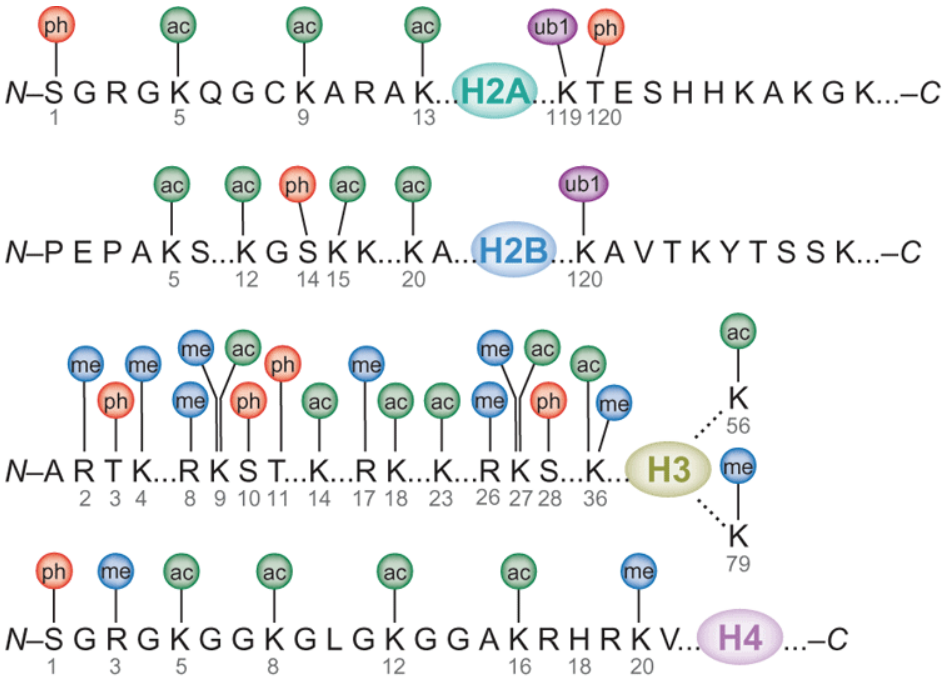
PTMs

Jon K. Lærdahl,
Structural Bioinformatics

Post-translational modifications (PTMs), *i.e.* chemical modification after translation, *e.g.*

- Glycosylation (addition of sugar groups to *e.g.* Asn, Ser, or Thr)
- Phosphorylation of Ser/Thr by kinases
- Methylation of Lys in histones
- Ubiquitination (addition of the protein ubiquitin to Lys)
- Methionine aminopeptidases may remove N-terminal Met
- *Many, many more!!*

Bhaumik *et al.*, *Nat. Struct. Mol. Biol.* **14**, 1008 (2007)



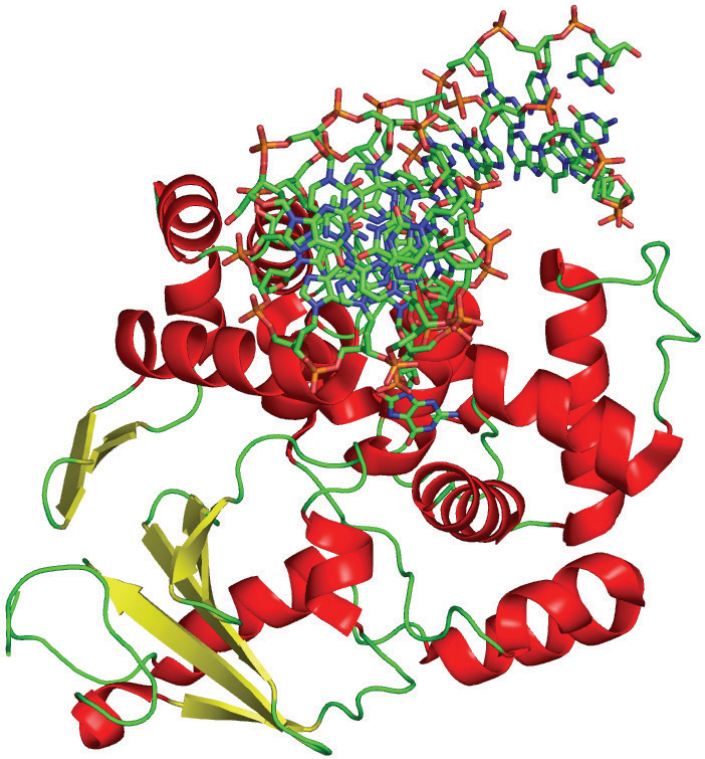
PTMs of human histones include acetylation (ac), methylation (me), phosphorylation (ph) and ubiquitination (ub1)

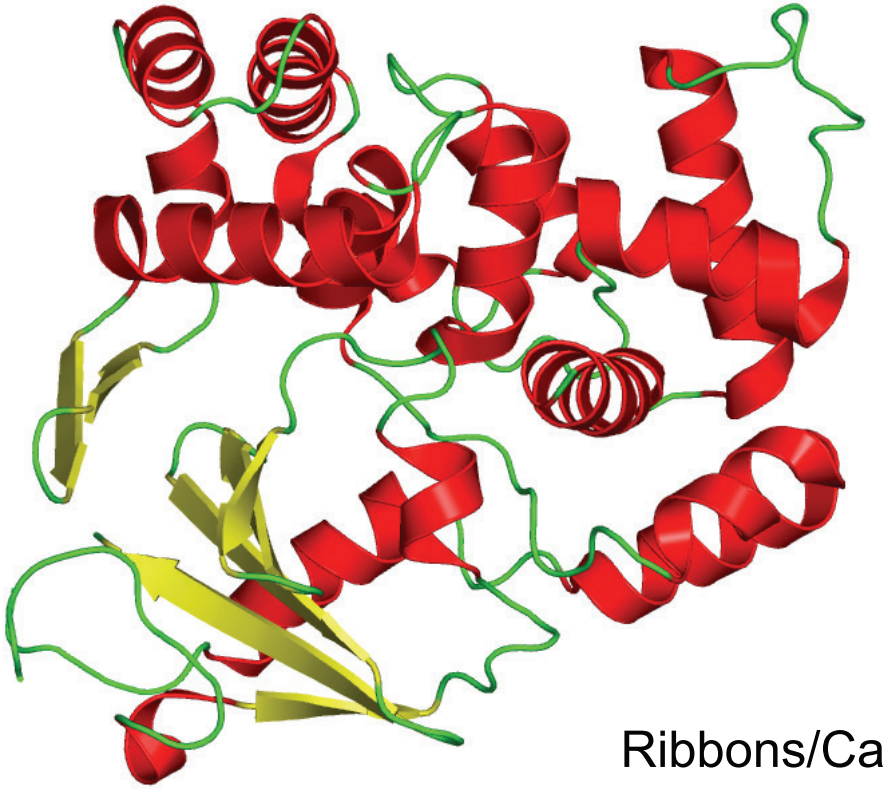
Even if you know the complete 3D structure of the apo-protein you may be unable to understand the function of the protein if you have no information about the PTMs!

Visualization of protein structure

Jon K. Lærdahl,
Structural Bioinformatics

Human OGG1, a DNA repair enzyme that recognizes and excises oxidized DNA bases





Ribbons/Cartoon

Software (advanced graphics rendering):

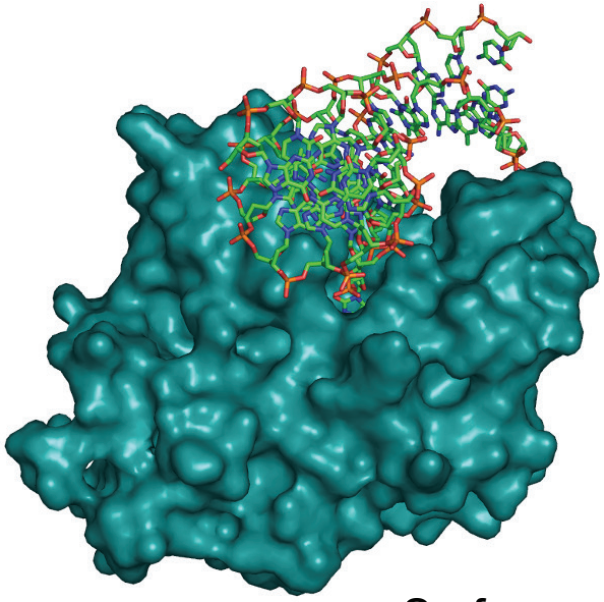
- RasMol
- Swiss-PDBViewer (freeware; also homology modeling)
- Molscript (command-line-based)
- Jmol (open-source Java viewer)
- PyMOL (open-source, user-sponsored)
- Many more both free and very expensive

We will use some of these at the Exercises!

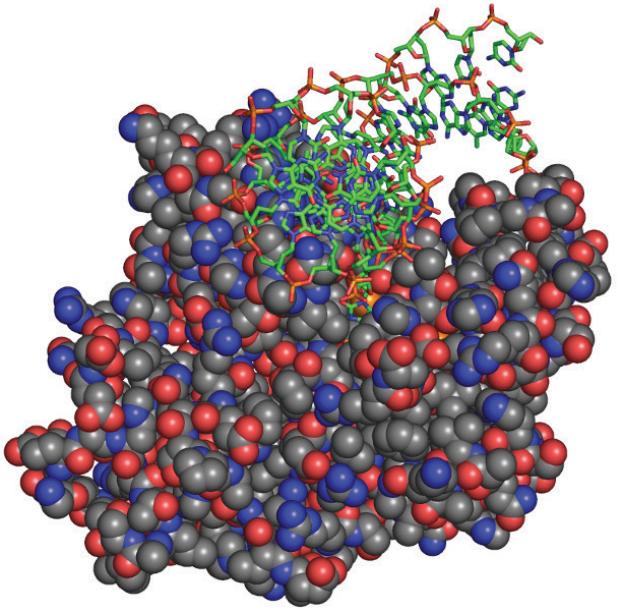
Visualization of protein structure

Jon K. Lærdahl,
Structural Bioinformatics

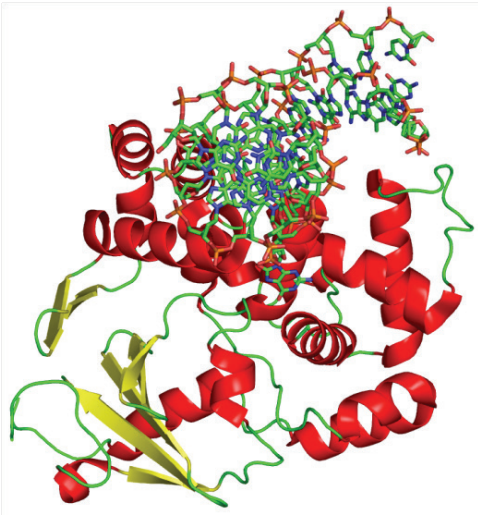
Human OGG1, a DNA repair enzyme that recognizes and excises oxidized DNA bases

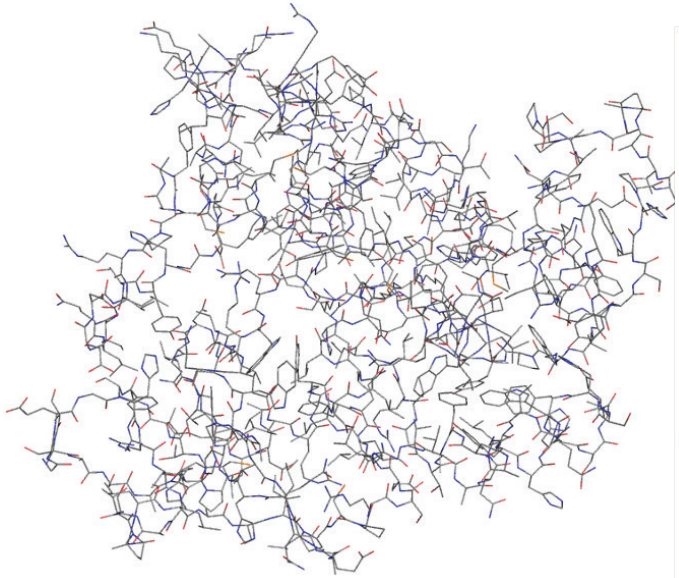


Surface

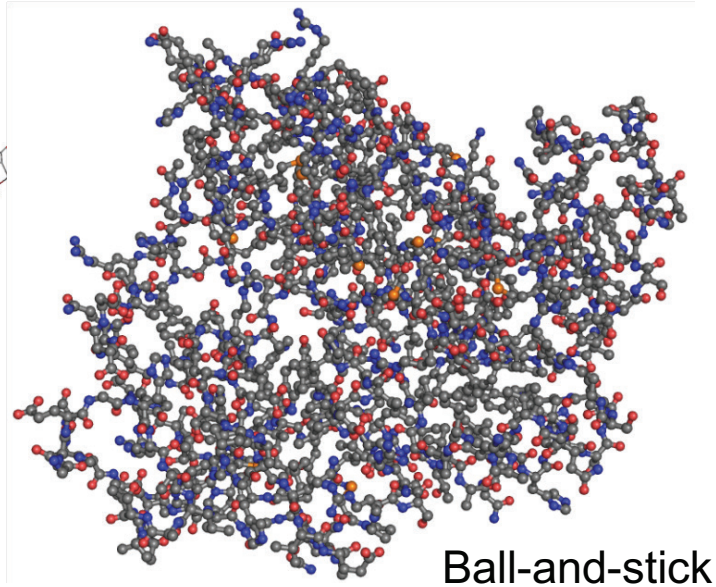


Space-filling spheres (CPK)





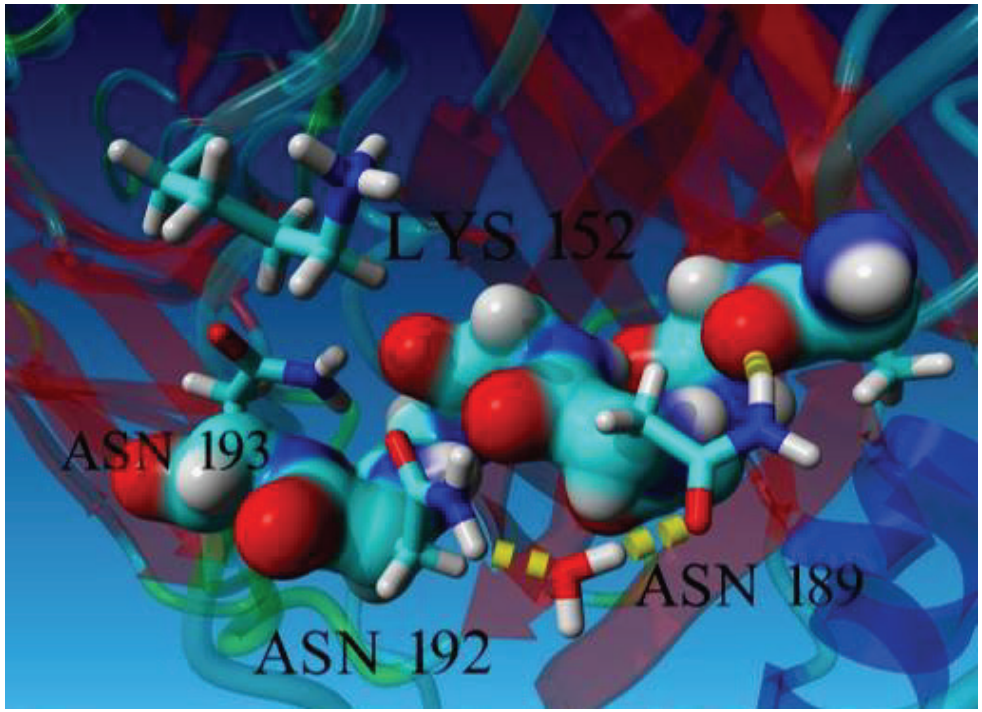
Wireframes

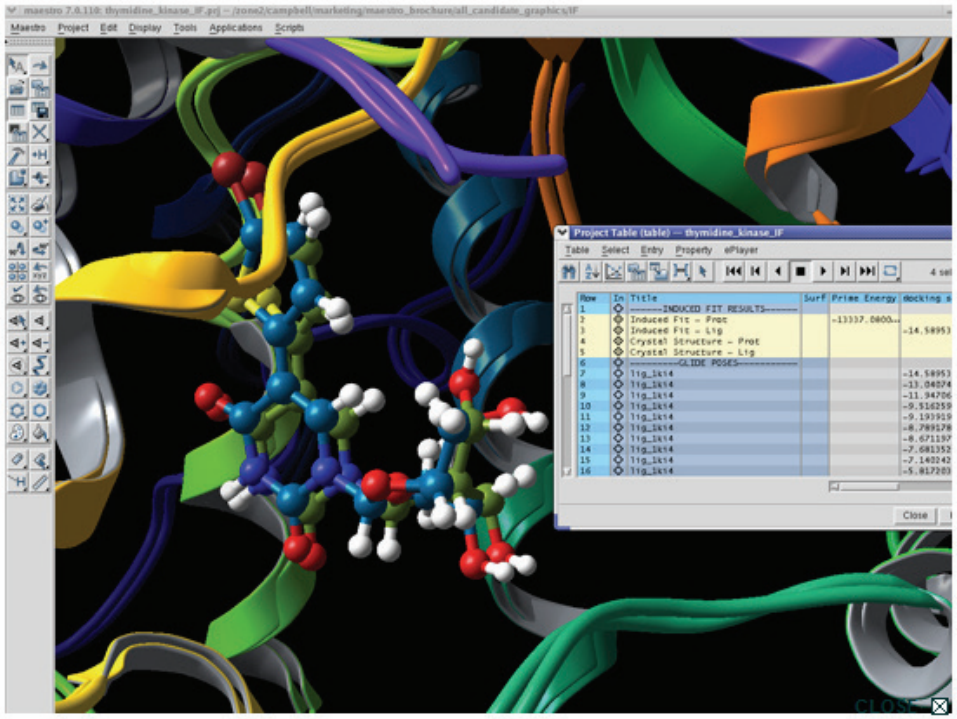


Ball-and-stick

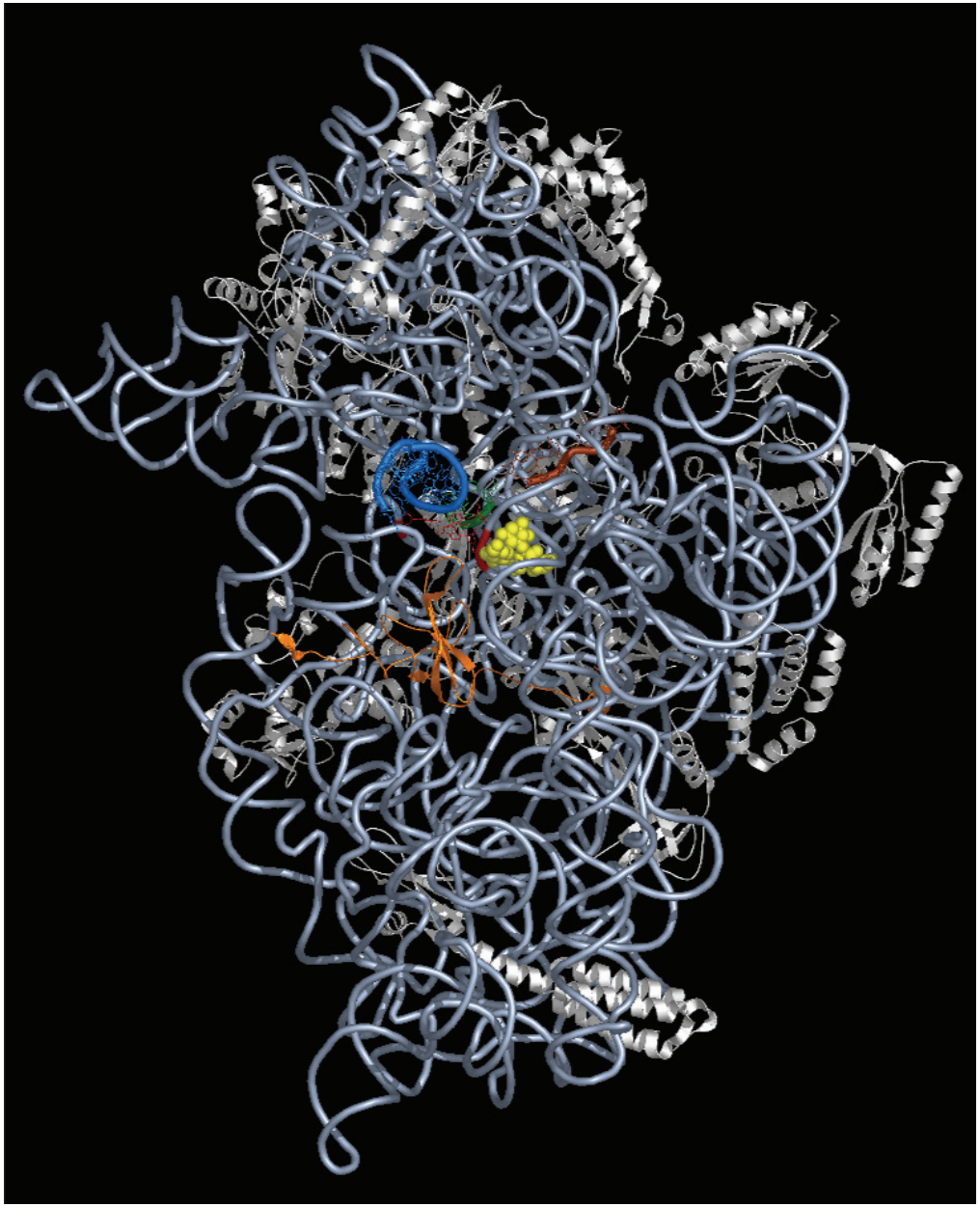
Visualization of protein structure

YASARA





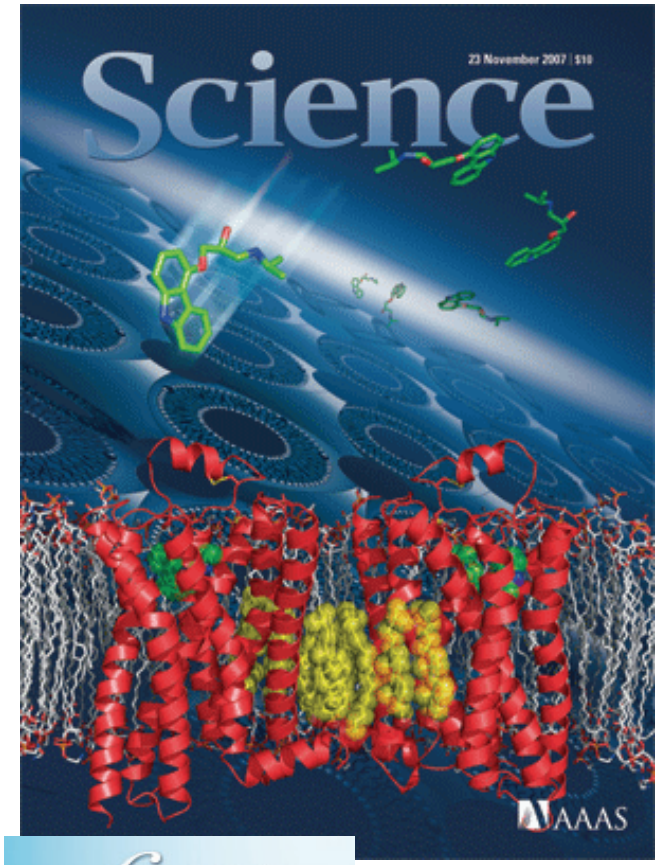
Maestro

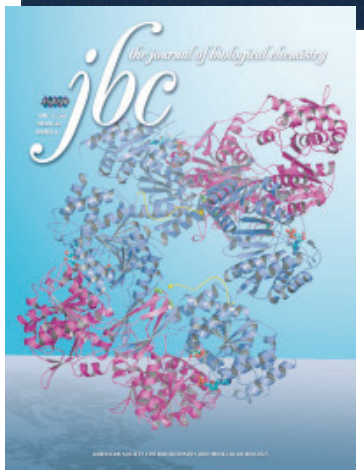


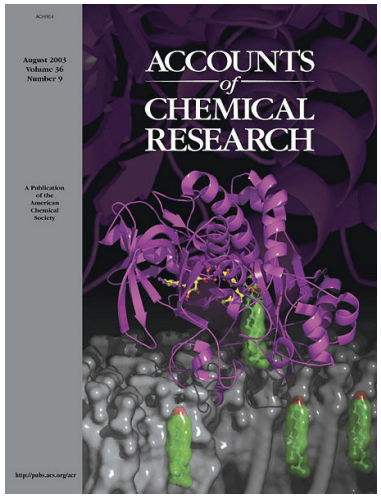
PyMOL

Visualization of protein structure

Jon K. Lærdahl,
Structural Bioinformatics



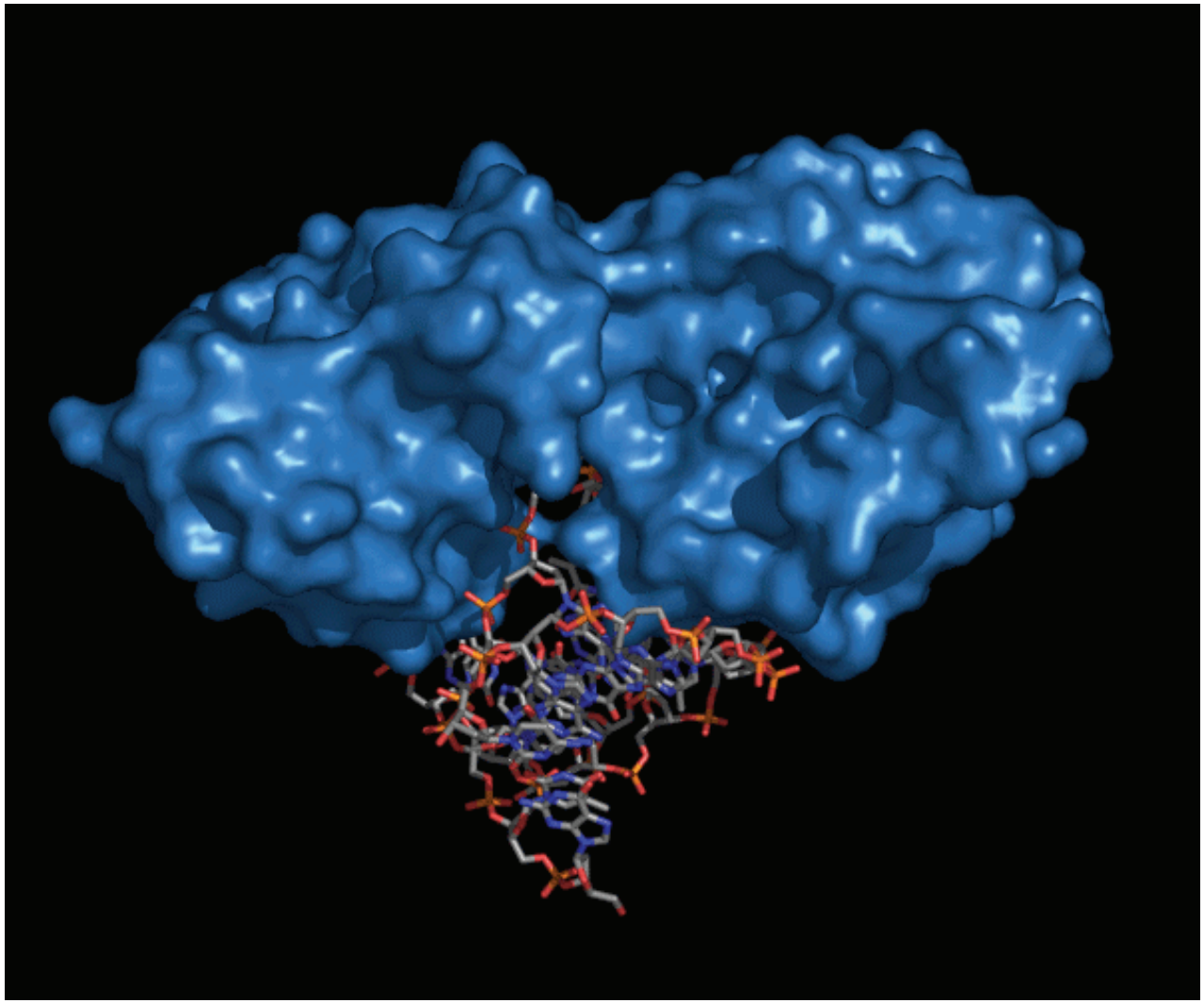




Publication quality graphics from PyMOL

Jon K. Lærdahl,
Structural Bioinformatics

Movies, interactivity etc.



The structure of *Bacillus stearothermophilus* Fpg protein borohydride-trapped with DNA oligo as determined by Fromme and Verdine, *Nat. Struct. Biol.* **9**, 544 (2002), PDB: 1L1Z.

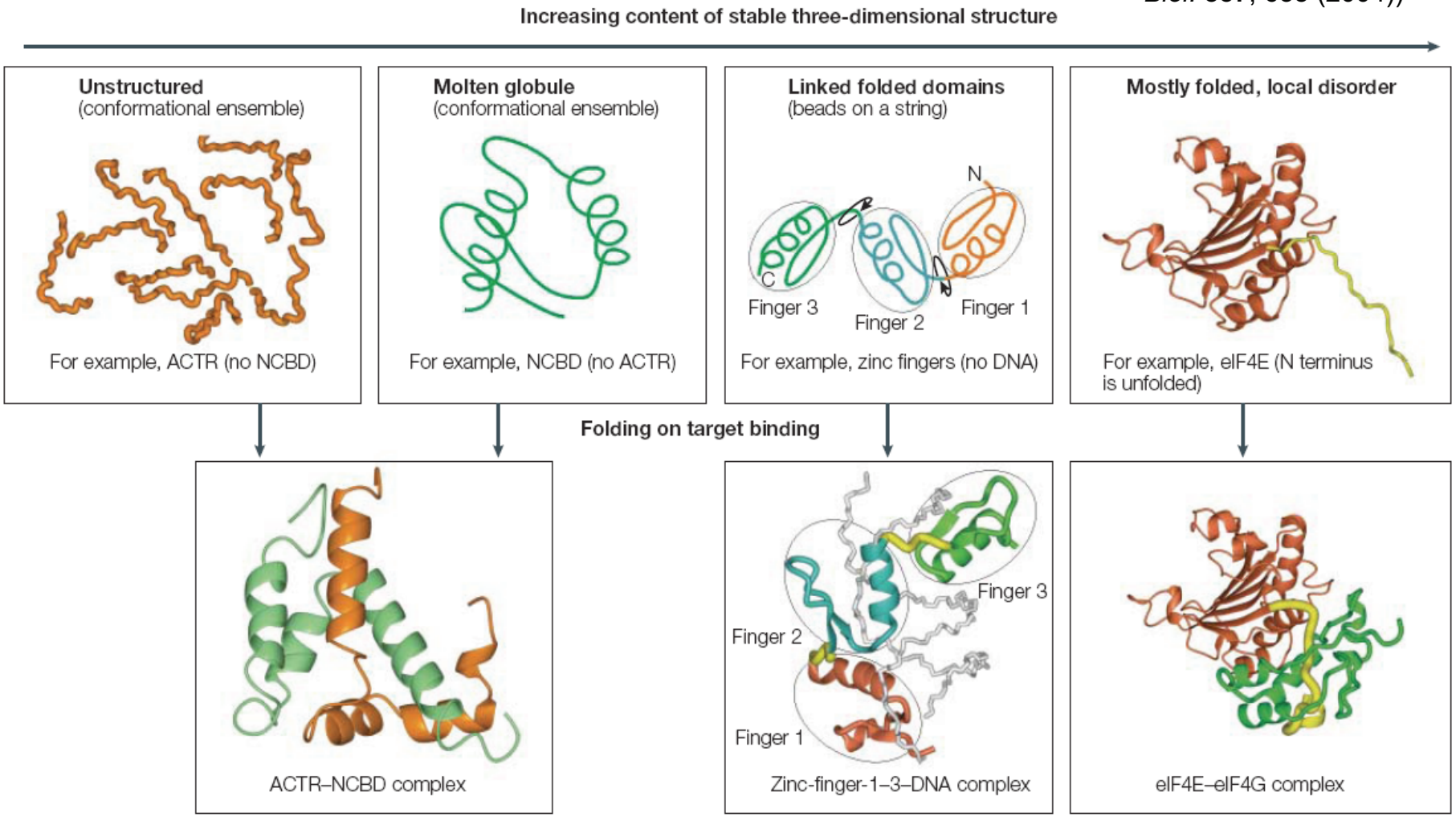
The graphics was generated with PyMOL

Structural disorder in proteins

Jon K. Lærdahl,
Structural Bioinformatics

- Not all proteins have a regular 3D structure for the full sequence
- The full protein, segments or small parts may be structurally disordered/intrinsically unstructured

Predicted 20% of human proteins have disordered segments of length >50 residues (1% in *E. coli*) (J.J. Ward *et al.*, *J. Mol. Biol.* **337**, 635 (2004))

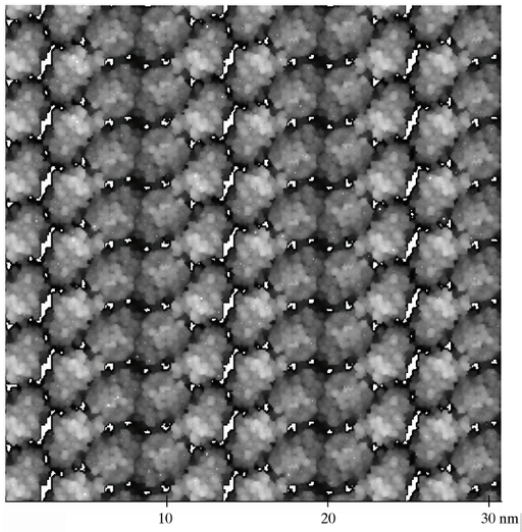


H.J. Dyson & P.E. Wright, *Nat. Rev. Mol. Cell Biol.* **6**, 197 (2005)

Experimental determination of protein structure – X-ray Crystallography

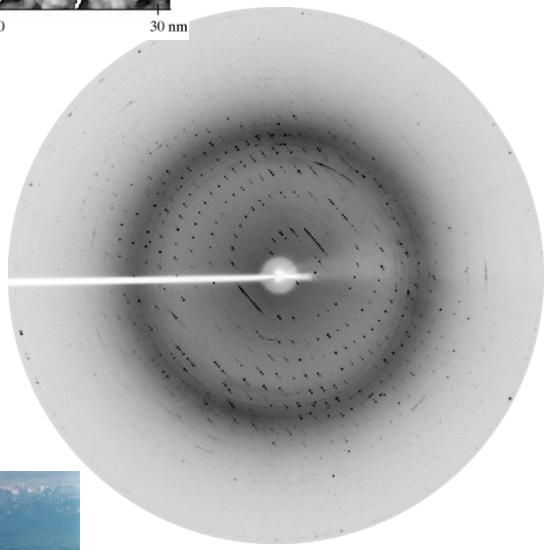
Jon K. Lærdahl,
Structural Bioinformatics

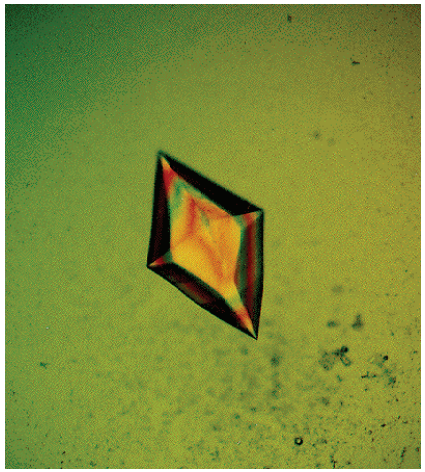
- Necessary to grow protein crystals
  - Often (extremely) difficult
- Diffraction in X-ray beam
- Must solve “phase problem” (due to unknown timing of diffraction waves hitting the detector):
  - Molecular replacement (use the known structure of similar protein)
  - Multiple isomorphus replacement (generate crystals with heavy atoms, *e.g.* by soaking)
- Strong X-ray source needed to get high accuracy (Synchrotron)

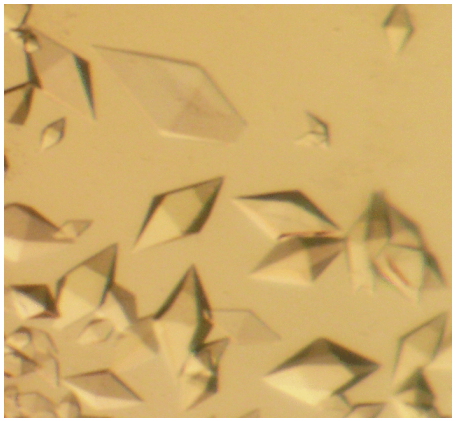


Li *et al.*, *Acta Cryst.* **D55**, 1023 (1999)

Proteins are located in a lattice, in a repeated and oriented fashion







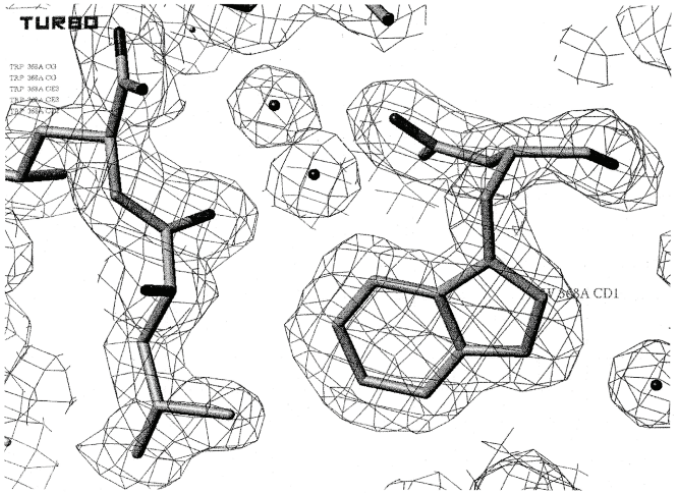
Experimental determination of protein structure – X-ray Crystallography

Jon K. Lærdahl,
Structural Bioinformatics

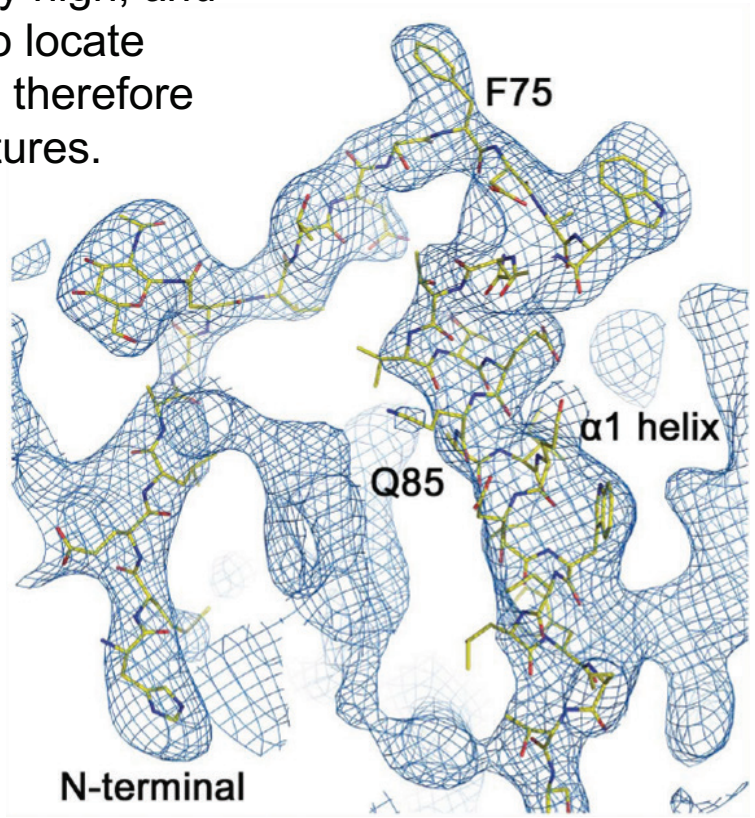
Diffraction pattern & solved phases: Electron density map ("electron cloud"):

- Model protein primary sequence into electron density map
- Resolution:
  - Low ~5.0 Å
  - Intermediate ~2.0-2.5 Å
  - High ~1.2 Å (Only at this very high, *and rare*, resolution it is possible to locate hydrogen atoms. H-atoms are therefore usually not visible in the structures.

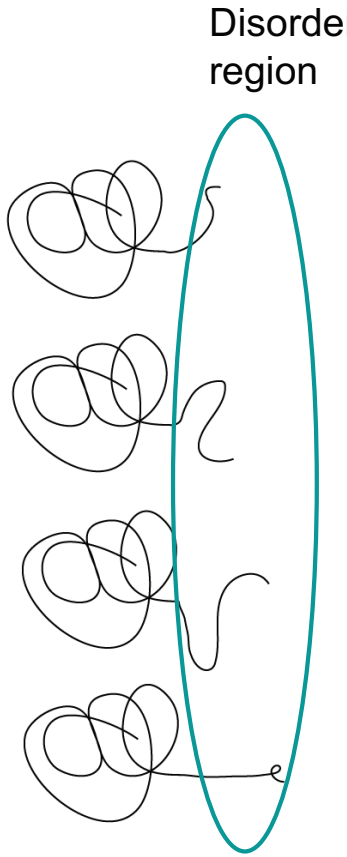
- Gives a *static* picture of the protein in the crystal which might not correspond closely to situation in solution
- Bottleneck: Crystallization (and phase problem)
- No electron density for structurally disordered regions



A.R. Slabas *et al.*, *Biochem. Soc. Trans.* **28**, 677 (2000) (1.9 Å resolution)



X. Chen *et al.*, *Acta Cryst.* **D65**, 339 (2009) (~3.5 Å resolution)

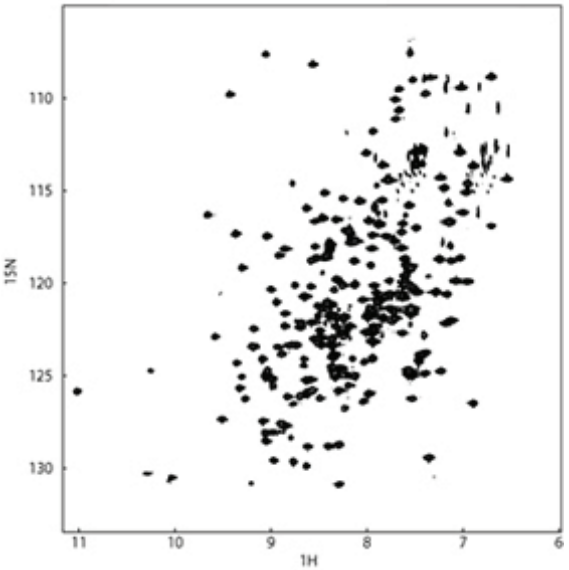


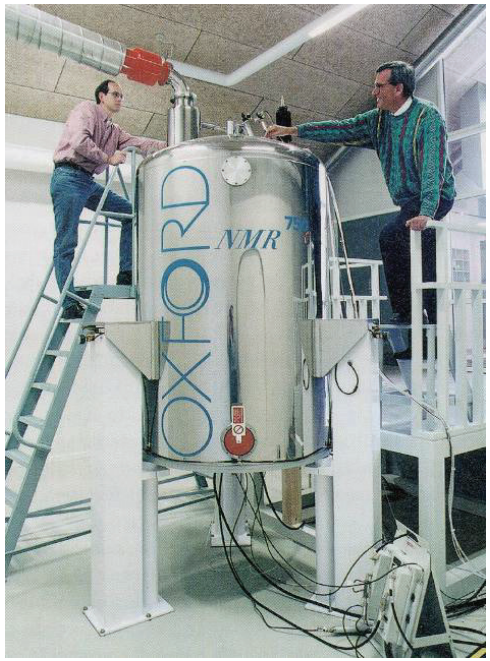
Experimental determination of protein structure – NMR Spectroscopy

Jon K. Lærdahl,
Structural Bioinformatics

Nuclear Magnetic Resonance (NMR) Spectroscopy:

- Based in energy levels of magnetic nuclei *(e.g.* ^{13}C and ^{15}N*)* in a *very* strong external magnetic field probed my a radio frequency signal
- Determines distances between all labeled atoms in a protein
- Structure model built from distances
- Structure solved in solution
    - No need to grow crystals
- Can be used to study proteins dynamics & behavior in solution
- Can currently only be employed for proteins of limited size (a few hundred residues)





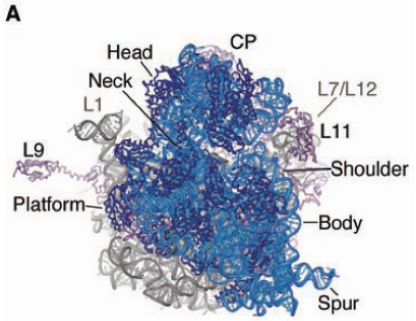
Experimental determination of protein structure

Jon K. Lærdahl,
Structural Bioinformatics

X-ray Crystallography:

Pros:

- Can be used for huge protein complexes
  - 10.000s of atoms in *e.g.* complete ribosomes



B.S. Schuwirth, *Science* **310**, 827 (2005)

- Can in fortunate cases give very high resolution (Atom position uncertainty ~0.2 Å or less)

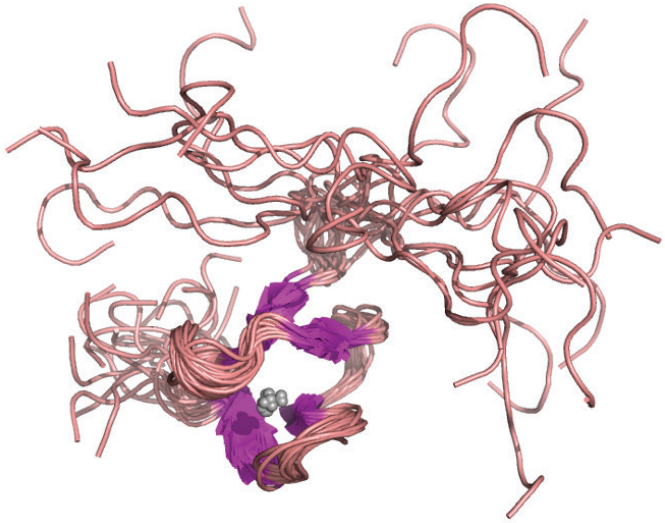
Cons:

- Usually (extremely!) tricky to grow crystals
  - Membrane proteins are particularly difficult
  - Proteins with disordered segments are difficult
- Need to solve phase problem
- Does not give insight into dynamics and protein disorder
- Large amounts of protein needed
- Usually missing H-atoms
- Disordered loops/regions are not visible

NMR Spectroscopy:

Pros:

- Can be used directly on proteins in solution
- No need for crystallization
- Dynamics studies
- Both ordered and disordered proteins (usually an ensemble of 20-40 models)



1N0Z

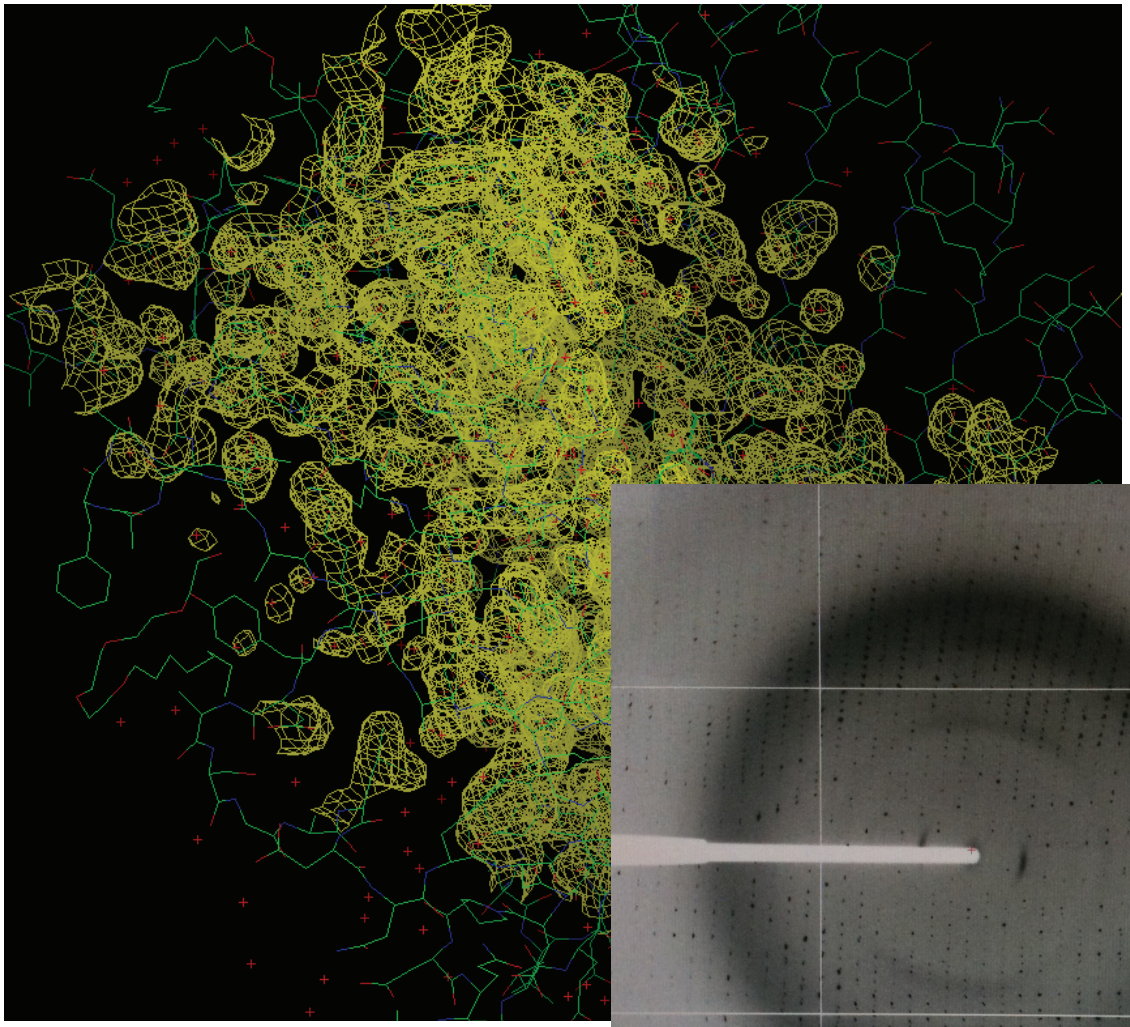
Cons:

- Only applicable for small proteins (<200 residues?)
- Huge amounts of protein needed

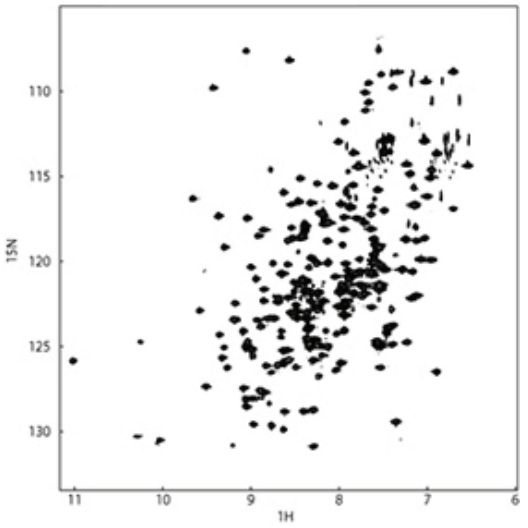
All experimental methods: Labor intensive and requiring (very) expensive instruments
Membrane proteins *extremely tricky*
The experimental structures are also models!

Modeling of atoms into electron density

Jon K. Lærdahl,
Structural Bioinformatics



X-ray crystallography

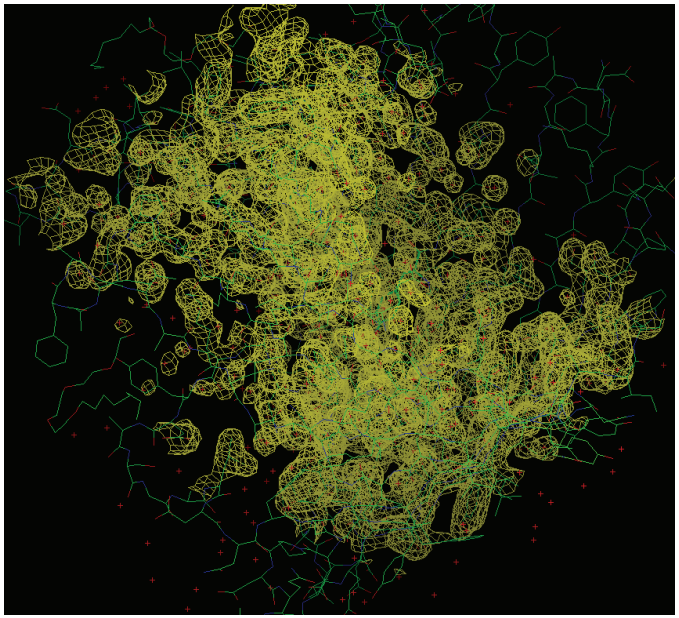


NMR

Modeling of atoms into electron density

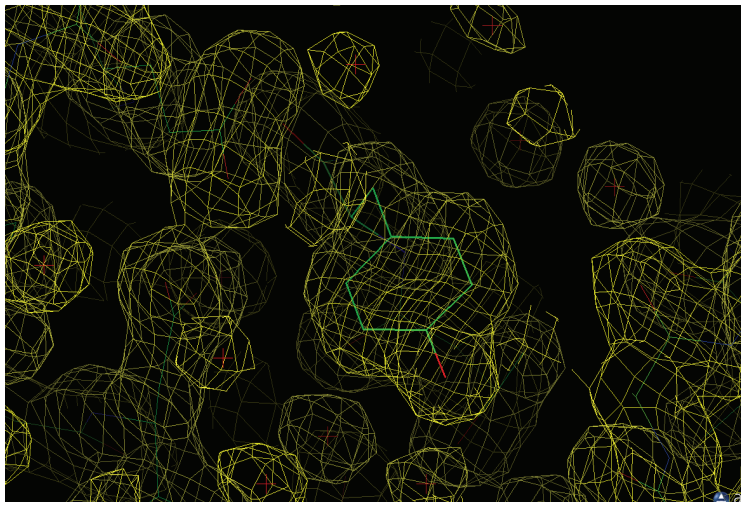
Jon K. Lærdahl,
Structural Bioinformatics

1PRN



The experimental structures are also "models"!

And heavily depends on computers/software



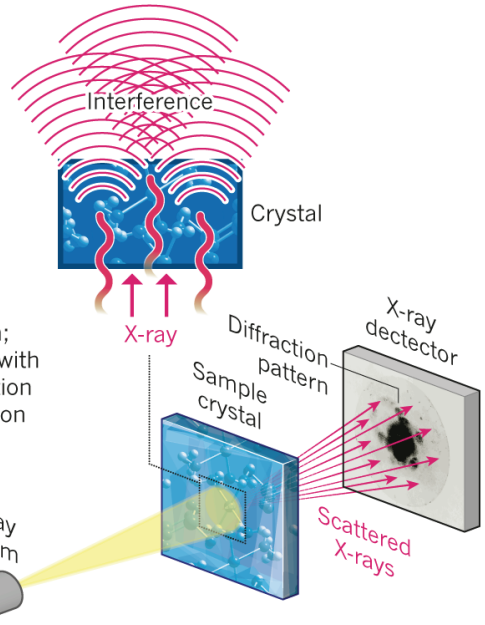
Remember, when looking at an ***experimental structure*** (X-ray):

- Resolution and R-factor gives you an idea about the quality of the experimental model
  - Resolution ~ 3 Å: side chains may be wrong rotamer or missing, main chain normally ok
  - Resolution ~ 2 Å: most side chains should be ok
  - Resolution < 1.5 Å: high accuracy structure
  - Resolution < 1.2 Å: may even be possible to determine positions for hydrogen atoms
- Due to structural flexibility or "problems" in crystals, some regions, typically loops or N-/C-terminus may have little visible electron density.
  - In some cases this gives gaps in the sequences or missing side chains
  - In other cases people put in residues/atoms anyway, in reasonable positions
  - The PDB-entry pages of the PDB in Europe (PDBe; http://pdbe.org/) may be useful – look at electron density!

Cryo-electron microscopy

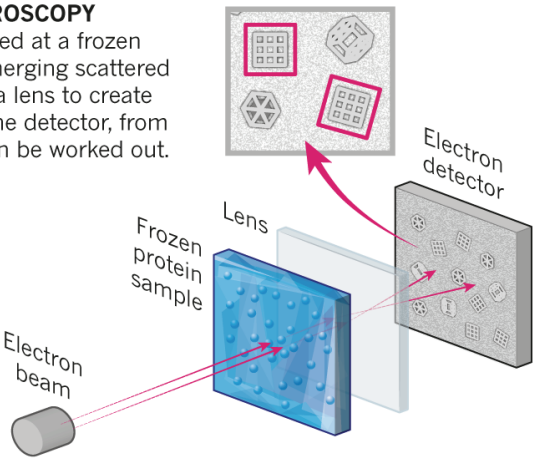
STRUCTURE SOLVERS

X-ray crystallography has long been the dominant method for deducing high-resolution protein structures, but cryo-electron microscopy is catching up.

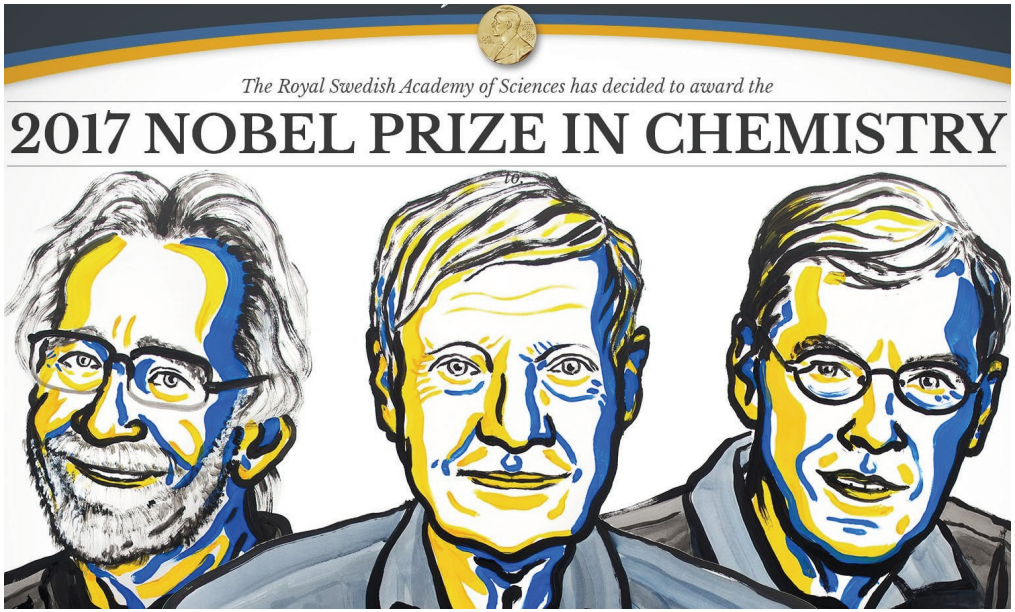


X-RAY CRYSTALLOGRAPHY
X-rays scatter as they pass through a crystallized protein; the resulting waves interfere with each other, creating a diffraction pattern from which the position of atoms is deduced.

CRYO-ELECTRON MICROSCOPY
A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.



E. Callaway, *Nature* **525**, 172 (2015)



The winners of the Nobel chemistry prize: Jacques Dubochet, Joachim Frank and Richard Henderson

Cryo-electron microscopy

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

THE NOBEL PRIZE IN CHEMISTRY 2017

POPULAR SCIENCE BACKGROUND

They captured life in atomic detail

***Jacques Dubochet**, **Joachim Frank** and **Richard Henderson** are awarded the Nobel Prize in Chemistry 2017 for their development of an effective method for generating three-dimensional images of the molecules of life. Using cryo-electron microscopy, researchers can now freeze biomolecules mid-movement and portray them at atomic resolution. This technology has taken biochemistry into a new era.*