Other ways to solve structure *ab initio*

*MXSchool2010*

Daniele de Sanctis
• History of RIP phasing

• Use of UV RIP phasing on ID23EH1

• Latest results and perspectives
• Radiation damage destroys your crystal

• Radiation damage prevents to get complete data

• Radiation damage depletes the anomalous signal

• The devil is not so black as he is painted
Specific Radiation Damage Can Be Used to Solve Macromolecular Crystal Structures

Prologue
The Structure Factor is dependent on the X-ray dose. With an appropriate experiment planning an $F_{\text{before}}$ and an $F_{\text{after}}$ can be identified.

If some particularly radiation sensitive site are identified, the difference between the two data collection can be treated as a derivative.
Relative photoelectric cross sections of carbon, nitrogen, oxygen, sulfur and selenium at 13.114 keV
SIR-like

\[ \mathbf{F}_{PH} \]

\[ \mathbf{F}_{P} \]

\[ \mathbf{F}_{H} \]

\[ \mathbf{F}_{PH} \]

\[ \mathbf{F}_{before} \]

\[ \mathbf{F}_{after} \]

\[ \mathbf{F}_{var} \]

\[ \mathbf{F}_{before} \]
Radiation-damage-induced phasing with anomalous scattering: substructure solution and phasing

Petrus H. Zwart, a Sankaran Banumathi, b Mirosława Dauter b and Zbigniew Dauter b

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Correspondence e-mail: dauter@bnl.gov

Substructure-solution and phasing procedures using a combination of anomalous scattering and radiation-damage-induced isomorphous differences have been investigated. The tyrosine residues in thaumatin were iodinated with N-iodosuccinimide in the crystalline form as well as prior to crystallization. Several data sets were collected from both forms and used for substructure solution and phasing using various protocols, employing anomalous, isomorphous or both these signals. It was shown that combination of the anomalous and isomorphous signals in the form of the RIPAS (radiation-damage-induced phasing with anomalous scattering) strategy is beneficial for both locating the substructure and subsequent phasing.

Keywords: anomalous scattering; radiation damage; RIP; RIPAS.
In the presence of an anomalous scatters, $F_{\text{before}}$ will contain the anomalous contribution, while $F_{\text{after}}$ will be collected away from the peak, or will contain much less anomalous signal because of depletion of the scatters

“(…) In fact, many structures have been solved unintentionally with a helping hand from RIP! In a MAD experiment, provided that the 'inflection point' dataset is collected last from the same crystal, the radiation damage has the effect of making $f'$ for the MAD element at this wavelength even more negative than usual, enhancing the dispersive part of the MAD signal.”

http://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/SHELX_C/D/E
“The best substructure were found by downscaling the ‘after’ data set in SHELXC...”
Is UV better?

Phasing Macromolecular Structures with UV-Induced Structural Changes

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France

Summary

Experimental phasing of macromolecular crystal structures relies on the accurate measurement of two or more sets of reflections from isomorphous crystals, where the scattering power of a few atoms is different for each set. Recently, it was demonstrated that X-ray-induced intensity differences can also contain phasing information, exploiting specific structural changes characteristic of X-ray damage. This method (radiation damage-induced phasing; RDP) has the advantage that using macromolecular crystallography; such a method would save on labor, synchrotron time, toxic heavy atom solutions, and costs. Interesting concepts such as brute force molecular replacement, iterated projections (Elser, 2003), free energy minimization (Castleden, 1992; Scheres and Gros, 2004), and three-beam X-ray diffraction (Weckert and Hummer, 1997) remain in rather early stages of development. In contrast, the use of weak anomalous scatterers within native proteins, sometimes enhanced by naturally bound heavy atoms, holds great promise as a generally useful method. Small anomalous differences for sulfur and phosphorus can be accurately measured from X-ray damage-induced phasing; RDP.
The ESRF Today

Public beamlines
CRG beamlines
Free bending magnet ports / insertion device ports
Instrumentation test and development beamlines
A UV laser is already installed on ID23EH1 since the early days...
Teem photonics laser

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Average Power (measured at sample position ~1.6 mW)
UV exposure is controlled through mxCuBE
No special intervention is needed by the user (except usual type&click)
Needed informations are exposing $\Delta \phi$ and time

At the beginning one oscillation was performed between chosen values
Now the two side of the crystal are exposed to maximize exposed volume
A shot in the dark
Test cases

As the experimental setup was not designed for this kind of experiment, we made some tests to verify its performance.

Proteins

- Elastase
- Thaumatin
- Lysozyme

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Elastase

UV-exposure in min

Scale-factor

% model built

UV-exposure in min
Thaumatin

![Graphs showing UV-exposure and scale-factor in relation to time of UV exposure and % model built.](image)
Lysozyme

UV-exposure in min

Scale-factor

Lysozyme

DSCA

0.92
0.94
0.96
0.98
1.00

CC weak

10
8
6
4
2
0

900 920 940 960 980

% model built

UV-exposure in min

Scalerfactor

0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

• All these test cases have disulphide bridges

• This commercial protein crystals are too good to really prove the technique

• Some minor UV damage was observed by Nanao&Ravelli on Methionine on PYP protein

• So we decide to play with Selenium labelled proteins....
Protocol

For all cases UV exposure was 50 mins around collecting rotation

1. RIP **before** and **after** datasets were collected @ 12 KeV

2. SAD/MAD A **pk** and **ip** datasets were collected before irradiation
SIRAS was done using **pk** and an **after** data from the same crystal

All data sets were processed with XDS and all structures were solved using Autorickshaw
FERULOYL ESTERASE DOMAIN OF XYN1 FROM CLOSTRIDIUM THERMOCELLUM  
1GKK

300 resi - 8 Selenium labelled methionine + 5 Cd ions  
Space group P212121  
2 mol/au  
58% solvent
Case 1

FAE Before

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

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FAE After 50 mins exposure

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Case 1

FAE - RIP - diso

FAE - SAD - dano

FAE - MAD - dano

FAE - SIRAS

29.92/17.78

42.46/28.13

48.25/29.14

25.41/18.80
Case 1

FAE - RIP

ARP/wARP (afterDM - ncs)
550/600 residues built

FAE - SAD

ARP/wARP (afterDM - ncs)
551/600 residues built

FAE - MAD

ARP/wARP (afterDM - ncs)
555/600 residues built

FAE - SIRAS-like

ARP/wARP (afterDM - ncs)
353/600 residues built

ARP/wARP (afterDM - ncs)
353/600 residues built

ARP/wARP (afterDM - ncs)
353/600 residues built
Mycobacterium tuberculosis Chorismate synthase
2011

400 resi - 11 Selenium labelled methionine
Space group P6422
1 mol/au
73% solvent
### ChSynt Before

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

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### ChSynt After 50 mins exposure

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

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<th>COMPLETENESS</th>
<th>R-FACTOR</th>
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<th>R-meas</th>
<th>Rmrgd-F</th>
<th>Anomal</th>
<th>SigAno</th>
<th>Nano</th>
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**Case 2**

A Light for Science

European Synchrotron Radiation Facility
Case 2

ChSynt - RIP - diso

ChSynt - SAD - dano

ChSynt - MAD Pk - dano

ChSynt - SIRAS diso/dano

26.89/14.24

54.98/33.86

51.41/33.50

34.56/25.58
### Case 2

#### ChSynt - RIP - shelxe

| ARP/wARP (after shelxe) | 346/400 residues built |

#### ChSynt - SAD - shelxe

| ARP/wARP (after shelxe) | 379/400 residues built |

#### ChSynt - MAD - shelxe

| ARP/wARP (after shelxe) | 379/400 residues built |

#### ChSynt - SIRAS - shelxe

| ARP/wARP (after shelxe) | 393/400 residues built |

**ARP/wARP (after shelxe)**

- 346/400 residues built
- 379/400 residues built
- 393/400 residues built
Case 3

100 resi - 7 Selenium labelled methionine
Space group P2₁2₁2₁
  4 mol/au
  56% solvent
## Case 3

### H35 Before

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<thead>
<tr>
<th>RESOLUTION LIMIT</th>
<th>NUMBER OF REFLECTIONS</th>
<th>COMPLETENESS</th>
<th>R-FACTOR</th>
<th>R-FACTOR</th>
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<th>Rmrgd-F</th>
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### H35 After 50 mins exposure

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---

European Synchrotron Radiation Facility
Case 3

H35 - RIP - diso

41.87/23.65

H35 - SAD - dano

38.75/19.29

H35 - MAD - dano

40.44/21.53

?
Case 3

H35 - RIP

H35 - SAD

H35 - MAD

?
Why H35 SIRAS didn’t work????

I already had exposed that crystal to UV and it looks like no additional damage is possible, but strange enough, the peak anomalous signal was still enough to phase....more work to do!!!
• the SIRAS approach is working at its best because damaged sites are the anomalous scatters

• More complicated (and maybe more interesting) scenario are foreseen in case of combination of independent phases

• Still there is room to investigate the effect of UV on
  • Metalloproteins
  • DNA
  • protein-DNA complexes
  • Nucleotide binding proteins
  • ....
Acknowledgment

Structural Biology Group

Matias Guijarro
Didier Nurizzo
Sasha Popov

Santosh Panjikar (EMBL HH)