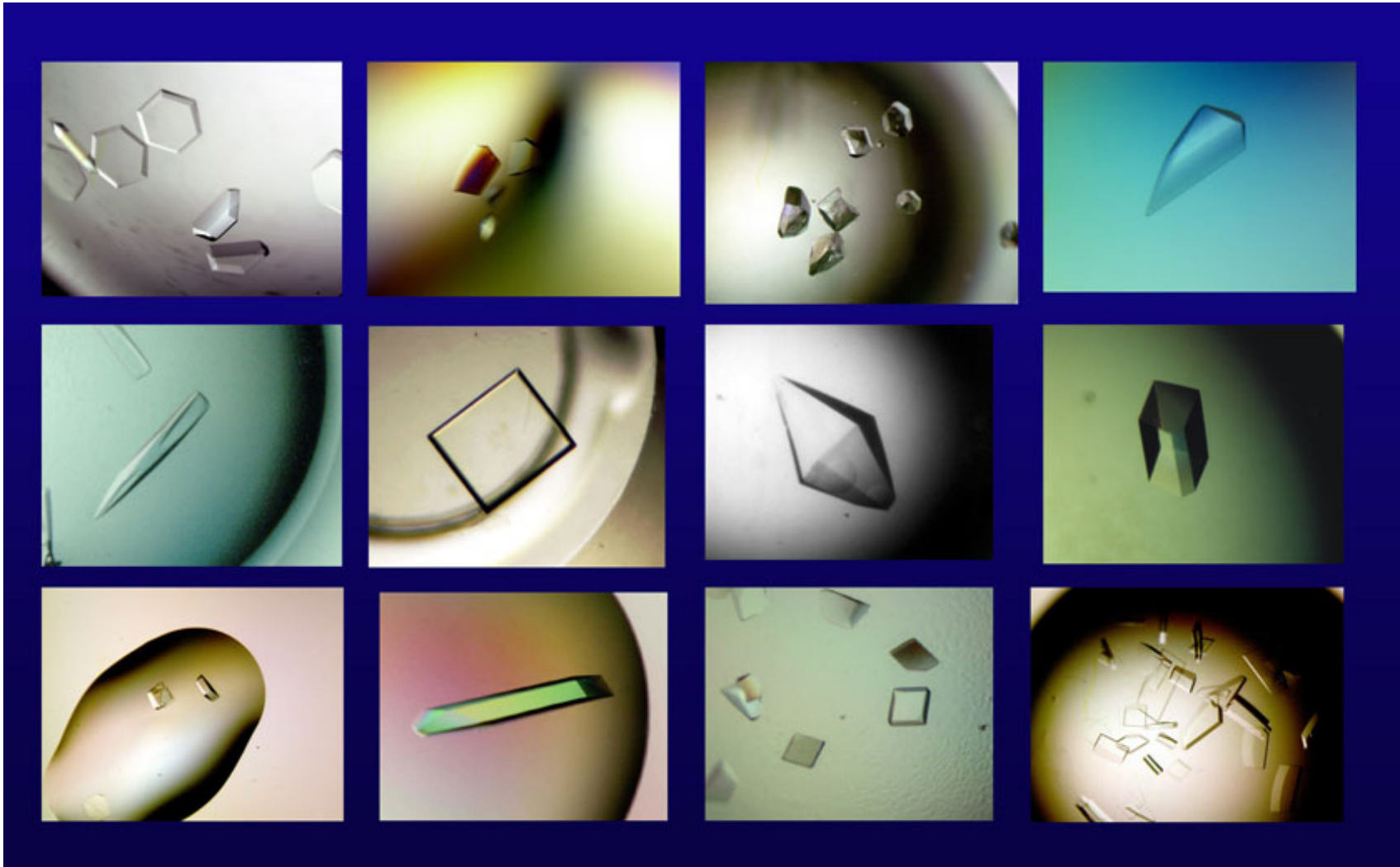
Check your spacegroup

Andrey Lebedev, CCP4



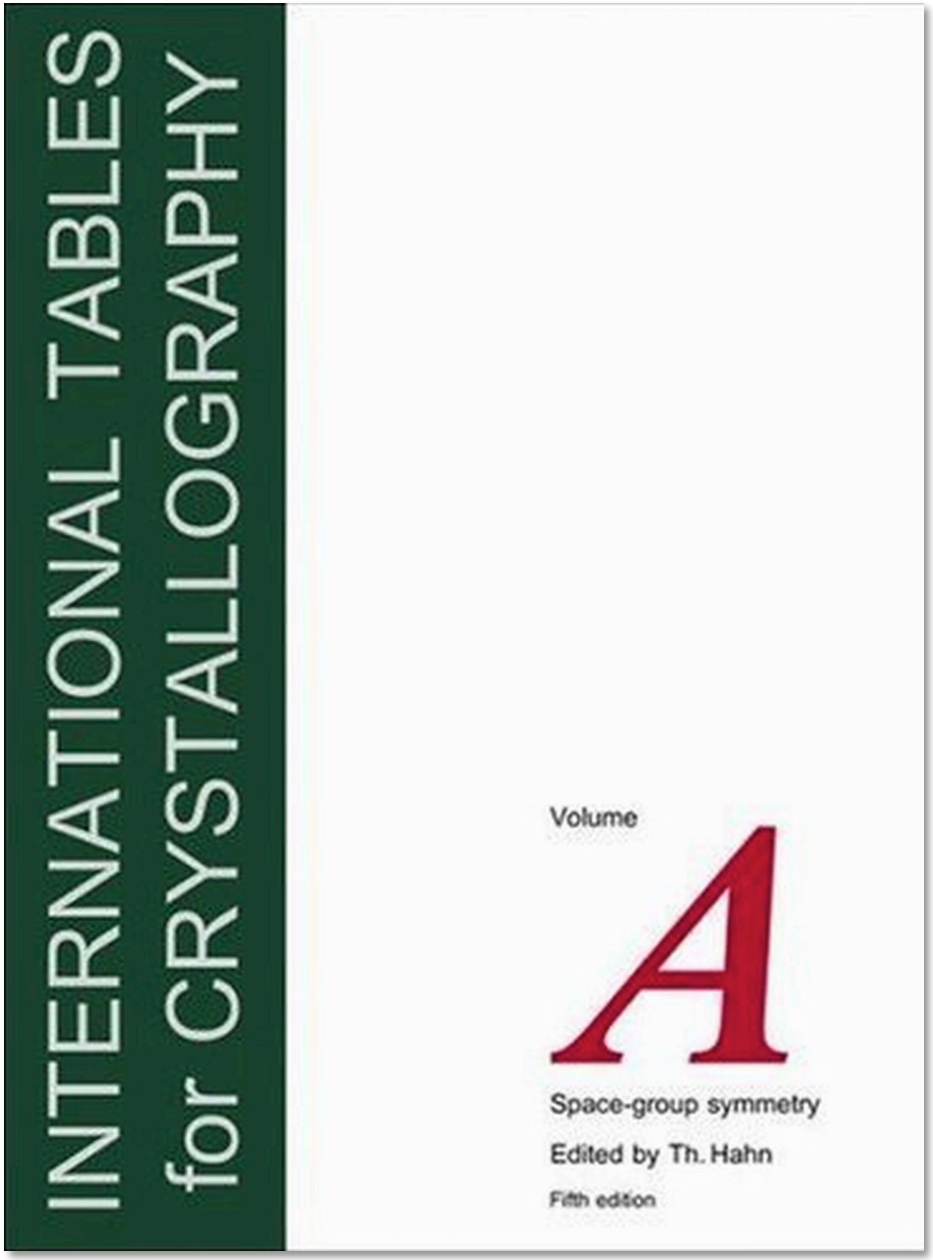
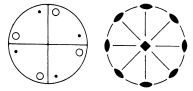


Table 10.1.2.2. *The 32 three-dimensional crystallographic point groups* (*cont.*)

TETRAGONAL SYSTEM (*cont.*)

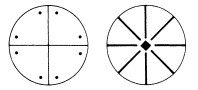


422 D_4

8	*d*	1	Tetragonal trapezohedron *Twisted tetragonal antiprism* (*p*)	(hkl) $(\bar{h}\bar{k}l)$ $(\bar{k}hl)$ $(k\bar{h}l)$ $(h\bar{k}\bar{l})$ $(\bar{h}kl)$ $(\bar{k}\bar{h}\bar{l})$ (khl)
			Ditetragonal prism *Truncated square through origin*	$(hk0)$ $(\bar{h}\bar{k}0)$ $(\bar{k}h0)$ $(k\bar{h}0)$ $(h\bar{k}0)$ $(\bar{h}k0)$ $(kh0)$ $(\bar{k}\bar{h}0)$
			Tetragonal dipyramid *Tetragonal prism*	$(h0l)$ $(\bar{h}0l)$ $(0hl)$ $(0\bar{h}l)$ $(h0\bar{l})$ $(\bar{h}0\bar{l})$ $(0h\bar{l})$ $(0\bar{h}\bar{l})$
			Tetragonal dipyramid *Tetragonal prism*	(hhl) $(\bar{h}\bar{h}l)$ $(\bar{h}hl)$ $(h\bar{h}l)$ $(hh\bar{l})$ $(\bar{h}\bar{h}\bar{l})$ $(\bar{h}h\bar{l})$ $(h\bar{h}\bar{l})$
4	*c*	.2.	Tetragonal prism *Square through origin* (*l*)	(100) $(\bar{1}00)$ (010) $(0\bar{1}0)$
4	*b*	..2	Tetragonal prism *Square through origin* (*j*)	(110) $(\bar{1}\bar{1}0)$ $(\bar{1}10)$ $(1\bar{1}0)$
2	*a*	4..	Pinacoid or parallelohedron *Line segment through origin* (*g*)	(001) $(00\bar{1})$

Symmetry of special projections

Along [001]	Along [100]	Along [110]
4*mm*	2*mm*	2*mm*



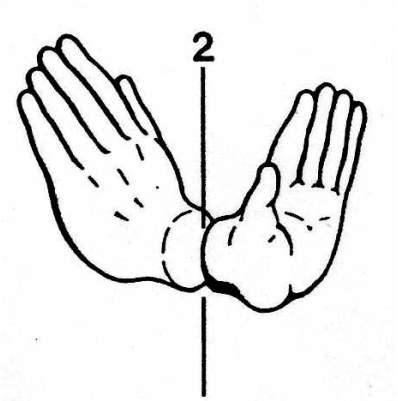
4*mm* C_{4v}

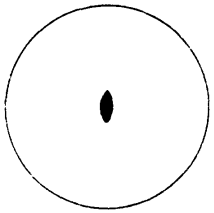
8	*d*	1	Ditetragonal pyramid *Truncated square* (*g*)	(hkl) $(\bar{h}\bar{k}l)$ $(\bar{k}hl)$ $(k\bar{h}l)$ $(h\bar{k}l)$ $(\bar{h}kl)$ $(\bar{k}\bar{h}l)$ (khl)
			Ditetragonal prism *Truncated square through origin*	$(hk0)$ $(\bar{h}\bar{k}0)$ $(\bar{k}h0)$ $(k\bar{h}0)$ $(h\bar{k}0)$ $(\bar{h}k0)$ $(\bar{k}\bar{h}0)$ $(kh0)$
4	*c*	.*m*.	Tetragonal pyramid *Square* (*e*)	$(h0l)$ $(\bar{h}0l)$ $(0hl)$ $(0\bar{h}l)$
			Tetragonal prism *Square through origin*	(100) $(\bar{1}00)$ (010) $(0\bar{1}0)$
4	*b*	..*m*	Tetragonal pyramid *Square* (*d*)	(hhl) $(\bar{h}\bar{h}l)$ $(\bar{h}hl)$ $(h\bar{h}l)$
			Tetragonal prism *Square through origin*	(110) $(\bar{1}\bar{1}0)$ $(\bar{1}10)$ $(1\bar{1}0)$
1	*a*	4*mm*	Pedion or monohedron *Single point* (*a*)	(001) or $(00\bar{1})$

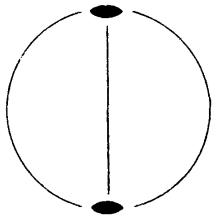
Symmetry of special projections

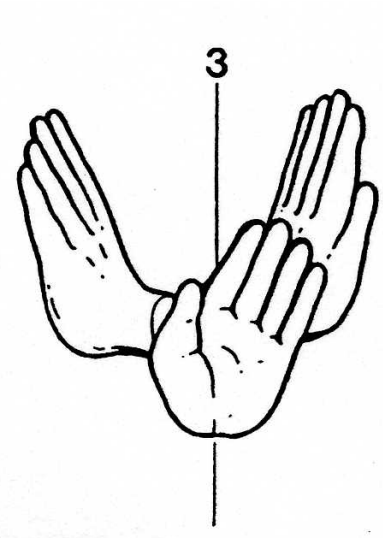
Along [001]	Along [100]	Along [110]
4*mm*	*m*	*m*

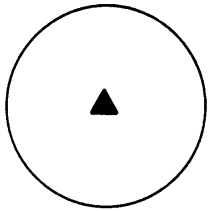
Point group symmetry (ignoring any translations)

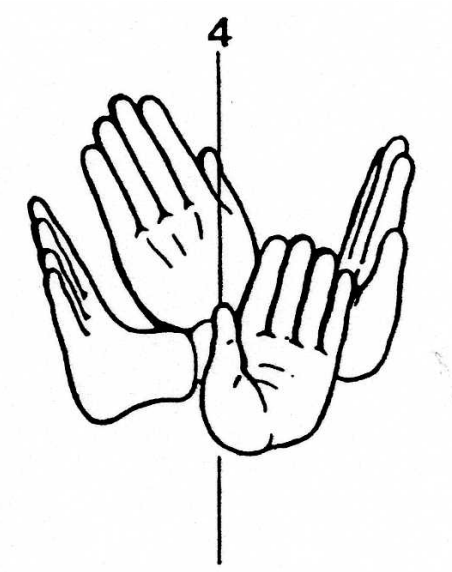


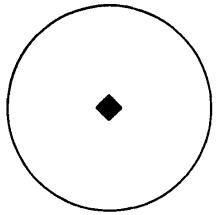


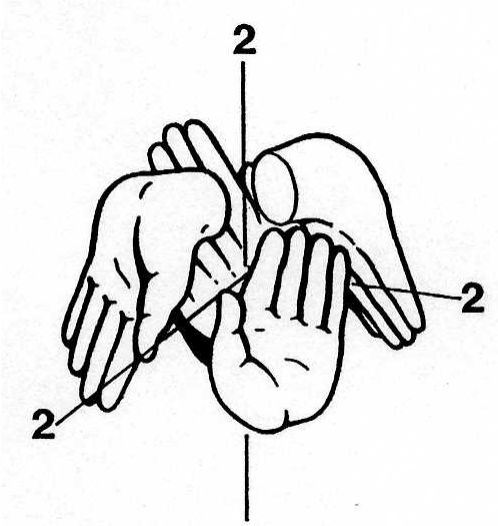


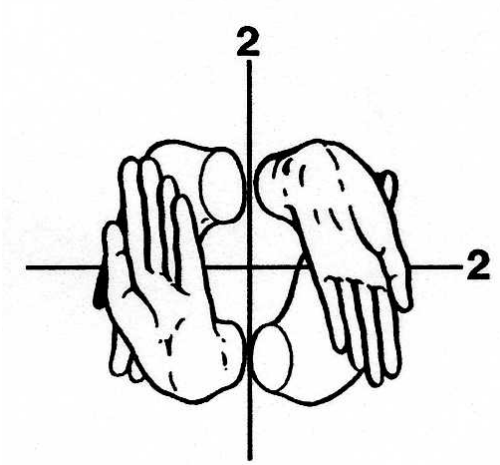


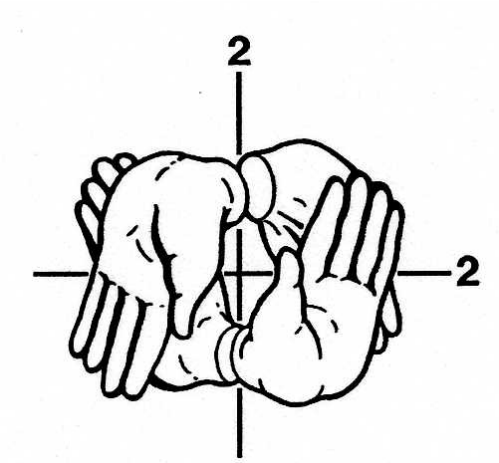


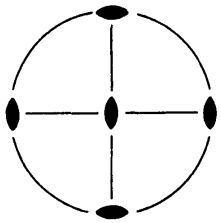




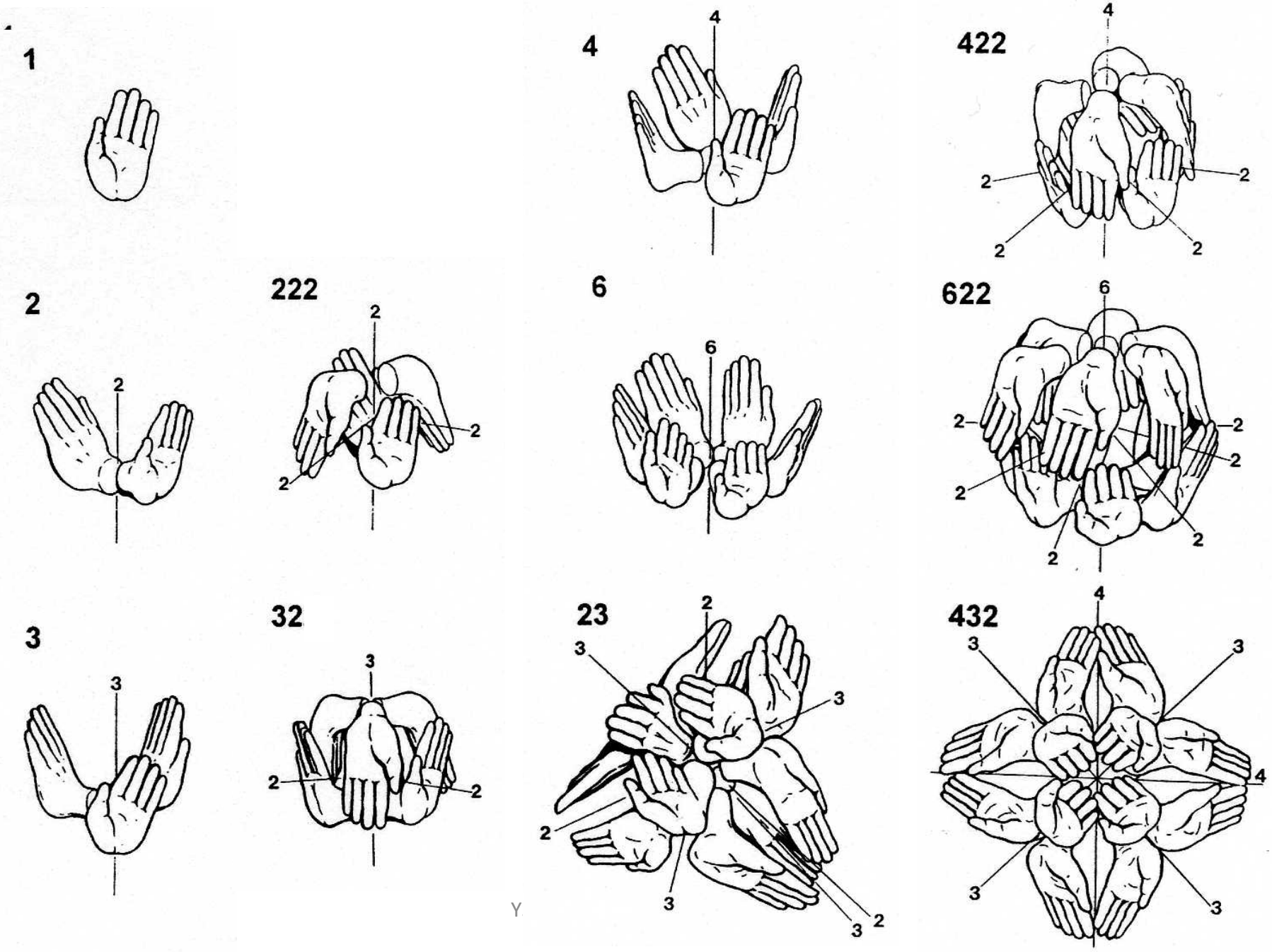

2
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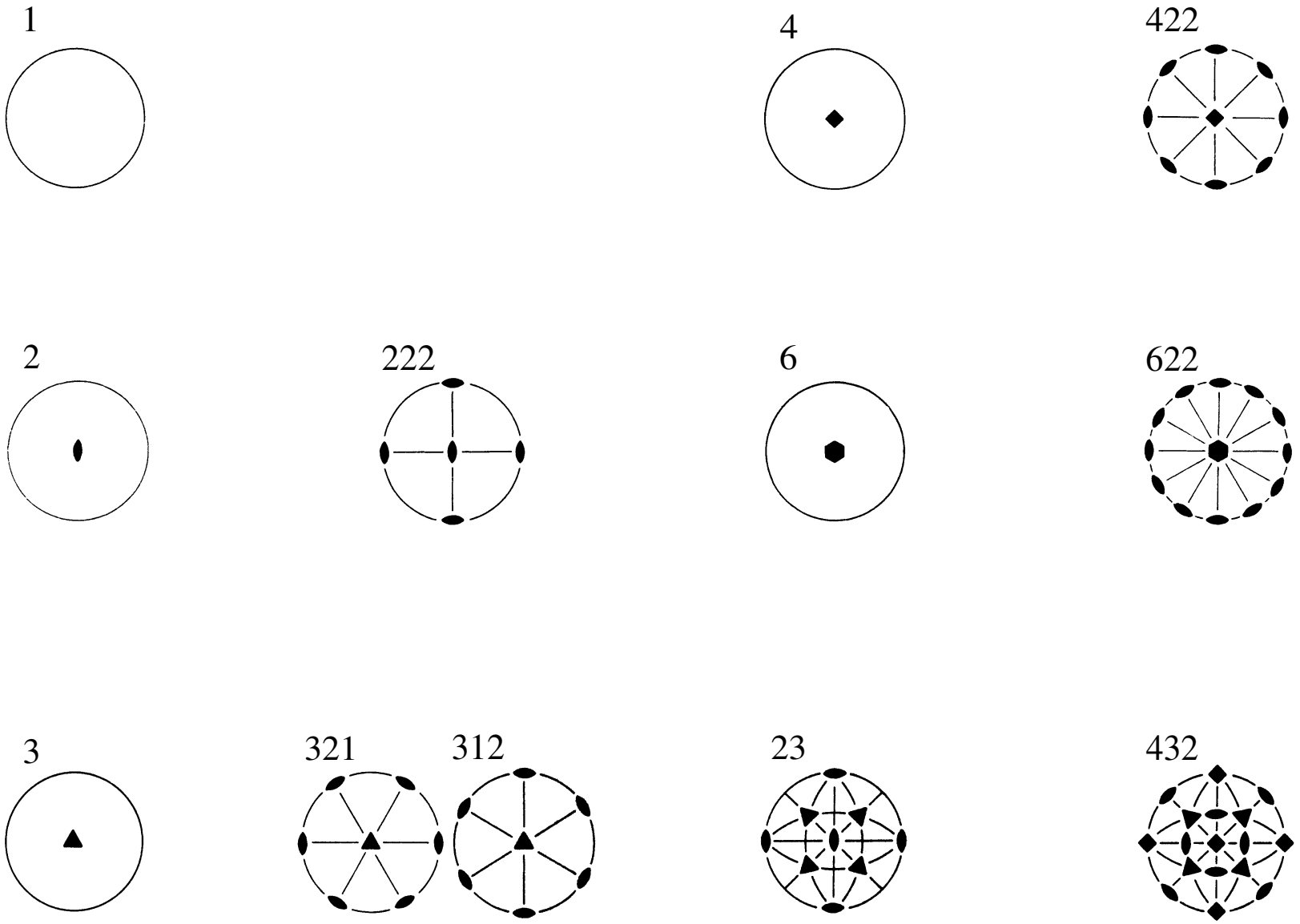

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2



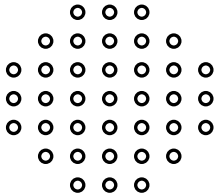
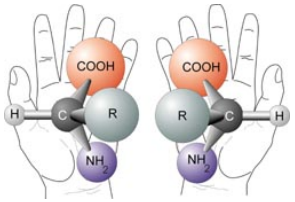
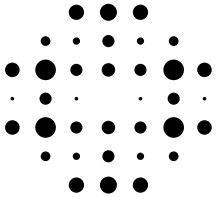
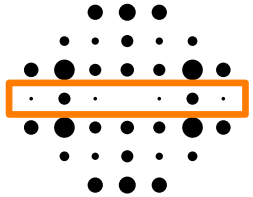
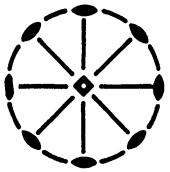
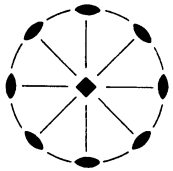
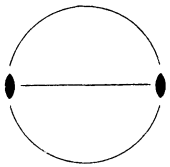
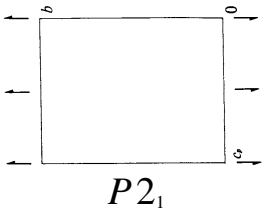
The point groups that can exist in protein crystals



The point groups that can exist in protein crystals

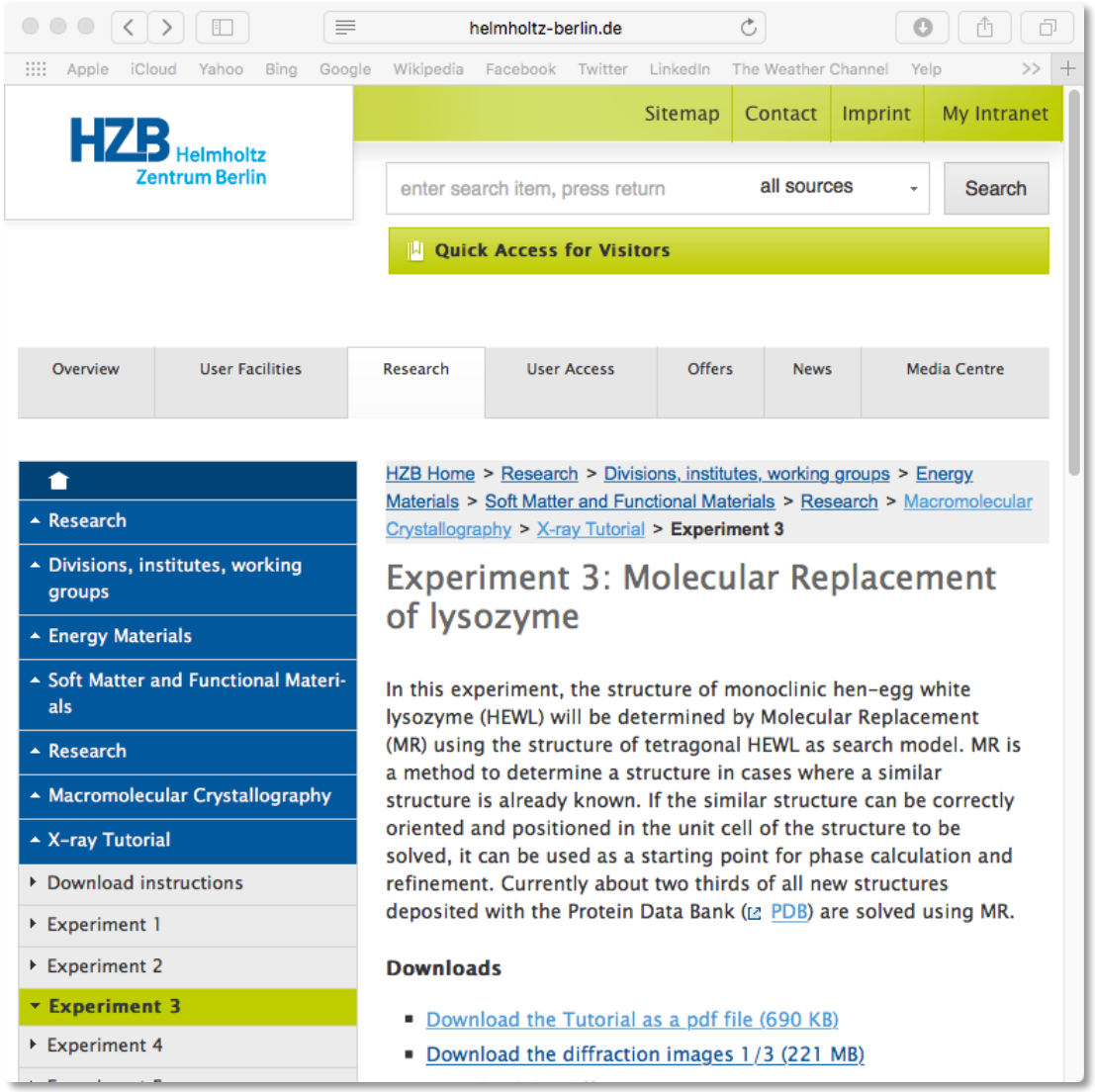


Space group assignment (*e.g. Pointless*)

Reciprocal space lattice (positions of reflections)	Mirror symmetry is <u>not</u> allowed in biological macromolecules	Intensities of reflections	Intensities of axial reflections
			
 $4/mmm$	 422	 2	 $P2_1$
(Approximate) lattice point group	Highest possible crystal point group	Probable crystal point group	Probable crystal space group

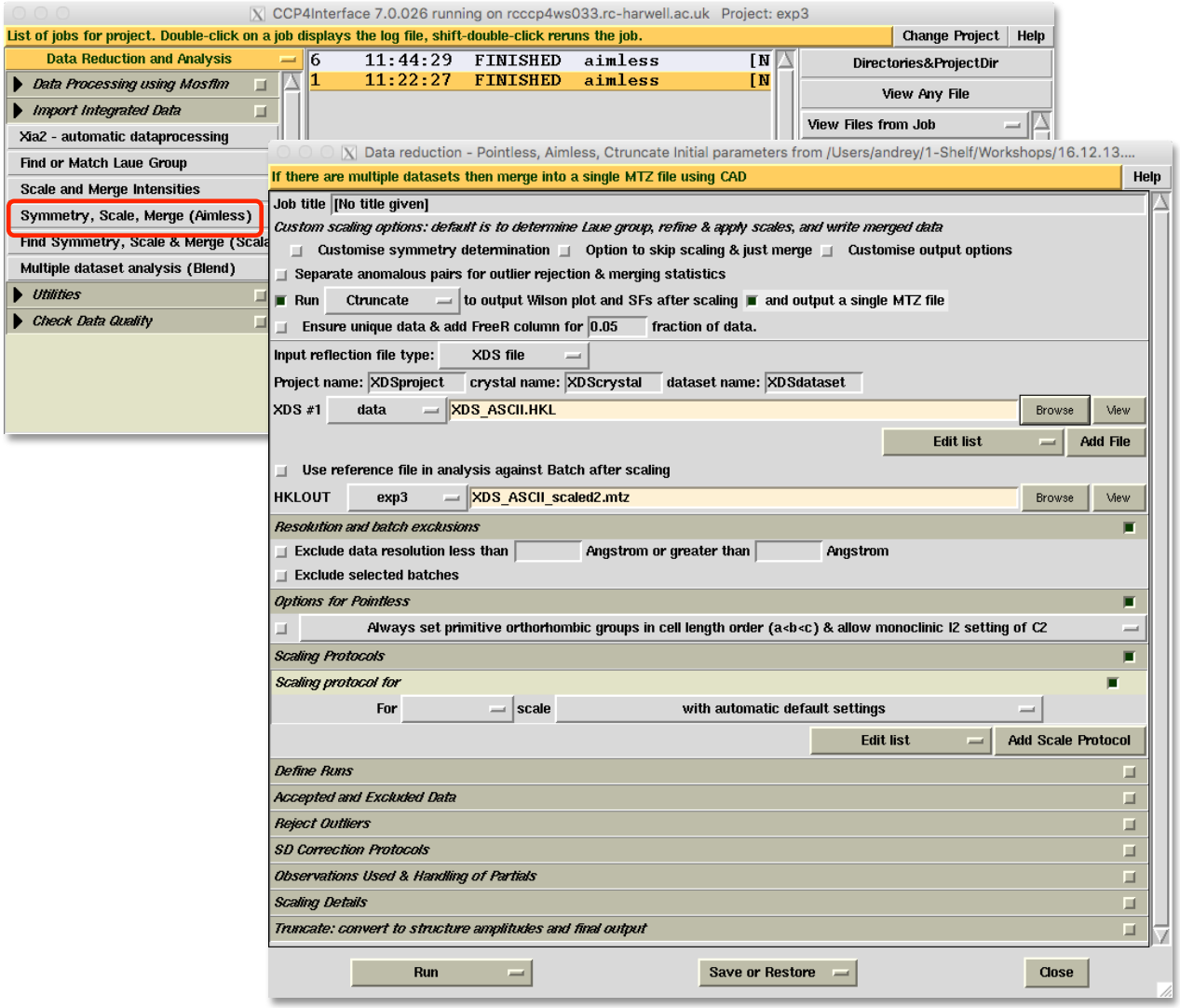
User: decision making

Example


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Apple iCloud Yahoo Bing Google Wikipedia Facebook Twitter LinkedIn The Weather Channel Yelp
HZB Helmholtz Zentrum Berlin
Sitemap Contact Imprint My Intranet
enter search item, press return
all sources
Search
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Overview User Facilities Research User Access Offers News Media Centre
Research
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Experiment 1
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HZB Home > Research > Divisions, institutes, working groups > Energy Materials > Soft Matter and Functional Materials > Research > Macromolecular Crystallography > X-ray Tutorial > Experiment 3
Experiment 3: Molecular Replacement of lysozyme
In this experiment, the structure of monoclinic hen-egg white lysozyme (HEWL) will be determined by Molecular Replacement (MR) using the structure of tetragonal HEWL as search model. MR is a method to determine a structure in cases where a similar structure is already known. If the similar structure can be correctly oriented and positioned in the unit cell of the structure to be solved, it can be used as a starting point for phase calculation and refinement. Currently about two thirds of all new structures deposited with the Protein Data Bank (PDB) are solved using MR.
Downloads
Download the Tutorial as a pdf file (690 KB)
Download the diffraction images 1/3 (221 MB)

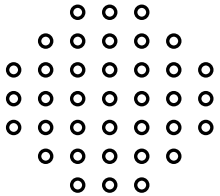
CCP4I

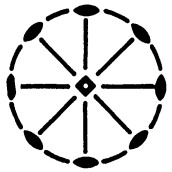
Aimless



Space group assignment (*e.g. Pointless*)

Reciprocal space lattice (positions of reflections)

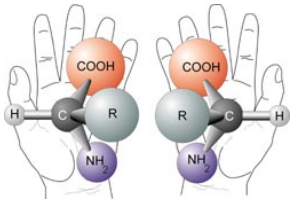


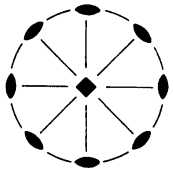


$4/mmm$

(Approximate) lattice point group

Mirror symmetry is <u>not</u> allowed in biological macromolecules

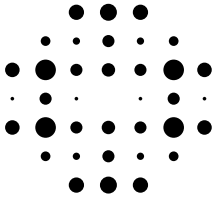


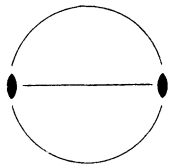


422

Highest possible crystal point group

Intensities of reflections

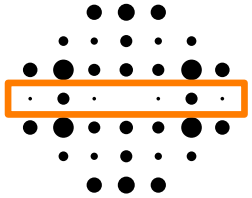


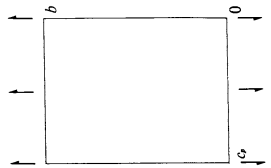


2

Probable crystal point group

Intensities of axial reflections





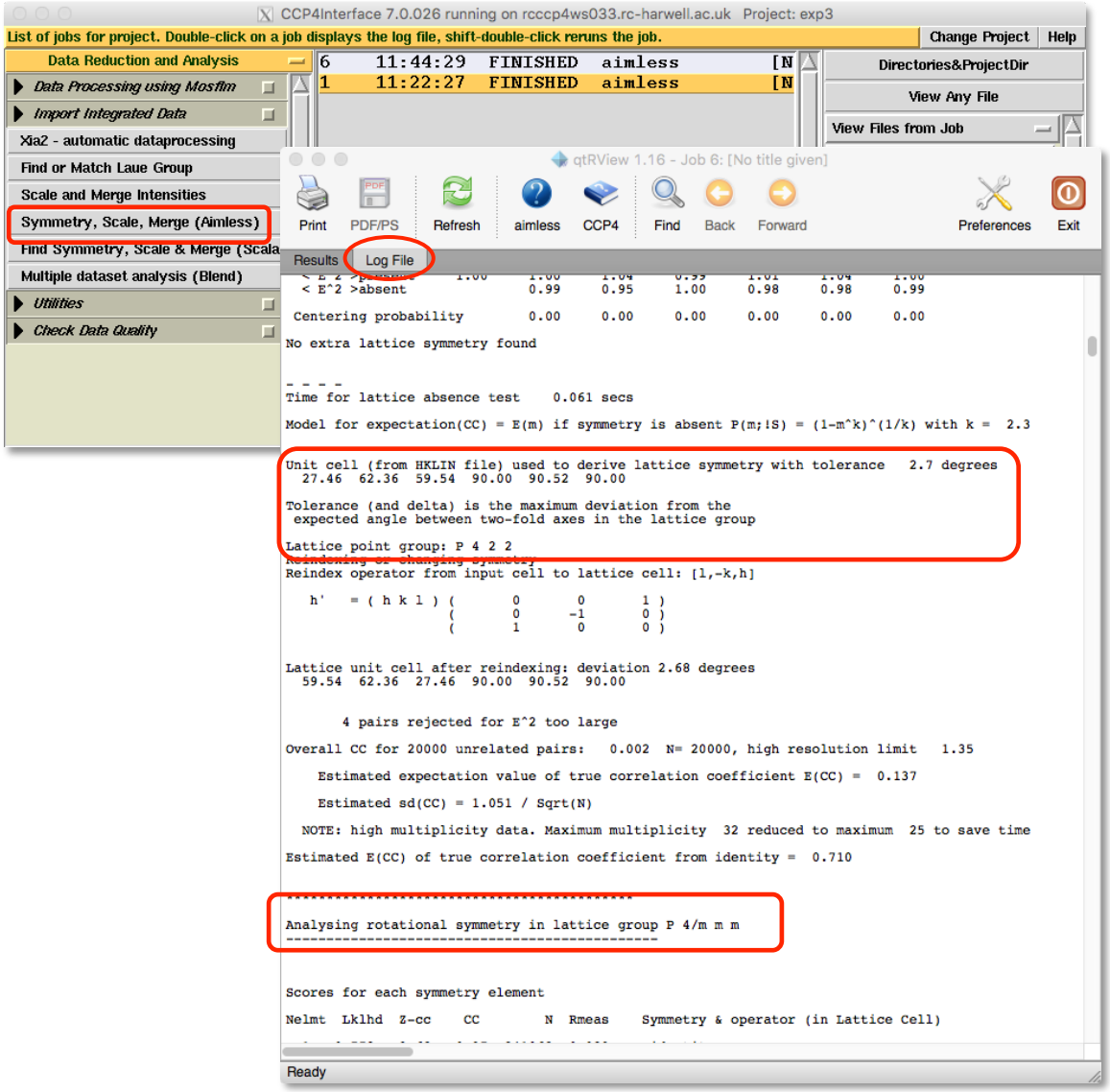
$P2_1$

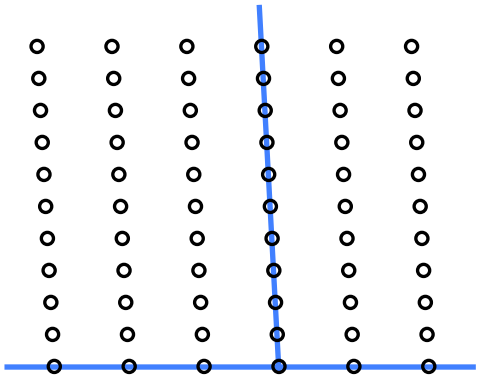
Probable crystal space group

User: decision making

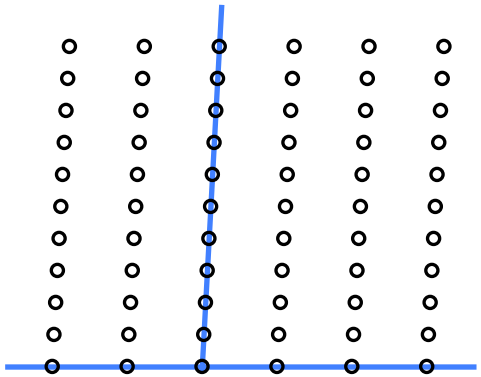
CCP4I

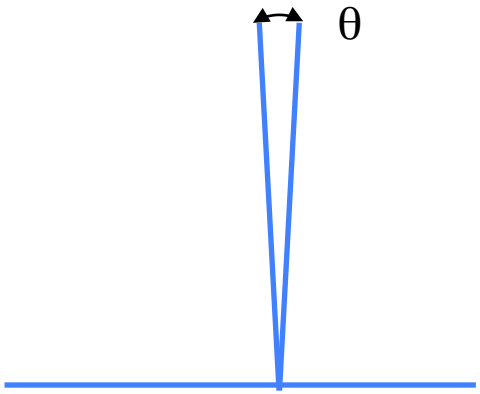
Aimless







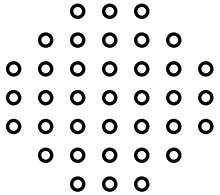


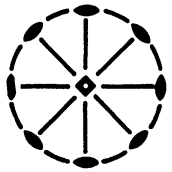


Geometrical score for lattice point group operations

Space group assignment (*e.g. Pointless*)

Reciprocal space lattice (positions of reflections)

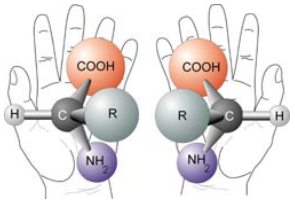


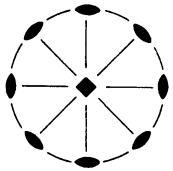


$4/mmm$

(Approximate) lattice point group

Mirror symmetry is <u>not</u> allowed in biological macromolecules

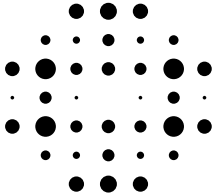


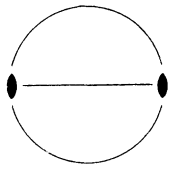


422

Highest possible crystal point group

Intensities of reflections

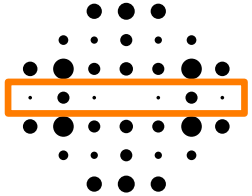


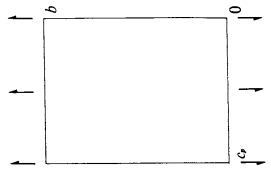


2

Probable crystal point group

Intensities of axial reflections





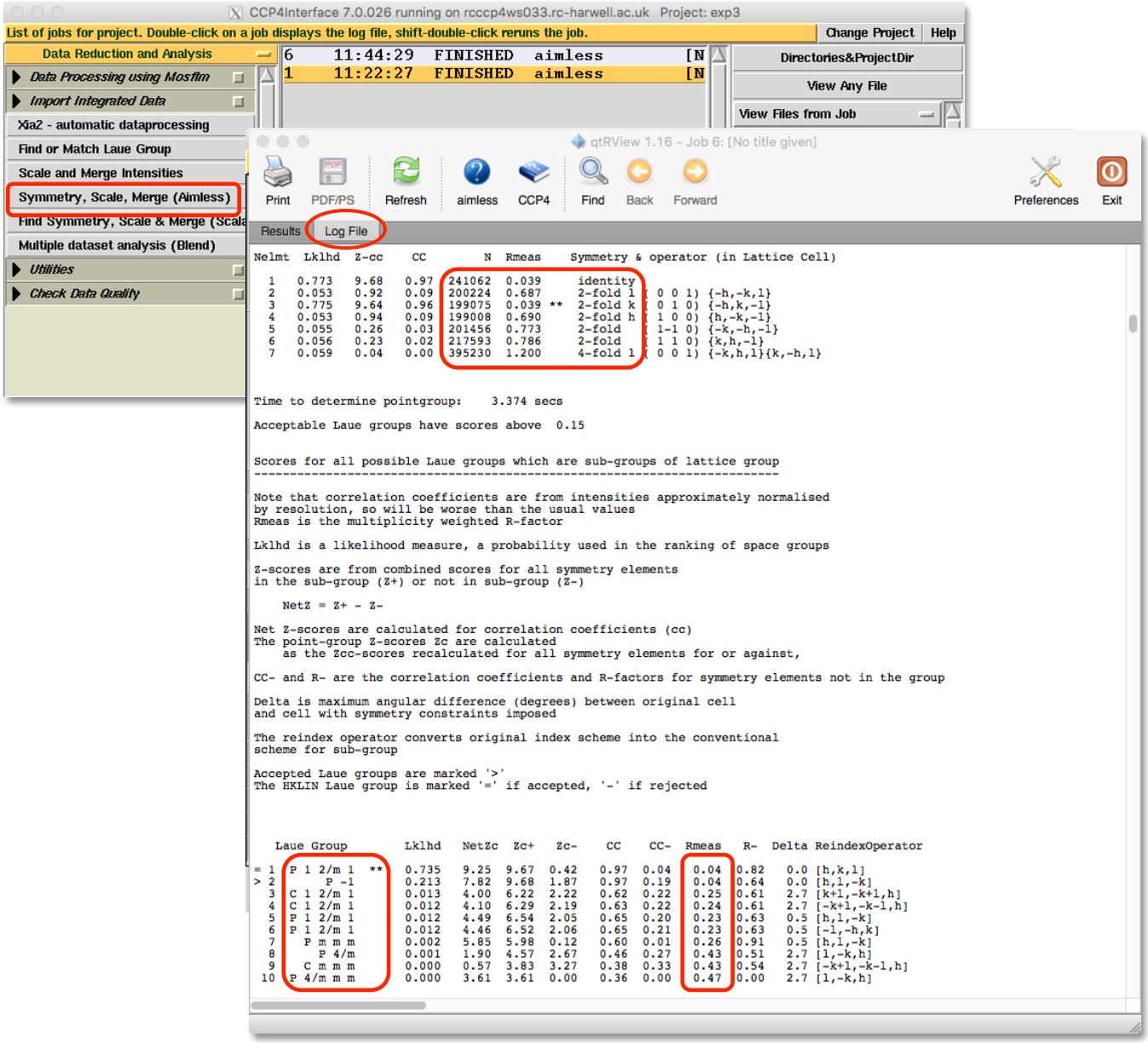
$P2_1$

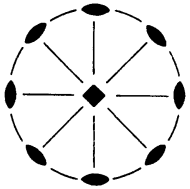
Probable crystal space group

User: decision making

CCP4I

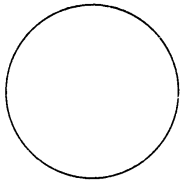
Aimless





422

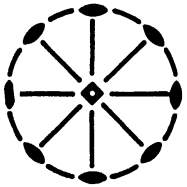
Rmeas		Symm operation
0.039		identity
0.687		2-fold l
0.039	**	2-fold k
0.690		2-fold h
0.773		2-fold
0.786		2-fold
1.200		4-fold l



1

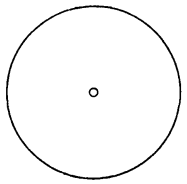
+ inversion

+ inversion

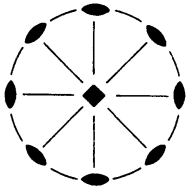


$4/mmm$

Laue Group		Rmeas
= 1 P 1 2/m 1	**	0.04
2 P -1		0.04
3 C 1 2/m 1		0.25
4 C 1 2/m 1		0.24
5 P 1 2/m 1		0.23
6 P 1 2/m 1		0.23
7 P m m m		0.26
8 P 4/m		0.43
9 C m m m		0.43
10 P 4/m m m		0.47

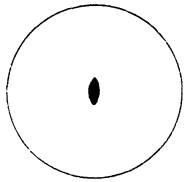


$\bar{1}$



422

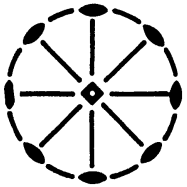
Rmeas		Symm operation
0.039		identity
0.687		2-fold l
0.039	**	2-fold k
0.690		2-fold h
0.773		2-fold
0.786		2-fold
1.200		4-fold l



2

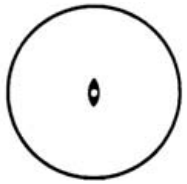
+ inversion

+ inversion

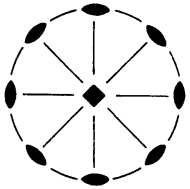


$4/mmm$

Laue Group		Rmeas
= 1	P 1 2/m 1 **	0.04
> 2	P -1	0.04
3	C 1 2/m 1	0.25
4	C 1 2/m 1	0.24
5	P 1 2/m 1	0.23
6	P 1 2/m 1	0.23
7	P m m m	0.26
8	P 4/m	0.43
9	C m m m	0.43
10	P 4/m m m	0.47

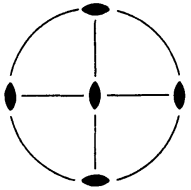


$2/m$



422

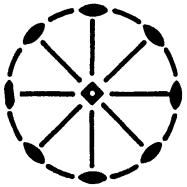
Rmeas		Symm operation
0.039		identity
0.687		2-fold l
0.039	**	2-fold k
0.690		2-fold h
0.773		2-fold
0.786		2-fold
1.200		4-fold l



222

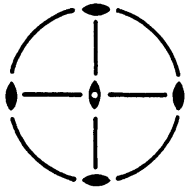
+ inversion

+ inversion



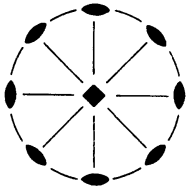
$4/mmm$

Laue Group		Rmeas
= 1 P 1 2/m 1	**	0.04
> 2 P -1		0.04
3 C 1 2/m 1		0.25
4 C 1 2/m 1		0.24
5 P 1 2/m 1		0.23
6 P 1 2/m 1		0.23
7 P m m m		0.26
8 P 4/m		0.43
9 C m m m		0.43
10 P 4/m m m		0.47



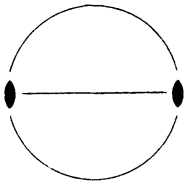
$m\ m\ m$

Probable crystal point group



422

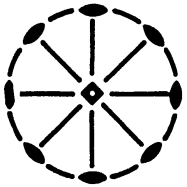
Rmeas		Symm operation
0.039		identity
0.687		2-fold l
0.039	**	2-fold k
0.690		2-fold h
0.773		2-fold
0.786		2-fold
1.200		4-fold l



2

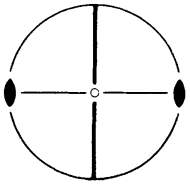
+ inversion

+ inversion



$4/mmm$

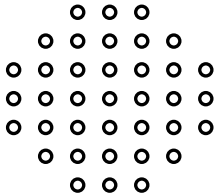
Laue Group		Rmeas
= 1 P 1 2/m 1	**	0.04
> 2 P -1		0.04
3 C 1 2/m 1		0.25
4 C 1 2/m 1		0.24
5 P 1 2/m 1		0.23
6 P 1 2/m 1		0.23
7 P m m m		0.26
8 P 4/m		0.43
9 C m m m		0.43
10 P 4/m m m		0.47



$2/m$

Space group assignment (*e.g. Pointless*)

Reciprocal space lattice (positions of reflections)

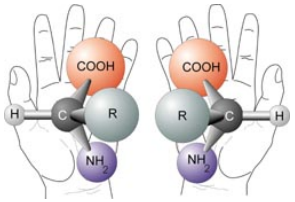


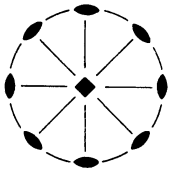


$4/mmm$

(Approximate) lattice point group

Mirror symmetry is <u>not</u> allowed in biological macromolecules

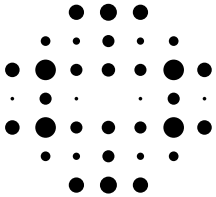


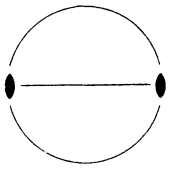


422

Highest possible crystal point group

Intensities of reflections

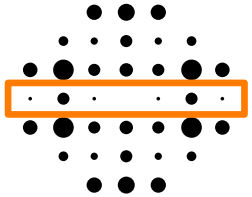


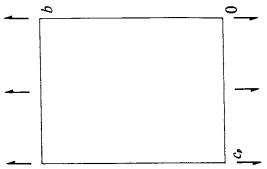


2

Probable crystal point group

Intensities of axial reflections





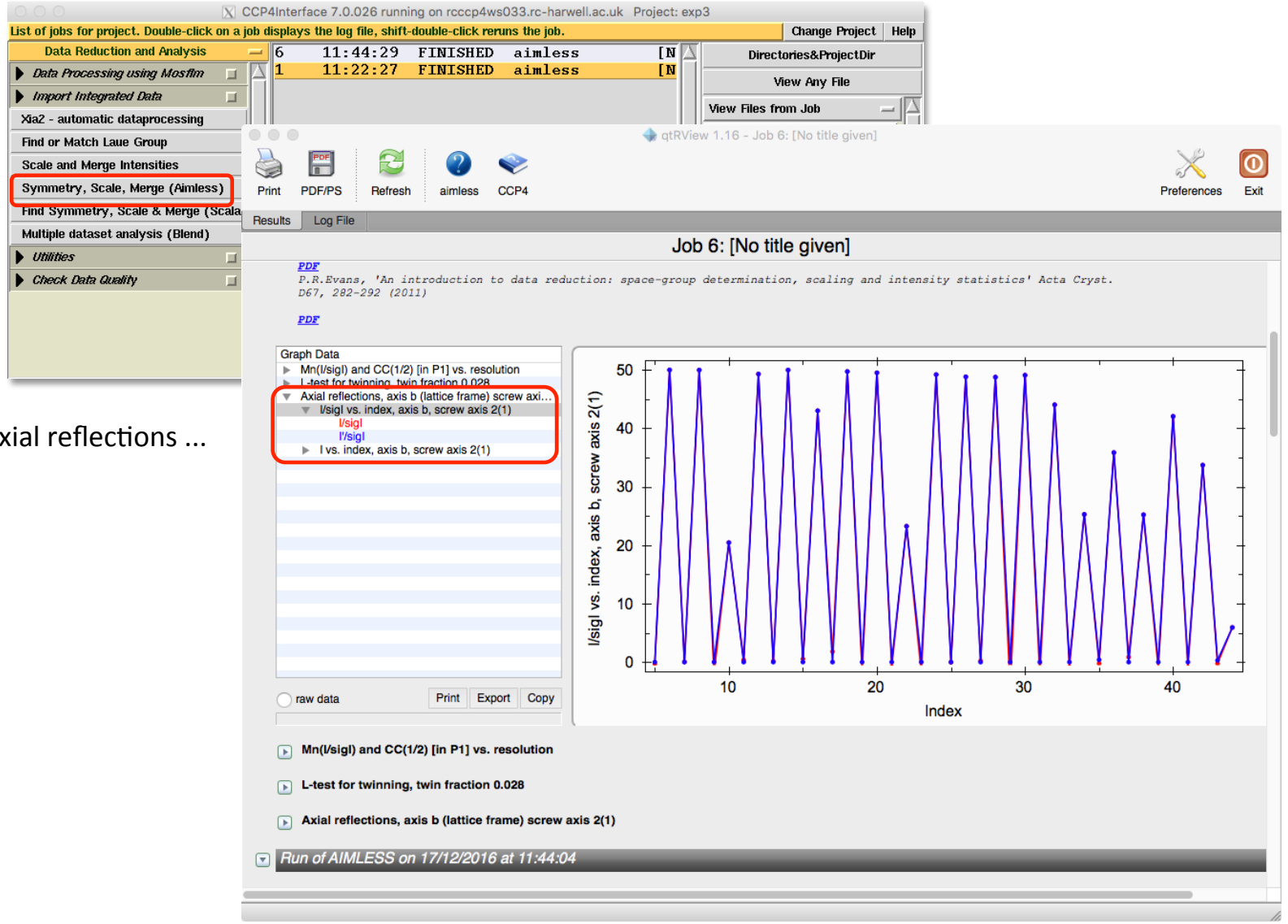
$P2_1$

Probable crystal space group

User: decision making

CCP4I

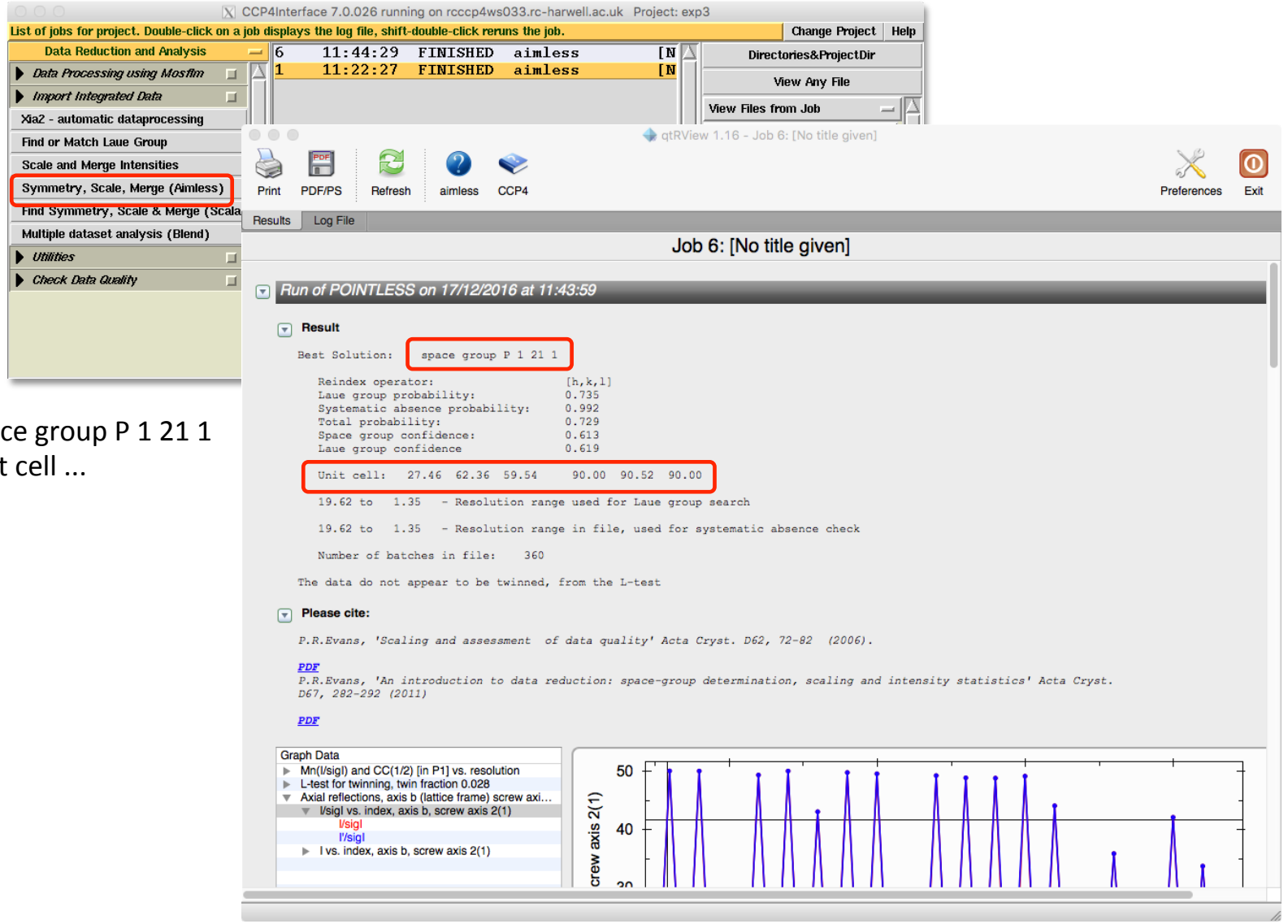
Aimless



Axial reflections ...

CCP4I

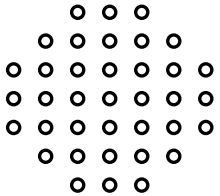
Aimless



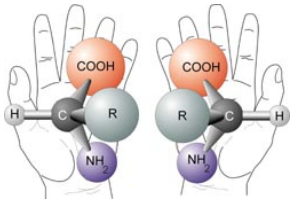
Space group P 1 21 1
Unit cell ...

Space group assignment (*e.g. Pointless*)

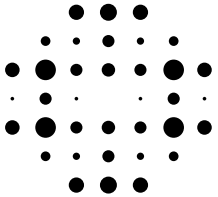
Reciprocal space lattice (positions of reflections)



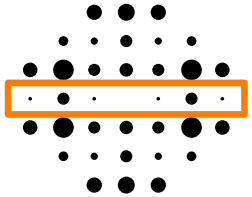
Mirror symmetry is <u>not</u> allowed in biological macromolecules

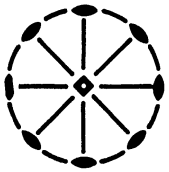


Intensities of reflections



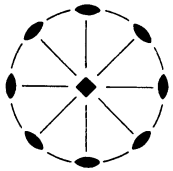
Intensities of axial reflections





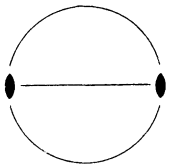
$4/mmm$

(Approximate) lattice point group



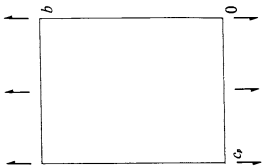
422

Highest possible crystal point group



2

Probable crystal point group



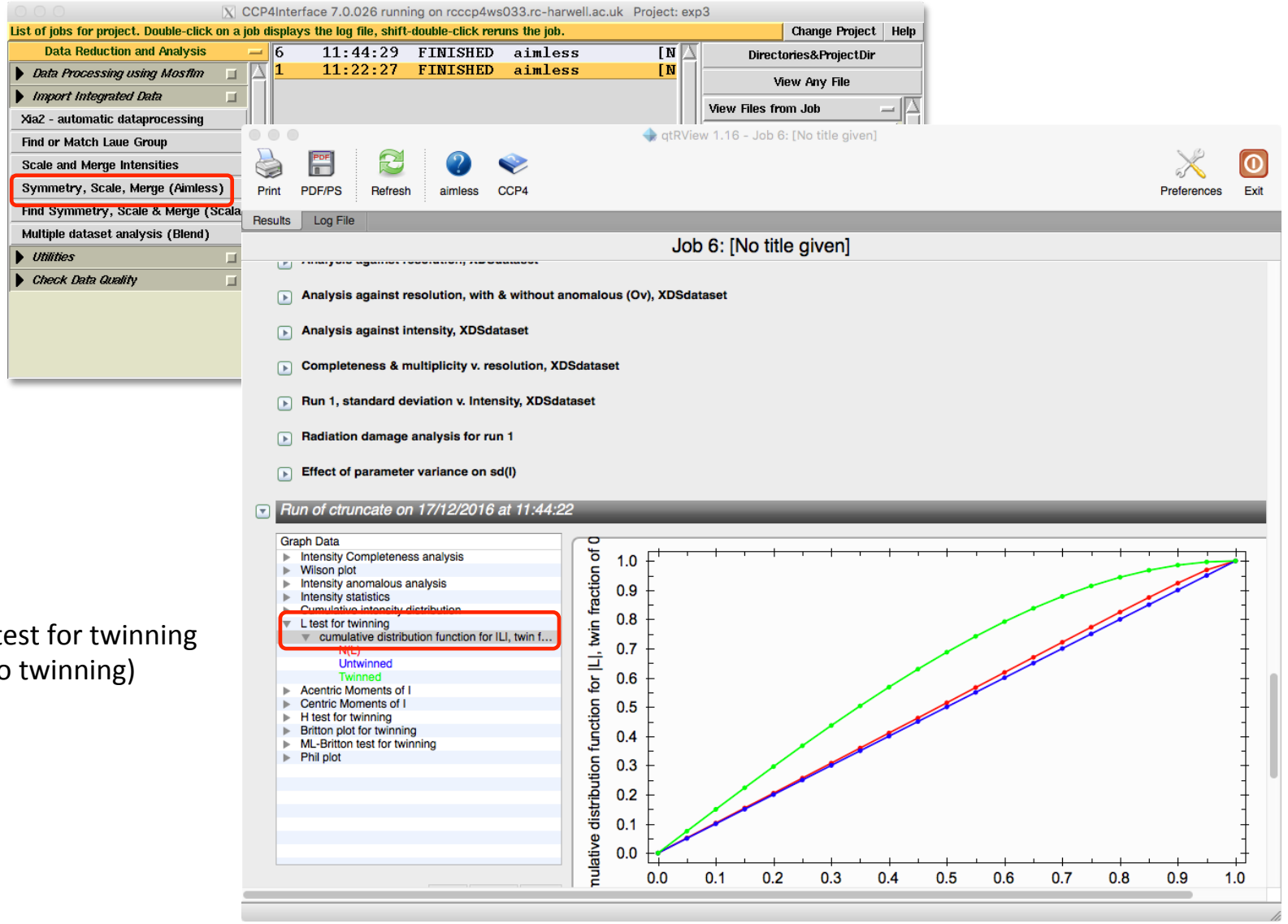
$P2_1$

Probable crystal space group

User: decision making

CCP4I

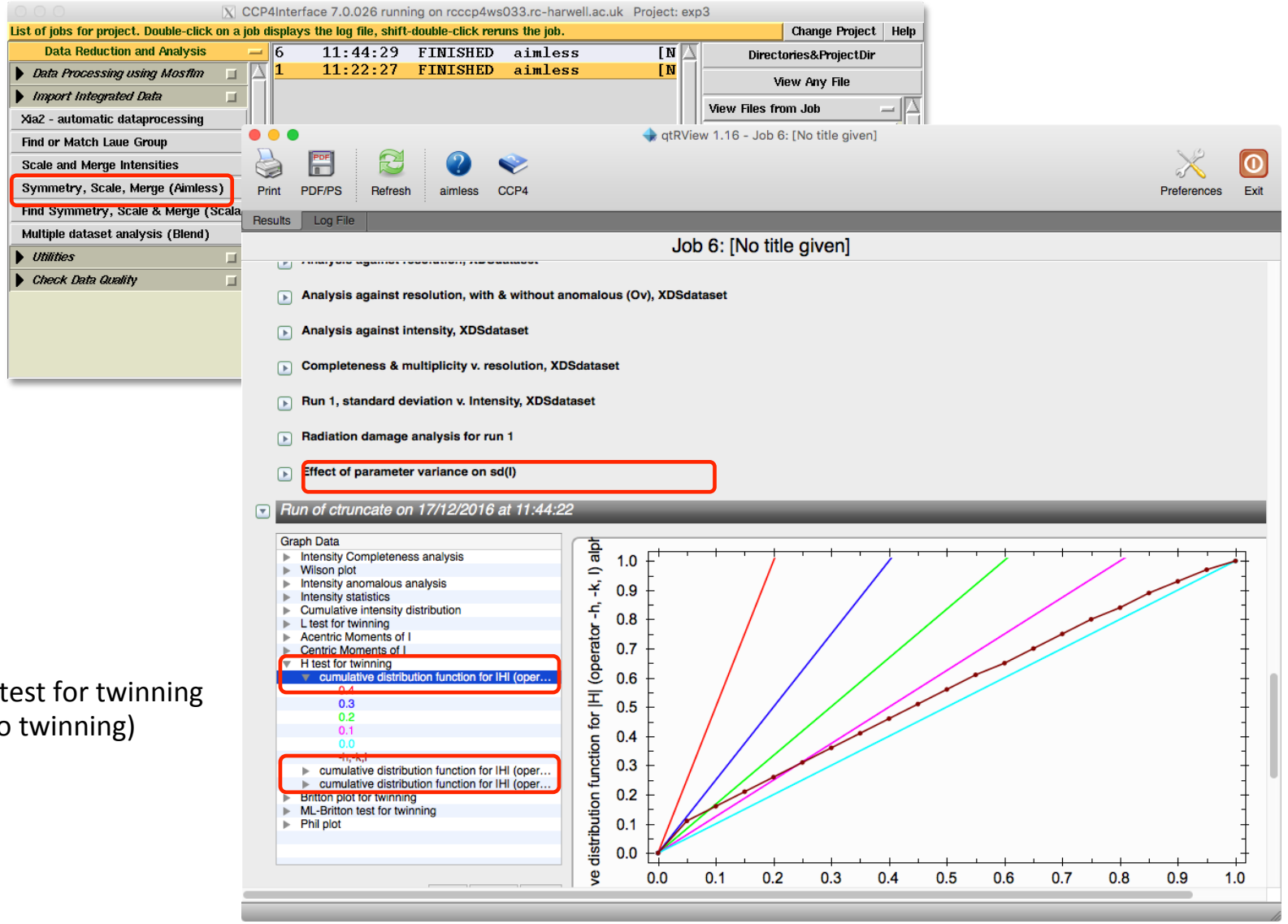
Aimless



L-test for twinning
(no twinning)

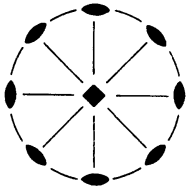
CCP4I

Aimless



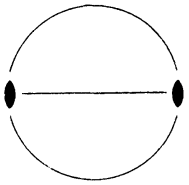
H-test for twinning
(no twinning)

Probable crystal point group



422

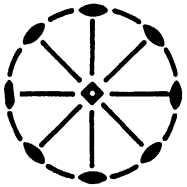
Rmeas		Symm operation
0.039		identity
0.687		2-fold l
0.039	**	2-fold k
0.690		2-fold h
0.773		2-fold
0.786		2-fold
1.200		4-fold l



2

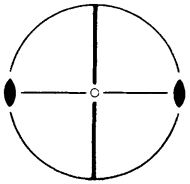
+ inversion

+ inversion



$4/mmm$

Laue Group		Rmeas
= 1 P 1 2/m 1	**	0.04
> 2 P -1		0.04
3 C 1 2/m 1		0.25
4 C 1 2/m 1		0.24
5 P 1 2/m 1		0.23
6 P 1 2/m 1		0.23
7 P m m m		0.26
8 P 4/m		0.43
9 C m m m		0.43
10 P 4/m m m		0.47



$2/m$

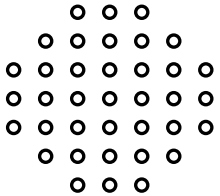
Space group assignment (*e.g. Pointless*)

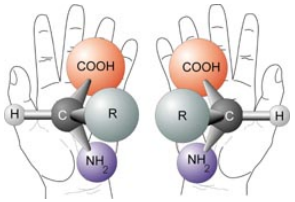
Reciprocal space lattice (positions of reflections)

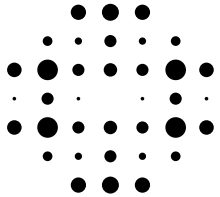
Mirror symmetry is <u>not</u> allowed in biological macromolecules

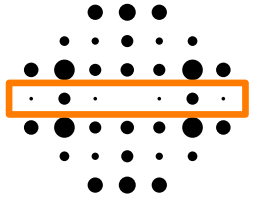
Intensities of reflections

Intensities of axial reflections

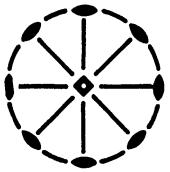




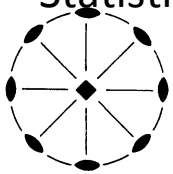




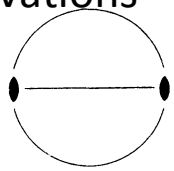
Statistics of two observations



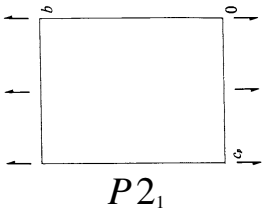
$4/mmm$



422



2



$P2_1$

(Approximate) lattice point group

Highest possible crystal point group

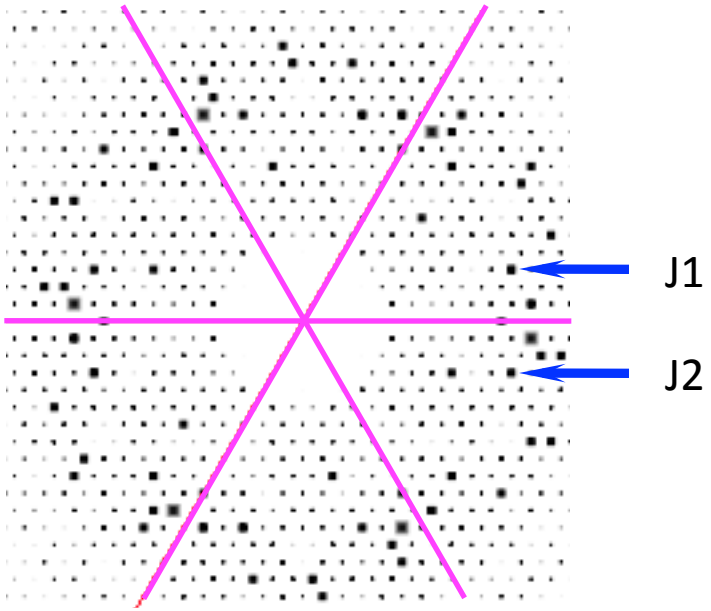
Probable crystal point group

Probable crystal space group

Space group: $P12_11$ Unit cell: 27.5, 62.4, 59.5, 90.0, 90.5, 90.0

H-test and L-test

$H = | J1 - J2 | / (J1 + J2)$



twin axes

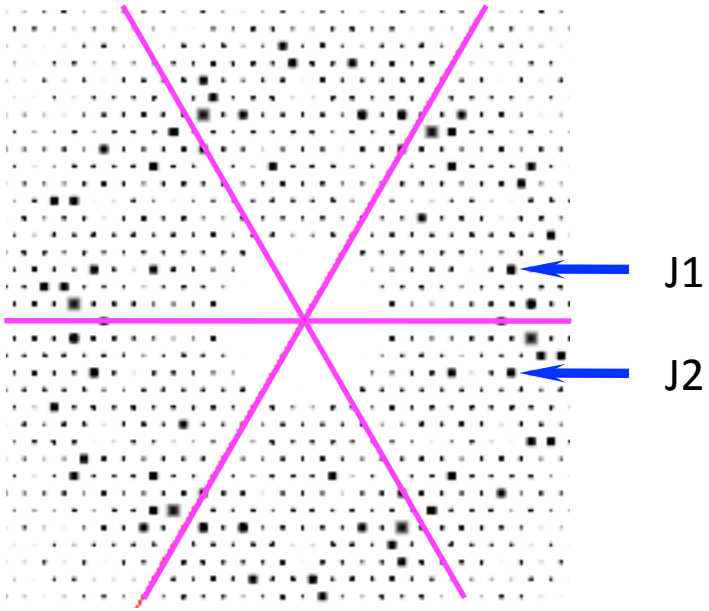
$L = | J1 - J2 | / (J1 + J2)$



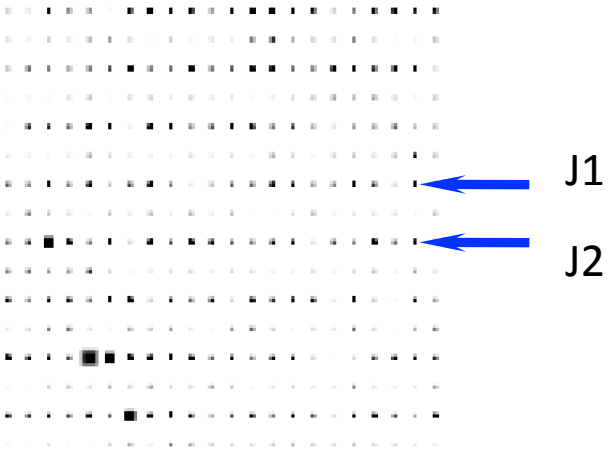
sublattices with strong and weak reflections (pseudotranslation)

H-test and L-test

$H = | J1 - J2 | / (J1 + J2)$



$L = | J1 - J2 | / (J1 + J2)$



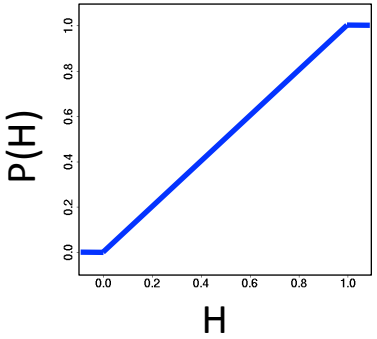
sublattices with strong and weak reflections (pseudotranslation)

Theoretical distribution of H

What is the tested operation?

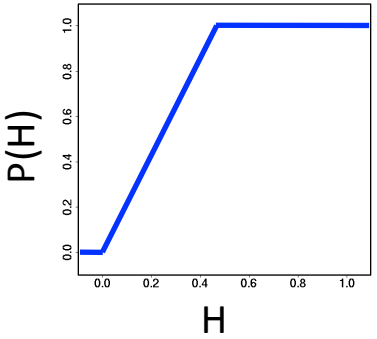
not a twin operation





twin operation
(partial twinning)



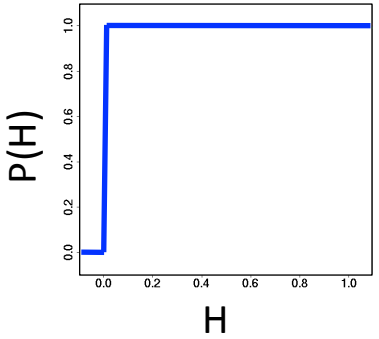


twin operation
(perfect twinning)

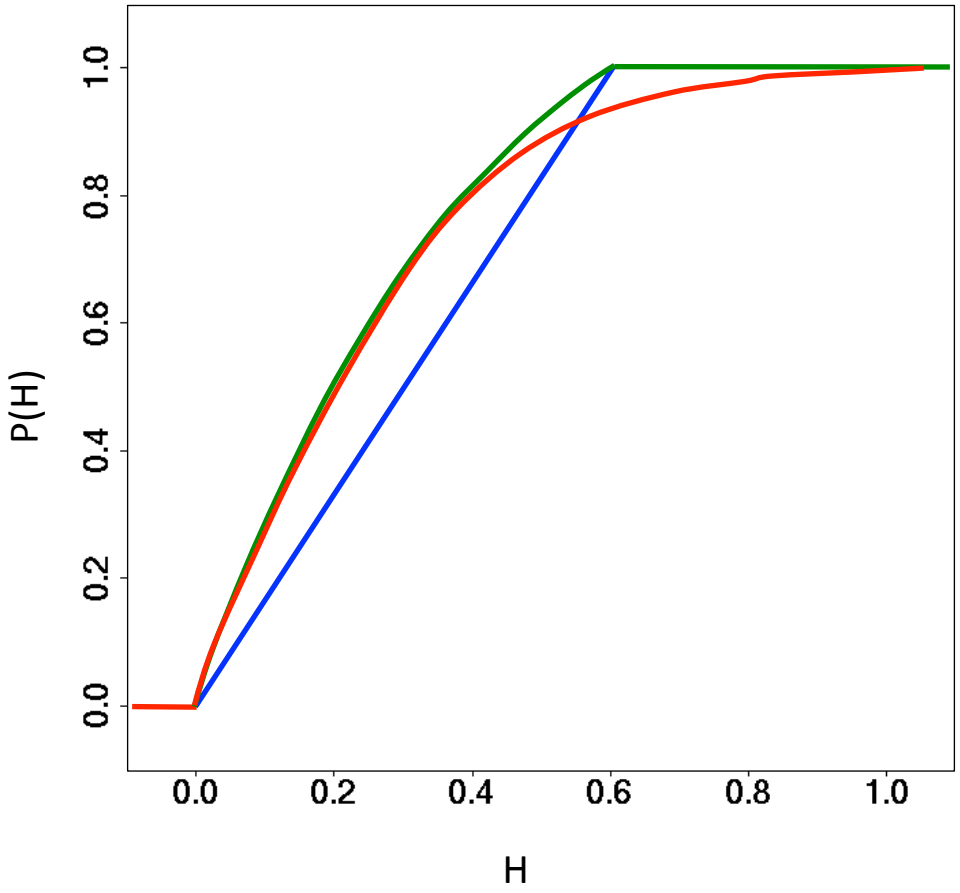


or operation of the
crystal point group





Distribution of H can be perturbed by NCS and weak observations

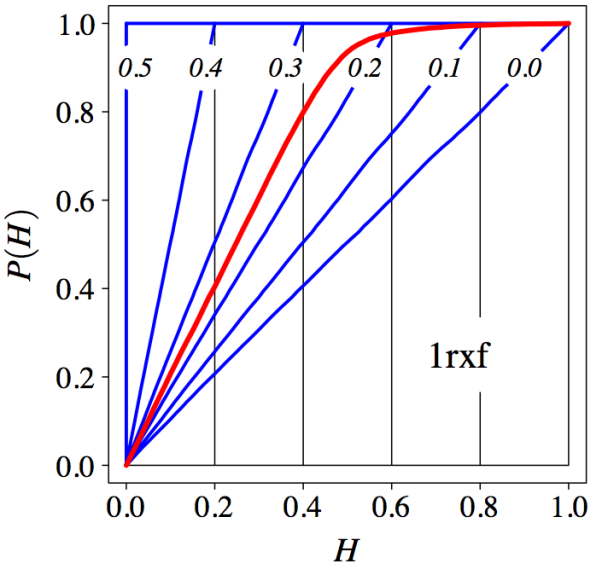


Blue:
ideal distribution for partial twin

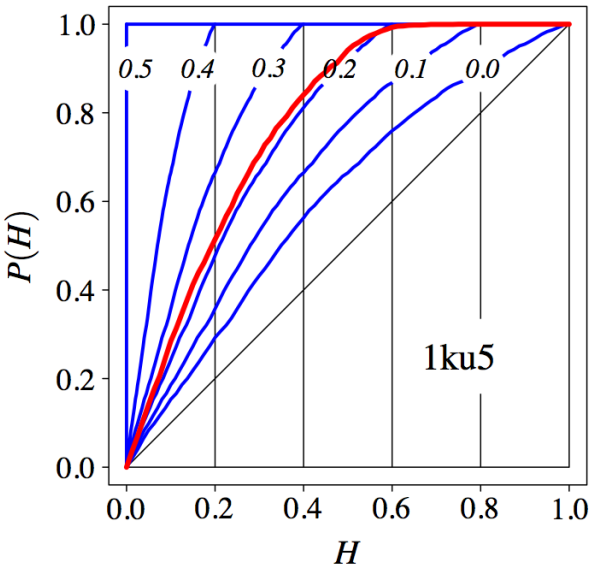
Green:
blue + effect of NCS axis || twin axis

Red:
green + effect of intensities with small I/ sig(I)

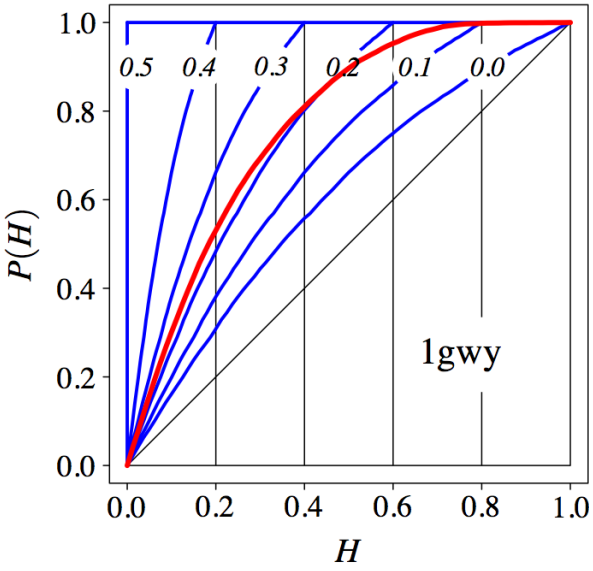
Examples of experimental P(H)



An almost ideal case



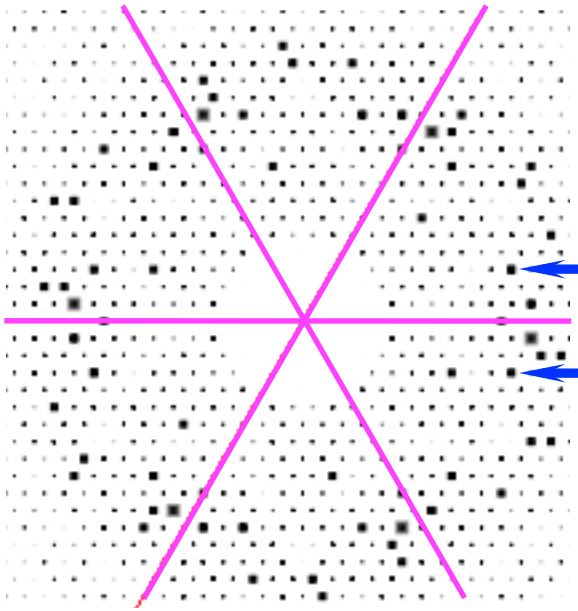
+ effect of
NCS axis || twin axis



+ effect of
intensities with
small I/ sig(I)

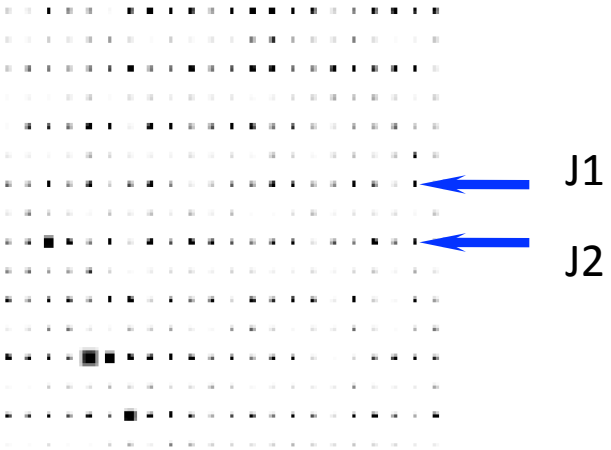
H-test and L-test

$H = | J1 - J2 | / (J1 + J2)$



twin axes

$L = | J1 - J2 | / (J1 + J2)$

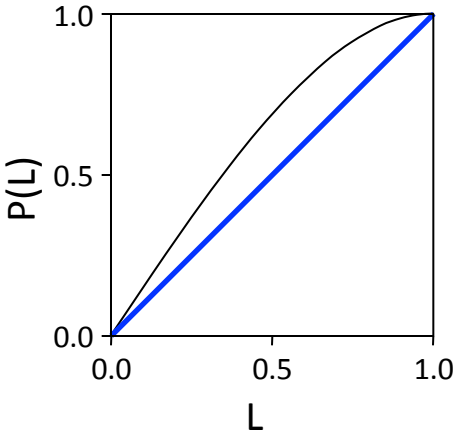


sublattices with strong and weak reflections (pseudotranslation)

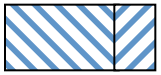
Theoretical distribution of L

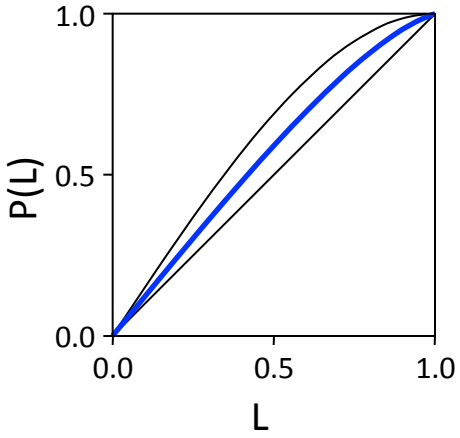
Single crystal



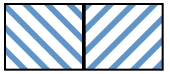


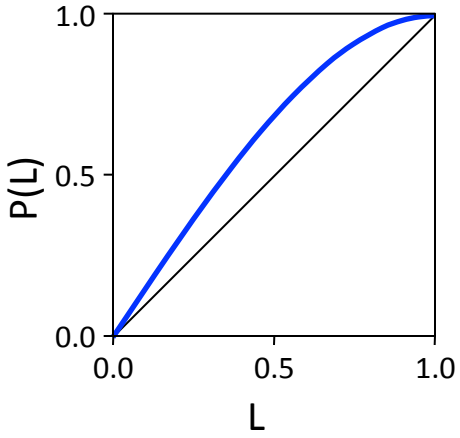
Partial twin or NCS || twin axis





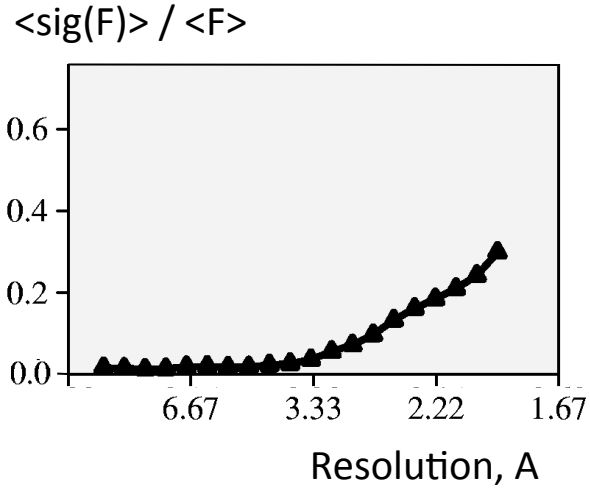
Perfect twin



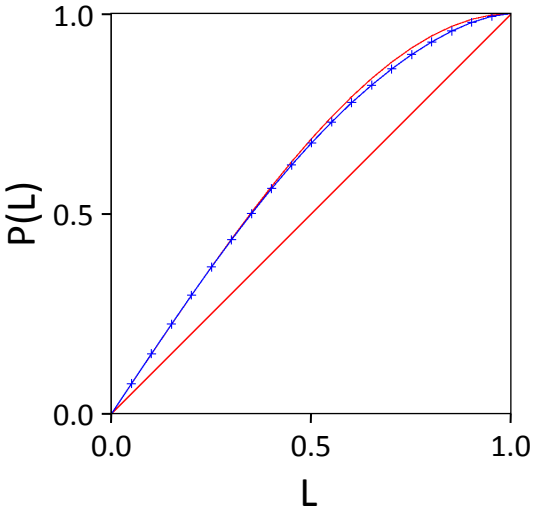


Distribution of L can be strongly perturbed by weak observations

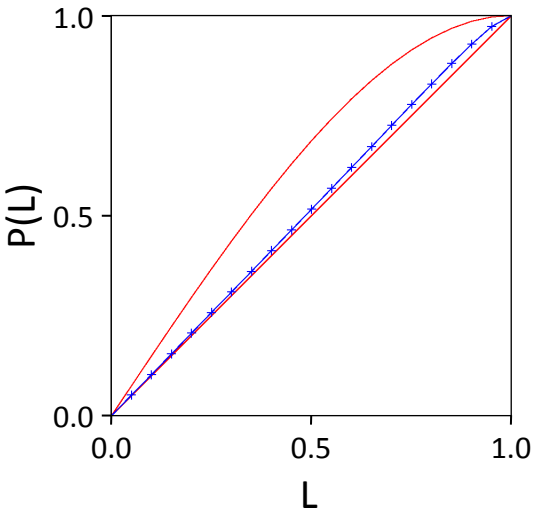
Cell: 64.2 109.2 100.2 90 93.8 90
Space group: $P2_1$
No pseudo symmetry
Pseudomerohedral twinning is impossible



All data:
as if a perfect twin

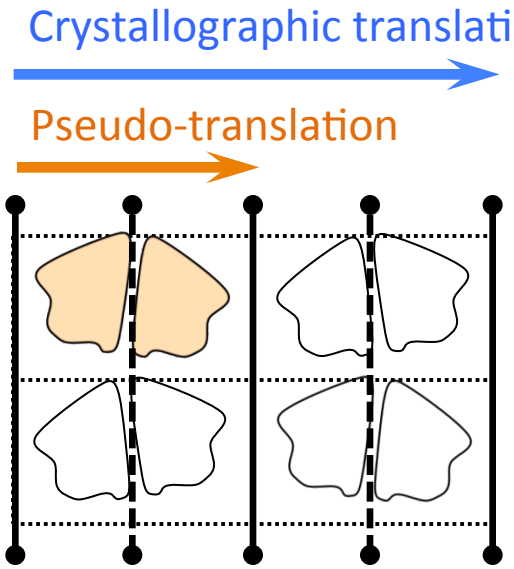


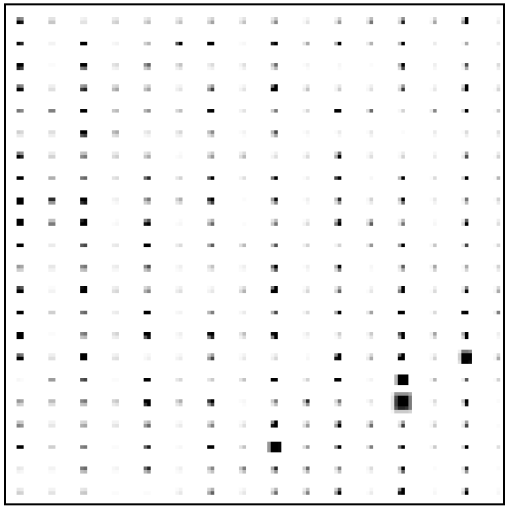
Data below 3A:
untwinned



Nevertheless the L-test is very useful when performed with right resolution range (or with several ranges)

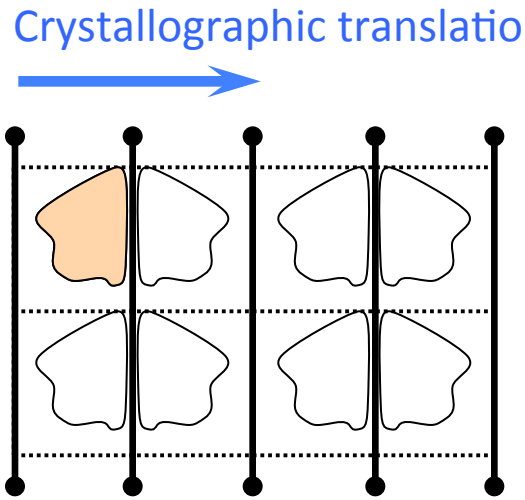
Pseudotranslation

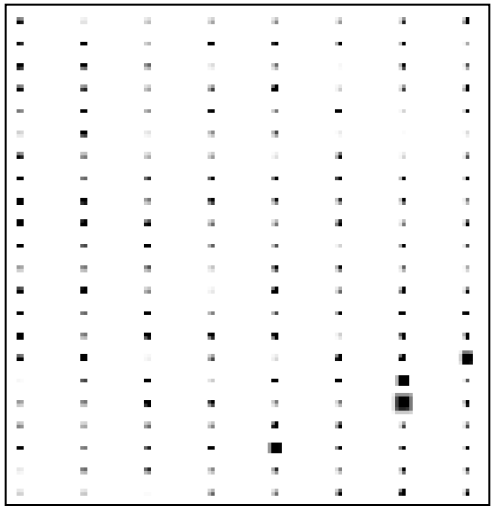




Pseudotranslation **C**/2

Planes 2L+1 contain weak reflections





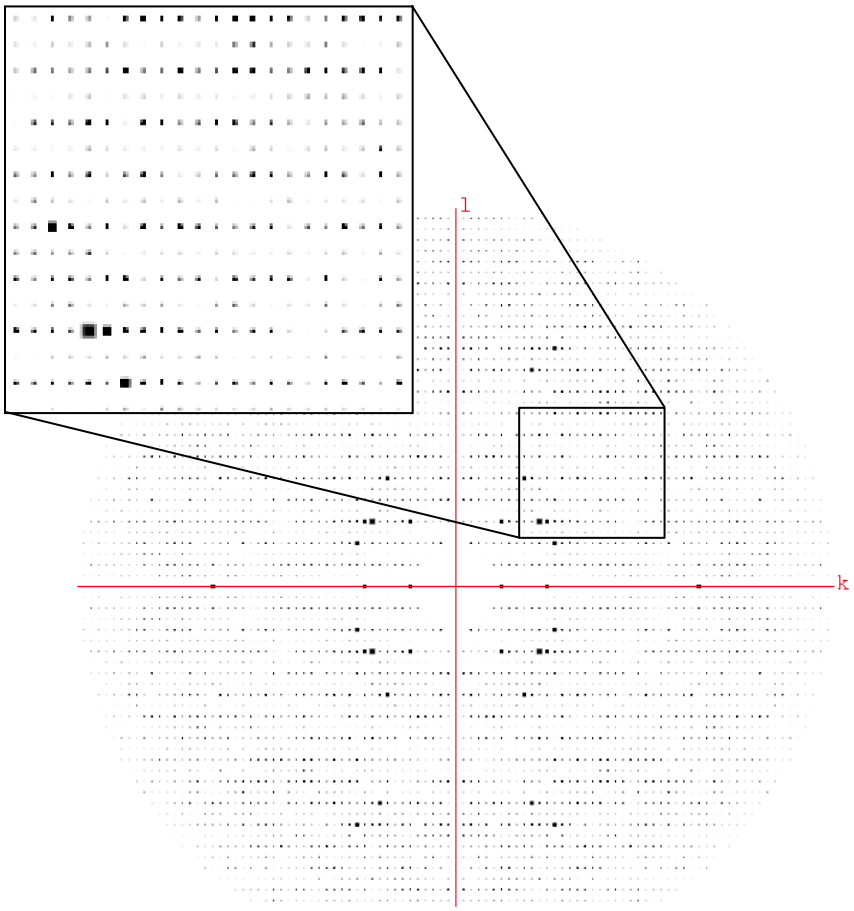
Limiting case, **C'** = **C**/2

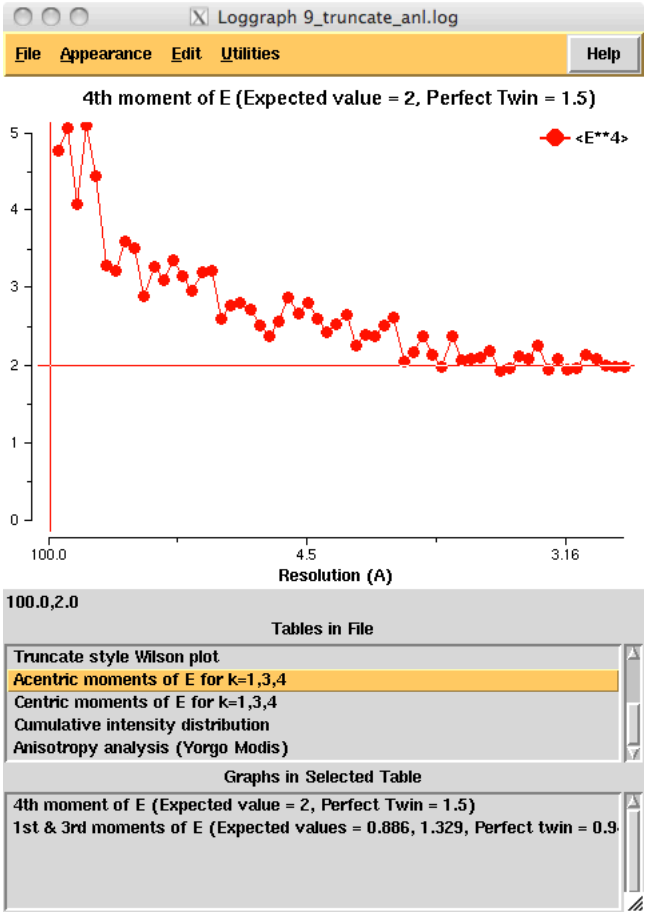
- Weak reflections vanish

Two times larger reciprocal lattice spacing

Statistics of one intensity are strongly affected by pseudotranslation

1jjk: Pseudotranslation results in alteration of strong and weak reflections

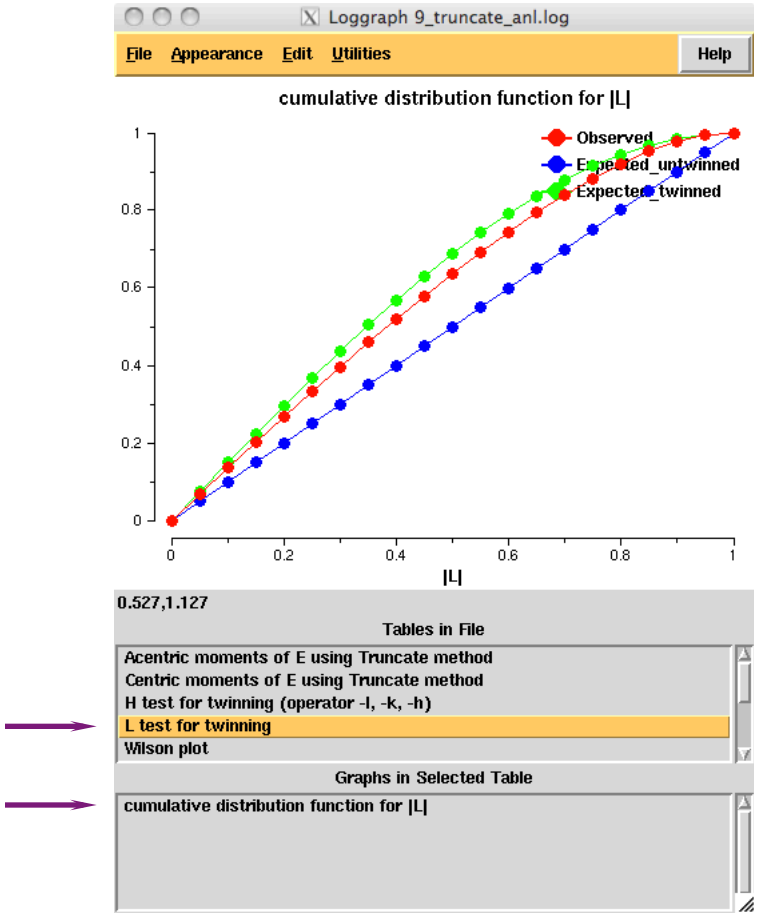


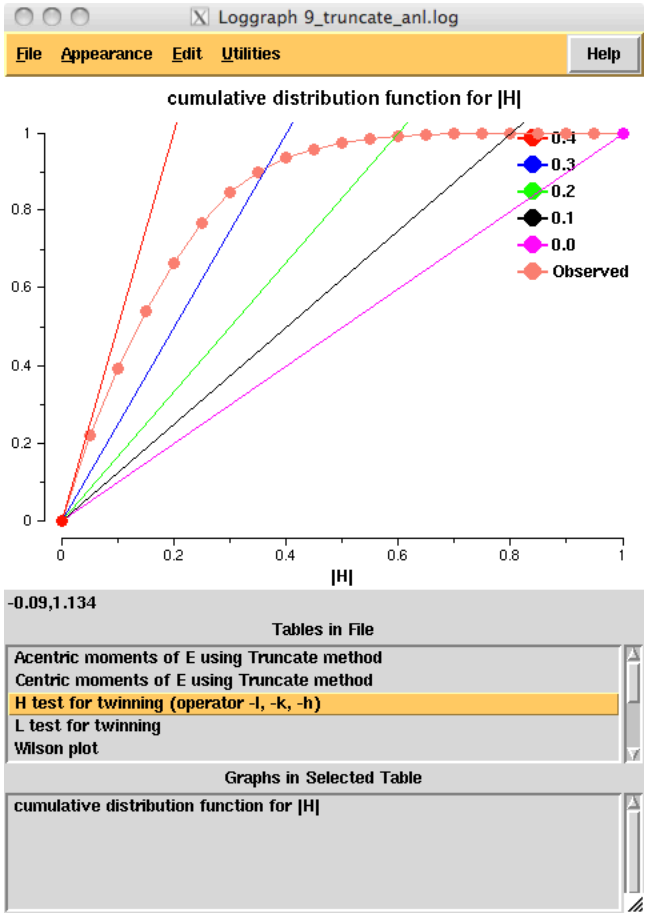


> Acentric moments of E for k=1,3,4

> 4th moments of E ...

L-test and H-test are not affected by pseudotranslation





> L test for twinning
> cumulative distribution function for |L|

> H test for twinning (operator ...)
> cumulative distribution function for |H|

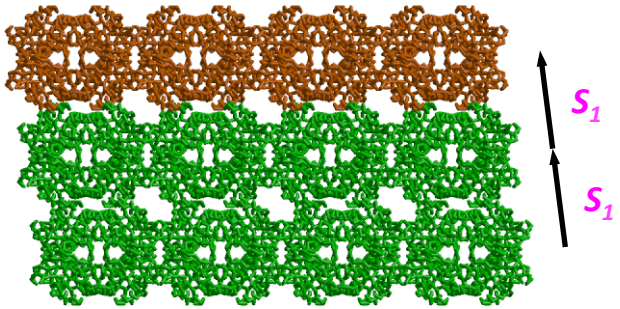
OD-structures

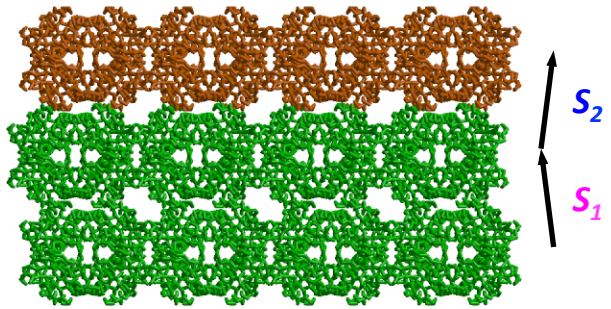
- Definition
- Example of an OD twin
- Demodulation of data
- Example of allotwin

Order-disorder structures (OD-structures)

- identical layers
- identical interfaces between the layers
- but: two or more ways of packing three adjacent layers

*) MX: "identical" means Ca r.m.s.d. < 1 A



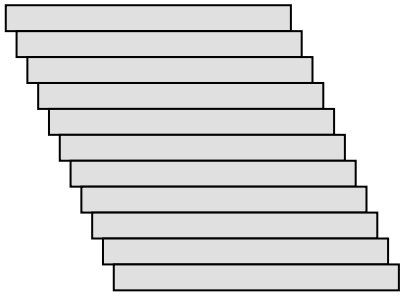


*) S_1 and S_2. are called stacking vectors

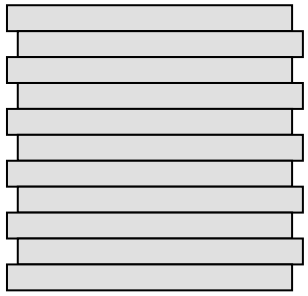
- two-dimensional periodicity
- a potential for disorder in the third dimension

OD-structures

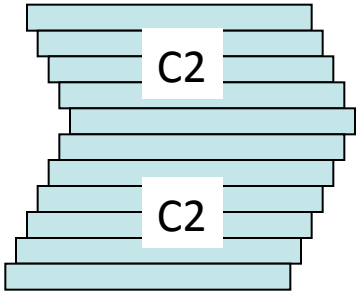
Single crystal



Single crystal

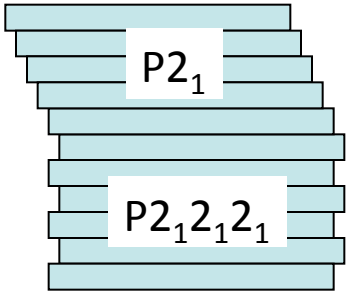


OD-twin



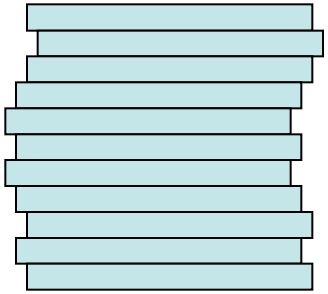
Example 1

Allotwin



Example 2

Partially disordered OD-structure

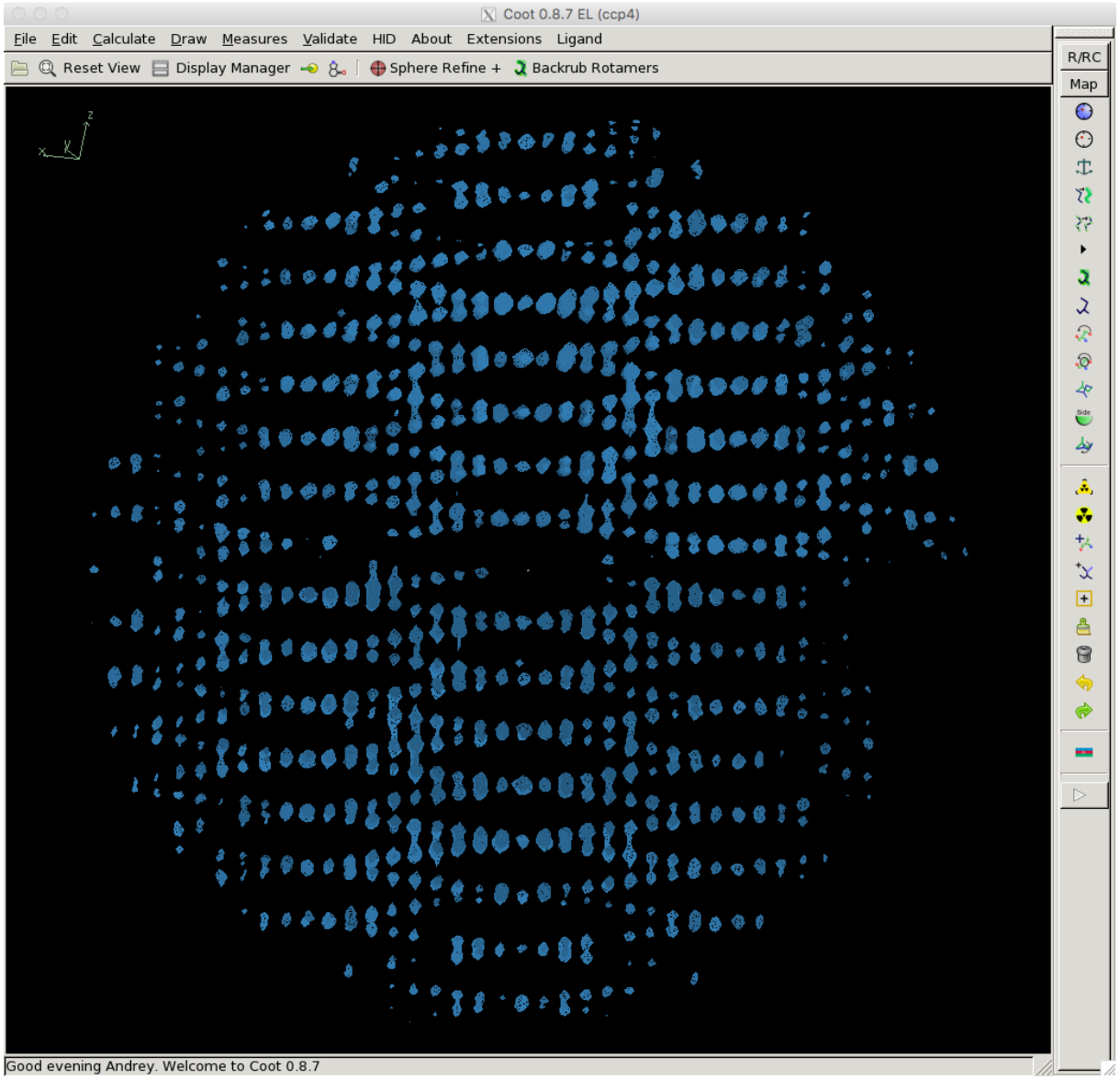


Examples in the next section

Example 1: OD-twin

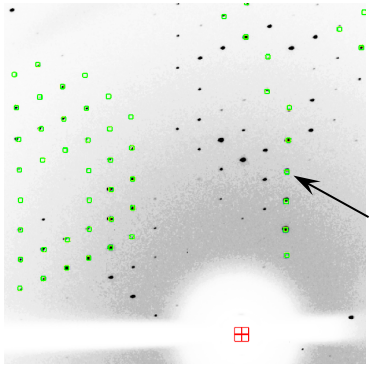
L-2-haloacid dehalogenase
from *Sulfolobus tokodaii*

Rye *et al.* (2007) *Acta Cryst.* **D**67

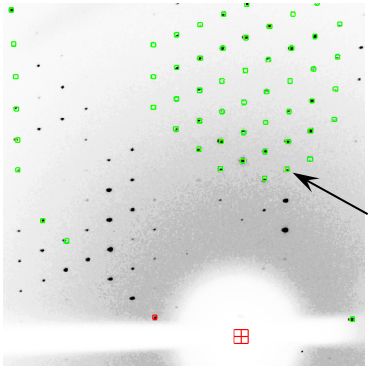


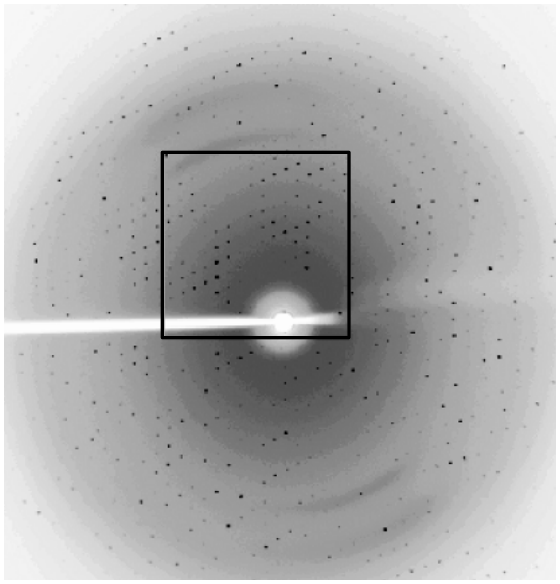
Example 1: OD-twin

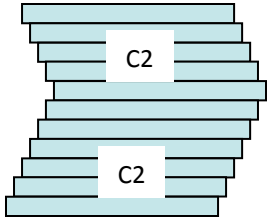
Indexing in C2



Indexing in C2





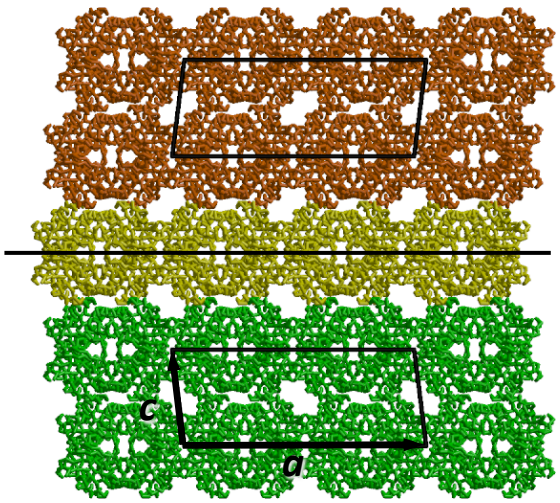


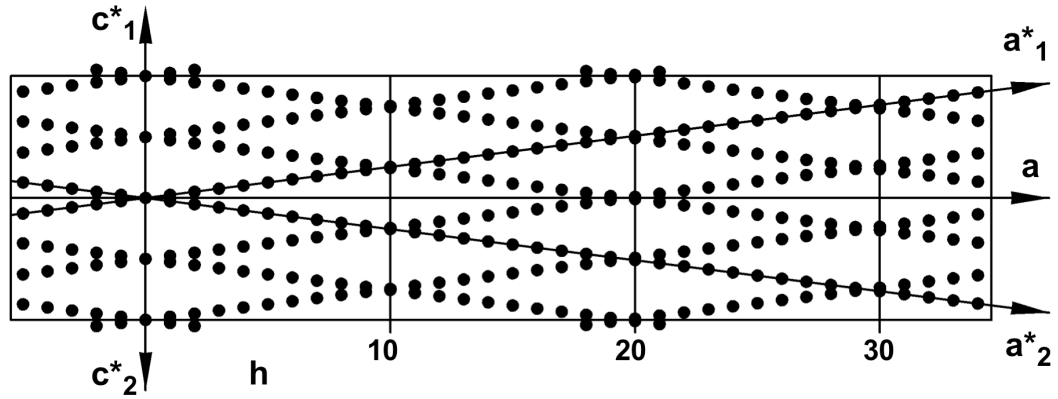
L-2-haloacid dehalogenase
from *Sulfolobus tokodaii*
Rye *et al.* (2007) *Acta Cryst.* **D**67

The diffraction images can be indexed in C2 with two different orientation of the crystal

Some reflections from two lattices overlap.

Real and reciprocal lattices





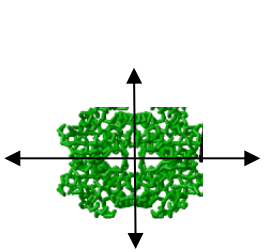
Twinning by reticular pseudo-merohedry

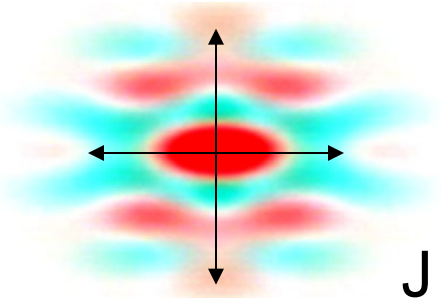
What to do?

- Process data from one lattice and ignore twinning
- Process data from one lattice and demodulate the data

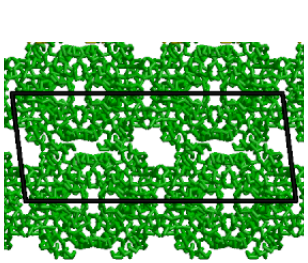
- Deconvolute overlapping sport during data processing
- Record total intensity of overlapping spots and deal with it at refinement

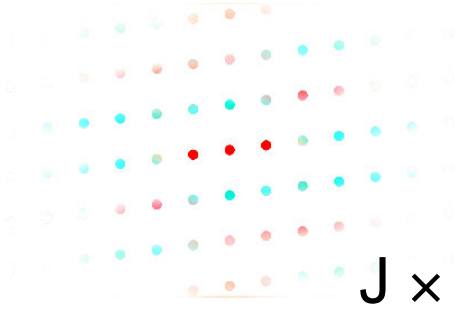
Intensities of the overlapping reflections



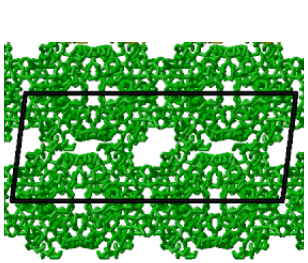


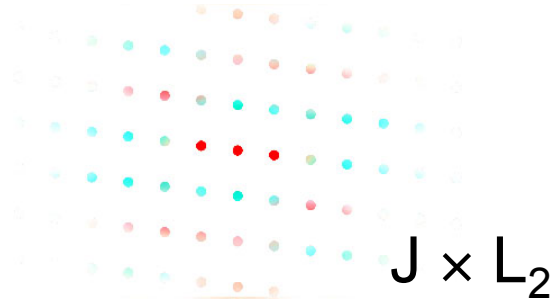
Fourier transform
of the tetramer





Diffraction pattern
of domain 1





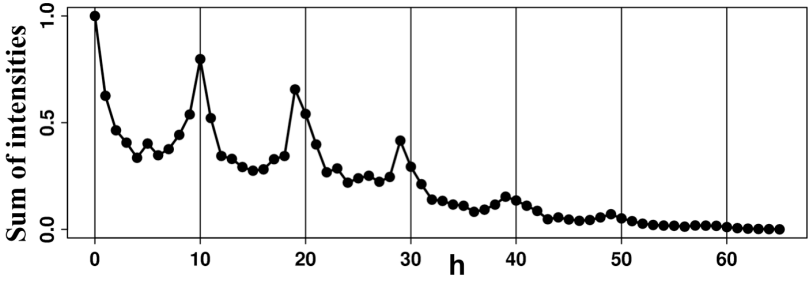
Diffraction pattern
of domain 2

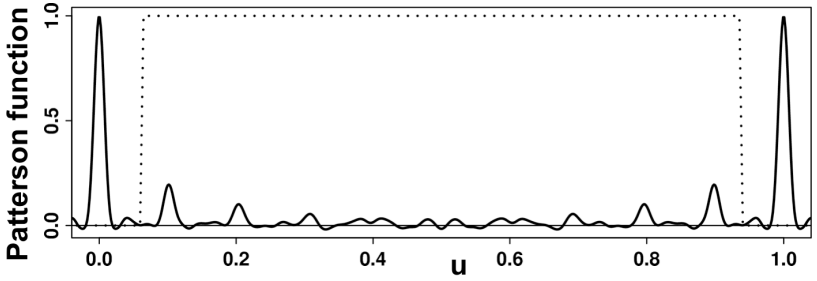
Tetramers in different twin domains are in the same orientation

Therefore, if reflections of the two lattices overlap, they have close intensities. The stronger the overlap, the closer the intensities are.

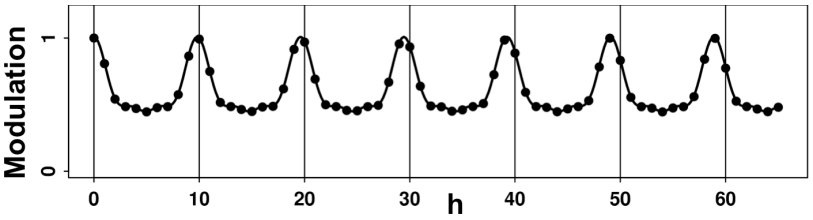
Demodulation

Original data: R / R-free = 0.21 / 0.27



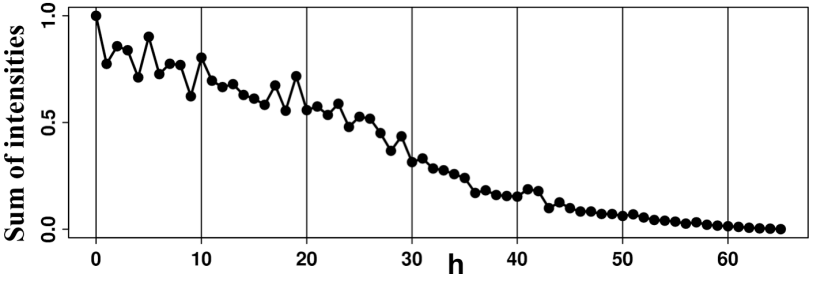


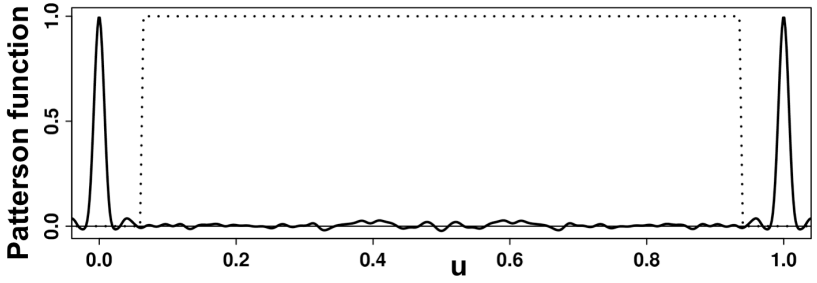
Modulation function



$$q'(h) = p_0 + p_1 \cos(2\pi th) + p_2 \cos(4\pi th) + \ldots$$

Corrected data: R / R-free = 0.16 / 0.23

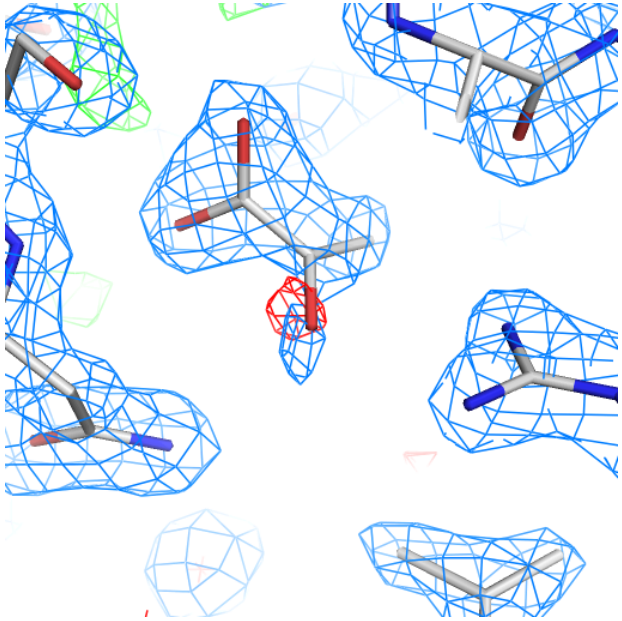


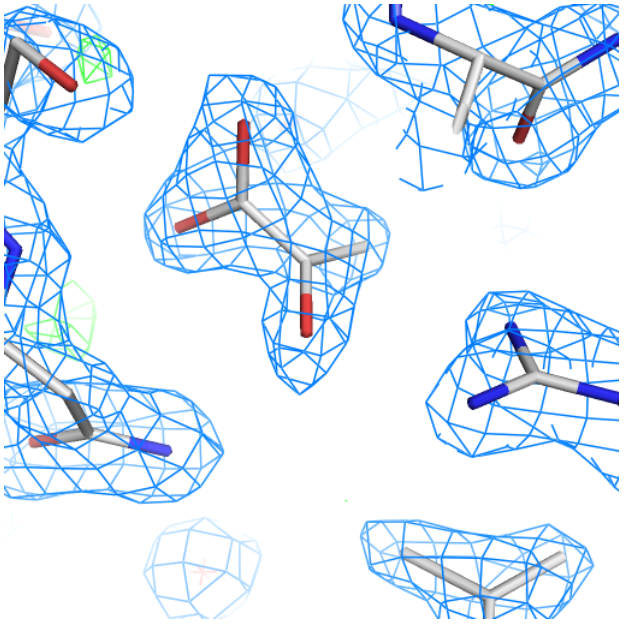


Improvement in the electron density

Visually, improvement occurred only for the electron density for solvent molecules
(Poor density for solvent was the original reason for data revision)

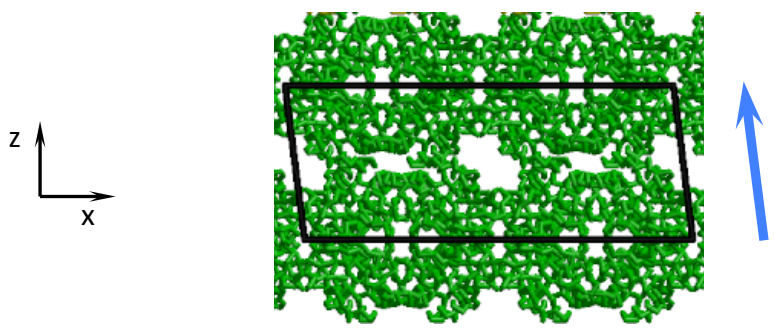
The electron density maps (2-1 at 1.5σ and 1-1 at 3σ)
around the pyruvate molecule before and after demodulation



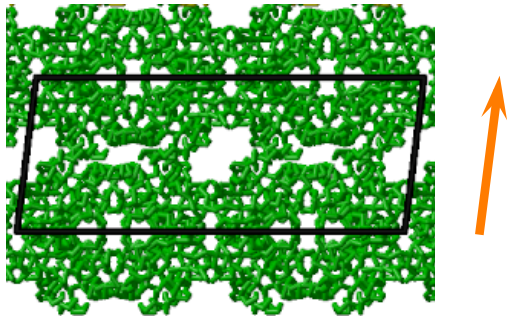


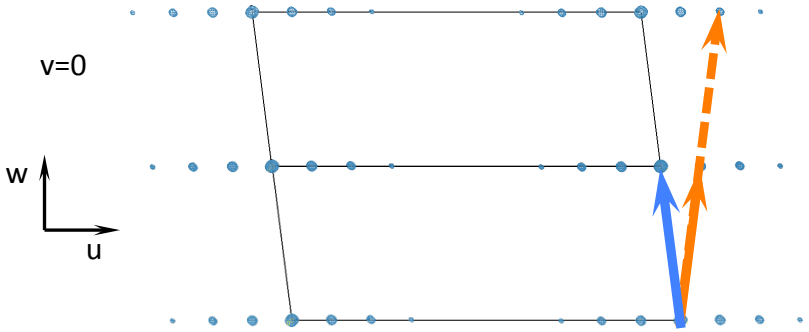
Diagnostics: Patterson Map

Indexed lattice



The second lattice



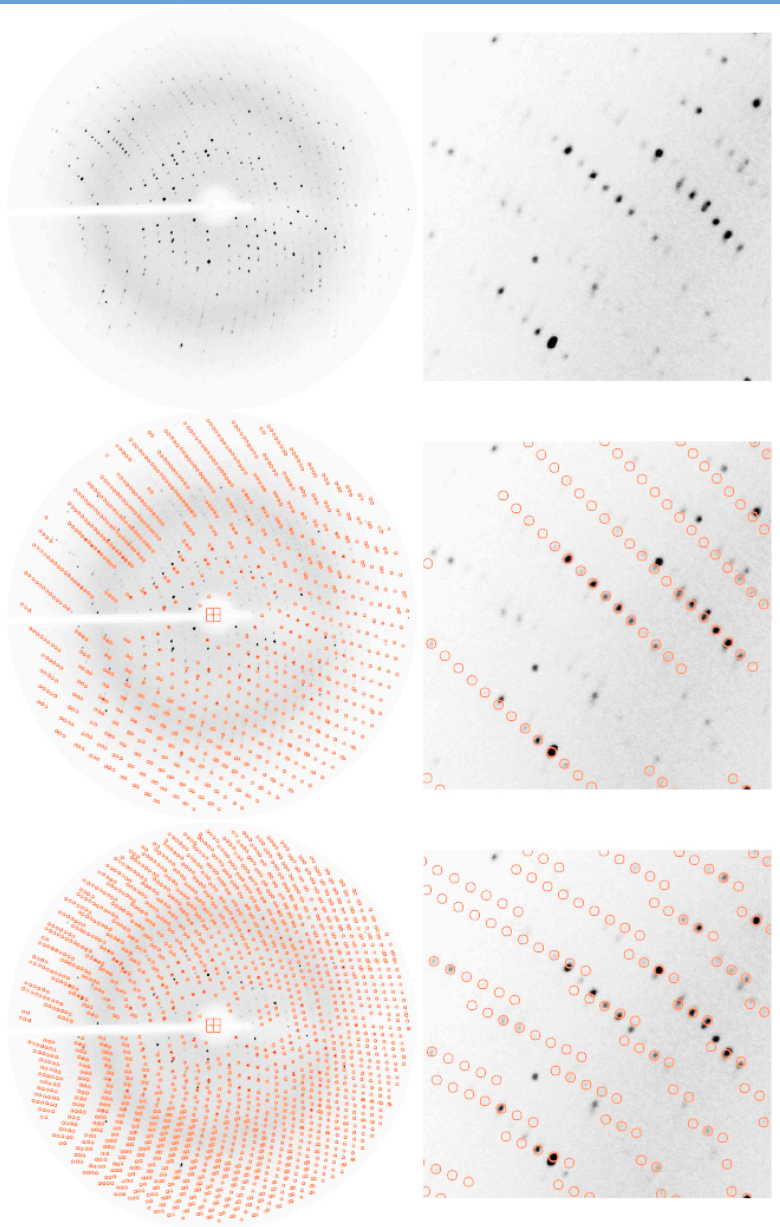


Non-origin peaks in the Patterson map:

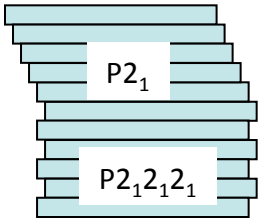
- contribution from the second lattice
- because of the overlapping spots

3D viewers provide more straightforward diagnostics

Example 2: allotwin



Crystals of Lon protease
Resolution 3Å



Dauter *et al.* (2005).
Acta Cryst. D**61**, 967-975.

$P2_1$

a = 48.5 Å
b = 86.3 Å
c = 138.0 Å
β = 92.3°

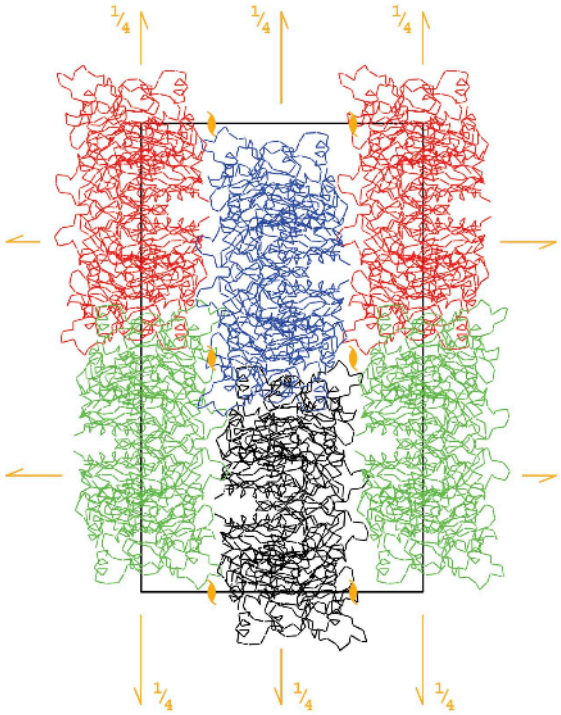
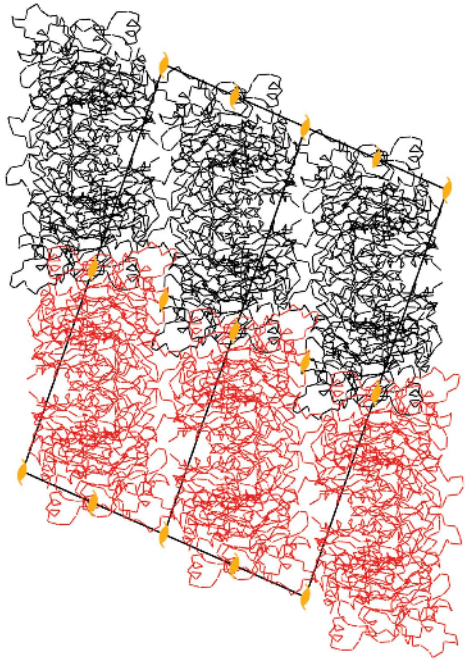
$P2_12_12_1$

a = 86.3 Å
b = 90.6 Å
c = 148.0 Å

Example 2: allotwin

Crystals of Lon protease
Resolution 3Å

Dauter *et al.* (2005).
Acta Cryst. D**61**, 967-975.

	PDB code 1z0t	PDB code 1z0v
		
Structures of both crystal forms were solved	$P2_12_12_1$	$P2_1$
R / R-free	0.19 / 0.35	0.21 / 0.31

Example 2: allotwin

- More frequently, the presence of very different indexing solutions means that the indexing program is struggling rather than existence of domains belonging to different space groups.

- 3D viewers will help to check what is actually happening.

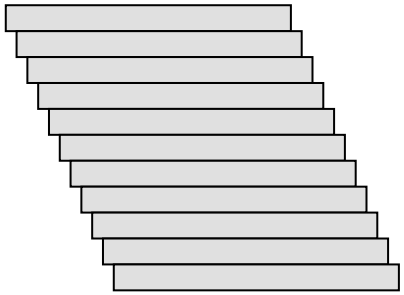
- Merging several fine-sliced images together may help indexing

Partially disordered OD-structures

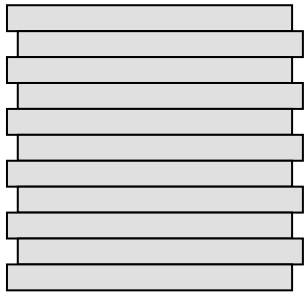
- Visualisation (detection)
- Ghost density
- Indexing
- Effect on structure solution and refinement

OD-structures

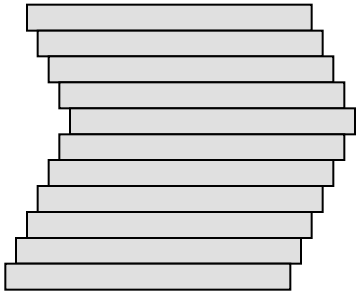
Single crystal



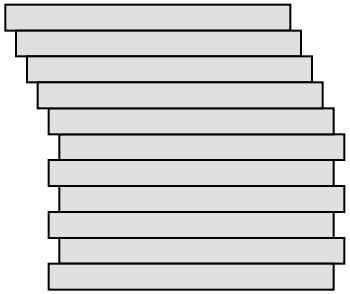
Single crystal



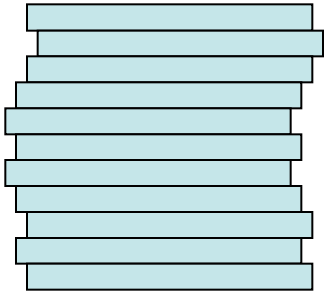
OD-twin



Allotwin



Partially disordered OD-structure

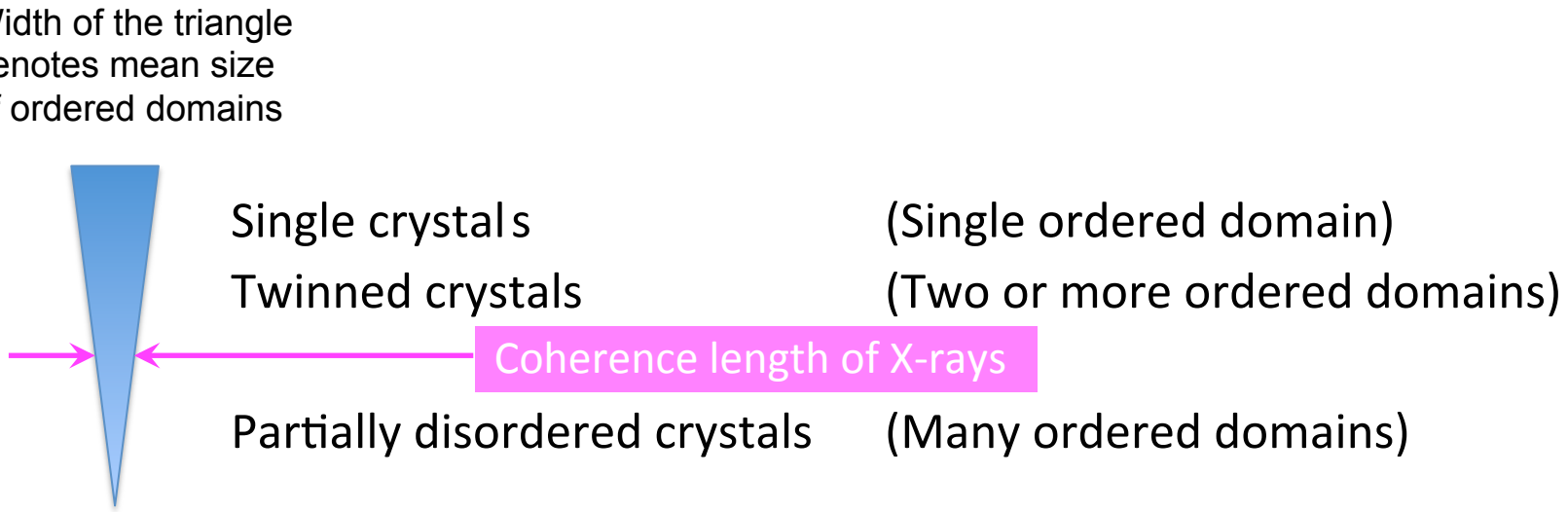


Examples 1,2 & 3

Partially disordered crystals

In both twins and partially disordered crystals:

- Global periodicity is missing in one or more directions
- There is local 3D periodicity in individual domains



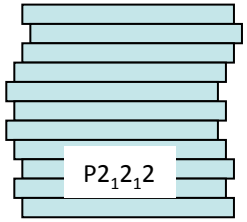
Example 1: ghost density

A "slice" of the reciprocal space
dials.rs_mapper + pymol



Direction in which
global periodicity is missing

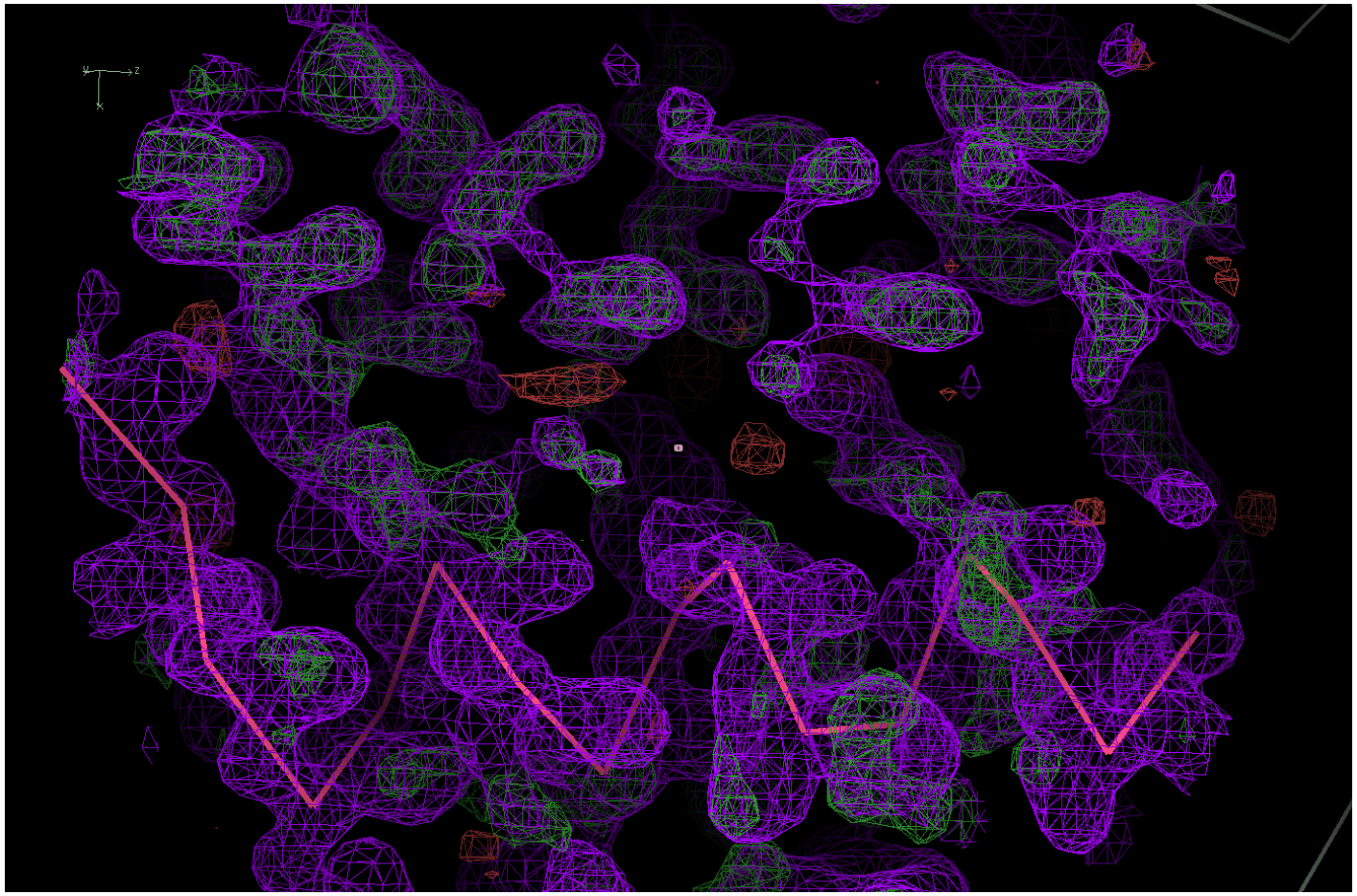
An example from **Rafael Ciges**,
Biomedical Institute of Valencia



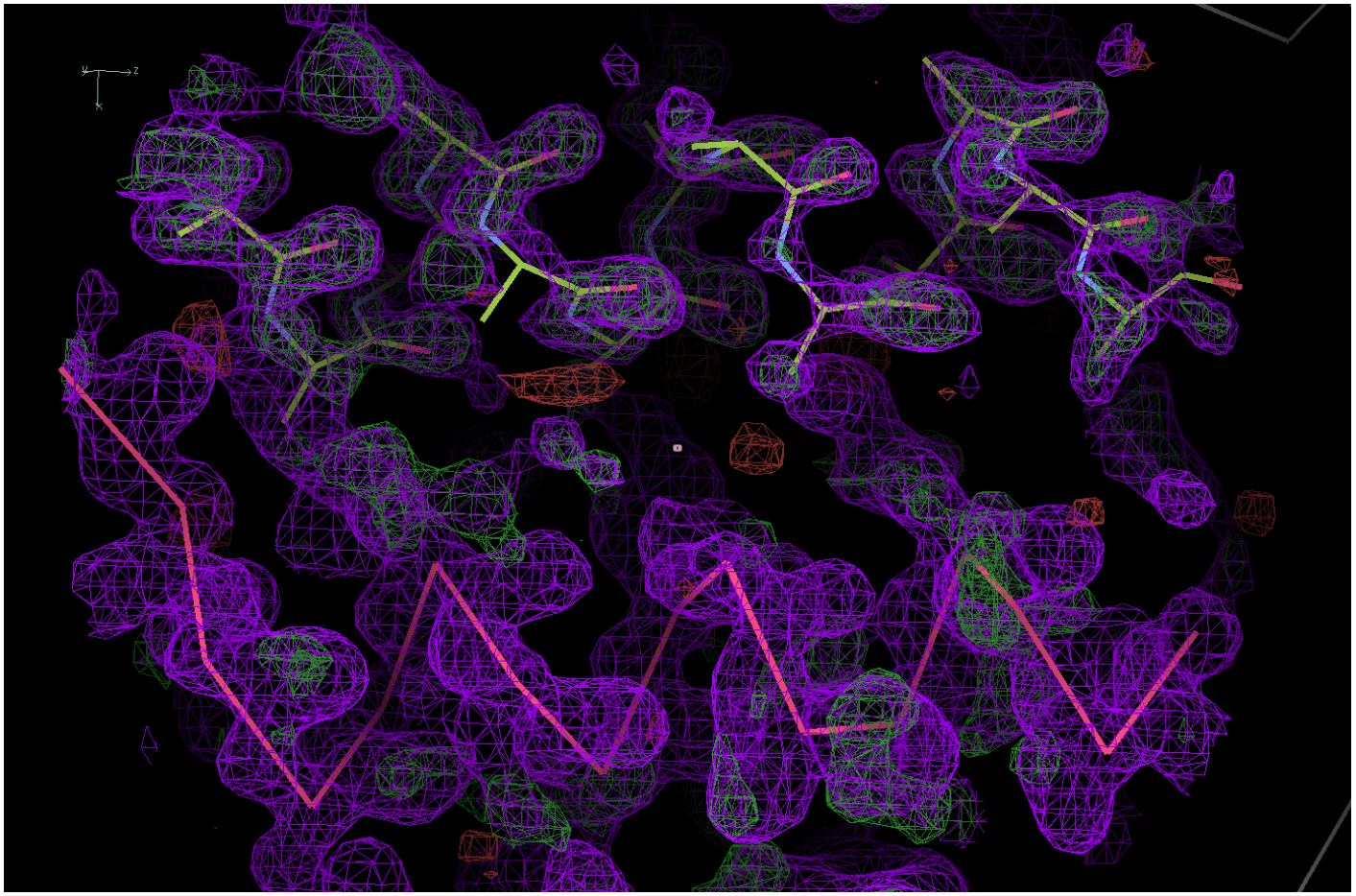
- Space group P21212
- Resolution 1.2Å
- The diffraction images were processed, and structure was solved and reffined.
- Later the data were demodulated and re-refined
  - » demodulation procedure was conceptually similar to the one used for OD-twin

	R	R-free
Original data	0.33	0.34
Corrected data	0.25	0.26

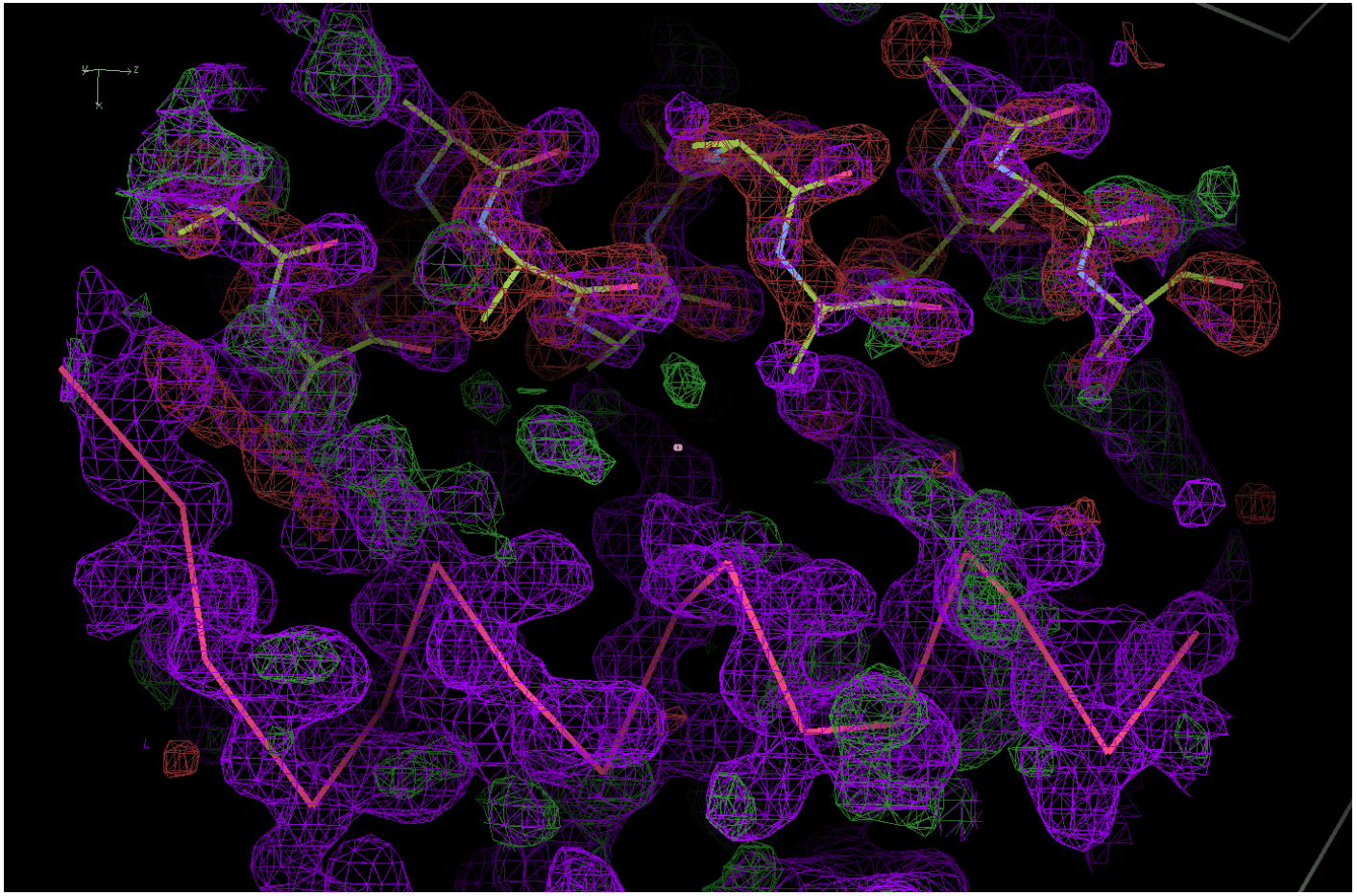
Example 1: after initial refinement



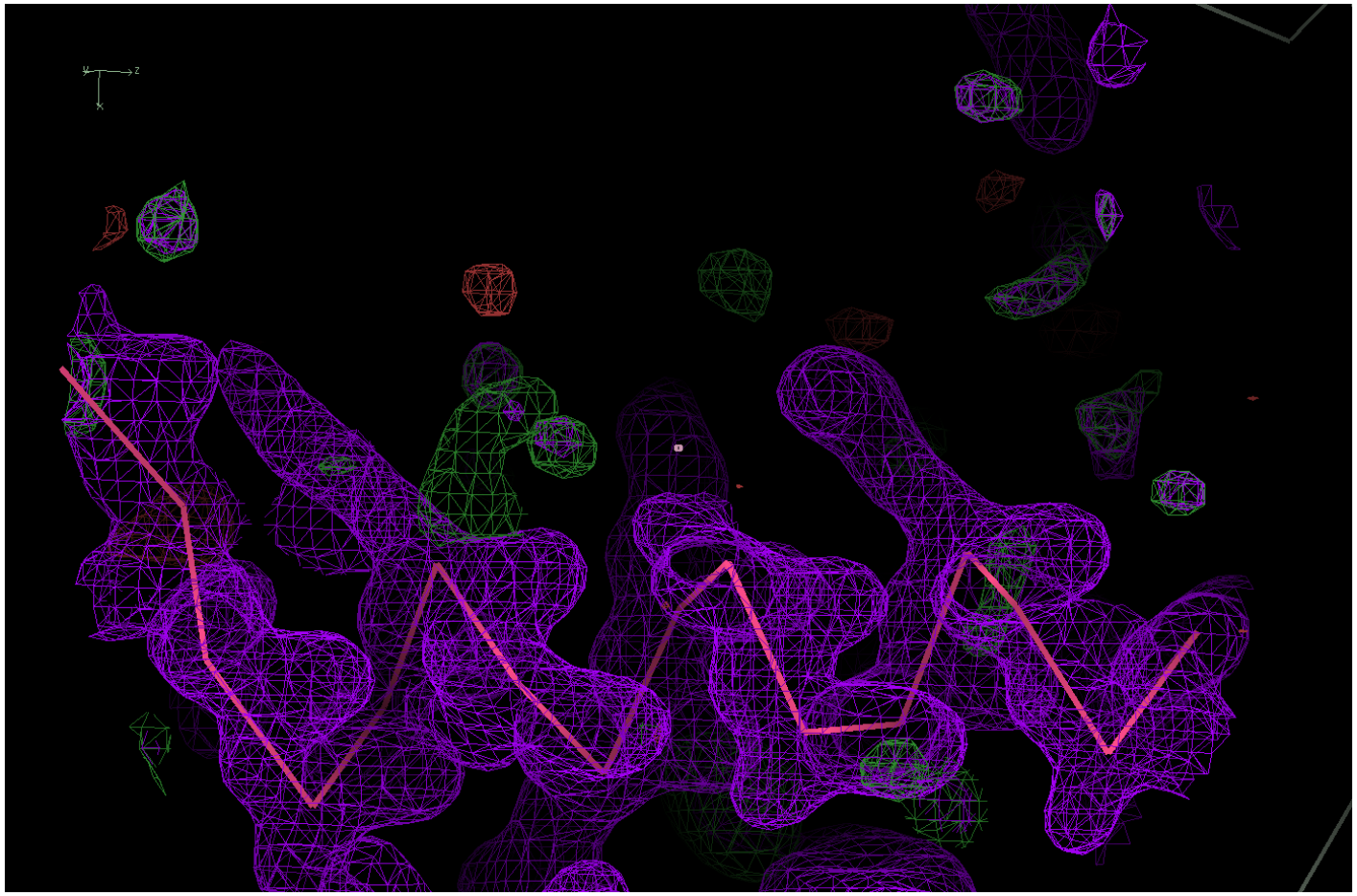
Example 1: helix added



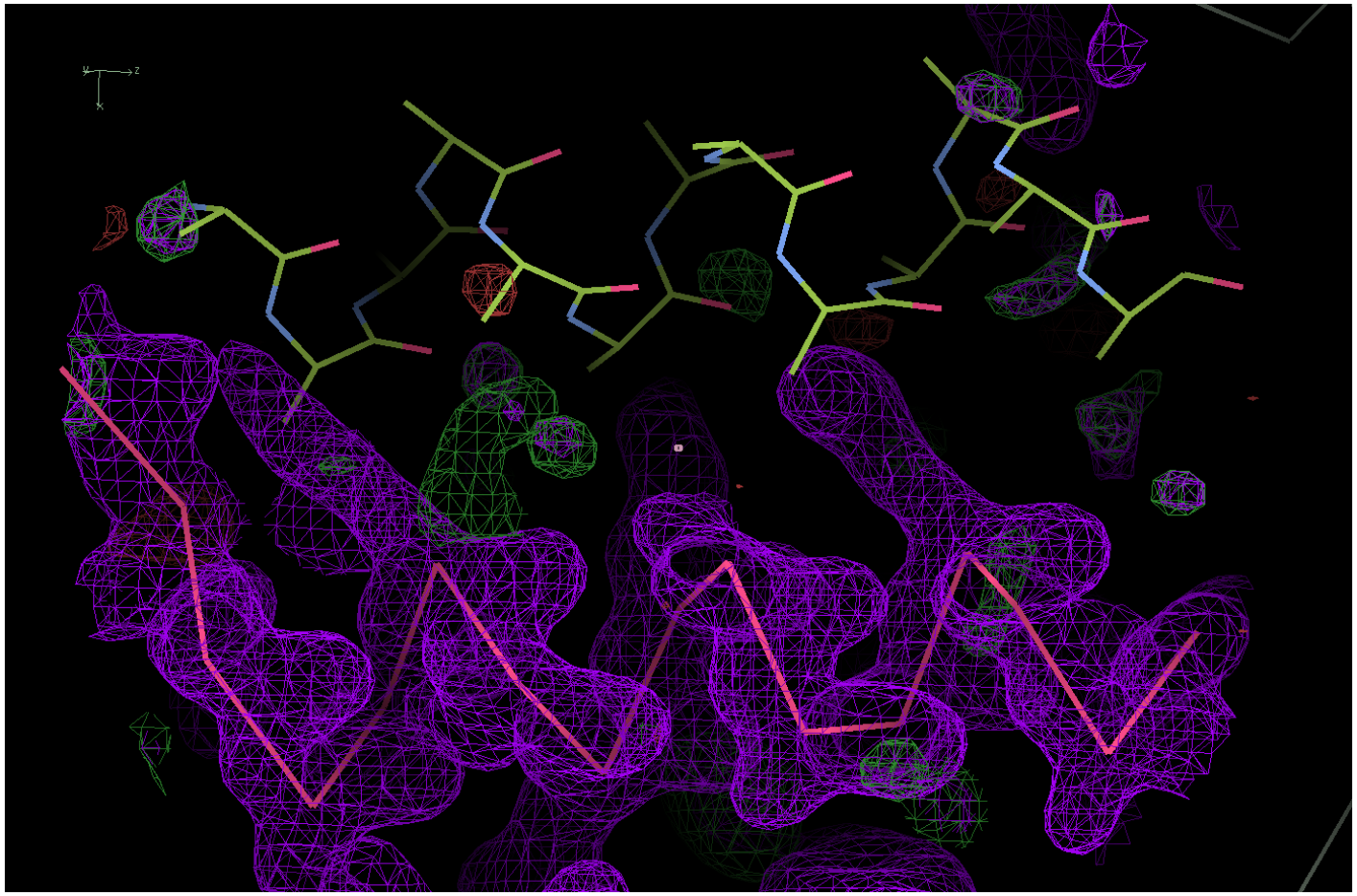
Example 1: after refinement with extra helix



Example 1: after refinement against demodulated data ...



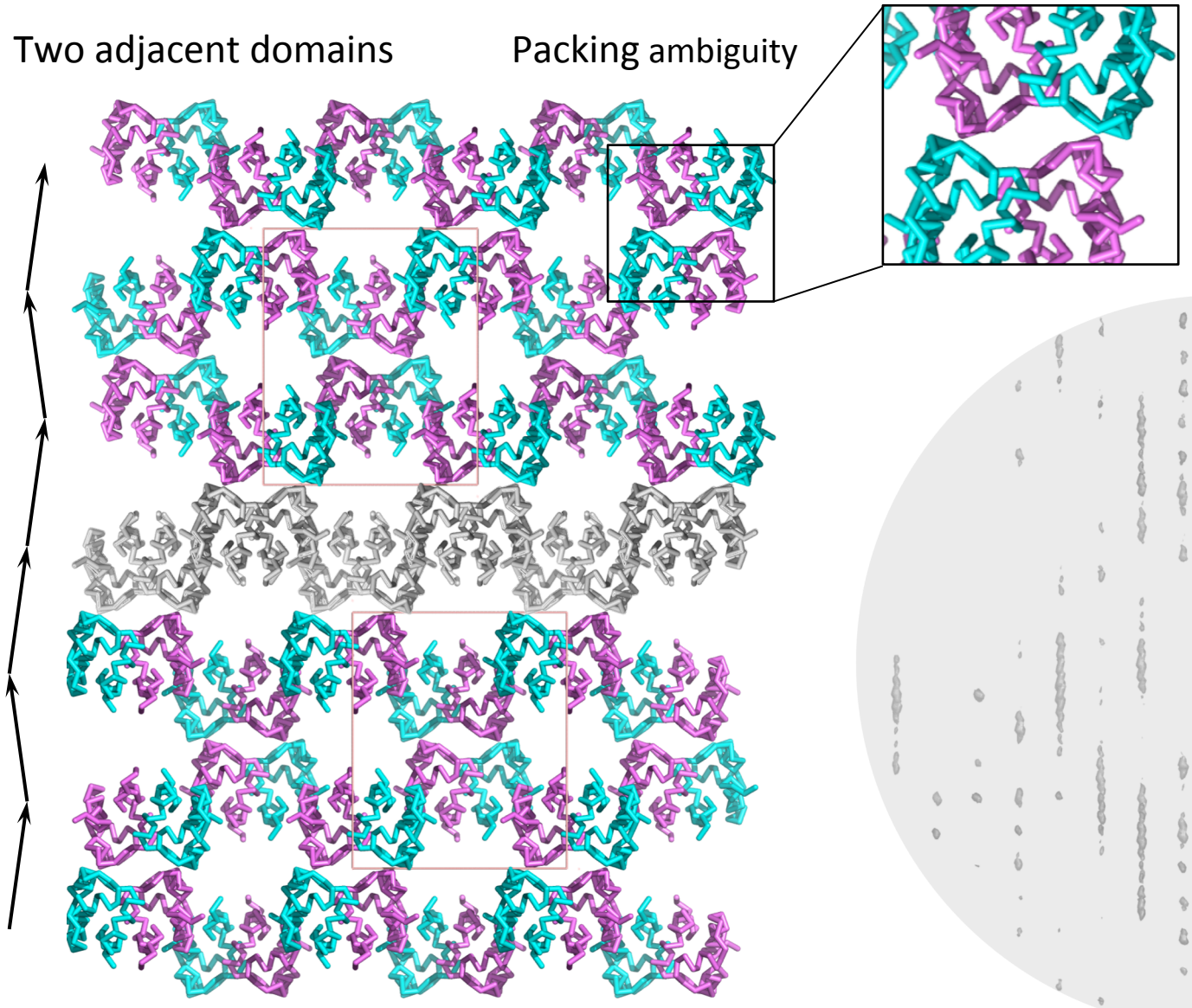
Example 1: ... there is no ED for the extra helix



Example 1: ghost density

Two adjacent domains

Packing ambiguity



Translation symmetry is only local and hence spots are smeared in vertical direction



Example 1: Summary

- Partial disorder in OD structures results in ghost density
- Structure can be solved and refined to the extent that general correctness of the solution (and identity of the crystallised protein) are beyond doubt
- Demodulation procedure removes ghost density and therefore helps with interpretation of the true density
  - » Not always badly needed

Example 2: auto-indexing failure

Fast DP @ DIAMOND

R_{meas} = 0.12 ?

Completeness = 96%

CC(1/2) = 0.3 at 1.56 Å

Refinement

R_{cryst} = 0.33 ?

R_{free} = 0.36

Molecular Replacement ✓

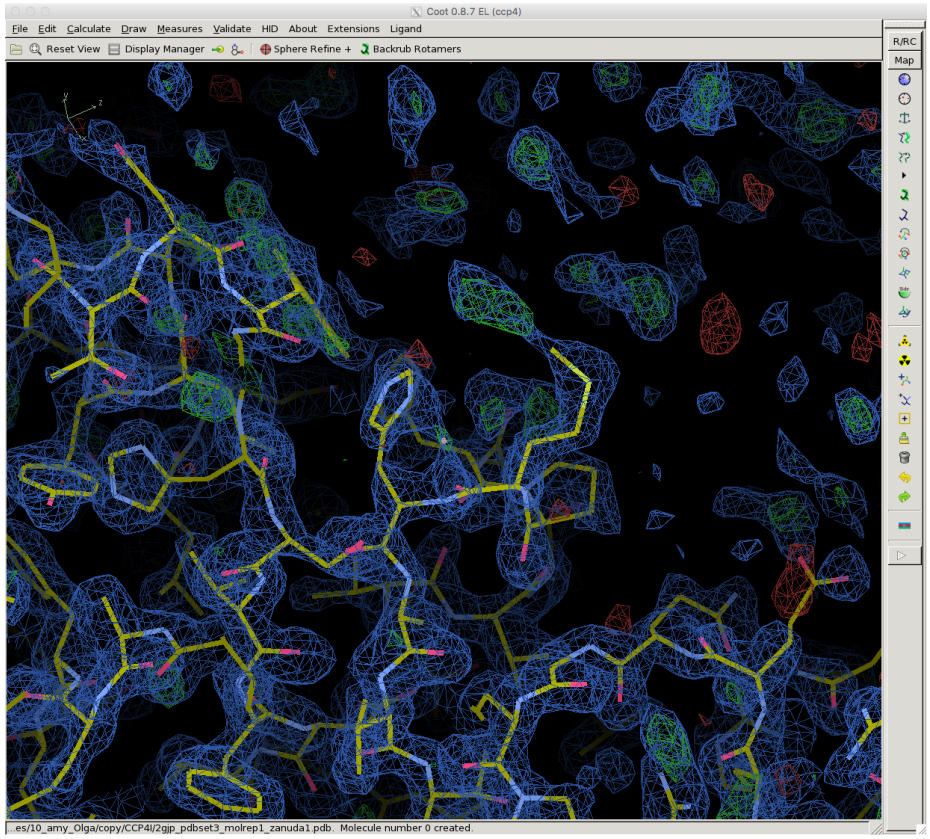
--- Peaks of Rotation Function ---

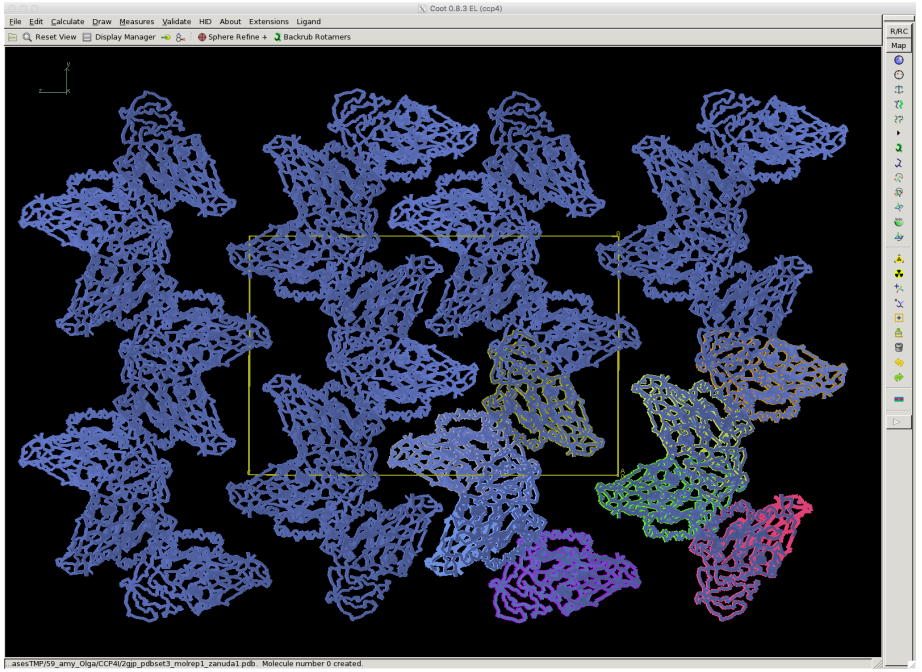
	theta	phi	chi	Rf/sigma
1	63.62	174.24	148.98	13.70
2	80.19	-58.05	61.61	13.63
3	149.48	-148.30	170.26	13.34
4	107.22	84.22	129.22	13.04
5	87.46	75.99	136.16	12.18
6	111.97	-14.20	175.28	12.10
7	157.20	173.73	153.99	11.25
8	58.77	-96.16	51.96	11.24
9	75.76	-63.11	54.46	6.21
10	102.46	82.67	133.90	5.83

Example 2: evidences of wrong indexing

Maps ?

Crystal Packing ✗



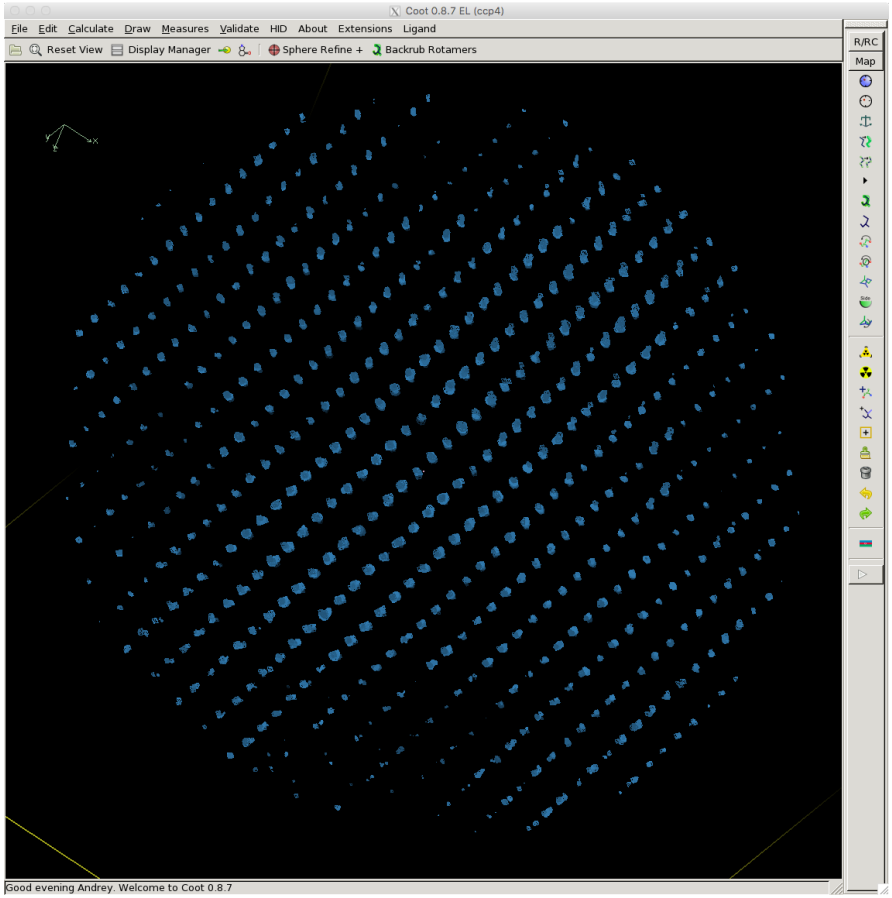


Wrong indexing?

Example 2: evidences of partial disorder

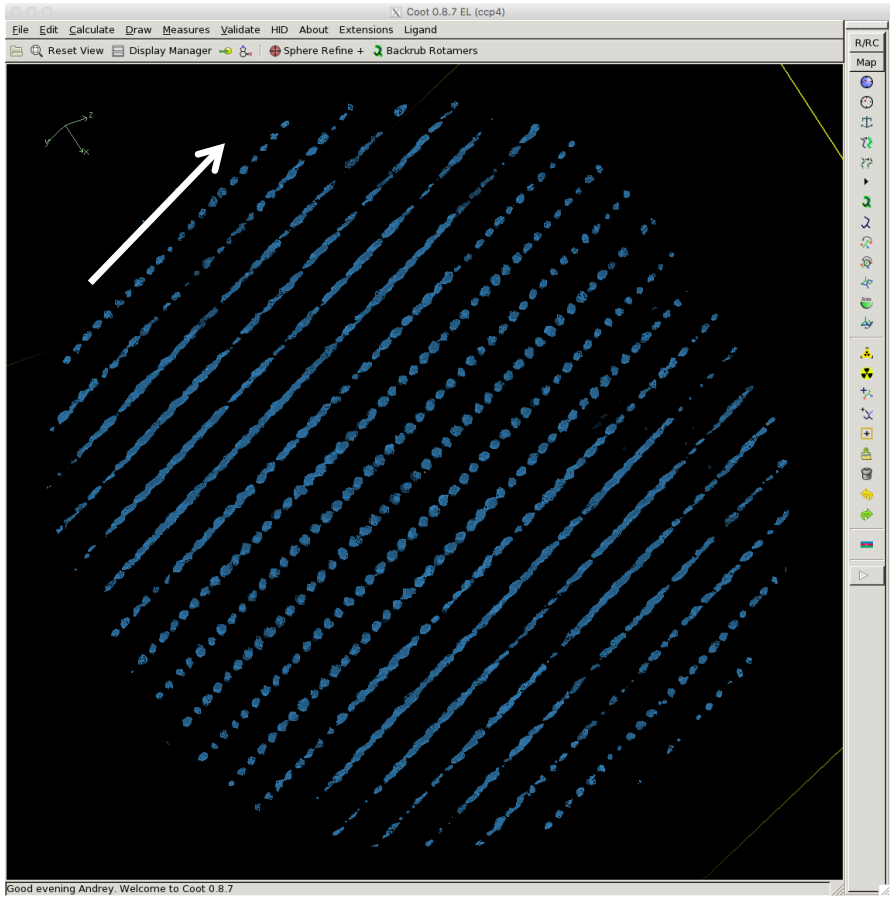
front view

There is global 2D translational symmetry in the plane of figure



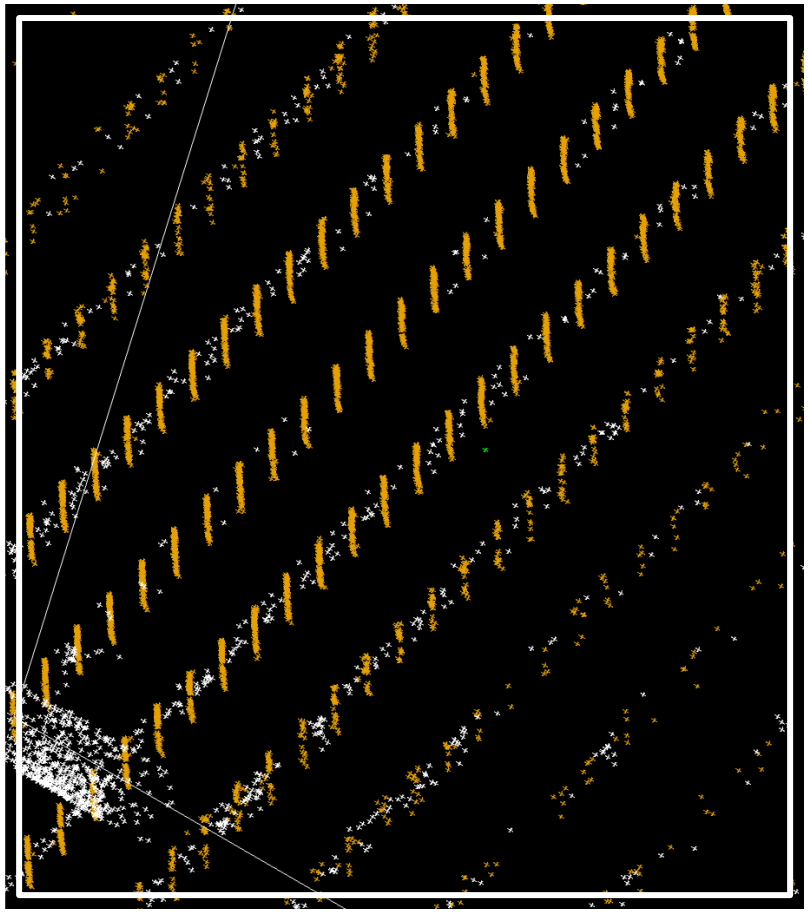
side view

White arrow indicates direction in which translational symmetry is not global (only within individual domains)



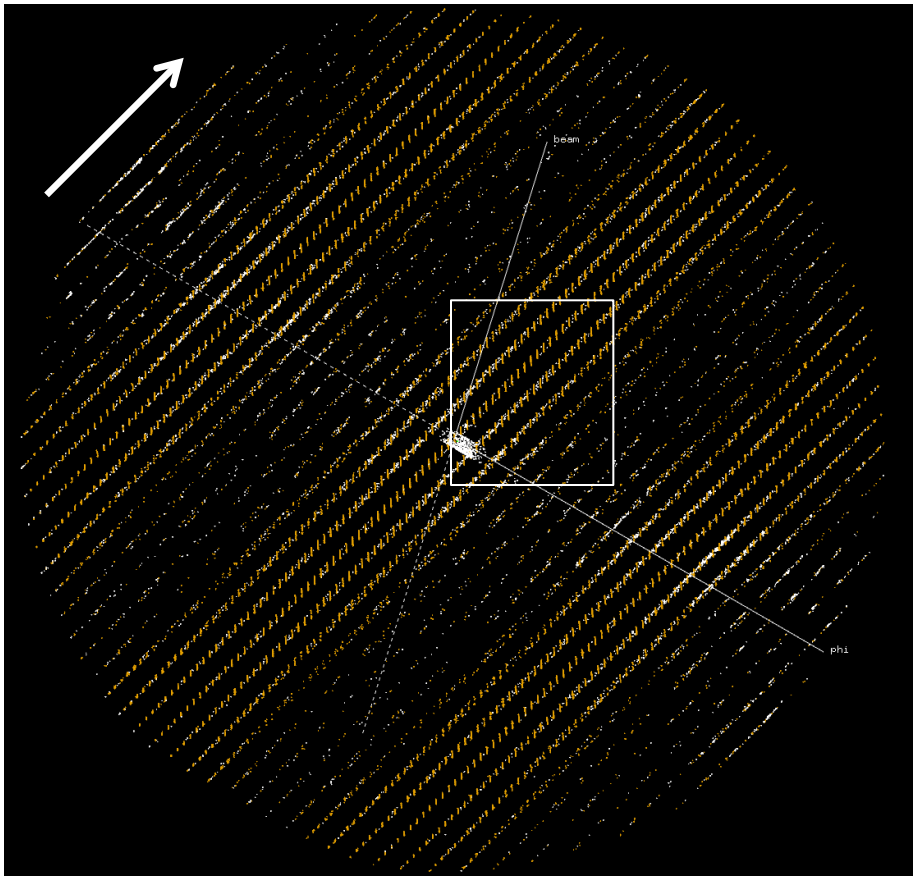
Example 2: correct indexing

White “spots” are not indexed; actually, these are tails of diffuse reflections



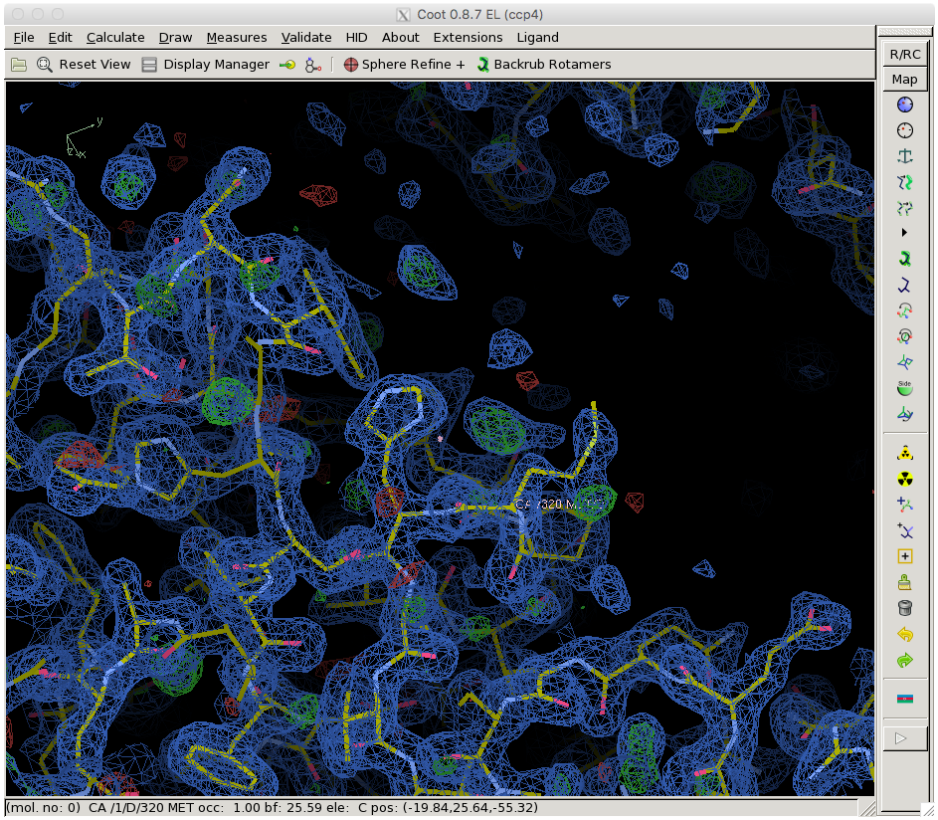
Automated indexing may easily fail (next example will show how)

White arrow indicates direction in which translational symmetry is not global (only within individual domains)
There are also areas with less spots

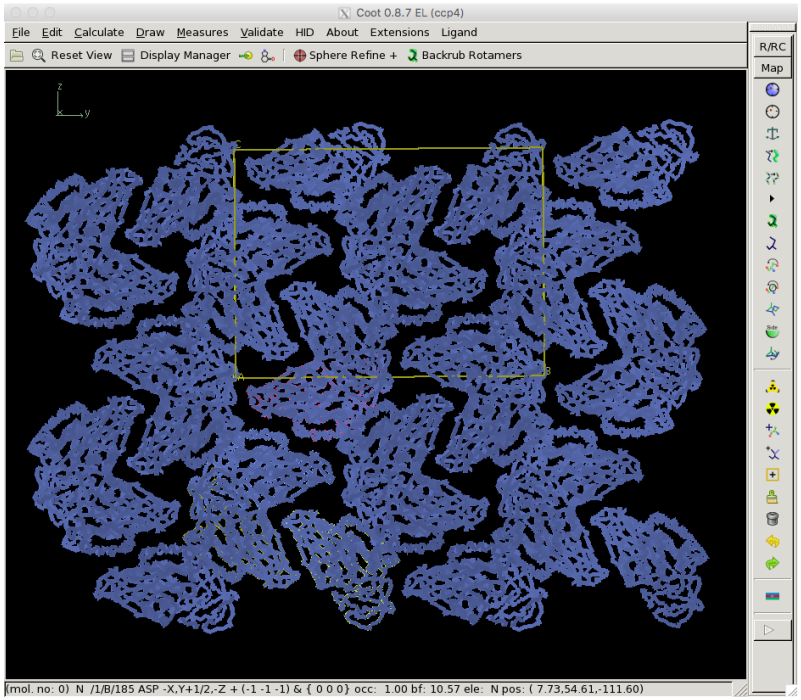


Example 2: happy end

Maps ✓



Crystal Packing ✓



Refinement ✓

R_{cryst} = 0.23

R_{free} = 0.26

Example 2: Summary

- Partial disorder a frequent reason of indexing failure
- Diffraction analysis in 3d is a good diagnostic tool for both partial disorder and for incorrect indexing
- Warning: high contrast in MR can be obtained even for wrongly indexed data provided that the search model is highly similar to the target
- Molecular replacement is quite tolerant to partial crystal disorder
  - » Especially RF
  - » In the next example this property of RF will be utilised

Example 3: unsolvable structure

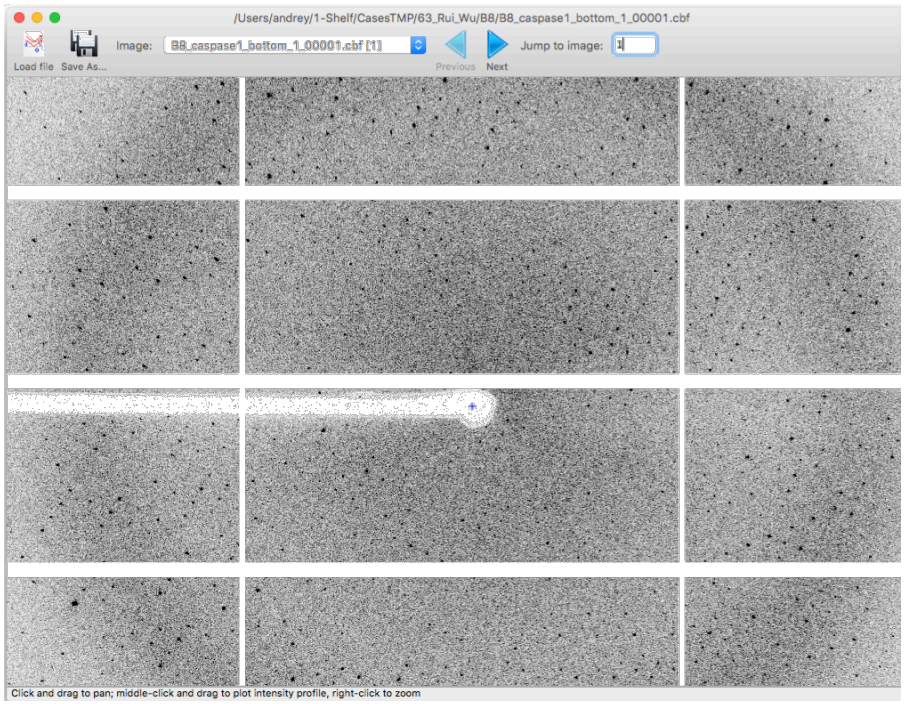
Input information:

- Images are good
  - But there a several different indexing solutions
- 99% homologue for Molecular Replacement
  - But no MR solution
  - Even more, no contrast on Rotation Function
- Twinning?

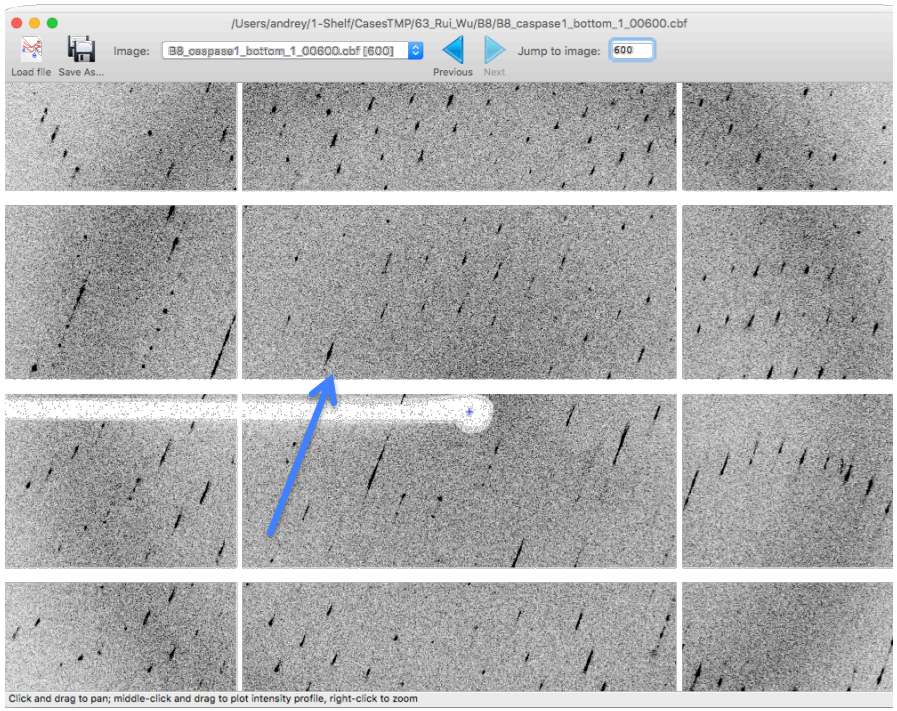
Example 3: first and last images

Partial disorder was not detected directly from images

first image



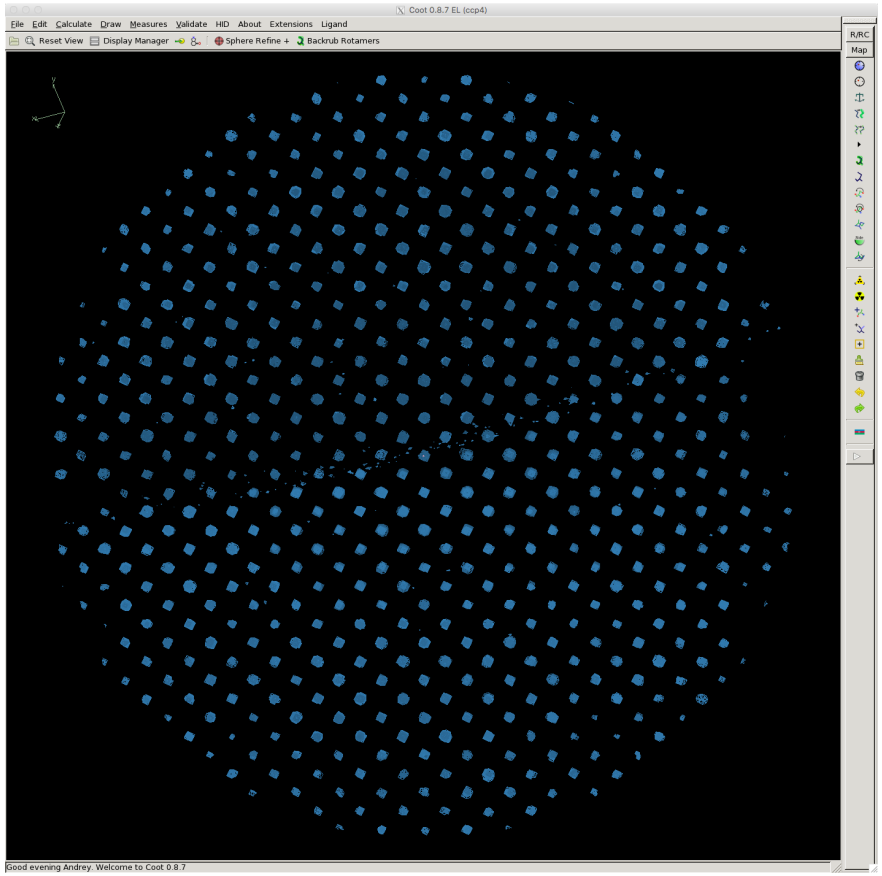
last image



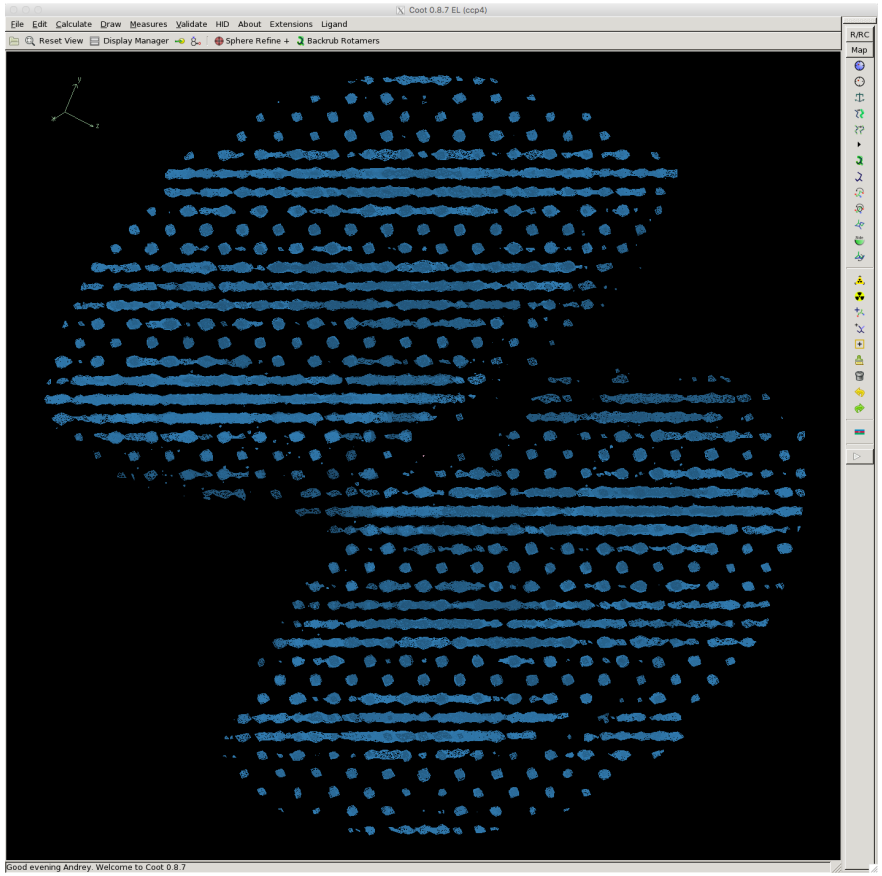
Blue arrow – direction of missing global translation

Example 3: checking diffraction in 3D

front view



side view



Clear partial disorder

Example 3: wrong and correct indexing

```
dials.index datablock.json strong.pickle
dials.refine_bravais_settings experiments.json indexed.pickle
```

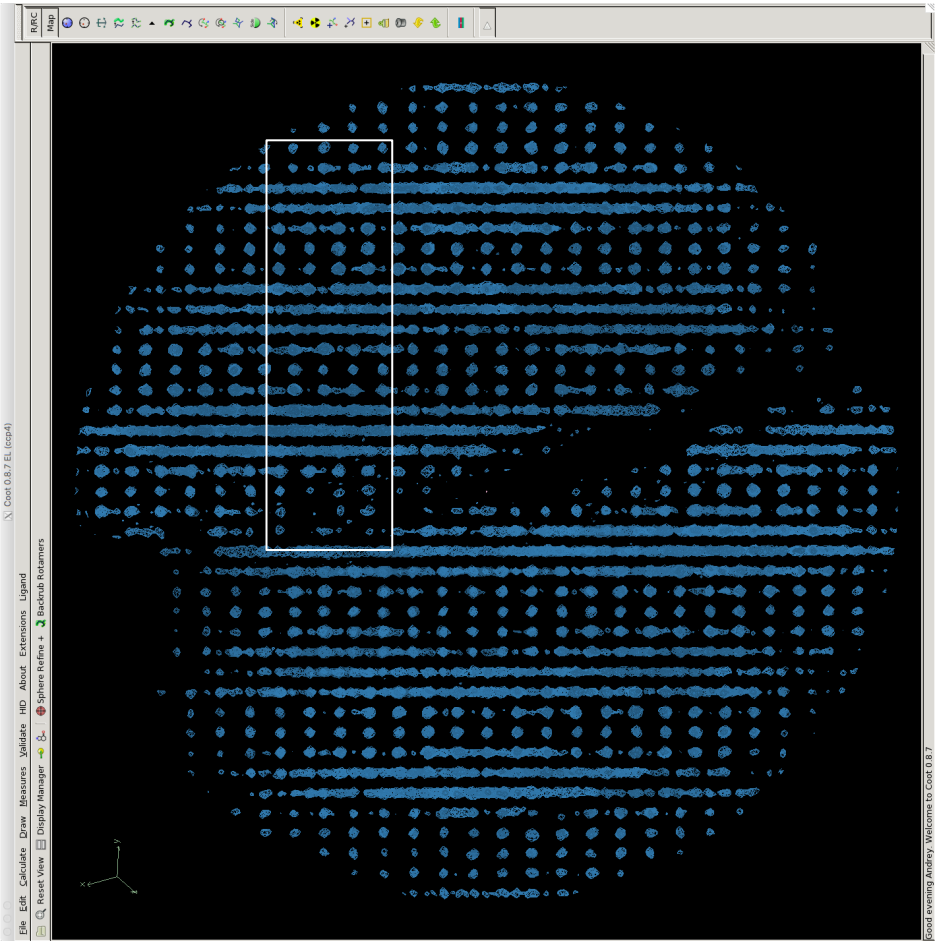
```
1_spots — -bash — 117×9
---------------------------------------------------------------------------------------------------------------------------------
Solution Metric fit   rmsd  min/max cc #spots lattice                                  unit_cell  volume          cb_op
---------------------------------------------------------------------------------------------------------------------------------
       5     2.9808 1.922 0.400/0.828  12000      oI  87.66 103.91 117.35  90.00  90.00  90.00 1068882   -c,a+b-c,a-b
       4     2.9808 1.873 0.400/0.400  12000      mI 103.84  87.53 117.25  90.00  89.69  90.00 1065729    a+b-c,c,a-b
       3     2.9805 1.924 0.425/0.425  12000      mI  87.50 103.84 117.17  90.00  90.13  90.00 1064596 -c,-a-b+c,-a+b
       2     0.0443 0.341 0.828/0.828  12000      mI  89.10 117.66 104.81  90.00  92.98  90.00 1097231  -c,a-b,-a-b+c
       1     0.0000 0.343         -/-  12000      aP  89.09  89.15  89.05  62.01  62.04  82.57  547871         a,b,c
---------------------------------------------------------------------------------------------------------------------------------
```

```
dials.index datablock.json strong.pickle unit_cell=118,134,139,90,90,90 space_group=C222
dials.refine_bravais_settings experiments.json indexed.pickle
```

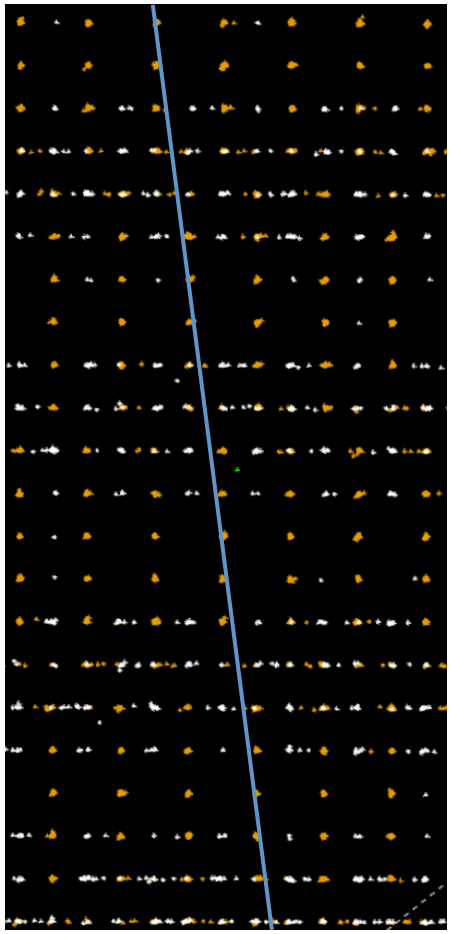
```
1_spots — -bash — 117×9
---------------------------------------------------------------------------------------------------------------------------------
Solution Metric fit   rmsd  min/max cc #spots lattice                                  unit_cell  volume          cb_op
---------------------------------------------------------------------------------------------------------------------------------
       5     0.0000 0.067 0.871/0.913  12000      oC 117.62 133.96 139.16  90.00  90.00  90.00 2192669 a+b,-a+b,c
       4     0.0000 0.066 0.913/0.913  12000      mC 133.96 117.62 139.14  90.00  90.02  90.00 2192369  a-b,a+b,c
       3     0.0000 0.067 0.871/0.871  12000      mC 117.62 133.96 139.16  90.00  90.00  90.00 2192772 a+b,-a+b,c
       2     0.0000 0.062 0.912/0.912  12000      mP  89.14 139.13  89.09  90.00  97.43  90.00 1095633   -a,-c,-b
       1     0.0000 0.060         -/-  12000      aP  89.14  89.09 139.12  89.98  90.01  97.43 1095521      a,b,c
---------------------------------------------------------------------------------------------------------------------------------
```

Example 3: wrong and correct indexing

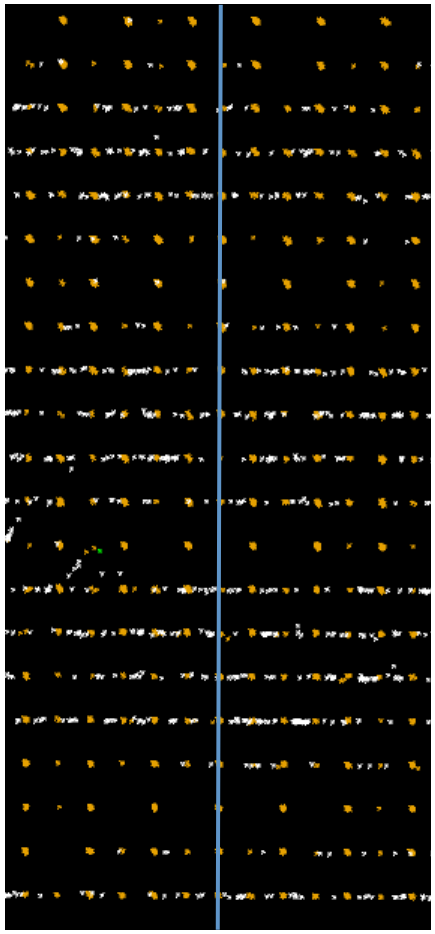
- Yellow spots are indexed, the white ones are not.
- Blue lines indicate crystallographic directions for a given indexing solution:
  - » they pass nearby yellow spots and avoid white spots



2, C (wrong)

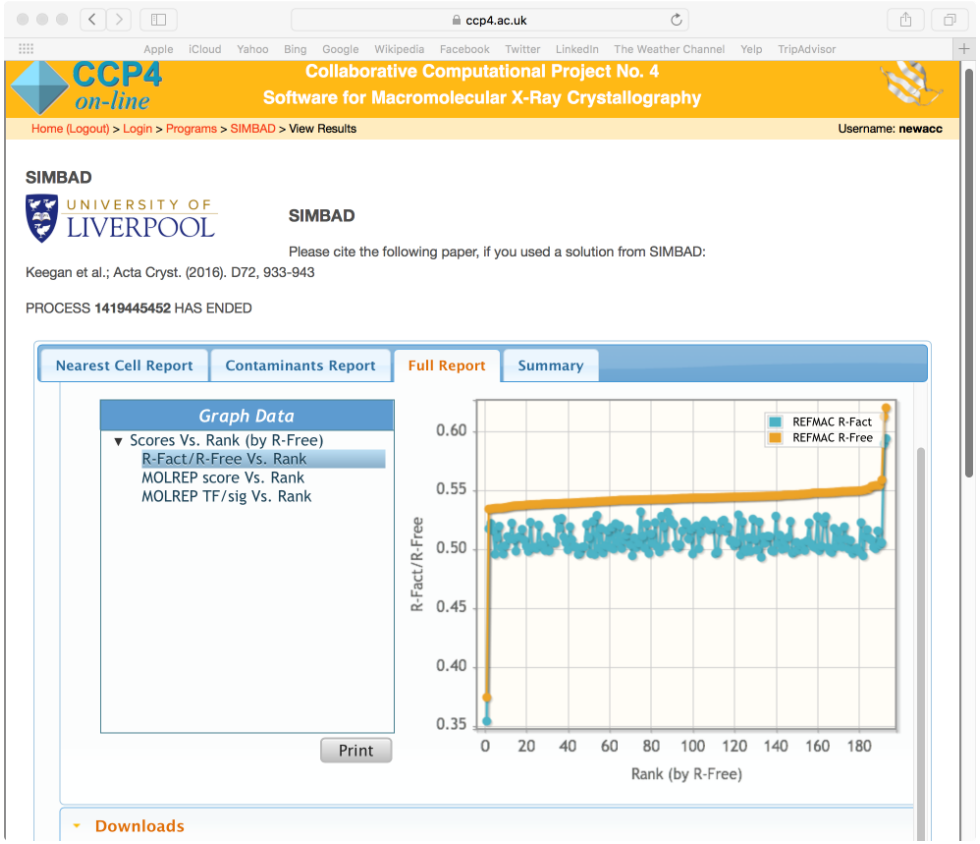


222, C (correct)



Example 3: MR against PDB (Simbad; happy end)

Despite very clean sample
a minor contaminant has crystallised:



Do not blame crystal defects for not finding a solution!

An example of misinterpreted structure of a contaminant protein is described here:

Manfred S. Weiss, M., S. et al. (2016). A critical examination of the recently reported crystal structures of the human SMN protein. *Hum. Mol. Genet.*

Partially disordered OD-structures: Summary

- Diffraction data from partially disordered OD-structures
  - are usually manageable as they are
  - in many cases may be adjusted if
    - » low R-factors are needed :)
    - » or if the ghost density prevents unambiguous interpretation of maps (usually for ligands)

- Merged data adjustment (also for non-merohedral OD-twins)
  - There are several bespoke scripts around
  - A general automated software solution is needed

- Ideal solution is integration of streaked spots
  - maybe implemented as reiteration of diffraction image processing based on structural information

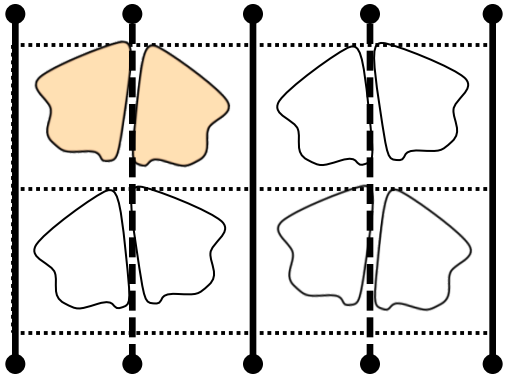
Pseudo-translation

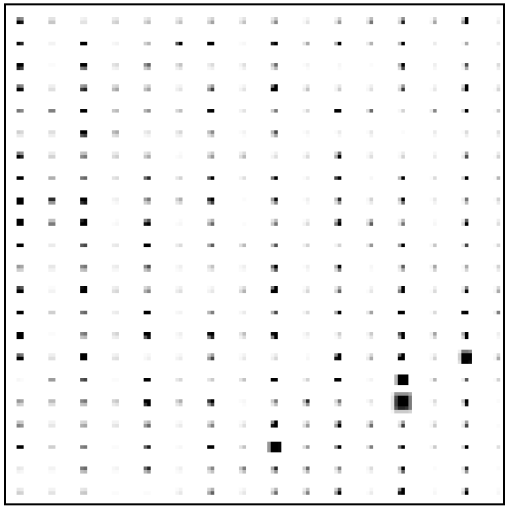
- Visualisation
- Effect on indexing
- Pseudo-origin MR solutions

Pseudotranslation

Crystallographic translation

Pseudo-translation

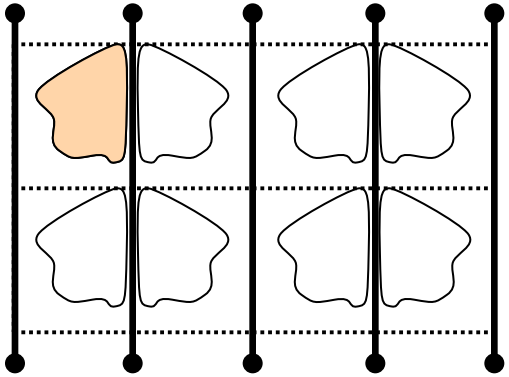


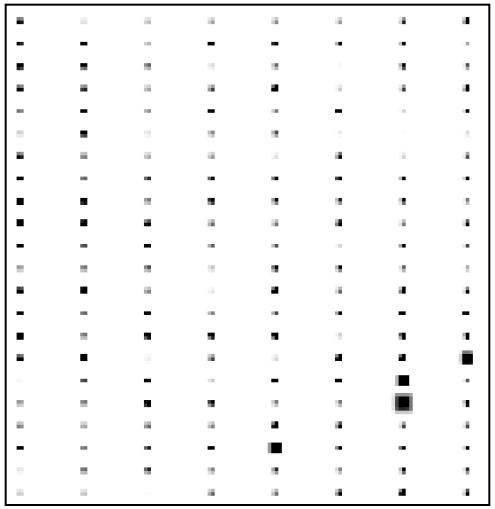


Pseudotranslation **C**/2

Planes 2L+1 contain weak reflections

Crystallographic translation





Limiting case, **C'** = **C**/2

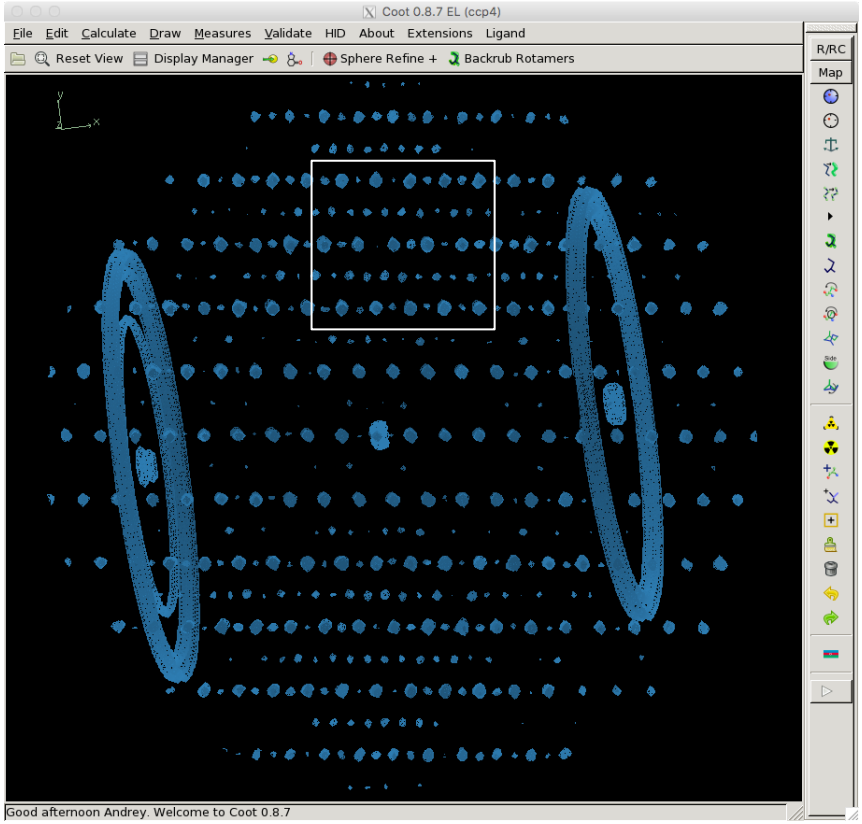
- Weak reflections vanish

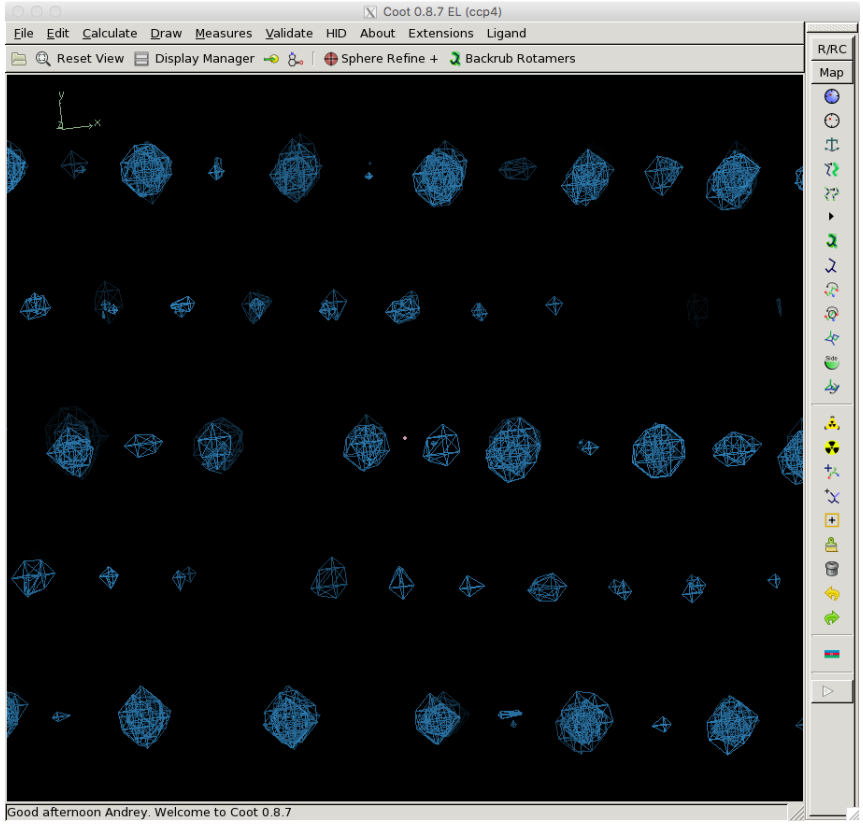
Two times larger reciprocal lattice spacing

Example: two pseudo-translation vectors

Example from Victor Lamzin, YSBL-DESY

	point group	lattice type	*a* (Å)	*b* (Å)	*c* (Å)
Space group	222	*C*	74.9	122.8	125.0
Pseudo-symmetry space group	222	*I*	37.5	61.4	125.0



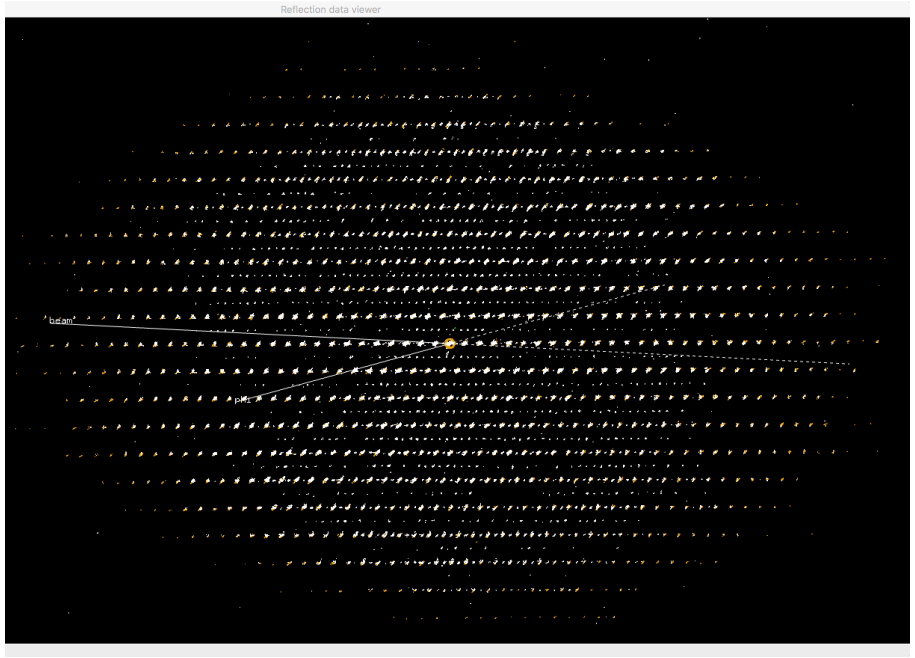


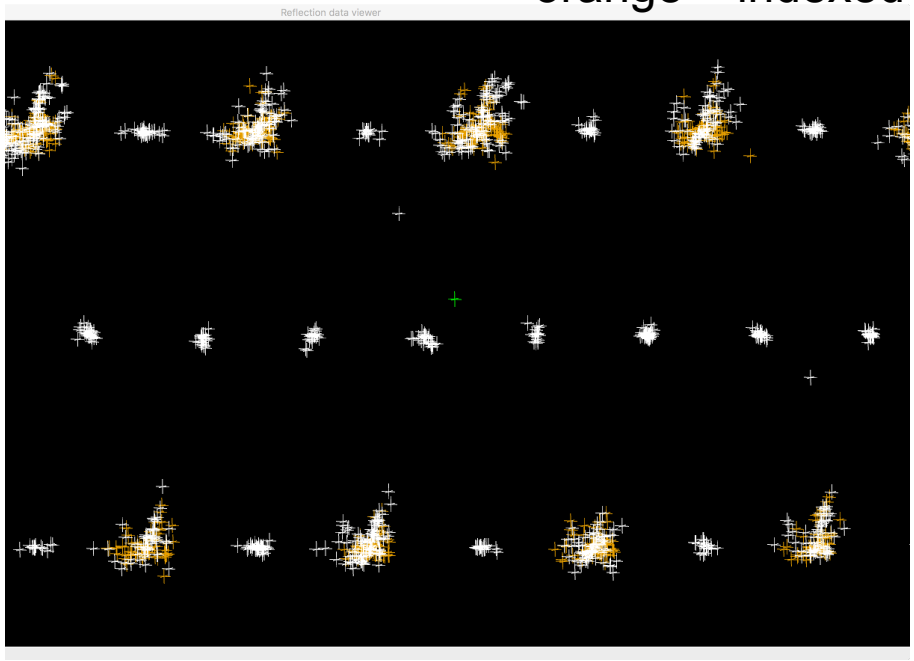
Example: two pseudo-translation vectors

Images imported as they were, oscillation 0.1°

```
dials.import template=images/SeMet_38_04_0####.cbf
dials.find_spots ...
dials.index ...
dials.refine ...
dials.reciprocal_lattice_viewer ...
```

white – not indexed
orange – indexed



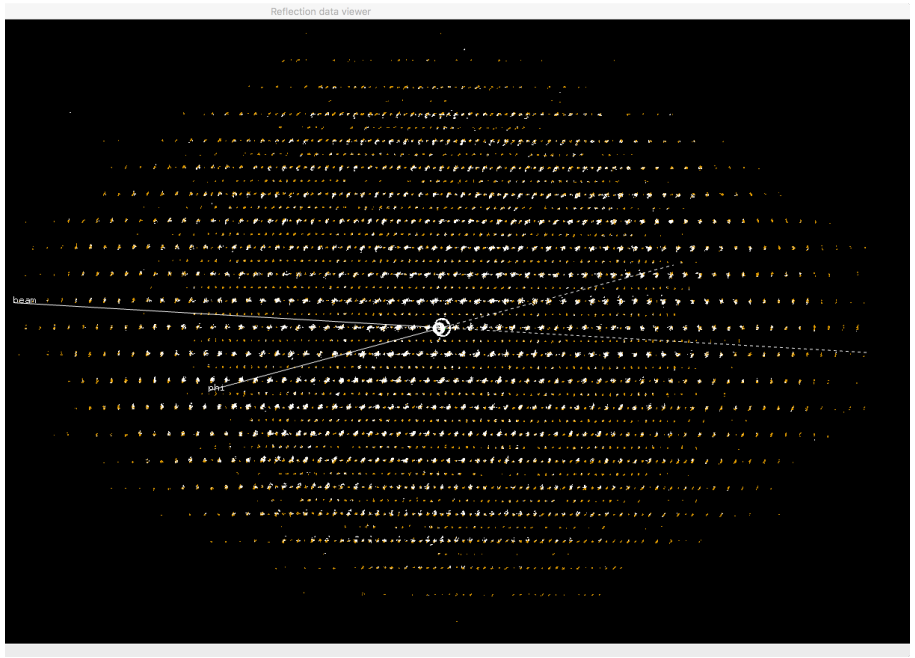


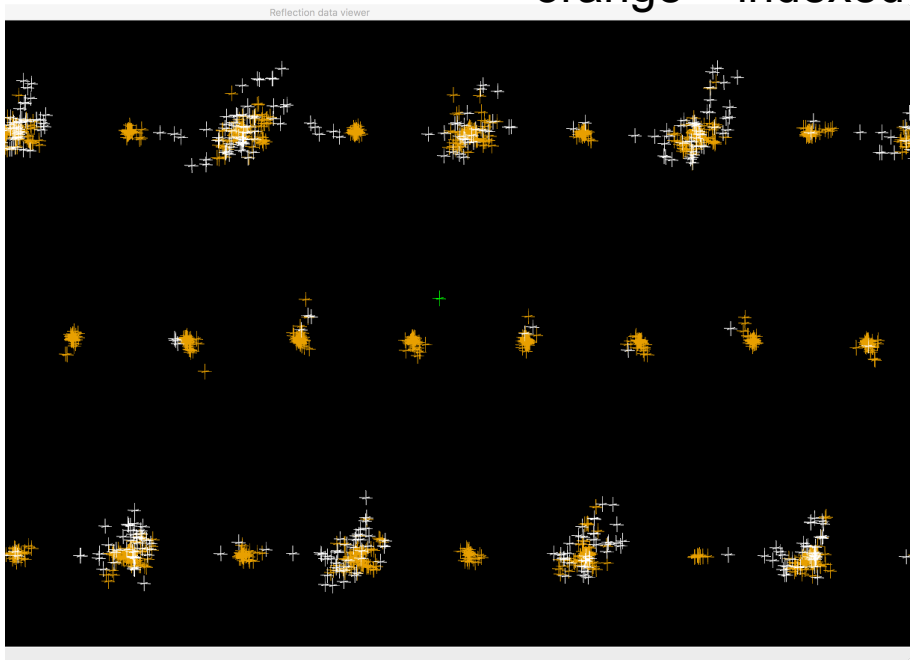
Example: two pseudo-translation vectors

Merged each 5 adjacent images to make oscillation 0.5°, then imported

```
dials.merge_cbf images/SeMet_38_04_0####.cbf merge_n_images=5
dials.import template=sum_####.cbf
dials.find_spots ...
dials.index ...
dials.refine ...
dials.reciprocal_lattice_viewer ...
```

white – not indexed
orange – indexed





Pseudo-translation and indexing

Example: structure solved using SAD; then native structure was solved by MR

Week reflections may confuse indexing programs

- A “sensible” error: weak reflections ignored = pseudo-translation(s) treated as proper crystallographic translations
  - Will not be critical for this example, as an approximate structure can indeed be determined after processing in smaller cell; the model then can be used in MR search against native data set
  - Sometimes the “cell reduction” is done intentionally to simplify the first steps of structure solution
- A serious error, e.g. monoclinic with $\beta \neq 90°$ instead of orthorhombic
  - This will not help with structure determination

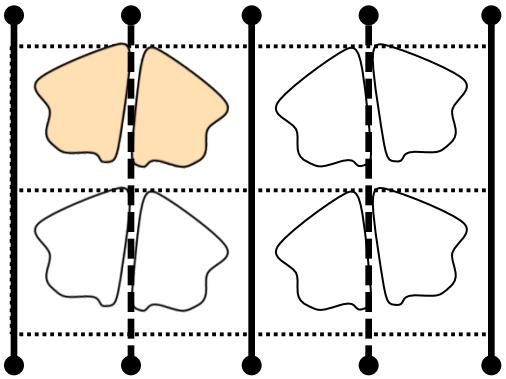
Visual control using 3D viewers is important

Pseudotranslation: what else can go wrong?

Cell and H-M symbol are the same

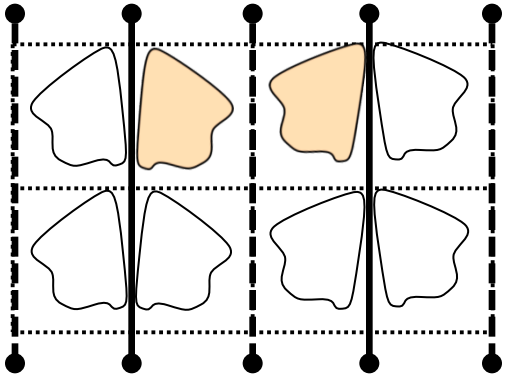
Crystallographic and pseudosymmetry axes are interchanged

P2 (a,b,c)



True structure

P2 (a,b,c)



False structure

Molecular Replacement:

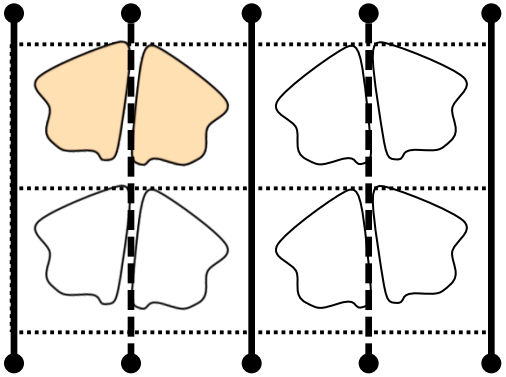
- If two structures are globally very similar (e.g. rmsd = 0.5A)
- MR can in some cases pick up a wrong solution

Pseudotranslation: what else can go wrong?

Cell and H-M symbol are the same

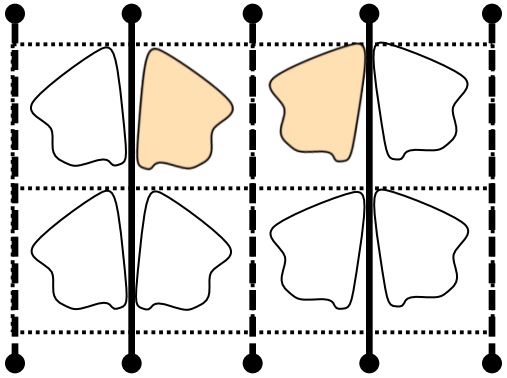
Crystallographic and pseudosymmetry axes are interchanged

P2 (a,b,c)



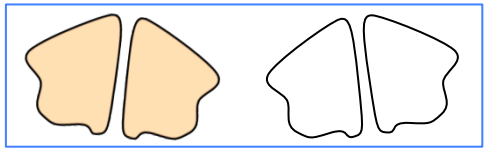
True structure

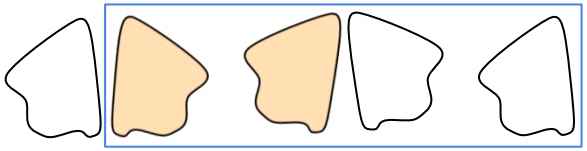
P2 (a,b,c)



False structure

Molecular graphics:
structure is shifted
relative to the unit cell



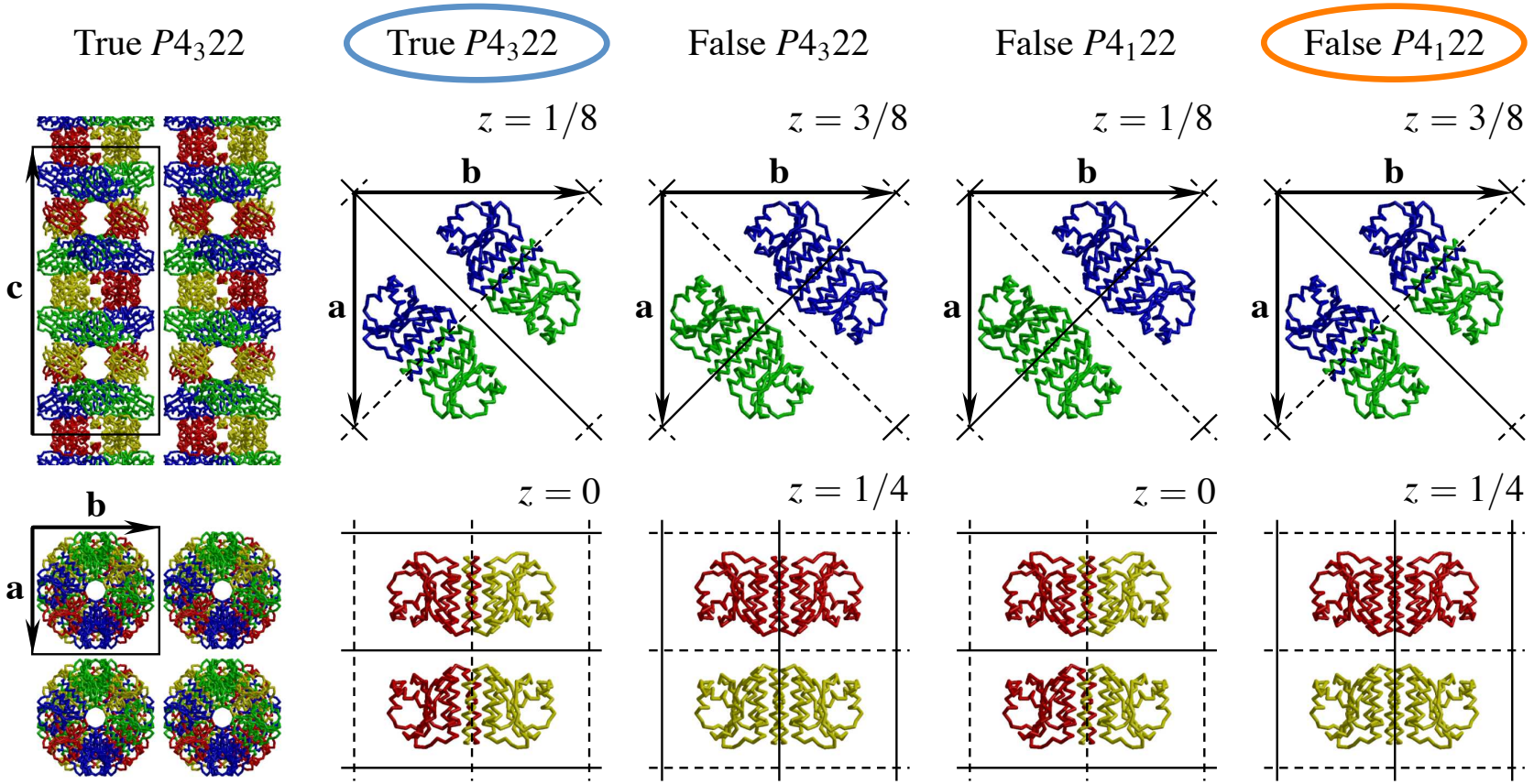


Four alternative solutions in two space groups

GAF (N-terminal) domain of CodY protein from Bacillus subtilis

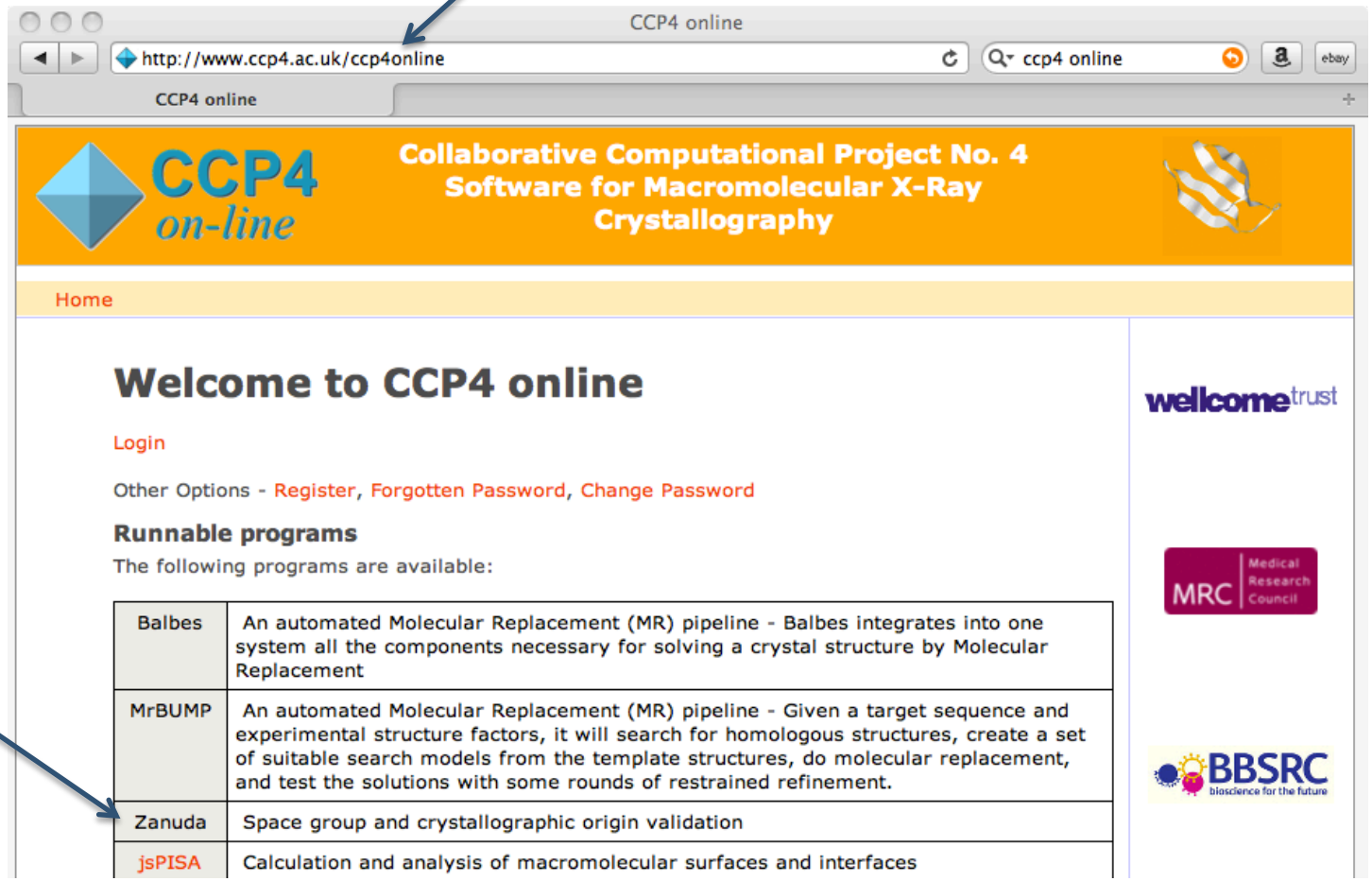
Levdikov, V. M. et al. (2006). J Biol Chem 281, 11366-73.

MR solution



CCP4 online

http://www.ccp4.ac.uk/ccp4online



Zanuda

Zanuda is also included in CCP4 program suite

Pseudo-translation: Summary

- A good reason to visually control indexing is to make sure that pseudo-translation
  - is not overlooked
  - is not an artefact
    - » Patterson map: non-origin peak almost as high as origin one (e.g. 95%)

- Pseudo-translation may result in pseudo-origin MR solutions
  This issue has to be addressed not before
  - the structure seems to almost match ED maps
  - R-free is well below 0.5, around 0.4

- Not discussed: pseudo-translation makes twinning tests difficult to analyse
  - L- and H-tests usually perform as normal