Structural techniques – Robert Gilbert

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Databases:
http://www.ebi.ac.uk/

http://www.ebi.ac.uk/pdbe
http://www.rcsb.org

Structural biology's aims

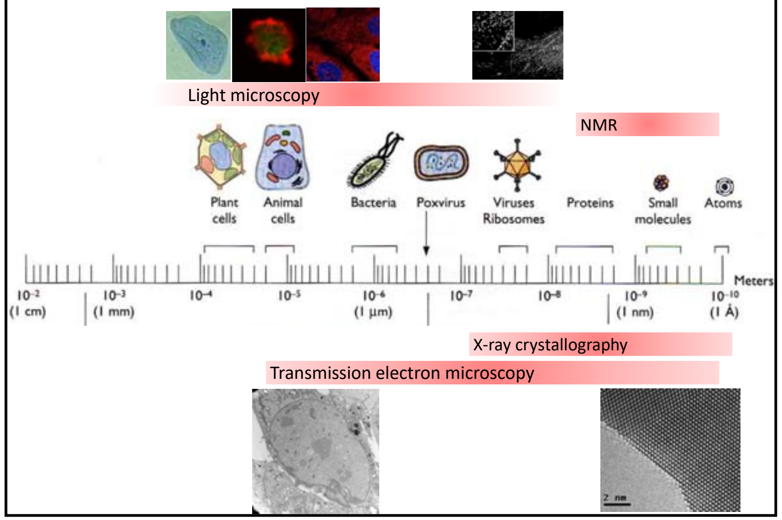
To discover the 3-dimensional arrangement in space of macromolecules, their complexes, and the functioning systems which they constitute.

e.g.
atomic structure of a protein – giving a spatial description of its chemistry (crystallography, NMR, electron microscopy (EM)).

electron density distribution of a larger complex – showing its shape, the way that its component proteins/lipids/nucleic acids come together (crystallography, EM).

tomograms of cell structure – showing the arrangement of protein complexes within the disordered environment of the cell (EM).

Resolving life at different levels



Nuclear magnetic resonance

X-ray crystallography

Cryo-electron microscopy

Structural biology techniques

Raman spectroscopy

Biophysical characterisation

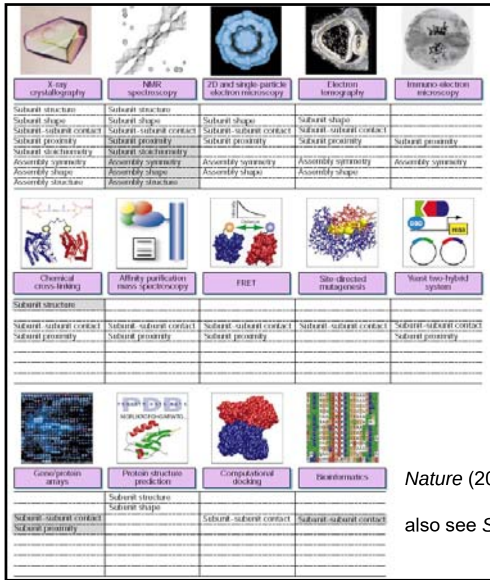
Analytical ultracentrifugation

Fluorescence/UV spectroscopy

Circular dichroism

Electron-spin resonance

Hybrid approaches can often be most helpful.

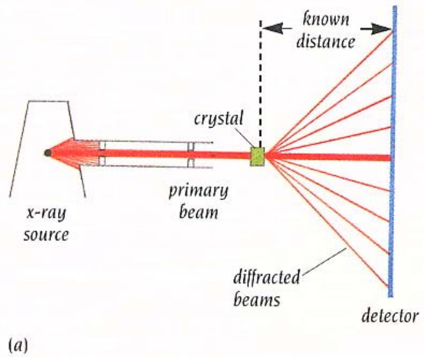


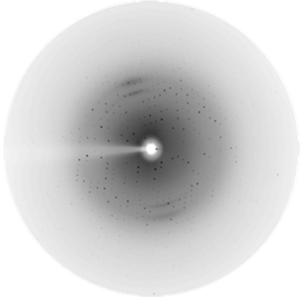
Nature (2003) **422**, 216-225

also see *Science* (2004) **303**, 2026-2029

X-ray crystallography

Diffraction - $n\lambda = 2d\sin\theta$ (Bragg's law)





X-rays generated using a synchrotron have λ = ~0.5- ~2.5Å. They are tuneable, bright and parallel. cf Diamond Light Source

X-ray crystallography

- is from planes of electrons. $n\lambda = 2d\sin\theta$

- is the physical equivalent of a mathematical Fourier transform into reciprocal (frequency) space, which is described by a reciprocal lattice.

- each diffraction spot corresponds to a point in the reciprocal lattice, and represents a diffracted wave with an amplitude and a relative phase.

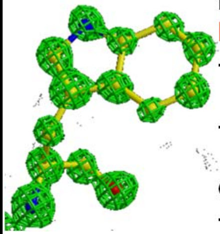
- the phase information is irretrievably lost when a diffraction image is captured.

- without this phase we cannot mathematically invert the Fourier transformation generated physically by diffraction to generate a real space description of the planes of electrons, *aka* electron density.

X-ray crystallography

So where do we get electron density from?

- we have to determine the phase of each scattered wave, whose amplitude we measure from the diffraction spot.



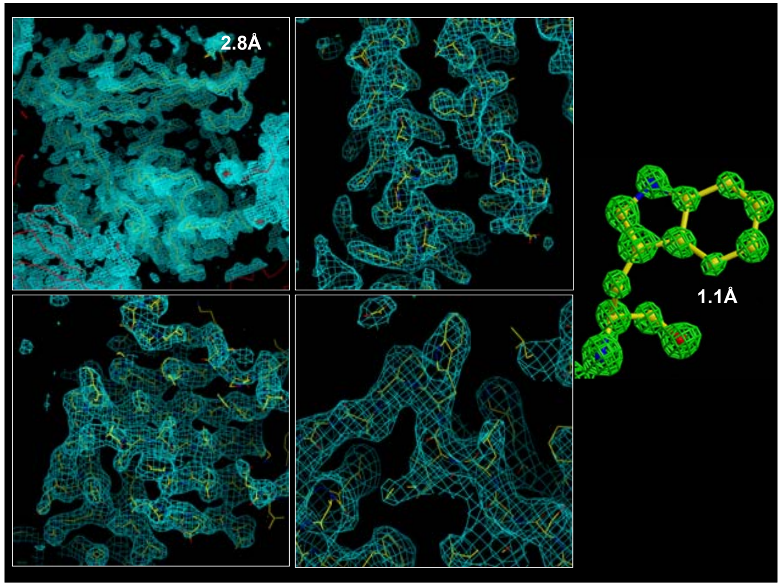
How?

-Molecular replacement
use structure of similar protein.

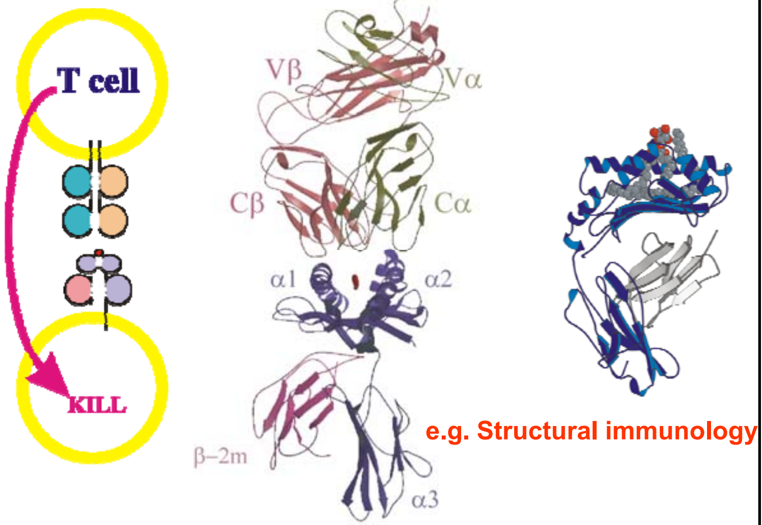
-Multiple isomorphous replacement
soak in heavy atoms to perturb the diffraction, *eg* W, U, Au, Ag, Pb, Hg, I, Xe …

-Multiwavelength anomalous dispersion (MAD)
use the anomalous signal of Se with selenomethionated protein, or of atoms that have been soaked in.

Once electron density map has been determined, we can build in an atomic model, to demonstrate chemistry of macromolecule.



X-ray crystallography



Nuclear magnetic resonance (NMR)

Some nuclei have magnetic spin, seemingly arising from a disparity in the number of protons *v* neutrons within them, *eg* ^{1}H, ^{13}C, ^{15}N, ^{19}F.

Magnetic spin can be ↑ or it can be ↓ (up or down, low or high).

↑ and ↓ have different energies – one has higher energy than the other.

In an NMR experiment the sample is bathed in a magnetic field, B_0. This results in vectorial alignment of the magnetic spins within a sample.

An additional magnetic pulse is applied to the sample, resulting in the excitement of some % of the nuclei from low to high spin.

Following the pulse, the high-spin-state nuclei relax back to a low-spin state, with an associated emission of electromagnetic radiation.

This can be measured.

The emission from any one nucleus is highly sensitive to its molecular environment and is quantified as the “chemical shift” of the resonating nucleus.

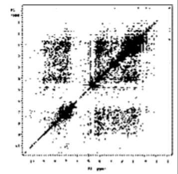
Nuclear magnetic resonance (NMR)

How can it give us a structure?

1. Through-bond (J) couplings of resonances from adjacent nuclei in polypeptide chain/adjacent bases in nucleic acid.

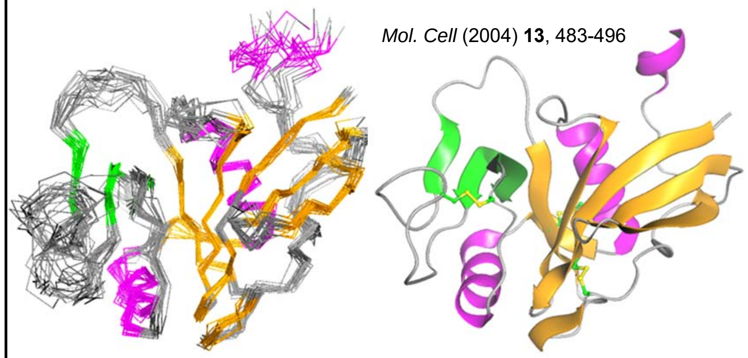
2. Through-space couplings of resonances from adjacent nuclei in fold – Nuclear Overhauser Effect.

3. Do 2D or 3D spectra, *eg* ^{1}H-^{15}N, ^{1}H-^{15}N-^{13}C. Use double or triple pulse sequence….



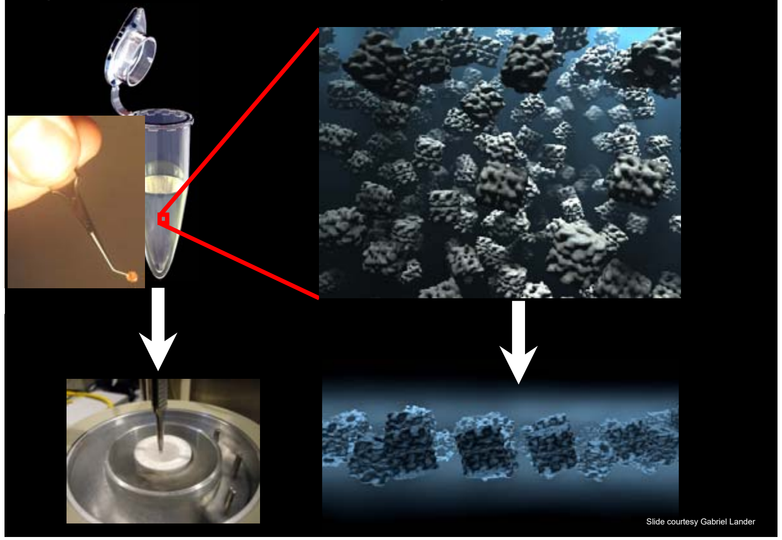
Nuclear magnetic resonance (NMR)

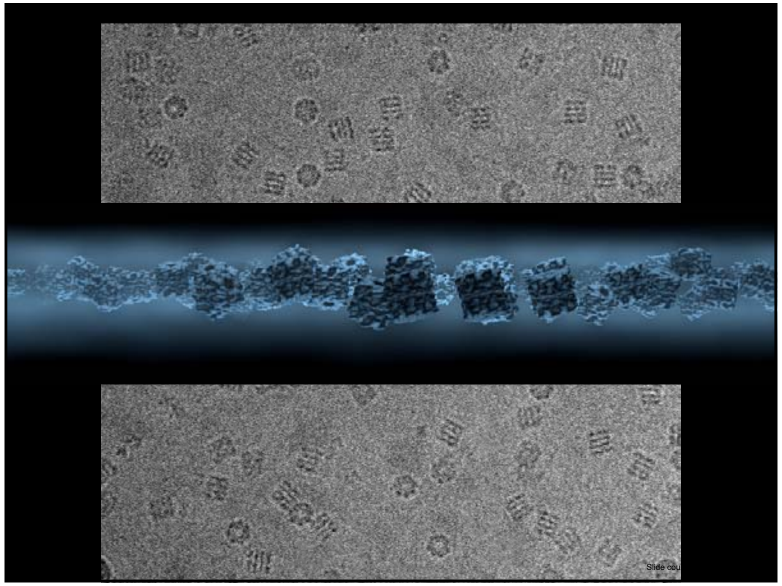
Mol. Cell (2004) **13**, 483-496

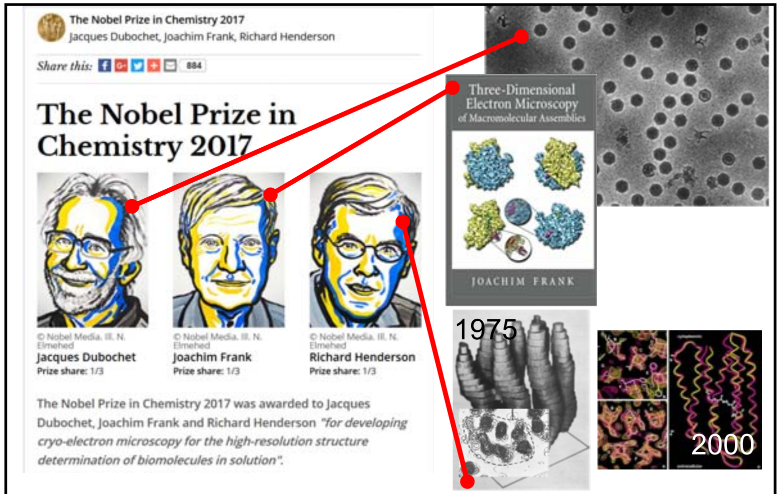


Hyaluronan-binding domain of CD44

Cryo-EM: sample preparation by vitrification

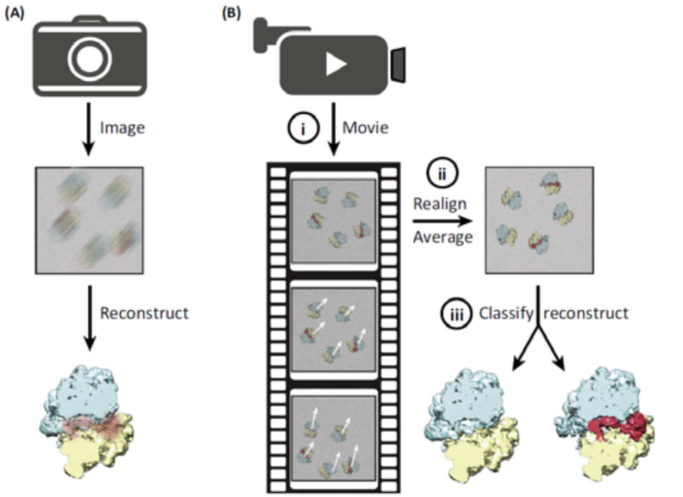






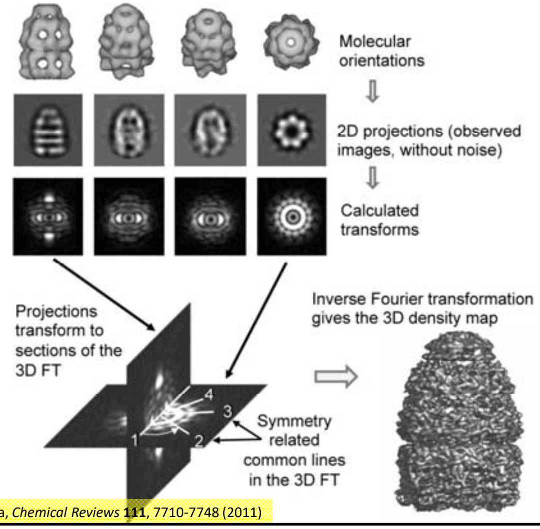
Why? Because they worked out how to vitrify thin films and how to analyse noisy (weak) images

Cryo-EM: a transformed technology



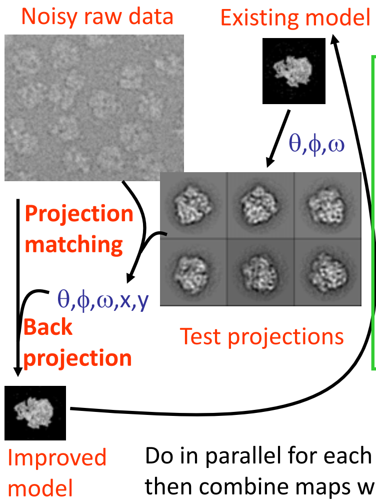
Bai et al., *TiBS* **40**, 49-57 (2015)

Cryo-EM, why it works: projection images, central sections

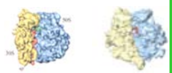
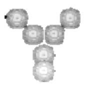


Saibil and Orlova, *Chemical Reviews* **111**, 7710-7748 (2011)

Cryo-EM: the single-particle approach



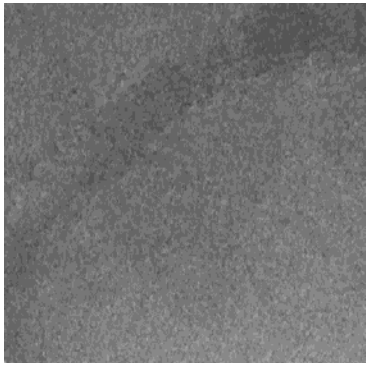
There are a number of ways to get your model to start with:

1. Use something similar 
2. Make one up (you think you know what it looks like) 
3. Use a "featureless" model
4. Use direct analysis of images grouped into class averages.

Do in parallel for each different defocus, and then combine maps with defocus correction.

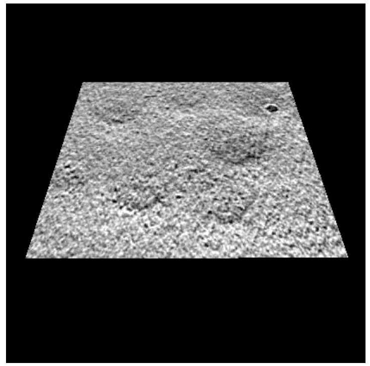
Large-scale/unique structures from electron tomography

MMTV cryo-electron tomography



Movie courtesy of John Briggs, MRC LMB, Cambridge

HIV cryo-electron tomography



John Briggs et al., *Structure* 14, 15-20 (2006)

…brought up to date: the atomic structure of HIV

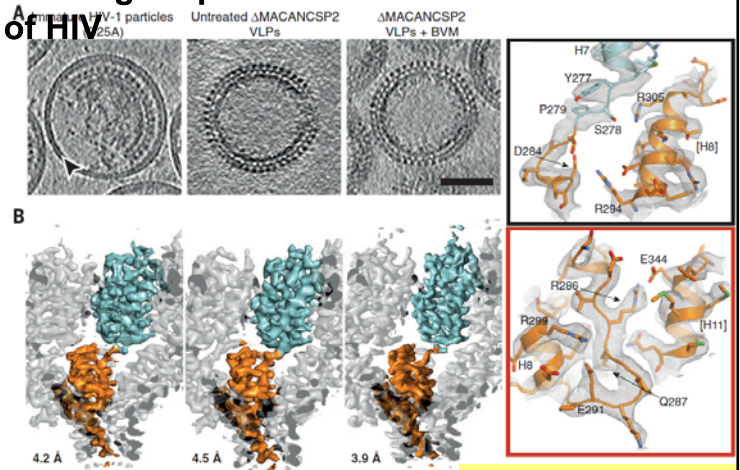
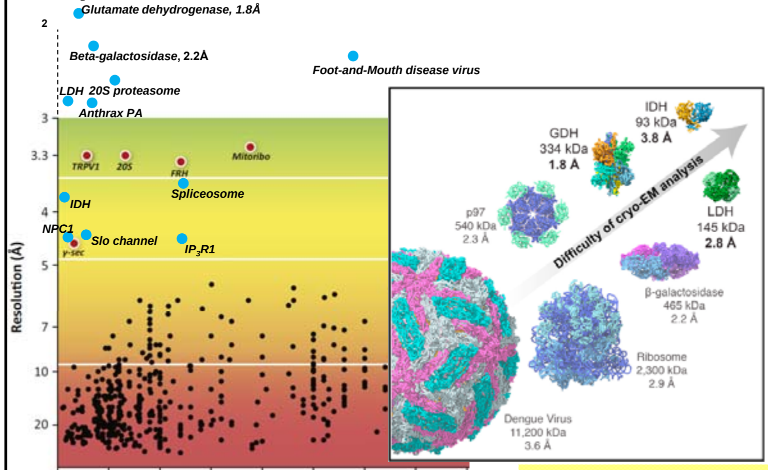


Fig. 1. Structure of the immature HIV-1 CA-SP1 lattice at 3.9 Å.

Schur et al., *Science* **353**, 506-508 (2016) and Mattei et al., *Science* **354**, 1434-1437 (2016)

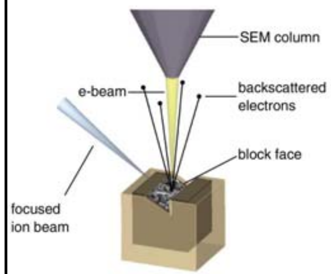
Cryo-EM: the resolution revolution

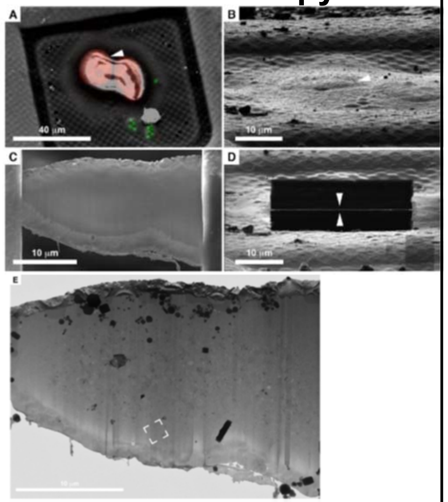


Bai et al., *TiBS* **40**, 49-57 (2015)

Merk et al., *Cell* **165**, 1698-1707 (2016)

FIB milling and correlative microscopy of cells

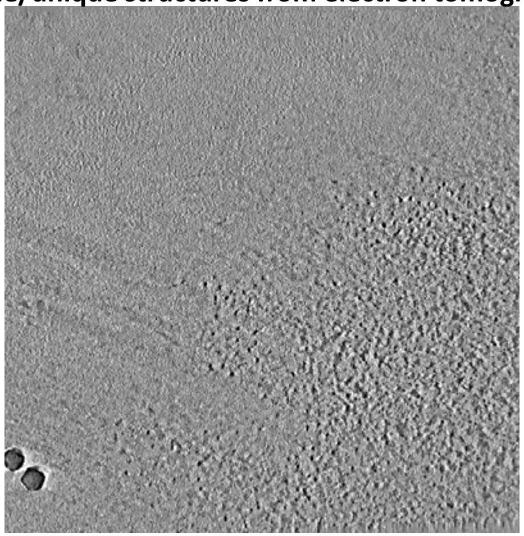




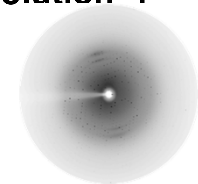
Mahamid et al., *Science* **351**, 969-972 (2016)

Large-scale/unique structures from electron tomography

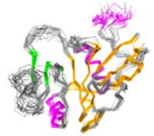
Mahamid et al., *Science* **351**, 969-972 (2016)



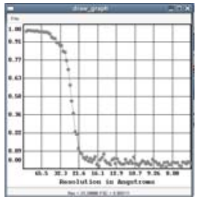
What do we mean by "resolution"?



Crystallography: how far you can measure your spots (I/σI, R_{merge}, multiplicity) and model quality, in Å.



NMR: how well your different models agree (root-mean-square deviation (RMSD) of models in Å).



Cryo-EM: how well do separate density maps of your structure agree with one another – to what resolution do they have a correlation coefficient of *X*?

Modelling

Ab initio?

By threading onto an homologue?

Using a variety of templates?

Phyre²

http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index

http://www.rcsb.org