BMB/Bi/Ch 173 – Winter 2018

Homework Set 4.1 – Assigned 1/30/2018, Due 2/6/18 by 10:30am

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Problem 1 - (60 points) Resolution in Electron Microscopy

1.a. (10 points) What factors limit resolution in a single particle cryoEM experiment? What is the fundamental resolution limitation in a cryo-EM experiment?

In cryo-EM the fundamental resolution limitation is electron dose. A biological specimen can tolerate 10 to 15 electrons per Å^2 before becoming useless as a source of structural information. Freezing the sample increases the tolerable electron dose, but it's still a major limitation.

Other factors: Radiation damage, quality of electron optics, defocus, CTF of microscope, signal to noise ratio, accuracy of alignment, particle homogeneity, MTF of camera, distortions, aberrations of microscope, beam-induced specimen movement, number of particles averaged

1.b. (30 points) Describe in a few sentences how, if at all, resolution can be assessed in electron crystallography, single particle analysis, and electron tomography.

Crystallography – Resolution can be determined by the distance of the diffraction spots from the unscattered beam in a diffraction pattern. The maximum possible resolution is determined by data collection parameters (like the diffraction length see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3856639/). Once you have solved a protein structure from your electron crystallography data, you can use Fourier Shell Correlation (FSC) to determine its resolution.

Single Particle – Fourier shell correlation (FSC). Resolution is assessed by splitting the image set in half and calculating independent reconstructions from the two half-data sets, and then assessing to what resolution do the two reconstructions agree

Tomography – No quantitative assessment of resolution. You can look for features you expect (can membranes be resolved? Can you see the bumps on the microtubules?) to get a qualitative assessment. Sub-tomogram averaging resolution can be assessed by FSC.

1.c. (10 points) What regions of reciprocal space are missing in:

Single---axis electron tomography (one tilt series) **--** missing a wedge

Two---axis electron tomography (two tilt series) **–** missing a pyramid

Single particle analysis **–** if you have random orientations, none missing

Many students commented on the limitations in Random Conical Tilt reconstruction in single particle analysis, which is not the typical workflow used

2D electron crystallography – missing cone

1.d. (10 points) What is the Fourier Shell Correlation (FSC) curve? Conceptually explain how it is solved and what is it used for in single particle cryoEM.

During the 3D refinement, the dataset is randomly split into two halves. Each half undergoes the reconstruction. At the end, the two electron densities are compared pixel by pixel.

For a given resolution shell, you compare the amplitudes and phases of each pixel with the corresponding pixel in the other reconstruction. To do the comparison, you take the real component of the dot product of the amplitudes and phases of one reconstruction with the amplitudes and phases of the other reconstruction. You do this for all shells. That sum is divided by the square root of the

product of the sum of the shell of the power in the first reconstruction by the sum of the power in that shell of the other reconstruction.

If the pixels are identical, the FSC equals 1.

Conceptually, the numerator of the FSC measures how similar the two reconstructions are in a given resolution shell while the denominator normalizes that value to representing how much weight that resolution shell has in the overall reconstruction.

1.e. (5 points) What does a low value on the FSC curve mean? What does this tell you about your reconstruction at that spatial frequency?

It means that the two reconstructions look very different at that spatial frequency. It's likely noise in each reconstruction that is varying randomly.

1.f. (5 points) Why was the ResMap software developed?

Electron microscopy resolutions have anisotropic resolution. ResMap maps the local resolution onto the electron density isosurface so that one can evaluate which areas of the structure we are more certain of.

Problem 2 – (30 points) Electron crystallography

2.a. (10 points) How is the convolution theory related to electron crystallography?

A crystal is the convolution of an object convolved with a lattice

FT of an object convolved with a lattice is equal to the FT of the object x FT of the lattice

FT of the lattice is another lattice L (represent with a series of discrete spots in the diffraction pattern

If you think of original lattice as a series of delta functions at each position, the FT of that lattice is another series of delta functions at the lattice.

So it's like you only see the FT of the object at places where L is one.

2.b. (10 points) What does the Fourier transform of a 2-D crystal look like? What about a 3D crystal?

The FT of a 3D crystal gives a series of discrete spots in all three dimensions. So you have an XY plane of discrete spots. You get the same pattern at every Z above that plane. Same effect in principle

In a 2D crystal, FT has discrete spots in XY plane but no discretization of spots in Z. Instead a column of A/P in Z. Since only 1 unit cell thick, need all the waves across that cell in Z to describe the info. So get a column instead of discrete spots.

2.c. (10 points) In an ideal 2D crystal, every unit cell would be identical. Real 2D crystals have subtle differences between unit cells. How do you find that information?

Look to the pixels between the diffraction spots

They are non-zero and contain info about how unit cells are different

The diffraction spots contain info about the average of the unit cells