MAE6291 2012 Midterm Exam

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| 1 If you assume most protein molecules have the same density as water, how would you expect their diffusion constants to vary with molecular weight? Hint: how does diffusion constant vary with radius; how does radius vary with molecular weight? (5points) | | | | | |
| 2 If the free ligand concentration is 10 times the KD, what fraction of capture molecules bind ligand at equilibrium? (5 points) | | | | | |
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| 3 What is the average distance between molecules whose concentration is 1.6nM? (5 points) | | | | | |
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| 4 What is the characteristic time to reach equilibrium for an antibody that binds ligand with kon = 106/Ms, KD = 10-9M when [L] = 1pM? Does this time change if [L] is much lower or higher? (5 points) | | | | | |
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| 5 On average, how long does it take a molecule with diffusion constant = 3x10-11m2/s to diffuse from the top to the bottom of a 100m tall flow cell (assume this is diffusion in 1 dimension)? (5 points) | | | | | |
| T=x2/2 |  |  | |  | |
| 6 What does the quasi-steady state depletion zone look like in a flow cell with H = 100m, W=1cm, L = 1cm, Q=1l/s, if the sensing surface covers the entire bottom of the flow cell and the ligand molecules have diffusion constant D=10-11m2/s? (10 points) | | | | | |
| 7 About how long should you have to wait for equilibrium in the flow cell of problem 6 if kon=106/Ms = 1.67\*10-21/[(molecule/m3)s], the surface density of antibodies on the sensor surface is 1016/m2, and the time to reach equilibrium for this antibody and ligand concentration in a bulk assay (where there is complete mixing) is rxn = 1000s? Pay attention to the units! (10 points)  8 What is the advantage of miniaturizing the volume of the last step of an ELISA reaction to  50fl? (5 points)  9 Why did Manalis et al go to the trouble of making a tiny flow channel *inside* their microcantilever instead of simply inserting the cantilever into a sample solution, since in the latter case they could easily assay much larger sample volumes? (5 points)  10 If the resonance frequency of a microcantilever with internal channel volume =10pl changes from 210,000Hz to 220,000Hz when it is filled with water, and from 220,000Hz to 220,001Hz when it is filled aqueous (water) sample containing ligand, about how many ligand molecules with molecular weight 100,000g/mole have been captured? (10 points)  11 Why does TIRF microscopy enhance sensitivity for detection of low amounts of fluorescent light in systems where the signal comes from analyte stuck to a surface? (5 points)  12 Why do higher Q-factors make it possible to detect lower analyte concentrations in sensors in which signal comes from changes in a resonant frequency? (5 points)  13 Why does increasing binding strength or capture molecule density on a sensor surface frequently improve sensitivity? What is the relationship between number of ligand molecules captured on the surface, number of molecules on the surface that do the capturing, free ligand concentration, and binding strength? (5 points)  14 What rate do you expect analyte molecules at 100 attomolar concentration (10-16 M) to bind capture antibodies on a microscale sensor with area = 100m2 if the antibodies are at a density of 1012/cm2 and the on rate (kon) is 105/Ms? (10 points)  15 Should you be skeptical or impressed if someone reports an antibody-based sensor that has half-maximum response when analyte concentration is femtomolar? Why? (5 points)  16 How can an ELISA assay, such as the one confining beads to 50fl volumes, detect ligand at 100aM concentration if it is based on antibodies that bind with a KD of only nM? (5 points) | | | | | |