X-ray crystallography

Some concepts

- experimental setup
- electron density map interpretation
- Cα-trace
- water of crystallisation
- model quality

Some issues for structural bioinformatics

- missing atoms
- alternative conformations
- crystal contacts
- Asn, Gln, His

Resolution

> “In X-ray crystallography, "2-Å model" means that the model takes into account diffraction from sets of equivalent, parallel planes of atoms spaced as closely as 2 Å in the unit cell. More closely spaced planes of atoms give rise to reflections farther from the center of the diffraction pattern. Presumably, data farther out than the stated resolution is unobtainable or too weak to be reliable.”

(“A Glossary of Terms from Crystallography, NMR, and Homology Modeling” by Gale Rhodes)

Reflections are the discrete spots that appear on the detector.
Each atom in the structure contributes to the intensity of each reflection.

The electron-density map for a high resolution structure (e.g. resolution value less than 1.5Å) is less blurred.

Occupancy

Ideally, all molecules in the crystal would be identical.

> "The occupancy n_j of atom j is a measure of the fraction of molecules in the crystal in which atom j actually occupies the position specified in the model. If all molecules in the crystal are precisely identical, then occupancies for all atoms are 1.00."

("A Glossary of Terms from Crystallography, NMR, and Homology Modeling" by Gale Rhodes)

Temperature factor

> “The temperature factor or B-factor can be thought of as a measure of how much an atom oscillates or vibrates around the position specified in the model. Atoms at side-chain termini are expected to exhibit more freedom of movement than main-chain atoms, and this movement amounts to spreading each atom over a small region of space. Diffraction is affected by this variation in atomic position, so it is realistic to assign a temperature factor to each atom ...
>
> From the temperature factors computed during refinement, we learn which atoms in the molecule have the most freedom of movement, and we gain some insight into the dynamics of our largely static model.”

(“A Glossary of Terms from Crystallography, NMR, and Homology Modeling” by Gale Rhodes)

Refinement

"The iterative process of improving agreement between the molecular model and the crystallographic data. An important element in refinement is a computationally massive least-squares adjustment of

1) the atomic positions in the model,
2) occupancies, and
3) temperature factors

in order to improve their agreement with

1) the data (reflection intensities), and
2) criteria of chemical reasonableness (structural parameters such as bond lengths and angles)."

("A Glossary of Terms from Crystallography, NMR, and Homology Modeling" by Gale Rhodes)

R-factor

> “A measure of agreement between the crystallographic model and the original X-ray diffraction data. The crystallographer calculates from the model the expected intensity of each reflection in the diffraction pattern, and then compares these calculated "data" with the experimental data, which consist of measured positions and intensities. The R-factor is used to assess the progress of structure refinement, and the final R-factor is one measure of model quality.”

(“A Glossary of Terms from Crystallography, NMR, and Homology Modeling” by Gale Rhodes)

Free R-factor

"The free R-factor, R_{free}, is computed in the same manner as R, but using only a small set of randomly chosen intensities (the "test set") which are set aside from the beginning and not used during refinement. They are used only in the cross-validation or quality control process of assessing the agreement between calculated (from the model) and observed data. At any stage in refinement, R_{free} measures how well the current atomic model predicts a subset of the measured reflection intensities that were not included in the refinement, whereas R measures how well the current model predicts the entire data set that produced the model."

("A Glossary of Terms from Crystallography, NMR, and Homology Modeling" by Gale Rhodes)

KBB057/KEM360 — Structure and dynamics of biomolecules

Course TDA507/DIT741 includes only a very light introduction to experimental methods for determining macromolecular structures, with emphasis on some of the issues that structural bioinformaticians should be aware of when using structures from the Protein Data Bank.

Course KBB057/KEM360 describes these experimental methods more thoroughly:

> “This course aims to provide an understanding of the methods that can be used the determination of protein structure and dynamics. The course will cover how X-ray crystallography and Nuclear Magnetic Resonance Spectroscopy, Electron Paramagnetic Resonance and Electron Microscopy can be used for structure determination. Students will be expected to understand the steps required to solve a protein structure, and the physical concepts which underpin these methods. They will get introduced to spectroscopic methods (based on NMR and vibrational spectroscopy) that can be used for studying protein dynamics at different timescales.”