
Spring School on Dynamical Modelling
of Biological Regulatory Networks
9–20 APRIL 2007, LES HOUCHES, FRANCE

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Differential Equation Modelling and Analysis of Metabolic Networks

Athel Cornish-Bowden & María Luz Cárdenas
(CNRS, Marseilles)

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Differential Equation Modelling and Analysis of Metabolic Networks

OUTLINE OF THE LECTURE

We start with a discussion of why classical enzymology — which has come to seem outmoded by many modern biochemists — continues to be essential for profiting from the genomic revolution and for many applications, including drug development.

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This will include the introduction of various important terms, the properties of the parameters implied by these terms, some more familiar ideas discussed in a less familiar context, and the relevance of all this to metabolic regulation, especially the idea that many metabolic processes are regulated according to the demand for their products, not according to the availability of starting materials.

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Methods available for modelling metabolic systems in the computer will be discussed rather more briefly, mentioning not only some of the principles involved, but also some of the principal tools currently in use.

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Understanding the different types of inhibition is crucial for understanding drug design, but it is remarkably poorly understood in practice.

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Understanding the different types of inhibition is crucial for understanding drug design, but it is remarkably poorly understood in practice.

In particular, one should realize that different kinds of inhibition are much more different *in vivo* than they are in the spectrophotometer, and one should understand why.

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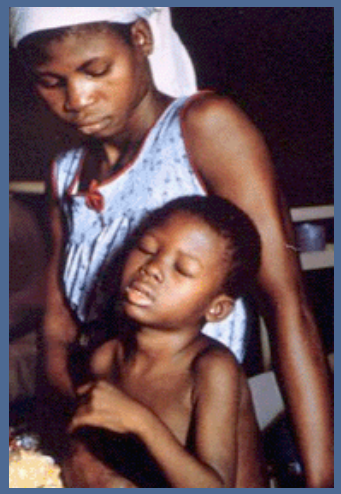
OUTLINE OF THE LECTURE

The glycolytic pathway in the parasite *Trypanosoma brucei* (responsible for African sleeping sickness) will provide the major example to illustrate the application of the main ideas to a real system.



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Some enzymes, such as pyruvate kinase, catalyse reactions with equilibrium constants so large that one might regard them as irreversible for practical purposes. To what extent is it safe to ignore reverse reactions entirely when setting up metabolic models?

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Differential Equation Modelling and Analysis of Metabolic Networks

OUTLINE OF THE LECTURE

The lecture will conclude with a brief discussion of the relevance of classical ideas of metabolic regulation (allosteric feedback inhibition, cooperativity, etc.) to a modern understanding of the subject.

RELEVANCE OF CLASSICAL ENZYMOLOGY

Classical enzymology

What use is it in the 21st Century?

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If Leonor Michaelis were still with us he would be 132 years old in 2007.

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Why should we continue teaching their ideas, which go back now almost a century?

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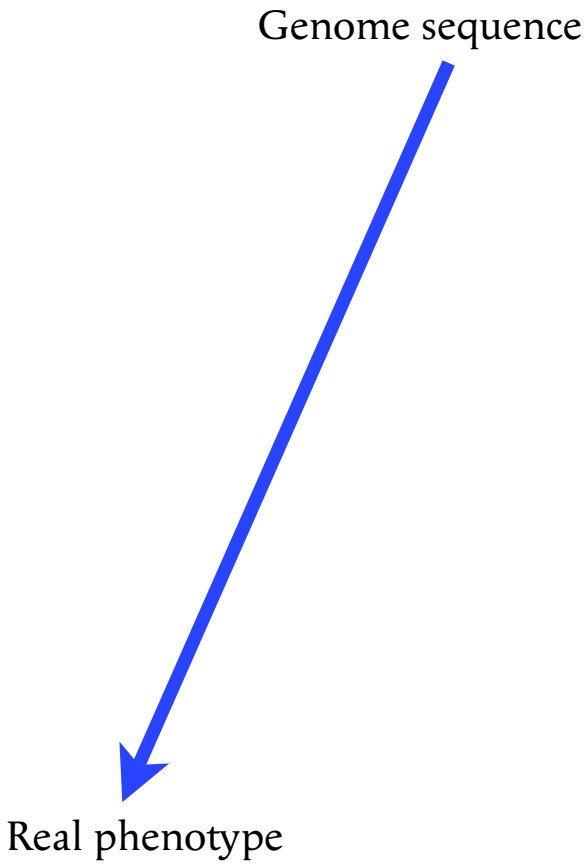

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Genome sequence

Real phenotype

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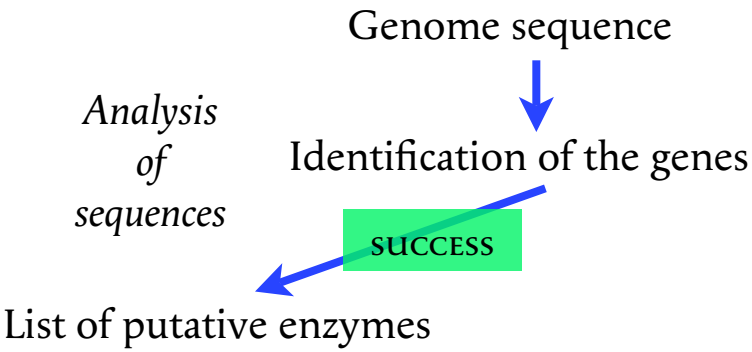

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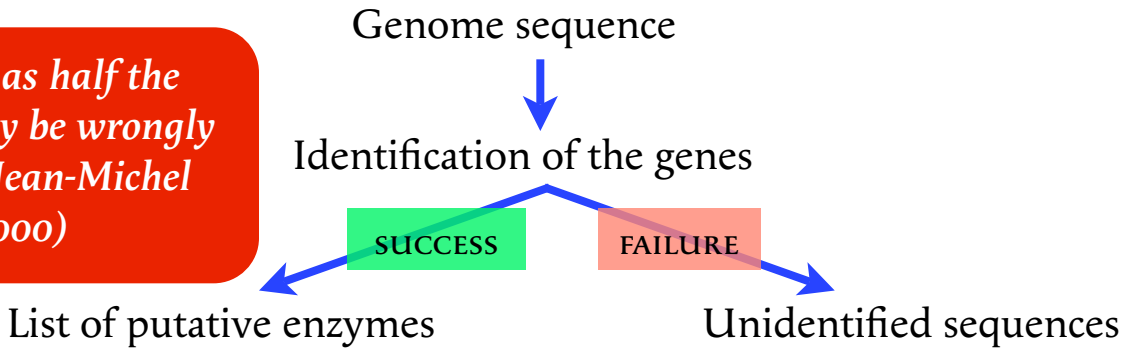
"As many as half the proteins may be wrongly identified" Jean-Michel Claverie (2000)



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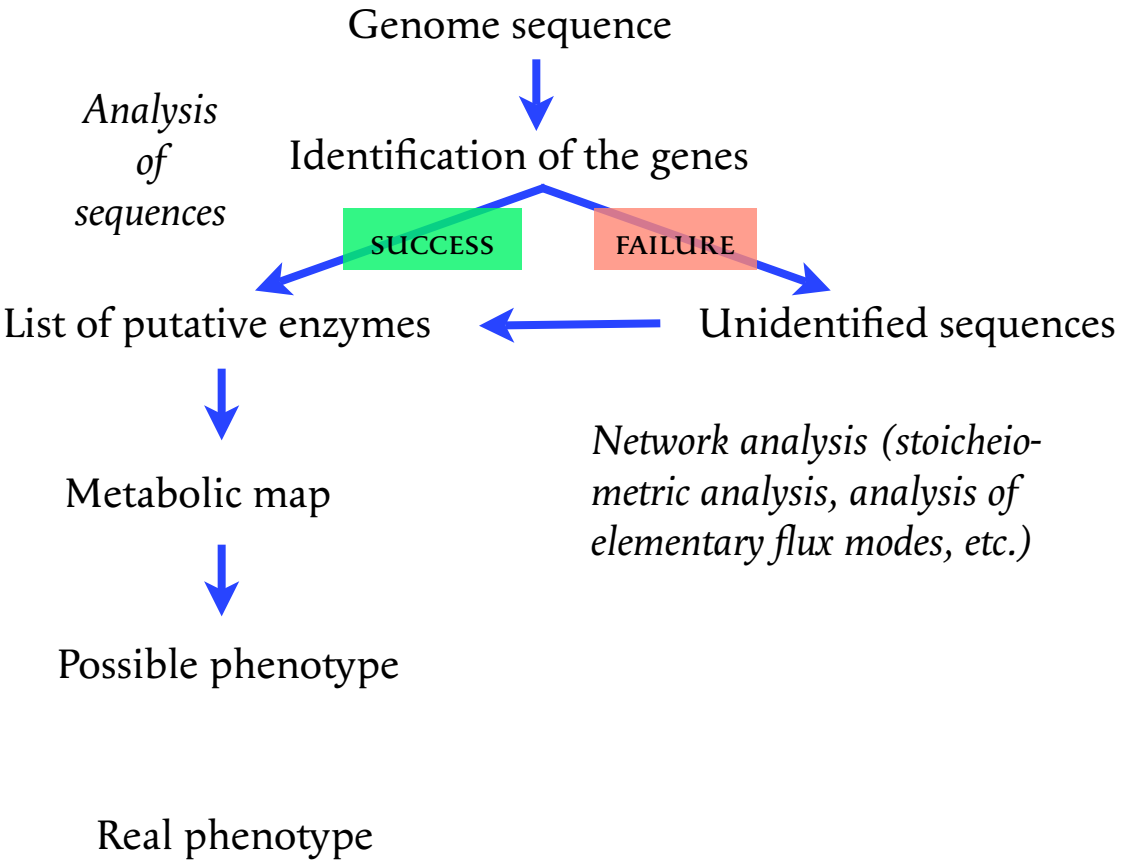

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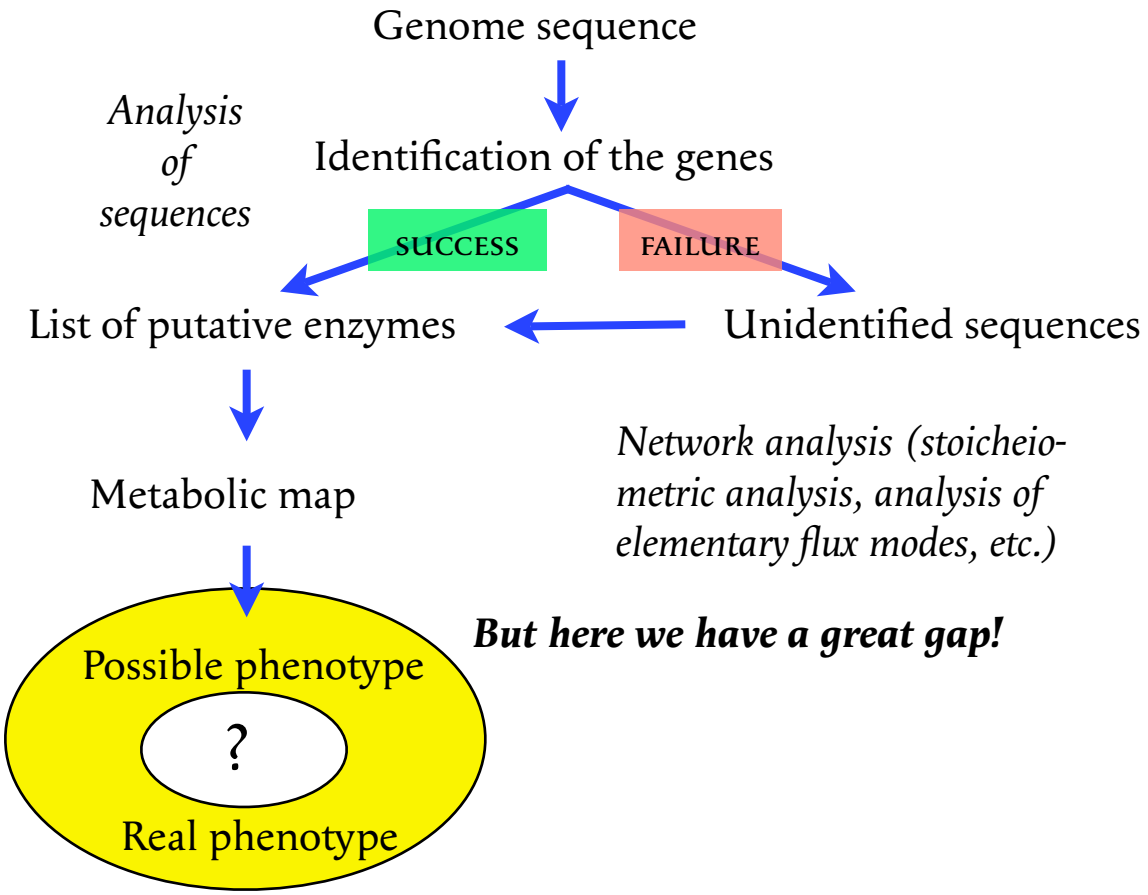

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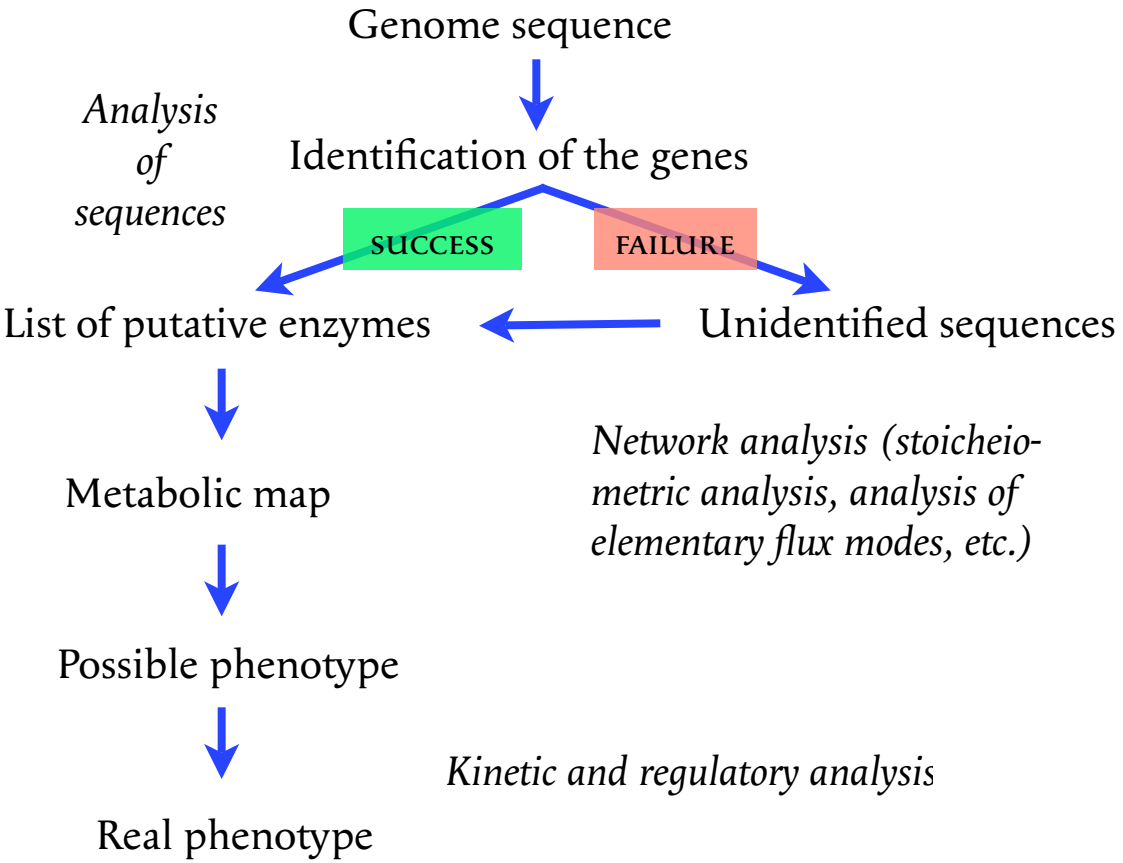

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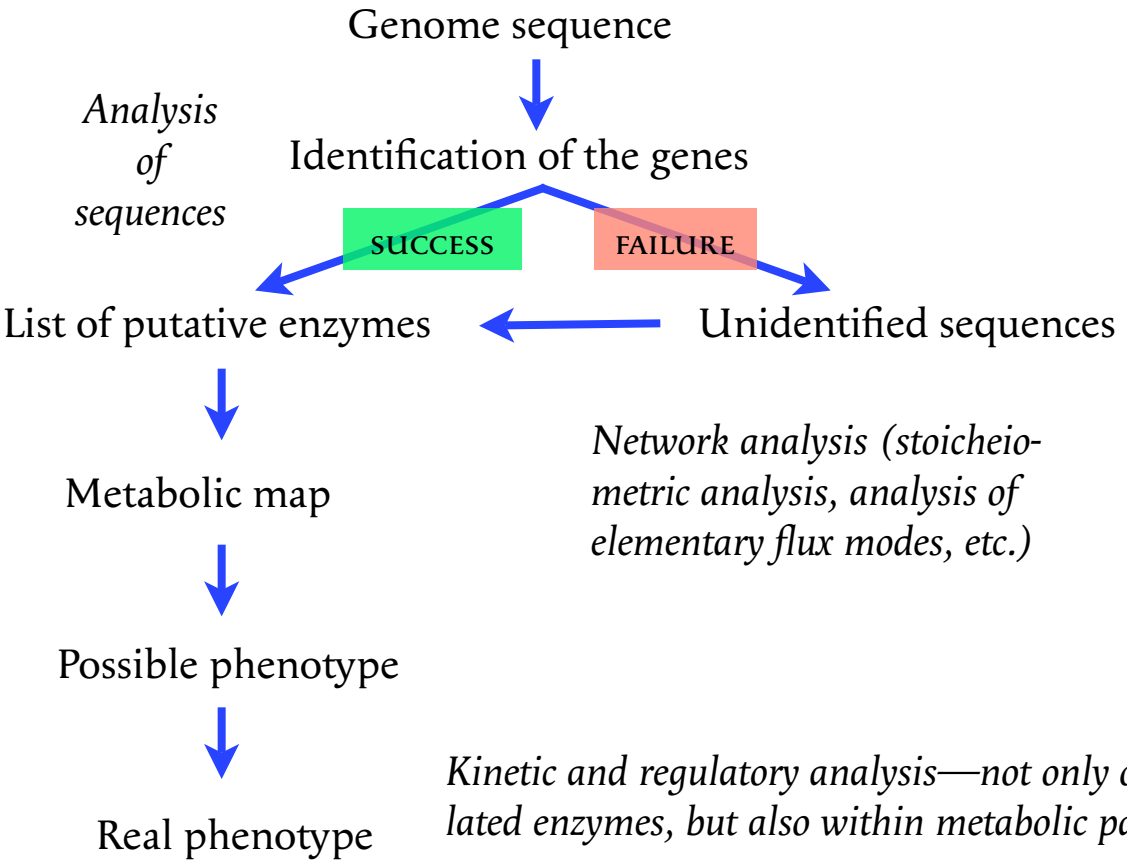

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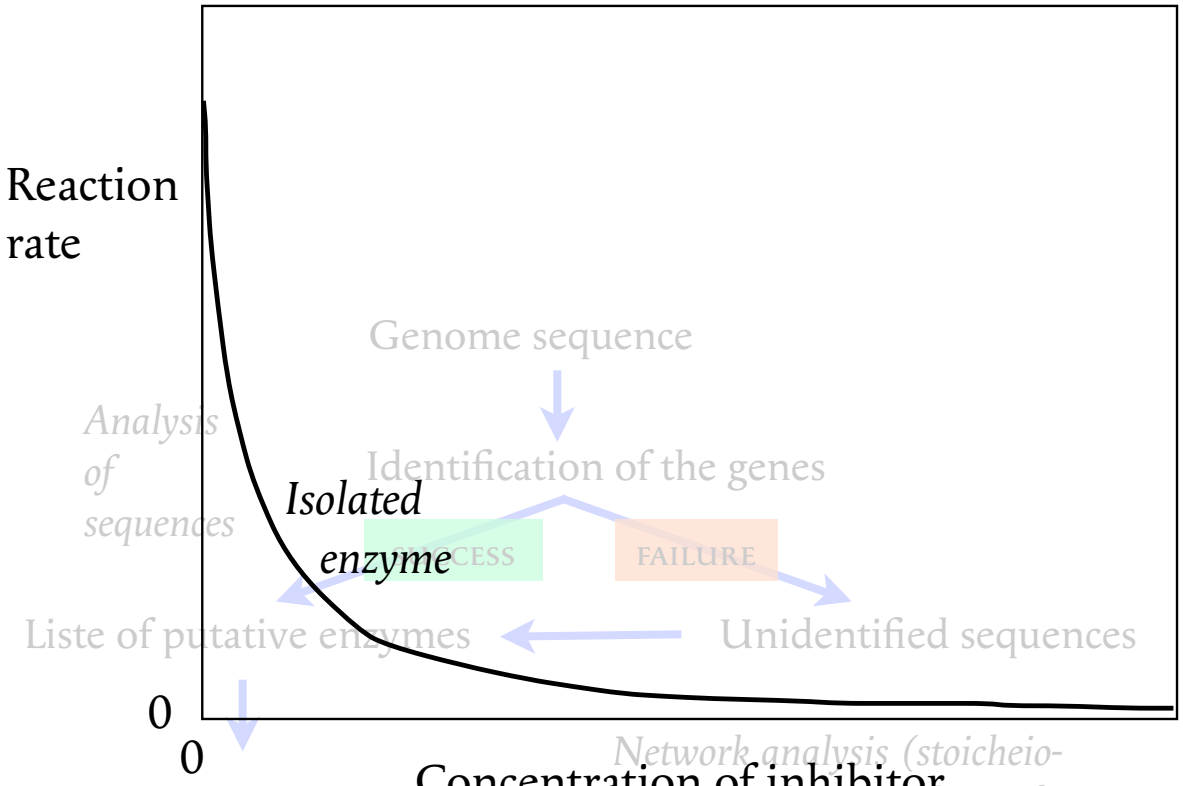

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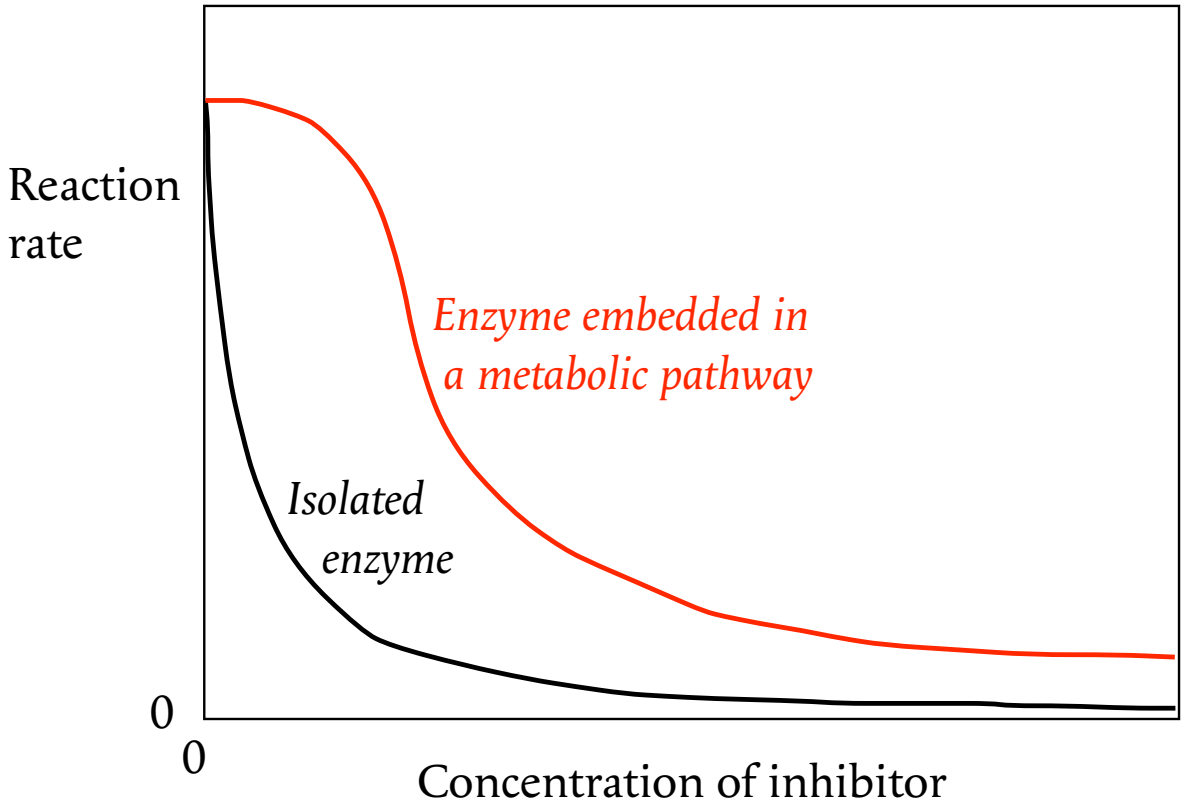

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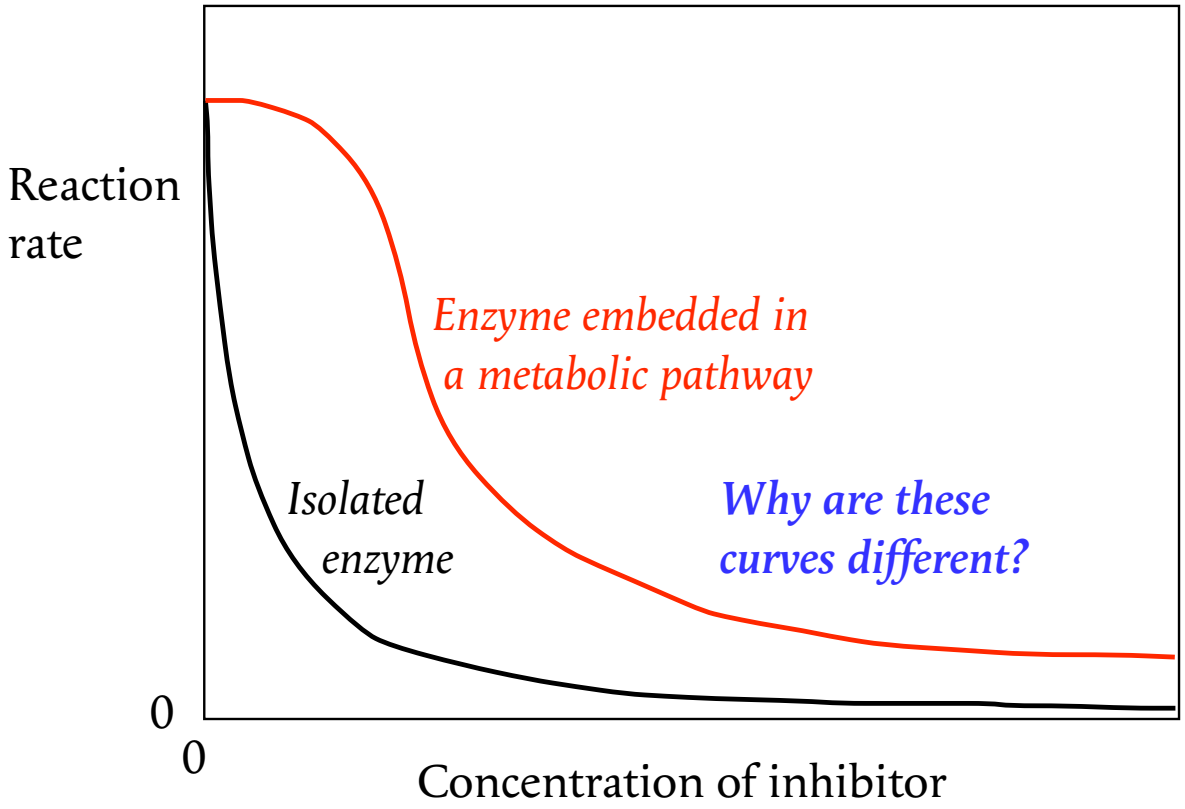

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*Drug design**

Many drugs work because of their effects on the kinetic properties of enzymes. To design them in a rational way one needs to know how to characterize an enzyme, and in particular how to measure its kinetic parameters and to characterize the effects of inhibitors.

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One must not think that good methods for evaluating the kinetic properties of an enzyme are less important today than they were in the past; on the contrary, they are ***much more important*** than they were in the past, because to understand the effects of a mutation one must be able to measure small changes in parameters with precision.

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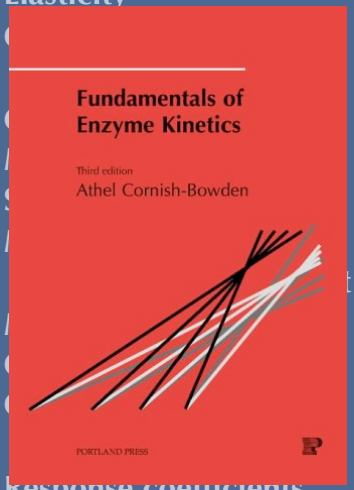

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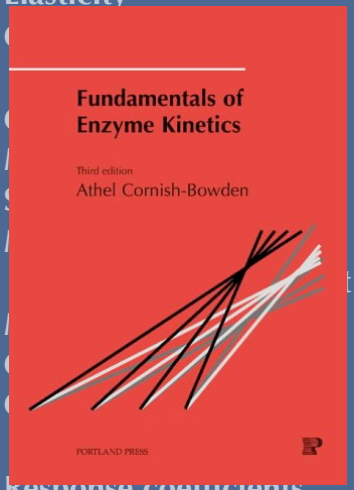

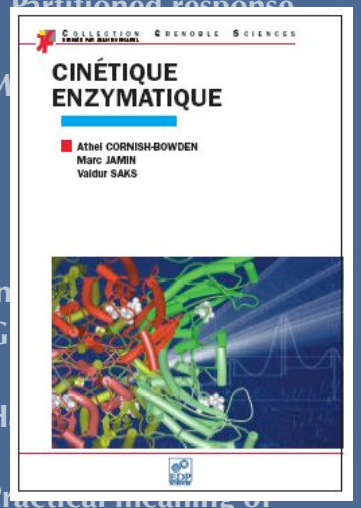

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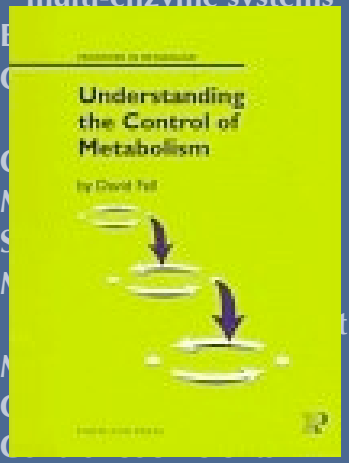

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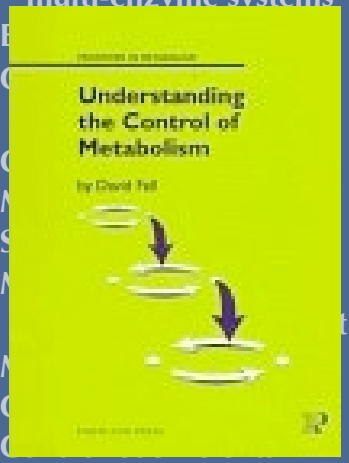

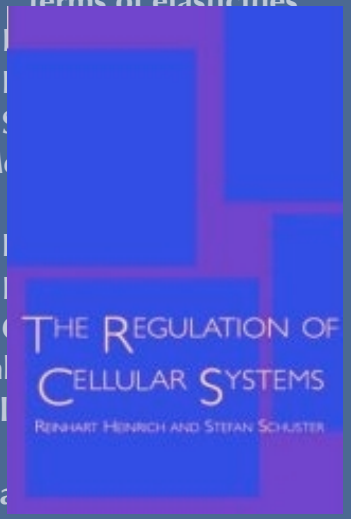

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KINETICS OF MULTIENZYME SYSTEMS

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Metabolic control analysis
Summation properties

In everyday enzyme kinetics we consider just one enzyme at a time:

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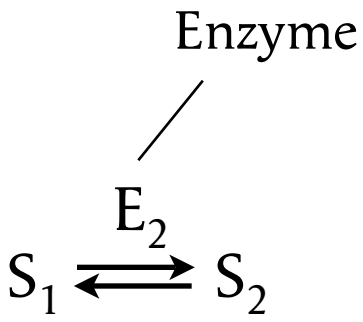

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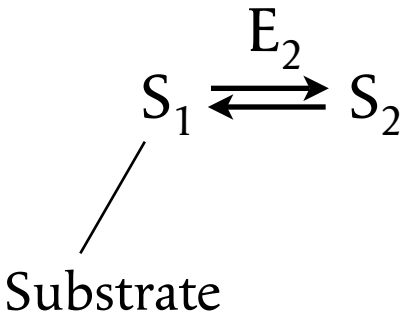

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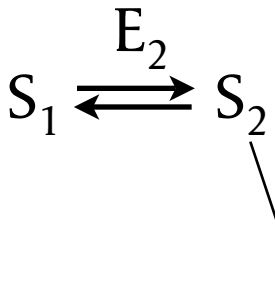

Product (why S_2 and not P? We shall see in a moment)

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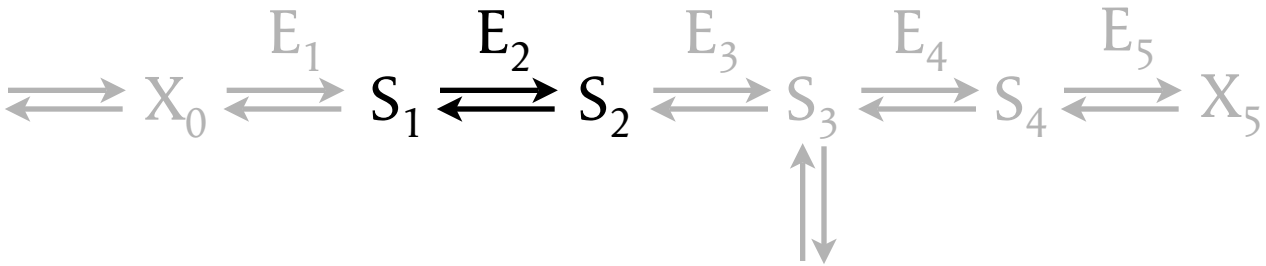
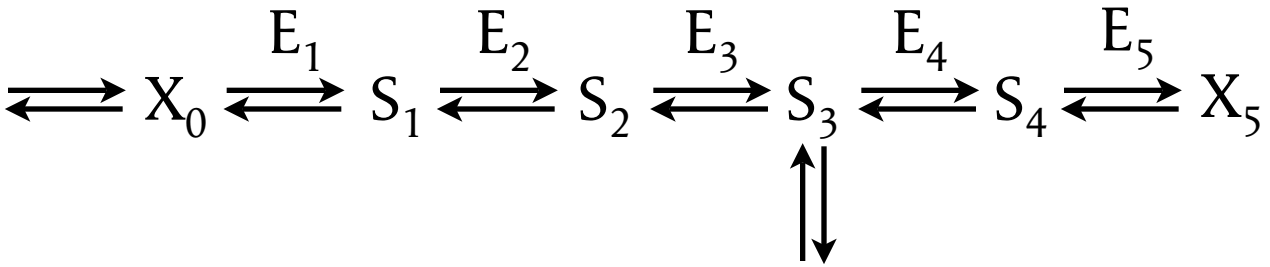


However, in physiological reality, this enzyme exists as a *component of a more extensive system*…

In such a system the distinction between substrates and products has a tendency to be obscured: almost all the products are substrates of other enzymes. So we don't need a symbol P.



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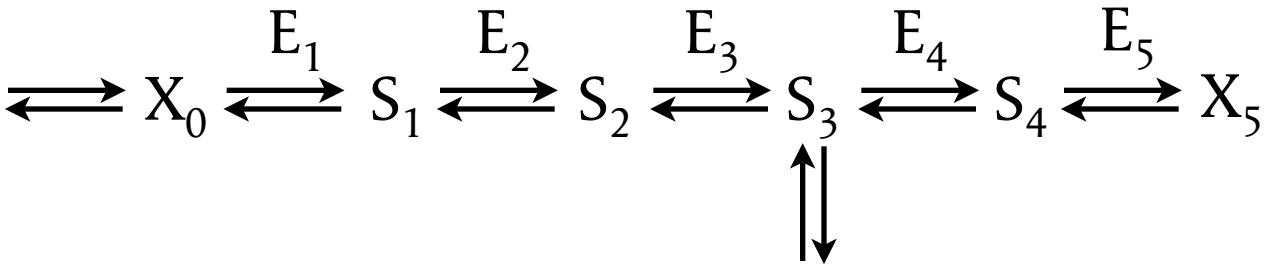
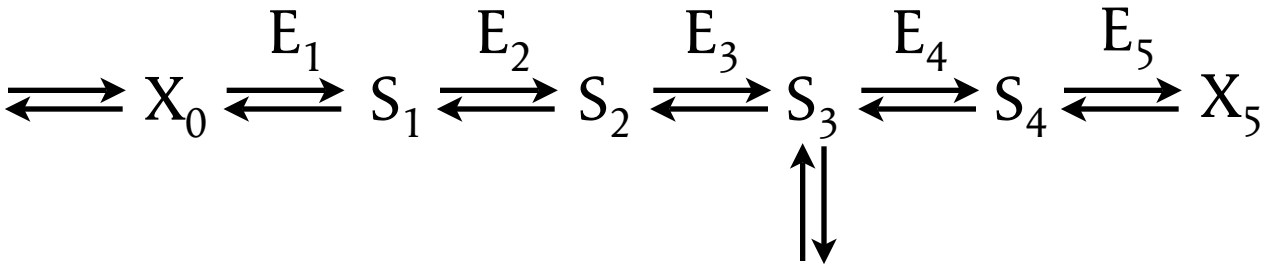


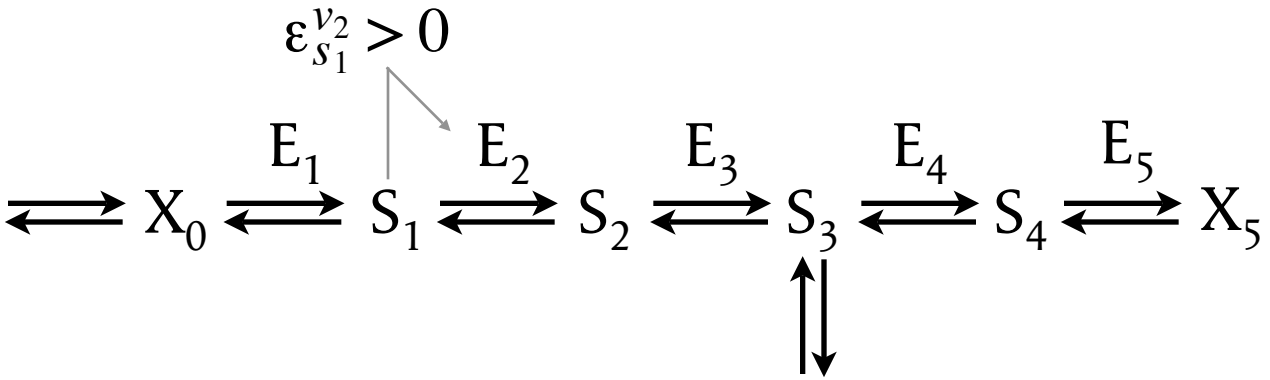
There is, however, an important difference between the metabolites *in* the system (S_1 to S_4), and those *external to the system* or at its limits (X_0 and X_5):

More generally, how can we understand the kinetic properties of the whole system in terms of the properties of the isolated enzymes?



More generally, how can we understand the kinetic properties of the whole system in terms of the zymes?

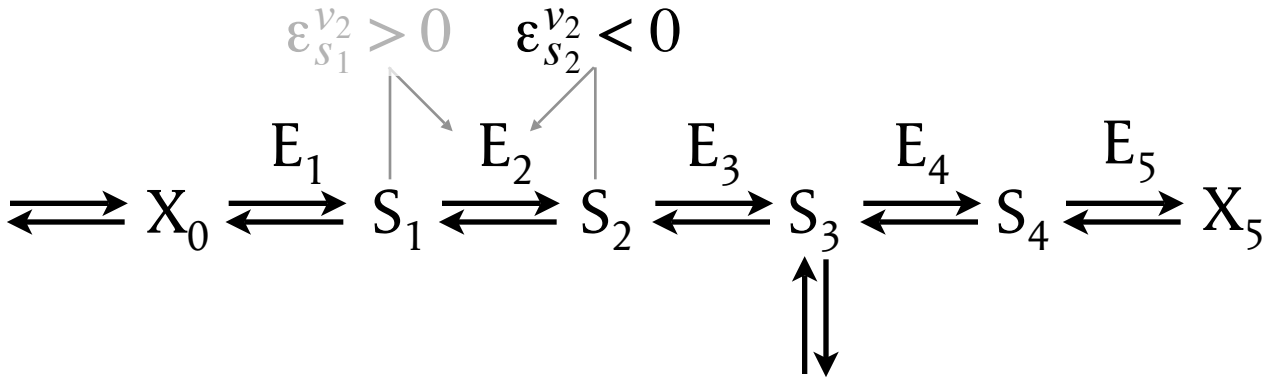
> 0 because *increasing* the concentration of the substrate implies *increasing* the rate.



For example, the enzyme we considered at the beginning, E_2, is certainly influenced by its substrate S_1

More generally, how can we understand the kinetic properties of the whole system in terms of the prope

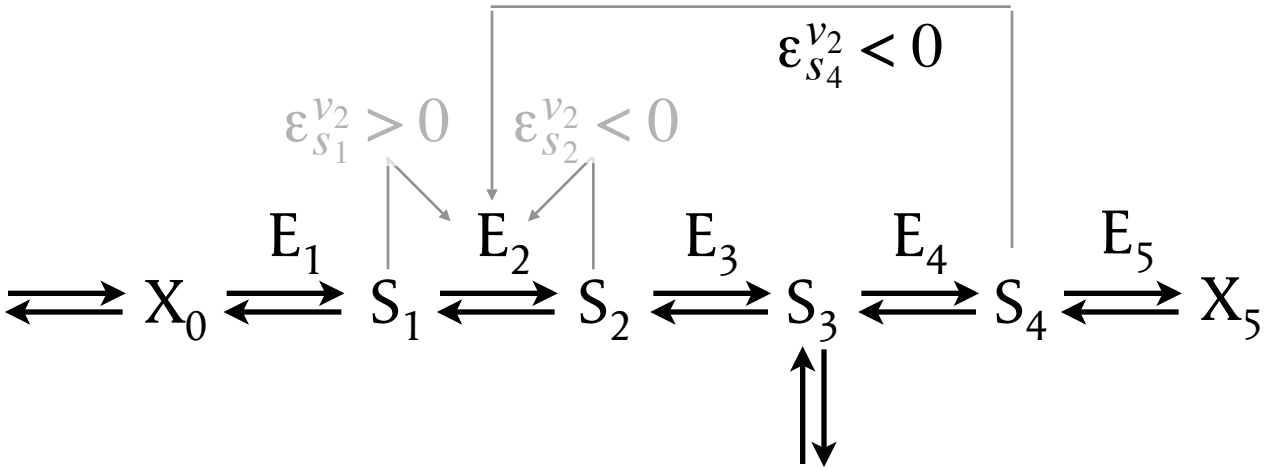
< 0 because *increasing* the concentration of the product implies *decreasing* the rate.



For example, the enzyme we considered at the beginning, E_2, is certainly influenced by its substrate S_1 and by its product S_2;

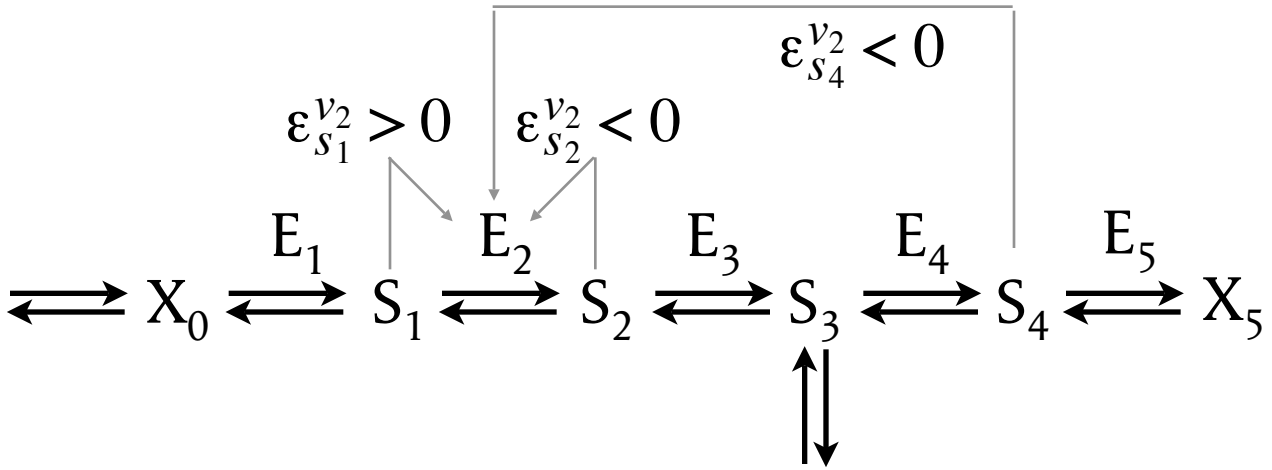
More general properties of properties of

< 0 because *increasing* the concentration of the feedback inhibitor implies *decreasing* the rate.



For example, the enzyme we considered at the beginning, E_2, is certainly influenced by its substrate S_1 and by its product S_2; and it may also be affected by feedback inhibition by another metabolite, S_4.

More generally, how can we understand the kinetic properties of the whole system in terms of the properties of the isolated enzymes?



The parameters ε are called the *elasticities* of the enzyme for the metabolites concerned. All the enzymes have elasticities for all the metabolites (though fortunately many of them are negligible).

…he
…substrate
…affected
…te, S_4.

More generally, how can we understand the kinetic properties of the whole system in terms of the properties of the isolated enzymes?

$\varepsilon_{s_1}^{v_2} > 0$ $\varepsilon_{s_2}^{v_2} < 0$ $\varepsilon_{s_4}^{v_2} < 0$

An elasticity is thus a measure of the *sensitivity of an enzyme rate to the concentration of a metabolite:* in a sense, therefore, measuring elasticities is what biochemists have been doing since the days of Michaelis and Menten, even if they didn't use the word.

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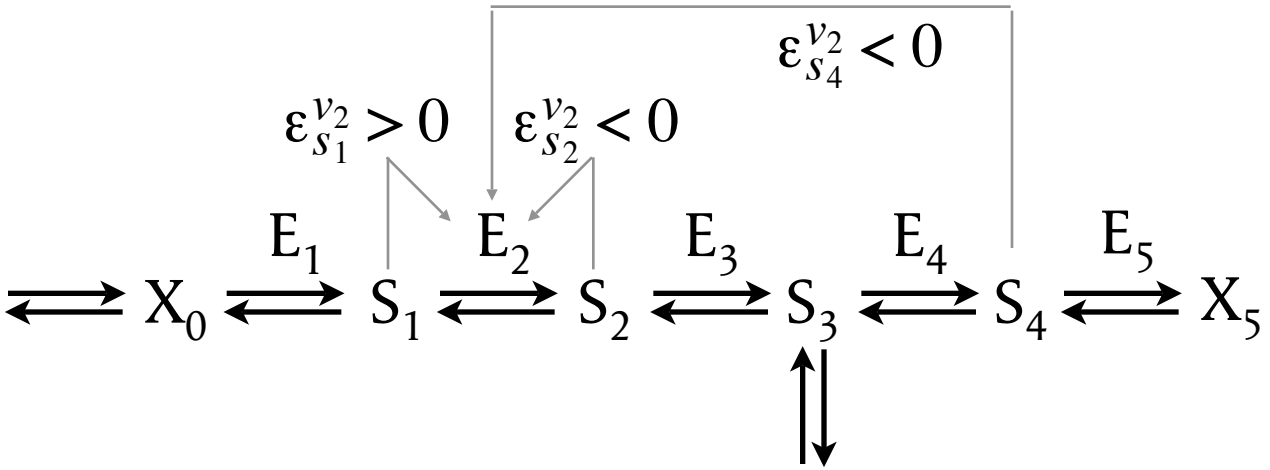
Now we need to define the limits of the system in a more exact way.



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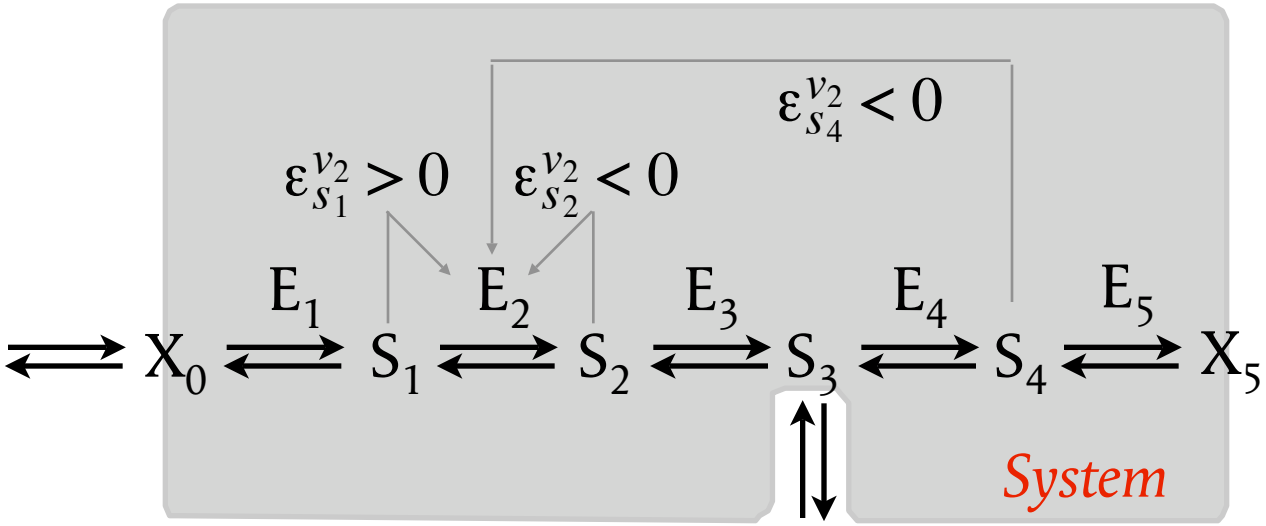

The *system*

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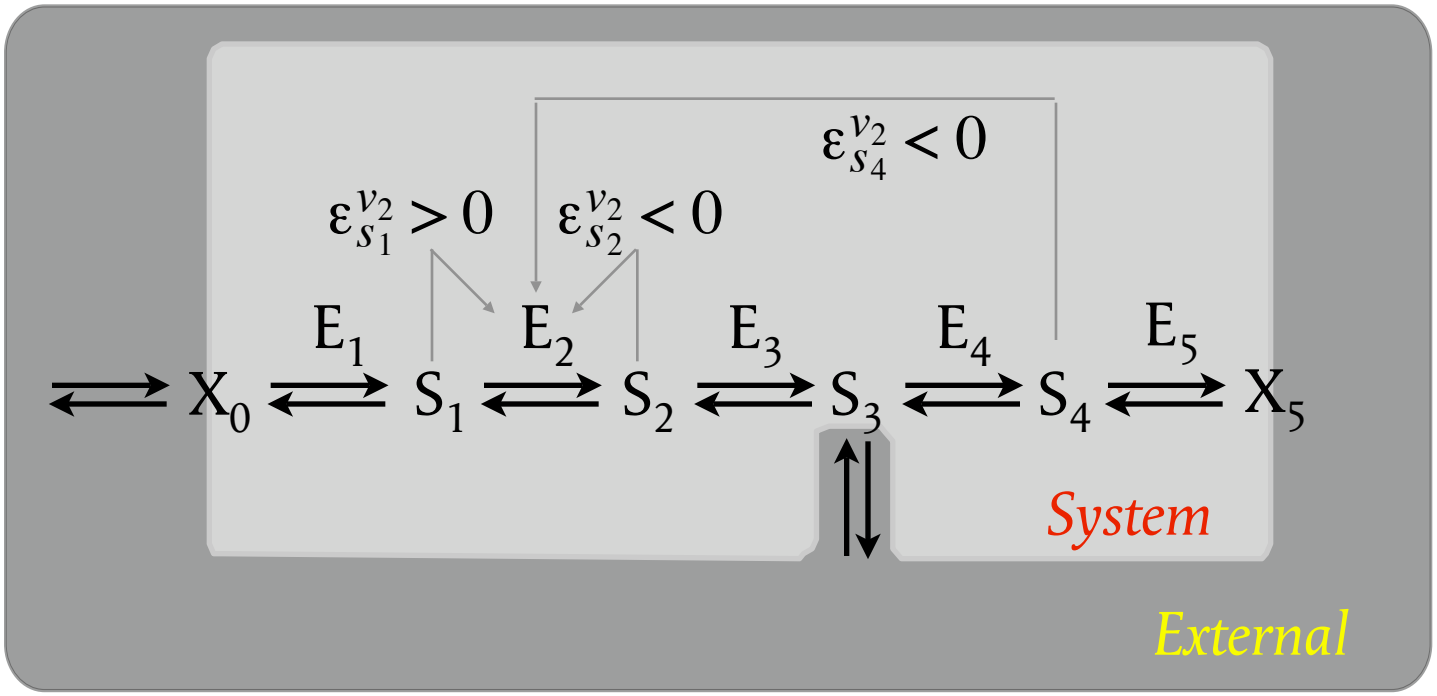
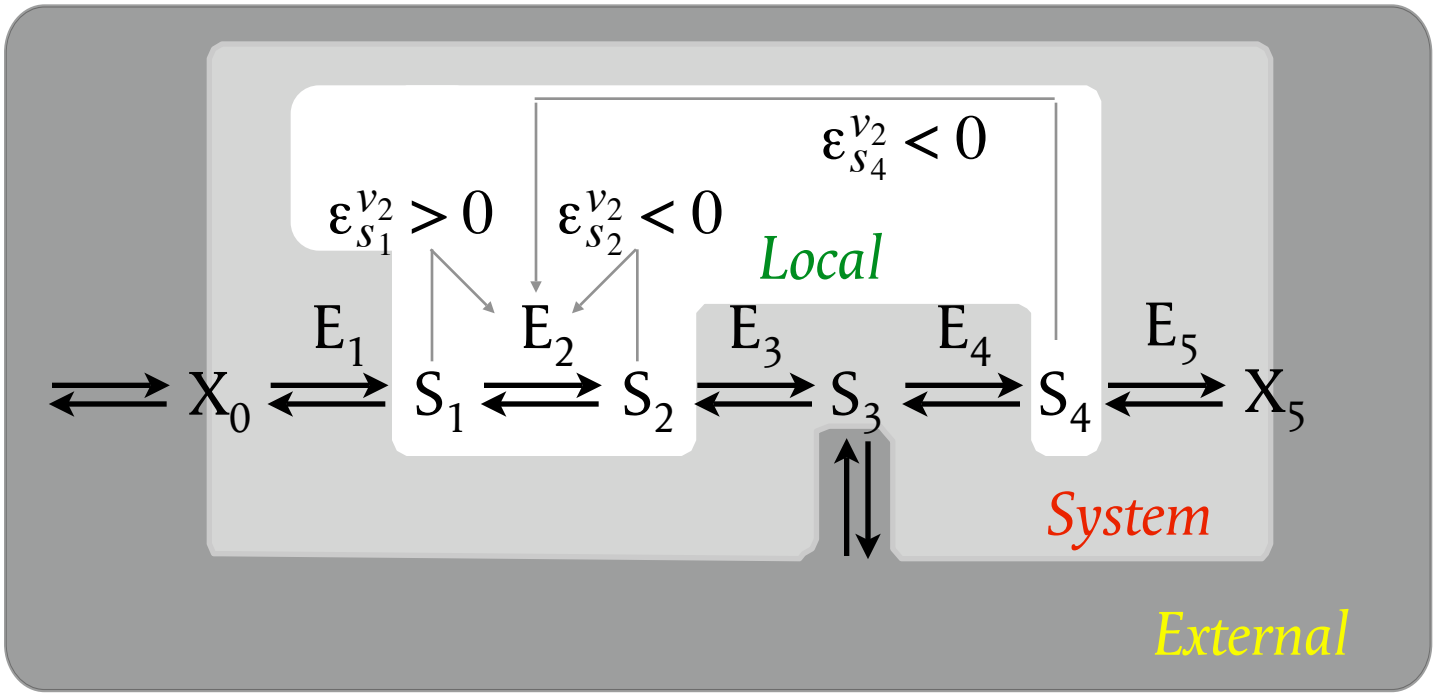


The *system* is embedded in an *external* environment,

Now we need to define the limits of the system in a more exact way.



The *system* is embedded in an *external* environment, and within the system we can define *local* regions in order to examine particular enzymes.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

Let us start with a typical equation for the rate of an enzyme-catalysed reaction in terms of the concentrations of its substrate, product and an inhibitor.

rate

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concentration of enzyme

Let us start with a typical equation for the rate of an enzyme-catalysed reaction in terms of the concentrations of its substrate, product and an inhibitor.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

concentration of substrate

Let us start with a typical equation for the rate of an enzyme-catalysed reaction in terms of the concentrations of its substrate, product and an inhibitor.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

concentration of product

Let us start with a typical equation for the rate of an enzyme-catalysed reaction in terms of the concentrations of its substrate, product and an inhibitor.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

concentration of inhibitor

Let us start with a typical equation for the rate of an enzyme-catalysed reaction in terms of the concentrations of its substrate, product and an inhibitor.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

…and various kinetic parameters

Let us start with a typical equation for the rate of an enzyme-catalysed reaction in terms of the concentrations of its substrate, product and an inhibitor.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

We can obtain the partial derivative with respect to any concentration by standard methods

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

$$\frac{\partial v}{\partial a} = \frac{k_A e_0 \left[1 + p\left(\frac{1}{K_{mP}} + \frac{k_P}{k_A K_{mA}}\right) + \frac{i}{K_i}\right]}{\left(1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}\right)^2}$$

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We can obtain the partial derivative with respect to any concentration by standard methods, and it is convenient to eliminate the dimensions of the result by multiplying by a/v.

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

$$\frac{\partial v}{\partial a} = \frac{k_A e_0 \left[1 + p\left(\frac{1}{K_{mP}} + \frac{k_P}{k_A K_{mA}}\right) + \frac{i}{K_i}\right]}{\left(1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}\right)^2}$$

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$$= \frac{1}{1 - \frac{k_P p}{k_A a}} - \frac{\frac{a}{K_{mA}}}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

$$= \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

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$$= \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

p/a at any state of the reaction

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

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$$= \frac{1}{1 - \frac{k_P}{k_A}} \cdot \frac{\frac{a}{K_{mA}}}{\left(1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}\right)}$$

$K_{eq} = k_A/k_P = p/a$ at equilibrium

$$= \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

$$v = \frac{k_A e_0 a - k_P e_0 p}{1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}}$$

$$\frac{\partial v}{\partial a} = \frac{k_A e_0 \left[1 + p\left(\frac{1}{K_{mP}} + \frac{k_P}{k_A K_{mA}}\right) + \frac{i}{K_i}\right]}{\left(1 + \frac{a}{K_{mA}} + \frac{p}{K_{mP}} + \frac{i}{K_i}\right)^2}$$

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Dimensionless concentrations

$$= \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

This is now the *definition* of the elasticity of the enzyme with respect to the substrate concentration.

$$\frac{\partial \ln v}{\partial \ln a} = \frac{a\,\partial v}{v\,\partial a}$$

$$= \frac{1}{1 - \Gamma/K_{\mathrm{eq}}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

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$$\varepsilon_a^v = \frac{\partial \ln v}{\partial \ln a} = \frac{a\,\partial v}{v\,\partial a}$$

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This is now the *definition* of the elasticity of the enzyme with respect to the substrate concentration.

Rearranging,

$$\varepsilon_a^v = \frac{\partial \ln v}{\partial \ln a} = \frac{a\,\partial v}{v\,\partial a}$$

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Rearranging,

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Rearranging,

$$\varepsilon_a^v = \frac{\partial \ln v}{\partial \ln a} = \frac{a\,\partial v}{v\,\partial a}$$

$$= \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

$$\varepsilon_a^v = \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

Rearranging, and doing the same operation for the other concentrations, we get…

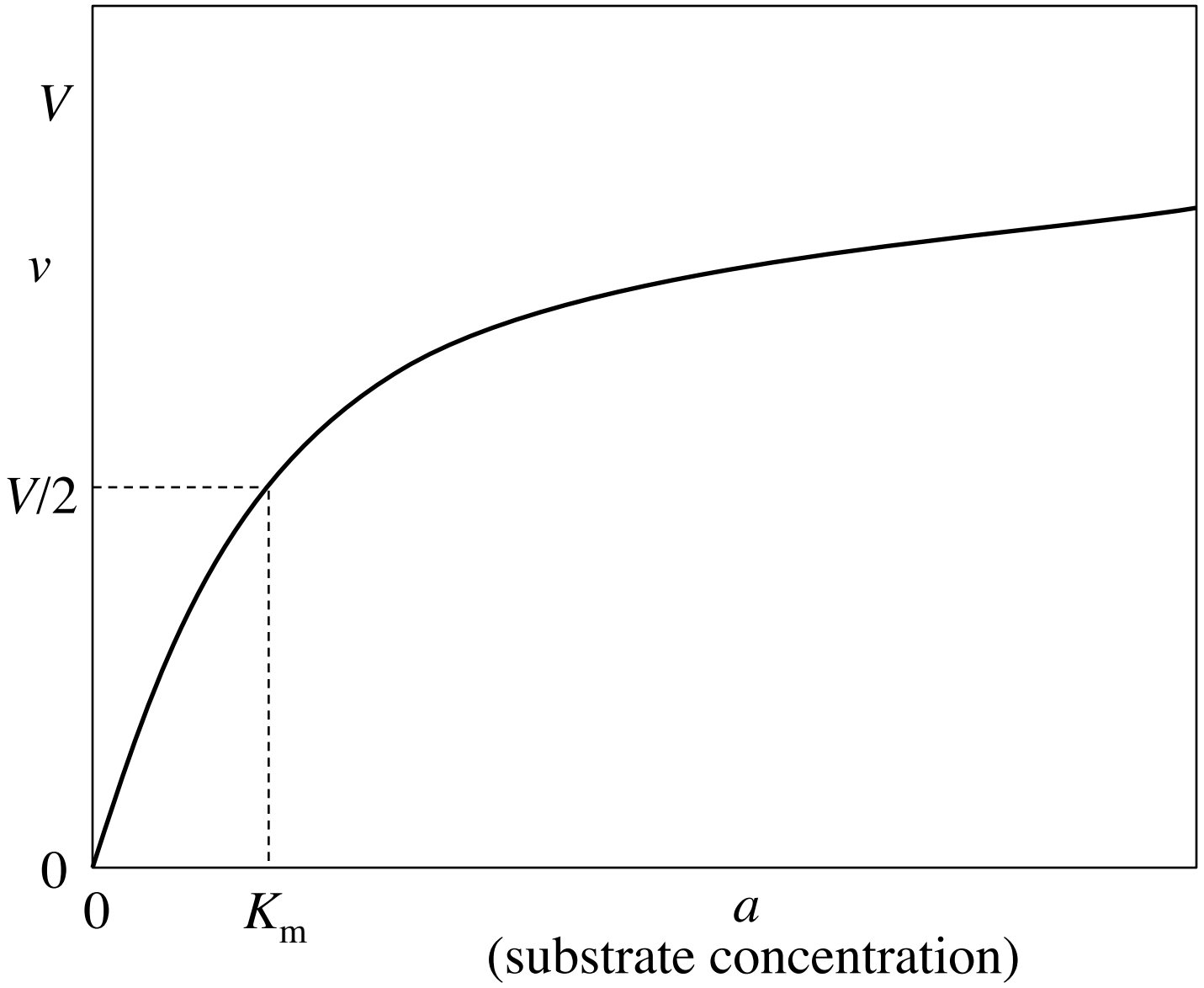
$$\varepsilon_a^v = \frac{1}{1 - \Gamma/K_{eq}} - \frac{\alpha}{1 + \alpha + \pi + \iota}$$

$$\varepsilon_p^v = \frac{-\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} - \frac{\pi}{1 + \alpha + \pi + \iota}$$

$$\varepsilon_{e_0}^v = \begin{cases} 1 & \text{if E catalyses the reaction} \\ 0 & \text{if E does not catalyse the reaction} \end{cases}$$

$$\varepsilon_i^v = -\frac{\iota}{1 + \alpha + \pi + \iota}$$

Elasticity or order of reaction?



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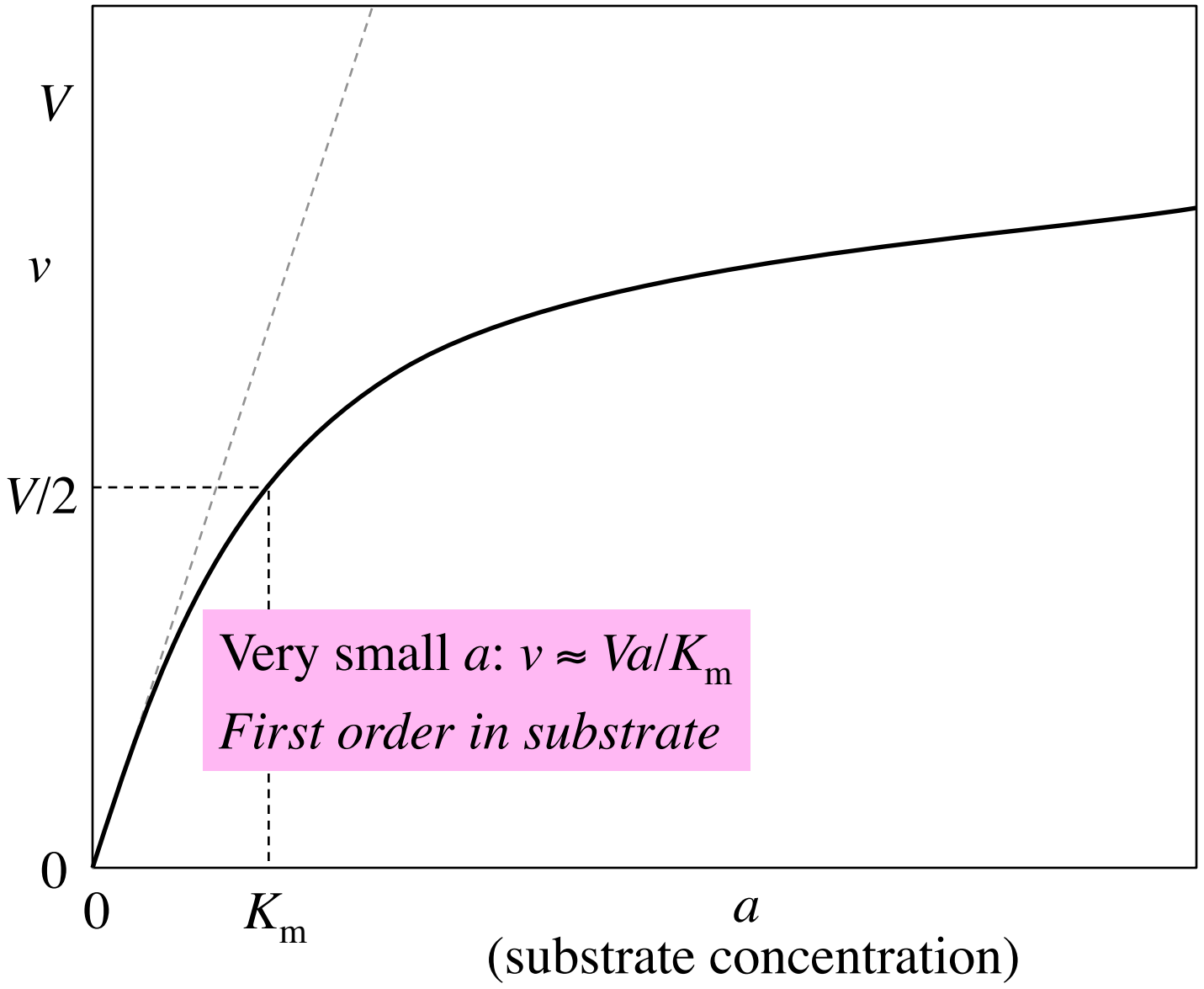

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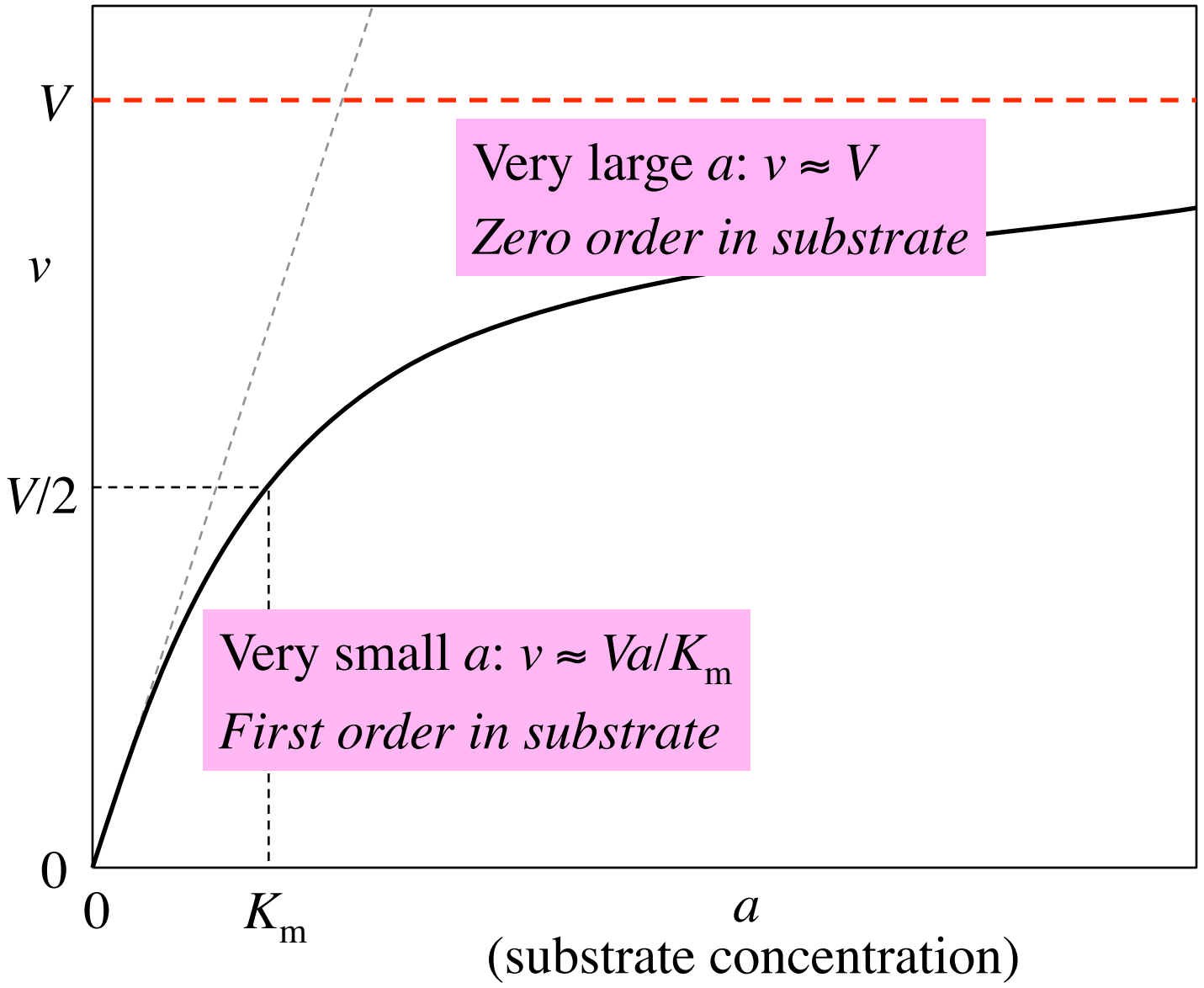

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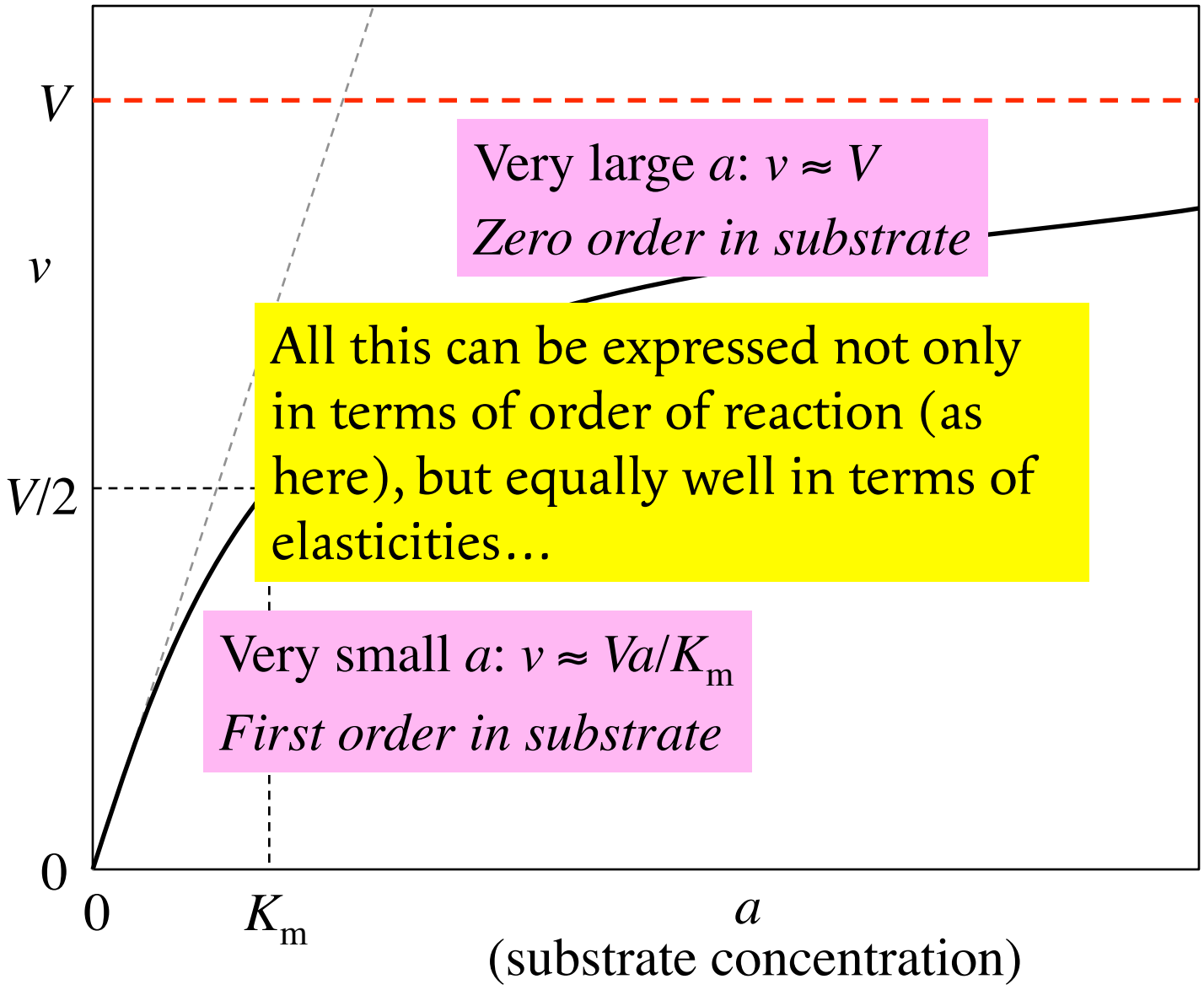

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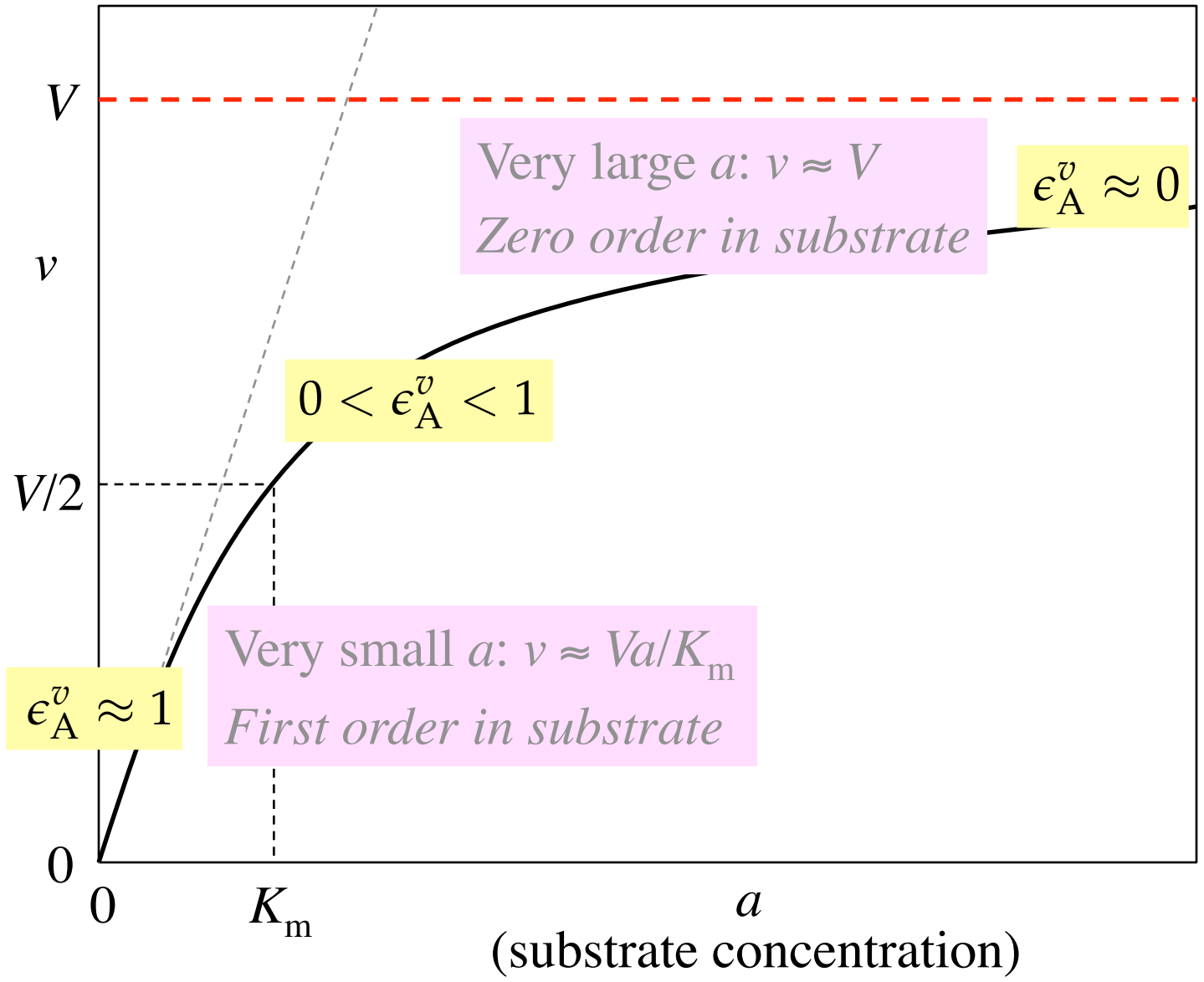

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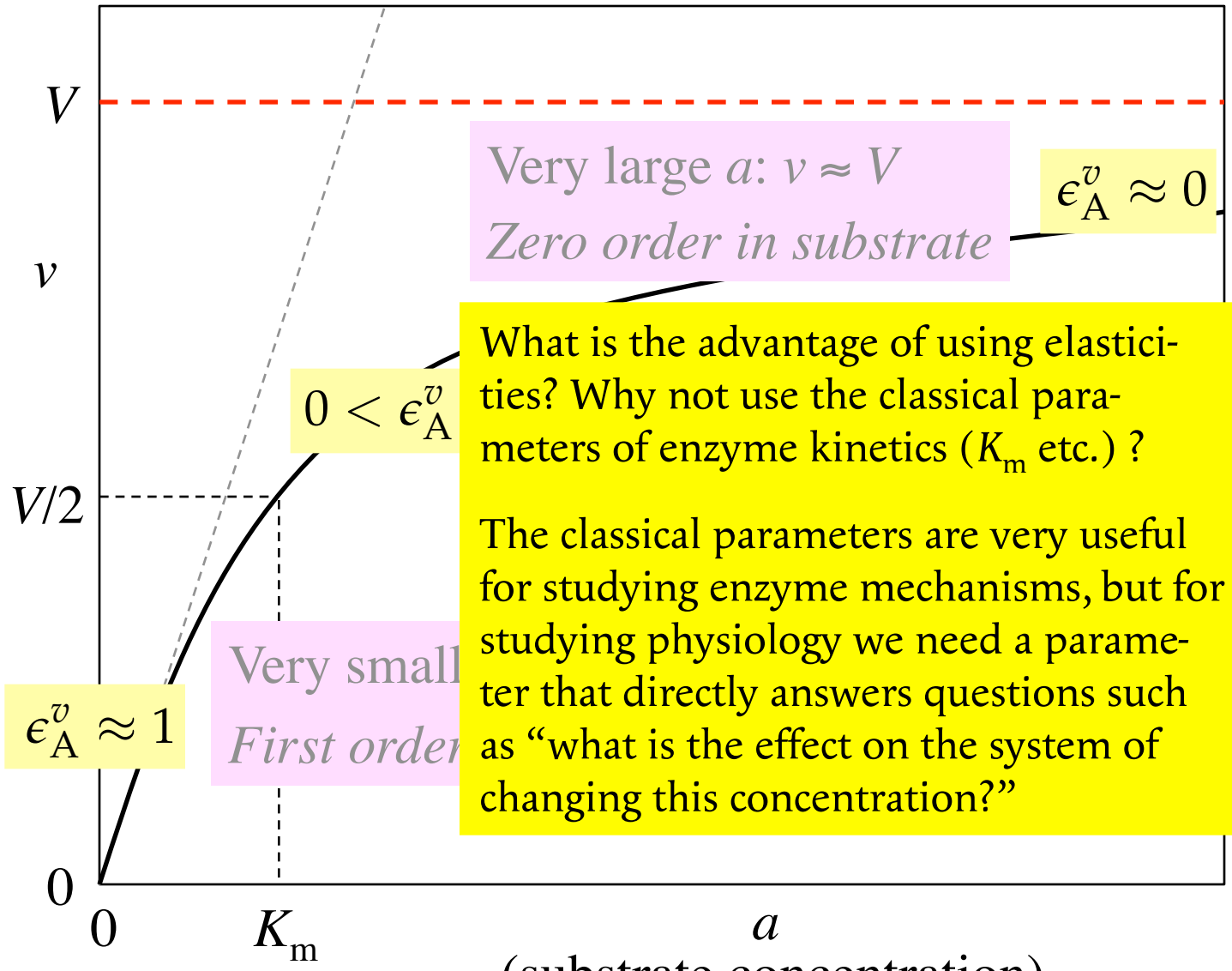
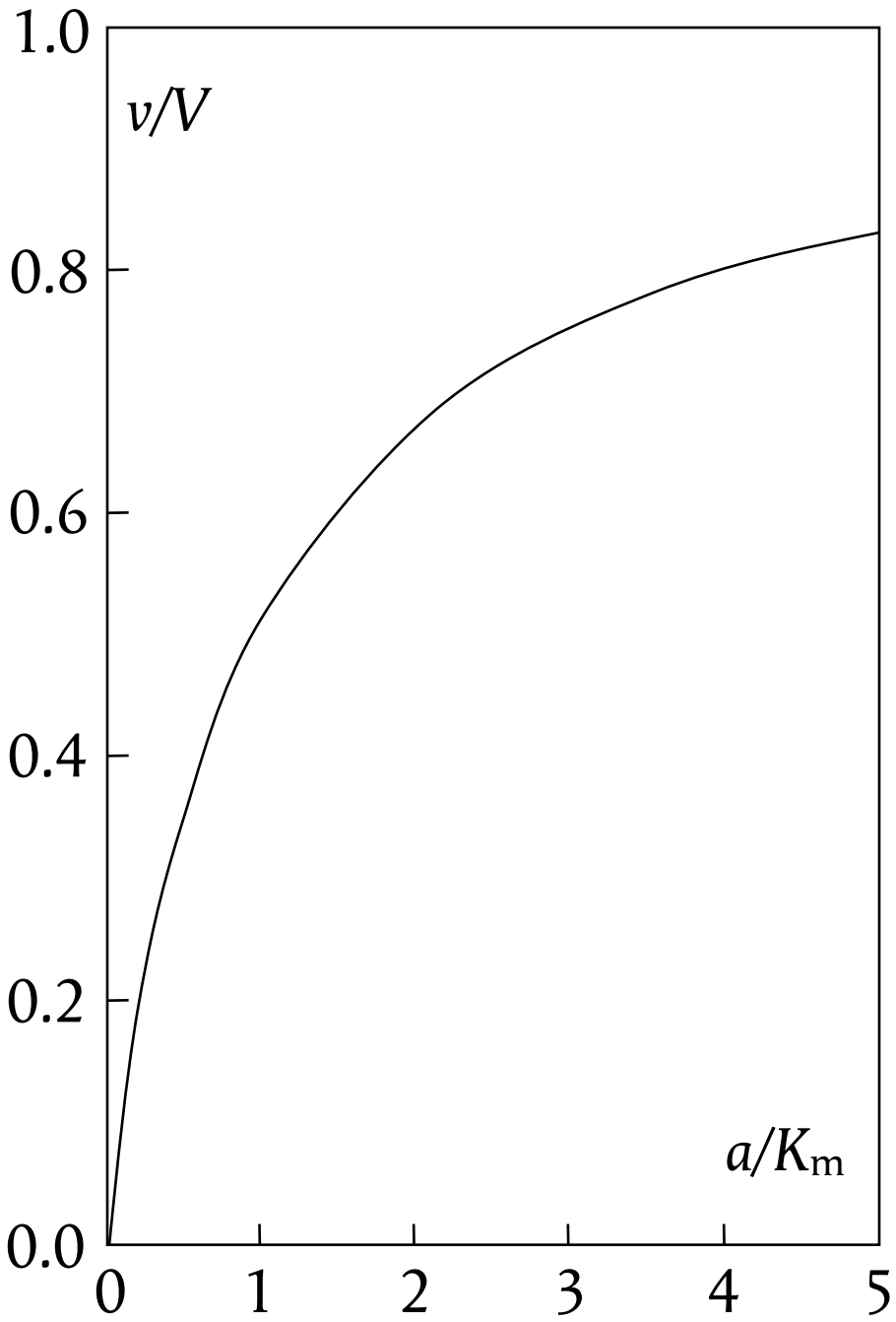
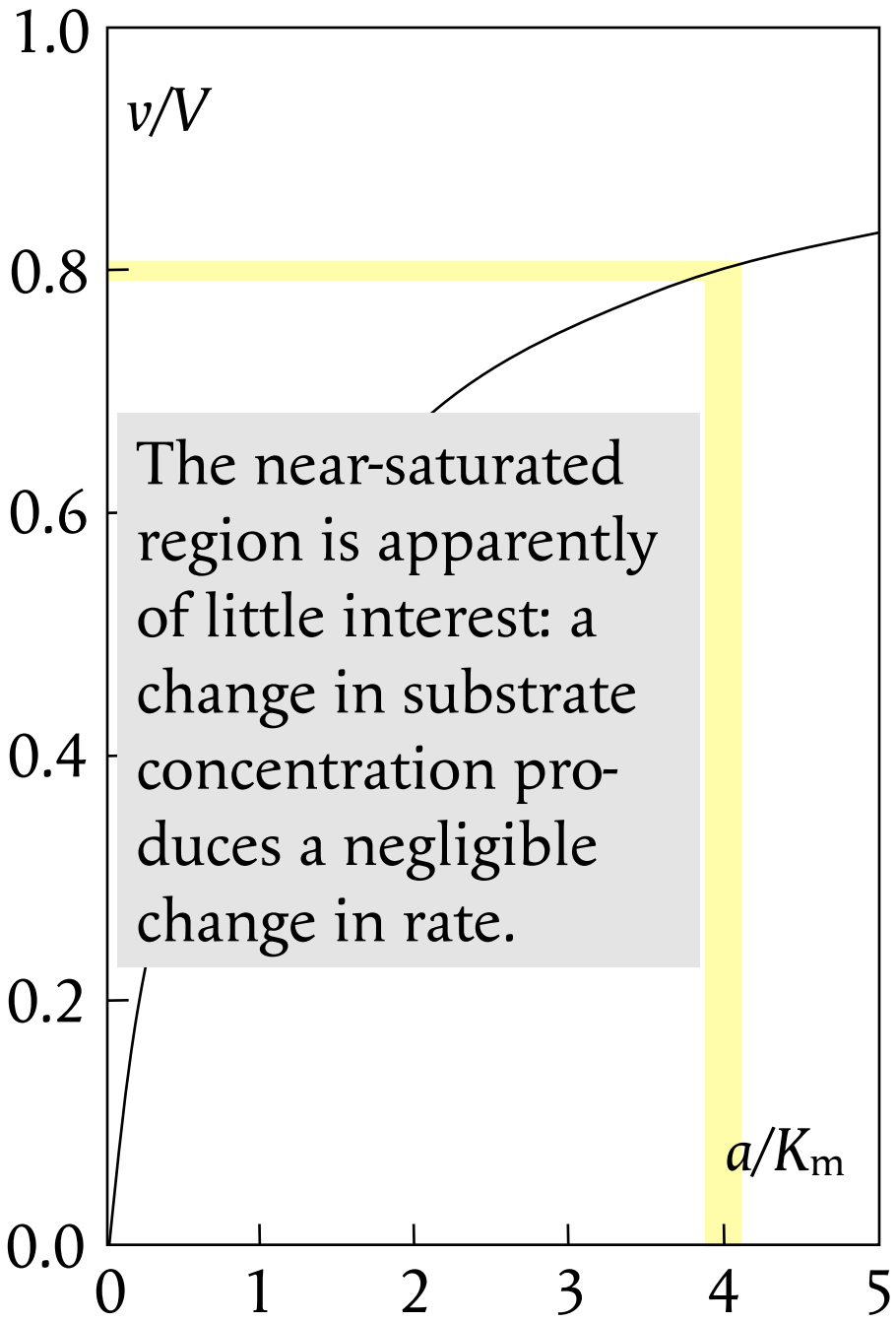


What is the advantage of using elasticities? Why not use the classical parameters of enzyme kinetics (K_m etc.) ?

The classical parameters are very useful for studying enzyme mechanisms, but for studying physiology we need a parameter that directly answers questions such as "what is the effect on the system of changing this concentration?"



The curve for the rate of an enzyme-catalysed reaction as a function of substrate is typically as shown at the left.



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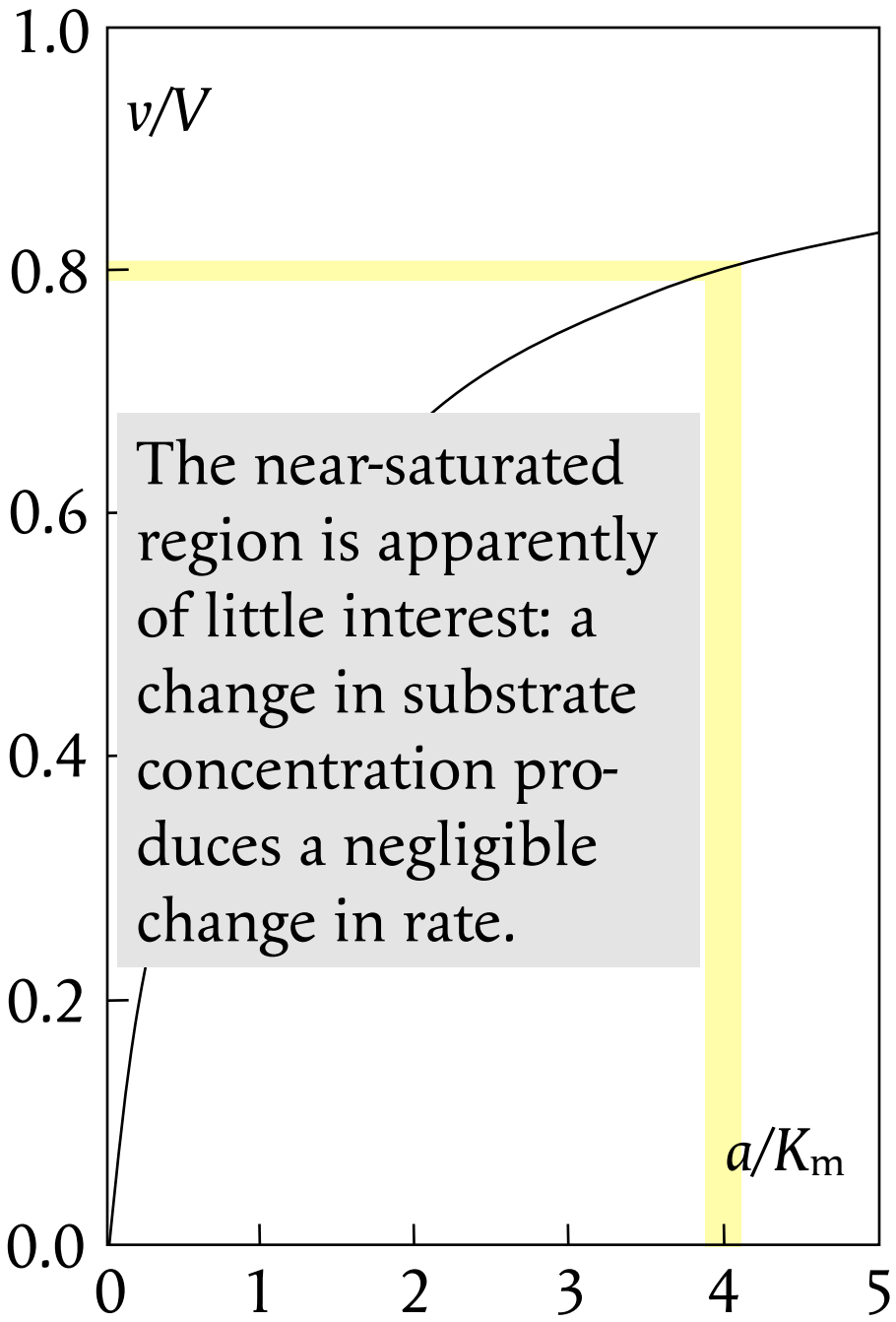
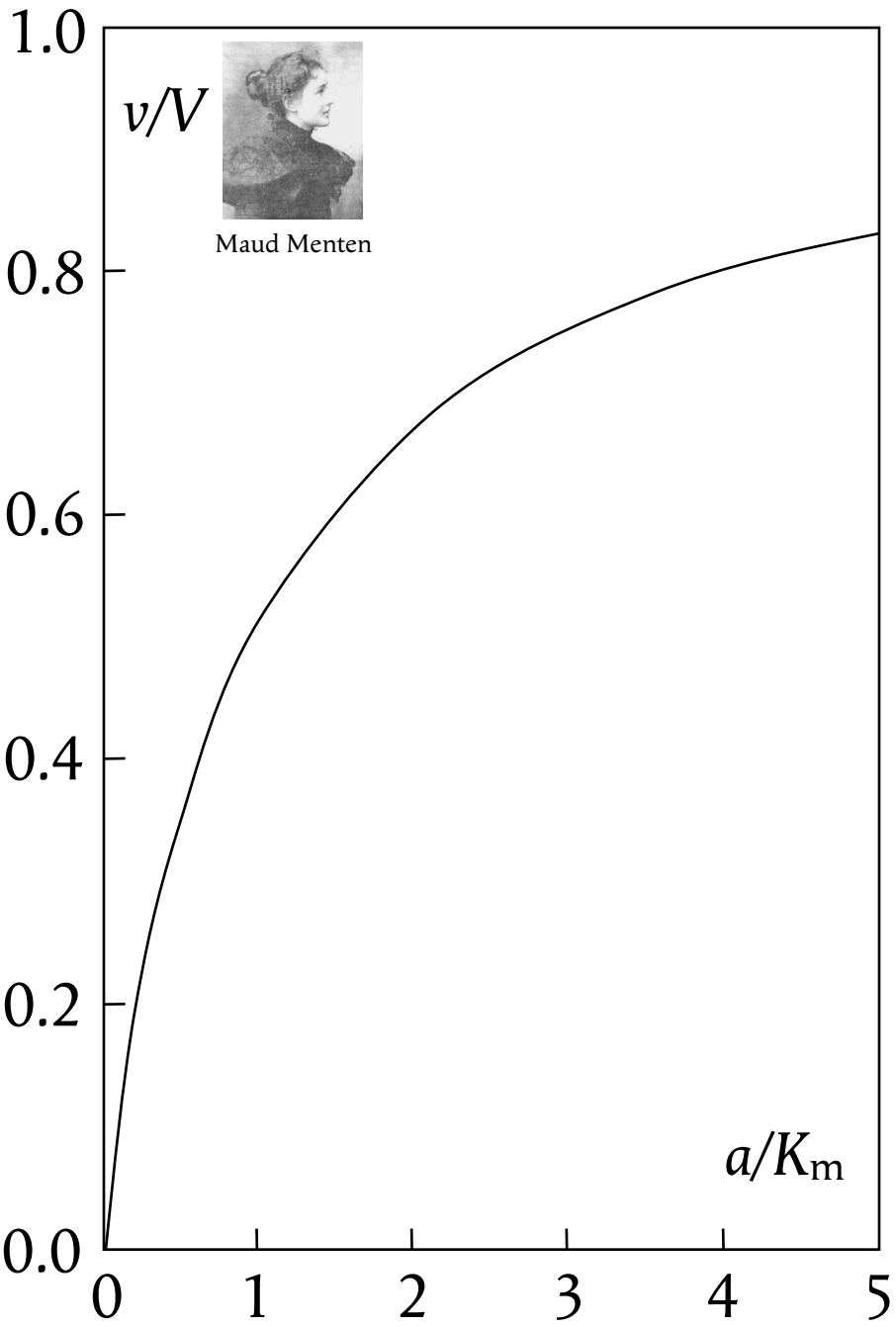


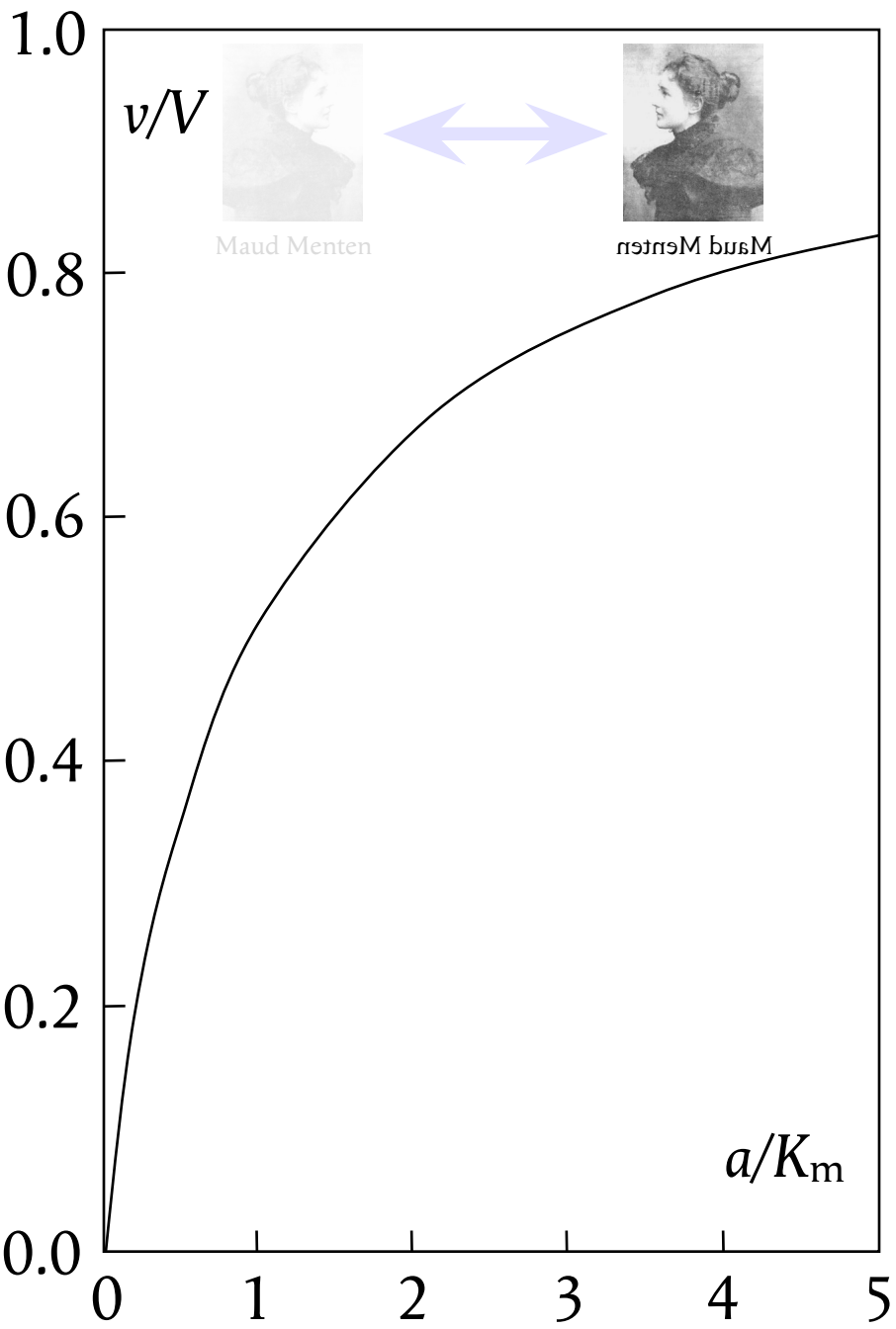
The curve for the rate of an enzyme-catalysed reaction as a function of substrate is typically as shown at the left.

However…



Maud Menten

It is still the same curve if we re-place it with its mirror image.



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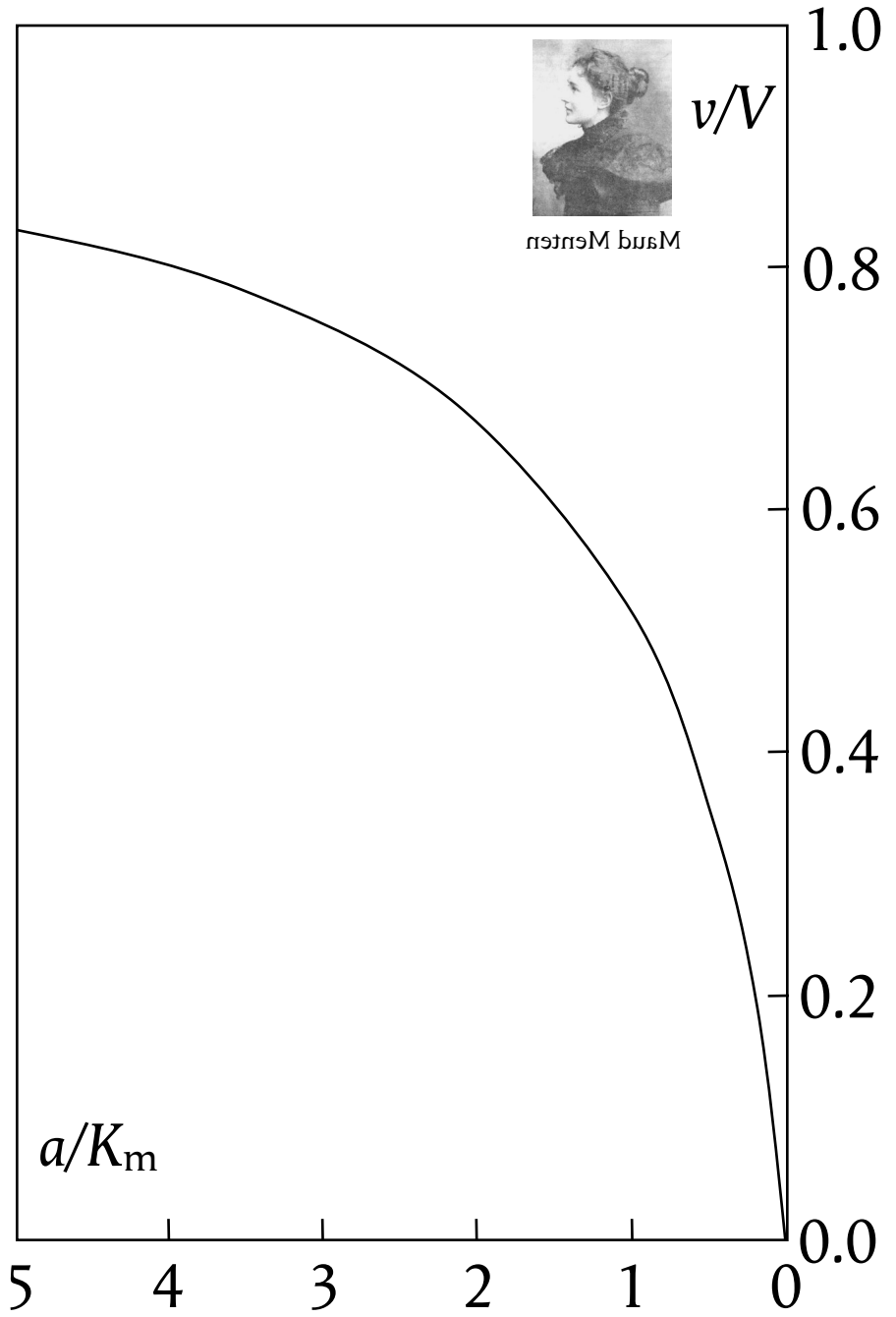
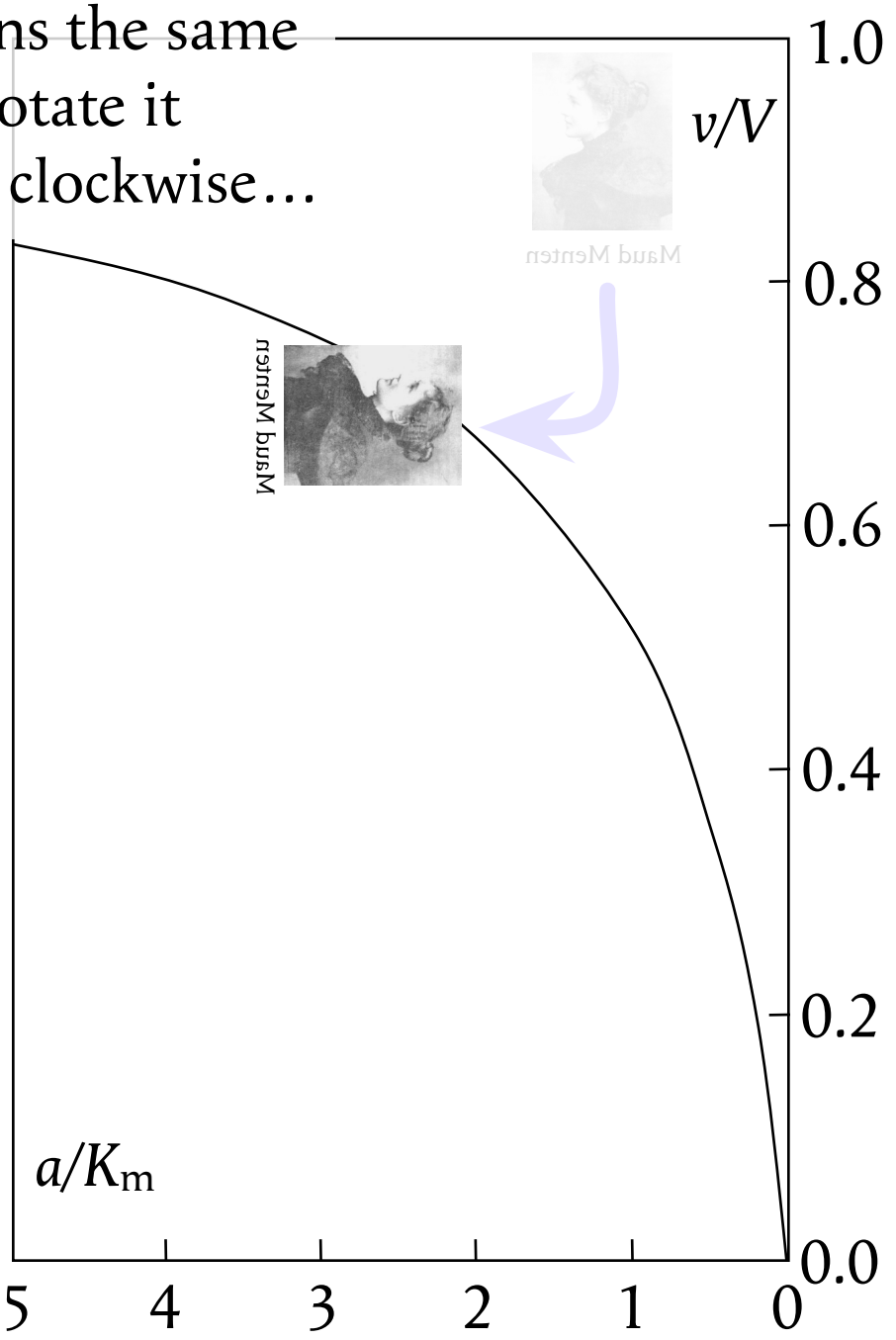


It is still the same curve if we re-place it with its mirror image.

and it remains the same curve if we rotate it through 90° clockwise…


Maud Menten

and it remains the same curve if we rotate it through 90° clockwise…



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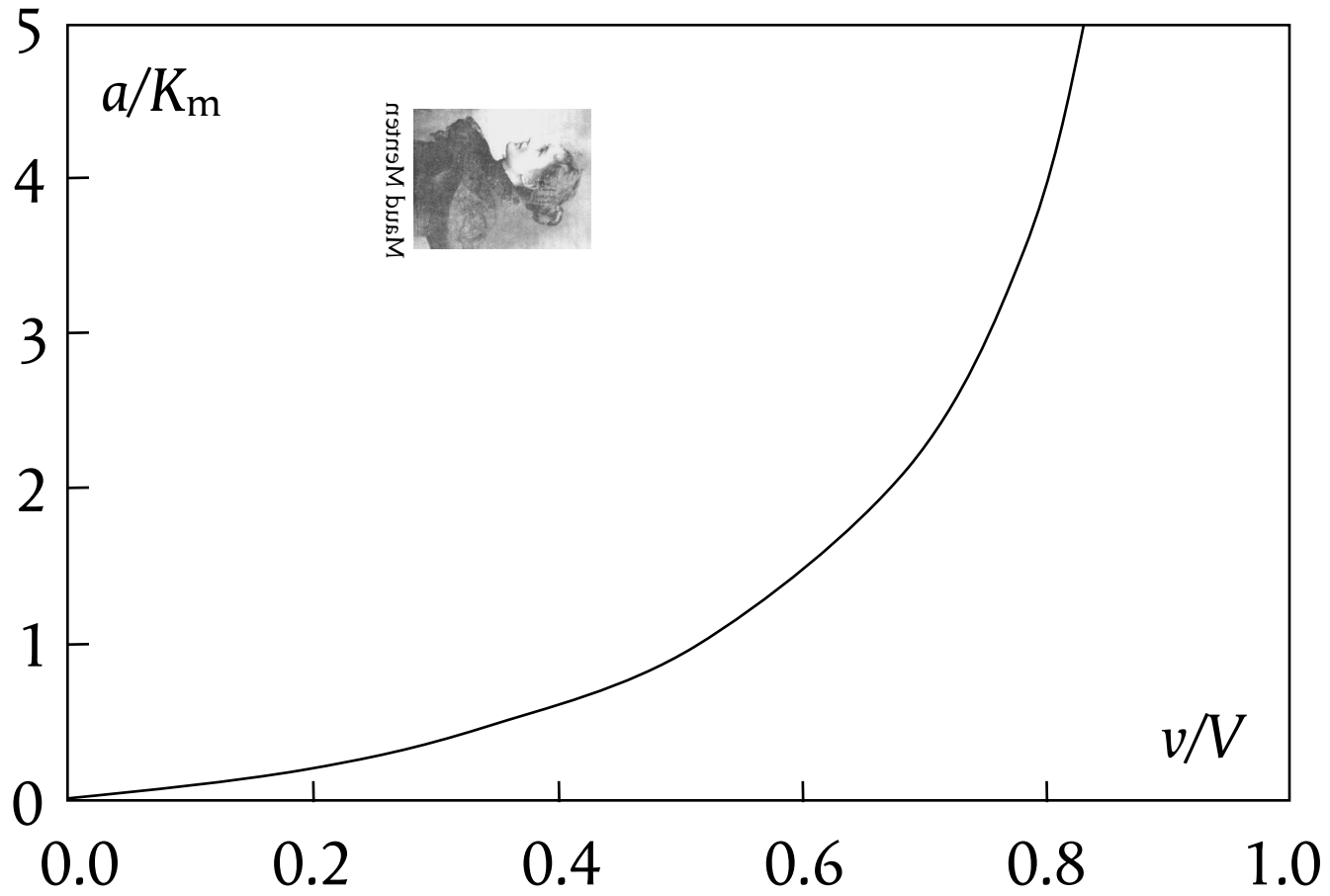

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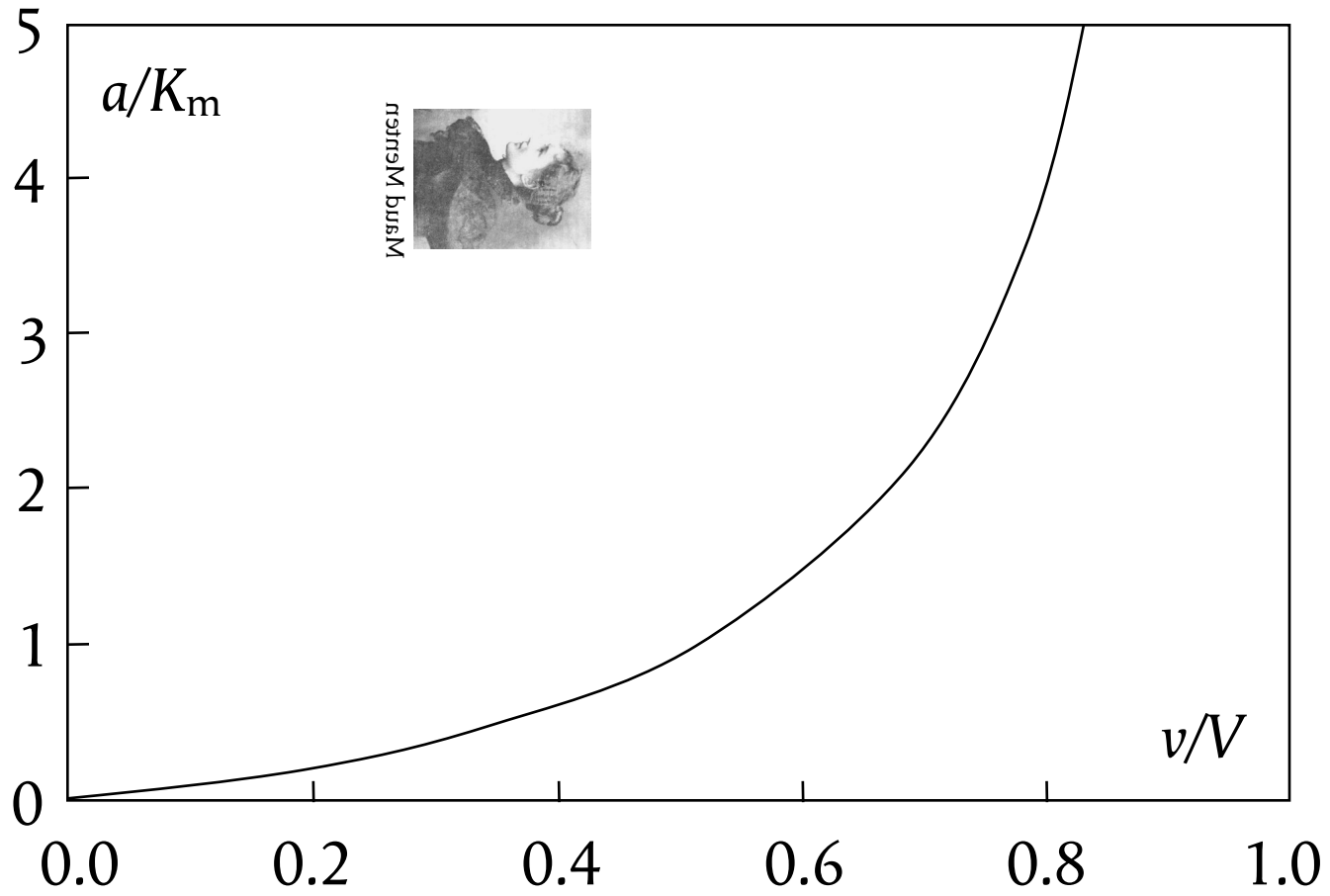

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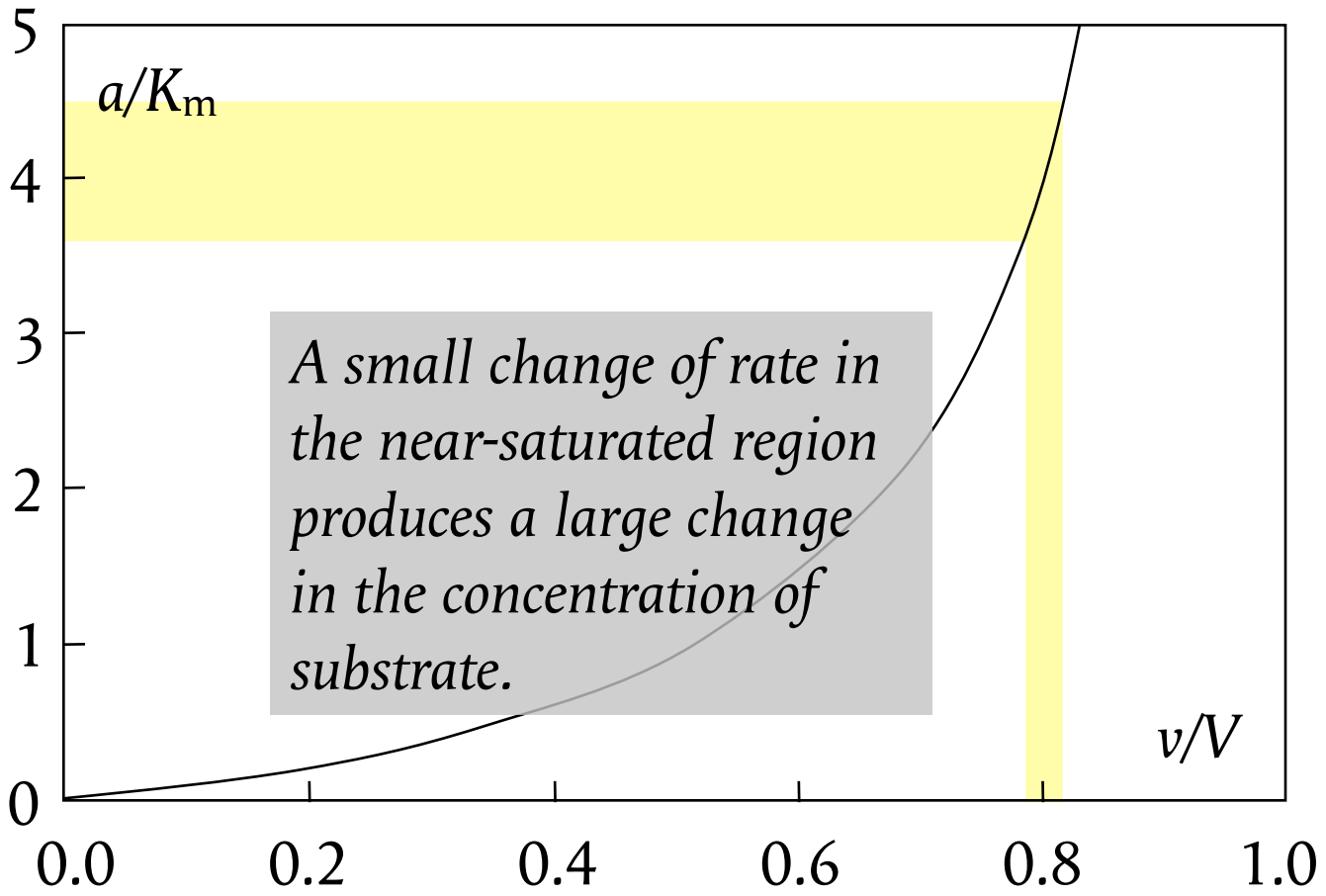

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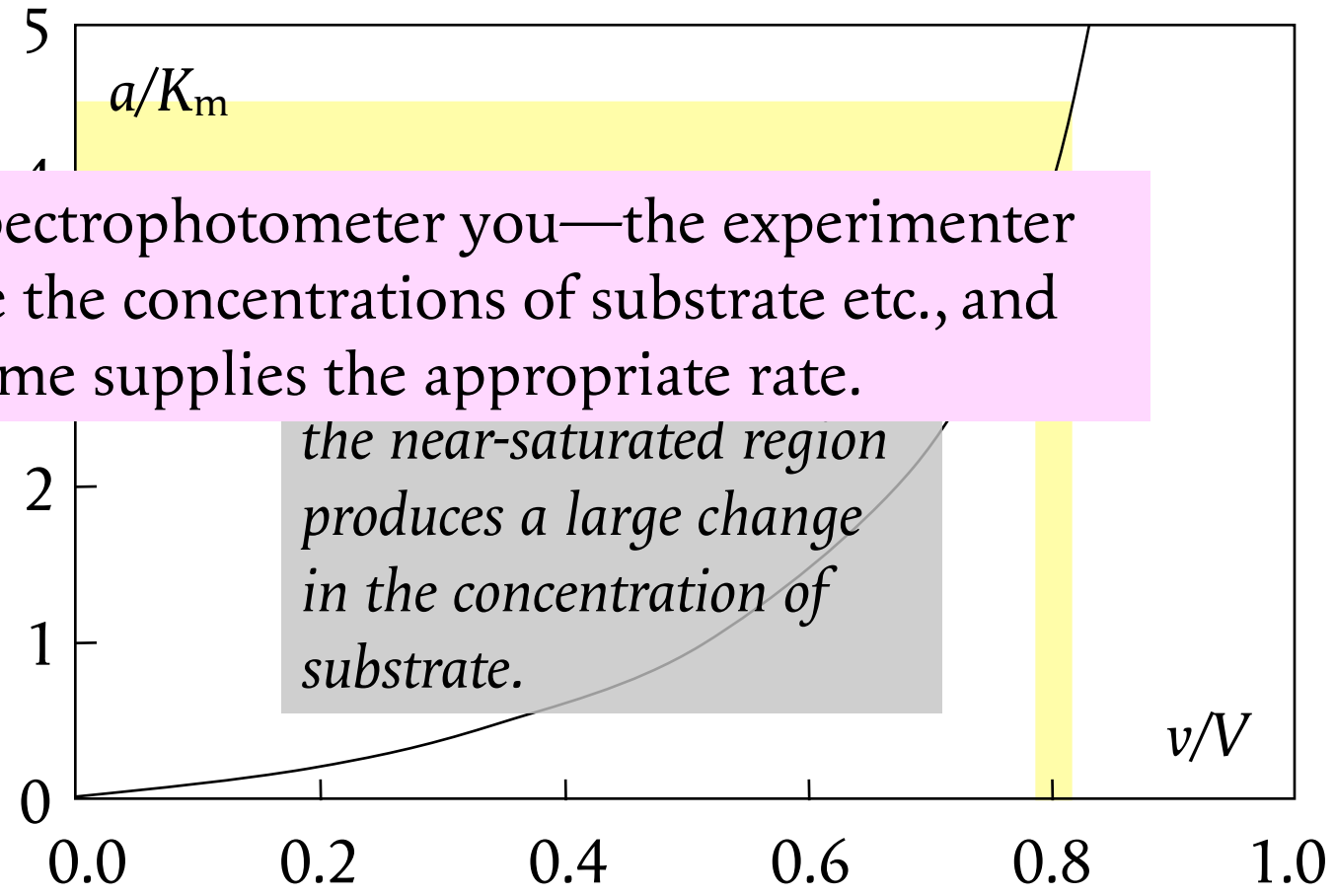

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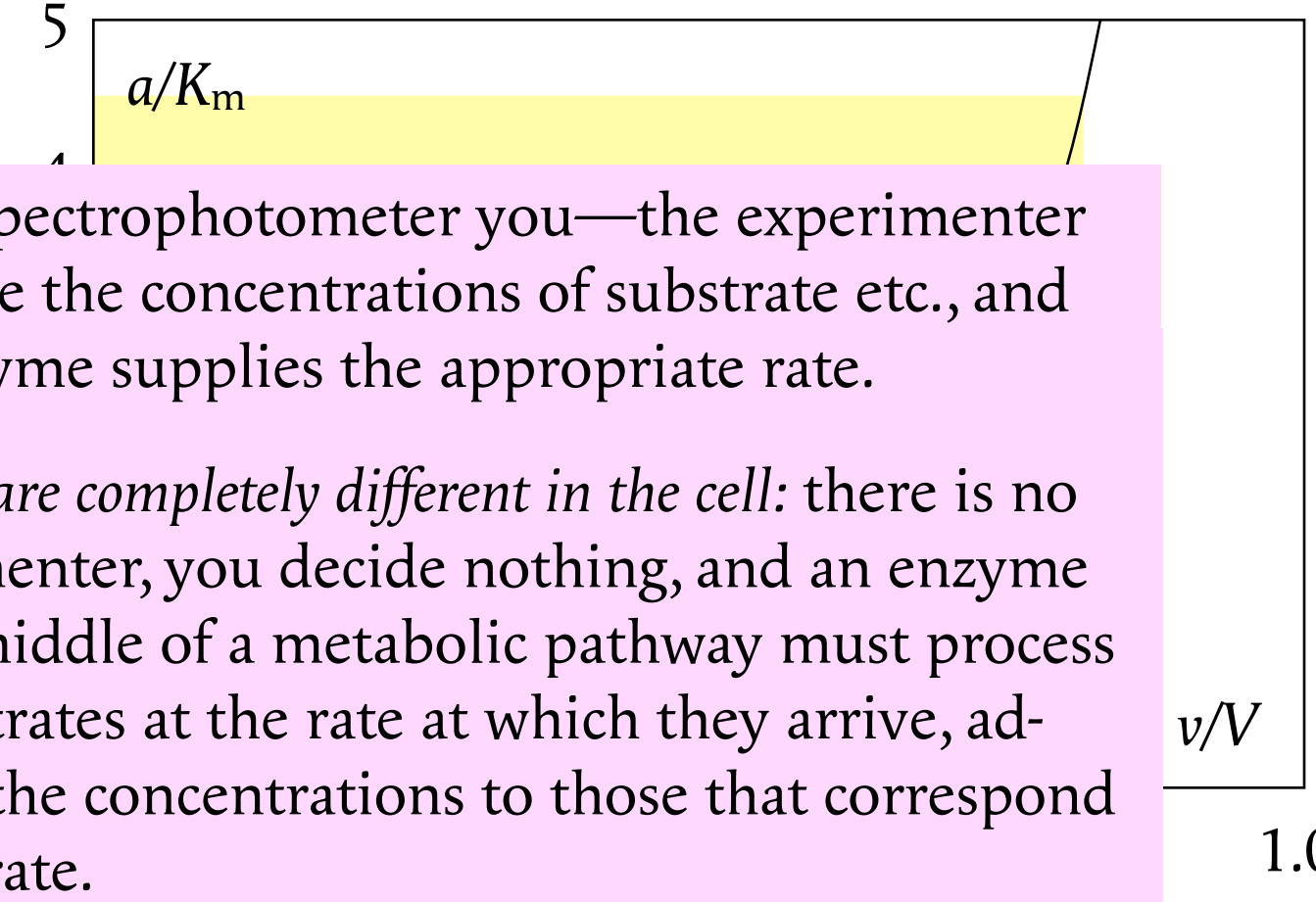

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Matters are completely different in the cell: there is no experimenter, you decide nothing, and an enzyme in the middle of a metabolic pathway must process its substrates at the rate at which they arrive, adjusting the concentrations to those that correspond to that rate.

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Nonetheless, treating the rates as the causes of the concentrations is not further from the truth than the assumption made in elementary kinetics courses that the concentrations determine the rates; in most circumstances it is *closer* to the truth.

When might we expect to see exceptions? Why?

(Understanding this has potentially great importance in drug design)

Control coefficients: how does a variable of the system change, for example the metabolic flux J, when the activity of an enzyme changes?

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(Anonymous) parameter that perturbs the system

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Simpler expression that is not, however, strictly correct, because v_i is not a true parameter of the system.

Control coefficients: how does a variable of the

In the early years of control analysis (1973–1989) the definition was often written in terms of the *concentration of the enzyme*: this remains acceptable as long as one does not forget that the real definition is more general.

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Concentration control coefficient

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Why 3.5?

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We shall come back to this example later

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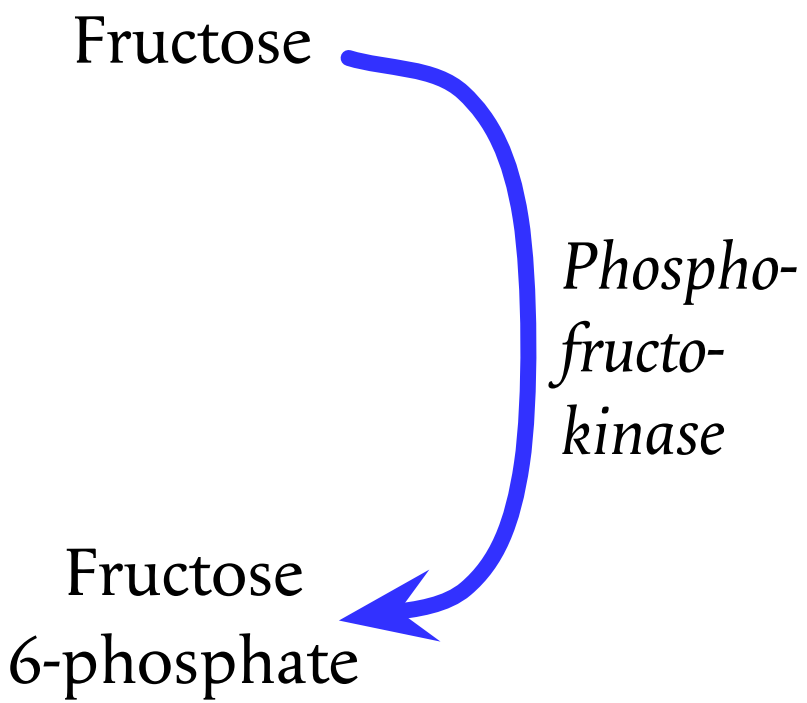
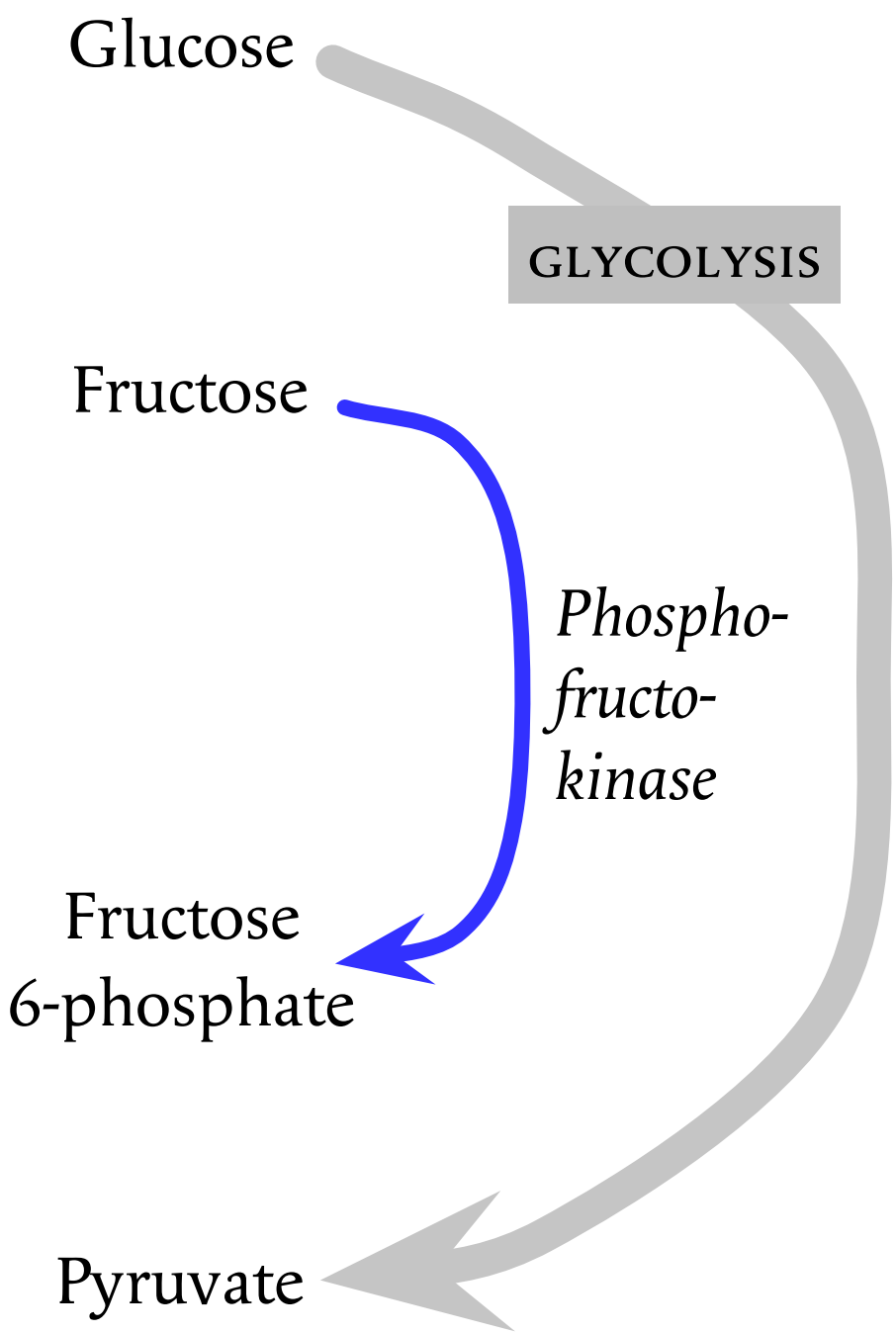
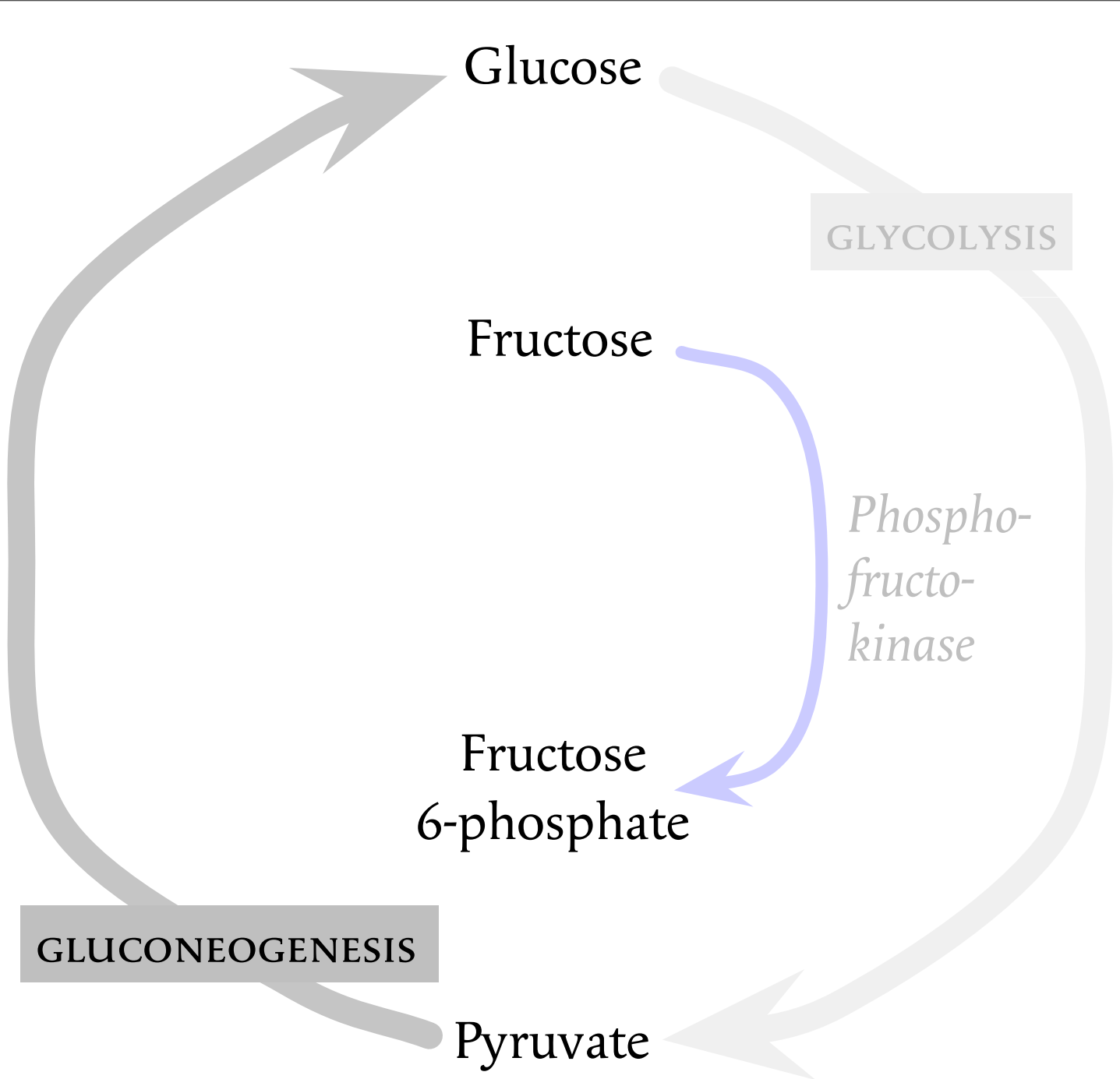
We can illustrate the difference with an example…

Fructose







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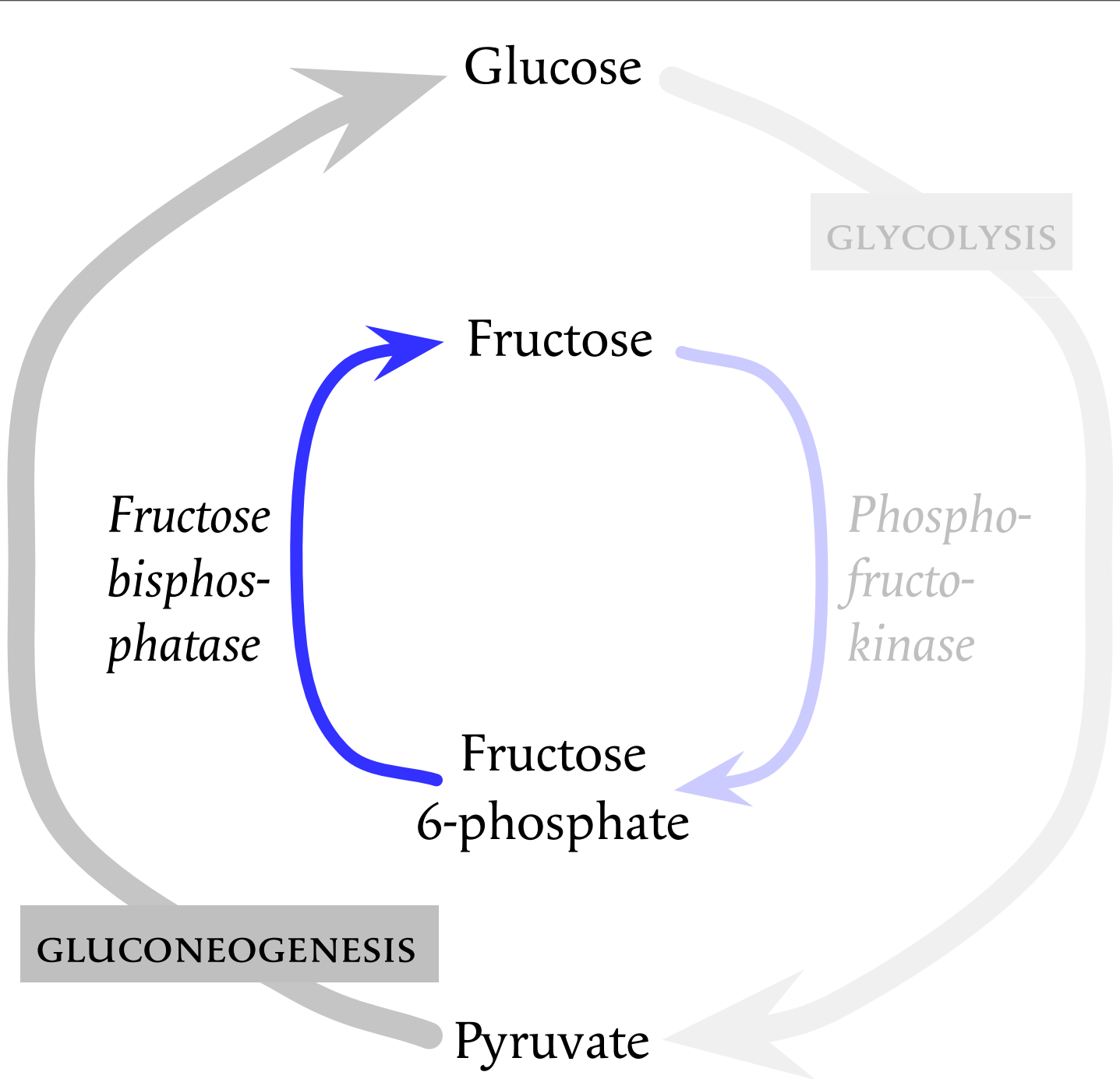

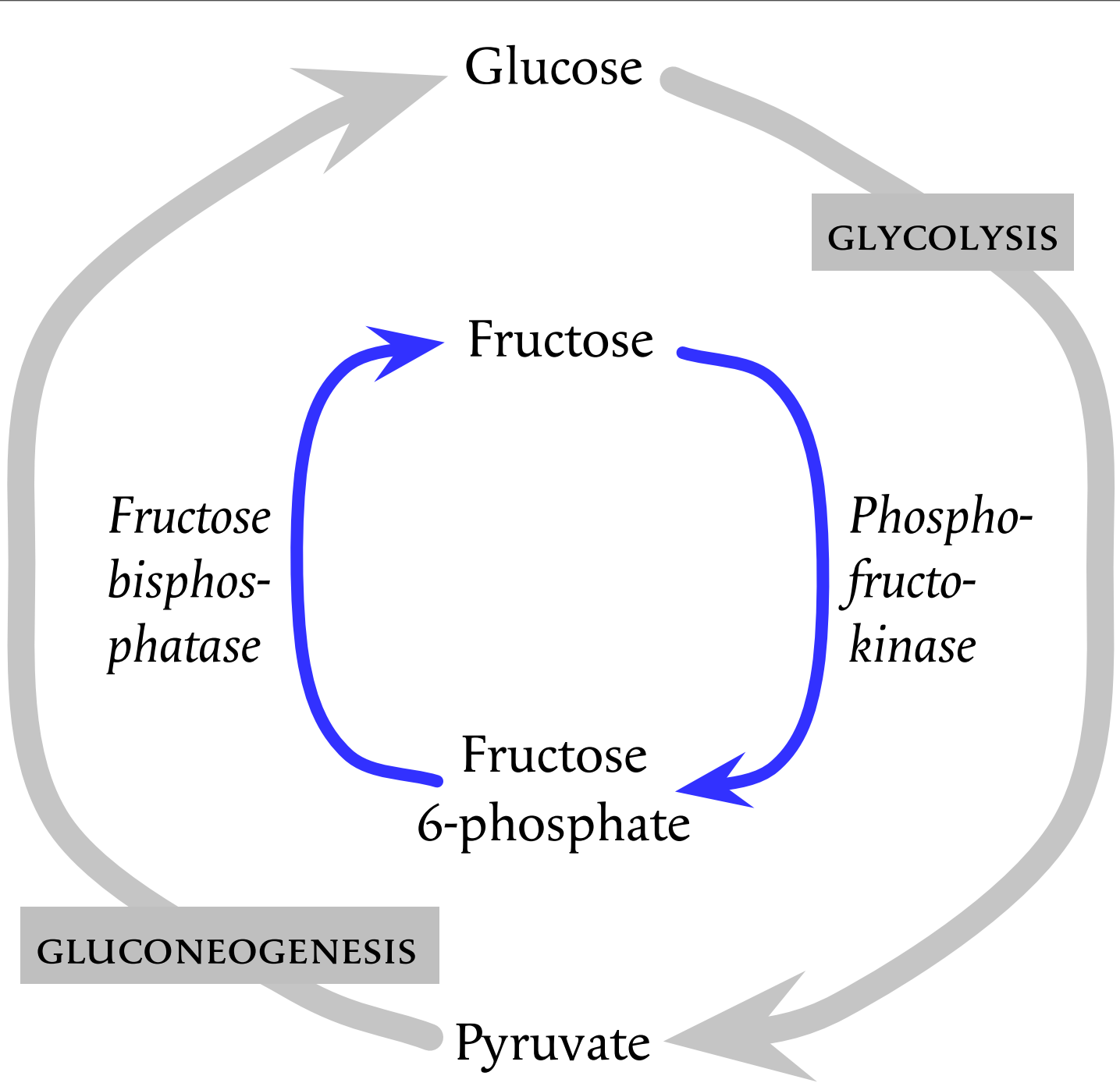

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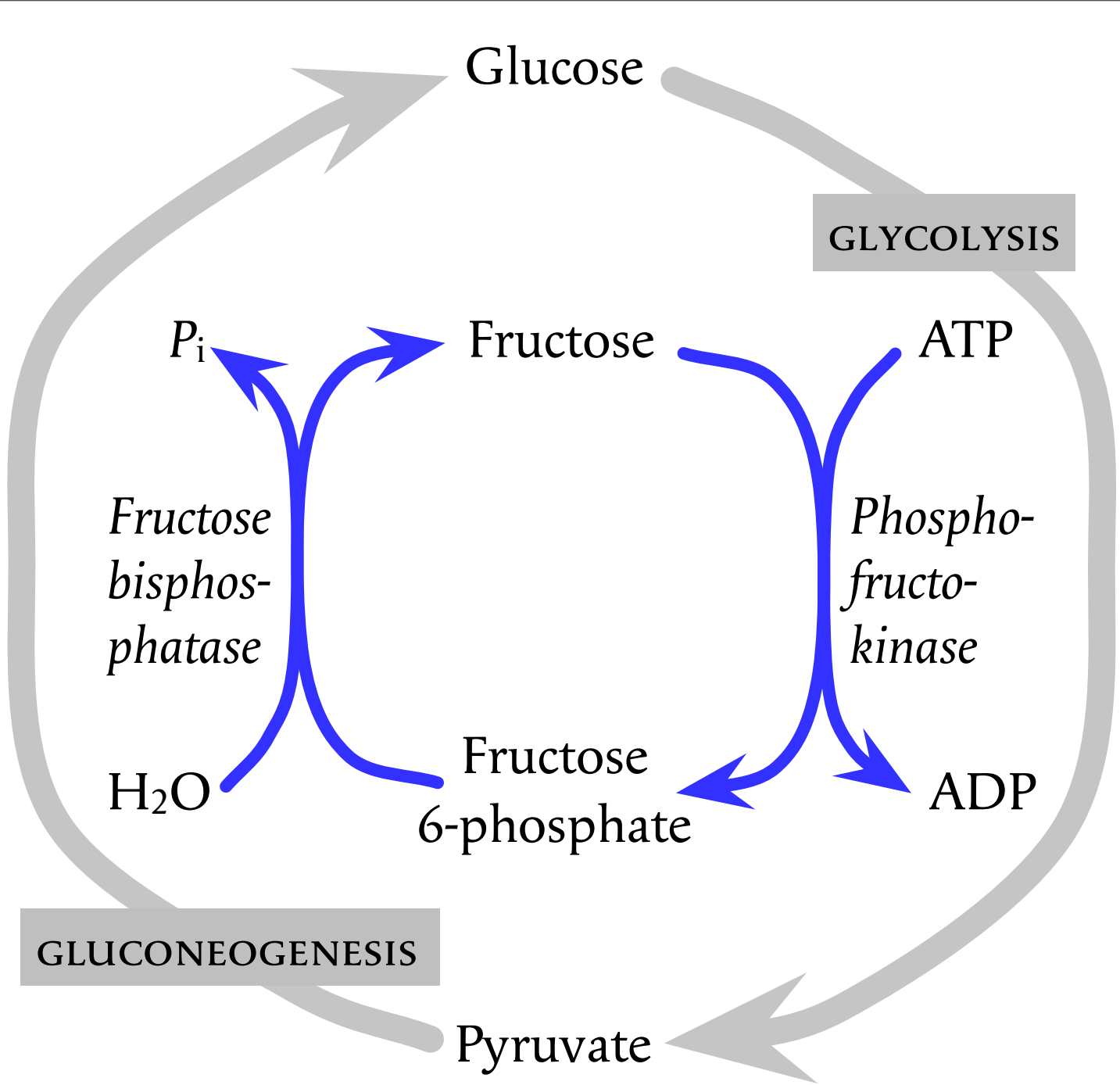

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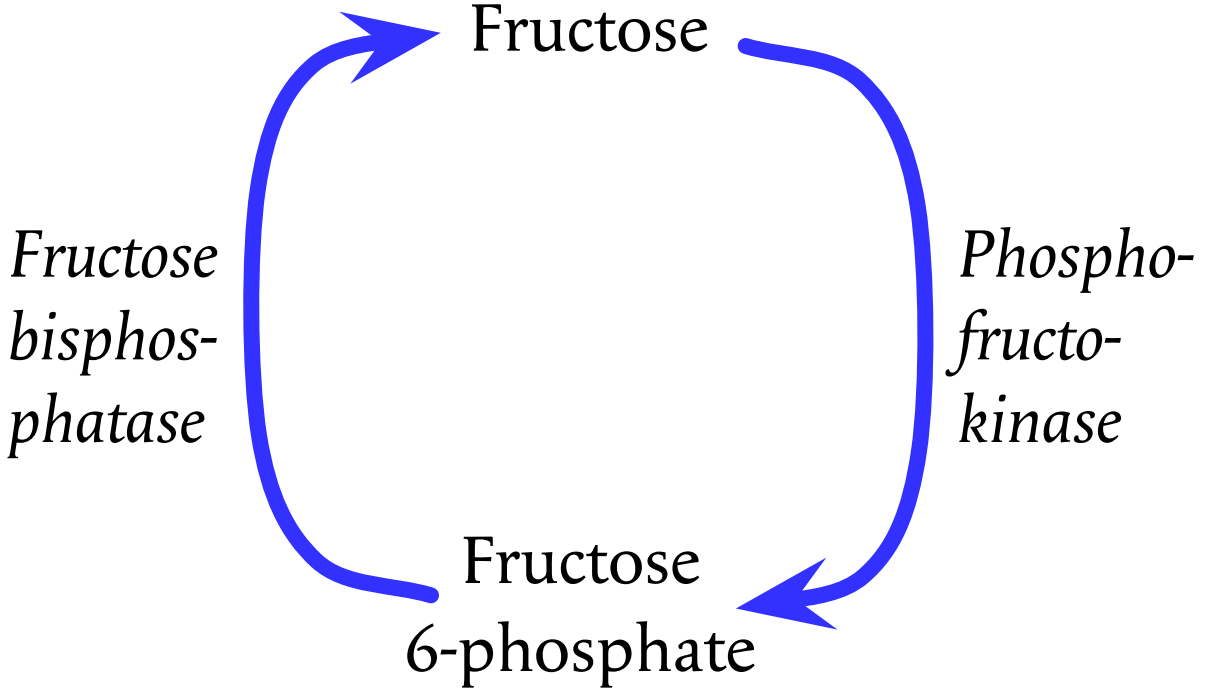

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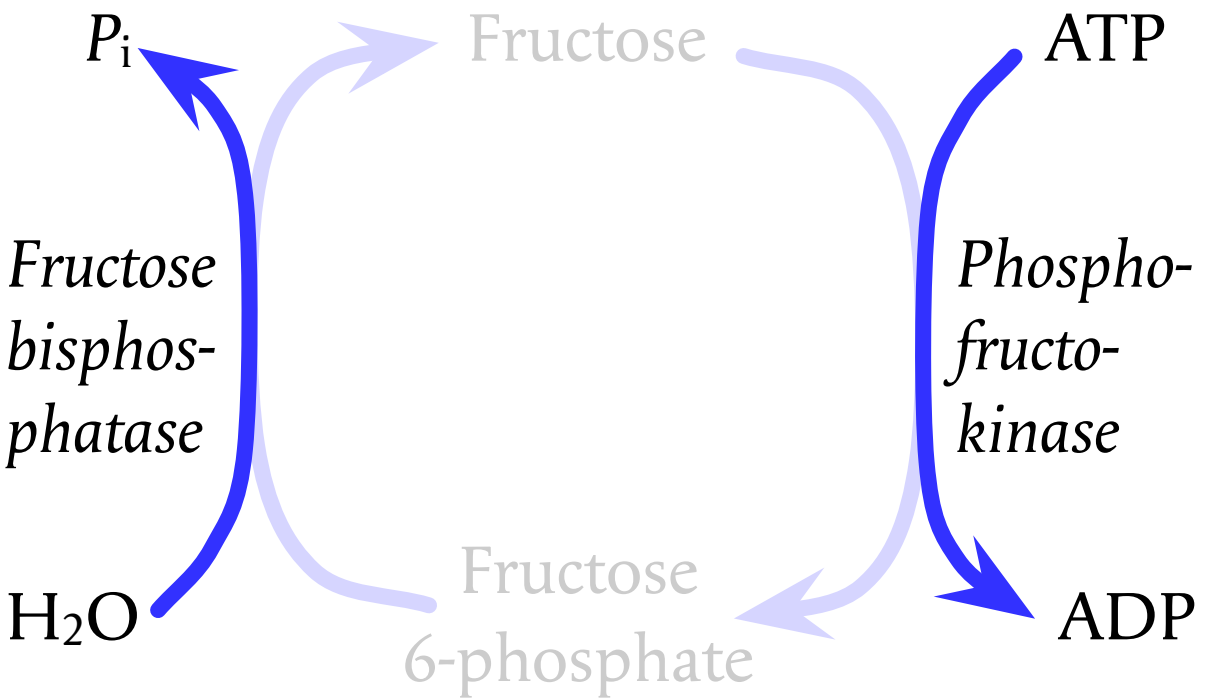

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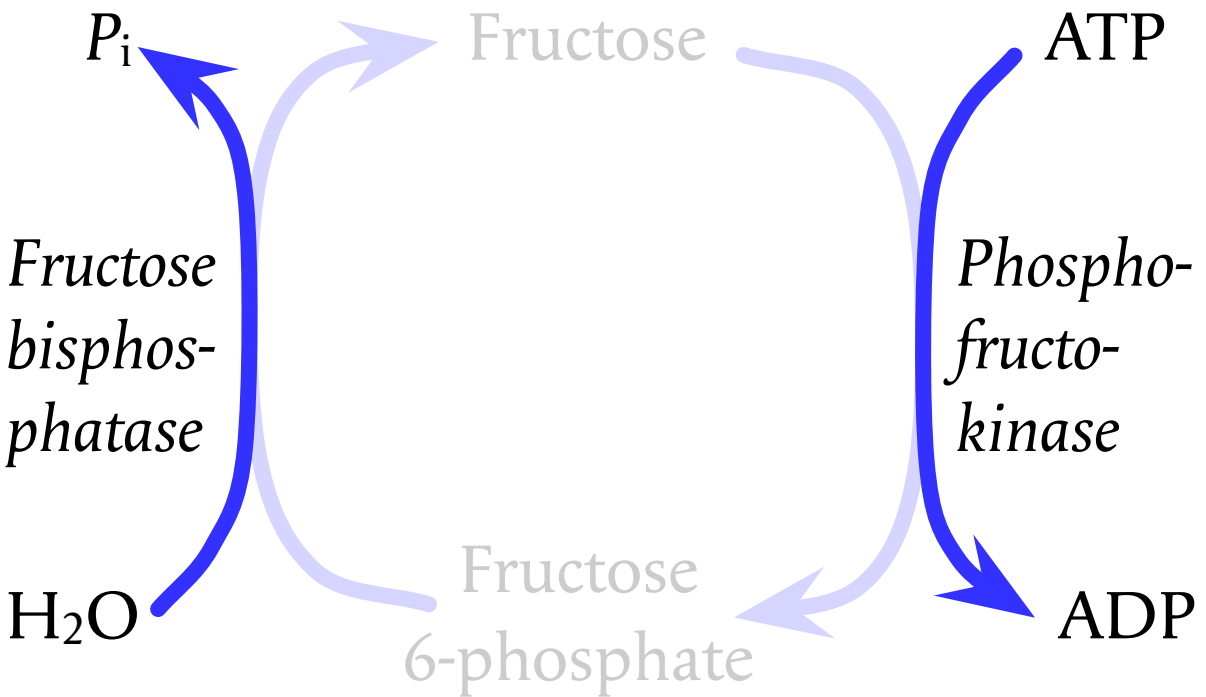

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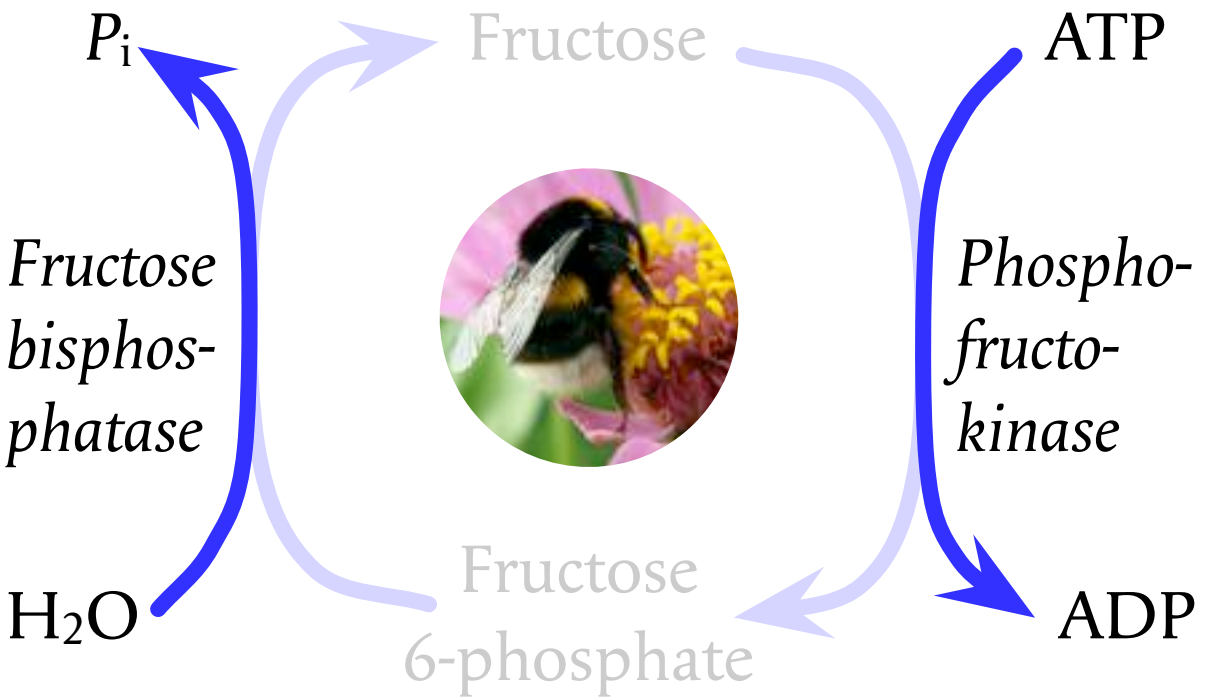

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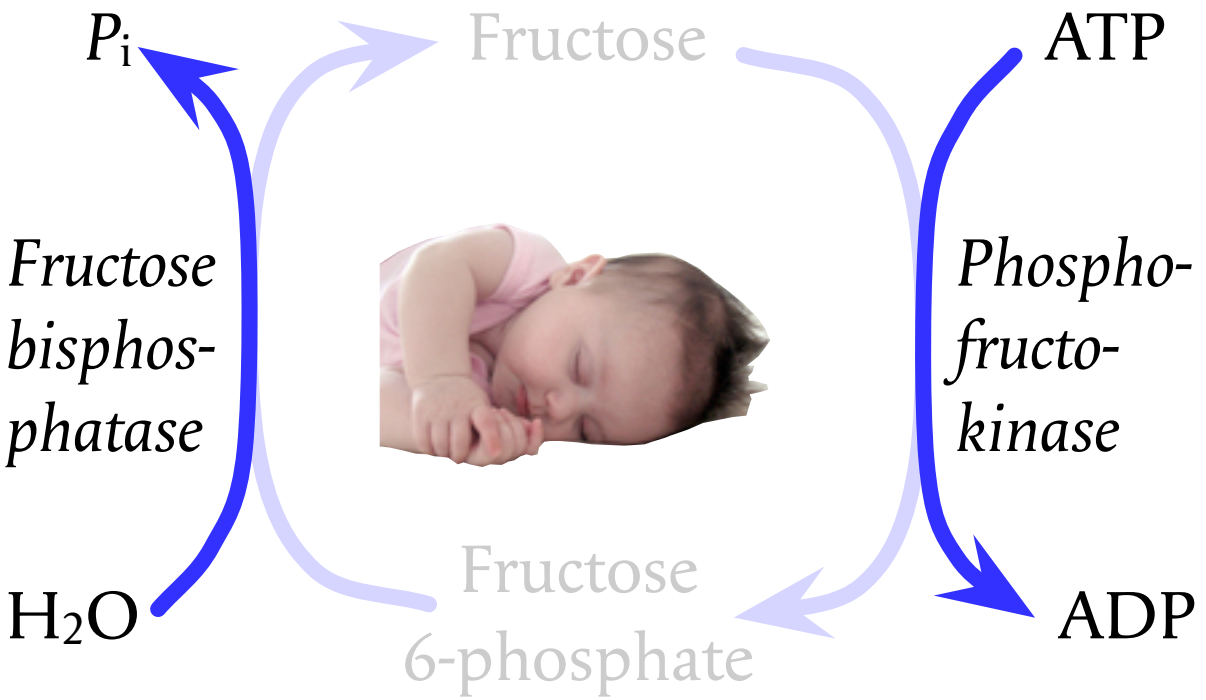

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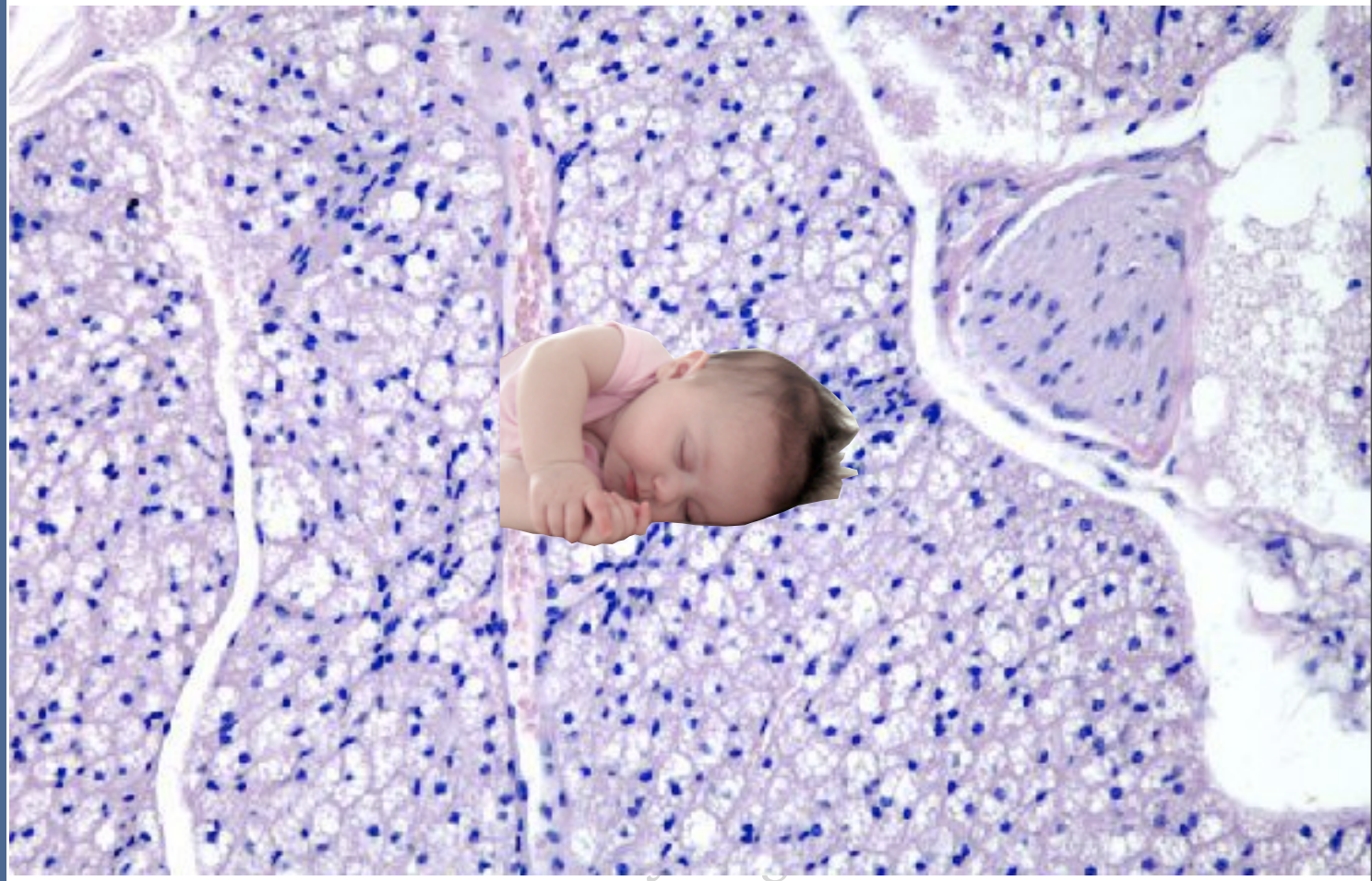

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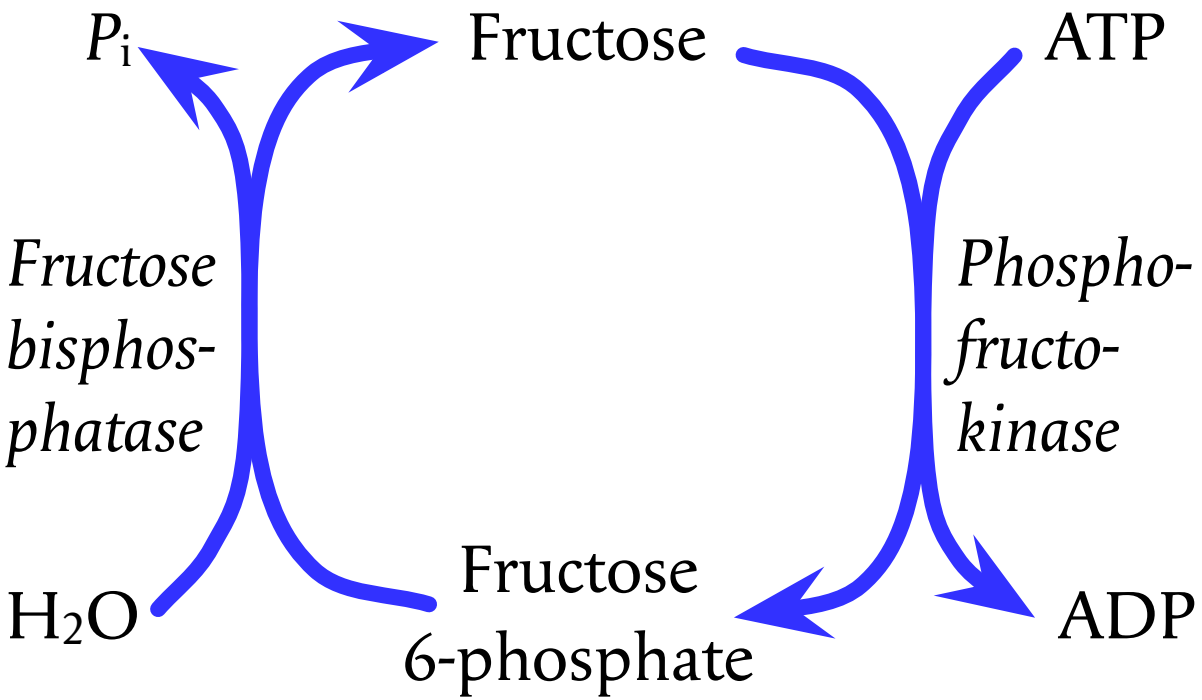
There are several regulatory mechanisms that act in opposite directions on the two enzymes



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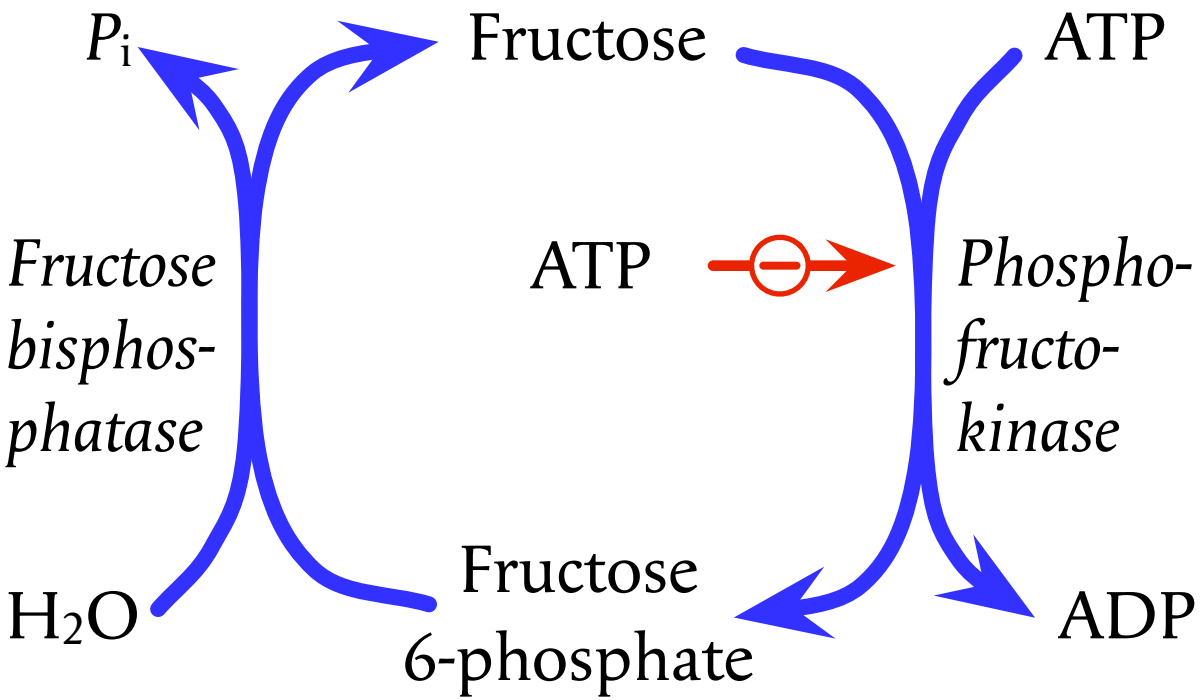

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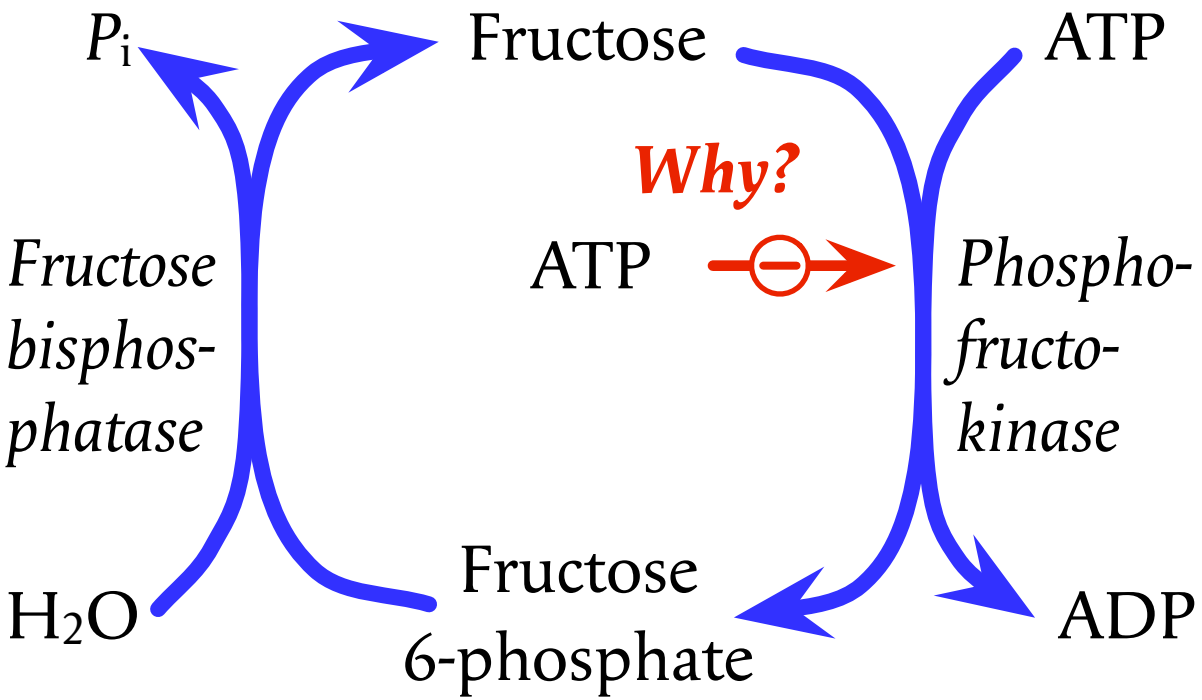

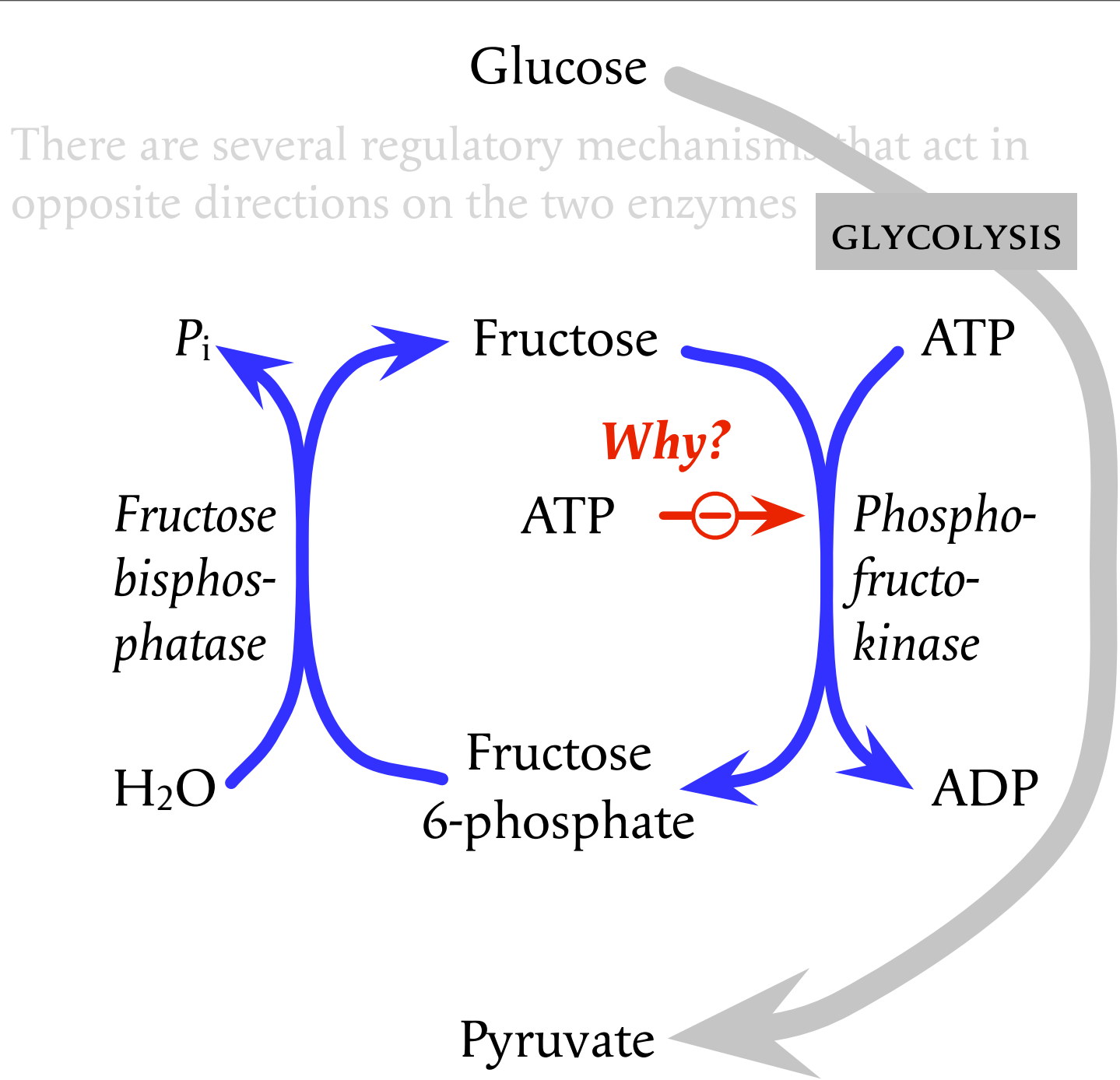

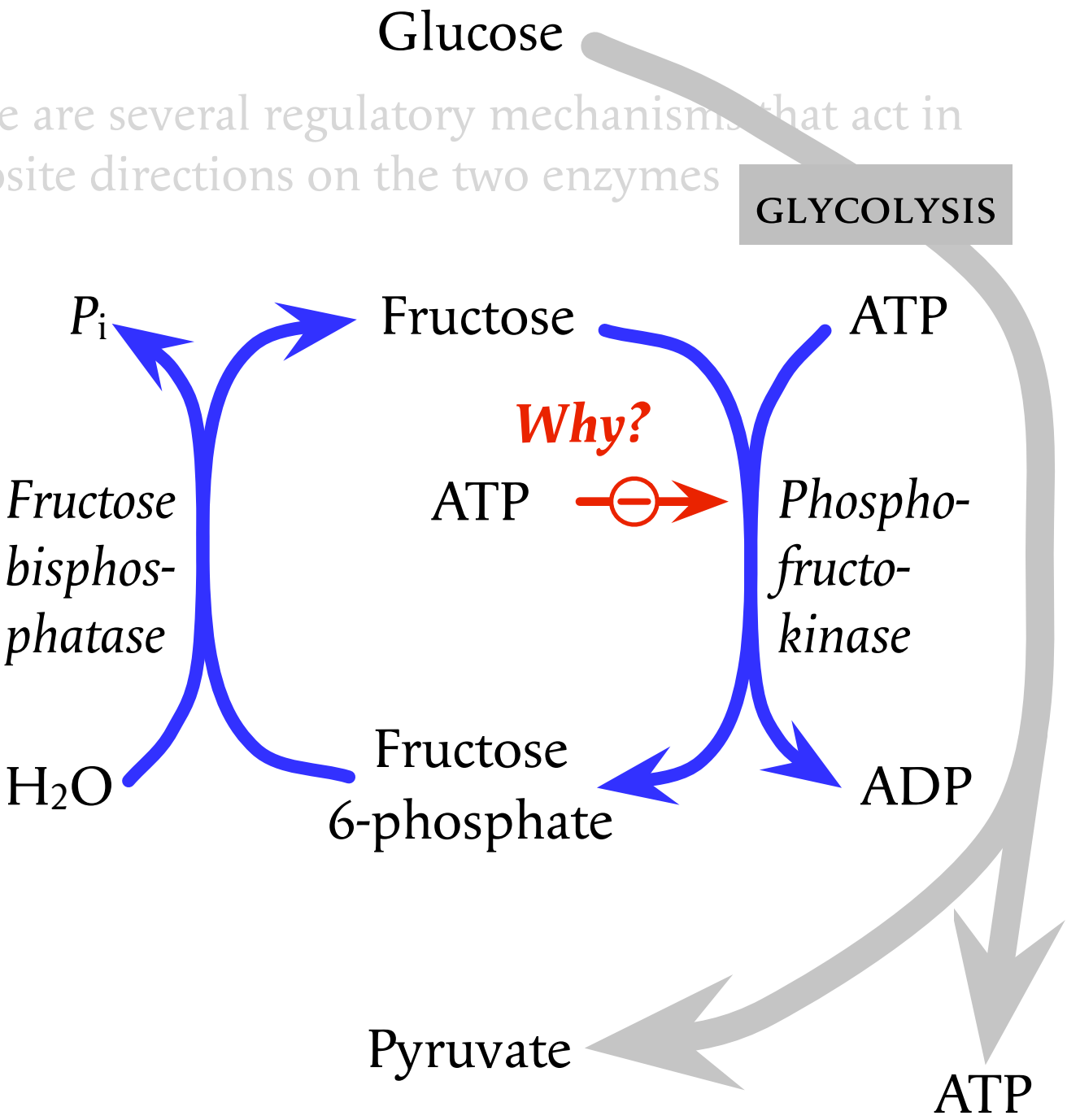

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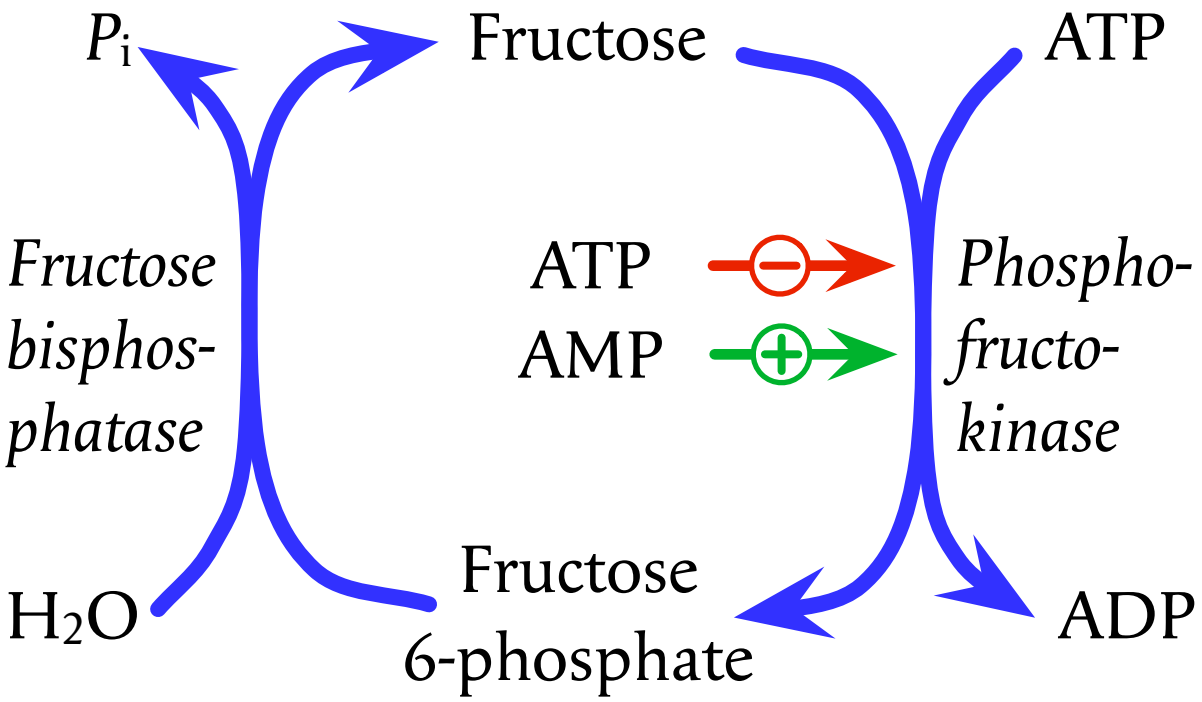

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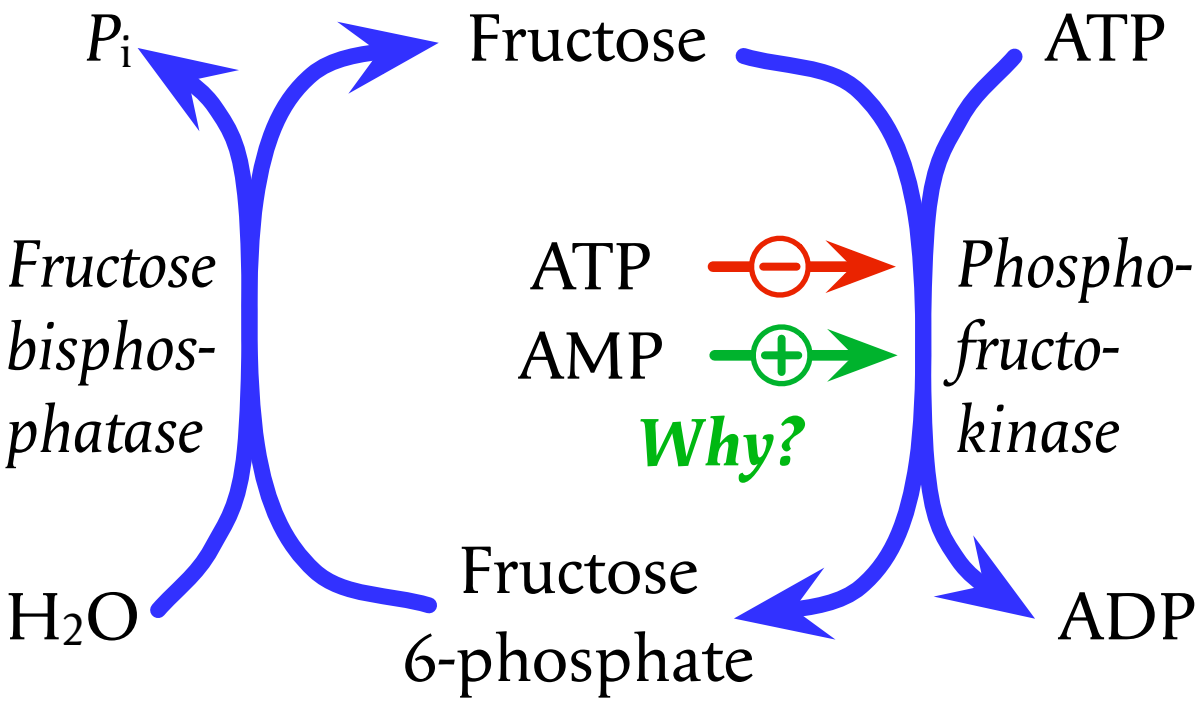

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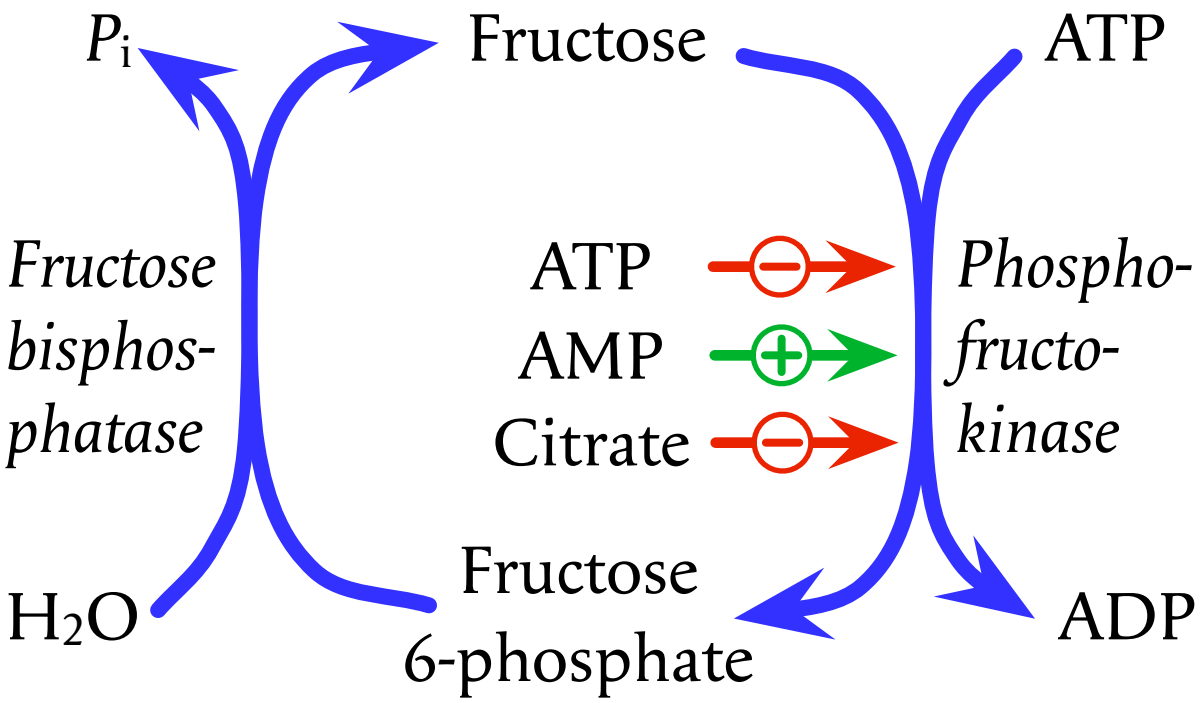

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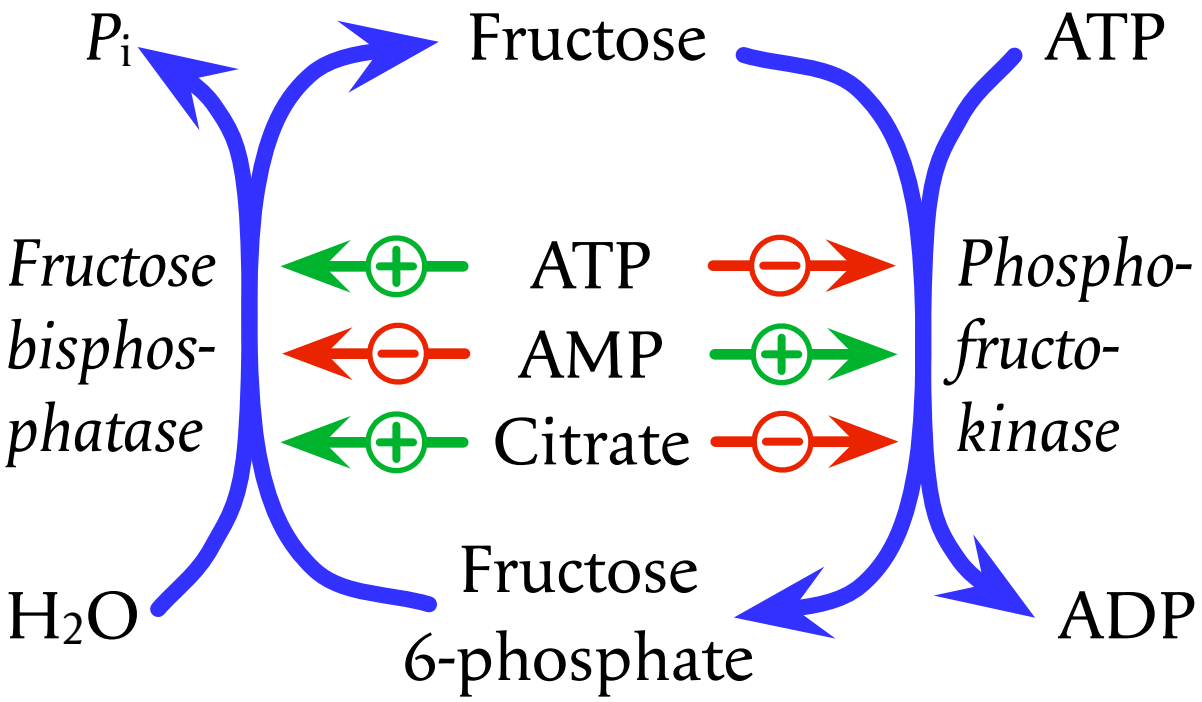

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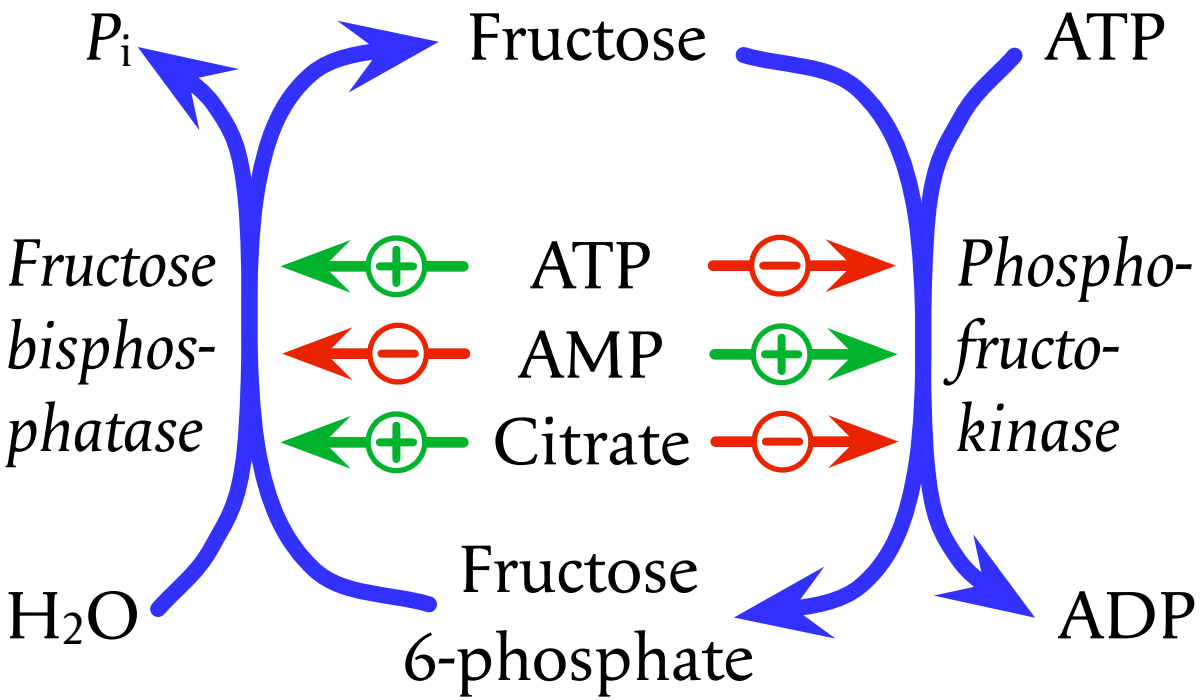
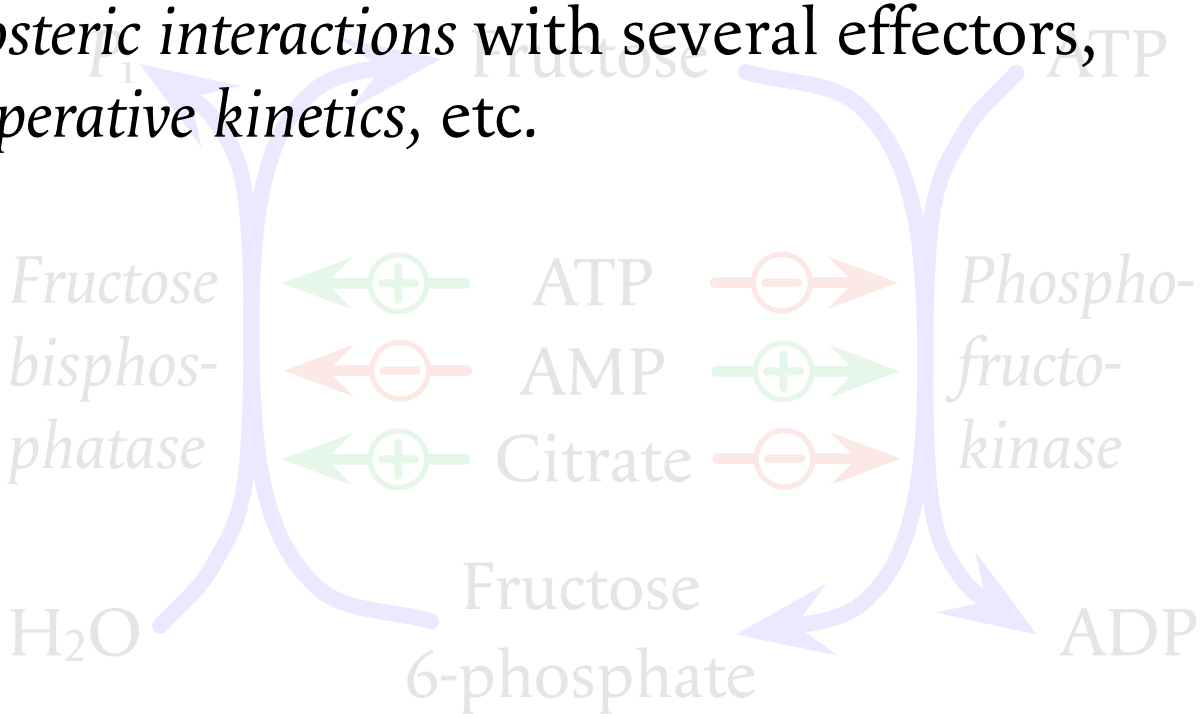


Why are there so many different effects?

Thus phosphofructokinase and fructose 1,6-bisphosphatase are *regulatory enzymes*, with *allosteric interactions* with several effectors, *cooperative kinetics*, etc.



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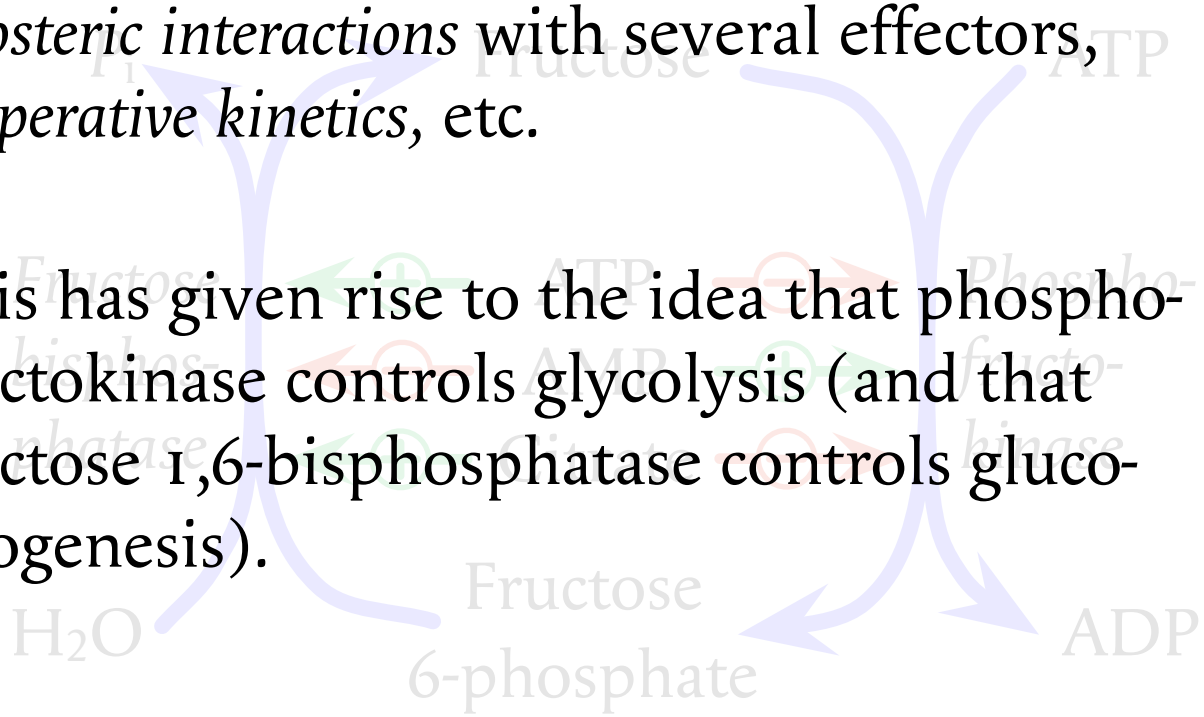
This has given rise to the idea that phosphofructokinase controls glycolysis (and that fructose 1,6-bisphosphatase controls gluconeogenesis).



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We shall see if this is really true…

Summation property: the fundamental property in the study of flux control.

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If you remember nothing else from this lecture, try to remember this! (That's why this equation appears on the sheet)

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What does this equation mean?

It means that flux control is *shared* between all the enzymes of the system, and given that a typical system contains many enzymes the average coefficient is very *small*.

A similar relationship applies to concentration control coefficients, except now the sum is zero:

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These two equations are fundamental: how can we show that they are correct?

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partial derivative of J with respect to e_1 (etc.)

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The vital point is to know the result:

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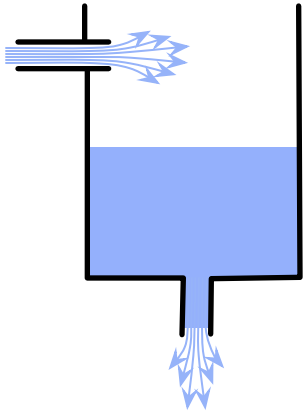

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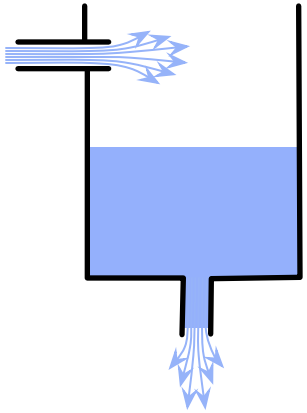

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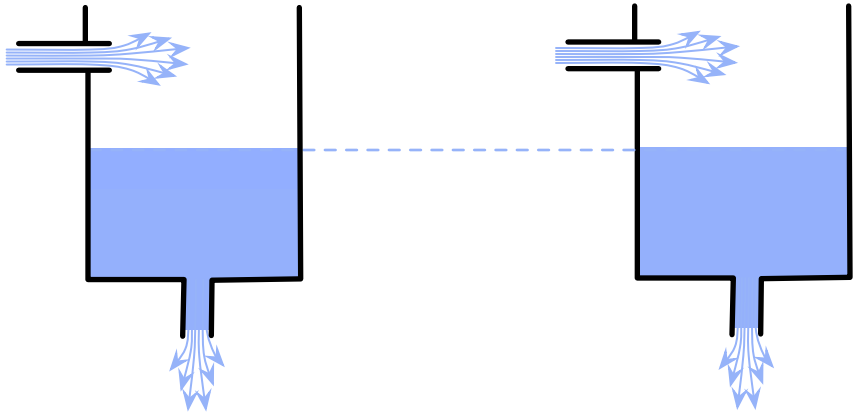

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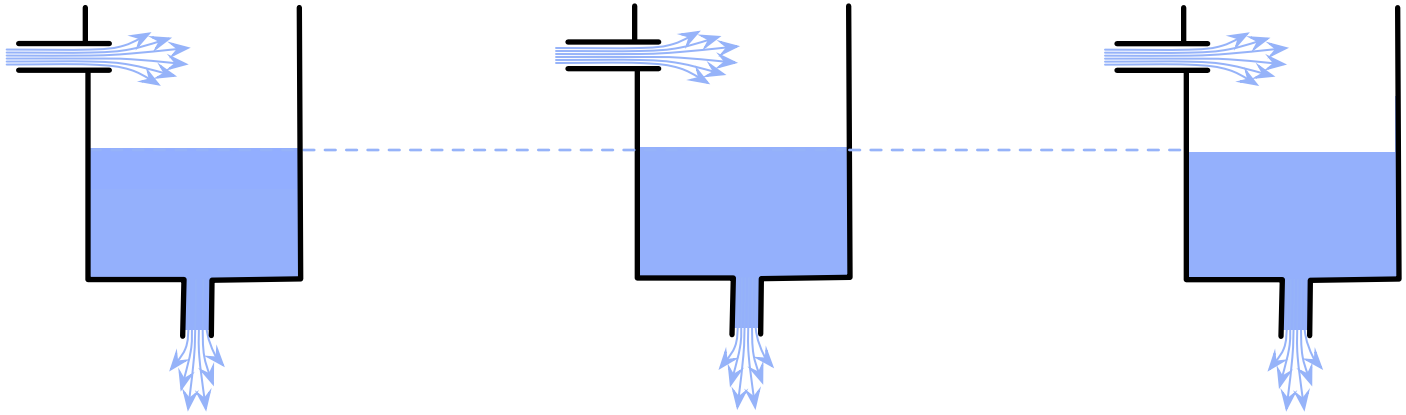

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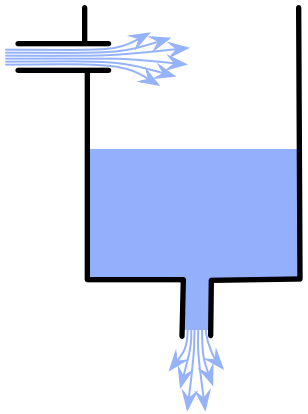
When the water level is too high the exit flow is greater than the entry flow, up to the moment when the steady state is reached.

A more intuitive way of arriving at the same result…



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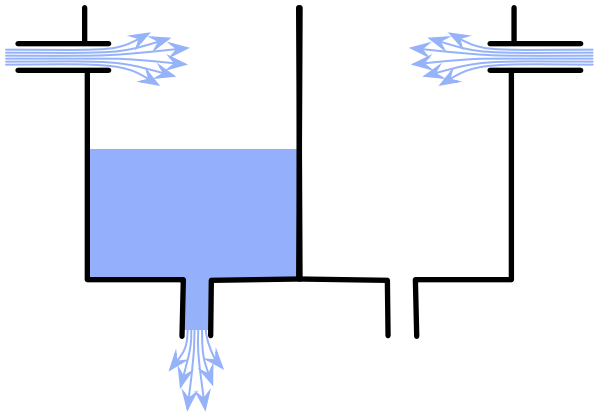

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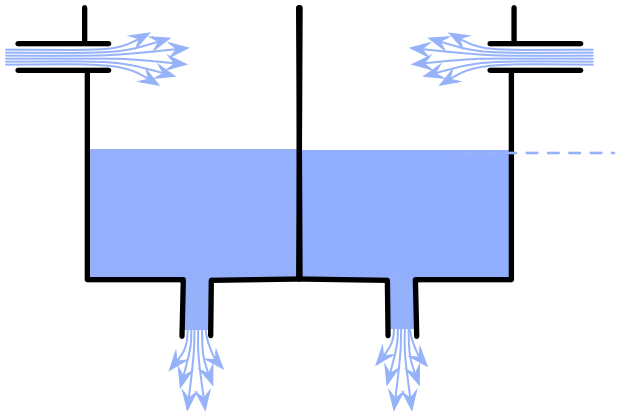
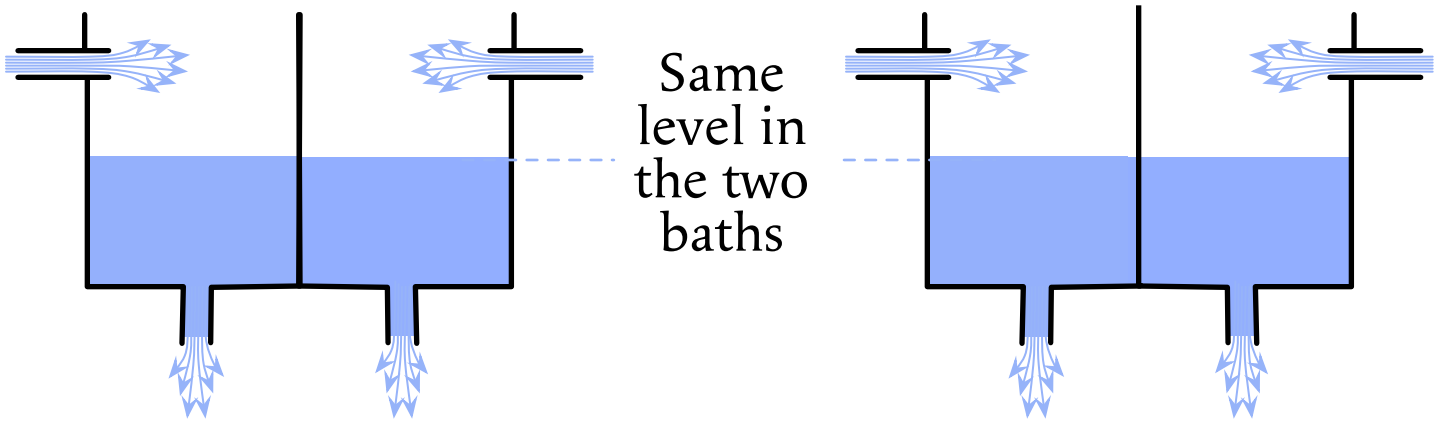


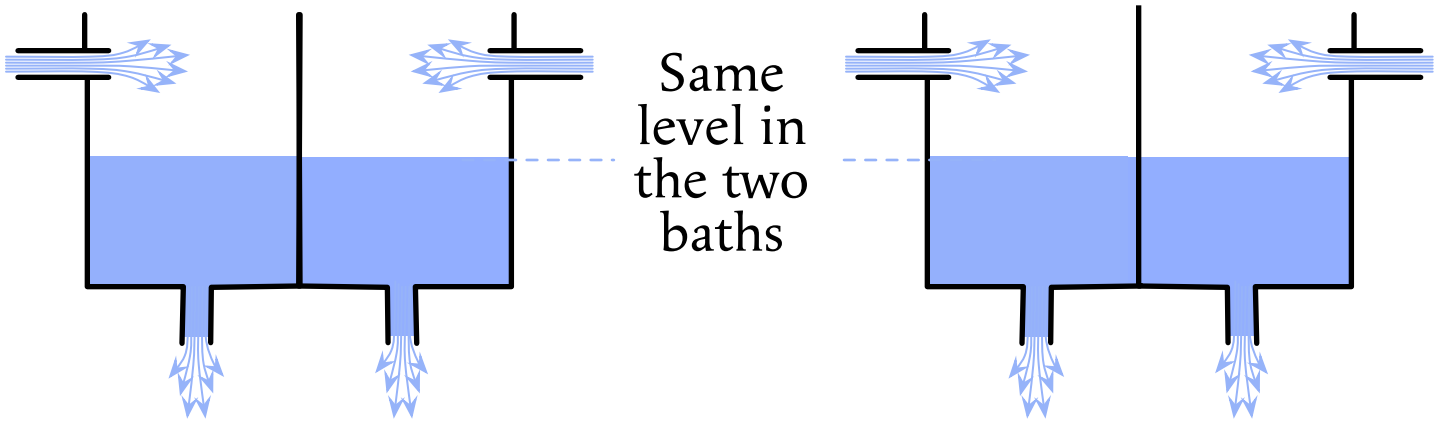
Same level in the two baths

A more intuitive way of arriving at the same result…



A more intuitive way of arriving at the same result...

What happens if we remove the partition?



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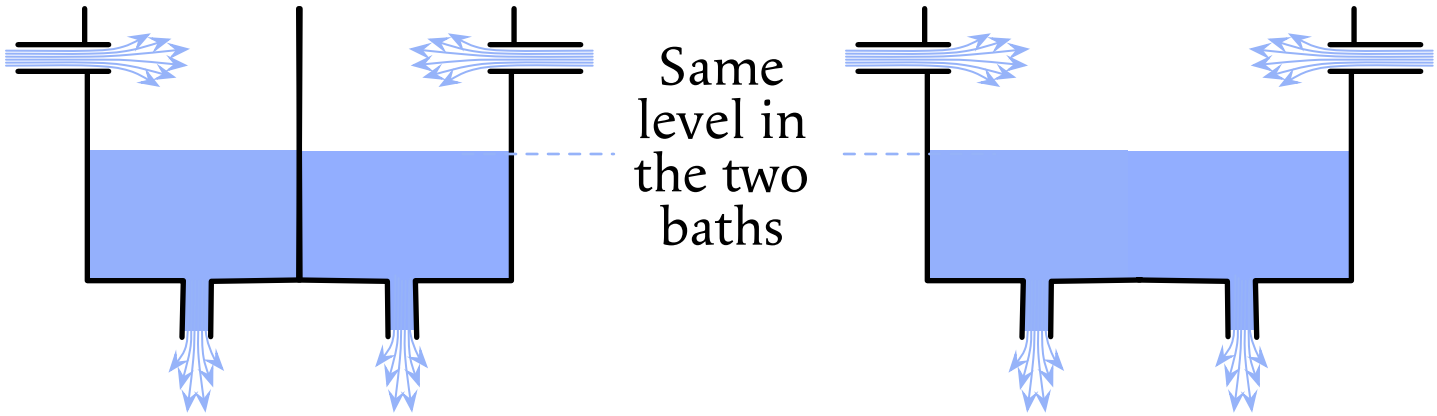

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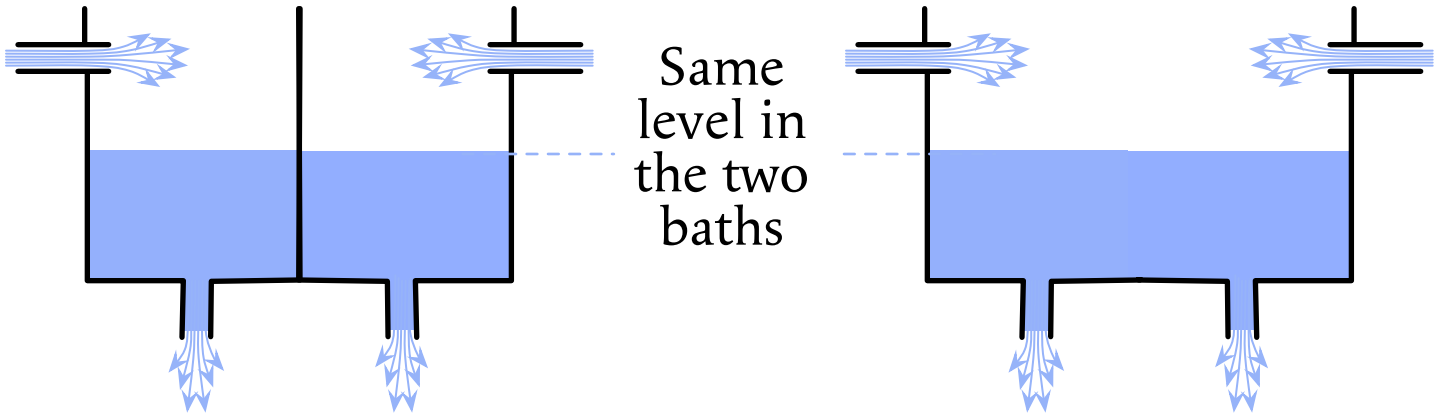

Double the capacity of the pipes (*double the activity of every enzyme in the system*): double the flow of water (*double the flux of metabolites*)

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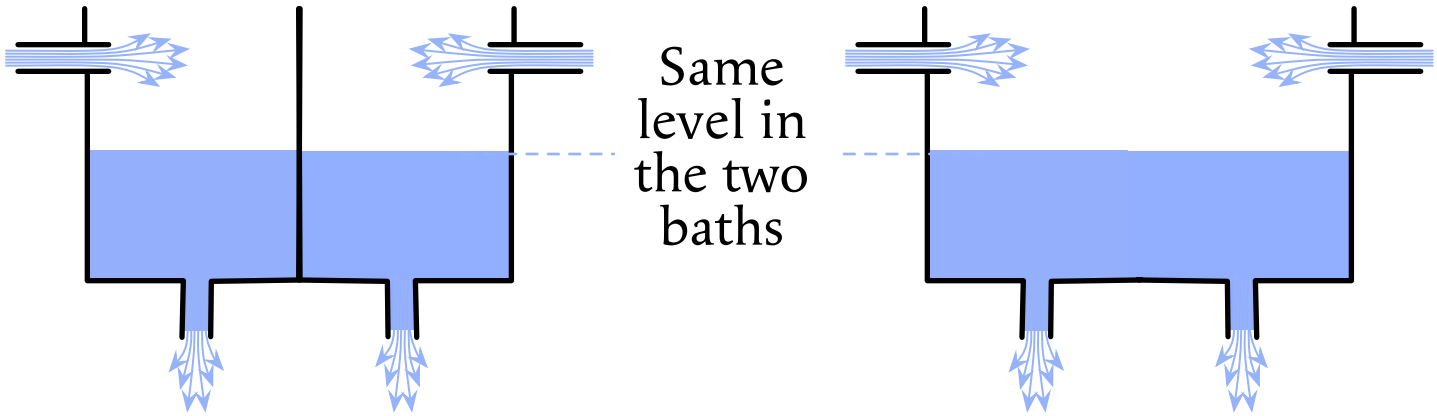

Double the capacity of the pipes (*double the activity of every enzyme in the system*): double the flow of water (*double the flux of metabolites*)

And if we did the experiment adding an extra bath to a set of 100 identical baths?

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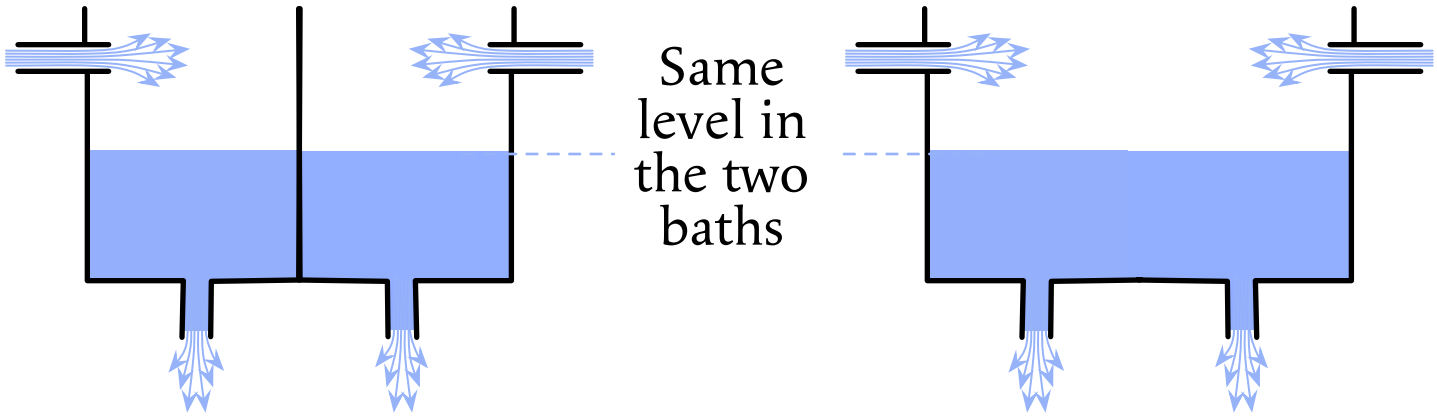

Double the capacity of the pipes (*double the activity of every enzyme in the system*): double the flow of water (*double the flux of metabolites*)

And if we did the experiment adding an extra bath to a set of 100 identical baths?

1% more capacity of the pipes (*1% more activity of every enzyme in the system*): 1% greater flow of water (*1% greater flux of metabolites*)

Yet another way of arriving at the same result…

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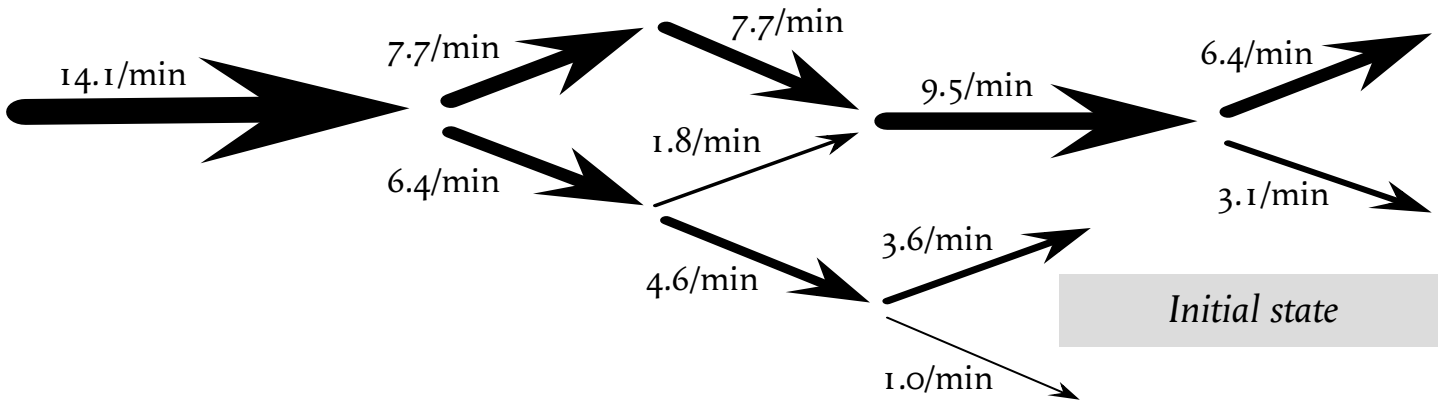

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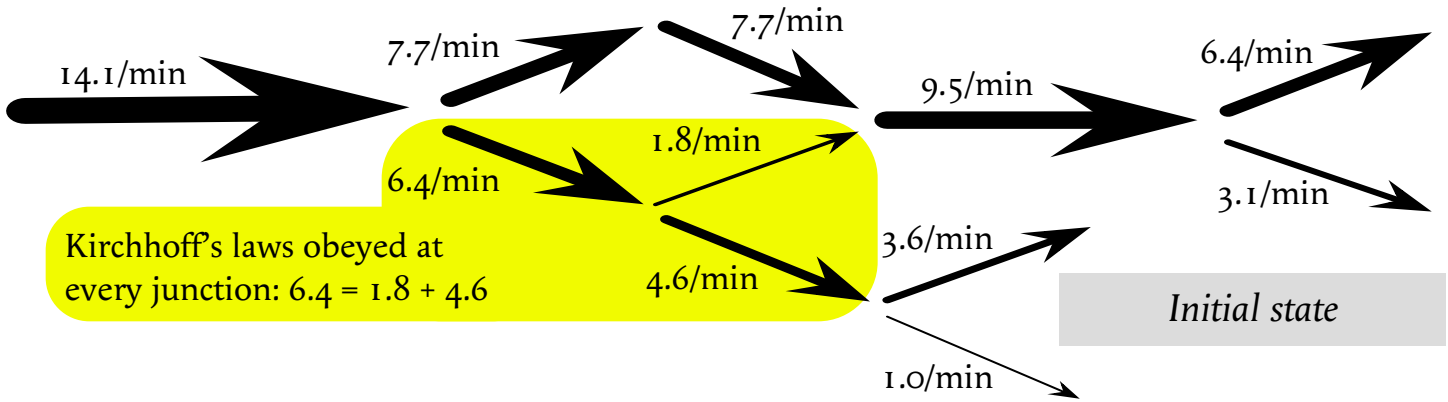

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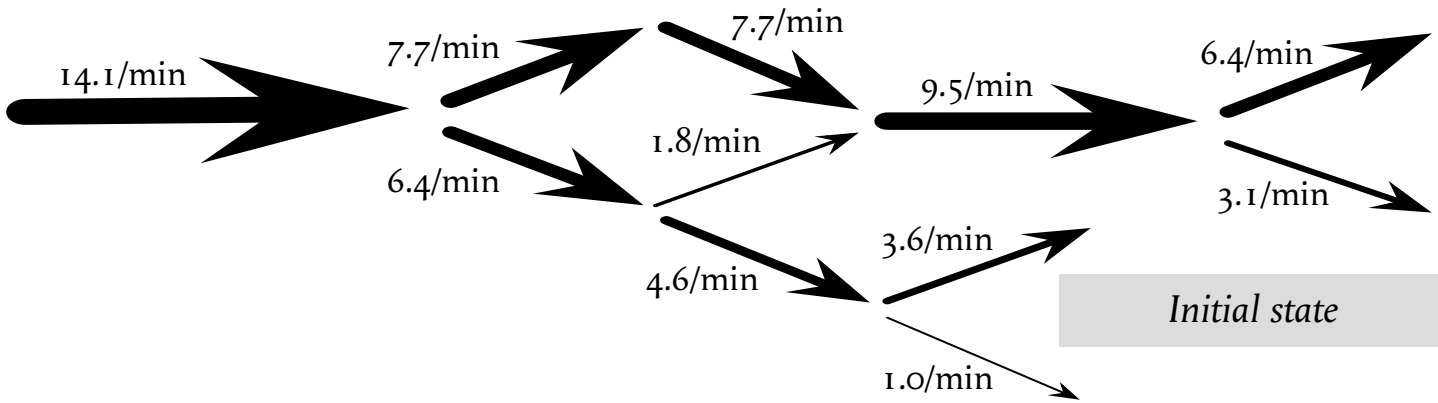

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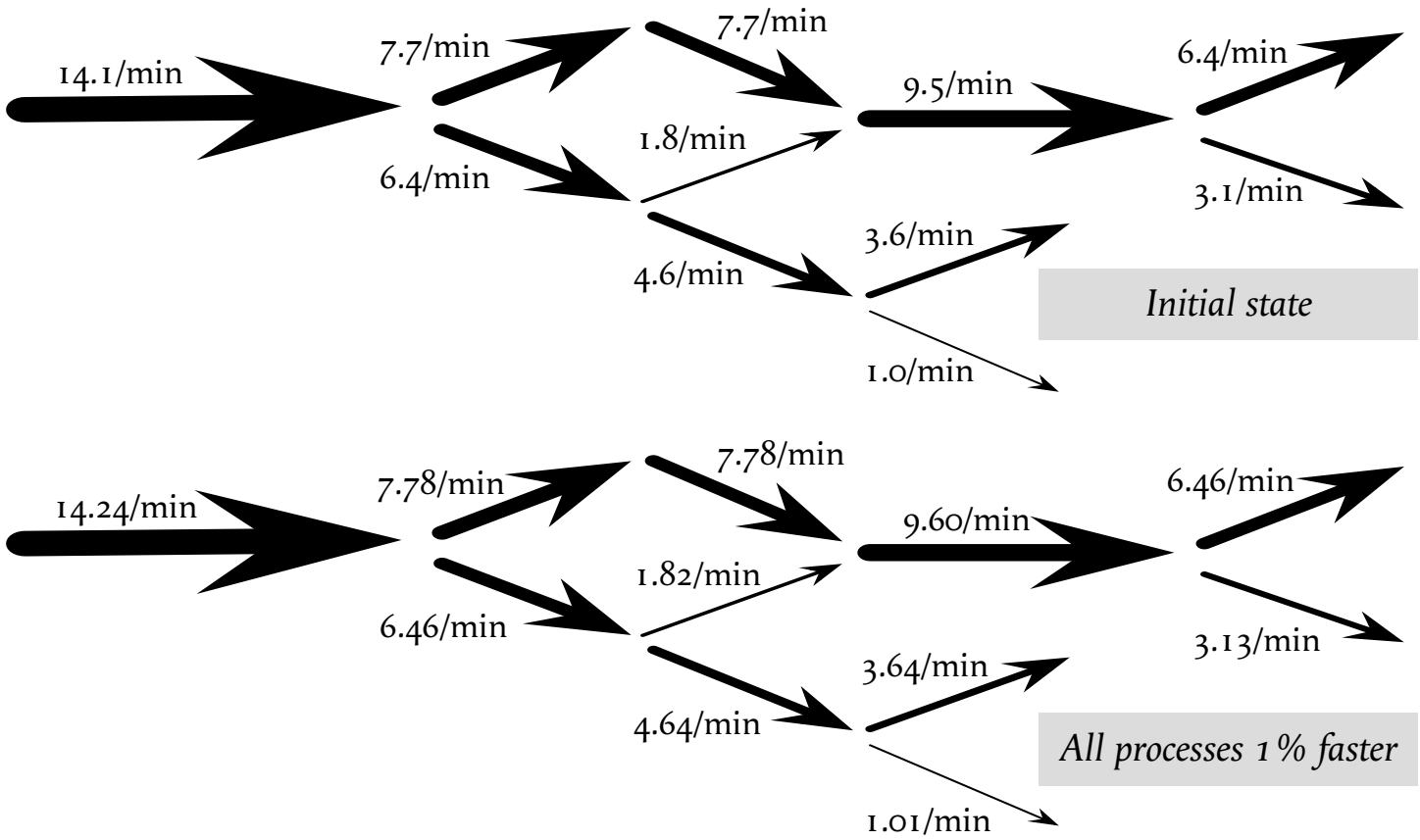

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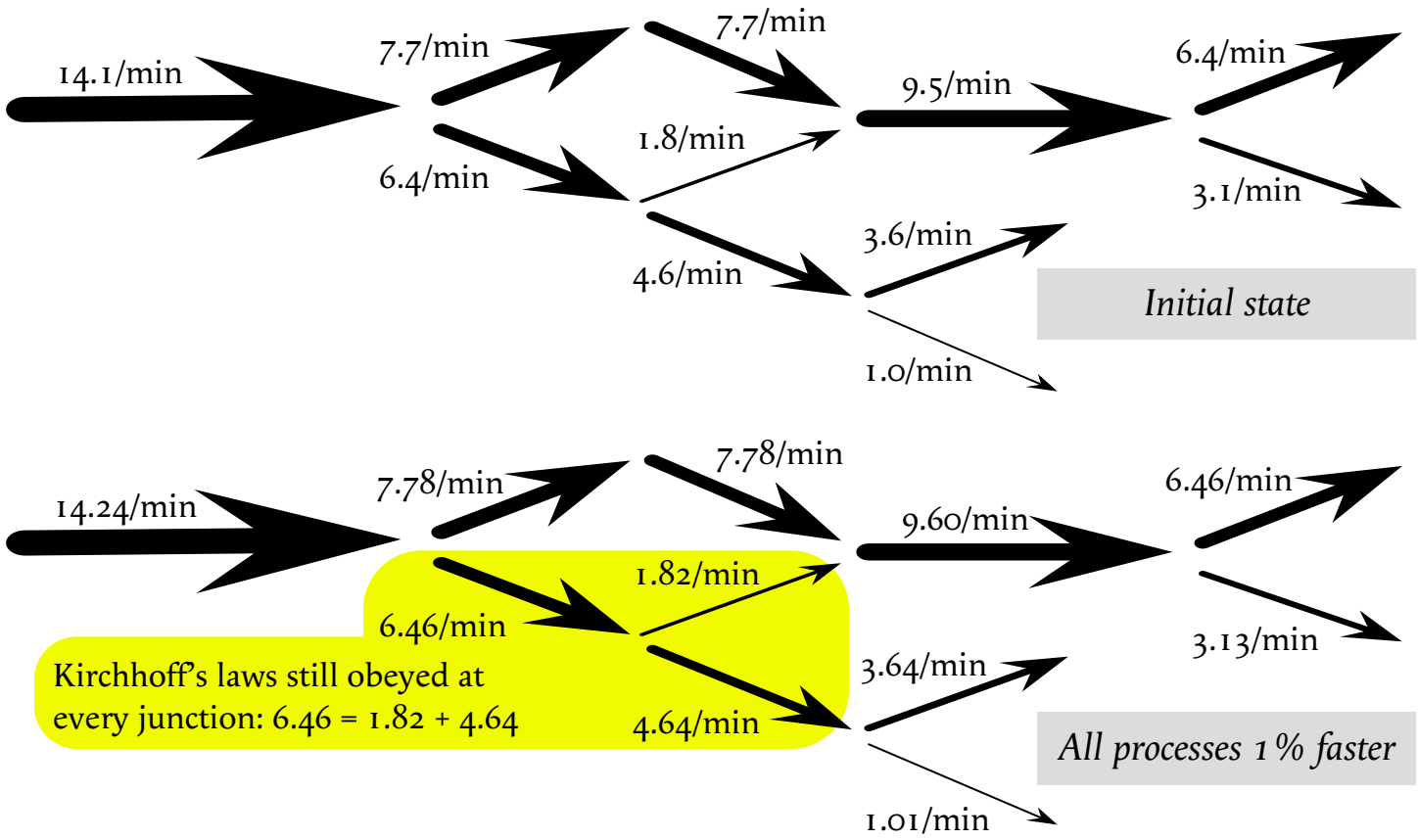

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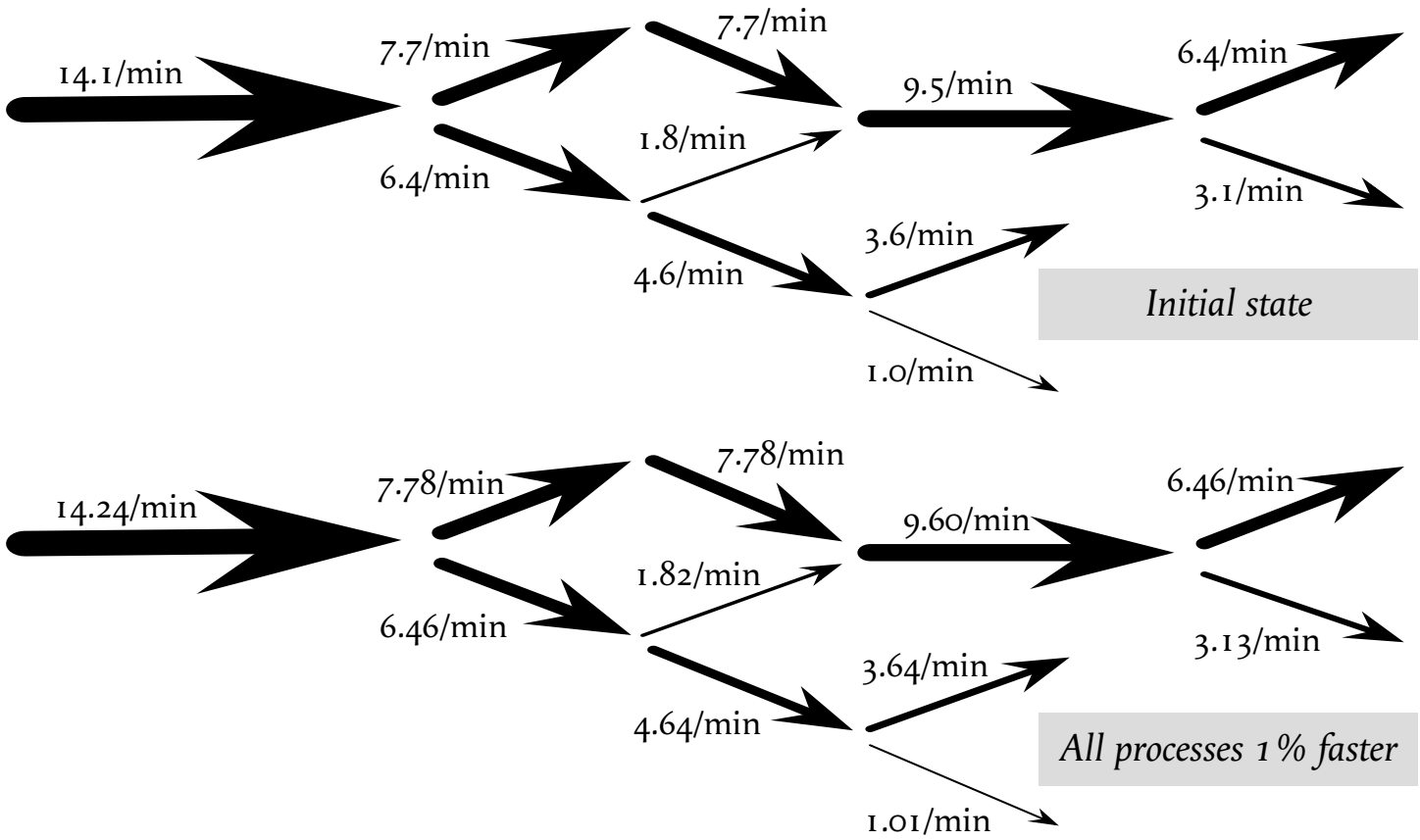

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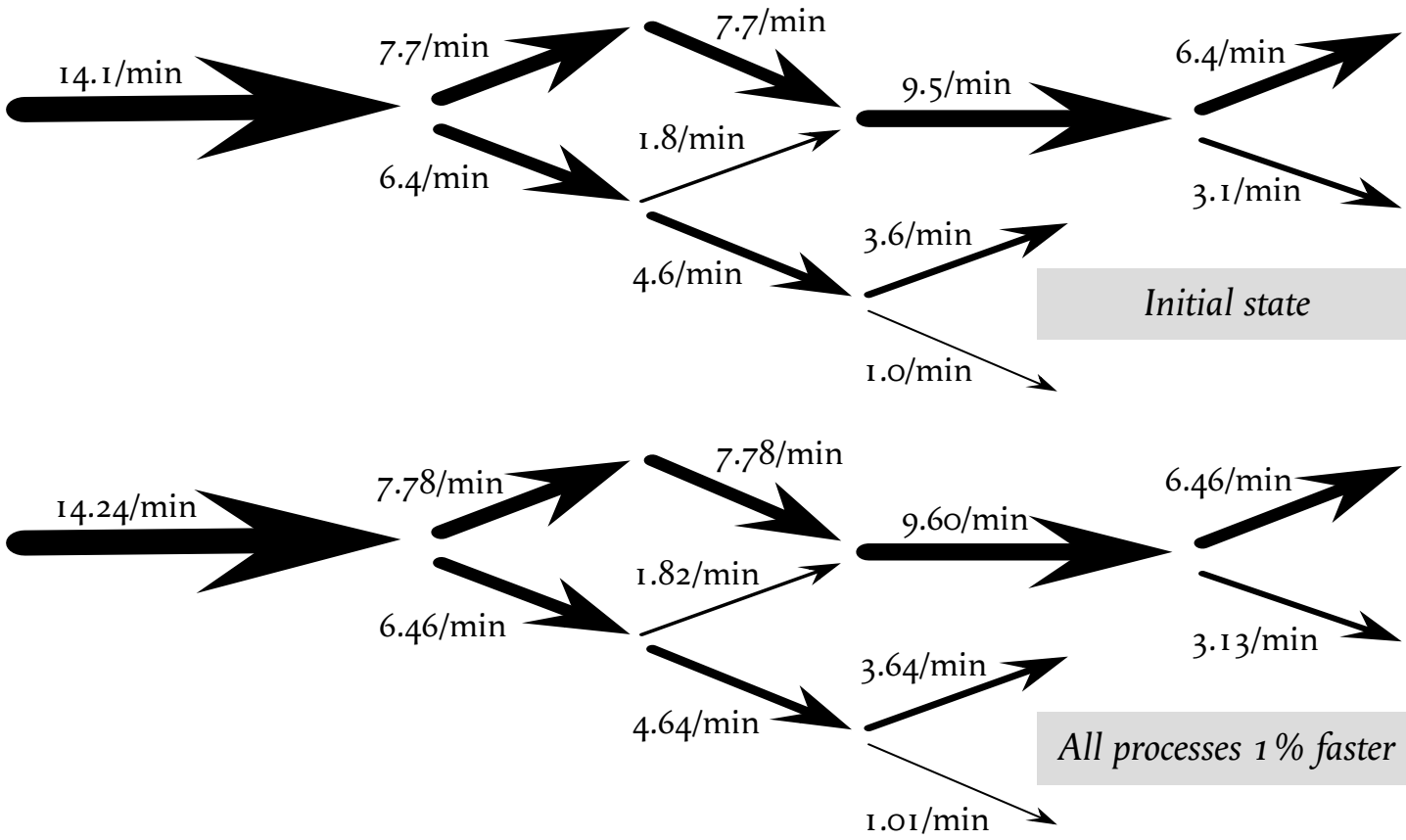

Change of time unit

1 minnit = 59.4 s

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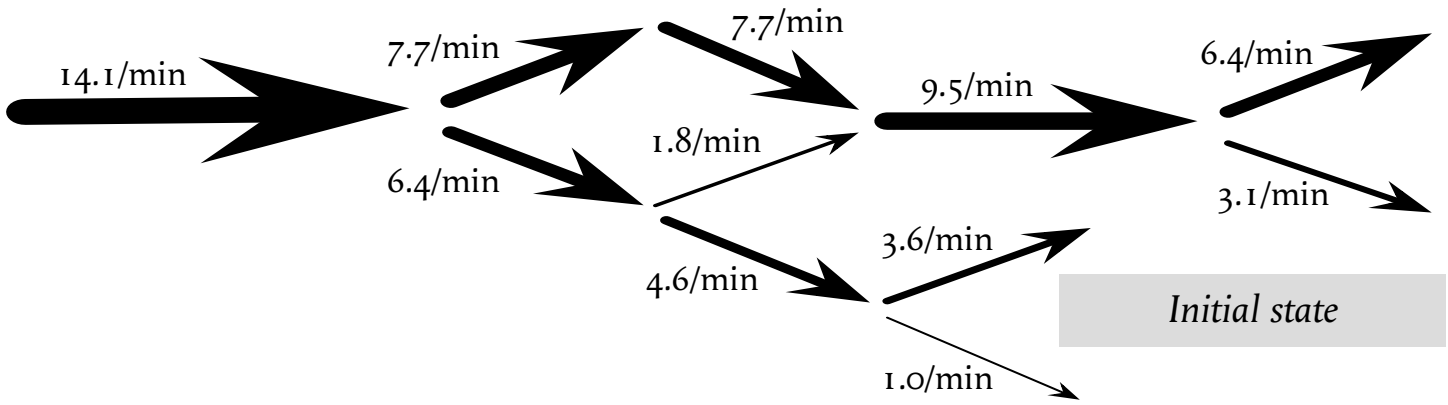
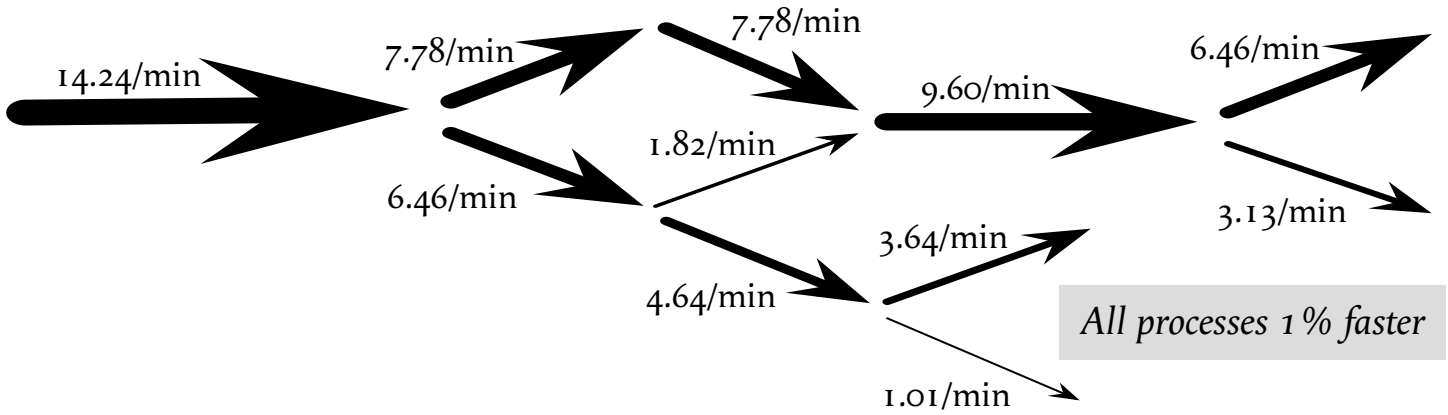
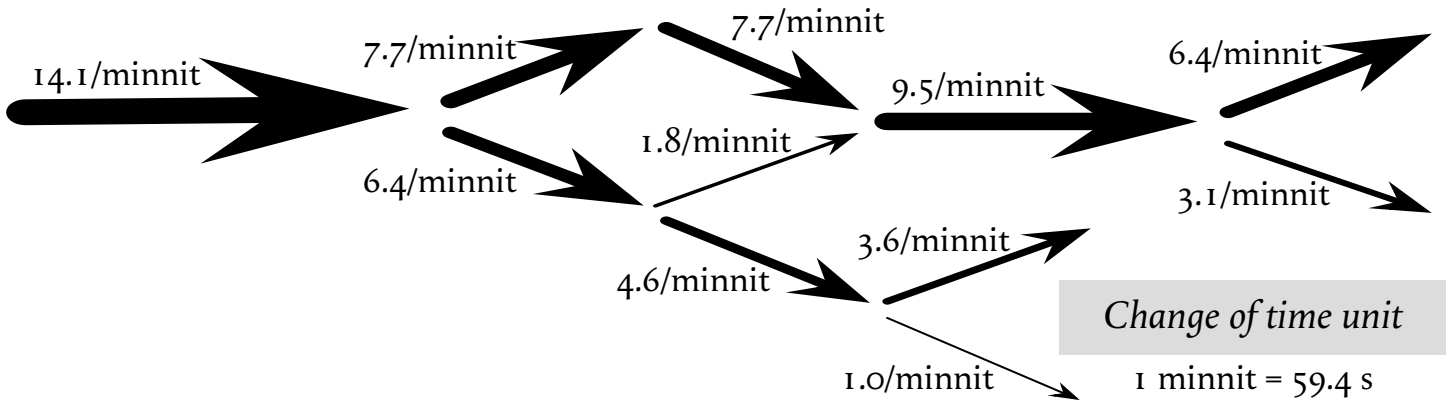


Initial state



All processes 1 % faster



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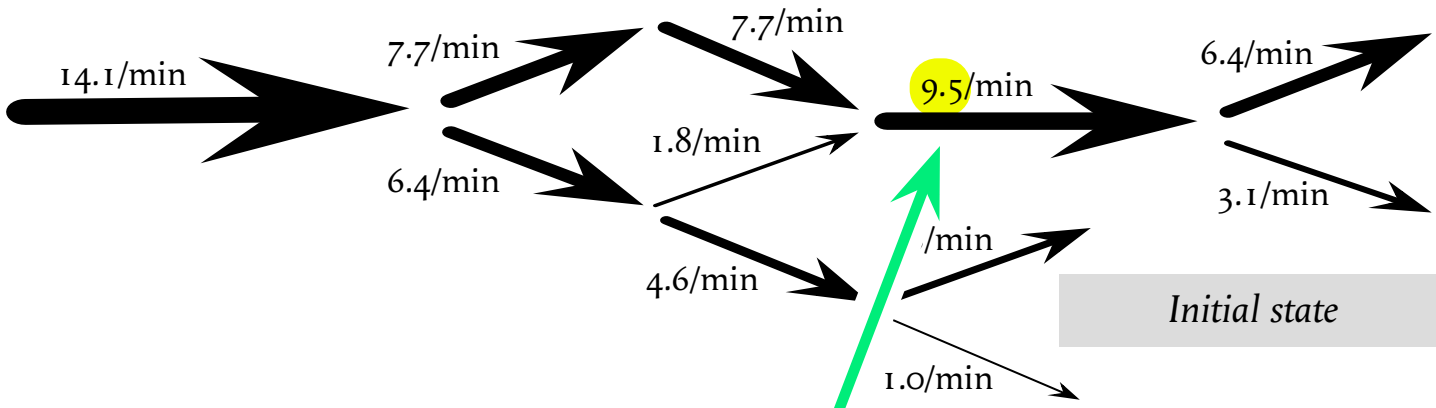
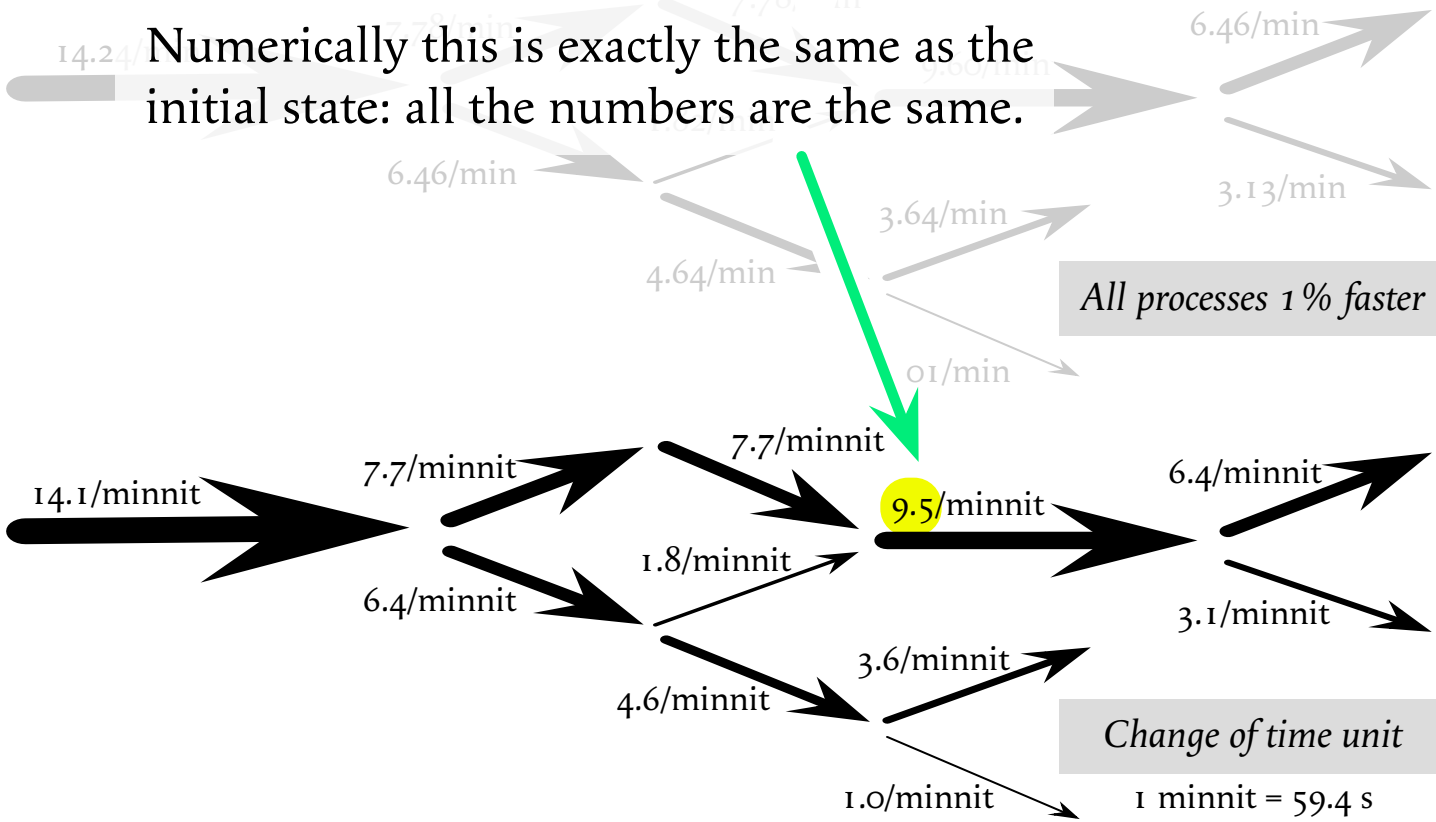


Numerically this is exactly the same as the initial state: all the numbers are the same.



Now a short test:

Stryer says: "*Phosphofructokinase is the key enzyme in the control of glycolysis*"

Is this true? And if it is true, what does it mean?

Let us take a practical example: if you use genetic manipulation to increase 3.5-fold the activity of phosphofructokinase in the cells of growing yeast (*Saccharomyces cerevisiæ*) what effects on the flux of ethanol production would you expect?

A. A 3.5-fold increase in flux?
B. An increase in flux of around 2-fold?
C. A decrease in flux?
D. No detectable effect on the flux?

Now a short test:

Stryer says: "*Phosphofructokinase is the key enzyme in th…*

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Fortunately the result is known beyond any doubt, because Heinisch did the experiment 20 years ago: we aren't required to use any theory to get to the answer.

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Let us t… …e genetic manipu… …ivity of phosph… …wing yeast (*Saccharo…* …n the flux of ethanol pro… …ct?

There is nothing special about yeast: similar results are now available in numerous different organisms. In general, the idea that phosphofructokinase controls glycolysis is completely false!

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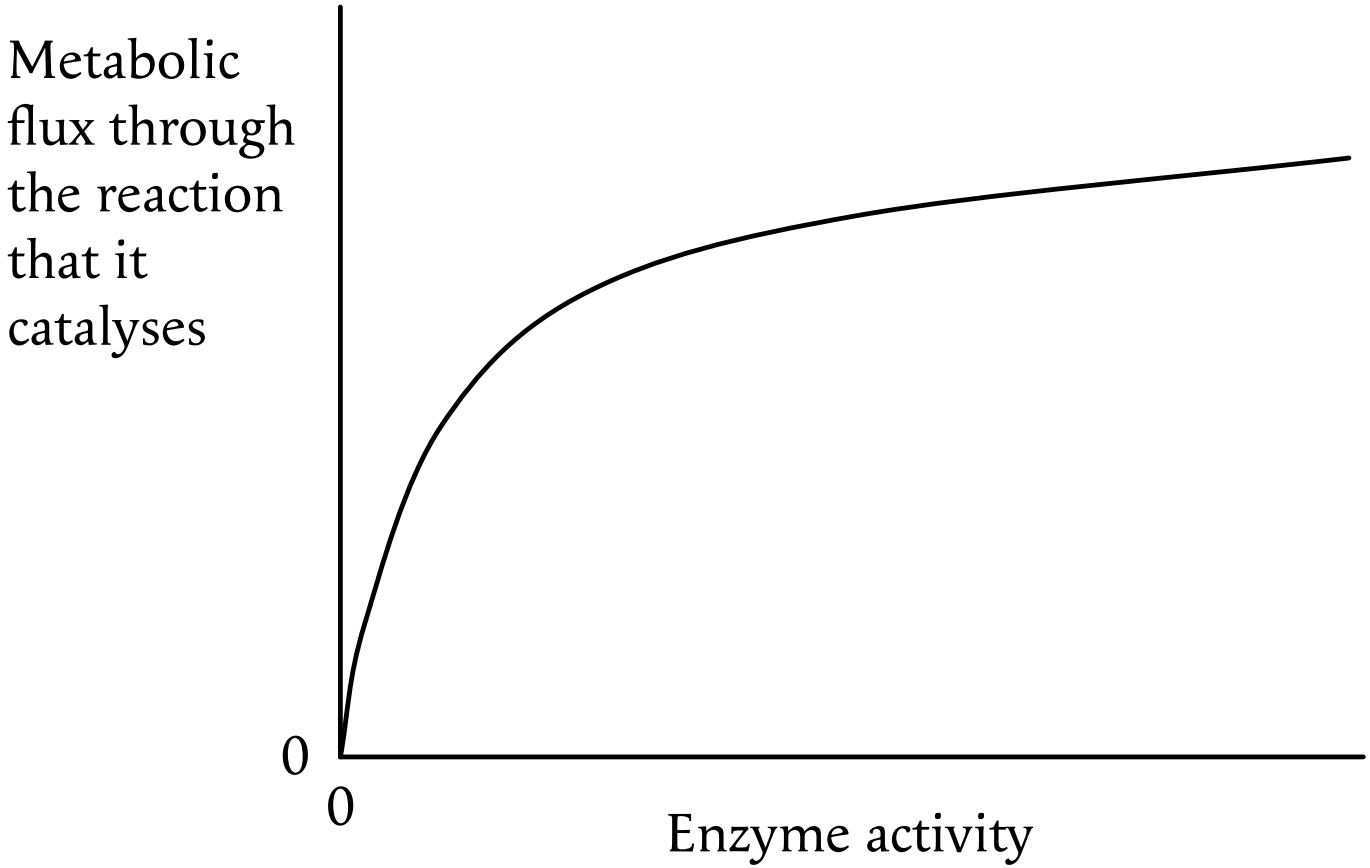
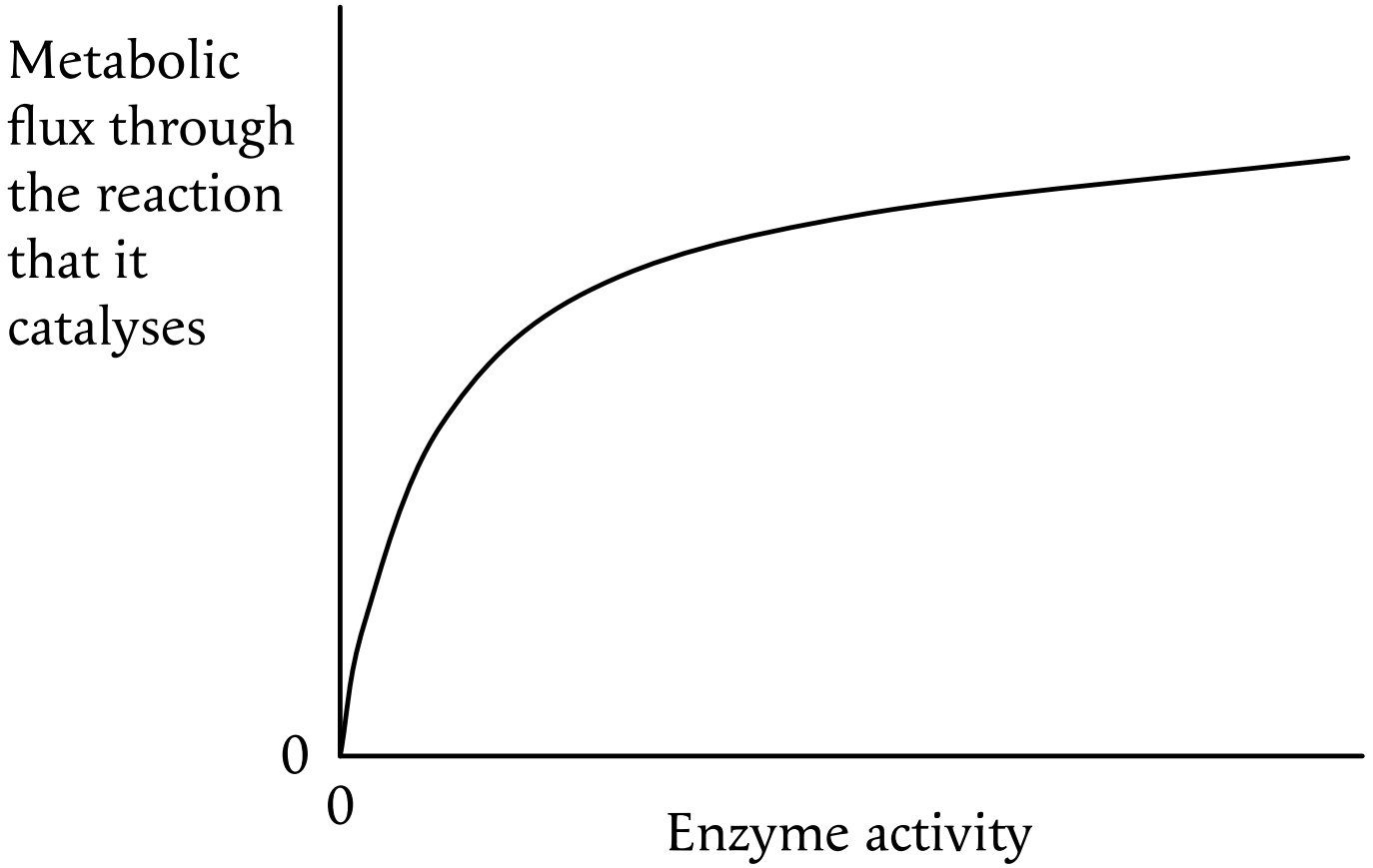
B. An increase in flux of around 2-fold? no!
C. A decrease in flux? no!
→ D. No detectable effect on the flux? yes

Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway



Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway

Where along this curve is an enzyme likely to be located in ordinary growth conditions in the wild type?



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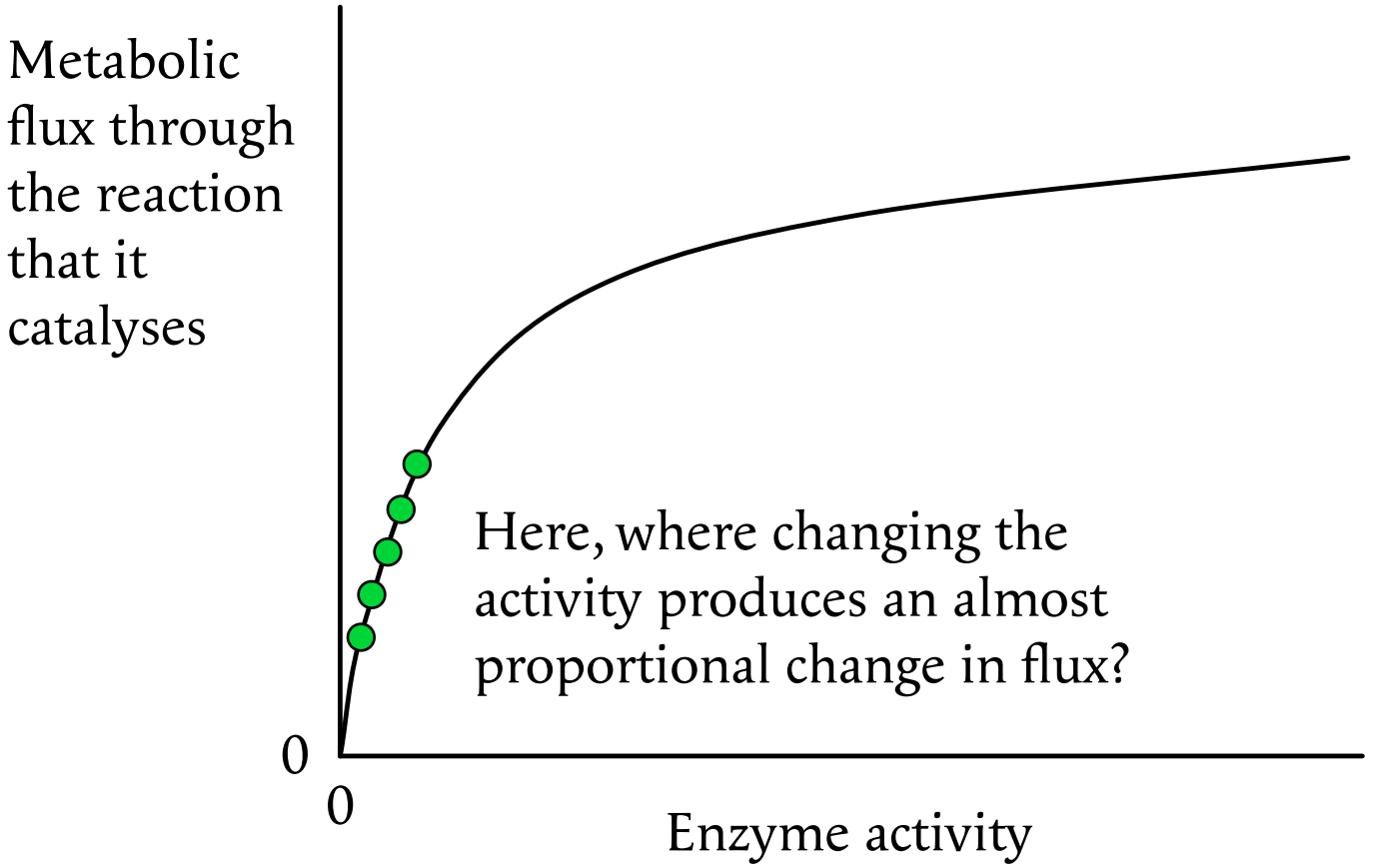

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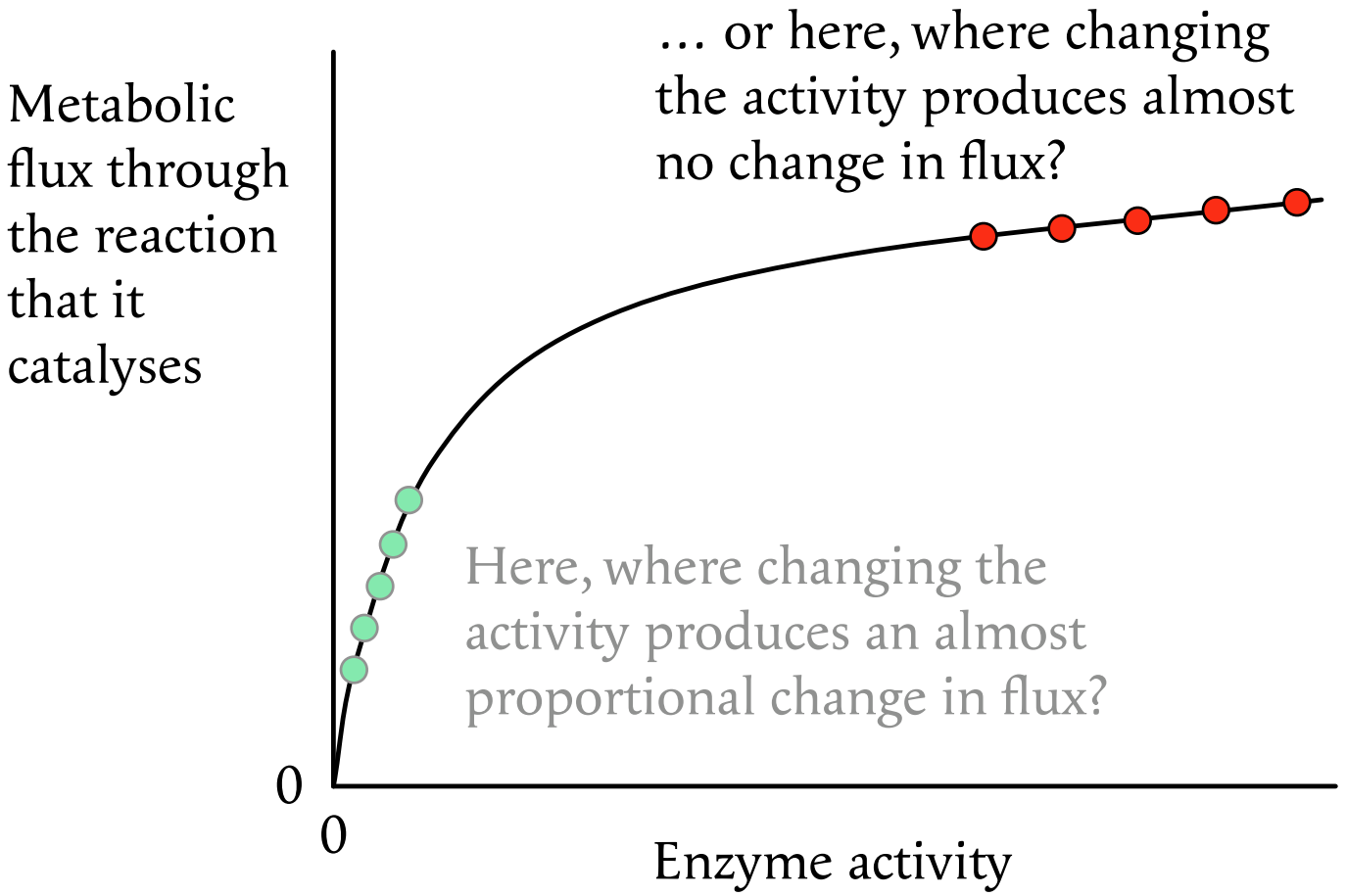

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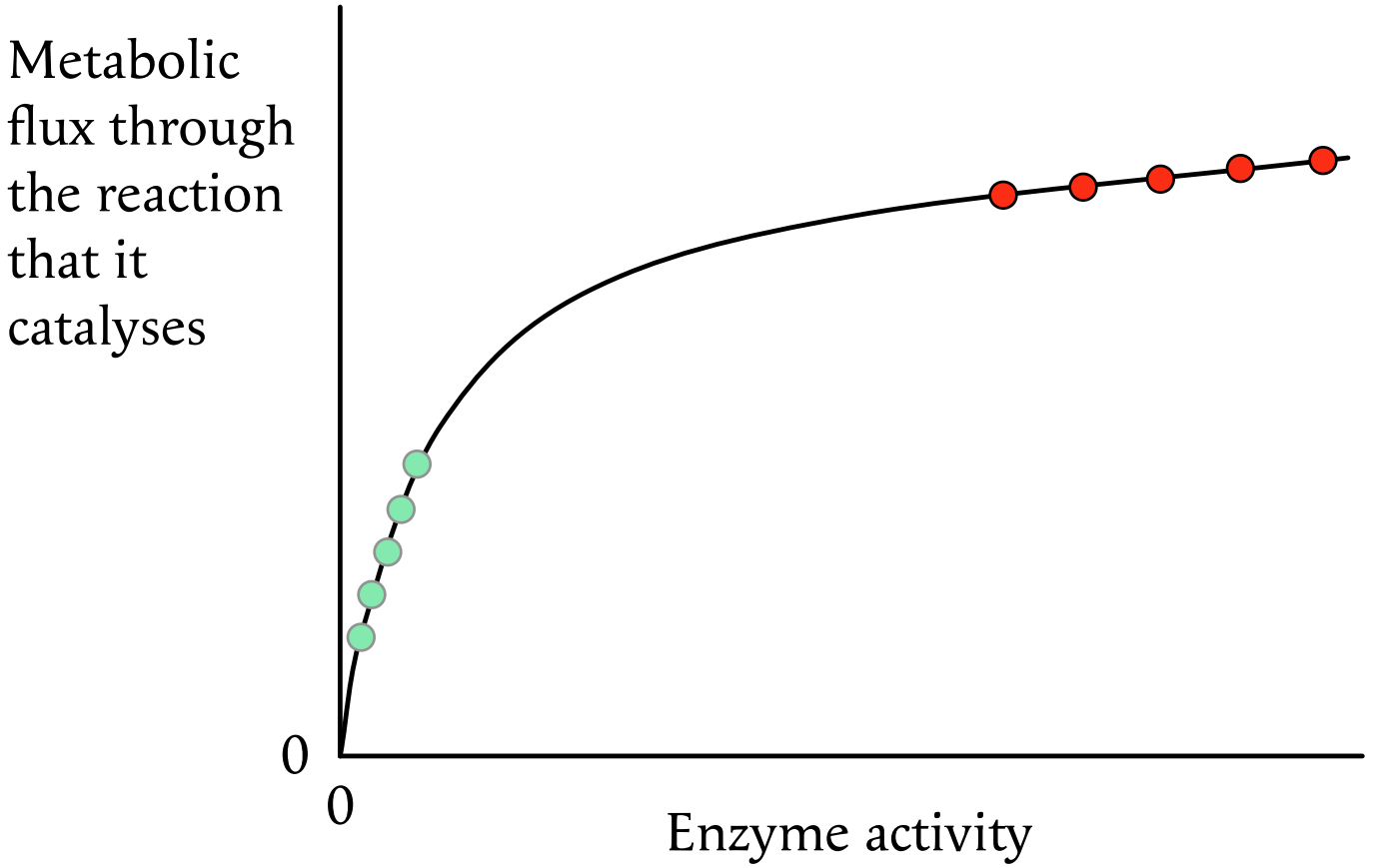

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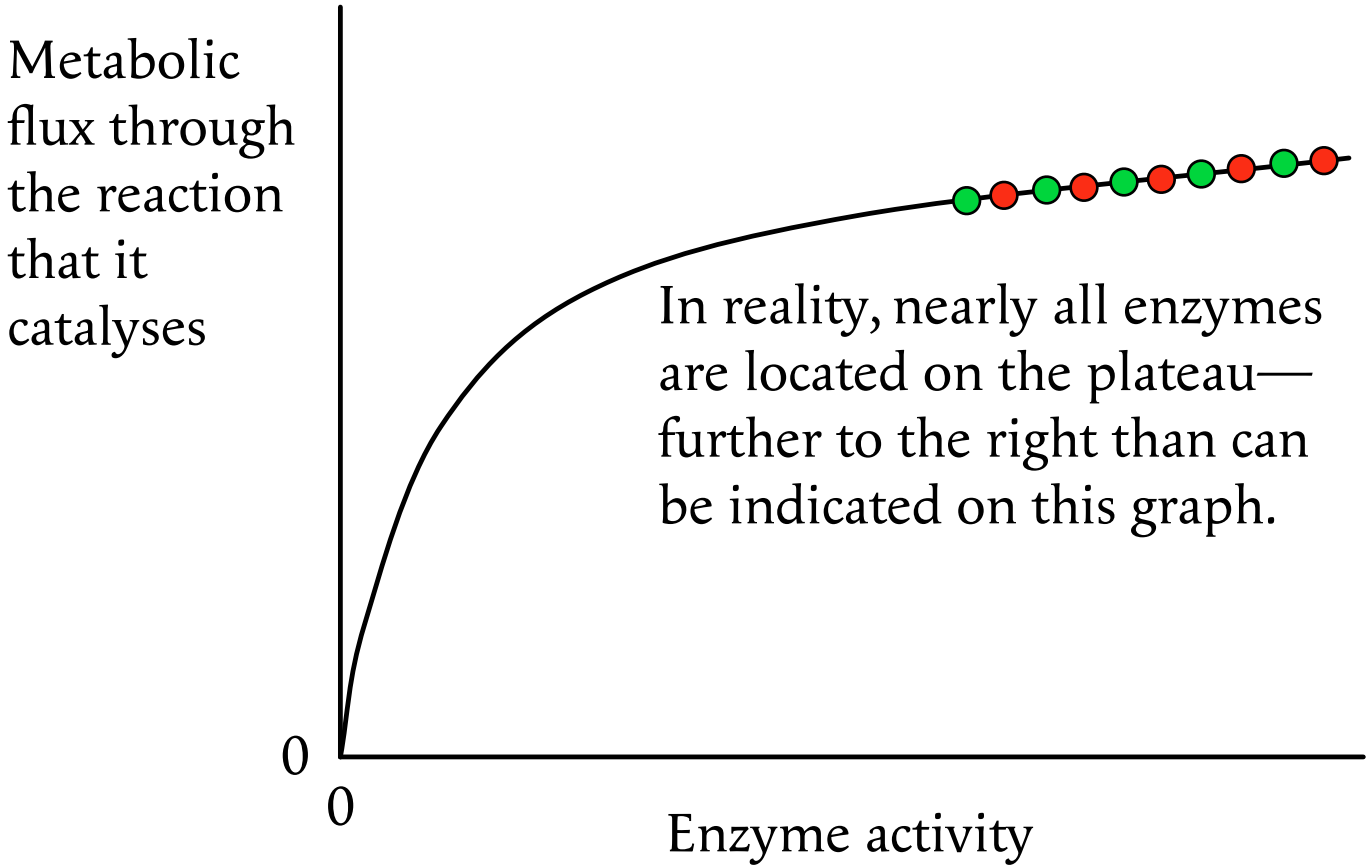

Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway

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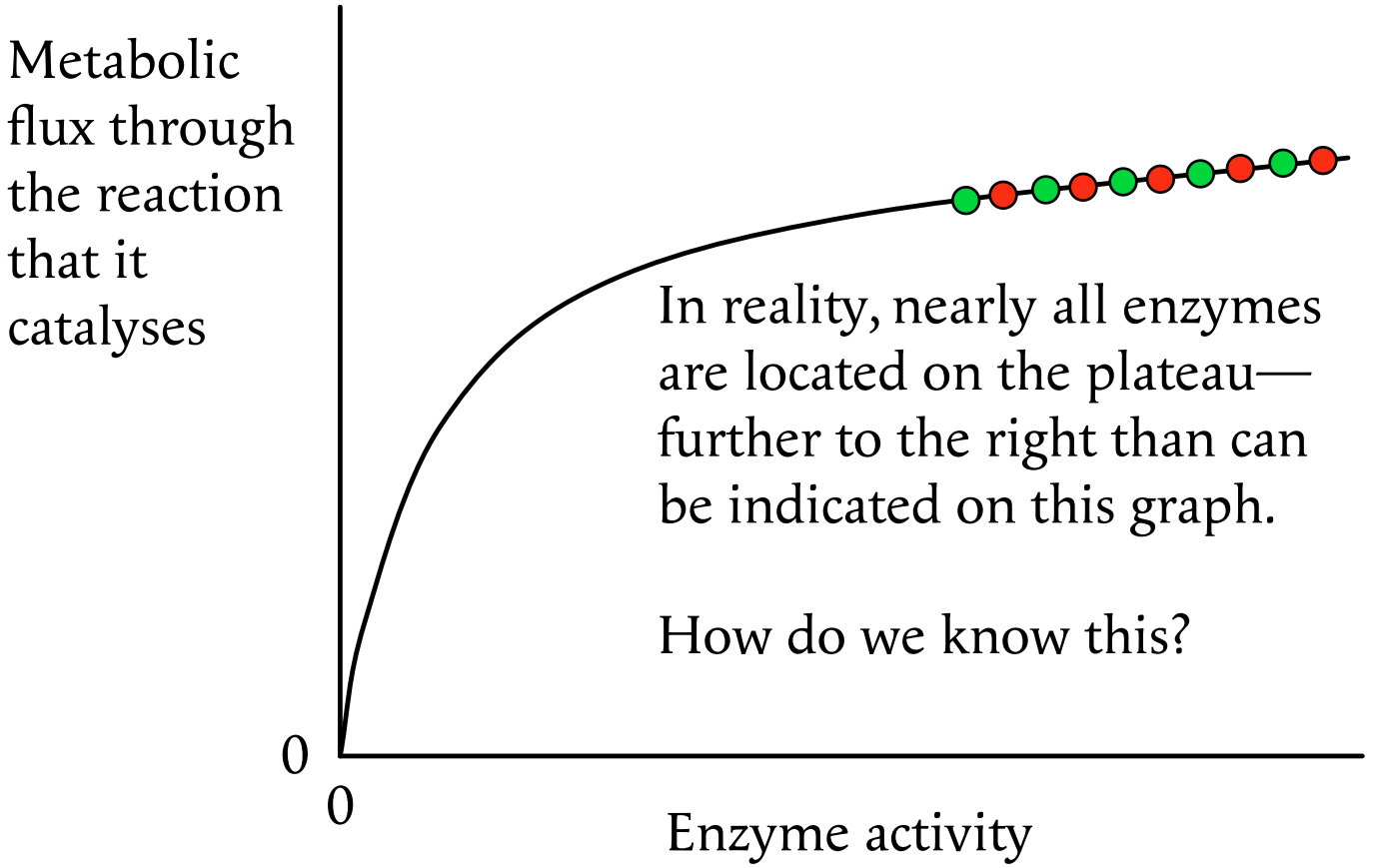


Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway

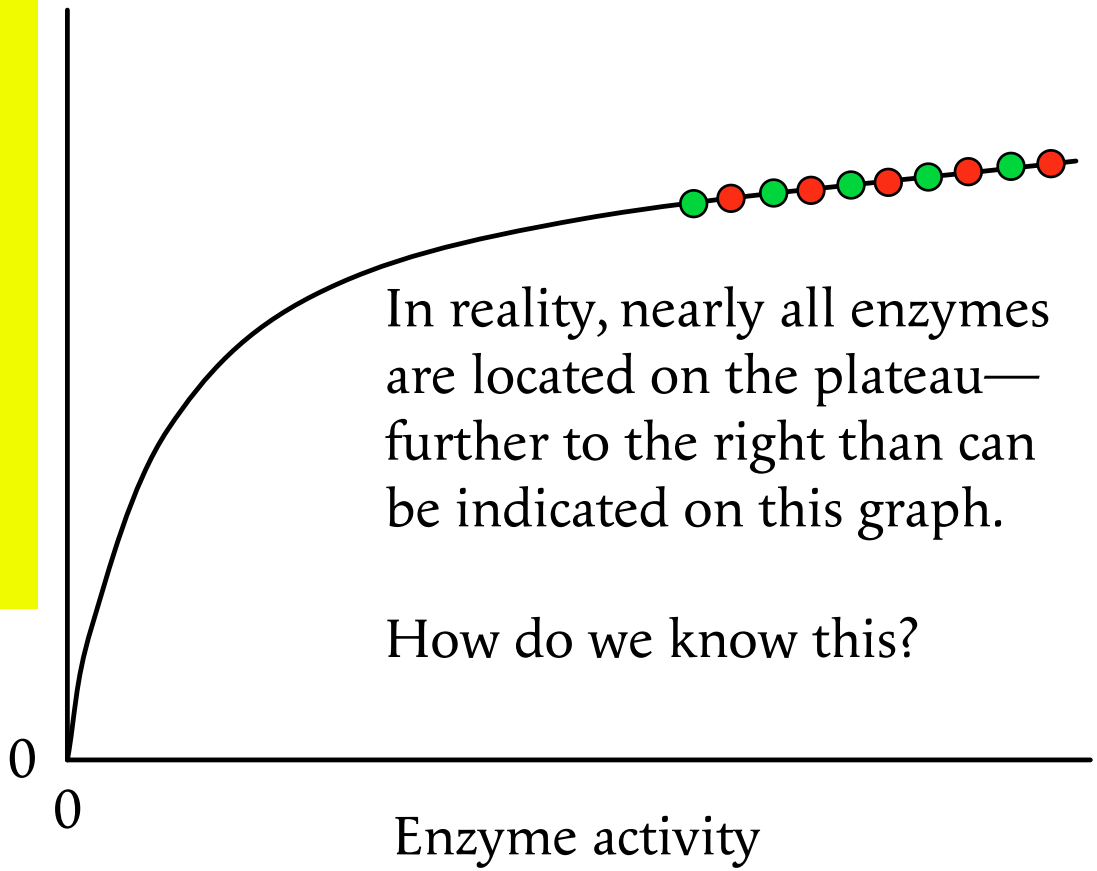
Where along this curve is an enzyme likely to be located in ordinary growth conditions in the wild type?

THREE KINDS OF EVIDENCE

1. Summation property

$$\sum_{i=1}^{n} C_i^J = 1$$

As n is typically large, the average flux control coefficient must be small.



In reality, nearly all enzymes are located on the plateau—further to the right than can be indicated on this graph.

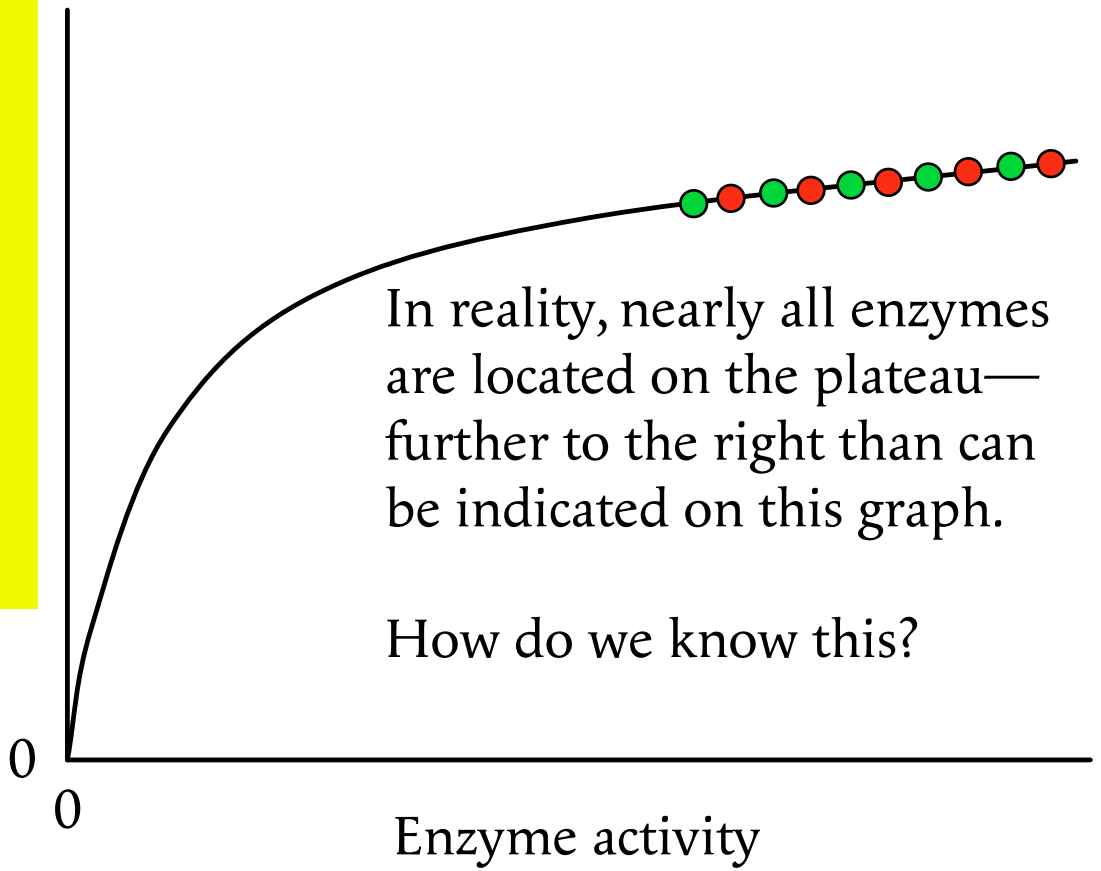
How do we know this?

Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway

Where along this curve is an enzyme likely to be located in ordinary growth conditions in the wild type?

THREE KINDS OF EVIDENCE

1. Summation property
2. Phosphofructokinase



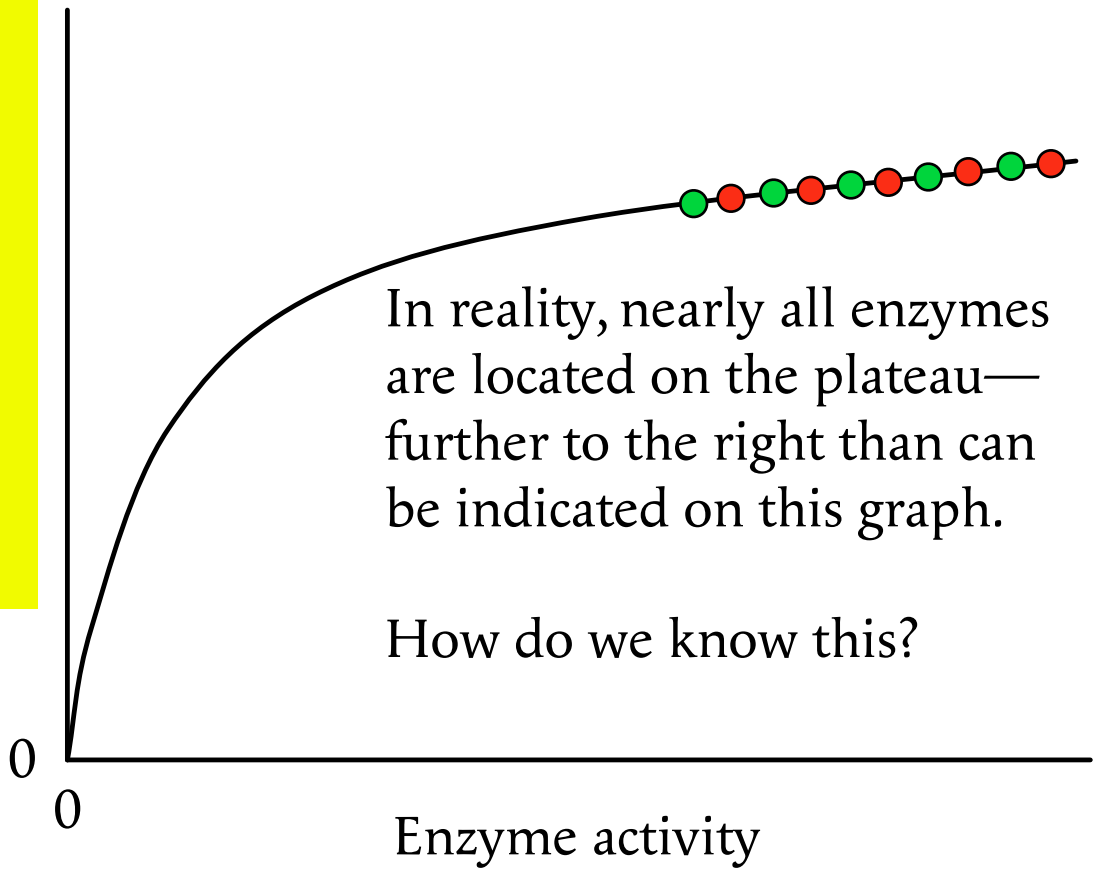
Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway

Where along this curve is an enzyme likely to be located in ordinary growth conditions in the wild type?

THREE KINDS OF EVIDENCE

1. Summation property
2. Phosphofructokinase

What happens to the flux to ethanol when its activity is increased 3.5-fold in fermenting yeast?



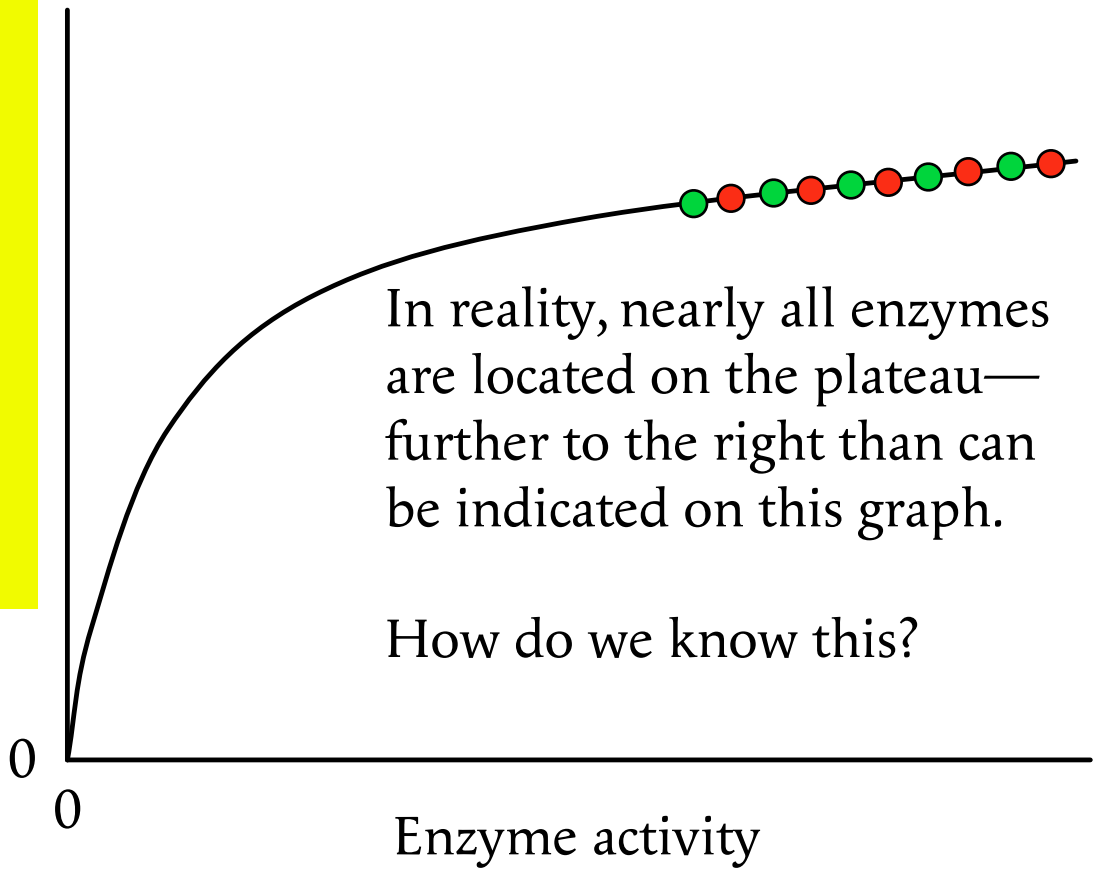
nothing

Typical curve for the dependence of metabolic flux on the activity of one enzyme in a pathway

Where along this curve is an enzyme likely to be located in ordinary growth conditions in the wild type?

THREE KINDS OF EVIDENCE

1. Summation property
2. Phosphofructokinase
3. Mendelian genetics



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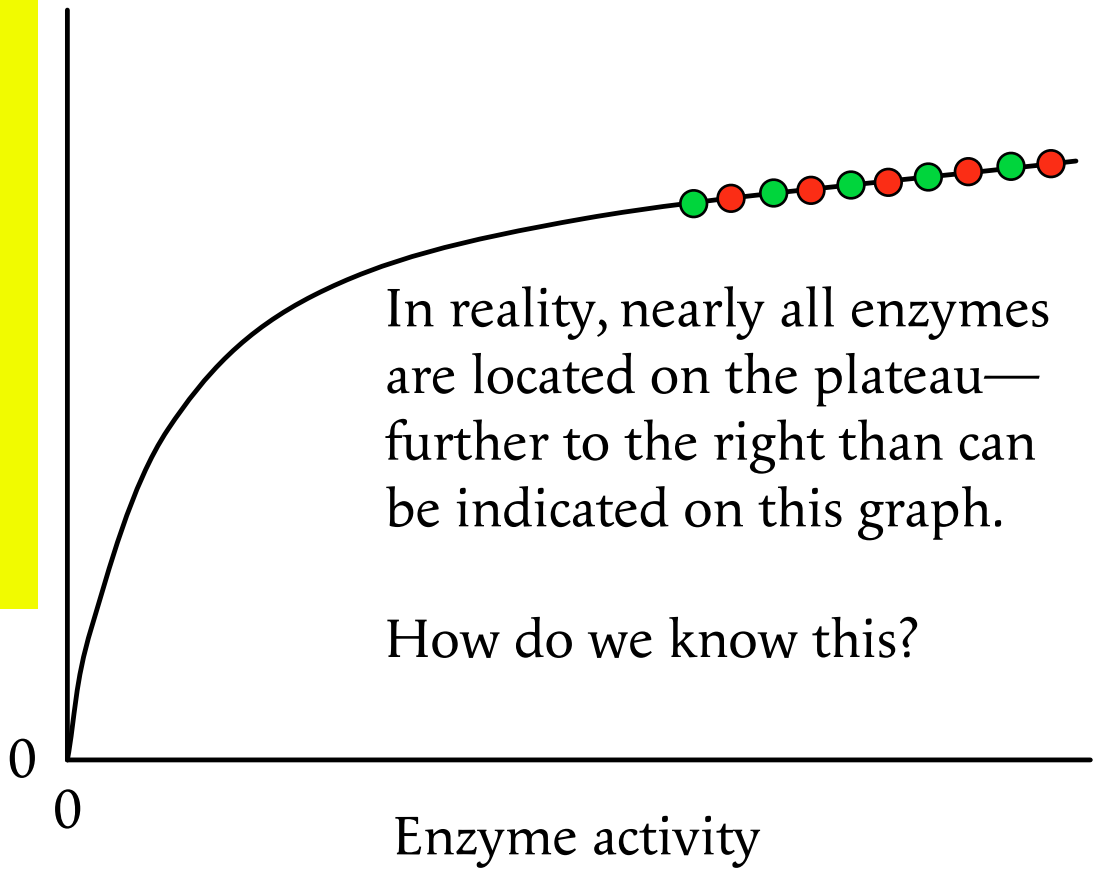
1. Summation property
2. Phosphofructokinase
3. Mendelian genetics

   Mutant alleles in diploid organisms are usually recessive.



Homozygotic brown-eyed parents have brown-eyed children

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In reality, nearly all enzymes are located on the plateau—further to the right than can be indicated on this graph.

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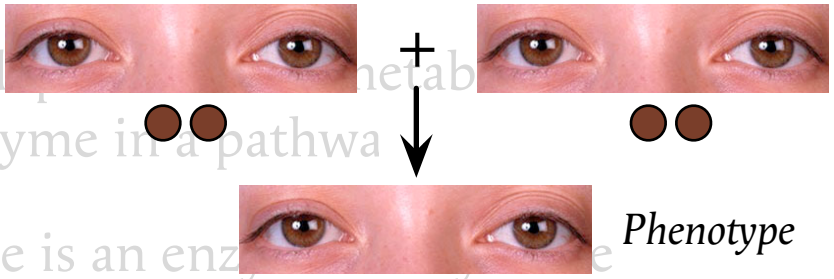

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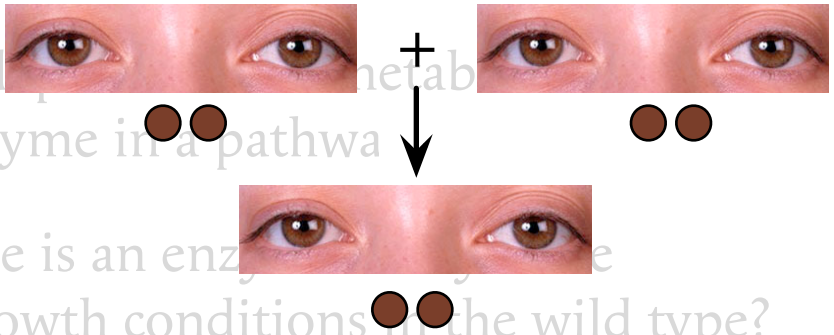

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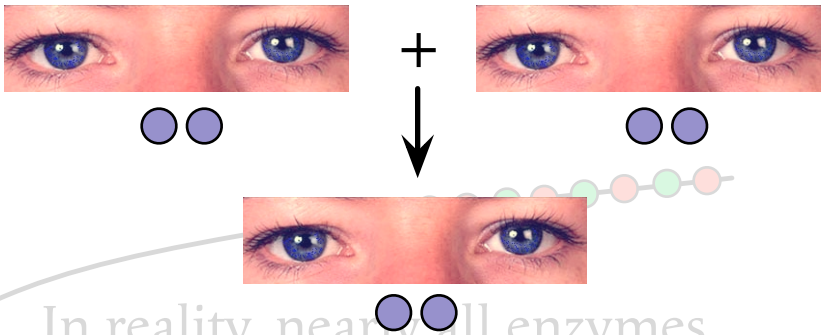

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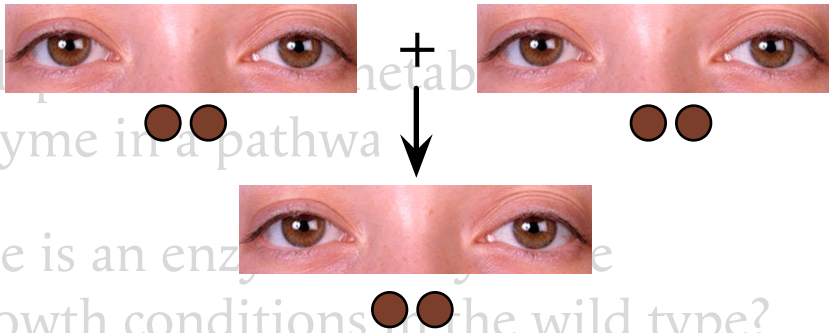

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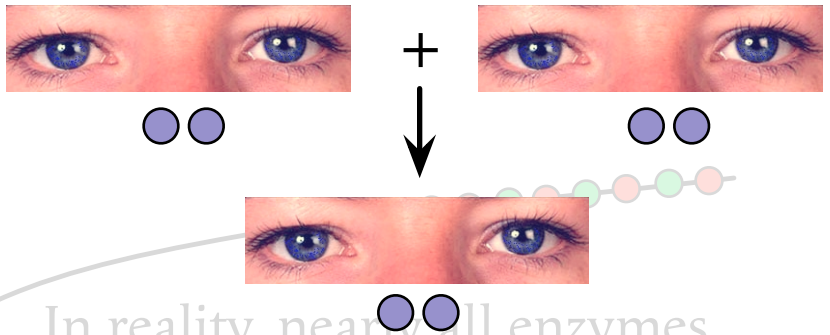
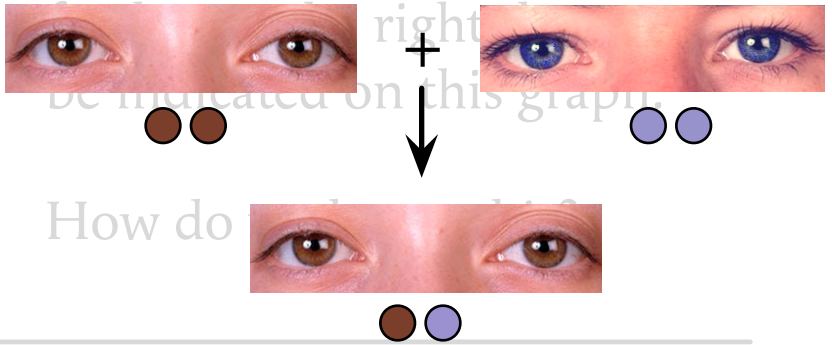


Homozygotic parents of different eye colours have brown-eyed children



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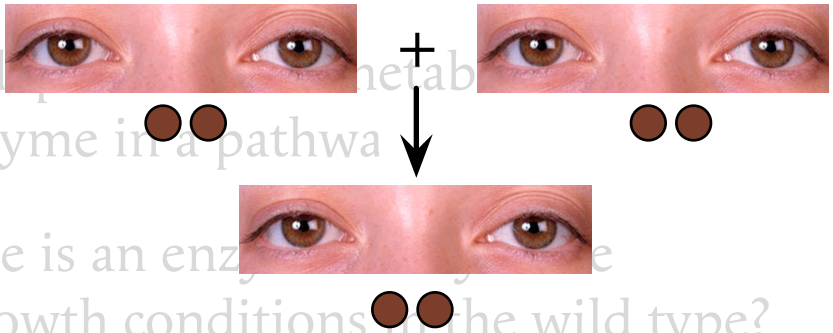

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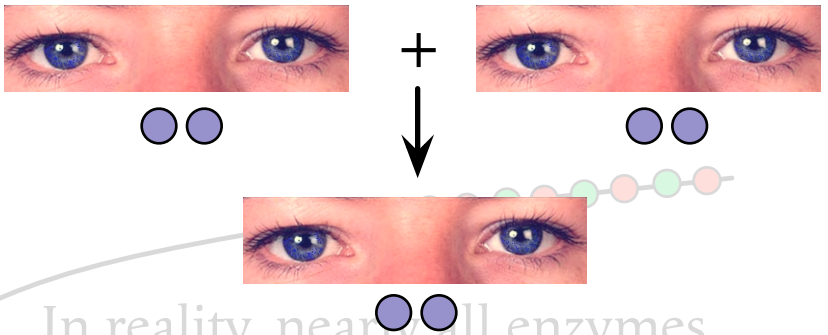

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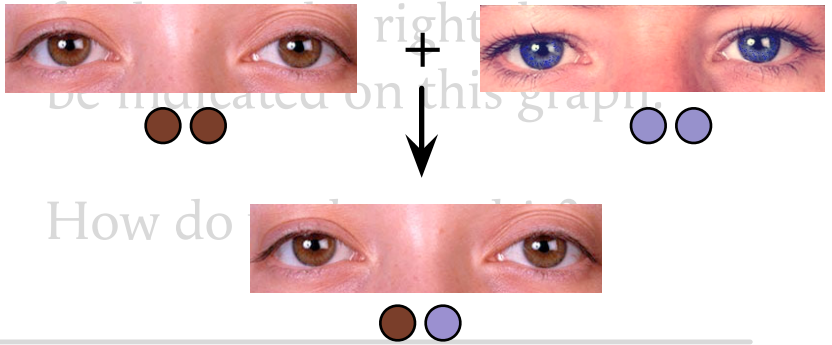

What is going on here?

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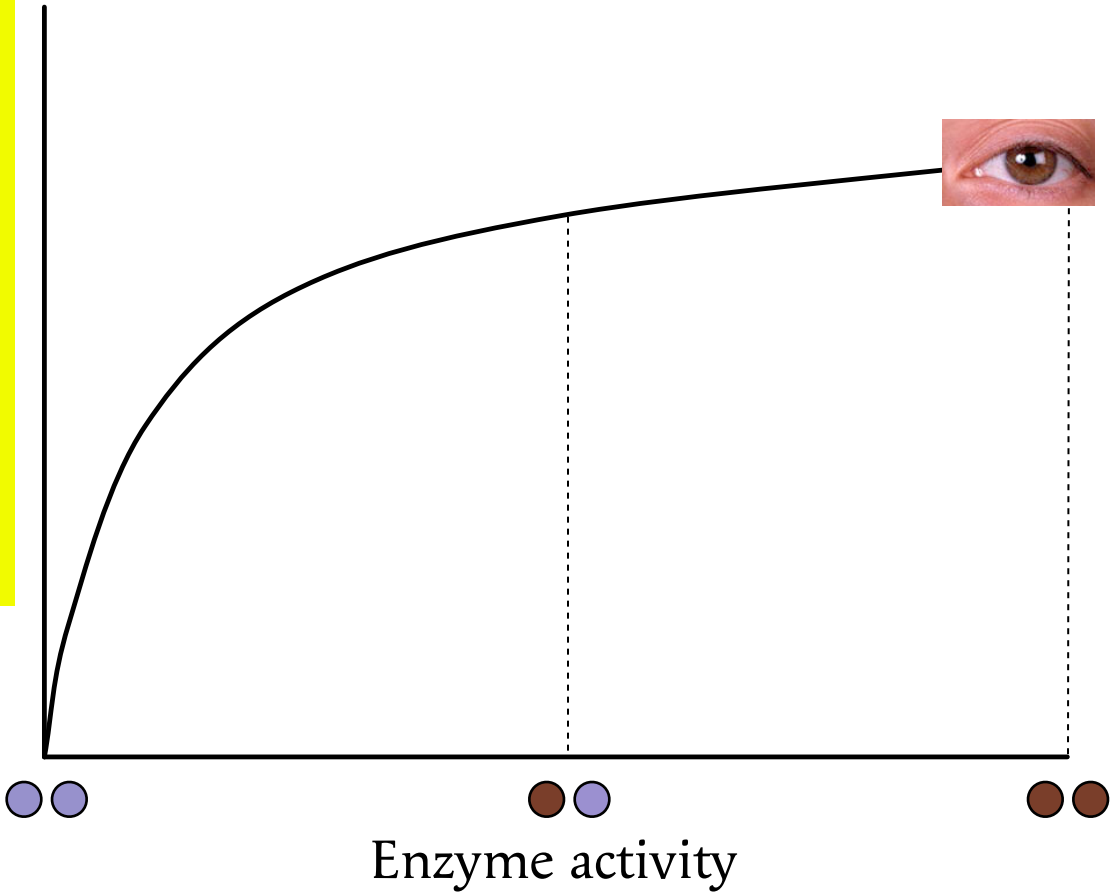

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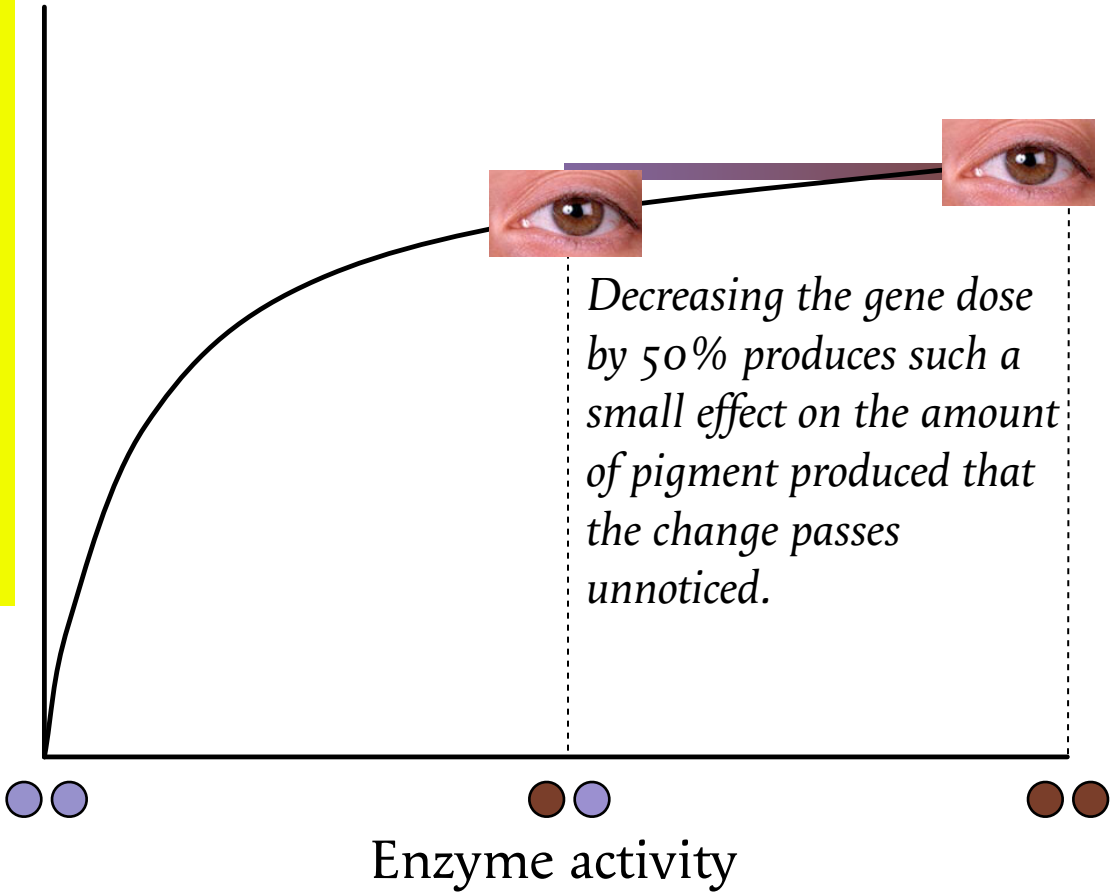

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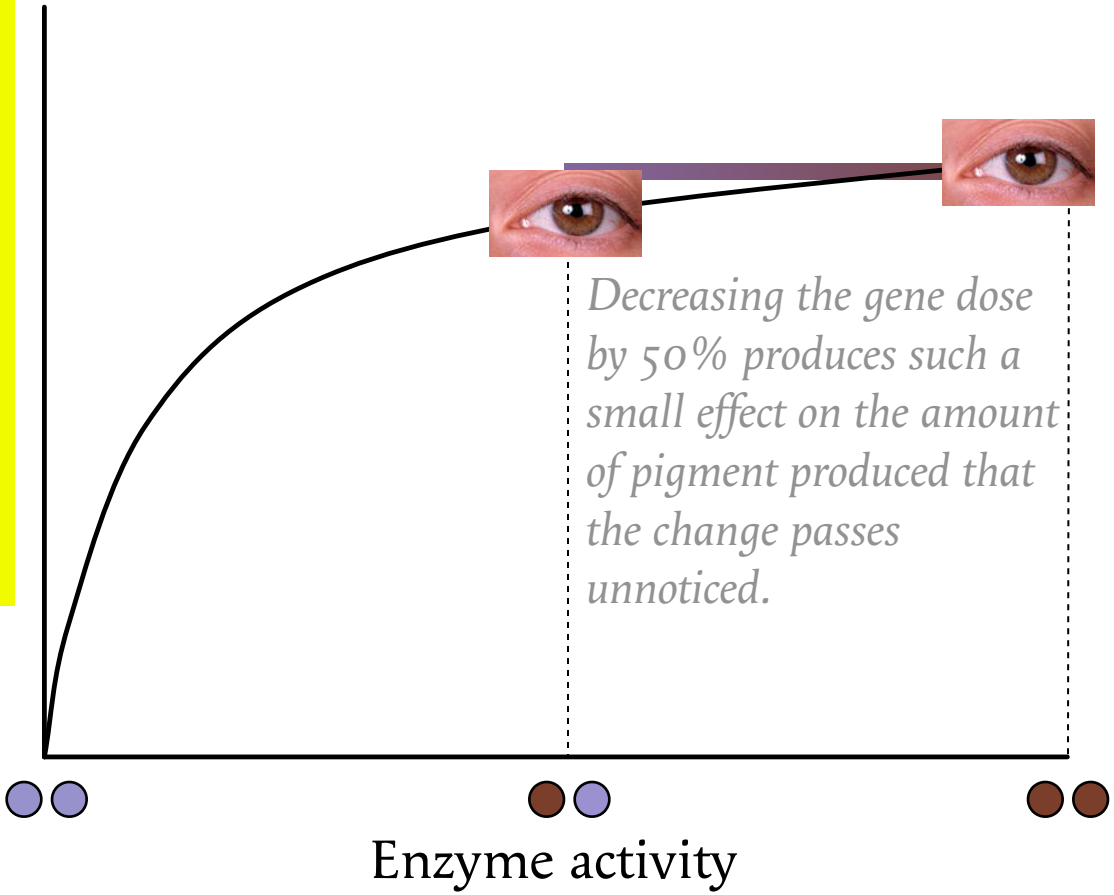

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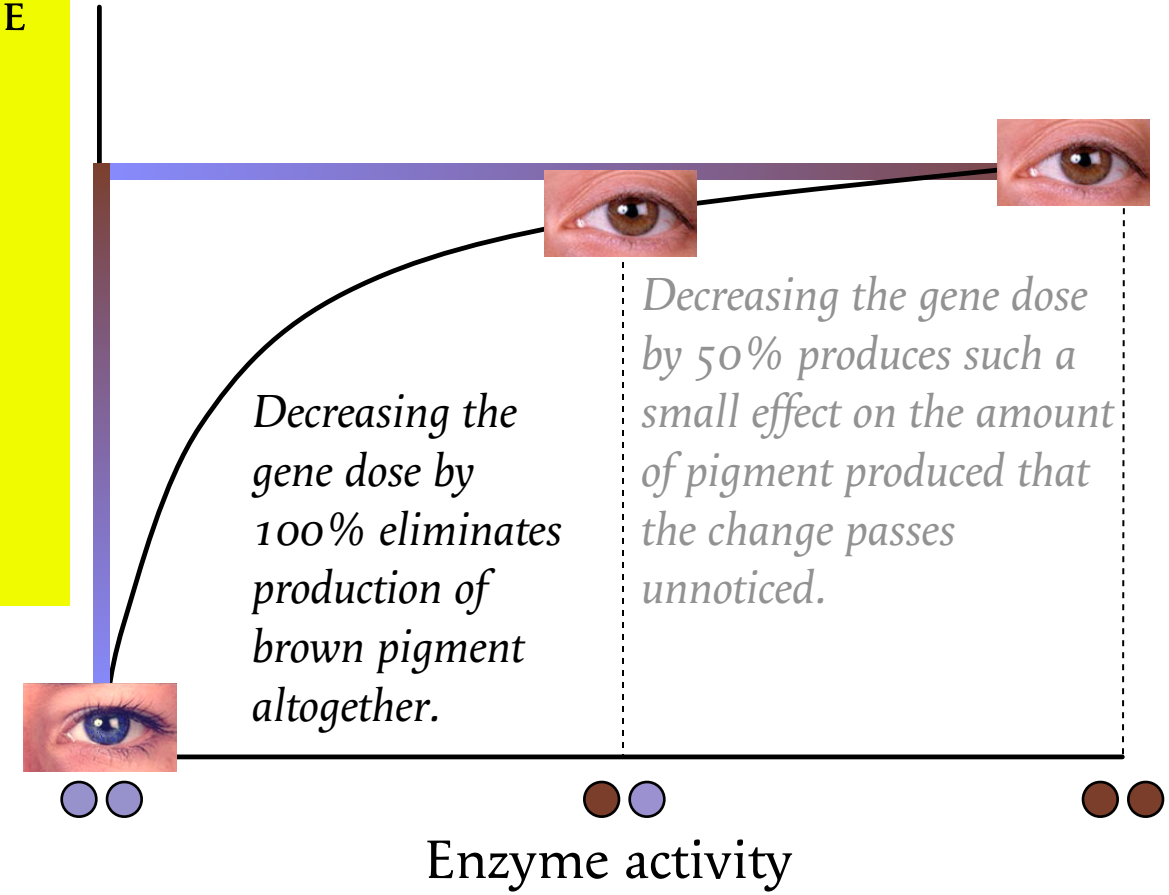

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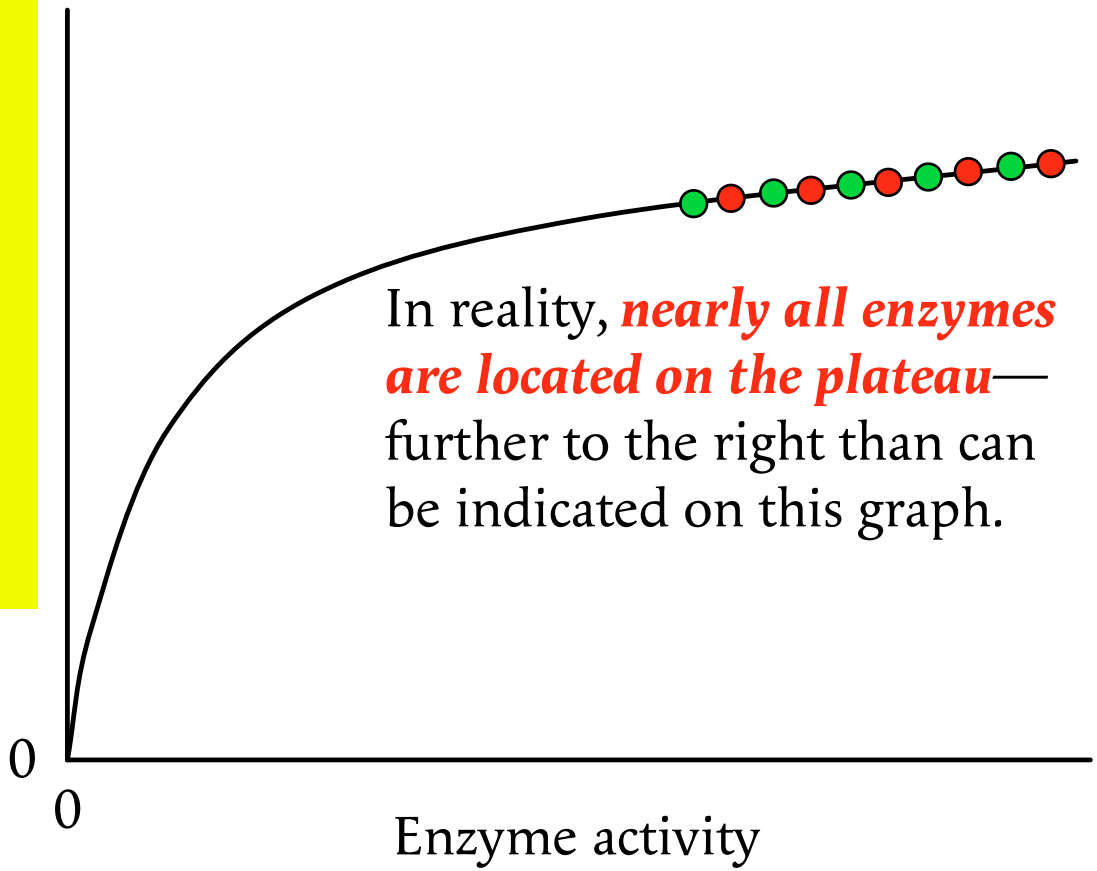

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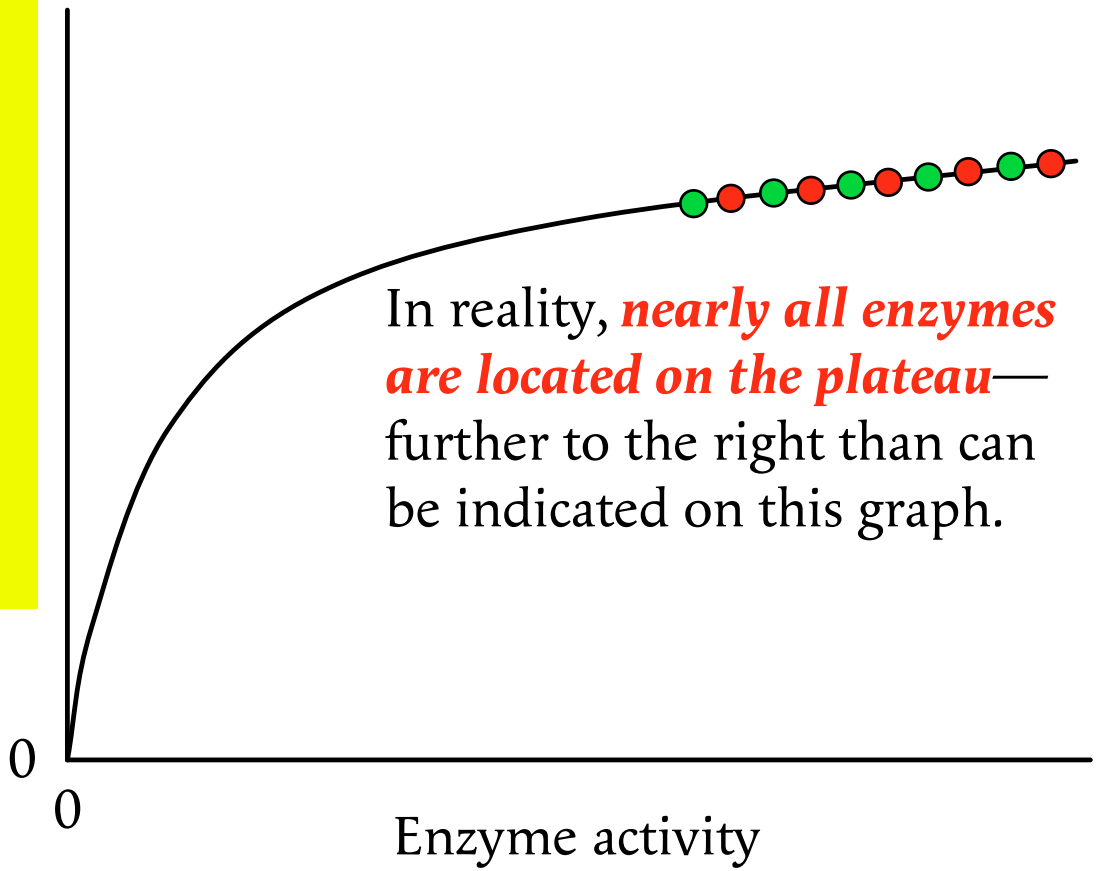

If the concentration e_i of an enzyme and the concentration s_j of a metabolite change simultaneously in such a way that there is no effect on the rate v_i of the enzyme concerned, then

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This equation expresses the *connectivity property* that relates the flux control coefficients and the elasticities.

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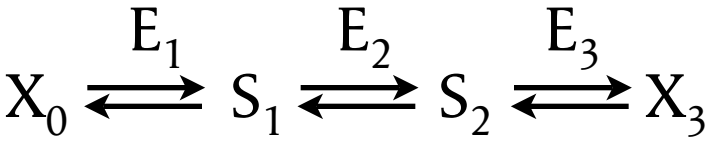
This equation expresses the *connectivity property* that relates the flux control coefficients and the elasticities.

Its importance lies in the fact that it expresses the idea that *the properties of a metabolic system depend directly on the properties of its components*: there is nothing mysterious about this!



$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

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$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

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$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

Each term in the denominator is the product of the elasticities for all the internal metabolites of the system.

The enzyme that appears here…

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

…does not appear in the numerator

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

Each term in the sum is "normally" positive

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

Each term in the denominator is the product of the elasticities for all the internal metabolites of the system.

The enzyme that appears here…

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

…does not appear in the numerator

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

Each term in the denominator is the product of the elasticities for all the internal metabolites of the system.

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \underset{}{\overset{E_1}{\rightleftharpoons}} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$\sum_{i=1}^{3} C_i^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$\sum_{i=1}^{3} C_i^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \underset{}{\overset{E_1}{\rightleftharpoons}} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$\sum_{i=1}^{3} C_i^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \underset{}{\overset{E_1}{\rightleftharpoons}} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^J = \frac{-\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^J = \frac{\epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$\sum_{i=1}^{3} C_i^J = \frac{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}} = 1$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^{s_1} = \frac{\epsilon_{s_2}^{v_3} - \epsilon_{s_2}^{v_2}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^{s_1} = \frac{\epsilon_{s_2}^{v_3} - \epsilon_{s_2}^{v_2}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^{s_1} = \frac{\epsilon_{s_2}^{v_1} - \epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^{s_1} = \frac{\epsilon_{s_2}^{v_2} - \epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

The enzyme that appears here…

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

…does not appear in the numerator

$$C_1^{s_1} = \frac{\epsilon_{s_2}^{v_3} - \epsilon_{s_2}^{v_2}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^{s_1} = \frac{\epsilon_{s_2}^{v_1} - \epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^{s_1} = \frac{\epsilon_{s_2}^{v_2} - \epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^{s_1} = \frac{\epsilon_{s_2}^{v_3} - \epsilon_{s_2}^{v_2}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^{s_1} = \frac{\epsilon_{s_2}^{v_1} - \epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

…does not appear in the numerator

$$C_3^{s_1} = \frac{\epsilon_{s_2}^{v_2} - \epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

The substrate that appears at the left…

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$C_1^{s_1} = \frac{\epsilon_{s_2}^{v_3} - \epsilon_{s_2}^{v_2}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_2^{s_1} = \frac{\epsilon_{s_2}^{v_1} - \epsilon_{s_2}^{v_3}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$C_3^{s_1} = \frac{\epsilon_{s_2}^{v_2} - \epsilon_{s_2}^{v_1}}{\epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_3} - \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_3} + \epsilon_{s_1}^{v_1}\epsilon_{s_2}^{v_2} - \epsilon_{s_1}^{v_2}\epsilon_{s_2}^{v_1}}$$

$$\sum_{i=1}^{3} C_i^{s_1} = 0$$

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$\begin{bmatrix} C_1^J & C_2^J & C_3^J \\ C_1^{S1} & C_2^{S1} & C_3^{S1} \\ C_1^{S2} & C_2^{S2} & C_3^{S2} \end{bmatrix}$$

If we write a matrix of control coefficients…

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$\begin{bmatrix} C_1^J & C_2^J & C_3^J \\ C_1^{S1} & C_2^{S1} & C_3^{S1} \\ C_1^{S2} & C_2^{S2} & C_3^{S2} \end{bmatrix}$$

The first row contains all the flux control coefficients

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

$$\begin{bmatrix} C_1^J & C_2^J & C_3^J \\ C_1^{S1} & C_2^{S1} & C_3^{S1} \\ C_1^{S2} & C_2^{S2} & C_3^{S2} \end{bmatrix}$$

The second row contains all the concentration control coefficients for S_1

$$X_0 \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} X_3$$

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The third row contains all the concentration control coefficients for S_2

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$$\begin{bmatrix} C_1^J & C_2^J & C_3^J \\ C_1^{s_1} & C_2^{s_1} & C_3^{s_1} \\ C_1^{s_2} & C_2^{s_2} & C_3^{s_2} \end{bmatrix} \cdot \begin{bmatrix} 1 & -\epsilon_{s_1}^{v_1} & 0 \\ 1 & -\epsilon_{s_1}^{v_2} & -\epsilon_{s_2}^{v_2} \\ 1 & 0 & -\epsilon_{s_2}^{v_3} \end{bmatrix} =$$

…and then premultiply the matrix of elasticities…

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The zero values imply that normally S_1 has no effect on v_3 and S_2 has no effect on v_1.

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This is called the *partitioned response.*

It says that the effect of an external parameter is the result of multiplying the elasticity representing its effect on a particular enzyme by the flux control coefficient of the same enzyme.

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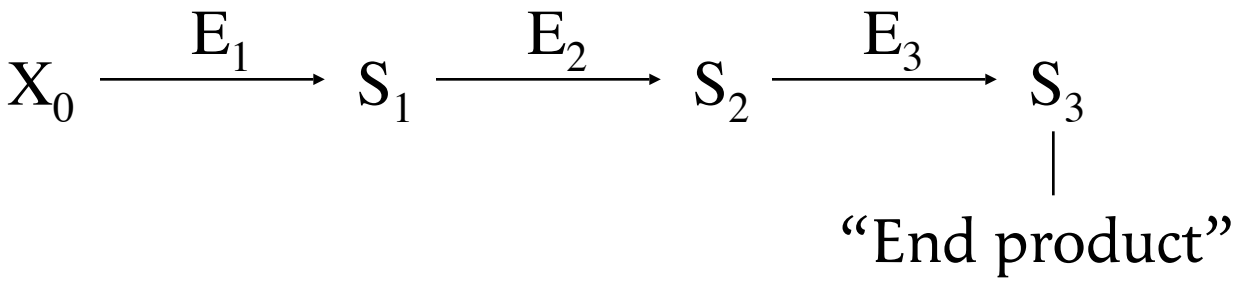
5. The law of supply and demand: does it always apply?

In biochemistry texts one often sees this sort of diagram:



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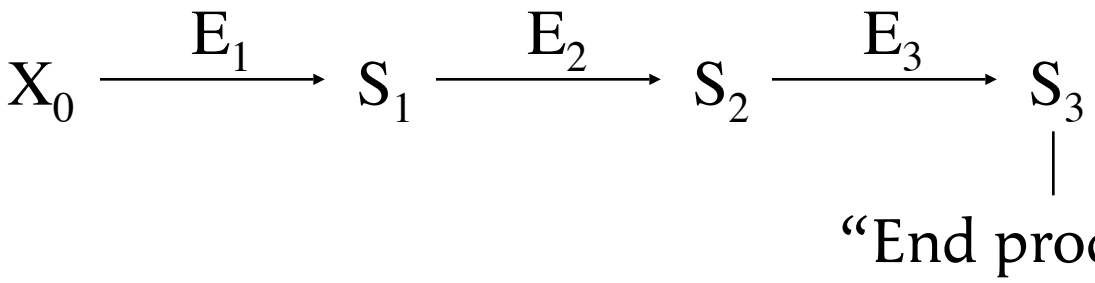

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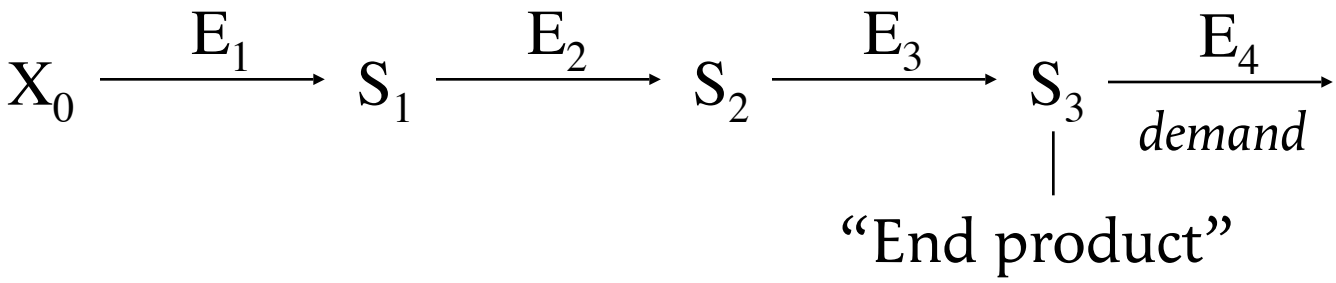

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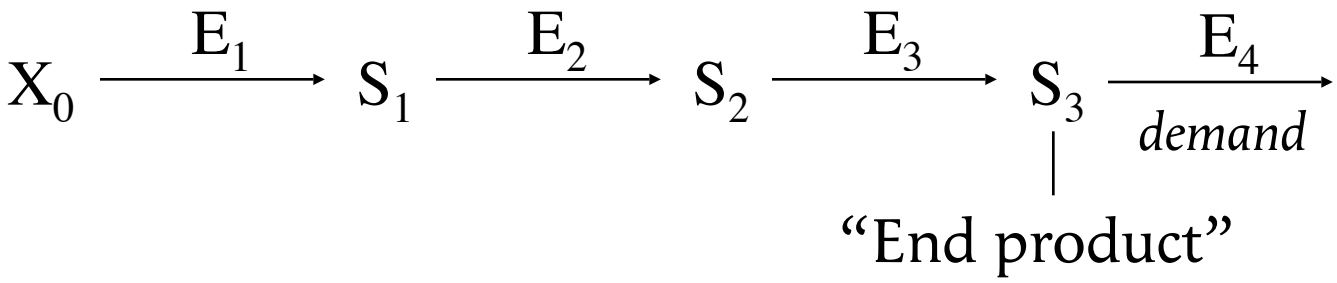

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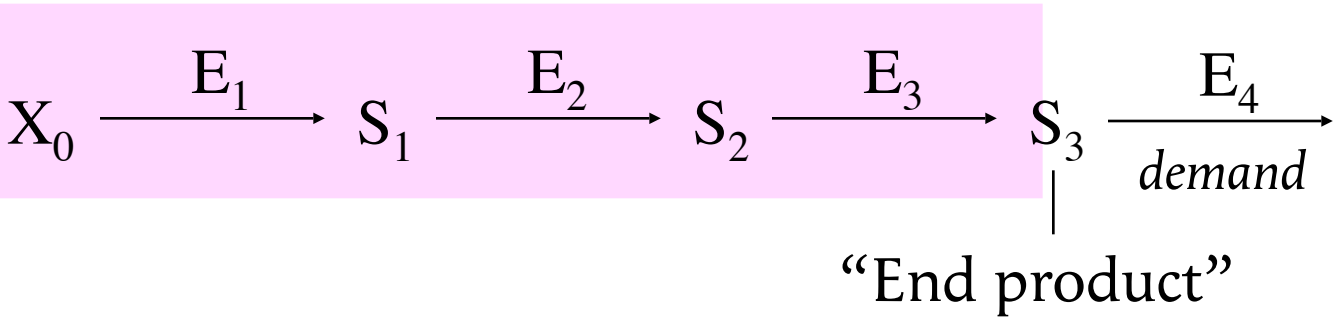

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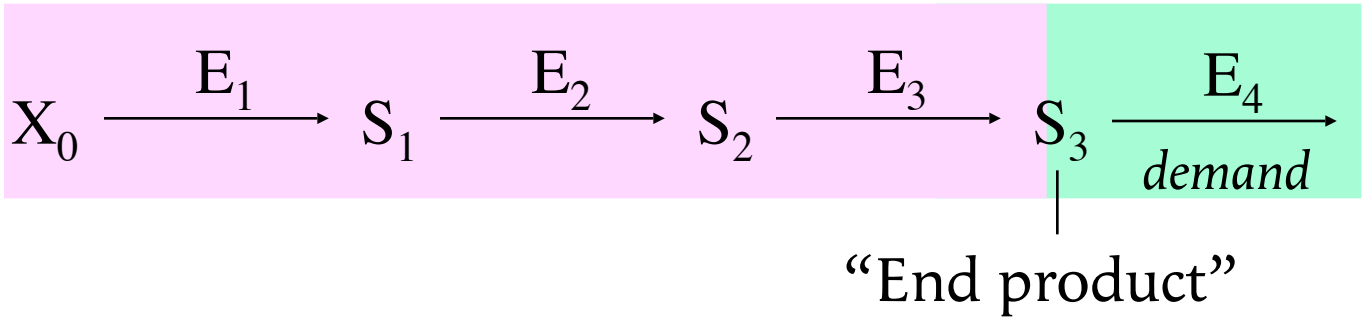

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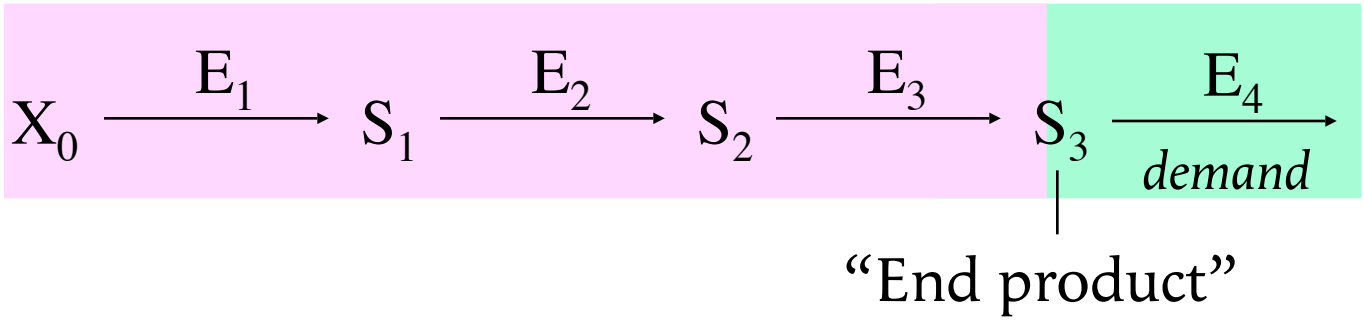

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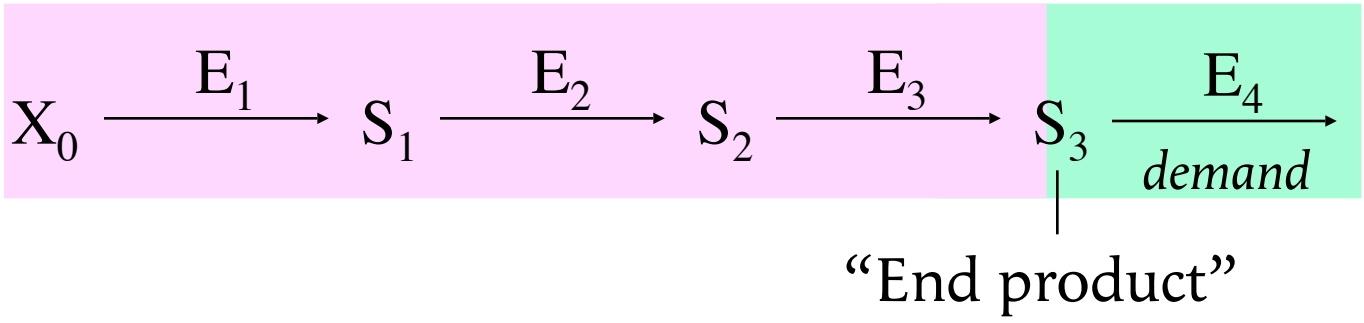

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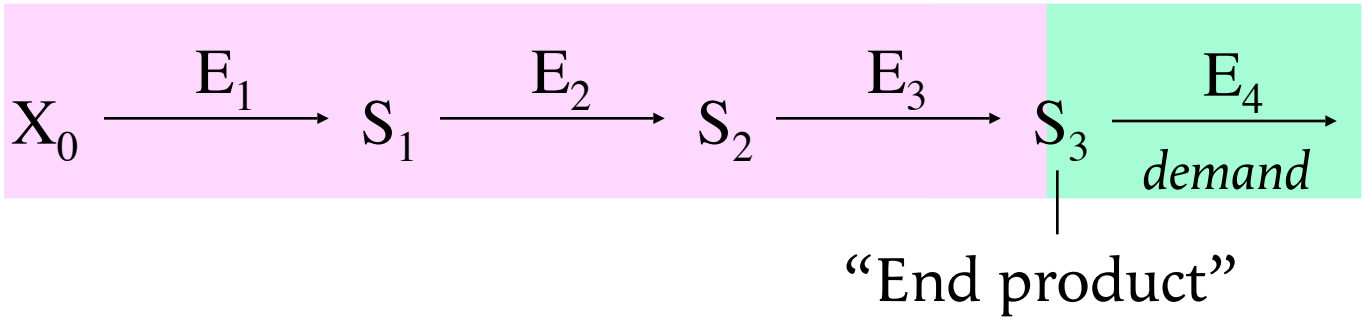

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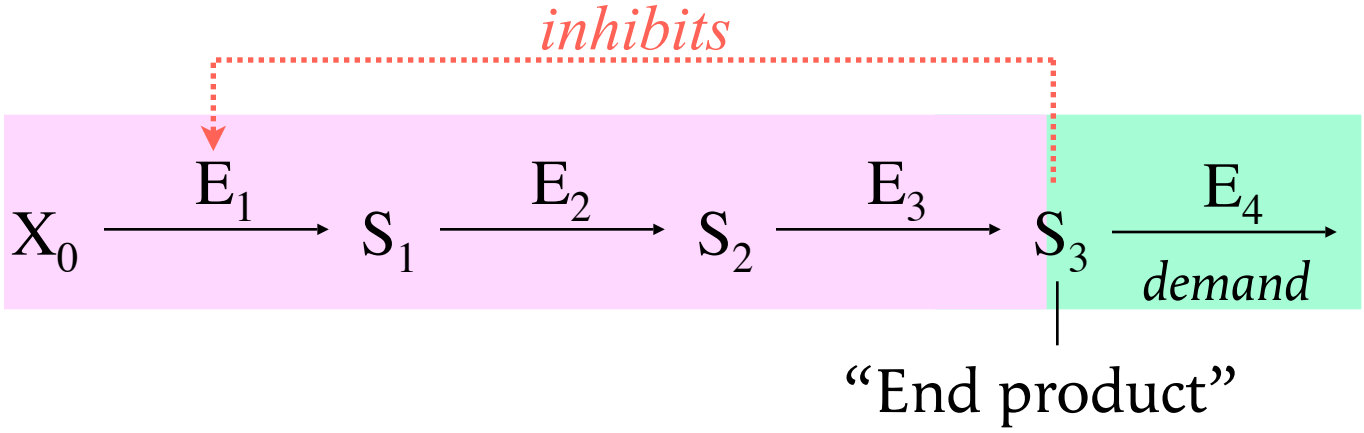

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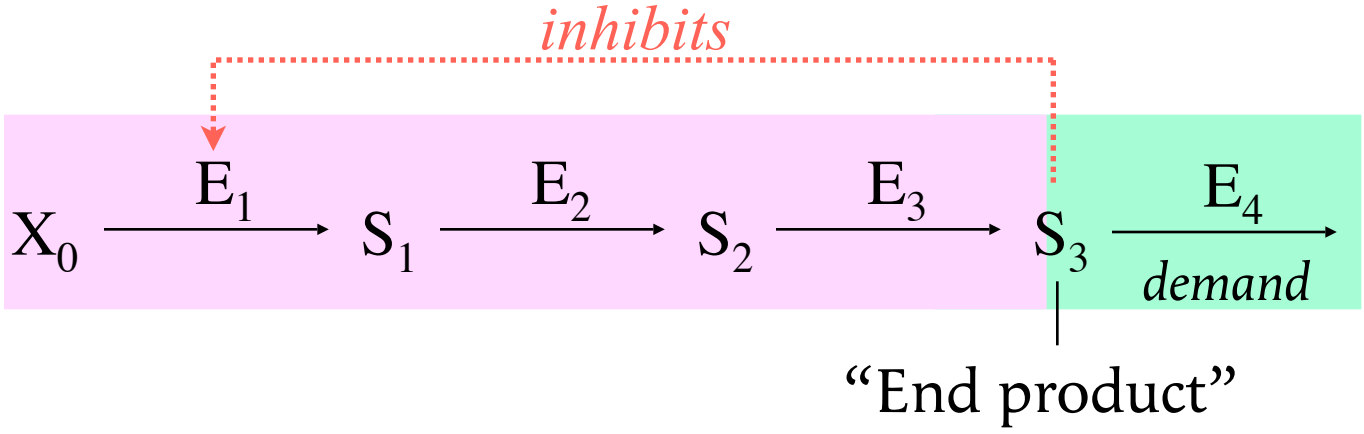

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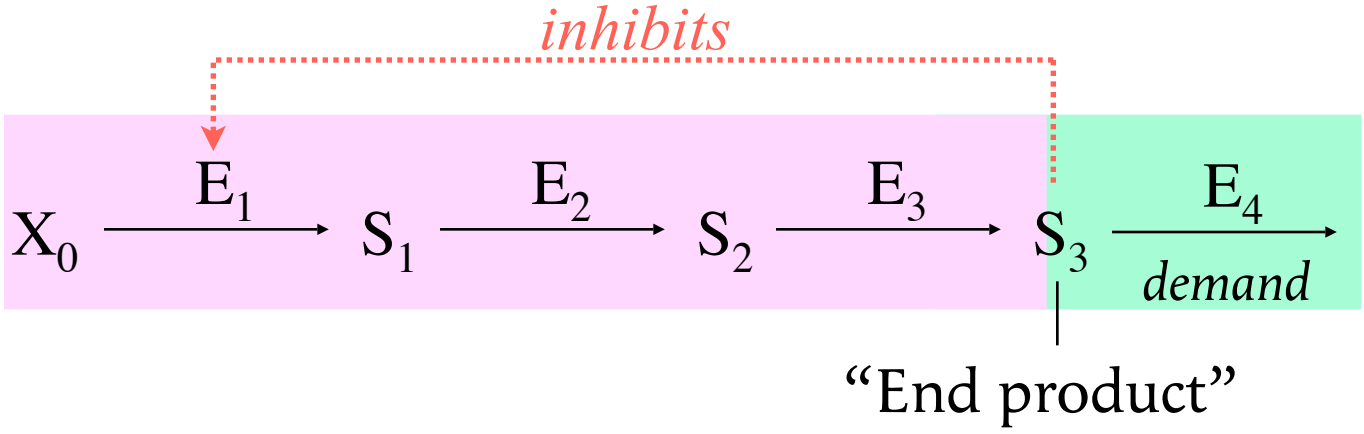

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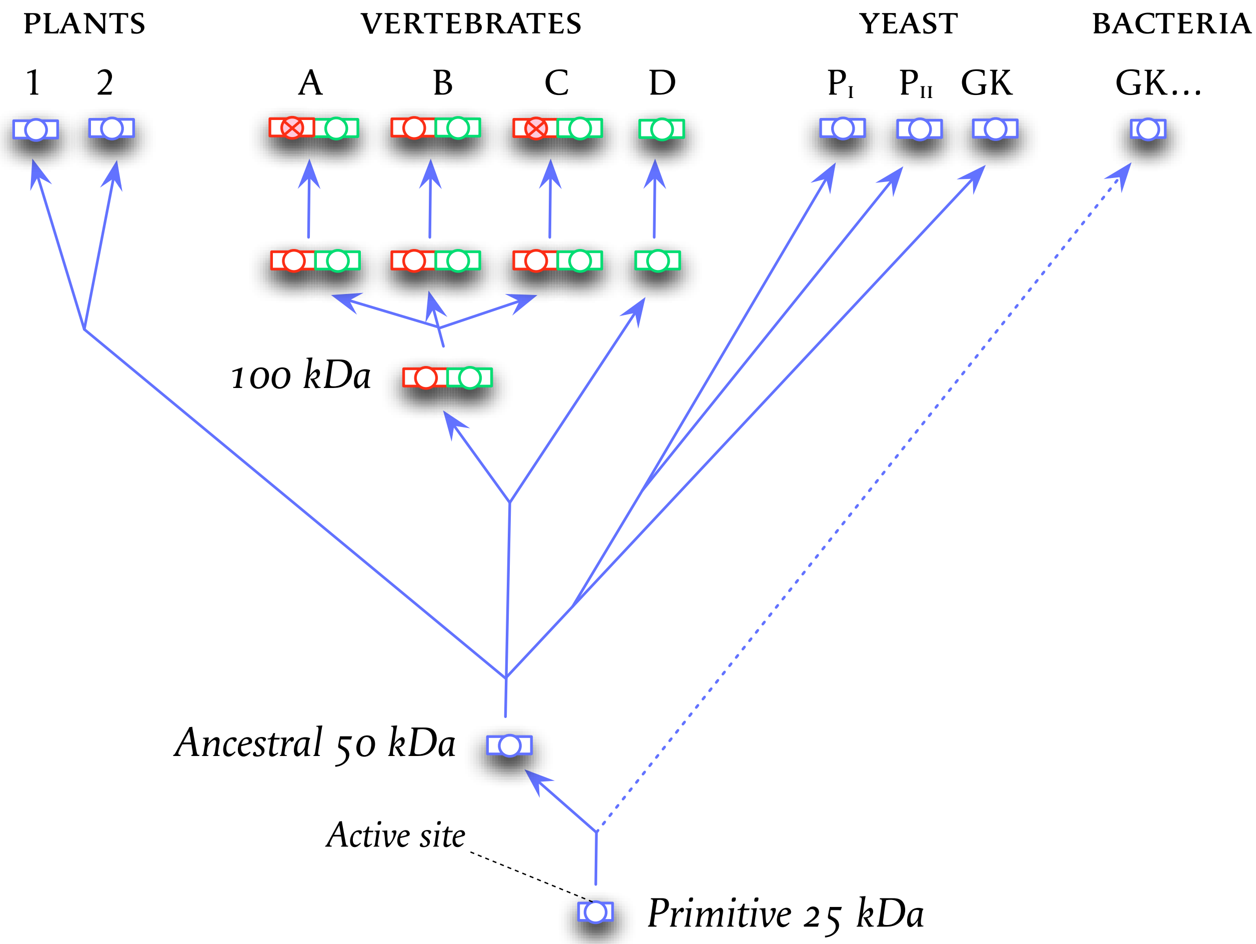

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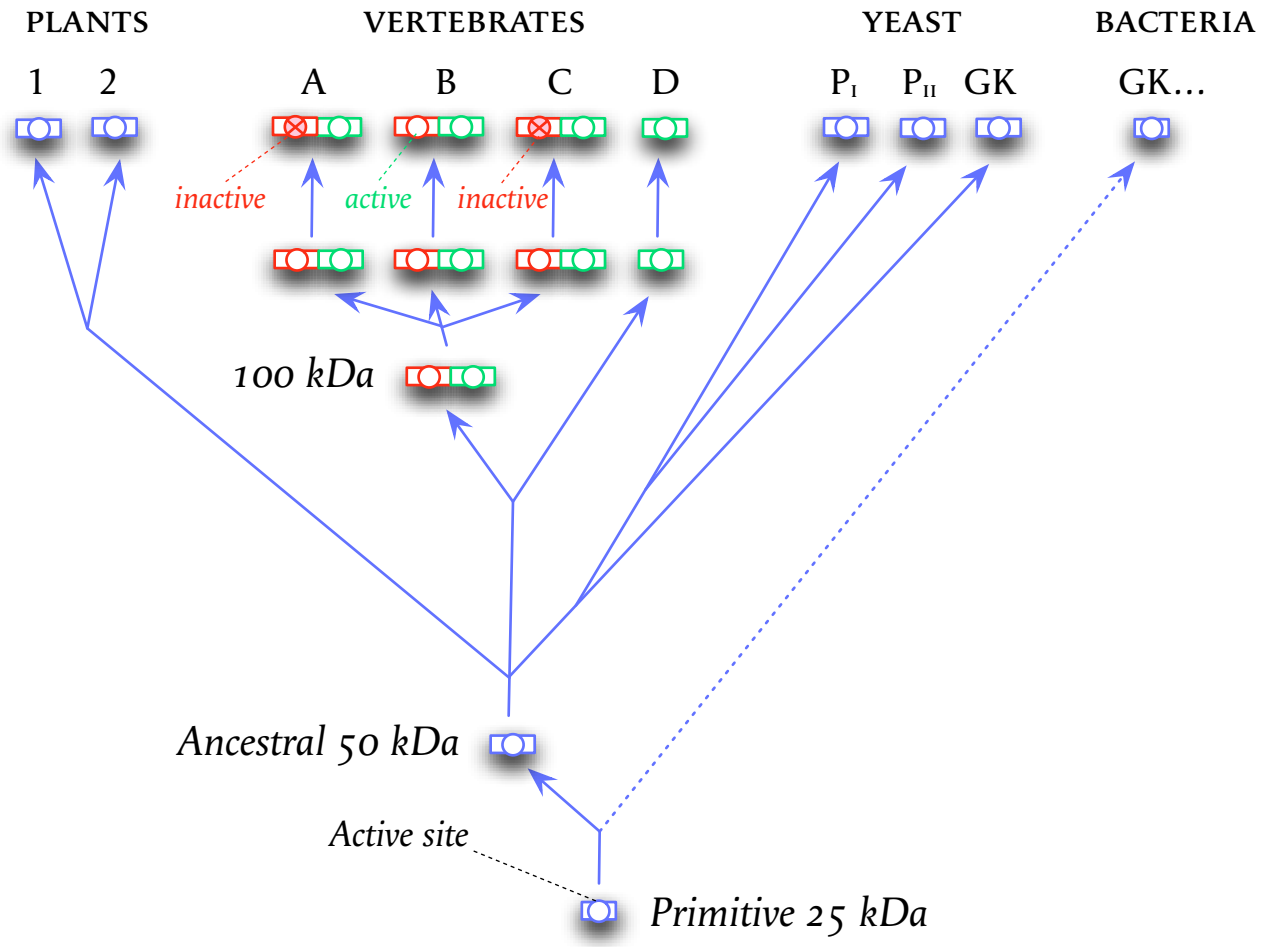

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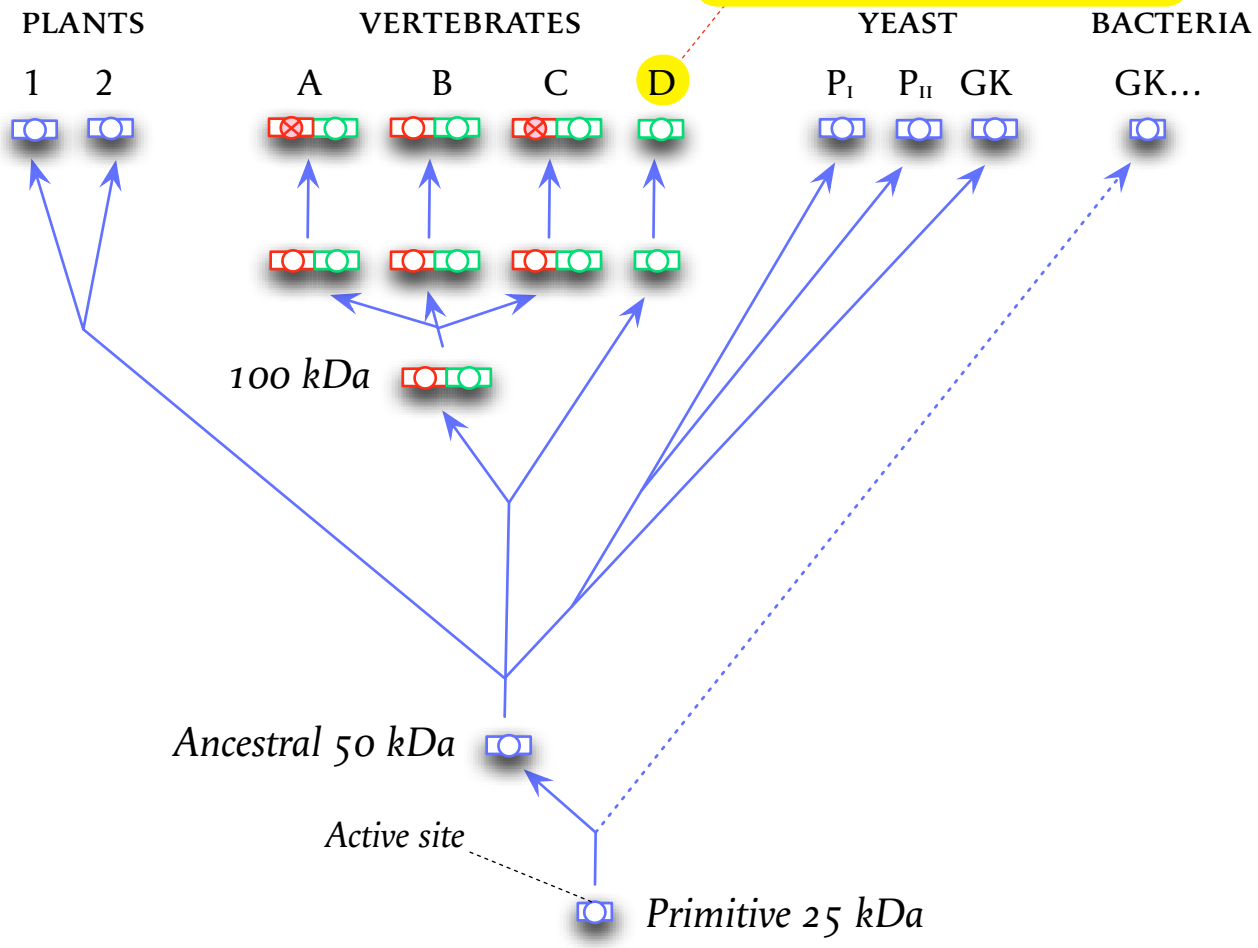

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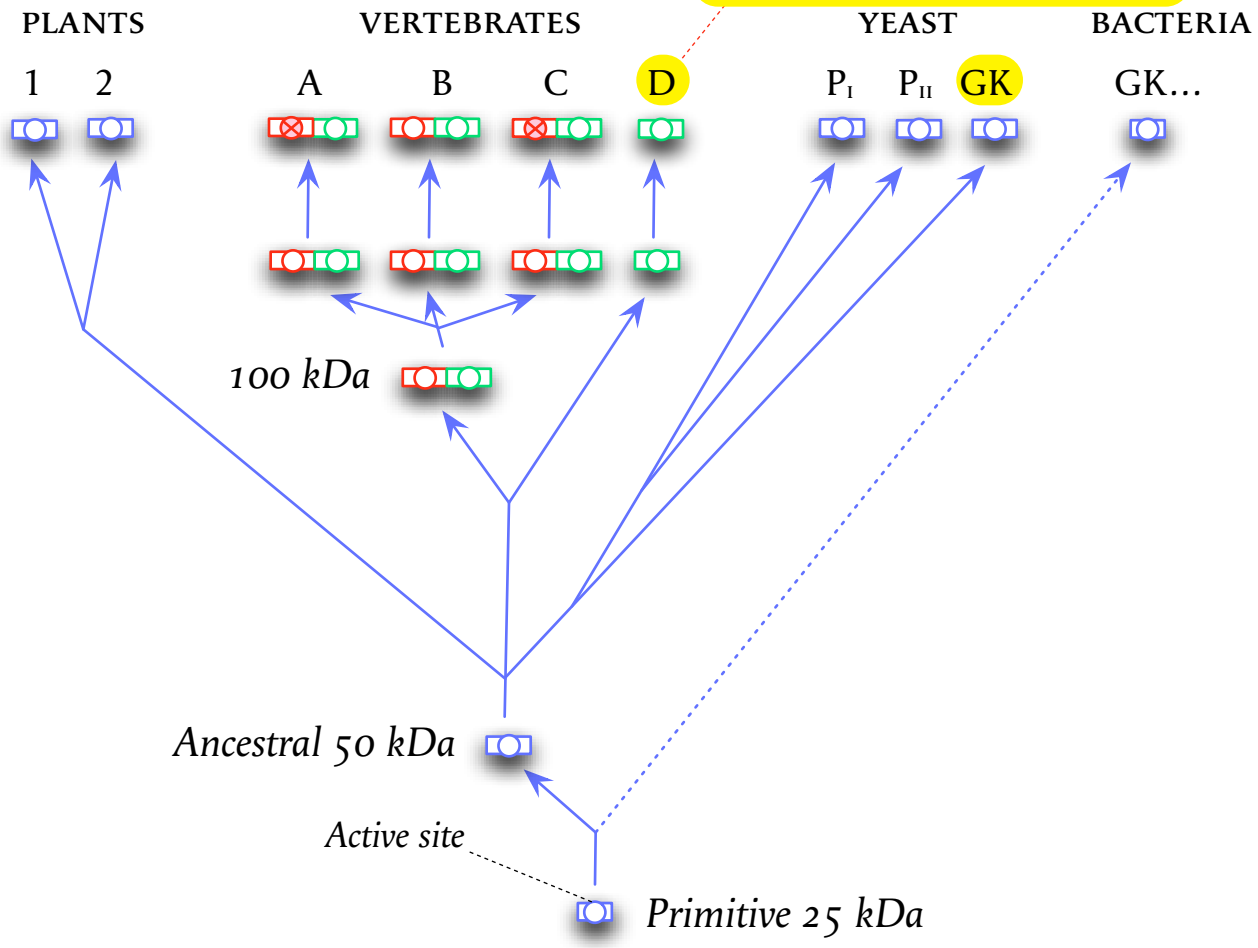

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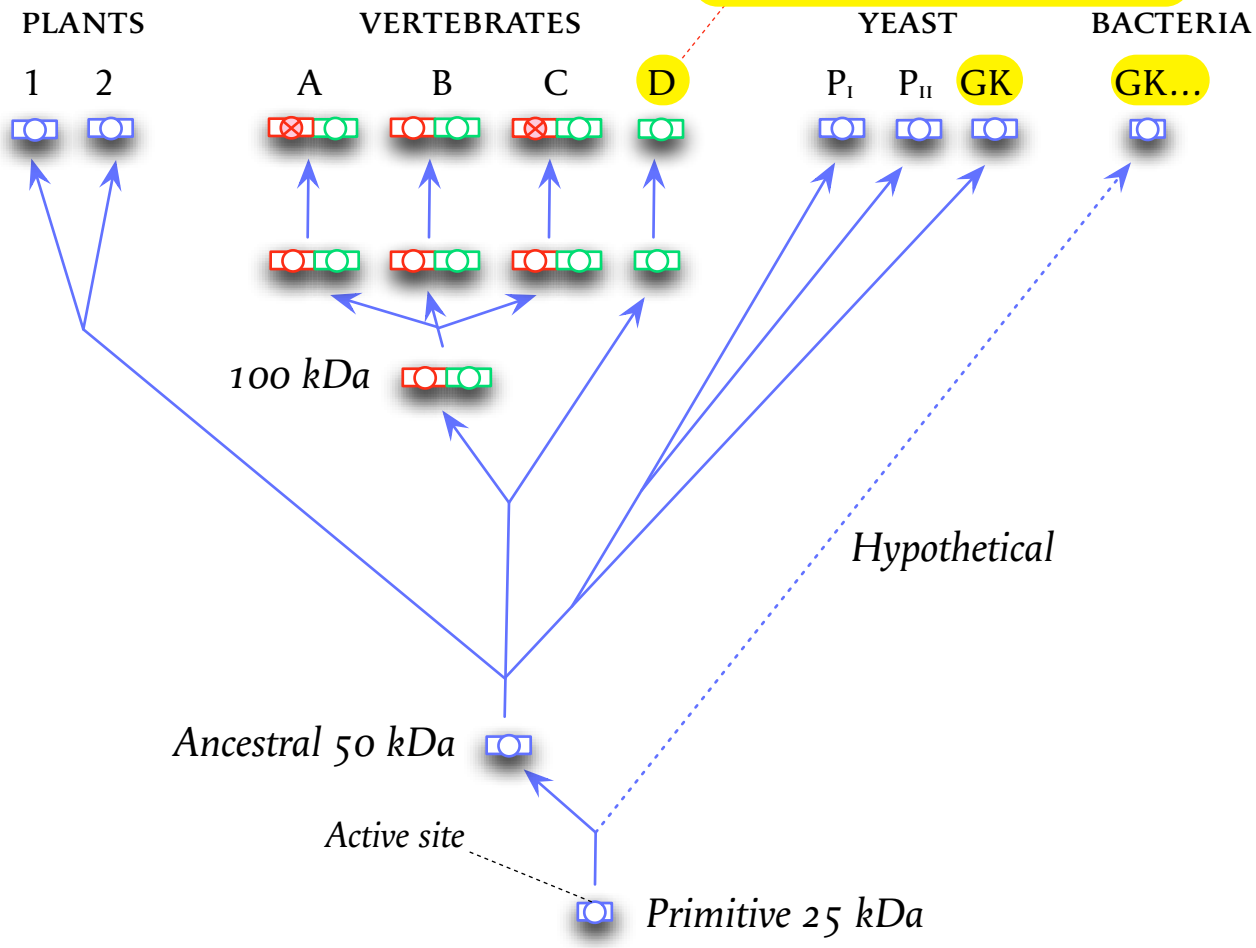

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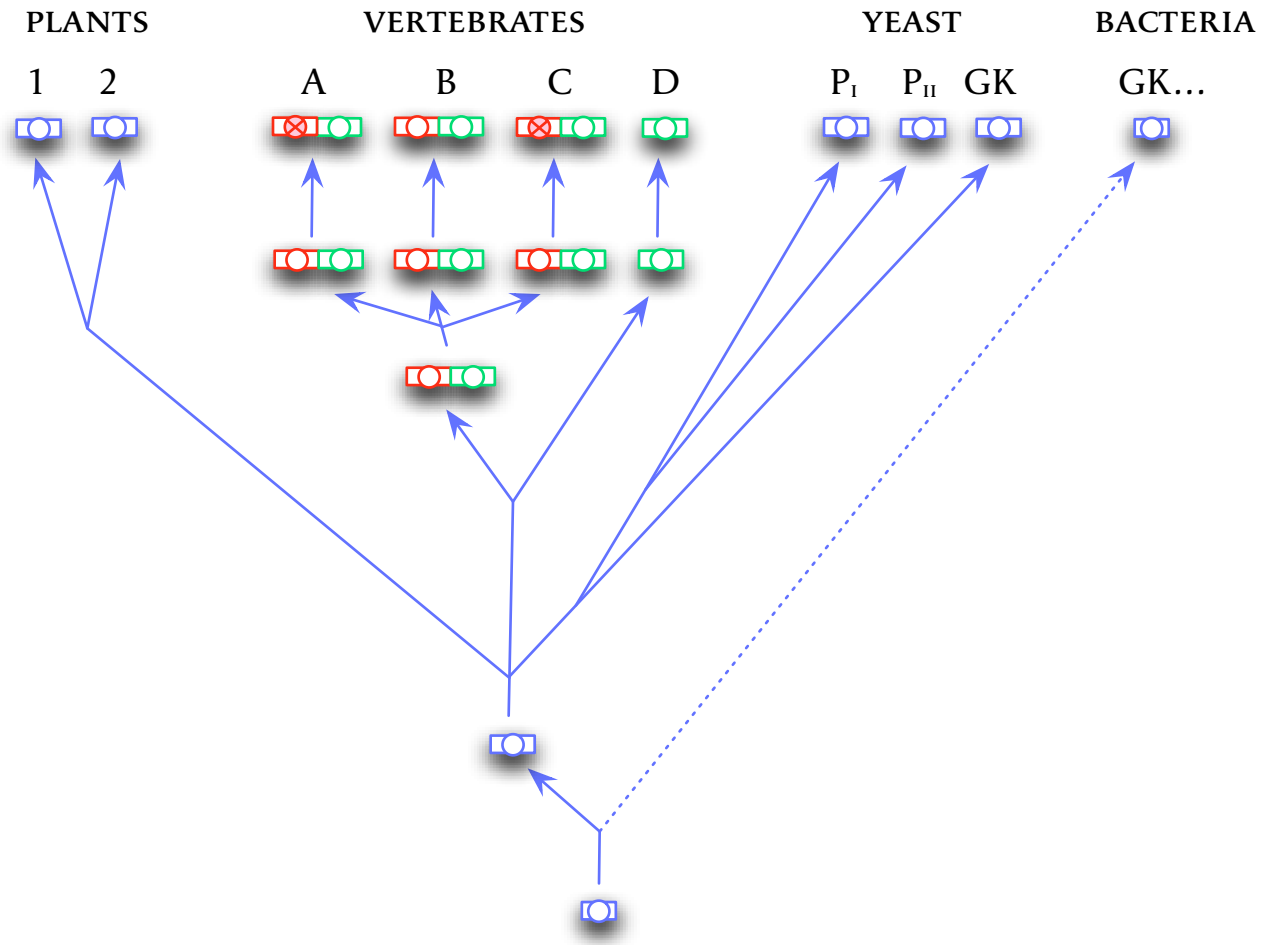

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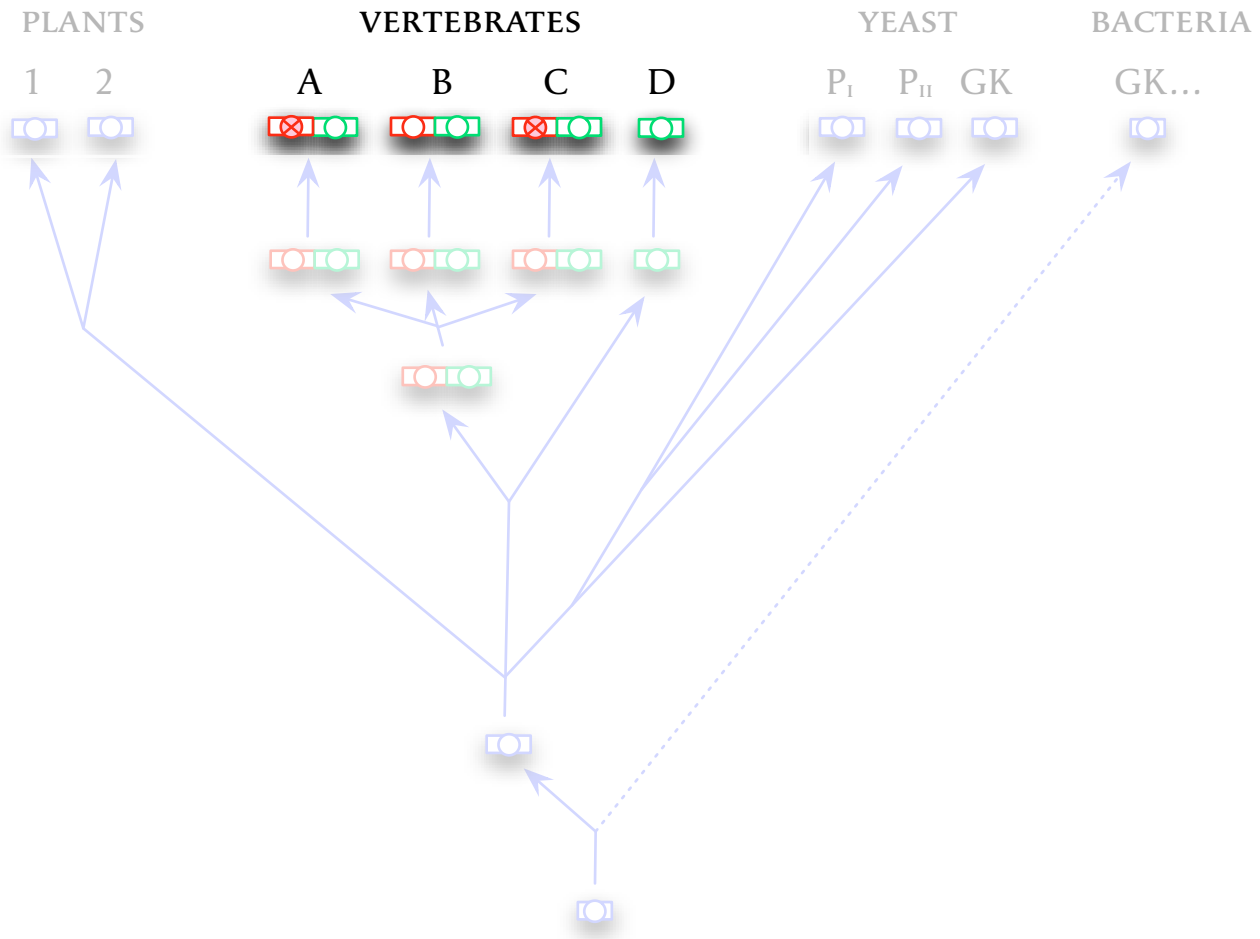

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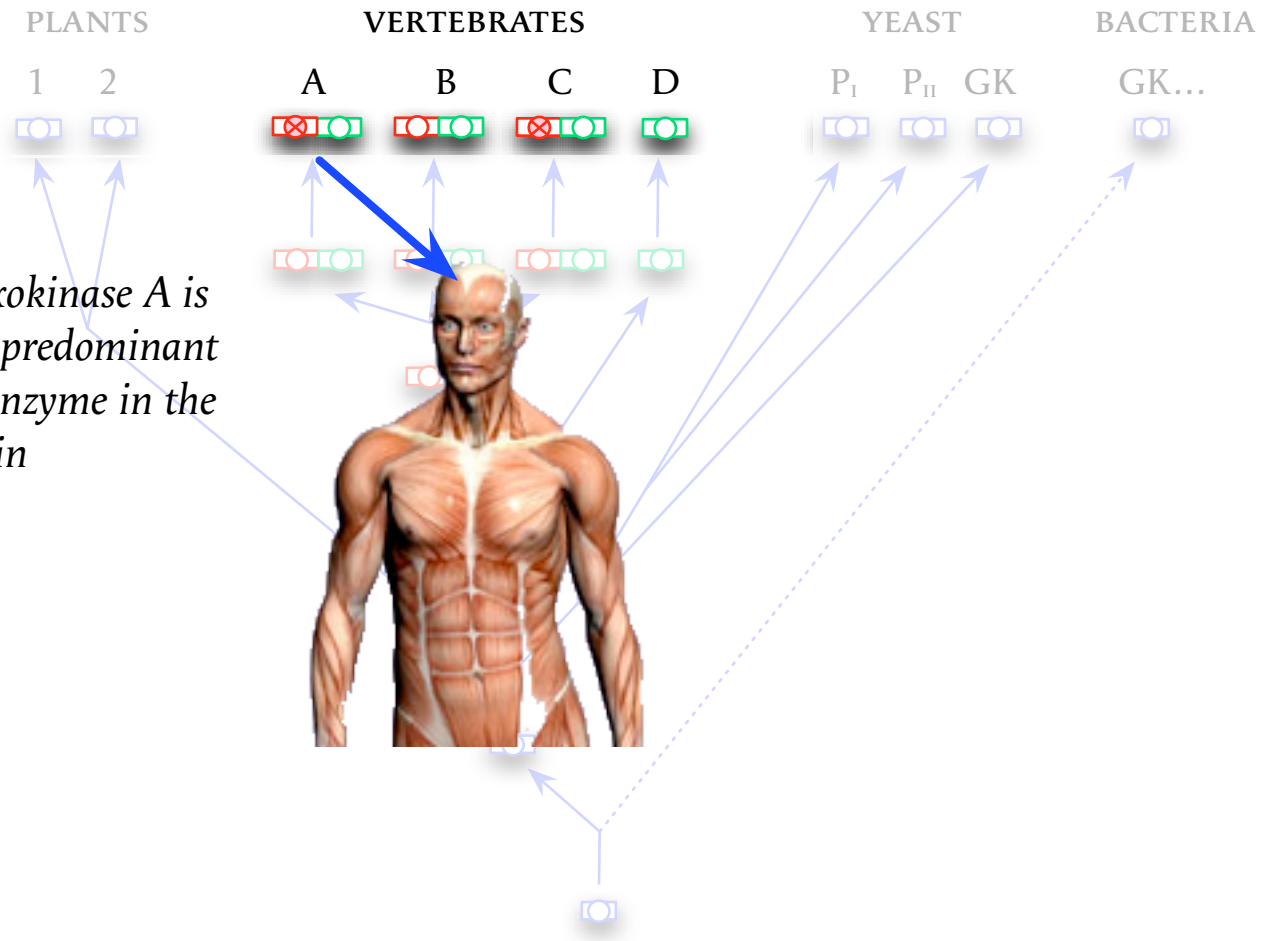

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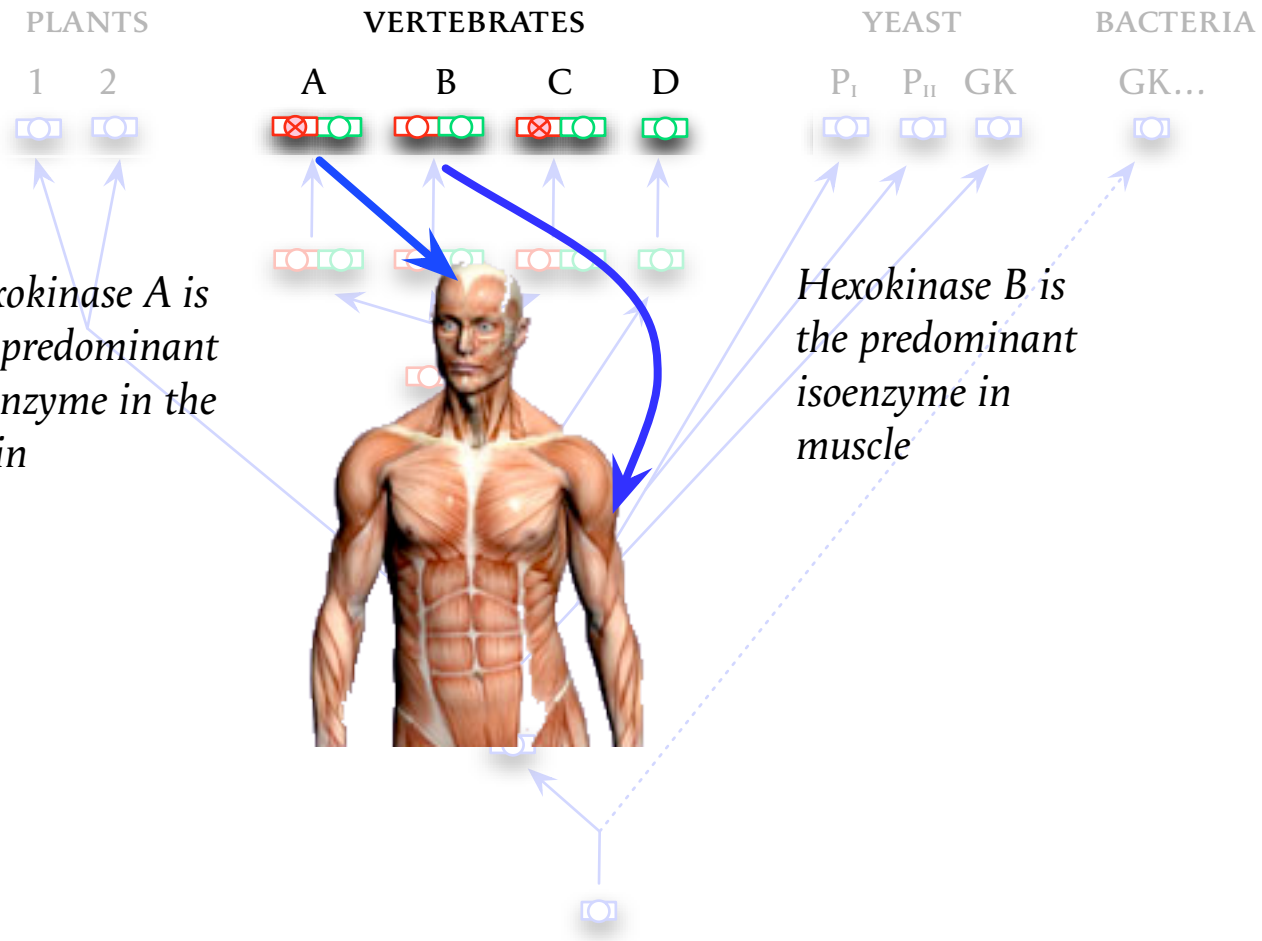

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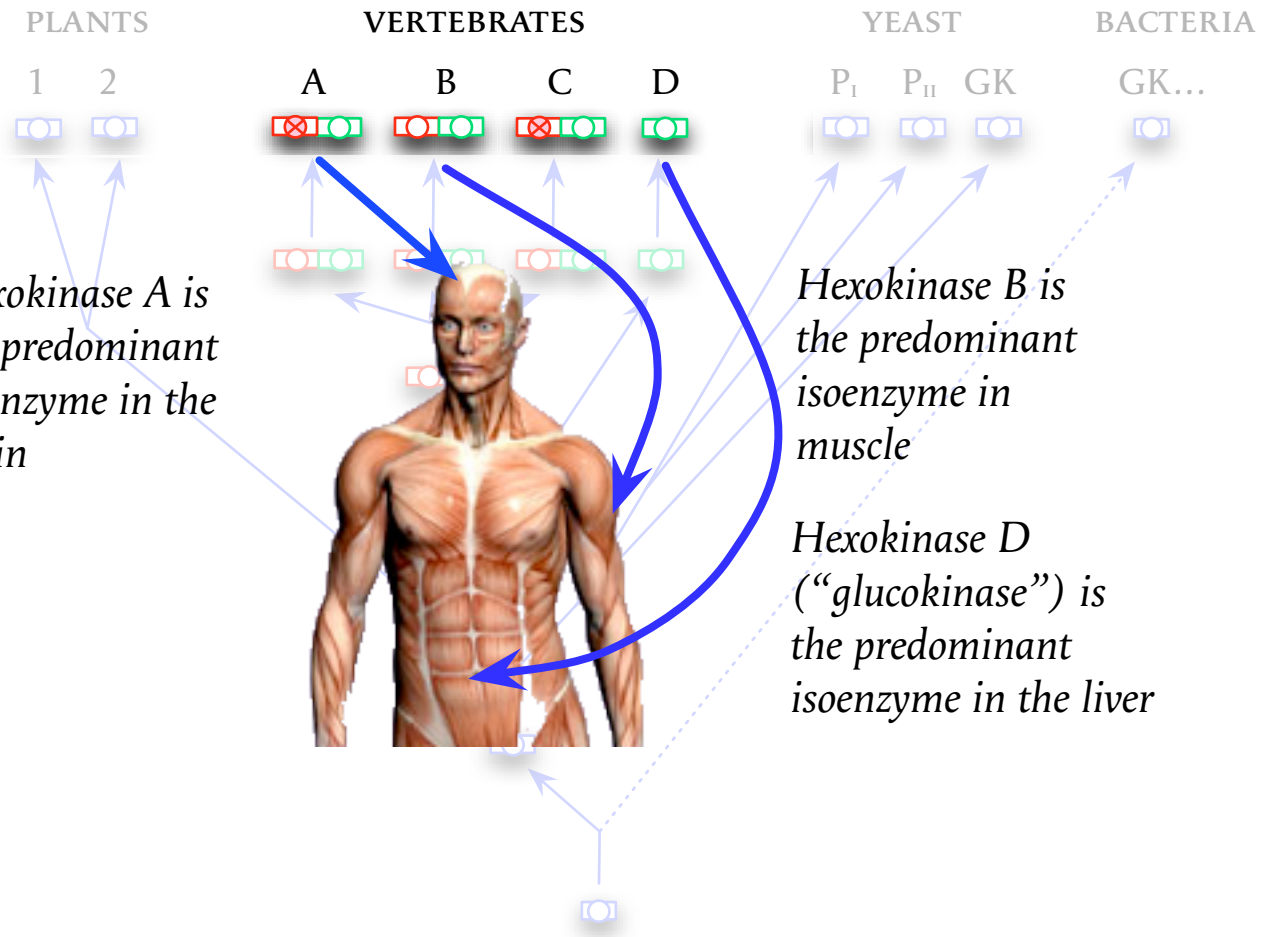

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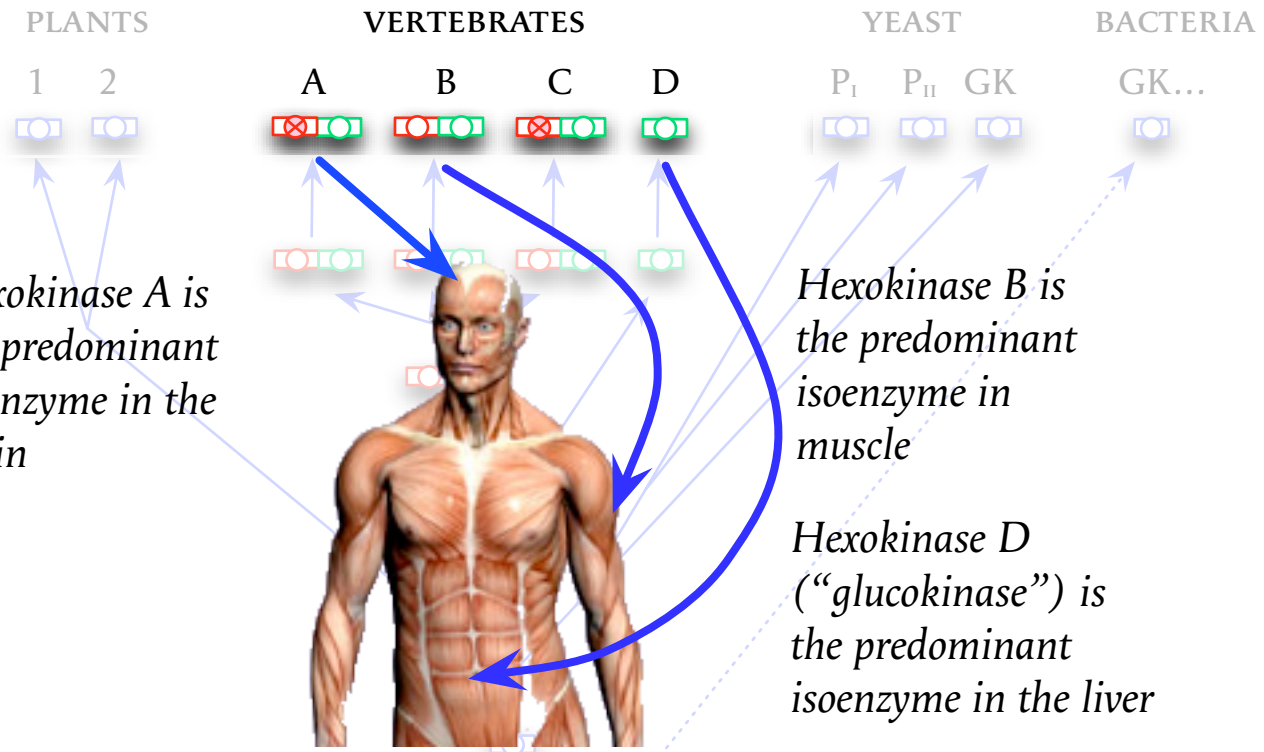
Hexokinase B is the predominant isoenzyme in muscle

Hexokinase D ("glucokinase") is the predominant isoenzyme in the liver

Hexokinases in mammals



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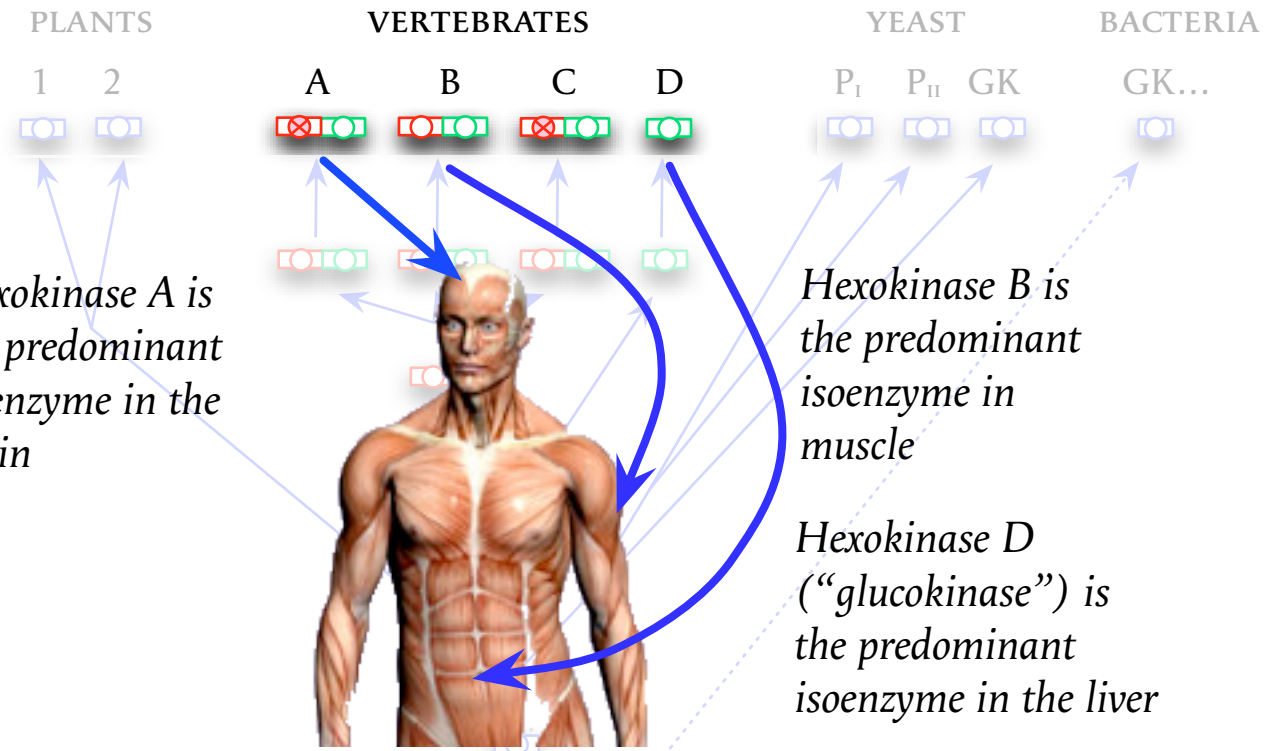

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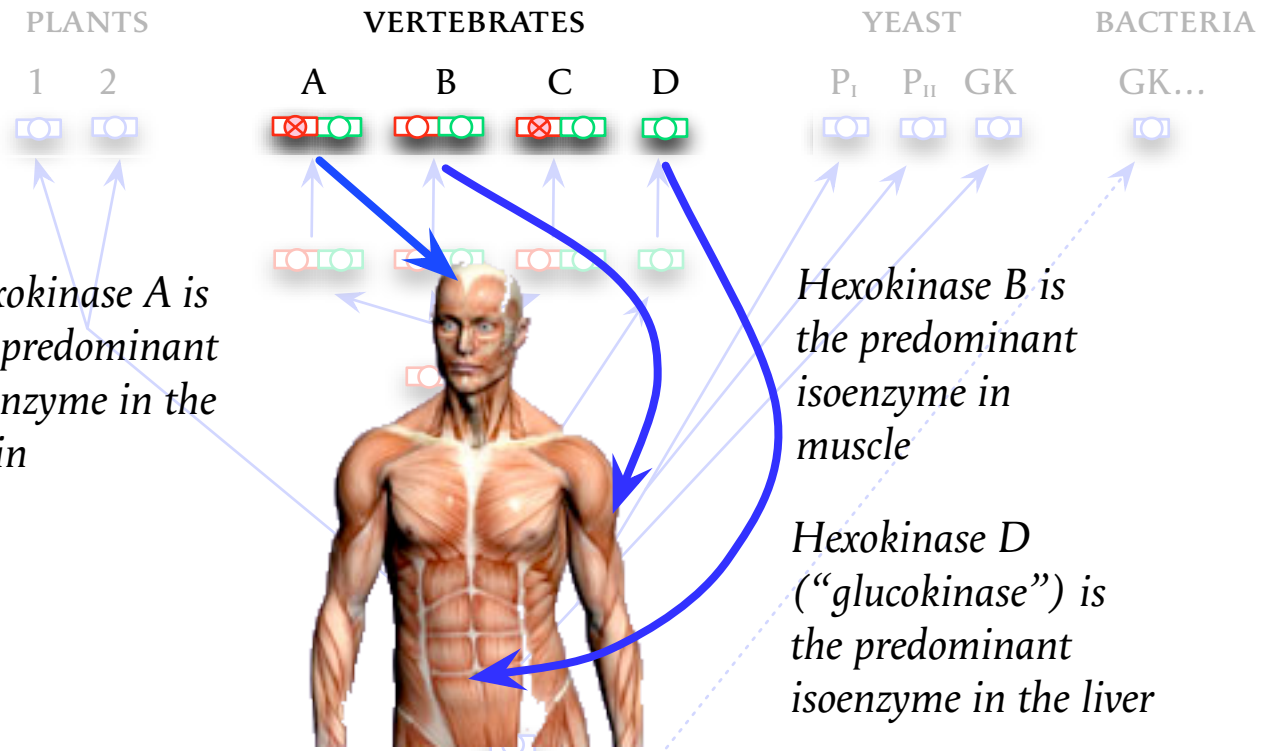

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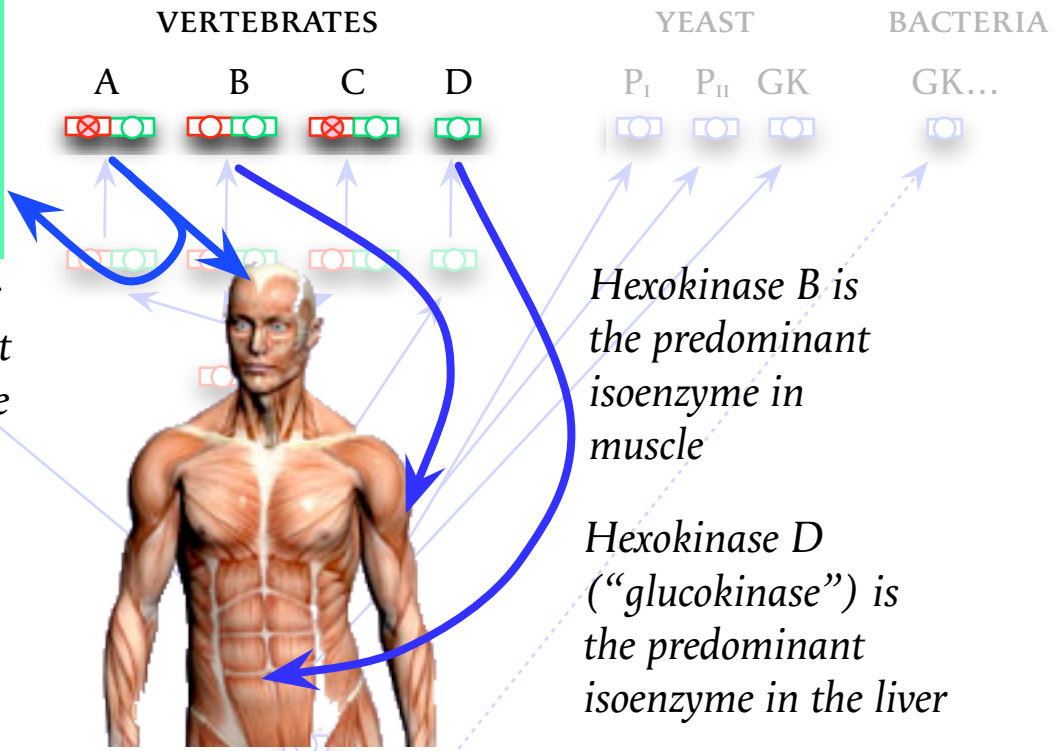

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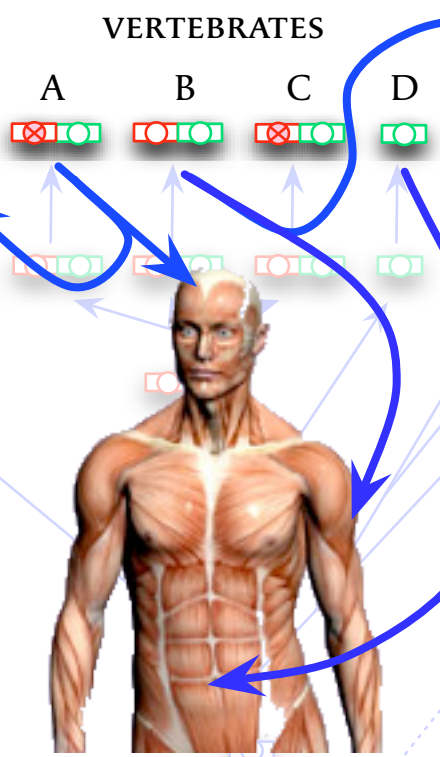

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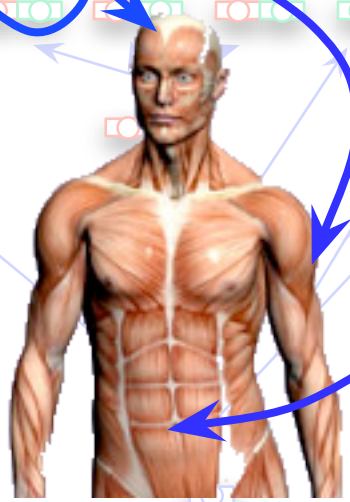

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demand

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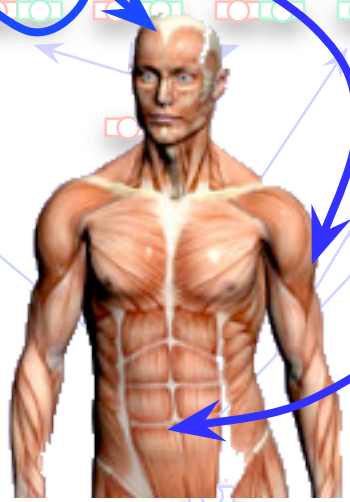

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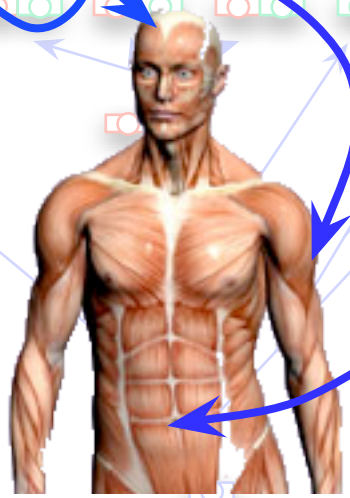

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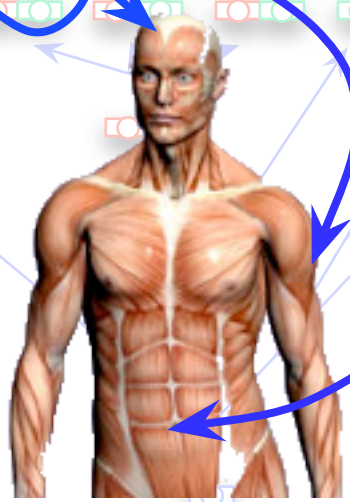

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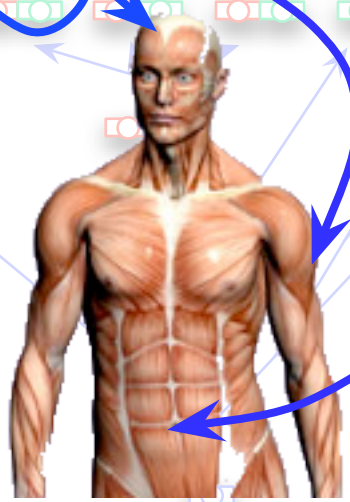

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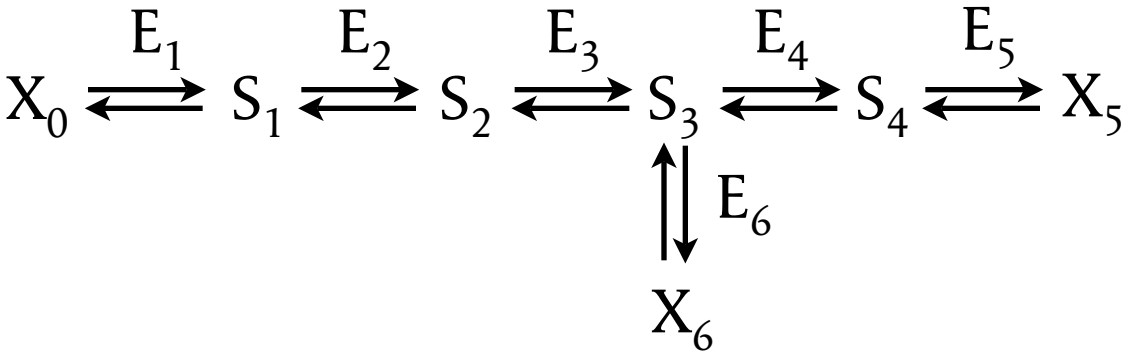
What implications do these properties have for drug development?

MODELLING A METABOLIC SYSTEM

To model an arbitrary metabolic system…



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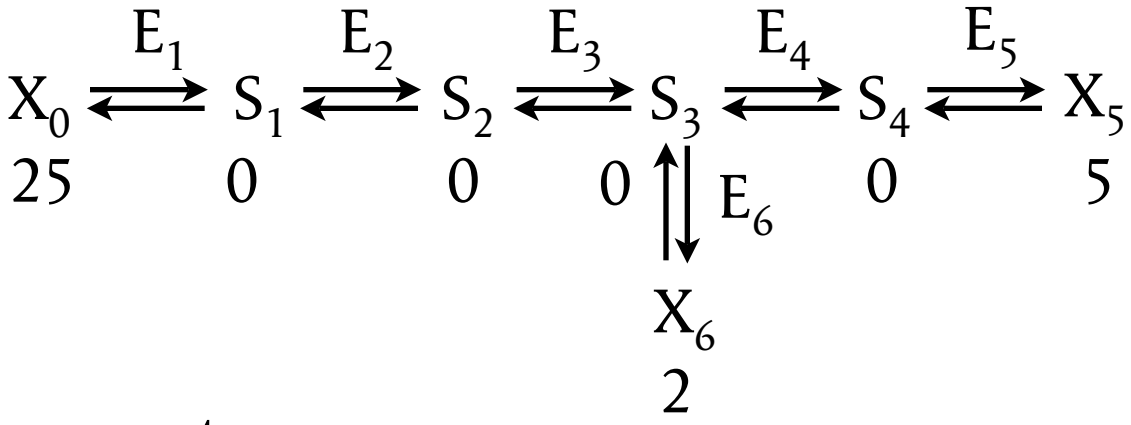

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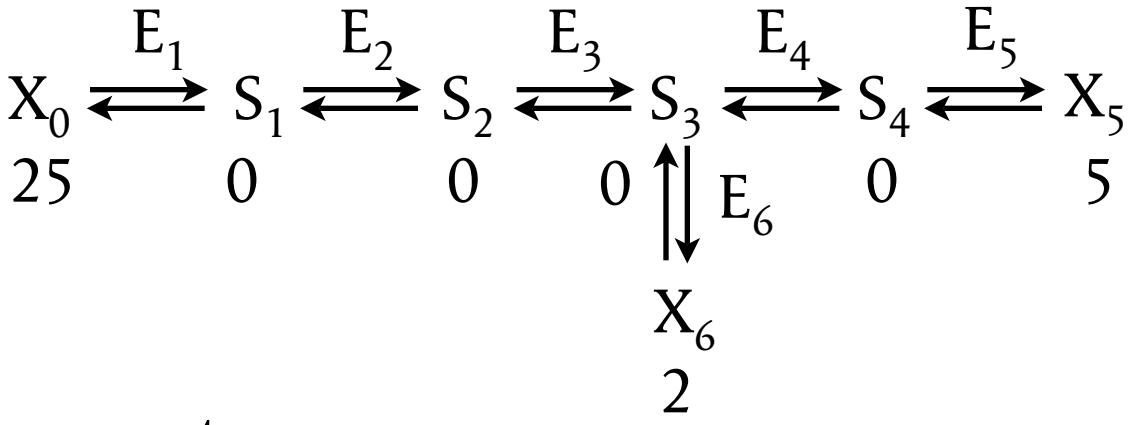

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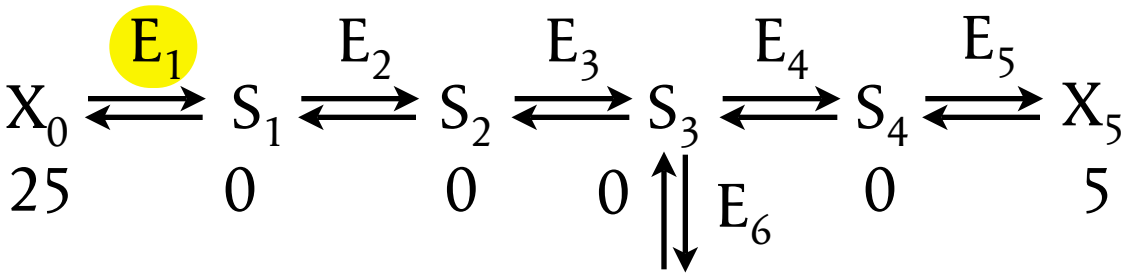

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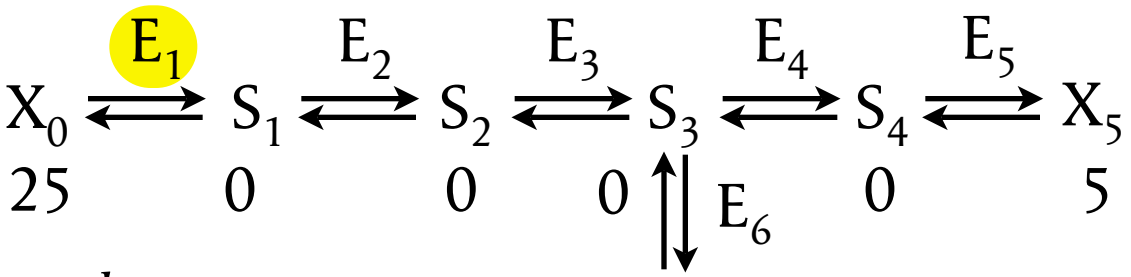

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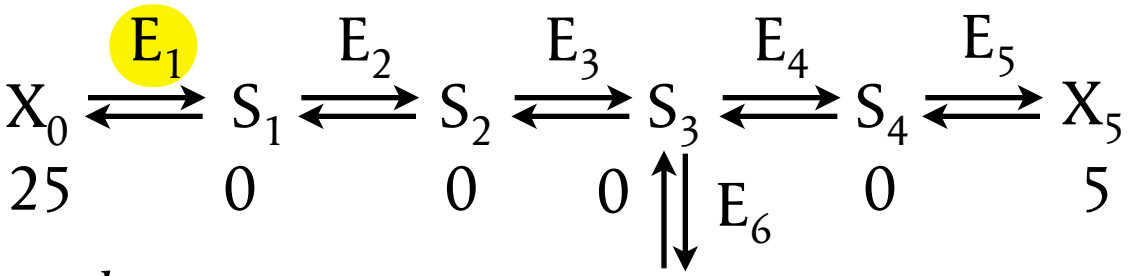

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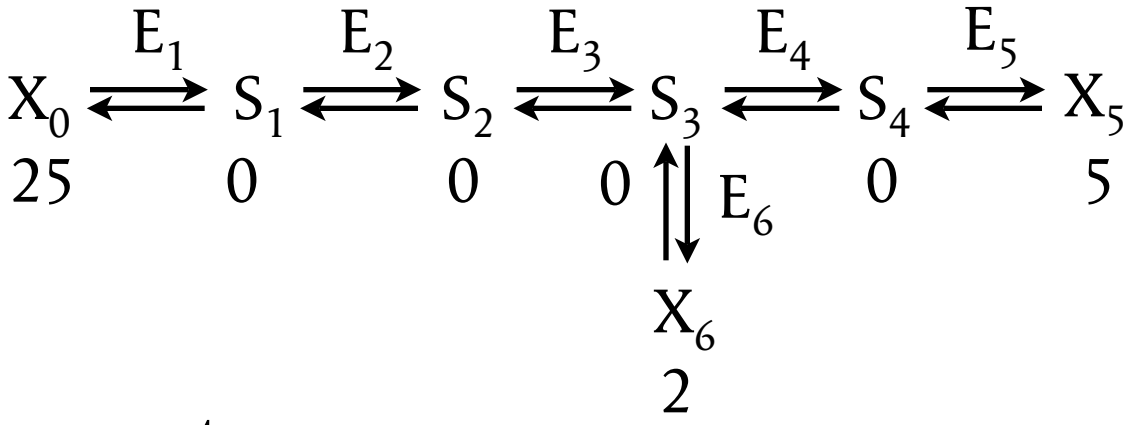

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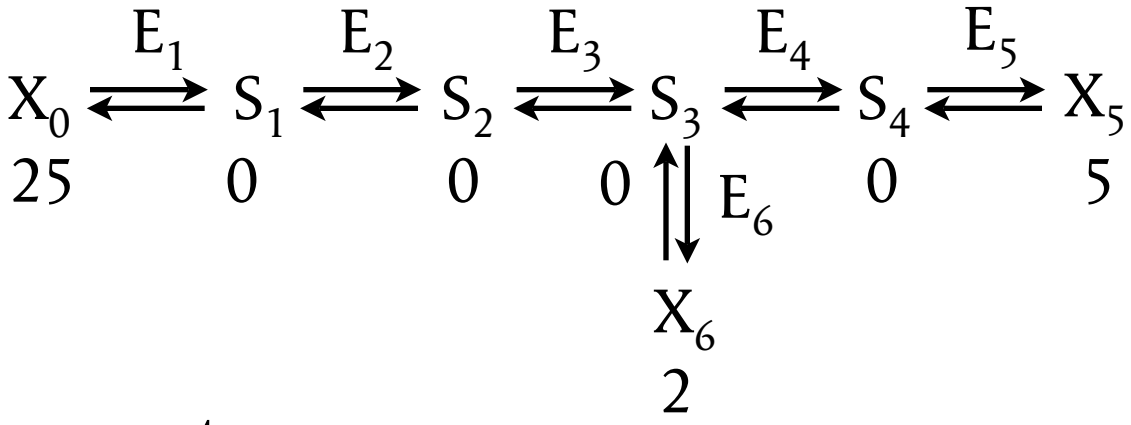

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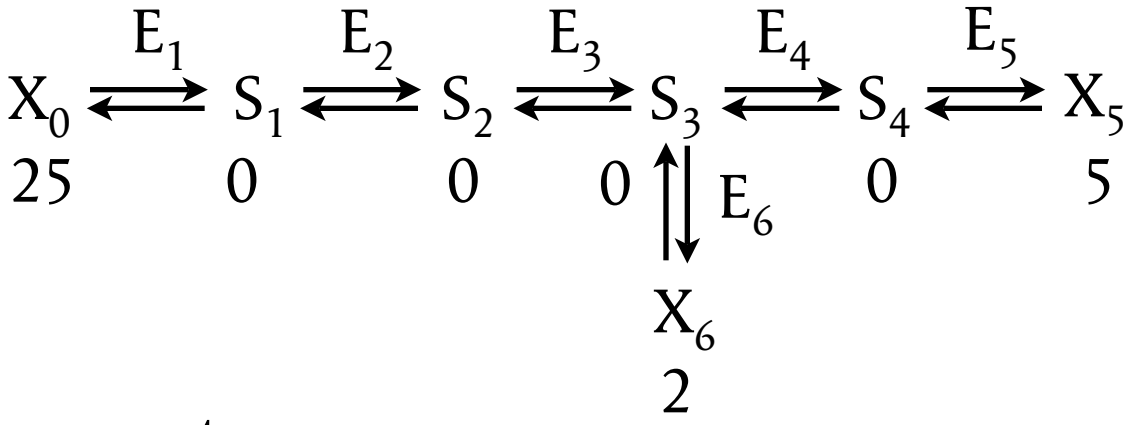

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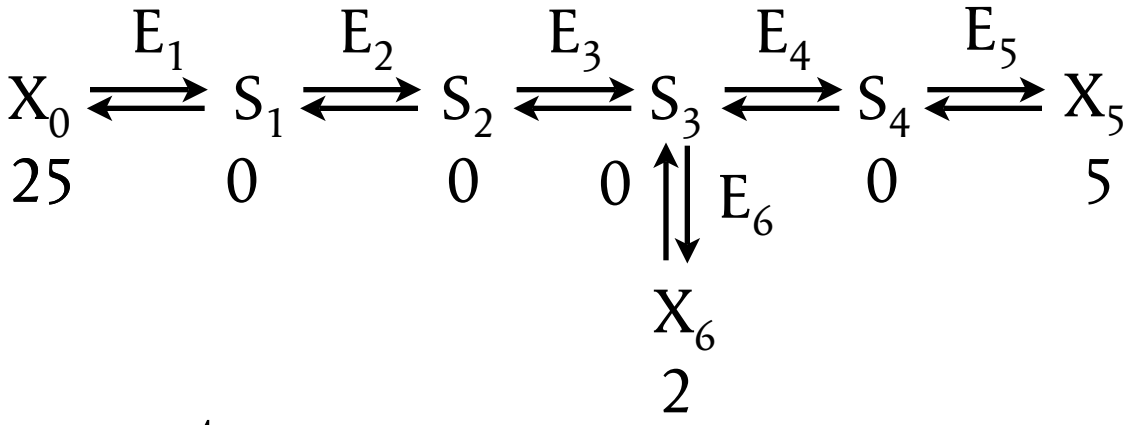

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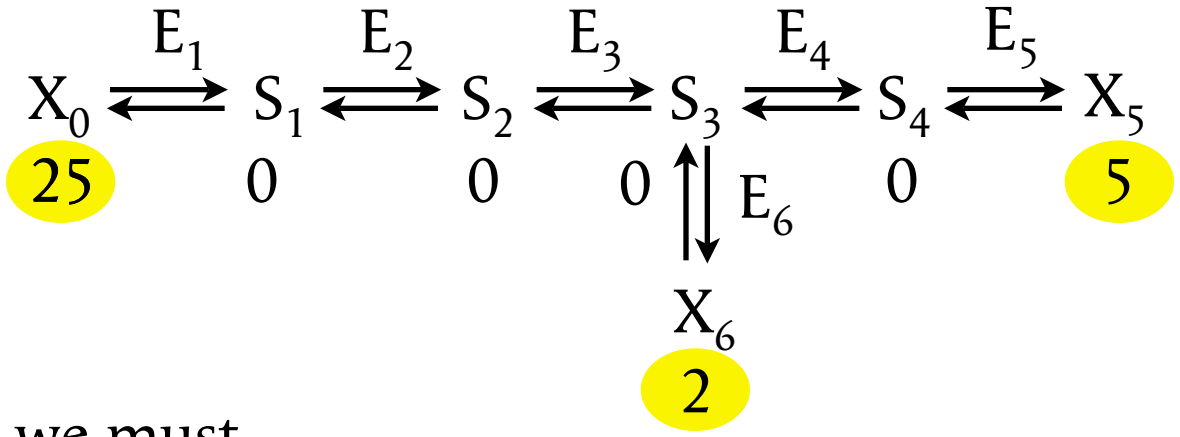

…we must

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2. *If the concentrations of the external metabolites are fixed, the system will evolve towards a steady state; otherwise, it will evolve towards equilibrium.*
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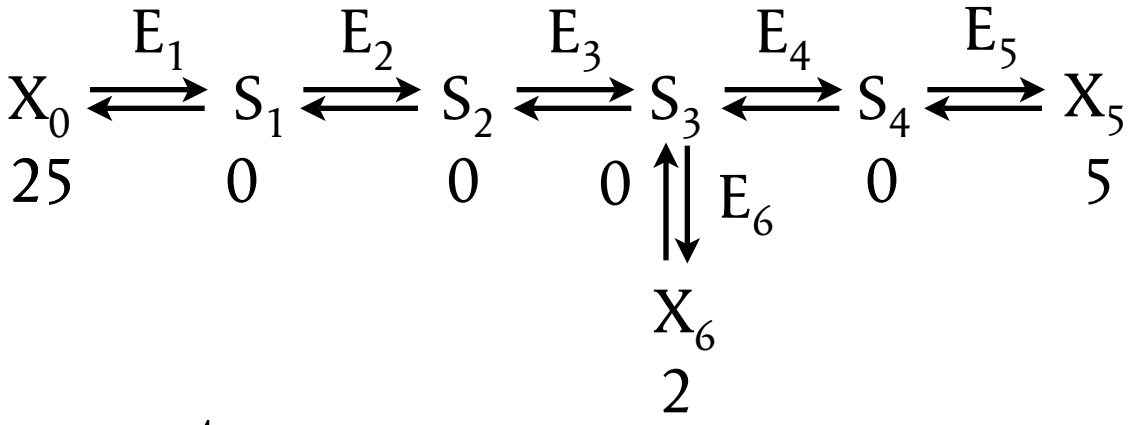

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But in any case, how are the calculations done?

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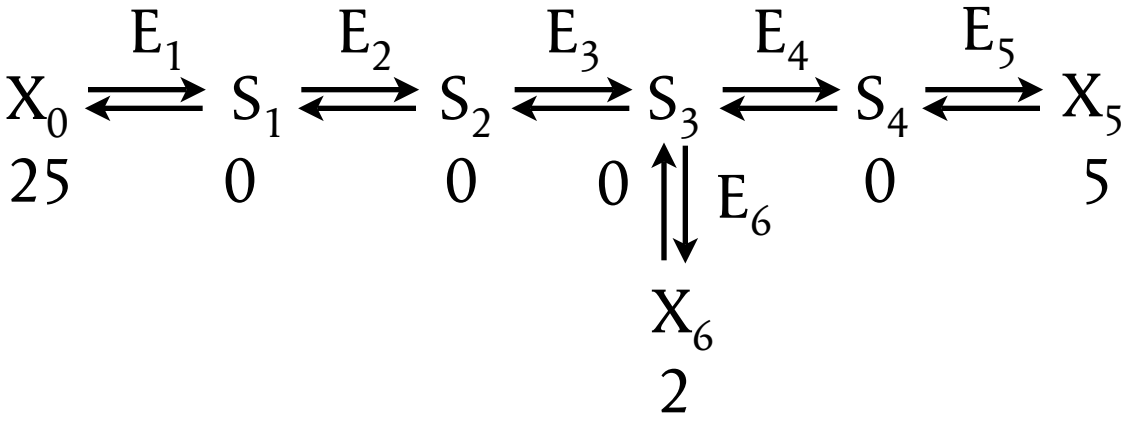

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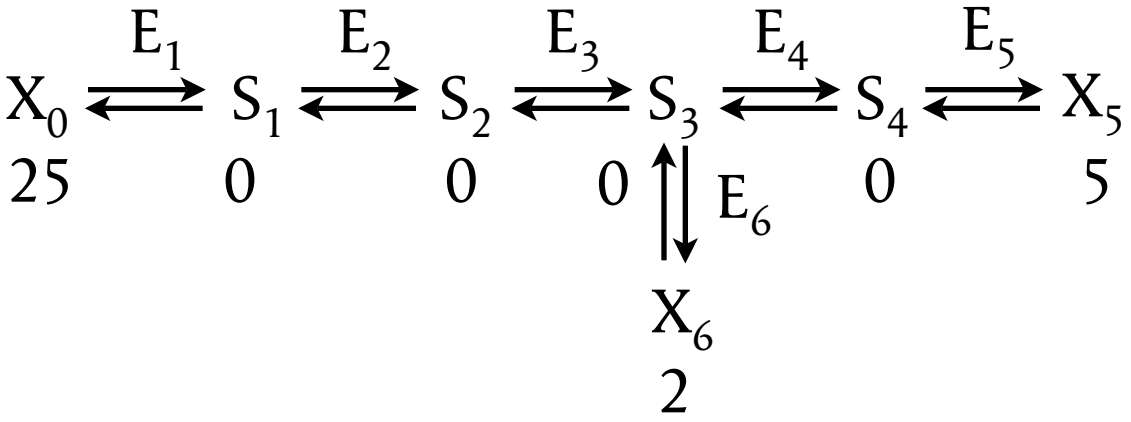

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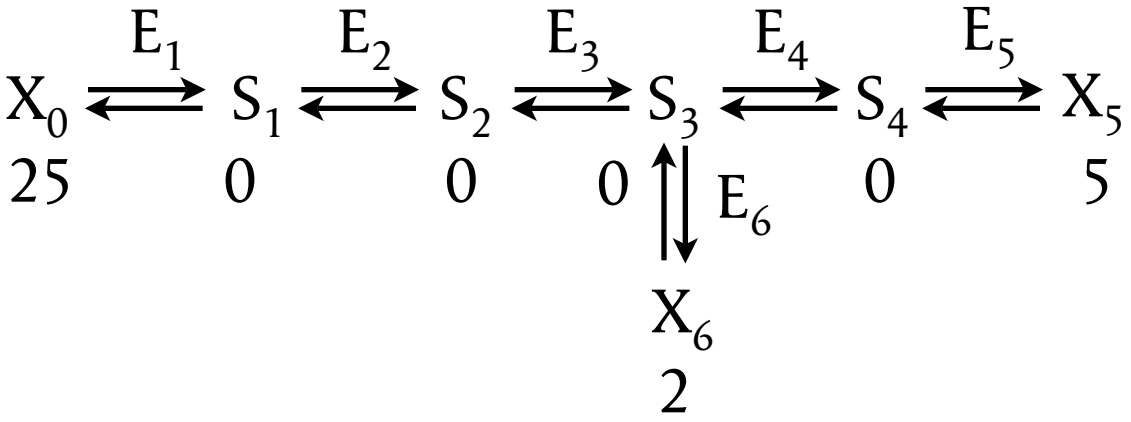

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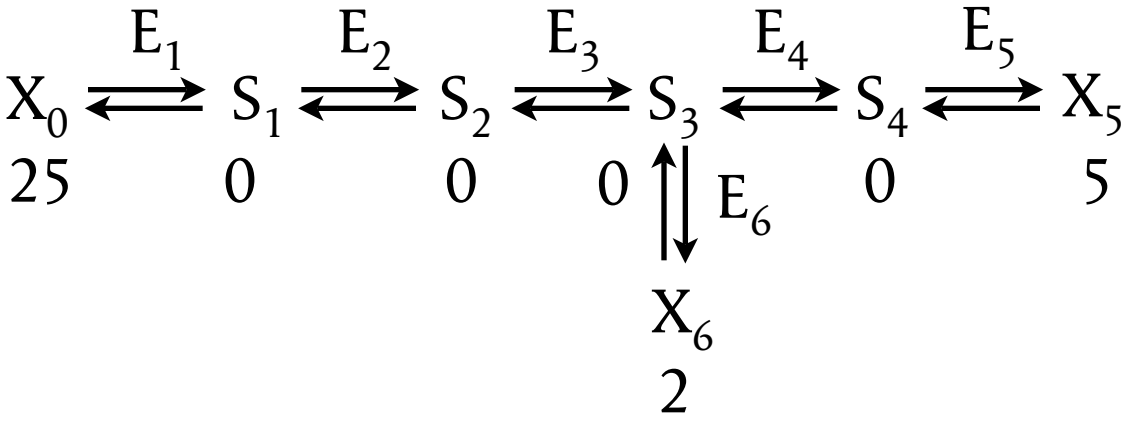

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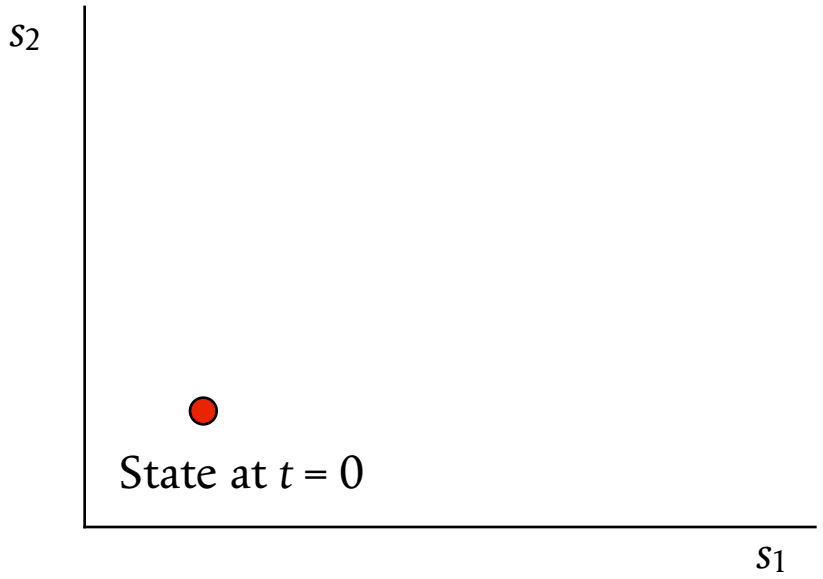

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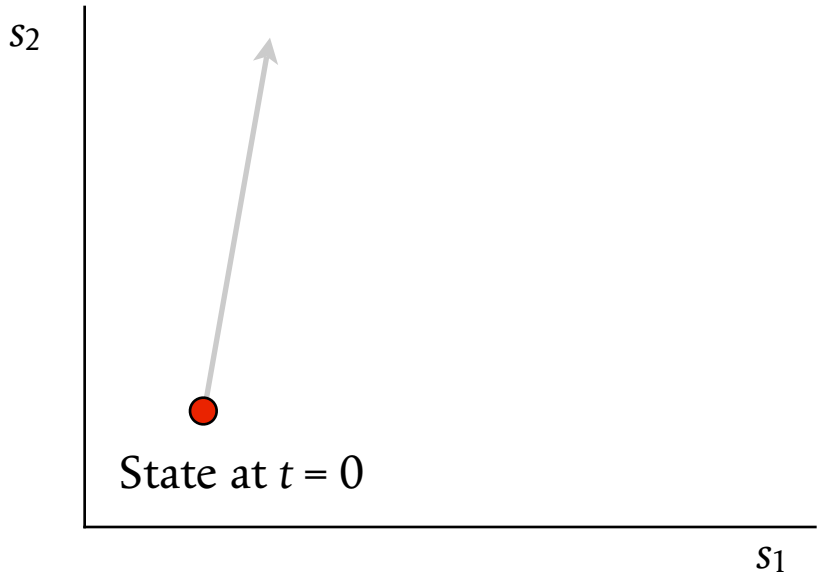

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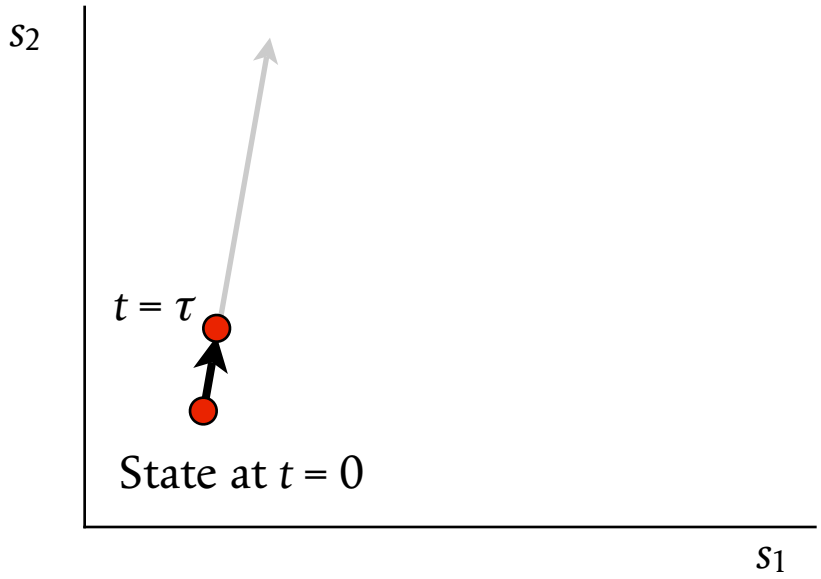

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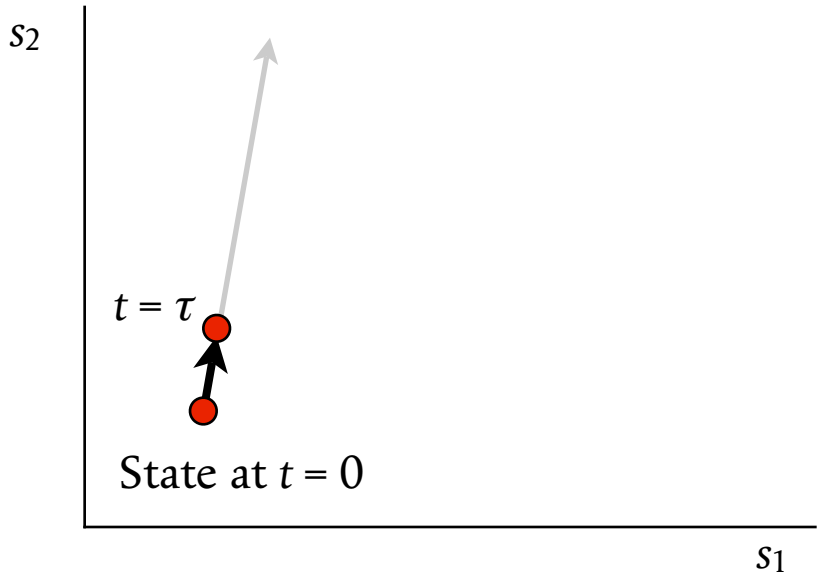

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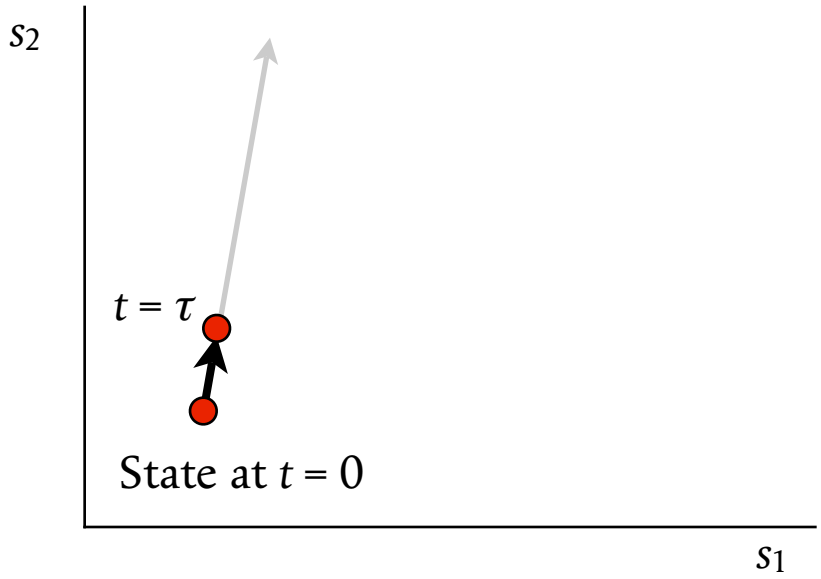

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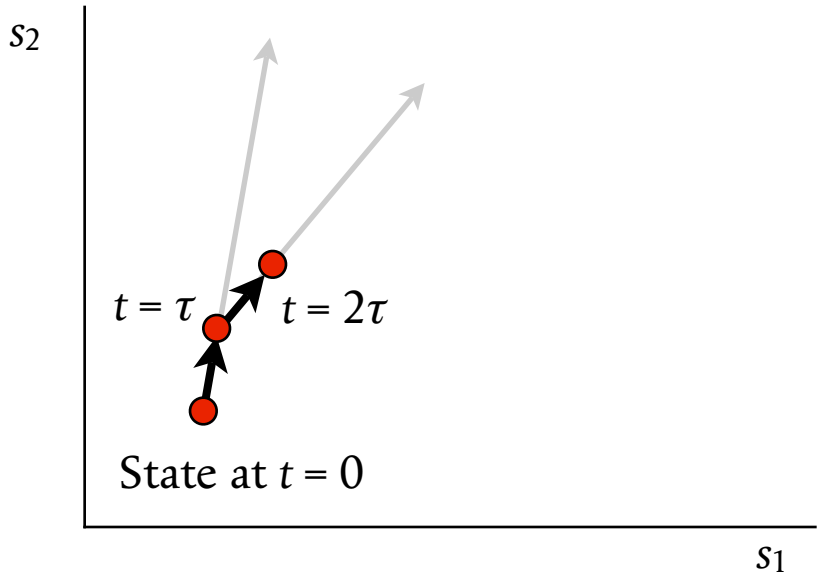

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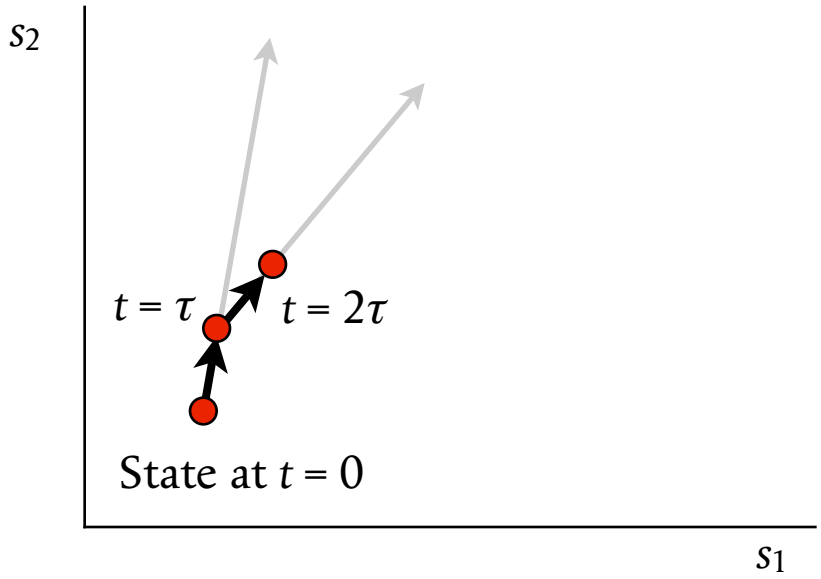

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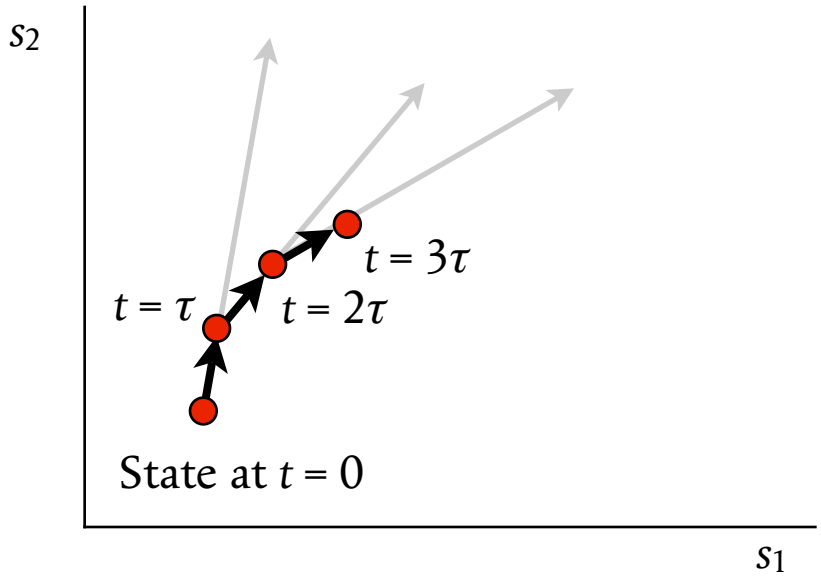

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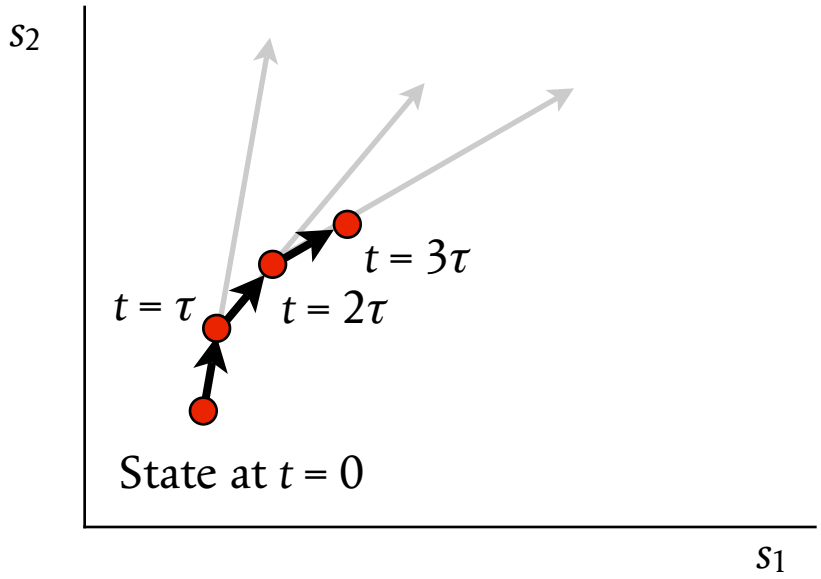

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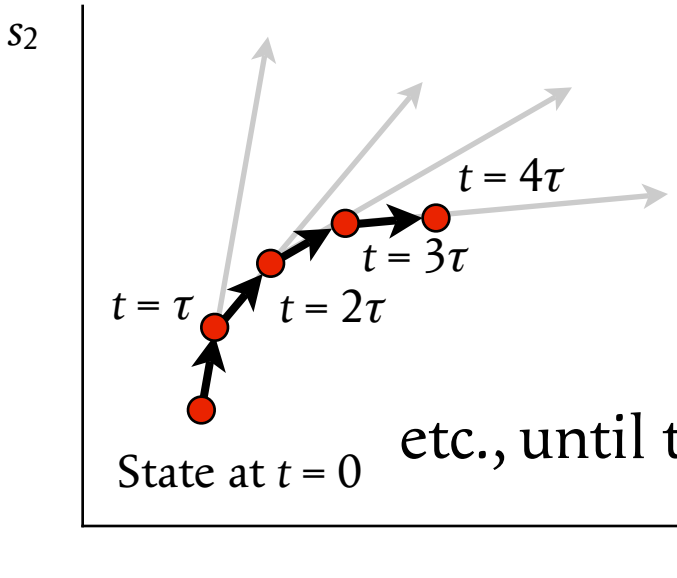

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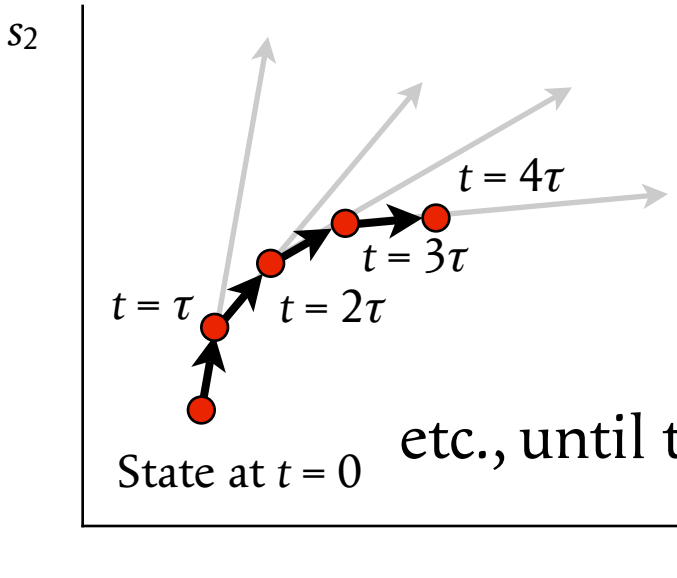

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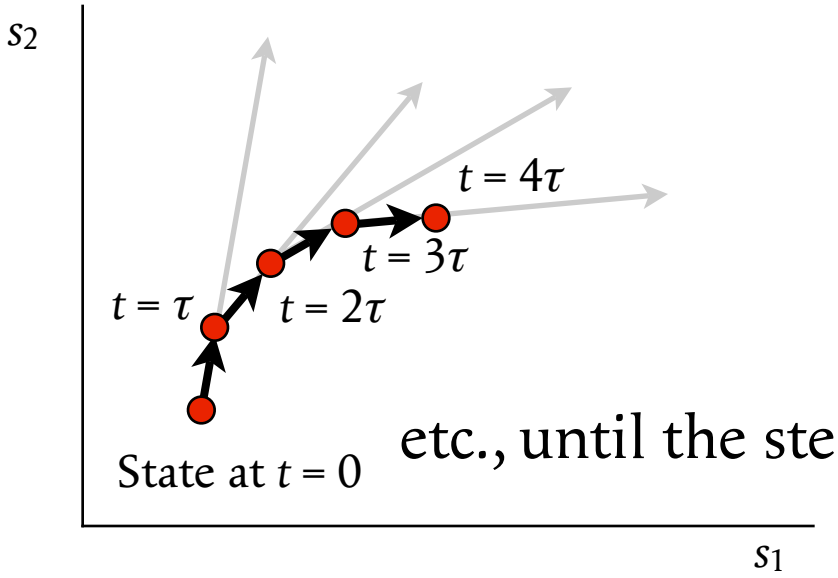

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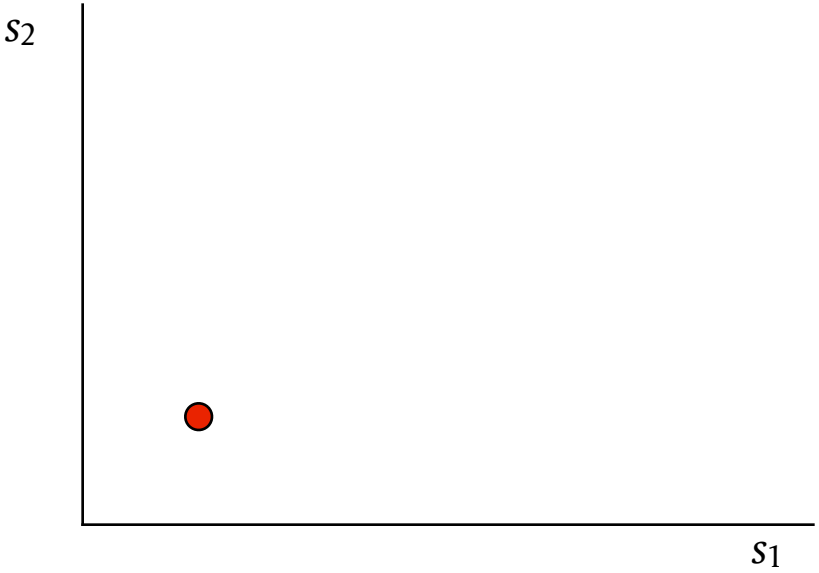

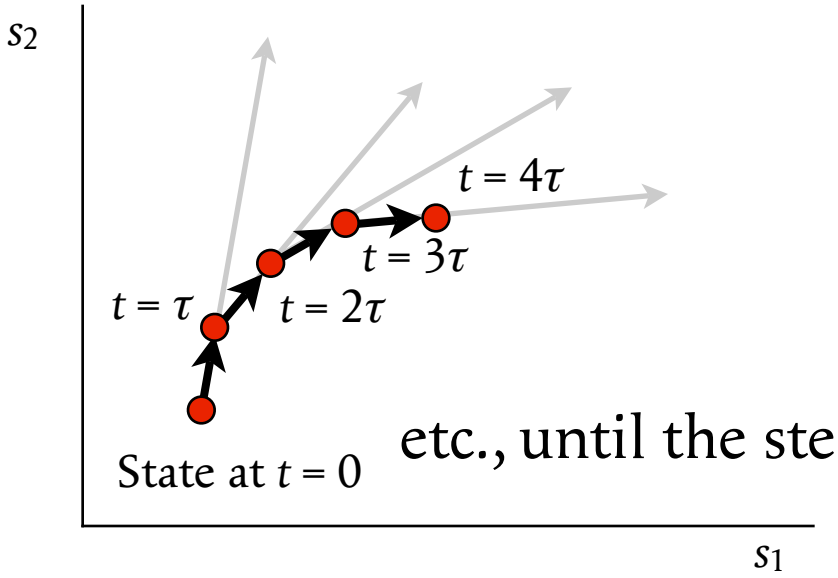

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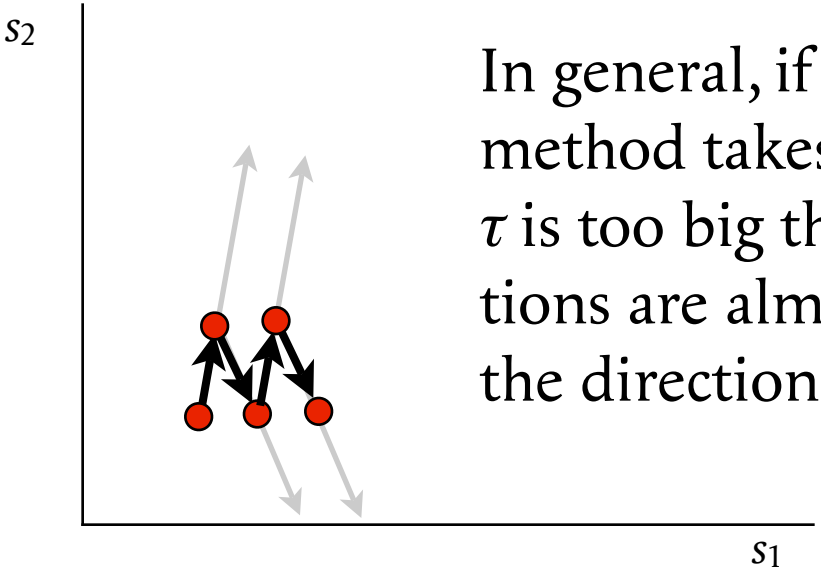
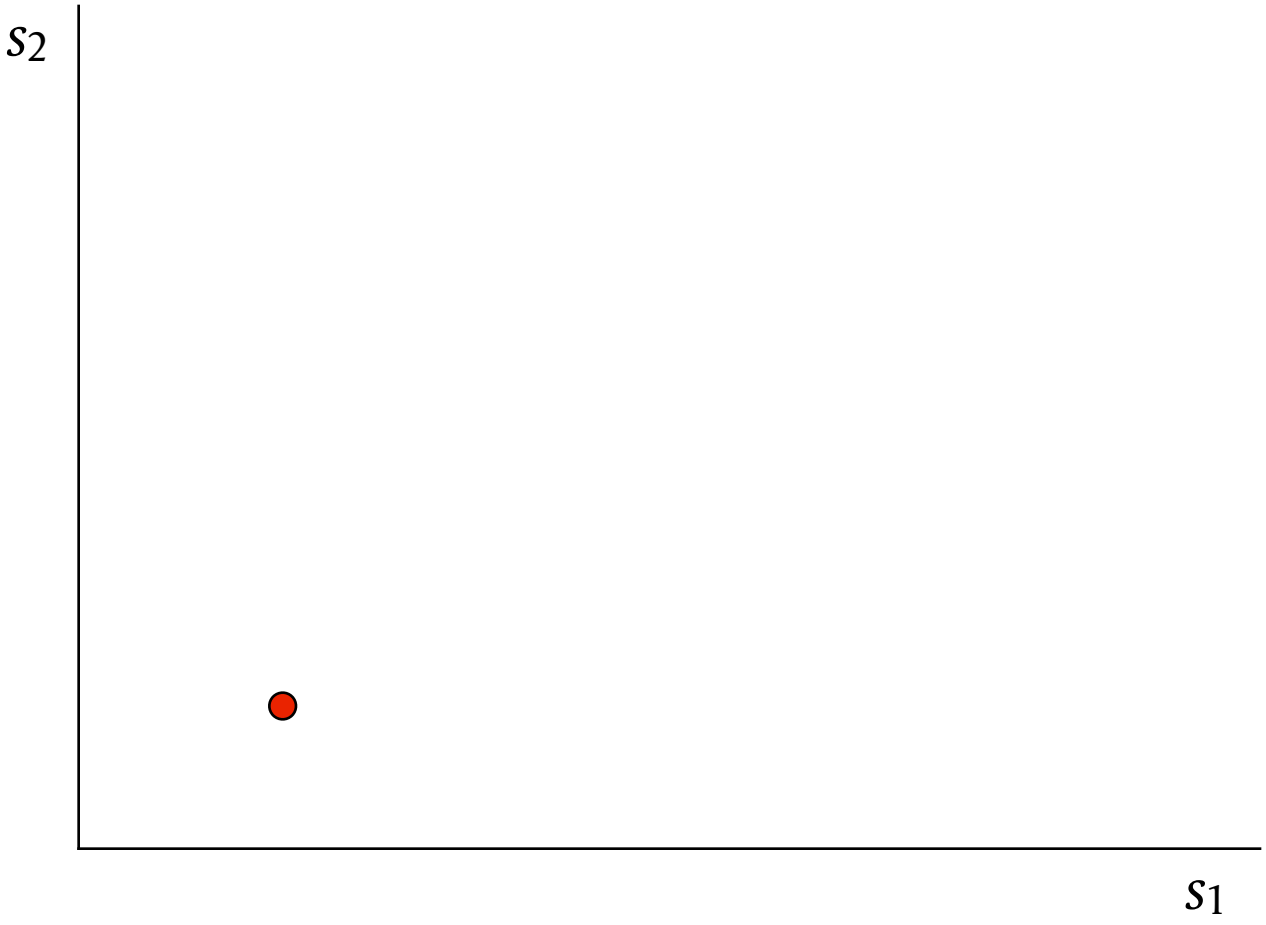


In general, if τ is too small the method takes too many steps; if τ is too big the calculated directions are almost orthogonal to the directions desired.

Euler's method is very poor: how might it be improved?



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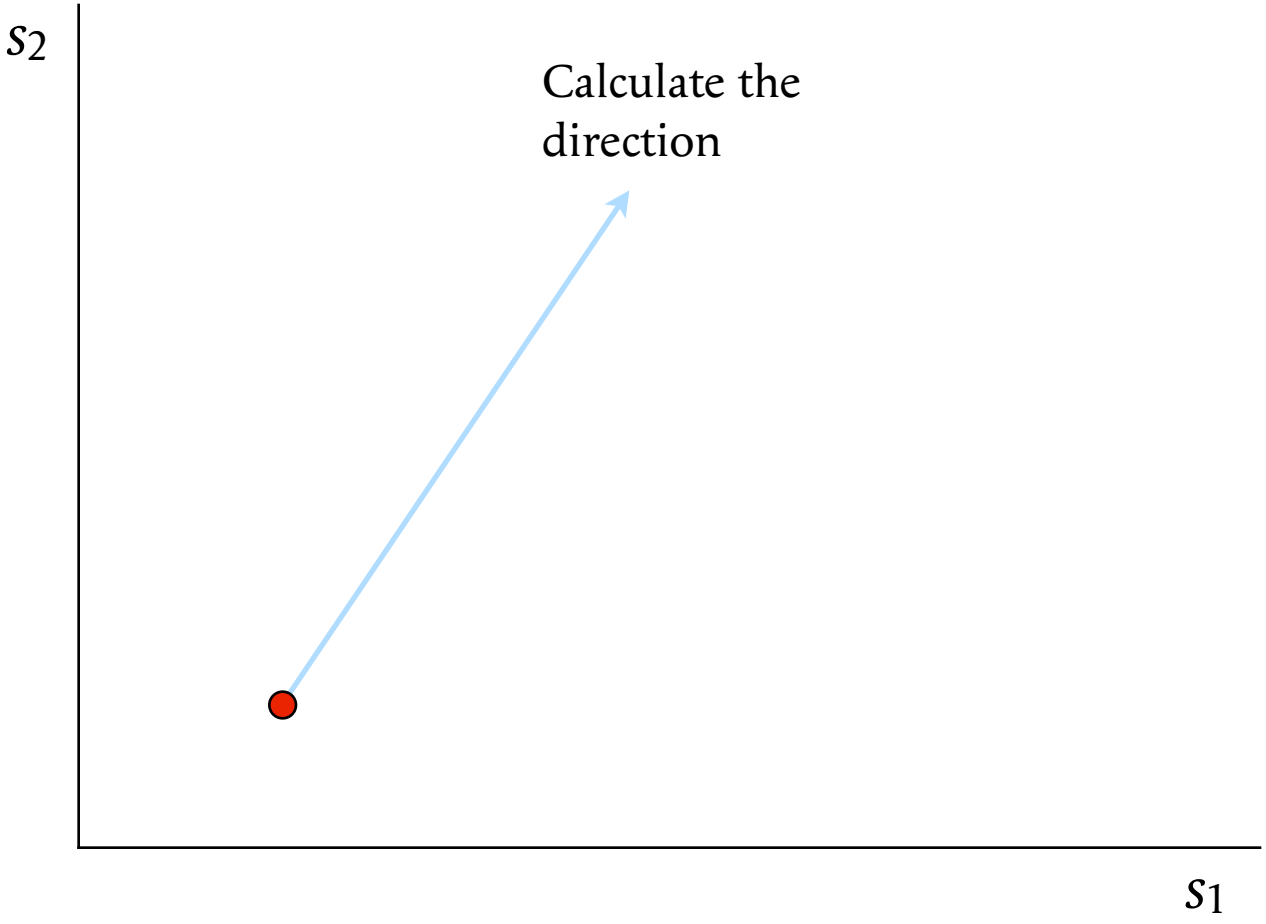

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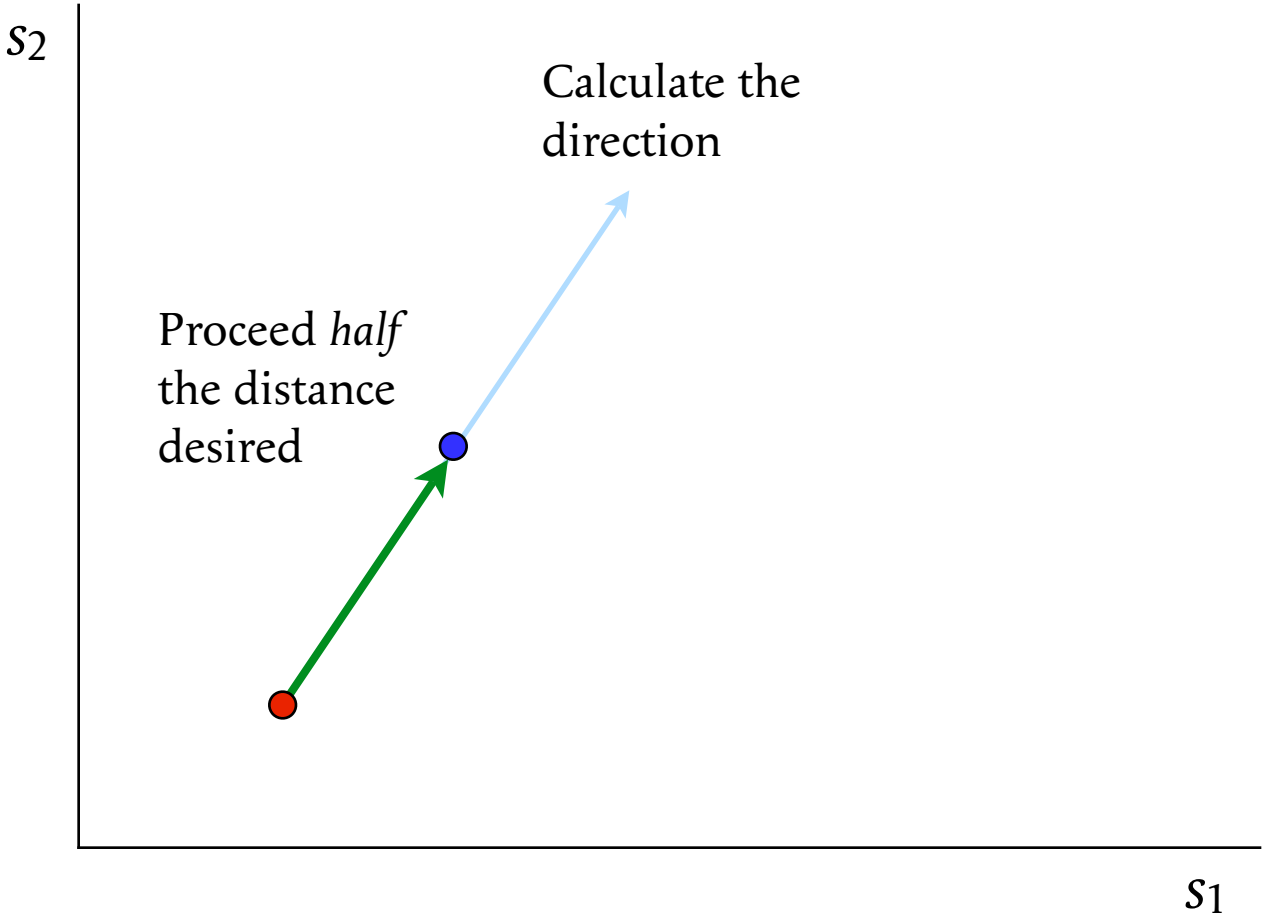

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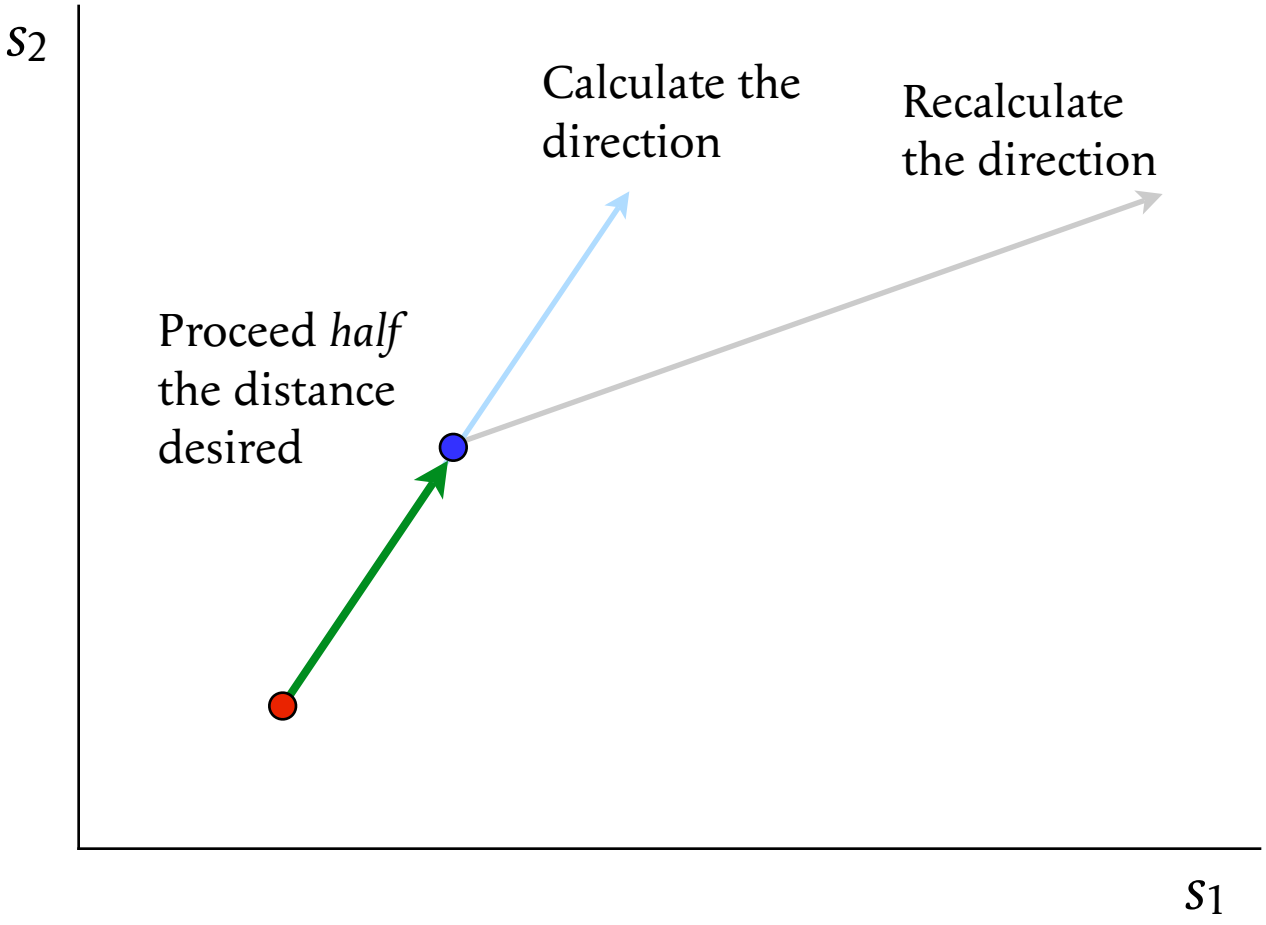

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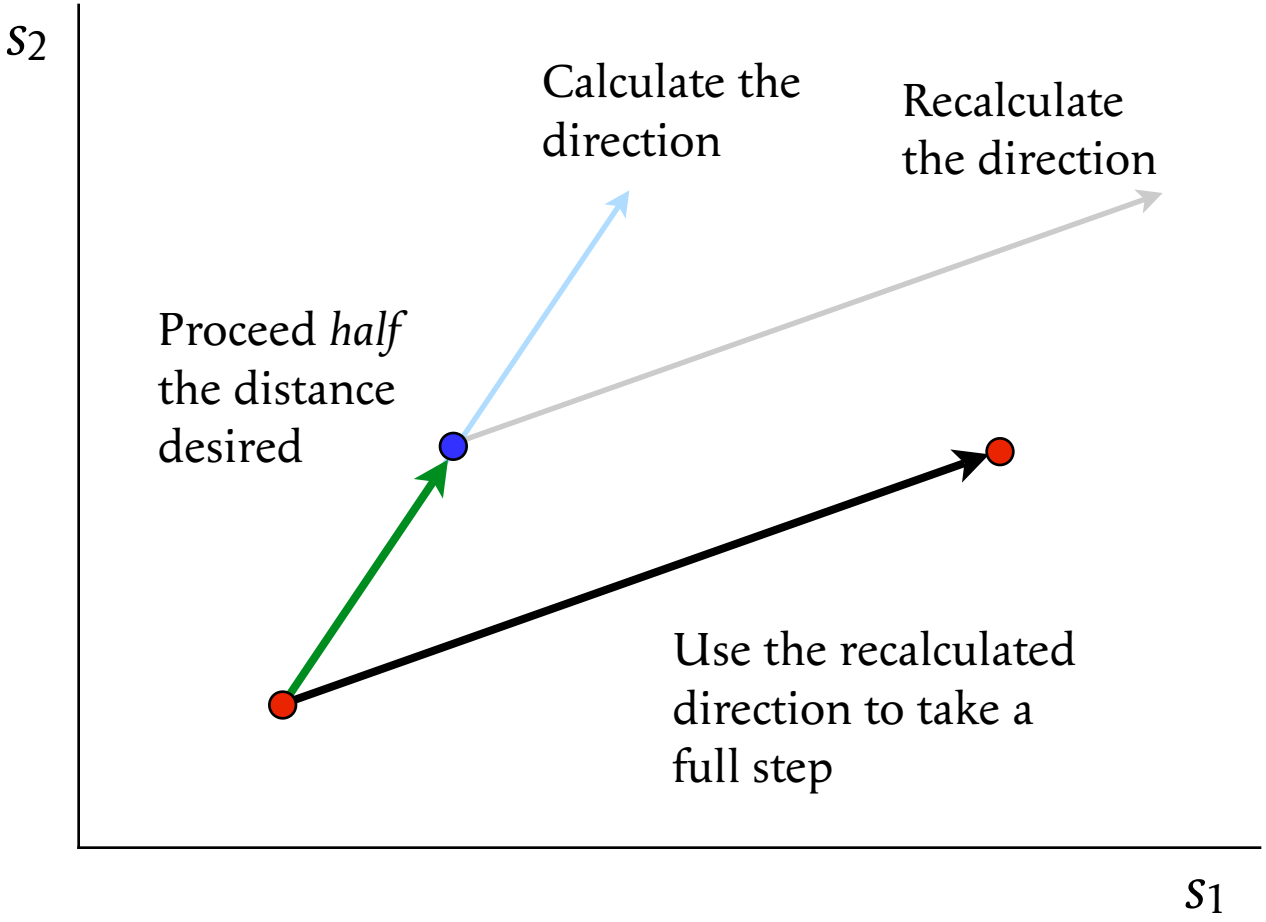

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So a good modern method includes the possibility of varying the value of τ during the calculation.

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In brief, one needs to calculate m values of s_j such that $\frac{ds_j}{dt} = 0$ for all $j = 1 \ldots m$.

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Both work well, but they are quite different, and users' preferences differ systematically with the type of user: people who plan to do one or two simulations without going very deeply into the subject prefer COPASI or GEPASI; people with a longer-term commitment prefer JARNAC or SCAMP. Why should this be?

9–20 APRIL 2007
LES HOUCHES

- Relevance of classical enzymology
- Kinetics of multi-enzyme systems
  - Elasticity
  - Concentration as a function of rate
  - Control coefficients
  - Metabolic regulation
  - Summation property
  - Magnitude of a typical flux control coefficient
  - Mendelian genetics
  - Connectivity
  - Control coefficients in terms of elasticities
  - Response coefficients
  - Partitioned response
  - Supply and demand
- **Modelling a metabolic system**
  - Euler's method
  - Runge–Kutta methods
  - COPASI and JARNAC
- Inhibition types
- Glycolysis in *Trypanosoma brucei*
- Handling of irreversible steps
- Practical meaning of feedback regulation

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INHIBITION TYPES

As we have seen for the relationship between rate and substrate concentration given by the Michaelis–Menten equation, kinetic behaviour at fixed rate can appear very different from behaviour at fixed substrate concentration: the latter corresponds to the usual case in the spectrophotometer, but the former may be closer to the reality in a living organism.

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- Mixed* (both V and V/K_m decreased)

*called "non-competitive inhibition" by some authors

The extreme cases

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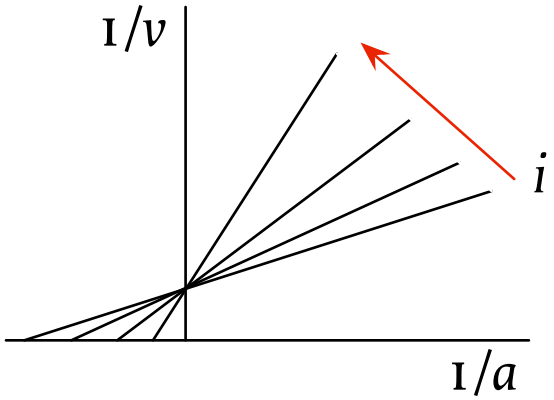

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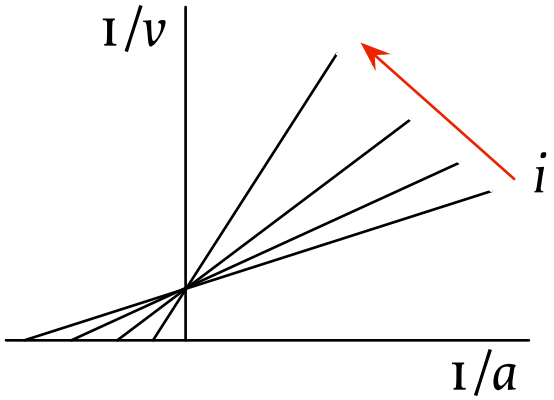

Straight lines intersecting on the ordinate axis in a double-reciprocal plot

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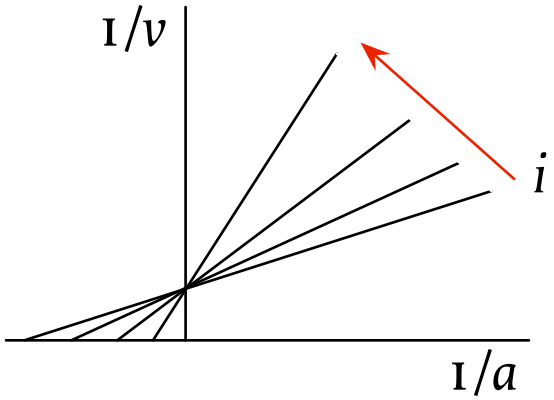

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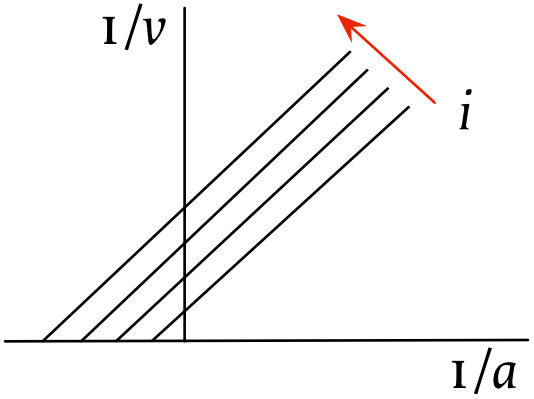

Parallel lines in a double-reciprocal plot

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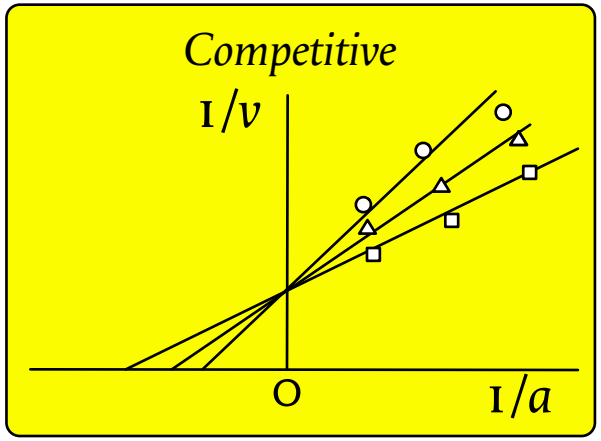

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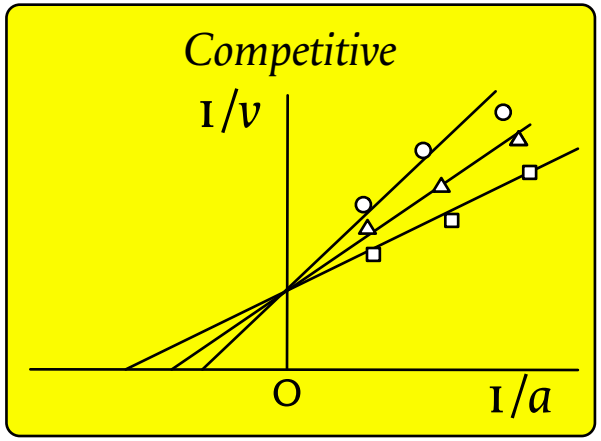

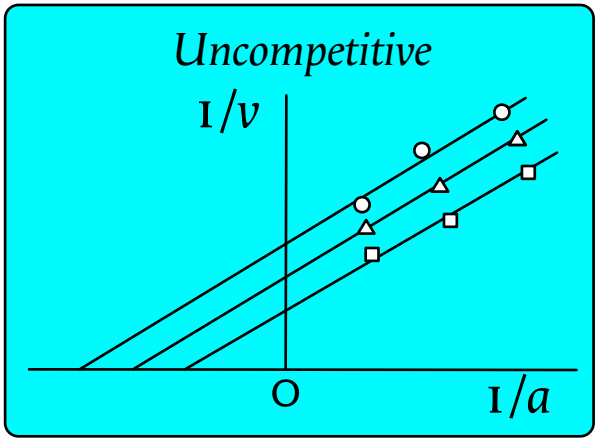

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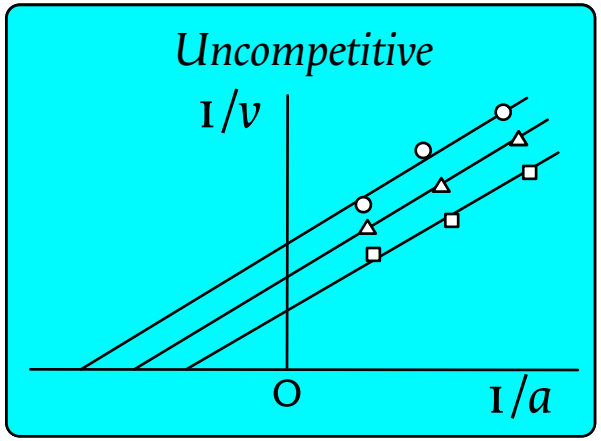

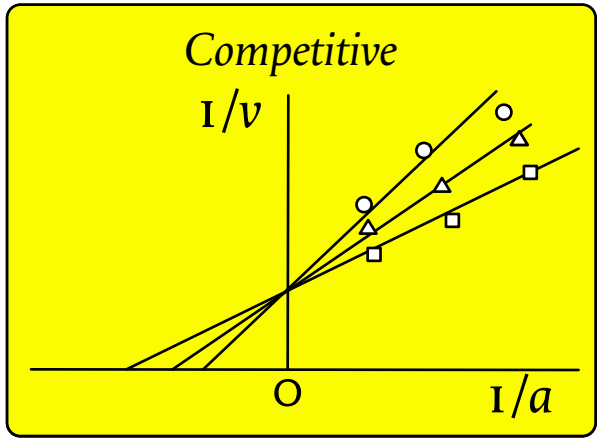

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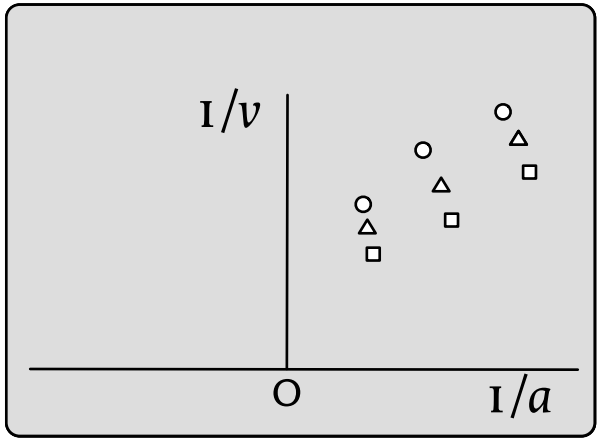

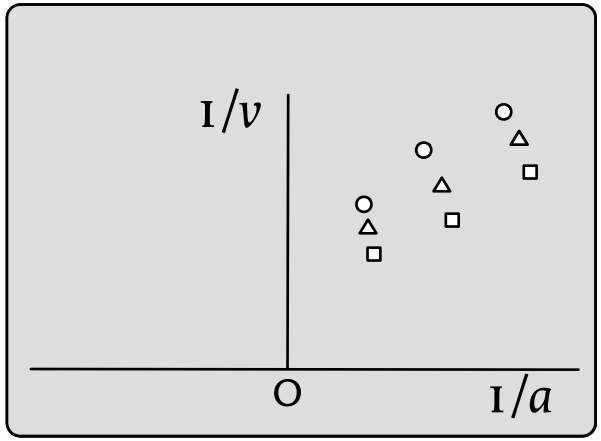

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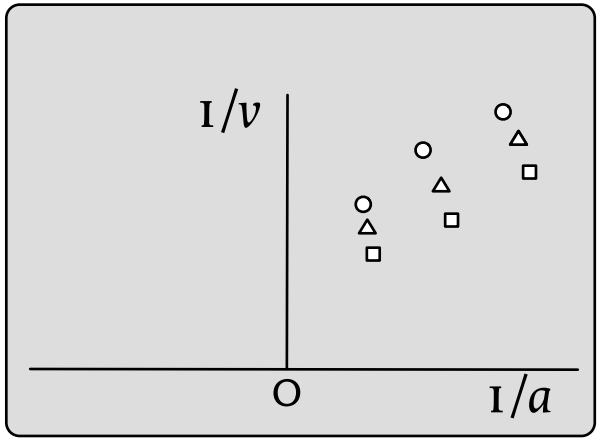

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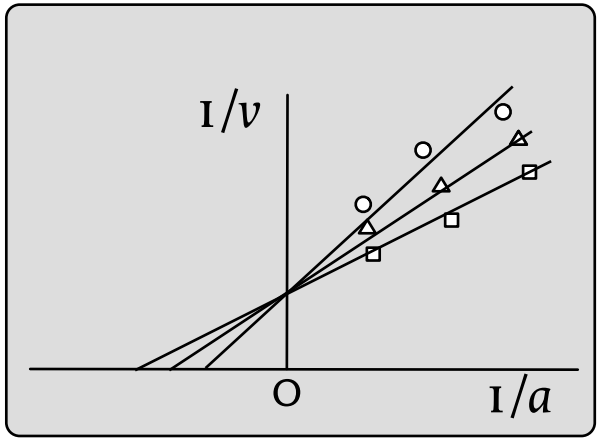

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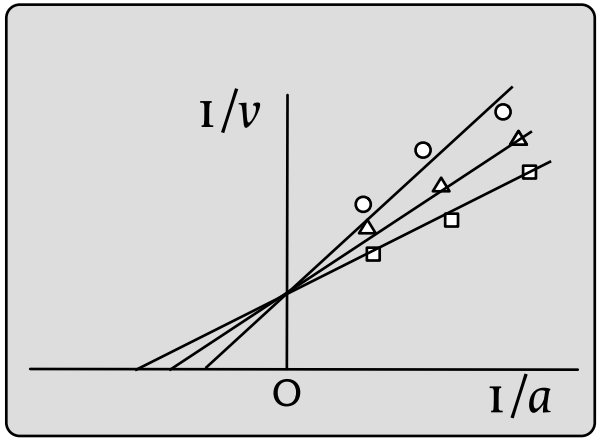

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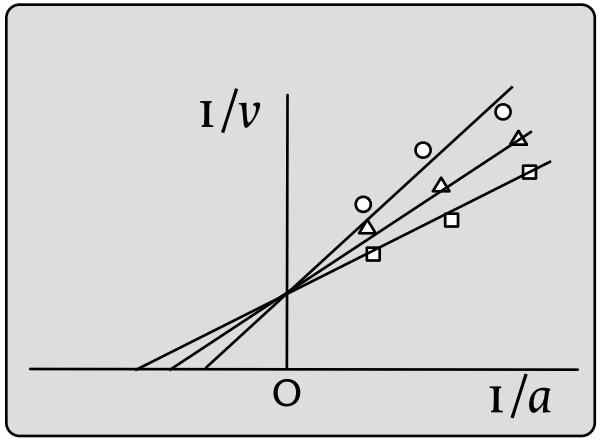

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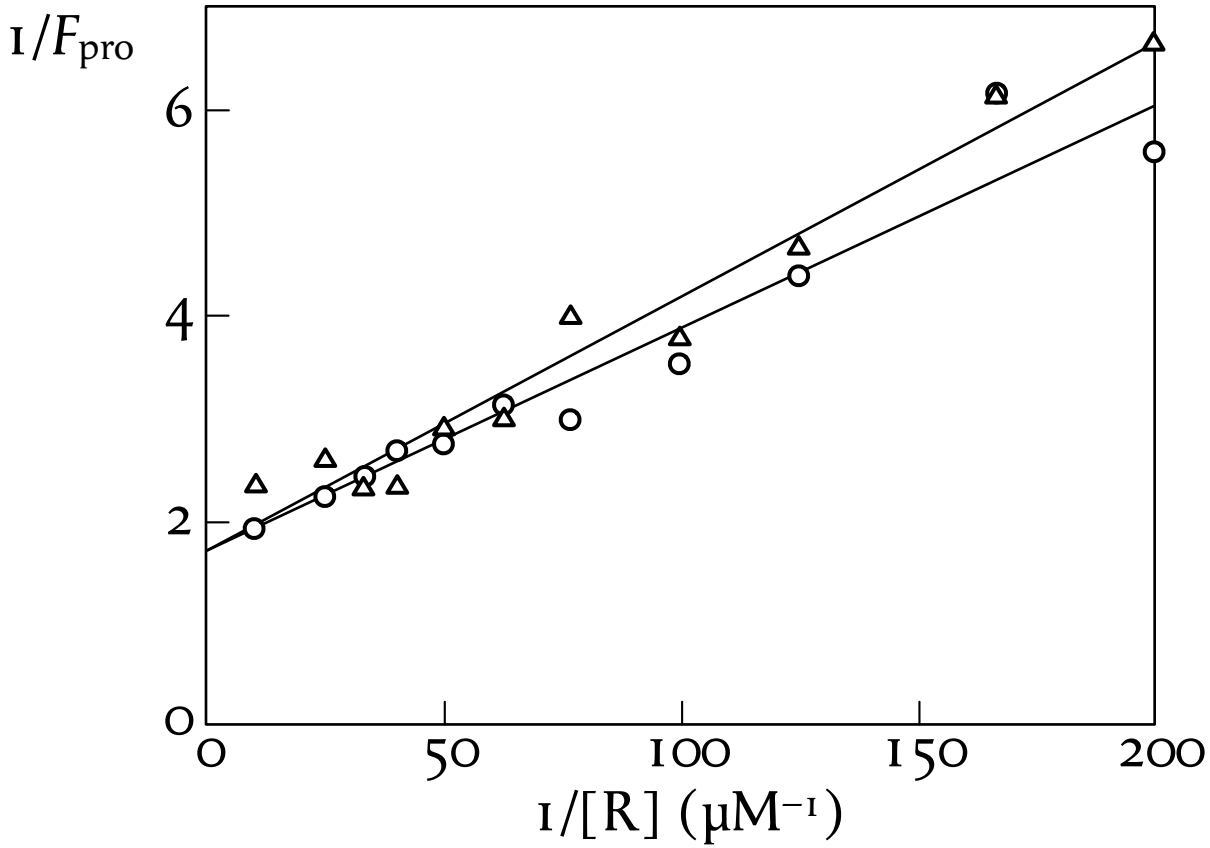

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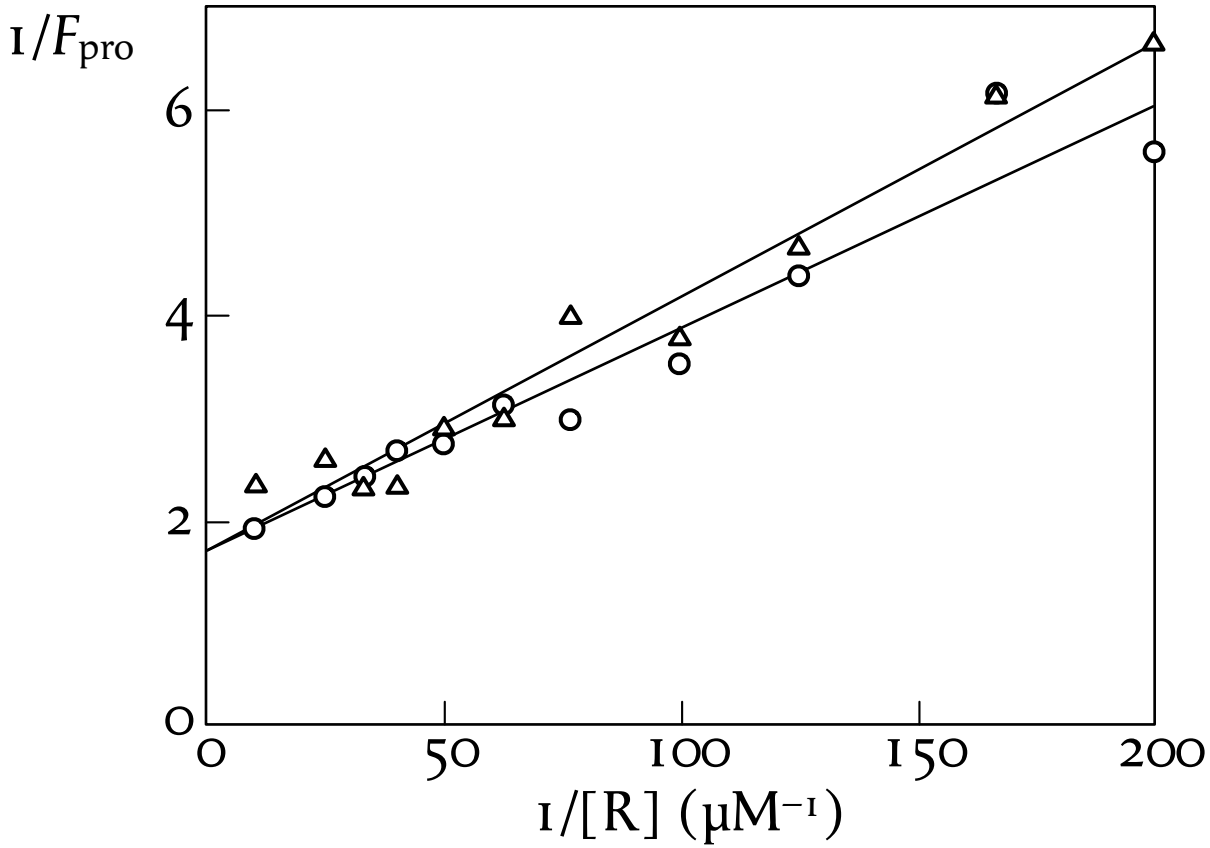

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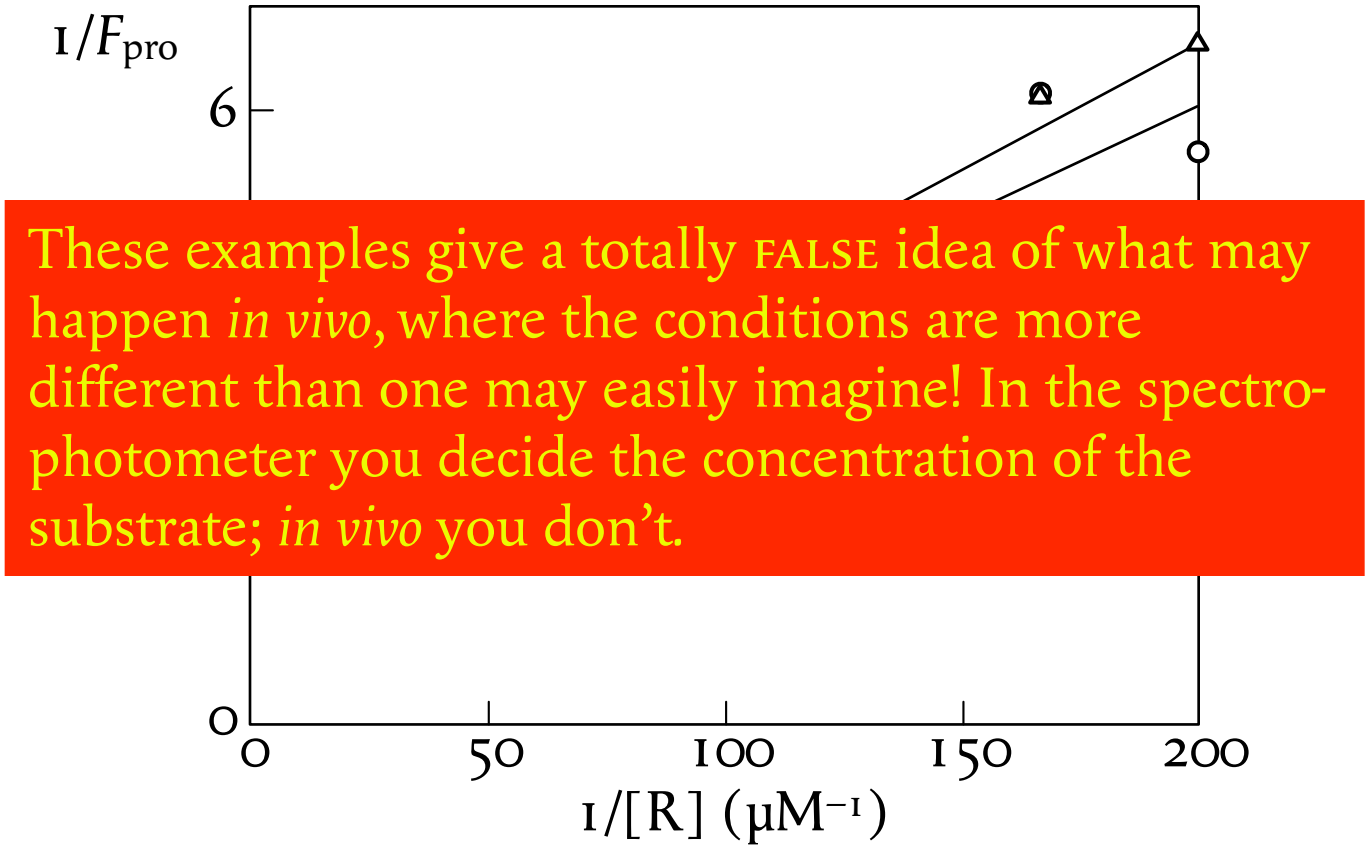

These examples give a totally FALSE idea of what may happen *in vivo*, where the conditions are more different than one may easily imagine! In the spectrophotometer you decide the concentration of the substrate; *in vivo* you don't.

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9–20 APRIL 2007
LES HOUCHES

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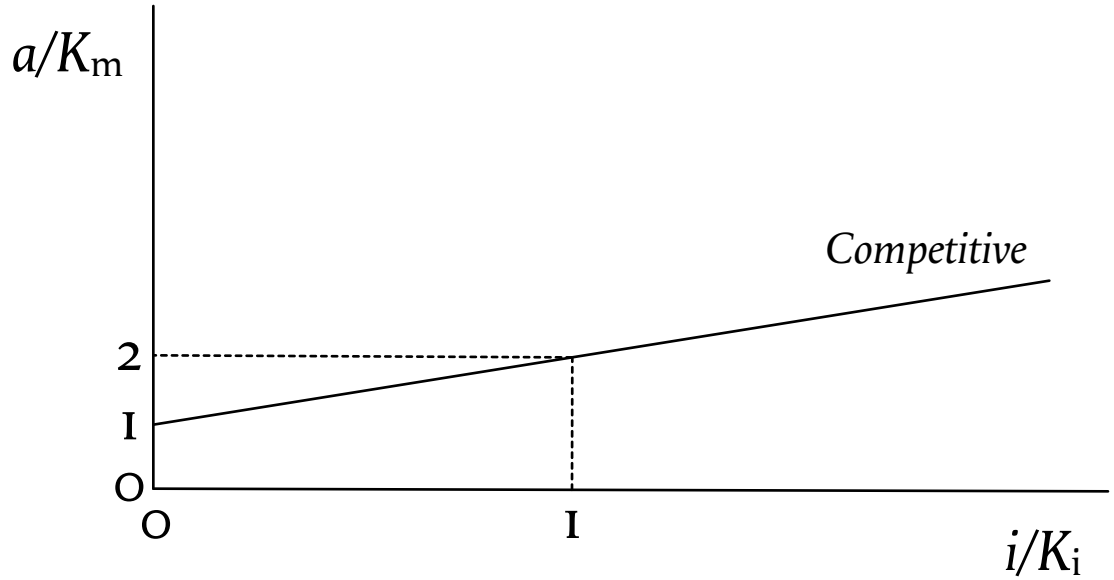

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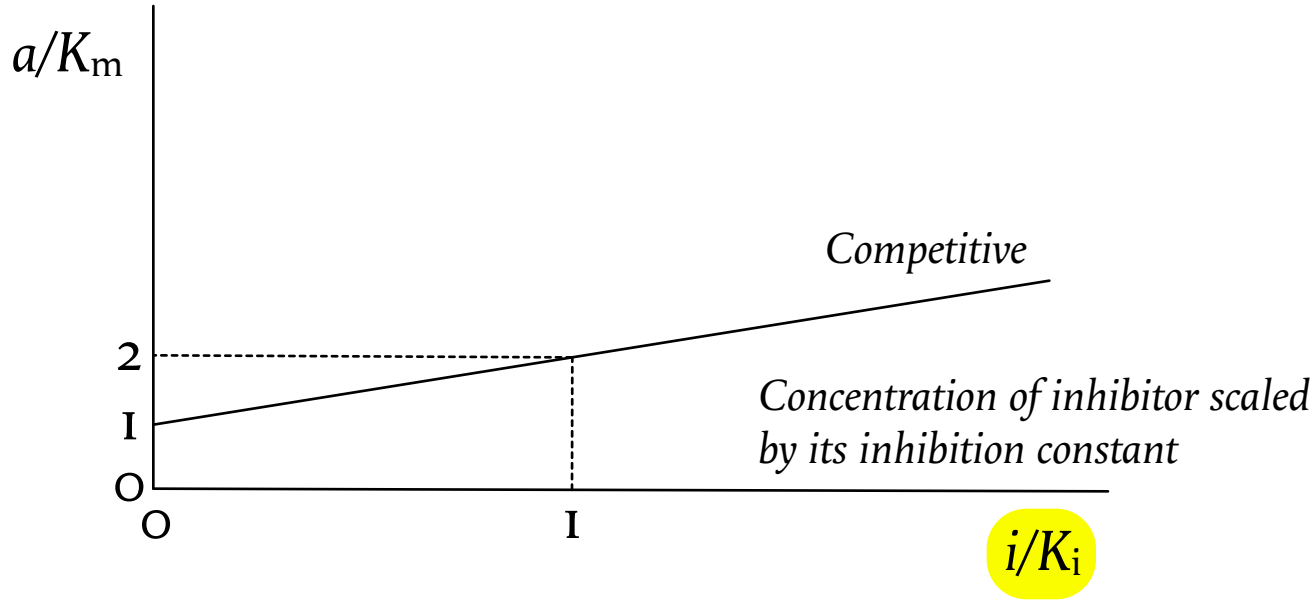

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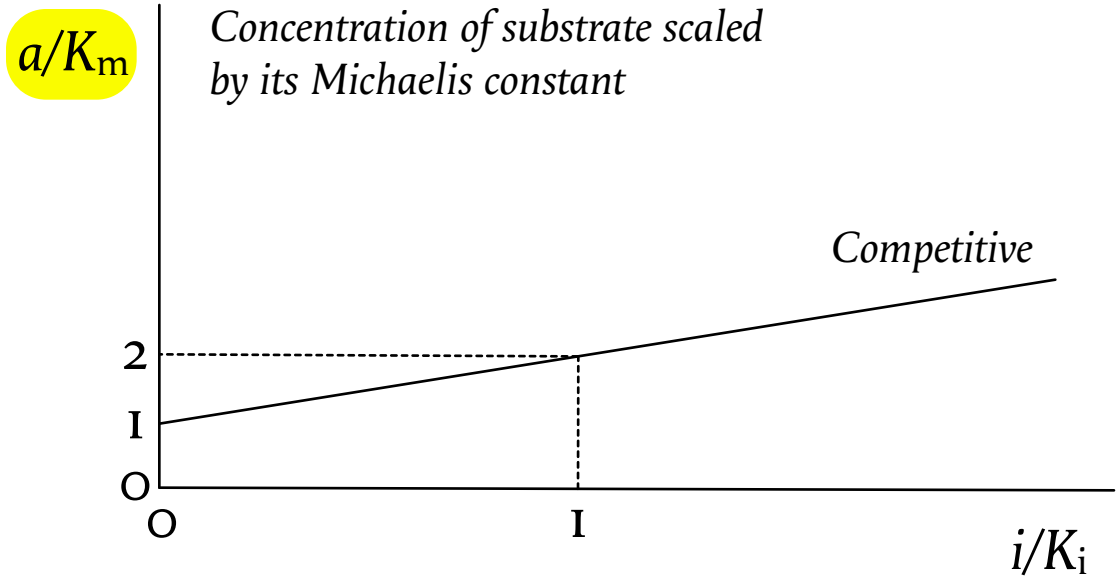

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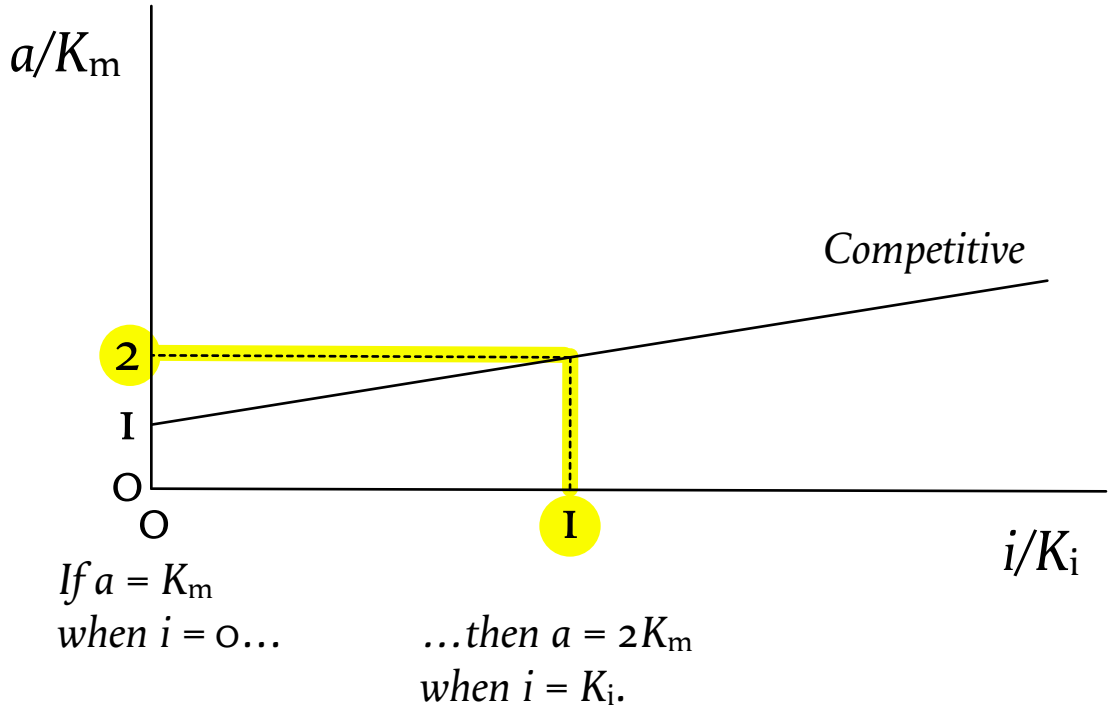

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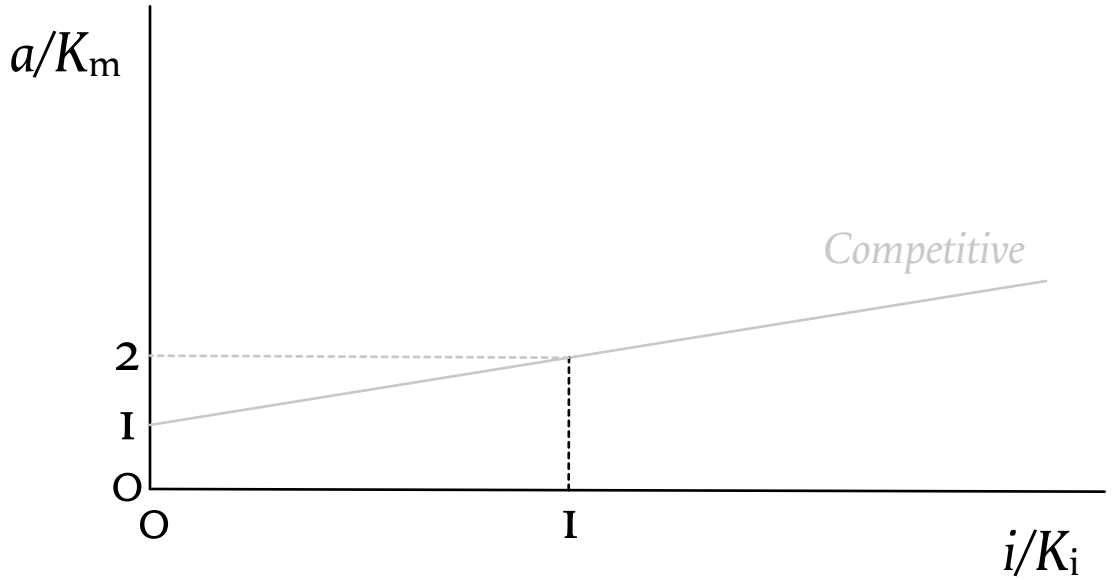

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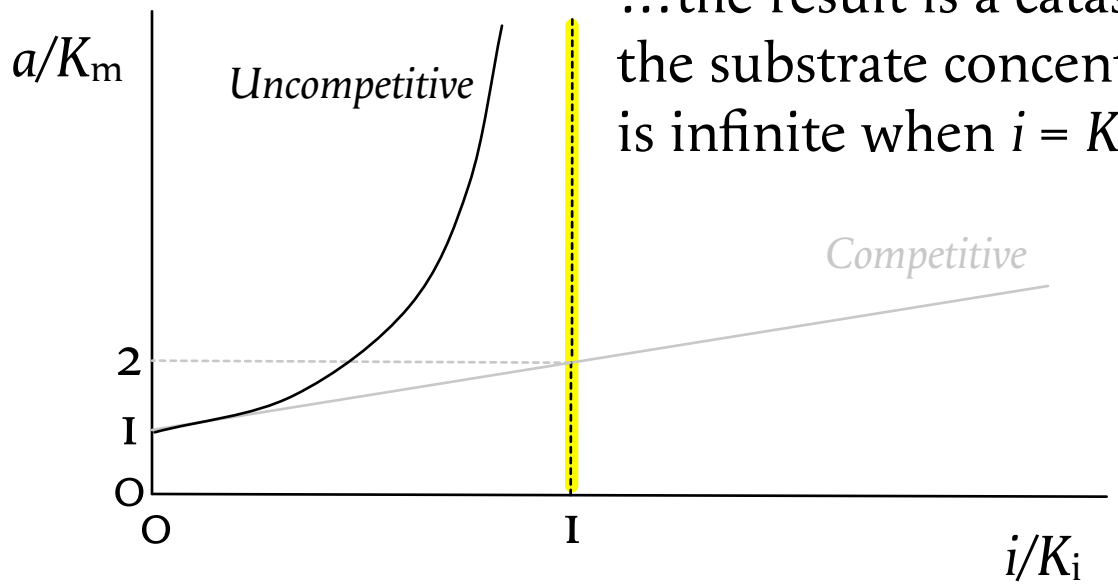

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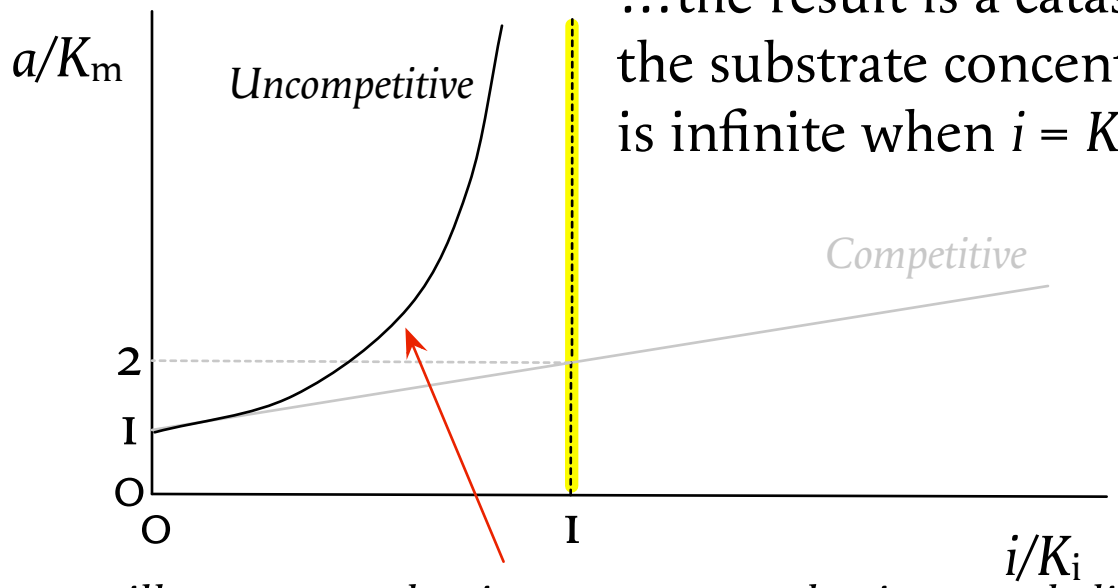

This curve illustrates another important general point: metabolite concentrations may change by very large amounts in conditions where fluxes do not change (or change very little).

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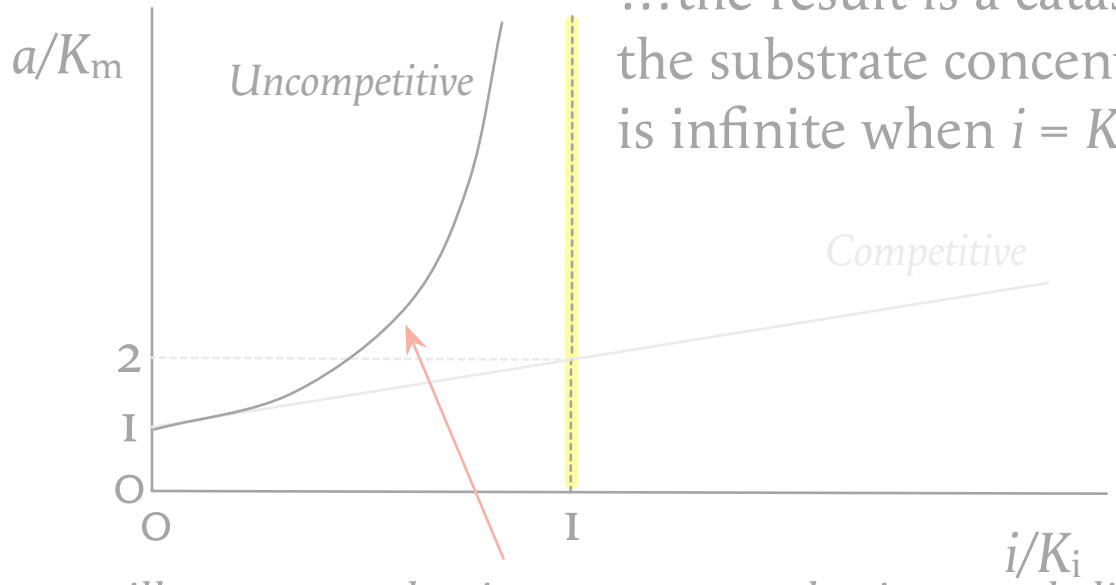

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"Roundup", or Glyphosate, is the herbicide with the greatest commercial success in history. Its effect is to increase the concentration of shikimate in the cells of treated plants by a factor of around 500, enough to kill the plant. It works because it is an *uncompetitive inhibitor*: why is that important?

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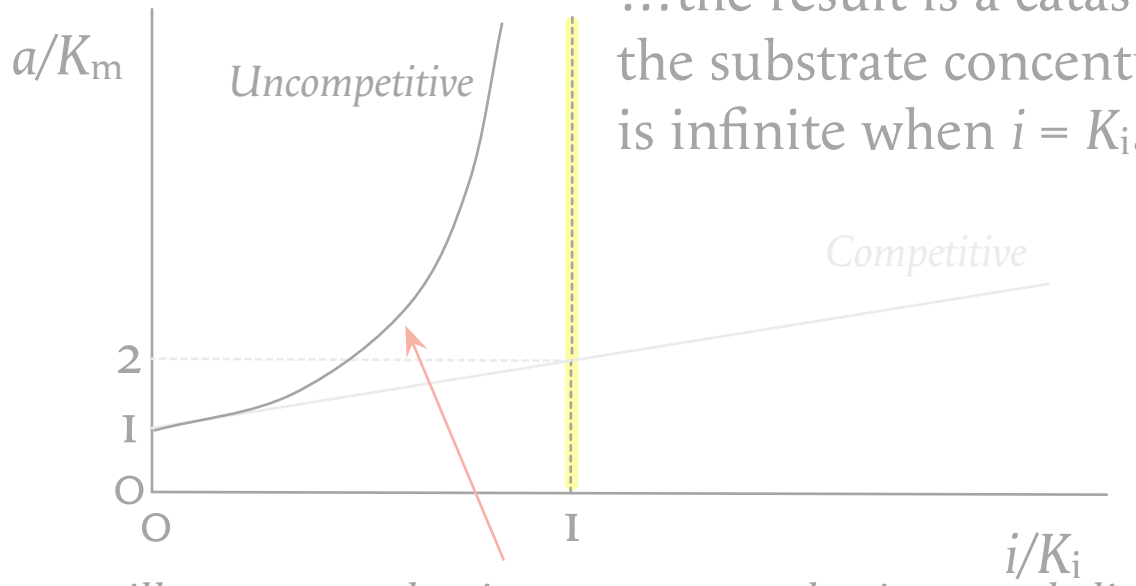

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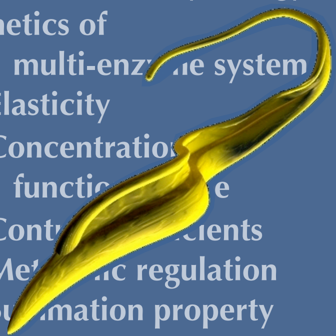
GLYCOLYSIS IN TRYPANOSOMA BRUCEI



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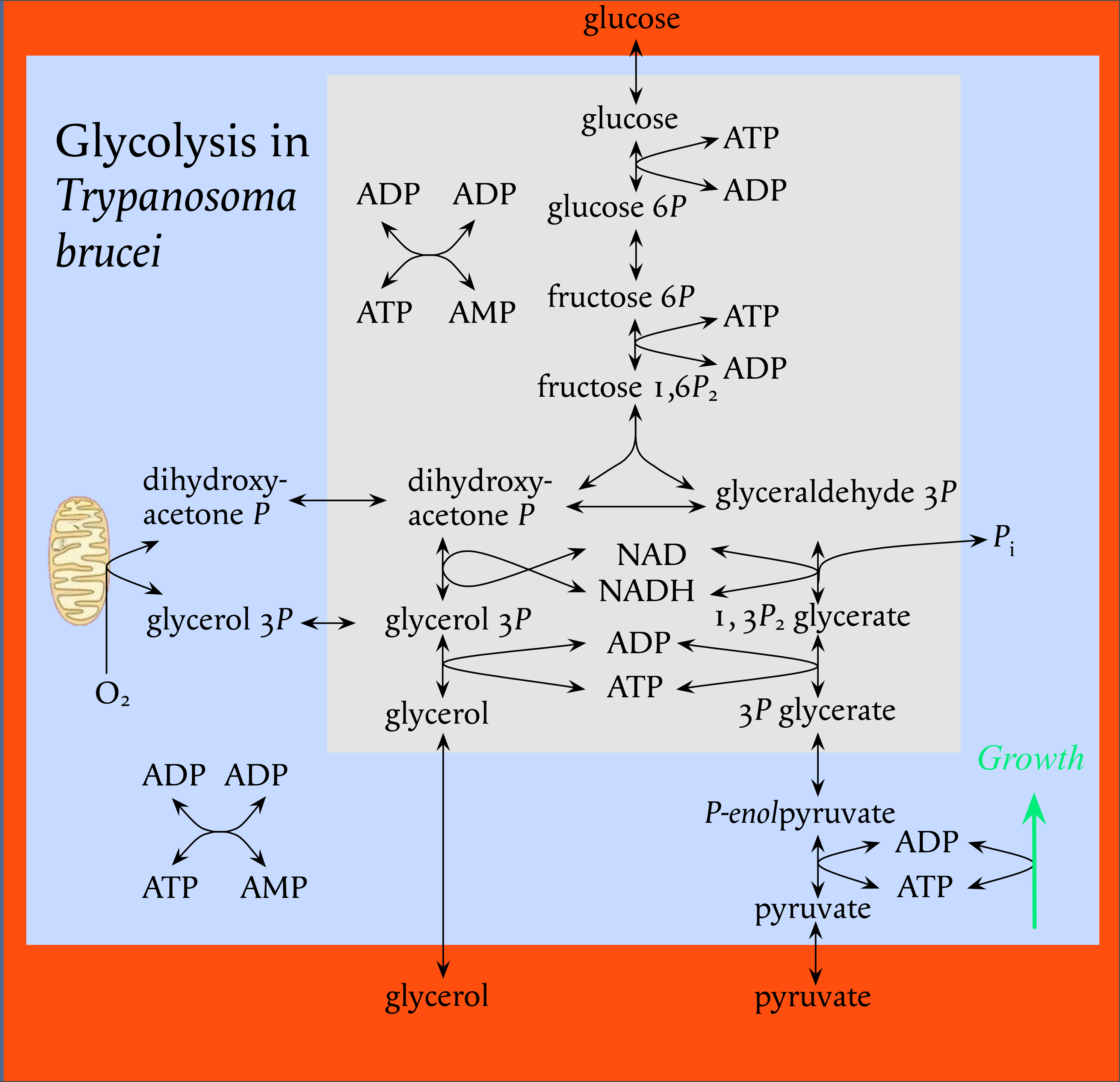

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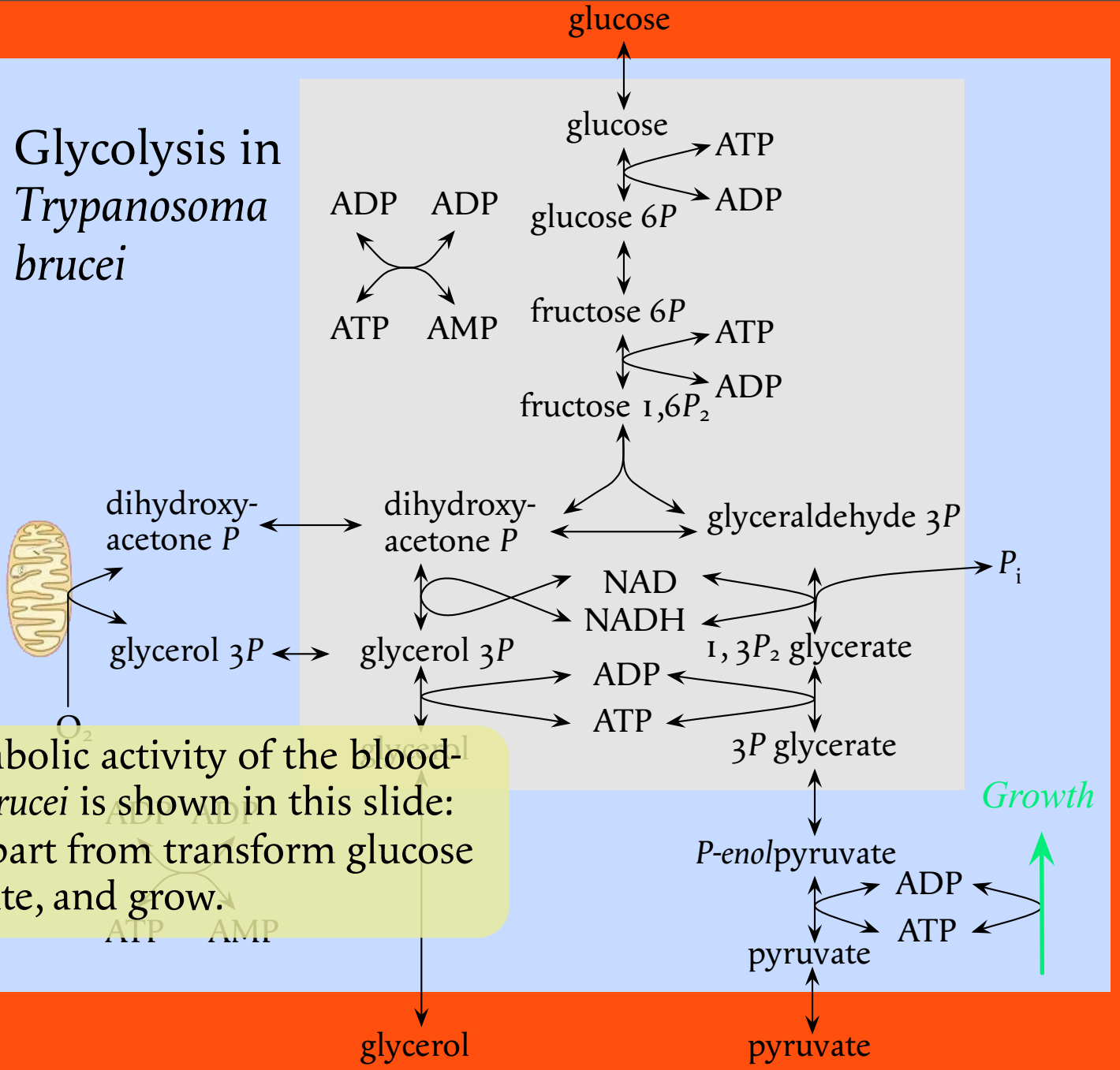

Almost all the metabolic activity of the blood-stream form of *T. brucei* is shown in this slide: it does very little apart from transform glucose rapidly into pyruvate, and grow.

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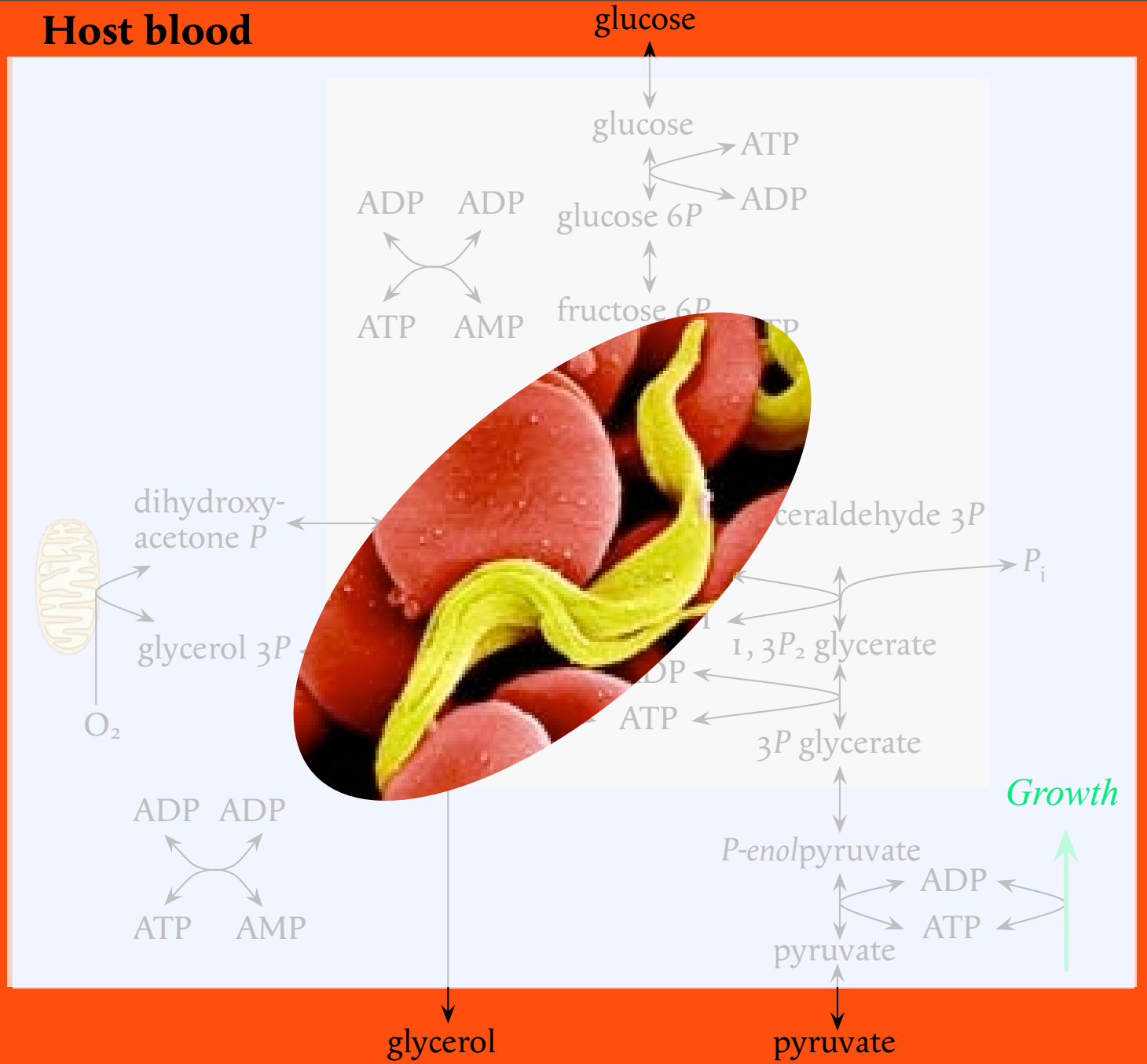

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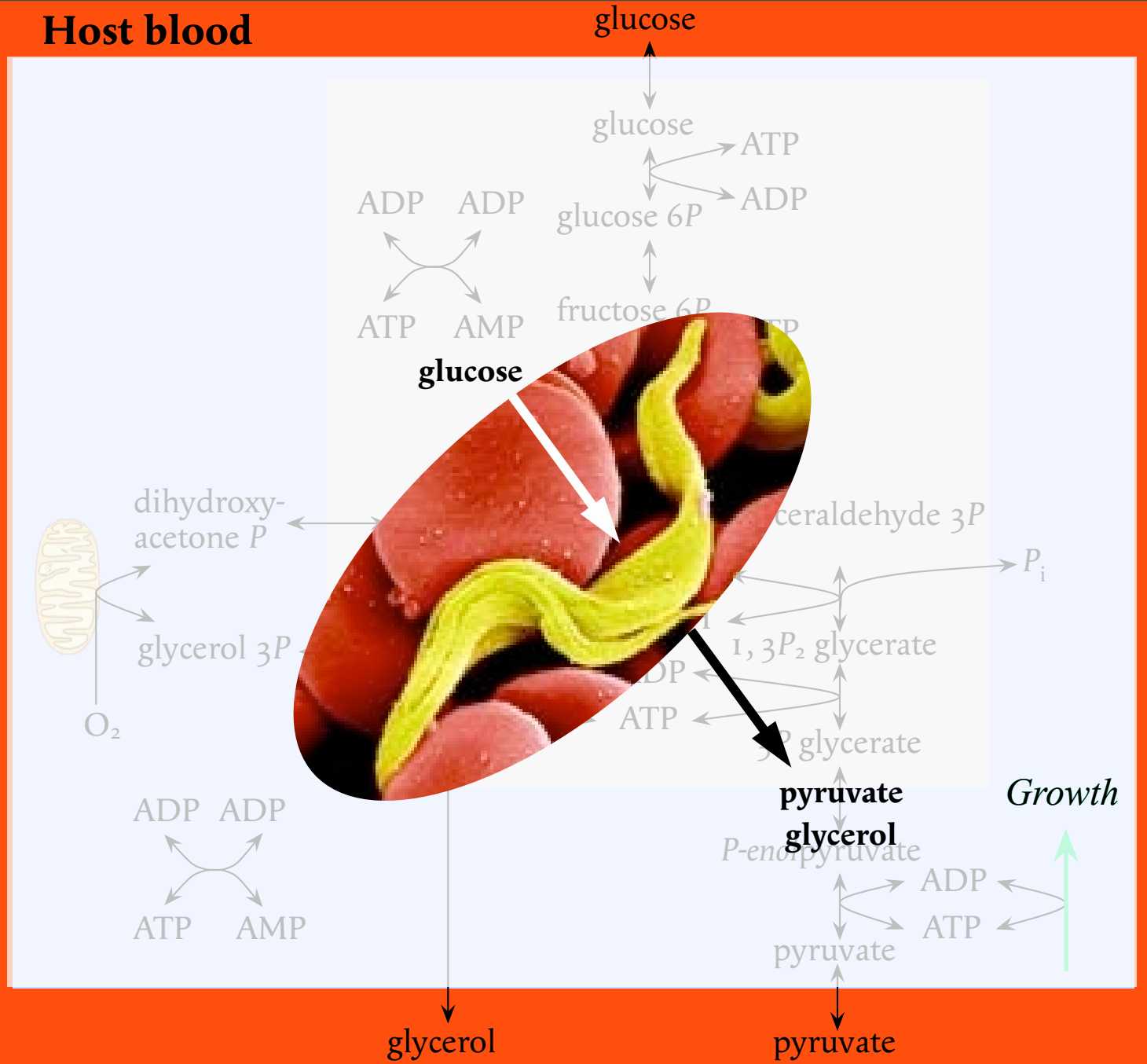
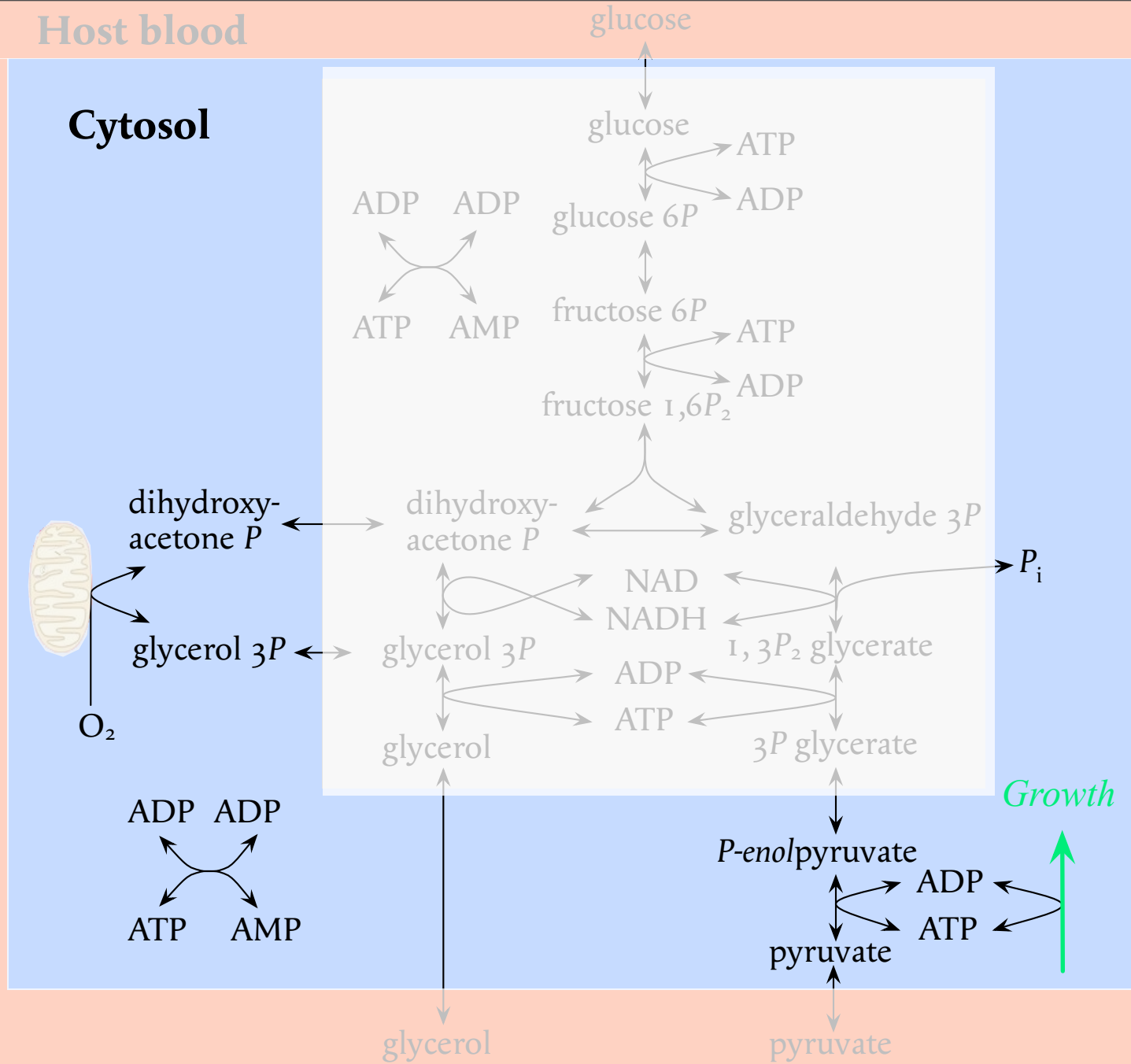
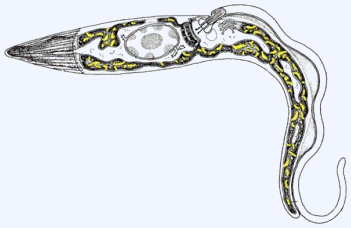
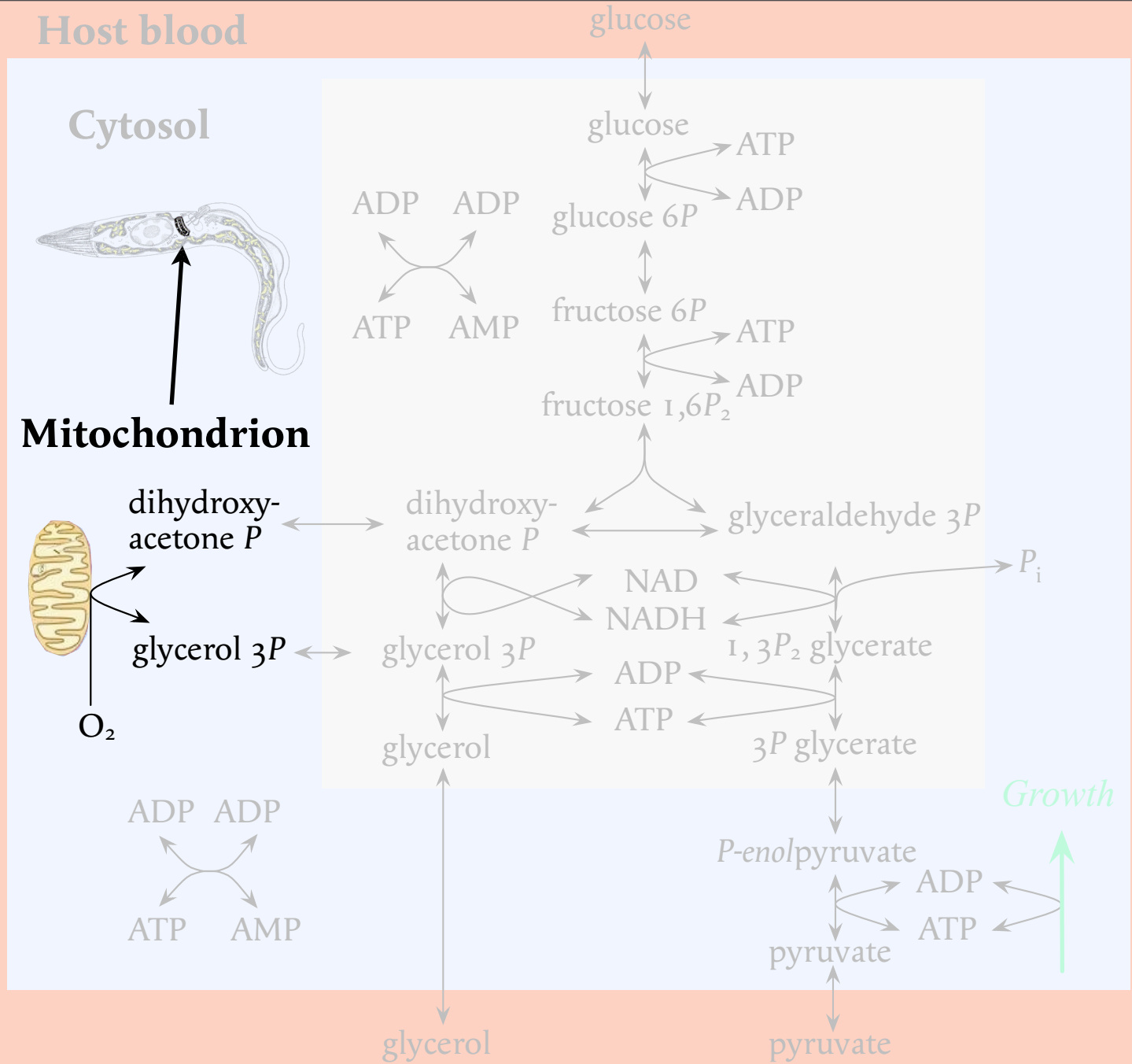
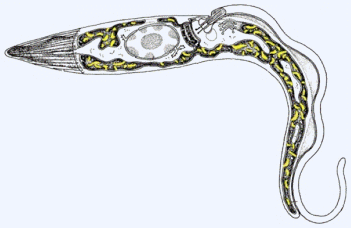
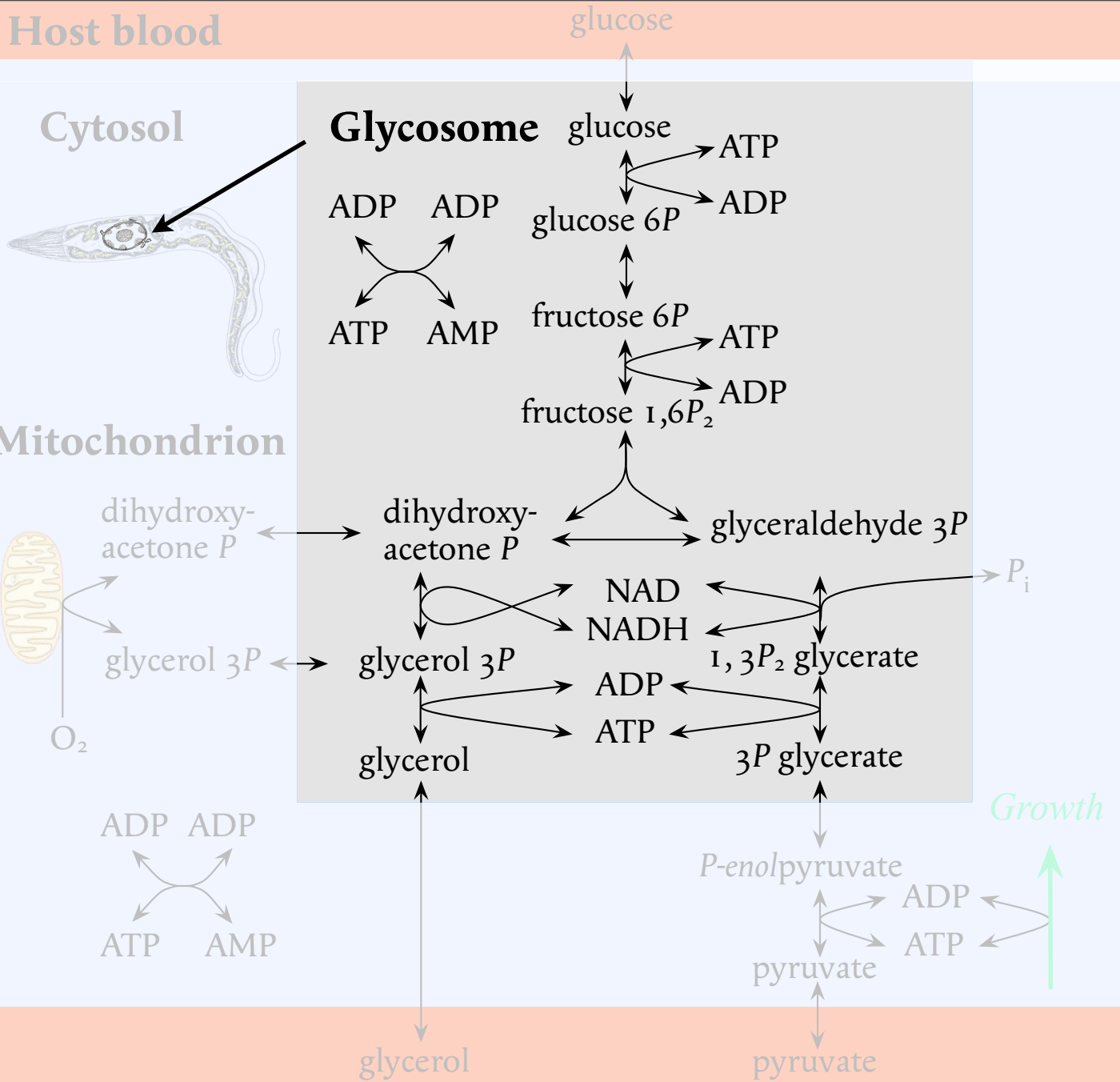


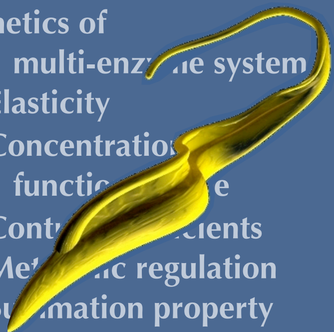






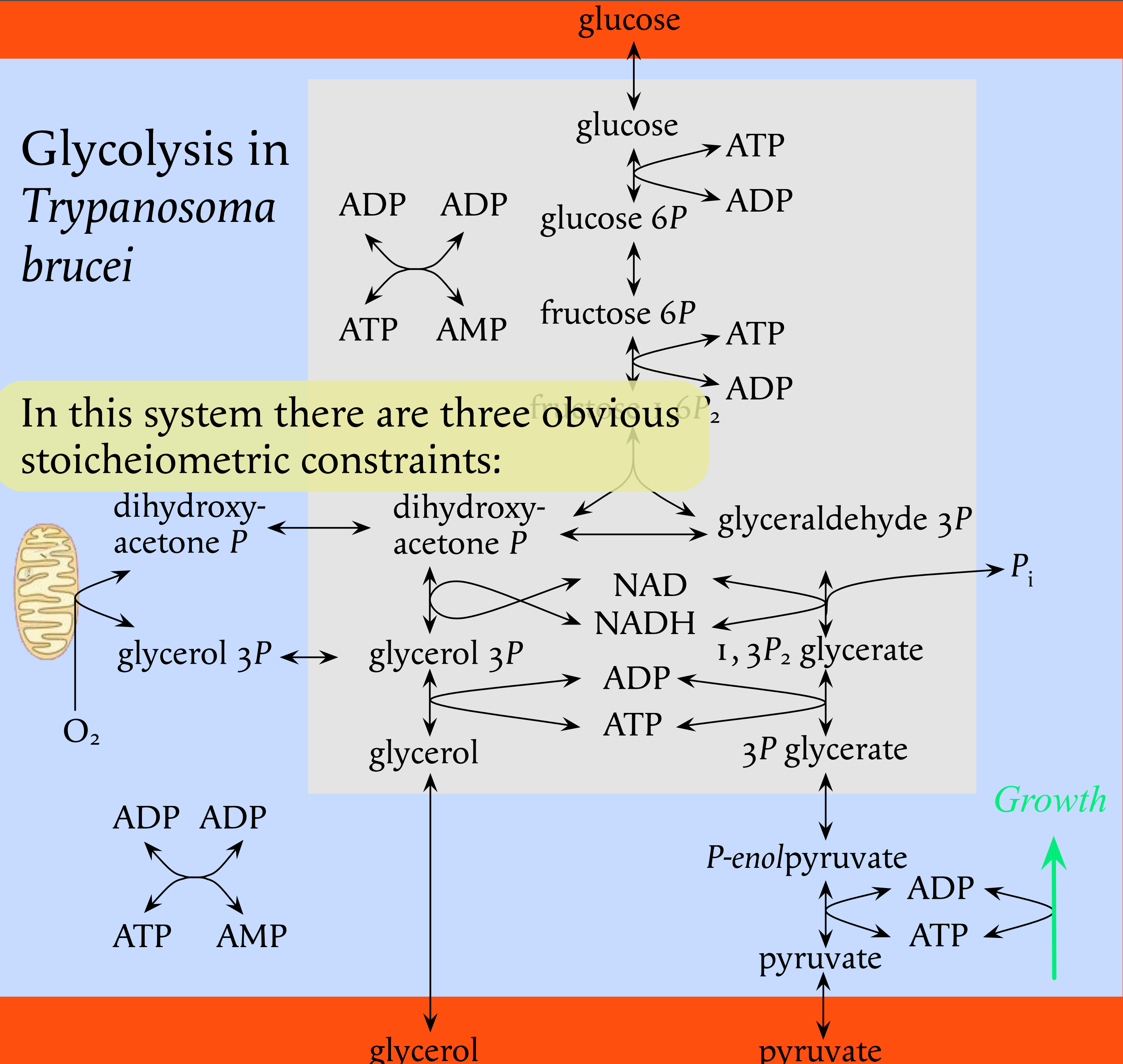






Glycolysis in *Trypanosoma brucei*

In this system there are three obvious stoicheiometric constraints:



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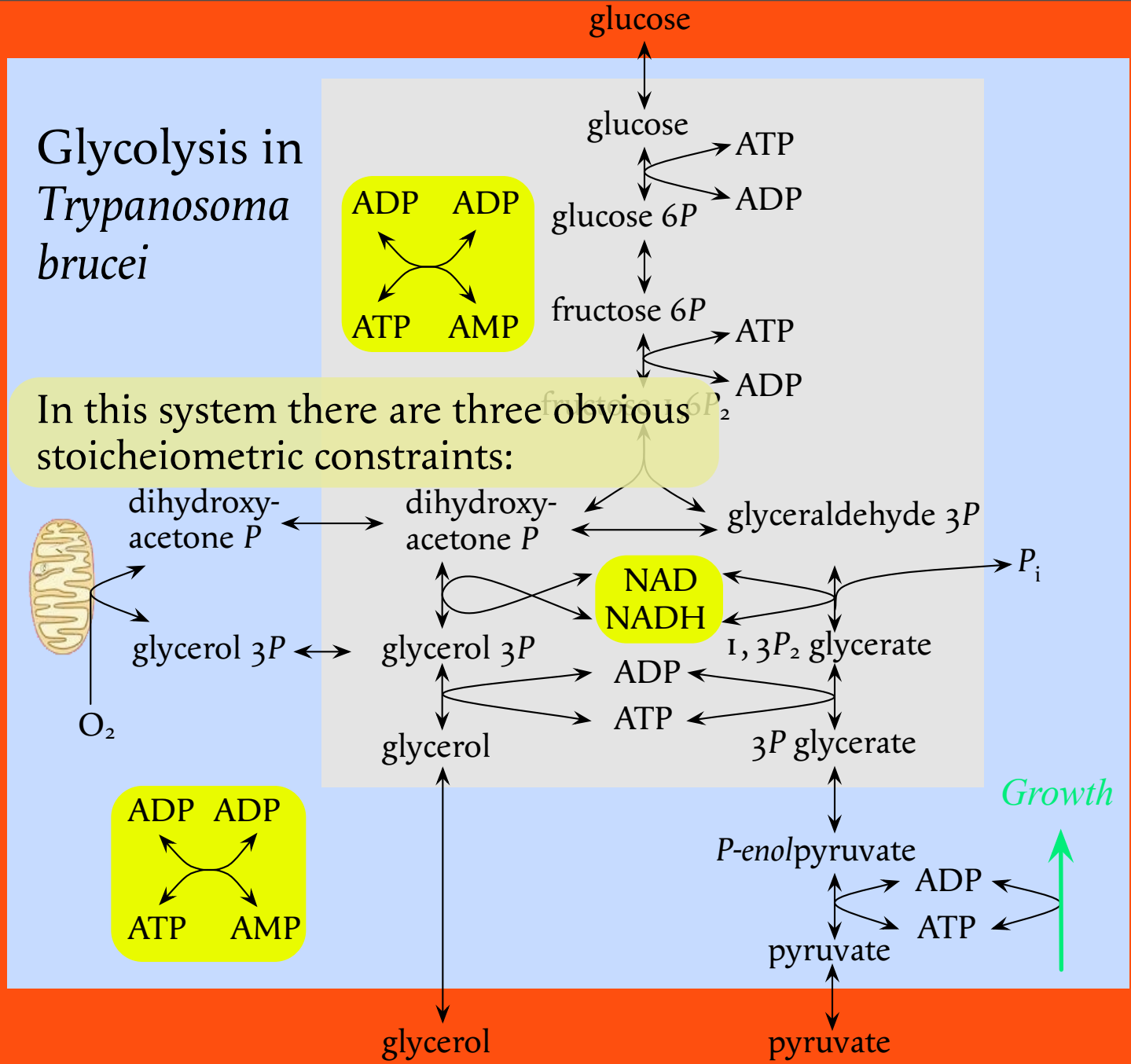

And a fourth that is not obvious at all:

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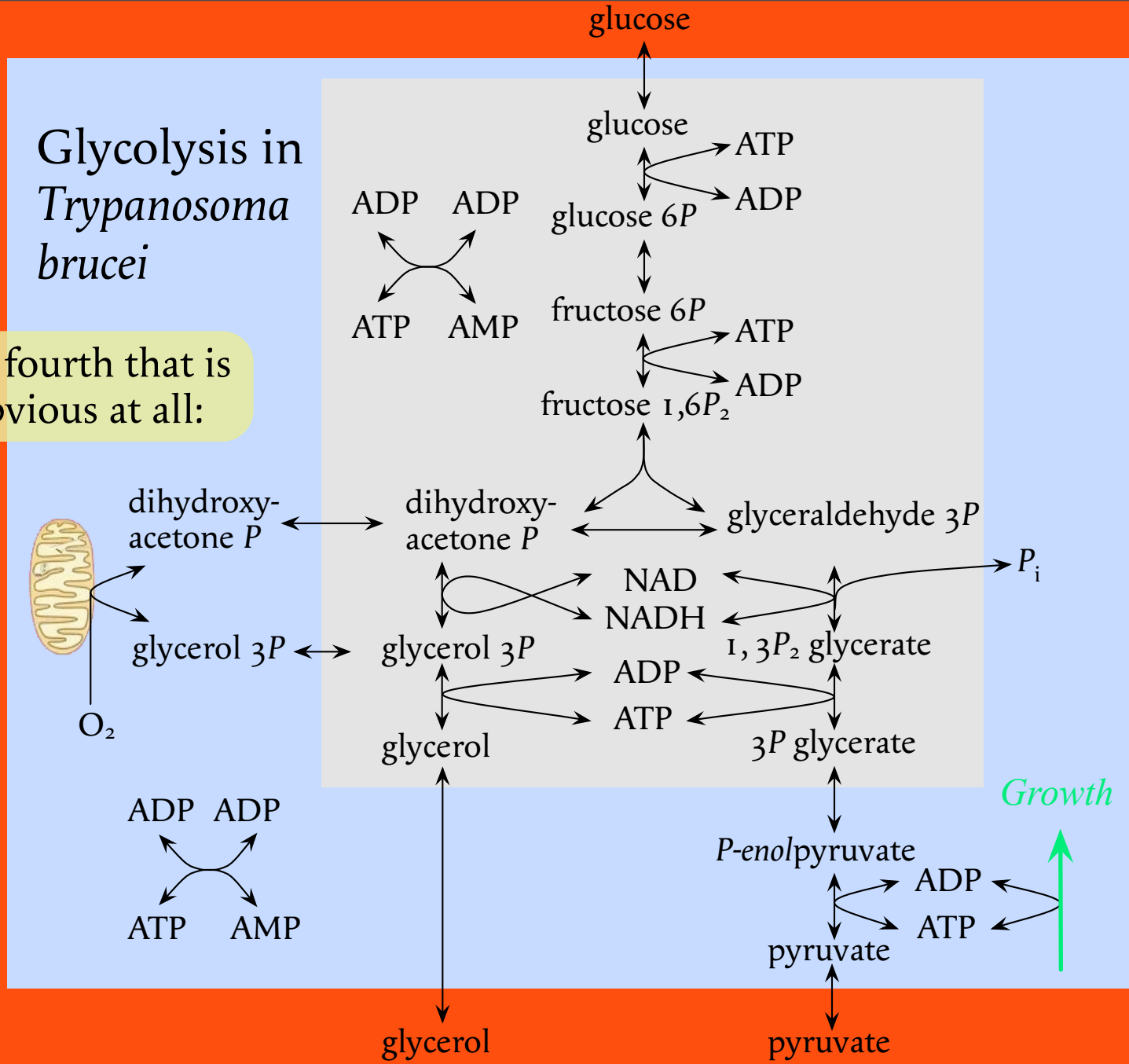

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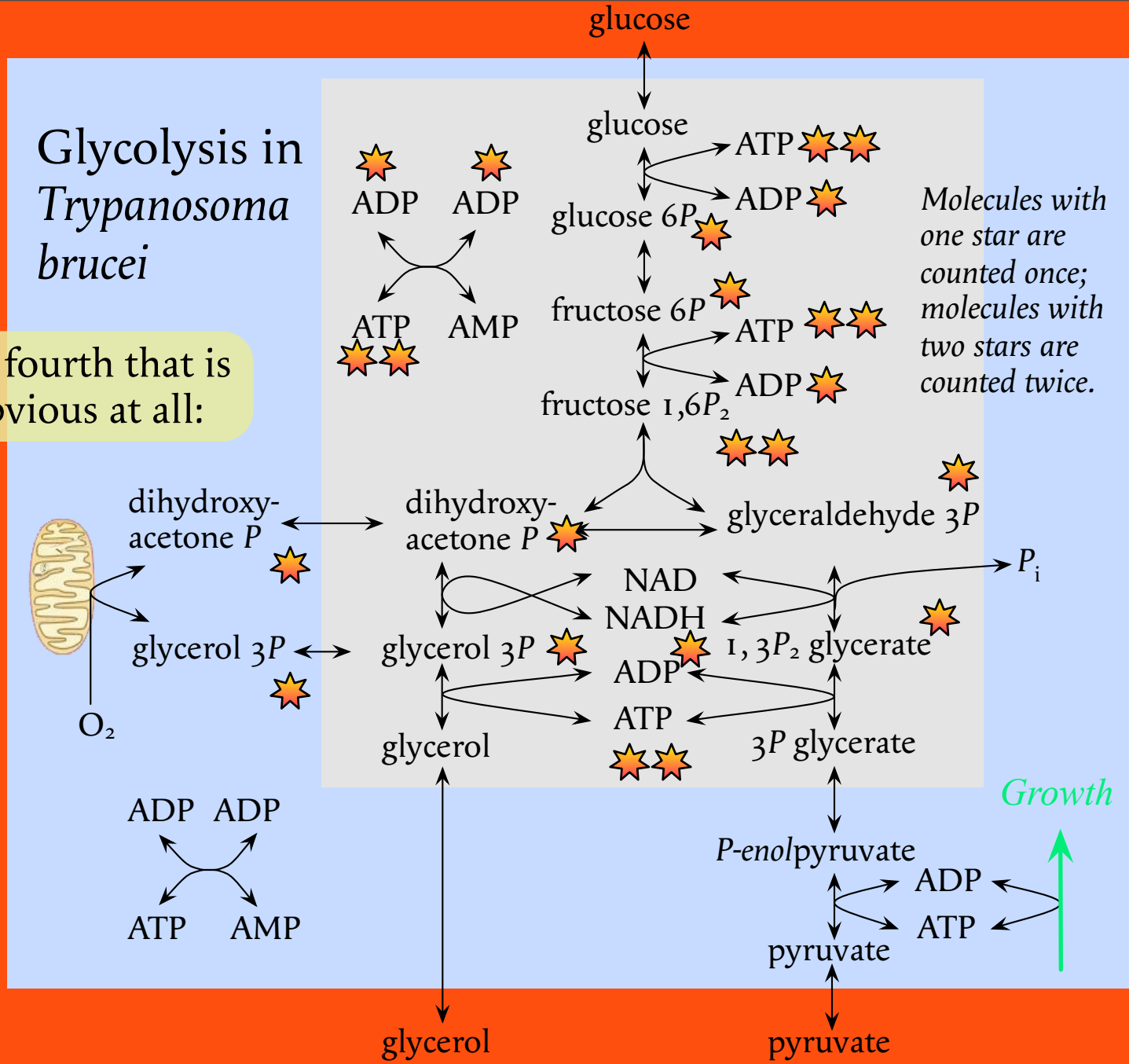
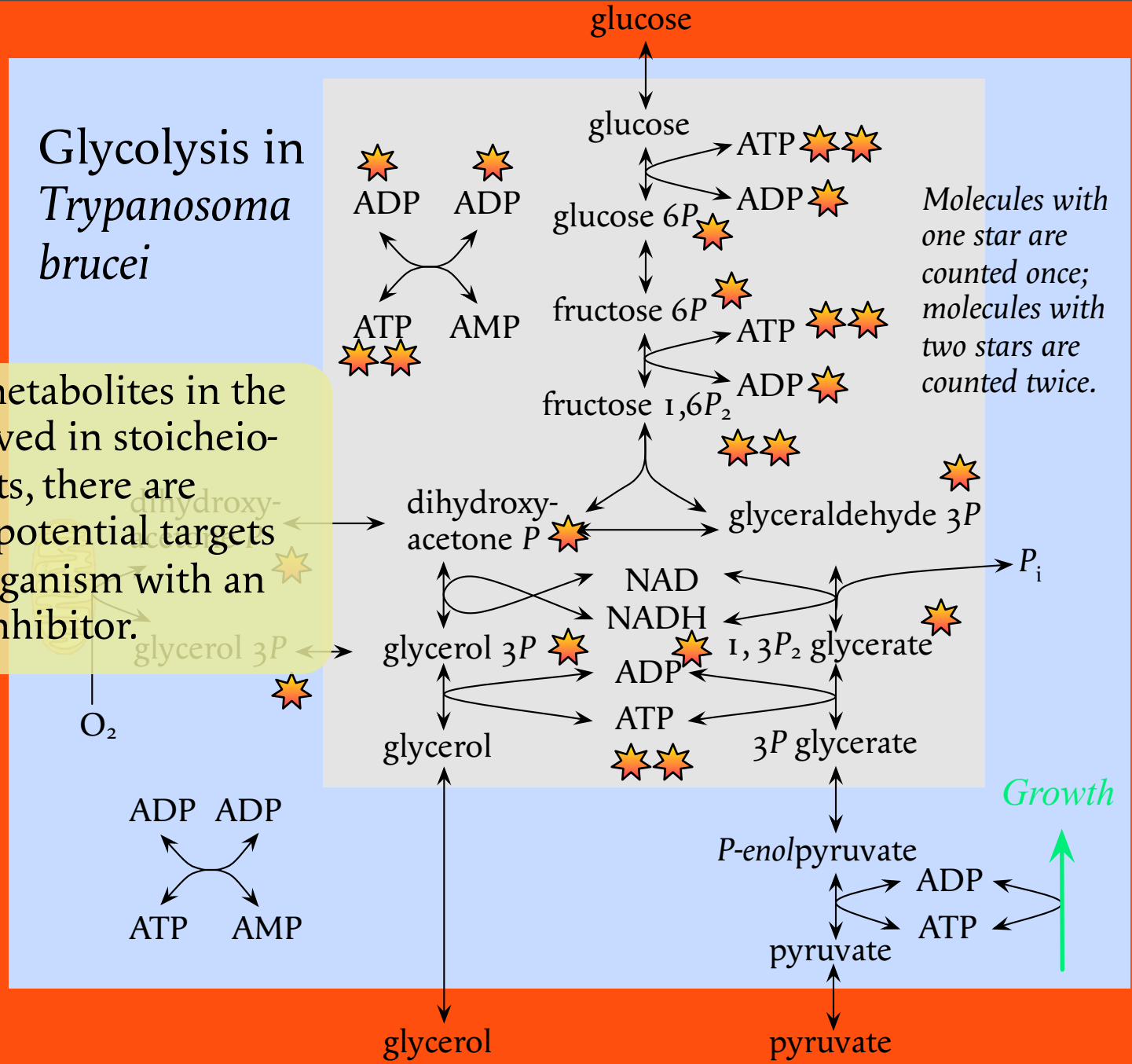


As most of the metabolites in the system are involved in stoicheiometric constraints, there are actually *very few* potential targets for killing the organism with an uncompetitive inhibitor.

Glycolysis in *Trypanosoma brucei*



Molecules with one star are counted once; molecules with two stars are counted twice.

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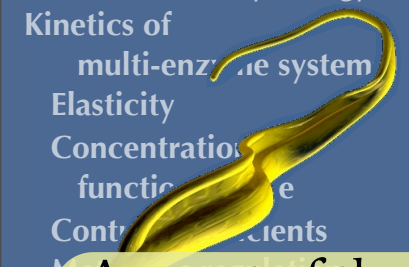

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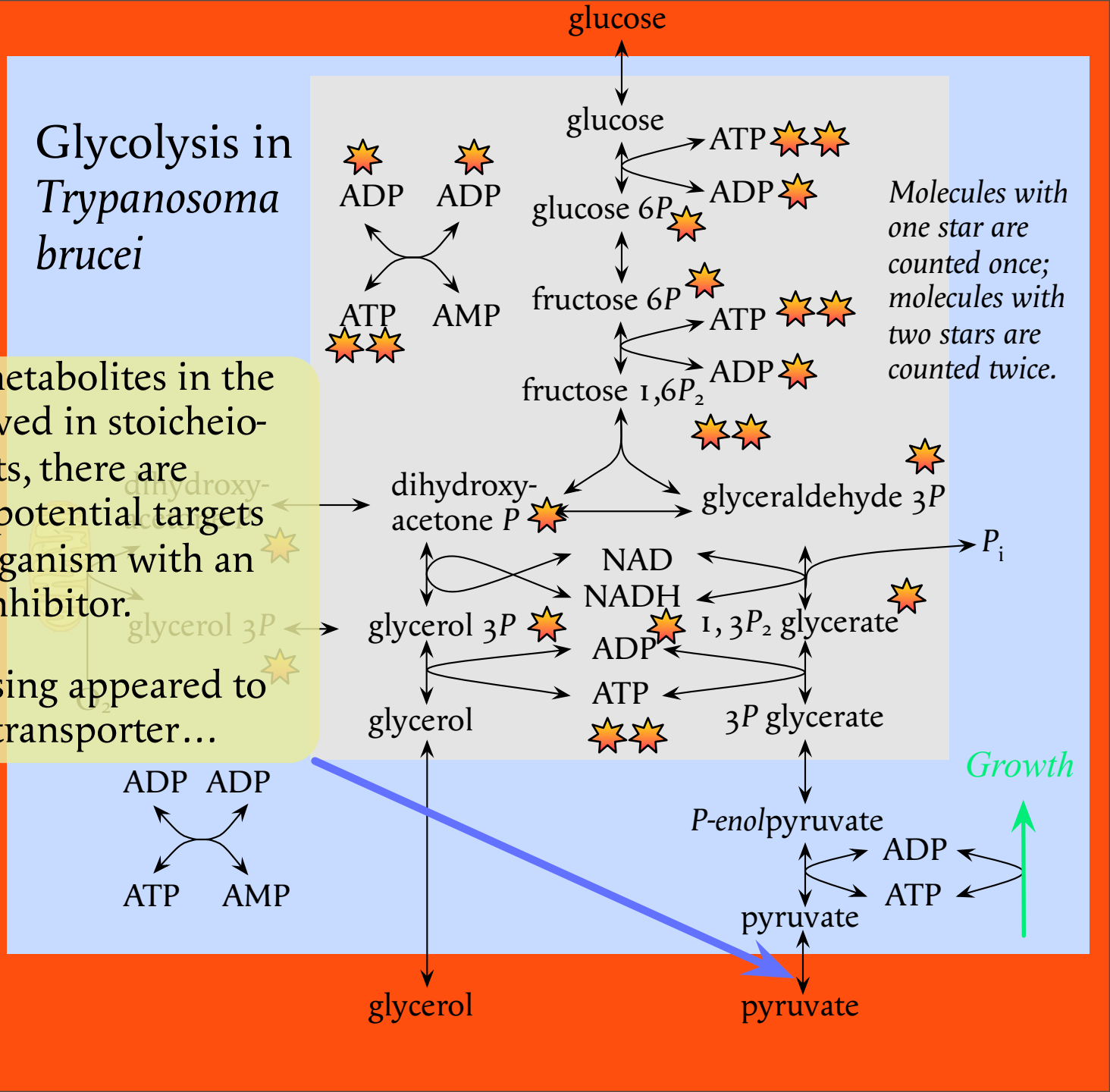
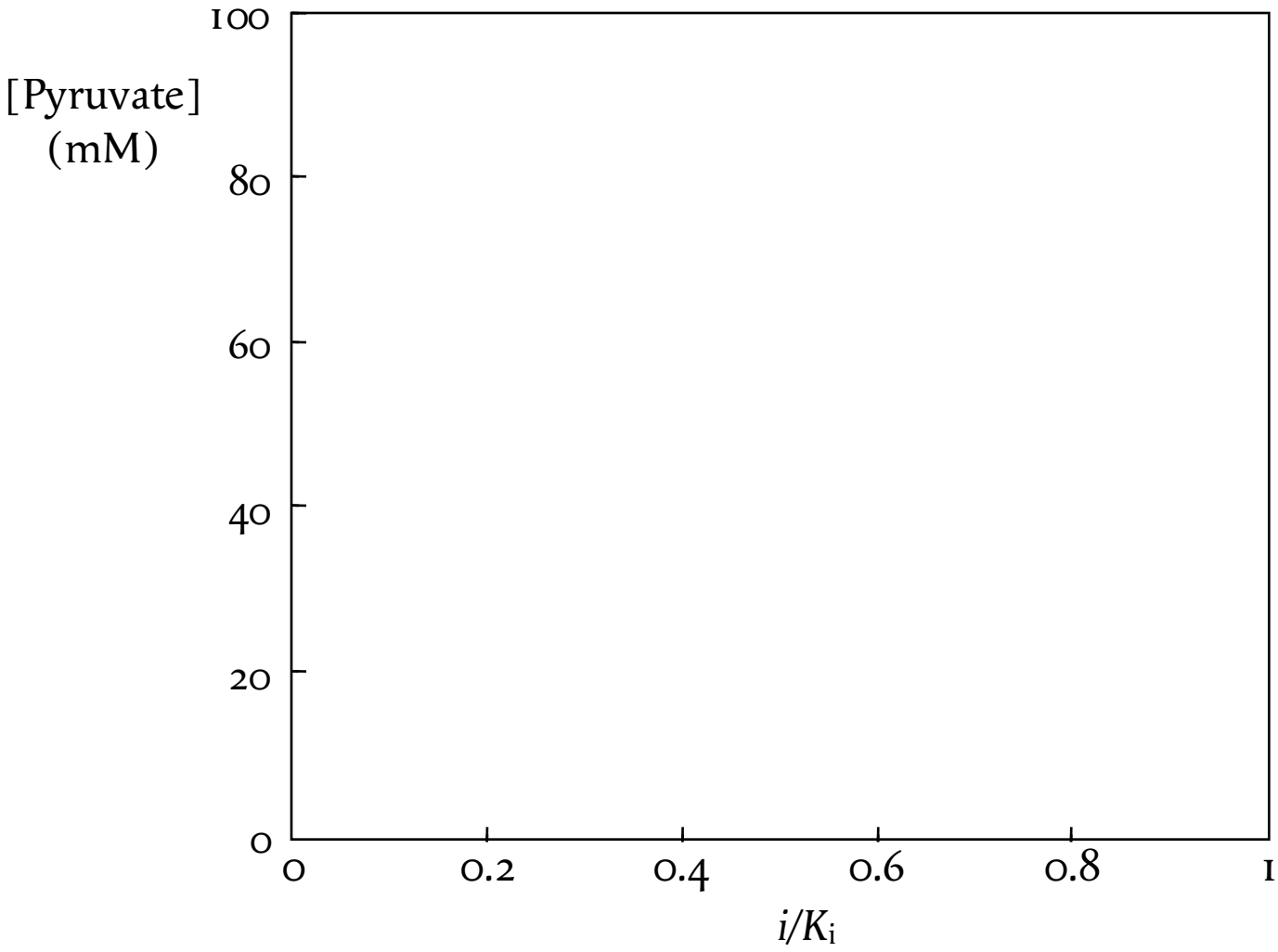
The most promising appeared to be the pyruvate transporter…



Simulation of the effect of inhibiting pyruvate export



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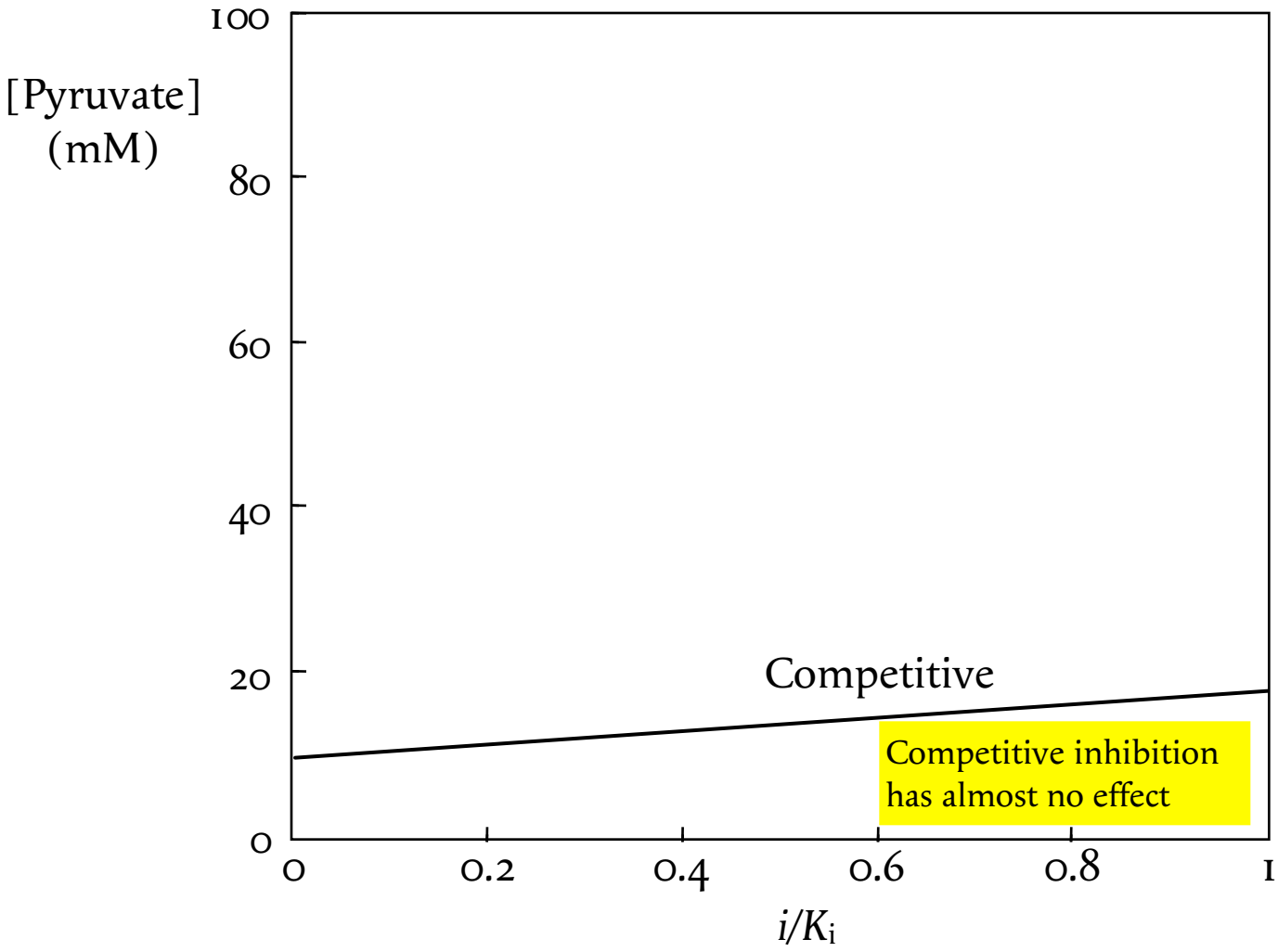
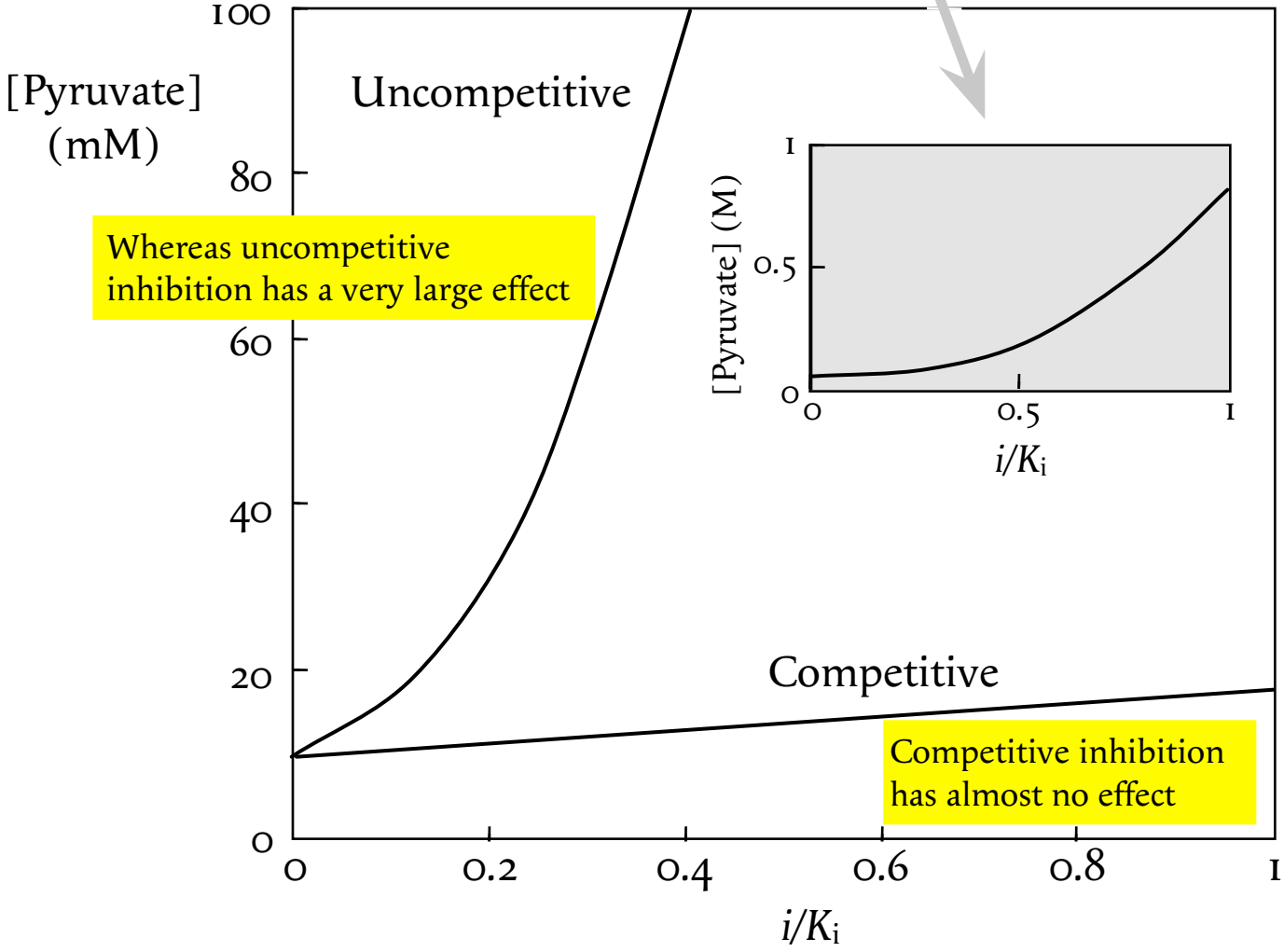


Simulation of the effect of inhibiting pyruvate export

continuing the curve on a smaller scale



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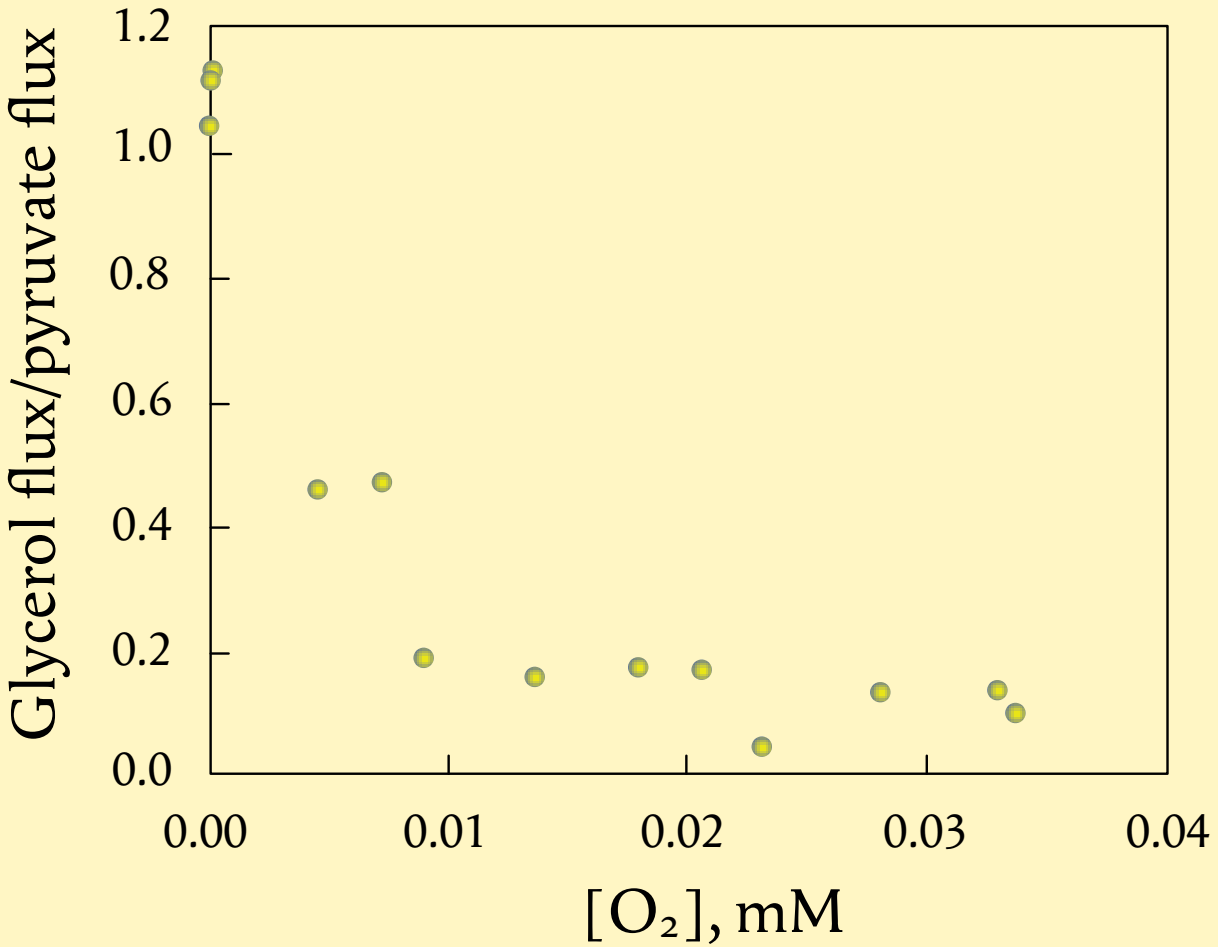
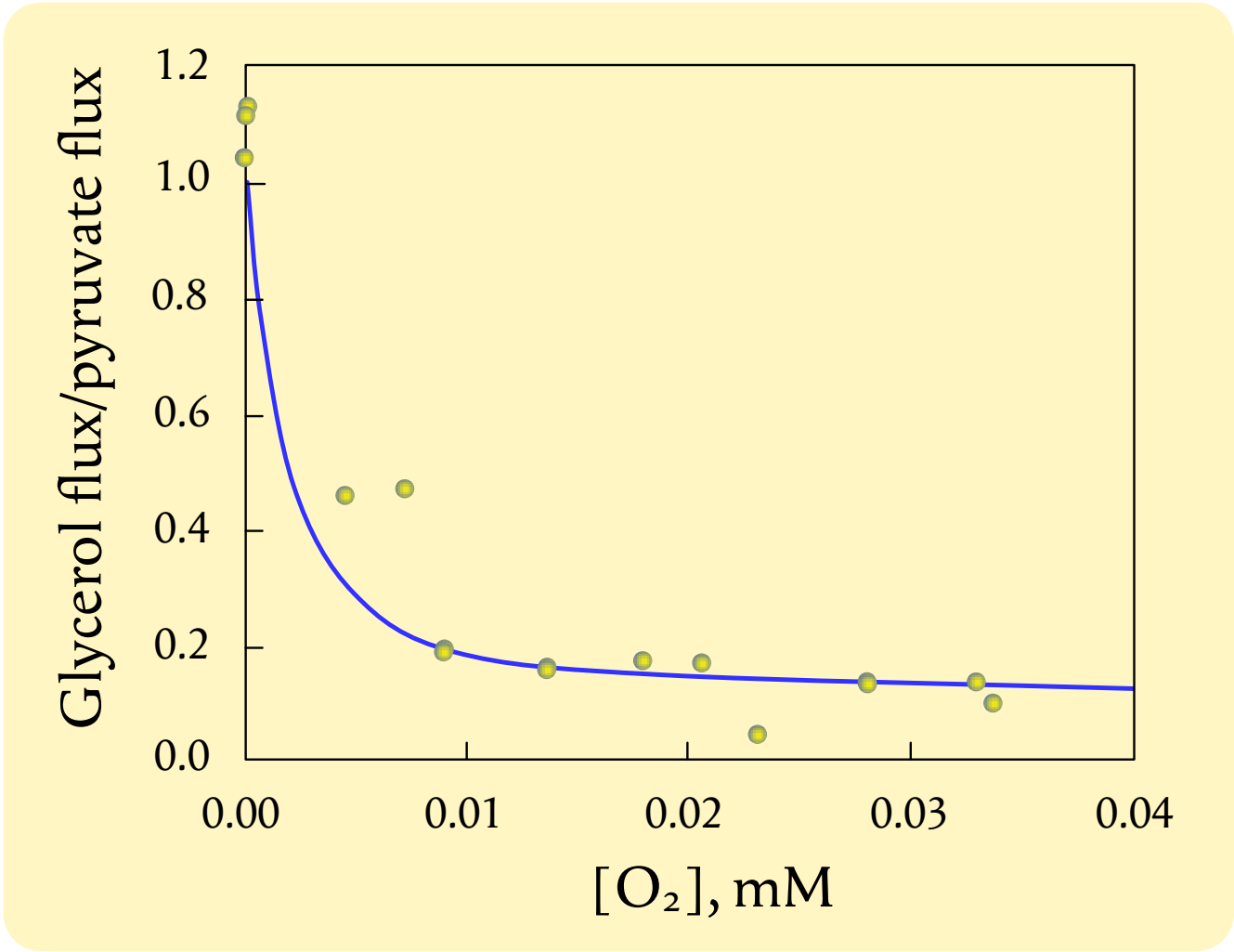
However, our experimental data had suggested that, even though glycerol efflux decreases greatly in aerobic conditions it does not reach zero.

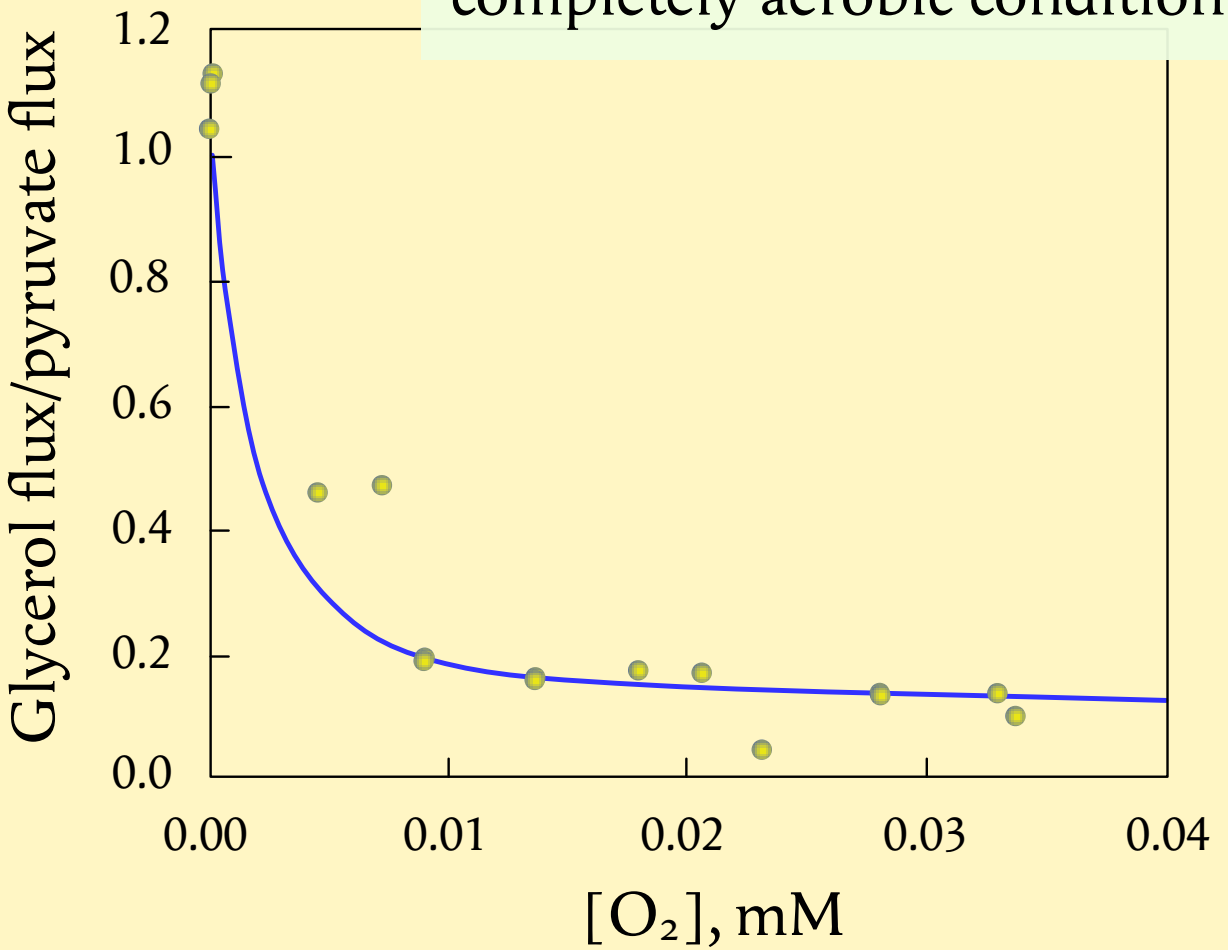
Results of Opperdoes and colleagues suggest

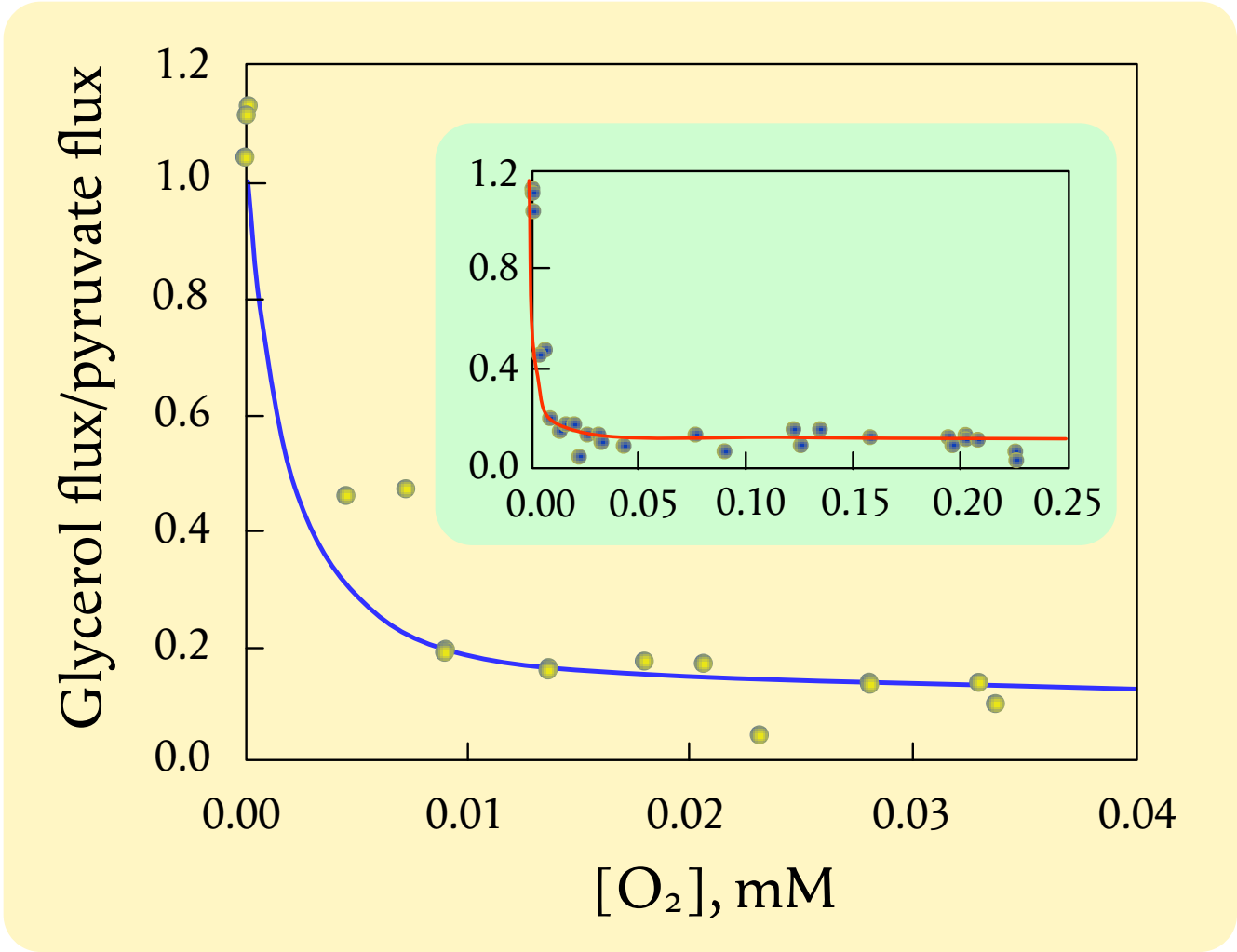


zero.



Not only is the agreement excellent, but it continues to completely aerobic conditions.





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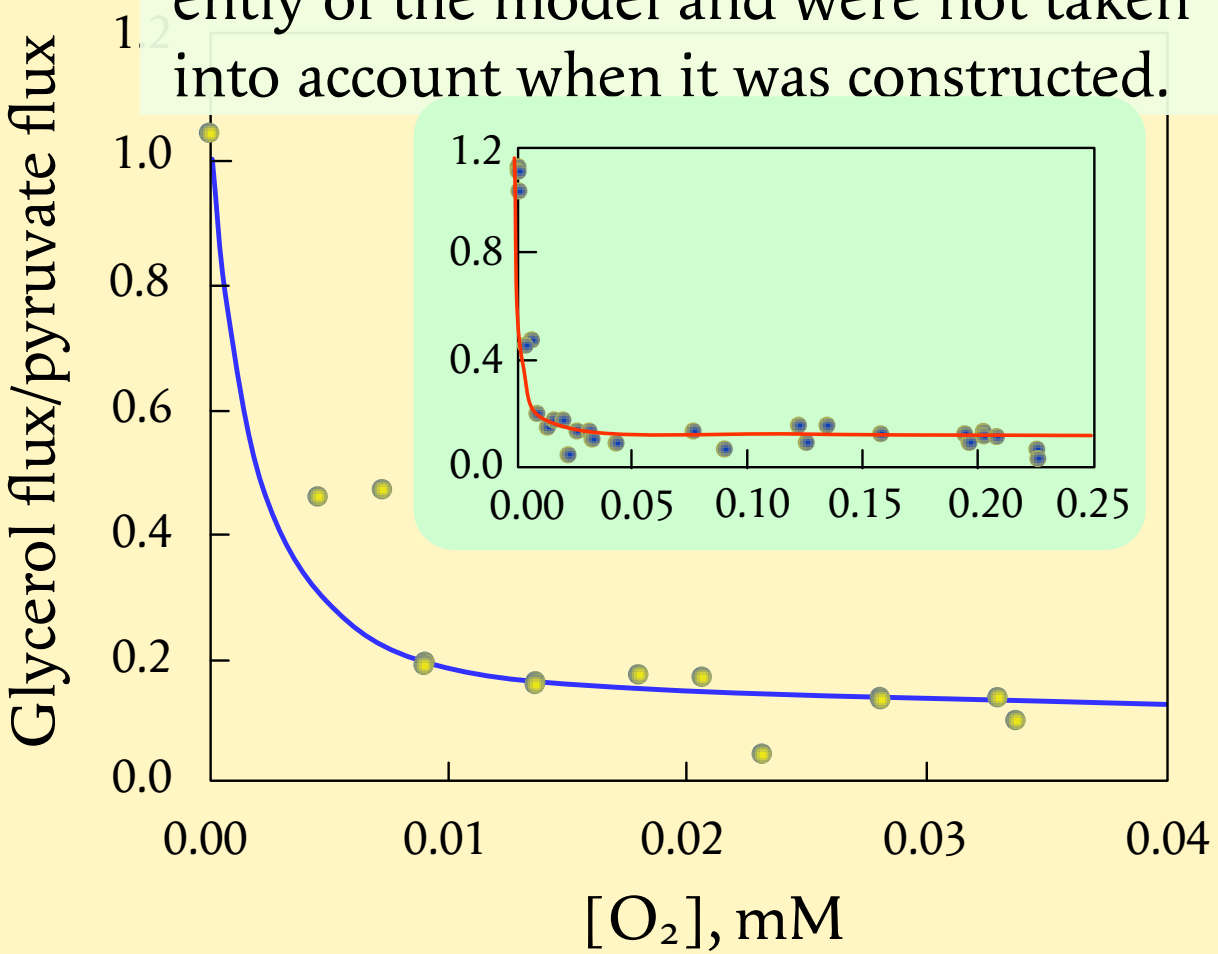

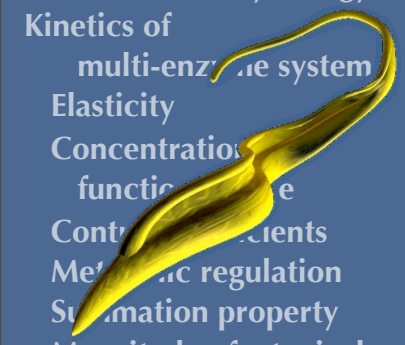

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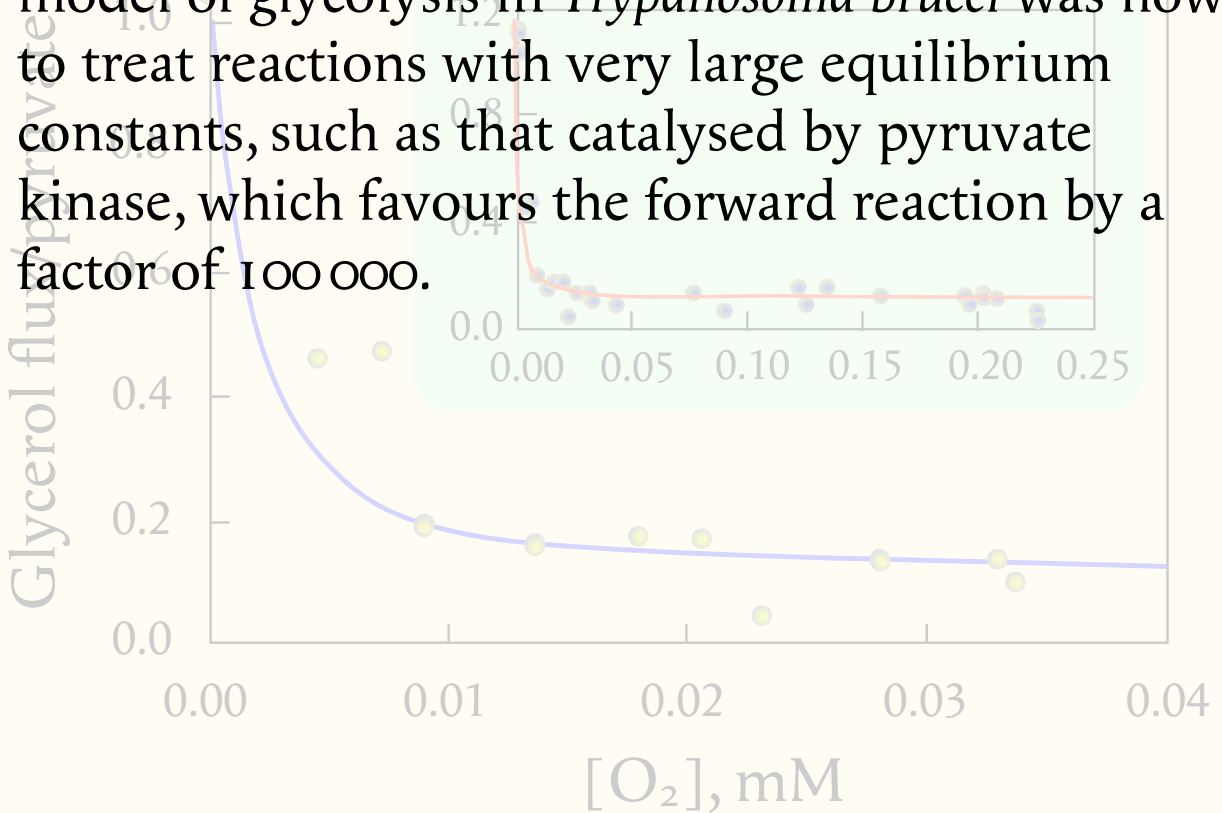

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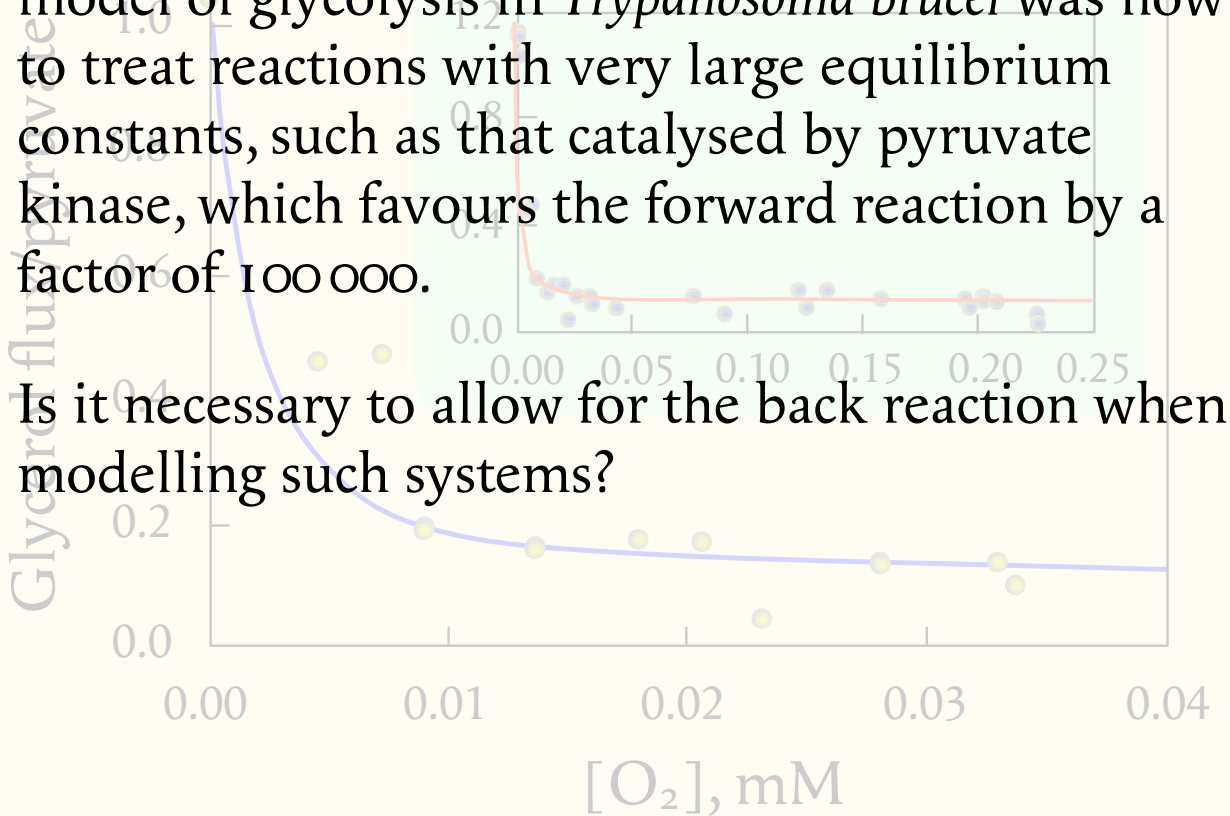
Is it necessary to allow for the back reaction when modelling such systems?



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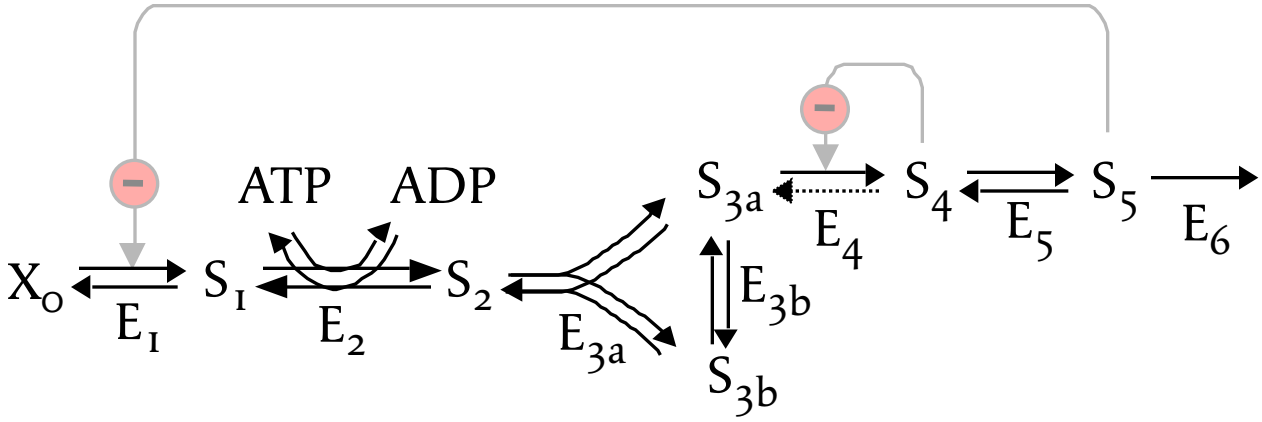
Including the reverse reaction for pyruvate kinase changed the distribution of flux control in the trypanosomal system drastically and it appeared important to understand why.

HANDLING OF IRREVERSIBLE STEPS IN METABOLIC MODELS

Trypanosomal metabolism is rather complicated for examining this question, and so we have studied a much simpler model (albeit complicated enough to avoid being trivial), which includes…



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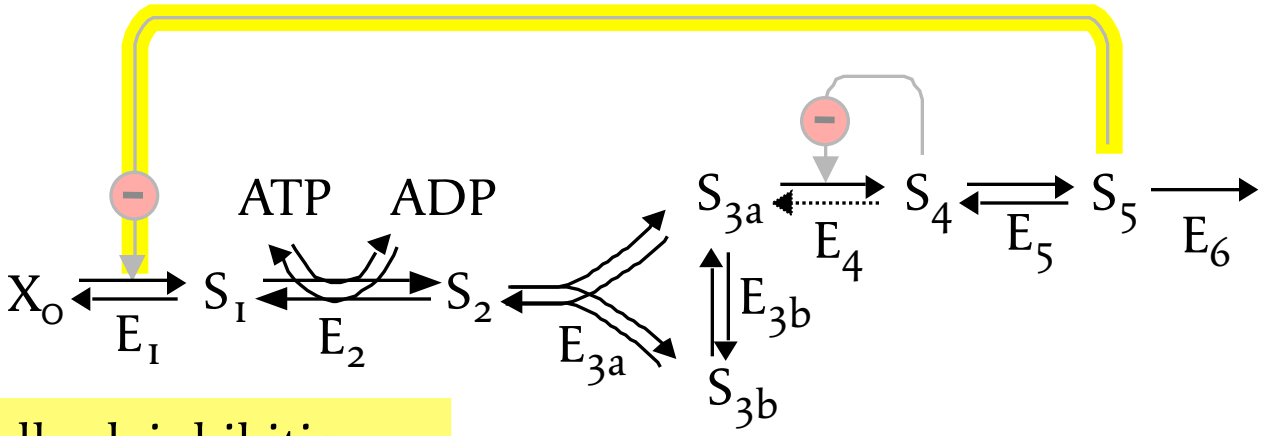

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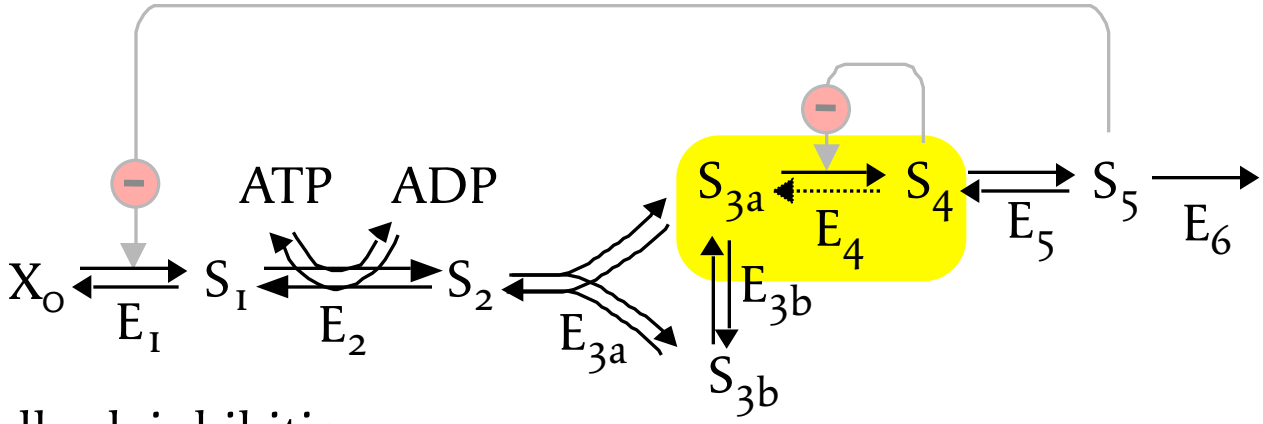

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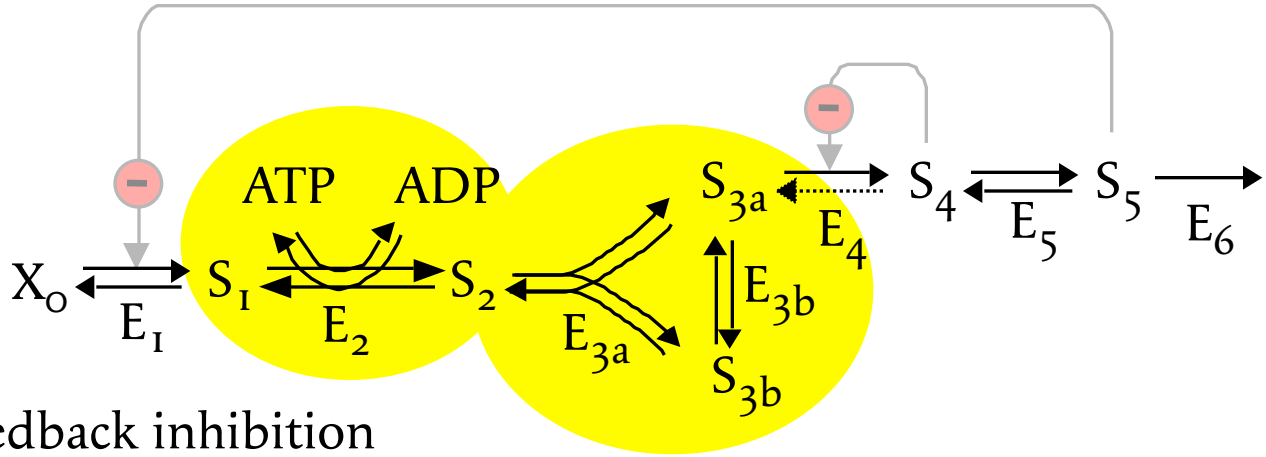

feedback inhibition of the first enzyme by the final product, a step with a high enough equilibrium constant to be considered irreversible, and other features that I shall not discuss.

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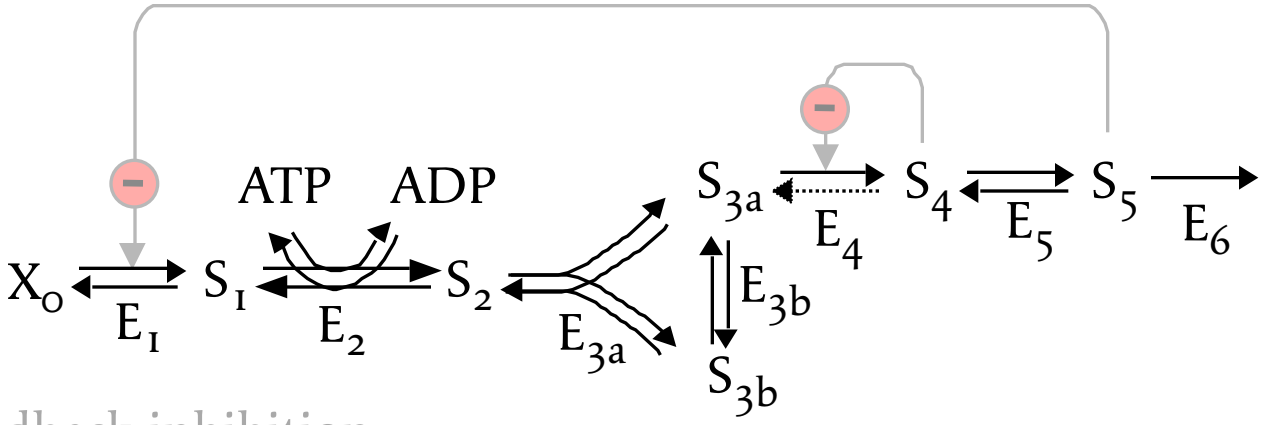
What happens to the fluxes and metabolite concentrations when the metabolic demand for the final product S_5 is varied by changing the activity of the final enzyme*?



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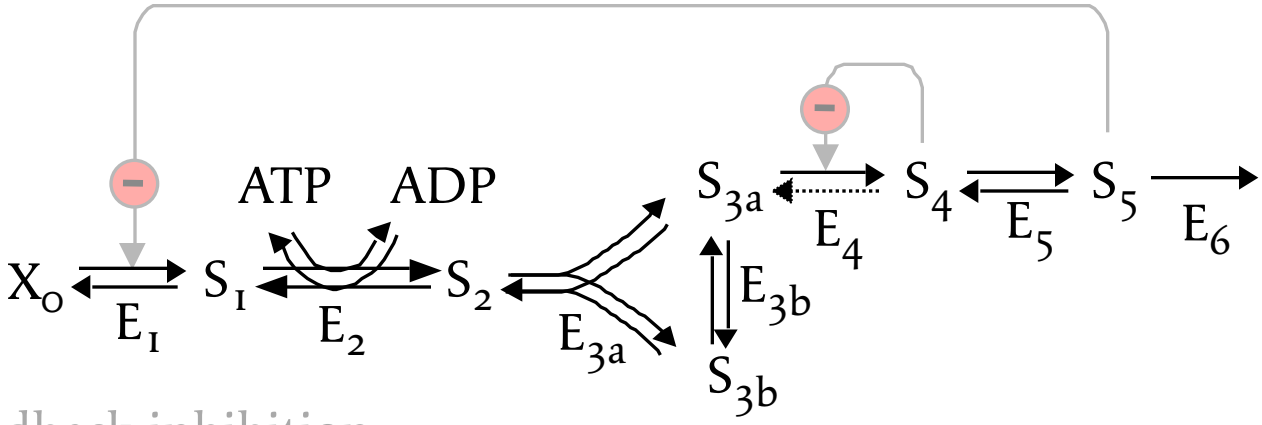

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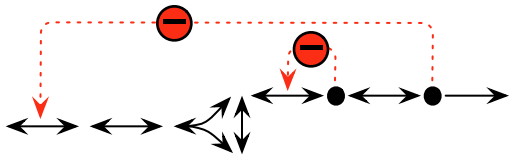

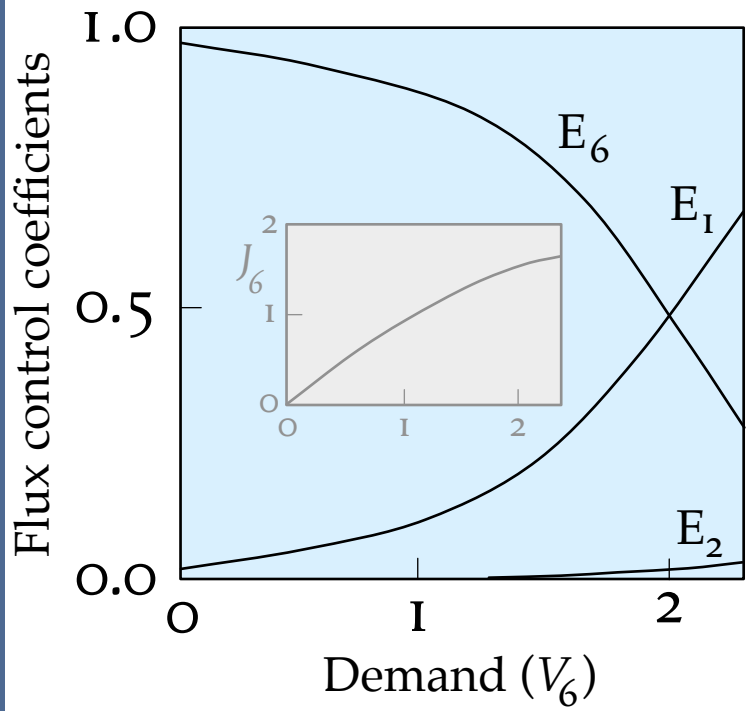

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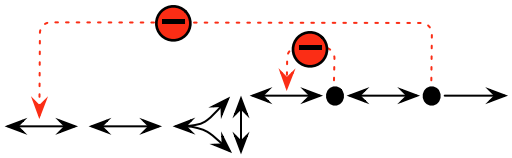
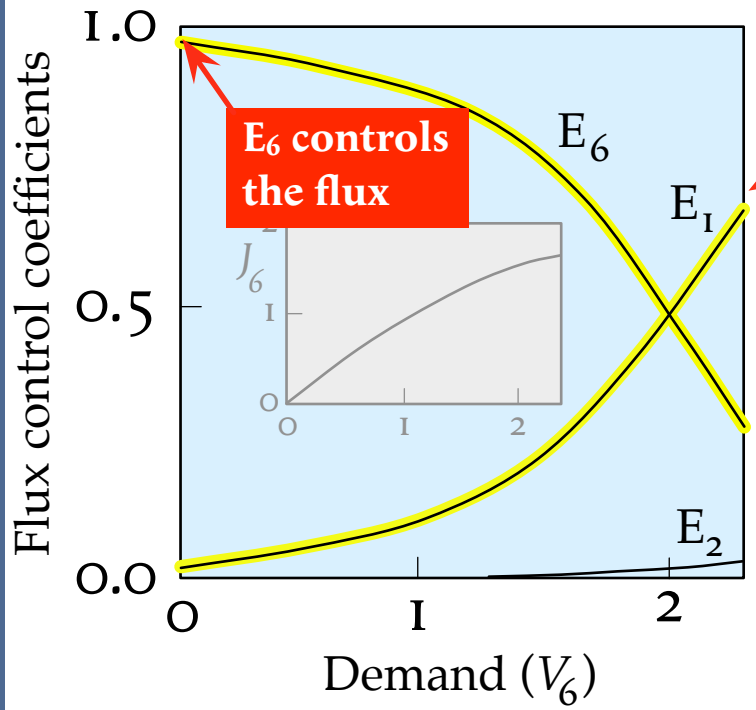
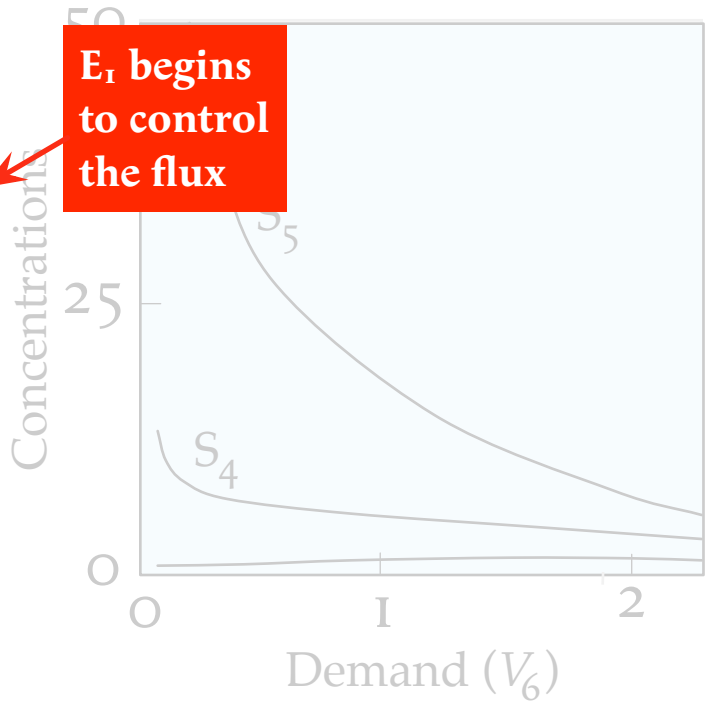
Flux control changes smoothly from E_6 to E_1 when the demand for the final product increases







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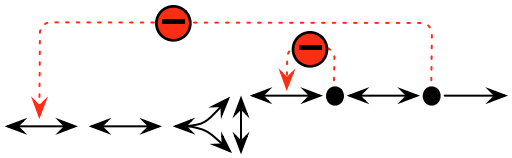

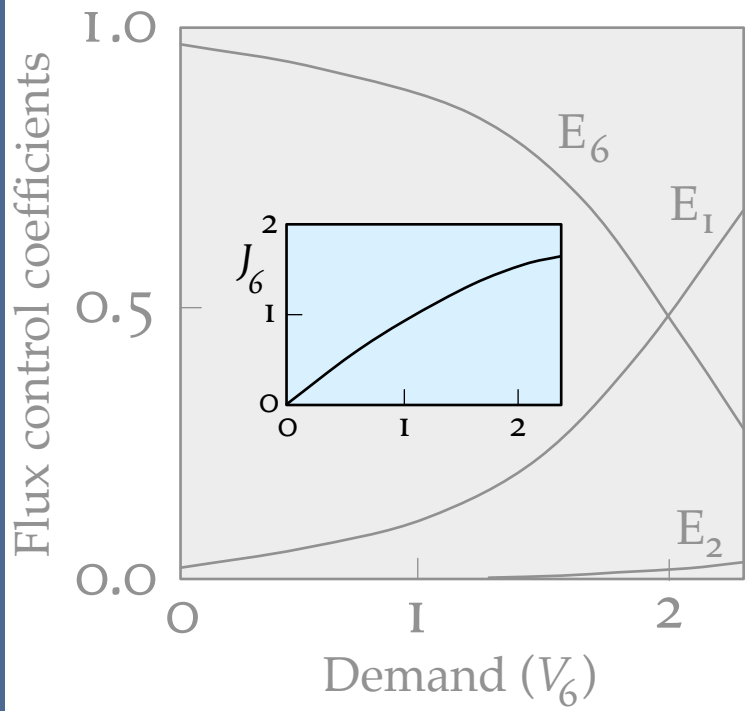

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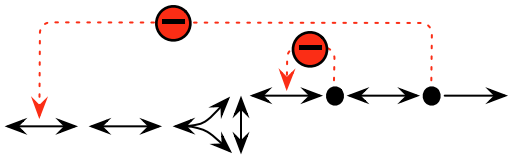

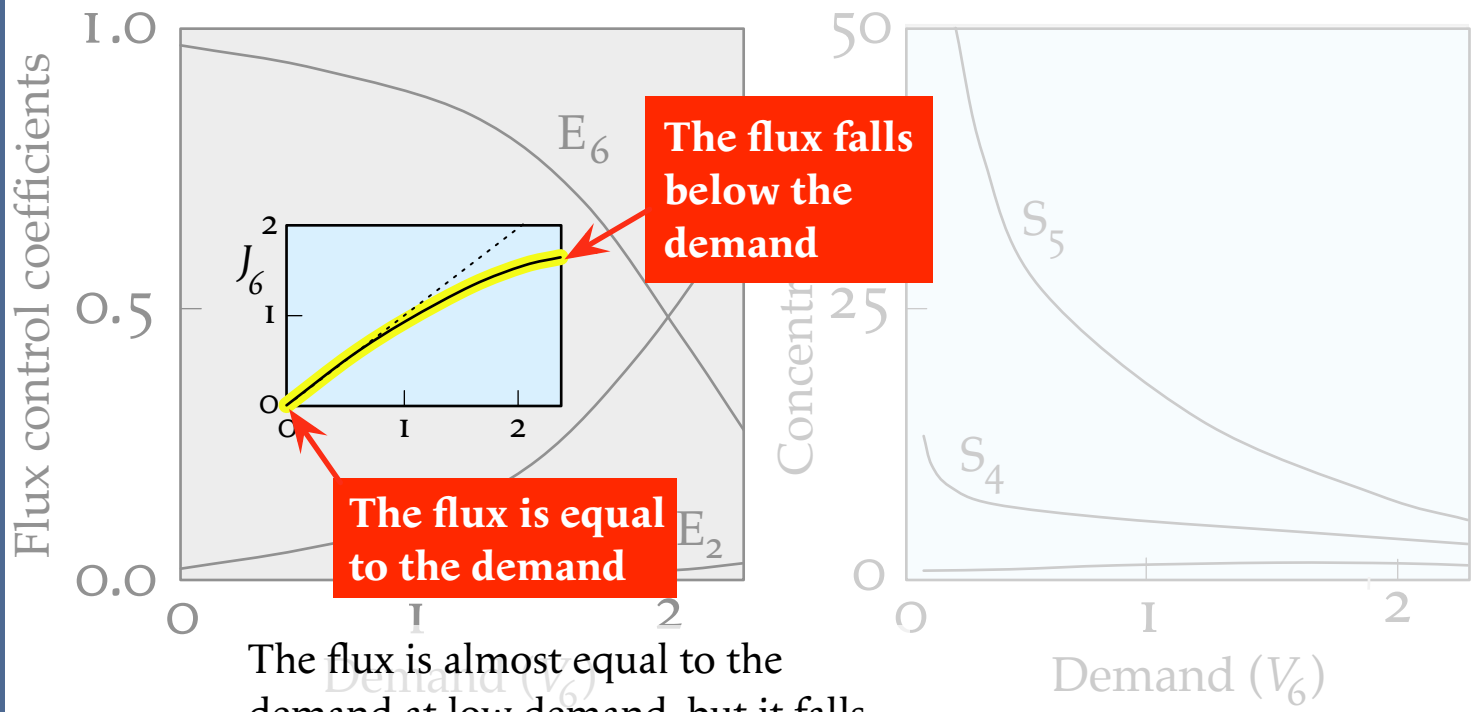

The flux is almost equal to the demand at low demand, but it falls below it when the demand increases

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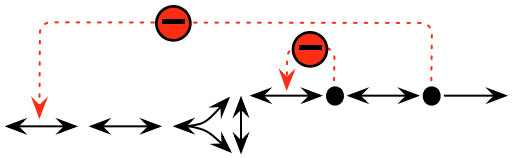

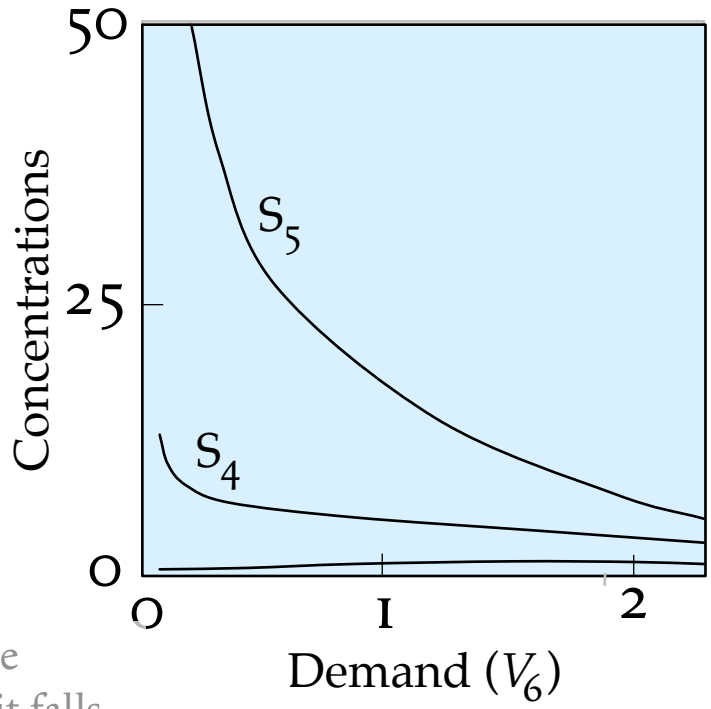

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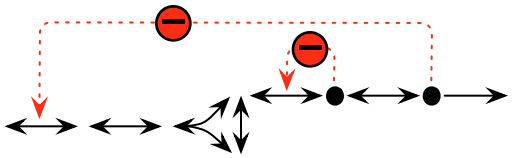
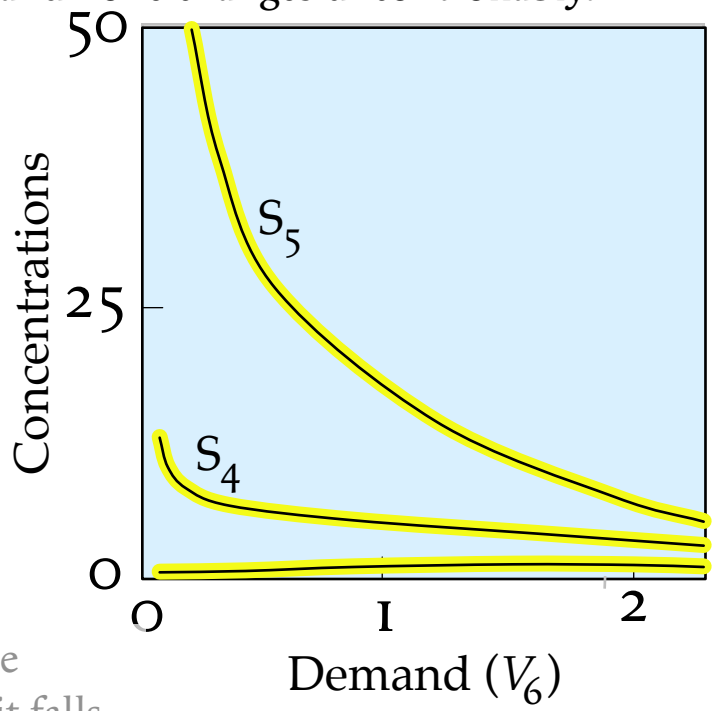


Most metabolite concentrations barely change when the demand decreases, and none changes uncontrollably.



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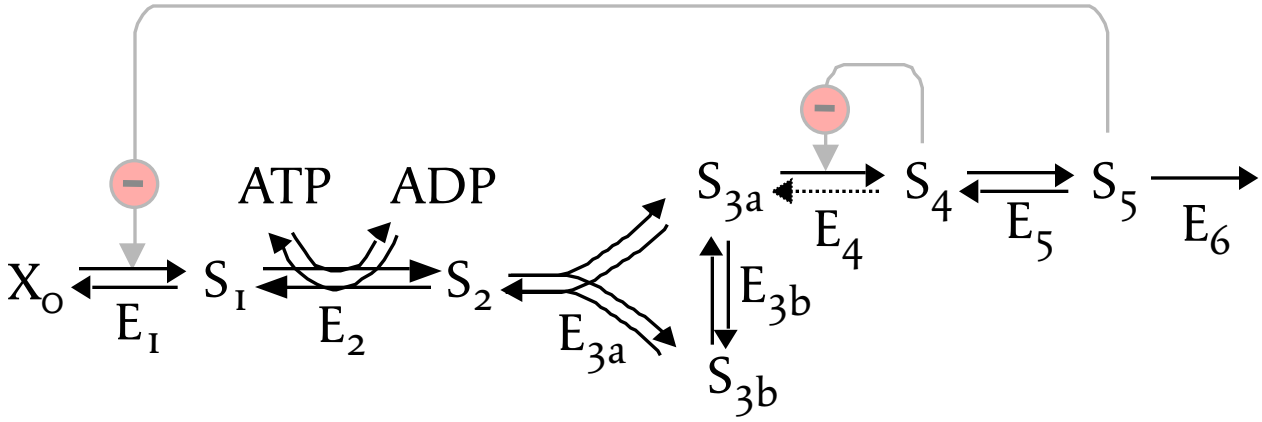
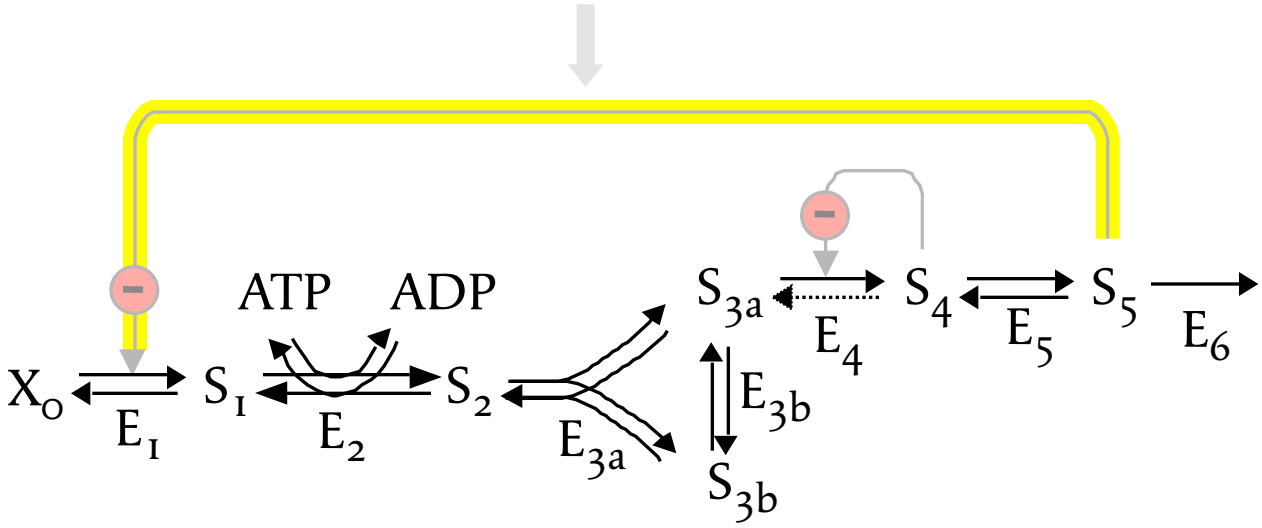


In this model the first step is subject to feedback inhibition by the final product,



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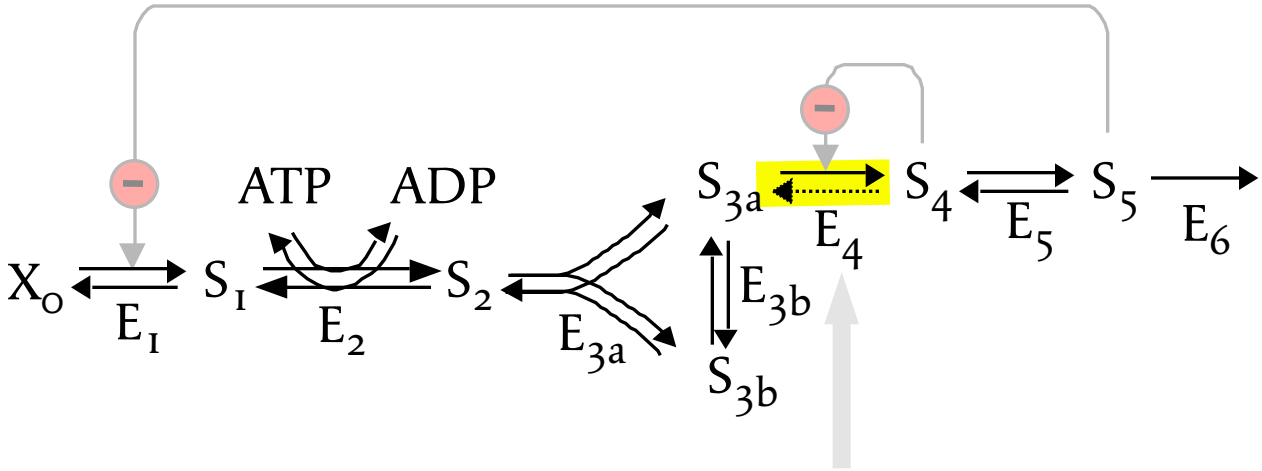

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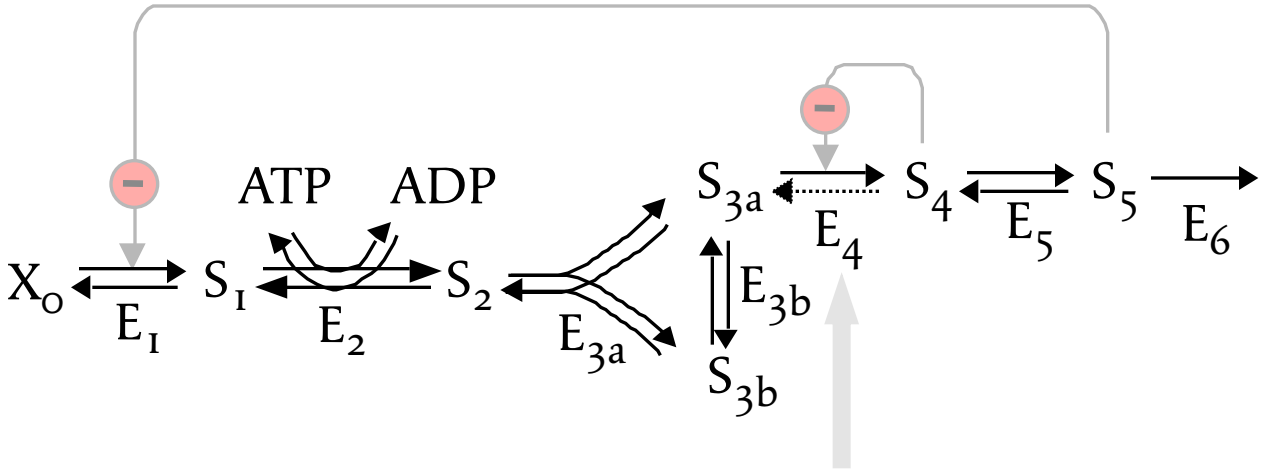
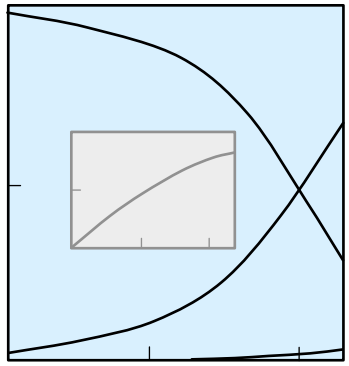
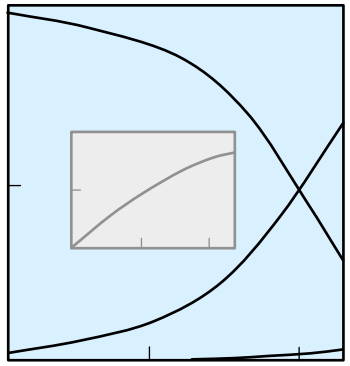


and the fourth step has a large equilibrium constant but is treated as reversible.

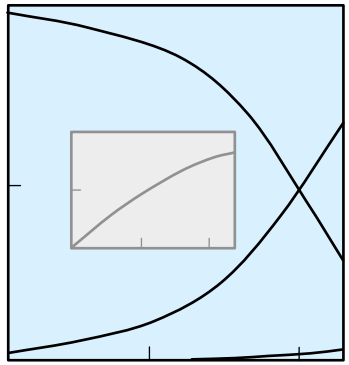
Which of the two properties is more important? The feedback inhibition? Or the reversibility (in all the steps)? Or are both essential?

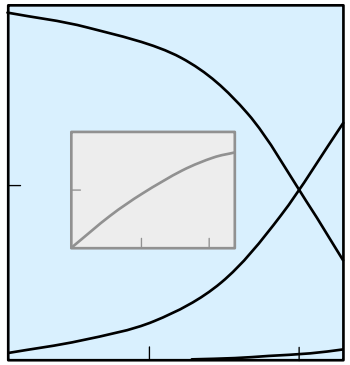
We know already what the first panel looks like.





Feedback inhibition and reversibility





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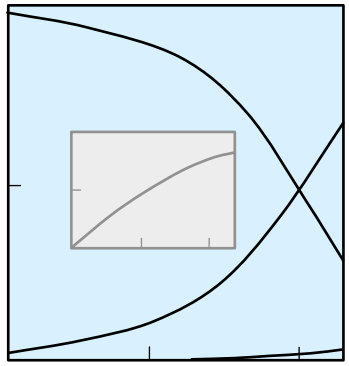
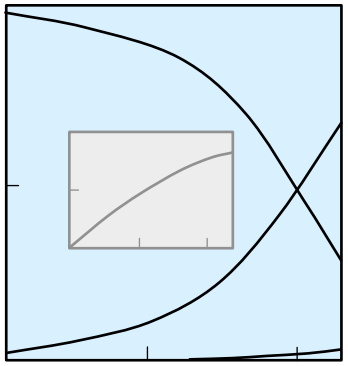
What about the other three?





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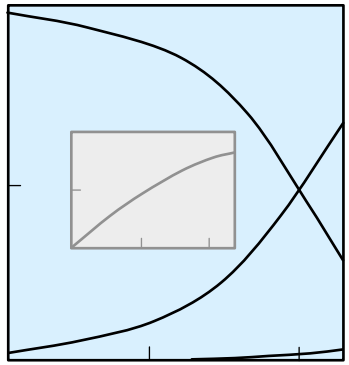

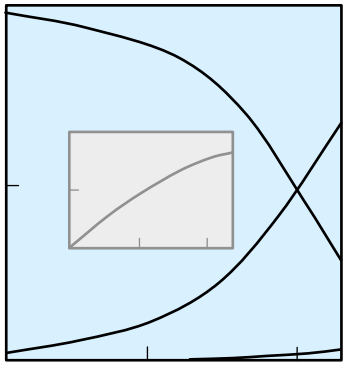

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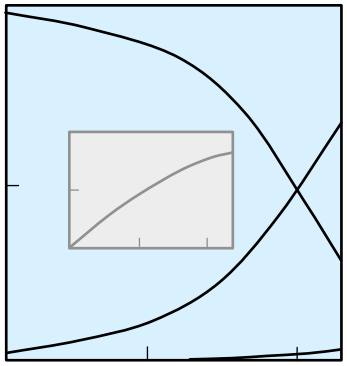

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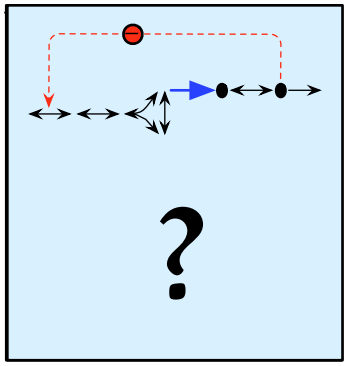
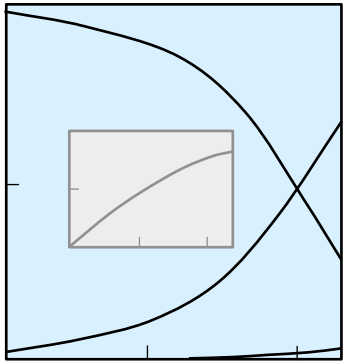
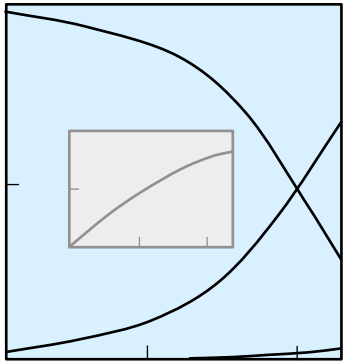


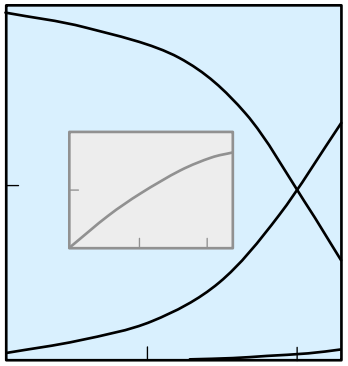
Feedback inhibition without reversibility



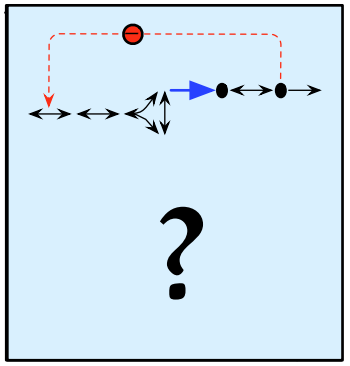


We know already what the first panel looks like.

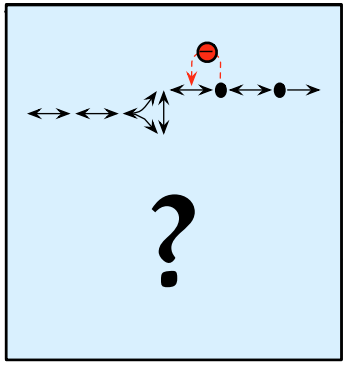
What about the other three?



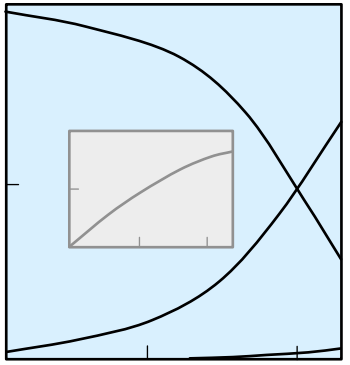
Feedback inhibition and reversibility



Feedback inhibition without reversibility

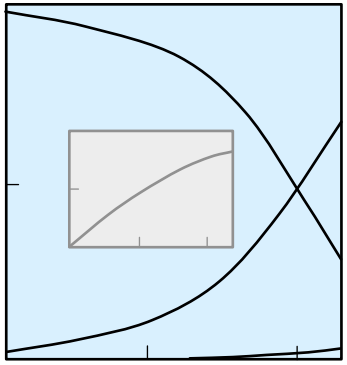


Reversibility without feedback inhibition

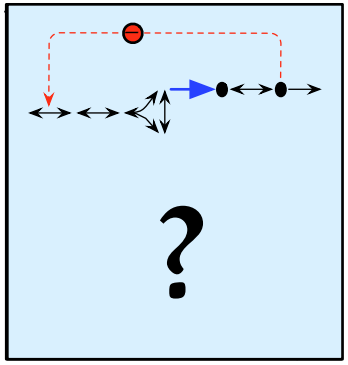


We know already what the first panel looks like.

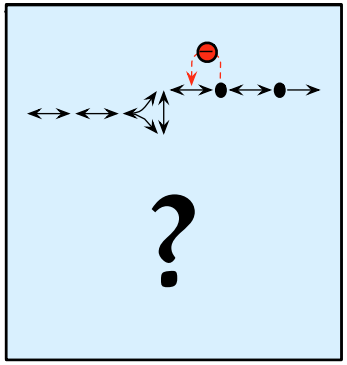
What about the other three?



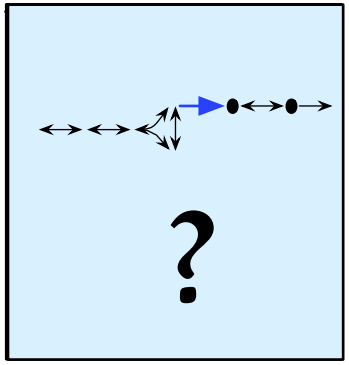
Feedback inhibition and reversibility



Feedback inhibition without reversibility

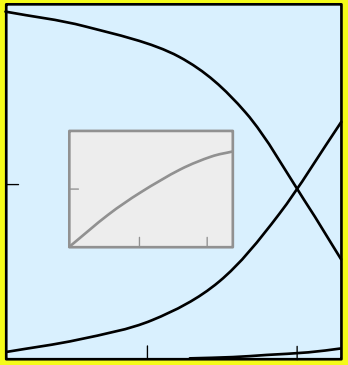


Reversibility without feedback inhibition

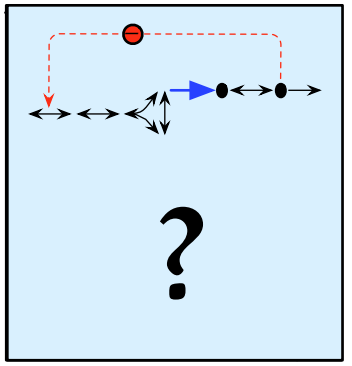


Neither one nor the other

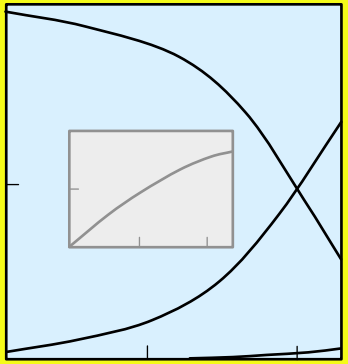
But if reversibility is essential and feed-back inhibition has no importance, the left-hand pair should be similar, the others different:



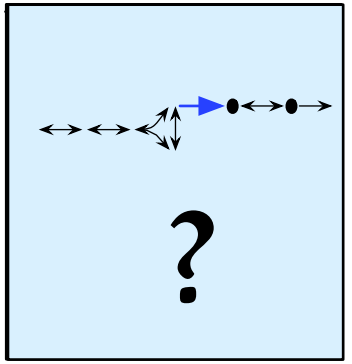
Feedback inhibition and reversibility



Feedback inhibition without reversibility

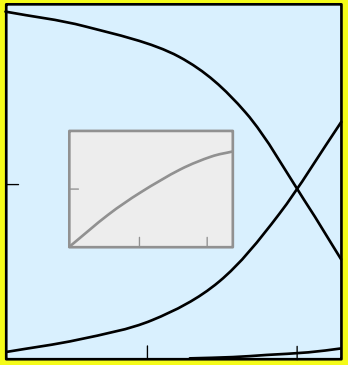


Reversibility without feedback inhibition

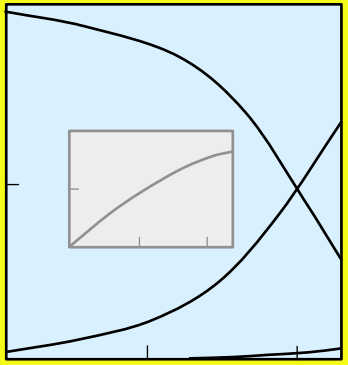


Neither one nor the other

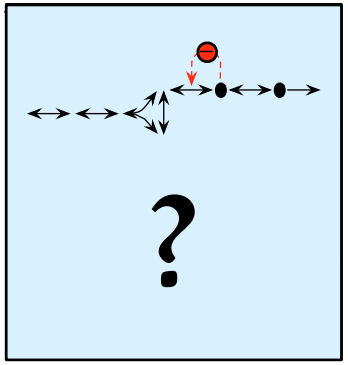
If feedback inhibition is essential and reversibility has no importance, the top pair should be similar, the others different:



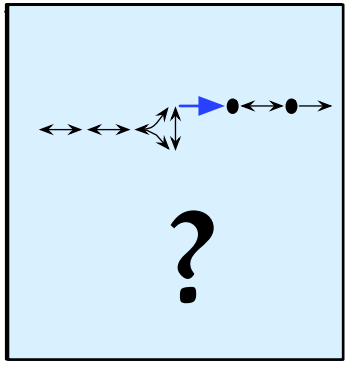
Feedback inhibition and reversibility



Feedback inhibition without reversibility

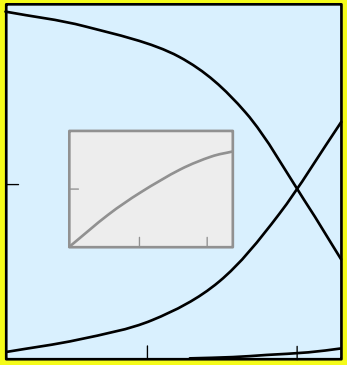


Reversibility without feedback inhibition

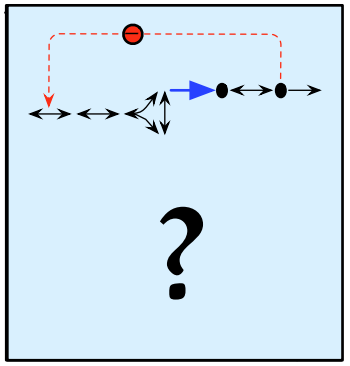


Neither one nor the other

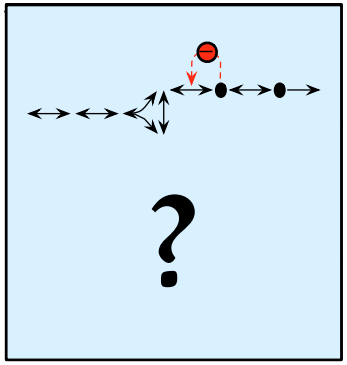
On the other hand, if reversibility and feedback inhibition are *both* essential, the top-left panel should be different from the other three:



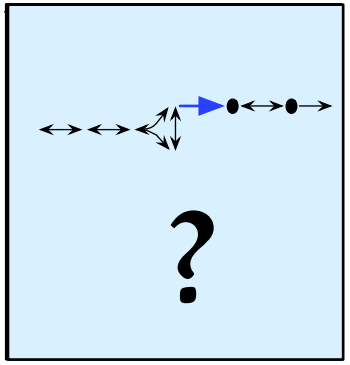
Feedback inhibition and reversibility



Feedback inhibition without reversibility

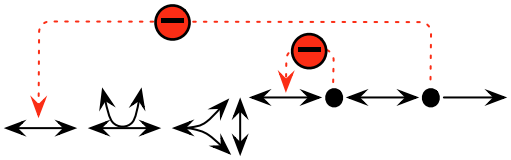


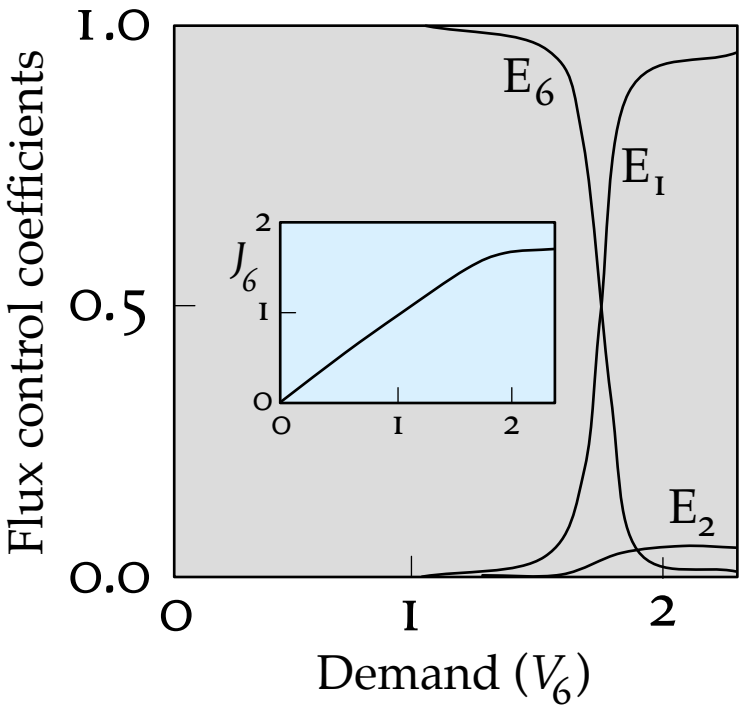
Reversibility without feedback inhibition



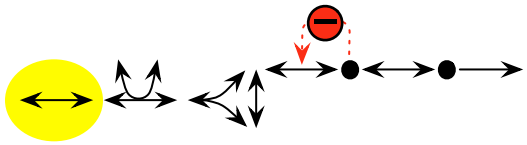
Neither one nor the other

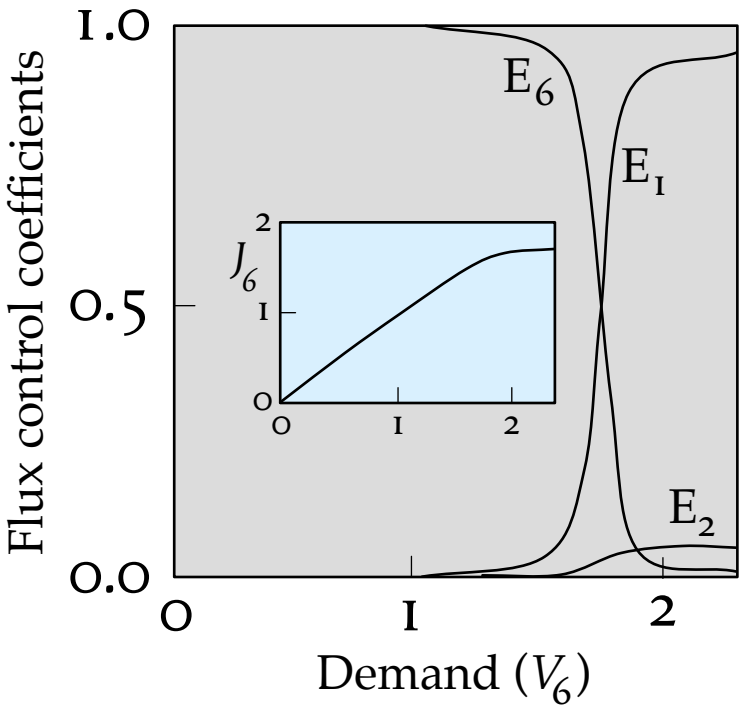
Feedback inhibition suppressed



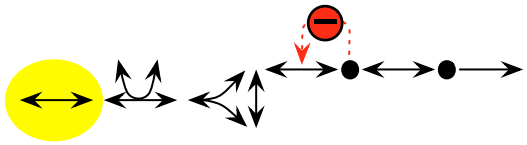


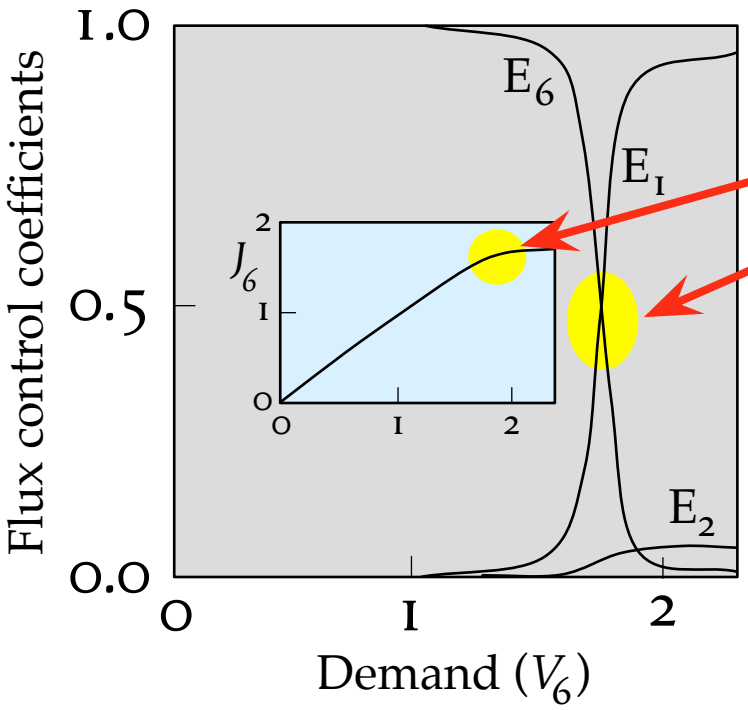
Feedback inhibition suppressed





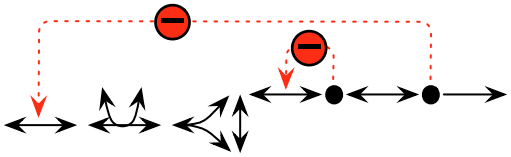
Feedback inhibition suppressed

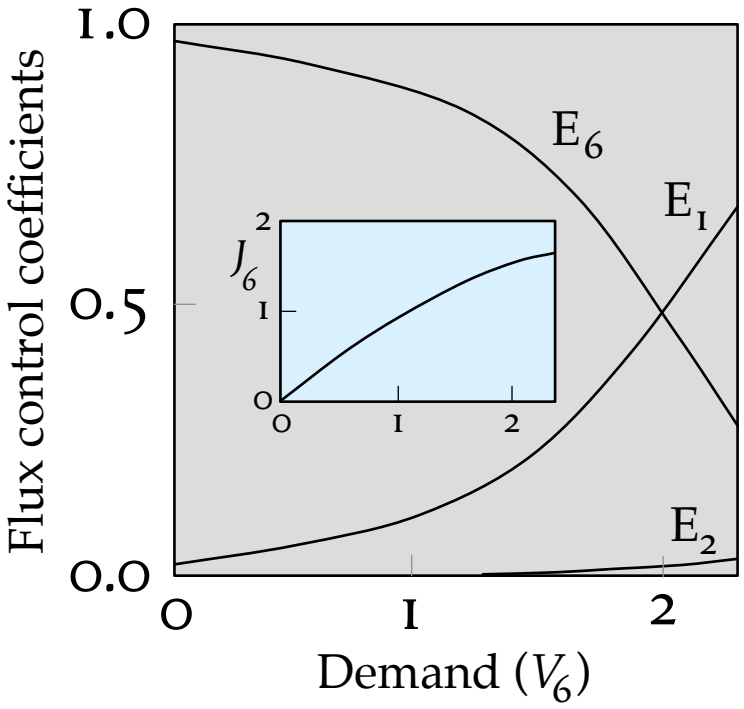




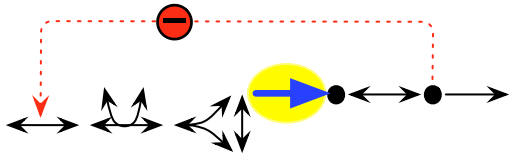
More abrupt transition, but qualitatively similar to the complete model.

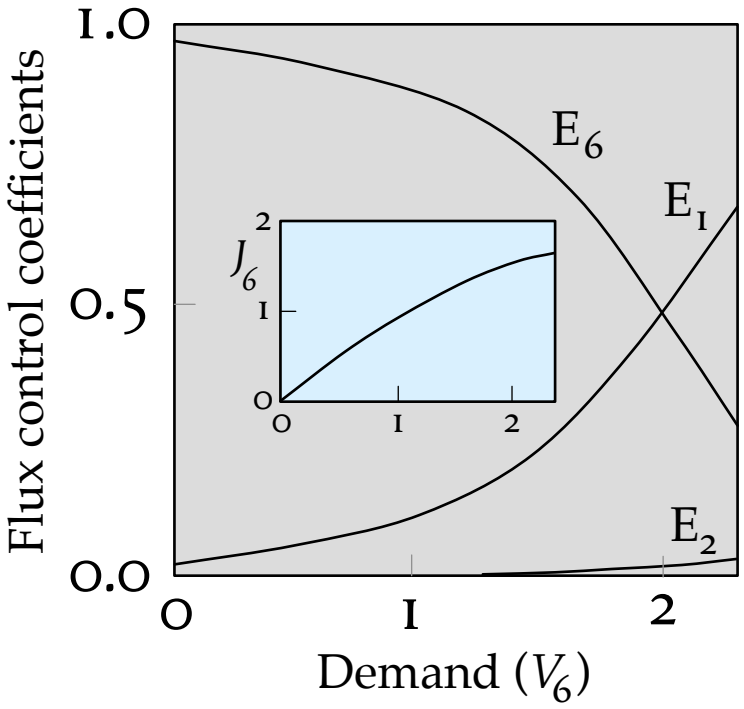
Reversibility suppressed





Reversibility suppressed

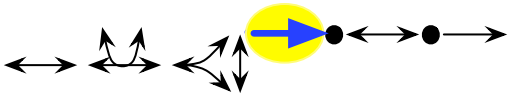


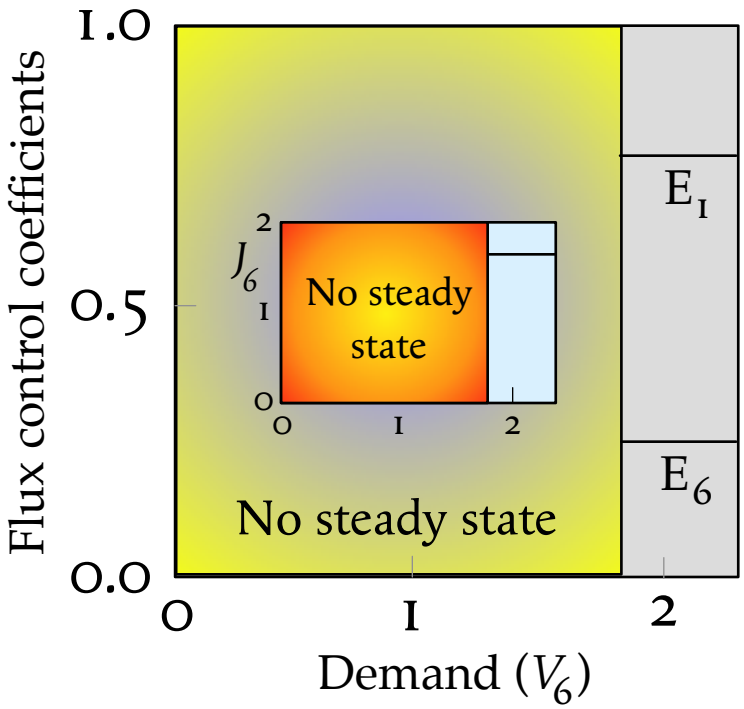


Indistinguishable from the results for the complete model

Feedback inhibition suppressed

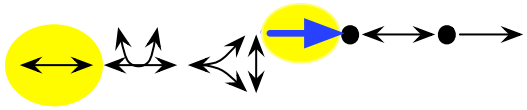
Reversibility suppressed

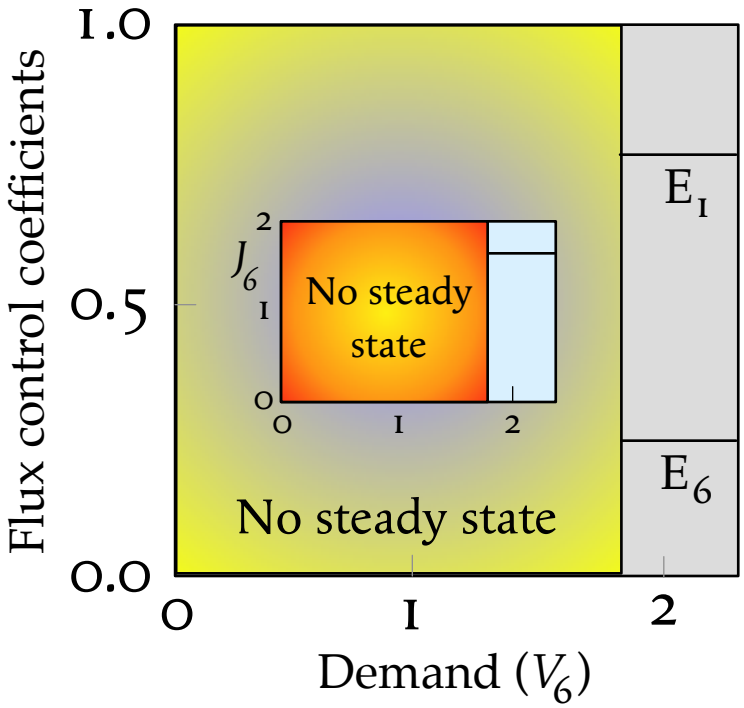




Feedback inhibition suppressed

Reversibility suppressed

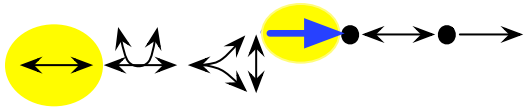


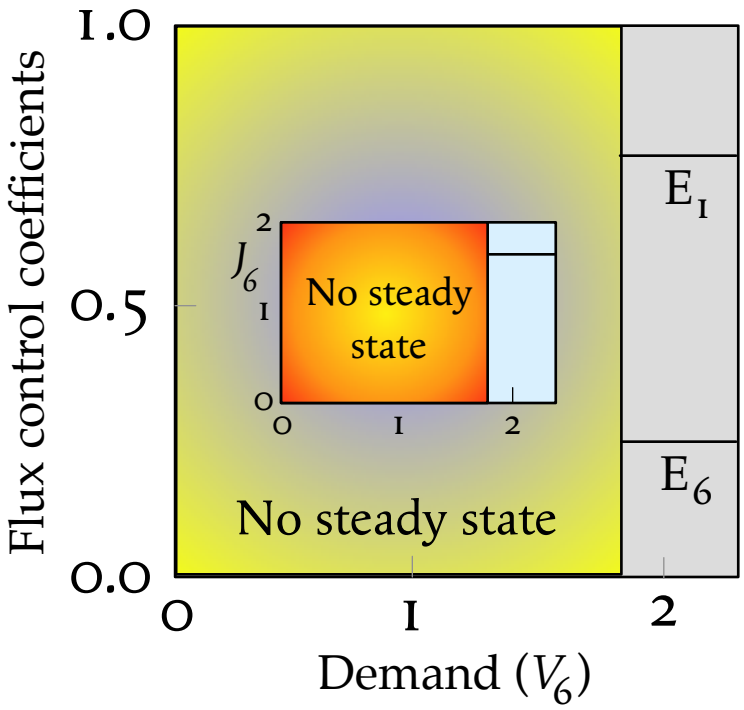


Completely different from the results for the complete model.

Feedback inhibition suppressed

Reversibility suppressed



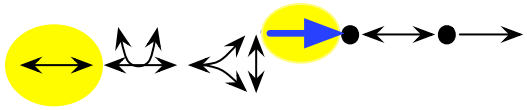


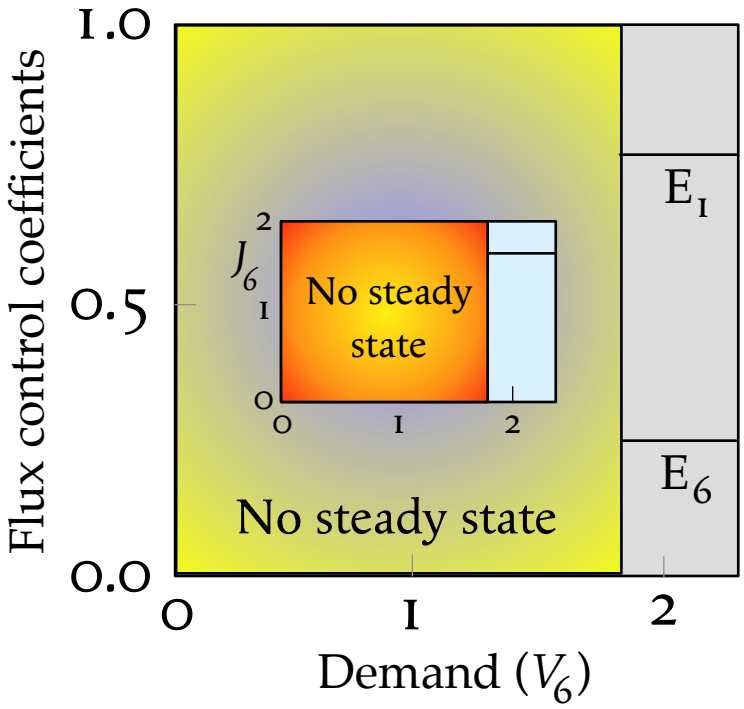
Completely different from the results for the complete model.

These results do not correspond to **any** of the possibilities considered beforehand!

Feedback inhibition suppressed

Reversibility suppressed





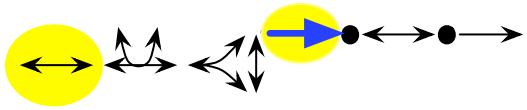
Completely different from the results for the complete model.

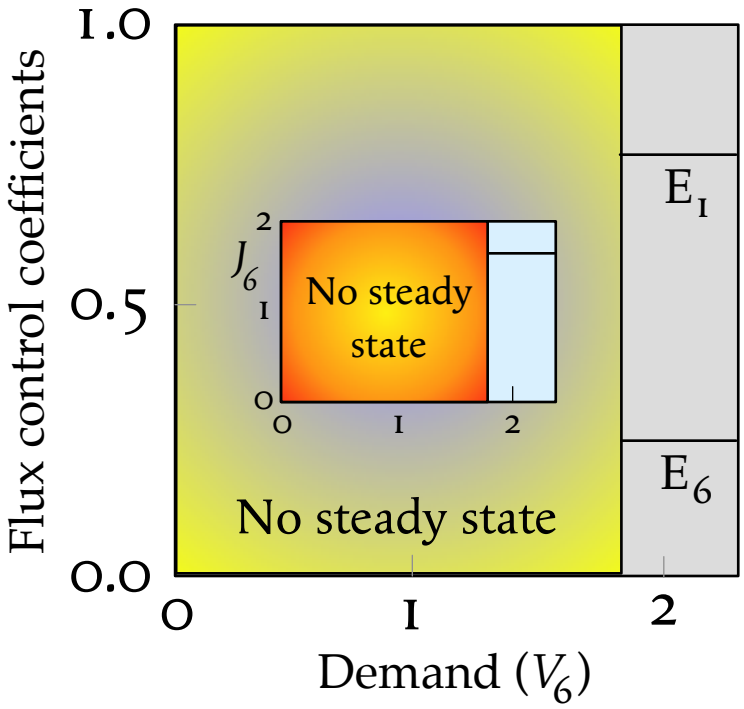
These results do not correspond to **any** of the possibilities considered beforehand!

Apparently one must have *either* feedback inhibition *or* reversibility, but it doesn't matter which!

Feedback inhibition suppressed

Reversibility suppressed



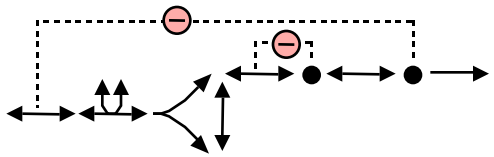


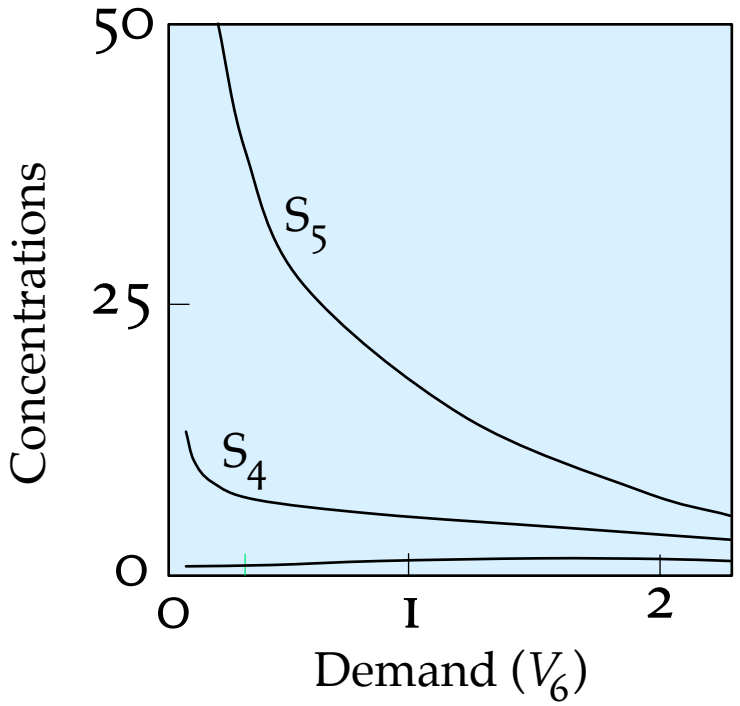
Completely different from the results for the complete model.

These results do not correspond to **any** of the possibilities considered beforehand!

Apparently one must have *either* feedback inhibition *or* reversibility, but it doesn't matter which! How can we rationalize this?

Before examining this question, let us note that the effects on the concentrations are simpler than those on the fluxes:





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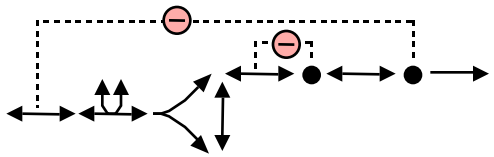
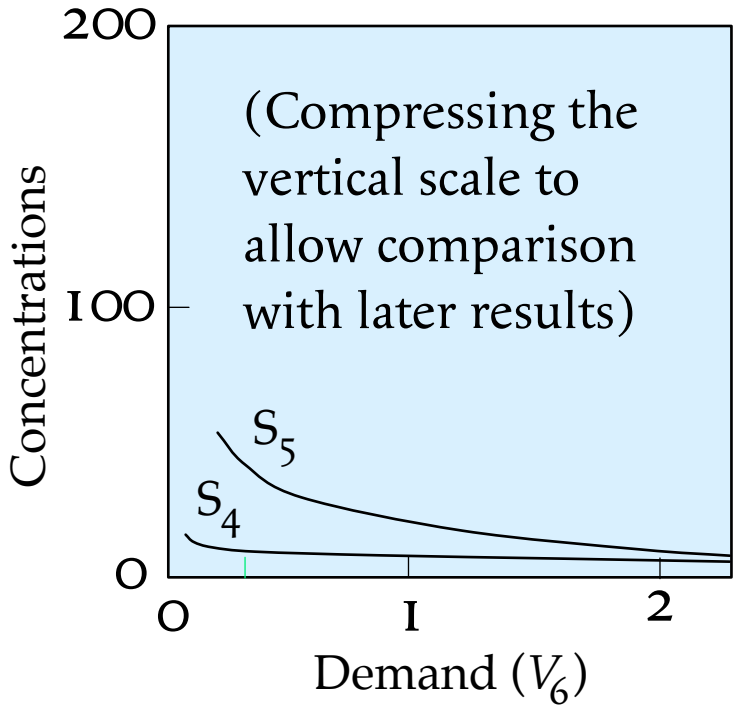
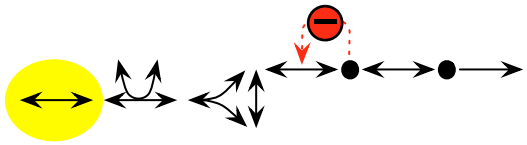
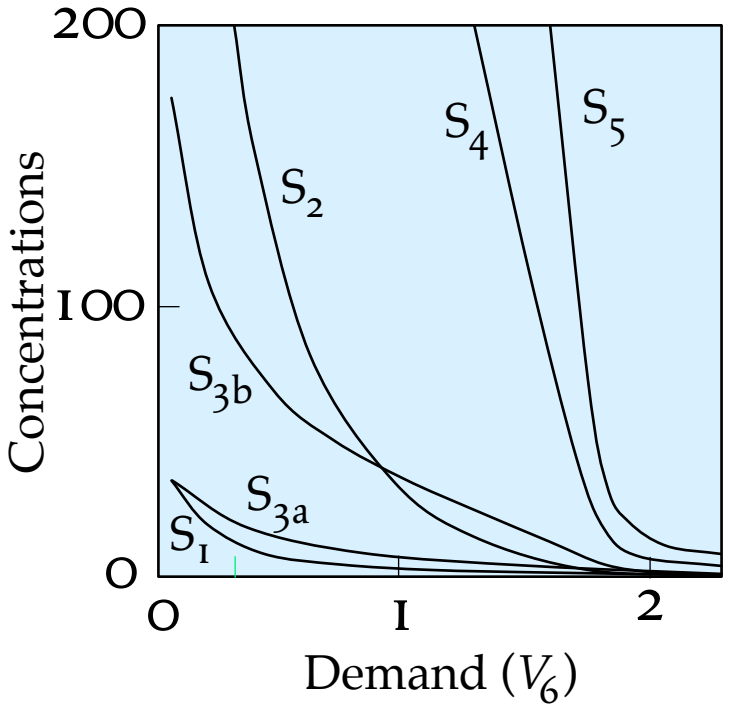




Feedback inhibition suppressed





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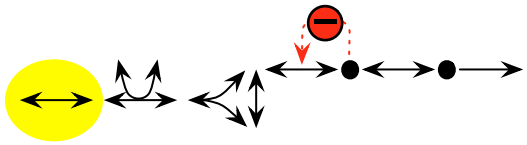
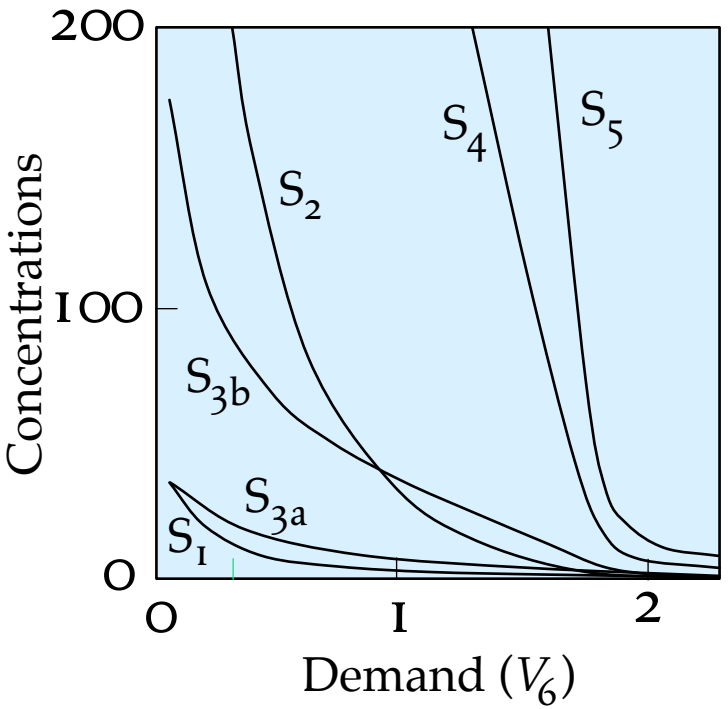


In the absence of feed-back inhibition the metabolite concentra-tions reach very high levels when the demand for end-product is low.



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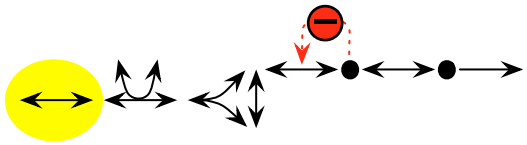

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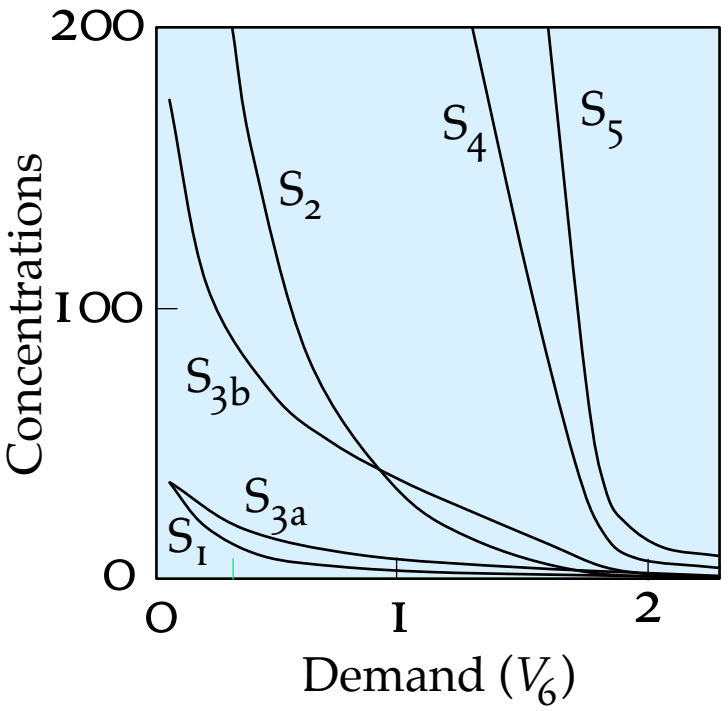
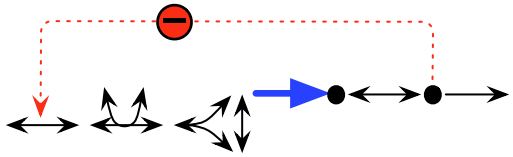
The feedback inhibition is primarily needed for stabilizing the metabolite concentrations.*



*J.-H. S. Hofmeyr and A. Cornish-Bowden (1991) "Quantitative assessment of regulation in metabolic systems" *Eur. J. Biochem.* **200**, 223–236; A. Cornish-Bowden, J.-H. S. Hofmeyr and M. L. Cárdenas (1995) "Strategies for manipulating metabolic fluxes in biotechnnology" *Bioorg. Chem.* **23**, 439–449

Reversibility suppressed



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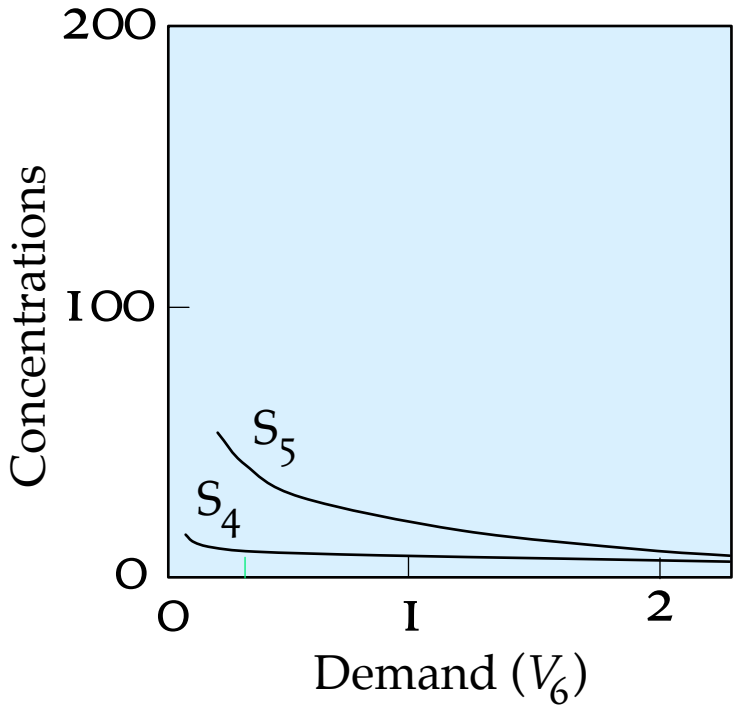

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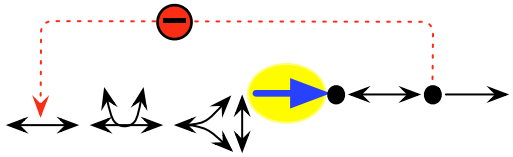

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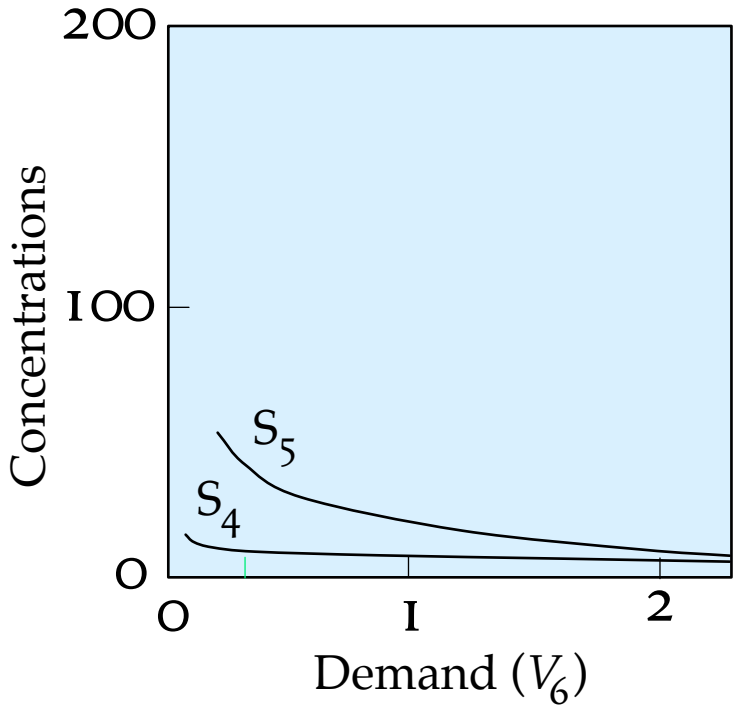

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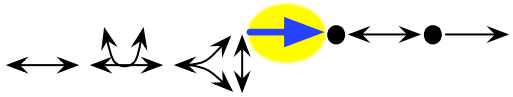

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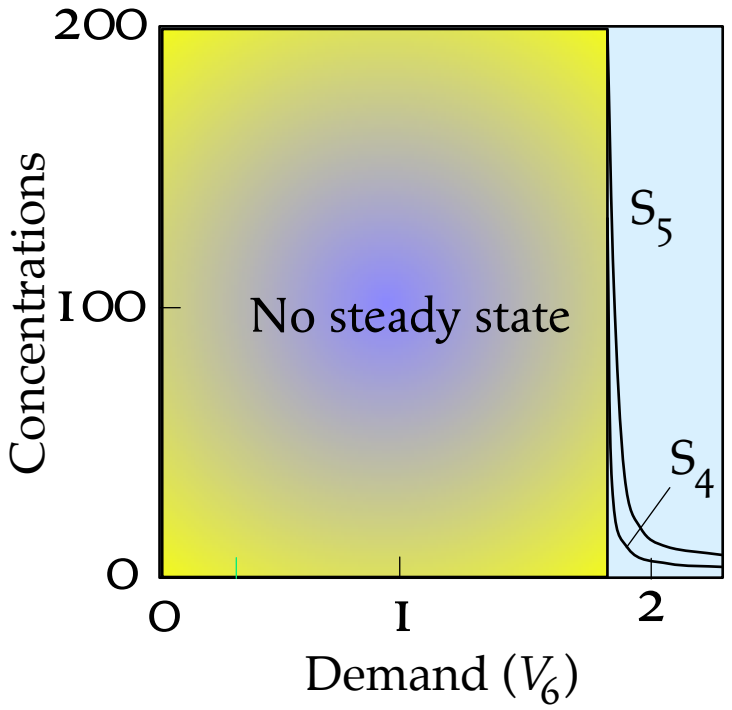

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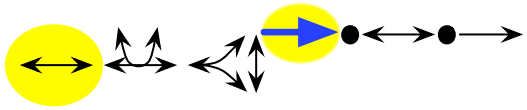

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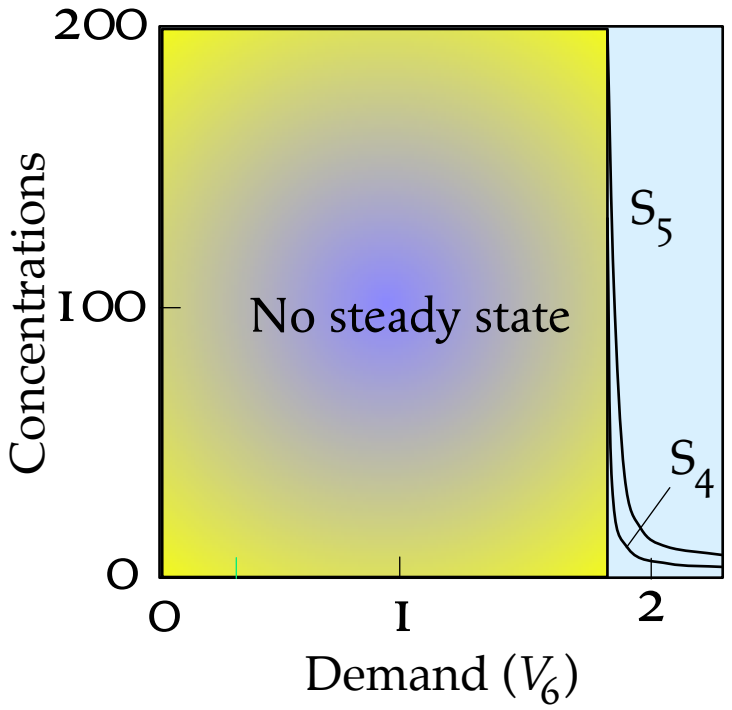

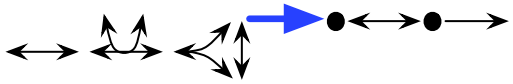

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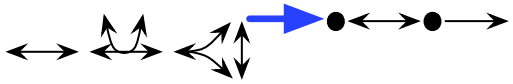

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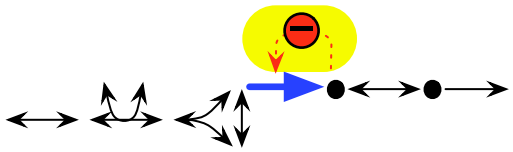

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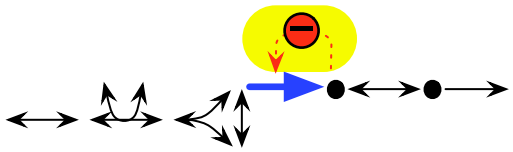

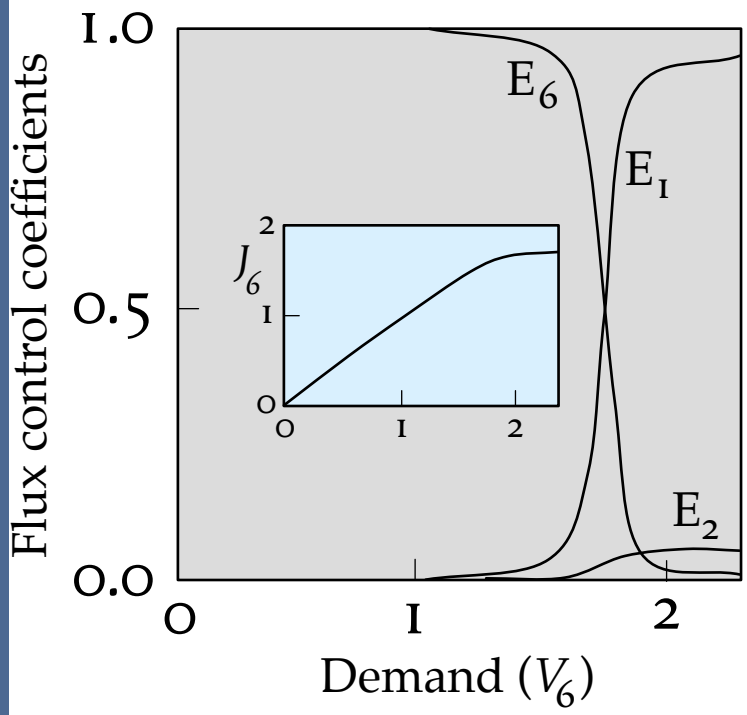

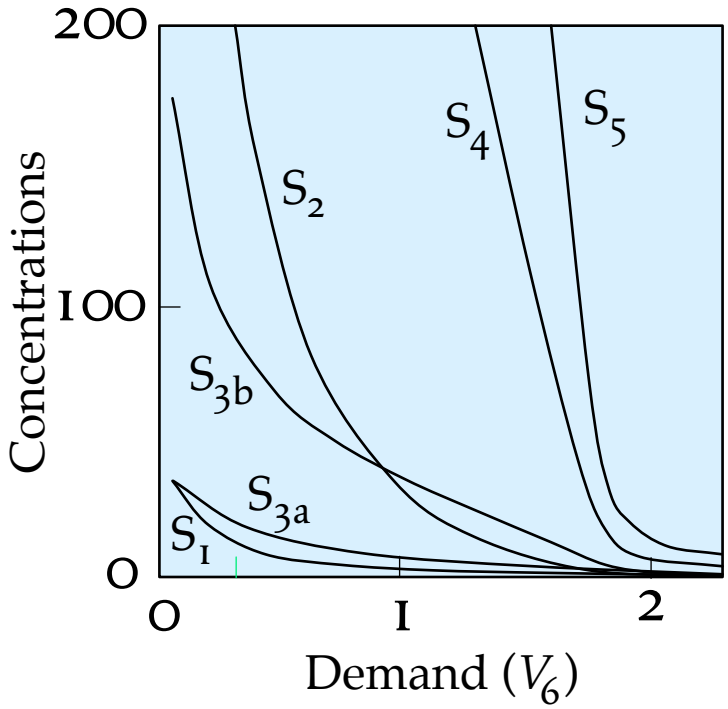

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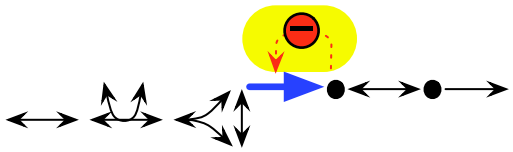
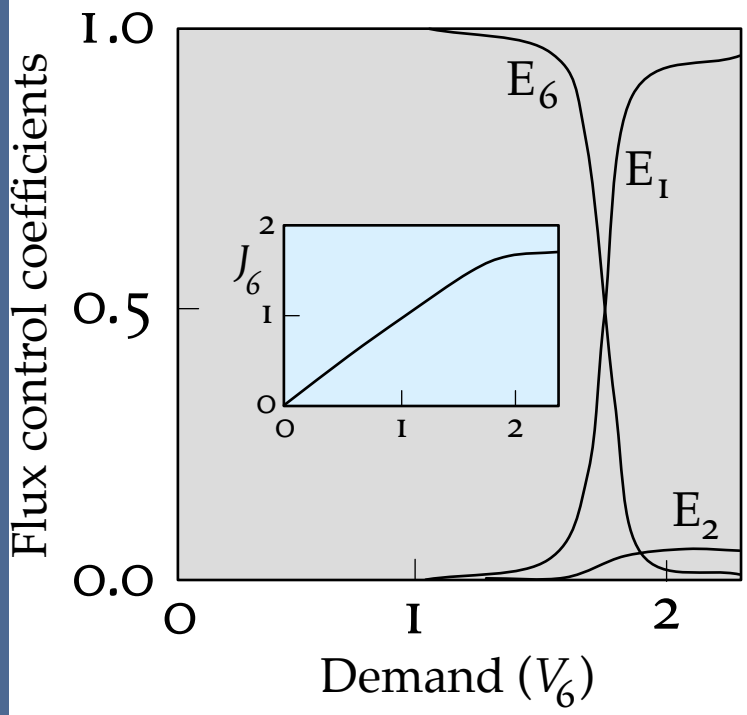
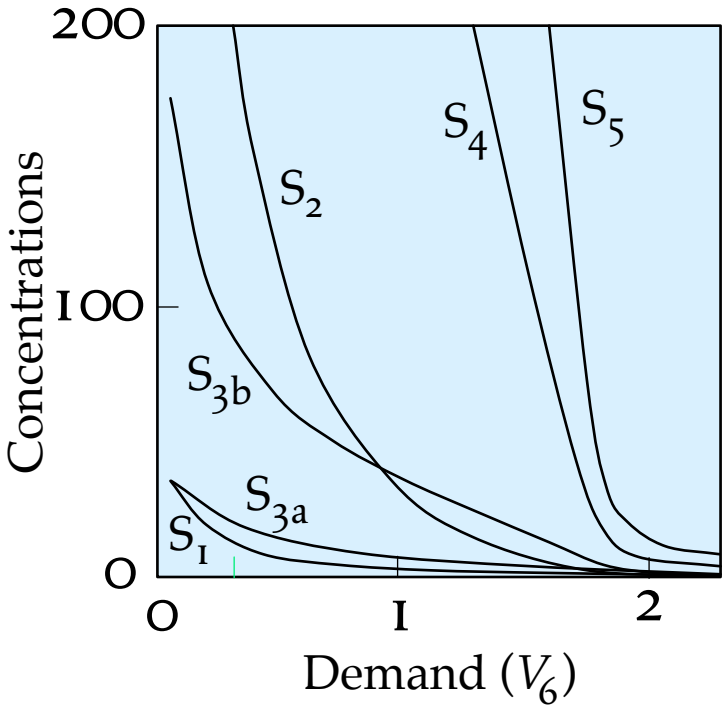
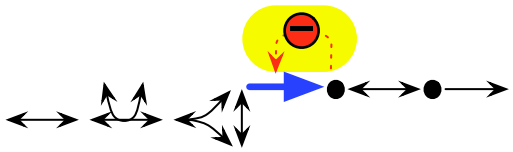
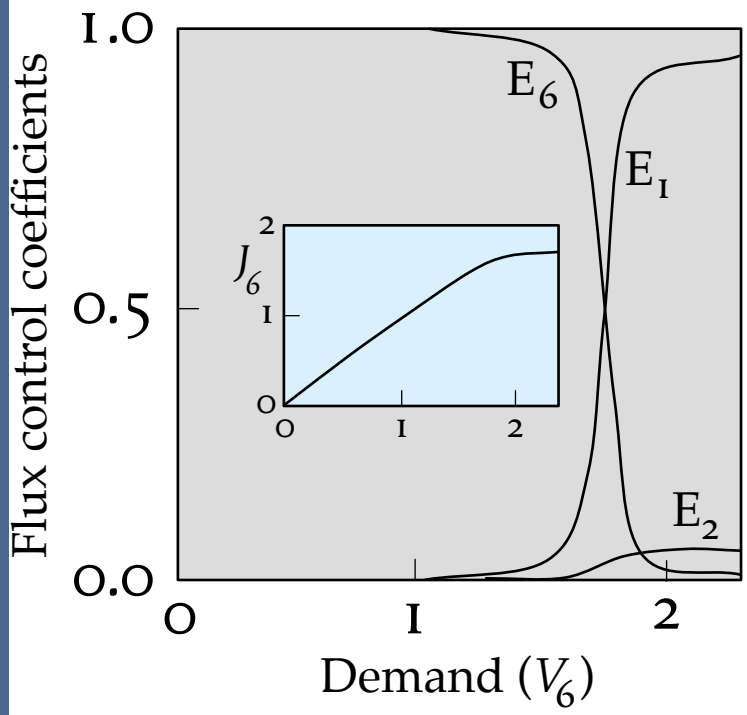


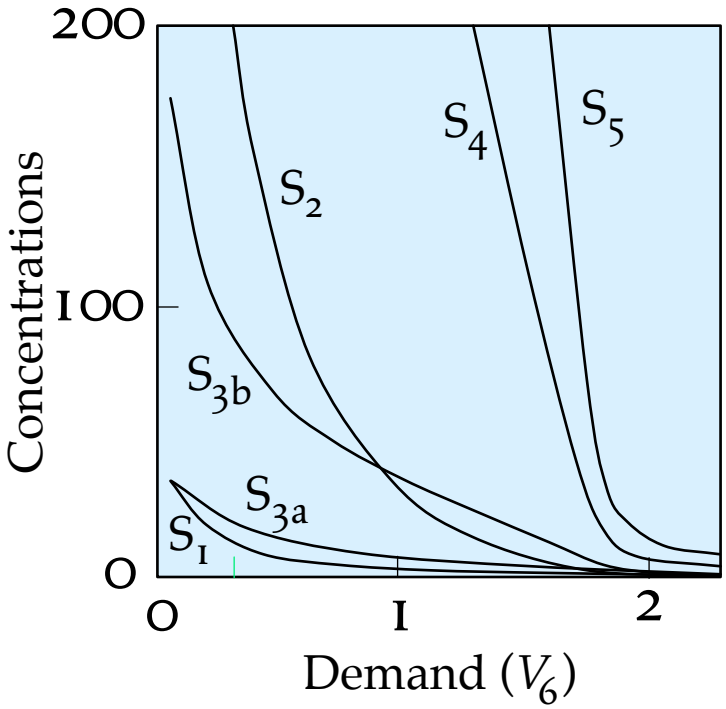




The results are essentially identical to those obtained with the same step reversible. This confirms that reversibility *as such* is not necessary: what is necessary is sensitivity to product.







Obvious? Maybe, but during 35 years of metabolic simulation nobody paid any attention to this obvious truth! Irreversible reactions were always treated as if being insensitive to their products.

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PYRUVATE KINASE IN FOUR DECADES OF METABOLIC SIMULATION*

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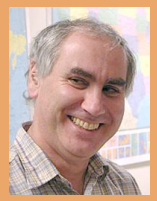

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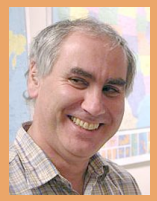

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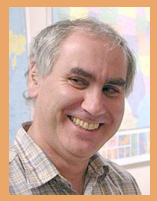

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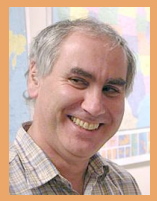

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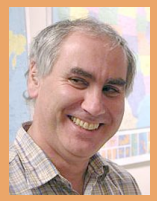

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PRACTICAL MEANING OF FEEDBACK INHIBITION AND COOPERATIVITY

Arthur Pardee

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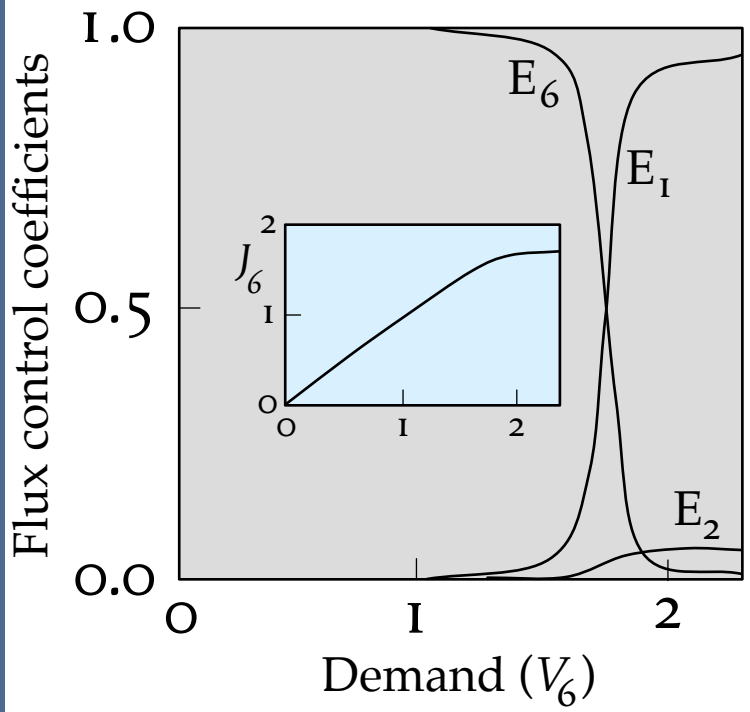

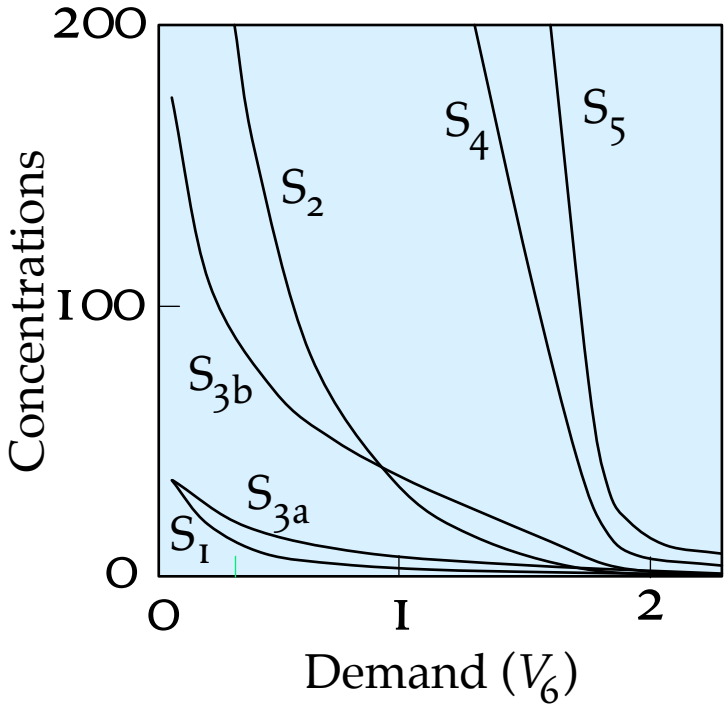

Arthur Pardee

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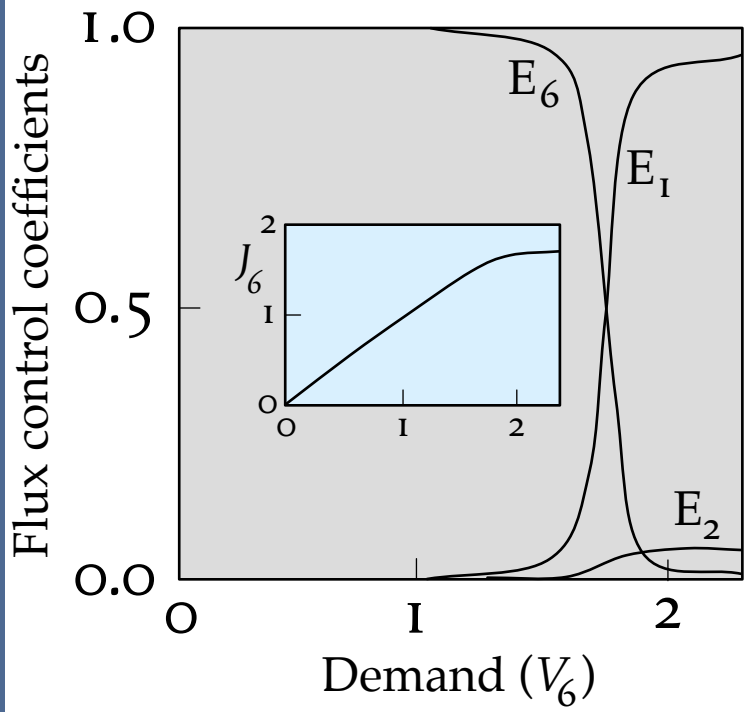

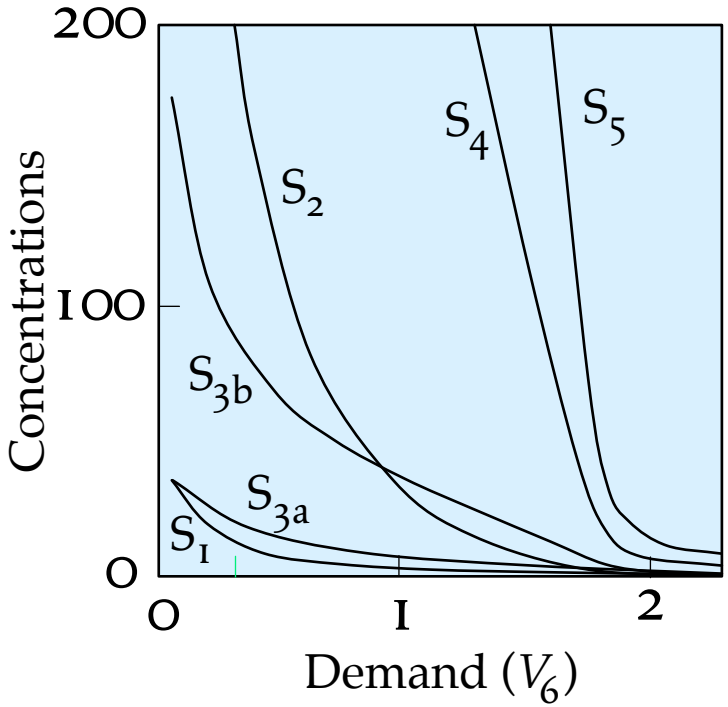

By no means! Only that its importance is different from what one has tended to think during more than 40 years.

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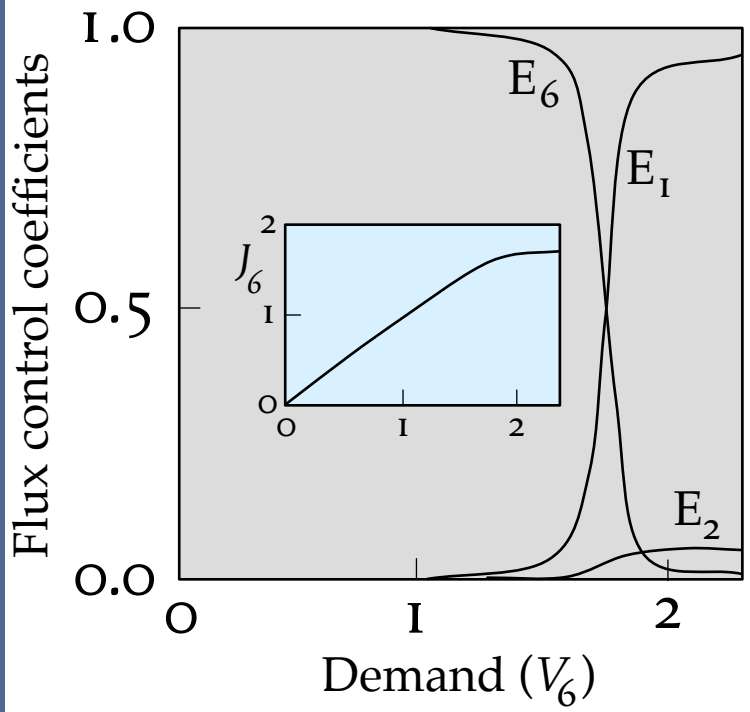

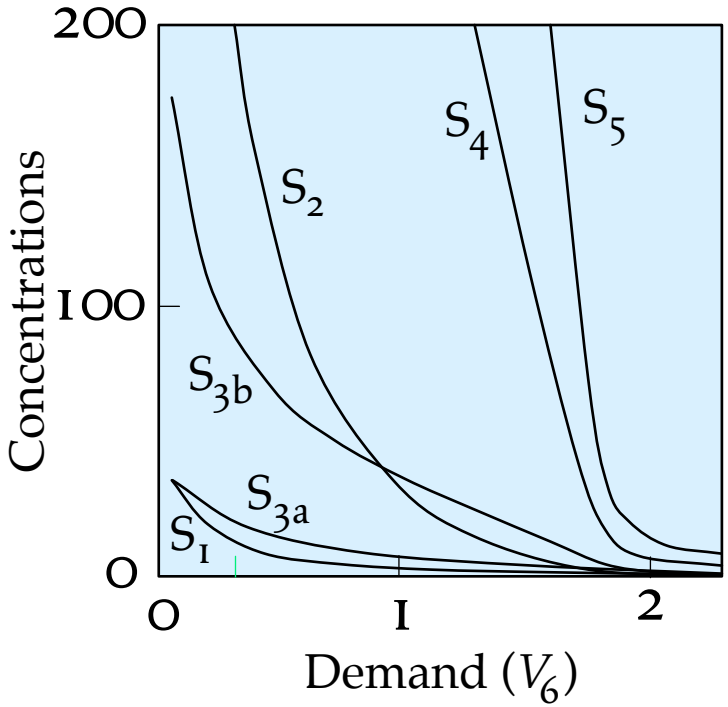

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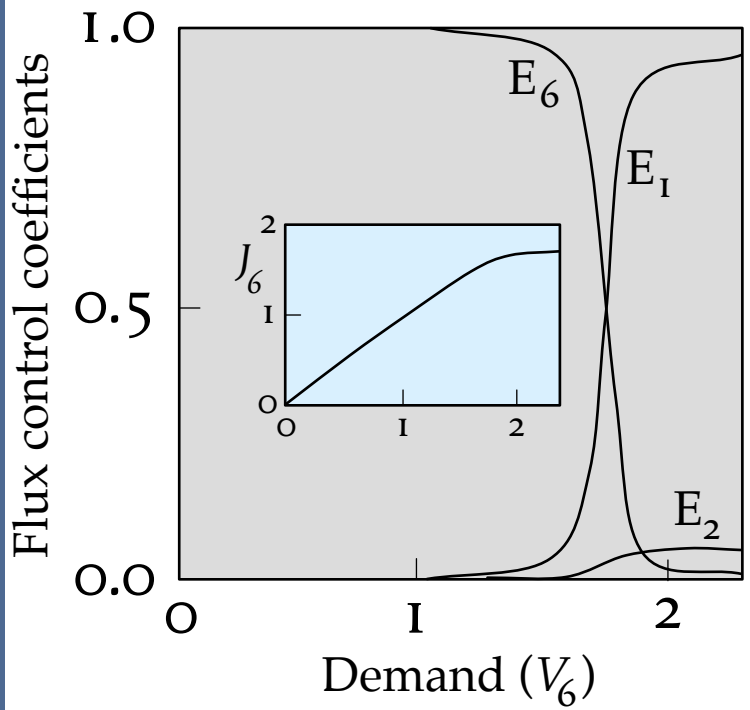

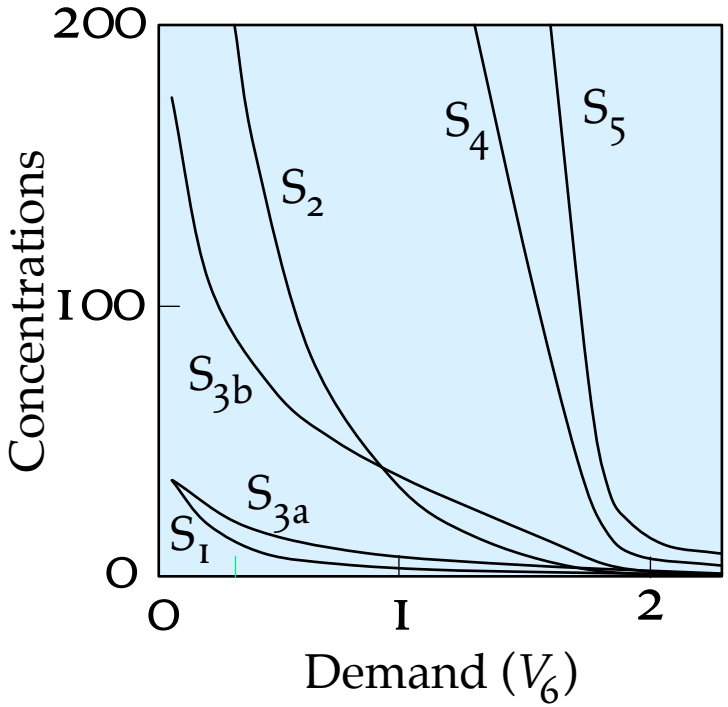

Fluxes can be regulated very well without feedback inhibition, but at the cost of uncontrolled variations in the metabolite concentrations. Feedback inhibition is needed for avoiding the huge variations in the concentrations of the metabolites that would otherwise accompany flux changes.

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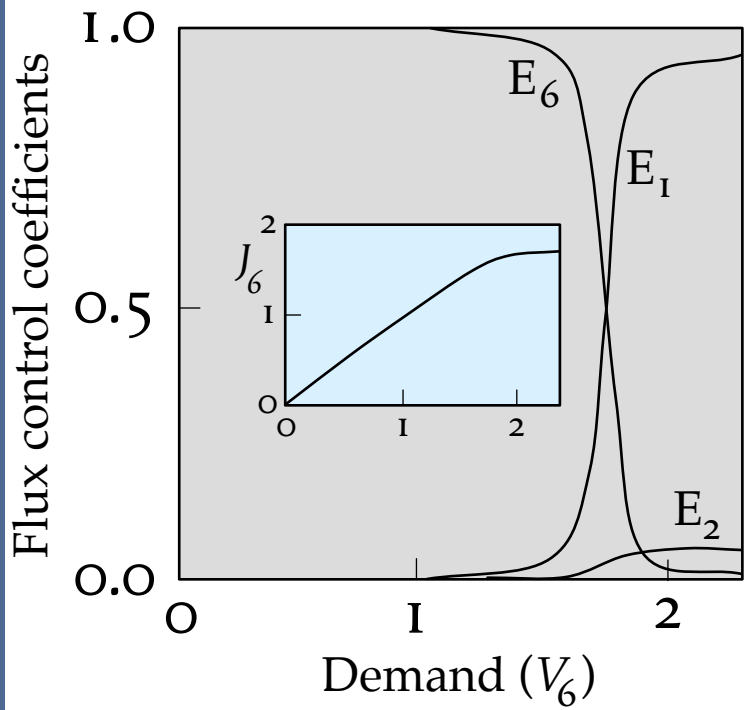
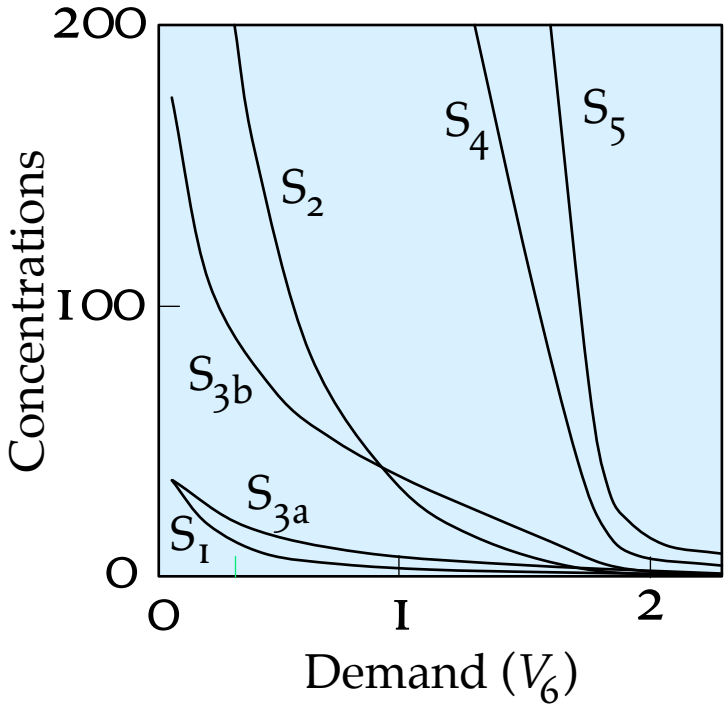
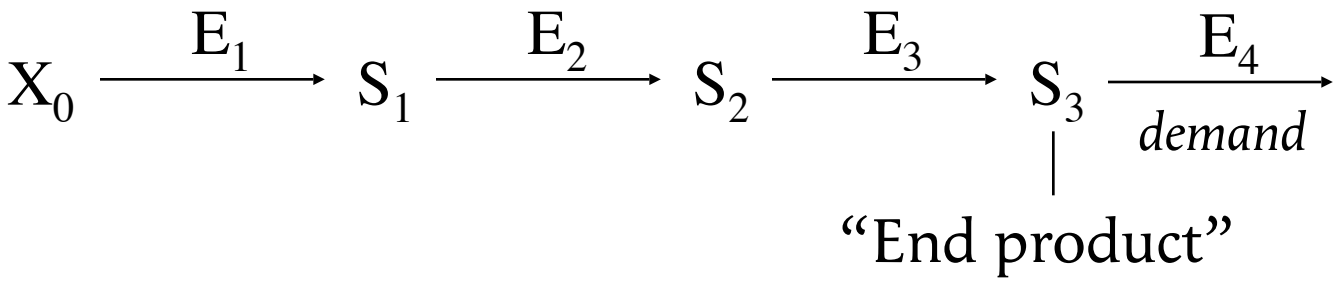




Fluxes can be regulated very well without feedback inhibition,* but at the cost of uncontrolled variations in the metabolite concentrations. Feedback inhibition* is needed for avoiding the huge variations in the concentrations of the metabolites that would otherwise accompany flux changes.

http://bip.cnrs-mrs.fr/bip10/leshouches/
acornish@ibsm.cnrs-mrs.fr

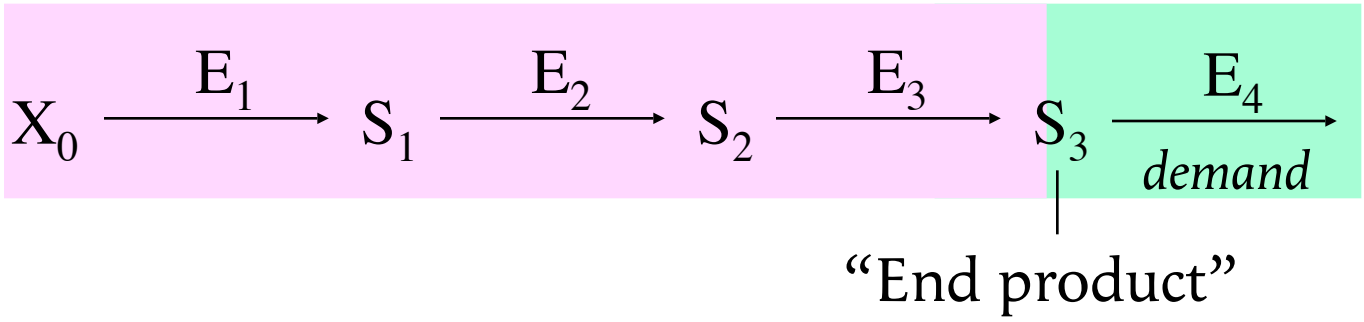
In biochemistry texts one often sees this sort of diagram:



But that is *bad* : a product is made in order to be used, and to understand the regulation of the pathway we must never forget the demand for the product. This demand must therefore be represented explicitly in the diagram.

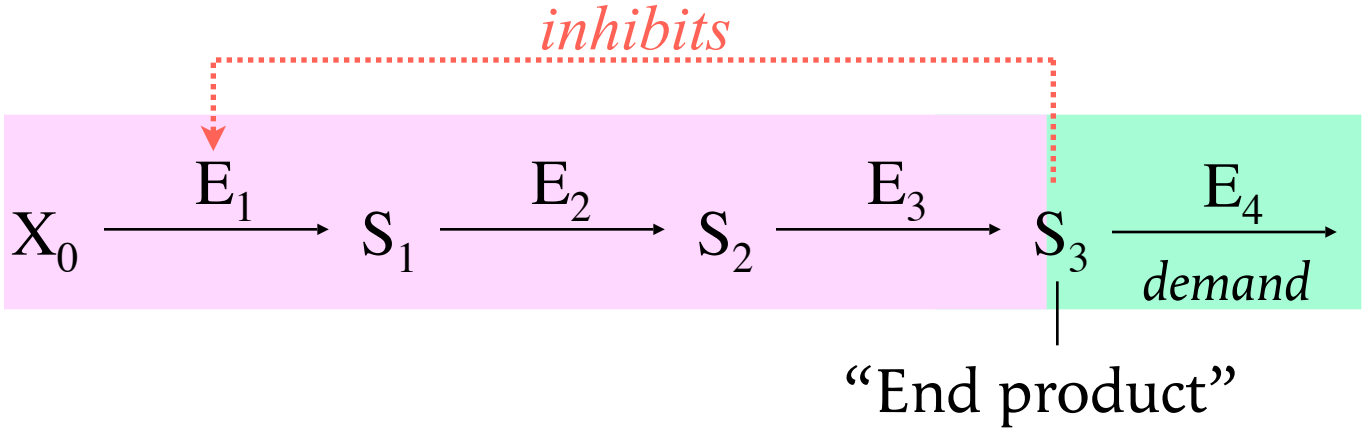
We can still use the term "end product", but now it is less obviously appropriate.

The system consists of a supply block and a demand block



A high proportion of work in biotechnology is based on the **false** notion that metabolic pathways respond to changes in supply, and the falsity of this idea goes a long way towards explaining the low level of success in this domain. *In reality most,* ***but not all****, biosynthetic pathways have evolved to respond to changes in demand.*

What function is served by feedback inhibition (for example of E_1 by S_3)? If E_1 does not control the flux, why should it be subject to regulatory mechanisms?



The feedback inhibition has the effect of *trans-ferring* the point of control out of the supply block (where it would not be useful) towards the demand block (where it is necessary).

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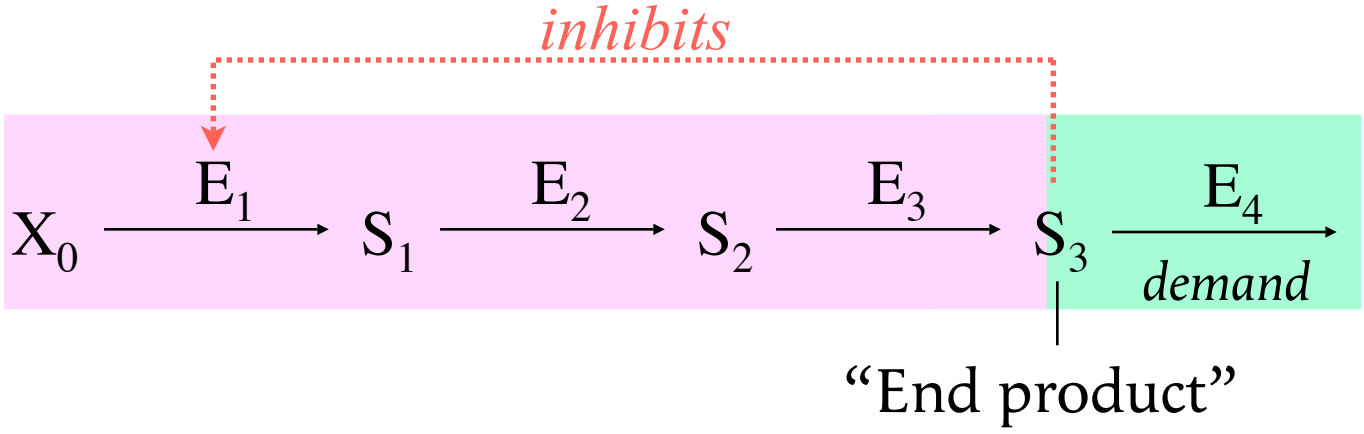
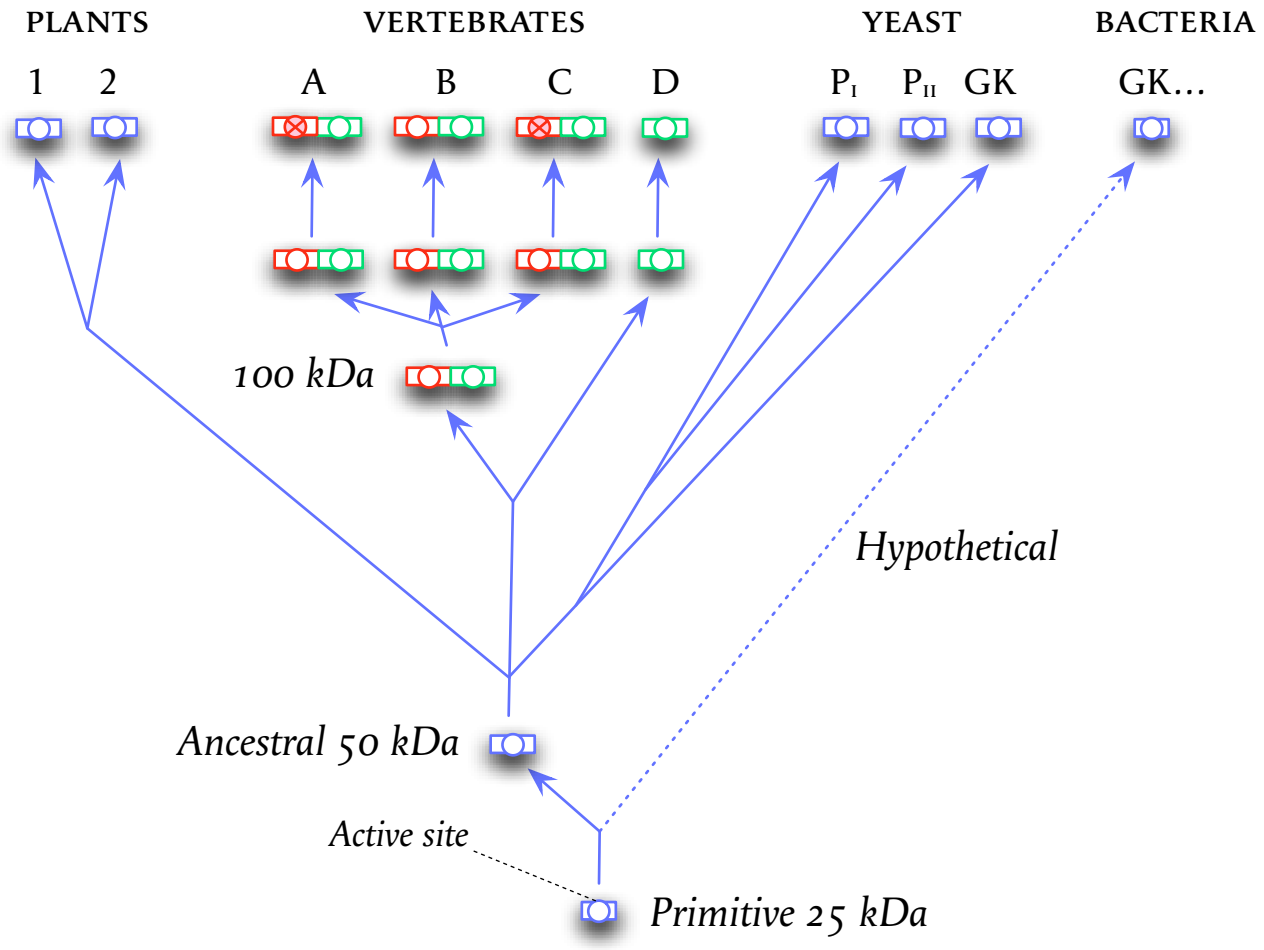


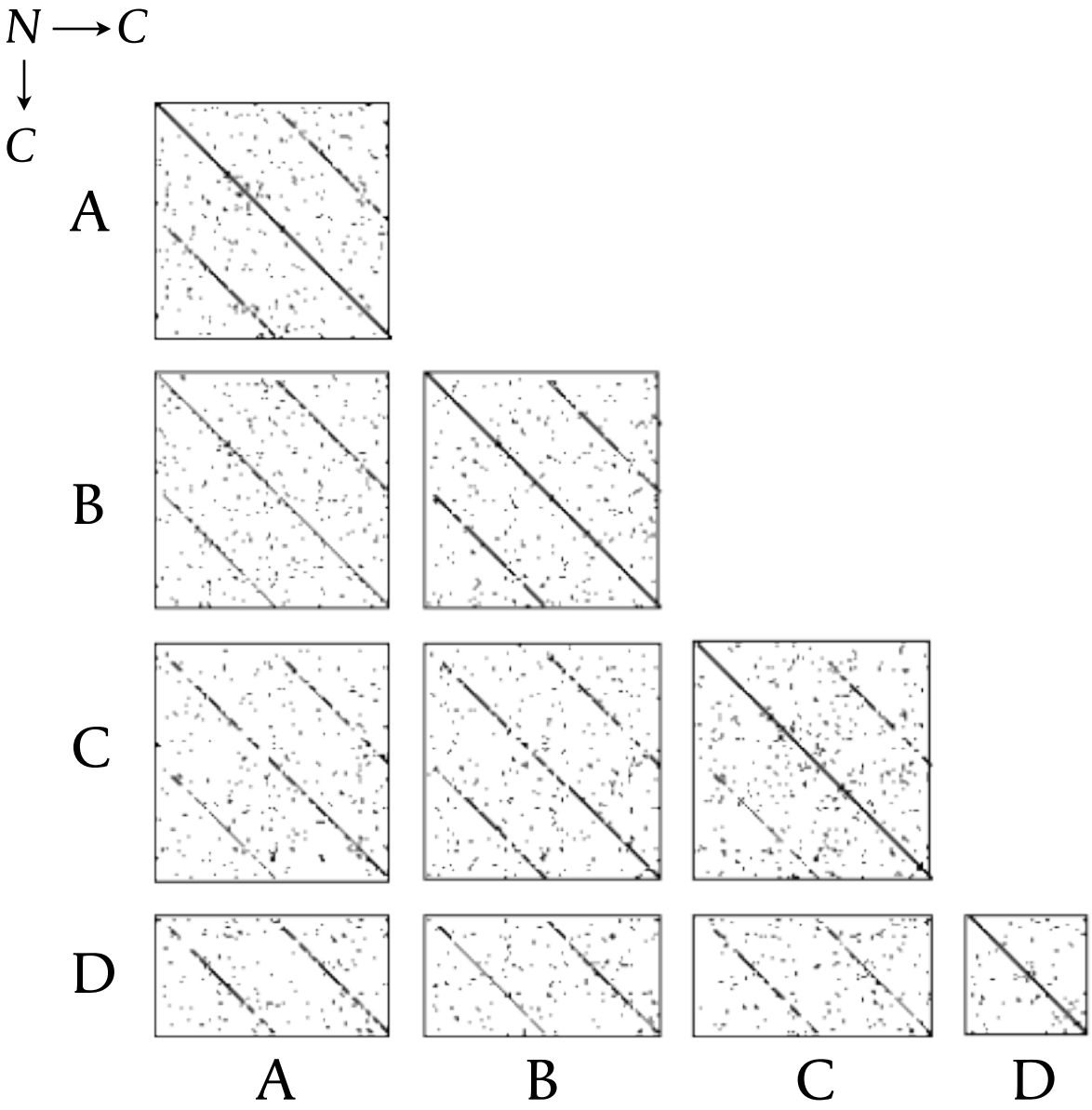
The feedback inhibition has the effect of *trans-ferring* the point of control out of the supply block (where it would not be useful) towards the demand block (where it is necessary).

This is not always desirable: when might regulation by supply be better?

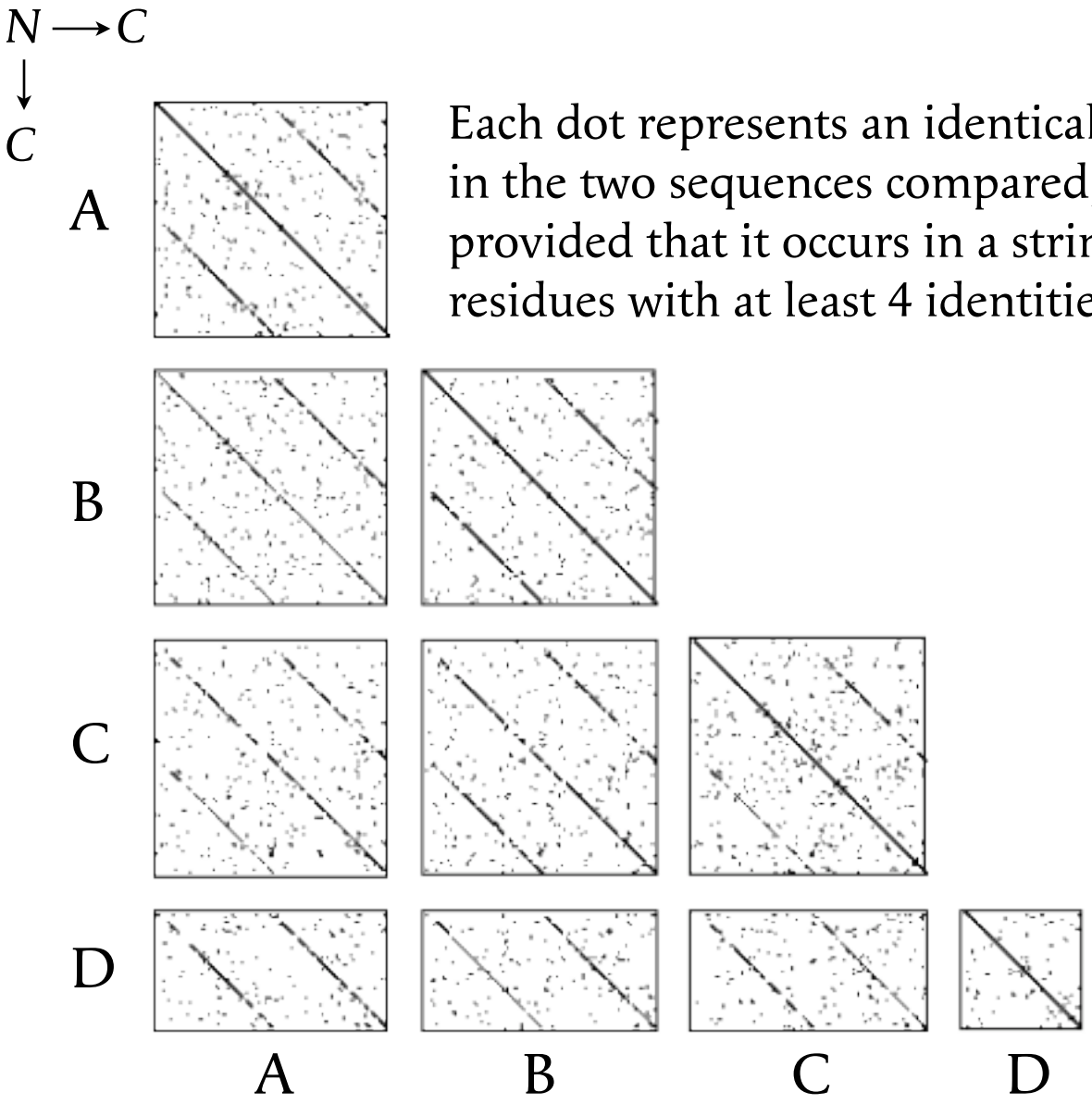
Hexokinases in mammals



Hexokinases in mammals

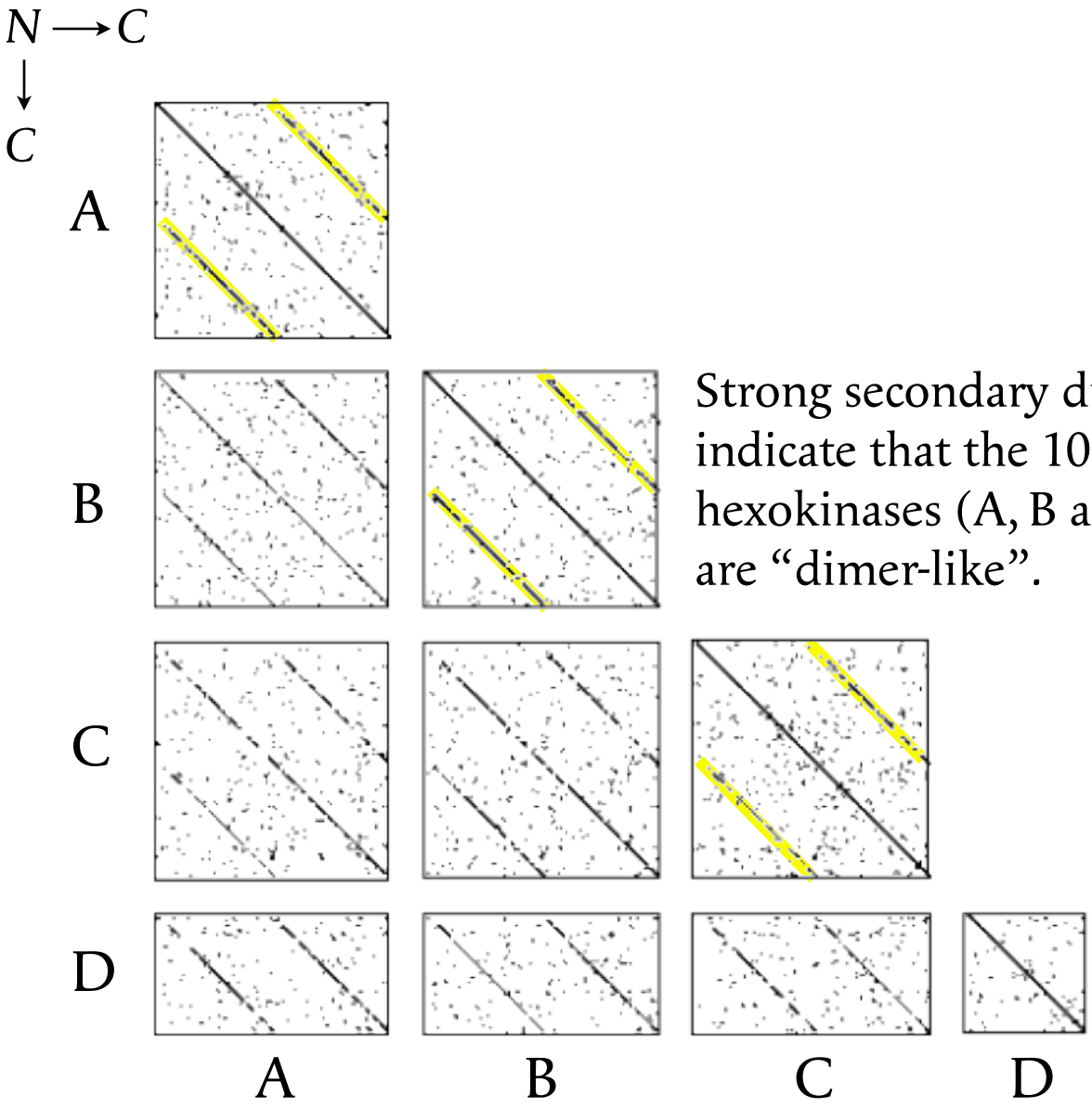


Hexokinases in mammals



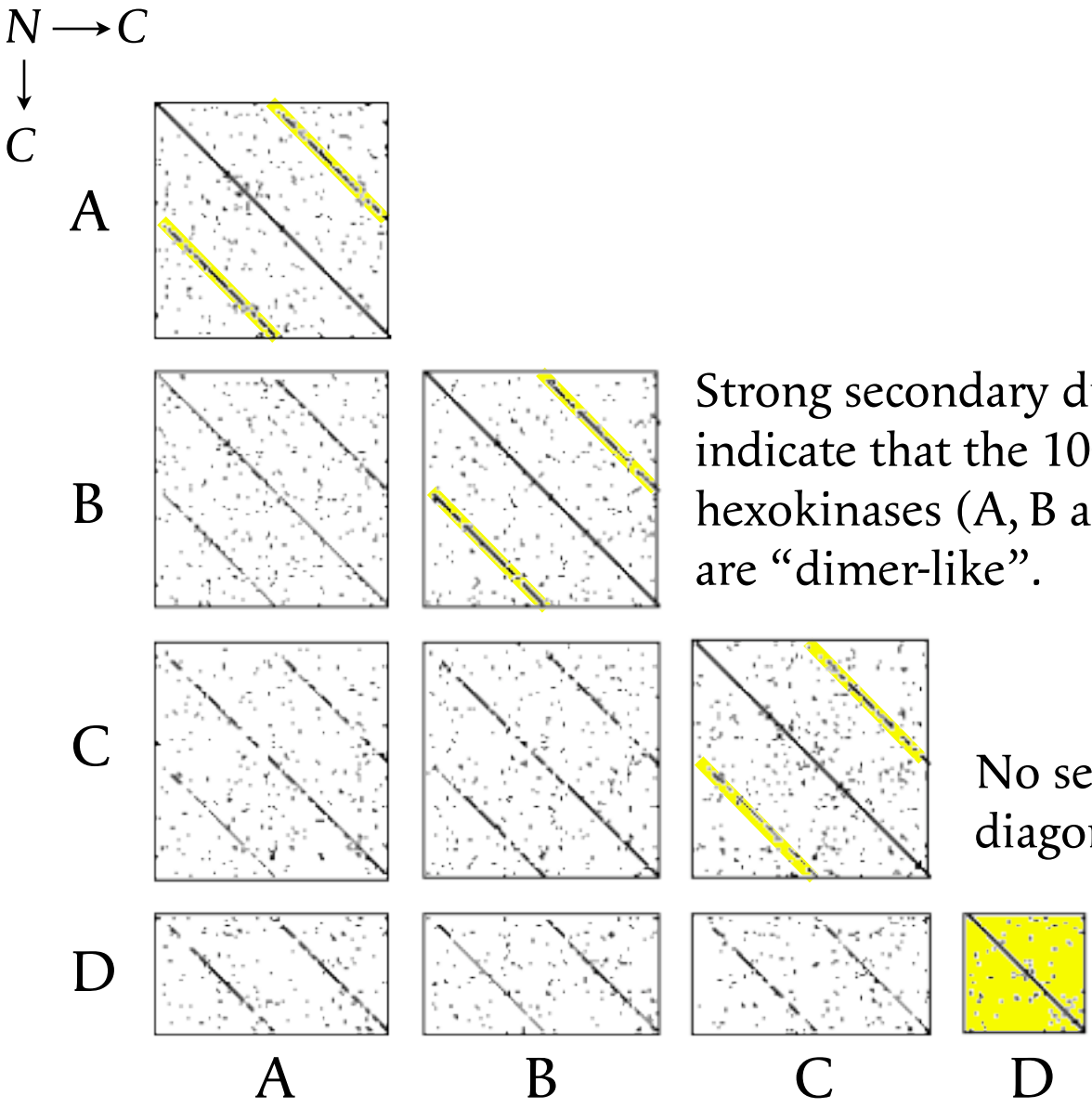
Each dot represents an identical residue in the two sequences compared, provided that it occurs in a string of 9 residues with at least 4 identities.

Hexokinases in mammals



Strong secondary diagonals indicate that the 100 kDa hexokinases (A, B and C) are "dimer-like".

Hexokinases in mammals



Strong secondary diagonals indicate that the 100 kDa hexokinases (A, B and C) are "dimer-like".

No secondary diagonal

$N \rightarrow C$
$\downarrow$
C

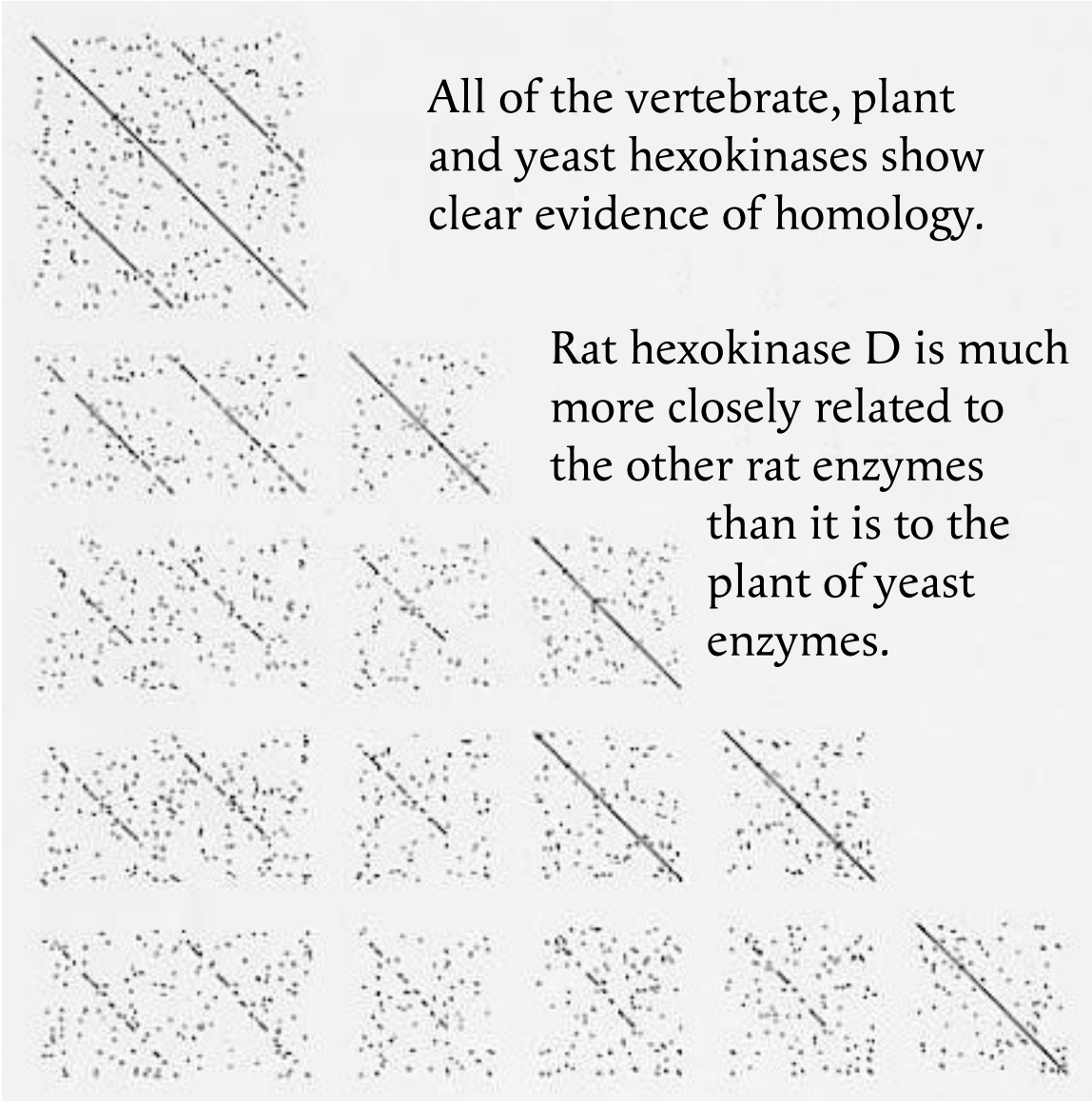
Rat hexokinase A

Rat hexokinase D (glucokinase)

Plant hexokinase 1

Plant hexokinase 2

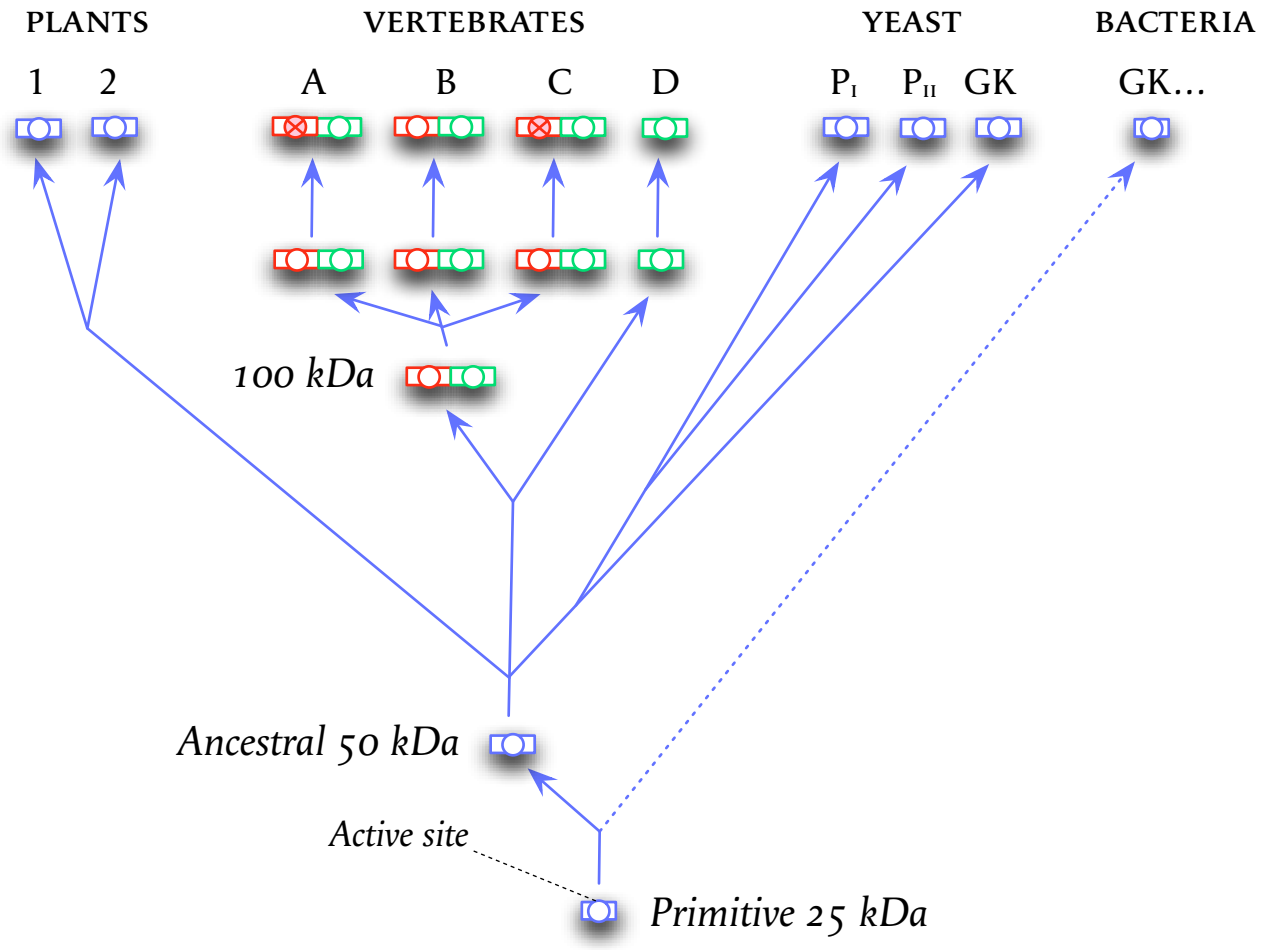
Yeast glucokinase



All of the vertebrate, plant and yeast hexokinases show clear evidence of homology.

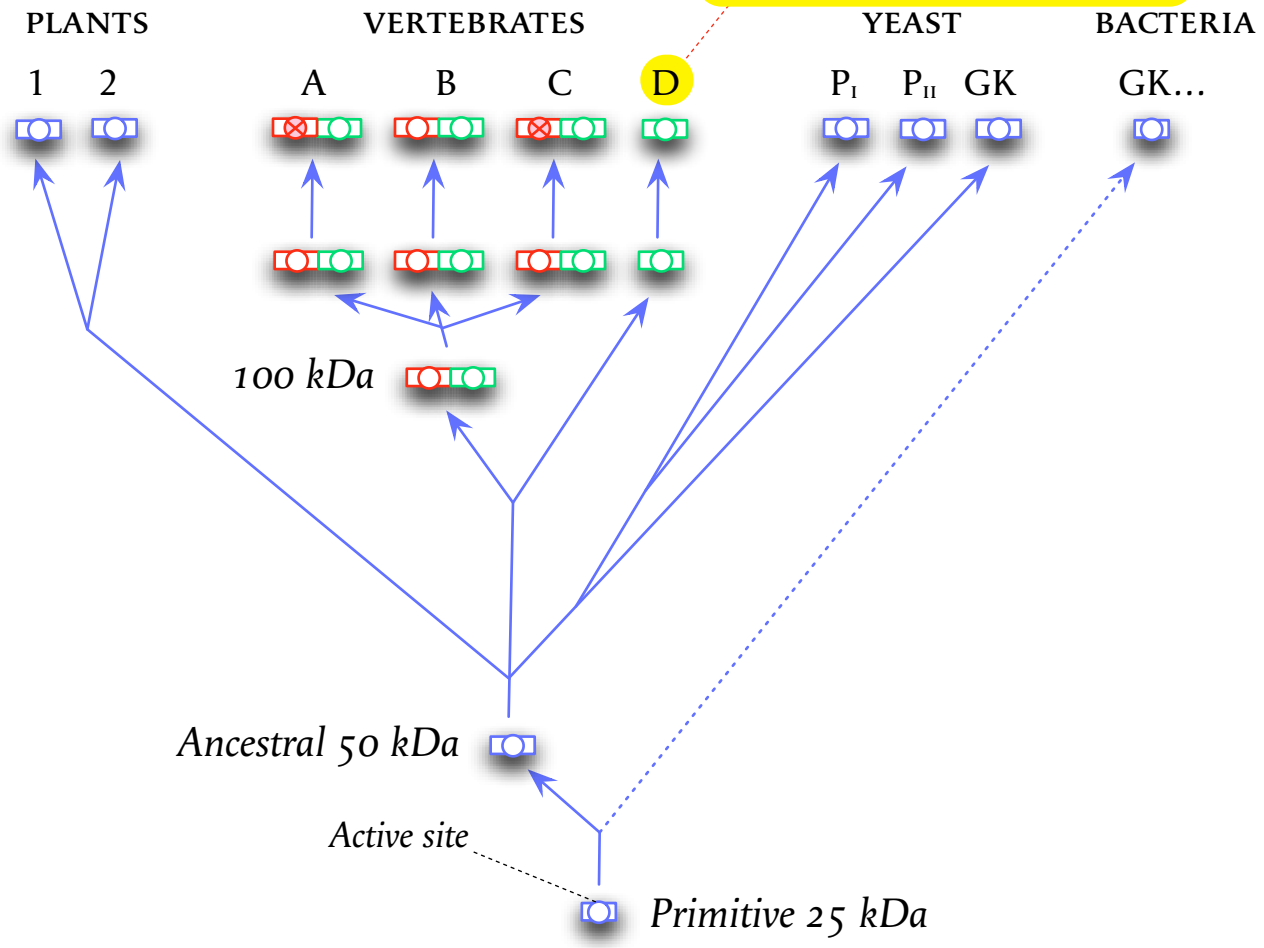
Rat hexokinase D is much more closely related to the other rat enzymes than it is to the plant of yeast enzymes.

Hexokinases in mammals



Hexokinases i

Often called "glucokinase", though no more specific for glucose than the other three



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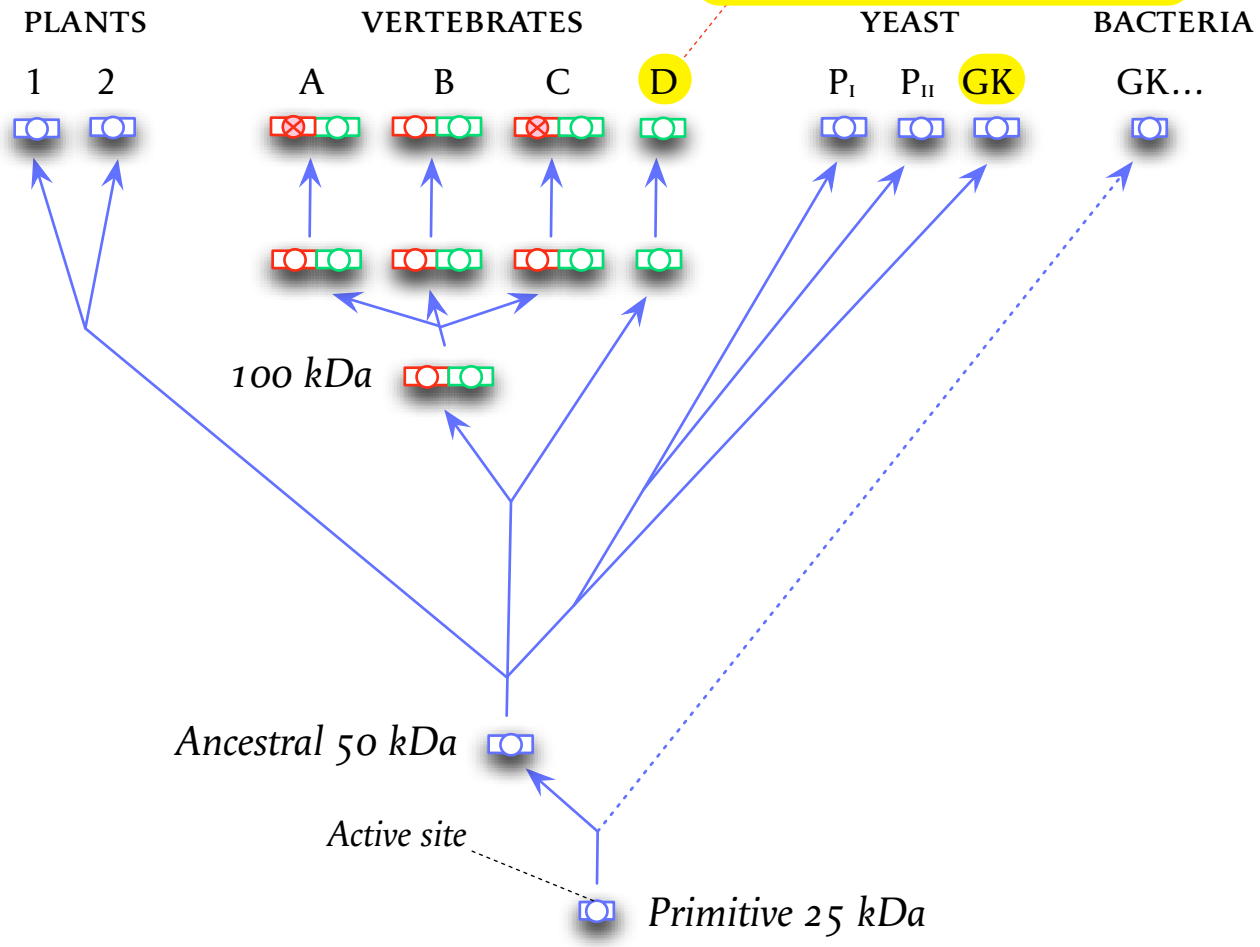

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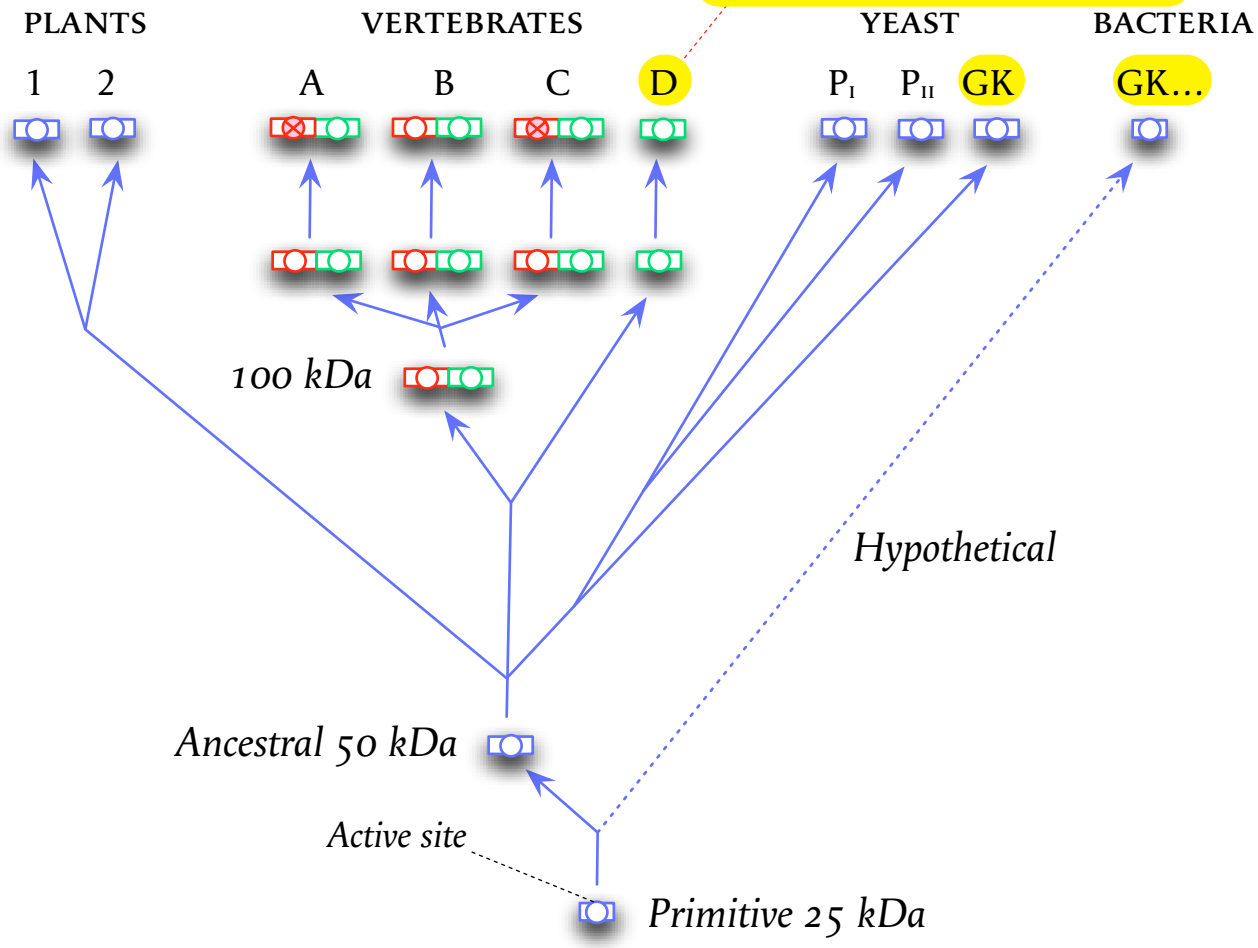

Kinetic parameters for liver hexokinase isoenzymes with glucose and fructose as substrates at pH 7.5.

Isoenzyme
Hexokinase A
Hexokinase B
Hexokinase C
Hexokinase D

M. L. Cárdenas, E. Rabajille and H. Niemeyer (1984) *Biochem. J.* **222**, 363–370

Kinetic parameters for liver hexokinase isoenzymes with glucose and fructose as substrates at pH 7.5.

Isoenzyme	$[Glc]_{0.5}$ (mM)
Hexokinase A	0.044
Hexokinase B	0.130
Hexokinase C	0.020
Hexokinase D	7.5

M. L. Cárdenas, E. Rabajille and H. Niemeyer (1984) *Biochem. J.* **222**, 363–370

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Isoenzyme	$[Glc]_{0.5}$ (mM)	$[Fru]_{0.5}$ (mM)
Hexokinase A	0.044	3.1
Hexokinase B	0.130	3.0
Hexokinase C	0.020	1.2
Hexokinase D	7.5	420

M. L. Cárdenas, E. Rabajille and H. Niemeyer (1984) *Biochem. J.* **222**, 363–370

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Isoenzyme	$[Glc]_{0.5}$ (mM)	$[Fru]_{0.5}$ (mM)	$[Fru]_{0.5}/[Glc]_{0.5}$
Hexokinase A	0.044	3.1	70.5
Hexokinase B	0.130	3.0	23.1 *smallest*
Hexokinase C	0.020	1.2	60.0
Hexokinase D	7.5	420	56.0

M. L. Cárdenas, E. Rabajille and H. Niemeyer (1984) *Biochem. J.* **222**, 363–370

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Isoenzyme	$[Glc]_{0.5}$ (mM)	$[Fru]_{0.5}$ (mM)	$[Fru]_{0.5}/[Glc]_{0.5}$	V_{Fru}/V_{Glc}
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Hexokinase B	0.130	3.0	23.1	1.2
Hexokinase C	0.020	1.2	60.0	1.3
Hexokinase D	7.5	420	56.0	2.4

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Isoenzyme	$[Glc]_{0.5}$ (mM)	$[Fru]_{0.5}$ (mM)	$[Fru]_{0.5}/[Glc]_{0.5}$	V_{Fru}/V_{Glc}	$\frac{V_{Fru}/[Fru]_{0.5}}{V_{Glc}/[Glc]_{0.5}}$
Hexokinase A	0.044	3.1	70.5	1.1	0.016
Hexokinase B	0.130	3.0	23.1	1.2	0.052
Hexokinase C	0.020	1.2	60.0	1.3	0.022
Hexokinase D	7.5	420	56.0	2.4	0.043

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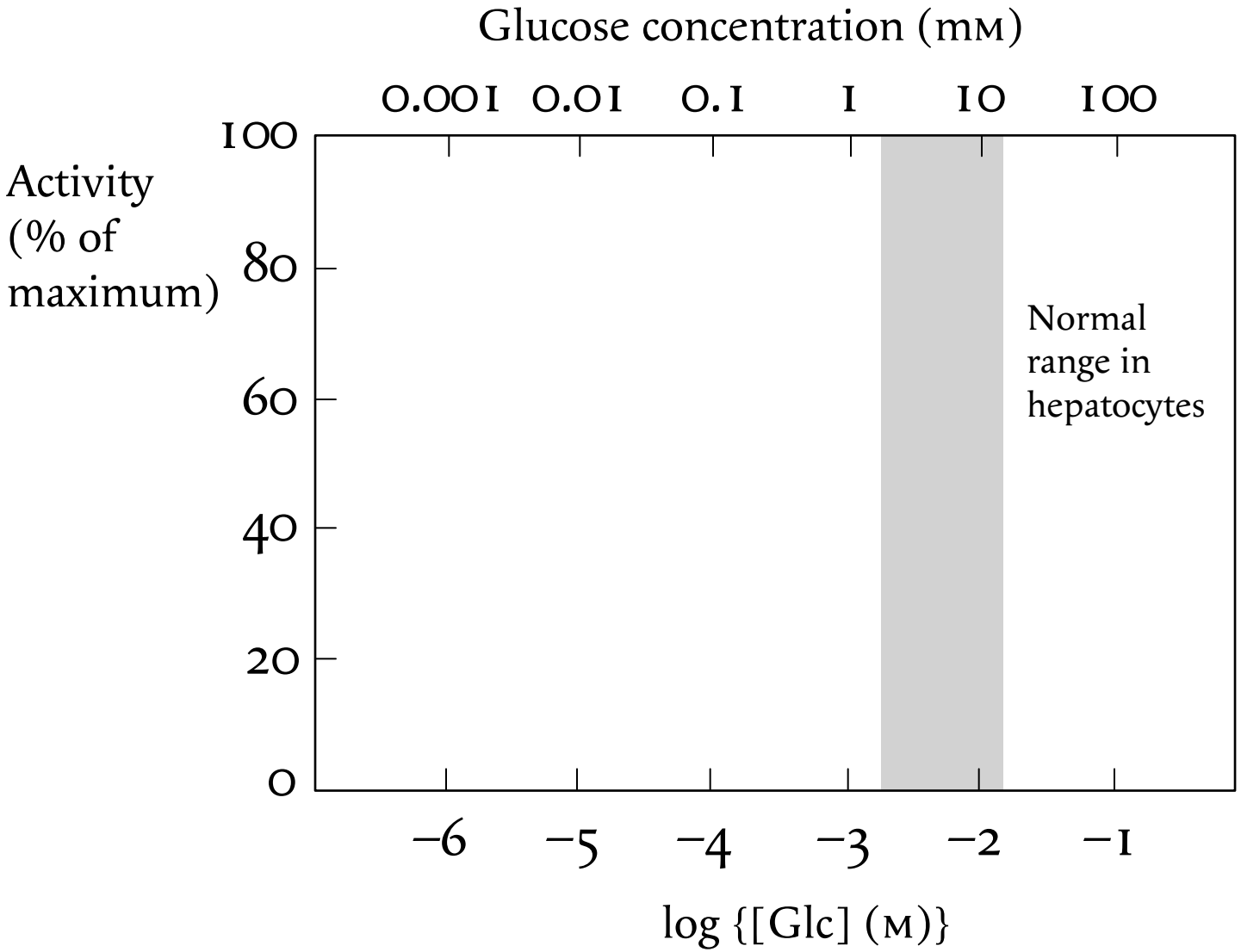
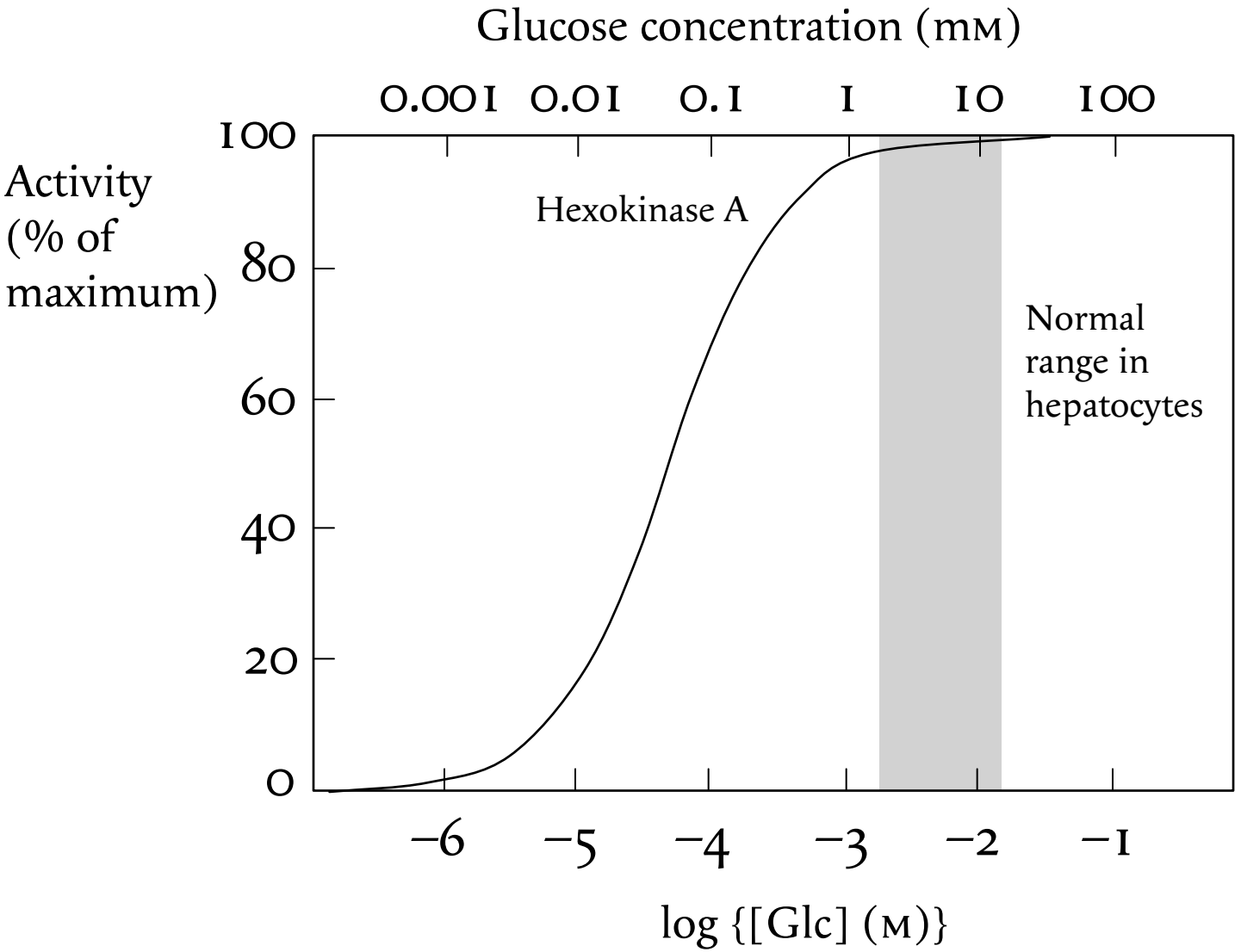
According to the best criterion of specificity (right-hand column), hexokinase D is within the range of the other three: by ***no*** criterion is it the most specific for glucose.

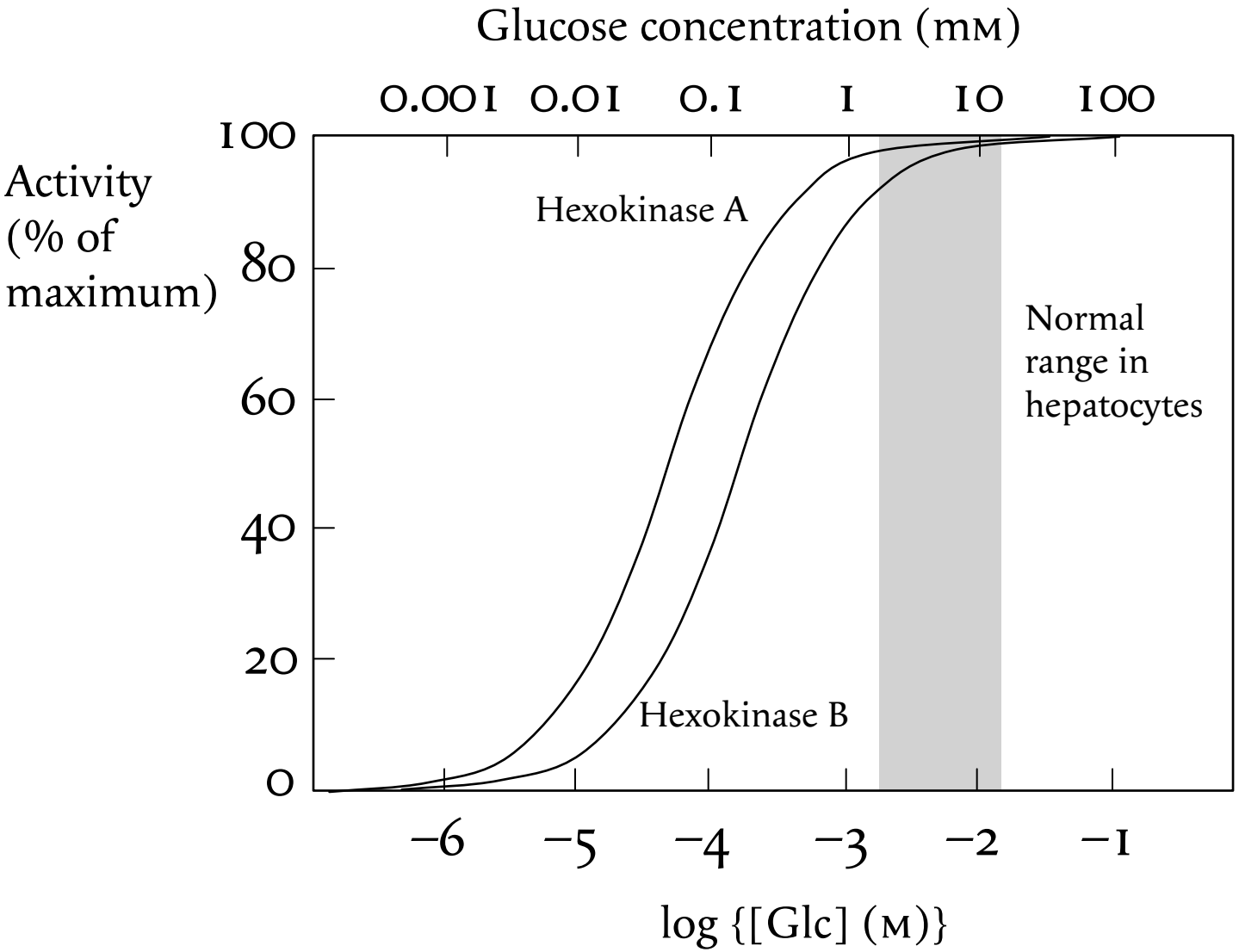
Hexokinases in mammals



Hexokinases in mammals



Hexokinases in mammals



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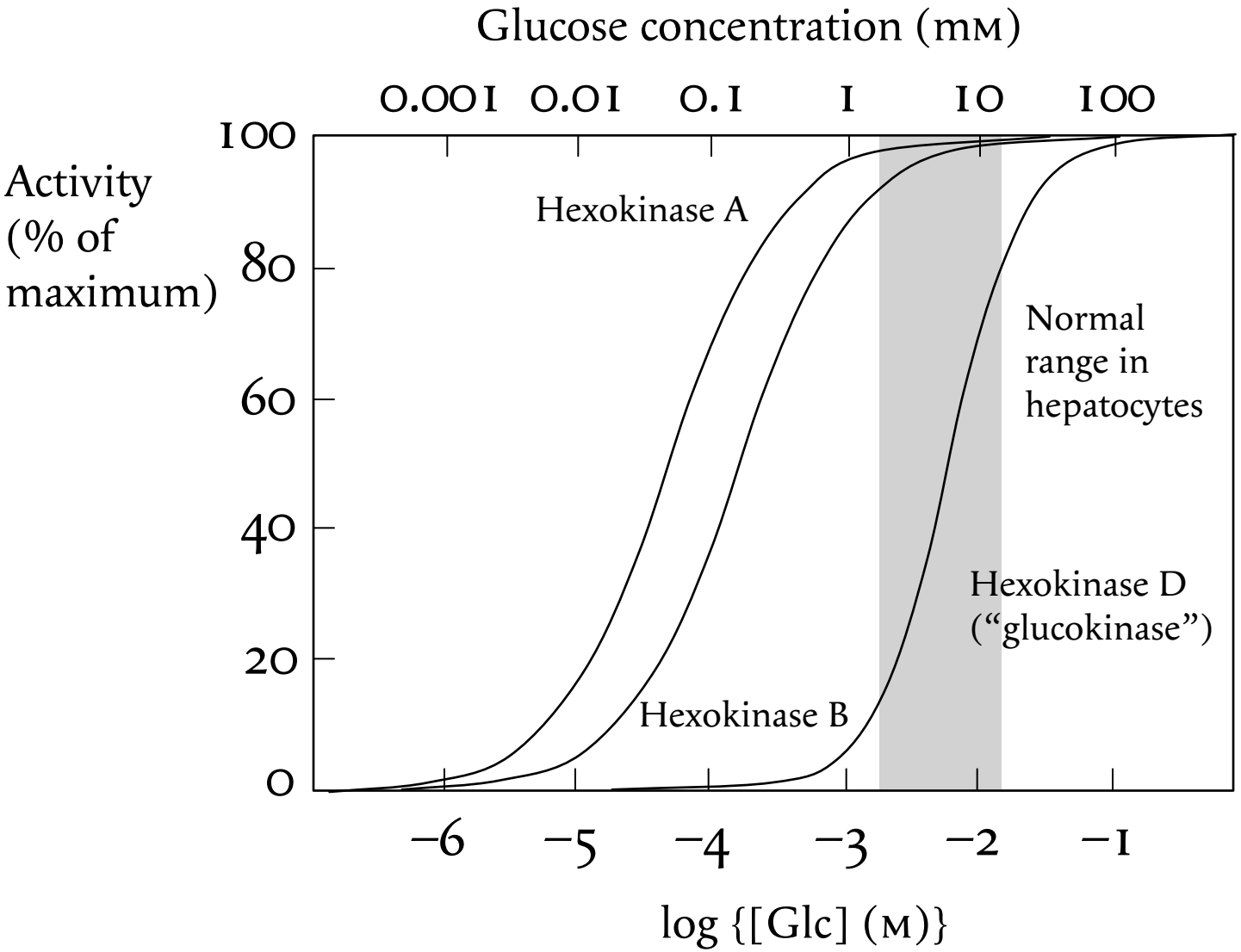
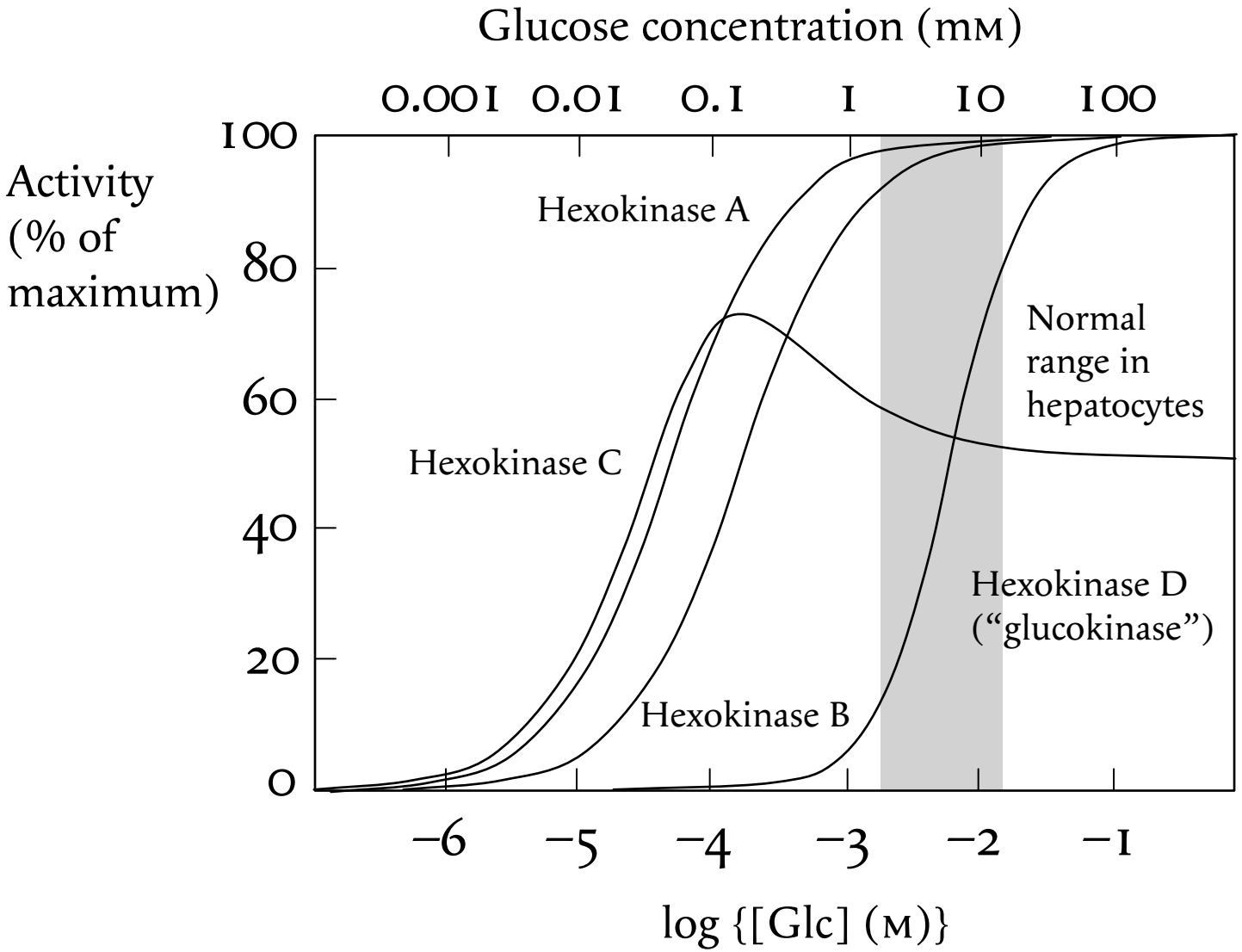


Hexokinases in mammals



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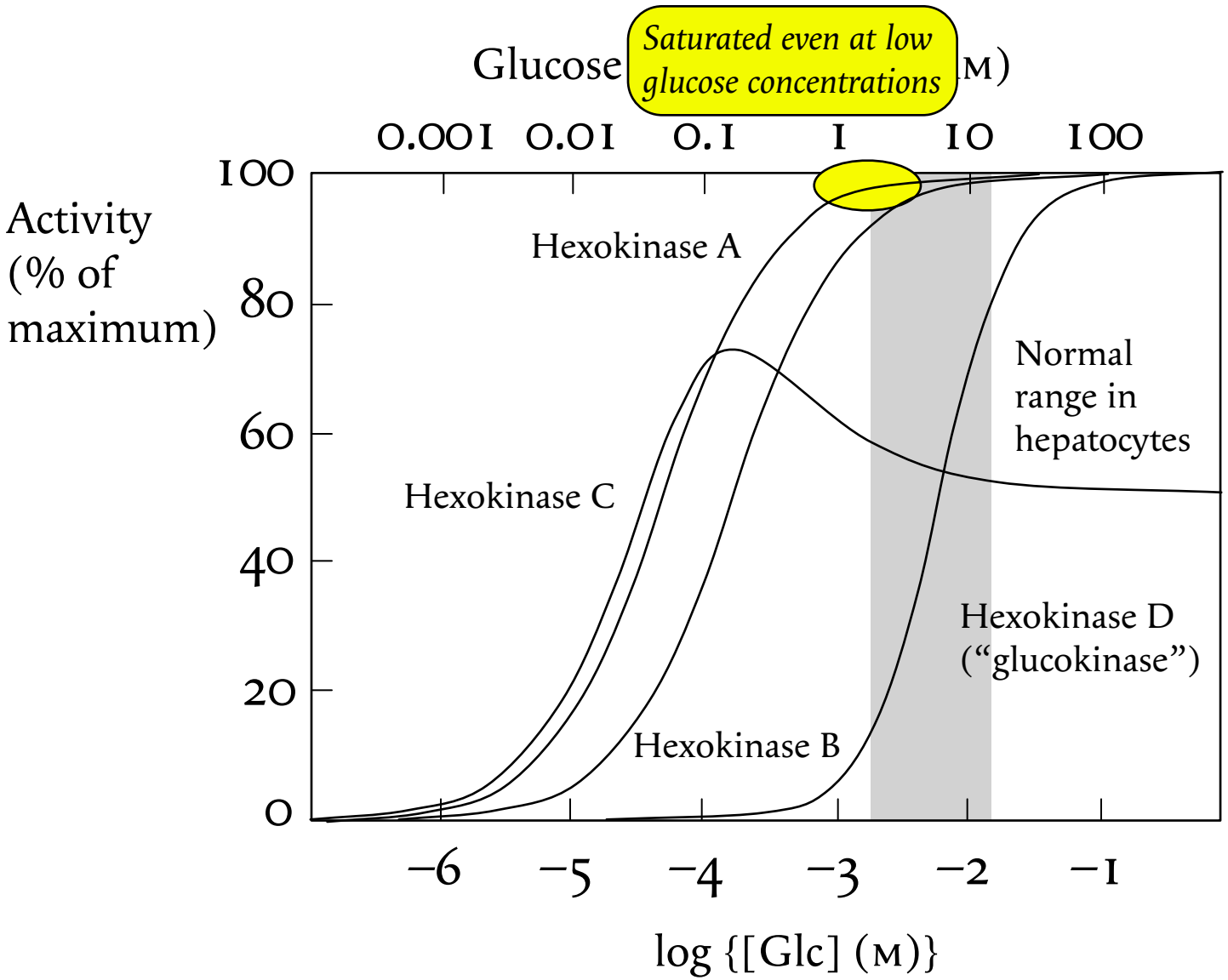

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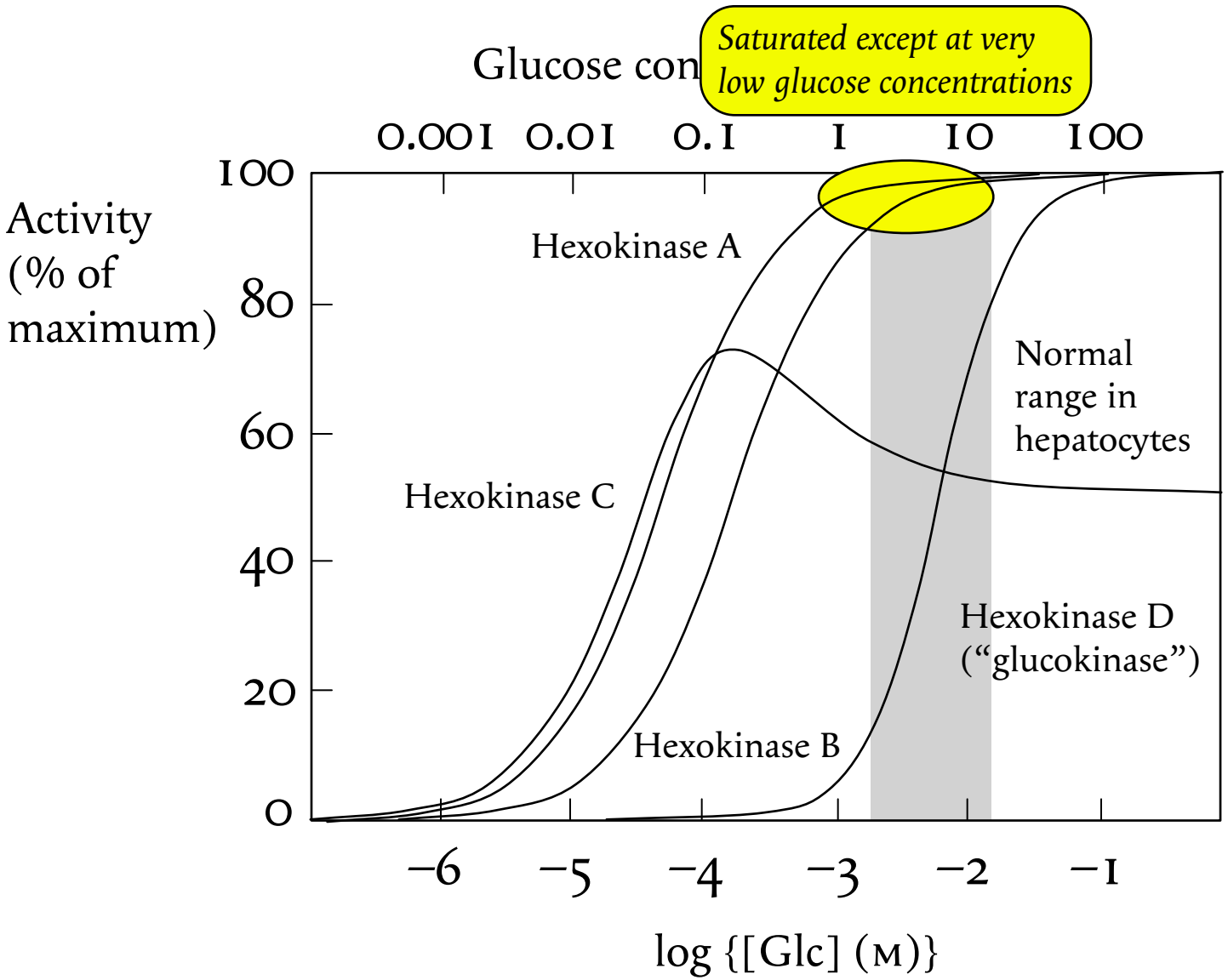
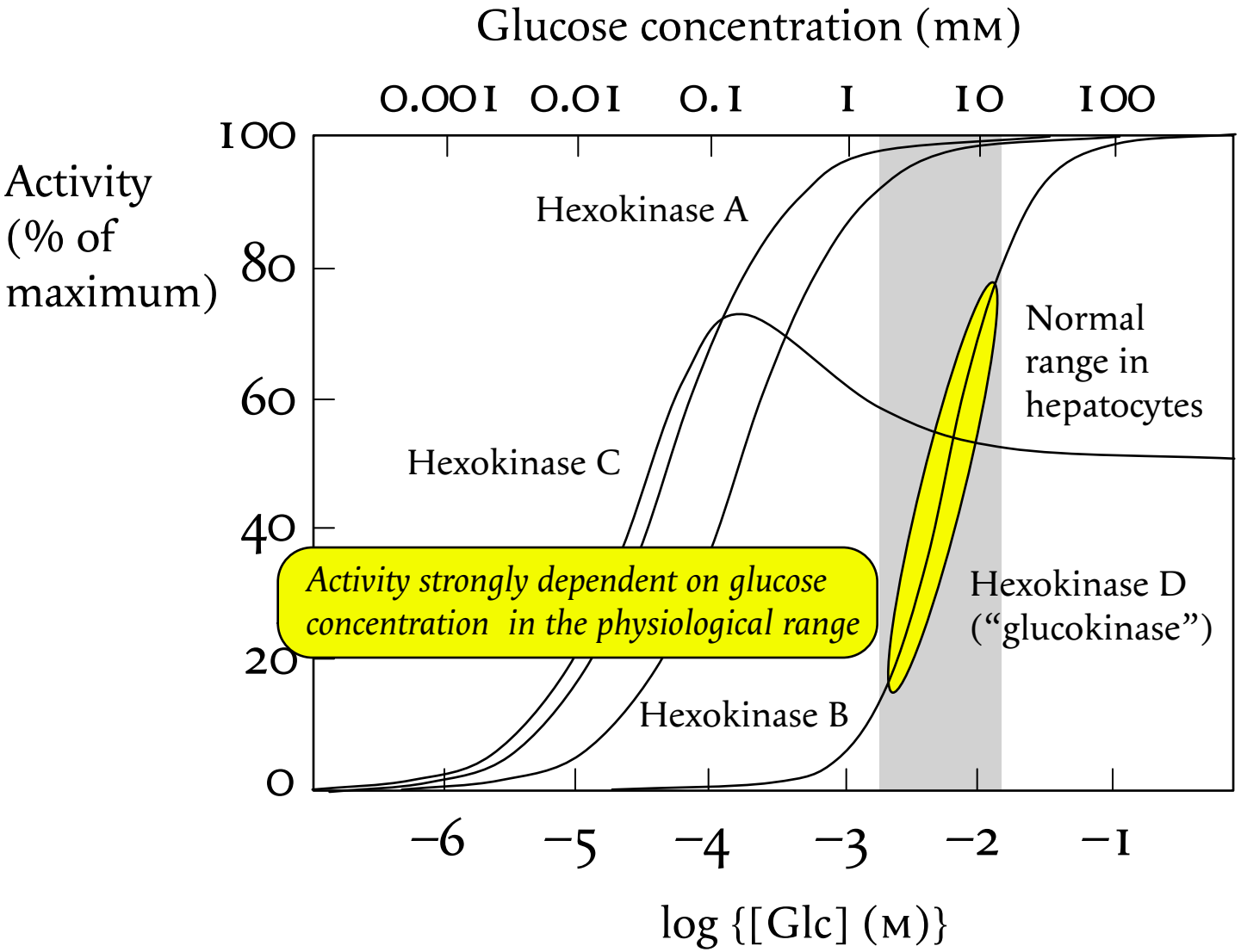
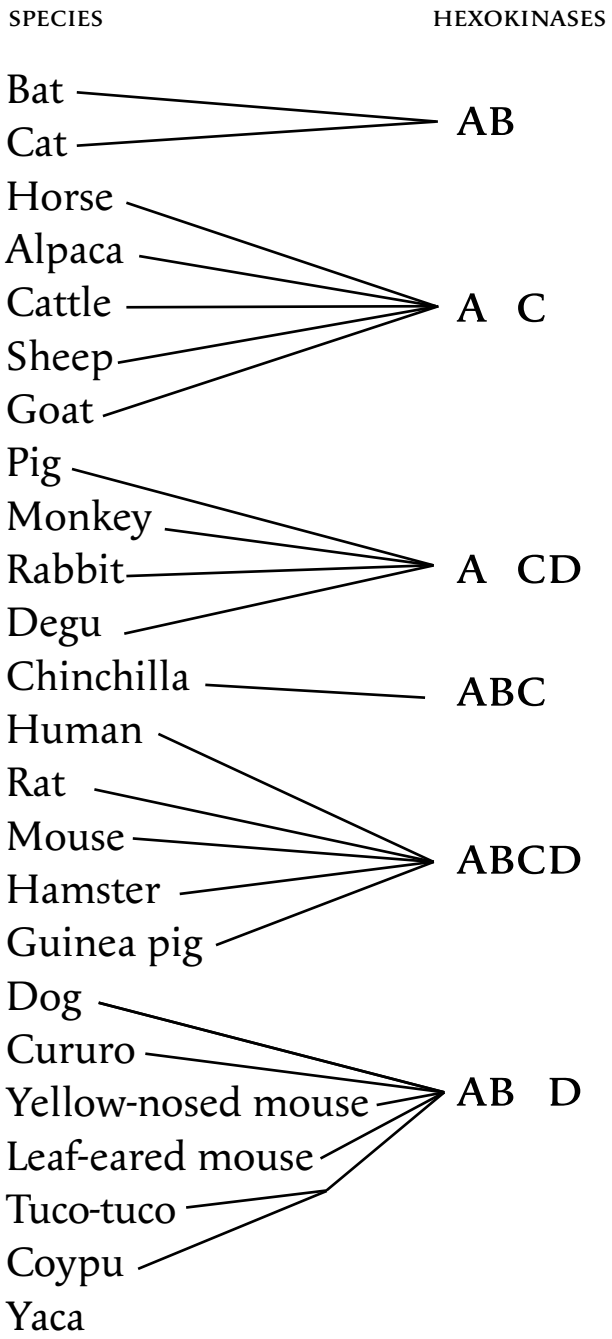


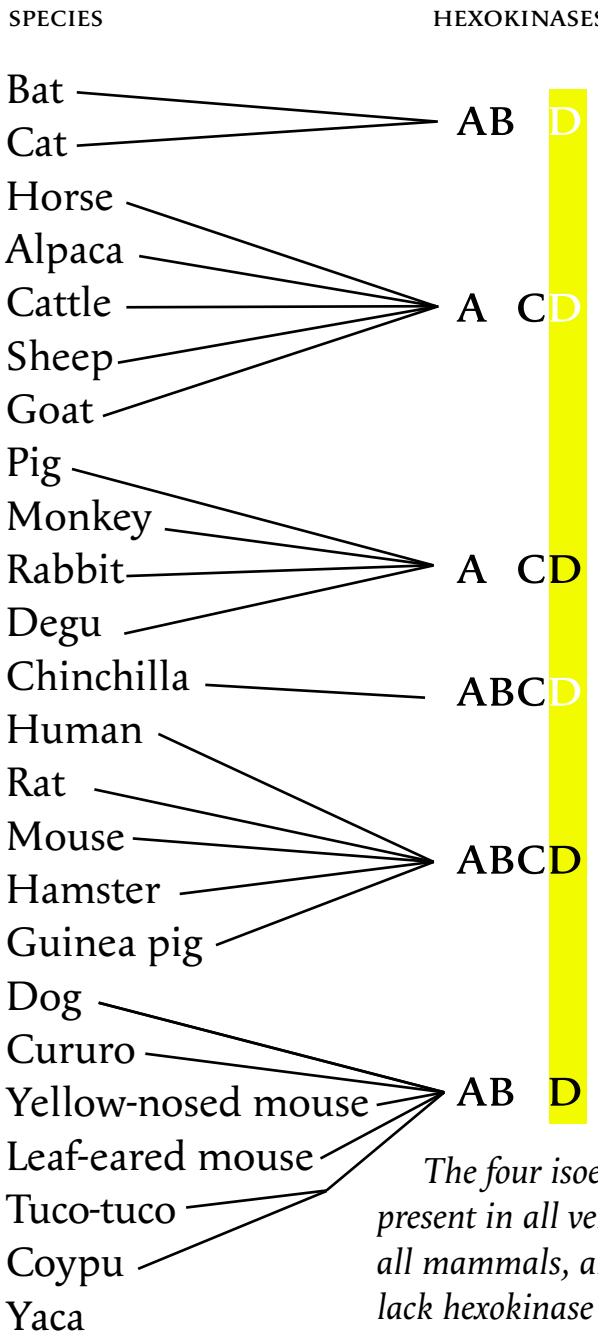
Hexokinases in mammals



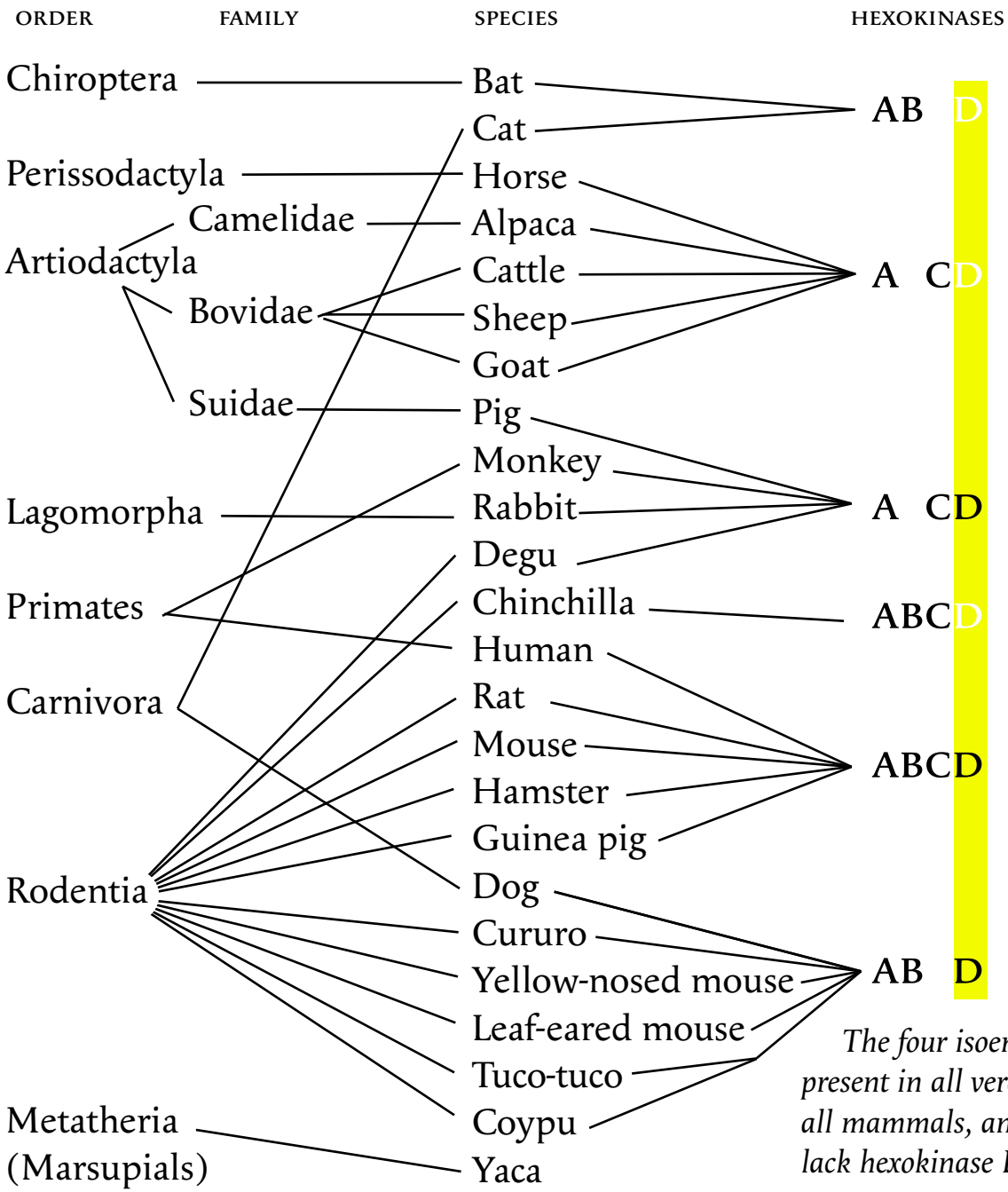
SPECIES

Bat
Cat
Horse
Alpaca
Cattle
Sheep
Goat
Pig
Monkey
Rabbit
Degu
Chinchilla
Human
Rat
Mouse
Hamster
Guinea pig
Dog
Cururo
Yellow-nosed mouse
Leaf-eared mouse
Tuco-tuco
Coypu
Yaca





The four isoenzymes are not all present in all verebrates, or even in all mammals, and several species lack hexokinase D.



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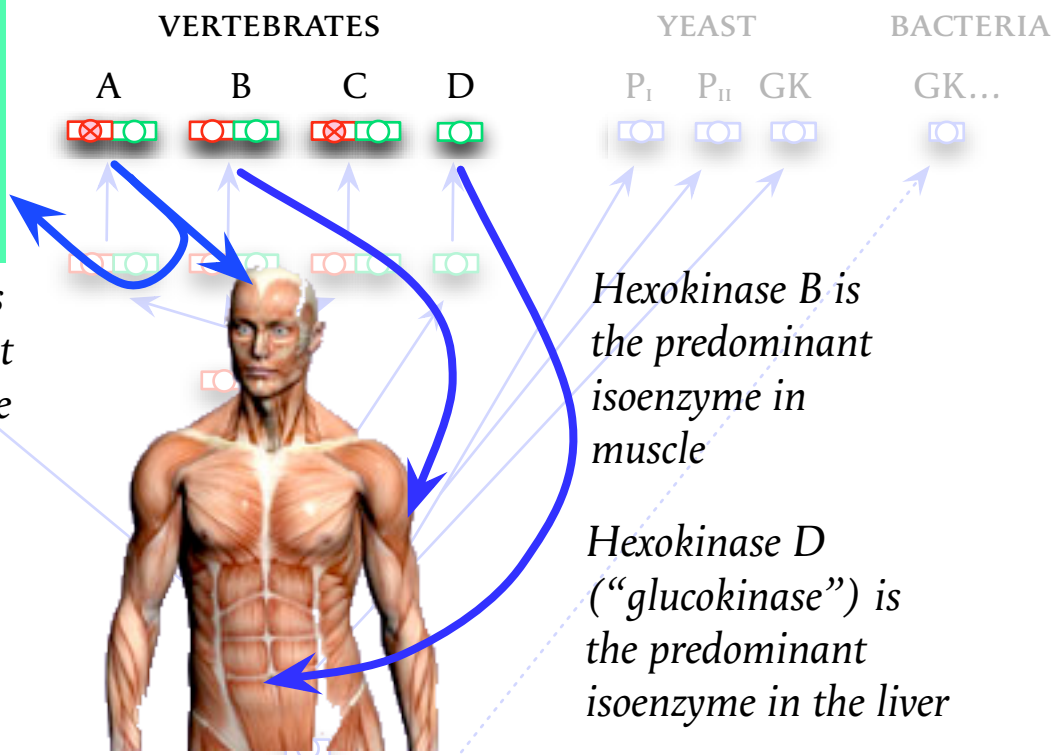
Hexokinases in mammals

Half-saturated at very low [glucose];

Michaelis–Menten kinetics with respect to glucose;

inhibited by glucose 6-*P*;

flux control coefficient very small.



Hexokinase A is the predominant isoenzyme in the brain

Hexokinase B is the predominant isoenzyme in muscle

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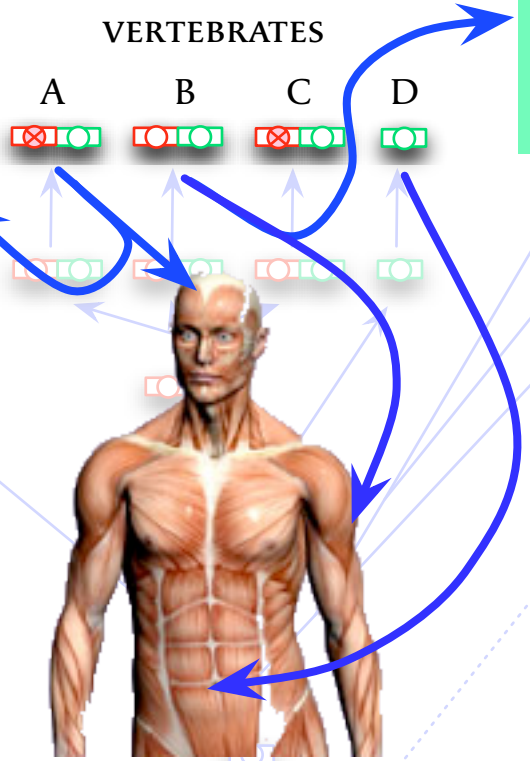

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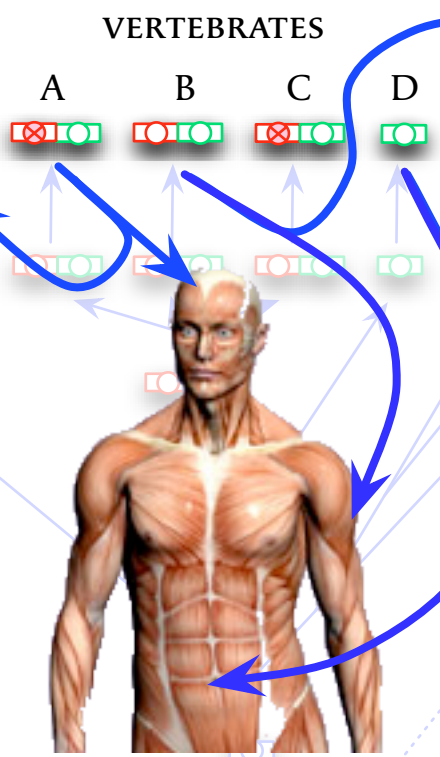

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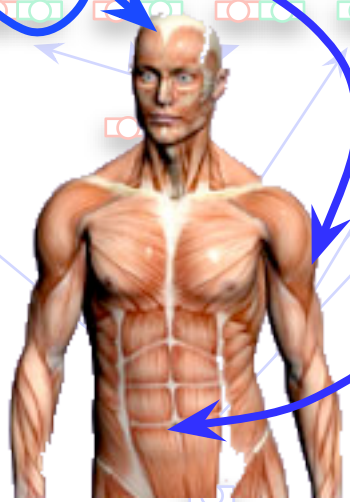
demand

VERTEBRATES

A B C D



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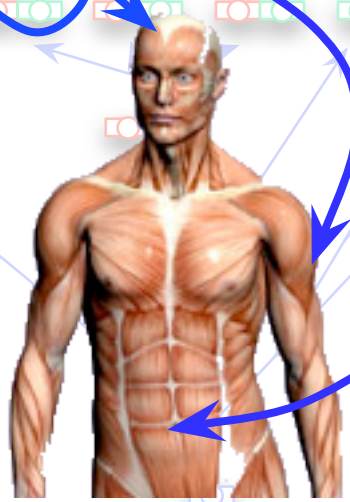

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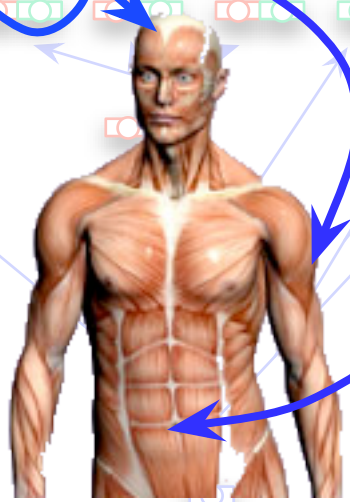

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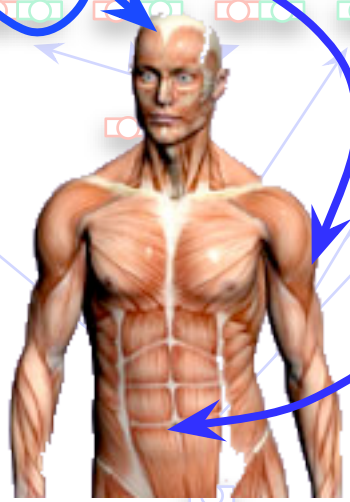

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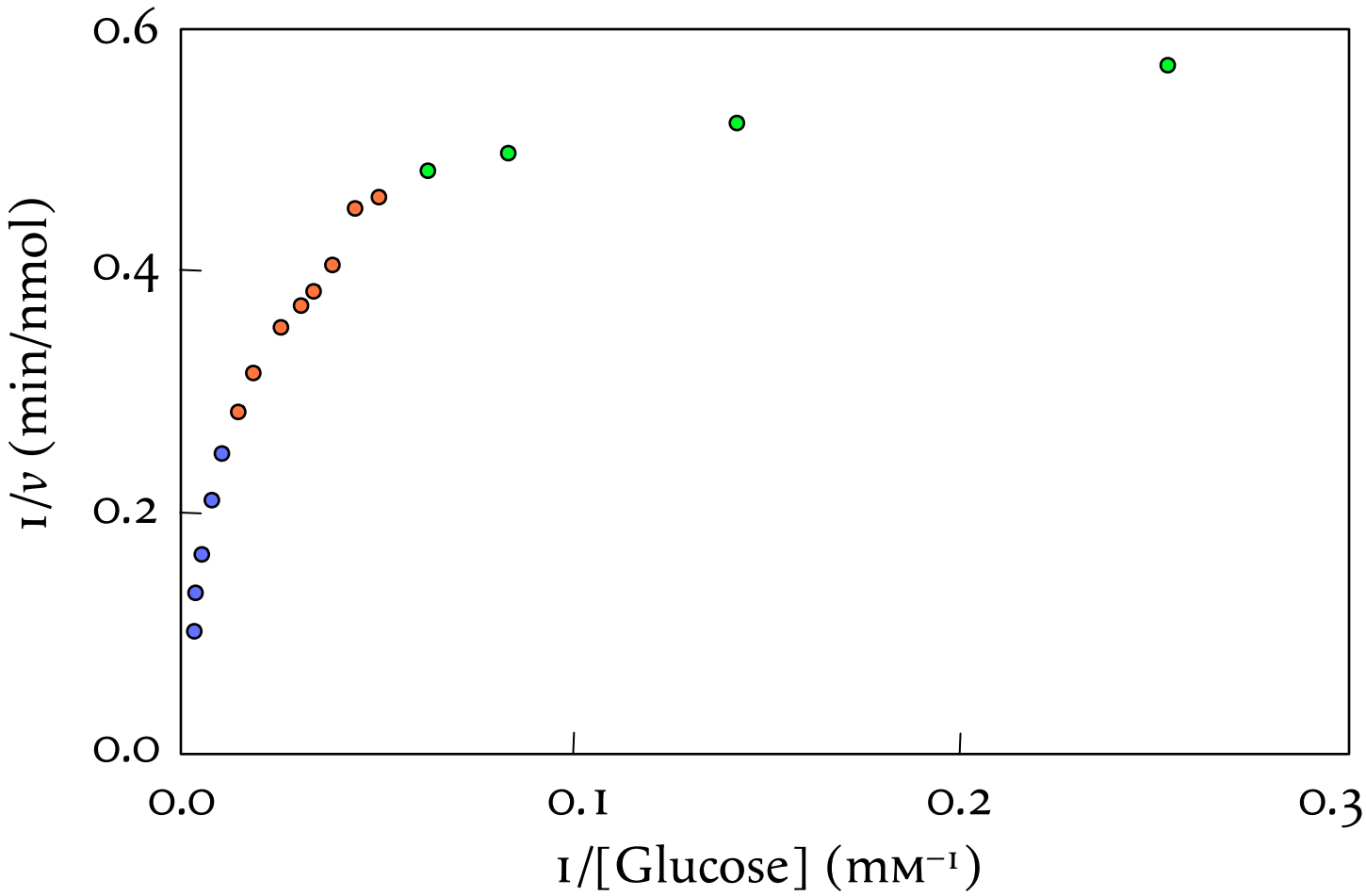
What implications do these properties have for drug development?

N-Acetylglucosamine kinase: sometimes mistakenly identified as hexokinase D



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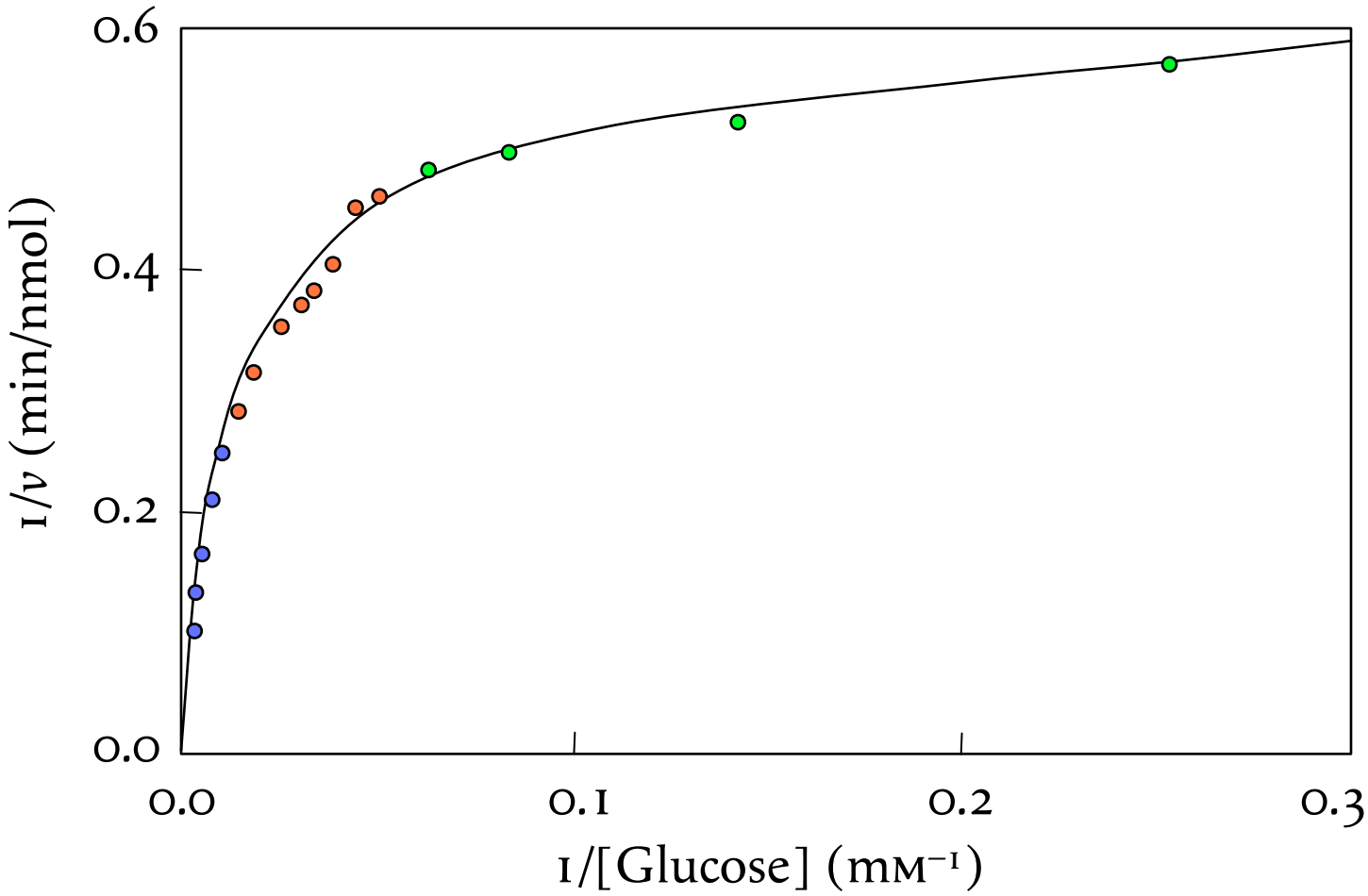

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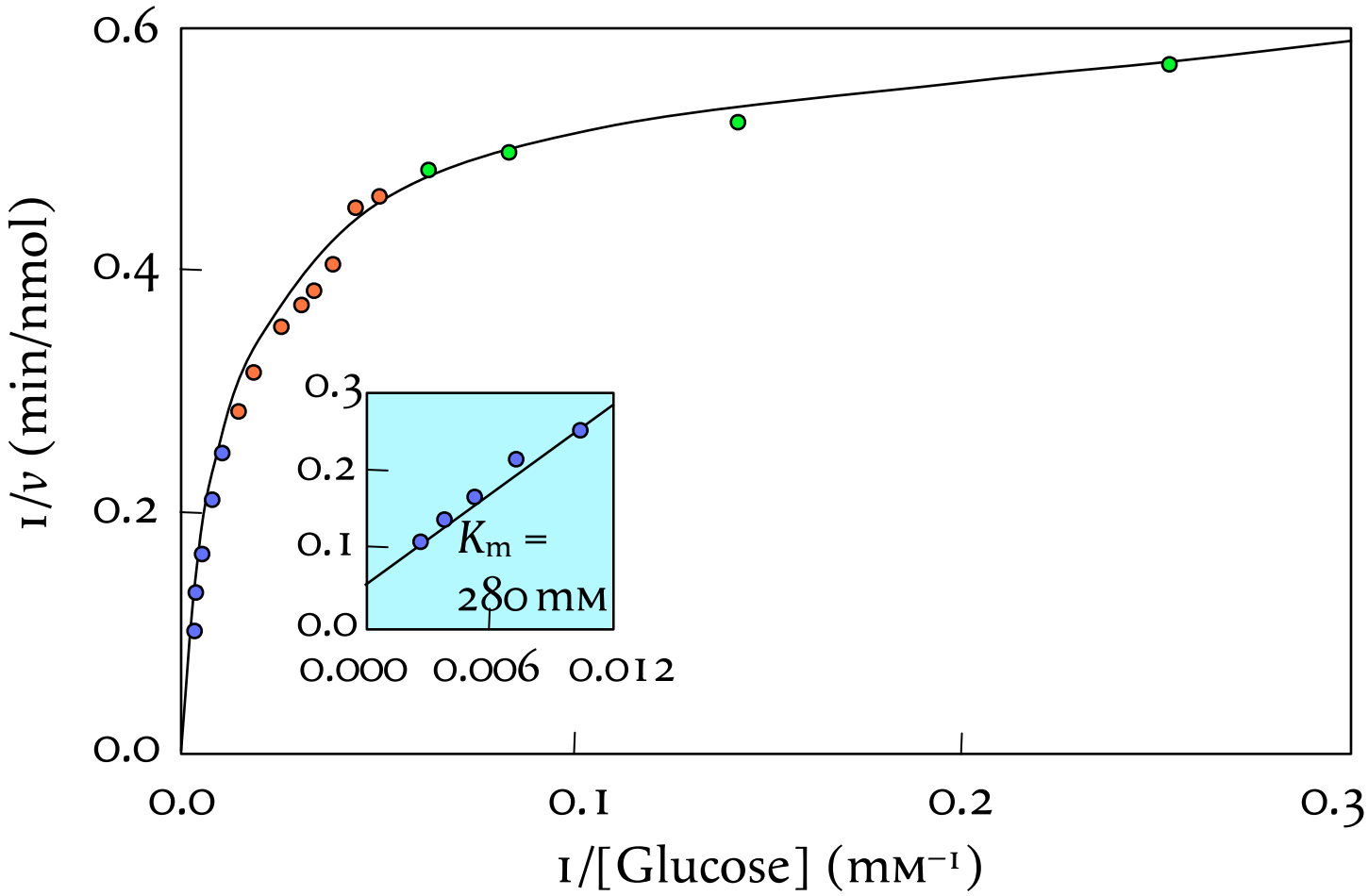

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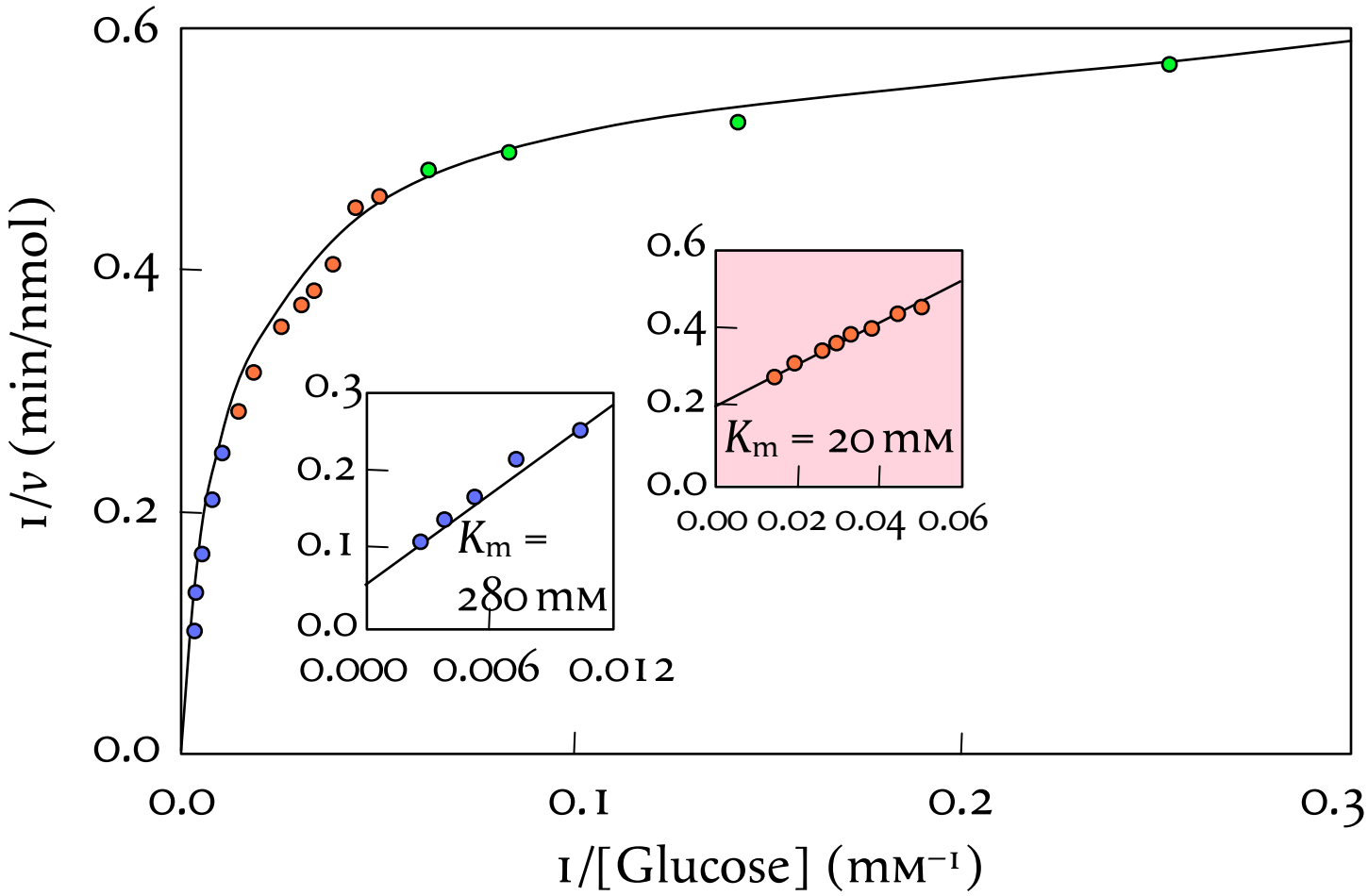

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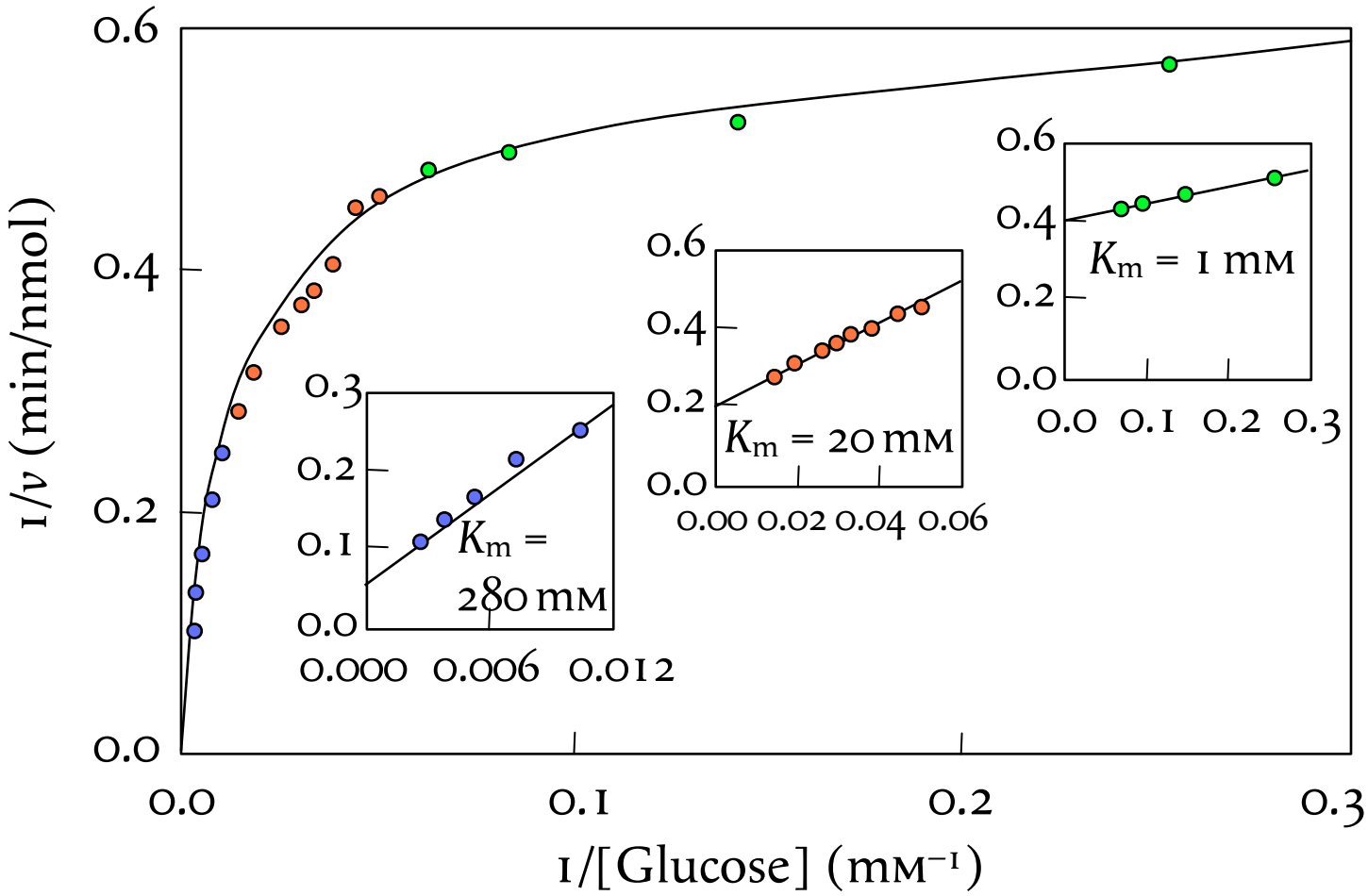

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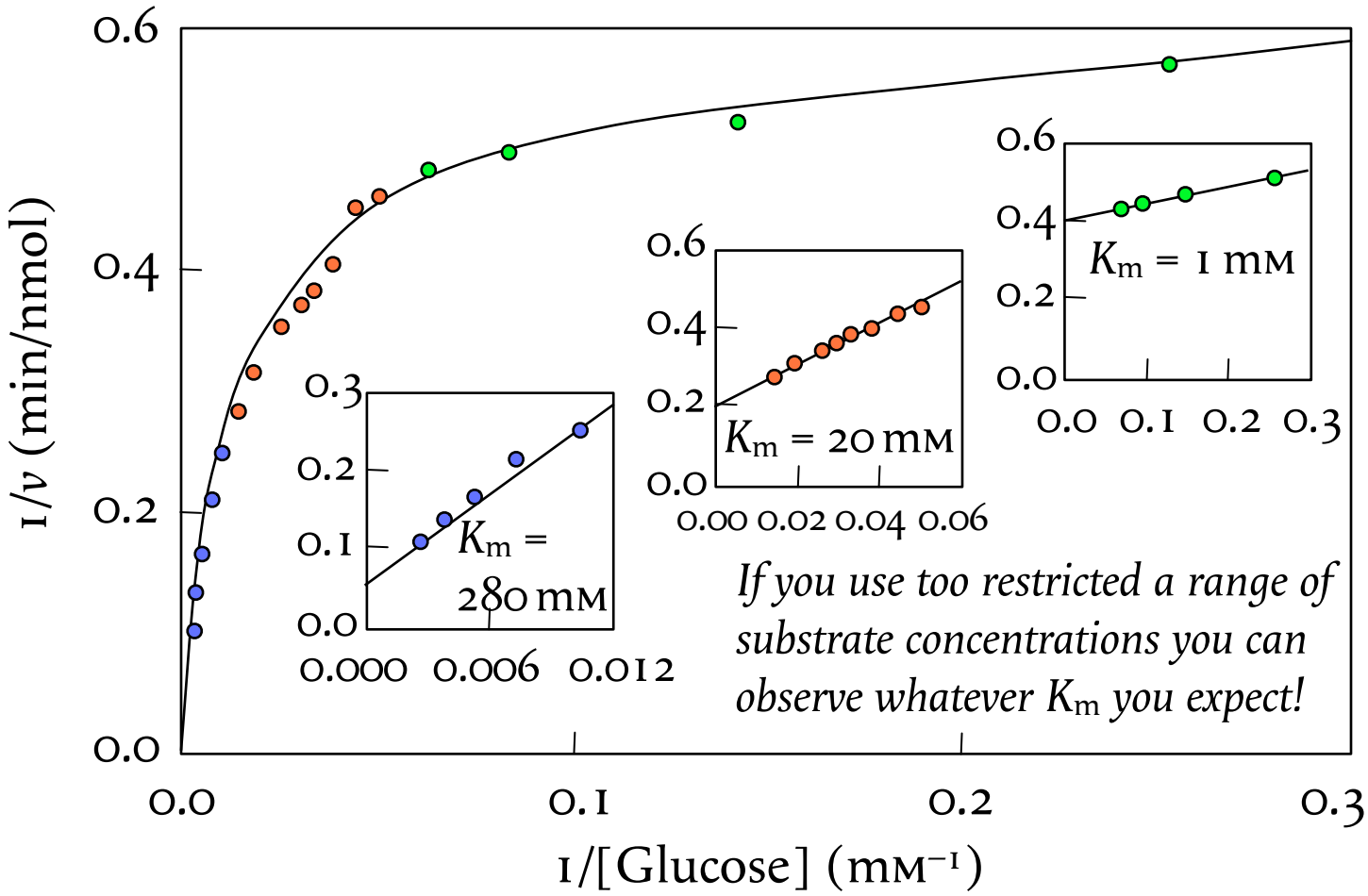

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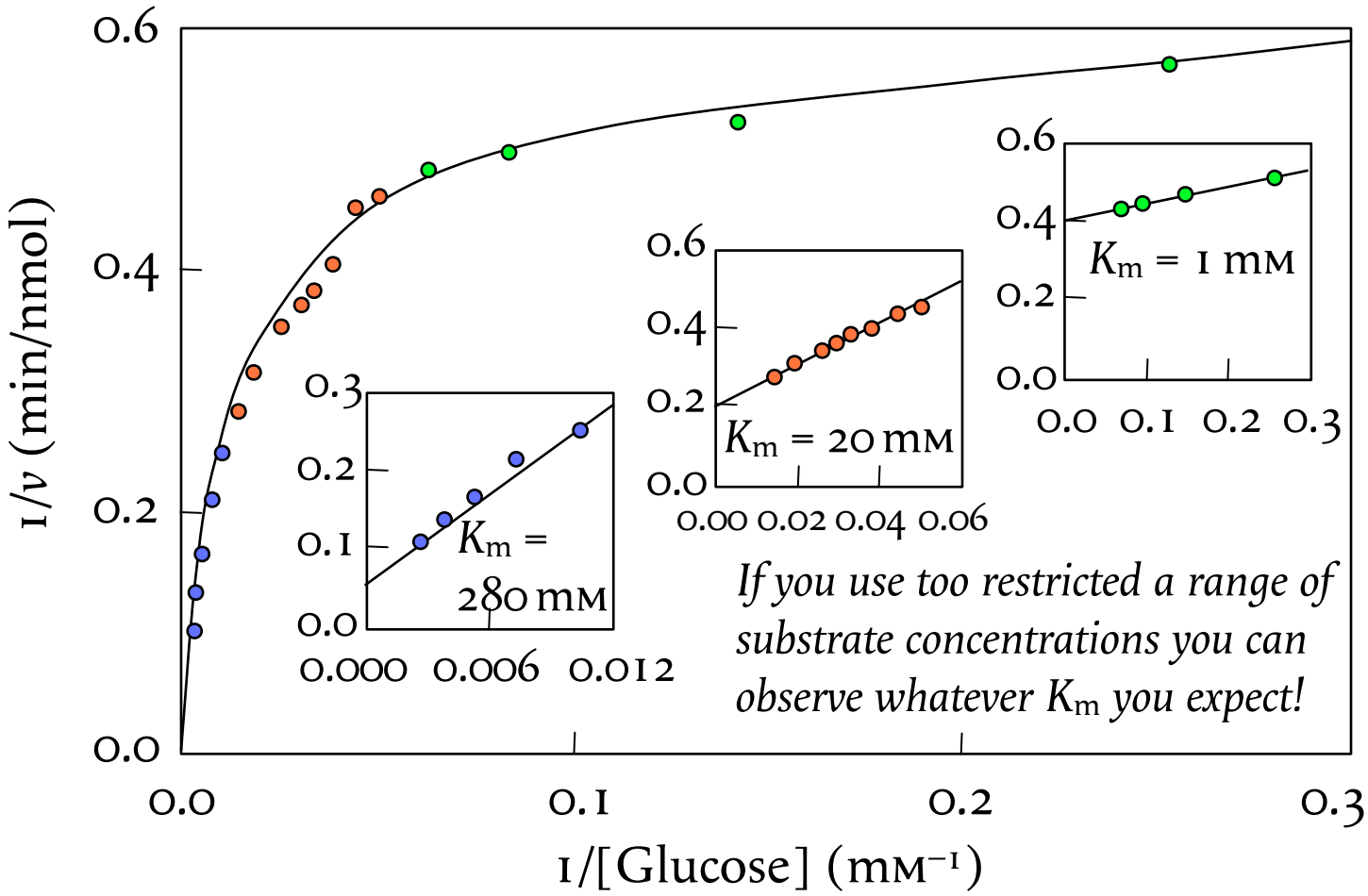
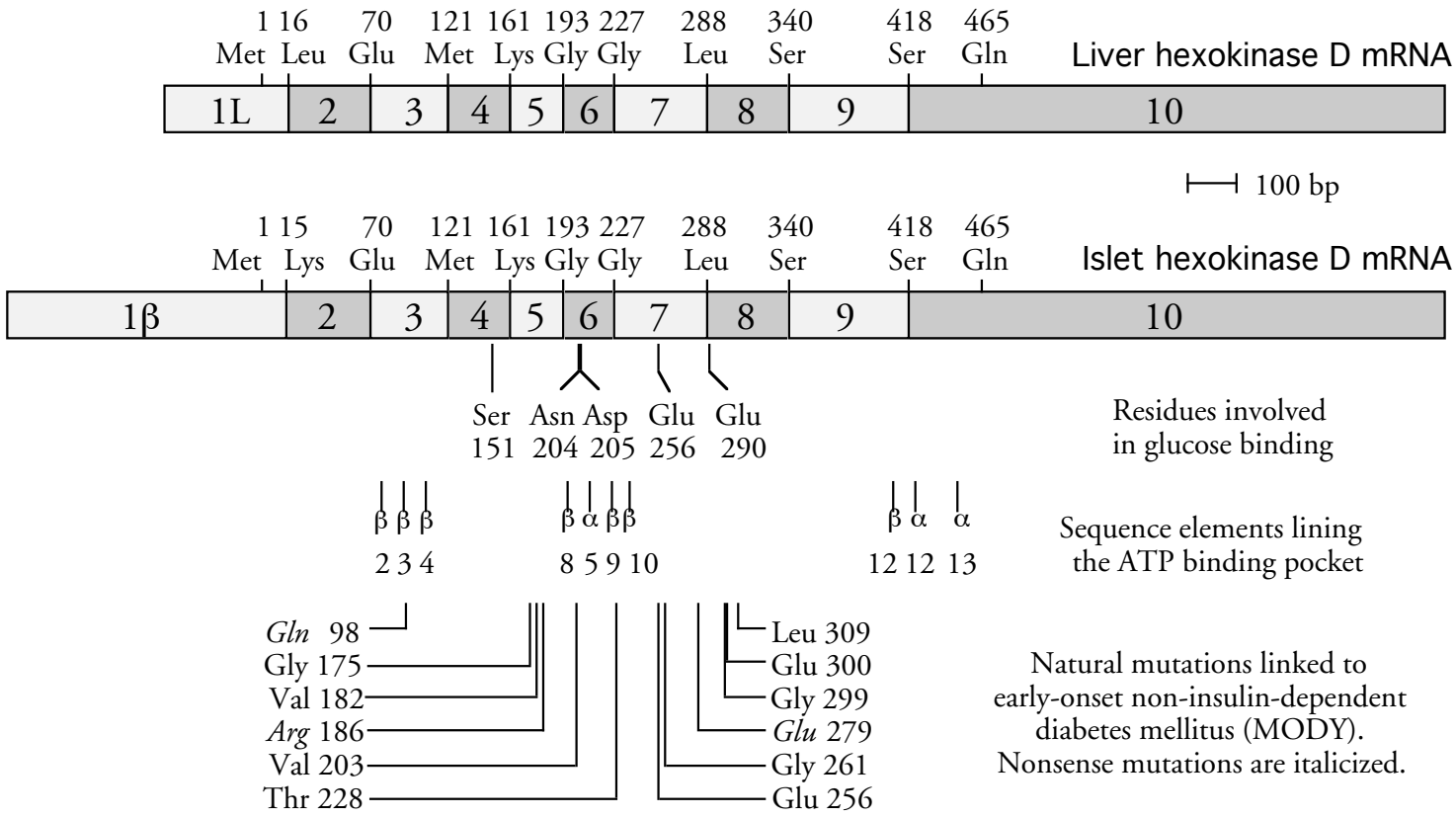


If you look for God you will find him, …but you will only find the God that you are looking for. Blaise Pascal

Functional assignments of amino acid residues in hexokinase D



María Luz Cárdenas (1995) "Glucokinase" Fig. 5.4: Adapted from Iynedjian (1993)

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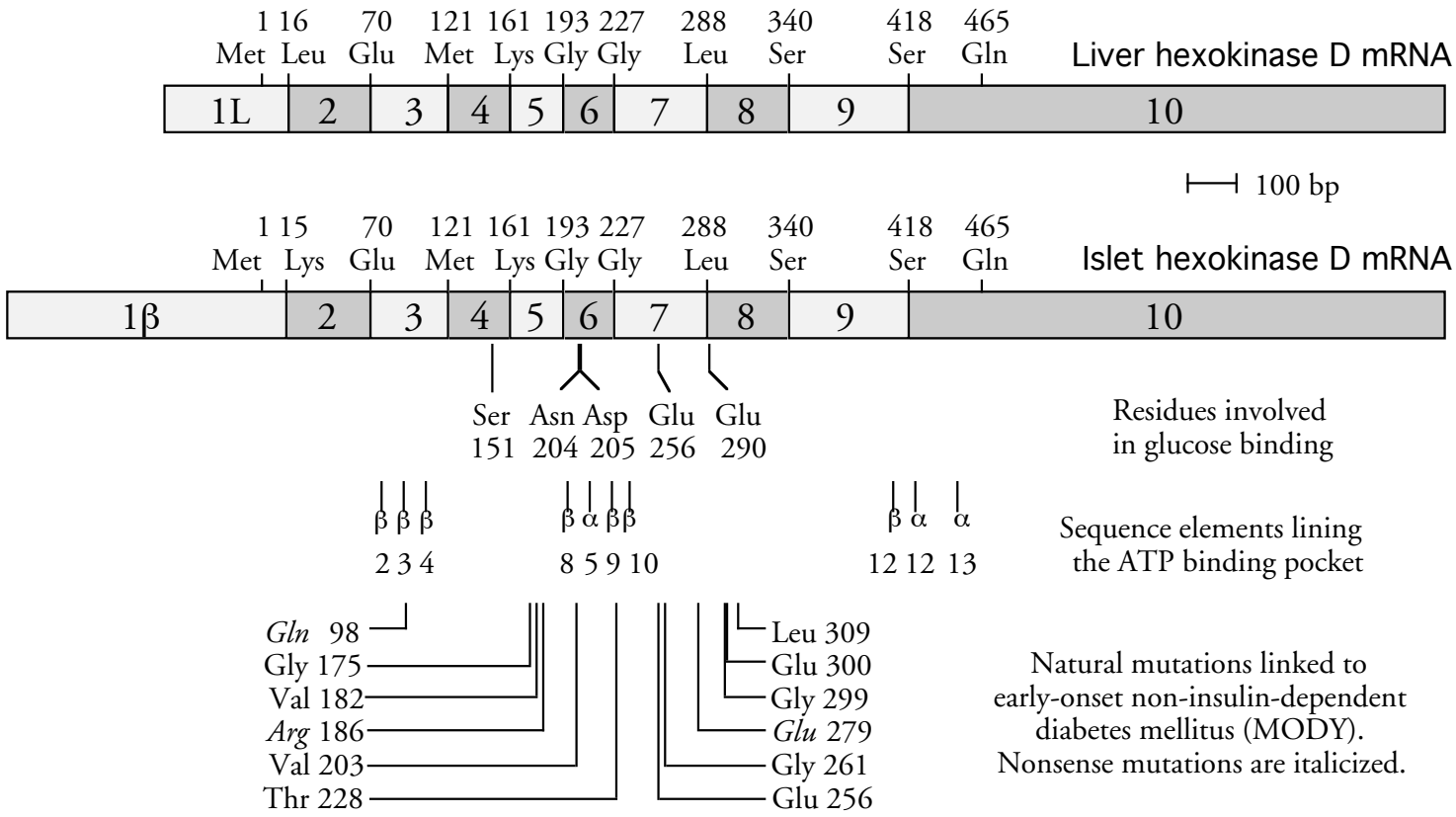

By 2004 the number of mutant forms of human hexokinase D identified had grown to more than 200

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