a systems approach to biology

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lecture 7
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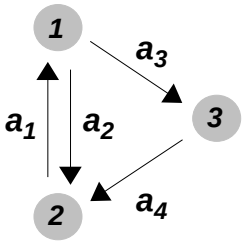
3. time-scale separation & the linear framework, continued

recap



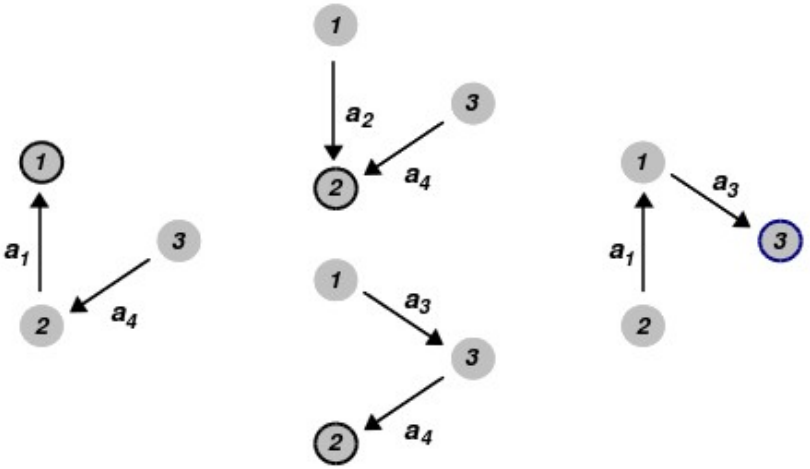
$$\frac{dx}{dt} = \mathcal{L}(G).x$$

if the graph is **strongly connected**, then the Laplacian dynamics has only a single steady state, up to a scalar multiple

$$\ker \mathcal{L}(G) = \langle \rho \rangle$$

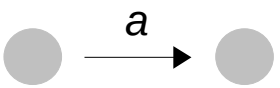
Tutte's **matrix-tree theorem**

$$\rho_i = \sum_{T \in \Theta_i(G)} \left(\prod_{j \xrightarrow{a} k \in T} a \right)$$



nonlinearity is encoded in the labels

labels are allowed to be complex algebraic expressions, involving rate constants and concentrations



$$a = \left(\frac{k_1[X_1] + k_2[X_2]}{k_3} \right) [X_3]$$

uncoupling condition: no concentration appearing in a label can be that of a vertex in the graph

$$\frac{dx}{dt} = f(x;k)$$

nonlinear system with simple rate constants

$$\frac{dx}{dt} = \mathcal{L}(G).x$$

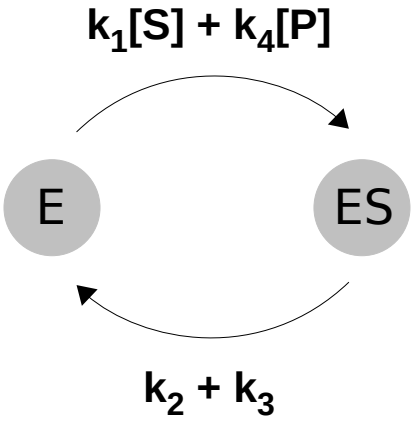
linear system with complex algebraic labels

reversible michaelis-menten

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

the vertices of the labelled directed graph are the enzyme forms

the labels are the aggregate rates of inter-conversion between the forms, with the slow variables, S and P, treated as constants



reversible michaelis-menten

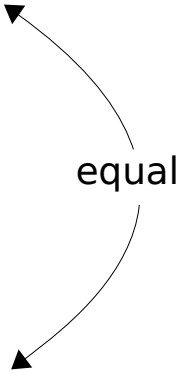
Laplacian dynamics

$$\frac{d}{dt}\begin{pmatrix}[E]\\[ES]\end{pmatrix} = \begin{pmatrix}-(k_1[S]+k_4[P]) & k_2+k_3\\ k_1[S]+k_4[P] & -(k_2+k_3)\end{pmatrix}\begin{pmatrix}[E]\\[ES]\end{pmatrix}$$

steady state of ES according to Laplacian dynamics

$$(k_1[S]+k_4[P])[E]-(k_2+k_3)[ES]=0$$

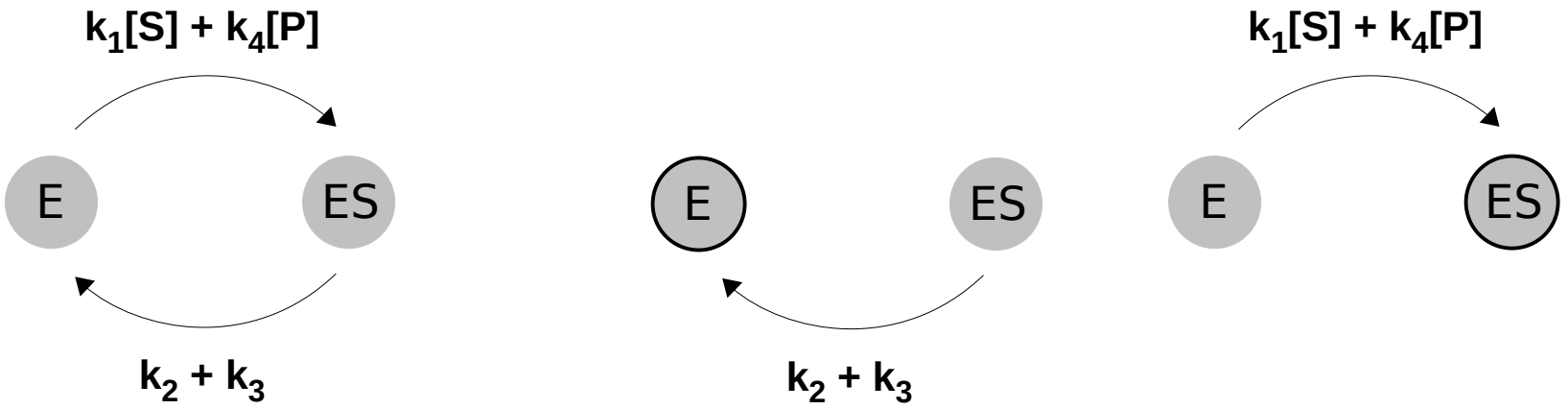
steady state of ES according to the full nonlinear dynamics



$$\frac{d[ES]}{dt} = (k_2+k_3)[ES]-k_1[E][S]-k_4[E][P]=0$$

reversible michaelis-menten

spanning trees



$$\rho = \begin{pmatrix} k_2 + k_3 \\ k_1[S] + k_4[P] \end{pmatrix}$$

elimination

$$[ES] = \left(\frac{k_1[S] + k_4[P]}{k_2 + k_3 + k_1[S] + k_4[P]} \right) E_{tot} \qquad [E] = \left(\frac{k_2 + k_3}{k_2 + k_3 + k_1[S] + k_4[P]} \right) E_{tot}$$

reversible michaelis-menten

rate formula

$$\frac{d[P]}{dt} = k_3[ES] - k_4[E][P]$$

$$\frac{d[P]}{dt} = \left(\frac{V_f[S]/K_f - V_r[P]/K_r}{1 + [S]/K_f + [P]/K_r} \right) V_{max}$$

$$V_f = k_3 E_{tot} \quad V_r = k_2 E_{tot} \qquad K_f = \frac{k_2 + k_3}{k_1} \quad K_r = \frac{k_2 + k_3}{k_4}$$

forward & reverse maxmial rates

forward & reverse michaelis-menten constants

Athel Cornish-Bowden, **Fundamentals of Enzyme Kinetics**, 2nd edition, Portland Press, 2001

thermodynamic equilibrium

Haldane relationship

$$\frac{[P]_{eq}}{[S]_{eq}} = \frac{V_f K_r}{V_r K_f} = \left(\frac{k_3}{K_f}\right)\left(\frac{k_2}{K_r}\right)^{-1}$$

set by thermodynamics

forward & reverse catalytic efficiencies

a high ratio of maximal velocities, V_f / V_r, must be compensated by the ratio of the M-M constants, K_f / K_r

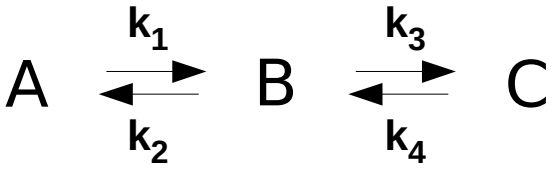
dissociation constants are bounded by the corresponding M-M constants

$$K_{D,S} = \frac{k_2}{k_1} \leq \frac{k_2 + k_3}{k_1} = K_f \qquad K_{D,P} = \frac{k_3}{k_4} \leq \frac{k_2 + k_3}{k_4} = K_r$$

an enzyme with a low K_r is more readily inhibited by its product

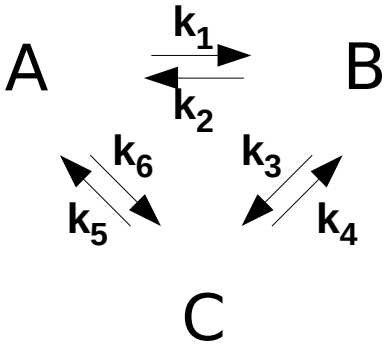
J B S Haldane, **Enzymes**, Longmans, 1930 (reprinted by MIT press in 1965)

detailed balance



at steady state each reversible reaction is separately at steady state

$$k_1[A] = k_2[B] \qquad k_3[B] = k_4[C]$$



at steady state each there may be net flux around the cycle

$$k_1[A] - k_2[B] = k_3[B] - k_4[C] = k_5[C] - k_6[A] \neq 0$$

detailed balance

at **thermodynamic equilibrium**, every reaction is reversible and each pair of reversible reactions is individually at equilibrium.

no net flux around a cycle

detailed balance is a consequence of microscopic reversibility

microscopic reversibility: the fundamental laws of physics, whether classical newtonian mechanics or quantum mechanics, exhibit time-reversal symmetry

Gilbert Lewis, *"A new principle of equilibrium"*, PNAS **11**:179-83 1925

Bruce Mahan, *"Microscopic reversibility and detailed balance"*, J Chem Edu **52**:299-302 1975

detailed balance

detailed balance imposes a constraint on the rate constants, whenever a system can achieve thermodynamic equilibrium

$$k_1[A] - k_2[B] = k_3[B] - k_4[C] = k_5[C] - k_6[A] = 0$$

$$[A] = \left(\frac{k_2}{k_1}\right)[B] = \left(\frac{k_2}{k_1}\right)\left(\frac{k_4}{k_3}\right)[C] = \left(\frac{k_2}{k_1}\right)\left(\frac{k_4}{k_3}\right)\left(\frac{k_6}{k_5}\right)[A]$$

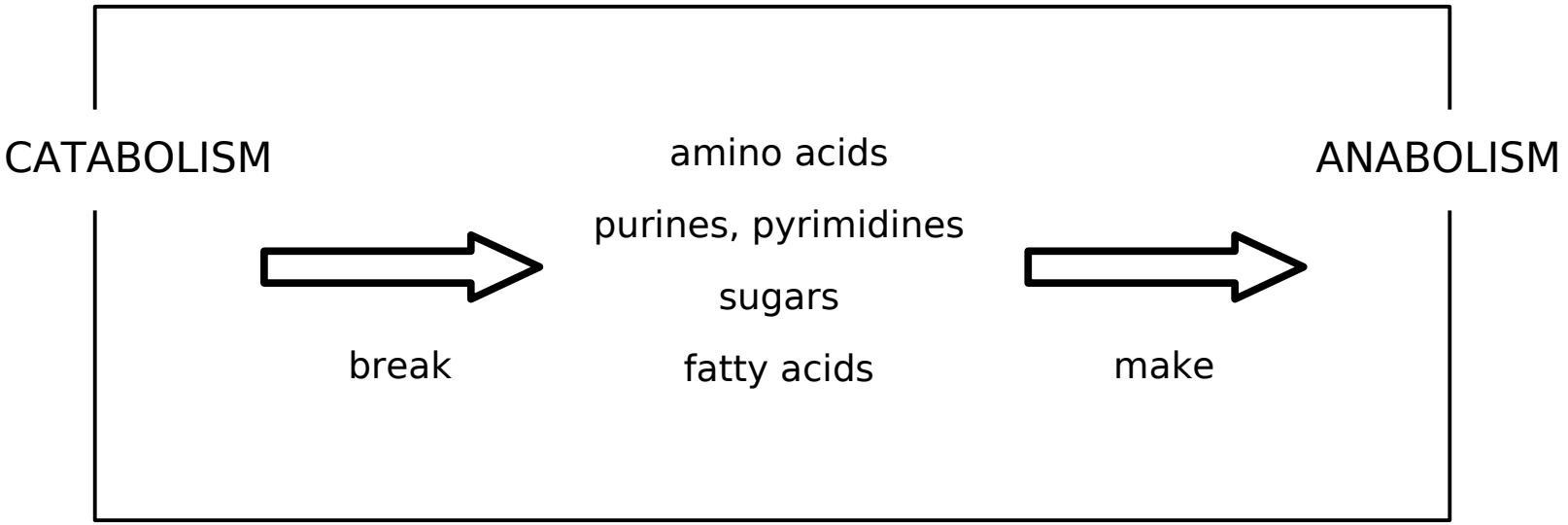
$$k_1 k_3 k_5 = k_2 k_4 k_6$$

***cycle condition**: the product of the rate constants in the clockwise direction equals the product in the counter-clockwise direction*

4. metabolism – balancing supply & demand

we are not what we eat

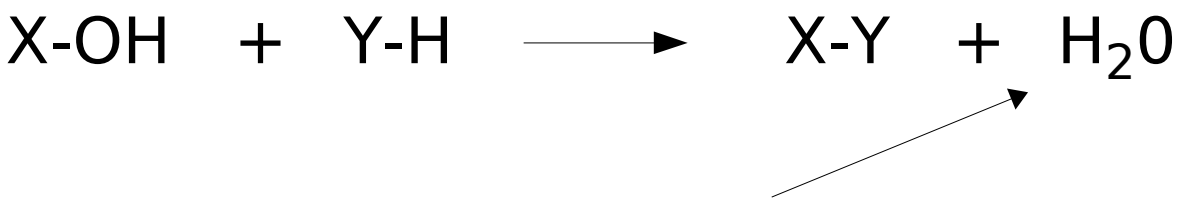
organisms like us break down complex bio-polymers acquired from other organisms and make new bio-polymers for own own use from the parts



another form of weak linkage?

this has biochemical implications

synthesis of biopolymers requires dehydrating condensations

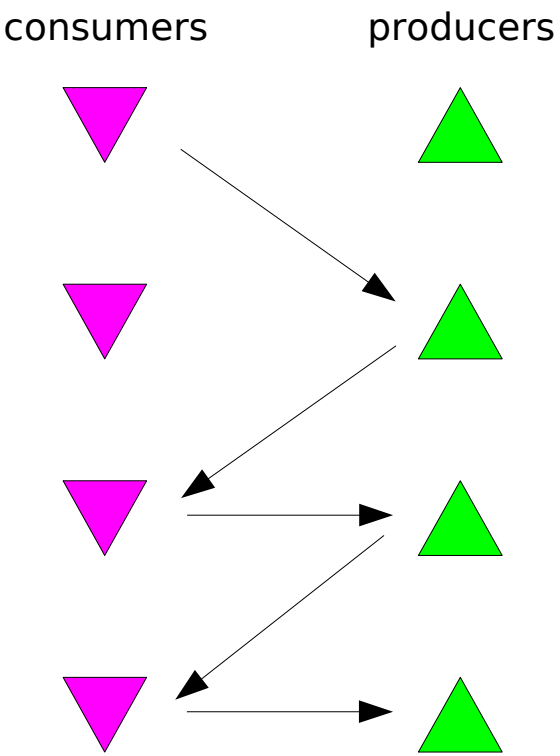


thermodynamically unfavourable in an aqueous environment

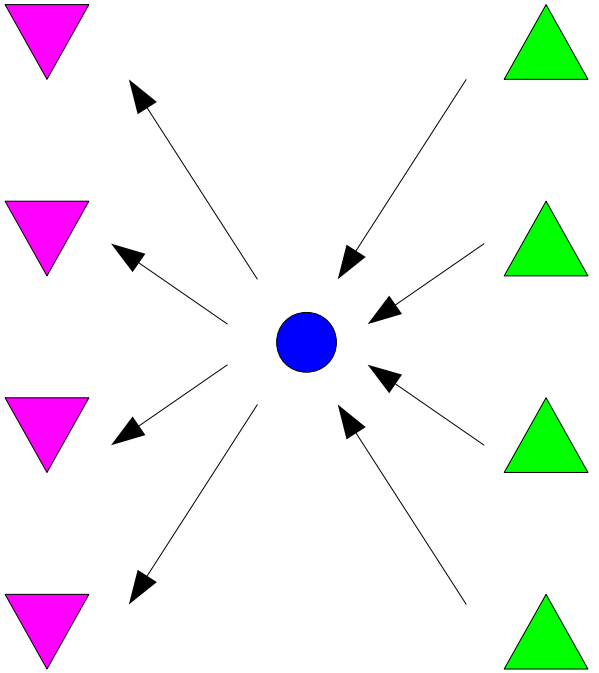
where does the energy come from?

energy consuming reactions have to be coupled to energy producing reactions

weak linkage, again



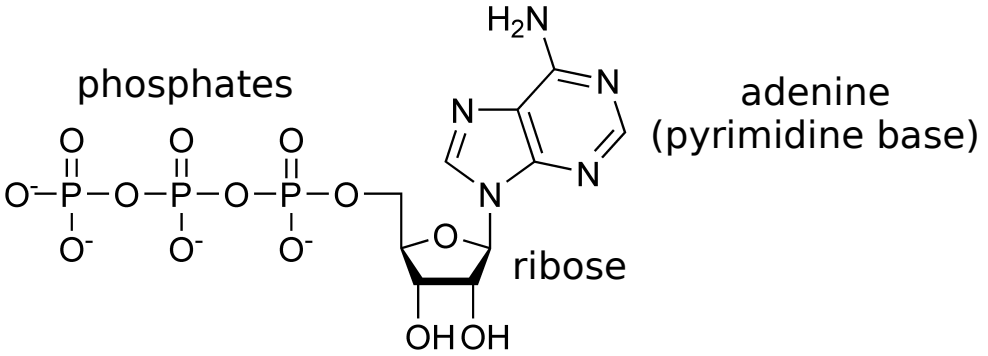
“barter economy”



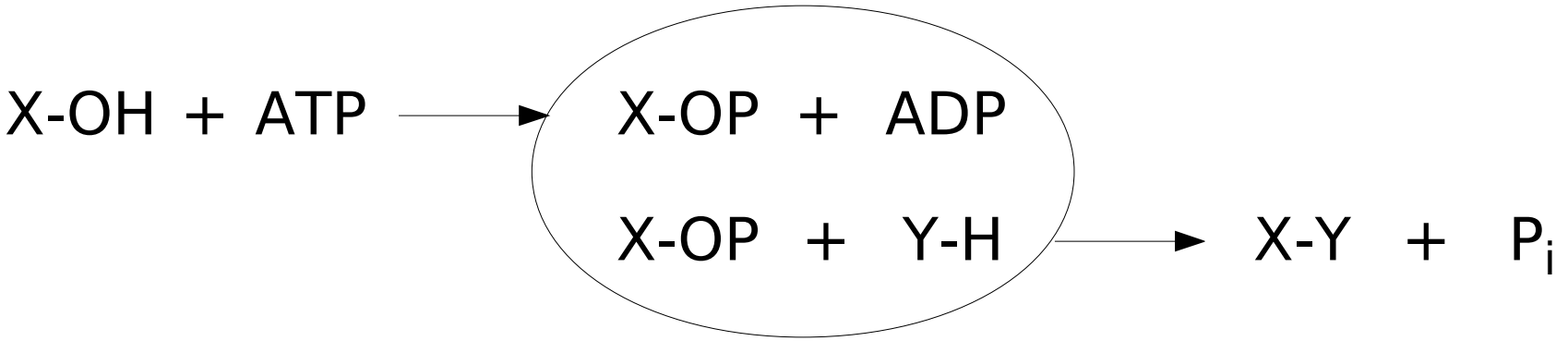
“invention of money”

universal energy currency

ATP

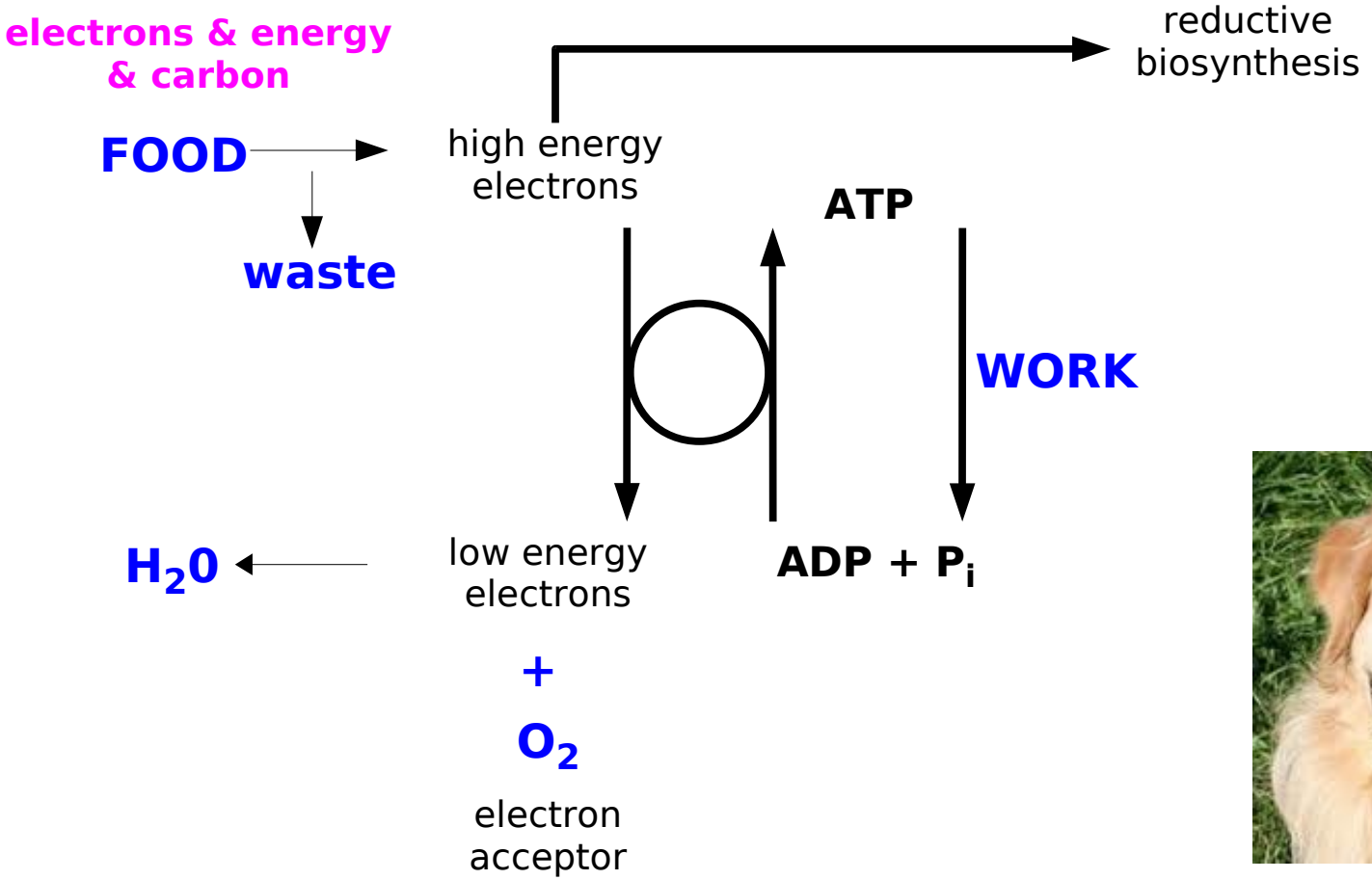


group transfer reactions do the coupling



ATP is generated by redox chemistry

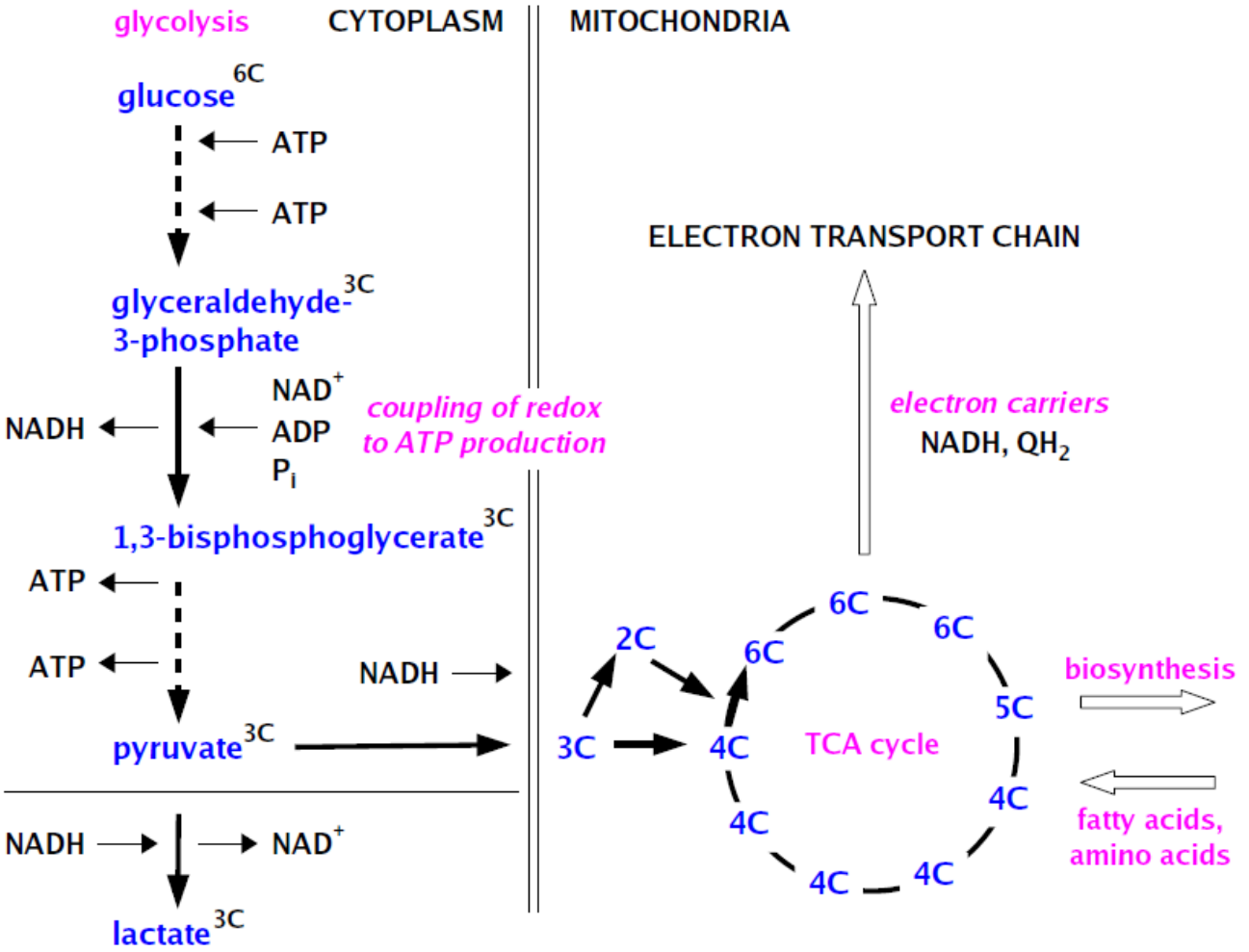
aerobic heterotroph



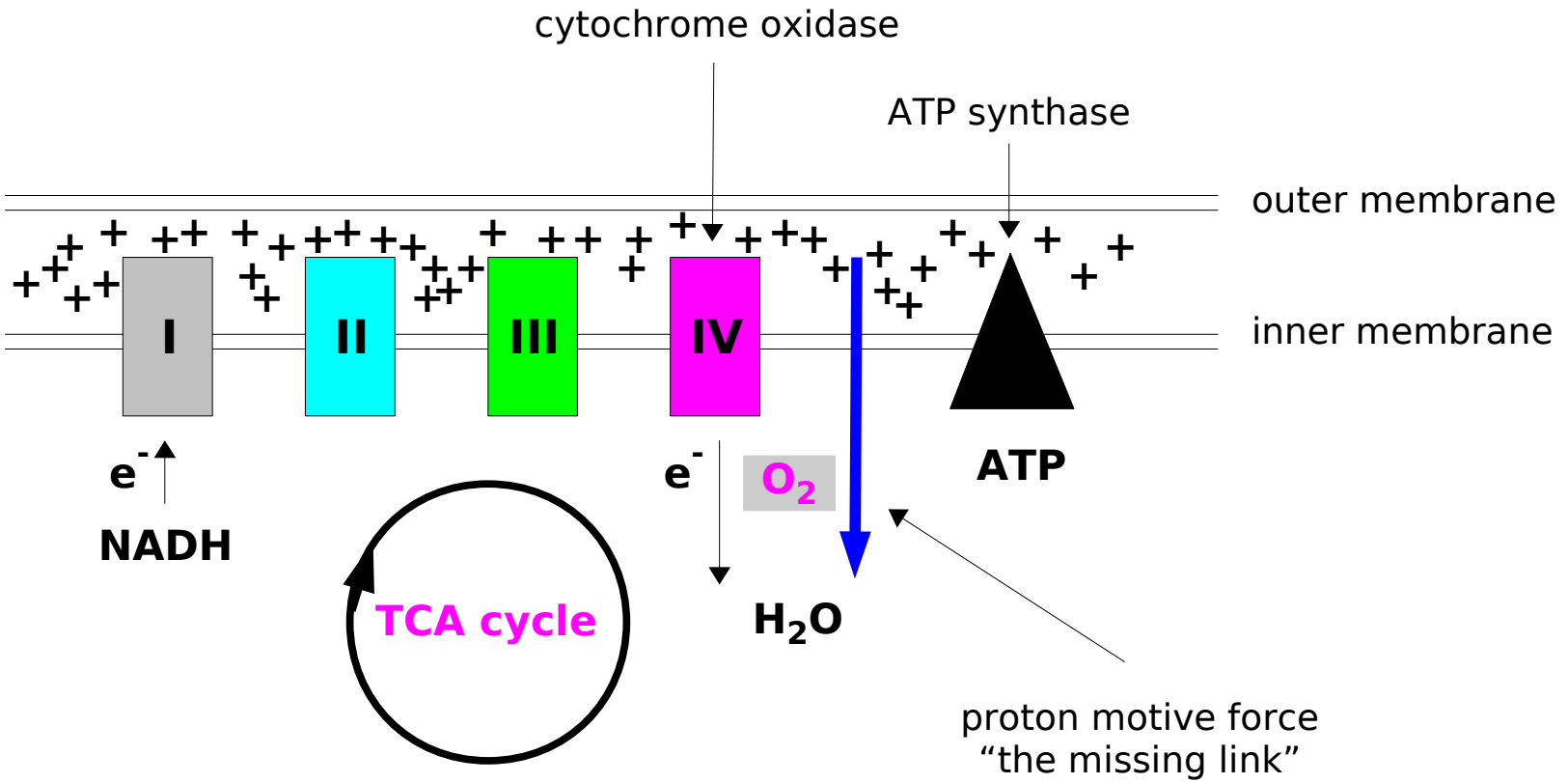
omnivore

glycolysis & tca cycle

2 ATP / glucose – fast but energy inefficient

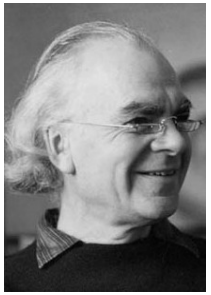


oxidative phosphorylation



1887-1963

1883-1970

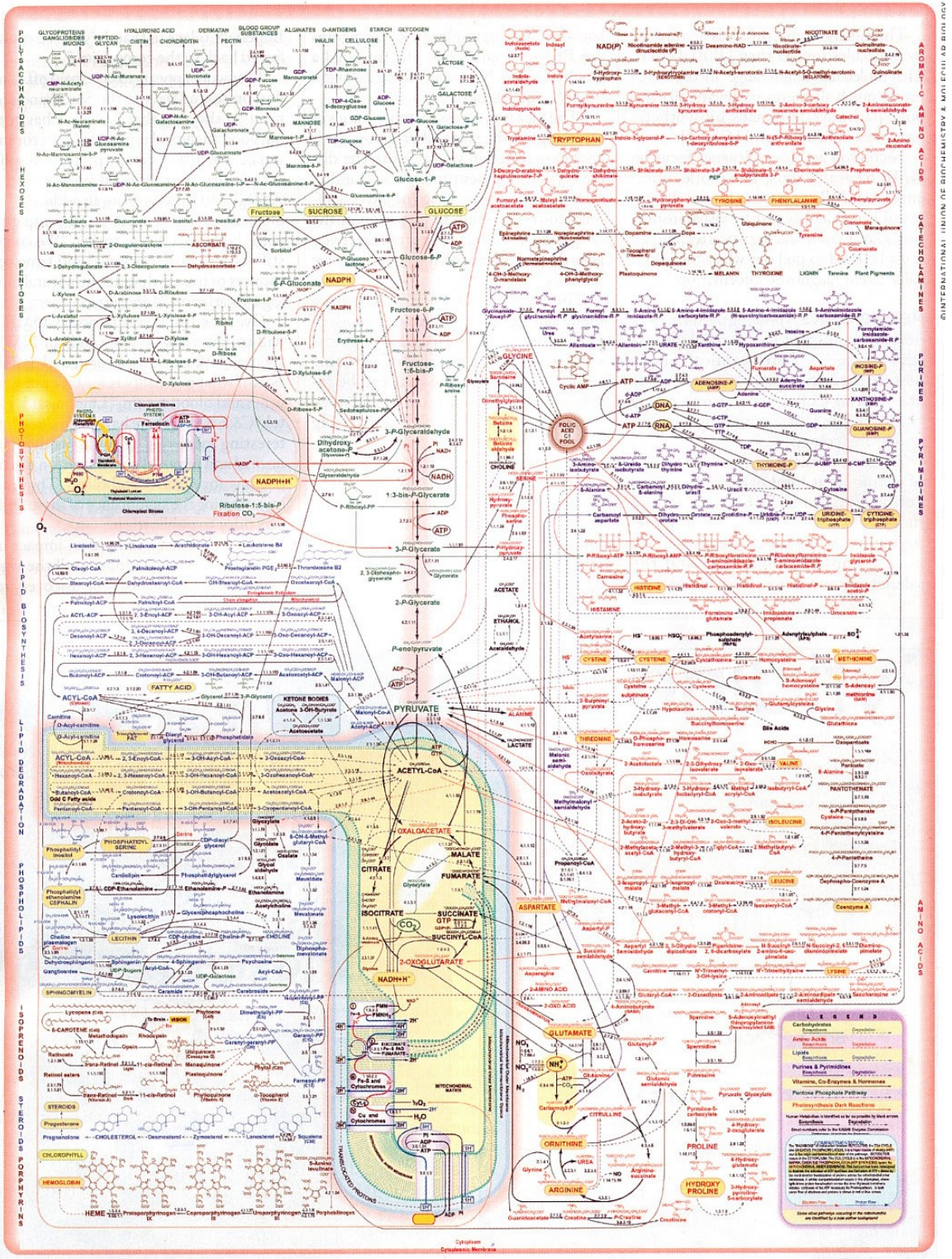


1920-1992

32 ATP / glucose – energy efficient but slow

metabolism

all worked out ...



or not?

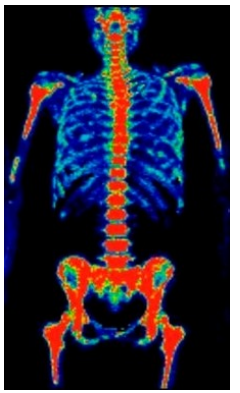
metabolic systems must balance nutrient supply against demand for energy & materials, although each may change independently on different time scales

metabolic paradox: flux can change by 10-100 fold, while concentrations of intermediates only change 3 fold

how do cells regulate metabolism to accomplish this?

Warburg effect: cancers use glycolysis, in preference to ox-phos, even when well supplied with oxygen

how (and why) do cells make the switch?



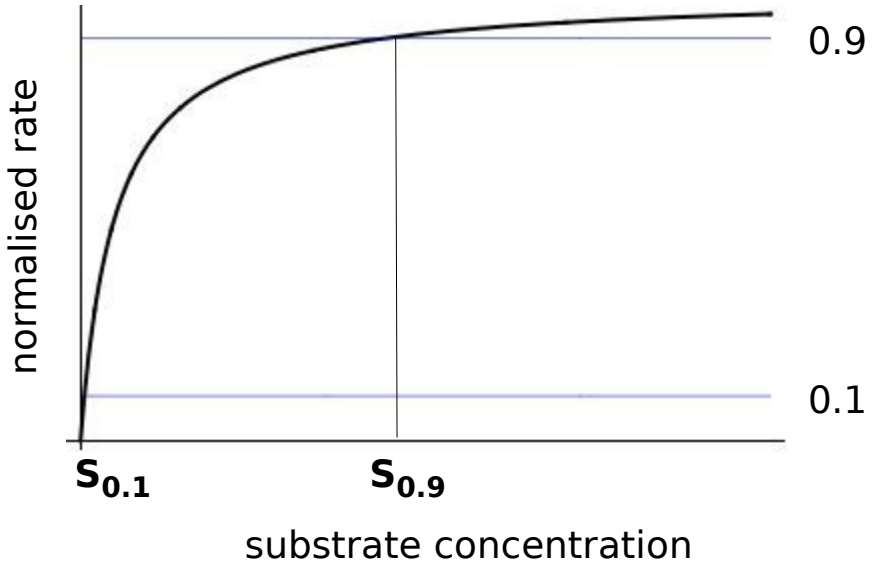
^{18}F-FDG PET scan

Hochachka, McClelland, *"Cellular metabolic homeostasis during large-scale changes in ATP turnover rates in muscles"*, J Exp Biol **200**:381-6 1997

Matt Vander Heiden, Lew Cantley, Craig Thompson, *"Understanding the Warburg effect: the requirements of cell proliferation"*, Science **324**:1029-33 2009

regulation of metabolism

standard Michaelis-Menten kinetics has poor sensitivity



normalised rate

$$(V_{max})^{-1}\left(\frac{dP}{dt}\right) = \frac{[S]}{K_M + [S]}$$

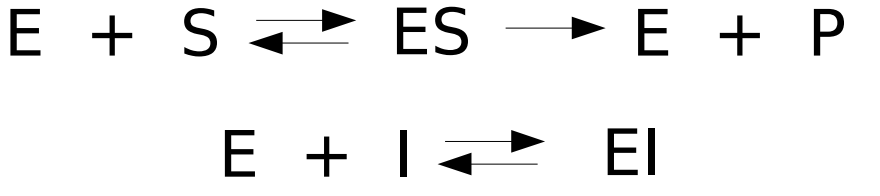
$$\frac{S_{0.9}}{S_{0.1}} = 81$$

cooperativity index (CI)

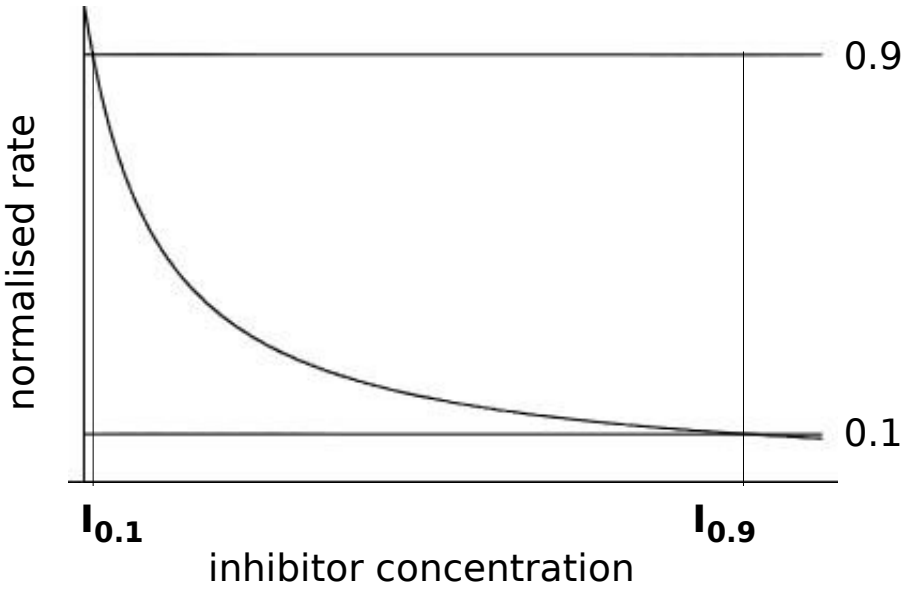
substrate concentration must increase 81 fold, to cause a 9-fold increase in flux

regulation of metabolism

similar insensitivity is found for competitive inhibition



$$\frac{dP}{dt} = \frac{V_{max}[S]}{K_M(1 + [I]/K_I) + [S]}$$



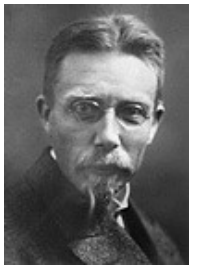
normalised rate

$$\left(\frac{V_{max}[S]}{K_M + [S]}\right)^{-1} \left(\frac{dP}{dt}\right)$$

$$\frac{I_{0.9}}{I_{0.1}} = \frac{1}{81}$$

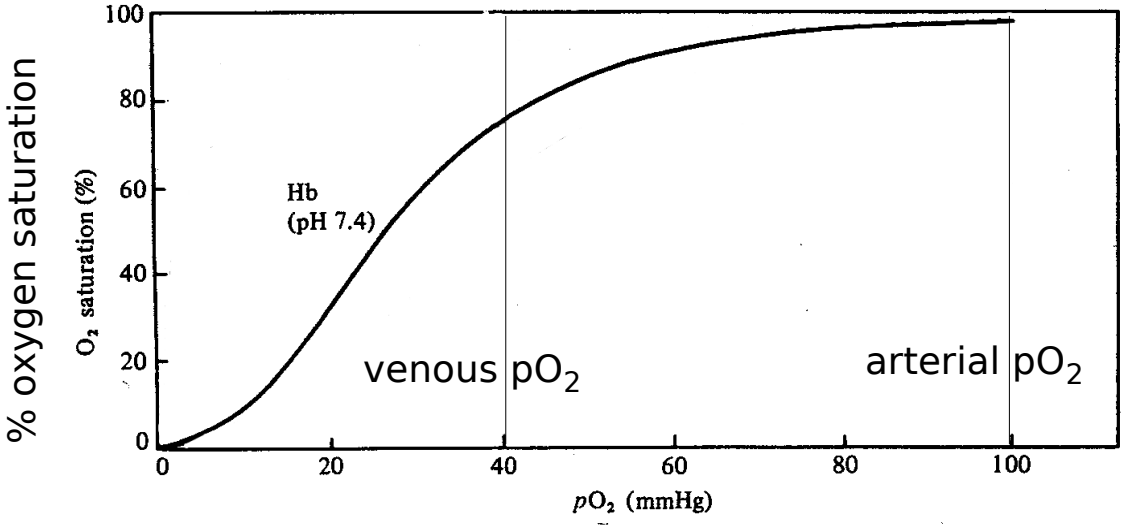
cooperativity

some proteins have a **sigmoidal** response



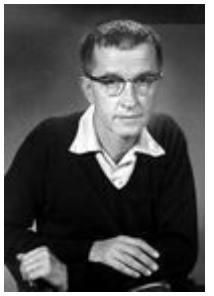
1874-1949

1855-1911



partial pressure of oxygen

1885-1962

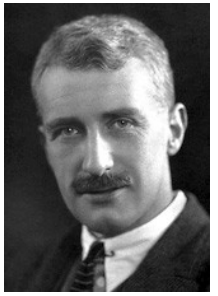


1906-1981

Christian Bohr, Boris Hasselbach, August Krogh, Skand Arch Physiol **16**:401-12 1904

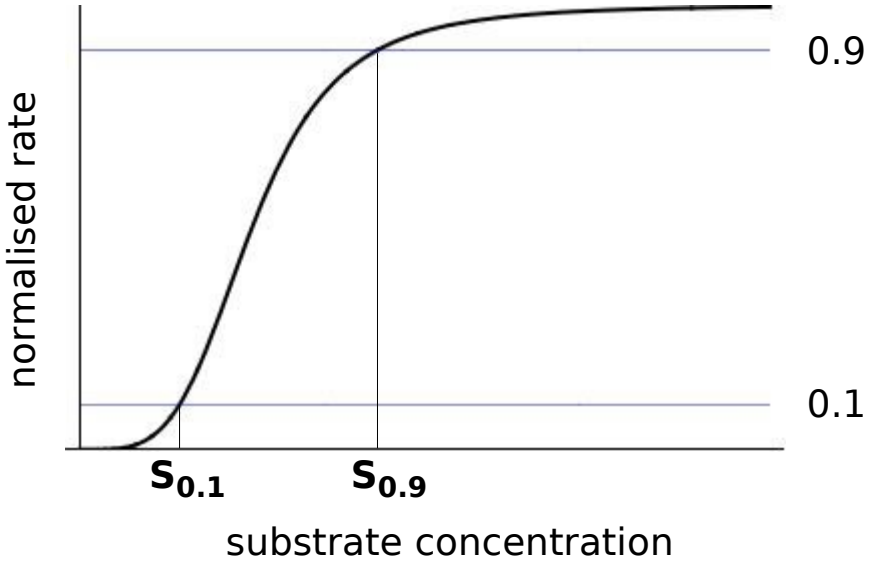
cooperativity index

Hill introduced a family of sigmoidal functions as an illustrative approximation for oxygen binding to hemoglobin



1886-1977

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

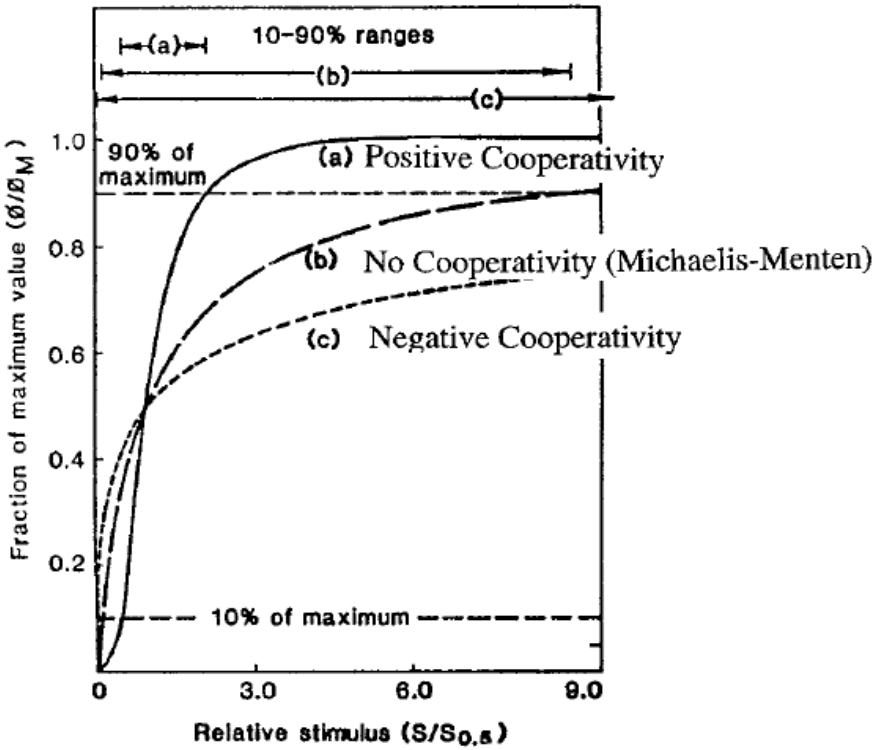


$$\frac{S_{0.9}}{S_{0.1}} = 81^{1/h}$$

Hill functions are widely used but have no mechanistic or biochemical significance

positive and negative cooperativity

positive cooperativity increases sensitivity; negative cooperativity increases range



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

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

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

Koshland, Hamidani, *"Proteomics and models for enzyme cooperativity"*, J Biol Chem **227**:46841-4 2002

Kolodziej, Tan, Koshland, *"Producing positive, negative and no cooperativity by mutations at a single residue at the subunit interface in the aspartate receptor of Salmonella typhimurium"*, Biochem **35**:14782-92 1996