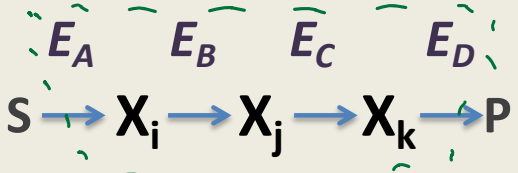
How do mutations affect metabolism?

Imagine this system being at a particular steady-state in WT. A mutation then occurs that doubles the k_{cat} for E_B.



→ ACTIVITY PER MOLECULE OF AN ENZYME (s^{-1})

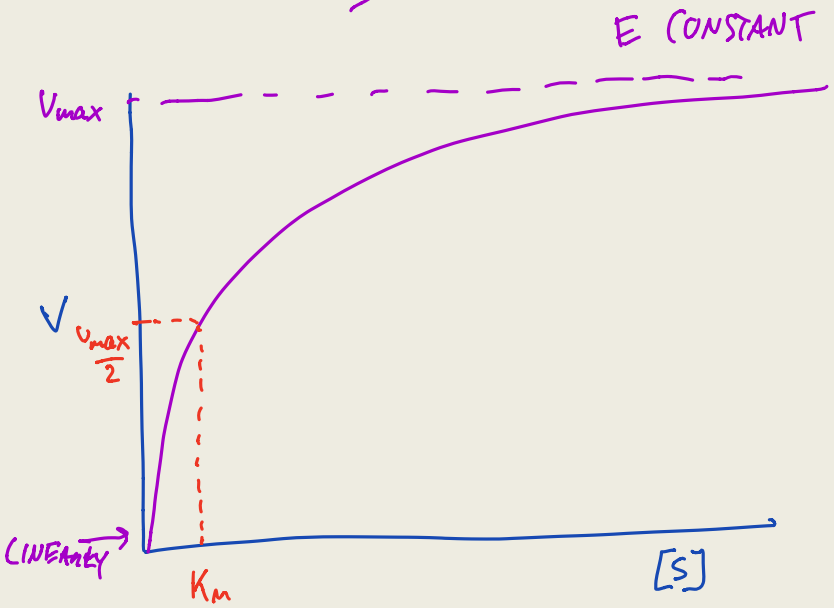
Relative to their initial values, would be the new steady-state values of the following be HIGHER/SAME/LOWER:

	UP	SAME	DOWN
Flux	10	7	~~0~~
X_i	1	1	6
X_j	11	~~0~~	~~0~~
X_k	5	2	~~0~~

One substrate MM kinetics

IRREVERSIBLE

$S \xrightarrow{E} P$



V_{max} = MAX CAPACITY AT HIGH [S]

ASSUMPTIONS:

- $[S] \gg [E]$, $[S]$ & $[P]$ ARE CONSTANT
- BINDING OF S (OR P) TO E IS MUCH FASTER THAN CATALYSIS

$$V = V_{max} \cdot \left(\frac{S}{S + K_M} \right)$$

V_{max}: $\frac{mM}{s}$

K_M: MICHAELIS CONSTANT (mM)

One substrate MM kinetics with P feedback

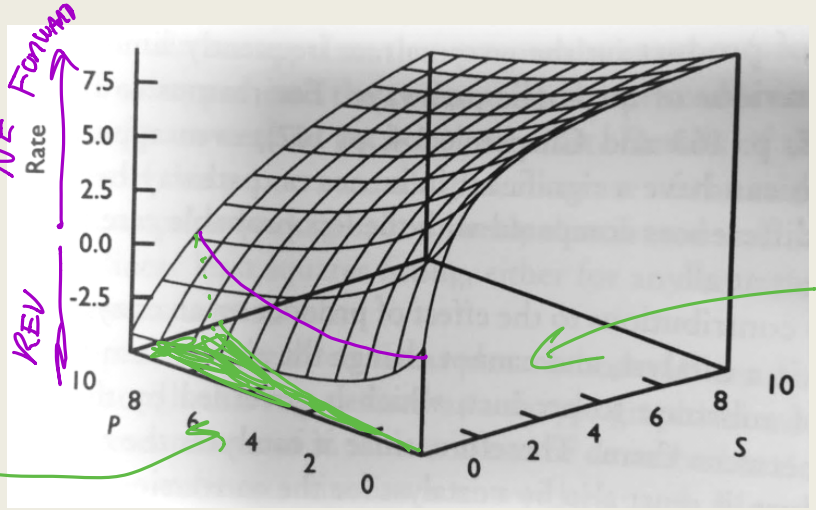
$$S \xrightleftharpoons{E} P$$

$$V = V_{max,f}\left(\frac{S}{S+K_S}\right) - V_{max,r}\left(\frac{P}{P+K_P}\right)$$

$$V_{max} = [E] \cdot K_{cat} \qquad mM \cdot s^{-1}$$

$$V = [E]\left(K_{cat,f}(\ ) - K_{cat,r}(\ )\right)$$

– TENSION btw/ BACKWARDS & FORWARDS



FORWARDS

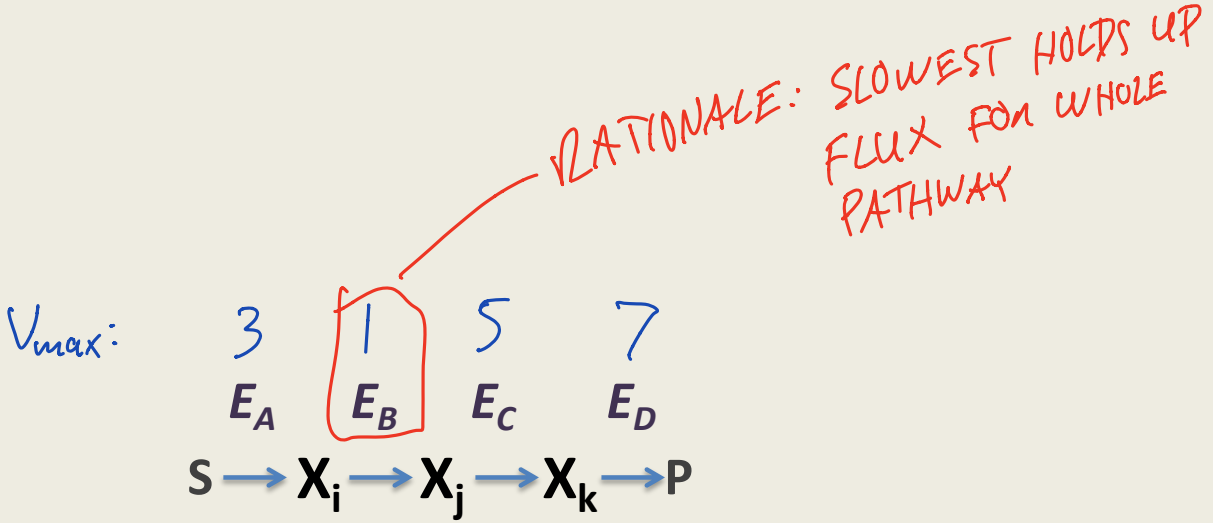
BACKWARDS

Two substrate MM kinetics

$$A + B \underset{}{\overset{E}{\rightleftharpoons}} C + D$$

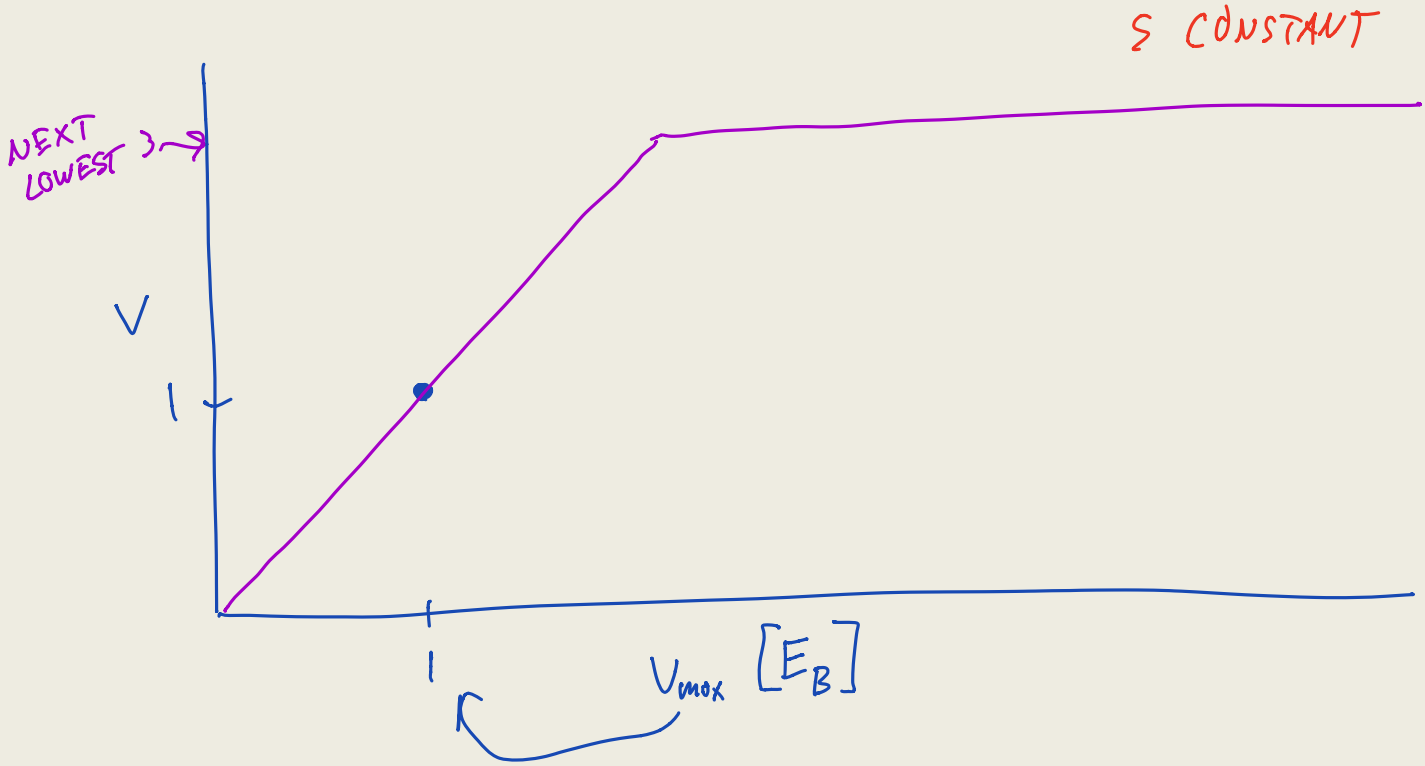
$$V = V_{max,f}\left(\frac{A}{A+K_A}\right)\left(\frac{B}{B+K_B}\right) - V_{max,r}\left(\frac{C}{C+K_C}\right)\left(\frac{D}{D+K_D}\right)$$

Rate-limiting enzyme



Rate-limiting enzyme

- *When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor* (Blackman, 1905. *Annals of Botany*)



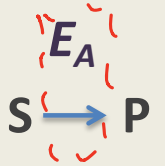
Single rate-limiting steps can exist

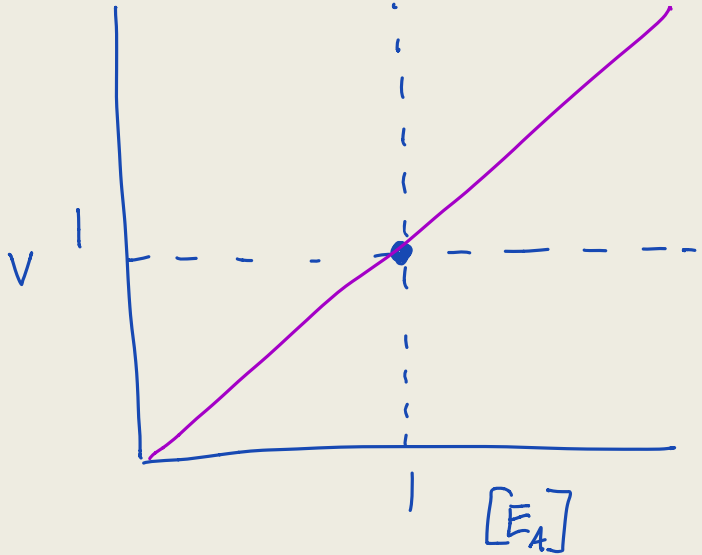
http://www.insidebainbridge.com/2013/12/03/an-unsettling-analysis-of-the-traffic-future-of-highway-305/

http://www.stepneyct.org/history/ht/stop17.html

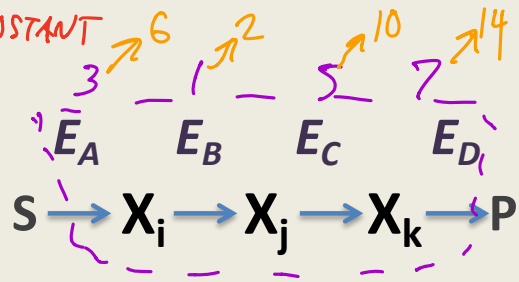
Dependence of flux upon [E]

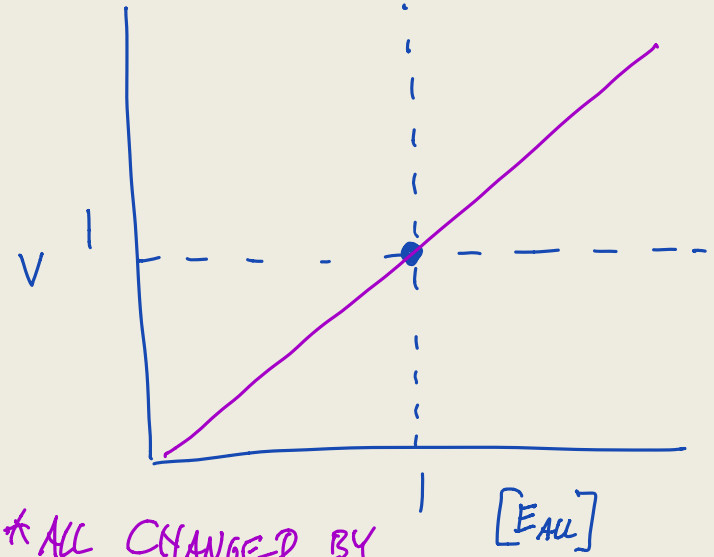
* ASSUMES [S], [P] CONSTANT





GET PROP. CHANGE IN v w/ E_A

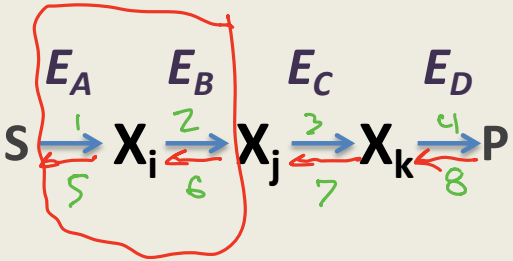




* ALL CHANGED BY SAME FACTOR → LINEAR

* WHAT HAPPENS TO $X_i \rightarrow X_k$?

Tutorial in R: ODEs of metabolism




```
state <- c(Xi=0.5,
           Xj=0.5,
           Xk=0.5)

times=seq(0,100,by=0.01)

MM_multi<-function(t,state,parameters.t) {
  with(as.list(c(state,parameters.t)), {
    #rate of change
    dXi = Ea(t)*(kcat_af*(S(t)/(S(t)+K_af))-kcat_ar*(Xi/(Xi+K_ar)))+Eb(t)*(-kcat_bf*(Xi/(Xi+K_bf))+kcat_br*(Xj/(Xj+K_br)))
    dXj = Eb(t)*(kcat_bf*(Xi/(Xi+K_bf))-kcat_br*(Xj/(Xj+K_br)))+Ec(t)*(-kcat_cf*(Xj/(Xj+K_cf))+kcat_cr*(Xk/(Xk+K_cr)))
    dXk = Ec(t)*(kcat_cf*(Xj/(Xj+K_cf))-kcat_cr*(Xk/(Xk+K_cr)))+Ed(t)*(-kcat_df*(Xk/(Xk+K_df))+kcat_dr*(P(t)/(P(t)+K_dr)))
```

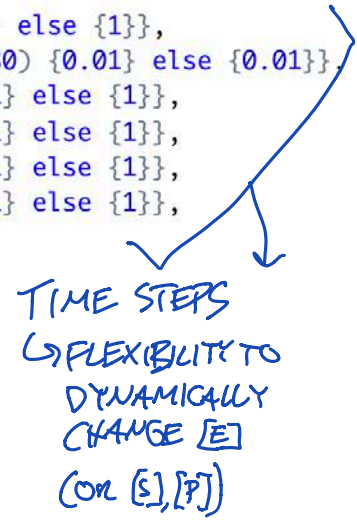
INITIAL CONDITIONS

MCA time dynamics v1 200201.R

Tutorial in R: ODEs of metabolism

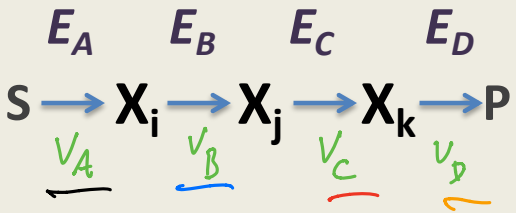
```
MM_multi<-function(t,state,parameters.t) {
  with(as.list(c(state,parameters.t)), {
    #rate of change
    dXi = Ea(t)*(kcat_af*(S(t)/(S(t)+K_af))-kcat_ar*(Xi/(Xi+K_ar)))+Eb(t)*(-kcat_bf*(Xi/(Xi+K_bf))+kcat_br*(Xj/(Xj+K_br)))
    dXj = Eb(t)*(kcat_bf*(Xi/(Xi+K_bf))-kcat_br*(Xj/(Xj+K_br)))+Ec(t)*(-kcat_cf*(Xj/(Xj+K_cf))+kcat_cr*(Xk/(Xk+K_cr)))
    dXk = Ec(t)*(kcat_cf*(Xj/(Xj+K_cf))-kcat_cr*(Xk/(Xk+K_cr)))+Ed(t)*(-kcat_df*(Xk/(Xk+K_df))+kcat_dr*(P(t)/(P(t)+K_dr)))
```

```
parameters.t <- c(S = function(t){if (t<40) {1} else if (t<60) {1} else if (t<80) {1} else {1}},
                  P = function(t){if (t<40) {0.01} else if (t<60) {0.01} else if (t<80) {0.01} else {0.01}},
                  Ea = function(t){if (t<40) {1} else if (t<60) {1} else if (t<80) {1} else {1}},
                  Eb = function(t){if (t<40) {1} else if (t<60) {1} else if (t<80) {1} else {1}},
                  Ec = function(t){if (t<40) {1} else if (t<60) {1} else if (t<80) {1} else {1}},
                  Ed = function(t){if (t<40) {1} else if (t<60) {1} else if (t<80) {1} else {1}},
                  kcat_af = 15,
                  kcat_ar = 15,
                  kcat_bf = 20,
                  kcat_br = 20,
                  kcat_cf = 15,
                  kcat_cr = 10,
                  kcat_df = 15,
                  kcat_dr = 5,
                  K_af = 0.2,
                  K_ar = 0.2,
                  K_bf = 2,
                  K_br = 0.2,
                  K_cf = 2,
                  K_cr = 0.2,
                  K_df = 0.2,
                  K_dr = 0.2)
```



Try increasing all enzymes by the same factor in R

`MCA time dynamics v1 200201.R`



Change all four of the E_i from 1 to 2 for the second time period, leaving the other time periods at 1.

What happened to fluxes in the second time period?

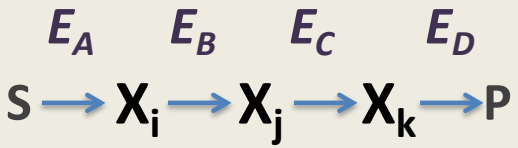
↳ EXACTLY DOUBLE

What happened to the concentrations at that time?

↳ NOTHING! NO CHANGES IN $X_{i,j,k}$

Which step controls flux?

```
kcat_af = 15,
kcat_ar = 15,
kcat_bf = 20,
kcat_br = 20,
kcat_cf = 15,
kcat_cr = 10,
kcat_df = 15,
kcat_dr = 5,
K_af = 0.2,
K_ar = 0.2,
K_bf = 2,
K_br = 0.2,
K_cf = 2,
K_cr = 0.2,
K_df = 0.2,
K_dr = 0.2)
```



Look at the parameters to the left and decide which enzyme has the most control over flux (closest to being "rate-limiting").

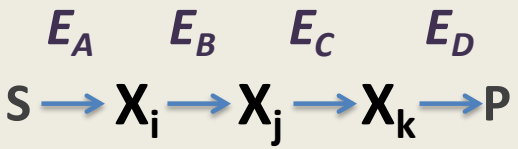
↳ SET 3 OF 4 BACK TO "1" FOR KCAT <60

WHICH IMPACT FLUX: A, B, C, D

↳ NONE GIVE 2x EFFECT

Which enzyme has the most control in our system?

```
kcat_af = 15,
kcat_ar = 15,
kcat_bf = 20,
kcat_br = 20,
kcat_cf = 15,
kcat_cr = 10,
kcat_df = 15,
kcat_dr = 5,
K_af = 0.2,
K_ar = 0.2,
K_bf = 2,
K_br = 0.2,
K_cf = 2,
K_cr = 0.2,
K_df = 0.2,
K_dr = 0.2)
```



Run `MCA v vs E 200201.R`

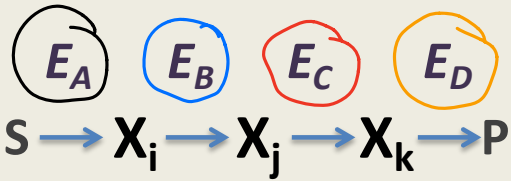
Look at the figure of v vs. E for each of the four enzymes to see how it behaves with changing each enzyme individually.

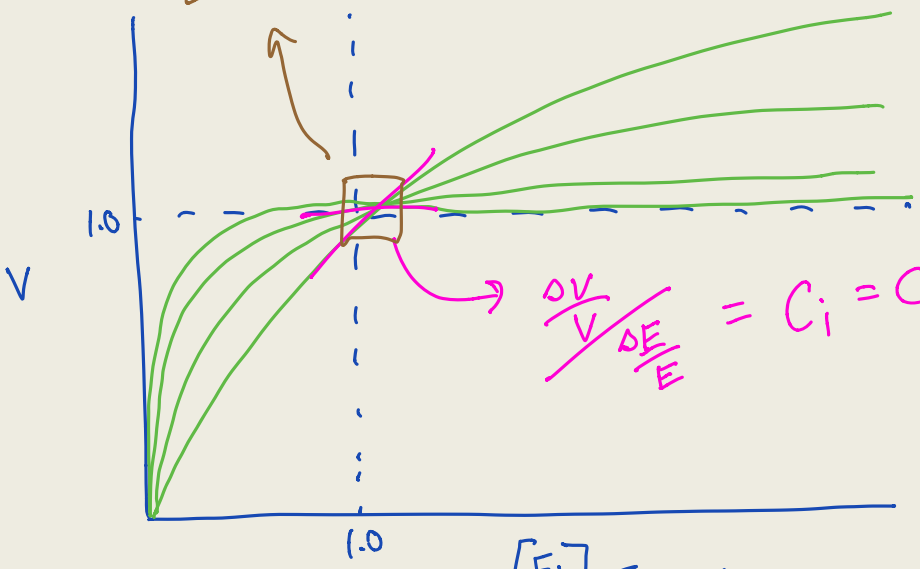
↳ RUNS LOOP TO EVALUATE $v_i, [X_i]$ FOR MANY VALUES OF $[E_i]$

[redacted]

Dependence of flux upon [E]

$\Sigma C_i = 1.0$ SUMMATION THEOREM $\Rightarrow$ AVERAGE C_i IN CELL $= \frac{\Sigma C_i}{\#ENZ} = \frac{1}{1000}$



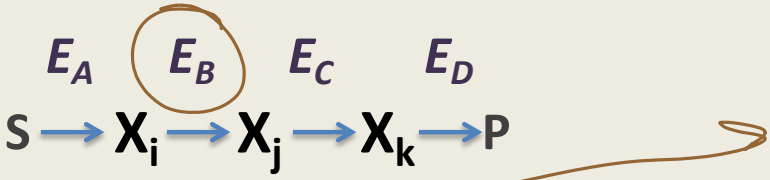


$\frac{\Delta V / V}{\Delta E / E} = C_i$ = CONTROL COEFFICIENT FOR ENZ i

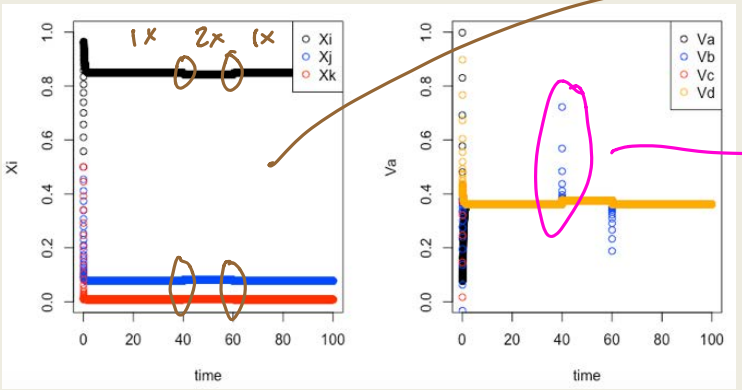
$[E_i]$ ← CHANGING JUST $[E_i]$, ALL OTHER $[E]$ STAY THE SAME

* METABOLIC CONTROL ANALYSIS (MCA)

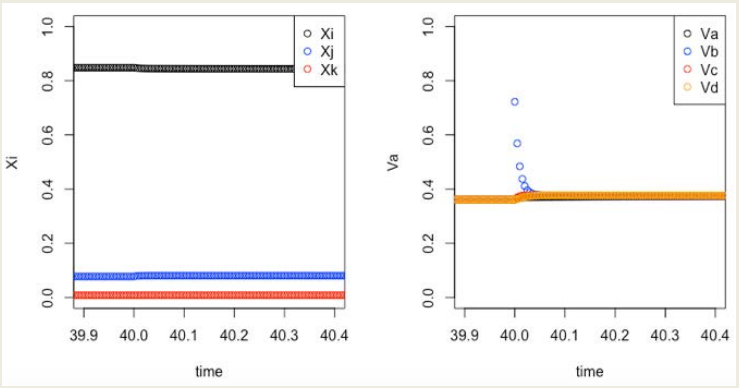
What happens upon changing an enzyme?



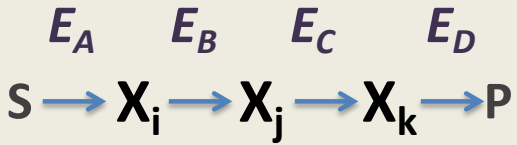
→ CONCENTRATION CHANGES



→ INSTANTANEOUS 2x FOR E_B
↳ QUICK DROP



Why so little effect upon changing activities?



1. REVERSIBILITY

2. SHIFT IN CONC.

Aspect #1: Metabolism flows both ways

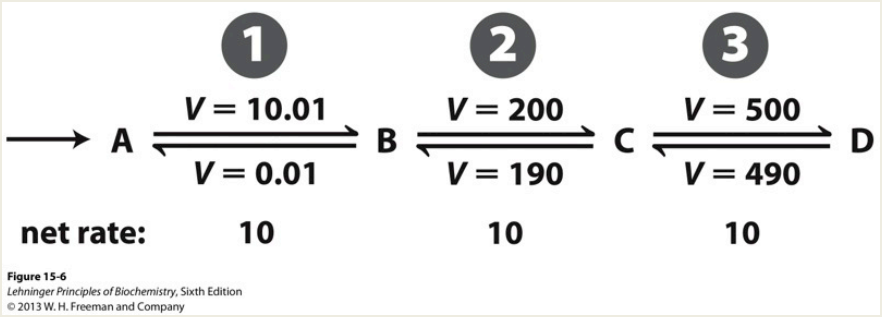


Figure 15-6
Lehninger Principles of Biochemistry, Sixth Edition

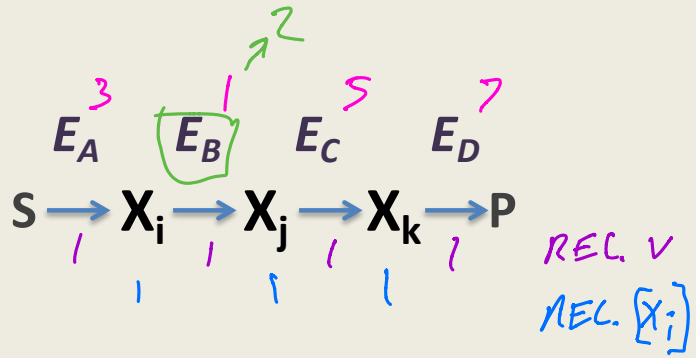
$$V_{NET} = [E_B]\left(k_{cat,f}(\ ) - k_{cat,r}(\ )\right)$$

q

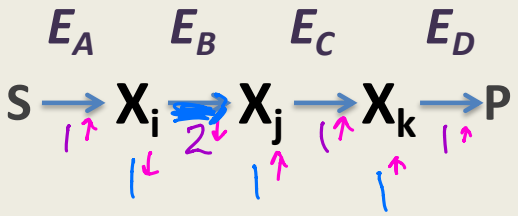
2x FOR BOTH

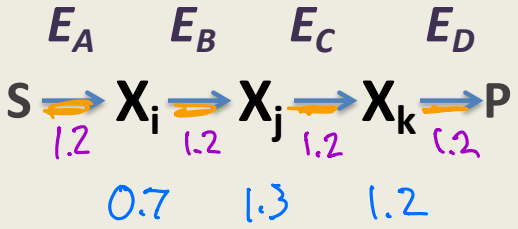
Aspect #2: changes in saturation

Thought experiment:
instantaneous doubling of k_{cat} for E_B



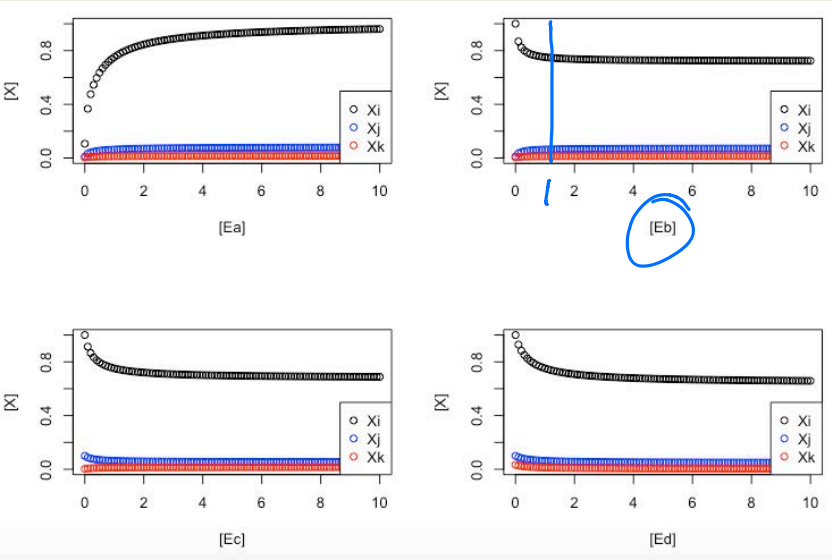
INSTANT AFTER CHANGE →



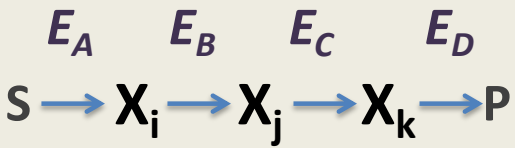


What changes with increases to one enzyme?

$$S \xrightarrow{E_A} X_i \xrightarrow{E_B} X_j \xrightarrow{E_C} X_k \xrightarrow{E_D} P$$



What happens to c_i values if one is changed?

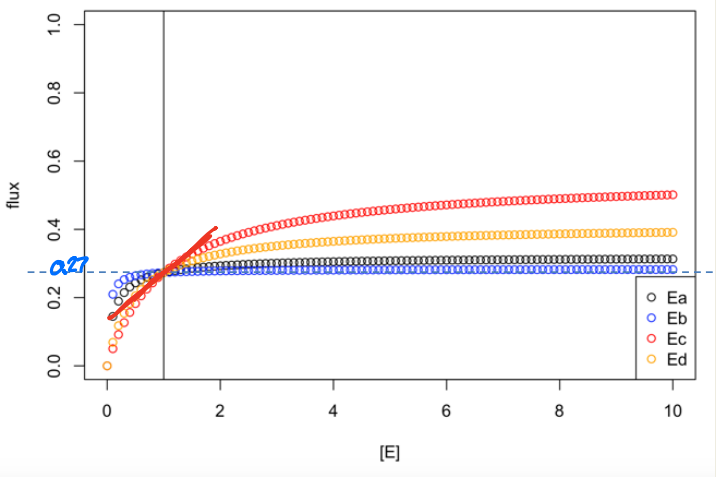


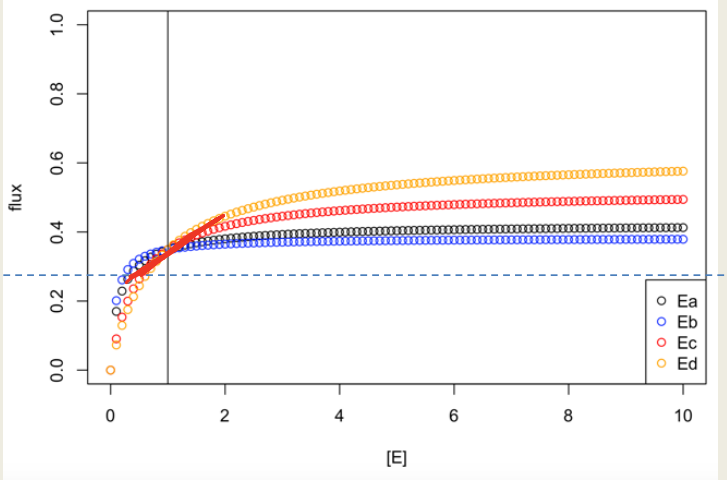
1. First guess: will increasing an enzyme raise or lower its own c_i? Will it change the other c_i values, and if so, will they go up or down?

2. Use `MCA v vs E 200201.R` and try this. Run once with original levels, and then pick an enzyme and increase both kcat values (f and r) by the same factor (a decent bit, i.e. 5-10 fold) and then run a second time.

What changes with increases to one enzyme?

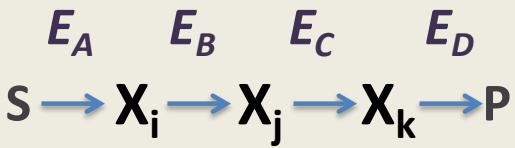
$$S \xrightarrow{E_A} X_i \xrightarrow{E_B} X_j \xrightarrow{E_C} X_k \xrightarrow{E_D} P$$





- C_i OF CHANGED ENZ DOWN; REST UP TO STILL ADD TO 1.0
- MULT. MUT. TO SAME ENZ., DIMINISHING RET. FOR BENEFICIAL
  " " DELETERIOUS

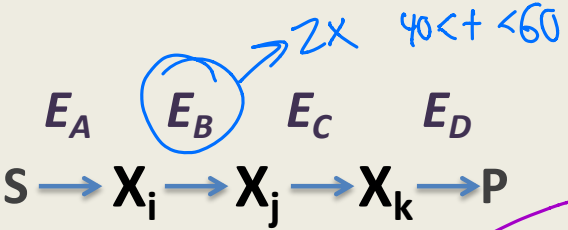
Instantaneous vs. steady-state behavior



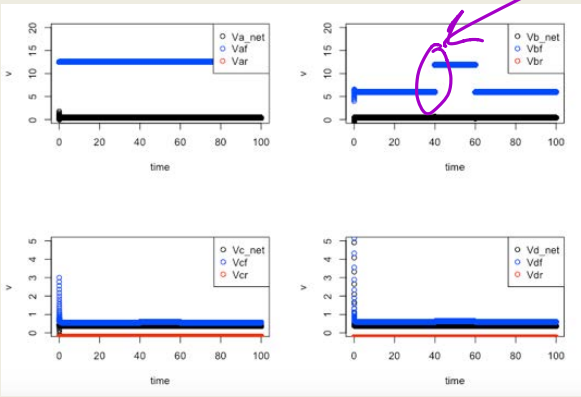
Go back and use other file: `MCA time dynamics v2 200201.R`

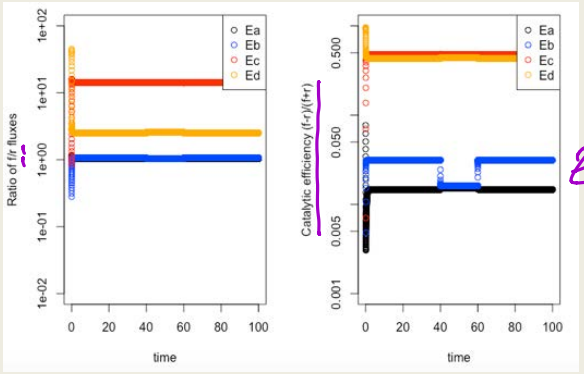
Pick an enzyme and double it. How did that affect the steady-state behavior? What about the immediate dynamics right around t=40?

What happens upon changing an enzyme?



v_f & v_r LARGER





VERY INEFFICIENT

Rate and saturation/balance (of fwd vs. rev) in control over flux

SATURATION TERMS

$$V = V_{max,f}\left(\frac{X_i}{X_i + K_i}\right) - V_{max,r}\left(\frac{X_j}{X_j + K_j}\right)$$

MAX CAPACITY FOR FLUX
↳ OFTEN TARGET OF MUTATIONS

$$S \xrightarrow{E_A} X_i \xrightarrow{E_B} X_j \xrightarrow{E_C} X_k \xrightarrow{E_D} P$$

ANY ENZ. RANGE OF ACTIVITIES POSSIBLE:

$V_{max,r} < V < V_{max,f}$

	FOR E_B	FOR E_C
AT ORIGINAL $[E_B]$ RELENZ: 1:1:1:1	$1 = 1 \cdot 1$	$1 = 1 \cdot 1$
w/ $2\times[E_B]$ 1:2:1:1	$1.2 = 2 \cdot 0.6$	$1.2 = 1 \cdot 1.2$

FLUX [E] SAT

What does this suggest about enzyme saturation?

THEORY: IMPOSSIBLE FOR ALL ENZ. TO BE HIGHLY SATURATED

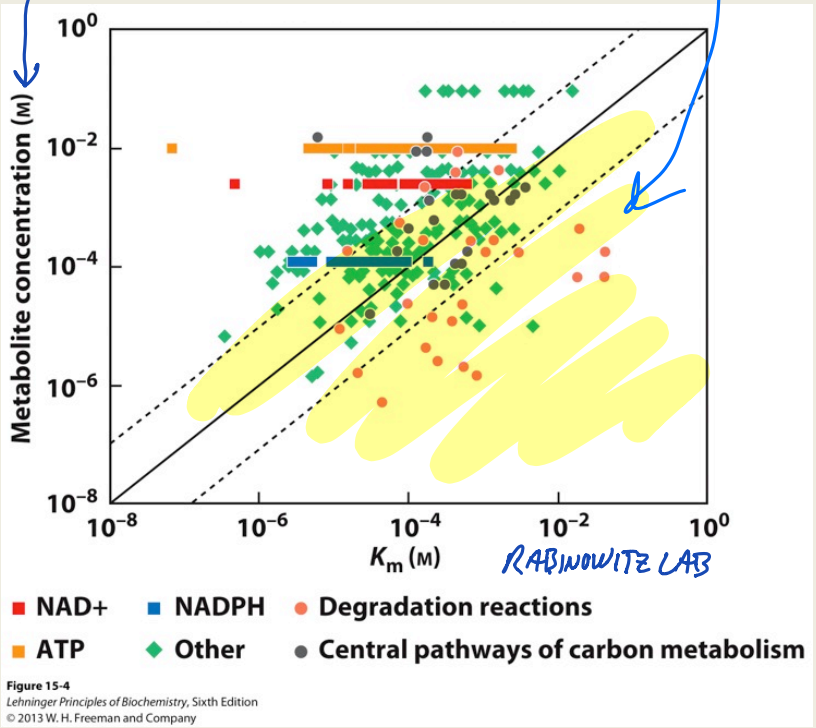
DATA: LC-MS

$$S \xrightarrow{E_A} X_i \xrightarrow{E_B} X_j \xrightarrow{E_C} X_k \xrightarrow{E_D} P$$

UNSATURATED

* MOST ENZ.-SUBSTATE PAIRS UNSATURATED

* THOSE W/ ONE SUBS HIGH, OFTEN HAVE SECOND SUBSTRATE LOW



$$r_{2a} = V_{2a}\left(\frac{[\text{me} - \text{H4MPT}]}{Km_{2a} + [\text{me} - \text{H4MPT}]}\right)\left(\frac{[\text{NAD}]}{Km_{2b} + [\text{NAD}]}\right)$$

$$r_7 = \frac{V_7 \frac{[\text{me–H4F}][\text{NADP}]}{Km_{7a}Km_{7b}} - V_{7r}\frac{[\text{mn–H4F}][\text{NADPH}]}{Km_{7ar}Km_{7br}}}{\left(1 + \frac{[\text{me–H4F}]}{Km_{7a}} + \frac{[\text{NADP}]}{Km_{7b}}\right)\left(1 + \frac{[\text{mn–H4F}]}{Km_{7ar}} + \frac{[\text{NADPH}]}{Km_{7br}}\right)}$$

(Marx et al., 2005. *PLoS Biology*)

How test MCA experimentally?

in vitro:

ADD ENZ. TO TEST TUBE

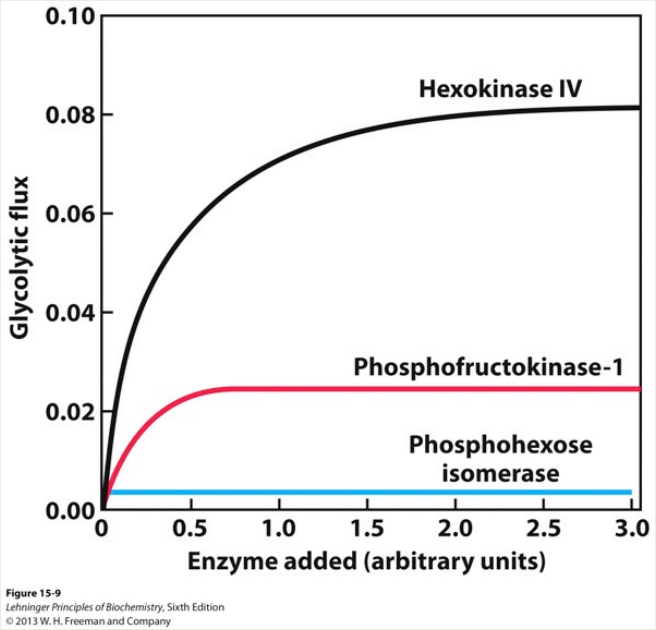
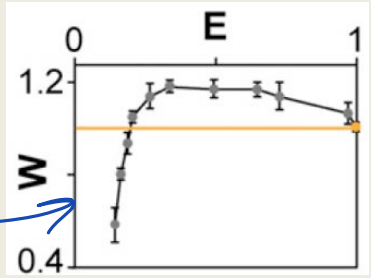


Figure 15-9
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company

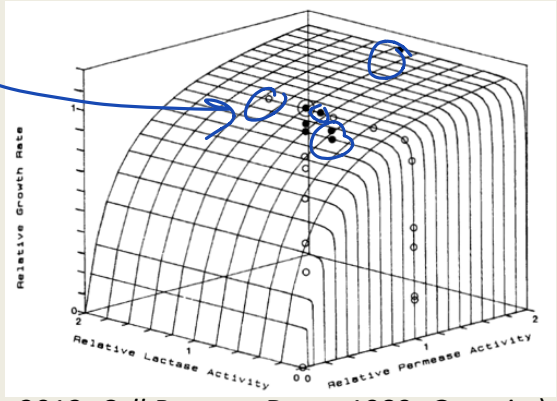
in vivo:

1. REGULATE E; w/ INDUCIBLE PROMOTER



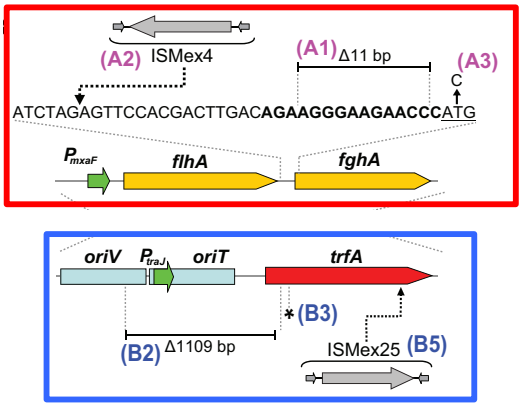
2. USE DISCRETE MUTATIONS

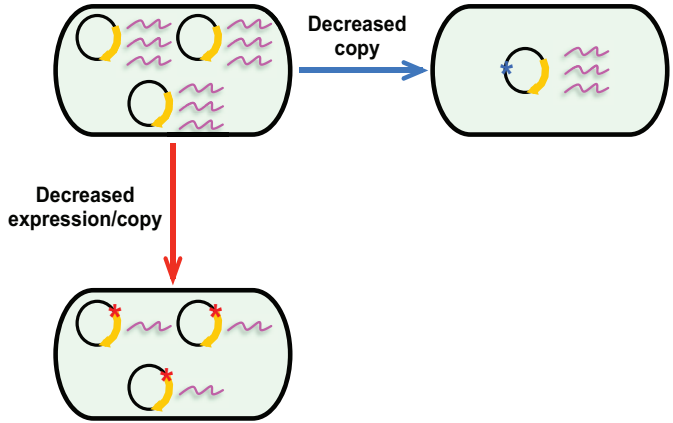
$$J'/J = j = \frac{\frac{1}{D} + \frac{K_t}{V_{\text{maxperm}}} + \frac{K_m}{V_{\text{max}\beta\text{gal}}\,K_{p,c}}}{\frac{1}{D} + \frac{K'_t}{V'_{\text{maxperm}}} + \frac{K'_m}{V'_{\text{max}\beta\text{gal}}\,K_{p,c}}}.$$



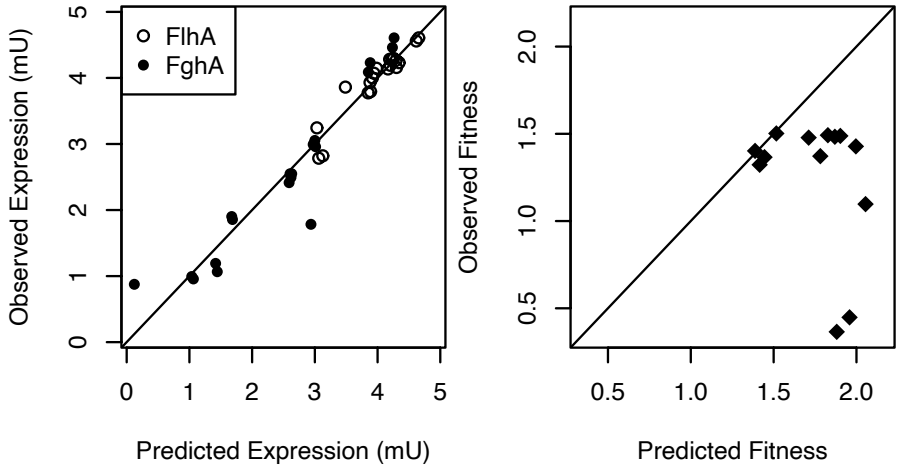
(Chou and Marx, 2012. *Cell Reports*; Dean, 1989. *Genetics*)

Independent effects upon expression



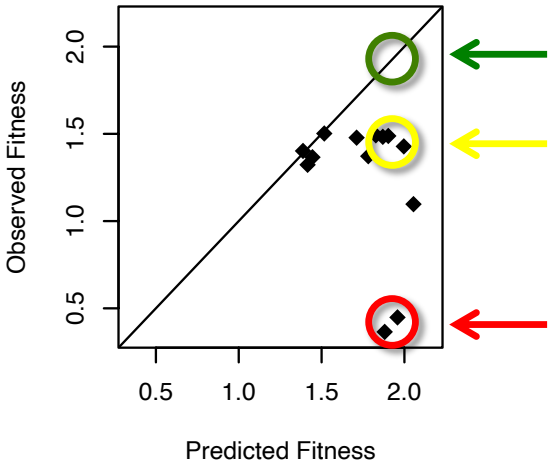


- Two classes should interact independently:
  - $E_{AB} = E_A \times E_B$
  - Yes
- Indep. upon fitness?
  - **No.**



(Chou et al., *PLoS Genetics*, 2014)

Why antagonism and sign epistasis?

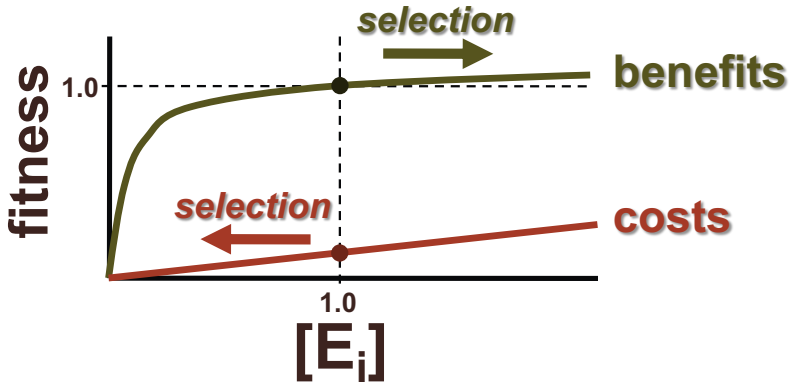


$W_{AB} = W_A \times W_B$; both provide benefit when together

$W_{AB} \approx \max(W_A, W_B)$; pair same as best individual

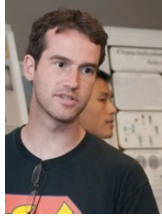
$W_{AB} < W_{ANC}$; pair much worse than individual mut.

- Caused by tension between benefits and costs?
  - Metabolic Control Analysis (MCA)

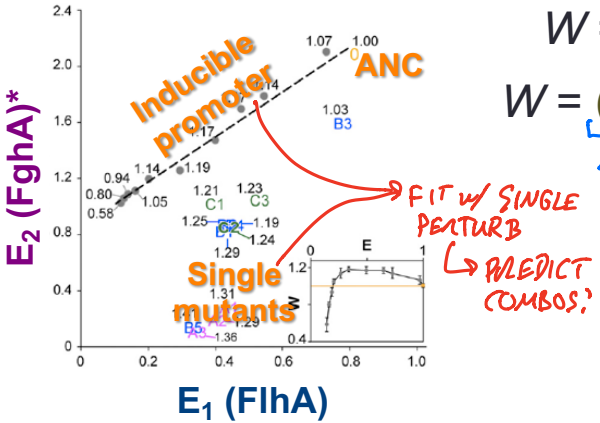


(Chou et al., *PLoS Genetics*, 2014)

Nigel Delaney



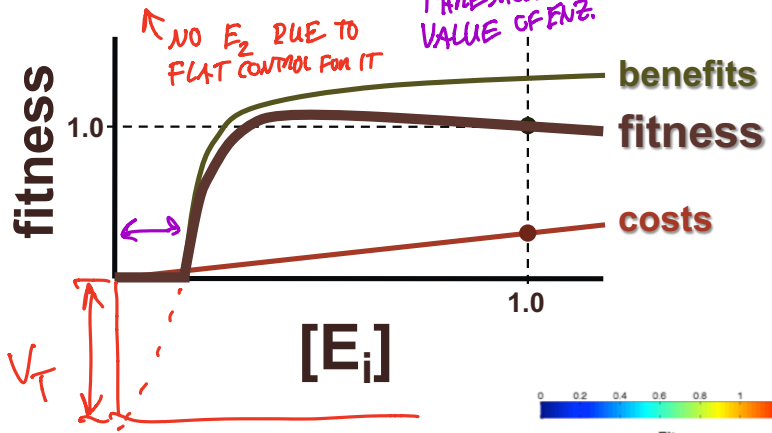
Map expression to fitness via model



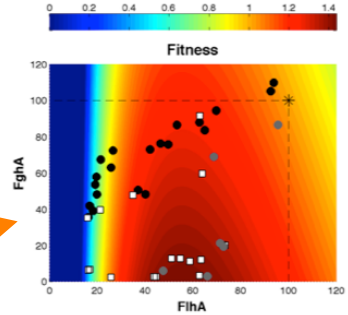
*FghA necessary for catalysis but not in benefit term because all data higher than threshold

$$W = \text{Flux above threshold} - \text{enzyme costs}$$

$$W = \underbrace{(v_{max} \times \mathbf{E_1}/(\mathbf{E_1} + E_{½\,max})}_{\text{APPROXIMATE MCA RESULT}} - \underbrace{v_T}_{\text{THRESHOLD VALUE OF ENZ.}}) - \underbrace{a \times \mathbf{E_1} - b \times \mathbf{E_2}}_{\text{COSTS}}$$

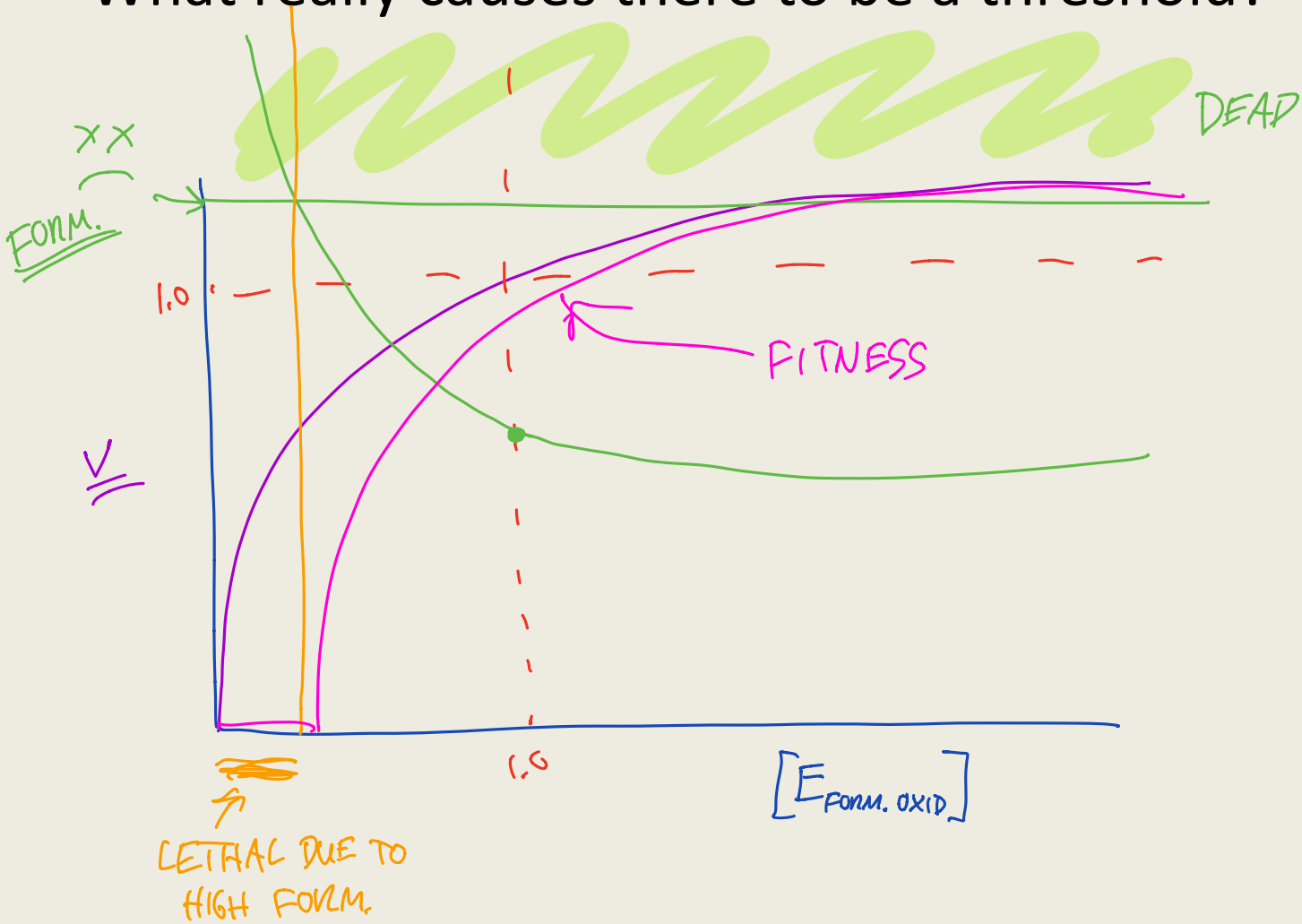


- ANC, single mutants, inducible promoter constructs (27 data points) to fit parameters
  - Try to predict 17 mutational combinations

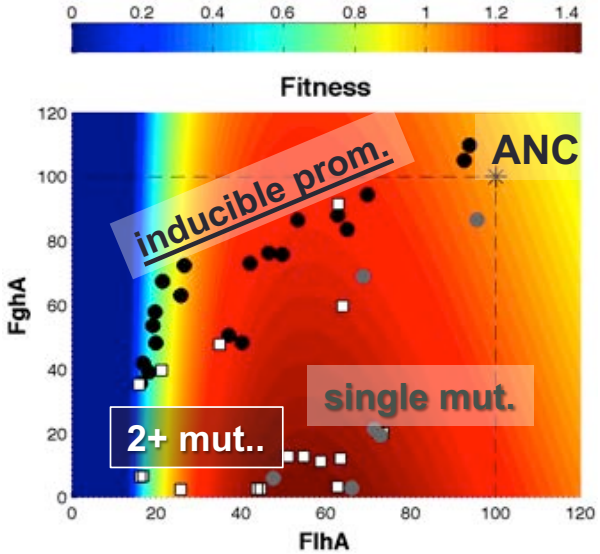


(Chou and Marx, 2012. *Cell Reports*; Chou et al., *PLoS Genetics*, 2014)

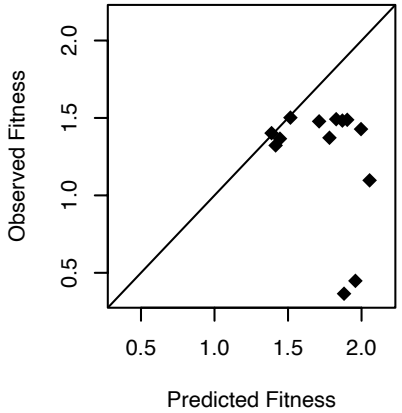
What really causes there to be a threshold?



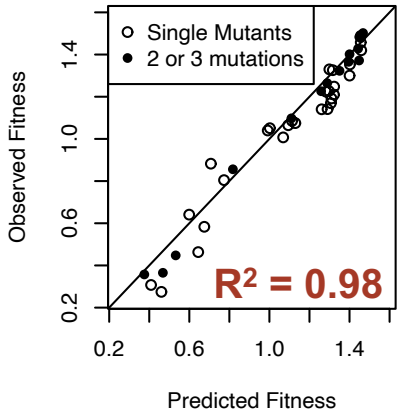
Model predicts mutational combos



$$W_{AB} = W_A \times W_B$$



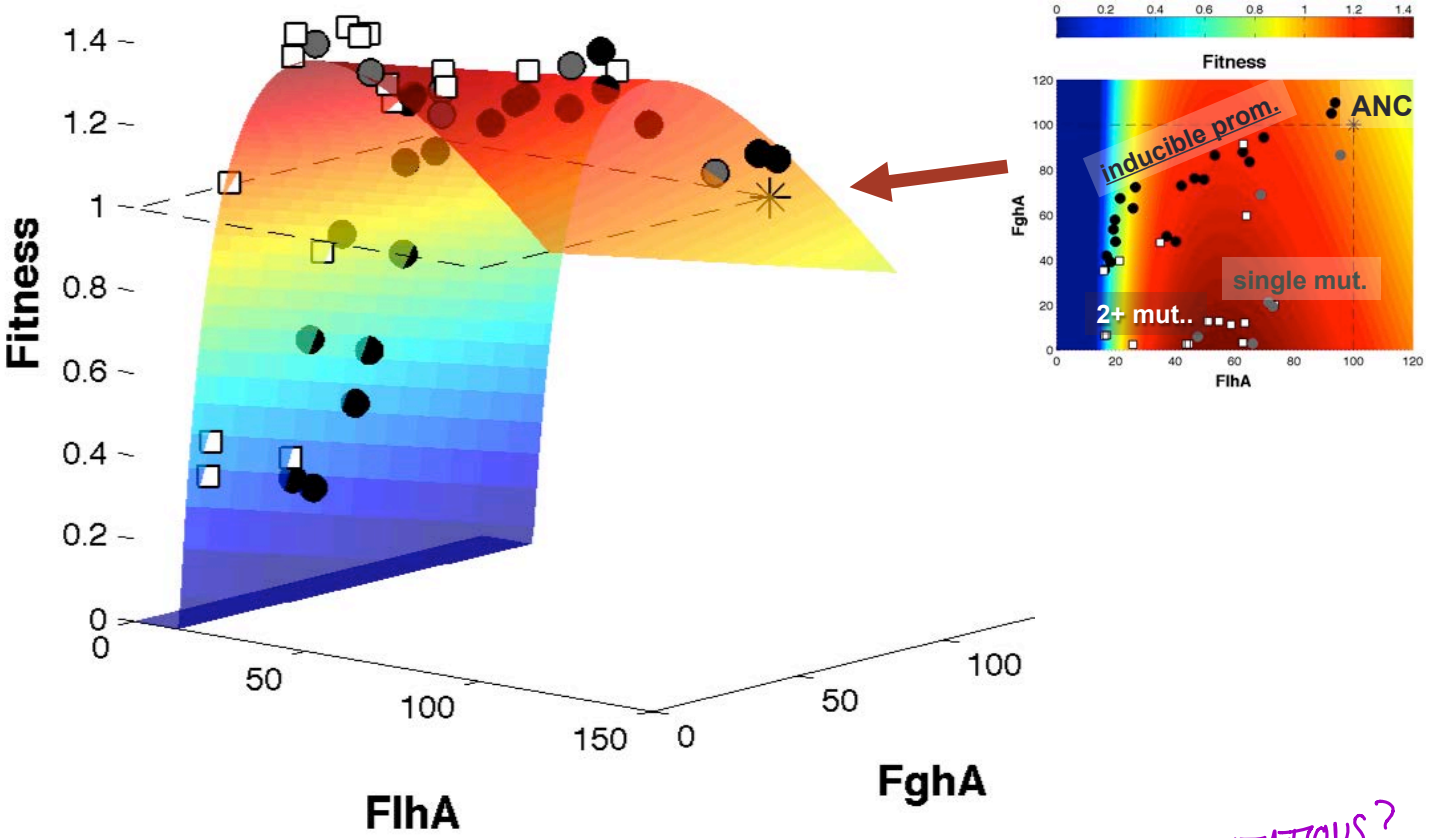
$$W = (v_{max} \times \mathbf{E_1}/(\mathbf{E_1} + E_{½\,max}) - v_T) - a \times \mathbf{E_1} - b \times \mathbf{E_2}$$



- Mechanistic model works quite well

(Chou et al., *PLoS Genetics*, 2014)

Interpret adaptation in light of model

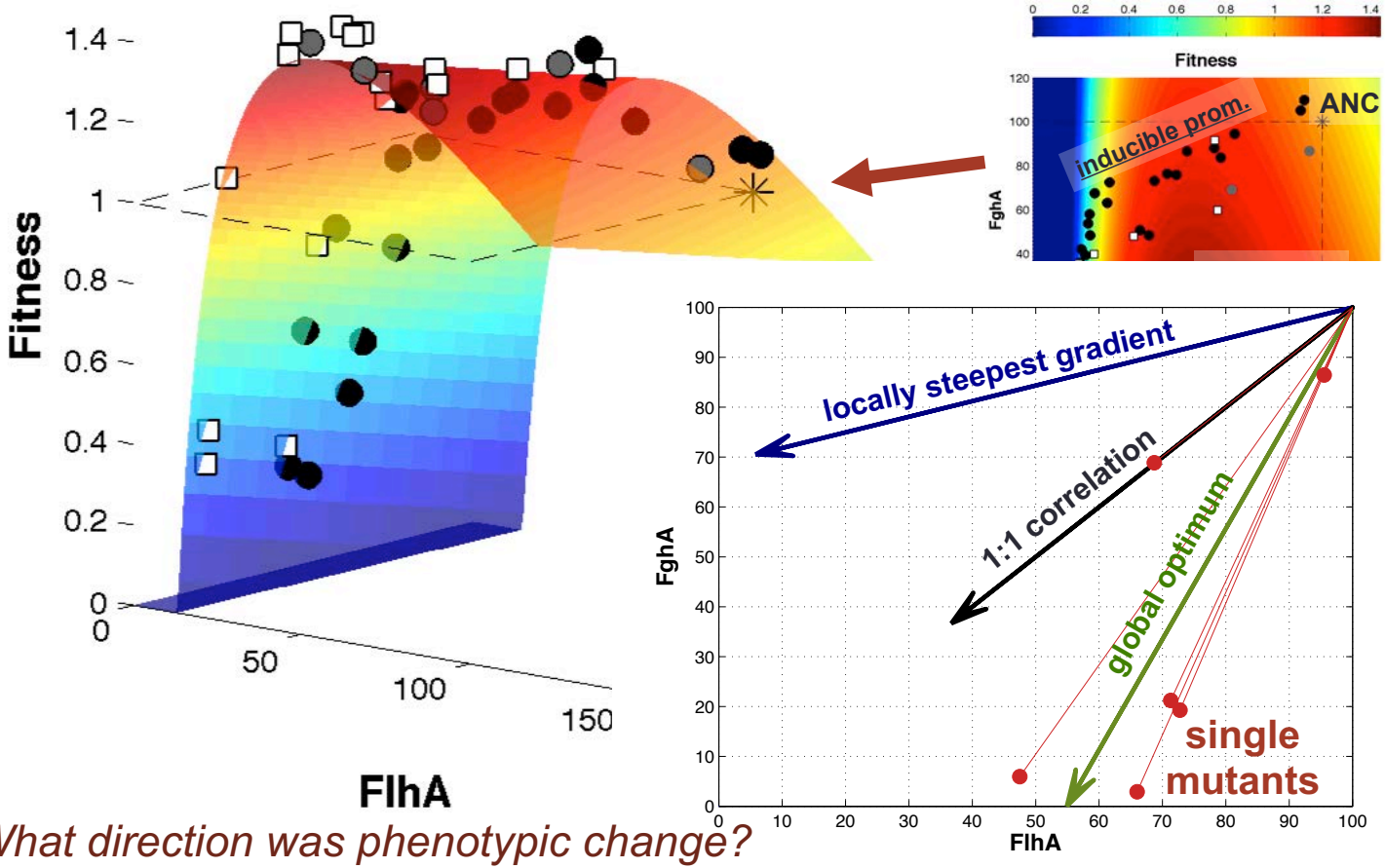


- *What direction was phenotypic change?*
- *How far to optimum was achieved?*

WHY NO PROMOTER MUTATIONS?

(Chou et al., *PLoS Genetics*, 2014)

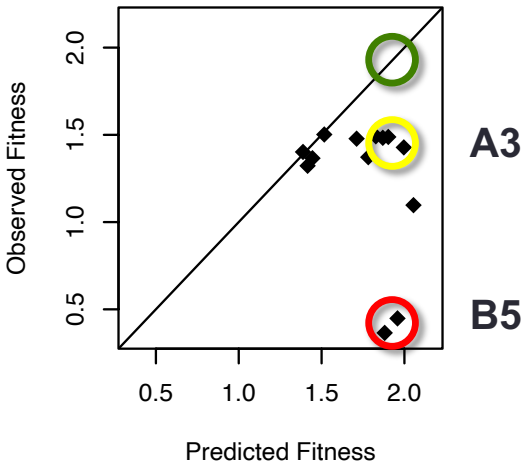
Interpret adaptation in light of model



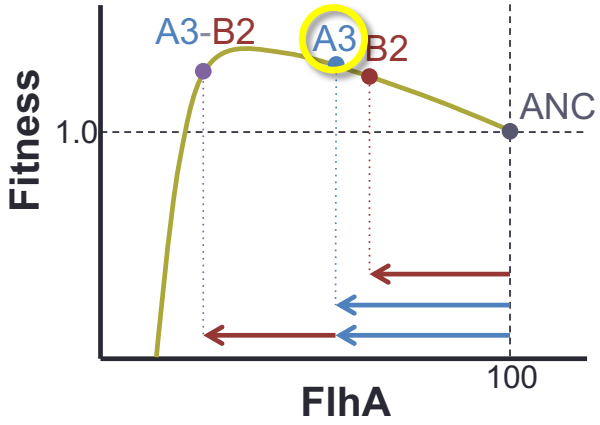
- *What direction was phenotypic change?*
- *How far to optimum was achieved?*

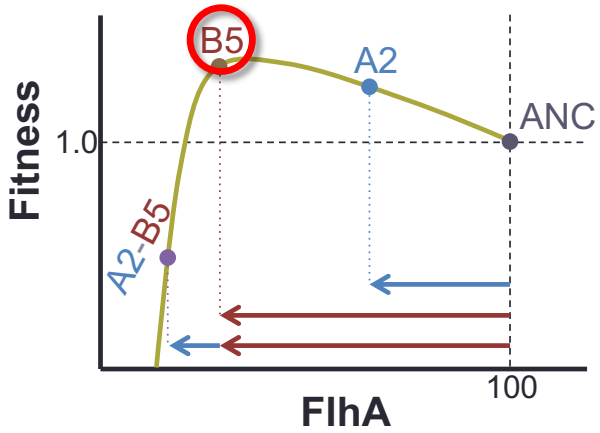
(Chou et al., *PLoS Genetics*, 2014)

Interpret interactions in light of model



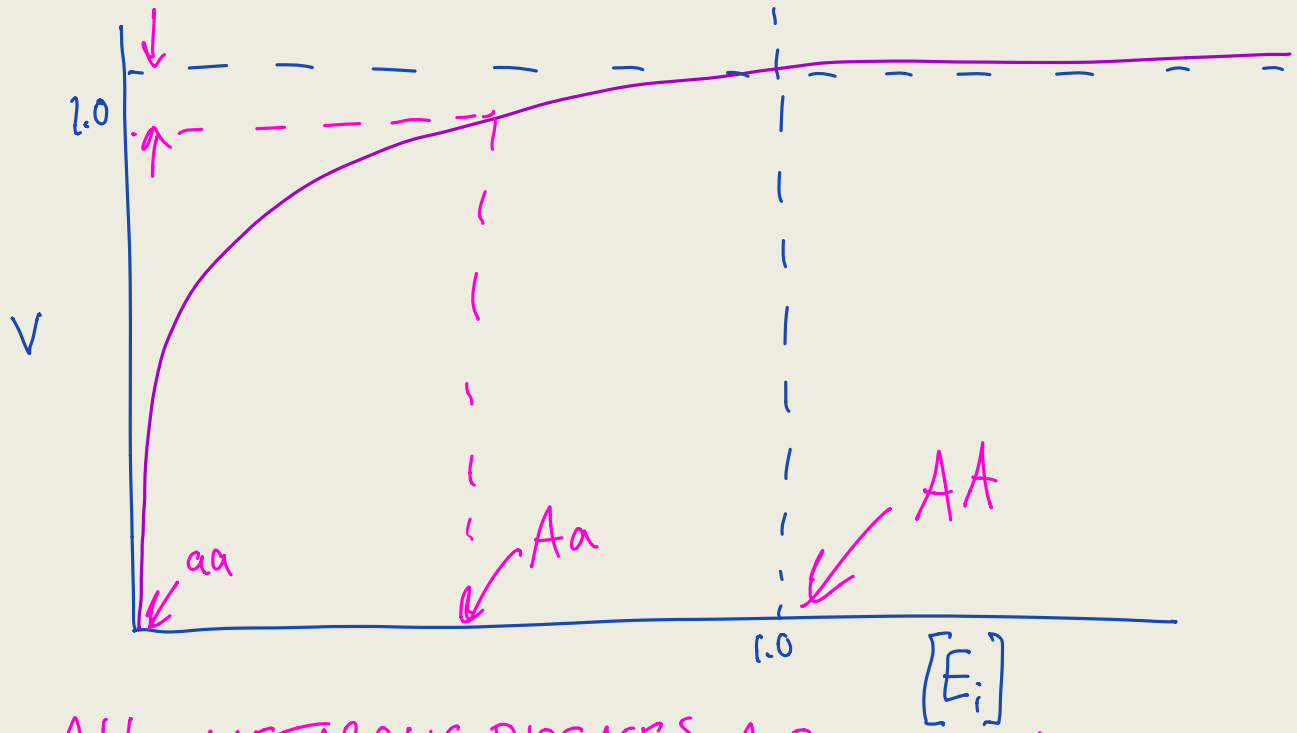
- A3, B5 same benefit (~0.45), but B5 has worse epistasis
- B5 on steep edge of peak…
- Combining expression-changing mutations may not speed adaptation





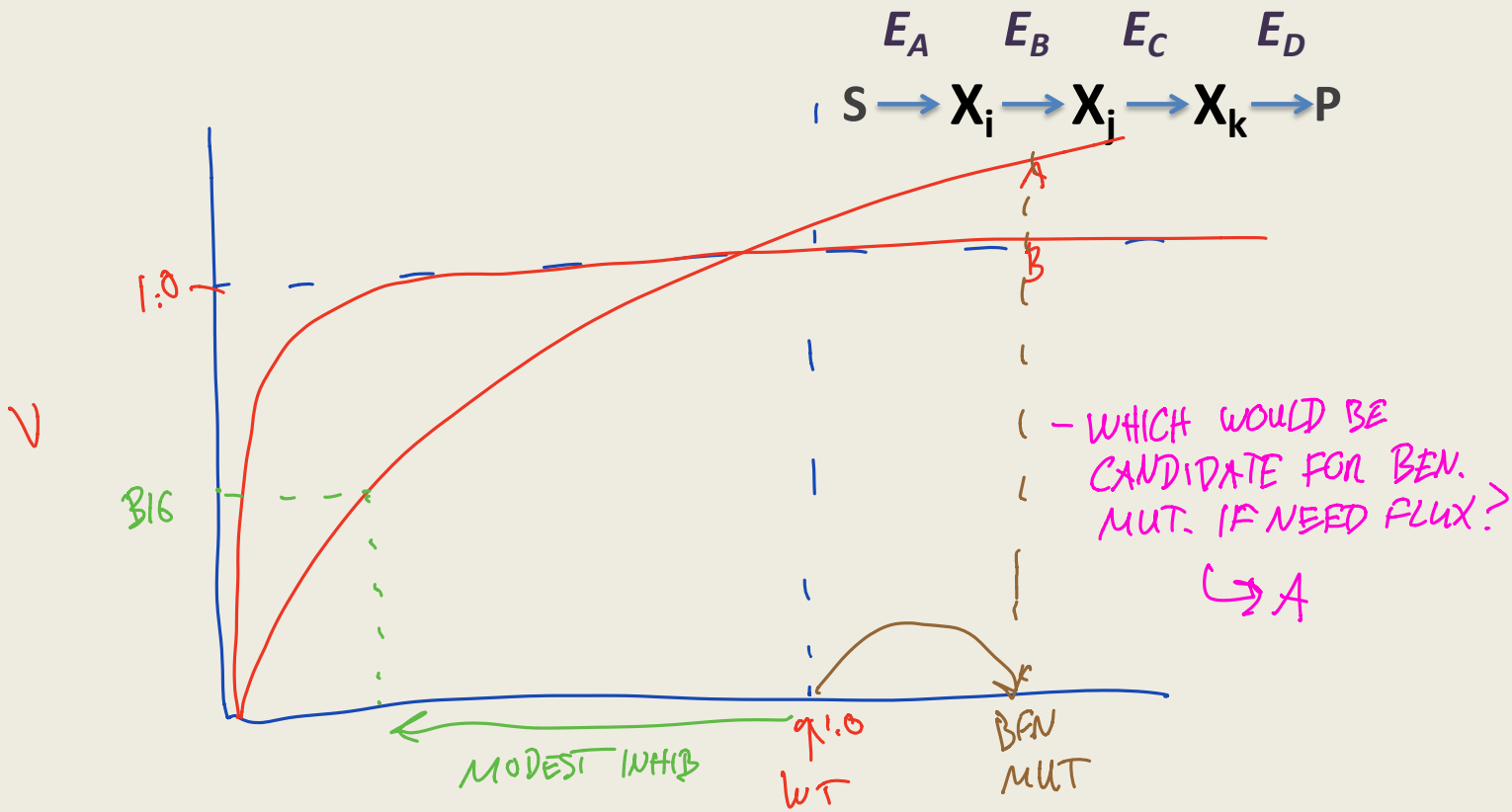
(Chou et al., *PLoS Genetics*, 2014)

Implication of MCA #1: dominance

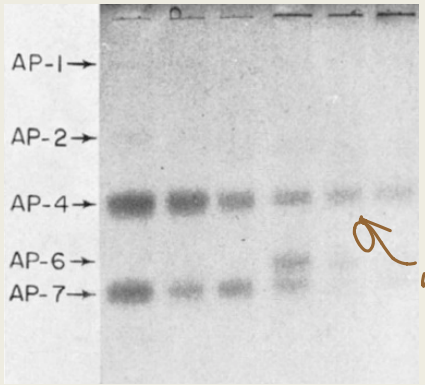


* ALL METABOLIC DISEASES ARE RECESSIVE
LOF ALLELES

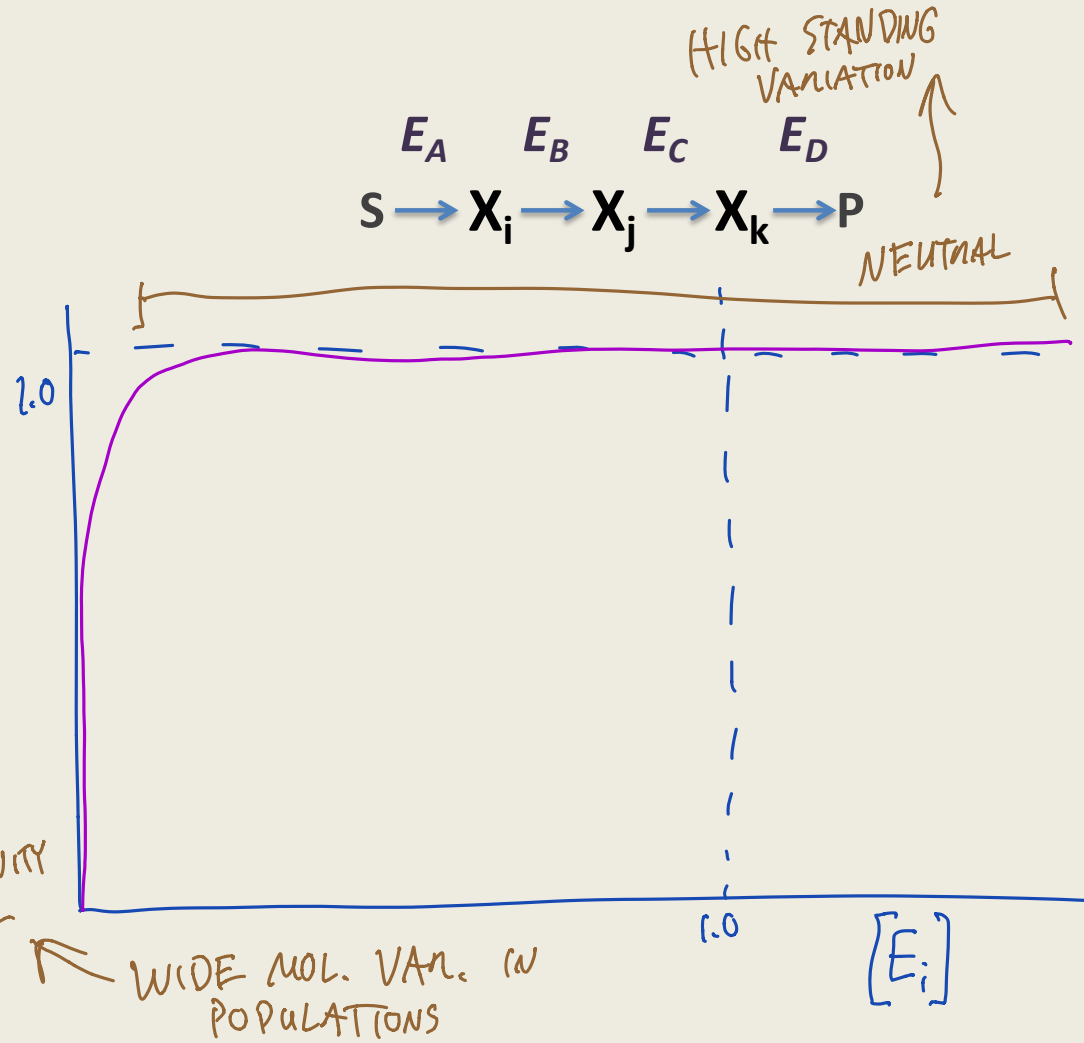
Implication of MCA #2: where to target drugs



Implications of MCA #3: selection vs. neutrality

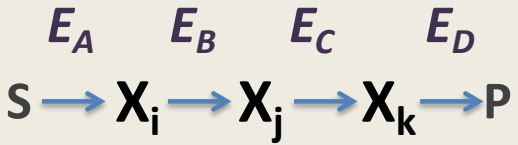


(Hubby and Lewontin, 1966. *Genetics*)



MCA: take-home messages

1. Because of saturation kinetics, there is not simply a single, rate-limiting enzyme.
2. Responsiveness of enzymes to [S] is set just by K_M and [S].
3. Flux responds linearly to changes in [E] if just one enzyme, or if all enzymes dialed up and down together.
4. Flux responds hyperbolically to changes in a single enzyme, the local slope of which is the "control coefficient", C_i.
5. Because $\Sigma C_i = 1$, most C_i are tiny.
6. Experimental tests in vitro and in vivo have confirmed MCA predictions.
7. Changes in one enzyme affect realized flux at other steps because of changing [Si] at the new SS flux.
8. Most enzymes are far from saturated, which is why each flux is responsive to alterations in other parts of the system.

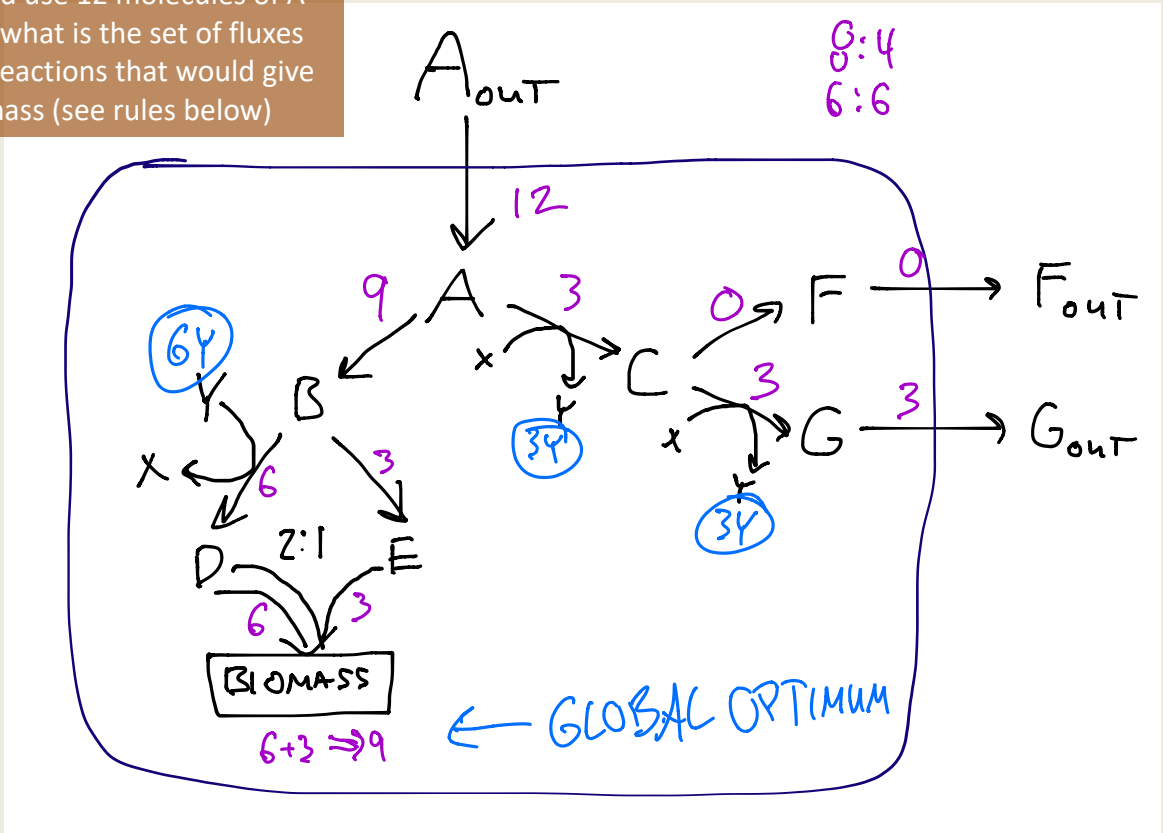


MCA explains:

1. Why metabolic diseases are recessive.
2. Why drugs effective on an enzyme often have little clinical effect.
3. Why much variation in populations is neutral.

A NEW CHALLENGE FOR YOU...

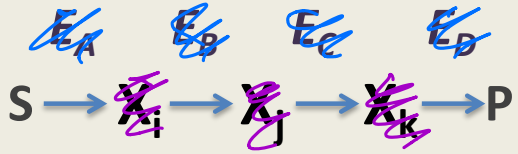
If this cell could use 12 molecules of A per unit time, what is the set of fluxes for the other reactions that would give the most biomass (see rules below)



Constraint: use no more than 12 molecules of A
Biomass: a 2:1 ratio of D:E
*Think of Y like ATP (X like ADP)

Flux balance analysis: what it isn't

What is ignored in FBA?



- NO ENZ. ACTIVITIES
- NO METAB. CONC.
- ONLY THING IS THE NET FLUXES

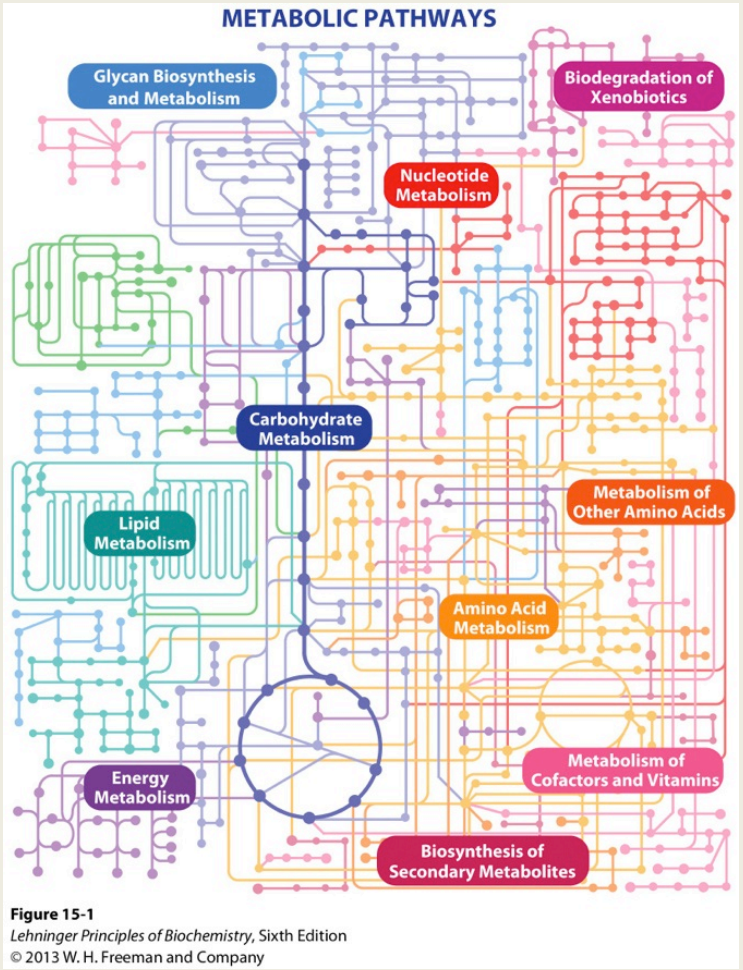
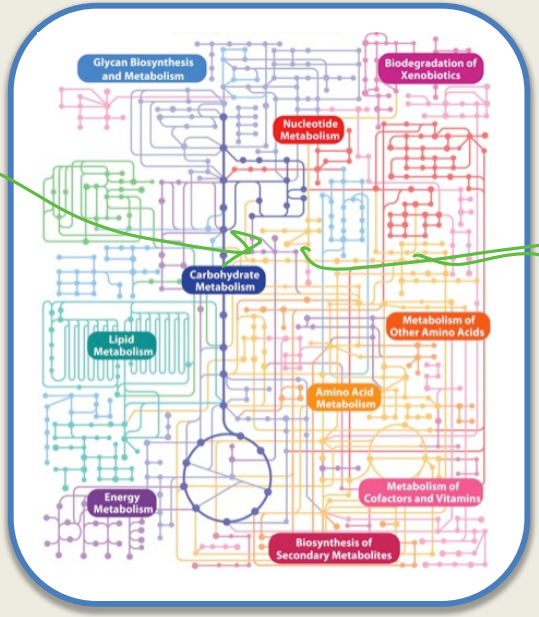


Figure 15-1
Lehninger Principles of Biochemistry, Sixth Edition

Flux balance analysis in a nutshell

FOOD

http://ant.seriouseats.com/archives/2009/04/mcdonalds.html



BIOMASS

http://uldissprogis.com/2014/05/

WASTE PROD.

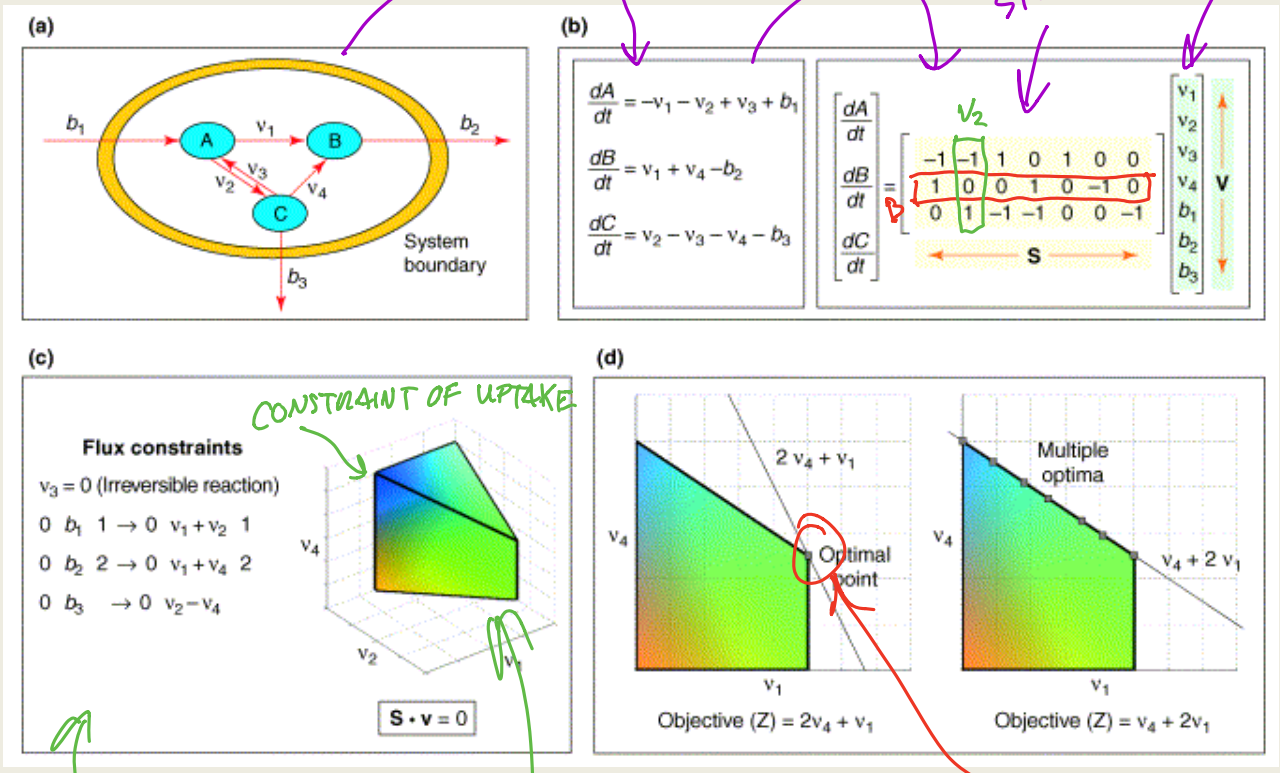
1. CONSTRAINTS UPON USE
   - USES KNOWN MET. RXNS
   - CAP AT LEAST ONE FLUX FOR TRANSPORT INTO CELL (USUALLY C SUBS.)

→ 2. NETWORK TOPOLOGY
   - BIOMASS COMPOSITION

3. OPTIMIZE BIOMASS ACCUM. W/IN FEASIBLE SPACE OF SS

Flux balance analysis: math

A, B, C ARE METABS.
v_i ARE FLUXES

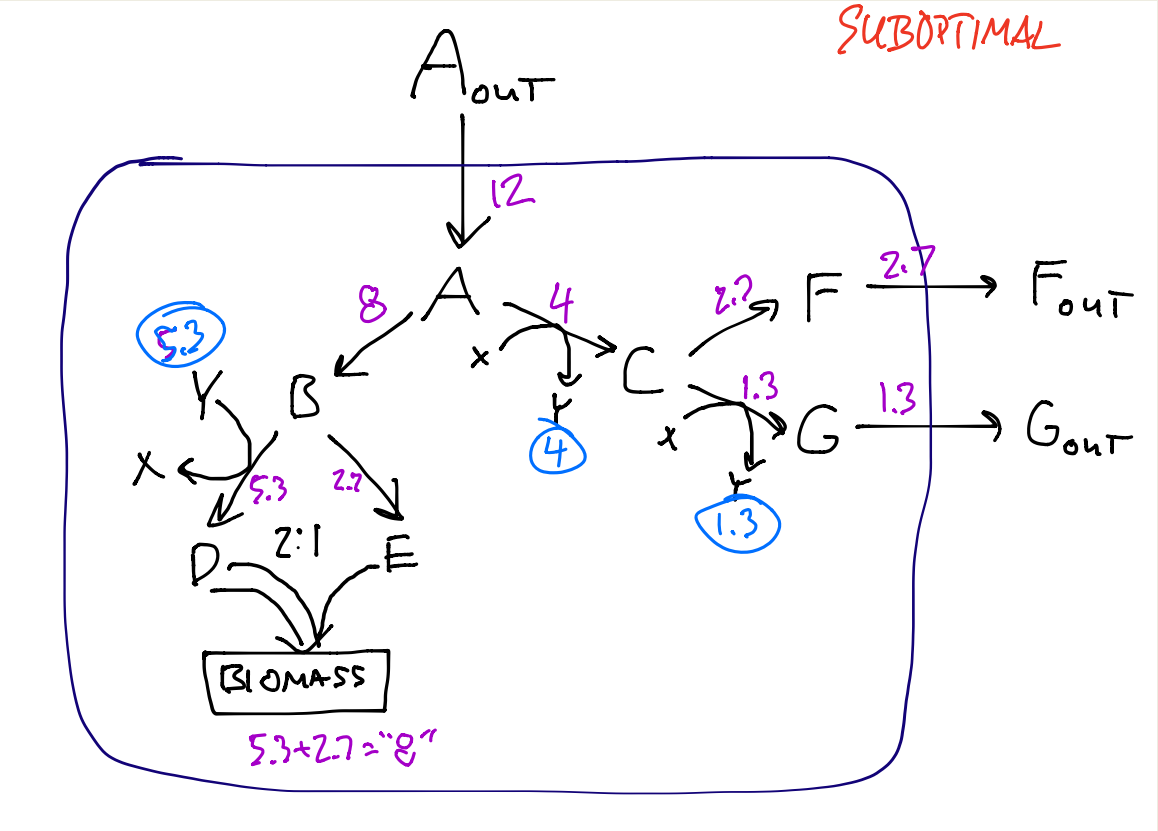


UNDERDETERMINED

FEASIBLE SS

PICK OPTIMUM
↳ ASSUMES PAST SELECTION FOR OPTIMALITY

Flux balance analysis: example #1

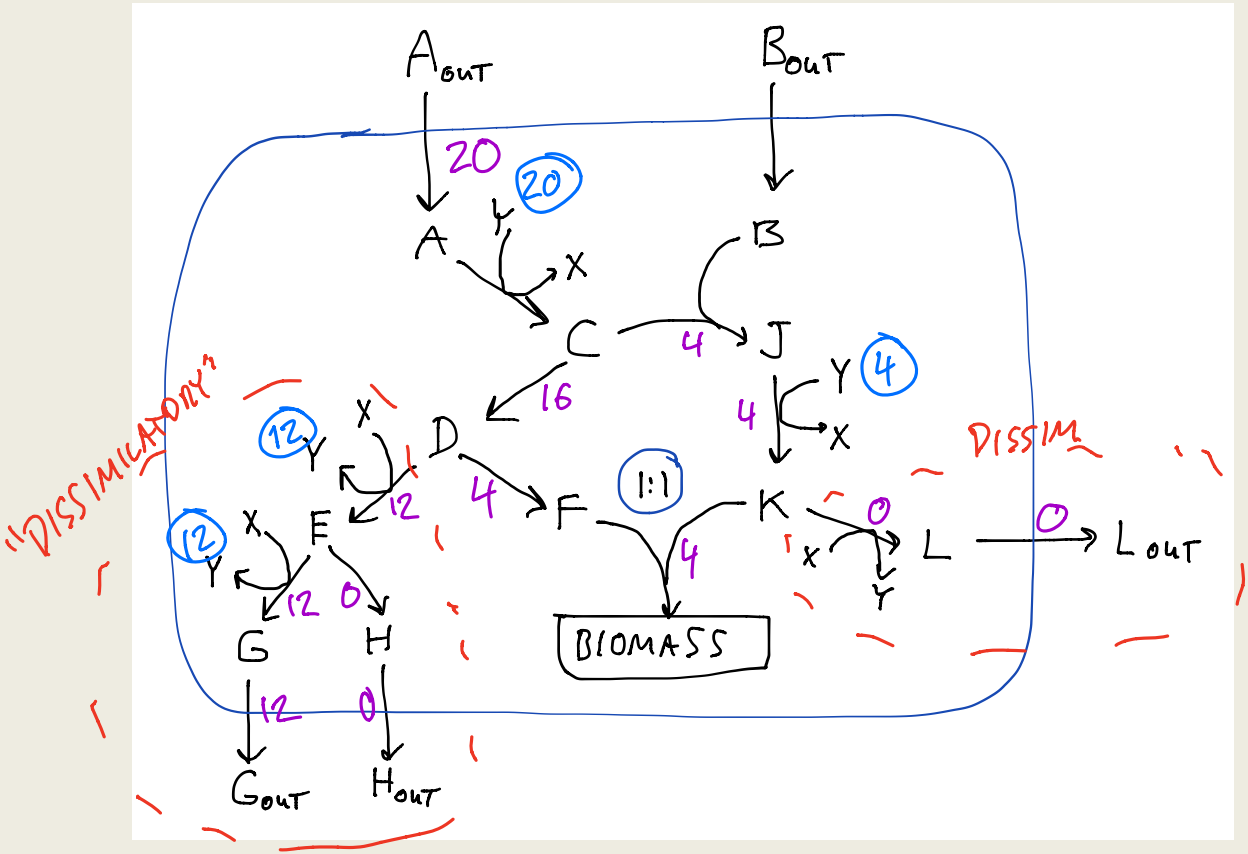


Constraint: use no more than 12 molecules of A

Biomass: a 2:1 ratio of D:E

*Think of Y like ATP (X like ADP)

Flux balance analysis: example #2

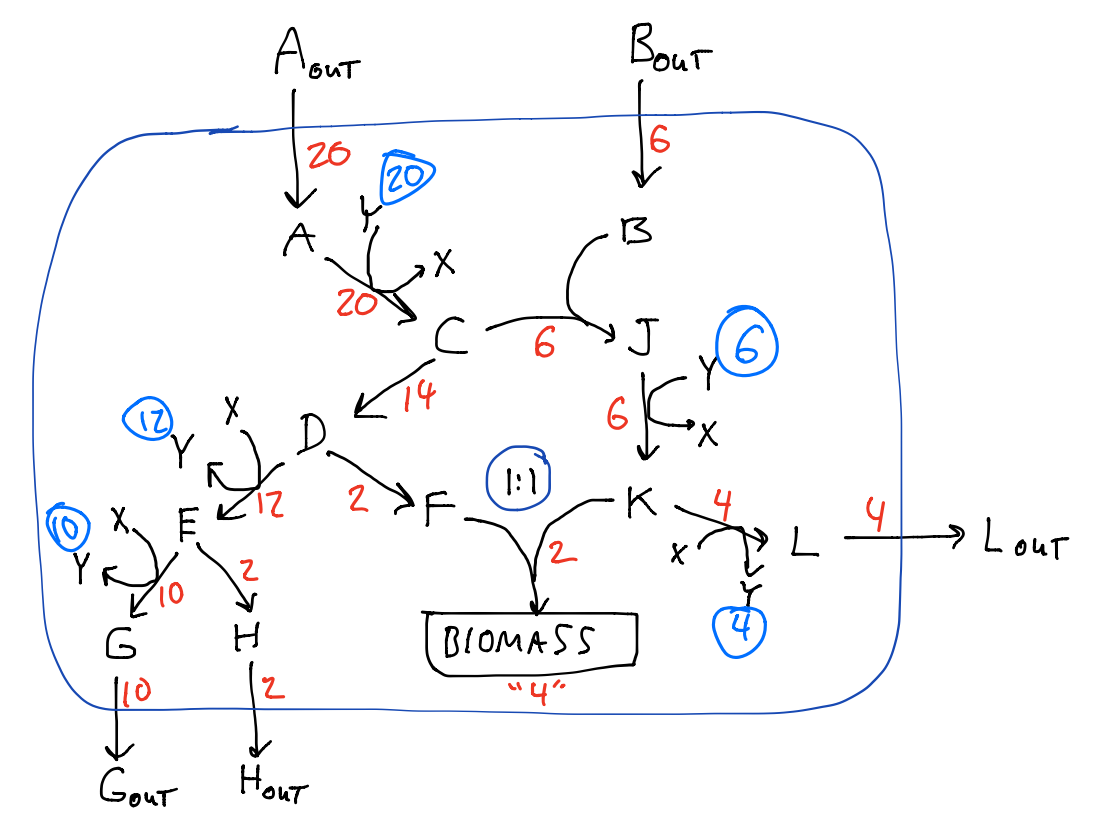


Constraint: use no more than 20 molecules of A; B is unconstrained

Biomass: a 1:1 ratio of F:K

*Think of Y like ATP (X like ADP)

Flux balance analysis: example #2 suboptimal

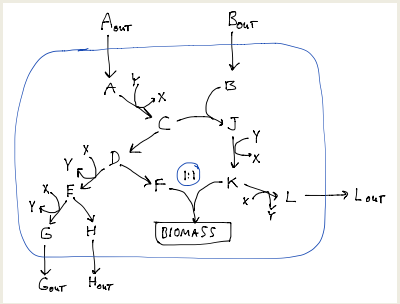


Constraint: use no more than 20 molecules of A; B is unconstrained

Biomass: a 1:1 ratio of F:K

*Think of Y like ATP (X like ADP)

FBA example #2 as stoichiometric network



Reactions:

1.) A+Y → C+X
2.) C → D
3.) B+C → J
4.) D+X → E+Y
5.) D → F
6.) E+X → G+Y
7.) E → H
8.) J+Y → K+X
9.) K+X → L+Y
10.) F+K → **biomass**

B1.) A_{out} → A
B2.) B_{out} → B
B3.) G → G_{out}
B4.) H → H_{out}
B5.) L → L_{out}

		reactions														
		1	2	3	4	5	6	7	8	9	10	B1	B2	B3	B4	B5
metabolites	A	-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	B	0	0	-1	0	0	0	0	0	0	0	0	1	0	0	0
	C	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	1	0	-1	-1	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	1	0	-1	-1	0	0	0	0	0	0	0	0
	F	0	0	0	0	1	0	0	0	0	-1	0	0	0	0	0
	G	0	0	0	0	0	1	0	0	0	0	0	0	-1	0	0
	H	0	0	0	0	0	0	1	0	0	0	0	0	0	-1	0
	J	0	0	1	0	0	0	0	-1	0	0	0	0	0	0	0
	K	0	0	0	0	0	0	0	1	-1	-1	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	1	0	0	0	0	0	-1
	X	1	0	0	-1	0	-1	0	1	-1	0	0	0	0	0	0
	Y	-1	0	0	1	0	1	0	-1	1	0	0	0	0	0	0
	biomass	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	A_{out}	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0
	B_{out}	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0
	G_{out}	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	H_{out}	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	L_{out}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

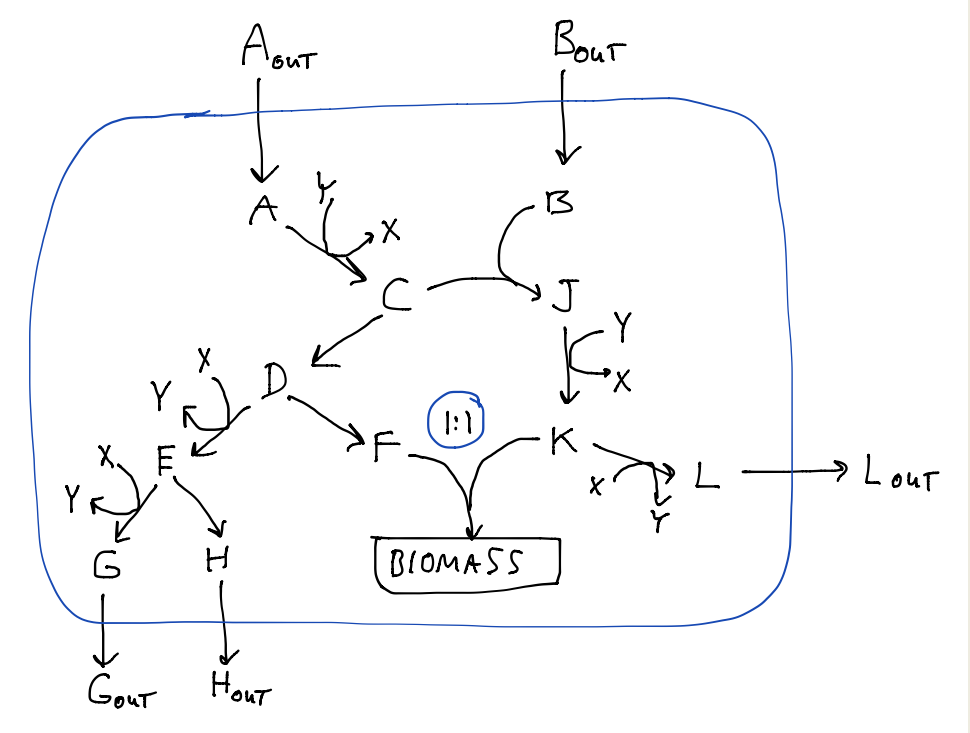
20
16
4
12
4
12
0
4
0
4
20
4
12
0
0

BOUNDARY FLUXES: TRANSPORT NOT $\frac{dx_i}{dt} = 0$ AT SS

FBA: test actual flux pattern

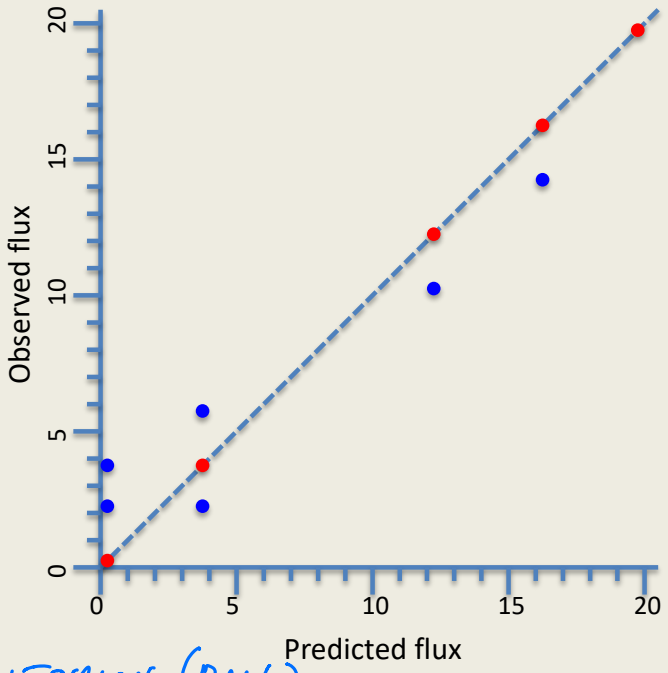
→ TEST KO MUTATIONS → VIABILITY

Deleted reaction	$flux_{opt}$	$flux_{sub\text{-}opt}$
1.) A+Y → C+X	20	20
2.) C → D	16	14
3.) B+C → J	4	6
4.) D+X → E+Y	12	12
5.) D → F	4	2
6.) E+X → G+Y	12	10
7.) E → H	0	2
8.) J+Y → K+X	4	6
9.) K+X → L+Y	0	4
10.) F+K → **biomass**	**4**	**2**
B1.) A_{out} → A	20	20
B2.) B_{out} → B	4	6
B3.) G → G_{out}	12	10
B4.) H → H_{out}	0	2
B5.) L → L_{out}	0	4



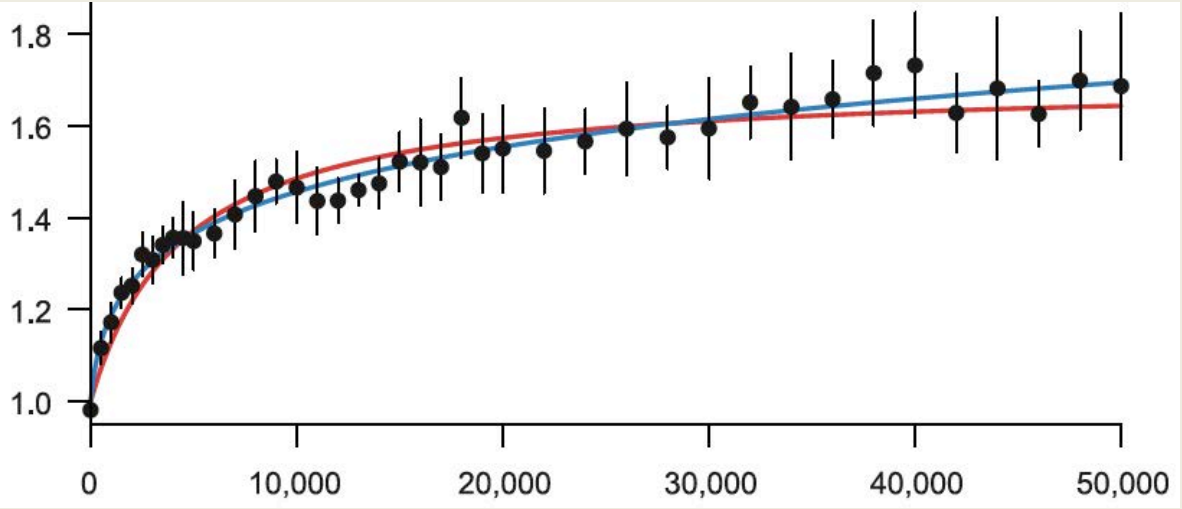
FBA: test actual flux pattern

Deleted reaction	$flux_{opt}$	$flux_{sub\text{-}opt}$
1.) A+Y → C+X	20	20
2.) C → D	16	14
3.) B+C → J	4	6
4.) D+X → E+Y	12	12
5.) D → F	4	2
6.) E+X → G+Y	12	10
7.) E → H	0	2
8.) J+Y → K+X	4	6
9.) K+X → L+Y	0	4
10.) F+K → **biomass**	**4**	**2**
B1.) A_{out} → A	20	20
B2.) B_{out} → B	4	6
B3.) G → G_{out}	12	10
B4.) H → H_{out}	0	2
B5.) L → L_{out}	0	4

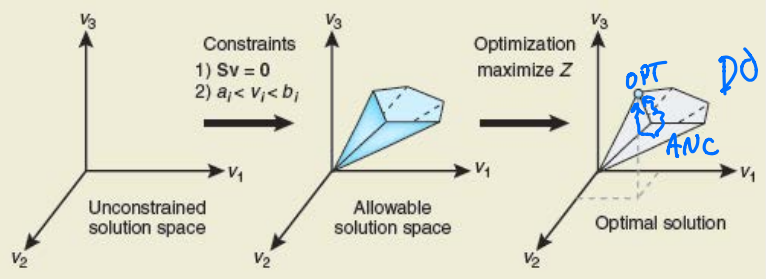


- RIGHT CRITERION (BM/S)
- SELECTION ON THAT SUBS TO REACH OPT.
- IS OPTIMUM POSSIBLE? (CONSTRAINTS, KINETIC PARAM)

FBA: tested in *E. coli* evolved for 25 years



(Wiser et al., 2014. *Science*)



DO THEY EVOLVE TO OPTIMUM?

Why might optimizing BM/S be an issue?

~GOOD CHOICE FOR OPTIMALITY CRITERION IN BATCH CULTURE?

- What FBA optimizes (BM/S, efficiency):

http://www.toyotainthenews.com/the-3rd-generation-prius/

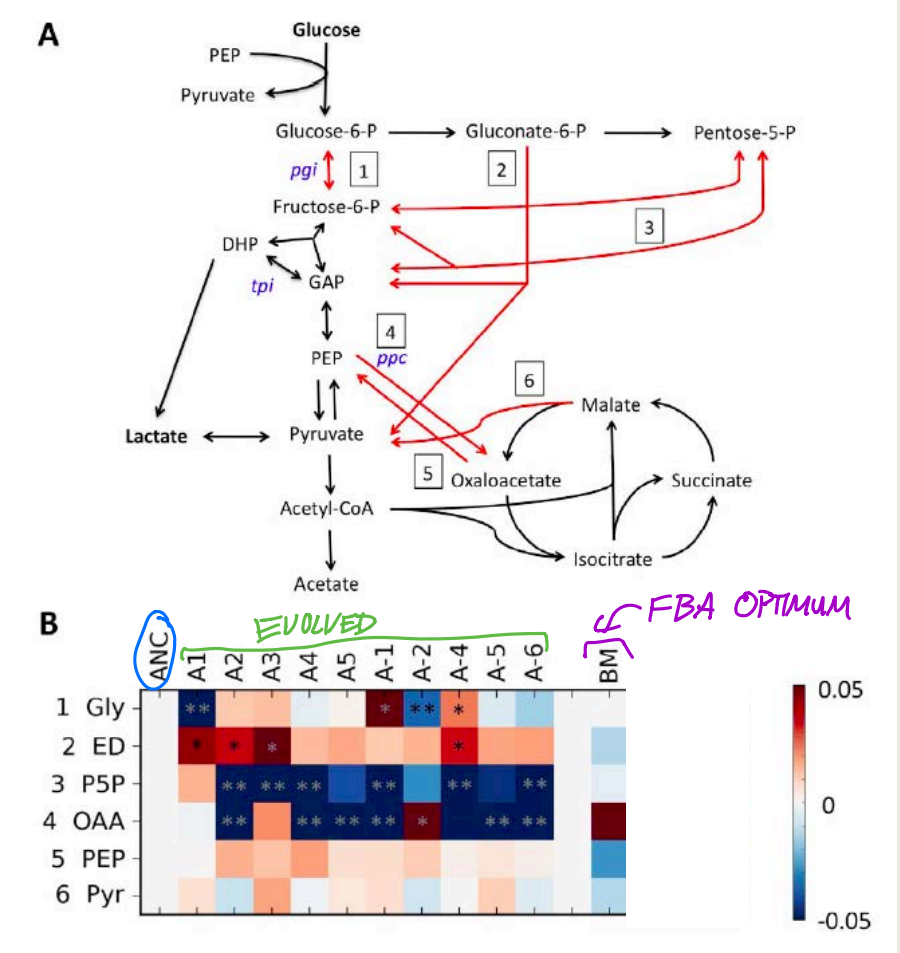
- What batch culture selects for (BM/time, rate):

http://flatsixes.com/porsche-motorsports/2010-volume-2/

FBA: tested in *E. coli* evolved for 25 years

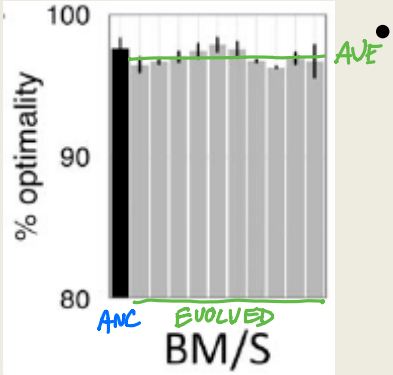
- **Rate** of glucose use increased in all evolved
- Measured **flux ratios** throughout central metabolism
- Assayed ancestor (ANC), many evolved strains.

1. WERE SIGNIFICANT CHANGES
2. ISOLATES DISTINCT FROM EACH OTHER
3. DID NOT LOOK LIKE PREDICTION



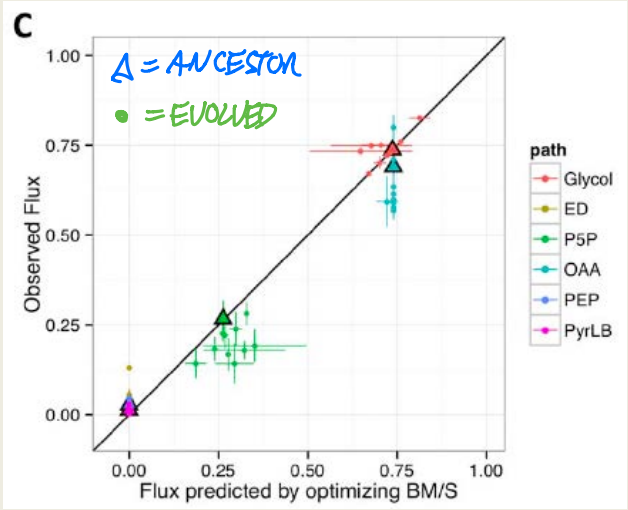
(Harcombe et al., 2013. *PLoS Computational Biology*)

E. coli evolved on glucose for 50K generations to have lower yield and less like FBA predictions



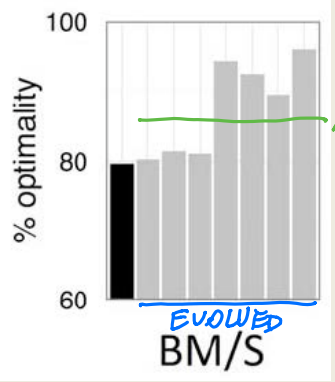
- Overall biomass yield:
  - ANCESTOR 98% OF OPTIMUM
  - EVOLVED 97% OPTIMAL

- Fluxes compared to predictions:
  - ANCESTRAL FLUXES WELL-PREDICTED
  - EVOLVED TO BE LESS WELL-PREDICTED



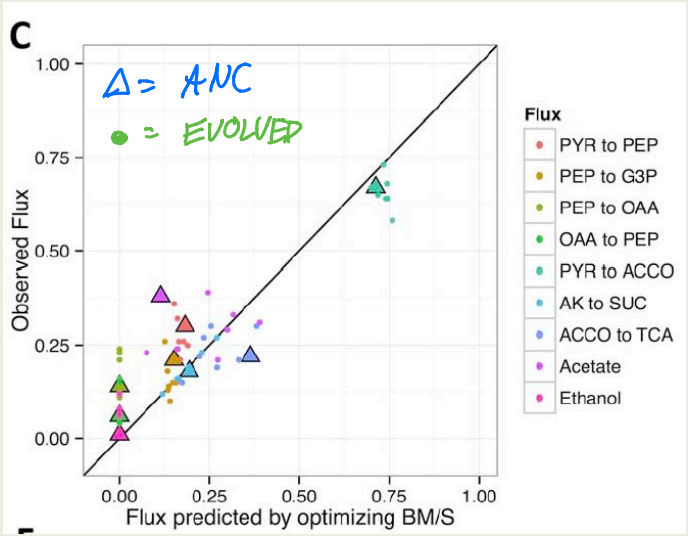
(Harcombe et al., 2013. *PLoS Computational Biology*)

E. coli evolved on lactate for <1K generations to have increase yield and less like FBA predictions



- Overall biomass yield:
  - ANCESTOR VERY SUB-OPTIMAL
  - EVOLVED STRAINS MORE OPTIMAL (FOR YIELD)

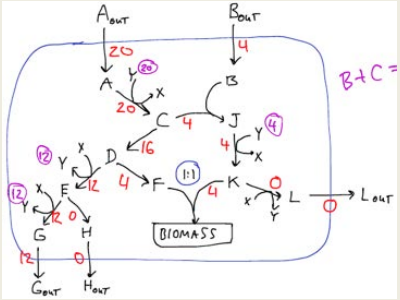
- Fluxes compared to predictions:
  - STRAINS EVOLVED TO BE BETTER PREDICTED



(Harcombe et al., 2013. *PLoS Computational Biology*)

FBA: take-home messages

1. FBA is an optimality model that seeks to predict how cells *should* use metabolism.
2. FBA only considers steady-state, balanced fluxes through reactions; there are no enzyme parameters or concentrations.
3. FBA first calculates all possible steady-states within uptake constraints, and then asks which of these maximizes biomass (assuming past selection for this).
4. FBA uses linear algebra (S matrix x flux vector = 0) to calculate the "feasible space" of steady-states, and then uses linear optimization to find the best solution.
5. Cells have to perform this "calculation" to be able to grow at a consistent rate.
6. Experimental evolution offers opportunity to test optimality assumption of FBA.



FBA explains:

1. Which enzyme deletions are lethal or detrimental.
2. Close to the actual use of fluxes in the cell and what is excreted from them.
3. Adapted cells may evolve to be more or less well-predicted, but have flux pattern close to suggested optimum.