a systems approach to biology

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lecture 8
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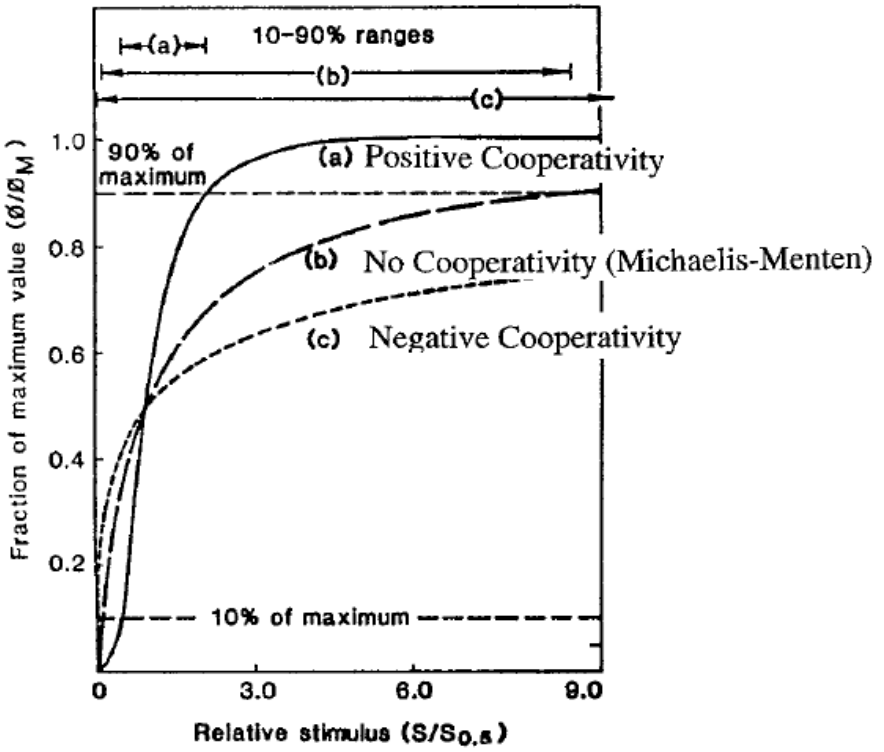
4. metabolism, continued

recap

$$\frac{[S]^h}{K^h + [S]^h}$$



$$\frac{S_{0.9}}{S_{0.1}} < 81$$

$$\frac{S_{0.9}}{S_{0.1}} = 81$$

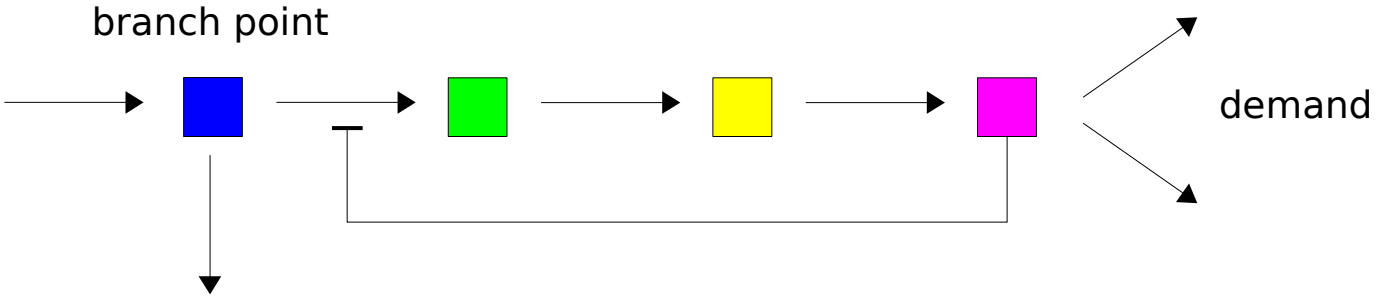
$$\frac{S_{0.9}}{S_{0.1}} > 81$$

warning: a single number, like the CI or the Hill coefficient, cannot adequately summarise the shape of a sigmoidal curve

JG, *"Multisite protein phosphorylation makes a good threshold but can be a poor switch"*, PNAS **102**:14617-22 2005

end-product feedback inhibition

the first committed step in a biosynthetic pathway is often inhibited by the terminal metabolite in the pathway



Novick, Szilard, in **Dynamics of Growth Processes**, Princeton Univ Press 1954

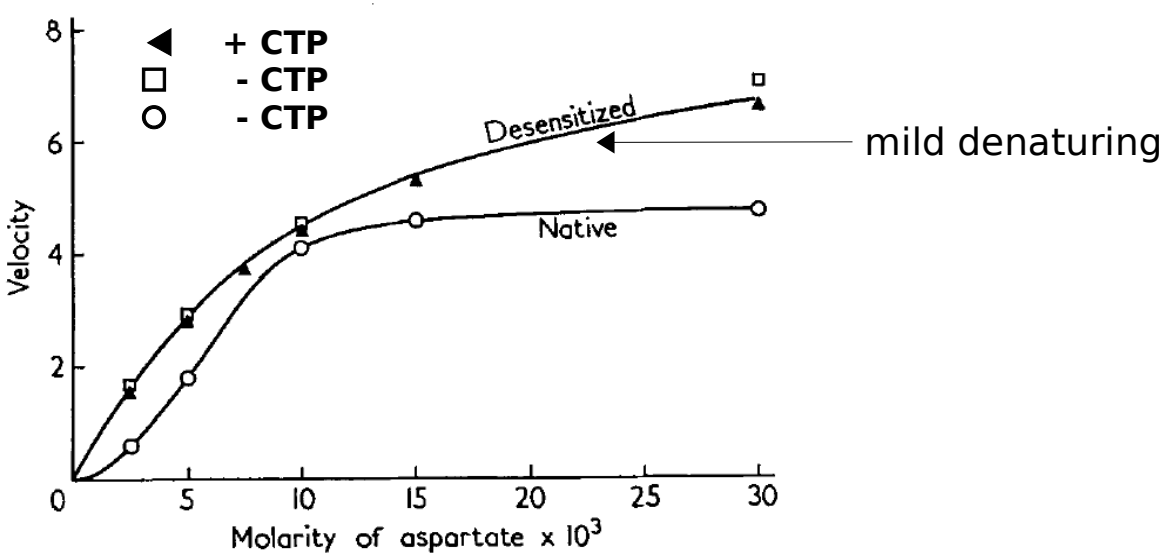
Pardee, Yates, *"Control of pyrimidine biosynthesis in Escherichia coli by a feed-back mechanism"*, J Biol Chem **221**:757-70 1956

Umbarger, *"Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine"*, Science **123**:848 1956

cooperativity in enzymes

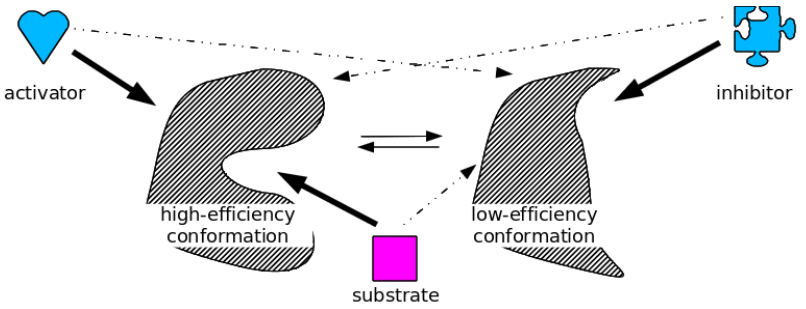
the regulated enzyme is often positively cooperative for both substrate and inhibitor

aspartate transcarbamylase (ATcase) is the first committed step in pyrimidine biosynthesis, ultimately yielding CTP



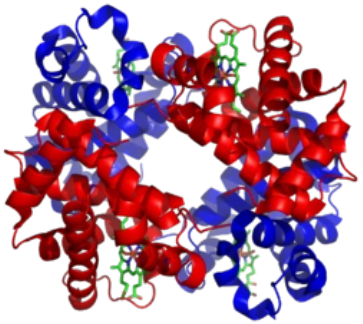
Gerhart, Pardee, *"Enzymology of control by feedback inhibition"*, J Biol Chem **237**:891-6 1962

allosteric enzymes

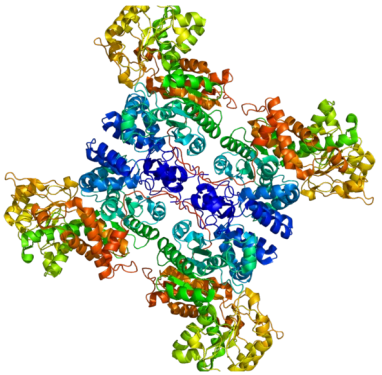


allostery explains feedback inhibition by ligands that are chemically distinct from the substrate – through weak linkage

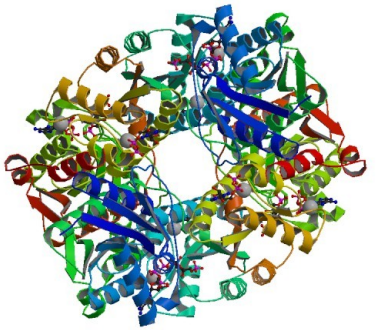
allosterically regulated enzymes are also found to be multimeric, with multiple binding sites for allosteric ligands – this gives rise to cooperativity



hemoglobin
PDB 1GZX



malic enzyme
PDB 1DO8



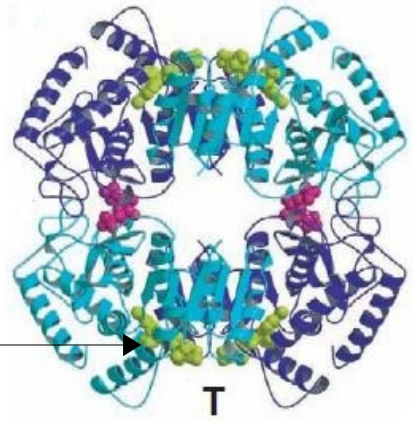
phosphofructokinase1
PDB 1PFK

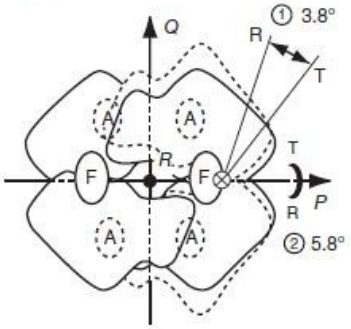
allosteric enzymes

allosteric ligands bind in subunit interfaces

conformational transitions can affect the quarternary structure of the multimer, not just the tertiary structure of the individual subunits

lactate dehydrogenase

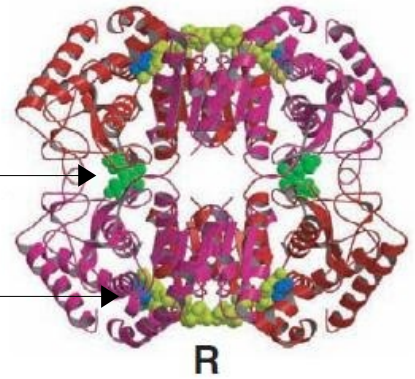


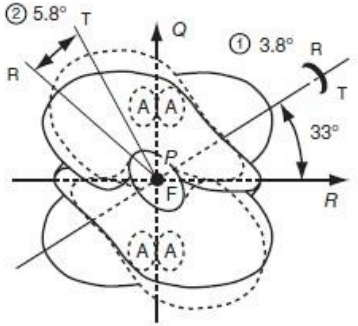


NADH
(light green)

F1,6-BP
(dark green)

oxaloacetate
(blue)





Iwata, Kamata, Yoshida, Minowa, Ohta, Nat Struct Biol **1**:176-85 1994

two models of allostery

Monod, Wyman, Changeux (MWC)

selective
symmetric or concerted – all subunits change together
independent binding
simple, popular

Koshland, Nemethy, Filmer (KNF)

instructive (induced fit)
sequential – subunits can change one at a time
non-independent binding
complex, unpopular

MWC gives a very good account of hemoglobin behaviour(*) but only KNF explains negative cooperativity

Monod, Wyman, Changeux,, *"On the nature of allosteric transitions: a plausible model"*, J Mol Biol **12**:88-188 1965

Koshland, Nemethy, Filmer, *"Comparison of experimental binding data and theoretical models in proteins containing subunits"*, Biochem **5**:365-85 1966

(*) Eaton, Henry, Hofrichter, Mozzarelli, *"Is cooperative oxygen binding by hemoglobin really understood?"*, Nature Struct Biol **6**:351-8 1999

ligand binding in the linear framework

a ligand, L, binding to a protein with k binding sites

protein conformations can be encoded by a letter *X*

X =	R	T
	relaxed	tense

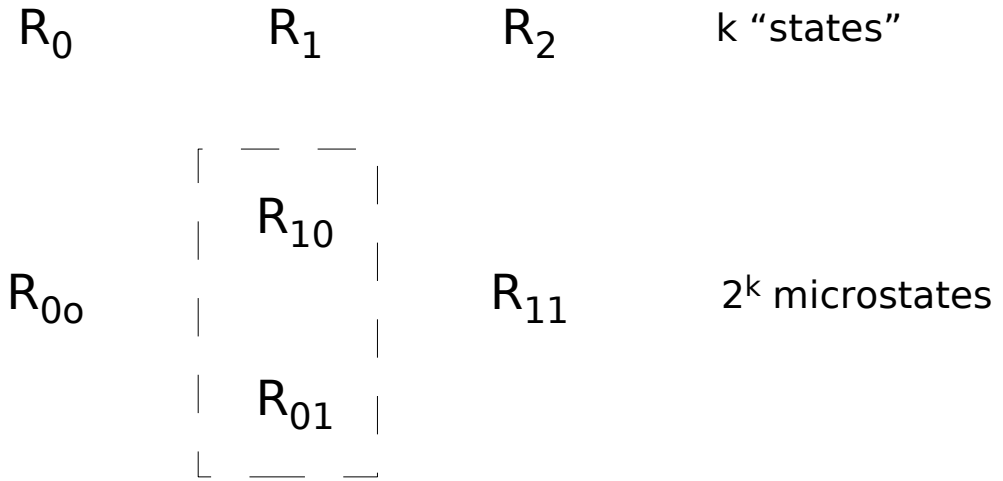
ligand binding can be encoded by a bitstring *u*

u =	00	10	01	11
	no ligand	ligand in site 1	ligand in site 2	ligand in both sites

protein **microstates** can be encoded by *Xu*

statistical factors

much of the biochemical literature(*) prefers to count numbers of bound ligands rather than keep track of where the ligands are bound



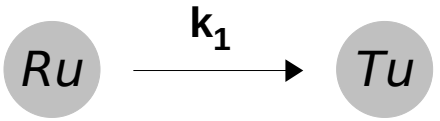
these “states” are not biochemically meaningful and require “statistical factors” (binomial coefficients) to keep track of the underlying microstates.

(*) Monod, Wyman, Changeux,, *“On the nature of allosteric transitions: a plausible model”*, J Mol Biol **12**:88-188 1965

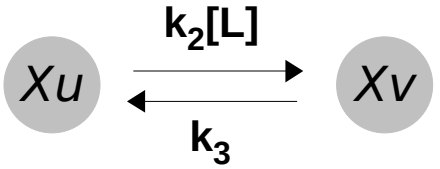
labelled, directed graph

protein microstates form the vertices of the graph

a conformational transition changes *X*, leaving *u* fixed (may be reversible)



ligand binding changes *u*, leaving *X* fixed (usually reversible)

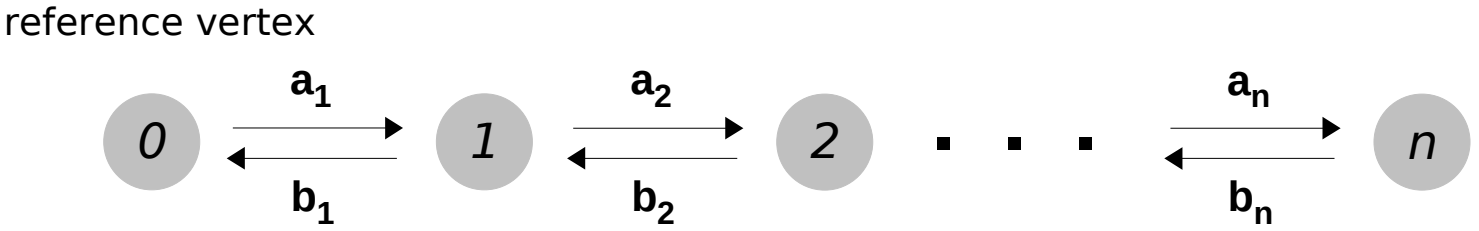


v has to differ from *u* in having only one bit changed from 0 to 1

thermodynamic equilibrium

detailed balance means that spanning trees are not needed to calculate equilibrium concentrations

choose any path of reversible edges from the reference vertex, 0, to the vertex whose concentration is to be calculated



$$x_1 = \left(\frac{a_1}{b_1}\right) x_0 \quad x_2 = \left(\frac{a_1}{b_1}\right)\left(\frac{a_2}{b_2}\right) x_0 \quad x_n = \left(\frac{a_1}{b_1}\right)\left(\frac{a_2}{b_2}\right)\cdots\left(\frac{a_n}{b_n}\right) x_0$$

the cycle condition ensures that the result is independent of the path taken

Monod-Wyman-Changeux

ligand binding to the allosteric protein is at thermodynamic equilibrium

in a given conformation, ligand binds independently to each site – the affinity of the ligand for a site does not depend on the other sites

protein activity is a function of **fractional saturation** – the proportion of sites bound by ligand

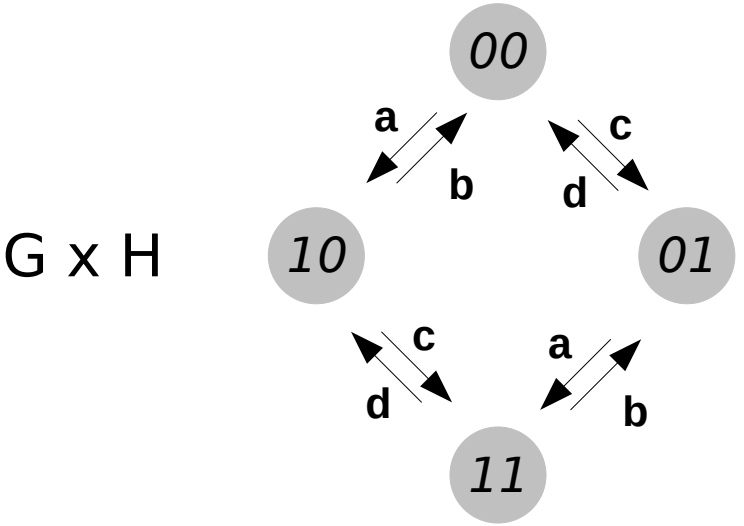
$$\frac{\text{number of bound sites}}{\text{total number of sites}} = \frac{\sum_\mu (\#\mu) x_\mu}{k(\sum_\mu x_\mu)}$$

$$\#\mu = \text{number of bound sites in microstate } \mu$$

$$= \text{number of 1's in the bitstring of } \mu$$

product graph

independent binding gives a product graph



product theorem

"partition function" – the total concentration of equilibrium states, normalised by the concentration of the reference state

$$\pi(G) = \left(\frac{1}{x_0}\right) \sum_{\mu} x_\mu$$

product theorem: if G and H both satisfy detailed balance then so does G x H and

$$\pi(G \times H) = \pi(G)\pi(H)$$

the reference vertex in G x H is taken to be the product of the reference vertices in G and H

Monod-Wyman-Changeux

fractional saturation

$$\frac{(1+x)^{n-1}x + L'(1+cx)^{n-1}cx}{(1+x)^n + L'(1+cx)^n} \qquad x = \frac{S}{K_R}$$

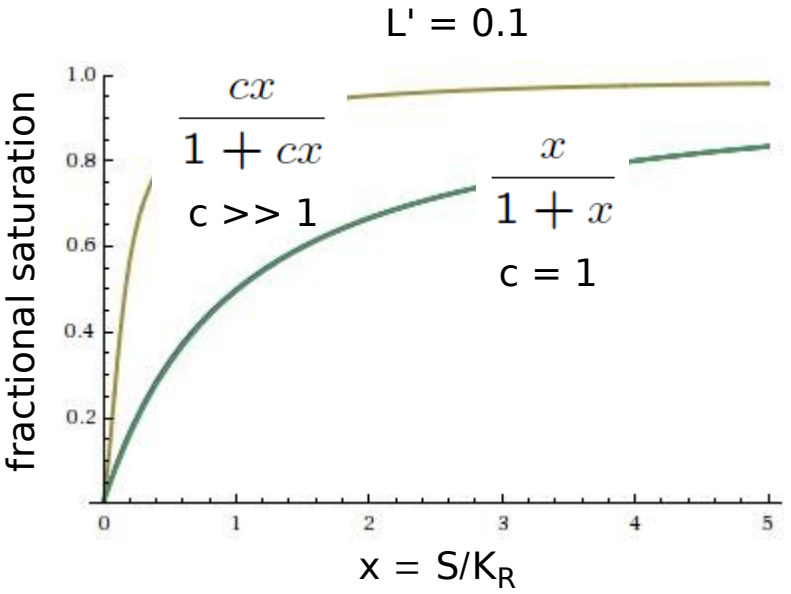
homotropic effects (substrate only)

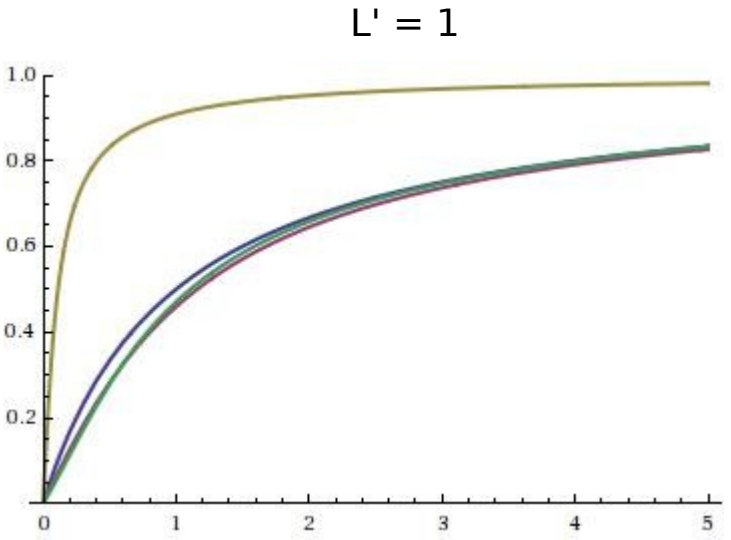
$$c = \frac{K_R}{K_T} \qquad L' = L = \frac{T_0}{R_0}$$

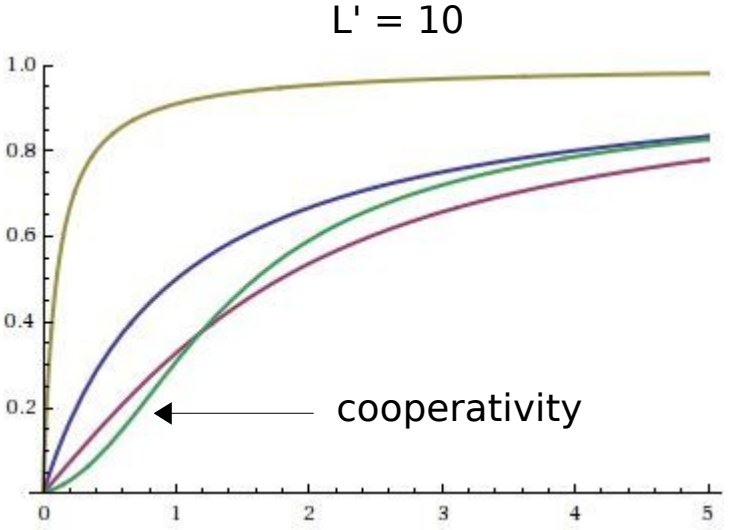
heterotropic effects (activator, A, binding only to R; inhibitor, I, binding only to T)

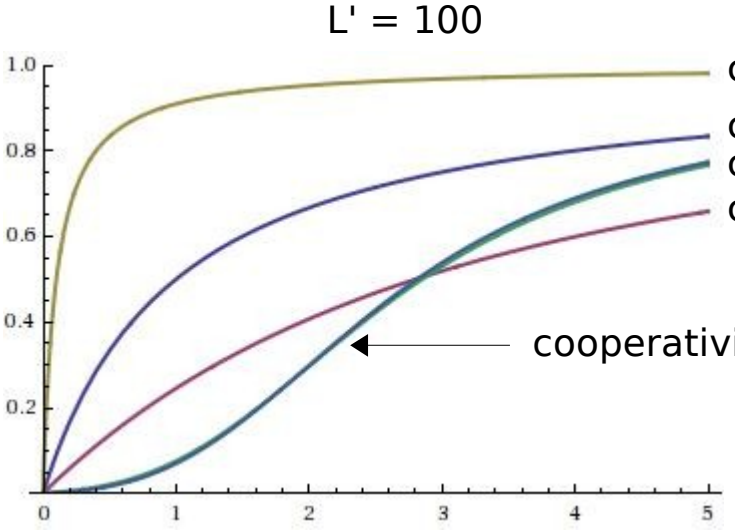
$$L' = \left(\frac{1+\beta}{1+\gamma}\right) L \qquad \beta = \frac{I}{K_I} \qquad \gamma = \frac{A}{K_A}$$

n = 4 sites, substrate only









c = 10
c = 1
c = 0.01, 0.001
c = 0.3

MWC cooperativity

requires an oligomer (n > 1)

the tense state favoured in the absence of ligand

$$L' = \frac{T_0}{R_0} \gg 1$$

and the ligand binding with higher affinity to the relaxed state

$$c = \frac{K_R}{K_T} \ll 1$$

when c << 1, the fractional saturation is $\frac{(1+x)^{n-1}x}{L'+(1+x)^n}$

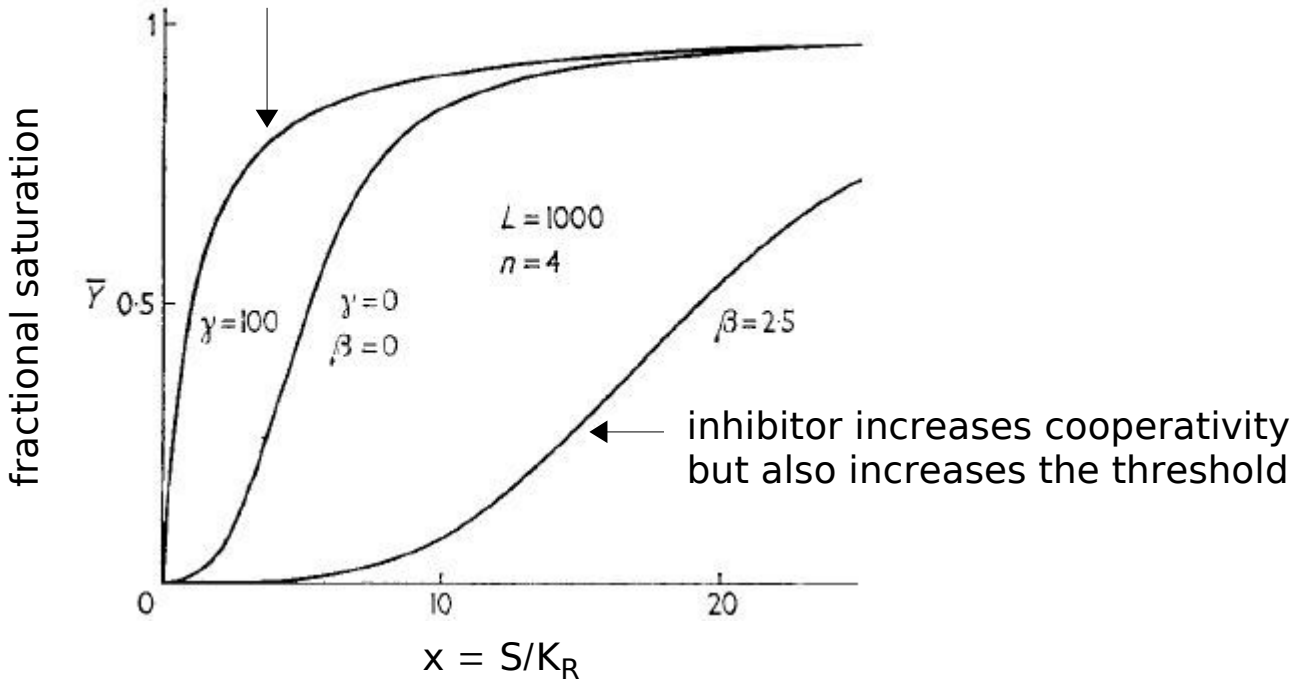
n = 4 sites

L'	$S_{0.9}$ / $S_{0.1}$	h
10	19.6	1.5
100	8.2	2.1

high cooperativity is limited by the number of monomers (sites)

n = 4 sites, activator & inhibitor

activator reduces cooperativity but also reduces the threshold

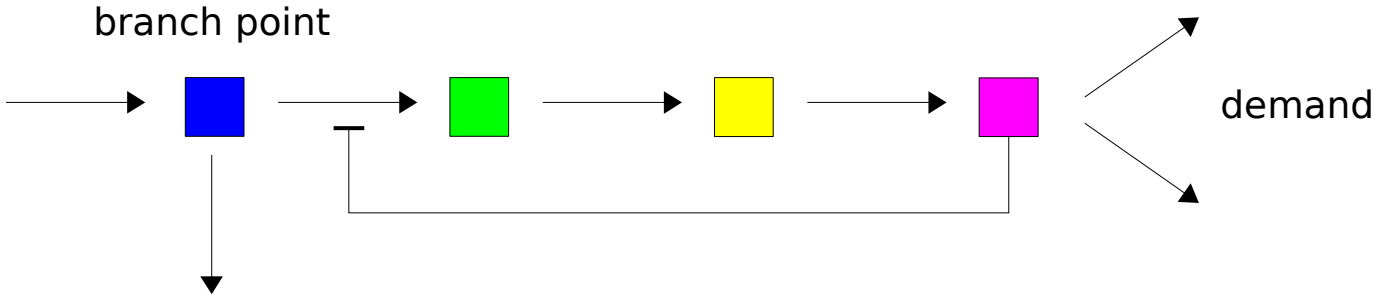


Monod, Wyman, Changeux, *"On the nature of allosteric transitions: a plausible model"*, J Mol Biol **12**:88-188 1965

Thomas Traut, **Allosteric Regulatory Enzymes**, Springer 2008

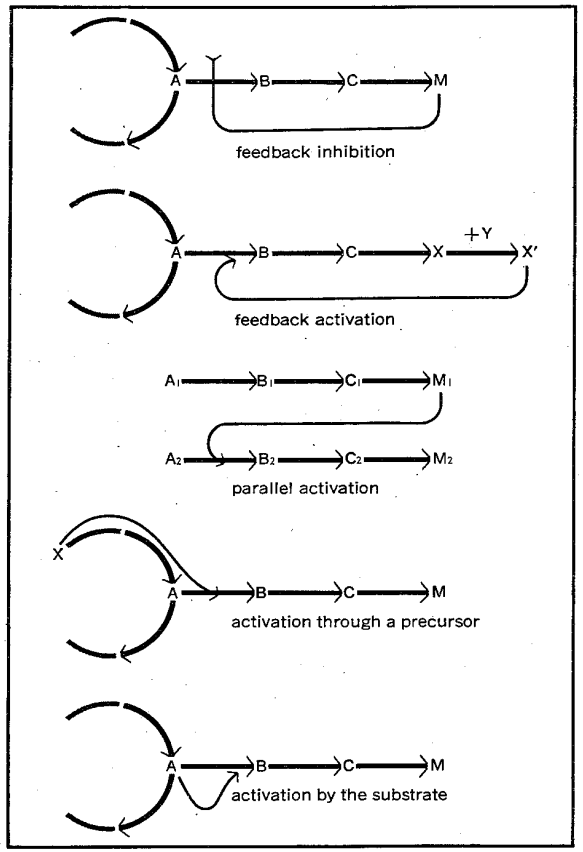
balancing supply & demand

how do allostery and cooperativity influence feedback inhibition?



how do they contribute to solving the metabolic paradox?

microscopic cybernetics (*)



Evidence for a Negative-Feedback Mechanism in the Biosynthesis of Isoleucine

Recent developments in automation have led to the use in industry of machines capable of performing operations that have been compared with certain types of human activity. In the internally regulated machine, as in the living organism, processes are controlled by one or more feedback loops that prevent any one phase of the process from being carried to a catastrophic extreme. The consequence of such feedback control can be observed at all levels of organization in a living animal—for example, proliferation of cells to form a definite structure, the maintenance of muscle tone, and such homeostatic mechanisms as temperature regulation and the maintenance of a relatively constant blood sugar level. Because of the complexity of so many biological systems, it is often difficult to postulate a mechanism on the molecular level that would serve in a regulatory function.

(*) Jacques Monod, **Chance and Necessity: on the Natural Philosophy of Modern Biology** Alfred Knopf, 1971 (French original, 1970); see Chapter 4

Edwin Umbarger, *"Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine"*, Science **123**:848 1956

still waiting for the revolution

unlike physiological homeostasis, metabolic regulation does not have a good analogy in engineering

balancing supply & demand is more analogous to what an economy does

> What corresponds in a nation to the internal environment of the body? The closest analogue appears to be the whole intricate system of production and distribution of merchandise, all the factors, human and mechanical, that produce and distribute goods in the vast circulatory system of exchange.

but very little effort has been expended on this perspective

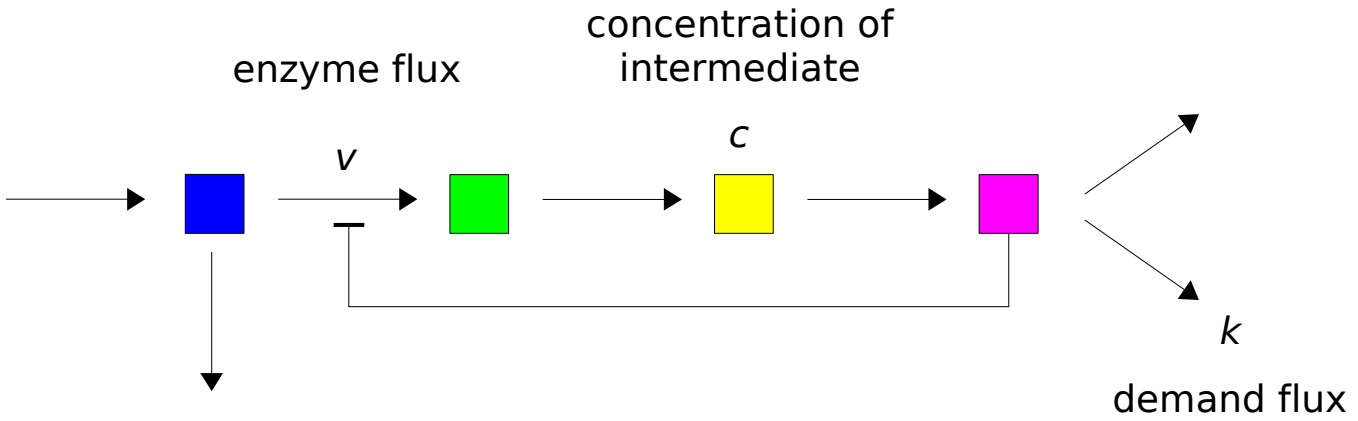
> How highly would one rate an economic analysis of a factory that ignored the consumer demand for its products? Ludicrous as it may sound, this is precisely what most metabolic studies of the past century have been doing.

Walter B Cannon, *"The body physiologic and the body politic"*, Science, **93**:1 1941, reprinted in Scientific Monthly, **79**:20-6 1954

Hofmeyr, Cornish-Bowden, *"Regulating the cellular economy of supply and demand"*, FEBS Letters, **476**:47-51 2000

classical systems approaches

Metabolic Control Analysis (MCA) & Biochemical Systems Analysis (MCA)



steady-state sensitivity analysis using logarithmic sensitivity coefficients

$$\frac{\partial \log v}{\partial \log k} = \left(\frac{k}{v}\right)\frac{\partial v}{\partial k} \qquad \frac{\partial \log c}{\partial \log k} = \left(\frac{k}{c}\right)\frac{\partial c}{\partial k}$$

flux control coefficient — concentration control coefficient

Savageau, *"Parameter sensitivity as a criterion for evaluating and comparing the performance of biochemical systems"*, Nature, **229**:542-4 1971

for references, see JG, *"Notes on metabolic control theory"*, http://vcp.med.harvard.edu/papers/

classical systems approaches

MCA tried to replace the “rate-limiting step” with the idea that rate control may be widely distributed across many steps

MCA provided a widely-used explanation for the evolution of dominance

> mutant would therefore be described as “recessive”. The widespread occurrence of recessive mutants is thus seen to be the inevitable consequence of the kinetic structure of enzyme networks. The *ad hoc* hypothesis of “modifiers” selected to

BSA was used to understand optimality and the dynamical aspects of feedback regulation

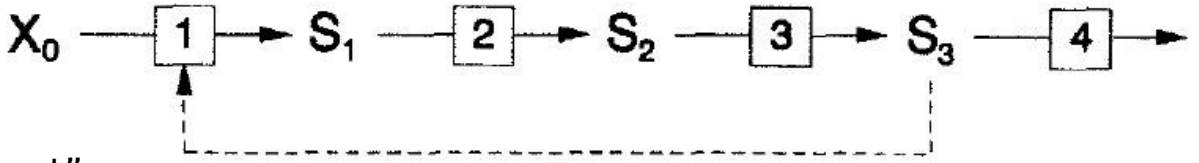
a bitter argument over priority did not help wider acceptance

David Fell, **Understanding the Control of Metabolism**, Portland Press, 1997

Kacser, Burns, *“The evolution of dominance”*, Genetics, **97**:639-66 1981

Savageau, *“Optimal design of feedback control by inhibition: dynamic considerations”*, J Mol Evol, **5**:199-222 1975

balancing supply & demand



X_0 clamped

"Hill coefficient"

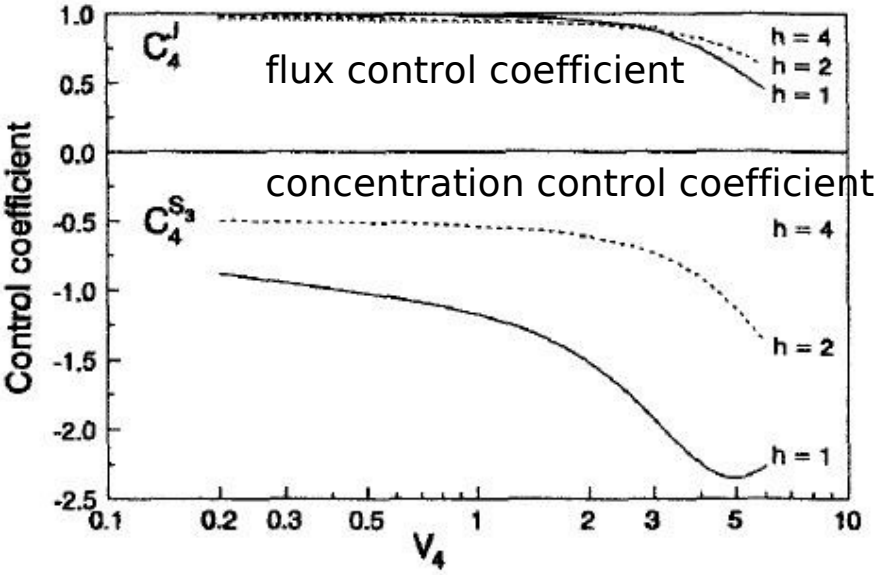
$$v_1 = \frac{10X_0 - S_1}{1 + X_0 + S_1 + S_3^h}$$

$$v_2 = \frac{10S_1 - 2S_2}{1 + S_1 + S_2}$$

$$v_3 = \frac{10S_2 - 2S_3}{1 + S_2 + S_3}$$

$$v_4 = \frac{V_4 \cdot S_3}{1 + S_3}.$$

V_4 varied



feedback inhibition gives control of steady-state flux to the demand

cooperativity helps keep substrate concentrations constant

Hofmeyr, Cornish-Bowden, *"Quantitative assessment of regulation in metabolic systems"*, Eur J Biochem, **200**:223-36 1991