Chemical Engineering 170B
Spring 2009
Midterm #2

Metabolism. The free energies of hydrolysis (in kcal/mol) for several phosphorylated compounds are given: **(12 points. 1 point each answer plus 2 points bonus for actually putting it in a table.)**

Compound	ΔG°'
Phosphoenolpyruvate	-14.8
Carbamoyl phosphate	-12.3
Acetyl phosphate	-10.3
Creatine phosphate	-10.3
Pyrophosphate	-8.0
ATP	-7.3
Glucose 1-phosphate	-5.0
Glucose 6-phosphate	-3.3
Glycerol 3-phosphate	-2.2

Assume the cellular ATP/ADP ratio is 20. (a) Calculate the equilibrium constant K'_{eq}, (b) predict the direction, and (c) determine the equilibrium ratios of reactants to products of the following reactions. Express your answer as a small table.
ATP + carbamate ⇔ carbamoyl phosphate + ADP
ATP + acetate ⇔ acetylphosphate + ADP
ATP + inorganic phosphate ⇔ pyrophosphate + ADP
ATP + glucose ⇔ glucose 1-phosphate + ADP

We work this problem out like the homework.

ATP + carbmate ⇔ carbamoyl phosphate + ADP
ATP ⇔ ADP + Pi -7.3
carbamate + Pi ⇔ carbamoyl phosphate 12.3
Sum: dG = 5

$dG = -RT \ln K_{eq}$

$K_{eq} = \exp(-dG/RT) = \exp(-5 \text{ kcal mol}^{-1}/(0.001985 \text{ kcal mol}^{-1} \text{ K}^{-1} * 310 \text{ K})$
= predict LEFT, Reactants
Equilibrium ratio of 5.92e-3 ($20*K_{eq}$)

Table:

Reaction	i	ii	iii	iv
a	2.96e-4	7.63e-3	3.21e-1	4.20e+1

b	**Reactants**	**Reactants**	**Reactants***	**Products**
c	**5.92e-3**	**1.53e-1**	**6.41**	**8.40e+2**

iii: The direction is actually changed by the number of ATP/ADP!

Partial credit was assigned if your answer was self-consistent. Here's my calculations:

Flux balance analysis. Below is a simple metabolic network in *E. coli.* (18 pts)

Write the stoichiometric balances for each of the numbered reactions. (5 points)

For instance,

1	**– ½ glucose + PEP + NADH = 0**
2	**– PEP – CO2 + oxaloacetate = 0**
3	**– PEP + pyruvate + ATP = 0**
4	**– pyruvate + lactate = 0**
5	**– pyruvate + acetyl–CoA + formate =0**
6	**– formate + CO2 + H2 = 0**
7	**– acetyl–CoA + acetate + ATP = 0**
8	**– acetyl–CoA – NADH + ethanol = 0**
9	**– oxaloacetate – NADH – NH3 + aspartate = 0**
10	**– aspartate – NH4 + asparagine = 0**
11	**– aspartate – NADH + NH3 + succinate = 0**

Express the balances above as a matrix equation at steady state in a continuous reactor. Use vectors of [substrates], [products], and [intracellular metabolites]. DO NOT SOLVE. **(5 points, the columns could be different orders dependent on how the student defined his/her scalars)**

Partial credit was assigned for (1) the proper setup of the matrix, (2) correct number of reactions (y-direction), (3) correct number of products/ intermediates (x-direction), and correct attempts to fill in the matrix.

Part (b) asks you NOT to solve the matrix equation. Why might this not be possible? Is the system overdetermined or underdetermined? (4 points)

First, it's way complicated. None of the matrices are square.
Number of reactions, m: 11
Number of metabolites, n: 18 (1+8+9)
F = m-n = -7. System is underdetermined. Additional constraints would be required to solve. Usually systems have a lot more reactions than metabolites, however.

You are interested in producing asparagine as a commercial product using this system. What can you do to encourage this? What must the system do in response? (4 points)

Might knock out or knockdown reaction 11 producing succinate; in which case the cells would have to make succinate some other way (e.g. overexpressing the TCA cycle or a portion thereof)

Might overexpress reaction 10, but may drain the cell of ammonium reserves

Might downregulate reaction 3, but then the cells will need to get their glycolysis or TCA cycle components otherwise.

3. A pathway for converting glucose-6-phosphate to artemisinin: **(20 points)**

The DXP pathway is native to *E. coli.* List at least two advantages and two disadvantages to using the native pathway in terms of downstream synthesis. (4 points)

Advantages: No cloning needed. More likely to work in native system since it already works.

Disadvantages: Side products may be used in other cellular reactions. Perturbation might cause deleterious effects.

The mevalonate pathway is native to yeast but was expressed in *E. coli*. List two advantages this incurred. (2 points)

Metabolites are not used in other pathways
More finely regulateable.

What were the two major drawbacks to using a mevalonate pathway? How did the researchers increase the flux through the pathway in spite of these? (6 points)

Cloning was necessary: codon optimized and regulated intergenic DNA regions to allow expression.

HMG-CoA intermediate was toxic. Downregulated its production and upregulated its uptake

Other answers are acceptable for partial credit

Diagram and describe a way to screen for mutants that confer increased flux through the mevalonate pathway. (4 points)

Biosensor fluorescent screen

Basically a mutated pathway in the first microbe that produced more mevalonate would upregulate more GFP using the mevalonate auxotroph. More GFP = better growth of the auxotroph = better mevaolonate production in the first strain.

Both the mevalonate and DXP pathways were also cloned for expression into yeast, but it resulted in lower titers. What advantage did this confer in terms of the overall picture? (4 points)

Yeast pathway makes a more native environment more amenable to act as host for screening a library of potential artemisinic acid enzymes.

4. *Microarray data.* Expression profiles of 657 *E. coli* stress response genes for nine different stress conditions. Color bar values correspond to $\log_2$-fold changes of gene expression values for stress versus controls. Box plot of the expression profile of the 222 genes that are highly up or down regulated under all nine stress conditions. (Stats refresher: Notches indicate 95% confidence interval for the median, and whiskers are two standard deviations) (**15 points)**

Describe the characteristics of each of the three clusters (by the black vertical lines).
(4 points)
Genes highly upregulated (dark blue)
Some significantly up/down regulated, mostly minor changes
Little dramatic change

Which experimental conditions had the most similar response, cold and uvb, or wounding and drought?

Cold and uvb (closer clustering), 2 points

(most people looked at bar plot instead of clustering)

Which stress condition had the most significantly down-regulated genes? Which had the most variance?

Looking at the box plot: (4 points)
Osmotic stress had the most downregulated genes (lowest average)
Heat stress had the most variance (widest 95% confidence interval)

Why do we use $\log_2$ ratios for this type of data?

(5 points) If condition A=1 and condition B=1, there is no change due to your treatment (a normal ratio of 1/1 = 1 results)

With $\log_2$ ratios, $\log_2(1)=0$, thus 0 = no change, which is what you observe biologically. This makes more sense than e.g. $\log_{10}$ ratios, largely because the changes in biological systems you see are on this order. Biological systems also tend to change on logarithmic, not arithmetic scales.

5. (20 points)

2 for each b/y ion (20 points among 10 ions)
1 for correct sequence (½ for 3 correct)
1 for justifying the choice of F instead of Mo since there were 64 Da losses, order D/F
3 correct b_2 ion pairs from Fig 4.2

25 points total

(10 points)
This question is very free form, just about anything goes so long as it is correct. Partial credit was assigned for the length and detail of your answer.

EMBED Equation.3