

# Long-wavelength crystallography

Armin Wagner

BAG training Dec 2021

Halina Mikolajek



Armin Wagner

# 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



# Why should you consider I23?

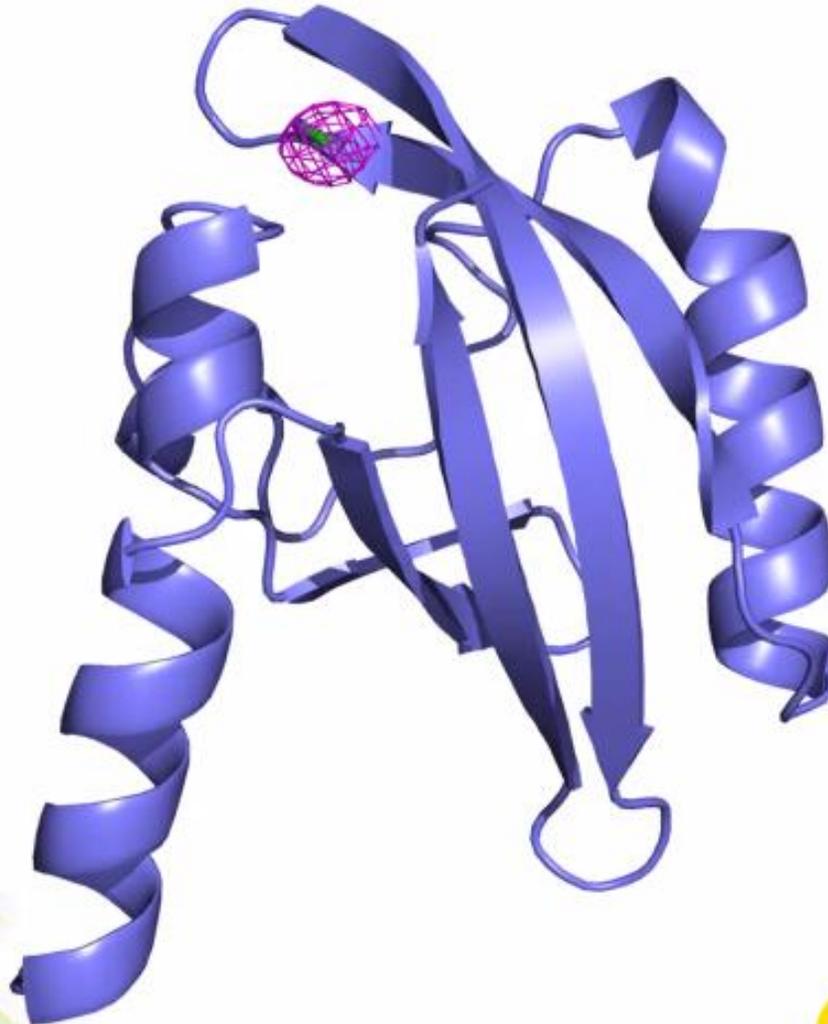
Anomalous contrast

Experimental phasing  
SAD / MAD

MR-SAD phasing

Assist model building

Element identification



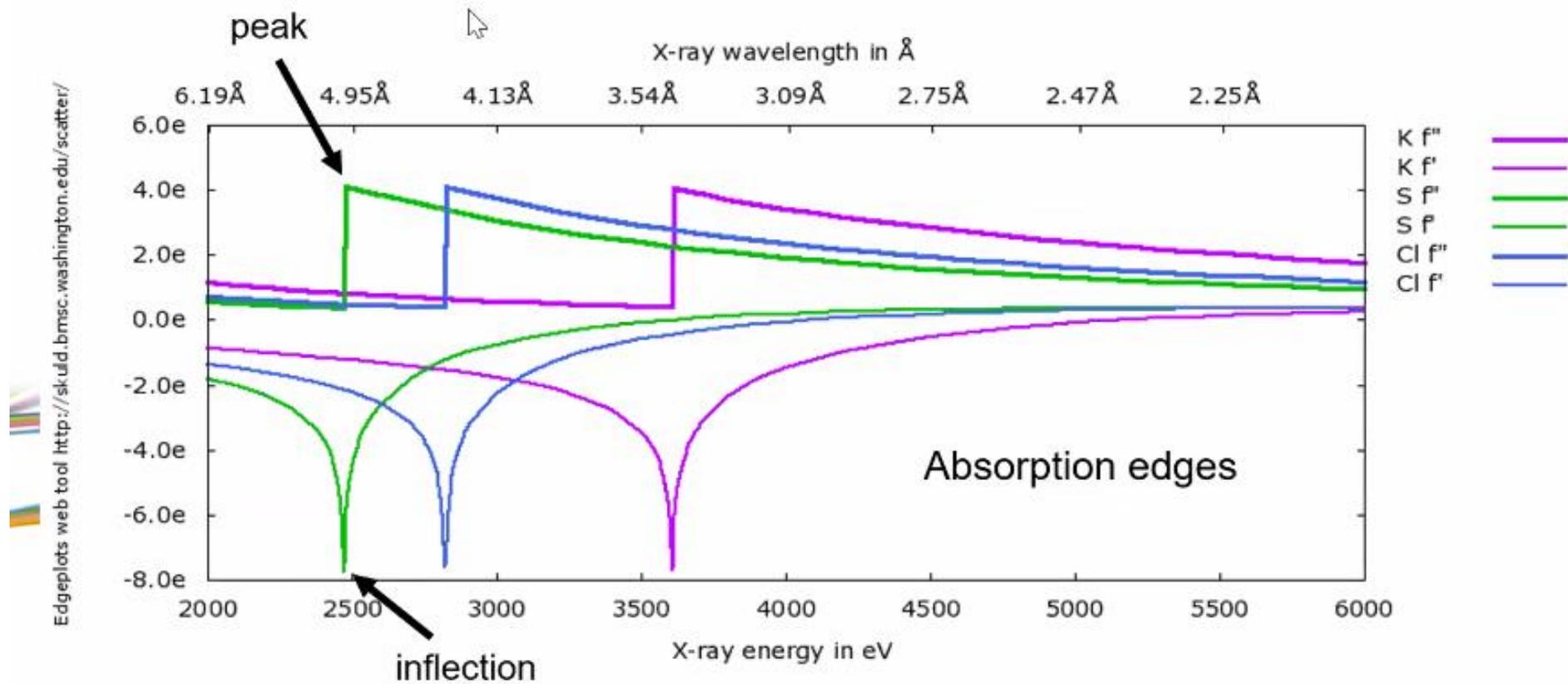
# Anomalous Contrast

- Atomic scattering factor

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

$f_0$  depends on Bragg angle

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

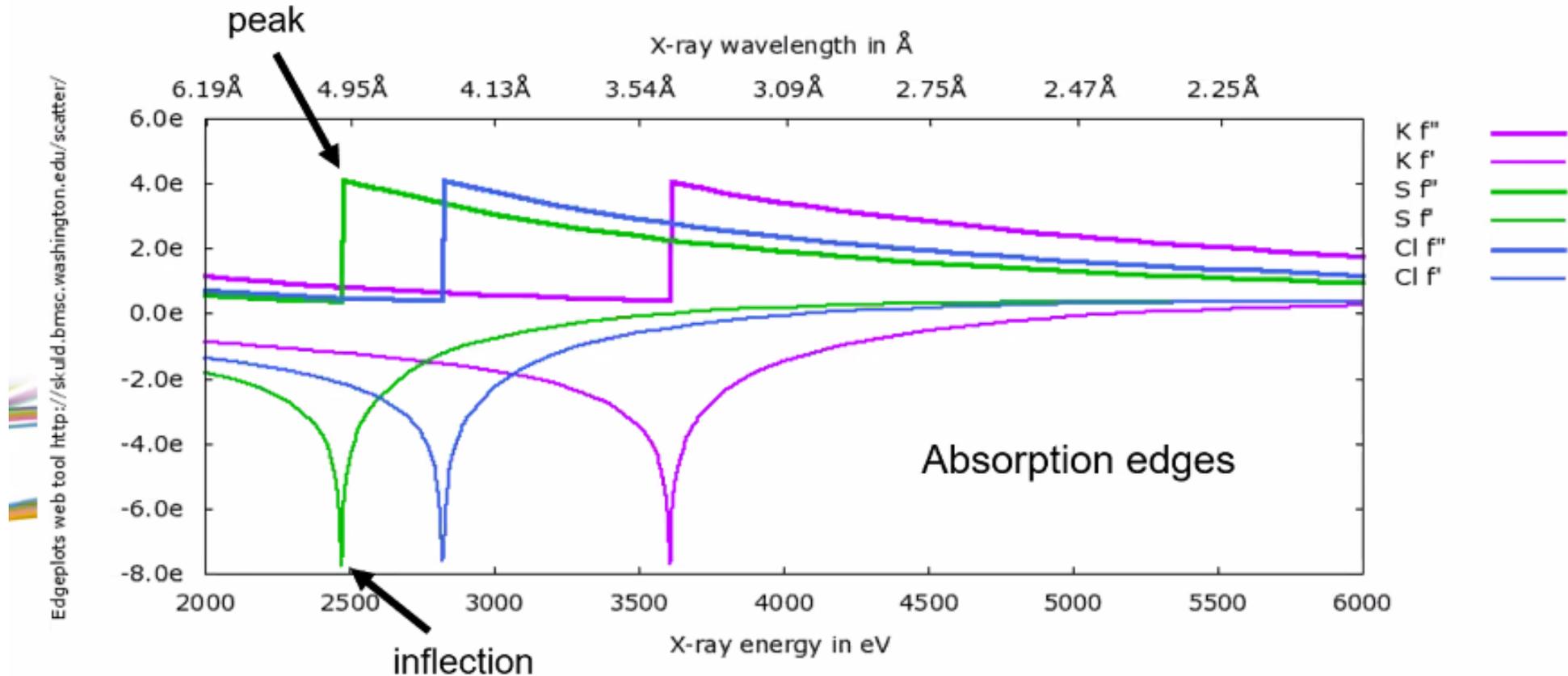


# Anomalous Contrast

Friedel's law breaks!

$$|F_{hkl}| \neq |F_{\bar{h}\bar{k}\bar{l}}|$$

$$\alpha_{hkl} \neq -\alpha_{\bar{h}\bar{k}\bar{l}}$$



# Periodic Table of the Elements

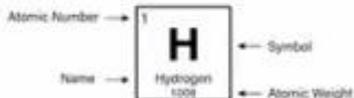
123

$$\lambda = 5.9 - 1.1 \text{ \AA}$$

103

$$\lambda = 2.3 - 0.7 \text{ \AA}$$

1 <b>H</b> Hydrogen 1.008																	2 <b>He</b> Helium 4.002602						
3 <b>Li</b> Lithium 6.94	4 <b>Be</b> Beryllium 9.012182																	5 <b>B</b> Boron 10.81	6 <b>C</b> Carbon 12.01	7 <b>N</b> Nitrogen 14.007	8 <b>O</b> Oxygen 15.999	9 <b>F</b> Fluorine 18.99840323	10 <b>Ne</b> Neon 20.1797
11 <b>Na</b> Sodium 22.98976928	12 <b>Mg</b> Magnesium 24.305																	13 <b>Al</b> Aluminum 26.9815385	14 <b>Si</b> Silicon 28.085	15 <b>P</b> Phosphorus 30.973761998	16 <b>S</b> Sulfur 32.06	17 <b>Cl</b> Chlorine 35.45	18 <b>Ar</b> Argon 39.948
19 <b>K</b> Potassium 39.0983	20 <b>Ca</b> Calcium 40.078	21 <b>Sc</b> Scandium 44.955908	22 <b>Ti</b> Titanium 47.867	23 <b>V</b> Vanadium 50.9415	24 <b>Cr</b> Chromium 51.9961	25 <b>Mn</b> Manganese 54.938044	26 <b>Fe</b> Iron 55.845	27 <b>Co</b> Cobalt 58.933194	28 <b>Ni</b> Nickel 58.6934	29 <b>Cu</b> Copper 63.546	30 <b>Zn</b> Zinc 65.38	31 <b>Ga</b> Gallium 69.723	32 <b>Ge</b> Germanium 72.630	33 <b>As</b> Arsenic 74.921595	34 <b>Se</b> Selenium 78.9718	35 <b>Br</b> Bromine 79.904	36 <b>Kr</b> Krypton 83.798						
37 <b>Rb</b> Rubidium 85.4678	38 <b>Sr</b> Strontium 87.62	39 <b>Y</b> Yttrium 88.90584	40 <b>Zr</b> Zirconium 91.224	41 <b>Nb</b> Niobium 92.90637	42 <b>Mo</b> Molybdenum 95.94	43 <b>Tc</b> Technetium (98)	44 <b>Ru</b> Ruthenium 101.07	45 <b>Rh</b> Rhodium 102.90550	46 <b>Pd</b> Palladium 106.42	47 <b>Ag</b> Silver 107.8682	48 <b>Cd</b> Cadmium 112.414	49 <b>In</b> Indium 114.818	50 <b>Sn</b> Tin 118.710	51 <b>Sb</b> Antimony 121.757	52 <b>Te</b> Tellurium 127.60	53 <b>I</b> Iodine 126.90447	54 <b>Xe</b> Xenon 131.29						
55 <b>Cs</b> Caesium 132.90545196	56 <b>Ba</b> Barium 137.327	57 - 71 Lanthanoids	72 <b>Hf</b> Hafnium 178.49	73 <b>Ta</b> Tantalum 180.94788	74 <b>W</b> Tungsten 183.84	75 <b>Re</b> Rhenium 186.207	76 <b>Os</b> Osmium 190.23	77 <b>Ir</b> Iridium 192.222	78 <b>Pt</b> Platinum 195.084	79 <b>Au</b> Gold 196.966569	80 <b>Hg</b> Mercury 200.59	81 <b>Tl</b> Thallium 204.38	82 <b>Pb</b> Lead 207.2	83 <b>Bi</b> Bismuth 208.9804	84 <b>Po</b> Polonium (209)	85 <b>At</b> Astatine (210)	86 <b>Rn</b> Radon (222)						
87 <b>Fr</b> Francium (223)	88 <b>Ra</b> Radium (226)	89 - 103 Actinoids	104 <b>Rf</b> Rutherfordium (261)	105 <b>Db</b> Dubnium (261)	106 <b>Sg</b> Seaborgium (266)	107 <b>Bh</b> Bohrium (270)	108 <b>Hs</b> Hassium (285)	109 <b>Mt</b> Meitnerium (276)	110 <b>Ds</b> Darmstadtium (281)	111 <b>Rg</b> Roentgenium (288)	112 <b>Cn</b> Copernicium (285)	113 <b>Nh</b> Nihonium (286)	114 <b>Fl</b> Flerovium (289)	115 <b>Mc</b> Moscovium (288)	116 <b>Lv</b> Livermorium (293)	117 <b>Ts</b> Tennessine (294)	118 <b>Og</b> Oganesson (294)						



57 <b>La</b> Lanthanum 138.9047	58 <b>Ce</b> Cerium 140.12	59 <b>Pr</b> Praseodymium 140.90766	60 <b>Nd</b> Neodymium 144.242	61 <b>Pm</b> Promethium (145)	62 <b>Sm</b> Samarium 150.36	63 <b>Eu</b> Europium 151.964	64 <b>Gd</b> Gadolinium 157.25	65 <b>Tb</b> Terbium 158.92535	66 <b>Dy</b> Dysprosium 162.502	67 <b>Ho</b> Holmium 164.93032	68 <b>Er</b> Erbium 167.259	69 <b>Tm</b> Thulium 168.93402	70 <b>Yb</b> Ytterbium 173.045	71 <b>Lu</b> Lutetium 174.967
89 <b>Ac</b> Actinium (227)	90 <b>Th</b> Thorium 232.0377	91 <b>Pa</b> Protactinium 231.03688	92 <b>U</b> Uranium 238.02891	93 <b>Np</b> Neptunium (237)	94 <b>Pu</b> Plutonium (244)	95 <b>Am</b> Americium (243)	96 <b>Cm</b> Curium (247)	97 <b>Bk</b> Berkelium (247)	98 <b>Cf</b> Californium (251)	99 <b>Es</b> Einsteinium (252)	100 <b>Fm</b> Fermium (257)	101 <b>Md</b> Mendelevium (288)	102 <b>No</b> Nobelium (289)	103 <b>Lr</b> Lawrencium (260)



# Periodic Table of the Elements

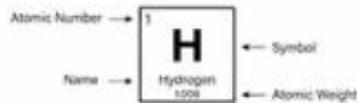
I23

$$\lambda = 5.9 - 1.1 \text{ \AA}$$

I03

$$\lambda = 2.3 - 0.7 \text{ \AA}$$

1 <b>H</b> Hydrogen 1.008																	2 <b>He</b> Helium 4.002602						
3 <b>Li</b> Lithium 6.94	4 <b>Be</b> Beryllium 9.012182																	5 <b>B</b> Boron 10.81	6 <b>C</b> Carbon 12.01	7 <b>N</b> Nitrogen 14.007	8 <b>O</b> Oxygen 15.999	9 <b>F</b> Fluorine 18.99847363	10 <b>Ne</b> Neon 20.1797
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19 <b>K</b> Potassium 39.0983	20 <b>Ca</b> Calcium 40.078	21 <b>Sc</b> Scandium 44.955912	22 <b>Ti</b> Titanium 47.867	23 <b>V</b> Vanadium 50.9415	24 <b>Cr</b> Chromium 51.9961	25 <b>Mn</b> Manganese 54.938044	26 <b>Fe</b> Iron 55.845	27 <b>Co</b> Cobalt 58.933194	28 <b>Ni</b> Nickel 58.6934	29 <b>Cu</b> Copper 63.546	30 <b>Zn</b> Zinc 65.38	31 <b>Ga</b> Gallium 69.723	32 <b>Ge</b> Germanium 72.630	33 <b>As</b> Arsenic 74.9216	34 <b>Se</b> Selenium 78.9718	35 <b>Br</b> Bromine 79.904	36 <b>Kr</b> Krypton 83.798						
37 <b>Rb</b> Rubidium 85.4678	38 <b>Sr</b> Strontium 87.62	39 <b>Y</b> Yttrium 88.90584	40 <b>Zr</b> Zirconium 91.224	41 <b>Nb</b> Niobium 92.90638	42 <b>Mo</b> Molybdenum 95.94	43 <b>Tc</b> Technetium 98	44 <b>Ru</b> Ruthenium 101.07	45 <b>Rh</b> Rhodium 102.9055	46 <b>Pd</b> Palladium 106.42	47 <b>Ag</b> Silver 107.8682	48 <b>Cd</b> Cadmium 112.411	49 <b>In</b> Indium 114.818	50 <b>Sn</b> Tin 118.710	51 <b>Sb</b> Antimony 121.757	52 <b>Te</b> Tellurium 127.6	53 <b>I</b> Iodine 126.90447	54 <b>Xe</b> Xenon 131.29						
55 <b>Cs</b> Cesium 132.90545196	56 <b>Ba</b> Barium 137.327	57-71 Lanthanoids	72 <b>Hf</b> Hafnium 178.49	73 <b>Ta</b> Tantalum 180.94788	74 <b>W</b> Tungsten 183.84	75 <b>Re</b> Rhenium 186.207	76 <b>Os</b> Osmium 190.23	77 <b>Ir</b> Iridium 192.222	78 <b>Pt</b> Platinum 195.084	79 <b>Au</b> Gold 196.966569	80 <b>Hg</b> Mercury 200.592	81 <b>Tl</b> Thallium 204.38	82 <b>Pb</b> Lead 207.2	83 <b>Bi</b> Bismuth 208.9804	84 <b>Po</b> Polonium 209	85 <b>At</b> Astatine 210	86 <b>Rn</b> Radon 222						
87 <b>Fr</b> Francium 223	88 <b>Ra</b> Radium 226	89-103 Actinoids	104 <b>Rf</b> Rutherfordium 261	105 <b>Db</b> Dubnium 262	106 <b>Sg</b> Seaborgium 263	107 <b>Bh</b> Bohrium 264	108 <b>Hs</b> Hassium 265	109 <b>Mt</b> Meitnerium 266	110 <b>Ds</b> Darmstadtium 267	111 <b>Rg</b> Roentgenium 268	112 <b>Cn</b> Copernicium 269	113 <b>Nh</b> Nihonium 270	114 <b>Fl</b> Flerovium 270	115 <b>Mc</b> Moscovium 271	116 <b>Lv</b> Livermorium 273	117 <b>Ts</b> Tennessine 274	118 <b>Og</b> Oganesson 274						



57 <b>La</b> Lanthanum 138.90547	58 <b>Ce</b> Cerium 140.12	59 <b>Pr</b> Praseodymium 140.90766	60 <b>Nd</b> Neodymium 144.242	61 <b>Pm</b> Promethium 145	62 <b>Sm</b> Samarium 150.36	63 <b>Eu</b> Europium 151.964	64 <b>Gd</b> Gadolinium 157.25	65 <b>Tb</b> Terbium 158.92532	66 <b>Dy</b> Dysprosium 162.50015	67 <b>Ho</b> Holmium 164.93032	68 <b>Er</b> Erbium 167.259	69 <b>Tm</b> Thulium 168.93032	70 <b>Yb</b> Ytterbium 173.05468	71 <b>Lu</b> Lutetium 174.967
89 <b>Ac</b> Actinium 227	90 <b>Th</b> Thorium 232.0377	91 <b>Pa</b> Protactinium 231.036888	92 <b>U</b> Uranium 238.02891	93 <b>Np</b> Neptunium 237	94 <b>Pu</b> Plutonium 244	95 <b>Am</b> Americium 243	96 <b>Cm</b> Curium 247	97 <b>Bk</b> Berkelium 247	98 <b>Cf</b> Californium 251	99 <b>Es</b> Einsteinium 252	100 <b>Fm</b> Fermium 257	101 <b>Md</b> Mendelevium 258	102 <b>No</b> Nobelium 259	103 <b>Lr</b> Lawrencium 260





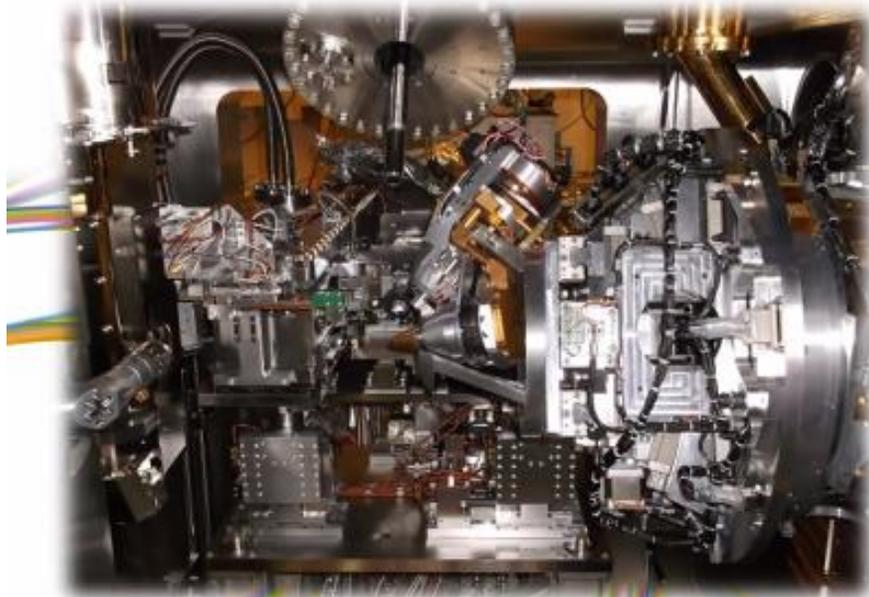
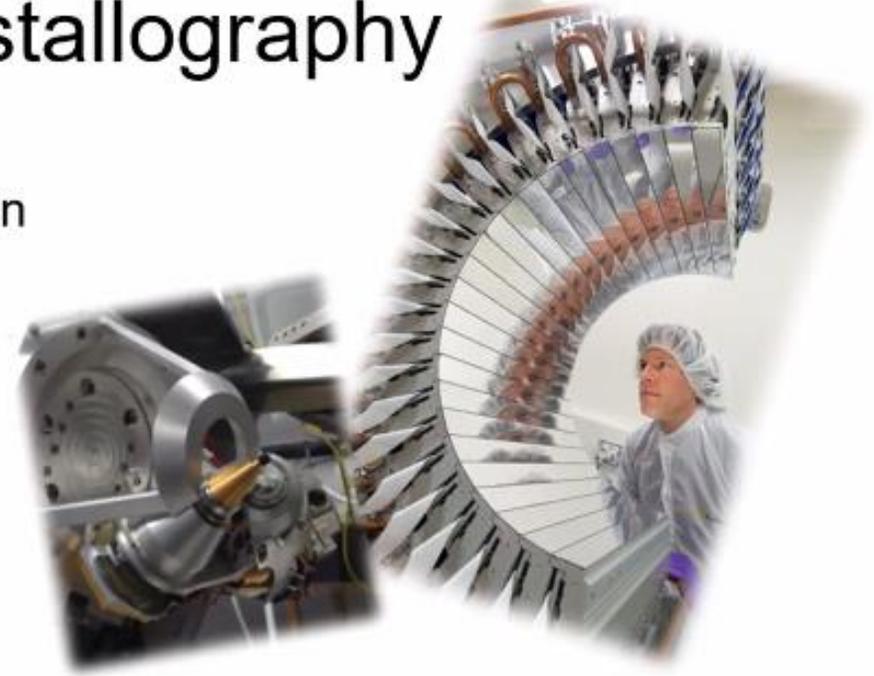
	E [keV]	$\lambda$ [Å]
P	2.146	5.779
S	2.472	5.016
Cl	2.822	4.393
K	3.607	3.437
Ca	4.038	3.070

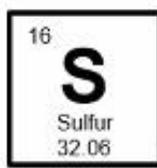
$$\lambda [\text{Å}] = 12.398 / E [\text{keV}]$$



# In-vacuum crystallography

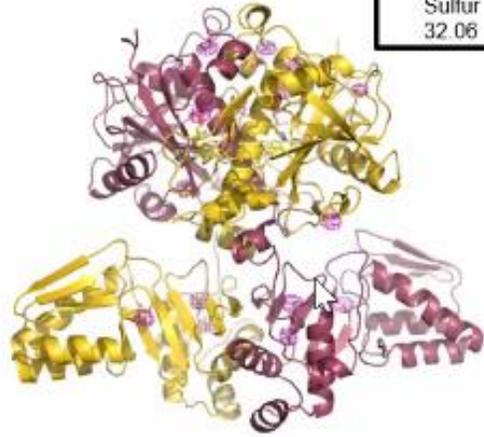
- Elimination of air scattering and absorption
- Several challenges had to be overcome
  - Conductive sample cooling
  - Design of conductive sample mounts
  - Sample transfer into vacuum
  - Sophisticated anti-collision system



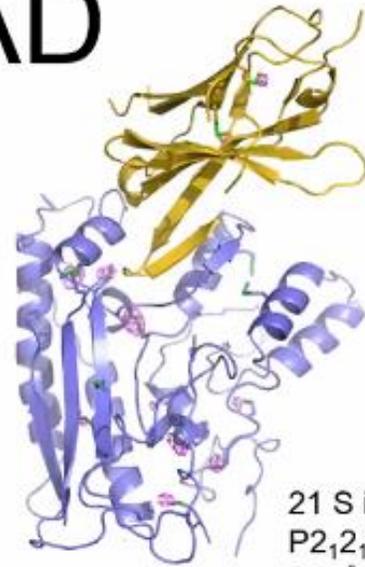


# - SAD

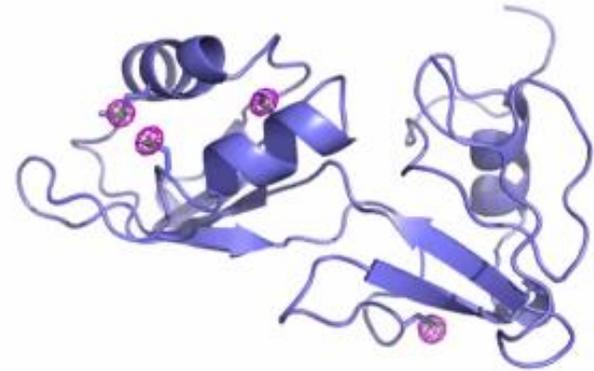
$\lambda = 2.75 \text{ \AA}$   
 Multiplicity: 10 - 20



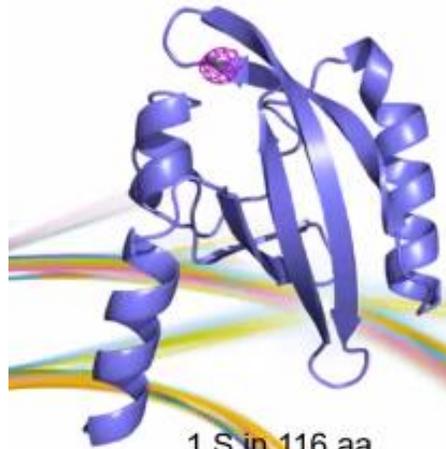
10 S in 477 aa  
 P4<sub>1</sub>2<sub>1</sub>2<sub>1</sub>  
 3.2 Å res



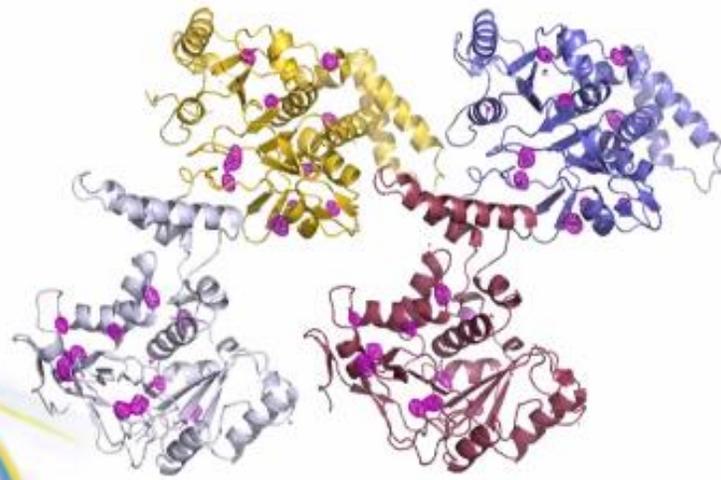
21 S in 427 aa  
 P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>  
 2.8 Å res



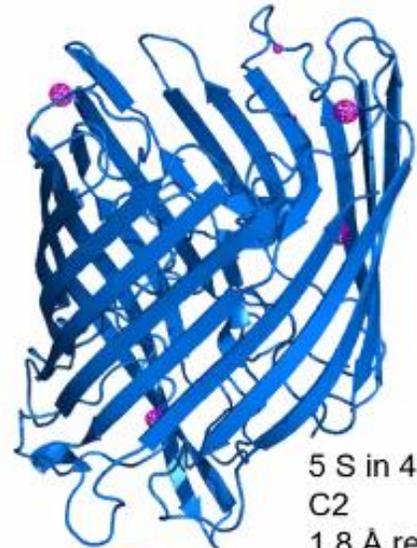
4 S in 157 aa  
 C2  
 1.8 Å res



1 S in 116 aa  
 P2<sub>1</sub>2<sub>1</sub>2  
 2.5 Å res



10 S in 322 aa  
 P2<sub>1</sub>  
 2.1 Å res  
 INCS



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

```
NLKMEIILTSQGLKYYGKILRLQLTLEEDTEGLEWCKRNLGLDCDDTFF
QKRIIEFFITGEGHFNEVLQFRTPGTLSTTESTPAGLPTAEPFKSYFAKGFLSIDSGYYS
AKCYSGTSNSGLQLINIRHSTRIVDTPGPKITNLKTINCINLKASIFKEHREVEINVLL
PQVAVNLSNCHVVIKSHVCDYSLDIDGAVRLPHIYHEGVFIPGTYKIVIDKKNKLNDRCT
LFTDCVIKGREVRKQSVLRQYKTEIRIGKASTGS
```

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

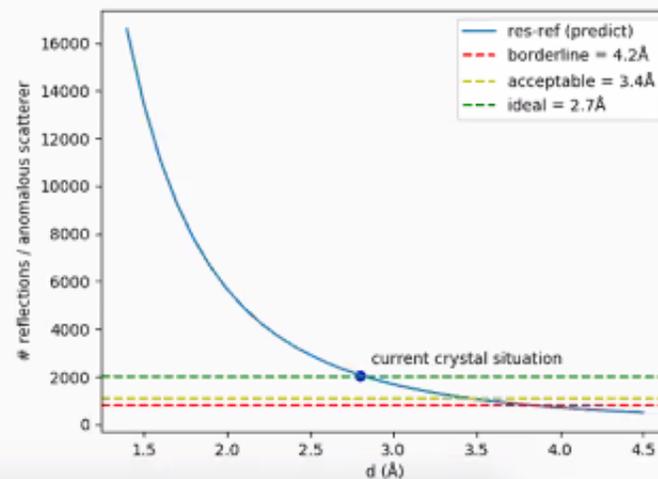
Cys: 16

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.

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

```
NLKMEIILTSQGLKYYGKILRLLQLTLEEDTEGLLEWCKRNLGLDCDDTFF
QKRIIEFFITGEGHFNEVLQFRTPGTLSTTESTPAGLPTAEPFKSYFAKGFLSIDSGYYS
AKCYSGTSNSGLQLINIRHSTRIVDTPGPKITNLKTINCINLKASIFKEHREVEINLL
PQVAVNLSNCHVVIKSHVCDYSLDIDGAVRLPHIYHEGVFIPGTYKIVIDKKNKLNDRCT
LFTDCVIKGREVRKGQSVLRQYKTEIRIGKASTGS
```

ASU PREDICT

Solvent content analysis

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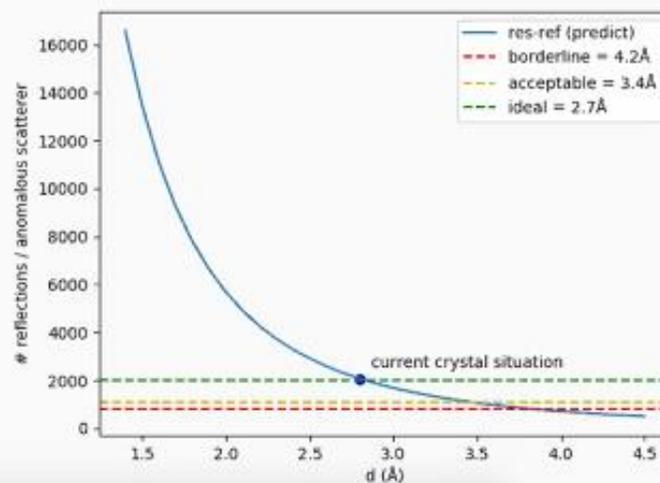
Cys: 16

Met: 2

CALCULATE

SAVE PLOT

RESET



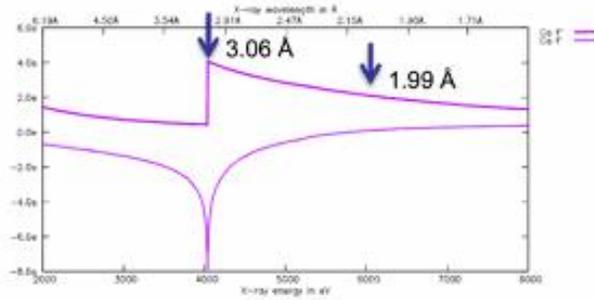
Based on:  
Terwilliger et al. "Can I solve my structure  
by SAD phasing? Anomalous signal in SAD  
Phasing" Acta D (2016) 346.

# Other SAD examples





- 1.5 Ca in ~900 amino acids
- Space group  $P6_522$
- $\lambda = 3.06 \text{ \AA}$ ,  $1.99 \text{ \AA}$
- Data to  $3.8 \text{ \AA}$  resolution



Anomalous peak height:  $15 \sigma$

Image removed as unpublished

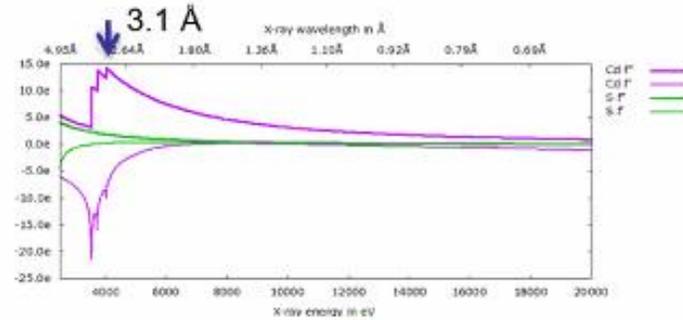


universität  
wien

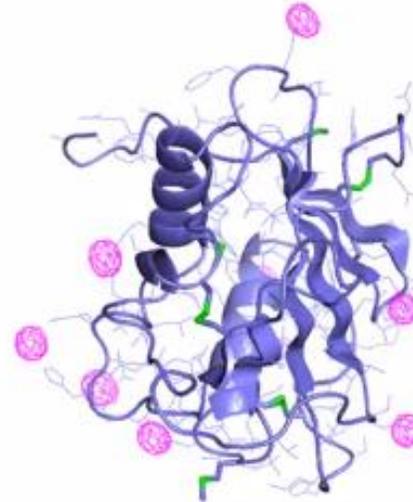
K. Djinovic-Carugo



- 11 S / 146 amino acids
- Space group  $P6_322$
- $\lambda = 2.75 \text{ \AA}$
- Inverse beam  $2 \times 180^\circ$
- Data to  $3 \text{ \AA}$  resolution



Anomalous peak height:  
 $10 - 17 \sigma$

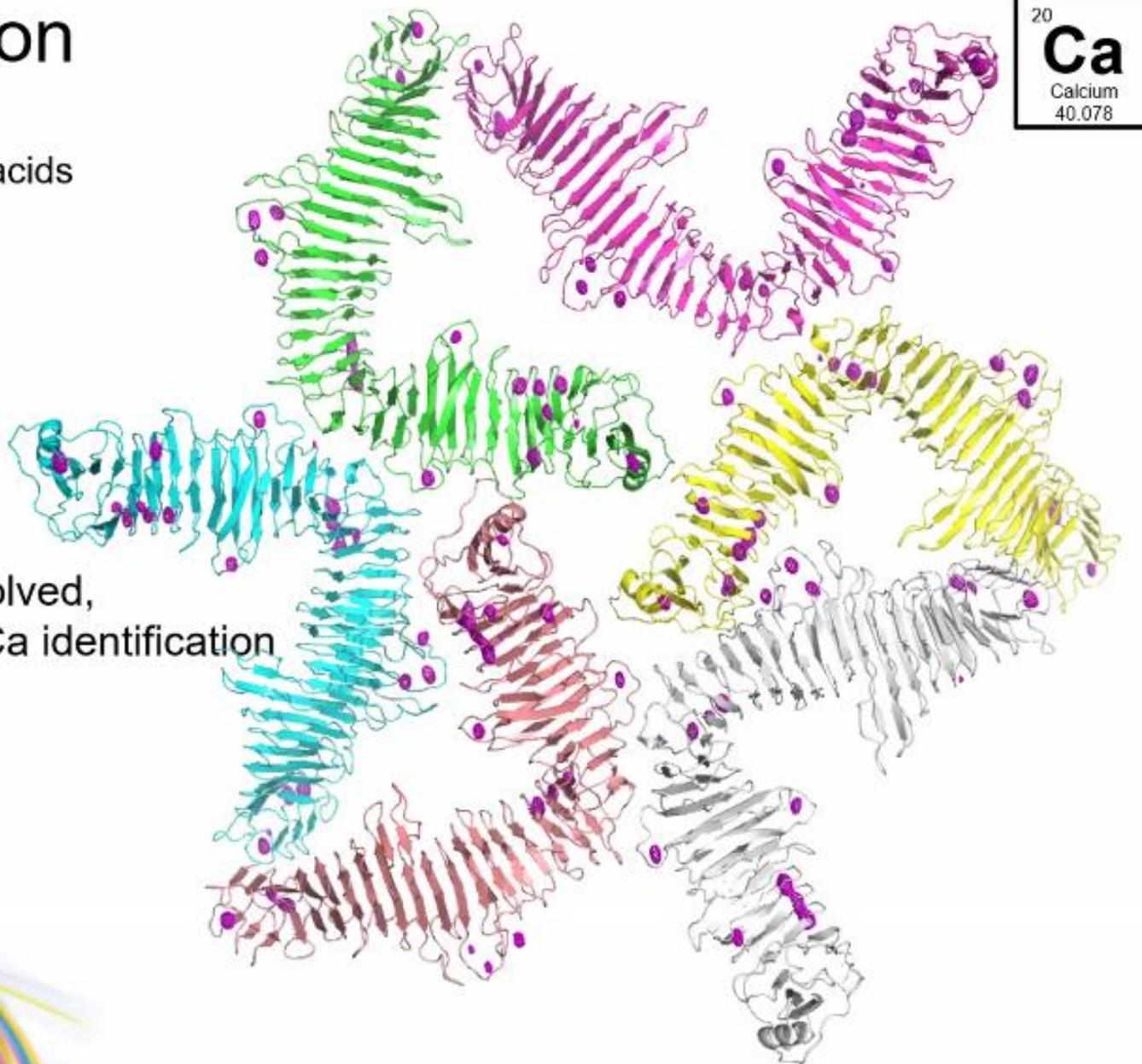


Pryce et al. J. Virol. (2019) 93 (1), e01048-18.

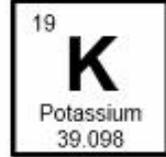
# Ion identification

- 19 Ca built in 471 amino acids
- Space group  $P2_12_12_1$
- $\lambda = 3.02 \text{ \AA}$
- Inverse beam  $2 \times 180^\circ$
- Data to  $3.5 \text{ \AA}$  resolution

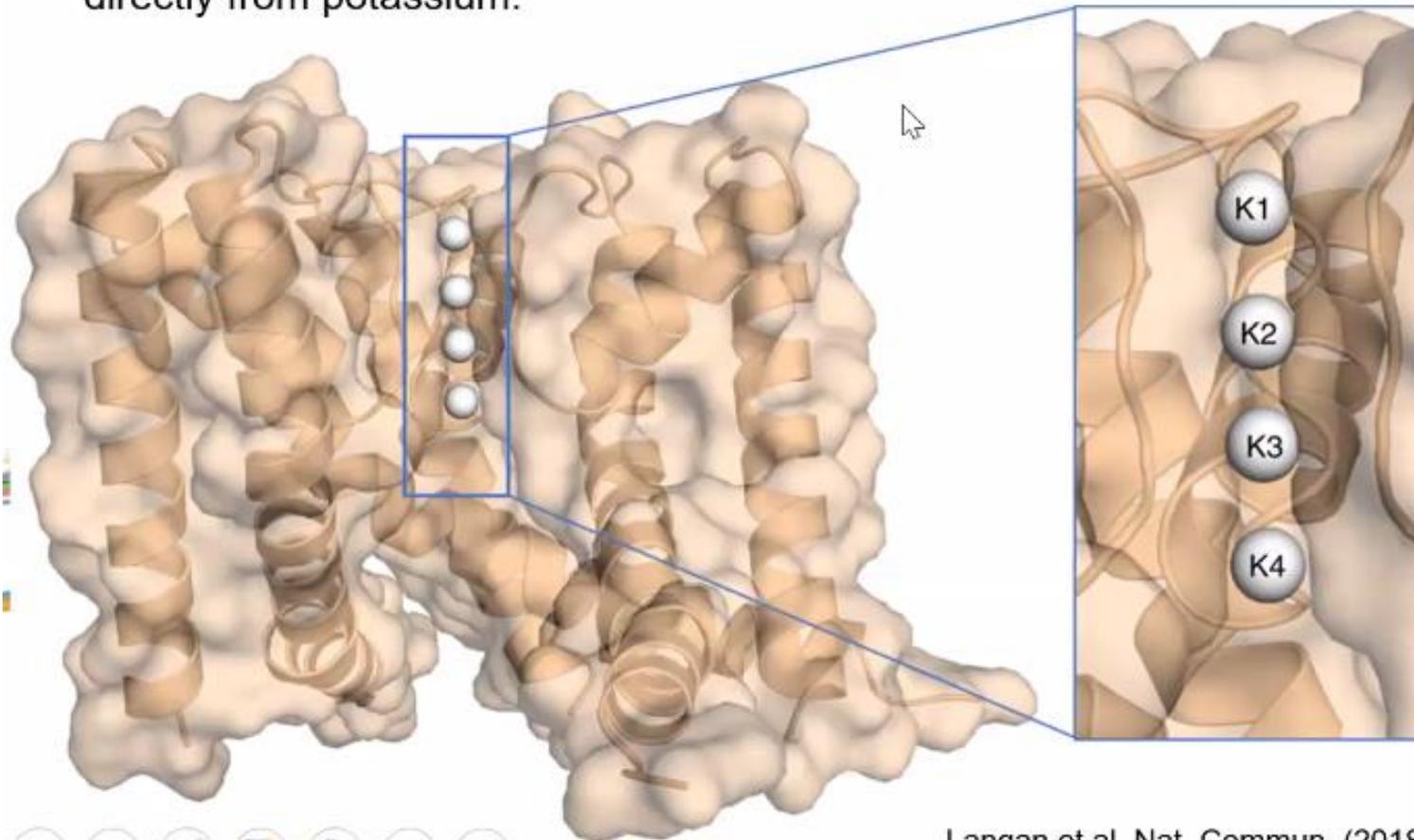
User request: Structure solved,  
reviewer commented on Ca identification



# K – binding in the selectivity filter

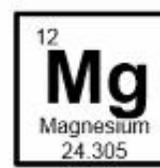


- In original work, based on substitution of  $K^+$  by  $Tl^+$ , alternating model was proposed (MacKinnon), either position 1 and 3, or 2 and 4 occupied.
- Data was reprocessed by Kopfer et al., full occupancies proposed.
- Data collected at I23 allowed to confirm full occupancy model based on anomalous data directly from potassium.

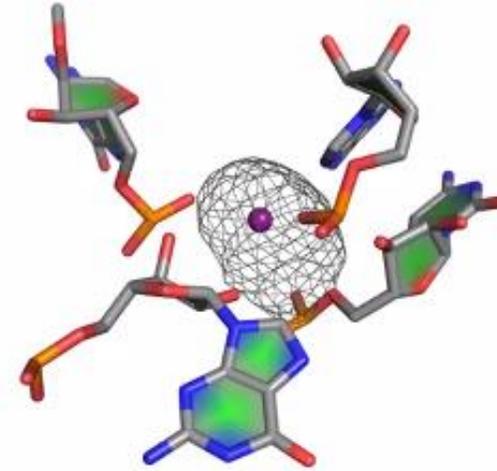
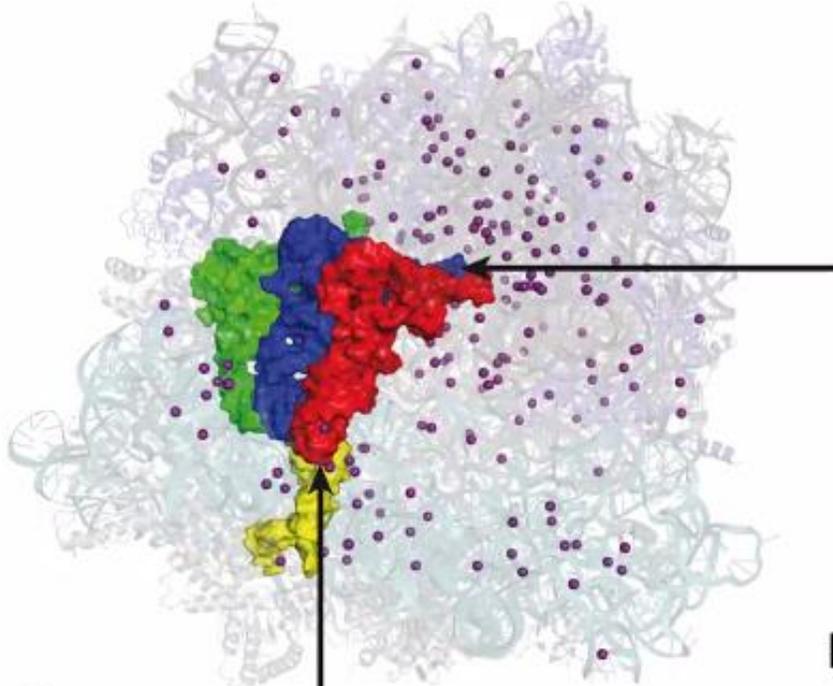
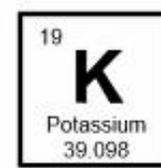




# 70S Ribosome

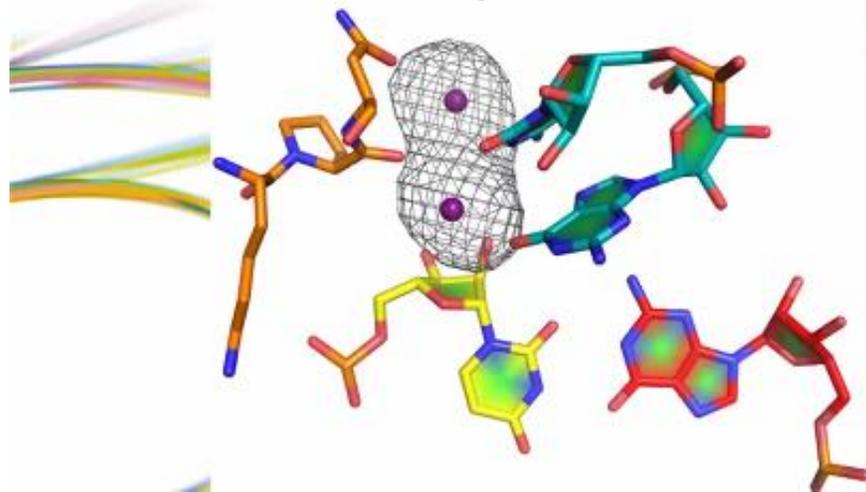


or



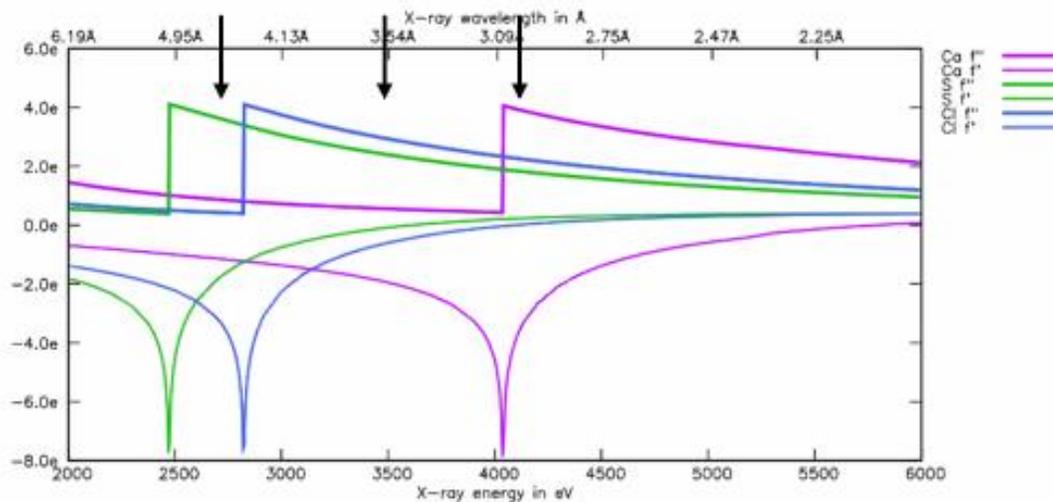
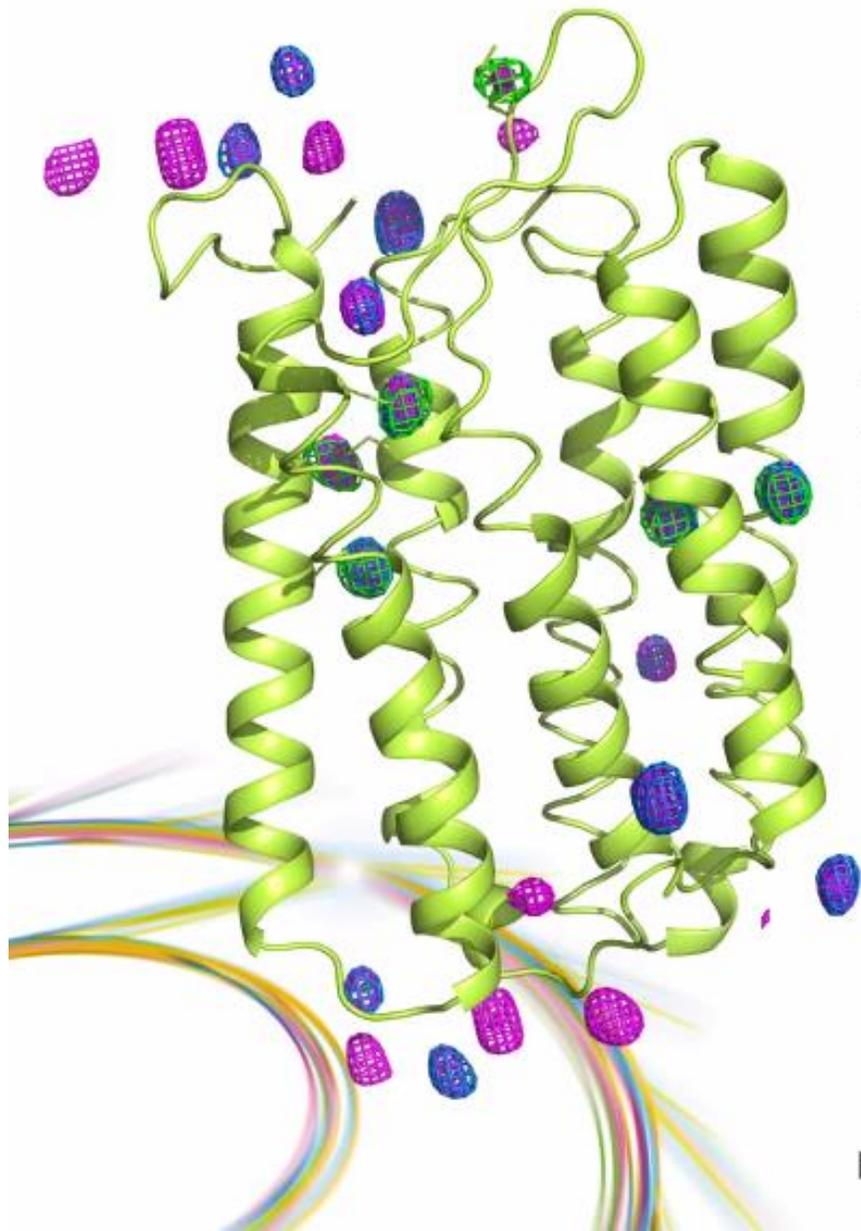
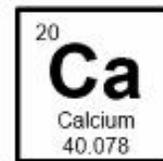
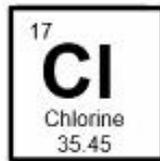
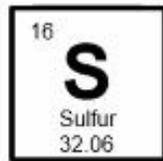
Peptidyl transferase centre

Decoding centre



	Mg (pdb)	K <sup>+</sup>	Mg <sup>2+</sup>	Mg(H <sub>2</sub> O) <sub>6</sub> <sup>2+</sup>
IC	3255	249	620	90
EC	1439	131	394	74

Rozov et al. Nat. Commun. (2019)



Anomalous difference  
Fourier maps:

green:  $\lambda = 4.428 \text{ \AA}$

blue:  $\lambda = 3.542 \text{ \AA}$

magenta:  $\lambda = 2.952 \text{ \AA}$

# 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**

