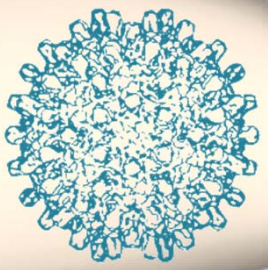
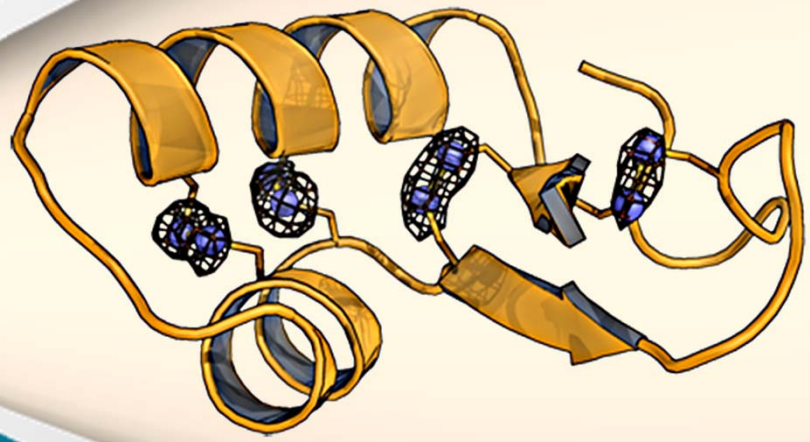
# CCP4 Diamond 2014

# SHELXC/D/E





**Andrea Thorn**

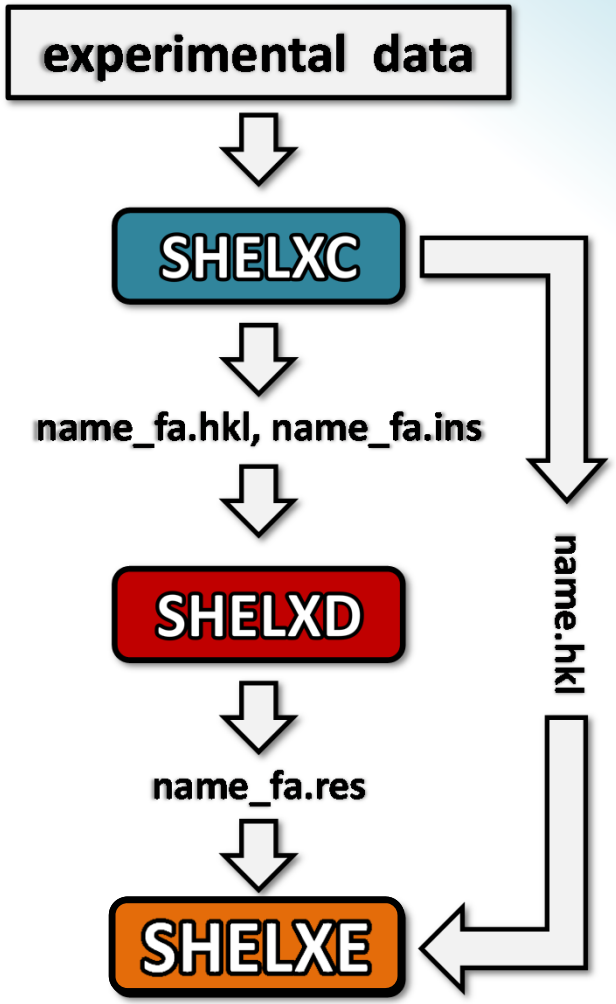
# SHELXC/D/E workflow

**SHELXC**: α calculation,
file preparation

**SHELXD**: *Marker atom search* =
substructure search

**SHELXE**: density modification

Maps and coordinate files are compatible with **COOT**




**SHELX citation: Sheldrick, Acta Cryst. (2008). A64, 112.**

**COOT: Emsley et al. (2010) Acta Crystallographica D66, 486.**

# What is experimental phasing?

Experimental phasing methods depend on intensity differences.

These differences are caused by a marker substructure of certain elements.

**MAD** and **SAD** exploit the anomalous signal from one or more data sets from the same crystal.

**SIR** (special case: **RIP**) and **MIR** utilizes several heavy-atom soaked derivative crystals. They have to be isomorphous to be utilized.

Theory

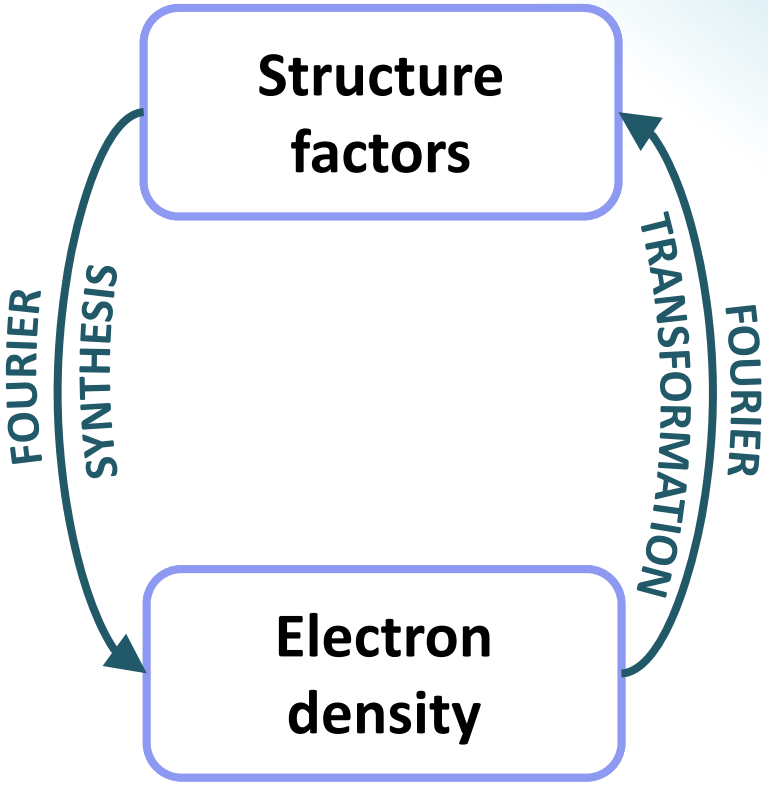
# STRUCTURE FACTORS

# Structure factors

For each reflection, there is a

**structure factor $F_{hkl}$**

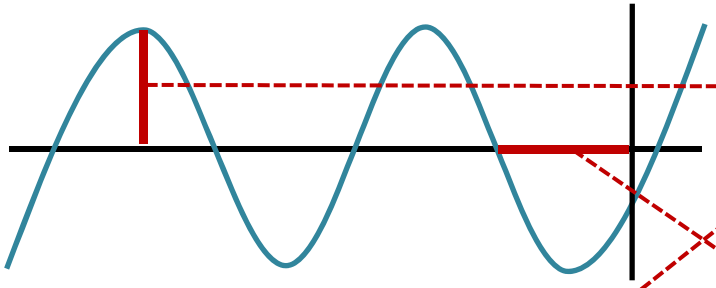
If we know the structure factors including their phases for all reflections, we can easily calculate the electron density map, and hence get the structure.



# Structure factors

**structure factor $F_{hkl}$**

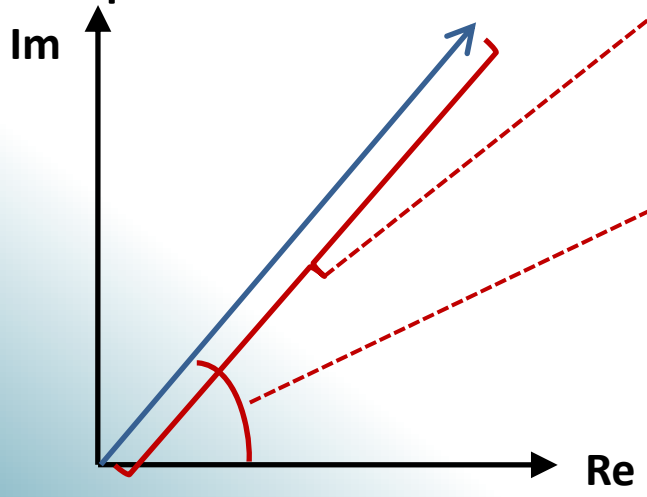
= a wave



**Amplitude = $|F_{hkl}|$**

$|F_{hkl}|^2 \sim I_{hkl}$ **Intensity ☑**

= a complex number




**Phase = $\phi_{hkl}$**

**cannot be measured... :-(**

# Structure factors

**Amplitude = $|F_{hkl}|$**

$|F_{hkl}|^2 \sim I_{hkl}$ **Intensity ☑**

**Phase = $\phi_{hkl}$**

**cannot be measured... :-(**

PHASE PROBLEM

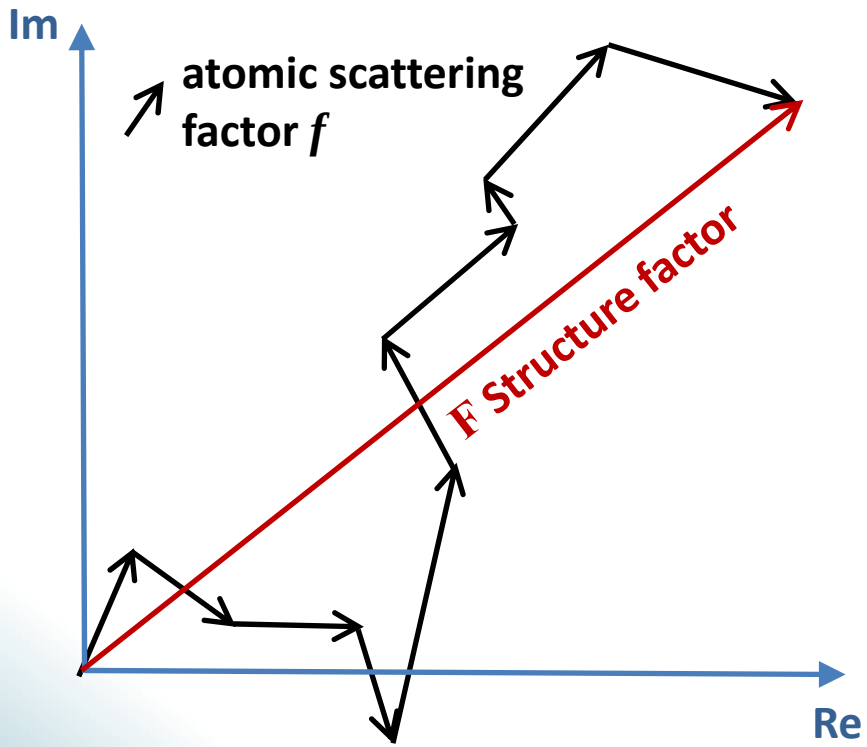
**The central problem of crystallography**

Theory

# ANOMALOUS SCATTERING

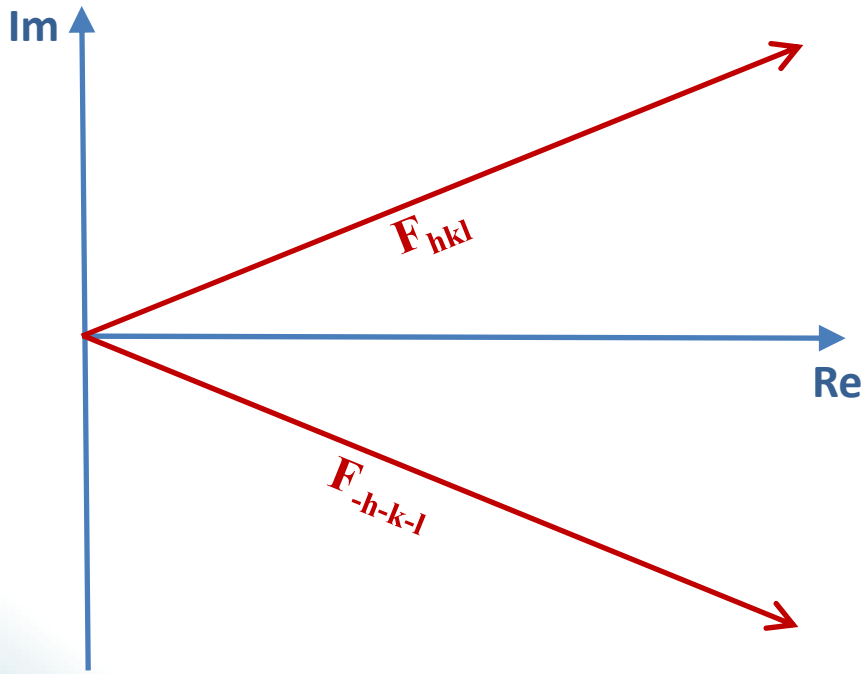
# The anomalous signal

Each structure factor is composed of contributions $f$ from each atom:



# The anomalous signal

Friedel's law: $|F_{hkl}| = |F_{-h-k-l}|$ $\phi_{hkl} = -\phi_{-h-k-l}$



# The anomalous signal

But in reality, there is **anomalous scattering** due to resonance with electronic transitions in the atom:

$$f = f_0 + f' + if''$$

**depends solely on resolution and element**

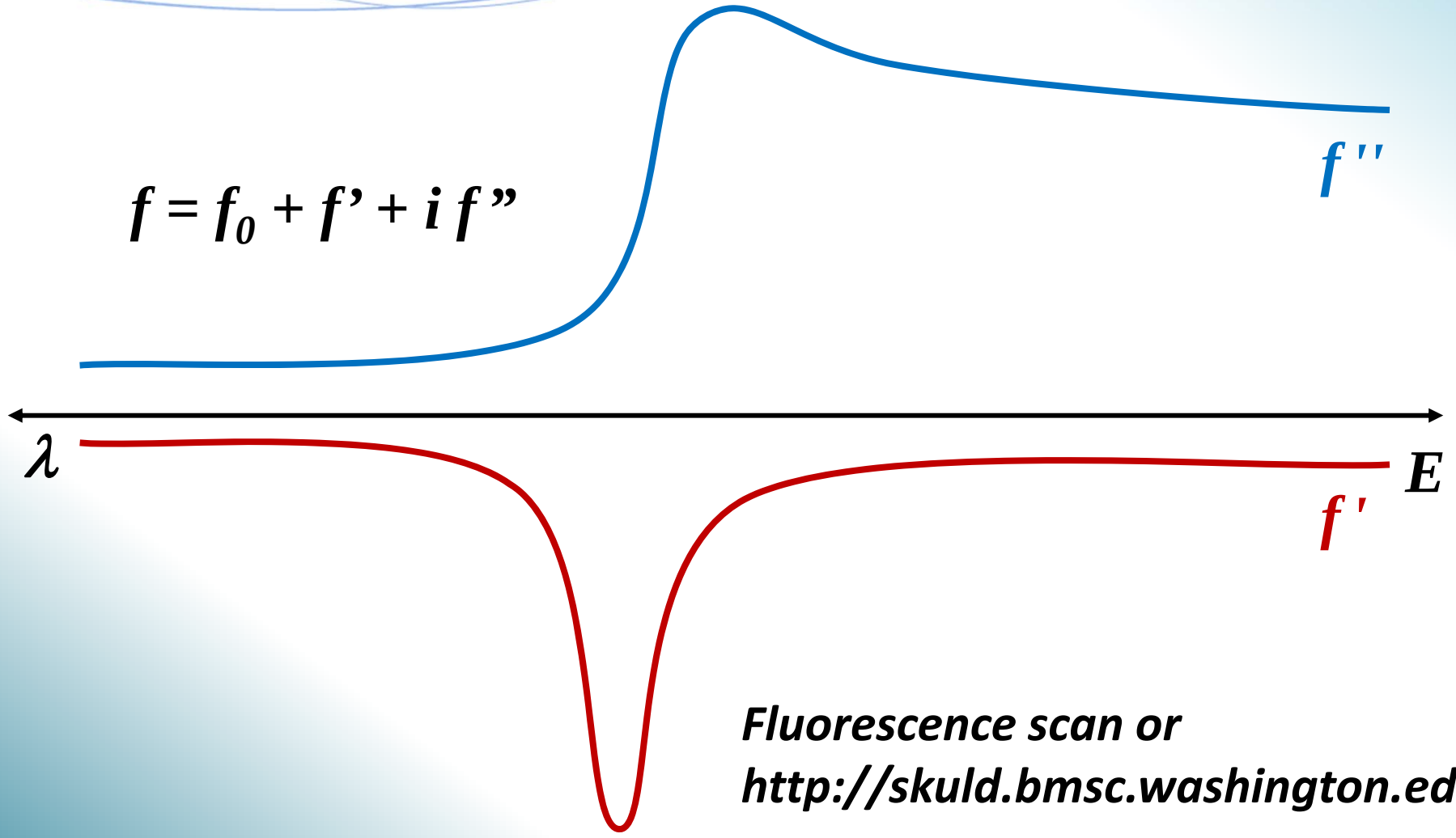
**real component**

**imaginary component**

*$f'$ and $f''$ are observed near absorption edges of the atom's element, and are $\lambda$-dependent*

# The anomalous signal

$f = f_0 + f' + i\,f''$



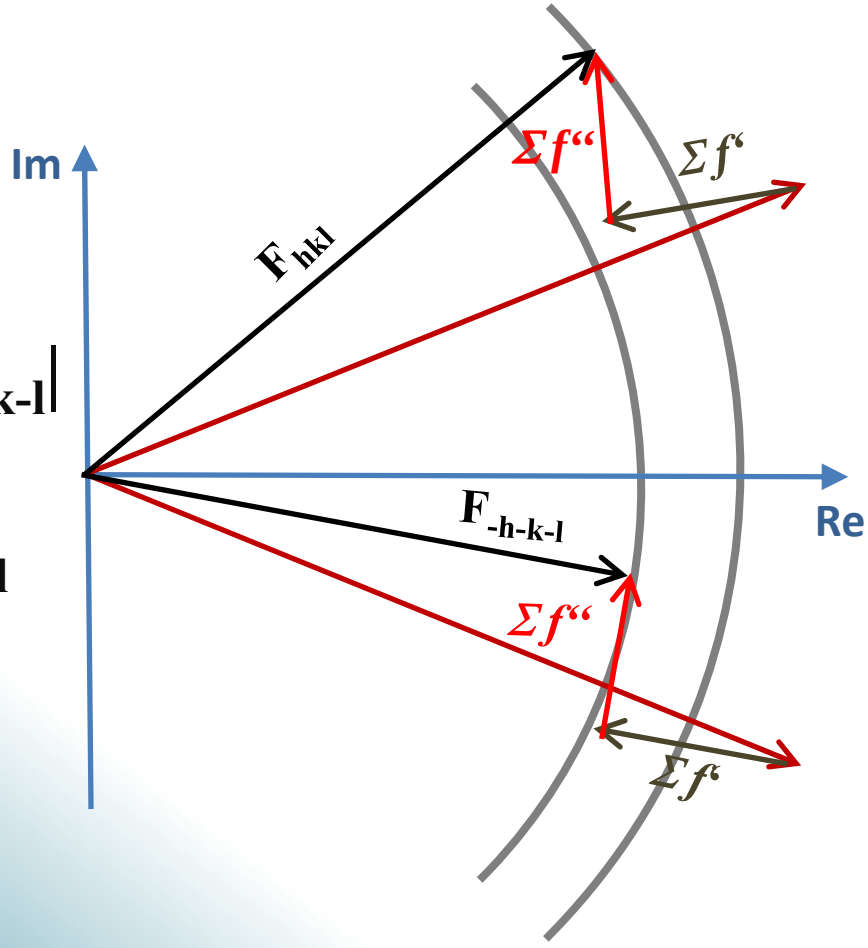

*Fluorescence scan or*
*http://skuld.bmsc.washington.edu*

# The anomalous signal

$f''$ breaks Friedel's law:

$$|F_{hkl}| \neq |F_{-h-k-l}|$$

$$\phi_{hkl} \neq -\phi_{-h-k-l}$$




**The intensities of Friedel pairs no longer have the same intensity!**

This can be used for the absolute structure determination and for experimental phasing!

How to...

# SUBSTRUCTURE SEARCH IN SHELXD

# Substructure search

An **overdetermined** problem with **noisy** data...

**Critical factors in substructure search:**

- **Resolution range** highly affects the outcome
- **Good data quality**
- Intensity **outliers** are problematic
- Scaling (also **anisotropic scaling**) is needed

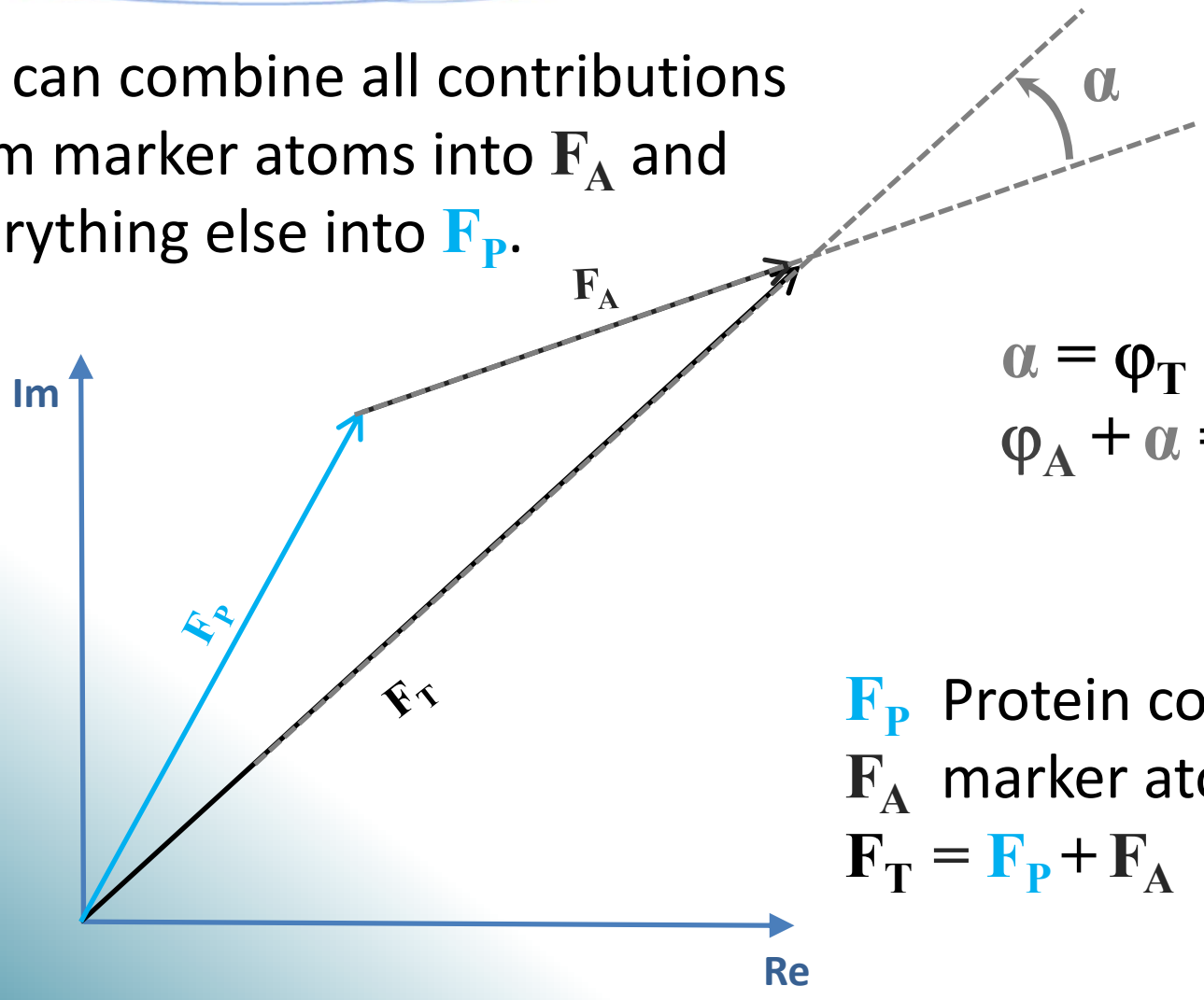
**BEWARE:** **Handedness is not resolved at this stage!**
(Density modification differentiates later.)

How to...

# PHASING THE REST (SHELXC)

# From substructure to structure

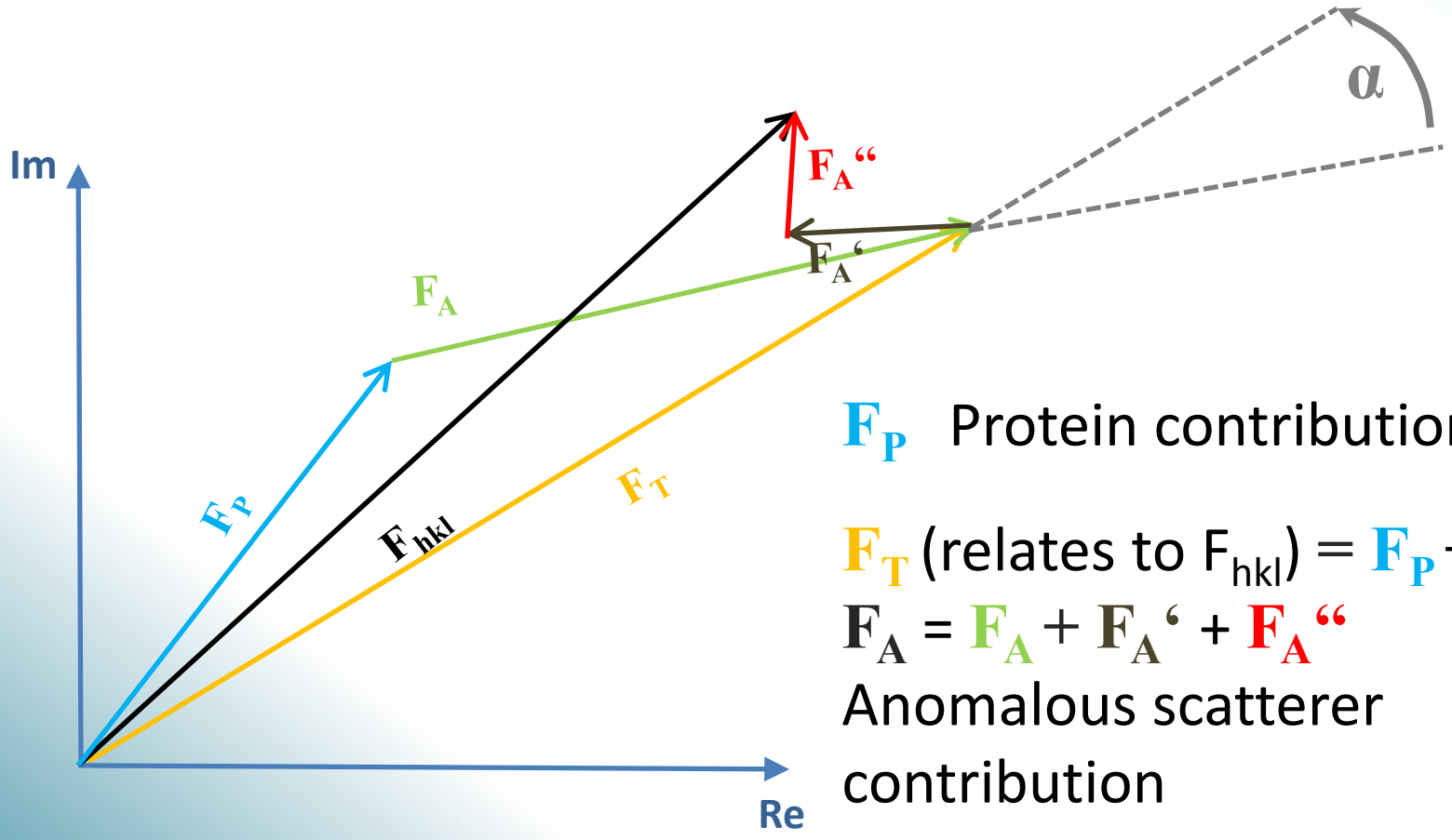
We can combine all contributions from marker atoms into $\mathbf{F_A}$ and everything else into $\mathbf{F_P}$.




$$\alpha = \varphi_T - \varphi_A$$
$$\varphi_A + \alpha = \varphi_T$$

$\mathbf{F_P}$ Protein contribution
$\mathbf{F_A}$ marker atom contribution
$\mathbf{F_T} = \mathbf{F_P} + \mathbf{F_A}$

# From substructure to structure



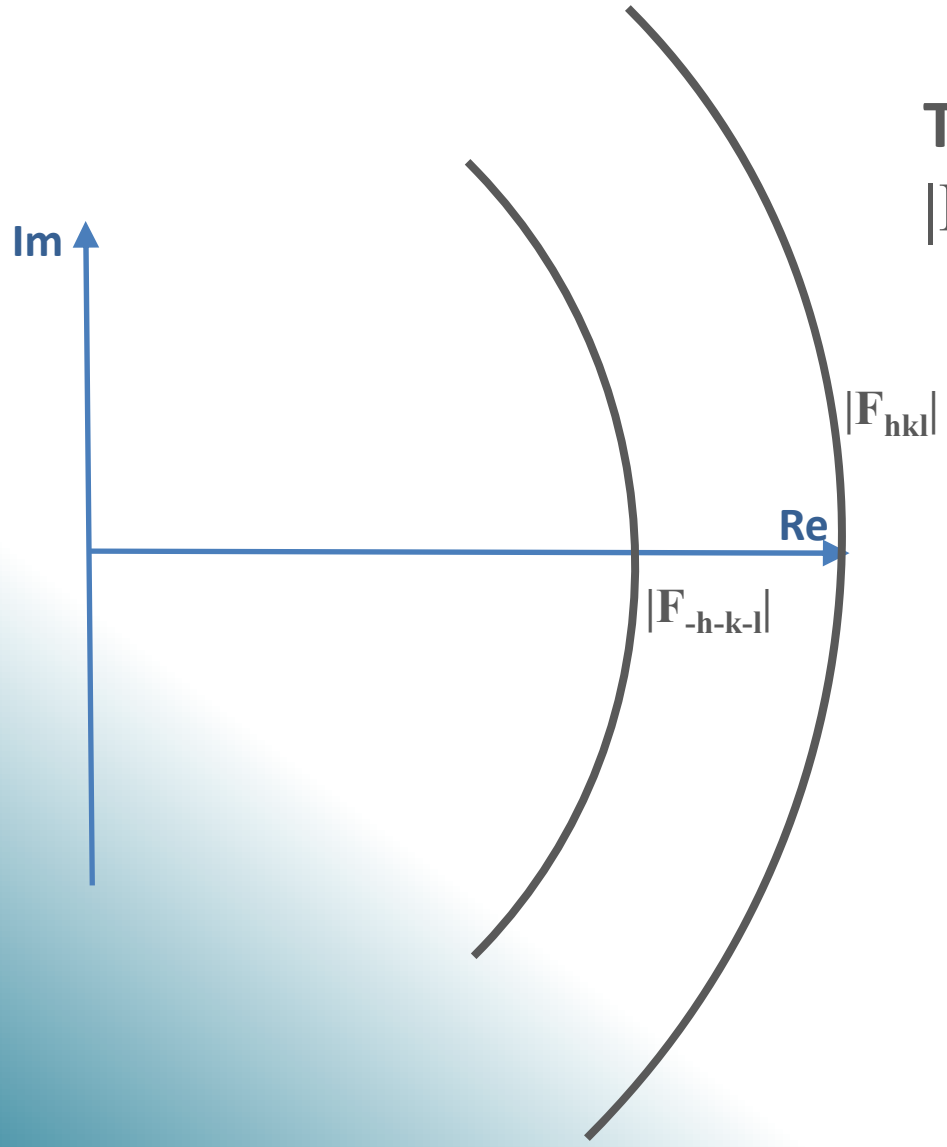

$F_P$ Protein contribution

$F_T$ (relates to $F_{hkl}$) = $F_P$ + $F_A$

$F_A$ = $F_A$ + $F_A$‘ + $F_A$“

Anomalous scatterer contribution

# From substructure to structure

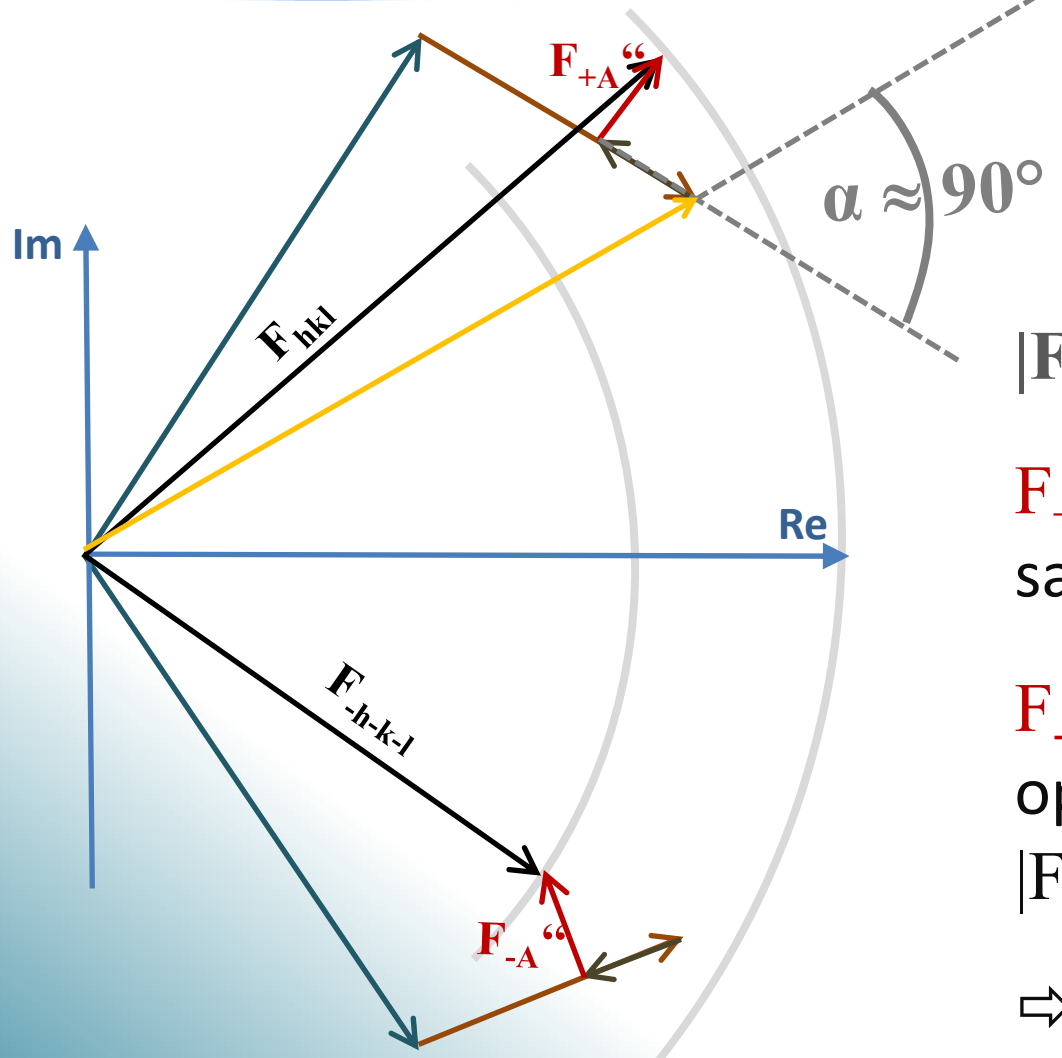



**This is what we know:**

$|F_{hkl}|$ **and** $|F_{-h-k-l}|$

$|F_{hkl}| >> |F_{-h-k-l}|$

# From substructure to structure



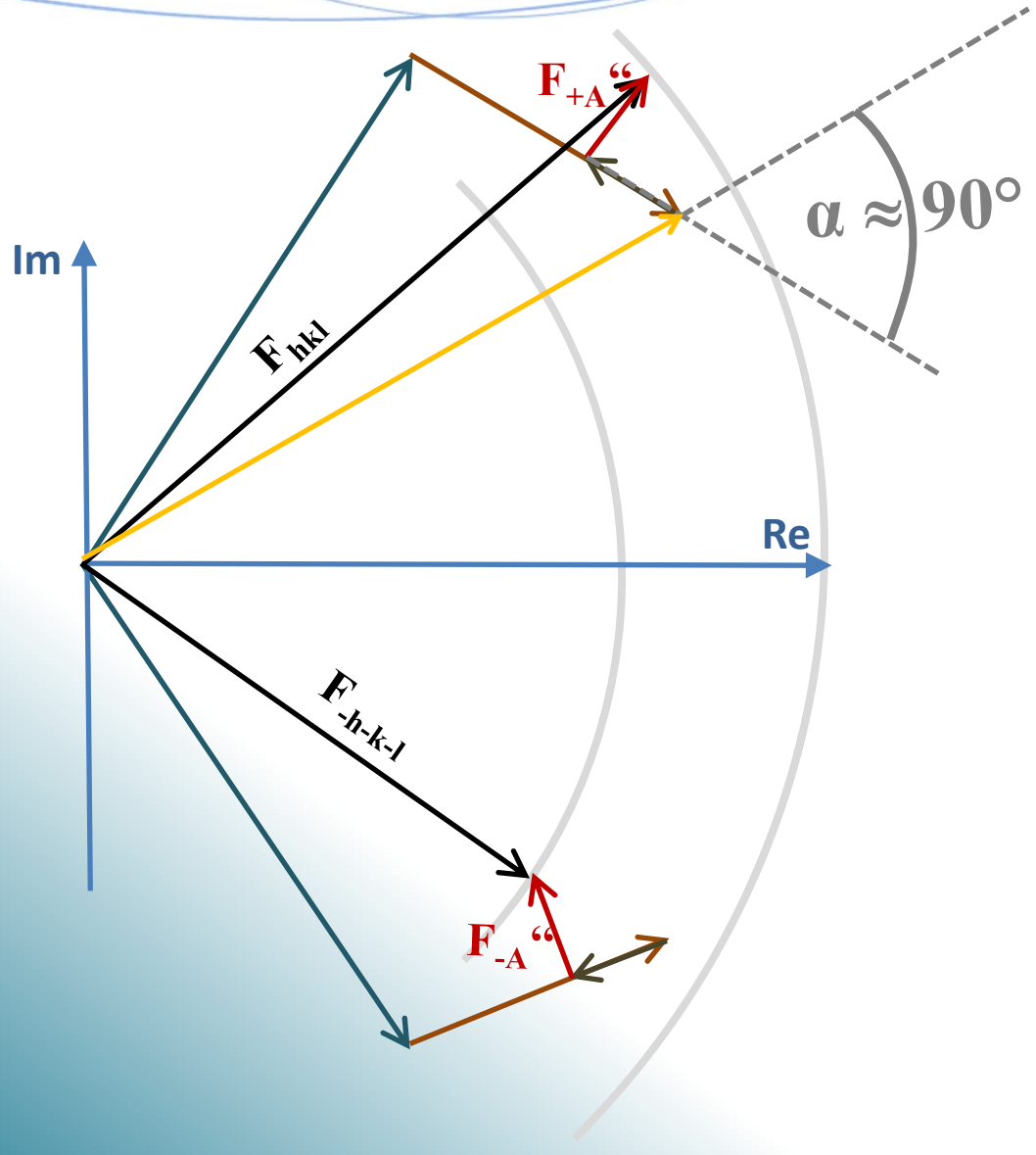

$|F_{hkl}| >> |F_{-h-k-l}|$

$F_{+A}$“ has to point in the same direction as $|F_{hkl}|$

$F_{-A}$“ has to point in the opposite direction as $|F_{-h-k-l}|$

⇨**α must be close to 90°!**

# From substructure to structure



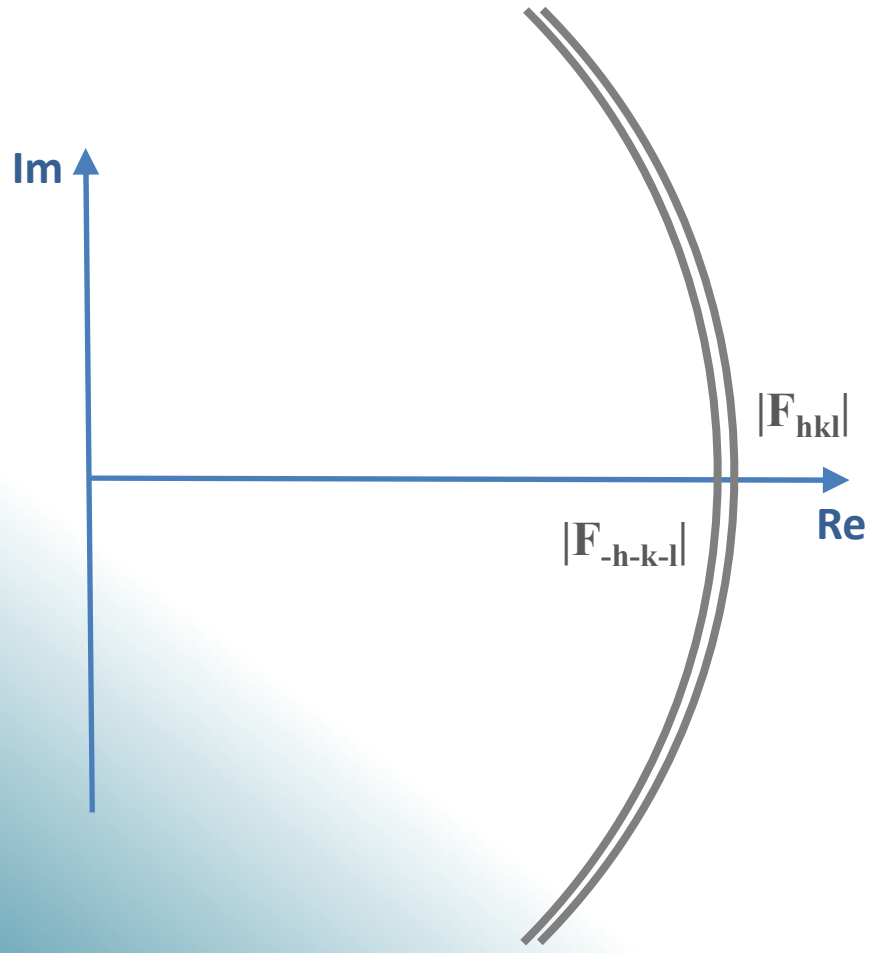

**If:** $|F_{hkl}| << |F_{-h-k-l}|$

⇨**α must be close to 270°!**

Reflections with the largest anomalous differences must be closest to $\alpha = 90°$ or $\alpha=270°$.

As you can easily see, estimation is rough.

# From substructure to structure




$|F_{hkl}| \approx |F_{-h-k-l}|$

$F_{+A}$‘‘ and $F_{-A}$‘‘ must be very small or almost perpendicular to $F_{hkl}$ or $F_{-h-k-l}$, respectively.

⇨**$\alpha$ must be close to 0° or 180°**

# Density modification

- $\varphi_T$ can now be computed from the phasing equations!

$$\varphi_A + \alpha = \varphi_T$$

Via Fourier synthesis, an initial map is gained.

- By $\Sigma_A$ coefficients the map is improved.
- But most important: **Density modification** is applied.

How to...

# DENSITY MODIFICATION IN SHELXE

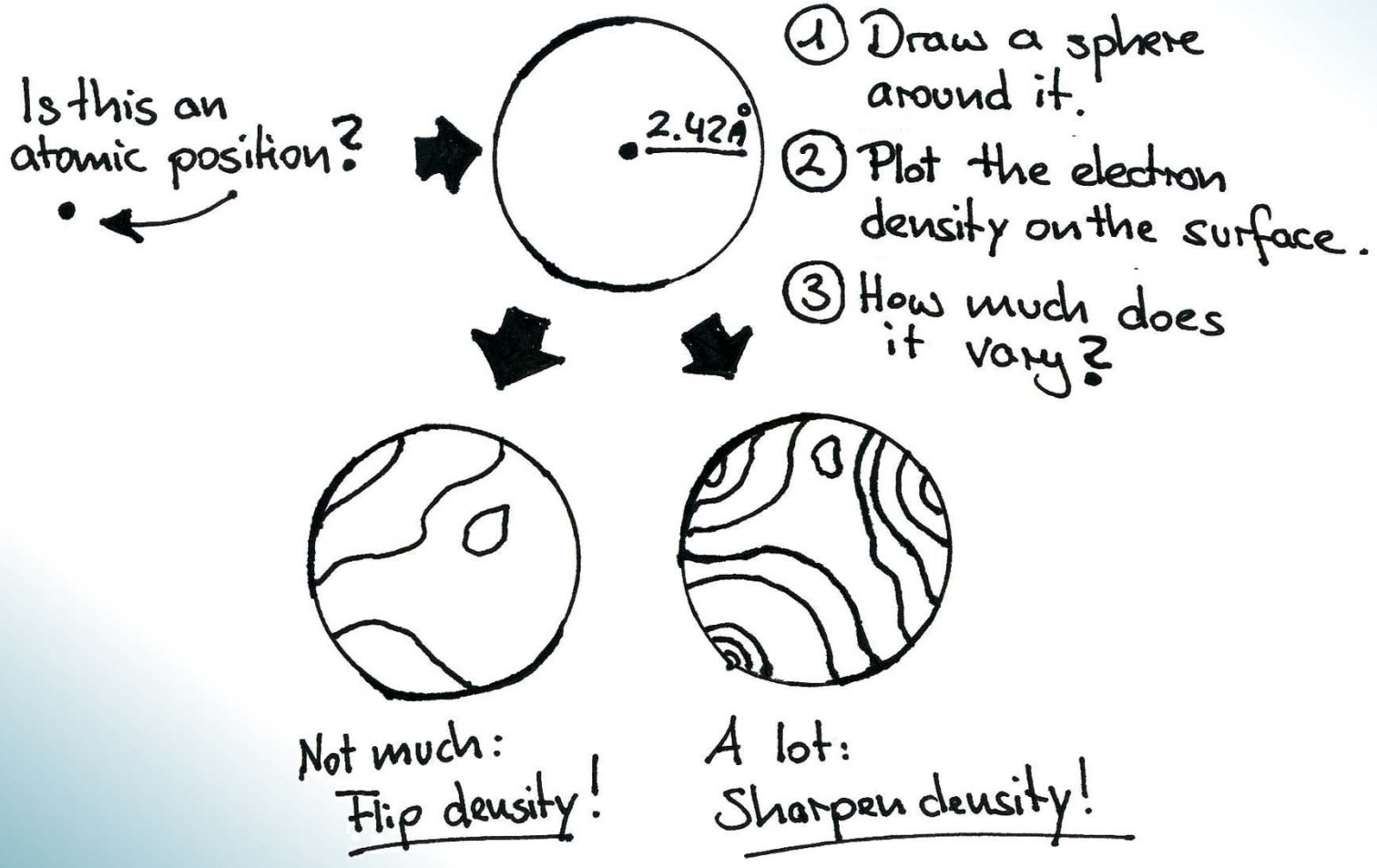
# Density modification

Especially SAD phases are still ambiguous as well as inaccurate. **Density modification** dramatically improves initial phases, electron density and **resolves handedness**!

- Based on areas filled by disordered solvent
- Solvent area is flattened or flipped
- NCS averaging can improve map quality
- High solvent content gives often better improvement

# Density modification

Most programs use a mask. SHELXE uses the sphere-of-influence method for density modification:



# Density modification

After several cycles, one of the two maps (one for each substructure enantiomer) looks ‚like protein‘.

The other has less connectivity and looks ‚ragged‘.

After density modification, the structure is solved! Experimental phasing has led to initial phases.

# SHELX workflow

**SHELXC**: α calculation, data analysis, file preparation

**SHELXD**: Substructure search

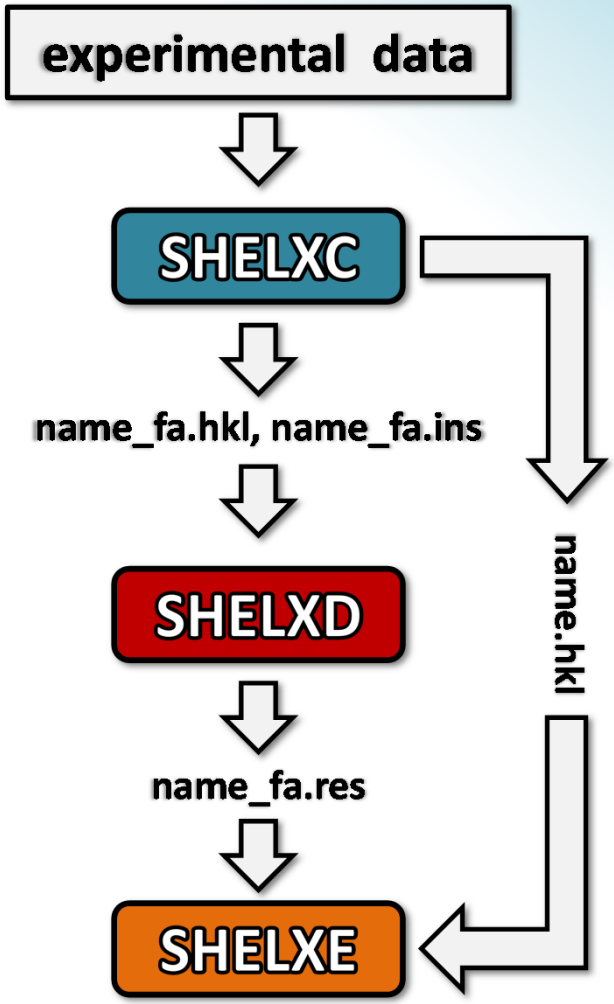
**SHELXE**: Density modification, tracing*

**** A traced structure is solved; CC (trace against native data) > 25% (for data < 2.5 Å)***

[**ANODE**: Validation]

**Pipeline?**

Other experimental phasing programs should be considered , in particular for ease of use or problem cases**.




** http://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/Experimental_phasing

Molecular Replacement

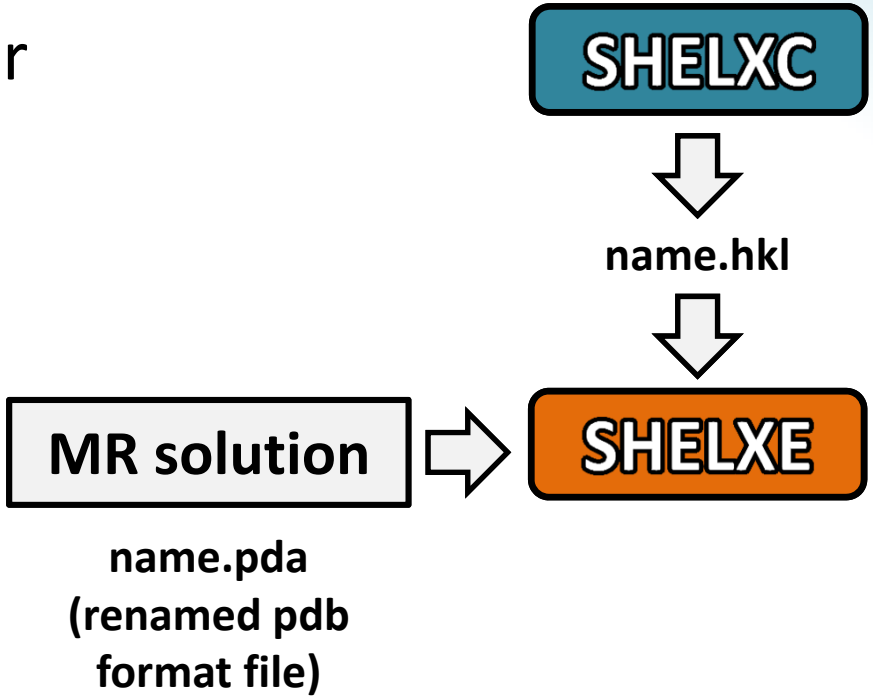
# SHELXE FOR MR

# Workflow for MR solutions

Input phases for SHELXE can be from a molecular replacement model:

**`shelxe XX.pda <options>`**

- Improve phases/map
- Extend structure
- Remove model bias




Data < 2.1 Å are required.*

***2.5Å for auto-tracing with experimental phase information.**

**Thorn, Sheldrick, Acta Cryst. D69 (2013), 2251-2256**

# SOFTWARE

The SHELXE can auto-trace a protein backbone.

**A structure that can be traced is a structure solved*.**

This proves particularly useful:

- in **borderline cases** of experimental phasing
- in **pipelines**, like ARCIMBOLDO, AMPLE or AUTORICKSHAW
- as **a quick indication** of a correct solution (no sequence)
- as a **step between MR and complete auto-building**

  *(If SHELXE does not work, the MR solution was not necessarily wrong.)*

*** Solved: CC(trace against native data) > 25% for data < 2.5Å!**

**AMPLE: Bibby et al., *CCP4 Newsletter* (2012) 48.**
**ARCIMBOLDO: Rodriguez et al., *Nature Methods (*2009) *6*, 651-653.**
**AUTORICKSHAW: Panjikar et al., *Acta Cryst.* (2005) D61, 449;**

# Example: Prp8

**Yeast Prp8 (residues 885–2413)**

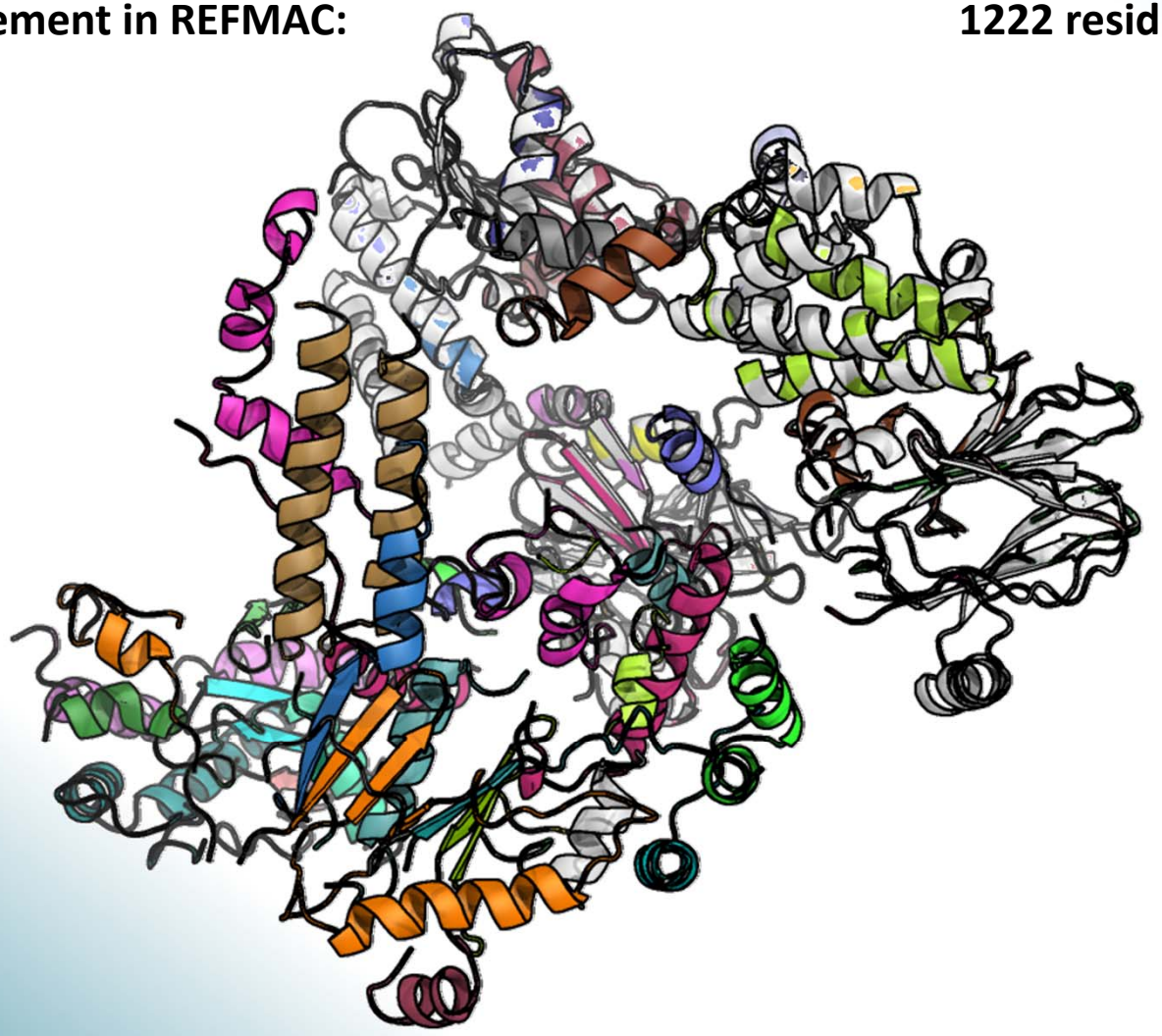
| | |
|---|---|
| **Data set resolution:** | 1.9 Å |
| **Space group:** | $C222_1$ |
| **Secondary structure:** | α-helices and β-sheet |
| **Residues/ASU:** | 1529 |
| **SHELXE version:** | 2012-1 |

**Galej, Oubridge, Newman & Nagai, Nature (2013) 493, 638-644**

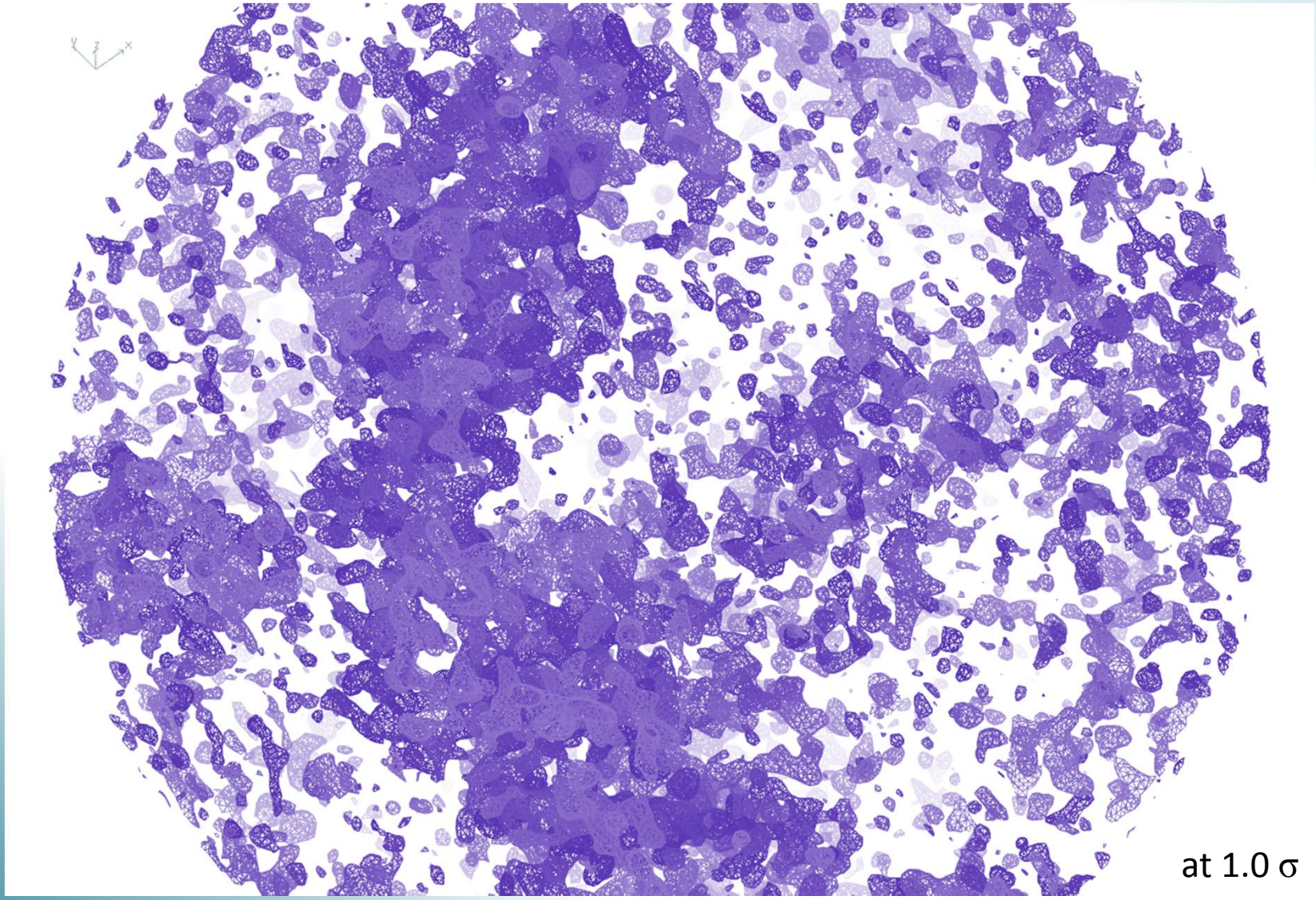
# Example: Prp8

**After MR with MolRep (contrast 17.27) and jelly body refinement in REFMAC:**
**$R_w$: 45.3**
**$R_{free}$ 48.6**
**695 residues**

**SHELXE CC : 32.27%**
**1222 residues**

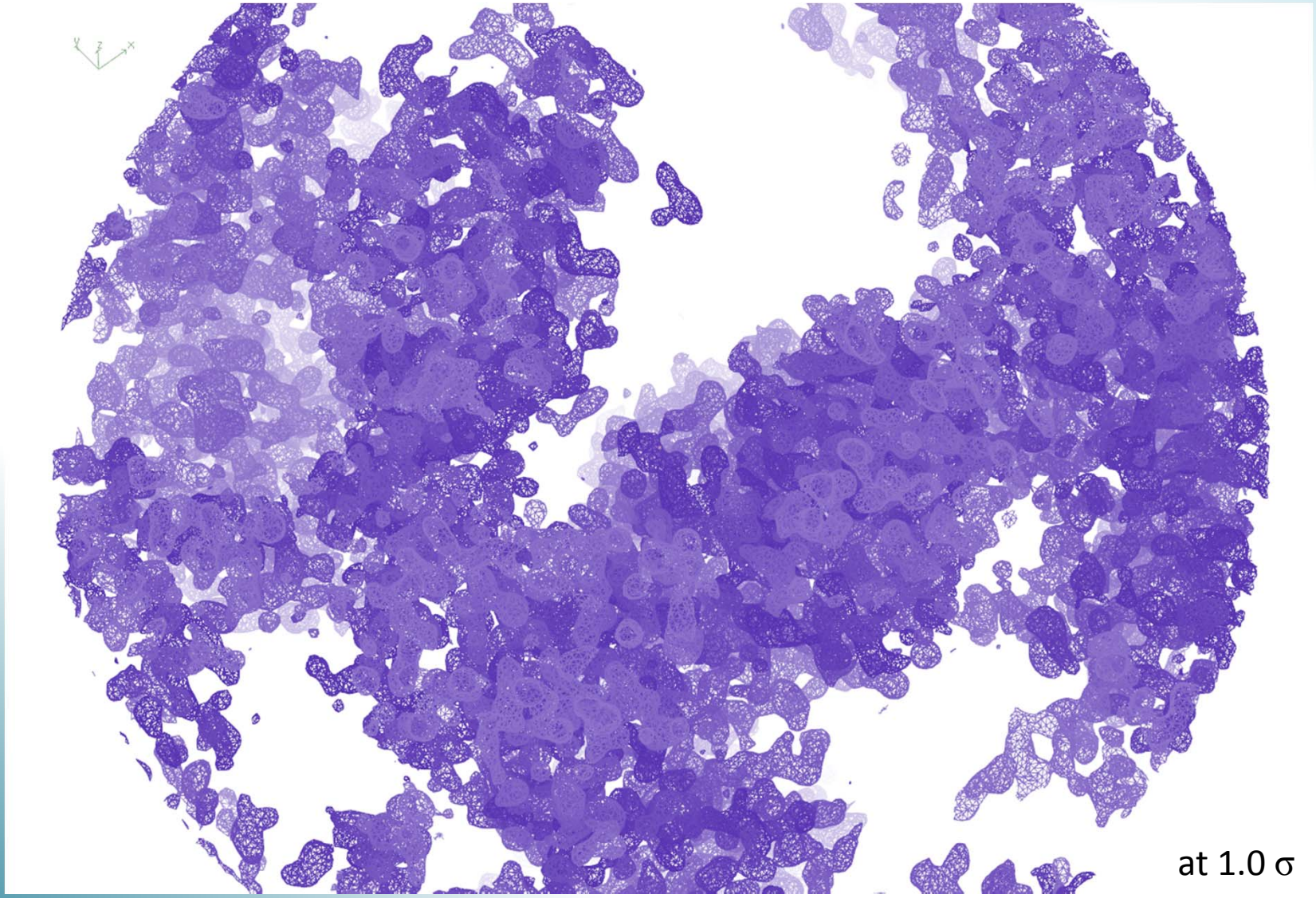


# Example: Prp8



at 1.0 σ

# Example: Prp8

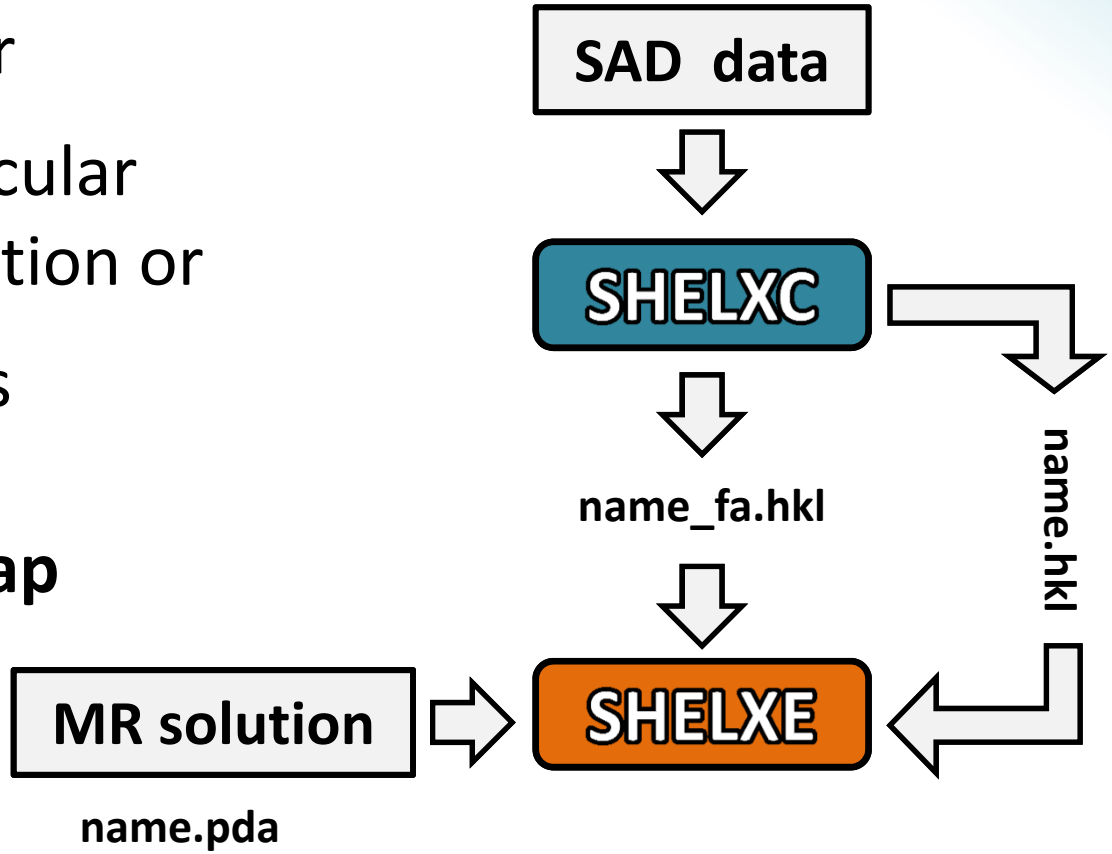


at 1.0 σ

# MR-SAD

- Not enough phase information from SAD alone or
- Only partial Molecular Replacement solution or
- Severe model bias

**Use MR to bootstrap SAD phases!**




**MR-SAD: Schuermann & Tanner, *Acta Cryst. D*59, 2003, 1731.**
**Thorn (2011). PhD thesis, University of Göttingen, Germany.**

Experimental phasing, for real

# PRACTICALITIES: PREPARATION, DATA COLLECTION & EVALUATION

# Things you want to have an idea about

- Space group? (Twinning?)
- How many marker atoms do you expect?
- Substructure: Which elements/molecules?
- What could be the best resolution cut-off?

  (SHELXC assumes data resolution + 0.5Å)
- Could any marker atoms ‚fuse' into bigger blobs of density because of resolution cut-off? Disulfides?
- Merging of data from different crystals/runs?
- Expected solvent content and residue numbers?

# Data collection

- High multiplicity is good.
- Radiation damage is often bad.
- Precise intensity measurements are good.
- Near to the absorption edge, the crystal absorbs most energy, therefore radiation damage is high.
- A **fluorescence scan** can prove the presence of anomalous scatterers in the crystal.
- Good low resolution completeness

Pictures courtesy of Airlie McCoy

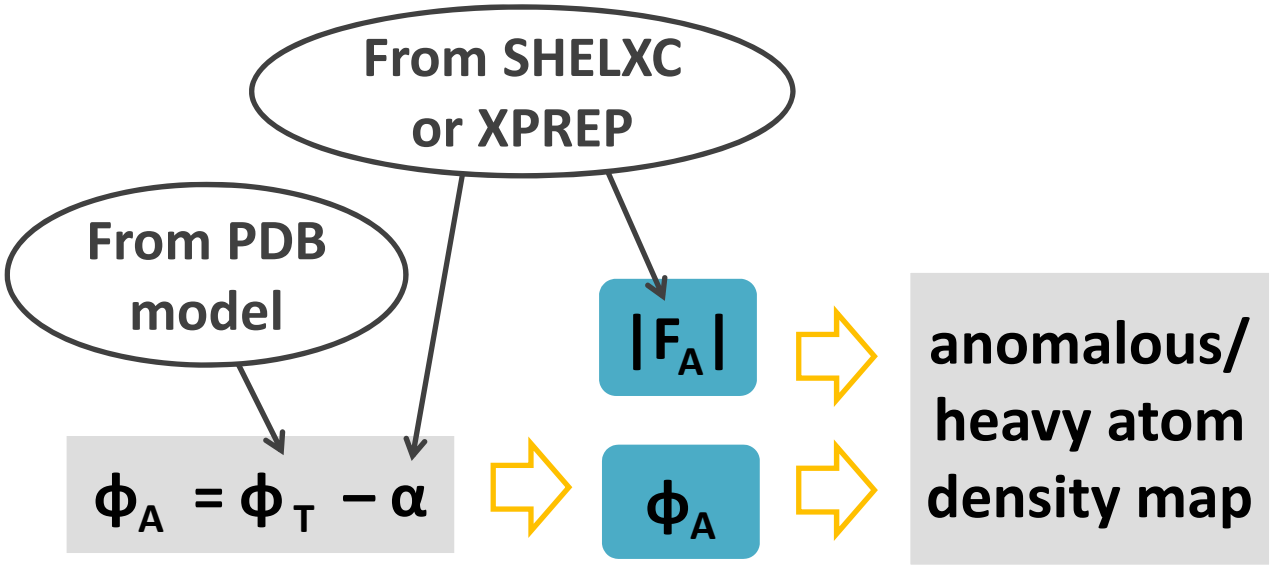
# Data evaluation

- The general data quality should be good – multiplicity, completeness, $R_{PIM}$ etc.
- If scaling was applied, check statistics.
- Check the mask; inner shell completeness?
- Data set files well distinguishable?
- If you have made a fluorescence scan, keep it.
- Is there an anomalous signal in the collected data?
  - Anomalous correlation within a data set: $CC_{anom(1/2)}$
  - <d”/σ> and/or <d’/σ>
  - Anomalous correlation of data sets: $CC_{anom}$

ANODE

# ANOMALOUS MAPS

# Introduction



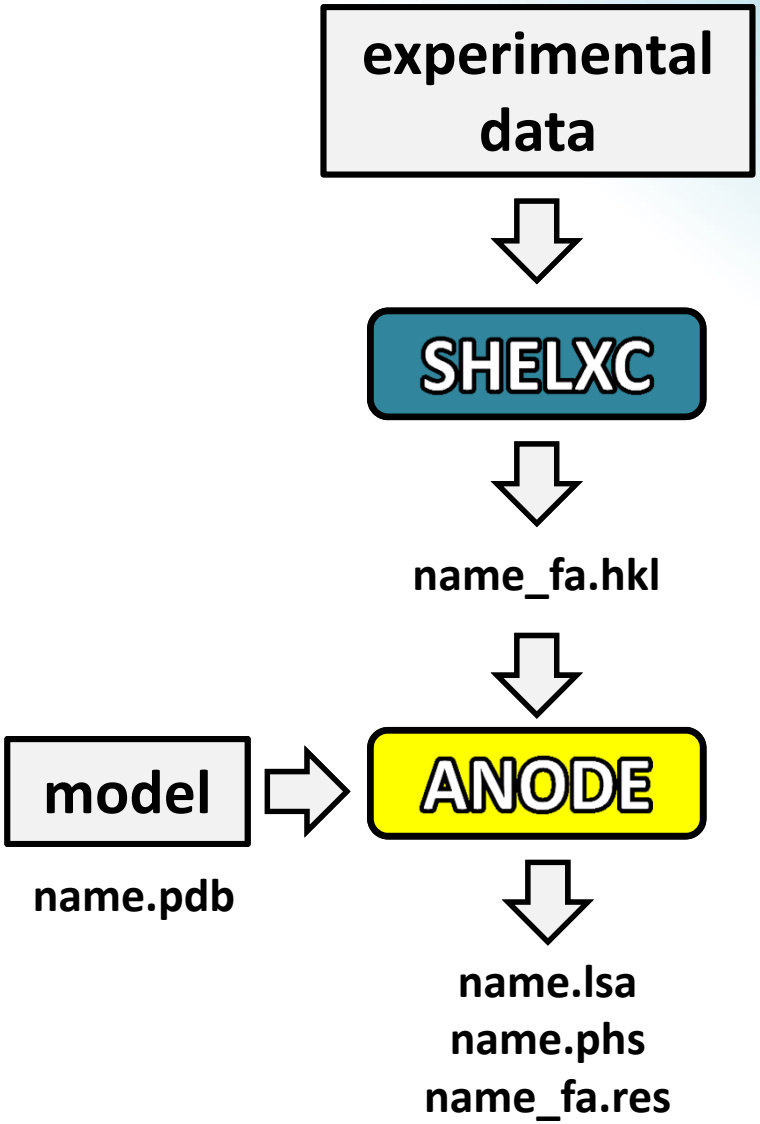

**ANODE** calculates **anomalous or heavy atom density**

If SHELX has been run input is straight forward.

# ANODE workflow

The program command:

**`anode name [options]`**



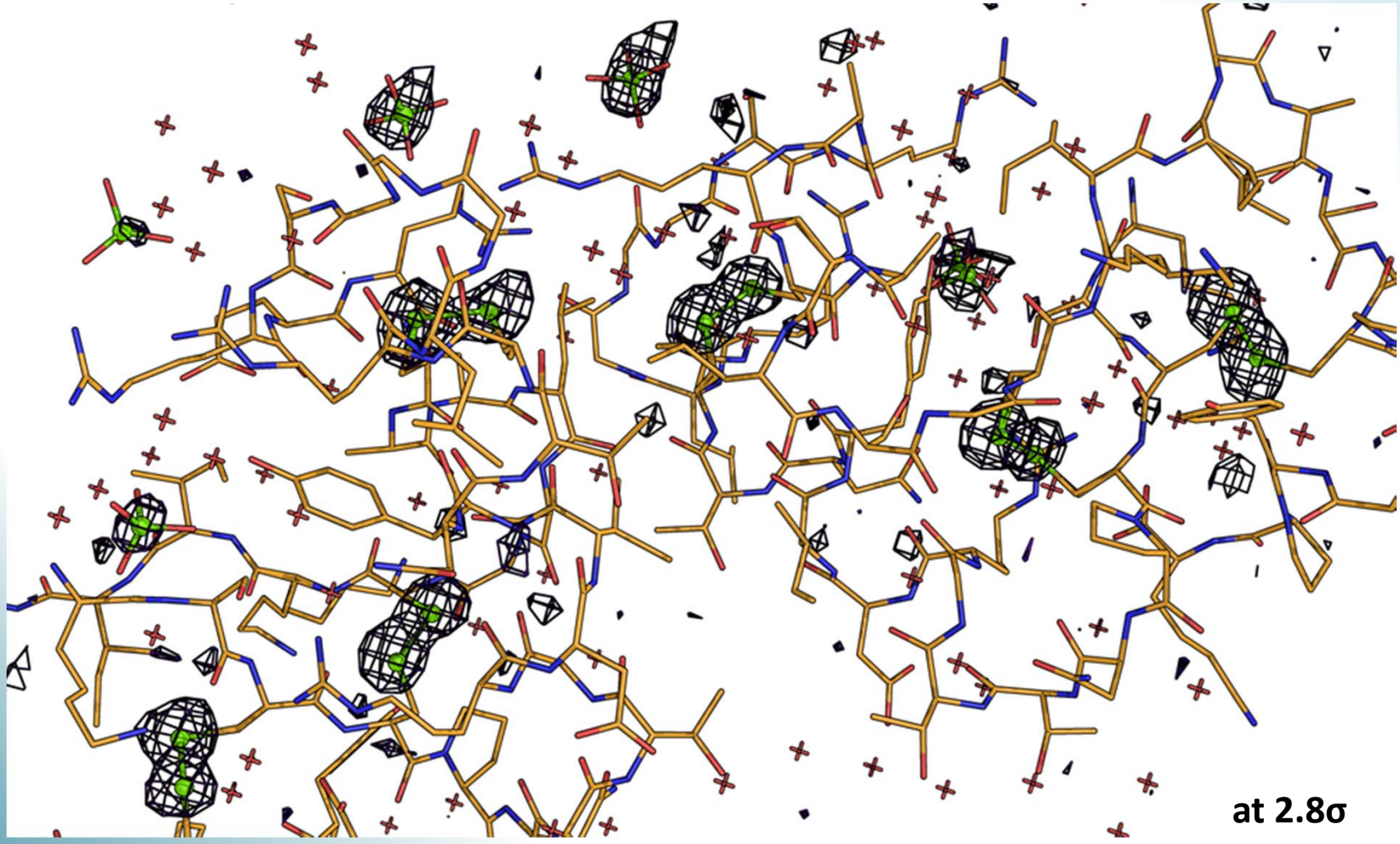

Thorn & Sheldrick, *J. Appl. Cryst.* 44 (2011), 1285-1287

# ANODE

Uses of maps calculated from intensity differences:

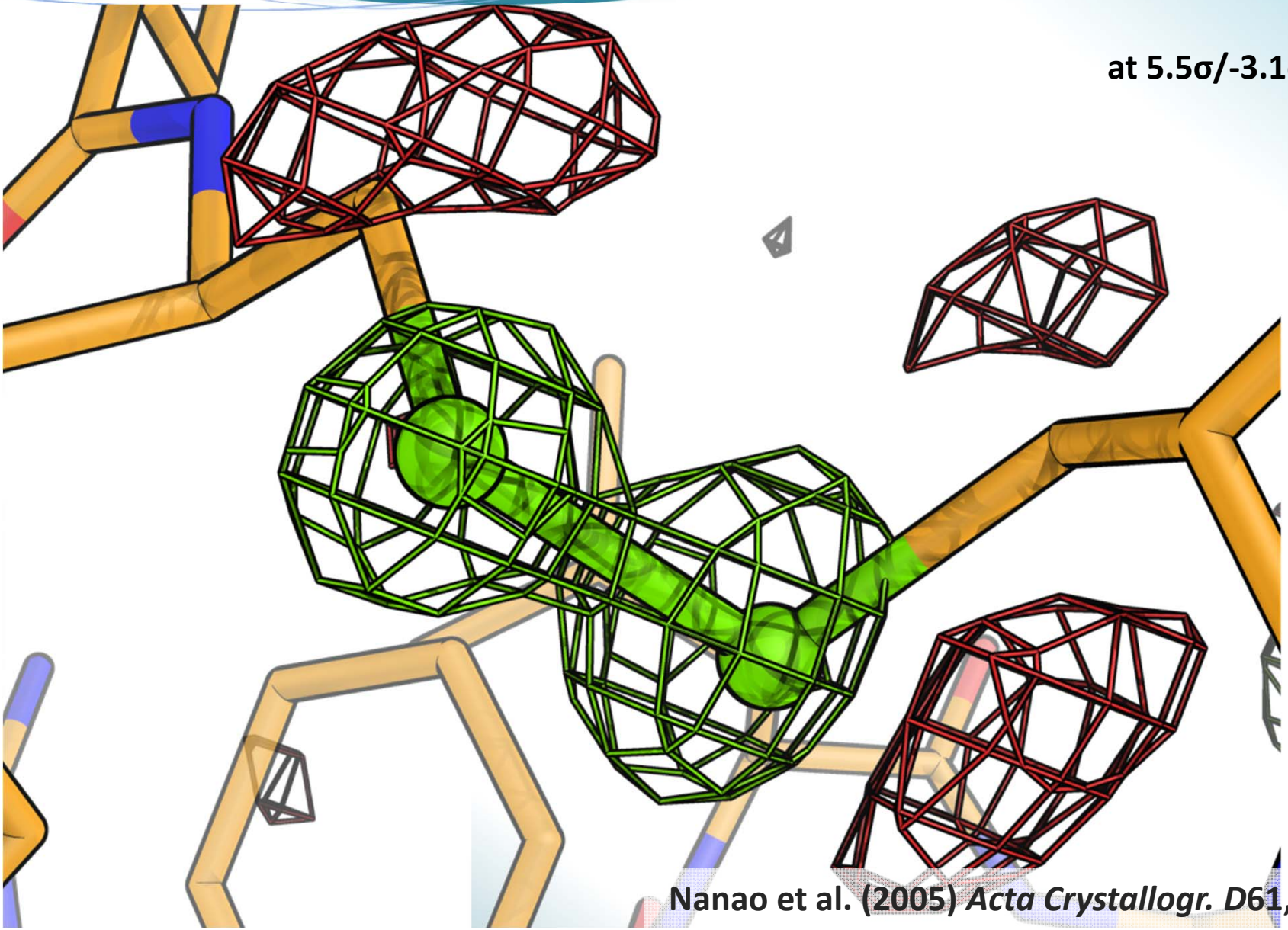
- **Ligand position and orientation**
- Structure **validation**
- Identification of **elements**
- **Analysis** of radiation damage
- Analysis of the anomalous signal

# Example: Viscotoxin B2




Viscotoxin B2; PDB 2V9B; Pal et al. (2008). *Acta Cryst. D*64, 985-992.

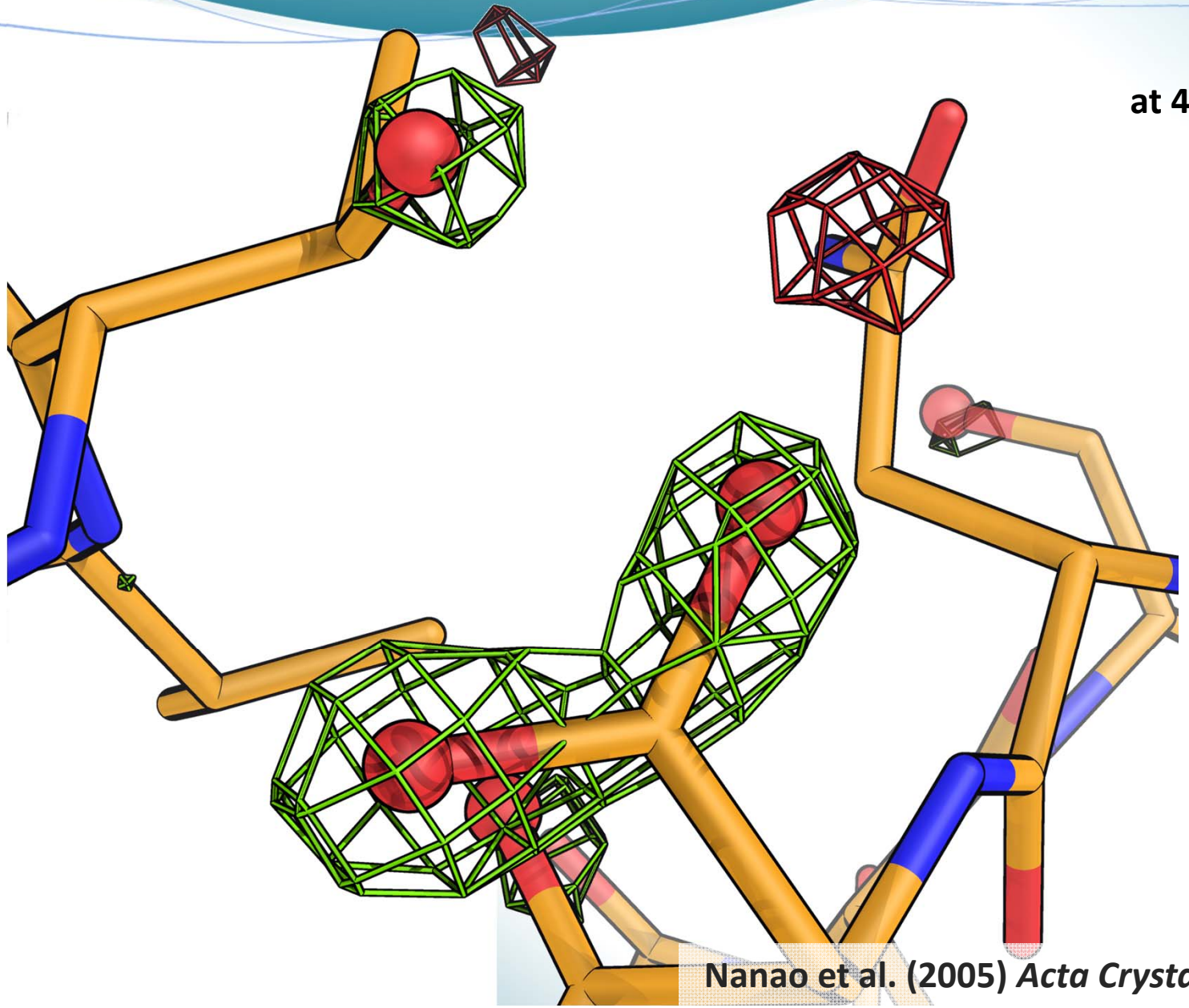
# RIP density maps



at 5.5σ/-3.1σ

Nanao et al. (2005) *Acta Crystallogr. D*61, 1227

# RIP density maps



at 4.8 σ / -3.1 σ

Nanao et al. (2005) *Acta Crystallogr. D*61, 1227

Final

# SUMMARY

# Summary

**SHELXC/D/E is a set of programs for experimental phasing.**

**SHELXC** estimates/calculates the α angle contribution

**SHELXD** finds the substructure

**SHELXE** distinguishes the hand by density modification and generates an initial electron density map.

SHELXE can also **auto-trace** high-resolution structures and be used for **MR solutions**

**ANODE** can be used to validate a structure and to analyse difference density.

# Acknowledgements & Literature

**George Sheldrick**

**Aritra Pal, Max Nanao, Isabel Usòn, Ronan Keegan, Wojtek Galej**

**The Murshudov lab**

MRC Laboratory of Molecular Biology

SEVENTH FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS

If you want to try SHELX or ANODE: It is free for academic use and available at: **http://shelx.uni-ac.gwdg.de/SHELX/**

This lecture: **http://shelx.uni-ac.gwdg.de/~athorn/**

Thorn & Sheldrick: **"Extending Molecular Replacement Solutions with SHELXE"** Acta Cryst. D69 (2013), 2251-2256

Thorn & Sheldrick: "**ANODE: ANOmalous and heavy-atom DEnsity calculation**" *J. Appl. Cryst.* 44 (2011), 1285-1287

# LITERATURE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- Kai Diederichs, P. Andrew Karplus, **Improved R-factors for diffraction data analysis in macromolecular crystallography.** Nat. Struct Biol. (1997). 4, 269-75.

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# LITERATURE


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- George M. Sheldrick (2002). **Macromolecular phasing with SHELXE**, *Z. Kristallogr*. 217:644-650.
- George M. Sheldrick, **Experimental phasing with SHELXC/D/E: combining chain tracing with density modification**, Acta Cryst. (2010). D66, 479-485
- A. Thorn & G.M. Sheldrick: "**ANODE: ANOmalous and heavy-atom DEnsity calculation**" *J. Appl. Cryst.* 44 (2011), 1285-1287


**More material: shelx.uni-ac.gwdg.de/~athorn/**

**http://shelx.uni-ac.gwdg.de/SHELX/**