

Beamline I23 – long wavelength X-ray crystallography

Ramona Duman

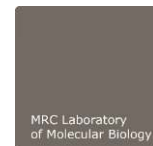
CCP4/DLS workshop
December 2021

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



I23 – In-vacuum long-wavelength MX



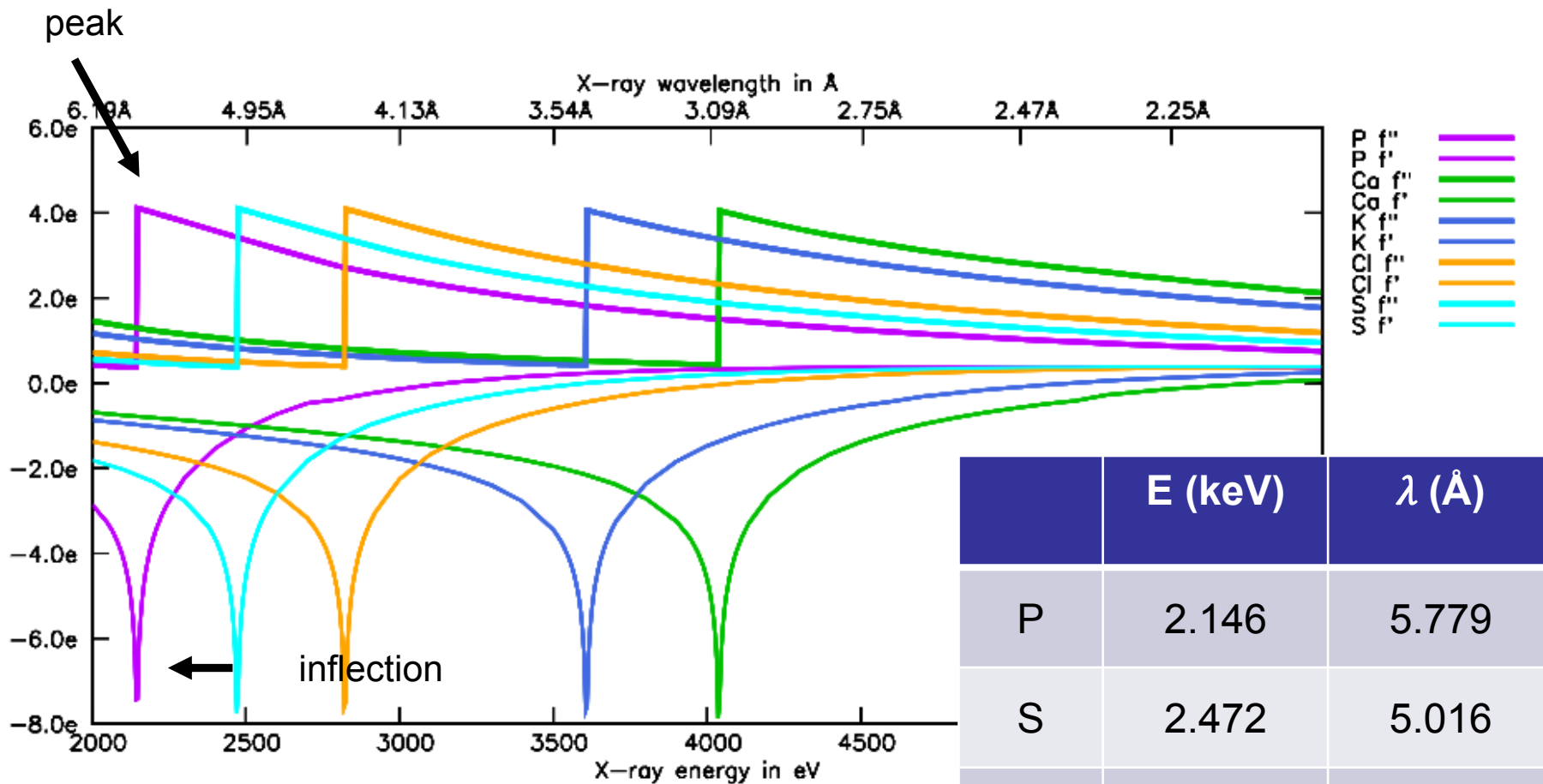
Beamline parameters:

Wavelength range: 1 - 5.9 Å

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

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

Accessible X-ray absorption edges of biologically relevant elements

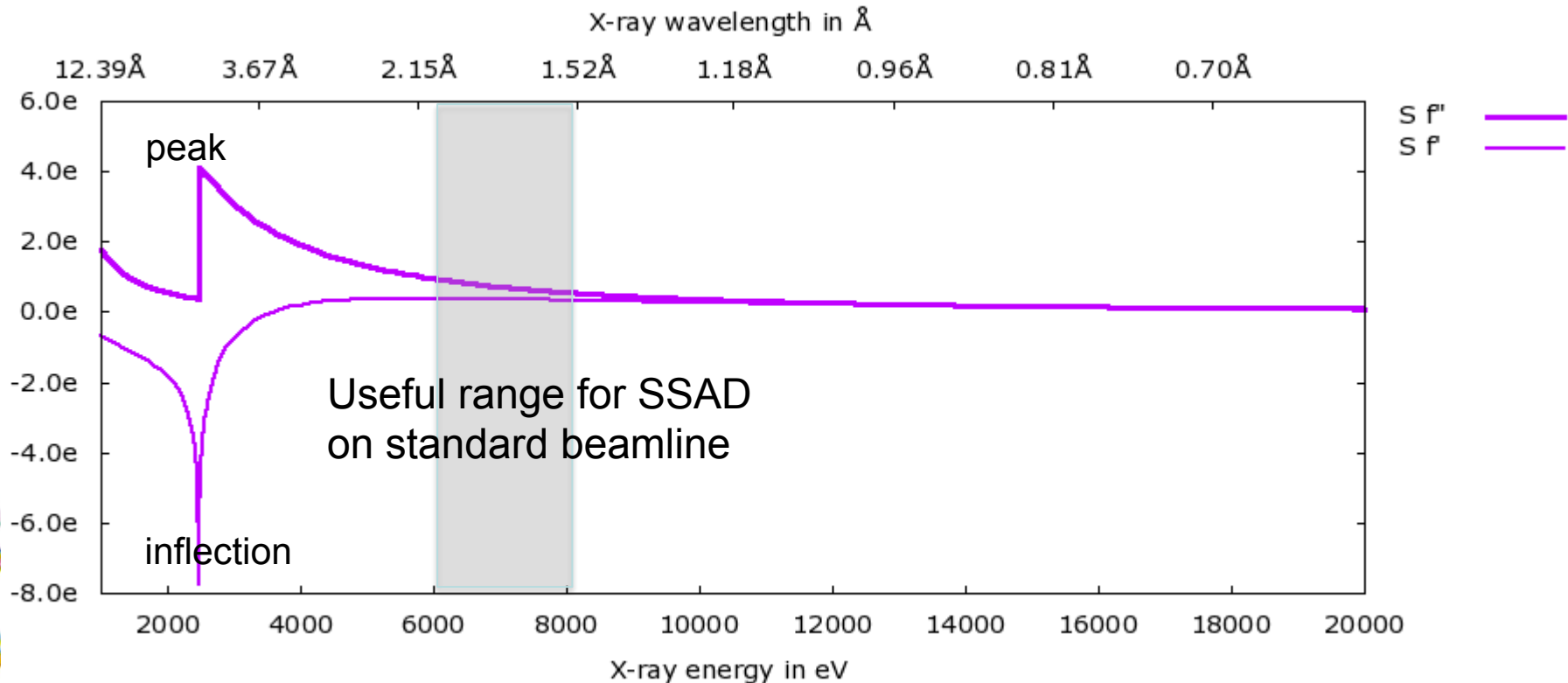


	E (keV)	λ (Å)
P	2.146	5.779
S	2.472	5.016
Cl	2.822	4.393
K	3.607	3.437
Ca	4.038	3.070

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

Why long wavelengths?

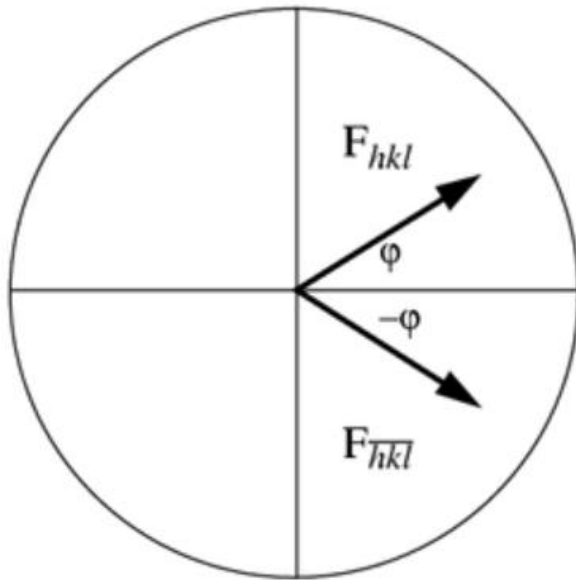
$$f = f_0 + \Delta f' + if'' = f' + if''$$



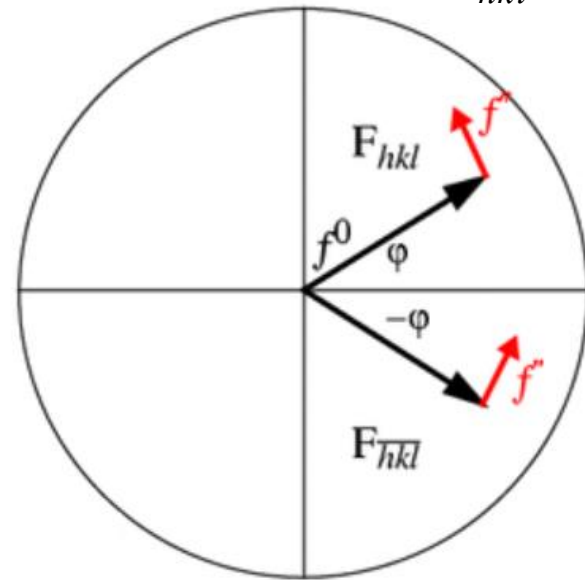
Anomalous scattering breaks Friedel Law

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

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



*Equal atom case:
all atoms have the same
scattering behaviour*



I23 – Long-wavelength MX

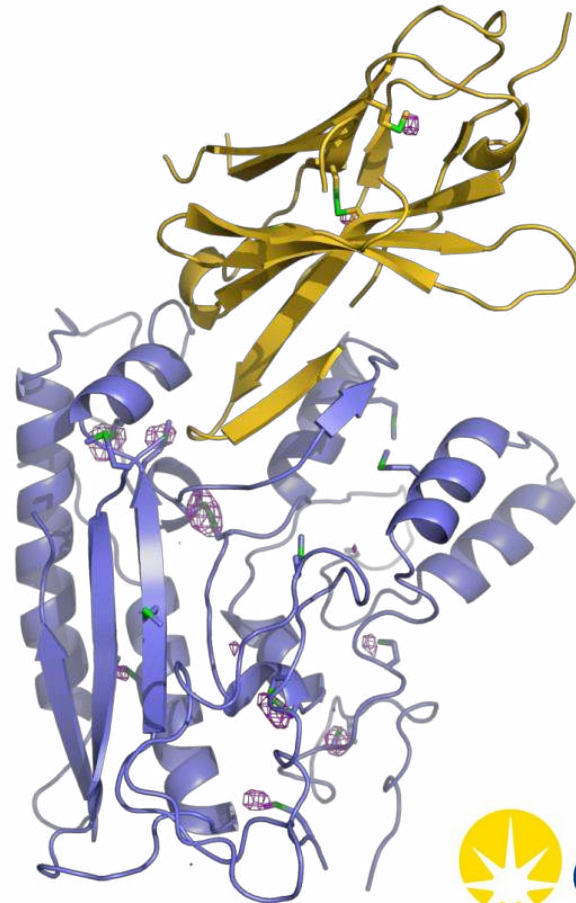
Experimental Phasing

- 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 Fourier maps

Assist model building



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

3

Li

Lithium

6.941

11

Na

Sodium

22.98976928

4

Be

Beryllium

9.012182

12

Mg

Magnesium

24.3050

19

K

Potassium

39.0983

20

Ca

Calcium

40.078

21

Sc

Scandium

44.955912

22

Ti

Titanium

47.887

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

(209.9824)

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)

1

H

Hydrogen

1.00794

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

47.887

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

(209.9824)

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)

1

H

Hydrogen

1.00794

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

47.887

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

(209.9824)

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)

1

H

Hydrogen

1.00794

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

47.887

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

(209.9824)

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)

1

H

Hydrogen

1.00794

2

He

Helium

4.002602

3

Li

Lithium

6.941

4

Design and Interface Copyright © 1997 Michael Dayah (michael@dayah.com). <http://www.ptable.com/>

Challenges at long wavelengths

- Limited resolution:

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

- Absorption:

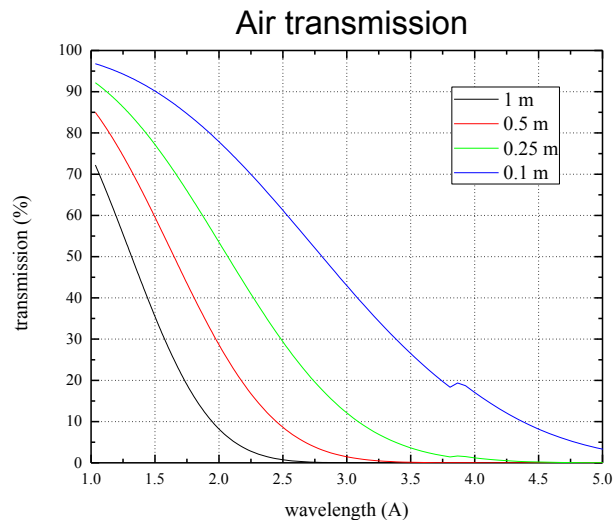
- Air absorption
- Crystal, solvent and loop absorption

Pilatus 12M (Dectris)

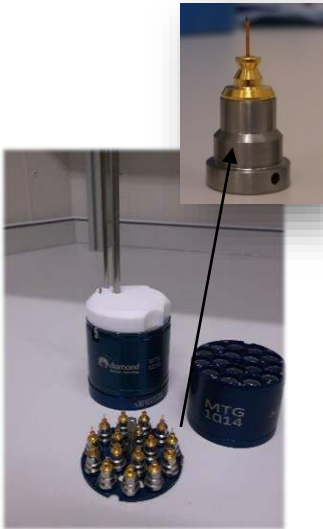


120 modules in vacuum
12,000,000 pixels
Half cylinder $r = 250$ mm
Width $z = 423.6$ mm
 $2\theta = \pm 100^\circ$ (40.3° laterally)

In-vacuum
crystallography:
Elimination of air scattering and
absorption



Sample preparation logistics



Tools sent to users



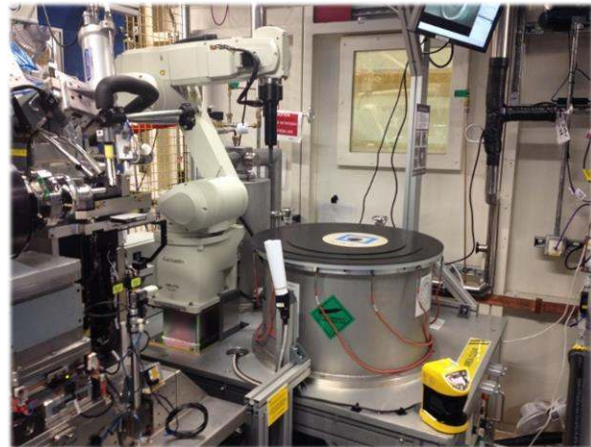
Users harvest and cryo-cool crystals



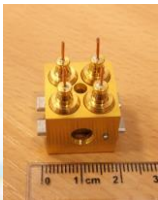
Samples sent to Diamond

Transfer to I23 vacuum endstation

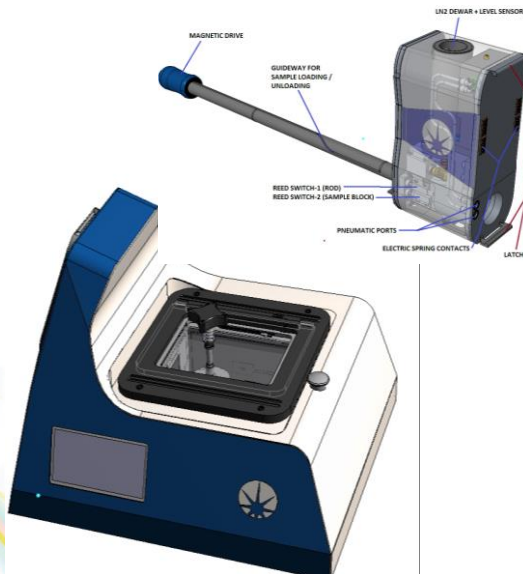
Pre-screening on other MX beamlines



I23 thermally conductive sample holders and blocks



Diamond Cryogenic Sample Transfer System



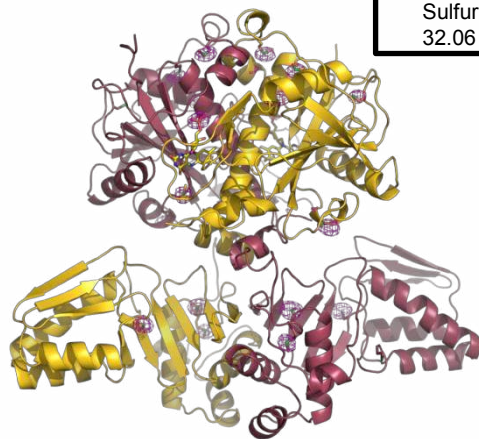
Sulfur SAD examples



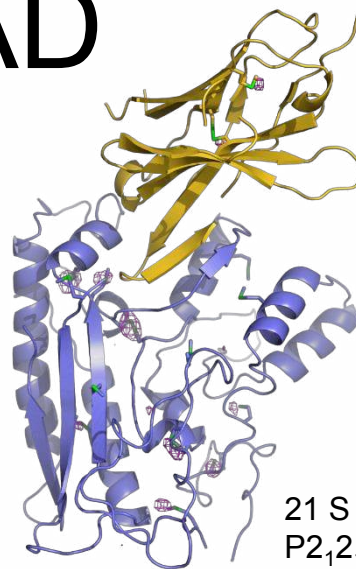
¹⁶
S
Sulfur
32.06

- SAD

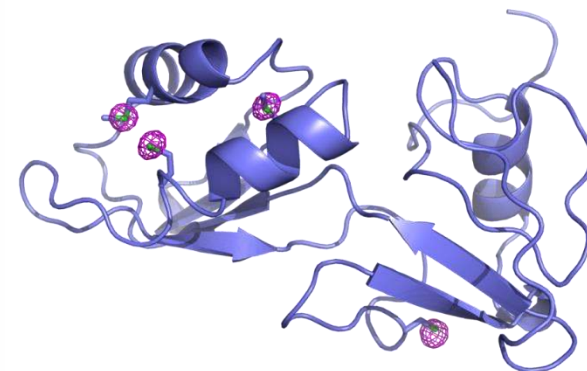
$\lambda = 2.75 \text{ \AA}$
Multiplicity: 10 - 20



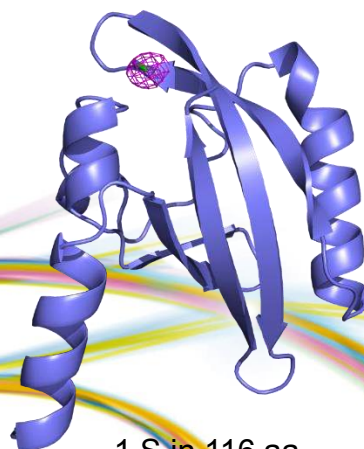
10 S in 477 aa
P4₁2₁2₁
3.2 Å res



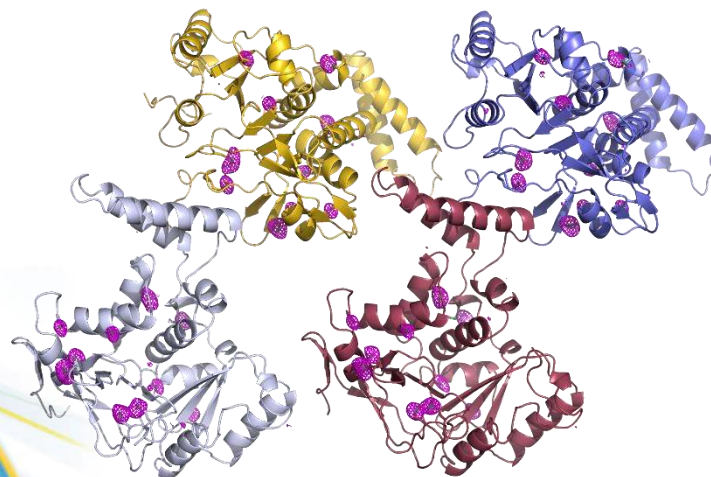
21 S in 427 aa
P2₁2₁2₁
2.8 Å res



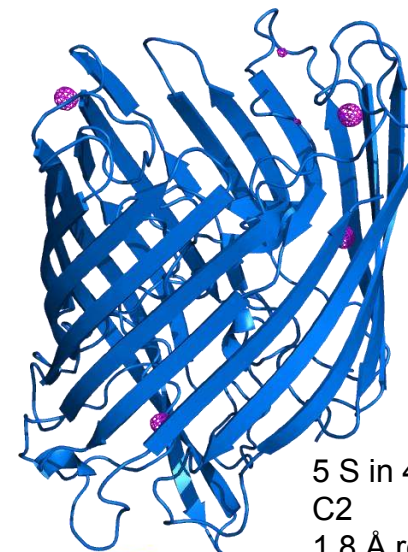
4 S in 157 aa
C2
1.8 Å res



1 S in 116 aa
P2₁2₁2
2.5 Å res

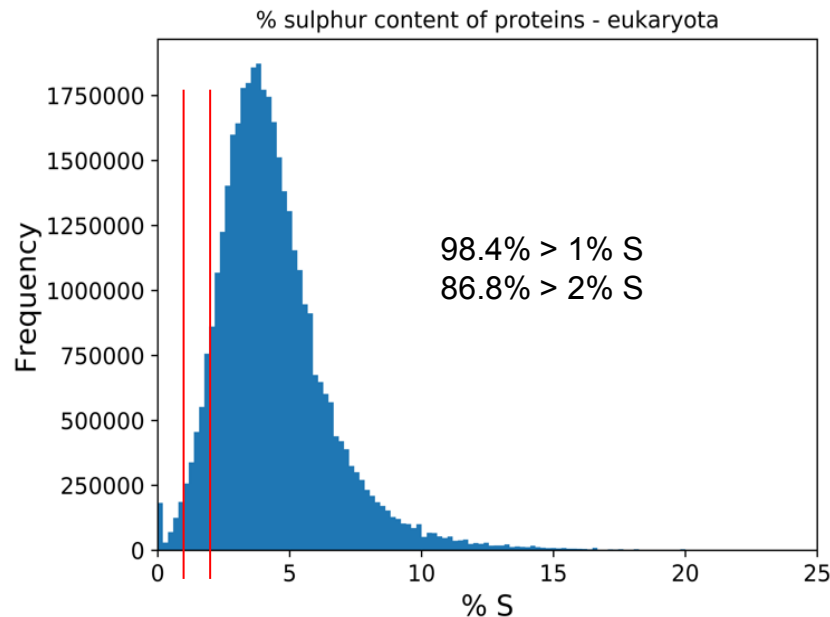


10 S in 322 aa
P2₁
2.1 Å res
tNCS



5 S in 453 aa
C2
1.8 Å res

Experimental phasing



- 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%

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

NLKMEIILTSQGLKKYYGKILRLLQLTLEEDTEGLLEWCKRNLGLDCDDTFF
 QKRIEEFFITGEGHFNEVLQFRTPGTLSTTESTPAGLPTAEPFKSYFAKGFLSIDSGYYS
 AKCYSGTSNSGLQLINIRHSTRIVDTGPKITNLKTINCINLKASIFKEHREVEINVLL
 PQVAVNLSNCHVVIKSHVCDYSLDIDGAVRLPHIYHEGVFIPGTYKIVIDKKNLNDRCT
 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	

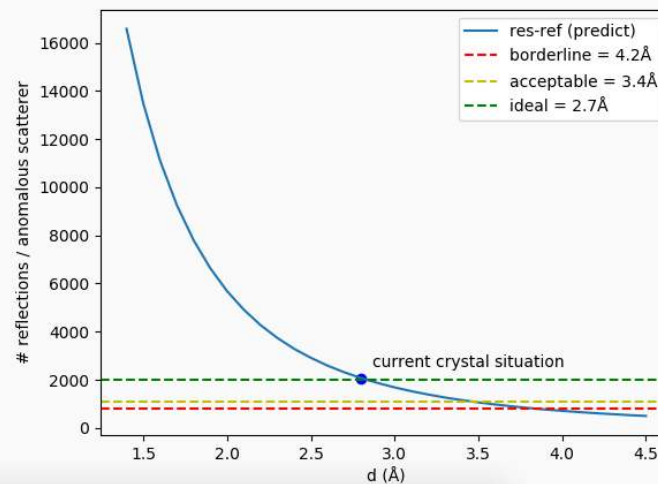
Molecules per ASU:

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

CALCULATE

SAVE PLOT

RESET

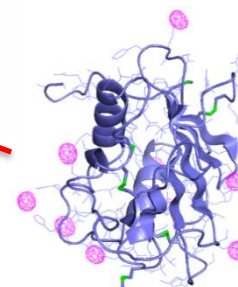
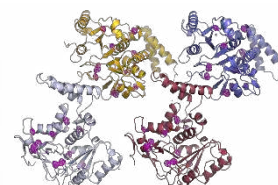
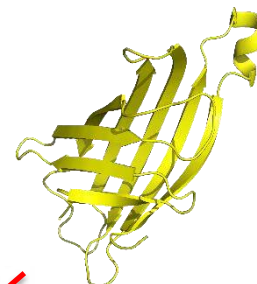
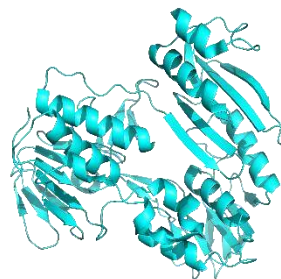
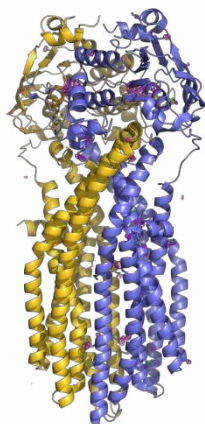
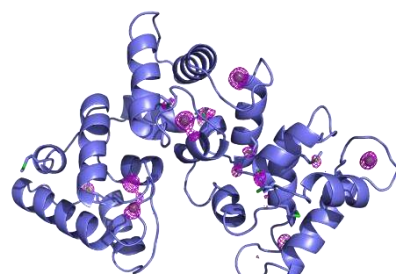


Based on:
 Terwilliger et al. "Can I solve my structure
 by SAD phasing? Anomalous signal in SAD
 Phasing" Acta D (2016) 346.

Other SAD examples



Other types of SAD phasing



1	2											13	14	15	16	17	18
1 H .008	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	3	4	5	6	7	8	9	10	11	12	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)

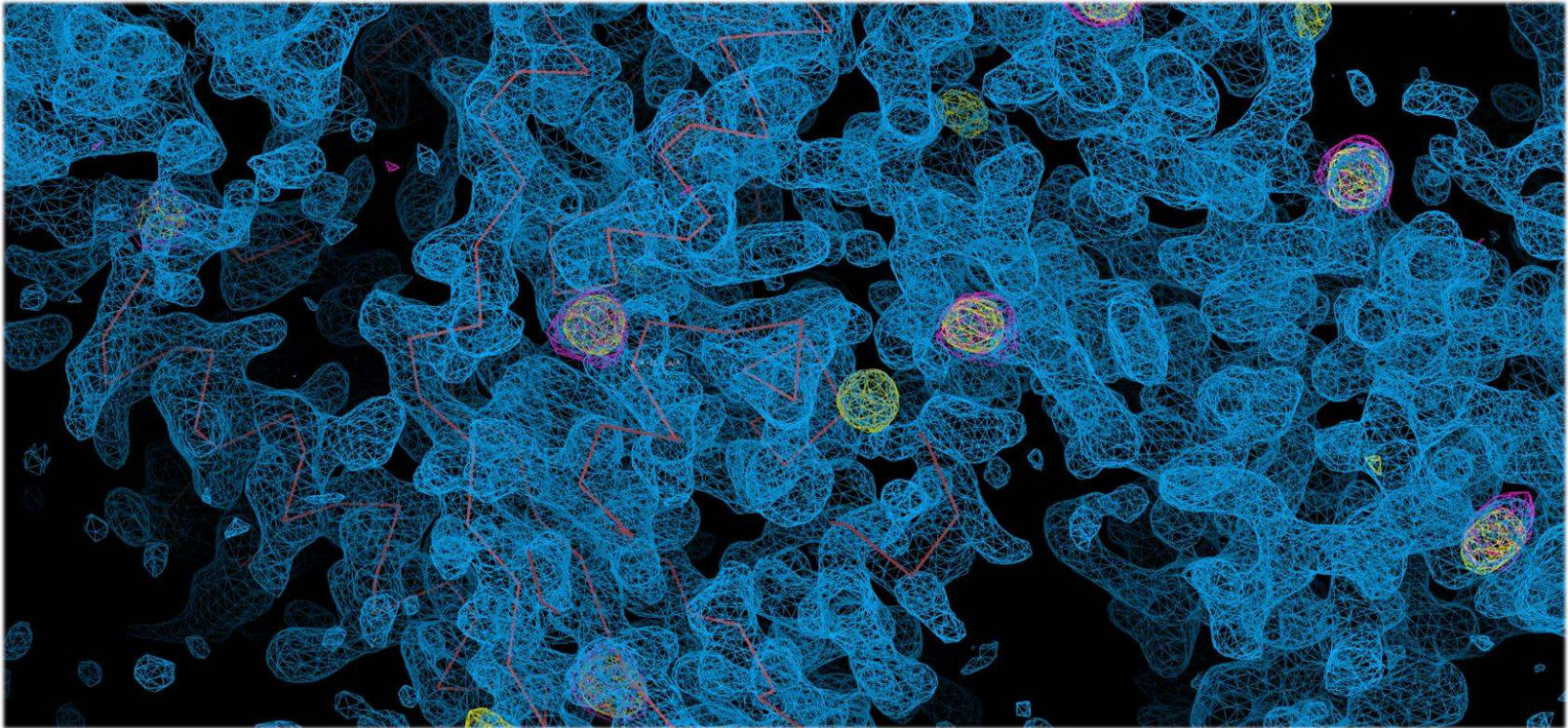
* Lanthanide series

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

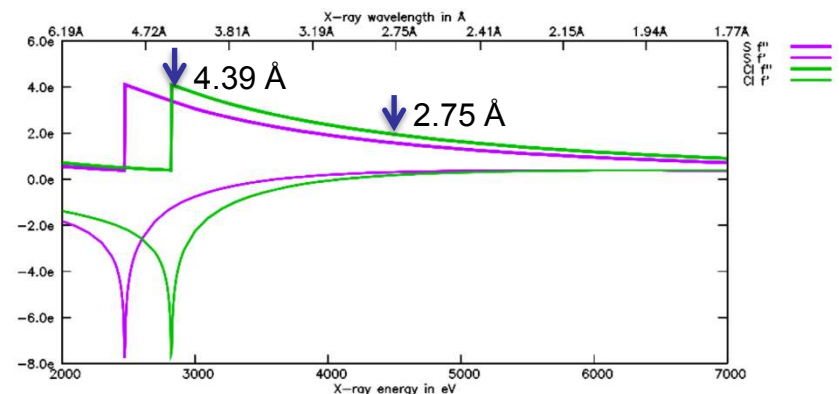
89 Ac (227)	90 Th 232.04	91 Pa 231.04	92 U 238.03	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (262)
-------------------	--------------------	--------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	--------------------	--------------------	--------------------	--------------------

S-CI SAD



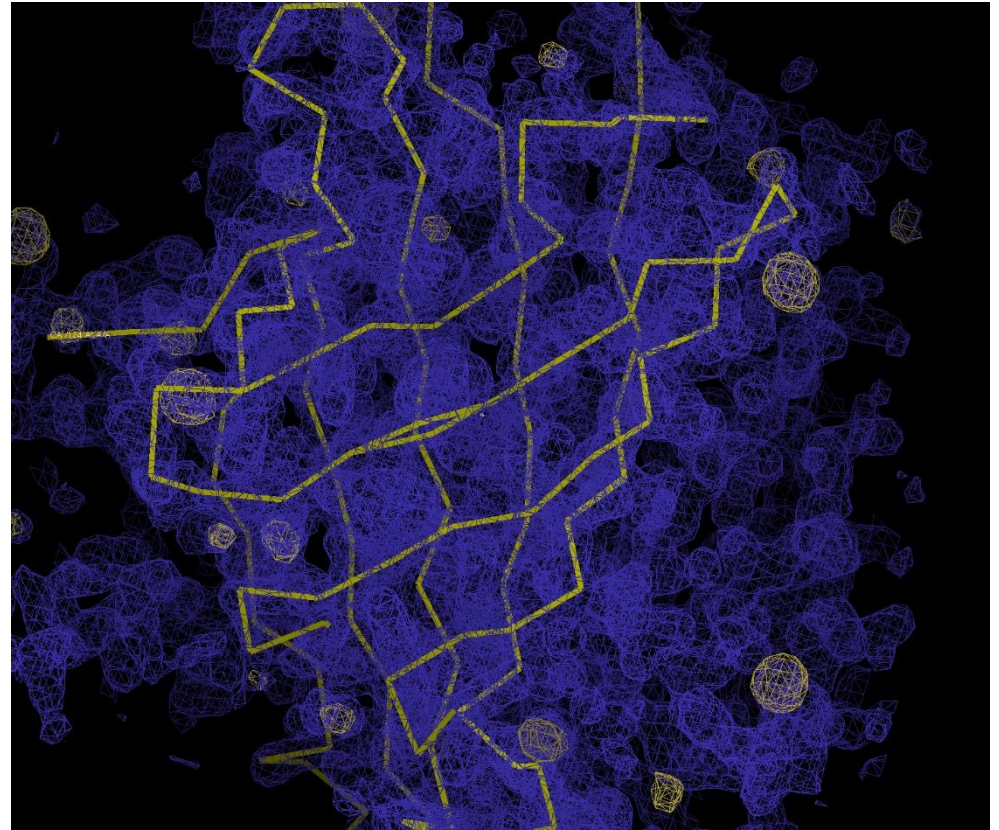
Crystals only grew after proteolytic cleavage

- 2 Å resolution data collected at $\lambda=2.75$ Å: 4 Cys (10-14 σ) and 3 Cl (6-15 σ) in 200 residues
- Dataset at $\lambda=4.42$ Å to confirm the Cl



CI-SAD

- When no S present ...
- Streptavidin – by accident
- 2.75 Å/ 4.5keV
- 124 aa, 1.8 Å res

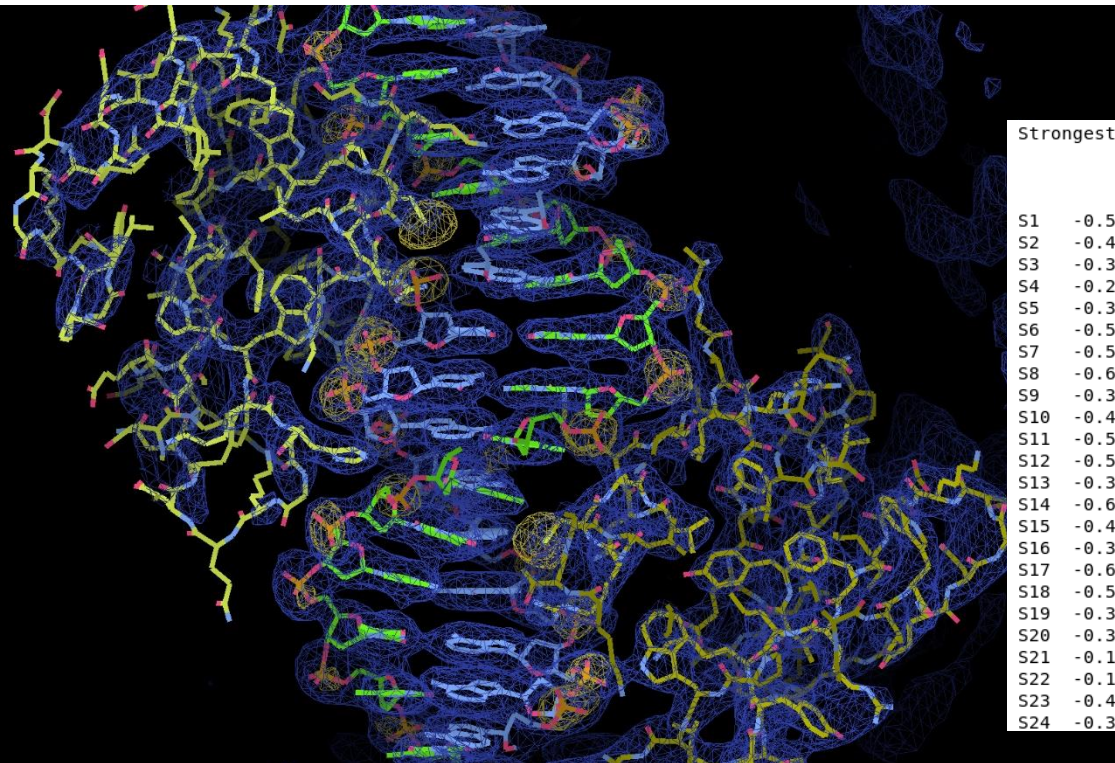
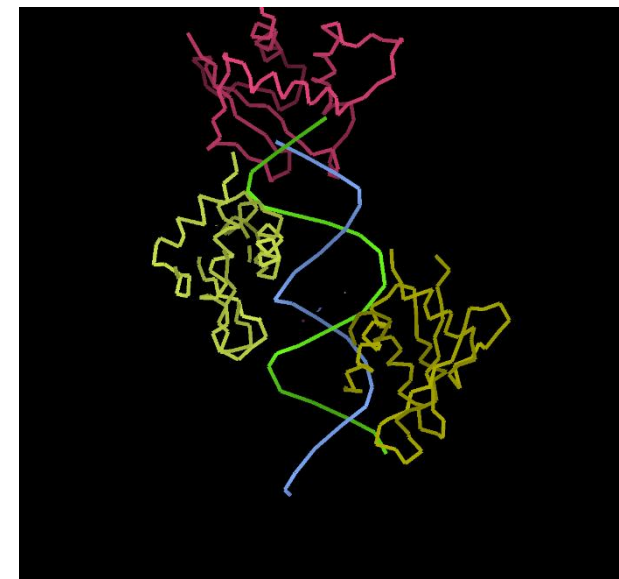


Strongest unique anomalous peaks

	X	Y	Z	Height(sig)	SOF	Nearest atom
CL1	0.12007	0.28673	0.20421	21.69	1.000	0.161 CL_A:CL154
CL2	0.25070	-0.11130	0.27163	18.22	1.000	0.147 CL_A:CL152
CL3	0.55049	0.15263	0.35564	17.03	1.000	0.035 CL_A:CL150
CL4	0.11652	0.20638	0.34395	11.53	1.000	0.366 CL_A:CL155
CL5	0.24511	-0.01222	0.30005	8.79	1.000	0.369 CL_A:CL151
CL6	0.30127	-0.12587	0.25139	8.75	1.000	0.164 CL_A:CL153
CL7	0.21334	0.06462	0.34445	8.02	1.000	0.336 CL_A:CL156

S-P SAD

- DNA-protein complex: transcription factor
- 3 copies of protein + 2 nucleotide chains
- 3 S + 42 P
- $\lambda = 2.7552 \text{ \AA}$
- 2.6 Å resolution

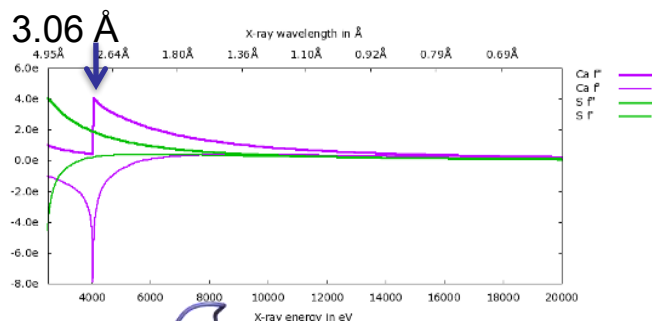


Strongest unique anomalous peaks

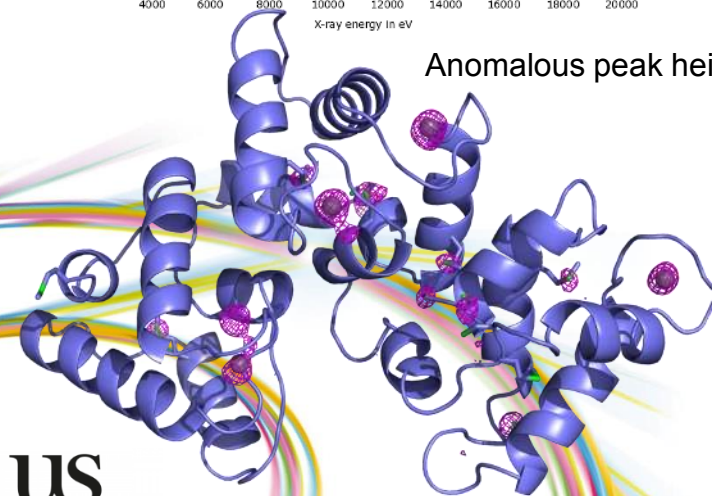
	X	Y	Z	Height(sig)	SOF	Nearest atom
S1	-0.50462	-0.07487	-0.61799	18.30	1.000	0.218 SG_A:CYS79
S2	-0.47304	-0.04165	-0.68116	13.56	1.000	0.058 P_E:DT13
S3	-0.32113	-0.03346	-0.66971	13.16	1.000	0.132 P_D:DC13
S4	-0.29208	0.03690	-0.64865	13.16	1.000	0.214 SG_B:CYS79
S5	-0.33852	0.01449	-0.68998	13.02	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

Ca-SAD

- 4 Calcium in 261 amino acids
- Space group $P2_12_12$
- $\lambda = 3.02 \text{ \AA}$
- Inverse beam $2 \times 200^\circ$
- Data to 2.1 \AA resolution



Anomalous peak height: $13 - 29 \sigma$



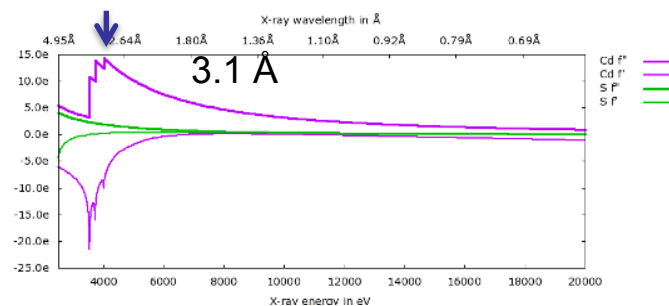
US
UNIVERSITY
OF SUSSEX

J.W. Noble

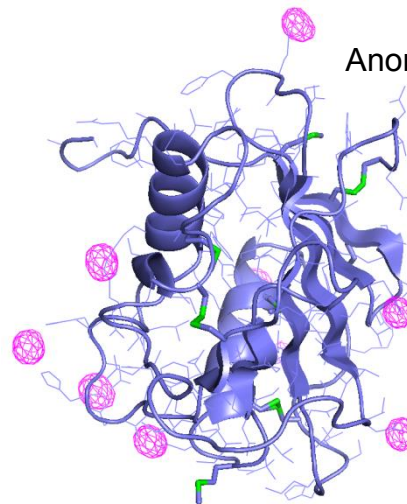
Noble, J. W., Almalki, R., Roe, S. M., Wagner, A., Duman, R. & Atack, J. R. (2018). Acta Cryst. D74, 1008-1014.

Cd-SAD

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



Anomalous peak height: $10 - 17 \sigma$



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

V-SAD

2.27 Å

X-ray wavelength in Å

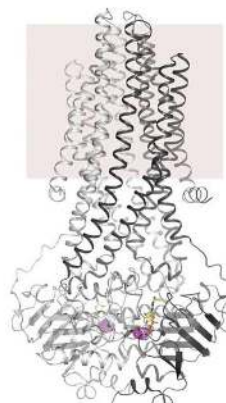
4.95 Å 2.64 Å 1.80 Å 1.36 Å 1.10 Å 0.92 Å 0.79 Å 0.69 Å

X-ray energy in eV

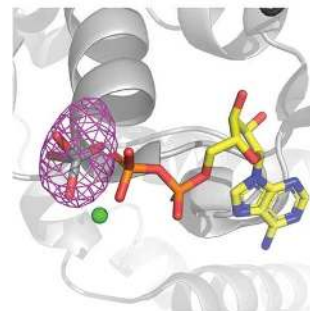
6.0e 4.0e 2.0e 0.0e -2.0e -4.0e -6.0e -8.0e

VP VP SP SP

McjD
1V/580 aa



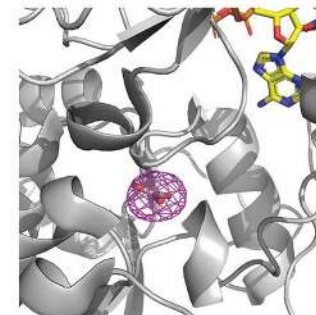
C2, 3.4 Å



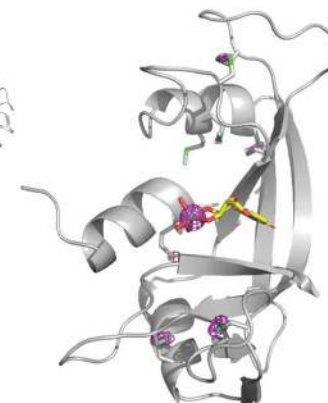
SERCA
1V/994 aa



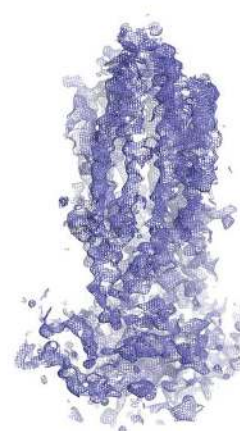
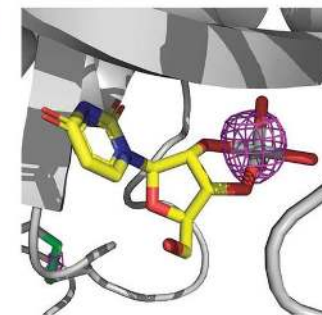
P21, 3.1 Å



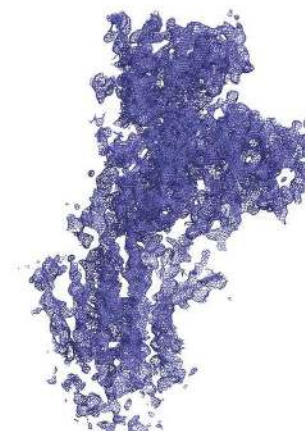
RNaseA
1V/292 aa



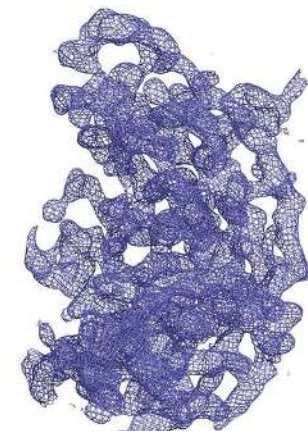
C2, 1.9 Å



(a)



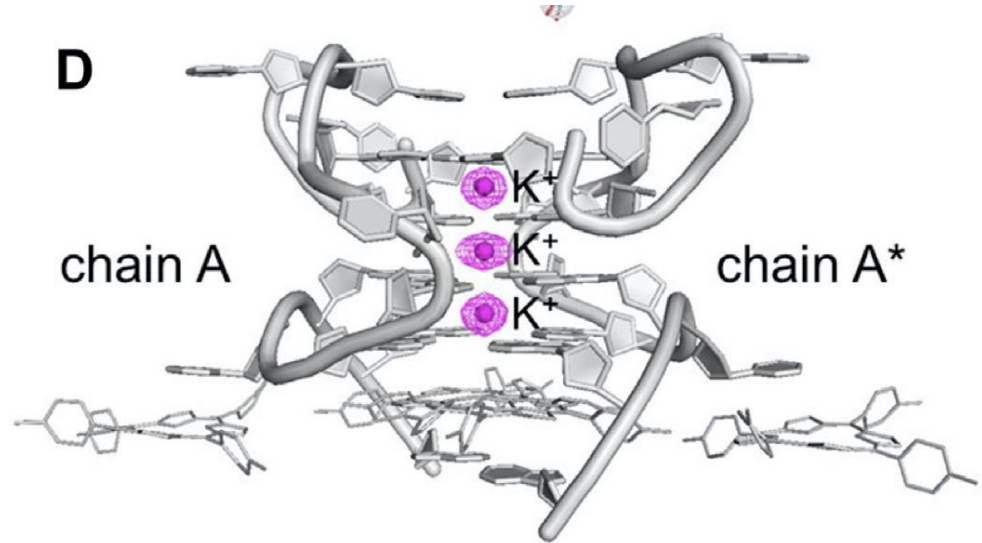
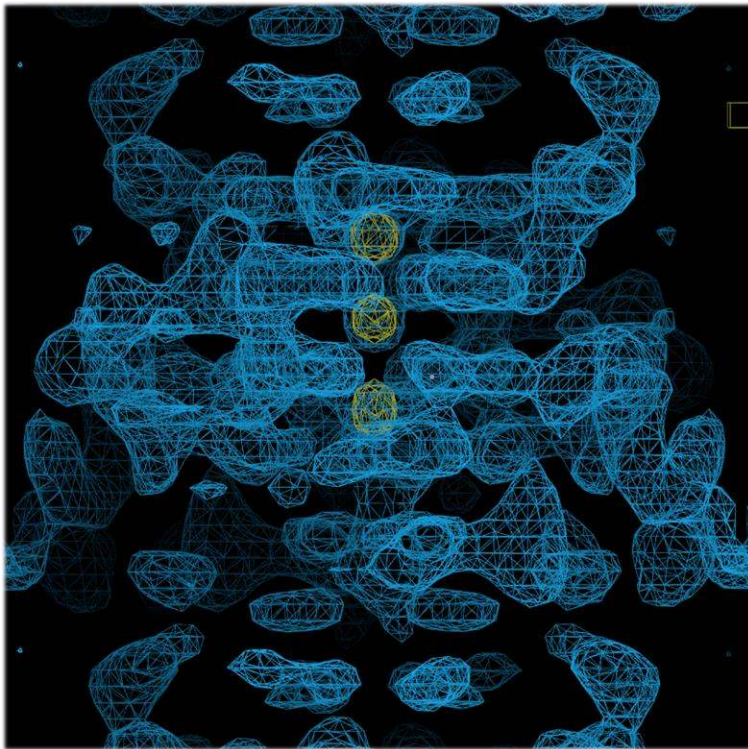
(b)



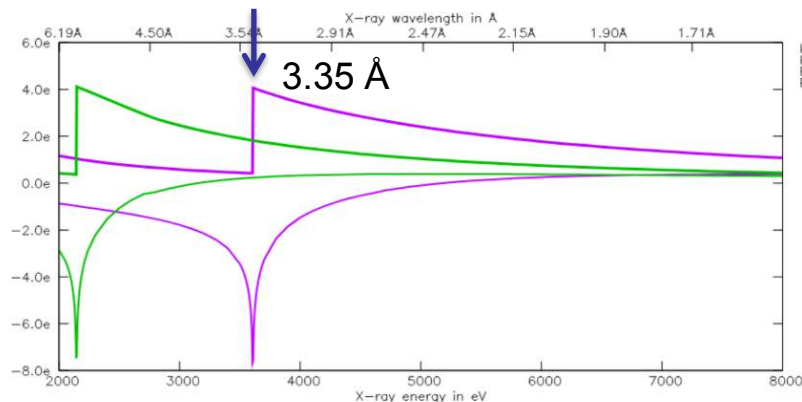
(c)

K-SAD

Stacked RNA G-tetrad



- $\lambda = 3.35 \text{ \AA}$ ($f' = -3.29$, $f'' = 3.9$)
- resolution 2.2 \AA , limited by detector
- P2: 30 24 31 90 107 90
- 14 RNA nucleotides/asu
- 3 K on 2 fold axis
- Anomalous peak heights 16σ



Zhang et al. Nucleic Acids Res. 48(1) (2020), 9886–9898.

Gary Parkinson



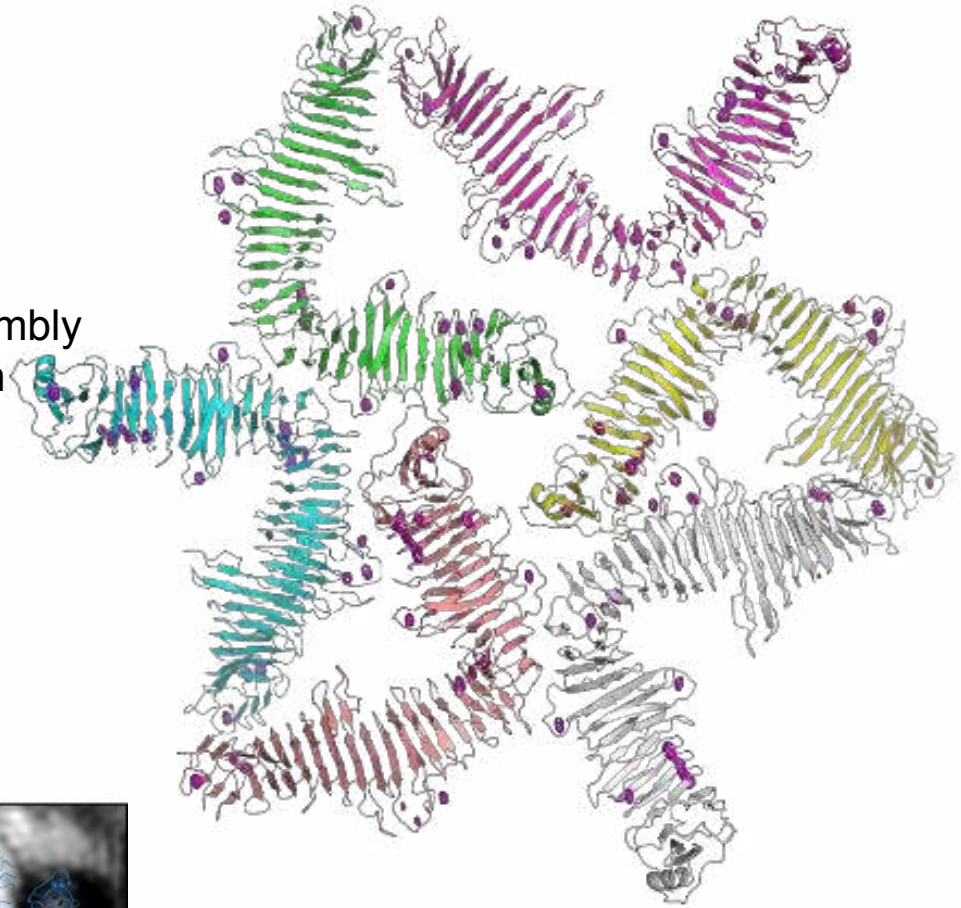
Element identification

Anomalous difference maps

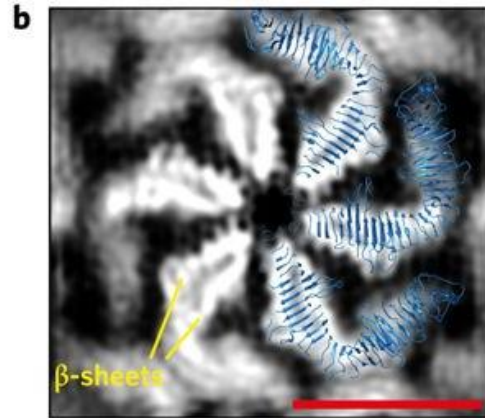
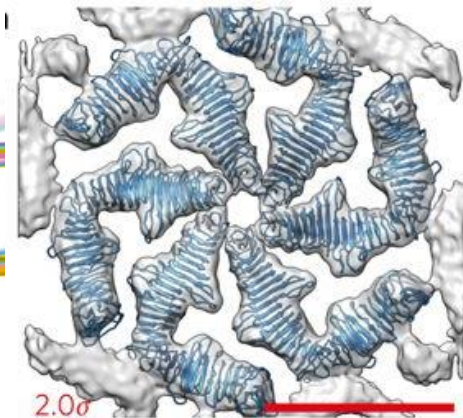


Ca identification

- Journal reviewer commented on Ca identification
- RsaA protein responsible for S-layer assembly
- Ca^{2+} important for S-layer lattice formation
- Data collected at $\lambda = 3.02 \text{ \AA}$
- 19 Ca built in 471 amino acids
- Space group $P2_12_12_1$
- 3.5 \AA resolution



X-ray structure of RsaA



Cryo-ET structure of the S-layer

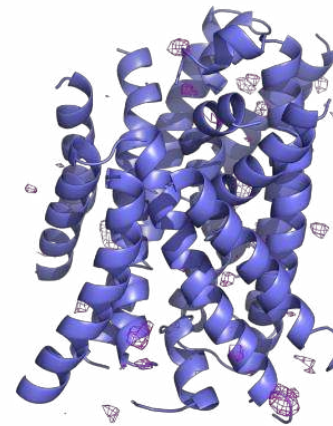
Bharat, T. A. M., et al. (2017). "Structure of the hexagonal surface layer on *Caulobacter crescentus* cells." *Nature Microbiology* **2**: 17059.

Tanmay Bharat
Jan Löwe



S identification

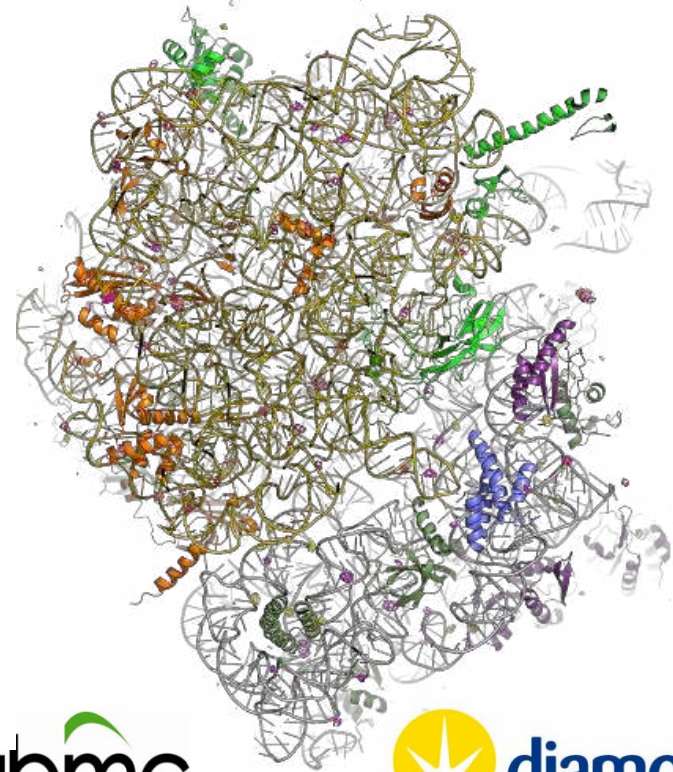
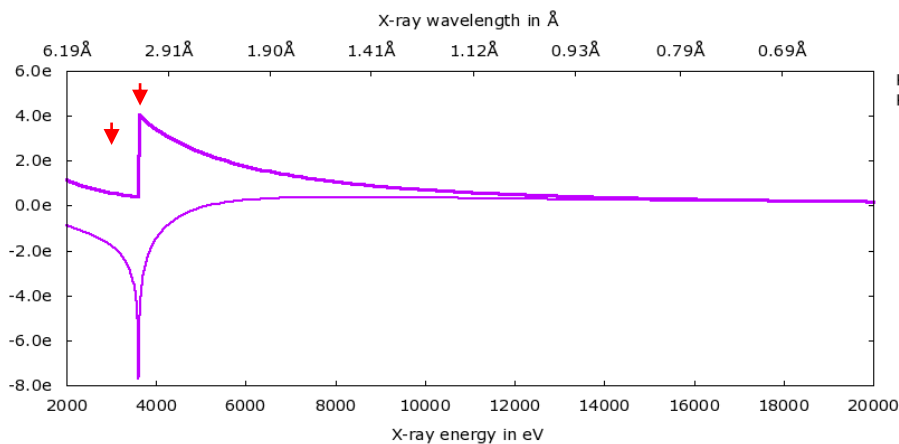
- Help with sequence assignment
- Membrane protein (LCP)
- 13 sulfurs in 471 amino acids
- Space group $P2_12_12_1$
- $\lambda = 3.1 \text{ \AA}$



S. Newstead

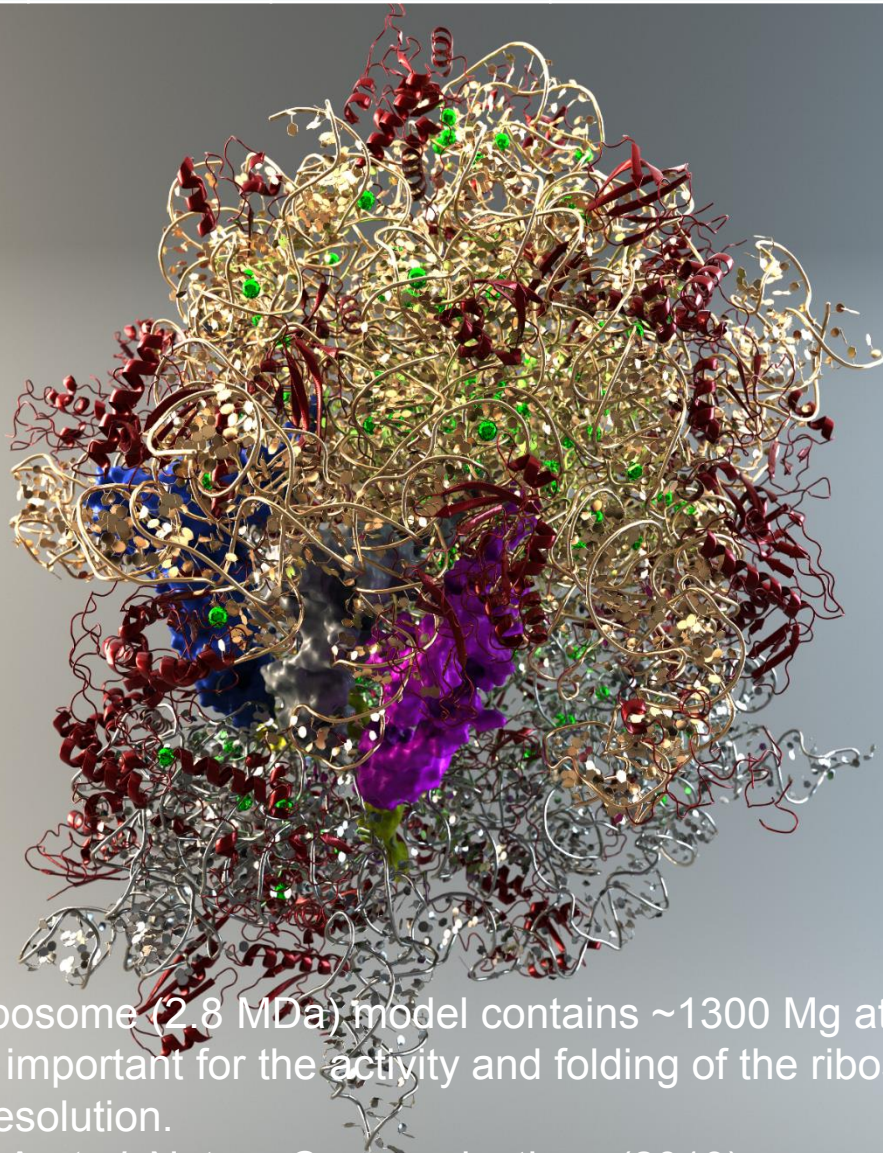
K identification

- Experiments above and below the K-edge ($\lambda=3.44 \text{ \AA}$).



A. Rozov & G. Yusupova

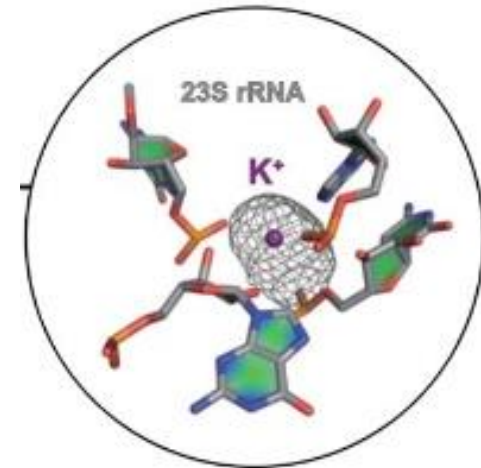
	Mg (pdb)	K ⁺	Mg ²⁺	Mg(H ₂ O) ₆ ²⁺
IC	3255	249	620	90
EC	1439	131	394	74



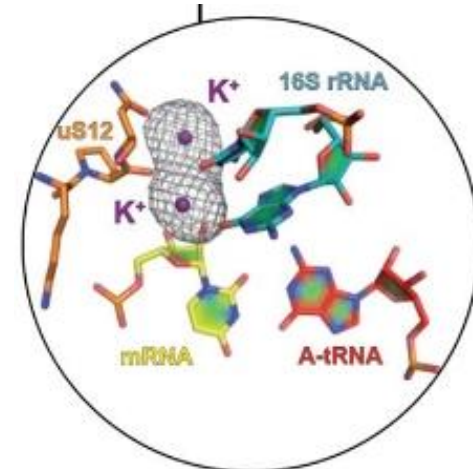
70S ribosome (2.8 MDa) model contains ~1300 Mg atoms.
K ions important for the activity and folding of the ribosome.
~4 Å resolution.

Rozov A *et al.* Nature Communications (2019).

Peptidyl transferase center



Decoding center

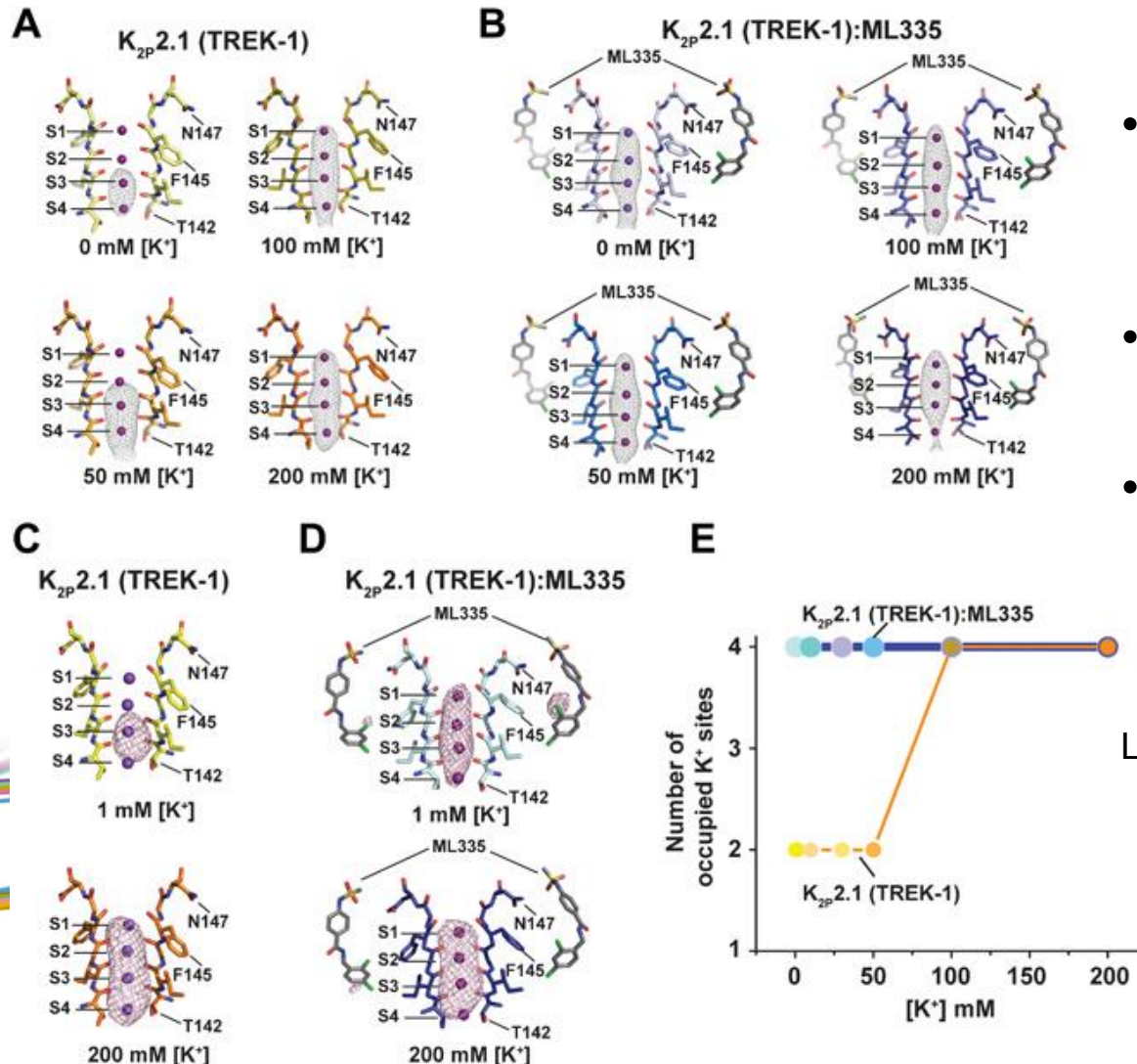


Gating mechanism in the selectivity filter

19

K

Potassium
39.098



- Anomalous difference Fourier maps
- Different K⁺ concentrations
- In presence and absence of ML335

Lolicato *et al.*, *Sci. Adv.* 2020; **6** : eabc9174

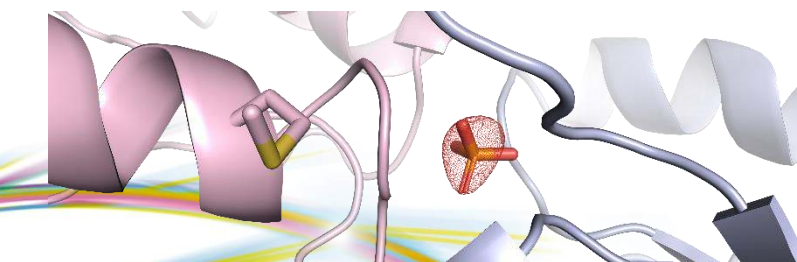
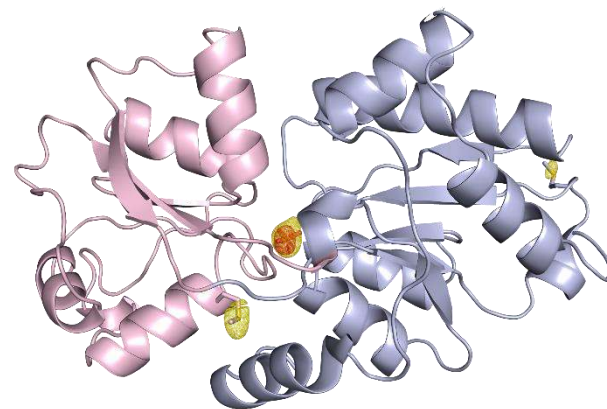
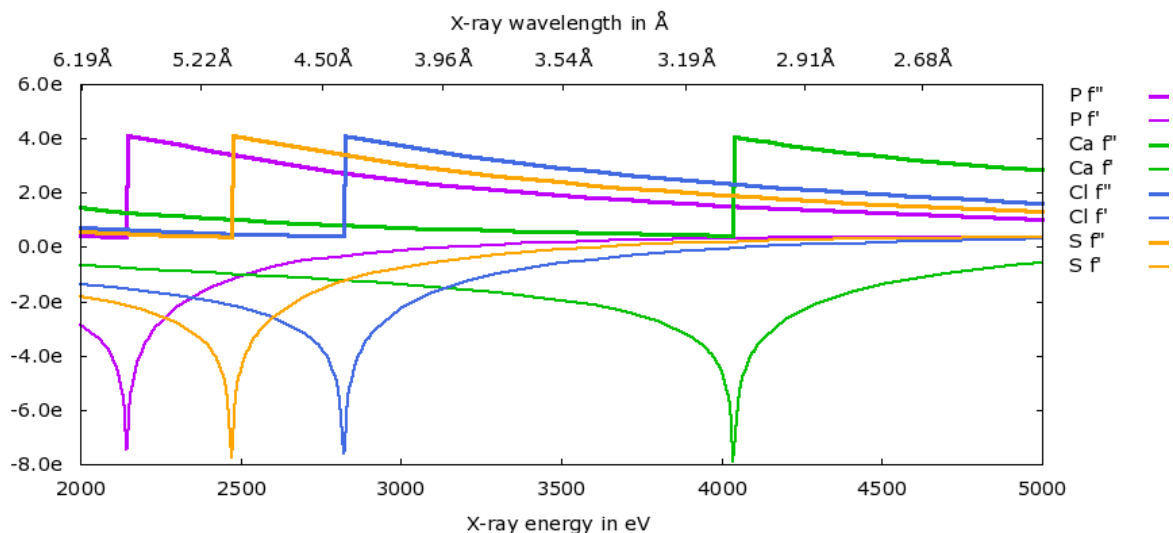
UCSF

University of California
San Francisco

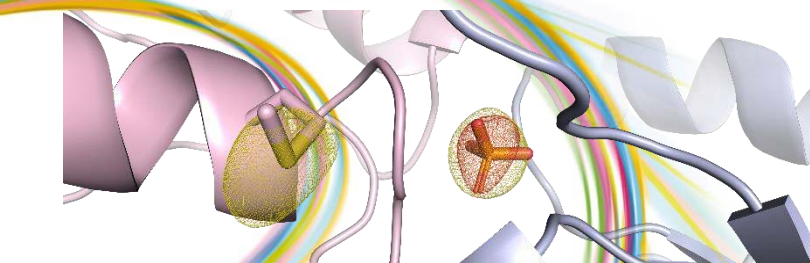
diamond

Discriminating between sulphate and phosphate

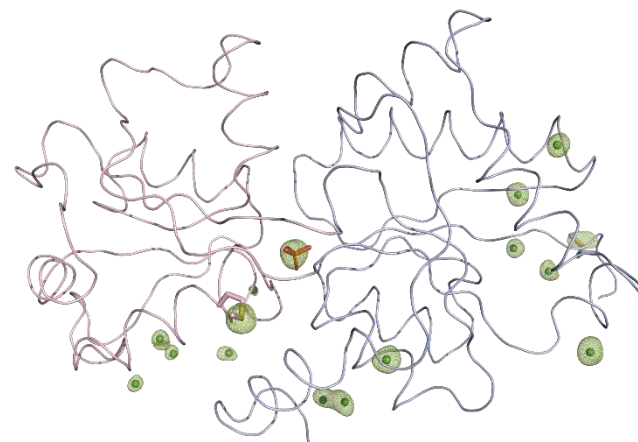
Edgeplots web tool <http://skuld.bmrc.washington.edu/scatter/>



E = 2.4 keV
Below sulphur edge



E = 2.4 keV
E = 2.55 keV
Above sulphur edge



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!
- Minimum of 1S / 116 residues (so far).
- 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>

