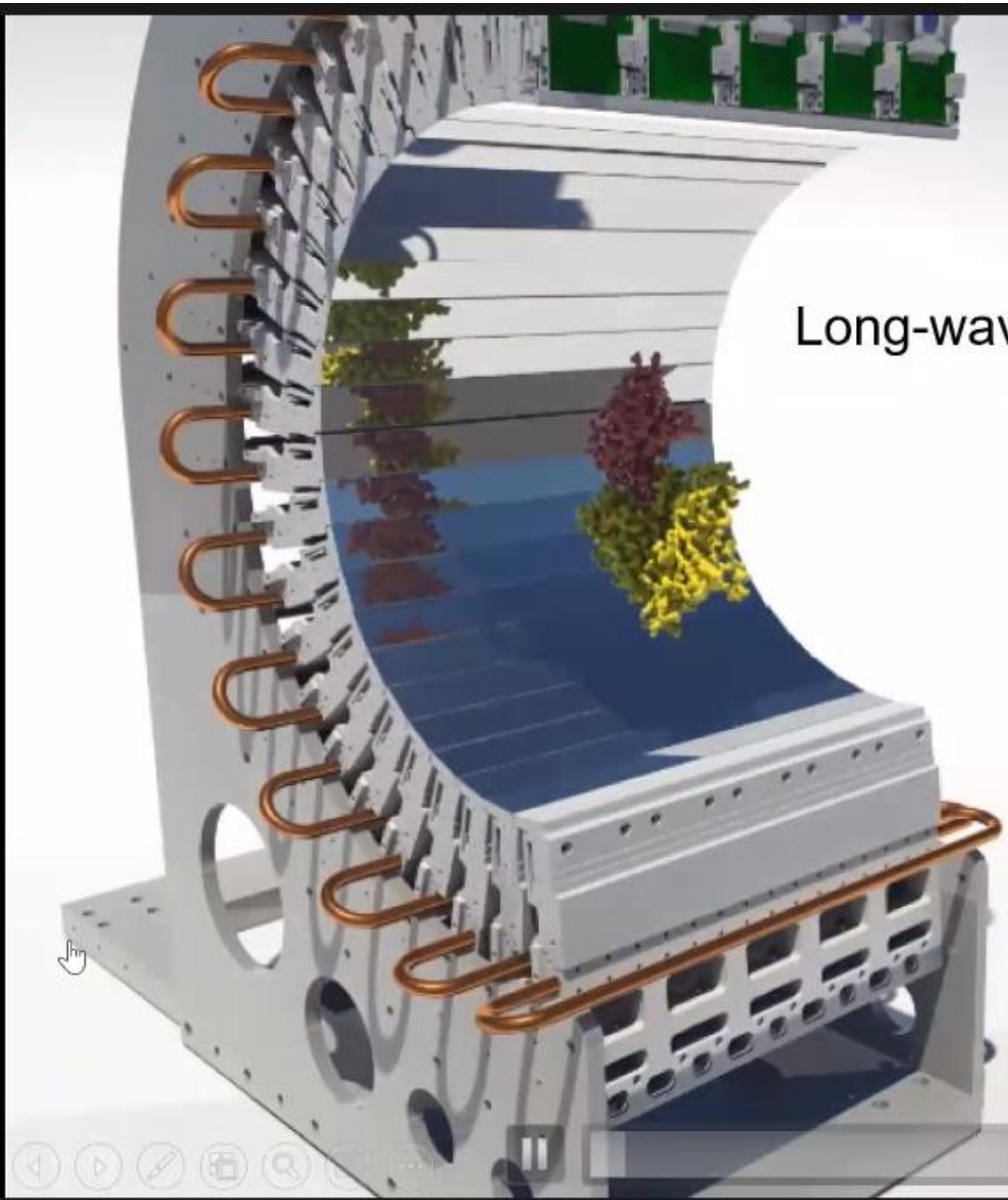
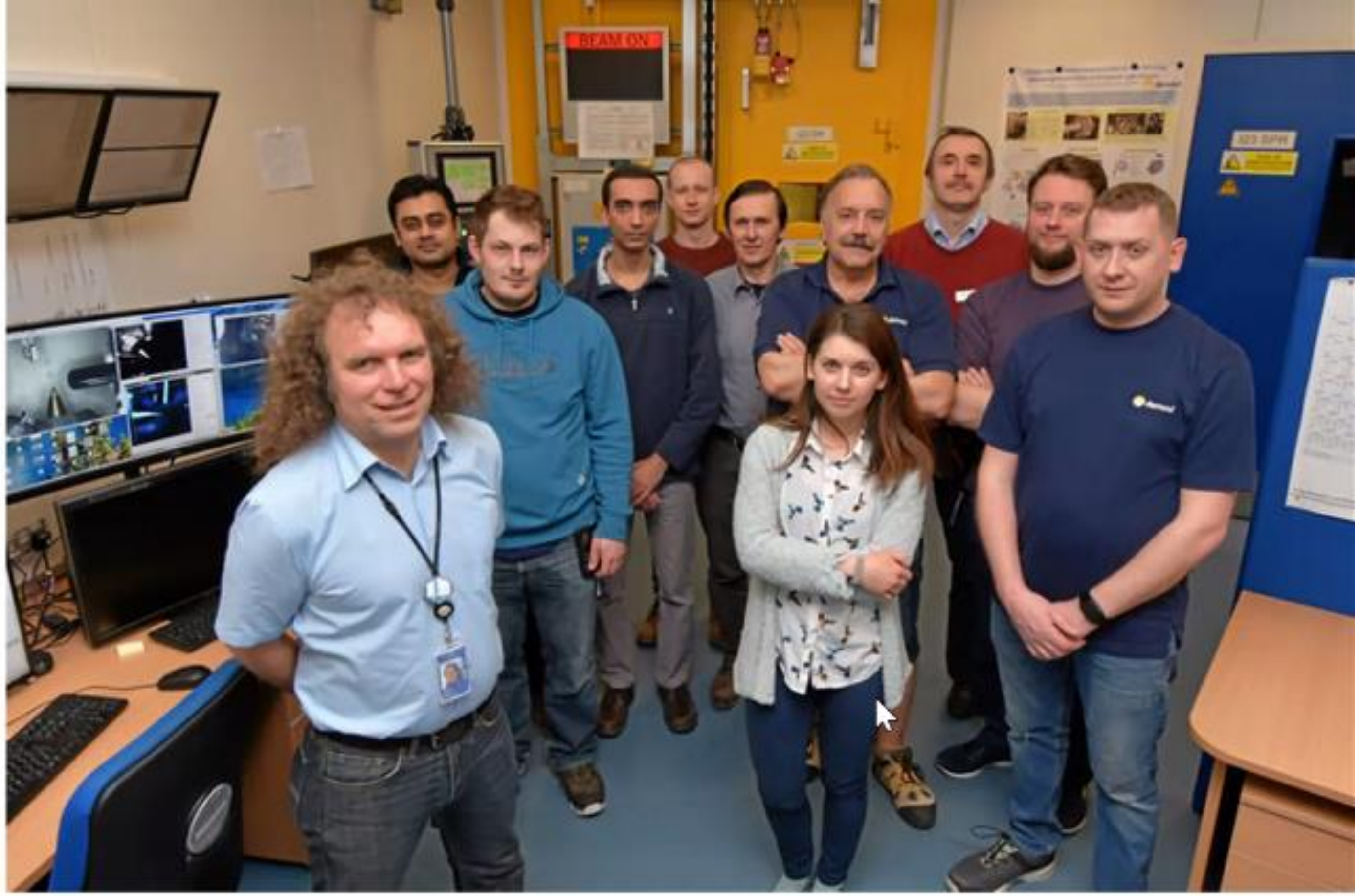


Long-wavelength crystallography

Armin Wagner

BAG training Dec 2021

I23 Team



- Kamel el Omari
- Ramona Duman
- Vitaliy Mykhaylyk
- Vinay Grama
- Adam Taylor
- Adam Prescott

- Arvinder Palaha
- James O'Hea
- Paul Hathaway
- Kevin Wilkinson

- Dave Stuart
- Andrew Leslie

- Wolfgang Kabsch
- Gerard Bricogne

University of Oxford
igbmc
GΦL
University of St Andrews
MRC Laboratory of Molecular Biology
University of Portsmouth
Research Complex at Harwell
OPPF-UK
Imperial College London
ICR The Institute of Cancer Research
The Francis Crick Institute
iNEXT Discovery
DIALS
diamond

Why should you consider I23?

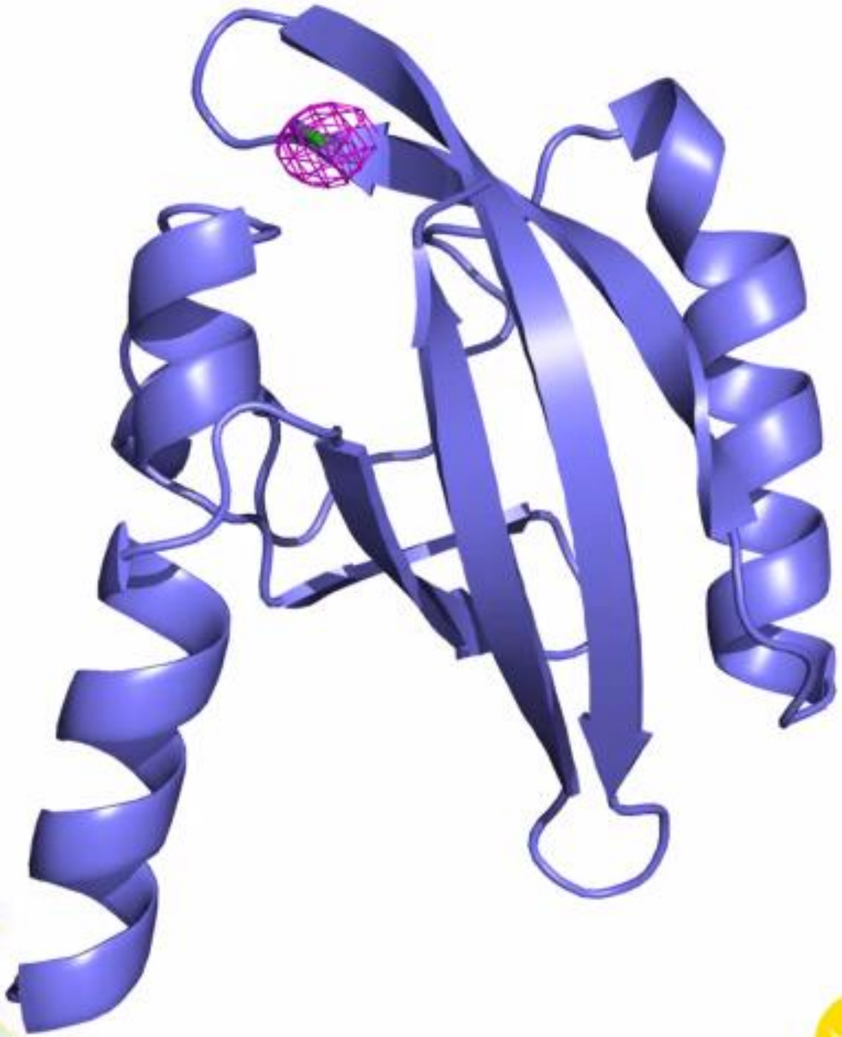
Anomalous contrast

Experimental phasing
SAD / MAD

MR-SAD phasing

Assist model building

Element identification



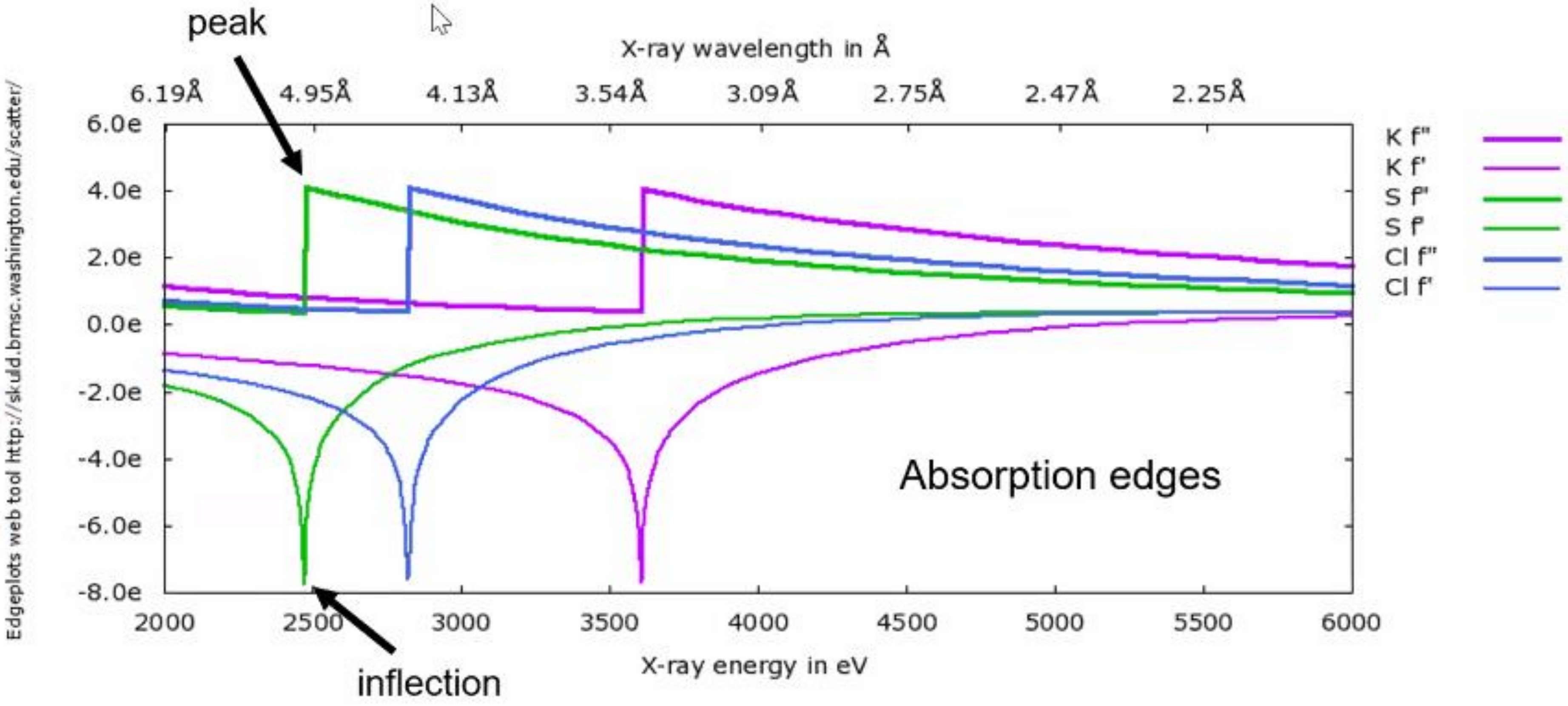
Anomalous Contrast

- Atomic scattering factor

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

f_0 depends on Bragg angle

$\Delta f'$ and f'' depend on energy

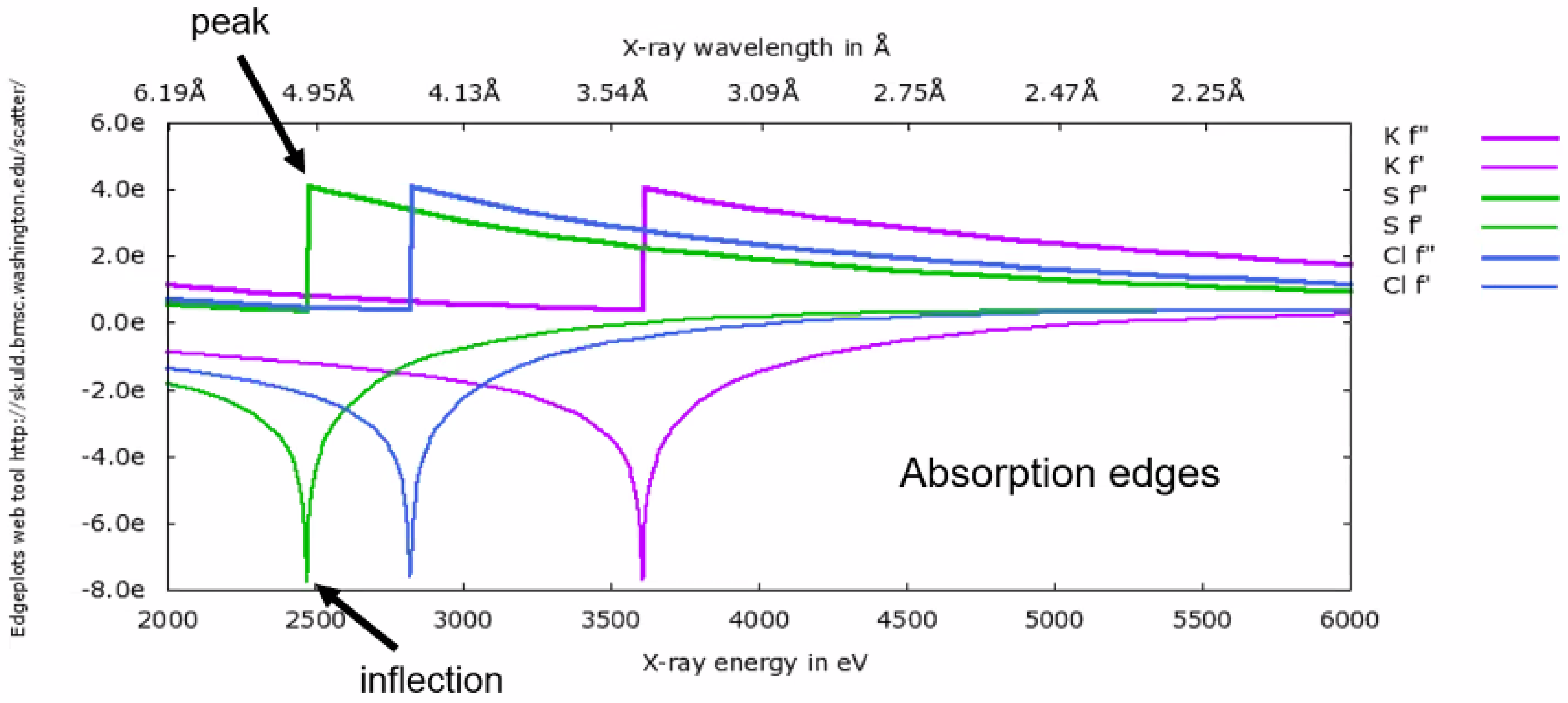


Anomalous Contrast

Friedel's law breaks!

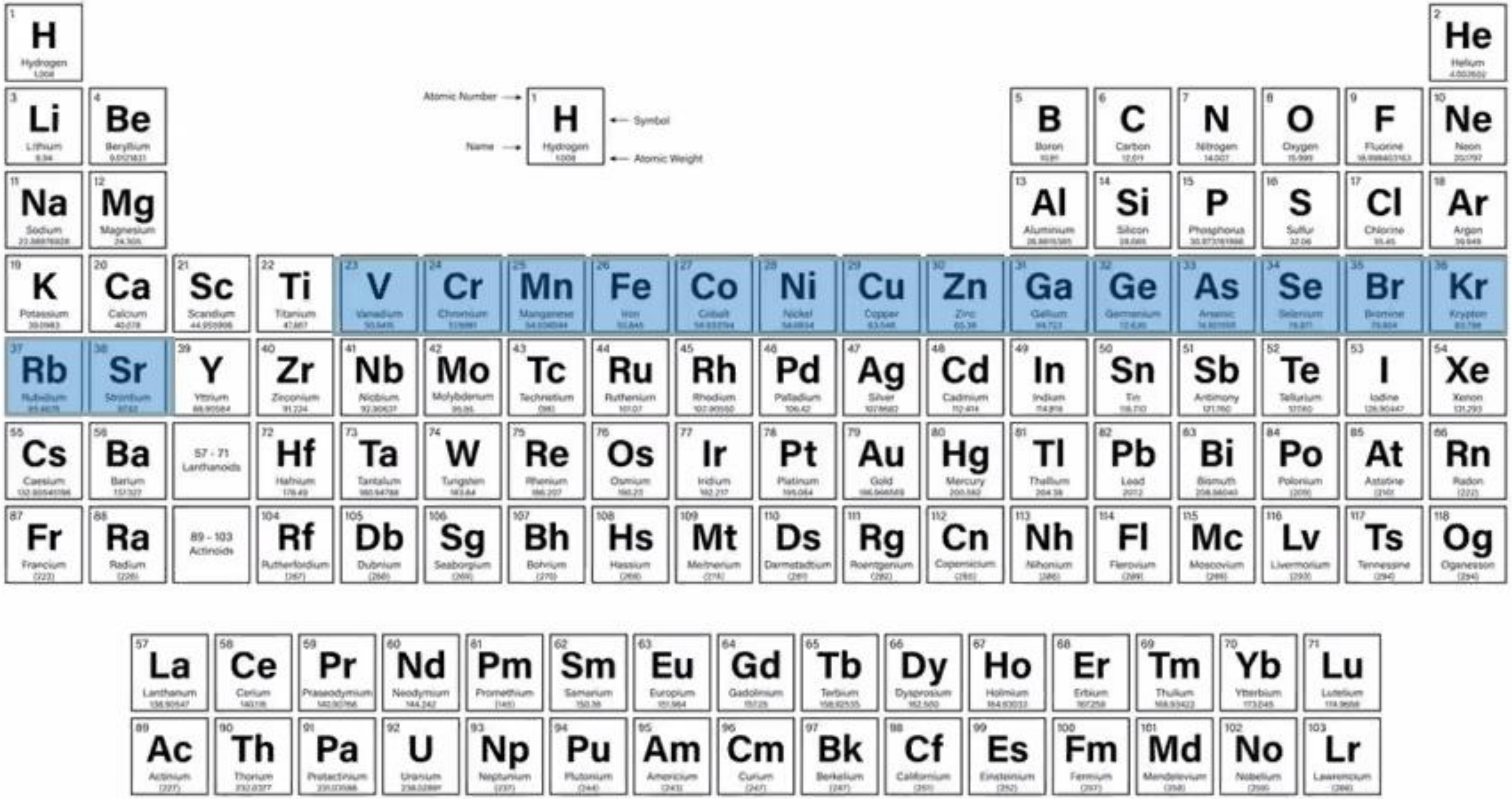
$$|F_{hkl}| \neq |F_{\overline{hkl}}|$$

$$\alpha_{hkl} \neq -\alpha_{\overline{hkl}}$$



Periodic Table of the Elements

I23	λ = 5.9 – 1.1 Å
I03	λ = 2.3 – 0.7 Å



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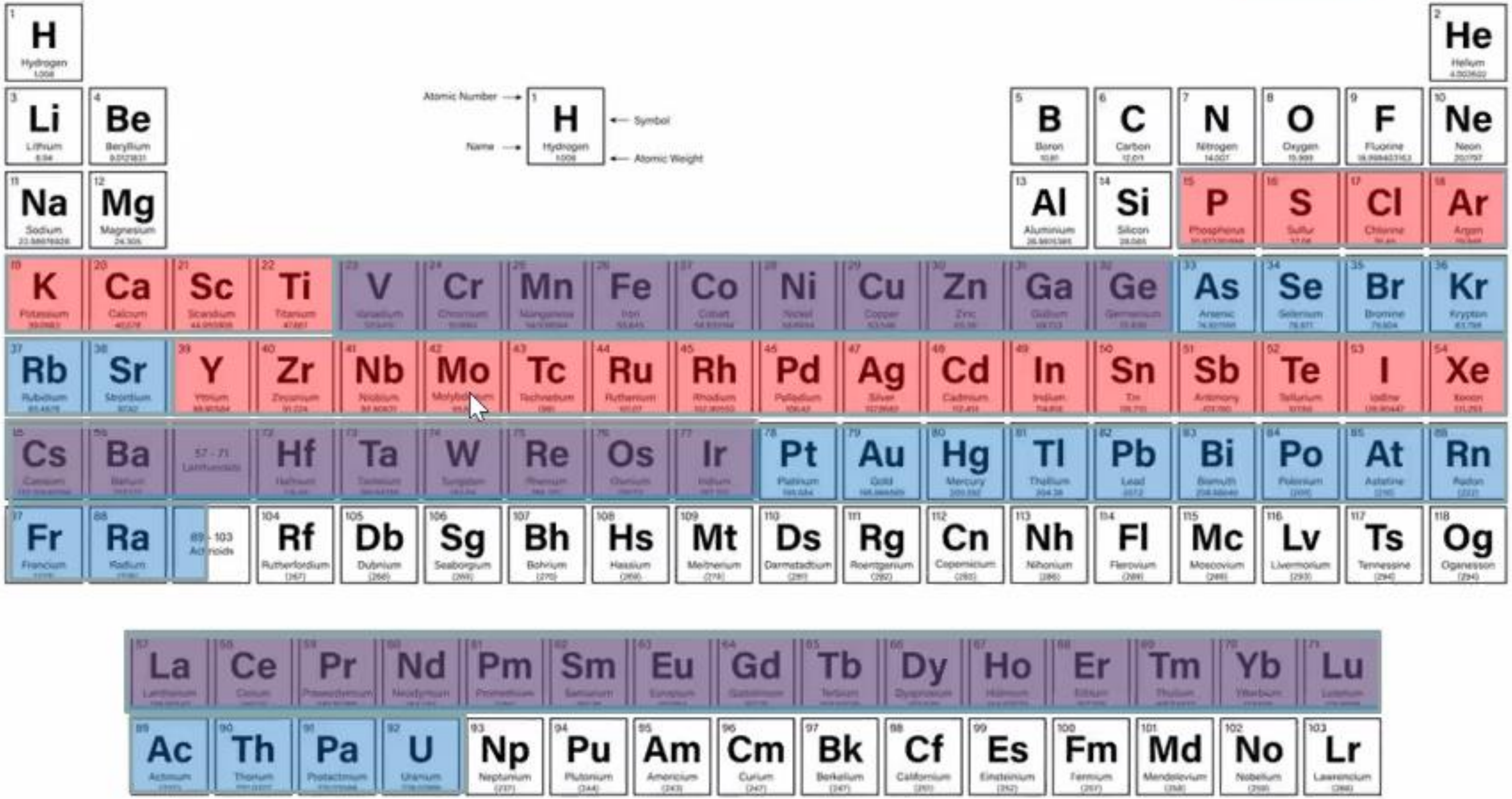

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	E [keV]	λ [Å]
P	2.146	5.779
S	2.472	5.016
Cl	2.822	4.393
K	3.607	3.437
Ca	4.038	3.070

λ [Å] = 12.398 / E [keV]

In-vacuum crystallography

- Elimination of air scattering and absorption

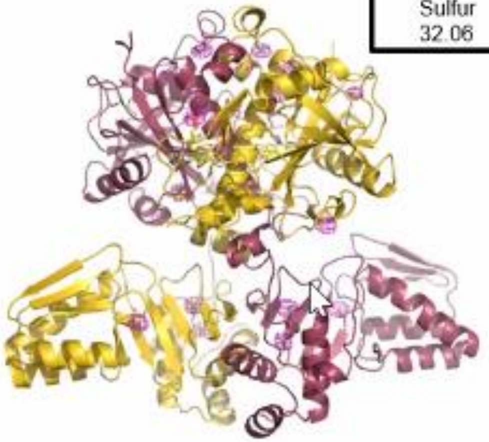
- Several challenges had to be overcome

  - Conductive sample cooling
  - Design of conductive sample mounts
  - Sample transfer into vacuum
  - Sophisticated anti-collision system

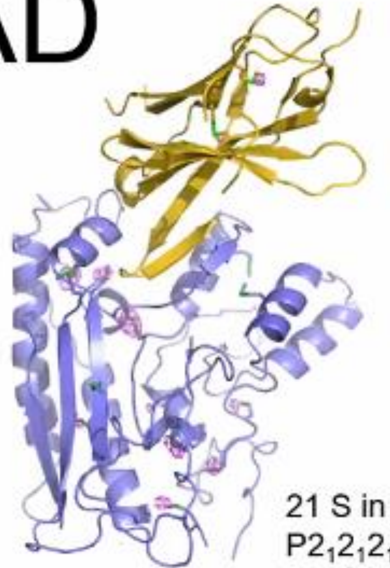
diamond

16 S Sulfur 32.06 - SAD

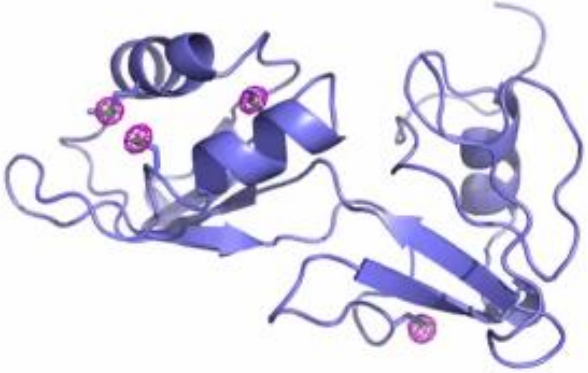
λ = 2.75 Å
Multiplicity: 10 - 20



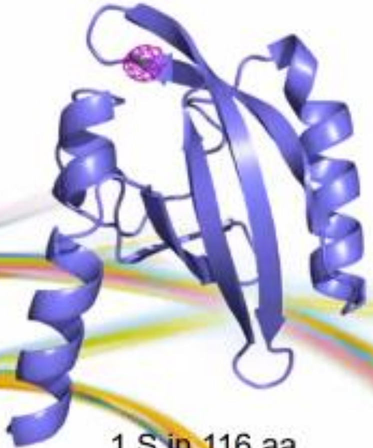
10 S in 477 aa
$P4_12_12_1$
3.2 Å res



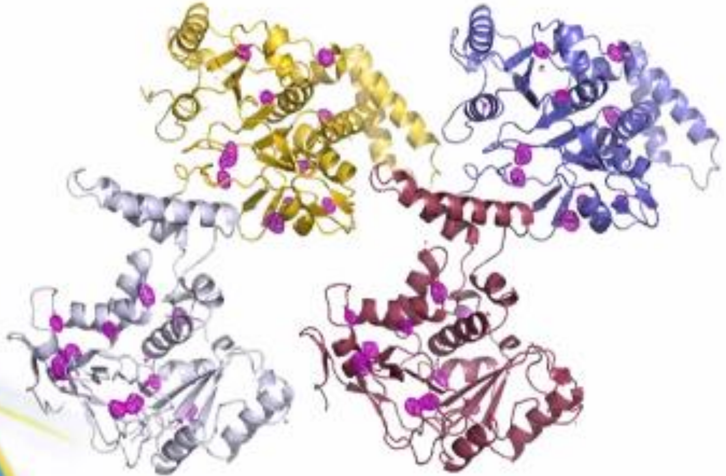
21 S in 427 aa
$P2_12_12_1$
2.8 Å res



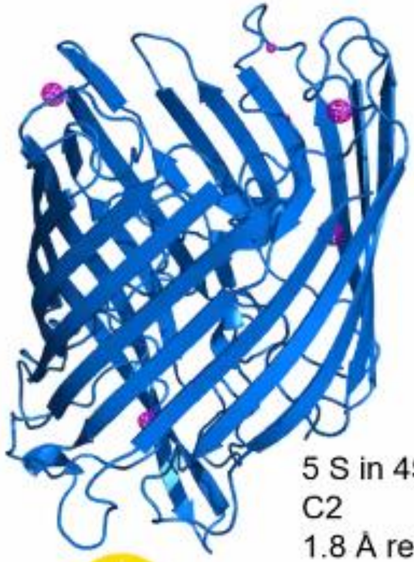
4 S in 157 aa
C2
1.8 Å res



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

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Yosoku - I23 Resolution Requirement for Phasing

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

ASU PREDICT

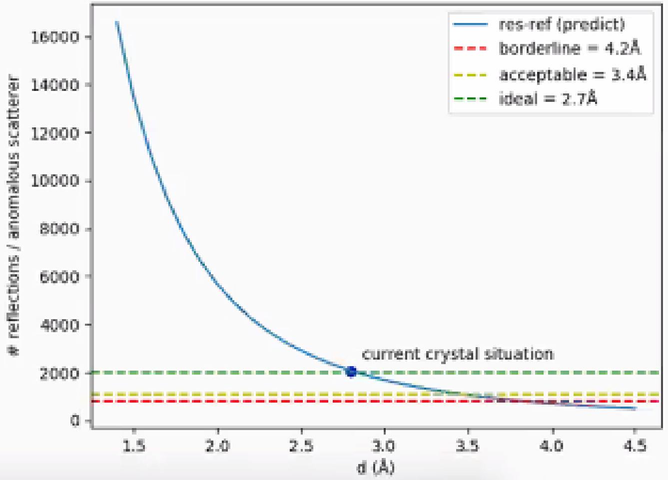
Solvent content analysis

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

CALCULATE | SAVE PLOT | RESET



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

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QKRIEEFFITGEGHFNEVLQFRTPGTLSTTESTPAGLPTAEPFKSYFAKGFLSIDSGYYS
AKCYSGTSNSGLQLINITRHSTRIVDTPGPKITNLKTINCINLKASIFKEHREVEINVLL
PQVAVNLSNCHVVIKSHVCDYSLDIDGAVRLPHIYHEGVFIPGTYKIVIDKKNKLNDRCT
LFTDCVIKGREVRKGQSVLRQYKTEIRIGKASTGS

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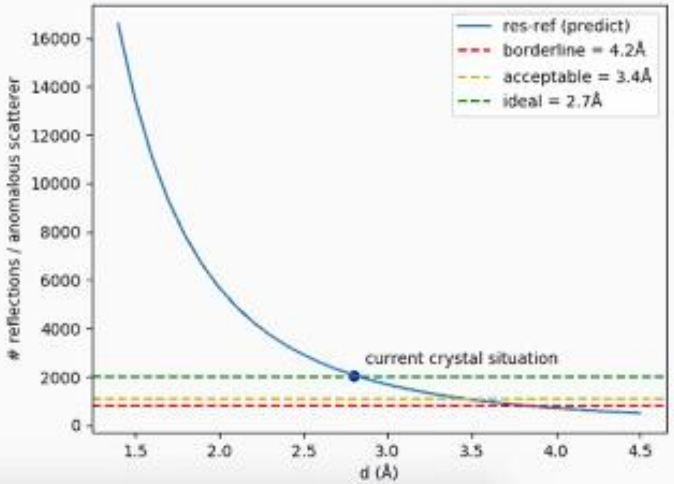
CALCULATE | SAVE PLOT | RESET

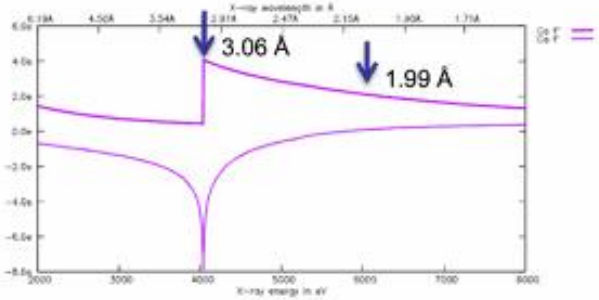


Based on:
Terwilliger et al. *"Can I solve my structure by SAD phasing? Anomalous signal in SAD Phasing"* Acta D (2016) 346.

Other SAD examples

20 Ca Calcium 40.078 - MAD

- 1.5 Ca in ~900 amino acids
- Space group $P6_522$
- λ = 3.06 Å, 1.99 Å
- Data to 3.8 Å resolution



Anomalous peak height: 15 σ

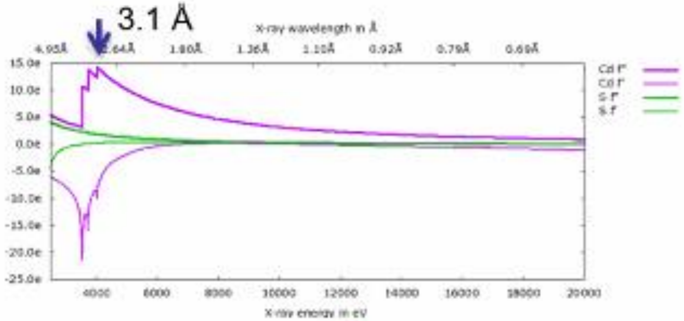
Image removed as unpublished

universität wien

K. Djinovic-Carugo

48 Cd Cadmium 112.41 - SAD

- 11 S / 146 amino acids
- Space group $P6_322$
- λ = 2.75 Å
- Inverse beam 2 x 180º
- Data to 3 Å resolution



Anomalous peak height: 10 – 17 σ

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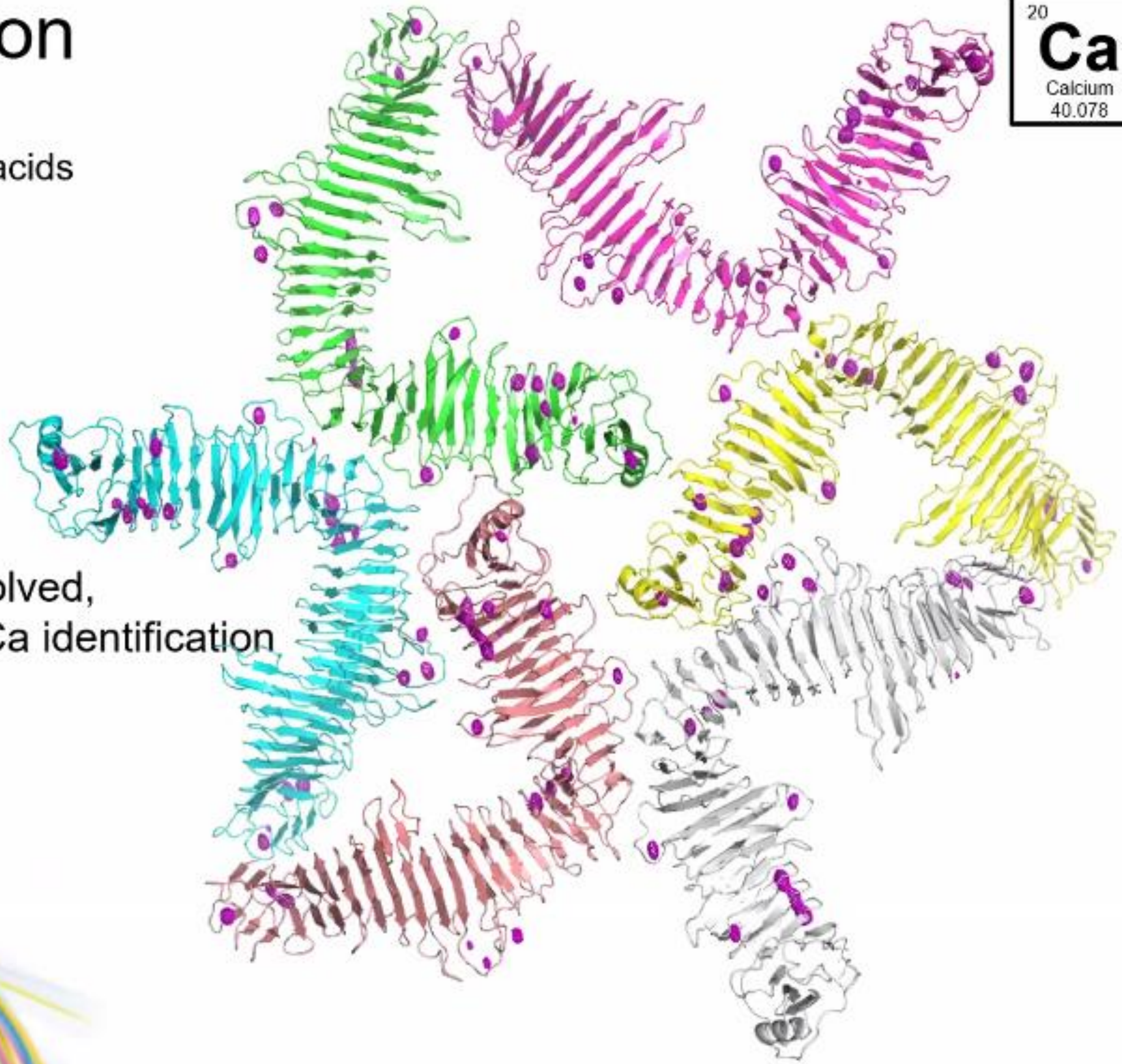
Pryce et al. J. Virol. (2019) 93 (1), e01048-18.

Ion identification

- 19 Ca built in 471 amino acids
- Space group $P2_12_12_1$
- λ = 3.02 Å
- Inverse beam 2 x 180º
- Data to 3.5 Å resolution

20
Ca
Calcium
40.078



User request: Structure solved, reviewer commented on Ca identification

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Tanmay Bharat

accepted in Structure

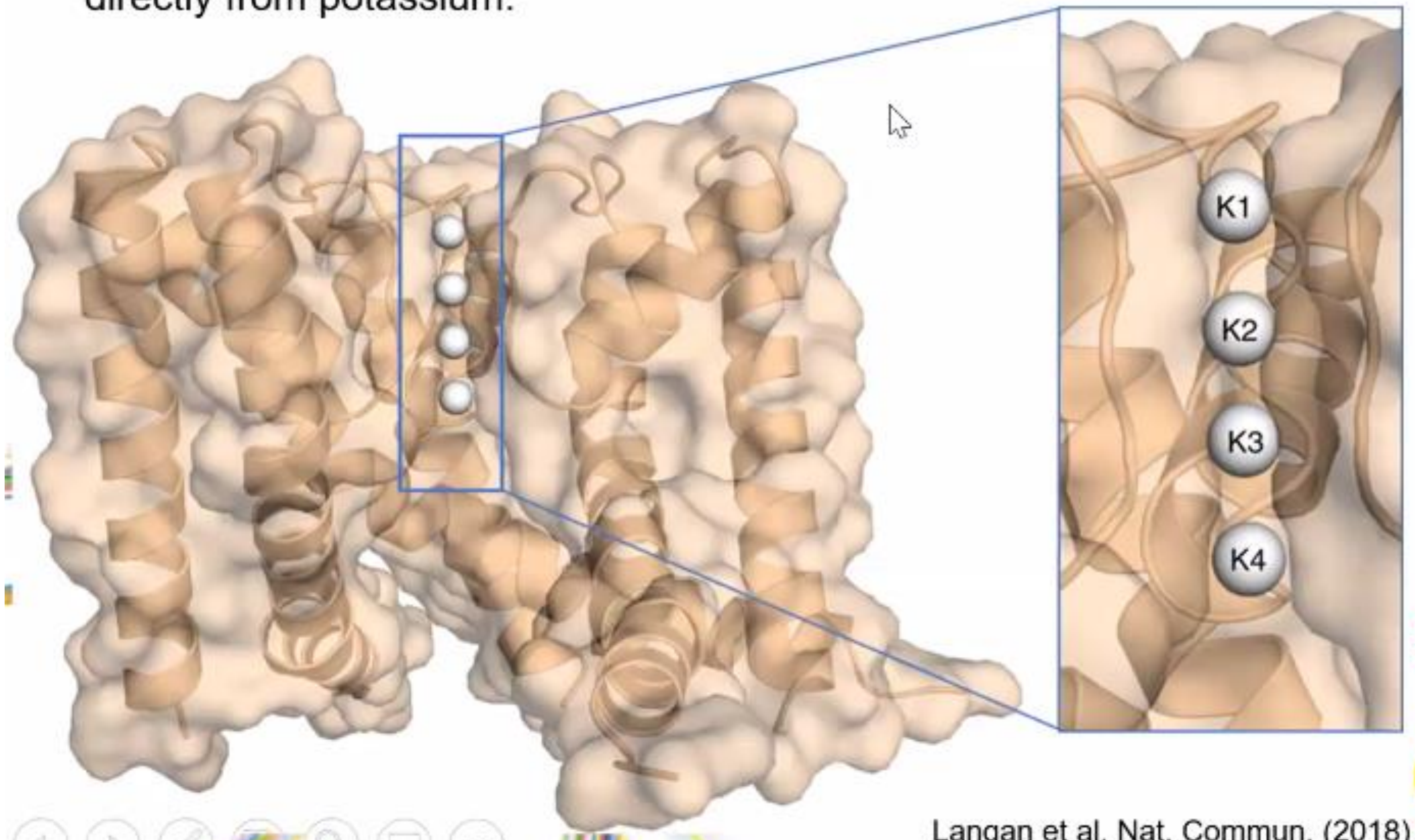
diamond

K – binding in the selectivity filter

19
K
Potassium
39.098

- In original work, based on substitution of K^+ by Tl^+, alternating model was proposed (MacKinnon), either position 1 and 3, or 2 and 4 occupied.
- Data was reprocessed by Kopfer et al., full occupancies proposed.

- Data collected at I23 allowed to confirm full occupancy model based on anomalous data directly from potassium.



OAK RIDGE National Laboratory

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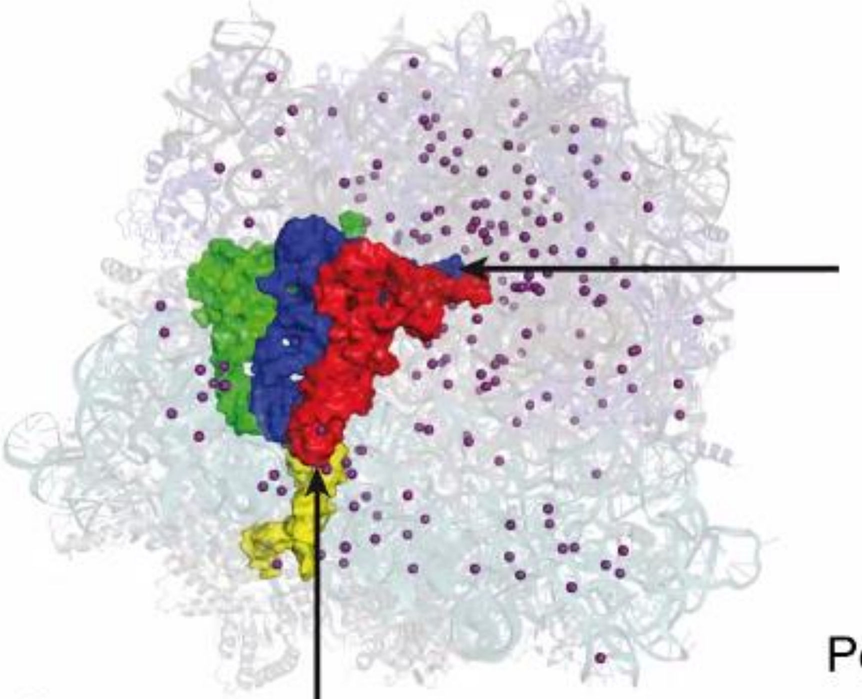
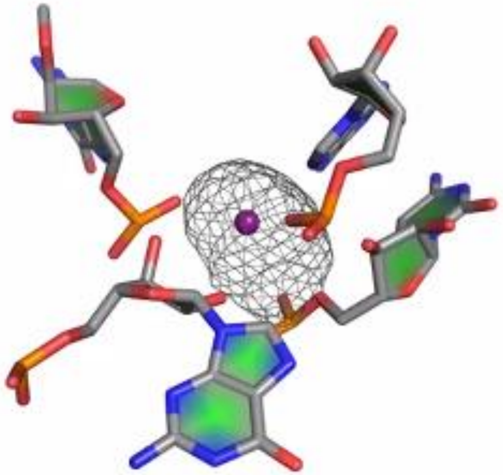
Langan et al. Nat. Commun. (2018) 9:4540.

70S Ribosome

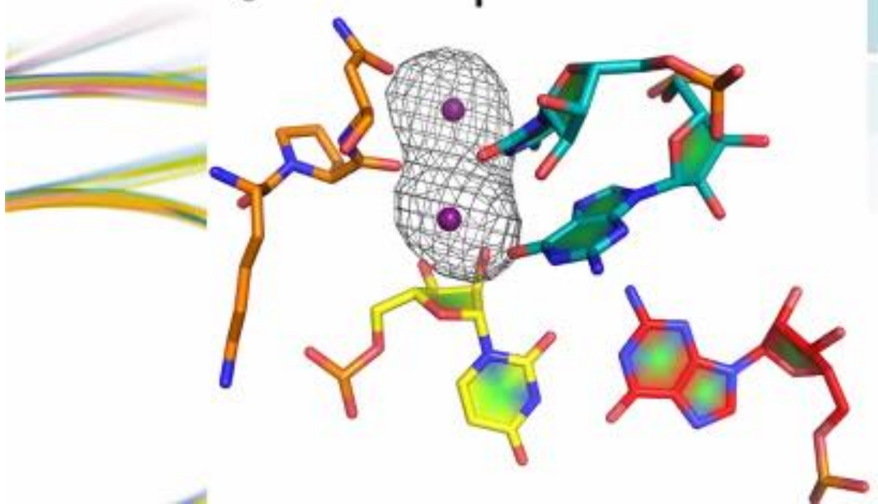
12 **Mg** Magnesium 24.305 or 19 **K** Potassium 39.098





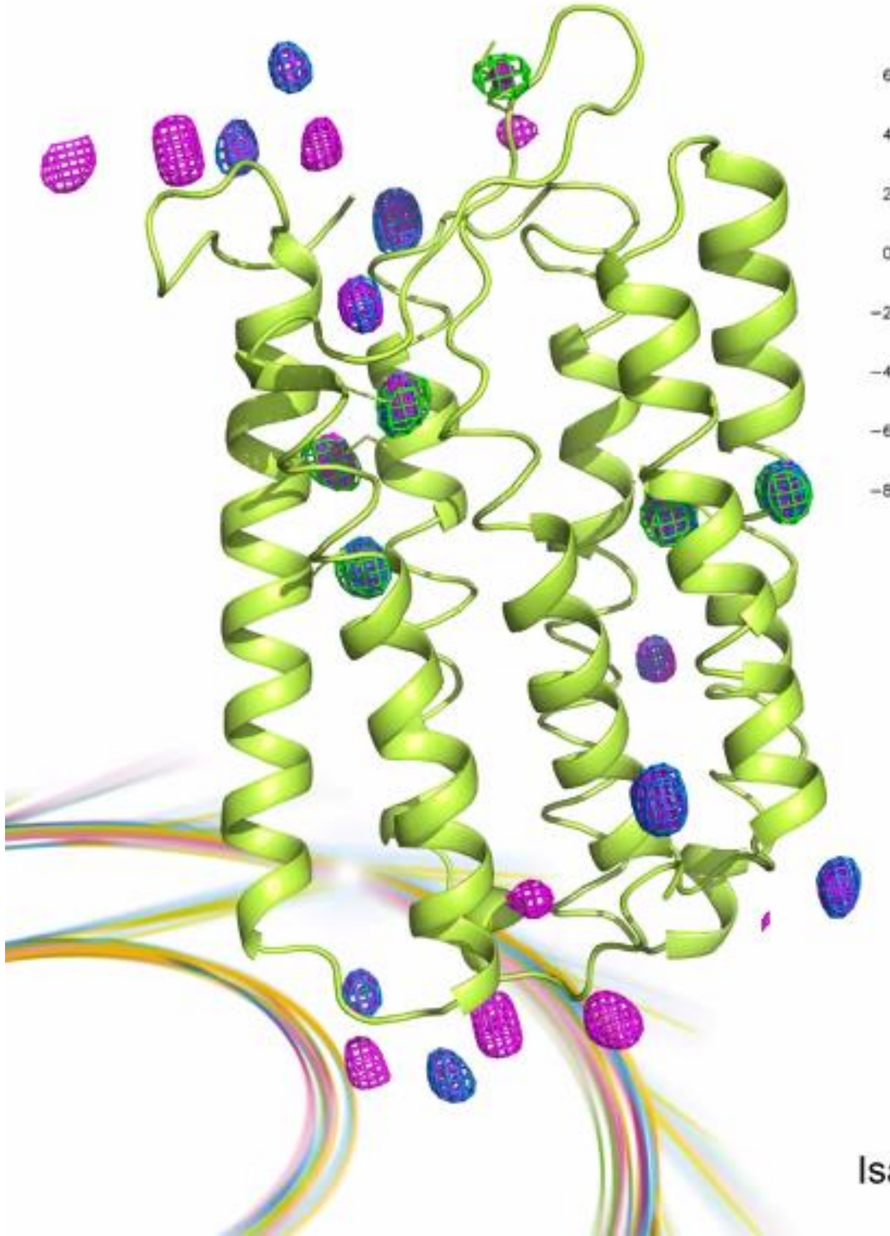
Peptidyl transferase centre

Decoding centre

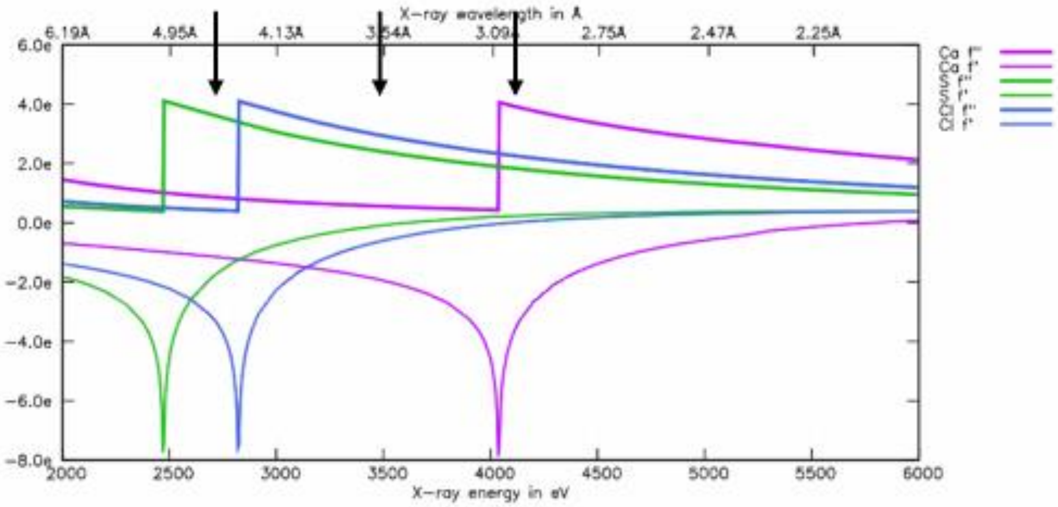


	Mg (pdb)	K^+	Mg^{2+}	$Mg(H_2O)_6^{2+}$
IC	3255	249	620	90
EC	1439	131	394	74

Rozov et al. Nat. Commun. (2019)



16 **S** Sulfur 32.06	17 **Cl** Chlorine 35.45	20 **Ca** Calcium 40.078



Anomalous difference
Fourier maps:

green: λ = 4.428 Å
blue: λ = 3.542 Å
magenta: λ = 2.952 Å

Isabel de Moras

NPL National Physical Laboratory

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How to access I23?

- Quick access for BAGs available, no additional proposals needed.
- Staff assists with sample transfer, setup of experiments and data analysis.
- Sample holders and tools will be shipped on request.

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

- **Armin.Wagner@Diamond.ac.uk**

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