Biochemistry 673 **Your Name:** Key

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

Midterm Exam (100 points total) **October 19, 2007**

You have 50 minutes for this exam.

Explanations should be concise and clear, but there is extra space on the last page.

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.

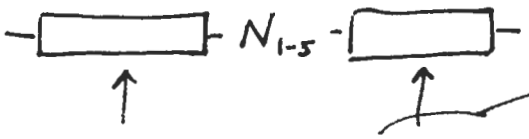
Honor Pledge: At the of the exam time, please write out the following sentence and sign it, or talk to me about it:

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

~

1. Nuclear Hormone Receptors and Hsp90 (50 pts):

(a; 10 pts) How is it that some nuclear hormone receptors can bind to their DNA sites even when the sites are wrapped in histone octamers? Why is this important for the function of NHRs?



(+3) for idea of spacing being important

For N = 3 or 4, (+3) centers of recognition elements are on one side of the DNA, so the binding face can be on the outer surface (+1) of the nucleosome.

Therefore the NHR can act as a "pioneer factor" to (+3) target remodeling machinery to the nucleosome, which then can allow other factors to bind.

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(b; 5 pts) Give a one-sentence summary of the Métivier paper on estrogen receptor signalling.

The ER binds DNA and initiates a round of chromatin remodeling and TBP/GTF binding, after which the ER is removed before a productive round of transcription initiation.

cyclR (+3) removal of ER or chromatin remodeling (+2)

Or full credit for discussing methods.

(c; 5 pts) Briefly describe how the RXR-TR heterodimer switches from being a transcriptional repressor to an activator upon thyroid hormone binding.

(+2) In the absence of ligand, the bound RXR-TR recruits a corepressor like HDAC. Upon ligand binding (+2) it recruits an acetylase (HAT) which helps initiate transcription. (+1) Ligand binding → movement of helix 12 alters interaction with ligands.

(d; 10 pts) Briefly describe one stage in the development of cancer, and the means by which Hsp90 unfortunately acts to potentiate passage through that stage.

– Many possible answers –

For example –

problem (+3) As a solid tumor grows, typically its rapid metabolism and poor vascularization provide inadequate oxygenation. The transcription factor HIFα is

response (+3) expressed in response – for example, it leads to angiogenesis.

role of Hsp90 (+4) Hsp90 is necessary to stabilize the HIFα factor.

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(e; 10 pts) What is the proposed means by which Hsp90 acts as a "genetic capacitor?" Briefly describe one experiment that supports that proposal.

(+2) (+2)

– The idea is that Hsp90 stabilizes mutant proteins so that the mutant can survive and then lead to further (+2) mutation. The resulting "cryptic variation" can be (+2) expressed under stress or when a new function is acquired.

+4 for either

– One experiment concerns arabidopsis – Hsp90 inhibition reveals a wide variety of different phenotypes. In yeast, Hsp90 ~~[illegible]~~ is required to enable short-term drug resistance.

(f; 10 pts) We discussed the Hsp90 inhibitor AAG (related to geldanamycin), which is in clinical trials as a chemotherapy agent. On the face of it, it is surprising that inhibition of a universally conserved protein would be sufficiently selective. What is the proposed explanation for AAG's specificity for cancer cells (i.e. why does it kill cancer cells and not normal cells)? We also discussed that Hsp90 inhibition could be a double-edged sword: what is one conceivable downside of Hsp90 inhibition in advanced cancers?

(+3) – Cancer cells are under stress (as above), and require Hsp90 for survival.

(+3) – Normal cells have mainly inactive/unoccupied Hsp90, so they can survive its inhibition.

(+4) – Advanced cancers are highly mutated – inhibition of Hsp90 could unleash new functions of mutant proteins that might, for example, lead to metastasis.

(+2 for Hsp90 could kill/damage normal cells)

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2. Chemotaxis (50 pts):

(a; 10 pts) What does bacterial chemotaxis look like? In other words, what does one see in observing bacteria moving up an attractant gradient? What is the evidence that bacteria respond to temporal signals rather than spatial ones, and why does this make biophysical sense?

(+2) - The bacterium traces out a biased random walk - it tumbles (+2) & swims, with swimming intervals of increased duration when [attractant] is increasing.

- The experiment where rapid mixing is used to expose an (+3) anchored bacterium to a change in attractant showed that the bacterium behaved as if it were in a gradient even at uniform attractant.

(+3) The changes in concentration to which the bug is sensitive is too small to be detected over the length of the bacterium.

(b; 10 pts) Chemotaxis is characterized by amplified responses to small concentration changes, over a wide range of initial concentration, followed by exact adaptation. Which of these properties must be explained by cooperative interactions among MCPs? Briefly describe some of the biochemical evidence for inter-receptor interactions (more space on next page if you need it).

(+3) - Cooperative interaction is needed to explain gain (MWC model for signalling) and also the wide range (+3) of concentration - ~~and~~ clustering allows for adjustable apparent Kd. [Should have specified "and how"]

Via ~~cluster~~ interaction of different receptor types

(+4) - The biochemical evidence for interaction is that expression of varying amounts of Tsr affects the response ~~of~~ mediated by the Tar receptor, and vice versa. This would not occur if the MCP's did not interact. Expressing more receptor gives a ~~better~~ greater response even if the added ~~recep~~ receptors don't bind attractant

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(c; 6 pts) We talked about the difference between robust and fine-tuned mechanisms for regulation. What is the difference? Which property of chemotaxis is believed to be robust, and what aspects of that same property are fine-tuned?

(+2) (+2) a robust mechanism, instead, is one that still operates correctly when parameters like Kd's, k's or protein concentrations change. A fine-tuned mechanism requires a particular set of values for [ ]'s, rate constants.

(+2) Exact adaptation is robust, but the level of activity (of CheY) and the speed with which adaptation occurs are fine-tuned parameters. (+2) for both

(d; 5 pts) Barkai and Leibler proposed a mechanism for robust exact adaptation. What is the fundamental proposed property of CheB that confers exact adaptation in their model? How has it been partially tested?

(+3) – CheB demethylates (+1) only active (+2) MCP's, so the level of activity is set by the CheB/CheR balance

(+2) – CheB that is unresponsive to phosphorylation still mediates exact adaptation – the MCP activity need not depend on CheA.

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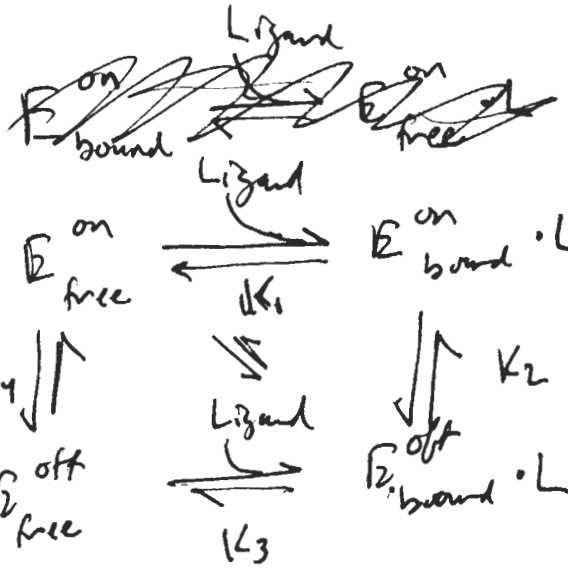
(e; 15 pts) We know that attractant binding to a receptor ends up suppressing the activity of CheA. In other words, it stabilizes the "off" state of the MCP receptor. Diagram why it must be true that the off state has a stronger affinity for attractant than the "on" state. Qualitatively, extend this idea to explain how bacteria can respond at a very wide range of attractant concentrations. How does the structural work that we discussed in class relate to this ability? (Hint: someone is always listening.)



Idea of thermodynamic cycle (+2) (+2)

– "Ligand turns off receptor" or "Ligand stabilizes off state" (+2) means that $K_2 > K_4$ (both written try ⇌ bottom)

– Since the K_{eq} for $E^{on}_{free} \rightleftharpoons E^{off}_{bound} \cdot L$ is independent of path, (+2) (+3) $K_1 \cdot K_2 = K_3 \cdot K_4$

Therefore $\frac{K_1}{K_3} = \frac{K_4}{K_2}$ < 1 and $K_3 > K_1$, i.e. ligand binds better to off state. (+2) (+8)

(+7) Exact adaptation leads to a set of inactive bound receptors and active free receptors. So methylation essentially tunes the apparent overall Kd for ligand by "mixing states." (+2)
– methylation stabilizes the "on" state, hence ↓ overall K.

The network of receptors means that there are MCP's in many different environments available, (i.e. mixtures of methylation states) hence a wide (+2) range of possible Kd's – therefore the system is always poised so that the θ vs. [attractant] curve shifts so the maximum slope is at the current [attractant]. (+2)

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(f; 4 pts) What was the most confusing concept we covered in these two sections, and how could it have been better explained?

Any comprehensible answer okay – +2 for problem, +2 for suggested improvement.

- Steroid hormone receptor spacing + specificities of ~~rece~~ receptors
- cooperativity and gain papers could be better
- fine tuning vs. robust & confusing nomenclature could be better
- Background requirements for Hill coefficients, cooperativity reading
- Mathematics of chemotaxis

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1	____/10
2	____/20
3	____/20
4	____/20
5	____/11
6	____/15
7	____/4
Total	____/100

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