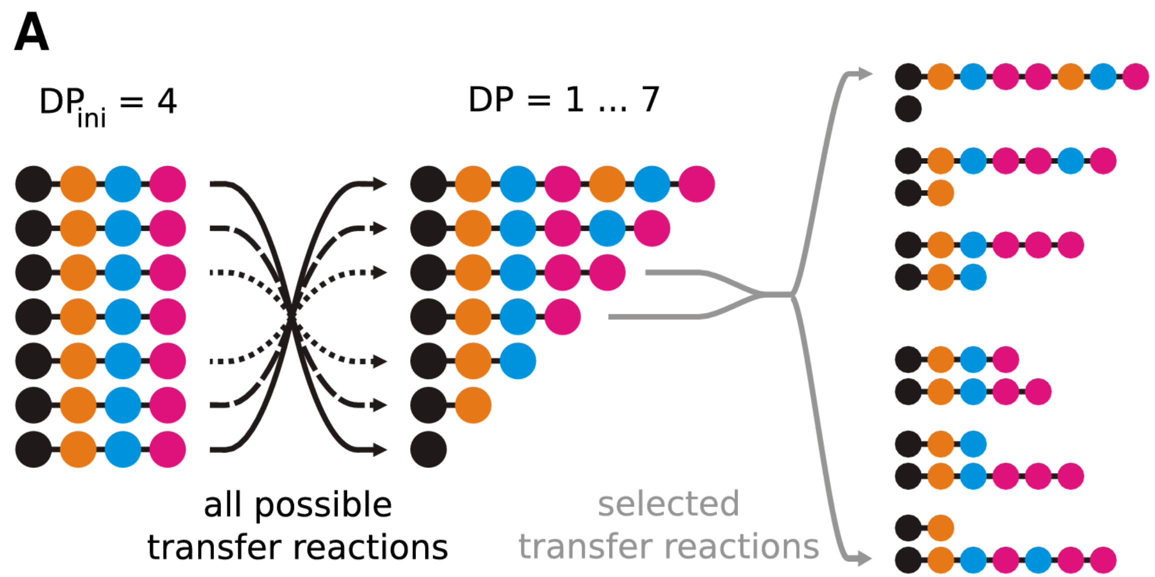
Disproportionating Enzyme randomises DPs



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

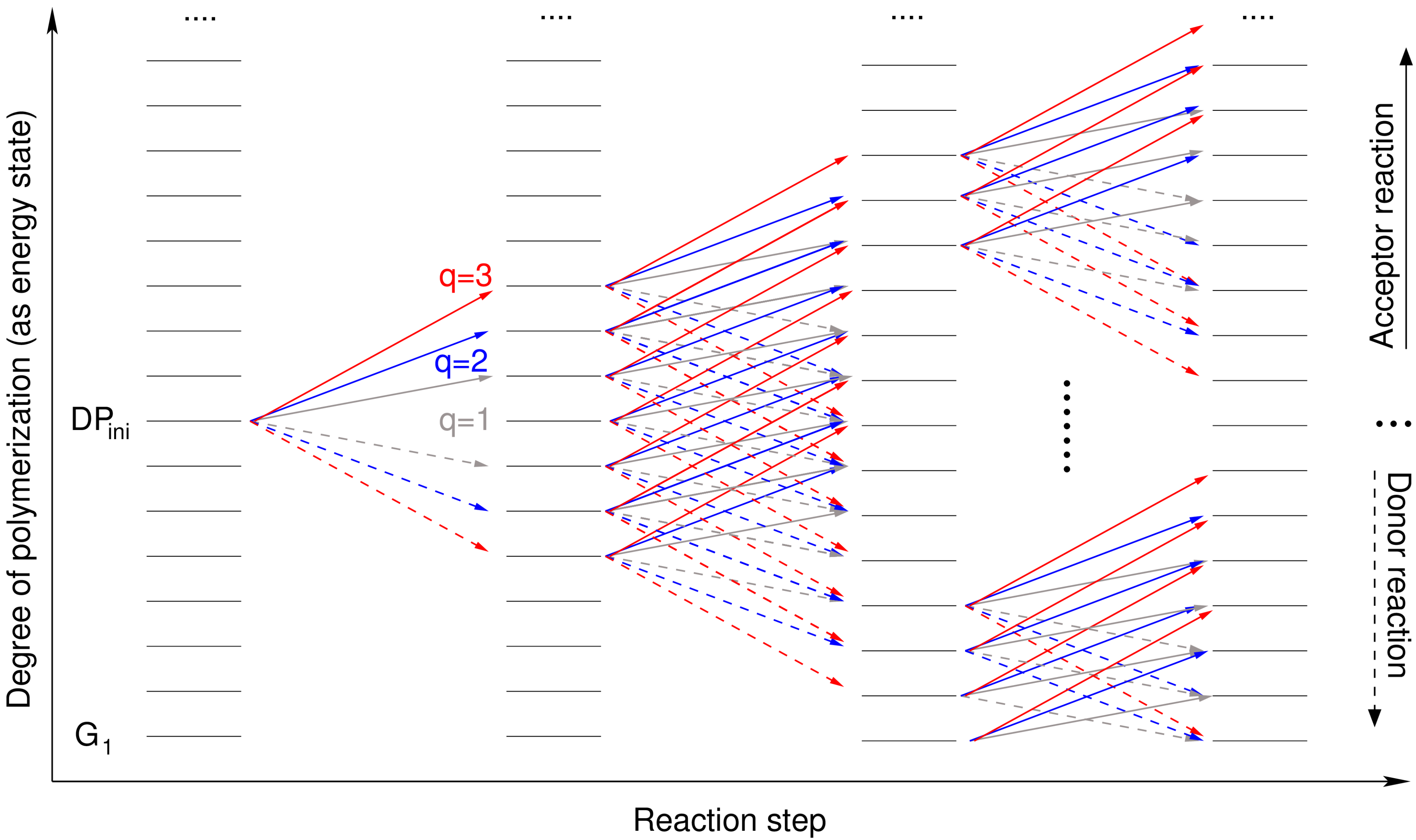
reaction must proceed towards a smaller Gibbs free energy : $\Delta G = \Delta H - T \Delta S < 0$

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

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

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

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

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

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

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

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

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

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

Entropic approach

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

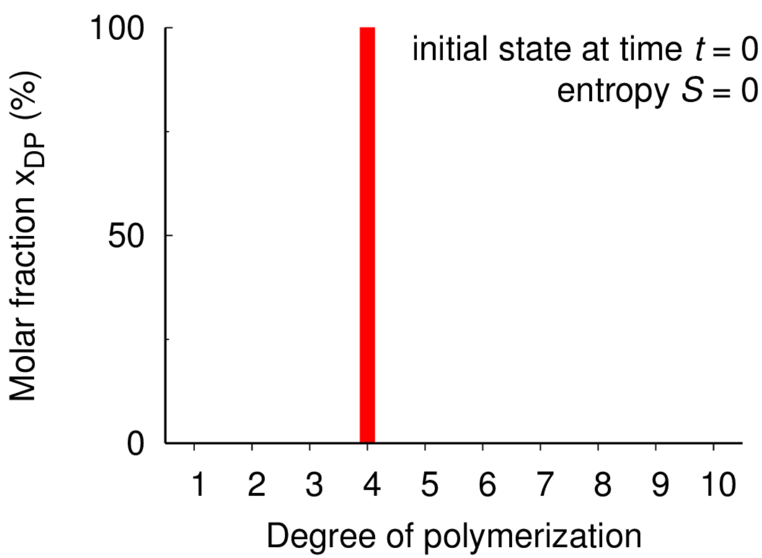
conservation of #molecules: $\sum x_k = 1$

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

implies

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

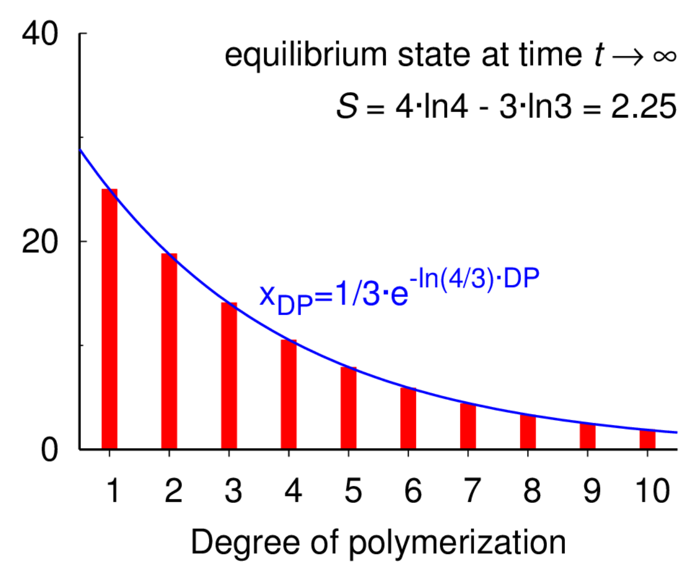
predicts



DPE1 action

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

with q = 1,2,3

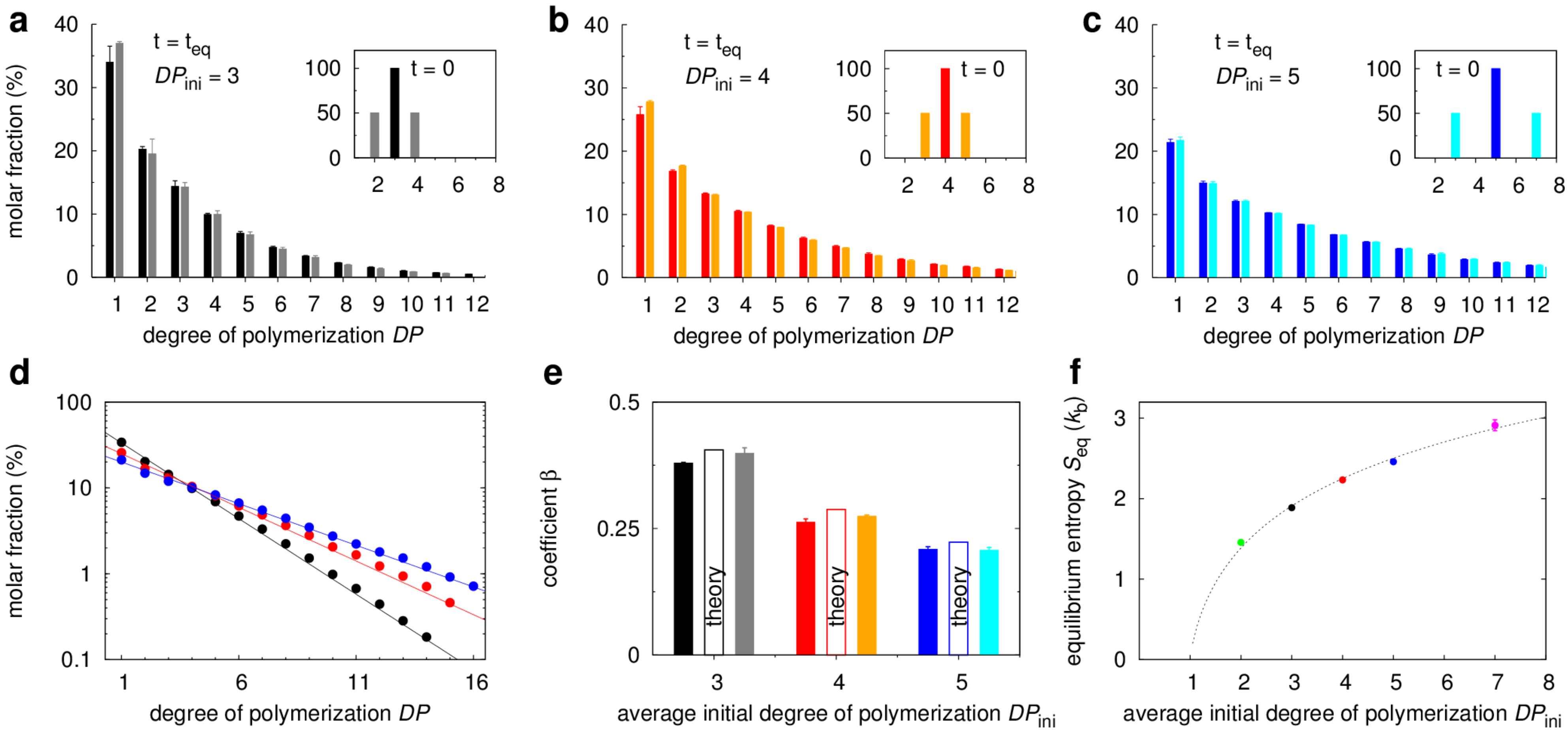


An instance of the 2nd law of TD!

DPE1 is entropy driven

Experiments with Martin Steup, University of Potsdam

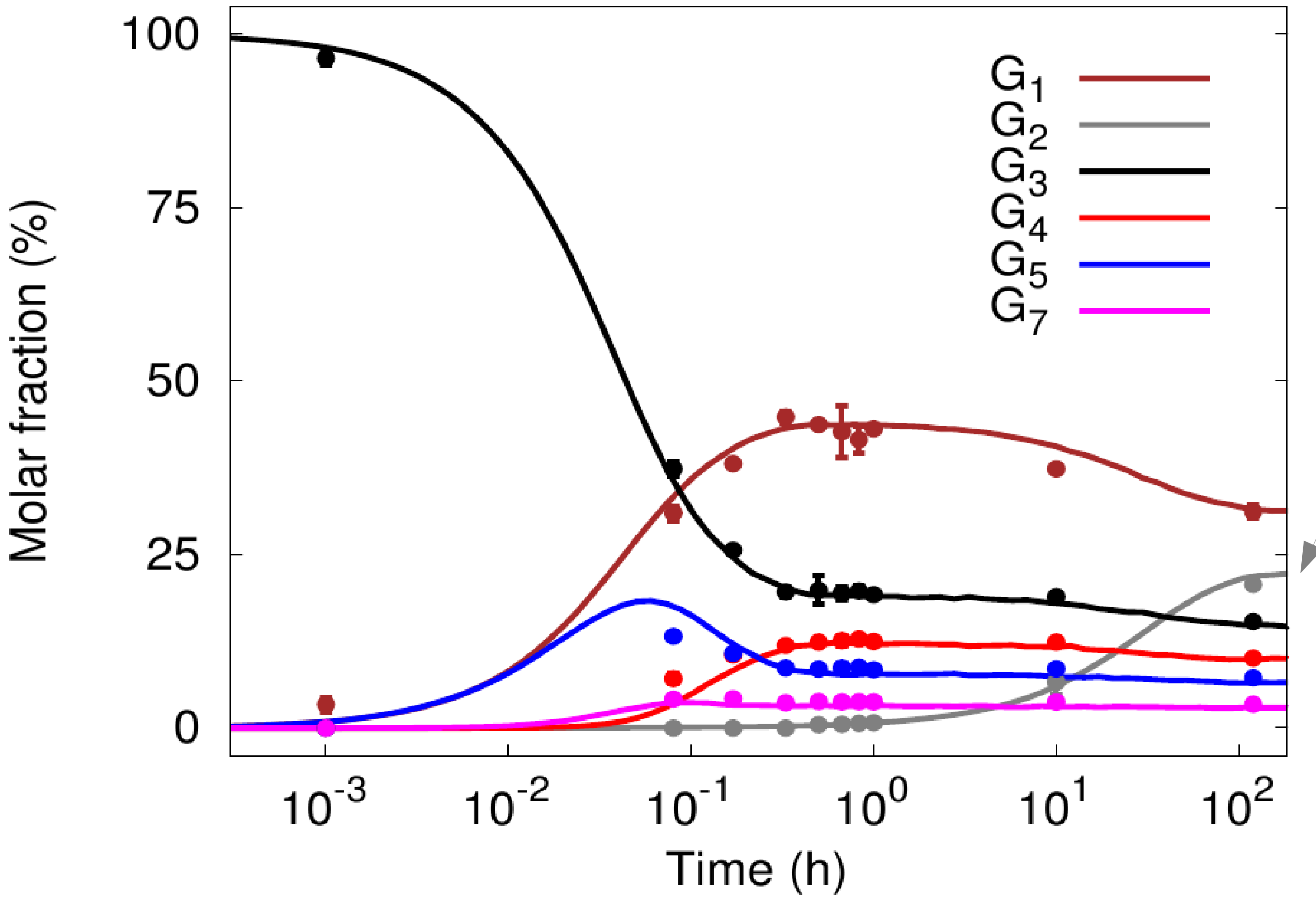
method: capillary electrophoresis



β is a generalisation of the equilibrium constant for polydisperse mixtures

(Kartal et al, 2011, Mol Syst Biol)

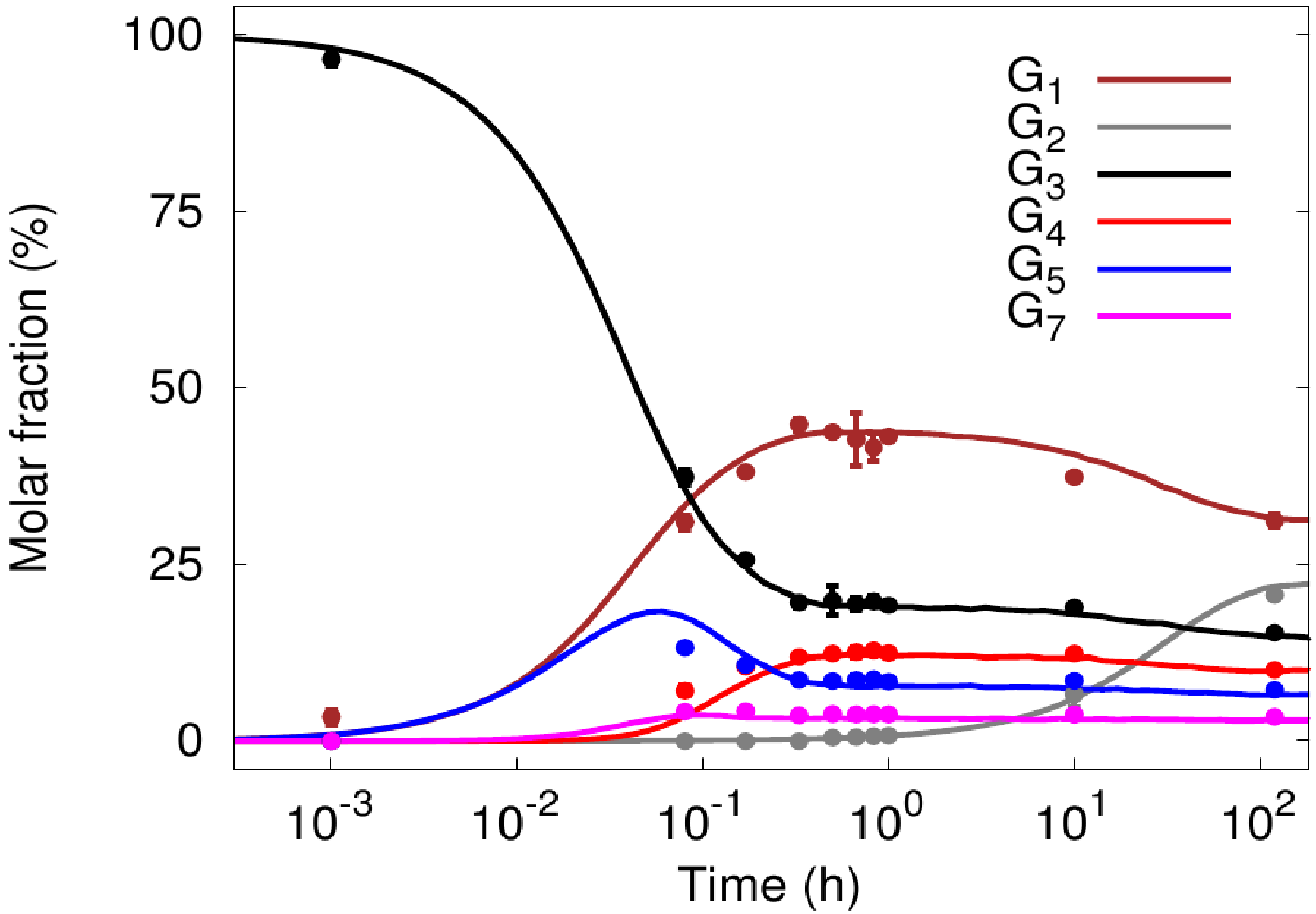
The dynamics of DPE1



maltose is formed late

Two time scales!

The dynamics of DPE1



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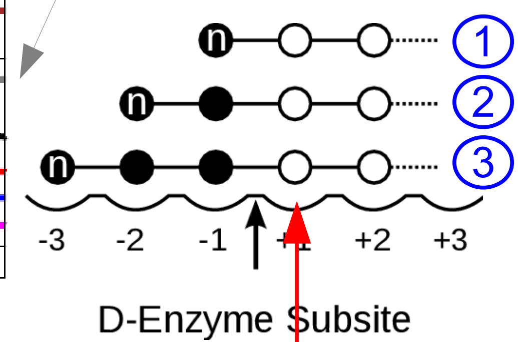


subsite +1 repellent
(binding of G2 unlikely)

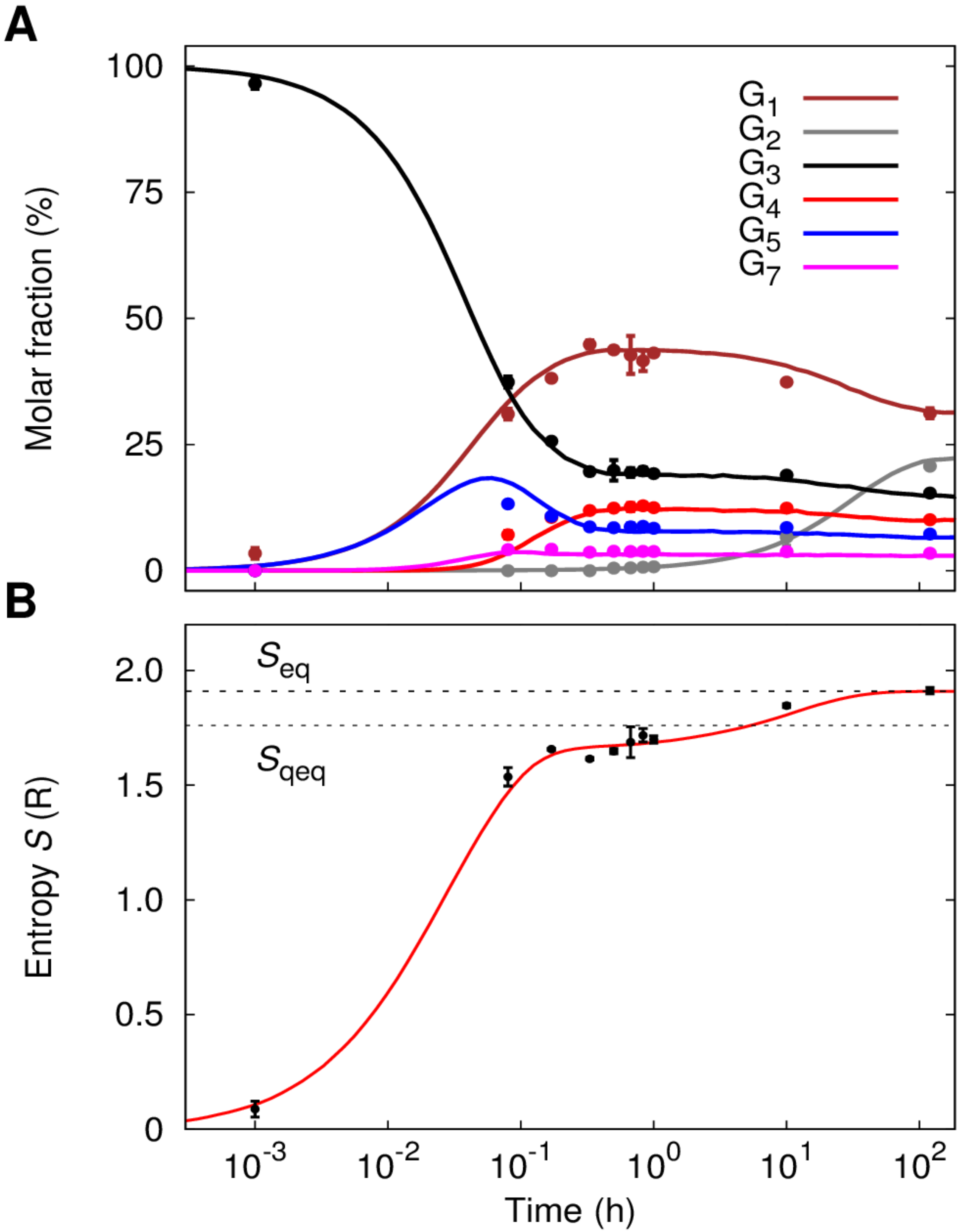
Two time scales!

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)

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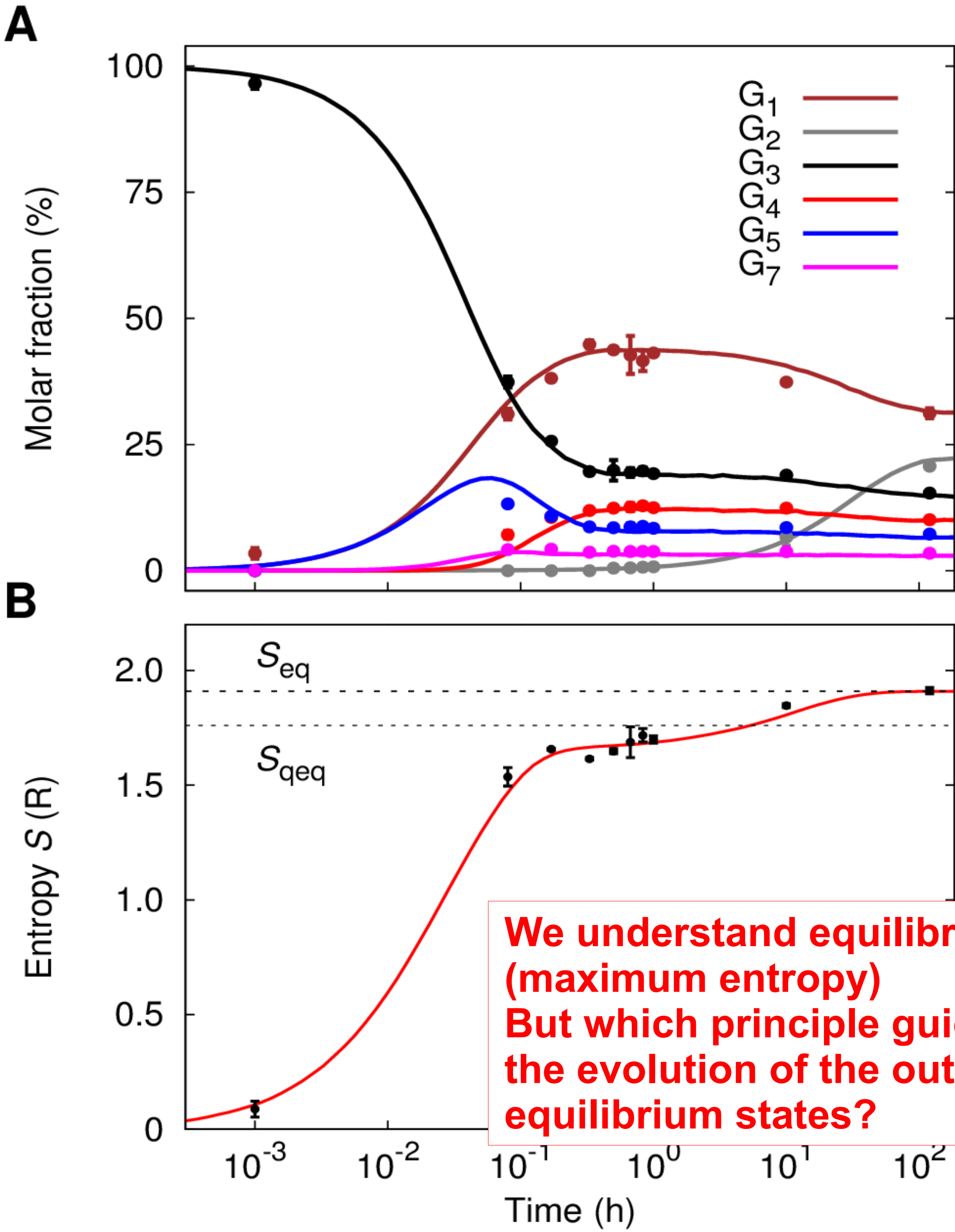

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We understand equilibrium (maximum entropy)
But which principle guides the evolution of the out-of-equilibrium states?

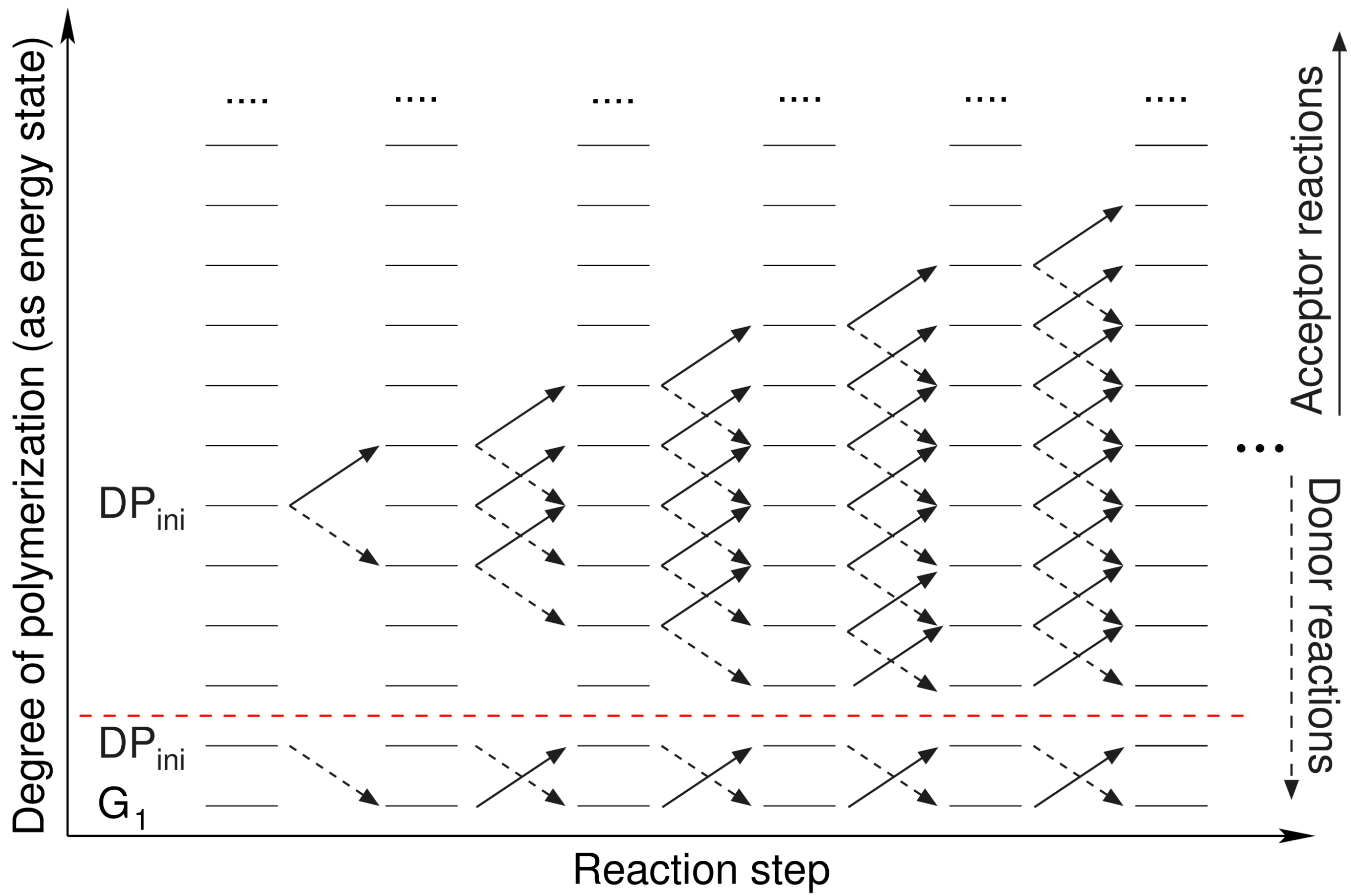
Theory is also confirmed by DPE2

DPE2 vs DPE1

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

<u>Generic reaction catalysed:</u>

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



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

with one additional side constraint

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

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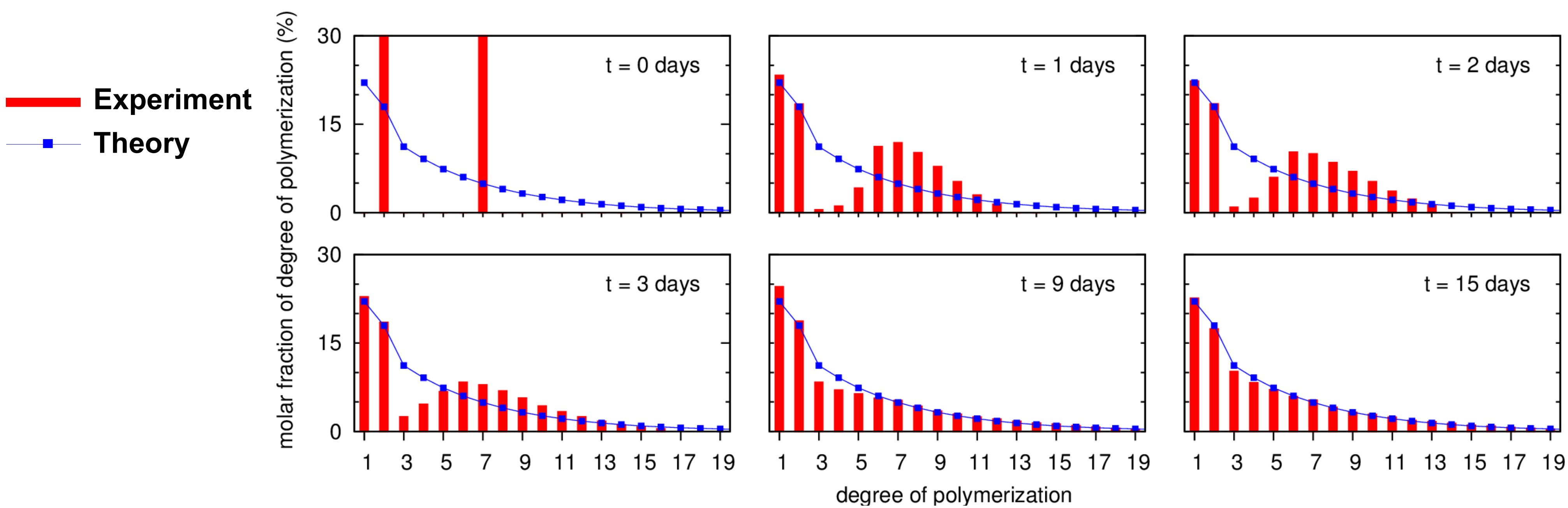

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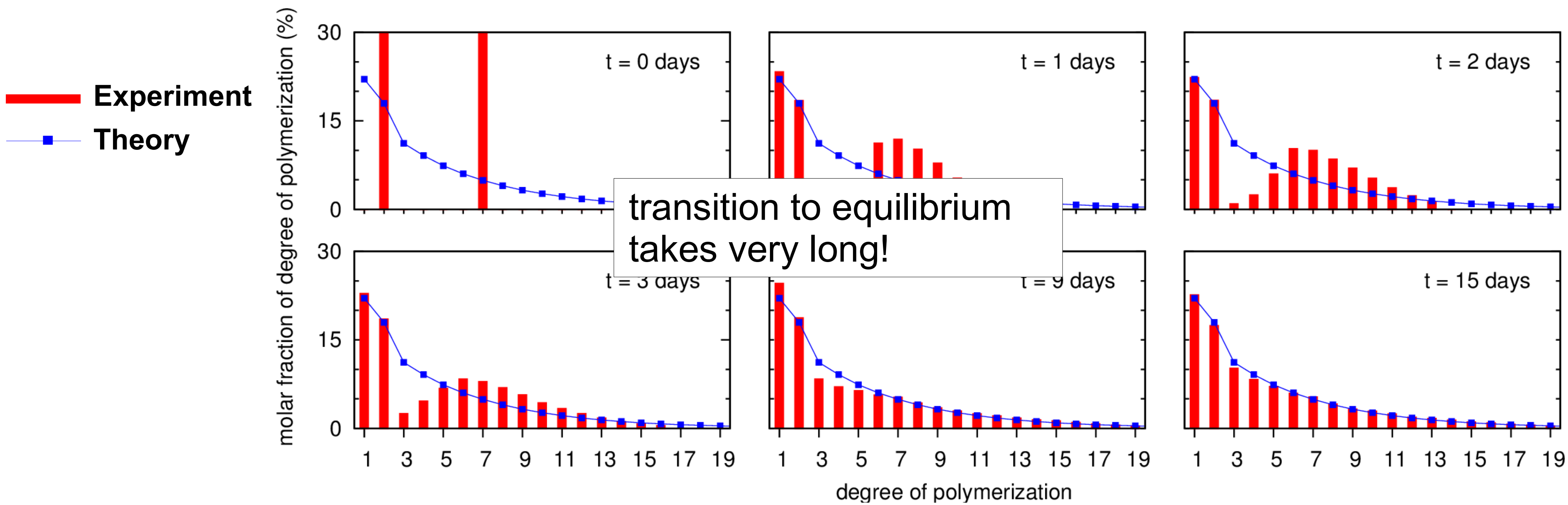


transition to equilibrium takes very long!

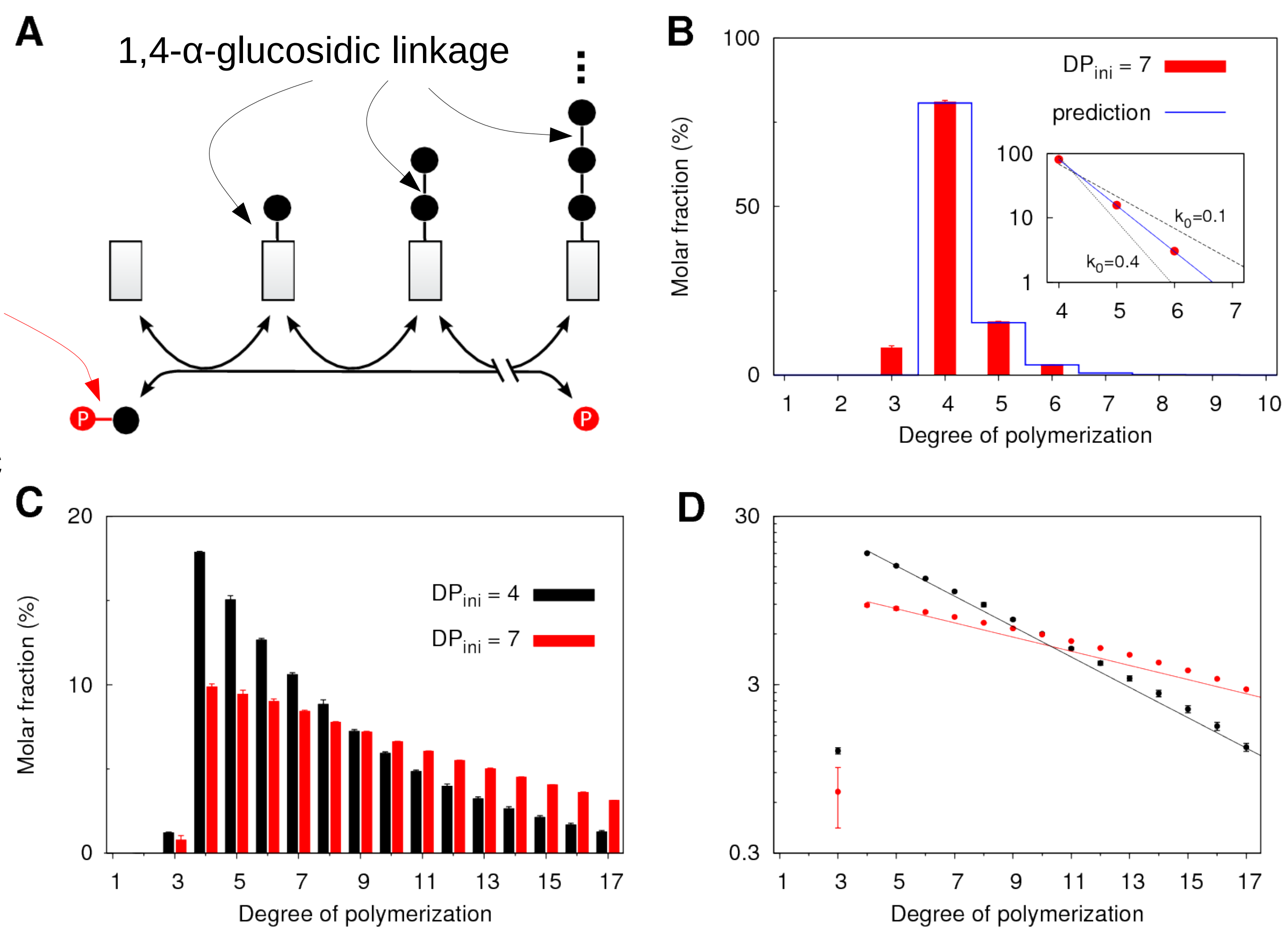
Generalisation to non-zero enthalpy changes

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:

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

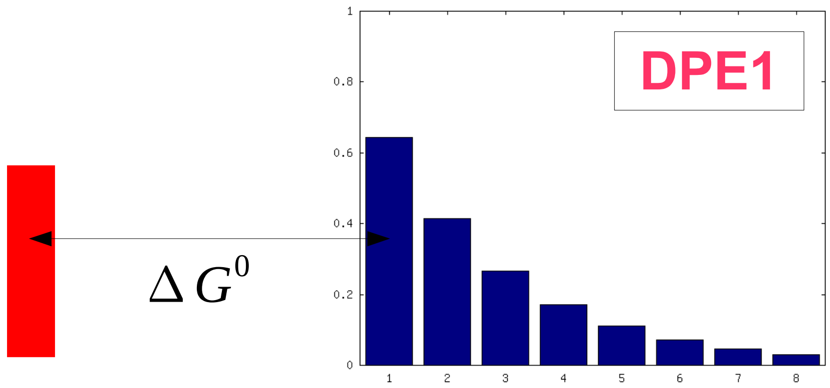
<u>Prediction</u>: Similar pattern as for DPE2

Experimentally confirmed.

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

The role of DPE1 in starch synthesis

During the day: no BAM activity, but GluEX is active



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- Bonds remain

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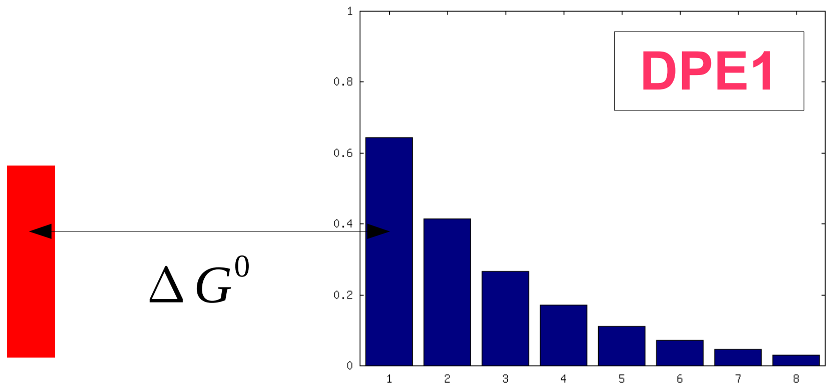

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Theoretical prediction:

equilibrium distribution is shifted to longer DPs! Stronger effect for larger energy differences.

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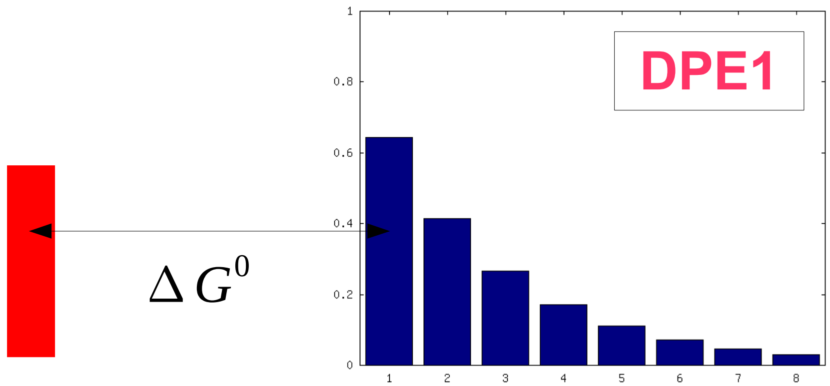

In vitro experiment: DPE1 + HXK

HXK: $\mathrm{ATP} + G_1 \longleftrightarrow \mathrm{ADP} + G6P$

Variation of [ATP] allows to control ΔG^0

$\mathrm{ATP}: a_3, \quad \mathrm{ADP}: a_2, \quad G6P: u, \quad G_k: x_k$

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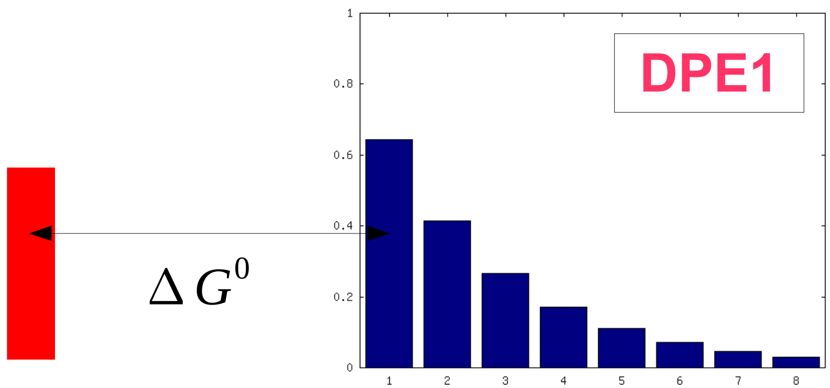
The role of DPE1 in starch synthesis

During the day: no BAM activity, but GluEX is active



In vitro experiment: DPE1 + HXK

HXK: $\mathrm{ATP} + G_1 \longleftrightarrow \mathrm{ADP} + G6P$

Variation of [ATP] allows to control ΔG^0

$\mathrm{ATP}: a_3,\ \ \mathrm{ADP}: a_2,\ \ G6P: u,\ \ G_k: x_k$

- Glucose residues are extracted from reaction mixture
- Bonds remain

Theoretical prediction:

equilibrium distribution is shifted to longer DPs! Stronger effect for larger energy differences.

Entropic principle:

$$G = u \cdot \Delta g - RT\left(a_2 \ln a_2 + a_3 \ln a_3 + u \ln u + \sum x_k \ln x_k\right) \rightarrow \min!$$

with 4 constraints:

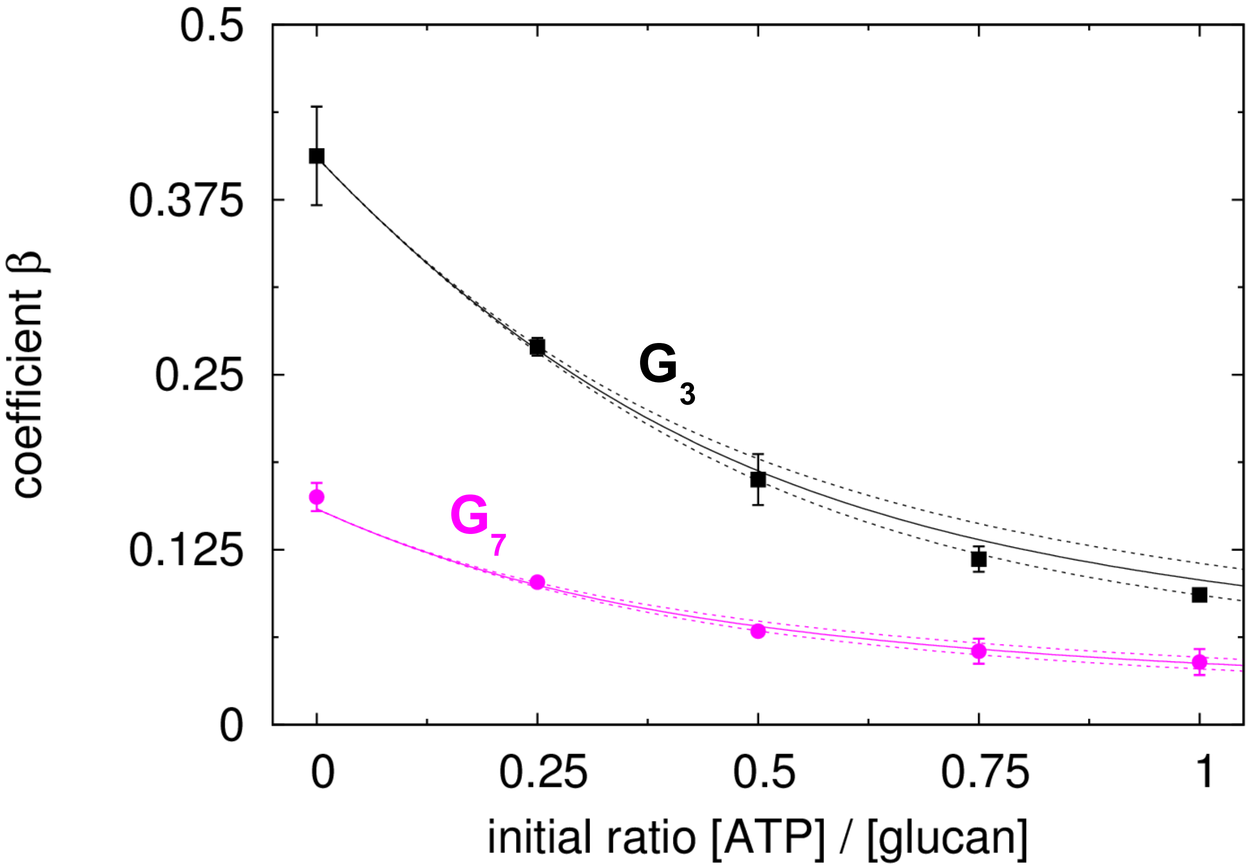
1) #molecules: $a_2 + a_3 + u + \sum x_k = 1$

2) #bonds: $\sum k \cdot x_k = b$

3) #adenine: $a_2 + a_3 = A$

4) ADP/G6P: $a_2 - u = B$

Experimental results

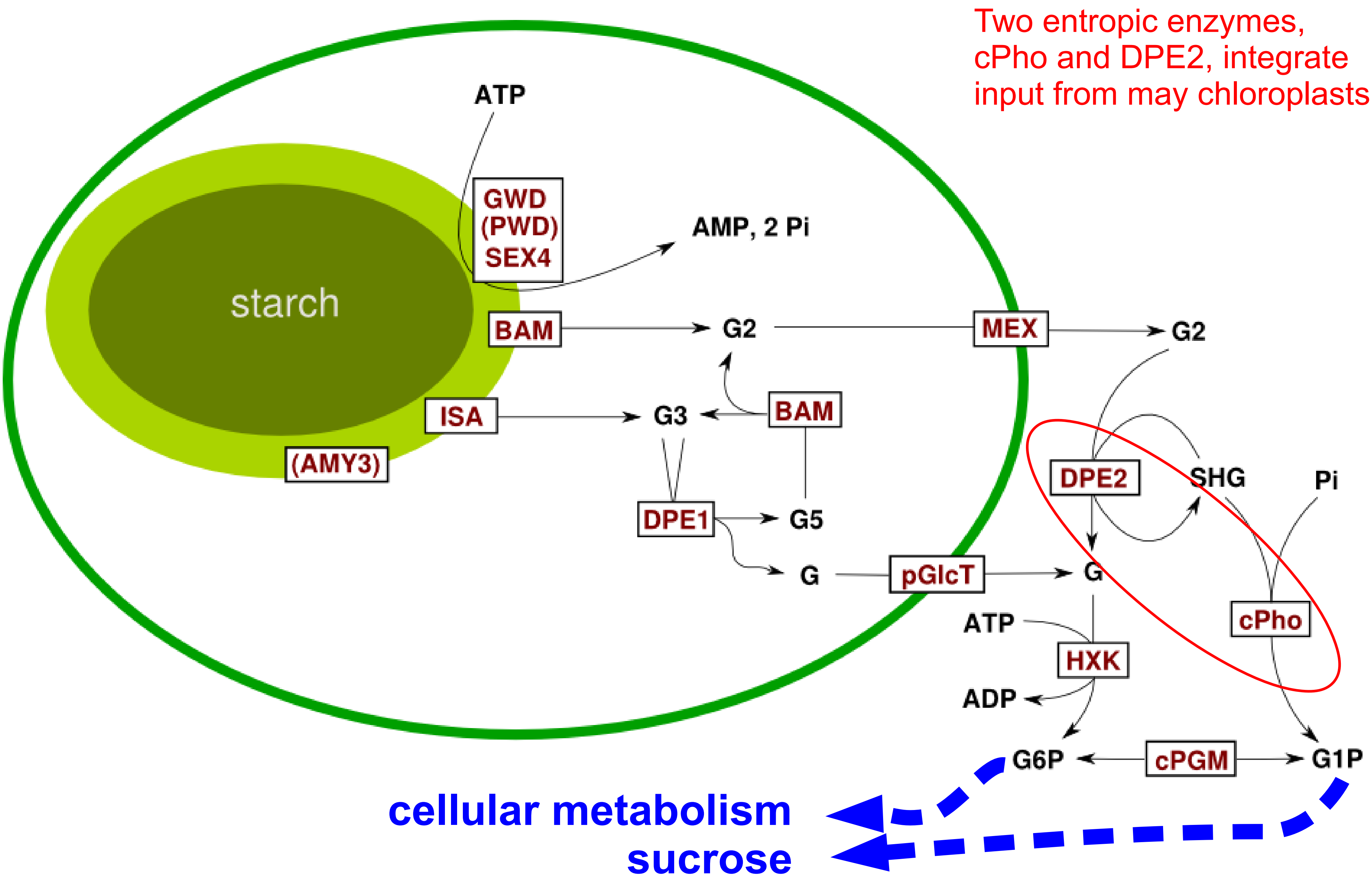


Theoretical prediction confirmed:

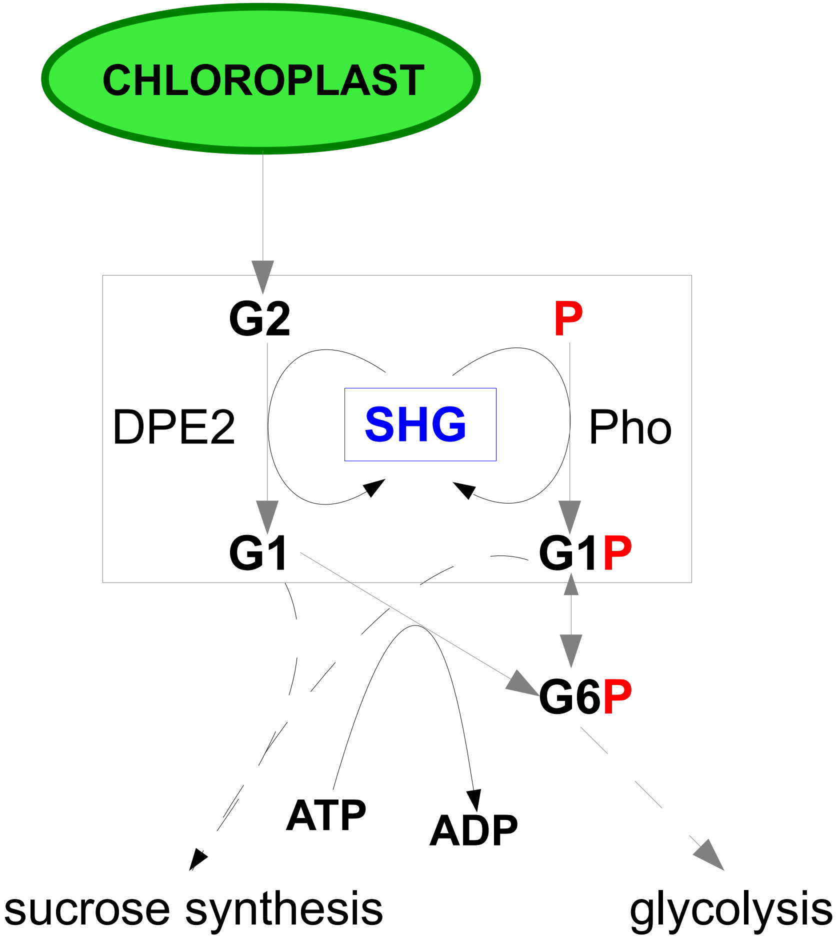
equilibrium distribution is shifted to longer DPs!

<u>Hypothesis</u>: DPE1 plays a role in starch synthesis. Entropic forces are exploited to provide 'primer' glucans.
<u>Hint</u>: *Chlamydomonas* mutants deficient in DPE1 are starchless!

An entropy-driven buffer



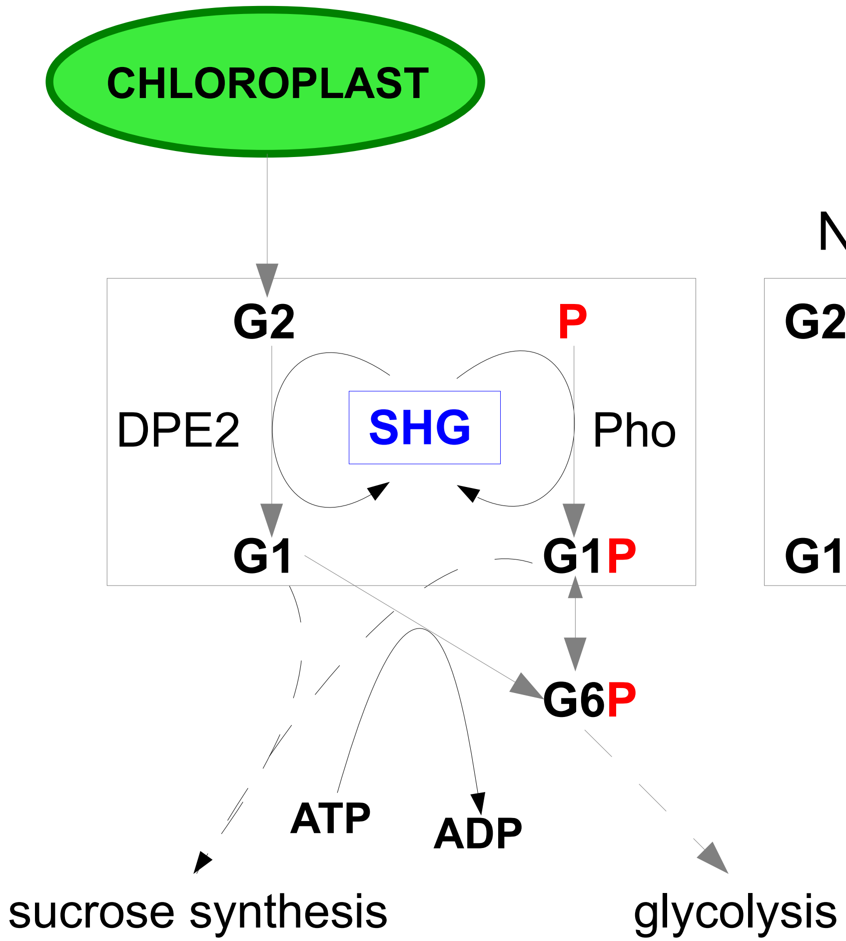
What is the role of the SHG pool?



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

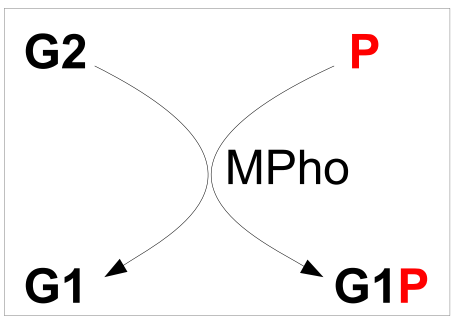
What is the advantage over other hypothetical systems?

What is the role of the SHG pool?

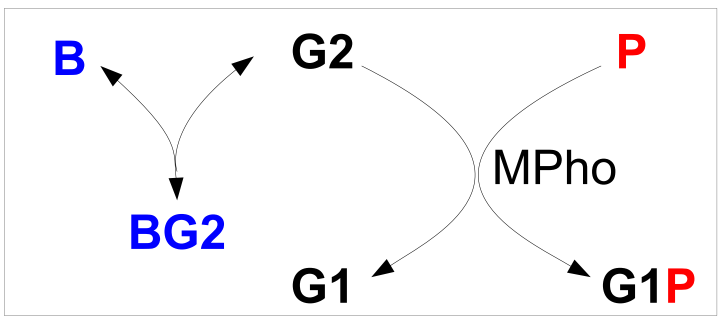


Comparison with two alternatives

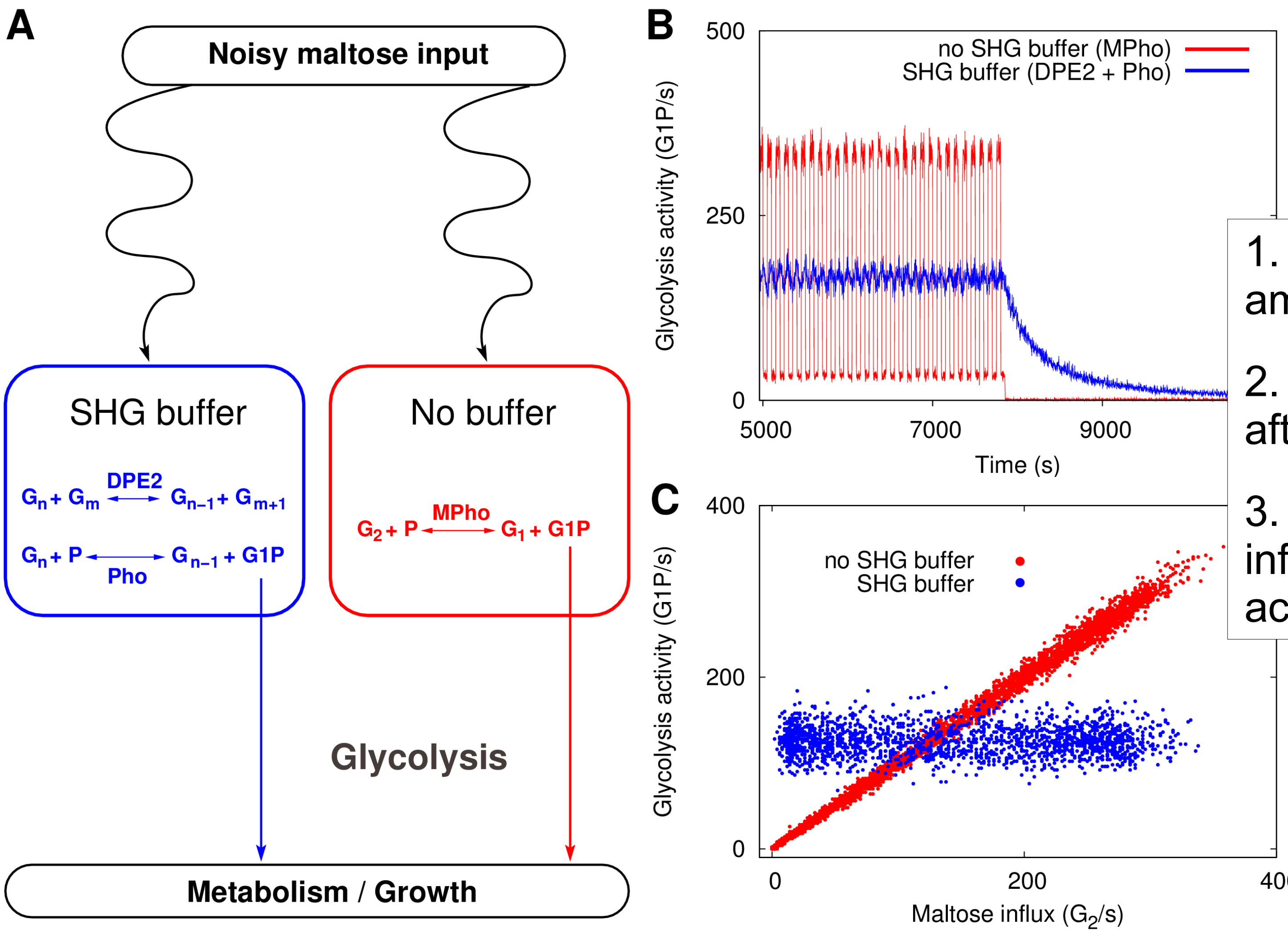
No buffer



Monodisperse buffer



Polydisperse SHG pools increases robustness *in vivo*

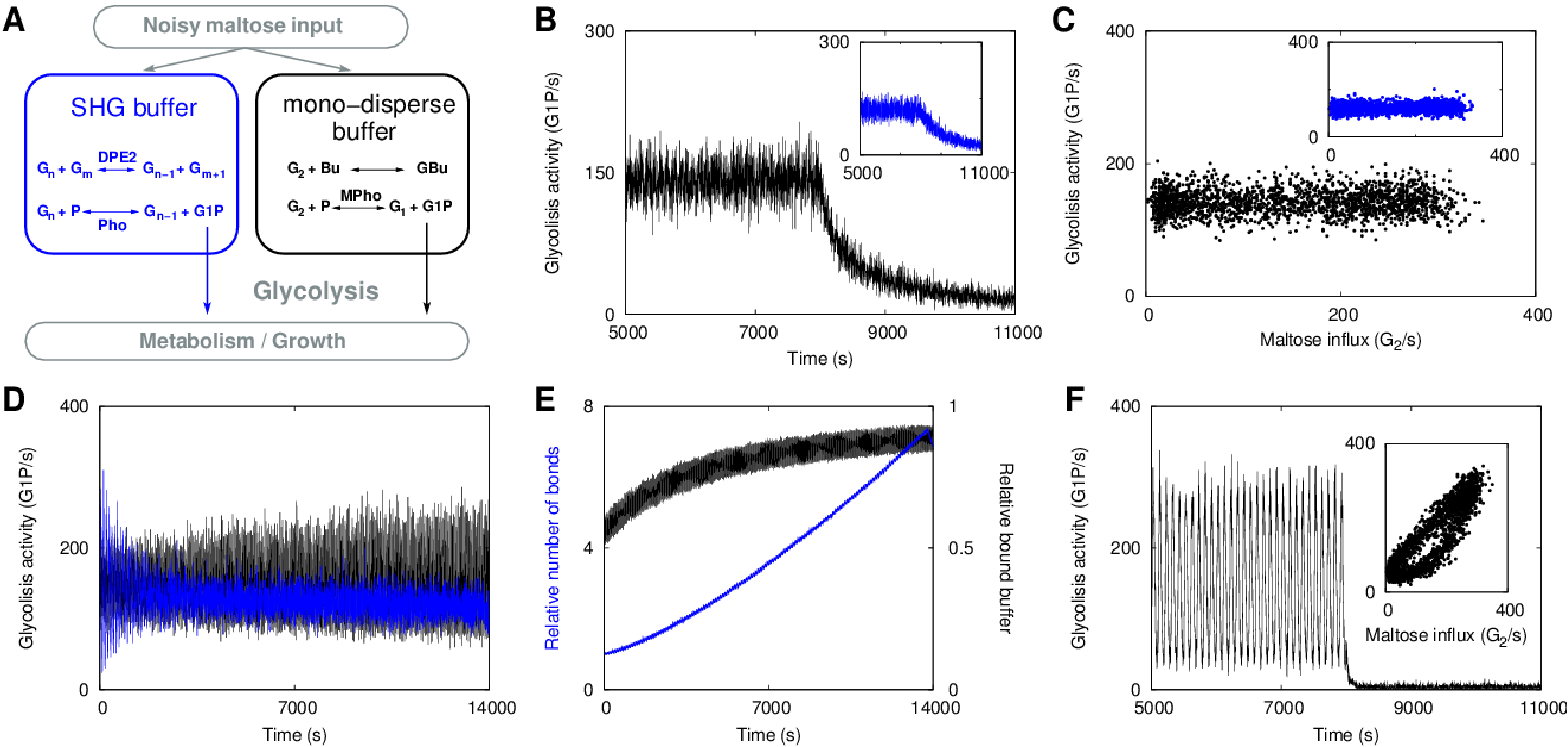


1. Attenuation of fluctuation amplitude (low-pass filter)

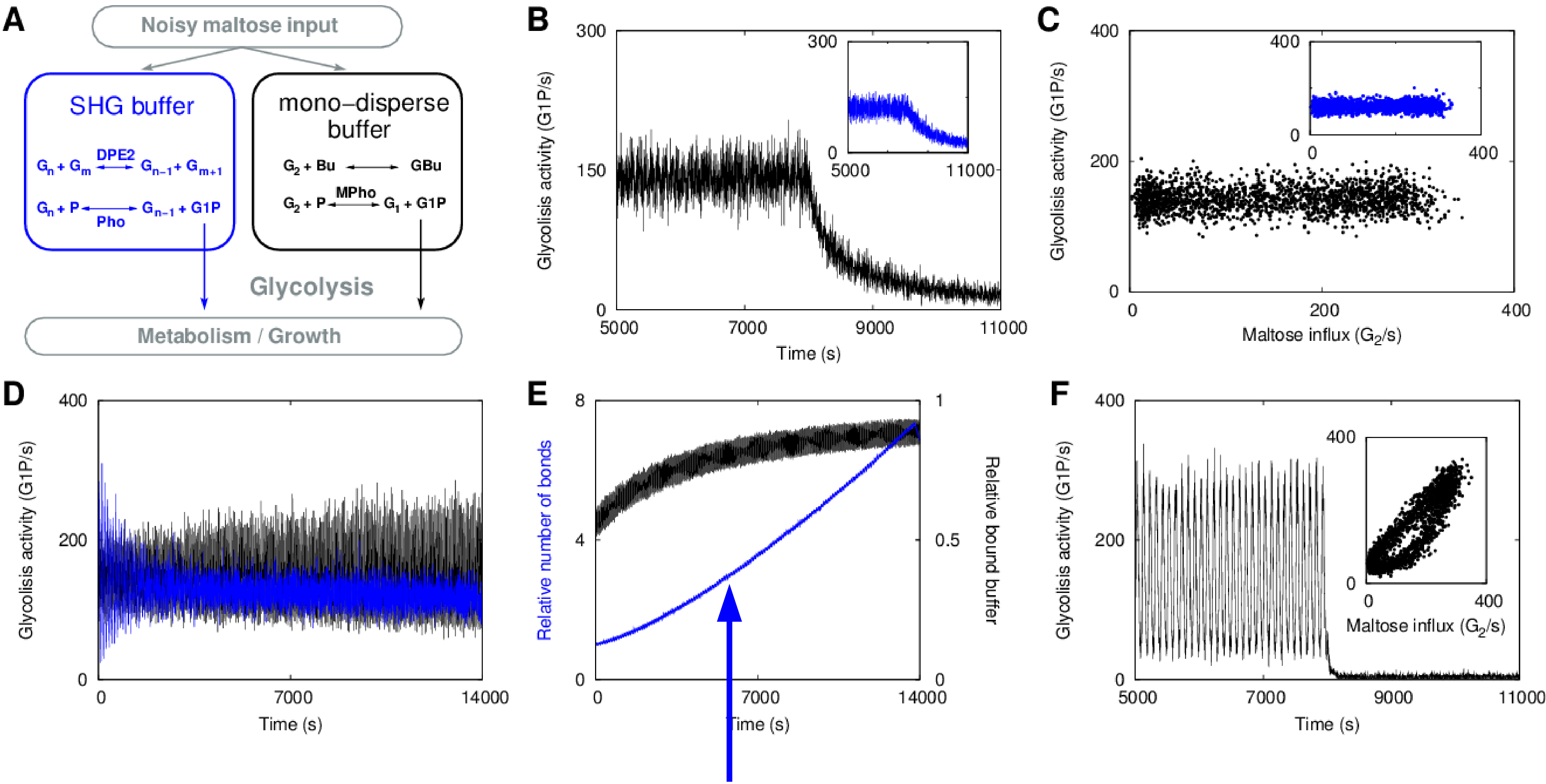
2. Transient support of activity after drop of maltose influx

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

Comparison to monodisperse buffer



Comparison to monodisperse buffer

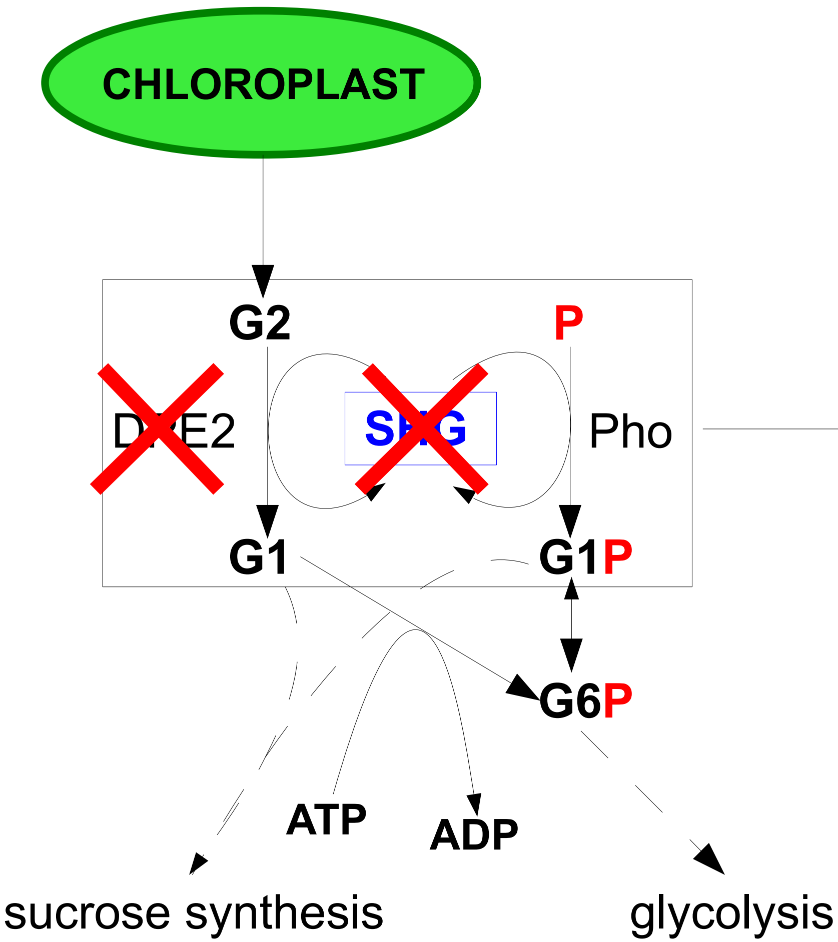


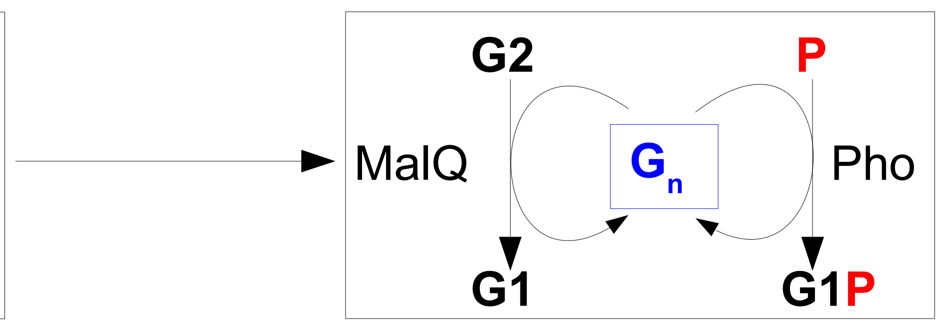
auto-adaptation of buffer size!

Replacing DPE2 by MalQ

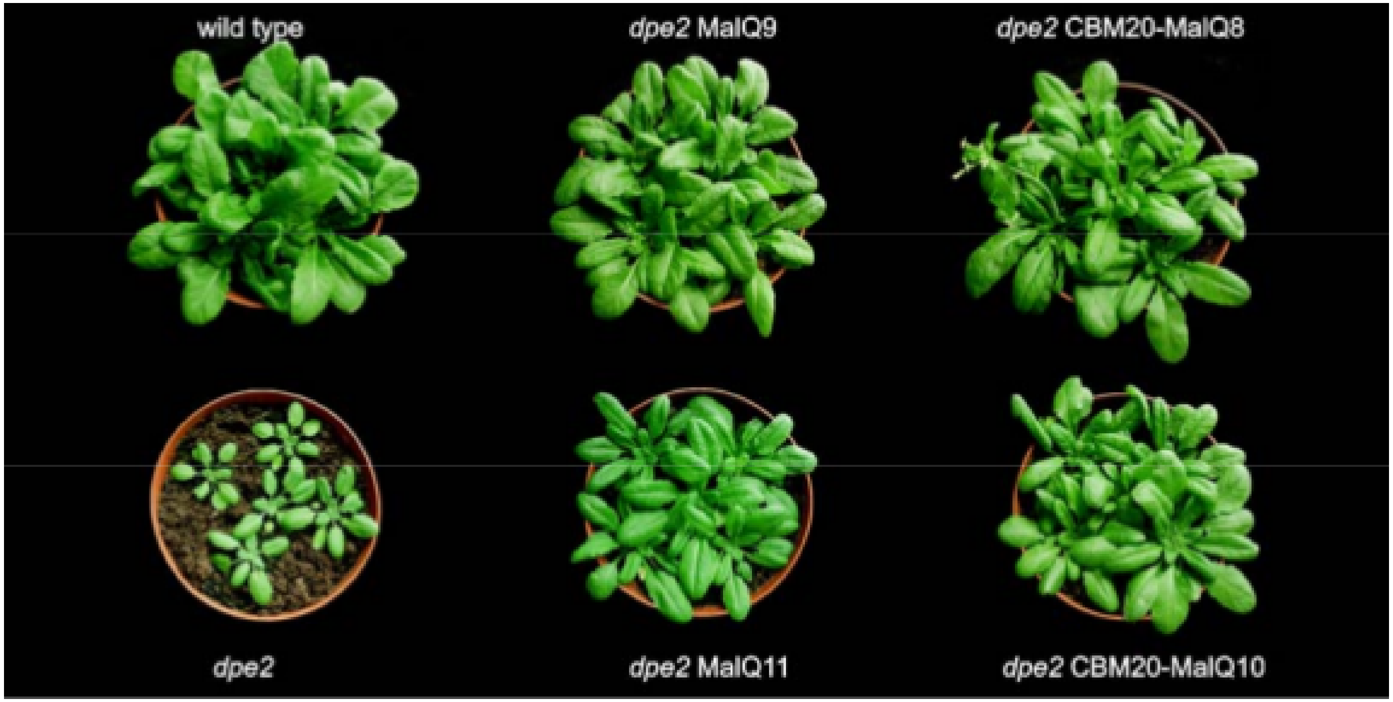
MalQ does the same as DPE2, but does not use SHG

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





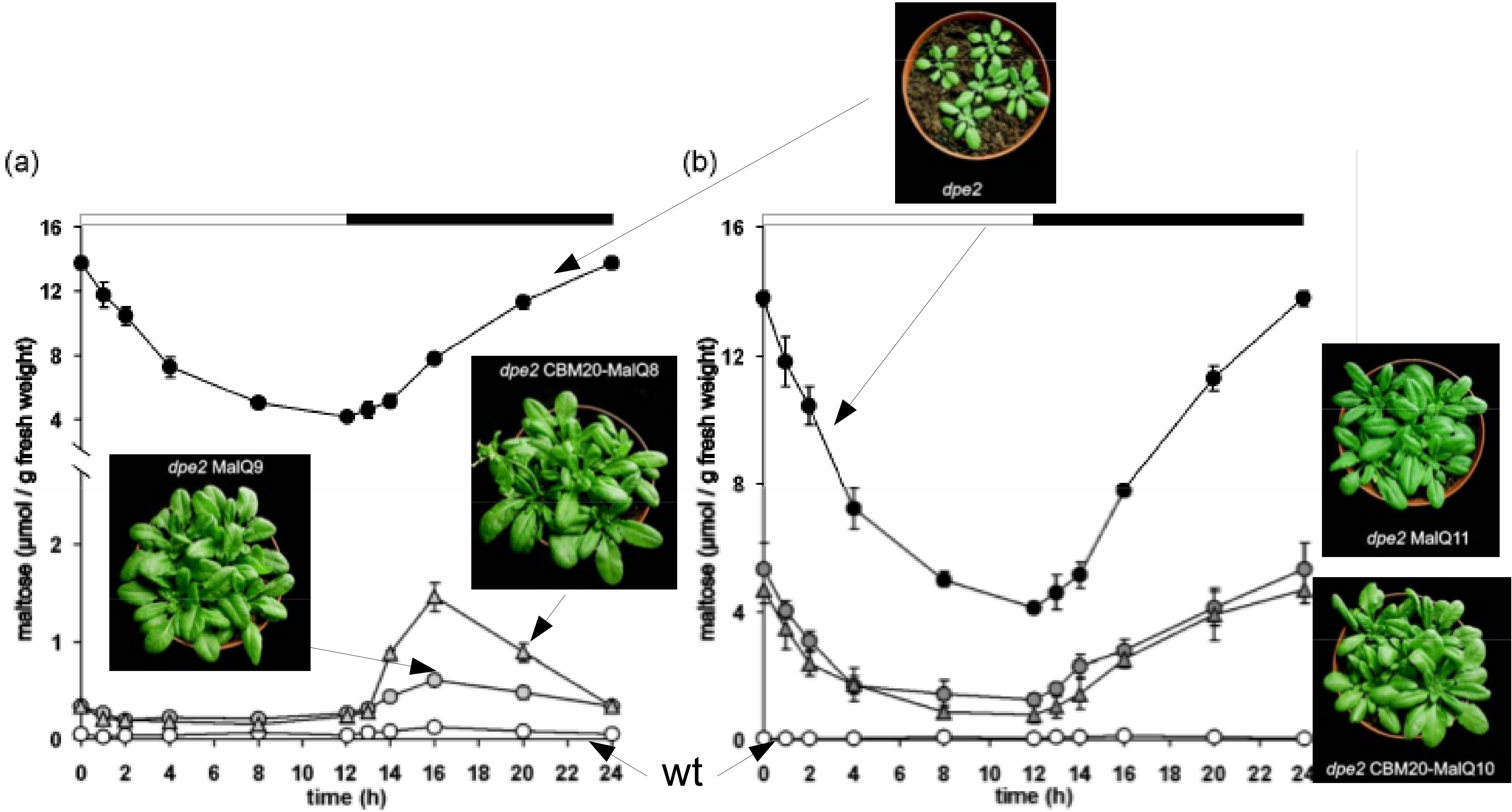
Moderate growth phenotype



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

complemented plants grow OK!

Maltose turnover

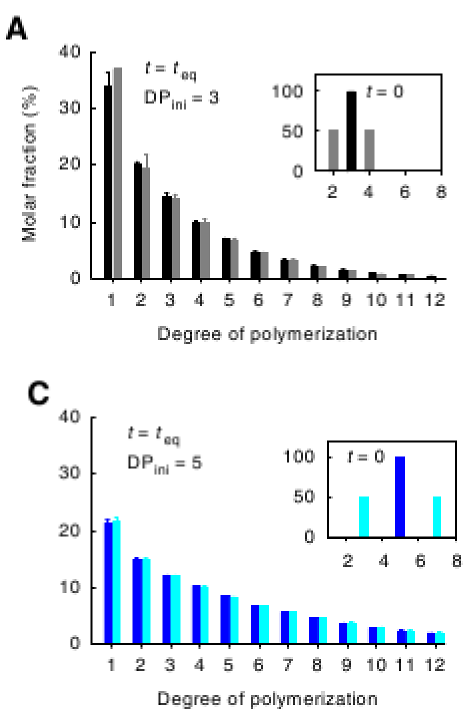


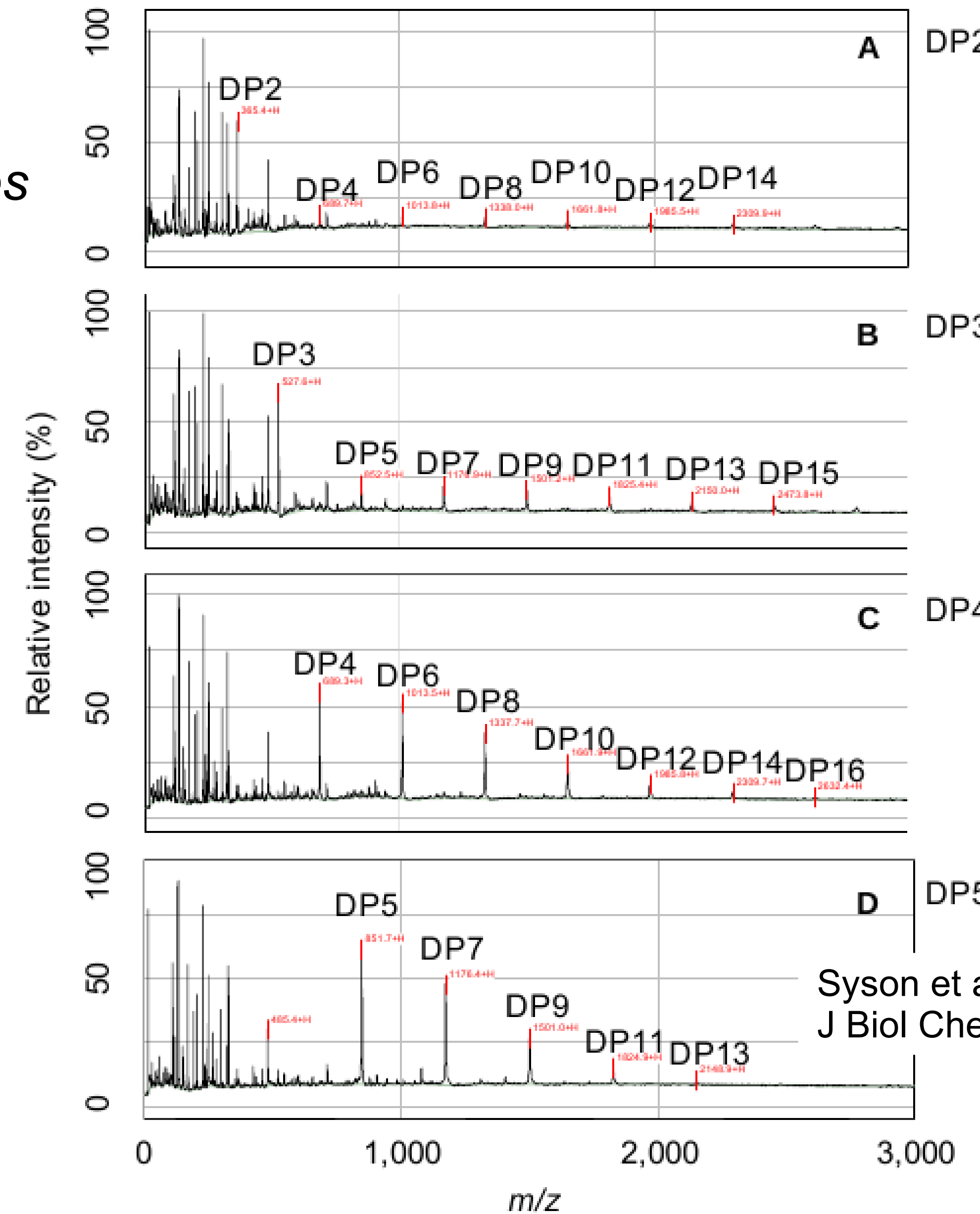
Where else do find entropic enzymes?

...for example

Maltosyltransferases in *Streptomyces*

"Acceptor specificity" can be explained by entropic principles





Syson et al, 2011, J Biol Chem

Food for thoughts

It appears that metabolism is organised as an interplay of 'entropic' and 'energetic' enzymes

- Why?
- Are there principles behind this organisation?
- How is this connected to resource allocation?

Postdoc wanted!

ERA-CAPS Project (ETH, JIC, HHU) : DesignStarch

Goal: To synthetically produce starch in yeast!

Postdoc: Tasks

- Simulate polymer systems and starch biogenisis
- Build theories

Profile

- Statistical physics
- Thermodynamics
- Programming Skills