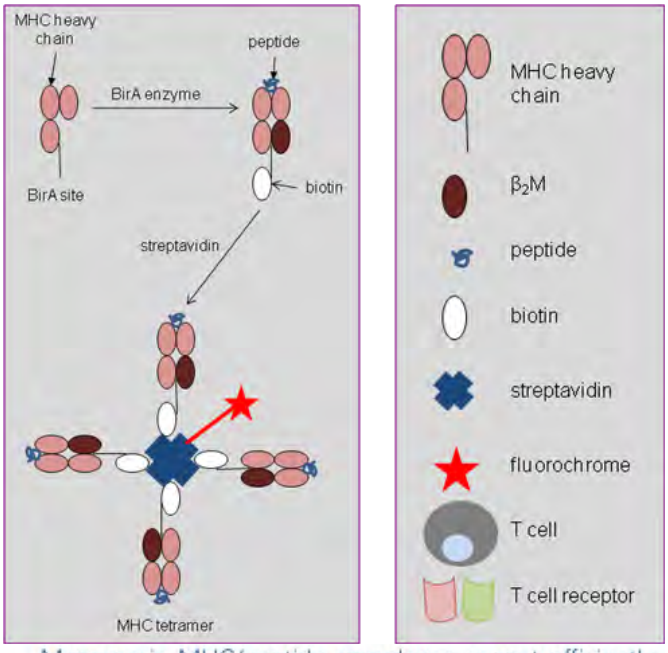
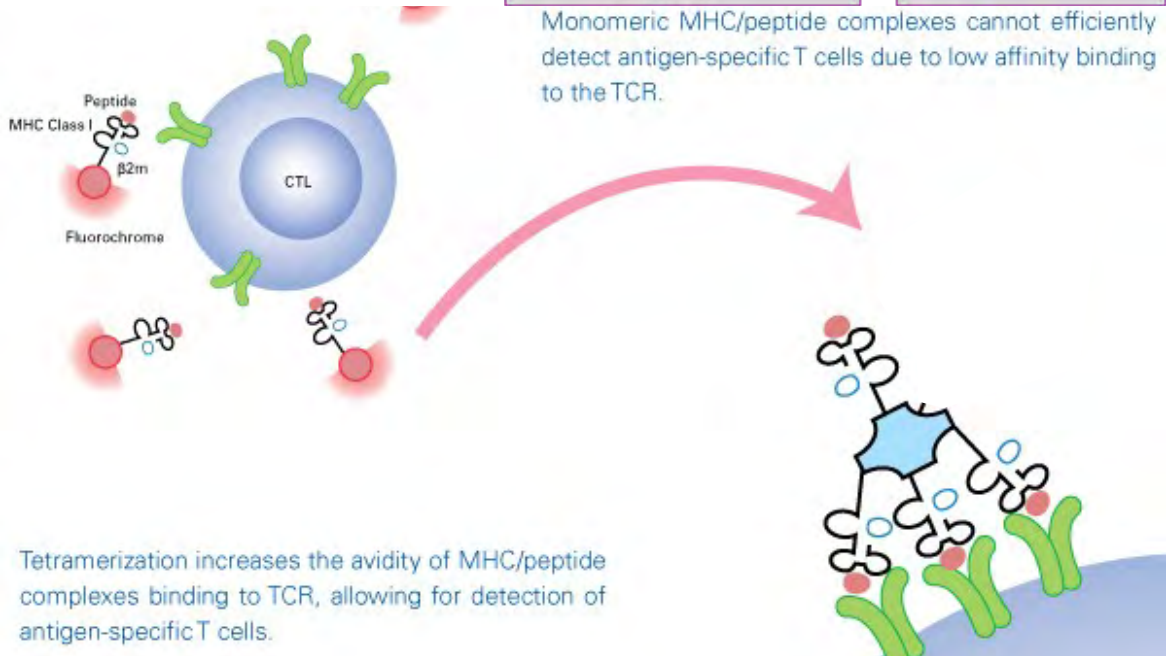
BE 167L: Bioengineering Laboratory — Exam 1
April 30, 2019
Please write your name and UID on all answer sheets.

Question 1 (12 pt). T cells bind to MHC-peptide complexes, but do so with weak affinity (off rates of ~10 seconds). To strengthen this binding interaction to the extent that binding T cells can be isolated and analyzed, Altman *et al*, *Science*, 1996 came up with a strategy to form labeled peptide-MHC "tetramers". Multiple binding interactions leads to avidity[1] and thus a more stable interaction.

You wish to simplify this strategy, using your knowledge of covalent protein conjugation. Instead of using streptavidin, which has four binding sites for biotin groups, you plan to use a multivalent molecule with four or more "arms" containing the reactive group of your choosing.





(a) On the MHC heavy chain, orientation is critical. We need to ensure the protein is oriented outward, and that conjugation only occurs on the tail of the protein. We are able to change the protein sequence wherever we would like, though. What could we do? Design a strategy for covalently conjugating this protein to a molecule with four arms and the reactive group of your choosing. Justify your answer.
(b) To provide fluorescent signal, we want to attach multiple fluorophores. Location doesn't really matter, as the MHC is large relative to the end portion that binds, and it's alright if a small number are made inactive. What reactive group could we use here?
(c) Sketch a spectra for a fluorophore, labeling the relevant properties of interest. What regions of the spectra indicate the wavelength of light we need for our light source and detector?
(d) Your colleague walks over to the medical school with your tube of assembled tetramers on a sunny day. While you checked that the complexes worked, they are unable to see any fluorescence. What happened? Can you fix this?

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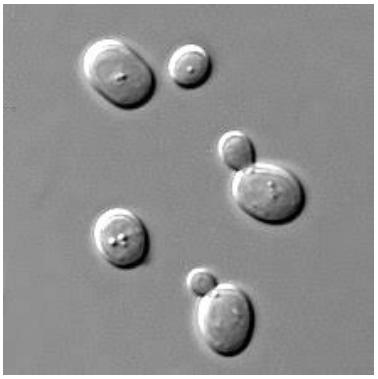
[1] Avidity is the stabilization of an interaction through multiple binding sites.

Question 2 (15 pt)

(a) Sketch the elements of a plot.
(b) What are three forms of data?
(c) List four ways to convey information in a plot. For which forms of data is each way effective?
(d) What is the principal reason to keep a lab notebook?
(e) What are three reasons to build a model?

Question 3 (13 pt). You are working in a lab trying to make microfluidic devices that automatically test sets of cancer drugs against cells taken out of a patient.

(a) Your first day, a colleague asks for help. A PTFE (Teflon) tube that injects media into the device is ending up with cancer cells growing in it, leading to contamination between samples. He's dumbstruck because "nothing sticks to Teflon." What are the cells sticking to? How could you fix this?
(b) Next he admits that the cancer cells grow better on the tubing that the glass surface of the device, where they are supposed to grow. What could you do to increase cell attachment here?
(c) You want to culture the cancer cells in the device after capture. What are four elements of the environment you must control to accomplish this?
(d) You place your first cells and start the device. The next day, your media is the wrong color, and there are little spots (see picture to the right) in the device. What happened? What should you do?



Question 4 (10 pt)

(a) You have a device where cells randomly fall into microwells. The distribution of cells can be modeled by a Poisson distribution:

$$p(k) = \frac{\lambda^k e^{-\lambda}}{k!}$$

where k is the number of cells in a well, and λ is a variable parameter. However, a Poisson distribution can take values $[0,\infty]$, while you know that only up to 2 cells can fit in a well (so your distribution should only go from 0 to 2). Given what you know about distributions, how might you need to adjust this expression?

(b) You want to maximize the number of wells with exactly one cell. What value of λ does this occur at? (You just need to give the expression you would solve, not the value.)
(c) What is the mean of your distribution for $\lambda = 1$?
(d) You measure the average number of cells per well on four different devices (5 wells per device). What are three things you can say (total) about the sampling distributions of the mean for $N = 1$ and $N = 5$ compared to the original distribution?