Biochemistry 673 **Your Name:** _____

Regulation of Metabolism **Assoc. Prof. Jason Kahn**

Final Exam (150 points total) **May 17, 2005**

You have 120 minutes for this exam.

Explanations should be concise and clear.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the exam time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. Glycogen Metabolism, Diabetes (50 pts):

(a; 12 pts) Sketch the bicyclic kinase cascade that activates glycogen breakdown and inhibits glycogen synthesis in response to glucagon. You need not include the regulation of protein phosphatases, but include the production of the classic second messenger cAMP.

(b; 8 pts) Name another second messenger, and briefly describe how it is produced and destroyed.

(c; 6 pts) How do excessive levels of circulating free fatty acids lead to type II diabetes, and why does the diabetic phenotype eventually become irreversible?

(d; 9 pts) What is a glucose tolerance test? Sketch typical test results for a normal mouse versus a diabetic mouse.

(e; 15 pts) Sketch the pathway leading from insulin signalling to activation of Akt/PKB. Speculate on why so much regulation occurs at the plasma membrane in general.

2. G-proteins and GPCRs. Miscellaneous (30 pts):

We have discussed the idea that turning activities and signals off is just as important as turning them on.

(a; 9 pts) How is PI3K signalling turned off? How is glycogen phosphorylase turned off? How is estrogen receptor signalling turned off, other than by removal of ligand?

(b; 10 pts) What is the built-in off switch for signalling by GTP-binding proteins such as Ras? In the language of G-proteins, what are GPCRs? Why do they tend to activate rather than repress activity? How do the $G_{\beta\gamma}$ subunits maintain the G_{α} G-protein in the off state?

(c; 6 pts) Describe how GPCR signalling is turned off after activation. Hint: how does this lead to a built-in time delay before the system is responsive again?

(d; 5 pts) Briefly discuss why the X-ray structure of rhodopsin is not as useful as one might have hoped for modeling other GPCR structures.

3. Apoptosis (35 pts):

(a; 10 pts) In general, what kinds of stimuli activate the intrinsic and extrinsic pathways of apoptosis? What are the two general kinds of caspases, and how are they activated?

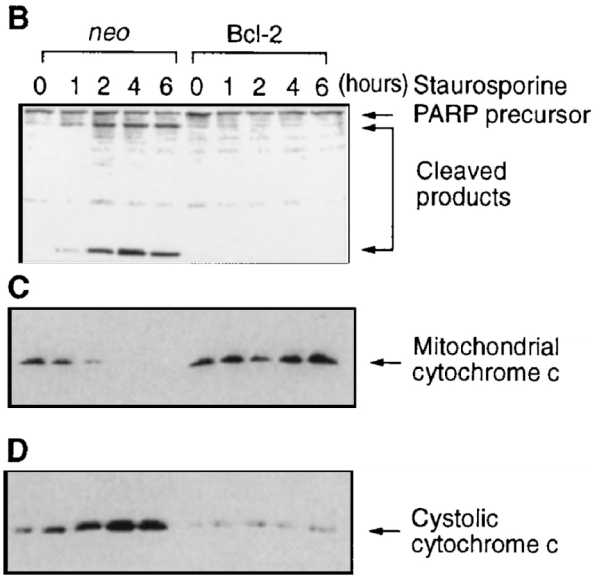
(b; 8 pts) What aspects of the mechanism of apoptosis contribute to its irreversibility, and why is it so important that the decision be irreversible?

(c; 6 pts) The idea of the selfish gene is that we are all just containers for our genes. How, then, can a pro-apoptotic gene like Bax or Bak arise? Why does it make sense that the default pathway for our cells is apoptosis rather than proliferation?

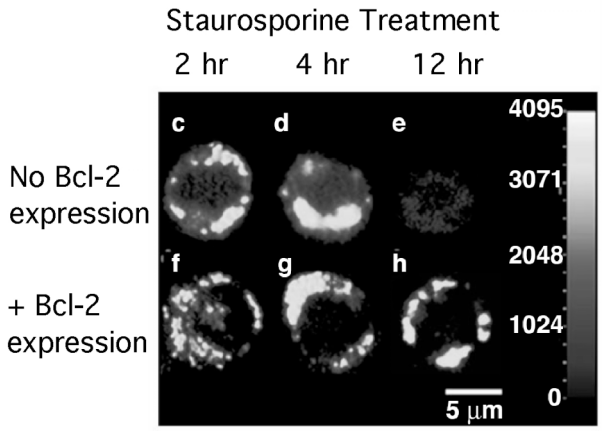
The figure at the right shows experiments designed to probe the role of Bcl-2 in controlling apoptosis.

(d; 2 pts) In one word, what does the cytochrome c release experiment tell us about the mechanism by which release causes apoptosis?

(e; 3 pts) Very briefly describe a common assay for apoptosis that isn't shown here.



(f; 6 pts) The bottom images (at right) show uptake of rhodamine followed by confocal imaging. Why is rhodamine taken up by active mitochondria and not those that have undergone the permeability transition? Why do these experiments suggest that the permeability transition is a downstream event rather than the trigger for apoptosis? (More space on next page if you need it)

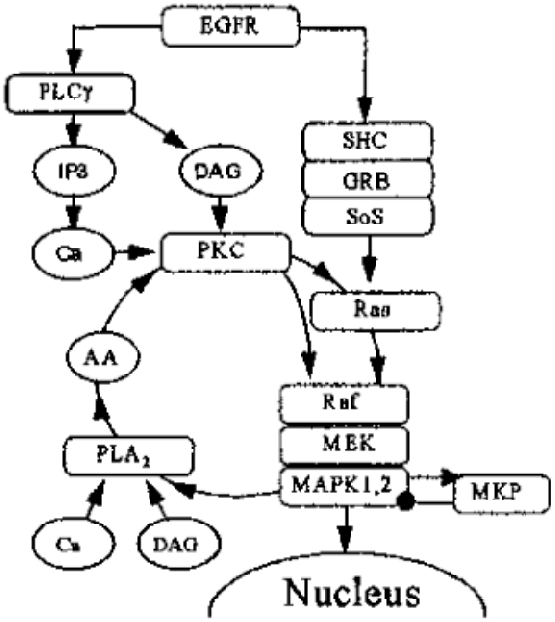


4. Network thinking (35 pts):

One example of an "emergent property" arising from a complex signalling network is the appearance of bistability, in which a network represses small variations in e.g. kinase activities, but a large enough imposed change snaps the system into a new stable state.

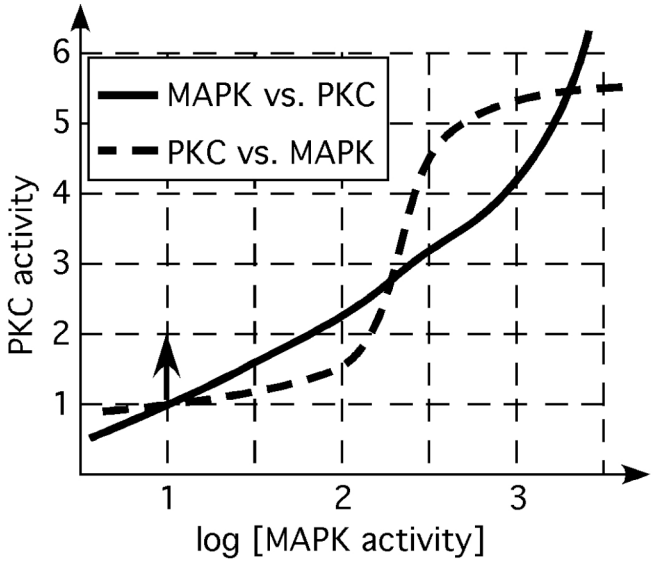
(a; 2 pts) We named at least one other "emergent property." What is it?

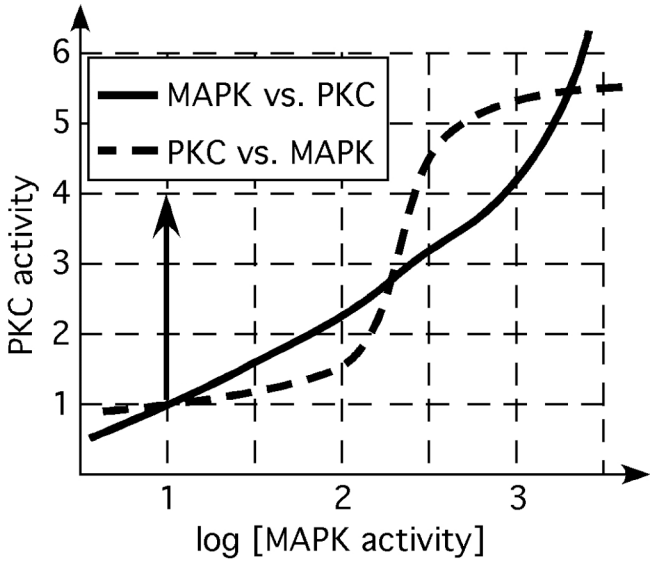
Parts b-d continue on the theme of emergent properties, referring to the signalling network diagrammed at the right.



The graphs below show the activity of PKC on the *y*-axis, the activity of MAPK on the *x*-axis. The dashed line is the level of PKC obtained if the MAPK activity is held fixed (in the computer). The solid line is the log(MAPK activity) at fixed PKC.

(b; 8 pts) If the system is originally stable at (PKC, log(MAPK) = (1,1) and a pulse of DAG is applied to raise the PKC activity to 2, sketch the system's response on the left-hand graph below. On the right-hand graph, sketch the response to a pulse of PLCγ sufficient to give a PKC activity of 4.





(c; 5 pts) In the absence of MAP kinase phosphatase (MKP) activity, would the system have a stable state? If so, what would it be?

(d; 5 pts) In the absence of phospholipase A_2 (PLA_2) activity, would bistability be observed? Why or why not?

E-mail spam filtering can be done in many ways. One way is simply to flag messages that contain particular words (examples are not needed…). These filters are easily fooled by alternative spellings and other tricks. "Bayesian" spam filters are an improvement. They calculate the probability of an e-mail being spam and then flag it if the probability exceeds some threshold. The Bayesian filters start with the programmer's rules and then "learn" by recording what an individual user flags as spam versus genuine e-mail.

(d; 8 pts) What fundamental characteristic of Bayesian methods forms the basis of both the spam filter above and the extraction of signalling network architectures from noisy data? Briefly explain why this idea suggests that you should switch doors in the game show scenario we discussed in class.

(e; 4 pts) Pursuing an analogy between signal network models and spam filtering, what is the spam-filtering analog of the idea that we perturb cell signalling in order to test and refine network models?

(f; 3 pts) What is the cell-signalling equivalent of the fact that some people actually are interested in Rolex replicas or right-wing German propaganda, or not interested in global warming?

Question	**Score**
1	_____/50
2	_____/30
3	_____/35
4	_____/35
Total	_____/150