10.555
Bioinformatics: Principles, Methods and Applications

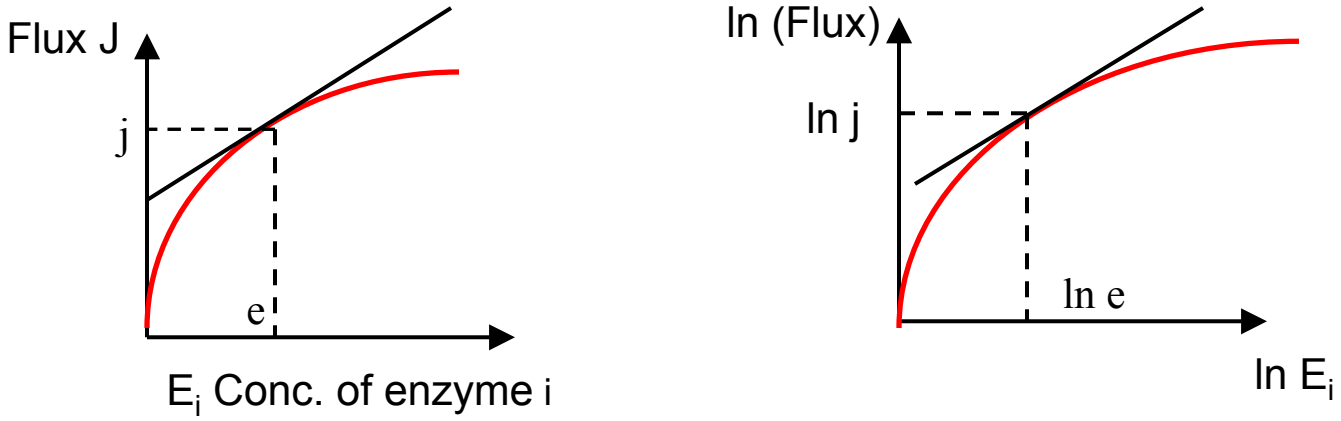
MIT, Spring term, 2003

Lecture 9:

- **Metabolic Control Analysis (MCA), or, Distribution of Kinetic Control**

Flux Control Coefficients (FCC)

- In a bioreaction network, how does the flux of a reaction J depend on the concentration of an enzyme E_i ?



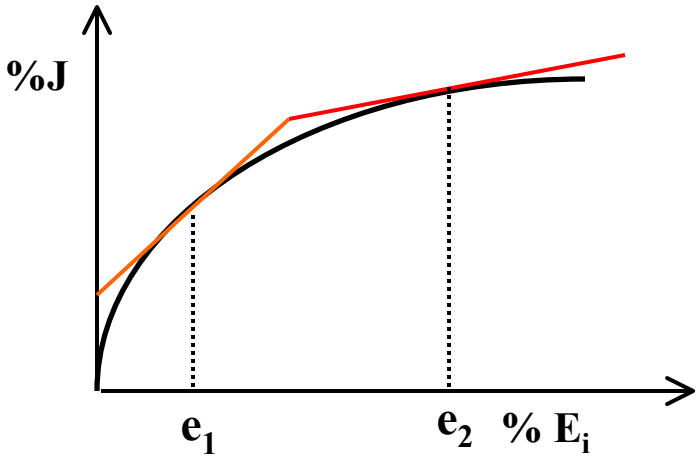
- Define: Flux Control Coefficient of flux J wrt enzyme E_i at (e,j)

$$C_{Ei} = \left(\frac{\partial J}{\partial E_i}\right)_{e,\,j} \frac{e}{j} = \frac{\partial(\ln J)}{\partial(\ln E_i)_{e,j}} = \frac{\delta J / J}{\delta E_i / E_i}$$

Flux Control Coefficients (FCC)

- FCCs extend the concept of the Rate Limiting Step
  - Obtained in the limit as $\delta E \rightarrow 0$
  - Independent of units
  - Defined at a steady state

  (different at different conditions, such as enzymes, environments)



Summation theorem

$$\sum_i C_{Ei}^J = 1$$

- Distribution of Kinetic Control vs. RLS
  - Kinetic control is shared but it is not shared evenly

Interpretation of FCC

- Think of a change in enzyme concentration small enough so that C_{Ei}^{J} is effectively constant:

$$C_{Ei}^{J} = \frac{\partial(\ln J)}{\partial(\ln E_i)} \approx \frac{\Delta \ln J}{\Delta \ln E_i} \Rightarrow \Delta \ln J = C_{Ei}^{J} \Delta \ln E_i$$

$$\therefore J = aE^{c}$$

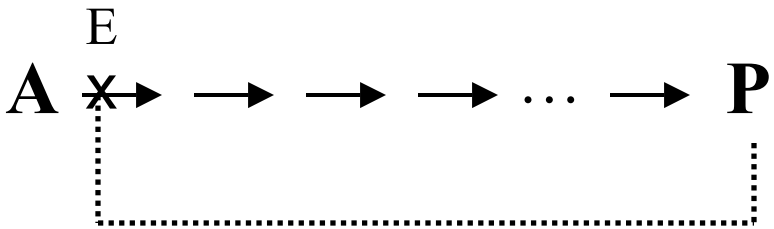
Local Power Law. Better approximation than:

$$\frac{\Delta J}{J} = C_{Ei}^{J} \frac{\Delta E_i}{E_i}$$

- FCC may take positive or negative values (what pathways?)
- FCC may take values greater than 1
- Most enzymes have small FCC. Diploids
- Interpretation of dominant vs. recessive genes in hetero-zygotes

Control Versus Regulation

Sensitivity of flux to enzymatic activity is not a measure of whether that enzyme is a control or a regulatory enzyme



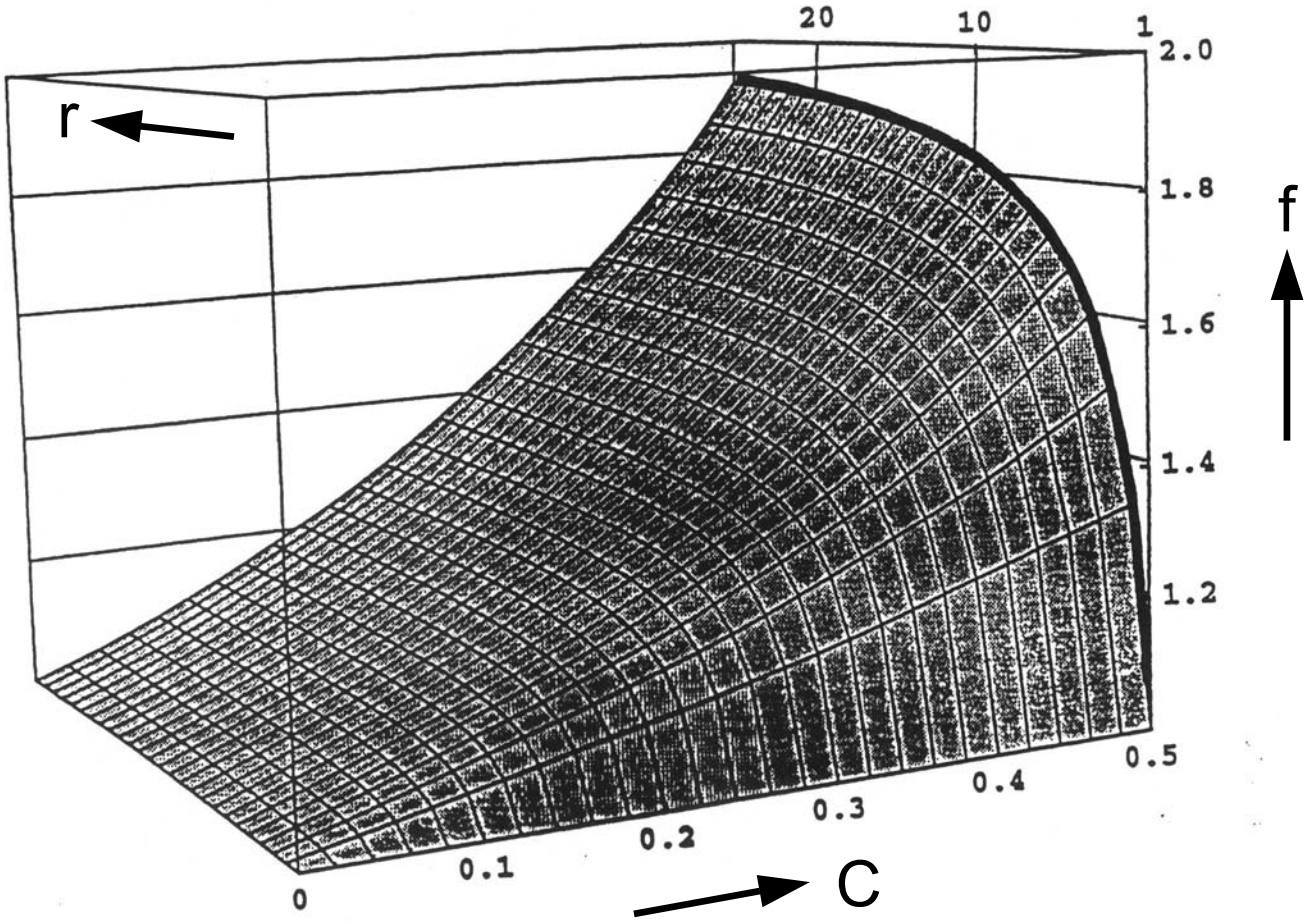
E is a regulatory enzyme but usually has small FCC. Why?

The implication that a regulatory enzyme is also the "rate limiting step" is nonsense

- Compare FCCs of enzymatic steps in the same pathway
- Never compare FCCs of different pathways

Predicting Flux Changes (cont'd)

C= FCC, r=amplification factor, f=flux amplification



Predicting Flux Changes (cont'd)

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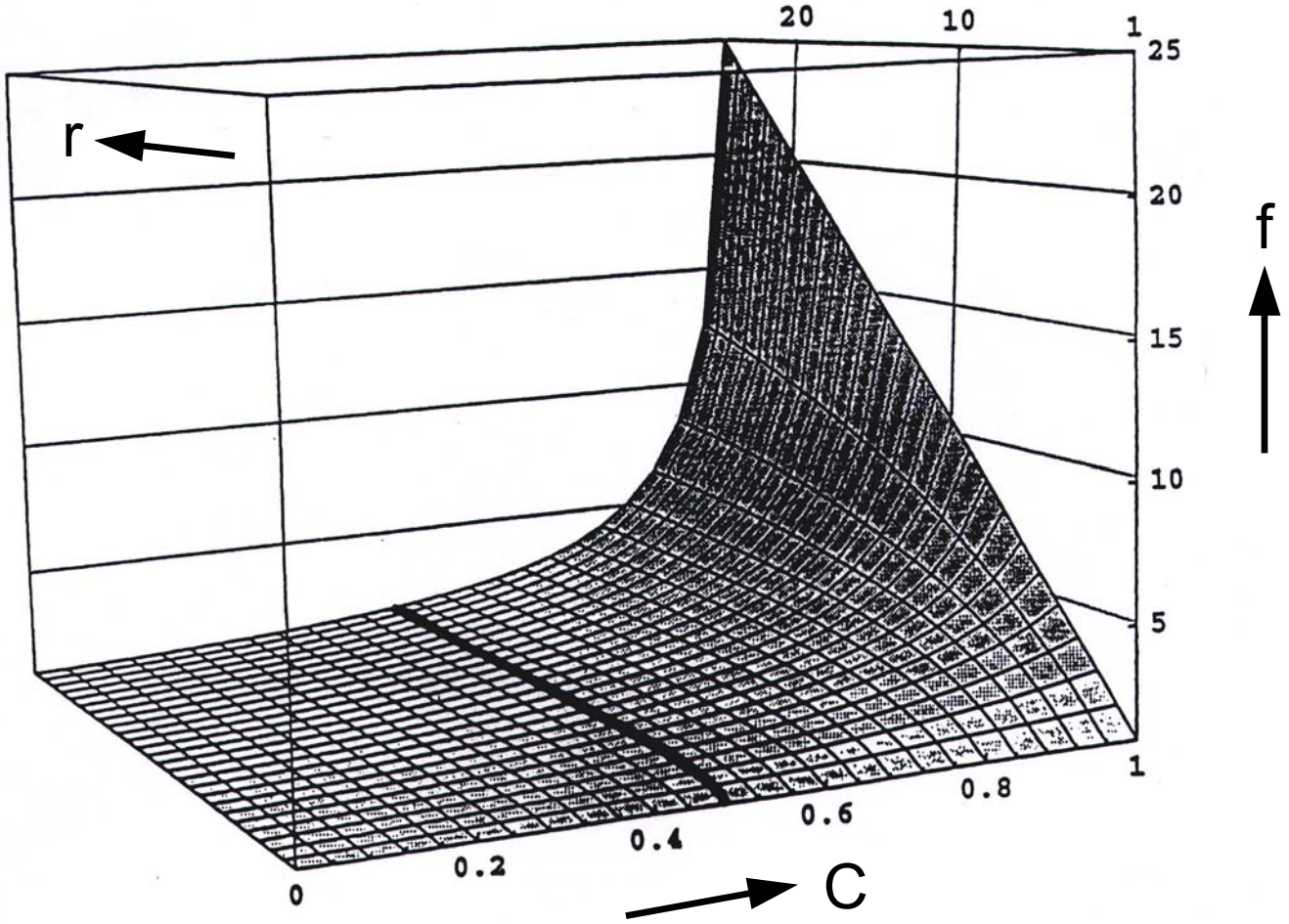
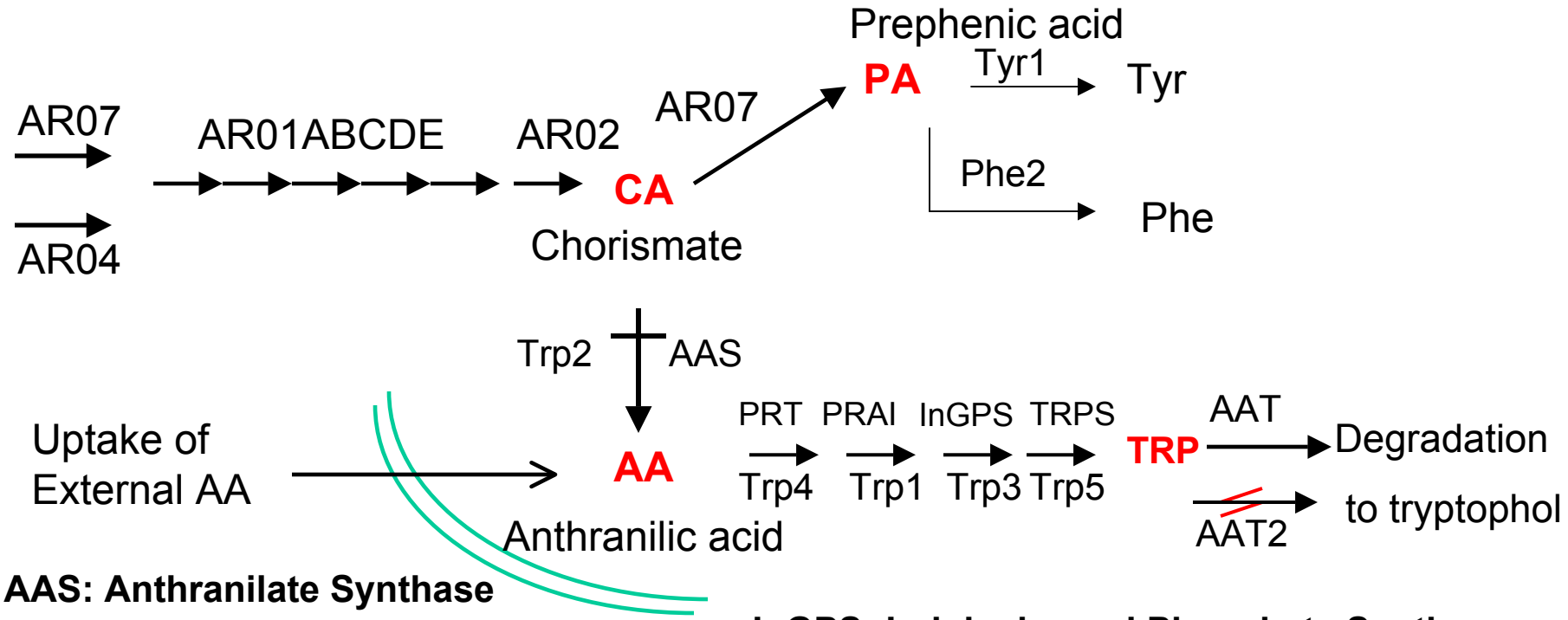


Distribution of kinetic control: Motivation

Aromatic aminoacid biosynthesis: (P. Niederberger et al., *Biochem J.* 287:473 (1992)



AAS: Anthranilate Synthase

PRT: AA Phosphorybosyl transferase

PRAI: Phosphorybosyl anthranilate isomerase

InGPS: Indoleglycerol Phosphate Synthase

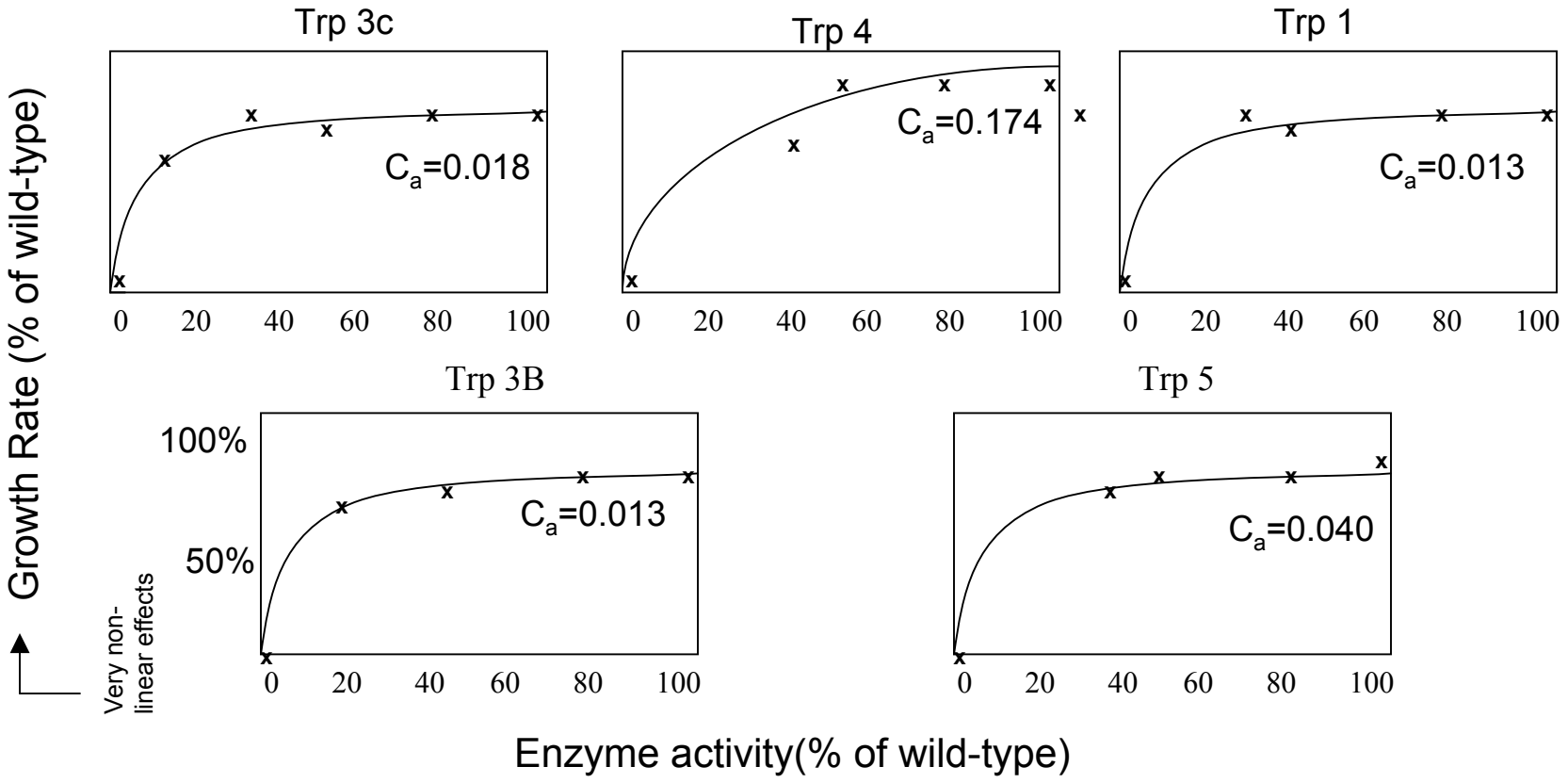
TRPS: Tryptophan Synthase

AAT1,2: Aromatic Amino Transferase

Case Study (cont'd.)
Trp accumulation and flux upon single TRP gene up-modulation

Up-modulated genes					Relative enzyme levels					Flux to Tryptophan		
					AA synthase (TRP2)	PR-transferase (TRP4)	PRA isomerase (TRP1)	InGP synthase (TRP3)	Trp synthase (TRP5)	Trp accumulation rate	Trp accumulation in protein	Total Trp flux
TRP2					1.0	1.0	1.0	1.0	1.0	0.1	0.33	0.43
	TRP4				1.2	19.9	1.2	1.6	1.2	0.27	0.3	0.57
		TRP1			1.5	1.2	10.6	1.6	0.7	0.2	0.3	0.5
			TRP3		1.5	0.8	1.8	51.5	1.1	0.13	0.31	0.44
				TRP5	1.3	0.8	1.7	1.6	37.1	0.23	0.29	0.52

Back to Trp synthesis



Tetraploid yeast strains with 4,3,2,1 and 0 doses of a TRP gene. Effect of Trp on μ (protein synthesis). Above effects are abolished in Trp-supplemented medium.

Case Study (cont'd.)

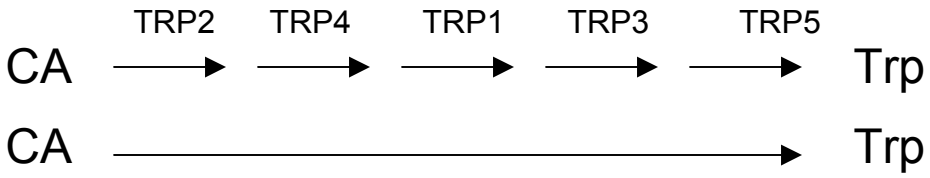
$C_a^\mu = 0.018, \quad C_b^\mu = 0.174, \quad C_c^\mu = 0.013 \quad C_d^\mu = 0.013$

$C_e^\mu = 0.040 \quad \rightarrow$ All very small

(Flux control coefficient evaluated at wild-type activity)

Suggestion: Trp flux relatively insensitive to TRP1-5

Note: $\sum_{i=a-e} C_i^\mu = 0.258$ $\leftarrow$ equal to a group coefficient



$\sum_{i=a-e} C_i^\mu = 0.258 \quad \Rightarrow$ Many enzymes contribute to flux control

- Large fraction of control residues outside the above chain of 5 reactions.

Case Study (cont'd.)
Trp Accumulation & Flux upon up-modulation of multiple TRP genes

Up-modulated genes					Relative enzyme levels					Flux to Tryptophan		
					AA synthase (TRP2)	PR-transferase (TRP4)	PRA isomerase (TRP1)	InGP synthase (TRP3)	Trp synthase (TRP5)	Trp accumula-tion rate	Trp accumula-tion in protein	Total Trp flux
TRP2					1.0	1.0	1.0	1.0	1.0	0.1	0.33	0.43
	TRP4				1.2	19.9	1.2	1.6	1.2	0.27	0.3	0.57
		TRP1			1.5	1.2	10.6	1.6	0.7	0.2	0.3	0.5
			TRP3		1.5	0.8	1.8	51.5	1.1	0.13	0.31	0.44
				TRP5	1.3	0.8	1.7	1.6	37.1	0.23	0.29	0.52
		TRP1	TRP3		1.5	0.6	58.6	56	0.5	0.58	0.29	0.87
TRP2	TRP4	TRP1d	TRP3		25.0	34.0	1.7	47.0	1.0	0.72	0.31	1.03
TRP2f		TRP1	TRP3		29.4	1.0	30.6	40.9	1.0	0.22	0.31	0.53
TRP2f	TRP4	TRP1	TRP3		26.5	26.4	30.3	37.4	0.8	0.59	0.32	0.91
TRP2	TRP4	TRP1d	TRP3	TRP5	20.0	15.2	1.6	20.7	18.1	3.25	0.29	3.54
TRP2f	TRP4	TRP1	TRP3	TRP5	24.0	19.6	22.1	26.9	23.9	3.51	0.28	3.79

Multiple up-modulations

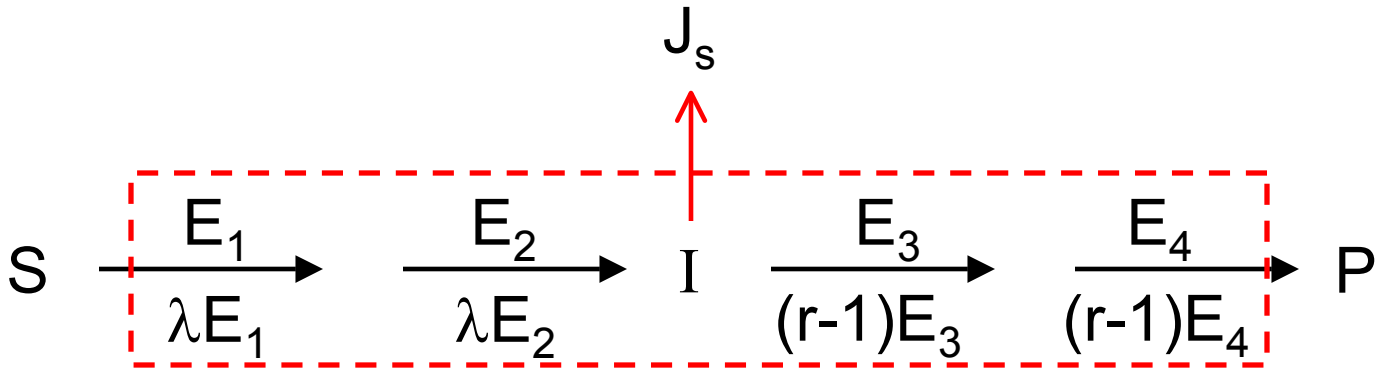
- Coordination theorem: If a system consisting of many enzymes sustains a flux J, then, if *all enzyme* concentrations are multiplied by r the resulting flux will be r.J (<u>any r)</u>
- <u>OR:</u>

$$\frac{\Delta J / J}{\Delta E_i / E_i} = 1$$

Stands for the simultaneous and equal elevation of all E_i

- <u>Implications:</u>
  - Chromosomal integration, multi-copy plasmid effects must be analyzed in terms of large-finite-change systems. These have the above property. Individual contribution of smaller group of enzymes are not additive.
  - Identify the group of enzymes whose control coefficients sum up to a substantial part.
  - The small groups must be modulated simultaneously

Universal Method of Flux Amplification (Henrik Kacser)



- Initial Flux J + J_S
- Final Flux rJ +J_S

$$\frac{\Delta J}{J_{initial}} = \frac{rJ + J_s - J - J_s}{J + J_s}$$

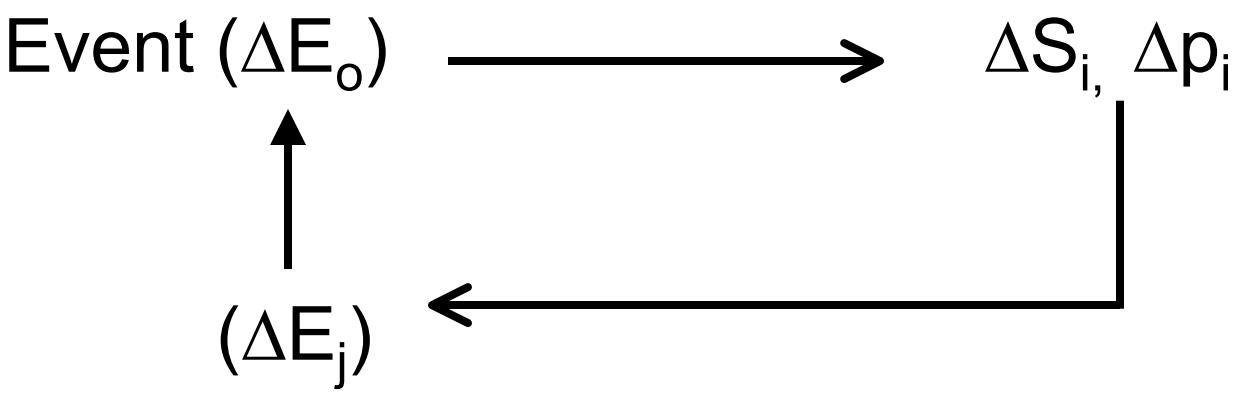
$$= \frac{r-1}{1+(J_s/J)} = \lambda$$

- Initial Flux J
- Final Flux rJ

$$\frac{\Delta J}{J_{initial}} = r - 1$$

Systemic vs. local effects

Metabolism is Complex :



The net result of an event (such as a genetic modification) is a Systemic property and not an isolated change

Elasticities

Definition:

Consider a small change δM is made in the amount of metabolite M affecting the rate of reaction v catalyzed by enzyme E and producing a change δv. All other metabolites affecting v are kept constant at the steady state values. (We use v instead of J to indicate that we are considering the response of an isolated enzyme, not the pathway).

Elasticity:

$$\varepsilon_M^v = \left(\frac{\partial v}{\partial M}\right)_{st.state} \frac{M}{v} = \left(\frac{\partial \ln v}{\partial \ln M}\right)_{st.state}$$

Note:

- ∂ : partial derivate, evaluated at steady state
- ε : depends on metabolite concentrations and rate parameters
- ε_E^v = 1 if E catalyzes reaction
  = 0 if E does not participate in reaction

Connectivity Theorem

- **How are FCC related to the kinetic properties of enzymes?**

Connectivity theorem (one for each metabolite)

$$C^J_{e_1}.\varepsilon^1_{M_k} + C^J_{e_2}.\varepsilon^2_{M_k} + ... + C^J_{e_n}.\varepsilon^n_{M_k} = 0$$

(M_k is any independent variable, metabolite, T, etc.)

For 2 enzymes

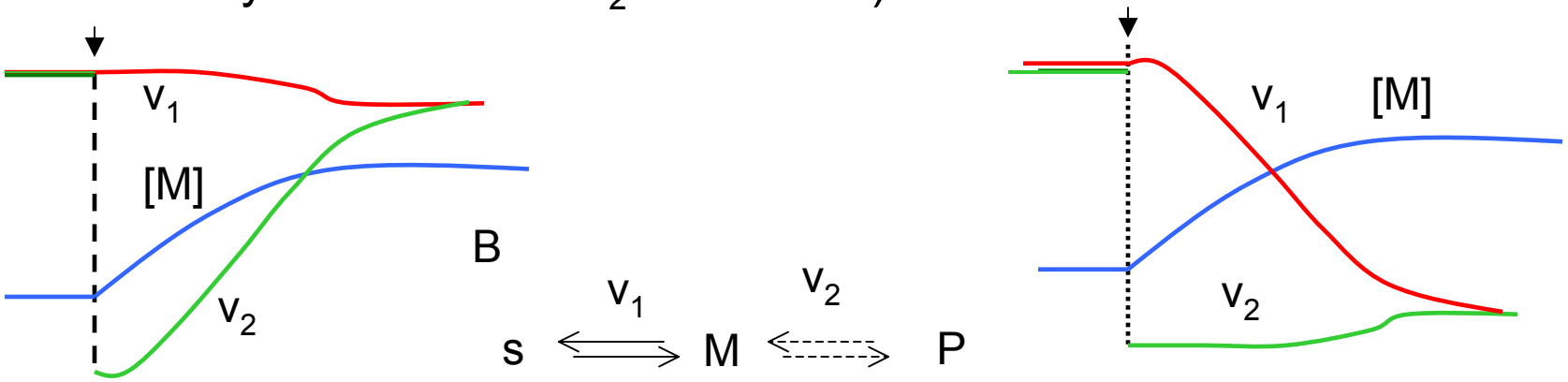
$$C^J_{e_1}.\varepsilon^1_M + C^J_{e_2}.\varepsilon^2_M \Rightarrow \frac{C^J_{e_1}}{C^J_{e_2}} = -\frac{\varepsilon^2_M}{\varepsilon^1_M}$$

Unresponsive (rigid) enzymes exert strong control on fluxes!

Enzymes whose rates do not change greatly when the concentrations of their reactants or products vary. Elasticities wrt a common metabolite are the correct measure.

Illustration of Connectivity Theorem

- After addition of the inhibitor(at the arrow) and the resulting instantaneous drop in v_2 (dashed line), the concentration of the intermediary metabolite M(blue) begins to rise. Depending on whether v_1 (red line) is less (left part of the figure) or more(right part) inhibited than v_2 is stimulated by this increase in [M], the point at which the steady-state condition $v_1 = v_2$ is re attained will be at high (left part) or low (right part) reaction rates (flux), respectively. Consequently, the flux control exerted by enzyme increases with an increase in the Elasticity Coefficient of v_2 towards M).



Response Coefficients

Define:

Response Coefficient R_X^J of flux J to an effector X:

$$R_X^J = \frac{X}{J}\frac{dJ}{dX}$$

If the effect of X is mediated by enzyme E_i

$$R_X^J = C_i^J \varepsilon_X^i$$

If an effecter acts upon more than one enzyme, the total response is the sum of responses from all individual enzymes

$$R_X^J = \sum_i C_i^J \varepsilon_X^i$$