

**Biochemistry 673**

**Your Name:** \_\_\_\_\_

**Regulation of Metabolism**

**Assoc. Prof. Jason Kahn**

**Midterm Exam (100 points total)**

**March 15, 2005**

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

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

Honor Pledge: At the 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. Nuclear Hormone Receptors (30 pts):**

(a; 5 pts) Sketch and briefly describe the protein domain organization of the glucocorticoid receptor.  
You don't need to give any residue numbers.

(b; 2 pts) What is an orphan nuclear receptor?

(c; 12 pts) Describe the process of transcriptional activation mediated by the glucocorticoid receptor in response to a ligand like dexamethasone. Include the following specific aspects: Where is the GR located in the presence and absence of ligand? What is the role of Hsp90 and its ATPase activity? What special features of the DBD allow it to bind to DNA wrapped on a nucleosome? What do the recruited transcriptional coactivators do?

(d; 2 pts) Give an example of a receptor for which the availability of different coactivators leads to different responses.

- (e; 9 pts) Briefly compare and contrast localization, DNA binding, and transcriptional activation by the GR with the corresponding processes carried out by the thyroid hormone receptor or other Class II heterodimeric nuclear hormone receptors.

**2. Fundamentals of Chemotaxis (25 pts):**

- (a; 12 pts) What are the roles of CheA, CheW, CheY, CheR, CheZ, and CheB in bacterial chemotaxis?

(b; 8 pts) Describe how the process of adaptation in chemotaxis works. Why does a bacterium need to have a memory?

(c; 5 pts) What would the phenotype be for a mutant that expressed much less than normal amounts of the demethylase CheB? What about an overexpression mutant?

**3. New techniques and observations on nuclear dynamics of NHR's (20 pts):**

(a; 6 pts) What is FRAP? Very briefly explain how it can be used to study the redistribution of proteins in a cell.

(b; 6 pts) Proteasome inhibitors dramatically slow down the observed redistribution of the estrogen receptor. What does this suggest about the amount of freely diffusing receptor under these conditions? How can degradation of the receptor possibly speed up its observed redistribution?

(c; 8 pts) The figure below shows the time-dependent occupancy of the GR and the Brg1 subunit of human SWI/SNF on the MMTV promoter reconstituted into chromatin, from the paper of Nagaich et al. (2004).

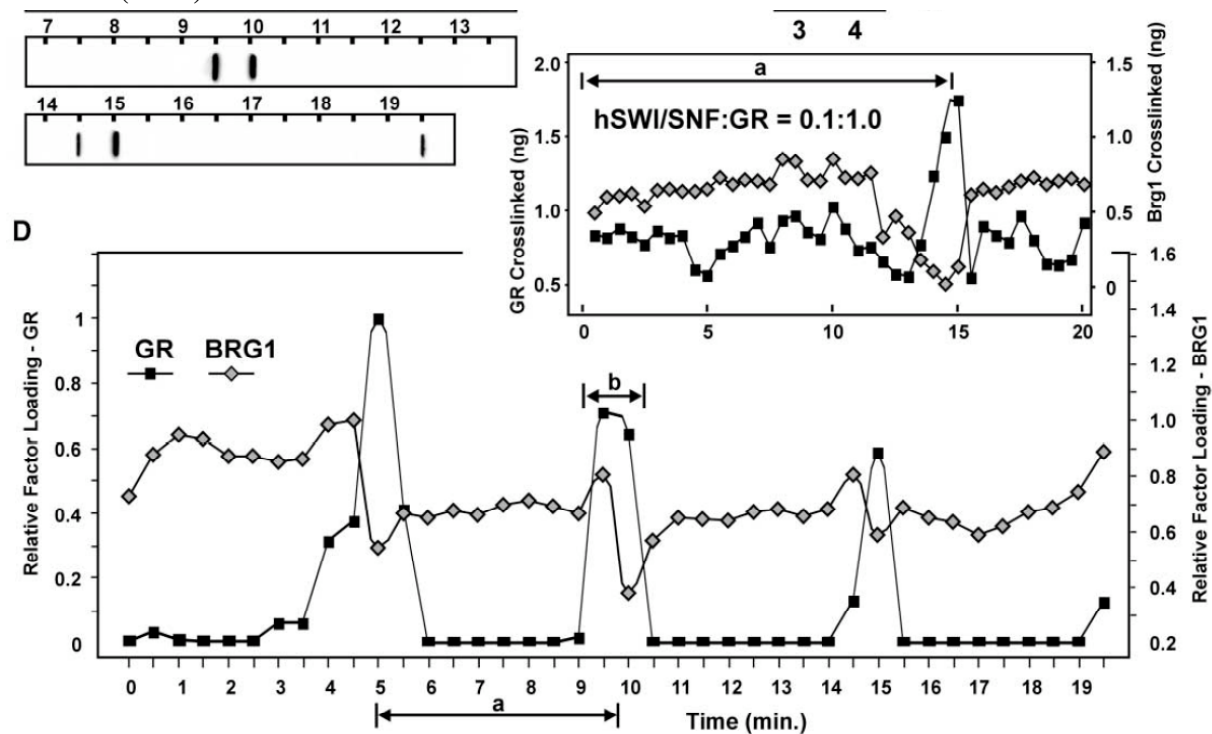


Figure 1. Periodic Binding Detected by UV Laser Crosslinking

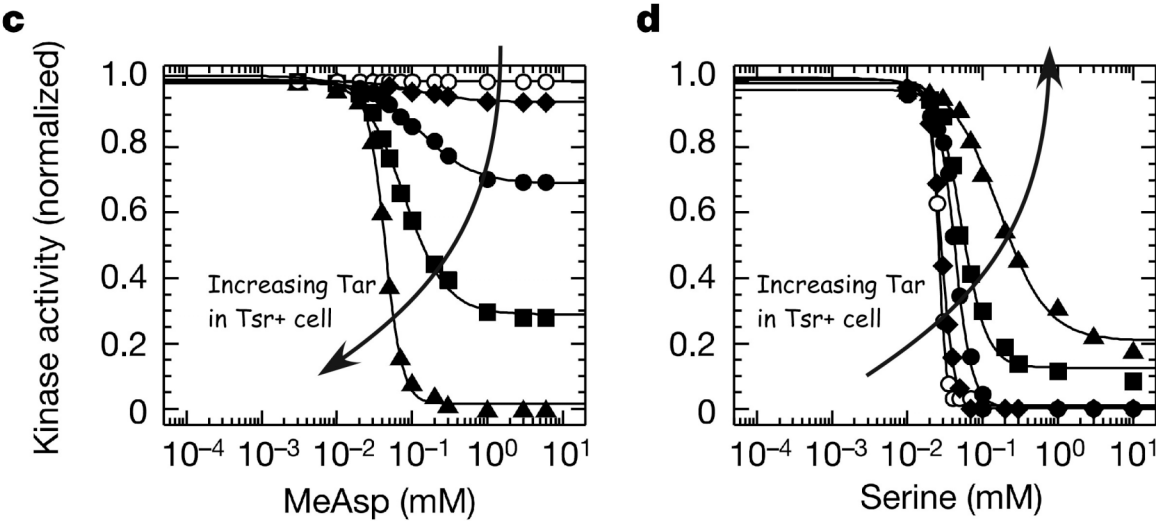
How did we interpret the sequence of events in panel D that starts at about 3.5 minutes? Where might periodic behavior come from in this reconstituted system? (As you recall, it is quite surprising). How might you test your idea?

**4. Chemotactic Receptor Clustering (25 pts):**

(a; 10 pts) Explain how Sourjik and Berg used FRET between CheZ and CheY~P fusion proteins as a rapid and sensitive in vivo assay for CheA activity.

(b; 6 pts) Give a physical rationale explaining why the Hill coefficient (or the MWC “*N*” parameter) for kinase activity vs. attractant concentration increases as the level of CheW increases (at least up to wild type levels). What would you predict at even higher levels of CheW?

(c; 9 pts) In the graph below from Sourjik and Berg, 2004, increasing levels of the aspartame-responsive Tar receptor are expressed in a cell with wild type levels of the serine-responsive Tsr receptor.



Why doesn't kinase activity go to zero at high levels of attractant? What is the evidence for increased cooperativity in uniform as opposed to mixed receptor populations? What is the reasoning suggesting that the Tsr and Tar receptors interact with each other in clusters?

Question	Score
1	____/30
2	____/25
3	____/20
4	____/25
Total	____/100