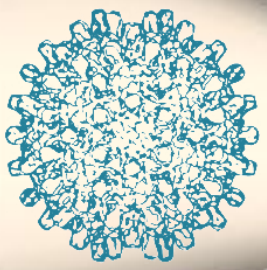
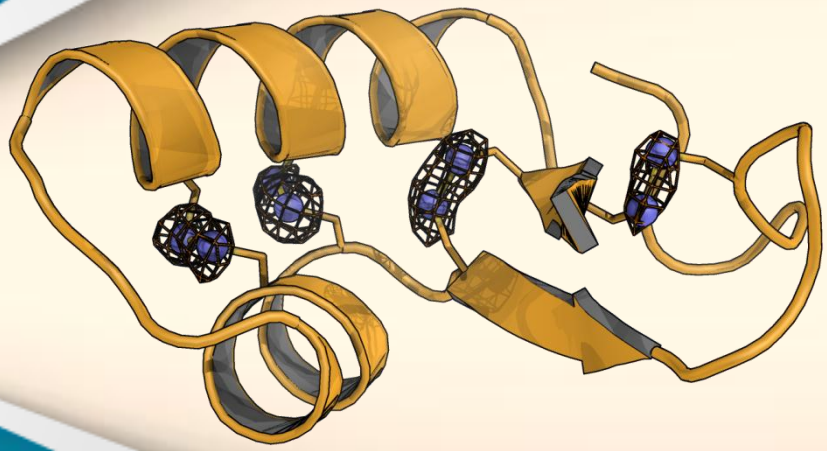
Bruker Webinar

ANODE: ANOmalous and heavy atom DEnsity



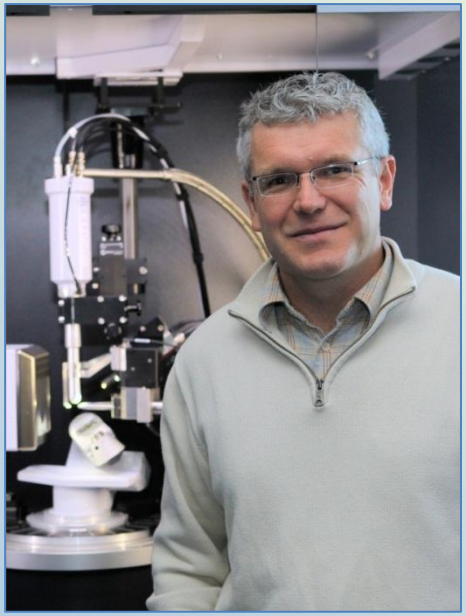


Andrea Thorn

03 December, 2013

Michael Ruf

Welcome



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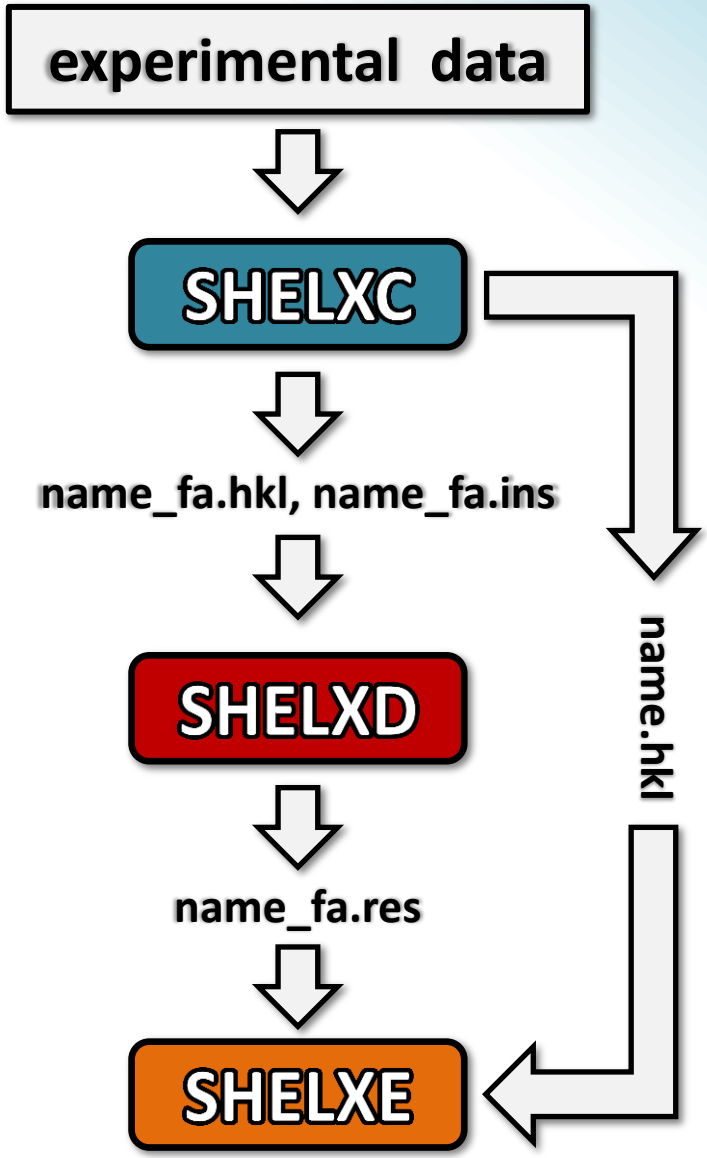
Bruker AXS Inc.
Madison, WI, USA

Andrea Thorn
Crystallographic Computing Group
Medical Research Council –
Laboratory of Molecular Biology
Cambridge, UK

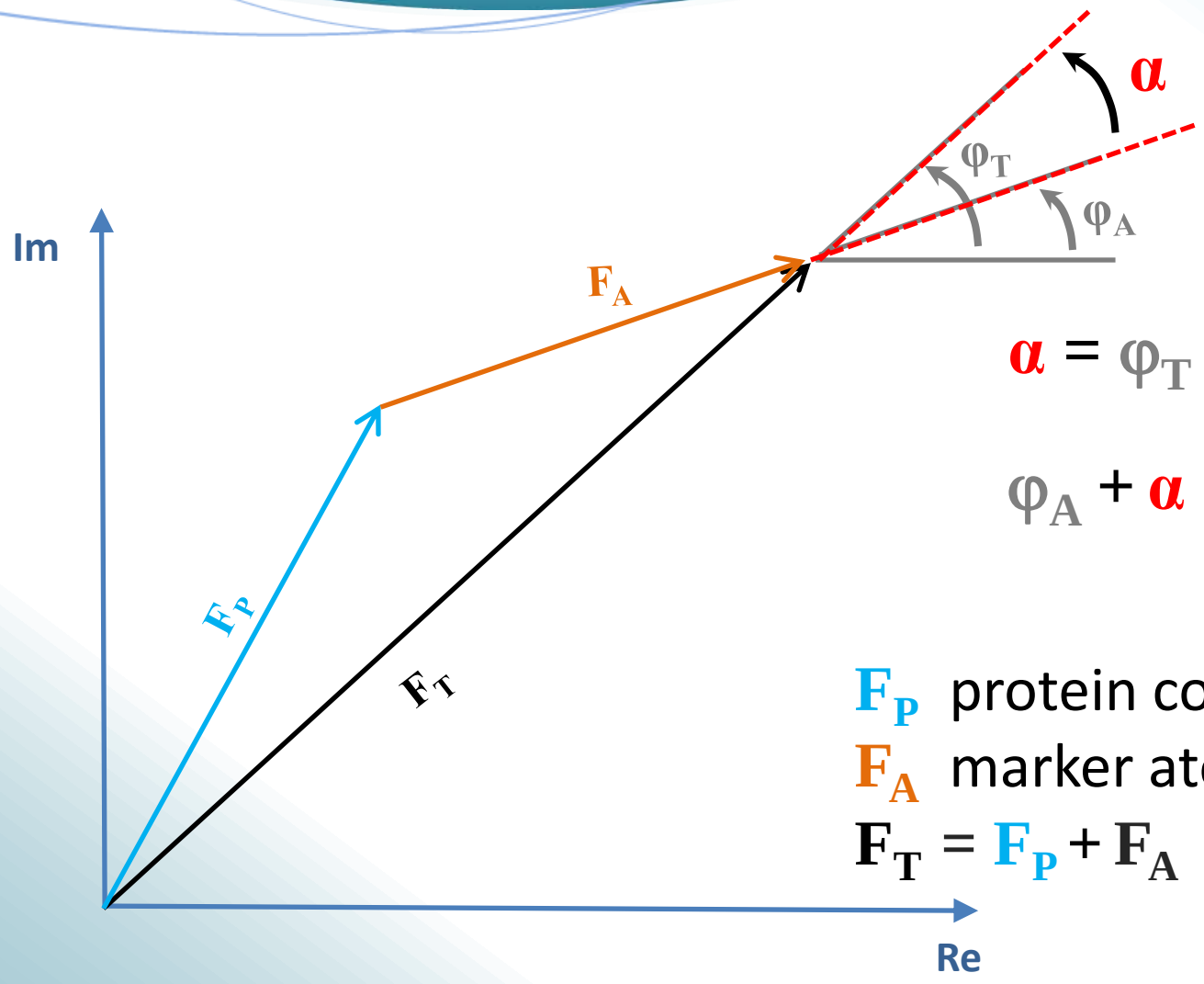
Typical SHELXC/D/E workflow

The anomalous or heavy atom signal is used to find the **substructure** of anomalous scatterers or heavy atoms.

With this information, the approximate phases of a macromolecular structure can be obtained.



The α angle



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

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

F_P protein contribution

F_A marker atom contribution

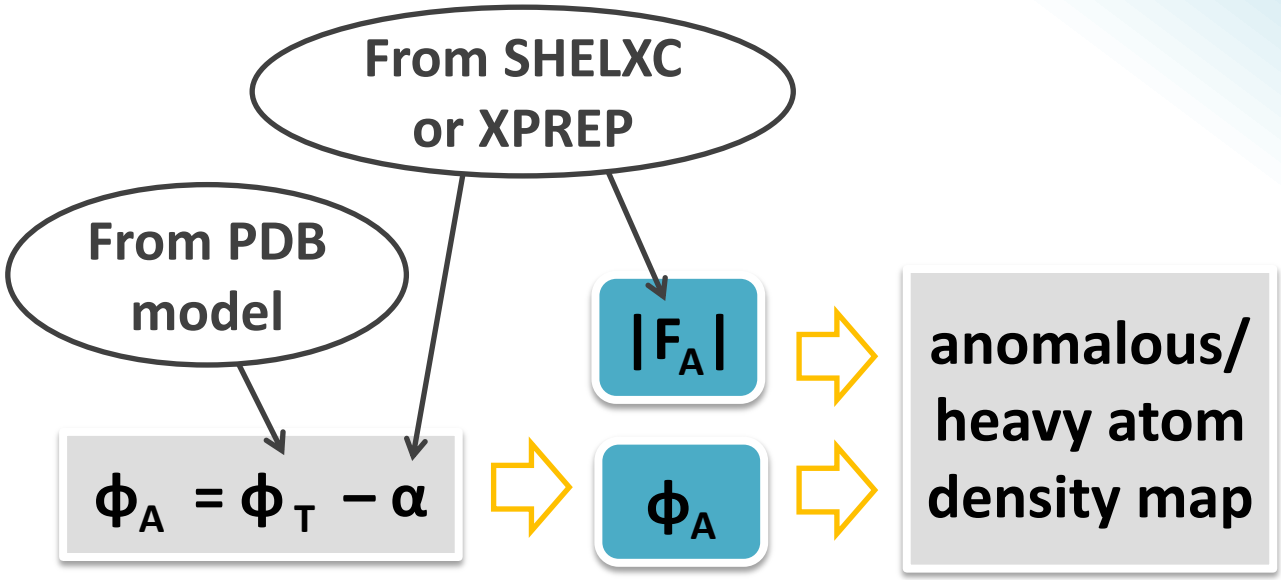
$F_T = F_P + F_A$

Introduction

Marker atom:
$|F_A|$ and ϕ_A

Macromolecule:
$|F_T|$ and ϕ_T

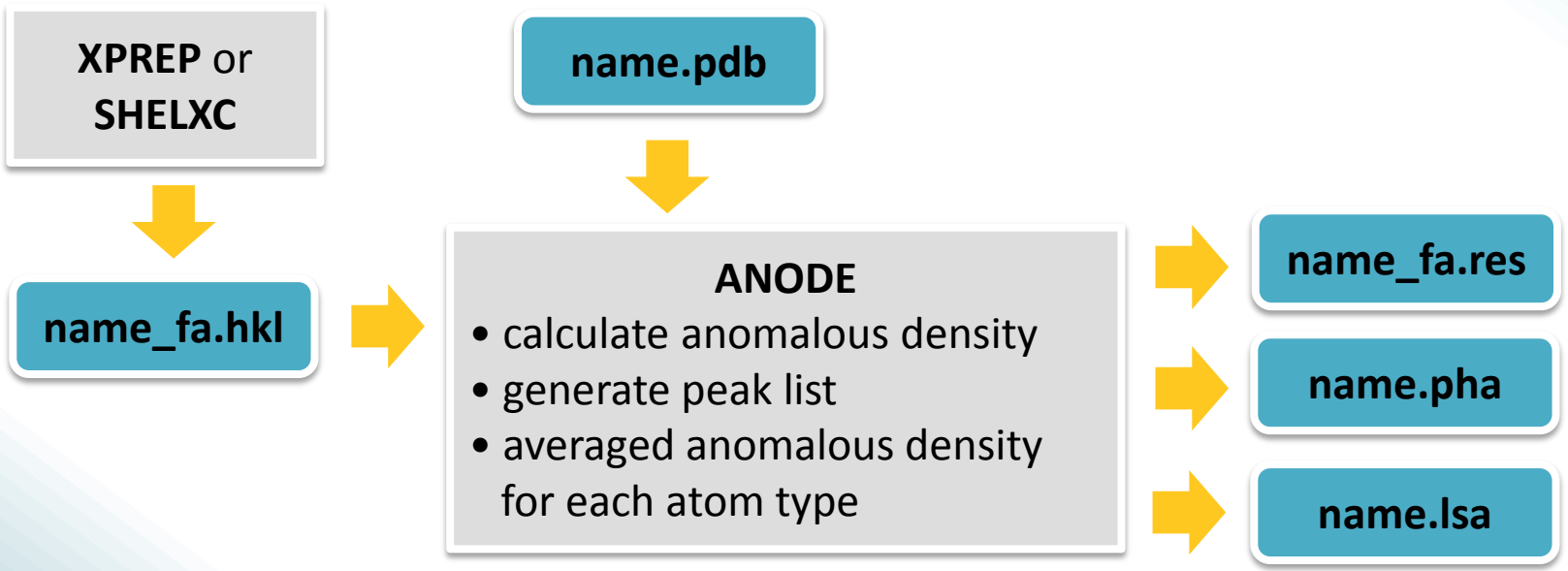
Phase relation:
$\phi_T = \phi_A + \alpha$



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

Strahs, G. & Kraut, J. (1968). *J. Mol. Biol.* 35, 503-512.

ANODE: general principle



Output and available options

ANODE calculates the density map by **F**ast **F**ourier **T**ransform.

The square root of the density variance σ is derived.

Output:

- **Averaged density for each site type**, for example S_Met
- Heights and coordinates of **unique peaks** and distance to the next atom in the PDB file.
- Map **name.pha** for COOT
- **name_fa.res** as written by SHELXD for testing with SHELXE
- **name.lsa** – listing file

Input and available options

The program is used with the command:

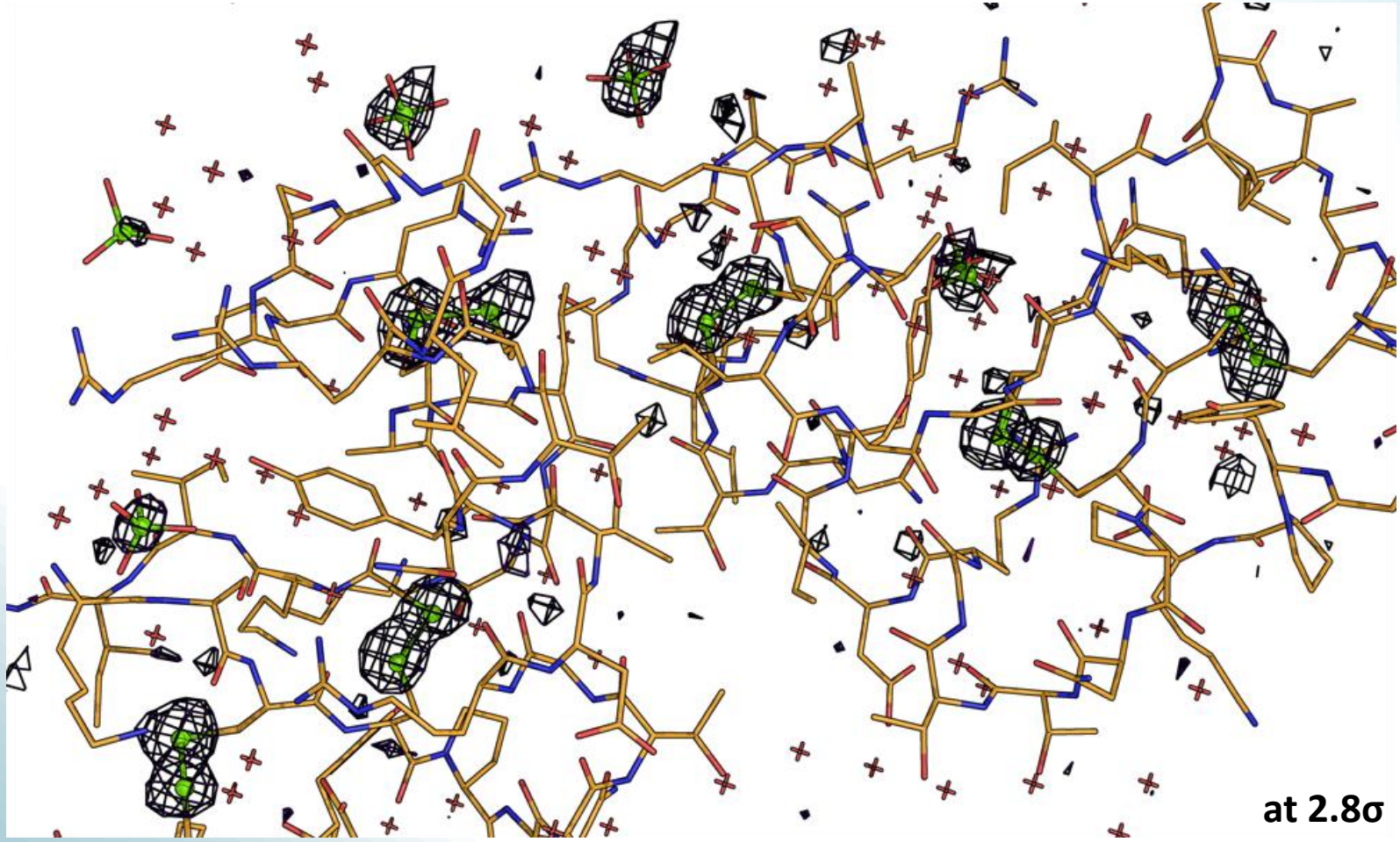
`anode name [options]`

reads *name.ent or name.pdb* and *name_fa.hkl*

If the data indices might be inconsistent with the PDB, the alternative orientation can be used by **`-i`**. For the space groups $P3_1$, $P3_2$ and P3 four indexing options exist and should be chosen by –i1,-i2 or –i3.

A maximum resolution for F_A can be given with a cut-off (**`-d`**) or damping can be applied (**`-b`**) which seems superior in our tests.

Demonstration: Viscotoxin B2



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

Examples

- Zn-MAD data: Chemical identities
- Hellethionin D: S-SAD data from Cu home source and MR-SAD
- SAD data: Radiation damage in a single data set
- RIP data: Seeing the effects of radiation damage
- Br-Se-DNA data from a Mo home source

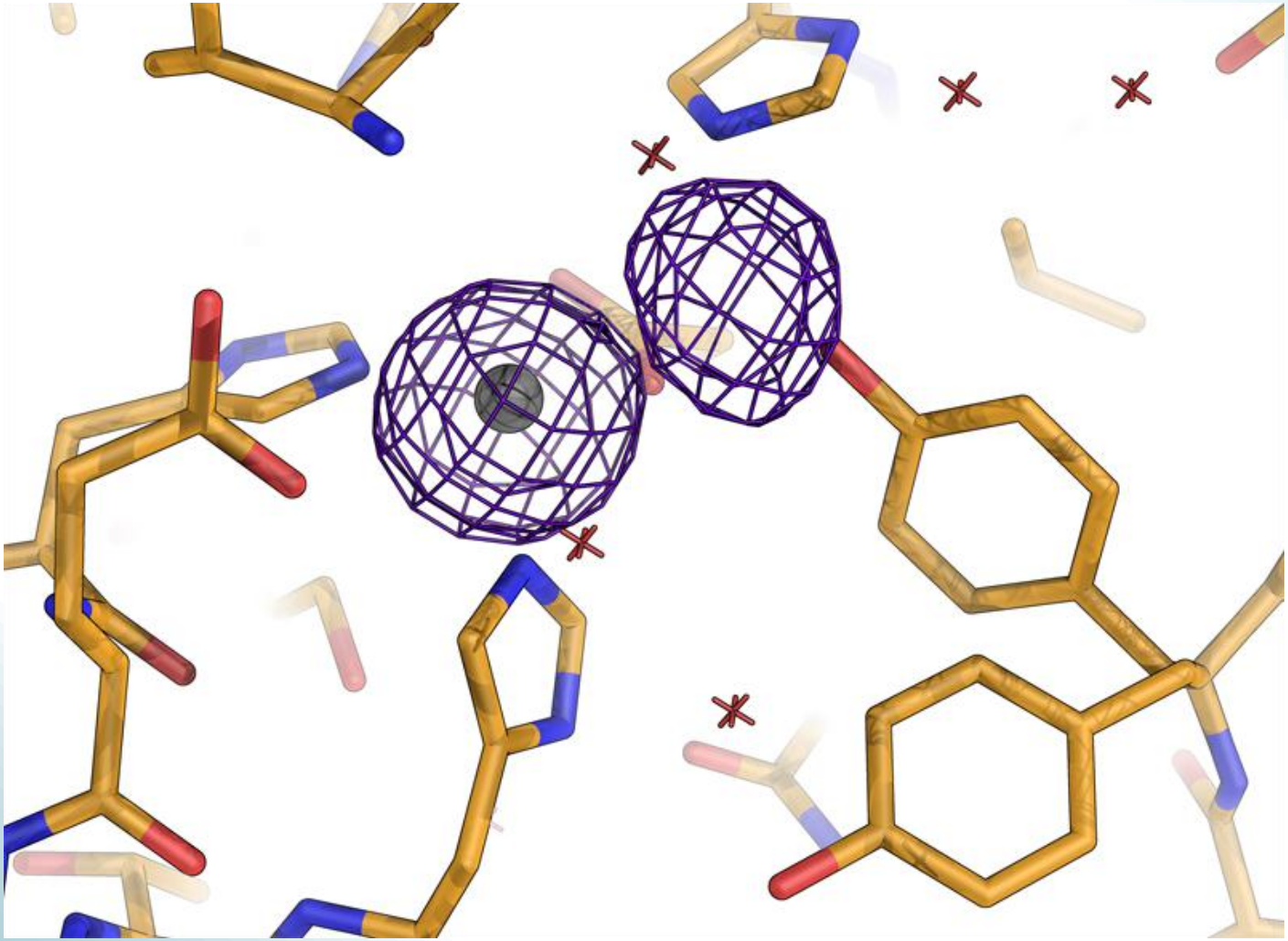
Zn-MAD

- Thermolysin measured by Marianna Biadene and Ina Dix
- excess zinc
- Three-wavelength MAD experiment
- BESSY beamline 14.2
- Resolution 2.06 Å
- Zinc, calcium and sulfur present

Unexpected peak *3.25 Å from the main zinc site*: Holland *et al.* (1995) argued on its nature based on the native density and chemical environment of the site.

Holland et al. (1995). *Protein Sci.* 4, 1955-1965.

Zn-MAD



at 3.5 σ

PDB 3FGD

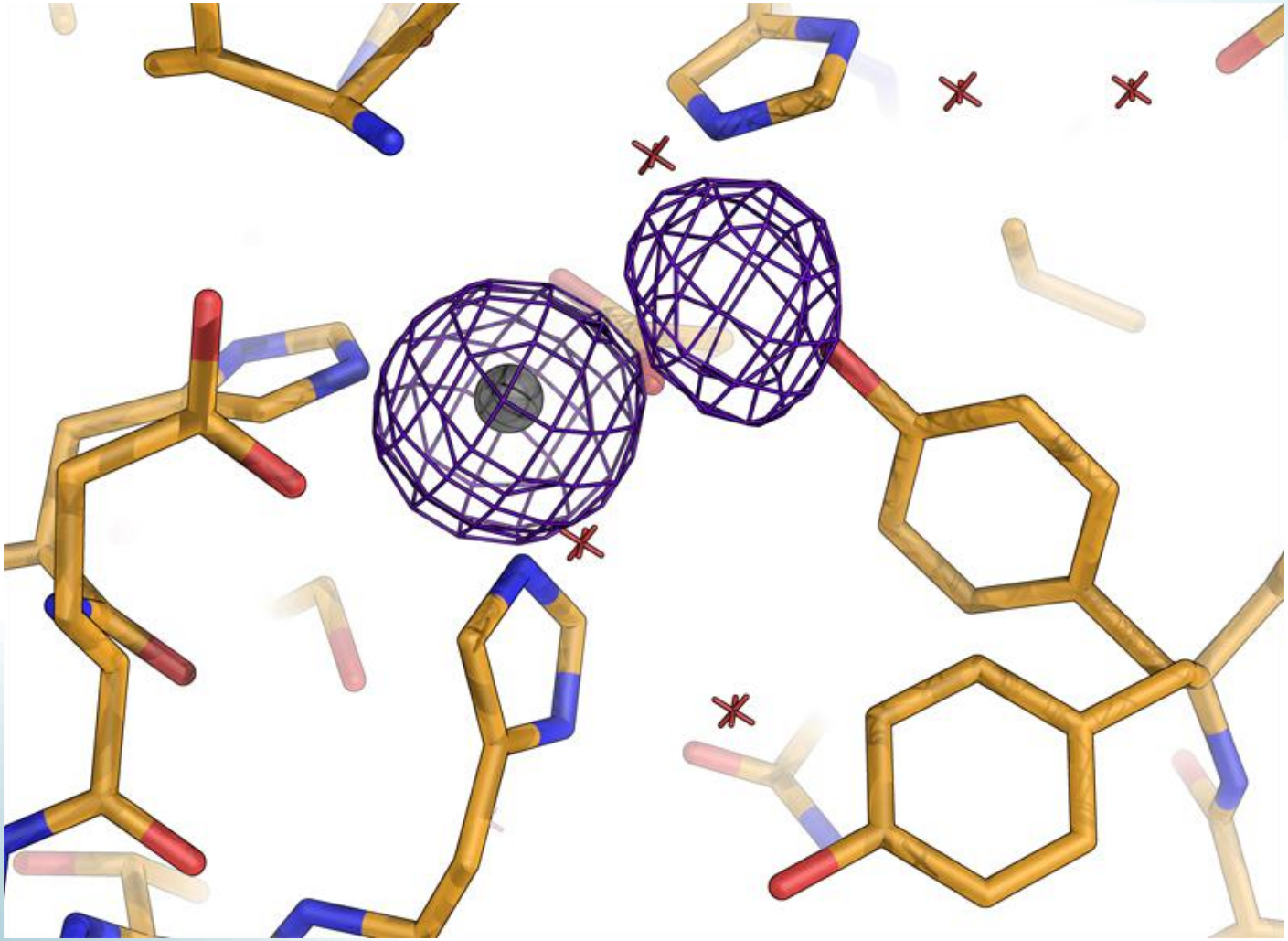
Zn-MAD

Peak height over σ as given by ANODE

Data	3-Wavelengths	Inflection point	Peak	High energy remote
Experiment	MAD	SAD	SAD	SAD
Zn^{2+}	82.5	55.7	66.4	56.0
Ca^{2+} (mean)	11.2	15.1	11.1	12.8
SD_Met (mean)	1.8	3.5	2.3	2.9
Unknown	28.5	18.2	24.7	20.1
Ratio Ca^{2+}/Zn^{2+}	0.136	0.271	0.167	0.229
Ratio Unk./Zn^{2+}	0.345	0.326	0.372	0.359

PDB 3FGD

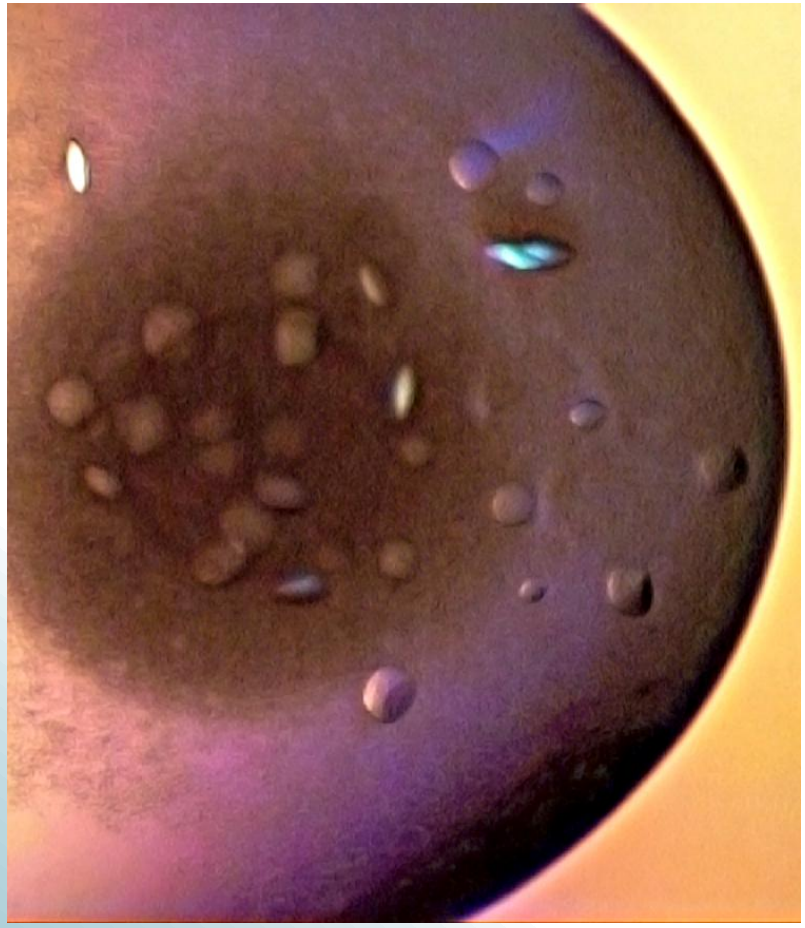
Zn-MAD



at 3.5 σ

PDB 3FGD

Hellethionin D (Cu home source)



Hellethionin D

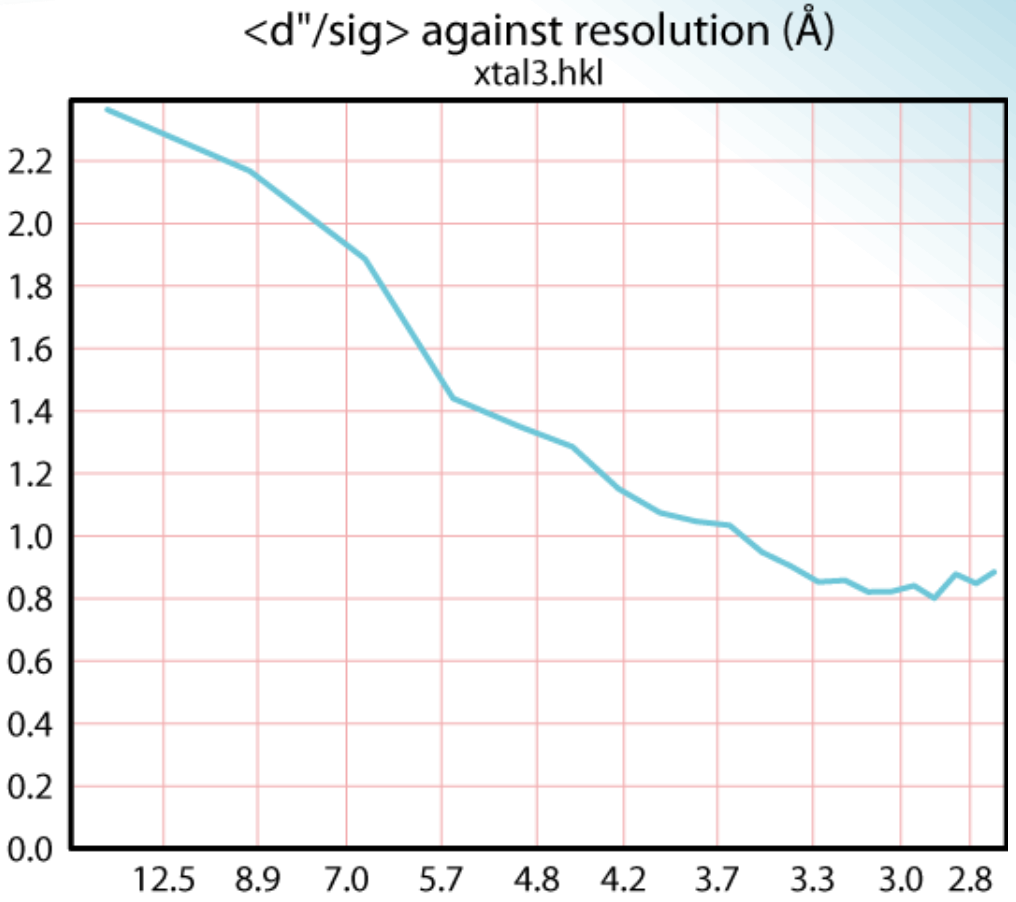
- 46 residues, 4 disulfide bridges
- ellipsoidal crystals in I422, 7 copies/ASU
- MacScience SRA Cu source with Incoatec Helios mirrors
- Chloride and sulphur present
- high multiplicity

The structure could not be solved by S-SAD.

Holland et al. (1995). *Protein Sci.* 4, 1955-1965.

Hellethionin D (Cu home source)

Wavelength (Å)	1.542
Resolution (Å)	34.10 – 2.70 (2.80 – 2.70)
Completeness (%)	98.6 (85.5)
Multiplicity	99.7 (85.1)
Friedel-compl. (%)	98.6 (85.1)
Mean I/σ	38.45 (17.39)
R_{int} (%)	15.21 (35.75)
R_{pim} (%)	1.51 (3.57)
d“/σ	1.08 (0.86)



Weak signal - not suitable for S-SAD phasing, high multiplicity.

PDB 3SZS, unpublished

Hellethionin D (Cu home source)

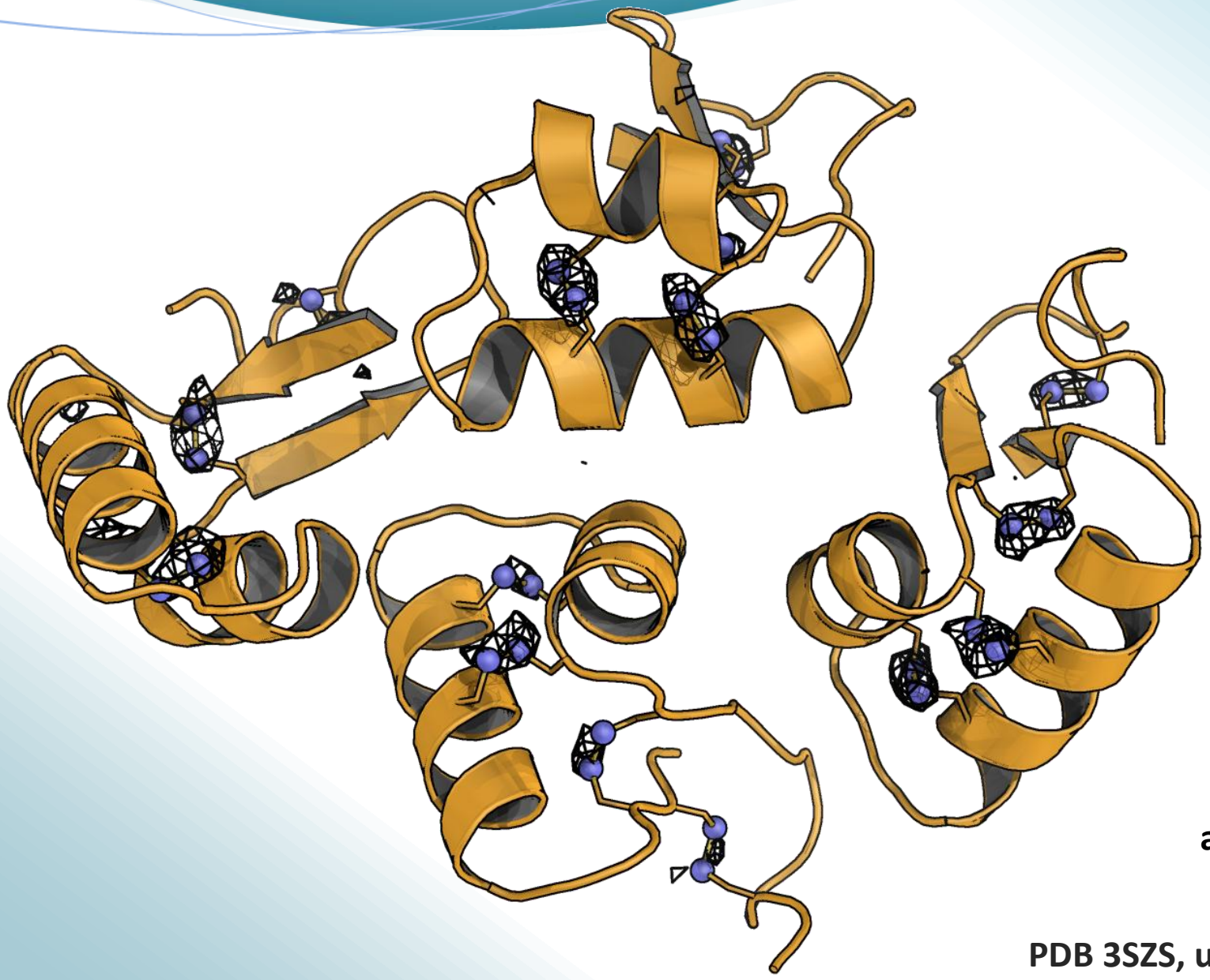
```
 Averaged anomalous densities (sigma)
   4.76  SG_CYS
   2.40  CL_CL
   0.65  BO_HOH
   0.22  NA_NA
(...)

 Strongest unique anomalous peaks
          X         Y         Z   Height(sig)   SOF     Nearest atom
 S1    0.50000   0.50000   0.50000    10.21     0.125   35.051  NZ_A:LYS1
 S2    0.60858   0.20837   0.18402     7.63     1.000    0.603  SG_E:CYS26
 S3    0.57538   0.26385  -0.04962     7.52     1.000    0.623  SG_C:CYS16
 S4    0.47052   0.22168   0.09210     7.38     1.000    0.417  SG_G:CYS26
(...)

S52   0.56305  0.41309  0.08752    4.10    1.000    1.899  SG_A:CYS40
   52 Peaks output to file xtal3_fa.res
```

PDB 3SZS, unpublished

Hellethionin D (Cu home source)

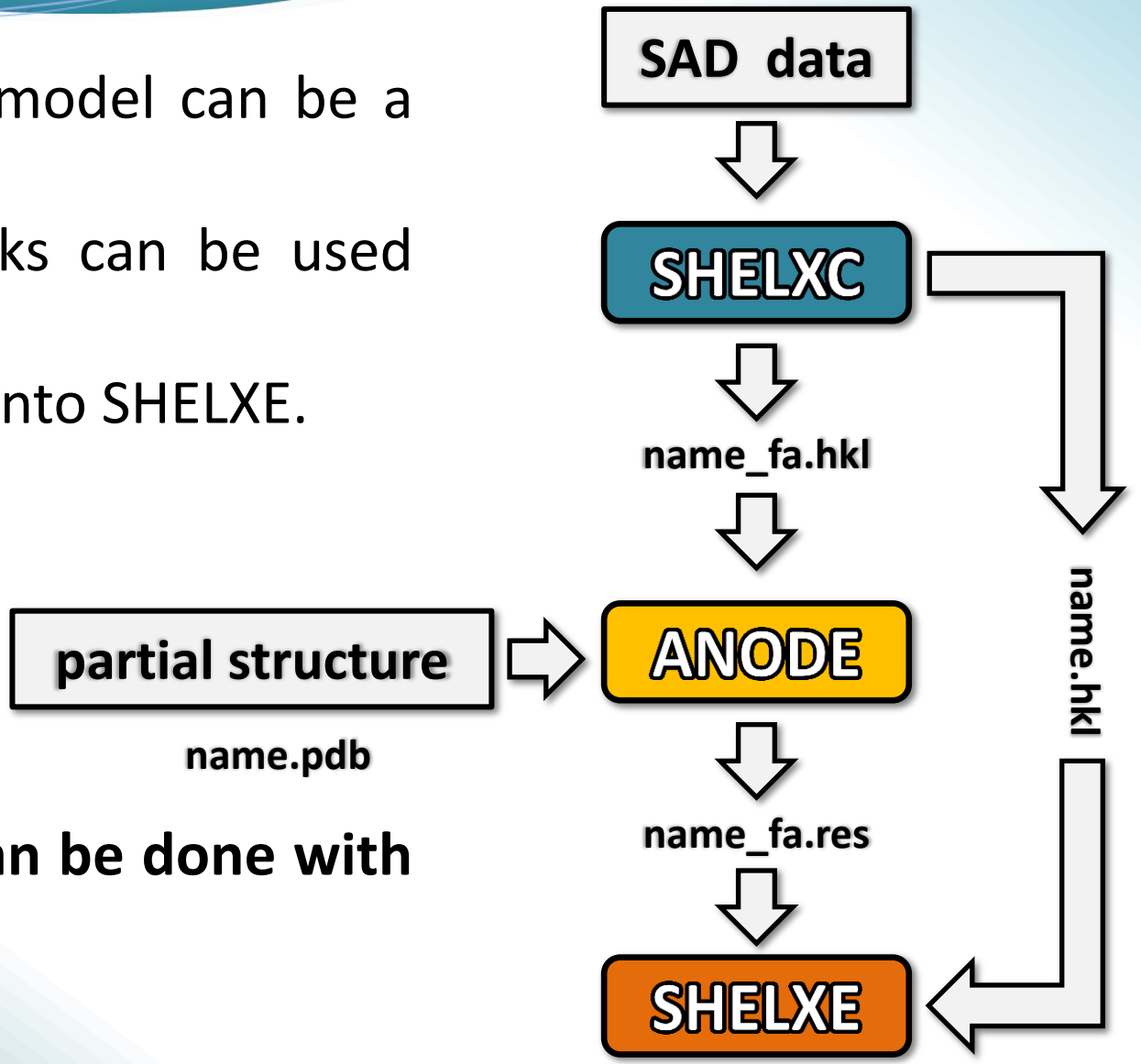


at 3.0 σ

PDB 3SZS, unpublished

MR-SAD

- The input PDB model can be a MR solution.
- Anomalous peaks can be used as substructure.
- This can be put into SHELXE.



Hence, MR-SAD can be done with ANODE.

MR-SAD

ANODE output with different models as input:

input	highest peak (σ)	correct	CC (SHELXE)*
PHASER solution	4.713	12	6.66%
ARCIMBOLDO solution	9.905	54	31.93%
final structure	12.273	60	32.10%

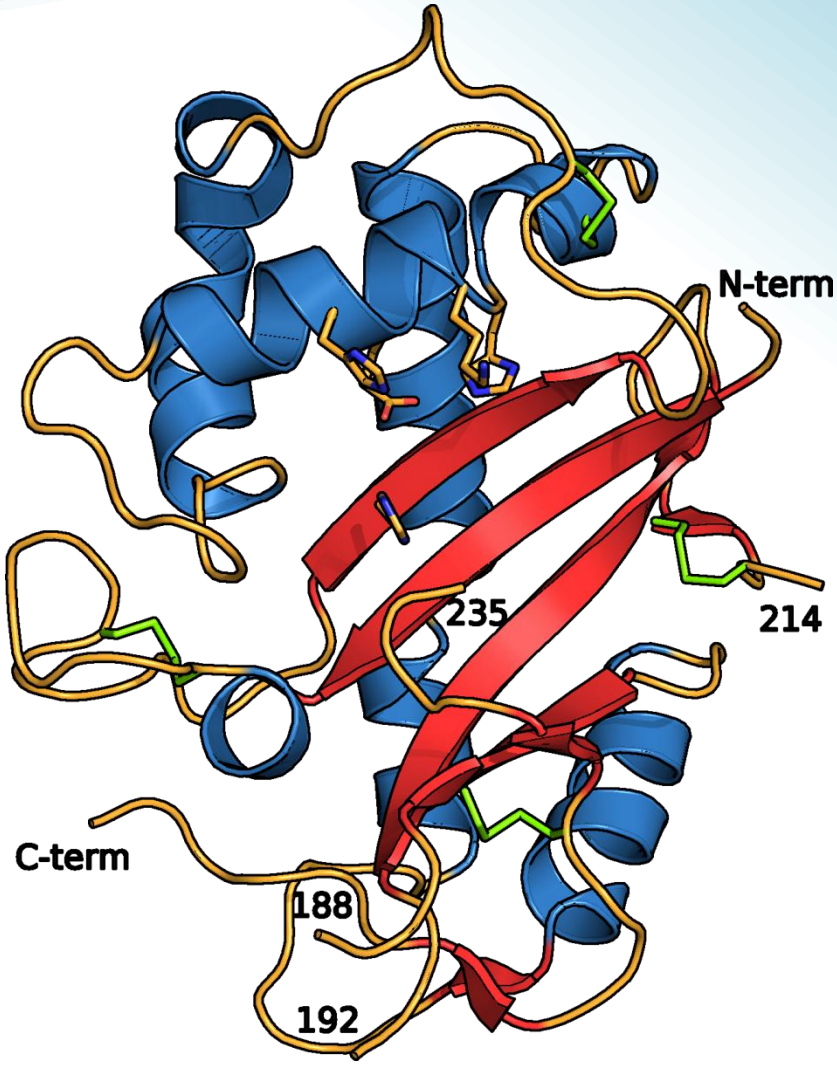
* A value over 25% usually indicates a correct structure solution.

Phaser: McCoy et al. (2007) *J. Appl. Cryst.* 40, 658
Arcimboldo: Rodriguez et al. (2010) *Nat. Methods* 6, 651

Radiation damage from one data set

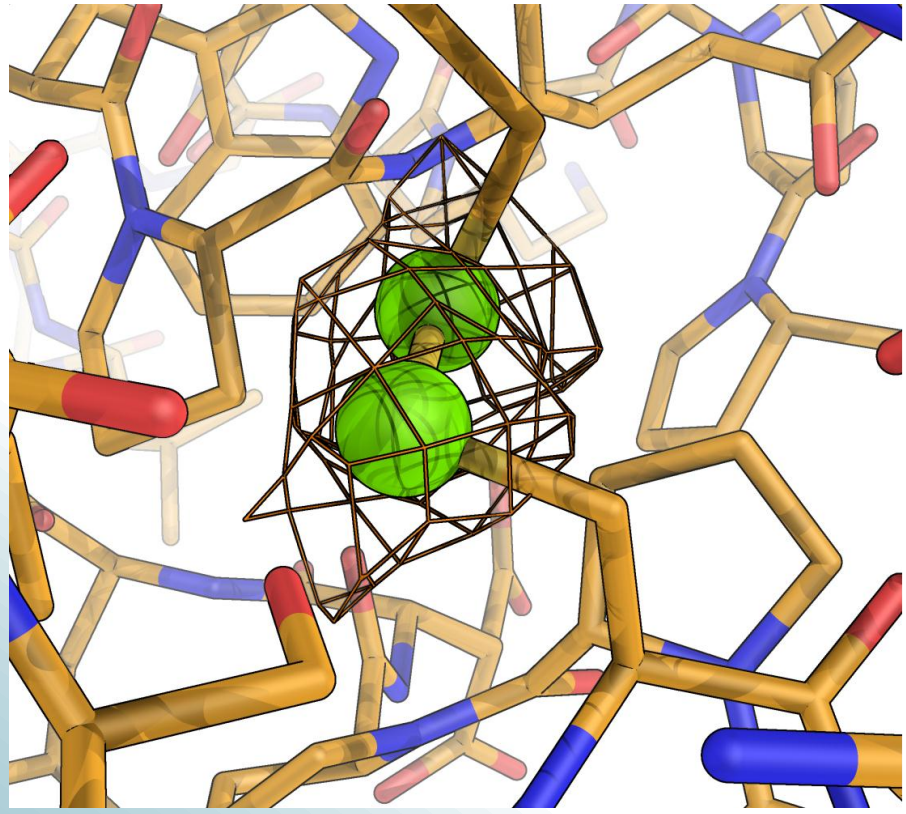
- Human RNase T2
- 252 residues
- SAD data set from BESSY 14.1
- $P2_1$; 1 monomer/ASU
- resolution 2.2 Å
- multiplicity 7.16
- four disulfide bridges

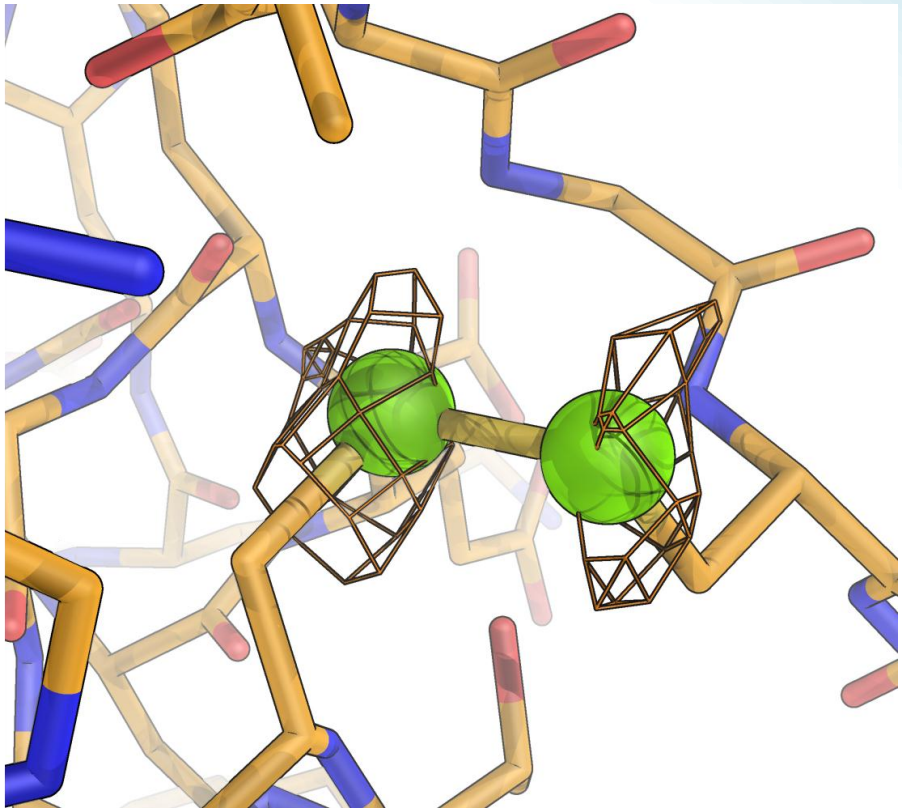
...which look different in ANODE.



PDB 3T0O, Thorn et al. (2012) Nuc. Acids Res., epub

Radiation damage from one data set





at 2.2 σ

PDB 3T0O, Thorn et al. (2012) Nuc. Acids Res., epub

Radiation damage from one data set

- Human Rnase T2
- 252 residues
- SAD data set from BESSY 14.1
- $P2_1$; 1 monomer/ASU
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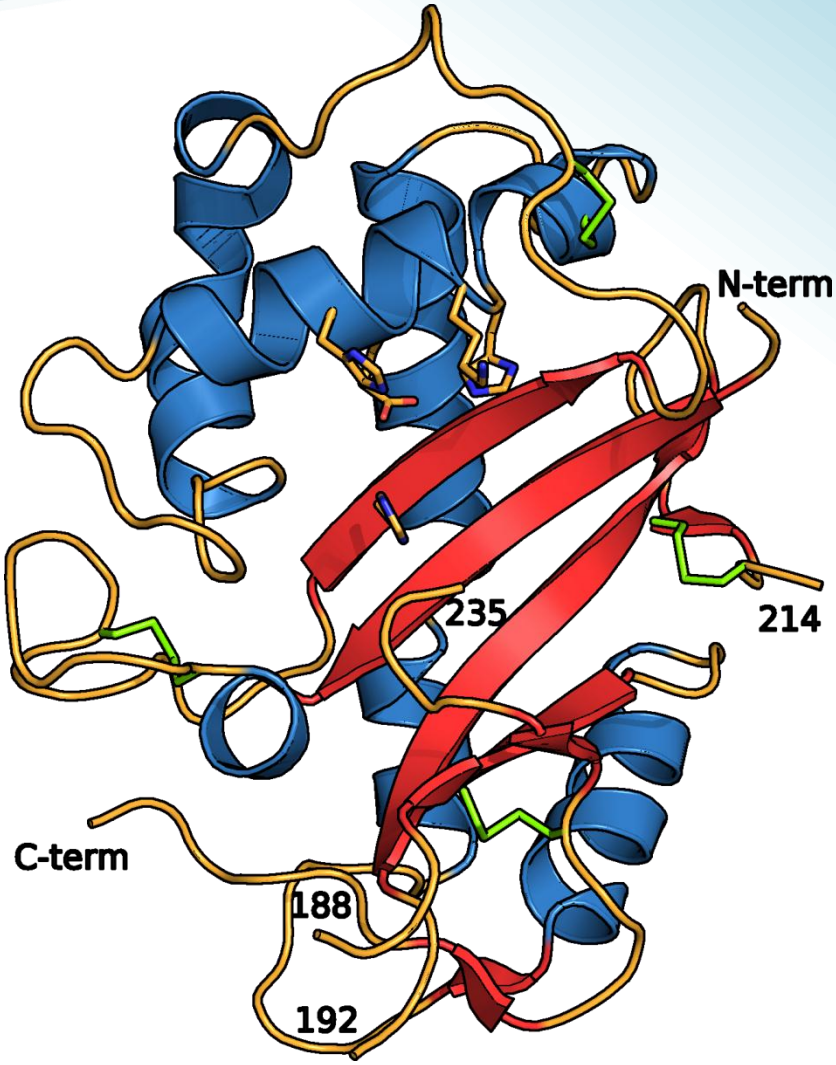
- four disulfide bridges

...which look different in ANODE.



PDB 3T0O, Thorn et al. (2012) Nuc. Acids Res., epub

RIP: Visualizing Radiation damage reactions

- Thaumatin RIP data from Max Nanao
- ESRF MAD beam line ID14-EH4
- Two data sets: Before and after radiation damage

How can the chemical changes by the radiation damage be assessed with ANODE?

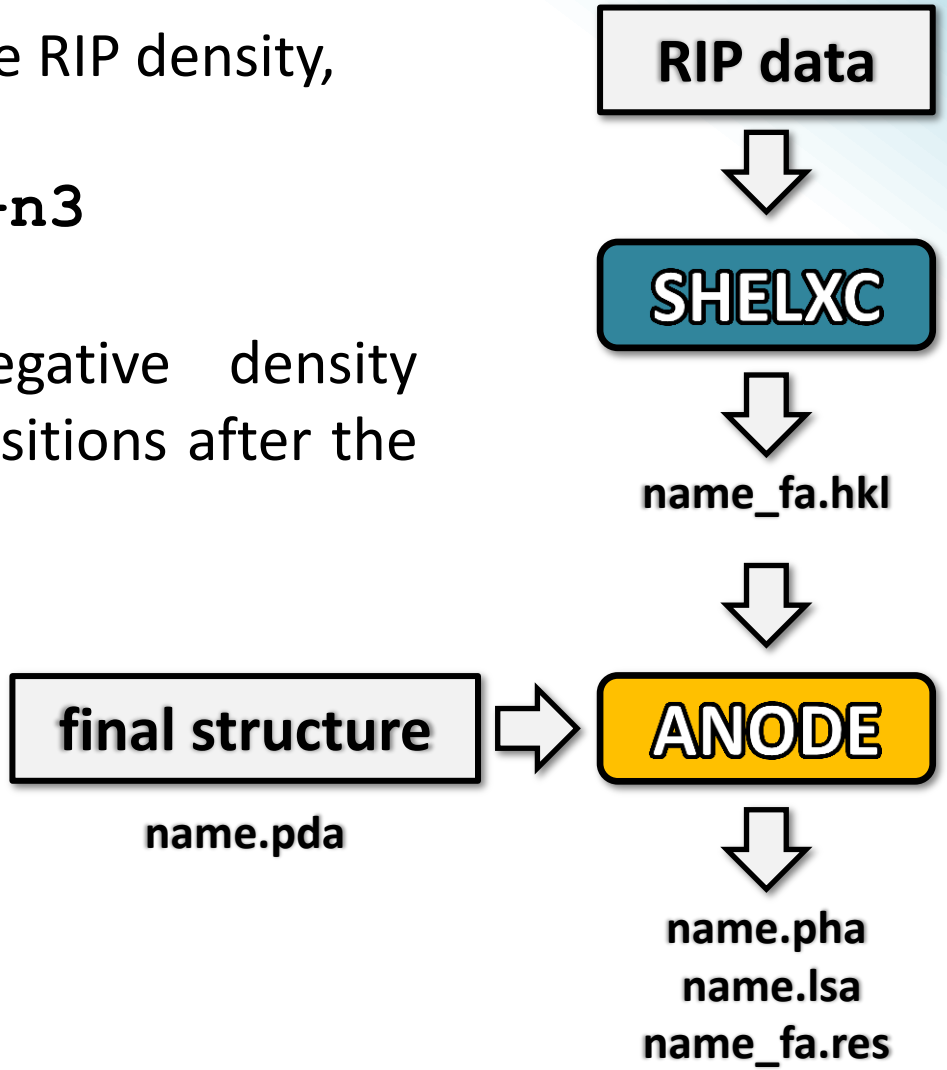
Nanao et al. (2005) *Acta Crystallogr. D*61, 1227

RIP: Visualizing Radiation damage

To obtain negative and positive RIP density,

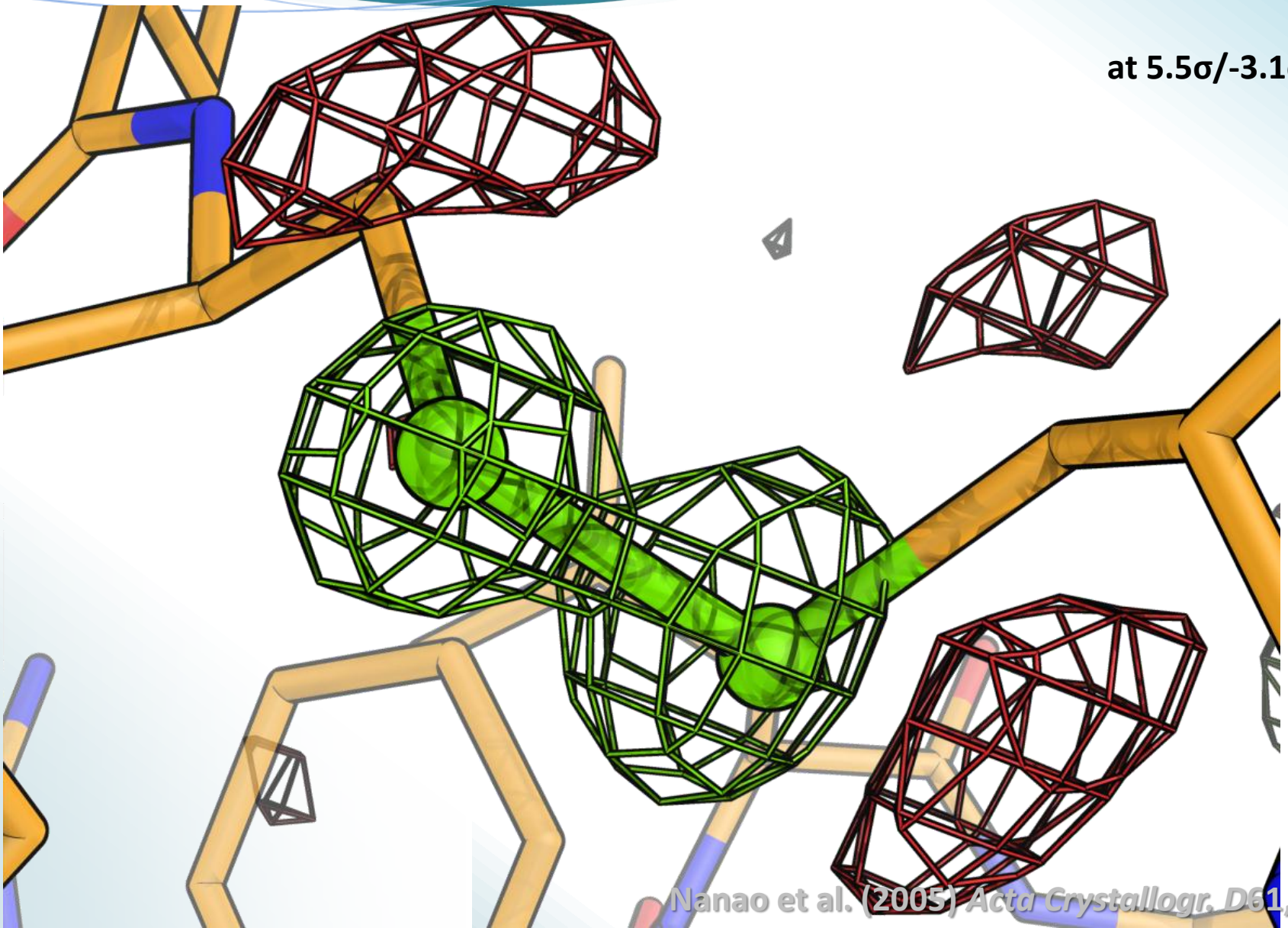
```
anode name -n3
```

has to be used. The negative density corresponds to the atomic positions after the radiation damage.



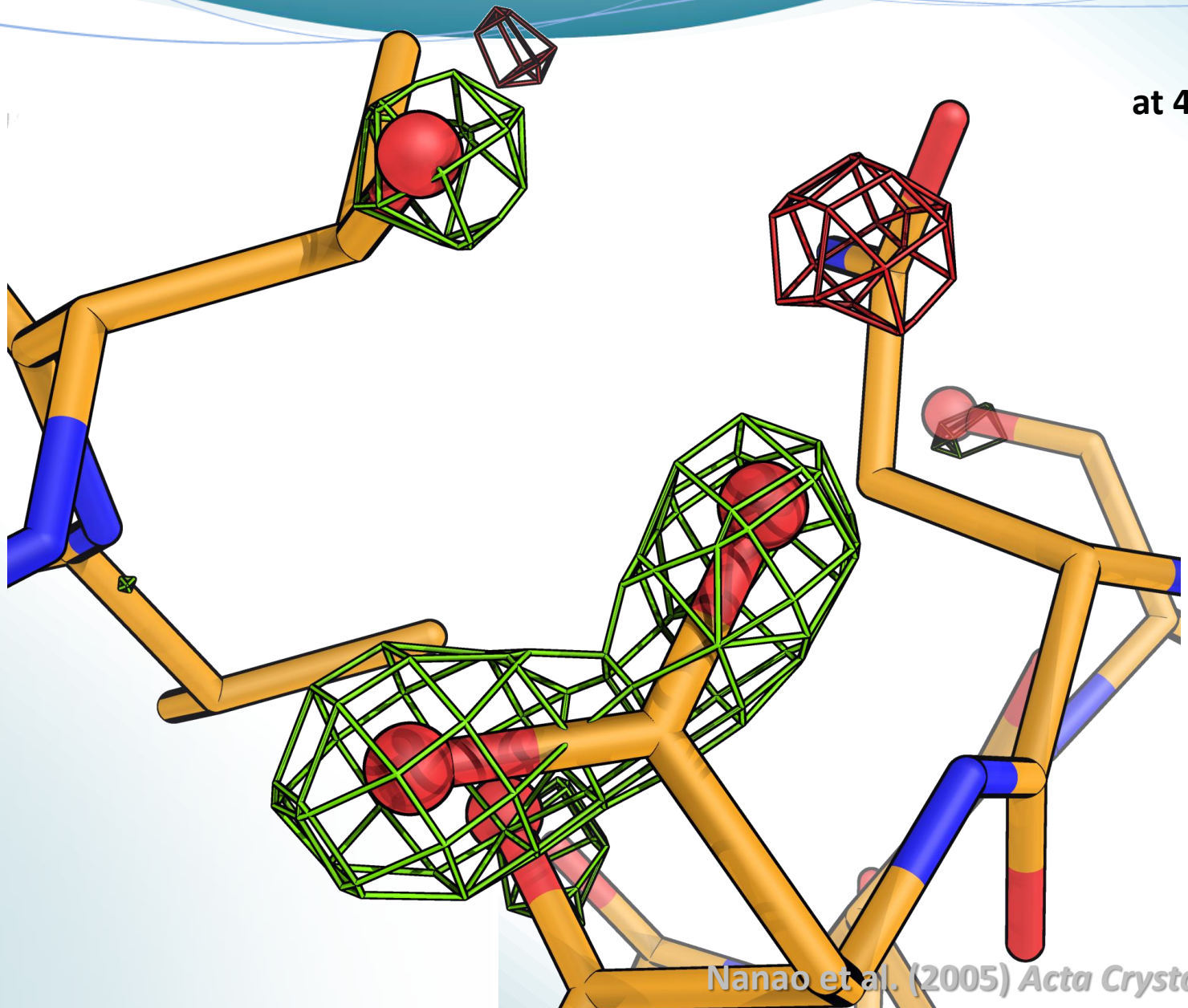
Nanao et al. (2005) *Acta Crystallogr. D*61, 1227

RIP: Visualizing Radiation damage



Nanao et al. (2005) *Acta Crystallogr. D61*, 1227

RIP: Visualizing Radiation damage



at 4.8 σ / -3.1 σ

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

Conclusion

- Effective and fast way to analyse/visualize anomalous scatterers, radiation damage and heavy atoms
- Works well with weak signal
- Identification of atom types and structure validation
- MR-SAD or validation of MR solutions
- Available at **http://shelx.uni-ac.gwdg.de/SHELX**
- The program ANODE is a standalone EXE file
- SHELXC or XPREP is needed to set up *_fa.hkl* files

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

ACKNOWLEDGEMENTS

George M. Sheldrick (gsheldr@shelx.uni-ac.gwdg.de)

Isabel Usón, Max Nanao, Christian Große, Kevin Pröpper, Tobias Beck, Marianna Biadene, Gabor Buncoczi, Judit Debreczeni, Ina Dix, Tim Gruene, Uwe Müller, Manfred Weiss

This lecture: **http://shelx.uni-ac.gwdg.de/~athorn/** or **http://shelx.uni-ac.gwdg.de/SHELX/** in a few days' time.

MRC | Laboratory of Molecular Biology

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Webinar	Content
Apr 02, 2013 **Fast, Intuitive Structure Determination II: Crystal Indexing and Data Collection Strategies**	Attend this one-hour webinar to learn skills for crystal indexing and data collection. Dr. Bruce Noll will cover the APEX2 tools that can be used to select reflections and sort them into arrays to facilitate indexing routine, twin and modulated structures. He will also examine the diffraction behavior of the sample to determine the best data collection parameters. Register now
Feb 26, 2013 **Advanced Crystallography: Data Collection and Data Reduction Techniques for Modulated Structures**	Drs. Michael Ruf and Charles Campana give a one-hour presentation demonstrating the steps required to collect and process data for modulated structures. Specific topics include: how to recognize modulated structures; introduction to q-vectors; APEX2 software tools; preparation of files for JANA2006; and examples of the solution of modulated structures from single-crystal diffraction data. View recording Download slides
Dec 05, 2012 **PROTEUM2 and Foreign Frame Formats**	View this 45-minute webinar to learn about Bruker's PROTEUM software, a suite of crystallographic data integration and reduction tools for macromolecules. Dr. Matt Benning and Dr. Michael Ruf will discuss how to reduce data from various detectors and show results from synchrotron beamlines. View recording Download slides
Nov 13, 2012 **Advanced Crystallography: Refinement of Disordered Structures**	Dr. Charles Campana gives a one-hour presentation demonstrating the steps required to refine disordered crystal structures. Along with specific examples, topics covered include SHELXL, SHELXTL, free variables, PART n and –n instructions, constraints vs restraints, and advanced refinement instructions. View recording Download slides
Dec 06, 2011 **High-Pressure Crystallography**	Dr. Francesca Fabbiani of Georg-August-Universität Göttingen takes you through the steps for performing a high-pressure single-crystal X-ray diffraction experiment with a diamond-anvil cell (DAC). You will learn DAC loading techniques and useful strategies for data collection, data reduction, and structure refinement. View recording Download slides
Oct 13, 2011 **Advanced Crystallography – Publication of Crystal Structures**	Publication of crystal structures is often a researcher's least favorite task. This 90-min webinar will help. It describes the steps required to prepare crystal structures for publication in chemical and crystallographic journals, including collection of high-quality intensity data, common refinement problems, data evaluation, error analysis, preparation of CIF files, and the use of CIF-checking programs. View recording Download slides
Jun 22, 2011 **CMOS: The Next Generation in X-ray Detector Technology**	CCDs have long been one of the most important detector technologies for X-ray crystallography. Recently, however, new detectors based on CMOS technology have begun to supplant CCDs. View this webinar to learn about Bruker AXS' new X-ray detector based on CMOS technology, the PHOTON 100. View recording Download slides
May 03, 2011 **Using APEX2 to Analyze Twinned Crystals**	Twinned crystals are common occurrences in many laboratories and pose special challenges in structure solution. View this 76-minute webinar to learn how to visualize, index, integrate, scale, solve and refine structure data from twinned crystals with the APEX2 software suite. (Note: playback begins at 14:00 minutes into the 1:30:20 hour presentation.) View recording Download slides
	Accurate 3D structures determined by X-ray diffraction require

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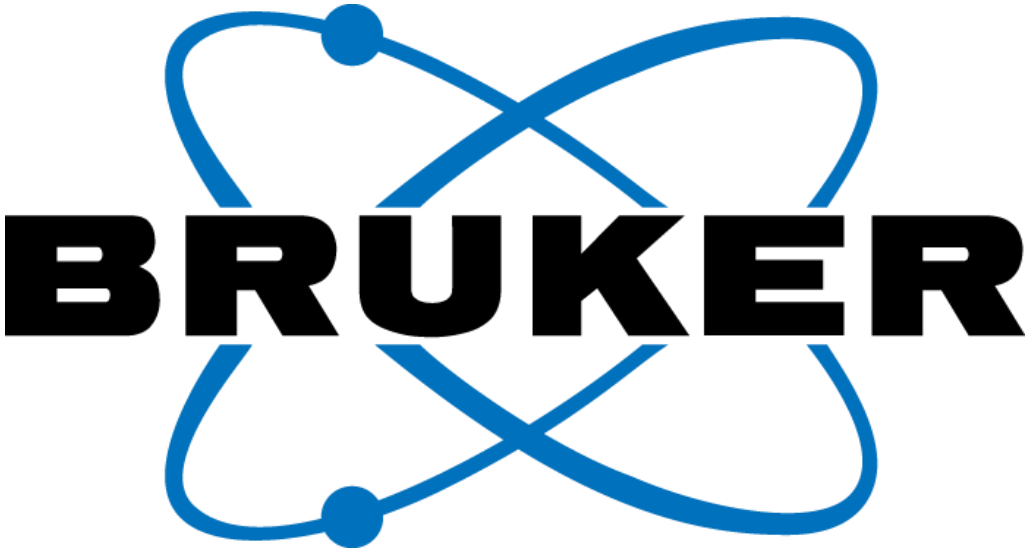

Innovation with Integrity