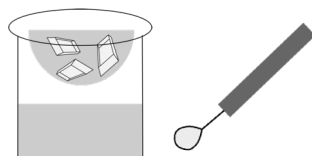
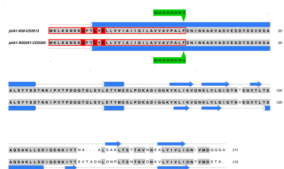


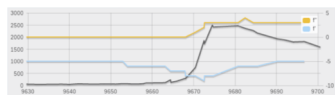
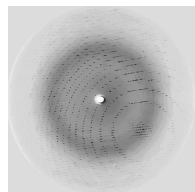
Experimental phasing: A practical perspective

Paula S. Salgado

DLS CCP4 Workshop2019



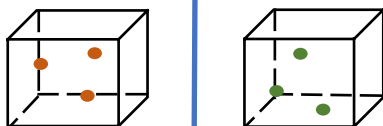
Derivatisation



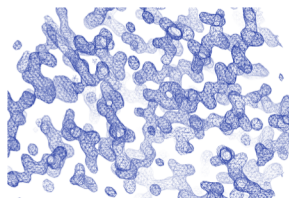
Data collection

Anomalous signal?

**Substructure
determination**



Phase calculation



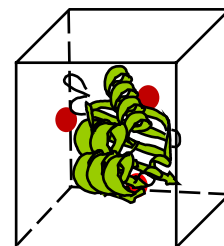
Density modification

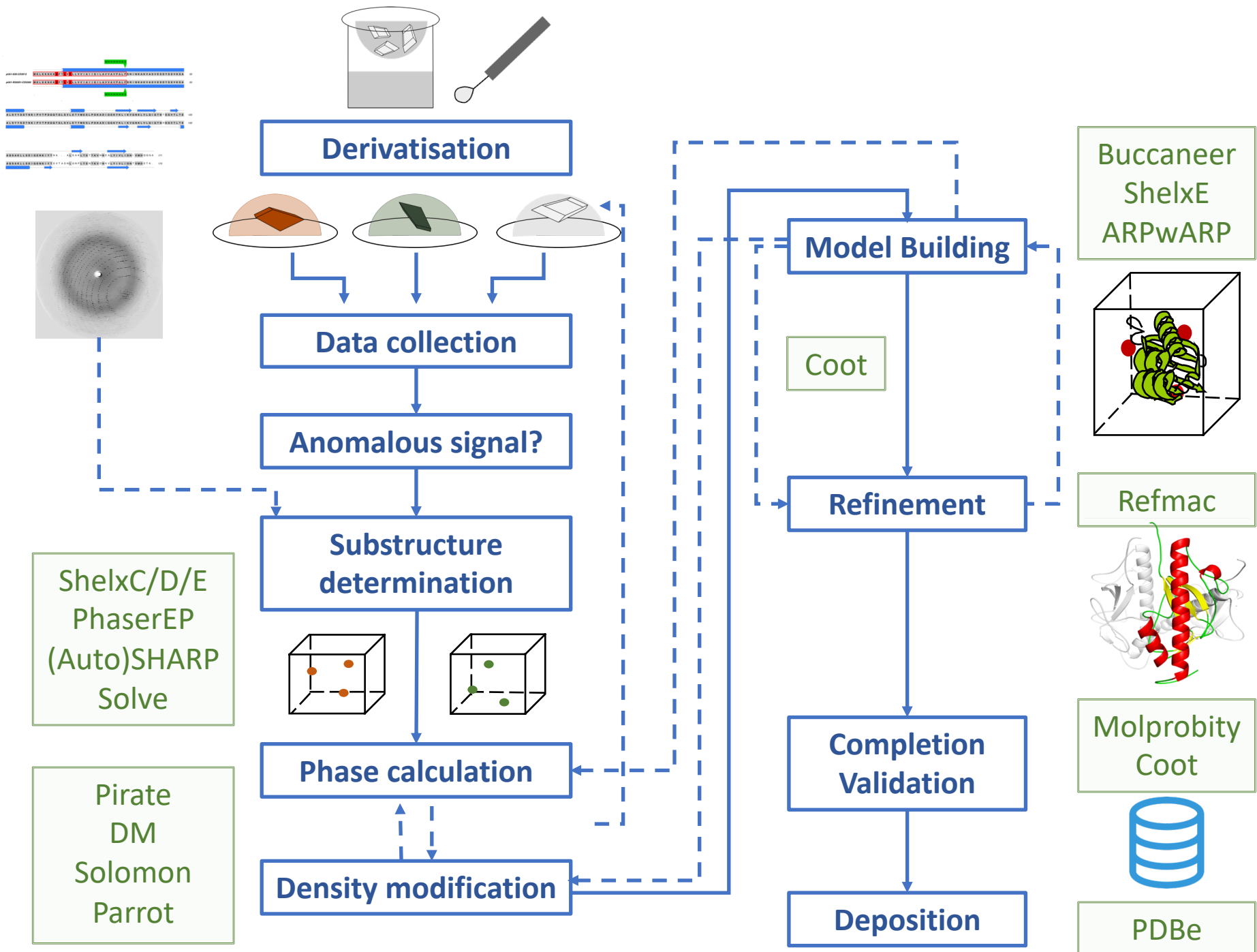
Model Building

Refinement

**Completion
Validation**

Deposition





Derivatisation



SIR/MIR (Single/Multiple Isomorphous Replacement)

SAD/MAD (Single/Multiple Anomalous Dispersion)

Elements accessible for fluorescence mapping at the :

K-edge

L-edge

M-edge

Bold characters: Elements accessible for XANES

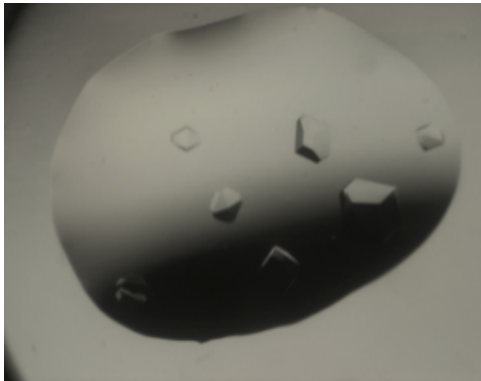
H																		He
Li	Be																	
Na	Mg																	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
Cs	Ba		Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	
Fr	Ra		Rf	Db	Sg	Bh	Hs	Mt	Uun	Uuu	Uub		Uuq		Uuh		Uuo	

Lanthanides

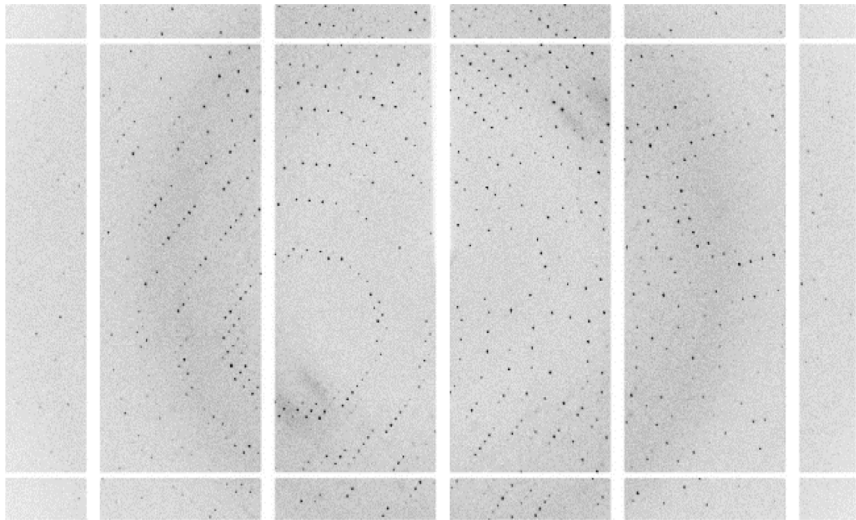
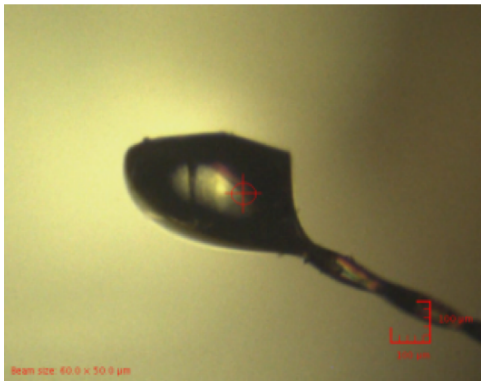
Actinides

La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw

(M)SIRAS Case study 1: CdPilA1 (R20291)

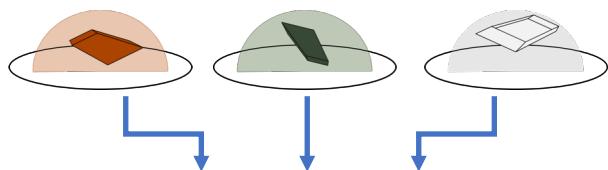


A. Crawshaw, C. Davies



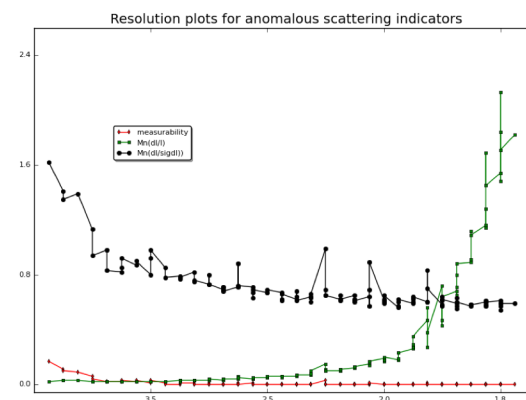
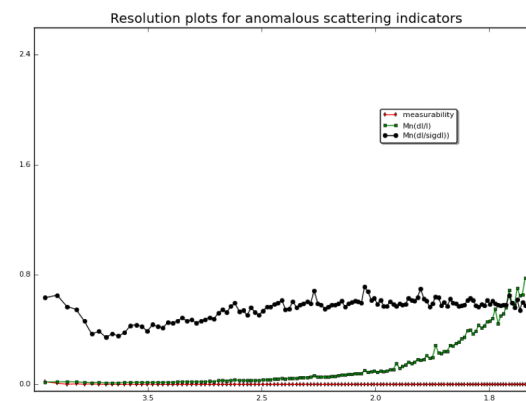
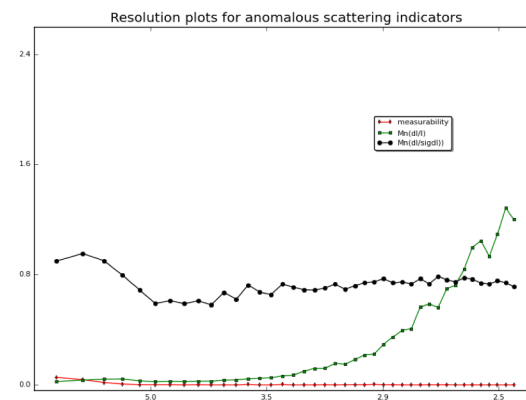
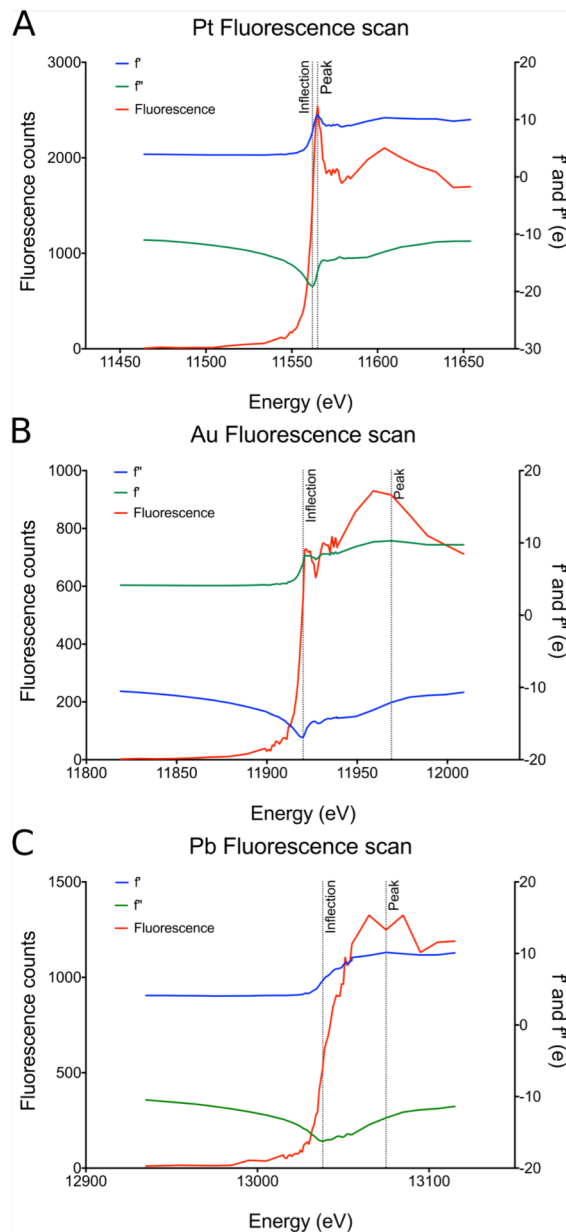
	R20291 Native
Resolution (Å)	51.21 - 1.65
Wavelength (Å)	
Unit cell	
a, b, c (Å)	102.42, 102.42, 104.21
α=β=γ (°)	90
Spacegroup	P4 ₁ 2 ₁ 2
R _{merge}	0.136 (1.887)
I/sig I	10.7 (1.5)
Mean intensity CC1/2	0.998 (0.323)

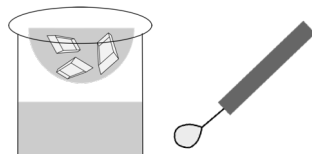
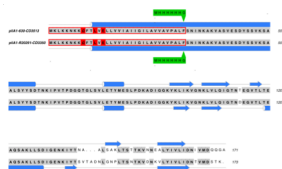
Derivatisation



Data collection

Anomalous signal?





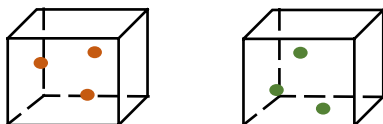
Derivatisation



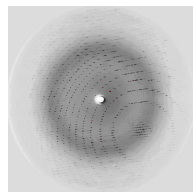
Data collection

Anomalous signal?

**Substructure
determination**

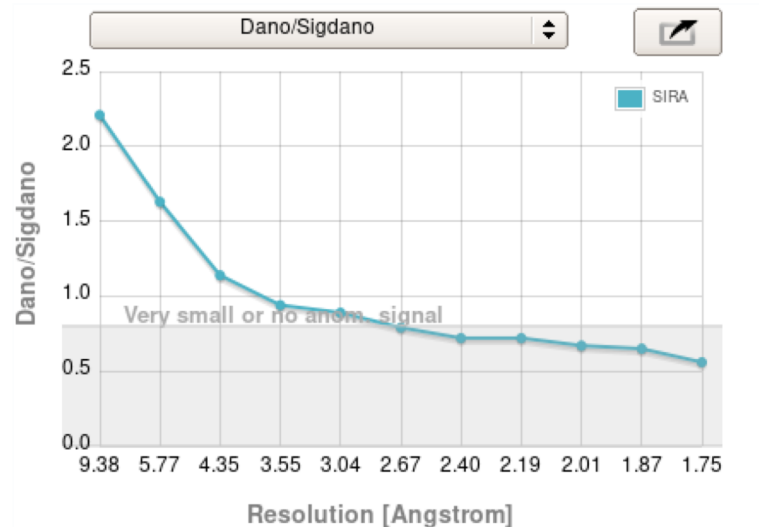


ShelxC/D
PhaserEP
(Auto)SHARP
Solve



ShelxC/D

Substructure determination



Programs used:

SHELXD

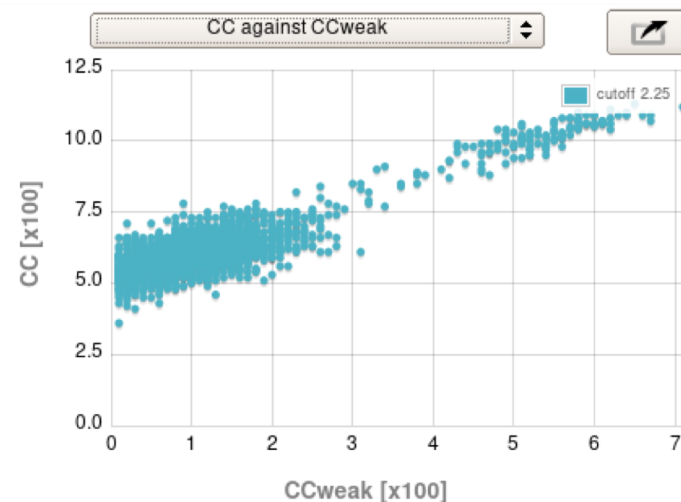
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 18.3 occurring at Trial 172.

The substructure has 9 atoms with occupancy of at least 25%, 13 in total.



Programs used:

SHELXD

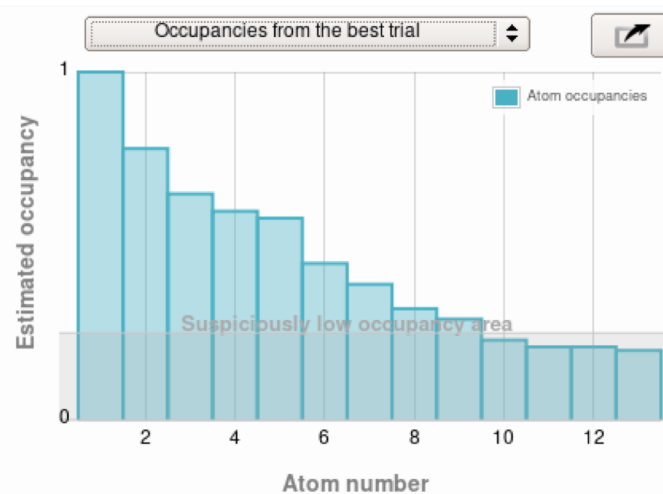
Stop:

Maximum number of trials reached.

Result:

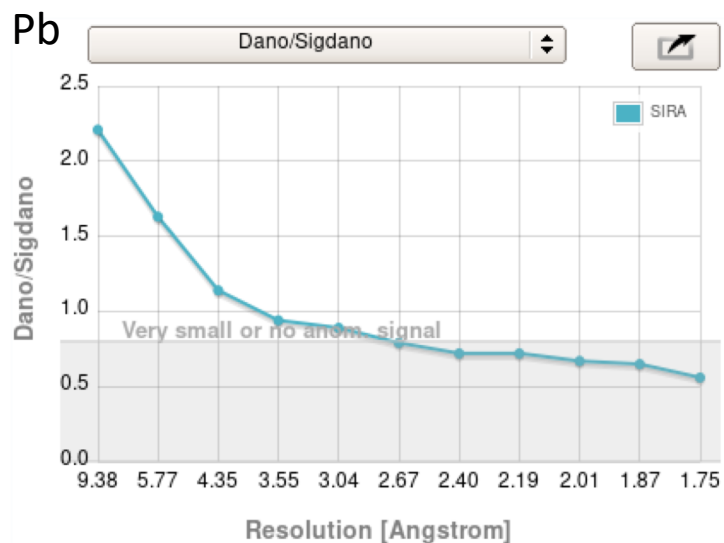
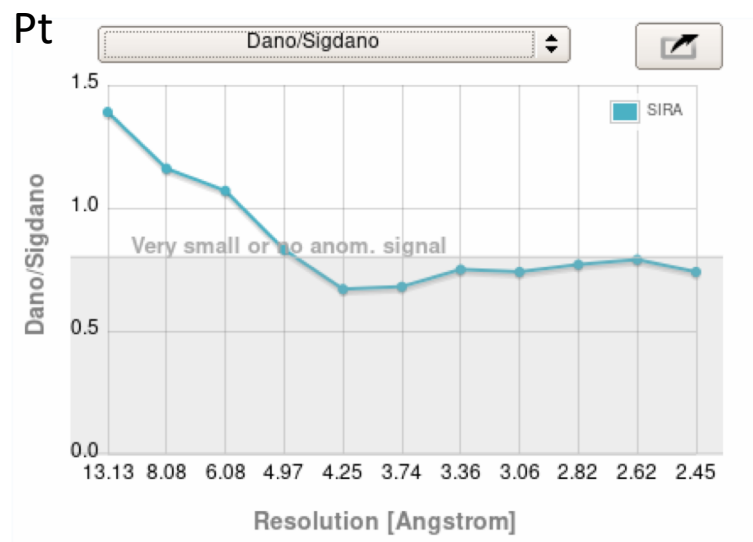
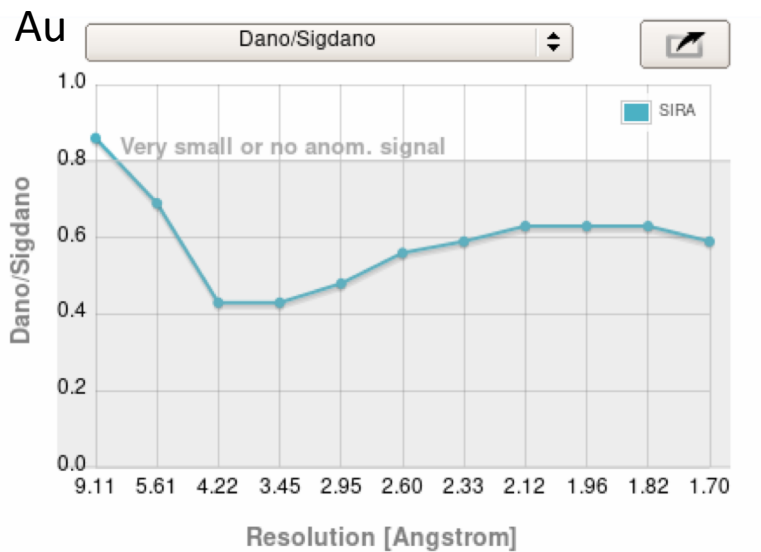
Maximum CFOM: 18.3 occurring at Trial 172.

The substructure has 9 atoms with occupancy of at least 25%, 13 in total.



ShelxC/D

Substructure determination



ShelxC/D

Substructure determination

Pt

Programs used:

SHELXD

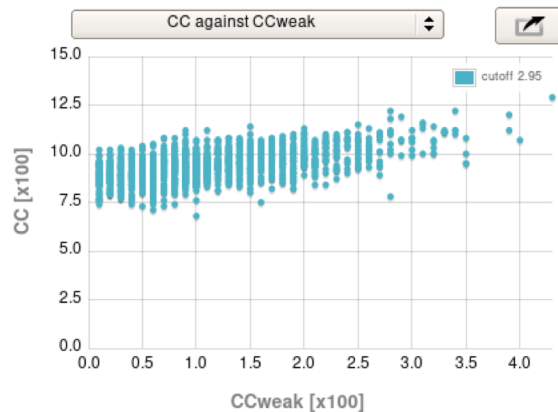
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 17.2 occurring at Trial 1054.

The substructure has 13 atoms with occupancy of at least 25%, 13 in total.



Au

Programs used:

SHELXD

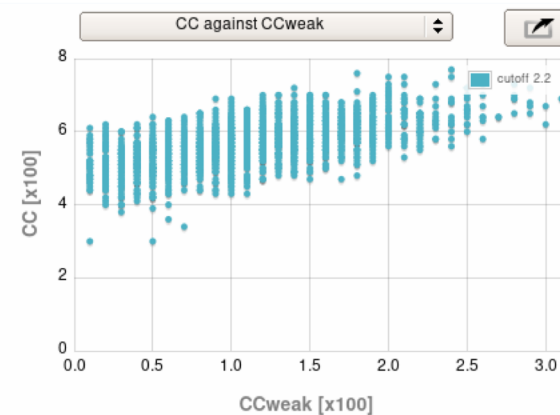
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 10.1 occurring at Trial 1617.

The substructure has 12 atoms with occupancy of at least 25%, 13 in total.



Pb

Programs used:

SHELXD

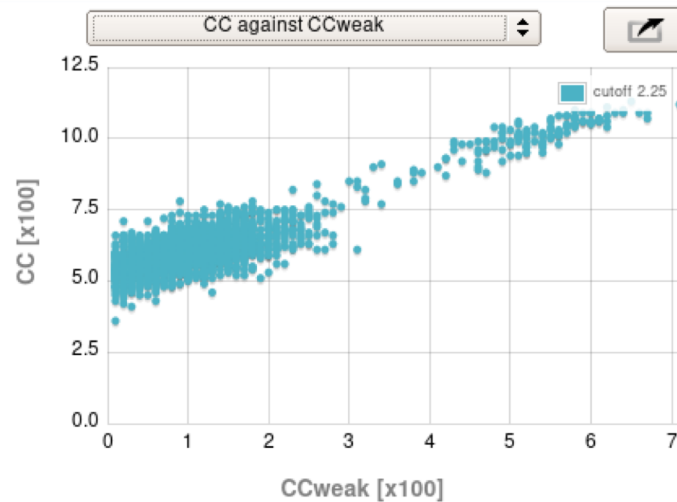
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 18.3 occurring at Trial 172.

The substructure has 9 atoms with occupancy of at least 25%, 13 in total.



ShelxC/D

Substructure determination

Pt

Programs used:

SHELXD

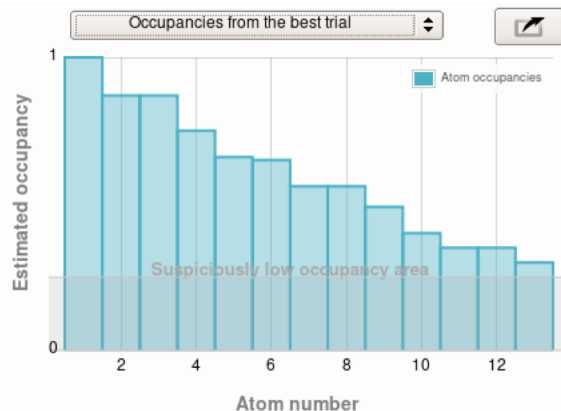
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 17.2 occurring at Trial 1054.

The substructure has 13 atoms with occupancy of at least 25%, 13 in total.



Au

Programs used:

SHELXD

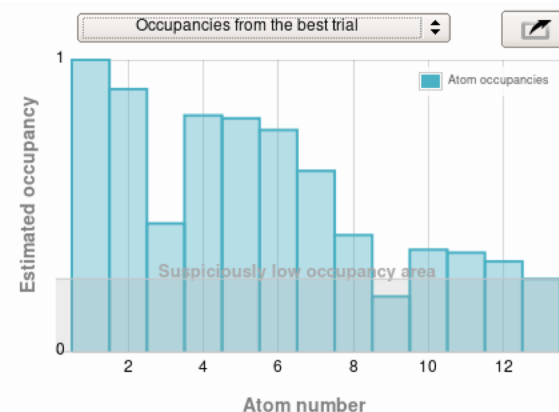
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 10.1 occurring at Trial 1617.

The substructure has 12 atoms with occupancy of at least 25%, 13 in total.



Pb

Programs used:

SHELXD

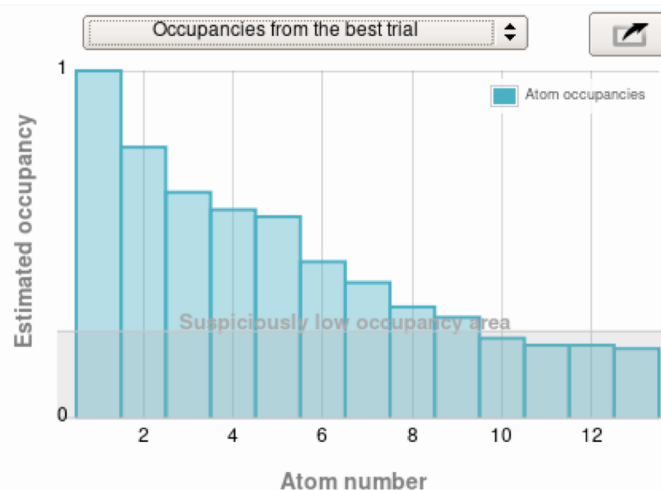
Stop:

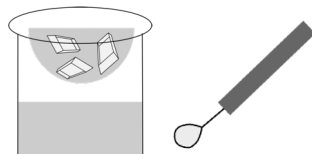
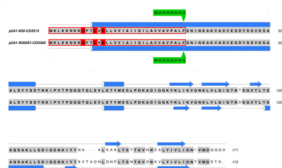
Maximum number of trials reached.

Result:

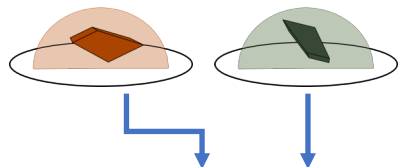
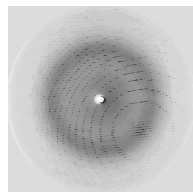
Maximum CFOM: 18.3 occurring at Trial 172.

The substructure has 9 atoms with occupancy of at least 25%, 13 in total.





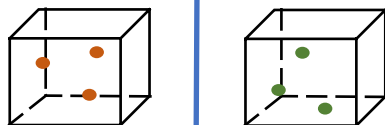
Derivatisation



Data collection

Anomalous signal?

**Substructure
determination**



Phase calculation

Density modification

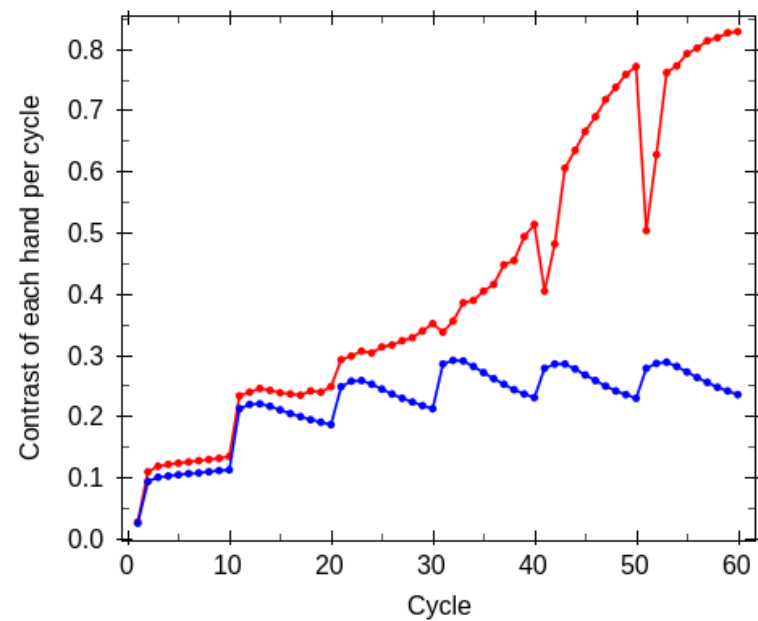
ShelxC/D/E
PhaserEP
(Auto)SHARP
Solve

Pirate
DM
Solomon
Parrot

ShelxE

Phase calculation

Density modification



Programs used:

SHELXE

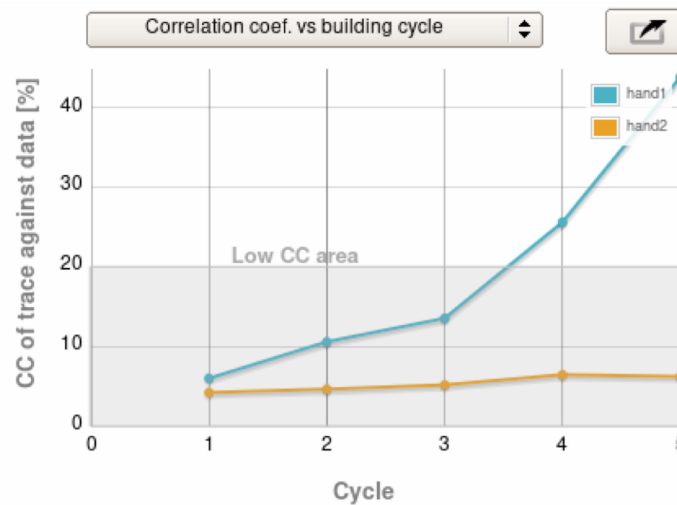
Stop:

CC parameter threshold (40.0) reached.
Stopping after the current cycle.

Result:

Maximum correlation coefficient for hand 1 is 43.81; max. correlation coef. for the other hand is 6.45. Hand 1 is chosen.

Max. number of residues built (for the chosen hand) is 380



ShelxE

Phase calculation

Density modification

Programs used:

SHELXE

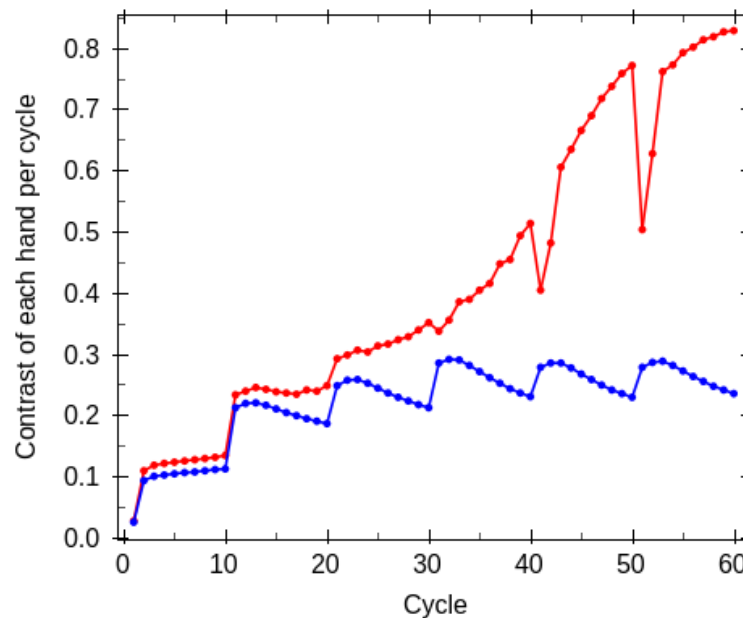
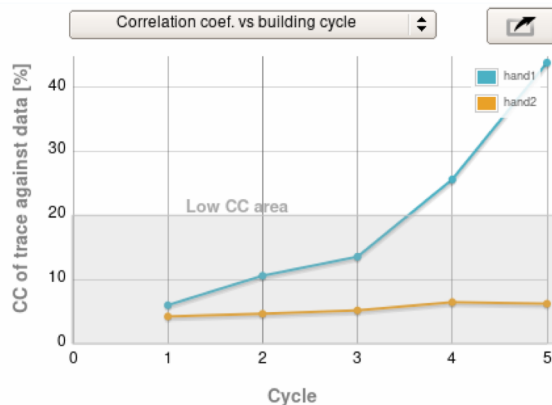
Stop:

CC parameter threshold (40.0) reached.
Stopping after the current cycle.

Result:

Maximum correlation coefficient for hand 1 is 43.81; max. correlation coef. for the other hand is 6.45. Hand 1 is chosen.

Max. number of residues built (for the chosen hand) is 380



Pt

Programs used:

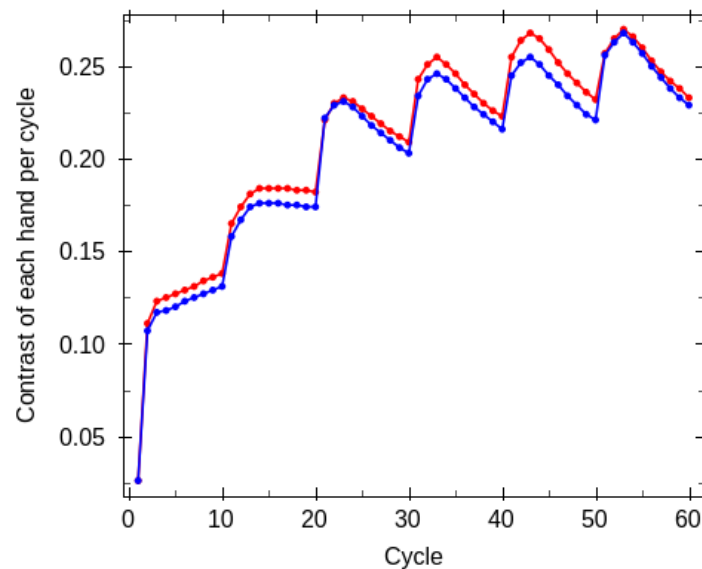
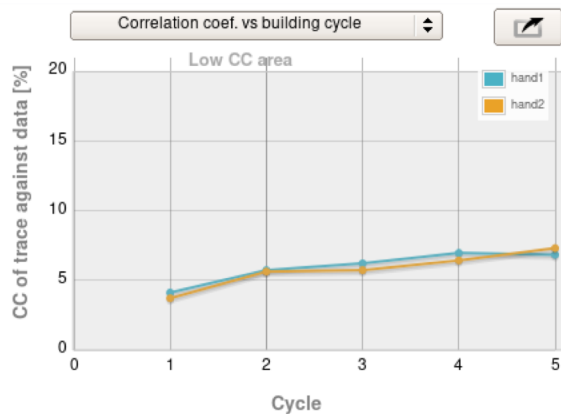
SHELXE

Result:

Maximum correlation coefficient for hand 1 is 6.98; max. correlation coef. for the other hand is 7.32. Hand 2 is chosen.

Max. number of residues built (for the chosen hand) is 237

Correlation coefficient too low, the traced model is most likely wrong.



ShelxC/D

Phase
calculation

Parrot

Density
modification

Buccaneer

Model building

Refmac

Refinement

Programs used:

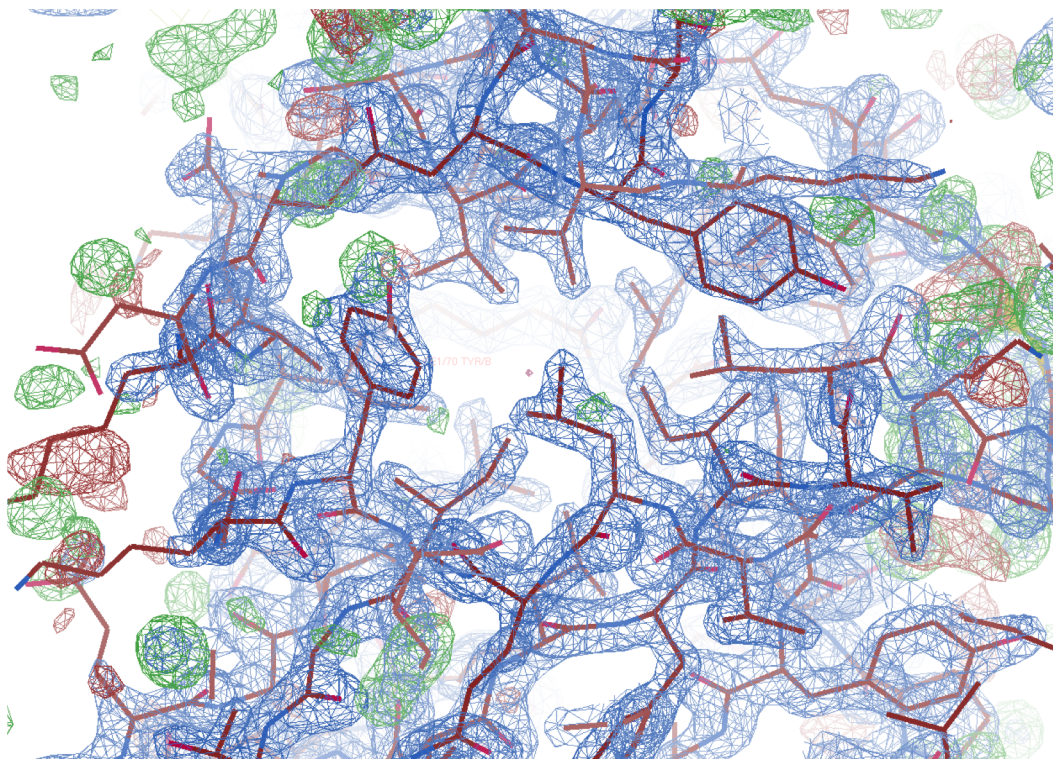
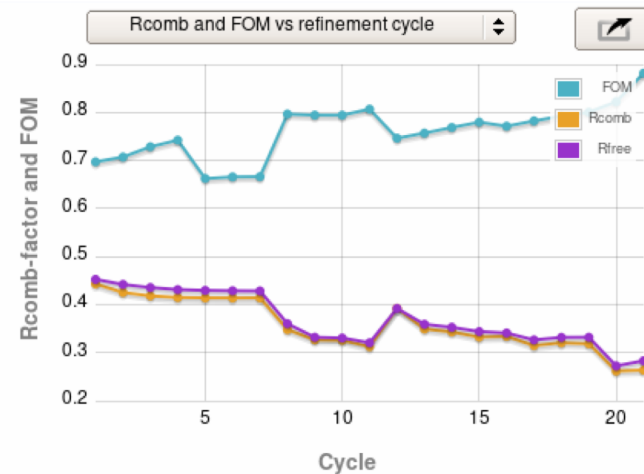
Parrot, REFMAC5, Buccaneer, SHELXE

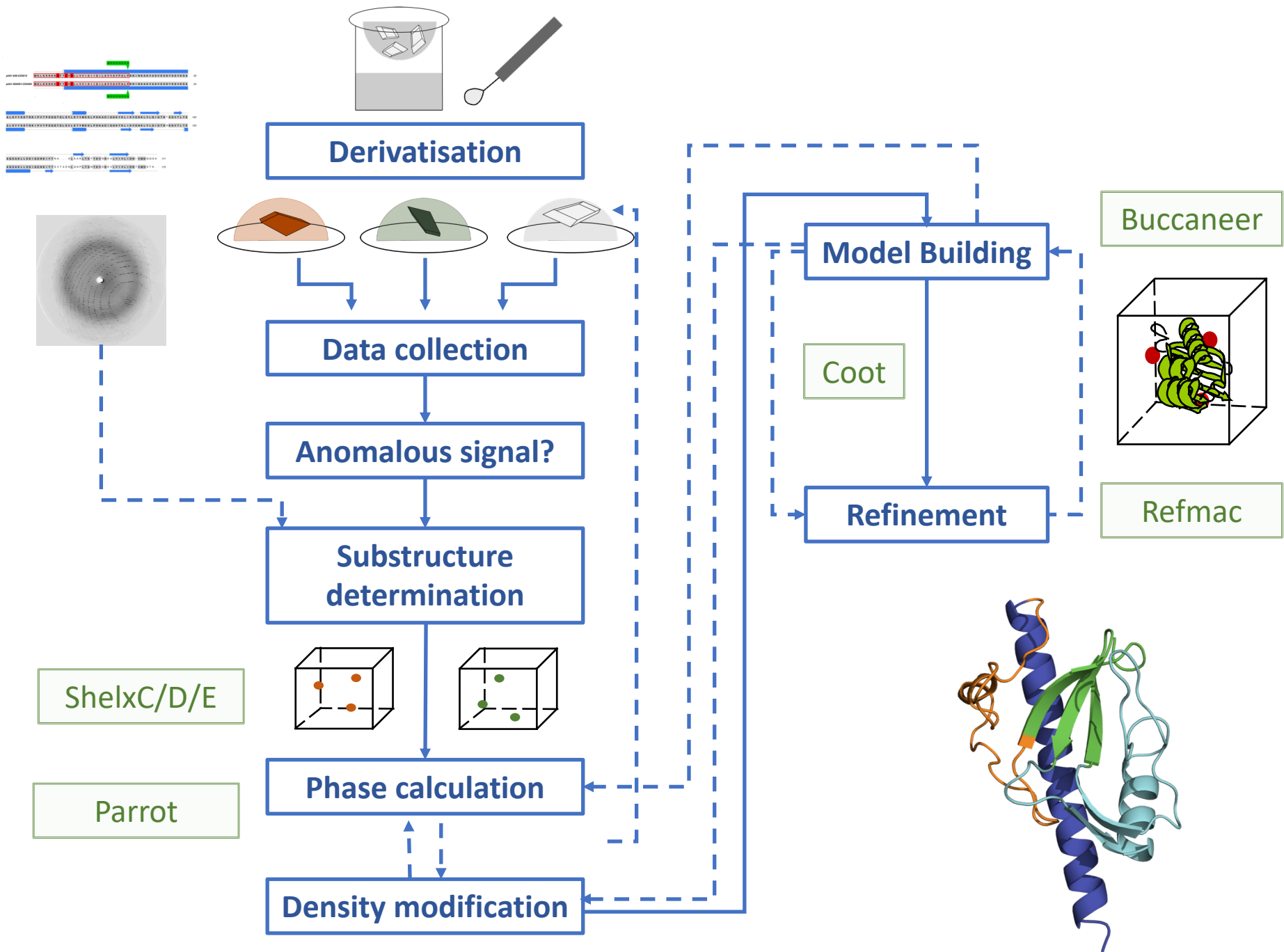
NCS detection:

4 NCS operators detected.

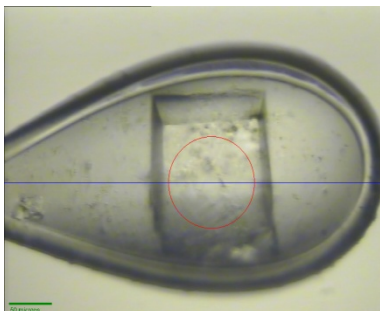
Result:

Majority of model was successfully built!

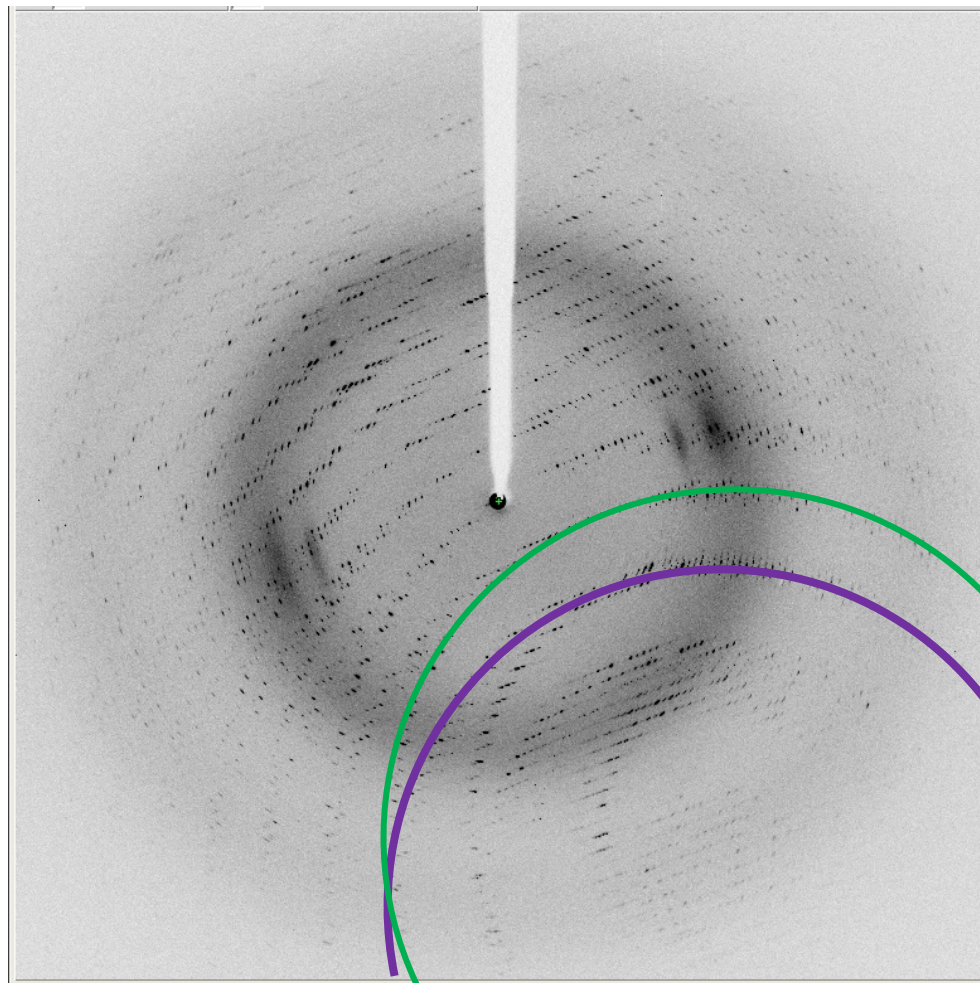




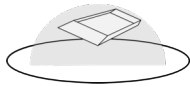
SIRAS case study 2:



Crystal Size	$\approx 100 \times 100 \times 50 \mu\text{m}$
Source	ESRF, Grenoble, BM14
Wavelength	0.9795 Å
Detector	ADSC Q315
No. Images	360
Unit Cell Dimensions	$a = 34.73, b = 68.71, c = 120.03 \text{ Å}$ $\alpha = \beta = \gamma = 90^\circ$
Space Group	$P2_12_12_1$
Resolution (Å)	50.0 – 2.0 (2.03 - 2.0)
Reflections total / unique	891062 / 20268
Completeness	99.7 %
Redundancy	8.5 (7.4)
R_{Sym}	0.12 (0.45)
$I/\sigma I$	19.8 (3.4)
Mosaicity	0.8
No. Molecules/A.u.	1
Solvent Content	48 %



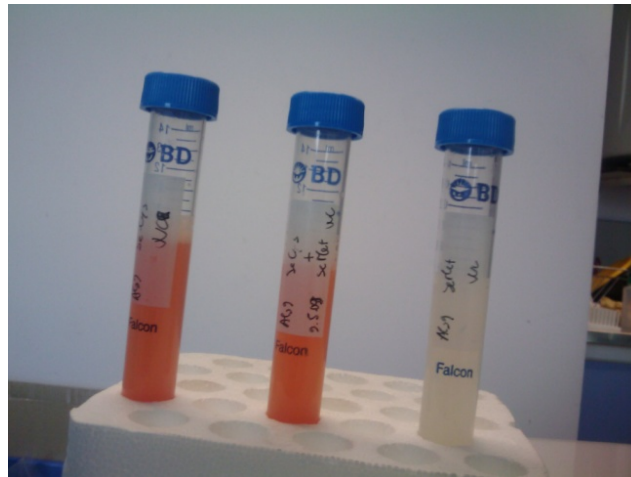
Derivatisation



KTITGVFNSFDSL TWTRSVEYVYKGPETPTWNAVLGWSLNSTTADPGDTFTL
ILP **C**VFKFITTTQTSVDLTADGVSYAT **C**DFNAGEEFTTFSSLS **C**TVNSVSVSY
ARVSGTVKLPITFNVGGTGSSVDLADSK **C**FTAGKNTVTF **M**DGDTKISTTVDF
DASPVSPSGYITSSRIIPSLNKLSSLFVVPQ **C**ENGYTSGI **M**GFVASNGATID
CSNVNIGISKGLNDWNFPVSSESFSYTKT **C**TSTSITVEFQNPAGYRPFVDA
YISAENIDKYTLTYANEYT **C**ENGNTVVDPFLLTWWGYKNSEADSDGDVIVV

- Only 2 **Met** but 8 **Cys**:

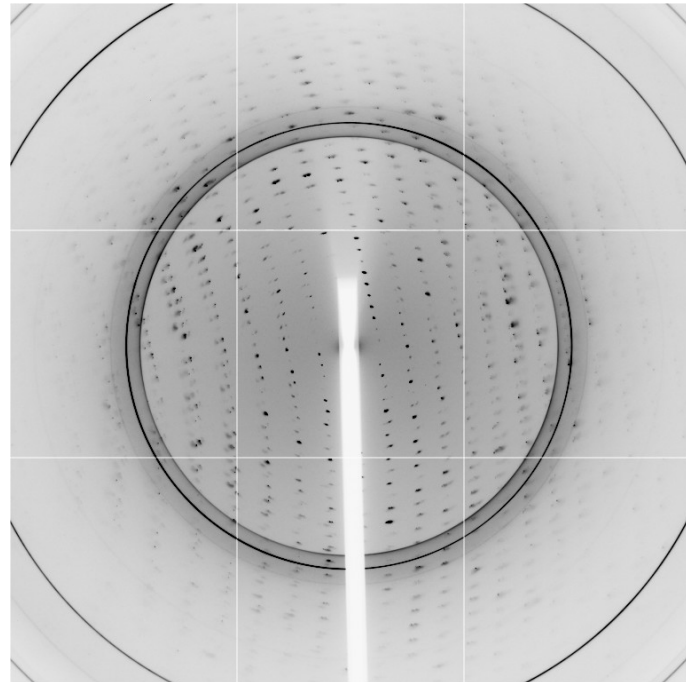
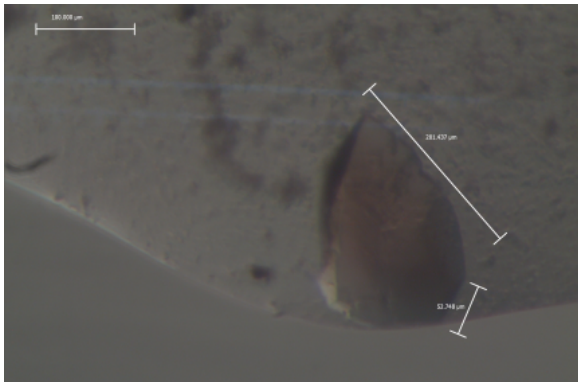
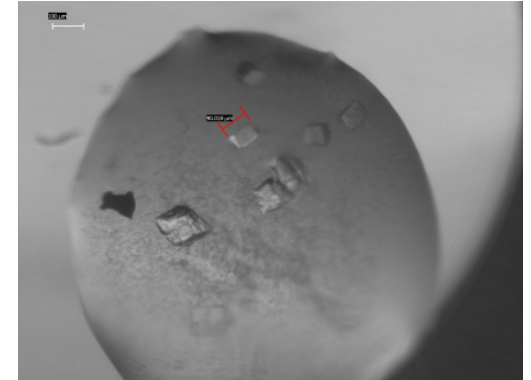
SeMet +SeCys – no expression (cells die)

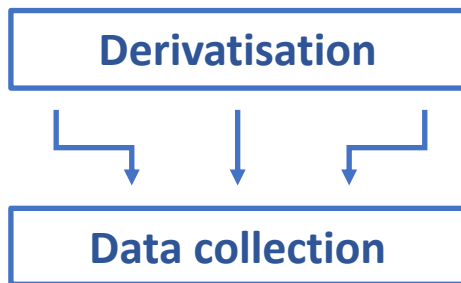


Derivatisation

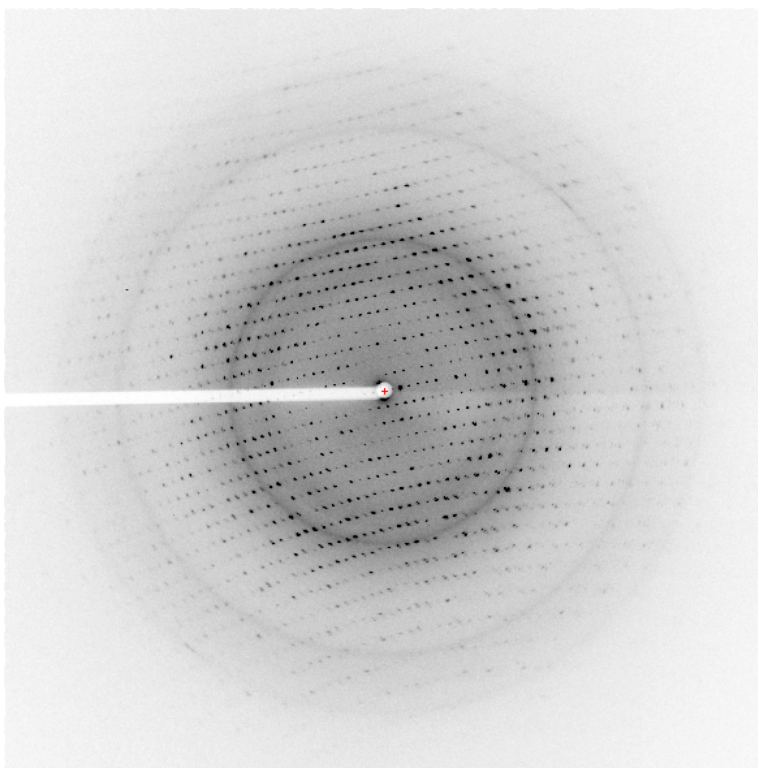


- Original crystals at pH 4.5 – no interaction with most HA
- Reoptimize, new crystals around pH 8
- OsCl₄ co-crystals
- Multiplicity problem
- Weak anomalous signal due to:
 - low occupancy
 - wavelength off peak

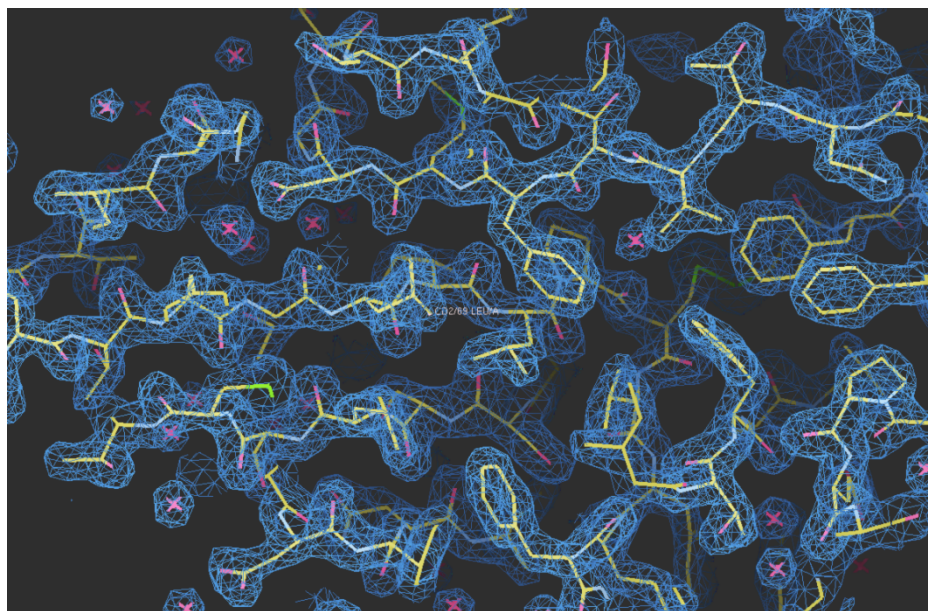
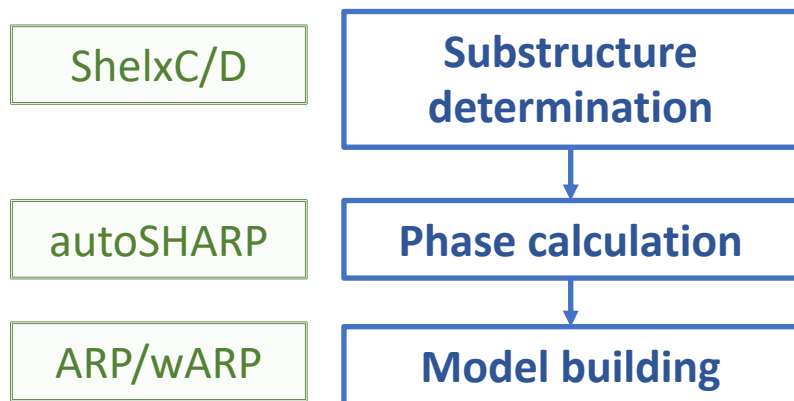




- Resoaked crystals at pH8 with excess PtCl_4
- Collected data at SLS, at precise peak wavelength
- Optimal data collection



Wavelength	1.068 Å
Unit cell	$a = 35.07$, $b = 68.99$, $c = 120.77$ Å $\alpha = \beta = \gamma = 90^\circ$
Space Group	P212121
Resolution (Å)	68.9 – 1.83 (1.93-1.83)
Reflections total/unique	366357/26698
Completeness	99.7 (98.2)
Redundancy	13.7 (12.5)
Rsym	0.08 (0.45)
$I/\sigma I$	24.9 (5.6)
Mosaicity	0.47
No. Molec./A.U.	1
Solvent Content	40.00%



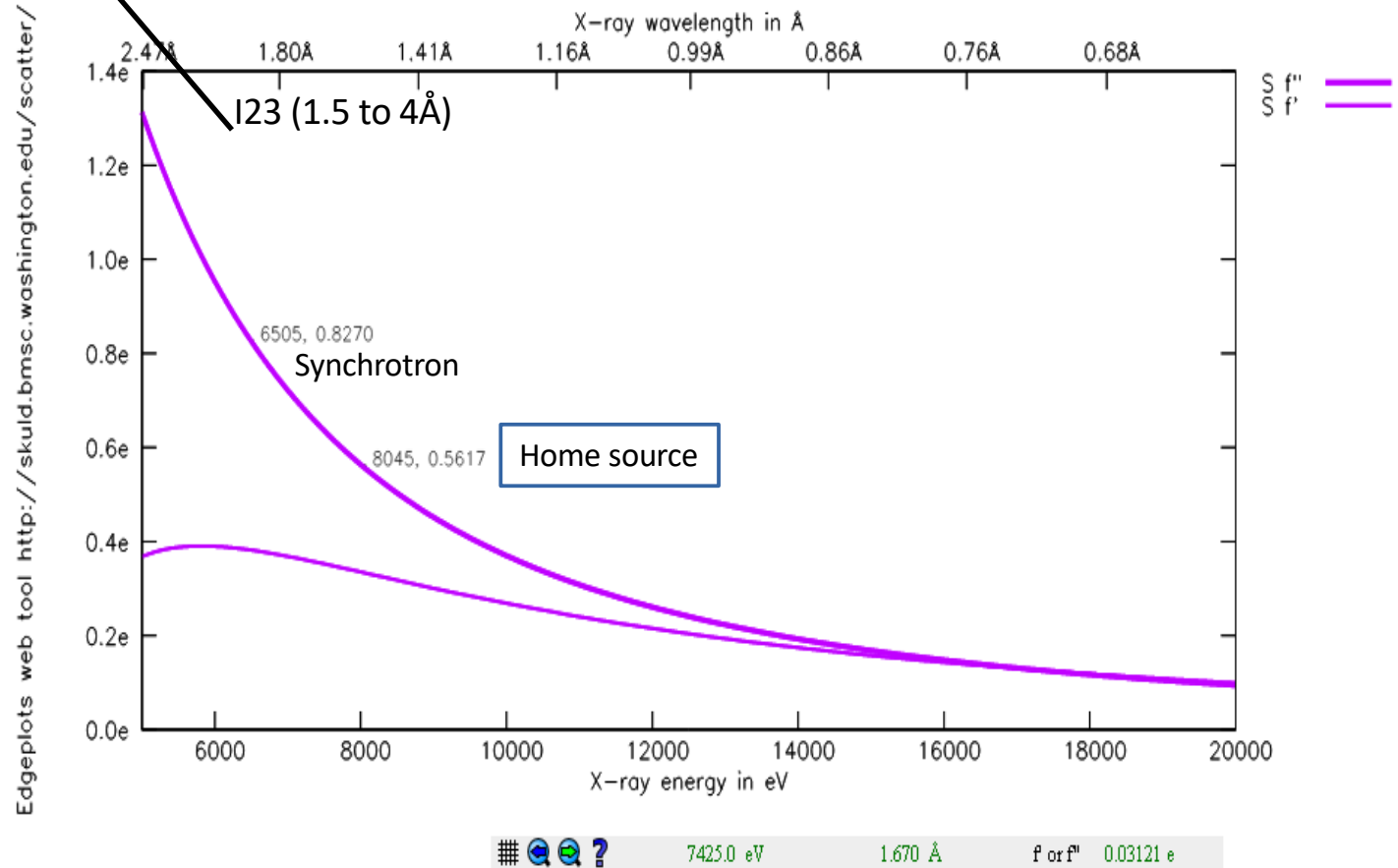
Salgado *et al.*, 2011

Substructure sol. - SHELX	
No. sites	2
CC	0.48
FOM	98.67
Phasing - SHARP	
FOM	0.38
Phasing Power Iso/Ano	1.04/1.7
Model Building - ARP/wARP	
No. residues built	282 (of 310)
Docked in sequence	279 (98%)
Score	2.26
Rwork	20.9
Rfree	27.3

S-SAD case study:

MGPVAGNYVHDFGTANGTSS...
(121 aa, 3 Met)

Anomalous
Scatterer – S in Met



S-SAD (Cu edge):

Data collection & processing

	90°	180°	360°	720°
Resolution	43.05 – 1.93 (1.98 – 1.93)			
Rmerge	0.054 (0.221)	0.068 (0.243)	0.062 (0.246)	0.062 (0.248)
Rmeas	0.057 (0.244)	0.070 (0.250)	0.062 (0.249)	0.062 (0.249)
Rpim	0.018 (0.076)	0.016 (0.055)	0.010 (0.039)	0.007 (0.027)
Total obs.	141180	282998	566986	1134844
Unique obs.	13948	13948	13948	13949
Mean I/ σ I	25.9 (8.1)	31.7 (11.8)	49.6 (40.4)	70.2 (23.3)
Mn (I) CC(1/2)	0.999 (0.979)	0.999 (0.989)	1.000 (0.995)	1.000 (0.997)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Multiplicity	10.1 (10.0)	20.3 (20.2)	40.6 (40.4)	81.4 (80.8)
Anomalous completeness	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Anomalous multiplicity	5.5 (5.3)	11.5 (10.7)	22.5 (21.7)	45.1 (43.4)

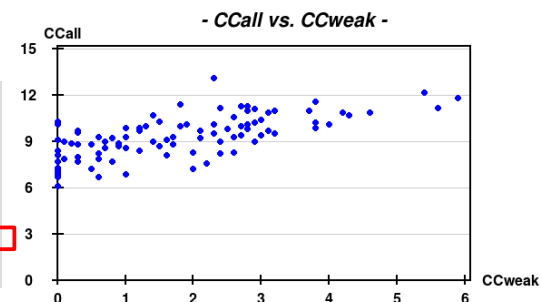
Anomalous signal?

Substructure
determination

ShelxC/D

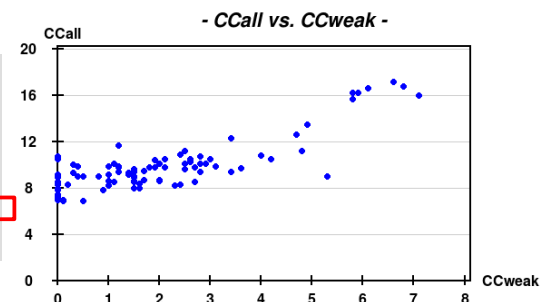
90°

Resl.	Inf.	4.29	3.41	2.98	2.70	2.51	2.36	2.24	2.15	2.06	1.99	1.93
N(data)		1465	1302	1265	1301	1233	1258	1258	1155	1341	1212	1158
Chi-sq		2.99	1.24	1.00	1.02	1.07	1.32	1.22	1.17	1.19	1.16	1.01
<I/sig>		43.9	50.3	41.7	31.0	24.7	20.8	18.5	16.2	13.3	10.2	8.4
%Complete		101.7	99.5	99.6	99.8	99.8	99.8	99.8	99.7	99.9	99.9	99.6
<d"/sig>		0.81	0.76	0.75	0.76	0.74	0.73	0.75	0.78	0.76	0.76	0.72
CC(1/2)		46.8	1.4	11.9	11.8	8.1	0.0	0.4	0.7	1.4	4.2	-7.3



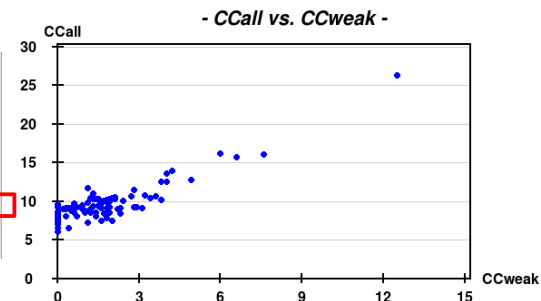
180°

Resl.	Inf.	4.29	3.41	2.98	2.70	2.51	2.36	2.24	2.15	2.06	1.99	1.93
N(data)		1465	1302	1265	1301	1233	1258	1258	1155	1341	1212	1158
Chi-sq		1.13	1.08	0.94	1.25	1.16	1.36	0.85	1.23	1.23	1.21	1.10
<I/sig>		48.4	56.1	49.0	38.5	31.8	27.6	24.7	22.1	18.4	14.5	12.1
%Complete		101.7	99.5	99.6	99.8	99.8	99.8	99.8	99.7	99.9	99.9	99.6
<d"/sig>		0.88	0.73	0.70	0.75	0.71	0.72	0.74	0.74	0.76	0.76	0.77
CC(1/2)		56.2	16.1	3.9	10.1	5.7	-1.2	0.4	-3.1	-1.6	-3.7	0.5



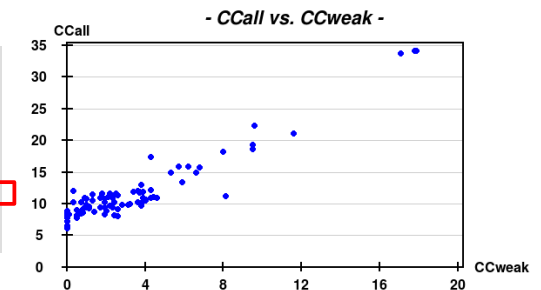
360°

Resl.	Inf.	4.29	3.41	2.98	2.70	2.51	2.36	2.24	2.15	2.06	1.99	1.93
N(data)		1465	1302	1265	1301	1233	1258	1258	1155	1341	1212	1158
Chi-sq		1.12	1.14	0.99	1.07	1.09	1.30	1.61	1.09	1.14	1.12	1.03
<I/sig>		80.8	93.3	79.0	59.9	48.2	41.0	36.4	32.2	26.4	20.5	17.0
%Complete		101.7	99.5	99.6	99.8	99.8	99.8	99.8	99.7	99.9	99.9	99.6
<d"/sig>		1.27	1.01	0.93	0.89	0.86	0.77	0.78	0.78	0.74	0.73	0.71
CC(1/2)		75.9	40.9	25.8	19.2	14.0	-0.2	3.8	0.4	-2.8	-4.2	-3.7



720°

Resl.	Inf.	4.29	3.41	2.98	2.70	2.51	2.36	2.24	2.15	2.06	1.99	1.93
N(data)		1465	1302	1265	1301	1233	1258	1258	1155	1341	1212	1159
Chi-sq		1.20	1.16	1.02	1.04	1.14	1.26	1.29	1.10	1.13	1.11	1.01
<I/sig>		114.4	132.0	111.9	84.9	68.4	58.1	51.5	45.6	37.3	29.0	23.9
%Complete		101.7	99.5	99.6	99.8	99.8	99.8	99.8	99.7	99.9	99.9	99.7
<d"/sig>		1.71	1.33	1.16	1.09	1.03	0.94	0.94	0.91	0.82	0.80	0.78
CC(1/2)		86.1	58.3	42.9	35.0	28.5	15.1	21.2	9.7	12.1	5.2	9.0



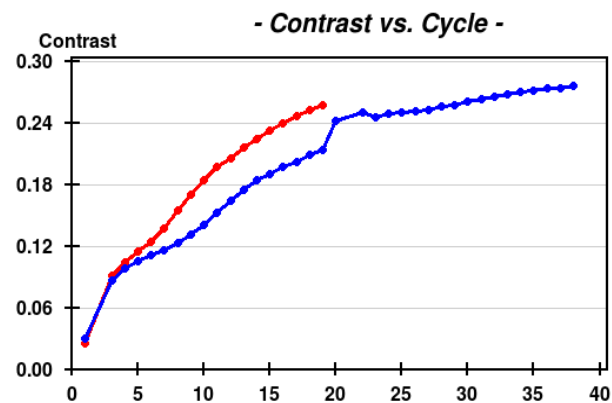
Phase calculation



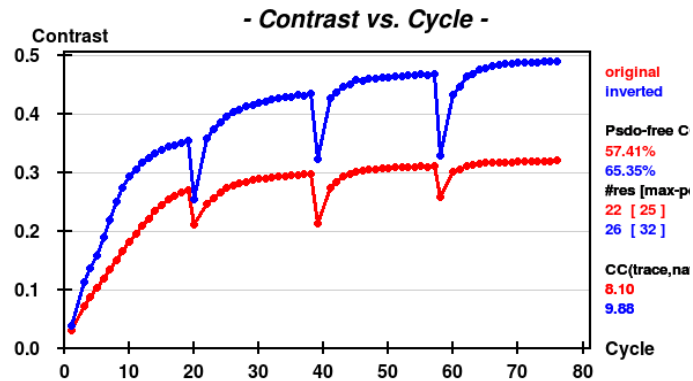
Density modification

ShelxE

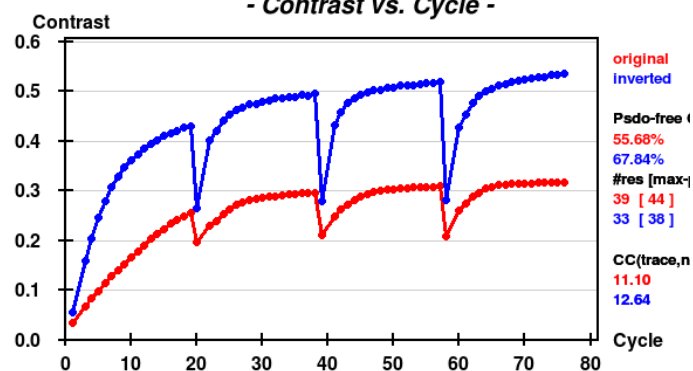
90°

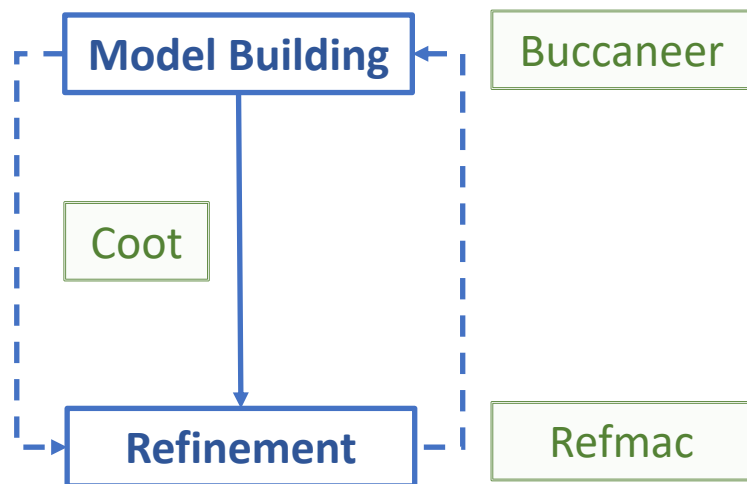


360°



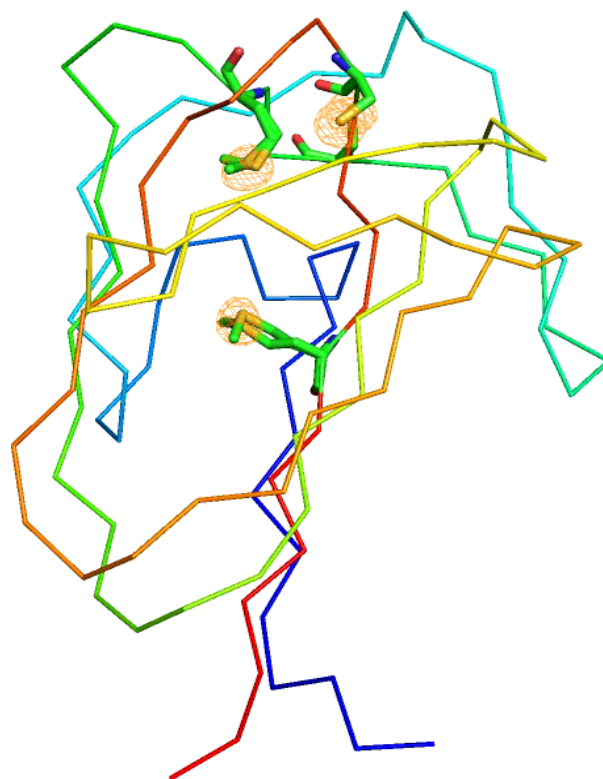
720°





Result

Initial	Final		
	R factor	0.1914	0.1862
	R free	0.2145	0.2100
	Rms BondLength	0.0099	0.0085
	Rms BondAngle	1.3979	1.3166
	Rms ChirVolume	0.0755	0.0691



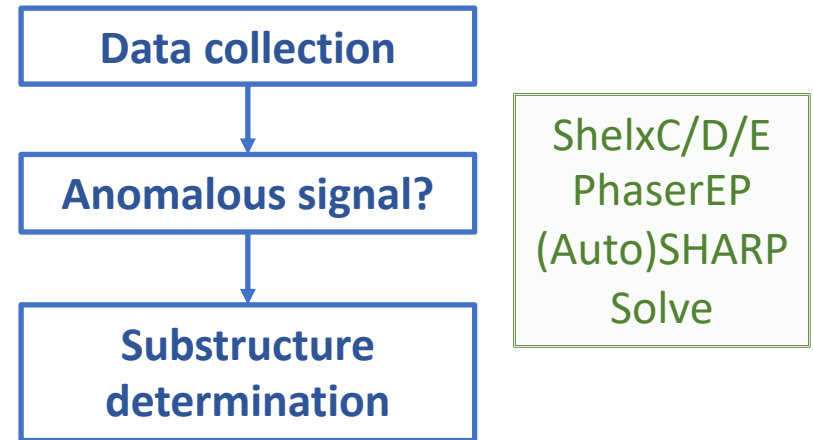
Soft X-ray SAD case study:

225 kDa protein asymmetric unit (three $\phi 6$ polymerase molecules) using the anomalous scattering signal from 3 Mn ions recorded near the Mn K edge.

Data-collection and processing statistics.

Values in parentheses refer to the highest resolution bin.

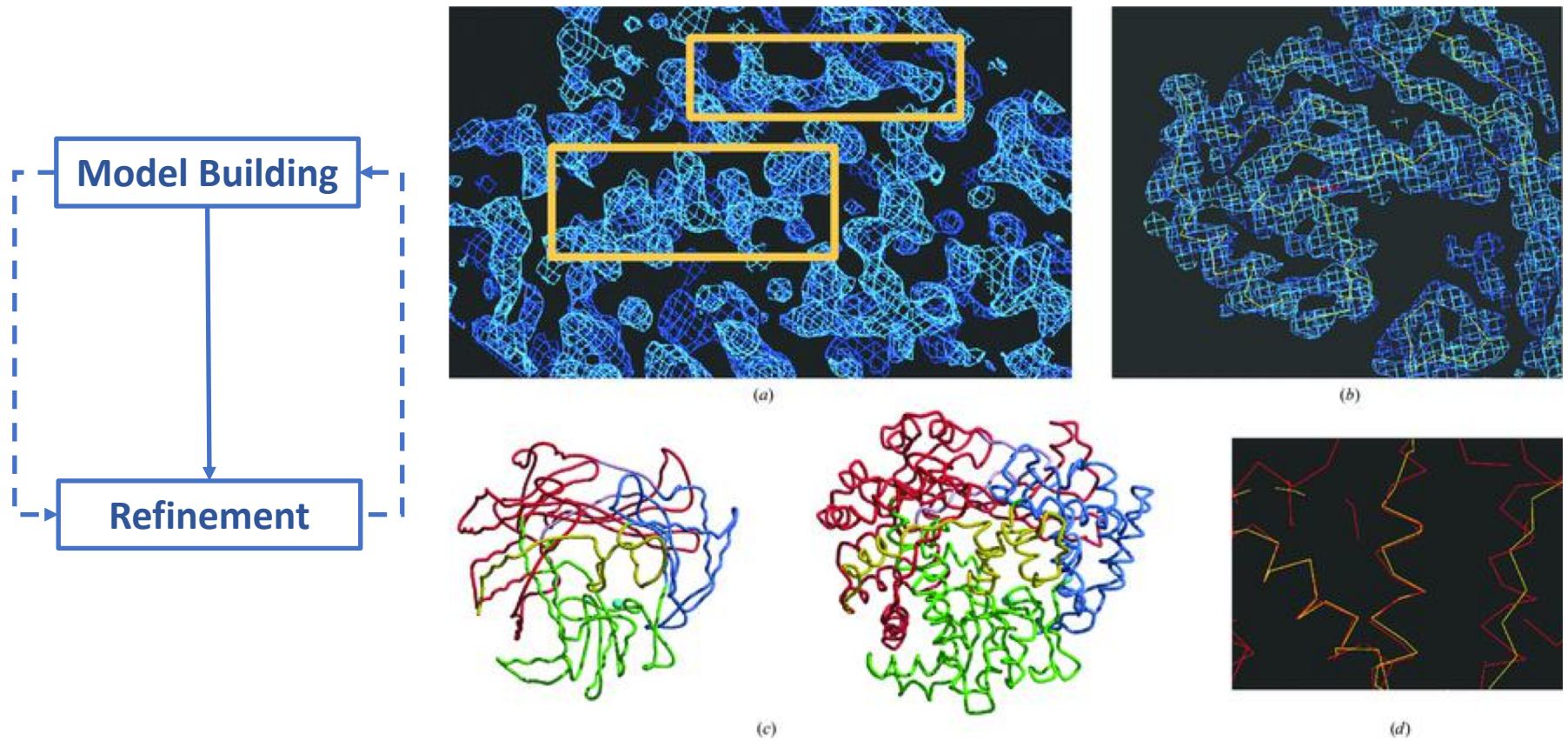
X-ray source	ESRF BM14
Wavelength (Å)	1.89
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 105.5$, $b = 93.6$, $c = 141.1$, $\beta = 101.1$
Resolution range (Å)	30.0–2.9 (3.0–2.9)
No. of images (1° oscillations)	647
Observations	1230575
Unique reflections	59598
Completeness (%)	99.2 (97.9)
$I/\sigma(I)$	29.6 (6.5)
R_{merge} (%)	9.5 (30.5)
Anomalous $I/\sigma(I)$	
∞–8.0 Å	2.2
8.0–6.0 Å	2.1
6.0–5.0 Å	1.9
5.0–4.5 Å	1.8
4.5–4.3 Å	1.7
4.3–4.1 Å	1.7
4.1–3.9 Å	1.5
3.9–3.7 Å	1.4
3.7–3.5 Å	1.3
3.5–3.3 Å	1.1
3.3–3.1 Å	1.0
3.1–2.9 Å	1.0
Solvent content (%)	60



Phase and map analyses.

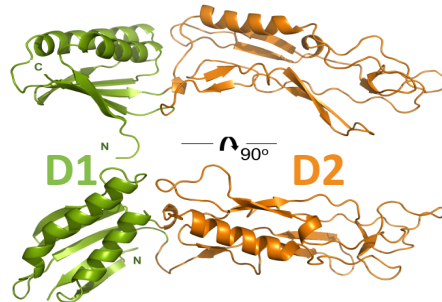
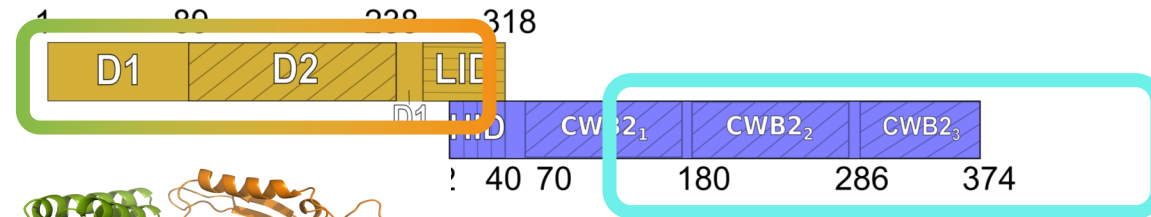
	Resolution	Mean phase error (Å)	Map correlation coefficient
<i>SOLVE</i>	30–3.5	67.0	0.69
<i>RESOLVE</i>	30–3.5	44.8	0.80
<i>RESOLVE</i> with NCS	30–3.5	34.7	0.85
<i>SOLVE</i>	30–2.9	71.2	0.68
<i>RESOLVE</i>	30–2.9	51.1	0.79
<i>RESOLVE</i> with NCS	30–2.9	37.4	0.89

Soft X-ray SAD at Mn edge:

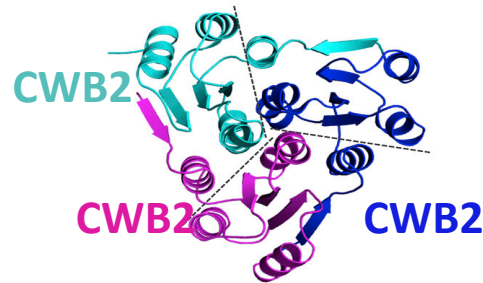


Salgado *et al.*, 2005

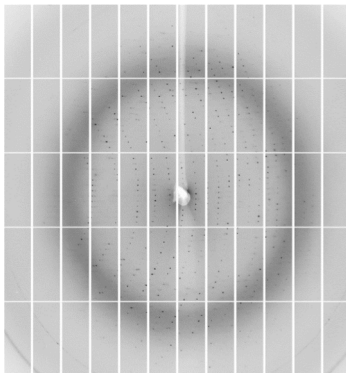
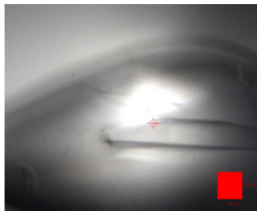
Difficult case study: MR-SAD, partial models, etc



Fagan *et al.*, Mol Microbiol. 2009



Usenik *et al.*, Structure, 2017



▼ Key summary

Selecting space group P 1 21 1
as there is a single space group with the highest score

Solution probability: 0.829, Confidence 0.807 (high resolution limit for symmetry testing 2.950Å)

Key statistics for Dataset: SlpA1/nat/1-4

Unit cell: 77.614 134.005 84.225 90.000 100.666 90.000, wavelength 0.979391Å

Resolution of input data: 2.95Å, resolution estimate: beyond 2.95Å

Anisotropic limits: - Along 1.00 a* - 0.06 c* 3.05Å CC(1/2), 3.24Å I/σ - Along k axis 2.95Å* CC(1/2), 3.23Å I/σ - Along -0.12 a* + 0.99 c* 2.95Å* CC(1/2), 2.95Å I/σ

Rmeas: overall 0.392, inner bin 0.144
In outer bin: Mean(I/σ) 2.2 CC(1/2) 0.928
Overall filtered Mean(chi^2): 1.03
Anomalous CC(1/2) in inner bin -0.295
No significant anomalous signal detected

SD correction information:

SEVERE WARNING: SD correction parameters are outside expected limits, see SD analysis panel below for more details

Asymptotic I/sigI, ISA: AllRuns: cannot determine ISA

✓ No evidence of twinning

✗ Warning: Possible translational non-crystallographic symmetry at coordinate (0.333 0.5 0.729), chance probability 0.0733, height 0.34 of origin

● Warning: Some anisotropy detected. This may have an effect on statistics.

● Warning: Completeness test shows some issues.

✗ Warning: Severe deviation from Wilson plot, 7.9% of bins deviate, please look at it

✓ No ice rings found.

A free-R set has been created, fraction of the data = 0.05

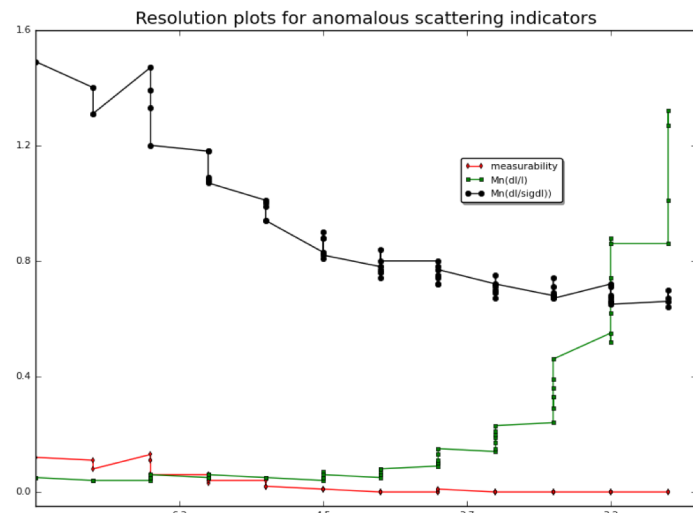
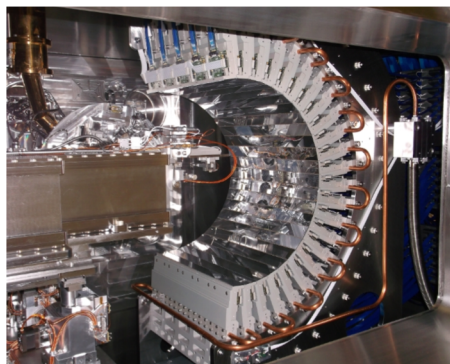
Derivatisation



HA	Soaks	Mounted	Screened	Collected	Anom. res. Å (overall res.)	Best res. data Å
Au	30	25	18	2	5.8 (3.4)	3.4
Hg	15	11	7	4	3.7 (2.9)	2.9
Br	30	20	9	2	no	3.1
I	30	25	9	2	4.1 (3.3)	2.9
Pb	30	27	22	4	3.6 (2.8)	2.8
Pt	15	14	8	4	5.2 (3.3)	3.2
TaBr	15	14	12	7	5.9 (3.4)	2.9
W	15	14	8	1	no	
Sm	15	8	5	0	-	
Cd	15	5	4	0	-	
Re	15	13	11	0	-	
Total	225	176	113	26		

HA	Soak	Screened	Collected	Anom. res. Å (overall res.)	Best res. data Å
Au	16	15	5	6.6 (3.3)	2.7*
Pt	9	5	2	4.7 (3.1)	3.2
TaBr	8	5	3	7.4 (3.5)	3.5
total	33	25	10		

Derivatisation



▼ Key summary

Selecting space group P 1 21 1

as there is a single space group with the highest score

Solution probability: 0.863, Confidence 0.861 (high resolution limit for symmetry testing 3.132Å)

Key statistics for Dataset: SlpA1/slpA/Ssad

Unit cell: 76.650 134.730 83.880 90.000 100.831 90.000, wavelength 2.755200Å

Resolution of input data: 3.00Å, resolution estimate: beyond 3.00Å

Anisotropic limits: - Along 1.00 a* - 0.02 c* 3.00Å* CC(1/2); 3.15Å I/σ - Along k axis 3.34Å CC(1/2); 3.52Å I/σ - Along -0.16 a* + 0.99 c* 3.00Å CC(1/2); 3.29Å I/σ

Rmeas: overall 0.070, inner bin 0.031

In outer bin: Mean(I/σdI) 1.3 CC(1/2) 0.716

Overall filtered Mean(chi^2): 1.06

Anomalous CC(1/2) in inner bin 0.541

Significant anomalous signal extends to a resolution of 4.98Å (above CCanom threshold 0.15)

NOTE: no scaling was done, just merging

SD correction information:

SD correction parameters were not refined

✓ No evidence of twinning

✗ Warning: Possible translational non-crystallographic symmetry at coordinate (0.312 0.5 0.729), chance probability 0.0482, height 0.367 of origin

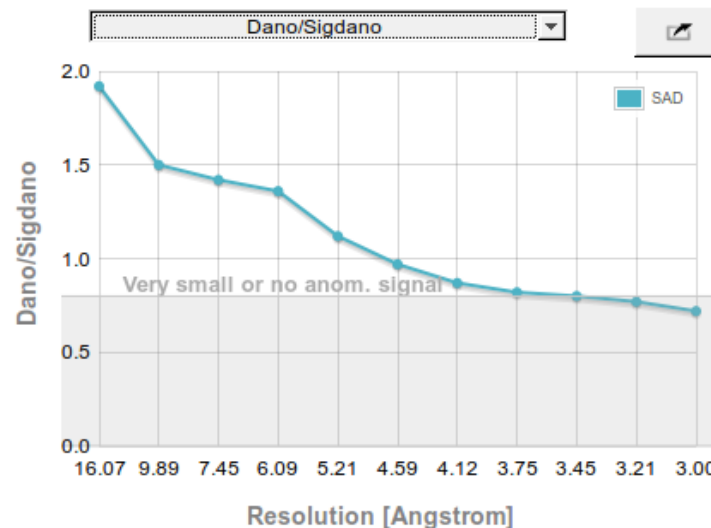
● Warning: Some anisotropy detected. This may have an effect on statistics.

● Warning: Completeness test shows some issues.

✗ Warning: Severe deviation from Wilson plot, 10.2% of bins deviate, please look at it

✓ No ice rings found.

A free-R set has been created, fraction of the data = 0.05



ShelxC/D

Substructure
determination

ShelxE

Phase calculation

Programs used:

SHELXD

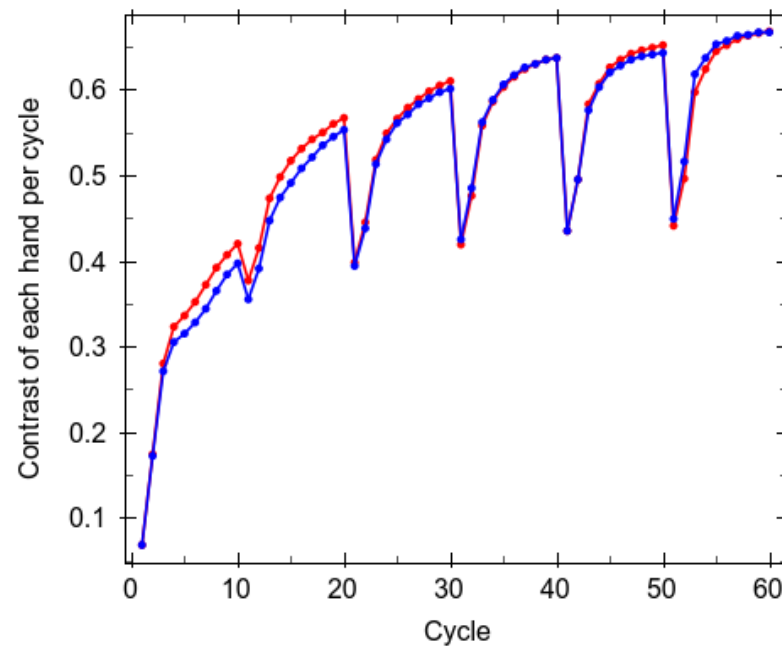
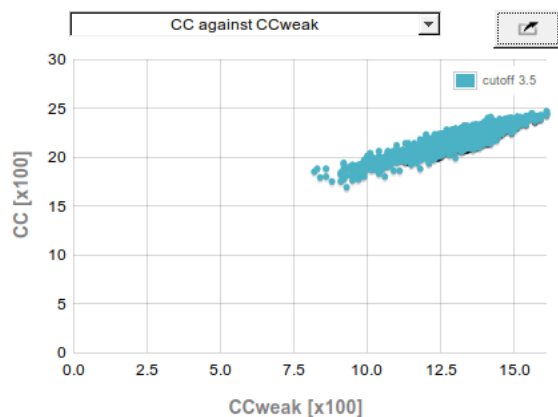
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 40.8 occurring at Trial 868.

The substructure has 3 atoms with occupancy of at least 25%, 22 in total.



Programs used:

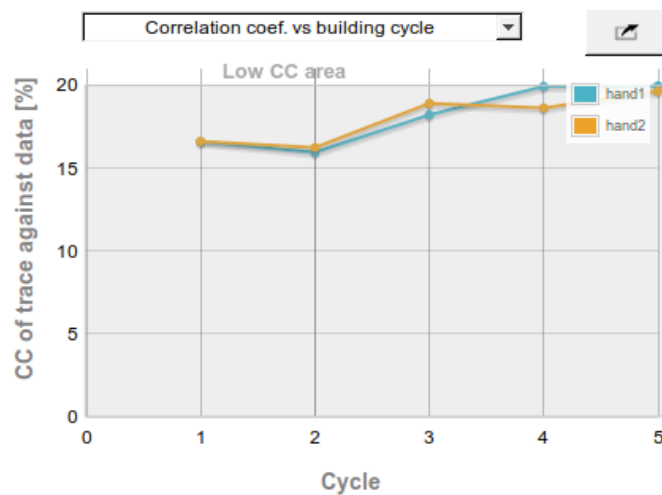
SHELXE

Result:

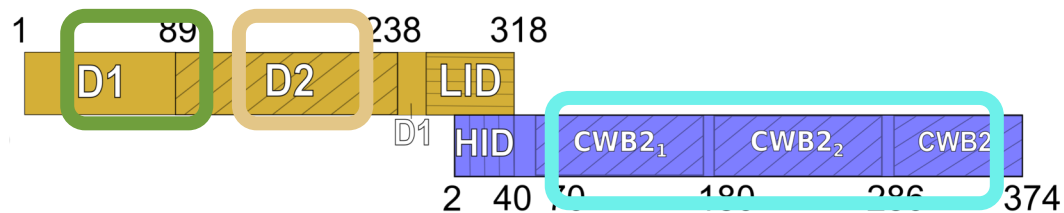
Maximum correlation coefficient for hand 1 is 19.96; max. correlation coef. for the other hand is 19.64. Hand 1 is chosen.

Max. number of residues built (for the chosen hand) is 464

Correlation coefficient too low, the traced model is most likely wrong.



MR-SAD



Job 4: build from S_Sad maps, no model after adding Rfree, correct seq

COWLAN K. (2006) Acta Cryst. D62, 1002-1011.

Result

1191 residues were built in 47 fragments, the longest having 111 residues.
 871 residues were sequenced, after pruning.
 780 residues were uniquely allocated to 4 chains.
 Completeness by residues built: 65.5%
 Completeness of chains (number): 56.5% (4)

Run of Refmac on 7/6/2017 at 11:46:57

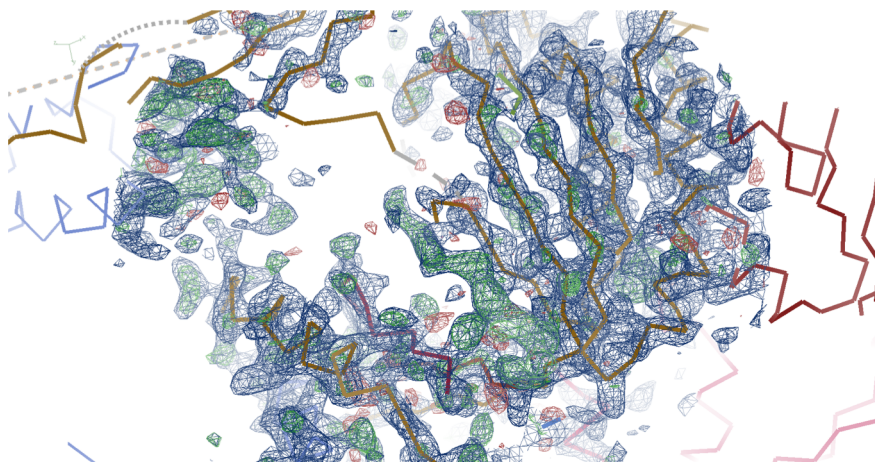
Please cite:

"REFMAC5 for the refinement of macromolecular crystal structures:"
 G.N.Murshudov, P.Skubak, A.A.Lebedev, N.S.Pannu, R.A.Steiner, R.A.Nicholls, M.D.Winn, F.Long and A.A.Vagin, (2011)
 Acta Crystallogr. D67, 355-367

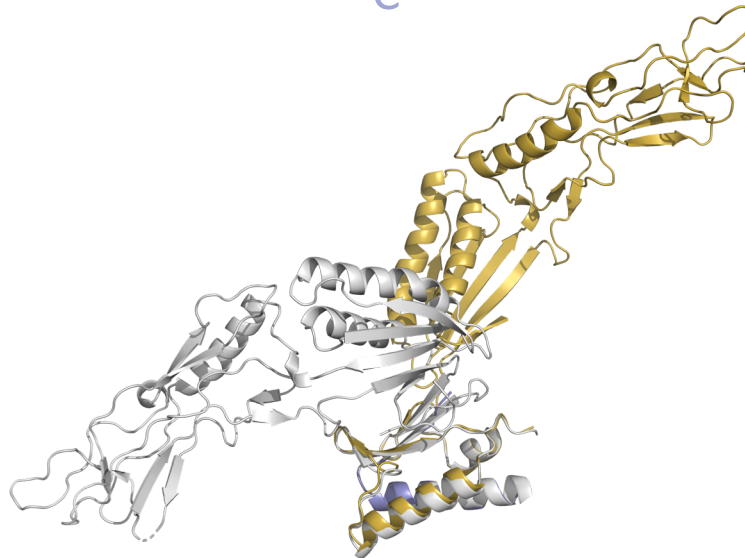
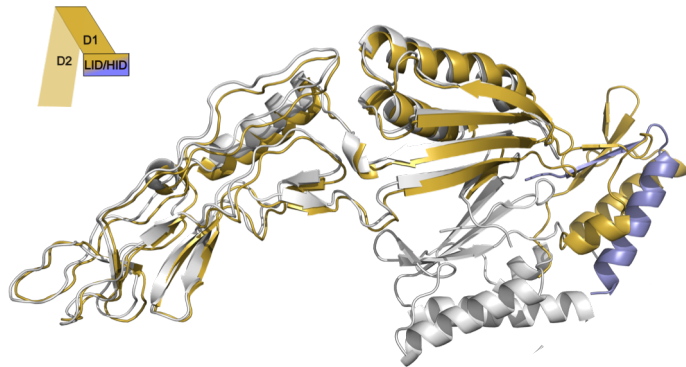
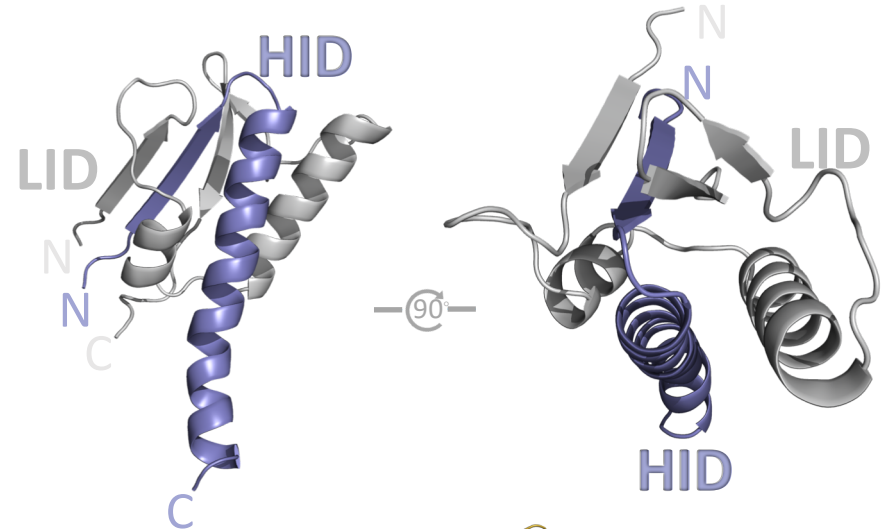
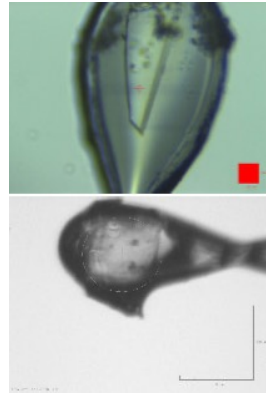
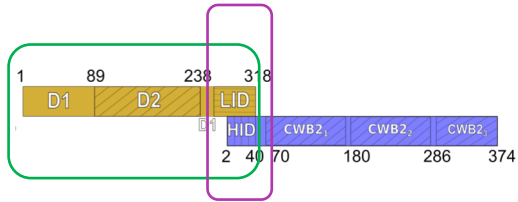
"Refinement of Macromolecular Structures by the Maximum-Likelihood Method:"
 G.N. Murshudov, A.A.Vagin and E.J.Dodson, (1997)
 Acta Crystallogr. D53, 240-255
 EU Validation contract: BIO2CT-92-0524

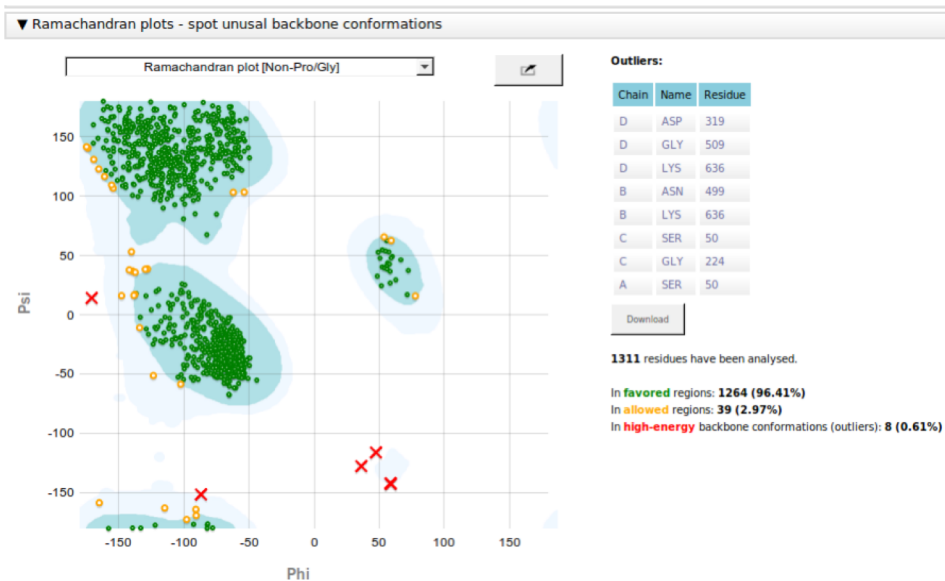
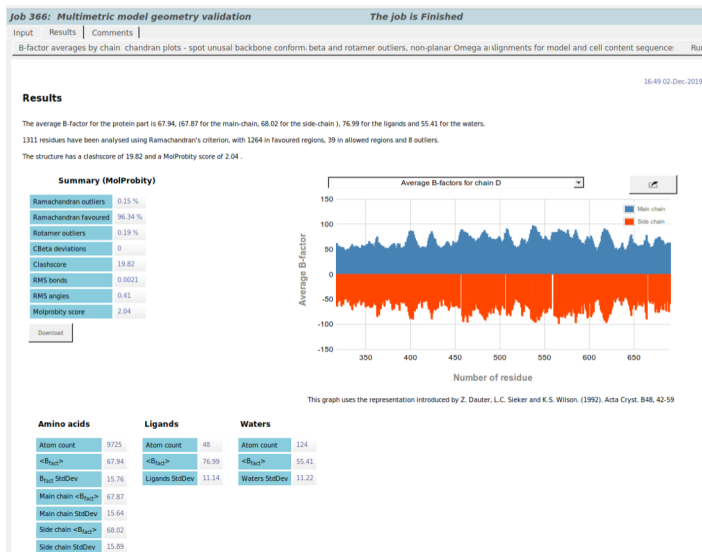
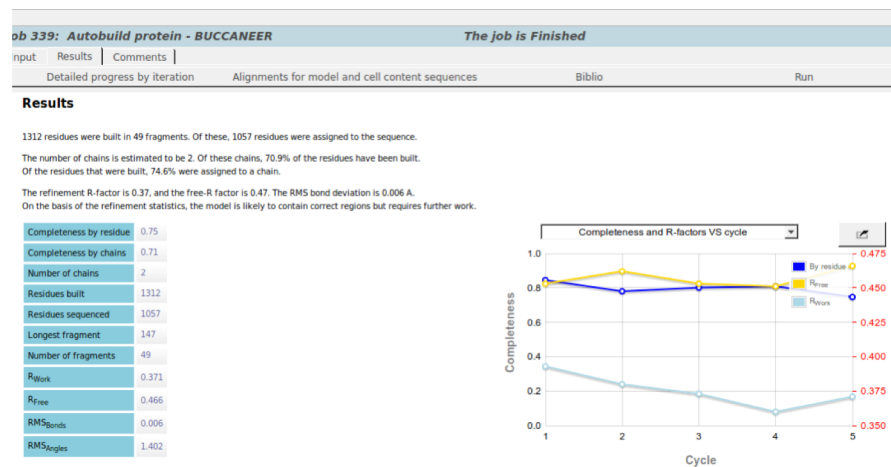
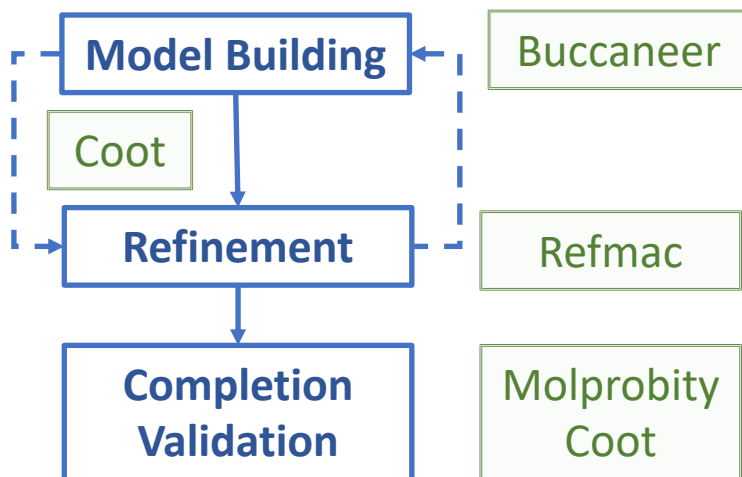
Result

Initial	Final
R factor	0.4568 0.4026
R free	0.4924 0.4458
Rms BondLength	0.0612 0.0115
Rms BondAngle	6.0762 1.9869
Rms ChirVolume	0.2428 0.1291



'Divide & conquer' – partial domains





Summary: isomorphous replacement

1. A good choice for the *de novo* structure solution of very large macromolecules or complexes
2. Signals can be (very) large
3. Non-isomorphism can be a problem and usual cause of failure
4. Search for suitable derivatives can be limiting (time, protein availability)

Summary: Anomalous scattering

1. No 'native' crystal when using heavy atom derivatives
2. No issues with isomorphism if data collected from single crystal
3. Se-Met can be introduced and used as a “magic bullet”*
4. Current 'method of choice' for *de novo* structure solution
5. Signals are small – data have to be good (thoughtful data collection)
6. For MAD, need to collect a lot of data from one crystal can lead to radiation damage (ie, non-isomorphism)

*Se-cysteine also a possibility

Difficult cases

1. No “magic bullet”
2. What does the data tell you?
3. “Know” your crystals: any issues? Twinning, pseudo-symmetry, tNCS, anisotropy?
4. Test and combine different phasing strategies
5. Combine information and models
6. Building can be as challenging as solving the phase problem
7. Try and try again – if data suggests it’s possible to solve!

Mn SAD case study



Division of Structural Biology

Dave Stuart

Jon Grimes



Human Frontier Science Program



UNIVERSITY OF HELSINKI

Dennis Bamford

Sarah Butcher

Minni Laurila

Eugene Makeyev

SIRAS case study 2

Imperial College London

- Ernesto Cota
- Rob Yan
- Jing Lin
- Fiona Rowan
- Lynn Buchnell
- Rhian Jones
- Steve Matthews
- Pete Simpson
- Matthews group
- Jeremy Moore



ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

- Lois Hoyer



Adam Crawshaw

Anna Barwinska-Sendra

Paola Lanzoni

Abbie Kelly

Gilly Wang

Zoe Wilkinson

Marcin Dembek

Emma Tarrant



Gill Douce
Filipa Vaz



The
University
Of
Sheffield.



Rob Fagan

Joe Kirk

Per Bullough

Oishik Banerji

Jason Wilson



Imperial College
London



Neil Fairweather



Arnaud Baslé



Kamel El Omari
Armin Wagner