Mathematical Models of Plant Energy Metabolism

Towards synthetic starch

Oliver Ebenhöh

www.accliphot.eu

aSSB Strasbourg, 25.3.2015

Why do we need mathematical models?

- Simplified representation of reality
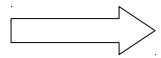- Reduction to the essentials

"Simplicity is the ultimate sophistication"

(Leonardo da Vinci)

Why do we need mathematical models?

- Simplified representation of reality

- Reduction to the essentials

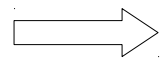### "Simplicity is the ultimate sophistication"

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Models help to discover general principles!

Why do we need mathematical models?

- Simplified representation of reality

- Reduction to the essentials

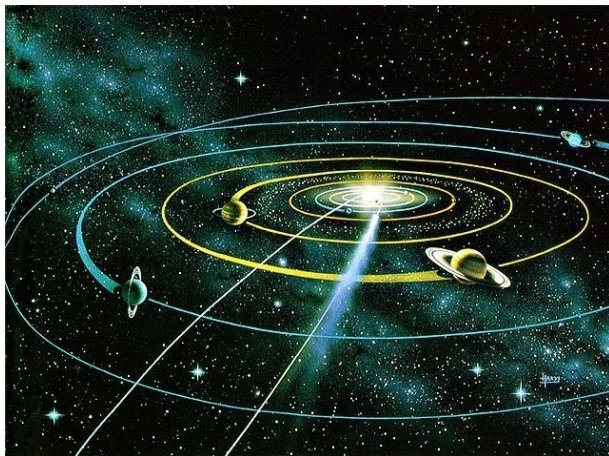"Simplicity is the ultimate sophistication"

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⟹ Models help to discover general principles!

Example from physics:

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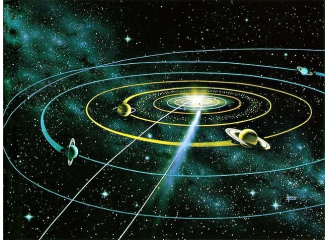

www.thehungryandfoolish.com
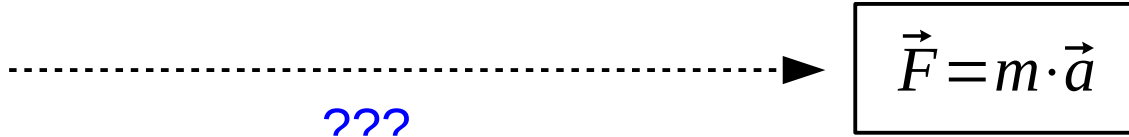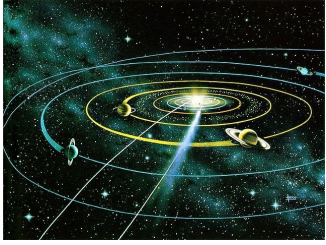
www.hh.schule.de

www.welt.de

How does one find principles (theory building)?

???

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

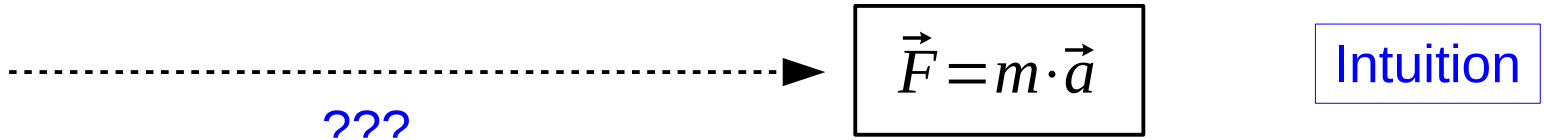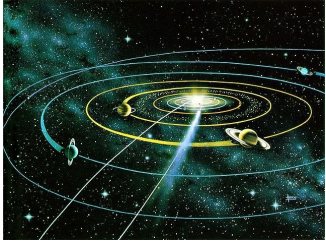# How does one find principles (theory building)?

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

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
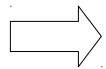- Improvement of experimental design

EXPERIMENT

Understanding

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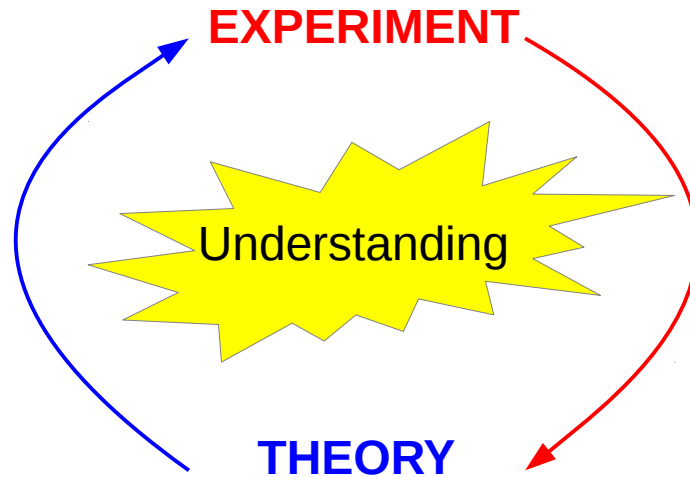
- Initial model formulation
- Confirmation / falsification of predictions
- New model assumptions

The Systems biology principle

What's special about plants?
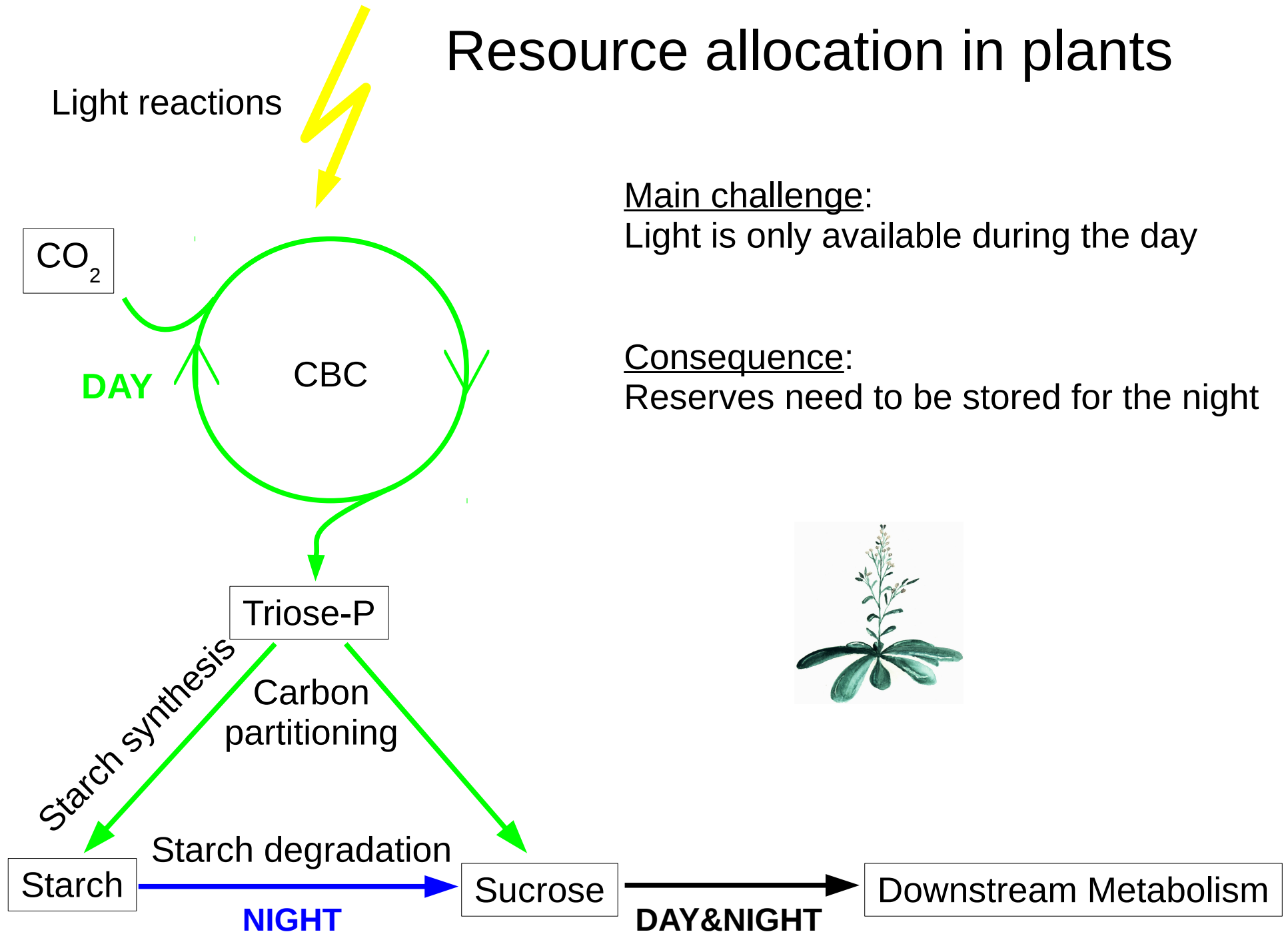
1. Photosynthesis

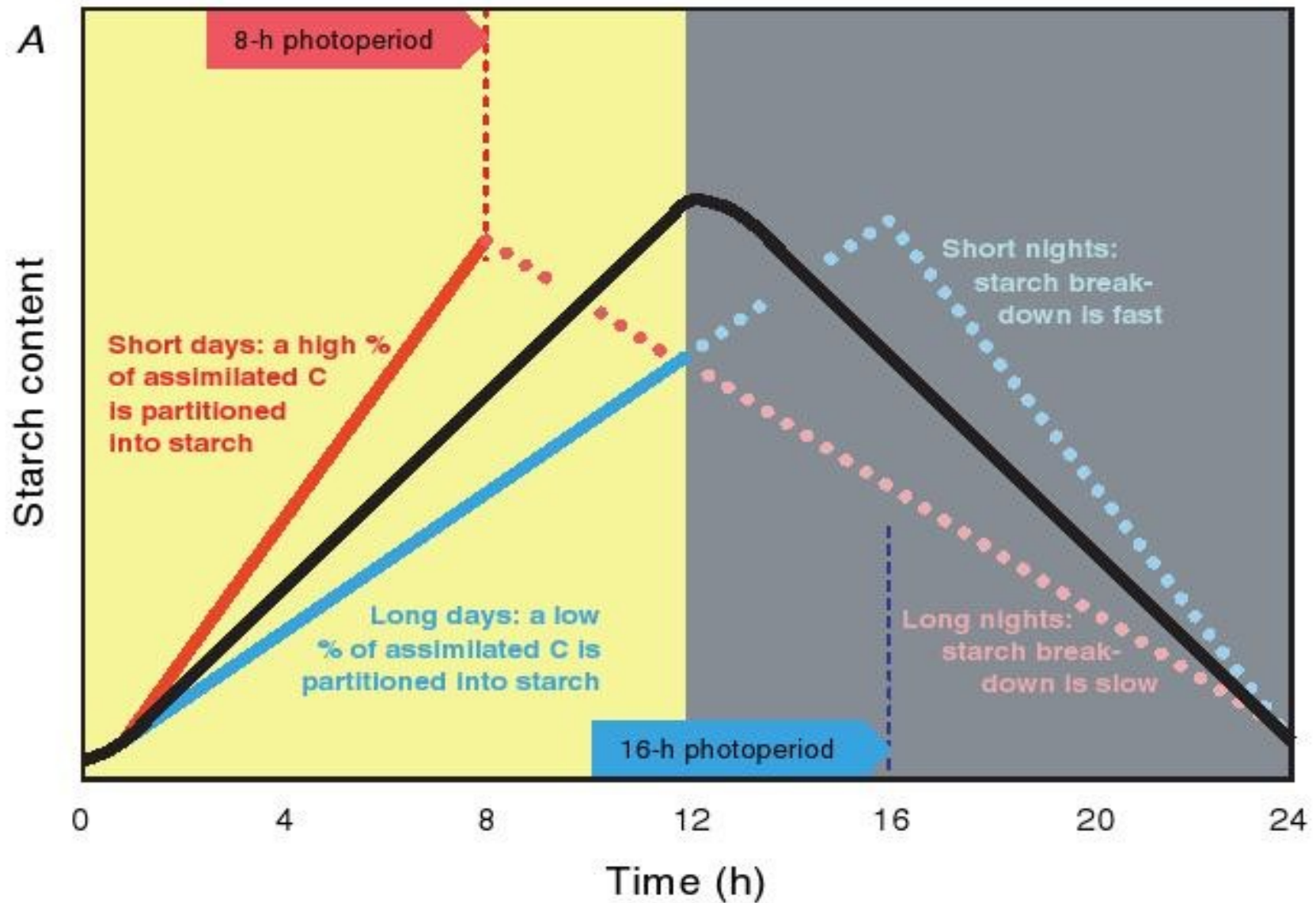2. Can't run away!

⟹   Experts in chemical warfare!

Estimated > 200,000 secondary metabolites!

Resource allocation in plants

Light reactions

CO$_2$

DAY

CBC

Triose-P

Starch synthesis

Carbon partitioning

Starch

Starch degradation

NIGHT

Sucrose

DAY&NIGHT

Downstream Metabolism

Main challenge:
Light is only available during the day

Consequence:
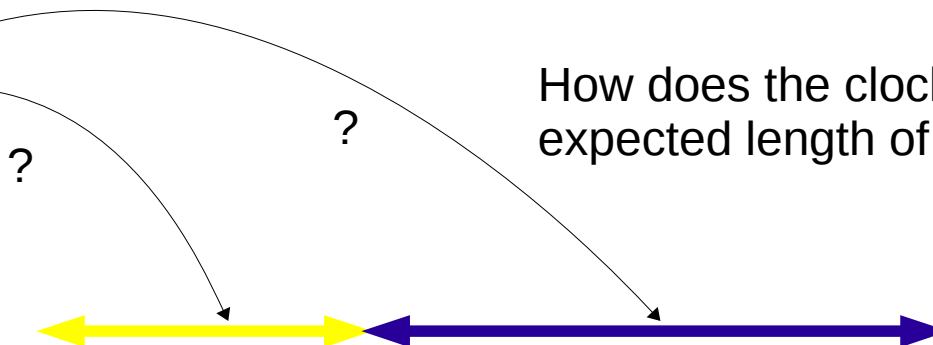Reserves need to be stored for the night
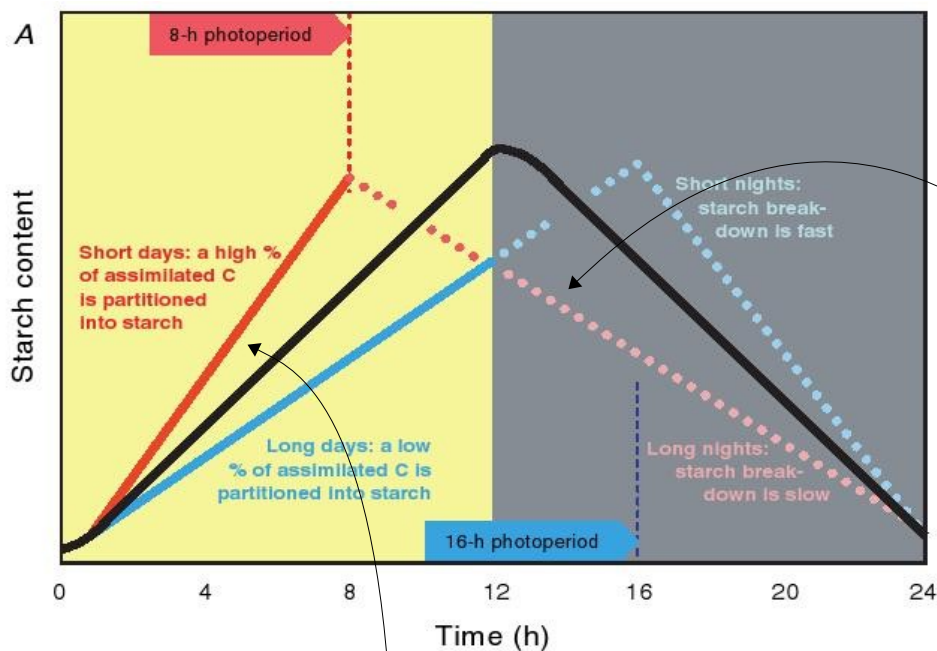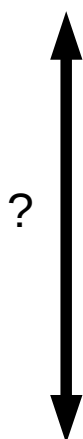
The diurnal turnover of starch

Open questions

How does the clock 'tell' expected length of day/night?
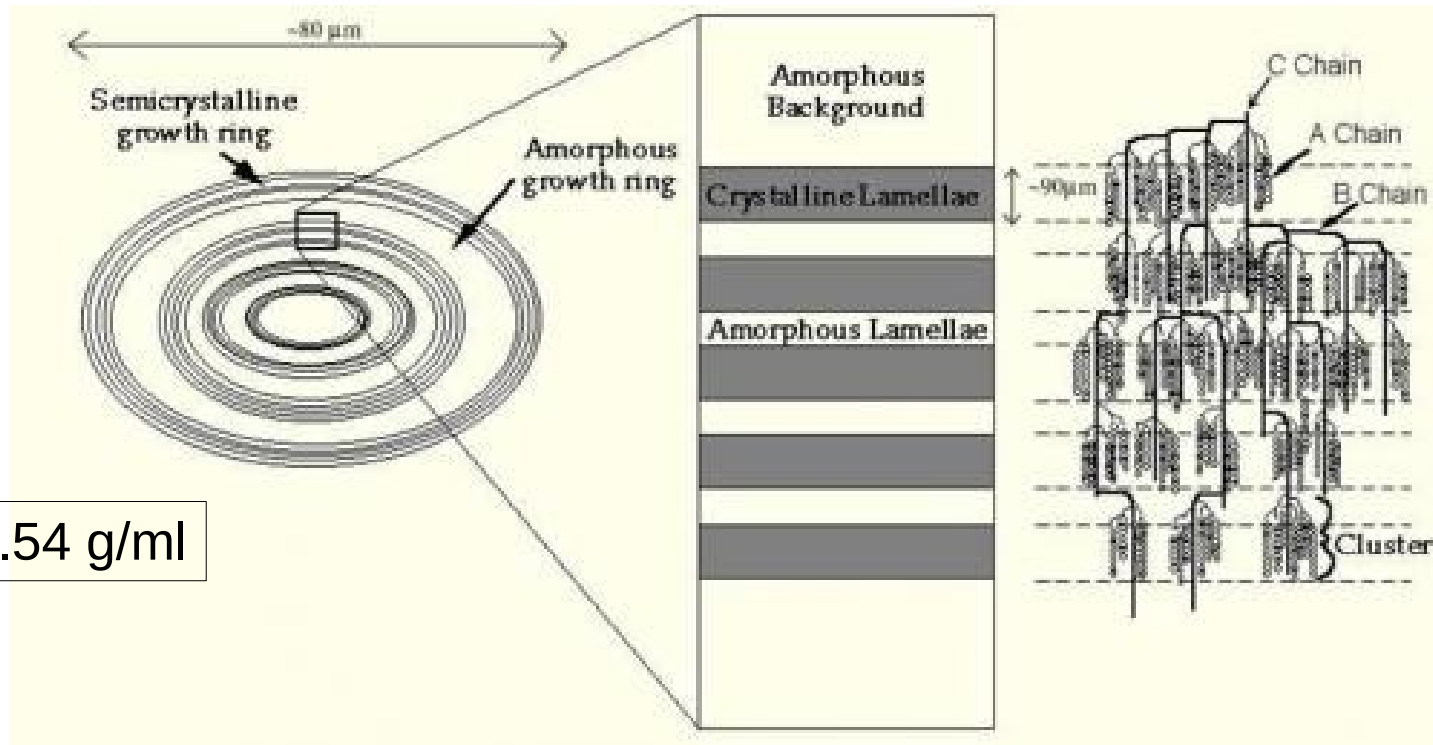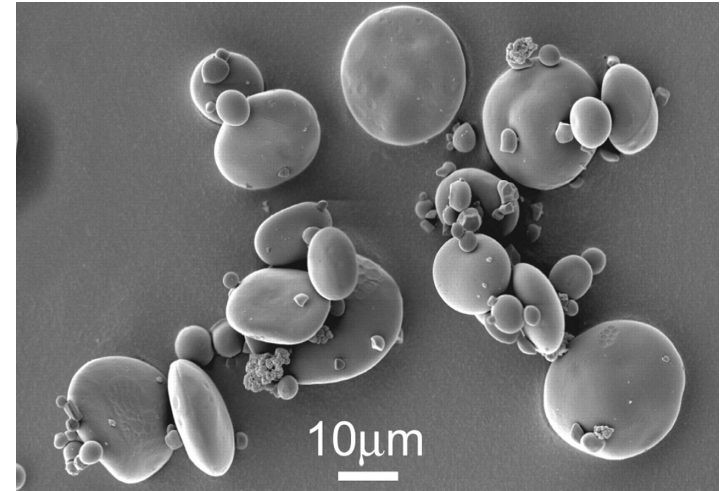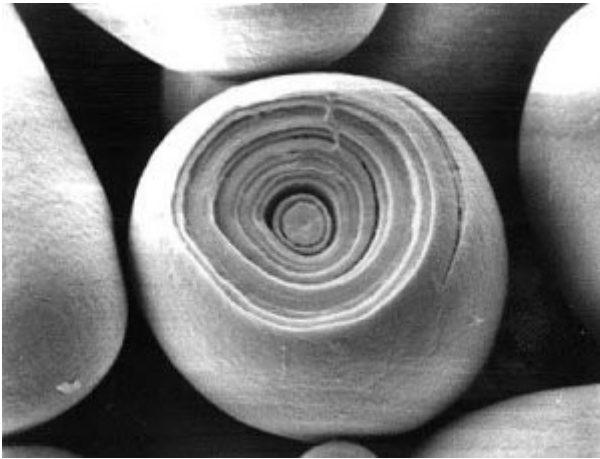
What measures the starch content?

How is the correct breakdown rate 'calculated'?

How is carbon partitioning controlled?

Why starch?

10μm

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

40µm
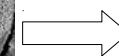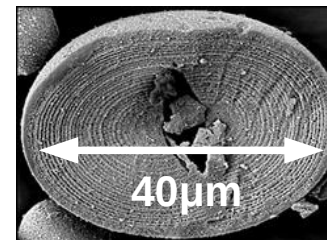
$\Longrightarrow$ $3 \cdot 10^{10}$ Da!!!

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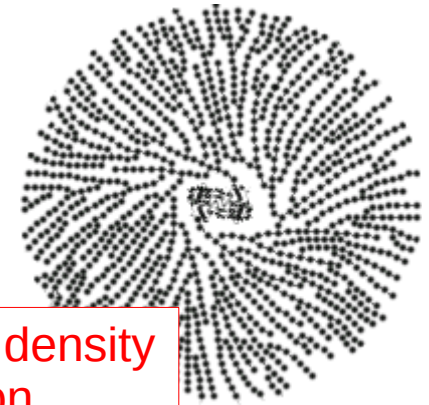
trade-off between storage density
and rapid mobilization

big molecule (up to 10 MDa)

Possible advantages of starch

- low osmolarity
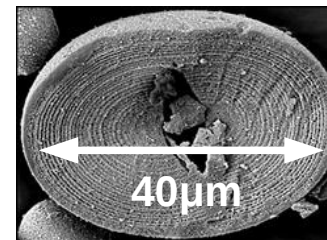- large size
- high density

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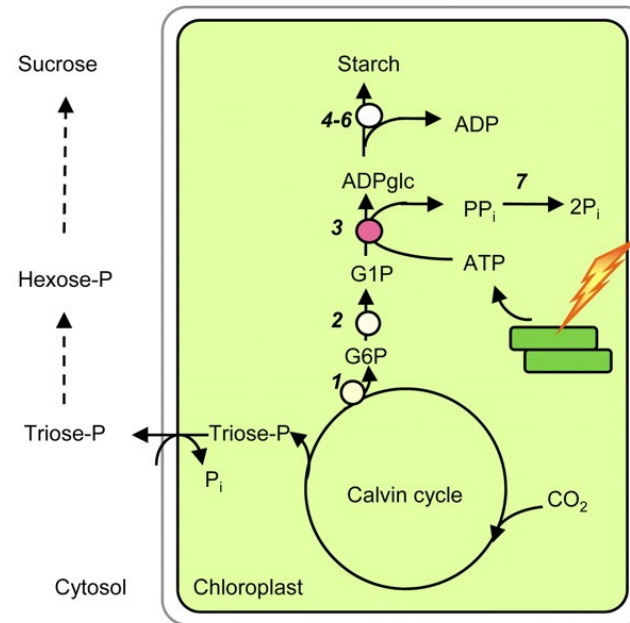$\Rightarrow$ $3 \cdot 10^{10}$ Da!!!

optimised for storage density,
slower deployment

How is starch made?
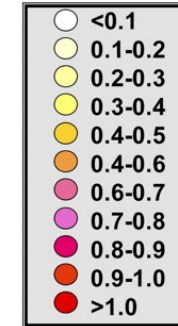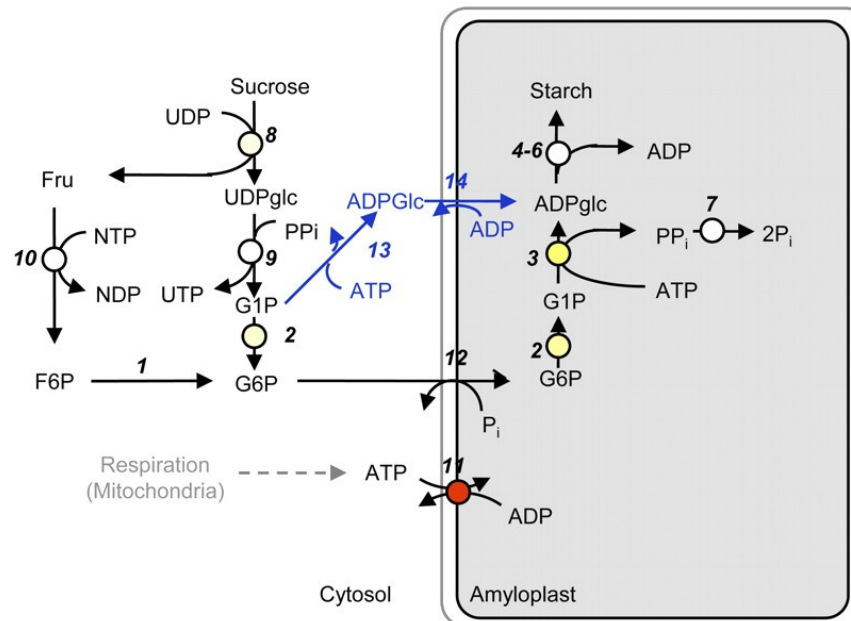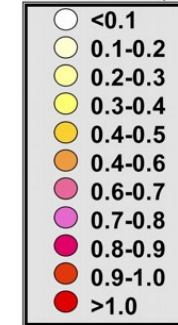
from: Geigenberger 2011 (*Plant Phys*)

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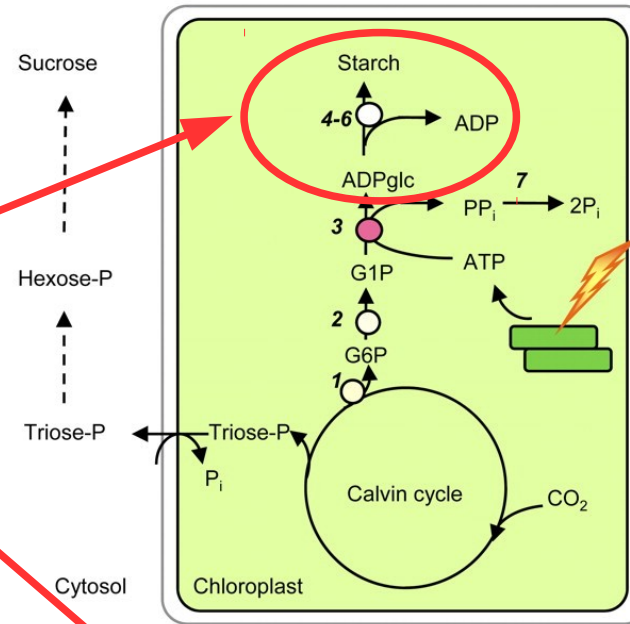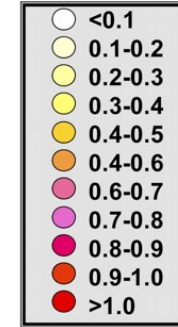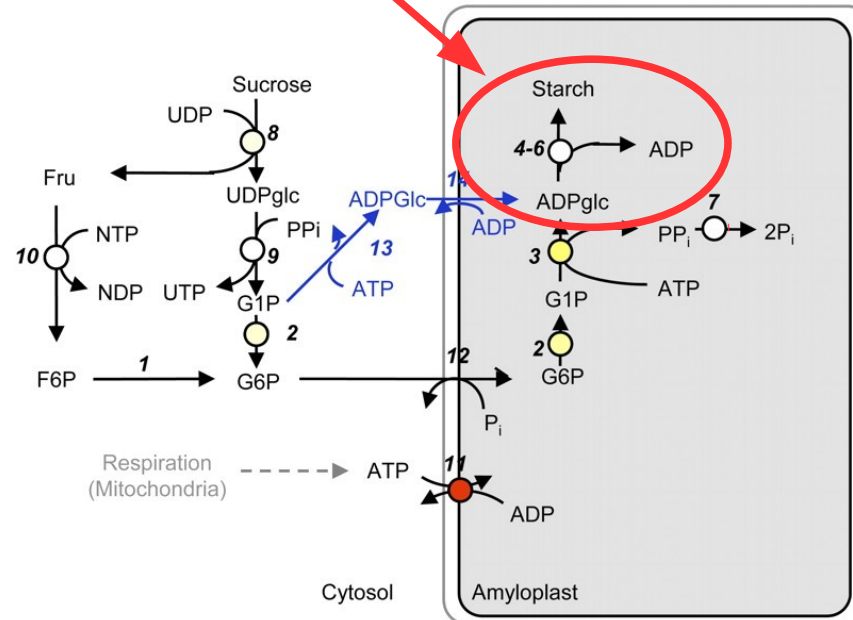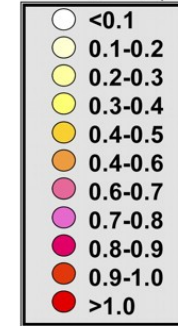
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Many enzymes are involved in starch synthesis
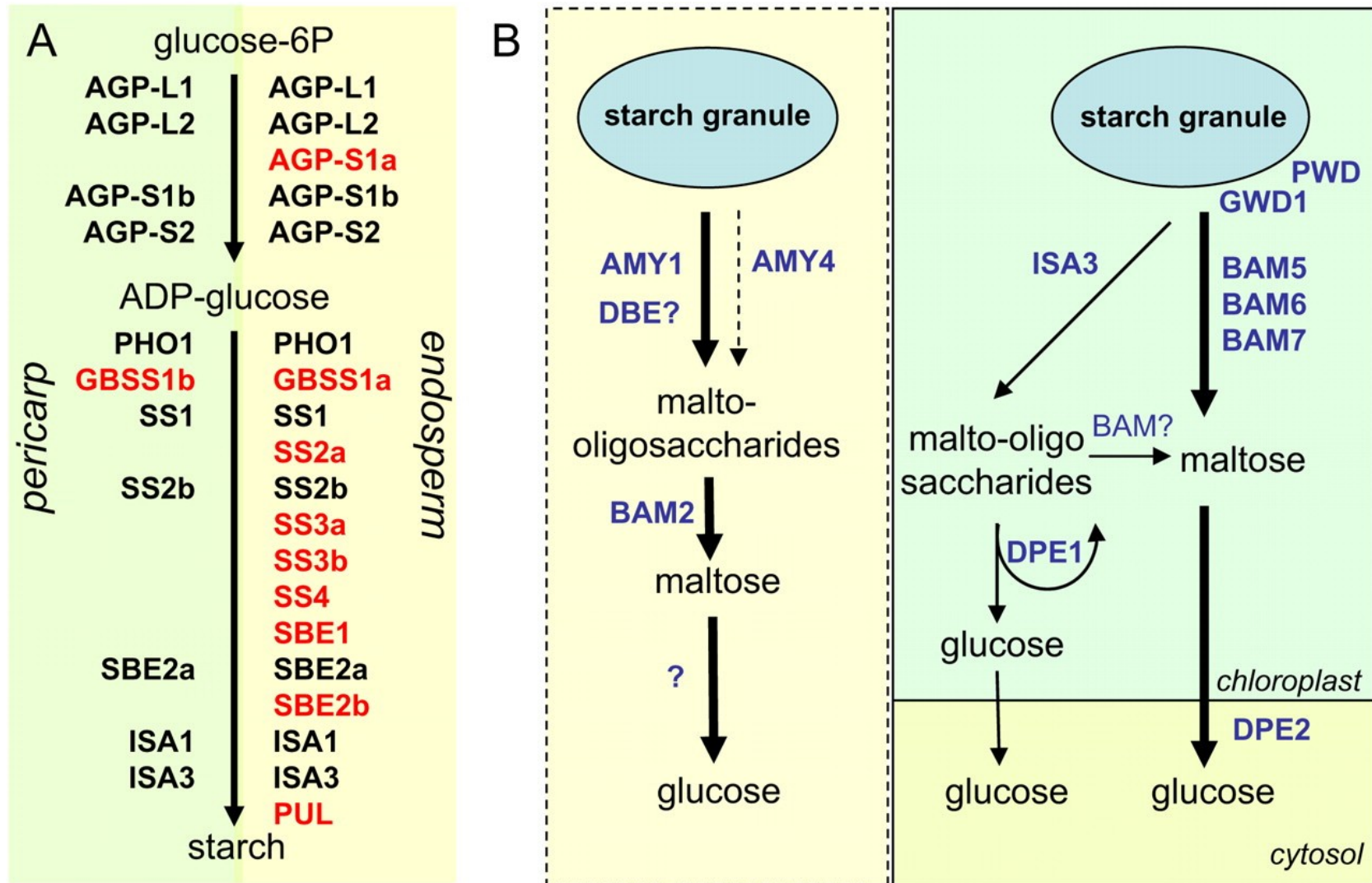
- starch synthases
- branching enzymes
- phosphorylases
- isoamylases

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

...and starch breakdown

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

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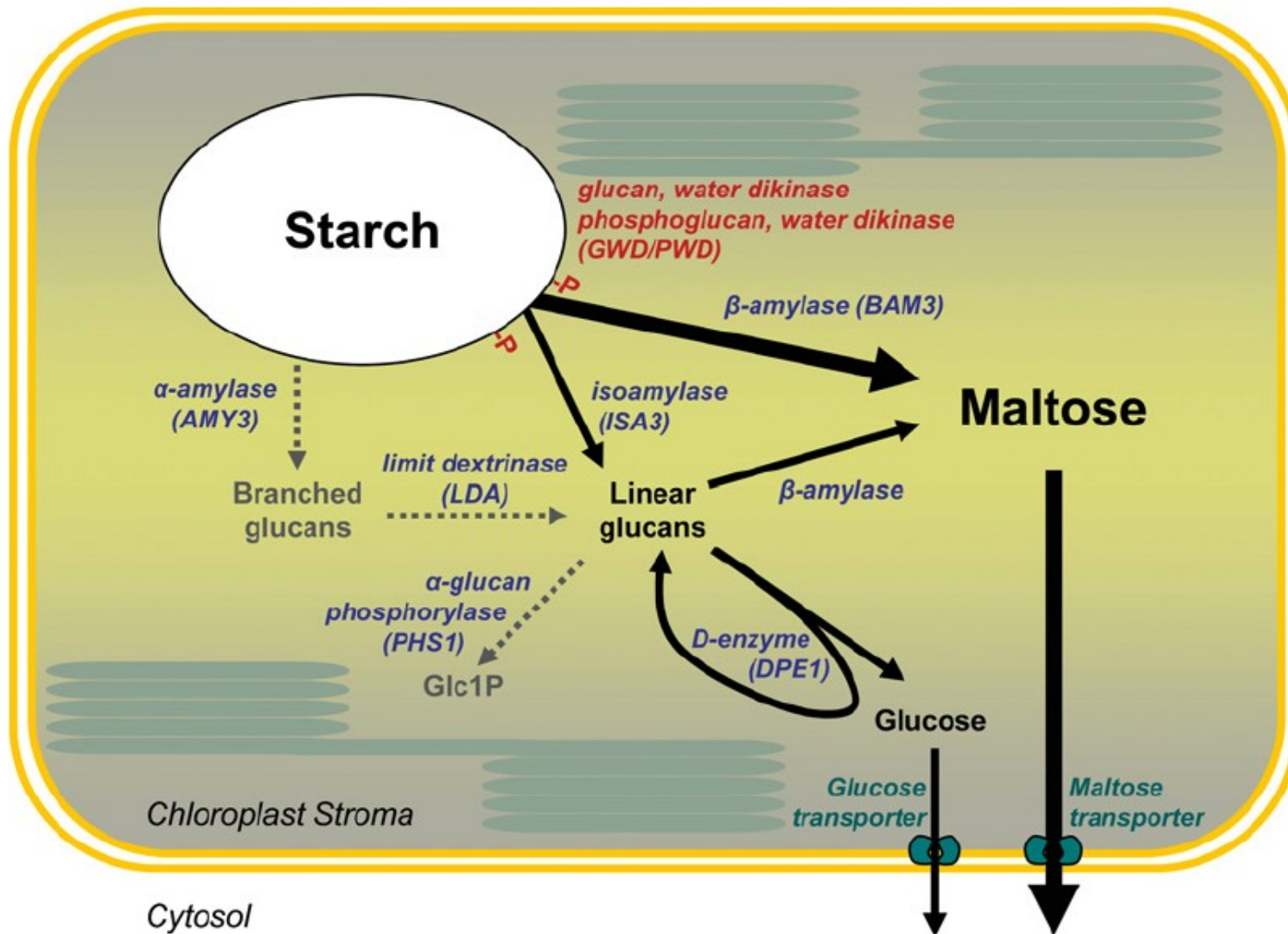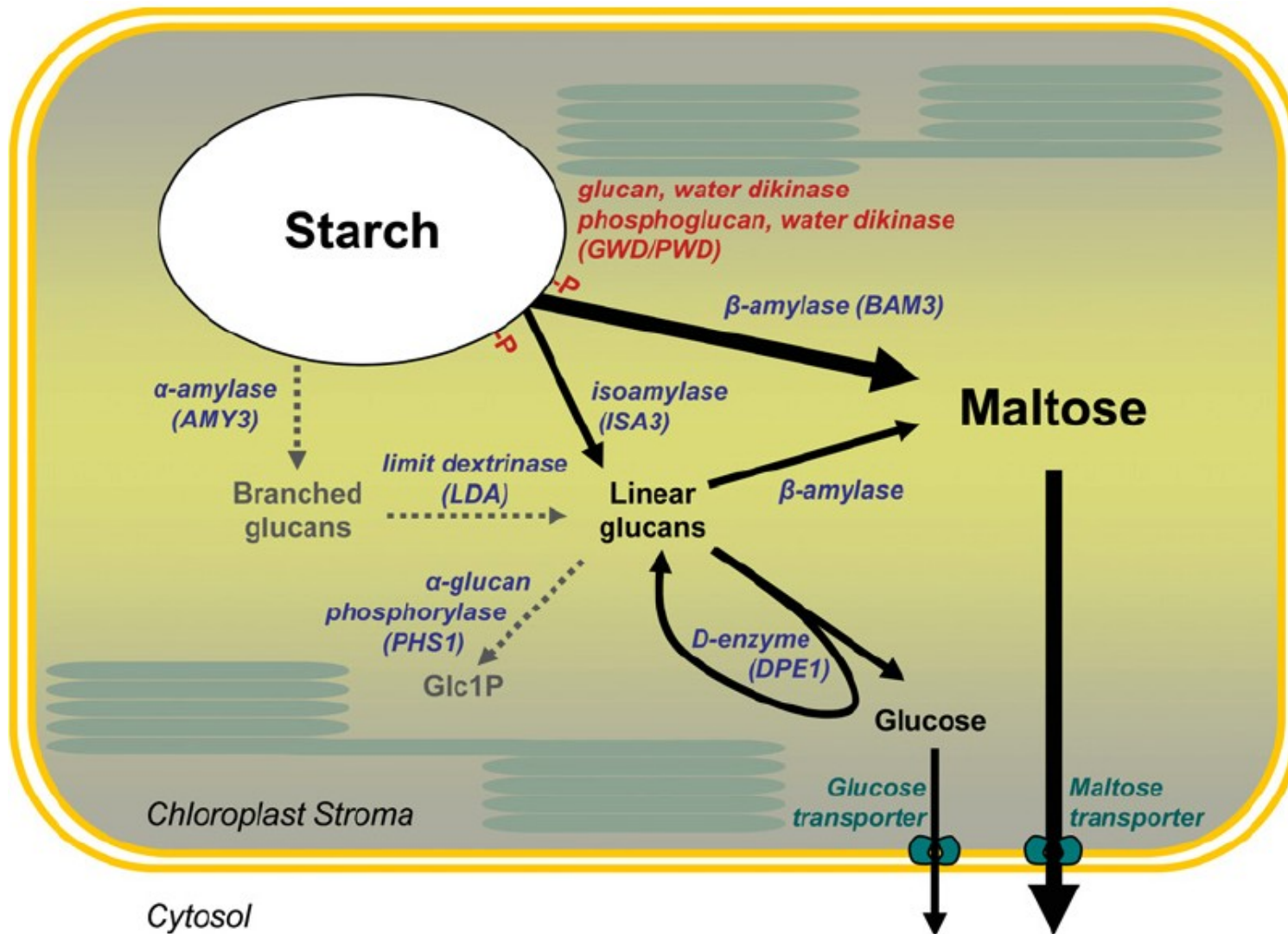
Many enzymes

- **are surface-active**

or

- **act on polymers**

⇩

hard to describe with traditional modelling approaches

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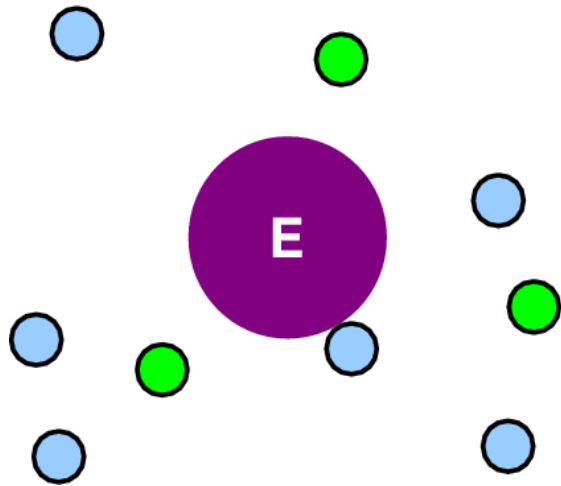
$$v = \frac{V_{max} S}{K_M + S}$$

aggregated substrate (with interfacial reaction space)

$$v = f(?)$$

Rate laws for surfactive enzymes

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Reaction space confined to 2D!

Rate laws for surfactive enzymes

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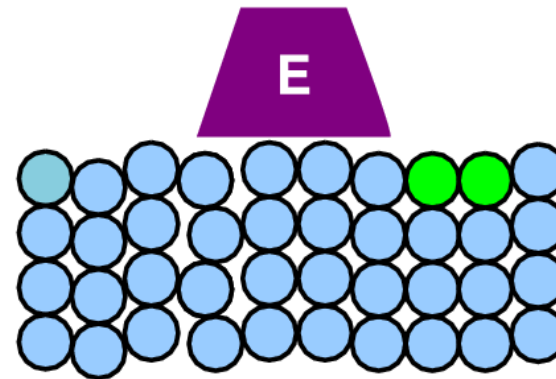
Reaction space confined to 2D!

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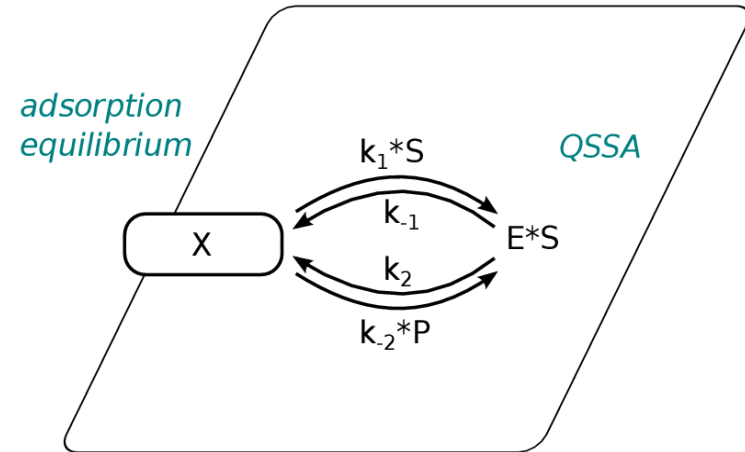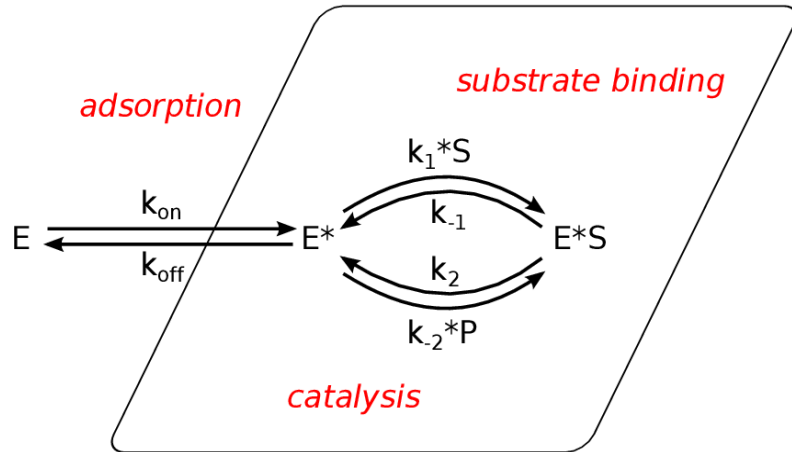
Kartal and Ebenhöh (2013) FEBS Letters – centenary issue commemorating Michaelis-Menten 'Kinetik der Invertinwirkung'

The adsorption equilibrium

The Langmuir isotherm
(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: $\quad r_a \;\propto\; c(E) \cdot (1 - \theta_E)$

Desorption rate: $\quad r_d \;\propto\; \theta_E$

The adsorption equilibrium

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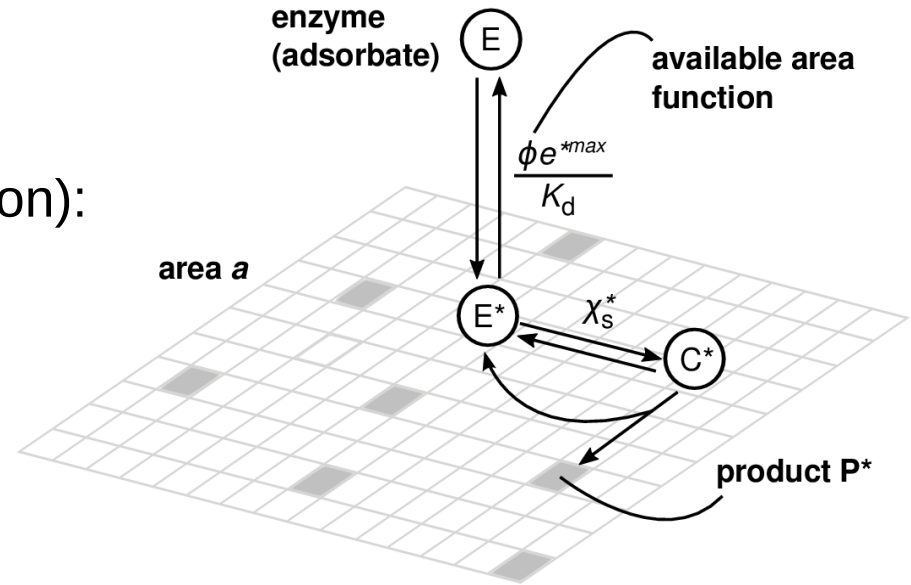
Available area function

The adsorption equilibrium

Other adsorption models can give quite different results:

Derivation of a generic surfactive rate-law

$$v = \frac{k_A a_s \Phi_{eq}[M][E_0](k_S\langle *S\rangle - k_P\langle *P\rangle)}{1 + k_A a_s \Phi_{eq}[M]\left(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\right)} = \frac{V_M^{app}\frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}$$
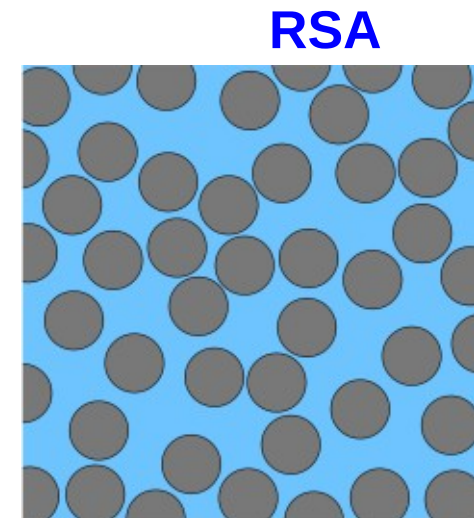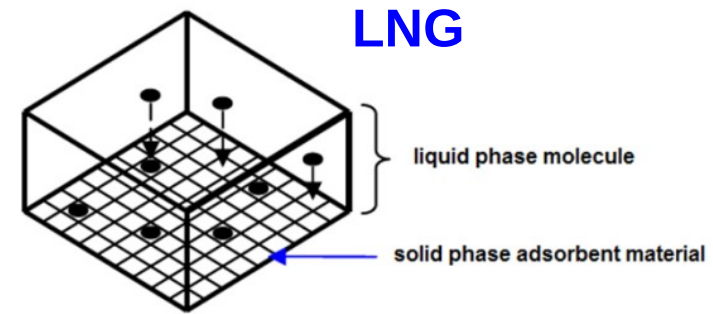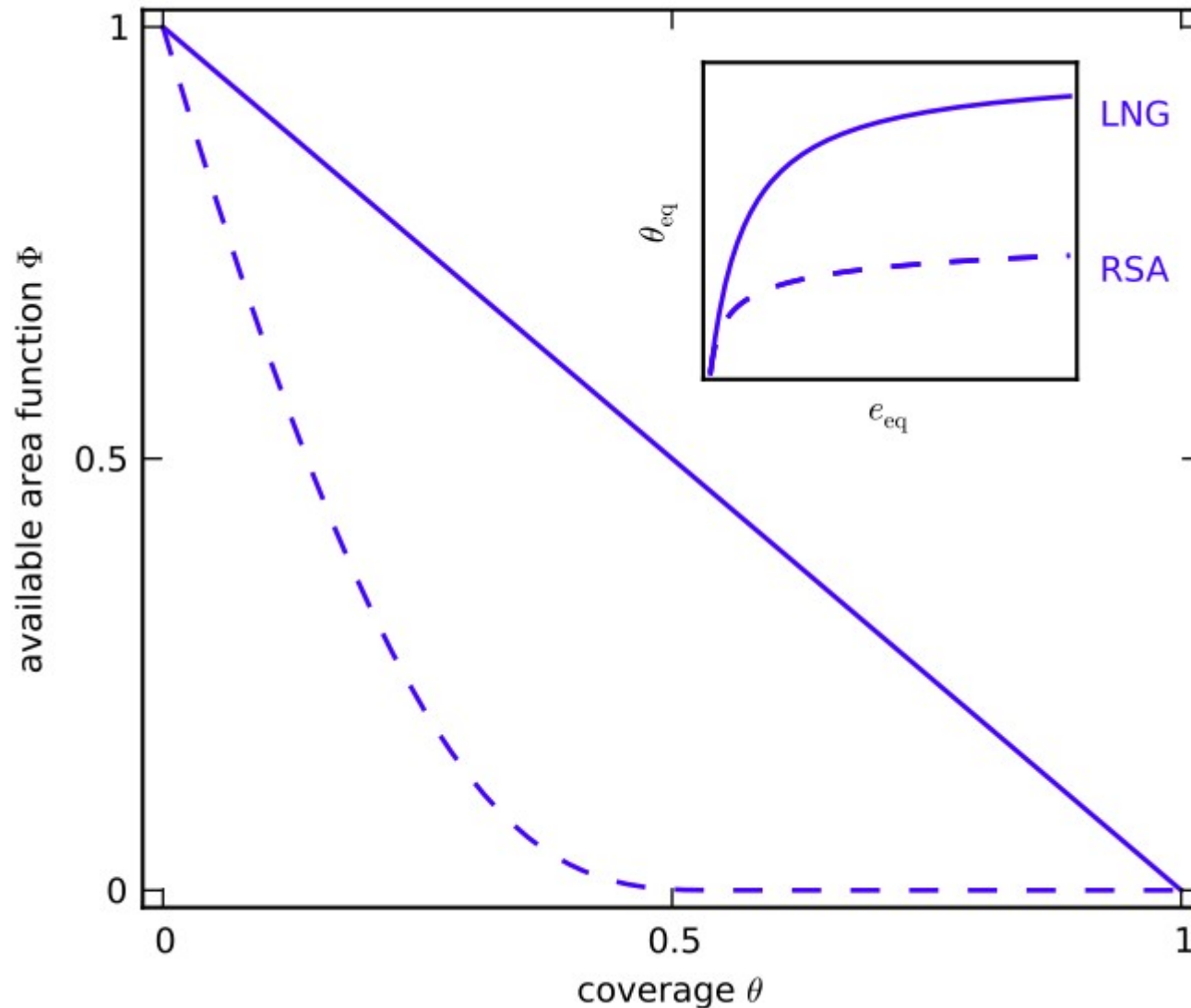
Derivation of a generic surfactive rate-law
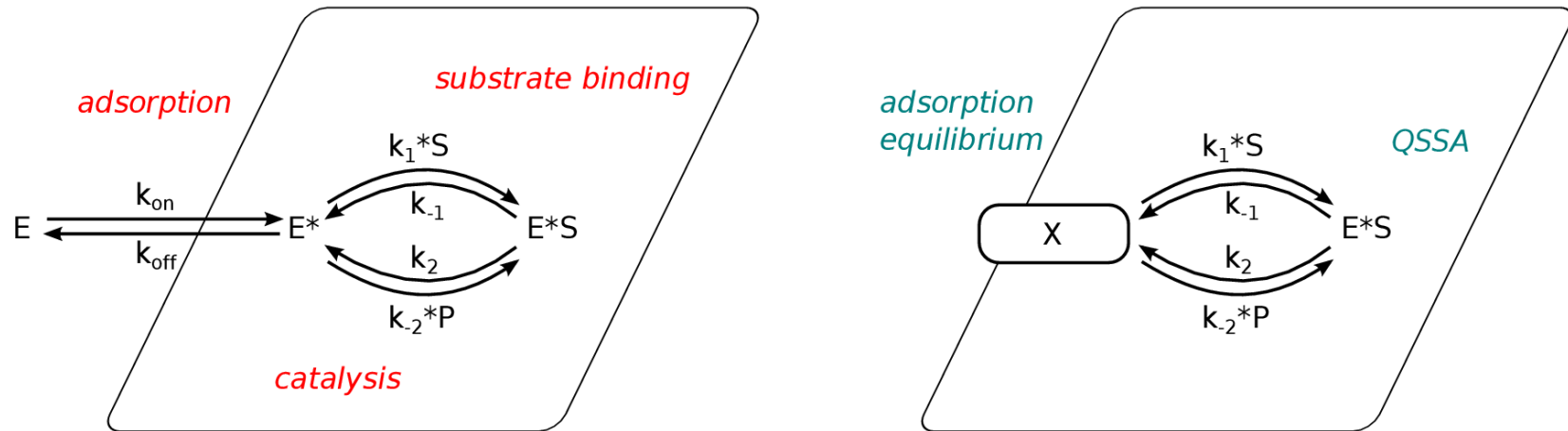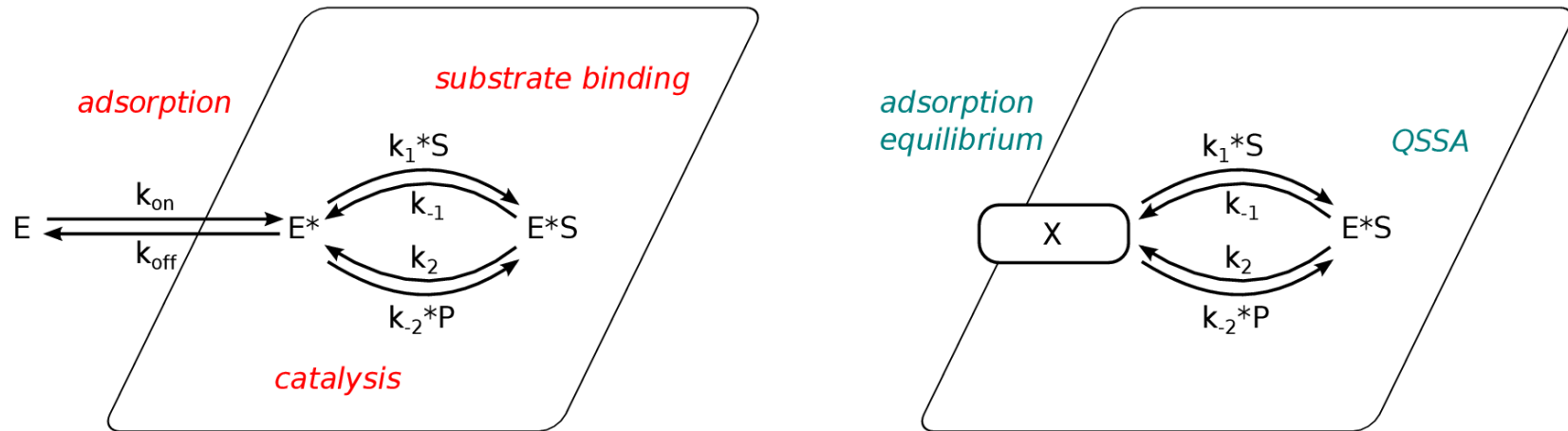
$$v = \frac{k_A\,a_s\,\Phi_{eq}\,[M][E_0]\big(k_S\langle *S\rangle - k_P\langle *P\rangle\big)}{1 + k_A\,a_s\,\Phi_{eq}\,[M]\Big(1 + \frac{\langle *S\rangle}{K_{mS}} + \frac{\langle *P\rangle}{K_{mP}}\Big)} = \frac{V_M^{app}\,\frac{[M]}{K_{mM}^{app}}}{1 + \frac{[M]}{K_{mM}^{app}}}$$

specific surface area   **available area function**

"few big objects behave different to many small objects"   "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
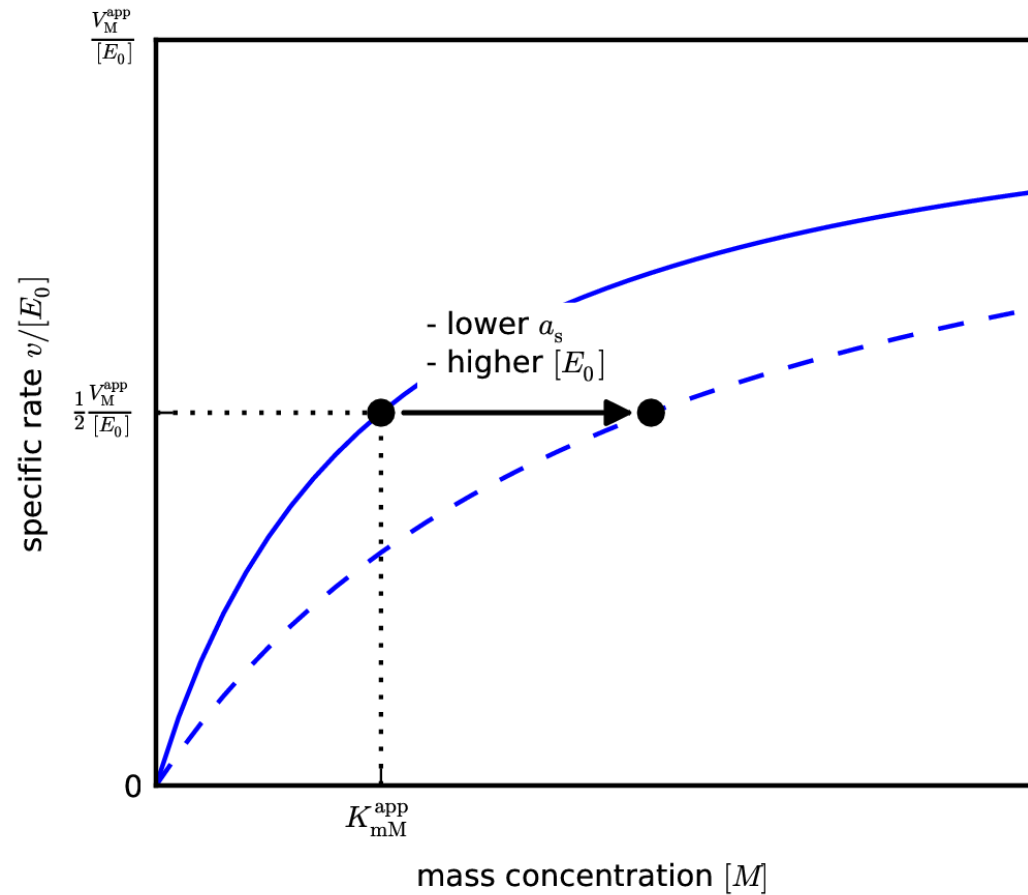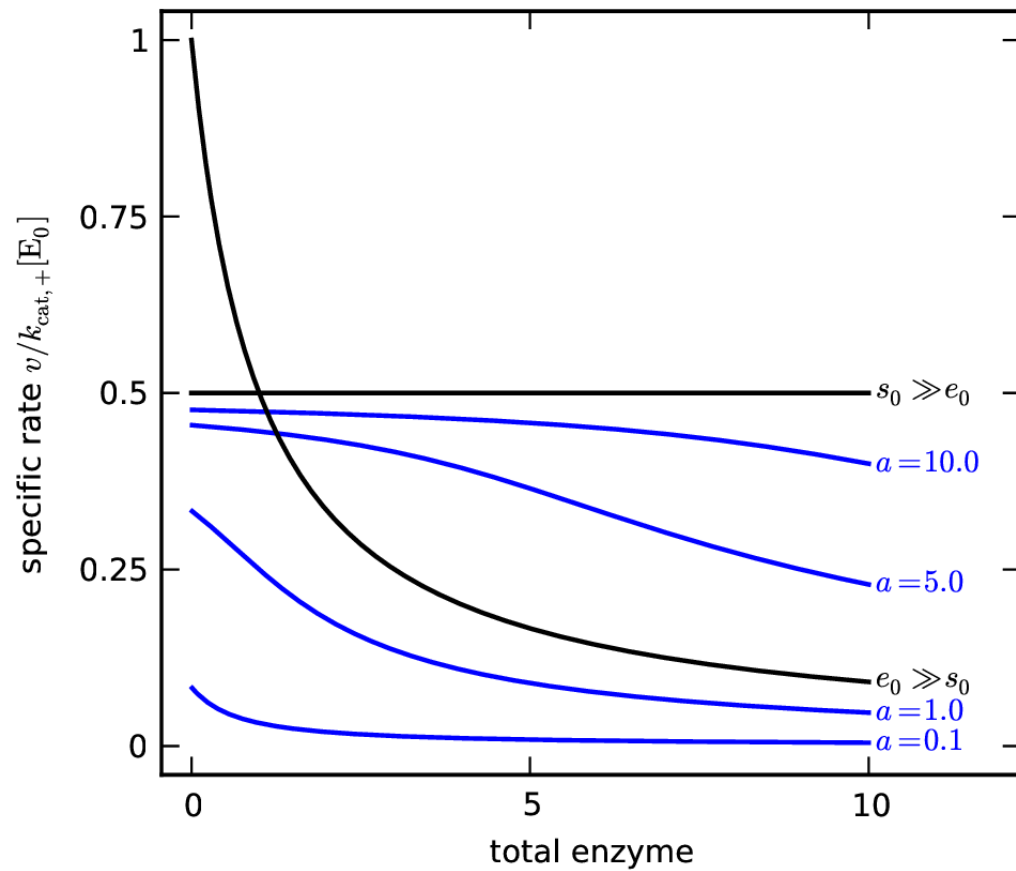
mass alone is insufficient!

A kinetic model of starch surface attack
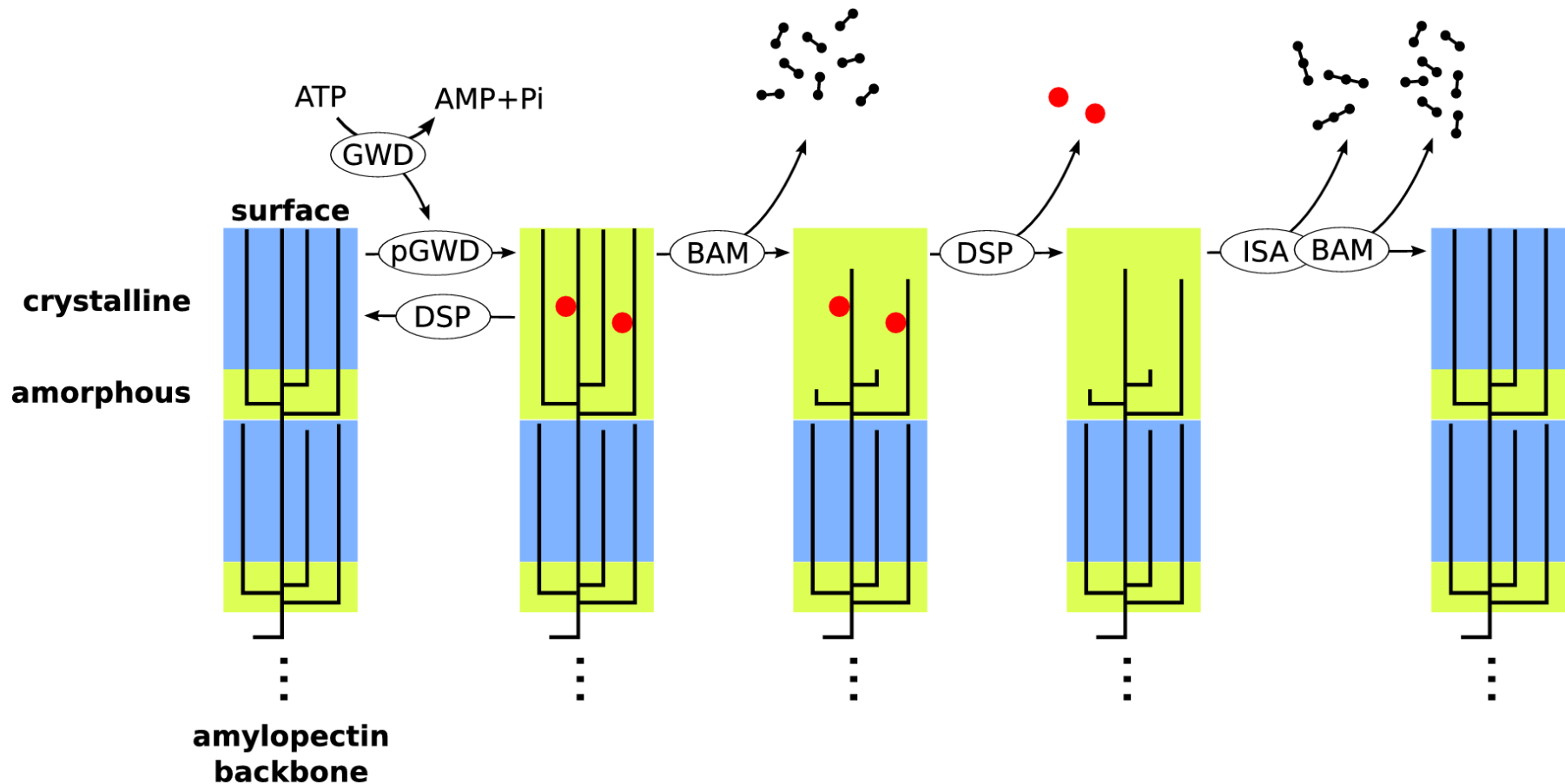
- Disruption of crystalline surface by phosphorylation allows access for BAM and ISA

- Dephosphorylation by DSP enables further degradation

Simulations compared to experiment
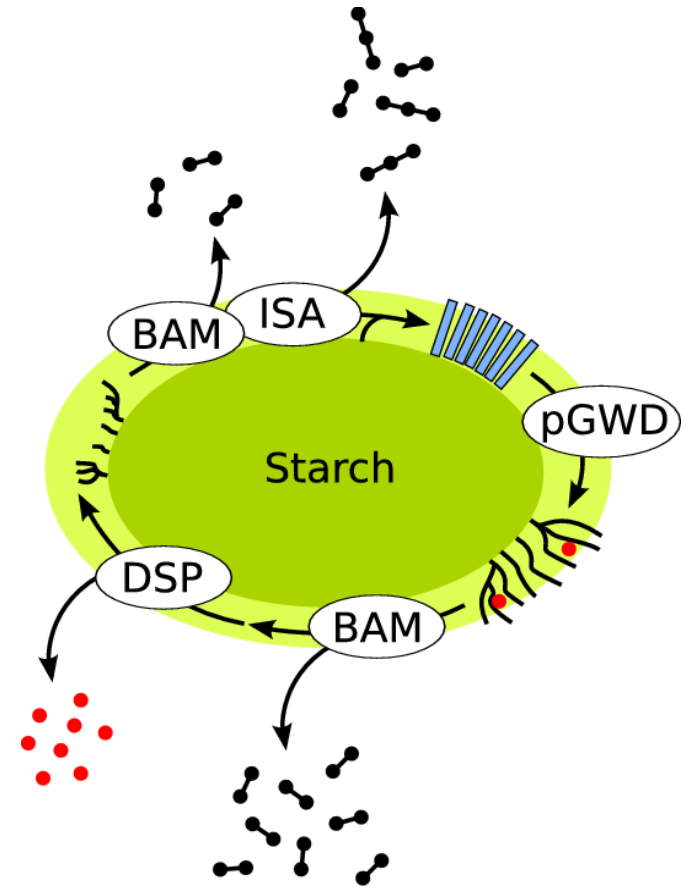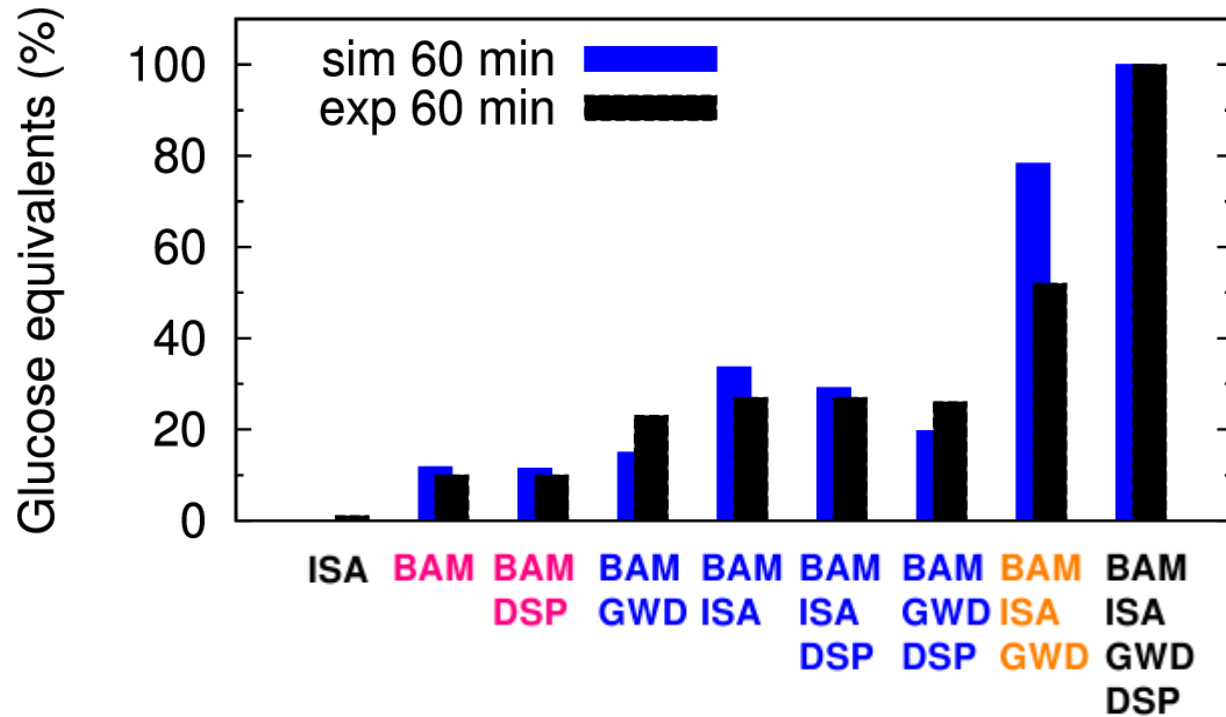
Good agreement with data from Kötting et al (2009) *Plant Cell*

But: only one time point!

2. Polymer Biochemistry

Why starch?
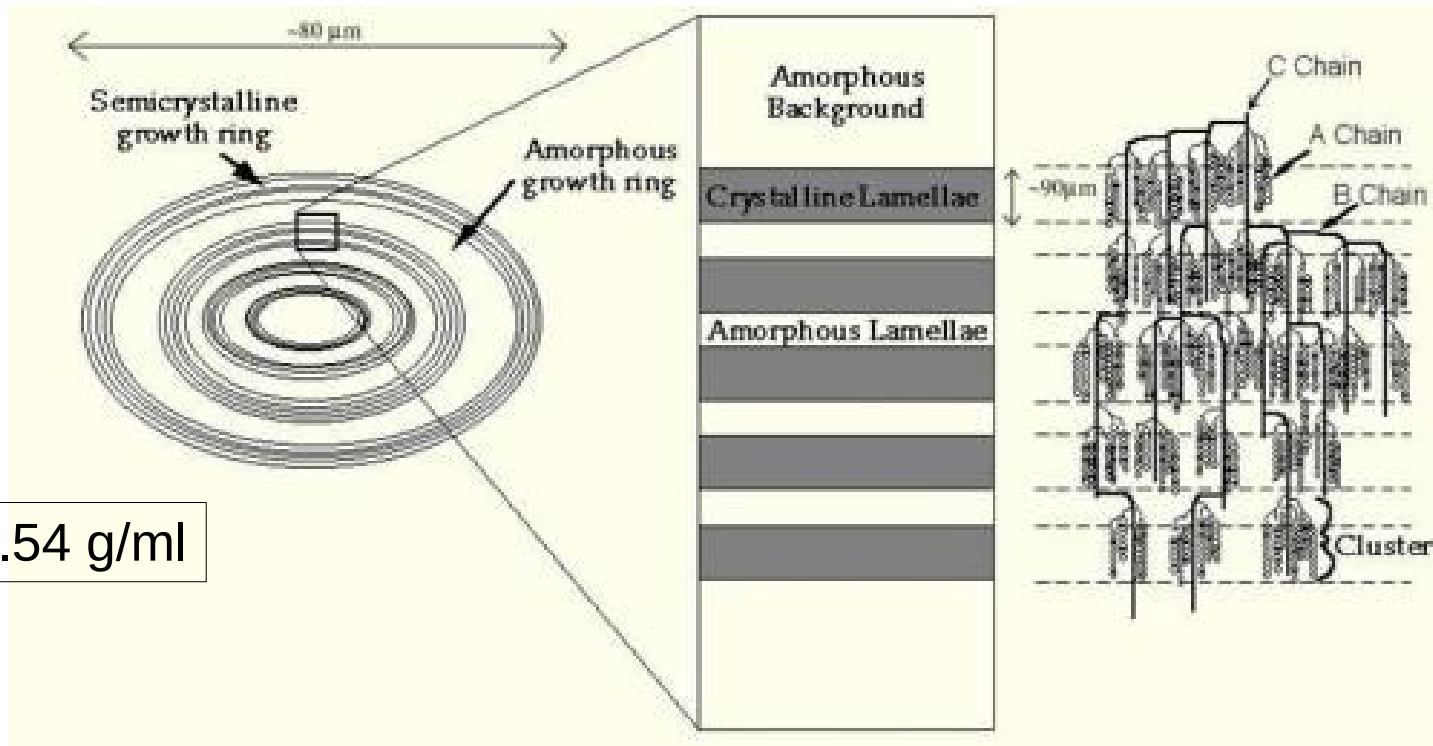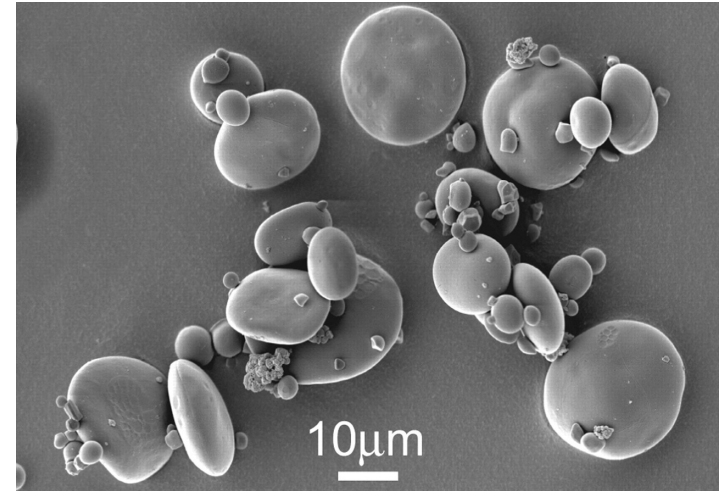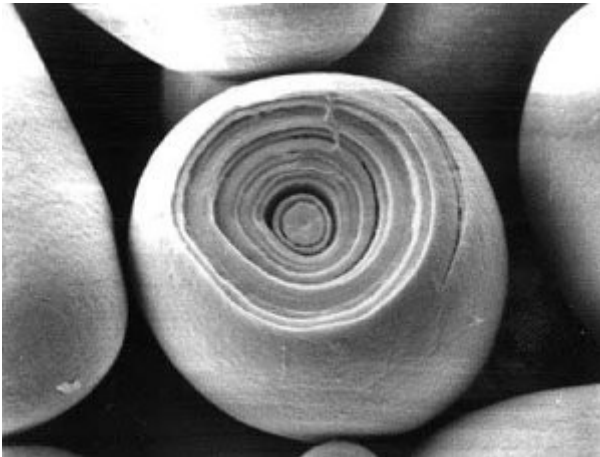
10μm

Density: 1.54 g/ml

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

Starch degradation - disproportionation

starch granule

?

malto-oligos

β-amylase

DPE1
(D-enzyme)

maltose

maltotriose

maltose
transporter

glucose
transporter

CHLOROPLAST

Disproportionating enzymes (D-enzymes)

DPE1   catalyses   2 maltotriose $\longleftrightarrow$ maltopentaose + glucose

EC: 2.4.1.25

$G3 + G3 \longleftrightarrow G5 + G1$

but not only!

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

(Takaha et al., JBC 1993)

Disproportionating enzymes (D-enzymes)

DPE1 catalyses $2\,\text{maltotriose} \longleftrightarrow \text{maltopentaose} + \text{glucose}$

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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}**???**

Disproportionating enzymes (D-enzymes)

DPE1

EC: 2.4.1.25

Disproportionating Enzyme
randomises DPs

A

$DP_{ini} = 4$ $DP = 1 ... 7$

all possible
transfer reactions

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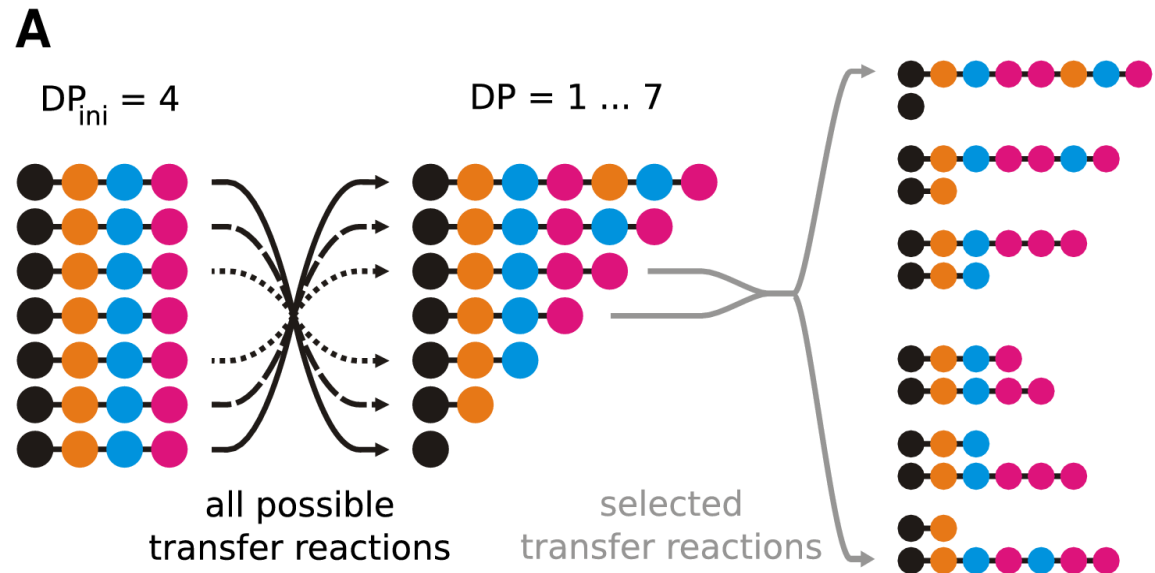
transfers glucosyl residues from one glucan to another: $G_n + G_m \longleftrightarrow G_{n-q} + G_{m+q}$

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)
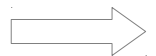
$\Longrightarrow$ DPE1 maximises the entropy of the polydisperse reactant mixture

The thermodynamic picture

- Different DPs are interpreted as different energy states (energy of formation)

- Enzymes mediate transitions between these states
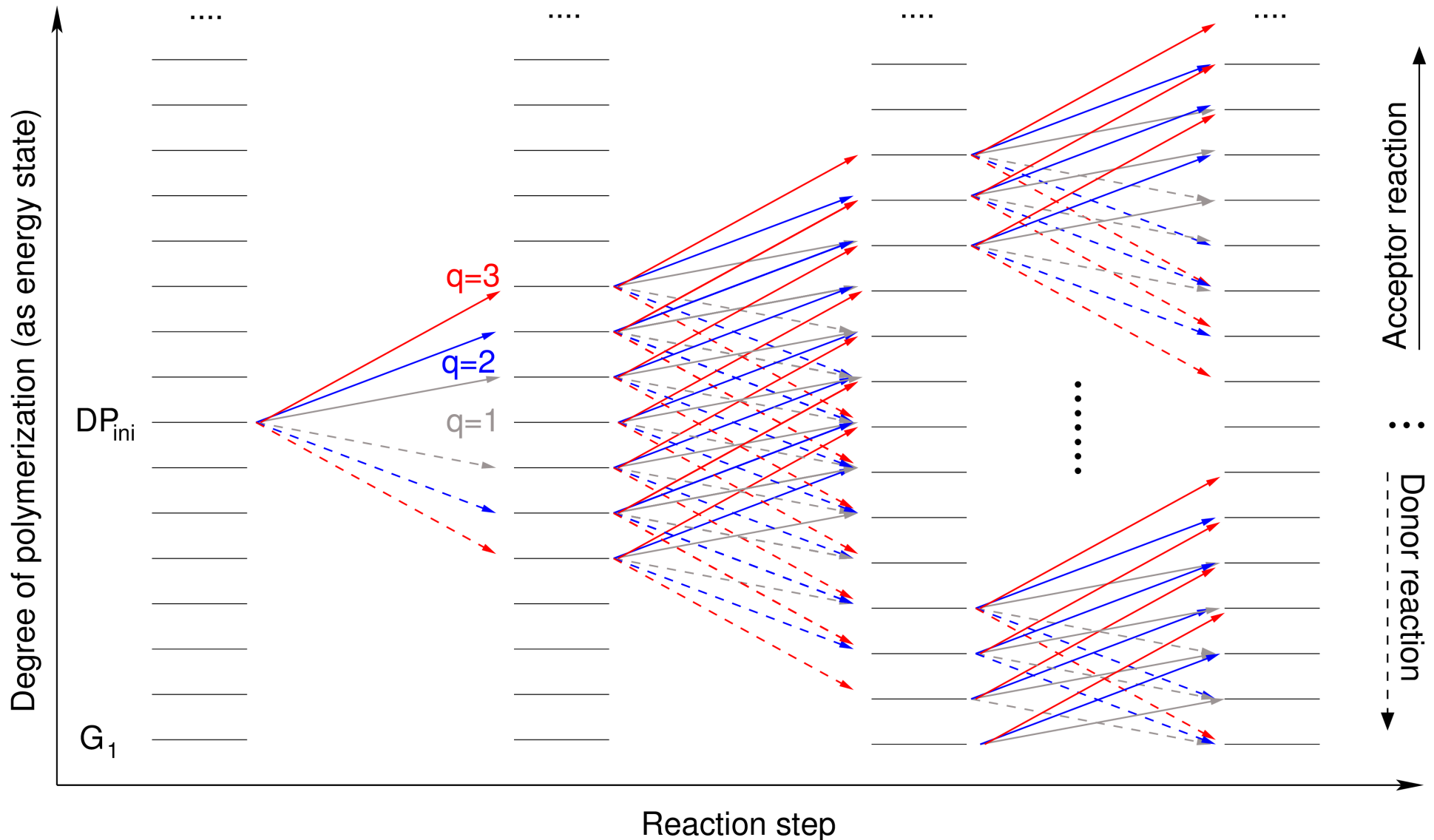
Polydisperse mixtures as statistical ensembles

x_i : molar fraction of glucans with length i

  corresponds to occupation number of state i

The distribution $\left[x_i \right]$ 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

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

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

Entropic approach

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

Entropic approach

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

Entropic approach

Solution using Lagrangian multipliers: Necessary conditions are given by

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

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

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

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

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

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

Entropic approach

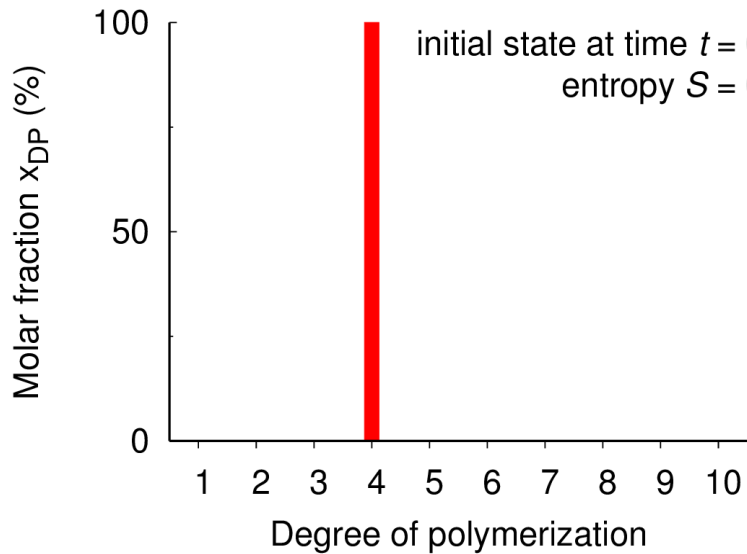$$S = -\sum x_k \ln x_k \rightarrow \max!$$

conservation of #molecules: $\sum x_k = 1$
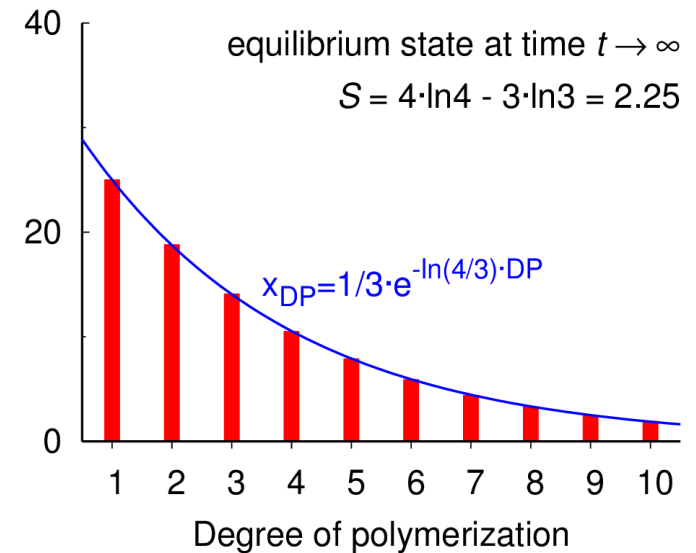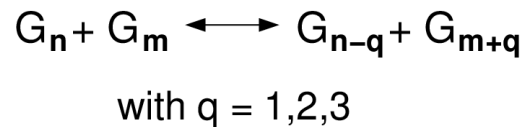
conservation of #bonds: $\sum k \cdot x_k = DP_{ini} - 1$

implies

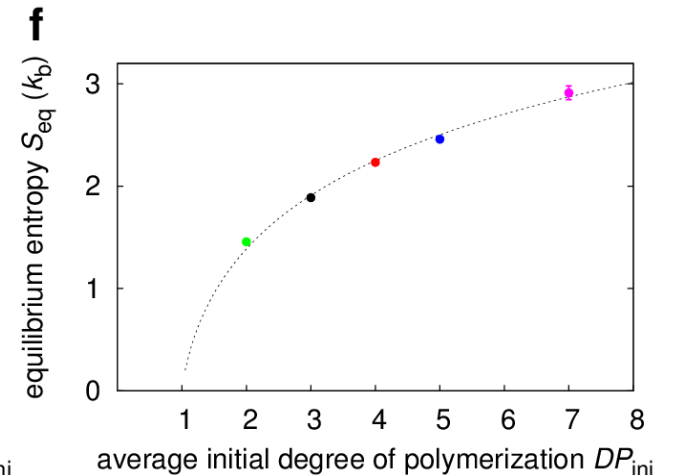$$x_i = \frac{1}{Z} e^{-\beta E_i}, \quad \beta = \ln \frac{DP_{ini}}{DP_{ini} - 1}$$

predicts

initial state at time $t = 0$
entropy $S = 0$

DPE1 action

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

with q = 1,2,3

equilibrium state at time $t \rightarrow \infty$

$S = 4 \cdot \ln 4 - 3 \cdot \ln 3 = 2.25$

$x_{DP} = 1/3 \cdot e^{-\ln(4/3) \cdot DP}$

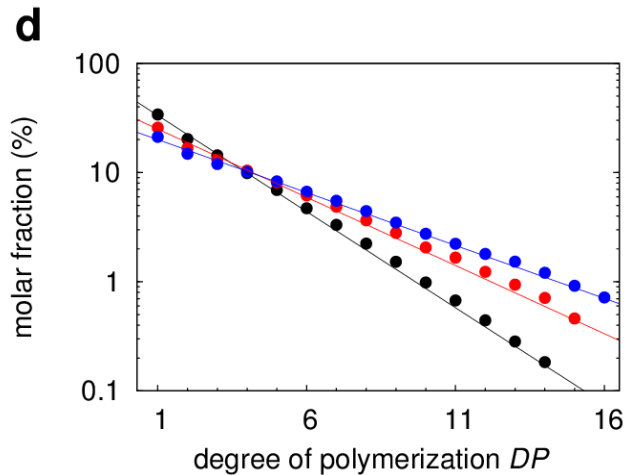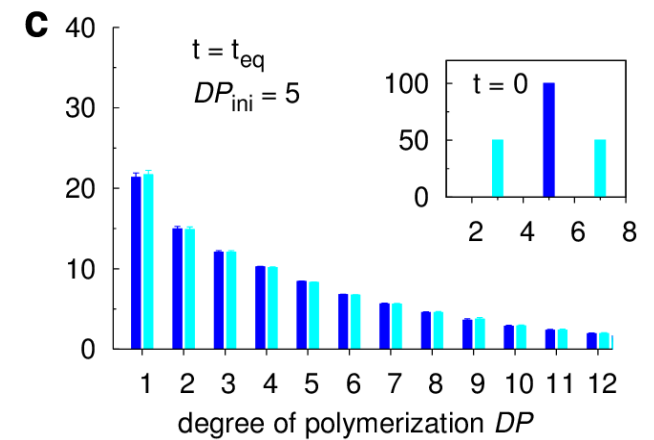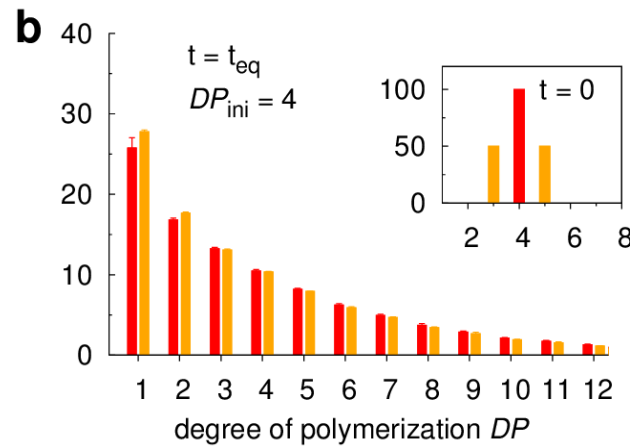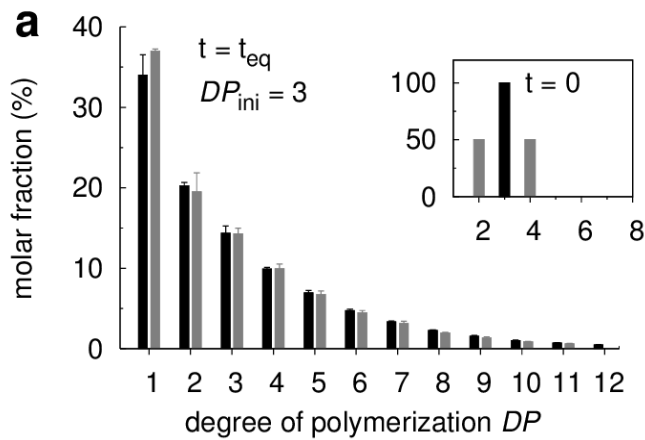Molar fraction x_{DP} (%)

Degree of polymerization

An instance of the 2nd law of TD!

DPE1 is entropy driven

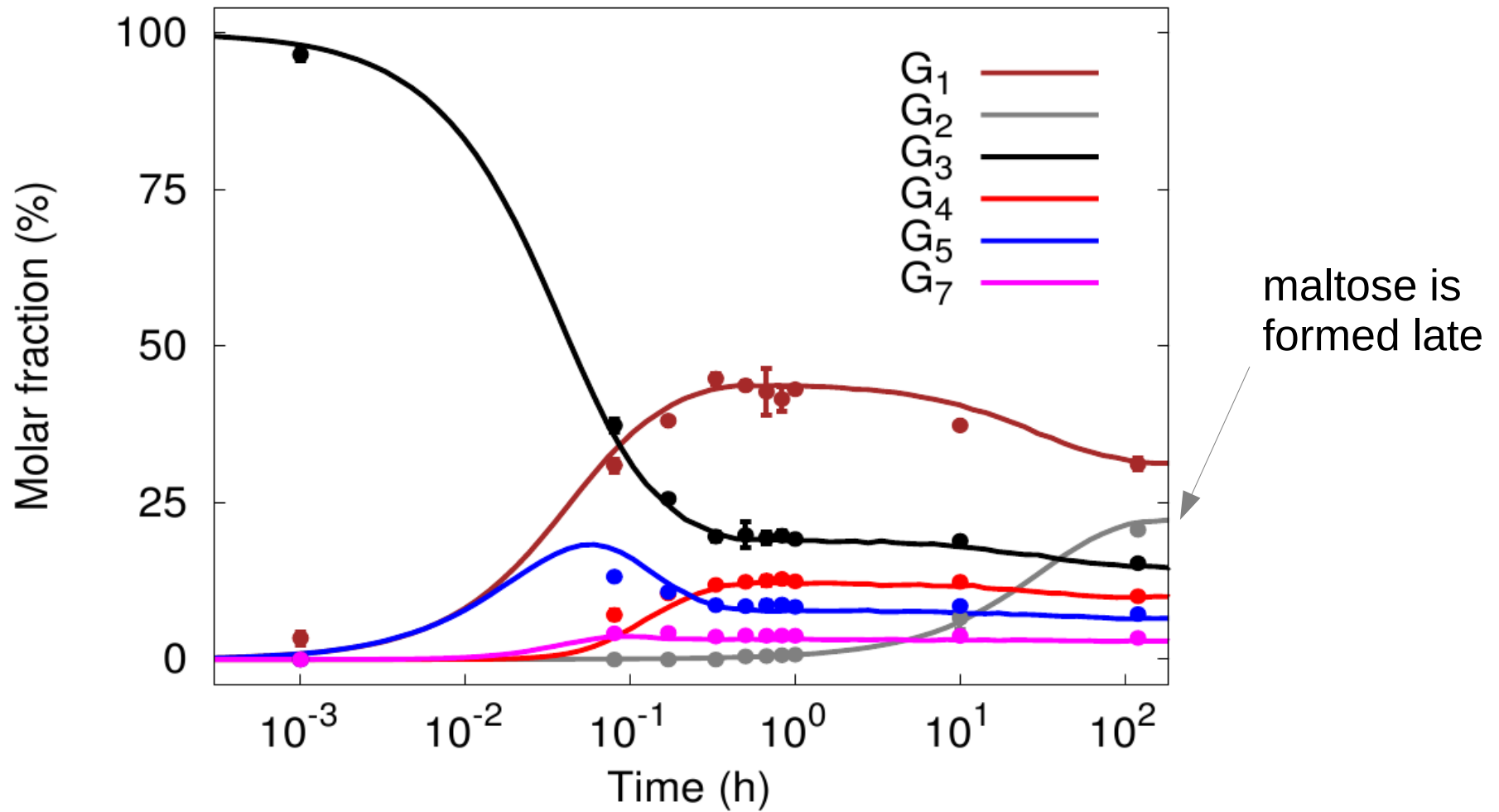<u>Experiments with Martin Steup, University of Potsdam</u>

method: capillary electrophoresis
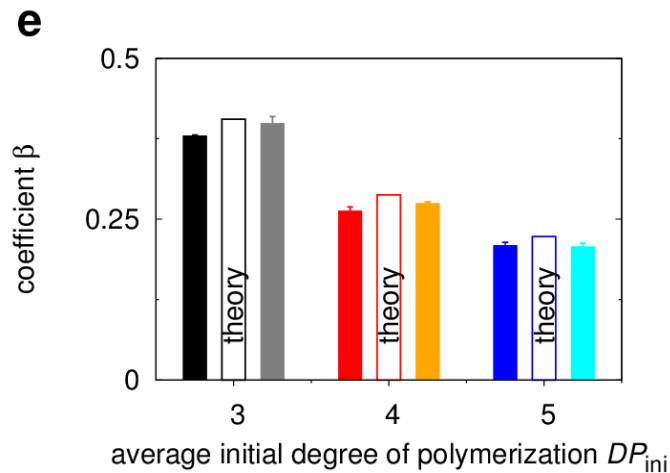
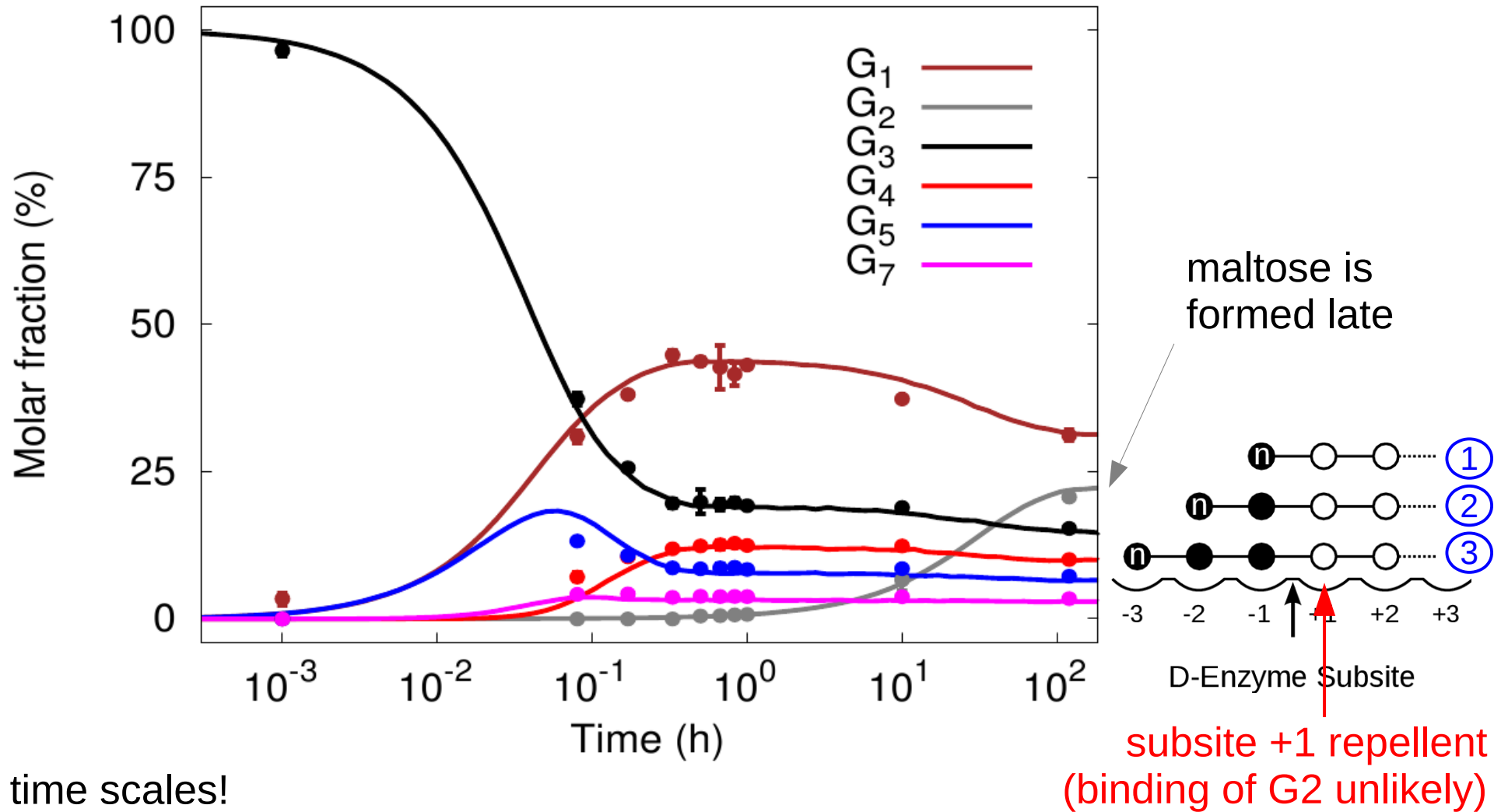β is a generalisation of the equilibrium constant for polydisperse mixtures

(Kartal et al, 2011, Mol Syst Biol)

The dynamics of DPE1

Two time scales!

The dynamics of DPE1

maltose is formed late

subsite +1 repellent
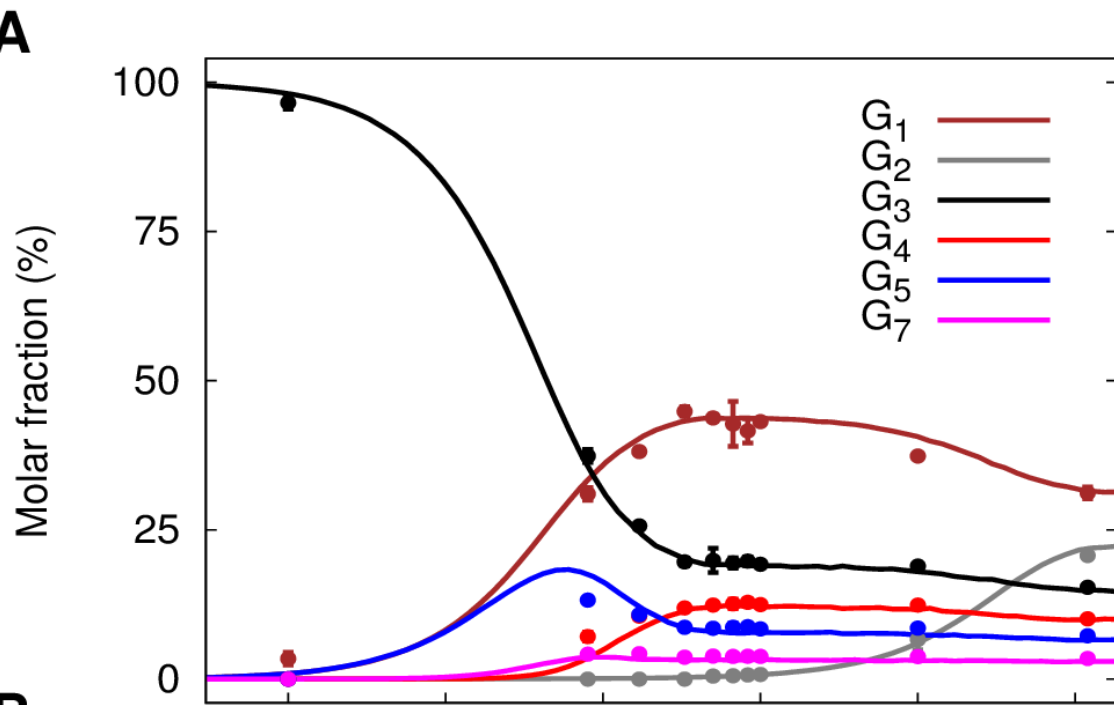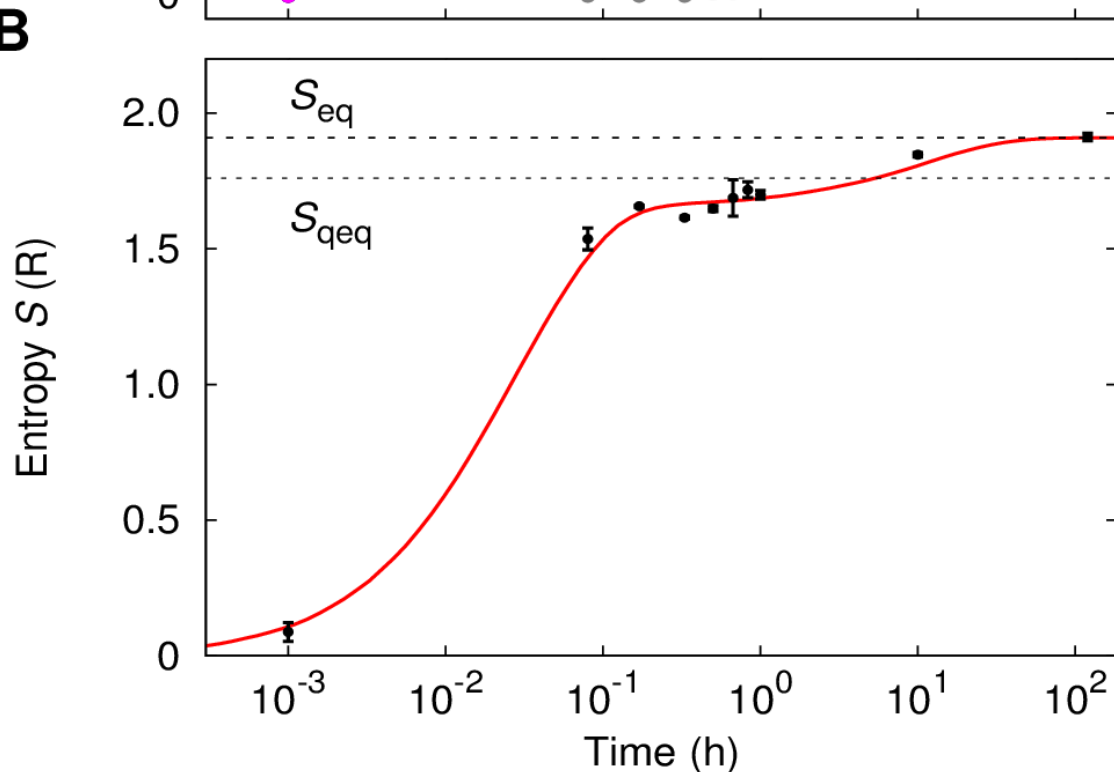(binding of G2 unlikely)

D-Enzyme Subsite

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

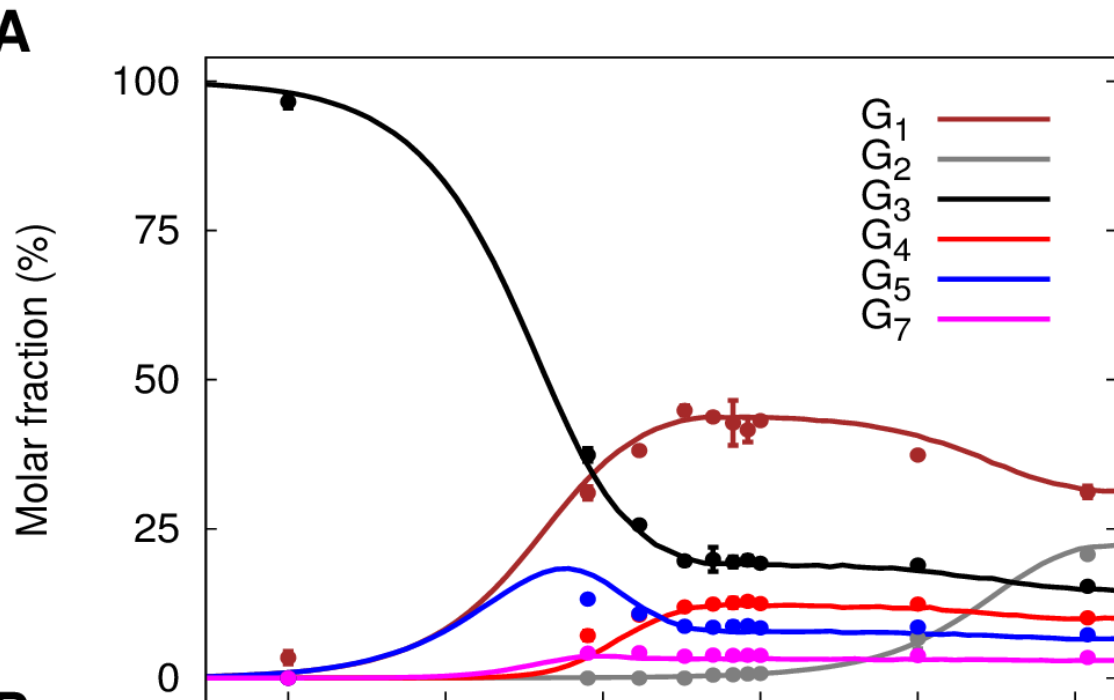**This system allows to follow the entropy *experimentally*!**
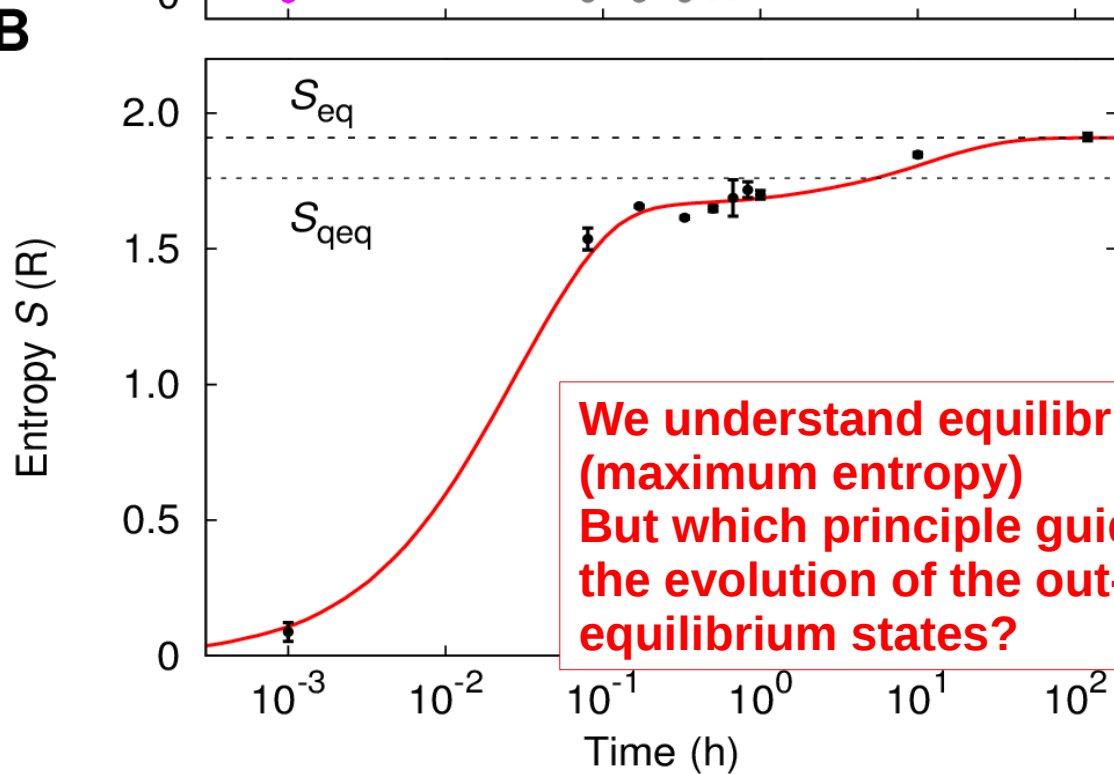
"true" equilibrium

(calculated as previously)

"quasi" equilibrium

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

A

Molar fraction (%)

G_1 — (brown)
G_2 — (gray)
G_3 — (black)
G_4 — (red)
G_5 — (blue)
G_7 — (magenta)

This system allows to follow the entropy *experimentally*!

B

Entropy S (R)

S_{eq}

S_{qeq}

Time (h)

"true" equilibrium

(calculated as previously)

"quasi" equilibrium

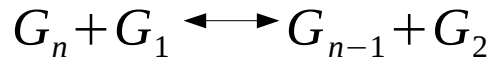(calculated with the same approach but omitting maltose from the statistical ensemble)

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:

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

with one additional side constraint

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

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

Theory is also confirmed by DPE2

DPE2 vs DPE1

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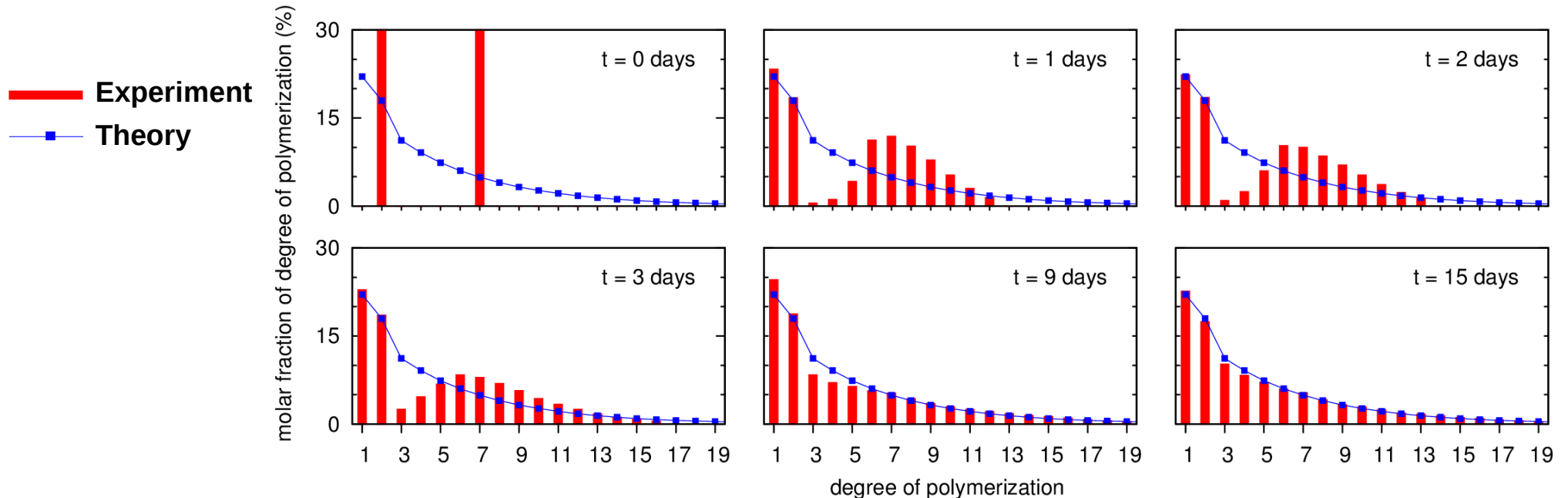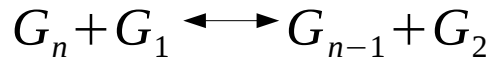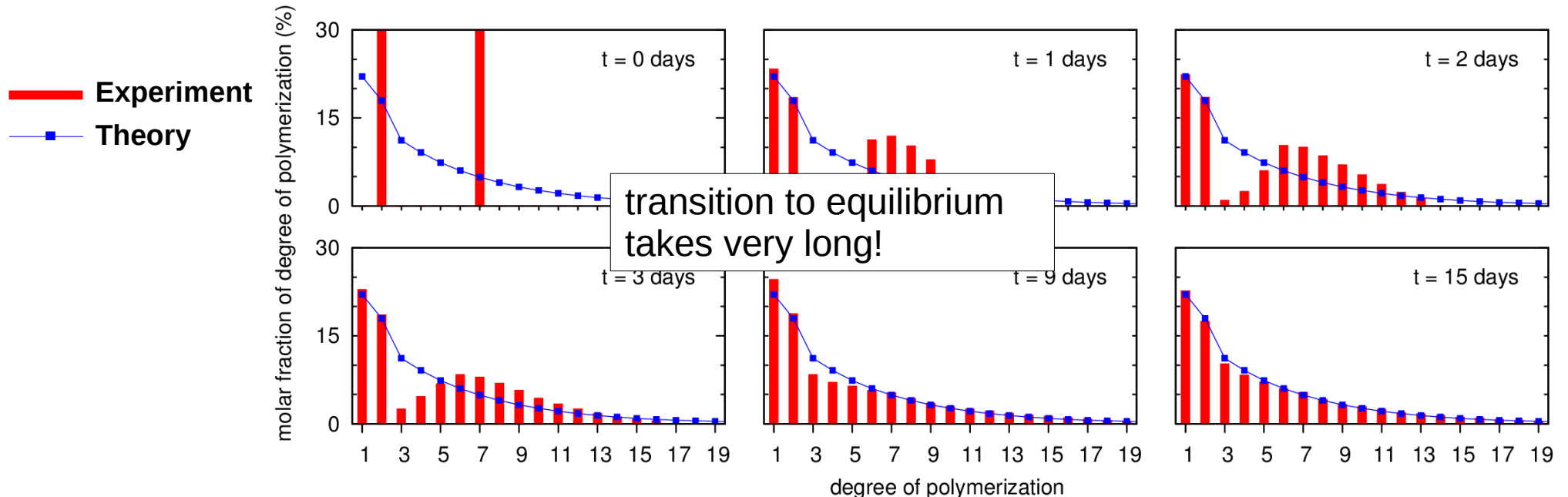- G3 only used as acceptor

Generic reaction catalysed:

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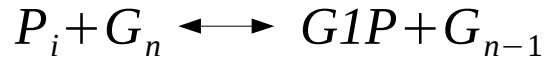
Entropic principle:

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

with one additional side constraint

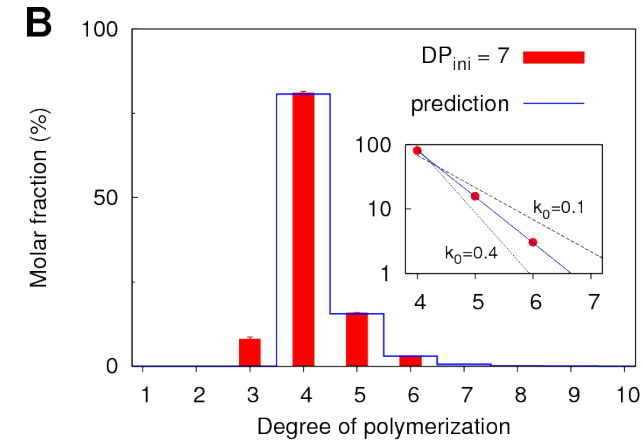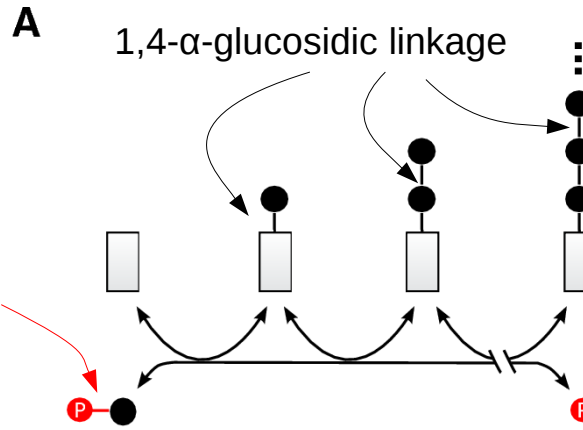$$x_1 + x_2 = m = \text{const.} \quad \left(\text{and} \sum x_k = 1 ; \sum k \cdot x_k = b \right)$$

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

transition to equilibrium takes very long!

Generalisation to non-zero enthalpy changes

Phosphorylase (cPho):

$$P_i + G_n \longleftrightarrow G1P + G_{n-1}$$
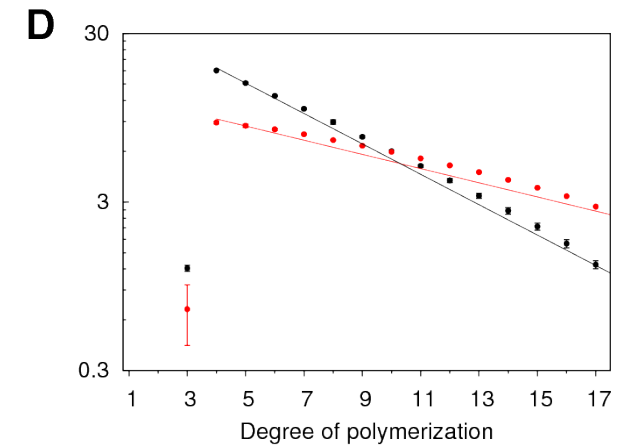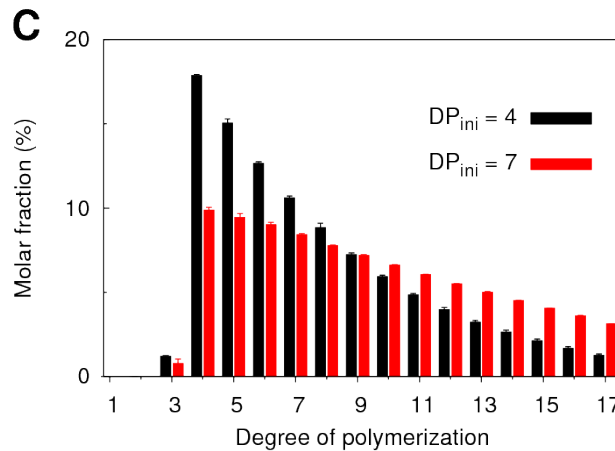
$$\Delta H \neq 0 !$$

Generalisation by including energetic and entropic contributions:

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

Gibbs energy of formation

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

A 1,4-α-glucosidic linkage

phosphoester bond

B Molar fraction (%)

DP$_{ini}$ = 7
prediction

k_0=0.1
k_0=0.4

Degree of polymerization

C Molar fraction (%)

DP$_{ini}$ = 4
DP$_{ini}$ = 7

Degree of polymerization

D

Degree of polymerization

Prediction: Similar pattern as for DPE2

Experimentally confirmed.

(Kartal et al, Supp to MSB 2011; Ebenhöh et al, Proc 5th ESCEC 2013)
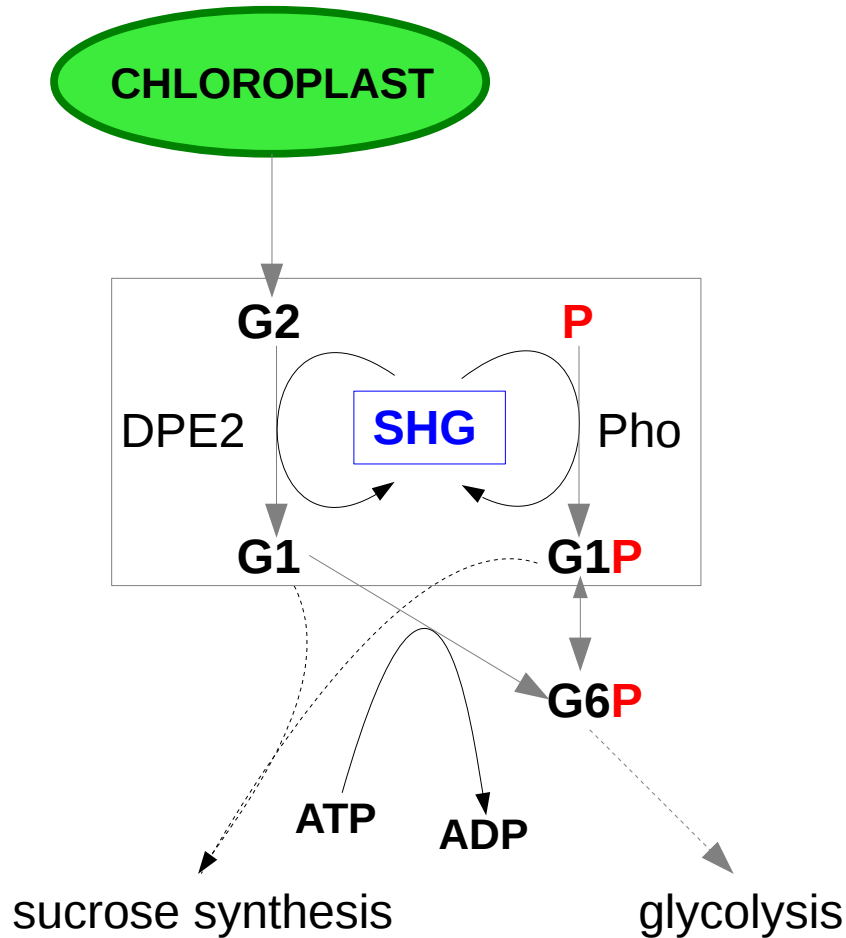
An entropy-driven buffer

Two entropic enzymes, cPho and DPE2, integrate input from may chloroplasts
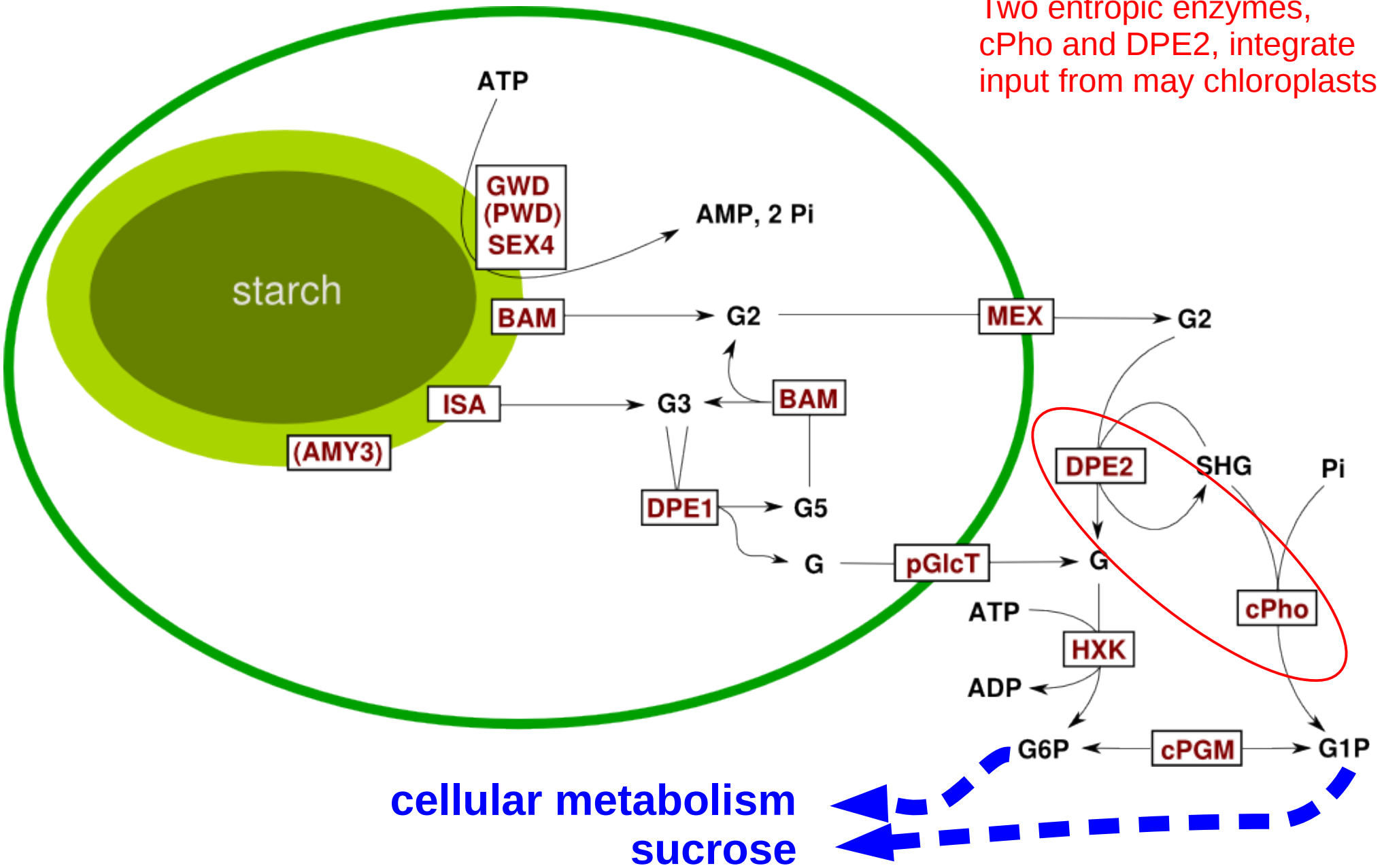
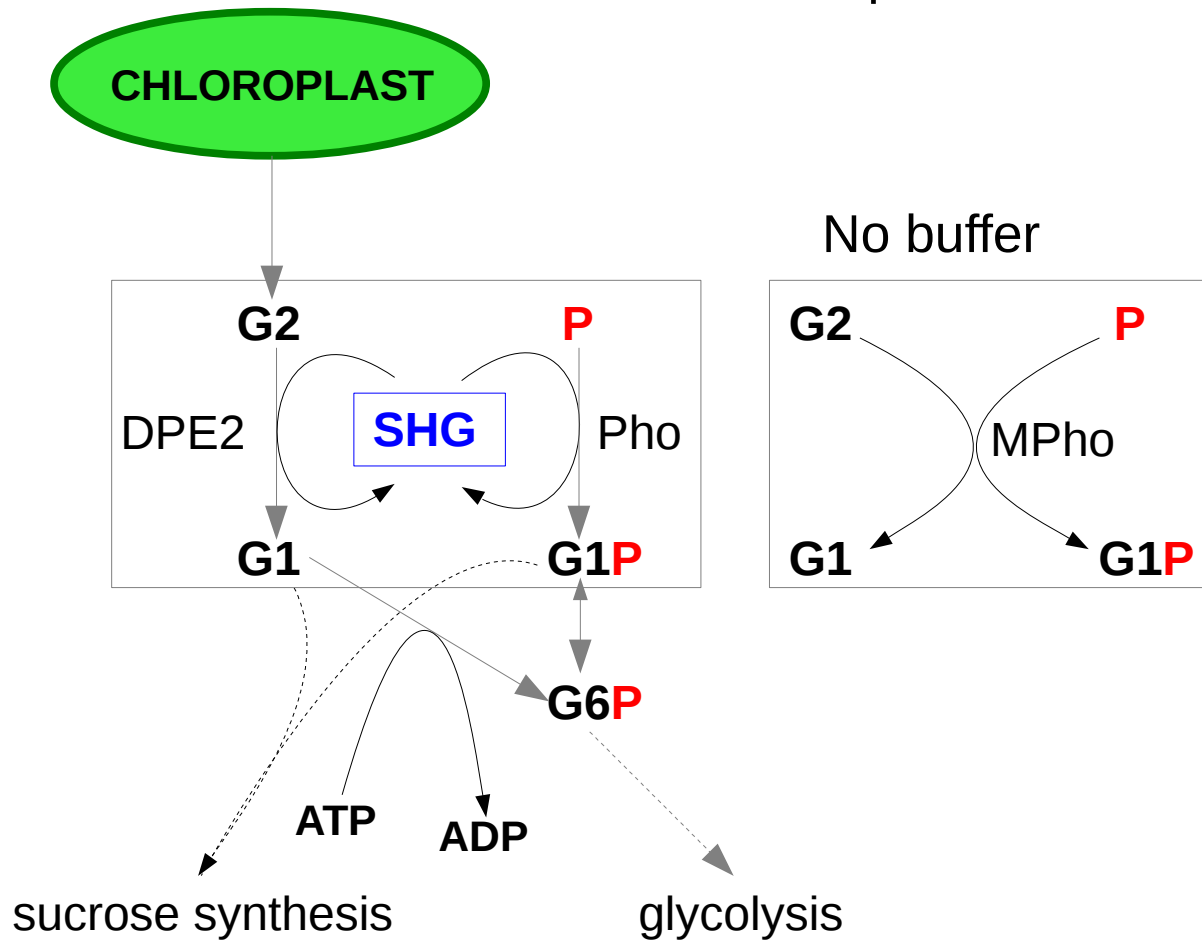# What is the role of the SHG pool?

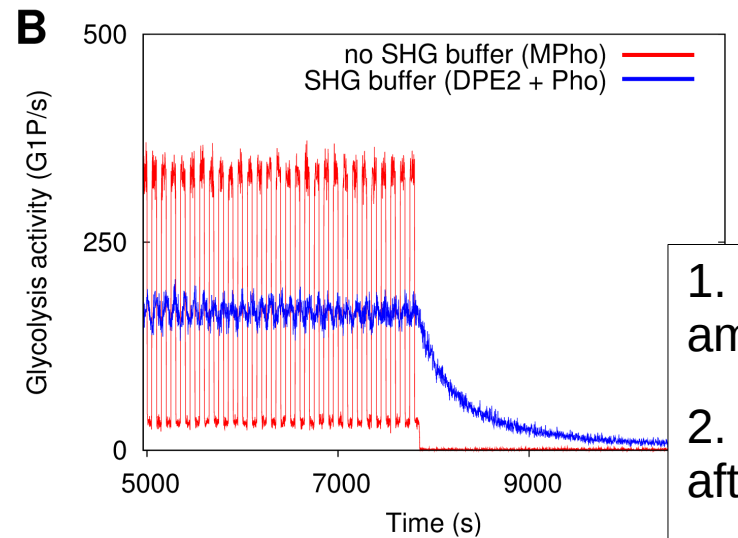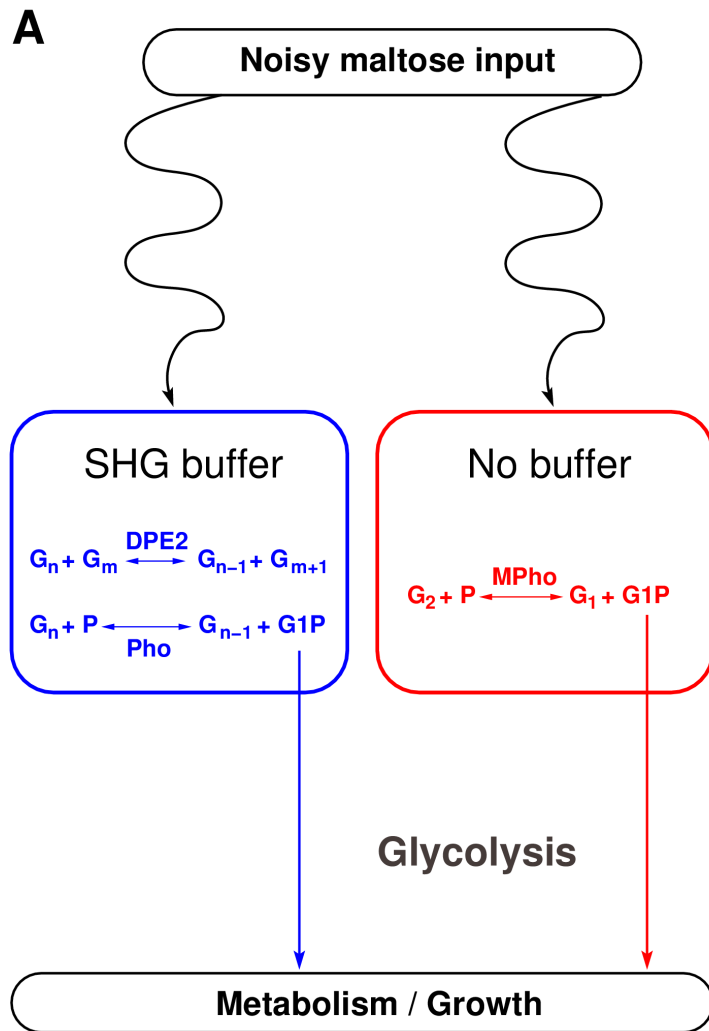Two 'entropic' enzymes mediate the turnover of a polydisperse pool

What is the advantage over other hypothetical systems?

What is the role of the SHG pool?

Comparison with alternative

CHLOROPLAST

No buffer

G2   **P**

DPE2   **SHG**   Pho

G1   **G1P**

G2   **P**

MPho

G1   **G1P**

G6P

ATP   **ADP**

sucrose synthesis   glycolysis

Polydisperse SHG pools increases robustness *in vivo*

A

Noisy maltose input

SHG buffer

$G_n + G_m \xrightleftharpoons[]{DPE2} G_{n-1} + G_{m+1}$

$G_n + P \xrightleftharpoons[Pho]{} G_{n-1} + G1P$

No buffer

$G_2 + P \xrightleftharpoons[]{MPho} G_1 + G1P$

Glycolysis

Metabolism / Growth

B

no SHG buffer (MPho) —
SHG buffer (DPE2 + Pho) —

Glycolysis activity (G1P/s)

Time (s)

C

no SHG buffer ●
SHG buffer ●

Glycolysis activity (G1P/s)

Maltose influx (G2/s)

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
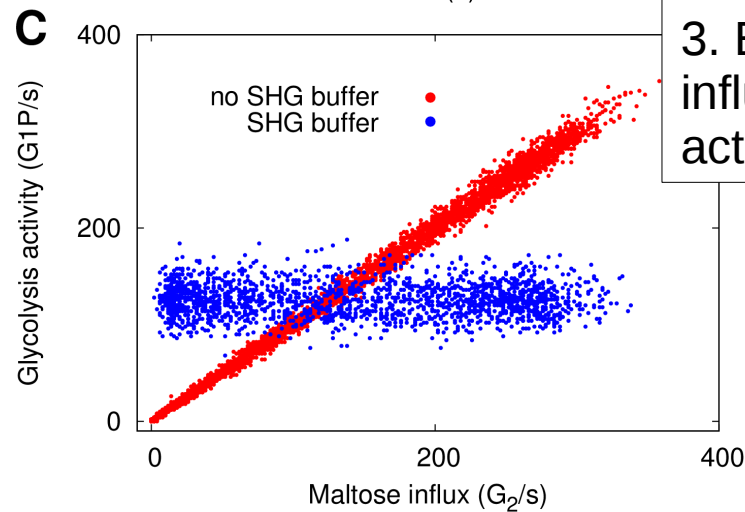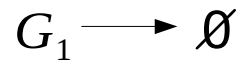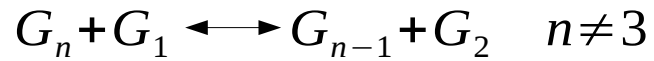
Replacing DPE2 by MalQ

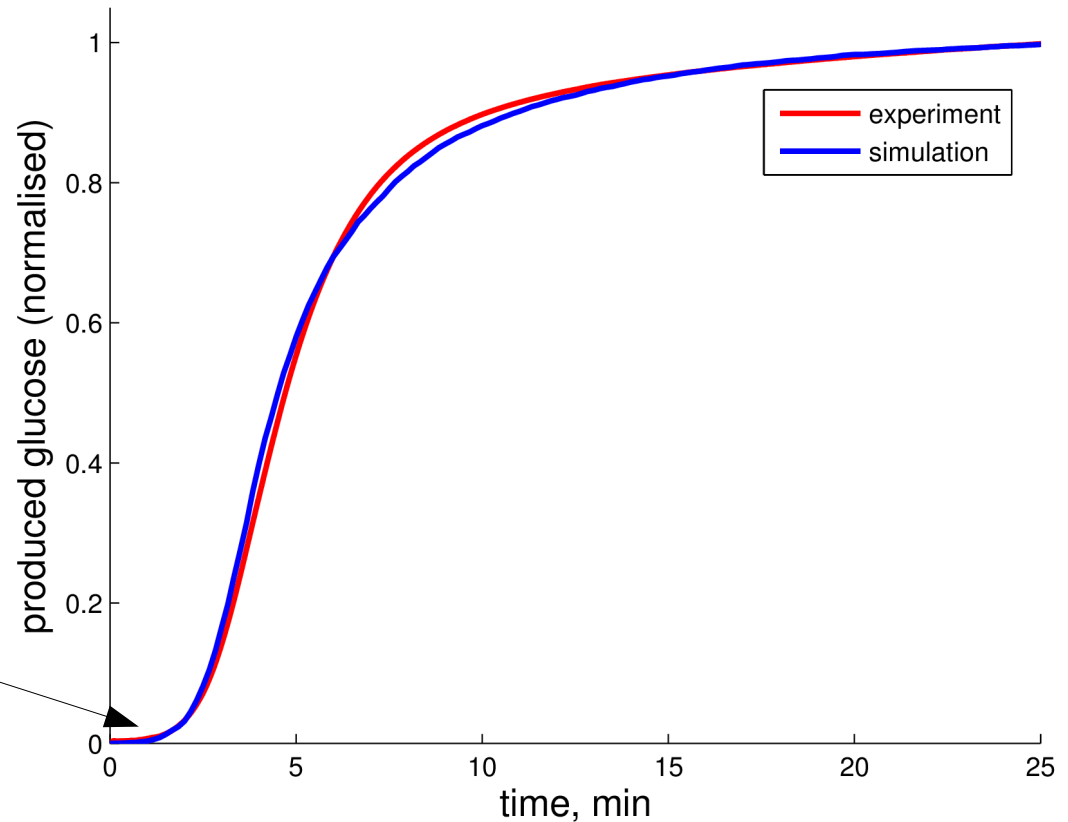MalQ does the same as DPE2, but does not use SHG

$$G_n + G_1 \longleftrightarrow G_{n-1} + G_2$$
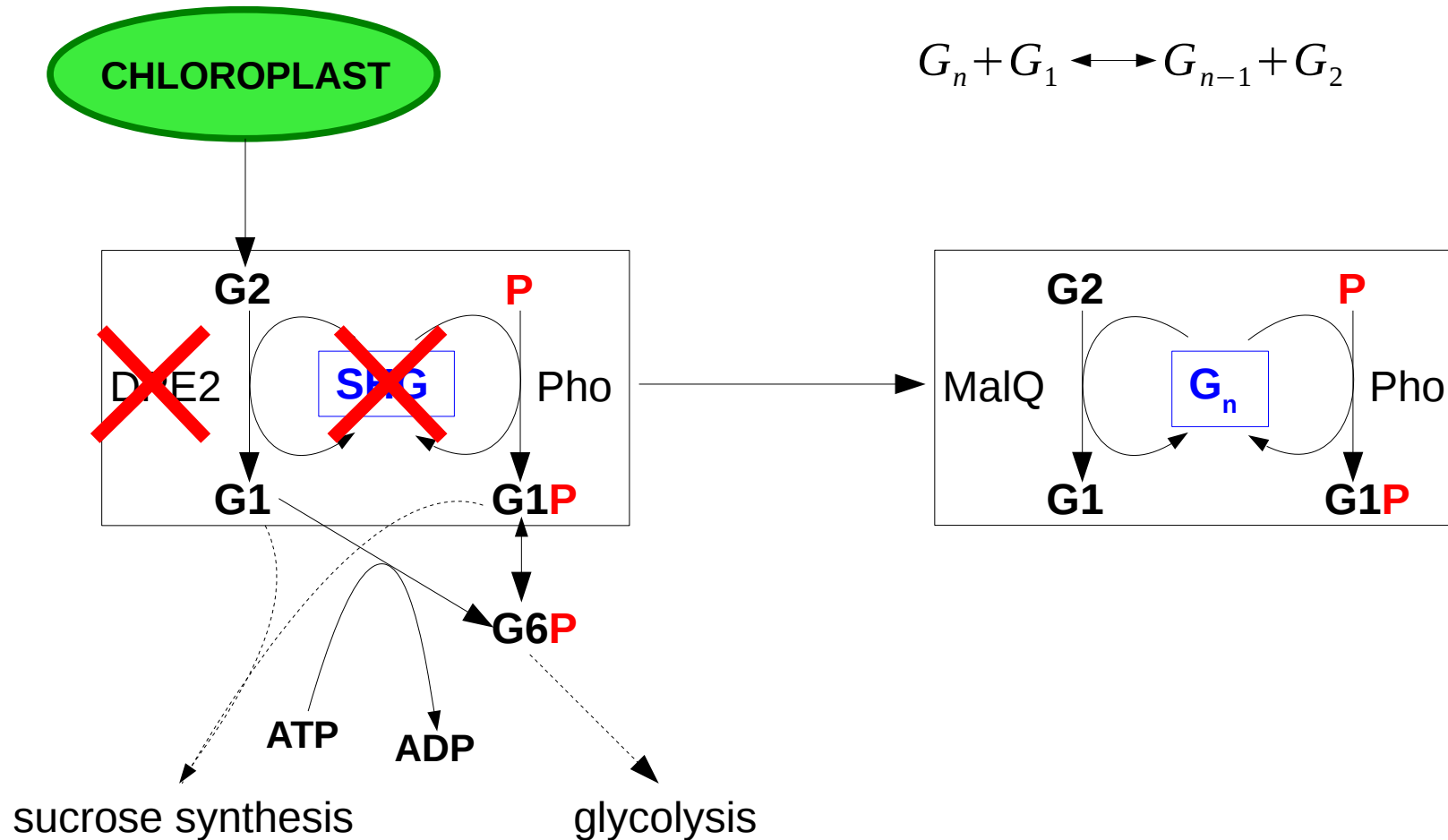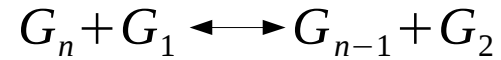
Simulating MalQ in vitro kinetics

In vitro system: DPE1 + HXK

Incubation with G_2 only!

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

$$G_1 \longrightarrow \emptyset$$

delayed start presumably
due to enzyme-bound
glucose residues

Moderate growth phenotype

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

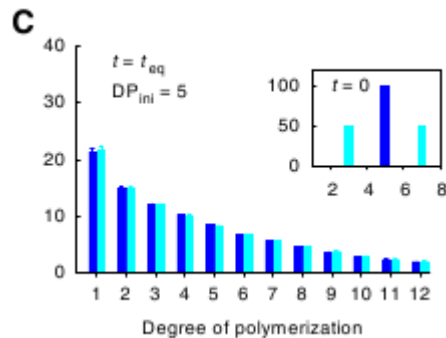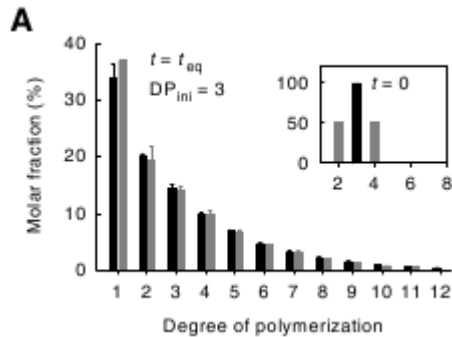complemented plants grow OK!

Maltose turnover
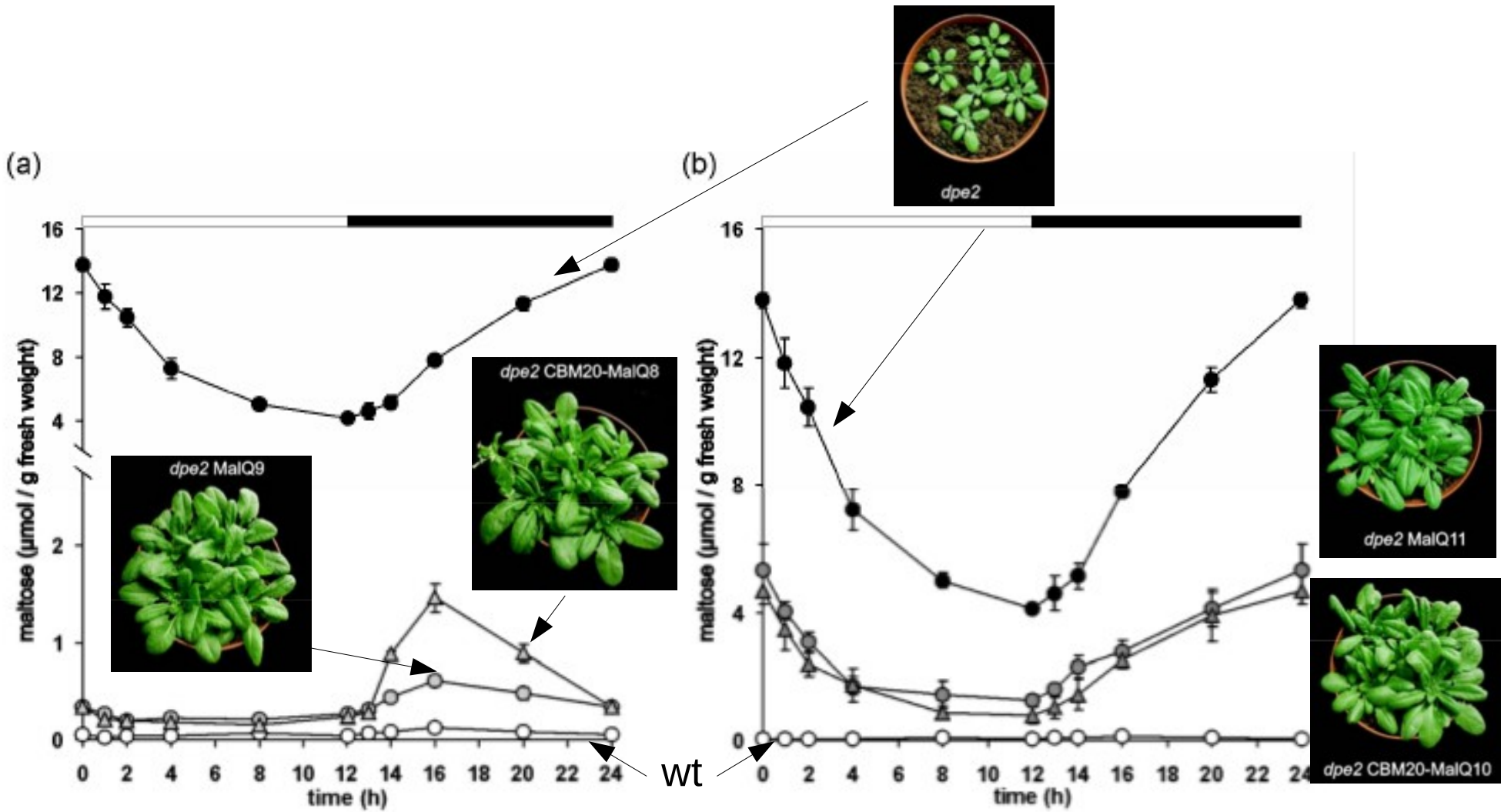
Where else do find entropic enzymes?

...for example

Maltosyltransferases in *Streptomyces*

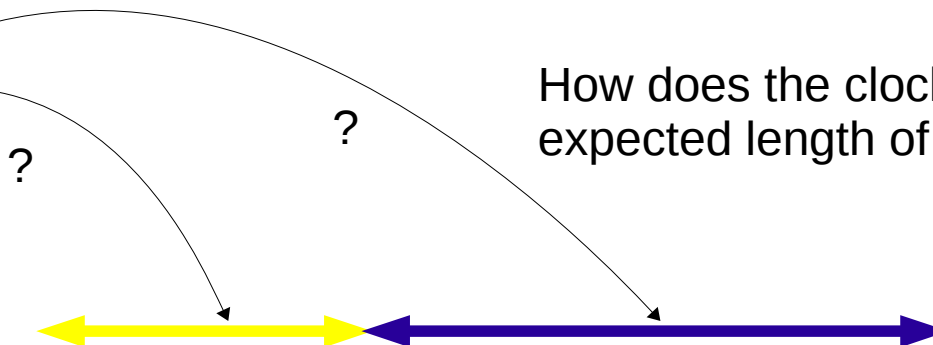"Acceptor specificity" can be explained by entropic principles

Syson et al, 2011, J Biol Chem
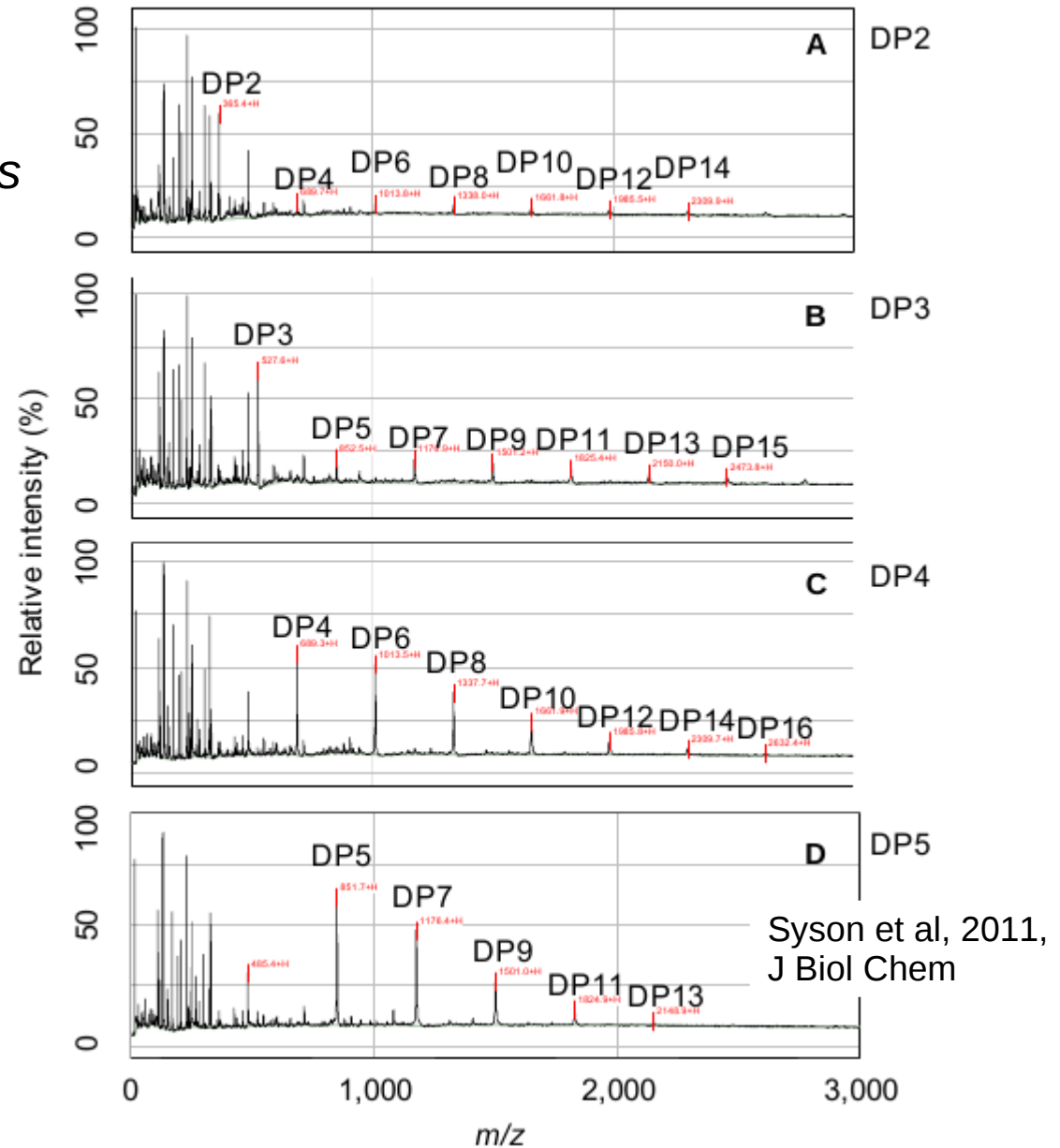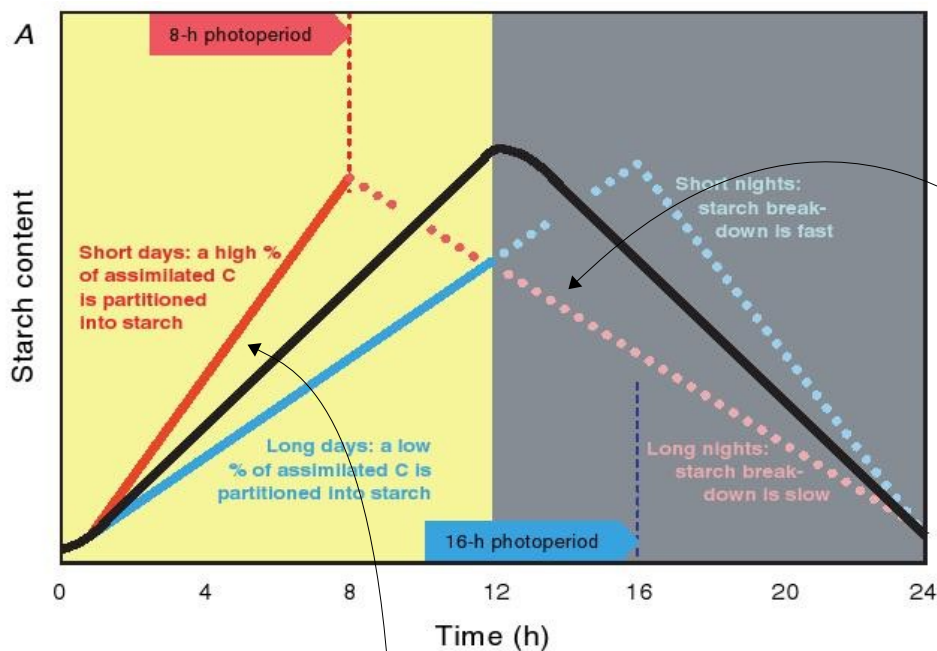
3. Timing of Metabolism

Open questions

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

What measures the starch content?
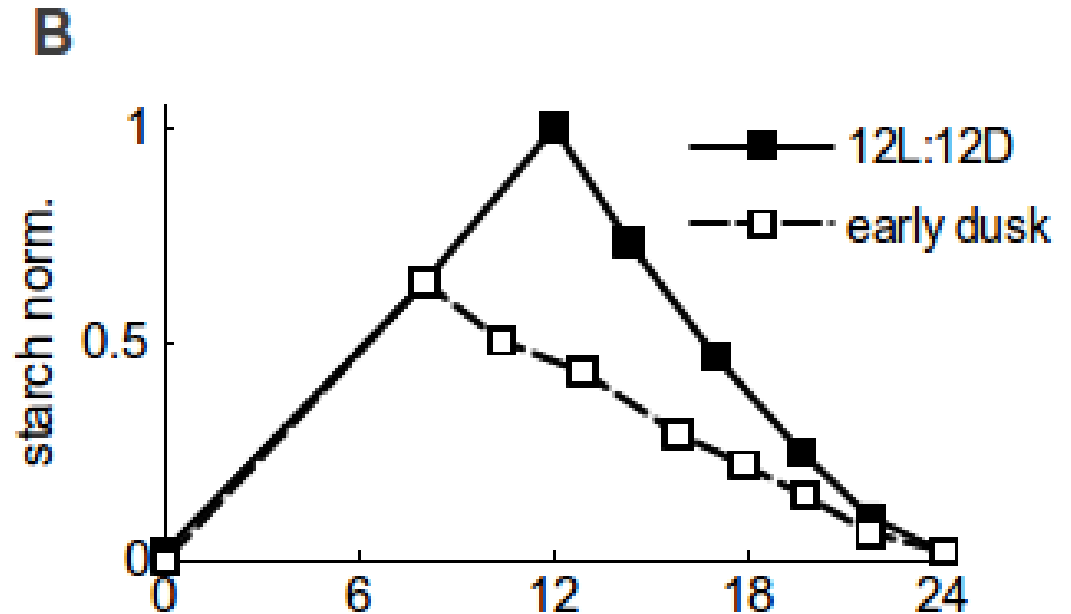
How is the correct breakdown rate 'calculated'?
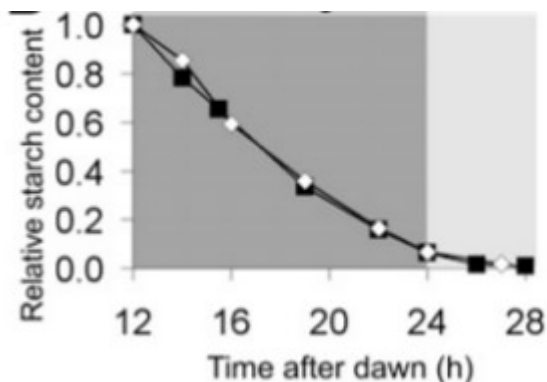
How is carbon partitioning controlled?

...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?
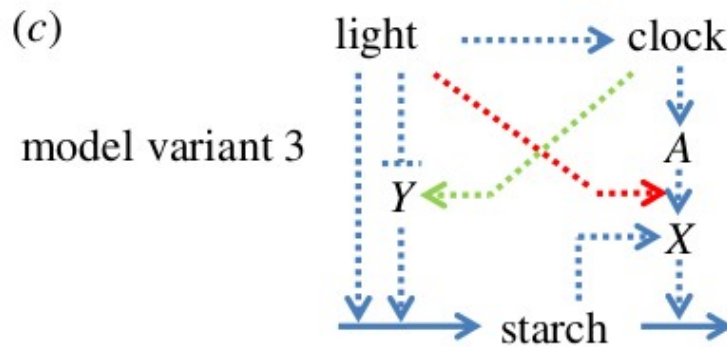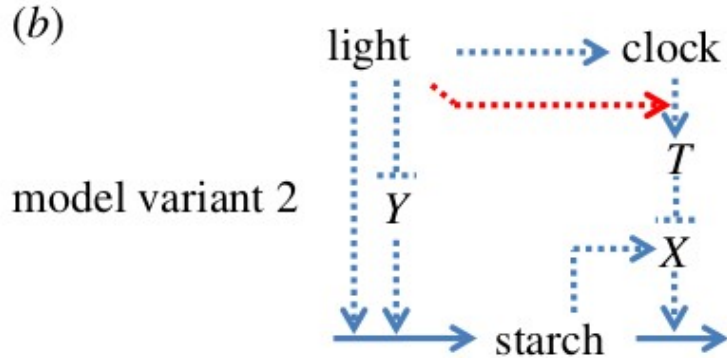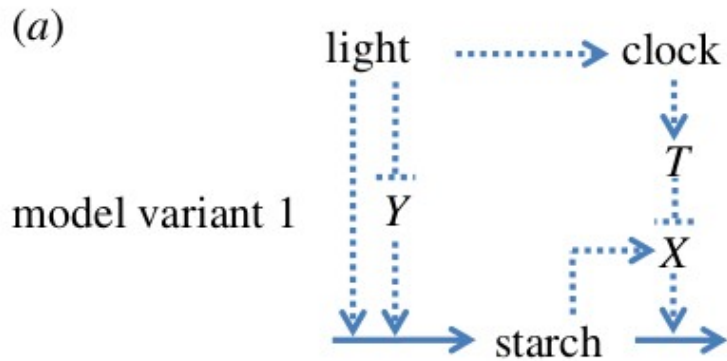
B

Arithmetic division

$$v = \frac{S}{T}$$
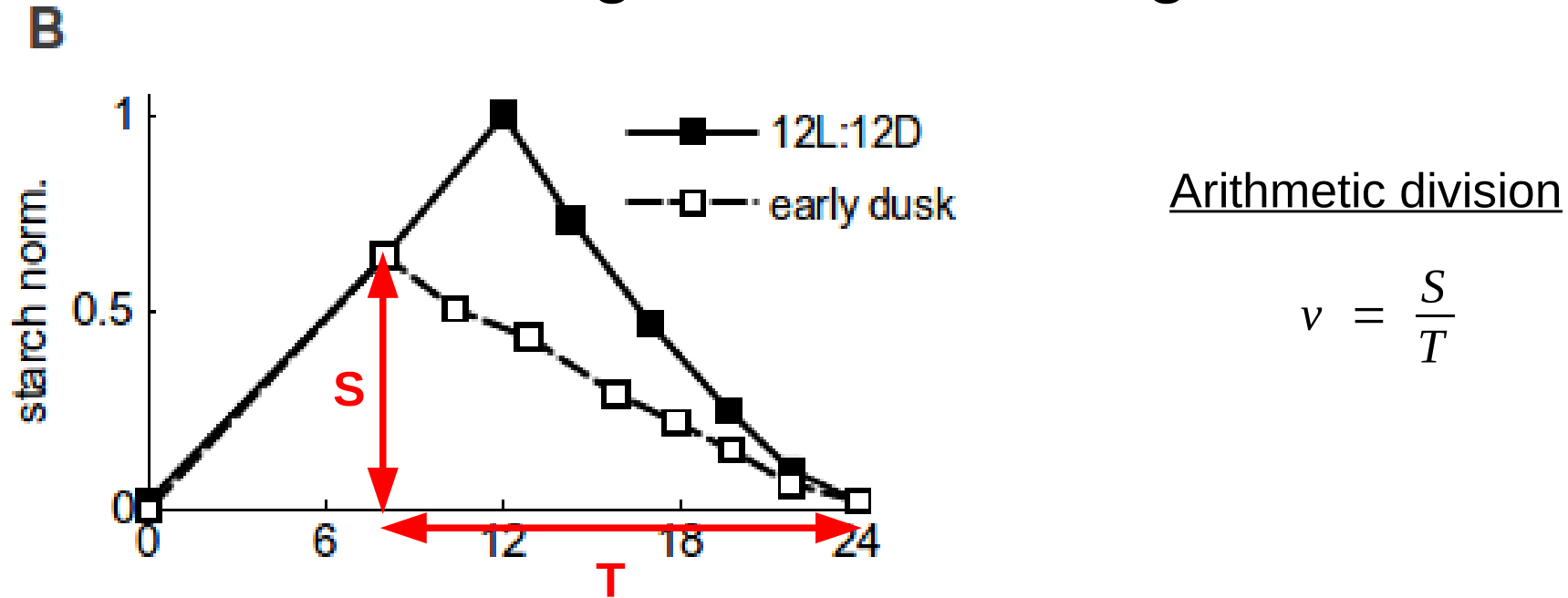
Simplest solution:

Auxiliary compound *X* (e.g. active form of starch degrading enzyme):
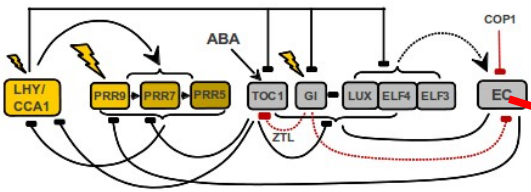
$$\frac{dX}{dt} = k_1 S - k_2 X T$$

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

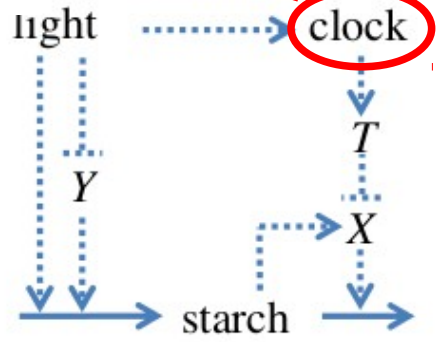Scaldione et al (2013), eLife: **Arabidopsis plants perform arithmetic division to prevent starvation at night**

The evolution of a model

In Seaton et al, 2013:

- Testing basic regulatory mechanisms

The evolution of a model

In Seaton et al, 2013:

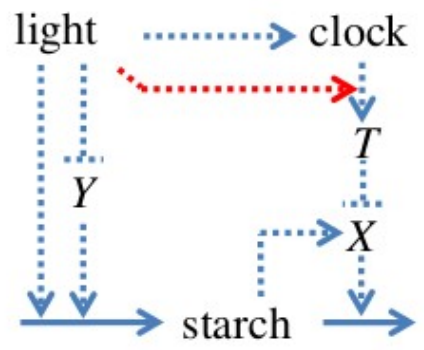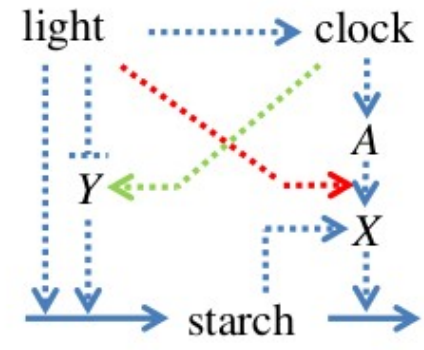- Testing basic regulatory mechanisms
- Replacing 'clock' by a detailed model

The evolution of a model

Pokhilko et al, 2011

In Seaton et al, 2013:

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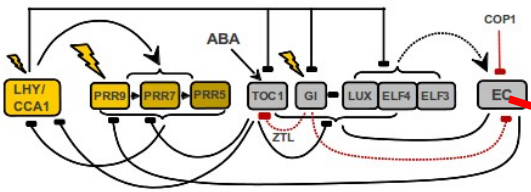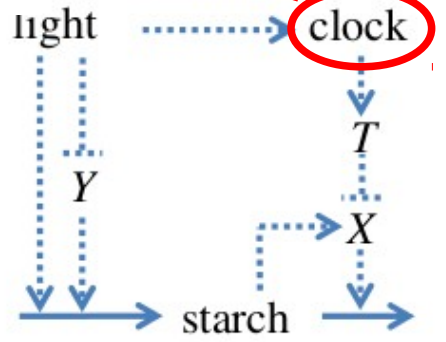- Replacing 'clock' by a detailed model
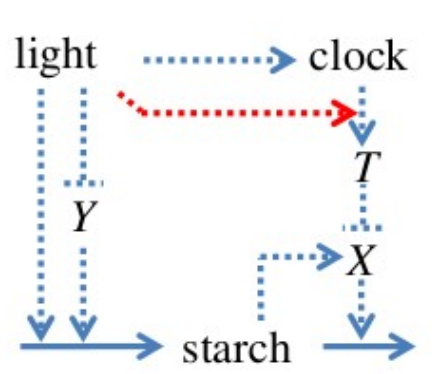- Simulate clock mutants

The evolution of a model
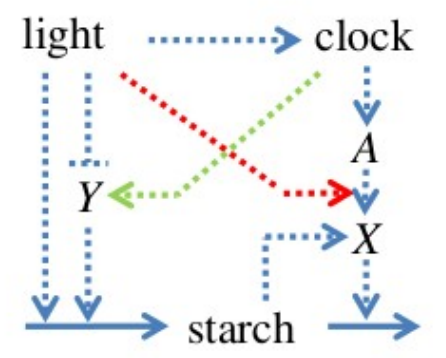
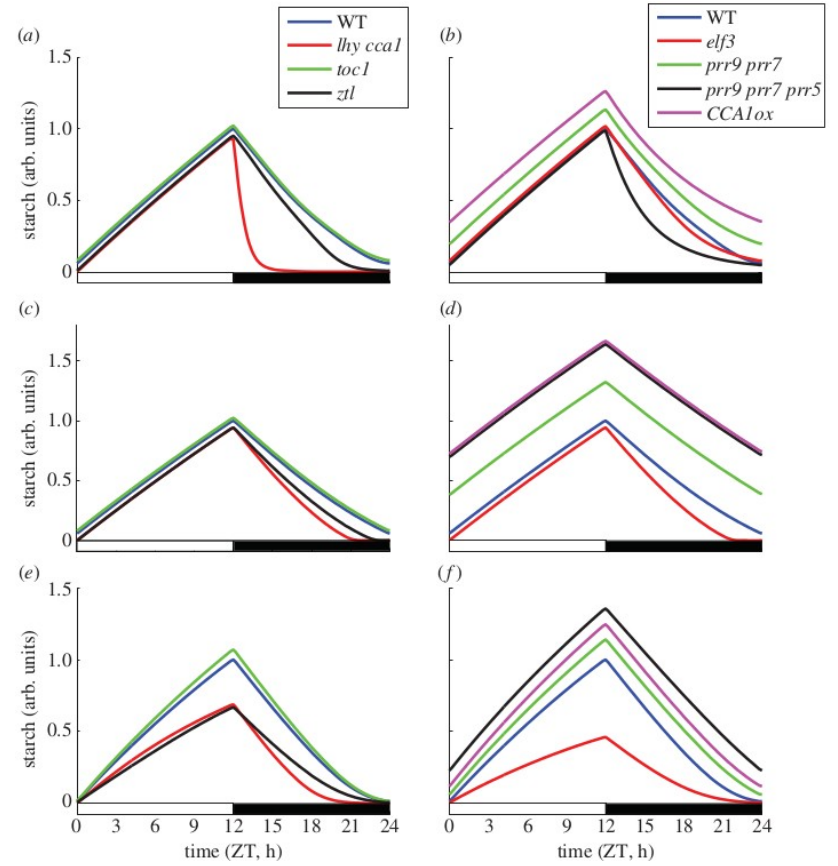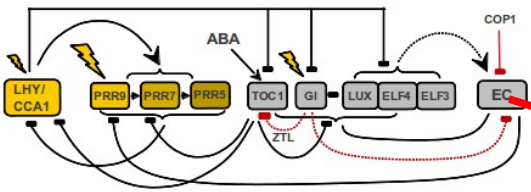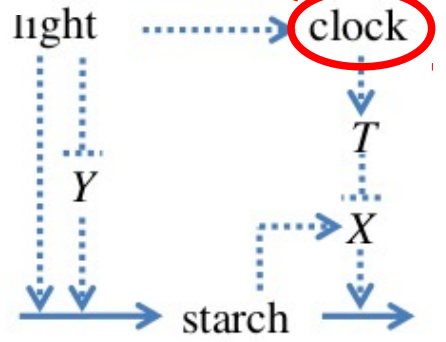**Pokhilko et al, 2011**

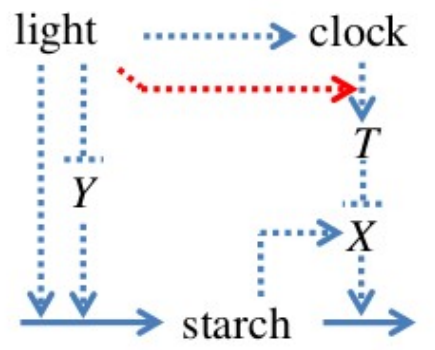model variant 1

(b) model variant 2

(c) model variant 3

In Seaton et al, 2013:

- Testing basic regulatory mechanisms
- Replacing 'clock' by a detailed model
- Simulate clock mutants
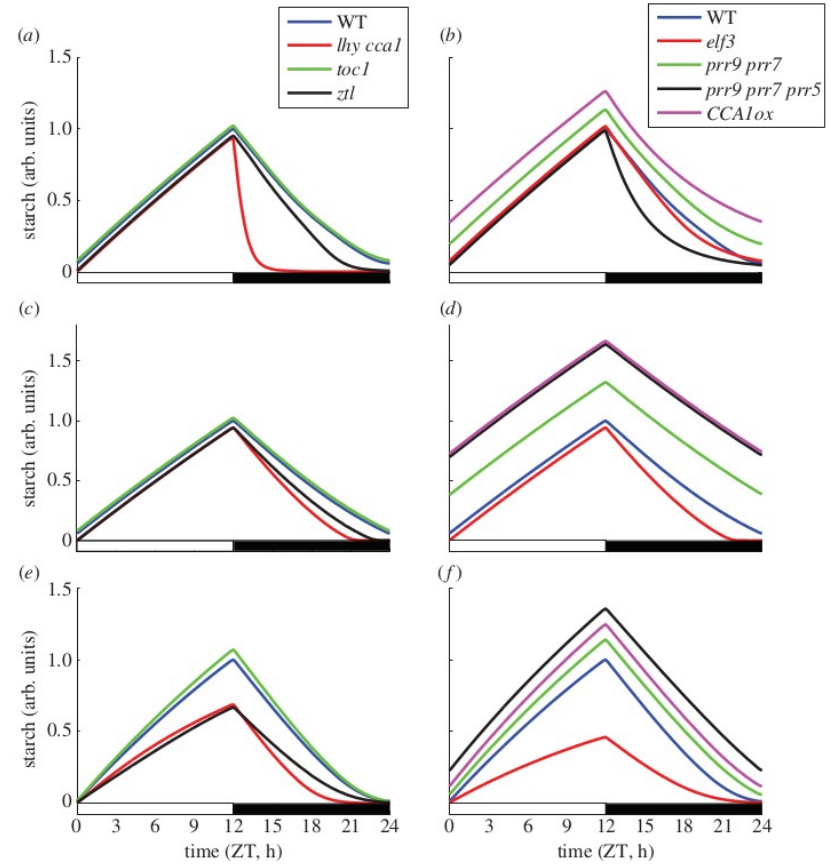
Conclusions:
- Variants 2 & 3 ok, more tests needed
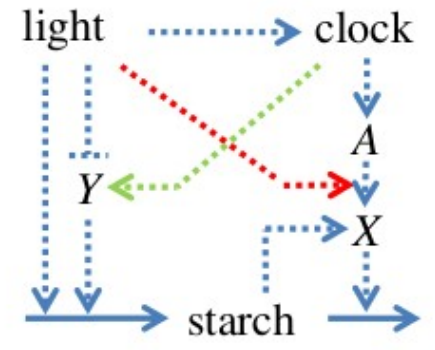- Components A,X,Y remain hypothetical

Adding more details of metabolism
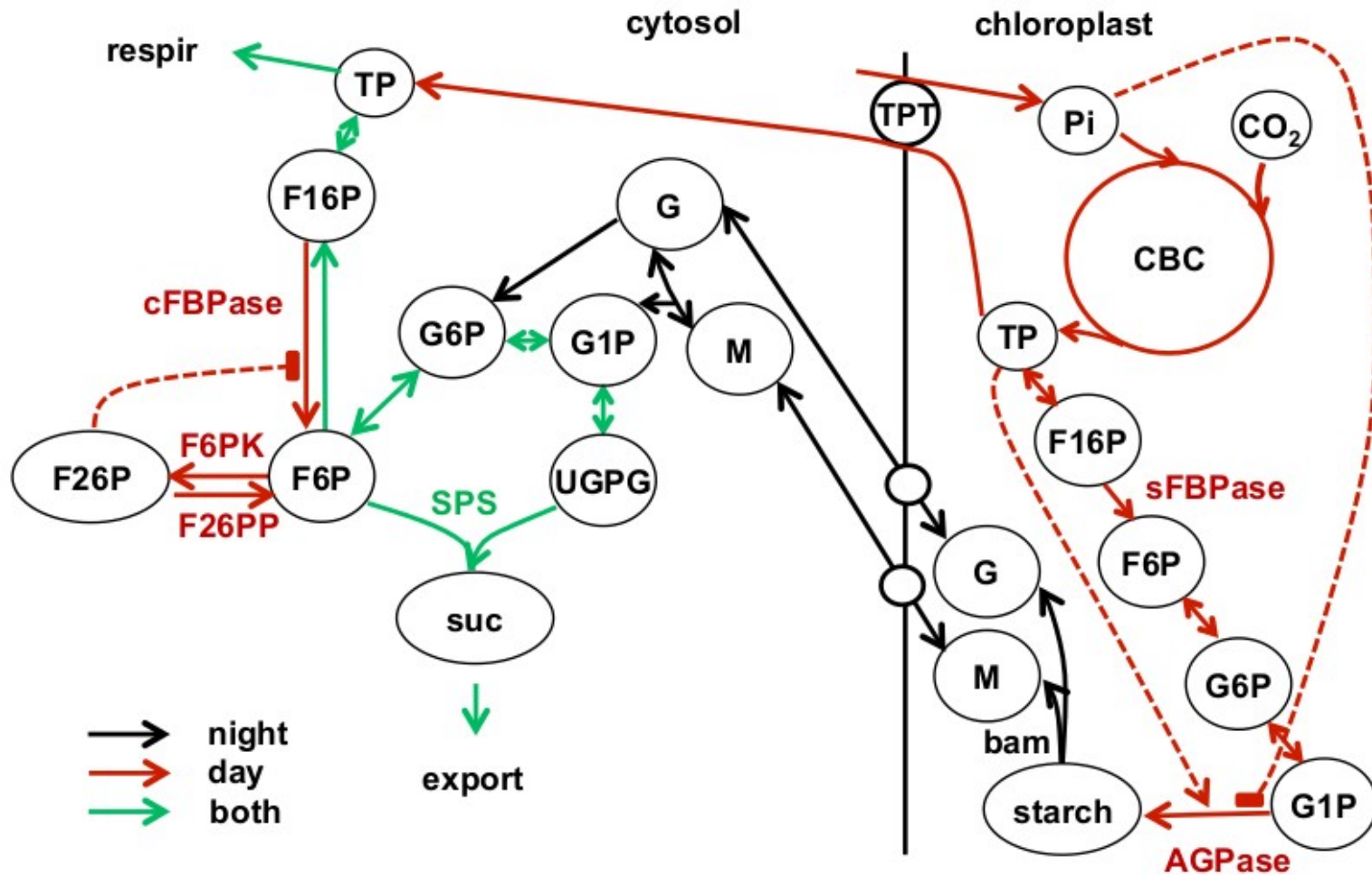
Pokhilko et al, 2014, Mol Biosystems

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

Include key steps but simplify pathways!

Model assumptions (postulates)

1. Key sensors:

Timer α   **dark sensor β**

time-to-dawn   carbon limitation

2. Global regulators:

Activator D   **Inhibitor I**
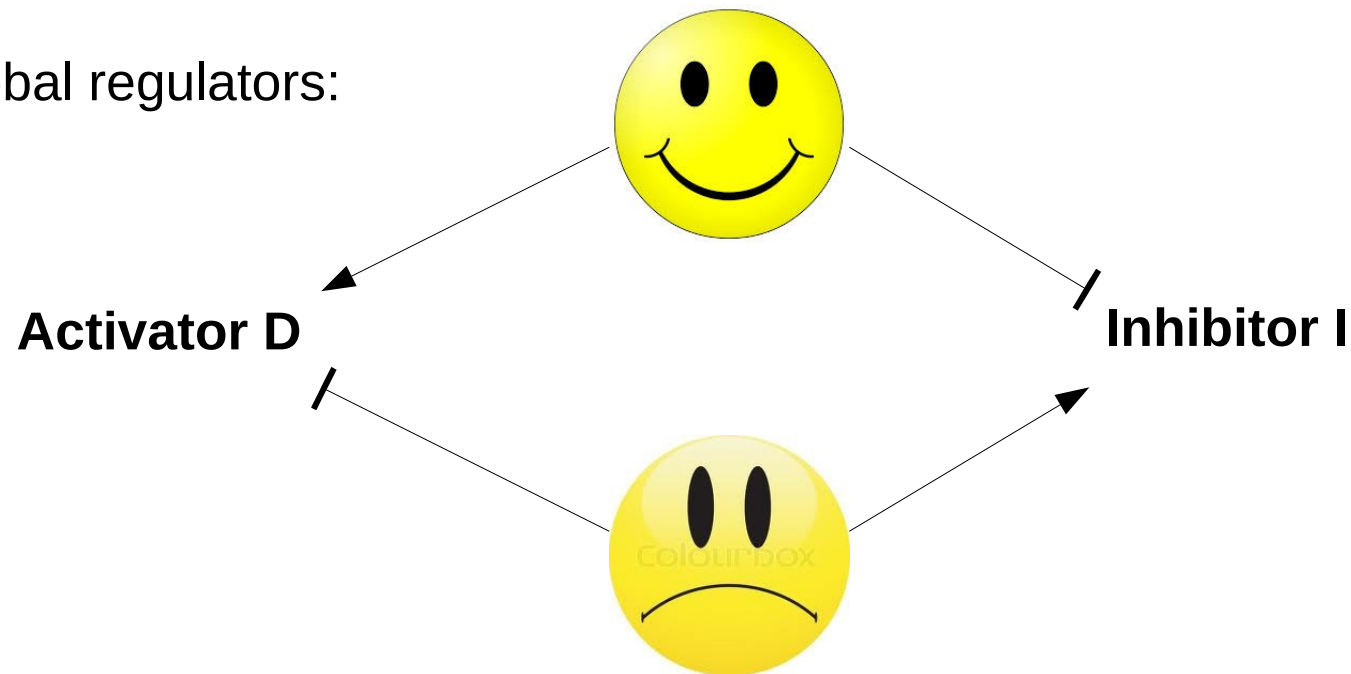
Regulatory principles
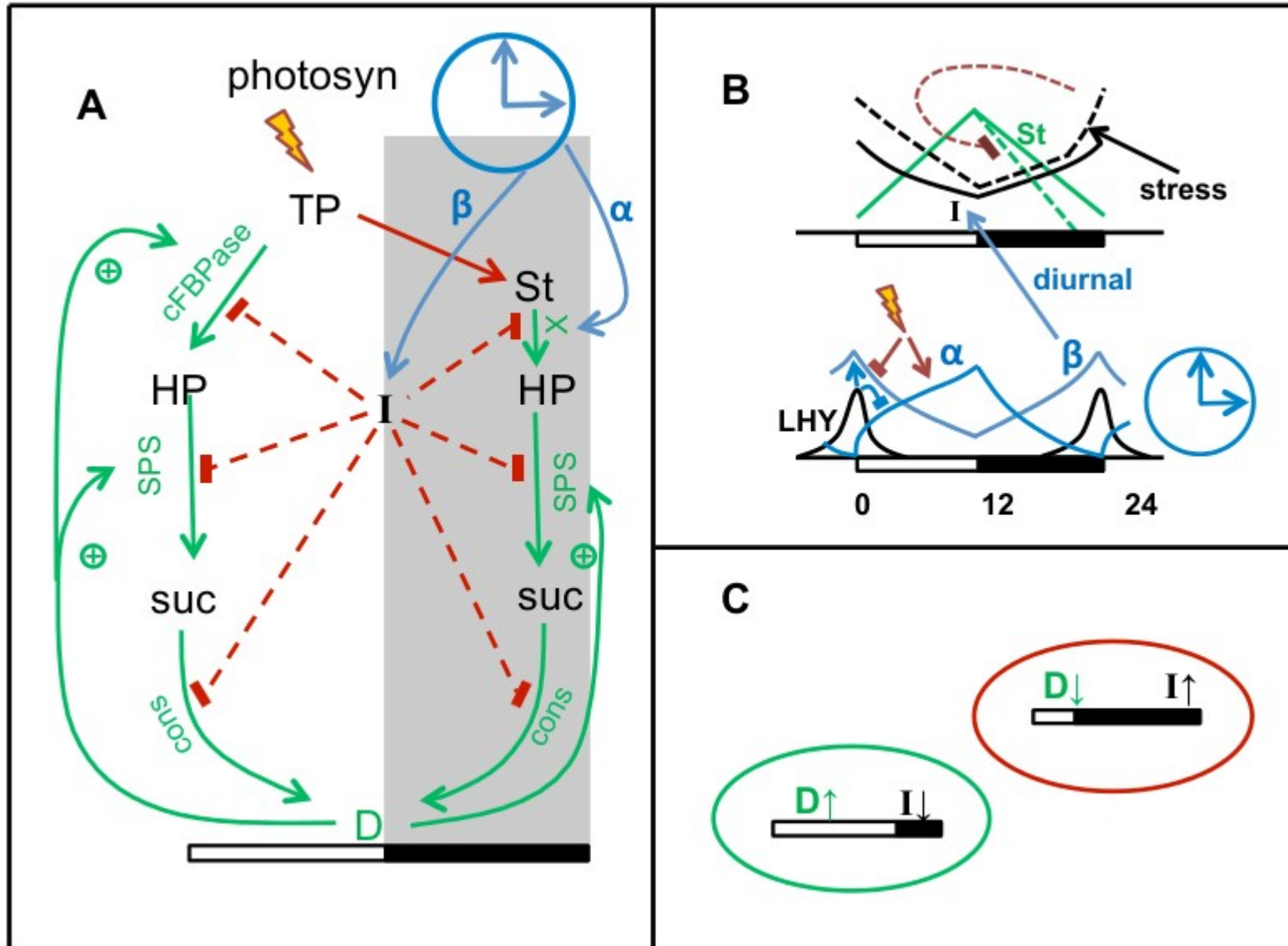
Simulations wild-type

Regulatory principles allow to explain wild-type starch turnover under various photoperiods
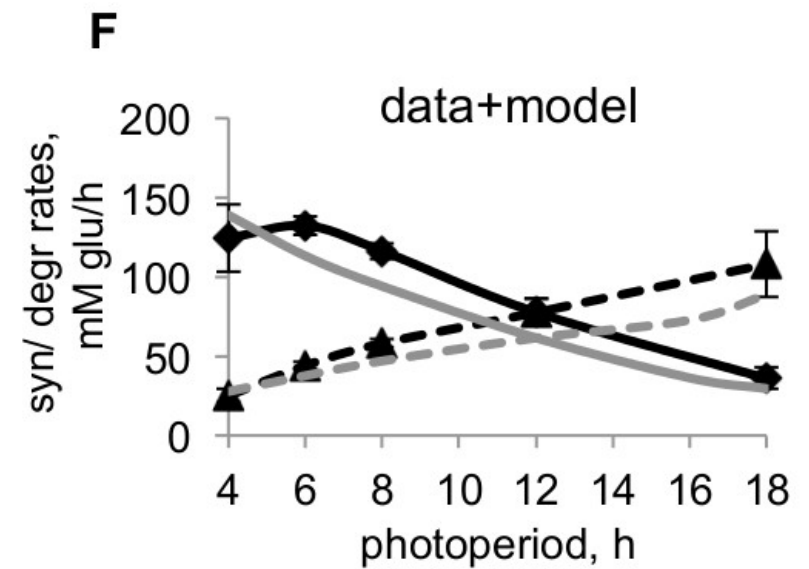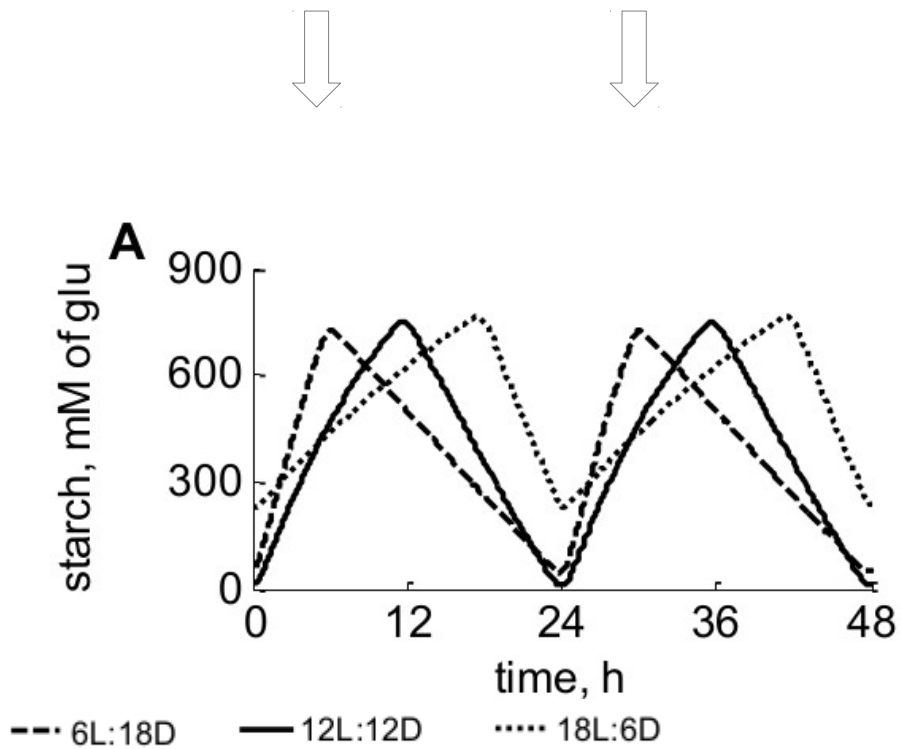
What are the unknown components?

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
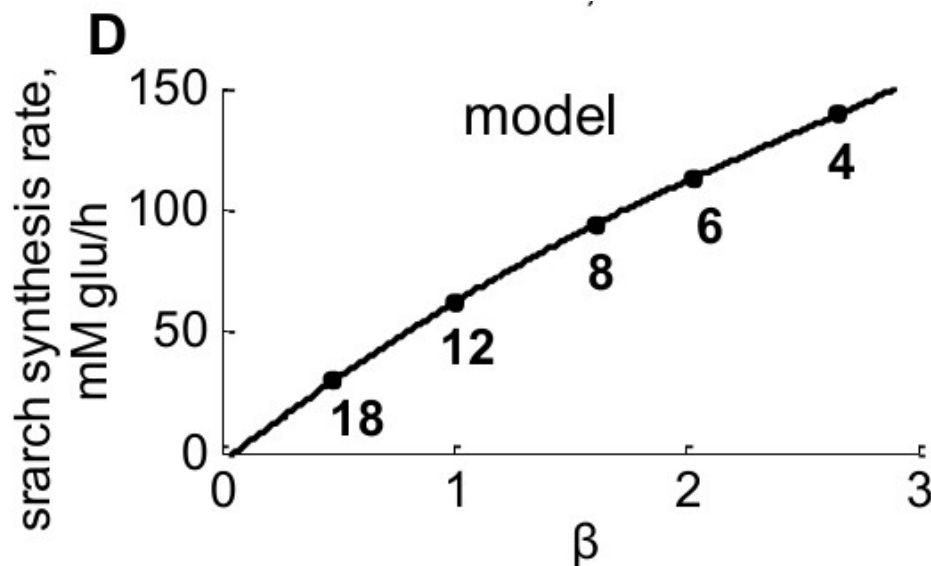
Microarray data for β-subunit of SNRK1
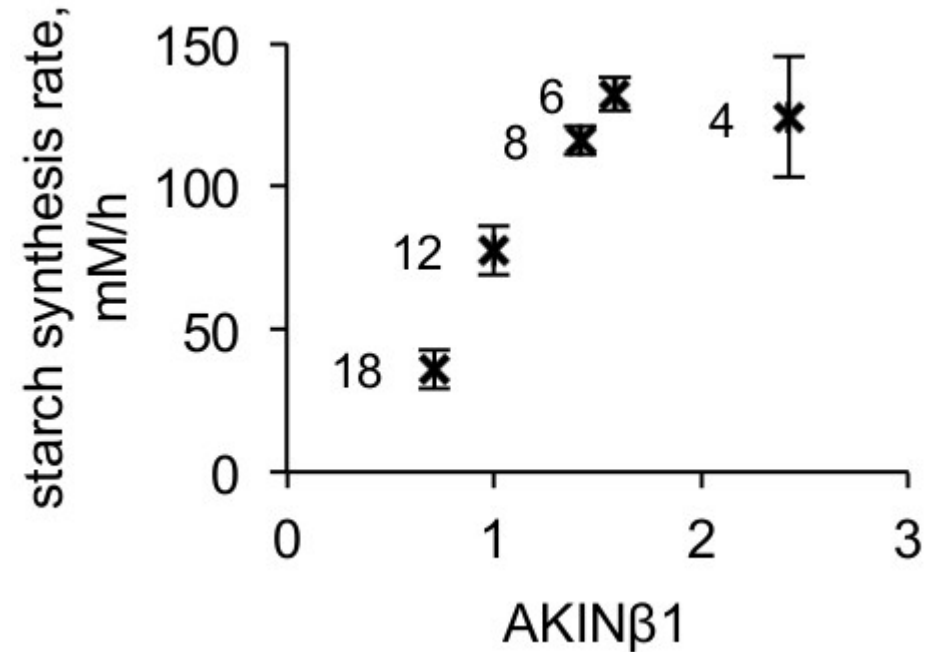
Promotor structure also supports AKINβ1 as good candidate for β

Other regulatory components still unknown!

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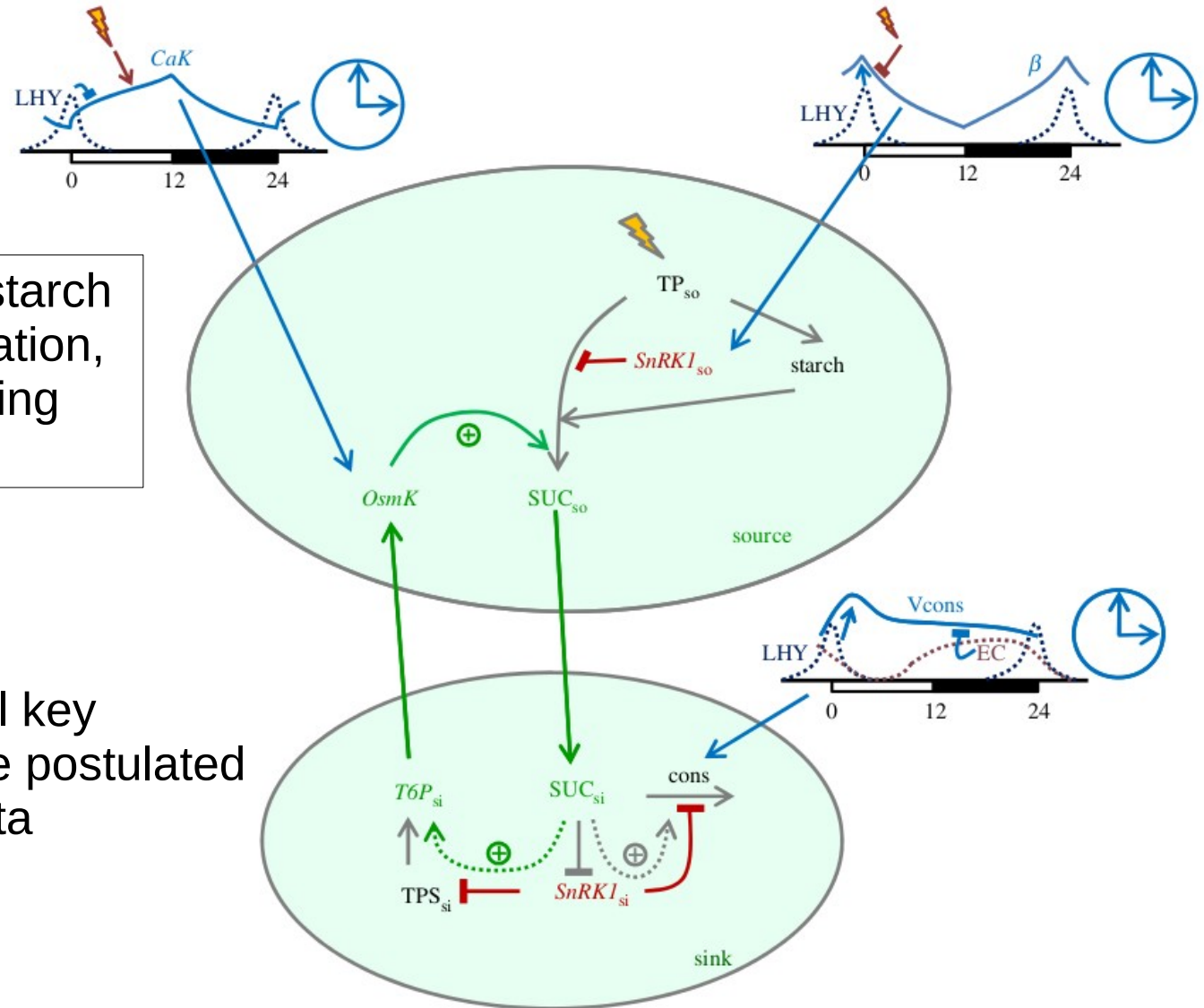
A combined regulation of starch turnover by demand regulation, carbon sensing, light sensing and timing (clock)

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
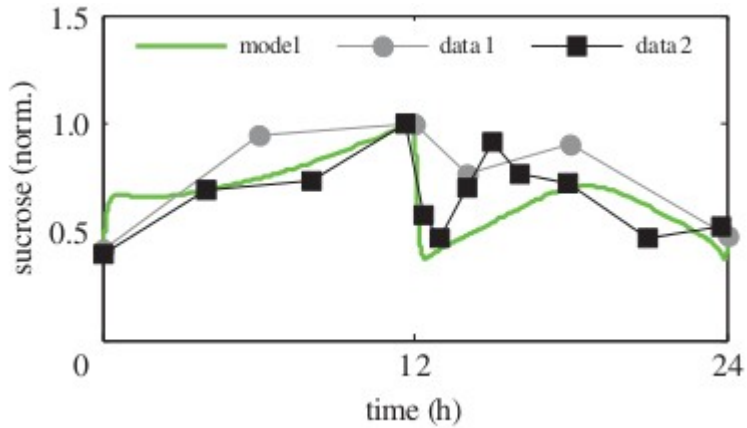
Comparison with experimental data

Theory/Experiment

Making new predictions
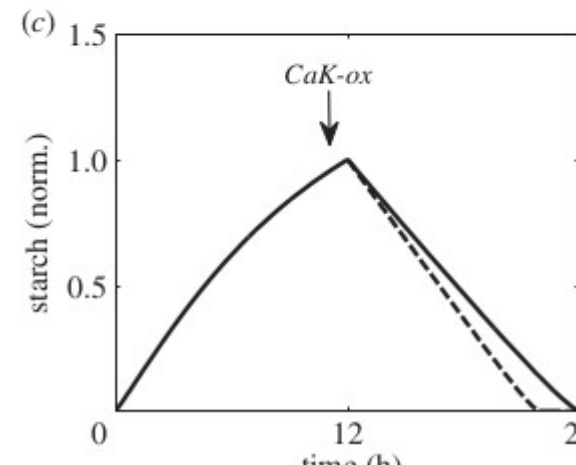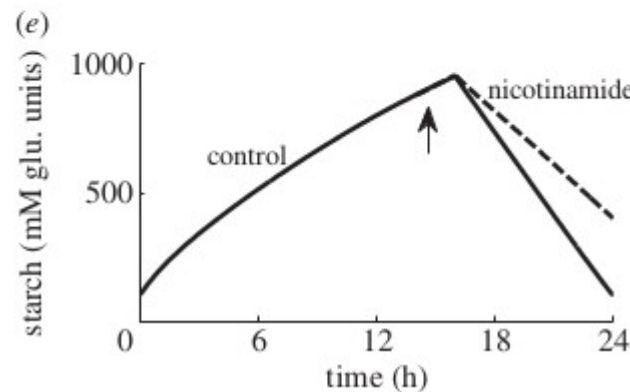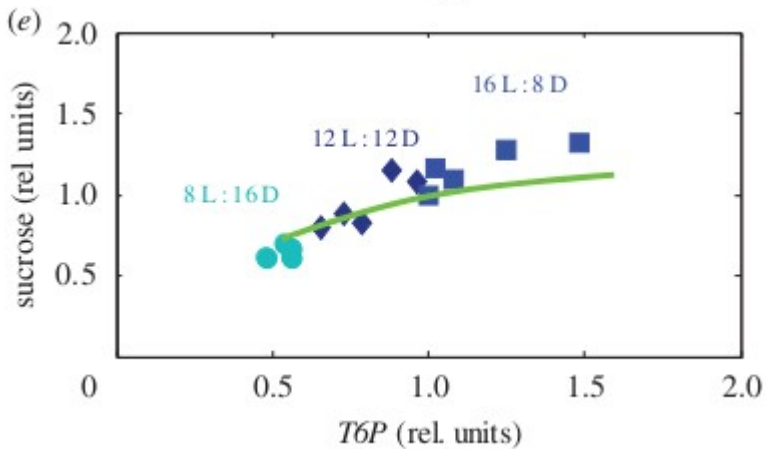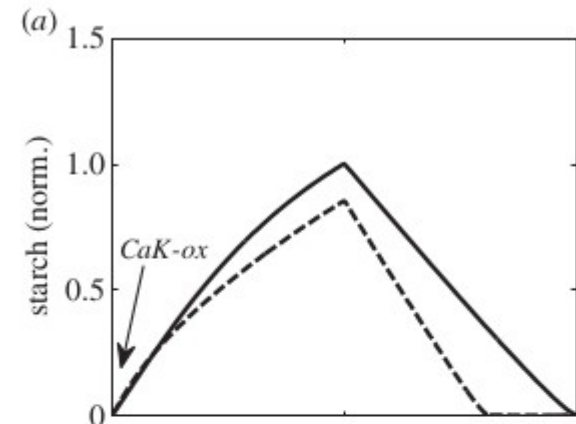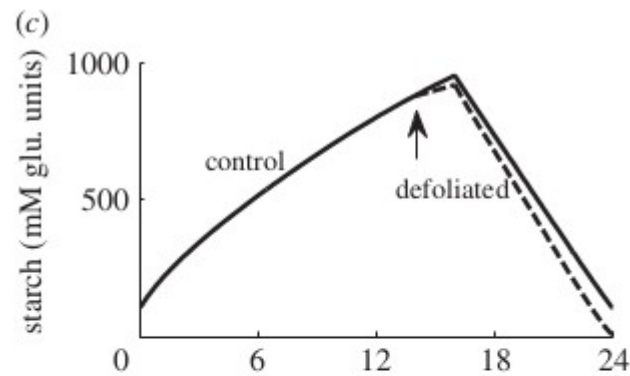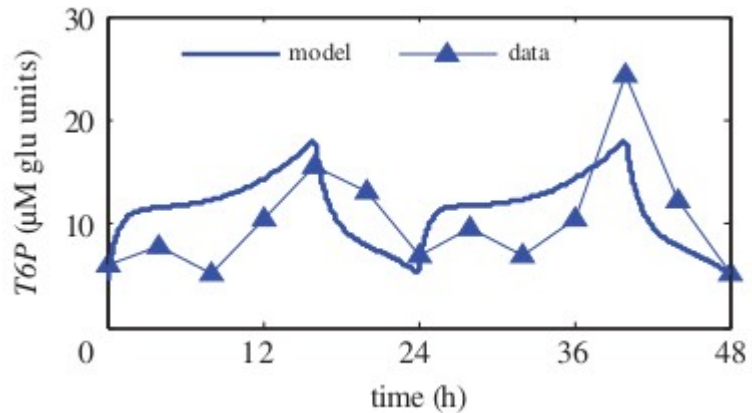
Outlook – towards designing starch

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
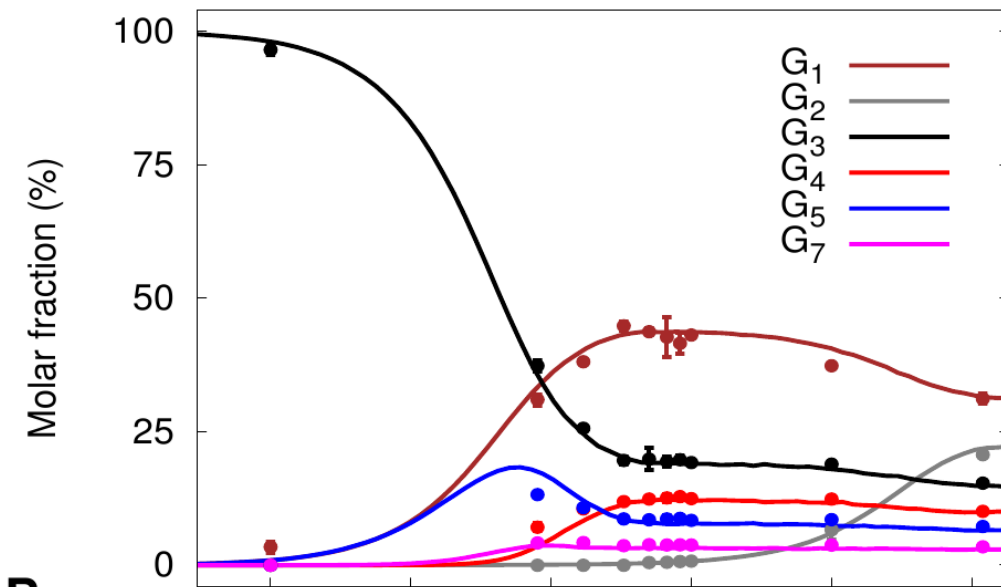
DPE1

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

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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!

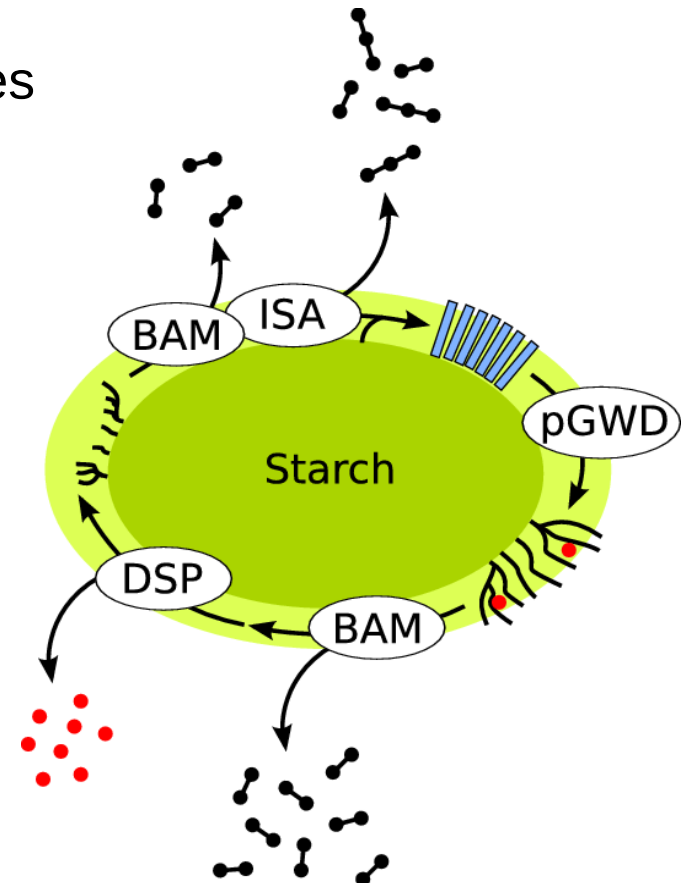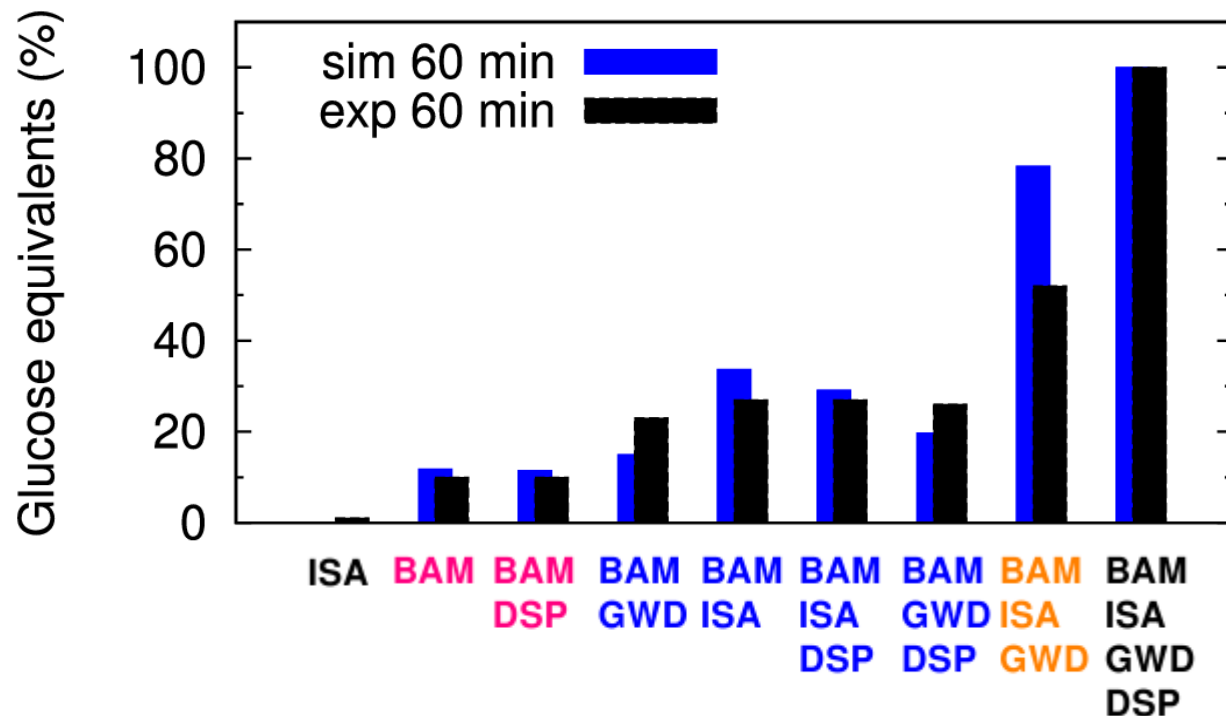# What do we need to model & design starch?

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      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

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)
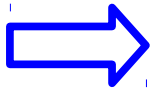
ERA-CAPS Project ***DesignStarch***

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

<u>Potsdam</u>
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<u>Edinburgh</u>
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Daniel Seaton

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Sam Zeeman

QTB

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THE UNIVERSITY OF EDINBURGH

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Swiss Federal Institute of Technology Zurich

DFG
CEPLAS
Cluster of Excellence on Plant Sciences

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