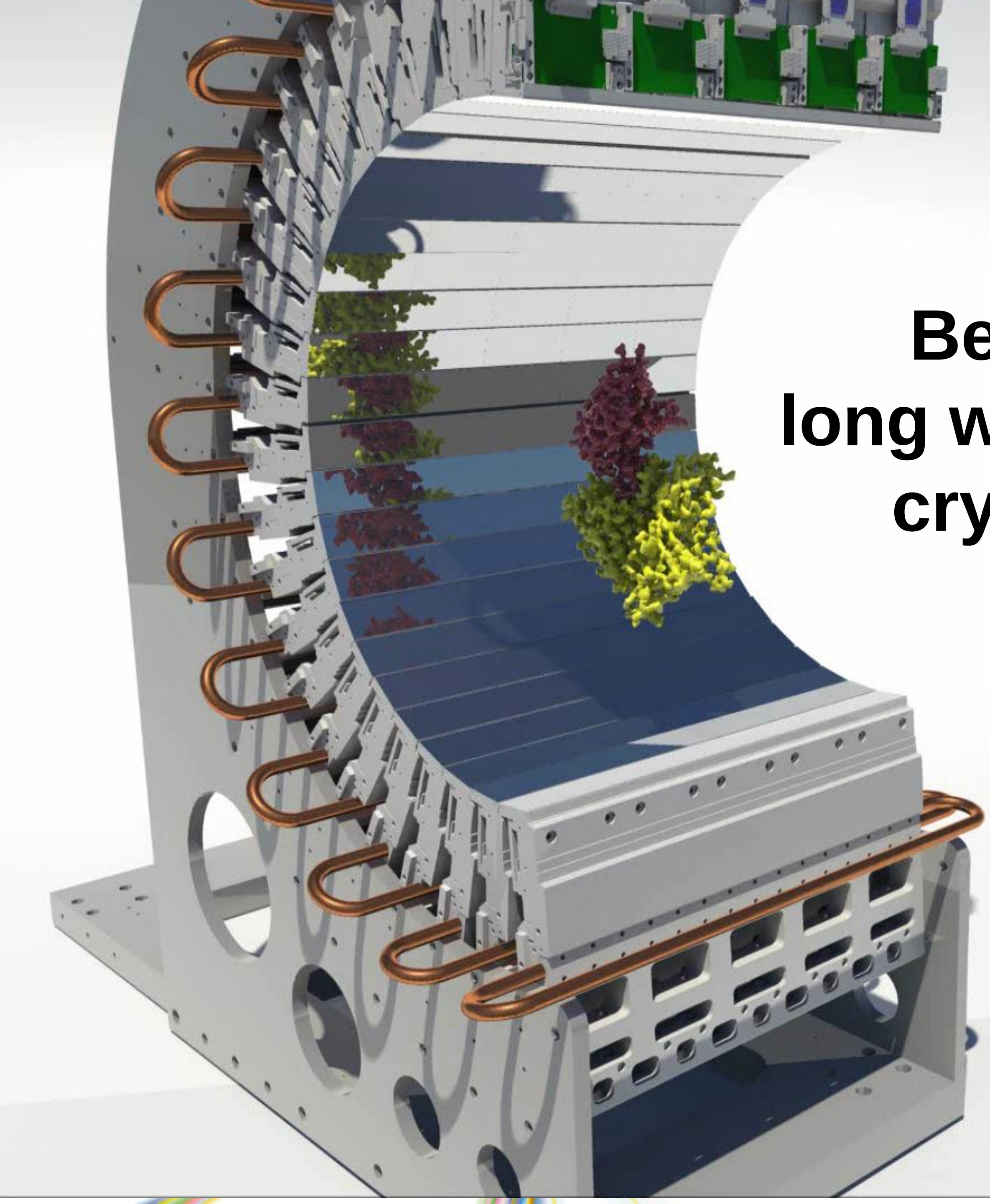


Beamline I23 – long wavelength X-ray crystallography

Ramona Duman

CCP4/DLS workshop
December 2022

I23 Team

- Armin Wagner
- Kamel El Omari
- Vitaliy Mykhaylyk
- Chris Orr
- Vinay Grama
- Adam Prescott
- Arvinder Palaha
- James O’Hea
- Paul Hathaway
- Kevin Wilkinson

- Dave Stuart

- Wolfgang Kabsch
- Gerard Bricogne

UNIVERSITY OF OXFORD

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

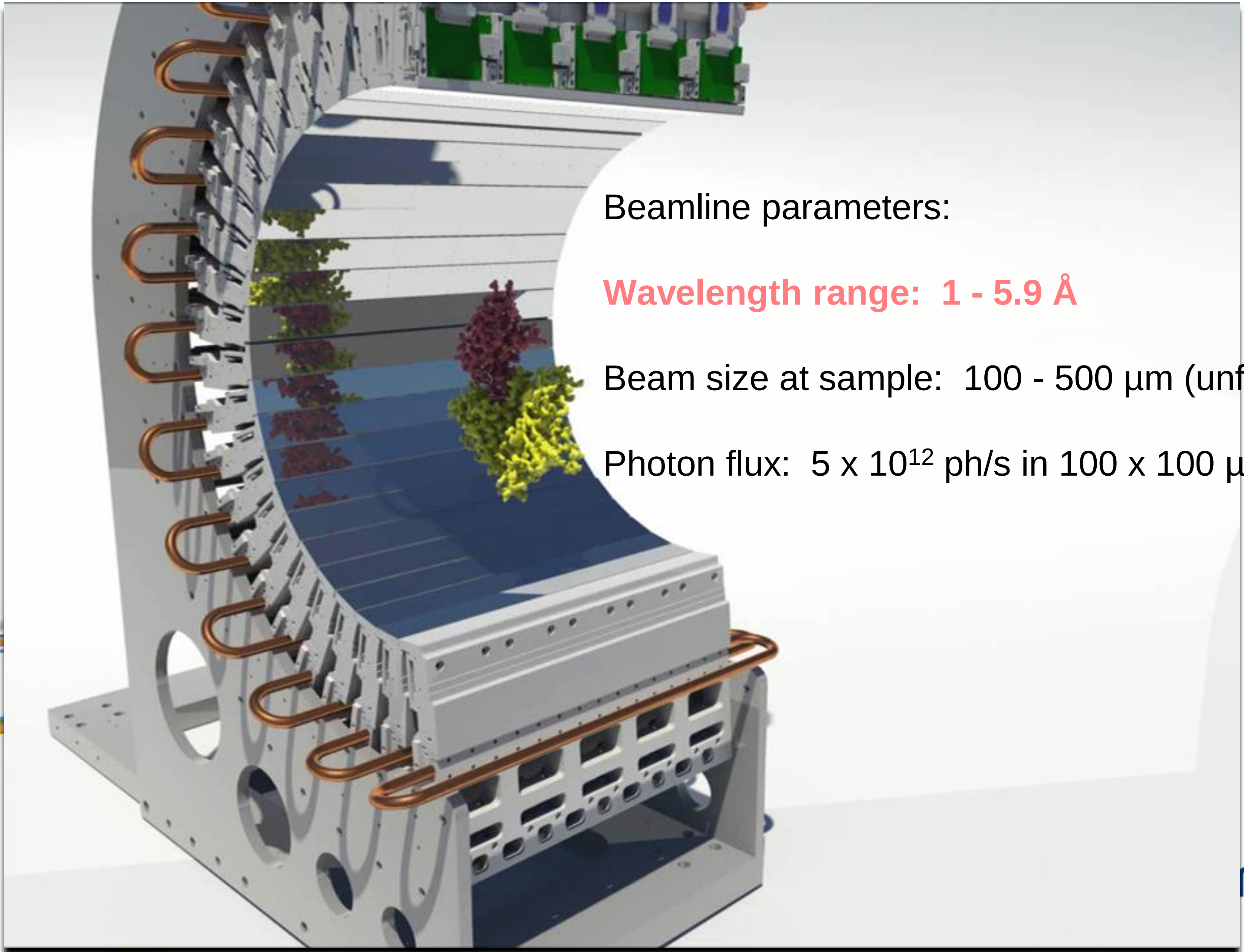
THE FRANCIS CRICK INSTITUTE

Newcastle University

DIALS Diffraction Integration for Advanced Light Sources

diamond

I23 – In-vacuum long-wavelength MX



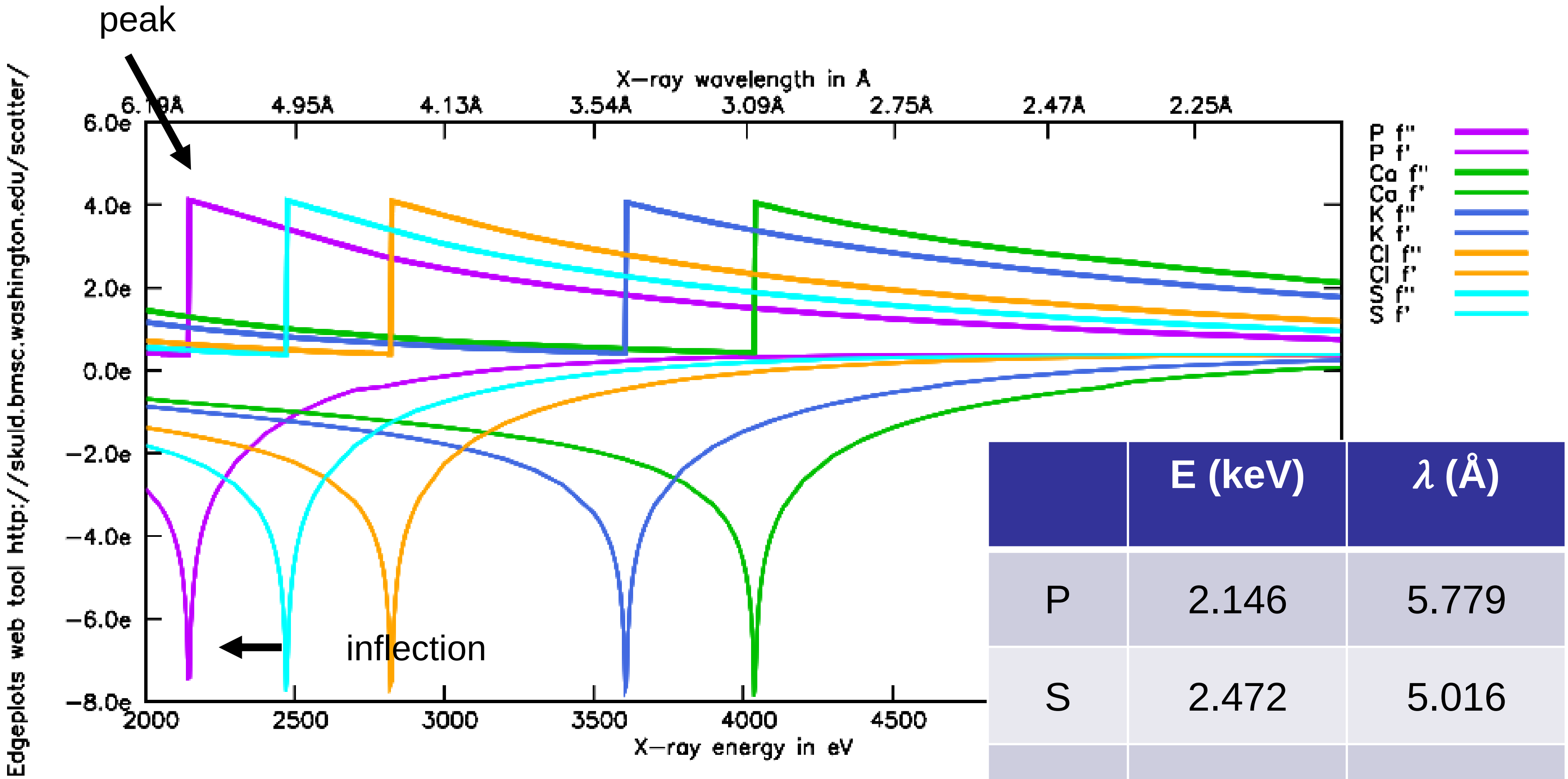
Beamline parameters:

Wavelength range: 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)

Accessible X-ray absorption edges of biologically relevant elements



peak

inflection

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

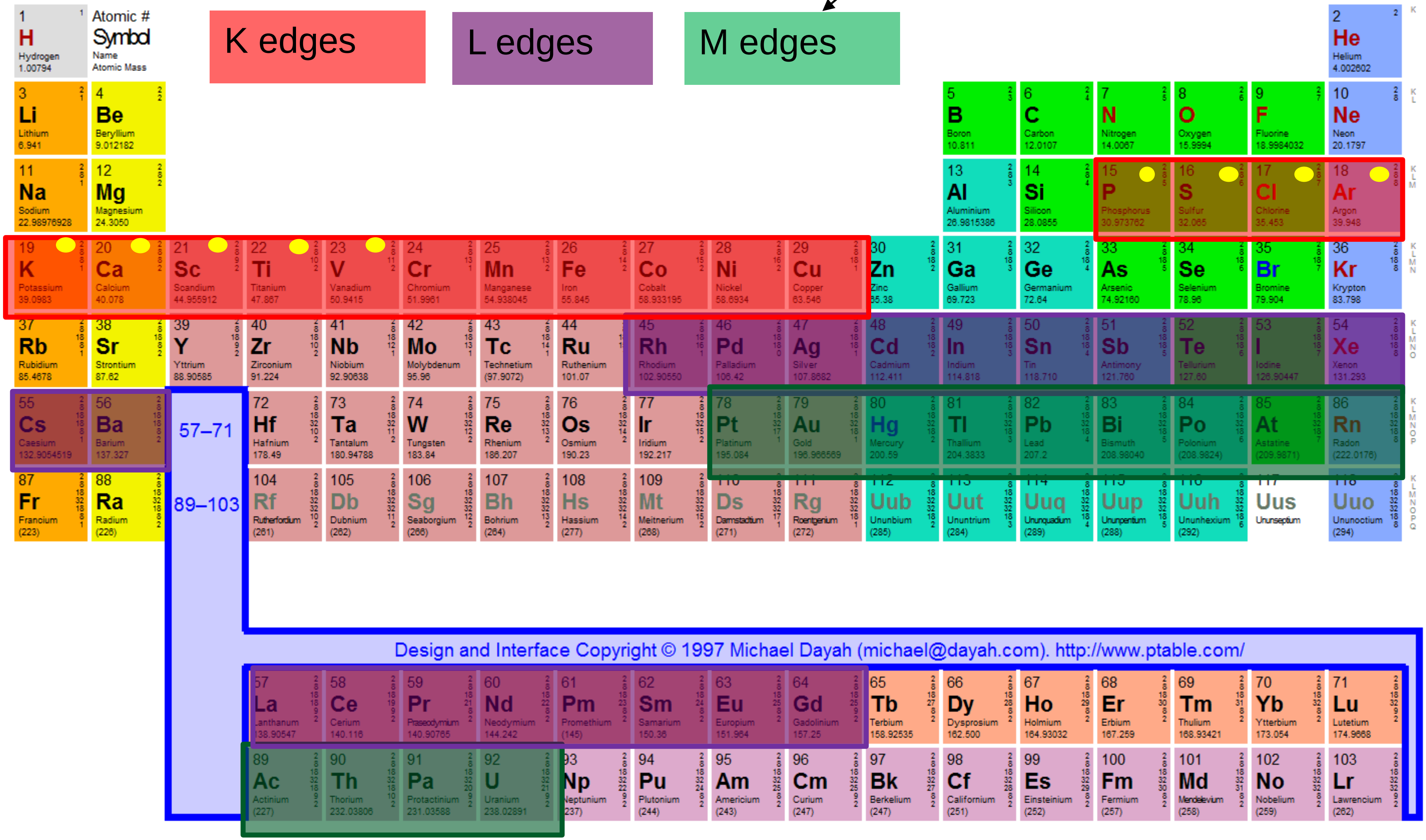
	E (keV)	λ (Å)
P	2.146	5.779
S	2.472	5.016
Cl	2.822	4.393
K	3.607	3.437
Ca	4.038	3.070

λ [Å] = 12.398 / E [keV]

Attainable X-ray absorption edges

● unique to I23

SAD and MAD phasing for large complexes by using anomalous signals from M-edges



K edges

L edges

M edges

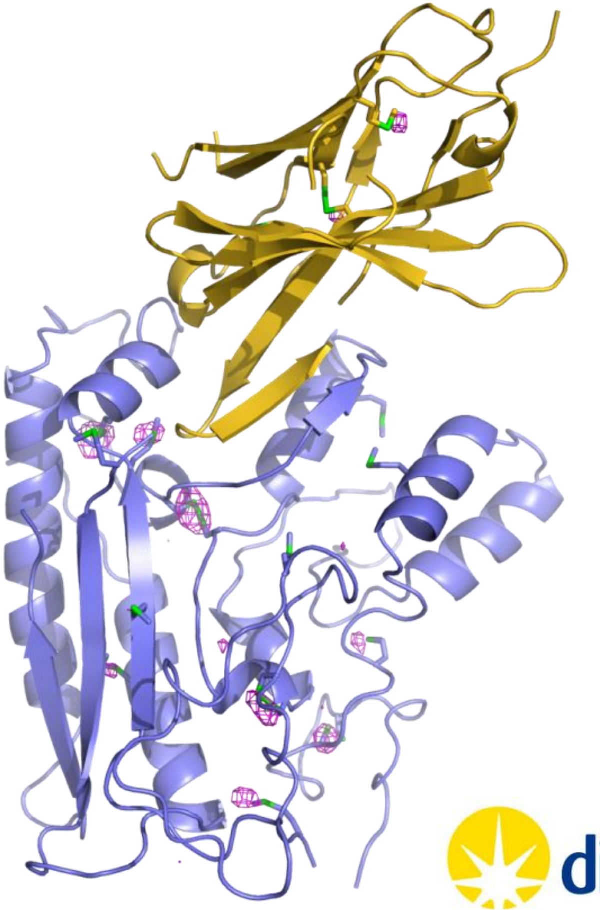
1 H Hydrogen 1.00794	Atomic # Symbol Name Atomic Mass																2 He Helium 4.002602
3 Li Lithium 6.941	4 Be Beryllium 9.012182											5 B Boron 10.811	6 C Carbon 12.0107	7 N Nitrogen 14.0067	8 O Oxygen 15.9994	9 F Fluorine 18.9984032	10 Ne Neon 20.1797
11 Na Sodium 22.98976928	12 Mg Magnesium 24.3050											13 Al Aluminium 26.9815386	14 Si Silicon 28.0855	15 ● P Phosphorus 30.973762	16 ● S Sulfur 32.065	17 ● Cl Chlorine 35.453	18 ● Ar Argon 39.948
19 ● K Potassium 39.0983	20 ● Ca Calcium 40.078	21 ● Sc Scandium 44.955912	22 ● Ti Titanium 47.867	23 ● V Vanadium 50.9415	24 Cr Chromium 51.9961	25 Mn Manganese 54.938045	26 Fe Iron 55.845	27 Co Cobalt 58.933195	28 Ni Nickel 58.6934	29 Cu Copper 63.546	30 Zn Zinc 65.38	31 Ga Gallium 69.723	32 Ge Germanium 72.64	33 As Arsenic 74.92160	34 Se Selenium 78.96	35 Br Bromine 79.904	36 Kr Krypton 83.798
37 Rb Rubidium 85.4678	38 Sr Strontium 87.62	39 Y Yttrium 88.90585	40 Zr Zirconium 91.224	41 Nb Niobium 92.90638	42 Mo Molybdenum 95.96	43 Tc Technetium (97.9072)	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.90550	46 Pd Palladium 106.42	47 Ag Silver 107.8682	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.710	51 Sb Antimony 121.760	52 Te Tellurium 127.60	53 I Iodine 126.90447	54 Xe Xenon 131.293
55 Cs Caesium 132.9054519	56 Ba Barium 137.327	57–71	72 Hf Hafnium 178.49	73 Ta Tantalum 180.94788	74 W Tungsten 183.84	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.217	78 Pt Platinum 195.084	79 Au Gold 196.966569	80 Hg Mercury 200.59	81 Tl Thallium 204.3833	82 Pb Lead 207.2	83 Bi Bismuth 208.98040	84 Po Polonium (208.9824)	85 At Astatine (209.9871)	86 Rn Radon (222.0176)
87 Fr Francium (223)	88 Ra Radium (226)	89–103	104 Rf Rutherfordium (261)	105 Db Dubnium (262)	106 Sg Seaborgium (266)	107 Bh Bohrium (264)	108 Hs Hassium (277)	109 Mt Meitnerium (268)	110 Ds Darmstadtium (271)	111 Rg Roentgenium (272)	112 Uub Ununbium (285)	113 Uut Ununtrium (284)	114 Uuq Ununquadium (289)	115 Uup Ununpentium (288)	116 Uuh Ununhexium (292)	117 Uus Ununseptium	118 Uuo Ununoctium (294)

57 La Lanthanum 138.90547	58 Ce Cerium 140.116	59 Pr Praseodymium 140.90765	60 Nd Neodymium 144.242	61 Pm Promethium (145)	62 Sm Samarium 150.36	63 Eu Europium 151.964	64 Gd Gadolinium 157.25	65 Tb Terbium 158.92535	66 Dy Dysprosium 162.500	67 Ho Holmium 164.93032	68 Er Erbium 167.259	69 Tm Thulium 168.93421	70 Yb Ytterbium 173.054	71 Lu Lutetium 174.9668
89 Ac Actinium (227)	90 Th Thorium 232.03806	91 Pa Protactinium 231.03588	92 U Uranium 238.02891	93 Np Neptunium (237)	94 Pu Plutonium (244)	95 Am Americium (243)	96 Cm Curium (247)	97 Bk Berkelium (247)	98 Cf Californium (251)	99 Es Einsteinium (252)	100 Fm Fermium (257)	101 Md Mendelevium (258)	102 No Nobelium (259)	103 Lr Lawrencium (262)

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

diamond

Challenges at long wavelengths

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

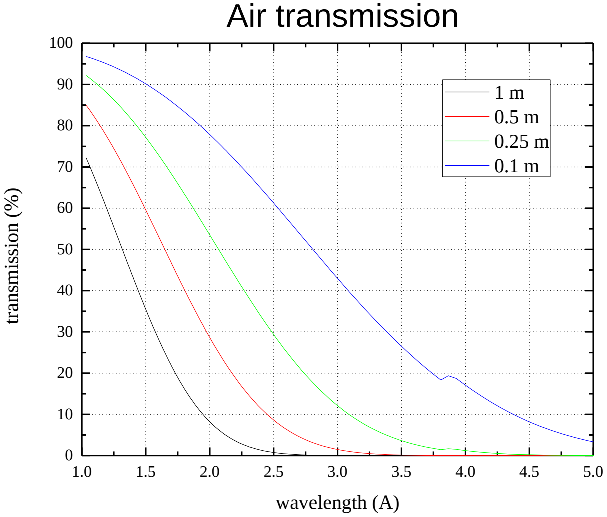
Pilatus 12M (Dectris)

120 modules in vacuum
12,000,000 pixels
Half cylinder r = 250 mm
Width z = 423.6 mm
2θ = ±100º (40.3º laterally)

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

In-vacuum crystallography:

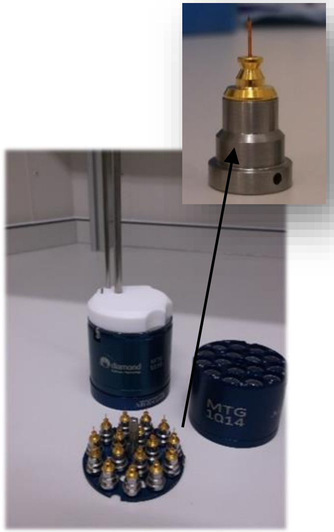
Elimination of air scattering and absorption



Air transmission

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

Sample preparation logistics

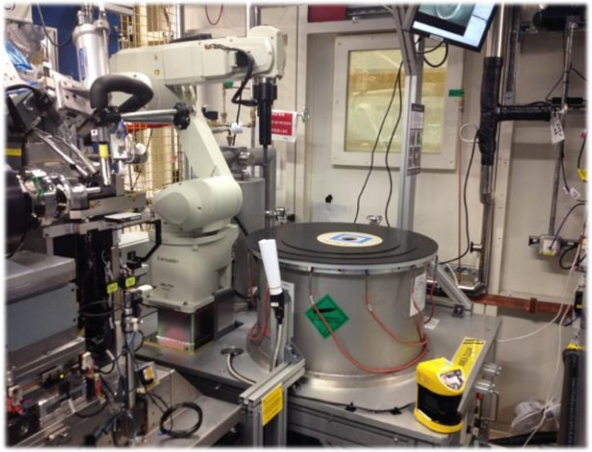


Tools sent to users

Users harvest and cryo-cool crystals

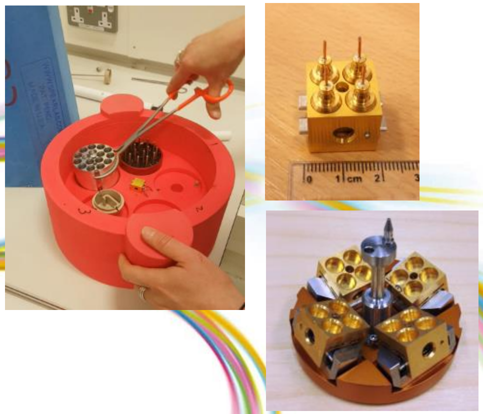
Samples sent to Diamond

Pre-screening on other MX beamlines



Transfer to I23 vacuum endstation

I23 thermally conductive sample holders and blocks

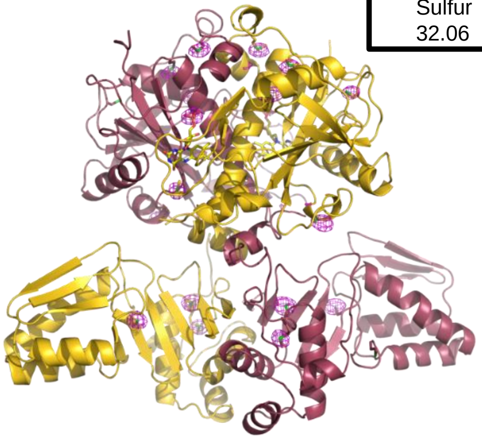


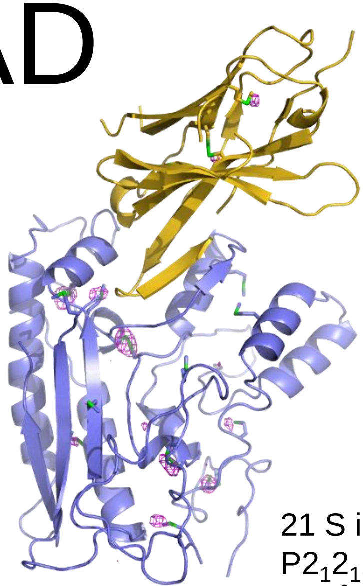
Diamond Cryogenic Sample Transfer System

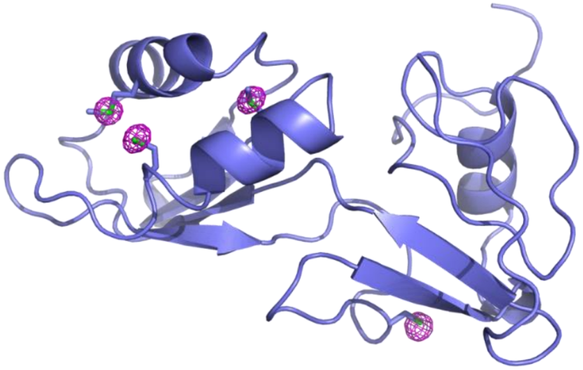
diamond

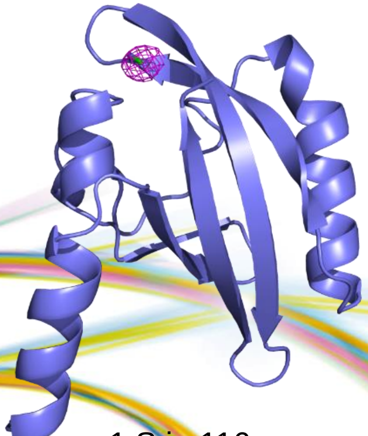
16 S Sulfur 32.06 - SAD

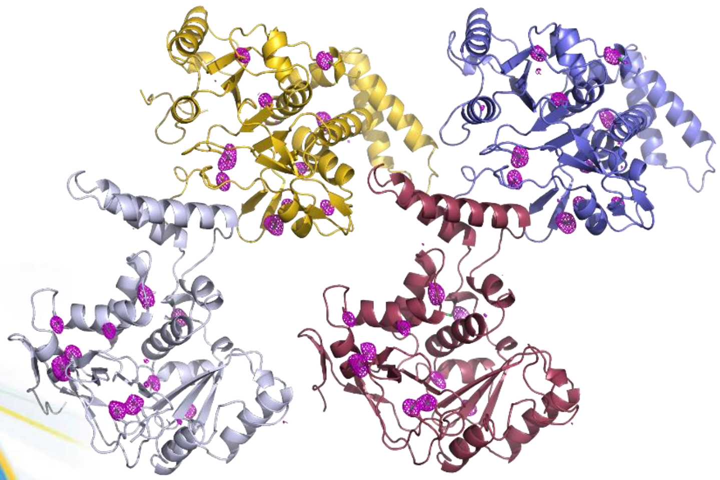
λ = 2.75 Å
Multiplicity: 10 - 20

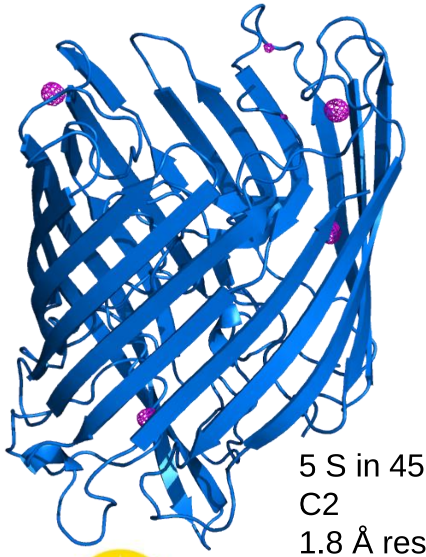

10 S in 477 aa
$P4_12_12_1$
3.2 Å res

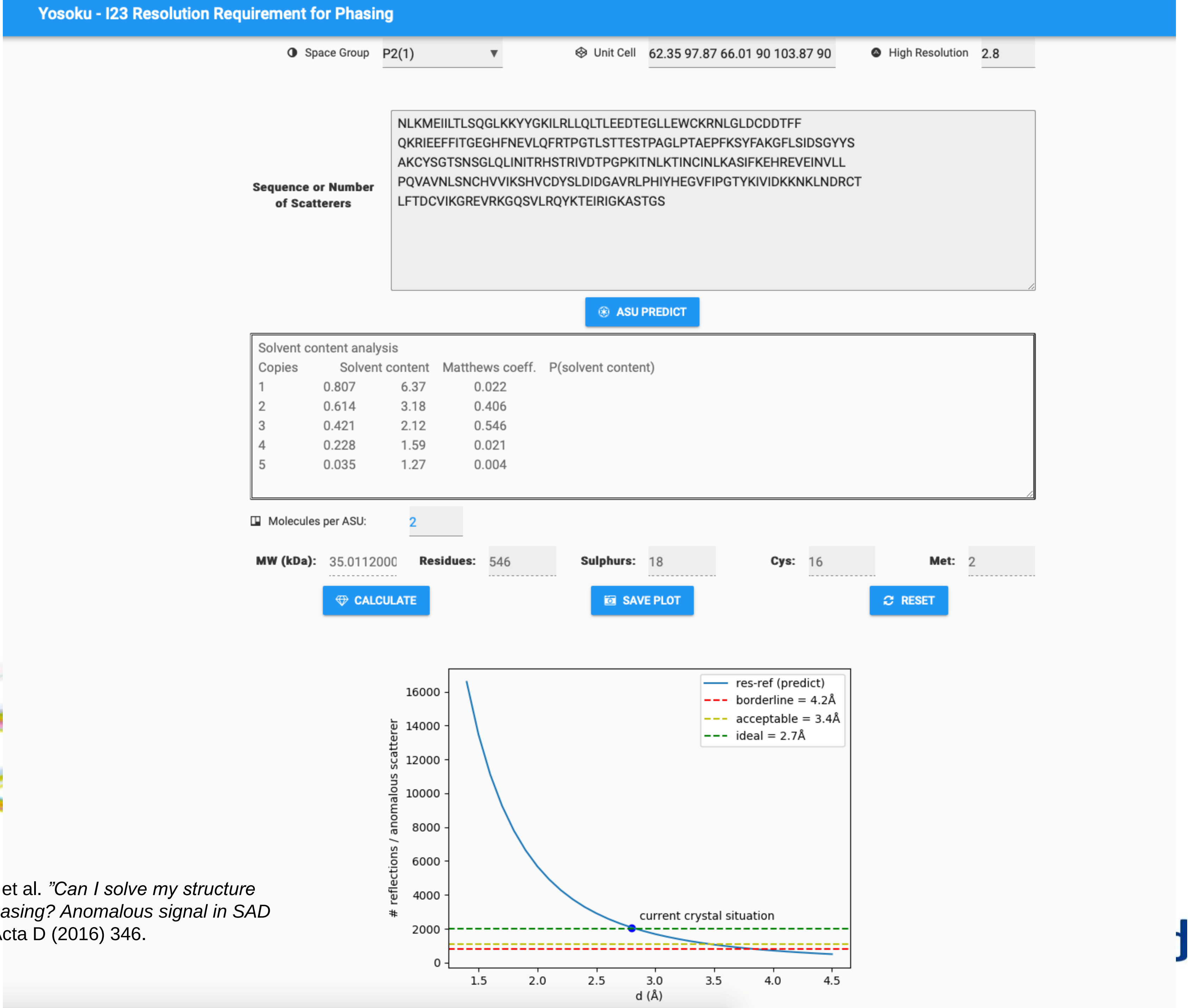

21 S in 427 aa
$P2_12_12_1$
2.8 Å res


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



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
```

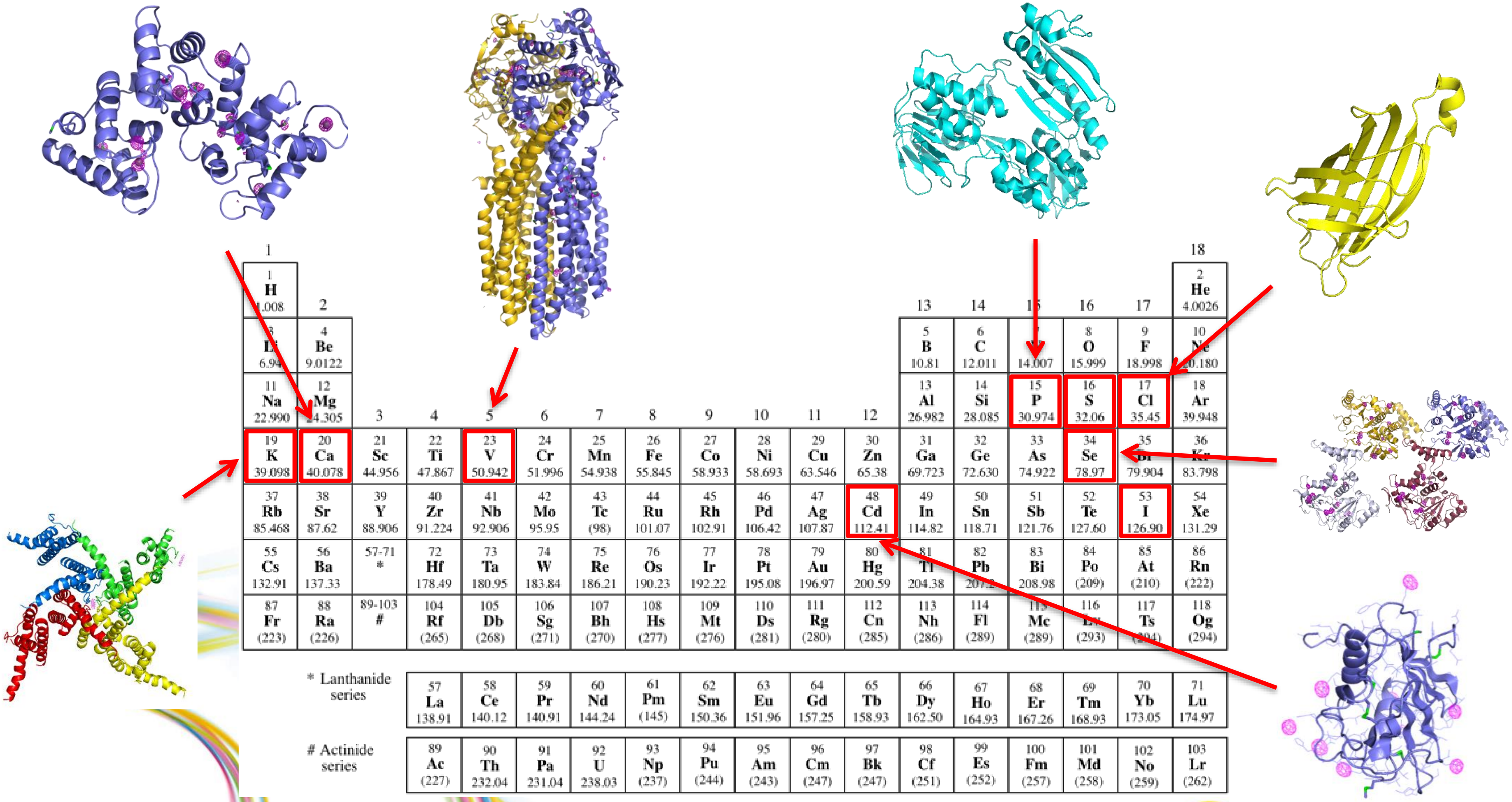
```
Solvent content analysis
Copies          Solvent content   Matthews coeff.   P(solvent content)
1         0.807          6.37          0.022
2         0.614          3.18          0.406
3         0.421          2.12          0.546
4         0.228          1.59          0.021
5         0.035     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

☐ Molecules per ASU: 2

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

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

Other types of SAD phasing



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 **H** 1.008																	2 **He** 4.0026
3 **Li** 6.94	4 **Be** 9.0122											5 **B** 10.81	6 **C** 12.011	7 **N** 14.007	8 **O** 15.999	9 **F** 18.998	10 **Ne** 20.180
11 **Na** 22.990	12 **Mg** 24.305											13 **Al** 26.982	14 **Si** 28.085	15 **P** 30.974	16 **S** 32.06	17 **Cl** 35.45	18 **Ar** 39.948
19 **K** 39.098	20 **Ca** 40.078	21 **Sc** 44.956	22 **Ti** 47.867	23 **V** 50.942	24 **Cr** 51.996	25 **Mn** 54.938	26 **Fe** 55.845	27 **Co** 58.933	28 **Ni** 58.693	29 **Cu** 63.546	30 **Zn** 65.38	31 **Ga** 69.723	32 **Ge** 72.630	33 **As** 74.922	34 **Se** 78.97	35 **Br** 79.904	36 **Kr** 83.798
37 **Rb** 85.468	38 **Sr** 87.62	39 **Y** 88.906	40 **Zr** 91.224	41 **Nb** 92.906	42 **Mo** 95.95	43 **Tc** (98)	44 **Ru** 101.07	45 **Rh** 102.91	46 **Pd** 106.42	47 **Ag** 107.87	48 **Cd** 112.41	49 **In** 114.82	50 **Sn** 118.71	51 **Sb** 121.76	52 **Te** 127.60	53 **I** 126.90	54 **Xe** 131.29
55 **Cs** 132.91	56 **Ba** 137.33	57-71 *	72 **Hf** 178.49	73 **Ta** 180.95	74 **W** 183.84	75 **Re** 186.21	76 **Os** 190.23	77 **Ir** 192.22	78 **Pt** 195.08	79 **Au** 196.97	80 **Hg** 200.59	81 **Tl** 204.38	82 **Pb** 207.2	83 **Bi** 208.98	84 **Po** (209)	85 **At** (210)	86 **Rn** (222)
87 **Fr** (223)	88 **Ra** (226)	89-103 #	104 **Rf** (265)	105 **Db** (268)	106 **Sg** (271)	107 **Bh** (270)	108 **Hs** (277)	109 **Mt** (276)	110 **Ds** (281)	111 **Rg** (280)	112 **Cn** (285)	113 **Nh** (286)	114 **Fl** (289)	115 **Mc** (289)	116 **Lv** (293)	117 **Ts** (294)	118 **Og** (294)

* Lanthanide series	57 **La** 138.91	58 **Ce** 140.12	59 **Pr** 140.91	60 **Nd** 144.24	61 **Pm** (145)	62 **Sm** 150.36	63 **Eu** 151.96	64 **Gd** 157.25	65 **Tb** 158.93	66 **Dy** 162.50	67 **Ho** 164.93	68 **Er** 167.26	69 **Tm** 168.93	70 **Yb** 173.05	71 **Lu** 174.97
# Actinide series	89 **Ac** (227)	90 **Th** 232.04	91 **Pa** 231.04	92 **U** 238.03	93 **Np** (237)	94 **Pu** (244)	95 **Am** (243)	96 **Cm** (247)	97 **Bk** (247)	98 **Cf** (251)	99 **Es** (252)	100 **Fm** (257)	101 **Md** (258)	102 **No** (259)	103 **Lr** (262)

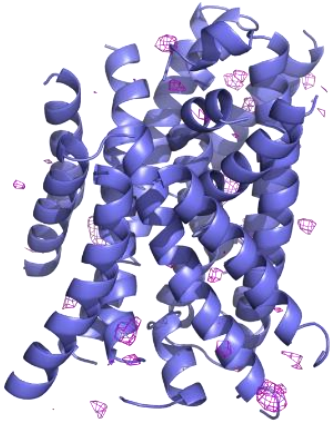
diamond

Element identification

Anomalous difference maps

S identification

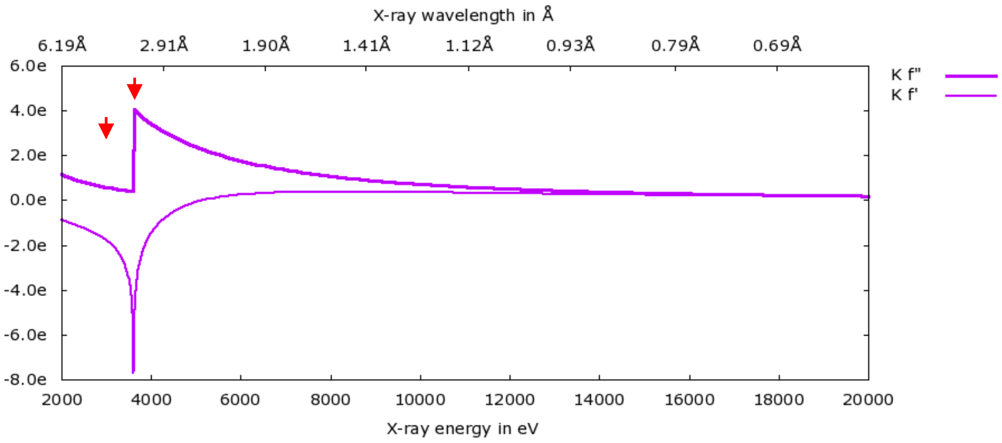
- Help with sequence assignment
- Membrane protein (LCP)
- 13 sulfurs in 471 amino acids
- Space group $P2_12_12_1$
- λ = 3.1 Å

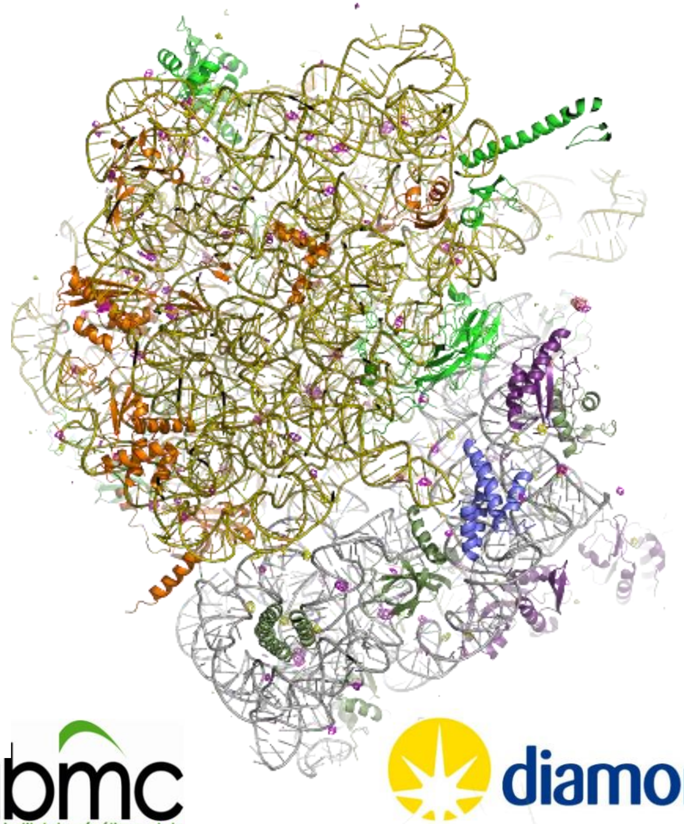


S. Newstead

K identification

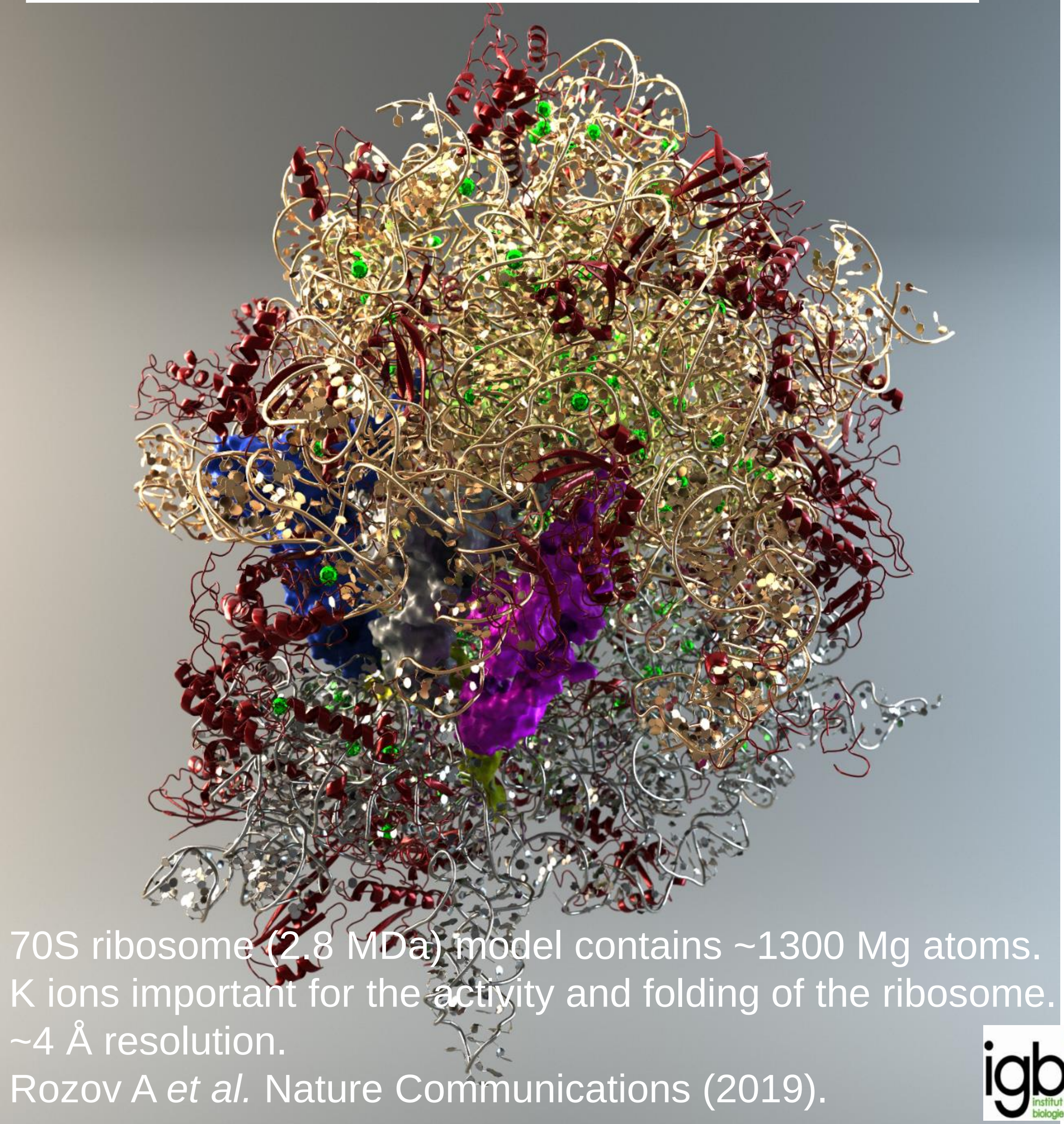
- Experiments above and below the K-edge (λ=3.44 Å).





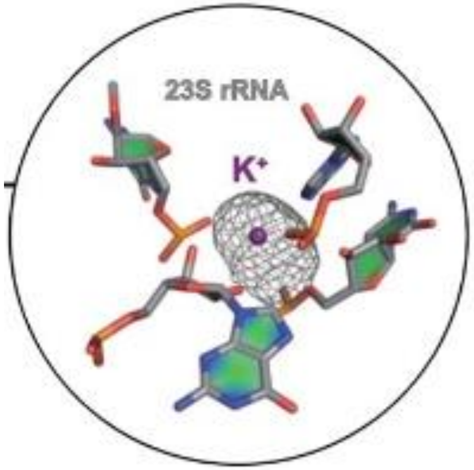
A. Rozov & G. Yusupova

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

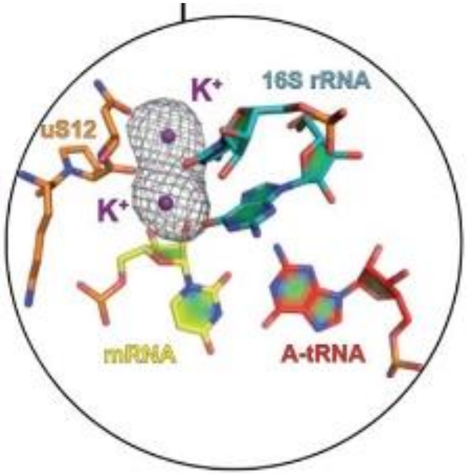


70S ribosome (2.8 MDa) model contains ~1300 Mg atoms.
K ions important for the activity and folding of the ribosome.
~4 Å resolution.
Rozov A *et al.* Nature Communications (2019).

Peptidyl transferase center

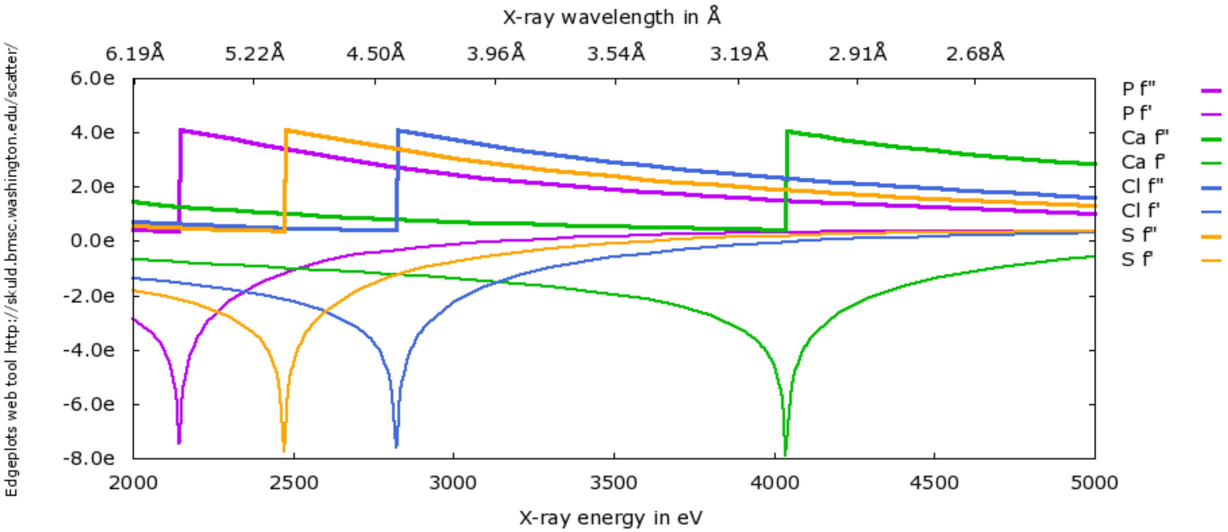


Decoding center

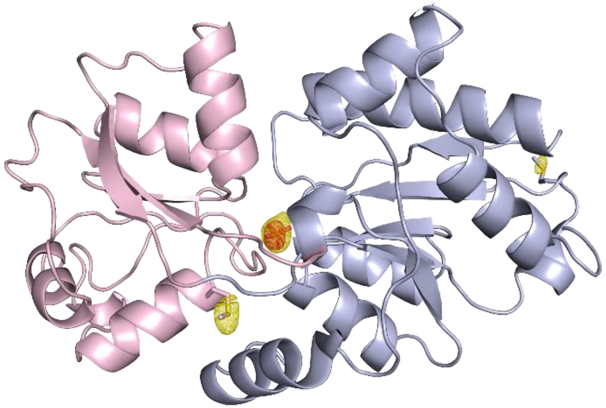


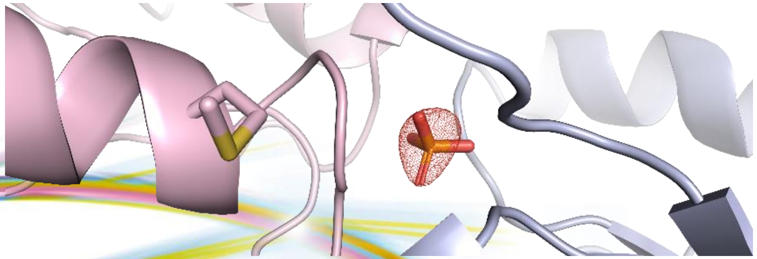
Alexey Rozov
Iskander Khusainov
Gulnara Yusupova

Discriminating between sulphate and phosphate

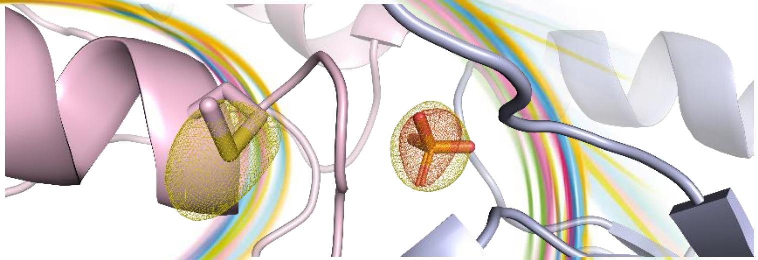


Edgeplots web tool http://skuld.bmsc.washington.edu/scatter/

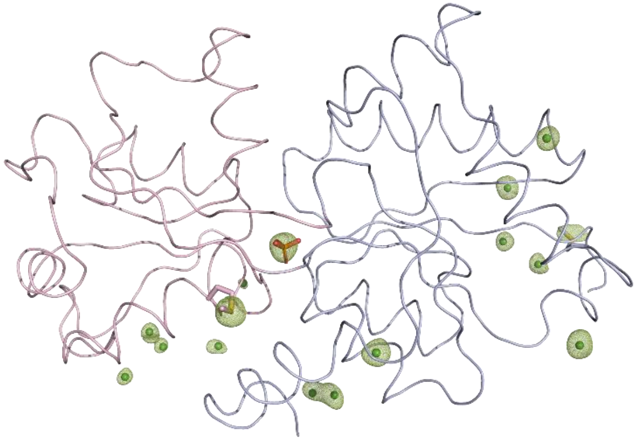




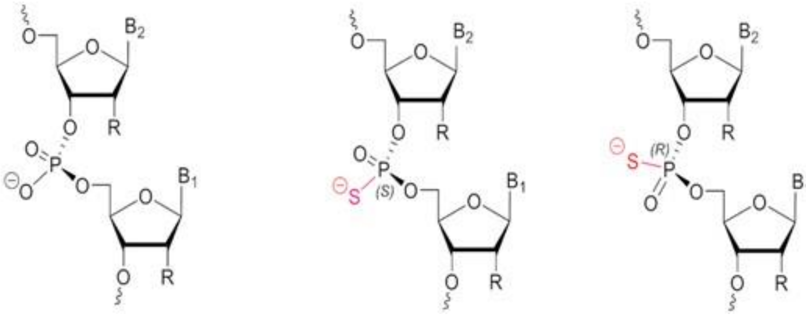
E = 2.4 keV
Below sulphur edge



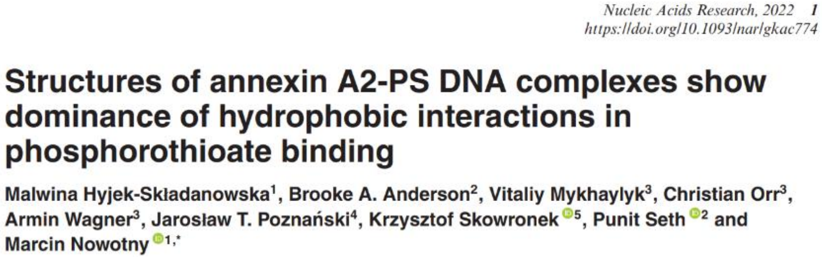
E = 2. 4 keV
E = 2.55 keV
Above sulphur edge



Chirality of phosphorothioate (PS) modifications of therapeutic oligonucleotides



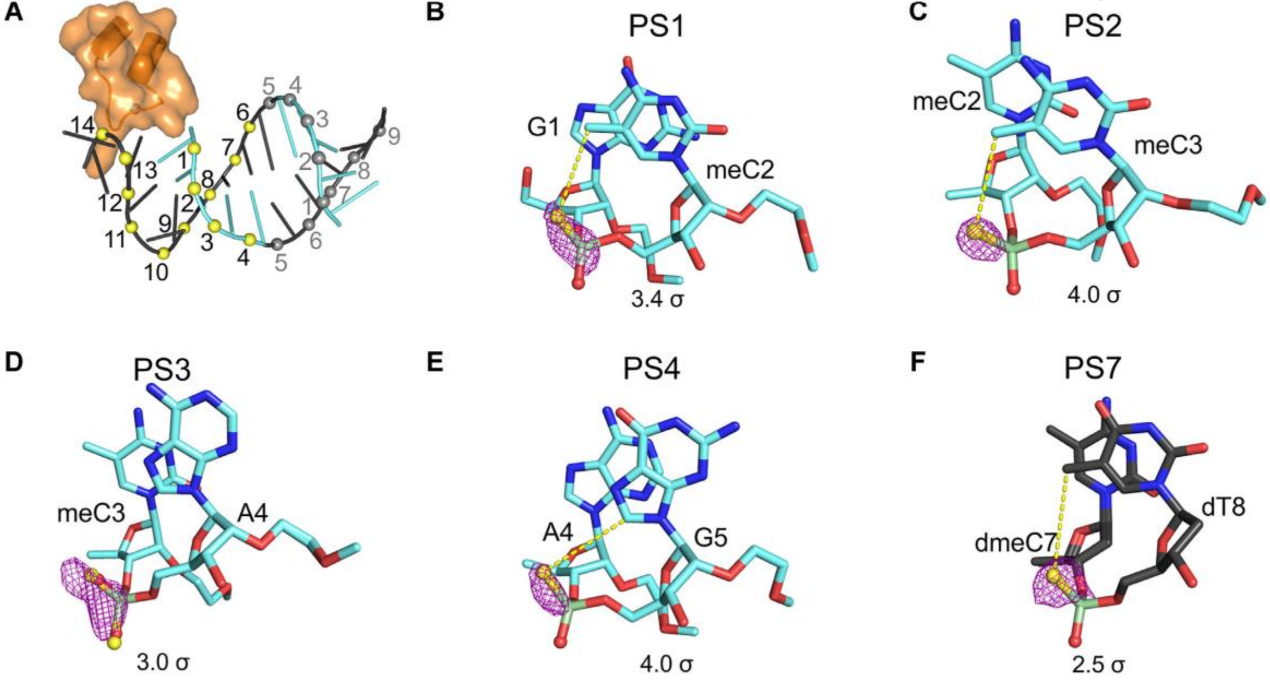
Jahns et al, Nucleic Acids Research, Volume 50, Issue 3, 22 February 2022, 1221–1240



Nucleic Acids Research, 2022 **1**
https://doi.org/10.1093/nar/gkac774

Structures of annexin A2-PS DNA complexes show dominance of hydrophobic interactions in phosphorothioate binding

Malwina Hyjek-Składanowska[1], Brooke A. Anderson[2], Vitaliy Mykhaylyk[3], Christian Orr[3], Armin Wagner[3], Jarosław T. Poznański[4], Krzysztof Skowronek[5], Punit Seth[2] and Marcin Nowotny[1,*]

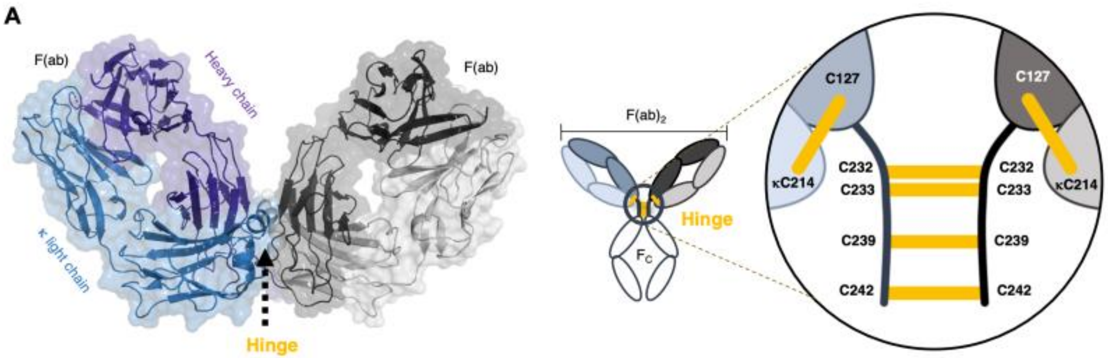


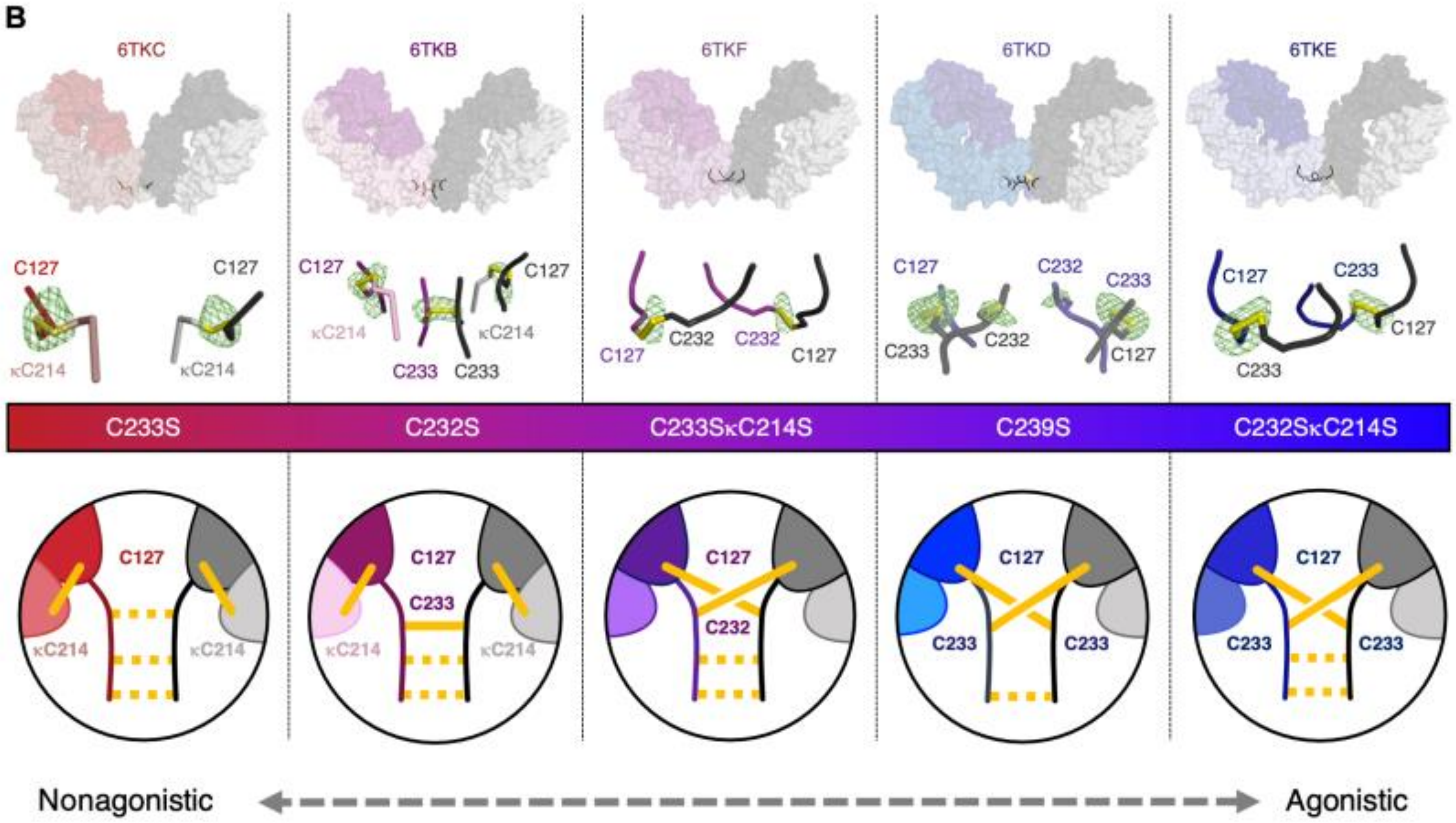
SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

ANTIBODIES

Hinge disulfides in human IgG2 CD40 antibodies modulate receptor signaling by regulation of conformation and flexibility

Christian M. Orr[1,2,3,4]†, Hayden Fisher[1,2]†, Xiaojie Yu[2], Claude H.-T. Chan[2], Patrick J. Duriez[2,7], Steven G. Booth[2], Isabel Elliott[1,2,8], Tatyana Inzhelevskay Christine A. Penfold[2], Armin Wagner[4], Martin J. Glennie[2], Ann L. White[2,9], Jo Arwen R. Pearson[3,5]‡, Mark S. Cragg[2,10]‡, Ivo Tews[1,10]*‡





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:

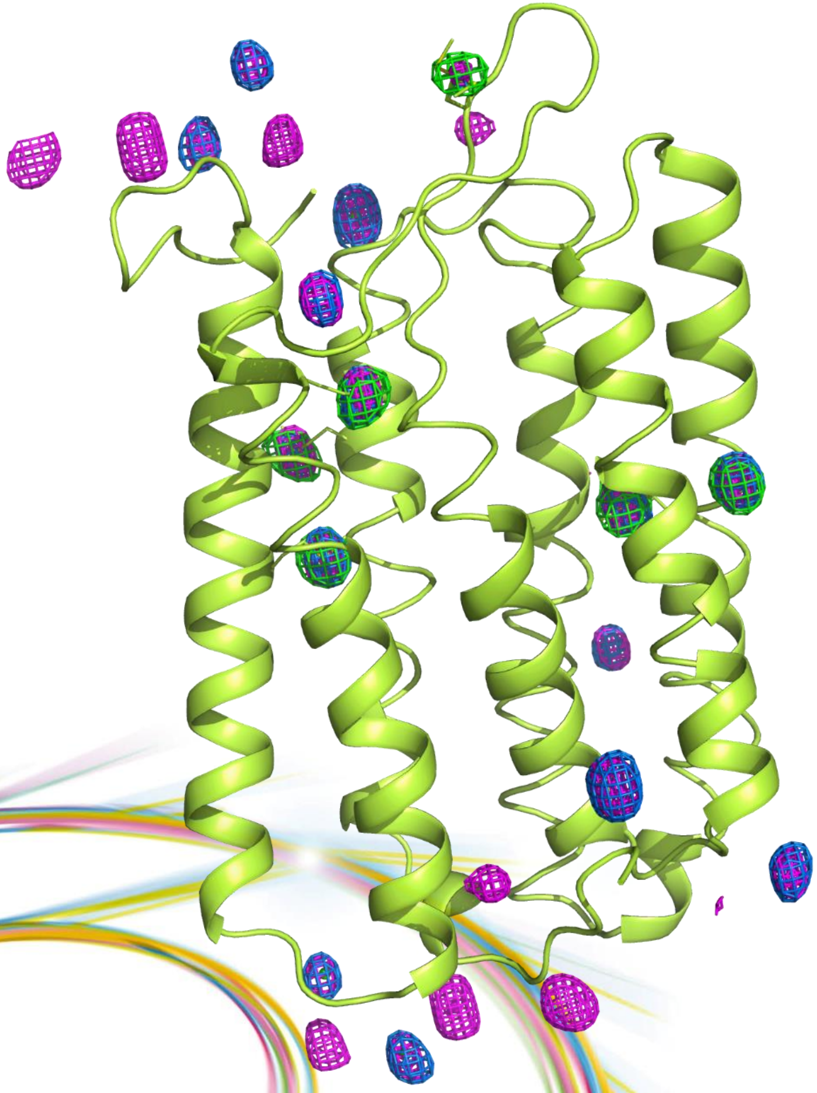
https://www.diamond.ac.uk/Instruments/Mx/I23.html

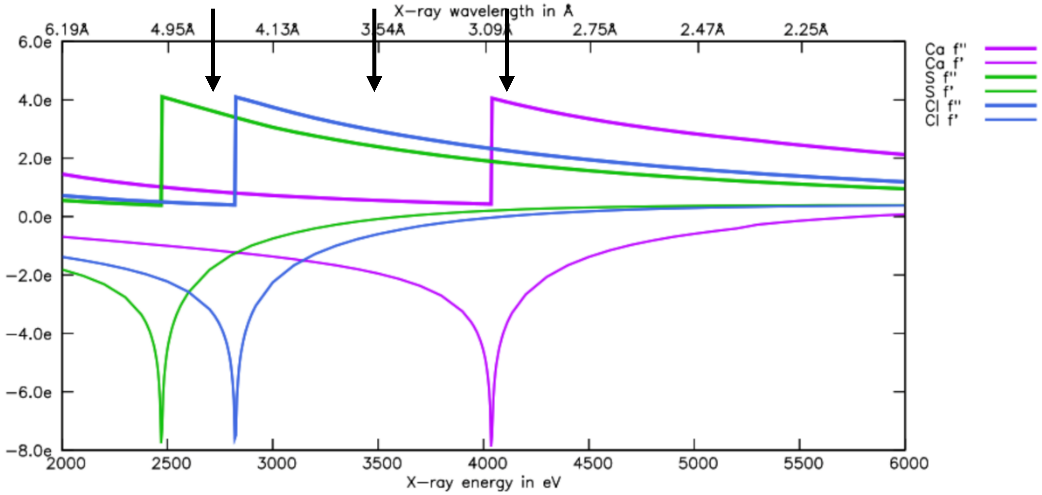
diamond

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

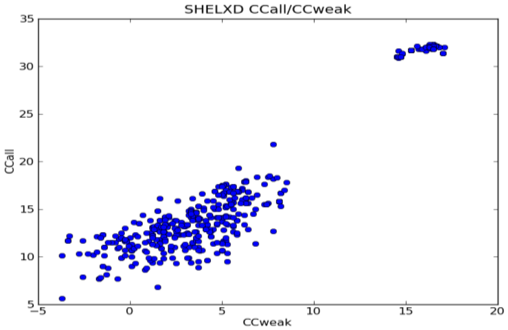
diamond

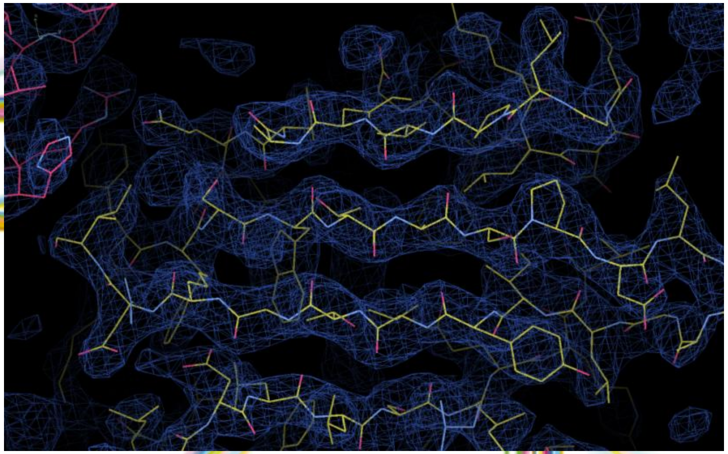
S-SAD 2

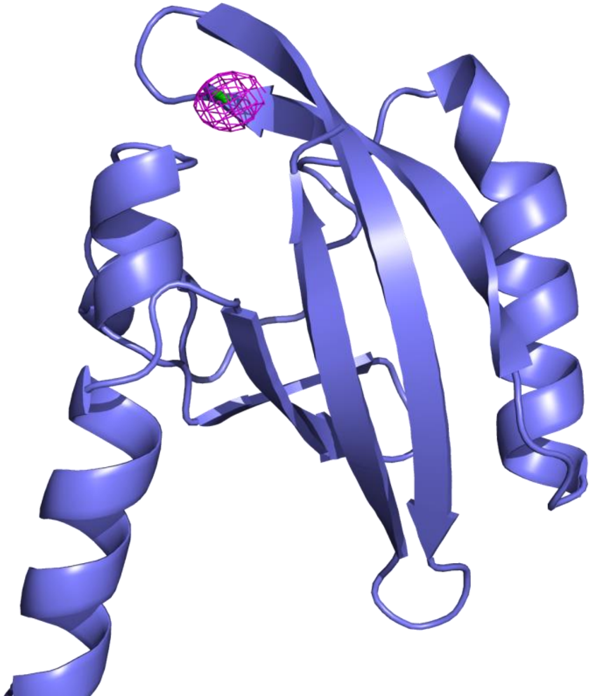
R. Owens group

- MR not working
- Se-Met not possible (no MET)
- **1 S in 116 amino acids**
- 3 molecules in the asu
- Space group $P2_12_12$

- λ = 2.75 Å
- Bijvoet ratio 1.35% (0.6% at λ = 2 Å)
- Inverse beam 4 x 180º (single crystal)
- Solvent content: 0.45
- Data to 2.5 Å resolution







Anomalous peak height: 12 – 17 σ

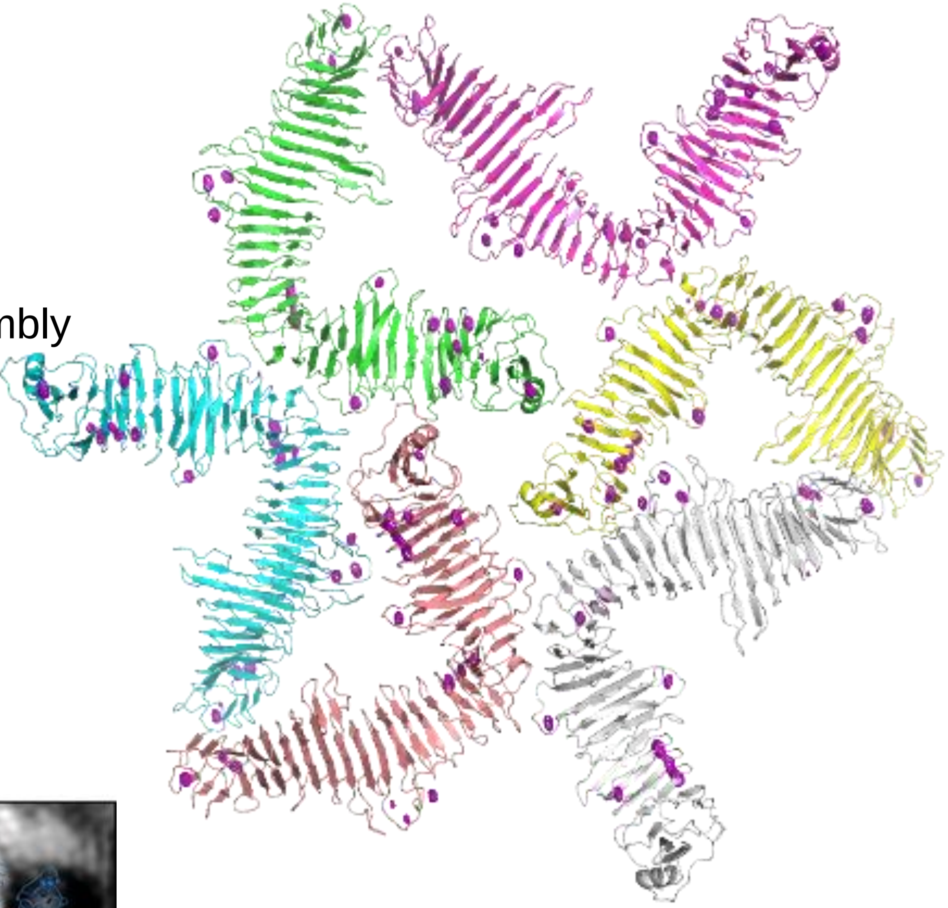
Phases improved with 3-fold averaging

Ca identification

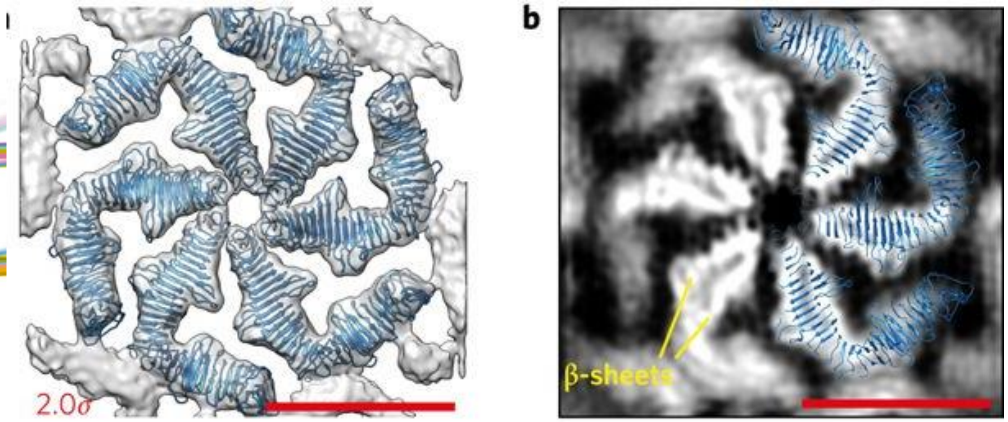
- Journal reviewer commented on Ca identification

- RsaA protein responsible for S-layer assembly
- Ca^{2+} important for S-layer lattice formation

- Data collected at $\lambda = 3.02$ Å
- 19 Ca built in 471 amino acids
- Space group $P2_12_12_1$
- 3.5 Å resolution



X-ray structure of RsaA



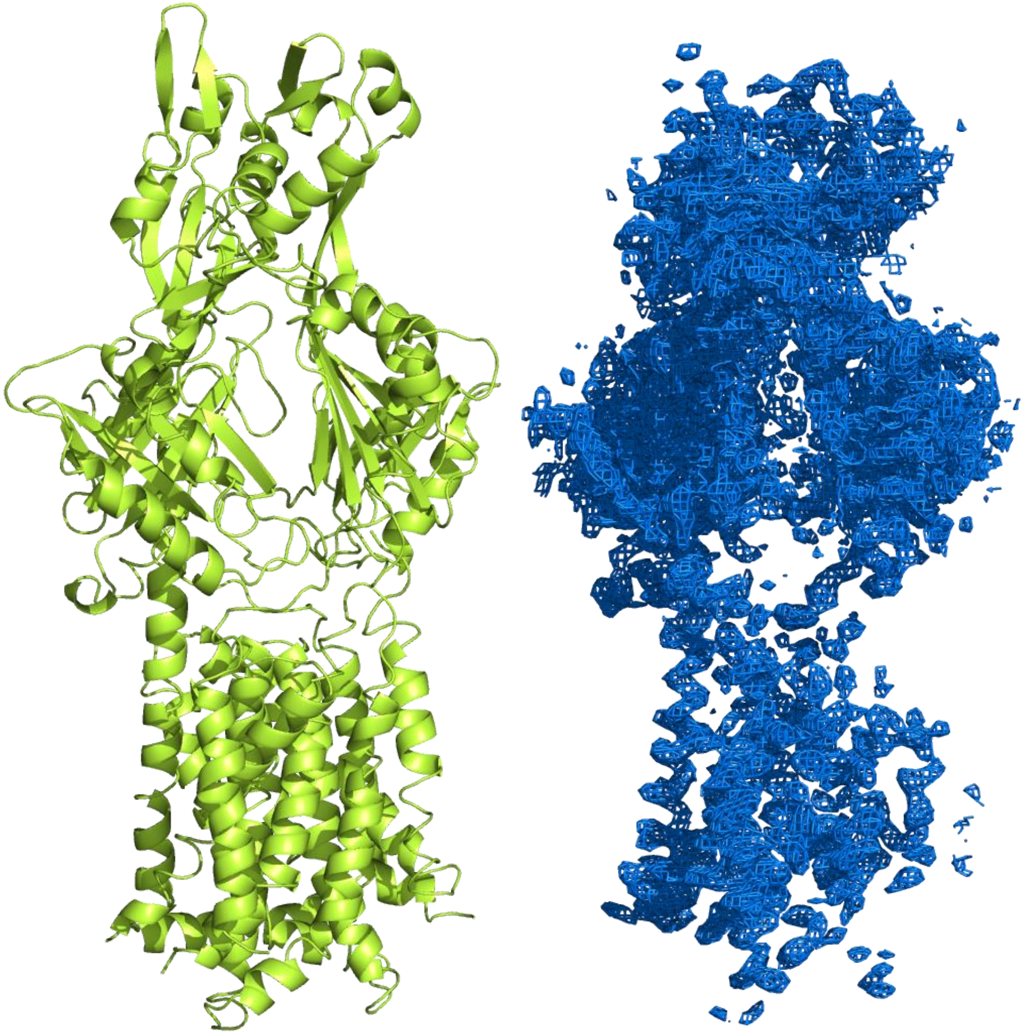
Cryo-ET structure of the S-layer

Bharat, T. A. M., et al. (2017). "Structure of the hexagonal surface layer on Caulobacter crescentus cells." Nature Microbiology **2**: 17059.

Tanmay Bharat
Jan Löwe

Membrane protein: ACRB

- 1049 residues
- 42 Met, 2 Cys
- Space group: *H*32
- Max. resolution 3.4 Å
- Multiplicity 80
- 72% solvent content
- λ = 3.02 Å

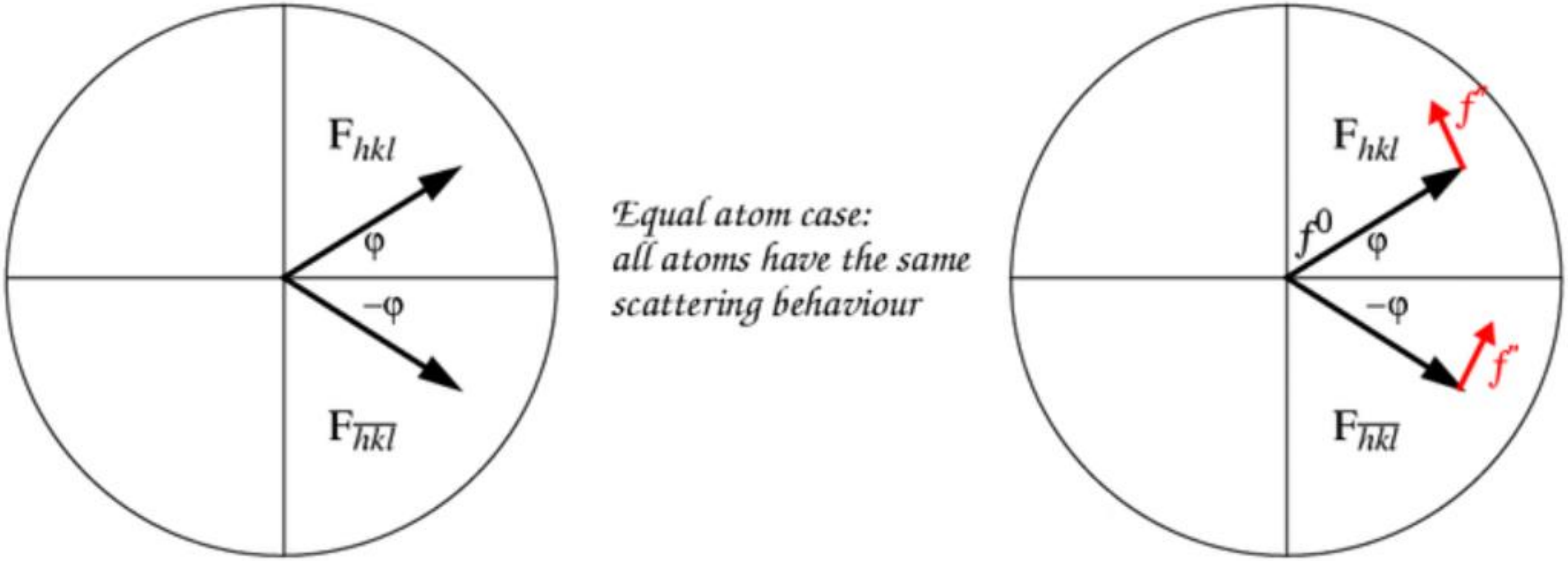


K. Beis

Anomalous scattering breaks Friedel Law

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

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



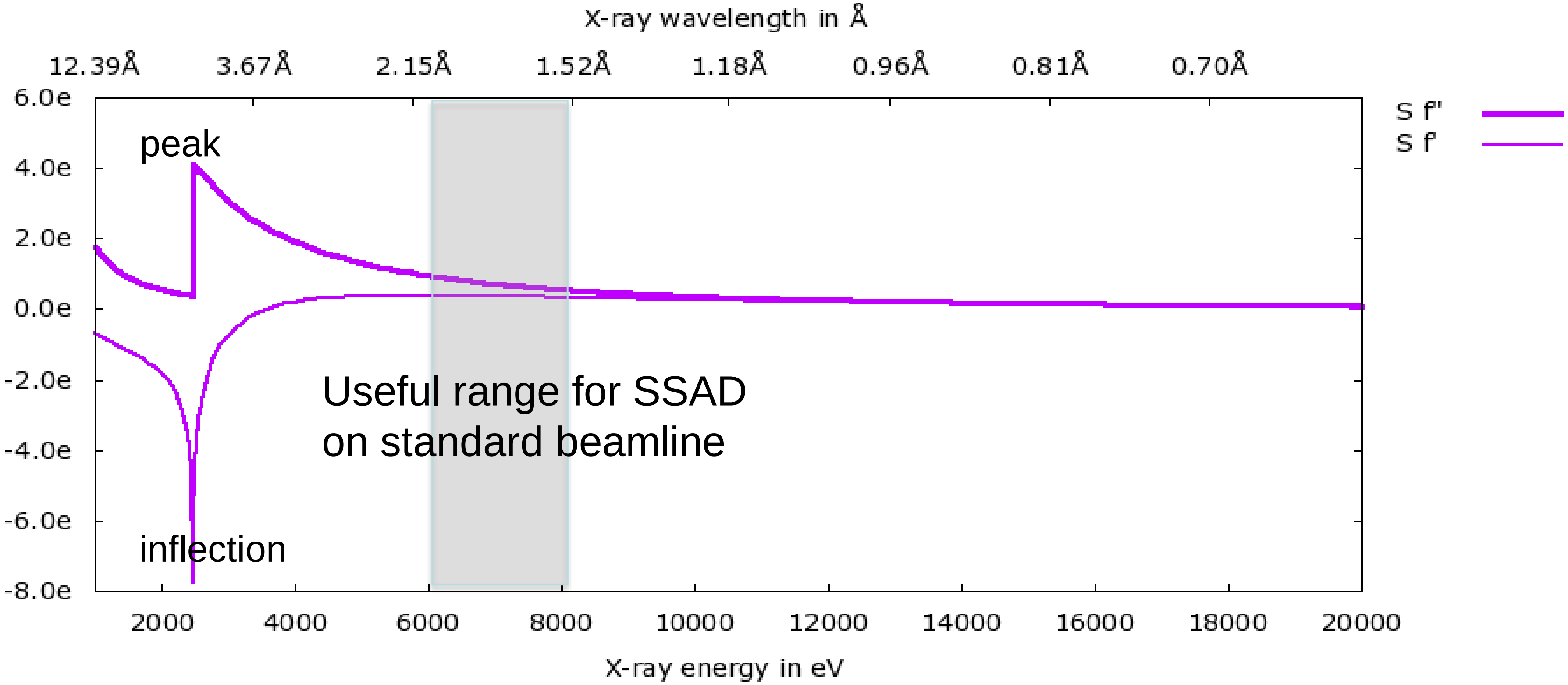
Equal atom case:
all atoms have the same
scattering behaviour

http://skuld.bmsc.washington.edu/scatter/AS_Friedel.html

Ethan A Merritt ©1996-2001/ merritt@u.washington.edu

Why long wavelengths?

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



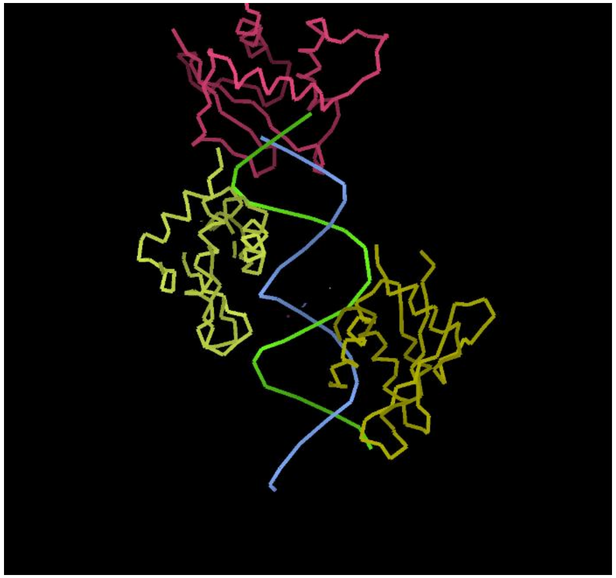
peak

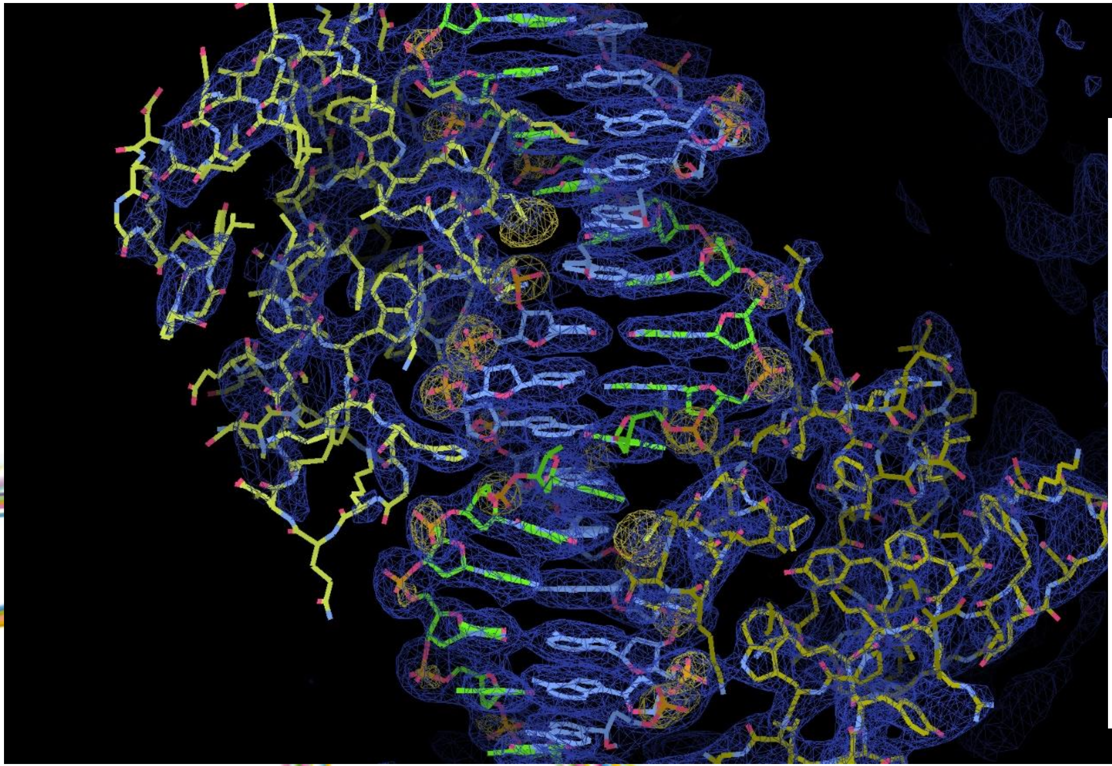
inflection

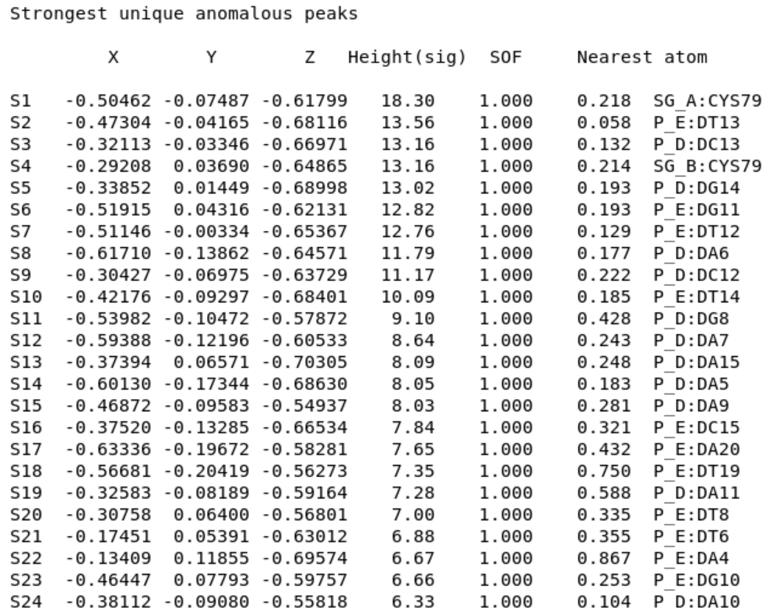
Useful range for SSAD
on standard beamline

S-P SAD

- DNA-protein complex: transcription factor
- 3 copies of protein + 2 nucleotide chains
- 3 S + 42 P
- λ = 2.7552 Å
- 2.6 A resolution







```
Strongest unique anomalous peaks

         X         Y         Z    Height(sig)  SOF      Nearest atom

S1   -0.50462 -0.07487 -0.61799   18.30     1.000     0.218  SG_A:CYS79
S2   -0.47304 -0.04165 -0.68116   13.56     1.000     0.058  P_E:DT13
S3   -0.32113 -0.03346 -0.66971   13.16     1.000     0.132  P_D:DC13
S4   -0.29208  0.03690 -0.64865   13.16     1.000     0.214  SG_B:CYS79
S5   -0.33852  0.01449 -0.68998   13.02     1.000     0.193  P_D:DG14
S6   -0.51915  0.04316 -0.62131   12.82     1.000     0.193  P_E:DG11
S7   -0.51146 -0.00334 -0.65367   12.76     1.000     0.129  P_E:DT12
S8   -0.61710 -0.13862 -0.64571   11.79     1.000     0.177  P_D:DA6
S9   -0.30427 -0.06975 -0.63729   11.17     1.000     0.222  P_D:DC12
S10  -0.42176 -0.09297 -0.68401   10.09     1.000     0.185  P_E:DT14
S11  -0.53982 -0.10472 -0.57872    9.10     1.000     0.428  P_D:DG8
S12  -0.59388 -0.12196 -0.60533    8.64     1.000     0.243  P_D:DA7
S13  -0.37394  0.06571 -0.70305    8.09     1.000     0.248  P_D:DA15
S14  -0.60130 -0.17344 -0.68630    8.05     1.000     0.183  P_D:DA5
S15  -0.46872 -0.09583 -0.54937    8.03     1.000     0.281  P_D:DA9
S16  -0.37520 -0.13285 -0.66534    7.84     1.000     0.321  P_E:DC15
S17  -0.63336 -0.19672 -0.58281    7.65     1.000     0.432  P_E:DA20
S18  -0.56681 -0.20419 -0.56273    7.35     1.000     0.750  P_E:DT19
S19  -0.32583 -0.08189 -0.59164    7.28     1.000     0.588  P_D:DA11
S20  -0.30758  0.06400 -0.56801    7.00     1.000     0.335  P_E:DT8
S21  -0.17451  0.05391 -0.63012    6.88     1.000     0.355  P_E:DT6
S22  -0.13409  0.11855 -0.69574    6.67     1.000     0.867  P_E:DA4
S23  -0.46447  0.07793 -0.59757    6.66     1.000     0.253  P_E:DG10
S24  -0.38112 -0.09080 -0.55818    6.33     1.000     0.104  P_D:DA10
```

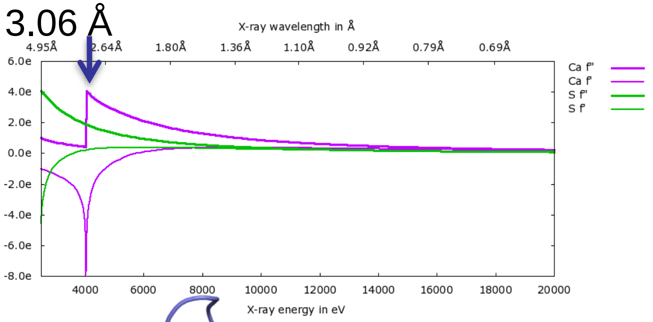
UNIVERSITY OF SUSSEX

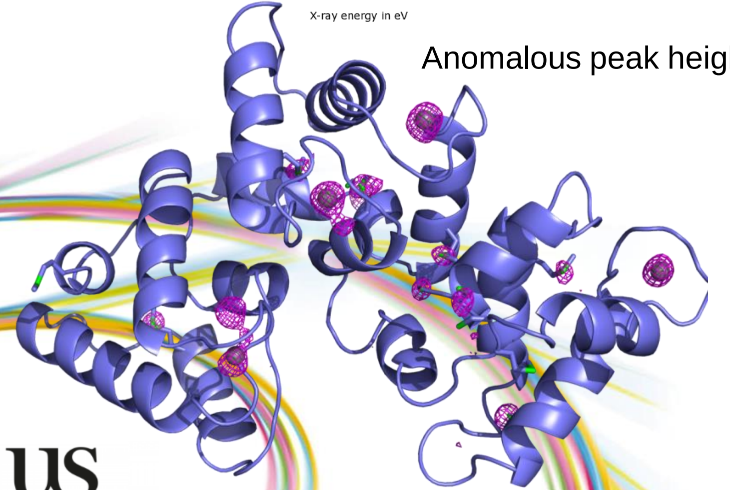
Erika Mancini

diamond

Ca-SAD

- 4 Calcium in 261 amino acids
- Space group $P2_12_12$
- λ = 3.02 Å
- Inverse beam 2 x 200º
- Data to 2.1 Å resolution





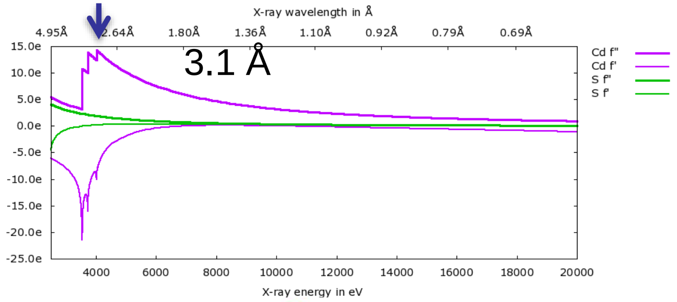
Anomalous peak height: 13 – 29 σ

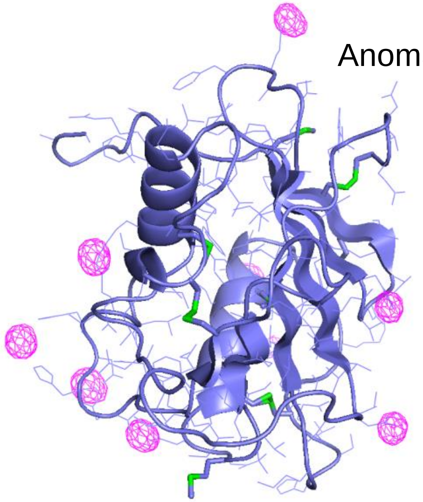
J.W. Noble

Noble, J. W., Almalki, R., Roe, S. M., Wagner, A., Duman, R. & Atack, J. R. (2018). Acta Cryst. D74, 1008-1014.

Cd-SAD

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





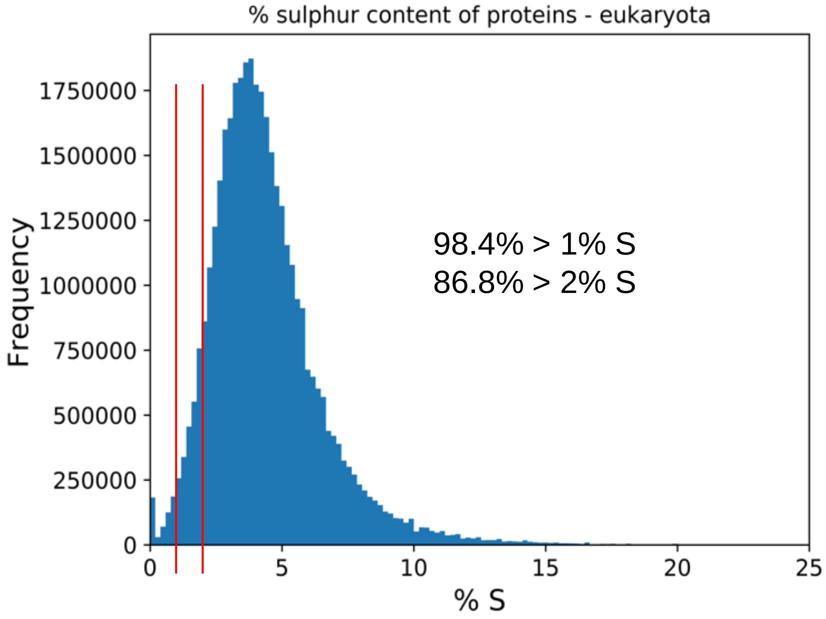
Anomalous peak height: 10 – 17 σ

T.A. Bowden

Pryce,R., Ng, W.M., Zeltina, A., Watanabe, Y.,El Omari,K.,Wagner, A., Bowden, T.A., Structure-based classification defines the discrete conformational classes adopted by the arenaviral GP1, Journal of Virology Dec 2018, 93 (1) e01048-18

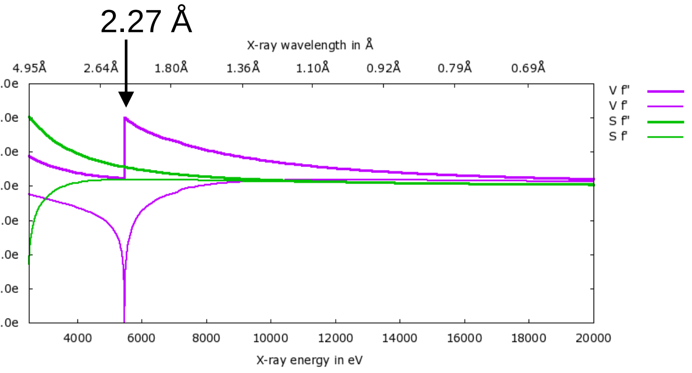
Experimental phasing

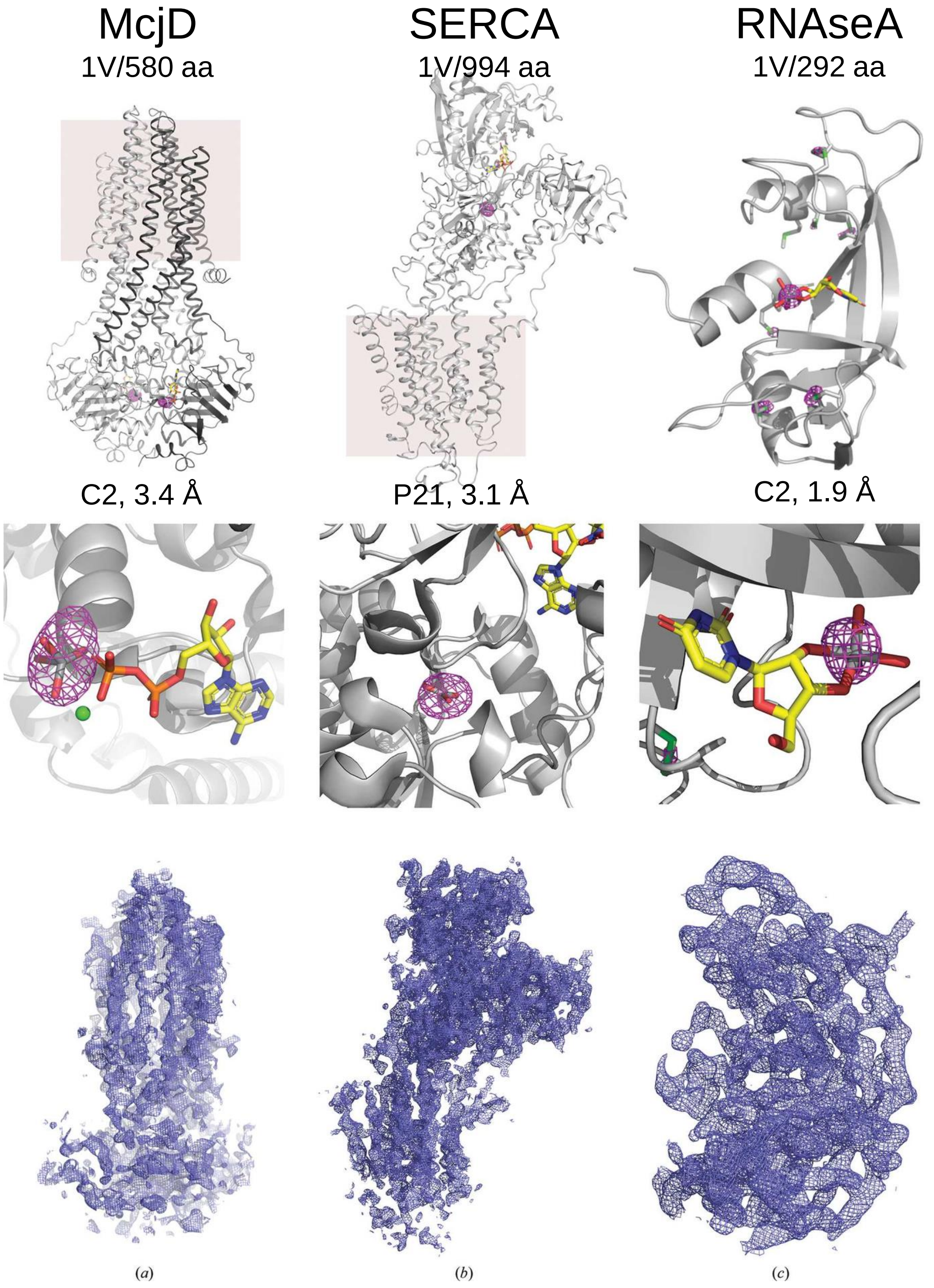
16
S
Sulfur
32.06



- Intrinsic anomalous signal from sulphur sufficient to solve phase problem
- For crystals diffracting to 2.5 Å, 1% sulphur content sufficient, for d_{min} = 3 Å, 2%

V-SAD





El Omari et al. *IUCrJ*, 2020, 7 (6):1092–1101