BMB/Bi/Ch 173 – Winter 2018

Homework Set 3.1 (100 Points) – Assigned 1-24-18, due 1-30-18 by 10:30 a.m.
TA: Sara Weaver (sjweaver at Caltech.edu).
Office hours: 3rd floor kitchen in Broad, Friday 1-26 from 4pm to 5pm and on Monday 1-29 from 5pm to 6pm, or by appointment.

Please note that the date of the EM facility tour has been changed to Tuesday, January 30th from 12-12:30pm.

Homework 3.1:

In graduate school you become fascinated by the way bacteria move. In 2006, Gavin Murphy, a BMB graduate student in Grant Jensen's lab, published the first sub-tomogram average of a flagellar motor in *Nature* (Murphy GE, Leadbetter JR, Jensen GJ. *In situ* structure of the complete *Treponema primitia* flagellar motor. *Nature* (2006) 442:1062–4). In 2007 he won the Ferguson Award for best dissertation in the Biology Option.

Part of Figure 3 from Murphy et.al 2006 is reproduced below:

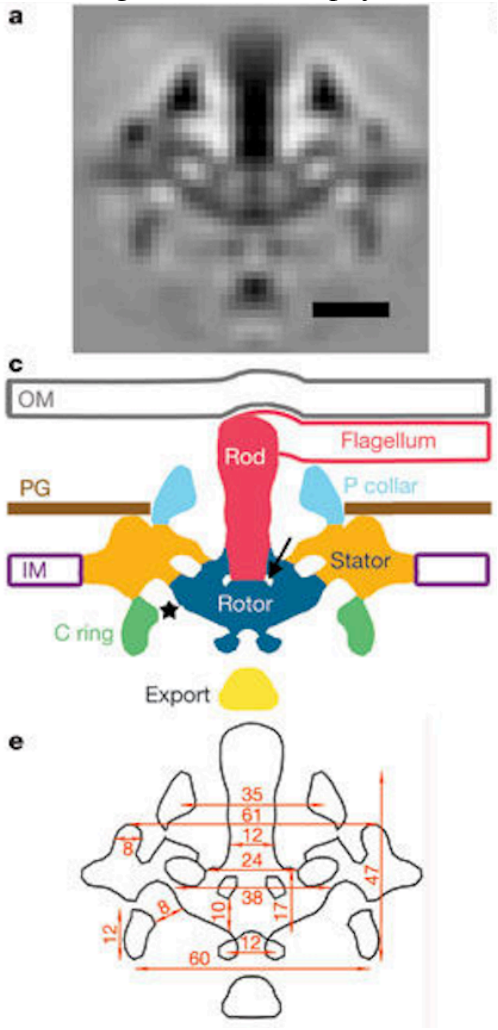


Figure 3: The *Treponema* motor

All objects are at the same scale. **a**, Axial slice through the *Treponema* flagellar motor. Scale bar, 20 nm. **c**, Cartoon interpretation of the *Treponema* motor with its components labelled. The arrows here point to the gap between the rotor and rod, whereas the stars indicate the gap between the C ring and rotor. The location of the peptidoglycan layer (PG) is conjectured. The outlined objects' locations are approximate. The flagellum actually bends more gradually over the P collar in presumably random directions but appears straight when averaged. The inner membrane (IM) and outer membrane (OM) both bulge more widely around the motor *in situ* than is pictured. **e**, Measurements (in nm) of various motor features.

In 2016, John Briggs' group reported the highest resolution sub-tomogram average to date: a 3.9 Å structure of the HIV Gag protein (http://science.sciencemag.org/content/353/6298/506.long). You want to try to beat that record with your sub-tomogram averages of flagellar motors. You decide to compare bacteria with polar flagella and peritrichous flagella.

Initially, you decide to take tomograms of a species with polar flagella (*Vibrio cholerae)* and a species with peritrichous flagella (*Bacillus cereus*) so that you can compare their structures by sub-tomogram averaging. Let's assume that the rod shaped *V. cholerae* is about 0.5 μm by 1.5 μm and the rod shaped *B. cereus* is about 1 μm by 3 μm.

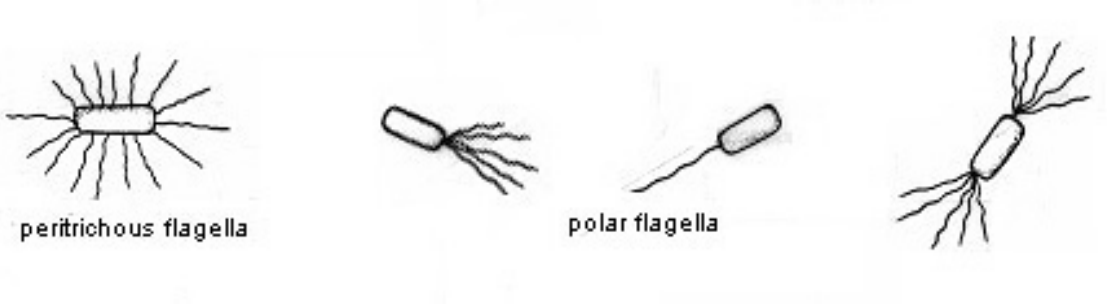
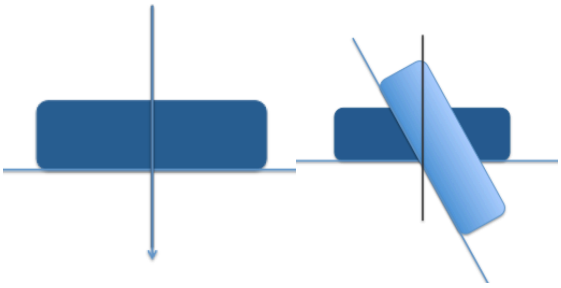


Image reproduced from http://textbookofbacteriology.net/structure_2.html

1.1. To collect a tomogram, you will tilt your sample to +/-60˚.
1.1.a. (5 points) Calculate the distance an electron passing straight through the center of the cell would travel at 0˚ tilt and at 60˚ tilt (see schematic).



1.1.b. (10 points) Which sample would you expect to give better tomograms? Are both samples tractably by whole cell electron cryotomography?

1.1.c. (10 points) We've discussed the idea that cryoEM samples thicker than 500 nm are not practical. What drawbacks will you face with a thicker sample?

1.2. (10 points) Let's say you decide one of your bacteria is too large to do whole cell cryotomography. What could you change in the sample prep or experimental design to improve the tomograms? (HINT: there are bacteria of various sizes with all types of flagella. Sometimes an experimental redesign involves switching species)

1.3. Now that you've selected your bacterial species and set a goal (high resolution sub-tomogram averaging the flagellar motors), let's design the tomography experiment. First you decide to set a resolution target by looking at Dr. Murphy's sub-tomogram average of the *Treponema* motor.

Assume that a flagellar motor is about 80 nm in diameter across its largest part. However, you're interested how the motor is constructed, so you're hoping to resolve objects within the motor that are 8 nm in diameter.

1.3.a. (10 points) Using the equation $N = \pi Ds$, determine how many projection images are required to resolve the 80 nm diameter of the flagellar motor and the 8 nm component in the *V. cholerae* sample using the cell diameter at 0˚ tilt calculated above.

1.3.b. (5 points) What is the minimum tilt increment would be required in each case?

1.3.c. (10 points) In the biological tomography literature, tomograms are typically taken with 0.5˚ to 2˚ tilt increments. Explain the pros and cons of taking a tomogram with 0.5˚ and 2˚ tilt increments.

1.4. You're a bit confused about what the appropriate tilt increment is for your data, so you look to Dr. Murphy's 2006 *Nature* paper. Dr. Murphy reports collecting a tilt series from -63˚ to +63˚ with a tilt increment of 1˚.

1.4.a. (5 points) Using the equation $N = \pi Ds$, calculate the predicted resolution of Dr. Murphy's tomogram.

1.4.b. (10 points) The equation $N = \pi Ds$ is an approximation. Explain how sub-tomogram averaging could be used to beat the apparent resolution "limit" suggested by the equation.

1.5. Now that you have some data, you're thinking about which flagellar motor could be more likely to go to high resolution.

1.5.a. (15 points) Explain what the missing wedge is and how it will affect your tomograms and your sub-tomogram averaging.

1.5.b. (10 points) Do you expect your sub-tomogram average of the polar flagellar motor to be more affected by the missing wedge than your sub-tomogram average of the peritrichous flagellar motor? Assume you have the same number of flagellar motors in your averages. Explain why or why not with a schematic.