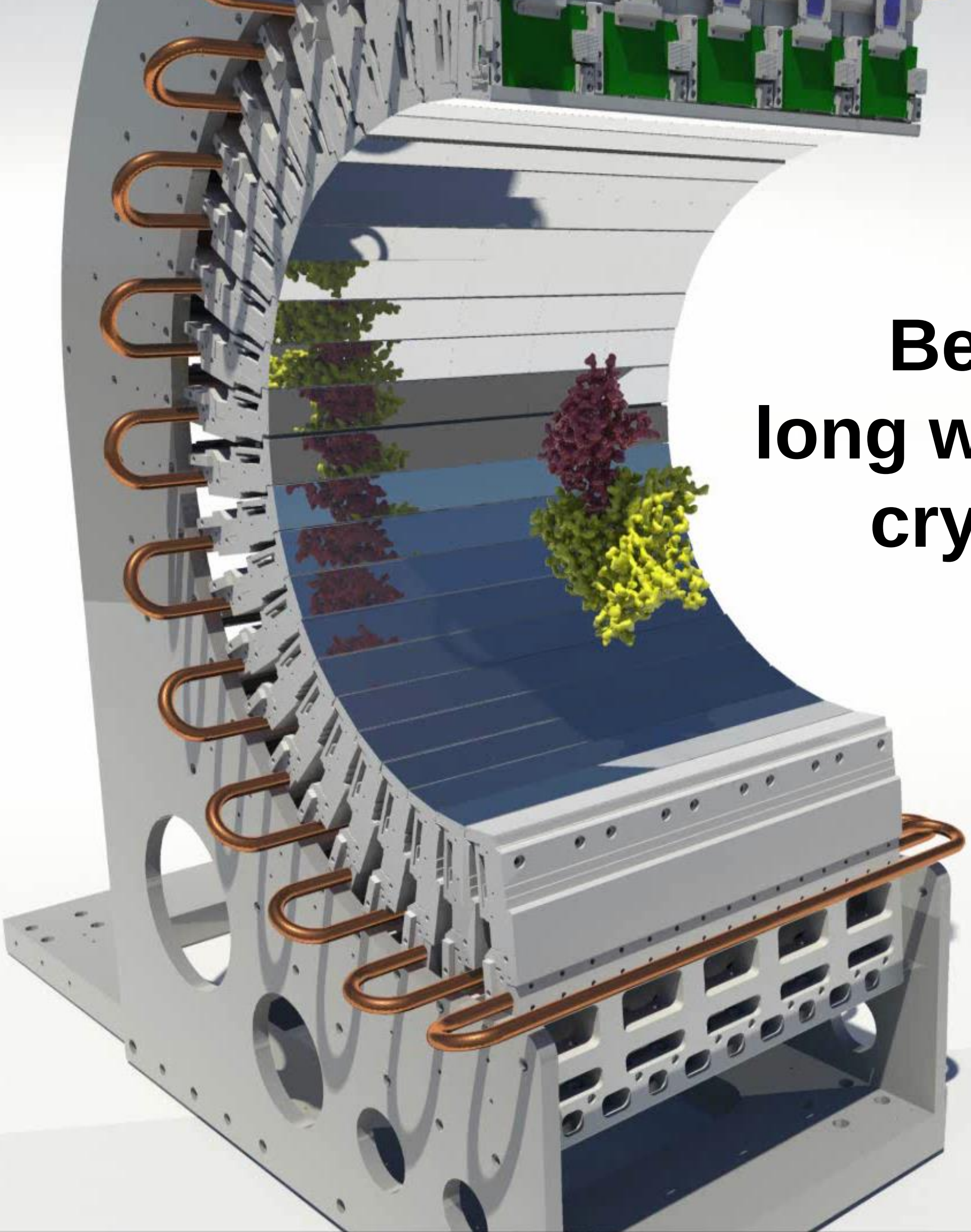
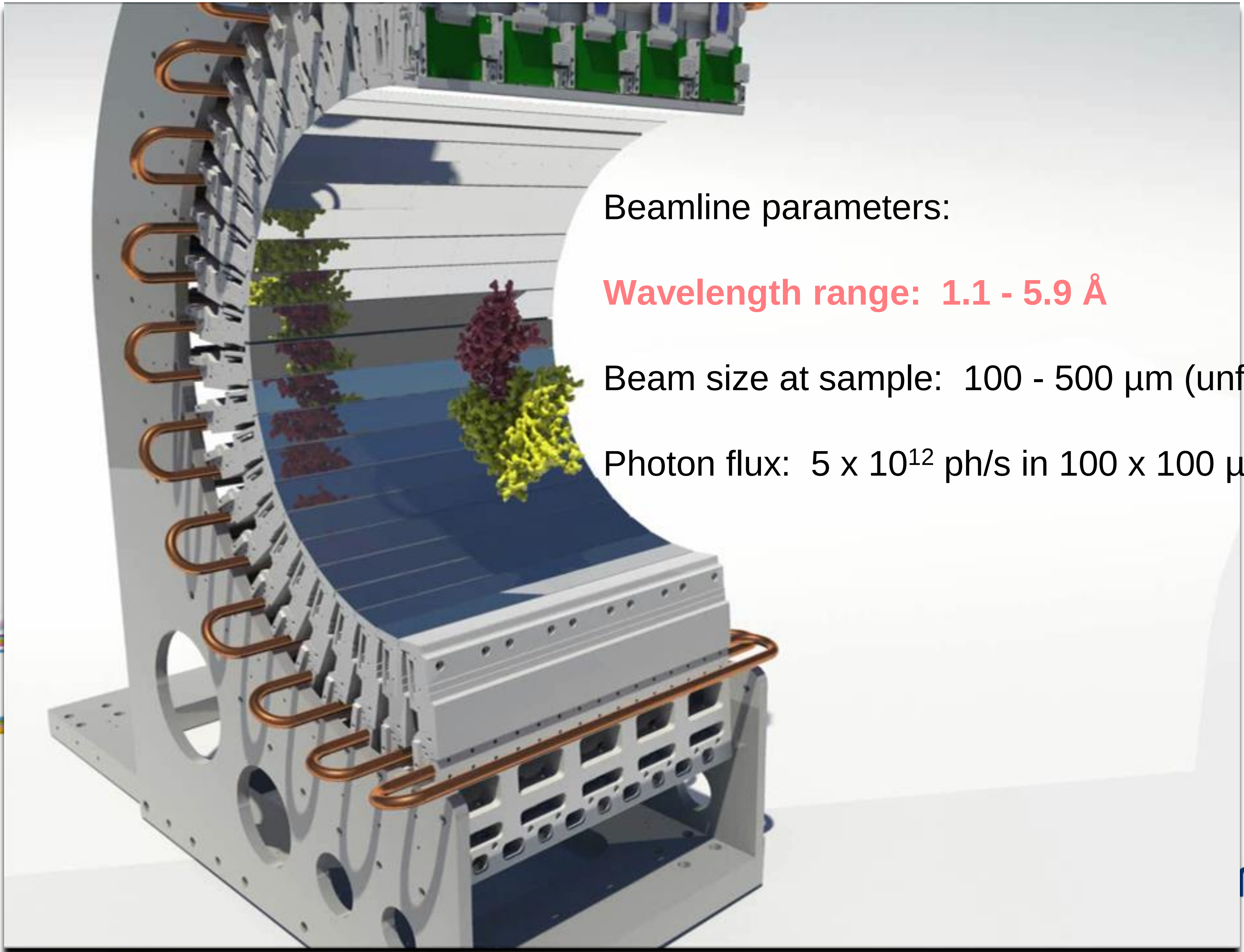


Beamline I23 – long wavelength X-ray crystallography

Ramona Duman

CCP4/DLS workshop
Nov/Dec 2023

I23 – In-vacuum long-wavelength MX



Beamline parameters:

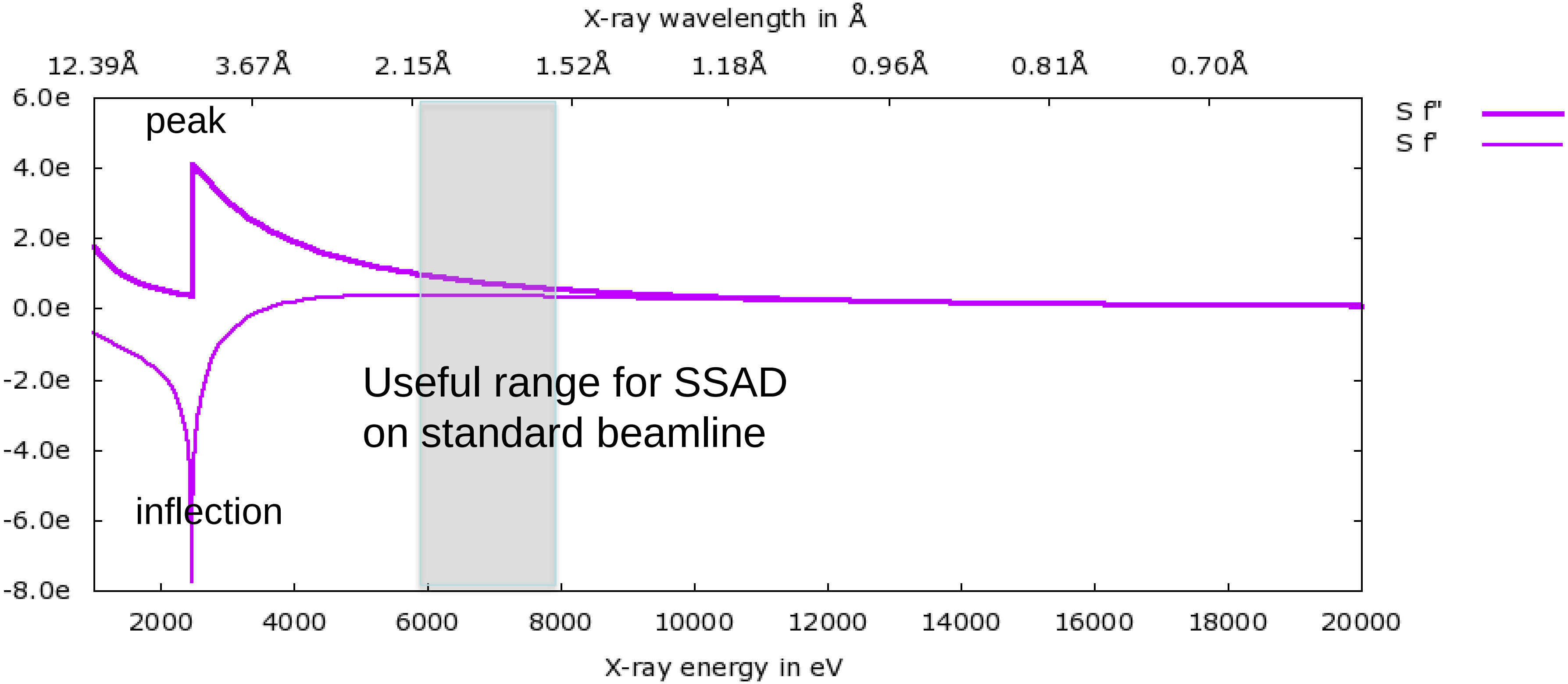
Wavelength range: 1.1 - 5.9 Å

Beam size at sample: 100 - 500 µm (unfocused)

Photon flux: 5 x 10^{12} ph/s in 100 x 100 µm (4 keV)

Why long wavelengths?

$$f = f_0 + \Delta f' + if'' = f' + if''$$

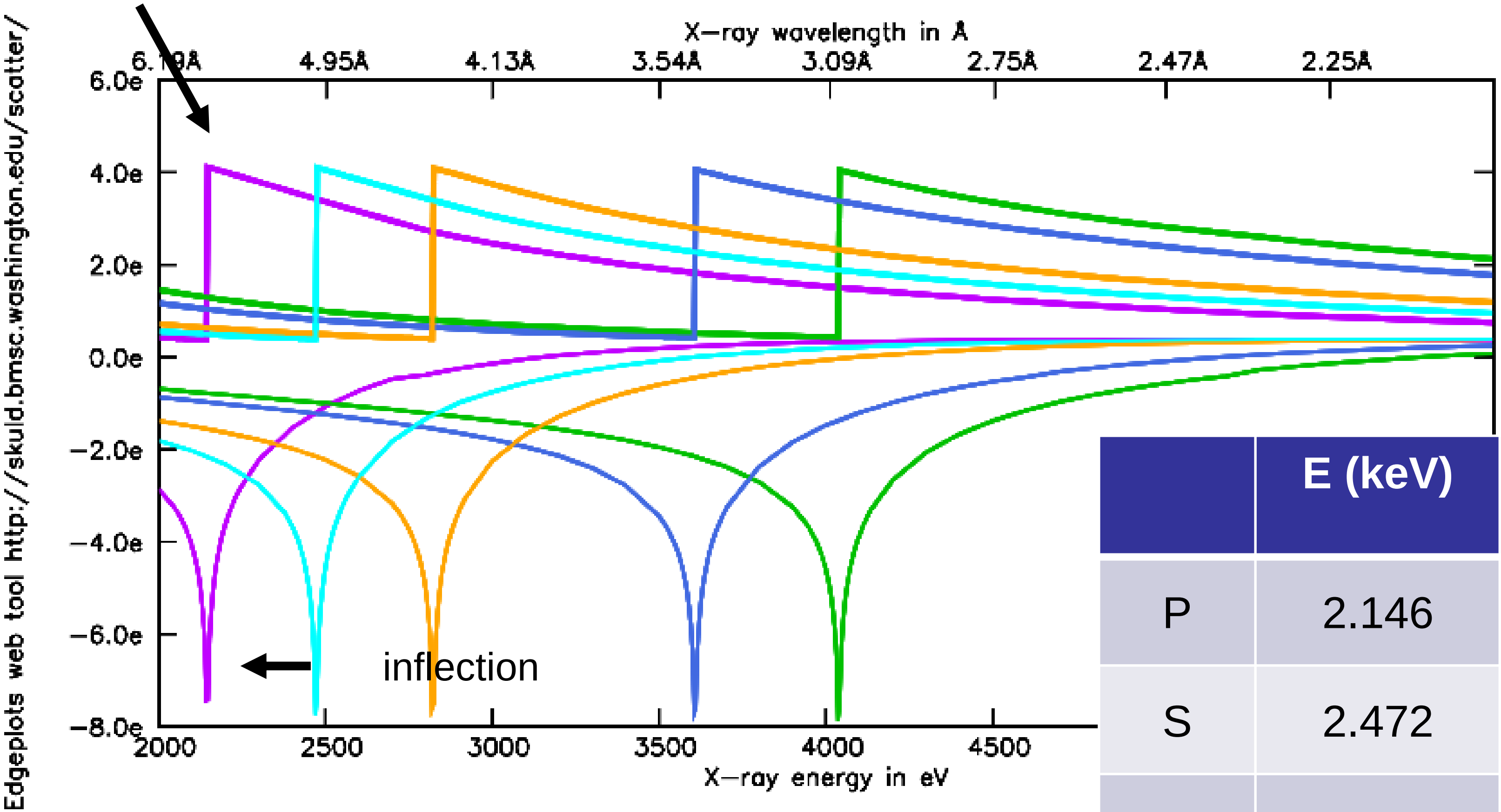


peak

Useful range for SSAD
on standard beamline

inflection

Accessible X-ray absorption edges of biologically relevant elements



peak

inflection

Edgeplots web tool http://skuld.bmsc.washington.edu/scatter/

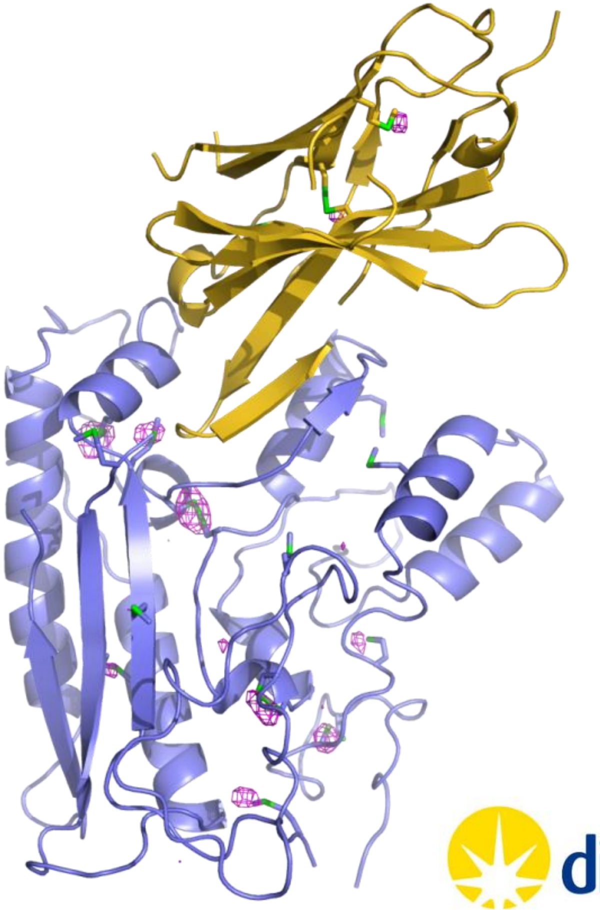
	E (keV)	λ (Å)
P	2.146	5.779
S	2.472	5.016
Cl	2.822	4.393
K	3.607	3.437
Ca	4.038	3.070

λ [Å] = 12.398 / E [keV]

I23 – Long-wavelength MX

Experimental Phasing

- SAD phasing from native proteins and DNA/RNA crystals by using anomalous signal from S/P/Ca/K/Cl
- MAD phasing



Element identification

- Anomalous difference Fourrier maps

Assist model building

In-vacuum sample environment

- Limited resolution:
  - – Bragg’s law: $n\lambda = 2d \sin\theta$

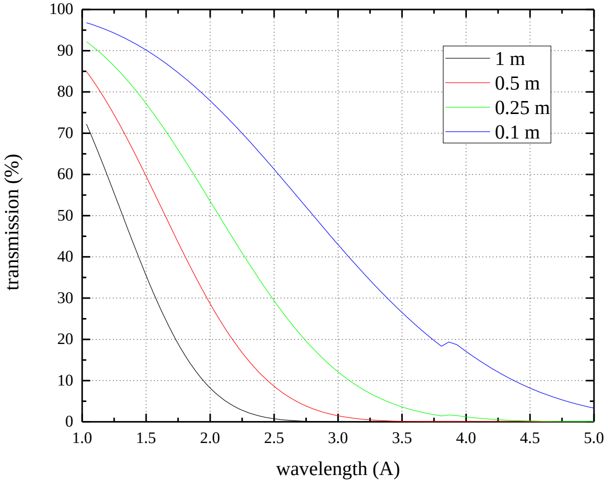
Pilatus 12M (Dectris)

120 modules in vacuum
12,000,000 pixels
Half cylinder r = 250 mm
Width z = 423.6 mm
$2\theta = \pm 100^\circ$ (40.3° laterally)

- Absorption:
  - Air absorption
  - Crystal, solvent and loop absorption

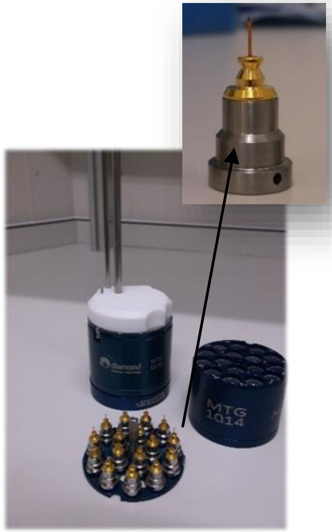
In-vacuum crystallography:
Elimination of air scattering and absorption

Air transmission



A. Wagner et al. *In-vacuum long-wavelength macromolecular crystallography* Acta Cryst. (2016)

Sample preparation logistics

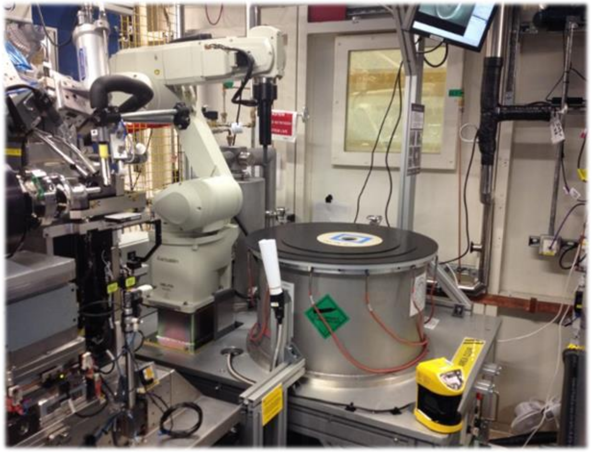


Tools sent to users

Users harvest and cryo-cool crystals

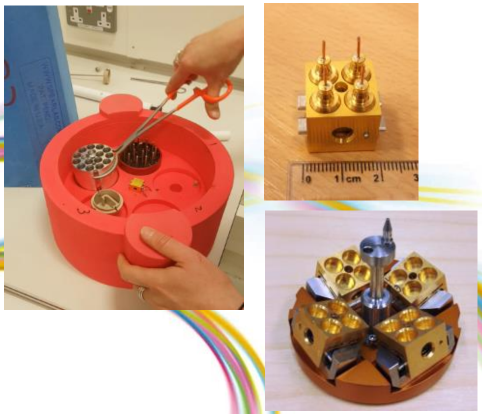
Samples sent to Diamond

Pre-screening on other MX beamlines



Transfer to I23 vacuum endstation

I23 thermally conductive sample holders and blocks

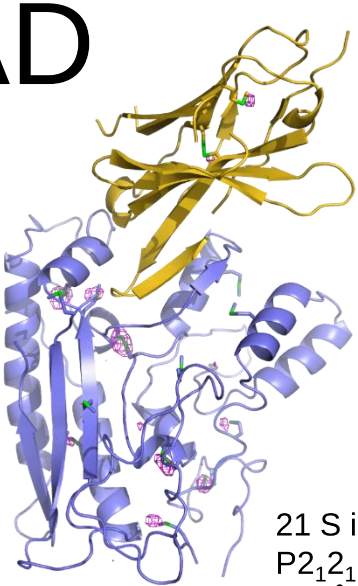


Diamond Cryogenic Sample Transfer System

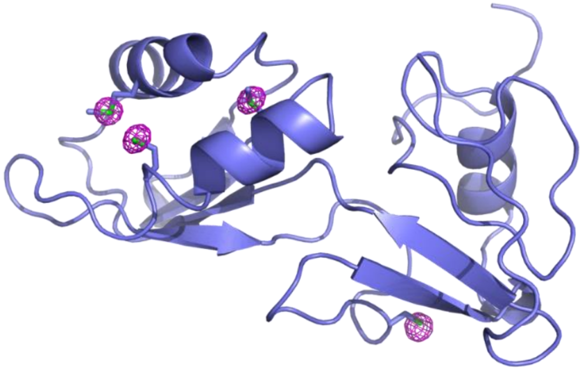
16 S Sulfur 32.06 - SAD

λ = 2.75 Å
Multiplicity: 10 - 20

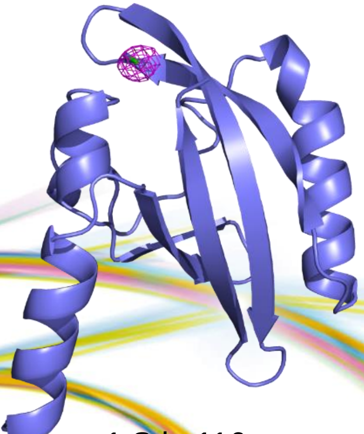
10 S in 477 aa
$P4_12_12_1$
3.2 Å res



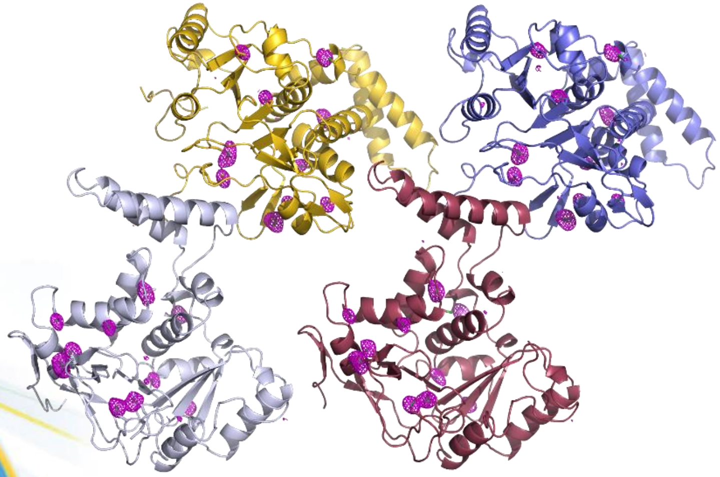
21 S in 427 aa
$P2_12_12_1$
2.8 Å res



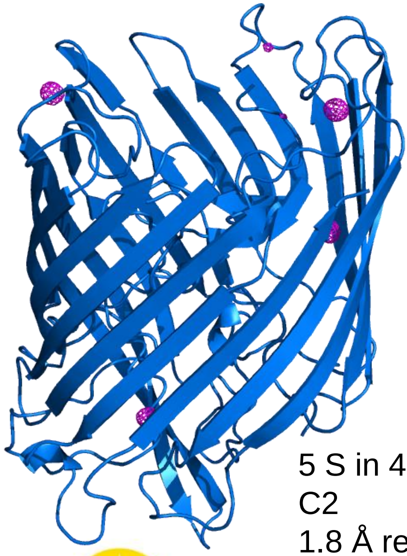
4 S in 157 aa
C2
1.8 Å res



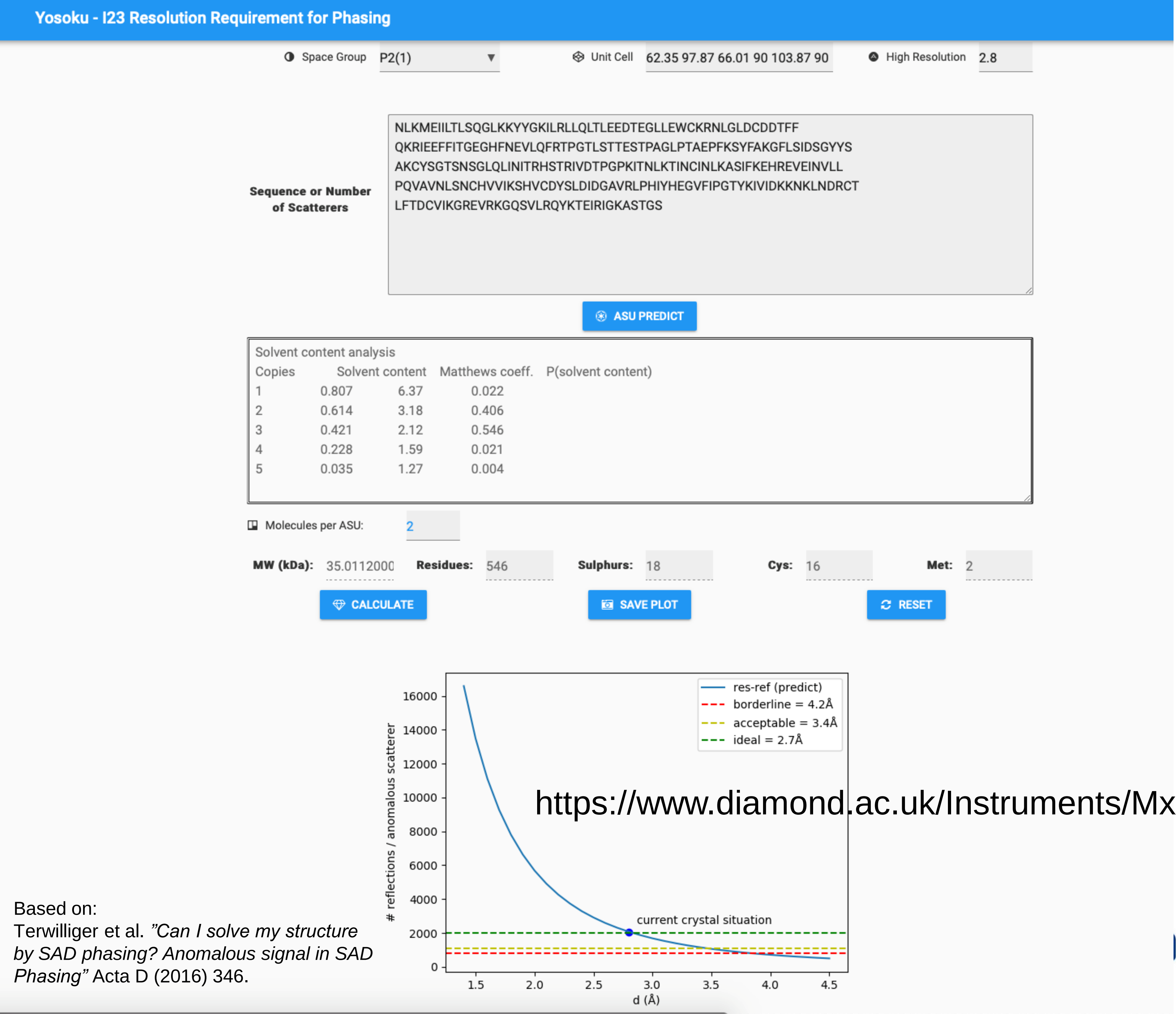
1 S in 116 aa
$P2_12_12$
2.5 Å res



10 S in 322 aa
$P2_1$
2.1 Å res
tNCS



5 S in 453 aa
C2
1.8 Å res



Space Group: P2(1)

Unit Cell: 62.35 97.87 66.01 90 103.87 90

High Resolution: 2.8

Sequence or Number of Scatterers

```
NLKMEIILTLSQGLKKYYGKILRLLQLTLEEDTEGLLEWCKRNLGLDCDDTFF
QKRIEEFFITGEGHFNEVLQFRTPGTLSTTESTPAGLPTAEPFKSYFAKGFLSIDSGYYS
AKCYSGTSNSGLQLINITRHSTRIVDTPGPKITNLKTINCINLKASIFKEHREVEINVLL
PQVAVNLSNCHVVIKSHVCDYSLDIDGAVRLPHIYHEGVFIPGTYKIVIDKKNKLNDRCT
LFTDCVIKGREVRKGQSVLRQYKTEIRIGKASTGS
```

```
Solvent content analysis
Copies        Solvent content   Matthews coeff.   P(solvent content)
1          0.807        6.37        0.022
2          0.614        3.18        0.406
3          0.421        2.12        0.546
4          0.228        1.59        0.021
5          0.035        1.27        0.004
```

Molecules per ASU: 2

MW (kDa): 35.0112000 **Residues:** 546 **Sulphurs:** 18 **Cys:** 16 **Met:** 2

https://www.diamond.ac.uk/Instruments/Mx/I23.html

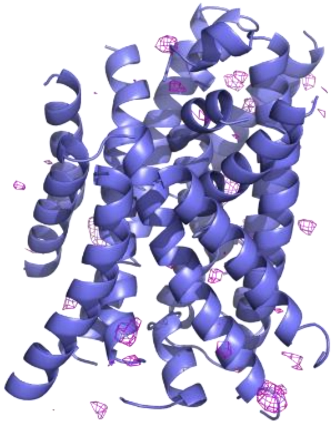
Based on:
Terwilliger et al. *"Can I solve my structure by SAD phasing? Anomalous signal in SAD Phasing"* Acta D (2016) 346.

Element identification

Anomalous difference maps

S identification

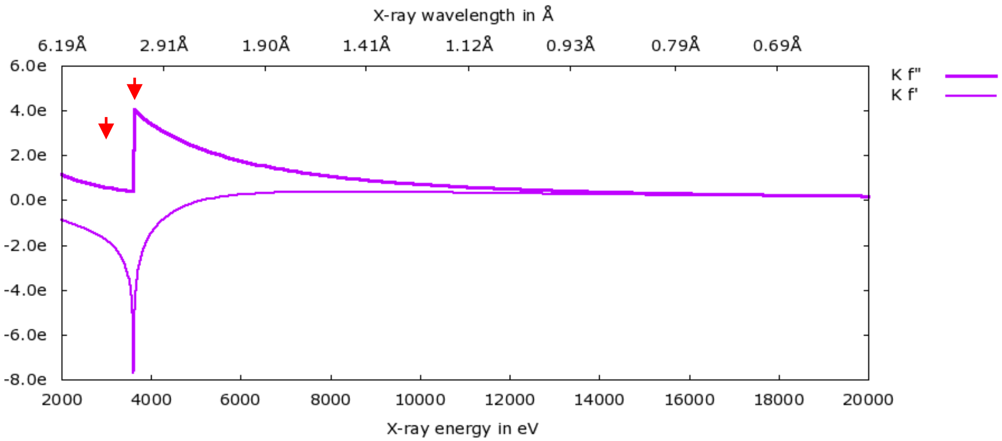
- Help with sequence assignment
- Membrane protein (LCP)
- 13 sulfurs in 471 amino acids
- Space group $P2_12_12_1$
- λ = 3.1 Å

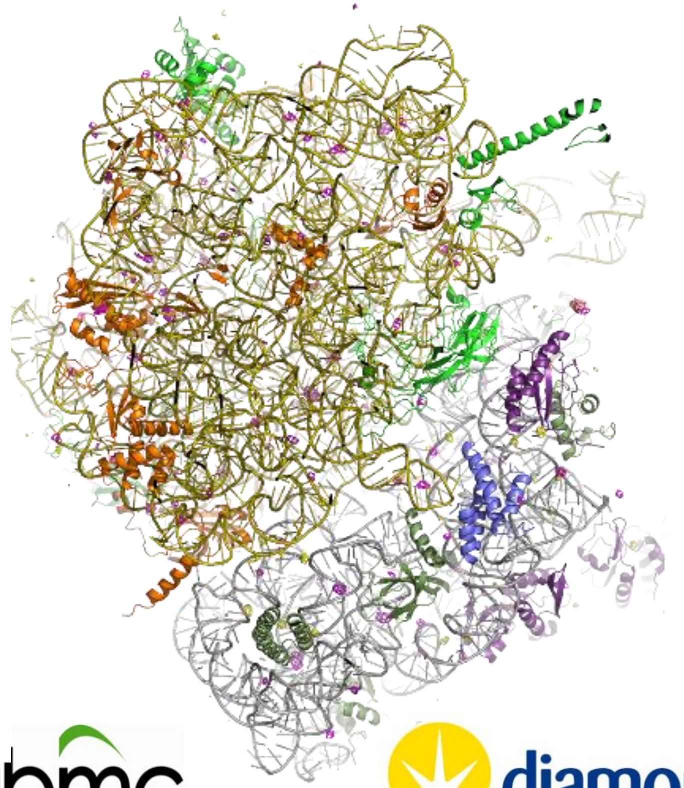


S. Newstead

K identification

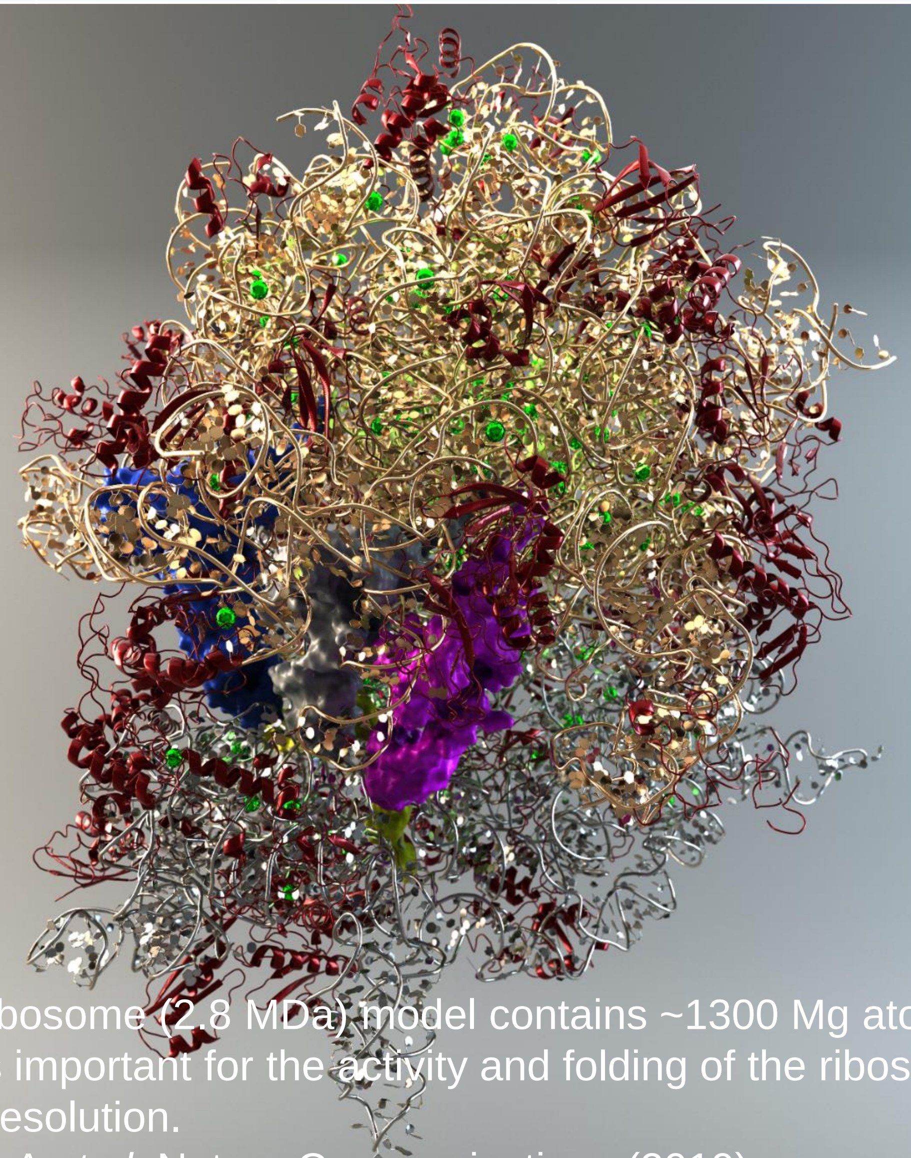
- Experiments above and below the K-edge (λ=3.44 Å).





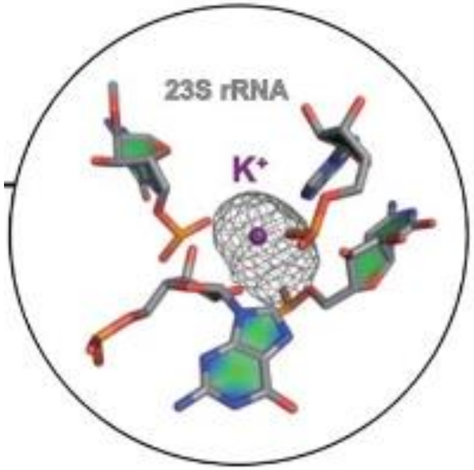
A. Rozov & G. Yusupova

	Mg (pdb)	K^+	Mg^{2+}	$Mg(H_2O)_6^{2+}$
IC	3255	249	620	90
EC	1439	131	394	74

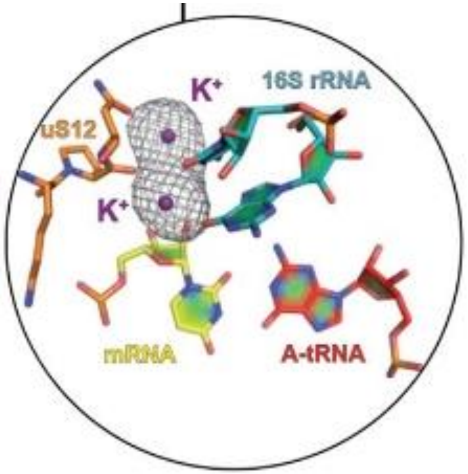


70S ribosome (2.8 MDa) model contains ~1300 Mg atoms.
K ions important for the activity and folding of the ribosome.
~4 Å resolution.
Rozov A *et al.* Nature Communications (2019).

Peptidyl transferase center

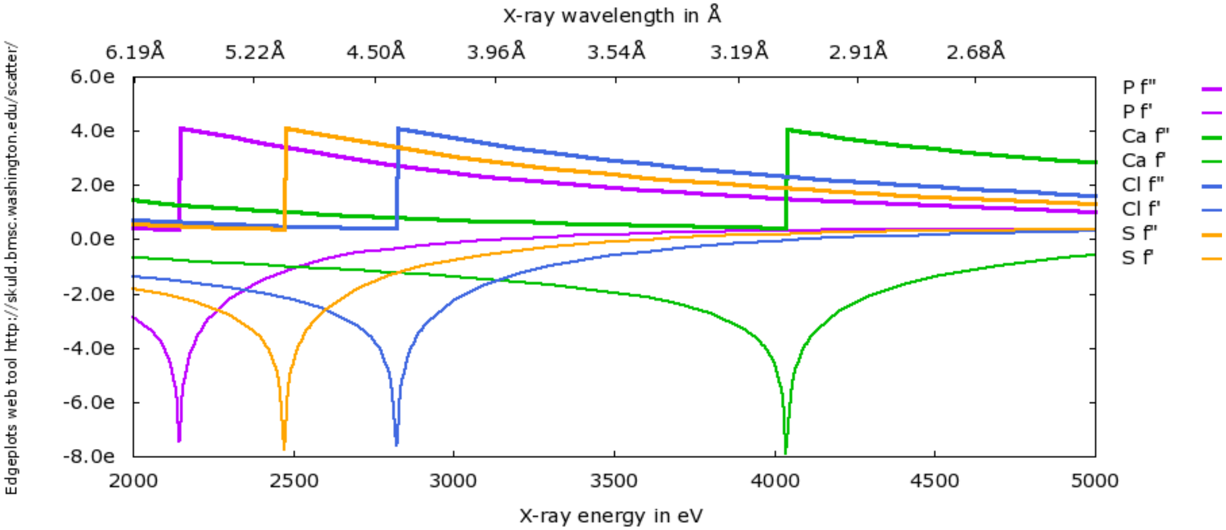


Decoding center

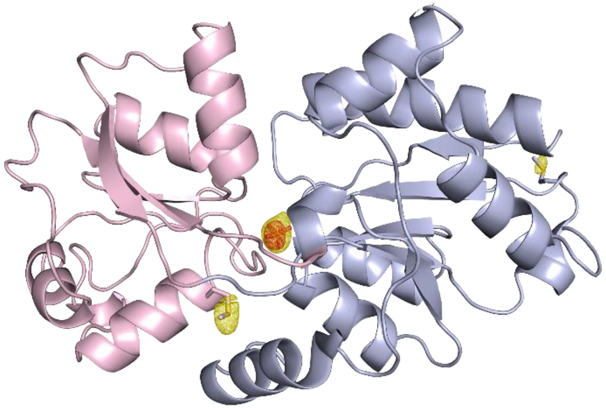


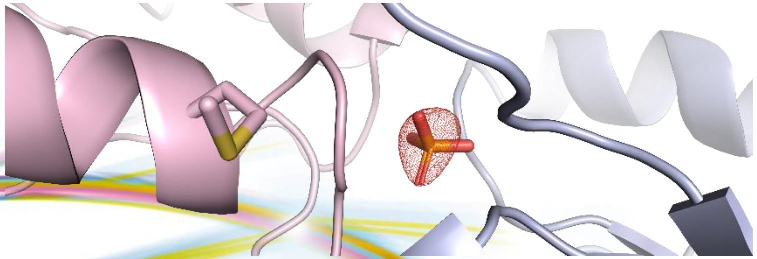
Alexey Rozov
Iskander Khusainov
Gulnara Yusupova

Discriminating between sulphate and phosphate

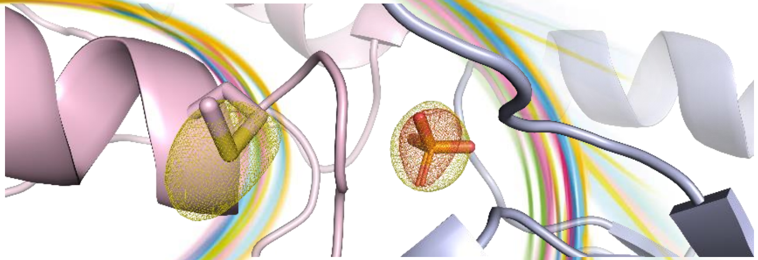


Edgeplots web tool http://skuld.bmsc.washington.edu/scatter/

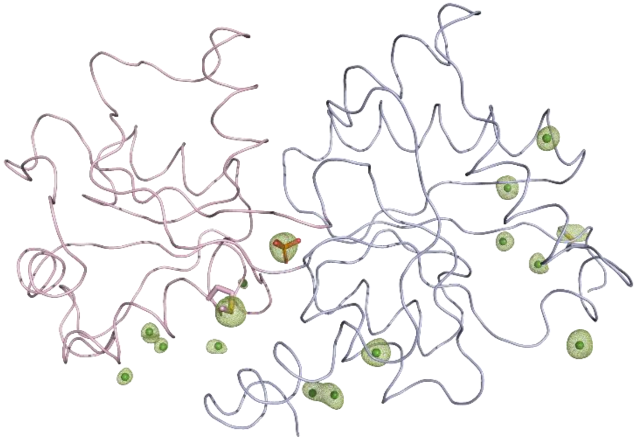




E = 2.4 keV
Below sulphur edge



E = 2. 4 keV
E = 2.55 keV
Above sulphur edge



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- Vinay Grama
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- Arvinder Palaha
- James O’Hea
- Paul Hathaway
- Kevin Wilkinson

- Dave Stuart

- Wolfgang Kabsch
- Gerard Bricogne

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igbmc institut de génétique et de biologie moléculaire et cellulaire

GΦL

University of St Andrews

MRC Laboratory of Molecular Biology

University of Portsmouth

OPPF-UK

Imperial College London

ICR The Institute of Cancer Research

Research Complex at Harwell

The Francis Crick Institute

Newcastle University

DIALS Diffraction Integration for Advanced Light Sources

diamond

Conclusions

- I23 delivers a high anomalous signal-to-noise ratios thanks to:
  - reduction of background (in vacuum)
  - Increase of anomalous signal (long wavelength)

- High multiplicity for S-SAD is not needed anymore even with low symmetry SG. Useful if non-isomorphous crystals!

- Successful SAD experiments with S, K, P, Cl, Ca, V, Cd, I, Au.

- Unique opportunities to identify Cl, K, Ca ions.

Beamline homepage for further information:

https://www.diamond.ac.uk/Instruments/Mx/I23.html

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