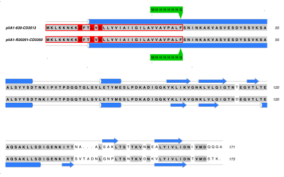
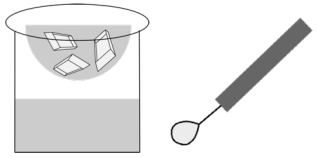
Experimental phasing: A practical perspective

Paula S. Salgado

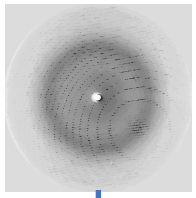
DLS CCP4 Workshop2019



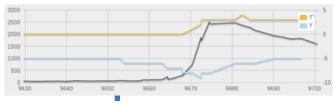


Derivatisation



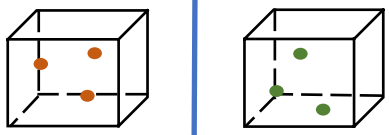


Data collection

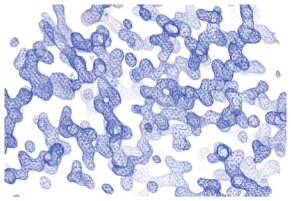


Anomalous signal?

Substructure determination

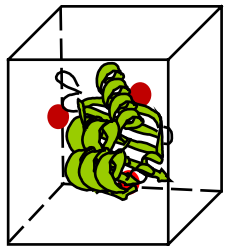


Phase calculation

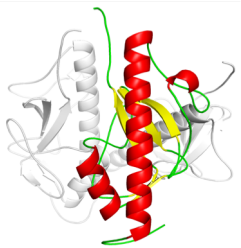


Density modification

Model Building



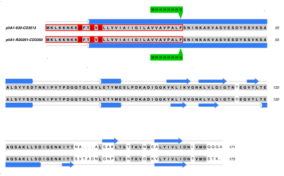
Refinement

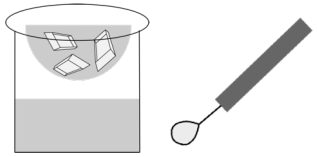


Completion Validation

Deposition



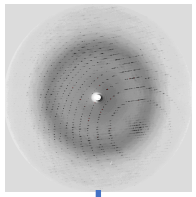




Derivatisation

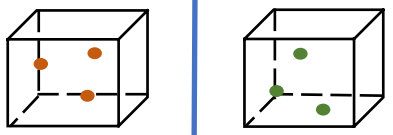


Data collection



Anomalous signal?

Substructure determination



ShelxC/D/E
PhaserEP
(Auto)SHARP
Solve

Phase calculation

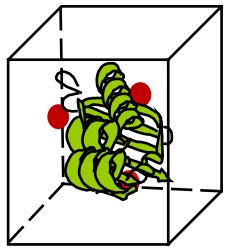
Pirate
DM
Solomon
Parrot

Density modification

Model Building

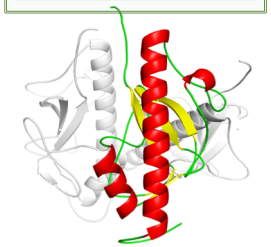
Coot

Buccaneer
ShelxE
ARPwARP



Refinement

Refmac



Completion
Validation

Molprobity
Coot



Deposition

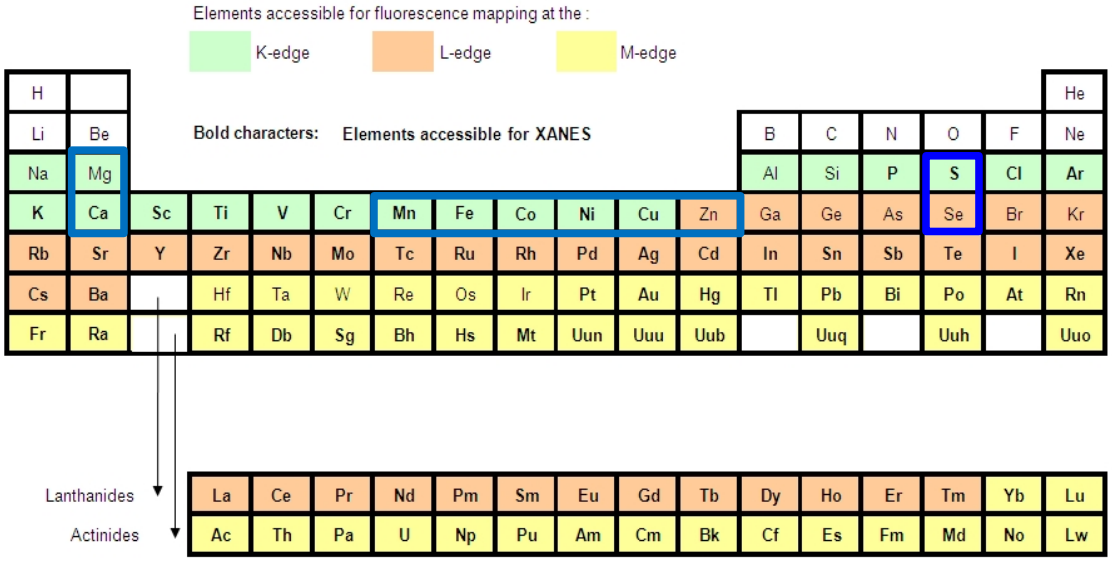
PDBe

Derivatisation



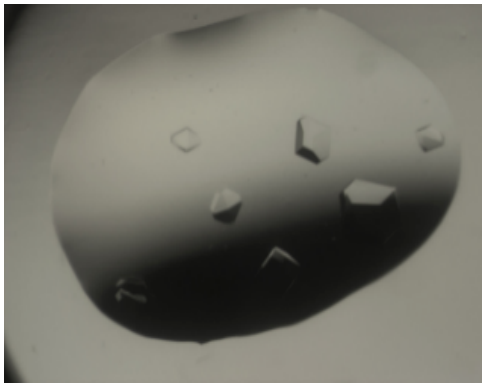
SIR/MIR (Single/Multiple Isomorphous Replacement)

SAD/MAD (Single/Multiple Anomalous Dispersion)

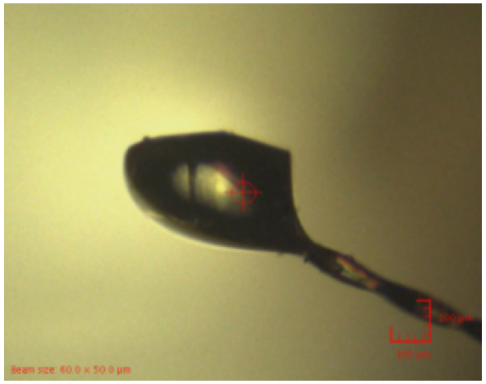


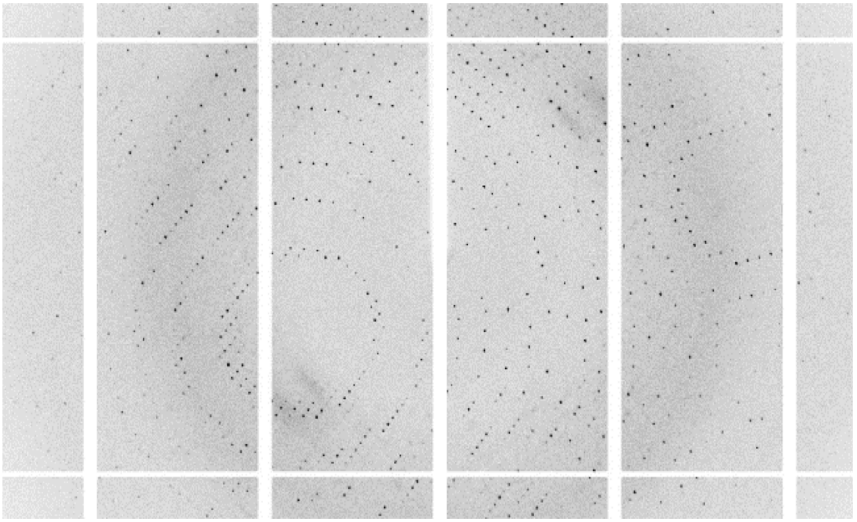
(M)SIRAS Case study 1: CdPilA1 (R20291)



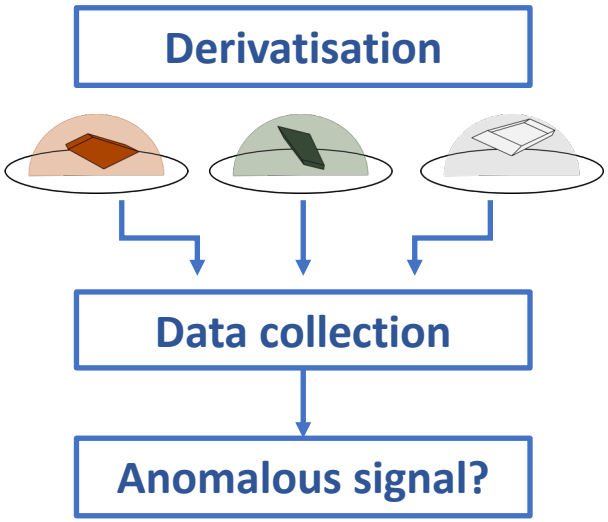


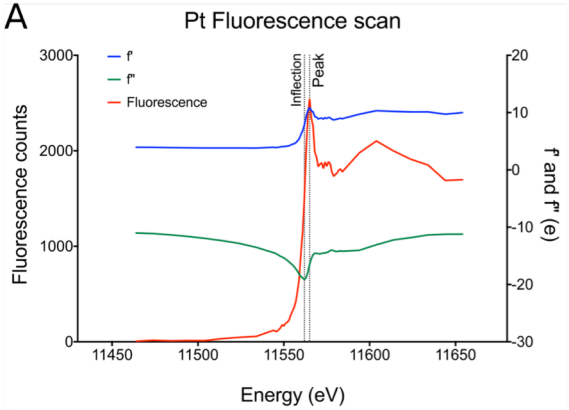
A. Crawshaw, C. Davies

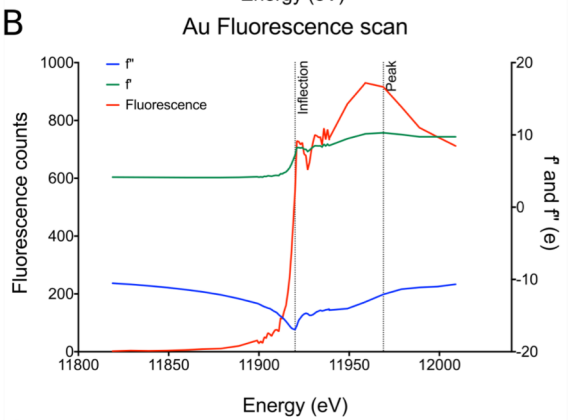


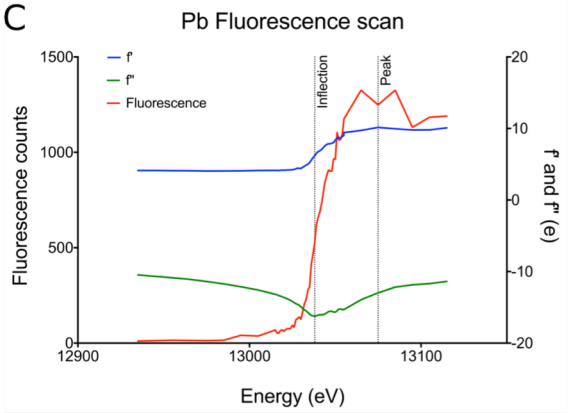


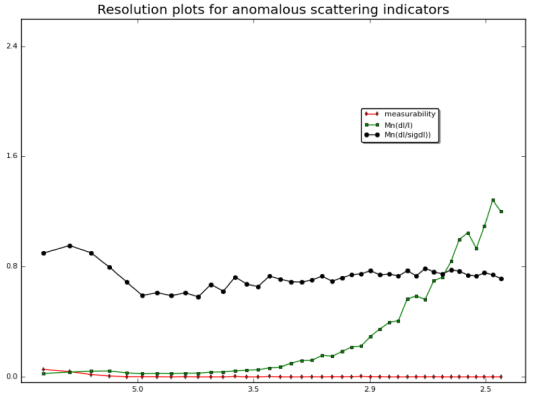
	R20291 Native
Resolution (Å)	51.21 - 1.65
Wavelength (Å)	
Unit cell	
a, b, c (Å)	102.42, 102.42, 104.21
α=β=γ (°)	90
Spacegroup	$P4_12_12$
R_{merge}	0.136 (1.887)
I/sig I	10.7 (1.5)
Mean intensity CC1/2	0.998 (0.323)

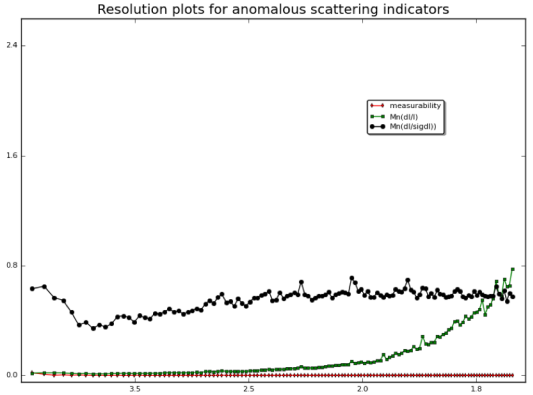

Derivatisation
Data collection
Anomalous signal?

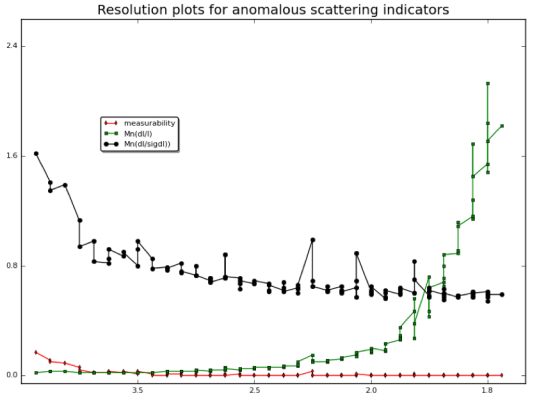

A
Pt Fluorescence scan
f'
f''
Fluorescence
Inflection
Peak
Fluorescence counts
f' and f'' (e)
Energy (eV)

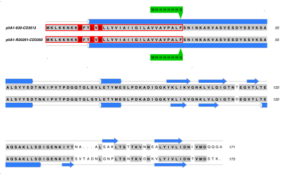

B
Au Fluorescence scan
f''
f'
Fluorescence
Inflection
Peak
Fluorescence counts
f' and f'' (e)
Energy (eV)

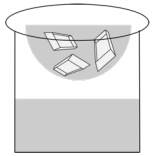

C
Pb Fluorescence scan
f'
f''
Fluorescence
Inflection
Peak
Fluorescence counts
f' and f'' (e)
Energy (eV)

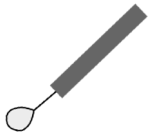

Resolution plots for anomalous scattering indicators
measurability
Mn(dI/I)
Mn(dI/sigdI))


Resolution plots for anomalous scattering indicators
measurability
Mn(dI/I)
Mn(dI/sigdI))


Resolution plots for anomalous scattering indicators
measurability
Mn(dI/I)
Mn(dI/sigdI))

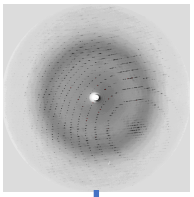






Derivatisation



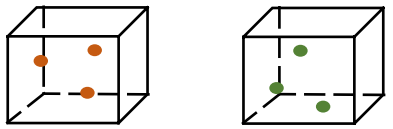


Data collection

Anomalous signal?

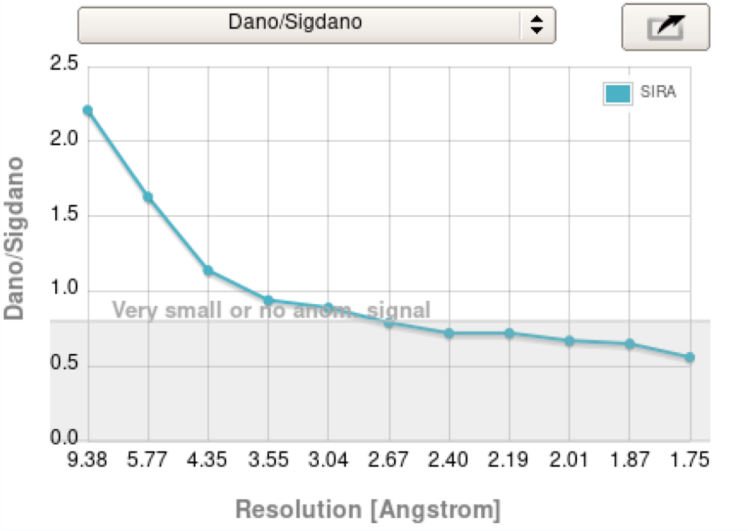
Substructure determination

ShelxC/D
PhaserEP
(Auto)SHARP
Solve



ShelxC/D

Substructure determination



Programs used:

SHELXD

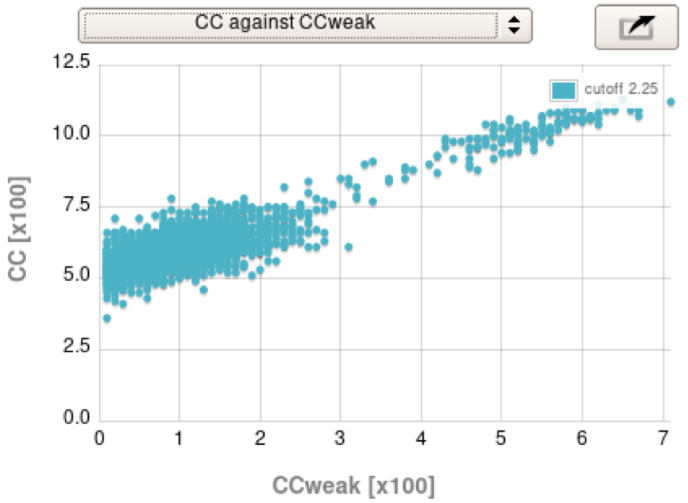
Stop:

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

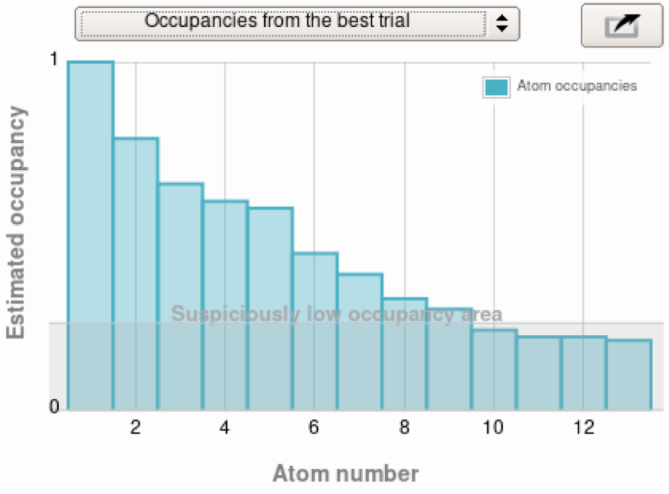
Stop:

Maximum number of trials reached.

Result:

Maximum CFOM: 18.3 occurring at Trial 172.

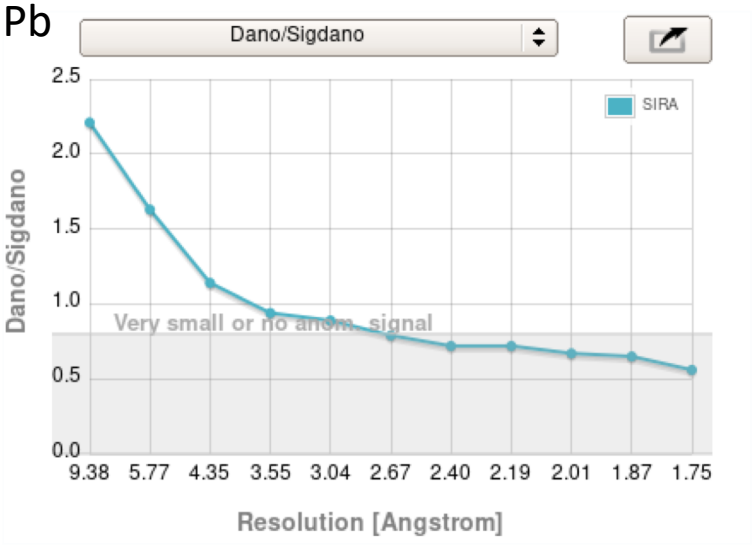
The substructure has 9 atoms with occupancy of at least 25%, 13 in total.



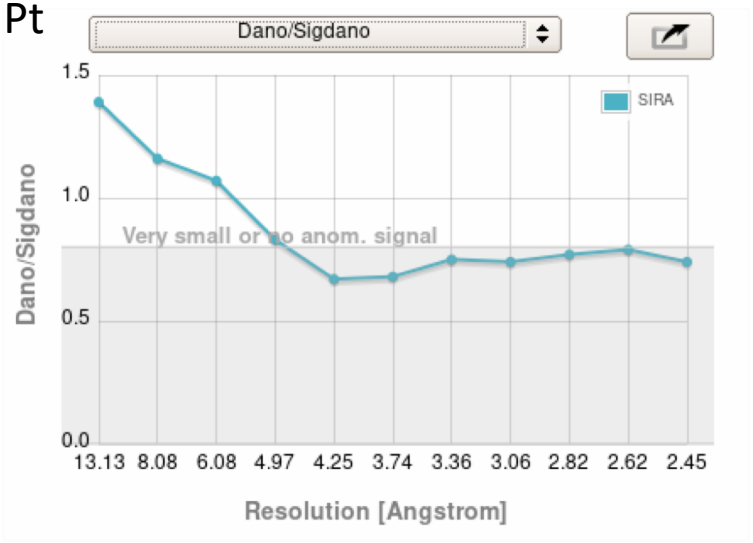
ShelxC/D

Substructure determination

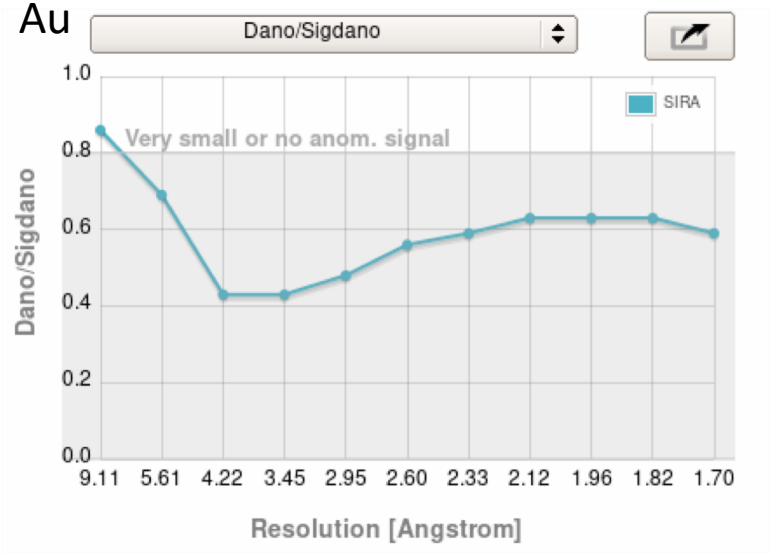
Pb



Pt



Au



ShelxC/D

Substructure determination

Pb

Programs used:

SHELXD

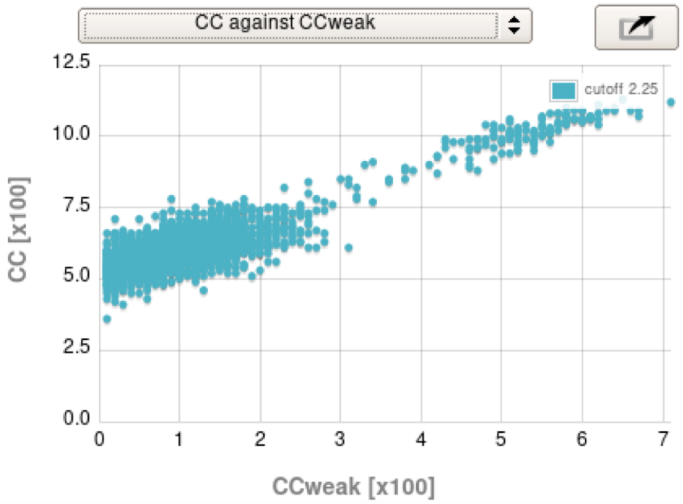
Stop:

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.



Pt

Programs used:

SHELXD

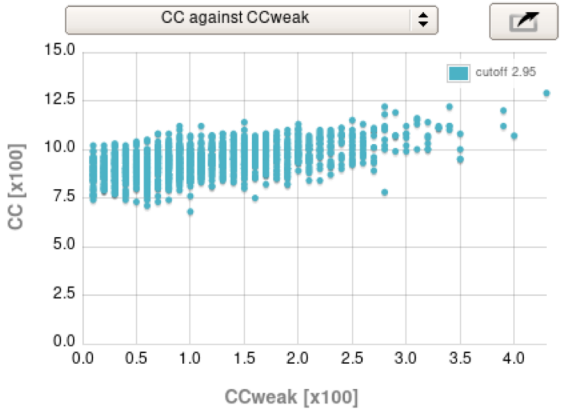
Stop:

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

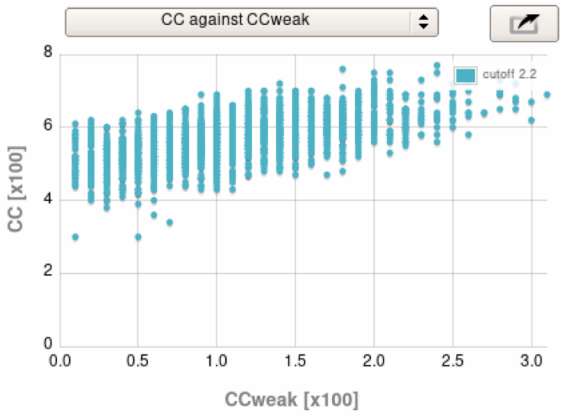
Stop:

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.



ShelxC/D

Substructure determination

Pt

Programs used:

SHELXD

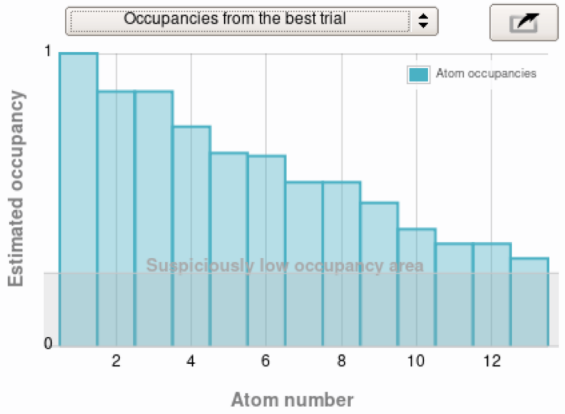
Stop:

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

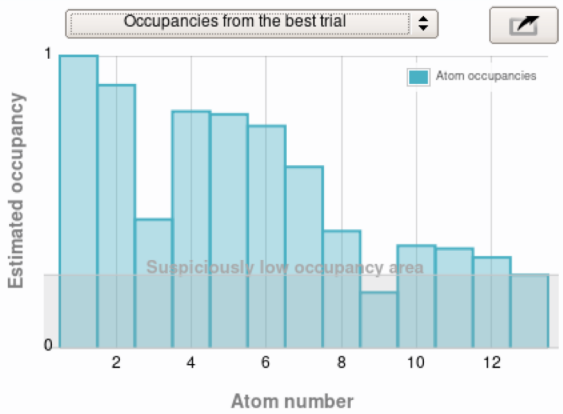
Stop:

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

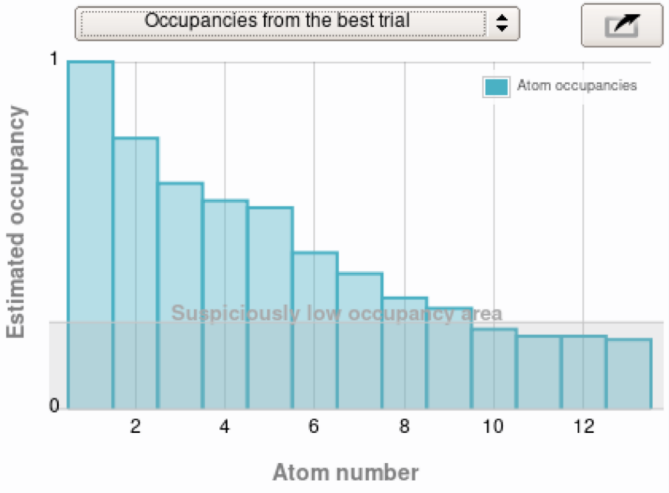
Stop:

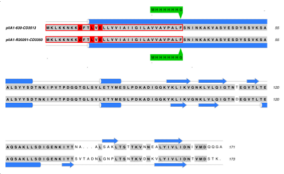
Maximum number of trials reached.

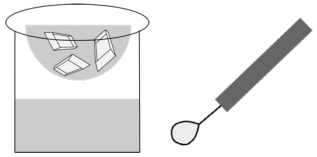
Result:

Maximum CFOM: 18.3 occurring at Trial 172.

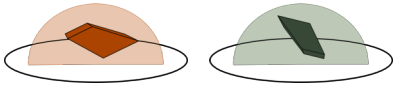
The substructure has 9 atoms with occupancy of at least 25%, 13 in total.







Derivatisation

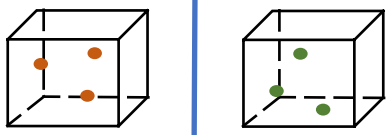


Data collection

Anomalous signal?

Substructure determination

ShelxC/D/E
PhaserEP
(Auto)SHARP
Solve



Phase calculation

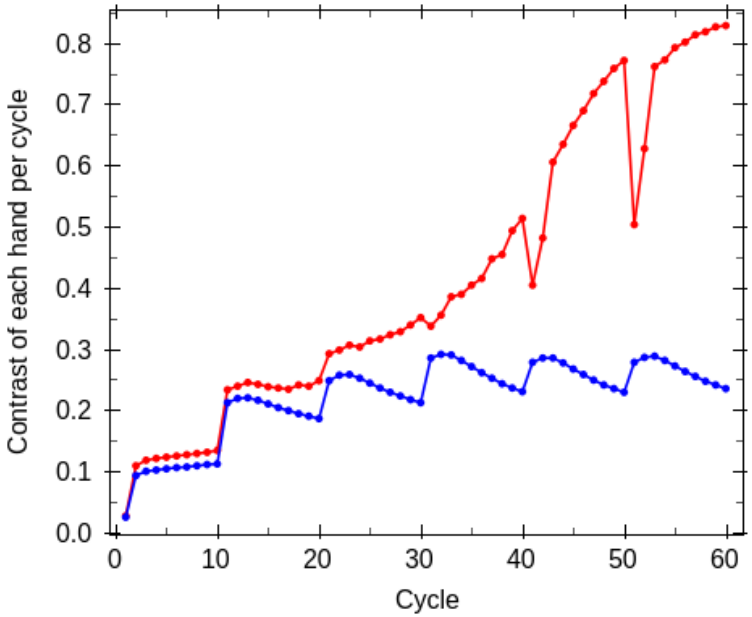
Density modification

Pirate
DM
Solomon
Parrot

ShelxE

Phase calculation

Density modification



Programs used:

SHELXE

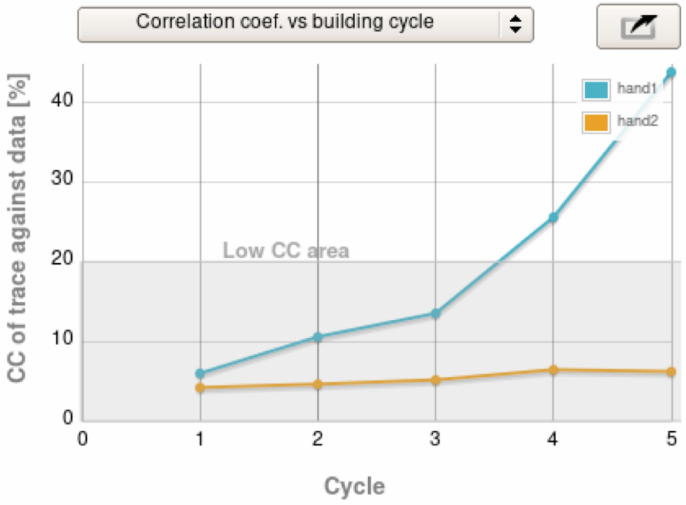
Stop:

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

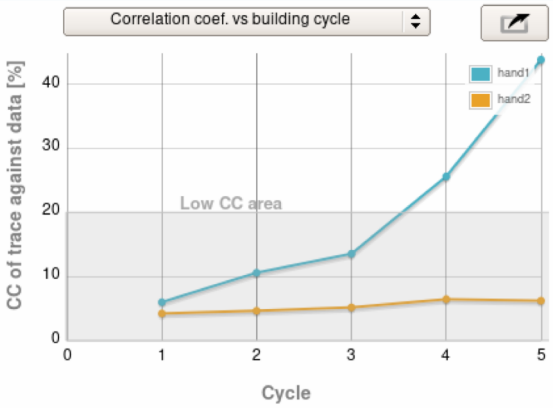
Stop:

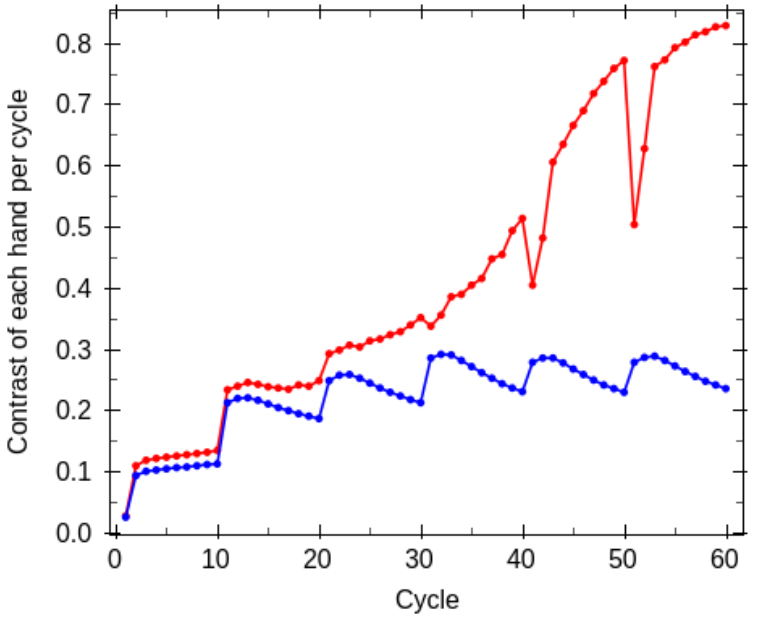
CC parameter threshold (40.0) reached. Stopping after the current cycle.

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:

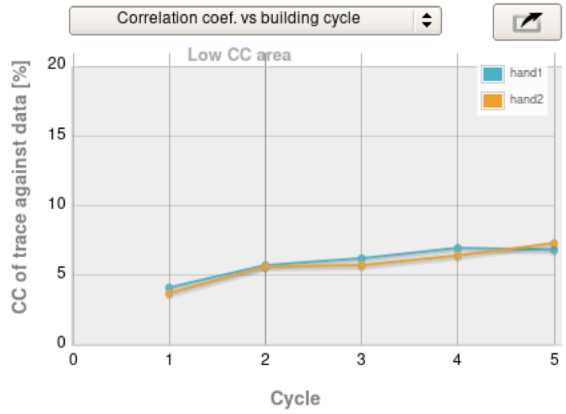
SHELXE

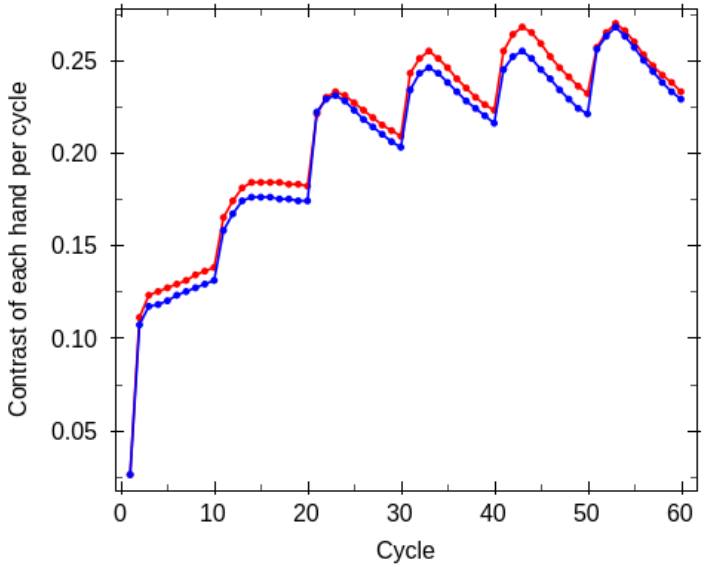
Result:

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.



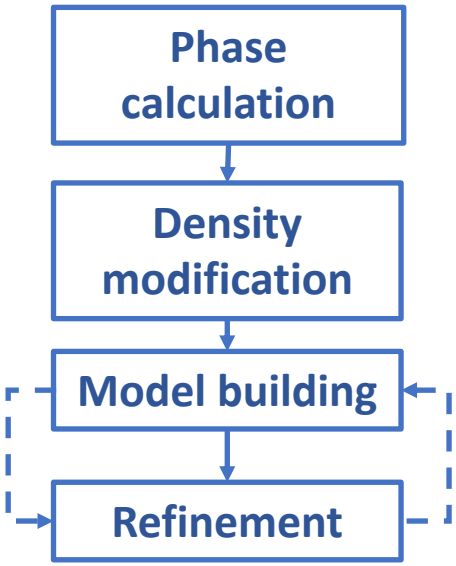


ShelxC/D

Parrot

Buccaneer

Refmac



Programs used:

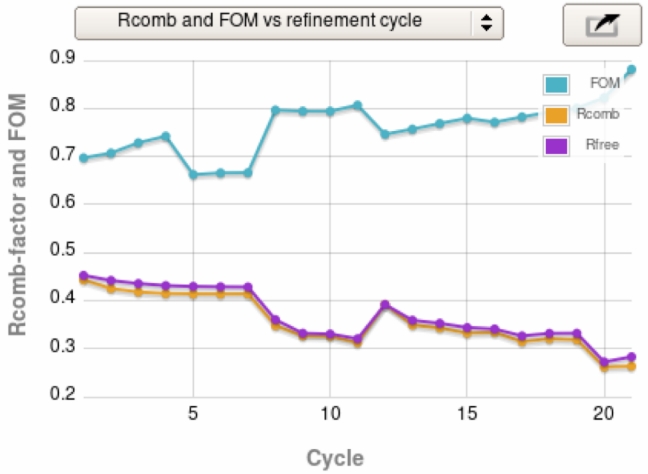
Parrot, REFMAC5, Buccaneer, SHELXE

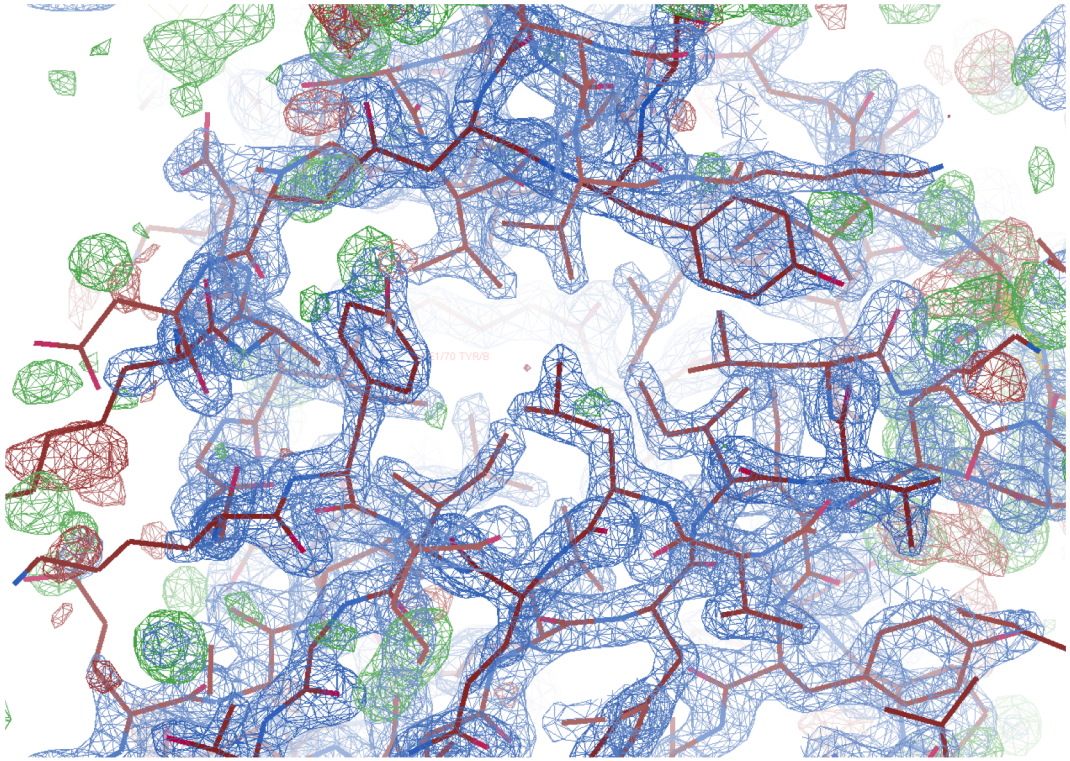
NCS detection:

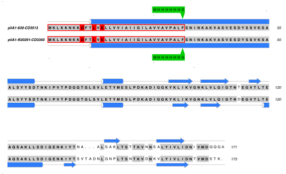
4 NCS operators detected.

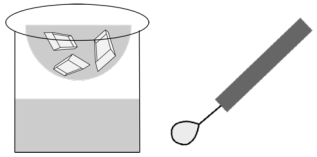
Result:

Majority of model was successfully built!

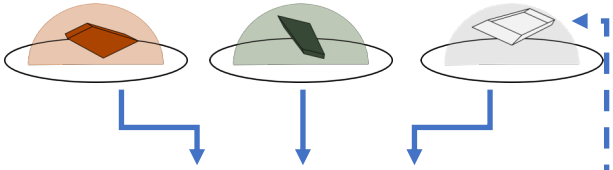




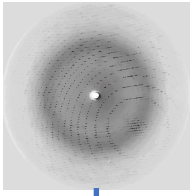




Derivatisation

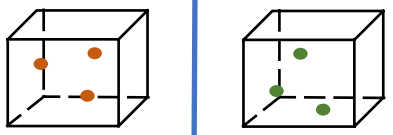


Data collection



Anomalous signal?

Substructure determination



ShelxC/D/E

Phase calculation

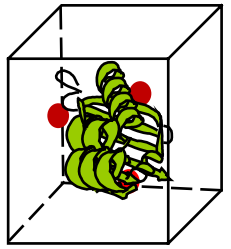
Parrot

Density modification

Model Building

Buccaneer

Coot

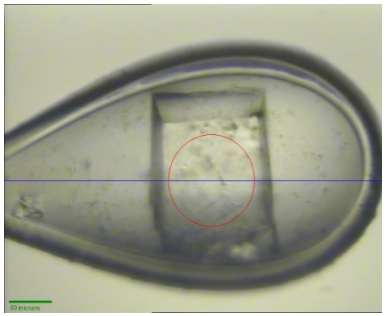


Refinement

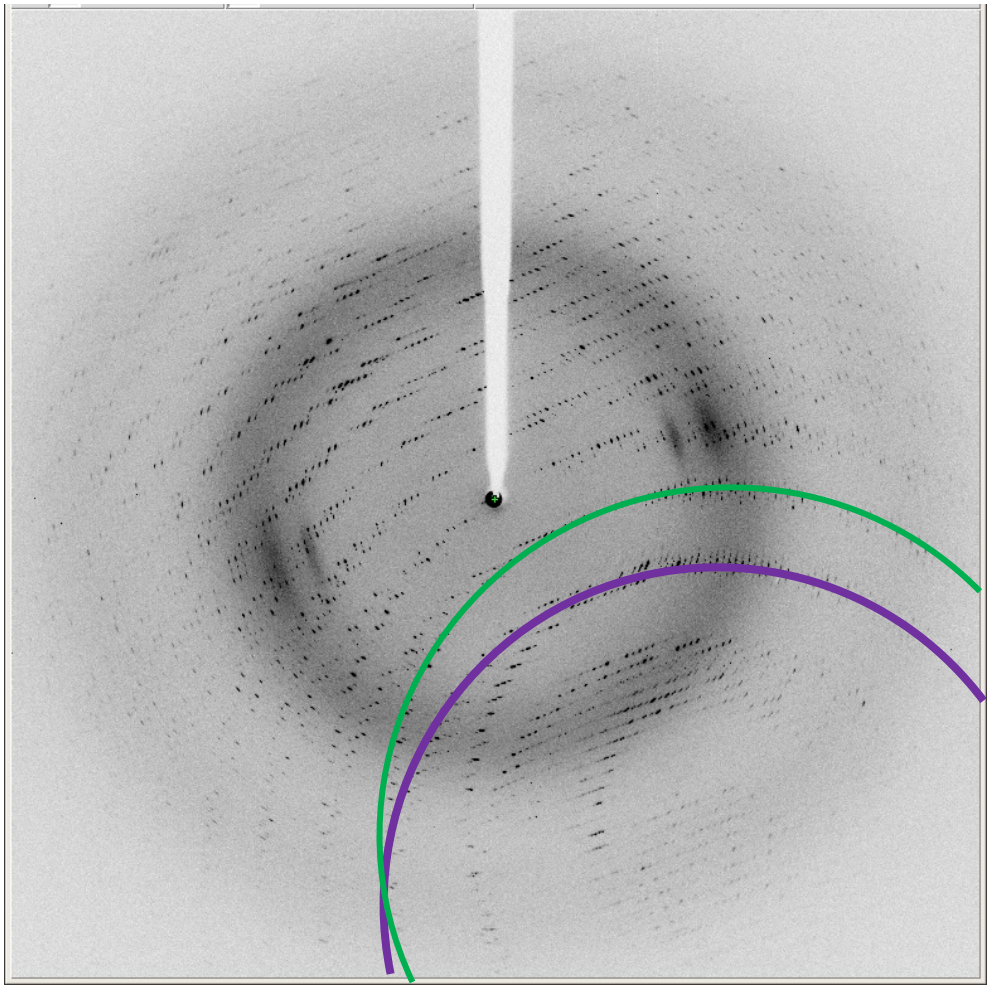
Refmac



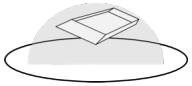
SIRAS case study 2:



Crystal Size	≈ 100 x 100 x 50 µm
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 Å α = β = γ = 90°
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/ δI	19.8 (3.4)
Mosaicity	0.8
No. Molecules/A.u.	1
Solvent Content	48 %



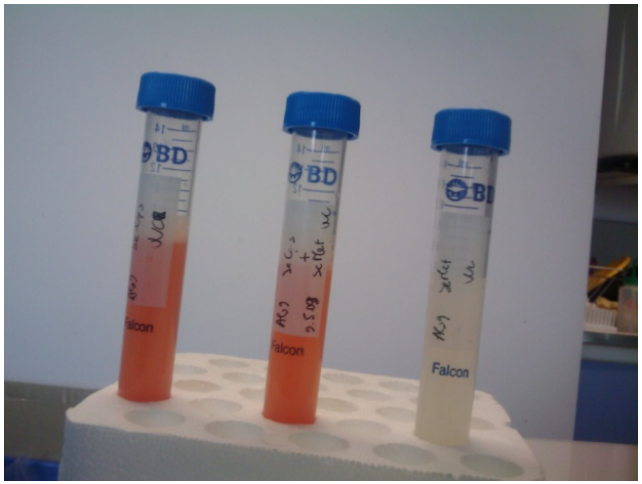
Derivatisation



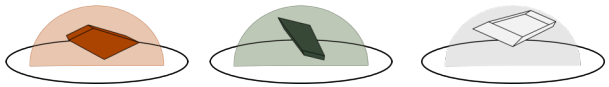
KTITGVFNSFDSLTWTRSVEYVYKGPETPTWNAVLGWSLNSTTADPGDTFTL
ILPCVFKFITTQTSVDLTADGVSYATCDFNAGEEFTTFSSLSCTVNSVSVSY
ARVSGTVKLPITFNVGGTGSSVDLADSKCFTAGKNTVTFMDGDTKISTTVDF
DASPVSPSGYITSSRIIPSLNKLSSLFVVPQCENGYTSGIMGFVASNGATID
CSNVNIGISKGLNDWNFPVSSESFSYTKTCTSTSITVEFQNVPAGYRPFVDA
YISAENIDKYTLTYANEYTCENGNTVVDPFTLTWWGYKNSEADSDGDVIVV

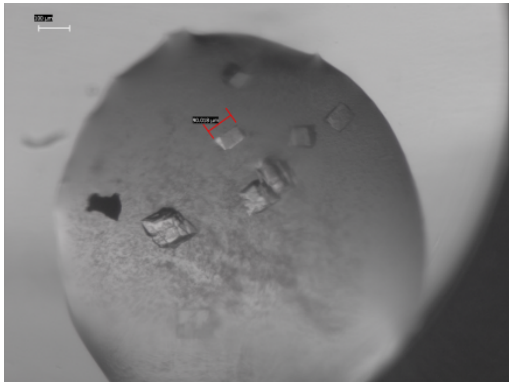
- Only 2 Met but 8 Cys:

  SeMet +SeCys – no expression (cells die)

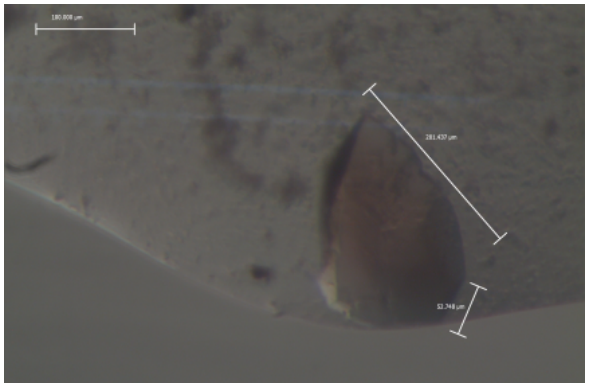


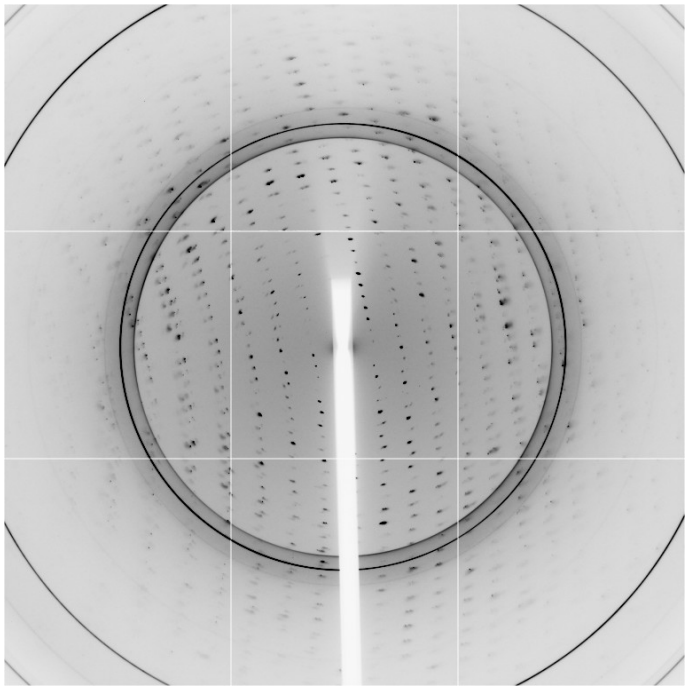
Derivatisation





- 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

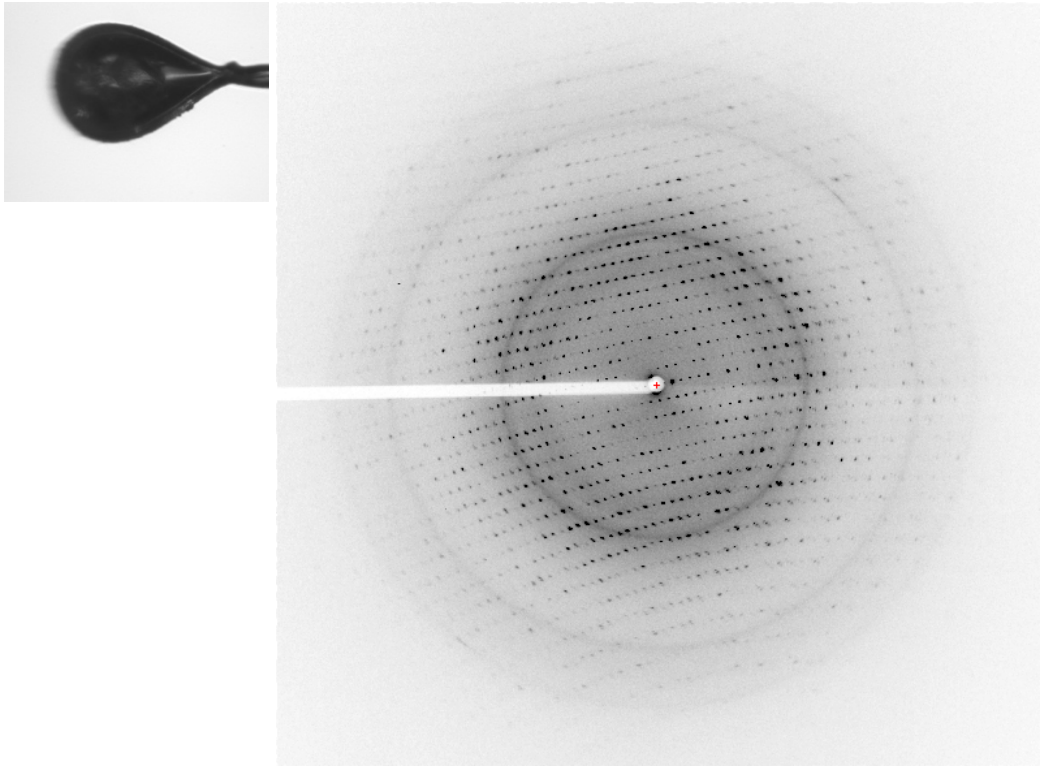




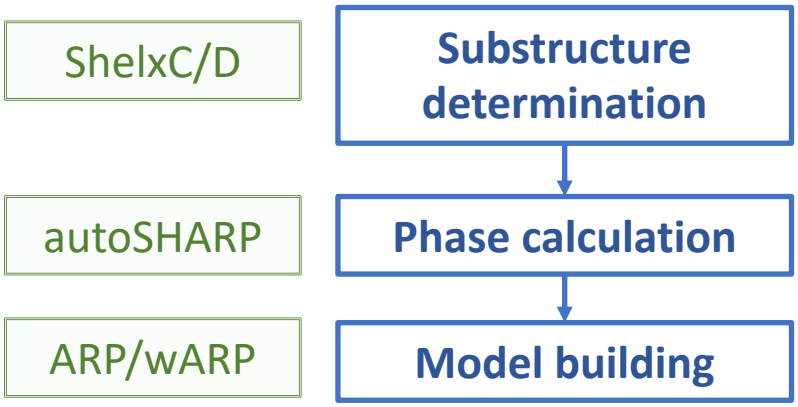
Derivatisation

Data collection

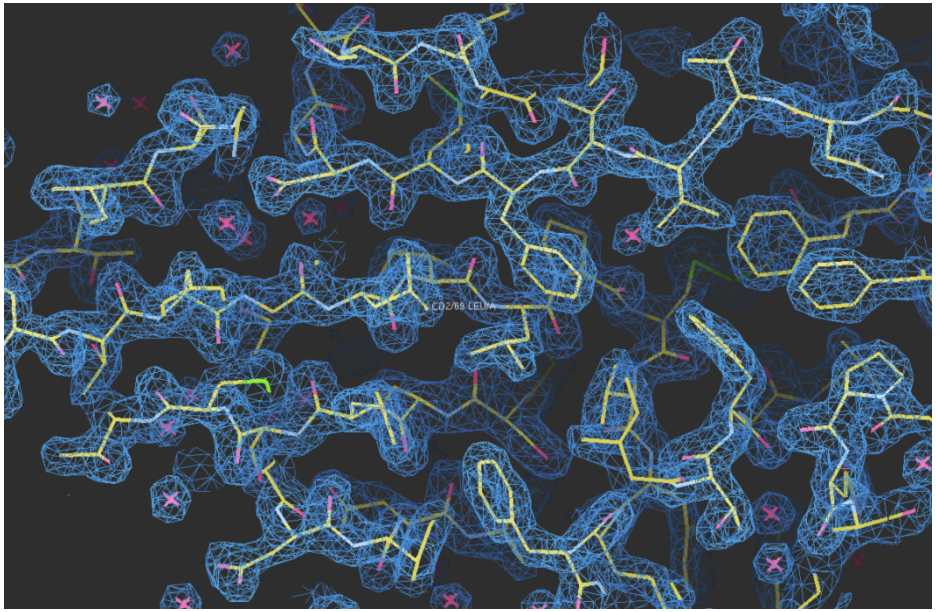
- Resoaked crystals at pH8 with excess PtCl4
- Collected data at SLS, at precise peak wavelength
- Optimal data collection



Wavelength	1.068 Å
Unit cell	a = 35.07, b = 68.99, c = 120.77 Å α = β = γ = 90°
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/δI	24.9 (5.6)
Mosaicity	0.47
No. Molec./A.U.	1
Solvent Content	40.00%



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

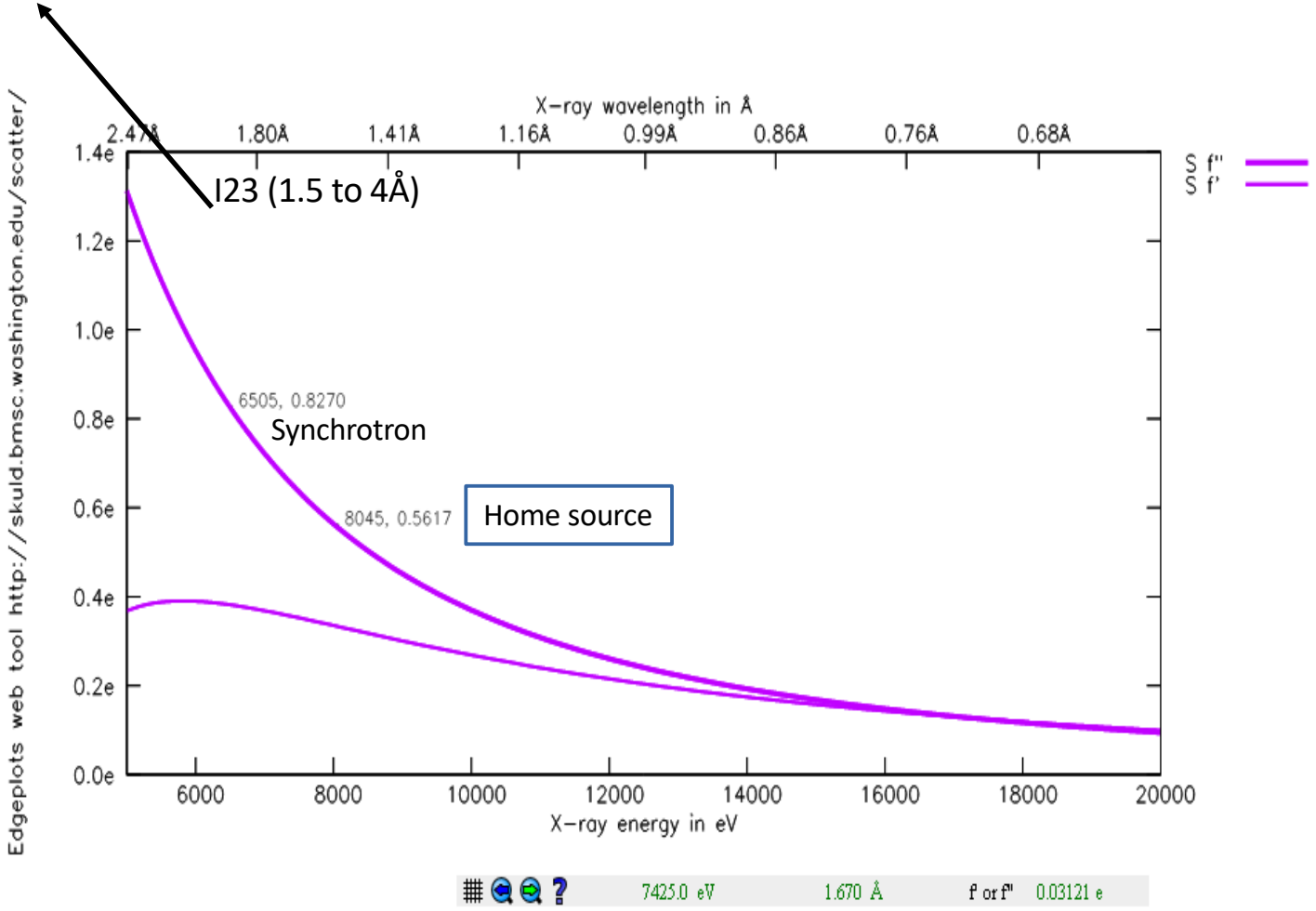


Salgado *et al*., 2011

S-SAD case study:

MGPVAGNYVHDFTANGTSS...
(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 (0244)	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/σI	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)**

A. Baslé, A. Luís, H. Gilbert
(ICaMB, Newcastle)

Anomalous signal?

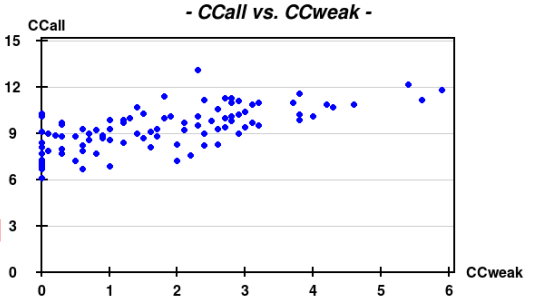
↓

Substructure determination

ShelxC/D

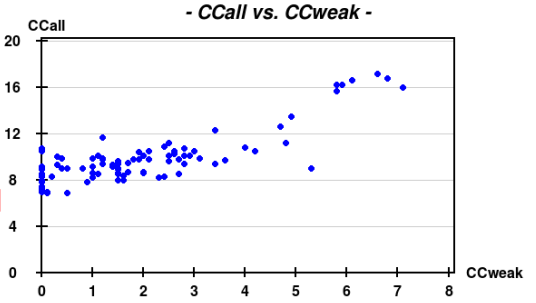
90°

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	



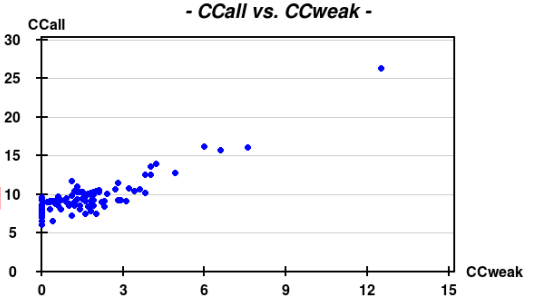
180°

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	



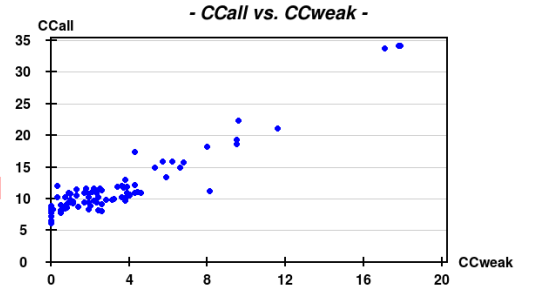
360°

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	

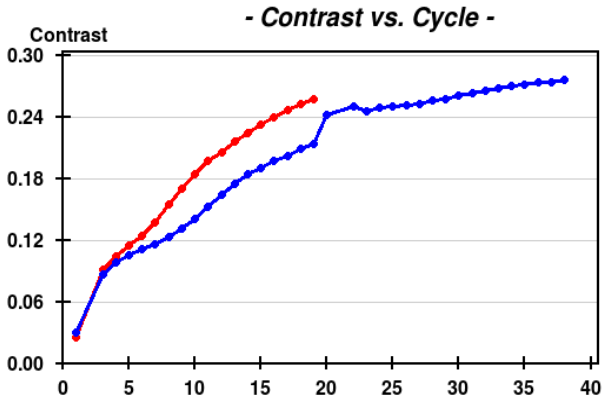


Phase calculation

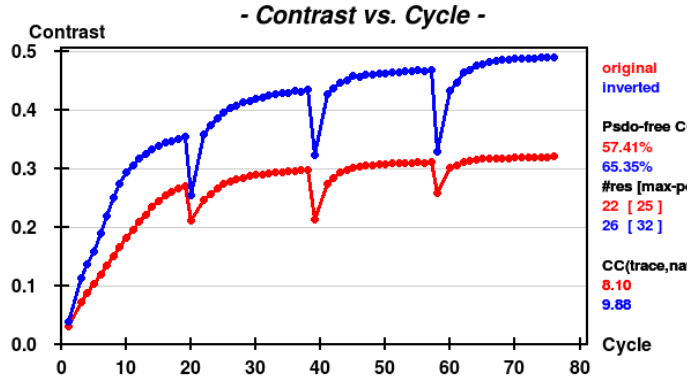
Density modification

ShelxE

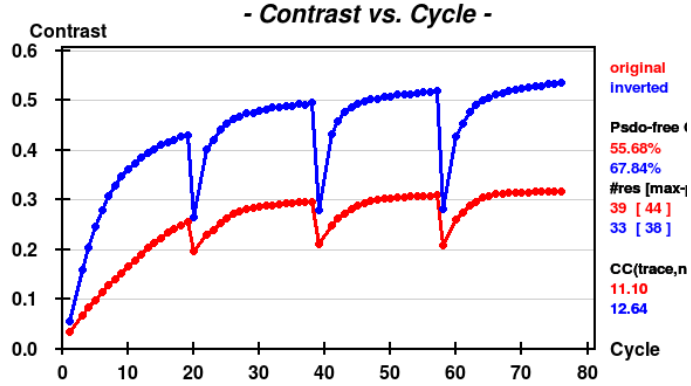
90°

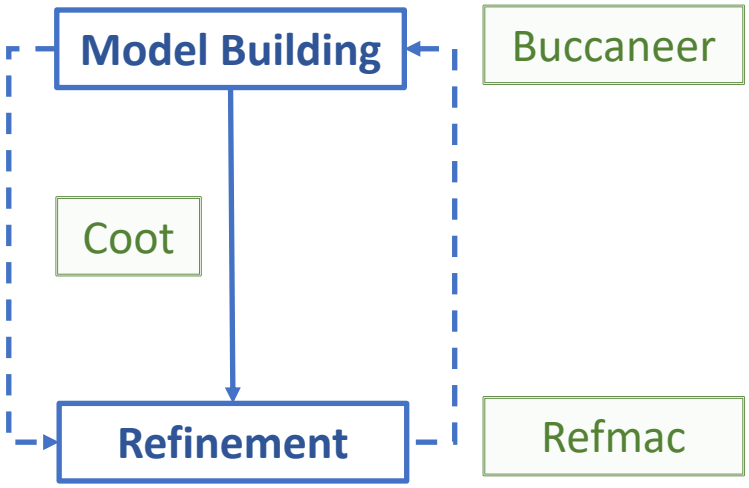


360°



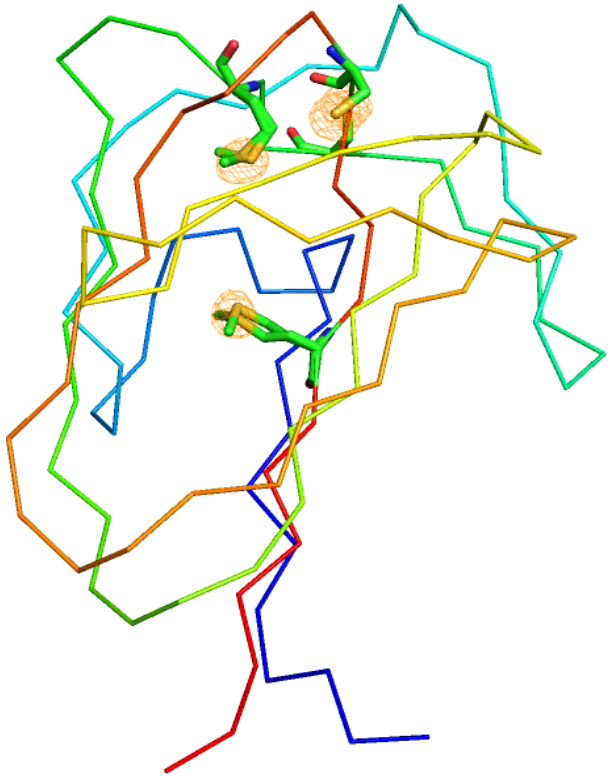
720°





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



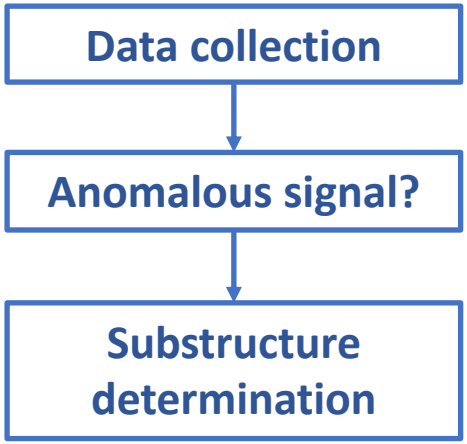
Soft X-ray SAD case study:

225 kDa protein asymmetric unit (three φ6 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, β = 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

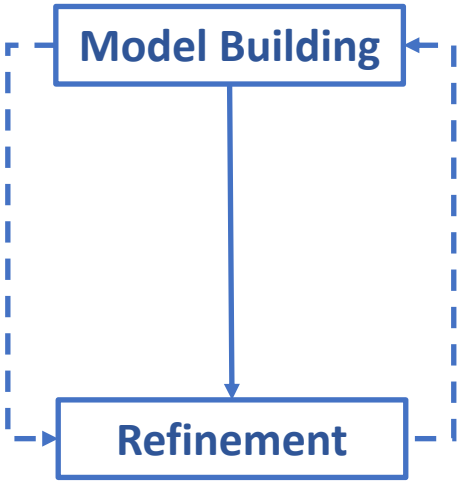


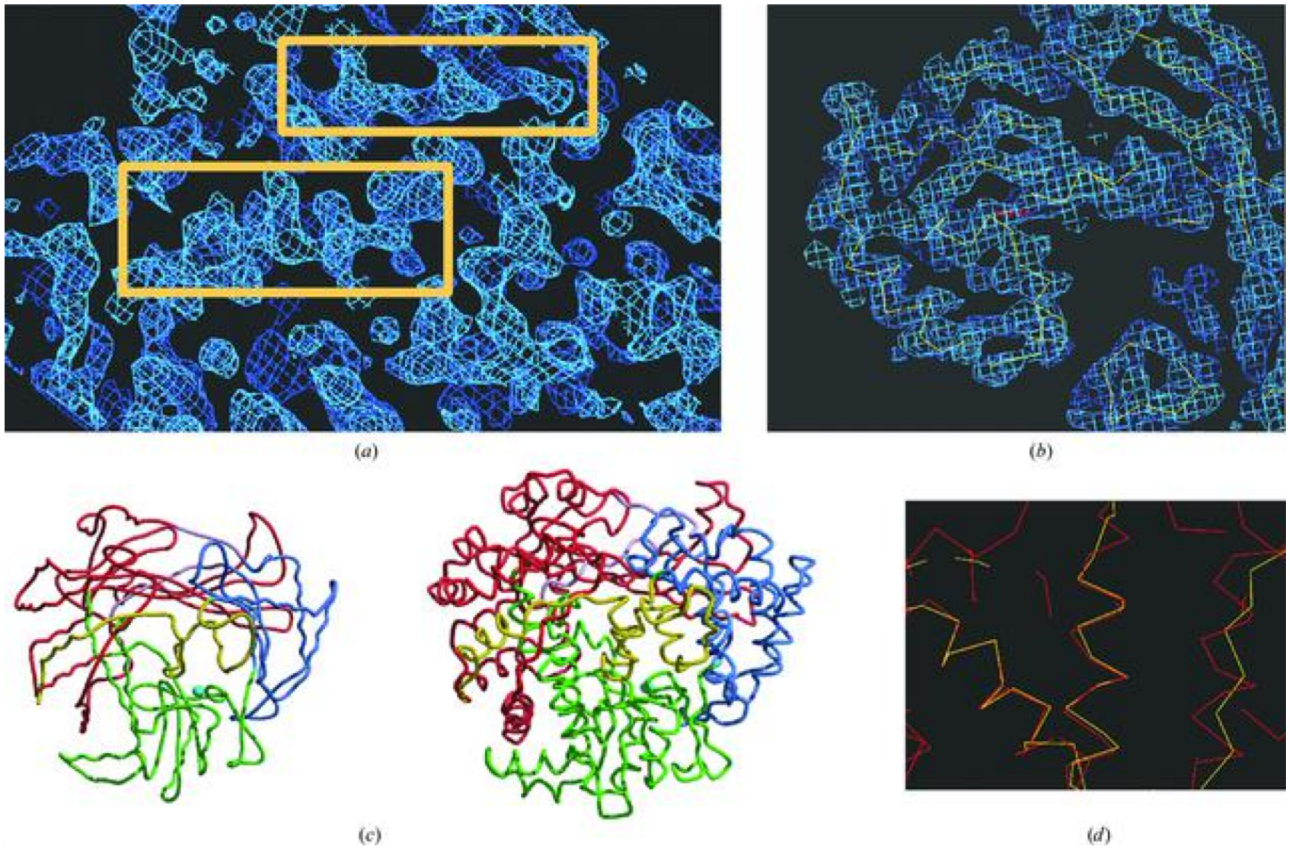
ShelxC/D/E
PhaserEP
(Auto)SHARP
Solve

Phase and map analyses.

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

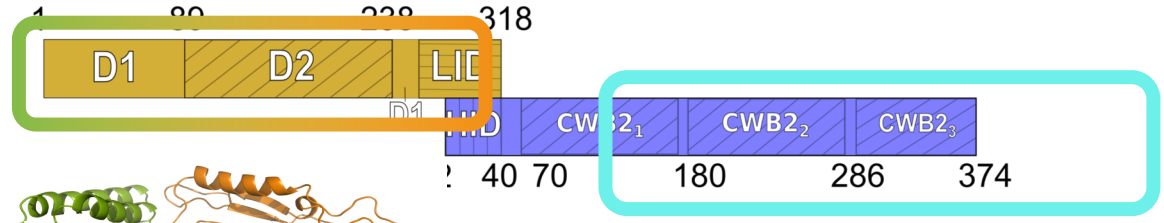
Soft X-ray SAD at Mn edge:

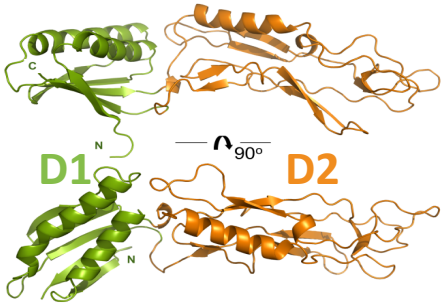




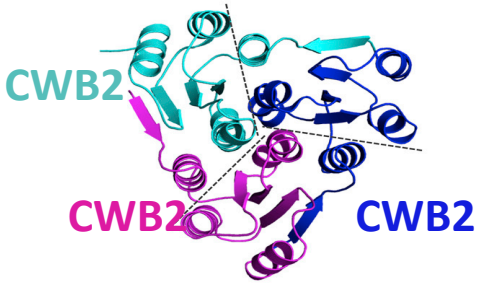
Salgado *et al*., 2005

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

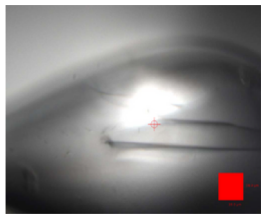


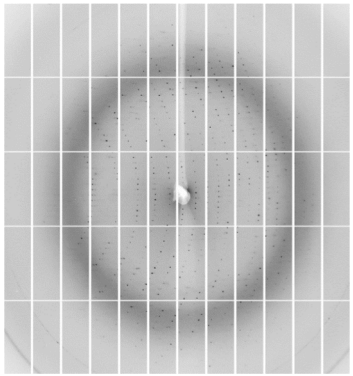


Fagan *et al.,* Mol Microbiol. 2009



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/sdI) 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.**

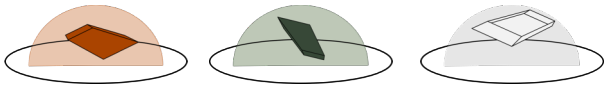
● **Warning: Completeness test shows some issues.**

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

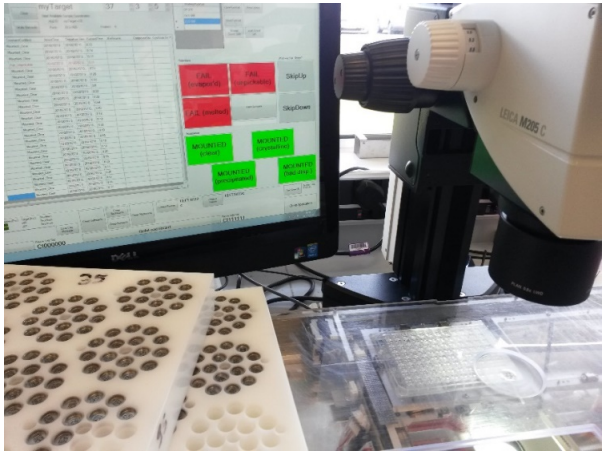
✓ **No ice rings found.**

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

Derivatisation



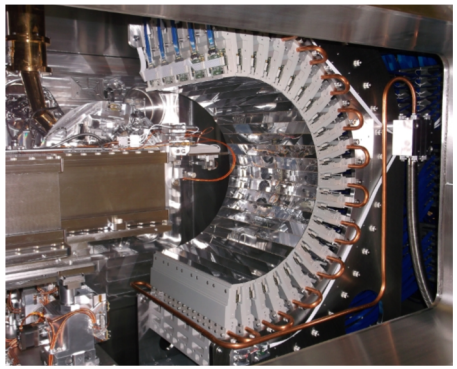


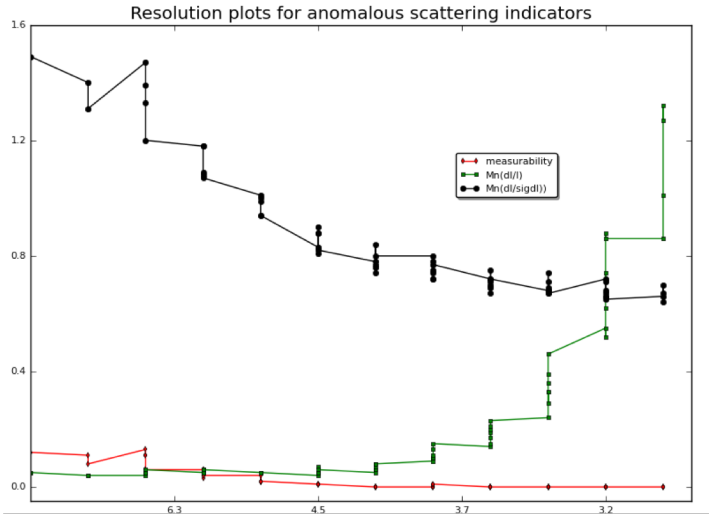


HA	Soaks	Mounted	Screened	Collected	Anom. res. Å (overall res.)	Best res. data Å
Au	30	25	18	2	5.8 (3.4)	3.4
Hg	15	11	7	4	3.7 (2.9)	2.9
Br	30	20	9	2	no	3.1
I	30	25	9	2	4.1 (3.3)	2.9
Pb	30	27	22	4	3.6 (2.8)	2.8
Pt	15	14	8	4	5.2 (3.3)	3.2
TaBr	15	14	12	7	5.9 (3.4)	2.9
W	15	14	8	1	no	
Sm	15	8	5	0	-	
Cd	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/sdI) 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 4.98A (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**

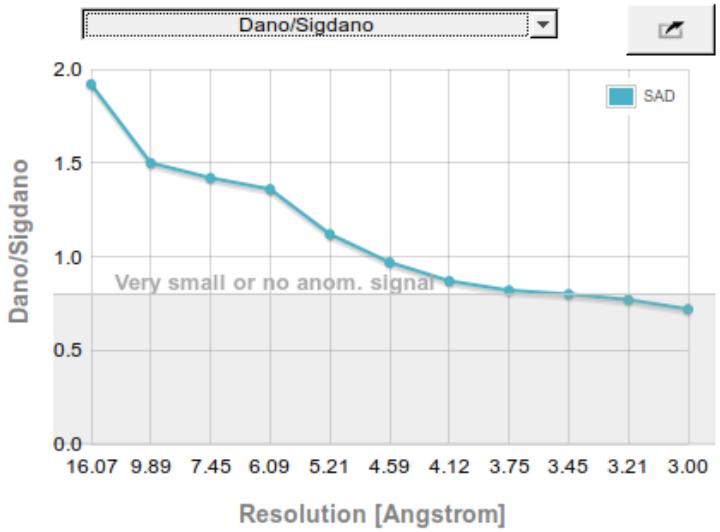
● **Warning: Some anisotropy detected. This may have an effect on statistics.**

● **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

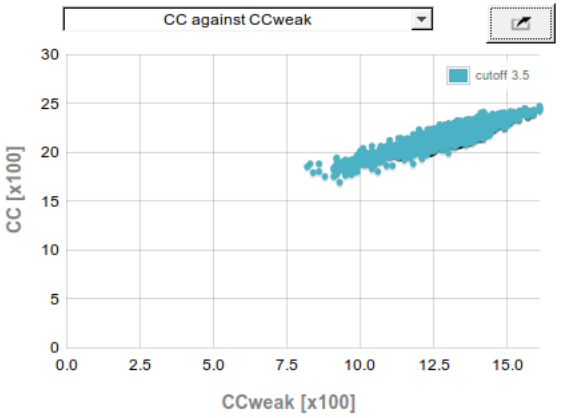
Stop:

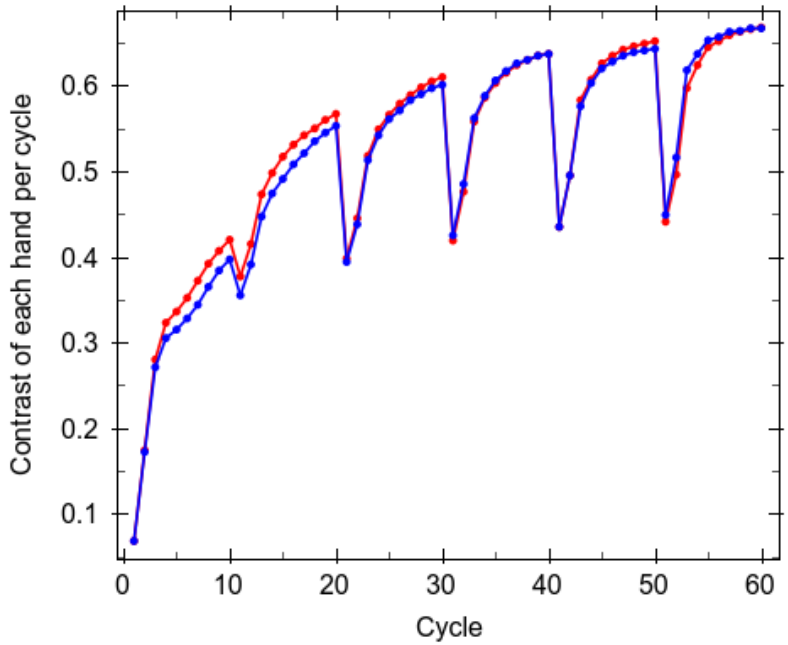
Maximum number of trials reached.

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:

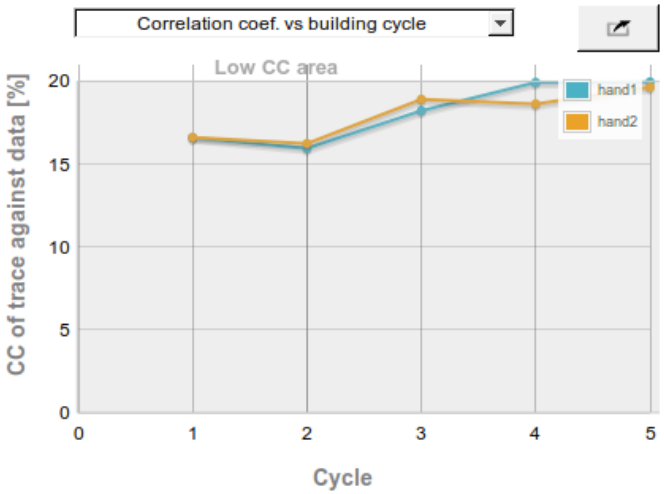
SHELXE

Result:

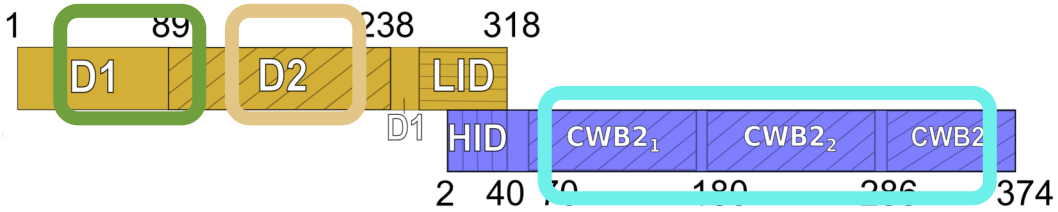
Maximum correlation coefficient for hand 1 is 19.96; max. correlation coef. for the other hand is 19.64. Hand 1 is chosen.

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

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

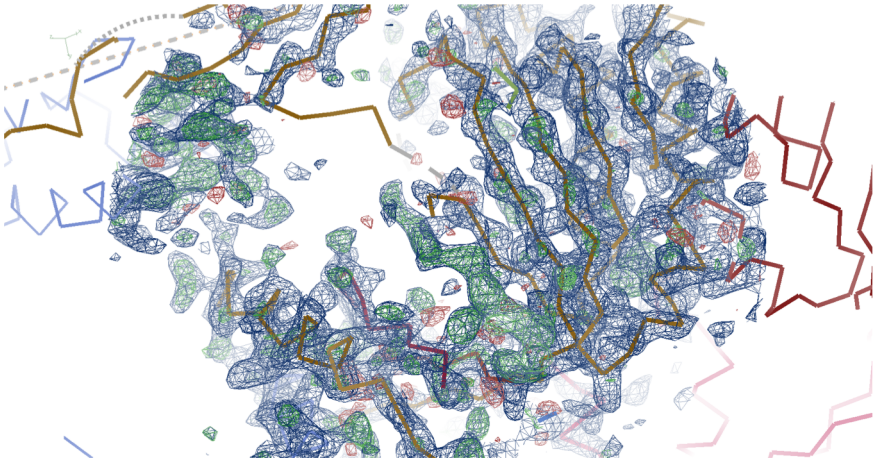
Please cite:

"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

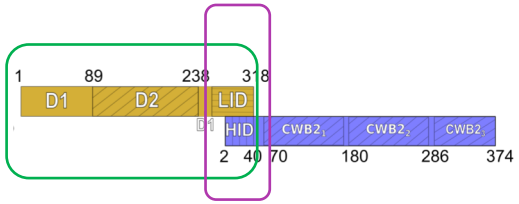
Result

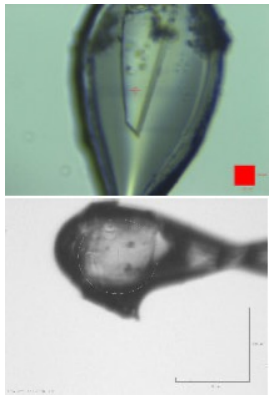
	Initial	Final
R factor	0.4568	0.4026
R free	0.4924	0.4458
Rms BondLength	0.0612	0.0115
Rms BondAngle	6.0762	1.9869
Rms ChirVolume	0.2428	0.1291

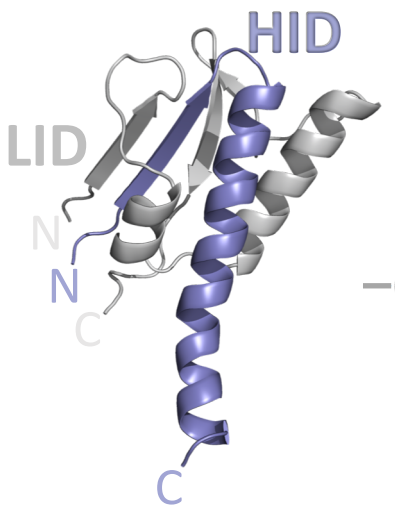


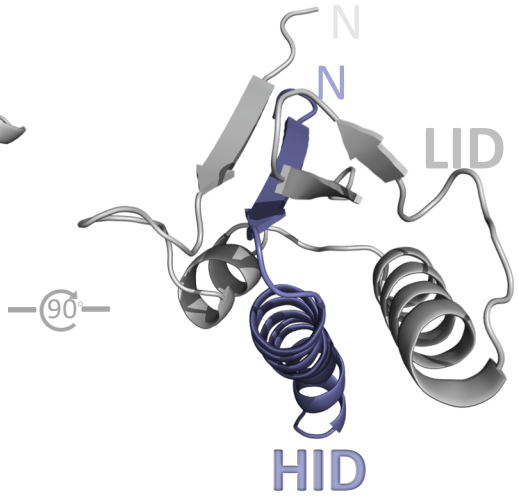


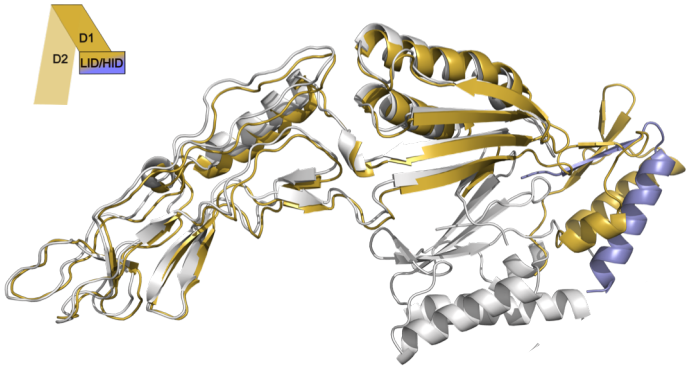
‘Divide & conquer’ – partial domains

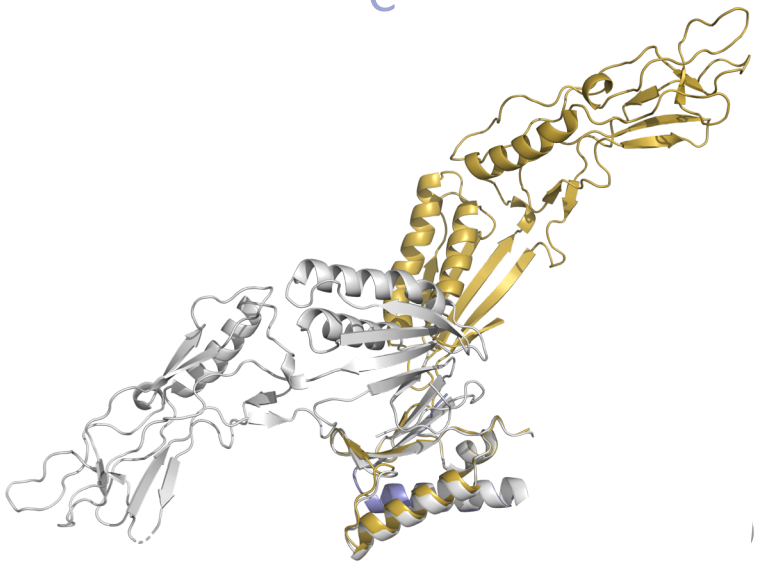




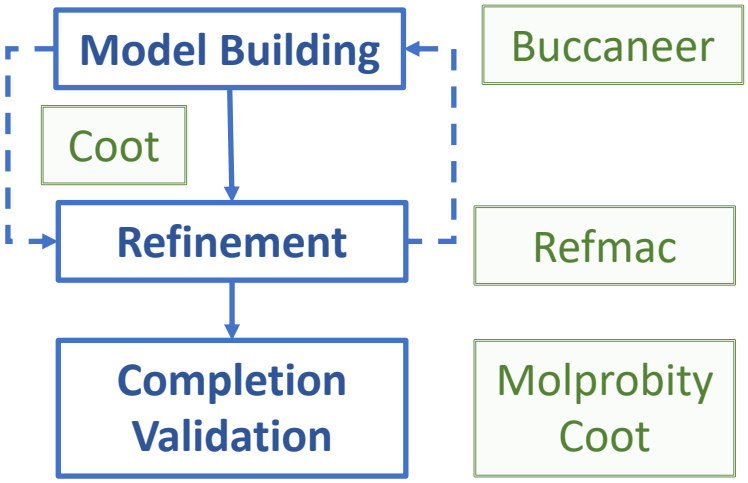


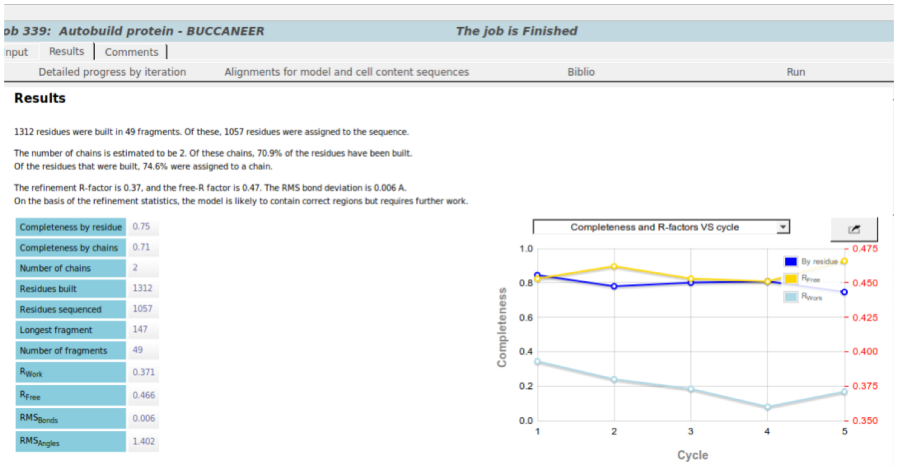


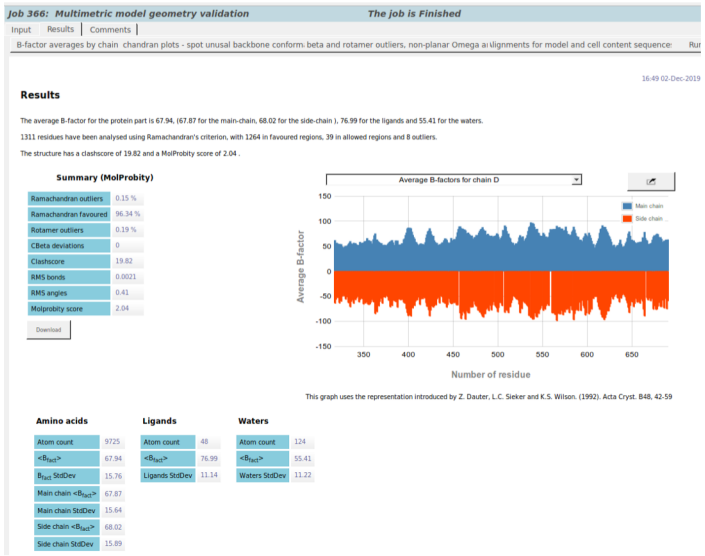


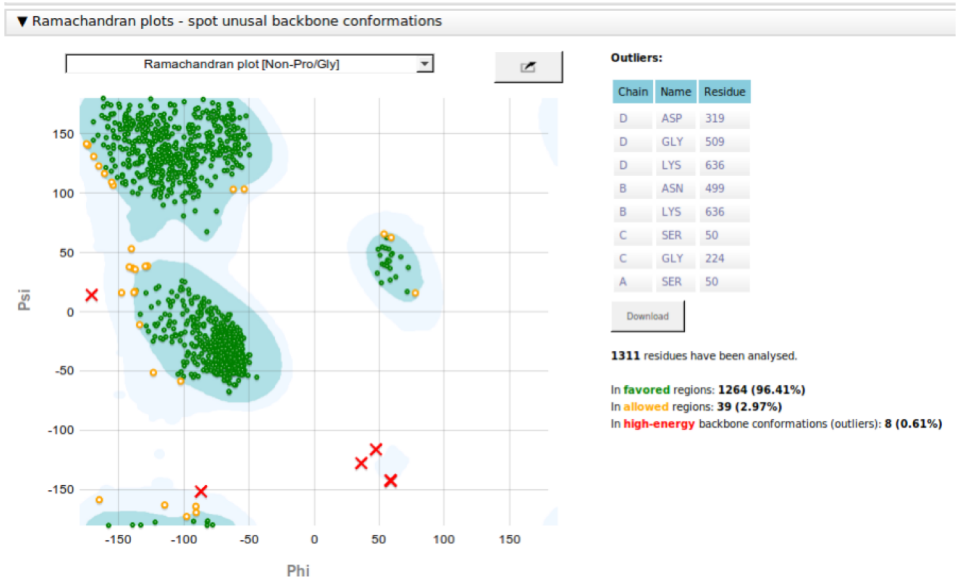


Anna Barwinska-Sendra









Paola Lanzoni-Mangutchi

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

HFSP 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

BBSRC bioscience for the future

BugSlayers Consortium

Adam Crawshaw
Anna Barwinska-Sendra
Paola Lanzoni
Abbie Kelly
Gilly Wang
Zoe Wilkinson
Marcin Dembek
Emma Tarrant







Arnaud Baslé

Kamel El Omari
Armin Wagner



Gill Douce
Filipa Vaz





Rob Fagan
Joe Kirk
Per Bullough
Oishik Banerji
Jason Wilson







Neil Fairweather