1-3, each 20 points, 4-7, each 10 points. Total 100.

1. What is the advantage of fluorescence imaging in biological application?

Fluorescence is relatively higher sensitivity than other optical detection method based on either looking at the light reflected on the surface of a molecule or absorbed by the molecule. Native biological tissue have only small amount of fluorescence so this also contributes to a higher sensitivity. Also, detection is essentially non-invasive.

2. What is the advantage and disadvantage of GFP? Compare with other methods to detect the spatial distribution of a protein of interest.

While fluorescence is advantageous for biological imaging, one still need to introduce fluorescence molecule somehow into the cell. For example, if you want to stain a protein one need to purify the protein, chemically conjugate, test the activity, and introduce back into the cells by injection and it is really a tedious work. Or one can use immunostaining but in that case, tissue has to be fixed and the cell membrane has to be permeabilized by detergent.

In contrast, GFP can label cells purely genetically. So once you make a fusion protein using recombinant DNA technology, one just need to introduce it into the cells and one can monitor the distribution of the protein in living cells.

There are, however, certain precautions. Fusion with GFP should not affect the activity or distribution of the protein. GFP should not detach from the protein it is fused to by protein degradation (one can test with western blotting). Also, overexpression of a protein which exist usually in small amount may impair a normal distribution.

3. What is the advantage and disadvantage of dissociated culture?

It is much easier to manipulate and observe compared with in vivo. However, because it has lost tissue architectures, It should be carefully tested

whatever happen in vitro actually happens in vivo.

4. In Figure 1B, there are dots which has red color (positive with PSD-95) but not green. How can it happen?

Transfection efficiency is not always 100%. Actually, in most methods, it is lower. The cells which shows only PSD-95 signal but not GFP signal are non-transfected cells.

5. In Figure 2B, what kind of experiment can you design to confirm that the loss of CaMKII spot is due to photobleaching of the GFP-CaMKII at synapse, not because the synaptic structure itself is lost?

If you can stain the neuron so that it shows structure in different color, you can confirm that the structure is not largely changed. For example, you can cotransfect with red fluorescent protein. If you do that, you can monitor the change in structure at red channel. You can also immunostain against synaptic marker such as Psd-95 later similarly to 1B. Also, other types of dye such as DiI also work.

6. There are many kinases which phosphorylate tyrosine, called tyrosine kinase as opposed to serine/threonine kinase, to which CaMKII belongs. If you want to block the phosphorylation by tyrosine kinase, into which amino acid can you change the tyrosine (hint look at the structure of different amino acids)? Also, can you design a phosphomimic mutation for tyrosine phosphorylation as in Figure 4F?

Tyrosine has a benzene ring and a –OH which receive phosphate. If you want to block phosphorylation, phenylalanine will be the best because it shares benzene ring but not –OH group. Phosphomimic mutation requires negative charge. For serine and threonine kinase, aspartate and glutamate are used because they have –COOH group which to some extent mimics phosphorylated status. But there is

no amino acid which has both benzene ring and negative charge. So the answer is NO.

7. The authors interpret that CaMKII is detached from F-actin by stimulation in Figure 4A. But just from this figure, an alternative interpretation is also possible. What is such possibility? How do other figures rule out such possibility? Can you design a more direct experiment to rule out such possibility?

It is possible that CaMKII keeps association with F-actin but F-actin itself changed the distribution from fibrous structure to homogeneous distribution by Ca2+ ionophore. If you just look at Figure 4A, such interpretation is also possible. However, in Figure 4C, even after application of ionophore, A303R mutant stays unchanged and looks like still bound to F-actin. This rules out that ionophore changes the distribution of F-actin. If one want to more directly demonstrate it, one can stain F-actin with antibody (actually, this is not usually done) or fluorescent phalloidin (a compound specifically binds F-actin. This is easier and more popular).