CEPLAS
Cluster of Excellence on Plant Sciences

HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

Entropy in Metabolism and the Emergence of Complex Structures

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QTB Institut für Quantitative & Theoretische Biologie

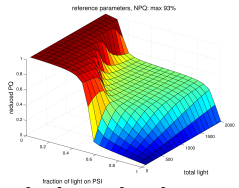
Luxembourg, 16.2.2017

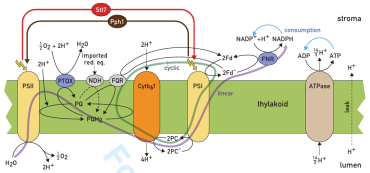
Overview: Ongoing research

AccliPhot

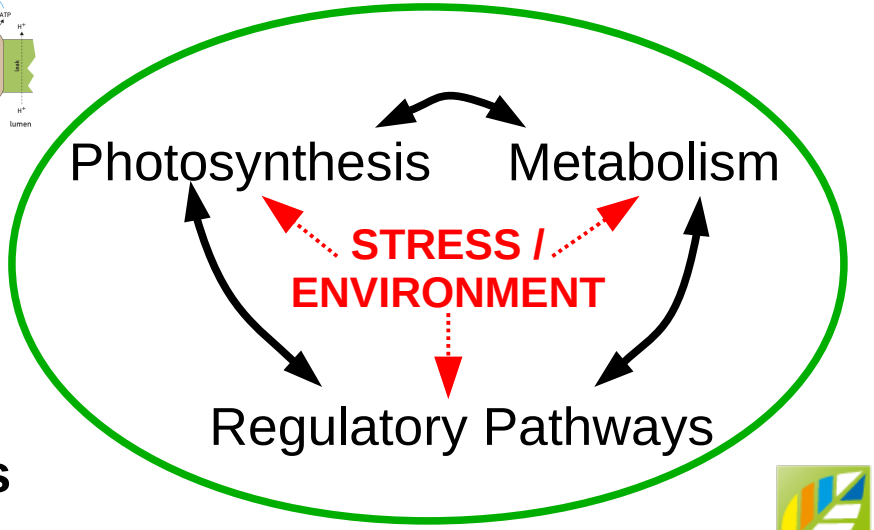
Photosynthetic Acclimation

- Understand the regulation of photosynthesis
- Nonphotochemical quenching, state transitions



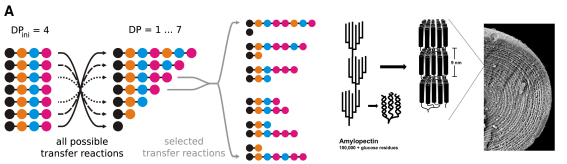


(Ebenhöh et al, 2011;
Ebenhöh et al, 2014;
Matuszyńska et al, 2015)



Designing Starch ERA-CAPS

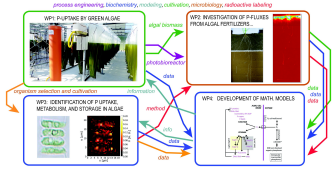
- Explain polymer biochemistry with statistical thermodynamics
- Understand the formation of a starch granule



(Kartal et al, 2011;
Ebenhöh et al, 2013;
Ruzanski et al, 2013)

BioSC
Bioeconomy Science Center

Algal Fertilizers



- Understand phosphate uptake and storage metabolism in plants and algae
- Use algae to extract P from waste-water and apply as fertilizer to soil

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Secondary metabolism

- Understand what controls the diversity of secondary metabolite structures
- Glucosinolates
- Fatty acids / designer oils

Algal Oils by Design

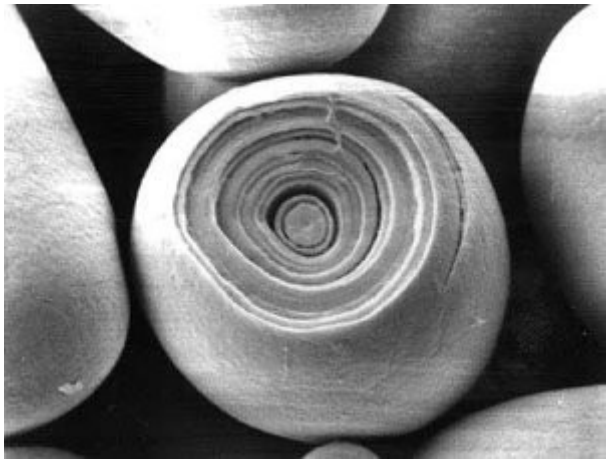
BBSRC

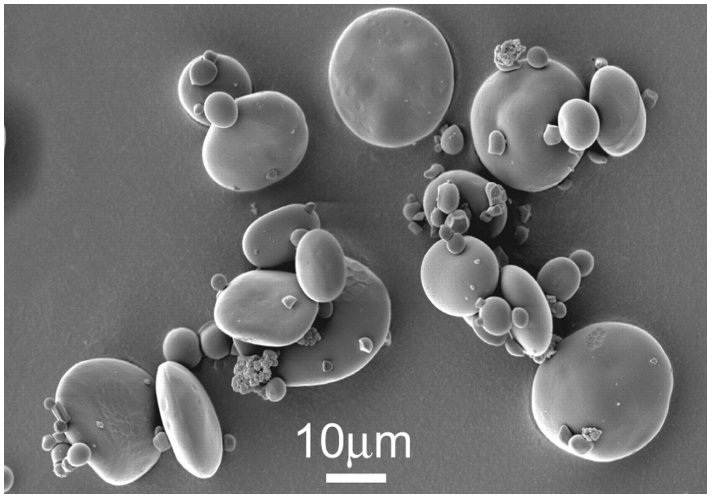
Starch – half the caloric uptake of humanity

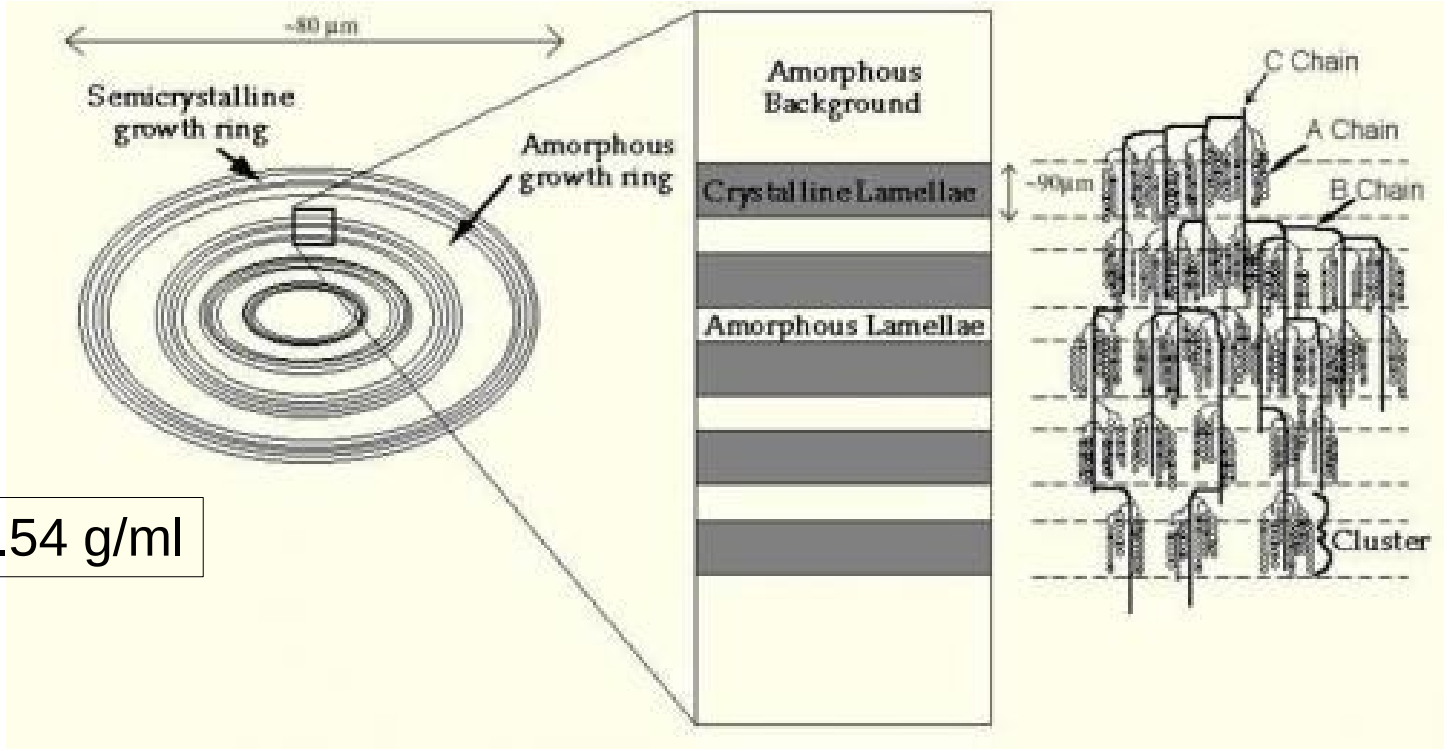
pictures from:

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)

Why starch?







Density: 1.54 g/ml

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

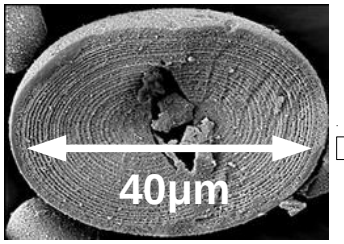
Alternatives

	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)

still small compared to starch



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

Possible advantages of starch

- low osmolarity
- large size
- high density

Alternatives

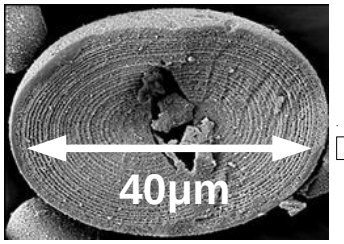
	energy content (kJ/g)
Carbohydrates	17
Lipids	38
Proteins	17
Alcohol	30

We (animals and fungi) predominantly use glycogen

trade-off between storage density and rapid mobilization

big molecule (up to 10 MDa)

still small compared to starch



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

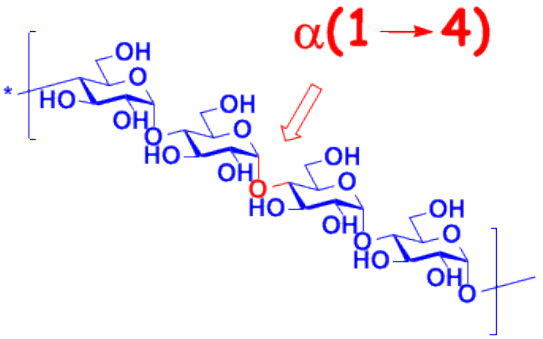
optimised for storage density, slower deployment

Possible advantages of starch

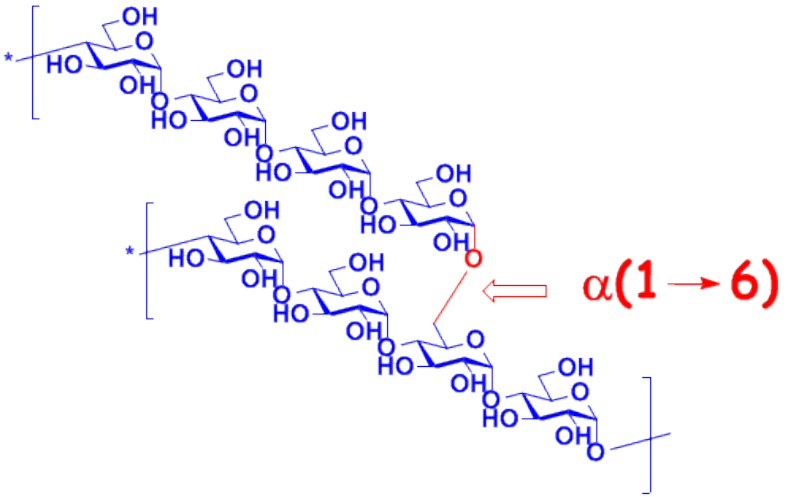
- low osmolarity
- large size
- high density

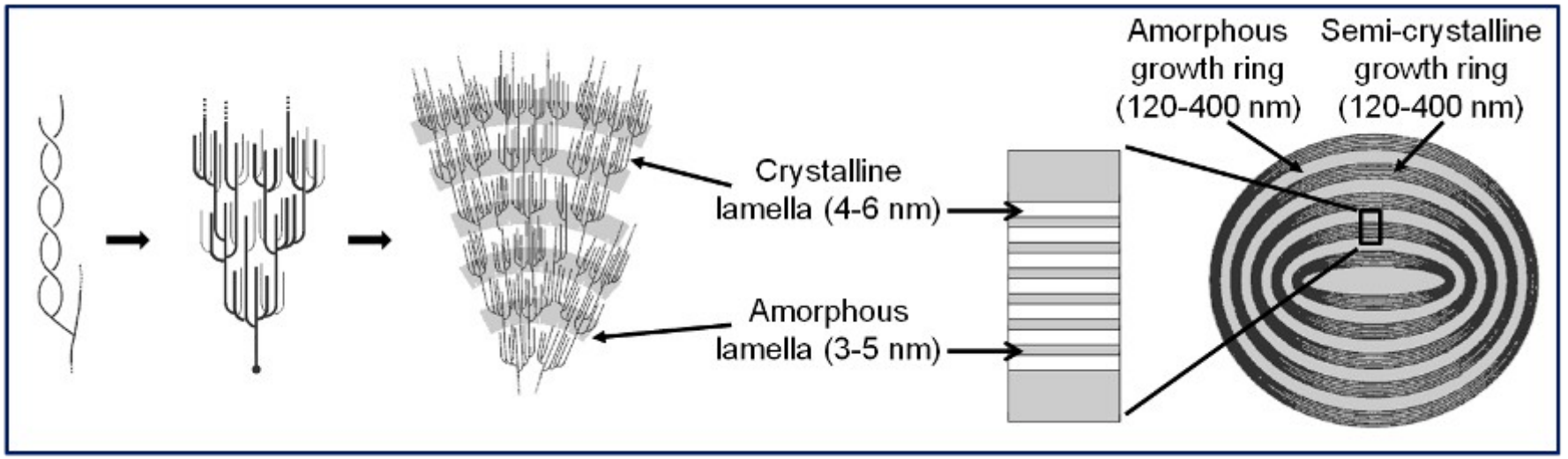
The structure of a starch granule

Amylose
(MW 32,000-113,000)



Amylopectin
(MW 10^7-10^9)

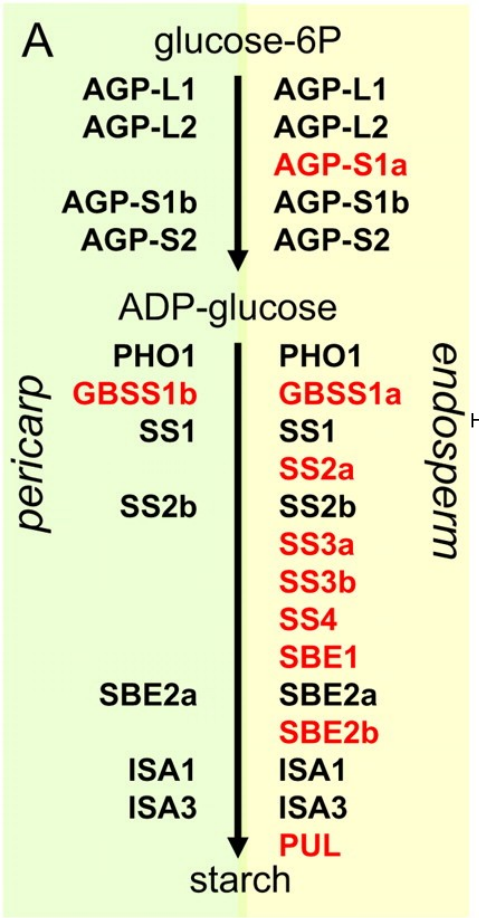




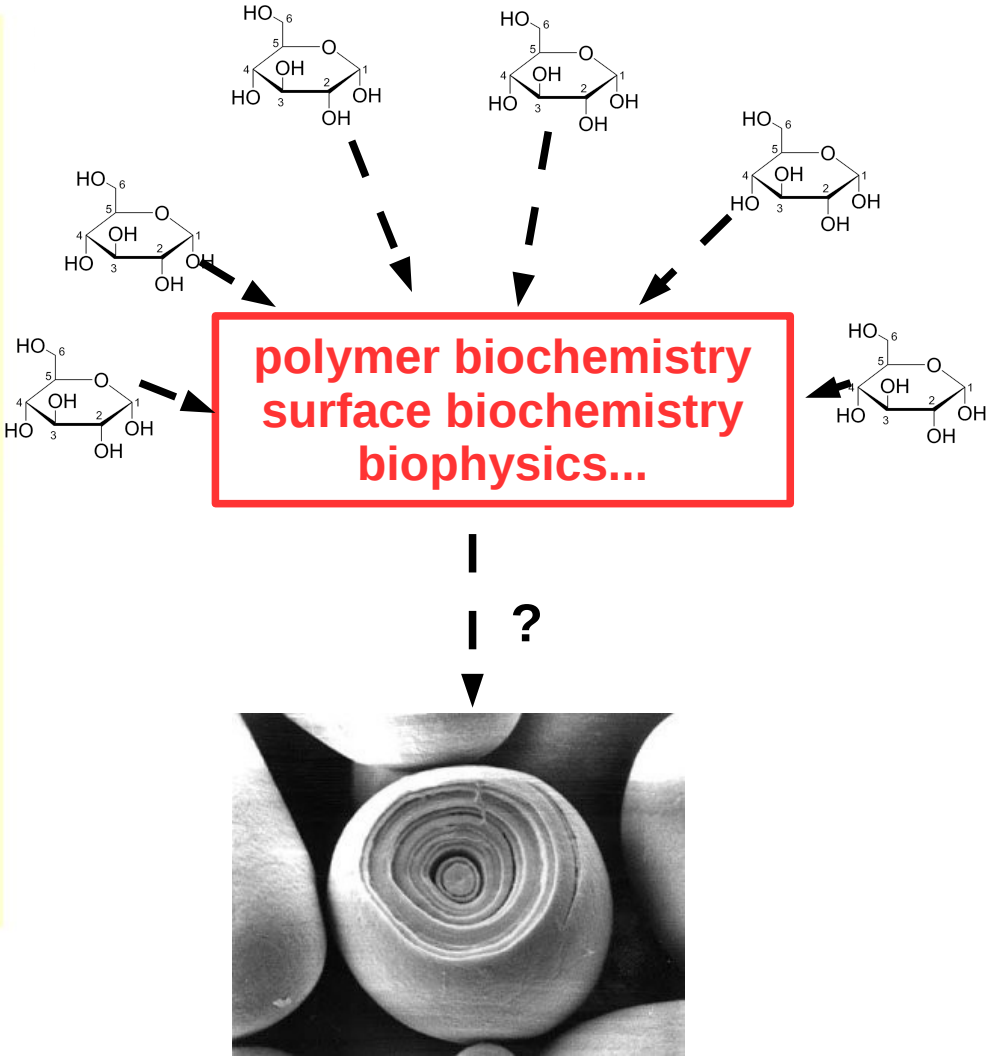
Wouldn't it be great...

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

But how do all these factors actually play together?



(Radchuk et al, 2009)



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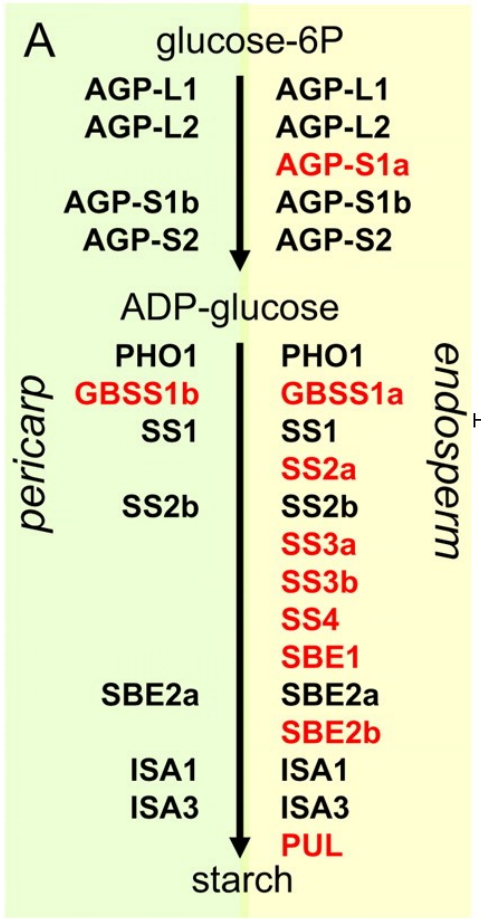
Most of the involved enzymes have homologs in glycogen metabolism!



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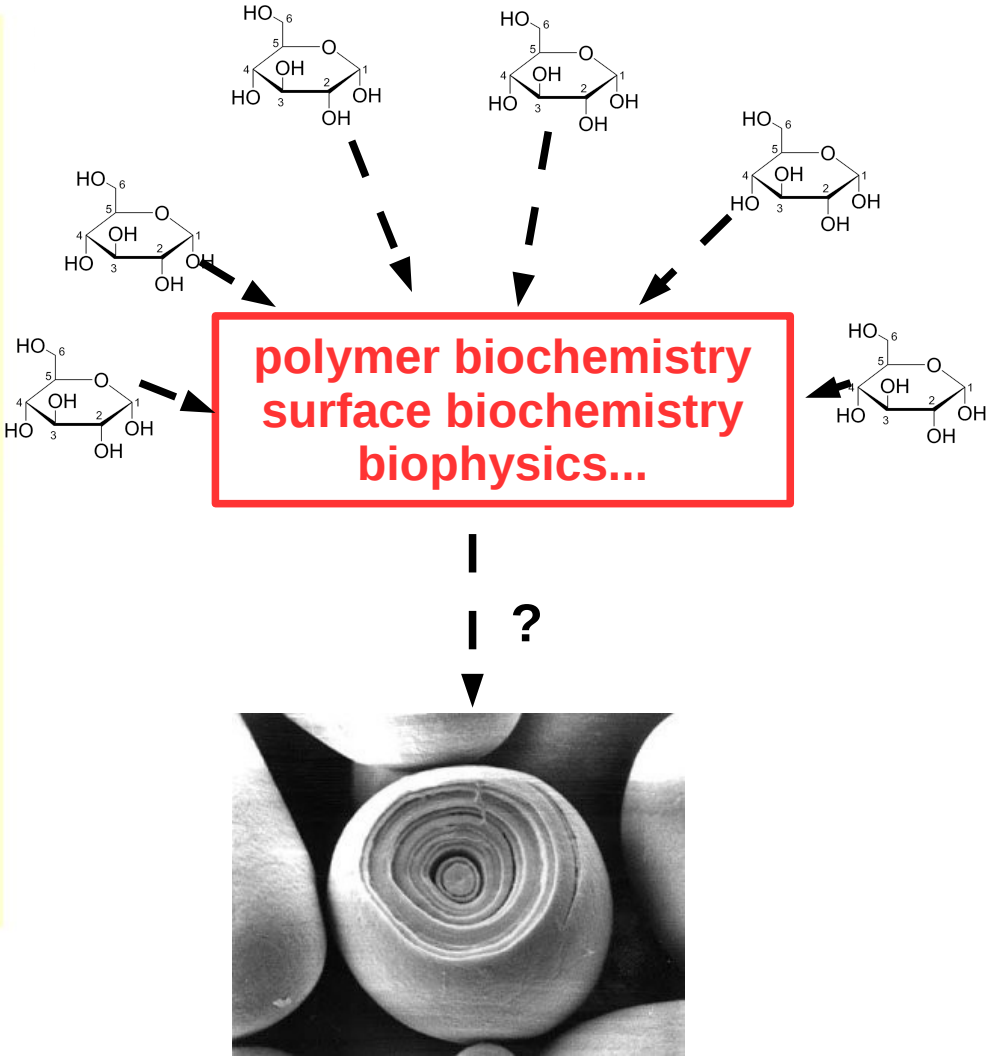
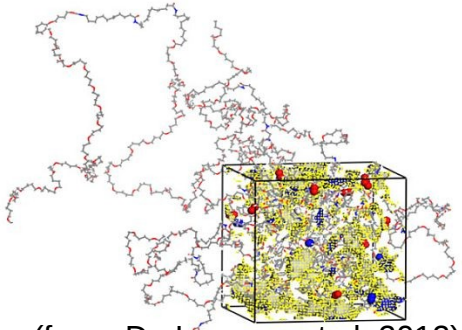


A classical physics problem

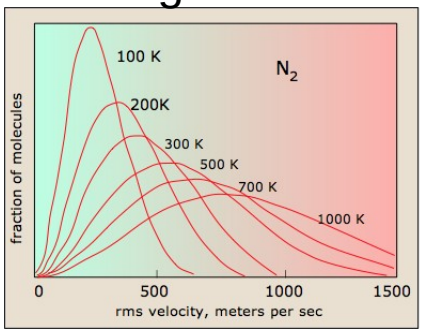
PHYSICS

molecular interactions



(from: De Lorenzo et al, 2012)

gas



(from: chemwiki.udavies.edu)

collective behaviour

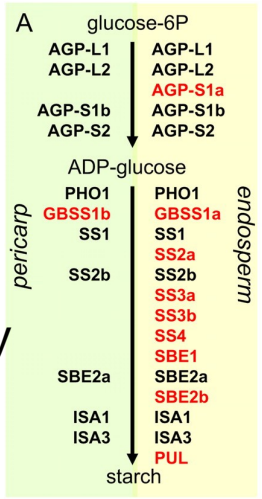
- pressure
- temperature

microscopic

macroscopic scale

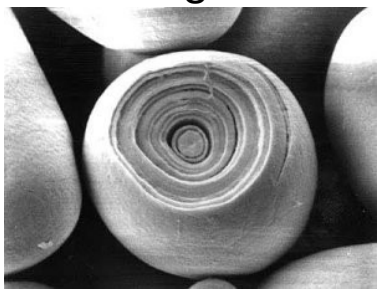
BIOLOGY

enzymes
surface physics
polymer chemistry



(from: Radchuk et al, 2009)

starch granule



(from: braukaiser.com)

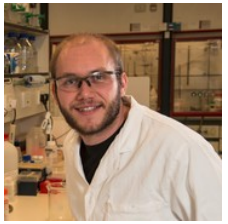
- chain length distr.
- branching pattern
- glycaemic index

TOP-DOWN OR BOTTOM-UP?

DesignStarch

ERA-CAPS

ERA-NET for Coordinating Action in Plant Sciences

Rob Field, Michael Rugen (JIC Norwich)

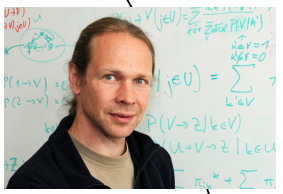
Biochemistry **WP1**
Isolation/characterisation of parts
Artificial, controlled in vitro systems

provides parts

informs

informs
parameterises

provides parts




Theory **WP4**
Mathematical models
Theoretical concepts
Computational predictions
Understanding of emergent properties

improves

guides
predicts

improves

informs
novel interpretations of results


Synthetic **WP2**
Expression of enzymes in yeast
Reconstitution of artificial systems

Systemic **WP3**
Genetic modifications in planta
Phenotypic characterisation

Sam Zeeman, Barbara Pfister (ETH Zurich)

Oliver Ebenhöh, Adélaïde Raguin (HHU Düsseldorf)

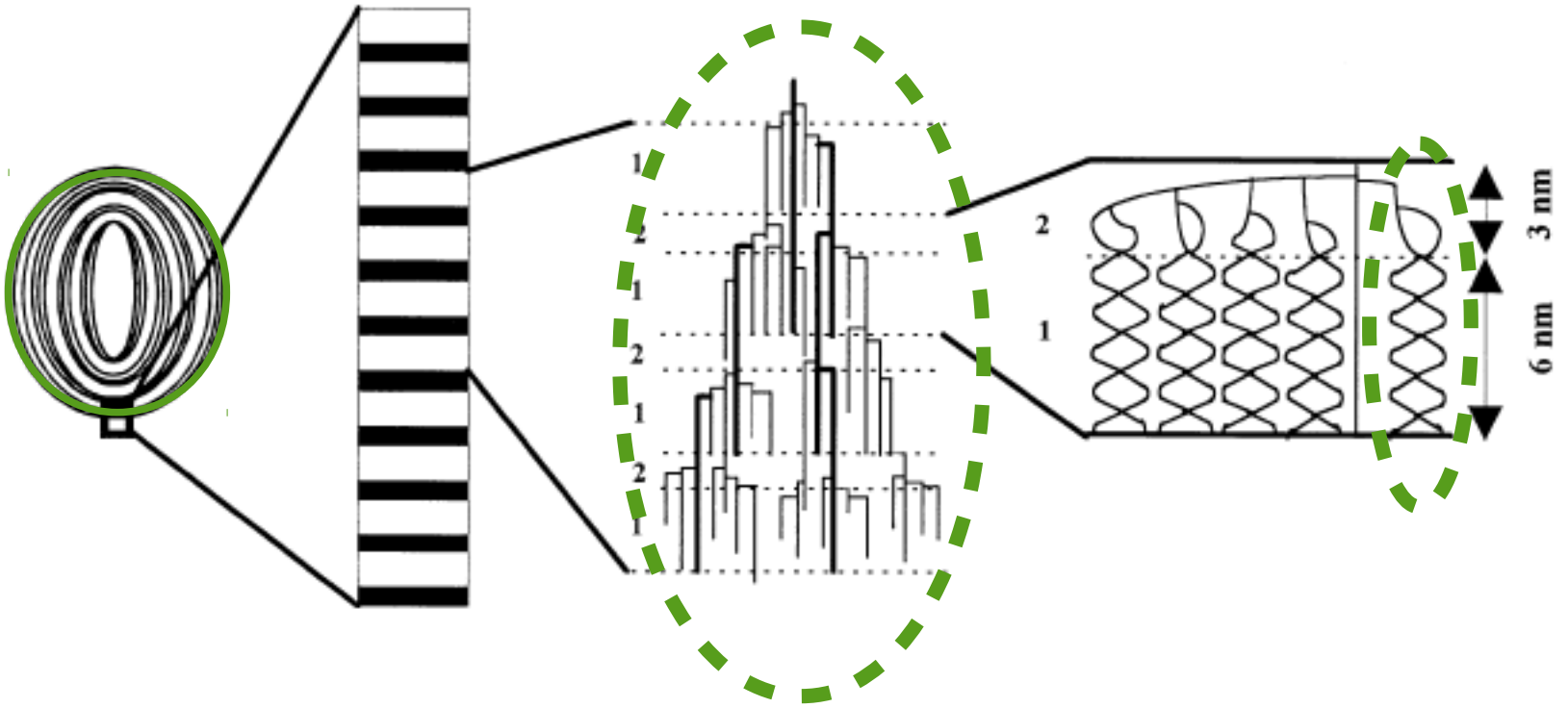
Starch metabolism: ingredients

A unique molecule

Ordered part

As a 2D tree

Double helices



Genealogy of the tree (mother-daugther connections)

Steven Ball *et al.* Cell 96

Starch metabolism: ingredients

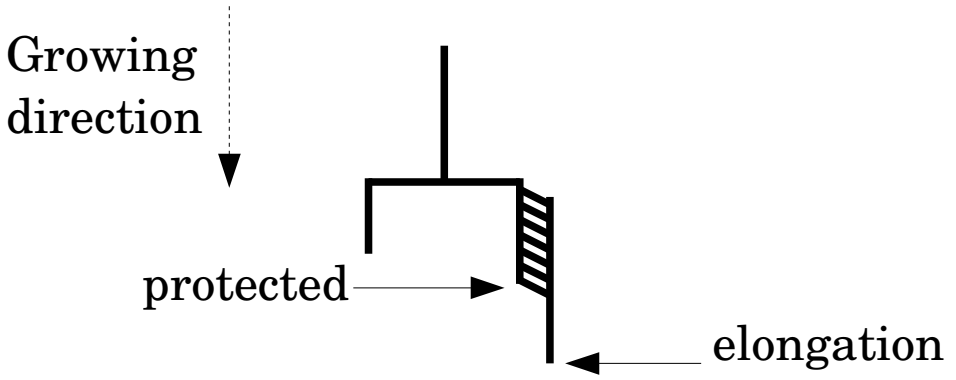
The main reactions

Elongation α-1,4 ⟶ α-1,4 (+1)

Branching (cut & re-branch) α-1,4 ⟶ α-1,6

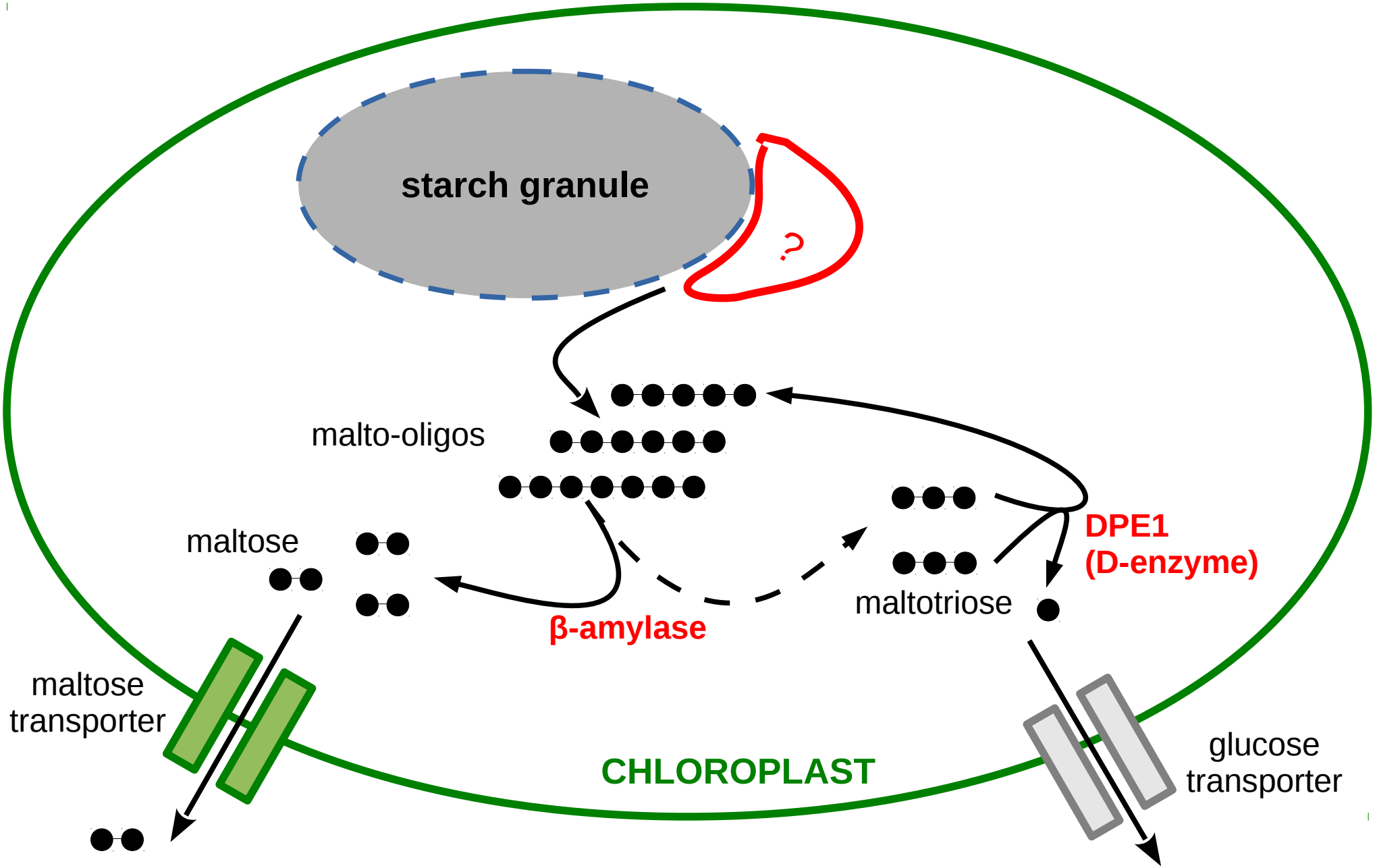
Debranching α-1,6 ⟶ Ø

Double helix formation



We need detailed kinetic descriptions of all involved enzymatic and biophysical processes!

Starch metabolism bottom-up



Disproportionating enzymes (D-enzymes)

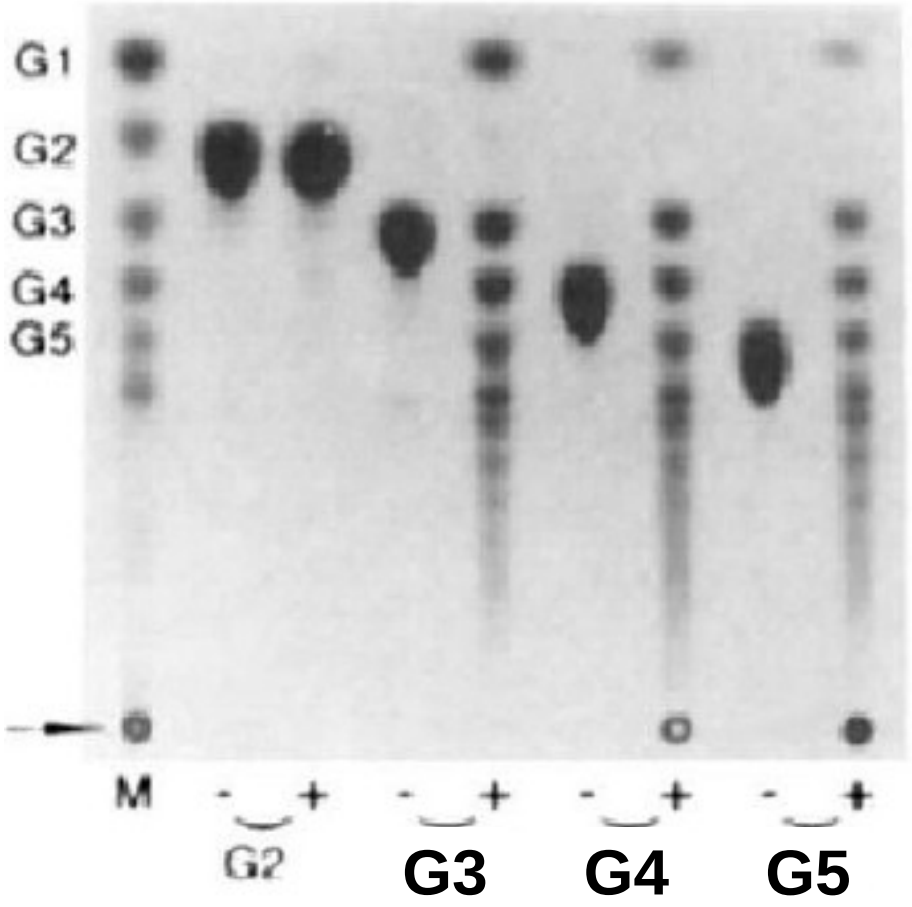
DPE1

EC: 2.4.1.25

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

$G3 + G3 \leftrightarrow G5 + G1$

but not only!



(Takaha et al., JBC 1993)

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

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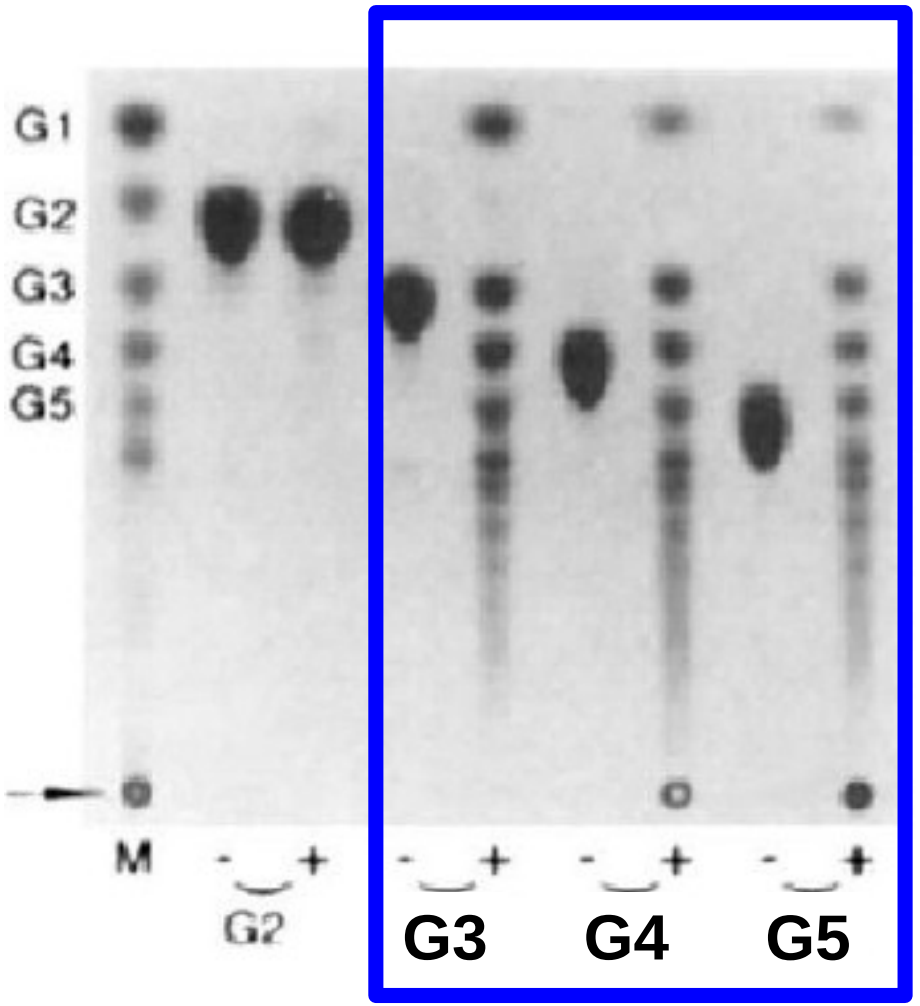

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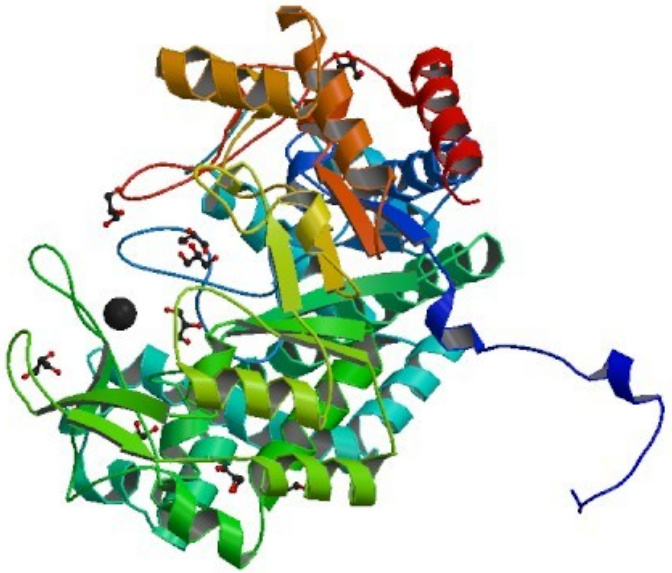
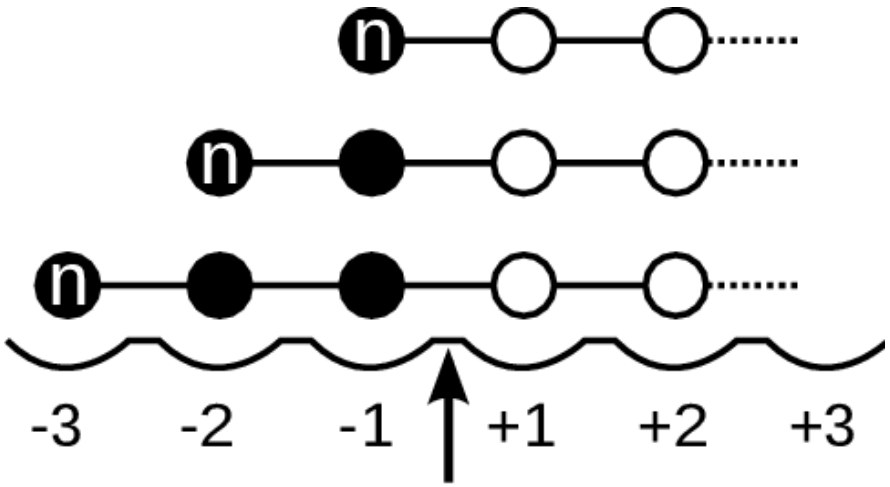
DPE1 produces a set of glucans of different length in *in vitro* assays.

Equilibrium distribution depends on initial conditions!

K_{eq} ???

Positional Isomers





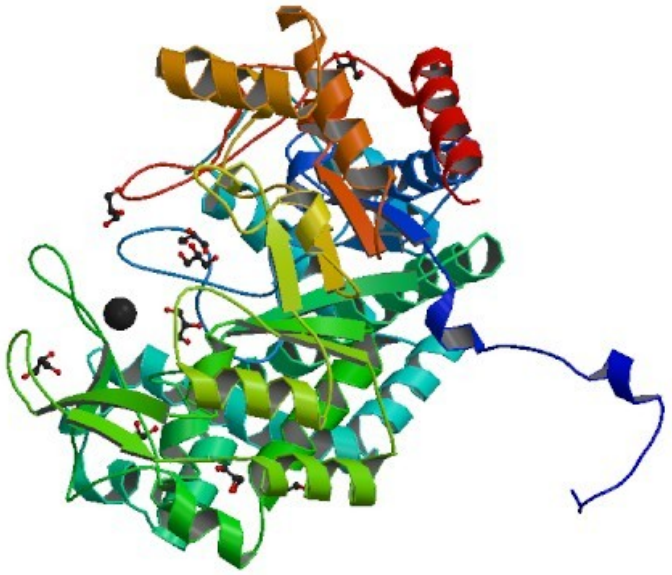
D-Enzyme Subsite

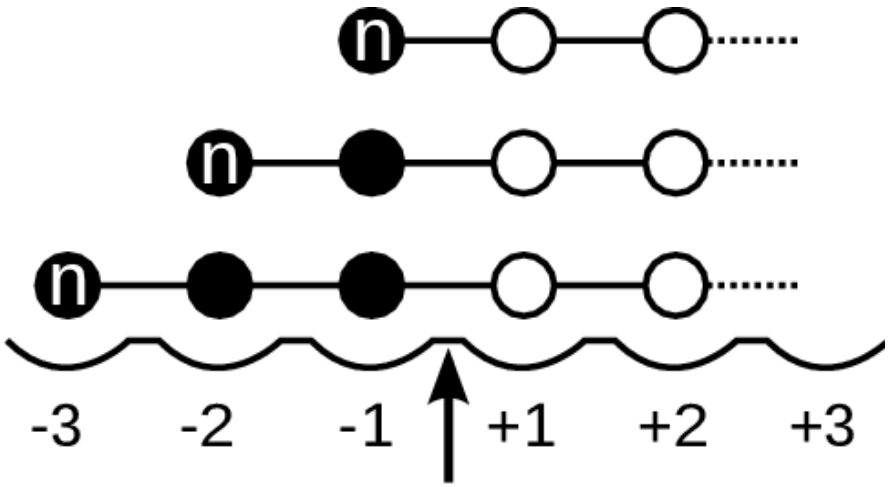
Different binding modes of the donor substrate exists

⇨ 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

Positional Isomers





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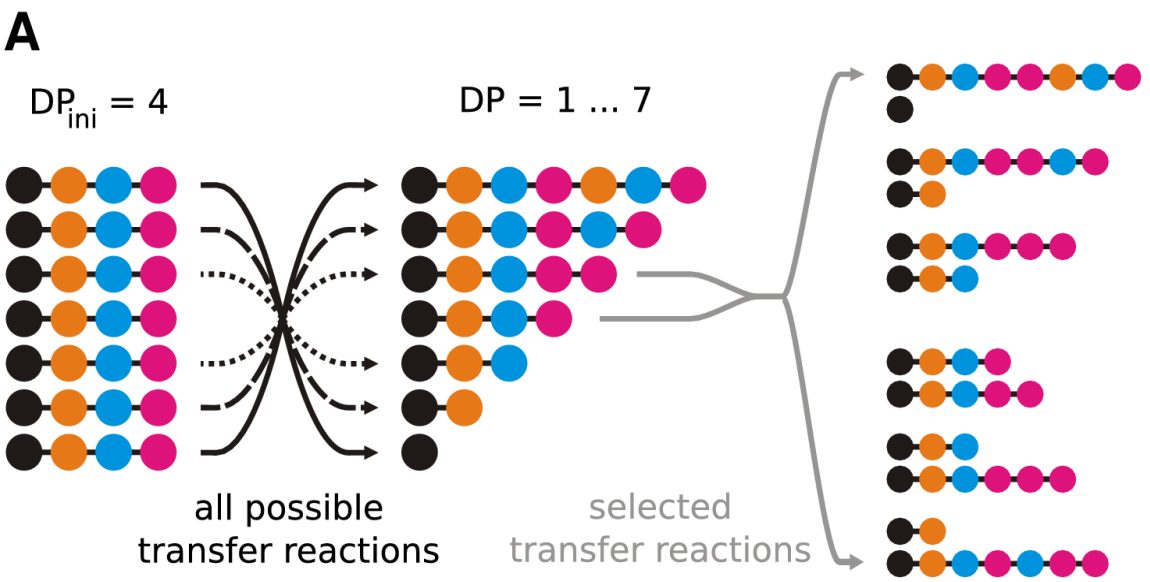
For such a reaction, what is the meaning of K_M???

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

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 $S = -\sum x_k \ln x_k$

Equilibrium is determined by maximal entropy:

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

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

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

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

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

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

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

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

Entropic approach

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

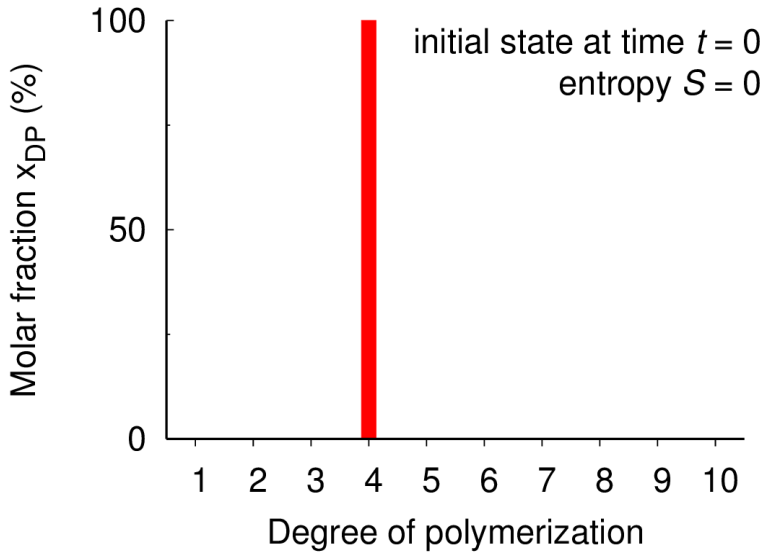
conservation of #molecules: $\sum x_k = 1$

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

implies

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

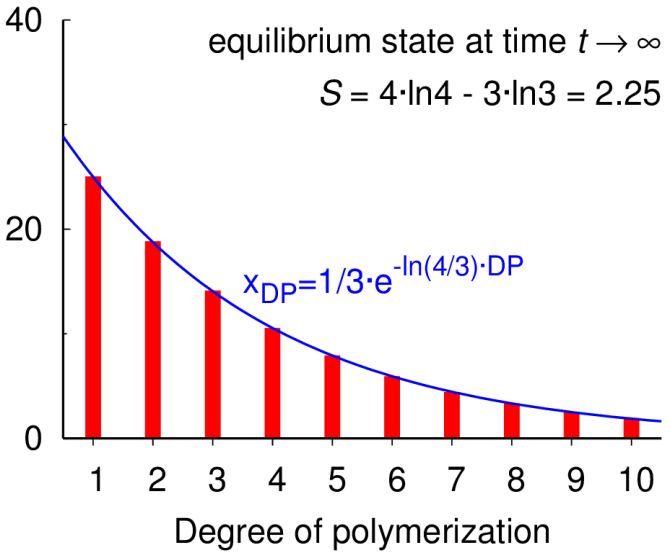
predicts



DPE1 action

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

with q = 1,2,3

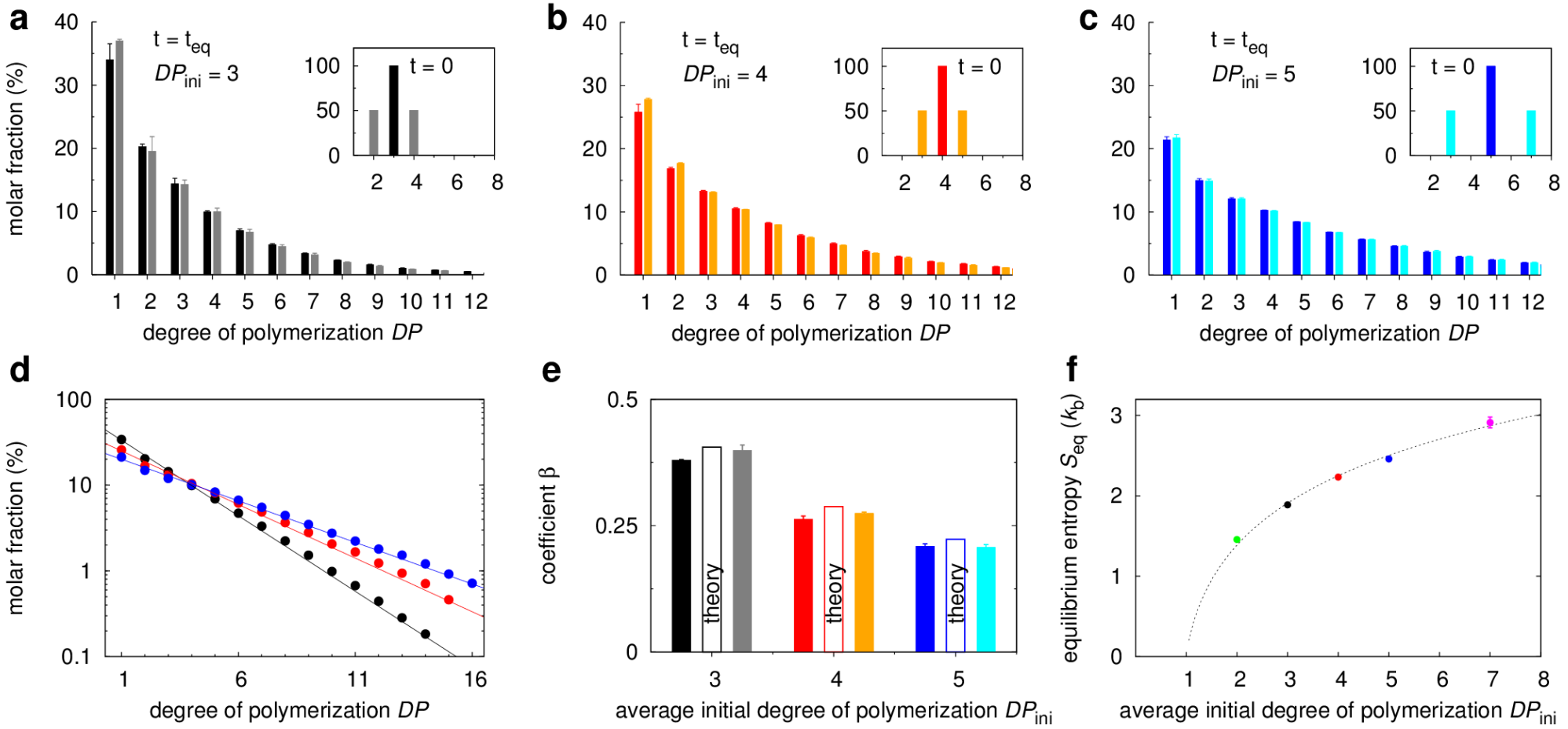


An instance of the 2nd law of TD!

DPE1 is entropy driven

Experiments with Martin Steup, University of Potsdam

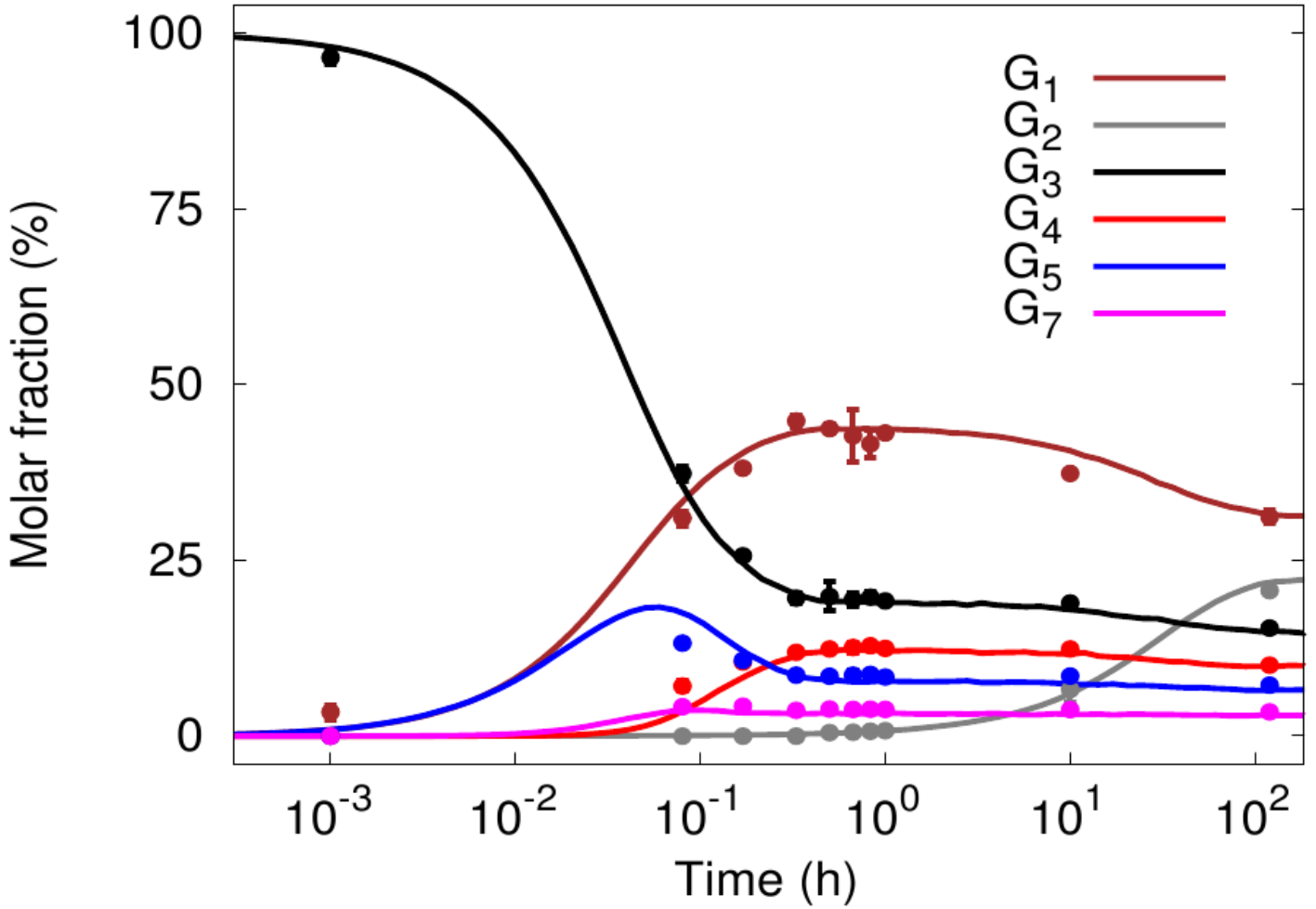
method: capillary electrophoresis



β is a generalisation of the equilibrium constant for polydisperse mixtures

(Kartal et al, 2011, Mol Syst Biol)

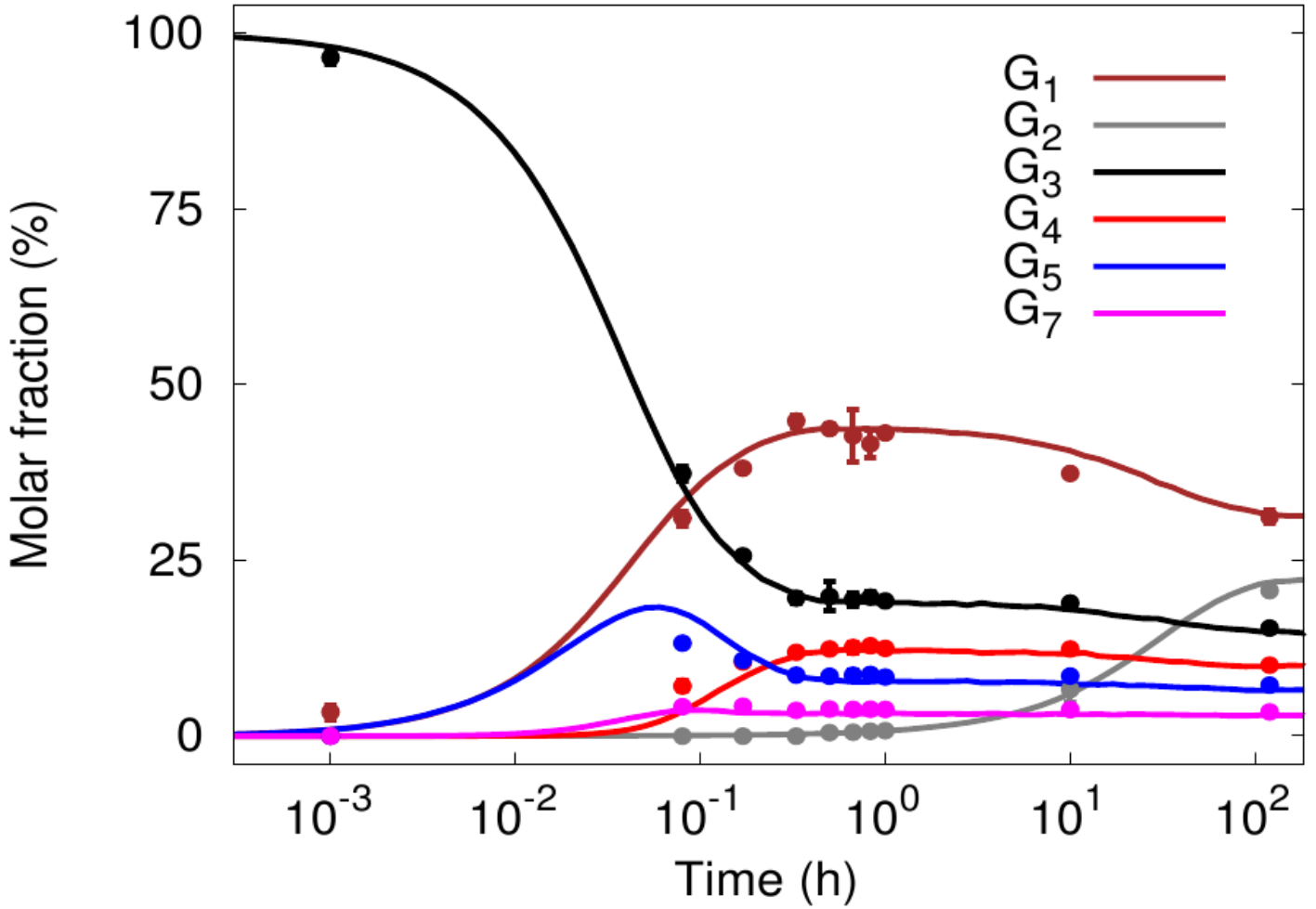
The dynamics of DPE1



maltose is formed late

Two time scales!

The dynamics of DPE1



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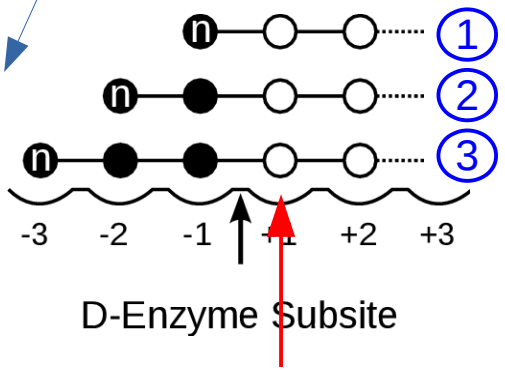


subsite +1 repellent (binding of G2 unlikely)

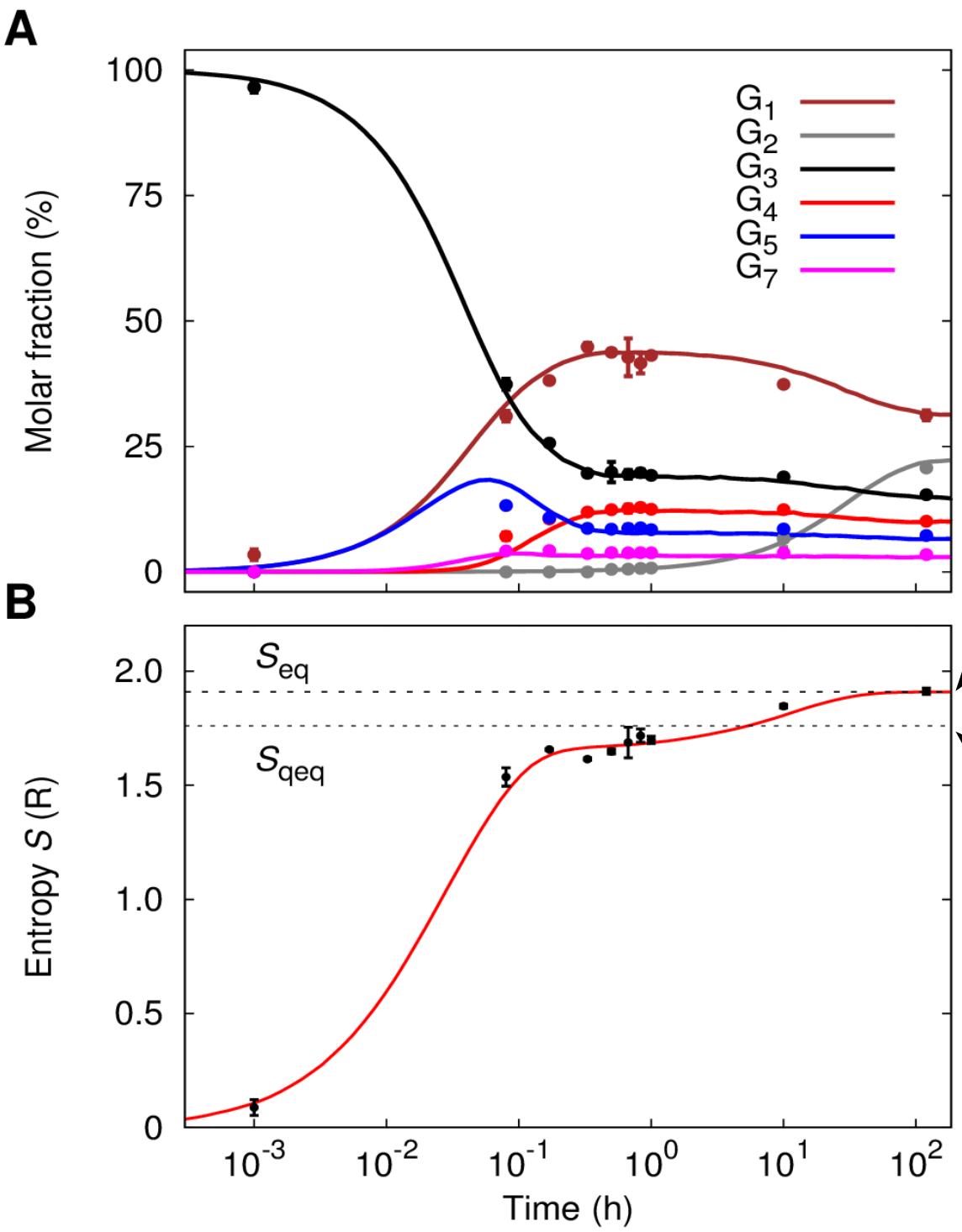
Two time scales!

The simulations used 3 parameters:

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

ratio 1:800

Affinities: K_M



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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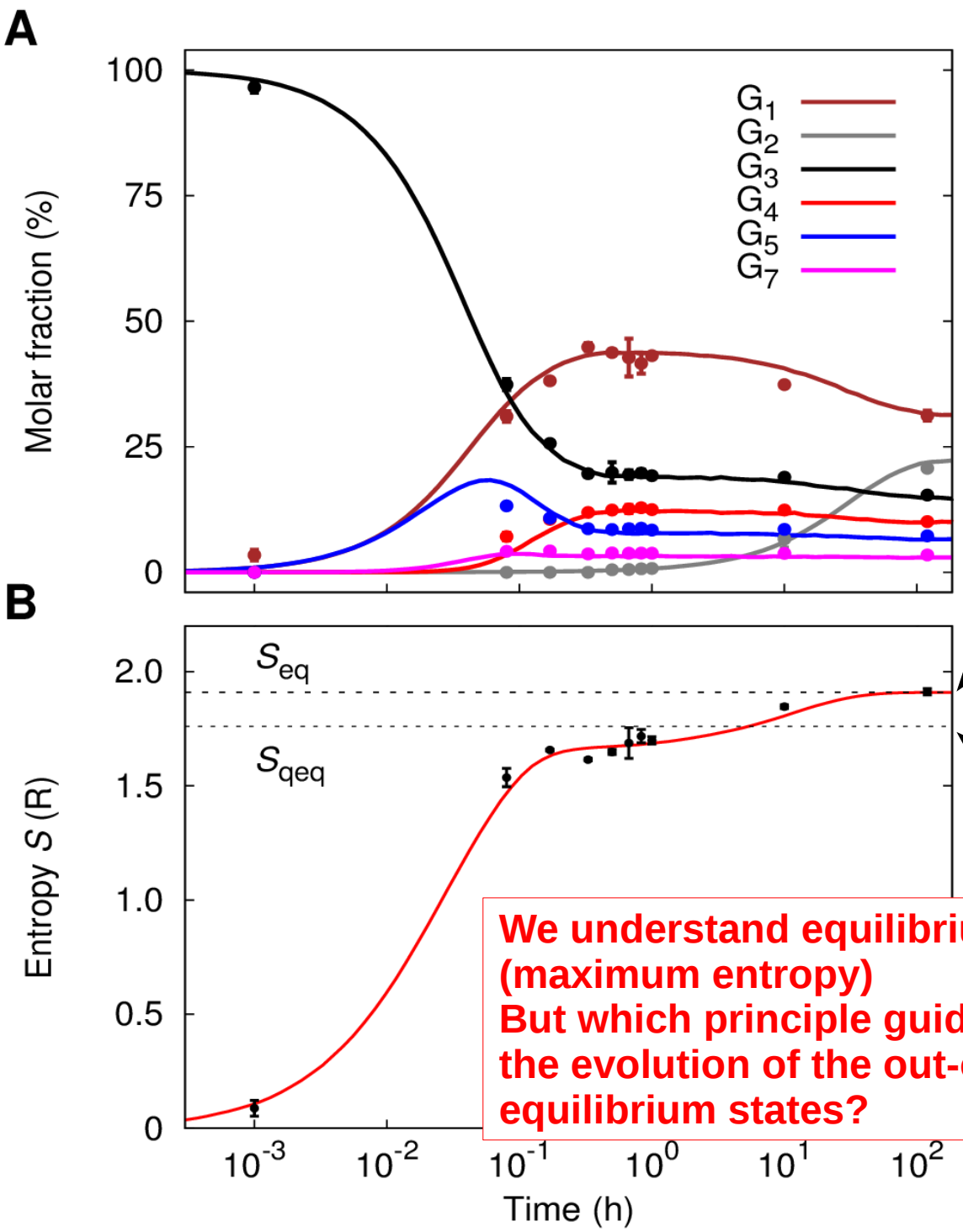


“true” equilibrium

(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

Generic reaction catalysed:

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

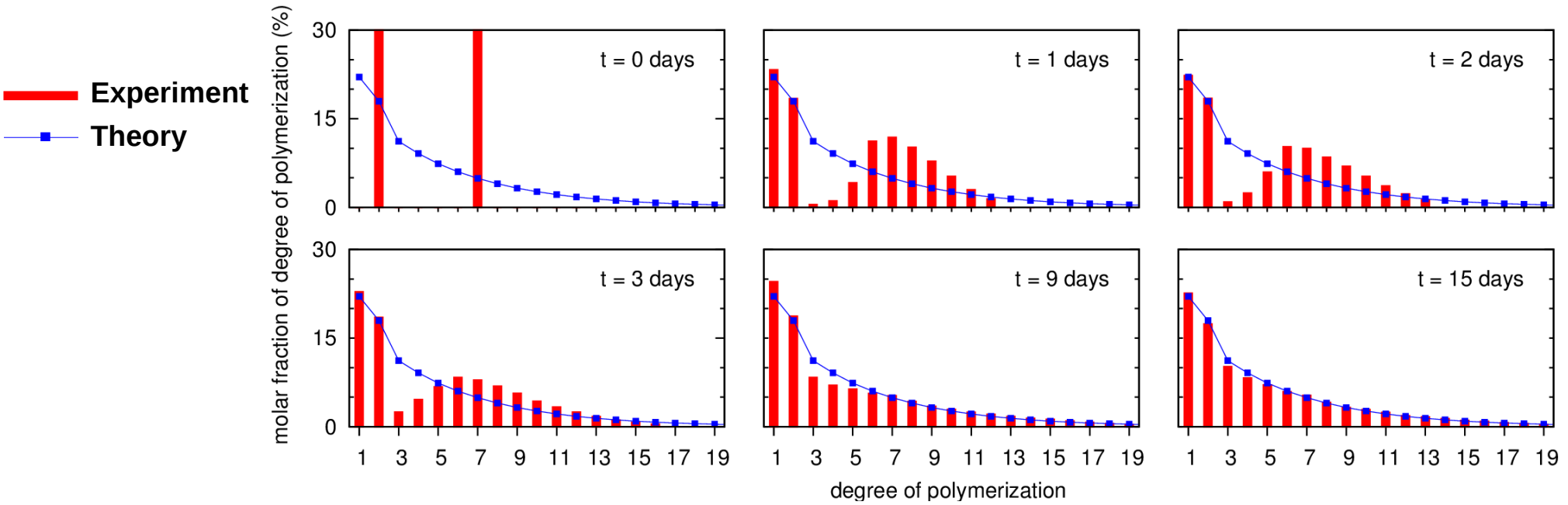
Entropic principle:

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

with one additional side constraint

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

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



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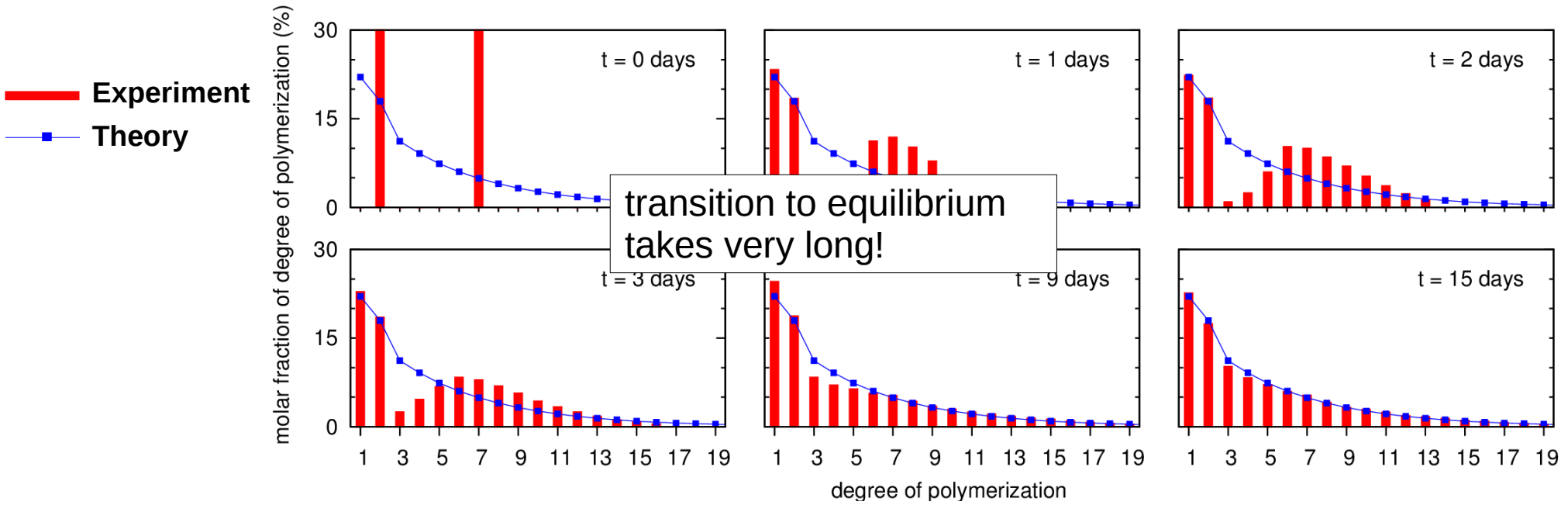


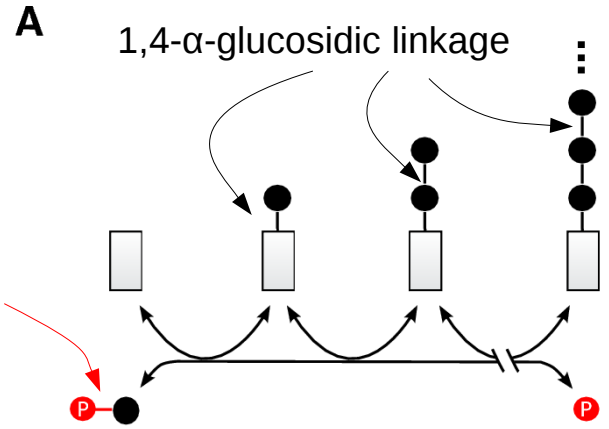
Generalisation to non-zero enthalpy changes

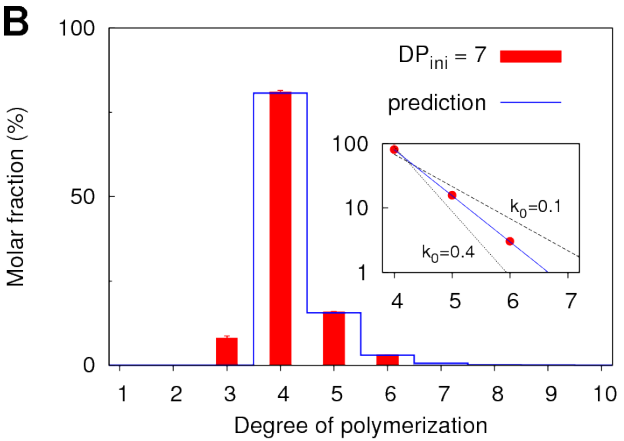
Phosphorylase (cPho):

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

$\Delta H \neq 0!$

phosphoester bond





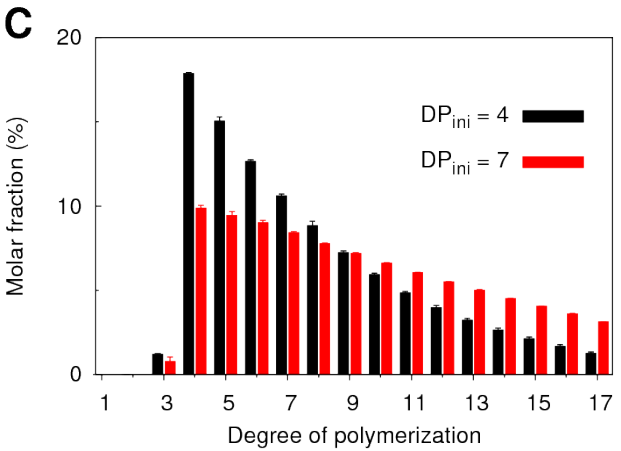
Generalisation by including energetic and entropic contributions:

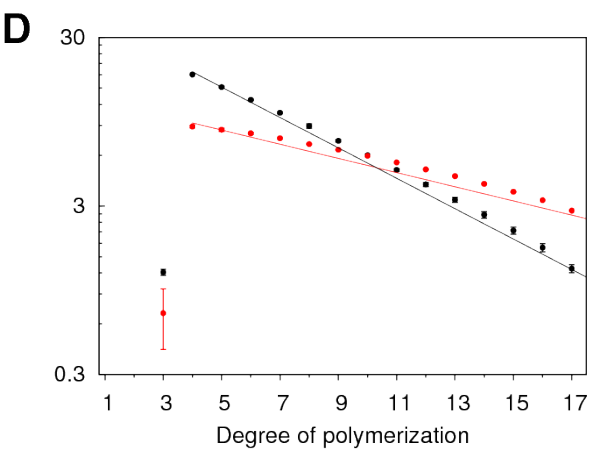
$$G = G^f - T \cdot S_{mix} \rightarrow \min!$$

Gibbs energy of formation

mixing entropy:

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



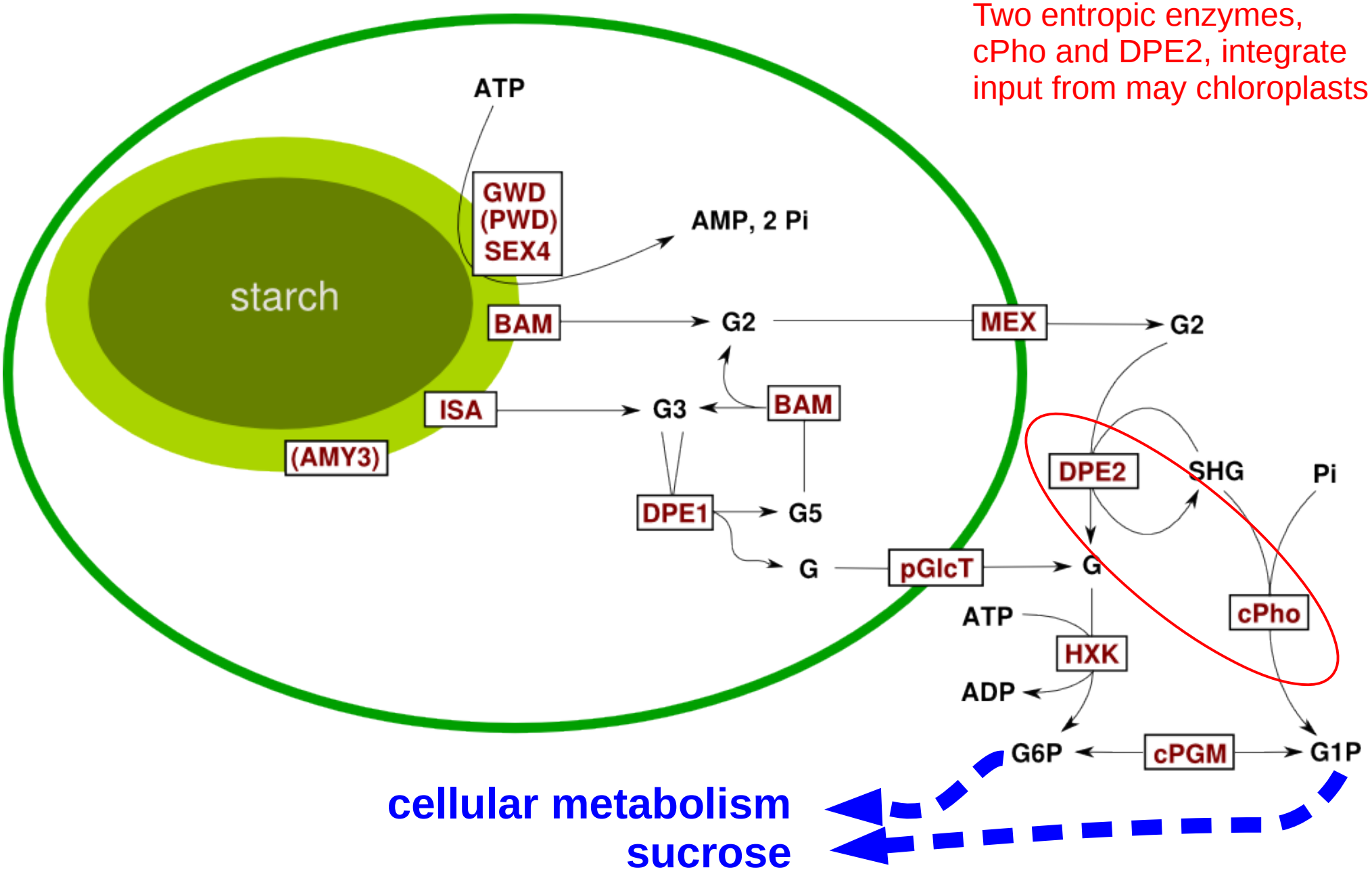


Prediction: Similar pattern as for DPE2

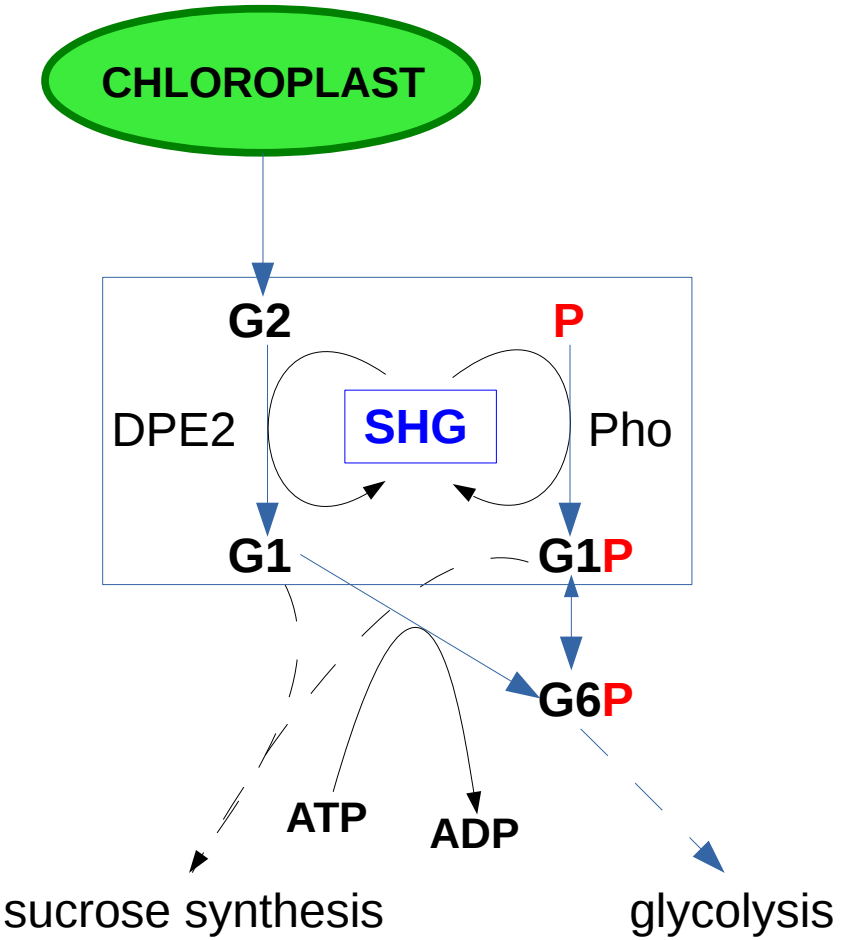
Experimentally confirmed.

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

An entropy-driven buffer



What is the role of the SHG pool?

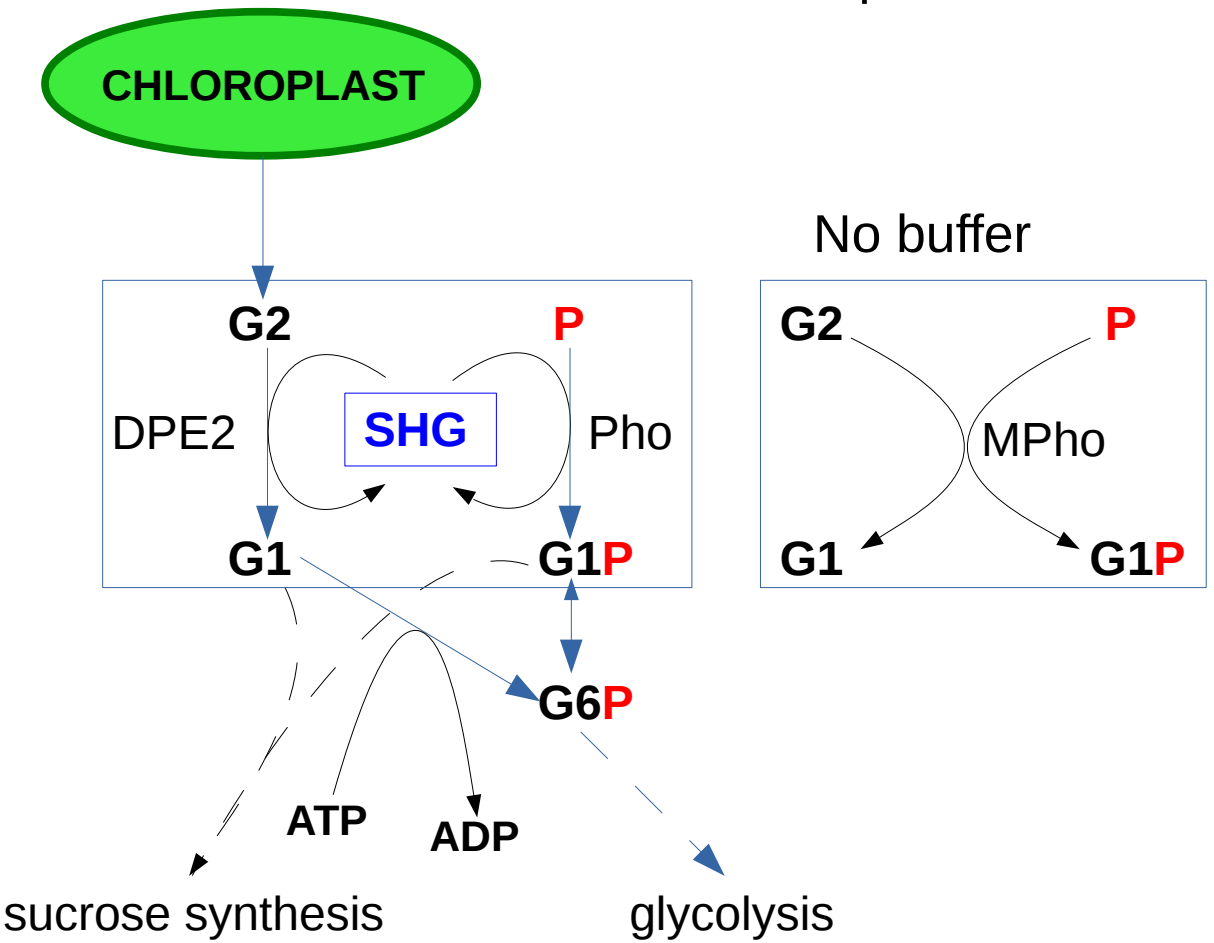


Two 'entropic' enzymes mediate the turnover of a polydisperse pool

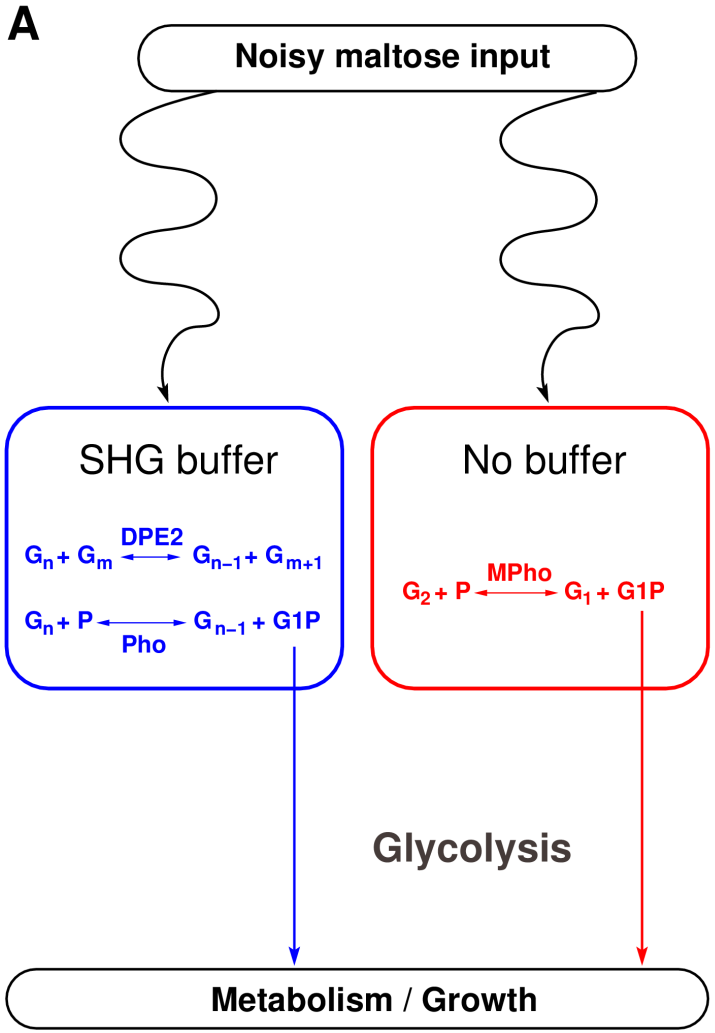
What is the advantage over other hypothetical systems?

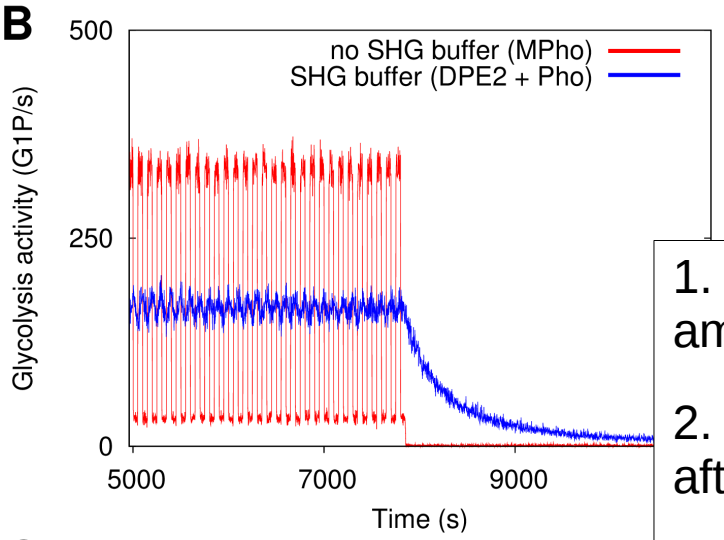
What is the role of the SHG pool?

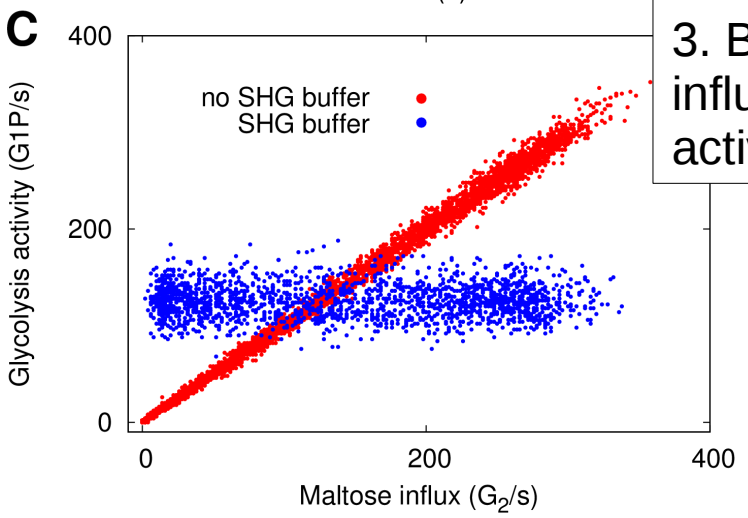
Comparison with two alternatives



Polydisperse SHG pools increases robustness *in vivo*





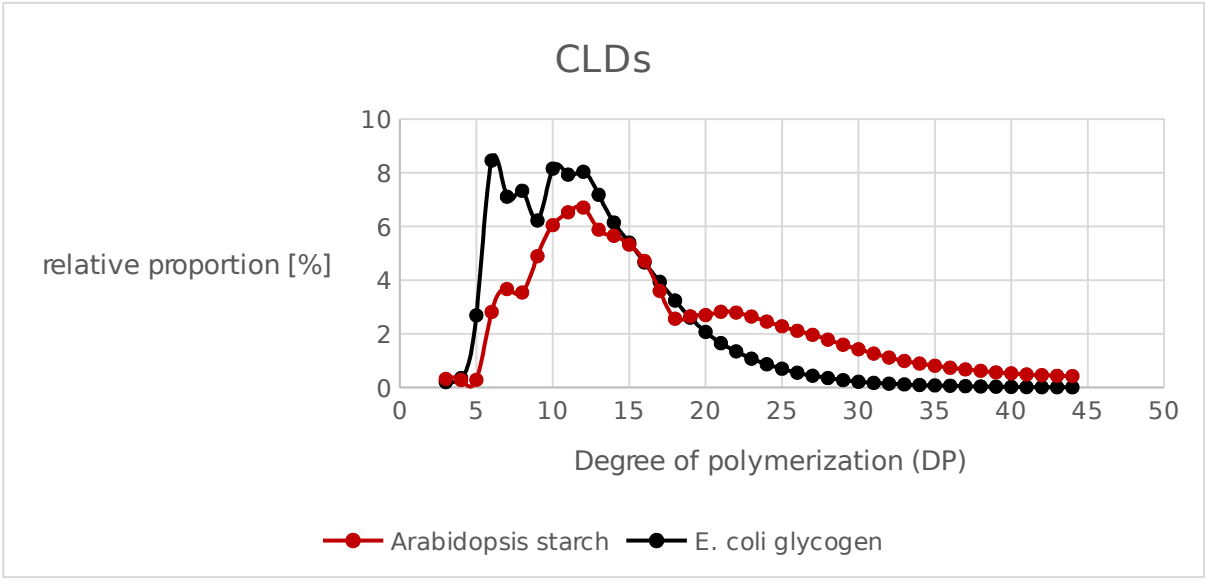


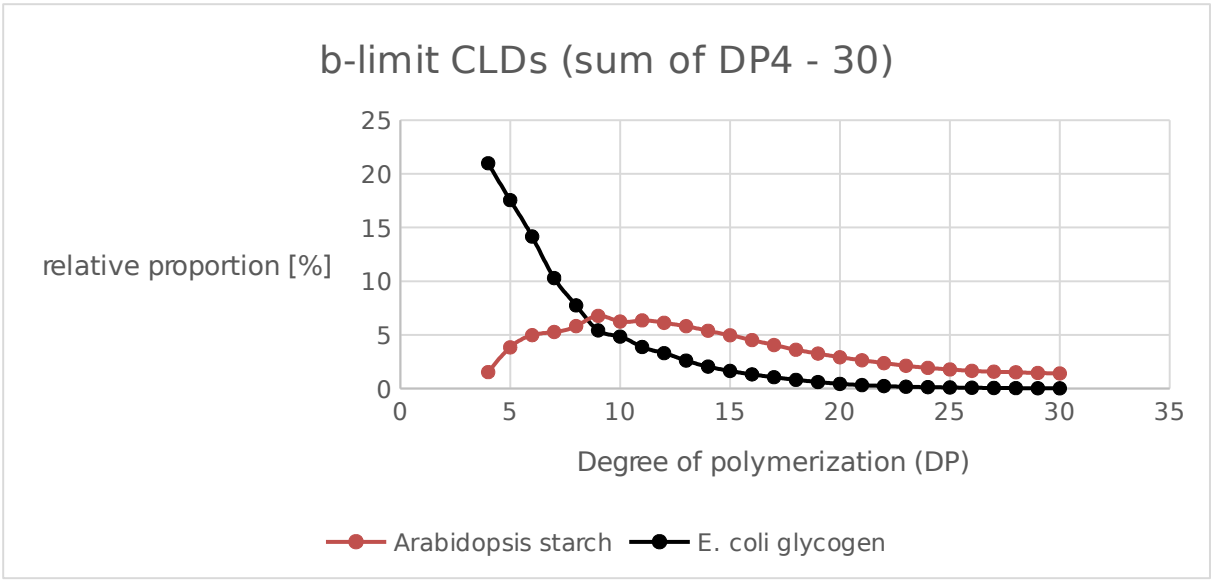
1. Attenuation of fluctuation amplitude (low-pass filter)

2. Transient support of activity after drop of maltose influx

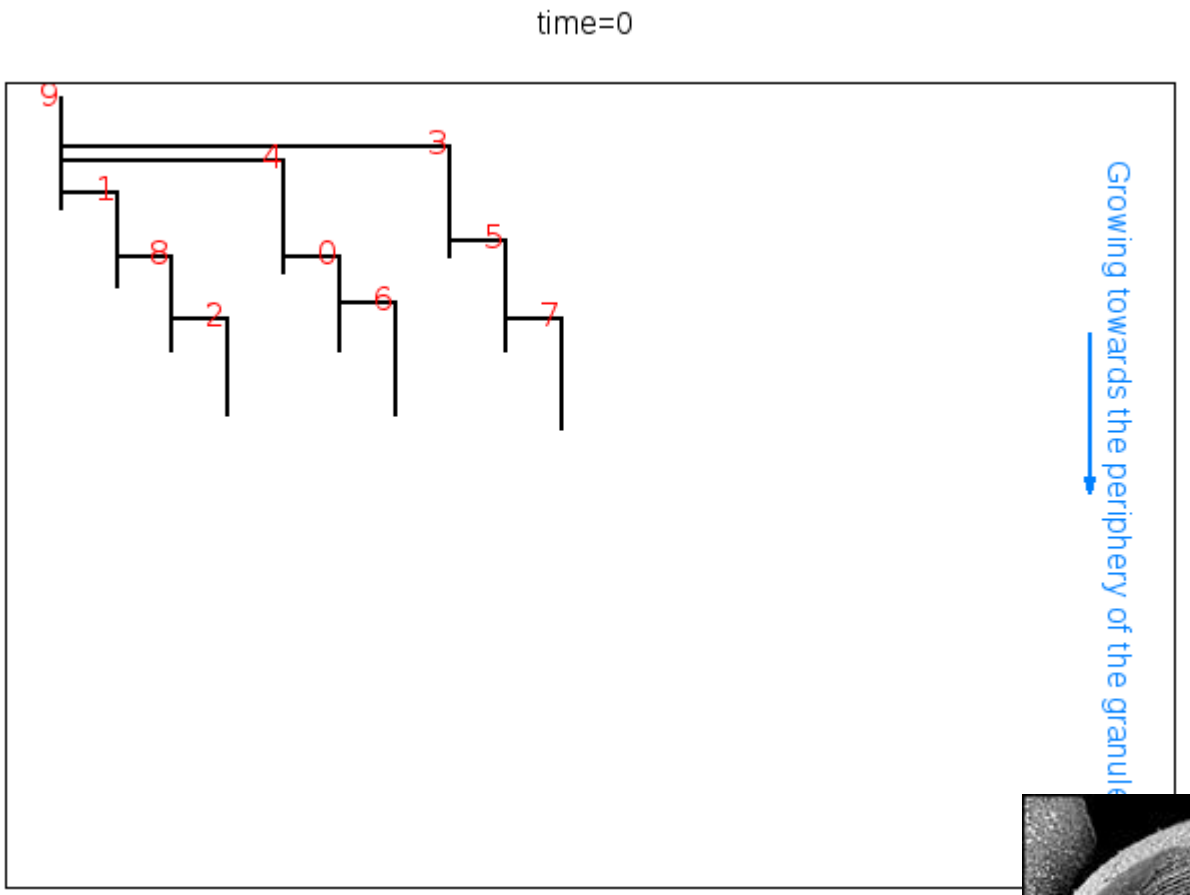
3. Buffering large variations in influx to provide robust output activity

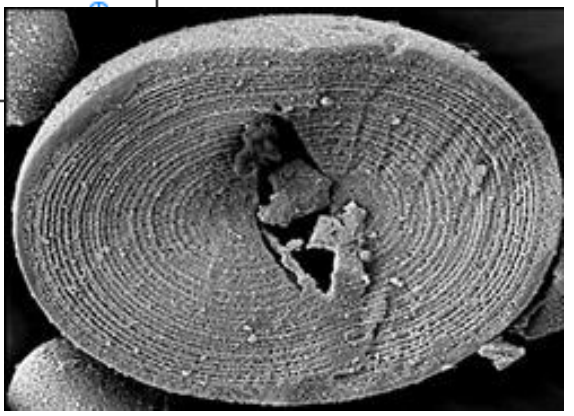
Challenge:
explain observations with bottom-up approach





Goal: reproduce emergent macroscopic properties with microscopic model



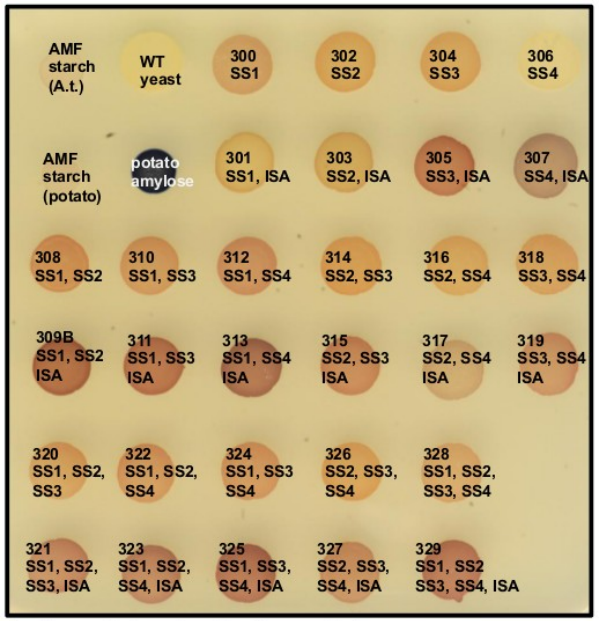


Top-down: expressing starch-like polymers in yeast

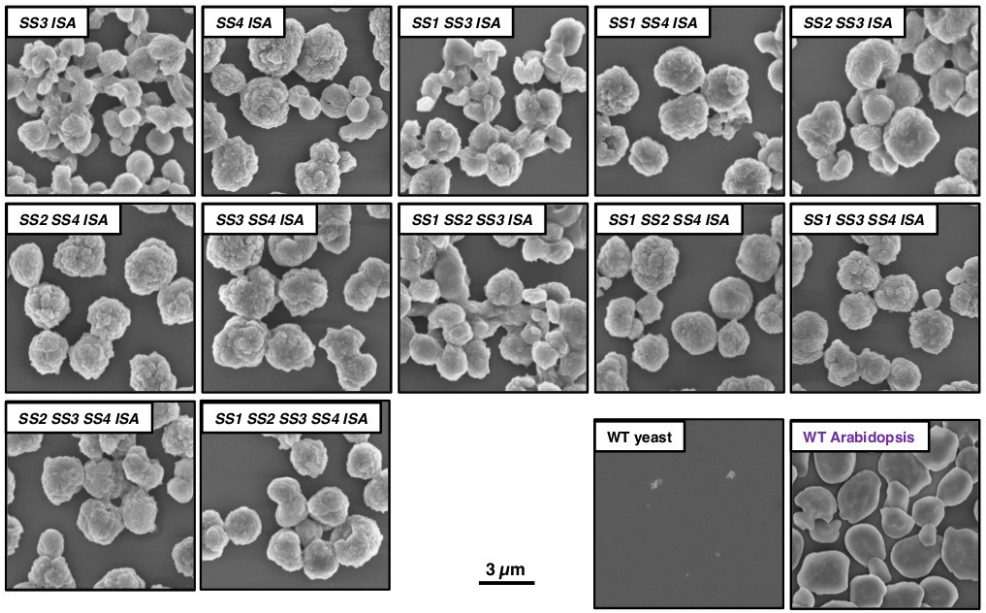
STARCH IN YEAST?

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



Conclusion & Outlook:

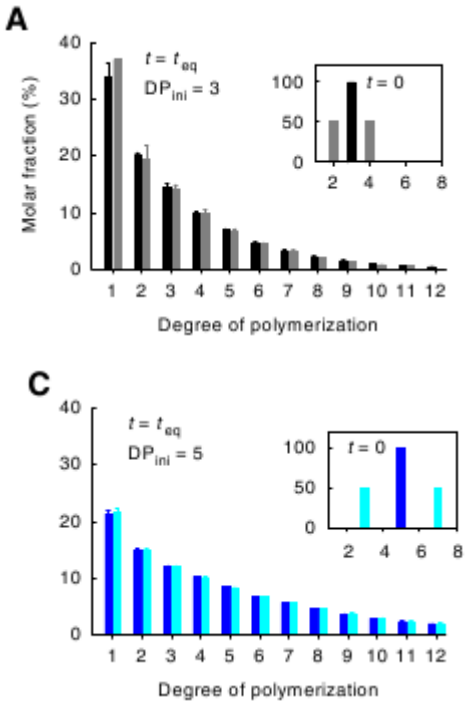
- We are only beginning to understand...
- We get something that looks like starch, but is not!
- How does this actually work?
- How can we control the properties of the insoluble glucans?

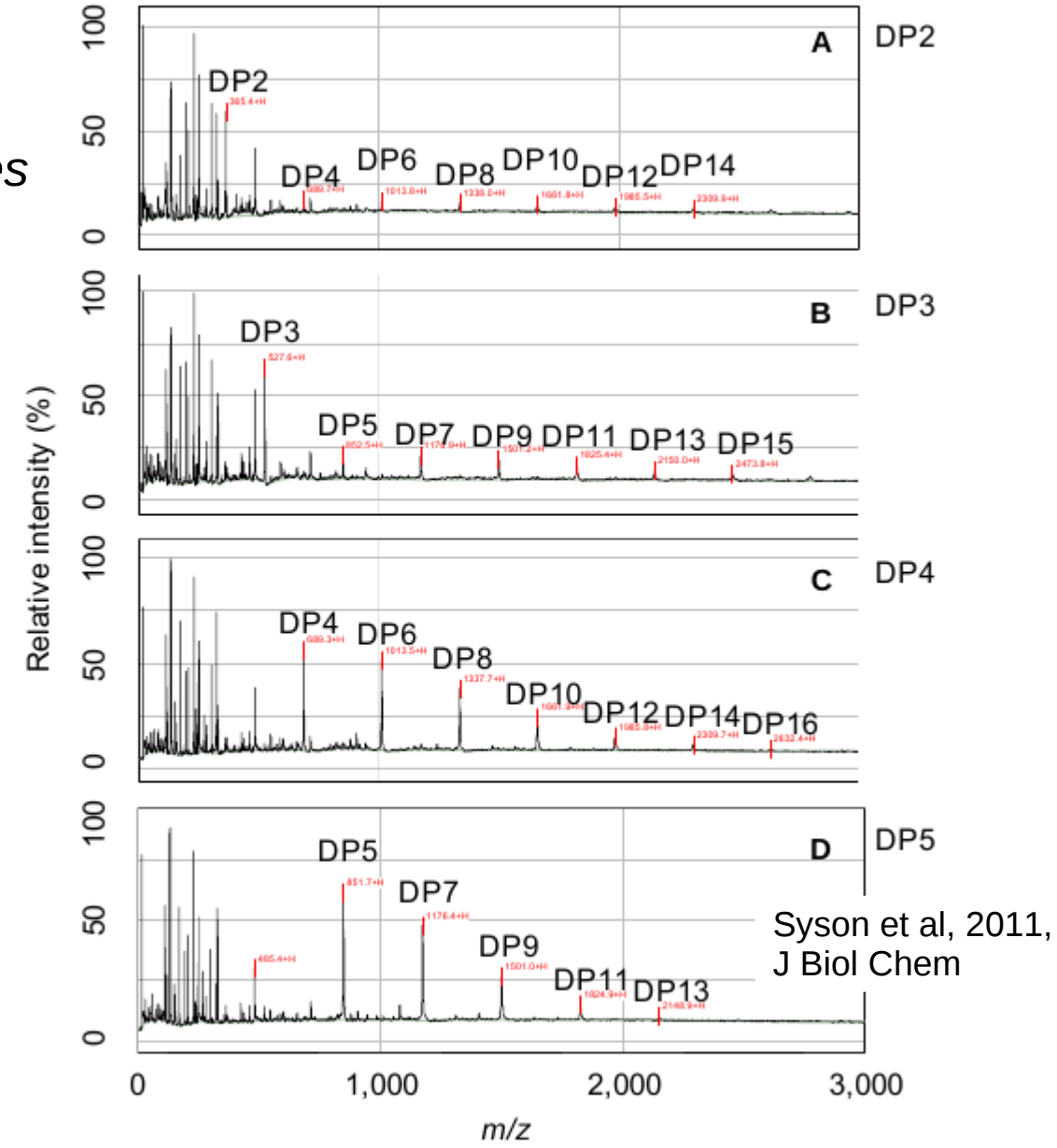
Where else do find entropic enzymes?

...for example

Maltosyltransferases in *Streptomyces*

"Acceptor specificity" can be explained by entropic principles





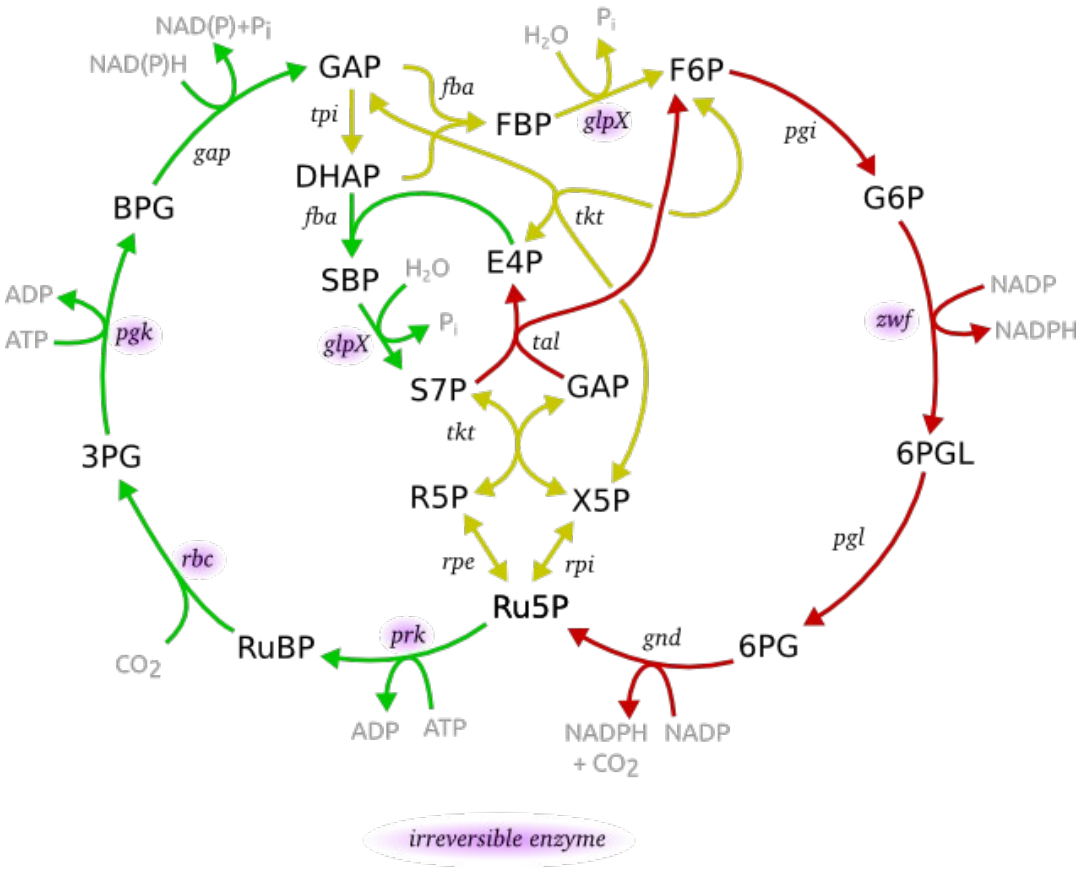
Syson et al, 2011, J Biol Chem

Where else do find entropic enzymes?

...or even in central metabolism?

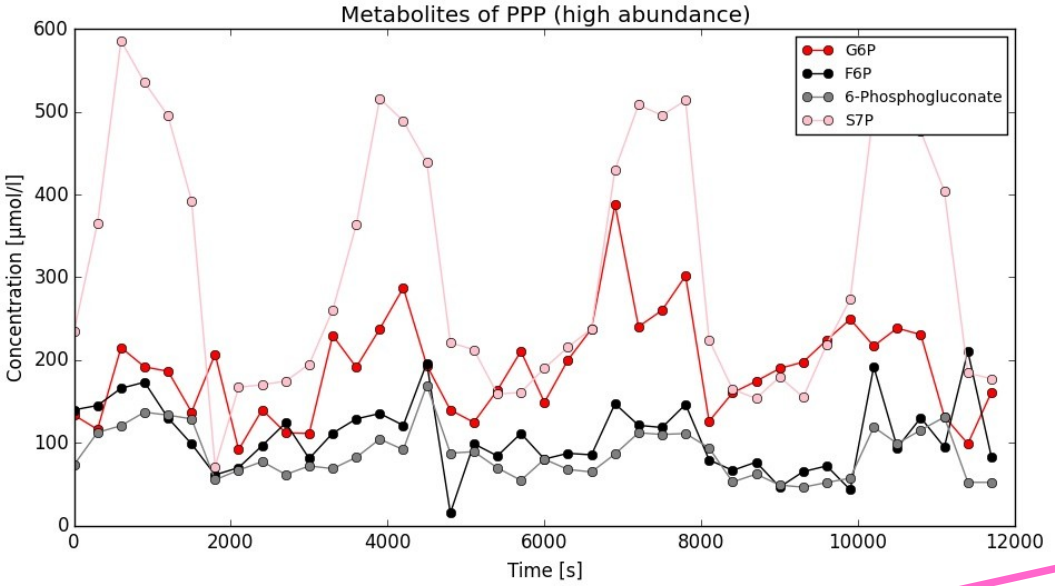
Transketolase? $K_n + A_m \Leftrightarrow A_{n-2} + K_{m+2}$

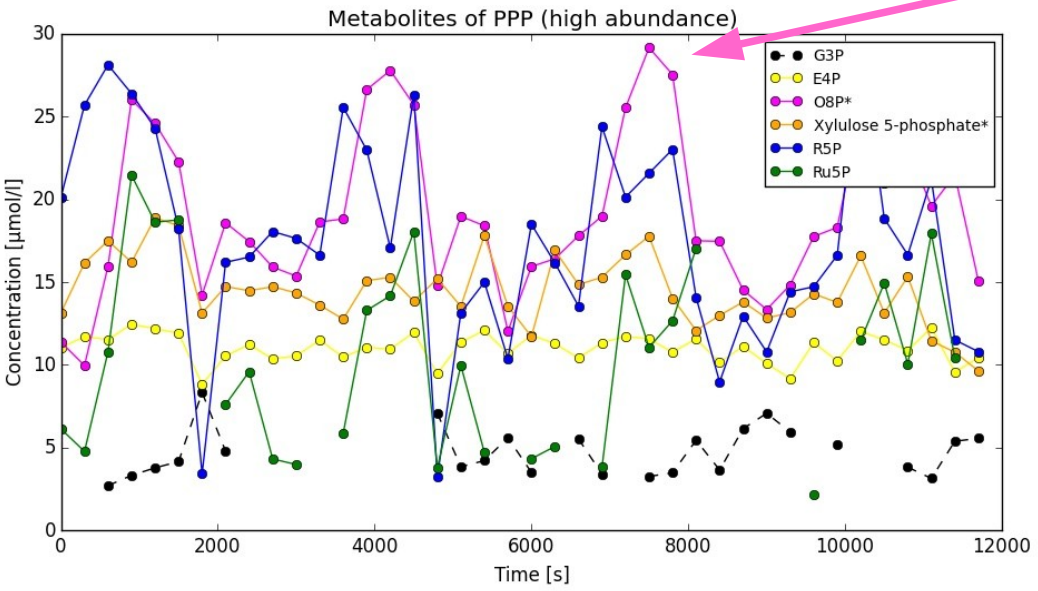
Why only *n*=5,6,7 und *m*=3,4,5?
Why should there be no octuloses / nonuloses...?



http://metamap.blogspot.de/2013/01/blog-post.html

Octulose-8P oscillates in respiratory cycle in yeast





O8P oscillations in phase with other PPP intermediates

Calvin cycle energetics

TABLE IV

FREE ENERGY CHANGES OF THE PENTOSE PHOSPHATE CYCLES IN *C. pyrenoidosa*

Reaction	$\Delta G'$ (*kcal*)	ΔG^s (*kcal*)		
Reductive cycle				
(A) $CO_2 + \text{Ribul-1,5-}P_2^{4-} + H_2O \rightarrow 2\ \text{3-}P\text{-glycerate}^{3-} + 2\ H^+$	−8.4	−9.8	R	
(B) $H^+ + \text{3-}P\text{-glycerate}^{3-} + ATP^{4-} + NADPH \rightarrow ADP^{3-} + \text{glyceraldehyde-3-}P^{2-} + NADP^+ + P_i^{2-}$	+4.3	−1.6		
(C) $\text{Glyceraldehyde-3-}P^{2-} \rightarrow \text{dihydroxyacetone-}P^{2-}$	−1.8	−0.2		Iso
(D) $\text{Glyceraldehyde-3-}P^{2-} + \text{dihydroxyacetone-}P^{2-} \rightarrow \text{Fru-1,6-}P_2^{4-}$	−5.2	−0.4		Ald
(E) $\text{Fru-1,6-}P_2^{4-} + H_2O \rightarrow \text{Fru-6-}P^{2-} + P_i^{2-}$	−3.4	−6.5	R	
(F) $\text{Fru-6-}P^{2-} + \text{glyceraldehyde-3-}P^{2-} \rightarrow \text{Ery-4-}P^{2-} + \text{Xyl-5-}P^{2-}$	+1.5	−0.9		TK
(G) $\text{Ery-4-}P^{2-} + \text{dihydroxyacetone-}P^{2-} \rightarrow \text{Sed-1,7-}P_2^{4-}$	−5.6	−0.2		Ald
(H) $\text{Sed-1,7-}P_2^{4-} + H_2O \rightarrow \text{Sed-7-}P^{2-} + P_i^{2-}$	−3.4	−7.1	R	
(I) $\text{Sed-7-}P^{2-} + \text{glyceraldehyde-3-}P^{2-} \rightarrow \text{Rib-5-}P^{2-} + \text{Xyl-5-}P^{2-}$	+0.1	−1.4		TK
(J) $\text{Rib-5-}P^{2-} \rightarrow \text{Ribul-5-}P^{2-}$	+0.5	−0.1		Iso
(K) $\text{Xyl-5-}P^{2-} \rightarrow \text{Ribul-5-}P^{2-}$	+0.2	−0.1		Iso
(L) $\text{Ribul-5-}P^{2-} + ATP^{4-} \rightarrow \text{Ribul-1,5-}P_2^{4-} + ADP^{3-} + H^+$	−5.2	−3.8	R′	
(M) $\text{Fru-6-}P^{2-} \rightarrow \text{Glc-6-}P^{2-}$	−0.5	−0.3		Iso
(N) $\text{Glc-6-}P^{2-} + H_2O \rightarrow \alpha\text{-D-Glc} + P_i^{2-}$	−3.3	(−7.2)*		

(Bassham and Krause, BBA 1969)

All 'close to equilibrium' reactions *shuffle*

Thermodynamic organisation of metabolism

tr.walls321.com – Pamukkale, Turkey

or

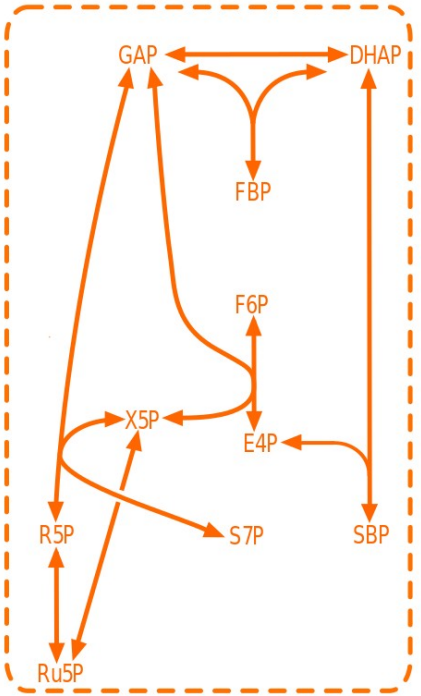
www.alamy.com – Loch Fyne, Scotland

?

CBB cycle energetics support this!

The Logic of the CBB

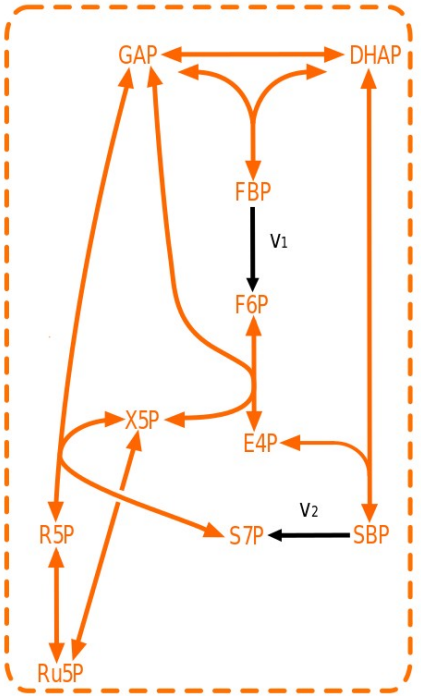
1) Near equilibrium reactions mix sugar phosphates, providing a range of substrates



Equilibrium Module

The Logic of the CBB

1) Near equilibrium reactions mix sugar phosphates, providing a range of substrates
2) De-phosphorylation as thermodynamic driving force (ΔG<0)



Equilibrium Module

The Logic of the CBB

1) Near equilibrium reactions mix sugar phosphates, providing a range of substrates
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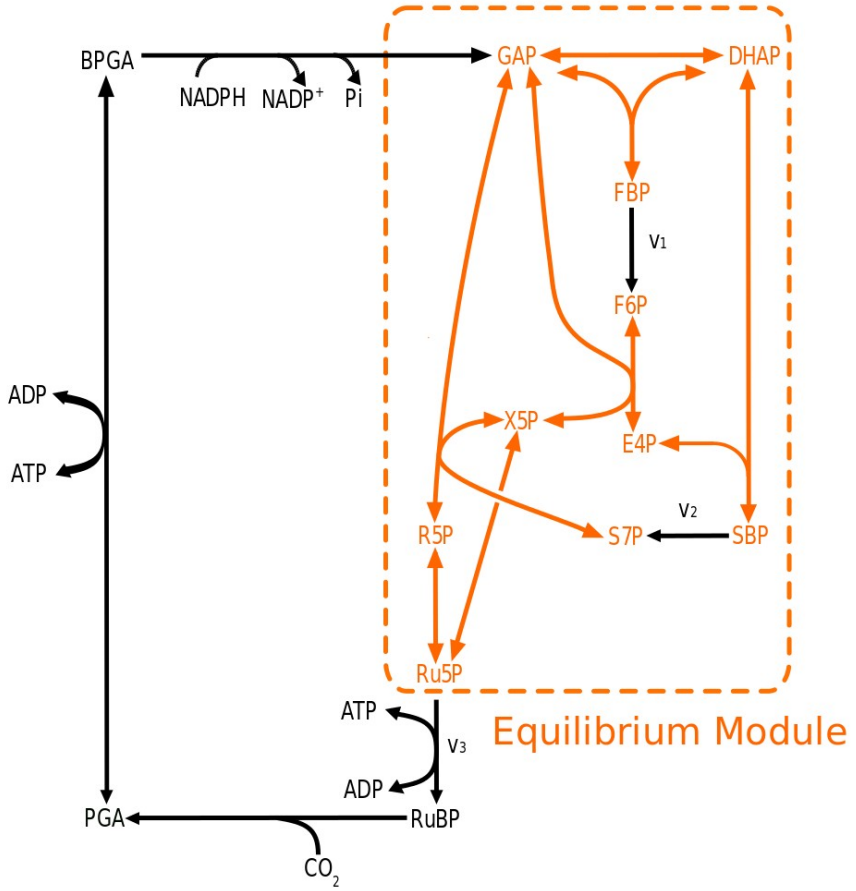
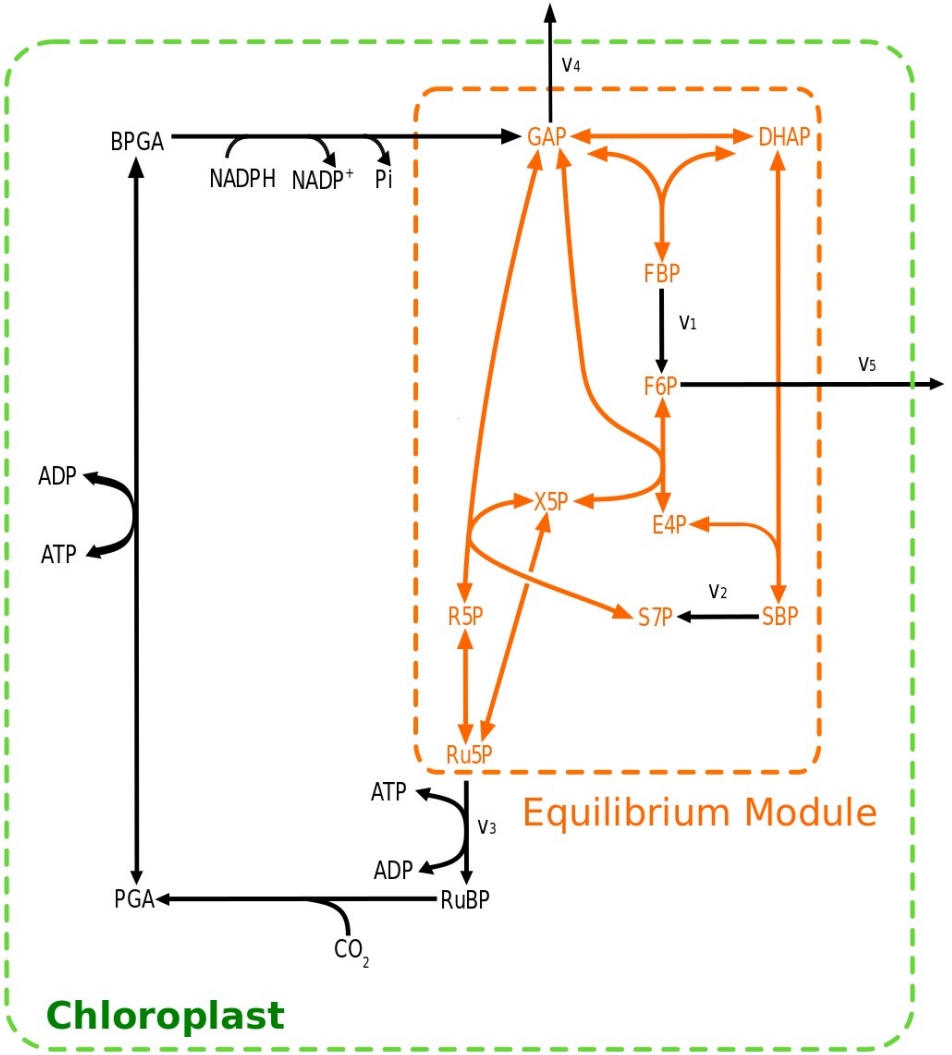
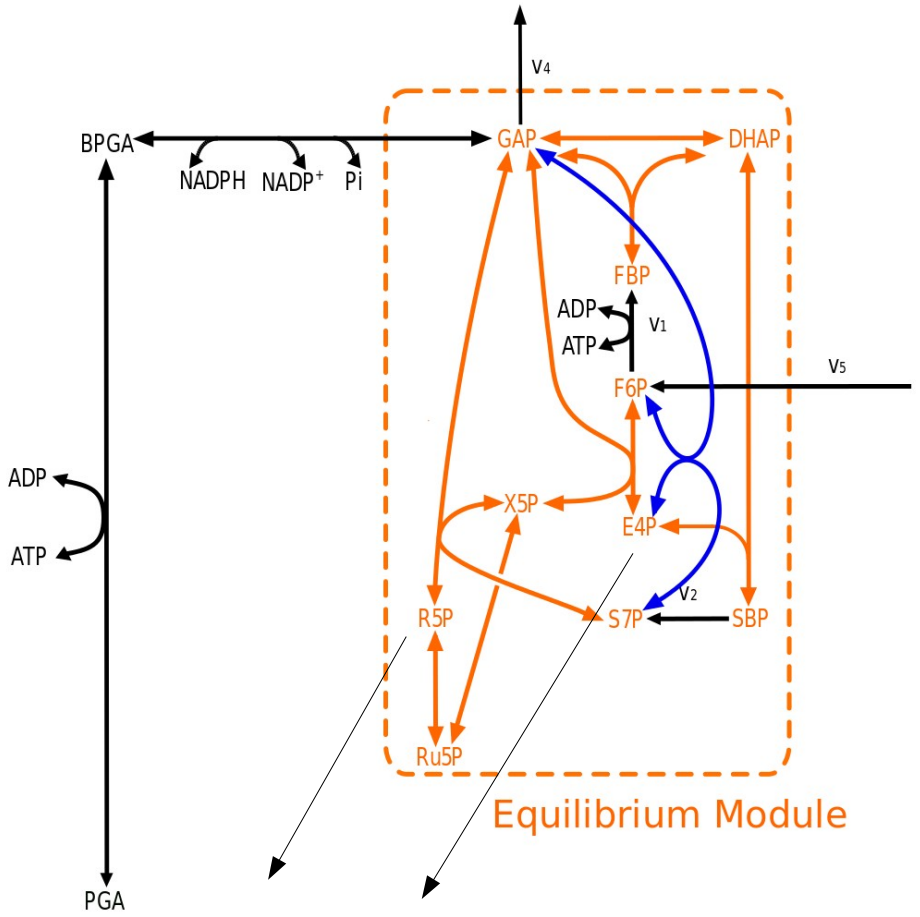


The Logic of the CBB

1) Near equilibrium reactions mix sugar phosphates, providing a range of substrates
2) De-phosphorylation as thermodynamic driving force (ΔG<0)
3) Activation, carbon fixation, reduction (overall ΔG<0)
4) Output

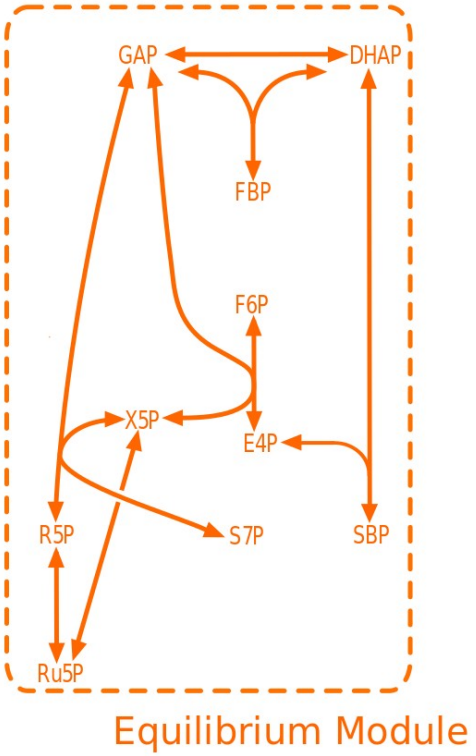


The pentose phosphate pathways uses the same equilibrium module



The Equilibrium Module

How to calculate the rapid equilibrium?

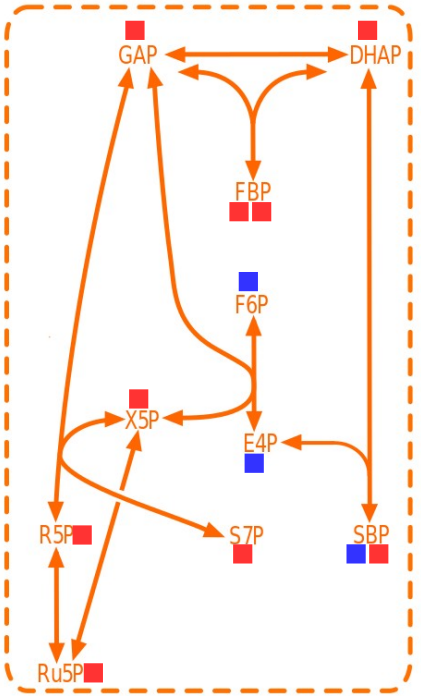


The Equilibrium Module

How to calculate the rapid equilibrium?

<u>Thermodynamics</u>
(see Supplementary to Kartal et al, 2011, MSB 7:542)

- Step 1: find conserved quantities



Equilibrium Module

3 conserved moieties:
2 from P, 1 from C

- P in odd-C sugars
- P in even-C sugars

Formally:

$$N_{eq} = \begin{array}{c} \\ GAP \\ DHAP \\ E4P \\ X5P \\ R5P \\ Ru5P \\ F6P \\ S7P \\ FBP \\ SBP \end{array} \begin{array}{c} \begin{array}{cc} \text{Iso / Epi} & \\ TPI & \end{array} \quad \begin{array}{cc} & \\ RPI & RPE \end{array} \quad \begin{array}{cc} TK & \\ & \end{array} \quad \begin{array}{cc} Ald & \\ & \end{array} \\ \begin{bmatrix} -1 & & & -1 & -1 & -1 & \\ 1 & & & & & -1 & -1 \\ & & & 1 & & & -1 \\ & & -1 & 1 & 1 & & \\ & & & & 1 & & \\ & & 1 & 1 & & & \\ & & & -1 & & & \\ & & & & -1 & & \\ & & & & & 1 & \\ & & & & & & 1 \end{bmatrix} \end{array}$$

Linearly independent solutions to $c \cdot N_{eq} = 0$:

$c_1 = (1,1,0,1,1,1,0,1,2,1)$
$c_2 = (0,0,1,0,0,0,1,0,0,1)$
$c_3 = (0,0,1,2,2,2,3,4,0,1)$

$P_1 = GAP + DHAP + X5P + R5P + Ru5P + S7P + 2FBP + SBP$
$P_2 = E4P + F6P + SBP$
$Q = E4P + 2(X5P + R5P + Ru5P) + 3F6P + 4S7P + SBP$

The Equilibrium Module

How to calculate the rapid equilibrium?

Thermodynamics

(see Supplementary to Kartal et al, 2011, MSB 7:542)

- Step 2: minimise Gibbs free energy

How to find the function

$$f : (P_1, P_2, Q) \longrightarrow \underbrace{(GAP, DHAP, E4P, X5P, R5P, Ru5P, F6P, S7P, FBP, SBP)}_{M} \; ?$$

THERMODYNAMIC APPROACH:

$$G = \sum_{j \in M} x_j \mu_j + RT \cdot \sum_{j \in M} x_j \cdot (\ln x_j - 1)$$

x_j : concentrations

μ_j : chemical potentials

Gibbs energies of formation

$T \cdot$ mixing entropy

Minimise G under constraints $C \cdot N = 0$

→ LAGRANGIAN MULTIPLIERS !

Solving the equilibrium module

3 equations with 3 unknowns:

GAP Lagrange multiplier **E4P**

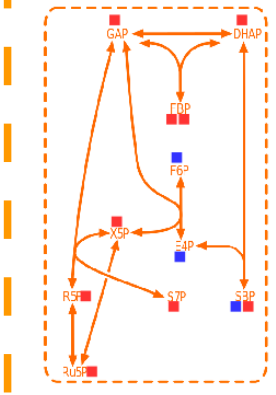
$$P_1 = x_0\left(f_0+\kappa_2 f_2 z+\kappa_4 f_4 z^2\right)+2g x_0^2+g_1 x_0 x_1$$

$$P_2 = x_1\left(1+\kappa_3 z\right)+g_1 x_0 x_1$$

$$Q = x_0\left(2f_2\kappa_2 z+4f_4\kappa_4 z^2\right)+x_1\left(1+3\kappa_3 z\right)+g_1 x_0 x_1$$

Notation:

x_k: compound with $k+3$ carbons



P_1 P_2

$Q = C - 3P$

$$\Longrightarrow \quad x_{k+2} = x_k \cdot e^{-\Delta\mu} \cdot z$$

A 3-variable model of the CBB cycle

Stoichiometry Matrix:

$$N = \begin{bmatrix} -2 & 0 & 1 & -1 & 0 \\ 1 & -1 & 0 & 0 & -1 \\ 3 & 3 & -2 & 0 & -3 \end{bmatrix} \quad \begin{matrix} P_1 \\ P_2 \\ Q \end{matrix}$$

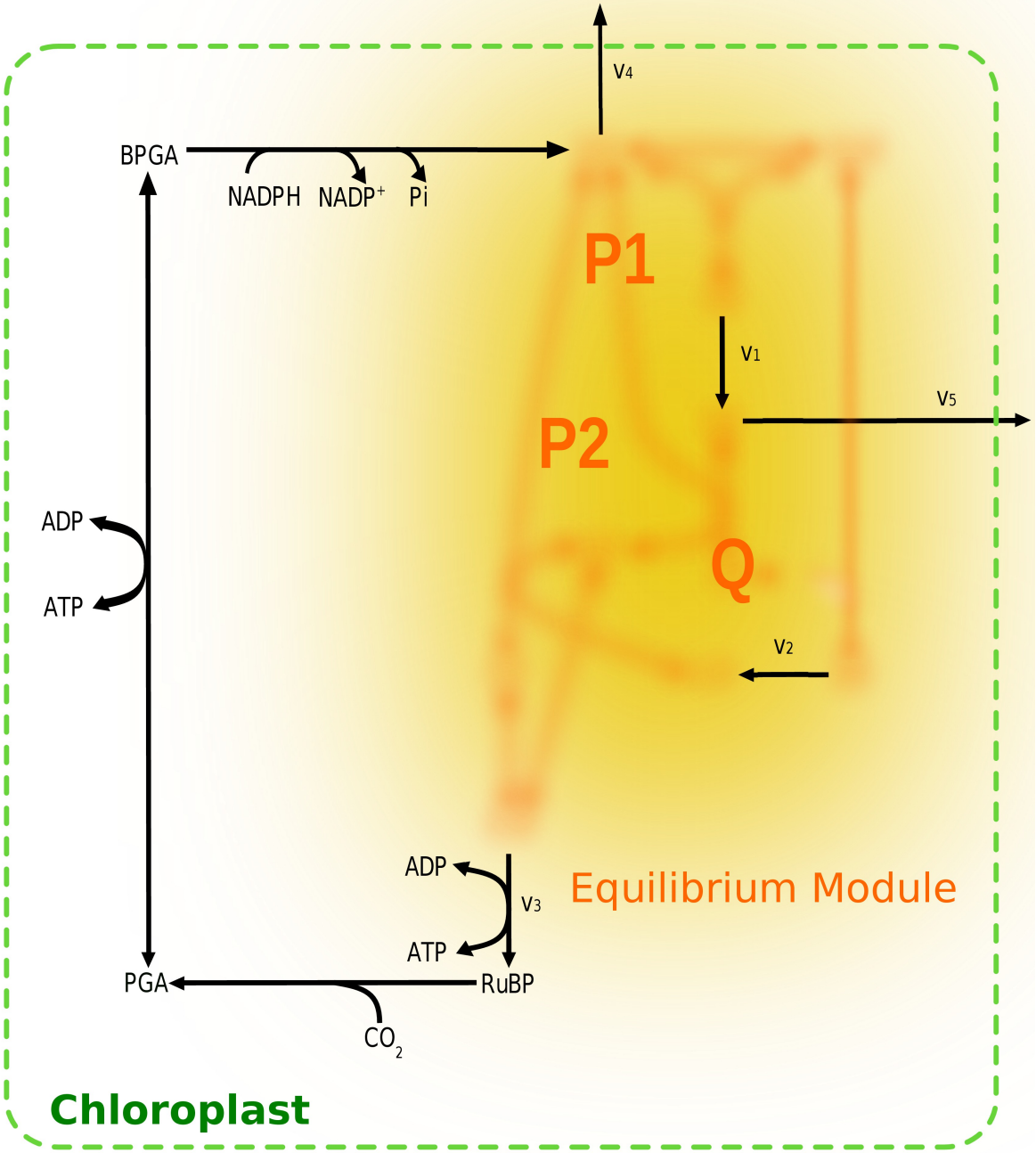
Differential Equations:

$$\dot{X} = N \cdot v(Y(X))$$

with:

$$X = \{P_1, P_2, Q\}$$

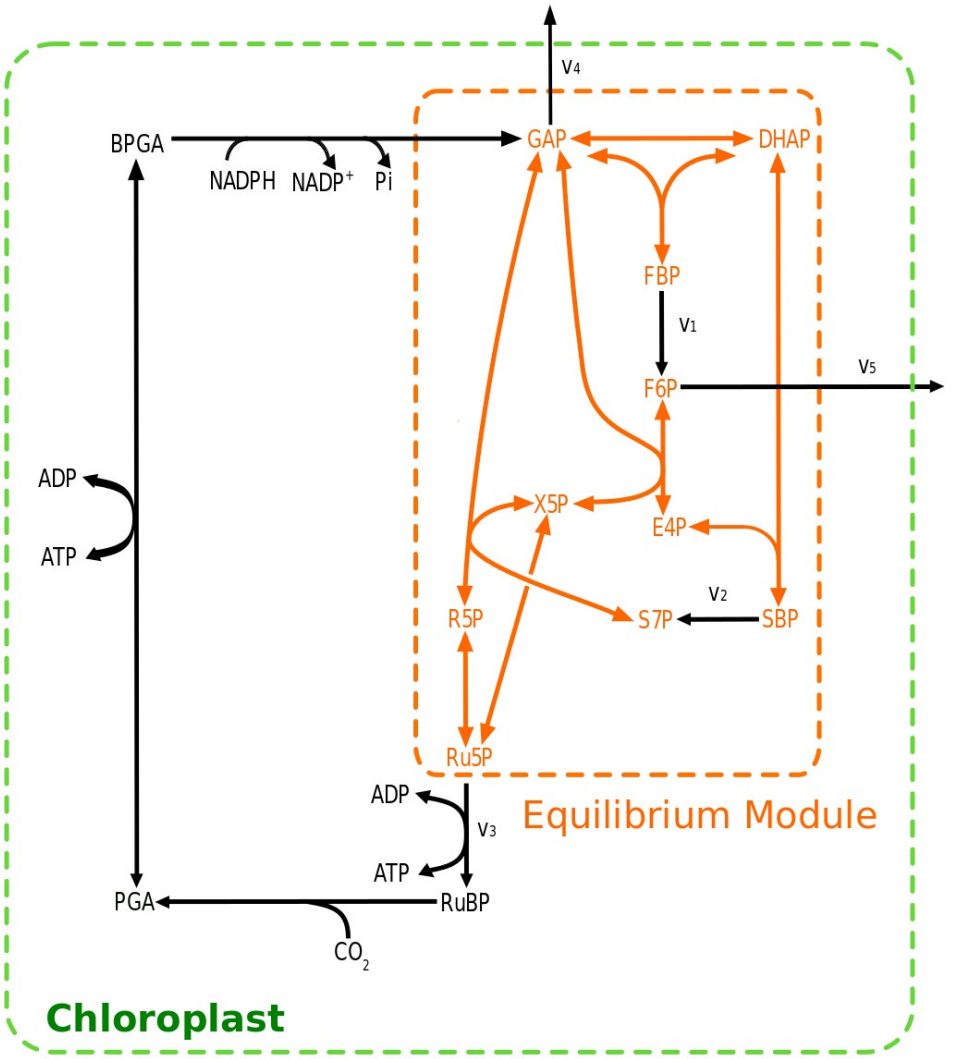
$$Y = \{GAP, DHAP, E4P \ldots SBP\}$$



Closing the cycle

First attempt: mass-action

$$v_1 = k_1[\text{FBP}]$$
$$v_2 = k_2[\text{SBP}]$$
$$v_3 = k_3[\text{Ru5P}]$$
$$v_4 = k_4[\text{GAP}]$$
$$v_5 = k_5[\text{F6P}]$$



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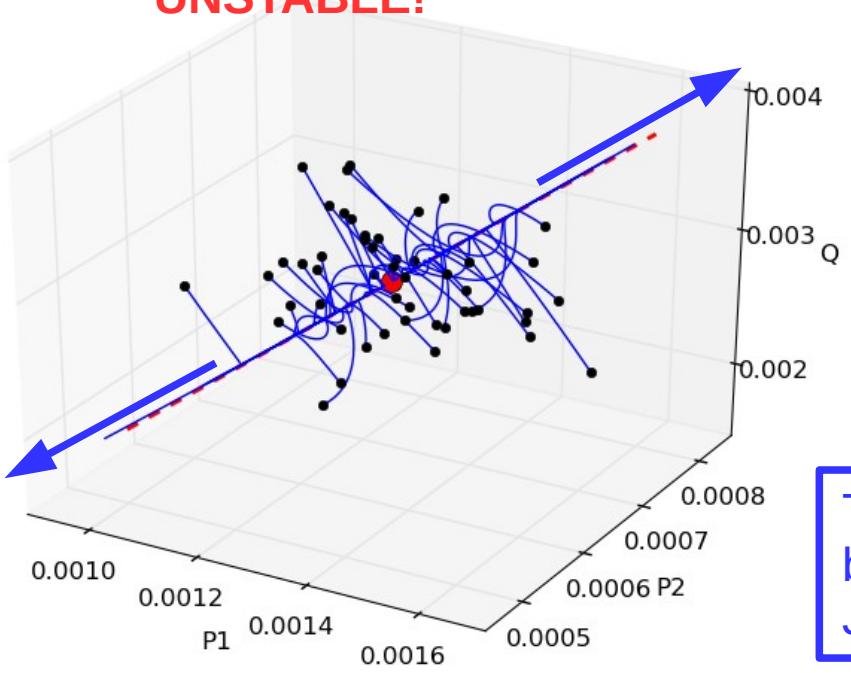
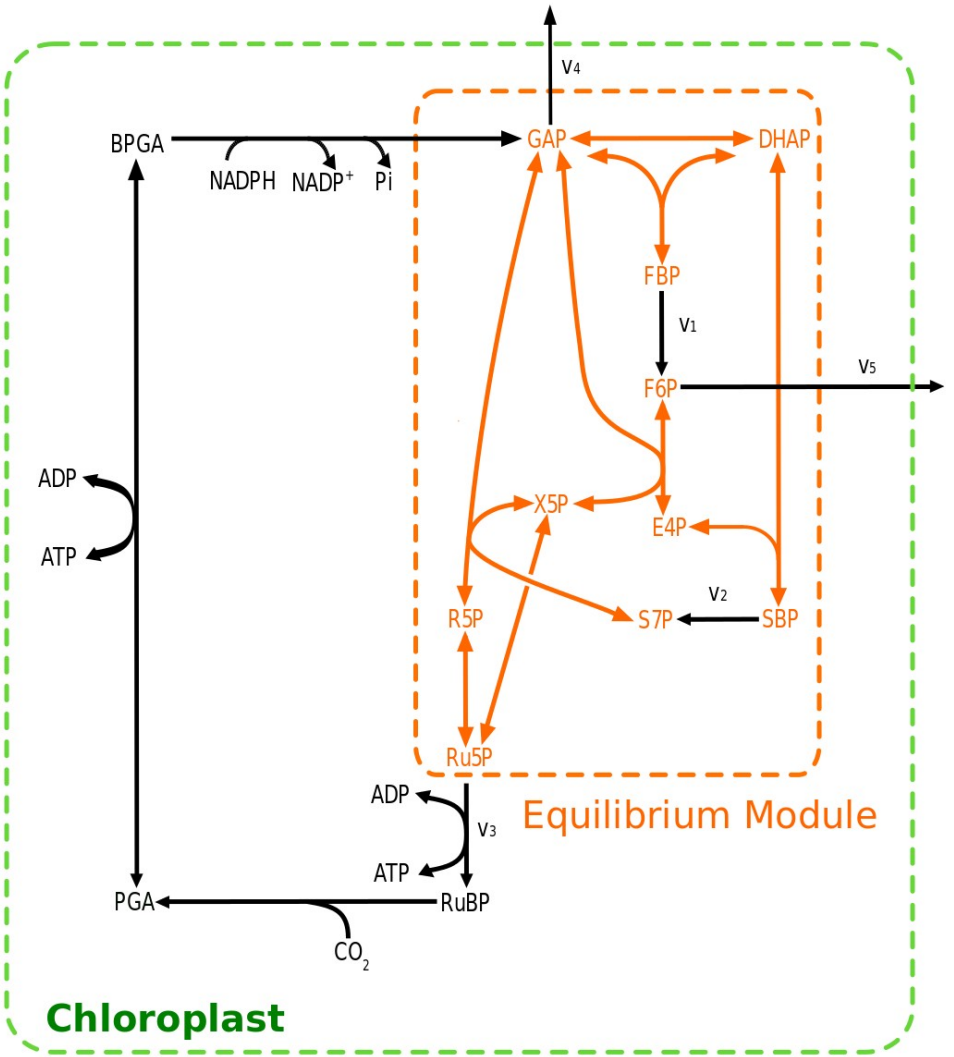
UNSTABLE!



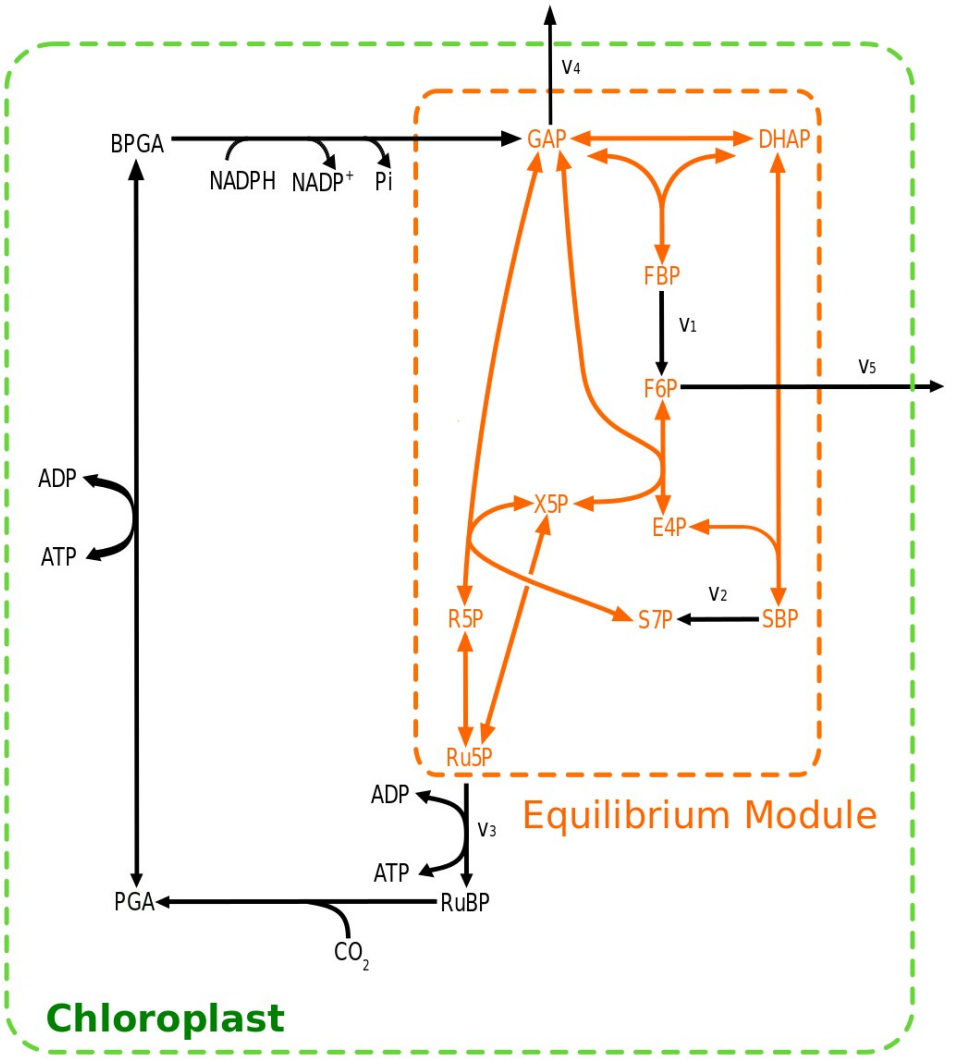


Trajectories diverge in direction of eigenvector belonging to (the only) positive eigenvalue of Jacobian

Closing the cycle

Second attempt: Michaelis-Menten

$$v_1 = V_{\max 1}[\mathrm{FBP}]/(K_{M1} + [\mathrm{FBP}])$$
$$v_2 = V_{\max 2}[\mathrm{SBP}]/(K_{M2} + [\mathrm{SBP}])$$
$$v_3 = V_{\max 3}[\mathrm{Ru5P}]/(K_{M3} + [\mathrm{Ru5P}])$$
$$v_4 = V_{\max 4}[\mathrm{GAP}]/(K_{M4} + [\mathrm{GAP}])$$
$$v_5 = V_{\max 5}[\mathrm{F6P}]/(K_{M5} + [\mathrm{F6P}])$$



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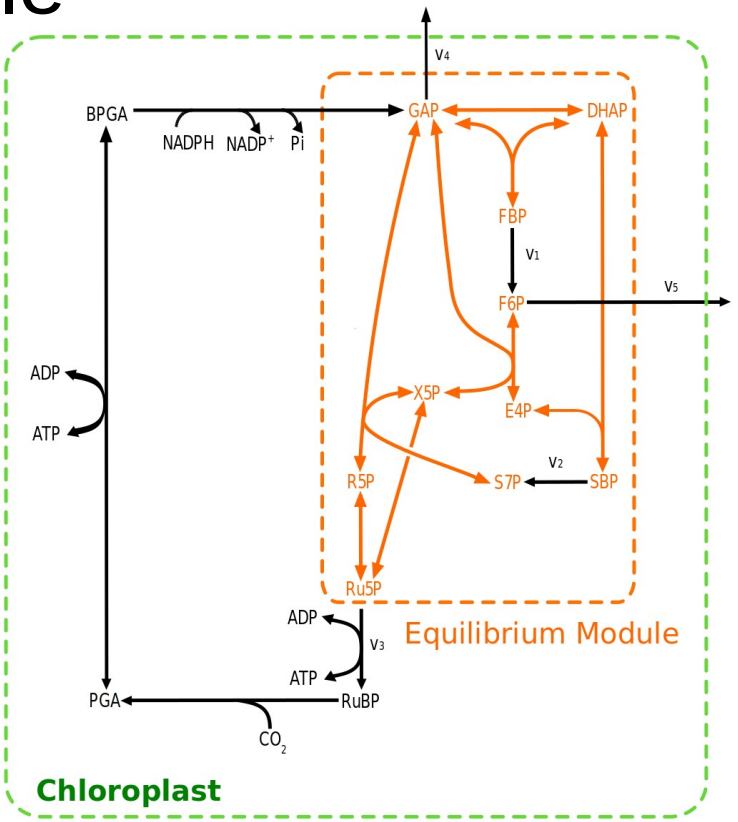

Finding 'good' $V_{\max}$ / K_M – values...

$$\dot{X} = N \cdot v(Y(X))$$

Jacobian $\quad J_{ik} = \sum_j n_{ij} \frac{\partial v_j}{\partial X_k}$ (this term: ε_{jk}) $\quad$ or $\quad J = N \cdot E$ ← elasticities ε_{jk}

$$\varepsilon_{jk} = \frac{\partial v_j}{\partial X_k} = \sum_e \frac{\partial v_j}{\partial Y_j} \cdot \frac{\partial Y_e}{\partial X_k}$$

$\frac{\partial v_j}{\partial Y_j}$ → η_{jk} $\quad$ $\frac{\partial Y_e}{\partial X_k}$ → Θ_{ek} (known)

or $\quad J = N \cdot H \cdot \Theta$ (3×5 · 5×10 · 10×3)

Optimising elasticities for stability

For irreversible reactions without allosteric regulation:

$$H = \begin{pmatrix} & & \varepsilon_1 \\ & \varepsilon_3 & \varepsilon_2 \\ \varepsilon_4 & \varepsilon_5 & \end{pmatrix}$$ only 5 non-zero

$$J = N \cdot H \cdot \Theta \quad (3\times5)\,(5\times10)\,(10\times3)$$

For mass-action kinetics $v_j = k_j \cdot X \quad : \varepsilon_j = k_j$

Define: $\Lambda(\varepsilon_j) := \max_{\lambda} \{ Re(\lambda) : \det(\lambda \cdot \mathbb{1} - J) = 0 \}$ maximal Eigenvalue

FIND STABLE SOLUTION BY MINIMISING Λ

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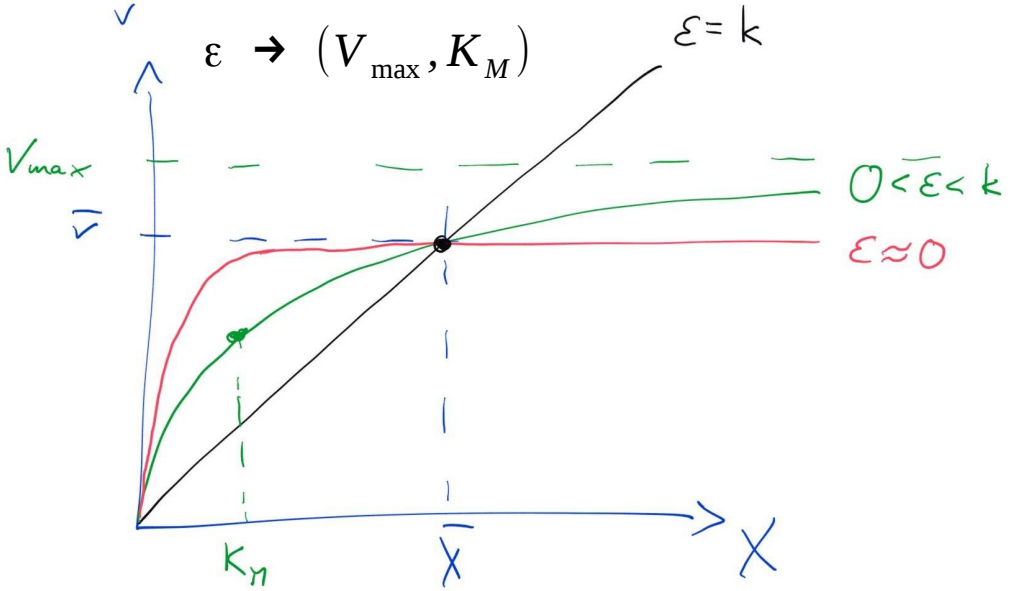

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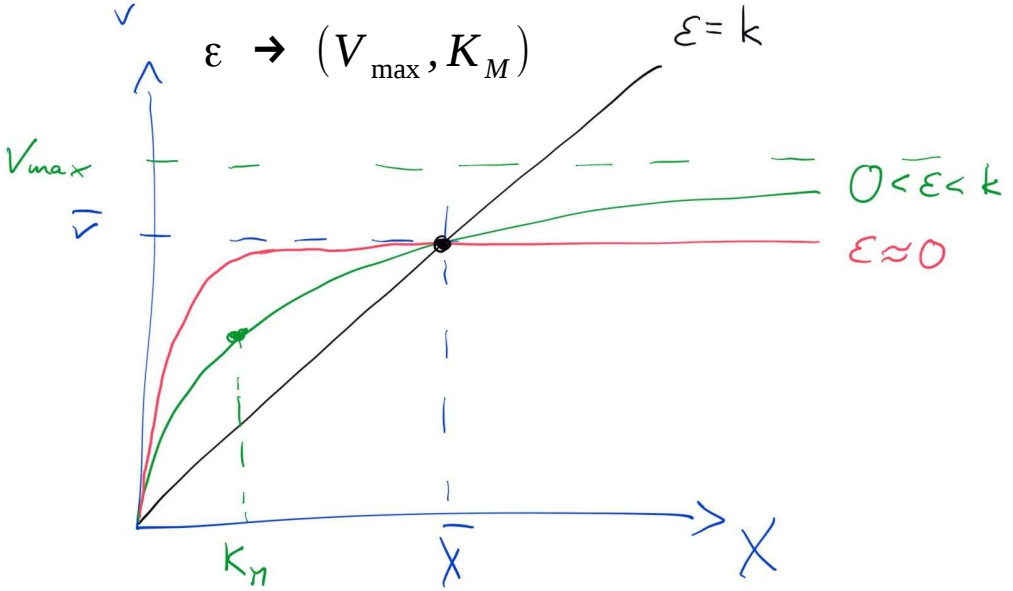
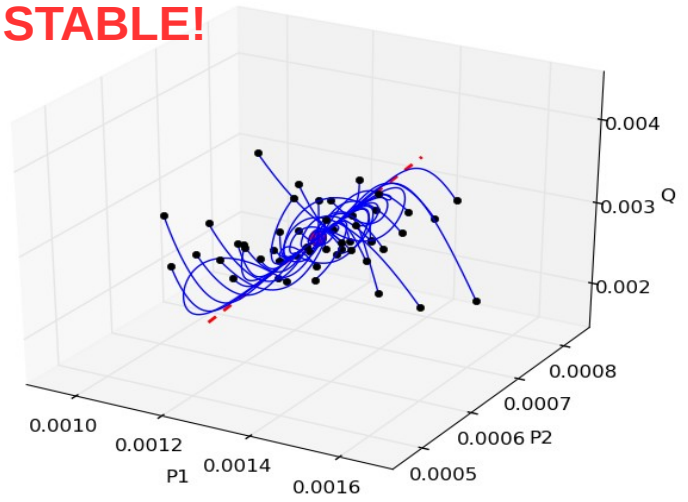
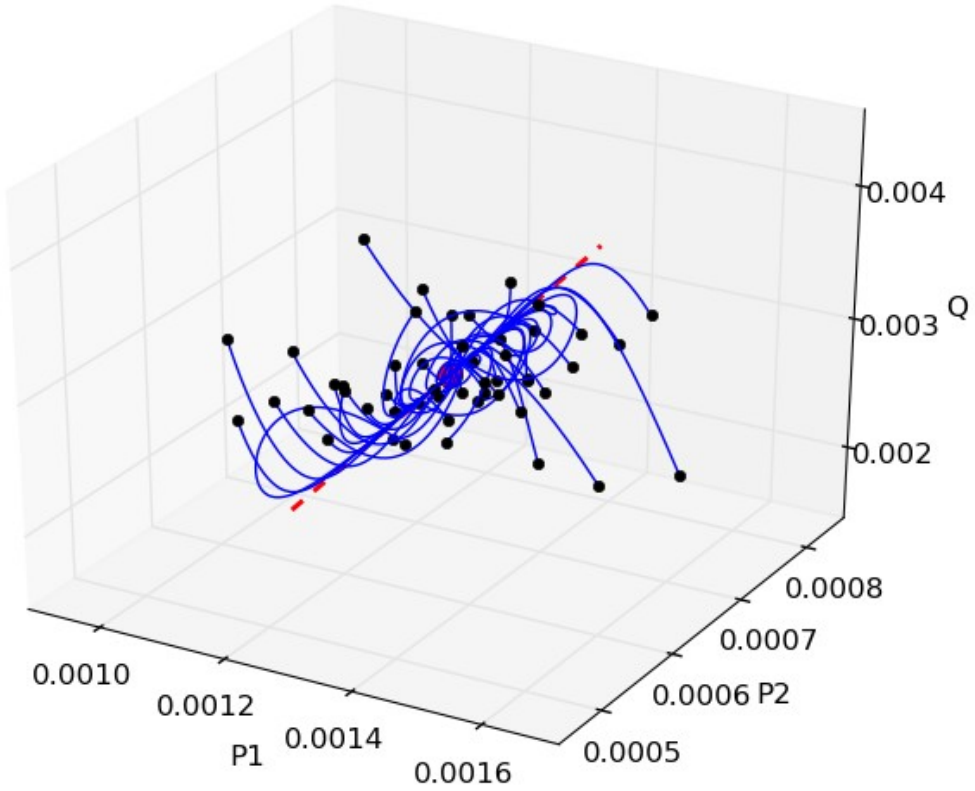
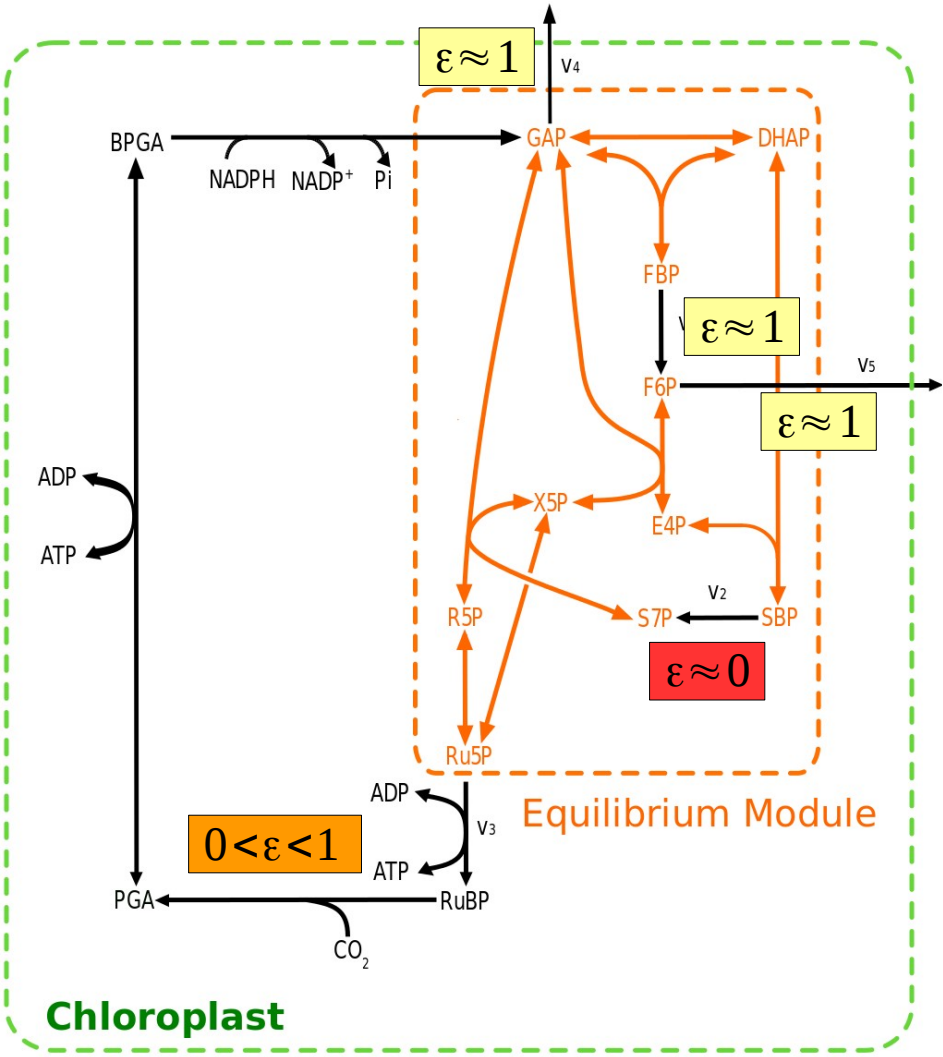


STABLE!

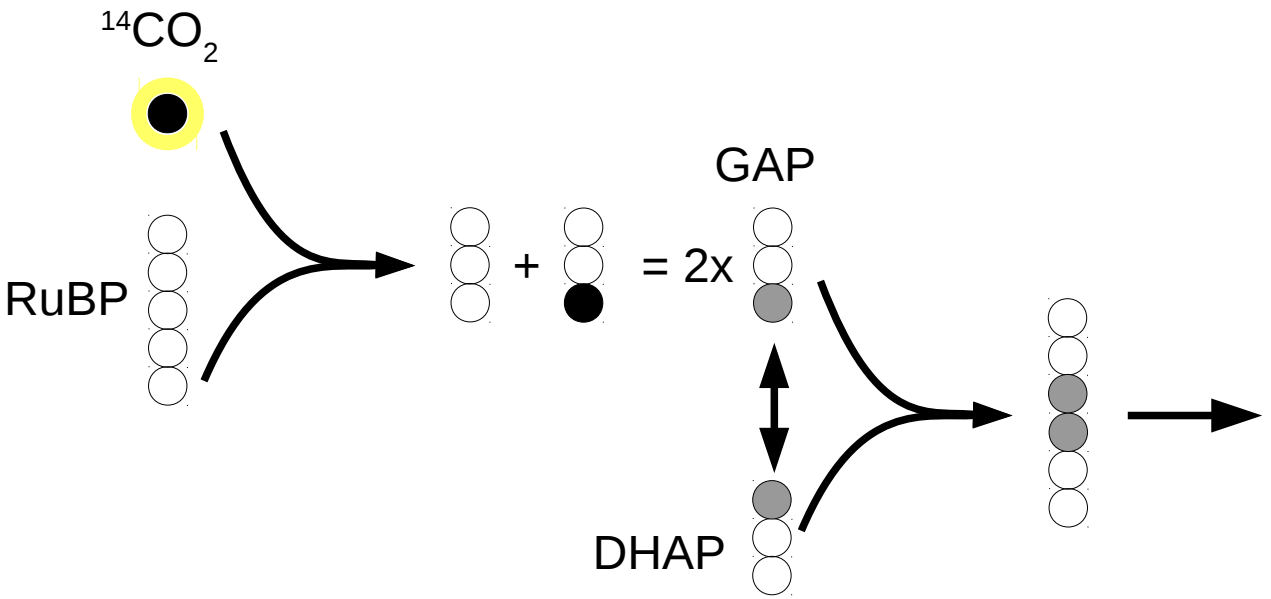


“Predicted” elasticities





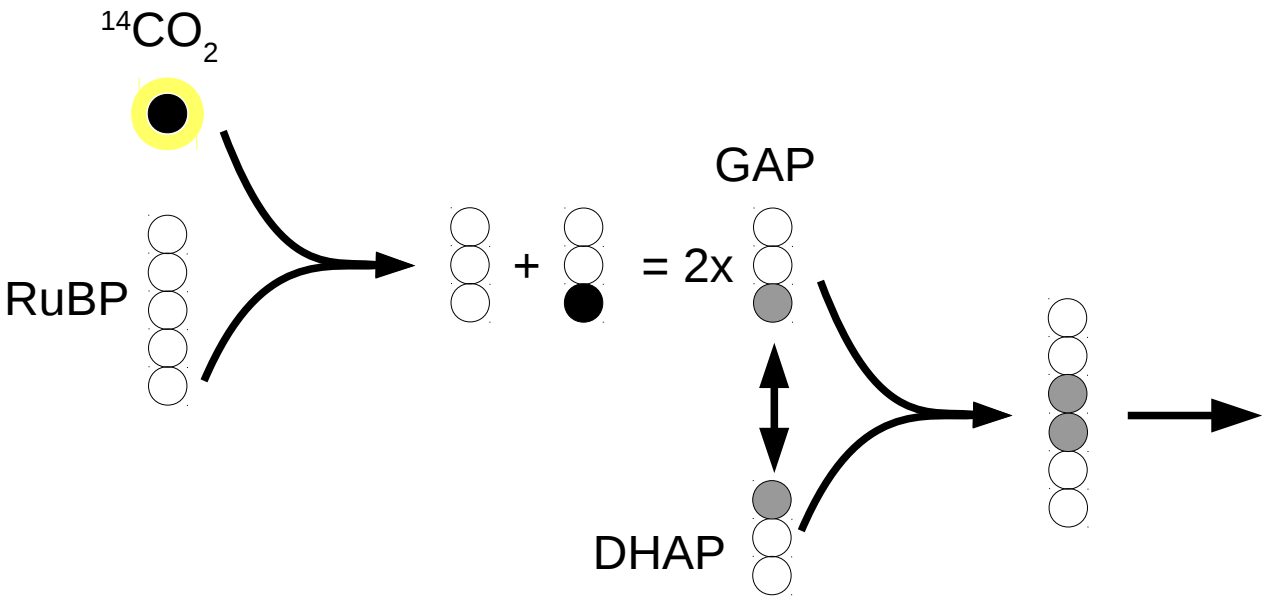
The photosynthetic Gibbs effect



Intuitive (naïve) assumption:

Label should appear symmetrically in position 3 and 4

The photosynthetic Gibbs effect



Intuitive (naïve) assumption:

Label should appear symmetrically in position 3 and 4

But (Gibbs & Kandler, 1957, PNAS): Label appears first in position 4!

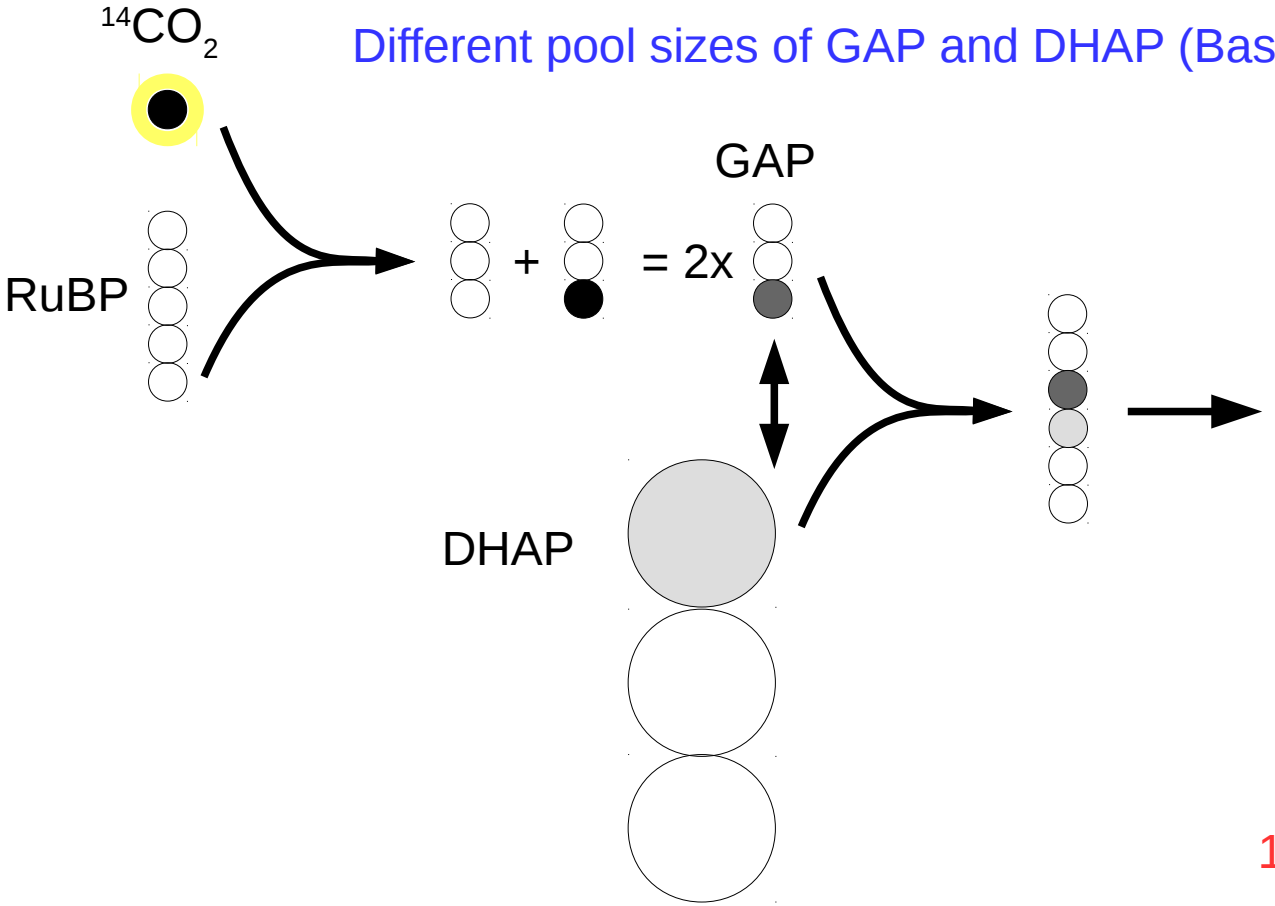
TABLE 1

DISTRIBUTION OF C^{14} IN GLUCOSE

PLANT	LIGHT INTENSITY (FOOT-CANDLES)	TIME	GLUCOSE SOURCE	TRACER CONTENT OF GLUCOSE CARBON ATOMS (MμC/MGC) 1	2	3	4	5	6
*Chlorella**	4,000	10 sec.	Starch	0.35	0.27	3.67	4.90	0.10	0.16
Chlorella†	4,000	60 sec.	Starch	1.16	1.15	5.16	7.00	0.42	0.46
Chlorella‡	700	45 min.	Starch	22.5	22.8	25.4	26.4	22.5	23.3
Tobacco§	4,000	50 sec.	Starch	2.69	4.30	11.0	18.6	1.17	2.99
Tobacco§	100	180 sec.	Starch	8.55	10.7	25.9	37.5	9.12	8.21
Sunflower§	70	15 min.	Sucrose	0.55	0.60	1.20	2.29	0.48	0.54
Canna	2,000	24 hrs.	Sucrose	5.36	5.16	5.19	5.08	5.08	5.12

Simple explanation for 3 and 4

Different pool sizes of GAP and DHAP (Bassham, 1964, Ann. Rev. Plant Phys.)



Label appears first in position 4

1>6, 2>5, 5 last

What about the other positions?

Bassham 1964:
"...because of the reversibility of transketolase..."

Glucose Source	Tracer Content of Glucose Carbon Atoms (MμC/mgC)					
	1	2	3	4	5	6
Starch	0.35	0.27	3.67	4.90	0.10	0.16
Starch	1.16	1.15	5.16	7.00	0.42	0.46
Starch	22.5	22.8	25.4	26.4	22.5	23.3
Starch	2.69	4.30	11.0	18.6	1.17	2.99
Starch	8.55	10.7	25.9	37.5	9.12	8.21

A dynamic model of isotope label distribution

Workflow

- stable Michaelis-Menten model, as developed above
- parameters to fit some measured steady-state
- multiply each metabolite by all possible isotope patterns ($2^{\#C}$):
  total 512 metabolites
- multiply each reaction by all possible isotope patterns of substrates:
  total 13368 rate expressions

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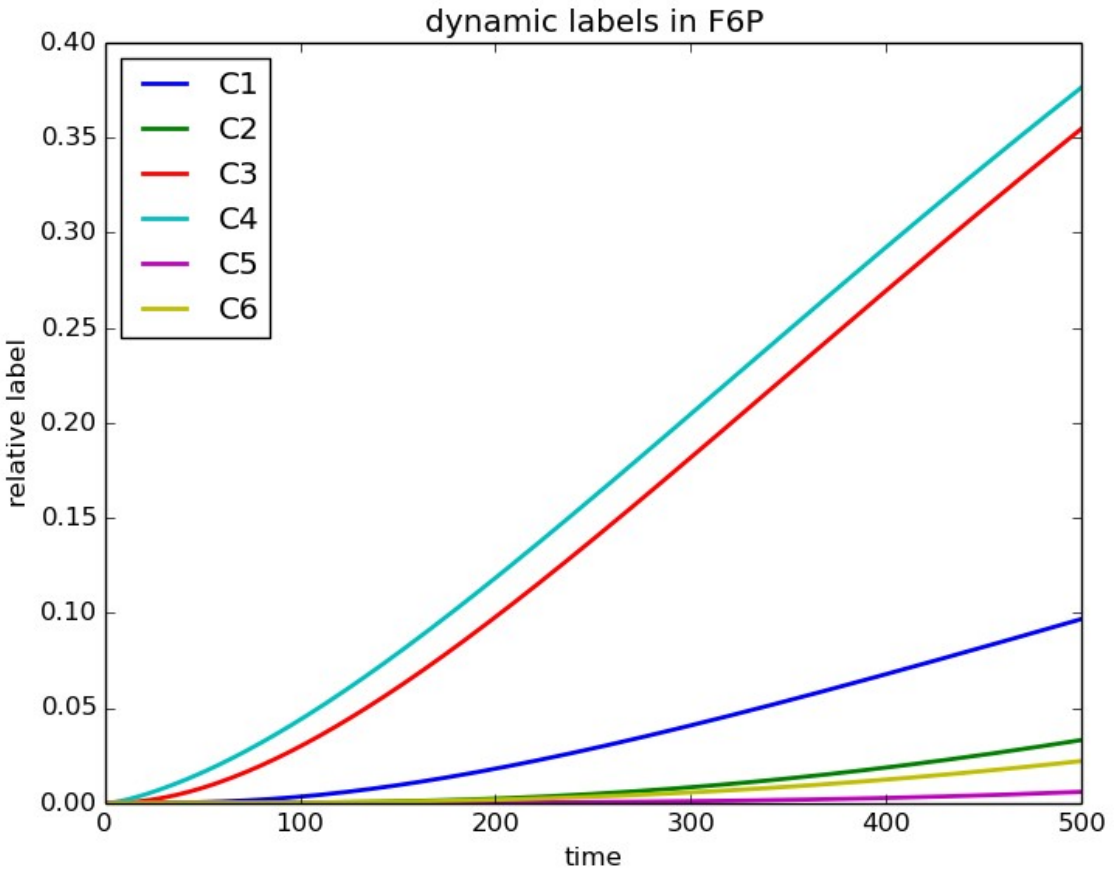
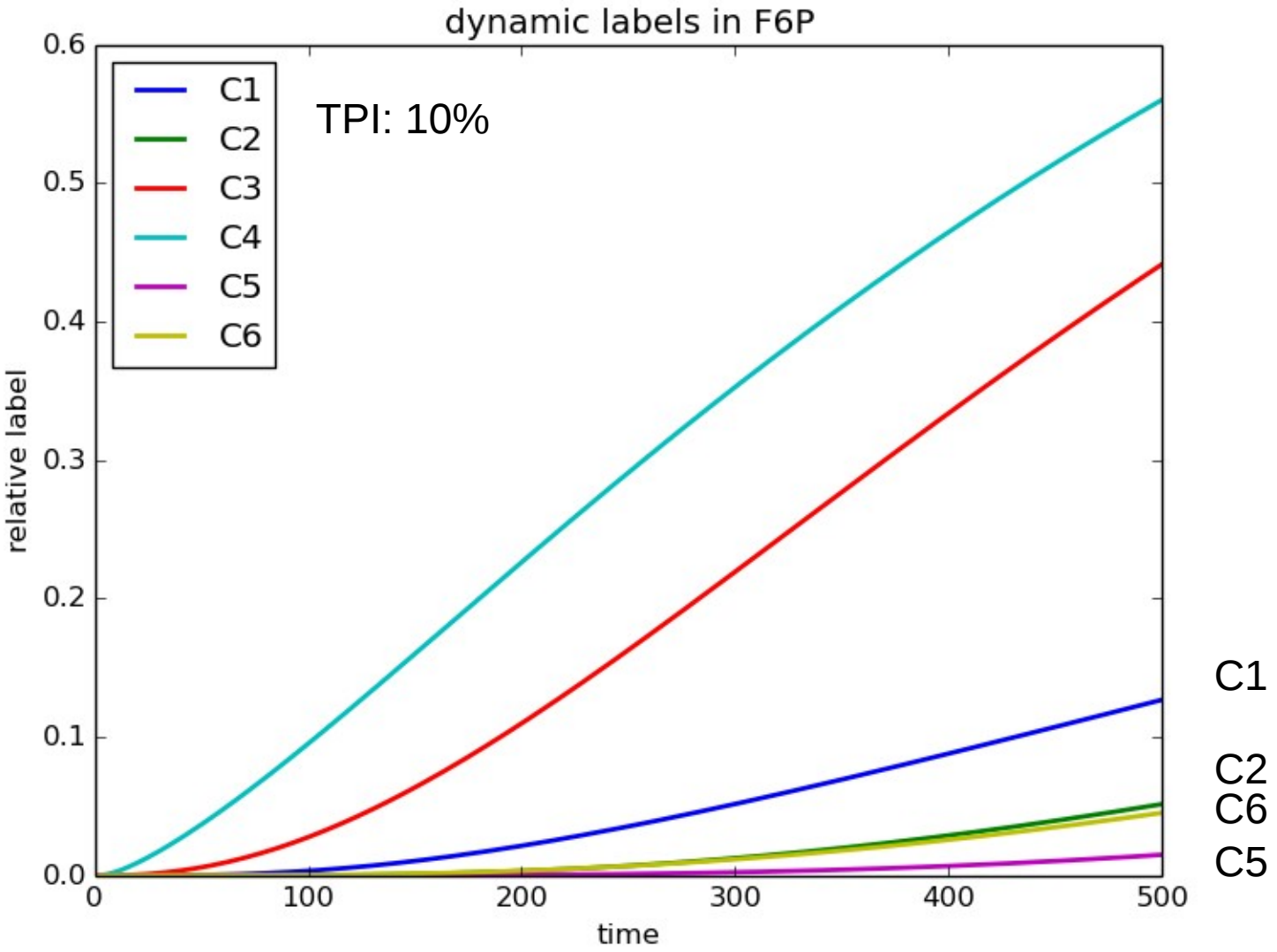
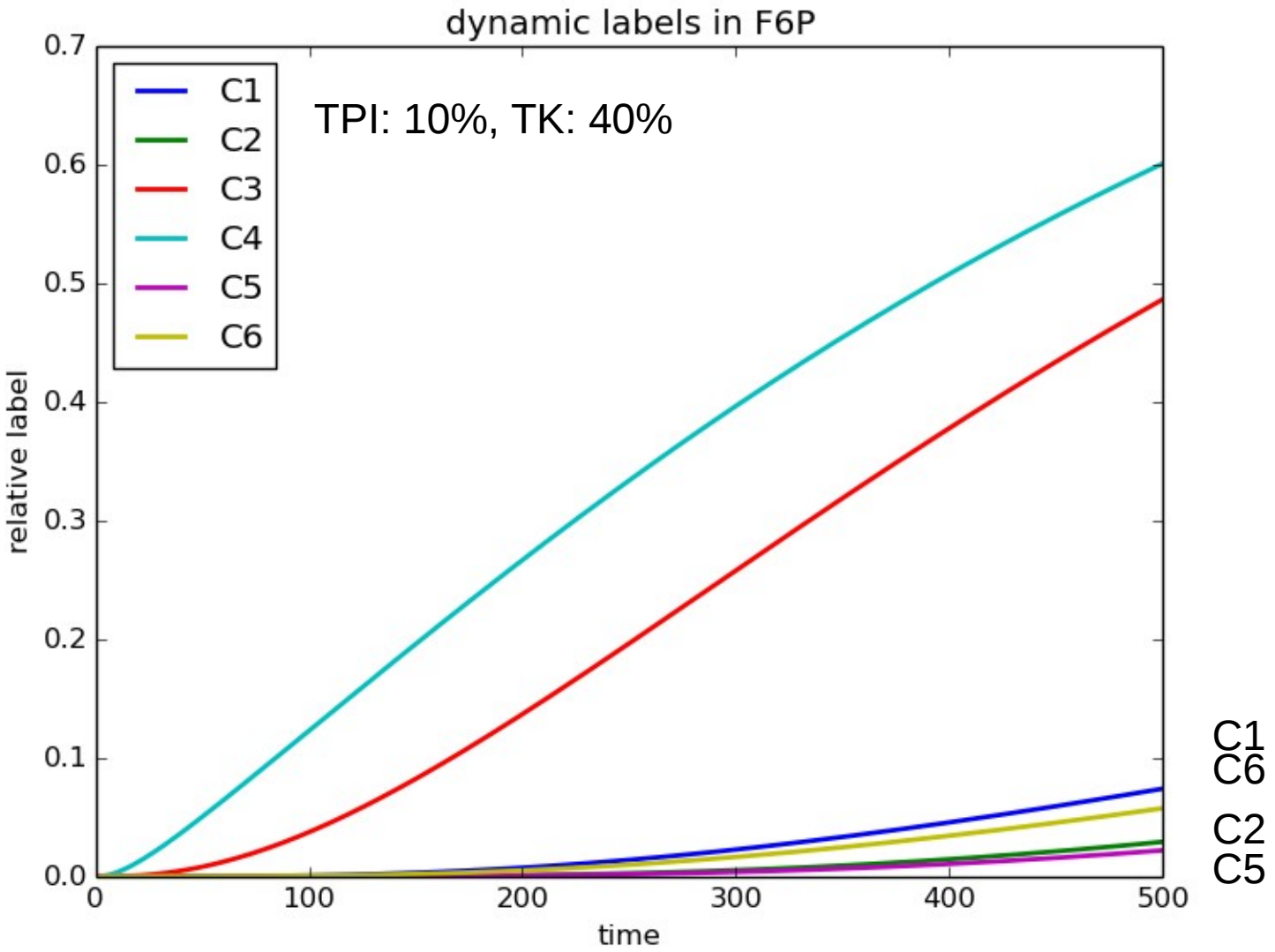


Slow TPI pronounces asymmetry



TK activity influences other labels



Conclusions

A minimal model of the Calvin-Benson-Bassham Cycle. Why bother?

- Modelling is simplification!
  - *“Simplicity is the ultimate sophistication”* (Leonardo da Vinci)
  - Simple designs allow for general conclusions and deeper understanding
- A (stable) minimal model serves as an easy-to-use module
  - more complex metabolic models
  - link with photosynthetic electron transport chain models
- Forms the basis for exploring dynamic isotope labelling
  - The Gibbs effect can be easily explained
  - It is an emergent property of the CBB cycle
  - We can understand which processes influence label dynamics

Thank you

Collaborators:

Experiments: Martin Steup (Potsdam)
Sebastian Mahlow
Sam Zeeman (Zurich)
Barbara Pfister
Rob Field (Norwich)
Mike Rugen
Douglas Murray (Tsuruoka)

Theory: Önder Kartal (Zurich)
Alexander Skupin (Luxemburg)

QTB Institut für Quantitative & Theoretische Biologie

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CEPLAS Cluster of Excellence on Plant Sciences

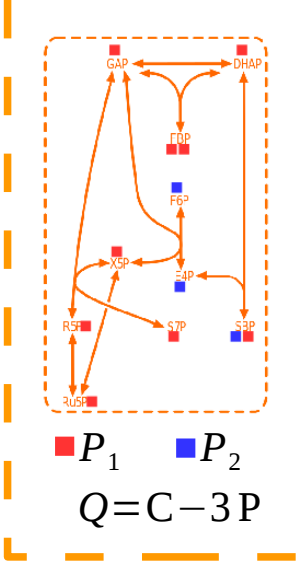
Internet: http://qtb.hhu.de
Public wiki: http://wiki.hhu.de/
Software & Models: http://github.com/QTB-HHU
@qtbduesseldorf

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?

Solving the equilibrium module



3 equations with 3 unknowns:

GAP Lagrange multiplier E4P

$$P_1 = x_0\left(f_0 + \kappa_2 f_2 z + \kappa_4 f_4 z^2\right) + 2g x_0^2 + g_1 x_0 x_1$$

$$P_2 = x_1\left(1 + \kappa_3 z\right) + g_1 x_0 x_1$$

$$Q = x_0\left(2 f_2 \kappa_2 z + 4 f_4 \kappa_4 z^2\right) + x_1\left(1 + 3\kappa_3 z\right) + g_1 x_0 x_1$$

Notation:

x_k: compound with $k+3$ carbons

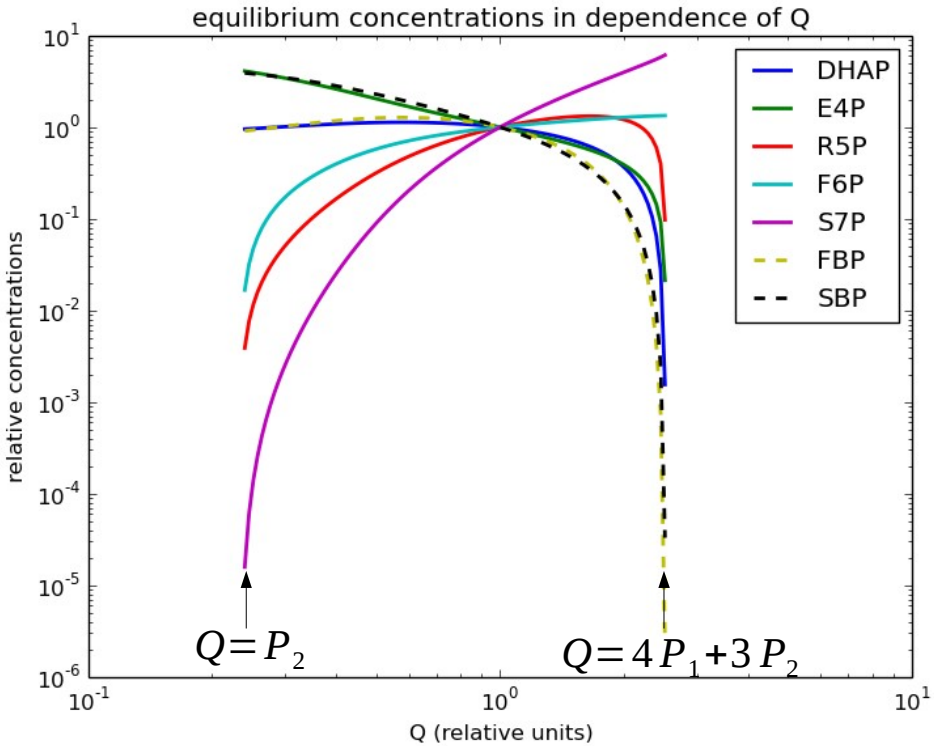
$$\Rightarrow \quad x_{k+2} = x_k \cdot e^{-\Delta\mu} \cdot z$$

Necessary condition: $P_2 < Q < 4P_1 + 3P_2$

What happens at the extremes?

$Q \rightarrow P_2$: accumulation of small sugars

$Q \rightarrow 4P_1 + 3P_2$: accumulation of large sugars



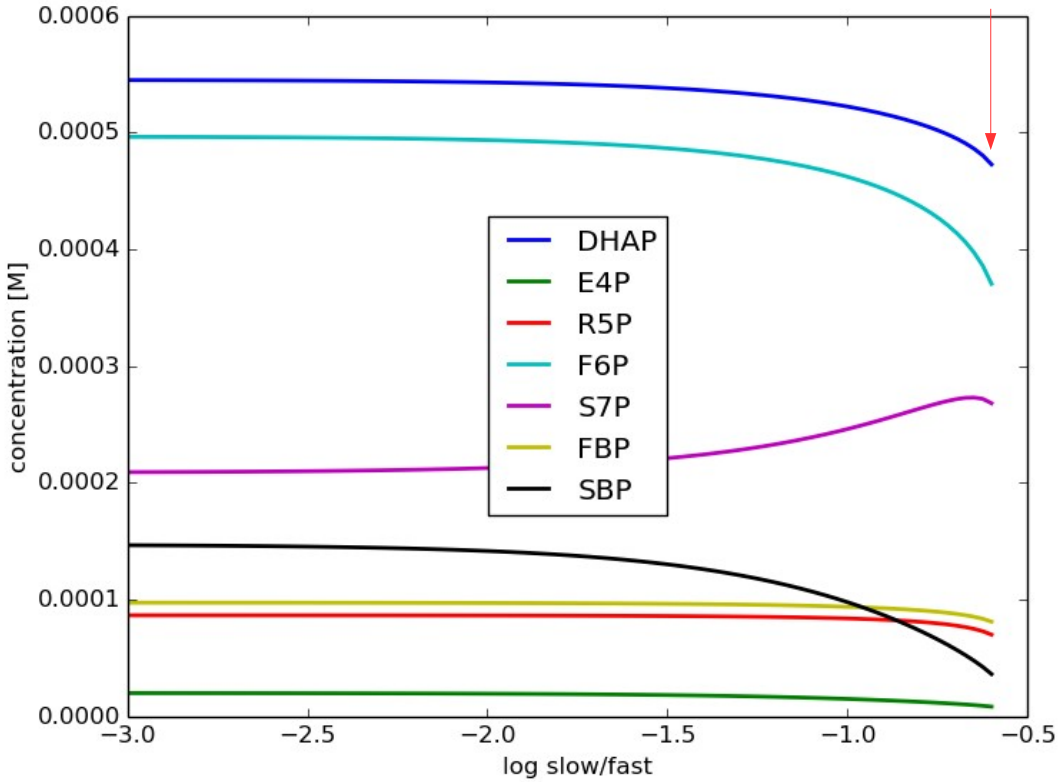
Back to the real world

What happens if the rapid equilibrium is not *exactly* fulfilled?

⇨

- Model the fast reactions as mass-action
- Tune the time-scale separation with one parameter

system breaks down



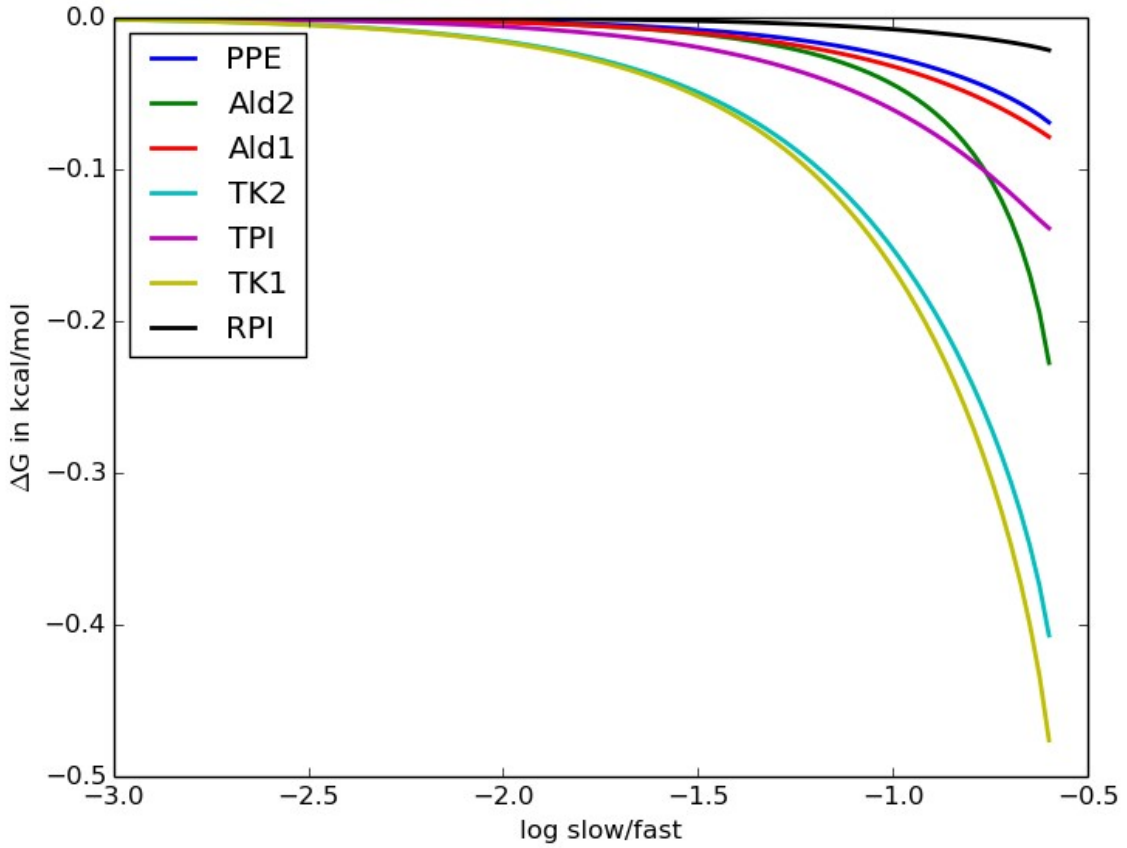
rapid equilibrium **similar time-scales**

When time-scales are not clearly separated, other regulatory mechanisms are necessary!

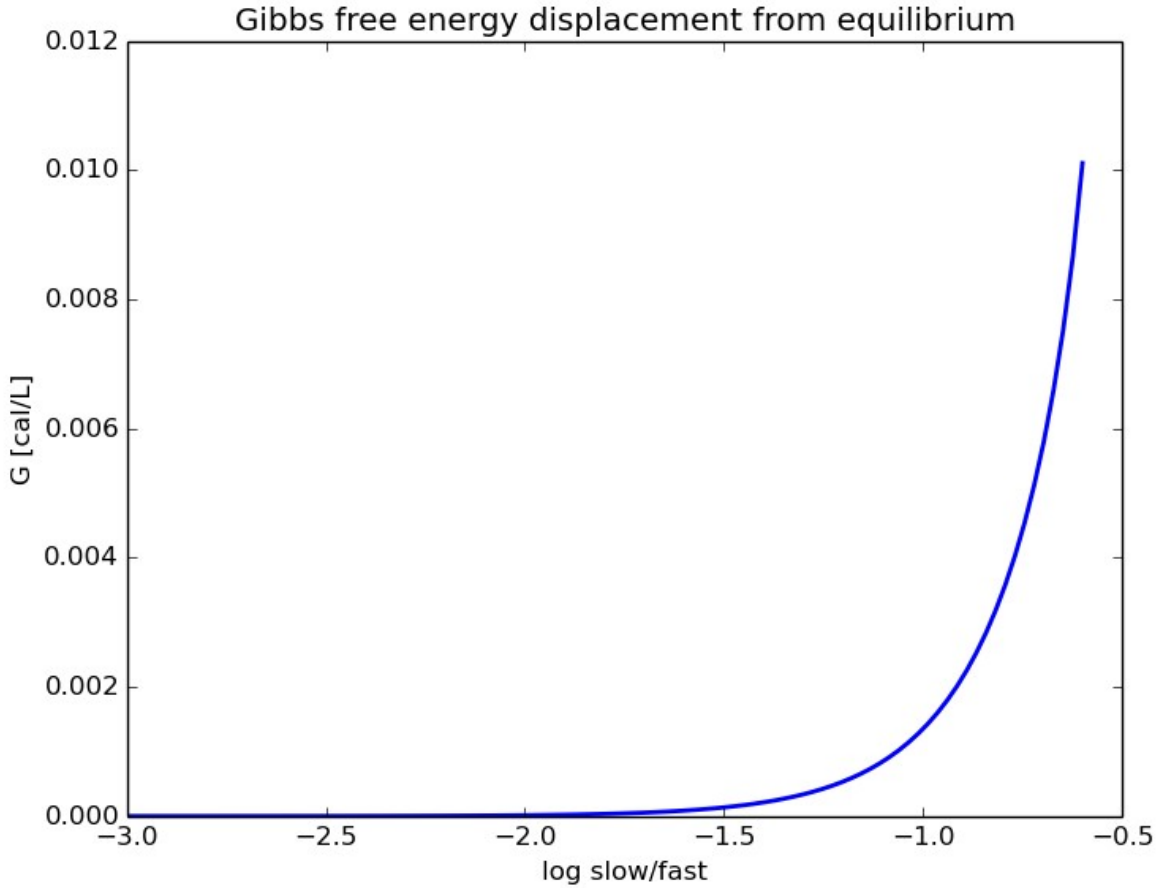
Displacement from equilibrium

The lowest ΔG is just -0.5 kcal/mol!

But Bassham measured -1.4...



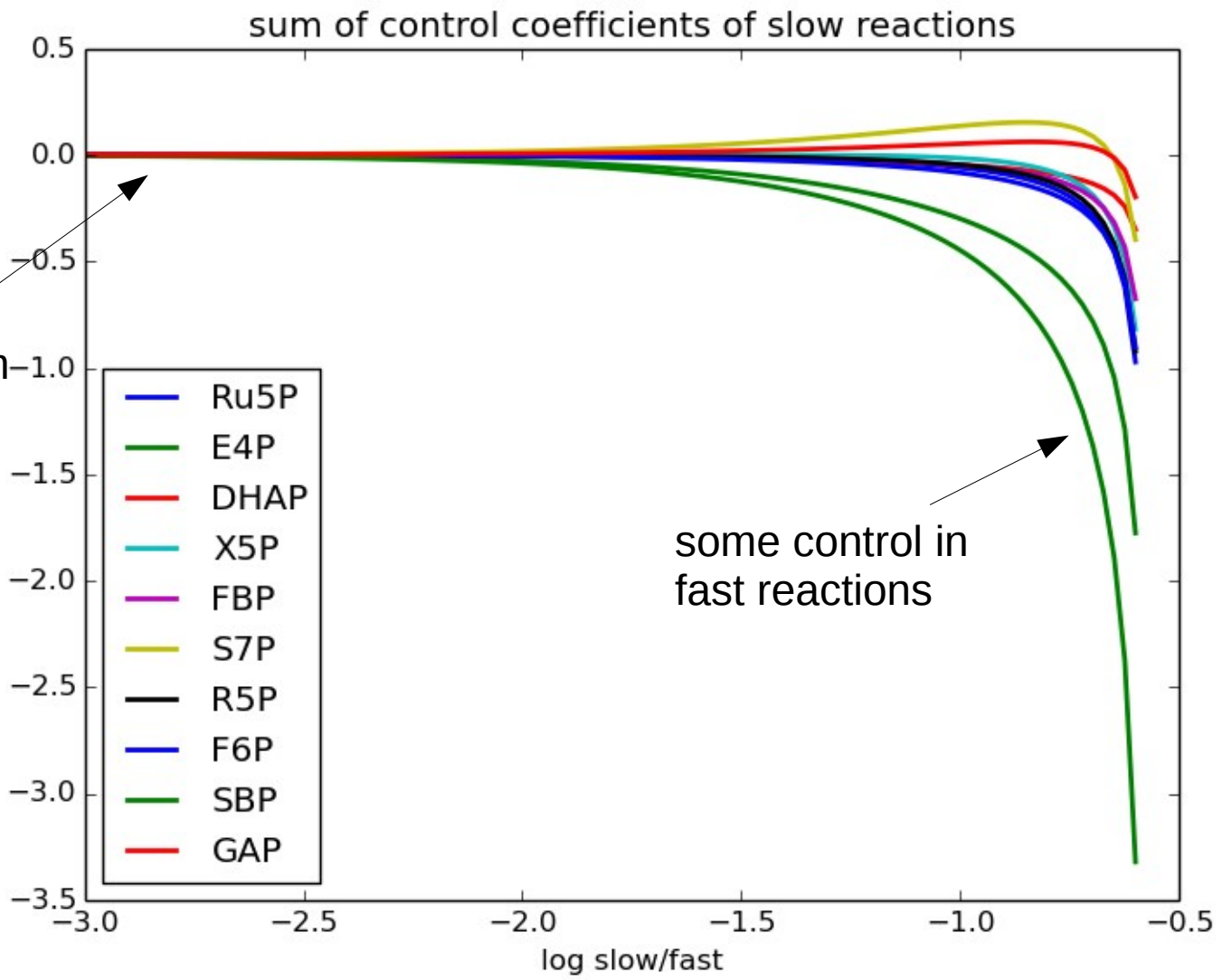
Total Gibbs free energy above equilibrium



$$G = \sum_{j \in M} x_j \mu_j + RT \cdot \sum_{j \in M} x_j \cdot (\ln x_j - 1)$$

Losing control

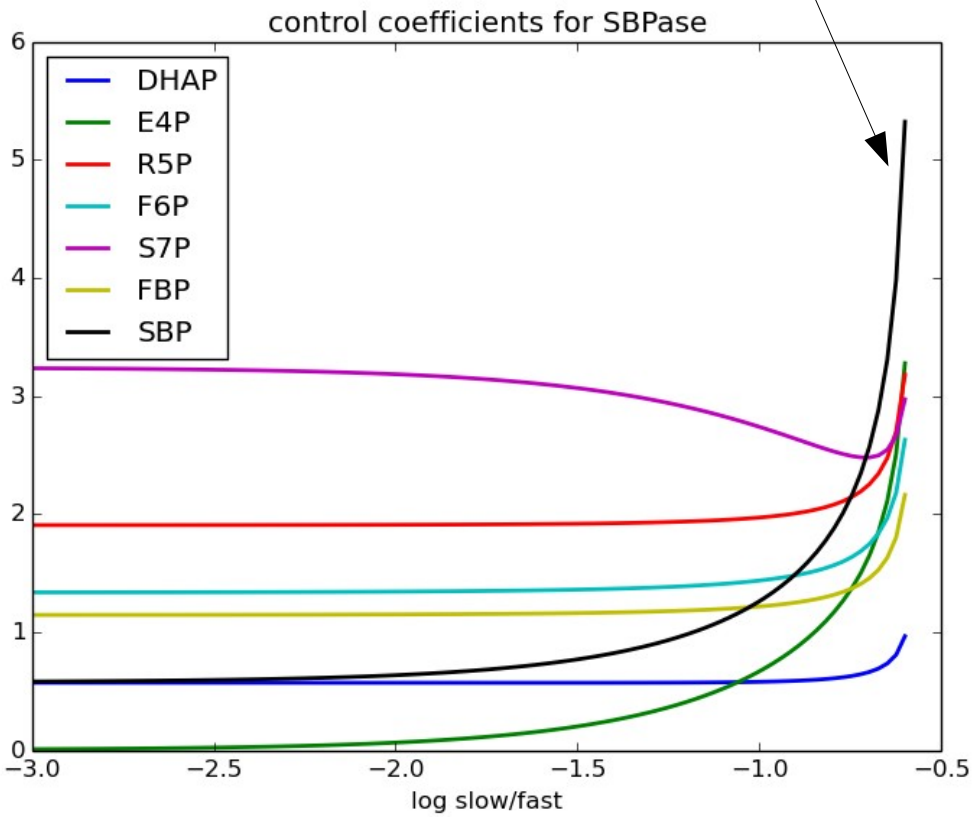
summation theorem fulfilled by slow reactions only!

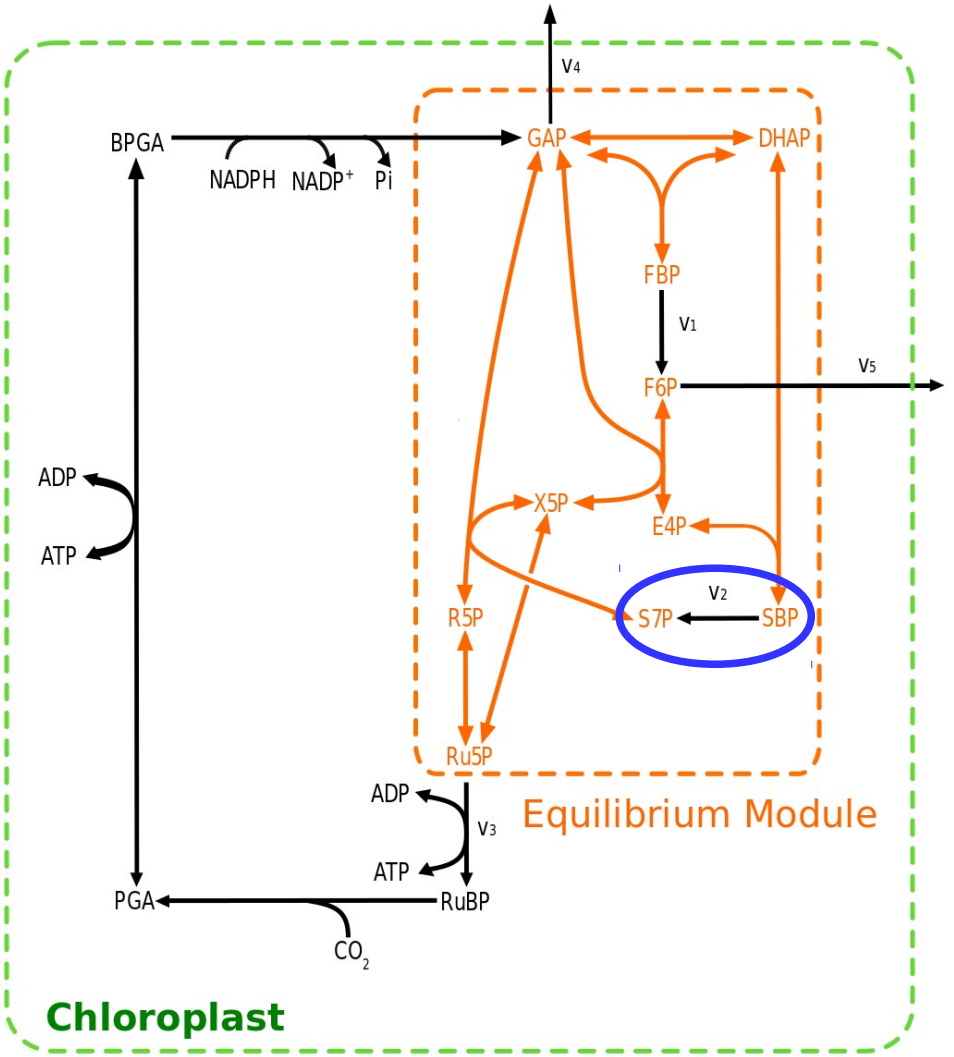


some control in fast reactions

The positive control of SBPase

Accelerating SBPase increases its substrate!!





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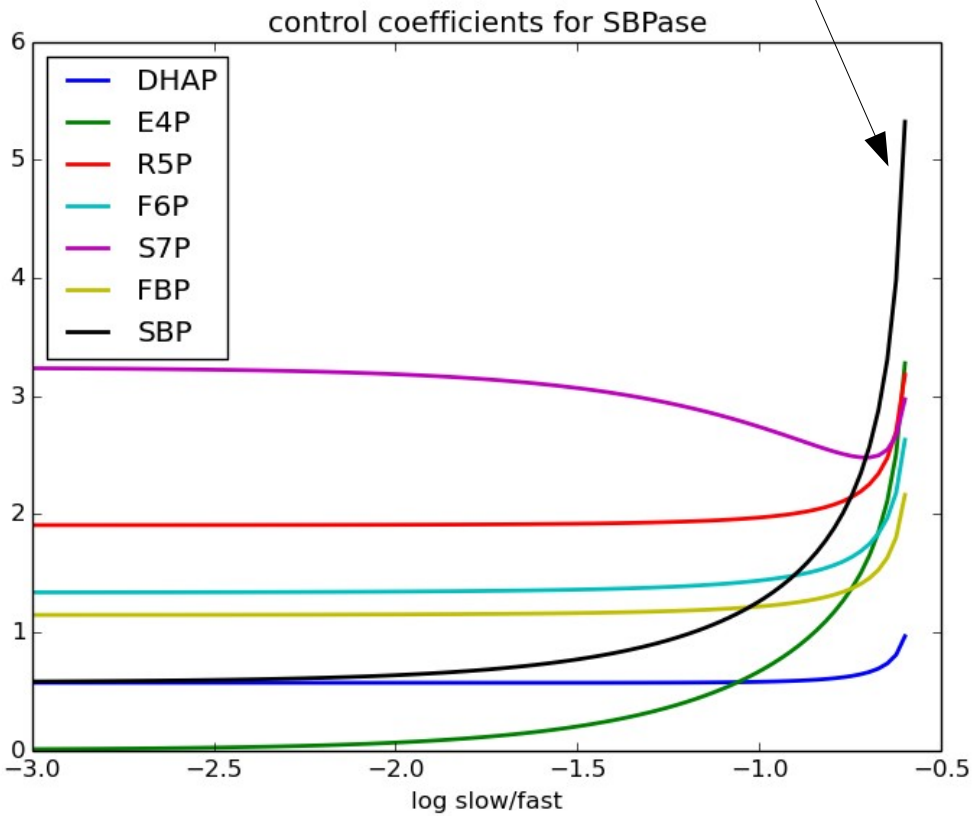

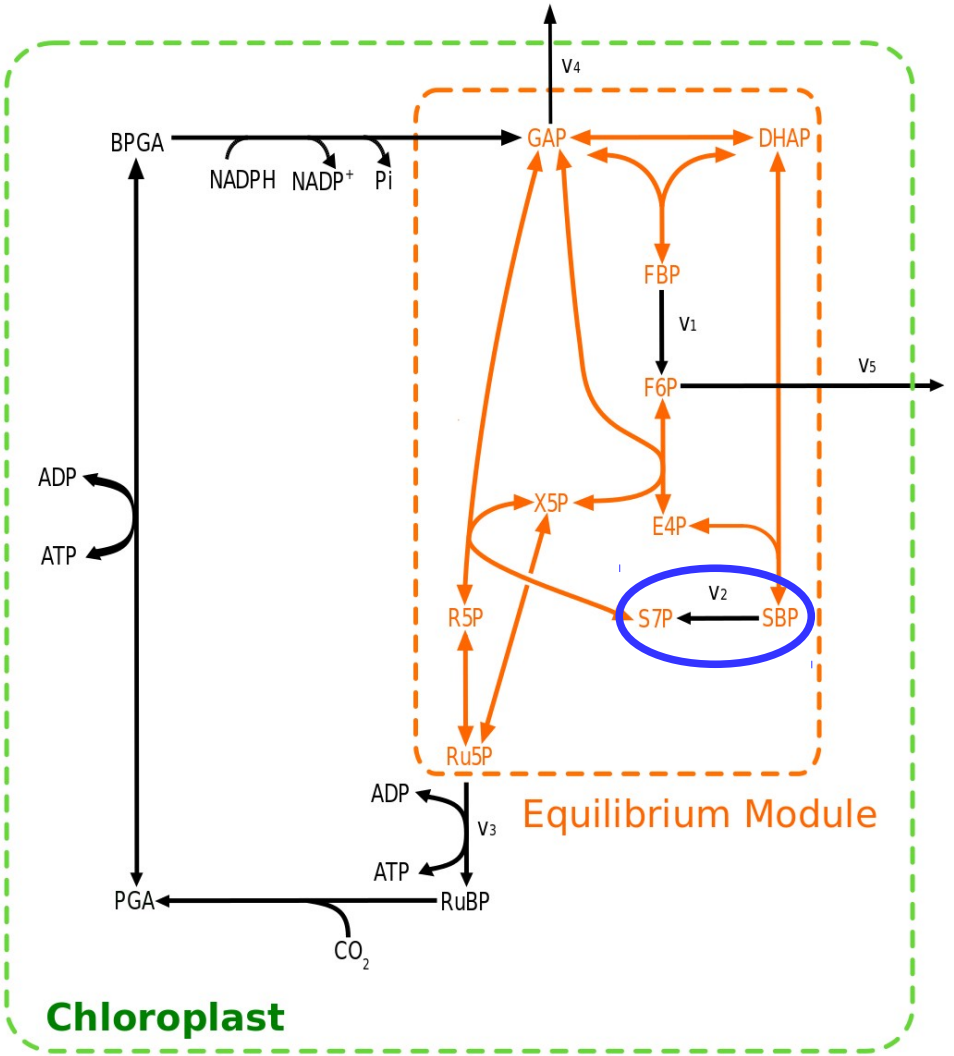

⇨ positive feedback! Stability problem...