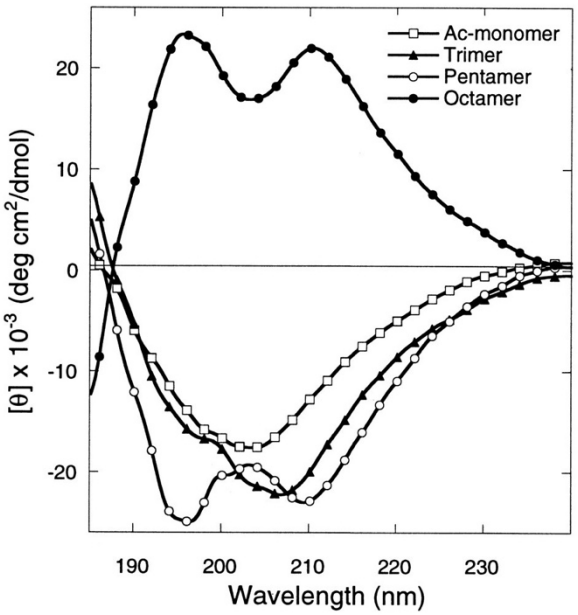
Homework Set 4.2 – Assigned 2/1/2018, Due 2/6/18 by 10:30am

TA - Sara Weaver sjweaver [a] Caltech.edu

Problem 3 (25 points) - Circular Dichroism

A novel family of biomimetic heteropolymers has been developed. In order to see the helical secondary structure, the CD spectra of the polymers of different lengths are obtained.



3.a. (5 points) What is the fundamental requirement that a molecule must have in order to yield a signal for a CD experiment?

The molecule must be chiral

3.b. (5 points) What do the peaks at 208 nm and 222 nm signify?

Alpha helical secondary structure

3.c. (5 points) How can you tell at what length does the polymer begin to show helical characteristics?

The characteristic double minima in the range of 203□240nm of a helix secondary structure are evident in the N□(p□nitrophenylethyl)glycine pentamer and octamer.

3.d. (5 points) If the octamer is helical, how can you account for its positive molar ellipticity?

The octamer is built from monomers with the opposite chirality and so displays positive bands.

3.e. (5 points) What experiments can you do using CD to test whether the helical structure in the polymer arises from backbone hydrogen bonding?

Because the helix secondary structure of this polymer is not driven by intramolecular hydrogen bonding, addition of denaturants such as urea, which usually competes with this interaction, would not "unfold" the polymer's secondary structure. If that were the case then the helix□like CD of the octamer would not have significant changes with the addition of such denaturants. <PNAS Zukermann 1998>

Problem 4 (40 points) - Circular Dichroism and nanodiscs

During graduate school, you decide to characterize the structure and function of a newly identified potassium channel. You've already shown that this membrane protein can be expressed recombinantly in *E. coli* and reconstituted into detergent micelles. Initially you tried single particle cryoEM with your protein in detergent micelles, but the image quality was poor due to the presence of the detergent.

You've read that membrane proteins can be isolated in "nanodiscs". In brief, membrane proteins integrated into a cell membrane can be extracted by amphipathic styrene-maleic acid copolymers (SMAs). The result is that the membrane proteins are then in a detergent-free nanodisc. For reference, consider figure 1 from Dörr et al PNAS 2014 (https://doi.org/10.1073/pnas.1416205112).

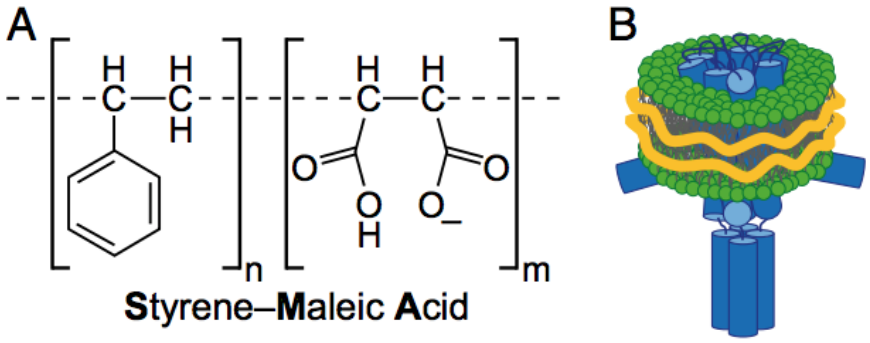


Fig. 1. (*A*) Chemical structure of SMA polymers at neutral pH. For this study, a polymer with an average SMA ratio of n:m = 2:1 was used. (*B*) Schematic representation of a native nanodisc containing a KcsA tetramer (blue) and native lipids (green). The outer hydrophobic surface of the lipids is shielded by SMA (orange).

Based on reading the literature about nanodiscs, you suspect your protein will be more stable in them than it is in detergent micelles. However, before your PI will approve more expensive cryoEM time, you decide to investigate the thermal stability of the nanodisk complexes by circular dichroism (CD) as a proxy for general protein stability.

(10 points) Your protein is a pain to purify, so you're hoping that you can freeze aliquots of protein at -80˚C for future use. How could circular dichroism be used to assess the resistance of your protein to freeze-thaw cycle degradation?

Take CD data before and after different numbers of freeze/thaw cycles and observe if the secondary structural elements disappear.

(5 points) What are the sample requirements for circular dichroism of proteins? Is there a size requirement?

You need ~100 µL of 0.25 mg/mL protein

No size requirement

Chiral

Buffer components that aren't CD active

Examine the CD data in Figure 3A reproduced below from Dörr et al PNAS 2014 (https://doi.org/10.1073/pnas.1416205112).

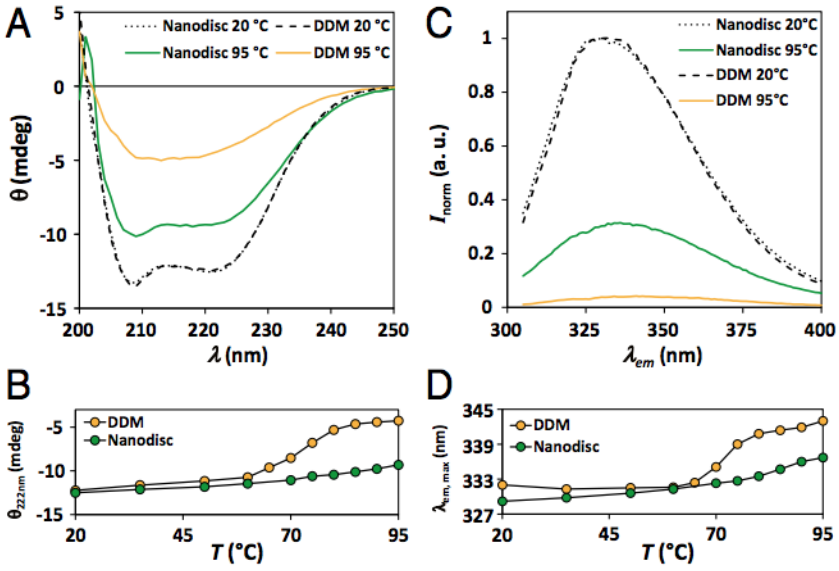


Fig. 3. Comparison of the thermal stability of KcsA in different environments. (*A*) Circular dichroism spectra of KcsA in native nanodiscs and DDM micelles at 20 °C and 95 °C. Data are offset corrected averages of 8–10 scans. (*B*) Corresponding thermal unfolding traces monitored by the ellipticity at 222 nm. Data are averages of two experiments with errors too small to be depicted. Solid lines are depicted to guide the eye. (*C*) Normalized fluorescence intensity of the intrinsic tryptophans of KcsA at 20 °C and 95 °C. Data are averages of three scans normalized to the intensity at 330 nm of the respective spectra at 20 °C. (*D*) Corresponding thermal unfolding traces monitored by the wavelength of maximum fluorescence emission. Solid lines are depicted to guide the eye.

(5 points) Compare the CD trace of the Nanodisc sample at 20˚C with the DDM sample at 20˚C. What does this tell you about the protein structure in either case?

The traces for Nanodisc and DDM look identical at 20˚C in figure 3A. This suggests they have the same secondary structure elements.

(5 points) Compare the CD trace of the Nanodisc sample at 95˚C with the DDM sample at 95˚C. What does this tell you about the protein structure in either case?

The traces for Nanodisc and DDM look different at 95˚C in figure 3A. The nanodisc sample shows some reduction in secondary structural elements. The DDM sample appears to have lost all alpha helical character upon heating.

(5 points) Explain the purpose of figure 3B.

Figure 3B shows the change in the signal at 222 nm (a characteristic peak in alpha helical proteins) as the temperature is gradually increased from 20˚C to 95˚C. Monitoring this allowed the researchers to identify the folding transition temperature in the nanodisc vs DDM environment.

(5 points) Could you directly use a CD protein sample for single particle cryoEM?

Yes, unless you diluted it too much. You don't have to add anything to a protein sample to perform CD, so the buffer should be fine for EM.

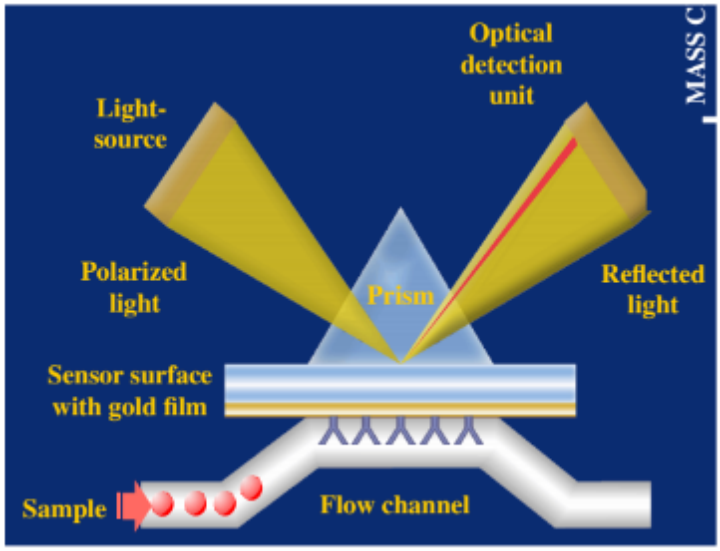
(5 points) One benefit of nanodiscs generated using SMAs is that some of the lipids from the native membrane are present in the nanodisk. Why might you want native lipids to be present in your sample?

Presence of native lipids could stabilize protein

Could discern native lipid/protein interactions in structural or functional studies

Problem 5 (50 points) - Surface Plasmon Resonance

5.a. (15 points) Draw the typical schematic of a Biacore SPR instrument and explain how signal is measured to produce affinity data for a biomolecular interaction.



In SPR, a wedge of polarized light is focused on a conductive slide (typically made of gold) under the conditions of total internal reflection. At a characteristic angle of light, energy transfer occurs between the light wave and the surface plasmons, resulting in a thin shadow in the reflected wedge of light. This angle depends on the refractive index of the material, which in turn depends on the mass of the film. When an analyte binds to a ligands on the SPR chip, the mass difference leads to a change in the angle of maximum light absorbance, which is the signal measured by the instrument.

5.b. (5 points) Why do SPR experiments include a surface regeneration step?

In a typical SPR experiment, different concentrations of analyte are flowed over the surface of the chip with the ligand. A regeneration step is necessary to remove all of the bound analyte so that the surface can be reused for the next analysis.

5.c. (10 points) Your undergraduate comes to you with an idea for an experiment and you need to decide if it makes sense. They would like to study the binding of a EF-Tu (elongation factor thermo unstable) to the *E. coli* ribosome by SPR. They plan to covalently immobilize the ribosome to the sensor chip using amine coupling and use EF-Tu as the analyte. Assume the *E. coli* ribosome is about 2.5 MDa and EF-Tu is about 40 kDa.

Do you expect a significant mass difference upon EF-Tu binding? Would EF-Tu behave better as an analyte or a ligand?

Answers vary. Logical answers accepted.

If there is a significant molecular weight difference, typically the smaller molecule is attached to the grid.

Additionally, proteins with molecular weight > 1 MDa or MW<500 Da are not usually attached to the grid.
https://cdn.gelifesciences.com/dmm3bwsv3/AssetStream.aspx?mediaformatid=10061&destinationid=10016&assetid=17633

EF-Tu would be a better choice for ligand.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3318685/ In this paper, SecYEG protein in an inner membrane vesicle is the ligand immobilized on a L1 sensor chip and ribosomes act as the analyte, so there is precedence for this type of experiment.

5.d. (10 points) Is the amine-based covalent immobilization strategy appropriate if the ribosome is the ligand? Comment on potential pitfalls of this design and suggest an alterative approach.

Answers vary. Logical answers accepted.

There is more than one free amine on the surface of the ribosome, so the ribosome will be immobilized in different positions on the grid surface. Since EF-Tu binds in a specific place, it's possible that some of these ribosome positions will sterically hinder EF-Tu binding. This hindrance could occlude binding, or it could change the kinetics in an unpredictable way, because you wouldn't know what ratio of ribosomes would be held in each capture orientation.

A capture-based immobilization strategy would be safer because you would know what approximate orientation the ribosome would be held at.

You can biotinylate nucleic acid instead.

Regeneration could destroy ribosome since it's non-covalently folded together with subunits

5.e. (10 points) Is the amine-based covalent immobilization strategy appropriate if EF-Tu is the ligand? Comment on potential pitfalls of this design and suggest an alterative approach.

Answers vary. Logical answers accepted.

There are probably many amines on the surface of EF-Tu, so it would be safer to select a capture-based immobilization strategy because you would know what approximate orientation the EF-Tu would be held at. The interaction between EF-Tu and the ribosome might be sensitive to the orientation.

5.f. (10 points) Sensor chips with different surface densities are available. Let's say you have low, medium and high density surfaces, which correspond to low, medium and high ligand density on the chip. Comment on the appropriate density for the scenario in which the ribosome is the ligand and for the scenario in which EF-Tu is the ligand.

Answers vary. Logical answers accepted.

A capture-based immobilization strategy would be safer because you would know what approximate orientation the ribosome would be held at.

Typically when the ligand is a small molecule, a high density is used. However, since the ribosome is large, if EF-Tu is at too high of density, there could be steric issues for ribosome binding.

If the ribosome was the ligand, you'd probably pick a low density chip (and also use short dextran layers) since it's a large protein. However, as stated above the ribosome is probably too large to immobilize on a chip.

There are also concerns that the linker needs to be long enough to facilitate the interaction.