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SHELXE for Molecular Replacement

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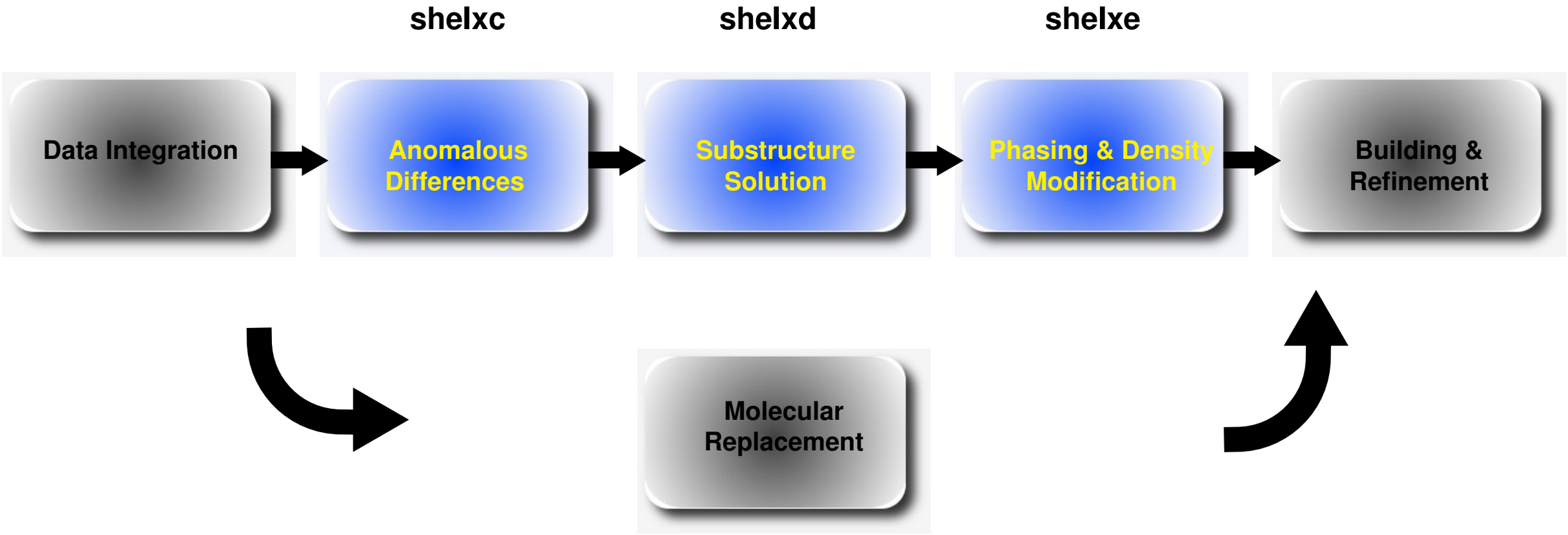
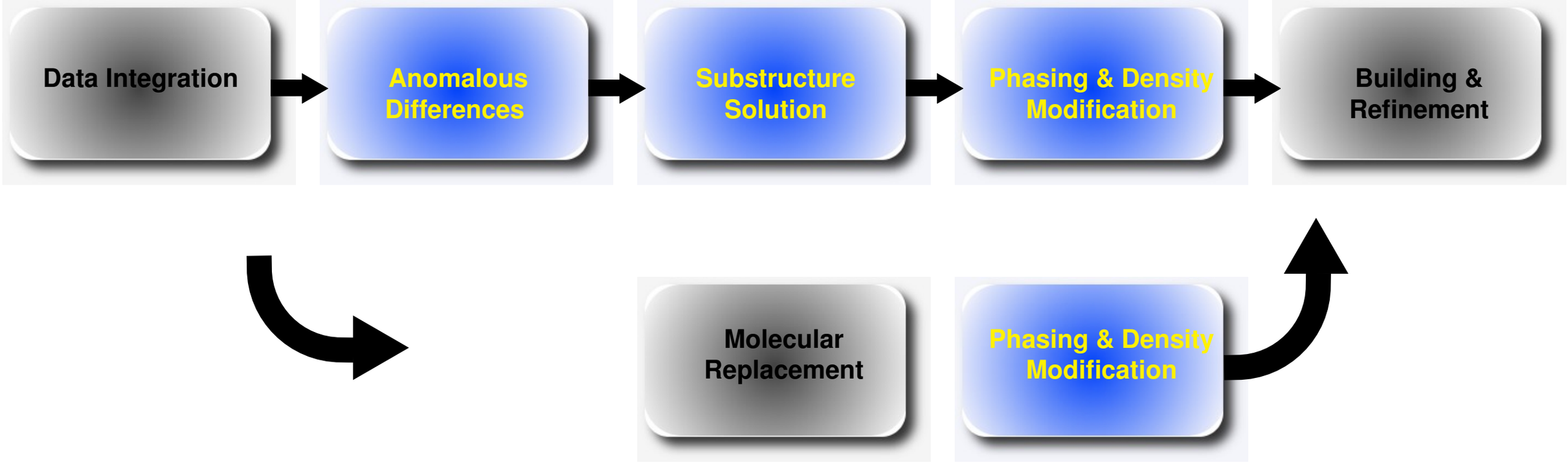
1 - Context within Structure Solution

SHELX C/D/E in Context with Structure Solution



Shelxe from Molecular Replacement?



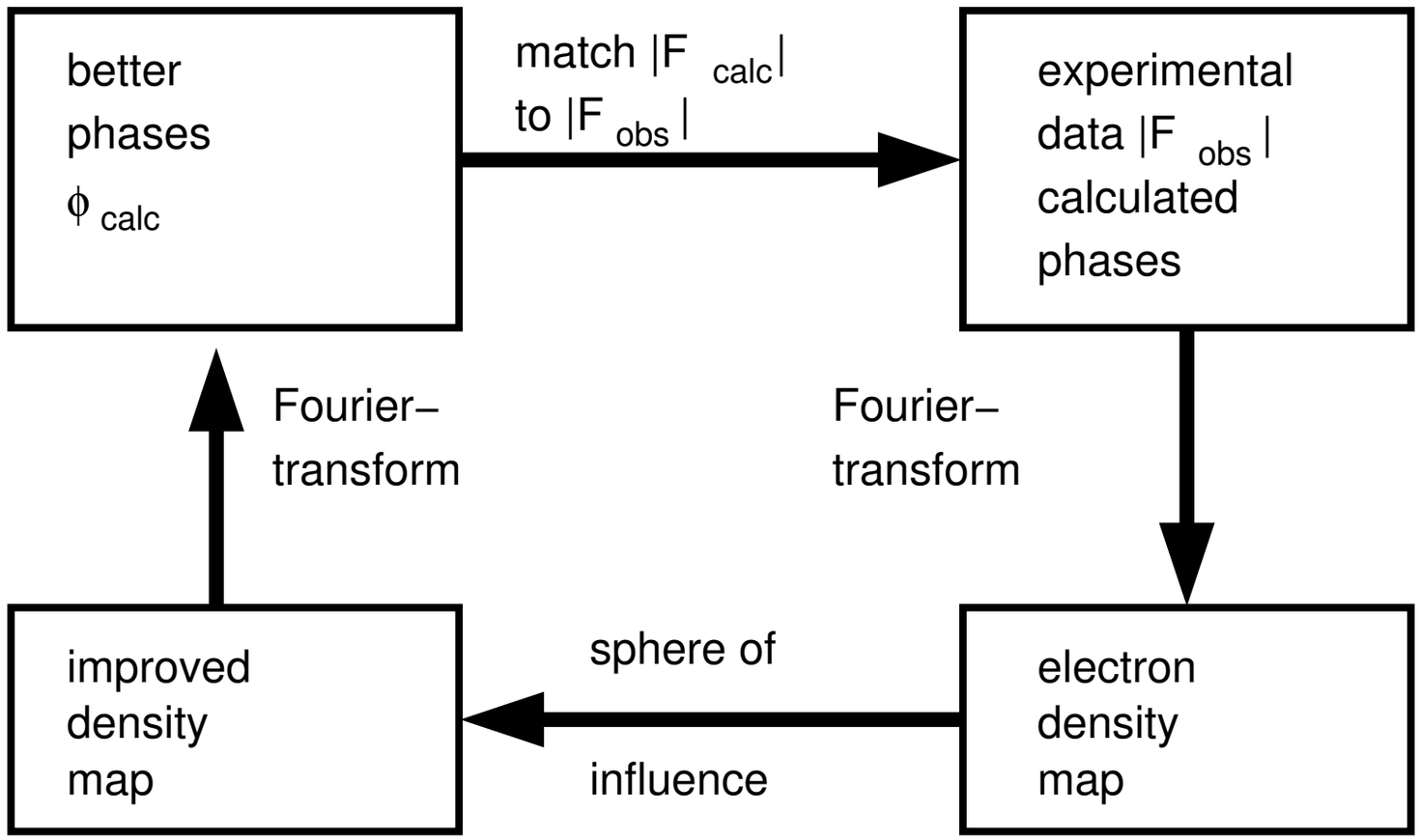
What can SHELXE offer for MR?

- The ability to expand from a very small starting fragment (as little as 3% of the final structure).
- Improvement of poor models that are basically correct but cannot be refined by conventional methods.
- Removal of model bias.
- The use of extra phase information from weak anomalous scattering (MRSAD).
- A reliable indication as to whether the solution is correct (CC>25% for native data better than 2.5Å resolution.

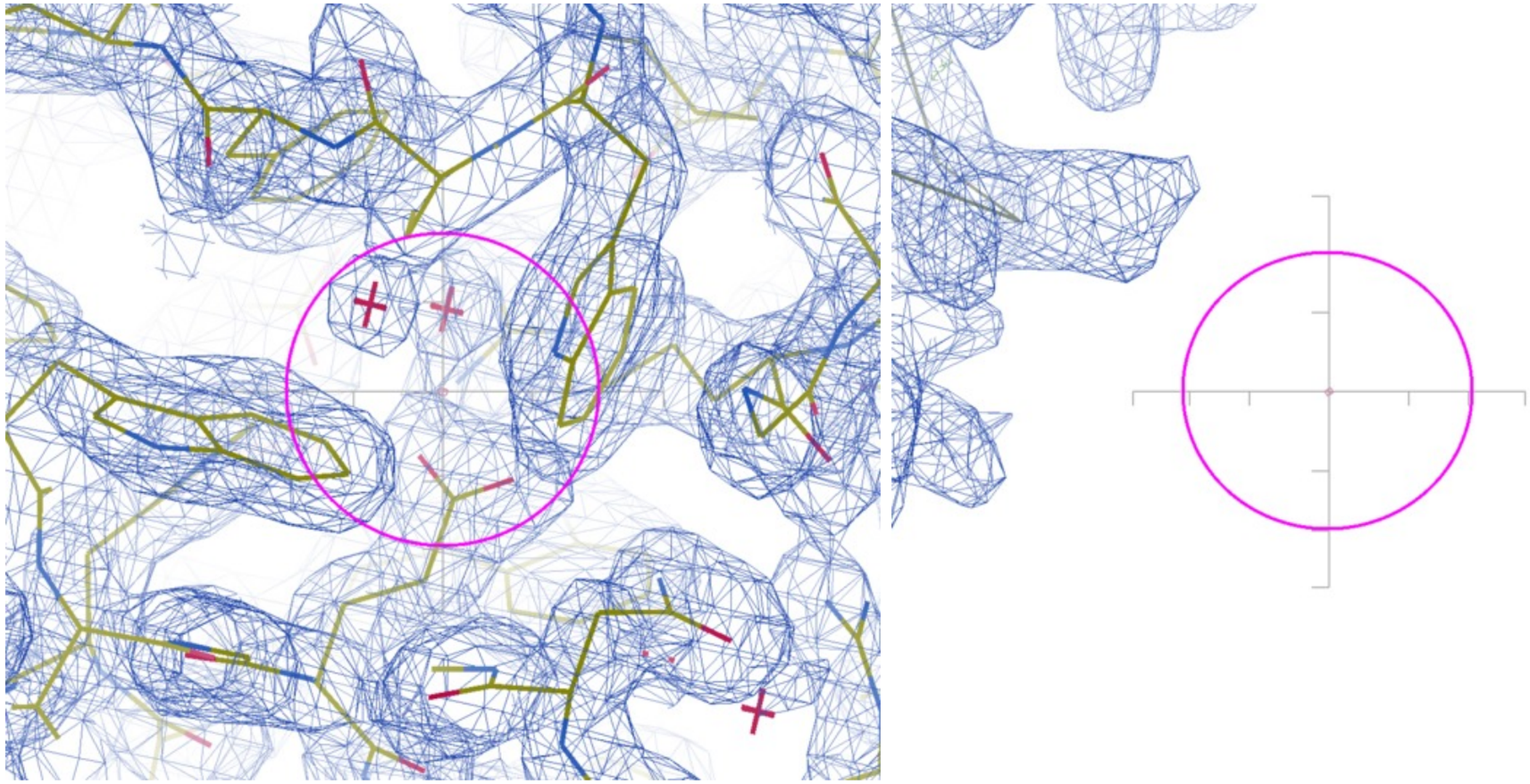
What is Shelxe?

- Shelxe = Density Modification program

- Originally: Fast and Reliable test for substructure solution (shelxd)



Shelxe: Sphere of Influence



- Any point in map: Calculate variance on 2.42Å sphere
- 2.42Å = 1,3-distance in proteins
- Large variance: protein, enhance
- Small variance: solvent, flatten

Starting phases from MR

- Final model = best phases

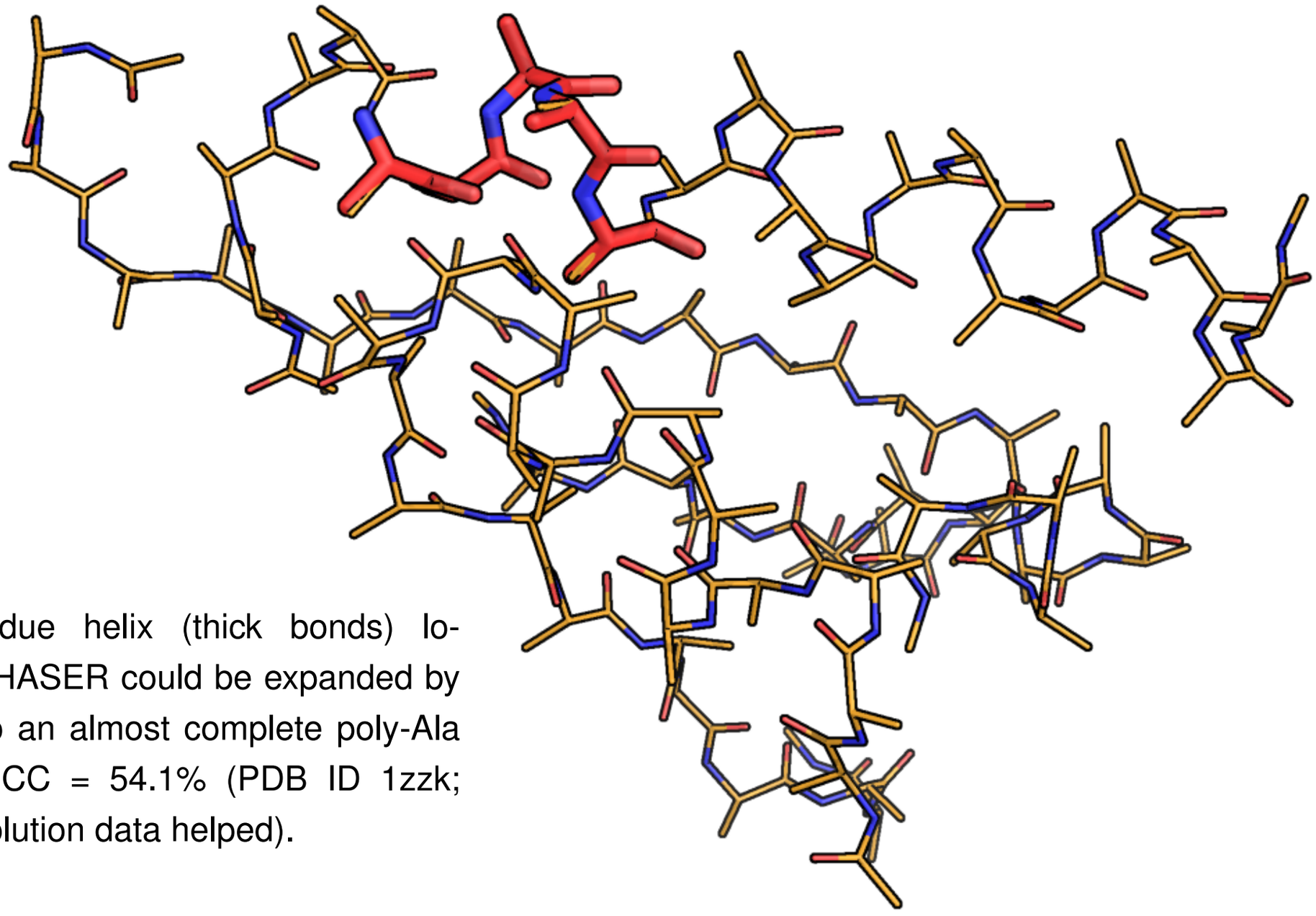
- Shelxe = combine experimental phasing with model building

- Shelxe: used to very poor starting phases

What happens when using Molecular Replacement solution as initial source of phases?

2 - Examples

Example: Poor starting phases for hnRNP K (89a.a.)



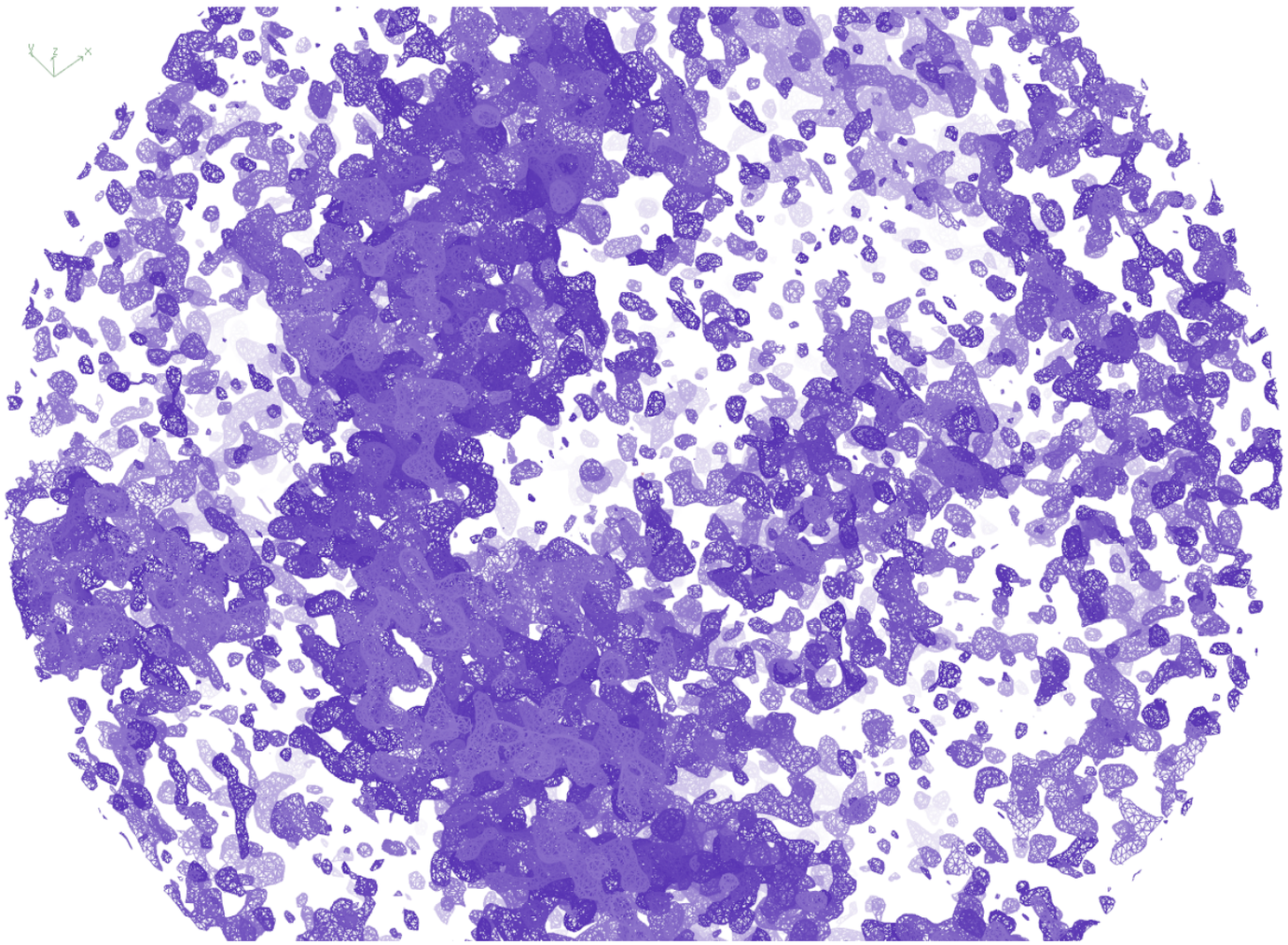
The 5-residue helix (thick bonds) located by PHASER could be expanded by SHELXE to an almost complete poly-Ala trace with CC = 54.1% (PDB ID 1zzk; atomic resolution data helped).

Prp8 – Improvement of a model that would not refine

Yeast Prp8 (residues 885–2413)	
Data set resolution:	1.9 Å
Space group:	C2221
Secondary structure:	α–helices and β–sheets
Residues/ASU:	1529
SHELXE version:	2012-1

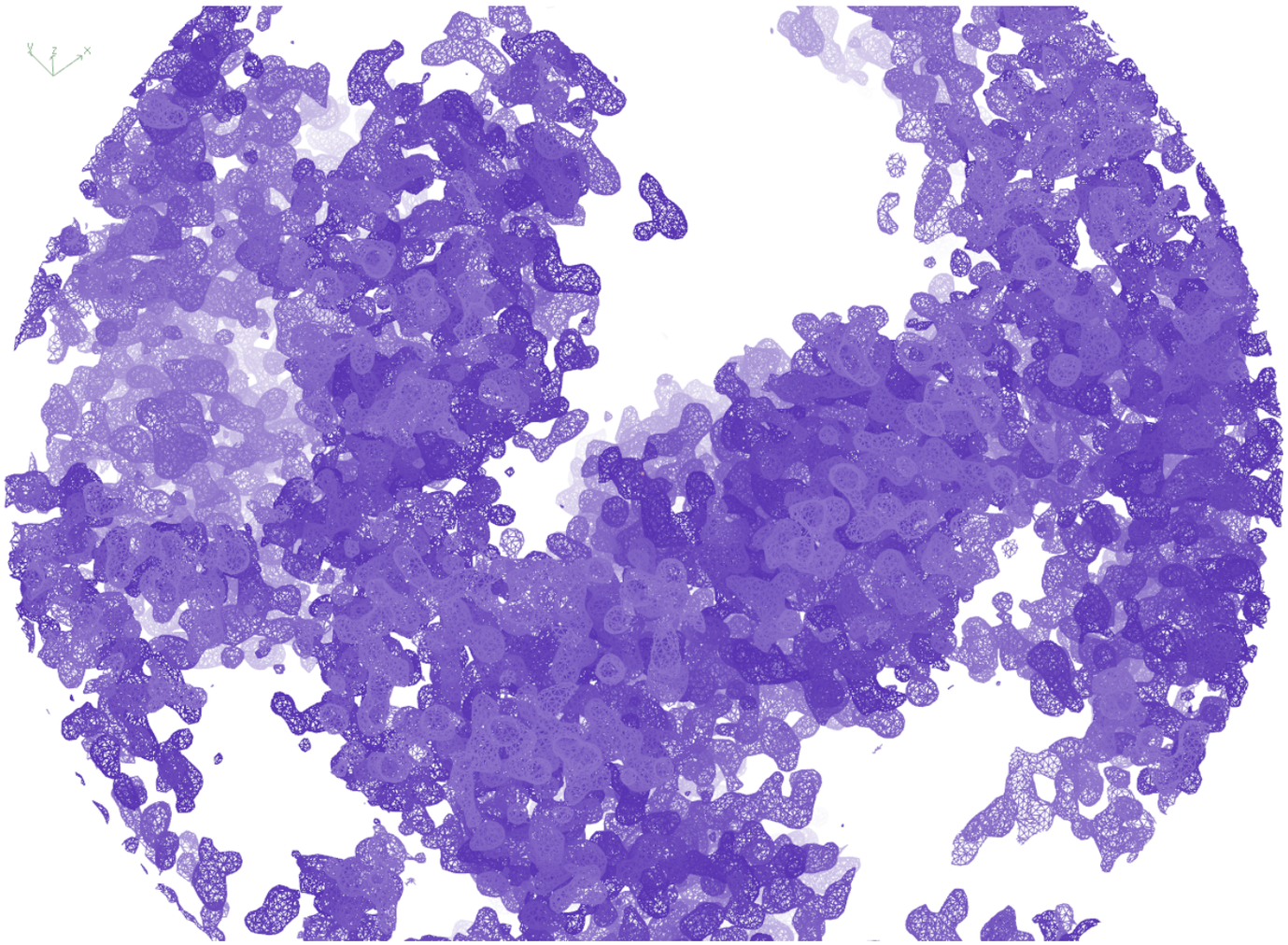
Galej, Oubridge, Newman & Nagai, Nature (2013) 493, 638–644 (PDB ID 4I43)

Prp8 – Improvement of a model that would not refine



Map from unrefinable structure

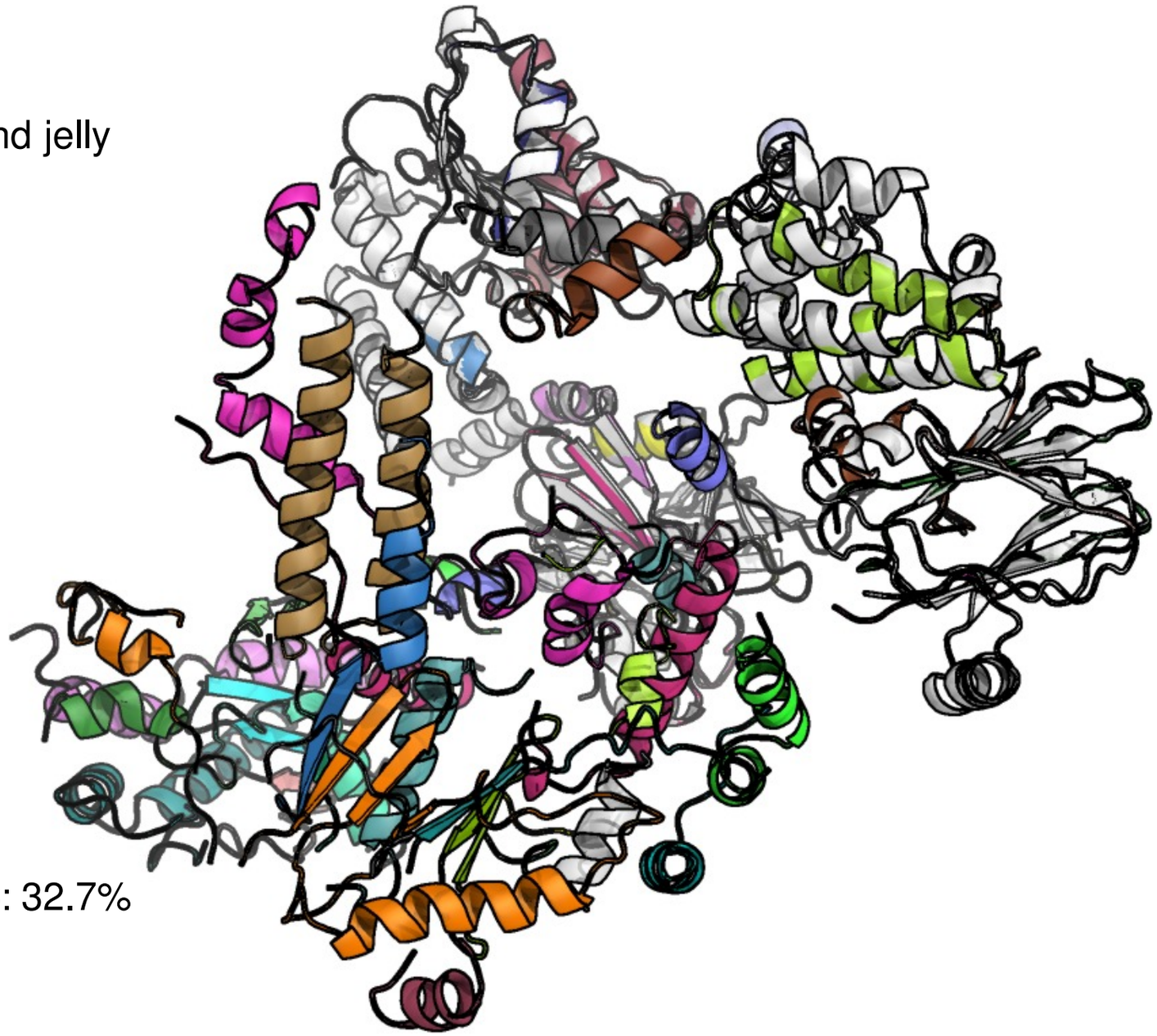
Prp8 – Improvement of a model that would not refine



Map after SHELXE

Prp8 – Improvement of a model that would not refine

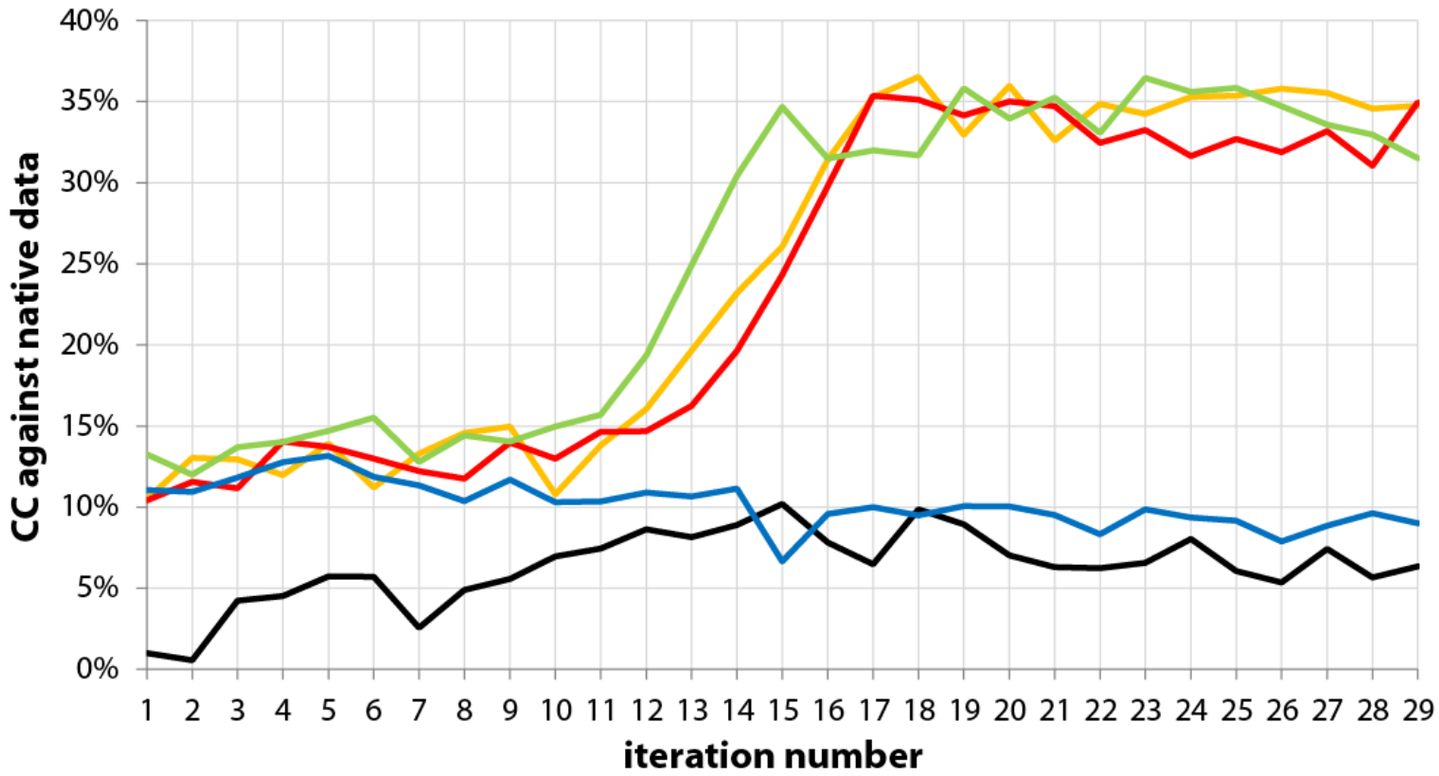
After MR with MolRep (contrast 17.27) and jelly body refinement in REFMAC:
Rwork: 45.3 %
Rfree 48.6 %
695 residues



SHELXE 1222 residues (out of 1529), CC: 32.7%
(final: Rwork: 19.5%, Rfree 24.9%)

When is a structure solved?

Instead of the CC for the trace against the native data gradually improving, it meanders randomly at a about 10% and then suddenly, in the course of four or five iterations, jumps to a value well above 25%, indicating a solved structure. This strongly resembles the behavior of small-molecule direct methods, with the important differences that they involve data to about 1Å or better, and start from random phases.



3 - SHELXE fragment extension

SHELXE fragment extension

- **Input**: PDB–file from molecular replacement, e.g. `phaser_output.pdb`
- rename to `phaser_output.pda`
- **Input**: (native) data file, *hkl*-format (use `mtz2hkl`)
- `shelxe phaser_output.pda -a30 -s0.5 -y2.0 -q -e1 -t10`
- Output: `phaser_output.pdb`, `phaser_output.phs` (map file for `Coot`)

If this still doesn't produce a structure that can be refined, it may be necessary to improve the MR solution with `ARCIMBOLDO_Shredder` before feeding it to `SHELXE`.

Fine–tuning the fragment extension

```
shelxe name.pda -a30 -s0.5 -y2.0 -q -e1 -t10
```

- A large number of tracing cycles may be needed (`-a30`)!
- `-s0.5` sets the solvent content (default 0.45).
- `-y2.0` sets the resolution up to which phases from the MR solution should be used. Experience suggests that -y2.0 is a good choice.
- `-q` requests a special α–helix search.
- `-e1` requests a “free lunch” extension of the observed data to 1.0Å
- `-t10` increases the search time by a factor of 10. This is slow, but often necessary.

The SHELXE autotracing algorithm

The poly–Ala chain tracing in SHELXE (Acta Cryst. D66 (2010) 479-485) is primarily designed for iterative phase improvement starting from very poor phases. The tracing proceeds as follows:

1. Find potential α–helices in the density and try to extend them at both ends. Then find other potential tripeptides and try to extend them at both ends in the same way

2. Tidy up and splice the traces as required, applying any necessary symmetry operations.

3. Use the traced residues to estimate phases and combine these with the initial phase information using σ_A weights, then restart density modification. The refinement of one B-value per residue provides a further opportunity to suppress wrongly traced residues.

4 - Uses and Limitations of SHELXE

Expansion from small fragments using SHELXE makes a major contribution to structure solution by both AMPLE and ACIMBOLDO. In general both require data to 2.1Å resolution for this, as opposed to 2.4Å when SAD phase information is input to SHELXE and 3.0Å when good MAD data can be used.

However SHELXE is also sometimes successful at lower resolution than this, especially when the solvent content is high. Predominantly β–sheet structures tend to require higher resolution data. But if SHELXE fails to expand a MR solution, it does not necessarily mean that it is wrong!

A highly parallel version is in preparation, it may be a little faster.

5 - Downloads & Acknowledgement

SHELX is available free for academic use via the SHELX homepage

see http://shelx.uni-goettingen.de/SHELX/tutorials.php

Extensive documentation and many links to useful programs may also be found there. There are plans to include SHELXC/D/E in future CCP4 distributions.

Acknowledgement George M. Sheldrick

References:

SHELXE for MR Thorn & Sheldrick, Acta Cryst. D69 (2013) 2251–2256

SHELX C/D/E Sheldrick, Acta Cryst. D66 (2010) 479–485