Thermodynamic and regulatory principles of the Calvin-Benson-Bassham Cycle
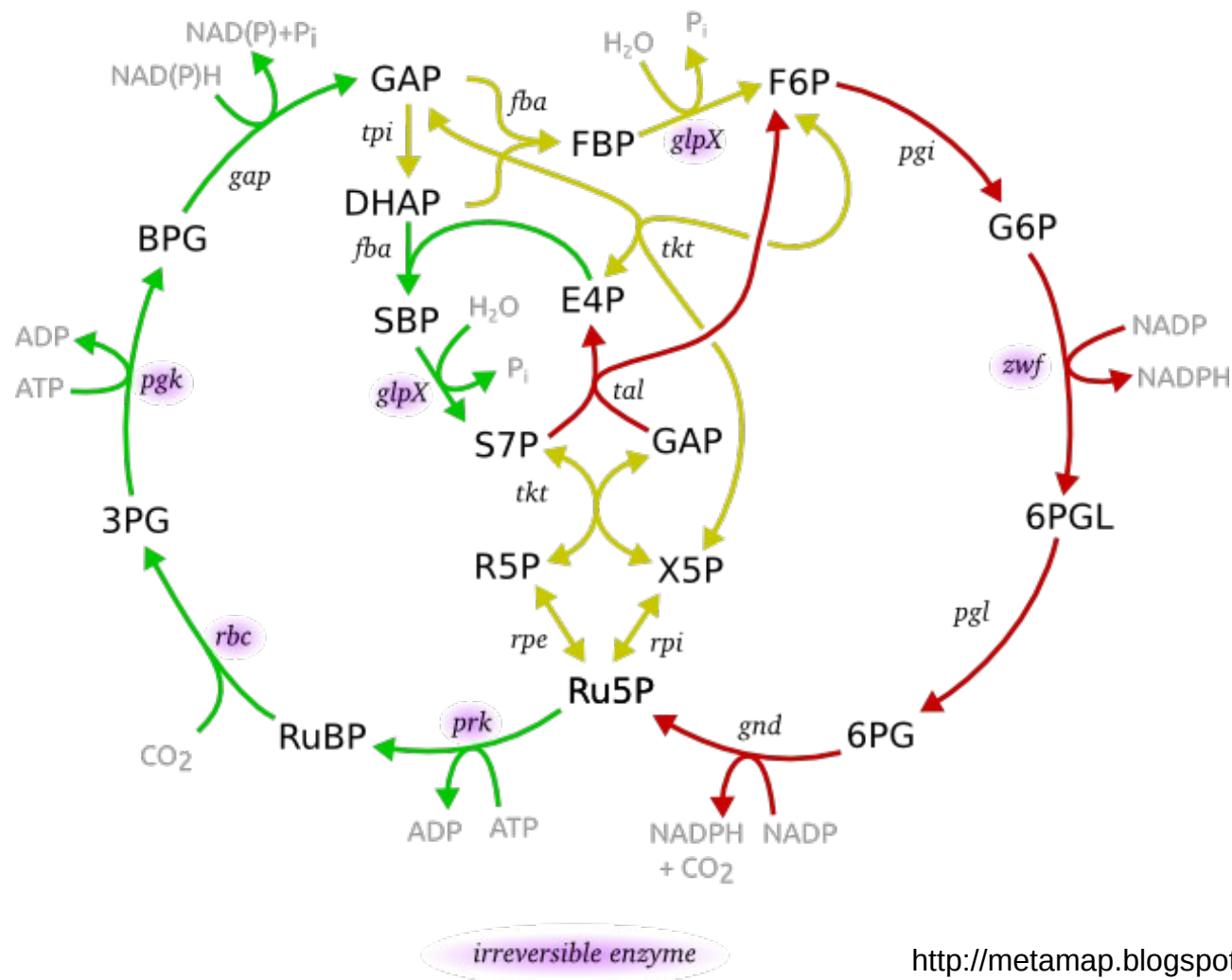
Oliver Ebenhöh

ISGSB Jena, 6.10.2016

The RuBisCO centered view
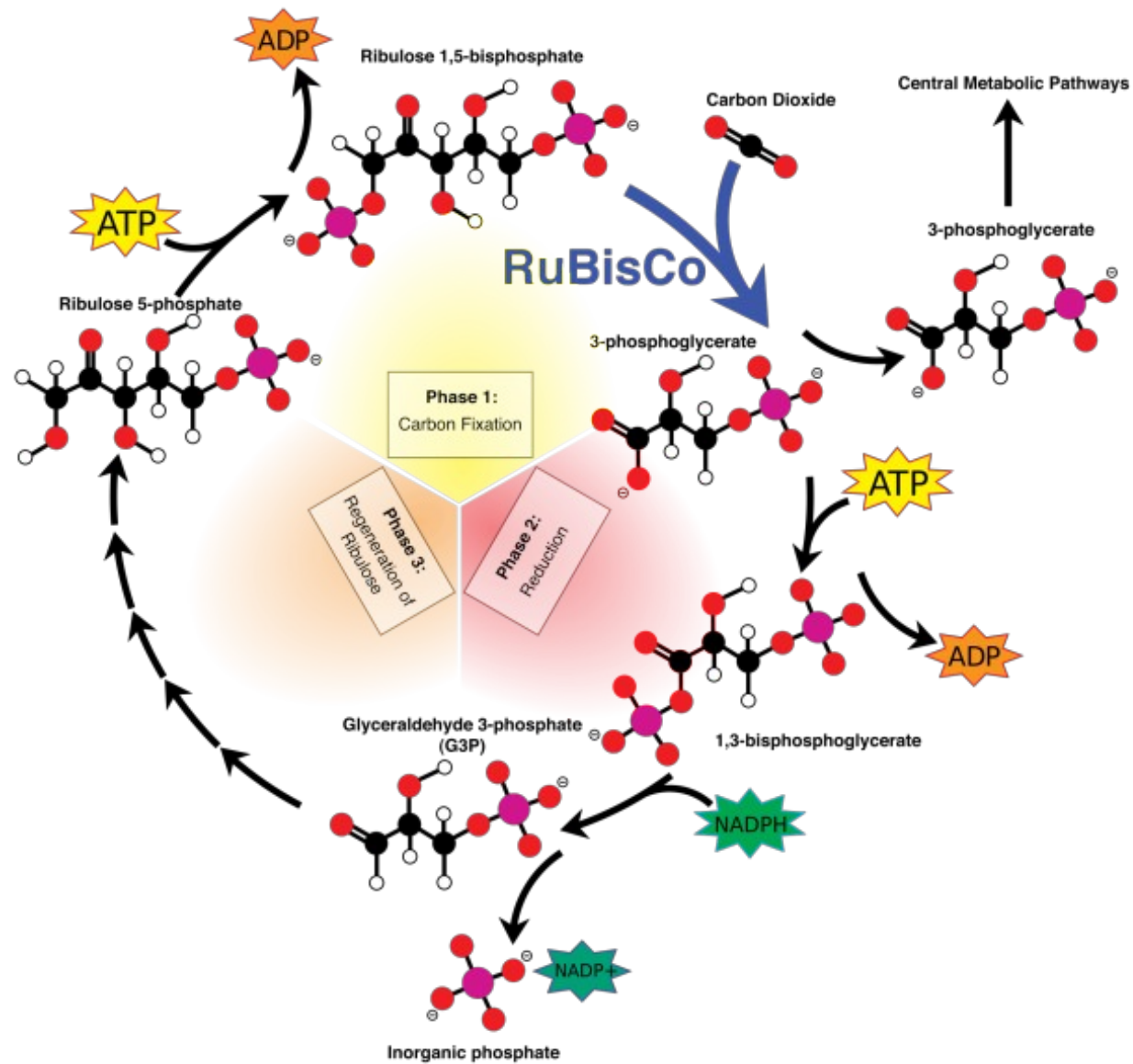
Changing the perspective

© Mike Jones, Creative Commons

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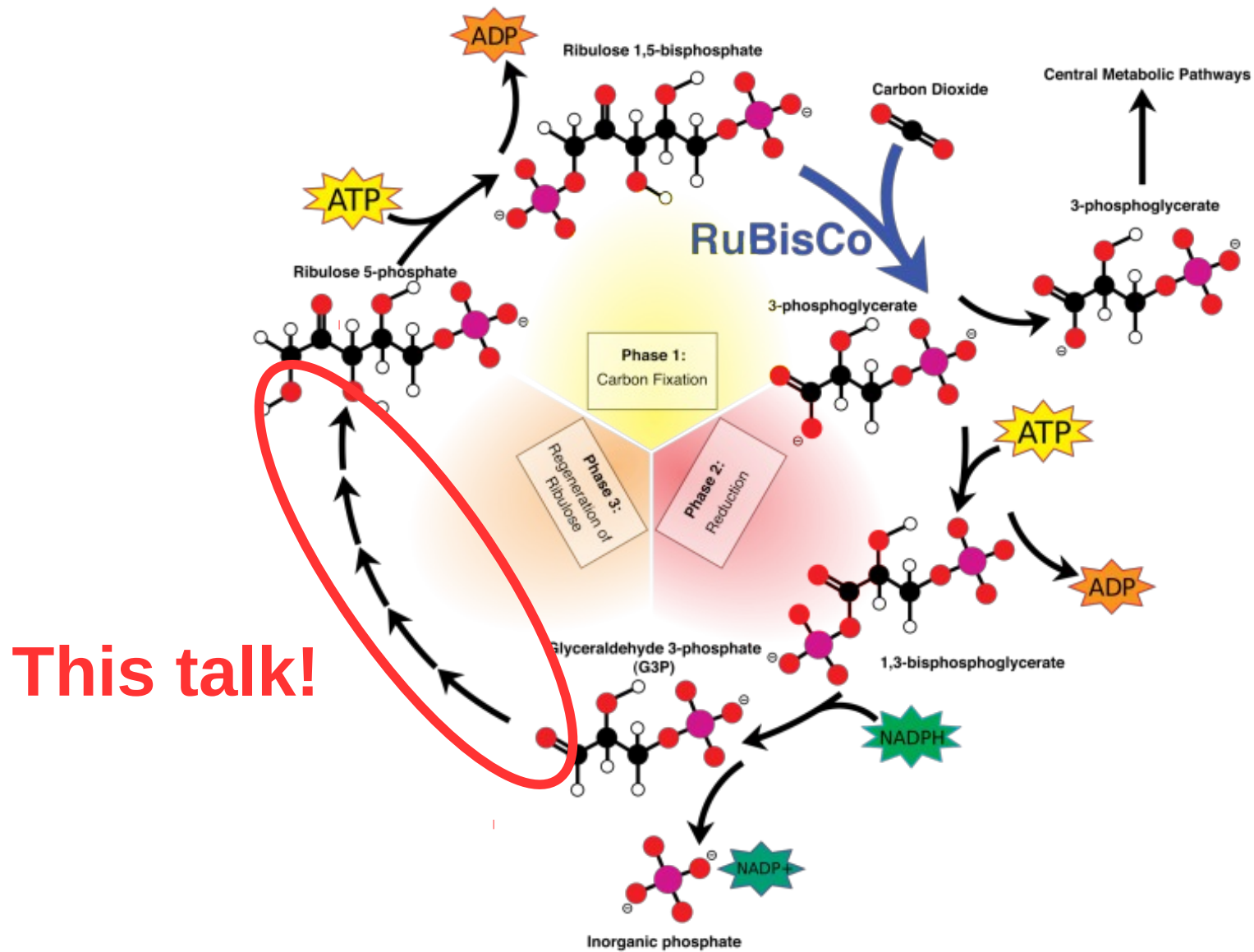
Calvin cycle energetics

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

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

(Bassham and Krause, BBA 1969)

Only a few reactions have a high energy gradient!

Conclusion by Bassham and Krause: this is where regulation must take place!

Gibbs energies of reaction in kcal/mol

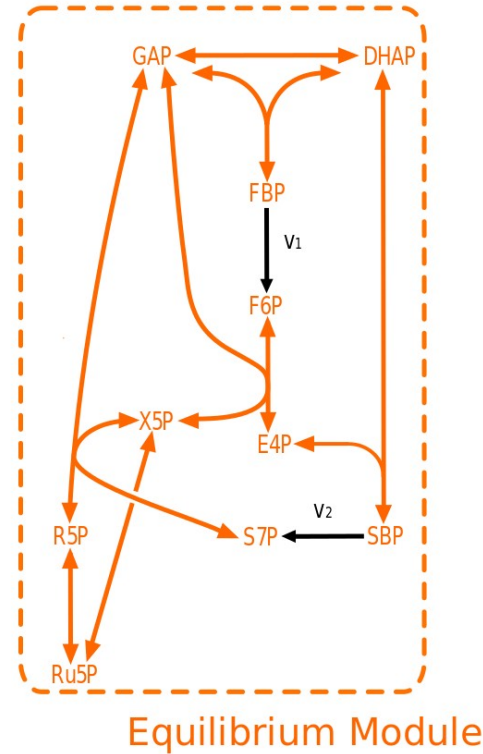from Bassham & Krause, 1969, BBA

The Logic of the CBB Cycle

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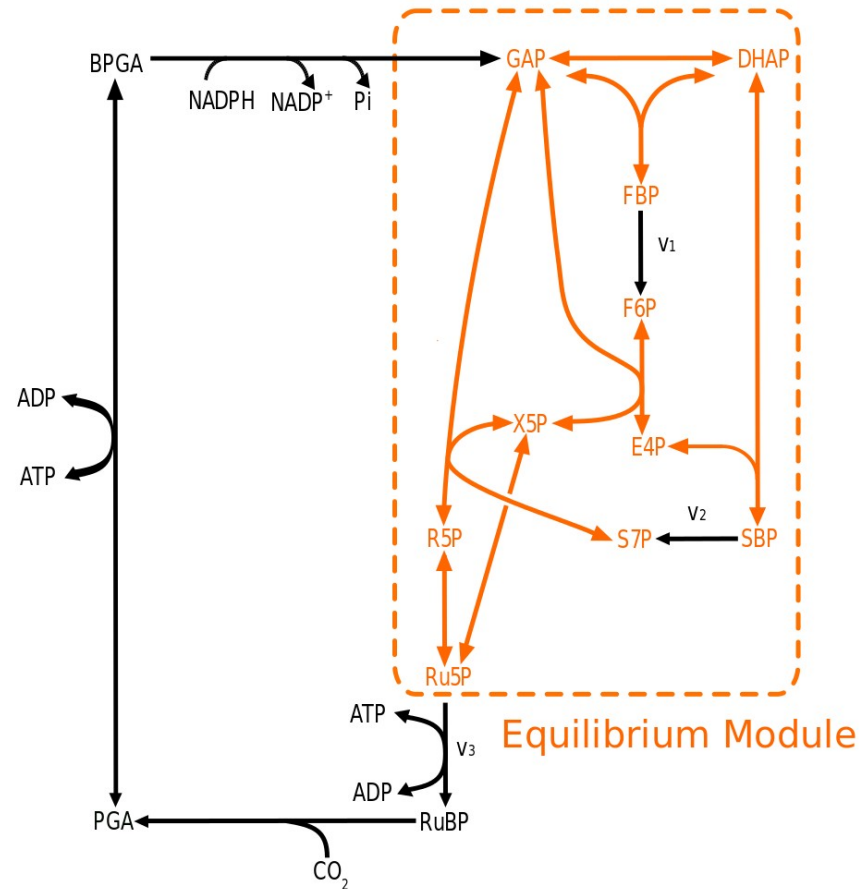

Equilibrium Module

The Logic of the CBB Cycle

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

2) De-phosphorylation as thermodynamic driving force (ΔG<0)

Equilibrium Module
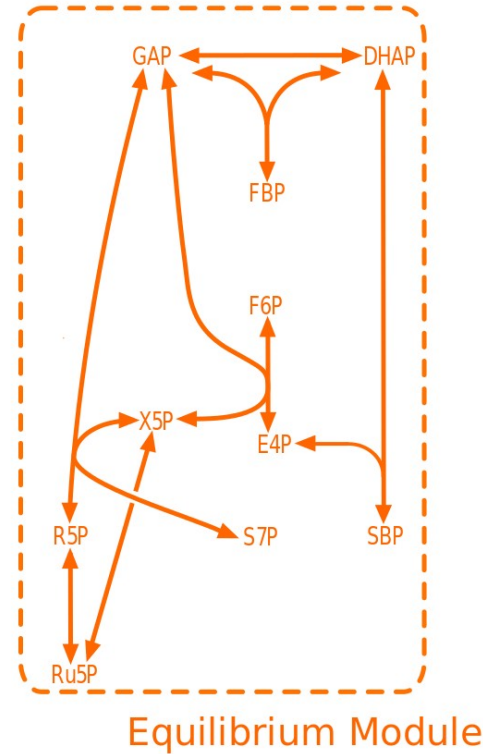
The Logic of the CBB Cycle

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

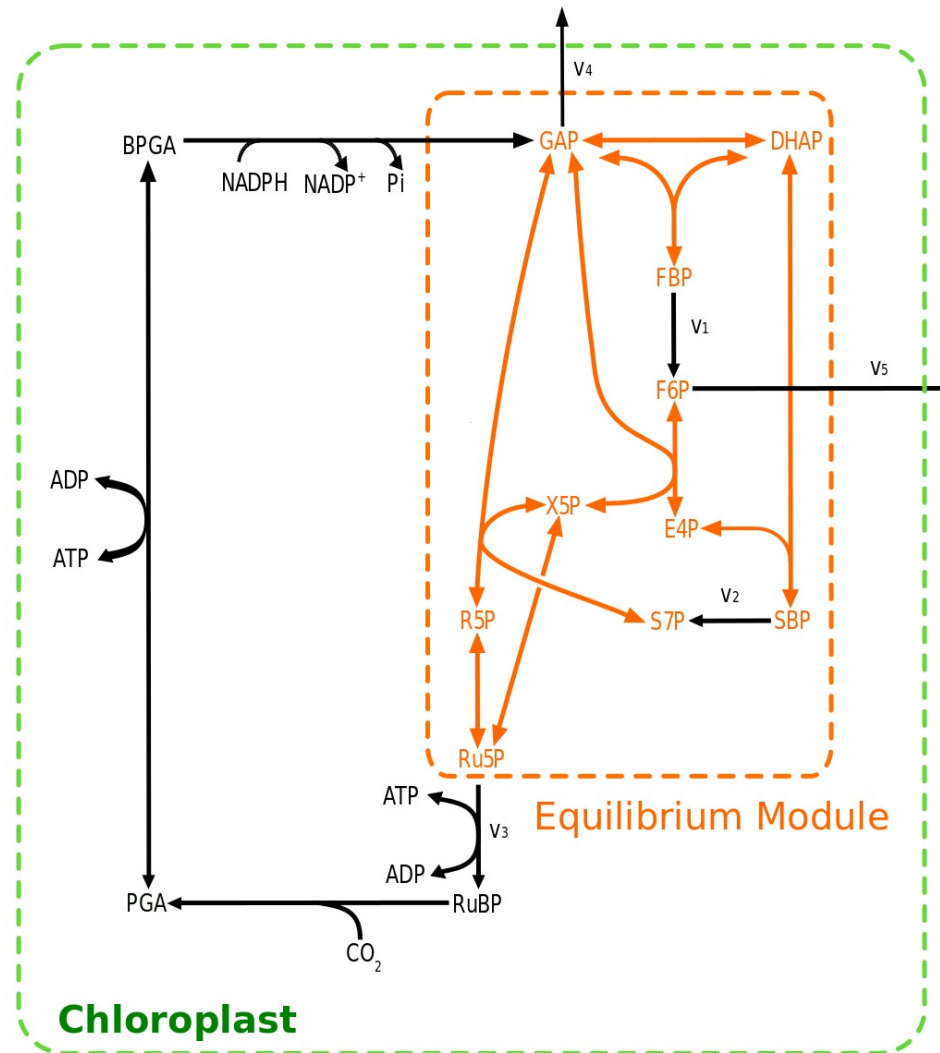2) De-phosphorylation as thermodynamic driving force ($\Delta G < 0$)

3) Activation, carbon fixation, reduction (overall $\Delta G < 0$)

The Logic of the CBB Cycle

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

2) De-phosphorylation as thermodynamic driving force (ΔG<0)

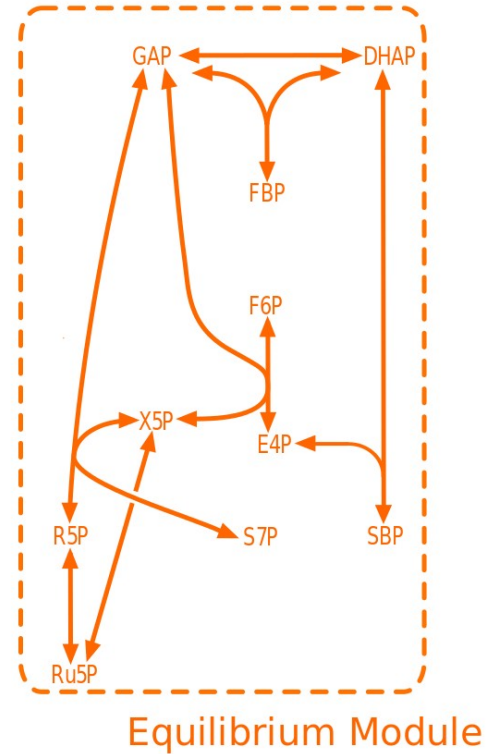3) Activation, carbon fixation, reduction (overall ΔG<0)

4) Output

The pentose phosphate pathways uses the same equilibrium module
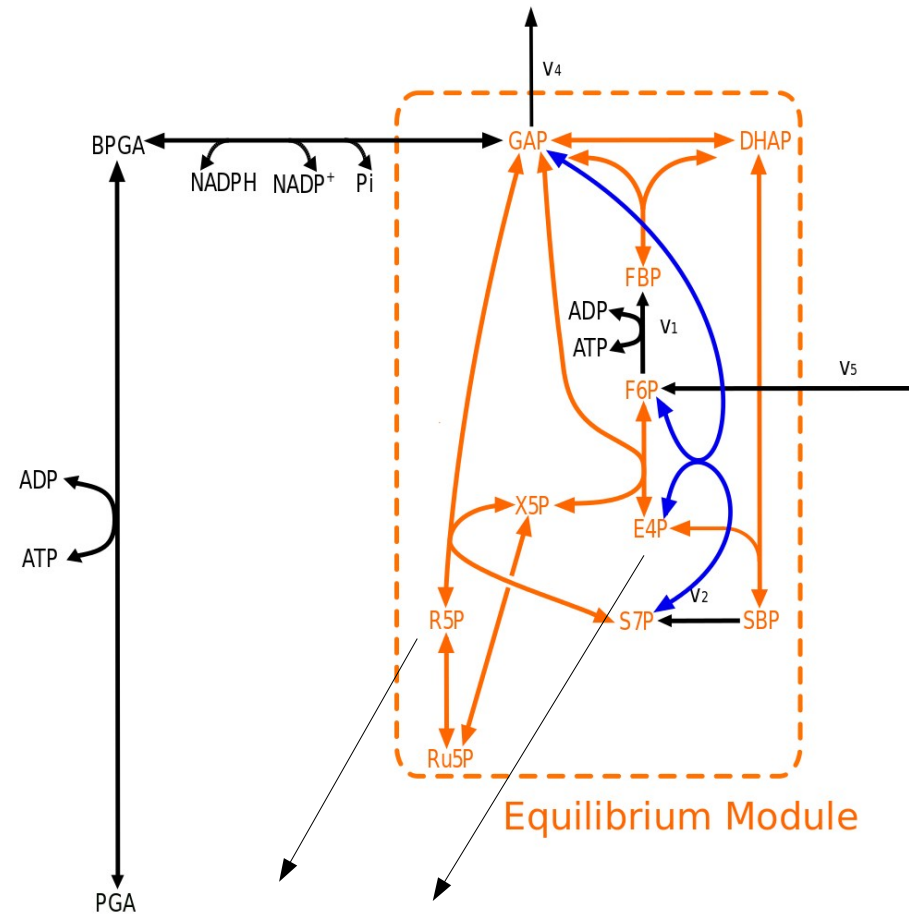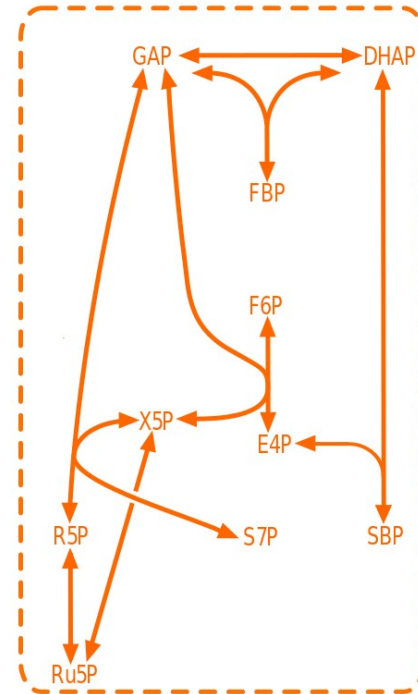
The Equilibrium Module

How to calculate the rapid equilibrium?

Equilibrium Module

The Equilibrium Module

How to calculate the rapid equilibrium?

Solution 1: Brute force

Equilibrium Module

see Pettersson 1988:

$$[BPGA][ADP] = q_2 [PGA][ATP] \quad (20)$$
$$[GAP][NADP^+][P_i] = q_3 [BPGA][NADPH][H^+] \quad (21)$$
$$[DHAP] = q_4 [GAP] \quad (22)$$
$$[FBP] = q_5 [GAP][DHAP] \quad (23)$$
$$[X5P][E4P] = q_7 [GAP][F6P] \quad (24)$$
$$[SBP] = q_8 [DHAP][E4P] \quad (25)$$
$$[X5P][R5P] = q_{10} [GAP][S7P] \quad (26)$$
$$[Ru5P] = q_{11} [R5P] \quad (27)$$
$$[Ru5P] = q_{12} [X5P]. \quad (28)$$

Coefficients A_{ji} in Eqn (59) are given by

$$A_{11} = 1$$
$$A_{j1} = 0; j = 2,\ldots,5 \quad A_{12} = A_{13} = 0$$
$$A_{14} = -2t_{11}$$
$$A_{15} = 2t_{13}$$
$$A_{22} = t_1(1 + 2q_8s_2)s_3$$
$$A_{23} = -\frac{2t_2s_4}{s_2}$$
$$A_{24} = 2(t_3s_3 + t_4) + t_5 + \frac{t_2s_4}{s_2} + \frac{t_7t_8}{t_6} - t_{10}t_{11}$$
$$A_{25} = (c_{ad} - s_5)\left(\frac{t_{10}s_2}{t_6 + t_9s_2} - \frac{t_7}{t_6}\right)$$
$$A_{32} = t_1(1 + q_8s_2)s_2 + t_{12}$$
$$A_{33} = t^{12}$$
$$A_{34} = t_3s_2 - t_1(1 + 2q_8s_2)s_2t_{11} - t_{12}(1 + t_{11})$$
$$A_{35} = t_{13}[t_{12} + t_1(1 + 2q_8s_2)s_2]$$
$$A_{42} = -\frac{t_1(1 + q_8s_2)s_2s_3}{s_4}$$
$$A_{43} = 4t_2 + t_{14}$$
$$A_{44} = t_{11}t_{15} - 2t_2 - \frac{t_1q_8s_2^2s_3}{s_4}$$
$$A_{45} = -t_{13}t_{15}$$
$$A_{52} = A_{53} = 0$$
$$A_{54} = \frac{c_{ad}t_7s_2(t_{11} - 1)}{t_6s_5} - t_{11}$$
$$A_{55} = 1 + t_{13} + \frac{c_{ad}t_7s_2(1 - t_{13})}{t_6s_5}$$

where

$$t_1 = \frac{q_7q_{12}}{q_4s_4}$$
$$t_2 = \frac{q_4s_4}{q_{10}q_{11}q_{12}s_2}$$
$$t_3 = \frac{q_7q_8q_{12}s_2}{q_4s_4}$$
$$t_4 = \frac{2q_5s_2}{q_4}$$
$$t_5 = 1 + \frac{1}{q_4}$$
$$t_6 = \frac{q_2q_3q_4s_5[NADPH][H^+]}{[NADP^+]}$$
$$t_7 = t_{16}[c_p\text{-}2s_1 - s_5 - (t_4 + t_5)s_2 - t_{12}s_3 - (t_2 + t_{14})s_4 - t_1(1 + 2q_8s_2)s_2s_3]$$
$$t_8 = c_{ad} + (q_2 - 1)s_5$$
$$t_9 = c_{ad} + (2q_2 - 1)s_5$$
$$t_{10} = 2t_4 + t_5 + t_1(1 + 4q_8s_2)s_3 + \frac{t_7t_9}{t_6} - \frac{t_2s_4}{s_2}$$
$$t_{11} = \frac{t_8t_{16}s_2}{t_6}$$
$$t_{12} = 1 + q_{14} + q_{14}q_{15}$$
$$t_{13} = \frac{t_{16}(c_{ad} - s_5)s_2}{t_6}$$
$$t_{14} = 1 + \frac{1}{q_{11}} + \frac{1}{q_{12}}$$
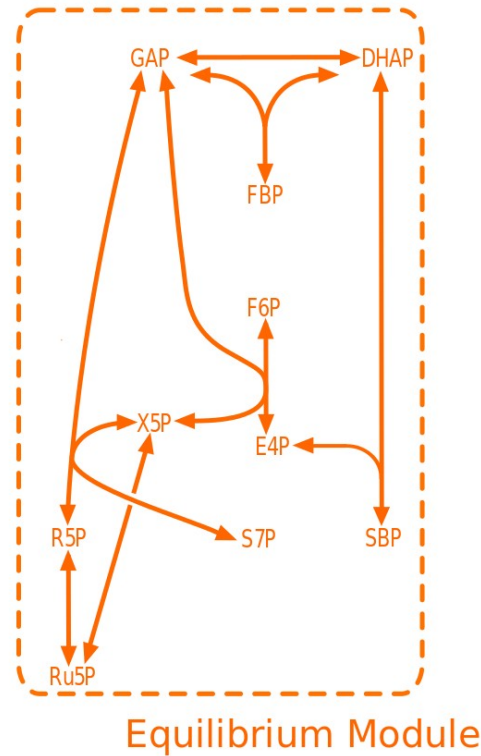$$t_{15} = \frac{t_1(1 + 2q_8s_2)s_2s_3}{s_4} - 2t_2 - t_{14}$$
$$t_{16} = \frac{t_6}{t_6 + t_9s_2}.$$

The Equilibrium Module

How to calculate the rapid equilibrium?

Solution 2: Brain and Thermodynamics
(see Supplementary to Kartal et al, 2011, MSB 7:542)

- Step 1: find conserved quantities

- Step 2: minimise Gibbs free energy

Equilibrium Module

The Equilibrium Module

How to calculate the rapid equilibrium?

<u>Solution 2: Brain and Thermodynamics</u>
(see Supplementary to Kartal et al, 2011, MSB 7:542)

- Step 1: find conserved quantities

Formally:

3 conserved moieties:
2 from P, 1 from C

- P in odd-C sugars (P_1)
- P in even-C sugars (P_2)

$$Q = \#C - 3 \cdot \#P$$

Equilibrium Module

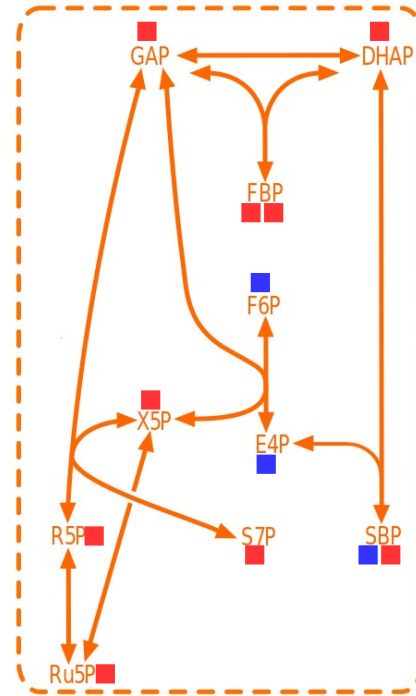Linearly independent solutions to $c \cdot N_{eq} = 0$:

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

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

The Equilibrium Module

How to calculate the rapid equilibrium?

Solution 2: Brain and 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 $C \cdot N = 0$

$\longrightarrow$ LAGRANGIAN MULTIPLIERS !

Solving the equilibrium module

3 equations with 3 unknowns:

Notation:

x_k: compound with $k+3$ carbons

GAP Lagrange multiplier **E4P**

P_1 P_2

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

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

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

$Q = C - 3 P$

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

Solving the equilibrium module

3 equations with 3 unknowns:

Notation:

x_k: compound with $k+3$ carbons

GAP   Lagrange multiplier   **E4P**

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

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

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

$\blacksquare P_1$ $\blacksquare P_2$

$$Q = C - 3\,P$$
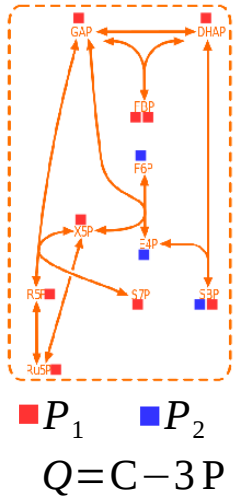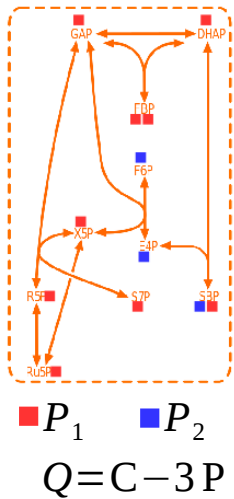
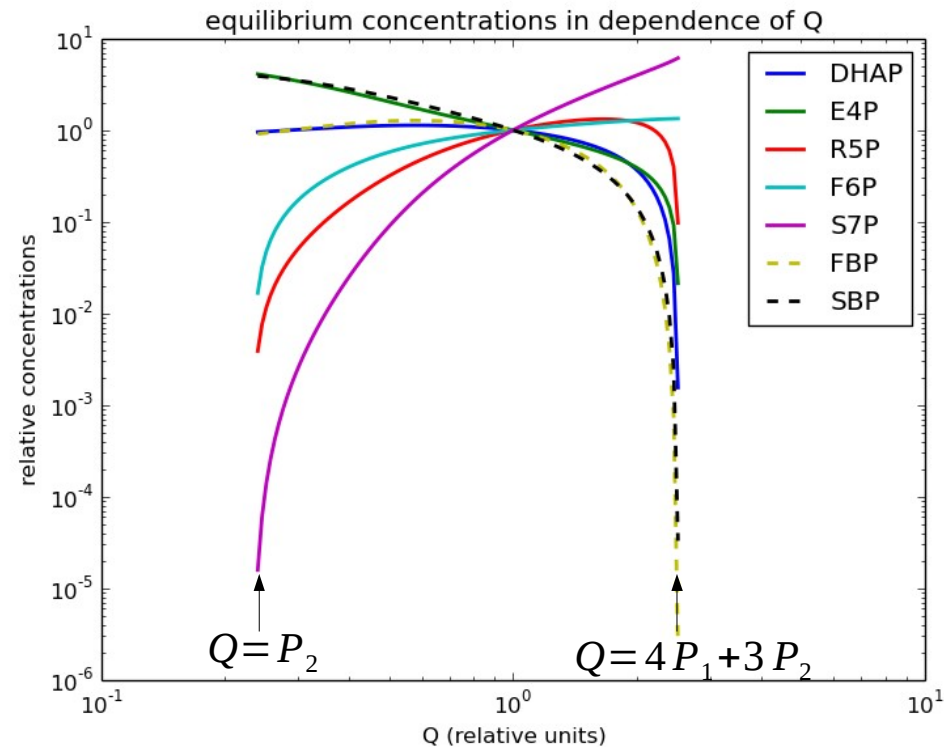$$\implies \quad x_{k+2}=x_k\cdot e^{-\Delta\mu}\cdot z$$

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

What happens at the extremes?

$Q \rightarrow P_2$:   accumulation of small sugars

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

A 3-variable model of the CBB cycle

Stoichiometry Matrix:

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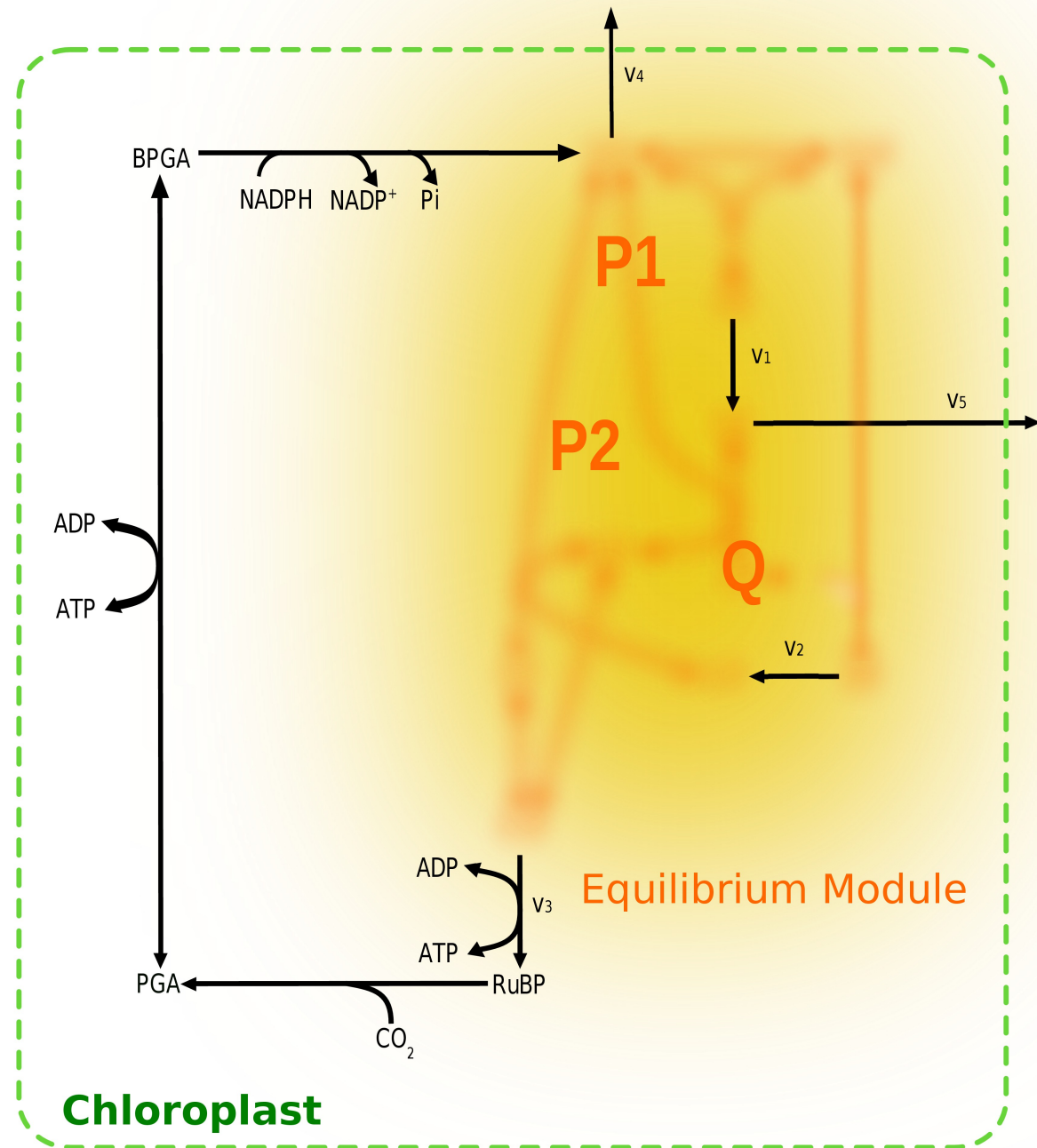
Differential Equations:

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

with:

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

$$Y = \{GAP, DHAP, E4P \dots SBP\}$$
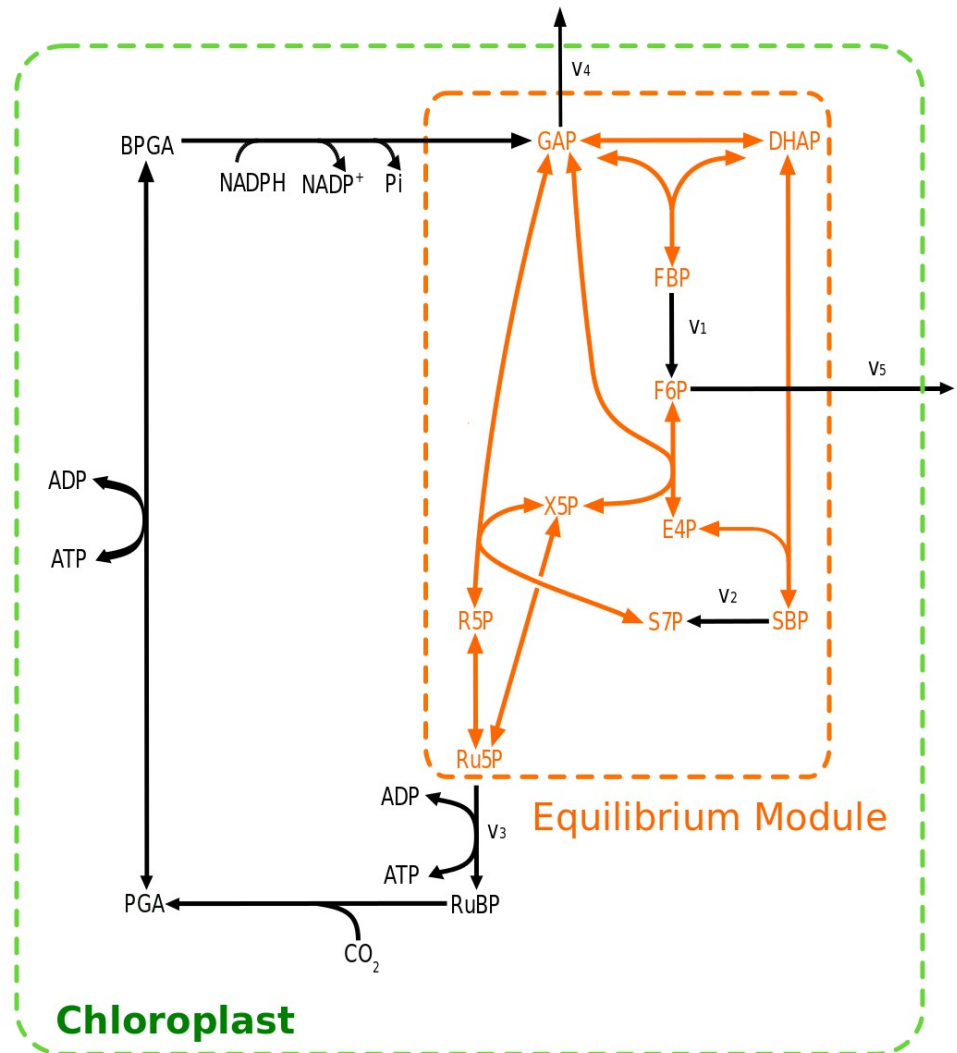
Closing the cycle

First attempt: mass-action

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

Closing the cycle

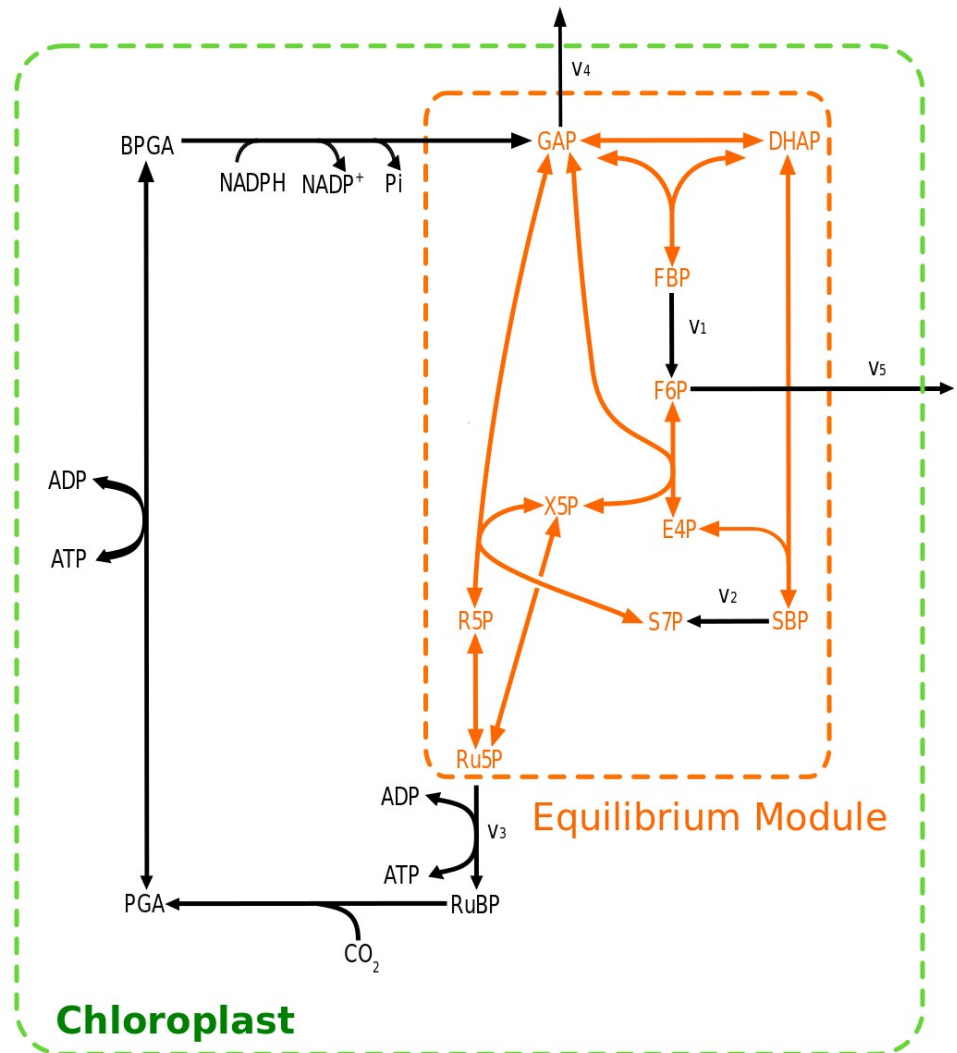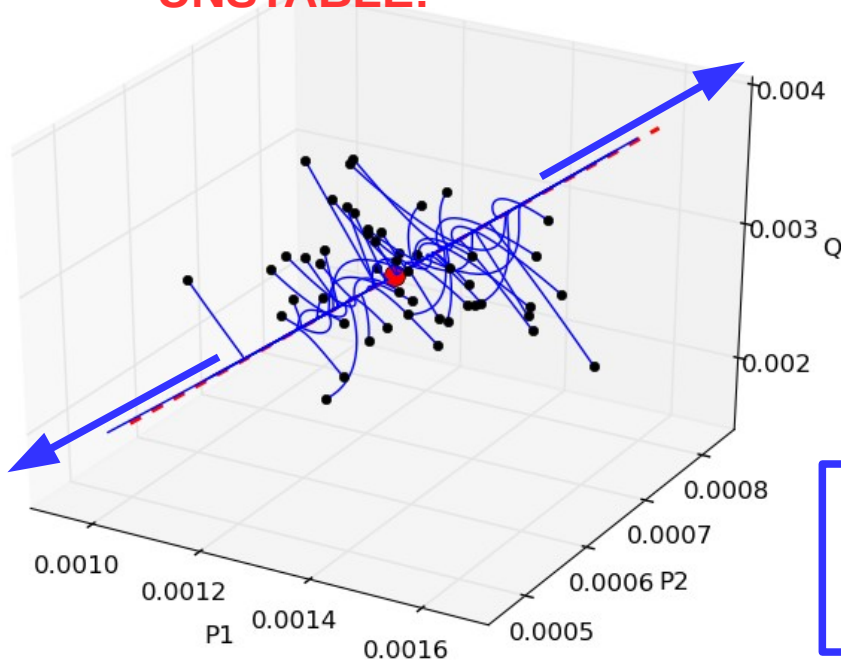First attempt: mass-action

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

UNSTABLE!

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

Closing the cycle

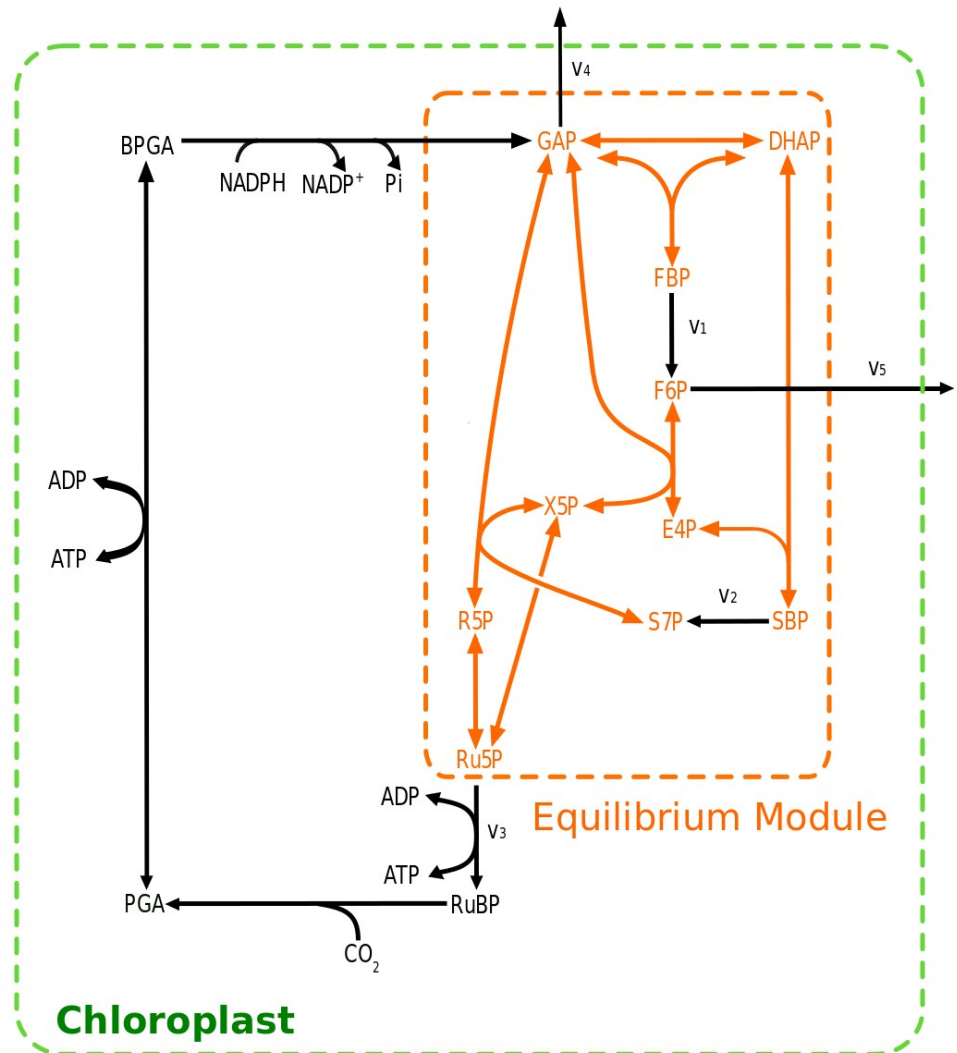Second attempt: Michaelis-Menten

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

Closing the cycle

Second attempt: Michaelis-Menten

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

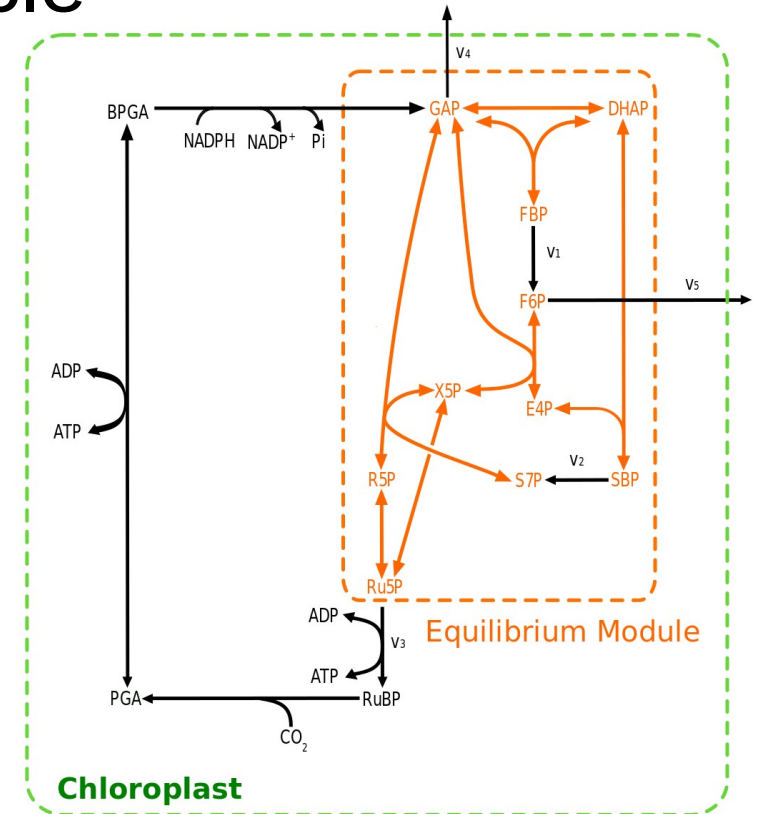Finding 'good' V_{max} / K_M – values...

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

Jacobian $\quad J_{ik} = \sum_j n_{ij} \overbrace{\left(\frac{\partial v_j}{\partial X_k}\right)}^{\varepsilon_{jk}}$ or $\quad J = N \cdot E \quad$ elasticities ε_{jk}

$$\varepsilon_{jk} = \frac{\partial v_j}{\partial X_k} = \sum_\ell \frac{\partial v_j}{\partial Y_j} \cdot \frac{\partial Y_\ell}{\partial X_k}$$

$\eta_{j\ell} \qquad \Theta_{\ell k} \text{ (known)}$

or $\quad J = N \cdot H \cdot \Theta$
$\quad\quad 3\times5 \quad 5\times10 \quad 10\times3$

Optimising elasticities for stability

For irreversible reactions without allosteric regulation:

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

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

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

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

FIND STABLE SOLUTION BY MINIMISING Λ

Optimising elasticities for stability

For irreversible reactions without allosteric regulation:

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

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$$\underset{3\times5}{} \quad \underset{5\times10}{} \quad \underset{10\times3}{}$$

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

FIND STABLE SOLUTION BY MINIMISING Λ

$\varepsilon \rightarrow (V_{max}, K_M)$ $\varepsilon = k$

$0 < \varepsilon < k$

$\varepsilon \approx 0$

V_{max}

$\bar{v}$

K_M $\bar{X}$ X

Optimising elasticities for stability

For irreversible reactions without allosteric regulation:

$$H = \begin{pmatrix} & & \varepsilon_1 \\ & & \varepsilon_2 \\ & \varepsilon_3 & \\ \varepsilon_4 & & \\ & \varepsilon_5 & \end{pmatrix} \quad \text{only 5 non-zero}$$
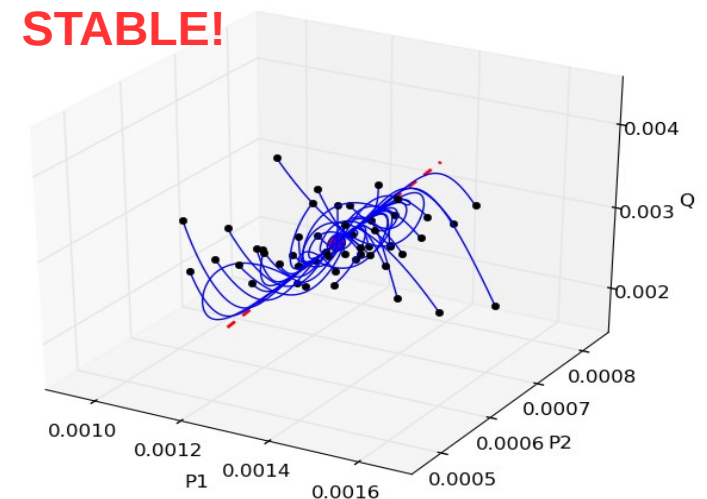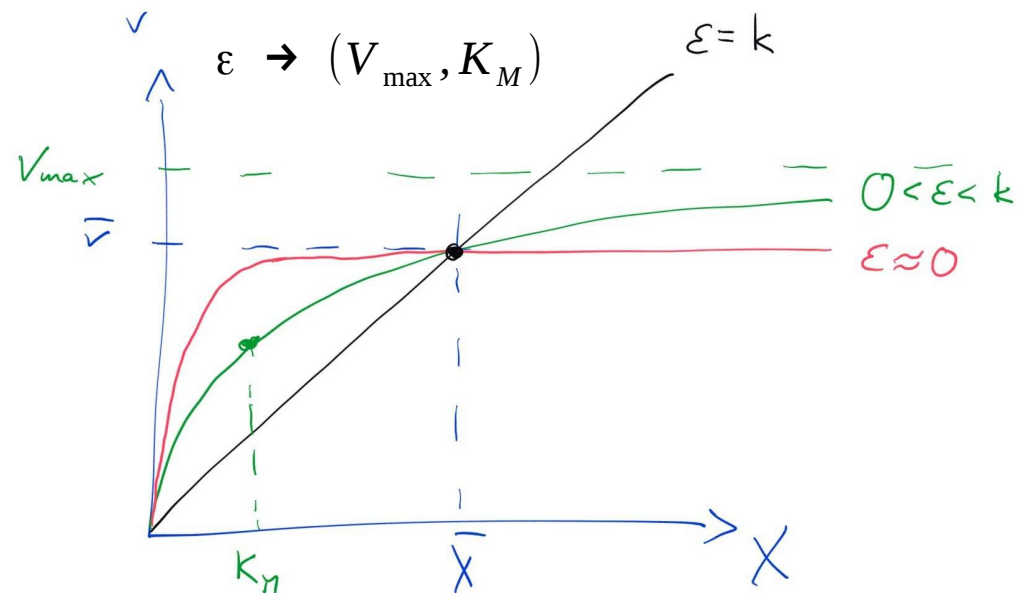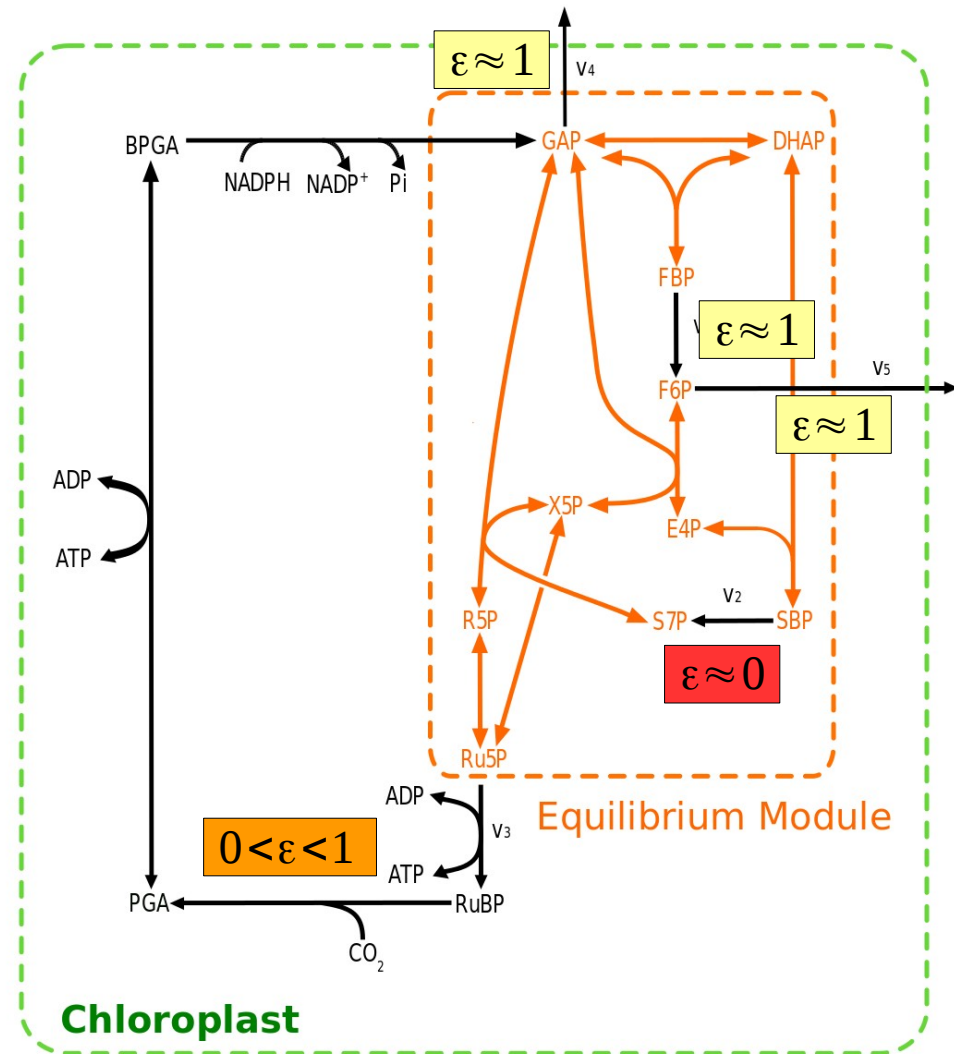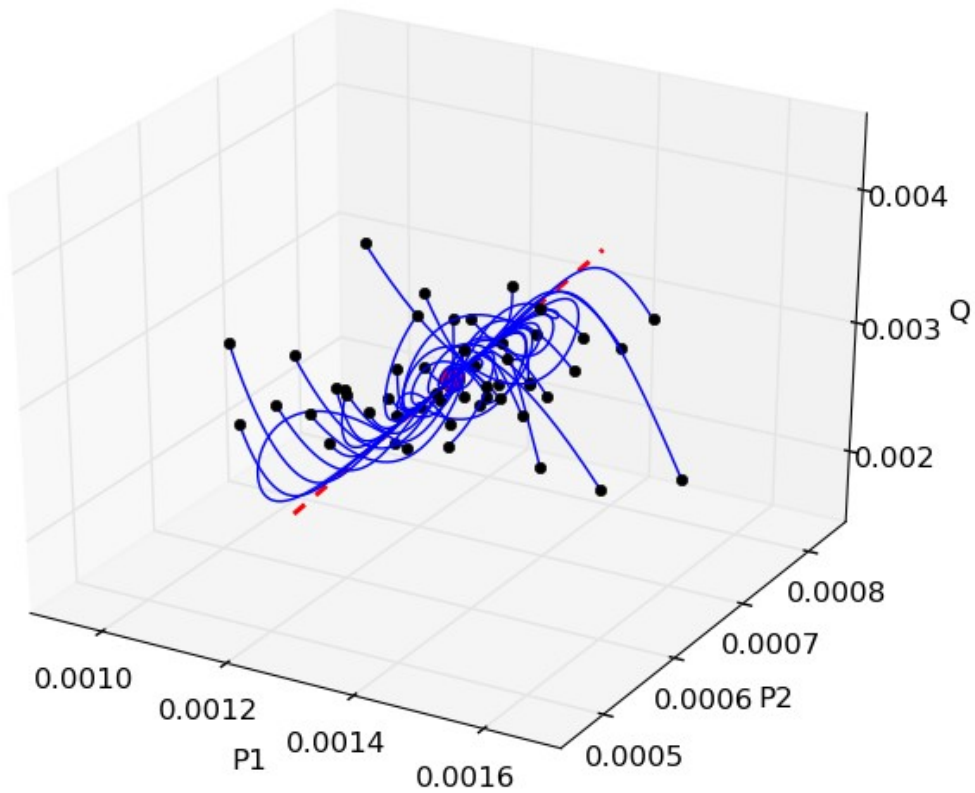
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Define: $\quad \Lambda(\varepsilon_j) := \max_\lambda \{ Re(\lambda) : \det(\lambda \cdot \mathbb{1} - J) = 0 \} \quad$ maximal Eigenvalue

FIND STABLE SOLUTION BY MINIMISING Λ

$\varepsilon \rightarrow (V_{max}, K_M)$

$\varepsilon = k$

V_{max}

$0 < \bar{\varepsilon} < k$

$\varepsilon \approx 0$

K_M $\quad \bar{X}$ $\quad X$

STABLE!
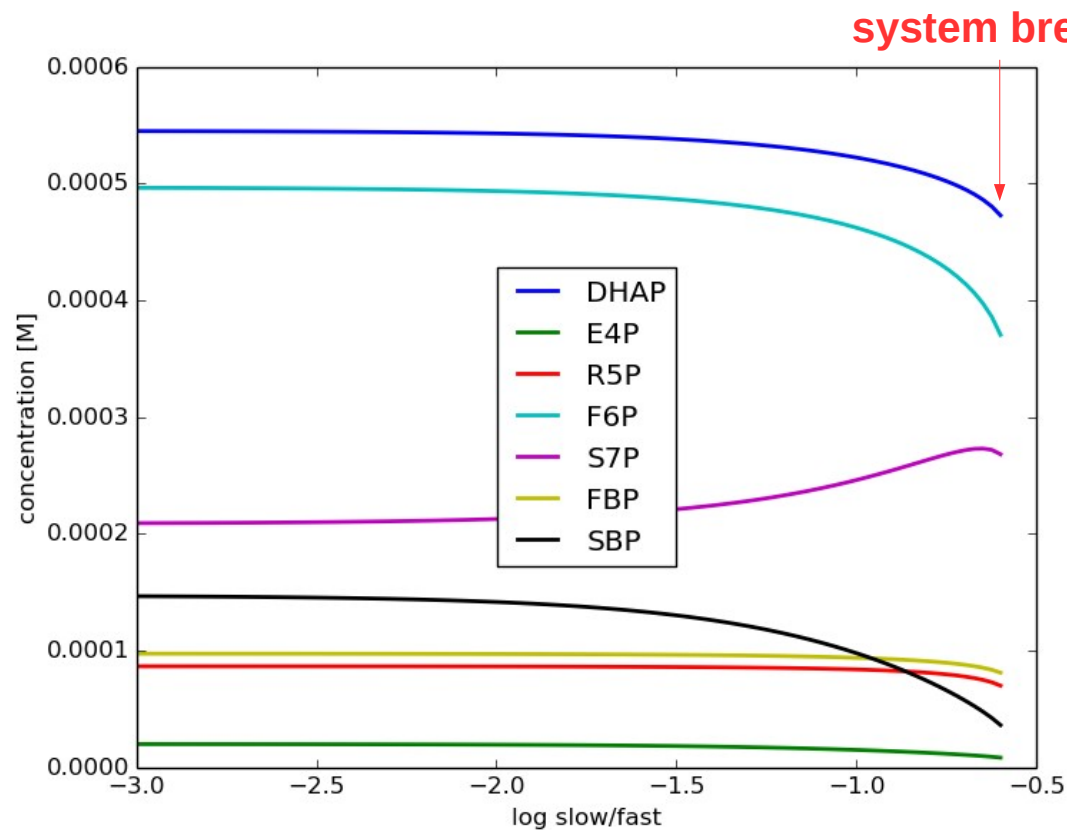
"Predicted" elasticities

Limitations of the 3-variable model

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

When time-scales are not clearly separated, other regulatory mechanisms are necessary!
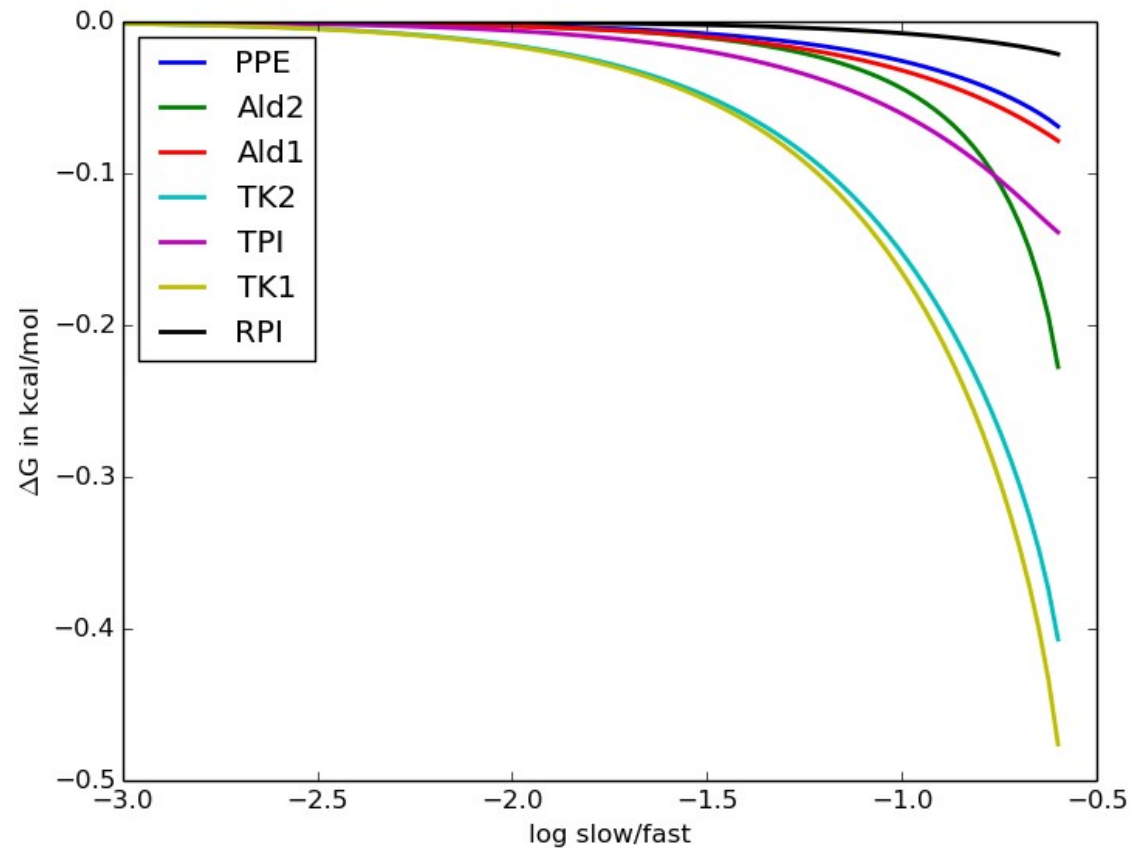
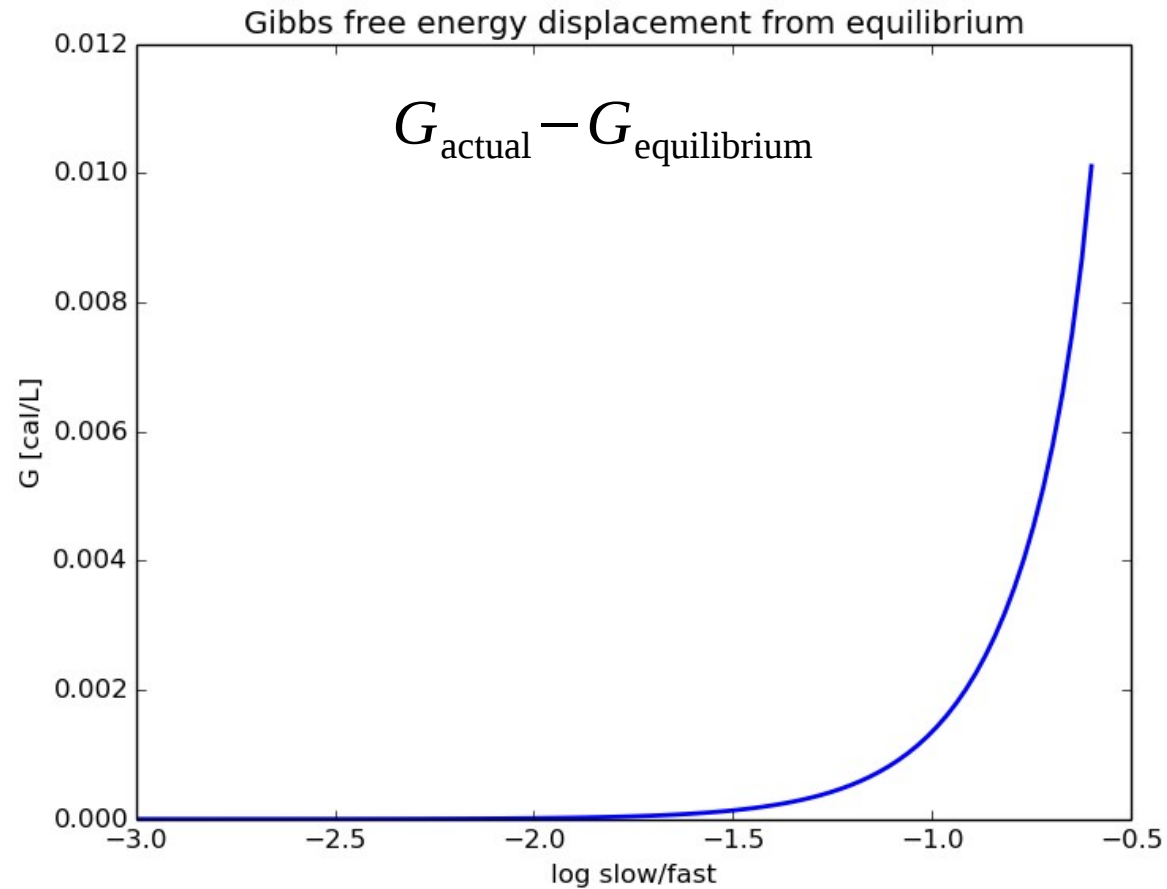**rapid equilibrium**   **similar time-scales**

Displacement from equilibrium
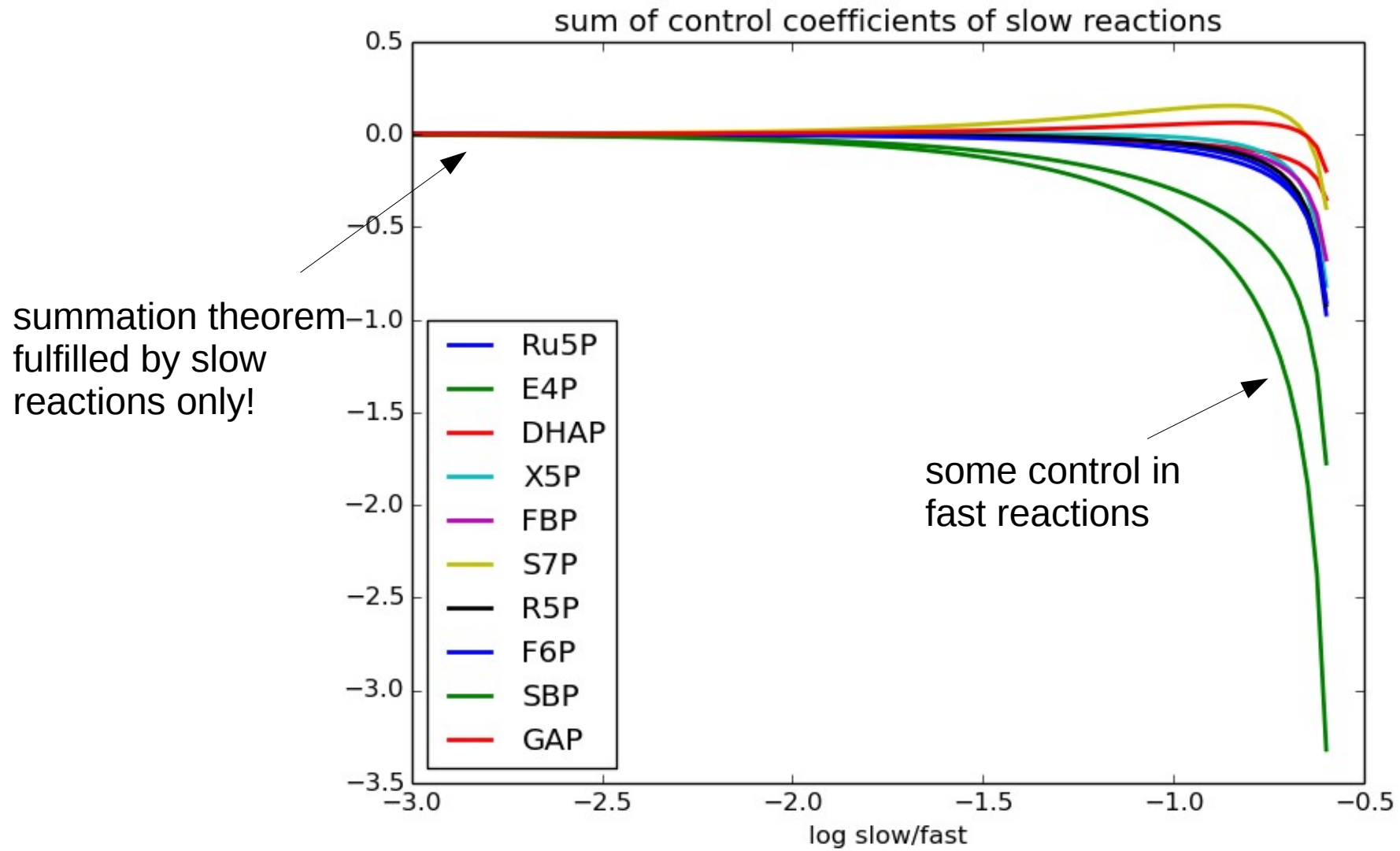
The lowest ΔG is just -0.5 kcal/mol!

But Bassham measured -1.4...

Total Gibbs free energy above equilibrium

Gibbs free energy displacement from equilibrium

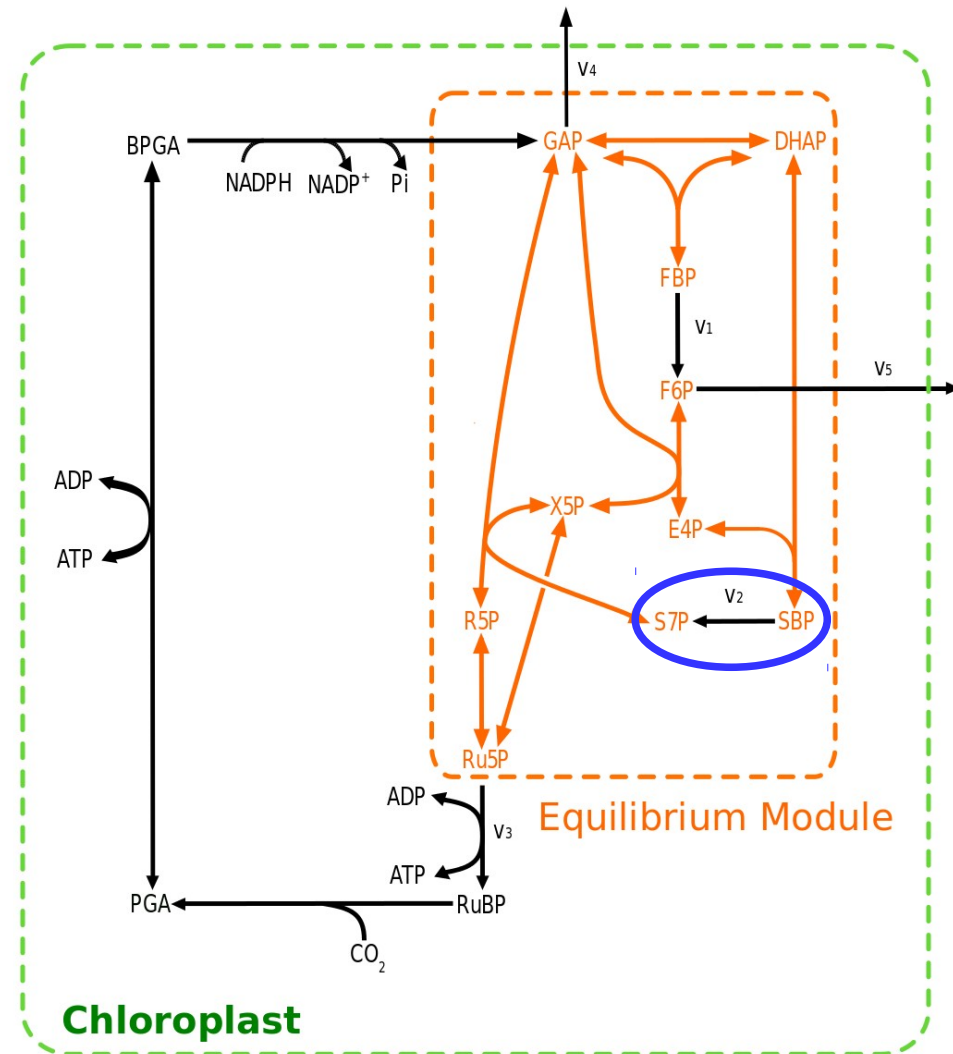$$G_{\text{actual}} - G_{\text{equilibrium}}$$

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

Losing control

summation theorem
fulfilled by slow
reactions only!

some control in
fast reactions

The positive control of SBPase

Accelerating SBPase increases its substrate!!

control coefficients for SBPase

Legend:
- DHAP
- E4P
- R5P
- F6P
- S7P
- FBP
- SBP

x-axis: log slow/fast

Chloroplast
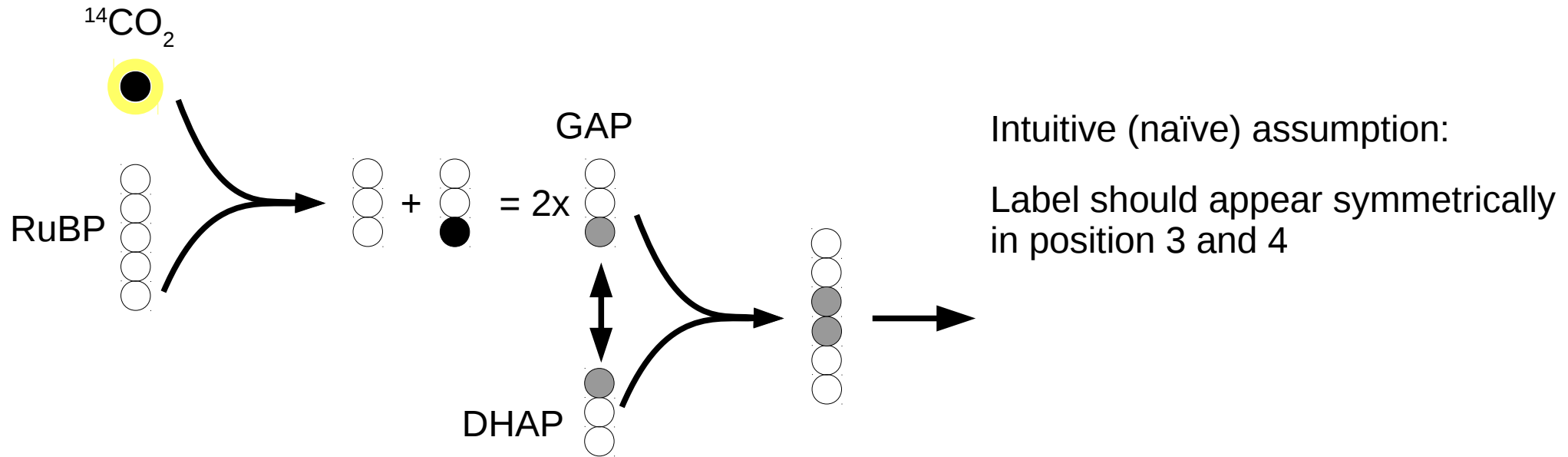
Equilibrium Module
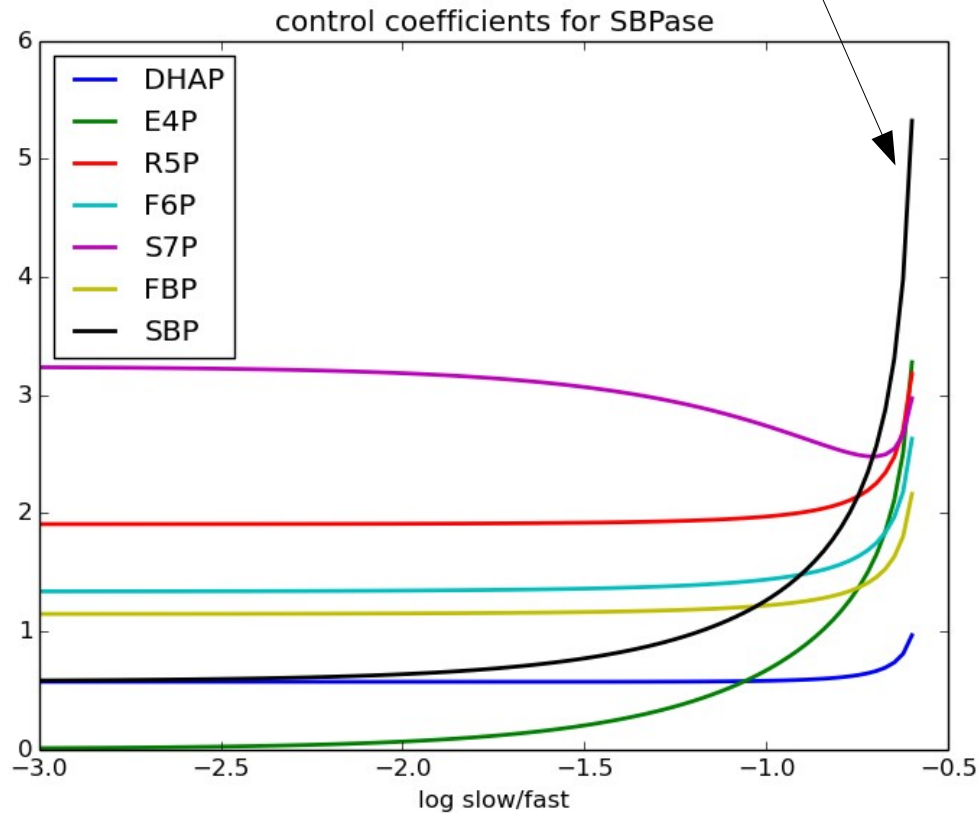
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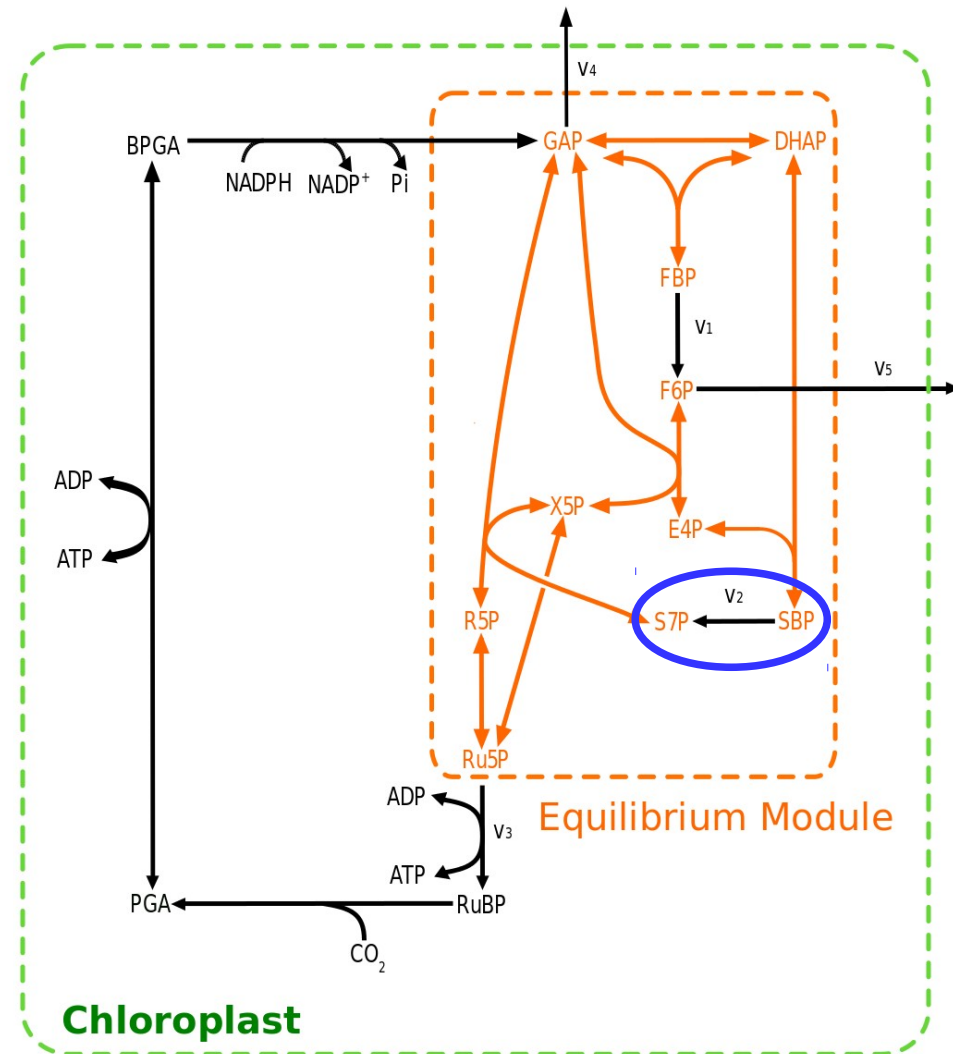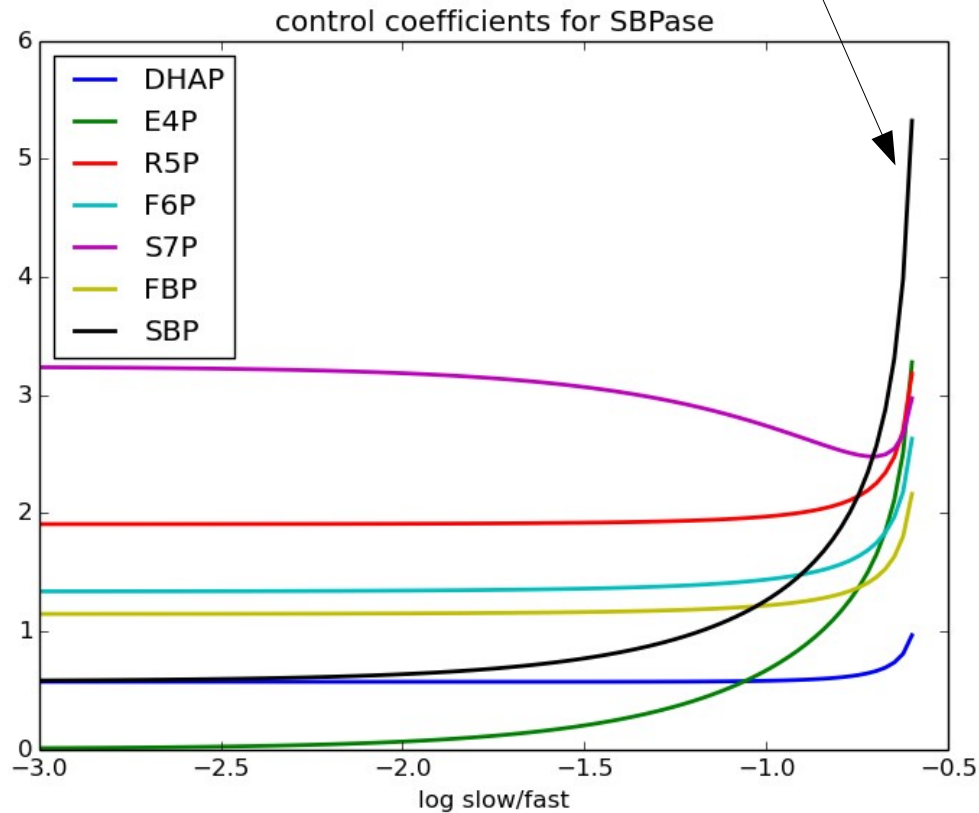
positive feedback! Stability problem...

The photosynthetic Gibbs effect

$^{14}CO_2$

RuBP

GAP

+ = 2x

DHAP

Intuitive (naïve) assumption:

Label should appear symmetrically in position 3 and 4

The photosynthetic Gibbs effect

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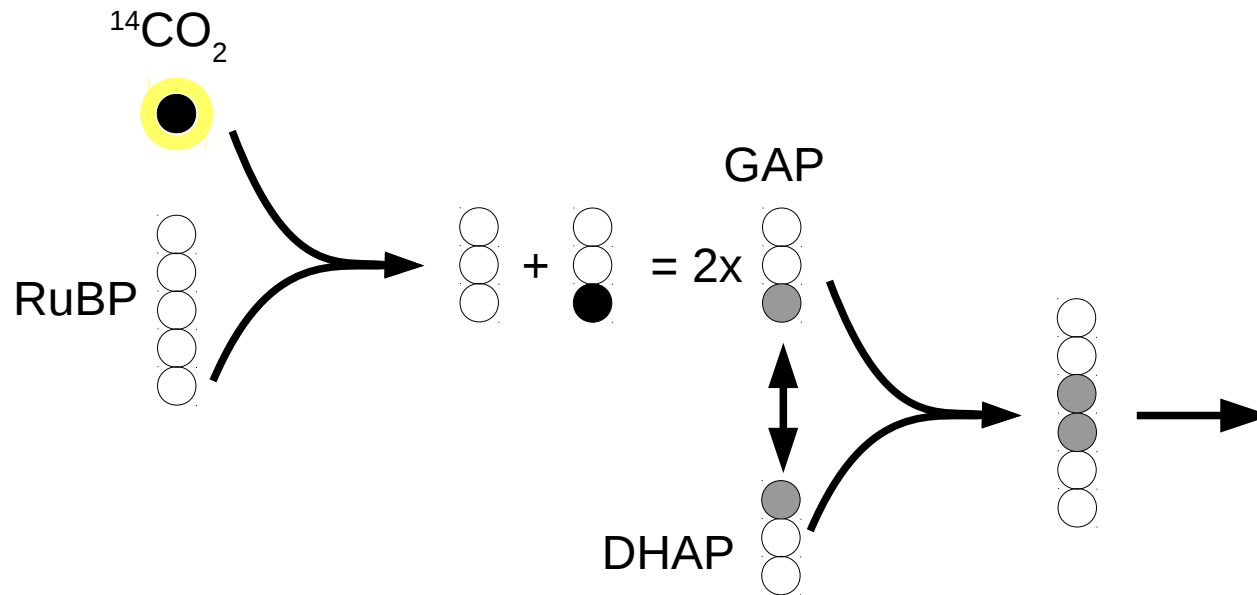
Intuitive (naïve) assumption:

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 ($M\mu C/MGC$)					
				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

$^{14}CO_2$

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

RuBP

GAP

+ = 2x

Label appears first in position 4

DHAP

1>6, 2>5, 5 last
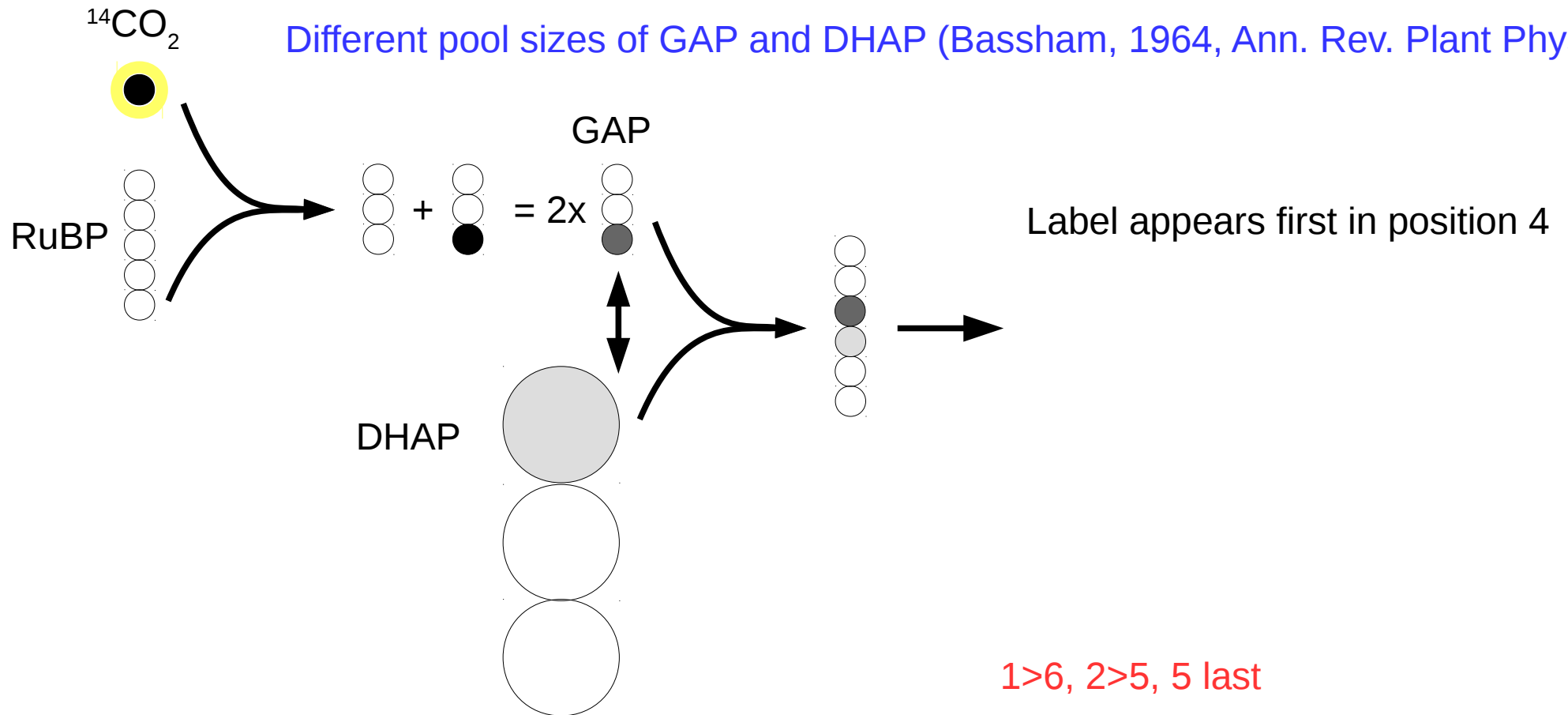
What about the other positions?

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

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# A dynamic model of isotope label distribution

<u>Workflow</u>

- 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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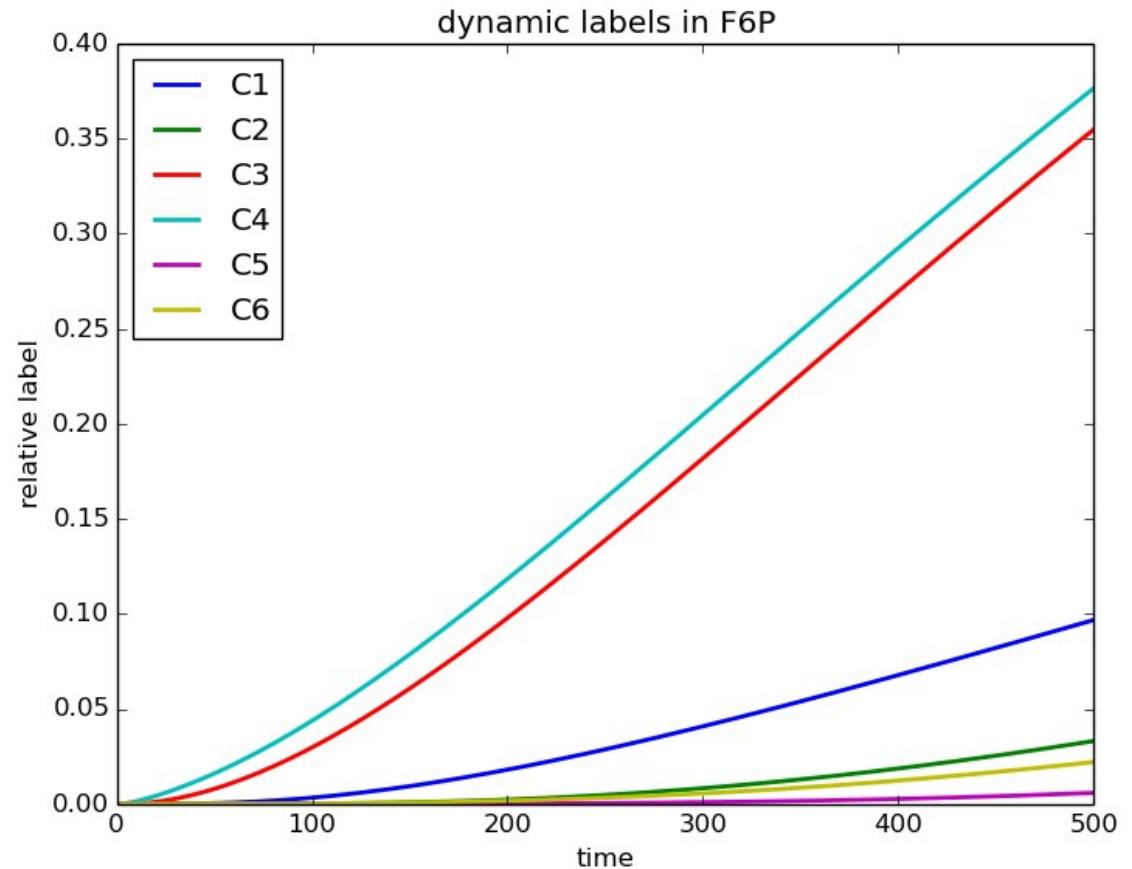
Slow TPI pronounces asymmetry
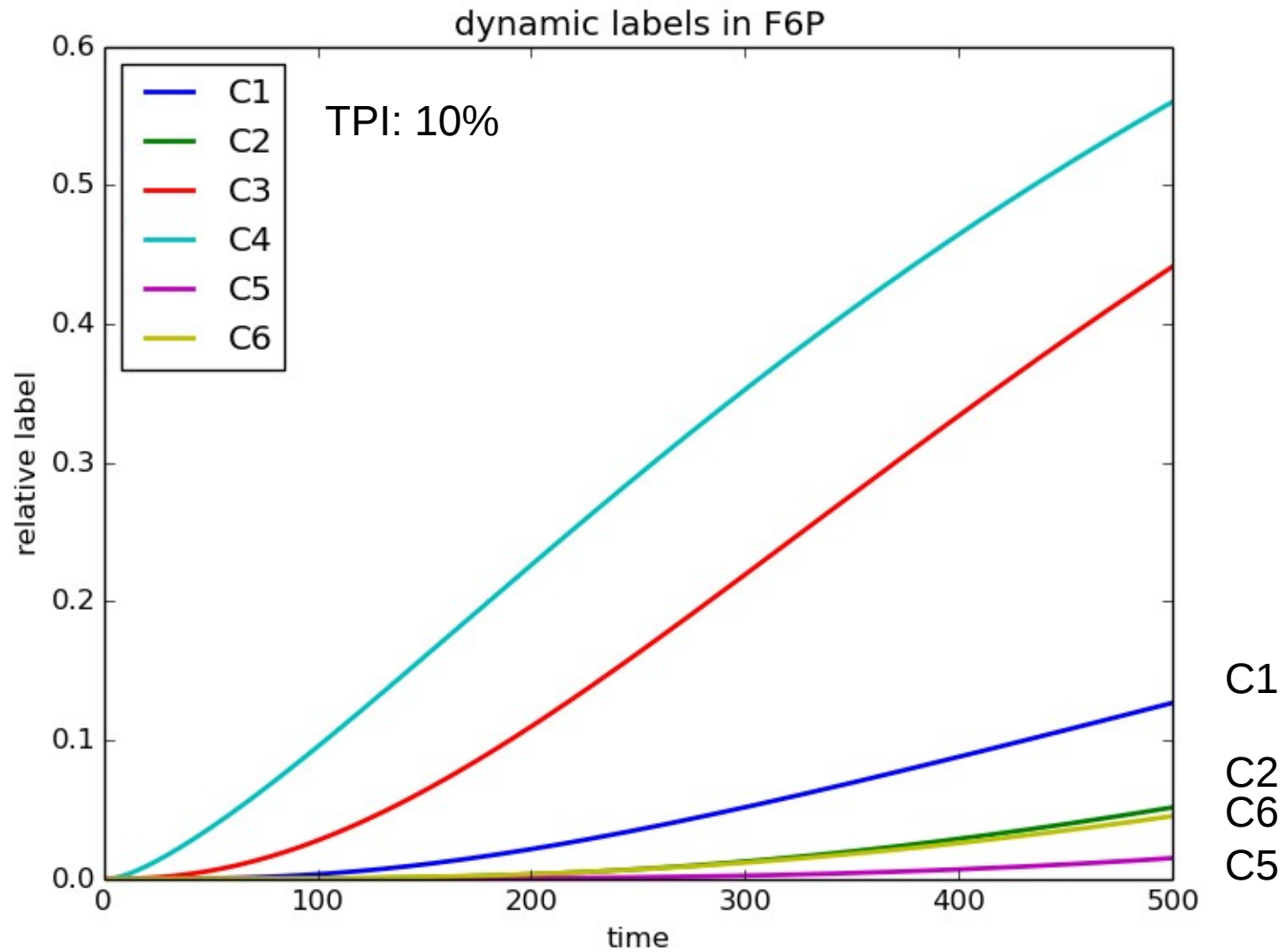
TK activity influences other labels

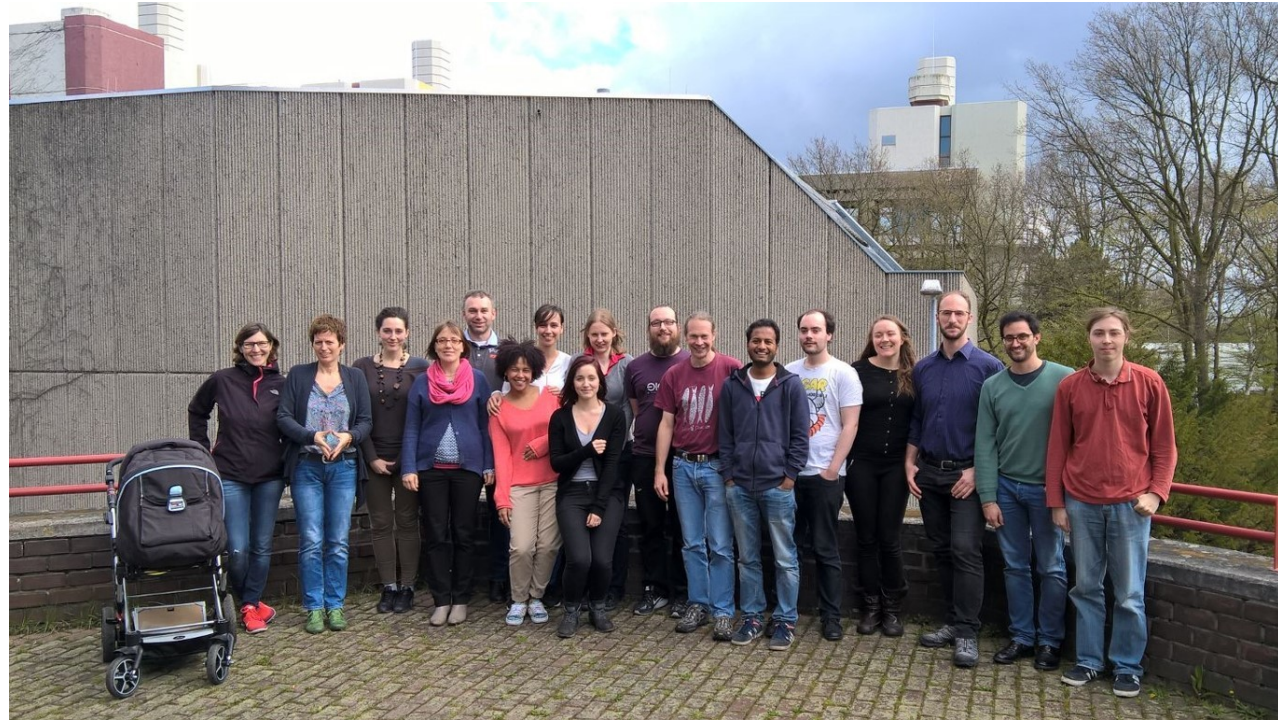dynamic labels in F6P

TPI: 10%, TK: 40%
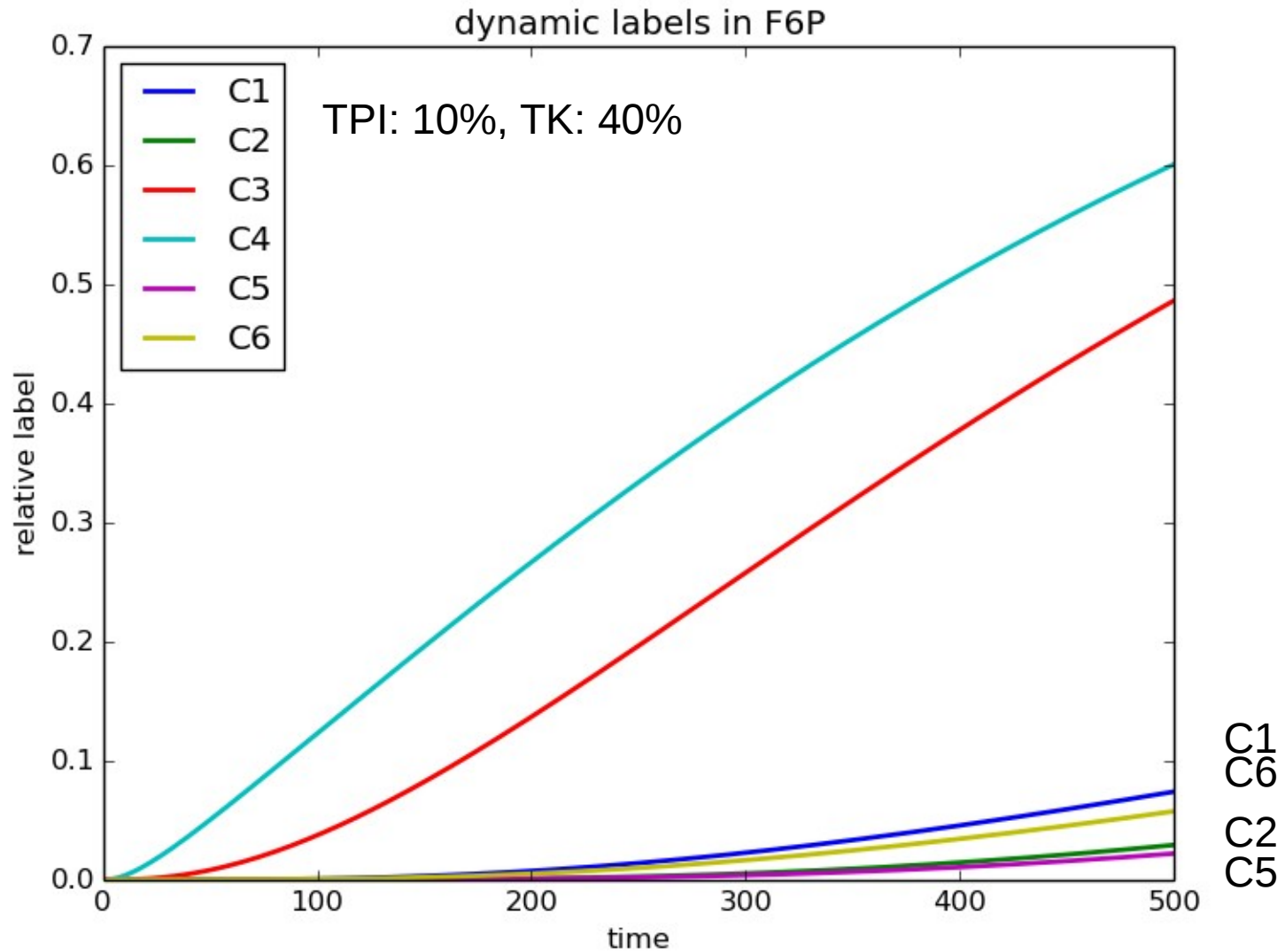
Conclusions

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

Financial Support