Question 1:

You are a scientist studying protein structure, function and interactions. You have access to X-ray crystallography, NMR and cryo-EM to solve some interesting problems from the world of protein biochemistry. Which of the 3 methods would you choose for each problem and why?

a) You are studying a protein receptor on cell surface (MW=150 kDa) as a potential drug target. Through cycles of drug discovery, you identified that ligand X binds to your cell receptor and exerts the desired functional effect (i.e., inhibiton of downstream signaling). Which method would you use to study the molecular details of ligand-receptor interactions?

b) In the pandemic time, scientists are intrigued by how the spike protein looks like on the virus particle itself in its native state. You decide to utilize the available facilities and expertise to answer that question. How would you solve the structure of the full COVID- 19 virus, knowing that it's molecular weight is approximately ~40MDa and the virus is pleomorphic (i.e., individual virions vary in size and shape)?

c) You have a protein with molecular weigh of 20 kDa, which interacts with ligand Y through a known mechanism and a well-stablished binding site (pocket). You would like to introduce point mutations in the pocket that disrupt the binding to this molecule. What structural methods could you use to assess how these mutations influence the ligand binding pocket?

d) You are trying to solve a structure of a protein (MW ~200kDa) which aggregates in solution at concentrations above 0.5 mg/ml. What method would be the most applicable in this scenario?

Question 2:

X-ray diffraction is one of the main methods for determination of macromolecular structures from crystals. However, there are some potential alternatives within the electromagnetic spectrum and within the subatomic particle space. Let's explore their applicability to this problem.

a) Microwave radiation – Can it be used in crystal diffraction experiments and allow to achieve atomic resolution? Explain.

b) Gamma radiation – Gamma (rays) have smaller wavelength compared to X-rays, and therefore a much lower diffraction limit. In theory, the maximum achievable resolution when using these rays would be superior. However they are not used for determination of biomolecular structures. Why do you think that is? Also, practically speaking, where could you perform such experiments?

c) Electrons - Can we use accelerated electrons instead of X-rays for crystal diffraction experiments? Do you think that crystal size would be an important factor for feasibility? Explain.

Question 3:

Multiple choice questions of Cryo-Electron Microscopy:

1. During EM, a vacuum is created inside the _____
    a) room of operation
    b) specimen
    c) microscope
    d) objective lens system

2. Which of the following component of EM collects the beam of electrons from the source and directs it towards the sample?
    a) ocular lens
    b) condenser lens
    c) stage
    d) projector lens

3. Image formation in electron microscope is based on _____
    a) column dimensions
    b) objective aperture size
    c) differential electron scattering
    d) specimen size

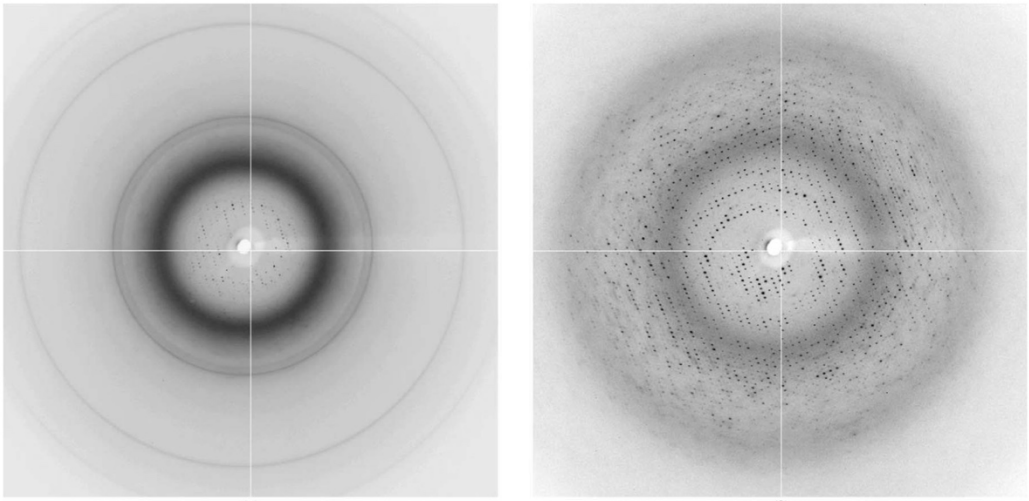
4. The biological materials have little intrinsic capability to scatter electrons compared to water/ice which is why it is essential to have _____
    a) low voltage in the microscope
    b) highly focused beam from the condenser lens
    c) thin ice layer surrounding the biomolecule
    d) very steady stage with minimal drift

5. _____ is the capacity to distinguish between two adjacent atoms or groups.
    a) Magnification
    b) Resolution
    c) Ionization
    d) Division

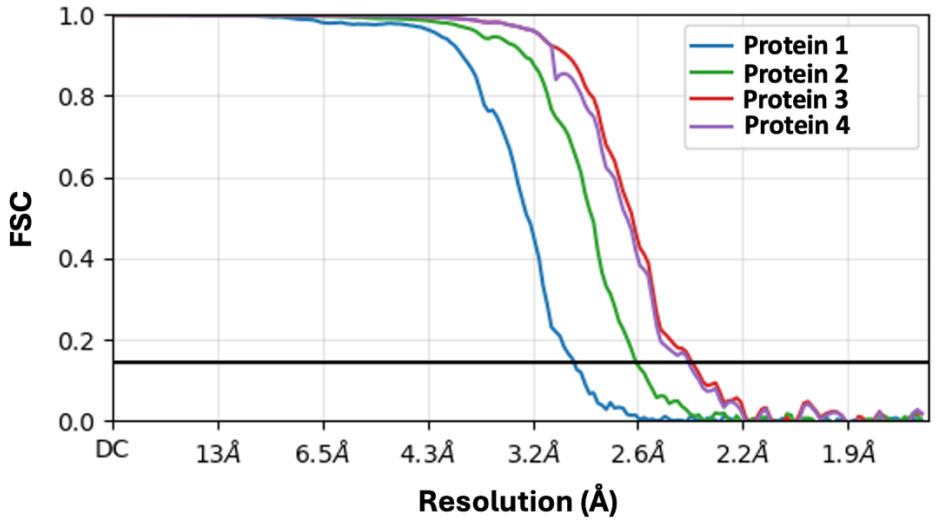
6. How is resolution calculated in cryo-EM?
    a) Fourier Shell Correlation of half-sets
    b) Contrast Transfer Function
    c) R-factors
    d) Model-to-map agreement

Question 4:

1) Below 2 pictures of X-ray diffraction pattern for the same protein. Which one has higher resolution? Why?



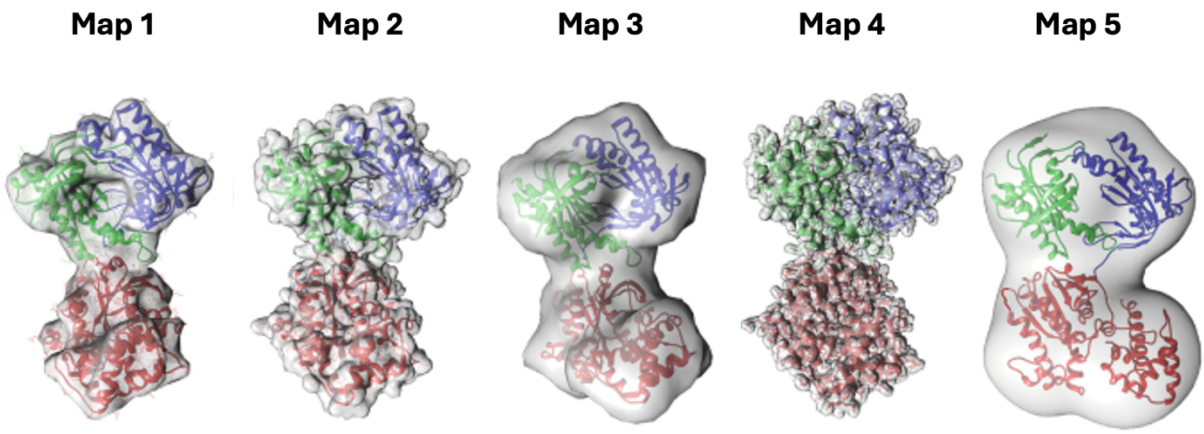
2) Below is the overlay of 4 FSC curves obtained by electron mciroscopy analysis of different proteins (1-4). The gold-standard line is shown in black. From these plots, estimate the resolution of each protein reconstruction and rank the maps from highest to lowest quality (in terms of resolution)?



Question 5:

Below are 5 maps of the same protein obtained by cryo-EM. The maps are presented as transparent gray surface and the corresponding model of the protein is shown with different domains colored in green, blue and red. The maps are all at different resolutions.

a) Can you rank the maps from highest to lowest resolution? Optional: Try to estimate the resolutions by comparing the level of detail in each map to the underlying protein models.
b) Which of the map(s) have sufficient quality to reliably place amino-acids into density?
c) Which of the map(s) only offer information on the level of protein domain but do not have sufficient resolution to accurately build amino-acids or secondary structure elements?



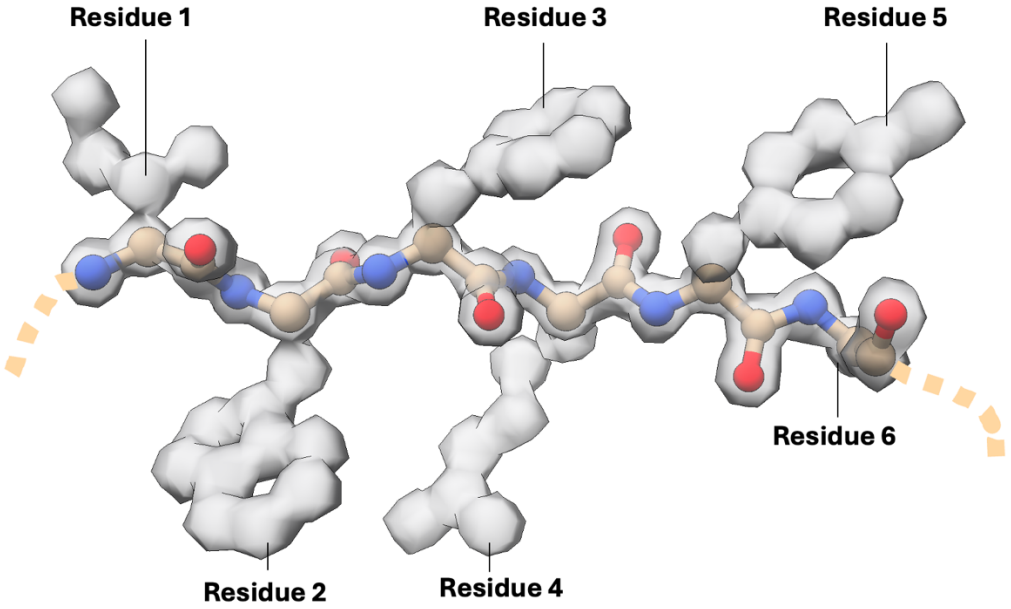
Question 6:

Jacques Dubochet, a Swiss scientist from Aigle, received the Nobel Prize in 2017 "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution". His contribution was the development of a method to rapidly freeze liquid protein sample in liquid ethane.

a) Why does the protein sample need to be frozen for cryo-EM?
b) Why does the protein sample need to be frozen rapidly in liquid ethane?
c) It is common practice to wear masks while freezing grids and to dry all instruments that come into contact with the liquid ethane and nitrogen. Why?

Question 7:

You have performed structural analysis of a new protein using cryoEM and recovered an excellent quality map that is at 1.5Å There are no previously published structures of related proteins and you have to build the atomic model from beginning. You arrive to this section of the map that consists of 6 connected amino-acids and you need to figure out which part of the protein it corresponds to. The polypeptide backbone is already included but the amino-acid identities (side-chains) are not known.



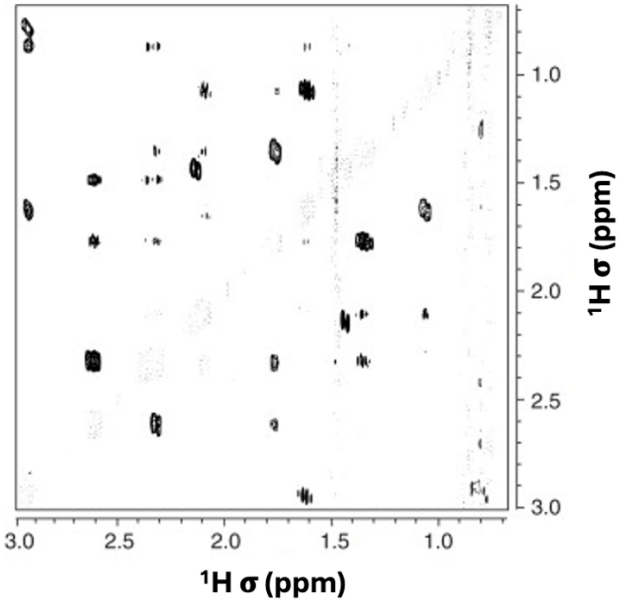
The sequence of the entire domain that also includes this region is:

----AFGRNTSWFRSSGIWFRYGTKEDGYYNPCLIHFNYRGSNTIWSRYGW----

Use your knowledge of amino-acid side-chains to infer which 6 consecutive amino-acids are shown in the map, and identify which section of the provided protein sequence it corresponds to. Note that at these resolutions you primarily see densities for heavy atoms (C, N, O) but not for hydrogens.

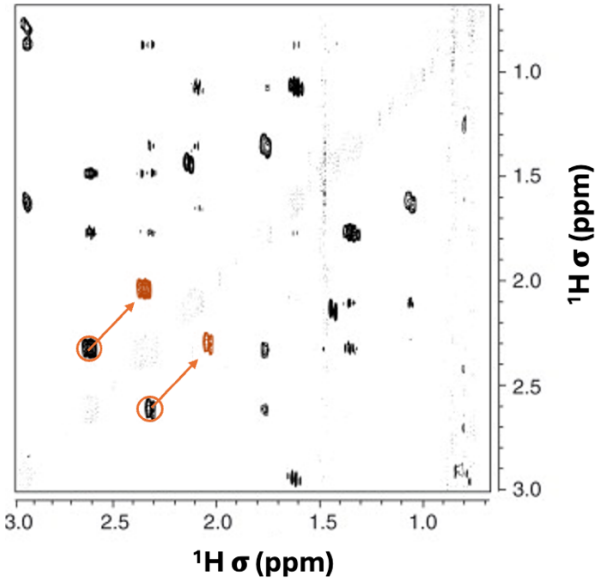
Question 8:

You are interested in resolving a structure of a small Calcium-binding protein by NMR. You collected a ^{1}H-^{1}H NOESY spectra of the protein in the absence of calcium and it looks like this:

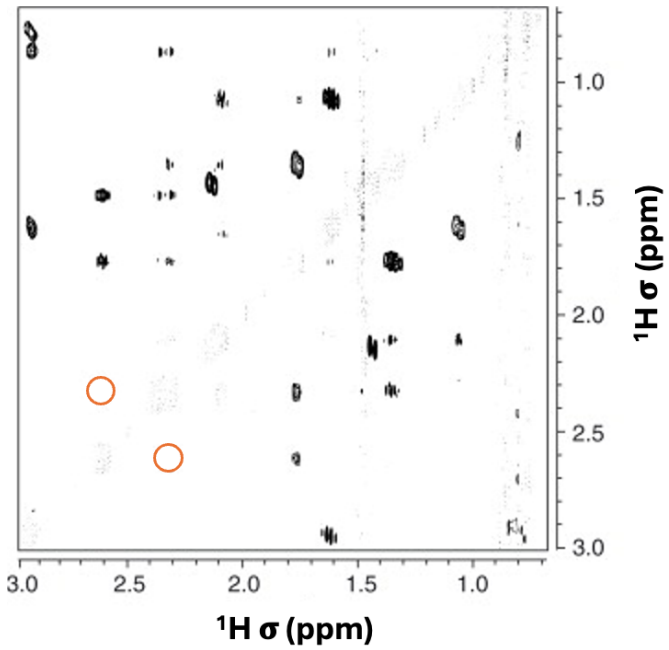


a) What do the peaks in the spectra correspond to? What do the peak intensities tell you about the corresponding ^{1}H atom pairs?

b) Scenario 1: You added calcium to the protein and that resulted in shift of several peaks in the ^{1}H-^{1}H NOESY spectra (see below). What do you think is the explanation? Why did these ^{1}H exhibit a change in chemical shifts and others did not?



b) Scenario 2: You added calcium to the protein and that resulted in loss of several peaks in the ^{1}H-^{1}H NOESY spectra (see orange circles below). What do you think is the explanation? What is this reduction of peak intensity telling you about the corresponding ^{1}H pairs?


1.0
1.5
2.0
2.5
3.0
^{1}H σ (ppm)
3.0
2.5
2.0
1.5
1.0
^{1}H σ (ppm)

Question 9:

Below is a small part of a PDB file from two different structures (A and B). Can you identify what is different in terms of atom composition? Considering the atom composition, which structural biology method could have these structures originated from?

Structure A:

```
ATOM   2270  N   THR A 364      37.053  61.679  55.135  1.00 45.18           N
ATOM   2271  CA  THR A 364      36.644  63.002  55.575  1.00 41.40           C
ATOM   2272  C   THR A 364      35.133  63.162  55.498  1.00 42.51           C
ATOM   2273  O   THR A 364      34.526  63.741  56.395  1.00 41.87           O
ATOM   2274  CB  THR A 364      37.351  64.100  54.751  1.00 39.60           C
ATOM   2275  OG1 THR A 364      38.772  63.954  54.915  1.00 36.65           O
ATOM   2276  CG2 THR A 364      36.957  65.486  55.238  1.00 38.36           C
ATOM   2277  N   GLN A 365      34.539  62.643  54.433  1.00 40.74           N
ATOM   2278  CA  GLN A 365      33.089  62.723  54.269  1.00 40.85           C
ATOM   2279  C   GLN A 365      32.396  61.991  55.413  1.00 42.74           C
ATOM   2280  O   GLN A 365      31.415  62.482  55.961  1.00 44.33           O
ATOM   2281  CB  GLN A 365      32.673  62.090  52.945  1.00 42.48           C
ATOM   2282  CG  GLN A 365      33.070  62.906  51.716  1.00 43.48           C
ATOM   2283  CD  GLN A 365      32.670  62.179  50.442  1.00 41.36           C
ATOM   2284  OE1 GLN A 365      31.508  62.163  50.065  1.00 43.05           O
ATOM   2285  NE2 GLN A 365      33.623  61.540  49.808  1.00 42.06           N
ATOM   2286  N   ILE A 366      32.898  60.810  55.761  1.00 46.58           N
ATOM   2287  CA  ILE A 366      32.307  60.043  56.861  1.00 46.20           C
ATOM   2288  C   ILE A 366      32.432  60.816  58.169  1.00 49.94           C
ATOM   2289  O   ILE A 366      31.492  60.866  58.955  1.00 50.63           O
ATOM   2290  CB  ILE A 366      33.008  58.673  56.996  1.00 43.57           C
ATOM   2291  CG1 ILE A 366      32.766  57.878  55.730  1.00 42.97           C
ATOM   2292  CG2 ILE A 366      32.495  57.932  58.231  1.00 42.86           C
ATOM   2293  CD1 ILE A 366      33.601  56.636  55.618  1.00 42.38           C
ATOM   2294  N   SER A 367      33.582  61.441  58.413  1.00 51.38           N
ATOM   2295  CA  SER A 367      33.704  62.192  59.647  1.00 53.17           C
ATOM   2296  C   SER A 367      32.759  63.380  59.676  1.00 53.23           C
ATOM   2297  O   SER A 367      32.302  63.772  60.740  1.00 54.16           O
ATOM   2298  CB  SER A 367      35.167  62.637  59.903  1.00 54.34           C
ATOM   2299  OG  SER A 367      35.575  63.694  59.063  1.00 56.93           O
ATOM   2300  N   SER A 368      32.457  63.962  58.512  1.00 50.86           N
ATOM   2301  CA  SER A 368      31.548  65.107  58.465  1.00 48.18           C
ATOM   2302  C   SER A 368      30.132  64.650  58.878  1.00 45.09           C
ATOM   2303  O   SER A 368      29.438  65.325  59.646  1.00 47.04           O
ATOM   2304  CB  SER A 368      31.536  65.712  57.052  1.00 49.16           C
ATOM   2305  OG  SER A 368      30.680  66.848  57.009  1.00 52.42           O
ATOM   2306  N   ALA A 369      29.710  63.503  58.361  1.00 44.98           N
ATOM   2307  CA  ALA A 369      28.404  62.945  58.712  1.00 44.29           C
ATOM   2308  C   ALA A 369      28.348  62.684  60.208  1.00 44.40           C
ATOM   2309  O   ALA A 369      27.358  63.001  60.862  1.00 43.34           O
ATOM   2310  CB  ALA A 369      28.162  61.625  57.956  1.00 44.57           C
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Structure B:

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ATOM   1007  CA  LEU A  97      -8.354   1.914  -3.884  1.00  0.22           C
ATOM   1008  C   LEU A  97      -7.637   2.570  -5.058  1.00  0.21           C
ATOM   1009  O   LEU A  97      -7.259   3.734  -4.992  1.00  0.25           O
ATOM   1010  CB  LEU A  97      -7.319   1.264  -2.966  1.00  0.25           C
ATOM   1011  CG  LEU A  97      -6.284   2.248  -2.408  1.00  0.38           C
ATOM   1012  CD1 LEU A  97      -6.926   3.157  -1.381  1.00  0.52           C
ATOM   1013  CD2 LEU A  97      -5.097   1.524  -1.799  1.00  0.62           C
ATOM   1014  H   LEU A  97      -9.265   0.002  -4.103  1.00  0.23           H
ATOM   1015  HA  LEU A  97      -8.885   2.676  -3.331  1.00  0.24           H
ATOM   1016 1HB  LEU A  97      -7.837   0.802  -2.137  1.00  0.28           H
ATOM   1017 2HB  LEU A  97      -6.796   0.500  -3.520  1.00  0.47           H
ATOM   1018  HG  LEU A  97      -5.921   2.867  -3.215  1.00  0.46           H
ATOM   1019 1HD1 LEU A  97      -6.208   3.895  -1.056  1.00  1.11           H
ATOM   1020 2HD1 LEU A  97      -7.778   3.652  -1.820  1.00  1.18           H
ATOM   1021 3HD1 LEU A  97      -7.247   2.570  -0.533  1.00  1.02           H
ATOM   1022 1HD2 LEU A  97      -5.412   0.988  -0.913  1.00  1.03           H
ATOM   1023 2HD2 LEU A  97      -4.690   0.829  -2.517  1.00  1.29           H
ATOM   1024 3HD2 LEU A  97      -4.340   2.248  -1.529  1.00  1.25           H
ATOM   1025  N   GLU A  98      -7.467   1.814  -6.136  1.00  0.18           N
ATOM   1026  CA  GLU A  98      -6.790   2.319  -7.326  1.00  0.19           C
ATOM   1027  C   GLU A  98      -7.507   3.553  -7.874  1.00  0.17           C
ATOM   1028  O   GLU A  98      -6.926   4.635  -7.956  1.00  0.20           O
ATOM   1029  CB  GLU A  98      -6.735   1.227  -8.394  1.00  0.21           C
ATOM   1030  CG  GLU A  98      -5.724   1.491  -9.495  1.00  0.27           C
ATOM   1031  CD  GLU A  98      -6.131   2.632 -10.405  1.00  0.88           C
ATOM   1032  OE1 GLU A  98      -5.528   3.721 -10.296  1.00  0.96           O
ATOM   1033  OE2 GLU A  98      -7.051   2.438 -11.226  1.00  1.87           O
ATOM   1034  H   GLU A  98      -7.838   0.907  -6.144  1.00  0.18           H
ATOM   1035  HA  GLU A  98      -5.783   2.591  -7.050  1.00  0.22           H
ATOM   1036 1HB  GLU A  98      -6.481   0.290  -7.921  1.00  0.27           H
ATOM   1037 2HB  GLU A  98      -7.711   1.136  -8.848  1.00  0.31           H
ATOM   1038 1HG  GLU A  98      -4.773   1.731  -9.045  1.00  0.85           H
ATOM   1039 2HG  GLU A  98      -5.625   0.595 -10.088  1.00  1.00           H
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