Structure Determination

- This lecture will be about how macromolecular structures are determined
  - via X-Ray Crystallography
  - via NMR
  - via Cryo-EM
- At the end of this mini-lecture, you should be able to answer the following questions:
  - What are the major ways in which we obtain the structures used for molecular modeling?
  - How do we utilize available technology to refine our structures?

Protein Data Bank

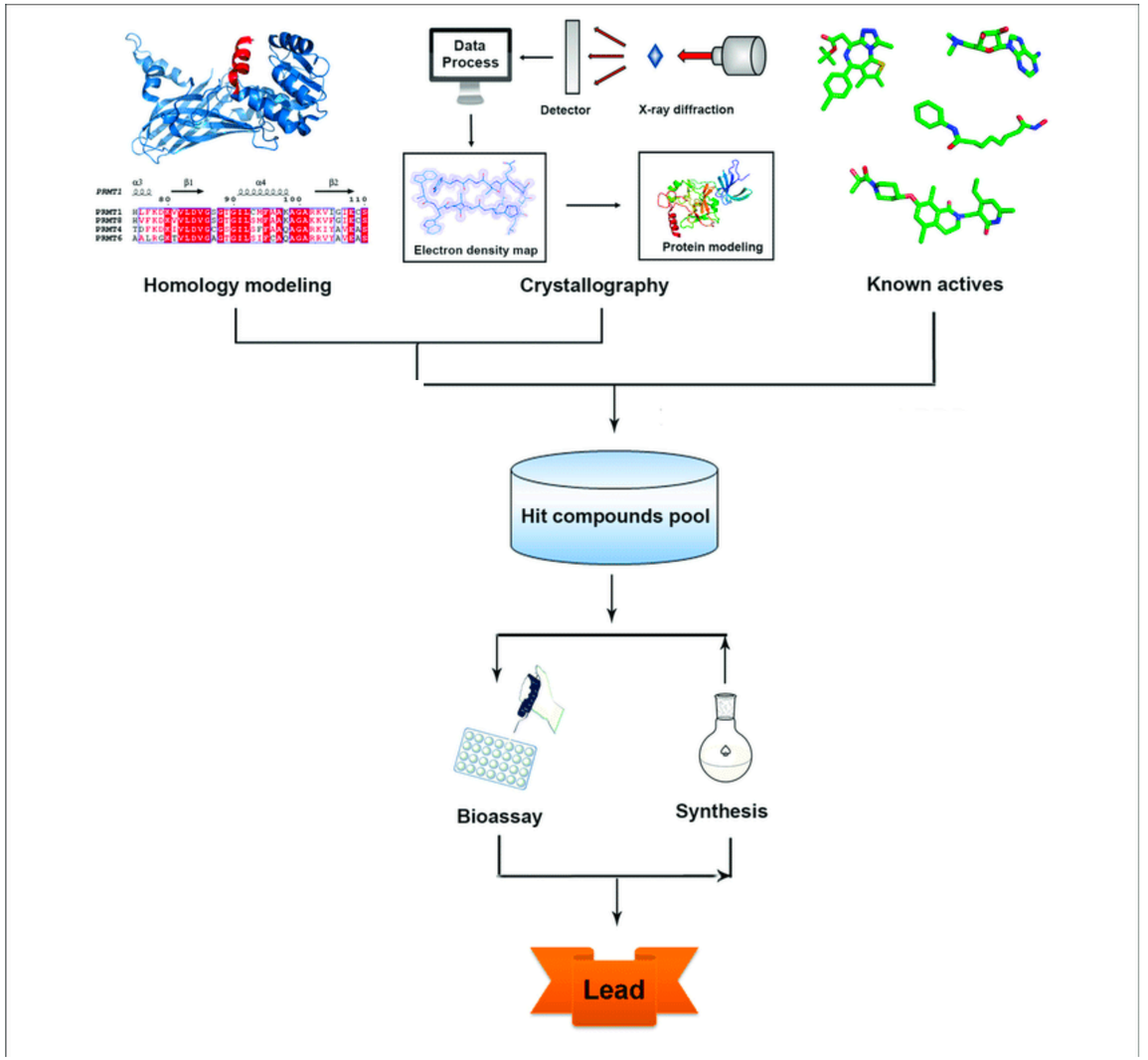
- About 180,000 macromolecular structures are in PDB
  - 90% proteins, 10% polynucleotides
- 89% are determined by X-Ray Crystallography
- CryoEM is rapidly increasing
- Used as input data for drug design

Applications to Drug Design

- Essential proteins in pathogens can be targeted
- Human proteins that are over expressed can be targeted
- Designing effective inhibitors (competitive, mixed, non-competitive) improved health
- Molecular dynamics simulations begin with these experimentally-determined structures
- Wet lab experiments are a subsequent step after molecular modeling and docking studies to assess the efficacy of designed pharmaceuticals

Relevance and Importance

- Info from atom arrangement in 3D structure:
  - Protein-protein interactions
  - Catalytic residues in active sites
- Allows us to perform site-directed mutagenesis to alter protein functions
- Computational chemists perform analysis on experimentally-determined structures
  - Ligand design, simulations



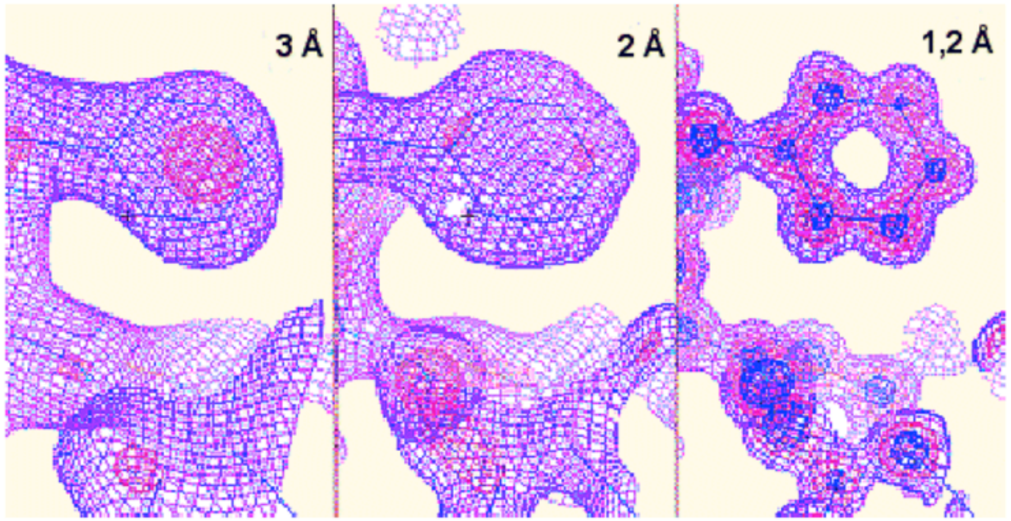
https://www.researchgate.net/publication/323700552_Computer-Aided_Drug_Design_in_Epigenetics

Experiments and Models

- Resolution is a critical property of 3D models for accuracy of downstream analysis
- Resolution: the size of the smallest observable detail in a model
  - High resolution: small value of resolution
  - Help us distinguish neighboring atoms, residues, subunits
  - Limited by diffraction limit

Needed Resolutions are Application-Dependent

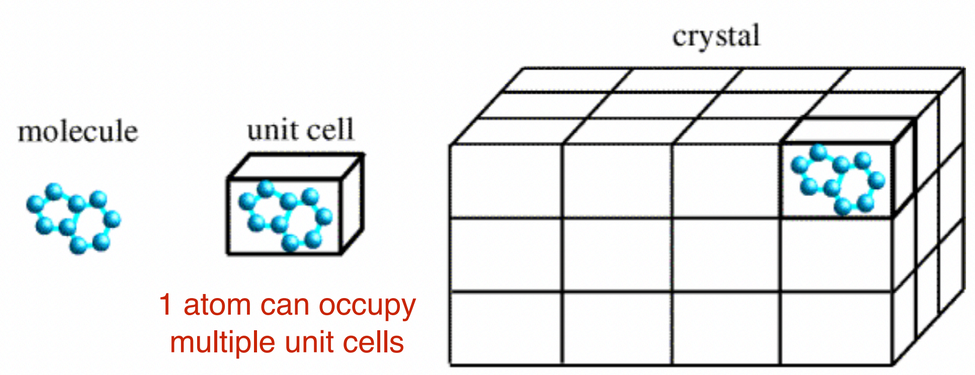
- For drug design we generally need near-atomic (~1.8Å resolution) so we can tell how the ligand is going to fit into the structure
- Alternate conformations or solvent dynamics might need better resolution
- For molecular dynamics, we need high resolution if we're trying to do QM in parts of the structure or if we want to begin the simulation with the atoms precisely located; but if we only want to model big changes, we can make do with 3Å or even 5Å resolution

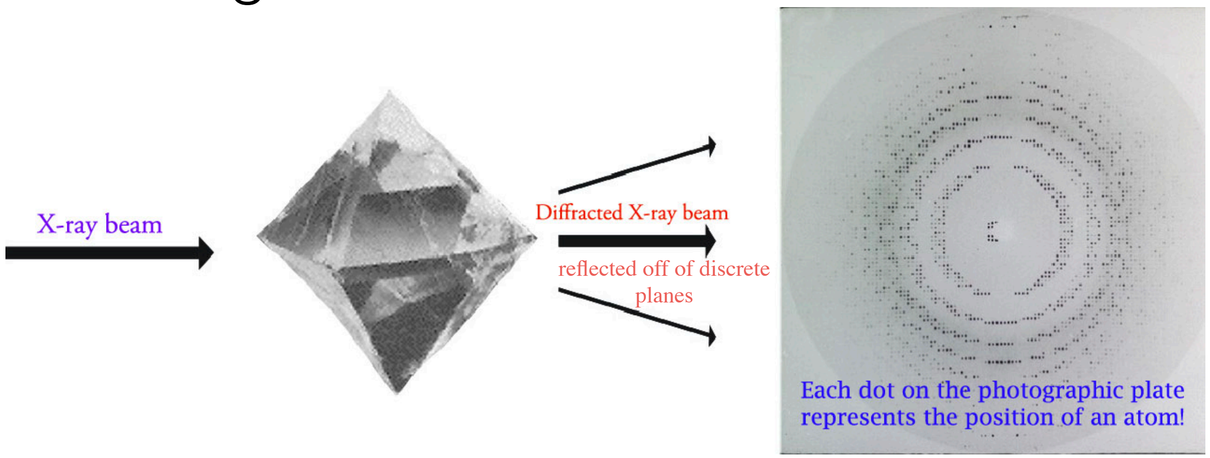


https://www.cambridgemedchemconsulting.com/resources/sbd.php

X-ray Crystallography

- X-ray Crystallography: angles and intensities of X-ray beams that reinforce each other allow electron density to be determined
  - atoms, chemical bonds, entropy of the crystal can be established
- Crystals are arranged in repetitive, organized patterns
  - Molecules are same distance apart and in the same orientation
- Positional disorder impacts resolution
  - Dynamic disorder: atoms migrate positions
  - Static disorder: atoms do not migrate during data collection





Crystal Structures

- Substance needs to be ~95% pure
- Interior of identified crystal structures is similar to interior of solution structure
  - Not impacted by crystallization process
- Structure-based drug design projects have created enzymatic inhibitors based on crystal structures that have proven effective

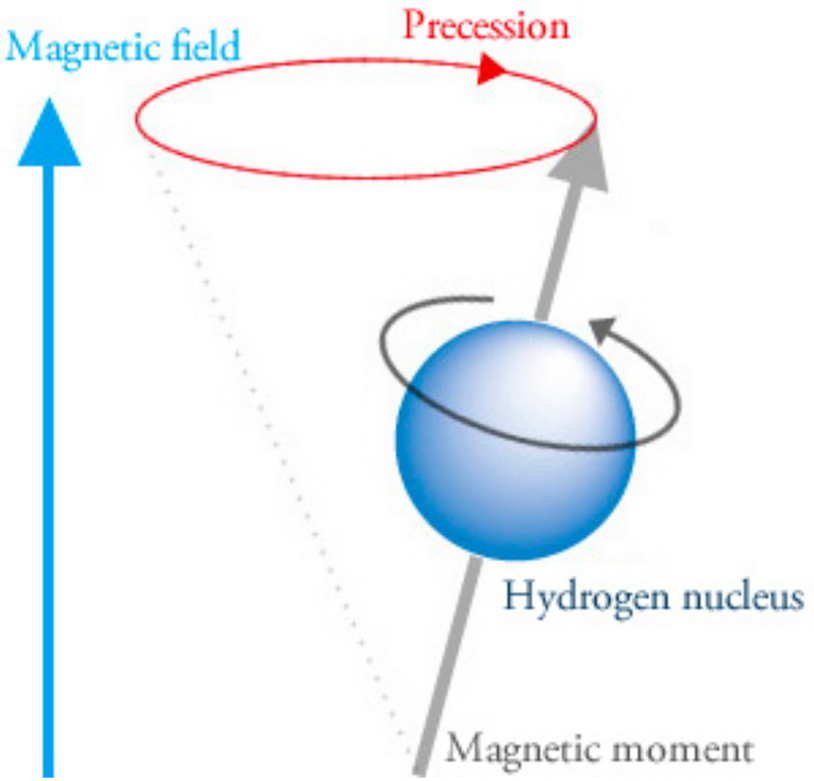
What do they tell us?

- X,Y,Z coordinates of atoms in macromolecules
- X,Y,Z coordinates of well-ordered water molecules

http://www.rcsb.org/3d-view/2HB2

Nuclear Magnetic Resonance

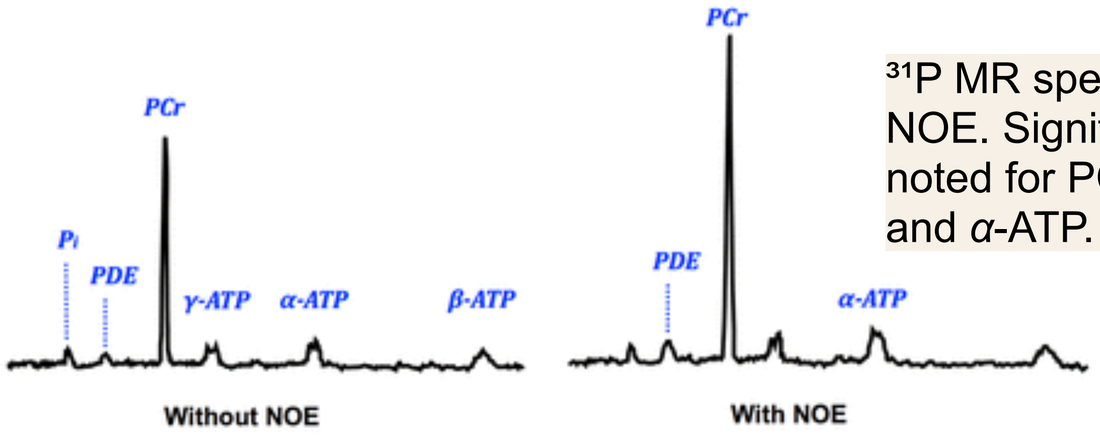
- Molecular structure via the interaction of nuclear spins within a powerful magnetic field
- Criteria for NMR
  - odd atomic number or mass
- Dependent on resonant interactions between microwave-frequency electromagnetic fields and unpaired nuclear spins in a given sample
- Small molecules have resonance arising from particular atoms/clusters
- Large molecules have peaks that overlap



https://www.jeol.co.jp/en/products/nmr/basics.html

NMR for Large Molecules

- To avoid overlapping peaks we examine resonating atoms and send different time sequences of pulses
  - Determine intra- and inter- molecular distances
- Nuclear Overhauser Effect (NOE) measurements determine resonance changes when one resonance is saturated
  - Allows measurement of interatomic distances between spins - a “molecular ruler”
  - Hypothesis testing is required to determine atoms associated with peak



^{31}P MR spectrum of calf muscle with and without NOE. Significant absolute signal increases are noted for PCr, PDEs (especially GPC) and α-ATP. The other peaks are not affected.

https://mriquestions.com/noe.html

X-Ray vs. NMR

- Ensembles of NMR structures are determined
  - Lack of specificity indicates heterogeneity in conformations while in solution
  - Not enough constraints (distances between atoms); many structures that satisfy this

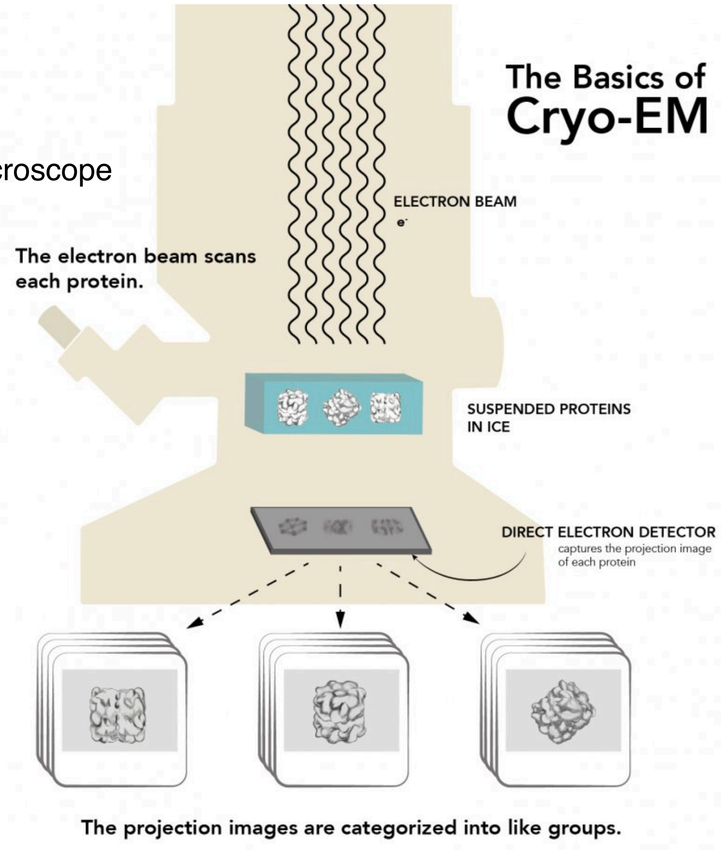
Limitations

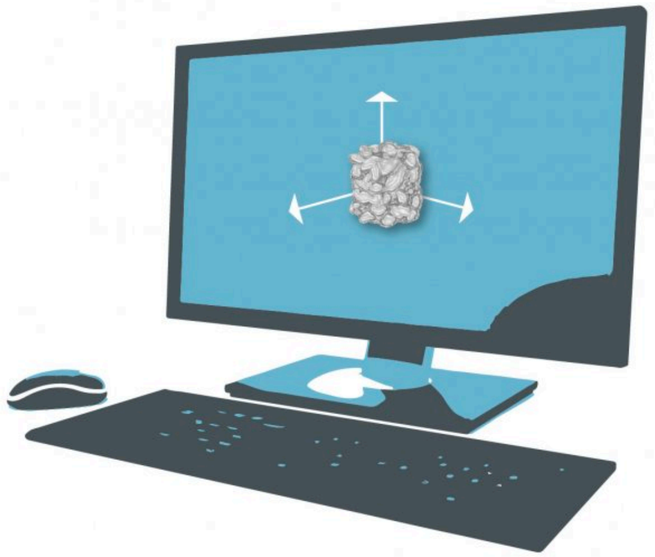
- X-Ray Crystallography can only be done for crystalline samples
  - 1 kDa - 500 kDa
  - H is not in X-ray crystallography because of no e- density
- NMR can be performed for samples within solutions
  - 5 kDa - 60 kDa
  - Reliable for H atoms

Cryogenic Electron Microscopy (Cryo-EM)

- Beams of electrons form 3D images of biomacromolecules at atomic resolution
  - Highly specific because electron wavelengths are short

Transmission Electron Microscope





https://cryoem.slac.stanford.edu/what-is-cryo-em

Cryo-EM

- Used for multi-component protein or protein-RNA complexes
- Starting substance must be ~85% pure
- Nearly atomic resolution
  - (2Å in ideal circumstances, 3Å in many cases, 4Å in most)
- Molecules are fixed in ice and projections of proteins in all directions can be obtained
- Cryo-EM and X-Ray Crystallography can be used in conjunction
  - Cryo-EM for the intact protein complex
  - X-Ray crystal structures of the individual components

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https://cryoem.slac.stanford.edu/what-is-cryo-em

Review Question

1. Cryo-EM
2. X-Ray Crystallography
3. NMR

1. Which method uses electrons to form images of biological macromolecules at atomic resolution?
2. Which method analyzes non-zero nuclear spin interactions within a magnetic field?
3. Which method analyzes the angles and intensities of X-ray beams to determine electron density?

Review Discussion Question

What are some of the benefits (positive aspects) and drawbacks (negative aspects) of X-Ray Crystallography, NMR, and Cryo-EM?

If you have a large (100 kDa) membrane protein that aggregates in aqueous solutions, which would be the ideal method to use for structural determination?

If you have a small, pure protein and you are interested in determining the atoms and chemical bonds of the protein, which method for structural determination is most ideal?