ESRF User meeting 2010 : MX School "Getting the most from the ESRF MX beamlines"
8–11 February 2010, ESRF, Grenoble, France

New frontiers in anomalous phasing : Phasing from unmerged data

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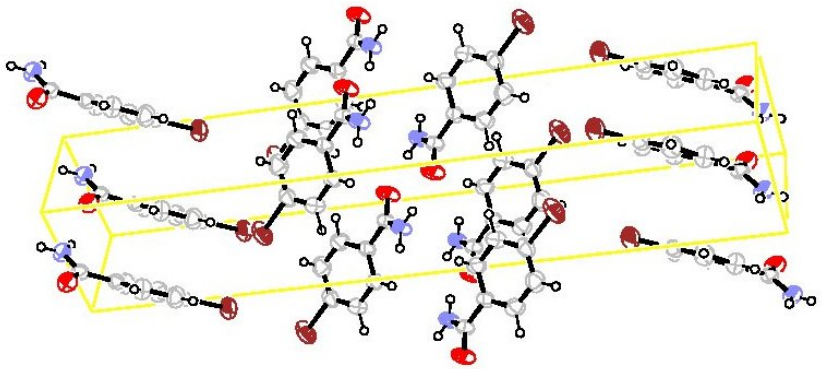
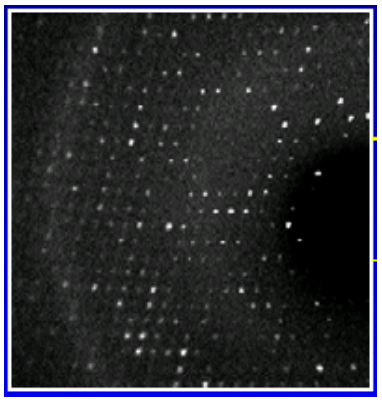
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Direct space and reciprocal space symmetries



Spacegroup



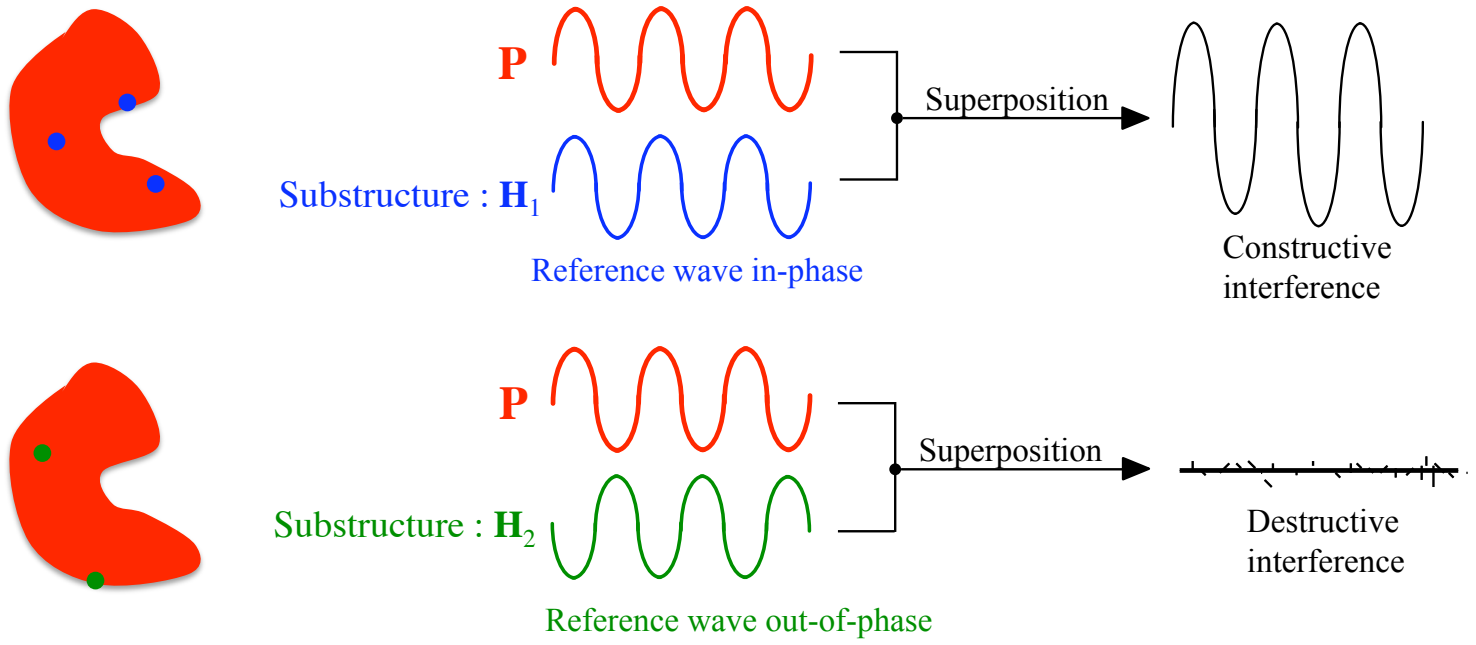
Laue group

- Symmetry-equivalent reflections
- 'Broken symmetries' : cases where symmetry-equivalent reflections are no longer equivalent
  - Radiation-induced processes (or other time-dependent phenomena)
  - Polarisation anisotropy of anomalous scattering

Basics of experimental phasing methods

'No wonder we lose the phase if there is nothing to compare with it ! Let us see what happens if we add a standard to it, a 'coherent background'. (…) The interference of the object wave and of the coherent background or 'reference wave' will then produce interference fringes. There will be maxima wherever the phases of the two waves were identical.' (Gabor, 1972)

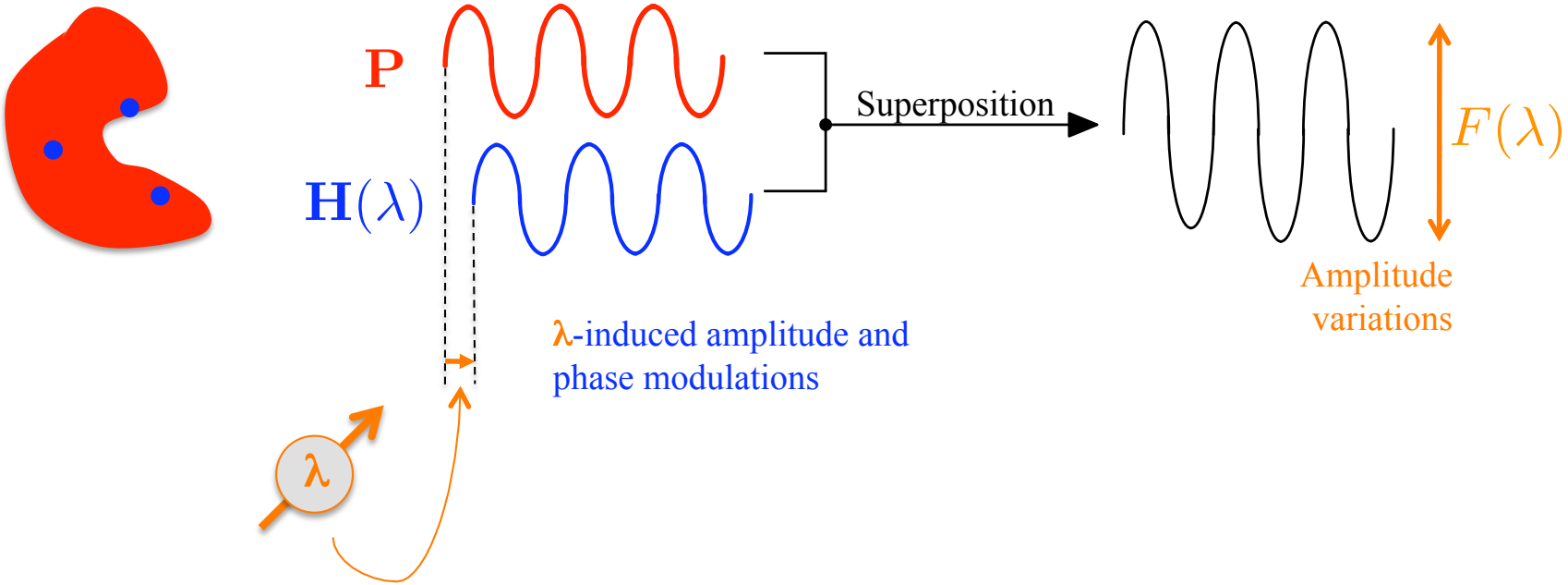
$$\mathbf{F}(\mathbf{h}) = \mathbf{P}(\mathbf{h}) + \mathbf{H}(\mathbf{h})$$



$$F^2(\mathbf{h}) = P^2(\mathbf{h}) + H^2(\mathbf{h}) + \underbrace{2\,P(\mathbf{h})\,H(\mathbf{h})\,\cos\{2\pi\,\mathrm{i}\,[\phi_P(\mathbf{h}) - \phi_H(\mathbf{h})]\}}_{\text{interference}}\ .$$

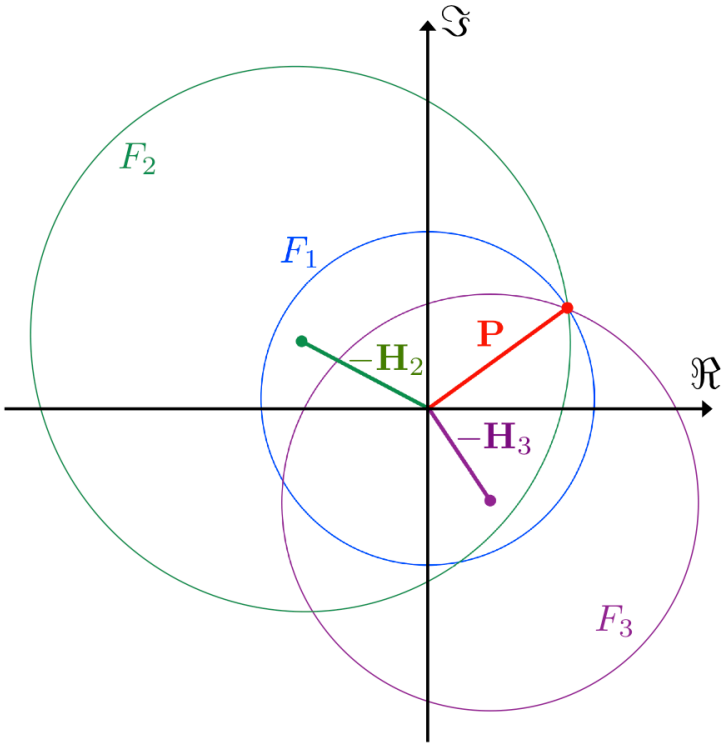
Basics of experimental phasing methods

'No wonder we lose the phase if there is nothing to compare with it ! Let us see what happens if we add a standard to it, a 'coherent background'. (…) The interference of the object wave and of the coherent background or 'reference wave' will then produce interference fringes. There will be maxima wherever the phases of the two waves were identical.' (Gabor, 1972)



Harker construction

- The traditional Harker construction holds for a *unique* (*hkl*) reflection
  - —Individual observations (circles) may come from
    - different heavy atom derivatives
    - different wavelengths



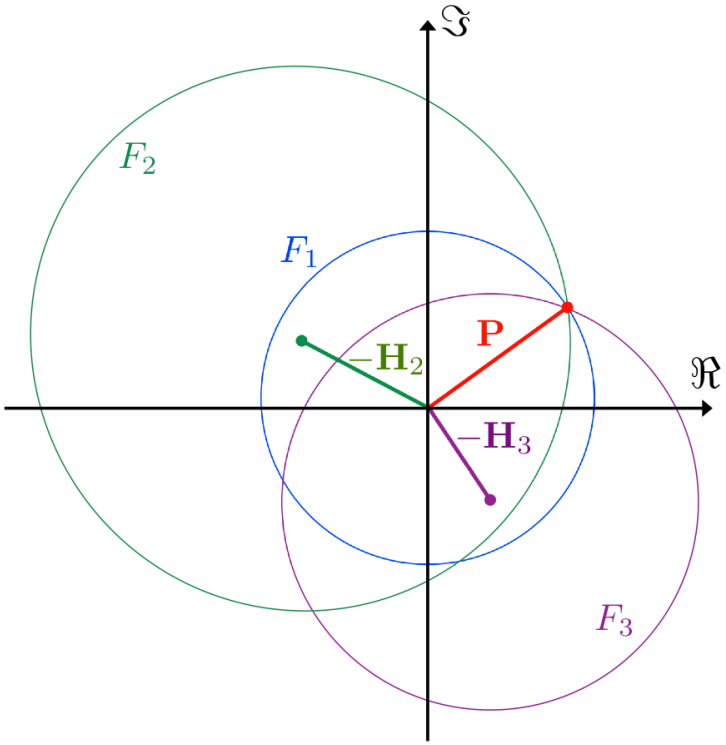
$$
\begin{aligned}
F_1 &= |\mathbf{P} + \mathbf{H}_1| \\
F_2 &= |\mathbf{P} + \mathbf{H}_2| \\
F_3 &= |\mathbf{P} + \mathbf{H}_3| \\
&\vdots \quad\quad \vdots \\
F_N &= |\mathbf{P} + \mathbf{H}_N|
\end{aligned}
$$

Common to all crystals, but unknown

Different in each crystal, but known

Extended Harker construction

- The Harker construction can be extended
  - – Individual observations (circles) may come from
    - symmetry-related observations of the same reflection
    - repeated measurements of the same reflection
  - – The differences must be genuinely structure-related (*i.e.* not noise or purely geometric factors)
  - – A model for the substructures (*i.e.* $\mathbf{H}_i$) must be available.

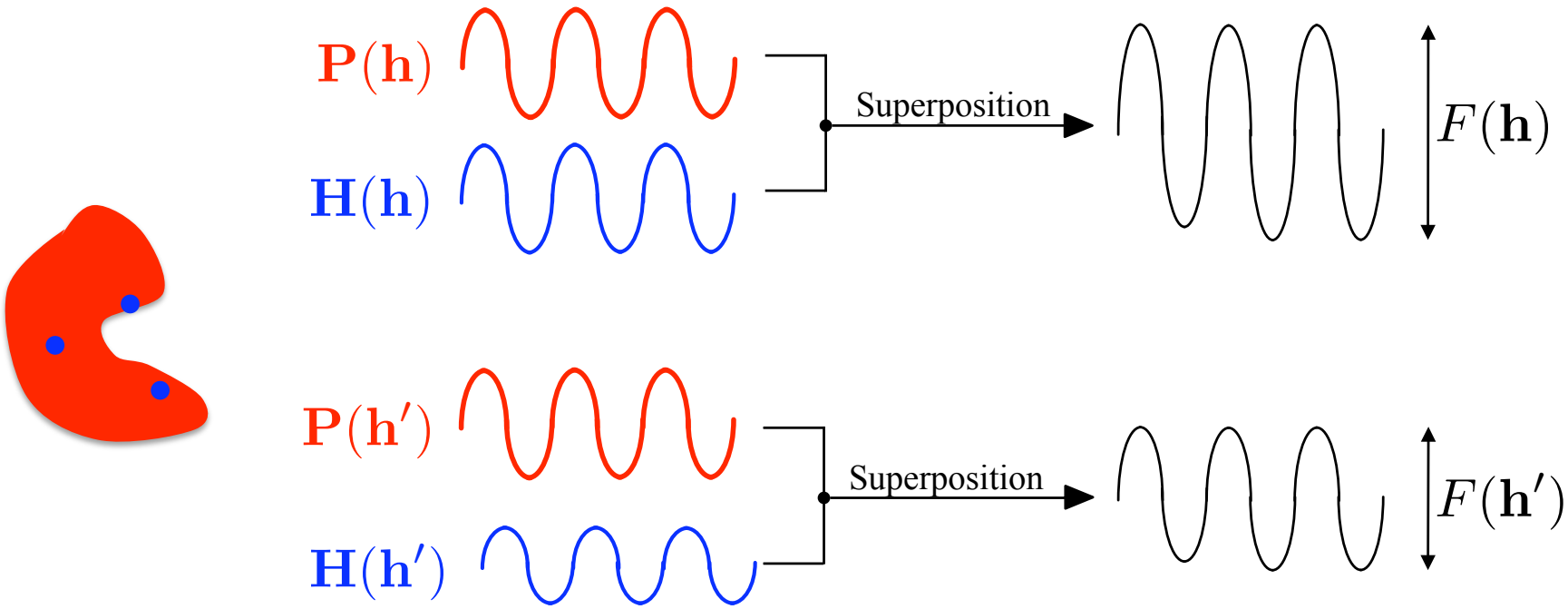


$$
\begin{aligned}
F_1 &= |\mathbf{P} + \mathbf{H}_1| \\
F_2 &= |\mathbf{P} + \mathbf{H}_2| \\
F_3 &= |\mathbf{P} + \mathbf{H}_3| \\
&\vdots \quad\quad \vdots \\
F_N &= |\mathbf{P} + \mathbf{H}_N|
\end{aligned}
$$

Common to all crystals, but unknown

Different in each crystal, but known

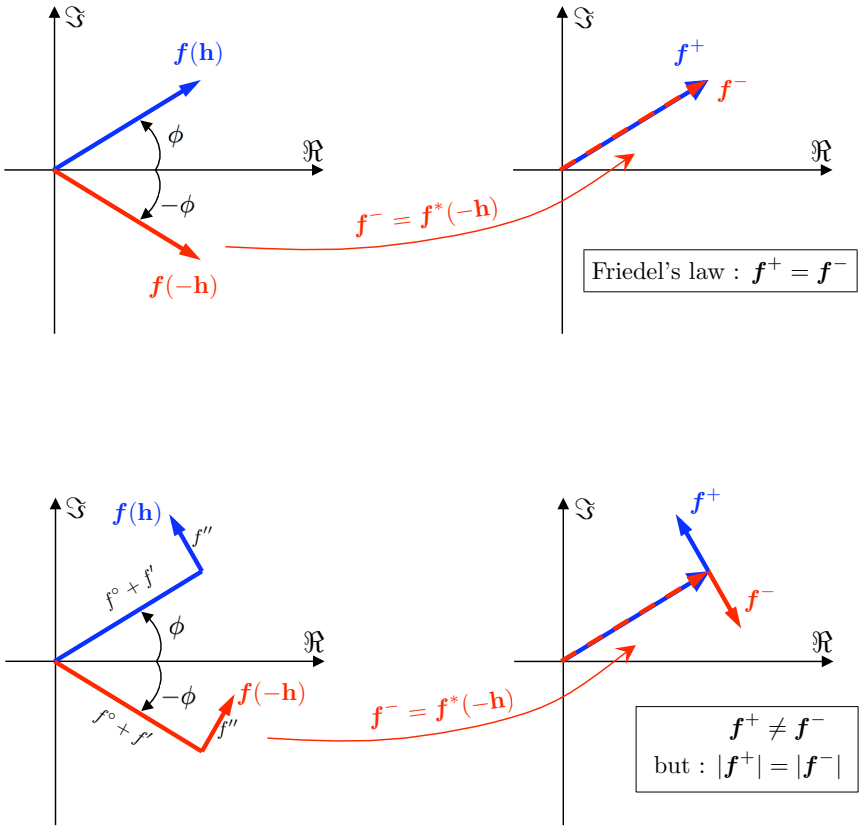
Generating phase information through symmetry-related reflections

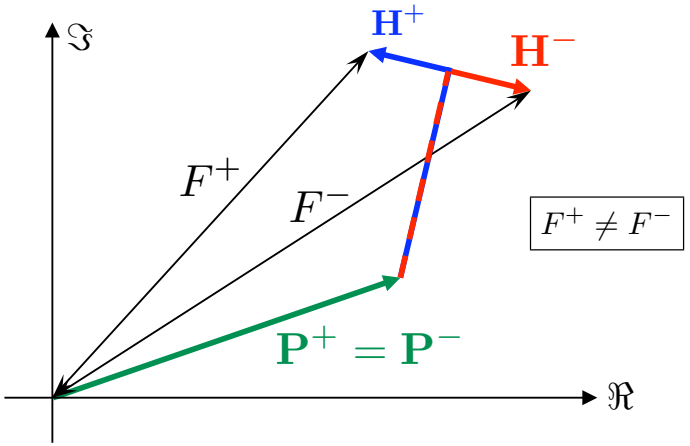


Generating phase information through symmetry-related reflections

- Anomalous scattering
  - Breaks the equivalence between F(**h**) and F(–**h**)
- Polarisation anisotropy of anomalous scattering
  - Can break the equivalence between all symmetry-related reflections
- Site-specific radiation damage
  - Breaks the equivalence between F(**h**) and F(**h'**) if **h** and **h**' (symmetry-equivalent) have been recorded at different times (X-ray doses)

Anomalous scattering : breaking the Friedel-equivalence





Site-specific Radiation Damage in Macromolecular Crystallography

- There is well-documented evidence for site-specific primary radiation damage whose onset is much faster than the overall damage to the crystal [Burmeister (2000) Ravelli *et al*. (2000), Weik *et al.* (2000), Ennifar *et al.* (2002), Weik *et al.* (2002)].

research papers

Acta Crystallographica Section D
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X-ray-induced debromination of nucleic acids at the Br *K* absorption edge and implications for MAD phasing

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Received 14 February 2002
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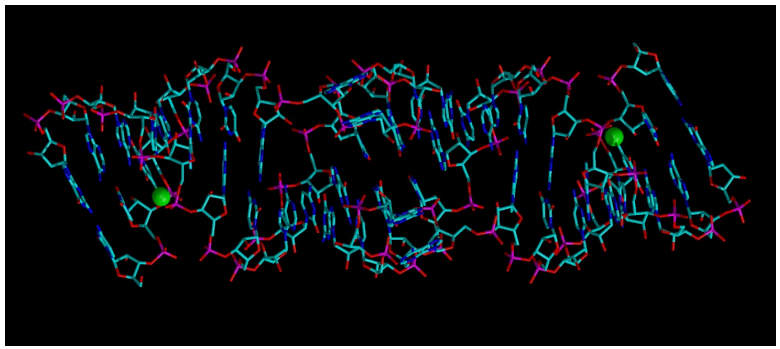
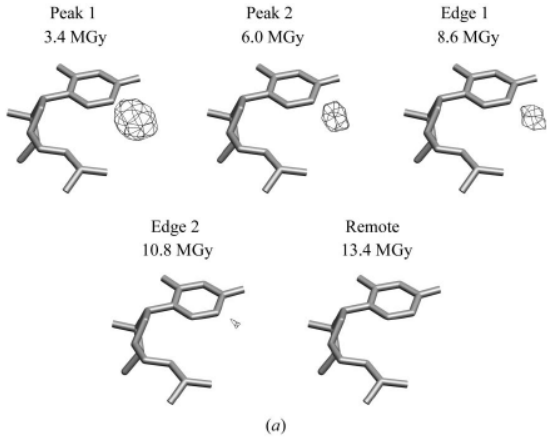
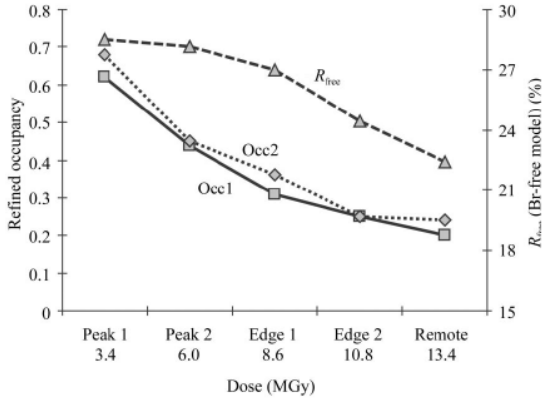
Multi-wavelength anomalous dispersion (MAD) using brominated derivatives is considered a common and convenient technique for solving chemically synthesized nucleic acid structures. Here, it is shown that a relatively moderate X-ray dose (of the order of 5×10^{15} photons mm^{-2}) can induce sufficient debromination to prevent structure determination. The decrease in bromine occupancy with radiation dose can be accounted for by a simple exponential, with an estimated rate constant at the absorption-peak wavelength, 7.4 (0.8) MGy, that is not significantly different from its value at the absorption-edge wavelength, 9.2 (2.6) MGy (the given e.s.d.s





(*a*)



(*b*)

Site-specific radiation damage
=
Phase information

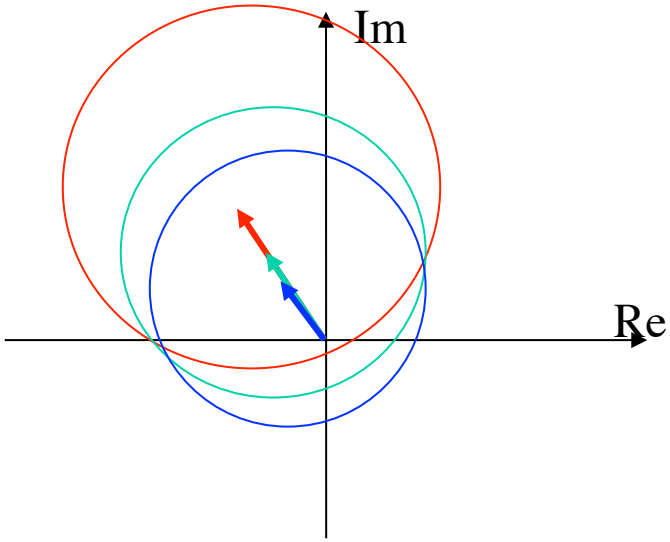
$$\mathbf{F}_h(d) = \mathbf{P}_h + \underbrace{\mathbf{H}_h \cdot \exp(-\beta \cdot d)}$$

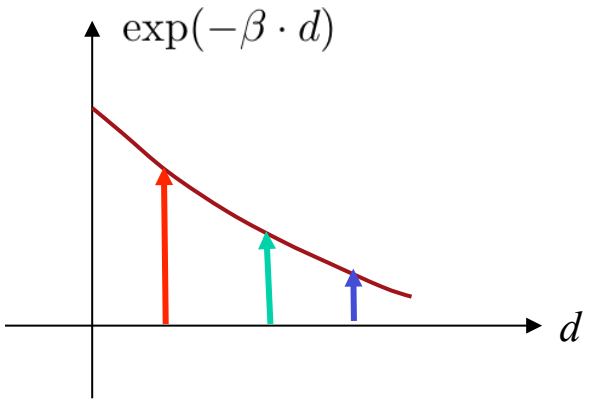
Stable part

Radiation-sensitive part

$$F_{h,i}^2 = P_h^2 + H_h^2 \exp(-2\beta \cdot d_i) + \underbrace{2P_h H_h \exp(-\beta \cdot d_i) \cdot \cos(\Delta\varphi_h)}$$

Interference term
--> **phase information**





How to deal with site-specific radiation damage ?

- Paradigm shift:
  - Keep the data unmerged !
  - Symmetry 'equivalent' reflections recorded at different X-ray doses correspond to different states of debromination, i.e. to different structures.
  - Data merging is deferred to the phasing stage and is effectively carried out on the complex plane, *i.e*. through the Harker construction: from all the symmetry-related intensities, a single is estimated, but as a complex value !
  - Model the dose-dependent evolution of substructure (*i.e*. the symmetry-breaking effects) :

$$Occ(d) = \mathbf{Occ}^{\circ} \cdot \exp(-\boldsymbol{\beta} \cdot d)$$

X-ray dose — Refineable parameters

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Modelling and refining site-specific radiation damage in SAD/MAD phasing

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Case studies and 'real cases'

research papers

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Phasing in the presence of severe site-specific radiation damage through dose-dependent modelling of heavy atoms

M. Schiltz,[a,b]* P. Dumas,[c] E. Ennifar,[c] C. Flensburg,[b] W. Paciorek,[b] C. Vonrhein[b] and G. Bricogne[b]

The case of a brominated RNA crystal structure determination in which standard three-wavelength MAD phasing was unsuccessful because of fast X-ray-induced debromination was reinvestigated [Ennifar *et al.* (2002), *Acta Cryst.* D58, 1262–1268]. It was found that if the data are kept unmerged

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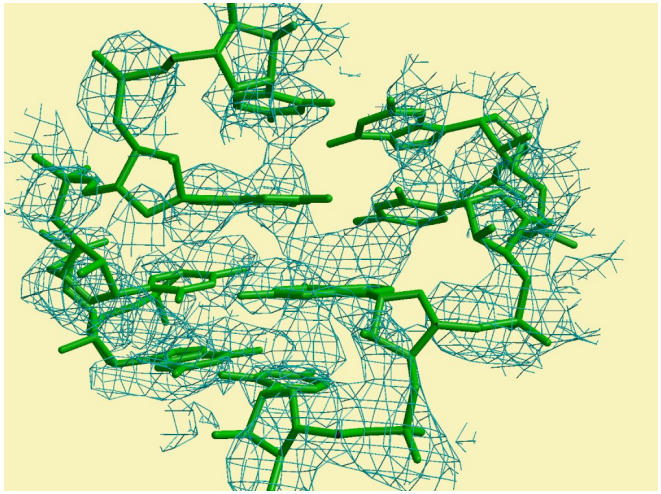
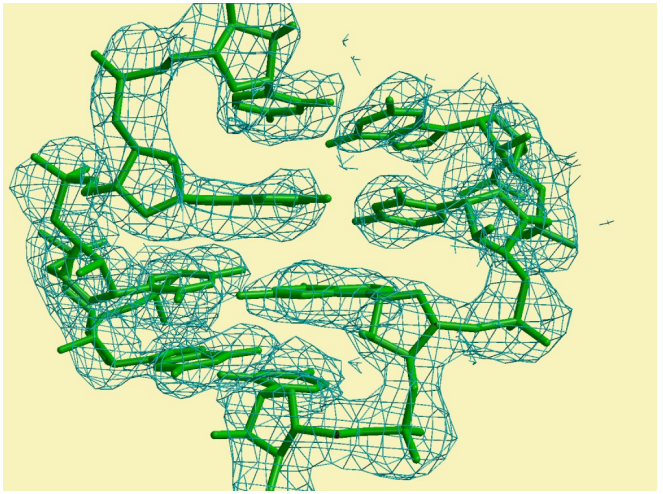
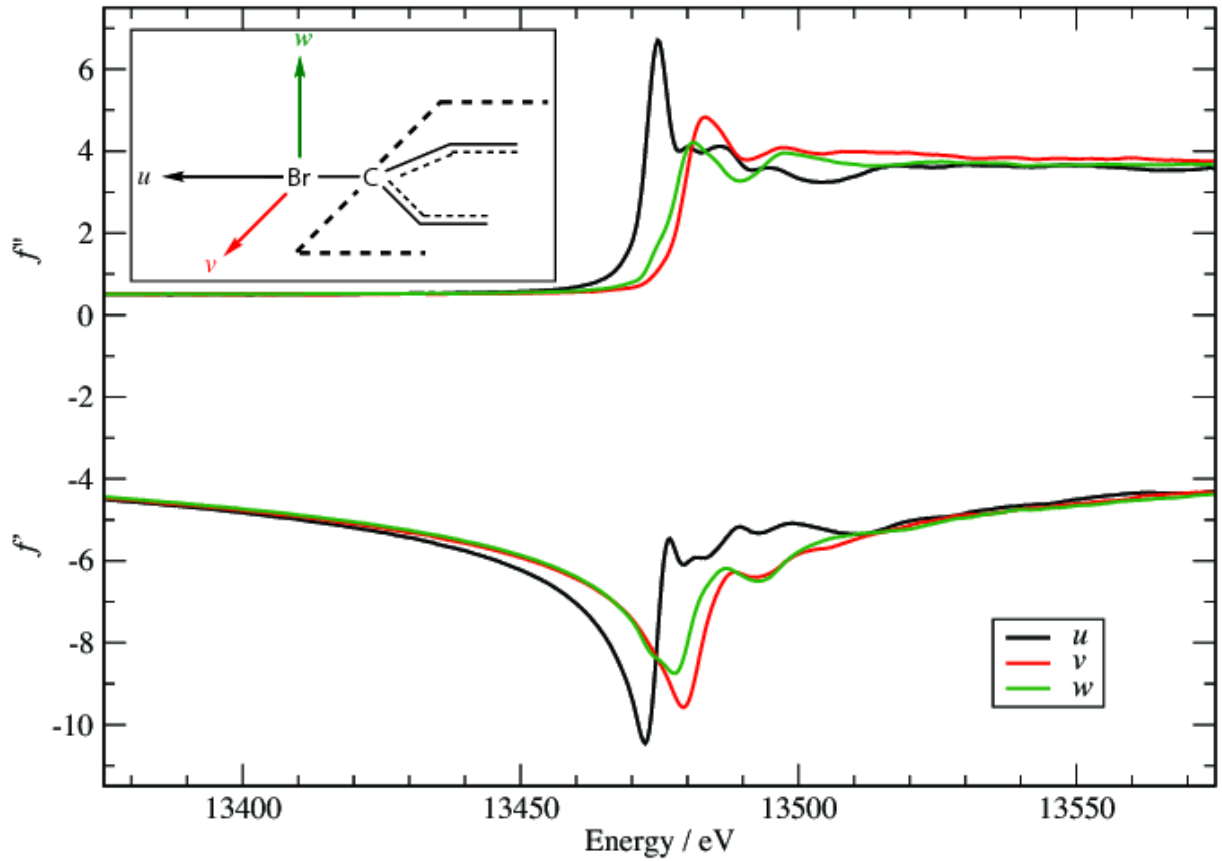


Standard SAD *CC=0.37*



Use of unmerged data *CC=0.78*

What is anisotropy of anomalous scattering (AAS) ?



⇒ Directional dependence of f' and f'' in the vicinity of an absorption edge

⇒ Polarisation anisotropy

What is anisotropy of anomalous scattering (AAS) ?

Acta Cryst. (1982). A38, 62–67

X-ray Dichroism and Polarized Anomalous Scattering of the Uranyl Ion

BY DAVID H. TEMPLETON AND LIESELOTTE K. TEMPLETON

Materials and Molecular Research Division, Lawrence Berkeley Laboratory and Department of Chemistry, University of California, Berkeley, California 94720, *USA*

⇒ Tensor description of f' and f''

$$f' \longrightarrow \mathbf{f}' = \begin{pmatrix} f'_{xx} & f'_{xy} & f'_{xz} \\ f'_{xy} & f'_{yy} & f'_{yz} \\ f'_{xz} & f'_{yz} & f'_{zz} \end{pmatrix} \qquad f'' \longrightarrow \mathbf{f}'' = \begin{pmatrix} f''_{xx} & f''_{xy} & f''_{xz} \\ f''_{xy} & f''_{yy} & f''_{yz} \\ f''_{xz} & f''_{yz} & f''_{zz} \end{pmatrix}$$

What is anisotropy of anomalous scattering (AAS) ?

"The polarization effects reported here **may be useful for phasing** but **can cause error** if not taken into account"
(Templeton & Templeton, 1988)

Acta Cryst. (1990). A**46**, 809–820

Effect of the Anisotropy of Anomalous Scattering on the MAD Phasing Method

BY ERIC FANCHON* AND WAYNE A. HENDRICKSON

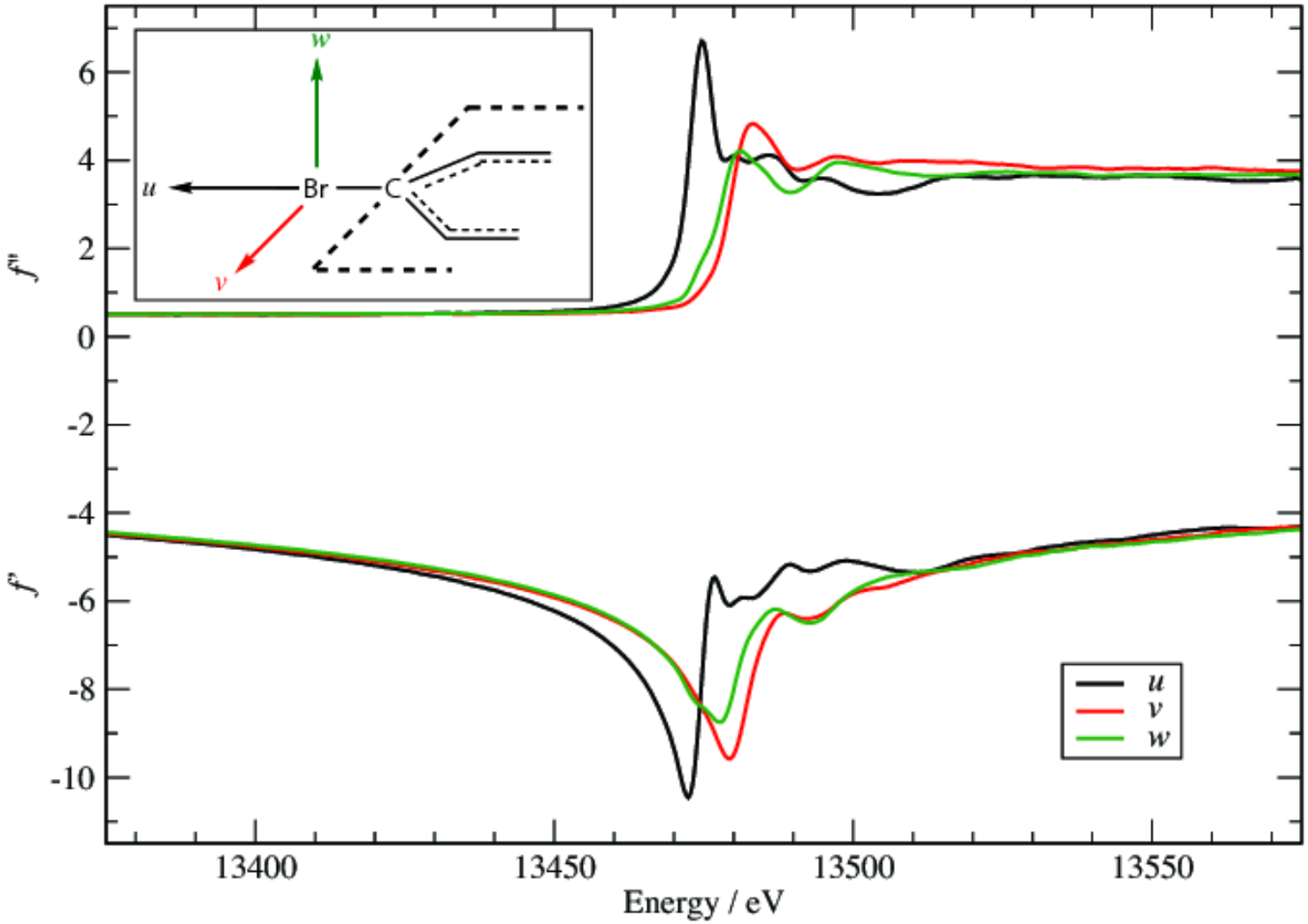
Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, New York 10032, USA

"(…) the results show that AAS does not cripple the MAD method, and that phases uncorrupted by these effects can be recovered."

Common misconceptions about AAS

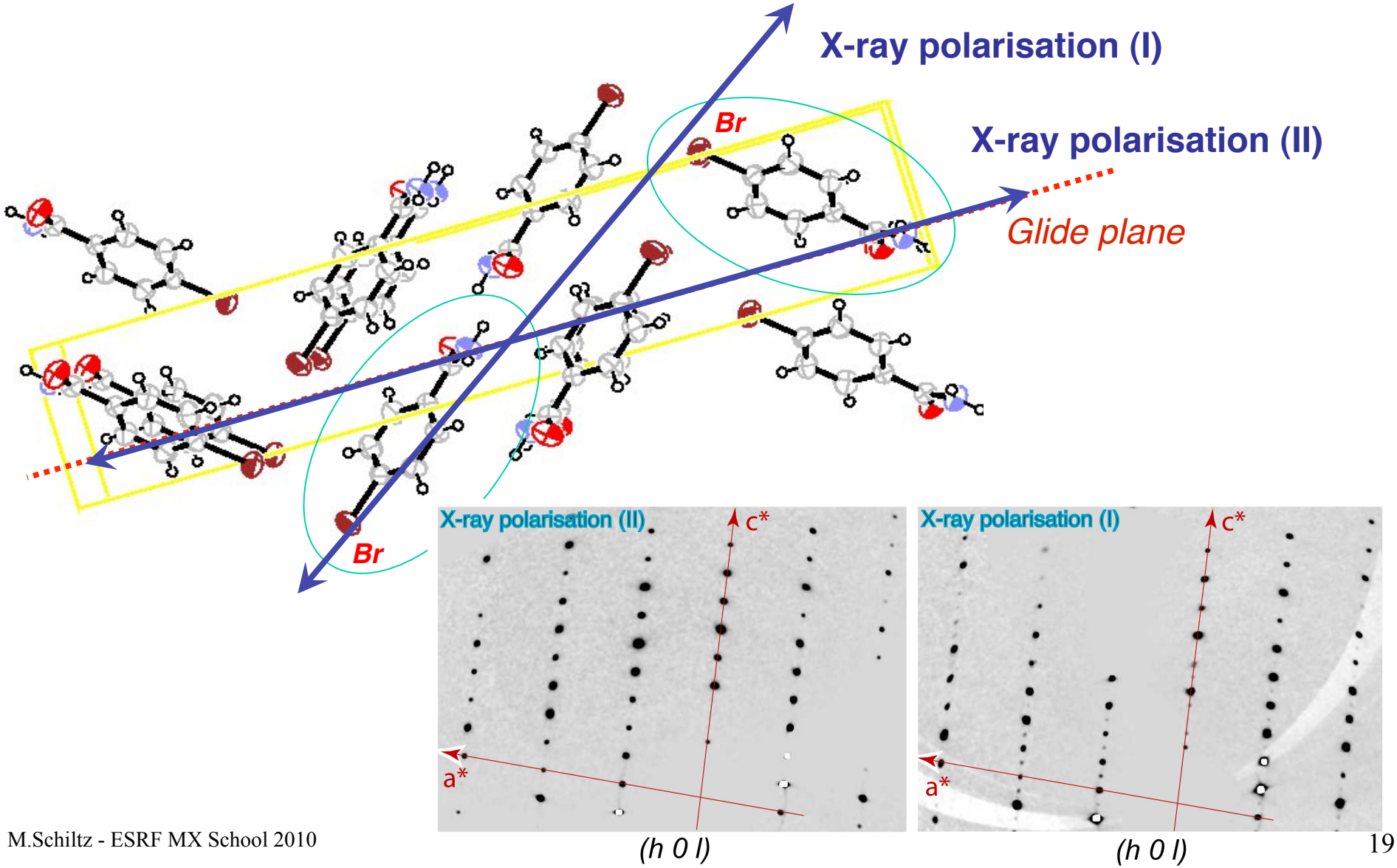
1. The effects of AAS are only present if all the molecules (or molecular groups) in the crystal are aligned
2. If there is a large number of sites with different orientations, the effects of AAS are "averaged out" to isotropy
3. The effects of AAS are only visible in high-resolution data (by "analogy" with anisotropic B factors)
4. Special scans (Ψ-scans) are necessary to reveal the effects of AAS
5. AAS only (or mainly) affects forbidden reflections
6. In cubic spacegroups, there can be no AAS except for higher-order (quadrupolar) effects

Broken symmetries due to AAS



Broken symmetries due to AAS

AAS in monoclinic crystals ($P2_1/c$) of p-Bromobenzamide



Broken symmetries due to AAS

⇒ AAS "breaks" the crystal symmetry

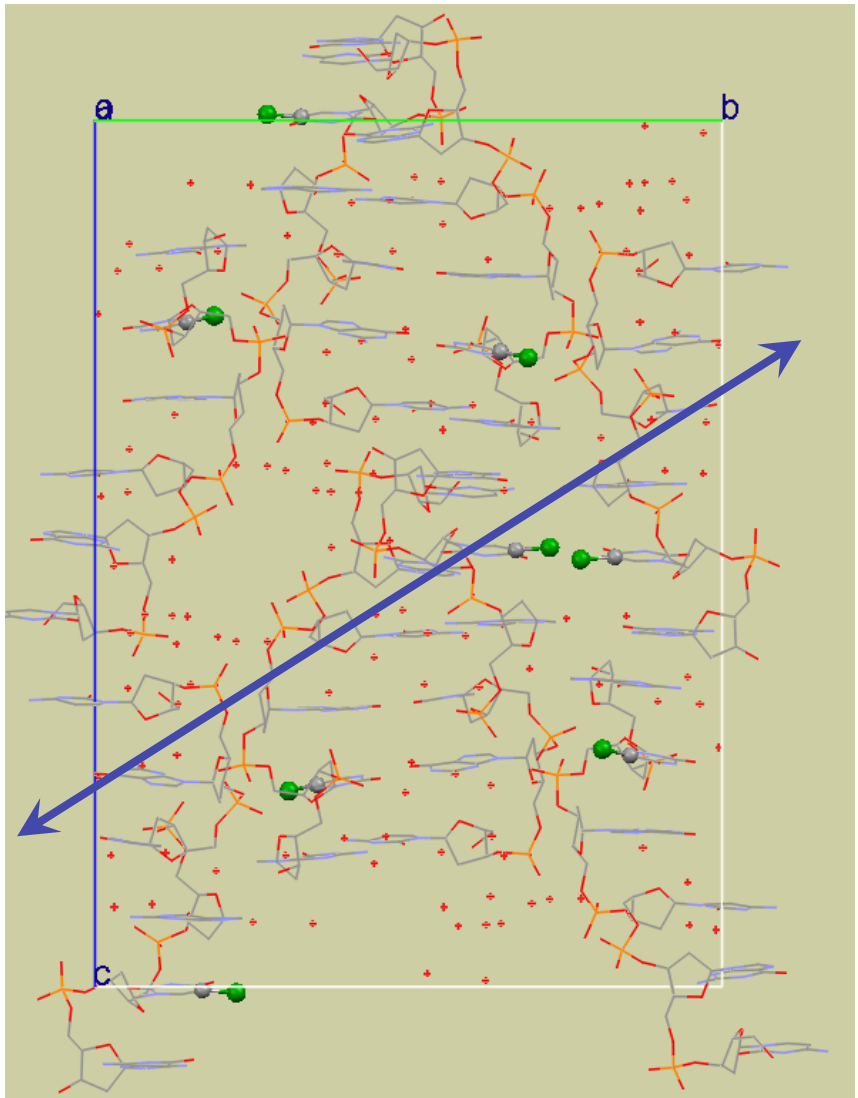
⇒ AAS shows up even if the molecules (or molecular groups) are not all aligned in the crystal

⇒ The effects of AAS show up in standard single-axis data collections

⇒ The effects of AAS depend on the orientation of the crystal w.r.t. the direction of X-ray polarisation

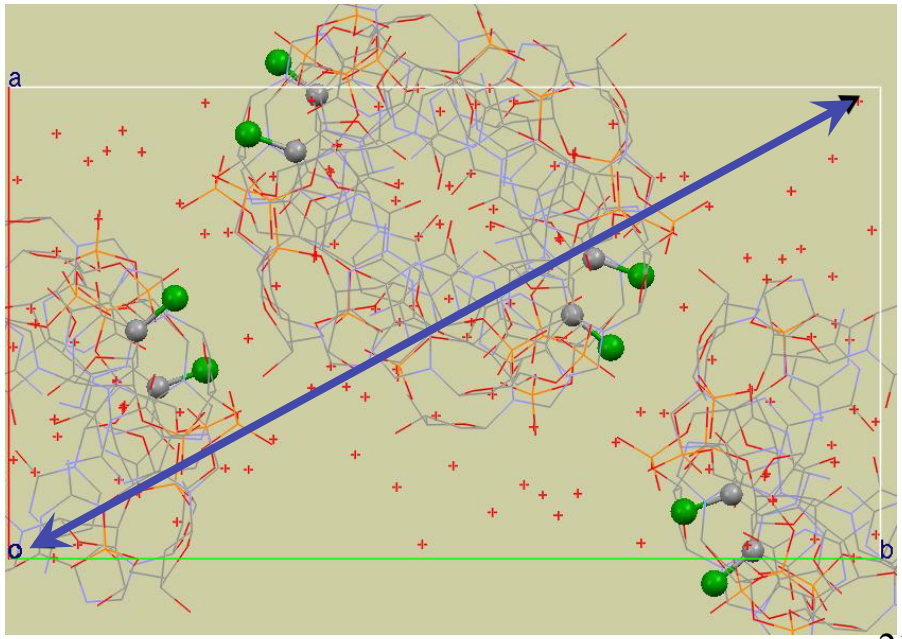
Broken symmetries due to AAS

AAS in a brominated DNA molecule



- Molecule : d(CGCG[BrU]G)
- Space group : $P2_12_12_1$
- Peak-λ data set collected at APS-19ID
- Single-axis data collection

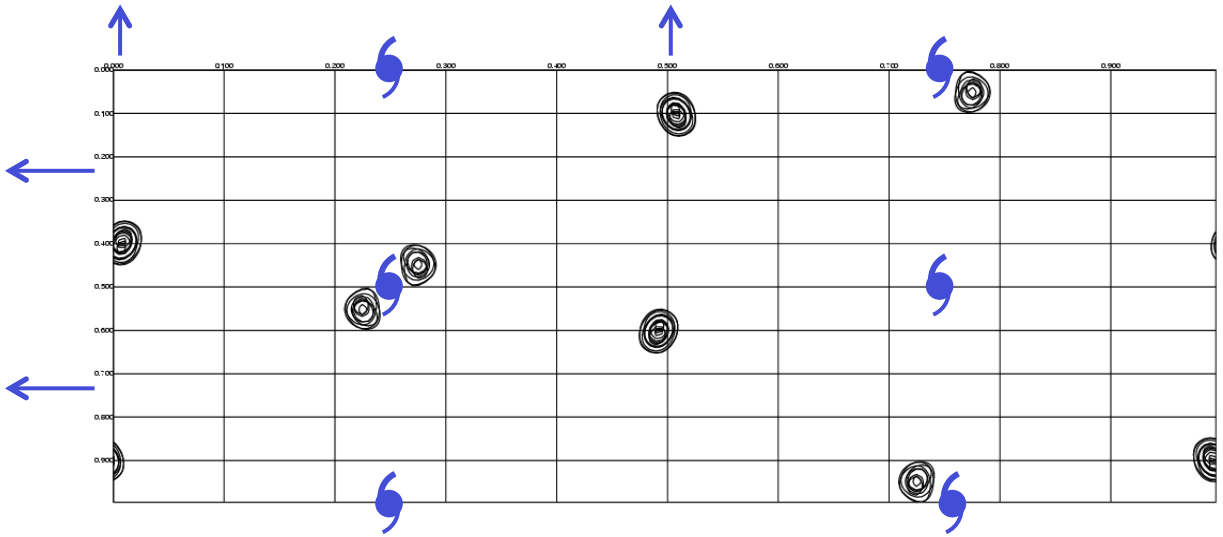
X-ray polarisation



Broken symmetries due to AAS

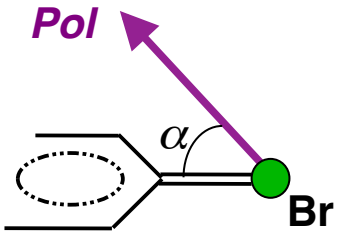
AAS in a brominated DNA molecule

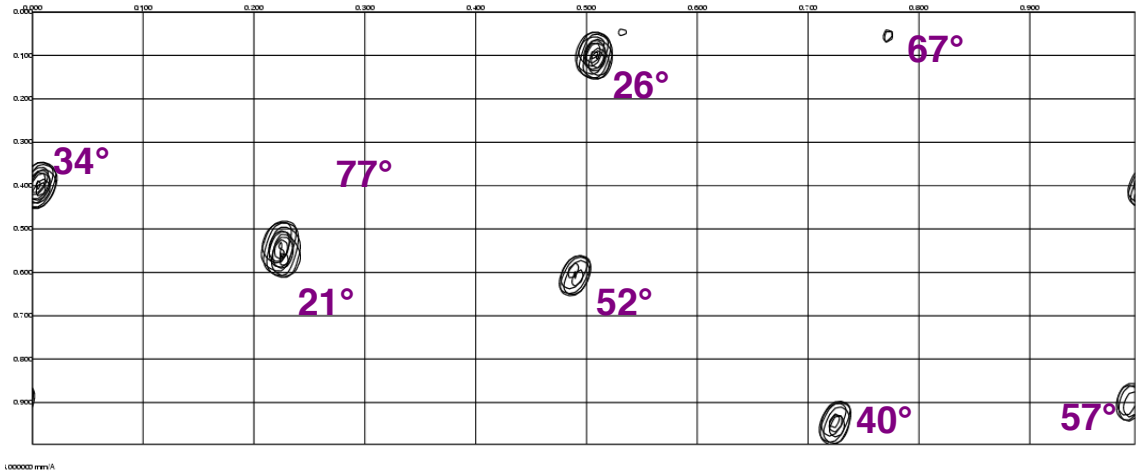
Data merged in point group 222
(crystal spacegroup: $P2_12_12_1$)



Anomalous difference Fourier maps (rojection along **c**)

Same data, treated as *P*1





Broken symmetries due to AAS

⇒ AAS induces measurable intensity differences between symmetry-related reflections

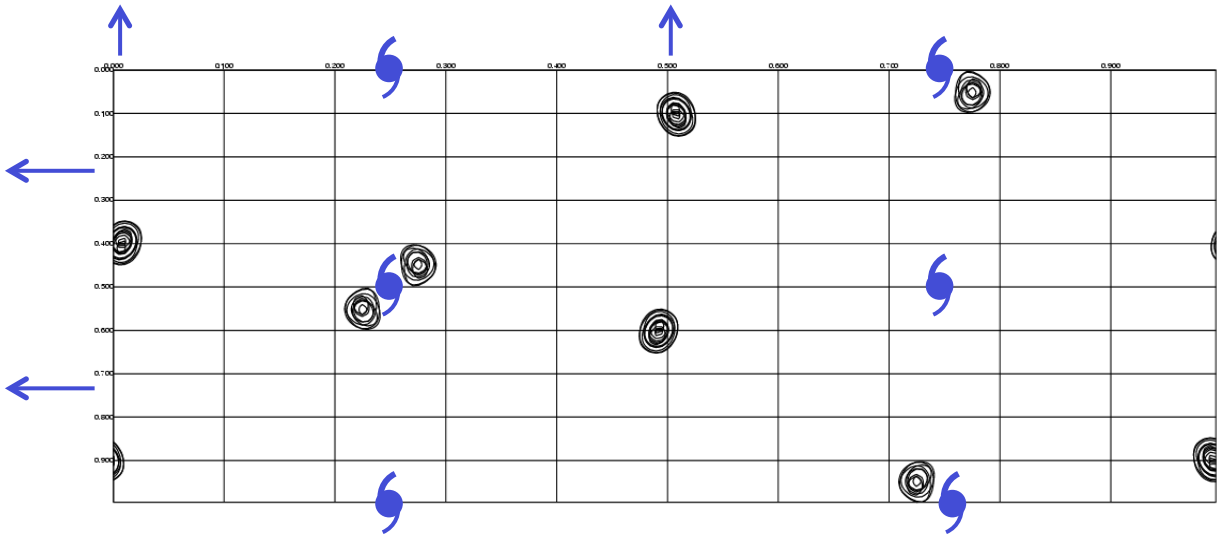
⇒ Data merging completely scrambles the effects of AAS

⇒ AAS affects all reflections (forbidden and non-forbidden)

Modelling and refining AAS

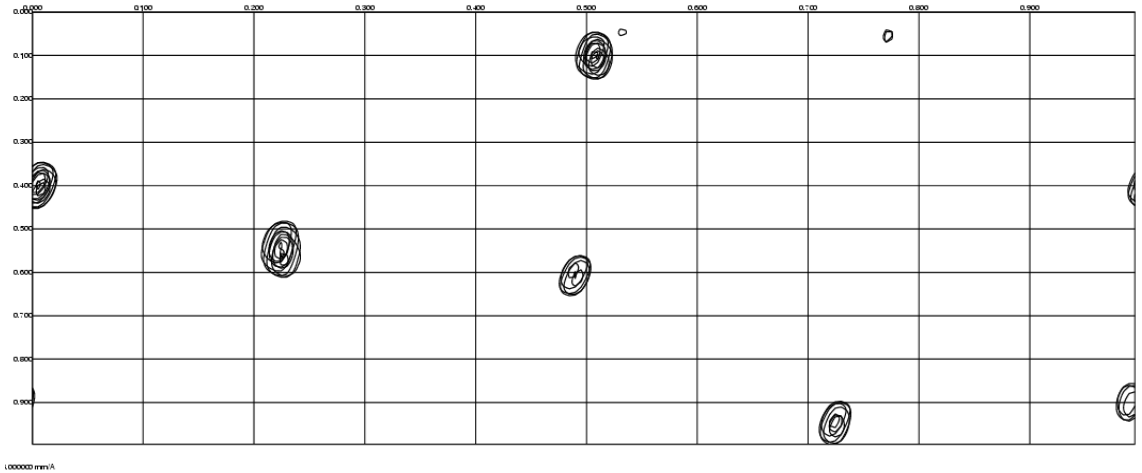
AAS in a brominated DNA molecule

Data merged in point group 222
(crystal spacegroup: $P2_12_12_1$)



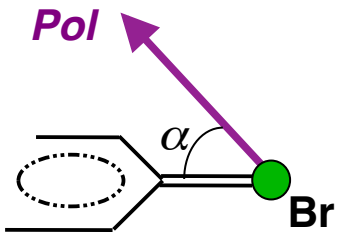
Anomalous difference Fourier maps (rojection along **c**)

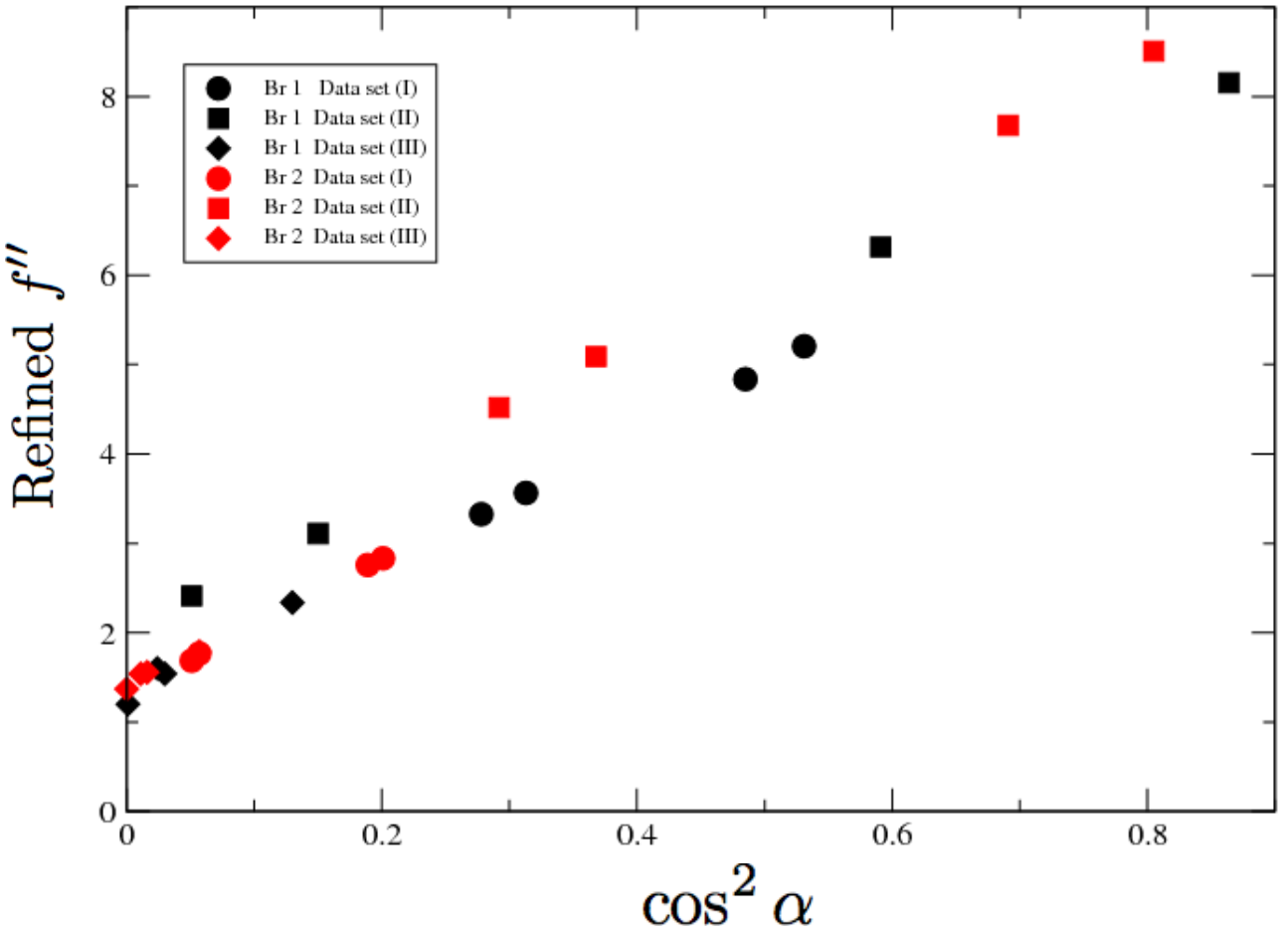
Same data, treated as *P*1



Modelling and refining AAS

- "Symmetry-unrolled" f' and f'' factors





Modelling and refining AAS

- "Symmetry-unrolled" f' and f'' factors
  - Refine individual *f'* and/or *f"* factors for symmetry-related sites (*i.e.* use *P*1 symmetry for *f'* and/or *f"* factors and the true spacegroup symmetry for all other parameters).
  - Useful approximation if the direction of X-ray beam polarization is constant for the whole data set (*i.e.* data collected with a single spindle axis // beam polarization)

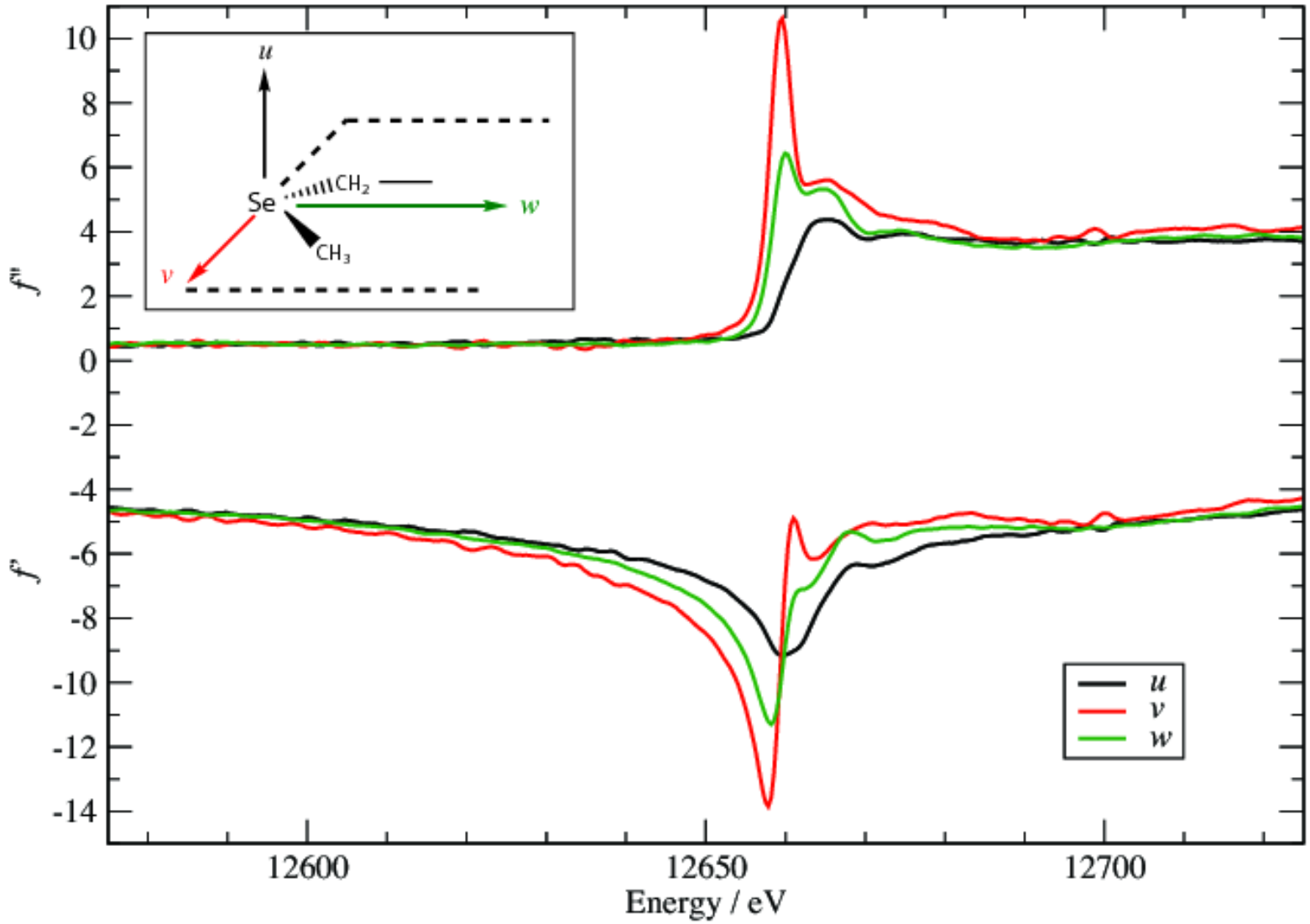
$$F(h) = \sum_{j}^{\text{sites}} f_j^{\circ} \Omega_j \exp(-Bs^2) \sum_{s}^{\text{sym}} (f'_{j,s} \pm i f''_{j,s}) \exp[2\pi i (h_s^T x_j + h t_s)]$$

- Refine f' and/or f'' factors as second-rank tensors

$$f''_{j,s} = \mathbf{p}^T \mathbf{R}_s^T \begin{pmatrix} f''_{xx} & f''_{xy} & f''_{xz} \\ f''_{xy} & f''_{yy} & f''_{yz} \\ f''_{xz} & f''_{yz} & f''_{zz} \end{pmatrix} \mathbf{R}_s \mathbf{p}$$

- Both methods are Implemented in *SHARP*

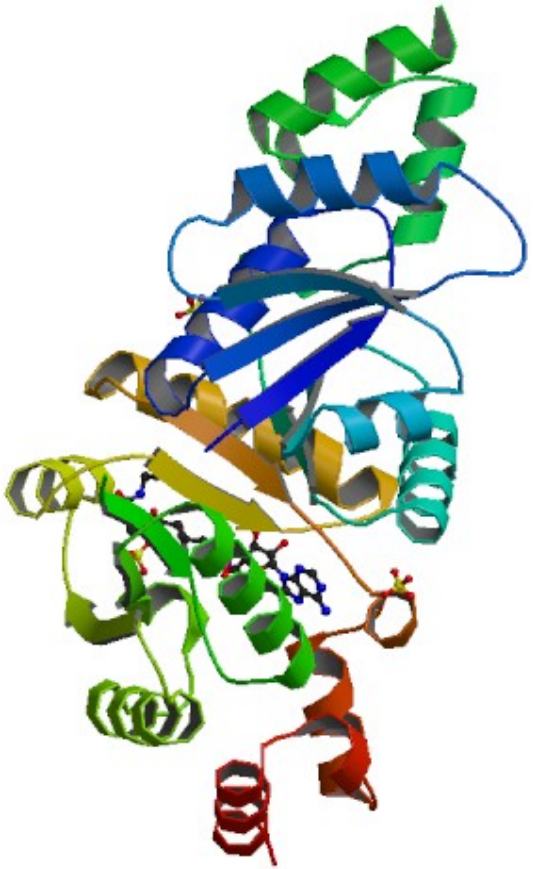
AAS in selenomethionine



Broken symmetries due to AAS

AAS in the selenated protein PPAT

- PPAT : Phosphopantetheine Adenylyltransferase
- 2 x 159 residues
- Selenomethionine samples: 2 x 8 Se sites per AU
- **Cubic** spacegroup *I*23
  - **384** Se sites in the unit cell !
  - No anisotropy in absorption and fluorescence scans
- Data collected at the Se *K* edge (peak λ), with the polarisation direction of the incident beam aligned with [0 1 0]

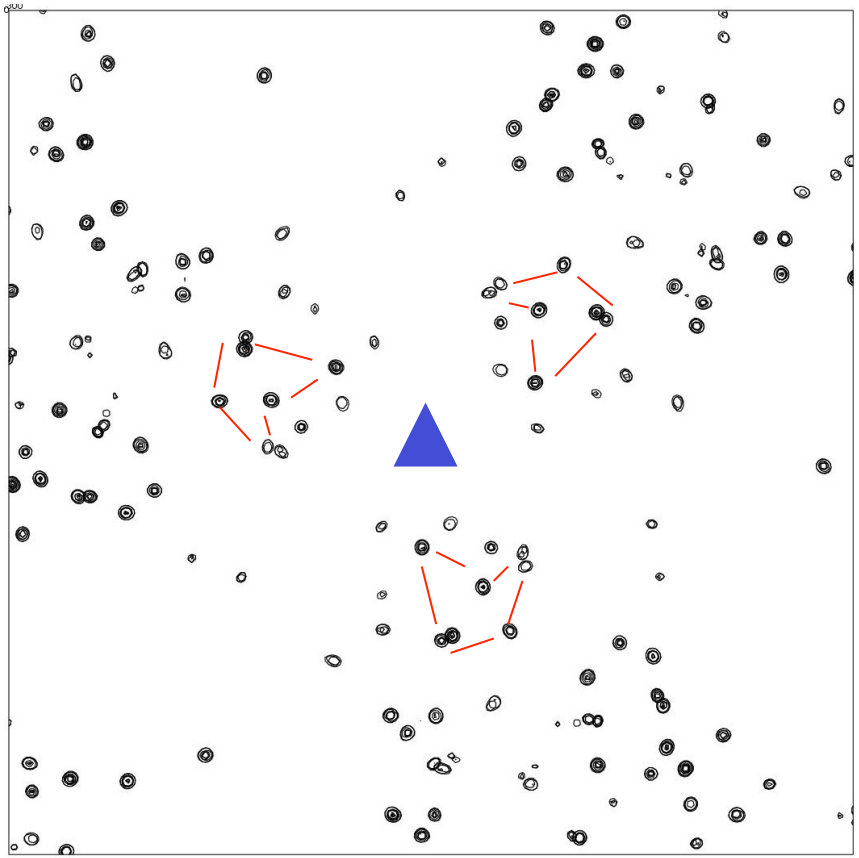


(Izard & Geerlof, 1999)

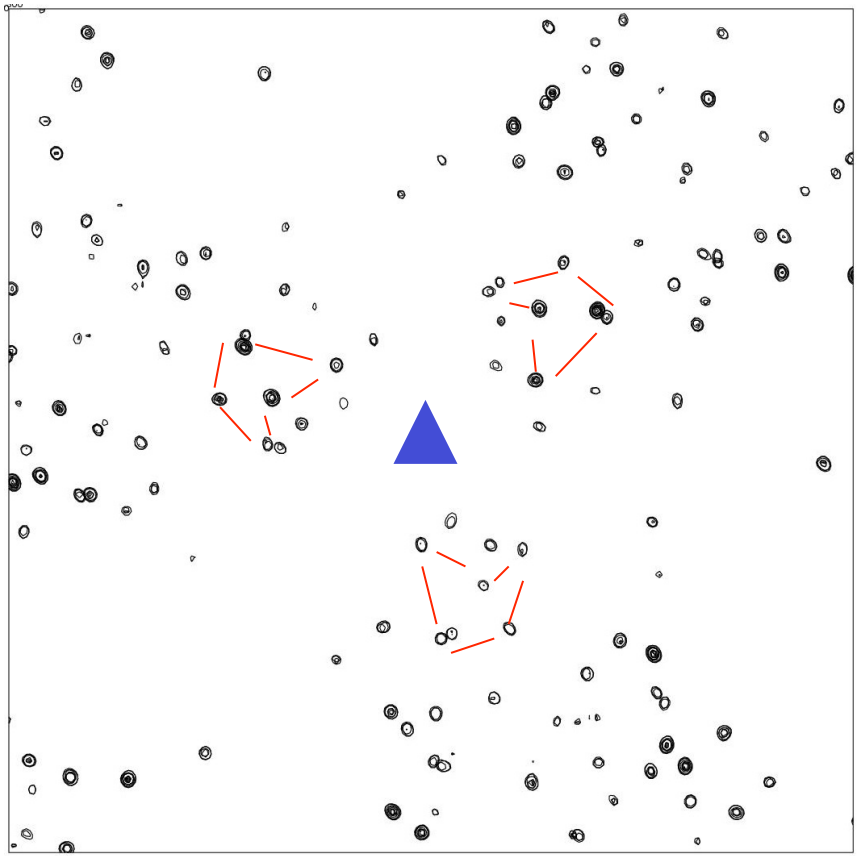
Broken symmetries due to AAS

AAS in the selenated protein PPAT

Anomalous difference Fourier maps : Projection along the [111] 3-fold axis



Data merged in point group 23



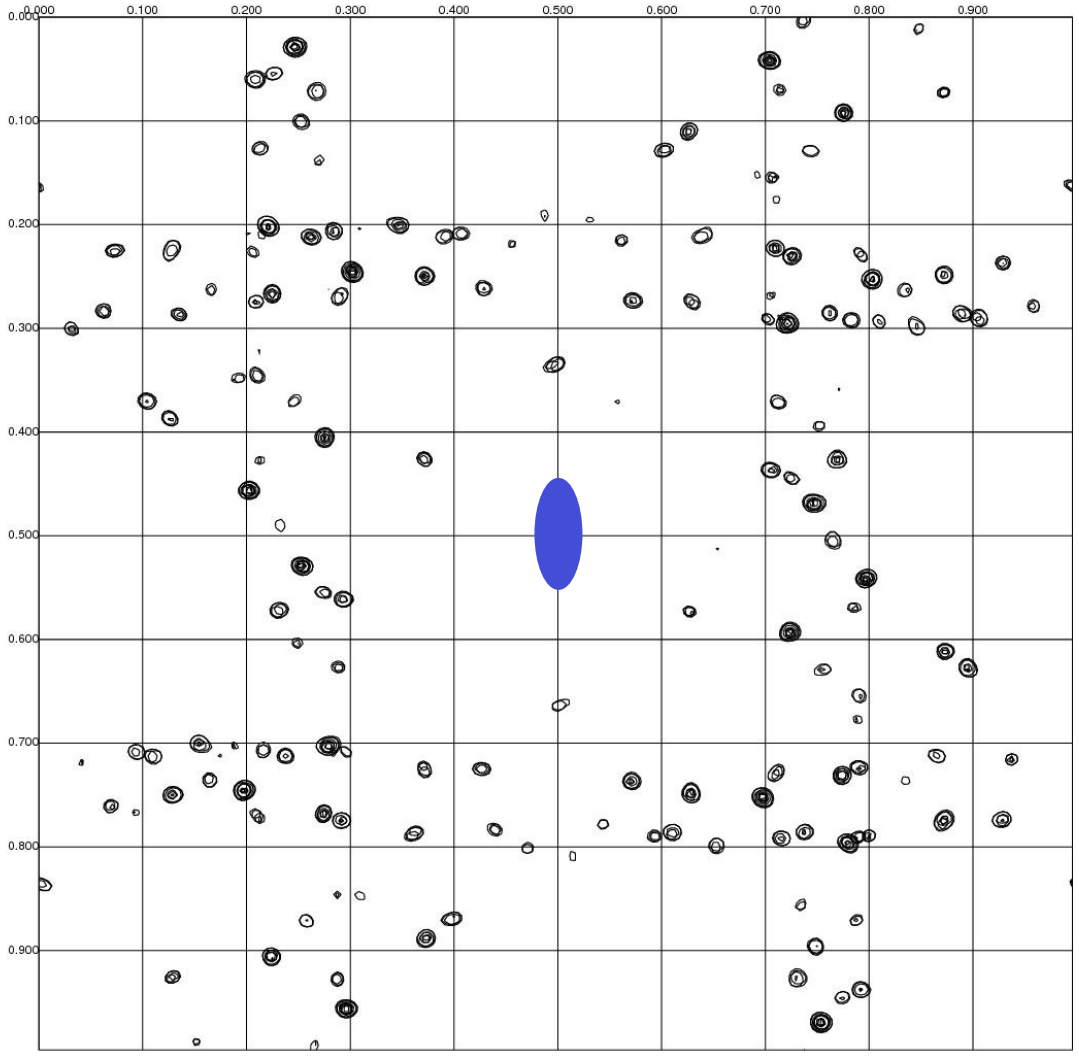
Same data, treated as *P*1

---> the 3-fold symmetry is broken !

Broken symmetries due to AAS

AAS in the selenated protein PPAT

Anomalous difference Fourier maps : Projection viewed along the [0 0 1] 2-fold axis



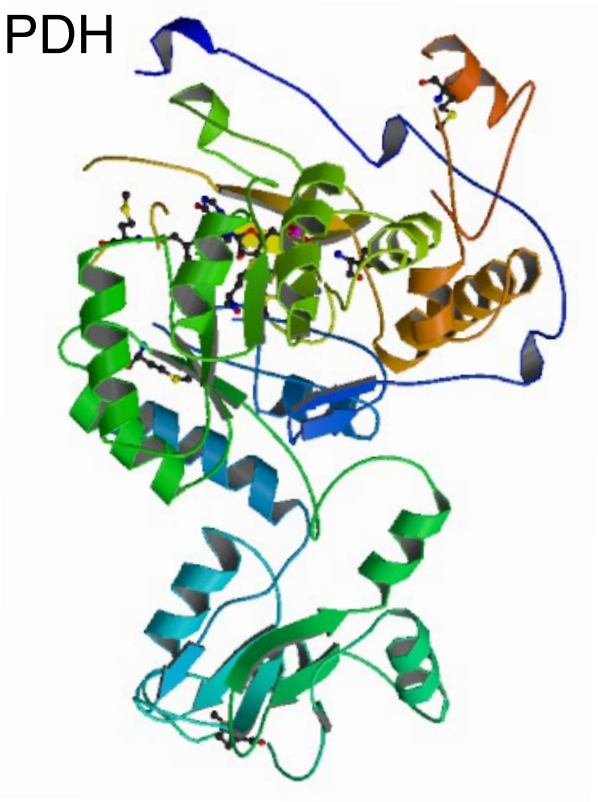
---> the 2-fold symmetry is preserved !

Broken symmetries due to AAS

⇒ A large number of Se sites and/or a high-symmetry space groups does not lead to an "averaging out" of the effects of AAS

Generating phase information through symmetry-related reflections

AAS in the selenated protein IMPDH

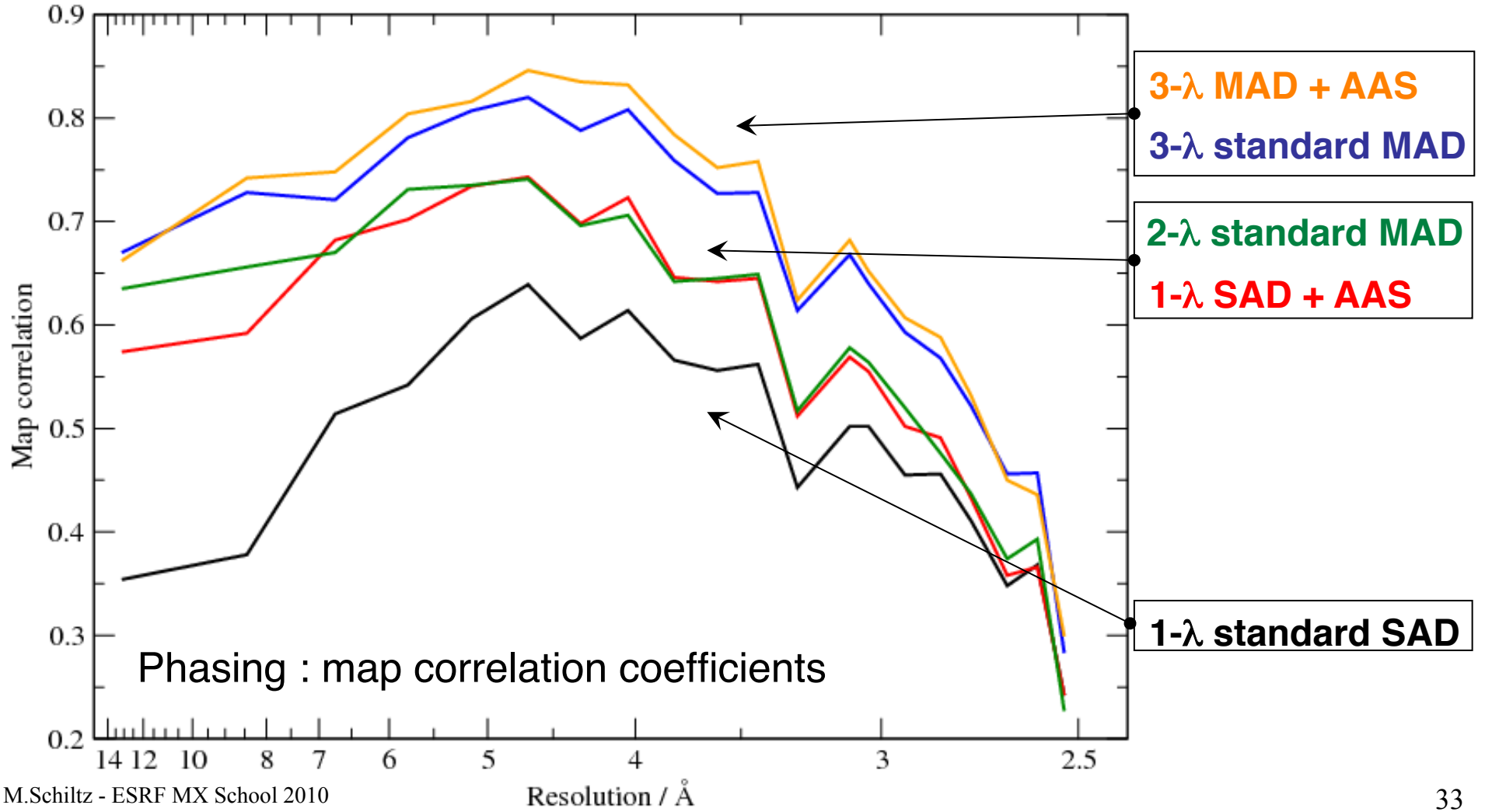


- IMPDH : Inosine-5'-monophosphate Dehydrogenase
- 52.7 kDa, 491 residues in the AU
- 13 Se sites per AU
- Spacegroup *I*422 (*a* = 151.49Å, *c* = 101.67 Å)
- Structure was solved by 3-wavelength MAD at the Se *K* edge (Zhang *et al.*, 1999)

- We re-used 1 of the 3 datasets (the peak wavelength dataset)
- Refinement of AAS tensors against the unmerged data
- Extracting phase information from the non-equivalence of symmetry-related reflections

Generating phase information through symmetry-related reflections

AAS in the selenated protein IMPDH



Generating phase information through symmetry-related reflections

⇒ Using the same data (one λ), the exploitation of AAS yields substantial improvements in the phases.

⇒ These improvements come essentially fro free, *i.e.* without collecting new data

⇒ AAS yields a two-λ map quality with a one-λ data set

Conclusion

- AAS is a ubiquitous effect that is present in data sets collected at the absorption edges of Se, Br,…
- There is still unused phase information in the data
  - Keep data unmerged. Exploit symmetry-breaking effects.
  - Better phases can be obtained without collecting more data.
  - Especially important for SAD cases where radiation damage prevents the collection of more than one wavelength
  - Implemented in the software package *SHARP*.
- AAS adds a new dimension of complexity and new opportunities to synchrotron macromolecular crystallography
  - New data collection strategies to maximize the symmetry-breaking effects of AAS
    - > Intentionally misalign the crystal
    - > Use a rotation axis which is not horizontal
    - > Multiple crystal orientations instead of multi-wavelength
  - Actively use and exploit the polarisation properties of synchrotron radiation

This effect adds a new dimension of complexity to the theory of X-ray scattering. By introducing an error into the conventional methods of computation, it offers a handicap to the use of anisotropic molecules like the uranyl ion to exploit the maximum effects at the absorption edges for solving the phase problem. Thus from a pessimistic point of view it is a setback. We adopt the opposite view: where there is a complication there is the opportunity of sharper, more penetrating methods for extracting information from diffraction experiments. There is much to look for in the exploration of this new region of diffraction optics.

Templeton & Templeton (1982)

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