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) Applications of chemical fixation to biological samples

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

Chemical fixation is used to preserve the structure of biological samples during other types of processing.

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? 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.

Chemical cross-linkers forge a covalent bond to link two or more molecules together. Chemical cross-linkers act like staples to literally bind nearby things together.

6.c. (5 points) What functional groups do typical chemical cross-linkers target? How specifically can chemical cross-linking target individual residues?

Typically cross-linkers target carbonyls, sulfhydryls, primary amines, and carboxyl groups.

Since the targets that these cross-linkers react with are ubiquitous in cells, they are non-specific. Not required, but FYI:

For example, an aldehyde in formaldehyde reacts with a primary amine in one protein, and then attacks a secondary amine in a nearby protein to form a methylene link.

Gluteraldehyde has an aldehyde group at each end of its carbon chain, and both react with primary amines in proteins. This links two proteins together.

Osmium tetroxide reacts with unsaturated lipids and sometimes proteins

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.

All reasonable answers will be accepted.

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

This ultrastructural observation is valid. It's unlikely that chemical fixation would completely rearrange the system

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

These are questionable because you don't know if the proteins were just nearby when fixation happened so they stuck there, or if this is a physiologically relevant interaction. Further experiments are required.

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

This is a valid observation because it would be hard to create an artifact like that

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

This is a questionable observation because most cytosolic proteins were probably lost during the fixation/dehydration process

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

The presence of the genome and its general location are probably ok.

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?

Pro: if you are studying a transient complex, a fixative might help you find more intact particles to further process

Con: you have to question if interactions you observe are physiologically relevant, because you don't know how the protein structure was altered by the fixative.

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?

-Access to microscopes--Either you can't get access or you can't afford too many cryo days because the nice cryoTEM is very expensive to use vs the older RT TEMs

-Sample too thick to image without sectioning (Cryo-sectioning is extremely challenging and few people are experts; FIB-milling is still a developing technique and hard to get access to)

-reasonable answers will be accepted