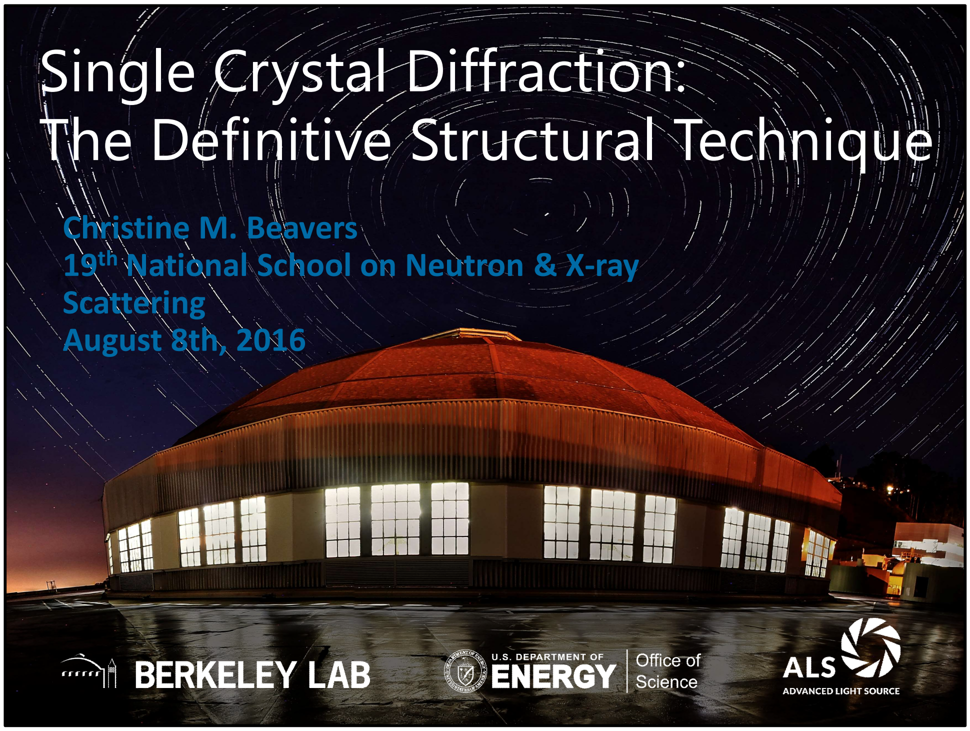

Single Crystal Diffraction: The Definitive Structural Technique

Christine M. Beavers
19th National School on Neutron & X-ray Scattering
August 8th, 2016

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Single Crystal Diffraction:
The Definitive Structural Technique

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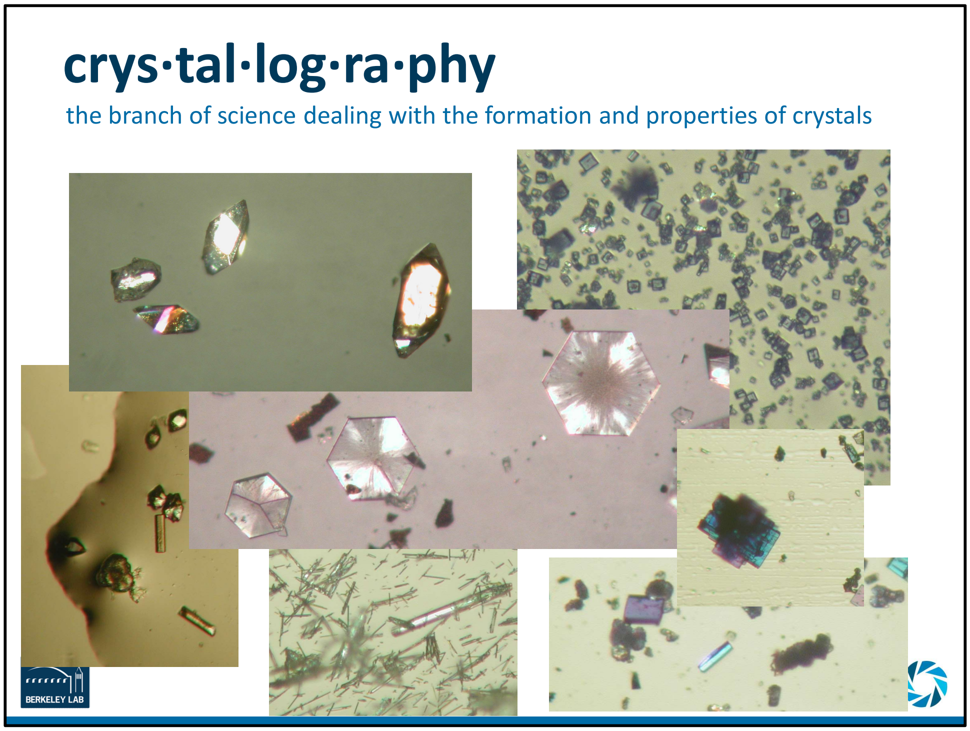
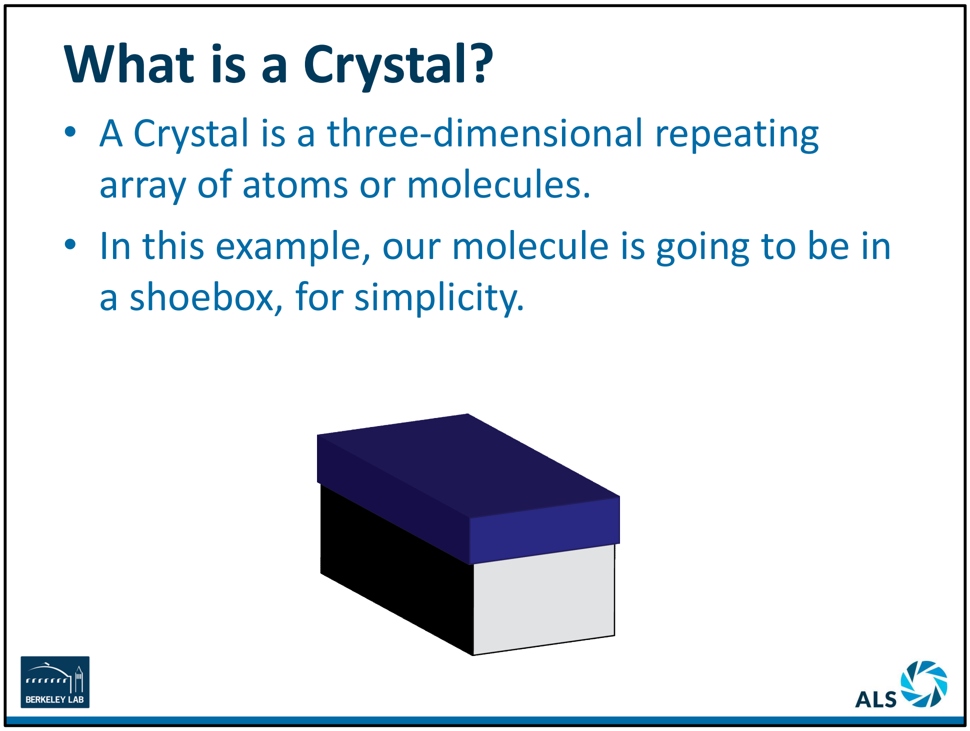


crys·tal·log·ra·phy

the branch of science dealing with the formation and properties of crystals

What is a Crystal?

- A crystal is a periodic arrangement of matter

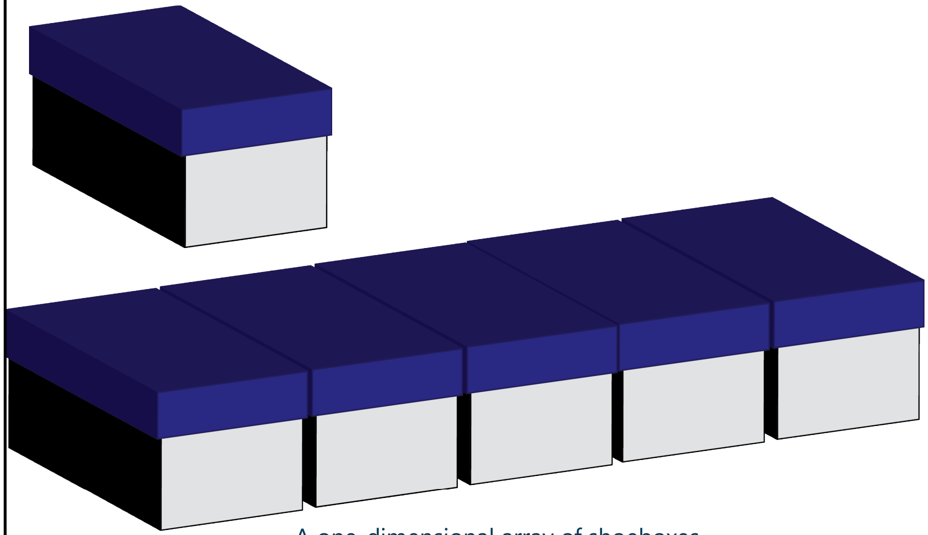


What is a Crystal?

- A Crystal is a three-dimensional repeating array of atoms or molecules.
- In this example, our molecule is going to be in a shoebox, for simplicity.

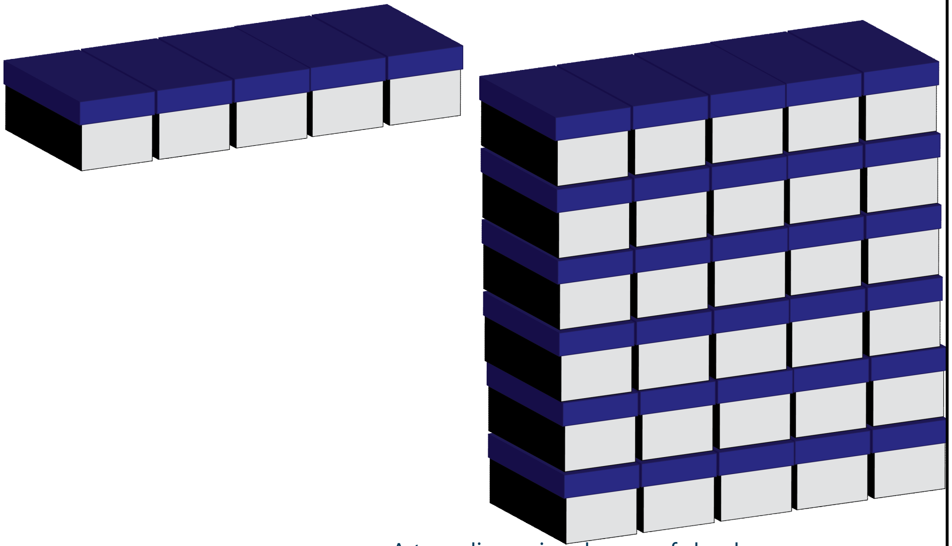
A crystal is a periodic arrangement of matter

Dimensionality



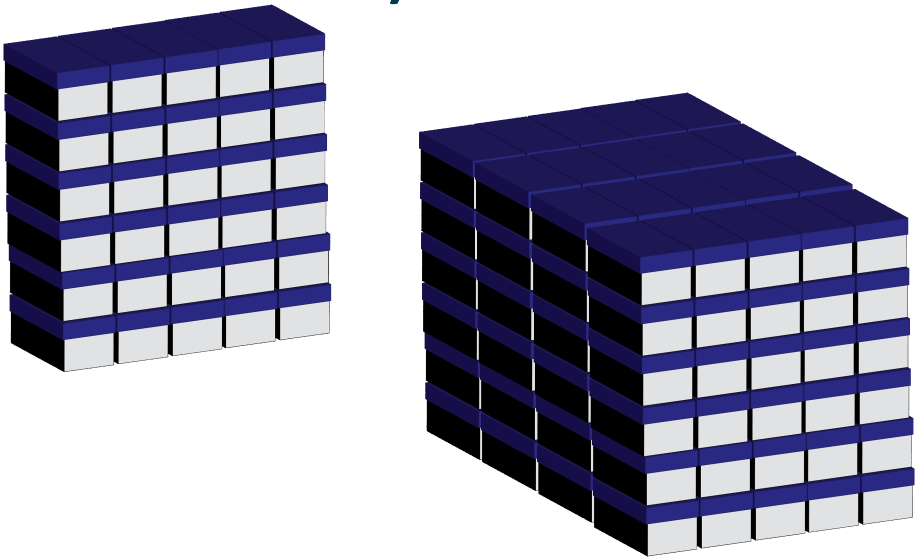
A one-dimensional array of shoeboxes

Dimensionality

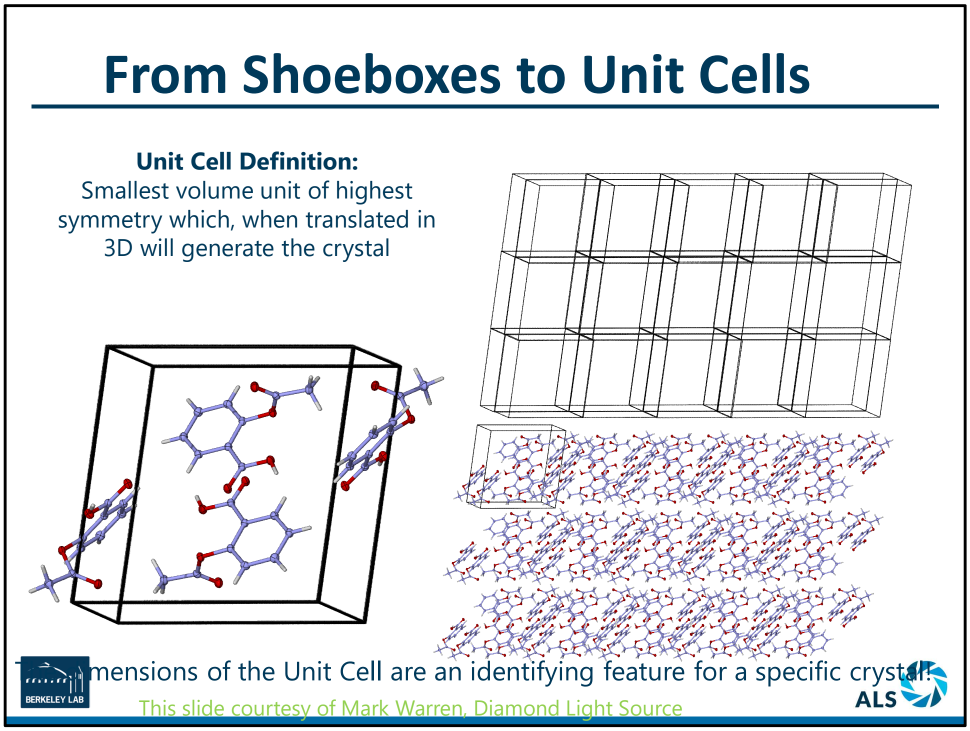


A two-dimensional array of shoeboxes

Dimensionality



A three-dimensional array of shoeboxes

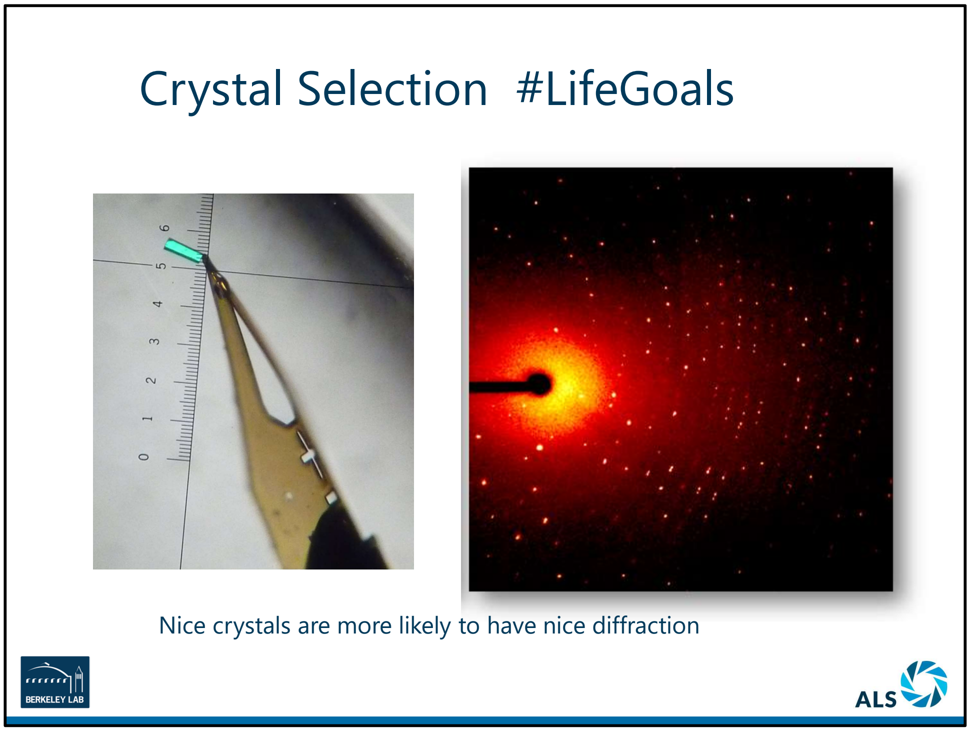


From Shoeboxes to Unit Cells

Unit Cell Definition:
Smallest volume unit of highest symmetry which, when translated in 3D will generate the crystal

T mensions of the Unit Cell are an identifying feature for a specific crystal

This slide courtesy of Mark Warren, Diamond Light Source

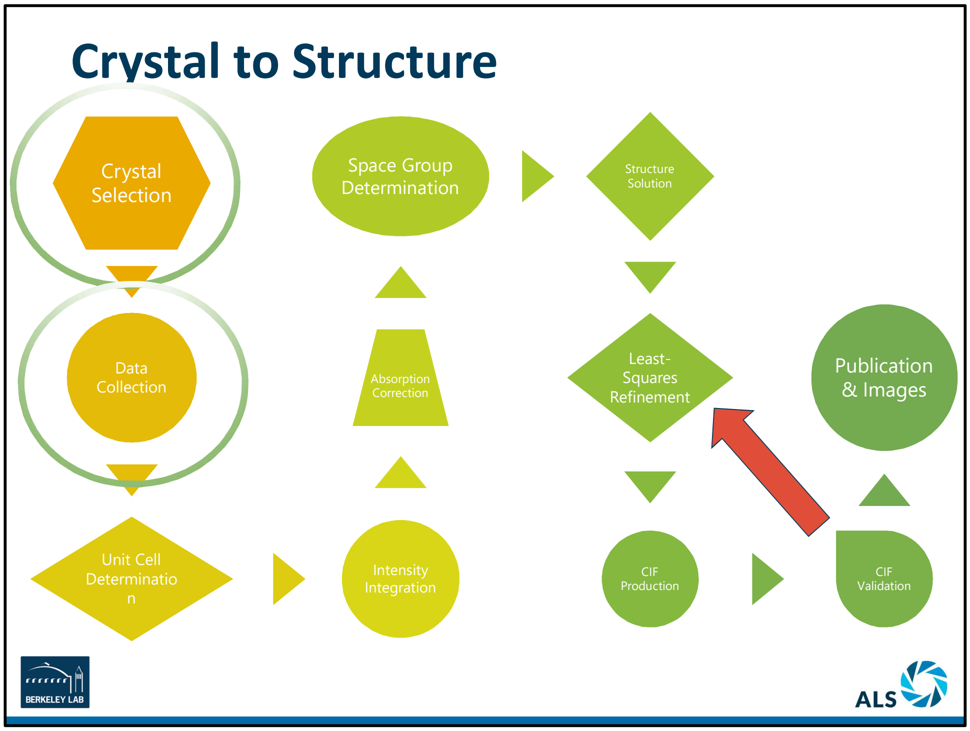


Crystal Selection #LifeGoals

Nice crystals are more likely to have nice diffraction

Faces and edges should be clean- this is best metric when dealing with opaque or dark crystals. Round things are not usually nice crystals- usually a collection of microcrystals with powder diffraction. Use a polarizing microscope when you have transparent crystals, and watch for extinction while rotating the stage. Nice crystals go extinct quickly- if different areas of the crystal go extinct (dark) at different times, you probably have a twin.

The image on the right shows some good and some troublesome characteristics. Lots of diffraction, and out to the edge of the image, but notice how the spacings between some spots are irregular. This is an acquired skill, but a trained eye can see that this crystal was a twin, and if you can avoid collecting data on a twin, your life will generally be easier!



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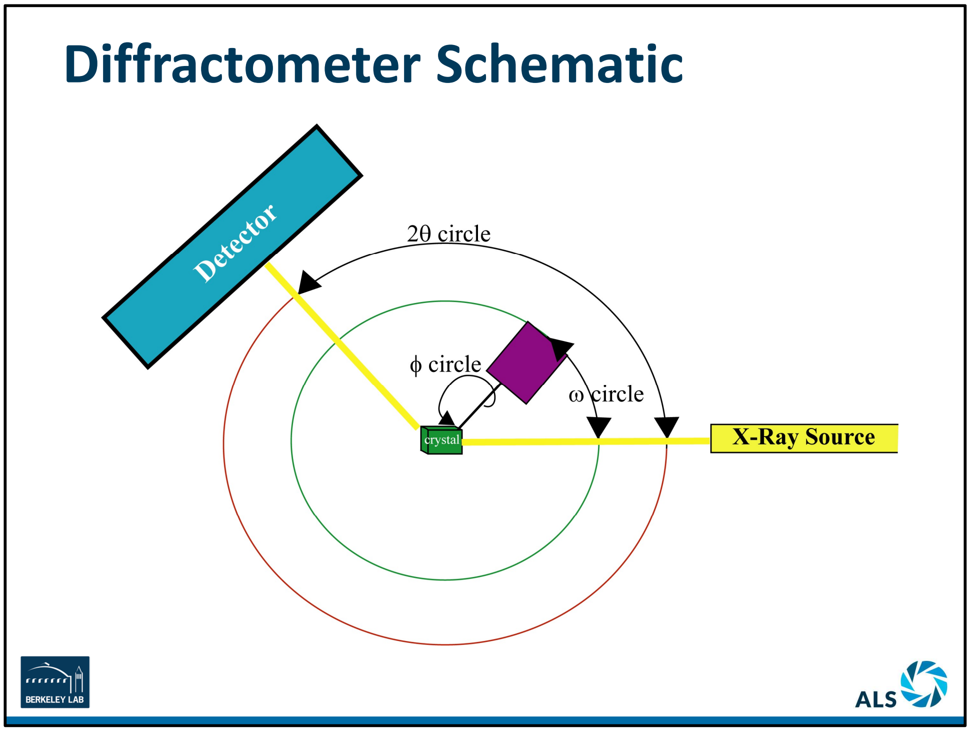
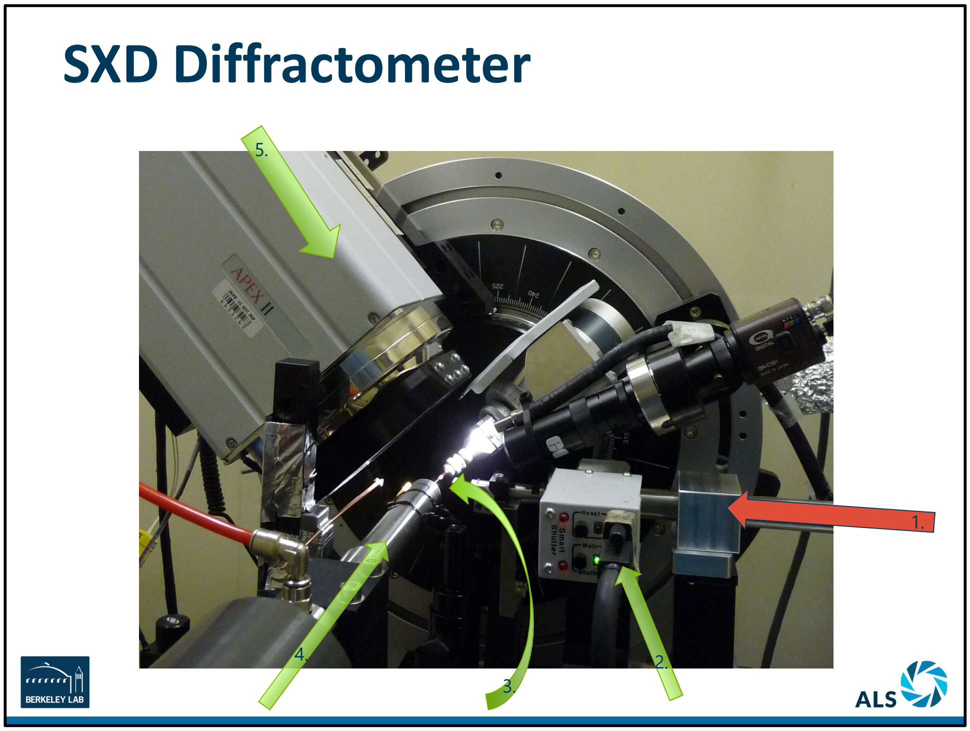


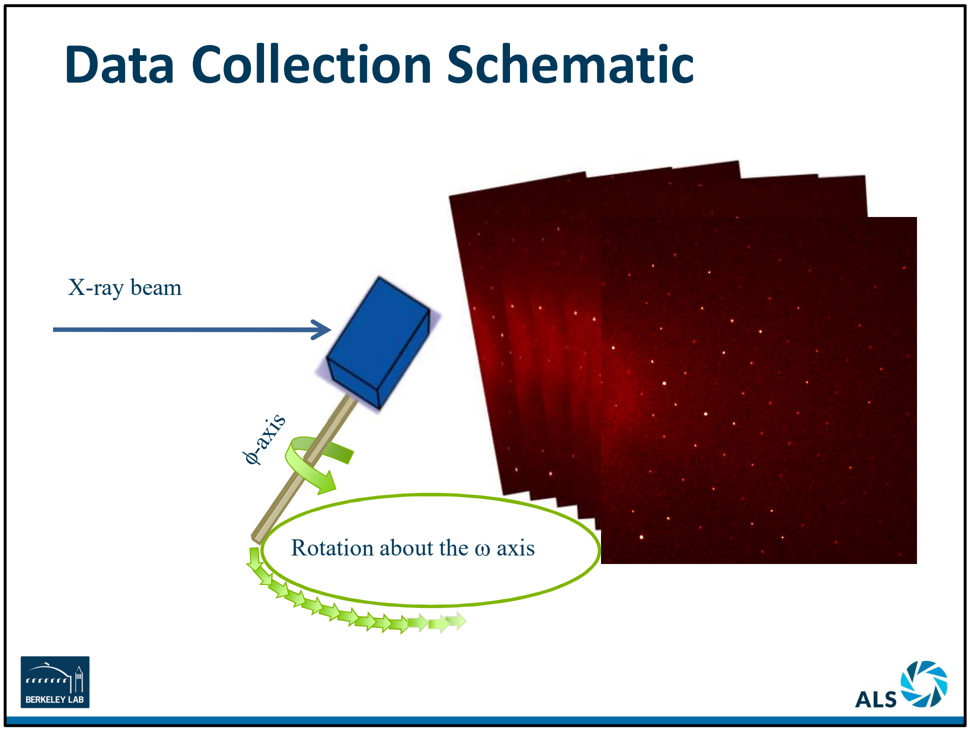
Diffractometer Schematic

We could spend months on diffractometry and the geometry of the diffraction experiment, but here is a very short version. Most diffractometers used currently operate under the formalisms developed by Busing & Levy (1937), where 2theta is the name of a rotation axis and the angle between the beam and the detector. Omega is coaxial to 2theta, and rotates the sample orienter. The sample orienter can consist of a Eulerian cradle(chi) carrying a phi axis, or as in a 3-circle device, chi can be fixed to the “Magic Angle” of 54.74degrees. Chi is defined by Busing & Levy as the angle by which the phi axis is rotated off the 2theta and omega axes. The goal of all of this is to have all of these axes converge upon one point in space, defined as the center of rotation. This is ideally where your crystal and the beam should interact. In this image we are looking down the 2theta/omega axis.



SXD Diffractometer

1. X-ray Beam
2. Rotary timing shutter- going to be less common as detector technology evolves. More on this later.
3. Crystal on X-ray transparent mount.
4. Liquid nitrogen Cryostream- reduces libration and improves sample diffraction quality
5. CCD based X-ray detector. This is the standard for X-ray detection at home labs, but at synchrotrons, pixel and CMOS X-ray detectors are becoming more common.



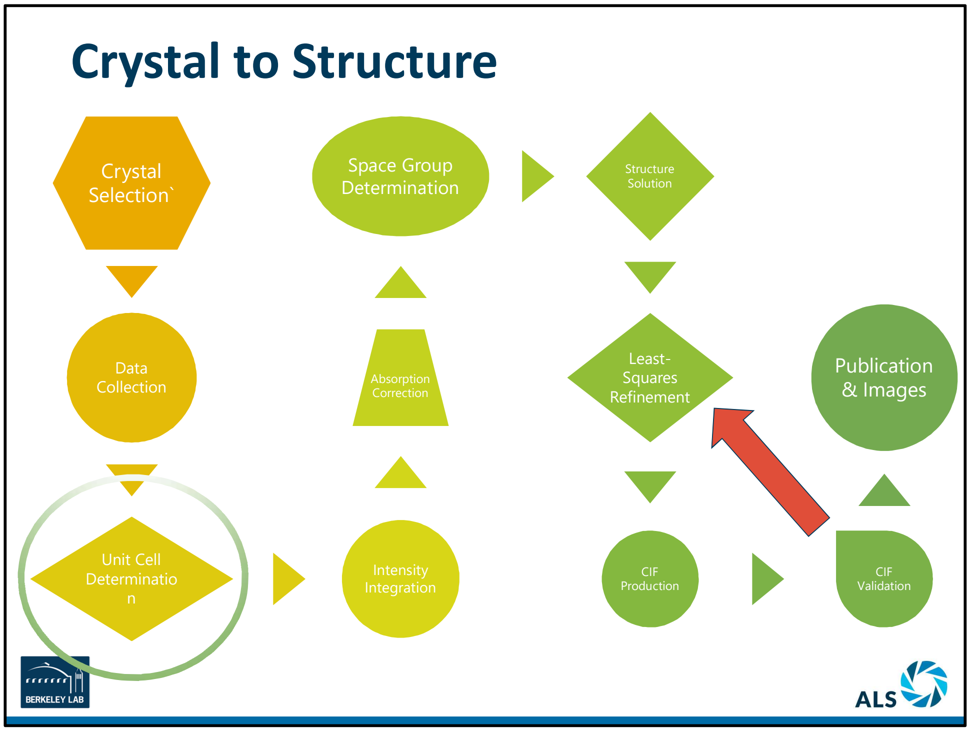
Data Collection Schematic

X-ray beam

ϕ-axis

Rotation about the ω axis

Most chemical crystallographers collect omega scans, where a phi setting is chosen, and the diffractometer scans in omega- the omega motor moves a set angular distance over a set exposure time. With a CCD, the X-ray shutter is closed after the appropriate move is made, and the detector is read out. With new fast detectors, the omega motor moves continuously at the pre-set rate, and the detector is triggered to read out at pre-determined time points. Omega scans are usually done with the detector at a non-zero 2theta value, and omega is rotated 180deg. To improve reciprocal space coverage, this is usually repeated at three or four phi settings. In Protein crystallography, the main difference is that phi scans are more common, and the 2theta angle is most commonly set at zero.



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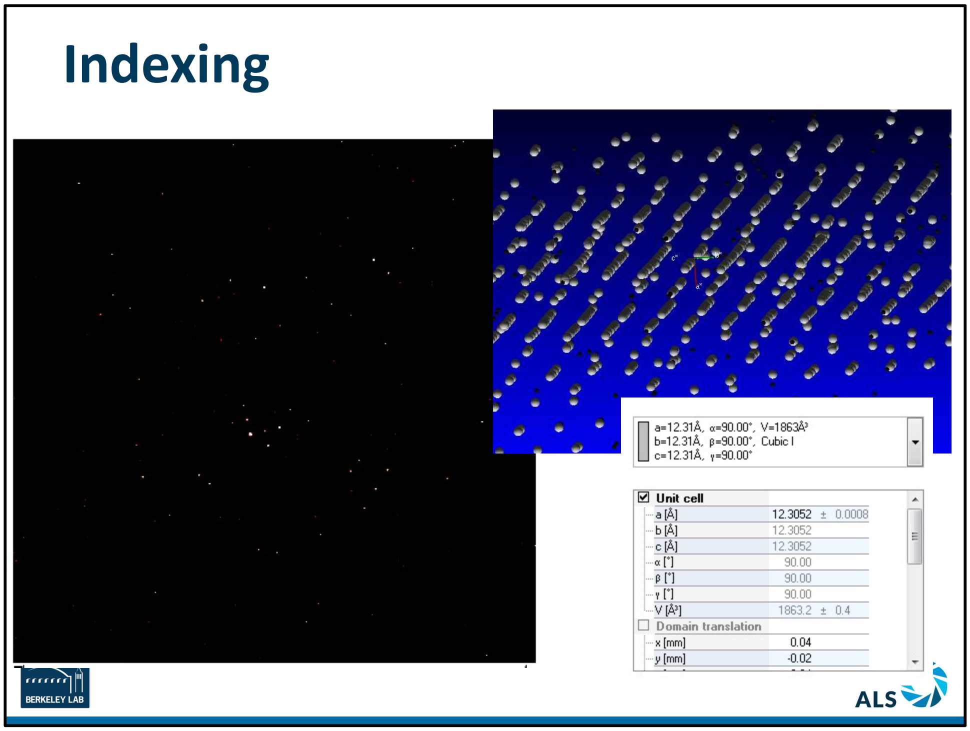


Indexing

a=12.31Å, α=90.00°, V=1863Å³
b=12.31Å, β=90.00°, Cubic I
c=12.31Å, γ=90.00°

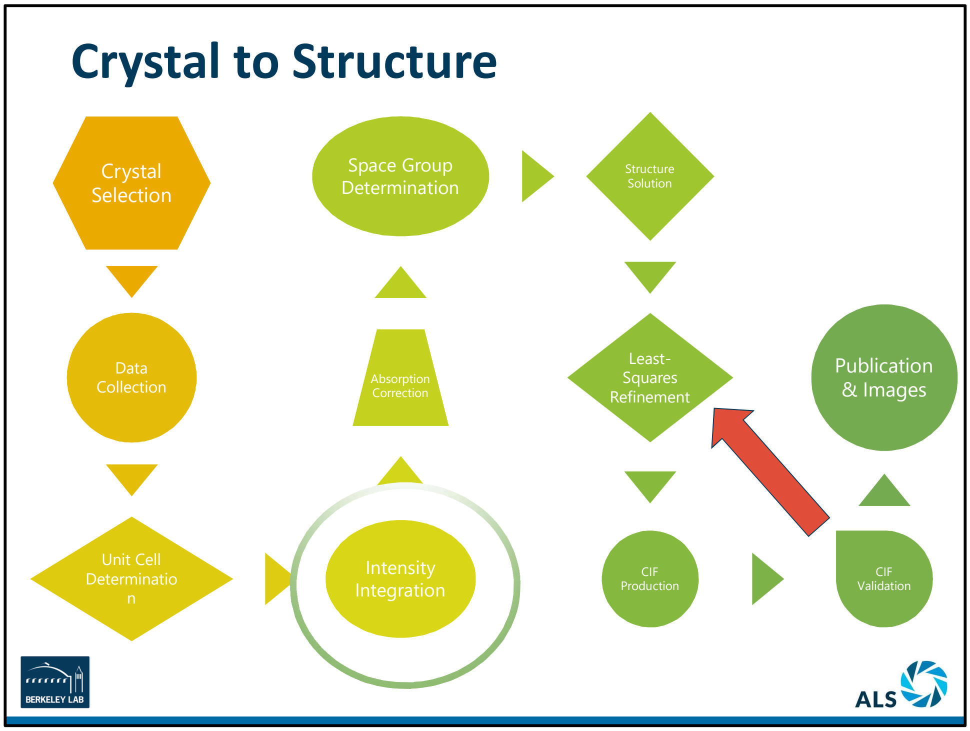
☑ **Unit cell**

a [Å]	12.3052	± 0.0008
b [Å]	12.3052	
c [Å]	12.3052	
α [°]	90.00	
β [°]	90.00	
γ [°]	90.00	
V [Å³]	1863.2	± 0.4

☐ **Domain translation**

x [mm]	0.04
y [mm]	-0.02

Also known as unit cell determination, there are multiple methods, but all strive for the same goal- to determine the unit cell dimensions and how the unit cell axes are defined in diffractometer coordinates. The matrix that relates the unit cell to the diffractometer is called the orientation matrix, and will be used later in indexing. The process is rought universal- reflections are located in the images from the data collection(one image, shown left), and projected into reciprocal space(shown right), where common vector directions are determined. The reciprocal space unit vectors are determined which are related to the real space unit cell lengths. After the unit cell is determined, the lattice type can be determined, and the unit cell can be refined, using the positions of the reflections.



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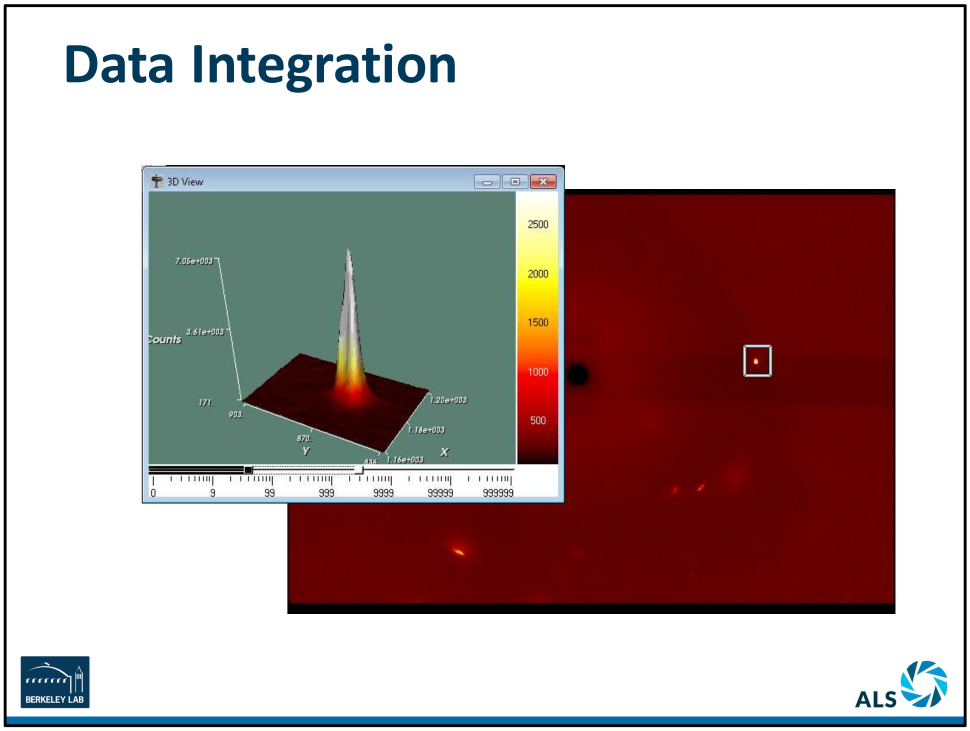
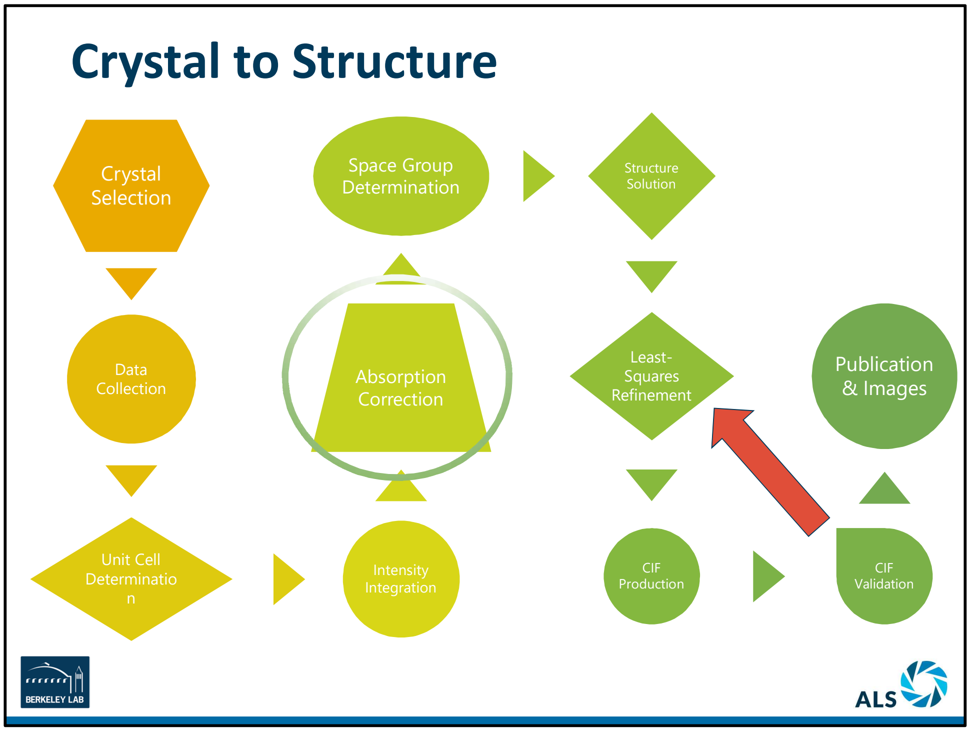


Data Integration

The process of integration is too complicated to explain here, but the main idea is to determine the number of X-rays responsible for each diffracted spot. The orientation matrix is used to determine where reflections should be, and the mosaicity of the crystal is calculated by measuring the angular spread of the reflections, which contributes to the refinement and error model of the unit cell. The output of the integration is a list of reflections, usually with directional cosines and other information about their location in the data set.



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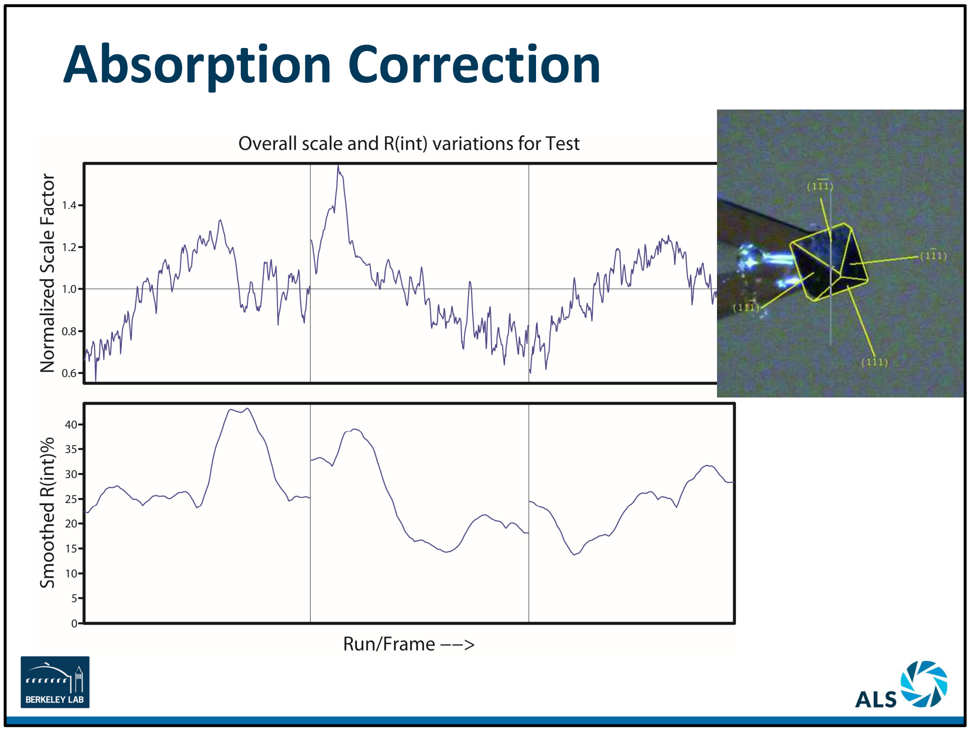
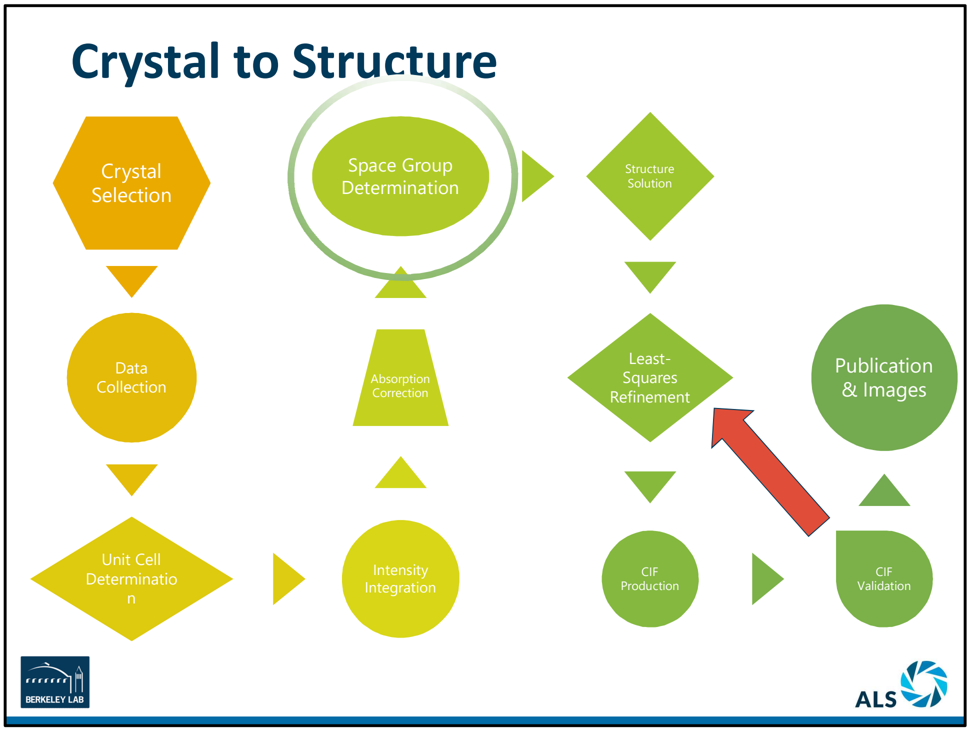


Absorption Correction

Since very few crystals are perfectly spherical, and fewer still match the size of the beam, a correction for the scattering volume at any image is needed. There are many ways of doing this, here is a list from the cif dictionary:

analytical - analytical from crystal shape
cylinder - cylindrical
empirical - empirical from intensities
gaussian - Gaussian from crystal shape
integration - integration from crystal shape
multi-scan - symmetry-related measurements**
none - no absorption correction applied
numerical - numerical from crystal shape**
psi-scan - psi-scan corrections
refdelf - refined from delta-F
sphere - spherical

On the left, I am showing the output from a multi-scan absorption correction, which uses equivalents to determine how much the overall scale has varied, and applies spherical harmonic based absorption models that approximate the changes in scale factor. On the right is an image of a crystal on the diffractomter, with the faces indexed, and used for a numerical absorption correction.



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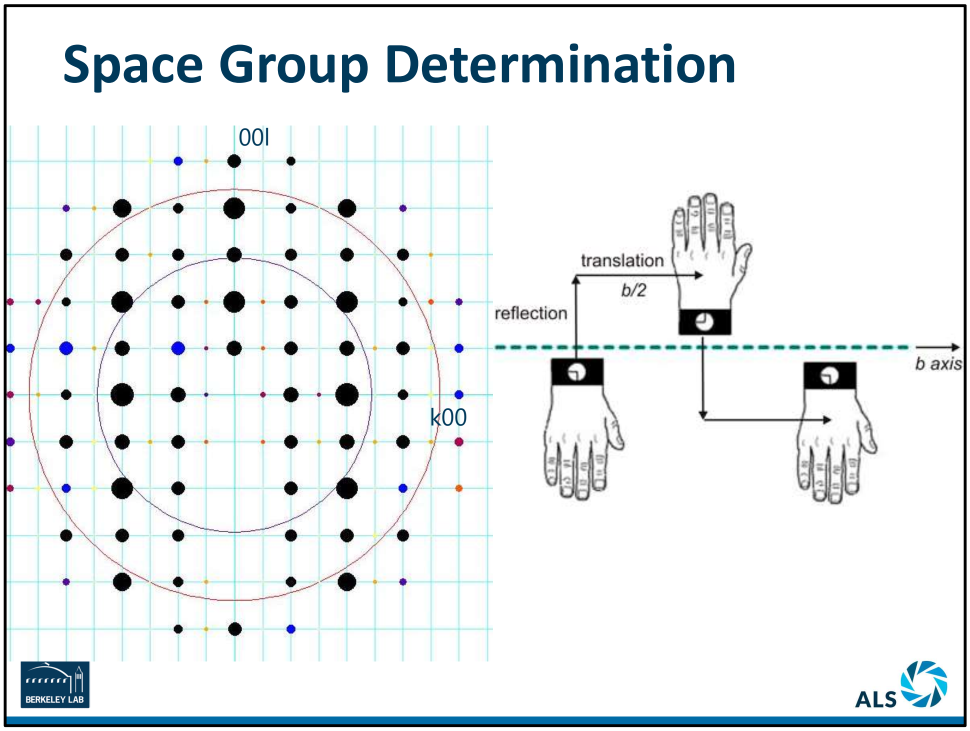


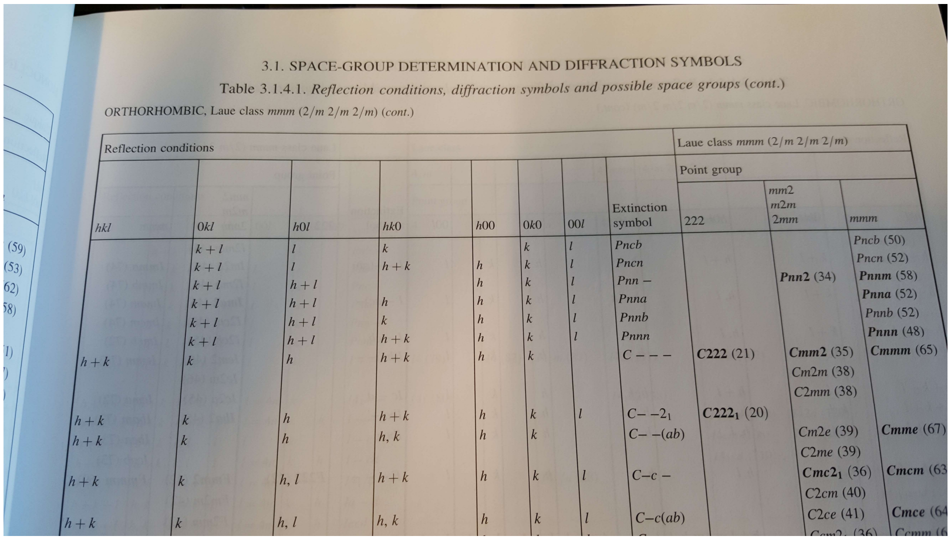
Space Group Determination

The h0l later of reciprocal space for a crystal with unit cell 7.316 22.824 4.307 90 90 90. Because of the interference properties of translation in some symmetry and centering operations, systematic absences will be seen in the lattice. These absences will present in different ways, depending on the operation present. In this case we have a glide plane present in the structure, which consists of a reflection then a translation. This symmetry element presents itself in the zonal plane related to the translation plane in real space. The absence looks to be only dependent on k=2n+1, the inverse being that the reflection conditions for k0l are k=2n.

Centering has a universal reflection condition- it will be seen in all areas of reciprocal space. Glides are zonal- they only show in planes where one axis is zero. Screw axis conditions are axial.

The name of the glide is the direction of translations (a, b, n (across a plane diagonal), d (across a body diagonal)). The position indicates the orientation of the mirror plane, i.e., the n glide in Pna2(1) would have the mirror plane orthogonal to the a axis.

Space Group Determination



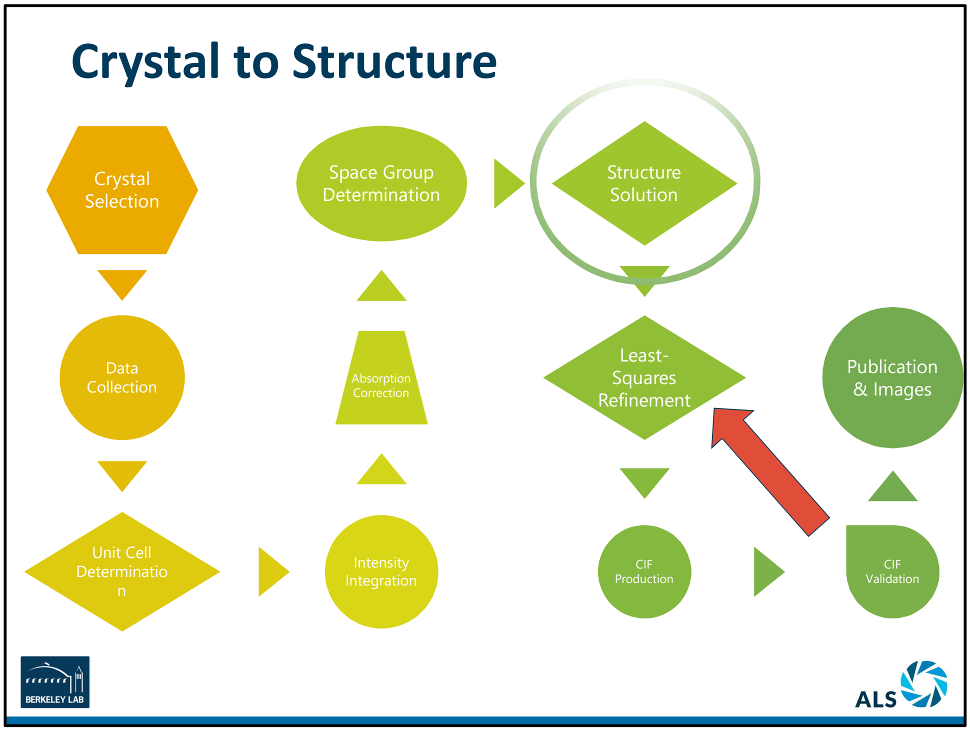
3.1. SPACE-GROUP DETERMINATION AND DIFFRACTION SYMBOLS

Table 3.1.4.1. *Reflection conditions, diffraction symbols and possible space groups* (*cont.*)

ORTHORHOMBIC, Laue class *mmm* (2/*m* 2/*m* 2/*m*) (*cont.*)

Reflection conditions								Laue class *mmm* (2/*m* 2/*m* 2/*m*)		
								Point group		
hkl	0*kl*	*h*0*l*	*hk*0	*h*00	0*k*0	00*l*	Extinction symbol	222	*mm*2 *m*2*m* 2*mm*	*mmm*
	k + *l*	*l*	*k*		*k*	*l*	*Pncb*			*Pncb* (50)
	k + *l*	*l*	*h* + *k*	*h*	*k*	*l*	*Pncn*			*Pncn* (52)
	k + *l*	*h* + *l*		*h*	*k*	*l*	*Pnn* –		***Pnn*2** (34)	***Pnnm*** (58)
	k + *l*	*h* + *l*	*h*	*h*	*k*	*l*	*Pnna*			***Pnna*** (52)
	k + *l*	*h* + *l*	*k*	*h*	*k*	*l*	*Pnnb*			*Pnnb* (52)
	k + *l*	*h* + *l*	*h* + *k*	*h*	*k*	*l*	*Pnnn*			***Pnnn*** (48)
h + *k*	*k*	*h*	*h* + *k*	*h*	*k*		*C* – – –	***C*222** (21)	***Cmm*2** (35) *Cm*2*m* (38) *C*2*mm* (38)	***Cmmm*** (65)
h + *k*	*k*	*h*	*h* + *k*	*h*	*k*	*l*	*C*– –2_1	***C*222$_1$** (20)		
h + *k*	*k*	*h*	*h*, *k*	*h*	*k*		*C*– –(*ab*)		*Cm*2*e* (39) *C*2*me* (39)	***Cmme*** (67)
h + *k*	*k*	*h*, *l*	*h* + *k*	*h*	*k*	*l*	*C*–*c* –		***Cmc*2$_1$** (36) *C*2*cm* (40)	***Cmcm*** (63
h + *k*	*k*	*h*, *l*	*h*, *k*	*h*	*k*	*l*	*C*–*c*(*ab*)		*C*2*ce* (41)	***Cmce*** (64

Here is a snapshot from the International table, vol A, where the reflection conditions are tabulated for all 230 of the space groups. The columns go from general (centering), to zonals, to axials. The standard settings are in bold type.



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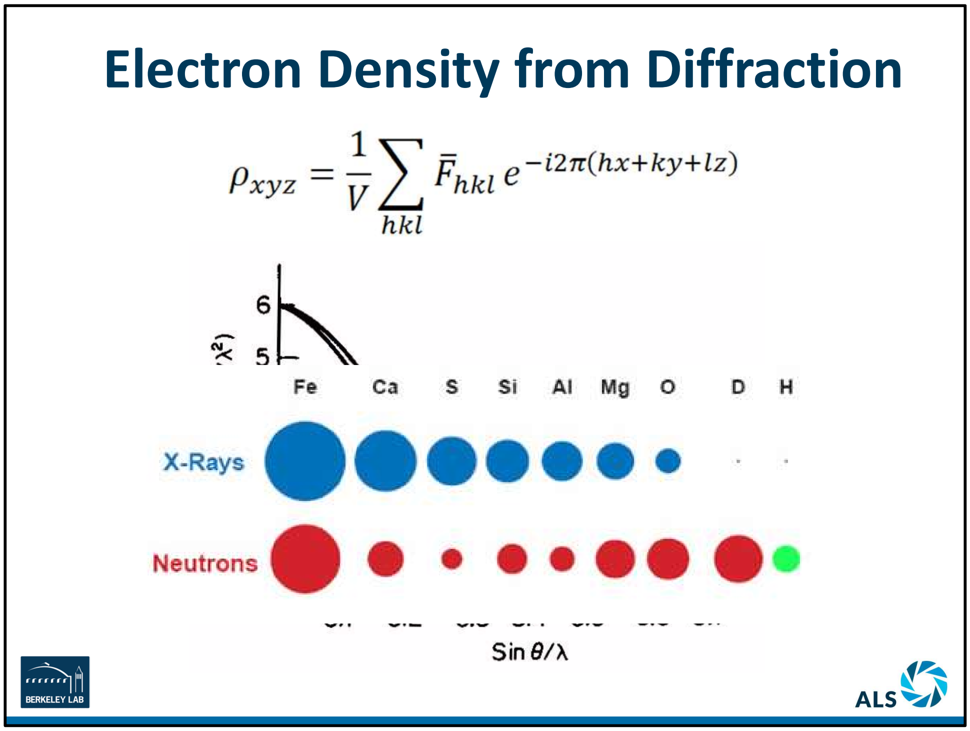
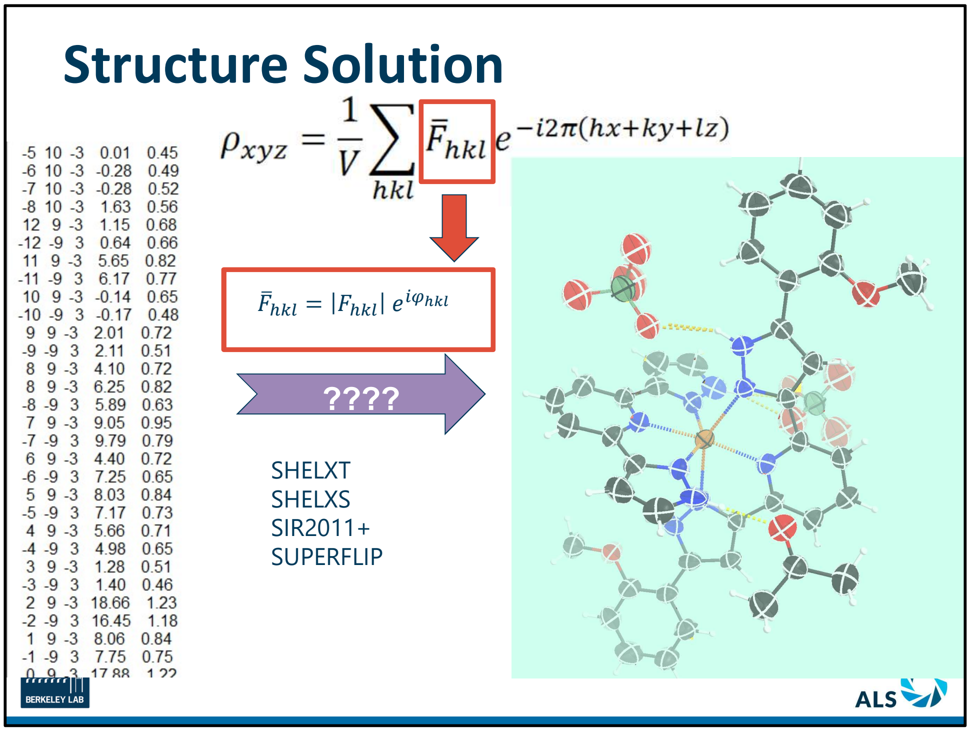


Electron Density from Diffraction

$$\rho_{xyz} = \frac{1}{V}\sum_{hkl} \bar{F}_{hkl}\, e^{-i2\pi(hx+ky+lz)}$$

Here is the short form. The equation at top is the Fourier synthesis for electron density from diffracted reflections. Fhkl is the structure factor which is "*The structure factor is the resultant of N waves scattered in the direction of the reflection hkl by the N atoms in the unit cell. Each of these waves has an amplitude proportional to f_i, the scattering factor of the atom, and a phase d_i with respect to the origin of the unit cell.*
(Stout, G. H.; Jensen, L. H. *X-ray Structure Determination: A Practical Guide*; Second ed.; John Wiley & Sons: New York, 1989, p 201.)"

The scattering factors for atoms are different for different probes. The image shown demonstrates that the scattering factor for Xrays increases with Z, but the neutron scattering factors do not. This difference leads to neutrons being more sensitive to some lighter elements. Also the X-ray scattering factors fall off at higher diffraction angle, but the neutron scattering factors are angle independent.



Structure Solution

$$\rho_{xyz} = \frac{1}{V}\sum_{hkl} \boxed{\bar{F}_{hkl}}\, e^{-i2\pi(hx+ky+lz)}$$

$$\boxed{\bar{F}_{hkl} = |F_{hkl}|\, e^{i\varphi_{hkl}}}$$

```
 -5  10 -3   0.01   0.45
 -6  10 -3  -0.28   0.49
 -7  10 -3  -0.28   0.52
 -8  10 -3   1.63   0.56
 12   9 -3   1.15   0.68
-12  -9  3   0.64   0.66
 11   9 -3   5.65   0.82
-11  -9  3   6.17   0.77
 10   9 -3  -0.14   0.65
-10  -9  3  -0.17   0.48
  9   9 -3   2.01   0.72
 -9  -9  3   2.11   0.51
  8   9 -3   4.10   0.72
  8   9 -3   6.25   0.82
 -8  -9  3   5.89   0.63
  7   9 -3   9.05   0.95
 -7  -9  3   9.79   0.79
  6   9 -3   4.40   0.72
 -6  -9  3   7.25   0.65
  5   9 -3   8.03   0.84
 -5  -9  3   7.17   0.73
  4   9 -3   5.66   0.71
 -4  -9  3   4.98   0.65
  3   9 -3   1.28   0.51
 -3  -9  3   1.40   0.46
  2   9 -3  18.66   1.23
 -2  -9  3  16.45   1.18
  1   9 -3   8.06   0.84
 -1  -9  3   7.75   0.75
  0   9 -3  17.88   1.22
```

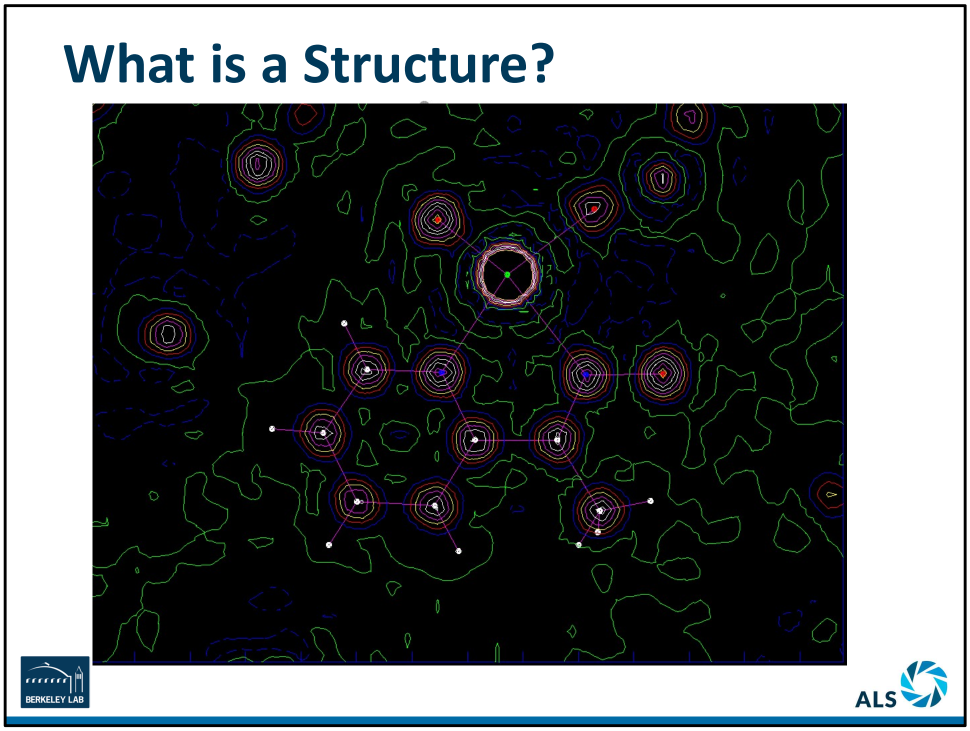
????

SHELXT
SHELXS
SIR2011+
SUPERFLIP

Here, again, is the short form. The equation at top is the Fourier synthesis for electron density from diffracted reflections. Fhkl is the structure factor which is "*The structure factor is the resultant of N waves scattered in the direction of the reflection hkl by the N atoms in the unit cell. Each of these waves has an amplitude proportional to f_j, the scattering factor of the atom, and a phase d_j with respect to the origin of the unit cell.* (Stout, G. H.; Jensen, L. H. *X-ray Structure Determination: A Practical Guide*; Second ed.; John Wiley & Sons: New York, 1989, p 201.)"

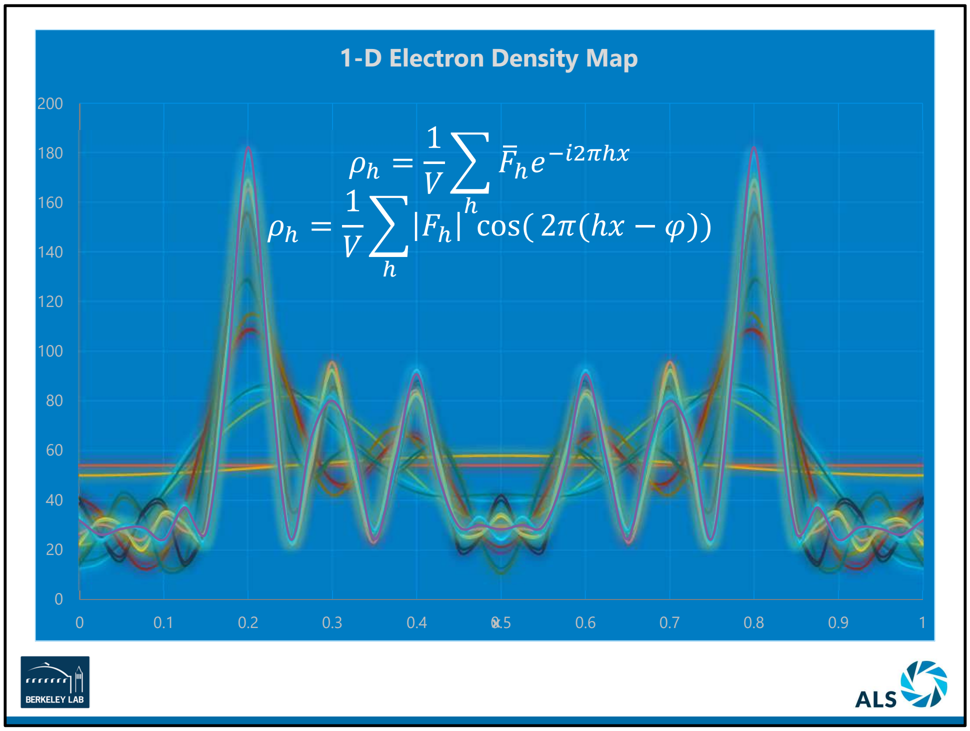
One slight problem- we don't know phases from X-ray measurements. We only know intensities, which are considered proportional to the magnitudes of the structure factor vectors. (this is what is known as "The Phase Problem" in crystallography) We have developed an elaborate number of coping strategies for this, most in the form of phase relationships. Modern structure solution programs take the list of reflection intensities on the right, and find reflections that are related, and use probablistic methods to build a set of phases that produce reasonable(non-negative) electron density maps.

"The intensity of a diffracted beam is directly related to the amplitude of the structure factor, but the phase must normally be deduced by indirect means. In structure determination, phases are estimated and an initial description of the positions and anisotropic displacements of the scattering atoms is deduced. From this initial model, structure factors are calculated and compared with those experimentally observed. Iterative refinement procedures attempt to minimise the difference between calculation and experiment, until a satisfactory fit has been obtained." -Online Dictionary of Crystallography, http://reference.iucr.org/dictionary/Structure_factor



What is a Structure?

A structure is the short way of saying that the atomic positions have been determined within the unit cell, and that the detected diffraction matches closely to the predicted diffraction from the determined atomic positions. This enables the crystallographer to determine the bond lengths and other interesting distances within the unit cell.



1-D Electron Density Map

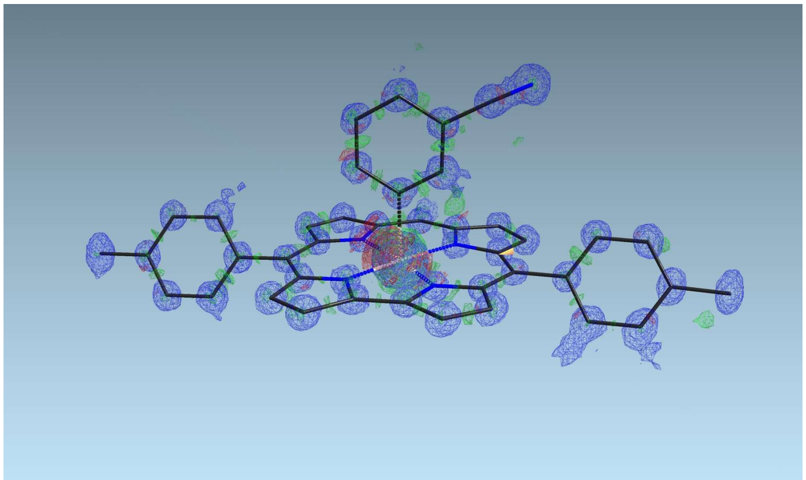
$$\rho_h = \frac{1}{V}\sum_h \bar{F}_h e^{-i2\pi hx}$$

$$\rho_h = \frac{1}{V}\sum_h |F_h| \cos(2\pi(hx-\varphi))$$

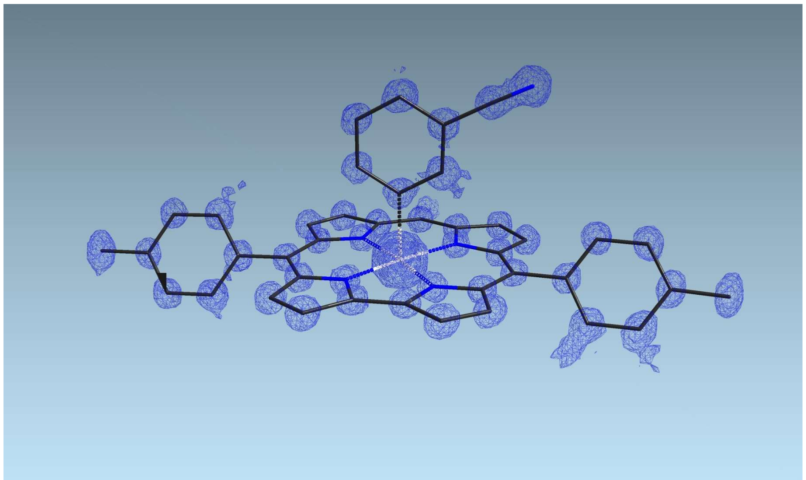
This structure is centrosymmetric, so that simplified the phase math greatly, with phases equaling either 0 or 180 degrees .

The take home message here is that the increased order of diffraction adds precision on the peak location, by decreasing the FWHM.

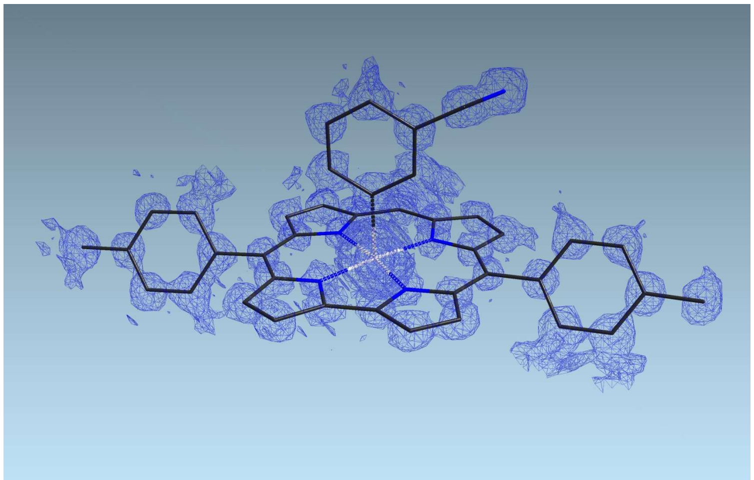
F_{obs} and Difference Map 0.5Å



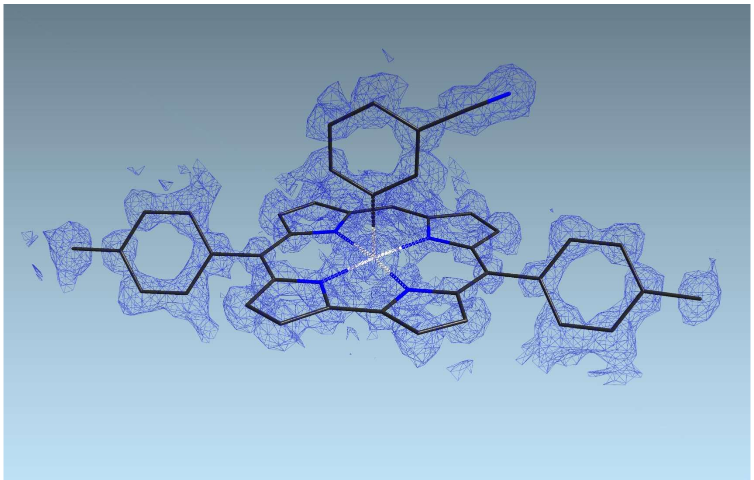
F_{obs} Map at 0.50Å



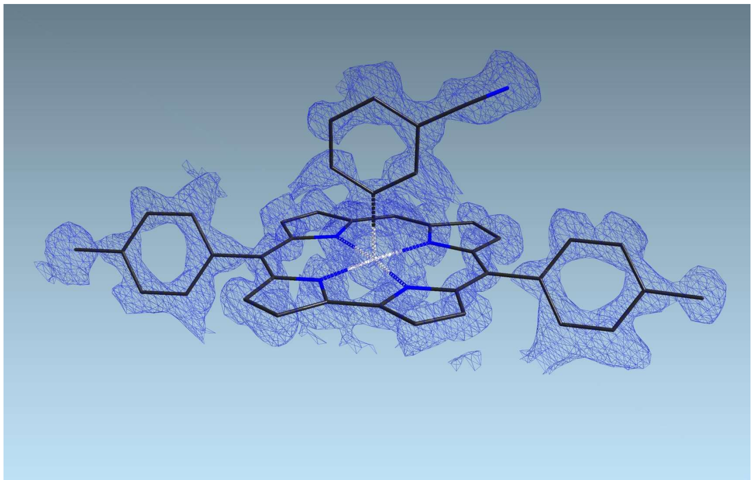
F_{obs} Map at 0.75Å



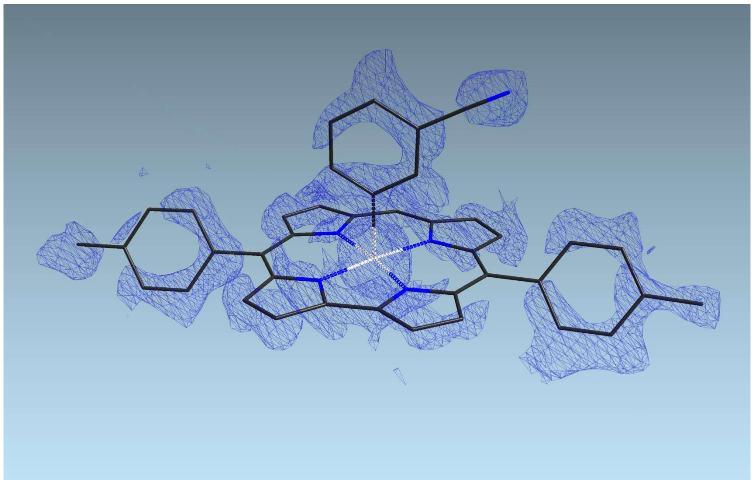
Fobs Map at 1.00Å



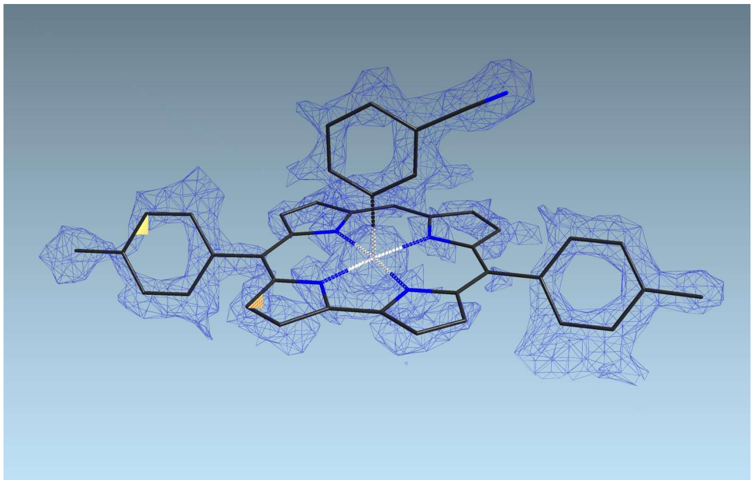
F_{obs} Map at 1.25Å



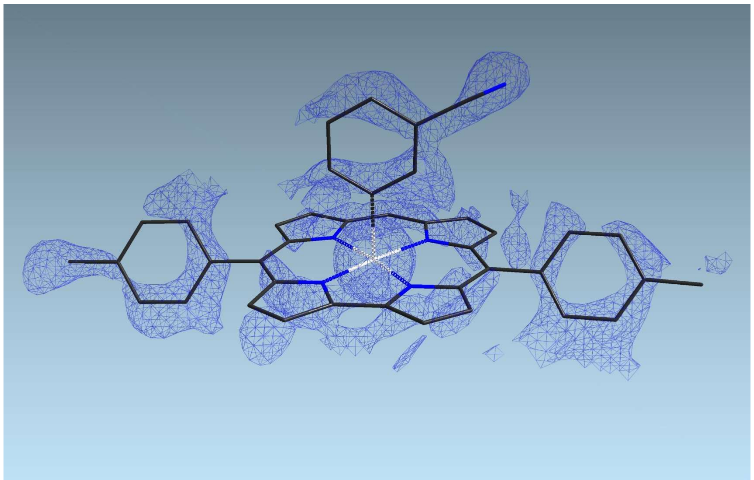
Fobs Map at 1.50Å



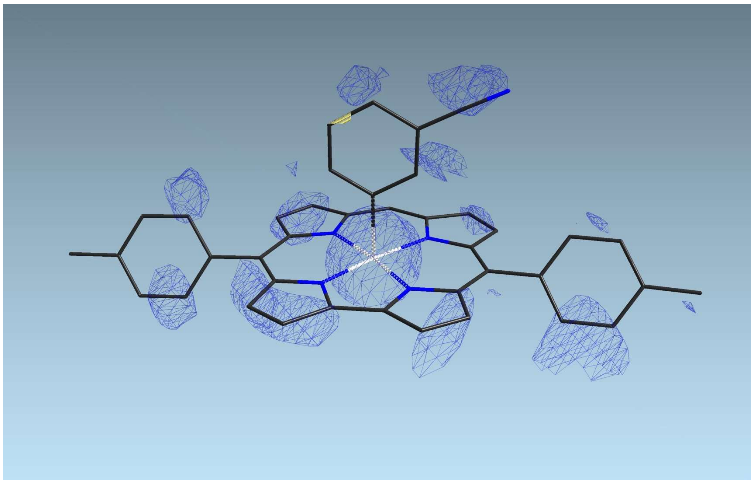
F_{obs} Map at 1.75Å



F_{obs} Map at 2.0Å



F_{obs} Map at 2.50Å



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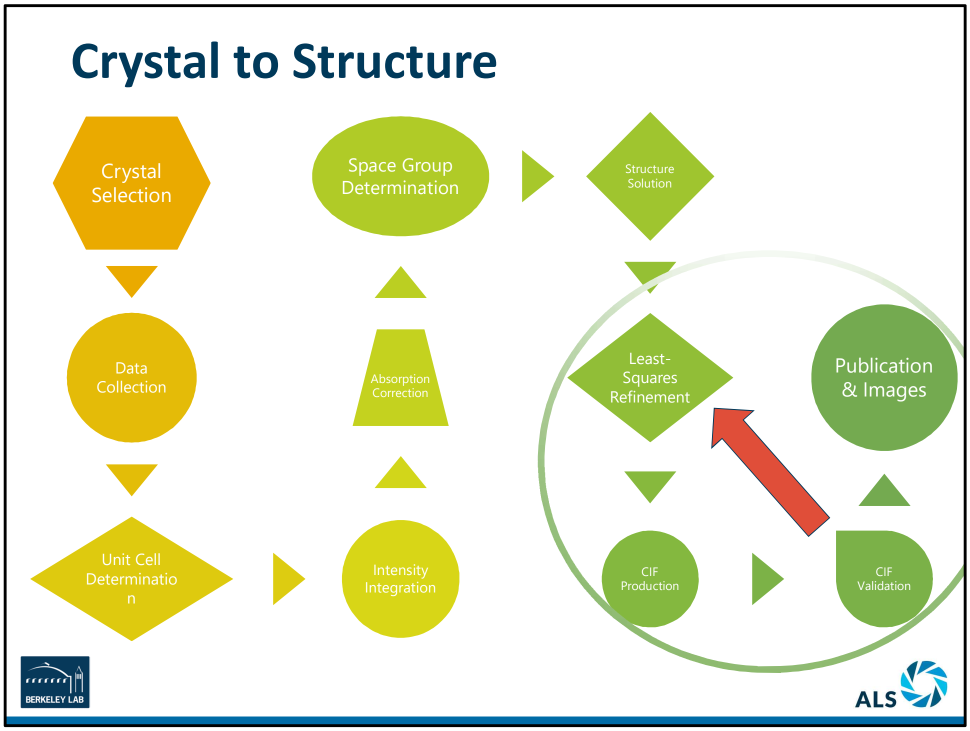
Crystal to Structure



Crystal Selection

Data Collection

Unit Cell Determination

Intensity Integration

Absorption Correction

Space Group Determination

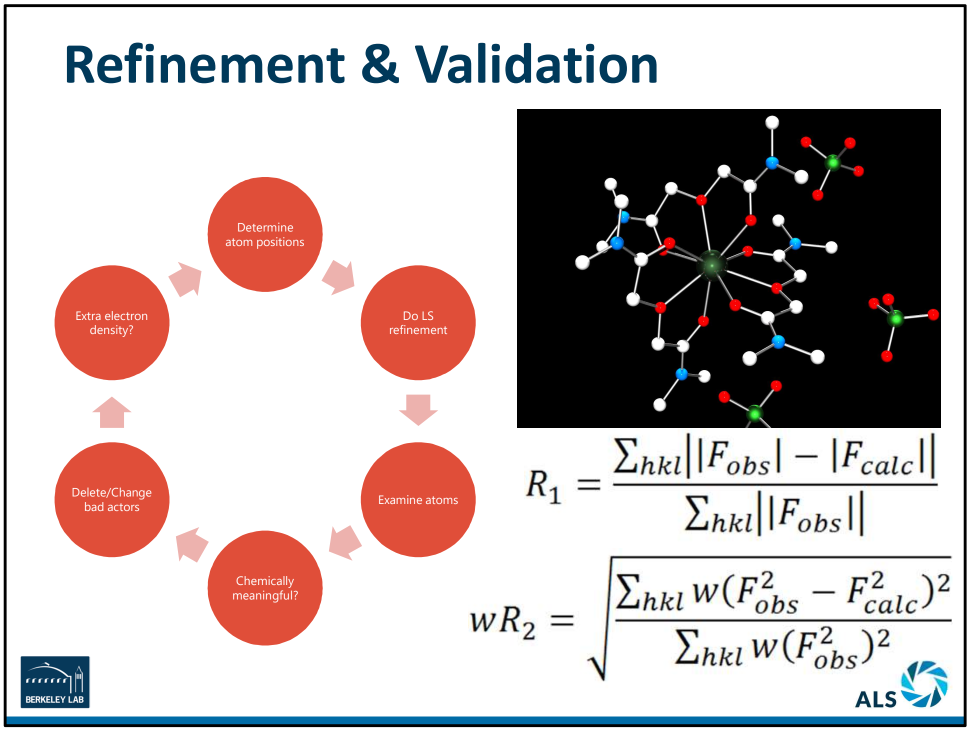
Structure Solution

Least-Squares Refinement

CIF Production

CIF Validation

Publication & Images



Refinement & Validation

Determine atom positions

Do LS refinement

Examine atoms

Chemically meaningful?

Delete/Change bad actors

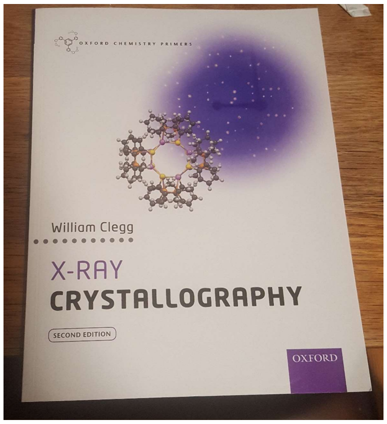
Extra electron density?

$$R_1 = \frac{\sum_{hkl} \left| |F_{obs}| - |F_{calc}| \right|}{\sum_{hkl} \left| |F_{obs}| \right|}$$

$$wR_2 = \sqrt{\frac{\sum_{hkl} w(F_{obs}^2 - F_{calc}^2)^2}{\sum_{hkl} w(F_{obs}^2)^2}}$$

Refinement of a structure is an iterative process. The structure solution result has to be inspected with a careful eye to chemical meaningfulness: do these bonds look like valid atom positions? Initial solutions can be messy with artifacts. The refinement process usually makes it clear which electron density peaks are false- either the refined position or the displacement elipsoid become poorly defined. As the model evolves, the R-values, which are commonly quoted measures of how well the model agrees with the data, should become smaller and smaller, as the model aligns with the data.

For More Information



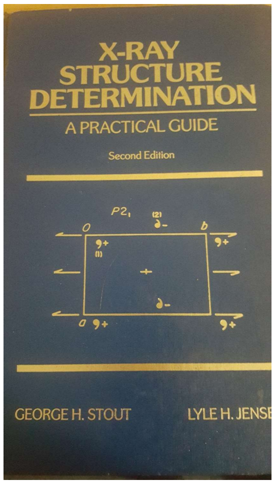
OXFORD CHEMISTRY PRIMERS

William Clegg

X-RAY
CRYSTALLOGRAPHY

SECOND EDITION

OXFORD

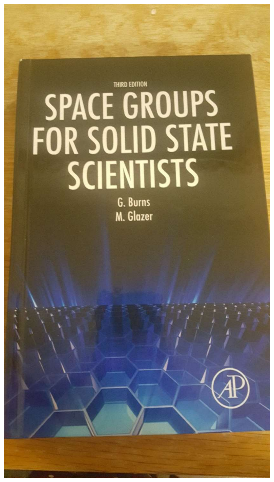


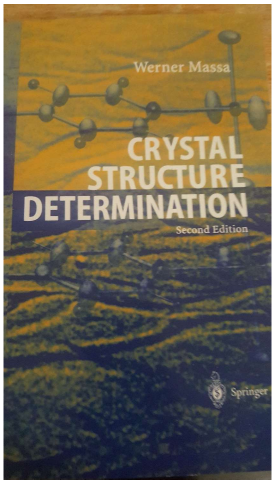
X-RAY
STRUCTURE
DETERMINATION
A PRACTICAL GUIDE

Second Edition

GEORGE H. STOUT LYLE H. JENSE

Even More Information





More Resources!!!

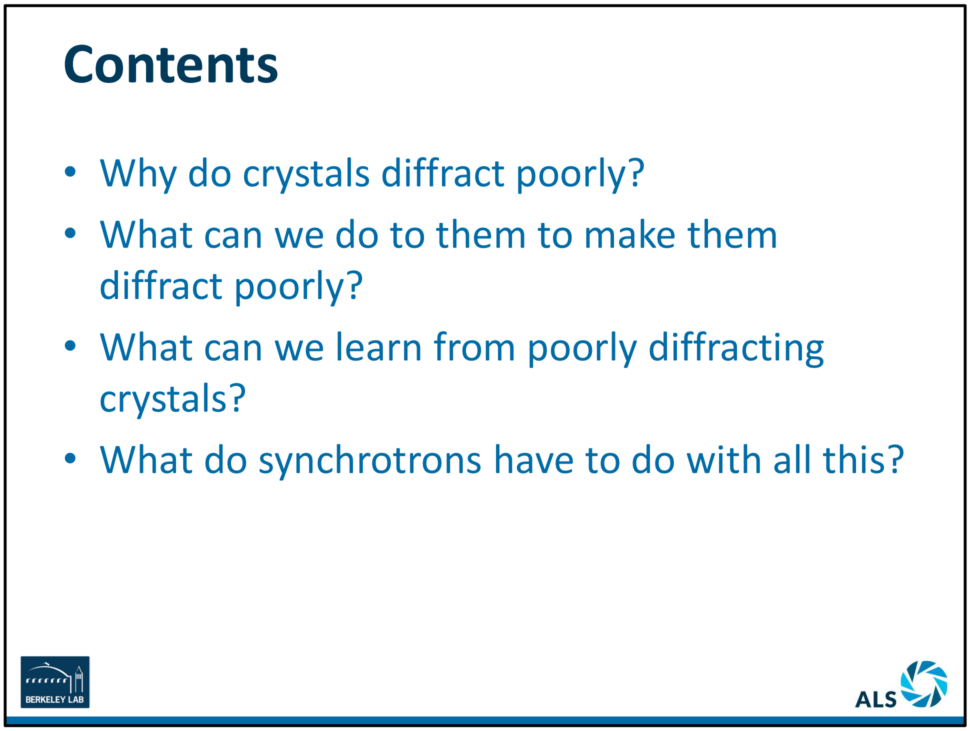
Internet

- X-ray Forum
  - www.xray**forum**.co.uk/
- IUCr Forum
  - forums.iucr.org
- CCP4
  - http://www.ccp4.ac.uk

Small Molecule Crystallography at a Synchrotron

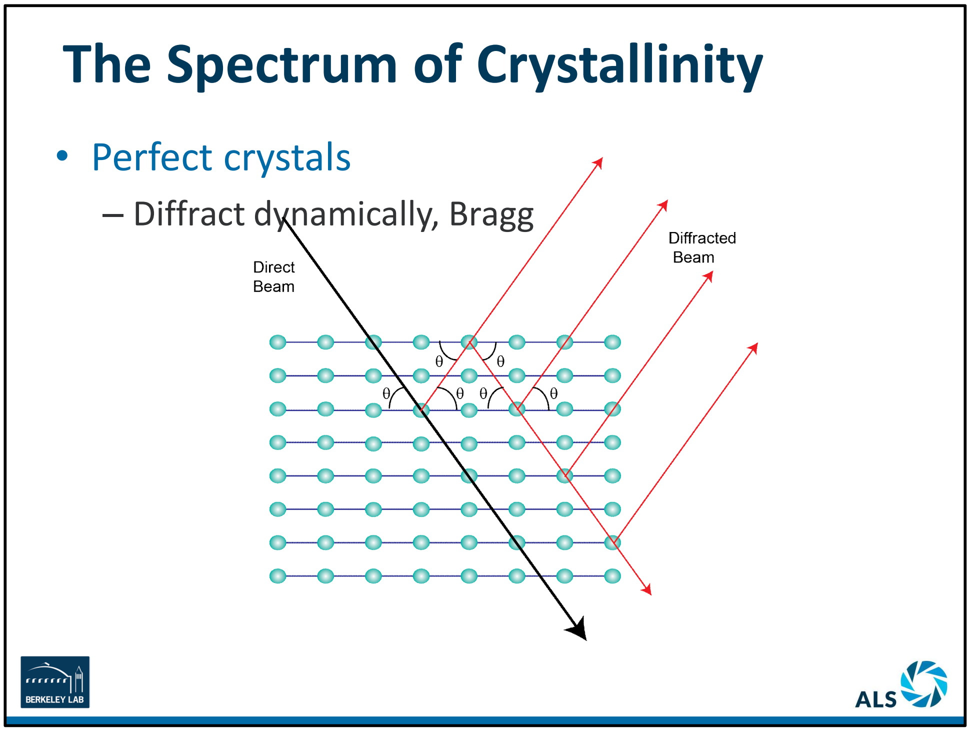
or

What can you do with more flux?



Contents

- Why do crystals diffract poorly?
- What can we do to them to make them diffract poorly?
- What can we learn from poorly diffracting crystals?
- What do synchrotrons have to do with all this?



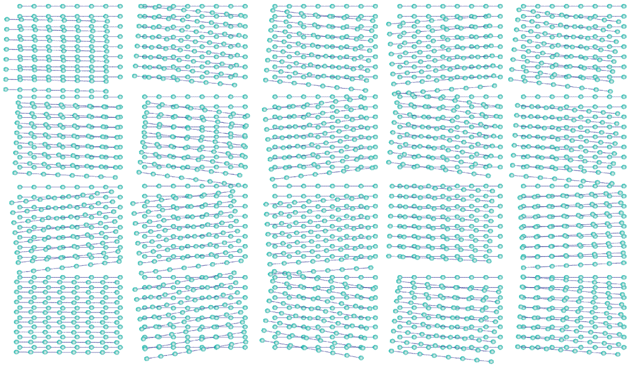
The Spectrum of Crystallinity

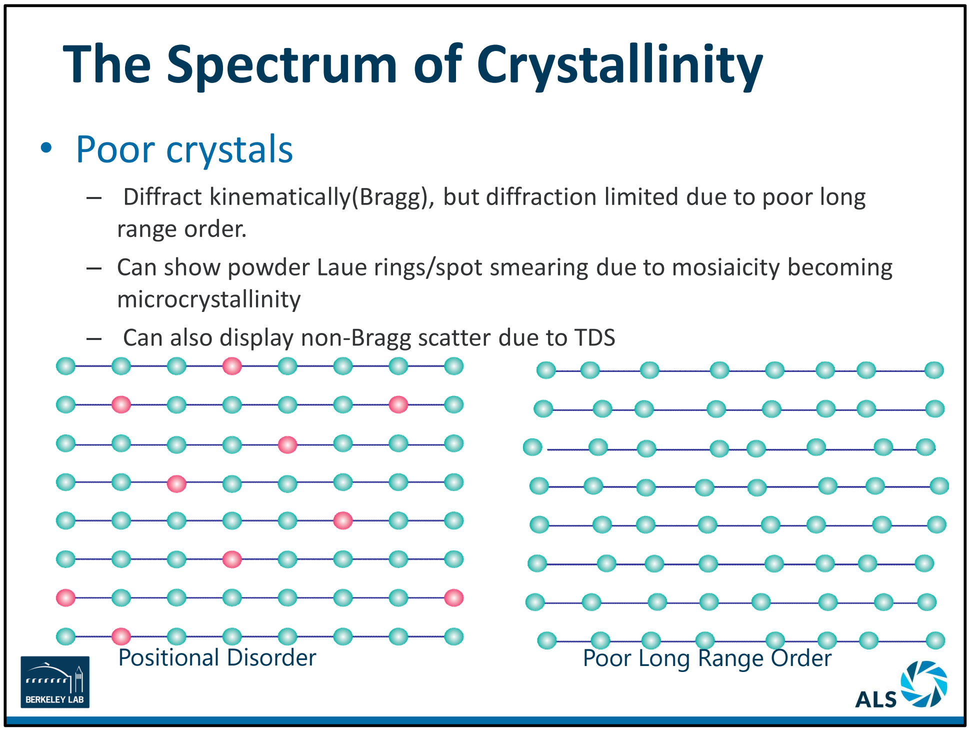
- Perfect crystals
  - Diffract dynamically, Bragg

Extinction a factor

The Spectrum of Crystallinity

- Good Crystals
  - Diffract kinematically(Bragg), due to mosaicity, but still have good long range order





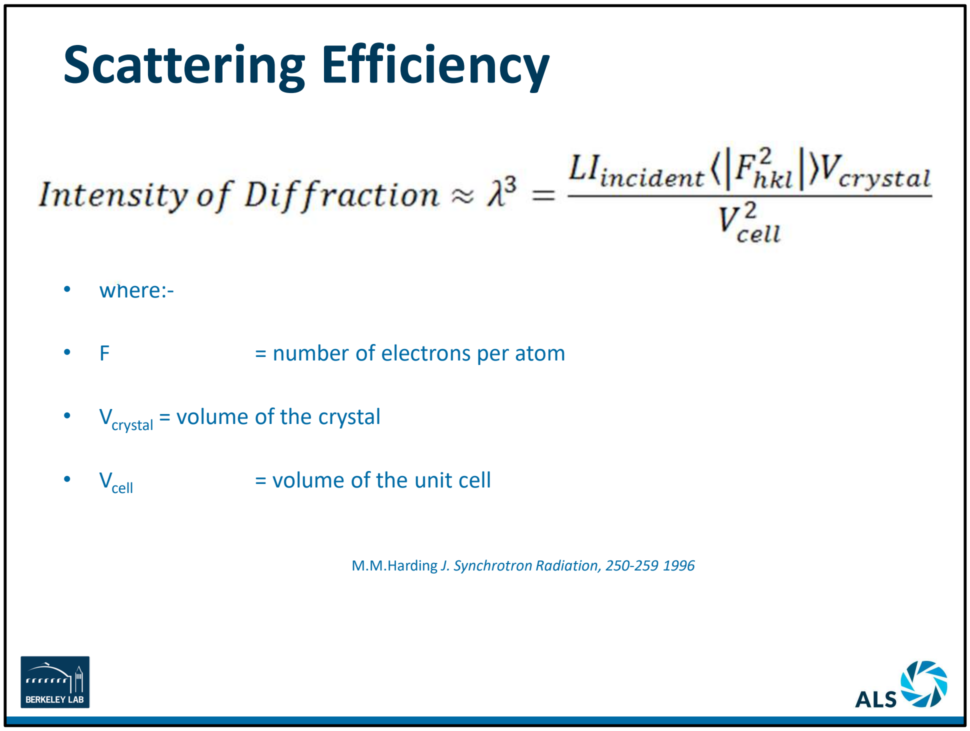
The Spectrum of Crystallinity

- Poor crystals
  - Diffract kinematically(Bragg), but diffraction limited due to poor long range order.
  - Can show powder Laue rings/spot smearing due to mosiaicity becoming microcrystallinity
  - Can also display non-Bragg scatter due to TDS

Positional Disorder

Poor Long Range Order

Disorder
Poor mosaicity
TDS = thermal diffuse scatter

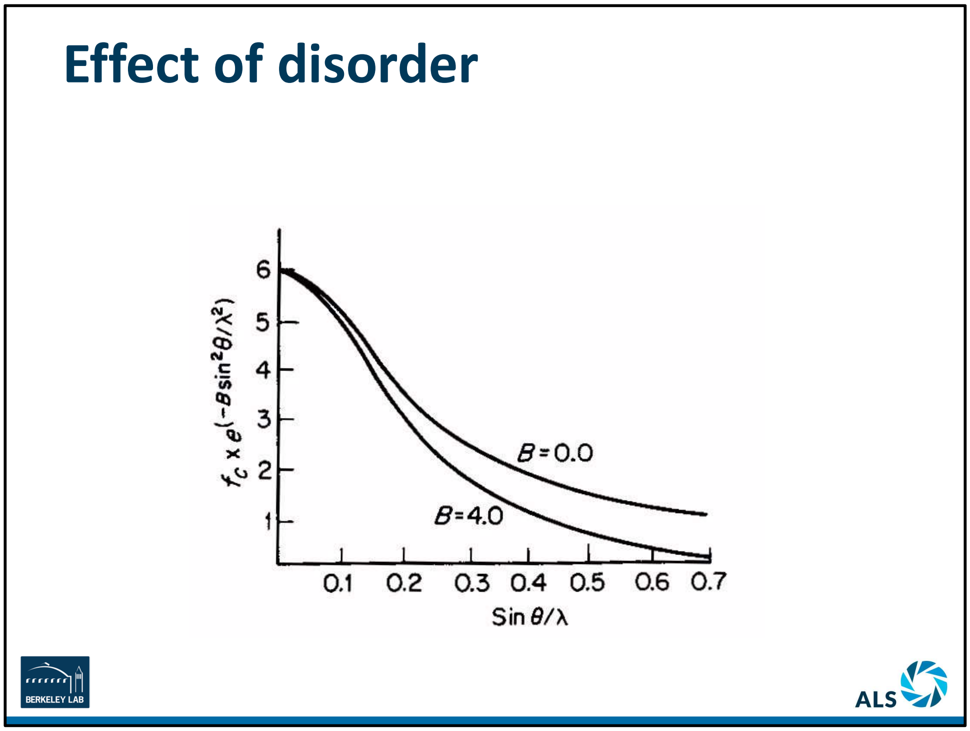


Scattering Efficiency

$$Intensity\ of\ Diffraction \approx \lambda^3 = \frac{L I_{incident} \langle |F^2_{hkl}| \rangle V_{crystal}}{V^2_{cell}}$$

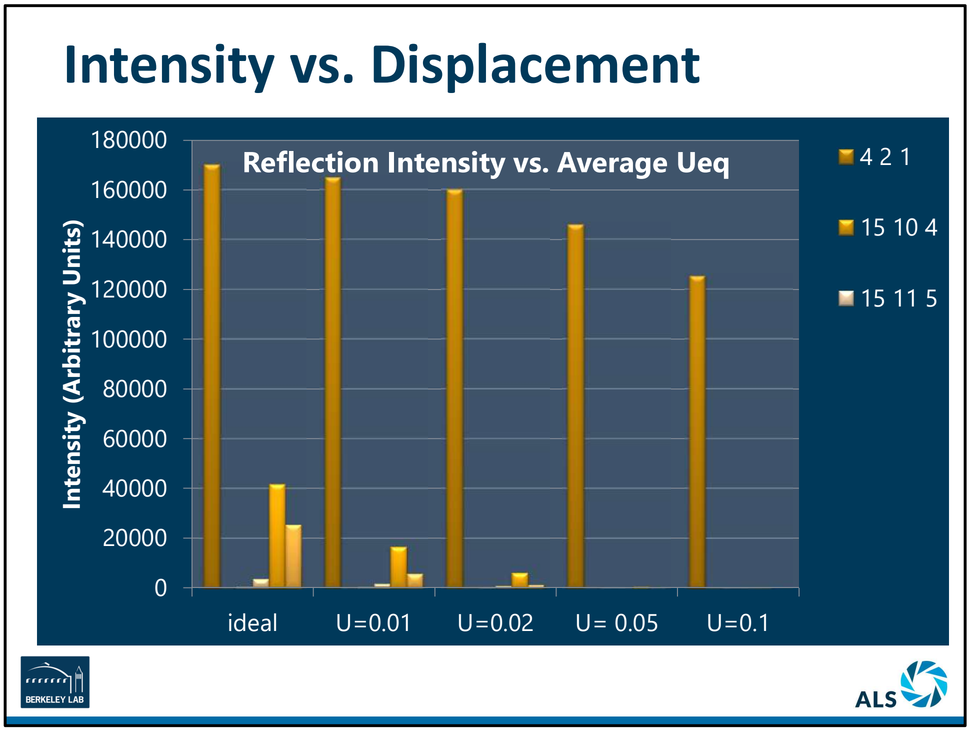
- where:-
- F = number of electrons per atom
- $V_{crystal}$ = volume of the crystal
- V_{cell} = volume of the unit cell

M.M.Harding *J. Synchrotron Radiation, 250-259 1996*



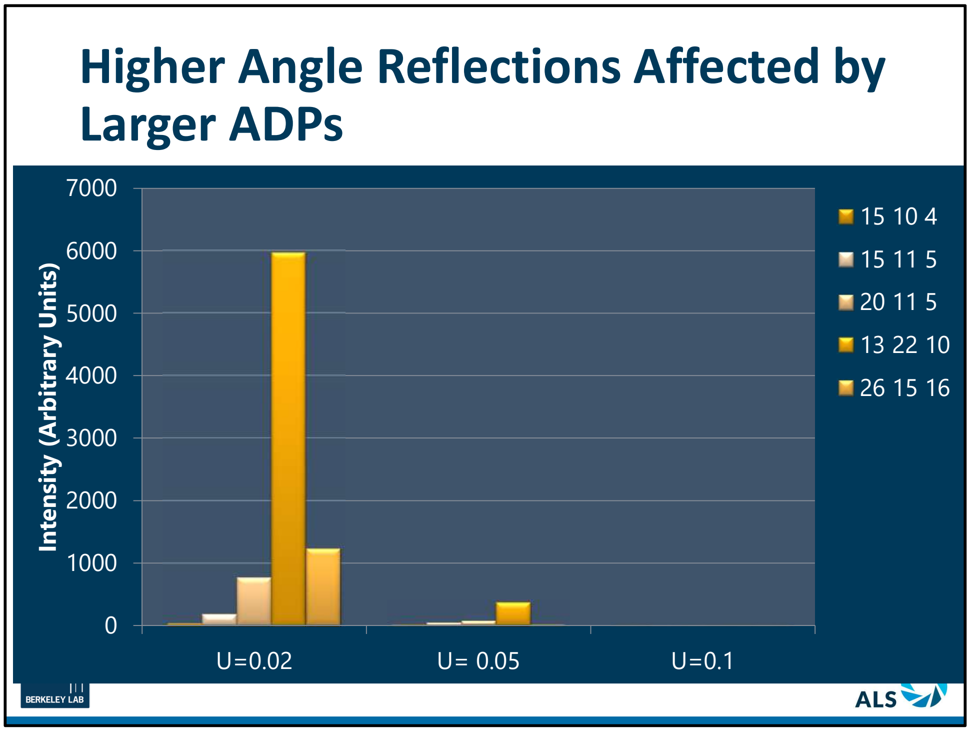
Effect of disorder

B values are the precursors to the U values- also called Debye-Waller factor, still used in protein world.



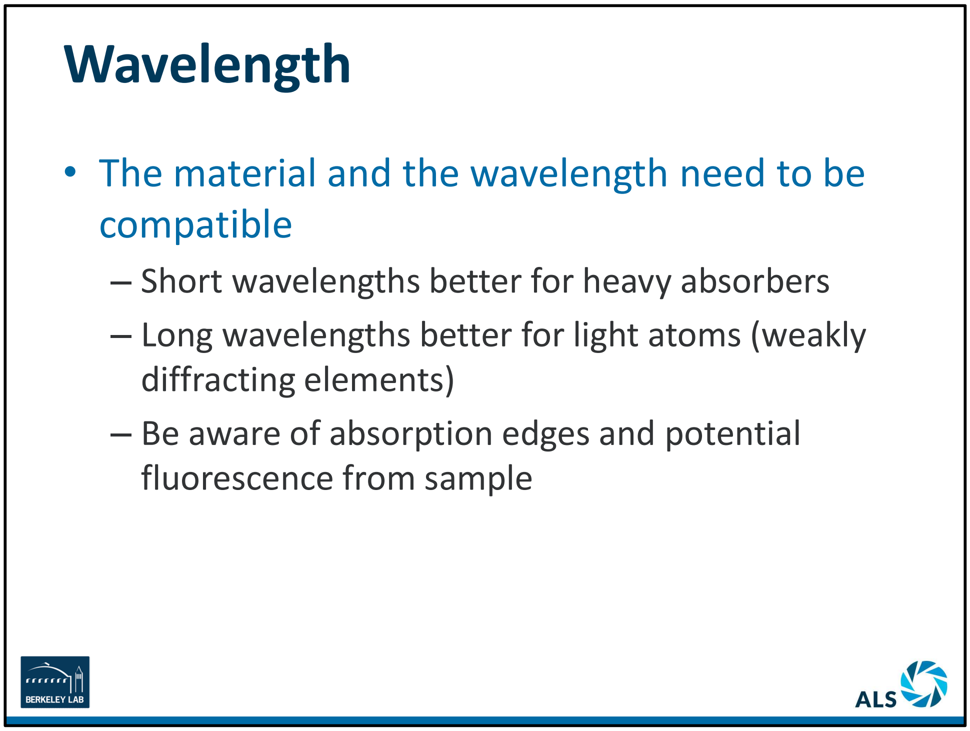
Intensity vs. Displacement

B= 8pi^2 U
U = mean square of vibration amplitude



Higher Angle Reflections Affected by Larger ADPs

Atomic Displacement parameters



Wavelength

- The material and the wavelength need to be compatible
  - Short wavelengths better for heavy absorbers
  - Long wavelengths better for light atoms (weakly diffracting elements)
  - Be aware of absorption edges and potential fluorescence from sample

Bigger isn't always better

- Large crystals aren't guaranteed to diffract better
- Crystal should match beam size
  - But if there is a choice, smaller than the beam is usually better
- Rocking width can be worse with large crystals due to poor mosaicity

Structures from change:

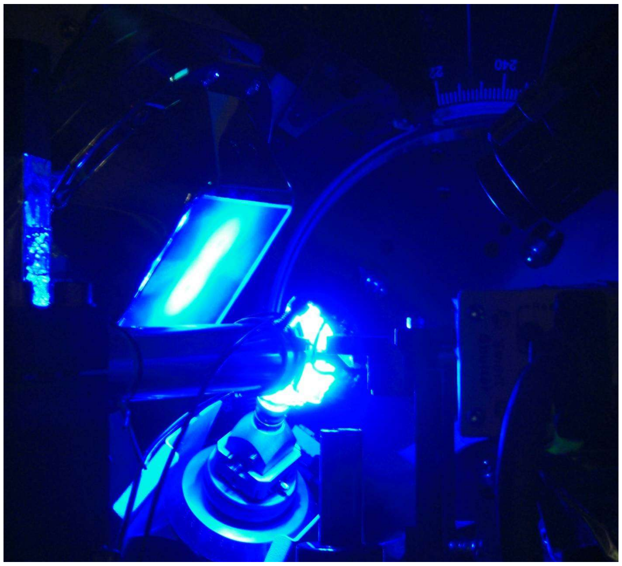
IN SITU EXPERIMENTS

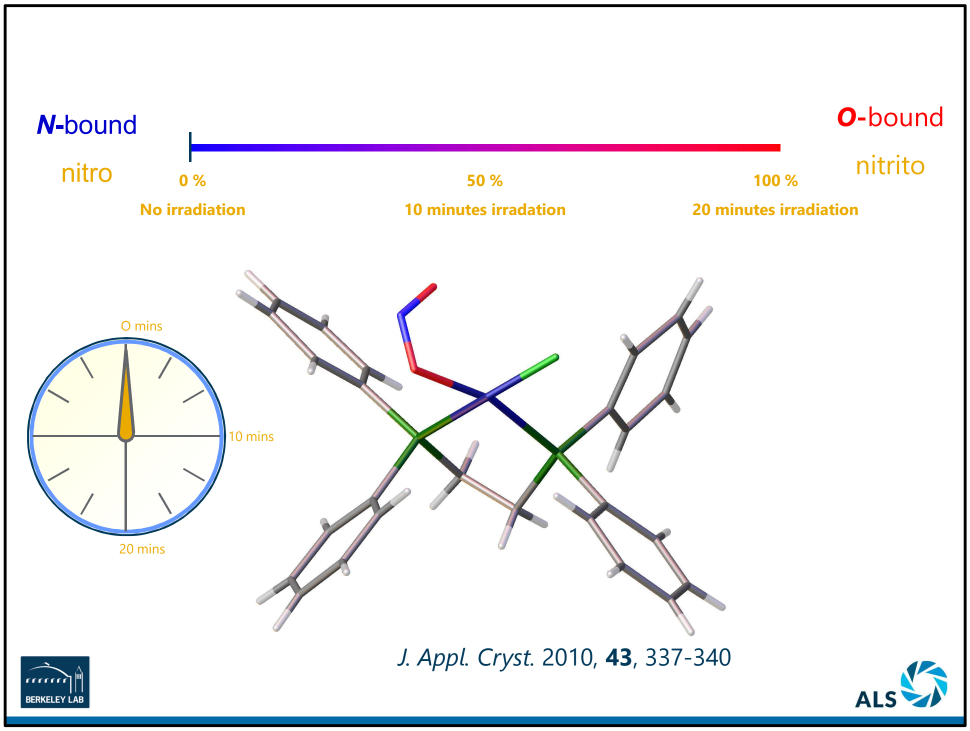
So you have super nice crystals that always work? Well, I am sure we can make them diffract poorly, and then learn about them!

In-situ Crystallography

- The application of a stimuli to produce structural change
    - Temperature
    - Pressure
    - Gas or Vacuum
    - Light
    - Electric or Magnetic Fields

Photocrystallography





***N*-bound**

nitro

0 %

No irradiation

50 %

10 minutes irradation

100 %

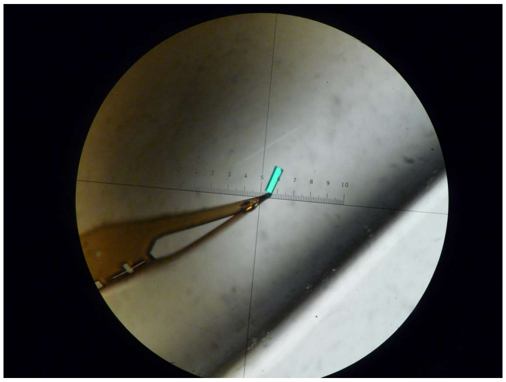
20 minutes irradiation

***O*-bound**

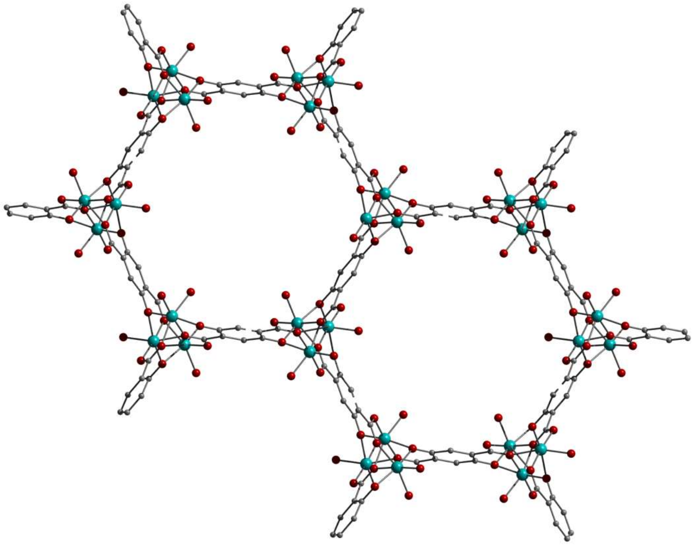
nitrito

J. Appl. Cryst. 2010, **43**, 337-340

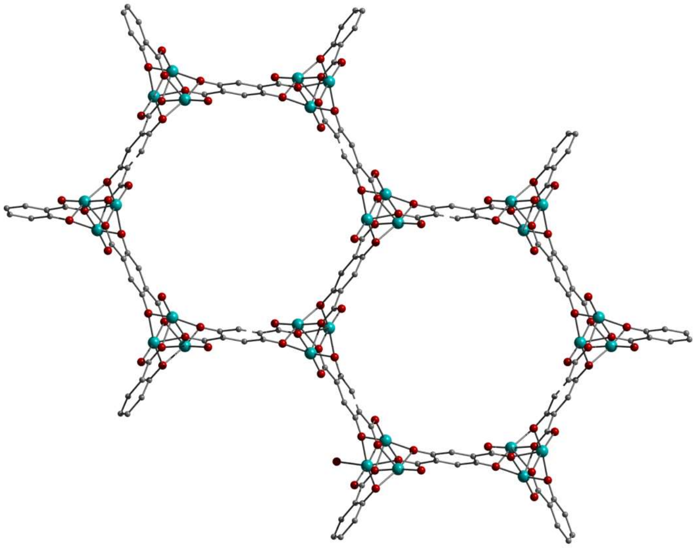
Gas Cell



Hydrated MOF



Dehydrated



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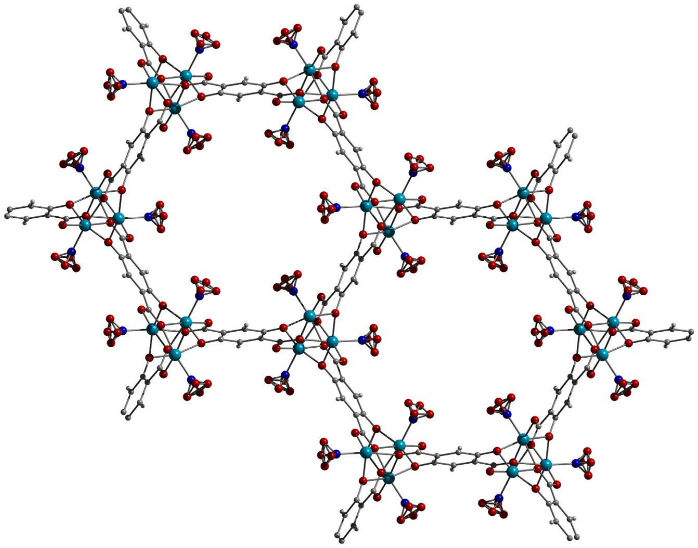
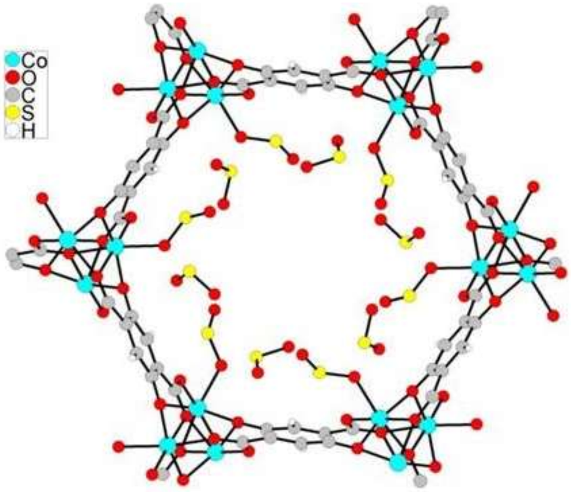
NO absorbed



ALS

SO_2 absorbed

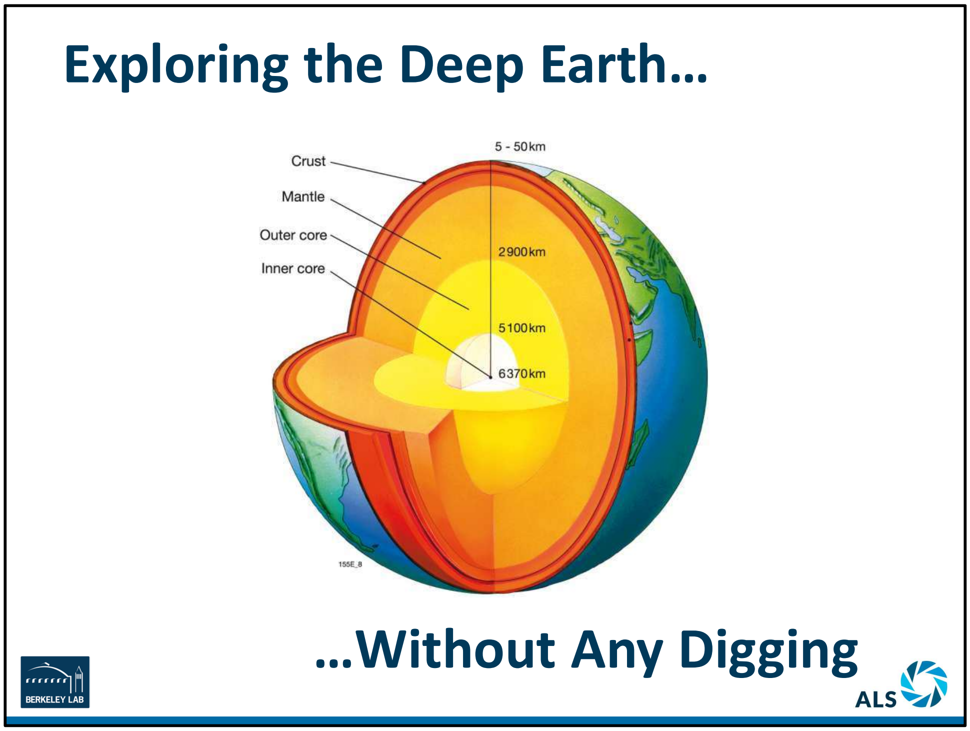


High Pressure with Diamond Anvil Cells

Why High Pressure?

*"Pressure is highly efficient for
generating phase transitions and new phases,
for triggering new chemical reactions,
conformational and structural transformations of molecules,
polymerization,
polymorphism
and determining structure–property relations
which are of interest to chemists and physicists."*

Katrusiak, A. *Acta Cryst., Sect. A,* **2007**, *64*, 135-148.



Exploring the Deep Earth…

…Without Any Digging

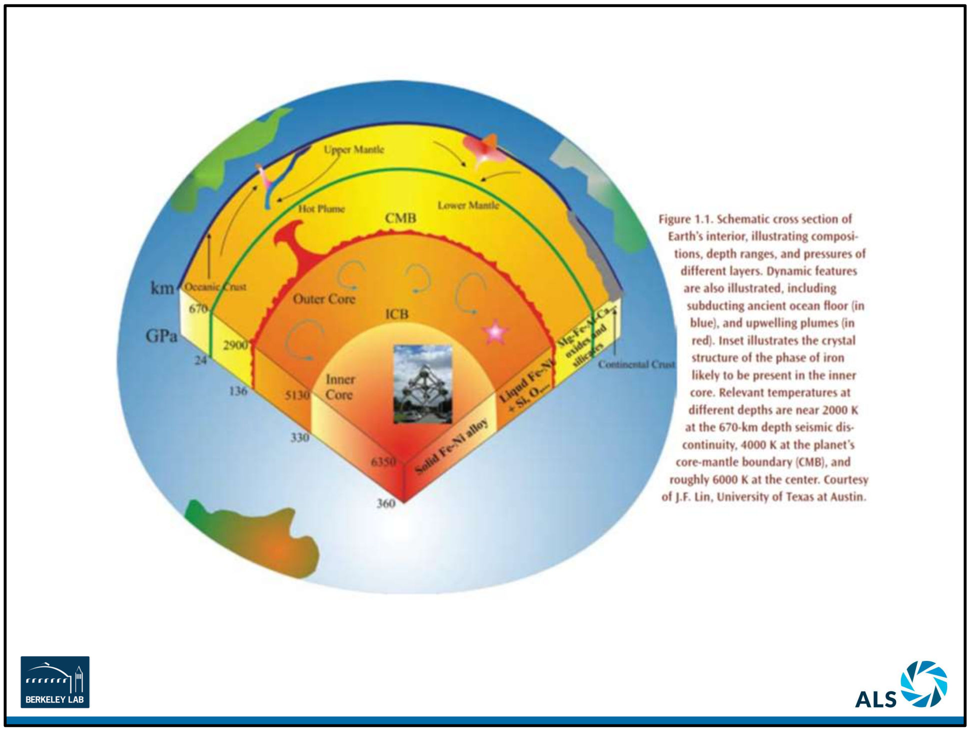
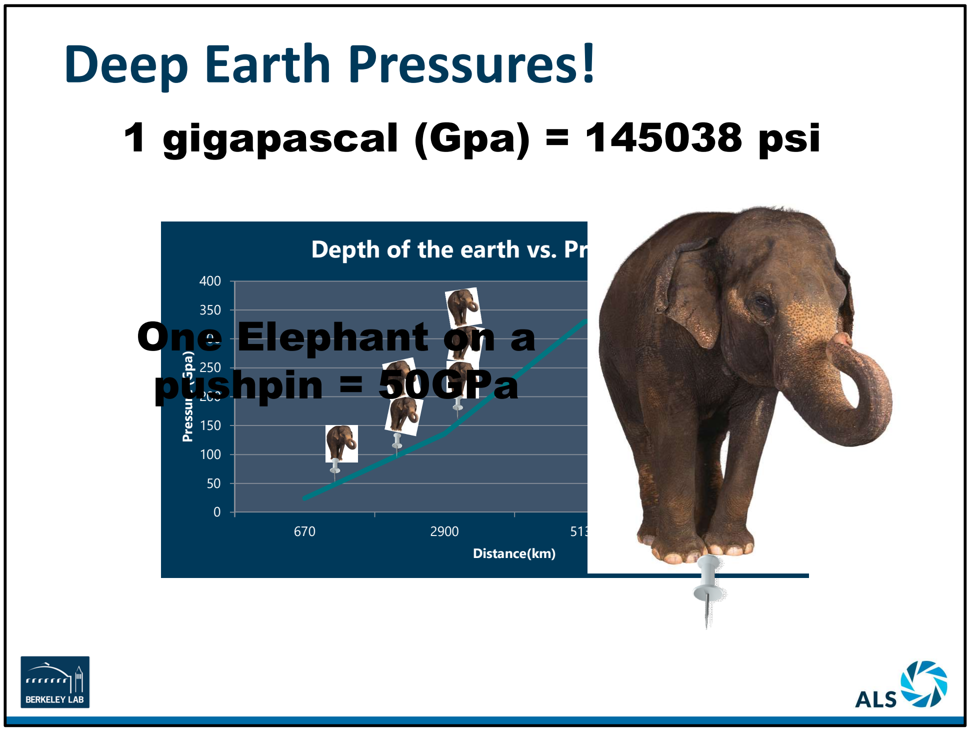


Figure 1.1. Schematic cross section of Earth's interior, illustrating compositions, depth ranges, and pressures of different layers. Dynamic features are also illustrated, including subducting ancient ocean floor (in blue), and upwelling plumes (in red). Inset illustrates the crystal structure of the phase of iron likely to be present in the inner core. Relevant temperatures at different depths are near 2000 K at the 670-km depth seismic discontinuity, 4000 K at the planet's core-mantle boundary (CMB), and roughly 6000 K at the center. Courtesy of J.F. Lin, University of Texas at Austin.

1 gigapascal = 145037.73773 psi



Deep Earth Pressures!

1 gigapascal (Gpa) = 145038 psi

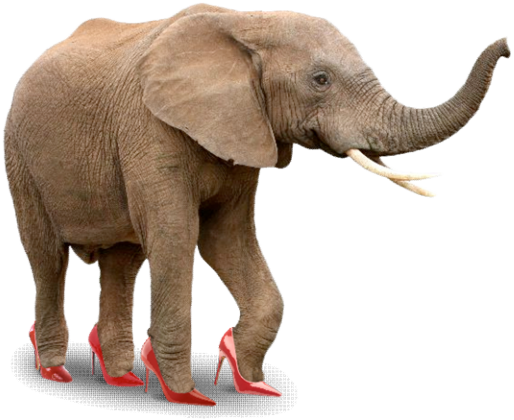
One Elephant on a pushpin = 50GPa

1 Gpa = 145038 psi

Imaginaing an elephant in 4 stilleto heels, the pressure produced by that elephant standing on just the pointy heels (1/4”) is about a half of a Gpa. But lets balance this elephant on the head of a pushpin… that’s about 50GPa

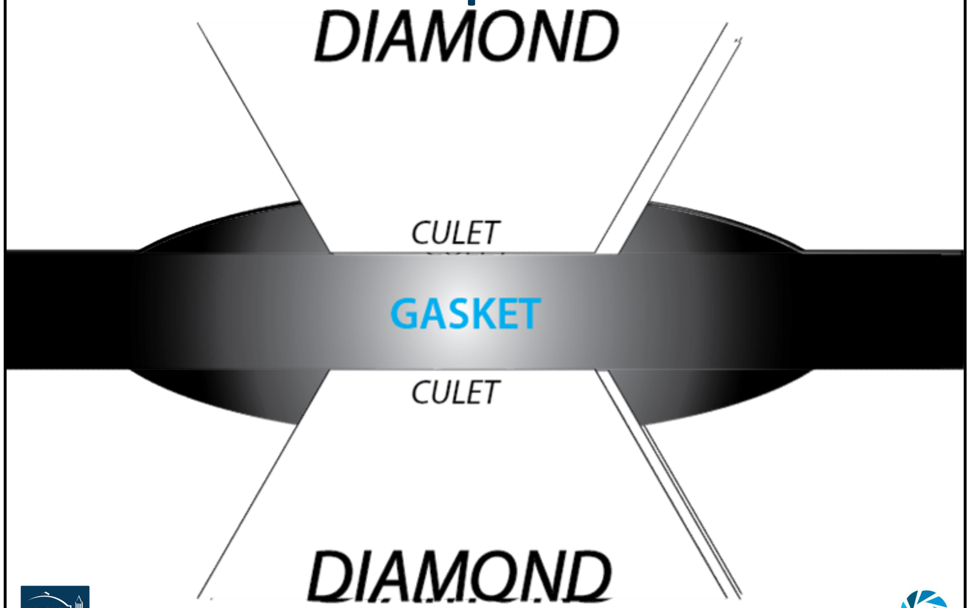
52 = 1 elephant
105= 2 elephants

Luckily I'm usually doing chemistry…



Thanks to Helen Maynard-Casely for this image!

Extreme Close-Up



Diamond Anvil Cell (DAC)

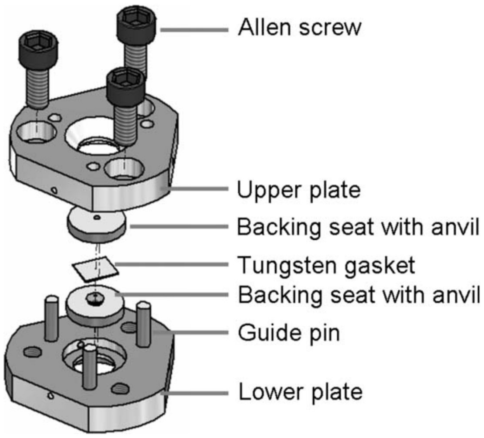
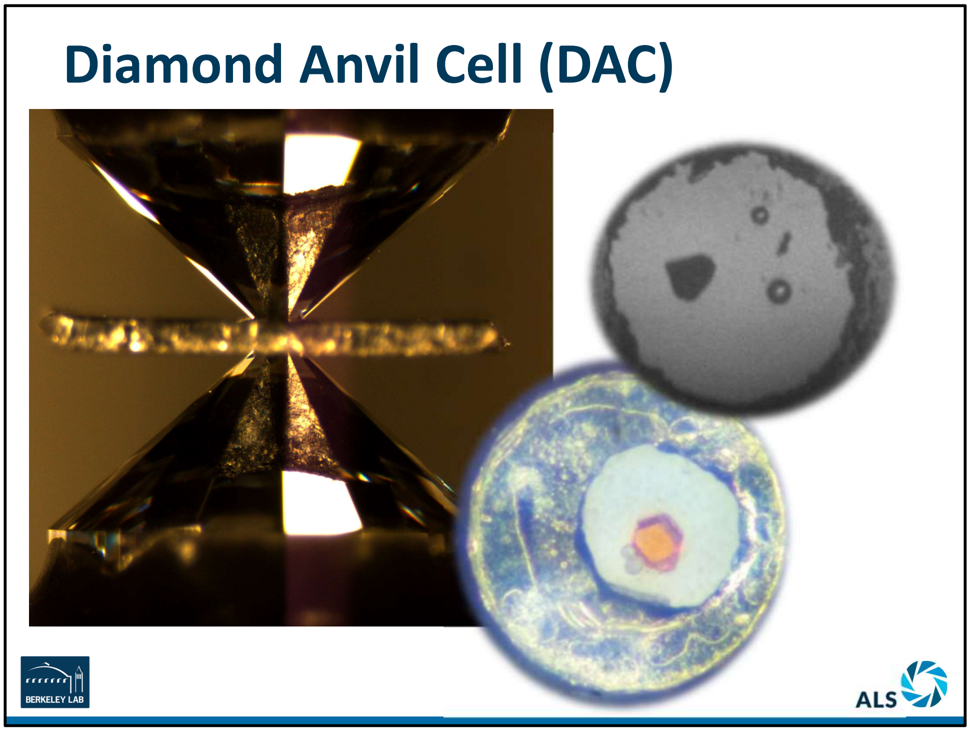
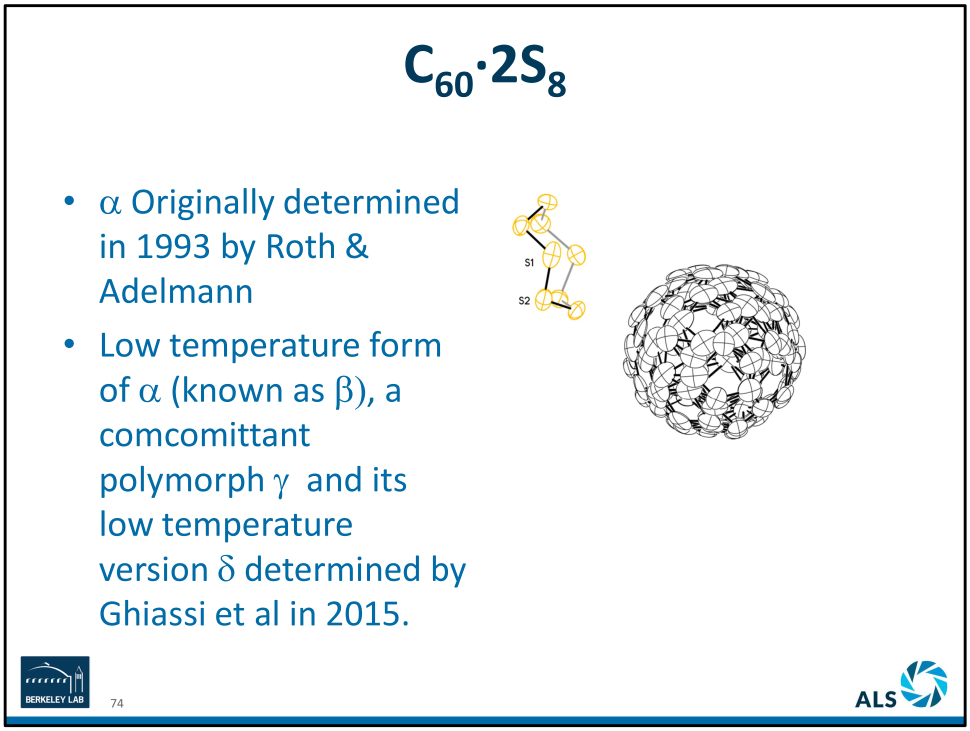


Figure 2
Exploded view of a Merrill–Bassett DAC.

Moggach, S. A. et al. *J. Appl. Crystallogr.* **2008**, *41*, 249-251.



Diamond Anvil Cell (DAC)

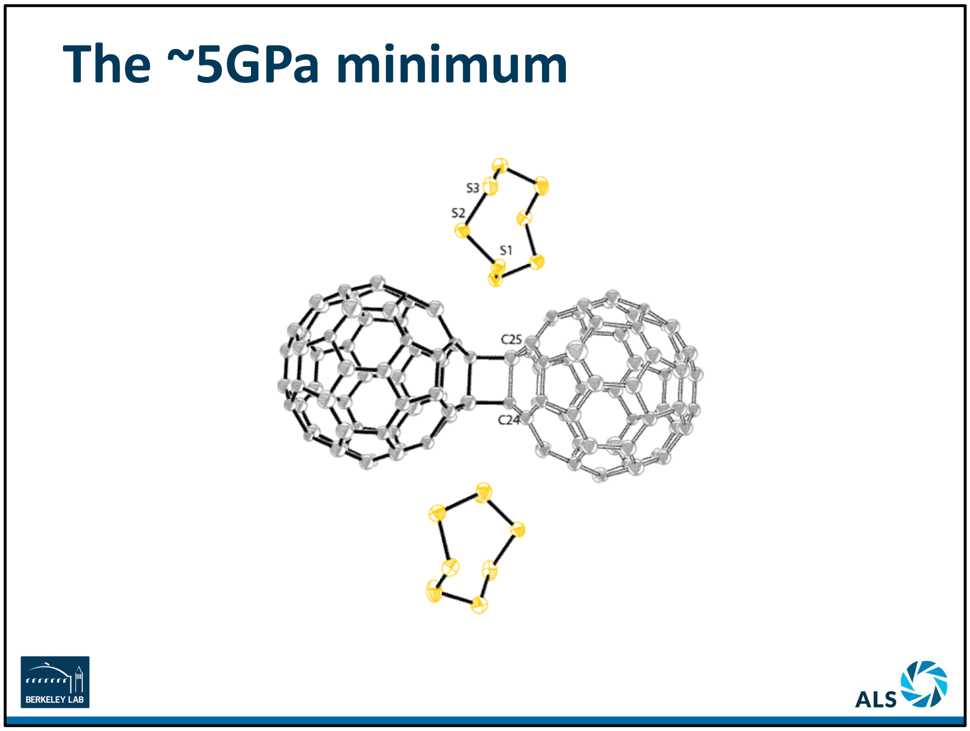


$C_{60} \cdot 2S_8$

- α Originally determined in 1993 by Roth & Adelmann
- Low temperature form of α (known as β), a comcomittant polymorph γ and its low temperature version δ determined by Ghiassi et al in 2015.

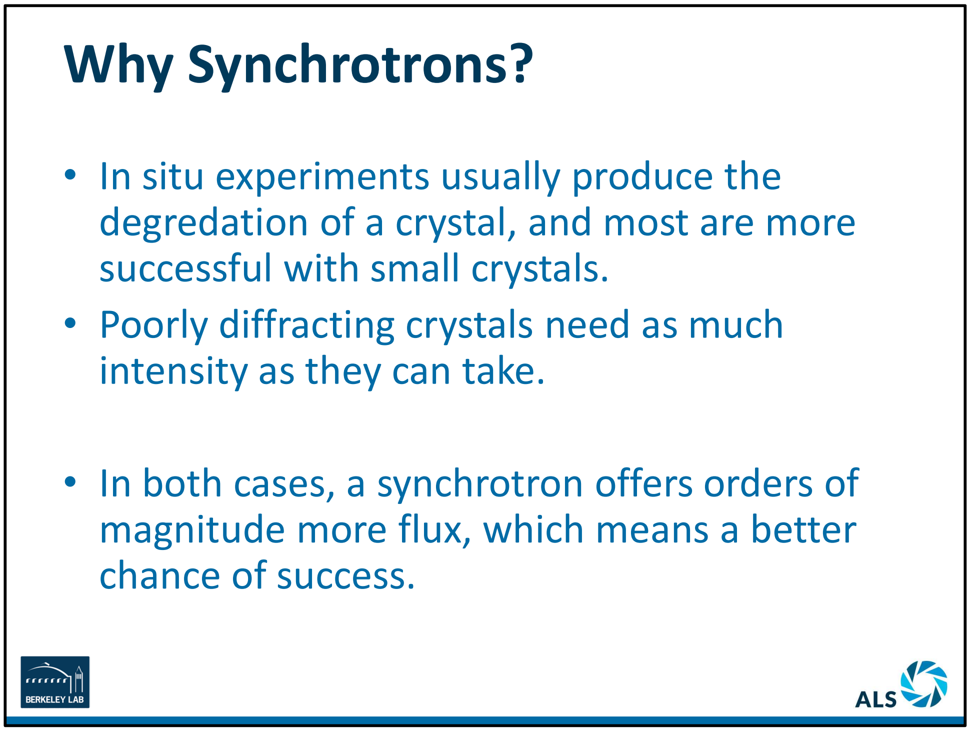
Alpha is mono C2/c, goes to beta (triclinic P-1). Gamma is mono P2(1)/c goes to Pc.

Compression Video



The ~5GPa minimum

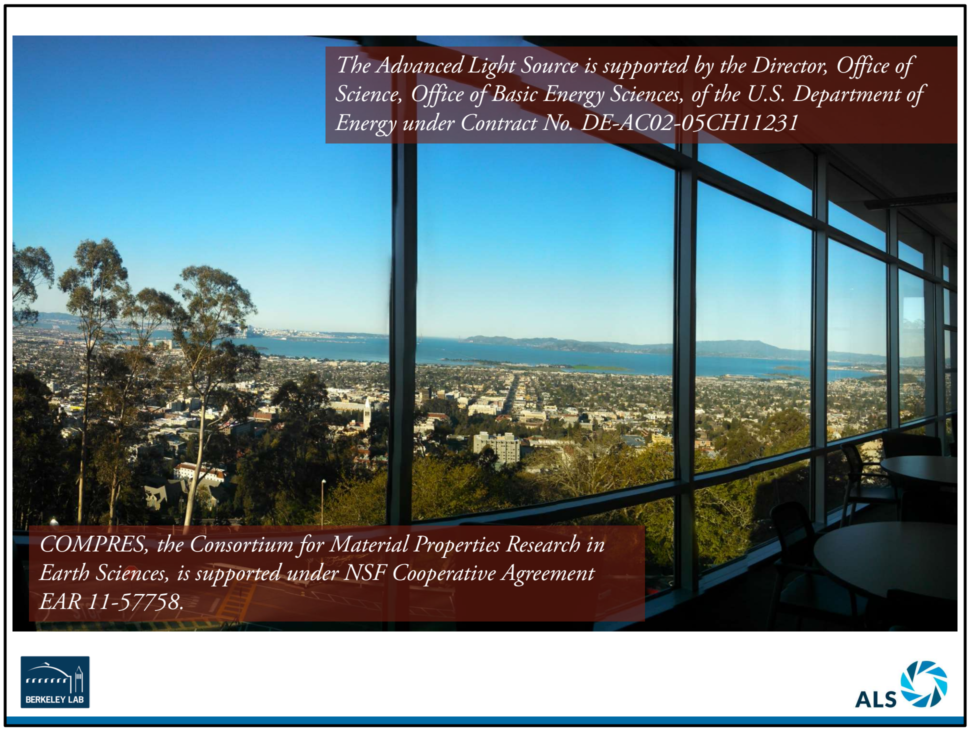
At 4p7GPa, the inter cage bond length is 1.68(3) Å with the 6:6 measuring at 1.70(3) Å, which is elongated, but gives a symmetric cyclobutene(R1= 7%). At 5.1GPa, the inter-cage bond length of 1.62(4) Å is comparable to that found in the dimer in the Wang et al Nature paper(1997) (1.575(7) Å), but the 6:6 bond length is hugely elongated- 1.78(4) Å. By 6.3GPa, the bond length has increased to 1.77(6) Å, with the 6:6 =??. What happens beyond 6.5 Gpa is unclear- attempts to pressurize and recover the crystal seem to indicate that long-range order degrades above 7GPa.



Why Synchrotrons?

- In situ experiments usually produce the degredation of a crystal, and most are more successful with small crystals.
- Poorly diffracting crystals need as much intensity as they can take.

- In both cases, a synchrotron offers orders of magnitude more flux, which means a better chance of success.



The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231

COMPRES, the Consortium for Material Properties Research in Earth Sciences, is supported under NSF Cooperative Agreement EAR 11-57758.