High Resolution Diffuse X-ray Scattering by Structurally-Defective Protein Crystals

Richard J. Matyi
Physics Laboratory
National Institute of Standards and Technology
Gaithersburg, MD 20899

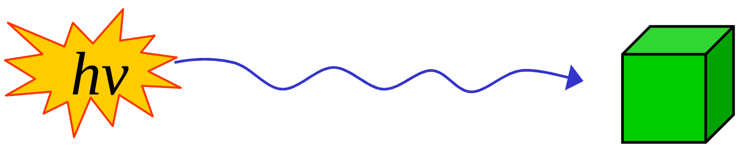
- Introduction to high resolution triple axis X-ray diffraction for reciprocal space mapping of diffuse scattering
- Experimental aspects of HRTXD analyses of organic crystals
- Application of HRTXD to radiation damage in protein crystals
- Prospects for small-angle X-ray scattering from grossly defective protein "crystals"
- Potential new directions and prospects for future work

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

NIST Physics Laboratory

Radiation damage and defects in protein crystals

- Protein crystals have large numbers of defects, and these defects adversely affect the quality of the structural determination
- Like all organic materials, protein crystals degrade when irradiated, thus reducing the minimum attainable structural resolution

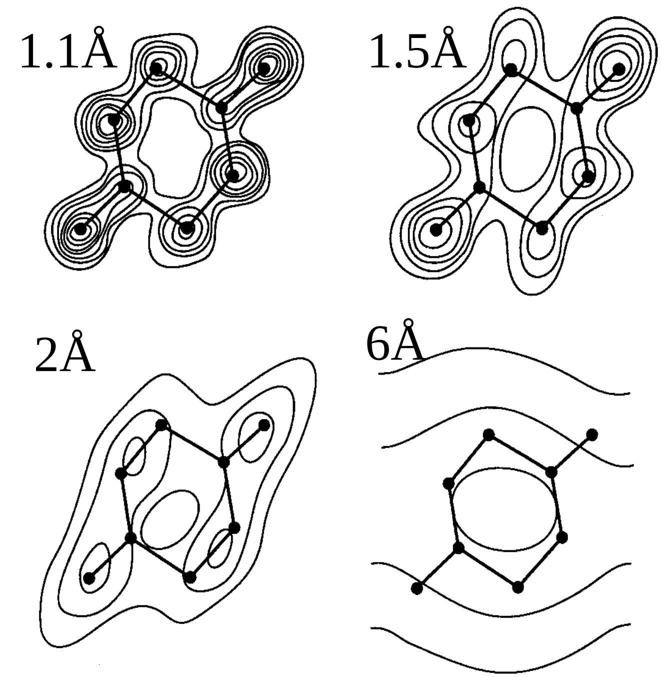


- Observed changes in structure with irradiation: increase in unit cell volume, breakage of disulphide bonds, alteration of amino acid residues

→ ***Dose dependency*** – photoelectric absorption

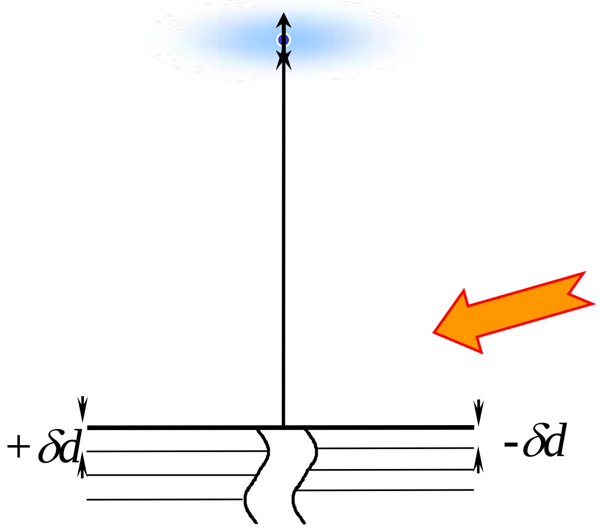
→ ***Time dependency*** – radicals diffuse through solvent to react

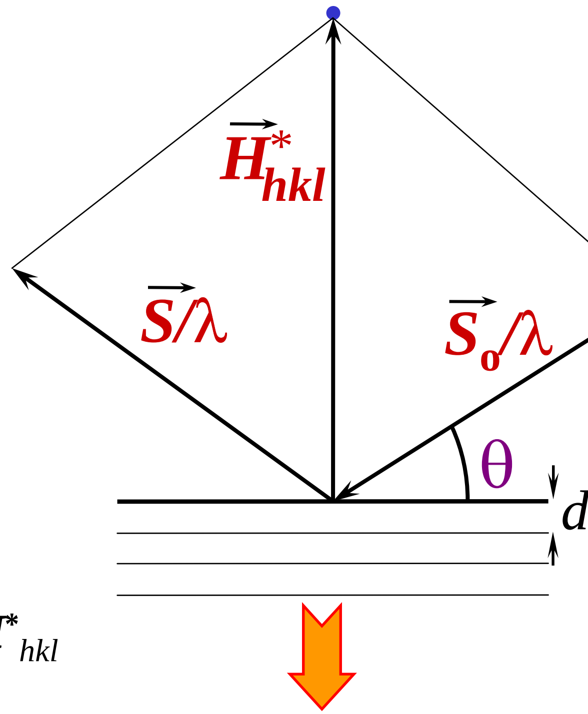
Electron density maps of diketopiperazine at resolutions ranging from 1.1Å to 6Å

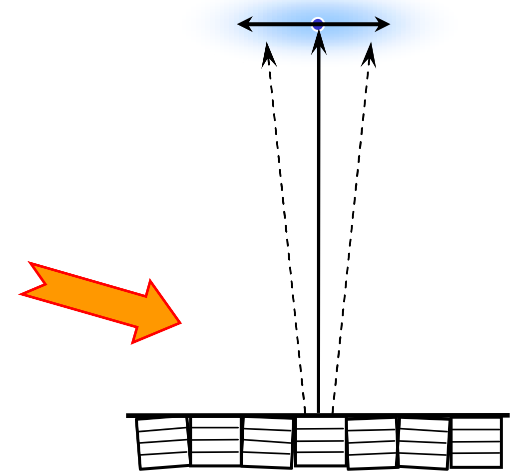


What is the relationship between chemical changes and physical structure?

Scattering around an *hkl* reciprocal lattice point: an indicator of long-range distortions

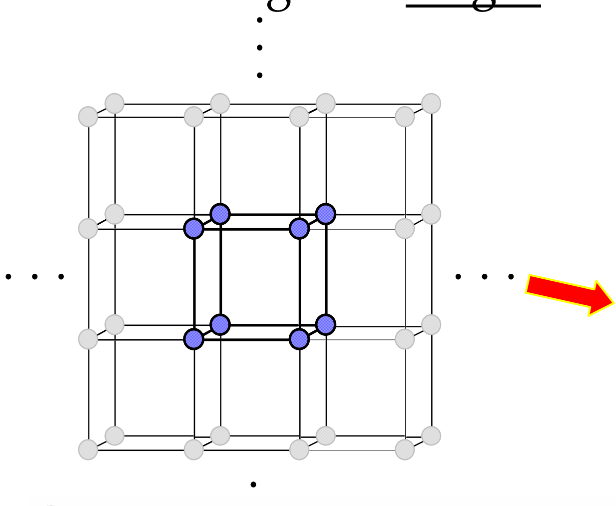




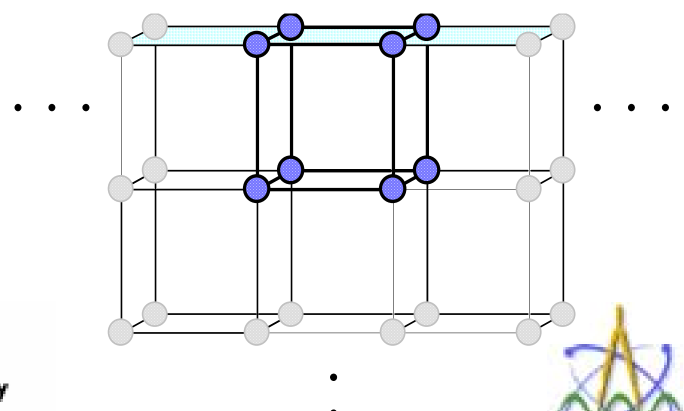


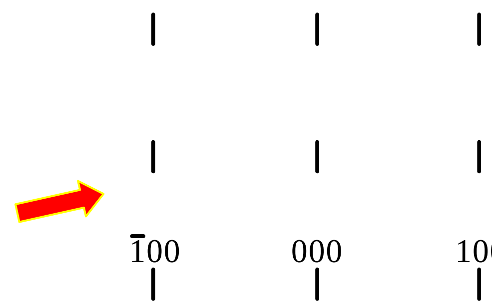
strains change the length of $\boldsymbol{H}^*_{hkl}$

misorientations change the direction of $\boldsymbol{H}^*_{hkl}$



the real crystal surface truncates the 3-D crystal





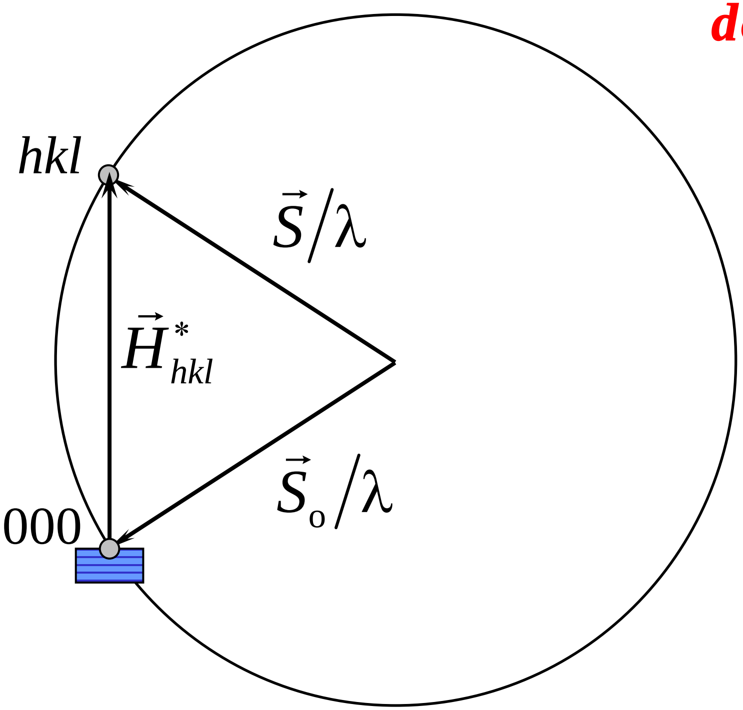
reciprocal lattice "points" become "rods"

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

NIST Physics Laboratory

Examining defect scattering with double- and triple-axis diffraction

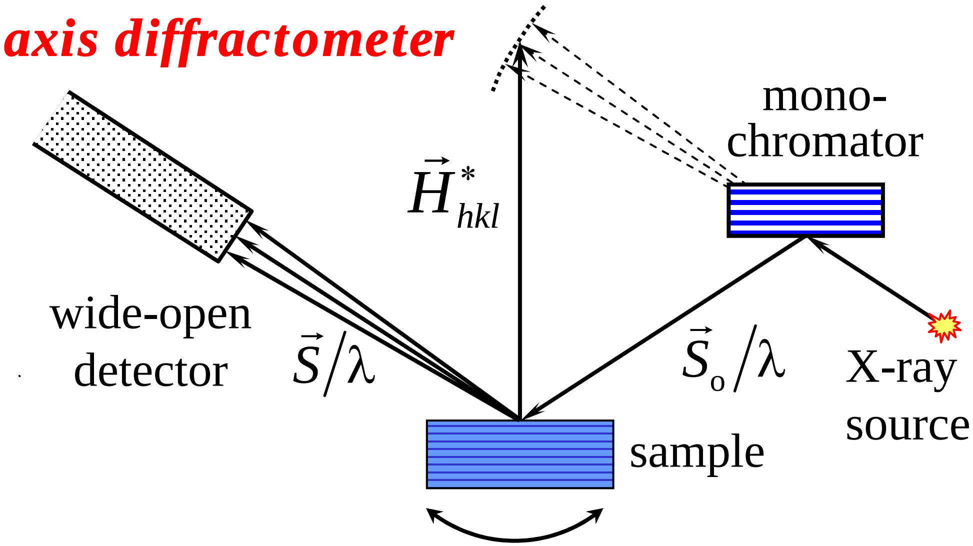
- Diffraction occurs if a reciprocal lattice point contacts the Ewald sphere



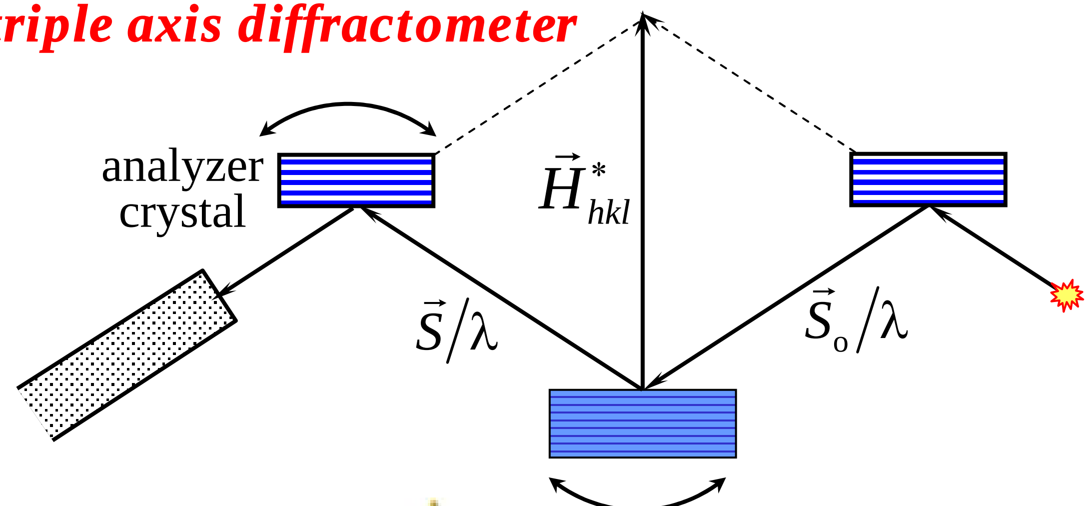
basic diffraction geometry

$$\vec{H}^*_{hkl} = \frac{\vec{S}-\vec{S}_o}{\lambda}$$
$$\Updownarrow$$
$$n\lambda = 2d\sin\theta$$

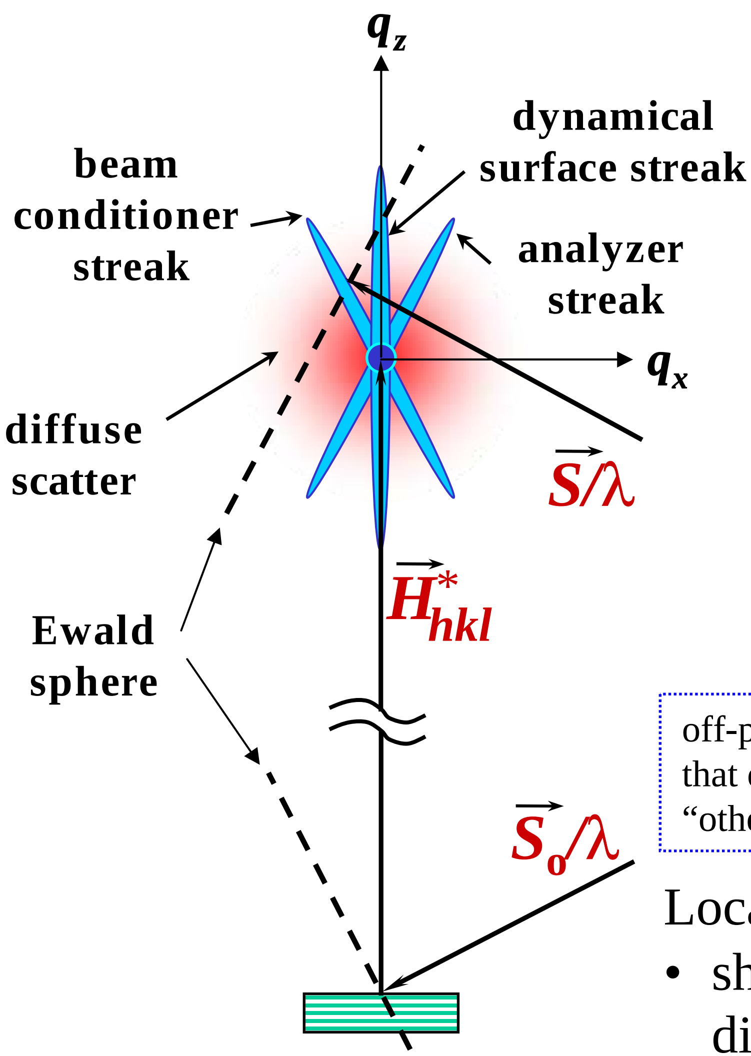
double axis diffractometer

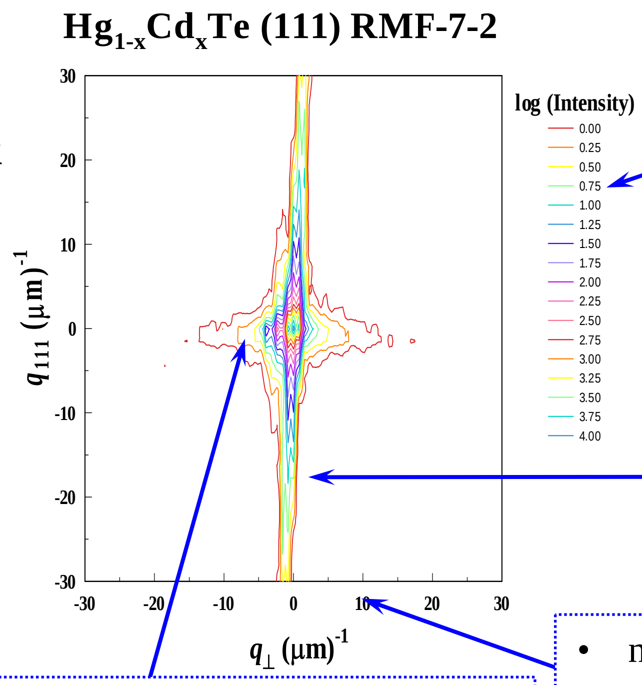


triple axis diffractometer



The appearance of a reciprocal lattice point in a triple axis experiment





plot as equal-intensity contours on a log scale (four contours per decade)

- surface streak evidence of dynamic "perfect crystal" diffraction → high perfection and good surface quality
- tilt due to miscut between (111) and crystal surface

off-peak scatter generated by defects that divert intensity from the peak to "other" locations in reciprocal space

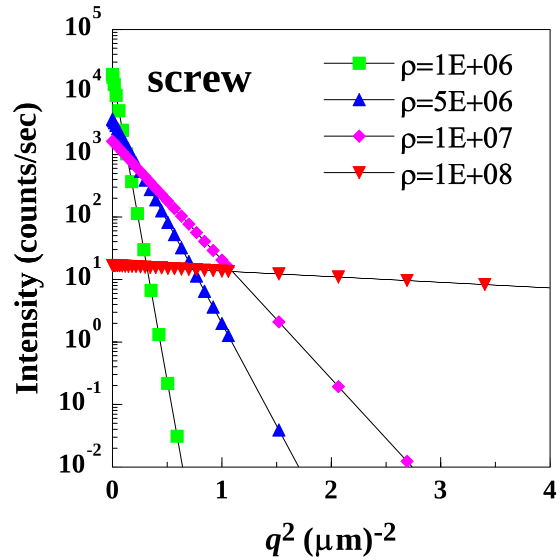
- note scale in reciprocal microns
  $q_x = (2\alpha-\beta)(\sin\theta/\lambda)$; $q_z = \beta(\cos\theta/\lambda)$
- length of H_{111} for CdTe is $1/d_{111} \approx 2670\ \mu m^{-1}$

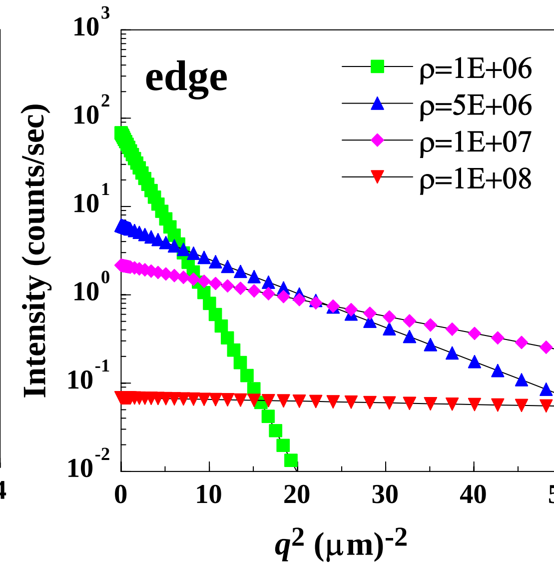
Location of diffuse scatter depends on type of defect:

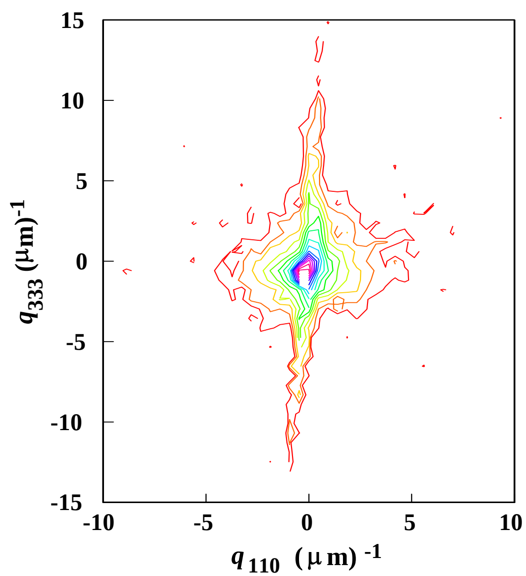
- short-range distortions → diffuse scatter at large distances in reciprocal space (between peaks)
- long-range distortions → "close-in" to Bragg peak

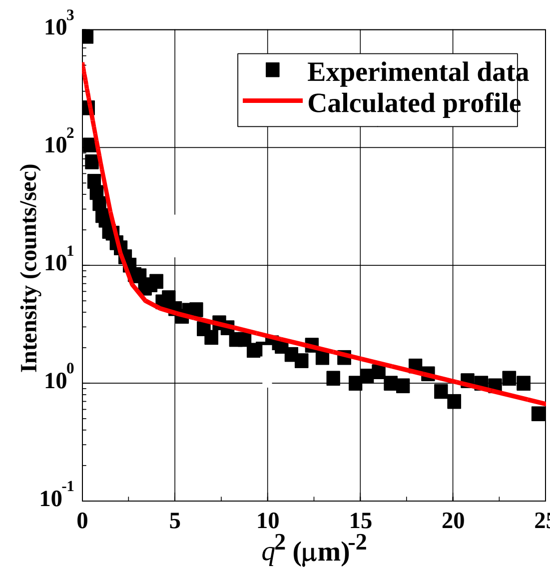
Calculated X-ray scattering from dislocations

- A model based on approach of Krivoglaz calculates the kinematic scattered intensity from dislocation strain fields
- Pure screw (left) and edge (right) dislocations give similar $\boldsymbol{q}^2$ dependence but large quantitative differences
- 333 reciprocal space map of USML-1 CdZnTe shows typical off-peak diffuse scatter
- Comparison of the transverse scan and the kinematic calculation shows agreement with an assumed dislocation density of 5×10^{6} cm^{-2}

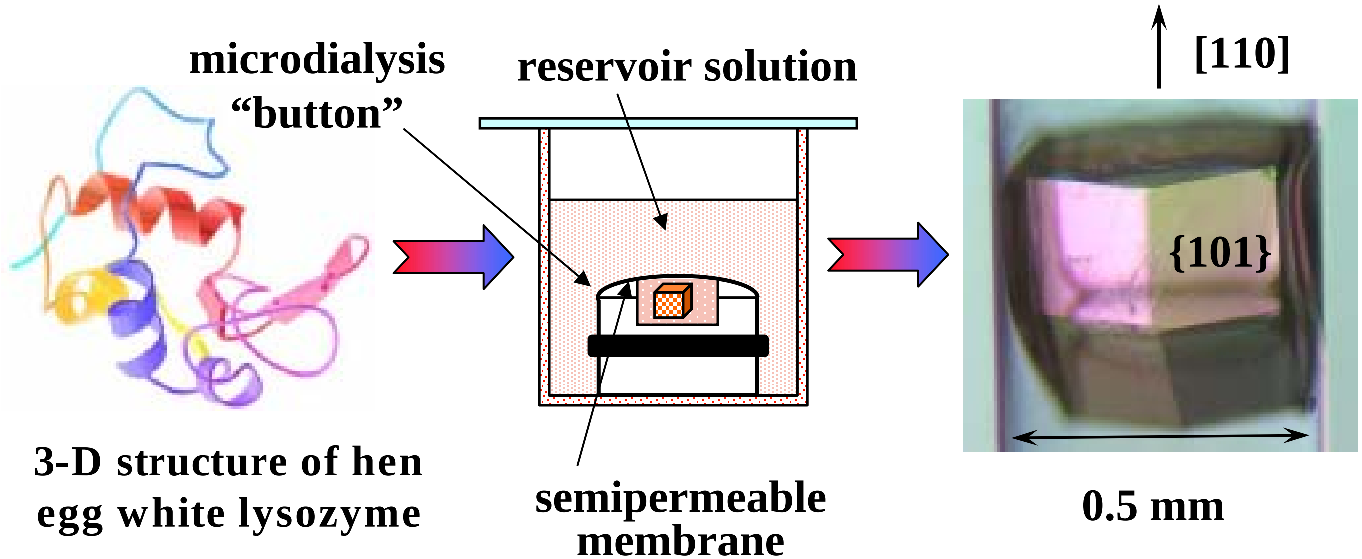








Growth and analysis of organic crystals

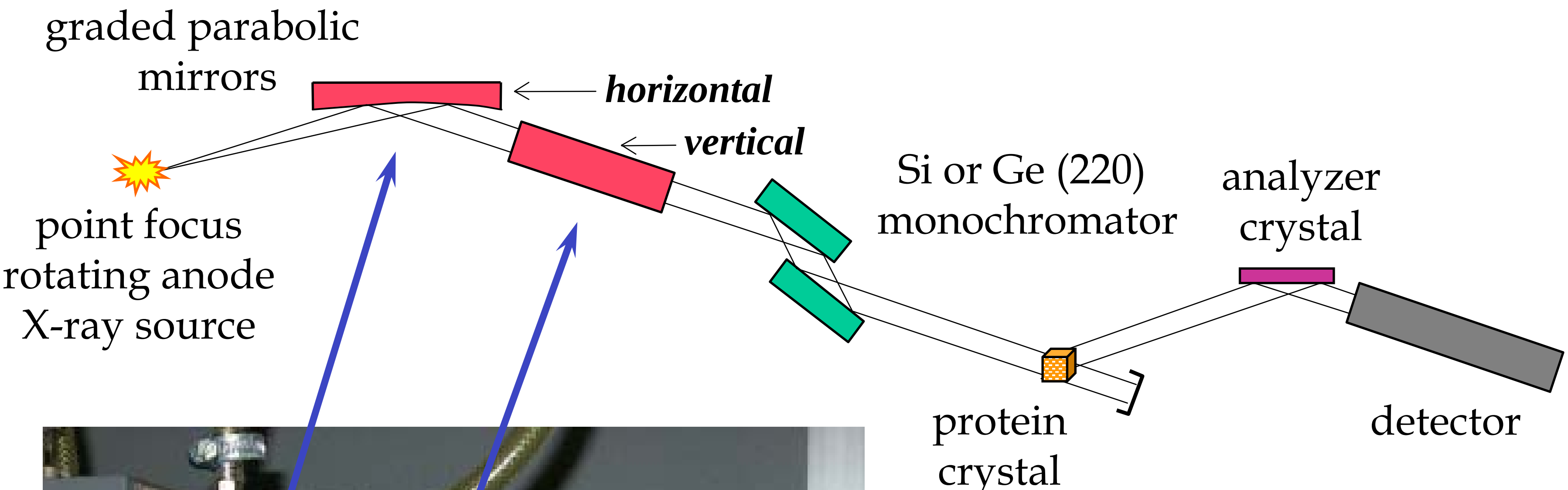


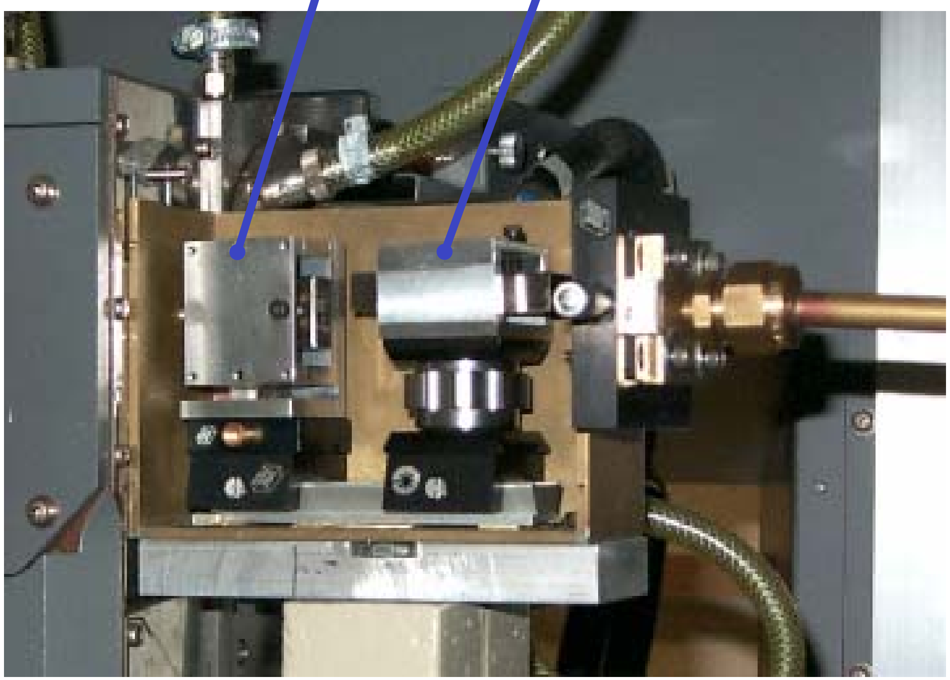
looks good, until you remember:

$$I(hkl) \propto \frac{|F_{hkl}|^2}{V_{\text{unit cell}}^2} e^{-2M}$$

- Inorganic crystals are relatively easy to study with X-rays: small unit cells, relatively high-Z materials, strong intermolecular bonding
- Organic materials are far more difficult to analyze with X-rays: large organic molecules crystallize in large unit cells (often hundreds of atoms in a basis), C, H, O, N have fewer electrons than Fe, Ni, Si, Cu, weak intermolecular bonding and solvent environment lead to large Debye-Waller factor
- High angular resolution measurements on protein crystals with extensive beam conditioning in a laboratory environment -- we need some help!

Triple axis X-ray diffraction system for protein crystals





- HEWL crystals were pre-oriented with a Syntex $P2_1$ diffractometer (sealed monochromated CuKα X-ray source) -- also used for irradiation experiments
- Sample mounted on a Huber 424/511 four-circle diffractometer (min. step size 4.5″)

High resolution triple axis X-ray diffraction from HEWL

- An early resolution triple axis reciprocal space map (HEWL-A) showed several features:

(1) a faint distribution of intensity in the transverse direction (horizontal streak in the Figure)

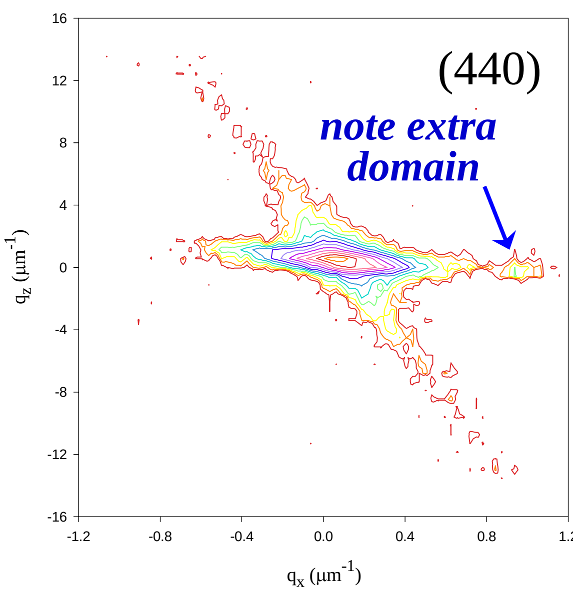
(2) a inclined, relatively intense distribution extending through the reciprocal lattice point from the upper left and towards the lower right

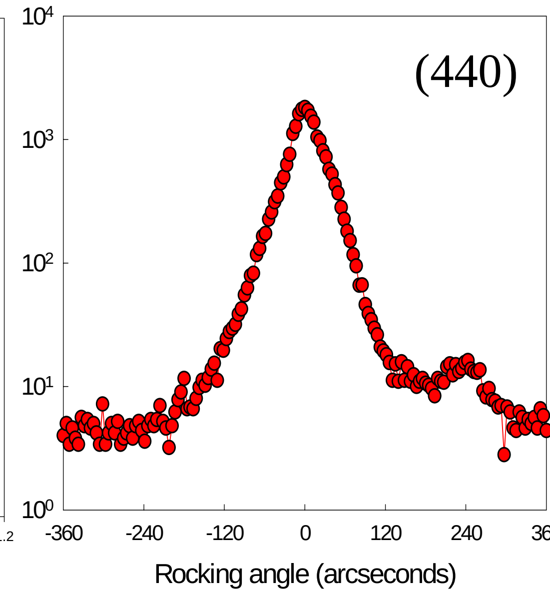
(3) a weaker intensity distribution also extending from upper left to lower right in the Figure, but at a steeper angle

H.M. Volz and R.J. Matyi, *Phil. Trans. Royal Soc. (London)*, **357**, 2789 (1999)

- (440) reflection from HEWL-D: isotropic intensity distribution in the transverse direction w/ extra domain
- Rocking curve FWHM ~45”(right); reciprocal space map (left) reveals much more information

H.M. Volz and R.J. Matyi, *Acta Cryst. D*, **34**, A64 (2001)

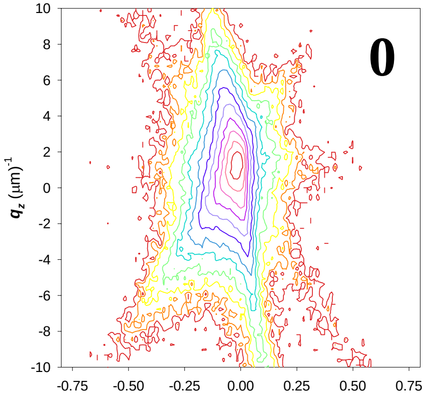




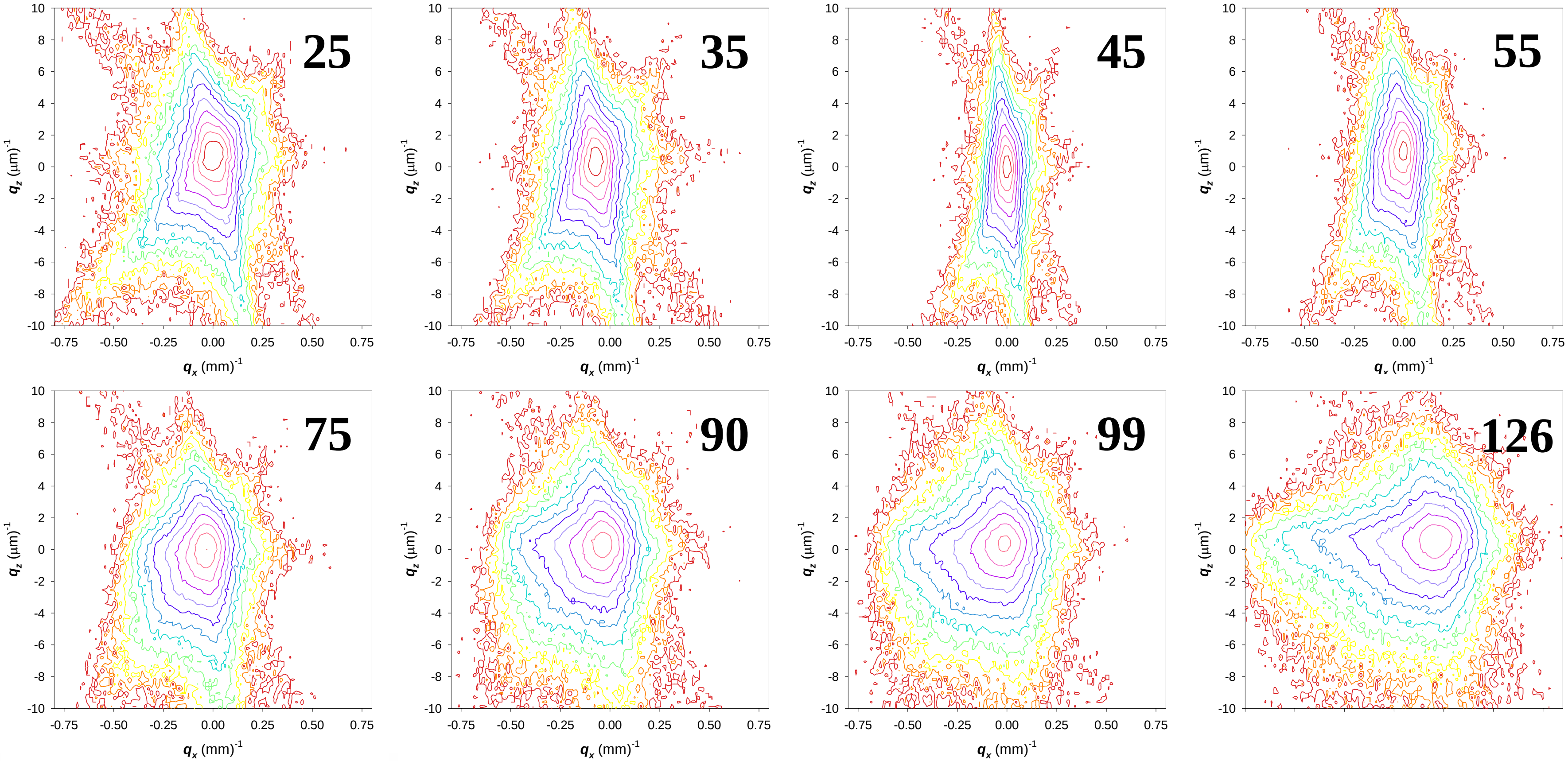
Effect of radiation damage on the structural perfection of HEWL crystals

- Radiation damage is a well-known degrading effect (via the formation of free radicals) in protein crystallography studies
- ***Question***: how are the ***chemical*** changes manifested in terms of ***structural*** changes (i.e. defects) in the protein crystals?
- We have experimentally monitored HEWL crystal degradation
  - a single crystal (HEWL-G) was irradiated for 5 to 10 hour increments at 45 kV/30 mA in our Syntex diffractometer (X-ray flux ~8.4×10^6 counts mm^{-2} s^{-1} at 20 kV/10 mA)
  - samples were transferred to our high resolution diffractometer
  - high resolution (440) reciprocal space maps were recorded
  - peak and integrated intensities, full width at half maximum (FWHM) and full width at 1/100 maximum ($FWC^{-1}M$) were measured as a function of Syntex irradiation time

Triple axis diffraction analysis of radiation damage in HEWL-G

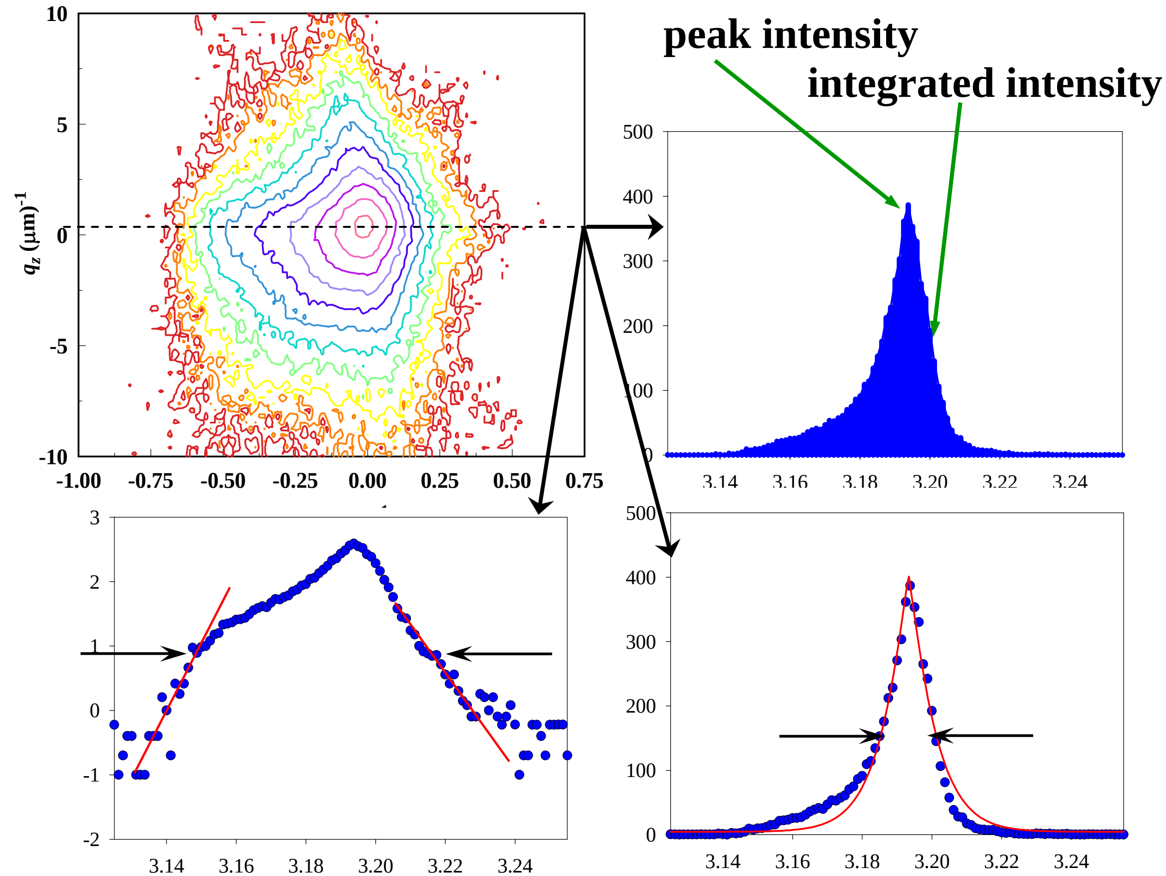


(440) reciprocal space maps recorded as a function of irradiation time (in hours) from HEWL-G showed a systematic change in the quantitative details of the distribution of the diffuse scattered intensity



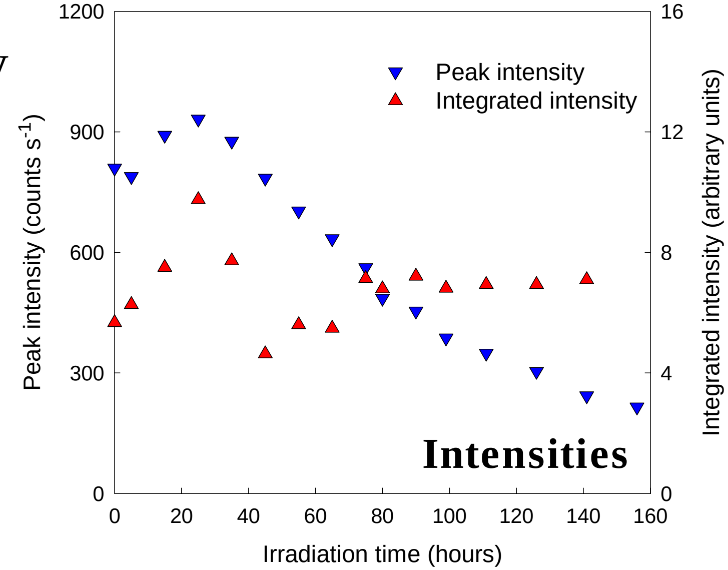
Triple axis X-ray diffraction analysis of radiation damage in HEWL-G

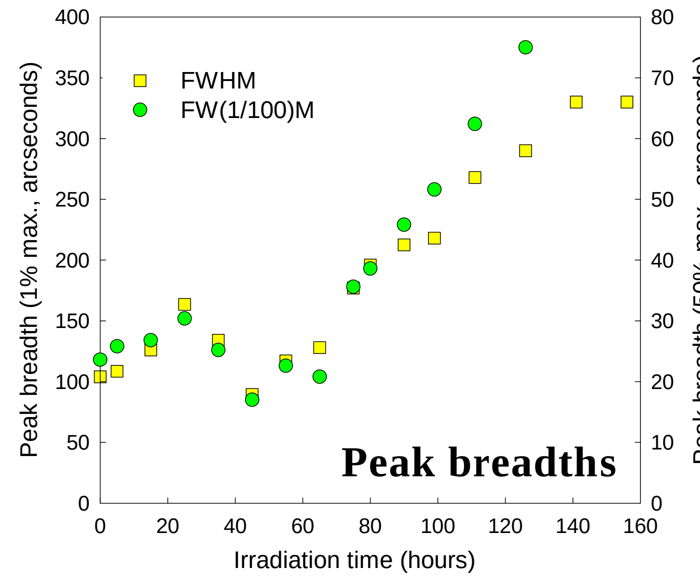
(440) reciprocal space map after 100 hours of X-ray irradiation (Cu Ka, 45kV/30mA)



full width at 1% maximum (FWC^{-1}M)

full width at 50% maximum (FWHM)



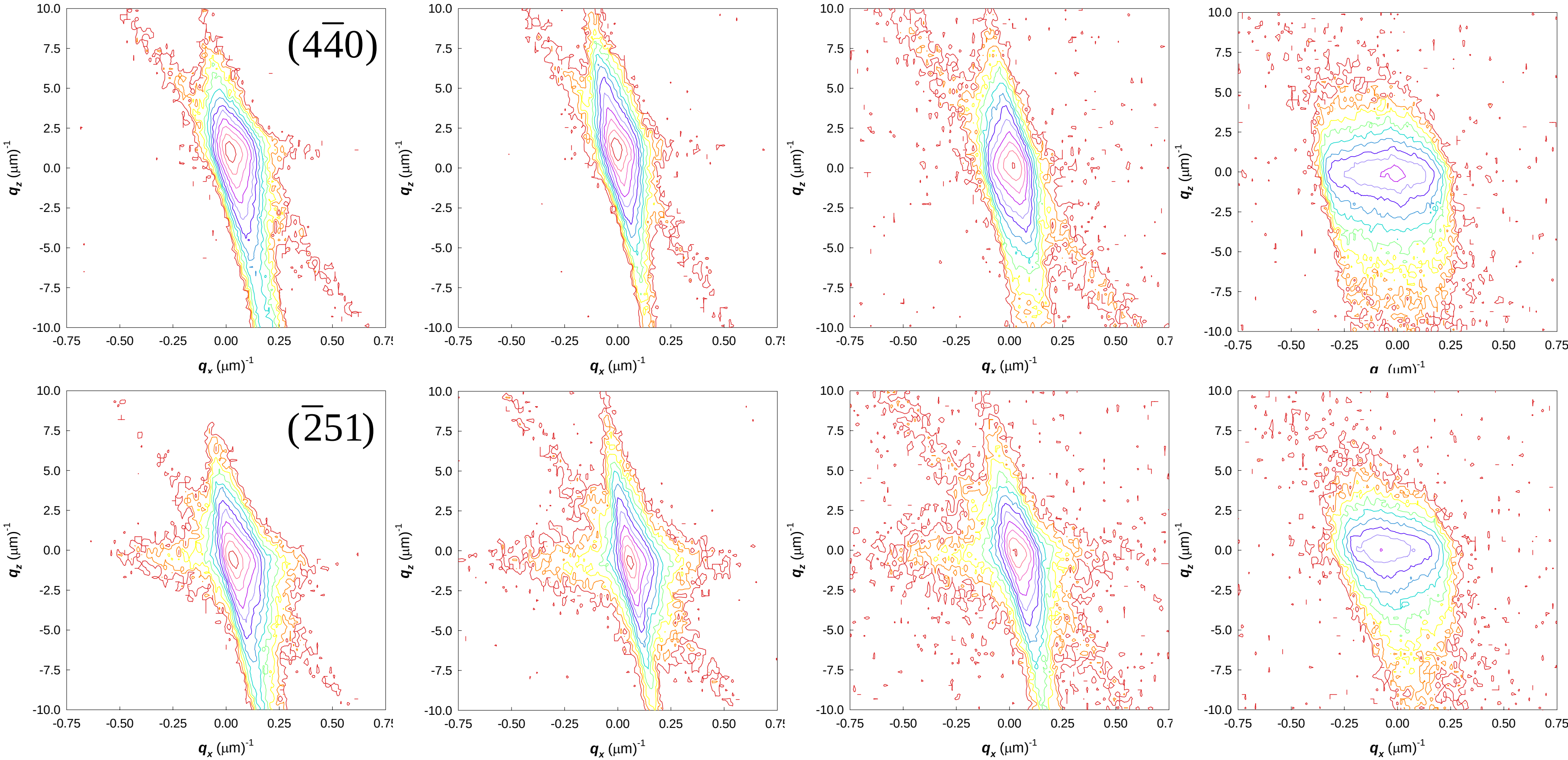


NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

NIST Physics Laboratory

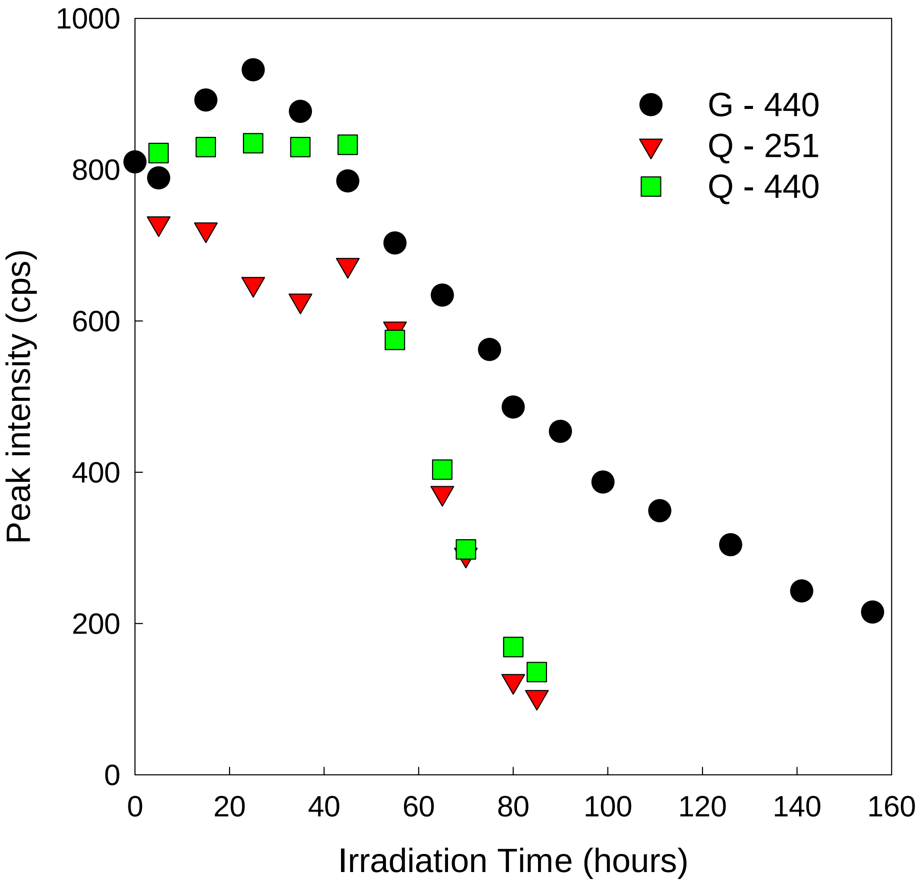
Triple axis diffraction analysis of radiation damage in HEWL-Q

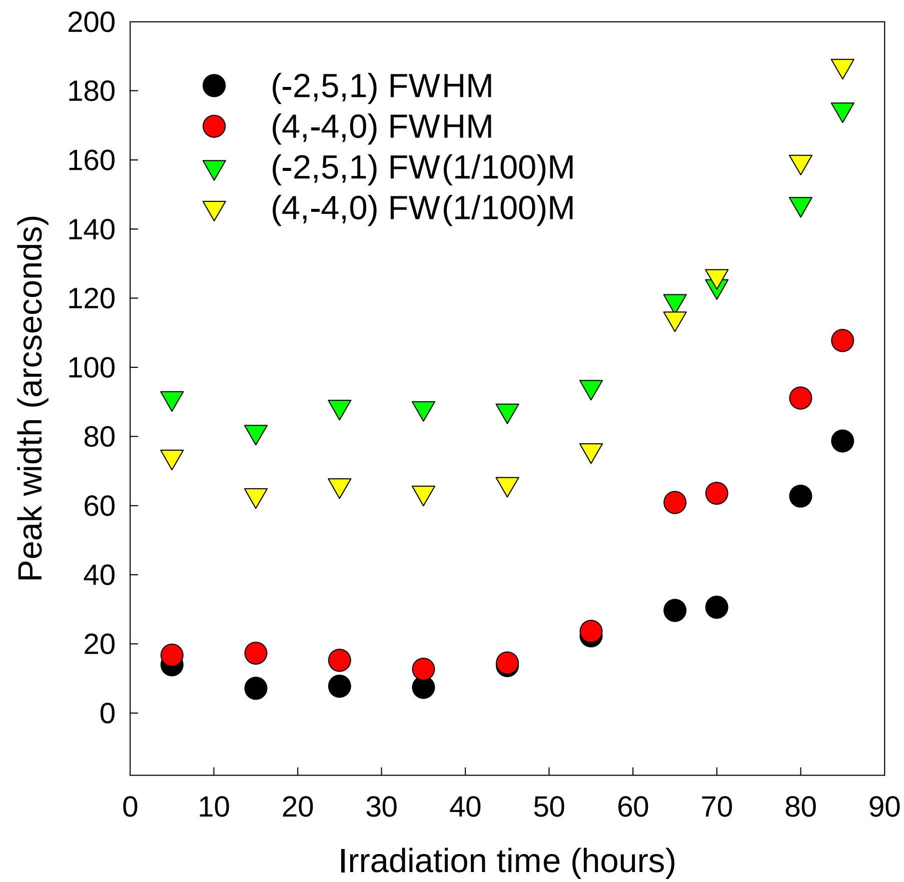
- The radiation damage experiment was repeated on HEWL-Q using both the $(4\bar{4}0)$ and the $(\bar{2}51)$ reflections



0 hours | 15 hours | 55 hours | 85 hours

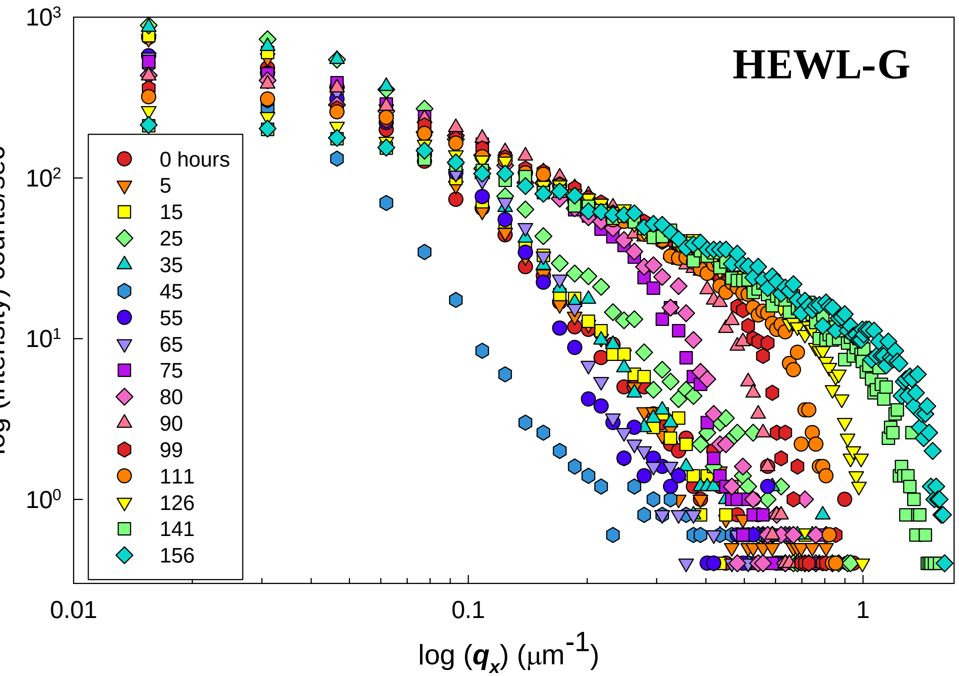
Peak intensities and breadths for HEWL-Q

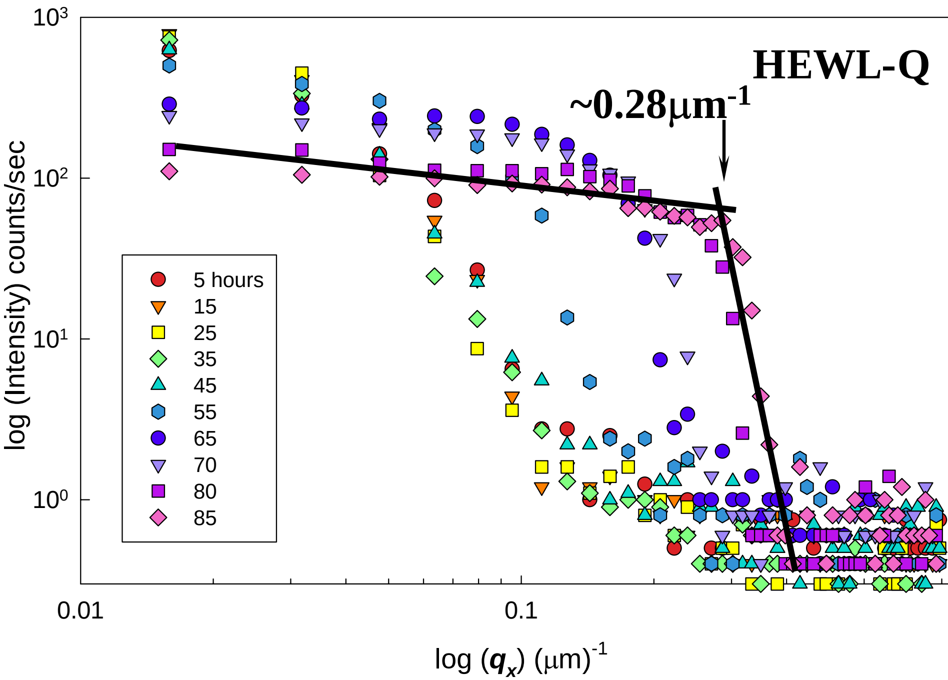




- Similar changes in the peak intensities and breadths were observed in crystal HEWL-Q as radiation damage progressed (some differences, but the overall results were reproducible)

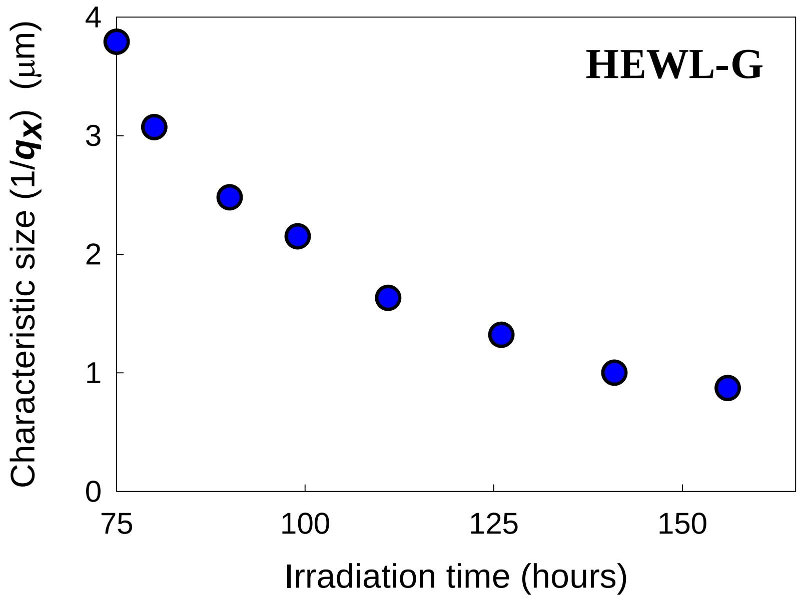
log I–log q_x transverse scans from HEWL-G and HEWL-Q

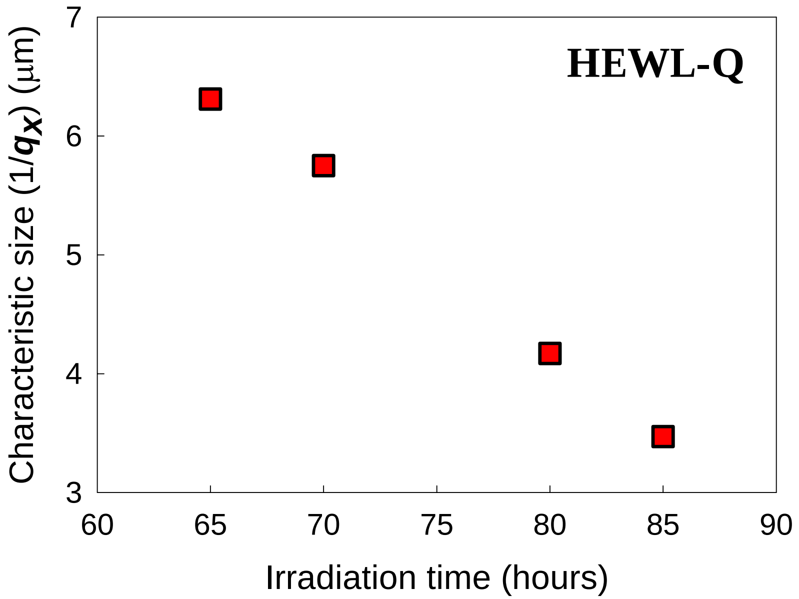




- Plots of log I versus log q_x perpendicular to a reciprocal lattice direction show roughly linear regions and definite breakpoints in slope at specific values of q (example: HEWL-Q at 85 hours)
- In semiconductor crystals, the transition from Huang (q^{-2}) to Stokes-Wilson (q^{-4}) scattering gives a characteristic defect size
- Is there a similar behavior in protein crystals?

Characteristic size in HEWL-G and HEWL-Q during irradiation





- Both HEWL-G and HEWL–Q show a characteristic size of a few µm from the log I - log q_x plots
- The decrease in characteristic size with increasing X-ray irradiation time may be indicative of structural modifications that accompany chemical changes
- Modeling of the observed behavior is in progress

Kinematic vs. dynamic X-ray diffraction conditions

- A crystal can diffract dynamically if it is structurally perfect and is large with respect to the extinction distance ξ:

$$\xi = \frac{V_C}{r_o C \left| F_h \right| \lambda}$$

with a structure factor of ~594 electrons and λ = 1.54Å the extinction distance is about 0.9 mm!

- In the dynamic limit the Laue-case rocking curve FWHM is

$$\Delta\theta = \frac{2\left|C\right|\left|\chi_h\right|}{\sin 2\theta} = \frac{2\lambda}{\pi\,\xi \sin 2\theta}$$

→ ***the predicted dynamical (440) FWHM for HEWL is 0.2"***

- A "kinematic" rocking curve FWHM can be estimated from:

$$J = \sum_{0}^{N_1-1} \exp\left(2\pi i N_1 q_1\right) \sum_{0}^{N_2-1} \exp\left(2\pi i N_2 q_2\right) \sum_{0}^{N_3-1} \exp\left(2\pi i N_3 q_3\right)$$

$$= \frac{\sin^2\left(\pi N_1 q_1\right)}{\sin^2\left(\pi q_1\right)} \frac{\sin^2\left(\pi N_2 q_2\right)}{\sin^2\left(\pi q_2\right)} \frac{\sin^2\left(\pi N_3 q_3\right)}{\sin^2\left(\pi q_3\right)}$$

→ ***~ 1.2" for a 0.5 mm HEWL crystal***

Defects in a dynamical formalism

- Kato has shown that defects can be included into a dynamical approach by including two parameters:
  - a ***long-range order parameter***: $E = e^{-K}$

    where K is a non-negative constant (static Debye-Waller factor)
  - a ***short-range order parameter:*** $\tau \equiv \int g(x)\, e^{-\pi i x} \mathrm{d}x$

    where g is the *correlation function* for a given defect
- These terms alter the Takagi-Taupin equations:

$$\frac{dA_O}{dz} = \kappa_{OO} A_O + \kappa_{OH} A_H$$
$$\frac{dA_H}{dz} = (\kappa_{HH} + \varphi) A_H + \kappa_{HO} A_O$$

no defects

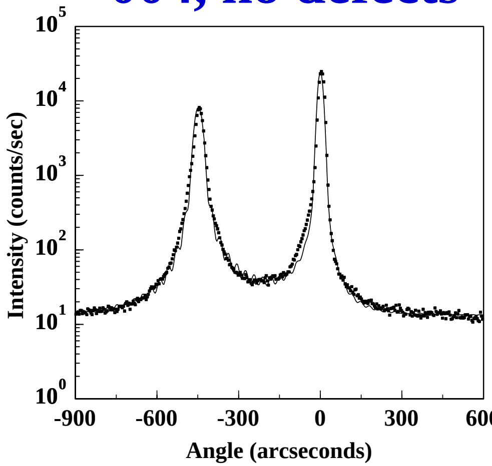
⇔

$$\frac{dA_O^c}{dz} = \left(\kappa_{OO} - \vartheta\left(1 - E^2\right)\tau^*\right) A_O^c + \kappa_{OH} E\, A_H^c$$
$$\frac{dA_H^c}{dz} = \left(\kappa_{HH} + \varphi + \vartheta\left(1 - E^2\right)\tau\right) A_H^c + \kappa_{HO} E\, A_O^c$$

with defects

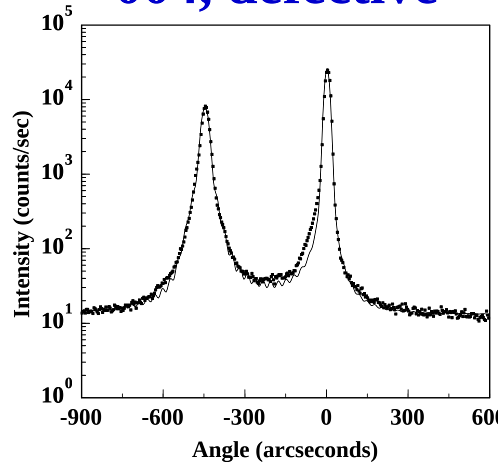
Single layer fits to 39.1%Ge-GaAs/GaAs (t_{true}=940nm)

004, no defects



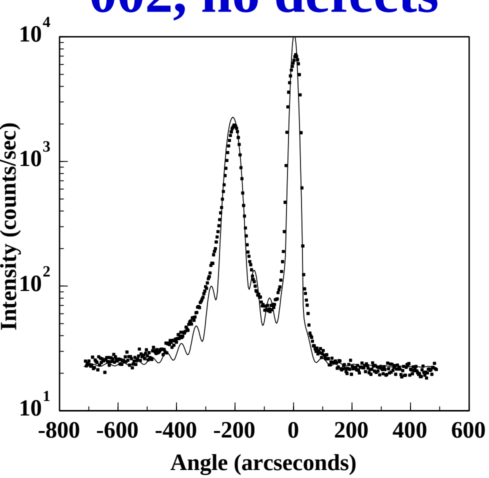
$\%\hat{\sigma}_{res}$= +110, t = 602nm

004, defective



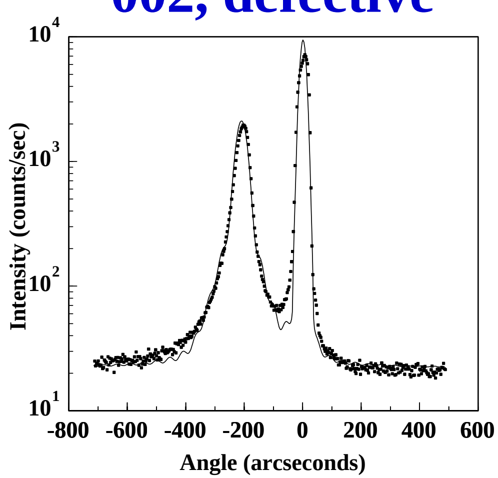
$\%\hat{\sigma}_{res}$= +15, t = 667nm, E = 0.71

002, no defects



$\%\hat{\sigma}_{res}$= +265, t = 330nm

002, defective



$\%\hat{\sigma}_{res}$= +156, t = 367nm, E = 0.39

(T.W. Staley, Ph.D. thesis, Univ. of Wisconsin (1997))

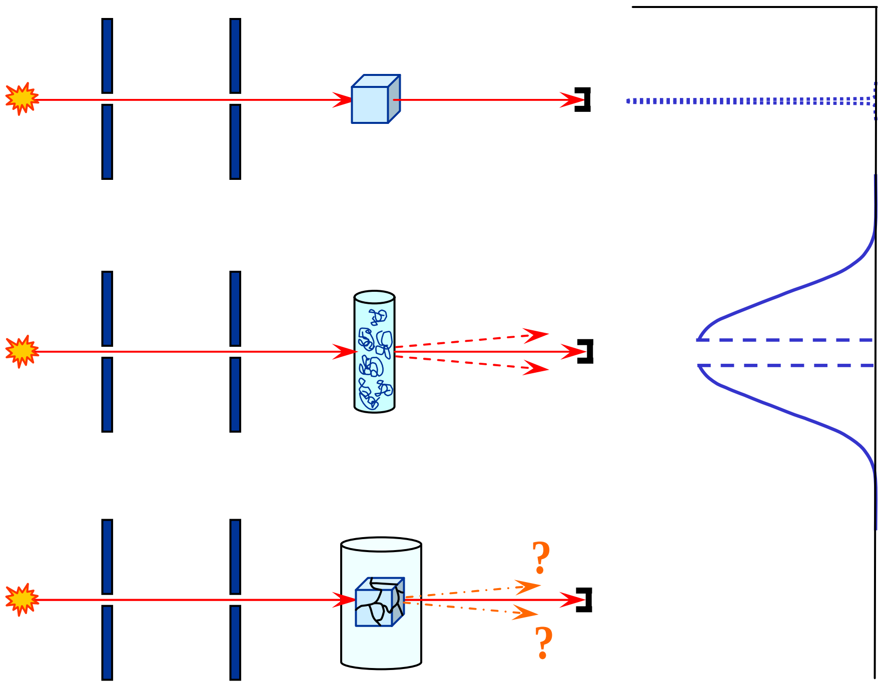
- The inclusion of defects into into a dynamical simulation significantly improves the statistical quality of the fit
- Thickness prediction for the 002 peak is about ½ that of the 004 peak due to defects
- The decrease in long-range order parameter E from the 004 to 002 reflections is consistent with the onset of the diamond ⇔ zincblende transition
- This approach is currently being adapted for use with protein crystals

High resolution reciprocal space mapping of protein crystals – a review

- ***The good news***:
  - ☺ High resolution reciprocal mapping of the diffuse X-ray intensity near an *hkl* reflection can be successfully accomplished in a laboratory setting
  - ☺ Analysis of the diffuse scatter may give new insights into the physical defect structure of protein crystals
- ***The bad news***:
  - ☹ The diffuse intensity is relatively weak, so relatively perfect crystals are desirable
  - ☹ Most protein crystals are "relatively lacking" in terms of structural perfection
  - ☹ The applicability of high resolution reciprocal mapping to highly defective crystals is problematic

➢ ***Small-angle X-ray scattering*** may provide an alternate or complimentary approach to the structural analysis of defective protein crystals

A proposed SAXS experiment



Ideal single crystal - the Fourier transform of the shape function should give a sharp, non-observable intensity "spike" at 2θ=0

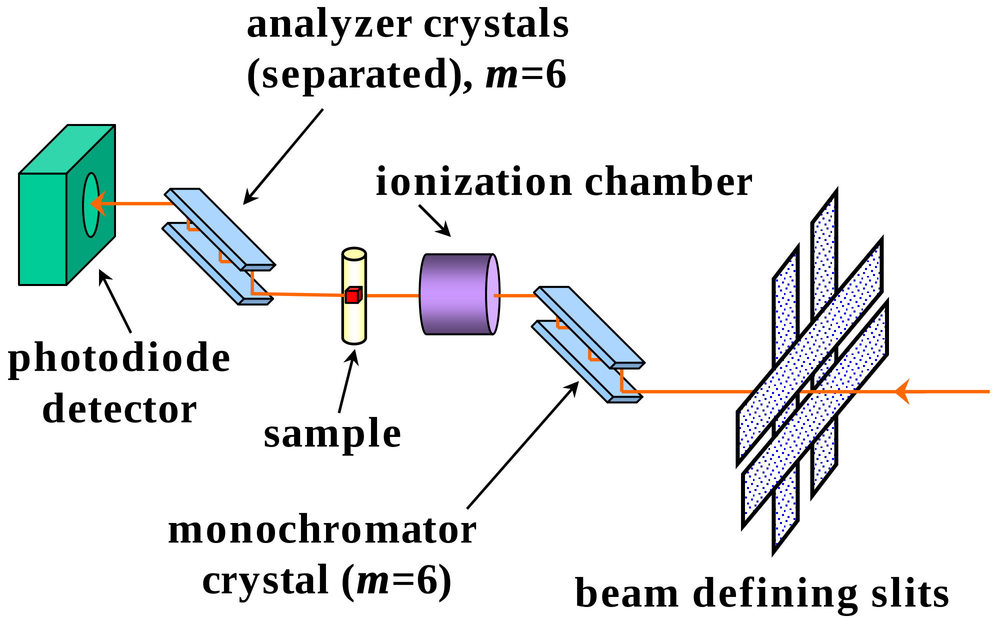
Protein molecules in water solution - the electron density difference generates SAXS intensity that is easily observed

Highly defective protein crystal in a water-saturated environment -- is there sufficient contrast and intensity to observe SAXS?

- After correction for extrinsic effects (e.g. capillary and air scatter) a structurally defective protein crystal <u>should</u> generate <u>greater</u> SAXS intensity than a highly perfect crystal
- The magnitude of the SAXS scattering should increase as the crystalline quality degrades (i.e. radiation damage)

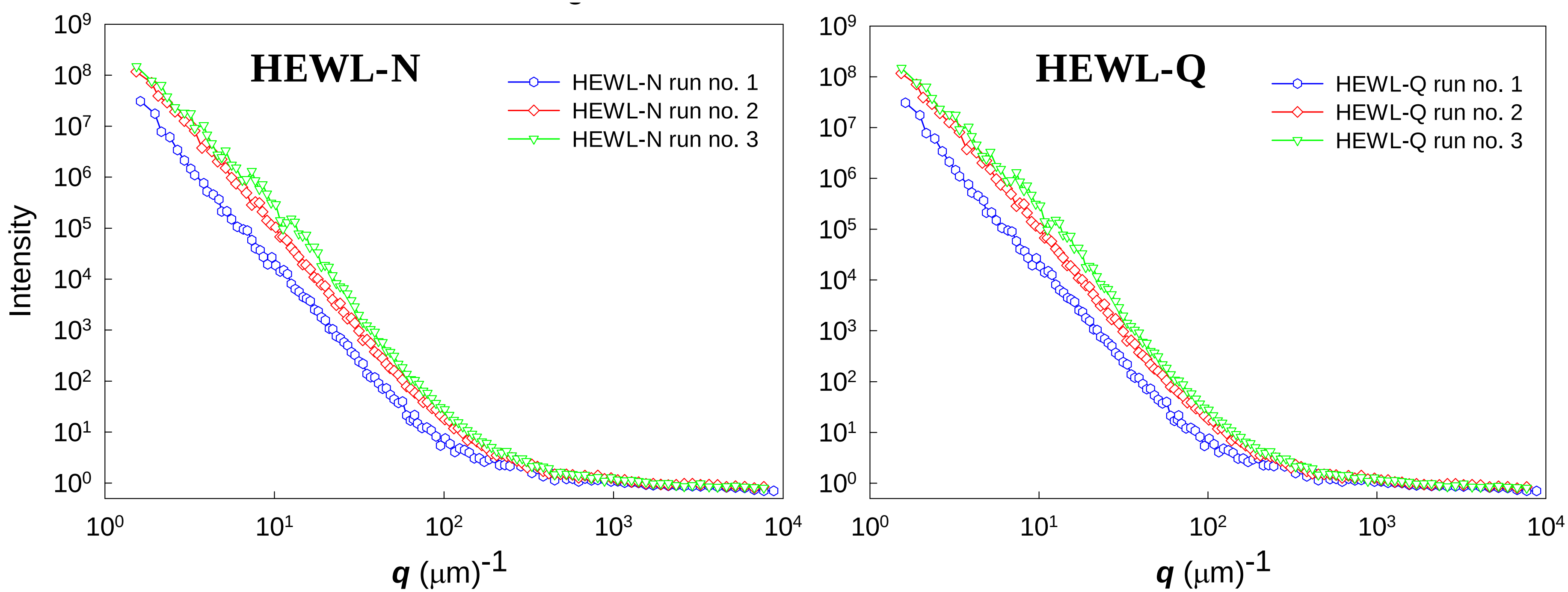
USAXS at the Advanced Photon Source

- USAXS experiments were performed at the APS on beamline 33-ID
- Used with a double crystal monochromator (photon selection) and two mirrors (harmonic rejection)
- Bonse-Hart design with $q_{min} = (4\pi/\lambda) \sin\theta = 0.00015 \text{Å}^{-1}$ or $1.5\ \mu m^{-1}$



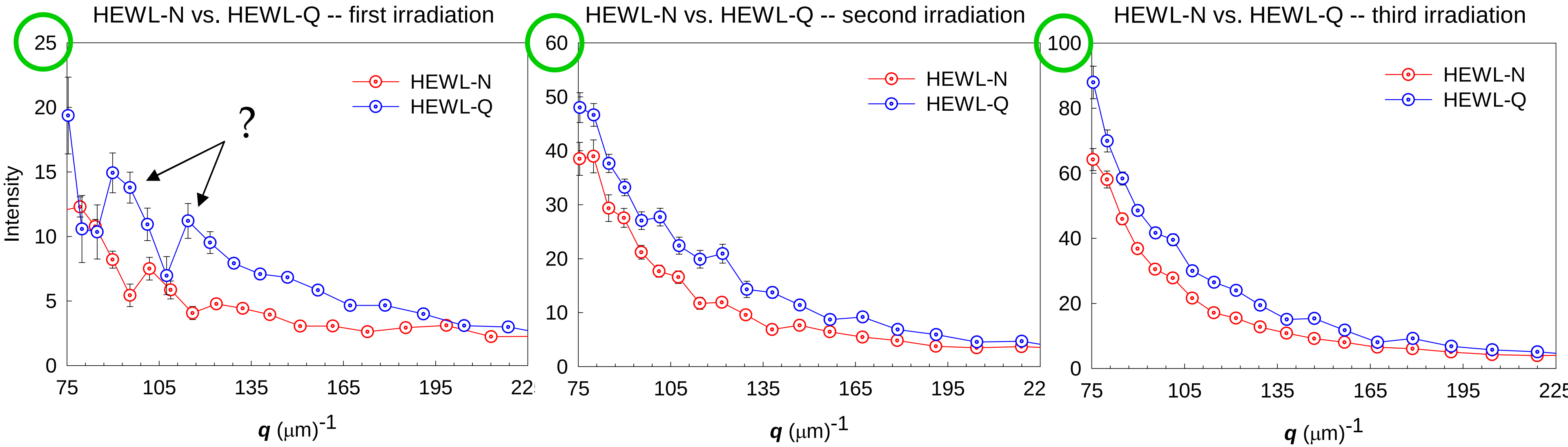
- X-ray beam size approx. 0.5 mm × 0.5 mm (size of crystal); beam flux $>1\times10^{12}\ s^{-1}$ in irradiated area at 10 keV
- Photodiode detector + electronics gives 10-decade linear range
- Data corrected for parasitic air and capillary scattering

SAXS analysis of HEWL crystals – effect of synchrotron irradiation on crystals N and Q



- HEWL crystals were subjected to cumulative synchrotron radiation: HEWL-N (initially unirradiated) and HEWL-Q (after radiation damage experiment)
- A consistent increase of the SAXS with synchrotron beam irradiation was observed!

USAXS analysis of synchrotron radiation effects – a side-by-side comparison of HEWL-N and -Q

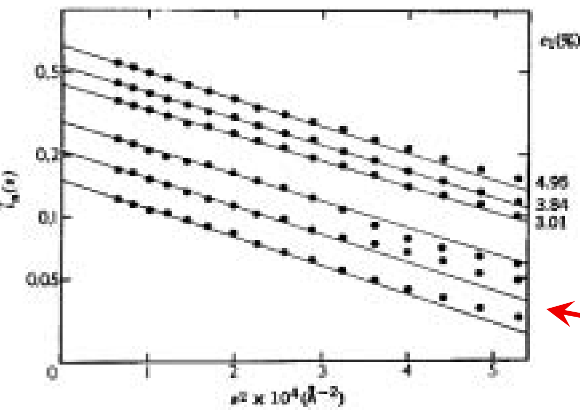


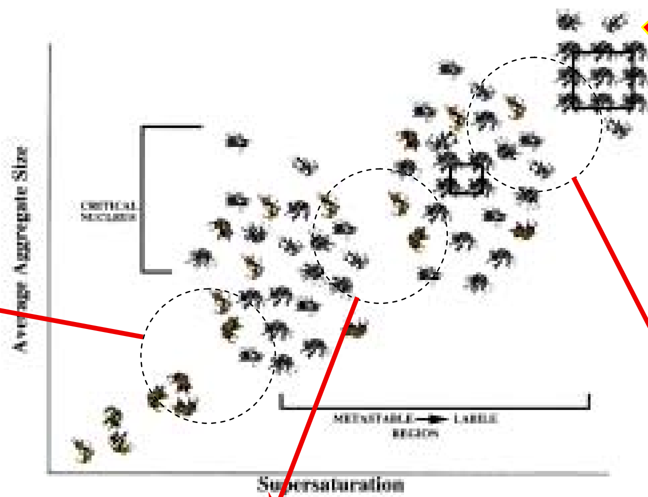
- The initially unirradiated HEWL-N consistently showed less scatter than the initially radiation-damaged HEWL-Q
- Observed scattering behavior is consistent with the thesis that ***increased structural disorder*** in protein crystals generates ***enhanced SAXS intensity***
- Preliminary results are encouraging, but more work is needed!

A proposal for using diffuse X-ray scattering as a diagnostic for the protein crystallization process

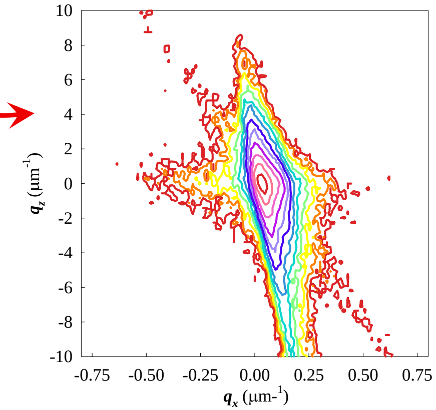
figure from McPherson, *Crystallization of Biological Macromolecules* (Cold Spring Harbor Press, 1999)

SAXS of lysozyme in solution
(Luzzati et al., *J. Molec. Biol.*, **3**, 367 (1961))

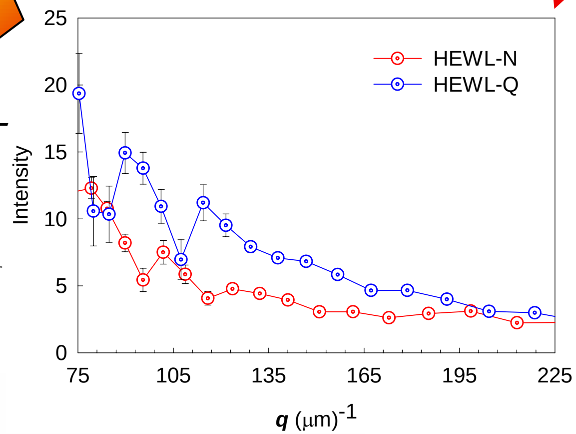


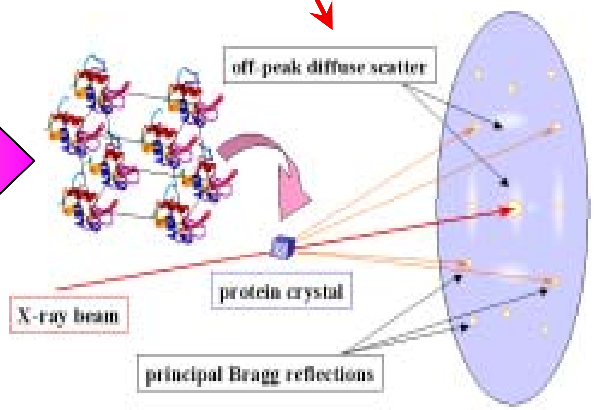


HRTXD reciprocal space map of the resultant crystal



USAXS of radiation-damaged lysozyme crystals or "pre-crystals" in solution





wide-angle diffuse scatter from early stages of protein crystal growth

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

NIST Physics Laboratory

Conclusions

- Methods of reciprocal space mapping developed for semiconductor crystals can be performed on protein crystals in a lab environment
- Complex physical changes accompany radiation damage
- First results of high resolution small-angle analyses confirm anticipated behavior and suggest that SAXS can be applied to highly defective protein crystals
- The time is right to combine a range of diffuse X-ray scattering methods to fully articulate the protein crystal growth process

Thanks to….

✓ H.M. Volz	Materials Science Program, UW – Madison (now at Los Alamos National Laboratory, Los Alamos, NM)
✓ G.G. Long	Materials Science & Engineering Laboratory, NIST (now at the APS/ANL)
✓ H. Holden, J. Thoden	Department of Biochemistry, UW – Madison
✓ A.A. Chernov	NASA Marshall Space Flight Center
✓ R.D. Deslattes	Physics Laboratory, NIST (deceased)