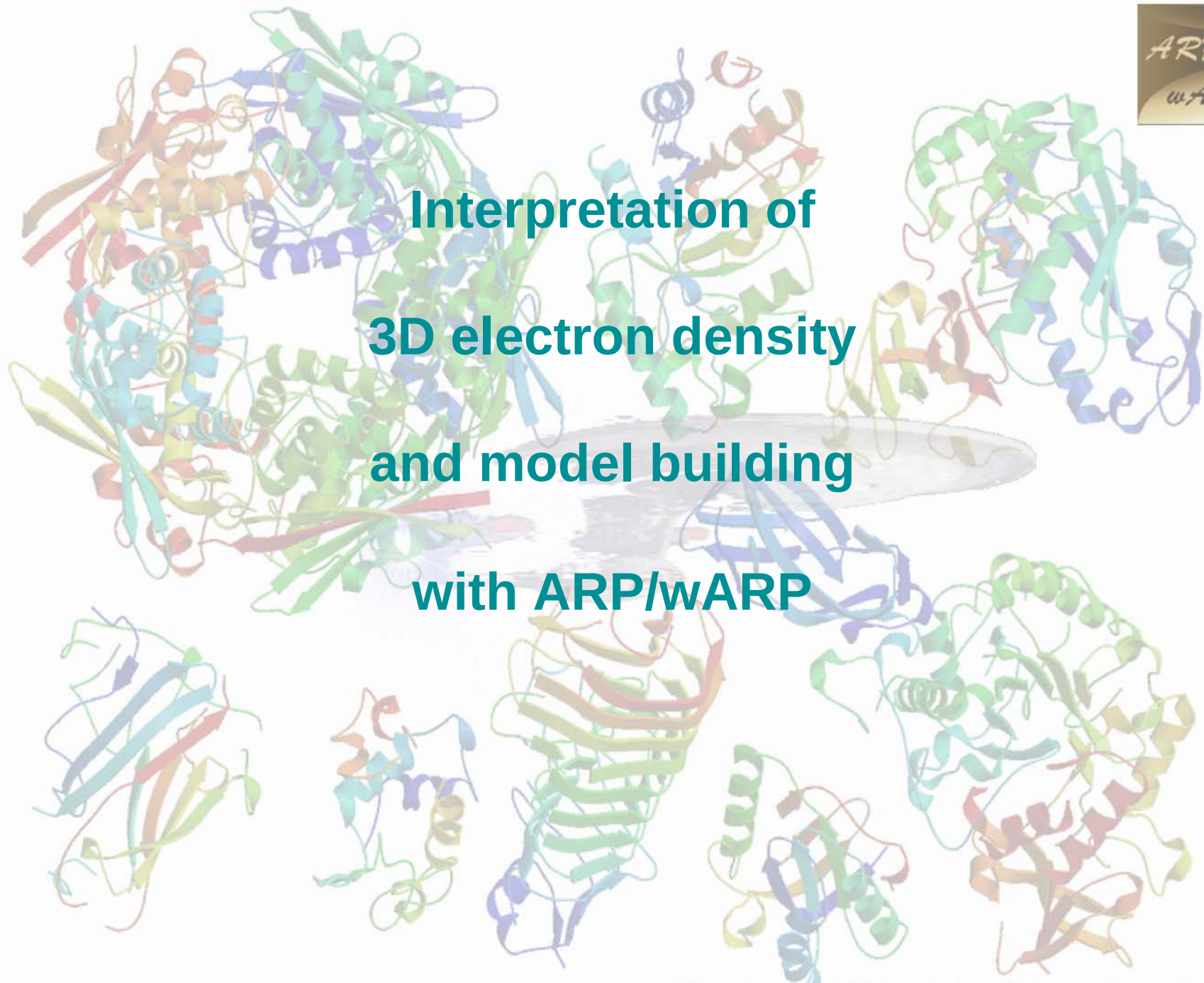


Interpretation of 3D electron density and model building with ARP/wARP

What is an Electron Density Map?

1. A result of an X-ray diffraction experiment

2. A result of an Electron Microscopy experiment

3. A molecular energy landscape

4. A distribution of electrons

5. A smeared representation of my protein structure

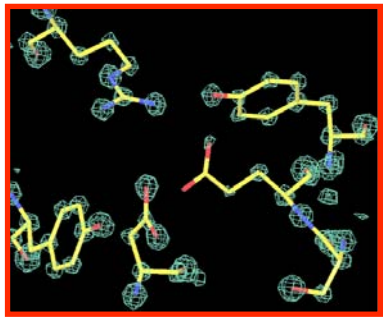
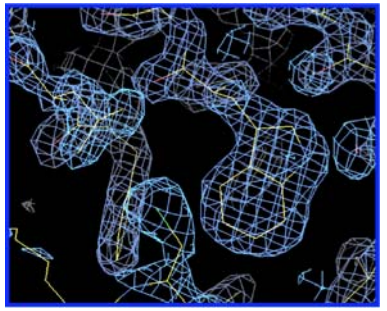
6. A probability density function

7. A nice picture that my boss told me to get

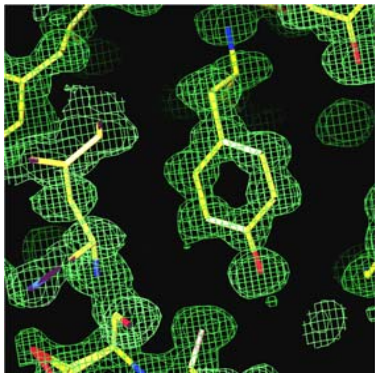
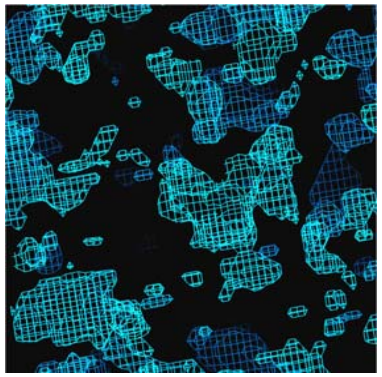
Fundamental Problems in Map Interpretation

1.0 Å | 2.0 Å | 3.0 Å

Limited resolution

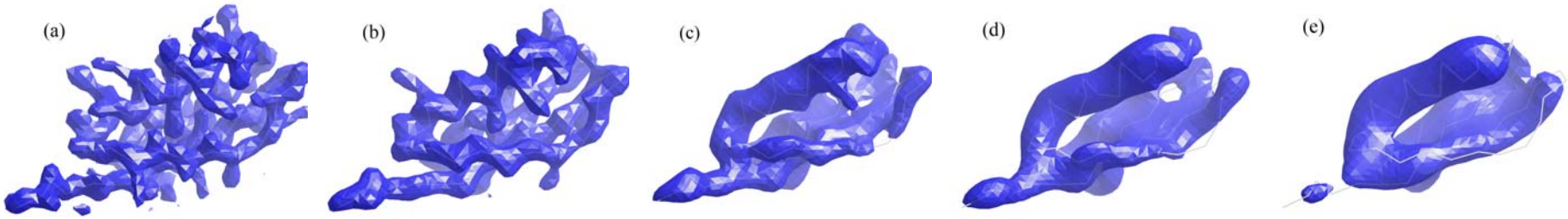

Errors in phases
(in general the amount and type of noise present)

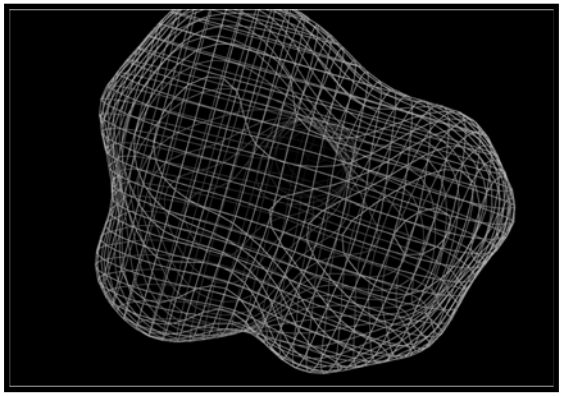
(Limited) Resolution of the X-ray Data

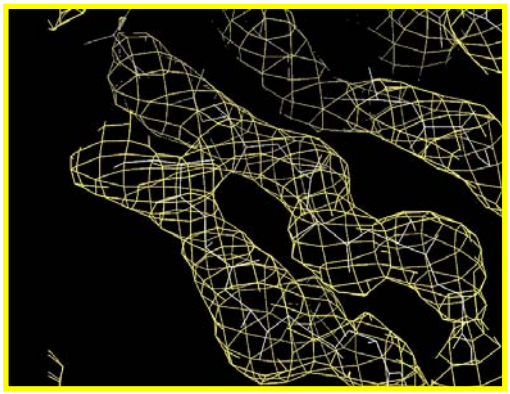
Calculated map of protein G

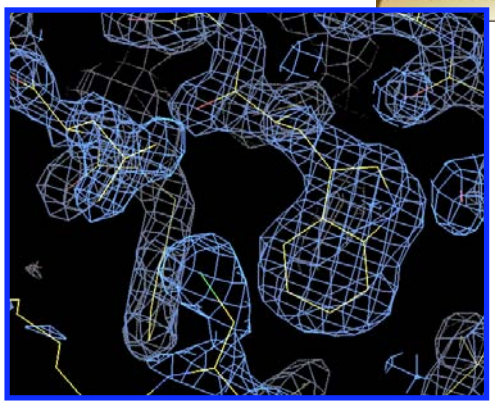
3 Å 4 Å 5 Å 6 Å 8 Å

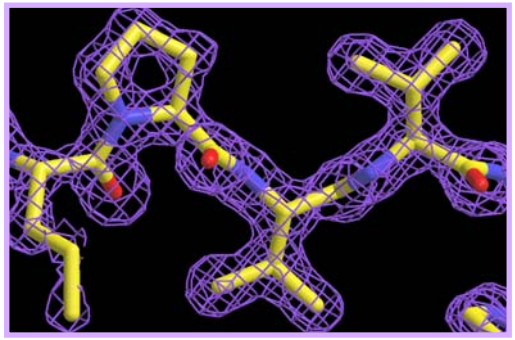


(Limited) Resolution of the X-ray Data

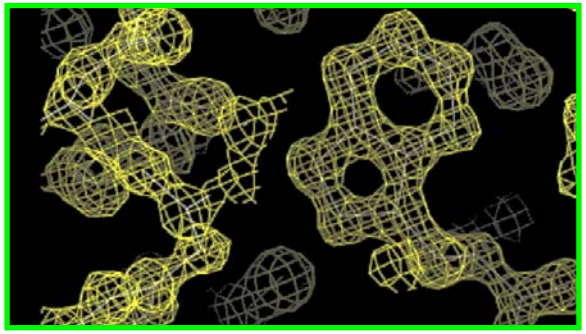


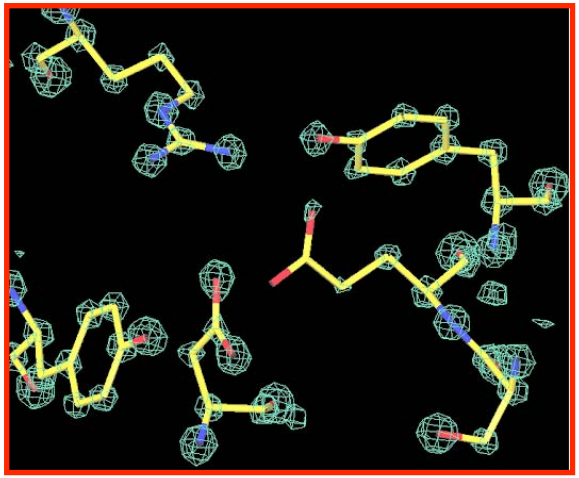


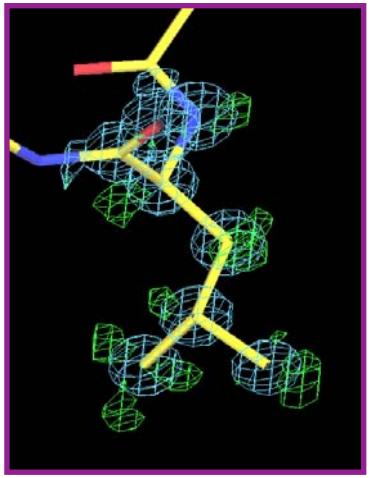


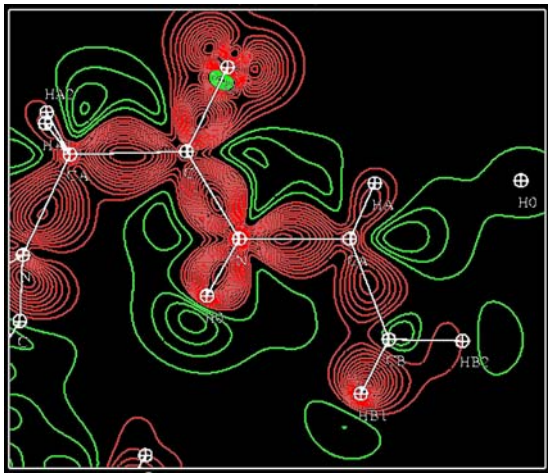


20 Å 3.0 Å 1.9 Å
1.6 Å 1.2 Å
1.0 Å 0.8 Å ultra

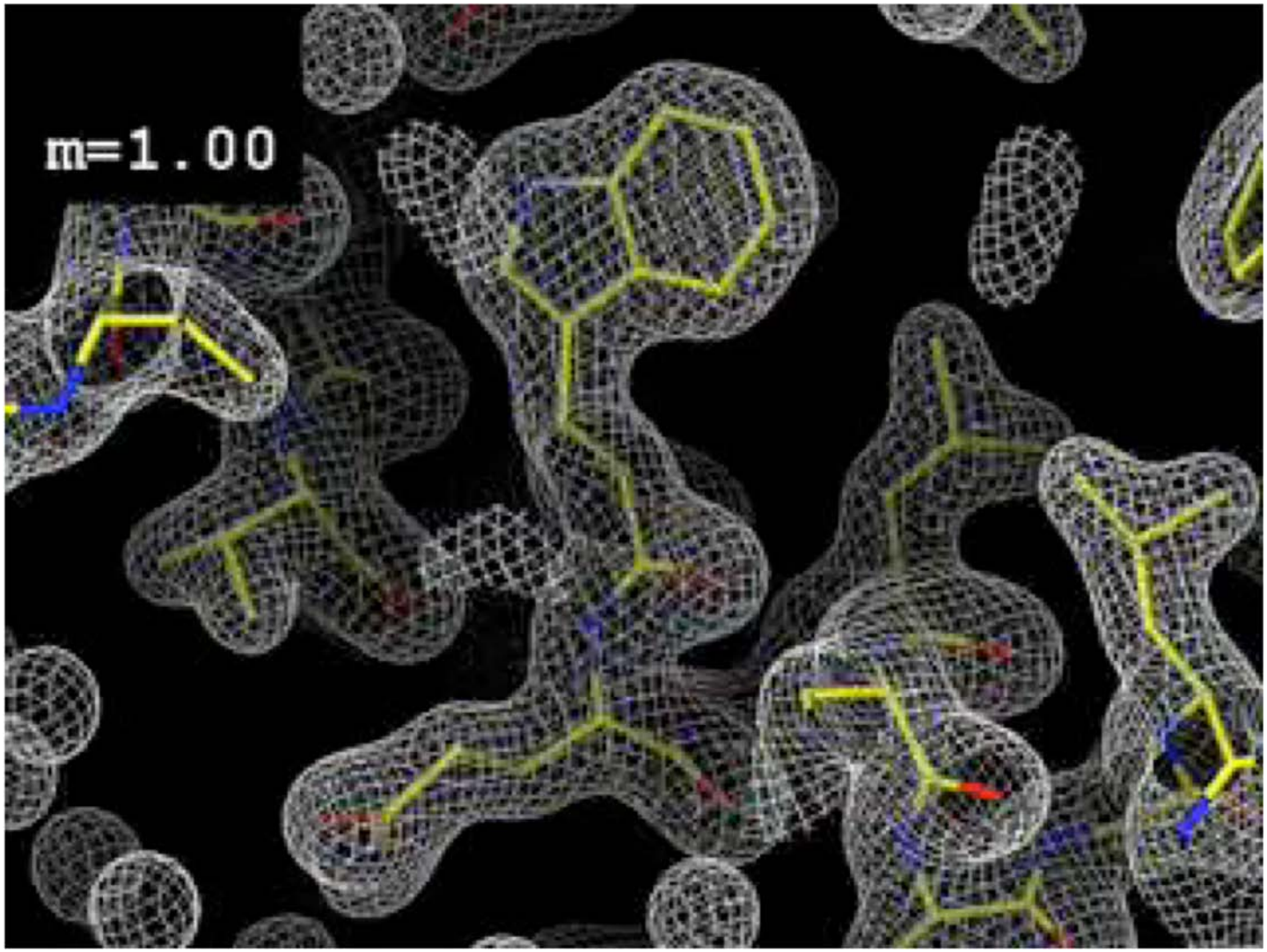




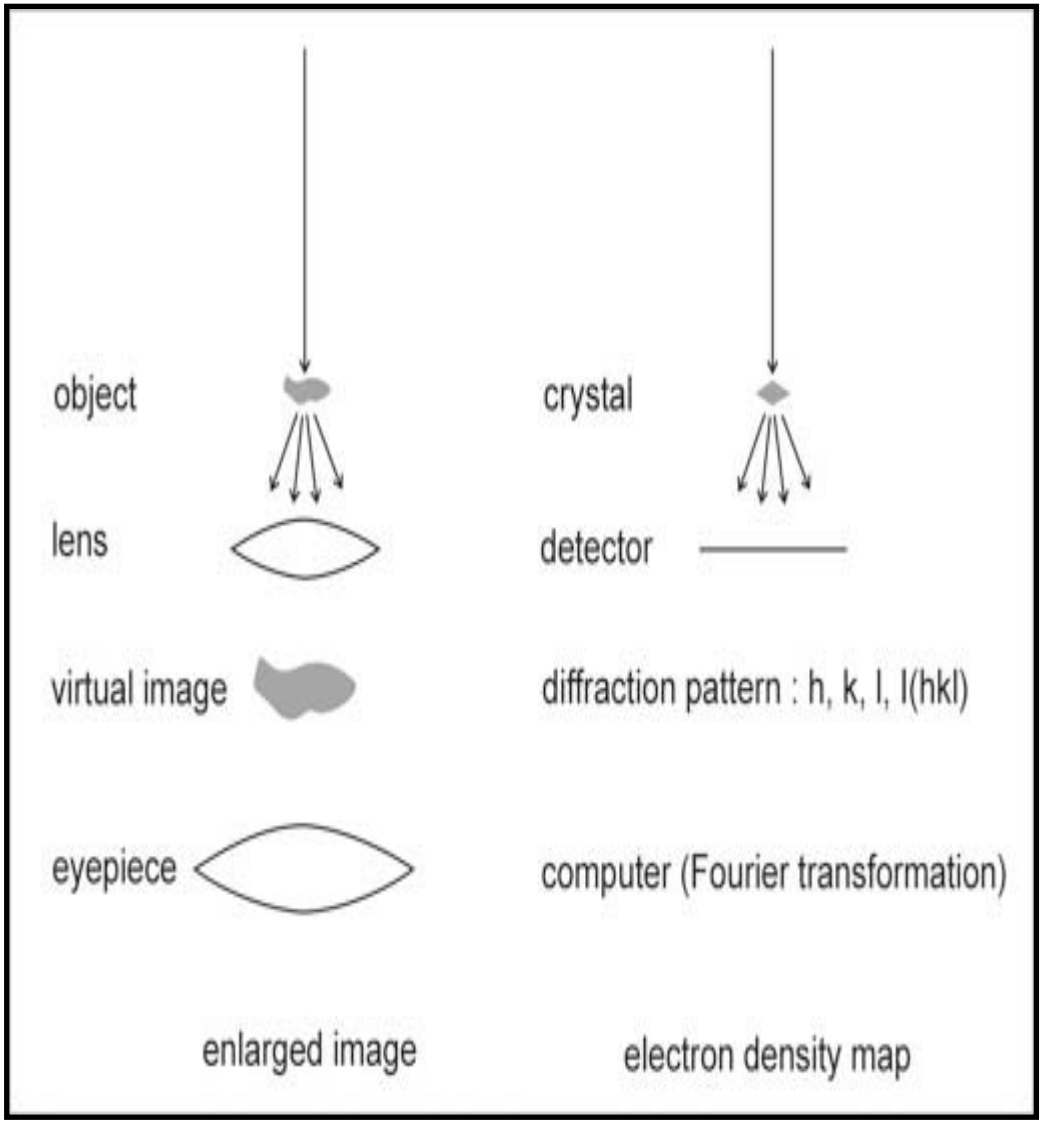


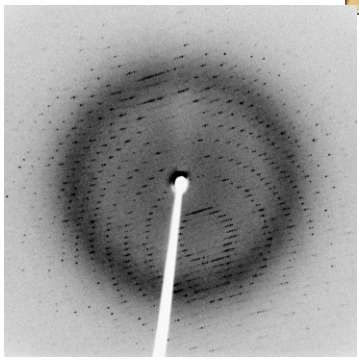


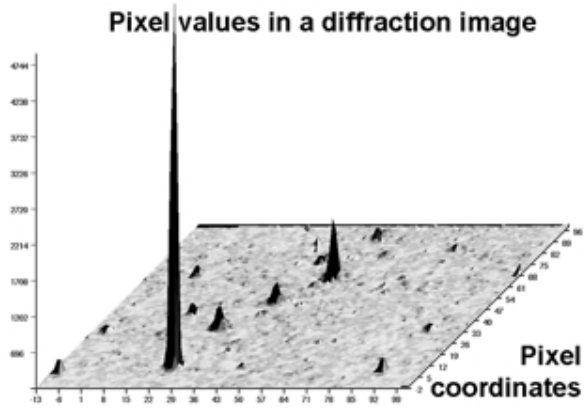
Importance of the Phases

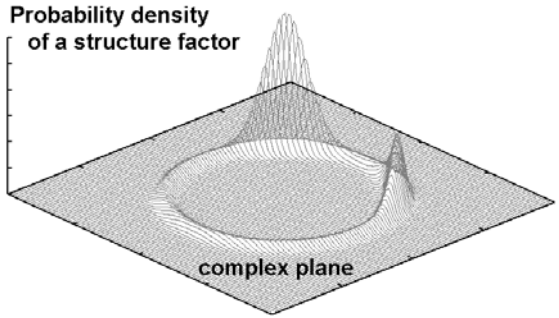


Why Do We Worry So Much About Phases?

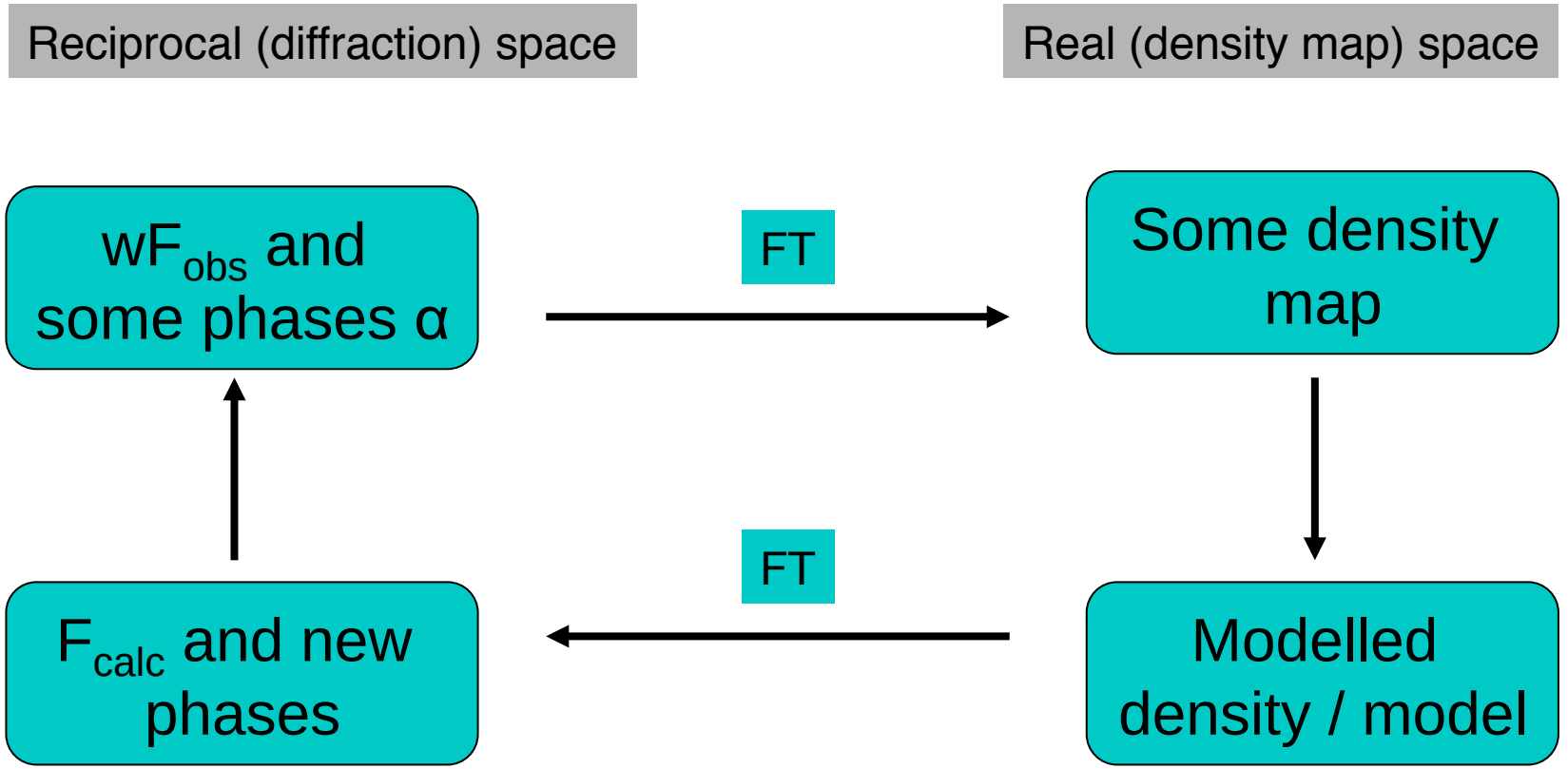








Iterative Solution to the Crystallographic Phase Problem



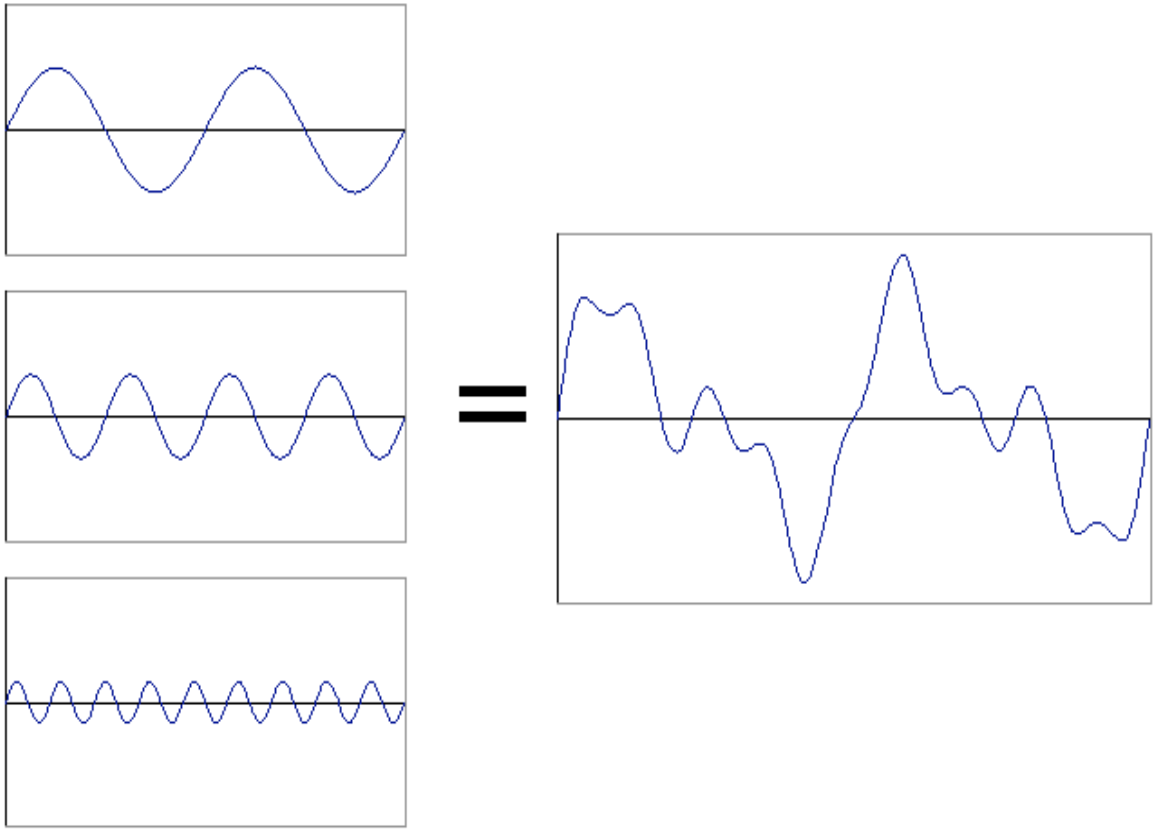
Interpretability and Information Content

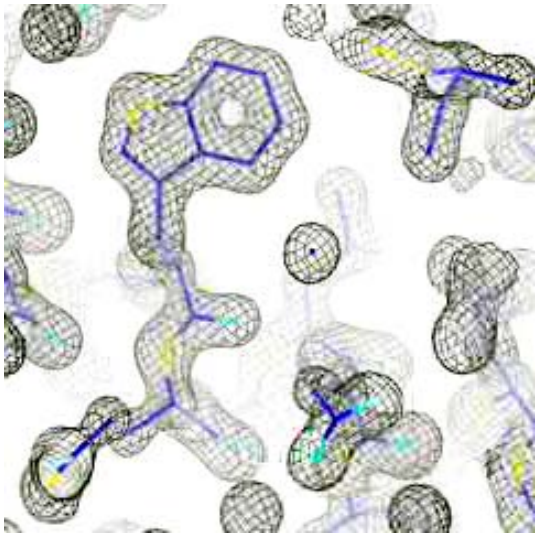
Interpretability of a density map is defined by its information content

The information content of our modelling should match the information content of the map

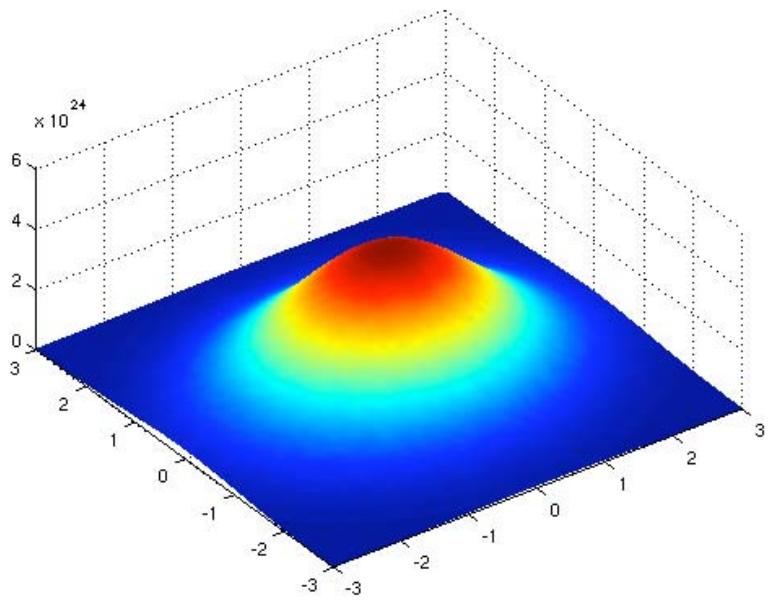
Modelling the Density: Fourier Transform

$$p(\mathbf{x}) = \sum_{\mathbf{h}} |F_{\mathbf{h}}| \cos(2\pi \mathbf{h}\mathbf{x} - \alpha_{\mathbf{h}})$$



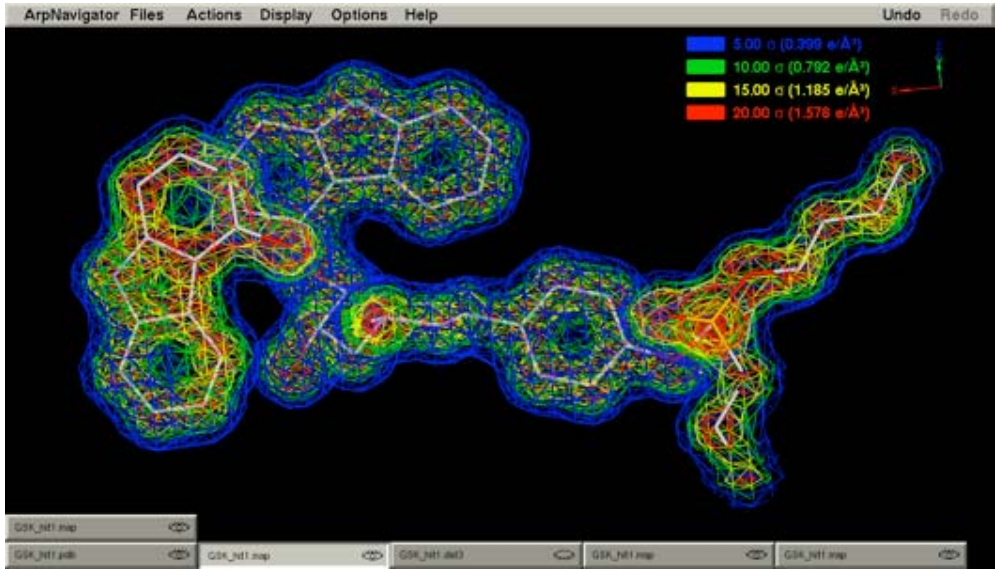


Modelling the Density: Radial Basis Functions



$$f(x) = \sum_i w_i \exp(-\frac{(x - x_i)^2}{2\sigma^2})$$

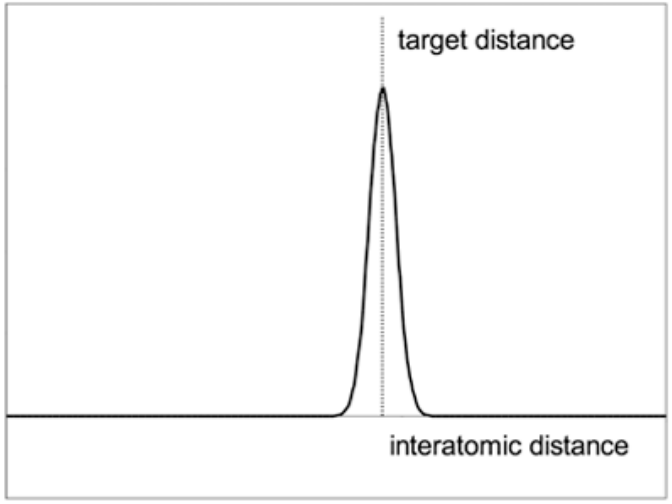
One per atom

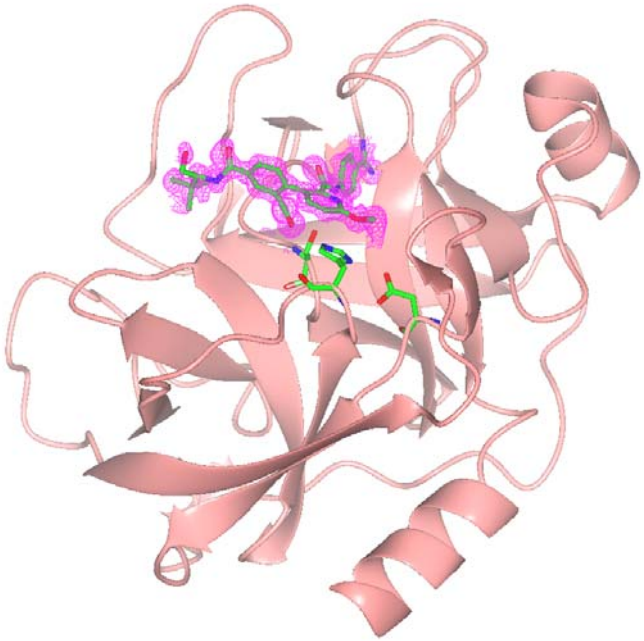


Advantage:
Gaussians in real space are Gaussians in reciprocal space, too!

Modelling the Density: Ball & Stick

xyz1 xyz2





Number of X-ray Observations

The number of diffracted X-ray reflections for a complete data in P1 lattice

$$N_{refl} = \frac{2\pi}{3d^3} V$$

Assuming 50% solvent content and an MW for an average residue of 110 Da, we arrive at:

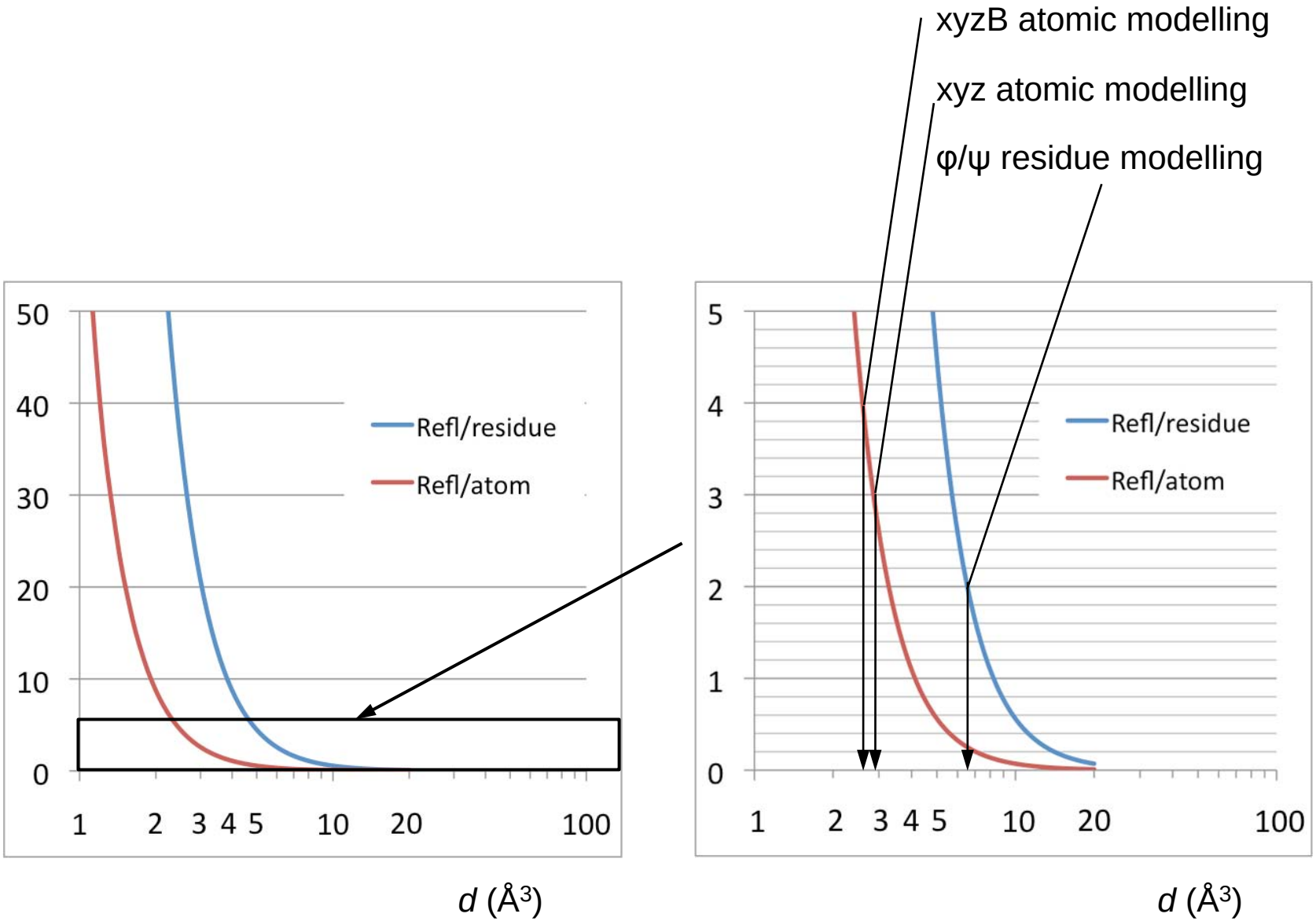
$$N_{refl} = \frac{560}{d^3} N_{res}$$

Or:

$$\frac{reflections}{residue} = \frac{560}{d^3}$$

$$\frac{reflections}{atom} = \frac{70}{d^3}$$

Number of X-ray Observations



Information Content

In the absence of additional data the information content of the model cannot exceed the information content of the map

One may try to increase the information content of the map by complementing it with additional data based on statistical grounds

Exercise: Children

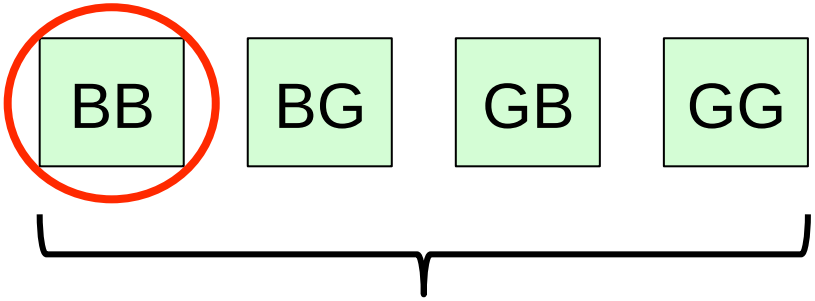
John has got 2 children
What is the probability that both children are boys?

1/4

$$f(x=k)=\frac{n!}{k!(n-k)!}p^{k}(1-p)^{n-k}$$

$$p=0.5;n=2;k=2$$

$$f(x=2)=\frac{2!}{2!0!}\left(\frac{1}{2}\right)^{2}\left(\frac{1}{2}\right)^{0}=\frac{1}{4}$$



$$f(x)=\frac{P\_success}{P\_all\_events}$$

Exercise: Children

John has got 2 children
At least one is definitely a boy
What is the probability that both children are boys?

1/3

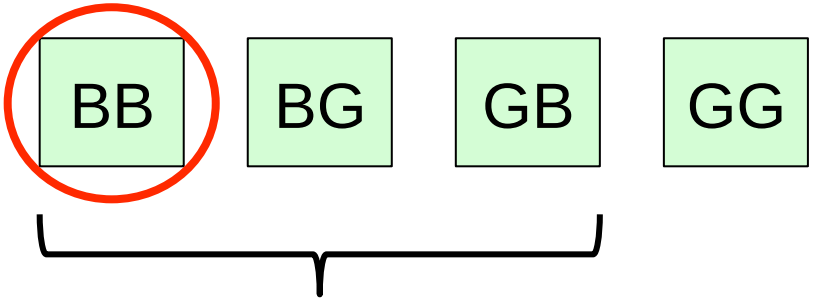
$$f(x=k)=\frac{n!}{k!(n-k)!}p^k(1-p)^{n-k}$$

$$p=0.5;n=2;k=2$$

$$f(x=2)=\frac{2!}{2!0!}\left(\tfrac{1}{2}\right)^2\left(\tfrac{1}{2}\right)^0=\tfrac{1}{4}$$

$$p=0.5;n=2;k=1$$

$$f(x=1)=\frac{2!}{1!1!}\left(\tfrac{1}{2}\right)^1\left(\tfrac{1}{2}\right)^1=\tfrac{1}{2}$$

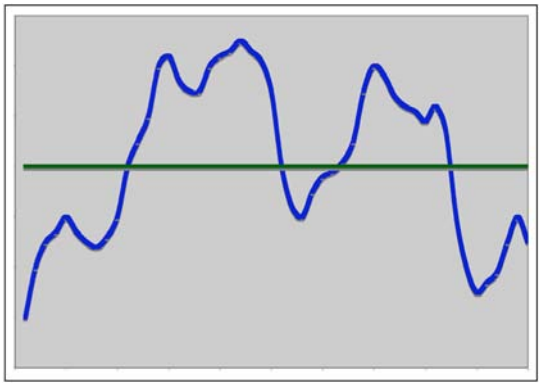


$$f(x)=\frac{P\_success}{P\_all\_events}$$

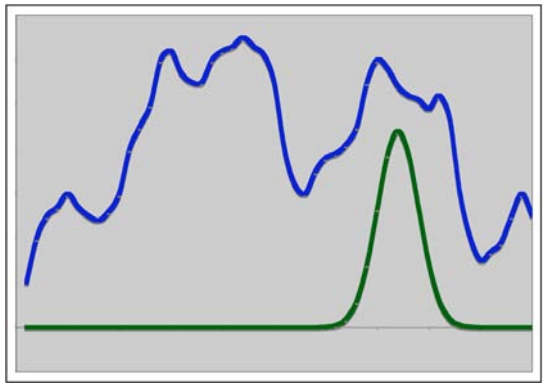
$$f(x=k\mid k\geq 1)=\frac{\tfrac{1}{4}}{\tfrac{1}{4}+\tfrac{1}{2}}=\tfrac{1}{3}$$

Knowledge/Model-Based Map Improvement

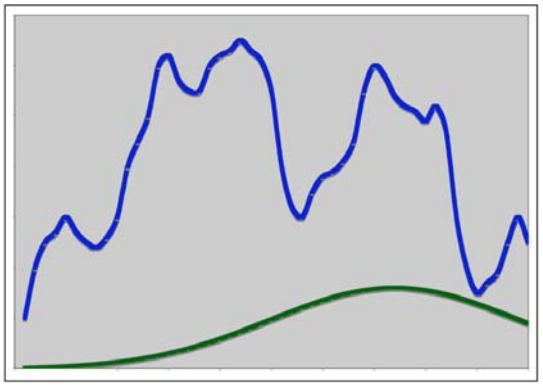
Uniform prior



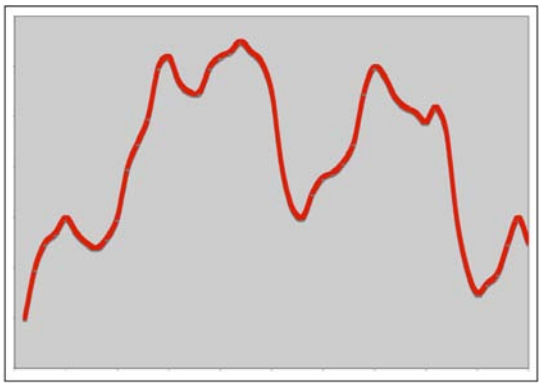
Tight prior (~constraint)

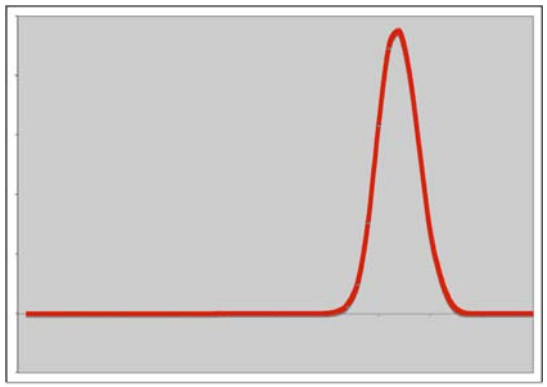


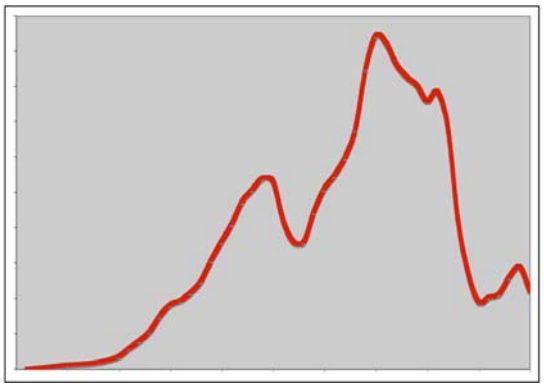
Smooth prior (restraint)



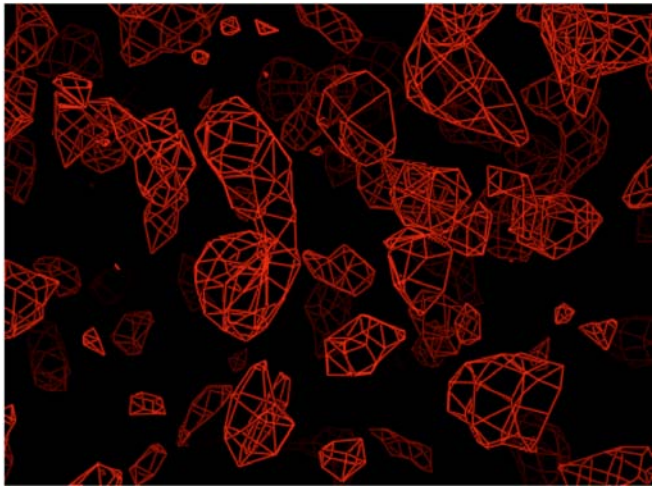
$p = p_1 p_2$

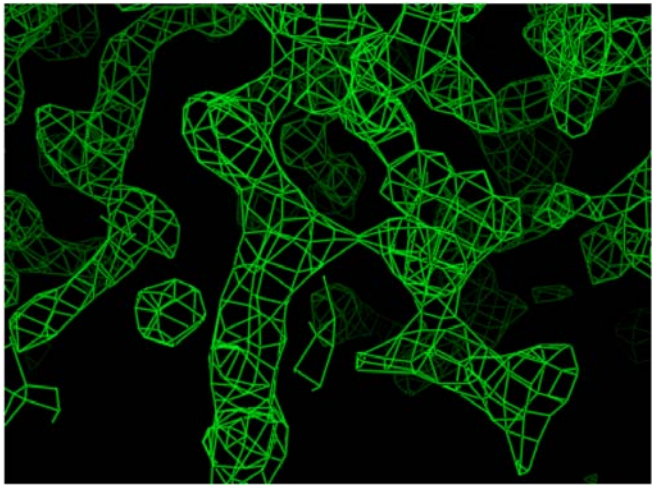






Improvement of a Density Map Prior to its Interpretation





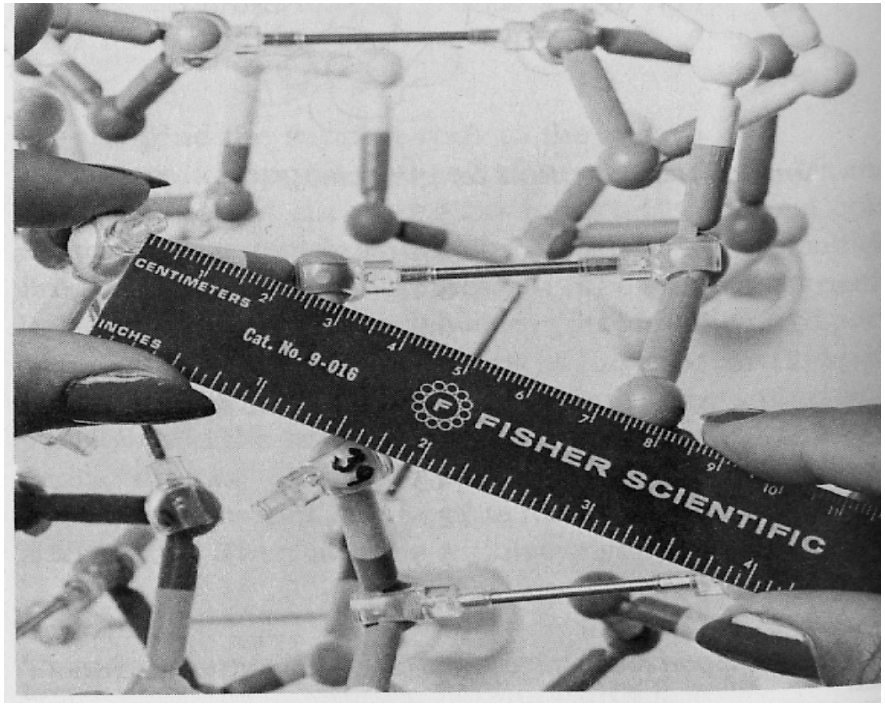
Successfull key-concepts

solvent flattening/flipping

histogram matching

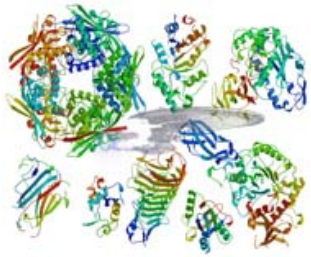
non-crystallographic averaging/symmetry

First Map Interpretations

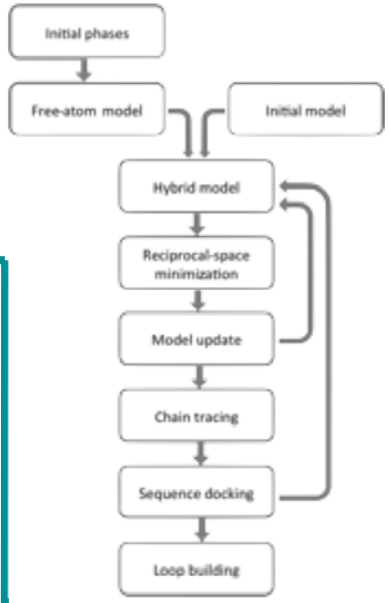


Victor Lamzin, CCP4 workshop, Okinawa, December 2011

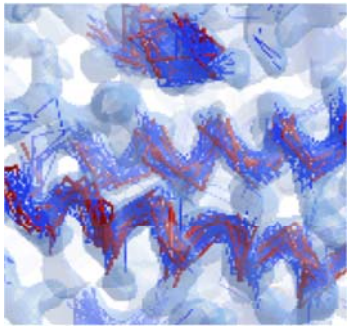
The ARP/wARP Project



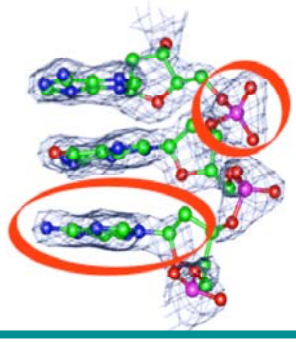
Iterative protein-model building

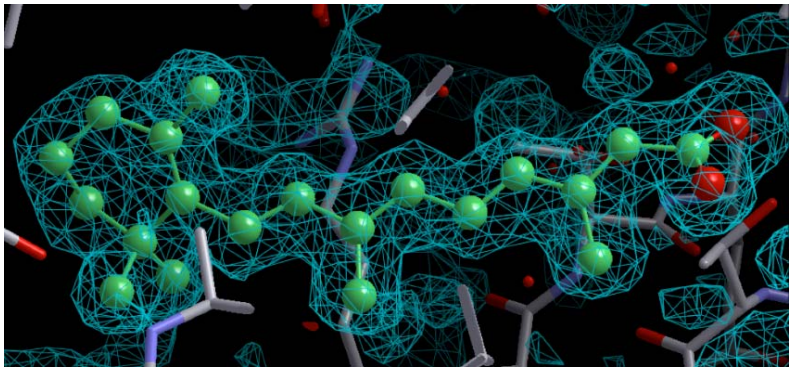


Recognition of secondary structure



Building polynucleotides



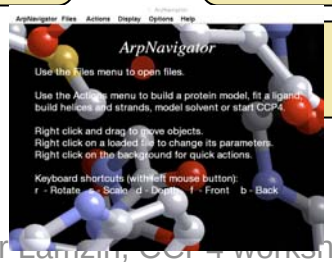


Ligand building and screening

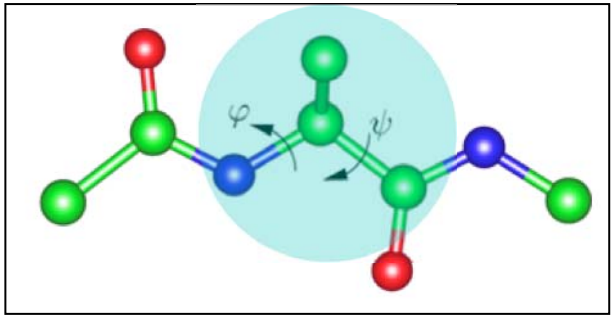
Major Software Releases

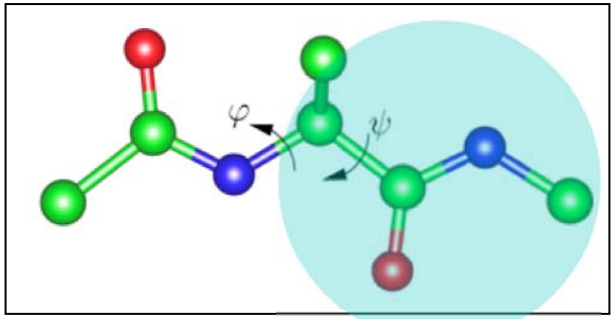
1998 1999 2002 2004 2007 2009 2011

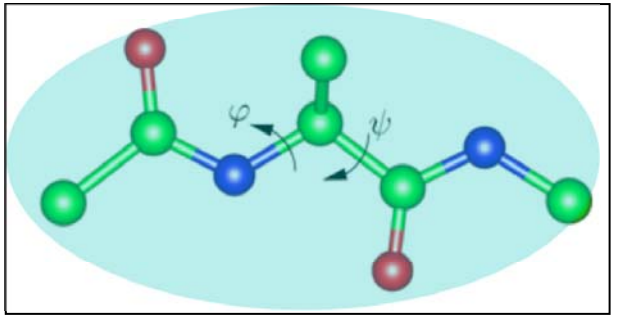
Protein chain tracing	Non-protein parts	Front End
Main chain NEW!	Ligands NEW!	Terminal / shell scr NEW!
Auto-NCS NEW!	Nucleotides	CCP4 GUI
Side chains	Solvent	Web service NEW!
Helices / strands	Refmac: twin, SAD, bulk solvent, jelly, etc	ArpNavigator NEW!
Loop completion		

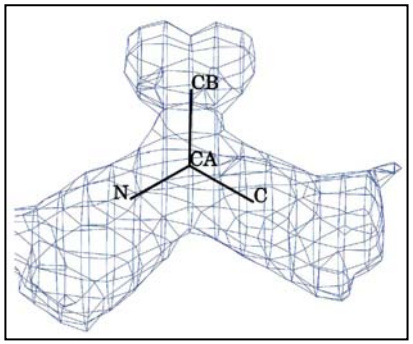


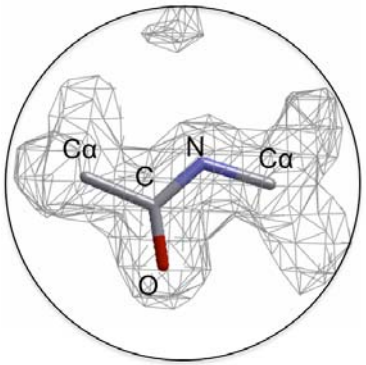
Methods for Protein Model Building

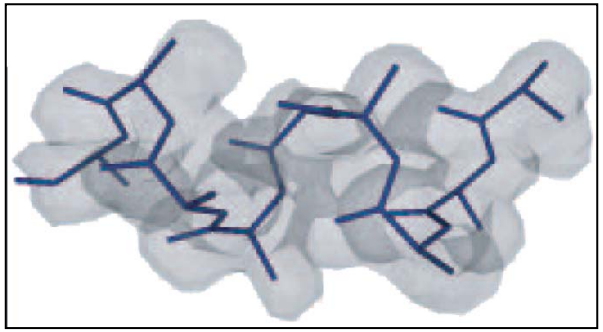






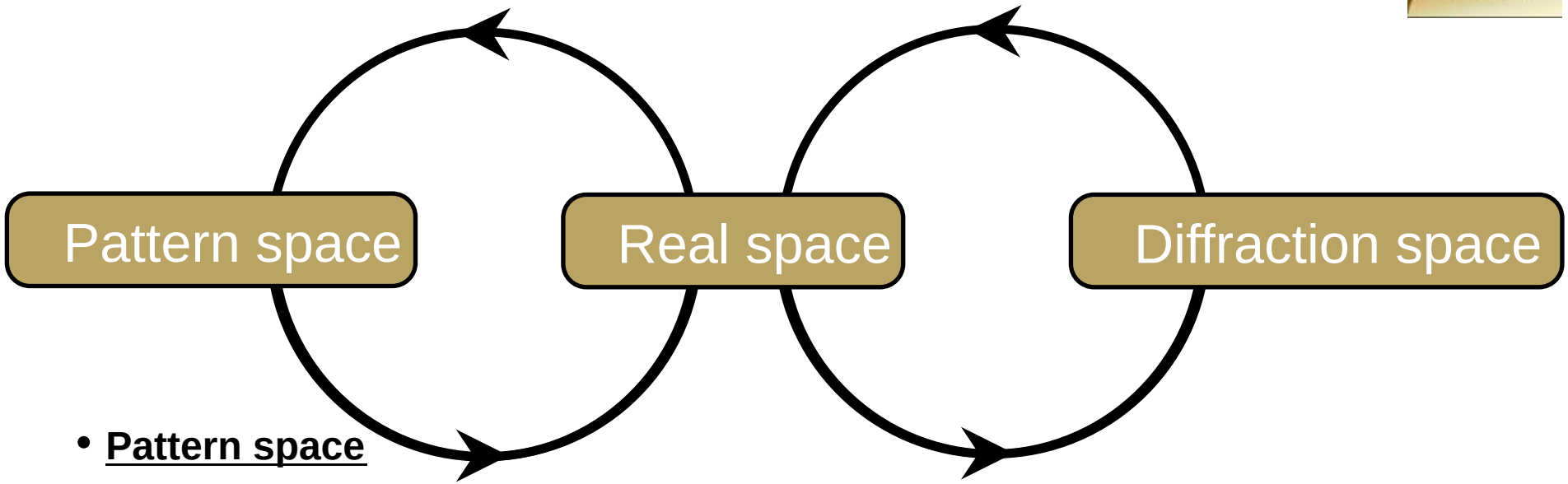






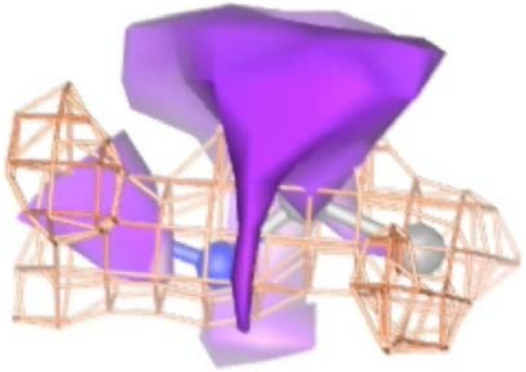
TEXTAL/Buccaneer	ARP/wARP	Resolve/ACMI
Cα atoms	Peptides/Dipeptides	Fragments

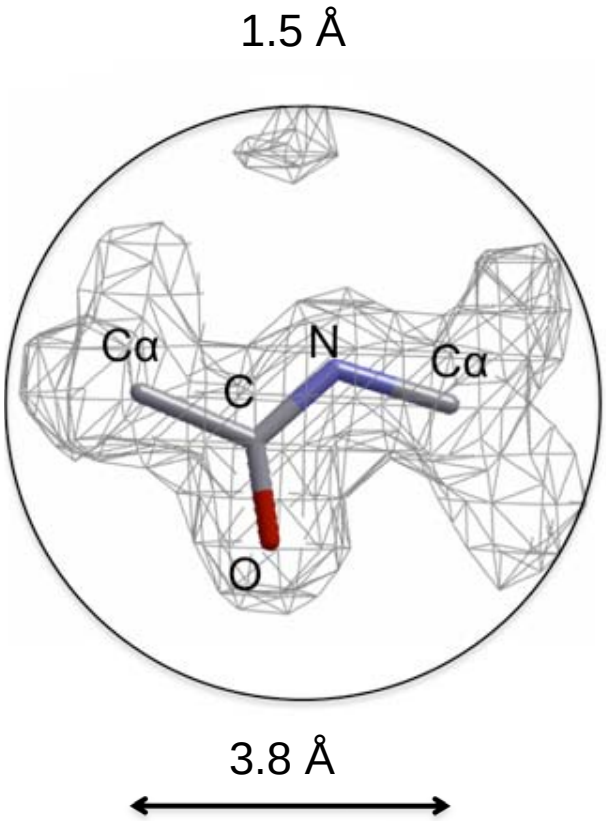
Fundamental ARP/wARP Concepts

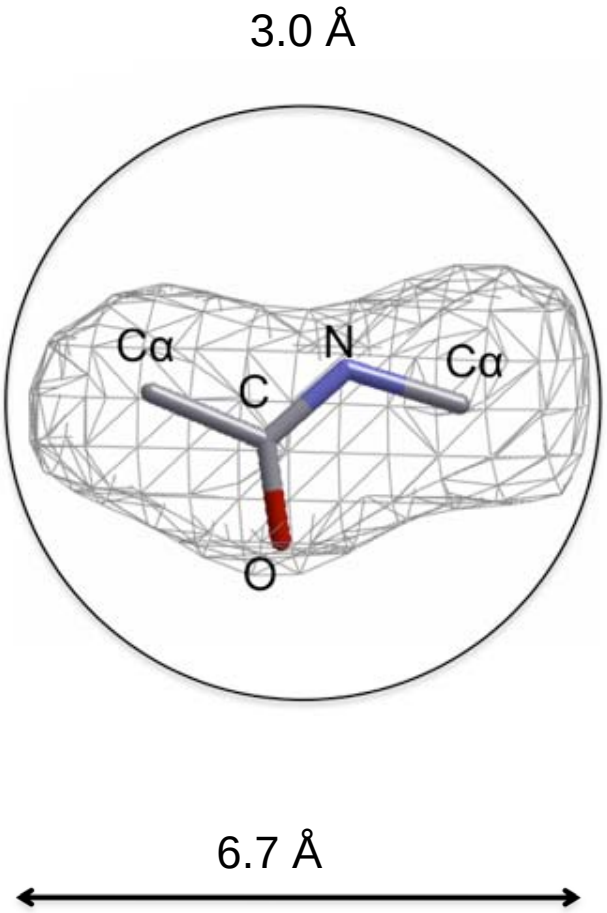


- **Pattern space**
  - **Electron density**: local object interpretation
  - **Hybrid Model**: local motif interpretation
- **Real space**
  - **Hybrid model**: atoms having chemical identity and free atoms
  - **Model update**: removing and adding parts of the model
- **Diffraction space**
  - **Unrestrained refinement** of free parts of the model
  - **Restrained refinement** of chemically assigned atoms

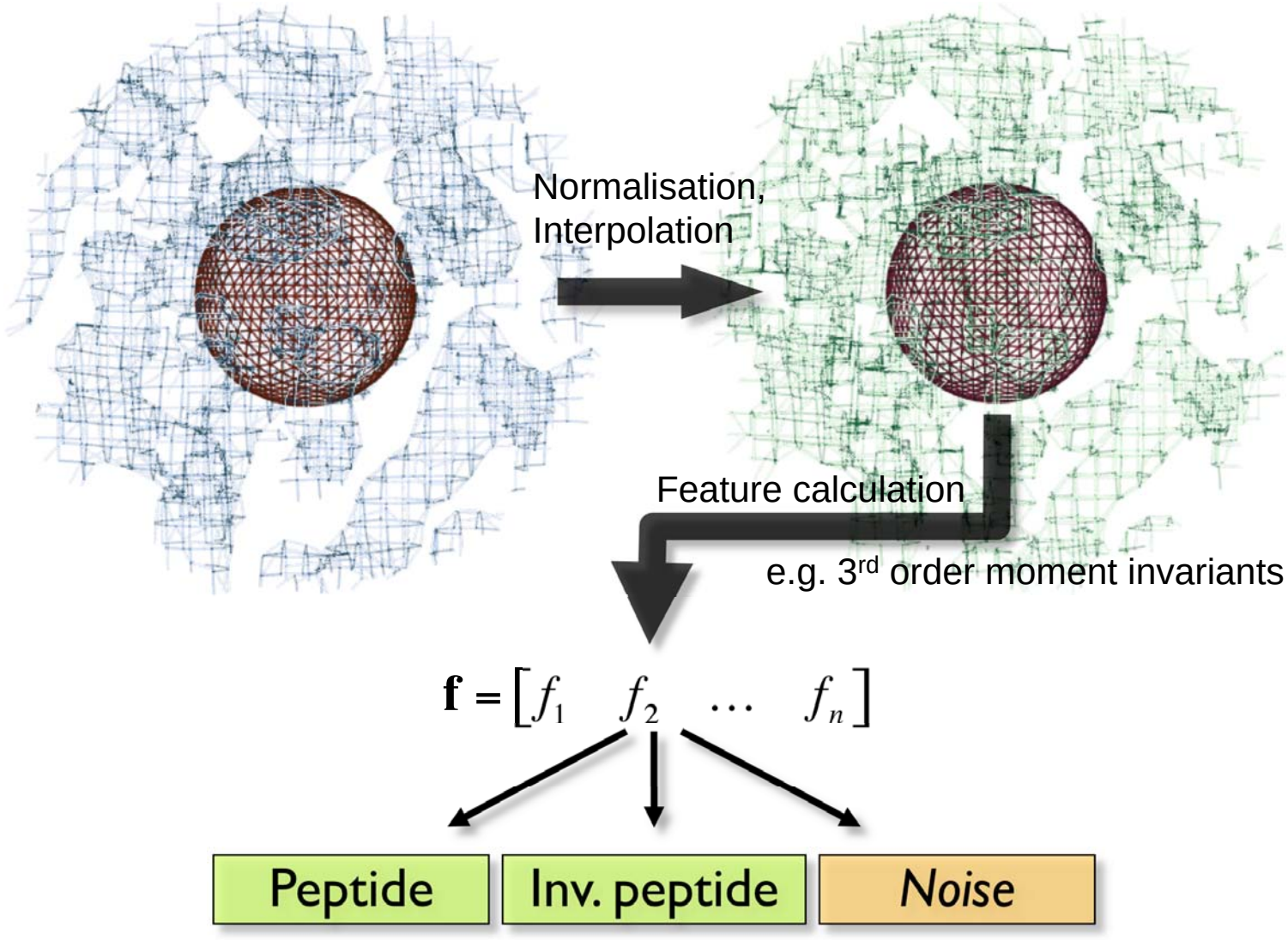
Concept #1: Pattern Recognition



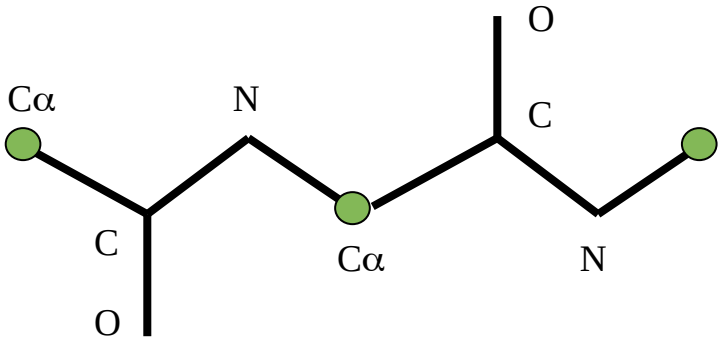


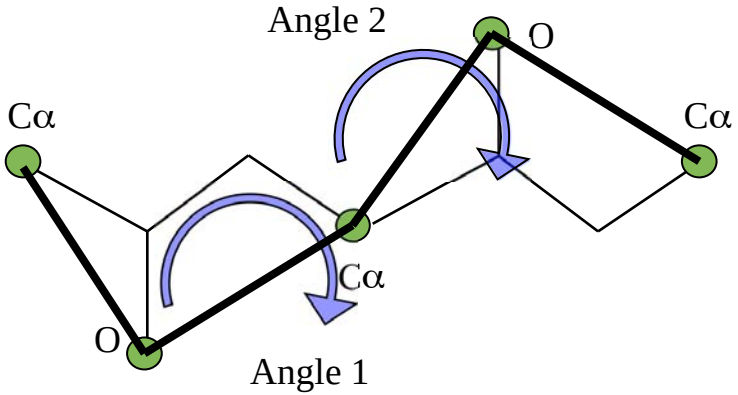


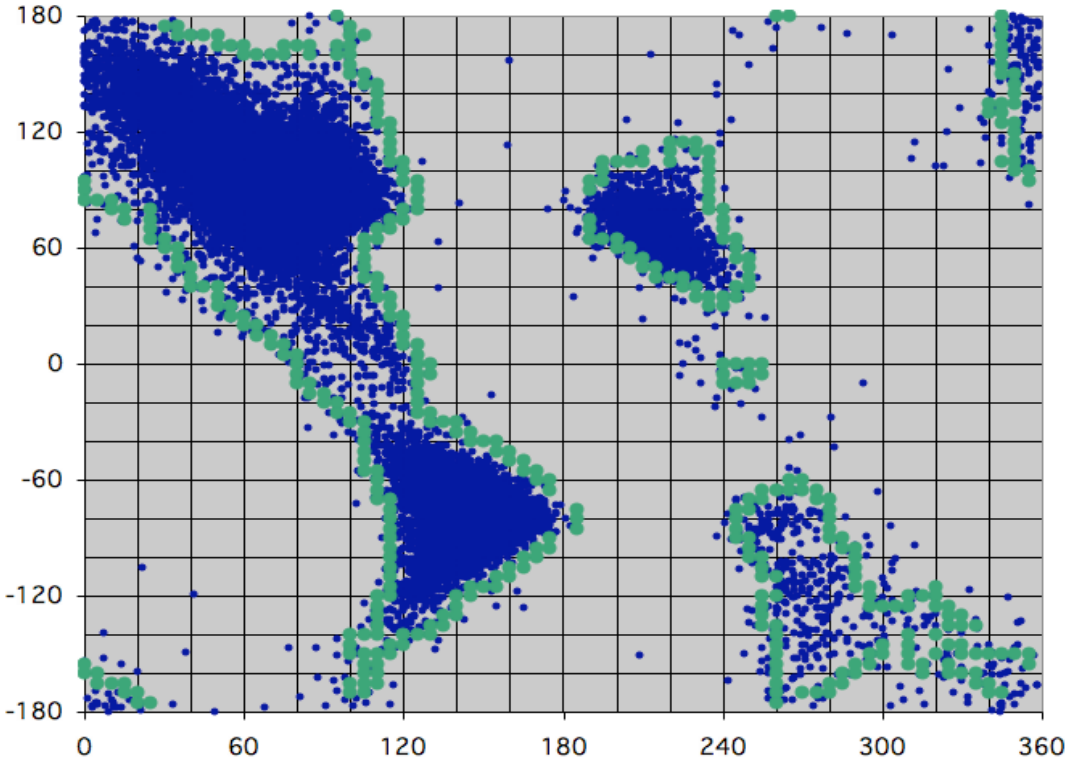
Local Pattern Recognition



Building Protein Chain: From Peptides to di-Peptides



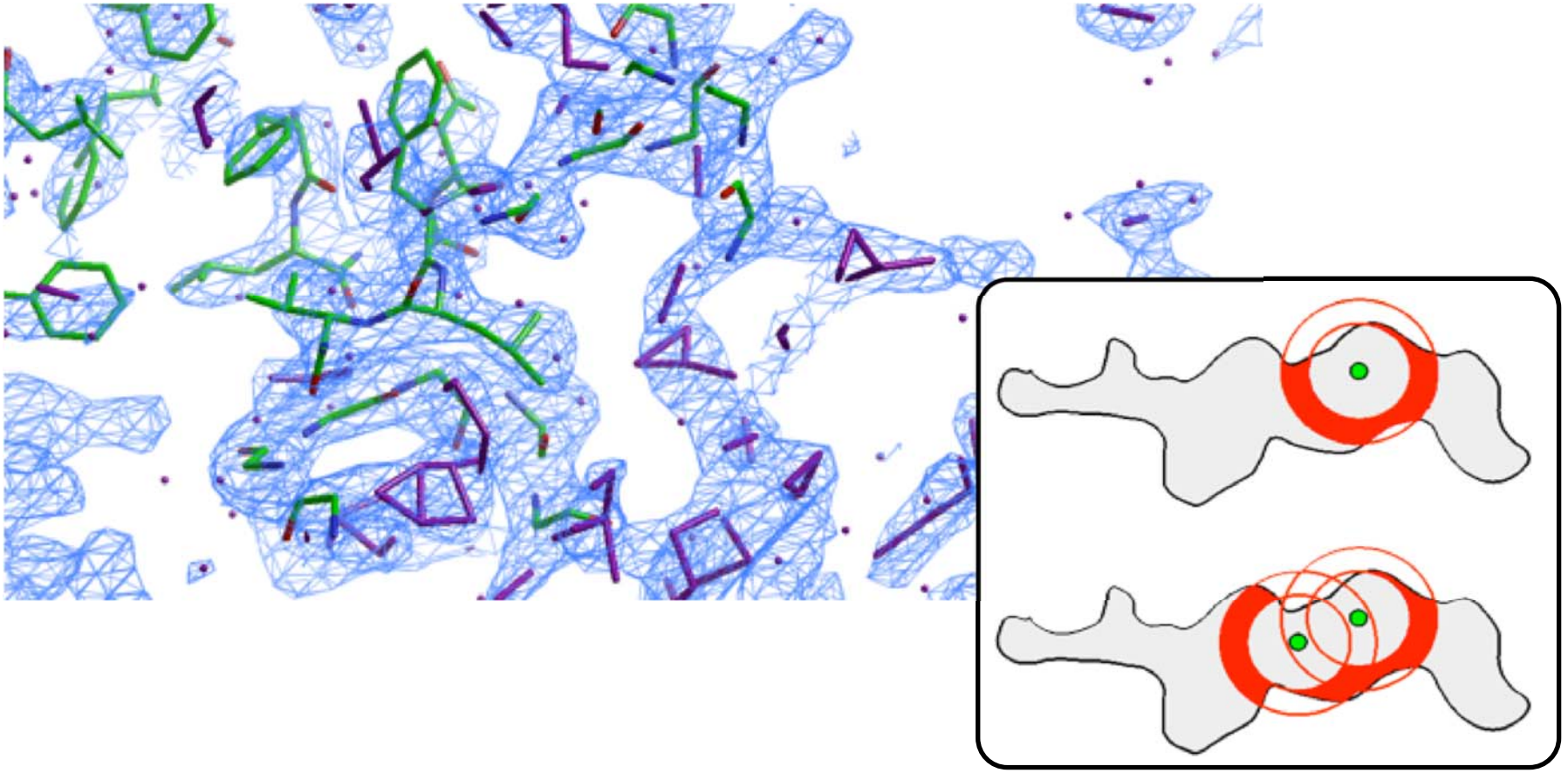




Restrict possible main-chain conformations

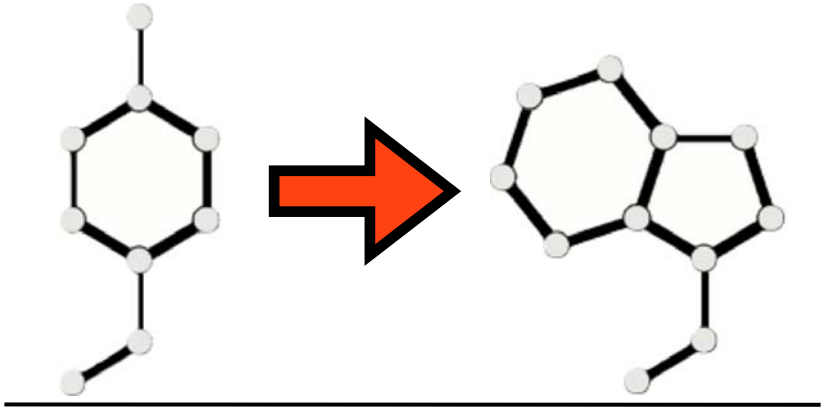
Concept #2: The Hybrid Model

- **Partial model** is used together with a **free-atom** model
- Chemically assigned parts provide **restraints** for refinement
- The **hybrid model** is converging to the **final model**



Concept #3: Iterative Update

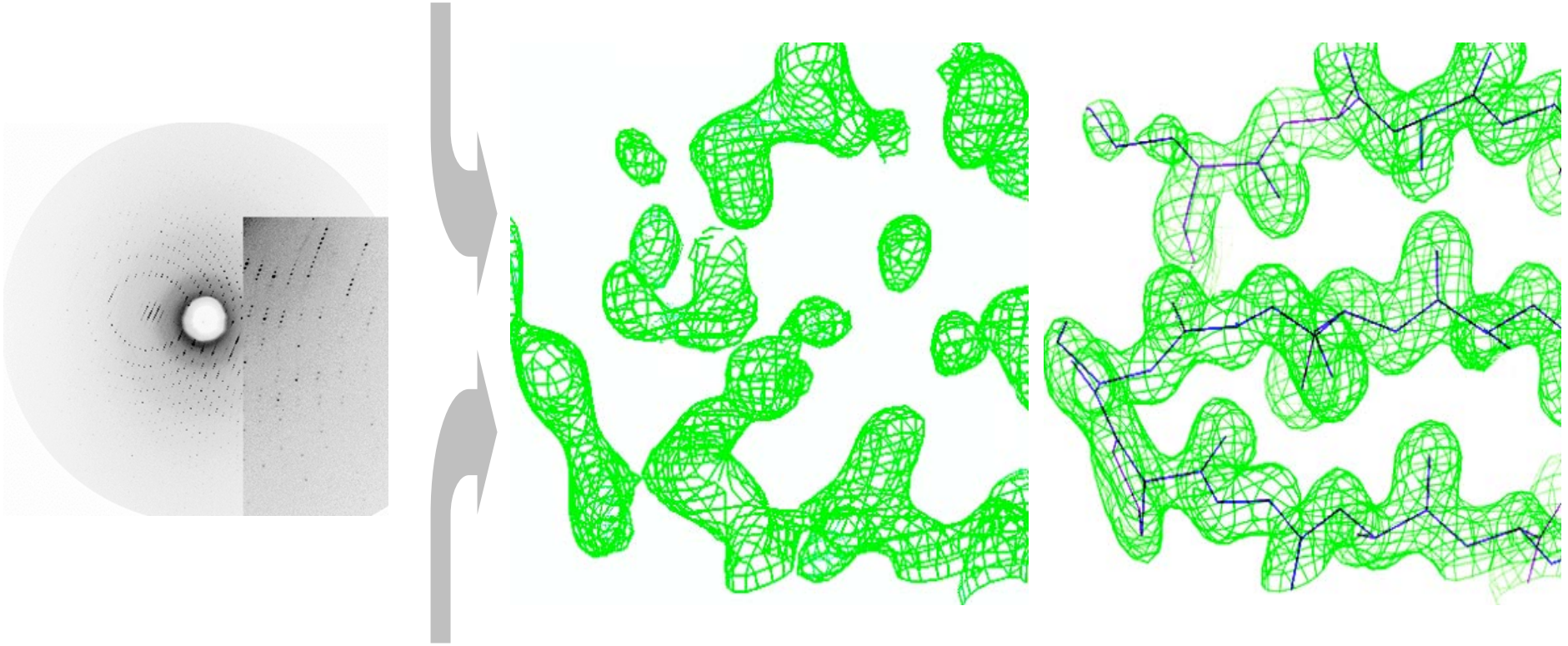
- **Iterative update** of parts of the model based on density and recognised patterns
- **Restraints** are re-assigned accordingly



More About the Iterations

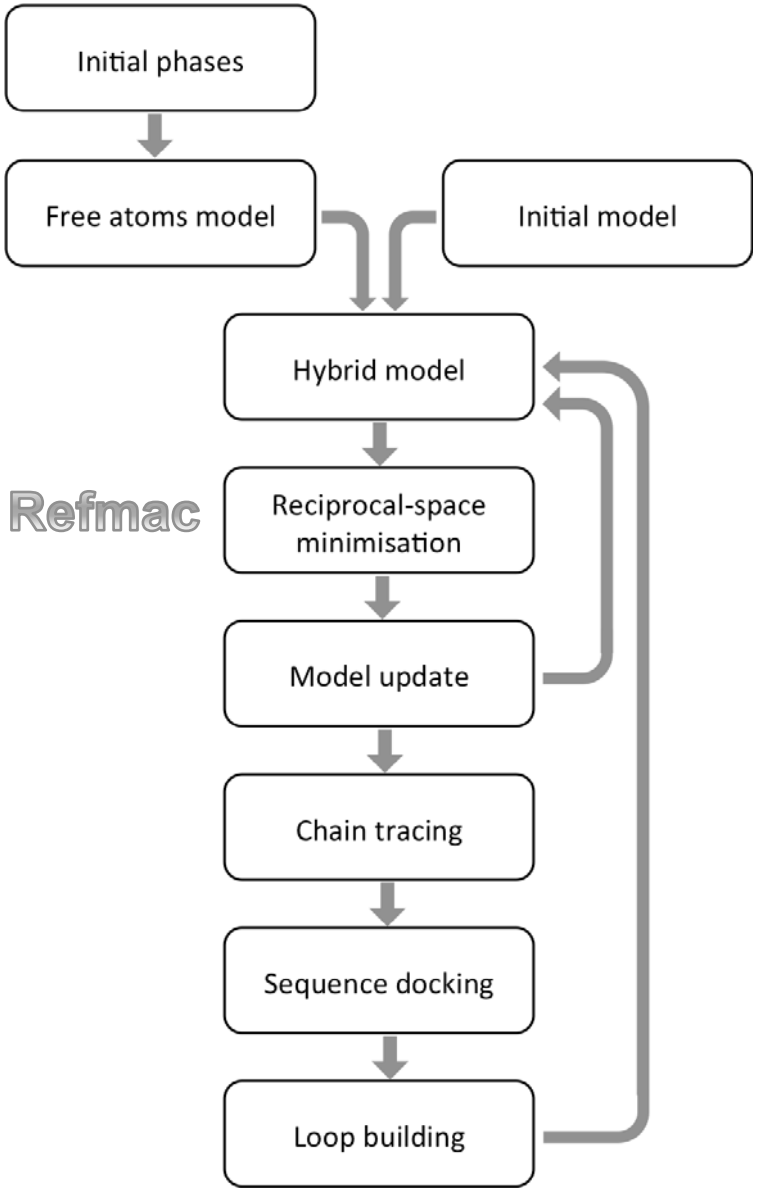
Experimental Phasing (MIR/MAD)

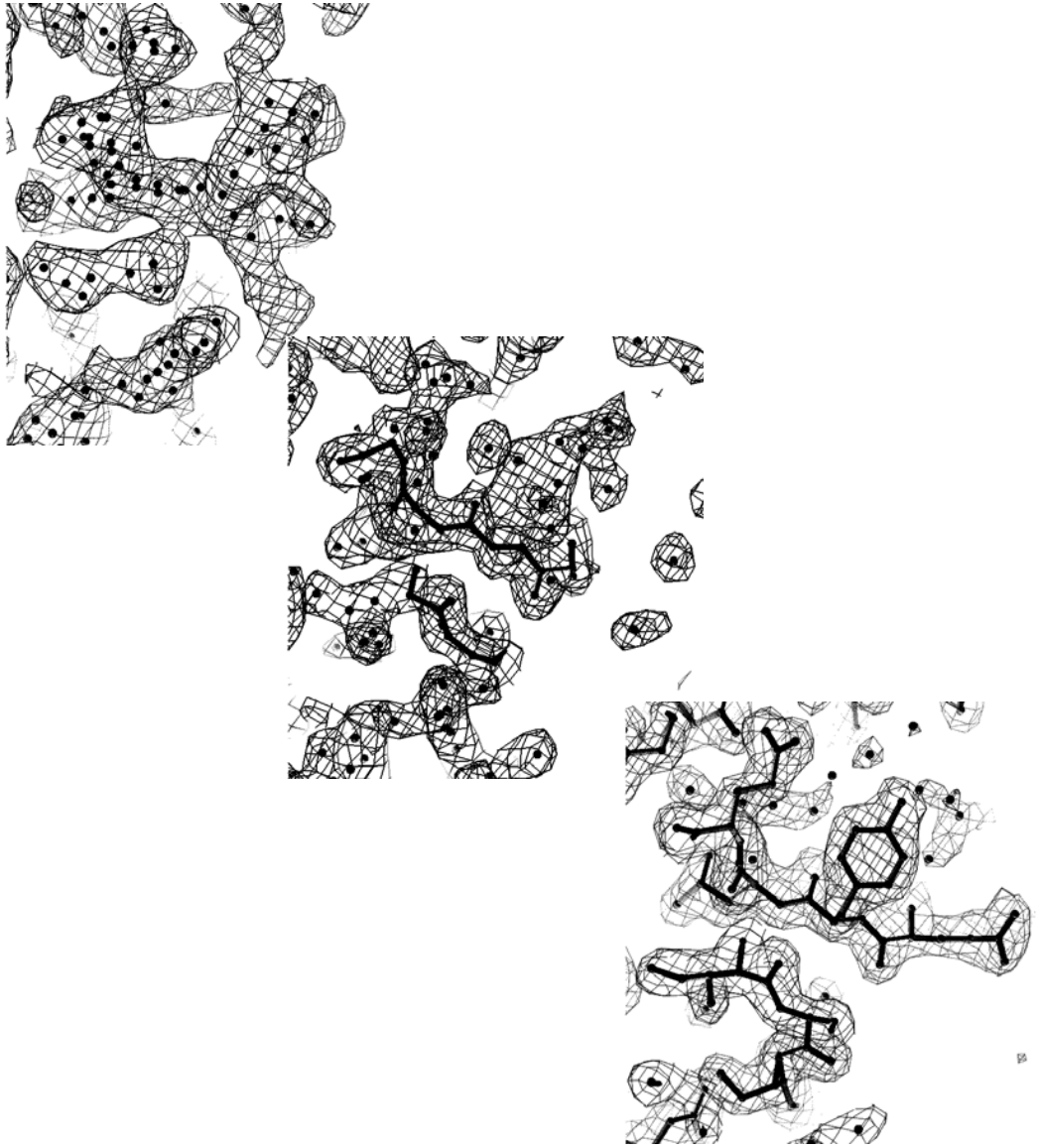
Different maps = different models



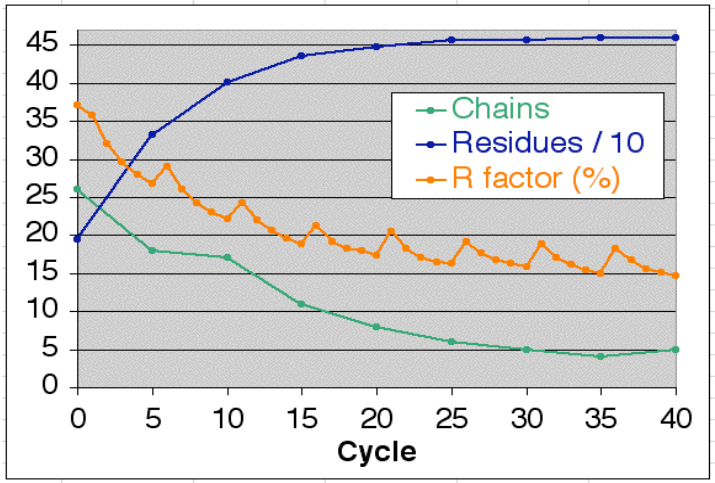
Homologous Model (MR)

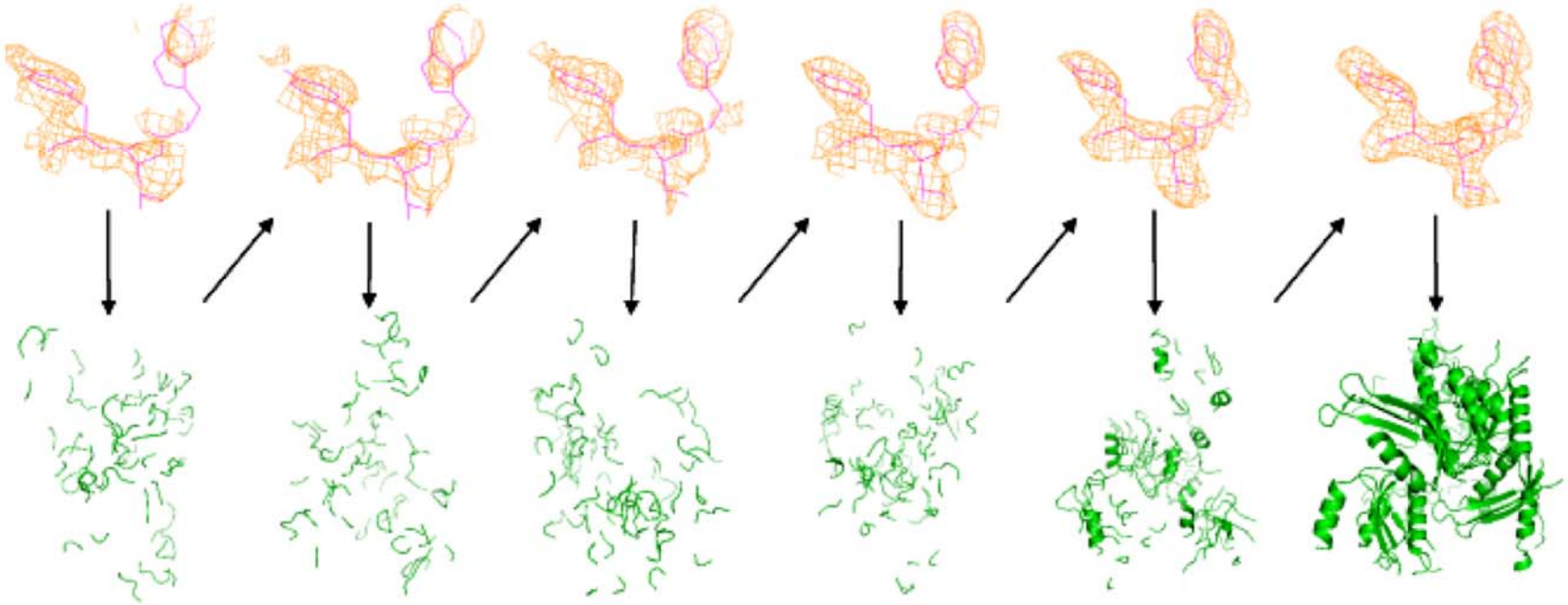
Iterative Protein Building in ARP/wARP





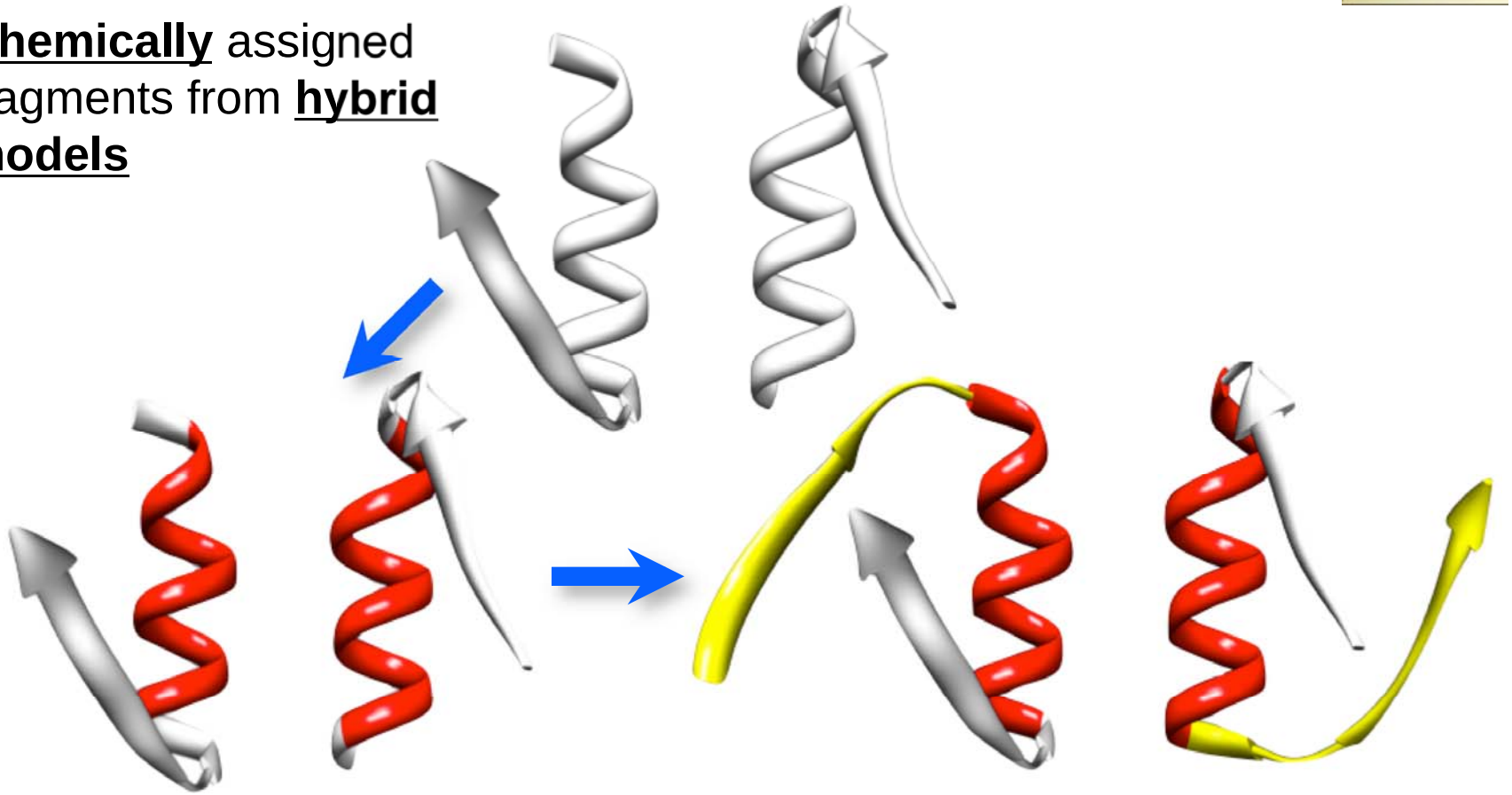
Iterative Protein Building in ARP/wARP





Auto-NCS Detection and Use

Chemically assigned fragments from **hybrid models**



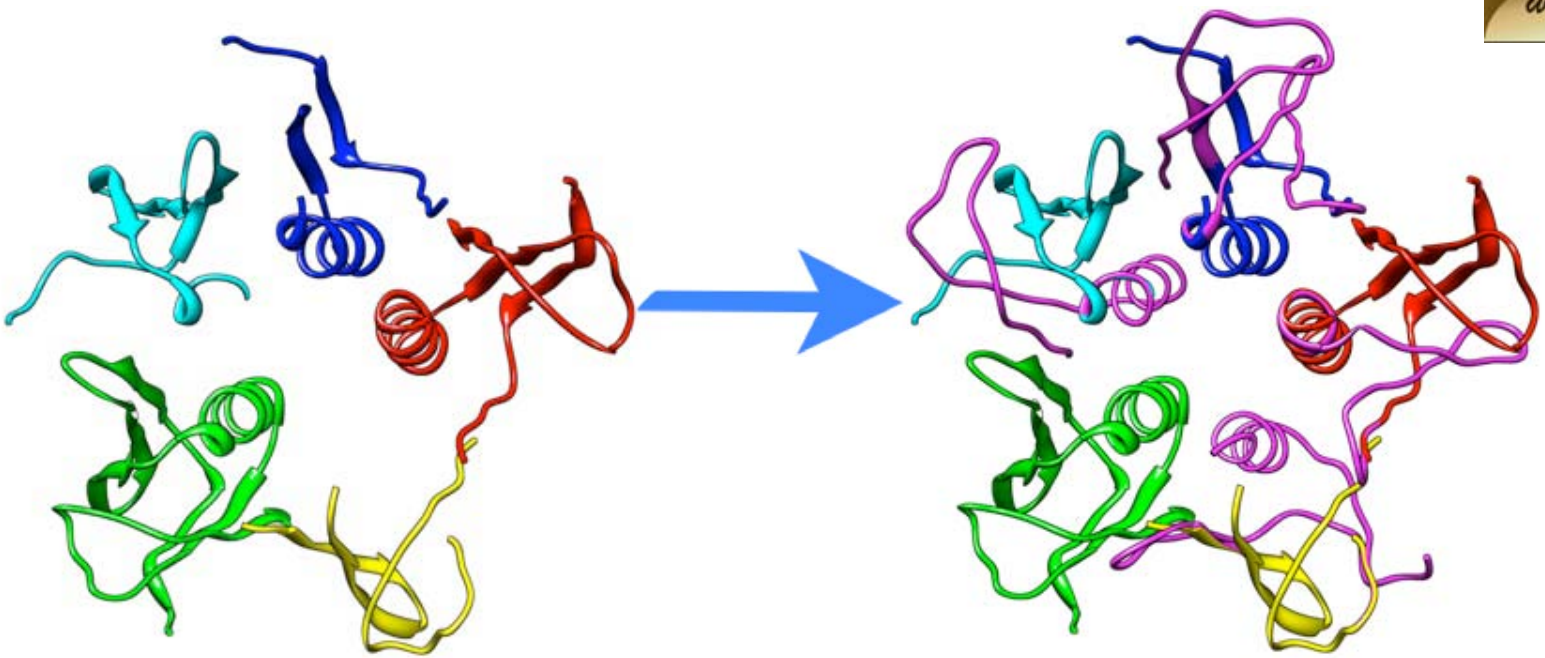
Superposition and **clustering** to identify NCS matches

Extended matches are **transformed** to related NCS operators

Seeds for further chain tracing

Restraints for refmac

Auto-NCS Detection and Use



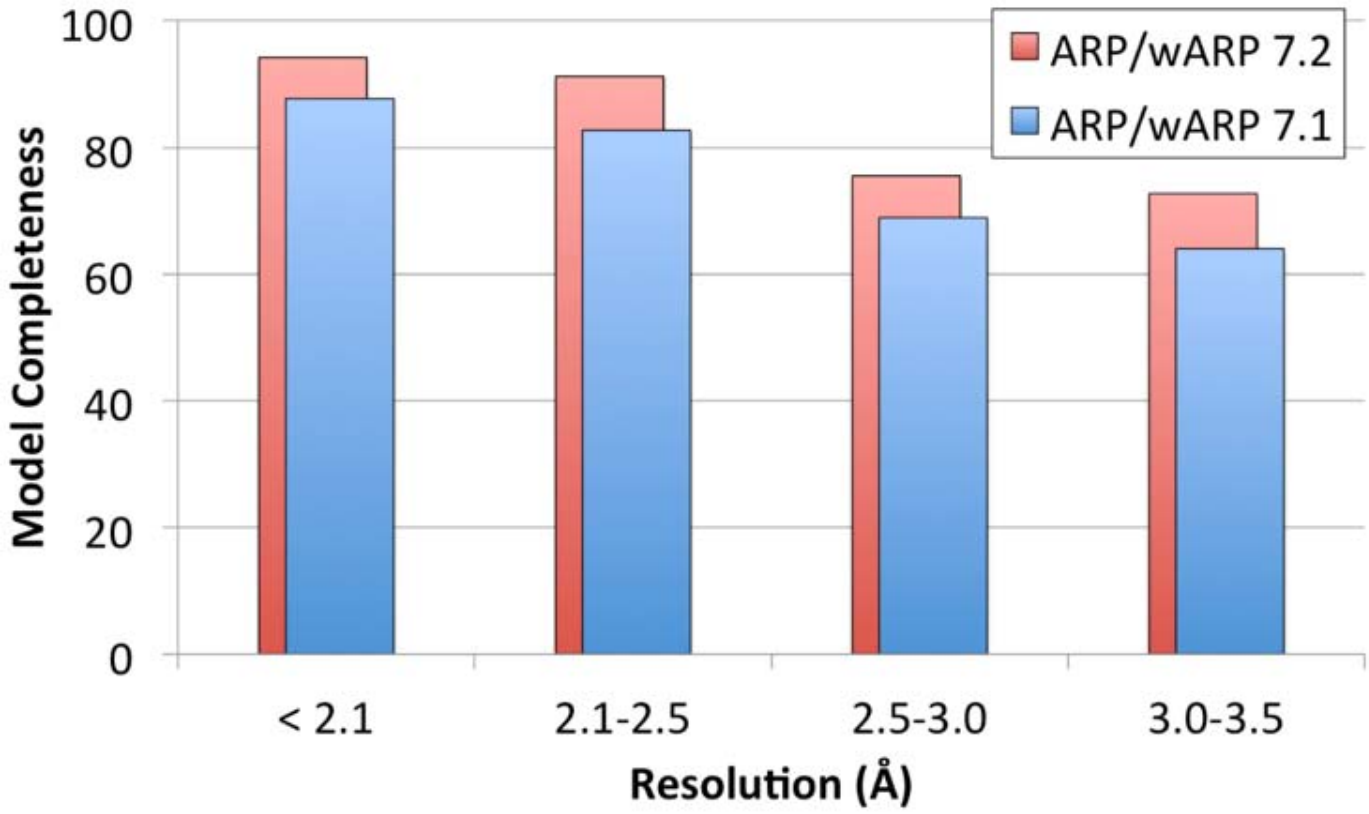
13 protein test structures with resolution 2.1 to 3.2 Å, NCS order 2 - 10

	Built Residues	Residues / Fragment	Sequence Coverage
Top Results	+15.3%	+ 220%	+ 56%
Average in 7.2	+ 4.5%	+ 25%	+ 10%

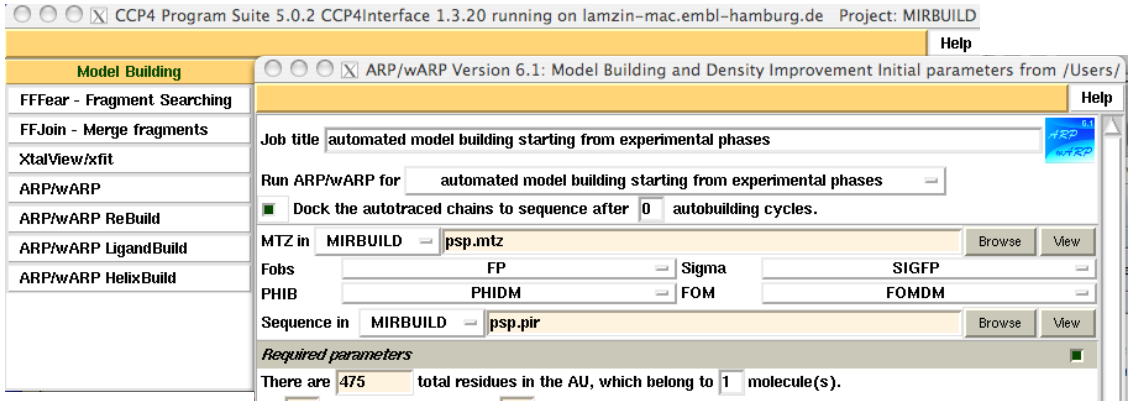
Dependence on the Resolution of the Data

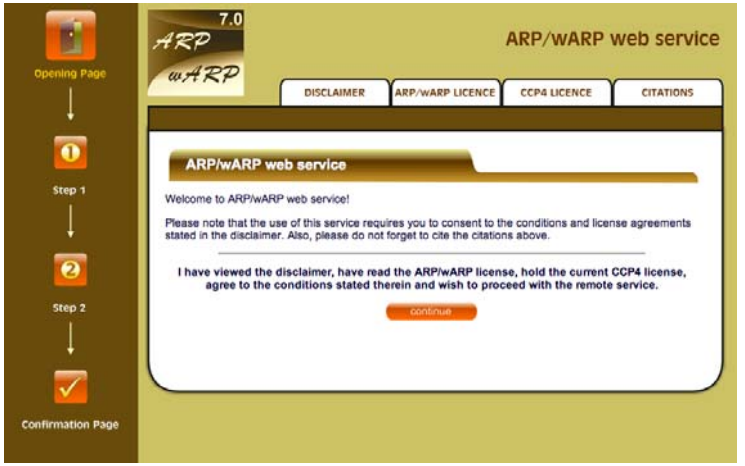
Resolution	Estimated fraction of automatically built protein structure (last updated on Aug 15, 2011)
2.1 Å and higher	over 90%
2.1 - 2.5 Å	75 - 90%
2.5 - 3.0 Å	60 - 75%
3.0 – 3.5 Å	up to 60%

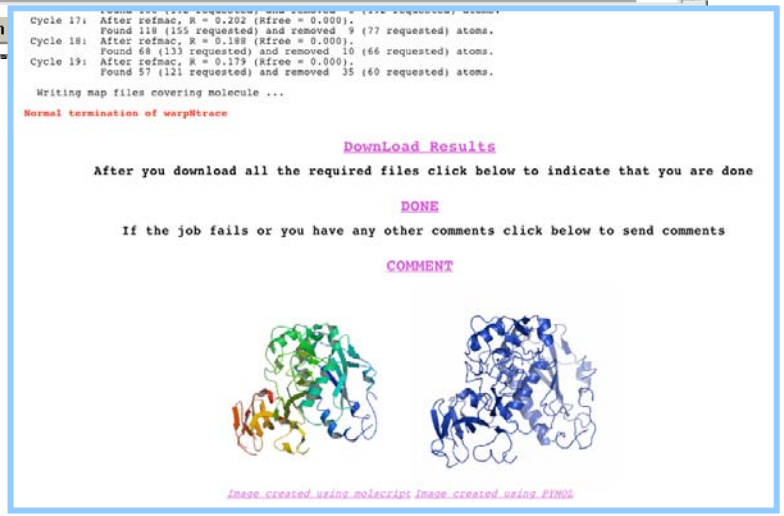
Dependence on the Resolution of the Data



Remote Computational Services



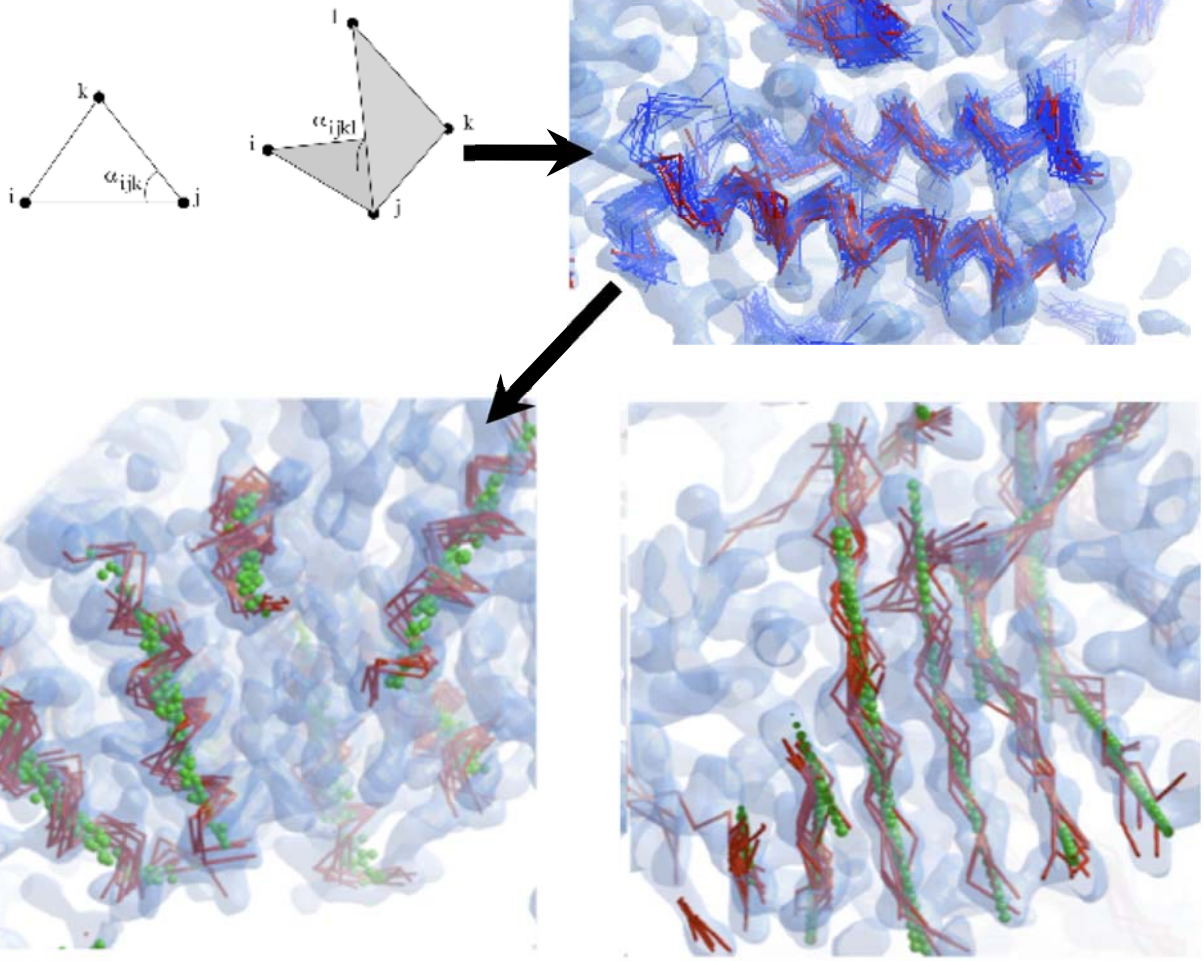


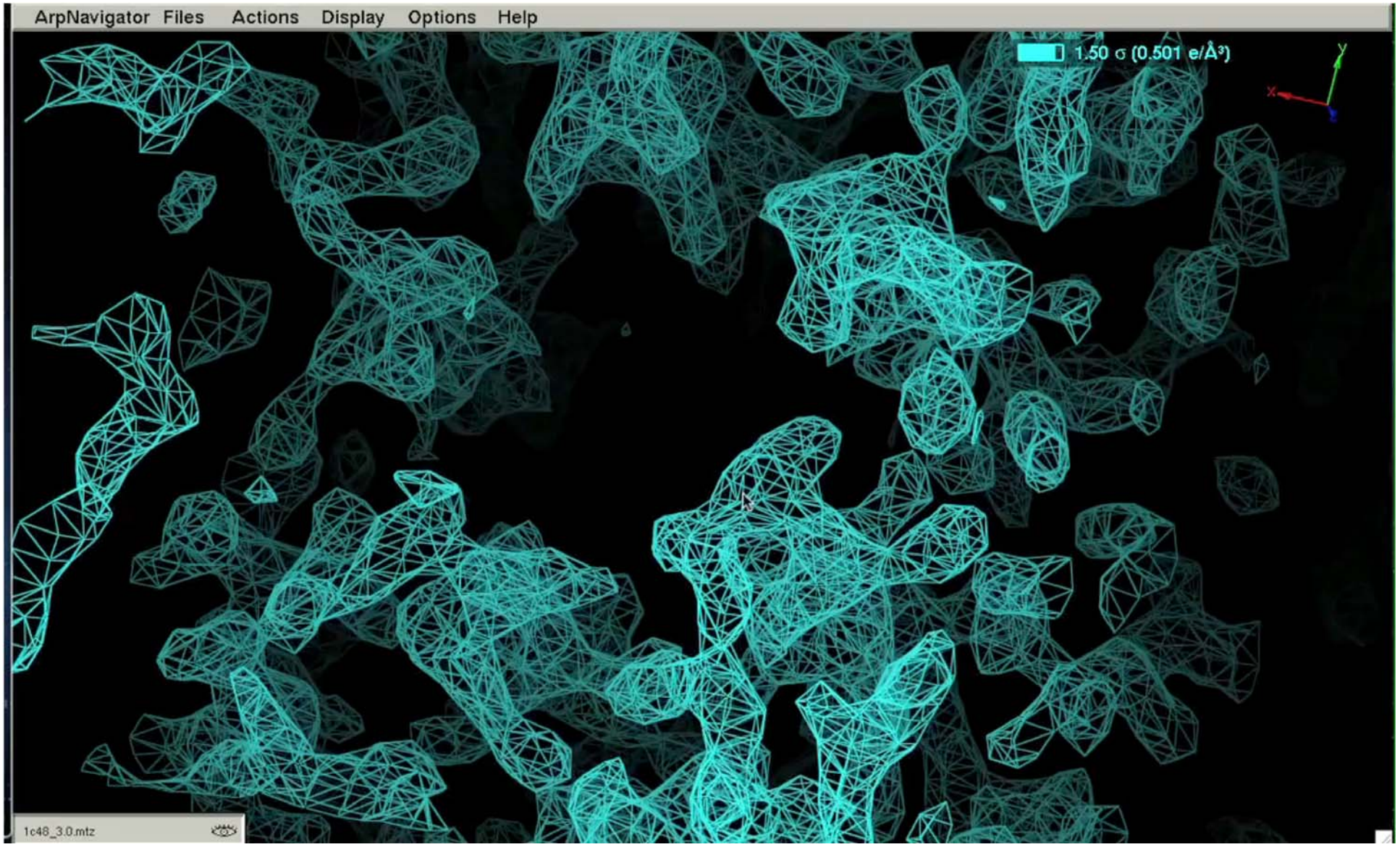


βαλβεσ

Modelling Secondary Structure

- **Short helix/strand fragments** (3 to 5 Cα candidates) are built.
- Longer traces are formed or which the **best are kept** (in red)
- Traces are **clustered**
- Assemblies are **averaged**





Helices for a 350-residue (3.0 Å) protein can be built in under 5 seconds on a modern MacBookPro

The people

Developers

EMBL Hamburg: Ciaran Carolan, Saul Hazledine, Philipp Heuser, Tim Wiegels, Victor Lamzin

NKI Amsterdam: Krista Joosten, Tassos Perrakis

Collaborators

Santosh Panjikar, Garib Murshudov's group, Raj Pannu's group, the CCP4 team

Former members

Serge Cohen, Helene Doerksen, Guillaume Evrard, Francisco Fernandez, Marouane Jelloul, Johan Hattne, Matheos Kakaris, Olga Kirillova, Gerrit Langer, Wijnand Mooij, Richard Morris, Venkat Parthasarathy, Tilo Strutz, Diederick De Vries, Peter Zwart

FAQ: Where Can I Get This?

http://www.embl-hamburg.de/ARP