Homework Set 7.2 – Assigned 2/22/2018, Due 2/27/18 by 10:30am

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Office hours – Broad 3rd floor kitchen – Friday 2/23 4:30-5:30pm, Monday 2/26 5-6pm or by appointment

Problem 5 (30 points) Fluorescent Labeling Methods

For your thesis, you decide to examine the localization of "your Favorite Protein" (yFavP).

5.a. (10 points) You are unsure of whether to study yFavP using immunofluorescence or a genetic fusion. Explain one advantage and one disadvantage for each of these two techniques.

You decide you would like to do correlated light and electron microscopy to determine the localization of yFavP.

5.b. (5 points) What additional information can electron microscopy provide as compared to fluorescence microscopy alone?

5.c. (10 points) Just like for light microscopy, electron microscopy can be done using genetic fusions (chemical reactions) or immunocytochemistry (immuno-EM, using gold-conjugated antibodies). Explain one advantage and one disadvantage for using a chemical reaction via a genetic fusion versus gold-labeled antibodies for localization in correlated light and electron microscopy.

5.d. (5 points) After considering your options, you decide you want to use a genetic fusion. Choose a genetic fusion for yFavP and explain how it can be used for both fluorescence and electron microscopy.

Problem 6 (20 points) – Microscopy Techniques and Troubleshooting:

Having chosen a labeling method, you begin to examine the localization of yFavP using confocal microscopy.

6.a. (10 points) You are using a fluorophore that photobleaches quite easily, and you find it hard to get quality images over time. Name another technique you could use instead of confocal microscopy and explain how it overcomes the issue of photobleaching.

6.b. (10 points) Your images suggest that yFavP is forming interesting structures in the cell, but these structures are too blurry in your images for you to come to any conclusions. Explain how deconvolution could be used to improve the images you have taken.

Problem 7 (30 points) –Subcellular Localization:

You want to determine the organelle in which yFavP is found using fluorescence microscopy.

7.a. (10 points) Dyes and marker proteins can be used to fluorescently label organelles or other cellular structures. To which cellular structures are each of the following specific?

i. DAPI

ii. Rab5

iii. RCAS1

iv. Lamin B1

v. LAMP1

vi. Calnexin

vii. COX IV

viii. PMP70

ix. MitoTracker Red

x. Fibrillarin

7.b. (10 points) What is fluorescent crosstalk/bleed-through, and how does it affect your choice of fluorophores in multicolor imaging?

7.c. (10 points) Based on your initial localization assays, you suspect yFavP interacts with a protein on the surface of the inner surface of the cell. Explain how intermolecular FRET could be used to study this investigation. How close do the fluorophores need to be for a successful FRET interaction? (suggested source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5038762/)

Problem 8 (20 points) –Fluorescence Imaging on Cell Surfaces

Your investigation of yFavP led you to another protein, "your second Favorite Protein" (yFavP2), which is found on the cell surface.

8.a. (10 points) Your labmate suggests using TIRF microscopy to study yFavP2. Describe how TIRF works and why it is well suited to your studies of yFavP2. What are the advantages of TIRF over other methods?

8.b. (10 points) You are particularly interested in the mobility of yFavP2 on the cell surface, so you choose to carry out a fluorescence recovery after photobleaching (FRAP) experiment. Explain how FRAP works, and draw an example trace of fluorescence over time during a FRAP experiment.