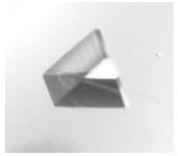
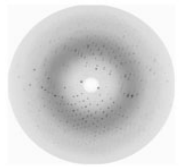
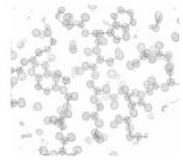
On the Use of Longer X-ray Wavelengths in Macromolecular Crystallography

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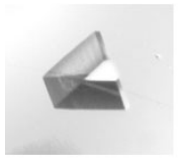
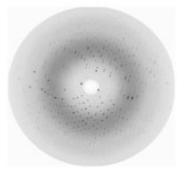
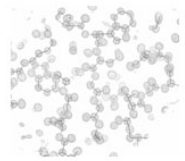
Longer X-ray Wavelengths ?

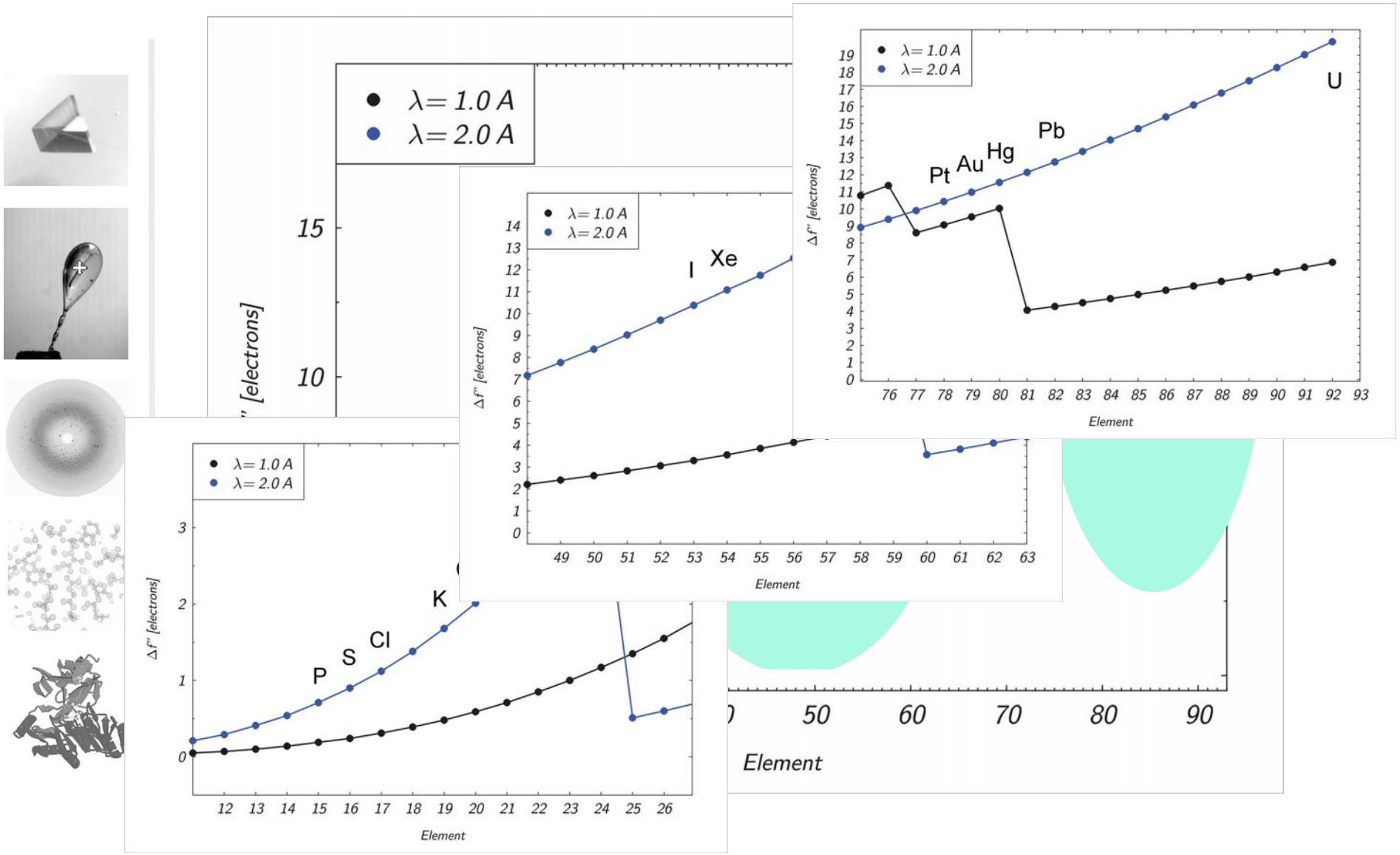


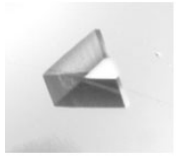

$\lambda \approx 1.5 – 3.0$ Å

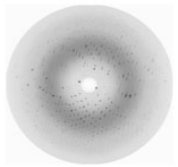
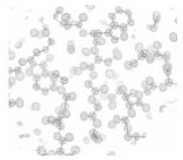
$E \approx 8 – 4$ keV

Why Longer X-ray Wavelengths ?



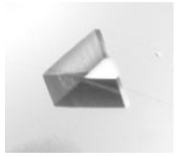
Candidate Experiments

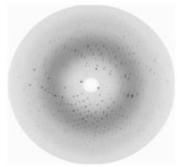
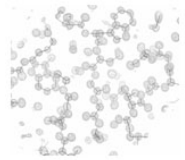



- Experimental Phase Determination
  - SAD on sulfur and other light elements
  - Pressure derivatization using xenon
  - Iodide, triiodide or I3C soaks
  - Quick-soaking with uranyl salts

Candidate Experiments

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  - MRSAD
  - Phased molecular replacement
  - Combined use of amplitudes and differences

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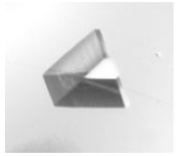

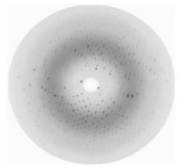
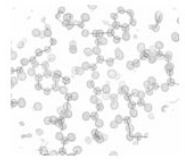


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- Molecular Replacement
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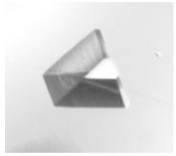
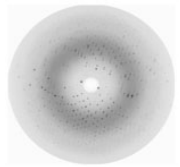
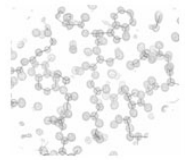
  - Phased molecular replacement
  - Combined use of amplitudes and differences
- Refinement

Candidate Experiments




- Experimental Phase Determination
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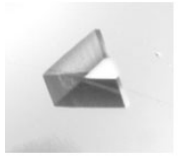
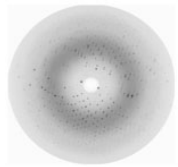
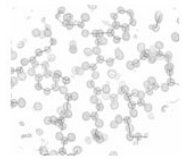
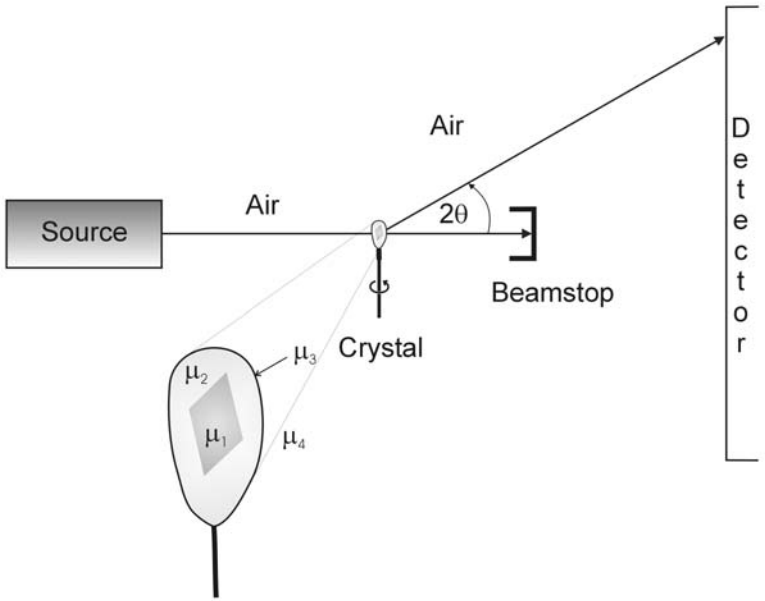
  - Iodide, triiodide or I3C soaks
  - Quick-soaking with uranyl salts
- Molecular Replacement
  - MRSAD
  - Phased molecular replacement
  - Combined use of amplitudes and differences
- Refinement
- Structure Validation
  - Verification of the chain trace
  - Localisation of ions, ligands, etc.

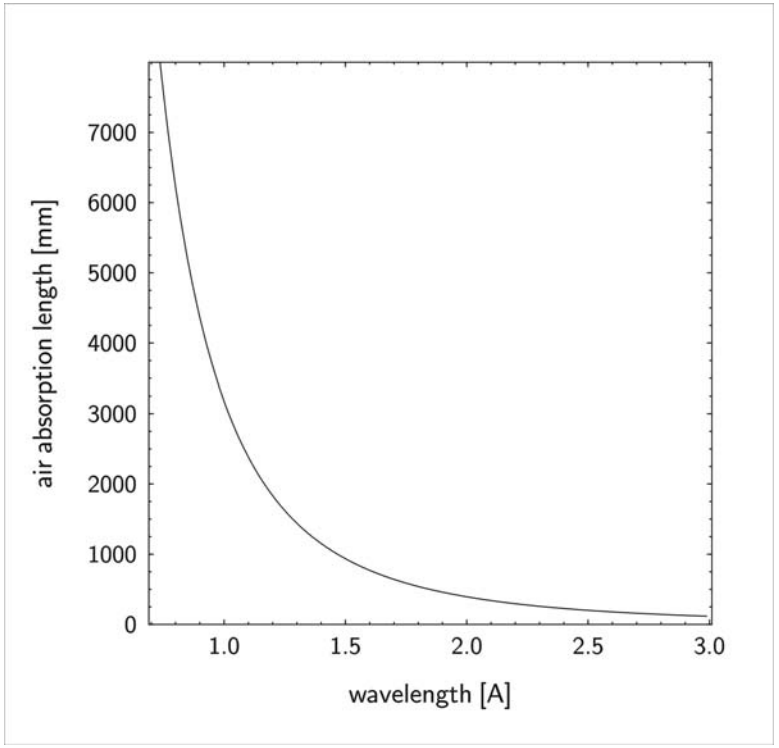
Challenges and Problems




- Absorption





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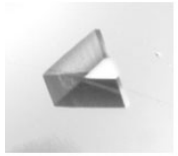
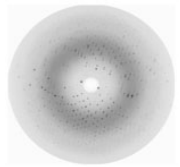
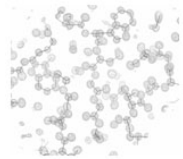
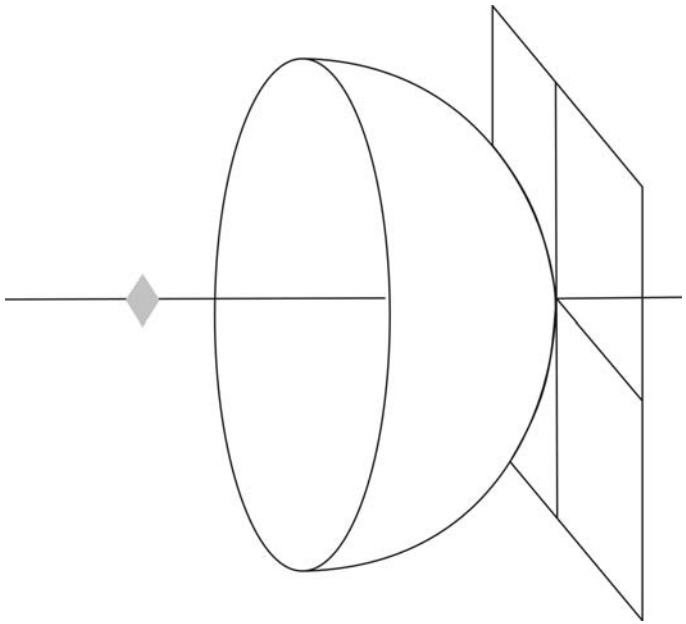
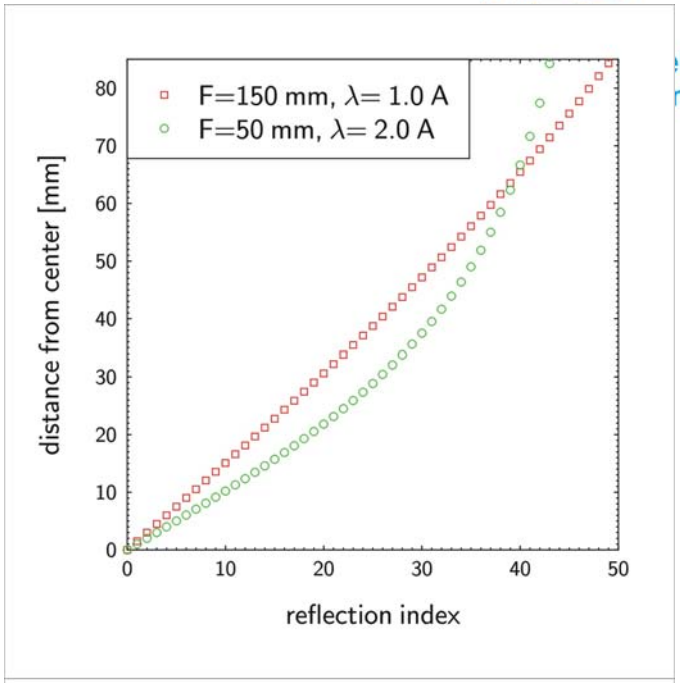
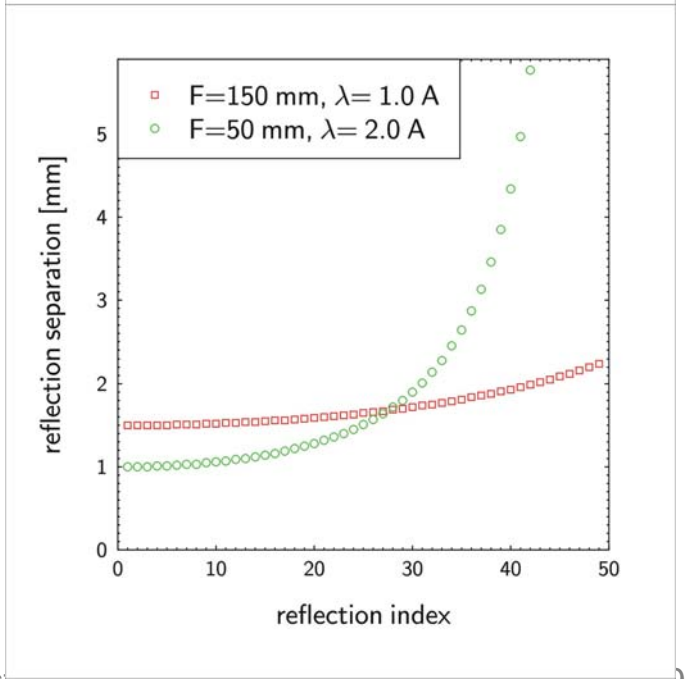



- Absorption
- Large scattering angles







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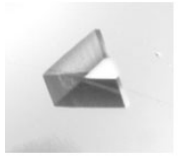
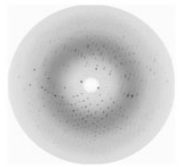
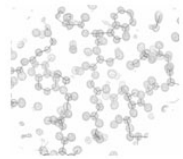
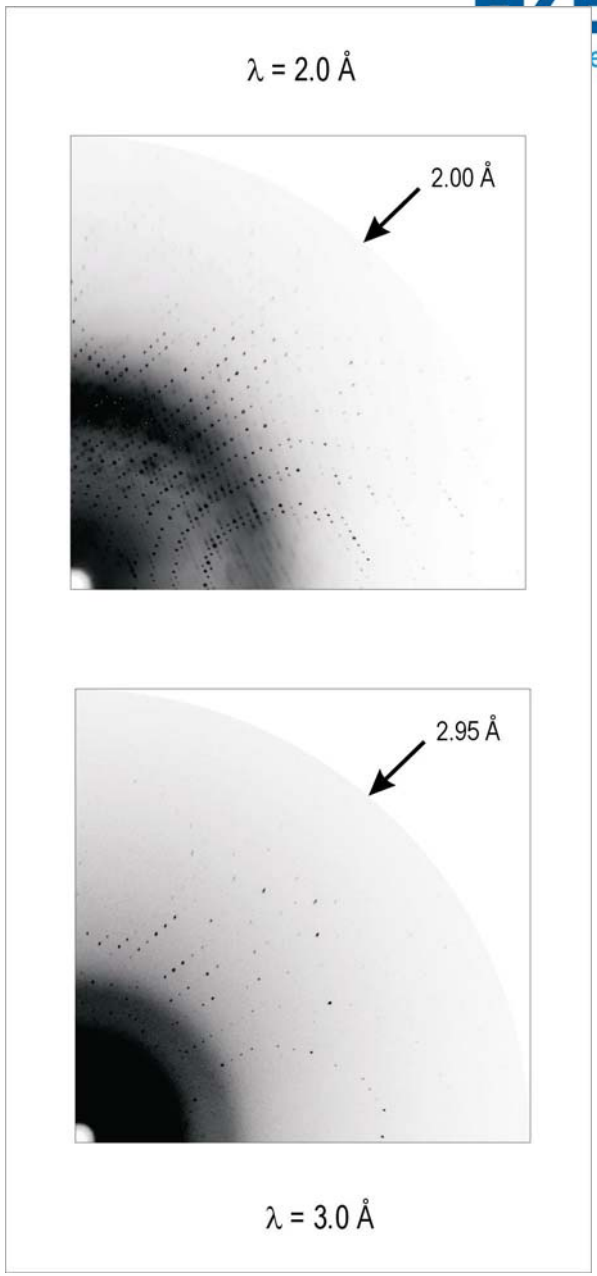



- Absorption
- Large scattering angles
- Air scattering



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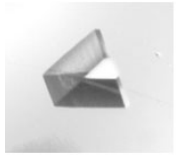
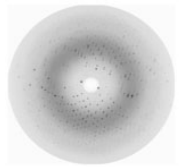
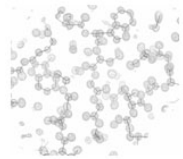
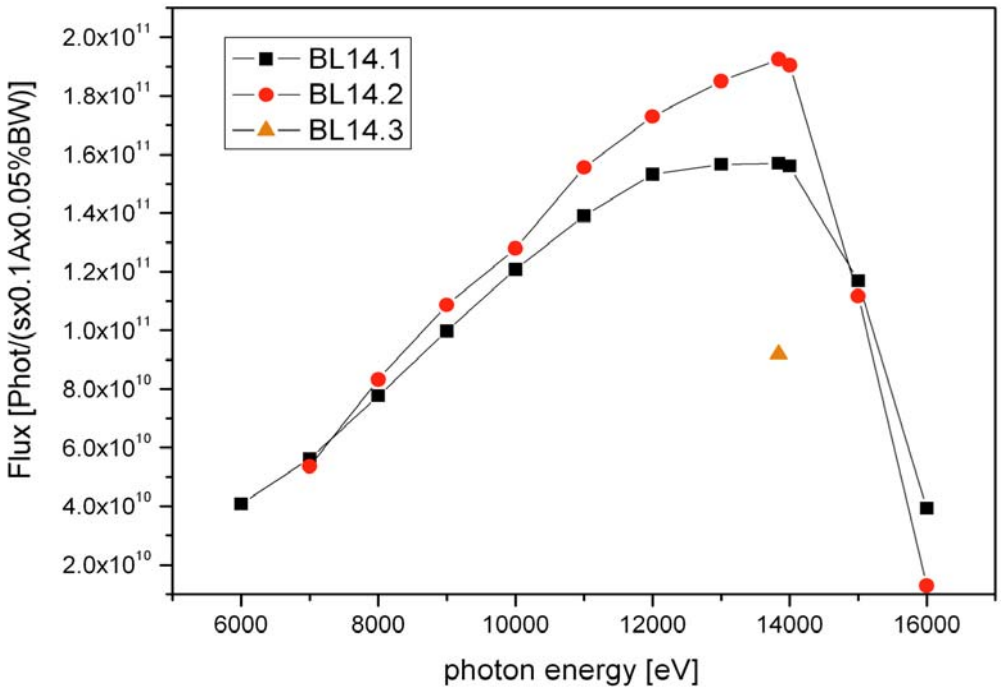



- Absorption
- Large scattering angles
- Air scattering
- Energy spectrum



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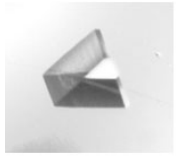

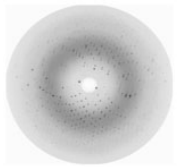

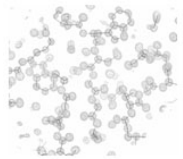

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- Air s
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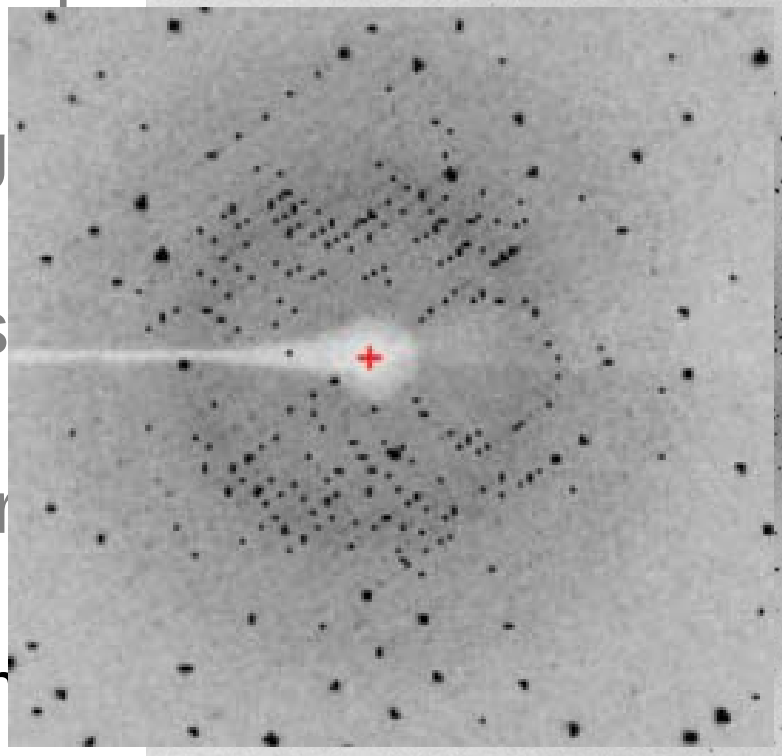

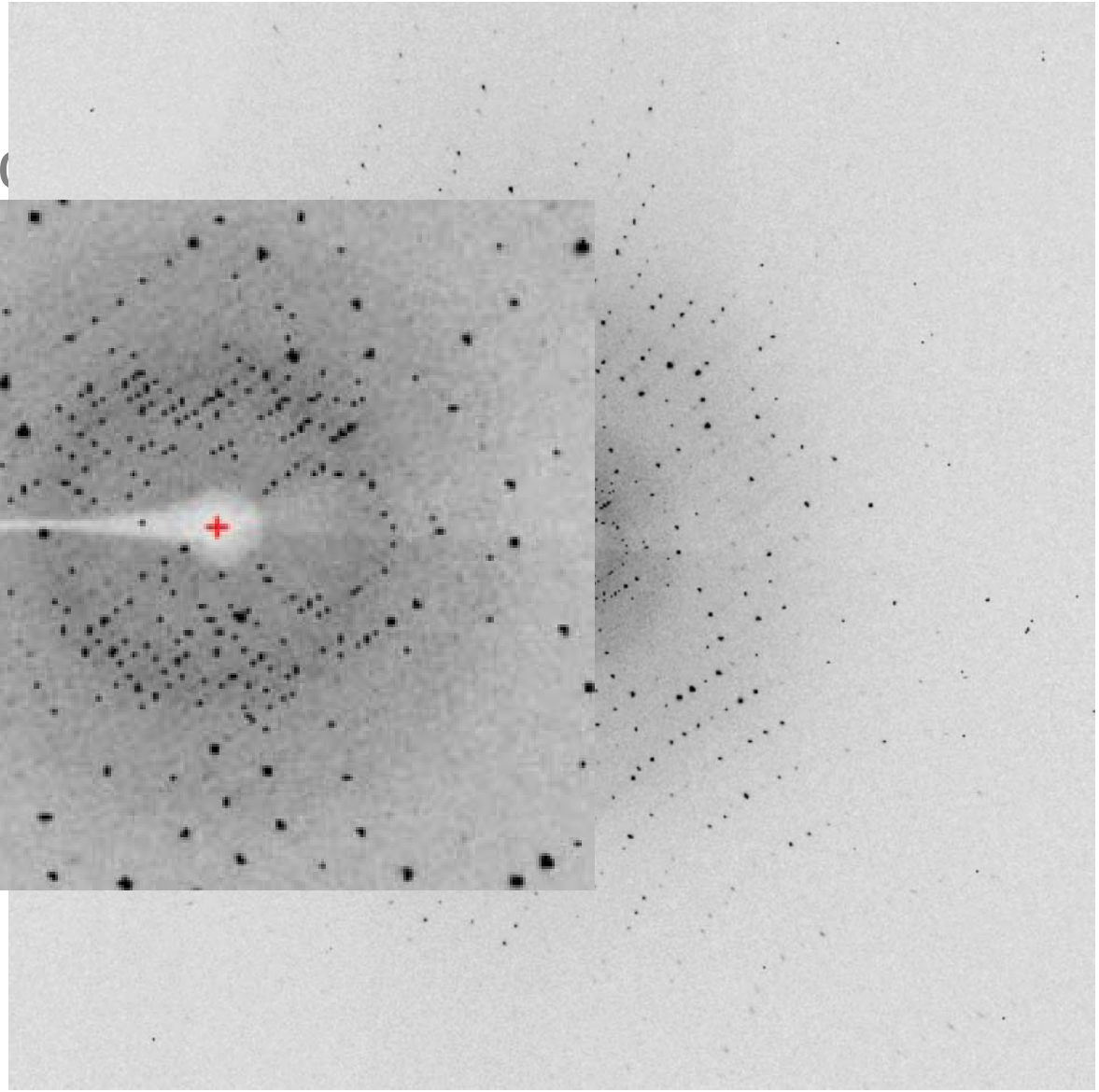

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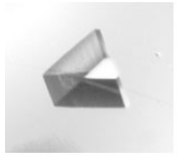
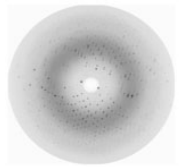
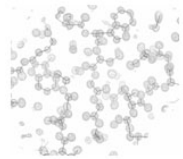
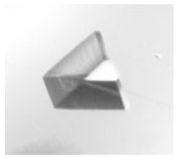
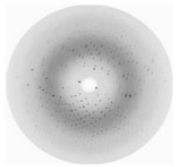


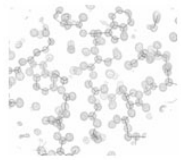




- Absorption
- Large scattering angles
- Air scattering
- Energy spectrum
- Harmonic contamination
- Monochromator limits

S-SAD Phase Determination







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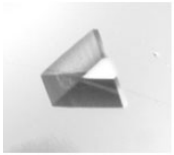
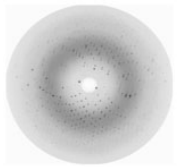
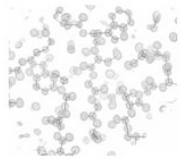
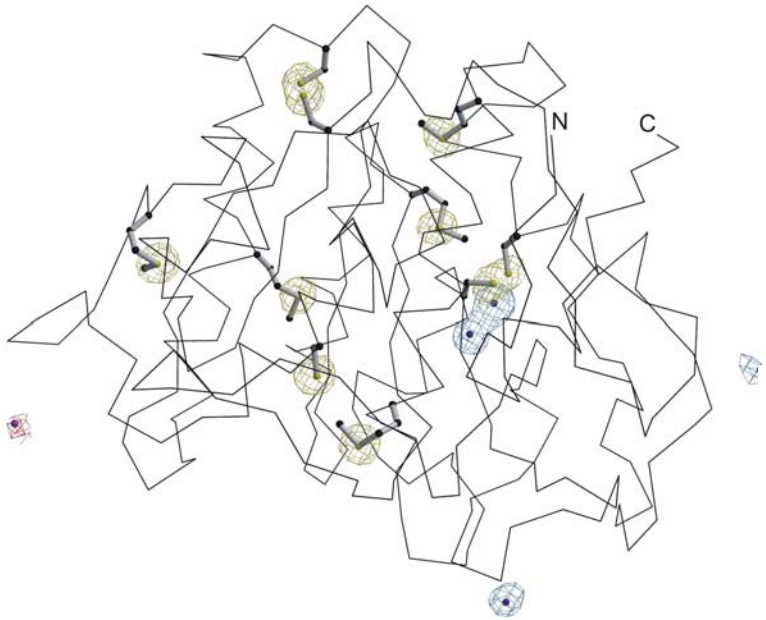
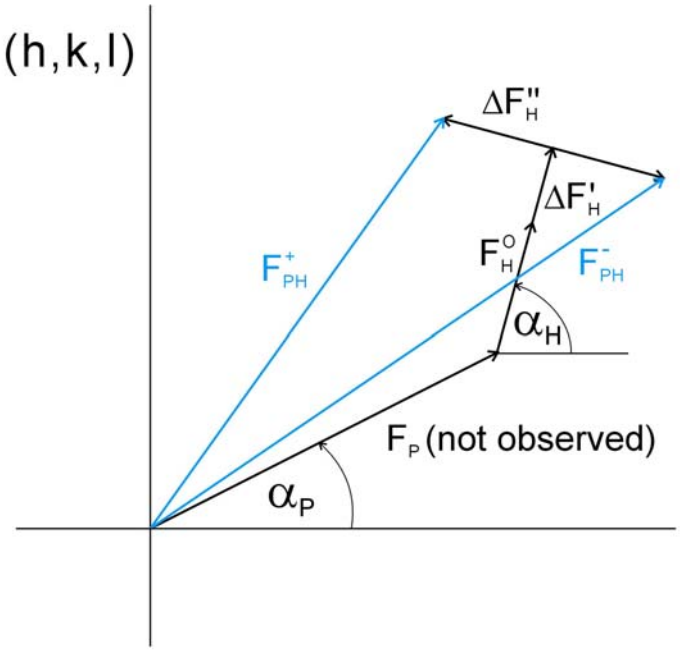
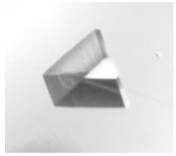


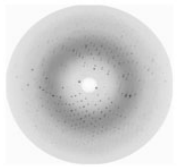


S-SAD – very small signal

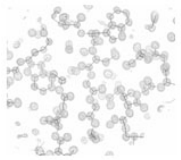


SIR: $R = 100 \bullet \Sigma_{hkl} \, | \, |F_{PH}| - |F_P| \, | \, / \, \Sigma_{hkl} \, |F_P|$ 15-30%

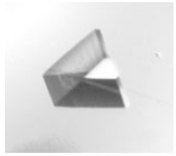
SAD: $R_{anom} = 200 \bullet \Sigma_{hkl} \, | \, I^+ - I^- \, | \, / \, \Sigma_{hkl} \, | \, I^+ + I^- \, |$ ~5%

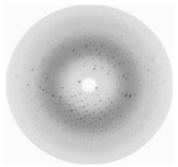
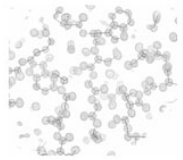


S-SAD: ~1%



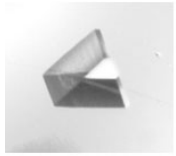
S-SAD – where are we now?

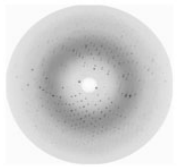
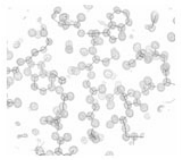



- 1981 Crambin (Hendrickson & Teeter)
- 1999 HEWL (Dauter *et al*.)
- 2000 Obelin (Liu *et al*.)
- 2001-2011: about 80 novel structures

S-SAD – 1999-2012

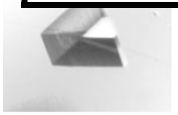


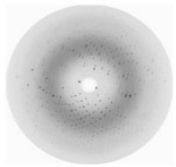



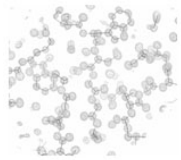

- about 50 methodological papers published
- Cr anode on the market (λ = 2.29 Å)
- more SR beam lines offer the possibility to collect data at longer wavelengths
- X-ray equipment companies often use an S-SAD diffraction data set as quality criterion
- significant improvement in structure determination software

S-SAD – 14 model systems

System	SG	CuKα 1.54 Å	CrKα 2.29 Å	Synchrotron Radiation 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3





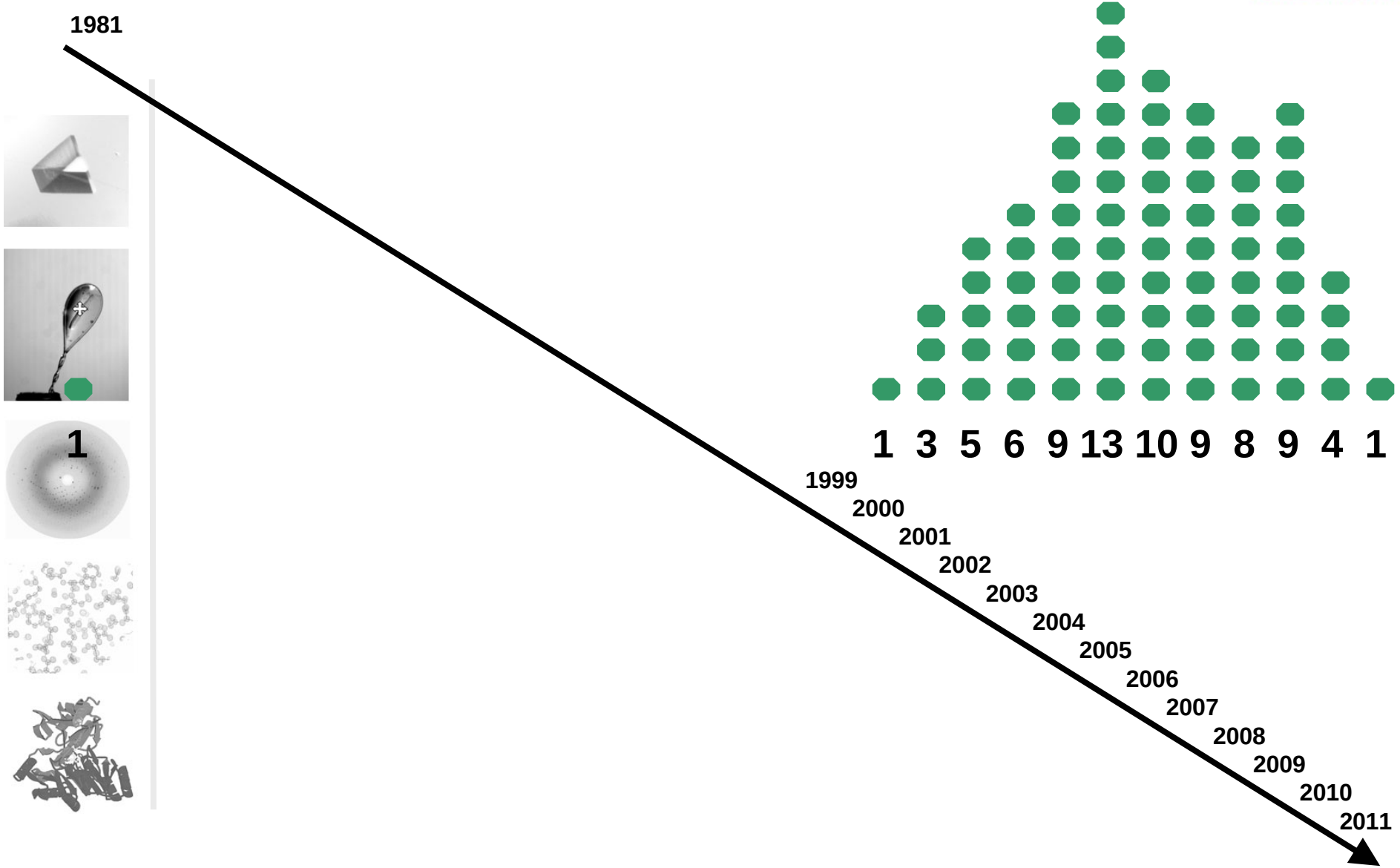


S-SAD – 14 model systems

HZB Helmholtz Zentrum Berlin

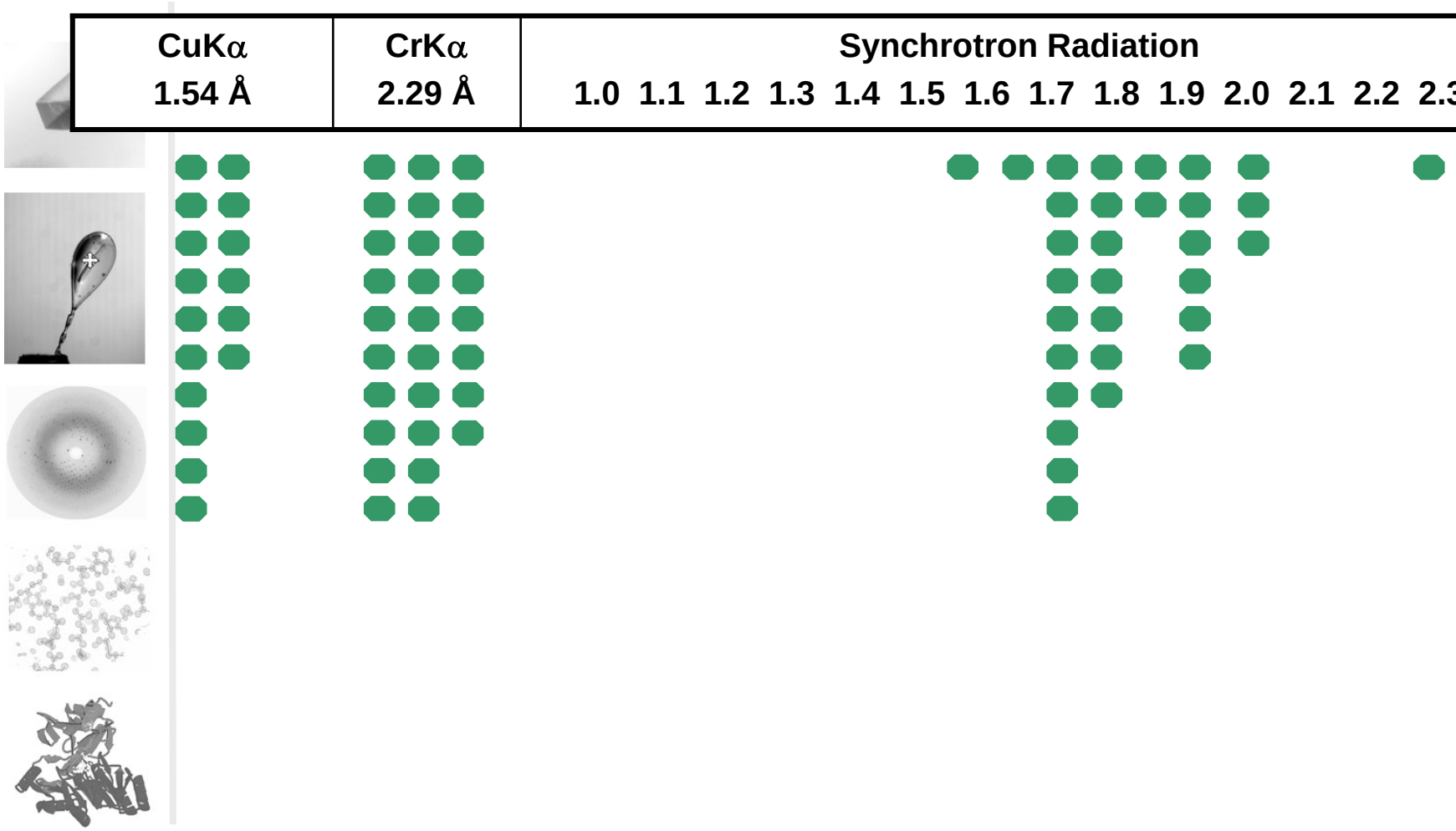
System	SG	CuKα 1.54 Å	CrKα 2.29 Å	Synchrotron Radiation 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3
Insulin	$I2_13$	X		X
Thermolysin	$P6_122$			X X X X
Lysozyme	$P4_32_12$	X	X	X X X X X X X X
Proteinase K	$P4_32_12$			X X
Thaumatin	$P4_12_12$	X	X	X X
Trypsin	$P3_121$	X	X	X
Insulin	R3	X		
Elastase	$P2_12_12_1$			X
Trypsin	$P2_12_12_1$	X	X	X
Subtilisin	$P2_12_12_1$	X		X
GFP	$P2_12_12_1$	X		
DNA	$P2_12_12_1$			X
Glucose isomerase	I222		X	X X
Xylanase	$P2_1$			X

S-SAD – 79 novel structures



S-SAD – 79 novel structures

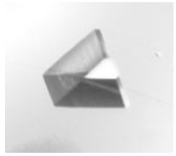
Radiation Source and Wavelength

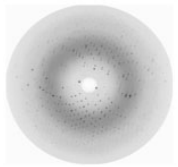


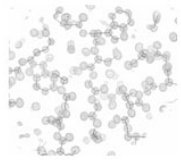
S-SAD – 79 novel structures

HZB Helmholtz Zentrum Berlin

Content per asymmetric unit







S-SAD – 79 novel structures

HZB Helmholtz Zentrum Berlin

Content per asymmetric unit

< 100 aa

100-150 aa

150-200 aa

200-250 aa

250-300 aa

300-350 aa

350-400 aa

> 400 aa

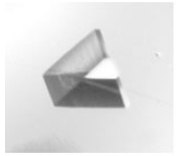
Largest structure:
2 x 738 aa
Watanabe *et al.*, 2008

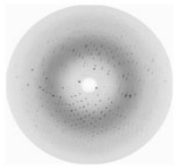
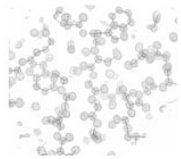
S-SAD – 79 novel structures

HZB Helmholtz Zentrum Berlin

Symmetry		
cubic	⬢⬢	2
hexagonal	⬢⬢⬢⬢⬢⬢⬢⬢⬢	9
tetragonal	⬢⬢⬢⬢⬢⬢⬢⬢⬢⬢ ⬢⬢⬢⬢⬢⬢	16
trigonal	⬢⬢⬢⬢⬢⬢⬢⬢	8
orthorhombic	⬢⬢⬢⬢⬢⬢⬢⬢⬢ ⬢⬢⬢⬢⬢⬢⬢⬢⬢ ⬢⬢⬢⬢⬢⬢⬢⬢⬢	30
monoclinic	⬢⬢⬢⬢⬢⬢⬢⬢⬢⬢ ⬢⬢	12
triclinic		0

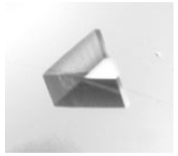
Data base of S-SAD structures

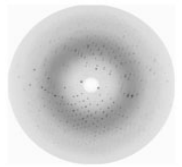
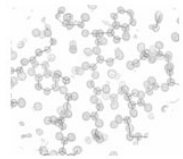



Initiative started by John Rose (U of Georgia)

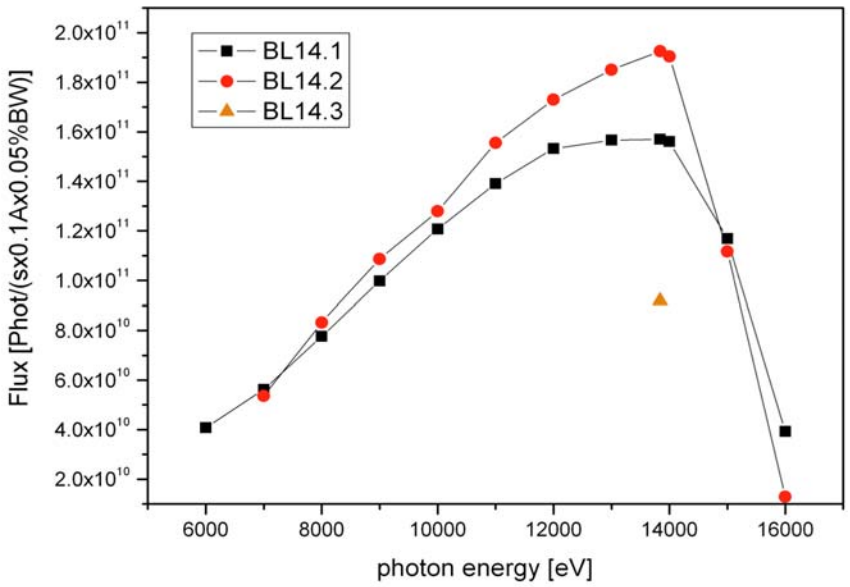
HZB Contribution – BL14.2



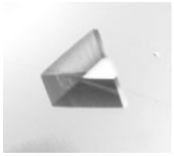



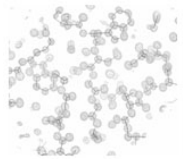

Long Wavelength Applications

- BL14.2 is particularly well suited
- long wavelengths
- small crystal-detector distance (45 mm)



Lysosomal 66.3 kDa Protein





S-SAD

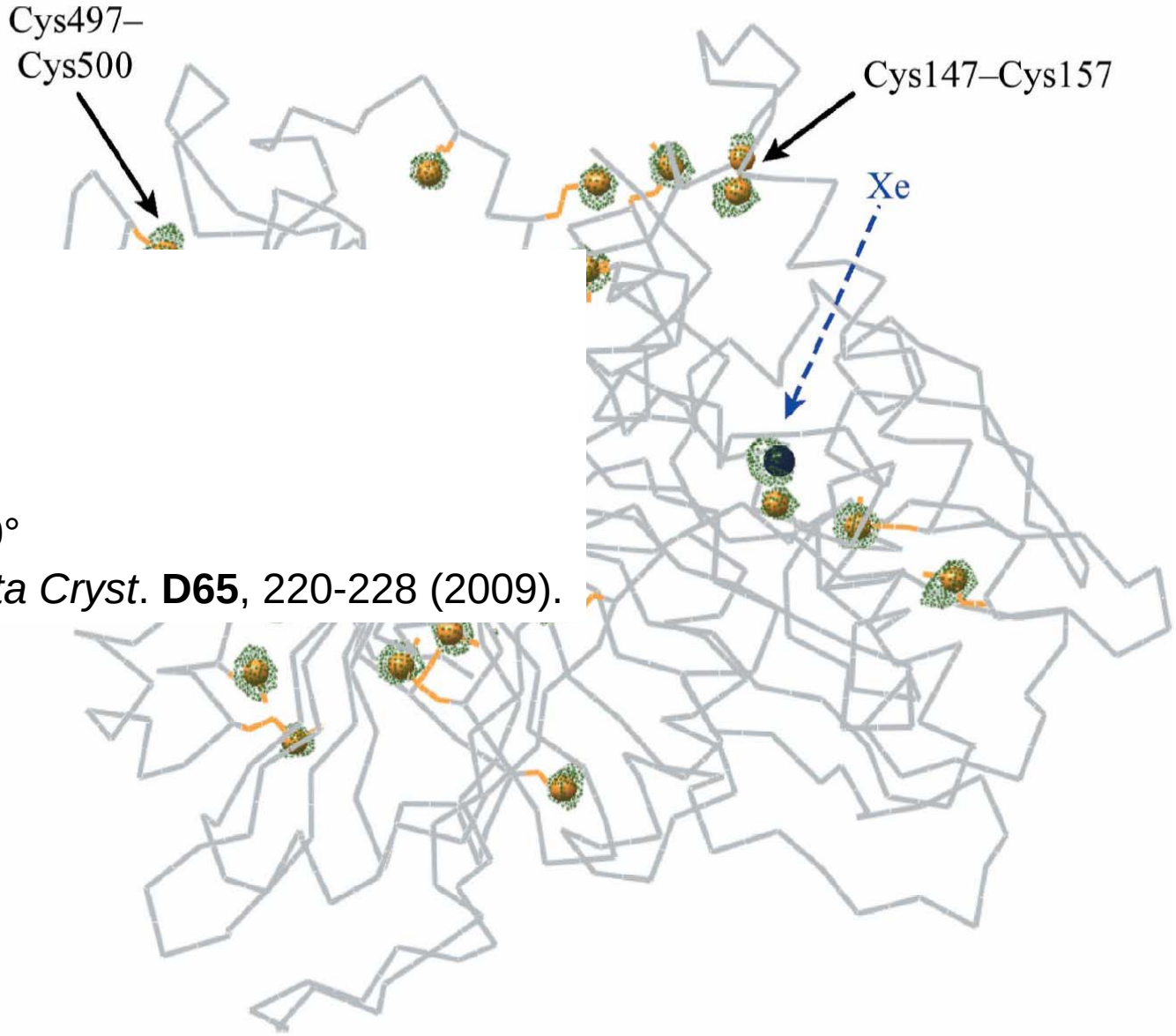
-space group C2

- λ = 1.9 Å

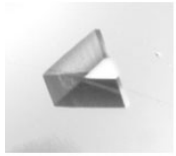
- BL14.2

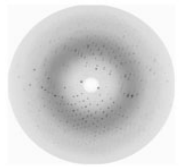
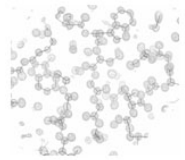
- 1120 images à 1.0°

- Lakomek *et al. Acta Cryst*. **D65**, 220-228 (2009).



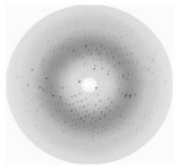
Where can we improve?

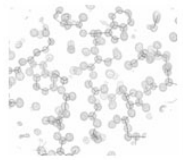


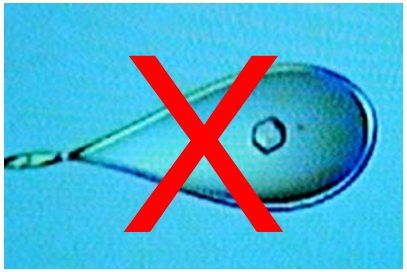

- Crystal mounting
- Data collection, better strategies
- Data processing / scaling

Crystal Mounting



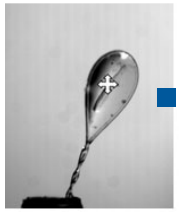


- Match loop size to crystal size

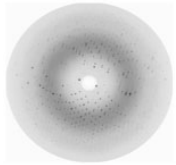


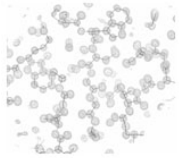
Crystal Mounting

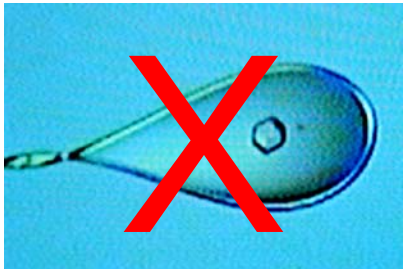
- Match loop size to crystal size



- Harden Loops

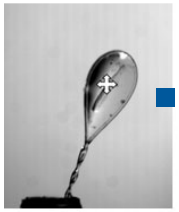




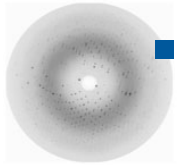


Crystal Mounting

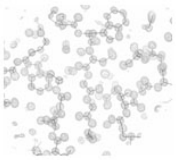
- Match loop size to crystal size

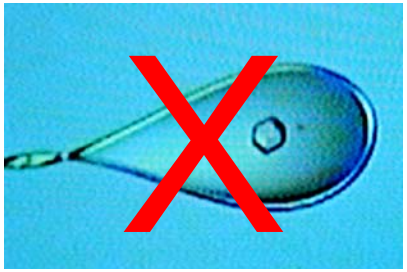


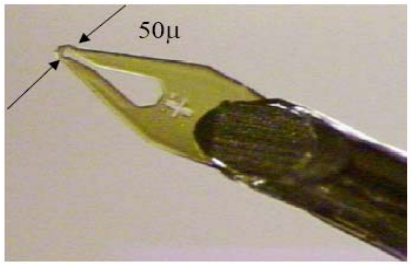
- Harden Loops

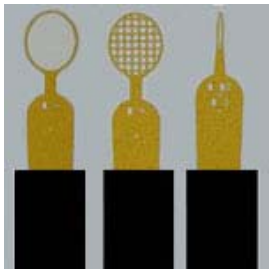


- Try Other Mounts



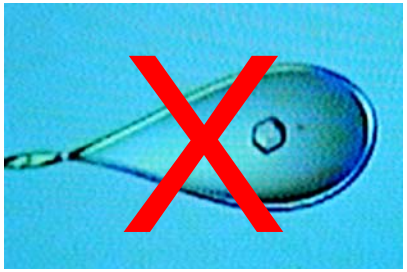


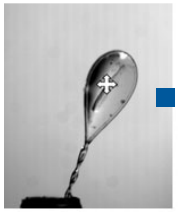




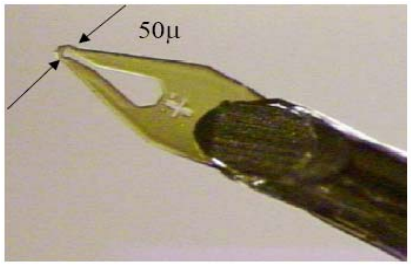
Crystal Mounting

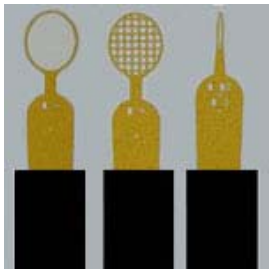
- Match loop size to crystal size

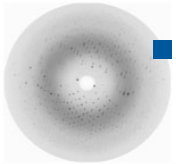




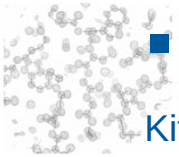
- Harden Loops





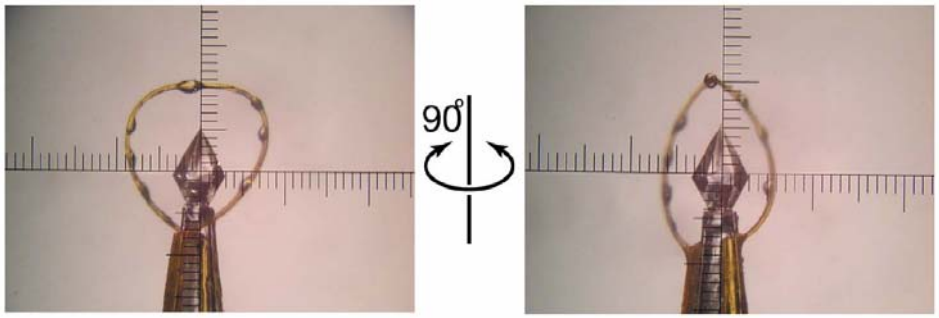


- Try Other Mounts

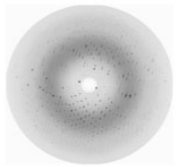


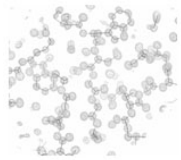
- Loopless Mounting

Kitago *et al*. (2005). *Acta Cryst*. **D61**, 1013-1021.
Kitago *et al.* (2010). *J. Appl. Cryst.* **43**, 341-346.

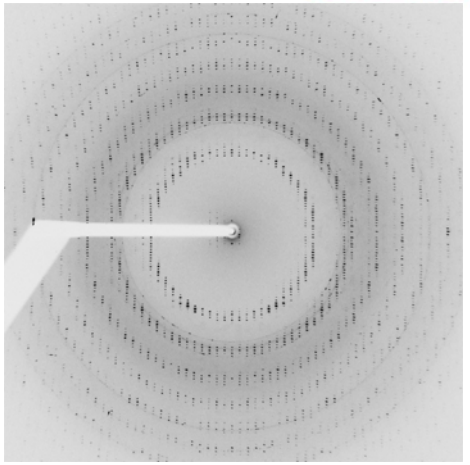


Data Collection Strategies - 1



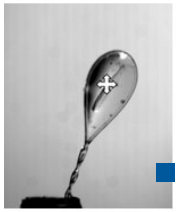


- Make Use of Kappa-Geometry

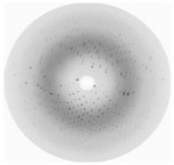


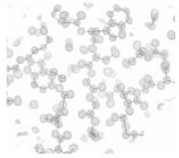
Data Collection Strategies - 1

- Make Use of Kappa-Geometry



- A Poor Man’s Kappa





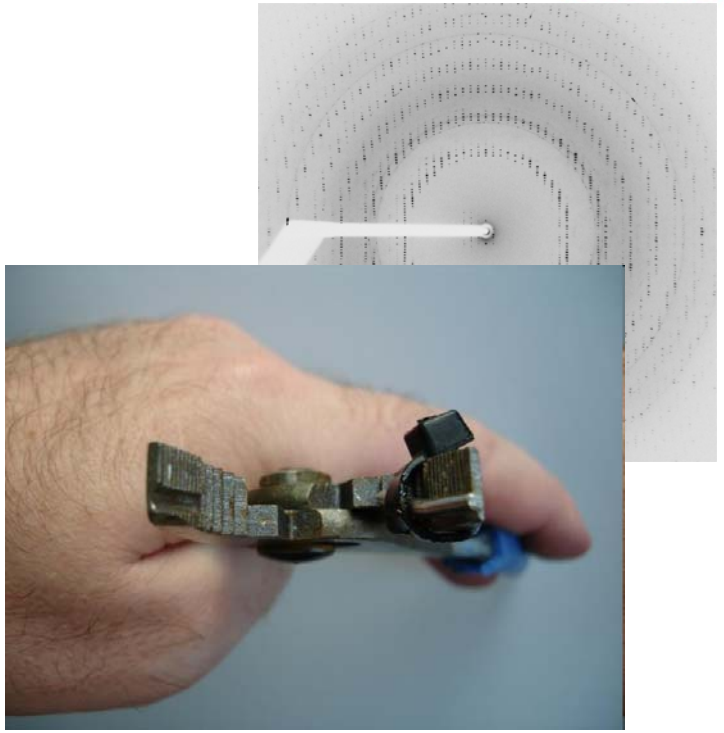
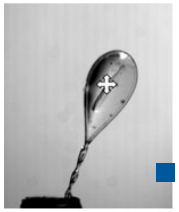


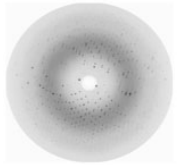
Foto: courtesy J. Holton

Data Collection Strategies - 1

- Make Use of Kappa-Geometry



- A Poor Man’s Kappa



- Helical Data Collection

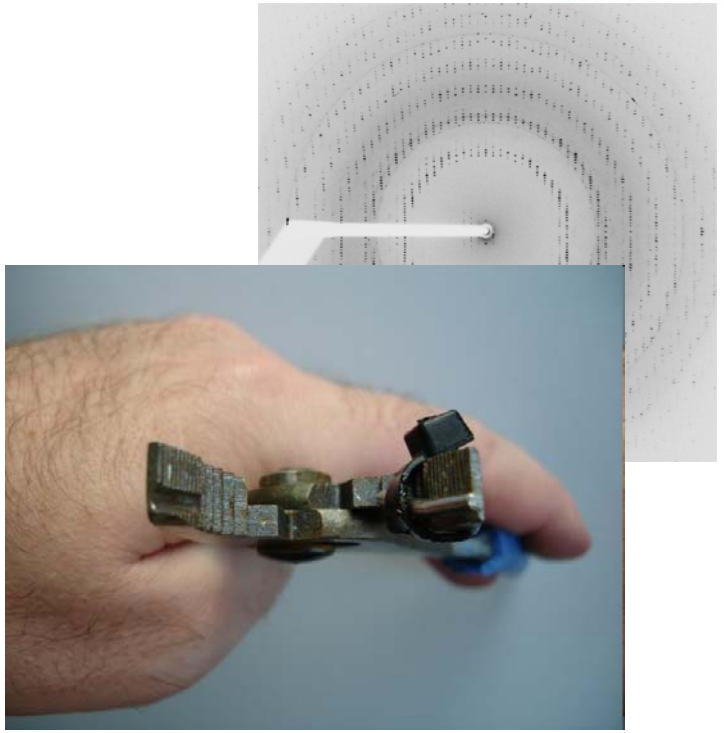
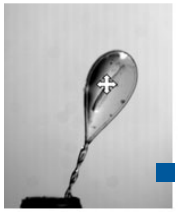


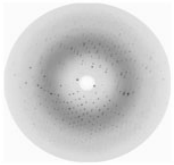
Foto: courtesy J. Holton

Data Collection Strategies - 1

- Make Use of Kappa-Geometry



- A Poor Man’s Kappa



- Helical Data Collection

- Multi-Crystal Data Collection

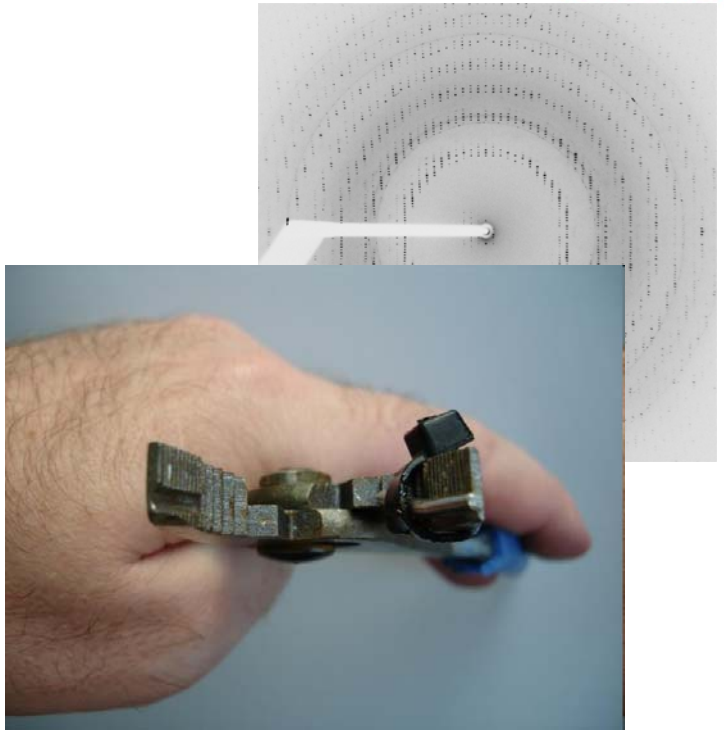
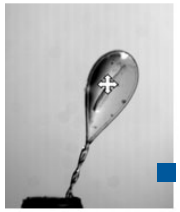


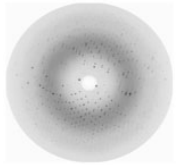
Foto: courtesy J. Holton

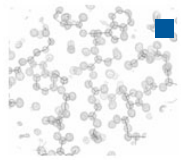
Data Collection Strategies - 2

- Helium Beam Path



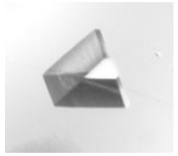
- Move the Detector

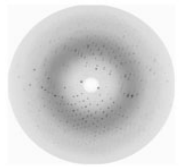
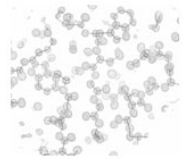




- MDS Approach (B.-C. Wang)

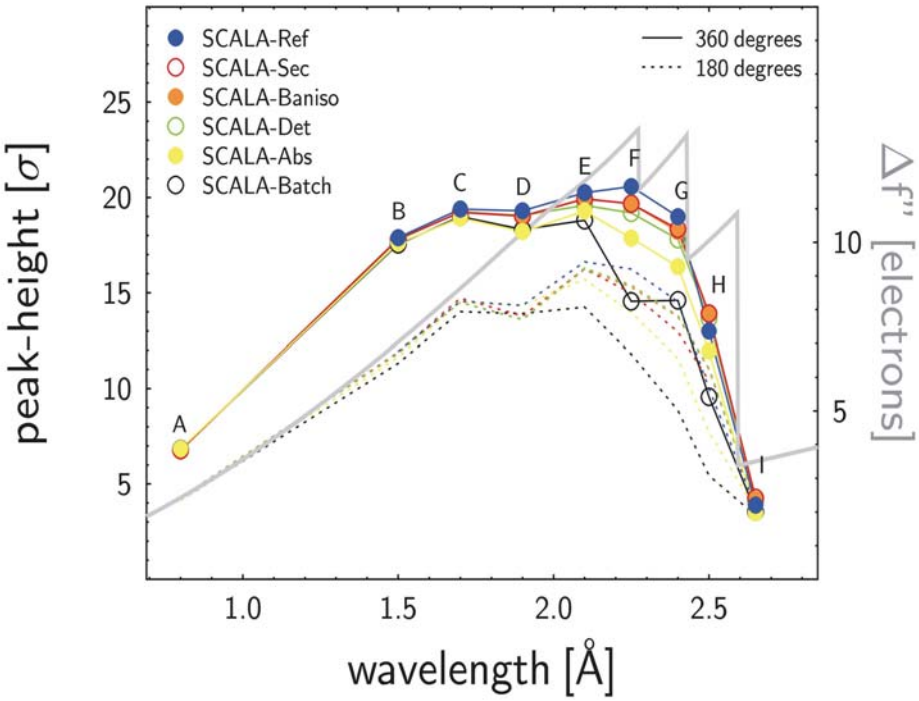
Scaling



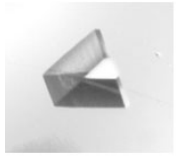



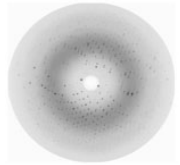
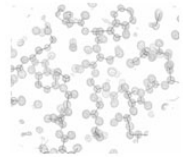

- 3D-Scaling Protocols

(Mueller-Dieckmann *et al*. (2004



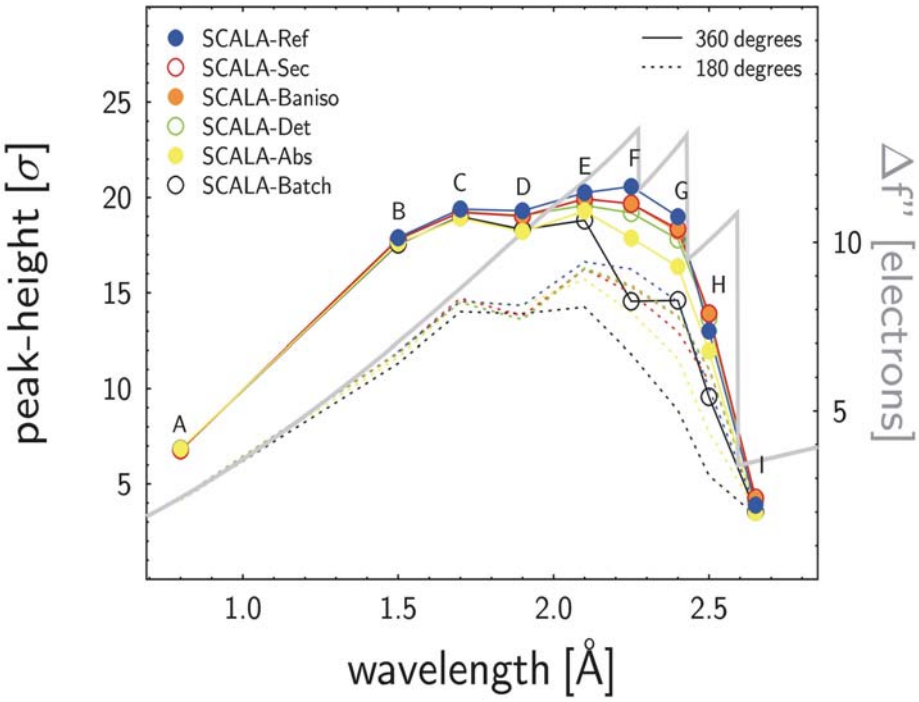
Scaling



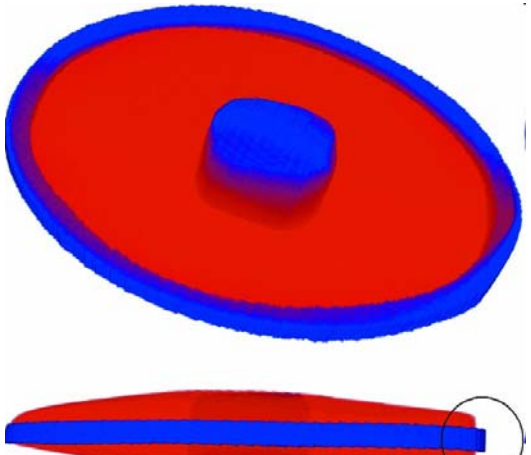

- 3D-Scaling Protocols

(Mueller-Dieckmann *et al*. (2004

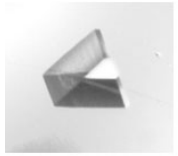


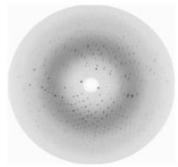
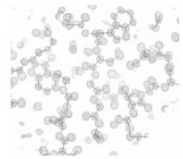
- Tomographic Sample Reconstruction

(Leal et al. (2008). *J. Appl. Cryst.* **41**,

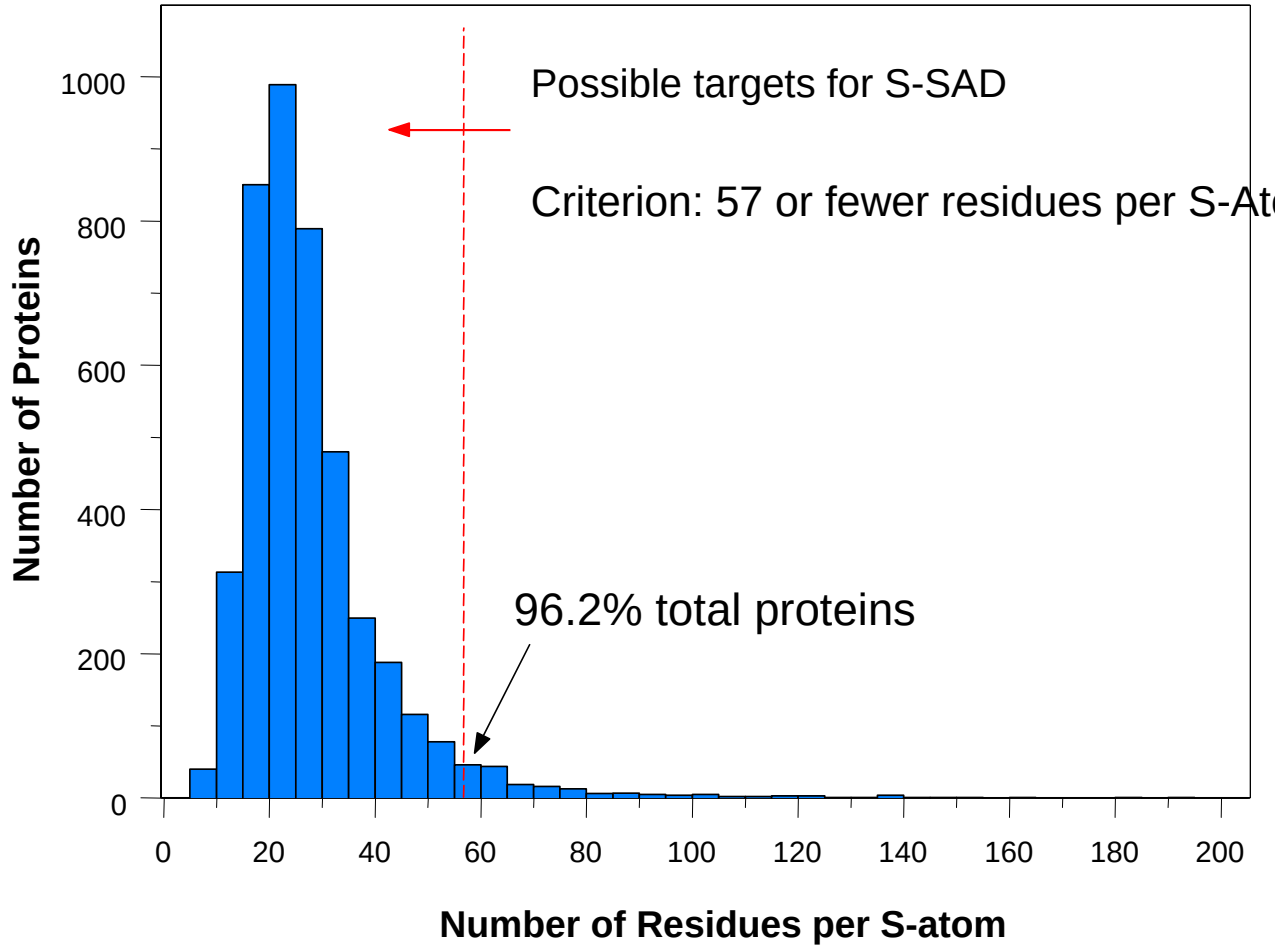


Possible Targets

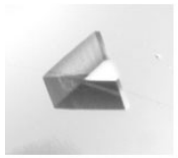


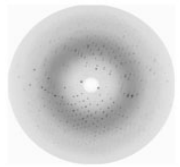
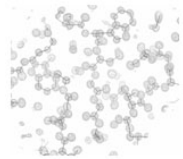




Sulfur Distribution in the *Escherichia coli genome*



Slide: courtesy B.-C. Wang




Thank you for your attention