Question 1

You are working in an organism with many genetic and molecular tools, including a complete genome sequence and easy transgenesis. You are curious to determine whether this organism has DNA methylation of any type.

A) Describe **TWO** experiments using non-overlapping methods with which you could determine if there is DNA methylation in this organism. (2 points)

B) You find that there is cytosine methylation present, and wish to determine the consequences of loss of cytosine methylation in this organism. Describe **one** simple experiment that could help to identify phenotypes associated with loss of cytosine methylation in this organism. (2 points)

C) You have identified a non-lethal phenotype associated with loss of cytosine methylation and are now interested in identifying the methyltransferase. Describe how you would accomplish this goal. Include sufficient details about experiments that are required to support your claim of having identified the methyltransferase responsible for methylating cytosines in the organism. Include potential problems or pitfalls and how you would address them. (3 points)

D) Another lab reports that they found that a mutation in a particular gene significantly reduced cytosine methylation in your organism. When you examine the sequence, you find no known homology to DNA methyltransferases and the other lab reports that *in vitro* assays for DNA methylation with the purified protein are negative. Suggest **TWO** plausible explanations for how this gene is involved in cytosine methylation and provide an experiment that would test your explanation/hypothesis for each. (3 points)

Question 2

You are interested in how gene expression is regulated by a specific growth factor in cultured neuronal cells. To address the question of post-transcriptional regulation of gene expression, you treat cells with growth factor and then use both RNA Sequencing (RNASeq) to identify transcripts and a proteomic approach to find proteins that show altered steady-state levels when you compare samples prior to treatment (Time=0h) to those following a 3-hour (Time =3h) treatment with the growth factor.

Your dual approach identifies one Big Really Actually Interesting New protein (BRAIN) that shows a significant decrease in steady-state level (at T=3h compared to T=0h) as well as one microRNA (miR-403) that is greatly increased (both changes occurring in response to treatment with growth factor). After examining the sequence of the microRNA as well as the sequence of the BRAIN mRNA transcript, you develop the **hypothesis:** that the BRAIN mRNA transcript is a target of miR-403.

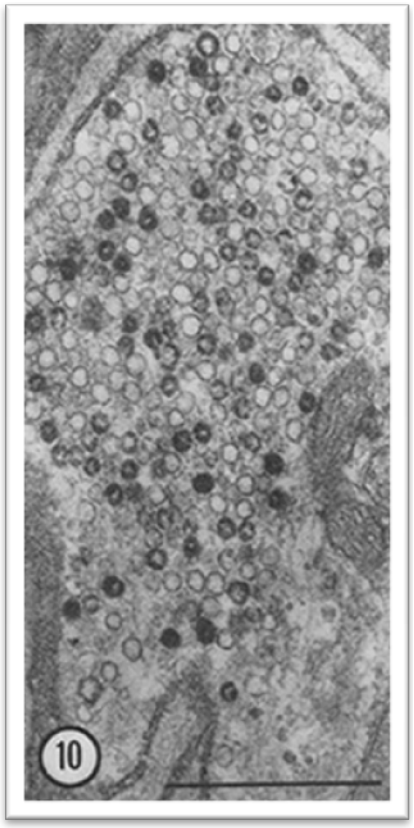
A) Briefly describe RNASeq. Mention another method that could be employed to identify changes in transcript levels on a genome-wide basis. Name **one** advantage and **one** disadvantage of RNASeq relative to your alternative method providing brief rationale for why RNASeq is better (advantage) or worse (disadvantage) (1 point)

B) Briefly describe the logic behind your hypothesis that the BRAIN mRNA is a target of miR-403 including information about how the data that you obtained combined with your analysis of the sequence of the miR-403 microRNA and BRAIN transcript might have prompted you to develop this hypothesis. (2 points)

C) You suspect that the BRAIN transcript is a direct target for regulation by miR-403. Provide a detailed experimental approach to test whether the BRAIN mRNA transcript is indeed a direct target for miR-403-mediated post-transcriptional regulation. Be sure to describe the experiment, the necessary controls, and describe what result you would expect if BRAIN mRNA IS a direct target of miR-403 or IS NOT a direct target of miR-403. (5 points)

D) Assume you find that BRAIN mRNA is NOT a direct target for regulation by miR-403; briefly describe **one** approach to test whether the observed decrease in steady-state BRAIN protein levels is dependent on miR-403 even though this mRNA transcript is not a direct target of this miR. (2 points)

Question 3



Heuser & Reese described the first mechanism of synaptic vesicle retrieval at the nerve terminal in Journal of Cell Biology (1973). These electron micrographs depict neuromuscular junctions that have been incubated in the presence of an electron-dense tracer that fills the extracellular space. This tracer reaches vesicles after electrical stimulation of the nerve attached to the muscle preparation. Synaptic vesicles labeled with the tracer appear dark (see insert). None of the vesicles becomes labeled if there is no electrical stimulation of the nerve.
Similar experiments conducted in cultured hippocampal or cortical neurons as well as neuroblastoma cells recapitulate these results indicating that this process is common to all neurons.

A) You hypothesize that endocytosis mediates the entry of the tracer into synaptic vesicles after electrical stimulation. Propose your **two** most interesting experimental predictions derived from this hypothesis and describe experimental approaches to test your predictions (3 points).

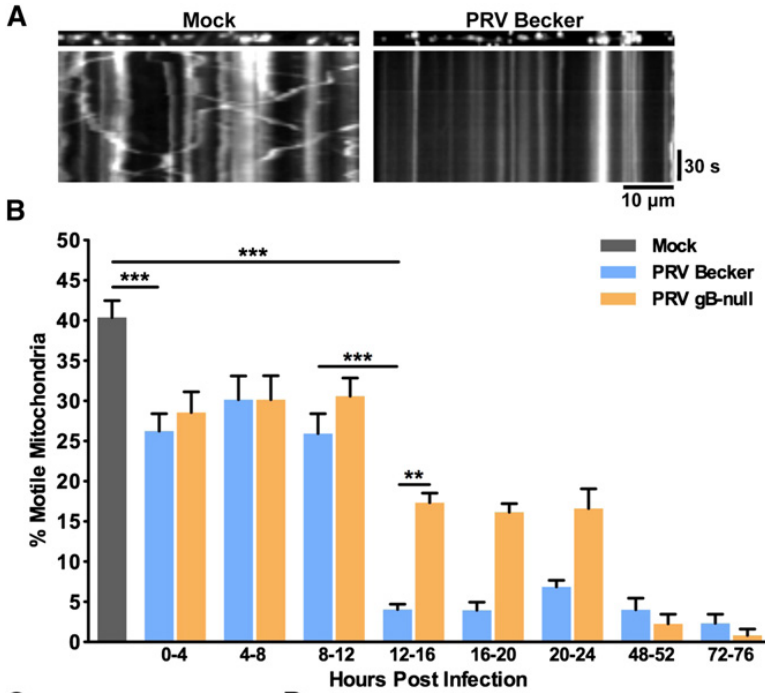
B) Only a few synaptic vesicles (~10%) are labeled with the electron-dense tracer, rather than all the vesicles in the terminal. How will you experimentally determine whether this partial labeling of vesicles corresponds to two functionally and biochemically distinct vesicle populations? Please provide enough detail of your approach to demonstrate feasibility. (3 points).

C) All vesicles are homogenous in size with an average diameter of 40 nm ± 3 nm (standard error). You are interested in what mechanism(s) or process(es) could account for this homogeneity in size. Propose **two** mechanisms that lead to such uniformity and briefly describe the rationale and experimental design to test your proposed mechanisms (4 points).

Question 4

Mammalian alpha-herpes viruses invade the peripheral and central nervous system of their hosts and produce severe neuropathology in humans. Human pathogenic herpes simplex virus 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV) infect a neuron in the peripheral or central nervous system, where they can spread transsynaptically. The mechanisms that lead to neuropathology by viral infection are not well understood but a recent report provides a central clue as to the cellular mechanism(s) being disrupted by the virus.

Figure 1 depicts a series of experiments to analyze mitochondrial dynamics in neuronal processes during alphaherpesvirus infection using primary cultured neurons. Panel A depicts Kymographs of MitoTracker-labeled mitochondria in axons from mock (left panels) and a wild-type virulent strain of herpesvirus PRV Becker (right panels)-infected neurons at 16 hour post infection. Vertical lines represent stationary mitochondria, and diagonal lines represent motile mitochondria. Cell bodies are located to the left of Kymographs. (Panel B) The percentage of motile mitochondria in axons from mock and from two herpes viruse strains PRV Becker- and PRV gB-null-infected neurons are presented.



Based on this information:

A) Describe the effects of Herpes virus infection upon mitochondrial motility (1 point).

B) Based on part A, what type of movement is or are affected. Anterograde or retrograde (1 point).

C) Propose **two** alternative hypotheses that could explain the effect of viral infection on mitochondrial motility along axons (2 points).

D) Select one of your hypotheses and propose an experimental strategy that tests **two** predictions derived from your hypothesis (3 points). Clearly state your predictions and design experiments that will address them.

E) Describe **one** experimental strategy to determine whether the effects of the virus are restricted just to mitochondria (3 points).

Question 5

You want to understand factors that control timing in the eukaryotic cell cycle. Specifically, you want to identify genes that mediate the speed of progression of yeast *S. cerevisiae* through the mitotic cell cycle.

As an open-minded approach to the question, you mutagenize an asynchronous clonal population of haploid yeast, mating type 'a', dilute the culture and pipette single cells into the wells of 96 well plates filled with dextrose growth medium. You grow the resulting cultures in a plate reader programmed to measure turbidity at 600nm every 15 minutes. Assume you test cultures representing more than 5000 mutagenized cells and also 100 non-mutagenized control cells, and identify 4 of the mutant cultures that demonstrate substantially faster-than-control proliferation rates and 15 that demonstrate substantially slower-than-control proliferation rates. Repeat testing of cells from these cultures demonstrates that the results are highly reproducible.

A) How will you determine which of your 19 mutant cultures harbor dominant mutations and which harbor recessive mutations? Be specific. (3 points)

B) How will you determine which mutant cultures harbor single locus mutations responsible for the altered proliferation rate phenotype? (2 points)

C) Assume you find 3 single locus recessive mutations in your collection of "fast growers" and 10 single locus recessive mutations in your collection of "slow growers." How will you divide these 13 strains into complementation groups? Explain. Why would you do this? (3 points)

D) Finally, how will you clone the mutated gene for any one of these 13 strains? How will you know you found the right gene and not a second site suppressor? Be specific. (2 points)

Question 6

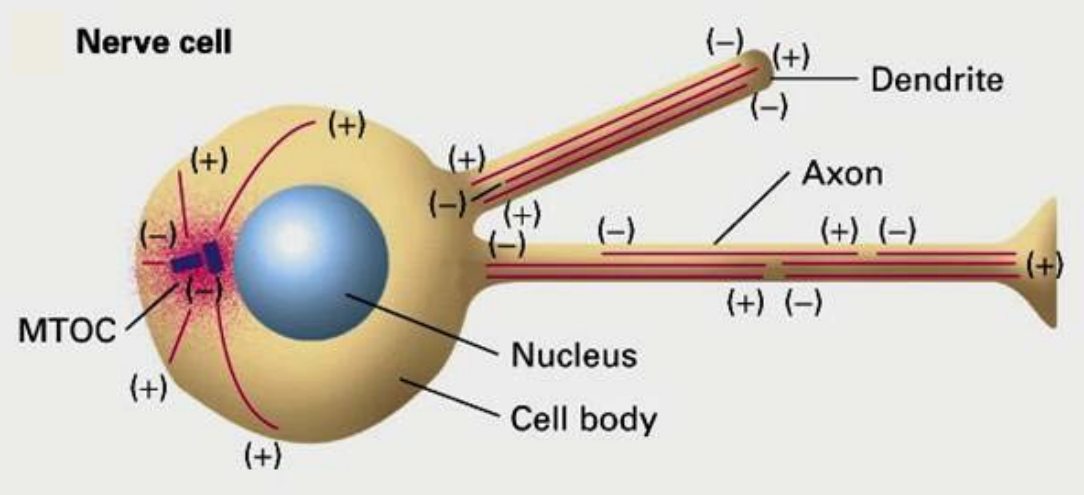


Figure 1. A simplified neuron is depicted in cartoon form. Major features including the Axon and Dendrite, as well as Microtubule Organizing Center (MTOC) are indicated. Centrioles are illustrated within the MTOC but not labeled. Microtubules are in red and microtubule ends are indicated by (+) and (-).

Your lab has recently identified a genetic mutation that results in the dying-back degeneration of axons of spinal cord motor neurons. Each motor neuron sends an elongated axon that can be up to 1 meter in length for conducting nerve impulses to skeletal muscles. Extension and maintenance of the motor axonal projection to muscle fibers are crucial for the daily functions of the body. You were asked to investigate the potential mechanisms of axonal degeneration. You have learned that the mutation occurs in a gene encoding an uncharacterized protein and expression of the mutant form of this protein in cultured neurons resulted in short axons. Initial analysis found that this protein is present in the fraction containing tubulin. You thus speculate that this novel protein may be involved in regulating axonal elongation by targeting the microtubule cytoskeleton.

Based on what you have learned from the microtubule module, you have decided to test one of three possible roles that this novel protein may play and to determine how the mutation could disrupt axonal growth. These possible roles include: (1) a microtubule-associated protein; (2) a microtubule end binding protein; and (3) a microtubule motor.

Please choose your favorite **one** and provide:

A) Hypothesis (1 point)

B) **Two** different experimental approaches to test your hypothesis (4 points total; 2 points/approach)

C) Discussion of the possible outcomes and data interpretation for each approach (4 points total, 2 points for discussion of each approach)

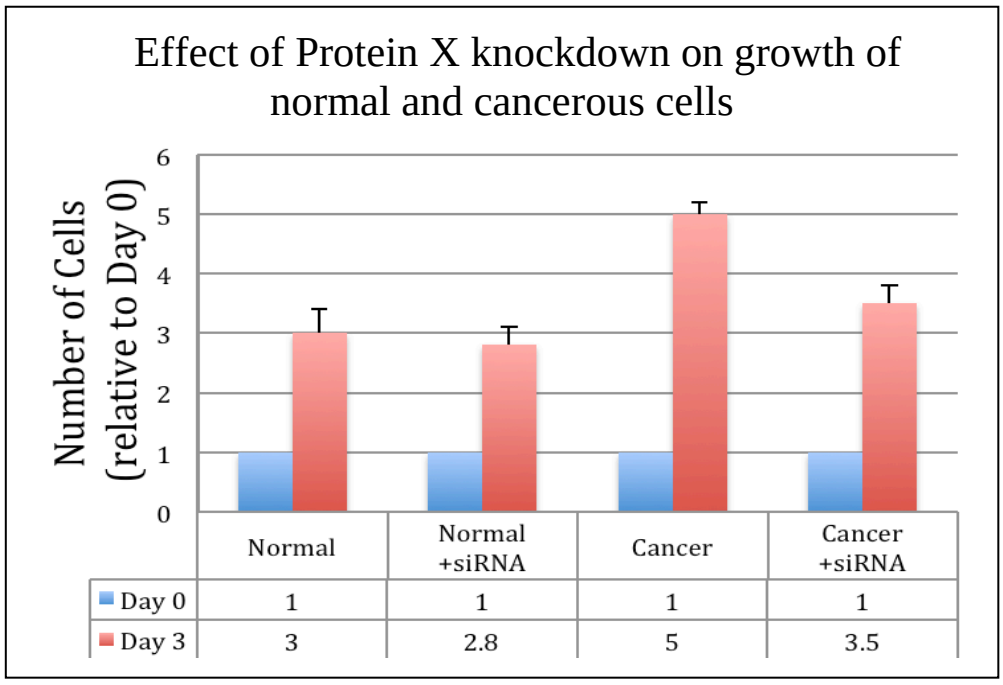
D) Please explain why the axons could be selectively affected by this mutation (1 point).

Question 7

You are studying signaling pathways involved in cancer. You perform a microarray experiment to determine what mRNAs are up- or down-regulated in lung cancer. For this experiment, you perform the microarray for multiple lung cancers and compare the results to microarrays done with normal lung tissue from the same patients. In many of the patients, you find an mRNA encoding a previously uncharacterized protein (Protein X) that has a twenty-fold increase in the cancer tissue relative to neighboring normal tissue. You make an antibody to the predicted Protein X protein and use it to find that, at the protein level, Protein X is essentially absent from the normal tissue but well-expressed in the cancer tissue.

A) Based on the information you were just given, propose **one** possible model for how Protein X expression might promote cancer. At this point in this question, keep your answer relatively simple (three sentences maximum), but clear. **(1 point)**

B) You obtain some cancer cells and normal cells from the primary tumor and surrounding tissue, respectively, and use siRNA to down-regulate Protein X in both cell types. The chart below shows the result from three experiments testing the effect of Protein X knockdown (Normal+siRNA vs Cancer+siRNA) on cell growth over the course of 3 days compared to controls with no knockdown (Normal vs Cancer).



From these data, what do we learn about the possible contribution of Protein X to growth of the cancer? **(1.5 point)**

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Question 7, continued

C) When you examined your Protein X in western blot results obtained using the lung cancer cells (mentioned in the stem of the question above), you see two bands of approximately 80 kDa and 83 kDa as estimated using protein size markers on SDS-polyacrylamide gel electrophoresis. Describe **two** controls that you would perform to make sure that both bands correspond to a form of Protein X and to rule out the possibility that one band is not a non-specifically recognized protein. Be specific but brief. Note: the most credit will be given for the most straightforward but useful approaches. **(2 points)**

D) Assume you determined that both bands are indeed Protein X. Propose **two** possible explanations for why there are two bands. **(1 points)** Briefly describe **one** experiment to differentiate between the two possibilities. Be sure to include necessary controls. **(2.5 points)**

E) Based on your original microarray analysis and follow-up experiments with western blotting, you find that some lung tumors do not express Protein X. Many of those tumors show much lower expression of an mRNA encoding another protein termed Protein Y. You believe that down-regulation of Protein Y promotes growth in the lung cancer cells much like increased expression of Protein X. Briefly describe **one** experiment you could do to test whether this was the case, including necessary controls and expected result **(2 points)**

Question 8

You are studying a eukaryotic RNA that you suspect is an mRNA. You want to follow the primary transcript as it matures into the final mRNA.

A) Your advisor suggests you use a radioactive substrate to label the primary transcript in living cells. What would be your choice for this radioisotopically-labeled precursor? Be specific in terms of your choice of the chemical AND the isotopic atom. (1 point)

B) What is meant by "specific activity" with respect to this radioactive precursor? What units (use of the SI International System of Units is preferred) are typically used to describe a radioisotope's specific activity? (2 points)

C) Assuming you have a suitable radiolabeled precursor, design an experiment to test that the mRNA is derived from a primary transcript. You can use any methods, assays, or reagents you wish but you must be specific. (4 points)

D) Name 3 enzymes (excluding RNA polymerase) you expect to be involved in the maturation of the mRNA from the primary transcript, what those enzymes do, and any substrates they may use other than the RNA itself. Be specific. (3 points)

Question 9

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 its 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) How would you express and purify β-glucuronidase for use in biochemical and/or structural studies? Describe your cloning strategy, purification, and quality control steps. (3 points)

B) You have identified two inhibitors that work by different mechanisms to inhibit β-glucuronidase. Assuming your enzyme reaction obeys normal Michaelis–Menten kinetics, you plot the effect of your inhibitors on the enzyme's reaction velocity with respect to substrate concentration (**Fig. 1**). Describe the difference between the two inhibitors in terms of their mode of action. Do you expect both inhibitors to bind in the enzyme active site? (2 points)

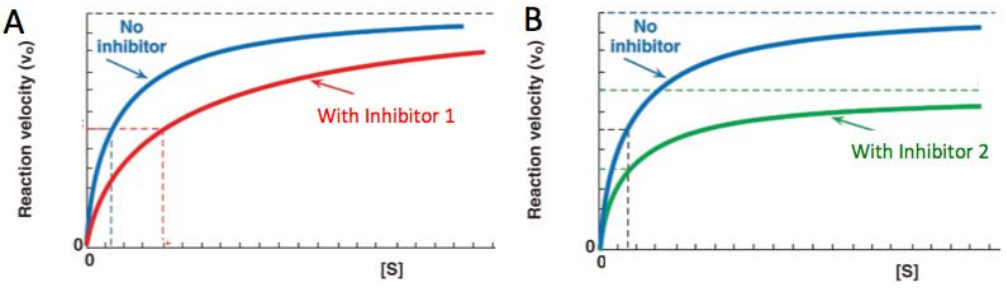

Figure 1. Reaction velocity plotted as a function of substrate concentration +/- Inhibitor 1 (A) and Inhibitor 2 (B).

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Question 9, continued

C) You determine the X-ray crystal structure of the β–glucuronidase:Inhibitor 2 complex (**Fig. 2**). Describe the types of interactions that contribute to the binding of this small molecule and suggest appropriate mutations you could make to test the relative contribution of these interactions to the formation of the Inhibitor 2:enzyme complex. (2 points)

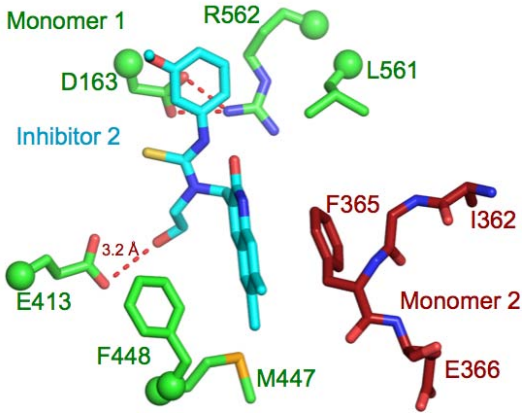
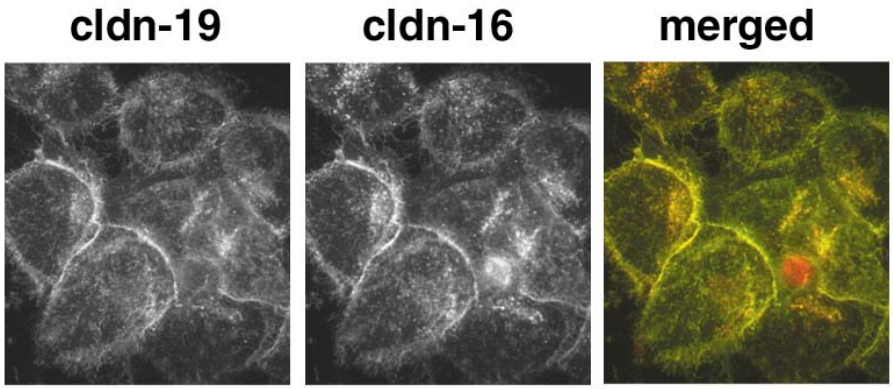


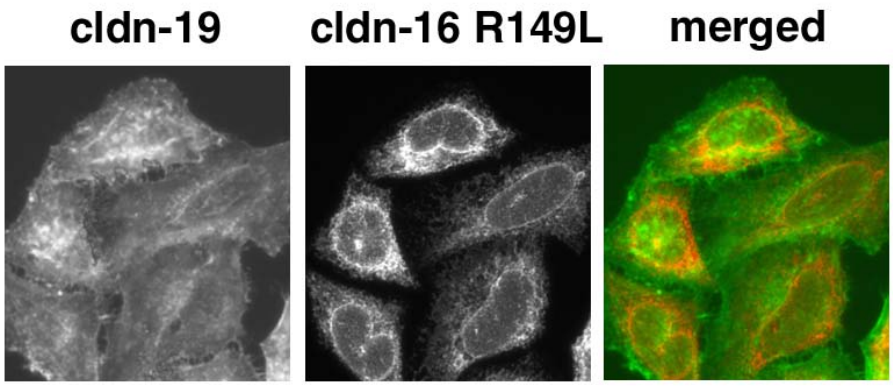
Figure 2. Close up view of the Inhibitor 2 binding site which is comprised of Monomers 1 and 2 (green and red, respectively) in complex with Inhibitor 2 (cyan). All residues shown are within 4.2 Å of the inhibitor. Monomer 1 side chains are depicted as sticks with the alpha carbon depicted as a sphere.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

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

Question 10

Mutations in either claudin-19 (cldn-19) or claudin-16 (cldn-16) have been shown to cause the inherited human renal disorder Familial Hypomagnesemia with Hypercalciuria and Nephro-Calcinosis (FHHNC). You isolate and immortalize renal tubule cells from a patient with normal kidney function and one from a patient with the R149L mutation in claudin-16 and see the following result from immunofluorescence microscopy (cldn-19 is green, cldn-16 is red):





A) Propose a model for how claudin-16 is disrupted by the R149L mutation. What is the likely identity of the compartment where claudin-16-R149L is localized? How would you identify this compartment? Include positive and negative controls. (4 points)

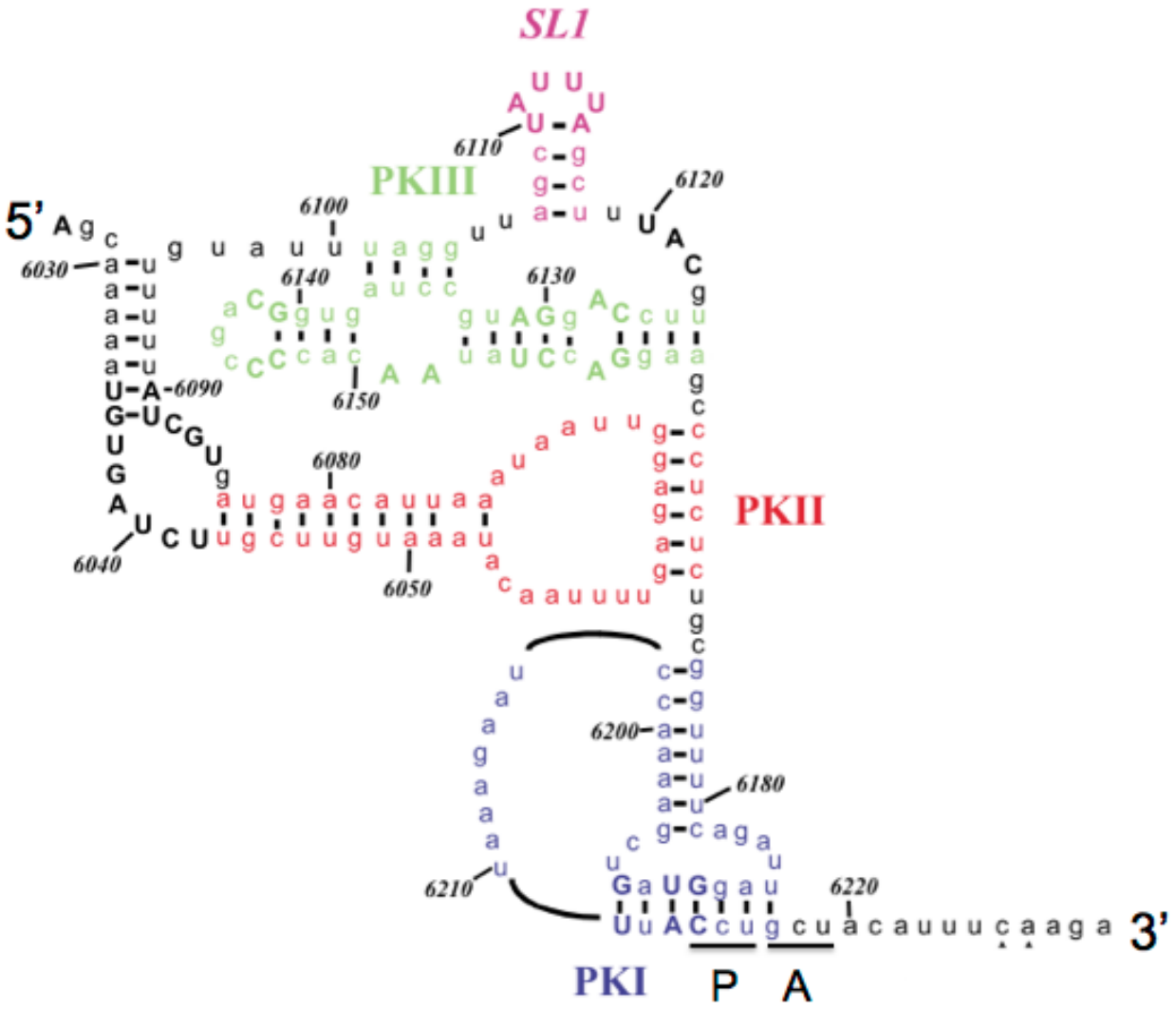
B) Propose a hypothesis for why the R149L mutation gives the immunofluorescence images shown above for claudin-19. Provide **two** distinct experimental approaches to test how claudin-16-R149L affects claudin-19 (4 points)

C) You see that the trafficking of kidney claudins other than claudin-19 is normal in cells expressing claudin-16 R149L. If the remaining claudins expressed by the kidney are unaffected and the cells produce a tight epithelial barrier, then how does the mutation cause FHHNC? (2 points).

Question 11

You are studying a virus that you believe contains a novel IRES (internal ribosome entry site). You came to this conclusion by using an RNA secondary structure program and then directly comparing your IRES to other known IRES. You believe that the beginning of the gene does not begin with a canonical AUG start site but instead begins with the first codon underlined below (P site).

A) What are the first five amino acids of this protein given the codon usage table on the next page? (1 point)



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Question 11, continued

First Position	Second Position				Third Position
	U	**C**	**A**	**G**	
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	Stop	Stop	**A**
	Leu	Ser	Stop	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**U**
	Ile	Thr	Asn	Ser	**C**
	Ile	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
G	Val	Ala	Asp	Gly	**U**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

B**)** You want to test if the RNA binds directly to the 40S subunit. Design an electrophoretic mobility shift assay (EMSA) to explore this possibility. Specifically list all the reagents you will need. Describe the appropriate control samples needed. Make a labeled schematic drawing showing the results of the assay (6 points).

C) Finally, you want to know that the IRES when bound to the ribosome positions the proposed CCU start codon in the P site. Describe an assay to test this, what starting molecules you need to perform it, what the appropriate controls are and what the readout looks like. (3 points)

Question 12

You are studying the mechanisms of activation and deactivation of a novel regulatory GTPase in the Ras superfamily, RAM1.You over-express and purify the protein from bacteria and also from human embryonic kidney (HEK) cells. You develop a radioligand binding assay to study the binding of [^{3}H]GDP or [^{35}S]GTPγS using a filter trapping assay to separate bound and free ligand.

A) Describe how you would determine the stoichiometry, on-rate, off-rate, and affinity for GDP binding to bacterially expressed RAM1. Include sufficient detail that another scientist can perform the experiment and calculations from your protocol/description. (3 points total)

B) Binding of small molecules is typically limited by diffusion and thus on-rates are typically much faster than off-rates. However, you determine that the on-rate and off-rates of GDP for RAM1 are identical. What is the simplest interpretation of the cause of this observation? (2 points)

C) You find that the HEK cell expressed protein binds GTPγS to higher stoichiometry and faster than does the bacterially expressed protein. Propose the most likely explanation and how you would test your hypothesis. (3 points)

D) Analysis of the amino acid sequence of RAM1 reveals that it is 93% identical to the GTPase K-Ras, a well characterized oncogene. As you know, K-Ras is a critical component of the mitogen-activated protein kinase (MAP kinase) signaling pathway involved with tyrosine kinase growth factor receptor (TKR) signaling. The prototype of this pathway is the EGF receptor (EGFR). Illustrate the EGFR-MAP kinase signaling pathway, starting with activation of the receptor and ending in the nucleus. Draw the pathway and identify each of the involved proteins and their function/signaling role using just a few words for each. (2 points)