BCDB Qualifying Exam Part I: May 26 & 27, 2015

Instructions:

The following pages contain six questions. You must answer five out of the six questions, on each of the two days. Do not answer extra questions; indicate which one you are skipping on each day. Any one question may ask for more than one response so please read each question carefully for specific instructions pertaining to that question.

All answers are to be typed on the provided computer and saved to a provided USB drive. Hand-written figure pages will be included with your typed answers when they are processed and distributed to graders. SAVE ANSWERS FREQUENTLY, in case of computer issues.

Start typing your answer after the end of its question. Do not make changes to the format, font, color, etc. Do not change the header or footer. For the question you choose not to answer, just type "SKIP". Use the provided pages for your figures.

Please remember as you answer the questions that you have approximately one hour per question. This means that we expect in depth answers unless otherwise noted. Use diagrams to illustrate your answer wherever possible. Although each question is worth the same number of points, not all questions will require the same amount of time. Allocate your time wisely. We recommend that you first make an outline of your answer, rather than just making up your answer as you write. Some questions ask you to propose experiments. Choose the most direct and realistic approaches and explain your experimental rationale as clearly as possible. Be sure to include controls, expected outcomes and possible problems and solutions.

Do NOT put your name on any of the question or answer sheets. To keep the exam anonymous, label your figure pages with the question number (*e.g.,* Question 1) and with a coded name using the code distributed by Susan.

PLEASE MAKE SURE THAT ALL FIGURE PAGES ARE NUMBERED and IDENTIFIED!!!!!

This is a closed book exam. Absolutely no discussion will be allowed between the students while the exam is in progress. Cell phones must be silenced and in a bag – NOT accessible on the desk! You will be held to an honor code by agreeing to not receive or give aid on this exam.

You will have from 8:00 a.m. until 2:00 p.m. to finish the exam. Lunch will be available from (about) 12:00-1:00. No exam materials can leave the room with you during lunchtime or any other time, and you may not refer to outside materials at any time. The exams will be collected no later than 2:00 p.m. sharp.

If you have questions during the exam please contact Sho Ono: (404) 727-3916. In case of emergency or no answer, call his cell (770) 940-9272.

If you need supplies, call Susan: 404 727-1594.

Be sure to save your final exam version to your USB drive. Put the drive and all papers into your envelope. If you have figure papers that are NOT to be used, just draw an X on them, fold them, or tear them in half.

Question 1

To investigate the effects of a novel growth factor (XGF) on human cells, you perform both an RNASeq analysis and a quantitative proteomic analysis comparing control HEK cells (control) to HEK cells treated with XGF (XGF treatment). Both approaches allow you to obtain approximate values for the level of individual RNAs and proteins which you plot as shown in the Figure 1.

(A) What are the points shown in gray indicative of? (**0.5 points**)

(B) What are the points shown in blue indicative of? (**0.5 points**)

(C) What result is indicated by the red data point circled in black (assume that the value is statistically significantly different from 0 for both the X and the Y value)? Suggest a hypothesis that could explain the molecular basis for this result. Describe an experimental approach to test your hypothesis. (**2 points**)

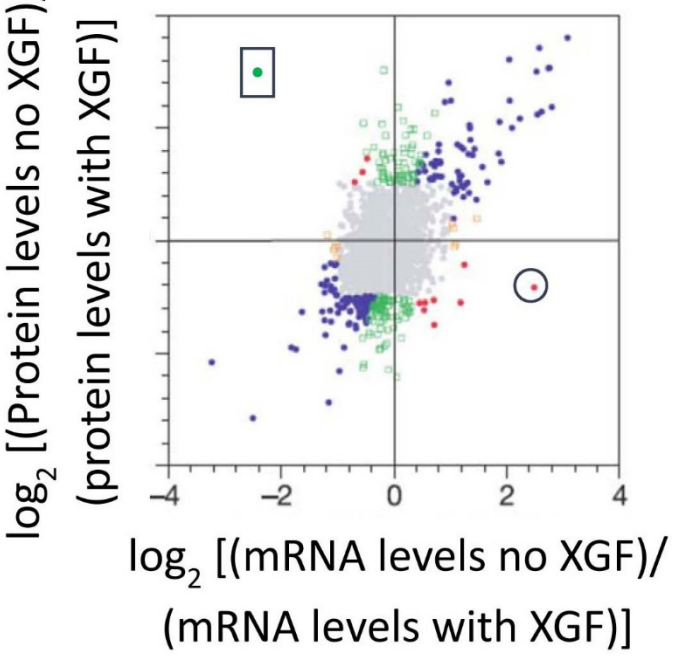

Figure 1

(D) What result is indicated by the green data point boxed in black (assume that the value is statistically significantly different from 0 for both the X and the Y value)? Suggest a hypothesis that could explain the molecular basis for this result. Describe an experimental approach to test your hypothesis. (**2 points**)

(E) When you analyze your data, you are intrigued by a previously undiscovered microRNA (which you name miR-9999) that is upregulated 50-fold in response to treatment with XGF. Describe a computational approach that you would take to identify candidate target genes that might be regulated by miR-9999. Of the data shown in the Figure, which color spots are most likely to represent possible targets of miR-9999? (**1 point**)

(F) You select three promising candidate genes as potential targets for miR-9999 regulation for further investigation (ABC1, ABC2, ABC3). Describe a wet bench experiment to test your hypothesis that they are regulated by miR-9999. Be sure to include all necessary controls. (**2 points**)

(G) One of your three candidates (ABC2) does appear to be regulated by miR-9999 based on the experiment that you designed. Describe another experiment that would demonstrate that the effects of miR-9999 on your candidate target are direct effects, and not indirect effects. Be sure to include all necessary controls. (**2 points**)

Question 2

You have identified an important yeast enzyme that you believe is a protein and RNA complex. You purify the native complex.

(A) Design ONE experiment to test that the complex has an RNA component. (**1 point**)

(B) You have successfully shown that there is RNA present. How do you determine its approximate size? (**1 point**)

(C) You next want to look at the RNA component of the complex during different times after initiation of stress that you think is important for the enzyme's activation. At this point, you now know the sequences of the protein and the RNA component. Design ONE experiment to determine if the RNA is present in the complex during this response. (**2 points**)

(D) Although you purified enough complex for in vitro experiments, the native purification protocol is long and demanding so you would like to be able to obtain large amounts of the purified RNA and protein. Explain how you would do this. Be specific. (**3 points**)

(E) Now with large amounts of a purified complex, you would like to know what secondary structure the RNA adopts when bound to the protein. Design ONE experiment to determine this. (**3 points**)

Question 3

Protein glycosylation on Asn residues (N-glycosylation) is universally found in all eukaryotes. When the process of N-glycosylation is disrupted, there is often cellular and organ pathology. Experimentally, consider the following situation. A young investigator examined the apparent molecular weights of glycoproteins in serum from a patient using SDS-PAGE separation (Note: All major serum proteins are glycoproteins and almost all have one or more N-glycans, except for serum albumin which is not a glycoprotein). In comparison to normal serum she noticed that several glycoproteins from the patient had lower apparent molecular weights (faster mobility), but that the total glycoprotein levels appeared similar between patient and normal. She suspects the patient may have a Congenital Disorder of Glycosylation (CDG). Describe a simple experiment to confirm your hypothesis. (**2 points**)

After confirming the diagnosis, describe experiments to identify whether the glycoproteins from the patient were (A) missing or reduced numbers of N-glycans (**4 points**) or (B) had altered structures of their N-glycans? (**4 points**)

Question 4

This story is fictional. Recently, I received a phone call from a person who had seen my website and wondered if I could help her. She told me that her family has an inherited form of macular degeneration where the central part of their retina degenerates at an early age. Because the macula is responsible for high-acuity vision, reading and driving are no longer possible. She says that in her immediate family, there are 18 people with this syndrome. Electrical recording from the surface of the eyeball (a type of electroretinogram) showed that affected family members lack a component of the electroretinogram believed to be generated by retinal pigment epithelial cells located at the back of the eye. A colleague of mine arranged to obtain skin cells from affected and non-affected individuals in her family and used them to make induced pluripotent stem cells that were then differentiated into retinal pigment epithelial cells. He sent these cells to me to voltage clamp to identify which ion channels might be affected. The cells were placed into a bathing solution of the following composition.

10 mM KCl, 100 NaCl, 10 mM Na-HEPES pH 7.4.

Assume that HEPES is not permeant through any channel and that these cells could have multiple species of ion channels. The resting membrane potential in the non-affected cells on average was -60 mV in the above solution. The resting potential in cells from affected patients was -40 mV.

(A) Suggest three ionic mechanisms to explain this result. (**2 points**)

(B) The cells were then whole-cell voltage clamped. The pipet solution (intracellular) was:

100 mM KCl, 10 mM NaCl, 10 mM Na-HEPES pH 7.4.

Calculate E_K, E_{Na}, and E_{Cl}. Describe calculated values and how you calculated each value. (**3 points**)

(C) Current-voltage relationships from the cells isolated from affected and non-affected individuals are shown in Figure 1. Explain these curves. (**2 points**)

(D) What is ONE possible molecular mechanism to explain this result? How would you test your hypothesis experimentally? You may use any technique that you have learned to approach this problem. (**3 points**)

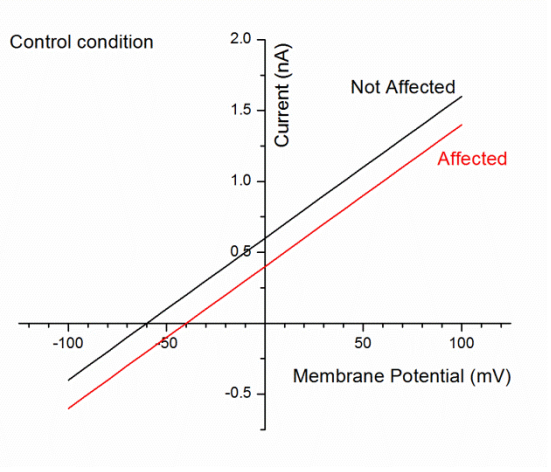

Figure 1

Question 5

A new cancer cell line (Can) and a normal control cell line (Cont) were established from epithelial tissues. You hypothesized that Can and Cont exhibit different dynamics of actin filaments and different responses to a growth factor. Can and Cont were cultured and incubated in culture medium in the absence or presence of the growth factor, fixed at several different time points, and stained with rhodamine-labeled phalloidin (a specific probe for filamentous actin [F-actin]). Unbound rhodamine-phalloidin was washed away. These cells were examined by fluorescence microscopy, and average fluorescence intensity per cell, which represents a relative amount of filamentous actin per cell, was quantified. The results are shown in Fig. 1 [black circles (Cont) and black reverse triangles (Can)]. There was no change in the total actin protein levels during the examined period.

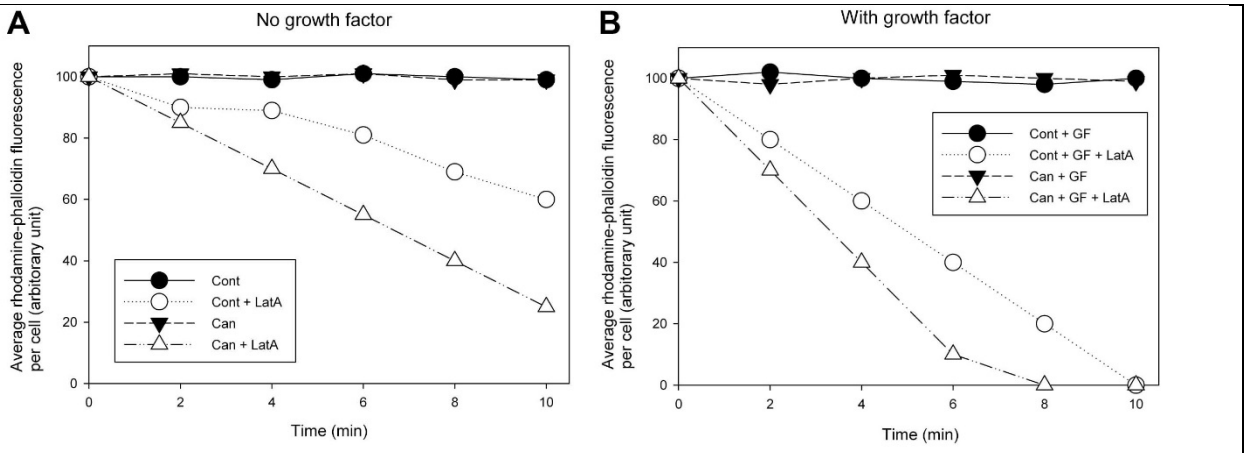

Fig. 1. Quantification of filamentous actin in Can and Cont. Can and Cont were treated in culture medium without growth factor (A) or with growth factor (B), fixed at different time points after the treatments, and stained with rhodamine-phalloidin. Average fluorescence intensity per cell was quantified by fluorescence microscopy and image analysis and plotted as a function of time after the treatments. Latruncluin A (LatA) was included in some experiments.

(A) Then, you did the same set of experiments in the presence of Latrunculin A (LatA), which sequesters actin monomers and prevents polymerization. The results are indicated by white circles (Cont) and white triangles (Can) in Fig. 1. Based on the data in Fig. 1, describe your interpretation of the states of actin filament dynamics in each cell line with or without the growth factor. In your answer, include the following essential components: any difference(s) between Can and Cont in the rates of actin polymerization and depolymerization (**2 points**), and any effect(s) of the growth factor on actin filament dynamics in each cell line. (**2 points)**

(B) The results in Fig. 1 suggest that activity of one or multiple actin-regulatory proteins is altered by the growth factor. Describe **ONE** type of actin-regulatory protein that could be involved in the observed growth factor-dependent changes. Briefly describe the function of the selected protein, and describe **ONE** hypothesis of how this protein could be regulated by the growth factor stimulation and contribute to the observed changes in Fig. 1. (**3 points**)

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Question 5 (Continued from the previous page)

(C) In the absence of Latrunculin A, the relative amounts of filamentous actin per cell were not altered in Cont and Can with or without the growth factor (Fig. 1). However, you hypothesized that dynamics of actin filaments might be different depending on subcellular compartments (for example, actin filaments in the lamellipodia vs. stress fibers). Describe **ONE** experimental approach to determine whether subcellular actin filament dynamics are different in Cont and Can with or without the growth factor. Include appropriate controls, and state **ONE** strength and **ONE** weakness of your selected approach. (**3 points**)

Question 6

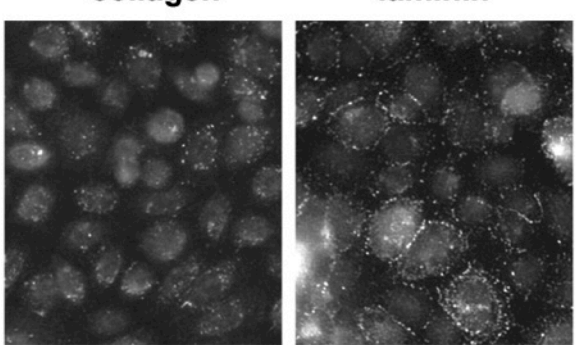

Figure 1. Immunofluorescence images of keratinocytes on collagen (left) or laminin (right) stained with anti-connexin43 antibody.

You are interested in wound healing in skin. Using a scratch wound assay to injure human keratinocytes in culture, you find that there is increased deposition of the extracellular matrix protein laminin at the wound edge and also an increase in gap junction mediated dye transfer between cells. In an effort to understand the molecular basis of your observations, you plate keratinocytes on plates coated with either collagen or laminin, stain for the gap junction protein connexin43 by an antibody and obtain the immunofluorescence results shown in Figure 1. By Q-RT-PCR (quantitative reverse-transcriptase polymerase chain reaction) and immunoblot, you find that keratinocytes on collagen or laminin have comparable steady-state levels of connexin43 mRNA and total protein.

(A) Interpret the images in Figure 1. Explain how the results account for the increase in dye transfer between cells on laminin? (**3 points**)

(B) You suspect that integrins are required for the increase in gap junctional communication by keratinocytes cultured on laminin. Since you know that laminin has high affinity for $\alpha 3\beta 1$ over other integrins, you hypothesize that $\alpha 3\beta 1$ plays a role in increasing gap junction communication. Propose **TWO** different distinct approaches to test your hypothesis. (**4 points**)

(C) Propose **ONE** mechanism of action linking $\alpha 3\beta 1$ activation and changes in intercellular communication. Describe **ONE** experimental approach to test this hypothesis? (**3 points**)

Question 7

During *Drosophila* development, a certain pool of stem cells gives rise to two distinct differentiated cell types (types A and B). Gene X is highly transcribed in cell type A but is silenced in cell type B.

(A) Develop a model to explain the transcriptional state of gene X in each of these two cell types. The model should invoke sequence-specific DNA binding proteins as well as histone modifications and should account for the observation that the daughters of cell types A and B maintain the parental activity state of gene X. (**3 points**)

(B) Provide two approaches that could be used to define the transcriptomes of cell types A and B and to determine how they differ from one another. For one of these approaches, describe the experimental approach you would employ to define the transcriptome. Provide one strength and one weakness for the approach you chose compared to the other approach mentioned. (**2 points**)

(C) You would like to map the epigenomes of cell types A and B in order to gain insight into the transcriptional differences between these two cell types. Which aspects of the epigenome would you examine and how? What would you learn from these experiments? (**2.5 points**)

(D) Describe an approach that would allow you to identify factors that are genetically required for the differentiation specifically of cell type A. (**2.5 points**)

Question 8

SN-38 (the active form of camptothecin) is a powerful chemotherapeutic used to treat colon and liver cancer. To clear SN-38 from the blood, liver enzymes glycosylate the drug by adding a sugar known as glucuronic acid, which enhances drug solubility for excretion via the intestine. However, a major problem facing chemotherapy is the "reactivation" of this drug by symbiotic bacteria residing in the intestines, which cleave glucuronic acid from SN-38 regenerating the active form of the drug. Such drug reactivation can lead to life-threatening side effects and is therefore dose-limiting, reducing the efficacy of chemotherapy.

You have recently identified the bacterial enzyme responsible for cleaving glucuronic acid from SN-38 as β-glucuronidase. This soluble enzyme exists in equilibrium between the inactive monomeric state and the active dimeric state in solution. Each β-glucuronidase monomer contains 605 amino acids (MW = 68,672 Da, pI = 5.82).

(A) Assuming that enzyme cannot tolerate an N- or C-terminal tag (e.g. His, GST, etc...), how would you express and purify *Escherichia coli* β-glucuronidase for use in structural studies? Describe your purification strategy including at least two orthogonal chromatographic steps. Also indicated the appropriate quality control steps for the purification of this enzyme. (**3 points**)

(B) To inactivate the bacterial enzyme, your lab has developed a β–glucuronidase inhibitor (Inhibitor 1). You determine the X-ray crystal structure of the β–glucuronidase:Inhibitor 1 complex (**Fig. 1**). Describe the two different types of interactions that contribute to the binding of this small molecule. Suggest mutations you could make to test the interactions you highlighted and describe how you would test the relative contribution of these interactions to the formation of the complex. (**4 points**)

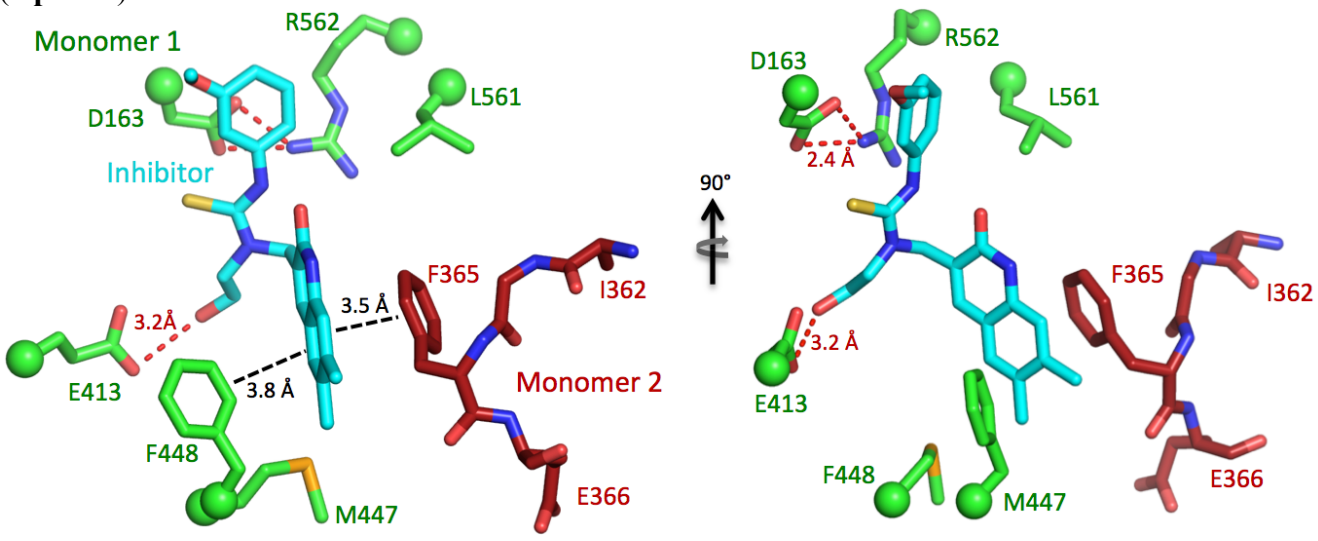

Figure 1. Close up view of the binding site, which is comprised of Monomers 1 and 2 (green and red, respectively) in complex with Inhibitor 1 (cyan). All residues shown are within 4.2 Å of the inhibitor. Monomer 1 side chains are depicted as sticks with the alpha carbon depicted as a sphere. Monomer 2 is depicted as sticks showing both the main chain and side chain bonds. Inhibitor 1 is depicted as sticks. Oxygen, nitrogen, and sulfur atoms are colored red, blue, and yellow, respectively.

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Question 8 (Continued from the previous page)

(C) Explain how you would probe the effect of the Inhibitor on: a) protein oligomerization and b) protein dynamics. You should suggest a distinct technique in each case and provide a brief (1-2 sentence) rationale for your choices. (**3 points**)

Question 9

You have just joined the lab in which you plan to spend the next few years and have been tasked with developing **two** new assays to study **two different** proteins that will be the focus of your research. These two assays are fundamentally different but must meet the generally agreed upon standards in the field for validity and reproducibility. You have been asked to develop these new assays and then write them up as a part of the Materials and Methods section for publication, thus you will need to document the key features of your assays. Below are two general descriptions of the types of assays needed.

Please provide for each assay:
(**1**) the features/characteristics of the assay that you think would be required to convince others that your assay is valid and publishable,
(**2**) the controls you would include along with the purpose of each control you list,
(**3**) how you would analyze and display your data
(**4**) what characteristics of your specific ligand and binding site would you propose to publish as part of your demonstration of the validity of your assay.

(A) The first of these assays will be a radioligand binding assay for a small molecule ligand binding to a known protein. You already have available milligram amounts of the purified protein and the ligand too is available in both radiolabeled and unlabeled forms. (**5 points**)

(B) The second assay will be an enzymatic assay. A protein (different from the radioligand binding protein) is also available in purified form and milligram amounts and is suspected of having the activity of converting substrate S into products P1 + P2. (**5 points**)

Question 10

In Foundations, we discussed 3 papers that indicated that the ciliary barrier, like the nuclear pore, permits free diffusion of "soluble" protein complexes less than about 50 kDa. However, large protein complexes, such as the dynein arms that assemble in the cytoplasm and have a mass ~1.5 MDa, can enter the ciliary compartment. Furthermore, unlike a fraction of membrane proteins and kinesin 2, no ciliary targeting sequence has been identified for soluble, axonemal complexes. The full complement of proteins at the junction between cilium and cell body is not yet defined. From what you have learned about trafficking through the nuclear pore complex and ciliary barrier:

(A) Propose **ONE** hypothesis for how large protein complexes such as the dynein arms might enter the cilium in a selective manner. Describe key machinery and explain how this functions in this process. (**2 points**)

(B) Design **ONE** experimental approach to test your hypothesis including appropriate controls, and discuss possible outcomes that will support your hypothesis and possible outcomes that will disprove your hypothesis. (**3 points**)

(C) In your model, is any type of energy required to drive selective transport of large protein complexes? Answer Yes or No. If Yes, describe the energy source and how the energy source is consumed to drive the transport. If No, describe how the transport is achieved without consuming energy. (**2 points**)

(D) Based on what you hypothesized and tested above, what might you propose as a possible molecular nature of the ciliary barrier? Briefly explain how your proposed barrier might function in selective transport. (**3 points**)

Question 11

Previous studies have demonstrated that Bcl-2 priming (also known as mitochondrial priming) is an excellent predictor of patient response in a variety of cancers. Please answer the following questions regarding Bcl-2 priming.

(A) Briefly explain what is Bcl-2 priming is and describe the difference between a primed mitochondria and an unprimed one. Feel free to write and/or diagram your answer. (**2 points**)

(B) What is the consequence of Bcl-2 priming to the apoptotic threshold of a cell? Explain why the level of priming causes a change to the apoptotic threshold. (**2 points**)

(C) In the previous work on Bcl-2 priming, the studies correlated priming levels to patient responses, however this approach could not be used to predict a patient's response to a specific drug. Therefore you set out to develop a new form of profiling where you get a patient sample, treat it with a variety of drugs for 16 hours and then measure level of priming induced by drug treatment and compare this to untreated levels (see Figure 1 describing the assay). You hypothesize that the percent change of priming (**Δ**% priming) would be a strong predictor of the death induced at 72 hours by the drug. You test this on 5 cell lines with 9 different potential chemotherapeutic agents and get the results in Figure 2:

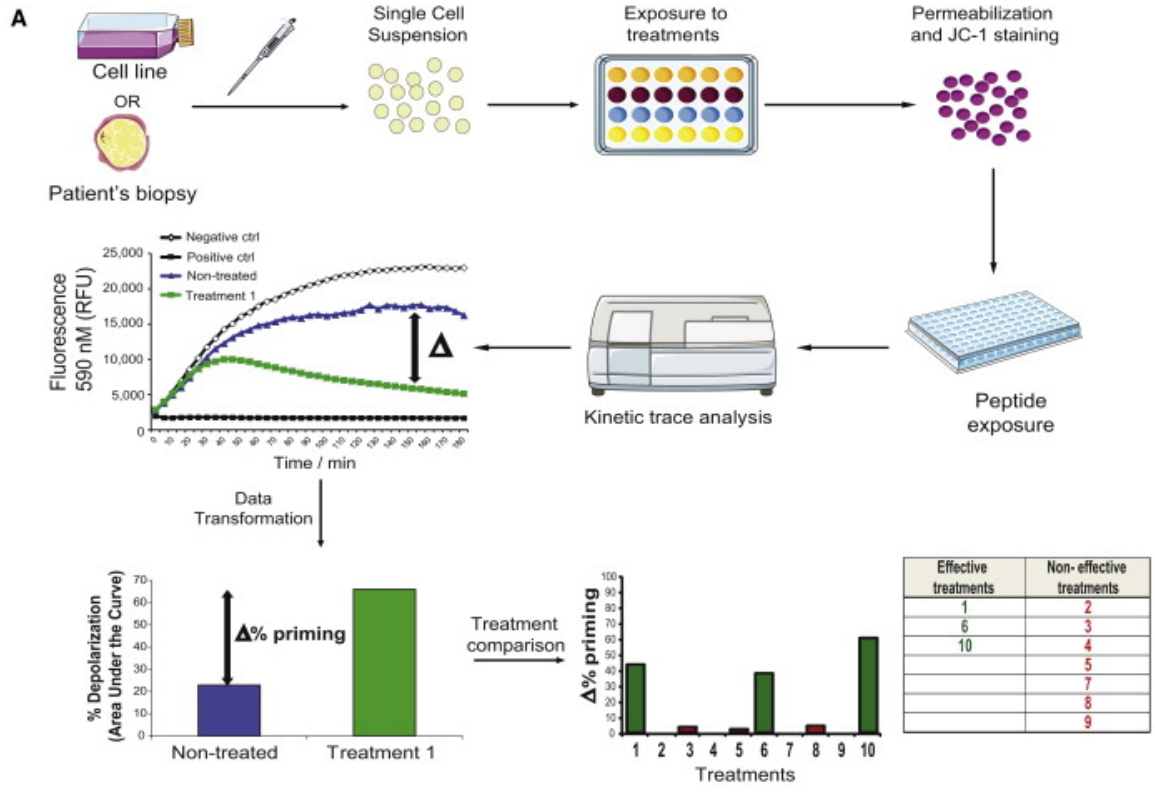

Figure 1

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Question 11 (Continued from the previous page)

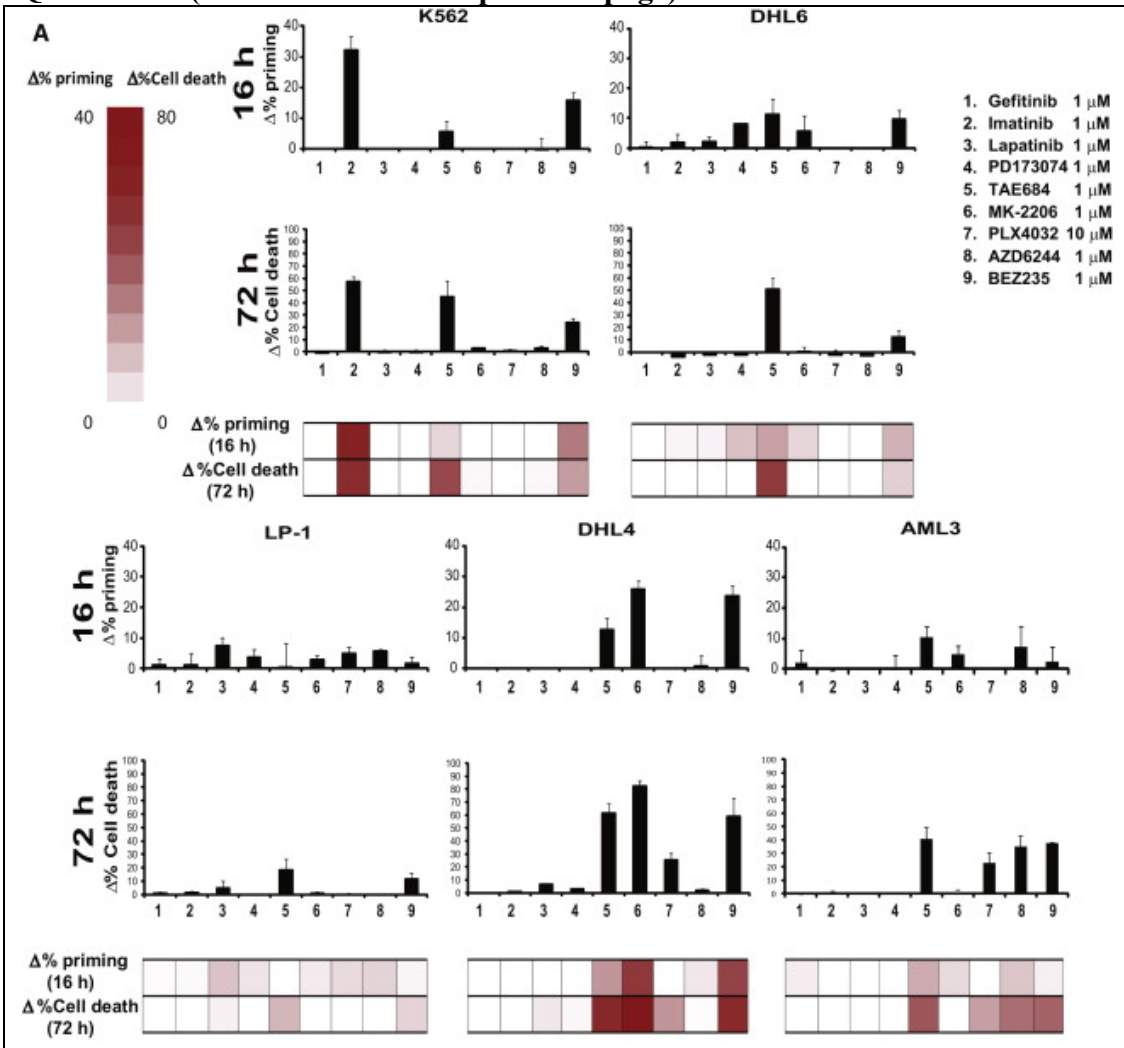


Figure 2

Answer the following questions about this assay and these data.

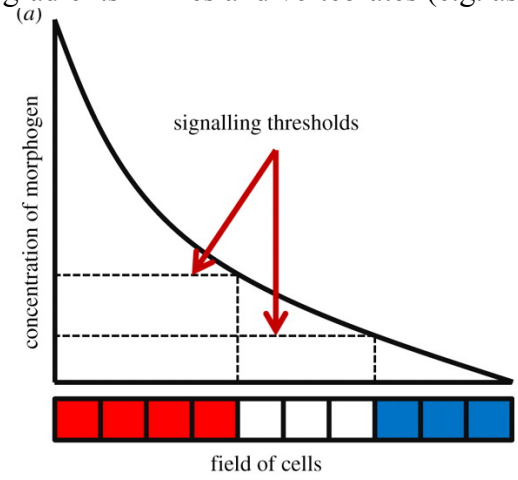
(C-1) Priming induced at 16 hours appears to accurately predict drug activity at 72 hours (predictive power is 83% for these data). Based on the information provided, can you predict which Bcl-2 protein is being primed by drug treatment? If yes, which one? If no, what additional information about the assay would you need to make this prediction? (**2 points**)

(C-2) These 5 cell lines are derived from 4 hematologic malignancies. Which drug appears to have the broadest spectrum activity across these diseases? Which drug has the most specificity for priming? (**2 points**)

(C-3) One line, LP1 does not appear to be primed particularly well by any of these drugs. This has you concerned that there maybe something wrong with the line, so you do next generation sequencing and find that it has a biallelic deletion in Bim. How would loss of Bim influence both the dynamic priming assay and induction of apoptosis? (**2 points**)

Question 12

Secreted factors that can diffuse across a field of cells and induce transcriptional responses in a graded fashion away from the source are termed "morphogens". The "French Flag model", which was discussed at length in your Development module, is a useful and robust way to think about how morphogens control gene expression using threshold effects on promoters. The French Flag model has proven to be particularly useful in studying the regulation and activity of Dpp/BMP morphogen gradients in flies and vertebrates (e.g. as in the Pentagone and Nodal papers covered in class).



The *Drosophila* larval wing disc is a prominent experimental setting to test and study morphogens. The disc contains two putative morphogen gradients shown below: one flowing out from a dorsal-ventral stripe of Dpp (blue stripe), and a second from a perpendicular anterior-posterior stripe of the proposed morphogen Wingless (Wg; red stripe), a secreted molecule that interacts with its receptor Frizzled on receiving cells and stimulates activity of the transcription factor Armadillo (ß-catenin in mammalian cells). A cartoon of these 'stripes' is shown below:

The dogma that Wg is a true morphogen was recently challenged by a paper in *Nature* which the only *Drosophila* Wingless protein, Wg, was fused to the transmembrane domain of the Neurotactin protein, which effectively and completely tethers all Wg in the *entire* fly to membranes of the cell that synthesizes it. To their great surprise, these flies are alive and look largely normal, suggesting that Wg does **not** need to diffuse to carry out all, or at least its most critical, developmental roles (see trimmed abstract below).

> ***Patterning and growth control by membrane-tethered Wingless***
> *C. Alexandre, A. Baena-Lopez, J-P. Vincent*
> *Abstract: Wnts are evolutionarily conserved secreted signaling proteins that, in various contexts, spread from their site of synthesis to form a gradient and activate target-gene expression at a distance. However, the requirement for Wnts to spread has never been directly tested. Here we use genome engineering to replace the endogenous wingless gene, which encodes the sole Drosophila Wnt, with one that expresses a membrane-tethered form of the protein. Surprisingly, the resulting flies are viable and produced normally patterned appendages of nearly the right size. We suggest that the spread of Wingless is dispensable for patterning and growth.*

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Question 12 (Continued from the previous page)

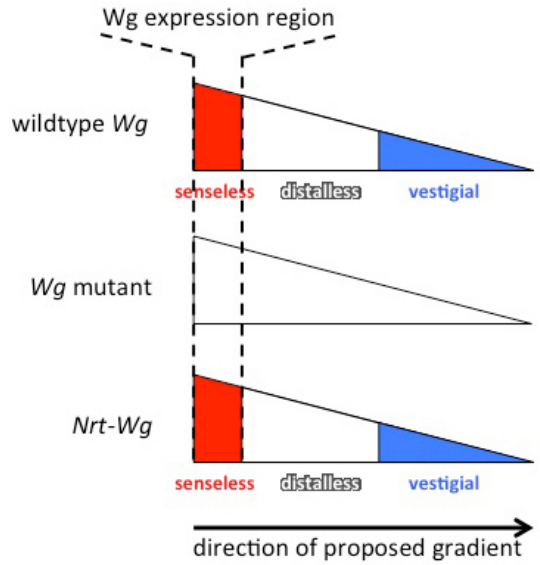
Shown below is a hypothetical set of data in which Alexandre et al also find that the normal pattern of gene expression of three genes that require Wg for their graded expression (Senseless, Distalless and Vestigial; see figure on the right) across the putative gradient is unaffected by substituting the non-diffusible Nrt-Wg into the normal Wg expression domain.

(A) Assuming that Nrt-Wg is truly **not** cleaved and Wg is **not** released into the extracellular space, propose ONE (1) hypothetical model of how Nrt-Wg expressed in the red domain can still induce expression of Senseless, Distalless and Vestigial in their normal domains in distant cells. (**5 points**)

Assume that all cells in the model express equal and sufficient levels of the Frizzled receptor and that the Nrt-Wg molecule remains an active ligand for Frizzled.

Hint: consider other ways in which one group of cells can provide a signal to more distant groups of cells.

(B) Propose an experimental approach to test your model; be sure to include controls and key readouts that will guide your interpretation of the results. (**5 points**)



Cartoon of a putative Wg gradient: (Top) Wg is expressed in the red domain cells. (Middle) Wg loss prevents expression of *senseless*, *distalless*, and *vestigial* in the indicated domains located progressively away from the Wg source. (Bottom) Substituting the Nrt-Wg protein into the normal Wg expression domain restores the normal pattern of gene senseless, distalless, and vestigial expression.