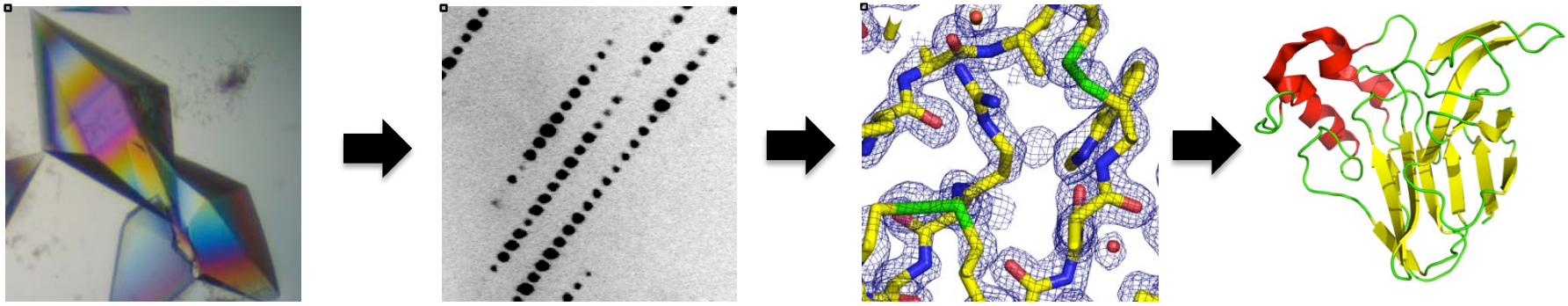
Soft X-rays in the Home Lab: Native SAD Phasing with Chromium and Copper Radiation

J.W. Pflugrath and Mark Del Campo
Rigaku Americas

Jim.Pflugrath@Rigaku.com



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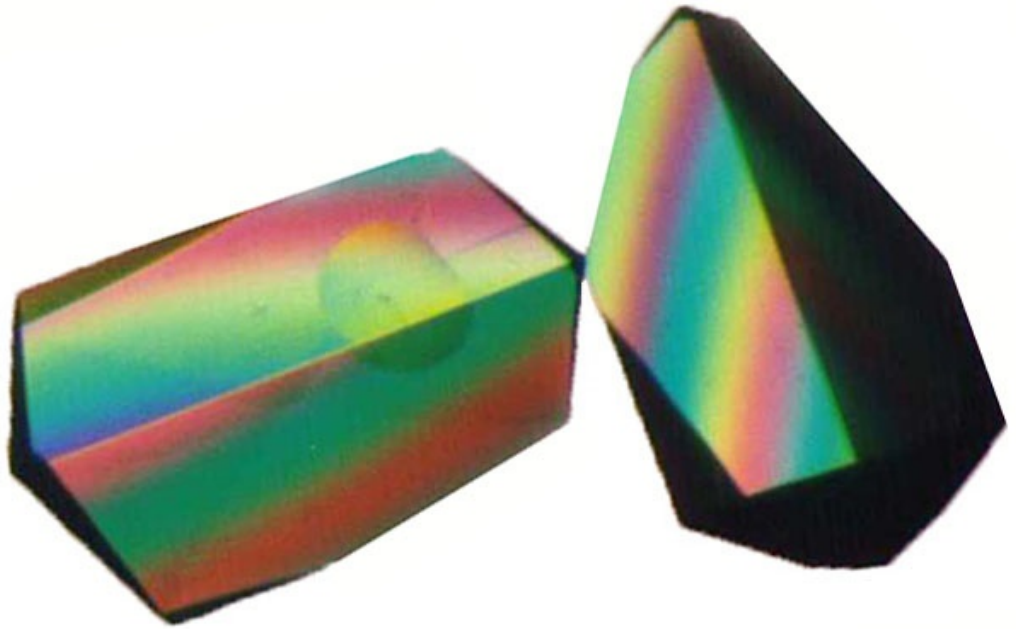
Outline of the seminar

- Hardware
- Software
- Examples
- Speculation



"… on the shoulders of giants."

- CuK_α or CrK_α radiation from a home source
  - Blow, D.M. Proc. of the Royal Society 247, 302-336 (1958).
  - Hendrickson,W.A. and Teeter, M.M. Nature 290,107-113 (1981).
  - Wang, B.C. Methods in Enzymology, 115, 90-112 (1985).
- Synchrotron radiation near the sulfur absorption edge
  - Stuhrmann, S., Bartels, K.S., Braunwarth, W., Doose, R., Dauvergne, F., Gabriel, A., Knöchel, A., Marmotti, M., Stuhrmann, H.B., Trame, C. and Lehmann, M.S. J. Synchrotron Rad. 4, 298-310 (1997).
  - Behrens, W., Otto, H., Stuhrmann, B. and Heyn, M.P. Biophys. J. 75, 255-266, (1998).
- Synchrotron radiation at 1.54 or 2.0 Å wavelength
  - Dauter, Z., Dauter, M., de La Fortelle, E., Bricogne, G. and Sheldrick, G.M. J. Mol. Biol. 289, 83-92 (1999).
  - Cianci, M., Rizkallah, P.J., Olczak, A., Raftery, J., Chayen, N.E., Zagalsky, P.F. and Helliwell, J.R. Acta Cryst. D57, 1219-1229 (2001).

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Background

History of Sulfur Phasing:

1981 : Hendrickson & Teeter (Nature), crambin (4.72kDa) with S-RAS (resolved anomalous scattering) method.

1985 : BC Wang (Methods in Enzymology), simulated a S-SAD phasing of protein RHE using iterative single-wavelength anomalous scattering (ISAS) method.

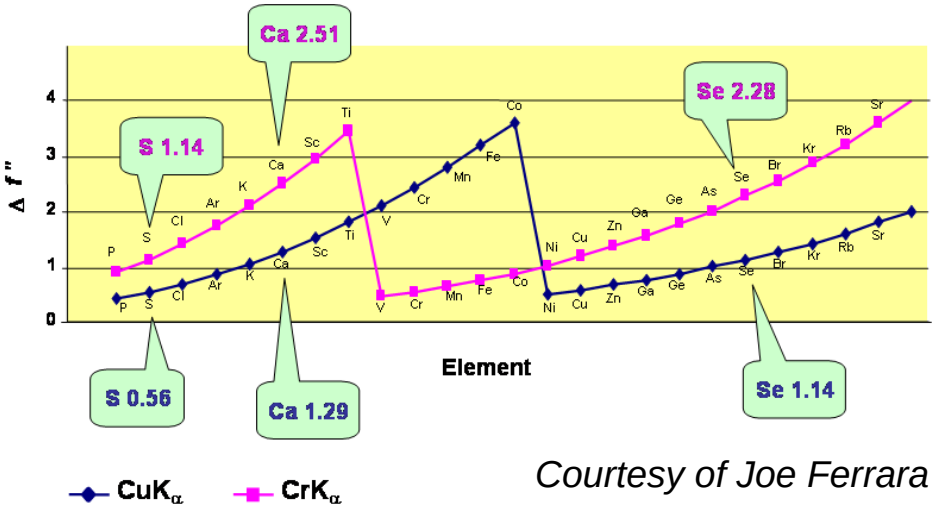
1999 : Dauter (Acta Cryst D), feasibility study on lysozyme.

2000 : Liu, etc (Protein Sci), new structure of obelin, λ=1.74Å, ISAS method.

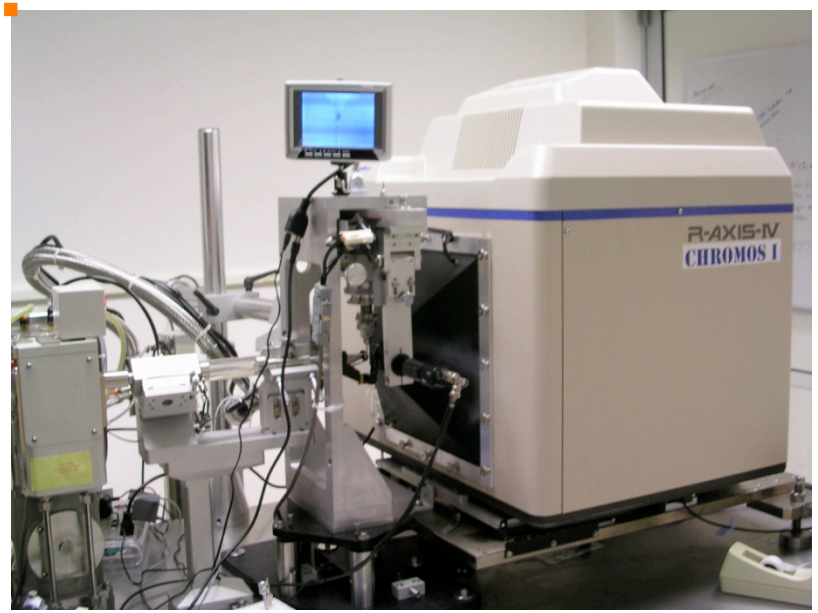
Courtesy of Zhi-Jie Liu, IBP

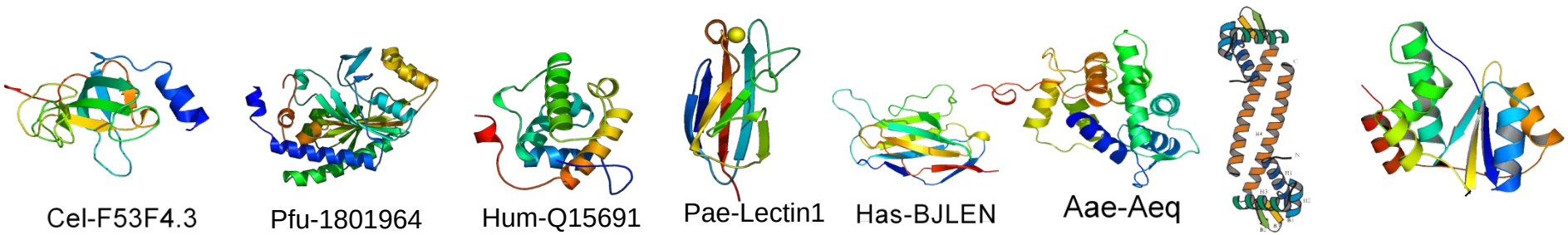
Background

Structures determined by Sulfur-SAD phasing using Cr X-rays



Courtesy of Joe Ferrara





Cel-F53F4.3 | Pfu-1801964 | Hum-Q15691 | Pae-Lectin1 | Has-BJLEN | Aae-Aeq | SSO10A | PFU 542154

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Courtesy of Zhi-Jie Liu, IBP

50 residues / sulfur

Our Home Lab Setup

- FR-E+ Dual Wavelength
- VariMax DW Multilayer Optic
- HTC Imaging Plate
  - Helium Beam Path

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X-ray Generator: FR-E+ SuperBright

- Highest brilliance microfocus rotating anode generator
  - 2.475 kW power (45 kV 55 mA)
  - 70 µm focal spot size
- Delivers **16 x 10^{10}** Cu *Kα* photons/ second/mm^2
  - for a 100 µm sample at 4.8 mR

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µXG Performance Comparison

	Rigaku MM-007 HF VariMax-HF	Rigaku FR-E+ VariMax-HF	Rigaku MM-003
Focal Spot Size (µm)	Ø70	Ø70	Ø30
Power (kW)	1.2	2.475	0.03
Beam Size at Sample (µm)	208	208	100
Divergence (mR)	4.8	4.8	7.3
Useable flux w/ 0.1 mm aperture (X-ray/sec/mm^2)	7.8×10^{10}	16×10^{10}	1.4×10^{10}
Normalized Useable Flux (RU + Blue)	**19.5**	**40**	**3.4**

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µXG Performance Comparison

micro-focus rotating anode

	Rigaku MM-007 HF VariMax-HF	Rigaku FR-E+ VariMax-HF	Rigaku MM-003
Focal Spot Size (µm)	Ø70	Ø70	Ø30
Power (kW)	1.2	2.475	0.03
Beam Size at Sample (µm)	208	208	100
Useable flux w/ 0.1 mm aperture (X-ray/sec/mm^2)	7.8 x 10^{10}	16 x 10^{10}	1.4 x 10^{10}
Divergence (mR)	4.8	4.8	7.3
Brilliance (X-ray/sec/mm^2/mR^2 x 10^9)	3.4	7.0	0.26
Normalized Brilliance (RU + Blue)	6.07	12.5	0.47

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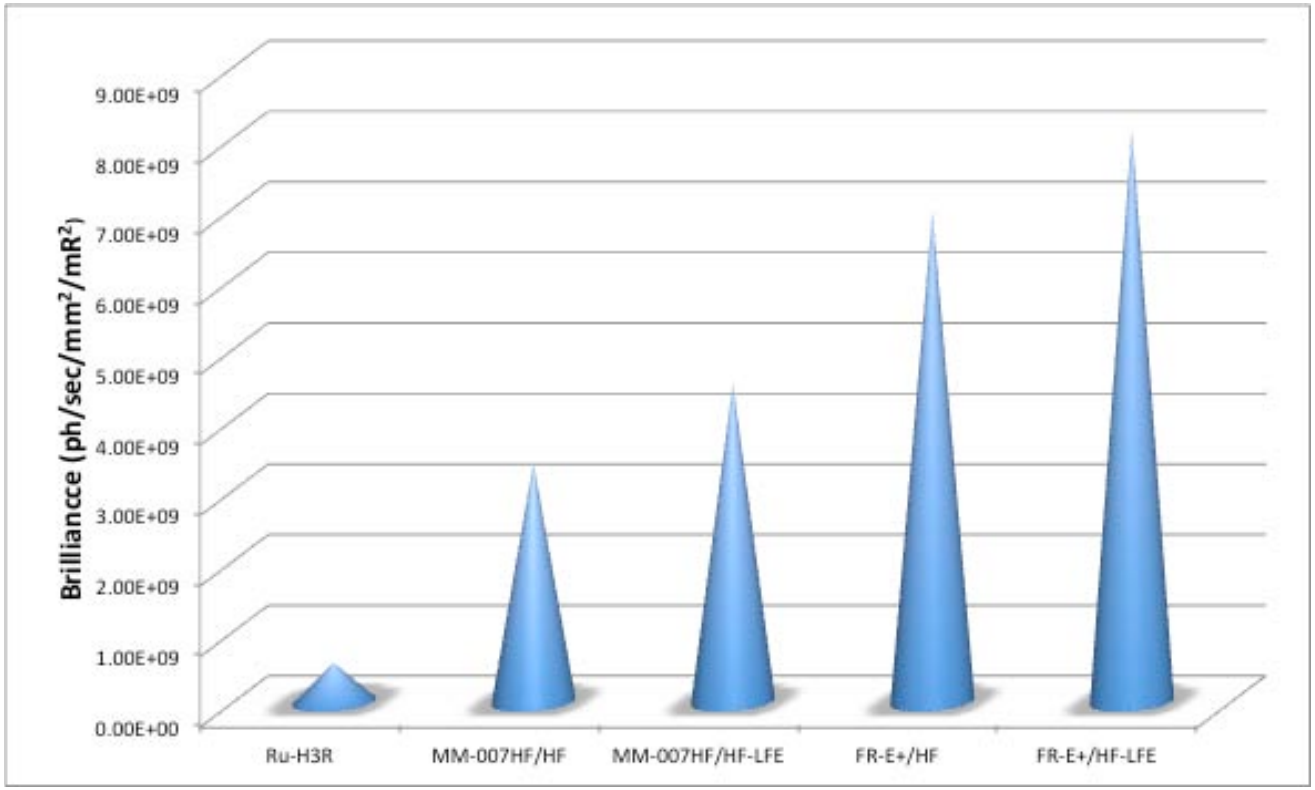
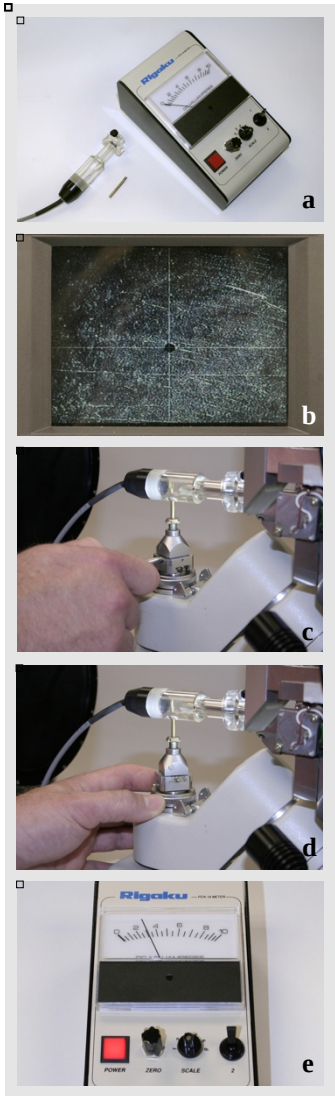
Home Lab: Like a Bending Magnet Source





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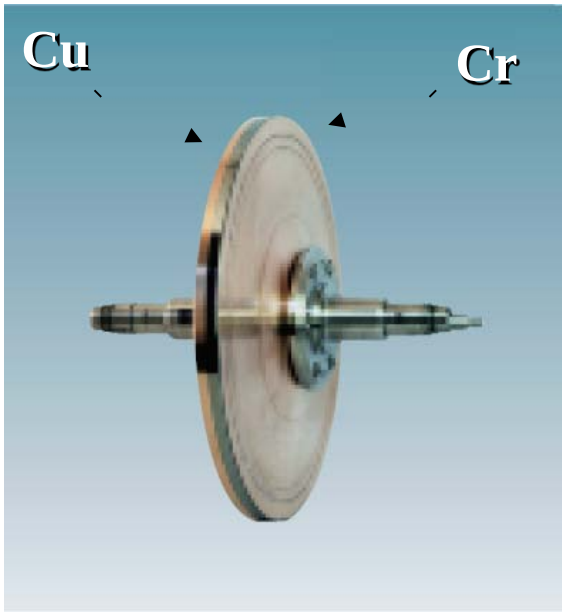
Generator: FR-E+ DW SuperBright

- **Two wavelengths in one generator**
  - Cr and Cu rotating anode
  - Switchable electron gun

Ultimate HomeLab w/ FR-E+ DW SuperBright



Chrome Phasing without Derivatization

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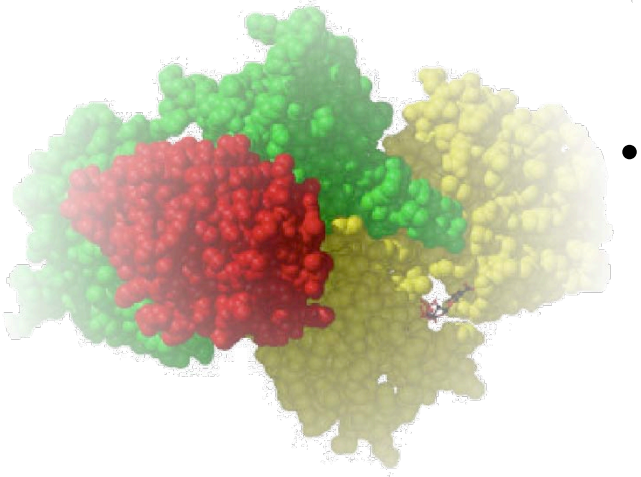
Why use Cr radiation?



- Problem
  - Scientists would like to speed up the process of solving structures and overcome the difficulty of expressing and crystallizing selenomet proteins
- Solution
  - Enhance the anomalous signal from sulfur, which is present in nearly all proteins (certainly present in the proteins for which we expect to use selenomet)
- In addition
  - Enhance the anomalous signal from some native metals, present in nearly 1/3 of all proteins
  - Enhance the anomalous signal from heavy atom derivatives, for example $\Delta f' \approx 12.6\ e^-$ for iodine

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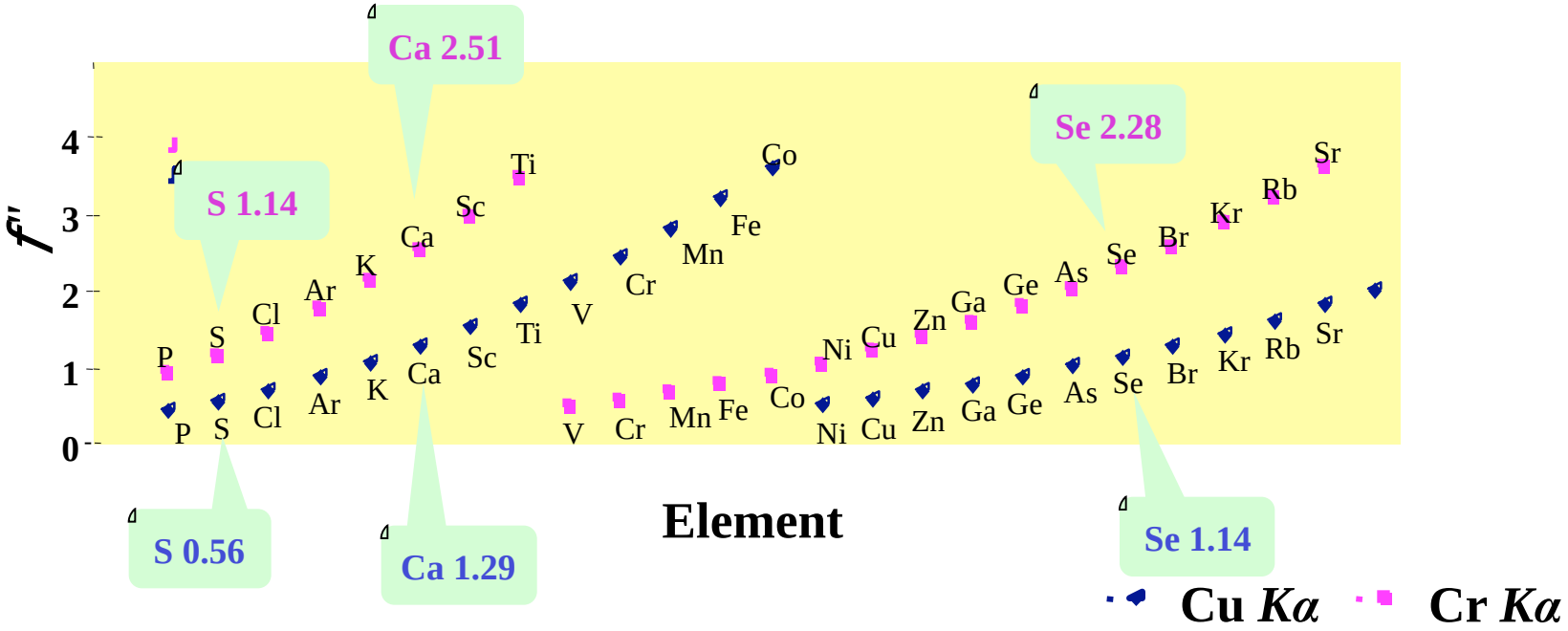
Native SAD Phasing with Cu or Cr

- Cr SAD phasing takes advantage of increasing of anomalous scattering of many intrinsic elements like S
- Obtain the phase of protein data without any derivatization.
  - Make the density map available before sending your crystal to synchrotron for the final high resolution data.



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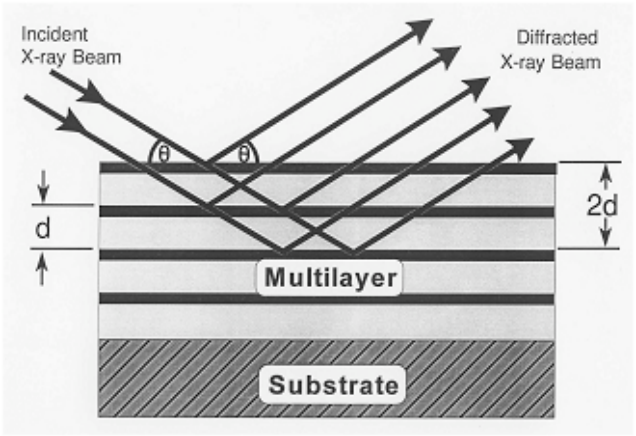
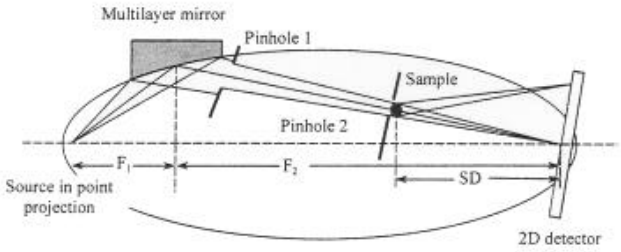
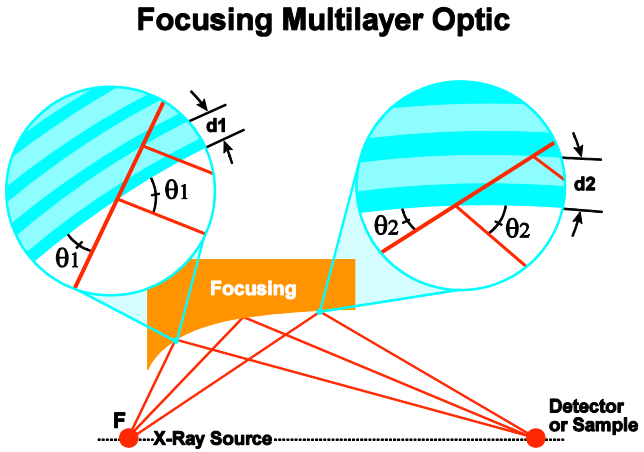
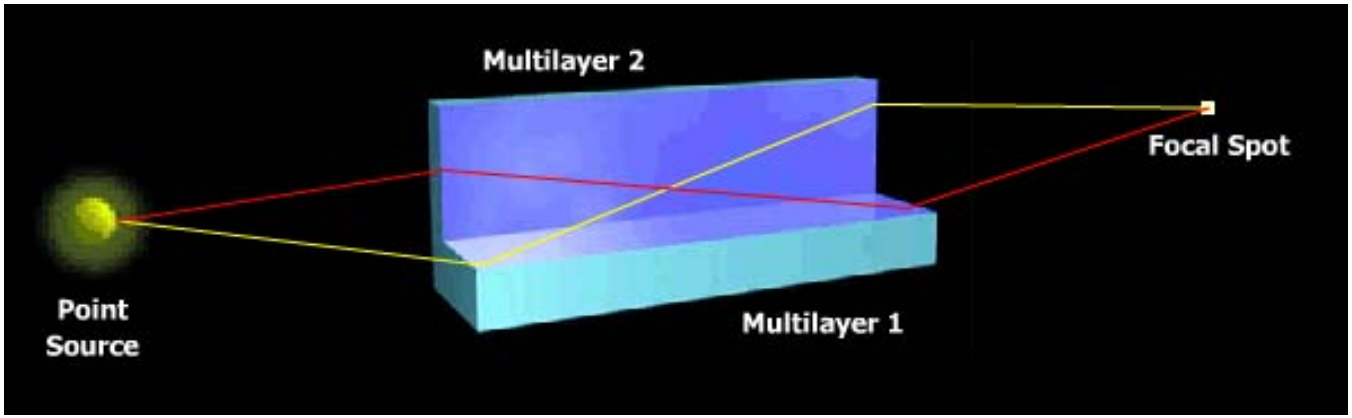
Cu $K\alpha$ 1.54 Å, 8.0 keV Cr $K\alpha$ 2.29 Å, 5.4 keV

Osmic Multilayer Optics









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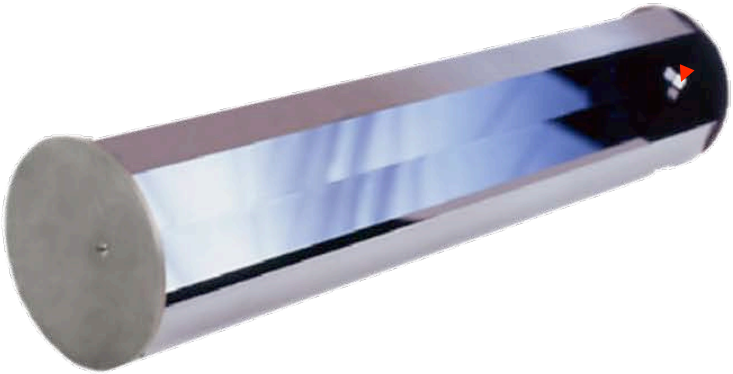
Osmic VariMax™ Optics

Variable
Divergence Slit



- VariMax HR
- VariMax HF
- VarMax VHF

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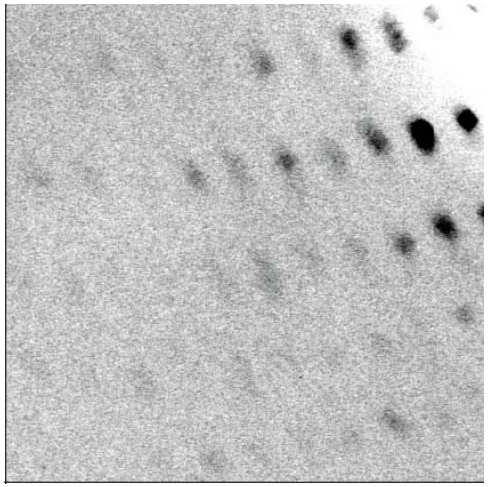
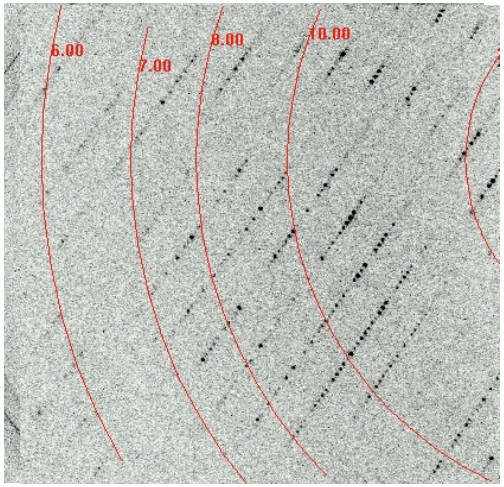
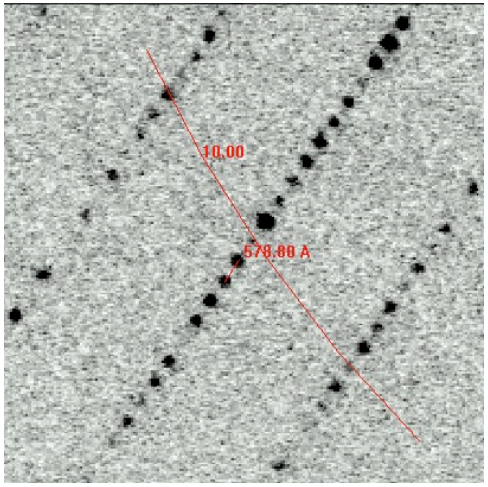
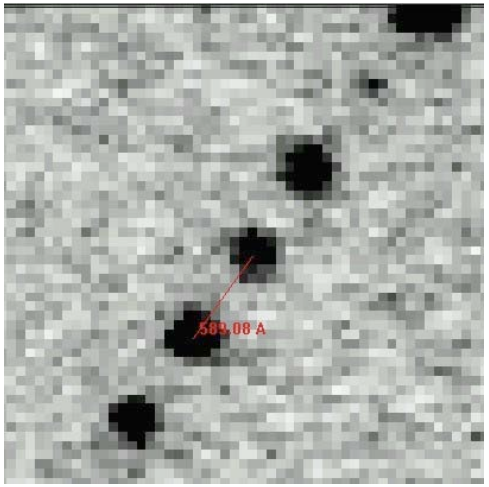
VariMax™ for Large Unit Cells



- 50S Ribosomal Subunit
  - Peter Moore, Tom Steitz and Martin Schmeing, Yale University
  - Space group: $C222_1$
  - Unit cell dimensions
    - 216.98 302.30 578.28
    - 90.00 90.00 90.00







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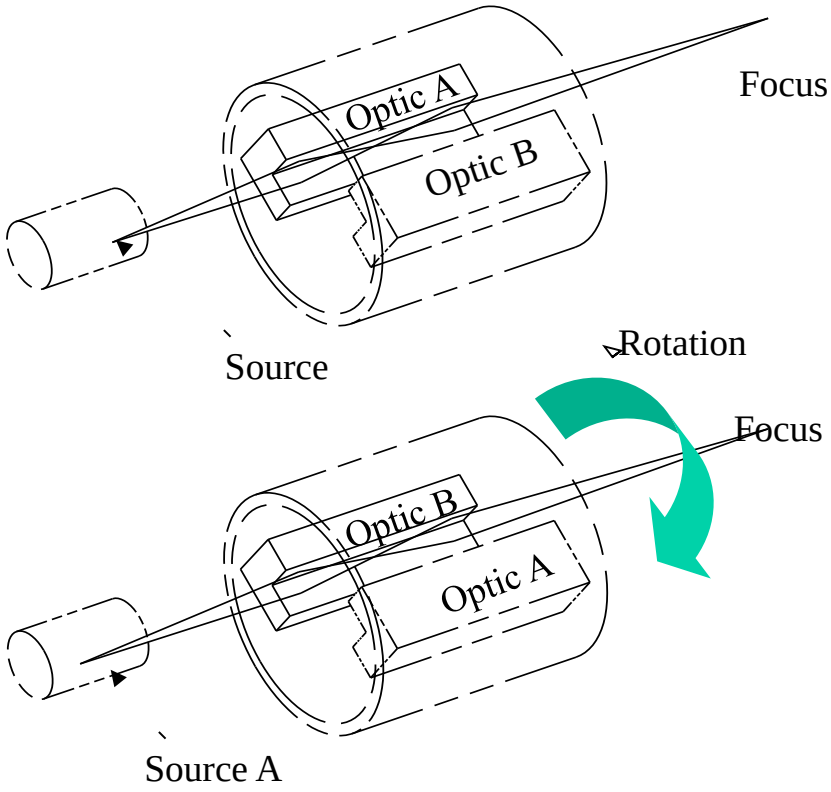
VariMax-DW

- Multiple wavelengths
- With preferred take-off angle
- Pre-aligned to each source spot
- Cross at sample position



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Rigaku patent pending

DualWave Mirror VariMax DW

Mirro
VariMax DW

Mo setting

Cu setting

Just rotate the handle

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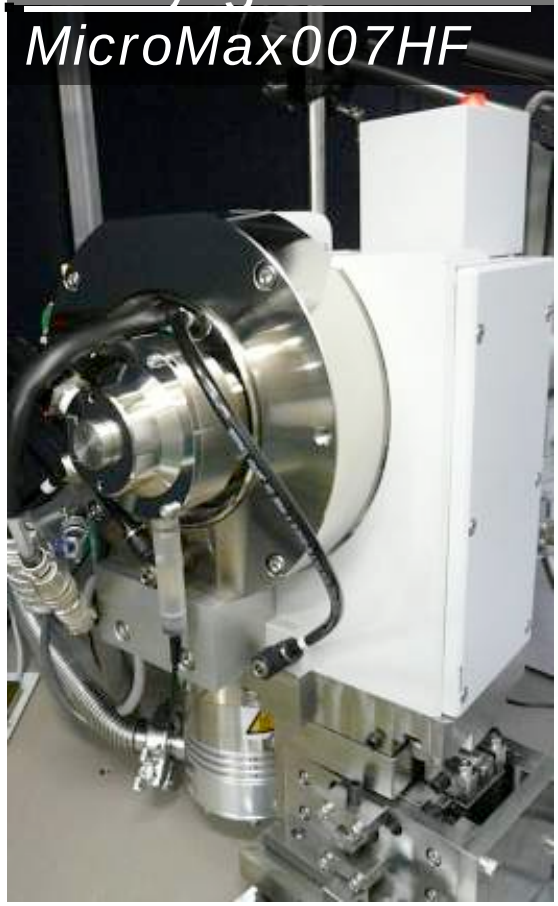
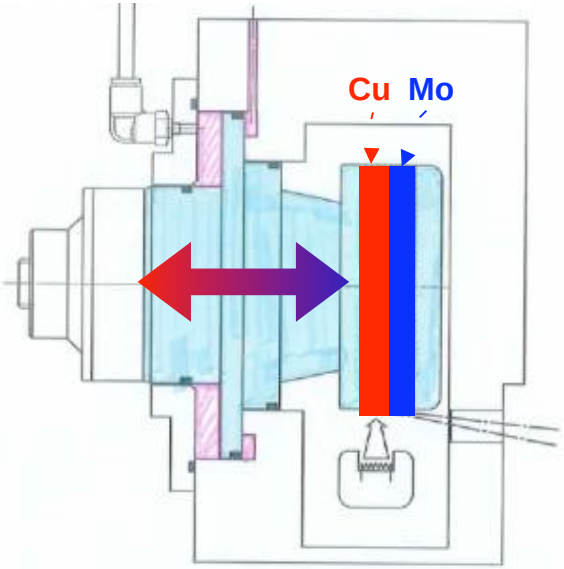
DualWave X-ray generator MicroMax007HF

X-ray generator
MicroMax007HF



Target
DW Target



Controller

<How to change>

1. X-ray OFF
2. Switch to position
3. X-ray ON

Unnecessary to remove target

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Imaging Plate Area Detectors

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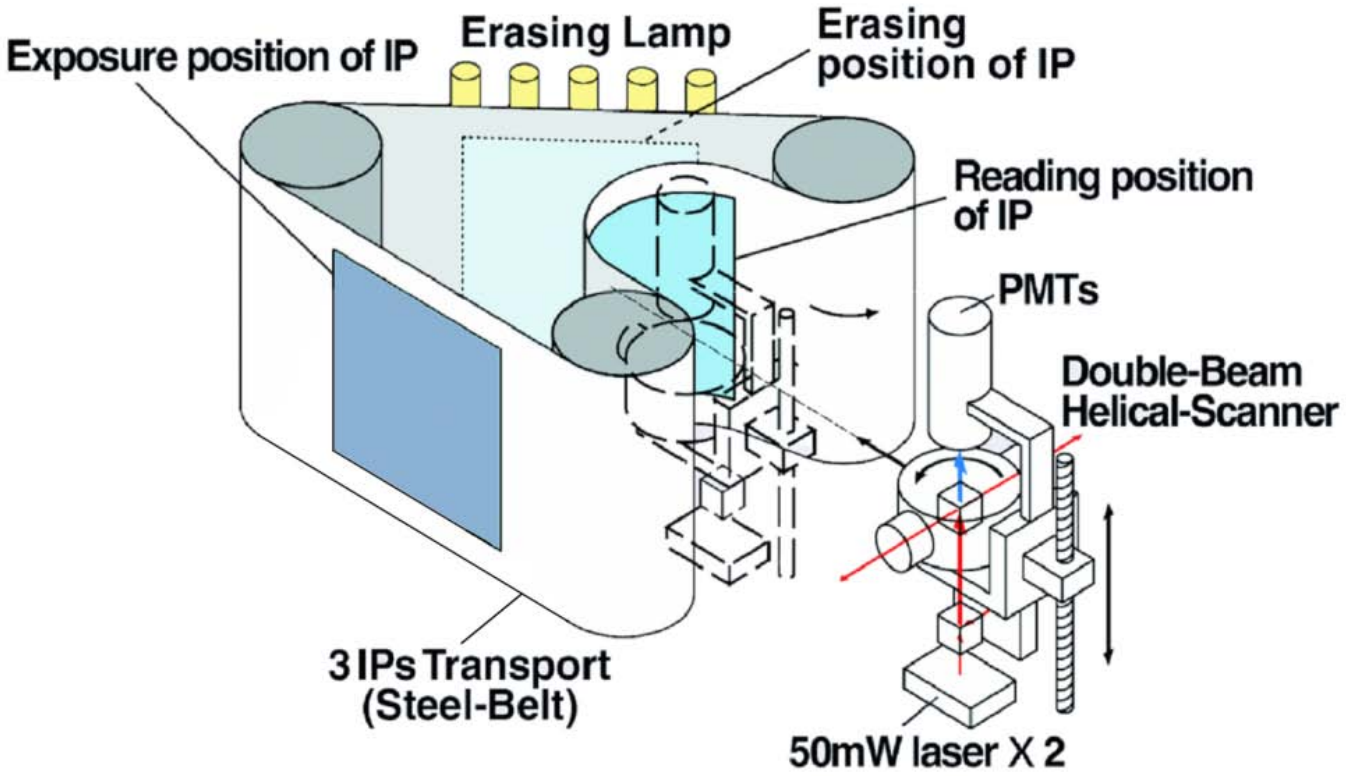
Imaging Plate Benefits

- Very low background noise
- Large solid angle
- No image distortion (no FOT)
- No calibration except for PMTs
- Wide dynamic range
- Available helium beam path
  - For chromium phasing applications
  - No large internal air gap behind the faceplate
- Supported by CrystalClear™, HKL2000™, MOSFLM and XDS

R-AXIS HTC



- 3 IPs: Expose, Read, Erase simultaneously
- As fast as 1st generation CCD detectors
- 30 s read time

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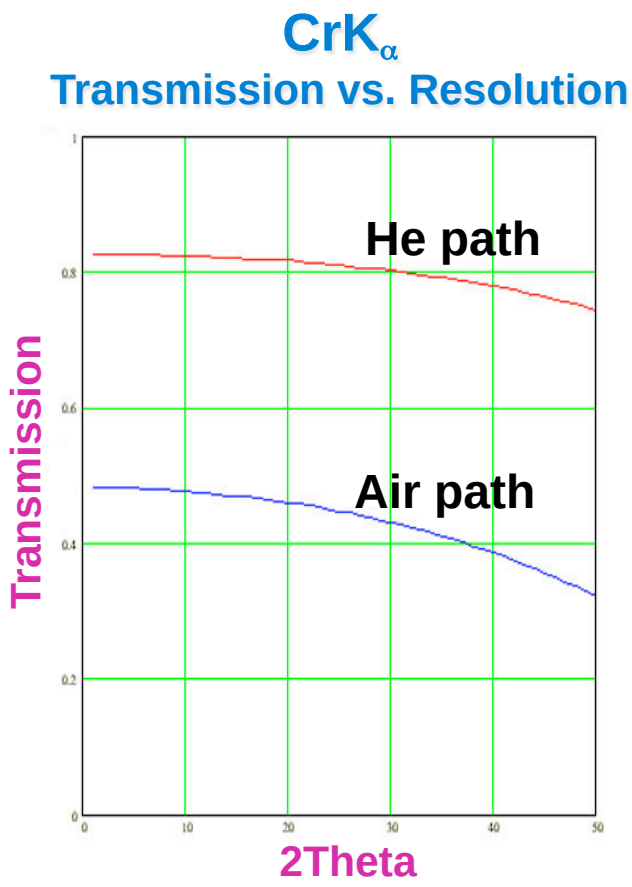
The Absorption Problem



- More than 50% of the flux is lost
  - Without $He_{(g)}$ path (d= 135 mm)
- Modified hardware setup
  - Essential to preserve small anomalous signals
  - $He_{(g)}$ path is critical to success
- Minimization of the free air path is important

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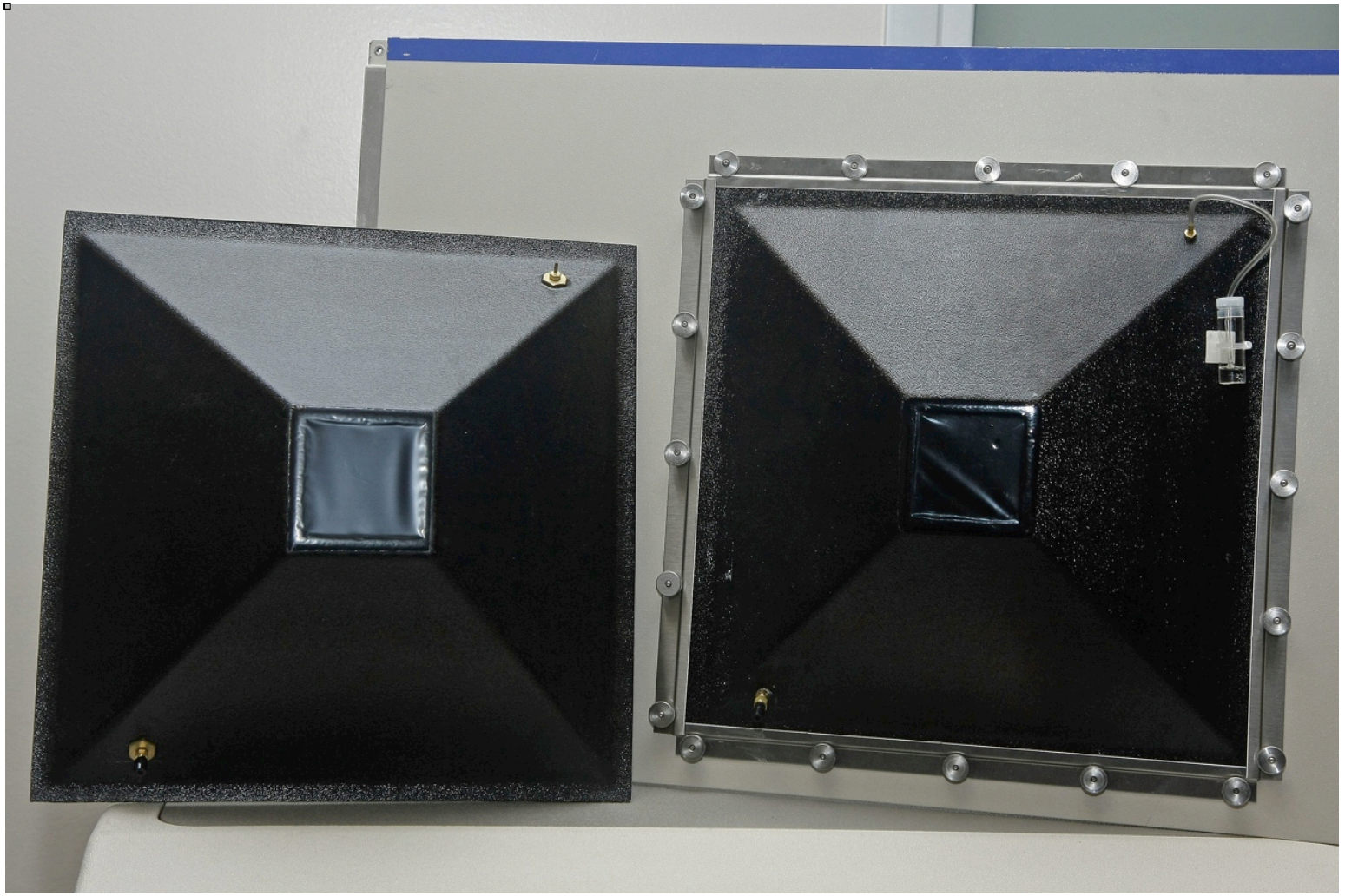
Helium Beam Path

Helium Beam Path



Our Home Lab Setup

- FR-E+ Dual Wavelength
- VariMax DW Multilayer Optic
- HTC Imaging Plate
  - Helium Beam Path

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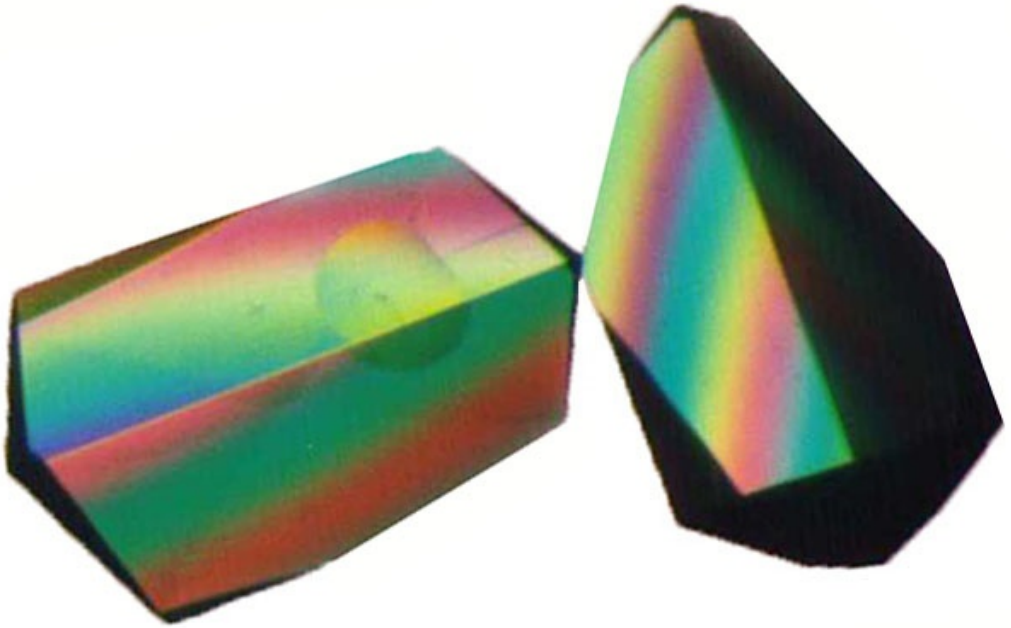
Outline of the seminar

- Hardware
- **Software**
- Examples
- Speculation



Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

HKL-3000: the integration of data reduction and structure solution – from diffraction images to an initial model in minutes

Wladek Minor,[a]* Marcin Cymborowski,[a] Zbyszek Otwinowski[b] and Maksymilian Chruszcz[a]

[a]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA 22903, USA, and [b]Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX 75235, USA

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Received 30 March 2006
Accepted 26 May 2006

A new approach that integrates data collection, data reduction, phasing and model building significantly accelerates the process of structure determination and on average minimizes the number of data sets and synchrotron time required for structure solution. Initial testing of the *HKL*-3000 system (the beta version was named *HKL*-2000_ph) with more than 140 novel structure determinations has proven its high value for MAD/SAD experiments. The heuristics for choosing the best computational strategy at different data resolution limits of phasing signal and crystal diffraction are being optimized. The typical end result is an interpretable electron-density map with a partially built structure and, in some cases, an almost complete refined model. The current development is oriented towards very fast structure solution in order to provide feedback during the diffraction experiment. Work is also proceeding towards improving the quality of phasing calculation and model building.

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Minor et al. (2006) Acta Cryst. D62, 859-866.

HKL3000 – Structure tab uses:

CCP4 suite --- Many authors
Collaborative Computational Project, Number 4. 1994.

"The CCP4 Suite: Programs for Protein Crystallography". Acta Cryst. D50, 760-763

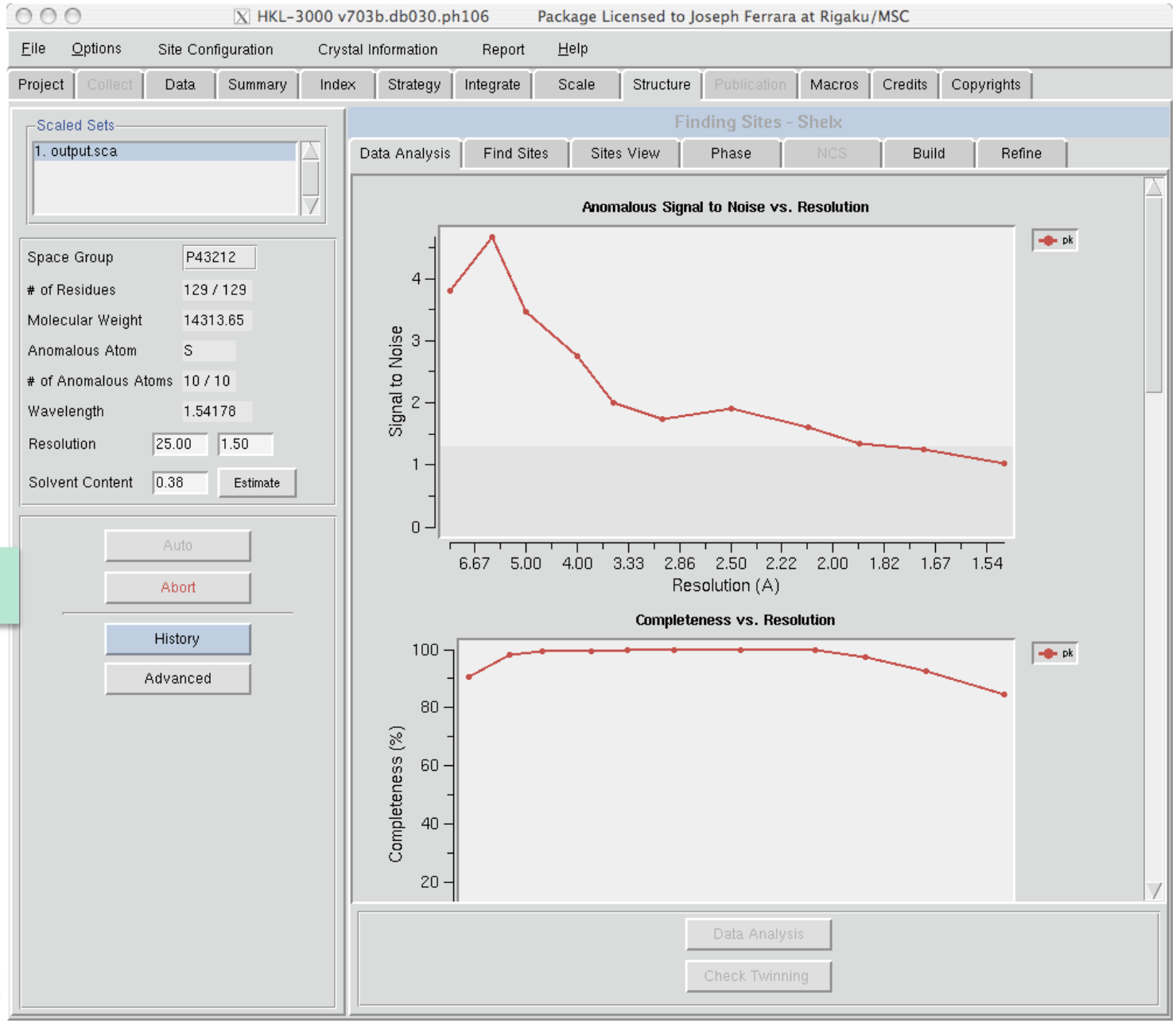
Data Analysis (signal high enough?)

Find sites: SHELXD --- Sheldrick

Phase: MLPHARE + DM --- Otwinowski; Cowtan

Auto-build: ARP/wARP --- Perrakis, Cohen, Lamzin, et al.
REFMAC: --- Murshudov, Vagin, Steiner, et al.

- Project
- Data
- Summary
- Index
- Strategy
- Integrate
- Scale
- **Structure**
- Macros



feature articles

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Accepted 7 September 2007

A short history of *SHELX*

George M. Sheldrick

Department of Structural Chemistry, University of Goettingen, Tammannstrasse 4, D-37077 Goettingen, Germany. Correspondence e-mail: gsheldr@shelx.uni-ac.gwdg.de

An account is given of the development of the *SHELX* system of computer programs from *SHELX*-76 to the present day. In addition to identifying useful innovations that have come into general use through their implementation in *SHELX*, a critical analysis is presented of the less-successful features, missed opportunities and desirable improvements for future releases of the software. An attempt is made to understand how a program originally designed for photographic intensity data, punched cards and computers over 10000 times slower than an average modern personal computer has managed to survive for so long. *SHELXL* is the most widely used program for small-molecule refinement and *SHELXS* and *SHELXD* are often employed for structure solution despite the availability of objectively superior programs. *SHELXL* also finds a niche for the refinement of macromolecules against high-resolution or twinned data; *SHELXPRO* acts as an interface for macromolecular applications. *SHELXC*, *SHELXD* and *SHELXE* are proving useful for the experimental phasing of macromolecules, especially because they are fast and robust and so are often employed in pipelines for *high-throughput* phasing. This paper could serve as a general literature citation when one or more of the open-source *SHELX* programs (and the Bruker AXS version *SHELXTL*) are employed in the course of a crystal-structure determination.

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Sheldrick (2008) Act Cryst A64, 112-122.

Structure

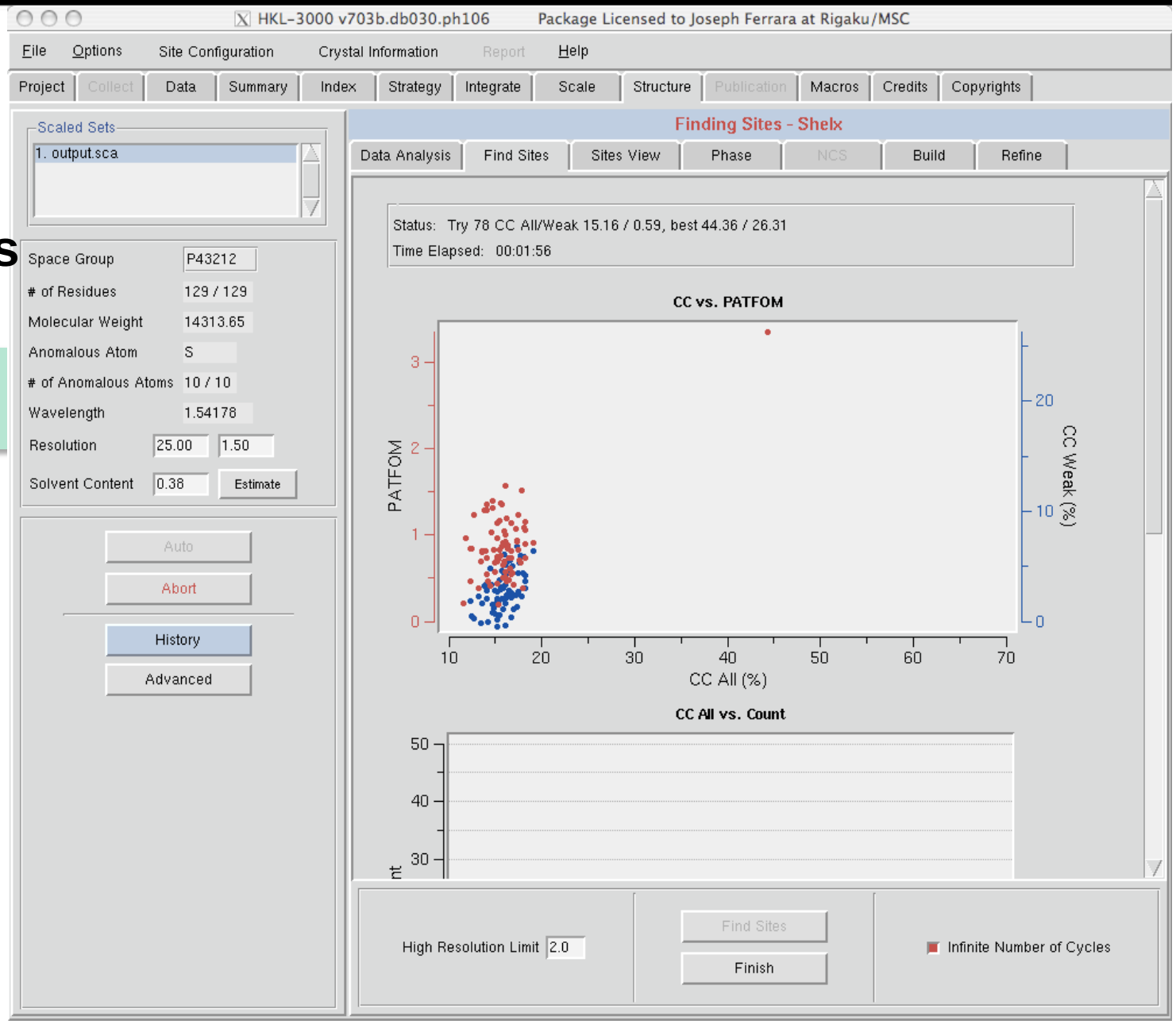
Data Analysis

Find Sites

Phase

Build

Refine



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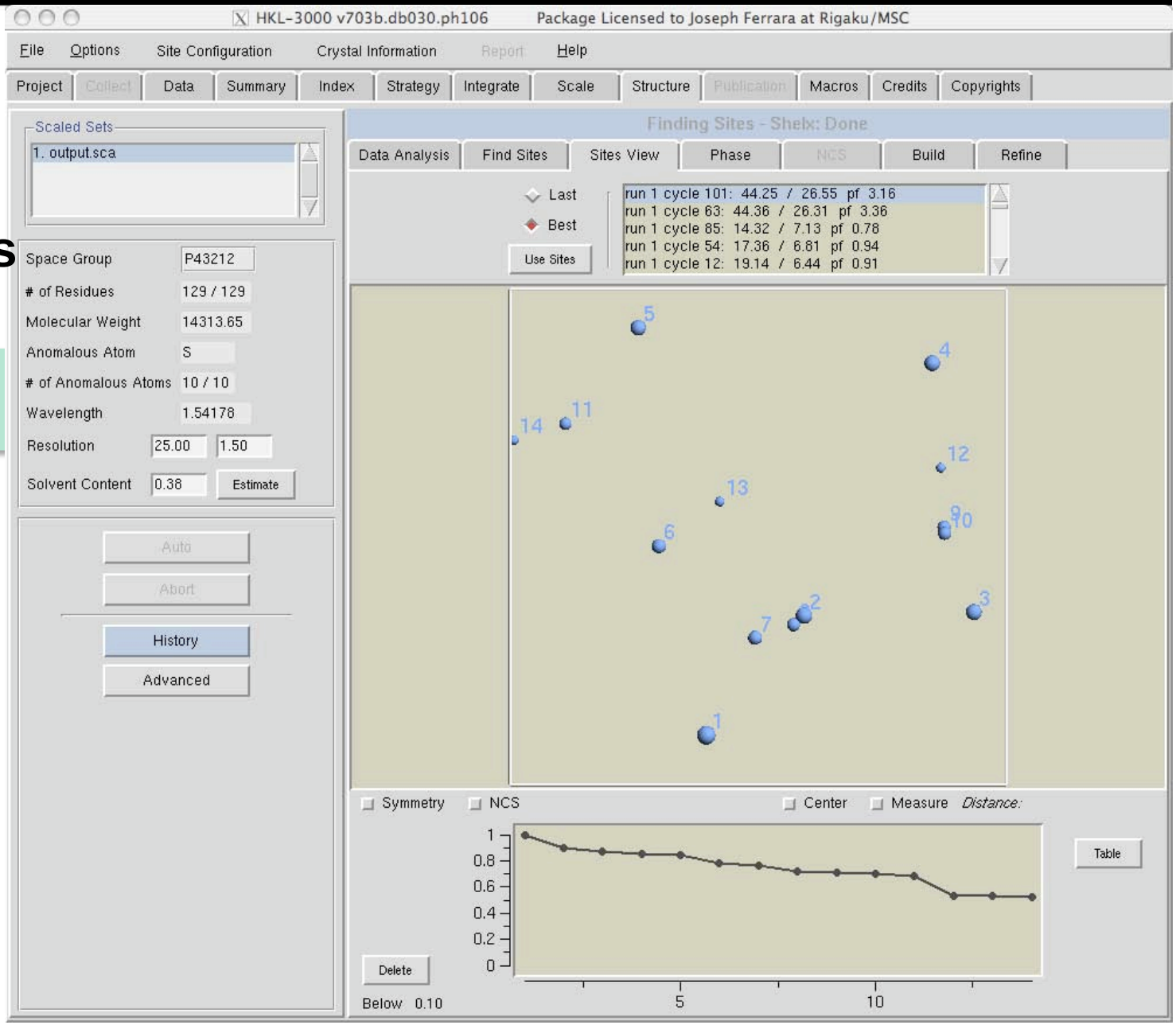

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MLPHARE

ISOMORPHOUS REPLACEMENT AND ANOMALOUS SCATTERING

Proceedings of the CCP4 Study Weekend
25-26 January 1991

Compiled by W. Wolf, P.R. Evans and A.G.W. Leslie

MAXIMUM LIKELIHOOD REFINEMENT OF HEAVY ATOM PARAMETERS

Zbyszek Otwinowski

Howard Hughes Medical Institute
and Department of Molecular Biophysics and Biochemistry,
Yale University, New Haven, CT 06514, USA

Introduction

Analysis of isomorphous replacement (MIR) data has two major steps. The first is refinement of the heavy atom substitution parameters. The second is application of the results of the first step to calculate probabilities of all possible phases and from that distribution the centroid phase and figure of merit.

Otwinowski (1991) Proc CCP4 SW 1991, 80-86.

DM

Daresbury Laboratory

JOINT CCP4 AND ESF-EACBM NEWSLETTER ON PROTEIN CRYSTALLOGRAPHY

An informal Newsletter associated with the BBSRC Collaborative Computational Project No.4 on Protein Crystallography and the ESF Network of the European Association of the Crystallography of Biological Macromolecules.

Number 31 November 1994

Contents

'dm': An Automated Procedure for Phase Improvement by Density Modification.

Dr. K. D. Cowtan, University of York, Heslington, York, YO1 5DD.

Density Modification: The Problem.

The techniques of protein structure solution by X–ray diffraction methods are now well established and are the method of choice for obtaining structural information about many types of biological macromolecule. In many cases however a structure solution by X–ray methods is still far from routine, and in particular the step of obtaining phases for the measured x–ray intensities can present problems. Experimental techniques, such as the measurement of intensities from single or multiple isomorphous derivatives or the differences due to anomalous scattering give estimates to some of the phase information, which may or may not be enough to deduce the structure of the molecule.

- Cowtan (1994) Joint CCP4 … 31, 34-38.

Structure

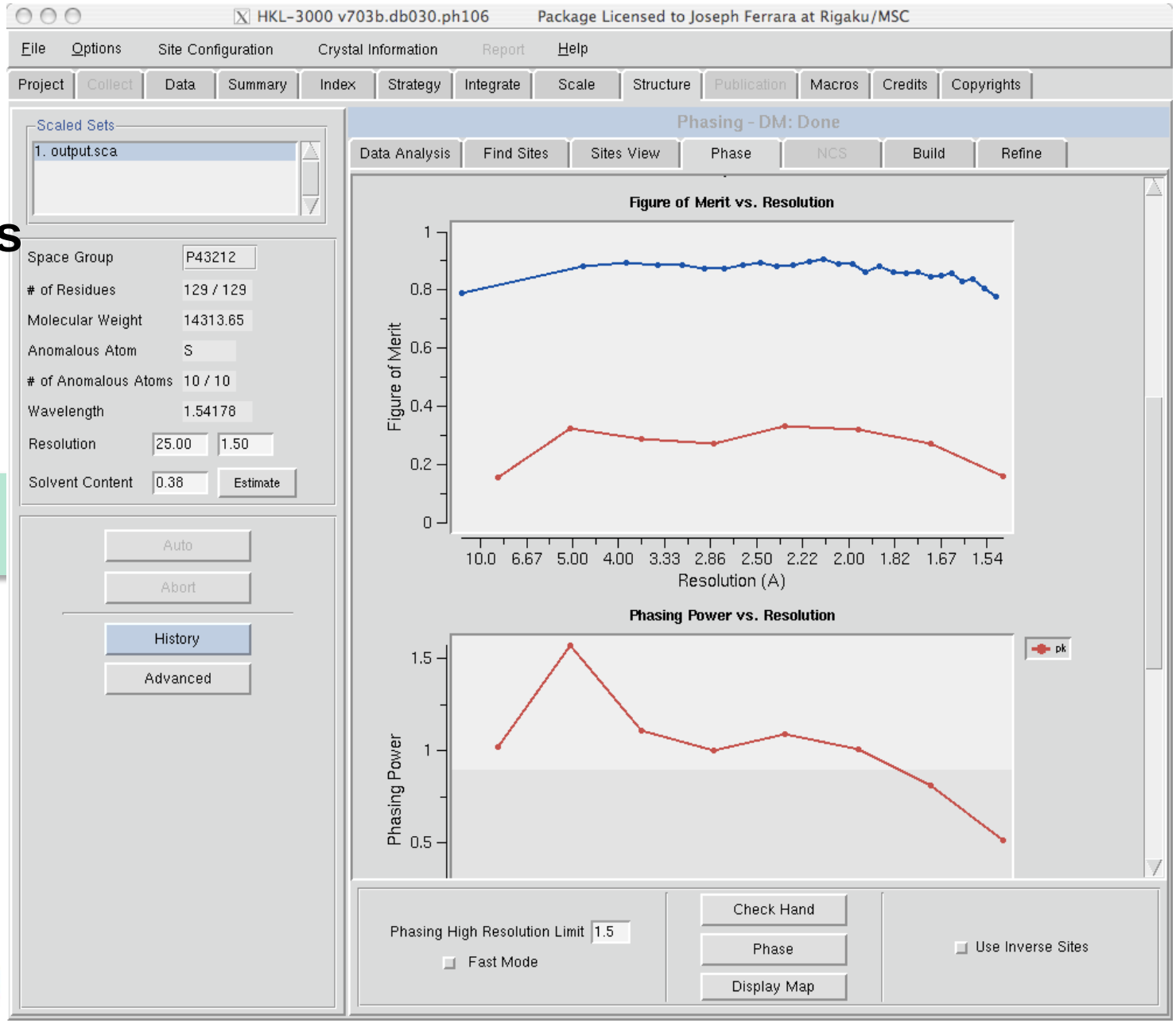
Data Analysis

Find Sites

Phase

Build

Refine



PROTOCOL

Automated macromolecular model building for X-ray crystallography using ARP/wARP version 7

Gerrit Langer[1,3], Serge X Cohen[2,3], Victor S Lamzin[1] & Anastassis Perrakis[2]

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ARP/wARP is a software suite to build macromolecular models in X-ray crystallography electron density maps. Structural genomics initiatives and the study of complex macromolecular assemblies and membrane proteins all rely on advanced methods for 3D structure determination. ARP/wARP meets these needs by providing the tools to obtain a macromolecular model automatically, with a reproducible computational procedure. ARP/wARP 7.0 tackles several tasks: iterative protein model building including a high-level decision-making control module; fast construction of the secondary structure of a protein; building flexible loops in alternate conformations; fully automated placement of ligands, including a choice of the best-fitting ligand from a 'cocktail'; and finding ordered water molecules. All protocols are easy to handle by a nonexpert user through a graphical user interface or a command line. The time required is typically a few minutes although iterative model building may take a few hours.

Langer et al. (2008) Nature Protocols 3, 1171-1179.

Rigaku

research papers

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*REFMAC*5 dictionary: organization of prior chemical knowledge and guidelines for its use

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One of the most important aspects of macromolecular structure refinement is the use of prior chemical knowledge. Bond lengths, bond angles and other chemical properties are used in restrained refinement as subsidiary conditions. This contribution describes the organization and some aspects of the use of the flexible and human/machine-readable dictionary of prior chemical knowledge used by the maximum-likelihood macromolecular-refinement program *REFMAC*5. The dictionary stores information about monomers which represent the constitutive building blocks of biological macromolecules (amino acids, nucleic acids and saccharides) and about numerous organic/inorganic compounds commonly found in macromolecular crystallography. It also describes the modifications the building blocks undergo as a result of chemical reactions and the links required for polymer formation. More than 2000 monomer entries, 100 modification entries and 200 link entries are currently available. Algorithms and tools for updating and adding new entries to the dictionary have also been developed and are presented here. In many cases, the *REFMAC*5 dictionary allows entirely automatic generation of restraints within *REFMAC*5 refinement runs.

Murshudov et al. (1997) Acta Cryst D53, 240-255.

Vagin et al. (2004) Acta Cryst D60, 2184-2195.

Rigaku

Structure

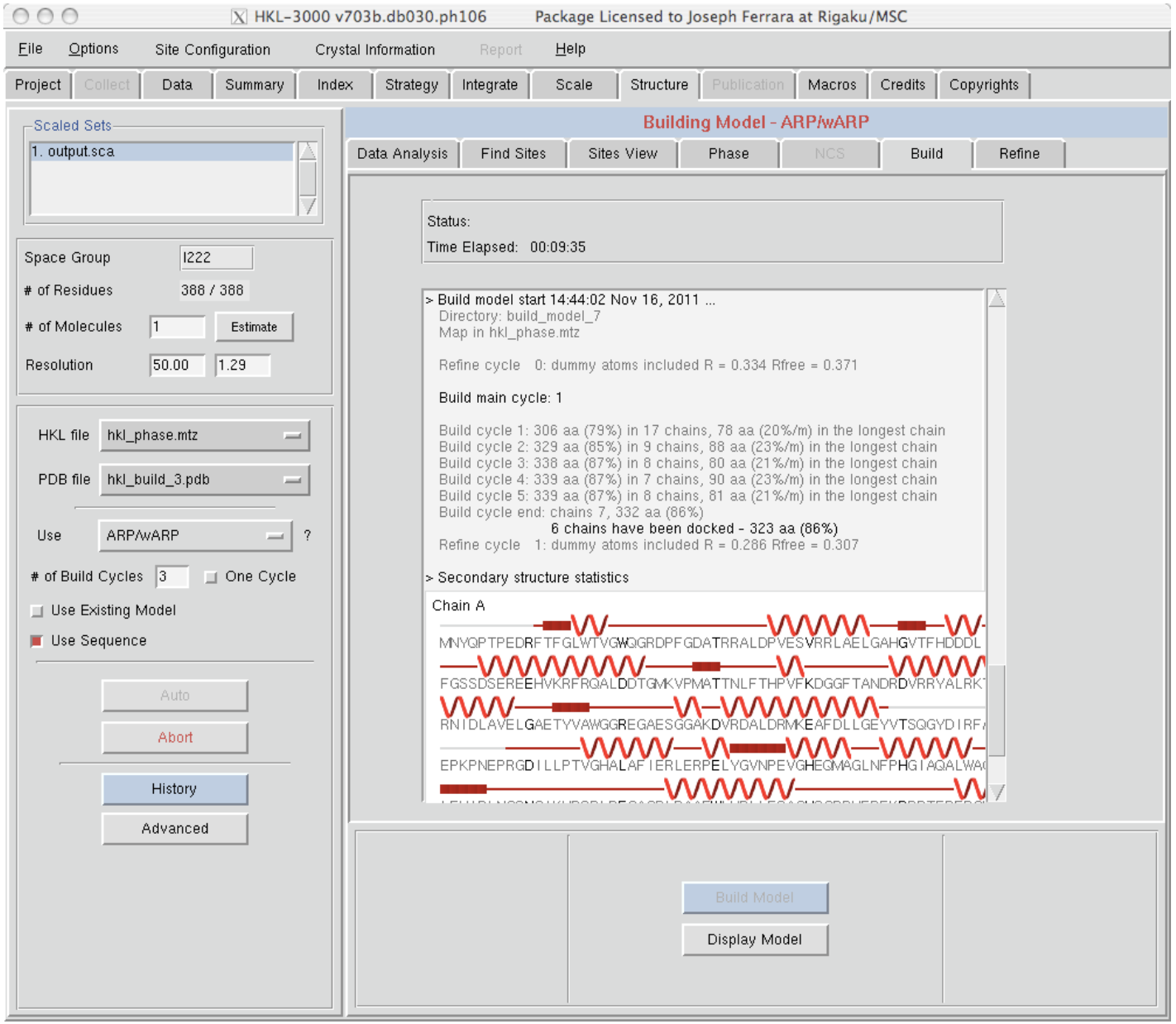
Data Analysis

Find Sites

Phase

Build

Refine



research papers

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Features and development of *Coot*

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Coot is a molecular-graphics application for model building and validation of biological macromolecules. The program displays electron-density maps and atomic models and allows model manipulations such as idealization, real-space refinement, manual rotation/translation, rigid-body fitting, ligand search, solvation, mutations, rotamers and Ramachandran idealization. Furthermore, tools are provided for model validation as well as interfaces to external programs for refinement, validation and graphics. The software is designed to be easy to learn for novice users, which is achieved by ensuring that tools for common tasks are 'discoverable' through familiar user-interface elements (menus and toolbars) or by intuitive behaviour (mouse controls). Recent developments have focused on providing tools for expert users, with customisable key bindings, extensions and an extensive scripting interface. The software is under rapid development, but has already achieved very widespread use within the crystallographic community. The current state of the software is presented, with a description of the facilities available and of some of the underlying methods employed.

Emsley et al. (2010) Acta Cryst D66, 486-501.

Thaumatin, Rigaku R-AXIS IV++, MM007HF

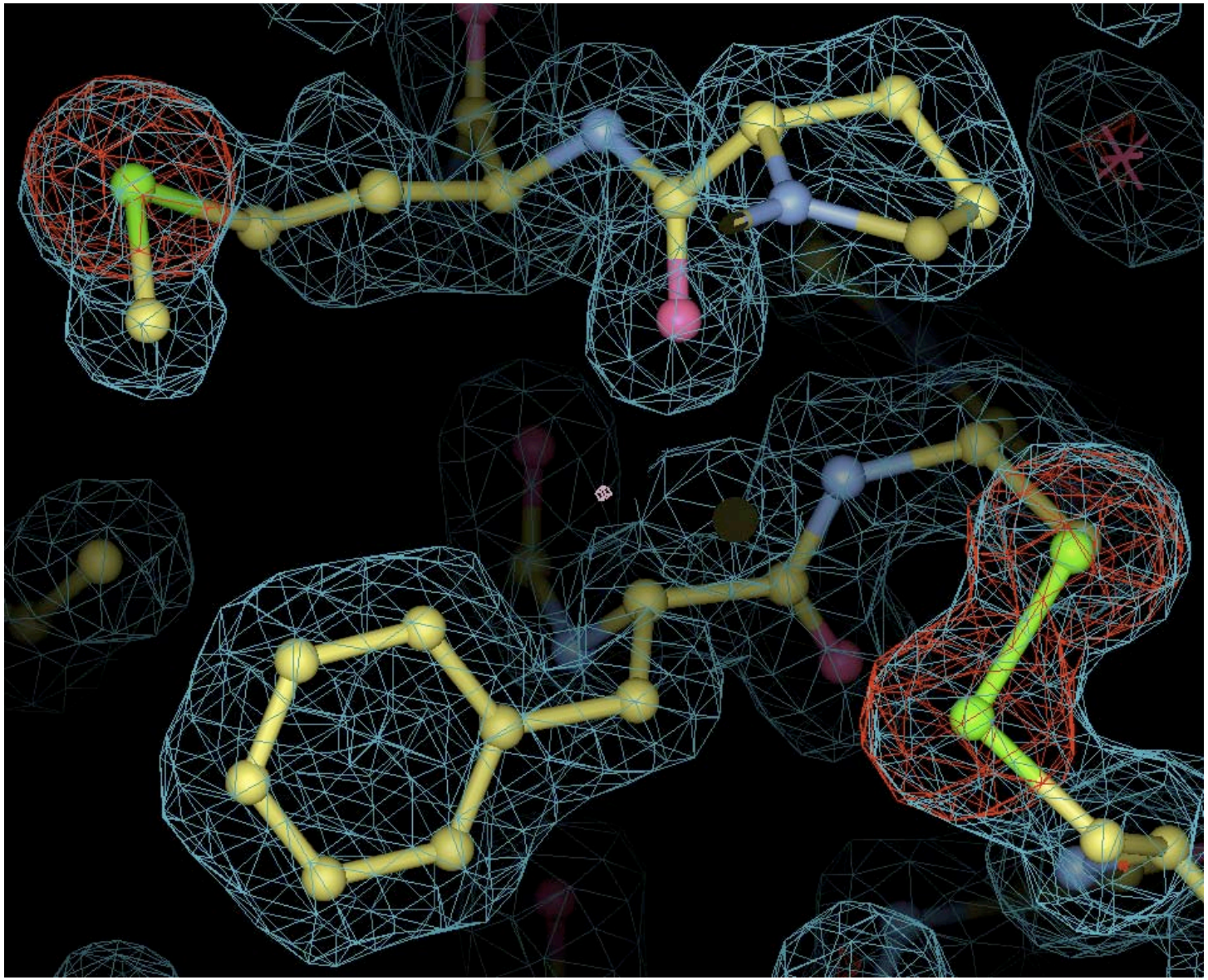
207 aa, 17 sulfurs, 300 images, 90 degrees, 10 hours

Summary of data collection statistics

Spacegroup	**$P4_12_12$**		
Unit cell dimensions	**57.81**	**57.81**	**150.10**
	90.00	**90.00**	**90.00**
Resolution range	**19.72 - 1.50**		**(1.55 - 1.50)**
Total number of reflections	**243628**		
Number of unique reflections	**40409**		
Average redundancy	**6.03**		**(2.48)**
% completeness	**96.8**		**(72.9)**
Rmerge	**0.029**		**(0.168)**
Rmeas	**0.031**		**(0.208)**
RmeasA (I+,I- reflns kept apart)	**0.030**		**(0.201)**
Reduced ChiSquared	**1.10**		**(0.80)**
Output <I/sigI>	**35.7**		**(5.4)**

Rigaku

Electron density of thaumatin map from ARP/wARP near phenylalanine residue Phe203. Nearby are Pro111, Met112, and the disulfide formed by Cys9 and Cys204.



Rigaku

Examples

- **Thaumatin**
  - $P4_12_12$ 58 x 58 x 150
  - 207 residues, 17 sulfurs
- **Lysozyme** (30 minute data collection)
  - $P4_32_12$ 77 x 77 x 38
  - 129 residues, 10 sulfurs
- **Glucose Isomerase** and a 2nd one
  - I222 93 x 98 x 102
  - 388 residues, 1 manganese & 1 calcium

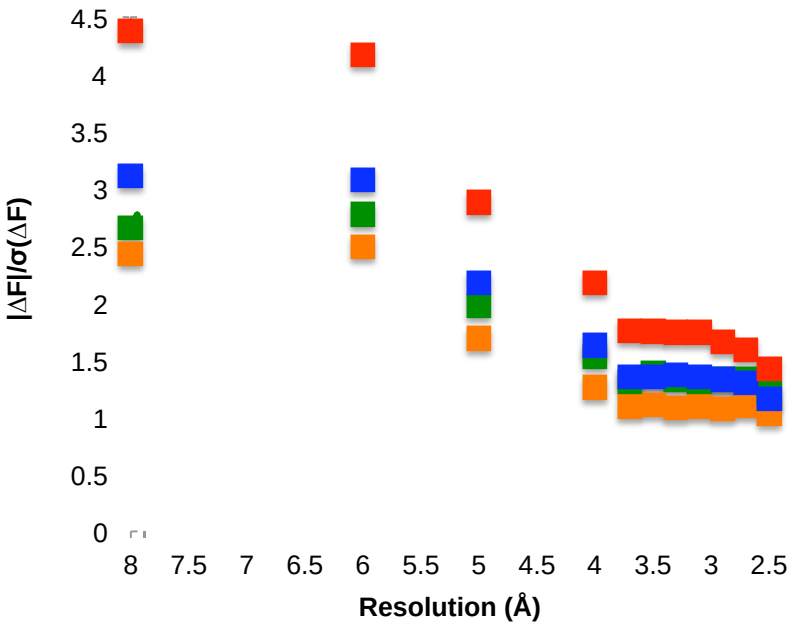
Thaumatin - Chromium radiation S-SAD

Spacegroup	$P4_12_12$			
Unit cell dimensions	57.95	57.95	150.10	
	90.00	90.00	90.00	
Mosaicity	0.53			
Resolution range	31.70 - 2.50			
Number of 0.3 deg images	600	266	200	150
Exposure time (hours)	5.00	2.21	1.67	1.25
Total number of reflections	119594			30759
Number of unique reflections	9436			9295
Average redundancy	12.67			3.31
% completeness	99.9			98.6
Rmerge	0.032			0.020
Rmeas	0.034			0.024
RmeasA (I+,I- reflns kept apart)	0.030			0.017
Output <I/sigI>	75.0			43.9

Rigaku

Thaumatin - Chromium radiation S-SAD

Anom S/N vs Reso



Chromium Phasing parameters

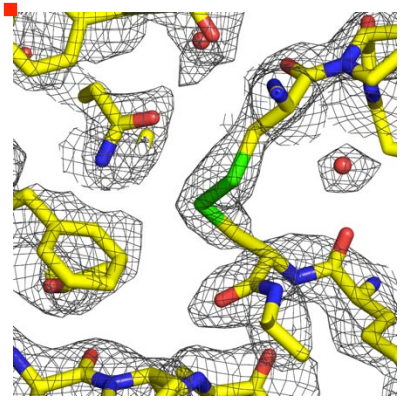
# of Img	Data Used	Avg. Redundancy	Find Sites Res. (Å)	FOM after DM
600	180°	12.7	2.5	0.85
266	79.8°	5.8	2.5	0.83
200	60°	4.4	2.5	0.83
150	45°	3.3	2.5	0.83

Rigaku

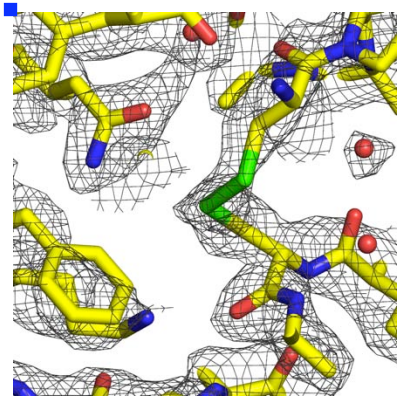
Thaumatin - Chromium radiation S-SAD

Maps after solvent-flattening
Models from ARP/wARP auto-build

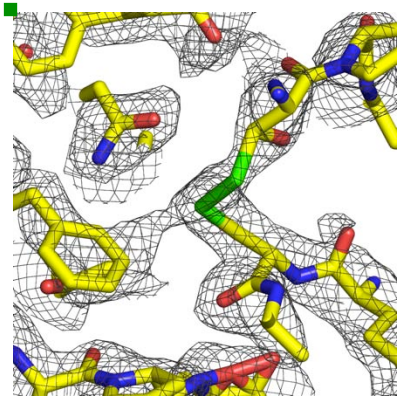
600 (5 hr)



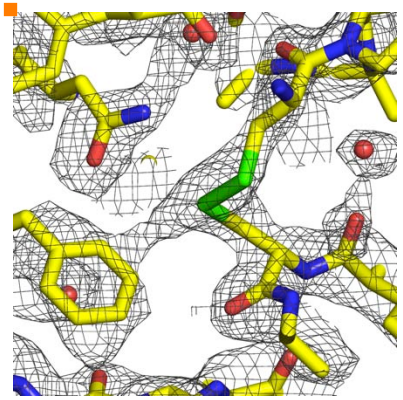
266



200



150 (1.25 hr)



Rigaku

Thaumatin - Copper radiation S-SAD

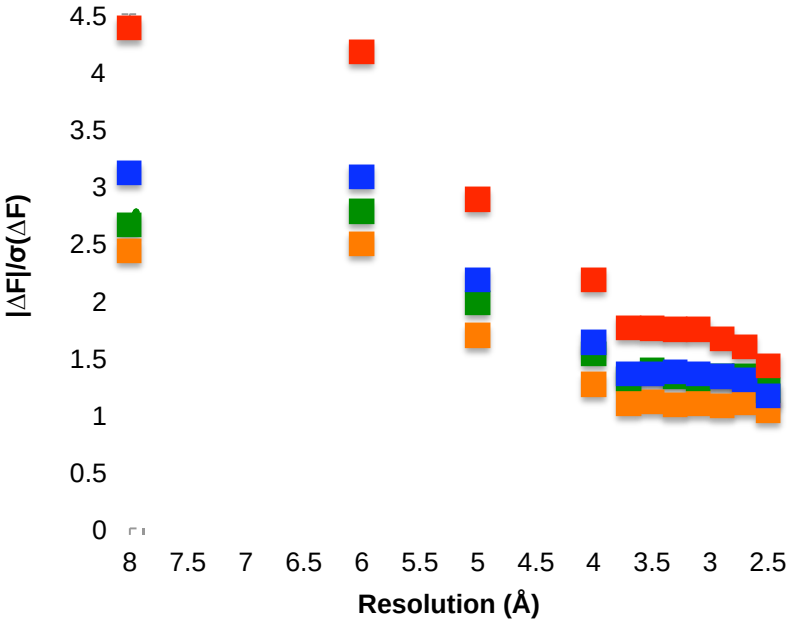
Spacegroup	$P4_12_12$		
Unit cell dimensions	57.88	57.88	150.0
	90.00	90.00	90.00
Mosaicity	0.45		
Resolution range	24.47 – 1.70		

Number of 0.3 deg images	600		150
Exposure time (hours)	5.00	2.21 1.67	1.25
Total number of reflections	384288		89184
Number of unique reflections	28883		28390
Average redundancy	13.30		3.14
% completeness	99.9		98.4
Rmerge	0.035		0.023
Rmeas	0.037		0.028
RmeasA (I+,I- reflns kept apart)	0.036		0.025
Output <I/sigI>	56.7		30.0

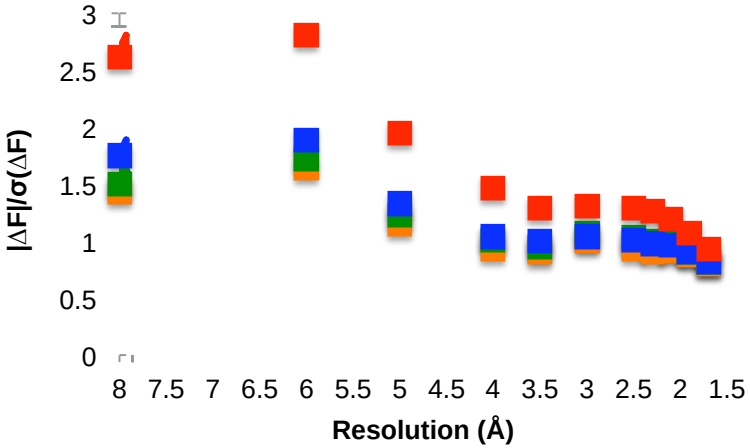
Rigaku

Thaumatin - Cr & Cu radiation S-SAD

Cr: Anom S/N vs Reso



Cu: Anom S/N vs Reso



Rigaku

Lysozyme, Rigaku R-AXIS HTC, FR-E+

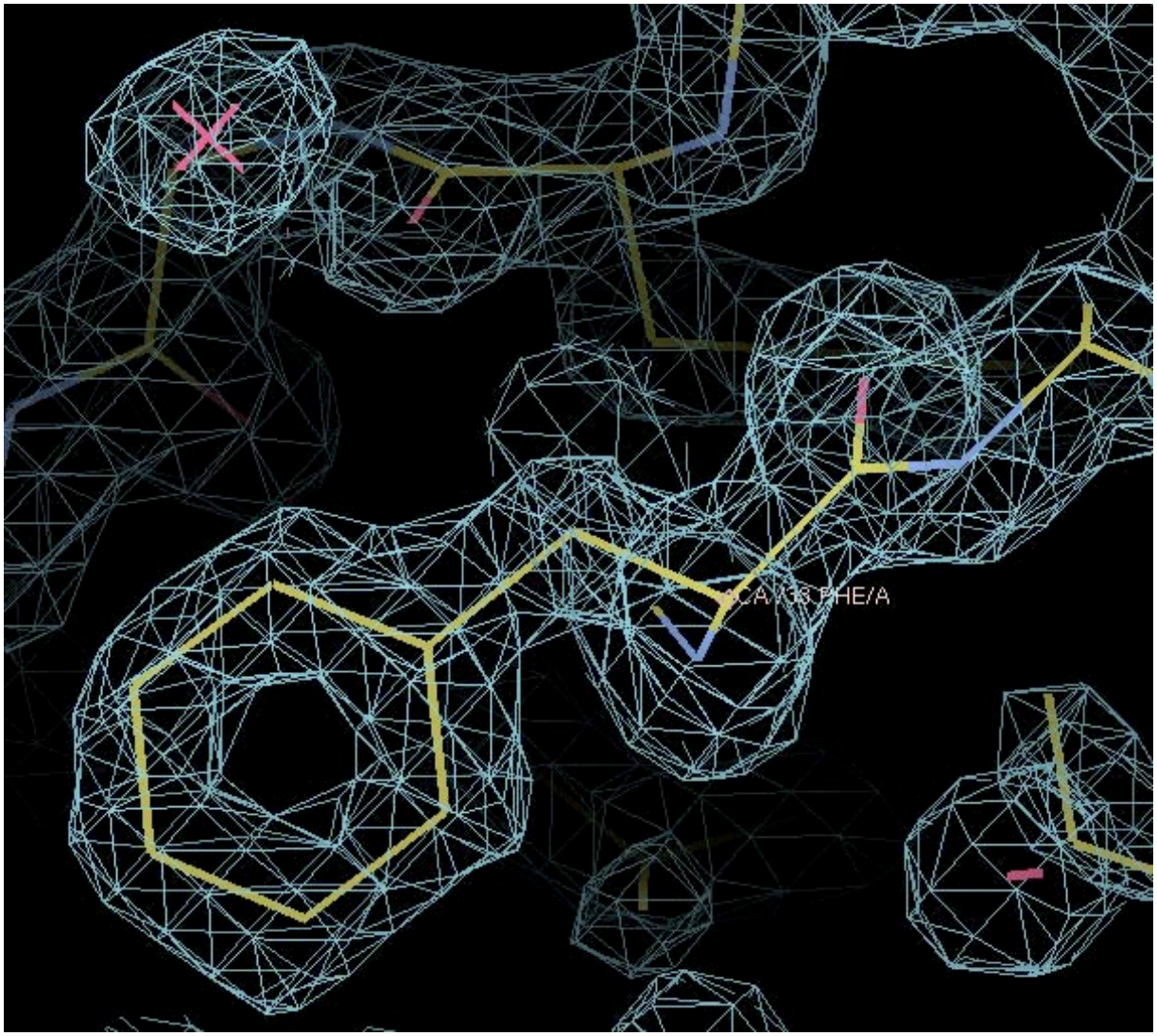
129 aa, 10 sulfurs, 60 images, 90 degrees, **30 minutes**

Summary of data collection statistics

Spacegroup	**$P4_32_12$**		
Unit cell dimensions	**77.56**	**77.56**	**38.04**
	90.00	**90.00**	**90.00**
Resolution range	**18.81 - 1.70**		**(1.76 - 1.70)**
Total number of reflections	**88041**		
Number of unique reflections	**13250**		
Average redundancy	**6.64**		**(6.39)**
% completeness	**100.0**		**(99.8)**
Rmerge	**0.035**		**(0.276)**
Rmeas	**0.038**		**(0.300)**
RmeasA (I+,I- reflns kept apart)	**0.036**		**(0.299)**
Reduced ChiSquared	**1.21**		**(1.04)**
Output <I/sigI>	**34.2**		**(6.0)**

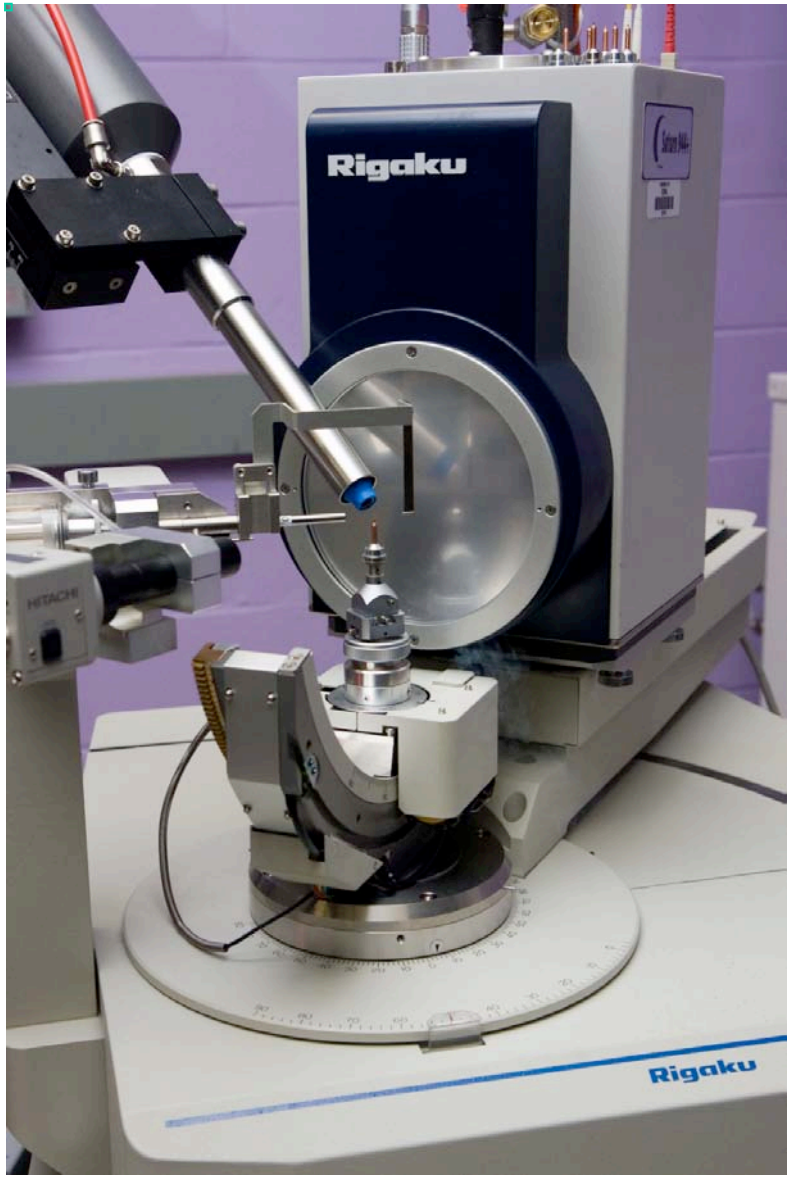
Rigaku

Lysozyme, Rigaku R-AXIS HTC, FR-E+



Rigaku

Example 3A Glucose isomerase, Rigaku MM007HF, Saturn 944+



Rigaku

Example 3 Glucose isomerase, Rigaku MM007HF, Saturn 944+

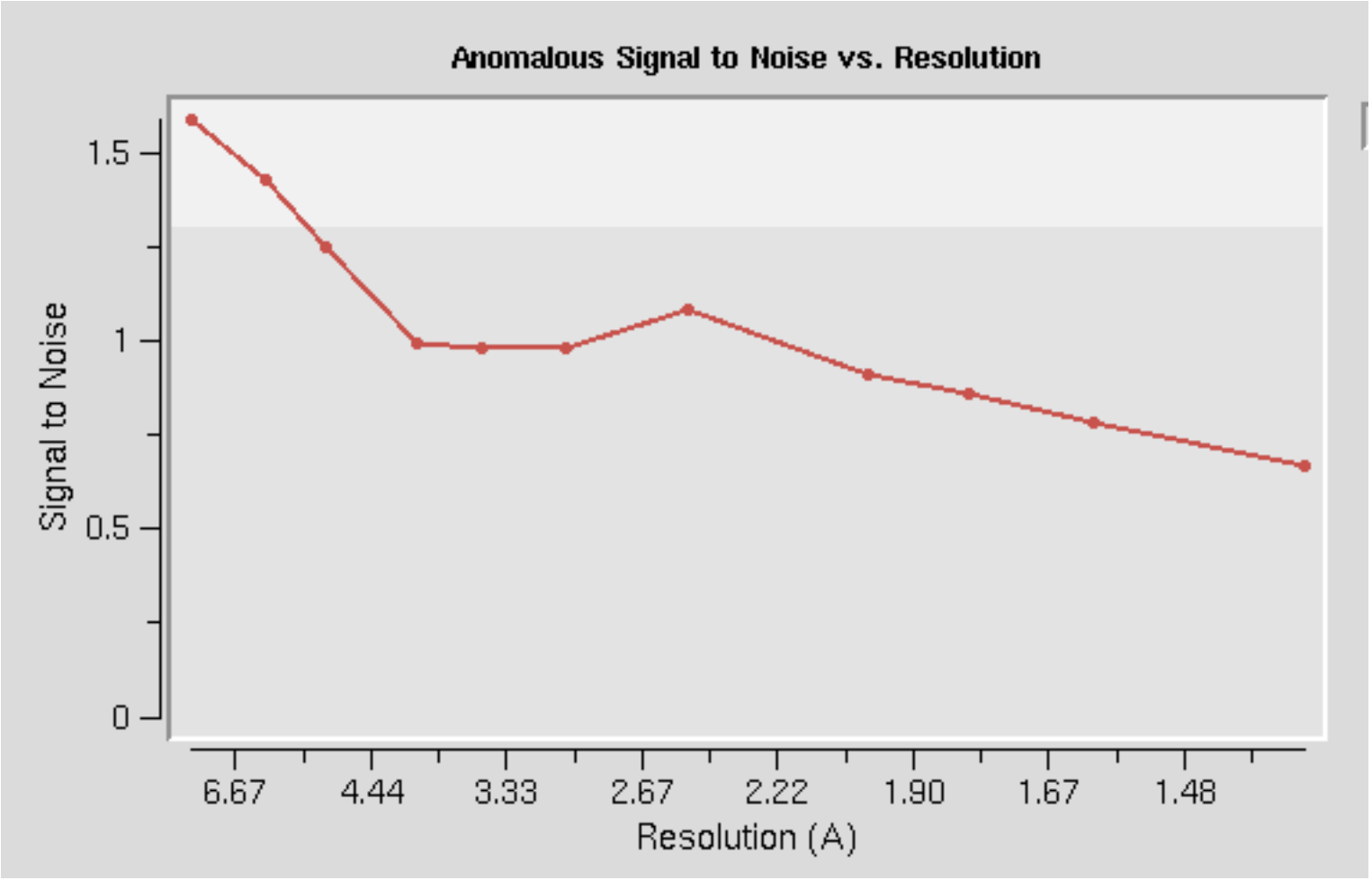
388 aa, 2 Mn?Ca, 1080 images, 540 degrees, 10 hours

Summary of data collection statistics

Spacegroup	I222		
Unit cell dimensions	92.64	98.36	102.46
	90.00	90.00	90.00
Resolution range	22.90 - 1.40		(1.45 - 1.40)
Total number of reflections	844698		
Number of unique reflections	92277		
Average redundancy	9.15		(6.64)
% completeness	99.8		(98.4)
Rmerge	0.047		(0.196)
Rmeas	0.050		(0.213)
RmeasA (I+,I- reflns kept apart)	0.050		(0.214)
Reduced ChiSquared	1.17		(0.65)
Output <I/sigI>	31.8		(6.8)

Rigaku

Example 3 Glucose isomerase, Rigaku MM007HF, Saturn 944+

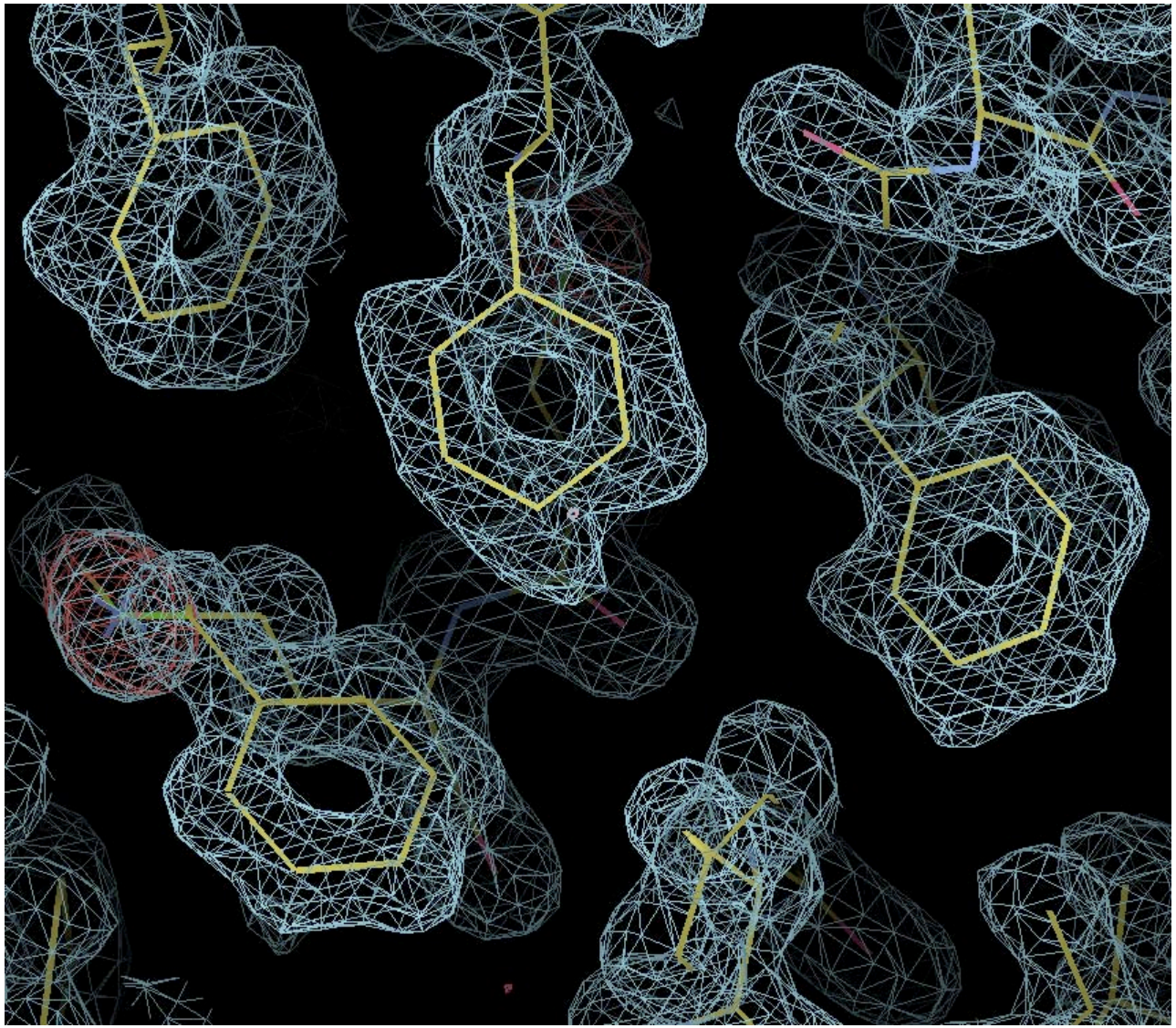


Rigaku

Example 3 Glucose isomerase, Rigaku MM007HF, Saturn 944+

Map after MLPHARE + DM

No refinement



Rigaku

Example 3B Glucose isomerase, Rigaku FR-E+, R-AXIS HTC

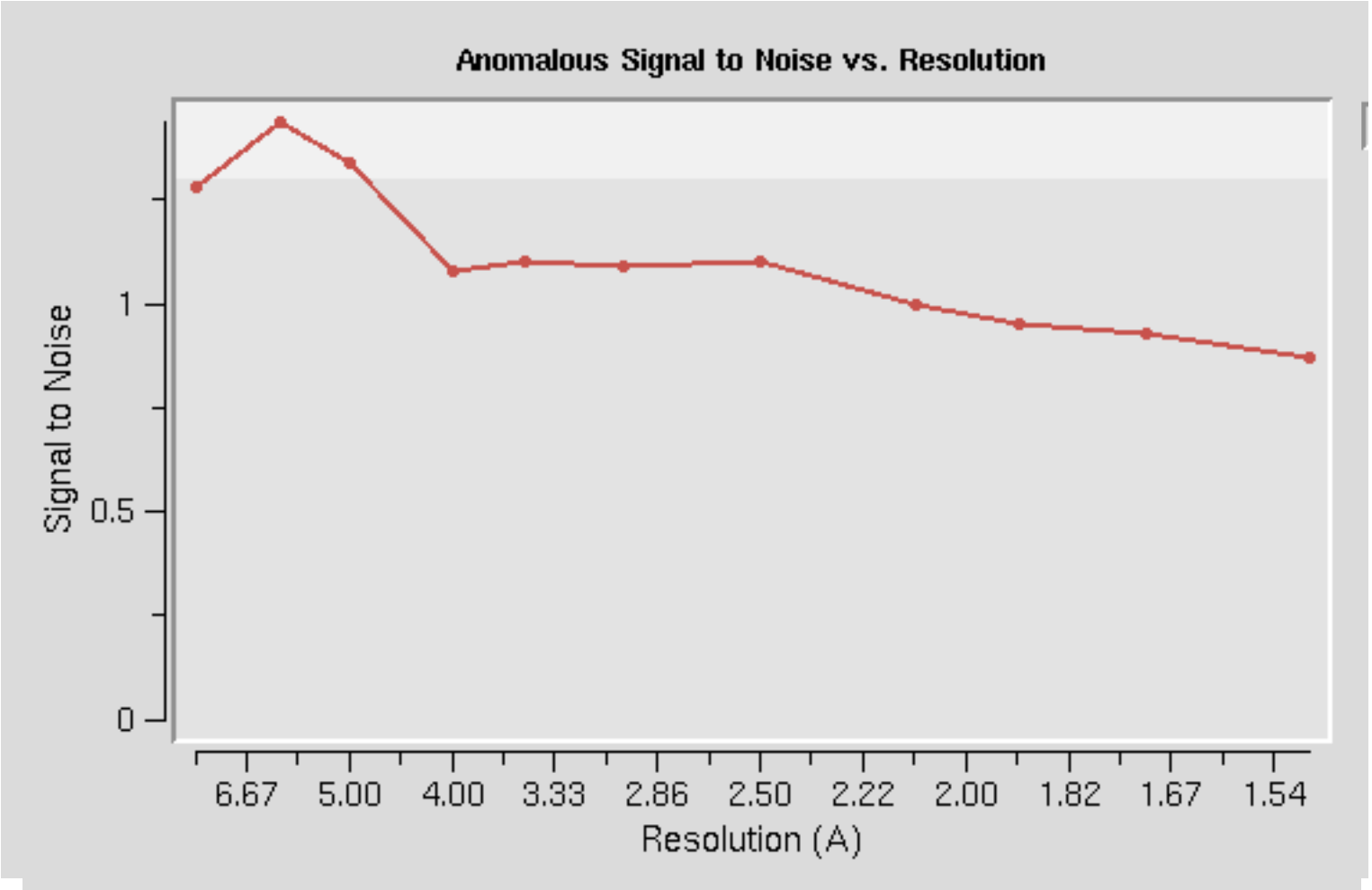
388 aa, 2 Mn?Ca, 468 images, 344 degrees, 3.9 hours

Summary of data collection statistics

Spacegroup	I222		
Unit cell dimensions	92.79	97.67	102.56
	90.00	90.00	90.00
Resolution range	21.61 - 1.50		(1.55 - 1.50)
Total number of reflections	968113		
Number of unique reflections	71594		
Average redundancy	13.52		(13.32)
% completeness	95.8		(91.3)
Rmerge	0.053		(0.345)
Rmeas	0.055		(0.359)
RmeasA (I+,I- reflns kept apart)	0.055		(0.362)
Reduced ChiSquared	1.24		(0.91)
Output <I/sigI>	34.5		(6.0).

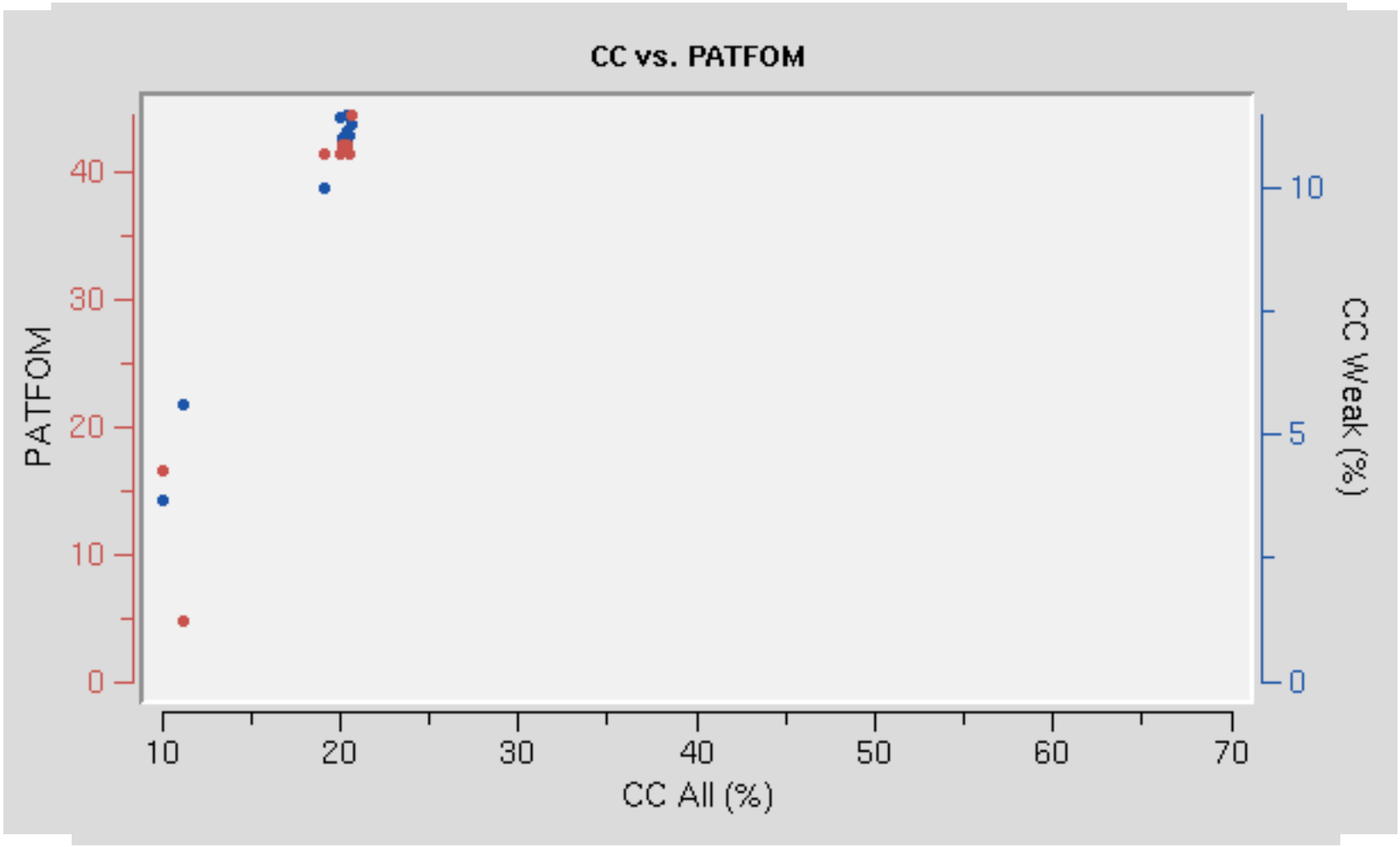
Rigaku

Example 4 Glucose isomerase, Rigaku FR-E+, R-AXIS HTC



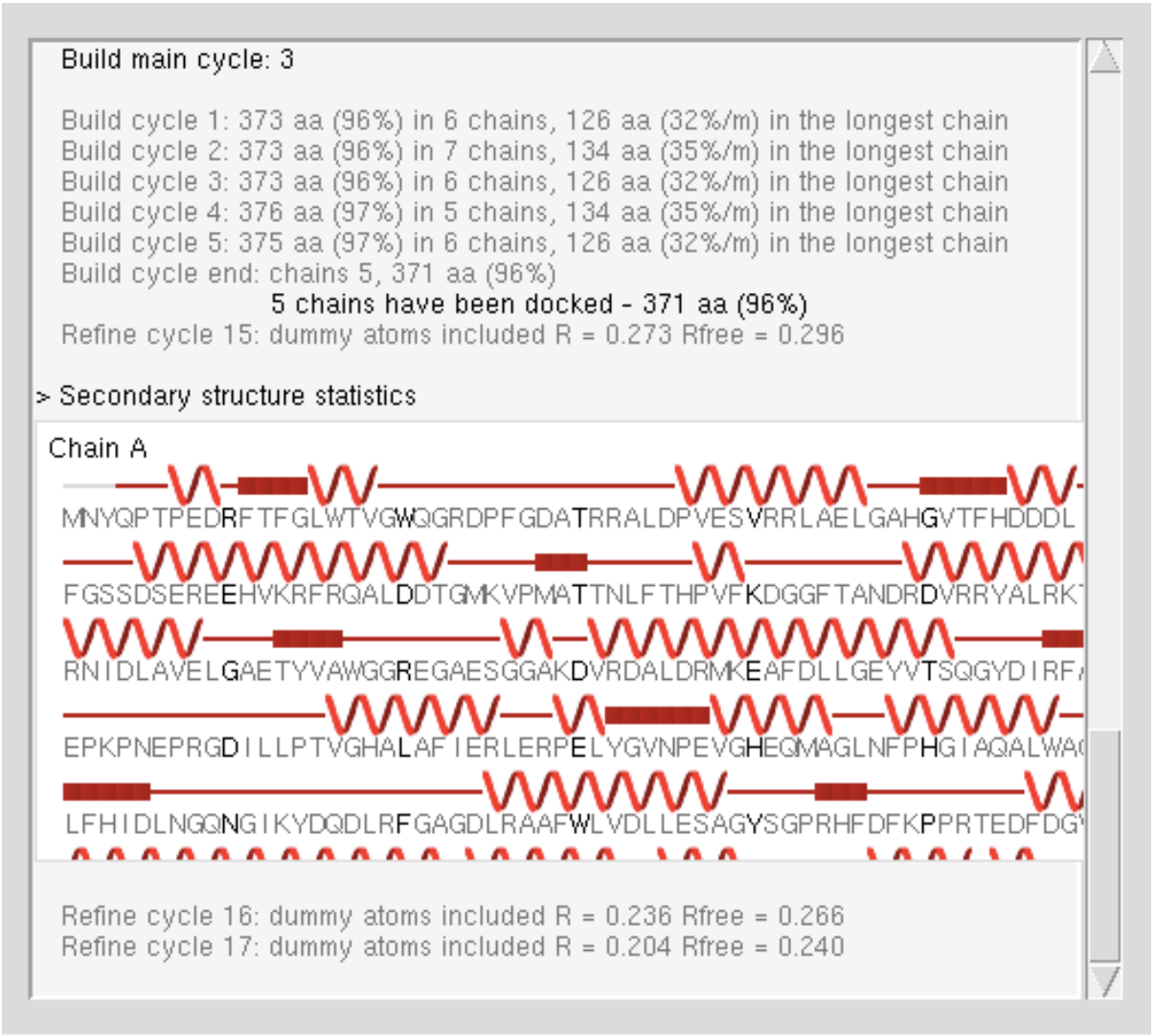
Rigaku

Example 4 Glucose isomerase, Rigaku FR-E+, R-AXIS HTC



Rigaku

Example 4 Glucose isomerase, Rigaku FR-E+, R-AXIS HTC



Rigaku

Conclusions

Do a careful experiment:

Helium beam path for Cr, not necessary for Cu

Redundancy as low as 3 to 4 on I+, I^- with Cu;
lower with Cr

Even low $\Delta F/\sigma_{\Delta F}$ can work

SHELXD is amazing!

Any phase improvement is helpful in auto-tracing

Speculation

Why does low-redundancy with a Home Lab and Imaging Plate work so well?

Stability?
Less demand on synchronization?
No funky detector calibration?

Dose: Does it matter if one can solve the structure with less than 1 minute of exposure?

Photons are photons

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- Rigaku Americas
  - Joe Ferrara
  - Vijaya Madakasira
  - Mark Pressprich
  - Angela Criswell
- HKL Research
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  - Marcin Cymborowski
- The Crystallographic Community and all those smart people who created great software!

Thank you!