If You Build it "We" Will Come

Past Experiences and Predictions

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University of Georgia

Conference on New Frontiers in Neutron Macromolecular Crystallography
July 12-13, 2005

Major Points for this Conference

- The scientific significance of neutron diffraction in structural biology.
- Building a facility - will people come to use it?

Outline

- Past and current contributions in structural research by neutron and X-ray diffraction at ORNL and APS/ANL
- Our own experiences with the SER-CAT beamline project
- Some predictions

Early Neutron and X-ray Advances at ORNL

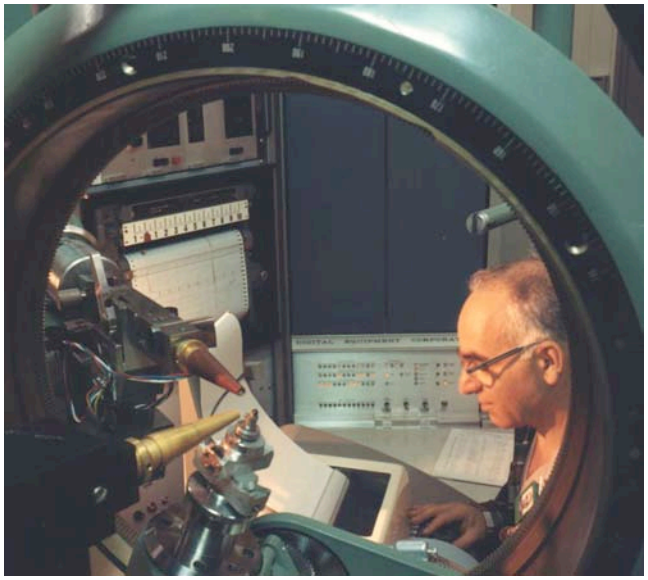
Neutron scattering studies on materials were pioneered in Oak Ridge National Laboratory.

Ernest Wollan installed a modified two-axis X-ray diffractometer at a beam port of the ORNL Graphite Reactor in November 1945, and was joined several months later by Clifford Shull.

Clifford Shull shared the 1994 Nobel prize in physics for his work in neutron scattering. At ORNL, the use of neutron scattering to determine the accurate positions for H atoms was developed.

The determination of hydrogen positions in materials was of such interest to ORNL crystallographers that a separate program was established in the Chemistry Division under Henri Levy to study hydrogen bonding in crystals. Levy and Selmer Peterson were pioneers in developing the neutron scattering technique for detailed structural analysis of single crystals. William Busing, Harold Smith, Ray Ellison, Dan Danford, George Brown, Carroll Johnson, Paul Agron, Bill Thiessen, and Al Narten joined the Chemistry Division program later and developed a very strong program in X-ray crystallography.

Henri A. Levy - President of ACA, 1965
William R. Busing - President of ACA, 1971
Carroll Johnson - President of ACA, 1977

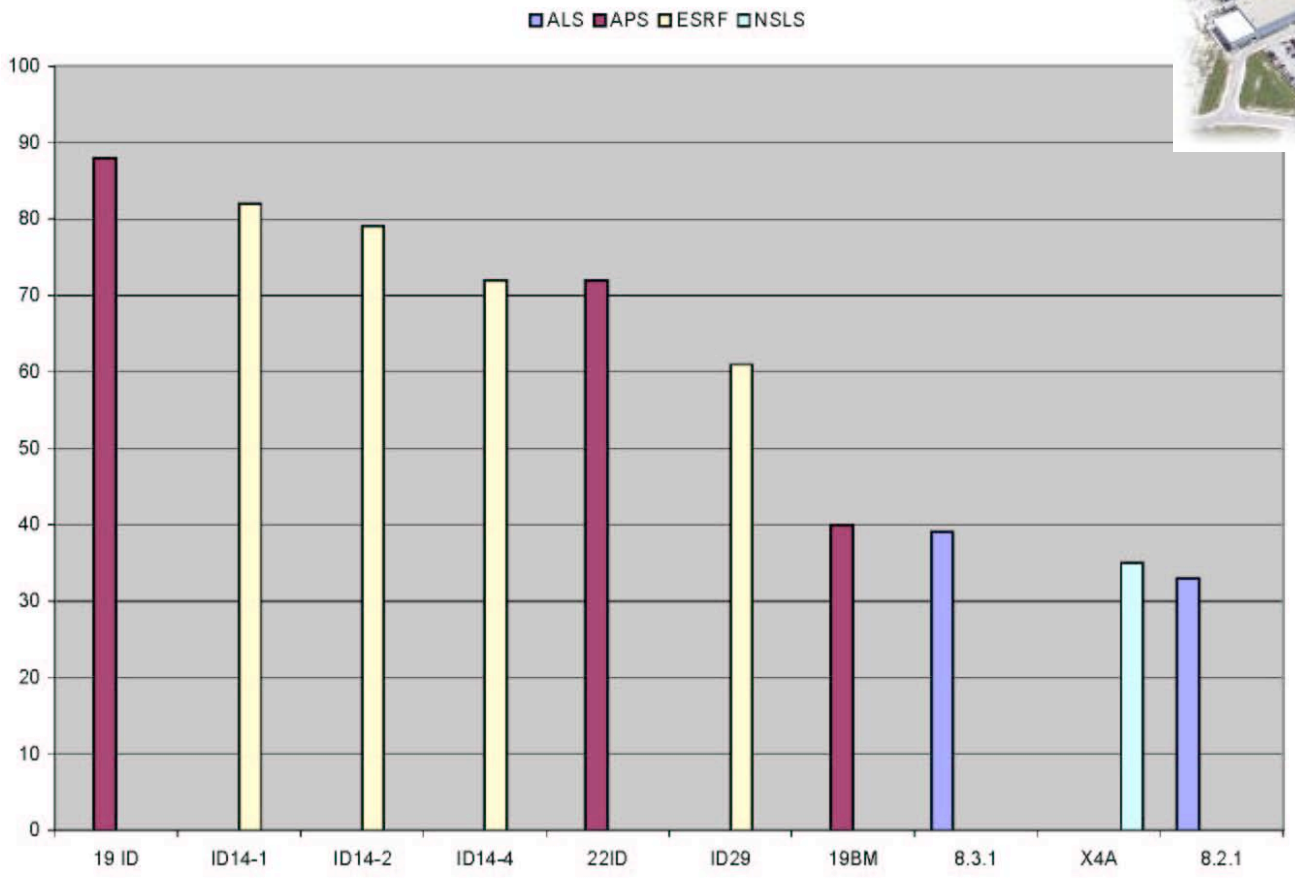


Levy at the Picker 4-circle diffractometer

Crystallography programs
- ORFLS
- ORTEP and others

Structural Biology at APS/ANL

2004 Publications



Statistics taken from each labs websit

Top 10 Producing Beamlines

As of 05/12/05

These advances in scientific discovery would not be possible without the appropriate facility to carry out the experimental work

Often it takes years for an idea to produce an impact

The SER-CAT 22-ID construction project is a good example

Southeast Regional Collaborative Access Team

Light when YOU need it!

History

1997

April: First discussions at UGA about the need for synchrotron access at APS

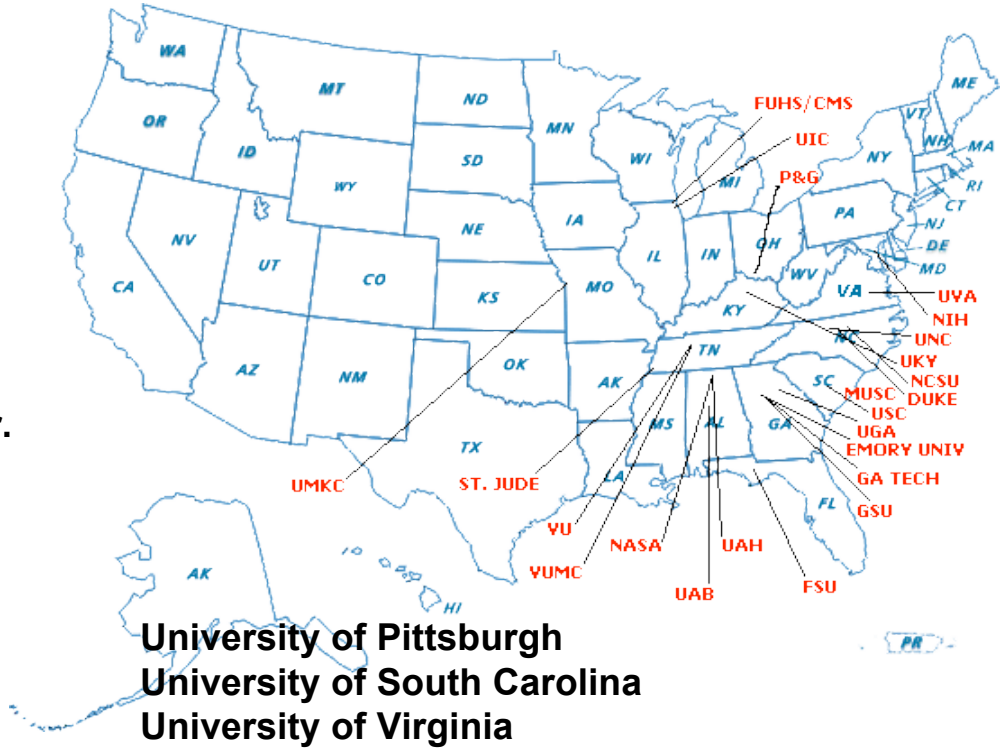
September - Received seed commitment from the Georgia Research Alliance

November - Received a commitment from UGA

1998	Proposal submitted to APS
1999	Construction on 22ID begins
	- 19 Member Institutions
2002	Construction on 22BM begins
2002	Ribbon cutting for 22ID
2004	X-ray commissioning 22ID
	- 23 Member Institutions
2005	X-ray commissioning 22BM
2005	General User program on 22ID begins
2005	- 25 Member Institutions

Southeast Regional Collaborative Access Team

Light when YOU need it!

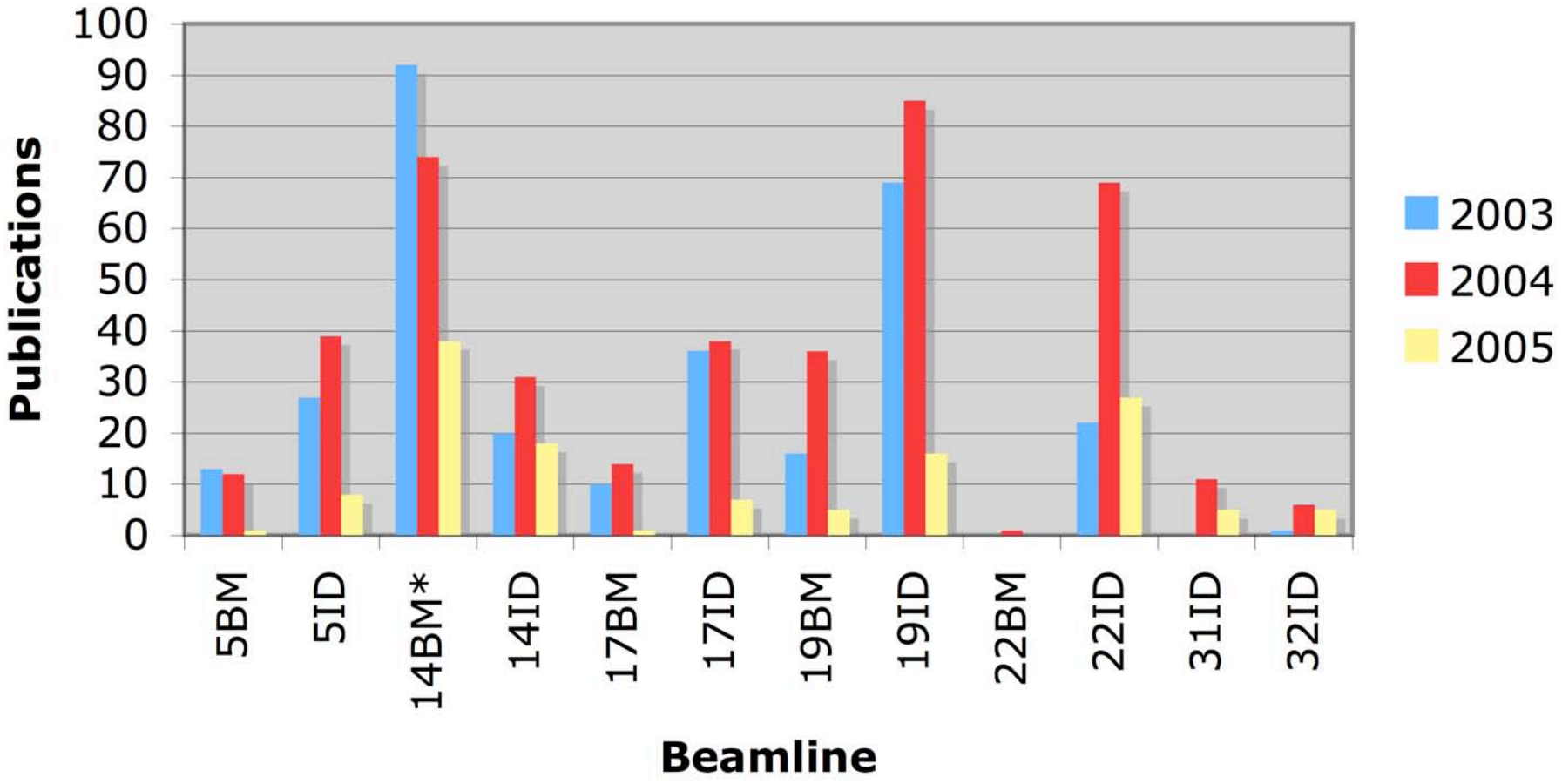


SER-CAT's current membership

- Duke University
- Emory University
- FUHS / The Chicago Medical School
- Florida State University
- Georgia State University
- Georgia Institute of Technology
- Medical University of South Carolina
- NASA George C. Marshall Space Flight Ctr.
- NIH Intramural Research Program
- North Carolina State University
- St. Jude Children's Research Hospital
- University of Alabama at Birmingham
- University of Alabama at Huntsville
- University of Georgia
- University of Kentucky
- University of Illinois at Chicago
- University of Missouri at Kansas City
- University of North Carolina at Chapel Hill
- University of Pittsburgh
- University of South Carolina
- University of Virginia
- Vanderbilt University
- Vanderbilt University Medical Center
- The Procter and Gamble Company
- Wyeth Pharmaceuticals

Yearly APS Publications by Beamline

As of July 8, 2005



*publications from 14BM-C and 14BM-D

Southeast Regional Collaborative Access Team

Light when YOU need it!

Member Users served 367

General Users served 58*

*Current run is over subscribed

Seed funds ($1.5 million) were obtained from the Georgia Research Alliance in 1997.

It took nearly 7 years to see the impact of the initial investment and effort.

Potential Impact of MaNDi
When should the investment in this resource begin?

Why Neutron Diffraction and why MaNDi?

Why Neutron Diffraction?

The ability to find accurate hydrogen positions is of fundamental importance in science!

All crystallographers know that hydrogen is the most difficult element to see by X-ray diffraction, but it has great importance in chemistry and in our lives overall.

If we combine X-ray crystallography with neutron diffraction, this partnership will be the last link to effectively see all of the elements in the periodic table.

Why are "We" Interested in Neutron Protein Crystallography Now?

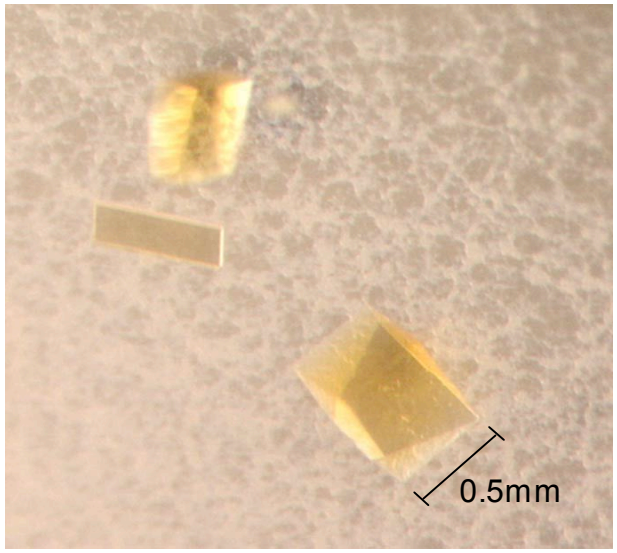
	X-ray	Neutron
Label	Se-MET ✔	D ✔
Crystal	0.2^3 mm ✔	0.5^3 mm ✔
Source	APS ✔	SNS

✔ Achievable

The reduction in the requirement of crystal size will make MaNDi an important cutting-edge resource to the structural biology community.

A Potential Project From Our Own Lab

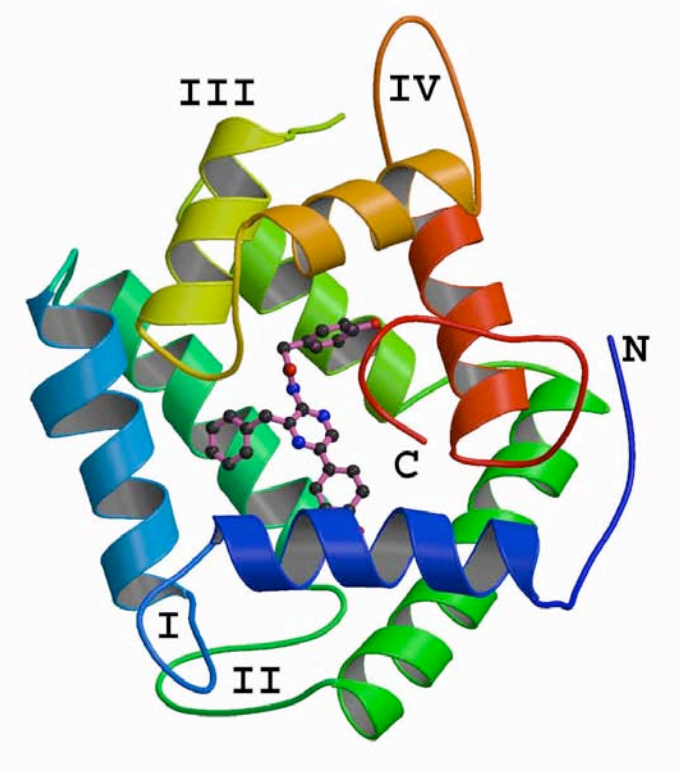
Calcium-regulated Photoprotein Obelin from *Obelia Longissima*



MW: 22.2kDa

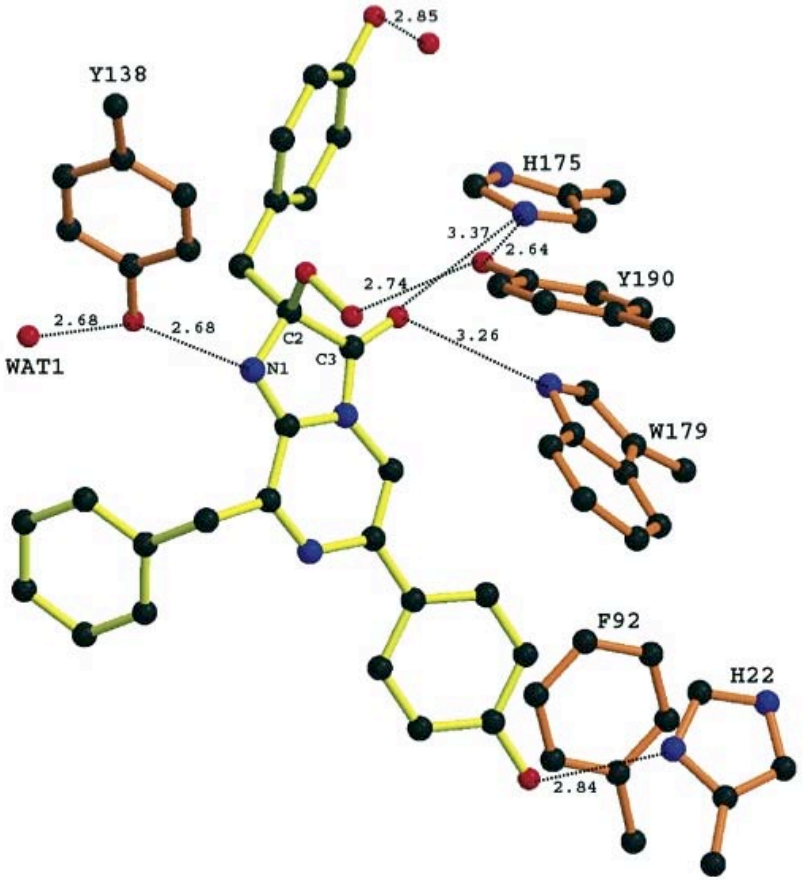
Space Group: C2

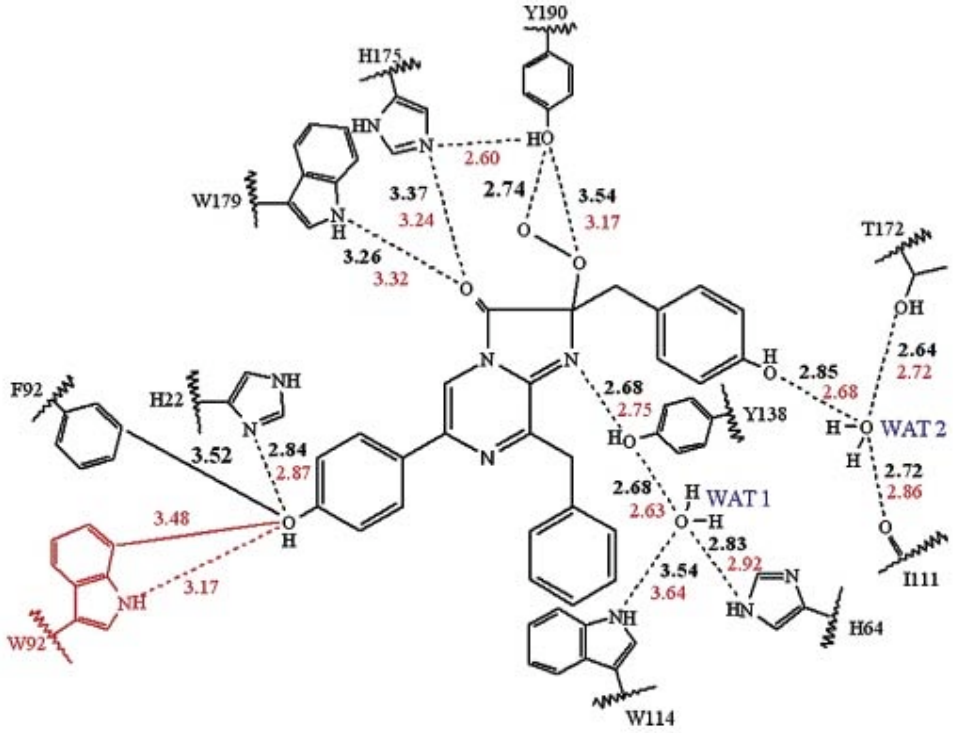
Resolution: 1.0Å



A proton-relay hypothesis has been proposed to explain how the Ca^{2+} binding triggers the bioluminescence reaction.

It is important to use the neutron diffraction to determine the location of protons and thus to experimentally prove the proton-relay hypothesis.





Predictions

"We", the community, will come, if MaNDi is built and functioning as it should

Another prediction on
A new potential

Long-wavelength X-ray beamlines for Direct crystallography

Diamond Synchrotron at UK
Is building a beamline
Optimized for long-wavelengths
(1.5 - 2.5 Å)

Prediction: people will come

Our Long-term Interest and Hypothesis:
Direct Crystallography Is Possible and Desirable

Use Unlabeled Native Crystals* and Single-wavelength X-rays

Metal atoms: **Fe, Co, Zn, Mn, Ca…., naturally present in metalloproteins, ~ 30% of all proteins contain metals (HHF for structural genomics)**

Sulfur atoms: **Nearly all proteins have sulfur**

If sulfur phasing is successful then virtually any other anomalous scatterer becomes available for phasing!

- **Can we use atoms that are already in the protein molecule as phasing probe?**
- **Can we use single wavelength X-rays?**

*The incorporation of selenomethionine in yeast, insect or eukaryotic cell lines, while possible, can present serious technical difficulties…" (page 8, Diamond Beamline Proposal 044)

Historical Perspective
Single-wavelength and Sulfur-SAS Phasing

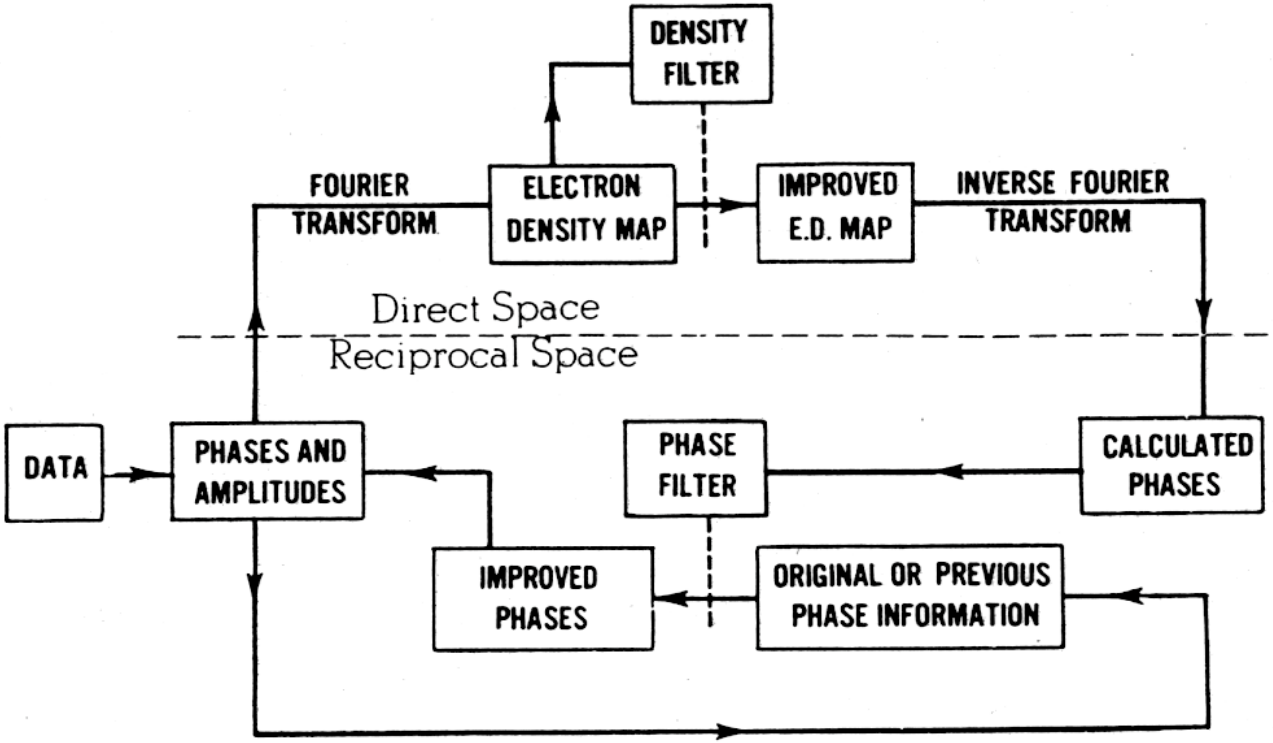
Two different theoretical approaches developed in 1980s

1981: **Crambin (4.7 kDa, 6 sulfurs)** (Hendrickson and Teeter, *Nature*, 289, 366, 1981). **A single-step process** called Resolved Anomalous (RA) phasing. Requires a **high percentage of sulfur atoms and high resolution data (1.5Å).**

1982: **Rhe (12.5 kDa, 2 sulfurs)** (Wang, *Method Enzymol*. 115, 90-122, 1985) **A multi-step processing** using "filters", Fourier transform and iteration, called **ISAS**. **Does not require high sulfur content or high resolution.** Simulation results showed that each sulfur atom can phase at least **57** residues.

An Iterative Algorithm

Noise Filtering (Solvent Flattening)

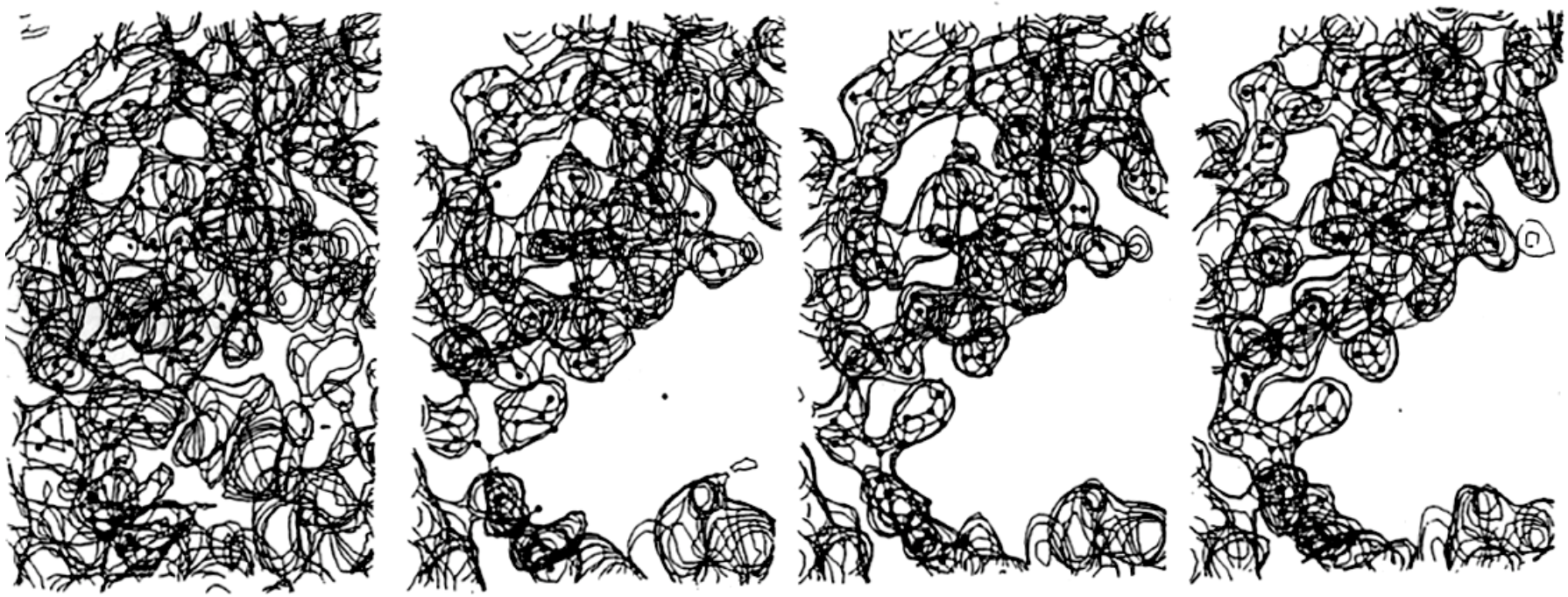


Wang, 1985

Computer Simulation on Rhe (113 res. 2 sulfurs) by Sulfur-ISAS Method

(Calculated in 1982 using simulated data)

SAS (Unfiltered) | Filter 1 Cycle 1 | Filter 3 Cycle 8 | F_{cal}

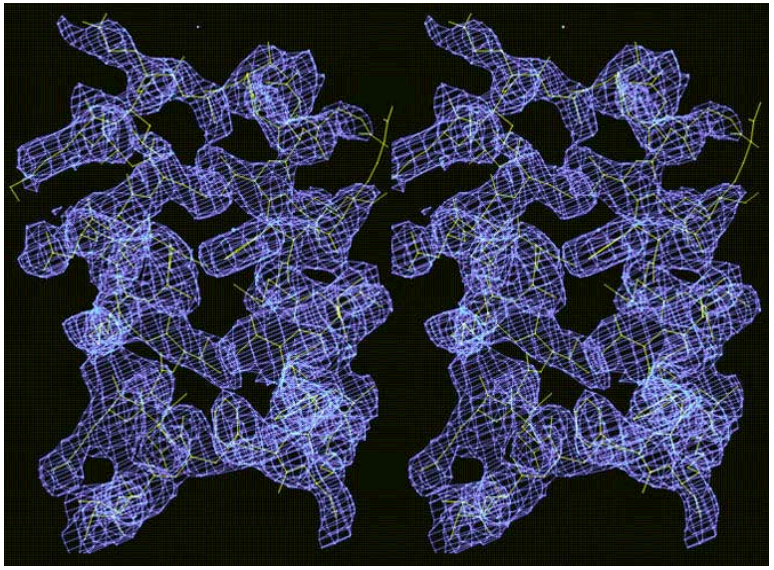


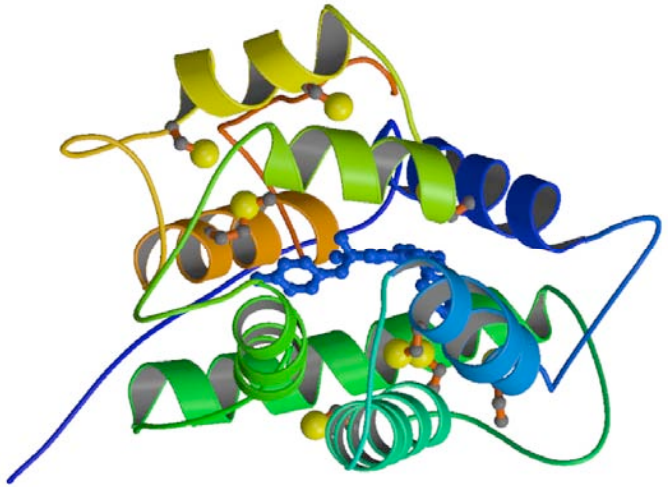
(Wang (1985), *Methods Enzym*, **115**, 90-112)

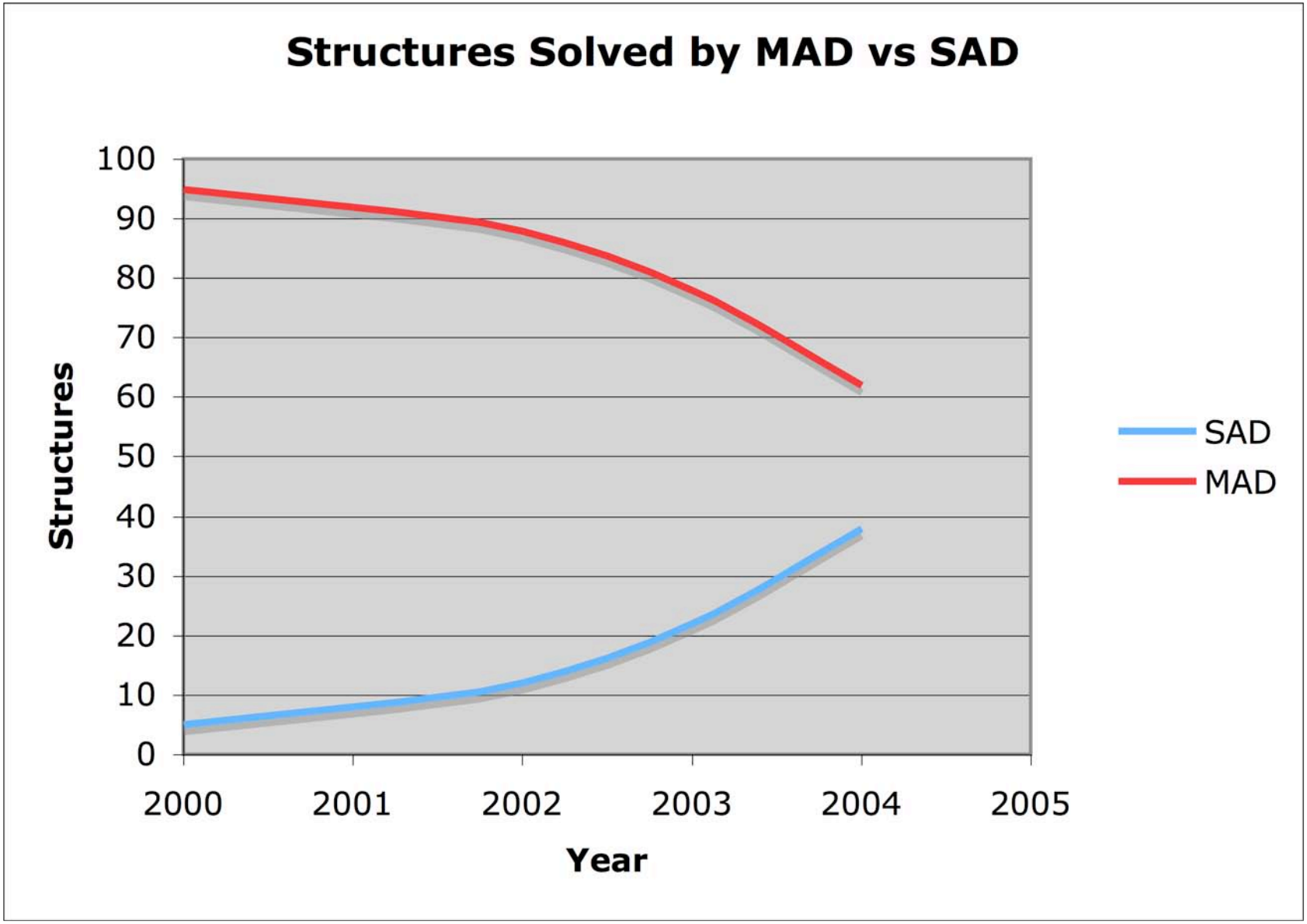
Photoprotein Obelin was Solved using the Weak Sulfur SAS Signal

Liu, etc, *Protein Science*. 9(11), 2085-93, (2000)
The first *de novo* structure solved using S-ISAS method (or solvent flattening).

M. W.	22.2 kD
# of S:	8
Phasing Resolution	3.0 Å

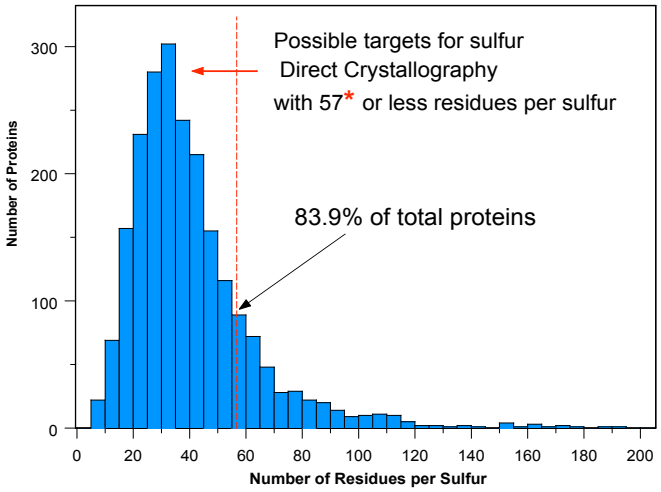




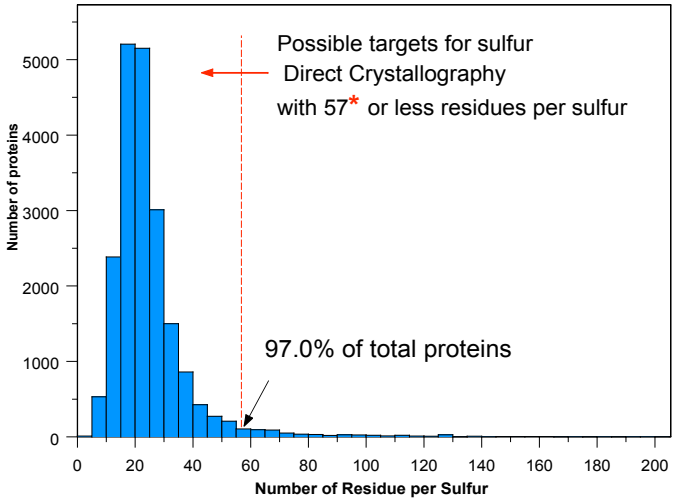

Structures Solved by MAD vs SAD
100
90
80
70
60
50
40
30
20
10
0
Structures
2000
2001
2002
2003
2004
2005
Year
SAD
MAD

Over 80% - 90% of Proteins Are Possible Targets for Sulfur Direct Crystallography

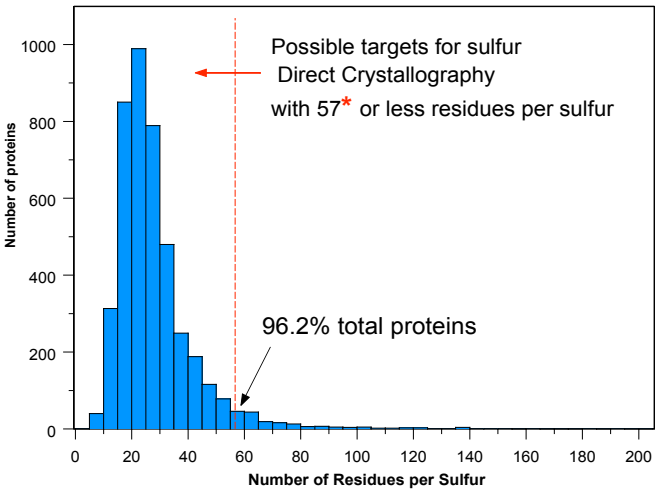
Sulfur Distribution in *Pyrococcus furiosus* Genome



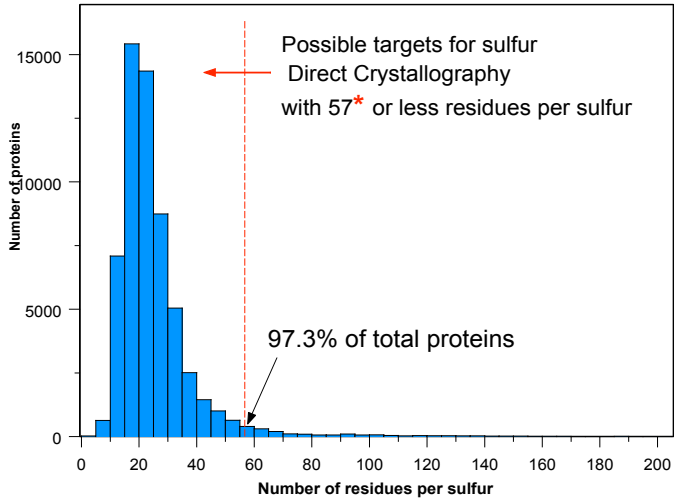
Sulfur Distribution in *Caenorhabditis elegans* genome



Sulfur Distribution in *Esherichia Coli* Genome



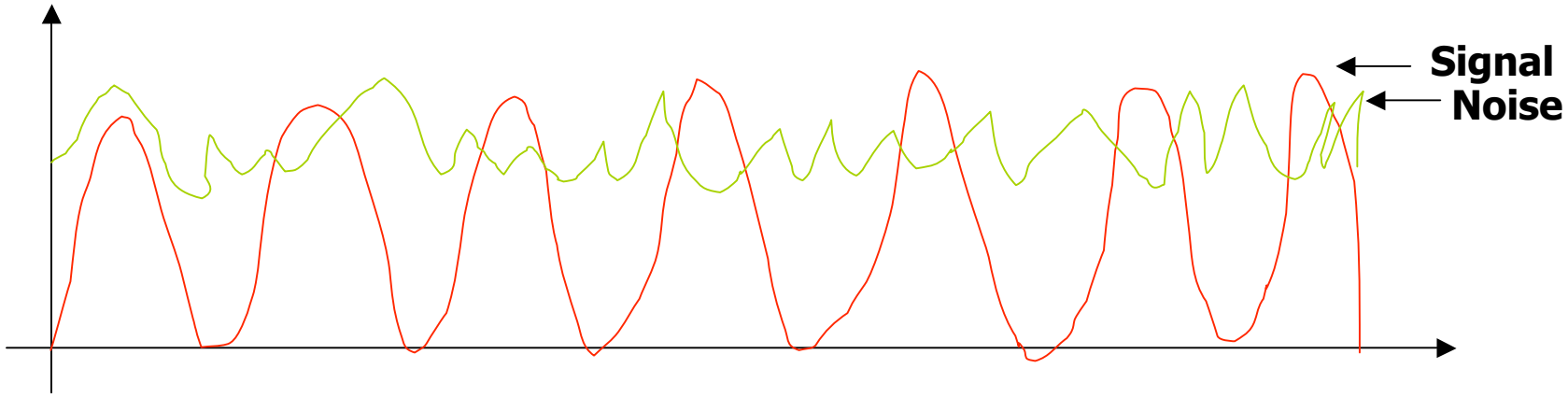
Sulfur Distribution in Human Genome



*** The number based on the Rhe simulation study by Wang (Methods Enzymol. 1985)**

Solution to Sulfur SAS Phasing Problems

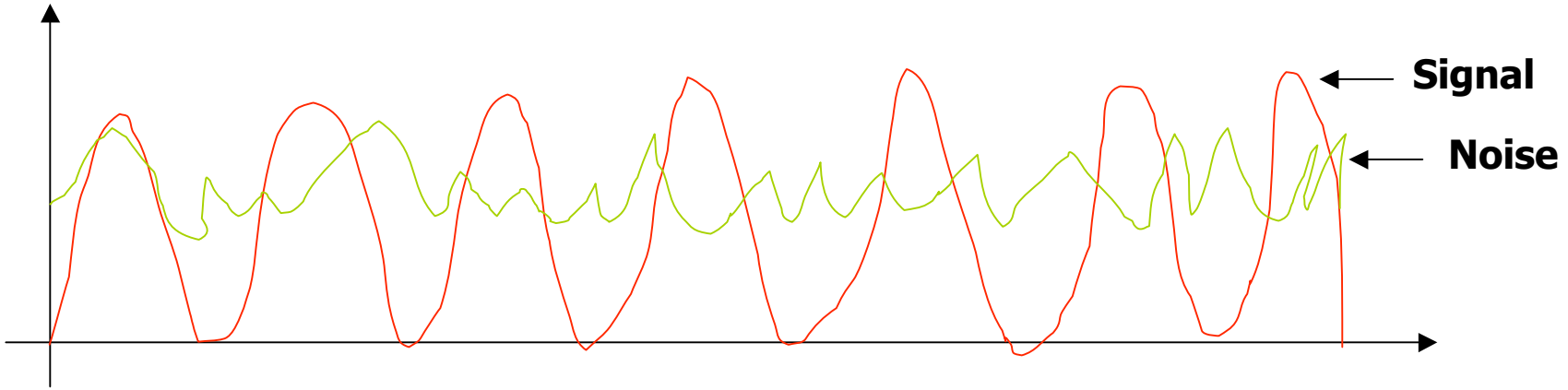
1. Reduce the noise to increase the signal/noise ratio



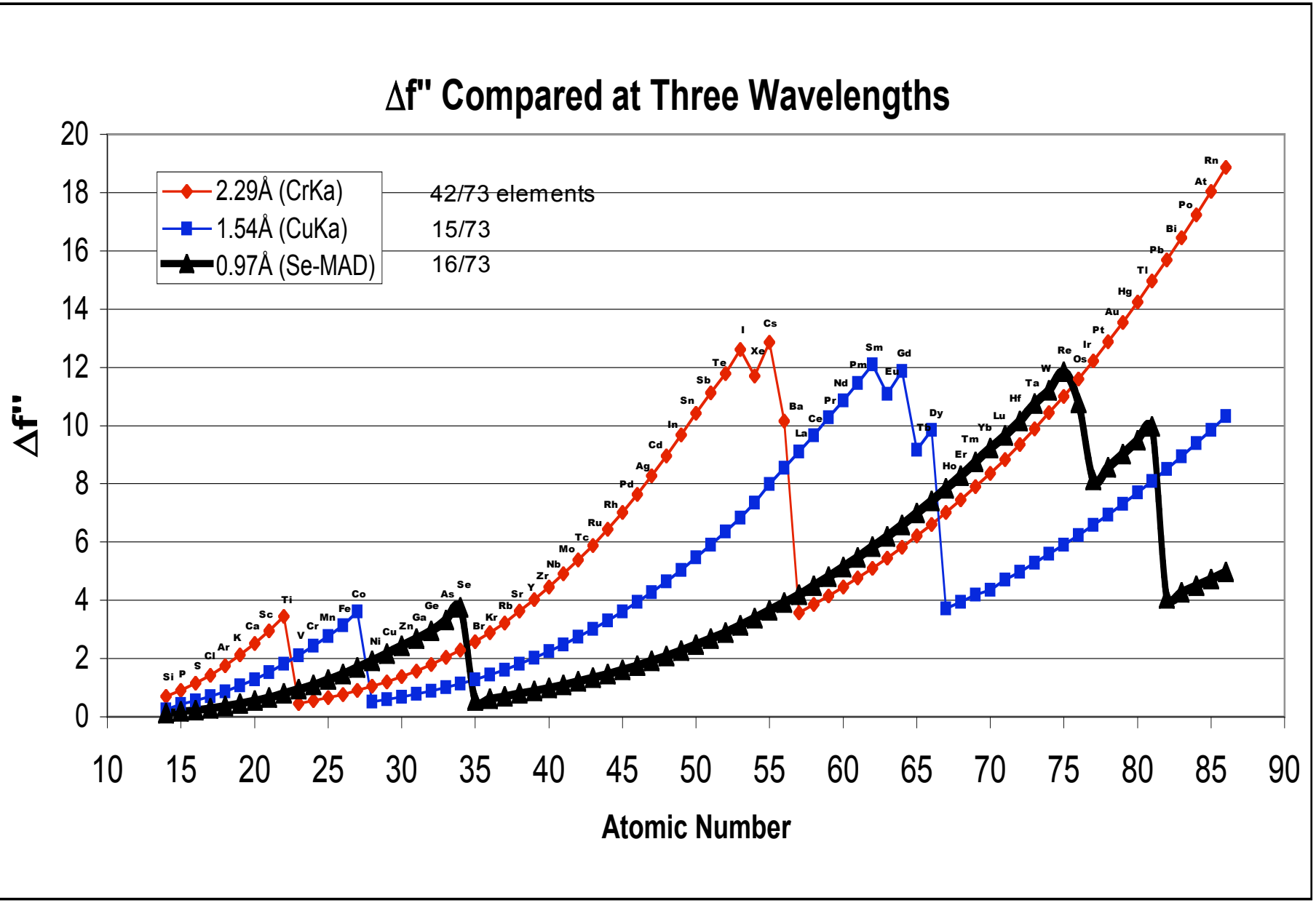
- Incorporation of improved X-ray optics and detector technologies
- Ensure that X-ray source is stable
- Accurate measurement of the diffraction data
- Multi-crystal averaging to increase the redundancy

Solution to Sulfur SAS Phasing Problems

2. Increase the signal to increase the signal/noise ratio

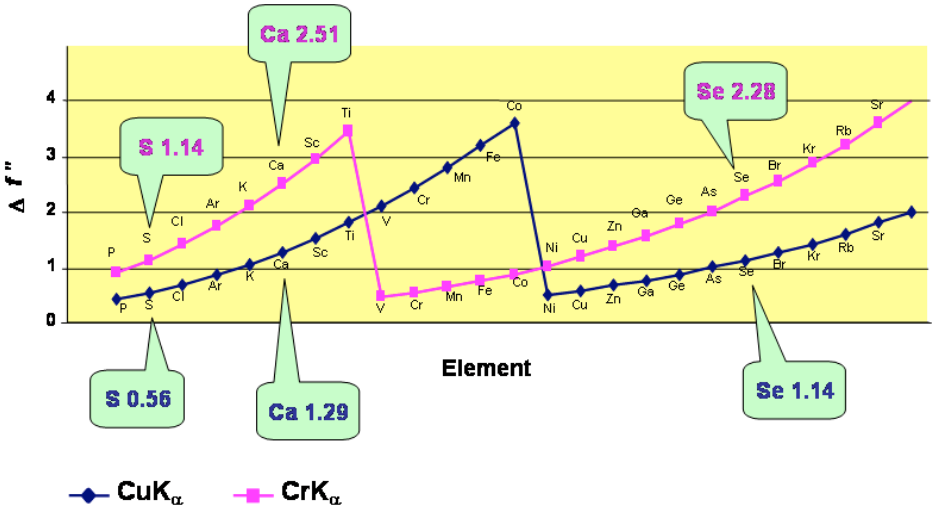


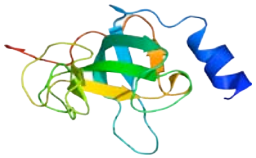
➢Using softer X-rays

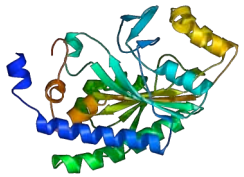


Dr. M. Gary Newton, University of Georgia

Sulfur SAS phasing on a Cr X-ray source by *Sca2Structure Pipeline*




Cel-F53F4.3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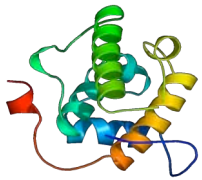
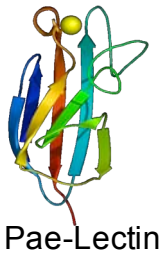
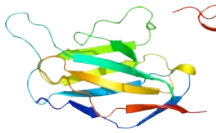
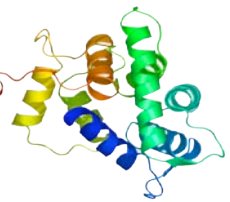
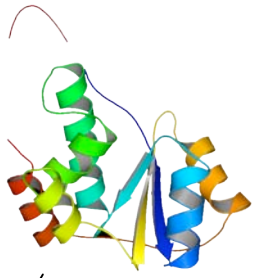

Hum-Q15691


Pae-Lectin1


Has-BJLEN


Aae-Aeq


SSO10A


PFU 542154

50 residues / sulfur

Three Cases of

If You Build it "We" Will Come

Acknowledgements

John Rose
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