BMB/Bi/Ch 173 – Winter 2017

Homework Set 2.2 – Assigned 1/19/2017, Due 1/24/17 by 10:30am

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Office hours – Broad 3rd floor kitchen - Friday 1/20 1:30pm-2:30pm, Monday 1/23 5pm-6pm, or by appointment

Problem 6 – (50 points) Chemical fixation in biology

6.a. (5 points) What is the purpose of chemical fixation in biology?

6.b. (5 points) There are several options for chemical fixation. Cross-linking fixatives (ex formaldehyde, gluteraldehyde, osmium tetroxide) are most common in electron microscopy. On a conceptual level, how do chemical cross-linkers work?

6.c. (5 points) What functional groups do typical chemical cross-linkers target? How specifically can chemical cross-linking target individual residues? Consider referencing an outside resource (https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-crosslinking-protein-modification.html) to increase your understanding.

6.d. Some insect cells have prokaryotes living inside of them. Both the insect cell and its endosymbiont are necessary for either cell to survive. Imagine you have isolated an insect cell and its endosymbiont. You use chemical fixation and dehydration before you embed the sample in plastic and cut thin sections to negatively stain for room temperature electron microscopy. Below is a list of observations you make. Which are valid, and which might be artifacts due to the sample preparation you followed? Explain your logic.

6.d.i. (5 points) You see where and how the endosymbiont is arranged inside of the host cell

6.d.ii. (5 points) You see what appear to be large proteins interacting with the endosymbiont cell membrane

6.d.iii. (5 points) You see that the endosymbiont inside of the insect cell has a bilayer membrane that you can trace all around the cell

6.d.iv. (5 points) You see relatively few cytosolic proteins inside of the endosymbiont

6.d.v. (5 points) You see genomic material inside of the endosymbiont appears to have a braided structure

6.e. (5 points) Sometimes scientists add small amounts of chemical fixatives to protein samples in single particle cryoEM experiments to prevent multi-protein complexes from falling apart. What are the pros and cons of this approach?

6.f. (5 points) What are some reasons people use "traditional" EM methods for biology in 2017 despite our great progress with the cryoEM techniques?