**FOR DISTRIBUITION IN JUNE 2022 MCD First Level Exam- PART ONE**
**Part 1: Essay Questions. ANSWER 4 QUESTIONS out of THE 6 CHOICES.**

**EACH ESSAY QUESTION IS WORTH 15 POINTS**
**General Essay on turn-it-in is opened from 9am until 12pm**

**Time yourself and make sure to submit BEFORE OR BY the deadline of 12pm. The turn-it-in link will close at 12 pm. If you do not turn it in before or by 12 pm you will receive a 0 grade for this part of the exam. Set a timer and submit whatever you have before or by the 12pm mark.**

**You must answer 4 of the 6 essay choices. Clearly indicate the number and the references for each of your essays. The essays must appear in numerical order in your written document. For example, if you answer choices 2, 4, 5, and 6 then they must appear in that sequential order.**

**Choice 1 Essay**
**References:**
1. Moore, A.R., et al., *RAS-targeted therapies: is the undruggable drugged?* Nat Rev Drug Discov, 2020. **19**(8): p. 533-552. (Moore, Rosenberg, McCormick, & Malek, 2020)
2. Zhao, Y., et al., *Diverse alterations associated with resistance to KRAS(G12C) inhibition.* Nature, 2021. **599**(7886): p. 679-683. (Zhao et al., 2021)

A) Please describe the process by which an inactive molecule of KRAS becomes active. (6 points)

B) How do approved covalent inhibitors of mutant KRAS(G12C) block the oncogenic function of mutant KRAS? (4 points) What is their effect on wild-type KRAS?

C) Recently a patient whose lung adenocarcinoma tested positive for KRAS(G12C) was prescribed an approved KRAS(G12C) targeted inhibitor as a single agent. One month ago, her lung tumor was down to 30% of its pre-treatment size. Unfortunately, at today's appointment her lung tumor had expanded to 120% of its pre-treatment size. Please describe several ways that these cancer cells might currently be resistant to this therapeutic. (5 points)

**Choice 2 Essay**

**References:**

1. Mateo, A.R., et al., *The p53-like Protein CEP-1 Is Required for Meiotic Fidelity in C. elegans.* Curr Biol, 2016. 26(9): p. 1148-58. (Mateo et al., 2016)
2. Pfister, N.T. and C. Prives, *Transcriptional Regulation by Wild-Type and Cancer-Related Mutant Forms of p53.* Cold Spring Harb Perspect Med, 2017. **7**(2). (Pfister & Prives, 2017)

A) (5 pts) Describe the earliest described roles of p53. How is the activity of the protein controlled and what is it best known functions and targets?

B) (4 pts) Mateo et al. describes two deletions that affect the *cep-1* locus and thus determined new roles for a p53 orthologue. What are the different alleles and what loss of activity to they have in common? What "p53 like domains" are deleted in the different alleles, and which deletion acts more like a complete loss of function? Why?

C) (6pts) In Mateo et al. they discovered two previously unknown roles for the p53-like protein CEP-1 in ensuring meiotic fidelity in the germline of *C. elegans*. How does CEP-1 act to maintain meiotic fidelity? What is the data that supports this conclusion?

**Choice 3 Essay**
**References:**

1. Tufariello, J.M., et al., *Separable roles for Mycobacterium tuberculosis ESX-3 effectors in iron acquisition and virulence.* Proc Natl Acad Sci U S A, 2016. **113**(3): p. E348-57. (Tufariello et al., 2016)
2. Roy, S., et al., *ESX secretion system: The gatekeepers of mycobacterial survivability and pathogenesis.* Eur J Microbiol Immunol (Bp), 2020. **10**(4): p. 202-209.(Roy, Ghatak, Das, & BoseDasgupta, 2020)
3. Chao, A., et al., *Iron Acquisition in Mycobacterium tuberculosis.* Chem Rev, 2019. **119**(2): p. 1193-1220. (Chao, Sieminski, Owens, & Goulding, 2019)

**Questions:**

A) What are the mycobacterial secondary metabolites known as mycobactin and carboxymycobactin, and
B) what is the role they are thought to play in M. tuberculosis? (4 pts)

C) What feature of the M. tuberculosis type VII secretion systems known as ESX-3 has hindered investigations of the roles of the system, and (D) how did Tuberuile et al. bypass this hurdle? (4 pts)

E) Describe the proposed functional relationship between the ESX-3 system and the mycobactin/carboxymycobactin system. (2 pts)

F) Provide an overview of the key experiment presented by Tufariello et al. that provided evidence for a role of the M. tuberculosis ESX-3 system in siderophore uptake/utilization of siderophore-bound iron and supported the authors' idea that a lower-affinity system for siderophore uptake/utilization of siderophore-bound iron functions in the absence of ESX-3 system. (5 pts)

**Choice 4 Essay**

**References:**

1. Klein, S., et al., *SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography.* Nat Commun, 2020. 11(1): p. 5885 (Klein et al., 2020)

2. Bracquemond, D. and D. Muriaux, *Betacoronavirus Assembly: Clues and Perspectives for Elucidating SARS-CoV-2 Particle Formation and Egress.* mBio, 2021. **12**(5): p. e0237121. (Bracquemond & Muriaux, 2021)

**Question:**

The cause of the current COVID-19 pandemic is the coronavirus SARS-CoV-2, a highly pathogenic β-coronavirus. While the virus replicates in the host cell cytoplasm and has a complex replication cycle the assembly of the virus structure occurs within intracellular membranes. While the entire mechanism is not yet understood it has been partially characterized by cryo-electron tomography as described in the assigned reference Klein *et al*. 2020.

A) Described the type of SARS-CoV-2 host cells utilized for the virus replication in culture and which cell surface receptor was required to bind the virus particles. (2.5 points)

B) Describe the nature of the viral replication compartment in regard to its membrane conformation and the intermediate replicative viral RNA. (5 points)

C) Describe the final viral assembly process within the unique membrane compartment that is associated with coronavirus release from infected host cells. What are the major virus structural proteins that must be correctly assembled to provide a particle? (7.5 points)

**Choice 5 Essay**
**References:**

1. Smith, A.J., et al., *Non-Coding RNA and Frizzled Receptors in Cancer.* Front Mol Biosci, 2021. **8**: p. 712546. (Smith, Sompel, Elango, & Tennis, 2021)
2. Naidoo, M., et al., *MicroRNA-1205 Regulation of FRYL in Prostate Cancer.* Front Cell Dev Biol, 2021. **9**: p. 647485. (Naidoo et al., 2021)

**Questions:**
In their 2021 article in Front. Mol. Biosci., Smith et al discussed three main non-coding RNA molecules.

A) What are the three main types of non-coding RNA molecules and how do they differ? (1 point)

B) Which miRNAs regulate Fzd8 expression, and how do they do it? (4 points)

C) How do long non-coding RNAs act as competing endogenous RNAs? (2 point

D) Which molecular target of miR-1205 was identified in the article by Naidoo et al, and how was it identified and confirmed? (8 points)

**Choice 6 Essay**
**References:**

1. Siebel, C. and U. Lendahl, *Notch Signaling in Development, Tissue Homeostasis, and Disease.* Physiol Rev, 2017. 97(4): p. 1235-1294. (Siebel & Lendahl, 2017)
2. Chen, X. and M.M. Emerson, *Notch signaling represses cone photoreceptor formation through the regulation of retinal progenitor cell states.* Sci Rep, 2021. 11(1): p. 14525. (Chen & Emerson, 2021)

Question:

In the vertebrate retina, inhibition of Notch signaling leads to an increase in cone photoreceptors. Recent evidence suggests that the role of Notch signaling in this process is mediated through a change in progenitor cell populations (Chen and Emerson 2021). Assume that there is a necessary role for ligand binding (such as Deltalike) to activate Notch signaling in this process.

A) Please discuss a protein, other than Notch itself, or a Notch ligand, that you could change the expression level of (either downregulate or upregulate) that would decrease the number of interactions of Notch with a ligand such as Deltalike. (5 points)
B) Please be sure to discuss why this change in expression level would affect Notch-ligand interactions and what you would predict would be the effect on Notch signaling and the number of multipotent retinal progenitor cells, restricted retinal progenitor cells, and cone photoreceptors. (5 points)
C) Discuss how you the evaluate the number of the different cell states are often determined in such an experiment. (5 points)

**MCD First Level Exam- PART TWO**
**Part 2: EACH QUESTION IS WORTH 20 POINTS**
**Turn-it-in opened from 2pm until 5pm**

**Time yourself and make sure to submit BEFORE OR BY 5 PM. The turn-it-in link will close at 5 pm. If you do not turn it in before or by 5 pm you will receive a 0 grade for this part of the exam. Set a timer and submit whatever you have before or by the 5 pm mark.**

**You must answer 1 of the 2 experimental design choices and 1 of the 2 data analysis choices. Clearly indicate the number and the references for your answers. The answers must appear in numerical order in your written document. For example, if you answer choices 7, and 10 then they must appear in that sequential order.**

**PART 2A: PICK ONE Experimental Design QUESTION, WORTH 20 POINTS**

**Choice 7. Experimental Design**
**References:**
1. Pluvinage, J.V., et al., *CD22 blockade restores homeostatic microglial phagocytosis in ageing brains.* Nature, 2019. 568(7751): p. 187-192. (Pluvinage et al., 2019)
2. Prinz, M., S. Jung, and J. Priller, *Microglia Biology: One Century of Evolving Concepts.* Cell, 2019. 179(2): p. 292-311.(Prinz, Jung, & Priller, 2019)

**Questions:**
A) Microglia are highly sensitive to perturbations to the microenvironment. In this paper, researchers isolate microglial cells and perform RNA sequencing and use a specific scientific approach. What type of gene expression can this approach lead to? Please discuss which experimental approaches may be affected by this pitfall and what methods you can use to overcome this limitation? Name all the methods that could be used. (5 points)

B) The authors mention that hypo-motility, increased lysosomal cargo, and chronic expression of pro-inflammatory signaling molecules, are features of aged microglia. If you wanted to address each of these cellular behaviors instead of phagocytosis, how would you have altered the design the experiments in Figure 1a? You can assume you have a live imaging system and qPCR system, in addition to flow cytometer. Please provide as much detail as possible, especially on your read out for each of the microglia functions. (8 points)

C) Please detail an experimental model that would allow testing the role of CD22 in microglia specifically. (6 points)

**Choice 8. Experimental Design**

**References:**

1. Ravotto, L., et al., *A Bright and Colorful Future for G-Protein Coupled Receptor Sensors.* Front Cell Neurosci, 2020. 14: p. 67. (Ravotto, Duffet, Zhou, Weber, & Patriarchi, 2020)
2. Sun, F., et al., *Next-generation GRAB sensors for monitoring dopaminergic activity in vivo.* Nat Methods, 2020. **17**(11): p. 1156-1166. (Sun et al., 2020)

**Questions:**

Genetically encoded, fluorescent biosensors based on G-Protein Coupled Receptors (GPCRs) have revolutionized the detection of neurochemical cell signaling molecules and other compounds of physiological importance. GPCRs form the largest family of cell surface receptors. For most GPCRs at least some of the natural ligands that bind to them are known. However, the ligands for some GPCRs remain to be discovered. These receptors are called orphan receptors.

A) Explain in detail what is a GPCR based fluorescent biosensor and what is their common mechanism? (5 points)

B) How would you go about constructing a biosensor based on an (orphan) GPCR? Please make sure to include all the experimental detail and steps required. (10 points)

C) What could you use your biosensor for? (5 points)

## PART 2B: PICK ONE Data Interpretation QUESTION, WORTH 20 POINTS

### Choice 9. Data Interpretation

**References:**

1. Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA synthesis in mammalian cells. PLoS Biol. 2006 Oct;4(10):e309. (Raj, Peskin, Tranchina, Vargas, & Tyagi, 2006)
2. Raser JM, O'Shea EK. Control of stochasticity in eukaryotic gene expression. Science. 2004 Jun 18;304(5678):1811-4. (Raser & O'Shea, 2004)
3. Nwokafor C, Singer RH, Lim H. Imaging cell-type-specific dynamics of mRNAs in living mouse brain. Methods. 2019 Mar 15;157:100-105.
Three papers describe different measurements of variability in gene expression. (Nwokafor, Singer, & Lim, 2019)

**Questions:**

A) (5 pts) Discuss how the variabilities measured in Raj et. al. (Fig.5) and Nwokafor et al. (Fig. 3d) are qualitatively distinct.
B) (5 pts) Suppose you want to elucidate the mechanism of an immediate early gene expression (e.g., *c-fos*), which occurs variably in multiple cell types of a mouse brain induced by sensory stimuli. What would be the challenges with the method of mRNA detection by FISH in Raj et al.?
C) (10 pts) Discuss any difference between data of Raser et. al. (Fig.1b) vs Nwokafor et al. (Fig. 3d). Explain why the former employs two-color dual reporters, while the latter uses a single reporter, to resolve the extrinsic and intrinsic noises. Describe the anticipated plots in case of a gene exhibiting allele-specific expression.

**Choice 10. Data Interpretation:**

**References:**

1. Sepich-Poore, G.D., et al., *The microbiome and human cancer.* Science, 2021. 371(6536). (Sepich-Poore et al., 2021)
2. Routy, B., et al., *Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors.* Science, 2018. **359**(6371): p. 91-97. (Routy et al., 2018)

**Questions:**

Recent findings have pointed to a role of the human microbiome in the effectiveness of certain anti-cancer therapies. Multiple cancer immunotherapies have been developed, and one example is the use of anti-PD1 (programmed cell death protein) monoclonal antibodies. These monoclonal antibodies fall into a class, or strategy, of immune checkpoint inhibitors (ICIs) and they function to prevent T lymphocytes (T cells) from being inactivated when targeting cancer cells. However, the ICI strategy is not effective for a large number of cancer patients, or the effectiveness drops off as cancer cells acquire resistance to the treatment. A number of reports have linked the host microbiome with the effectiveness of ICI treatments for a number of cancers, and specifically, Routy *et al.* (2018) reports on the role of the gut microbiome in influencing the effectiveness of PD-1-based immunotherapy against epithelial tumors.

Consider the data in Figure 1A-C.

A) (4pts) In mice with RET or MCA-205 tumors, what affect did administration of broad-spectrum antibiotics have on the effectiveness of anti-PD1 antibodies on tumor size?

B) (4pts) In Figure 1A, there appears to be a difference in effect of the iso-control antibodies administered with or without antibiotics on RET tumor size (and this difference is not seen in MCA-205 tumor size) – what might explain this? And what additional control could be used to test this explanation?

C) (2pts) Is the effect of antibiotics seen in mice (Figure 1A and 1B) also seen in human patients with NSCLC, RCC, and UC cancers undergoing anti-PD1 and anti-CTLA4 therapies? Support your answer with data from Figure 1C.

Consider the data in Figure 2A-2C.

D) (4pts) The researchers looked at patient gut microbiome samples and measured the gene count and metagenomic species (MGS). What can you say about patients with >6 months cancer progression-free survival (PFS) in regards to gene count and MGS from their gut microbiomes?

E) (4pts) Which groups (or taxa) of bacteria are enriched in patients classified as responders (partial response or stable disease) relative to those patients classified as non-responders (progressing disease or death)? Use the data in Figure 2B and 2C to support your answer (and provide at least three bacterial groups or taxa).

F) (2pts) Speculate on one or more potential mechanisms that might explain how these groups (or individual bacterial species or strains) within these groups could result in better responses to PD-1 or other ICI treatments for their cancers.