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

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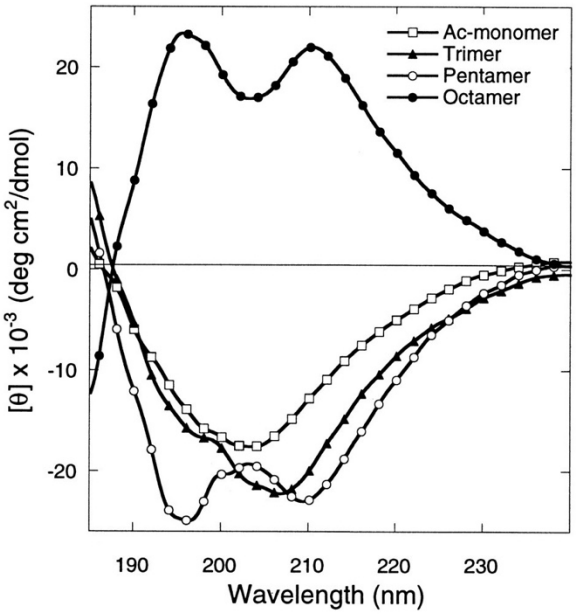
Office hours – Friday 2-5pm in BI EM Lab – Arctica Room and Monday Feb 5 11am-12pm BI EM lab computer room, or by appointment

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?

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

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

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

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?

Problem 4 (50 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).

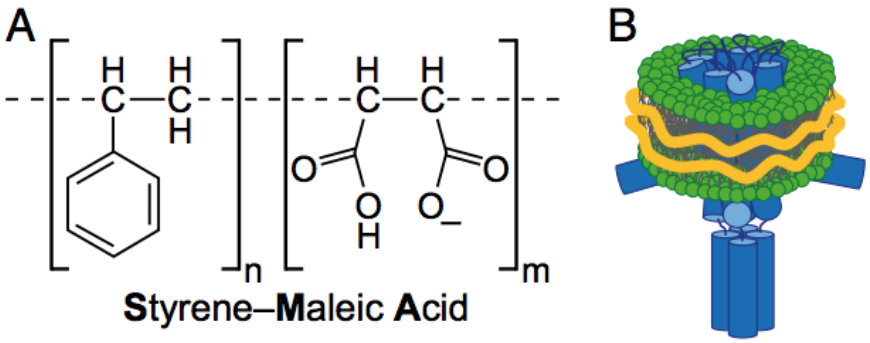


Fig. 1. (*A*) Chemical structure of SMA polymers at neutral pH. For this study, a polymer with an average SMA ratio of n:m = 2:1 was used. (*B*) Schematic representation of a native nanodisc containing a KcsA tetramer (blue) and native lipids (green). The outer hydrophobic surface of the lipids is shielded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

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

4.b. (5 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?

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

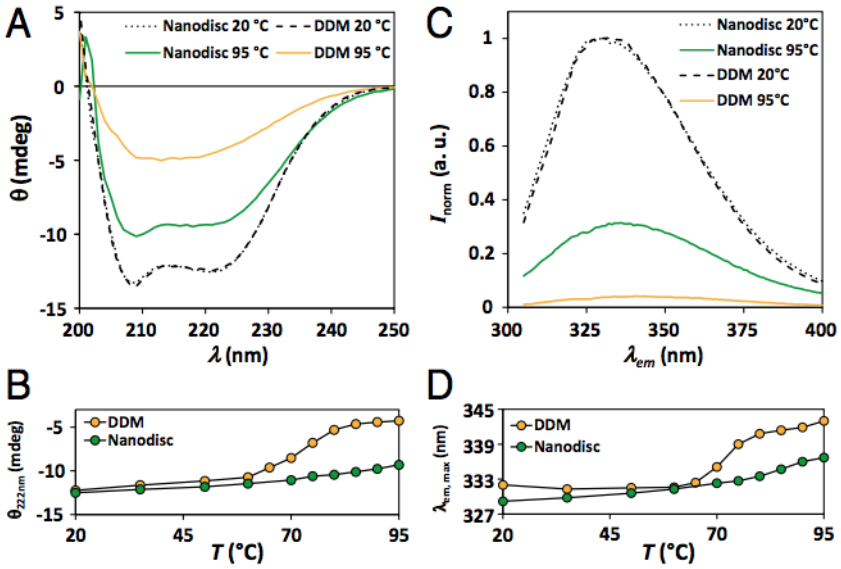


Fig. 3. Comparison of the thermal stability of KcsA in different environments. (*A*) Circular dichroism spectra of KcsA in native nanodiscs and DDM micelles at 20 °C and 95 °C. Data are offset corrected averages of 8–10 scans. (*B*) Corresponding thermal unfolding traces monitored by the ellipticity 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.

4.c. (10 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?

4.d. (10 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?

4.e. (10 points) Explain the purpose of figure 3B.

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

4.g. (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?

Problem 5 (65 points) - Surface Plasmon Resonance

Hint:
https://cdn.gelifesciences.com/dmm3bwsv3/AssetStream.aspx?mediaformatid=10061&destinationid=10016&assetid=17633

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.

5.b. (10 points) Why do SPR experiments include a surface regeneration step?

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?

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.

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.

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.