Twinning and other pathologies

Andrey Lebedev

CCP4

OD-structures

Twinning by (pseudo)merohedry

Statistics of one observation

Statistics of two observations

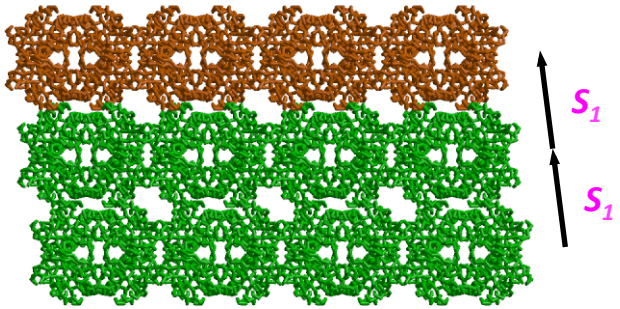
Twinning tests summary

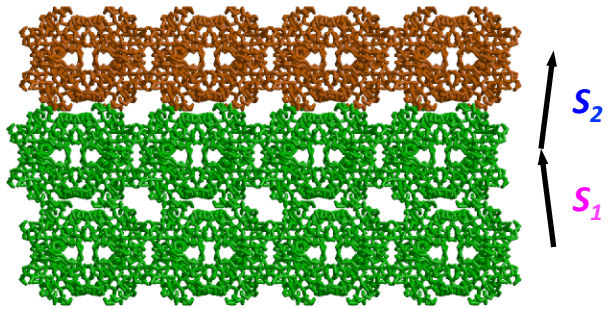
Space group validation

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



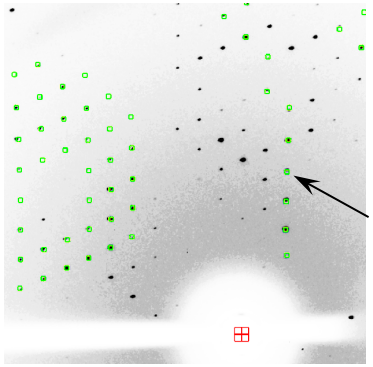


*) S_1 and S_2. are called stacking vectors

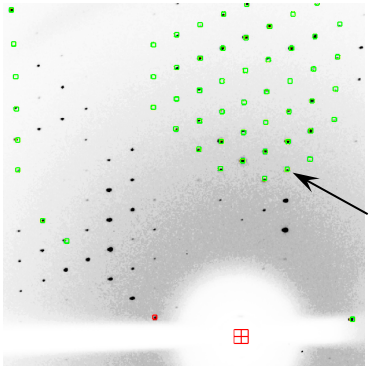
- two-dimensional periodicity
- a potential for disorder in the third dimension

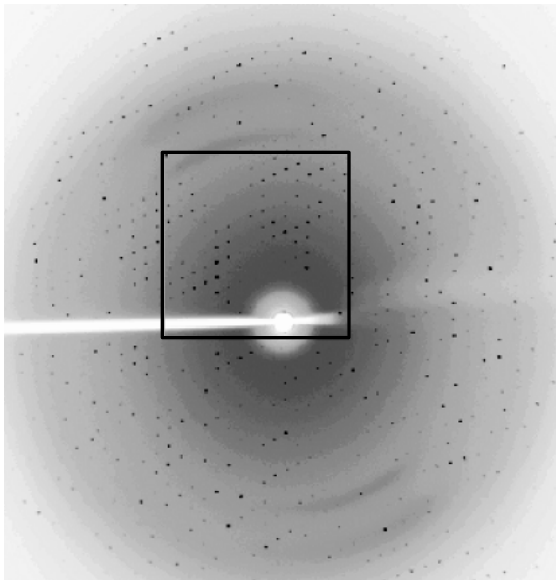
Example 1: OD-twin

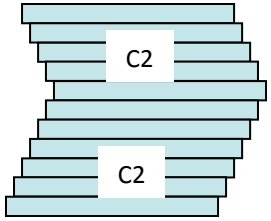
Indexing in C2



Indexing in C2





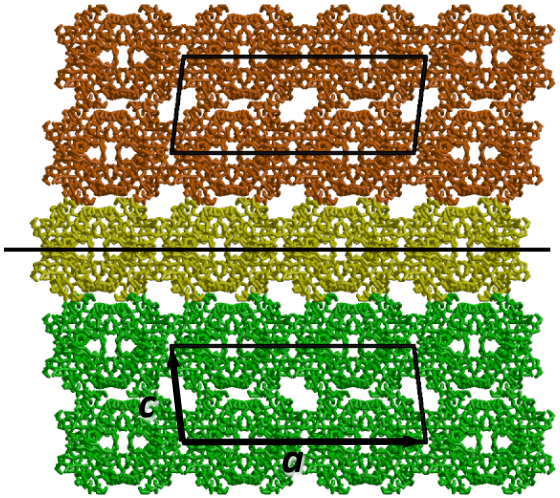


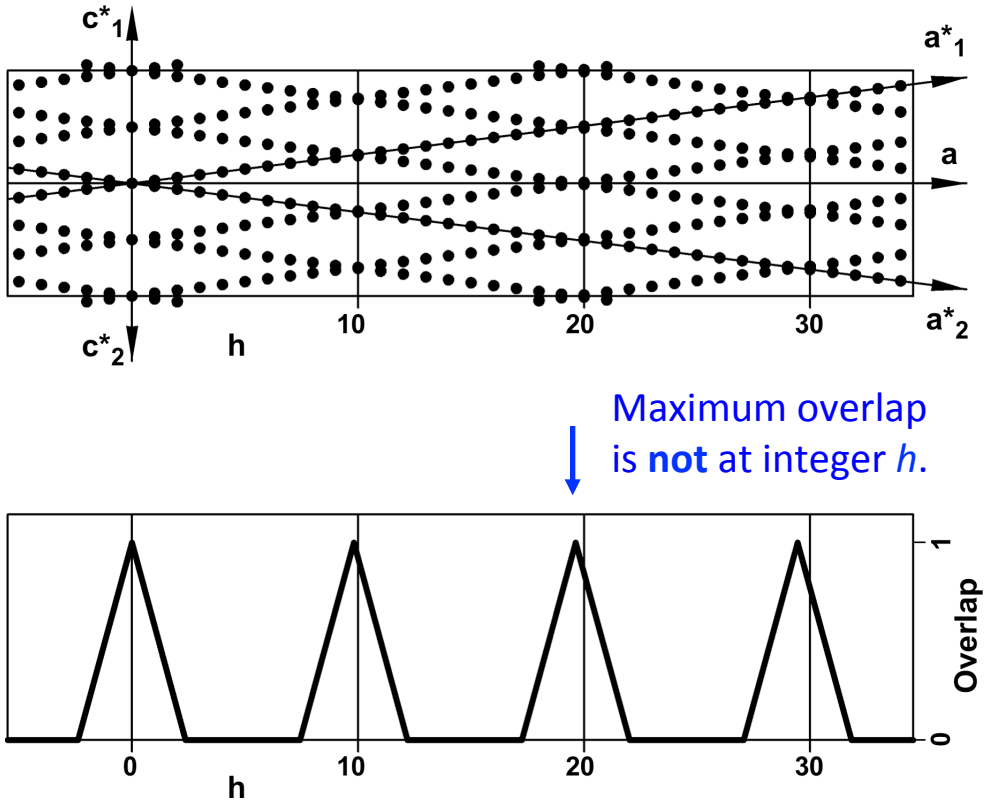
L-2-haloacid dehalogenase
from *Sulfolobus tokodaii*
Rye *et al.* (2007) *Acta Cryst.* **D**67

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

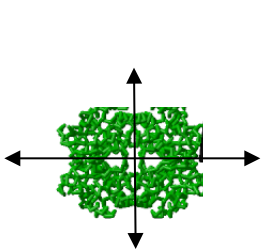


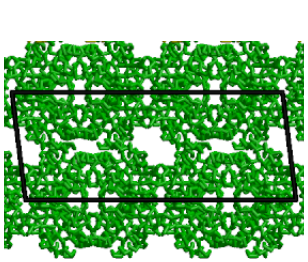


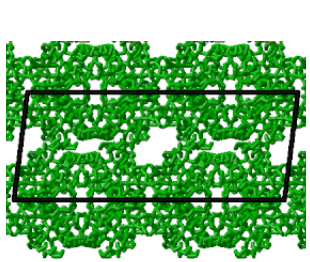
Twinning by reticular merohedry with twin index 10 and obliquity 0.1°

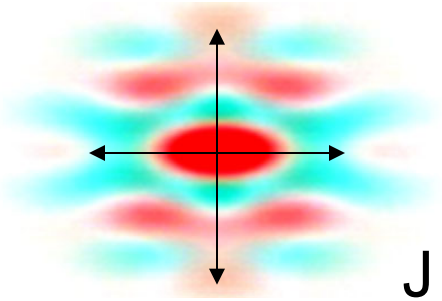
Integration of a single lattice: in effect, twinning coefficient depends on h

Intensities of the overlapping reflections

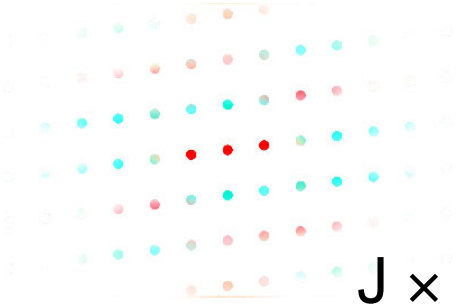




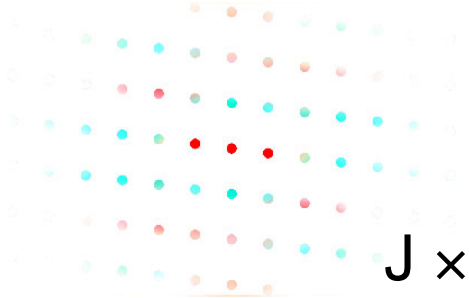




J



$J \times L_1$



$J \times L_2$

Fourier transform of the tetramer

Diffraction pattern of domain 1

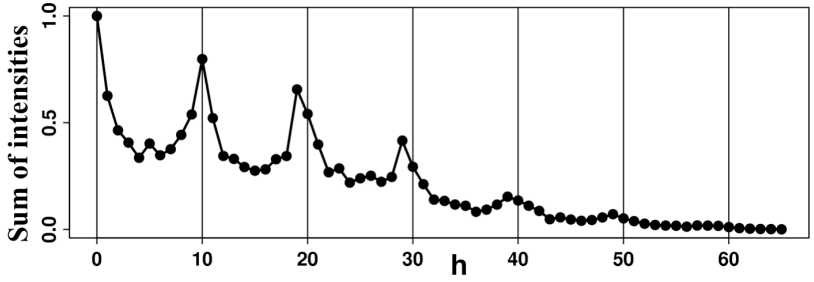
Diffraction pattern of domain 2

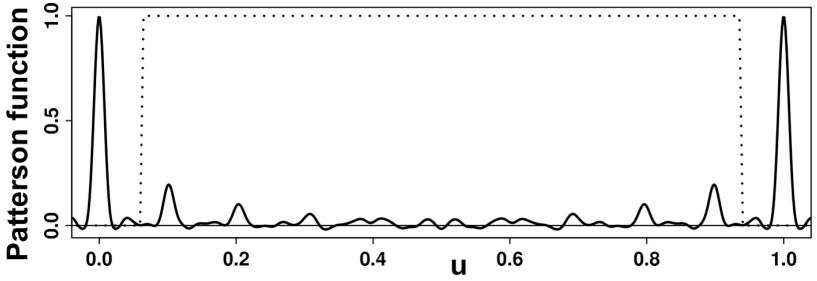
Tetramers in different twin domains are in the same orientation

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

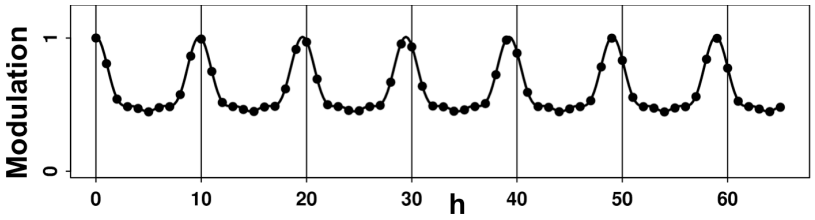
Demodulation

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



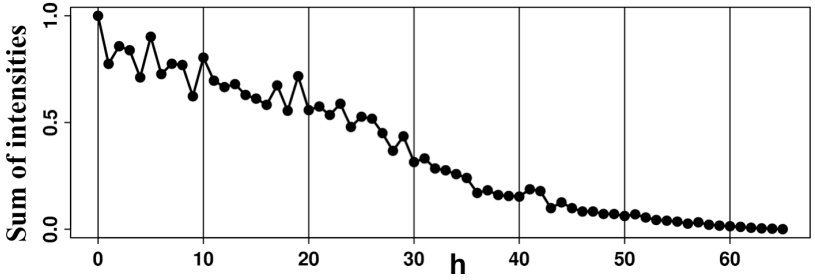


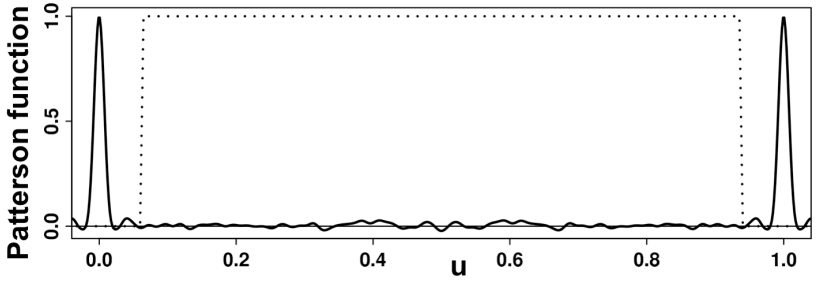
Modulation function



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

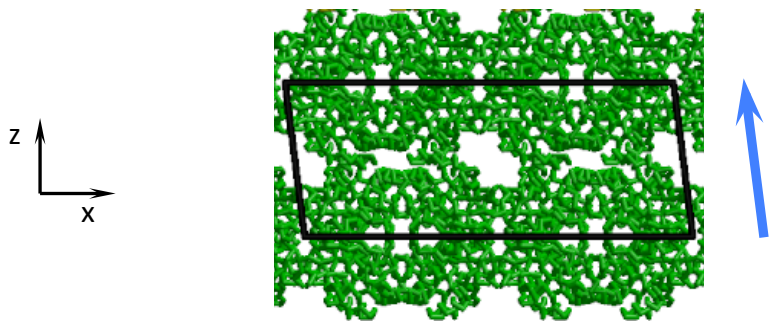
Corrected data: R / R-free = 0.16 / 0.23



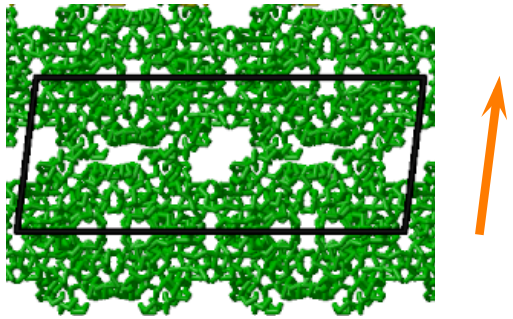


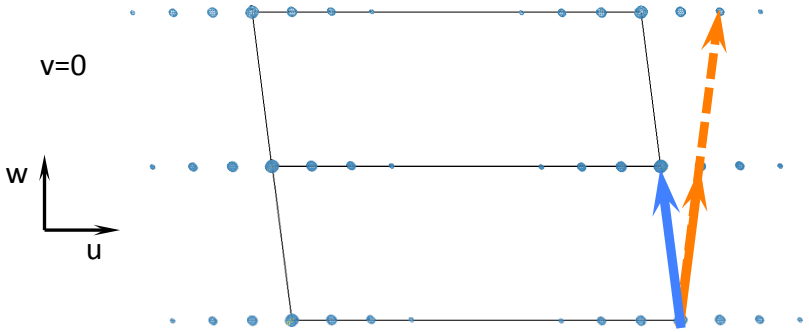
Patterson Map

Indexed lattice



The second lattice





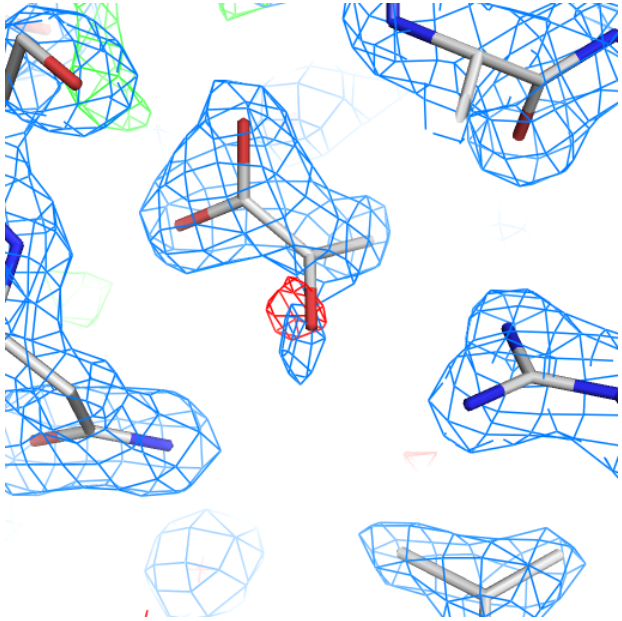
Non-origin peaks in the Patterson map:

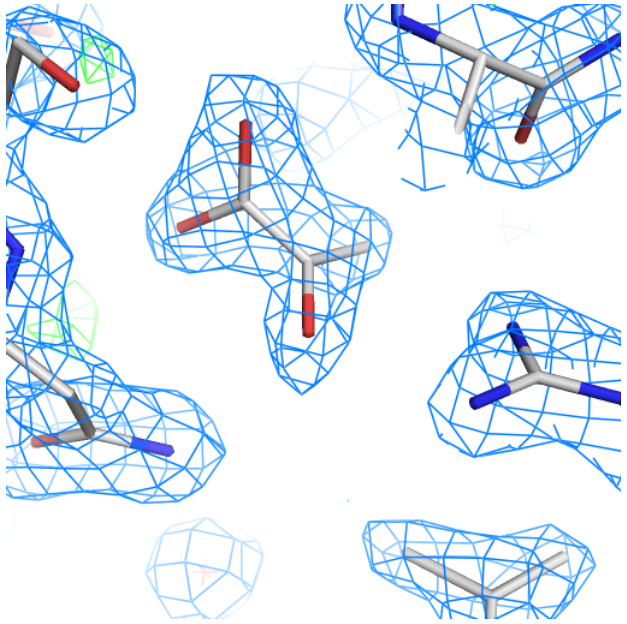
- contribution from the second lattice
- because of the overlapping spots

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)

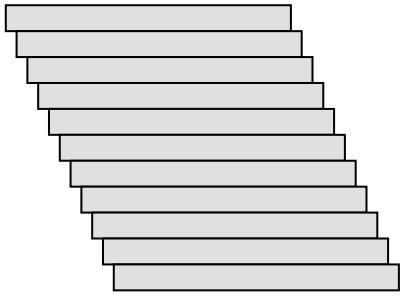
The electron density maps (2-1 at 1.5σ and 1-1 at 3σ)
around the pyruvate molecule before and after demodulation



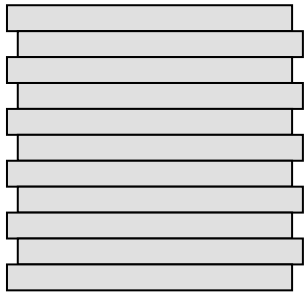


OD-structures

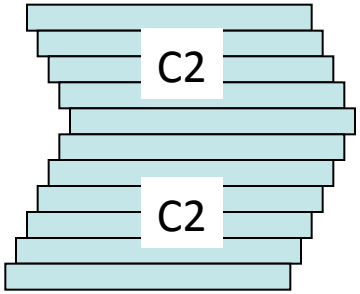
Single crystal



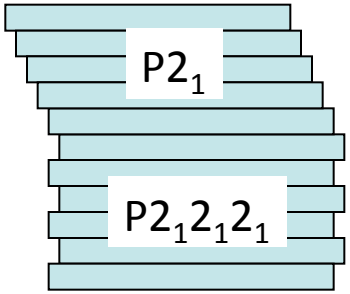
Single crystal



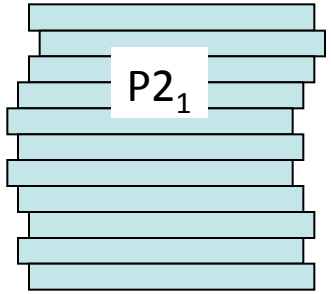
OD-twin



Allotwin



Partially disordered OD-structure



Examples 1 & 2

Example 3 & 4

Examples 4 & 5

Classification: OD-structures *vs.* twins

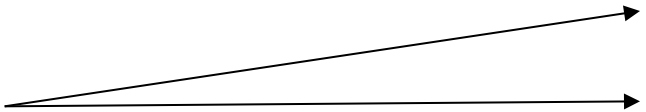
OD-structures:

Single crystals

allotwin

OD-twin

(partially) disordered OD-structure



Twinning:

by (pseudo)merohedry

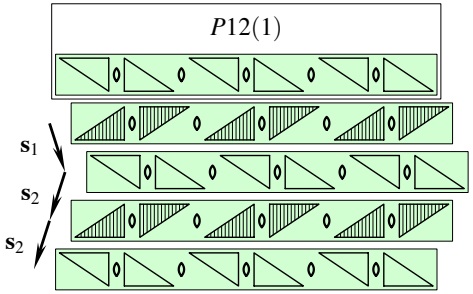
by reticular (pseudo)merohedry

...

This is structure based classification of a specific class of structures

This is geometry based classification accounting for crystal and lattice symmetries.

Symbols for groupoid symmetry



$P \quad 1 \quad 2 \quad (1)$

$\{ \quad 2_P \quad 1 \quad (2_2) \quad \}$

In 2_P, P is a non-integer subscript.

Special values of P correspond to space group symmetry or specialised groupoid symmetry

The following types are possible
(I) two surfaces of a single layer are identical;
(II) two surfaces of a single layer are different and contacts are made by different surfaces.
(III) two surfaces of a single layer are different but contacts are made by identical surfaces.

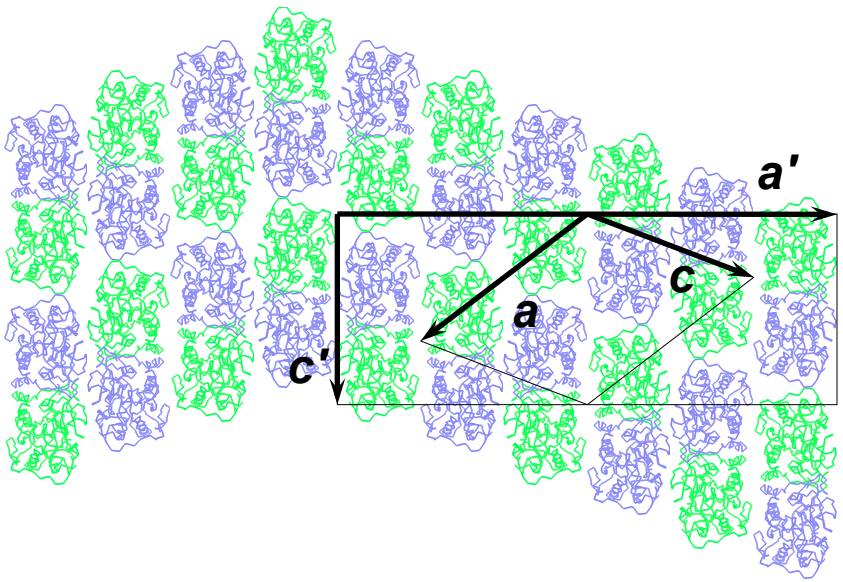
An example of symbol for groupoid of type (III):

$P \quad 1 \quad 1 \quad (4) \quad 1 \quad 1$

$\{ \quad 2_P \quad 2_Q \quad (1) \quad 2_U \quad 2_V \quad \}$

$\{ \quad 2_{P'} \quad 2_{Q'} \quad (1) \quad 2_{U'} \quad 2_{V'} \quad \}$

Example 2: OD-twin with zero obliquity



Uppenberg *et al*. (1995).
Biochemistry **34**, 16838-51.

Molecule: Lipase B from *Candida antarctica*

PDB code 1lbs

Space group: C2

a = 95.9 Å, b = 95.6 Å, c = 81.8 Å

β =122.2°

OD layer: $P(2)2_12_1$

- The data were processed in C2 but in the twin lattice (twin index = 3)

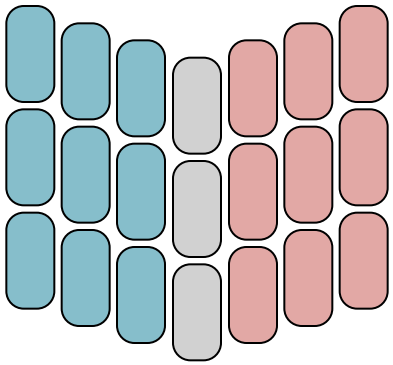
  a'=229.5 Å, c'=86.8Å, β =90°

- non-overlapping reflections from the minor twin component were removed

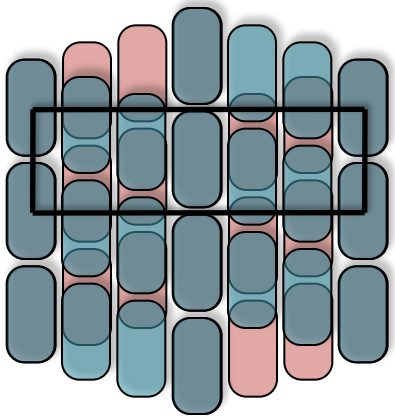
- overlapping reflections were detwinned

Example 2: OD-twin with zero obliquity

This packing could be assumed by similarity with the previous example



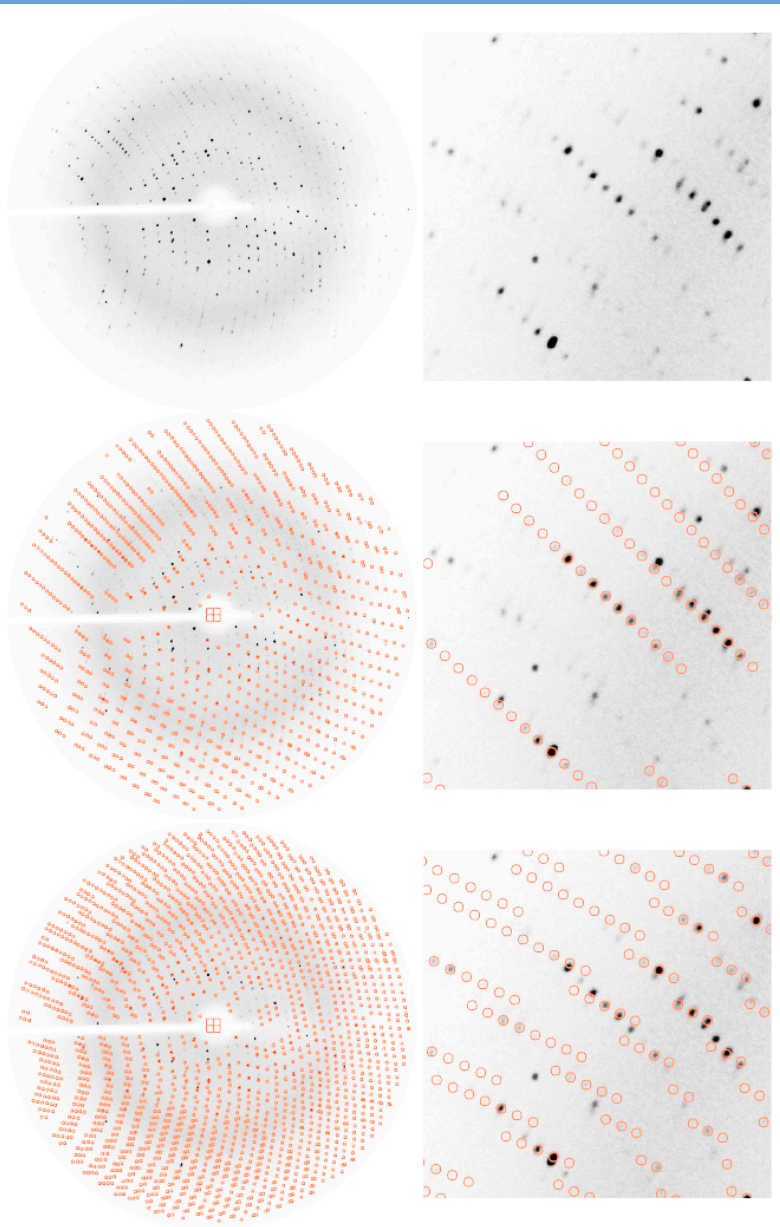
This packing is more likely to occur as it explains the exactly orthorhombic twin lattice



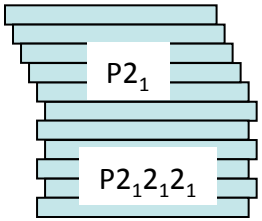
The previous example:	twin index 10	obliquity 0.1°
This example:	twin index 3	obliquity 0°

In general, protein OD-twins frequently have zero obliquity (twins by metric merohedry)

Example 3: 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°

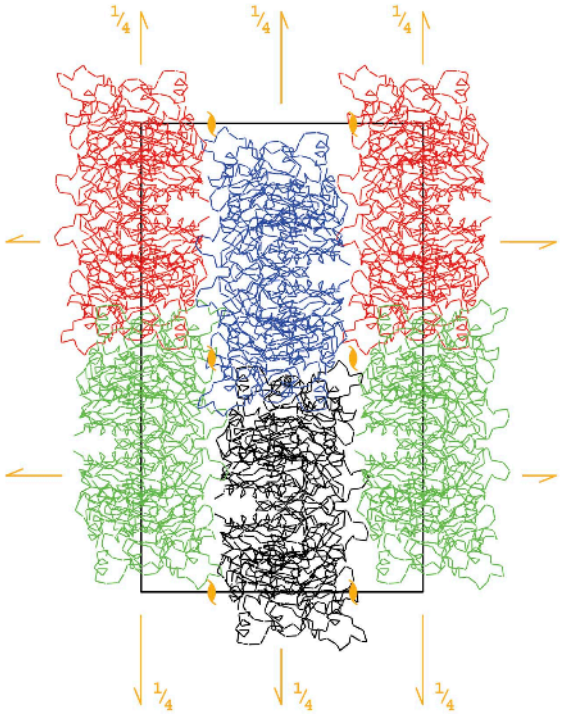
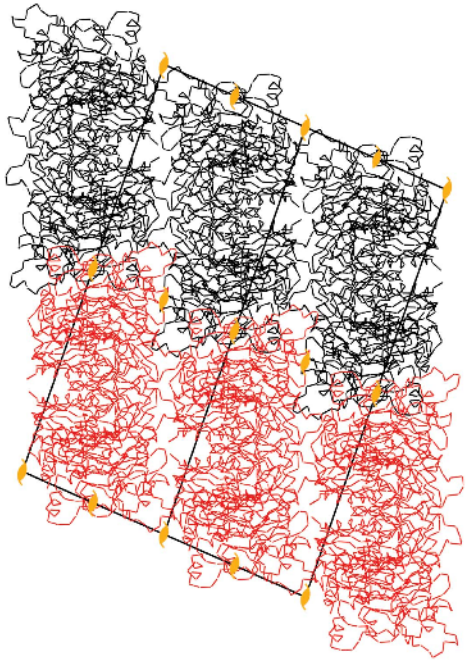
$P2_12_12_1$

a = 86.3 Å
b = 90.6 Å
c = 148.0 Å

Example 3: allotwin

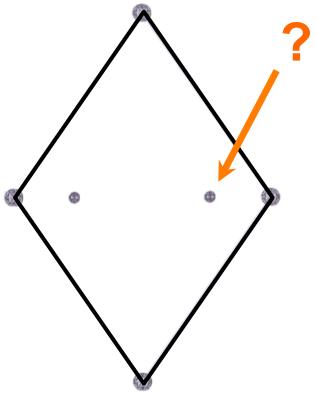
Crystals of Lon protease
Resolution 3Å

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

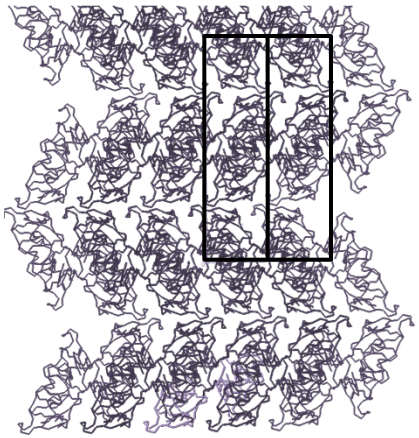
	PDB code 1z0t	PDB code 1z0v
		
Structures of both crystal forms were solved	$P2_12_12_1$	$P2_1$
R / R-free	0.19 / 0.35	0.21 / 0.31

Four types of domains

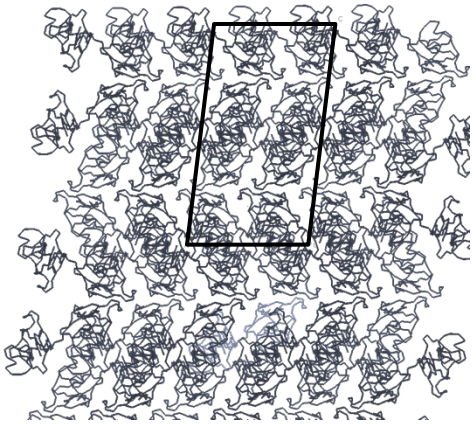
Patterson maps at W=0



$P2_1$ structure (1k7v)



Putative C2 structure



PDB	1k7v
R	23.2
R-free	31.0

Interpretation of the Patterson map for: four types of domains

- $P2_1$ (orientation 1)
- $P2_1$ (orientation 2)

} Twinned $P2_1$ data set

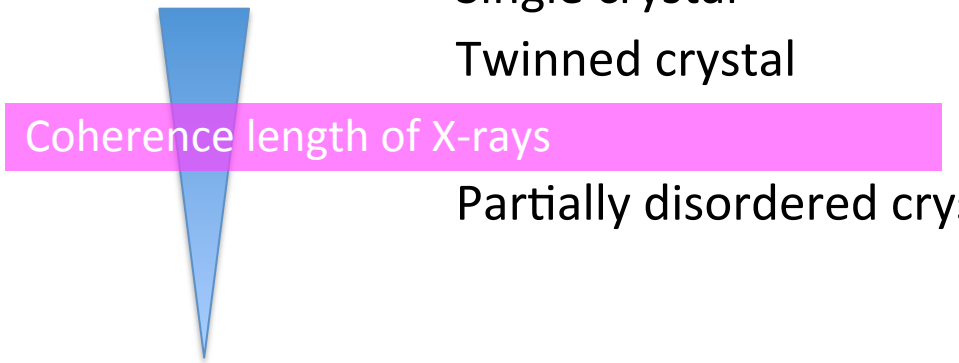
- C2 (orientation 1)
- C2 (orientation 2)

} contribute to some of the $P2_1$ spots, hence non-origin Patterson peaks

Crystal disorder

Twinning, partial disorder: Missing global periodicity

size of ordered domains

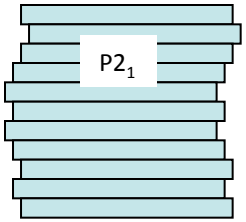


Single crystal (Single ordered domain)

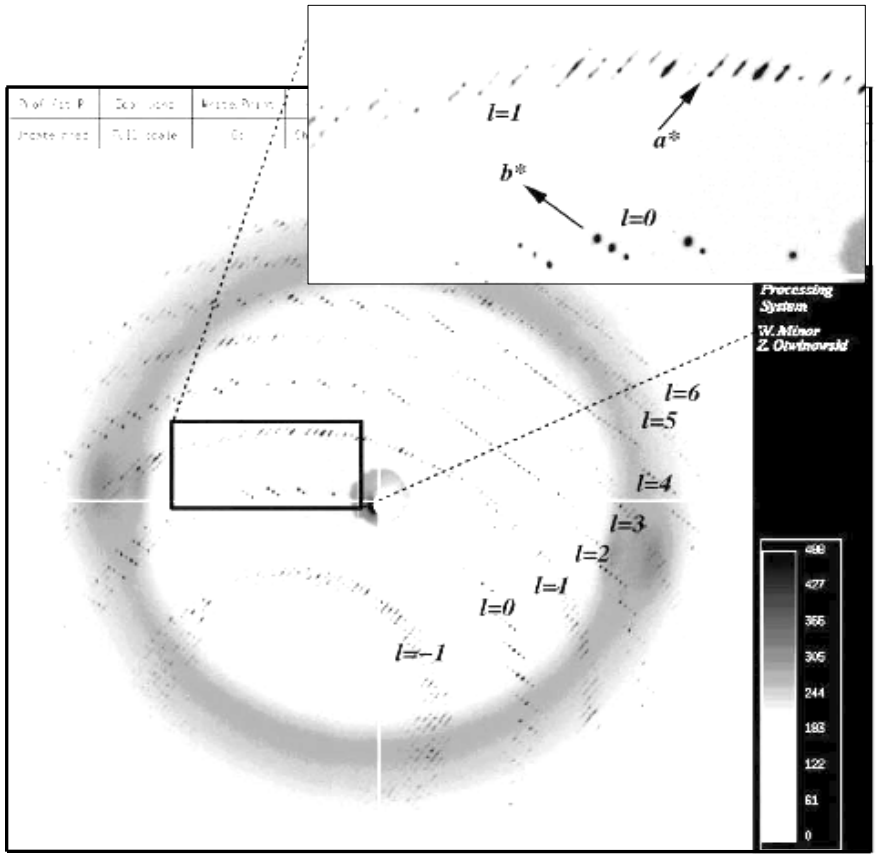
Twinned crystal (Two or more ordered domains)

Partially disordered crystal (Many ordered domains)

Example 4: partially disordered OD-structure



a* Wang *et al.* (2005). *Acta Cryst.* D**61**, 67-74.



Crystals of Phi29 DNA polymerase
Resolution 2.2Å

The translation symmetry is not global in the direction ***a****.

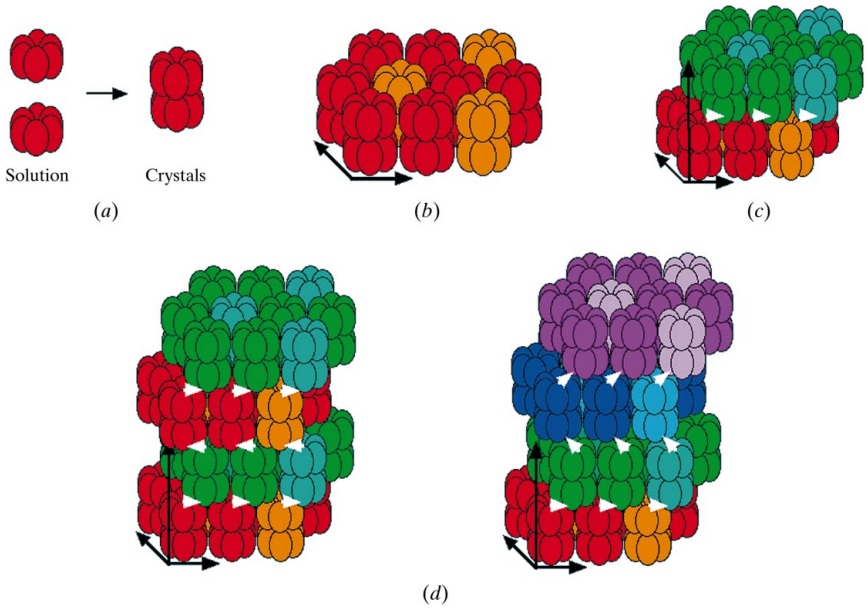
The diffraction pattern is characterized by the presence of the diffuse streaks along ***a****.

The structure was solved using demodulated data and experimental phasing

Refinement against corrected data: R=0.28

Example 5: Partial disorder with several stacking vectors

Trame, C. B. & McKay, D. B. (2001).
*ActaCryst. D***57**, 1079–1090.



model of $P222_1$ single crystal

model of disordered crystal

Heat-shock locus U protein from *Haemophilus influenzae* and its complexes

Several crystal forms,
all partially disordered OD
belonging to different OD-families.

Data:

Resolution	2.3Å
Processed in	P622

a = 110.6, c = 335.8

OD layer: P(6)22

OD-structures

Twinning by (pseudo)merohedry

Statistics of one observation

Statistics of two observations

Twinning tests summary

Space group validation

Twinning by (pseudo)merohedry

Twins by reticular merohedry (inc some OD-twins), allotwins, disordered structures

- Can be readily seen in diffraction images (with spot predictions shown)

Important special case: twinning by (pseudo)merohedry

- All spots overlap with related spots from another individual crystal

- Detection requires analysis of intensity statistics

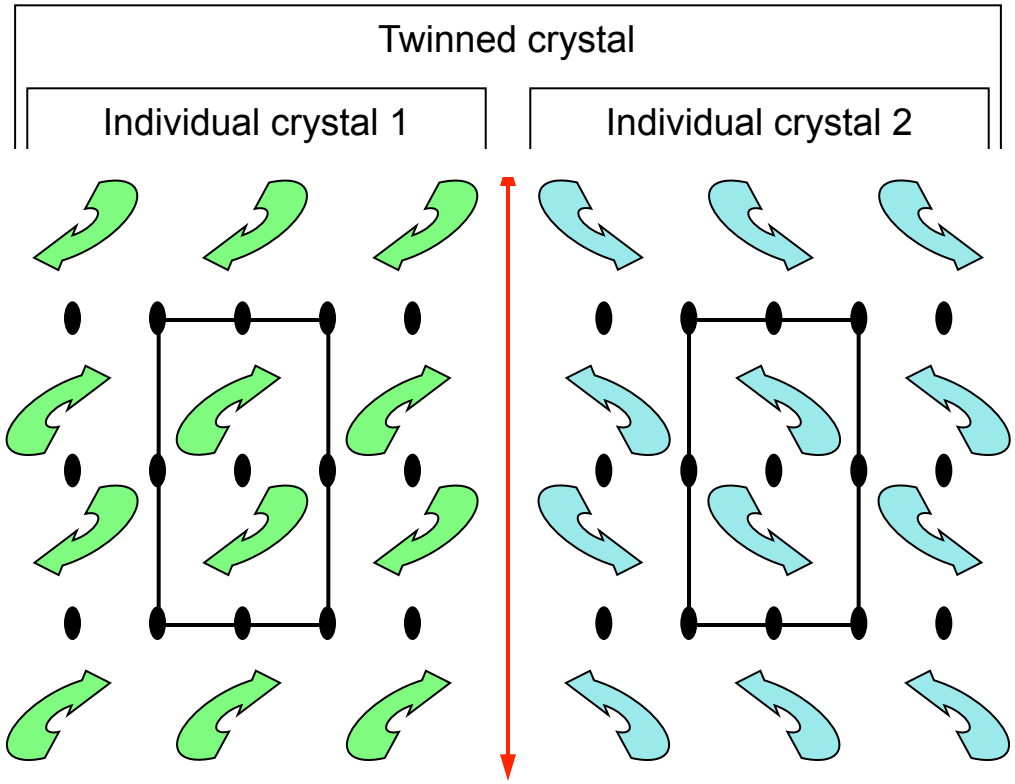
- More significant effect on model if ignored

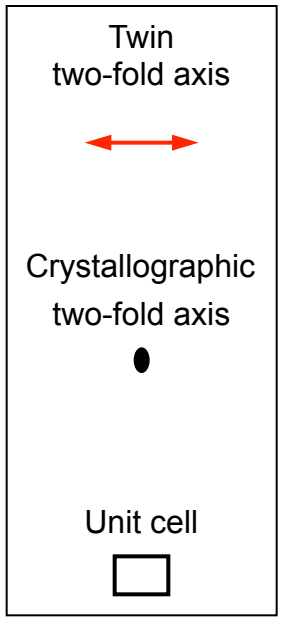
- Point group and, consequently, space group determination may be a problem

Twinning by (pseudo)merohedry

lattice in orientation 1	=	lattice in orientation 2		
crystal in orientation 1	≠	crystal in orientation 2		
crystal in orientation 1	+	crystal in orientation 2	=	Twinned crystal

Example: P2, $\beta = 90$





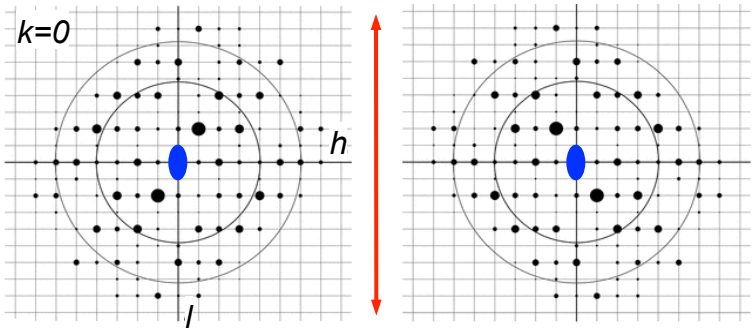
Twinning by (pseudo)merohedry

lattice in orientation 1	=	lattice in orientation 2		
crystal in orientation 1	≠	crystal in orientation 2		
crystal in orientation 1	+	crystal in orientation 2	=	Twinned crystal

P121, β = 90

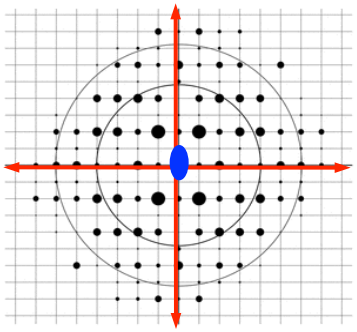
Intensities from individual crystal 1

Intensities from individual crystal 2



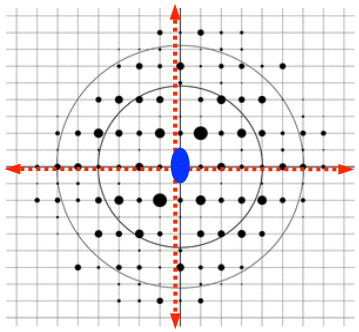
Perfect twin

(individual crystal of equal size)



Partial twin

(individual crystal of different size)

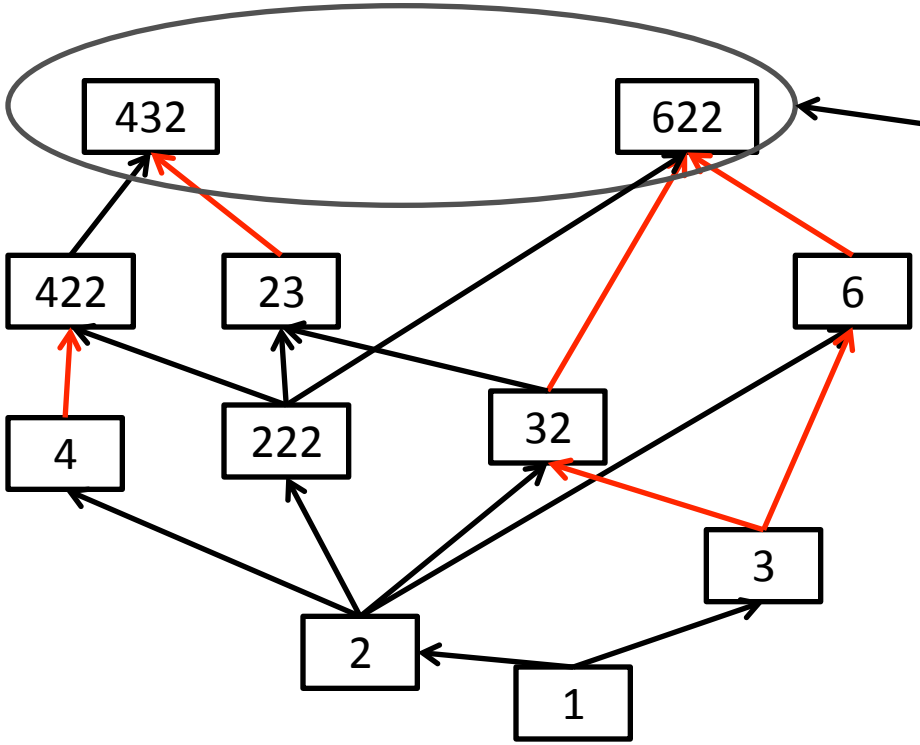


some weak reflections vanish

Twin two-fold axis

Crystallographic two-fold axis

Twinning by (pseudo)merohedry



(Pseudo)merohedral twining is impossible

supergroup

↑

subgroup

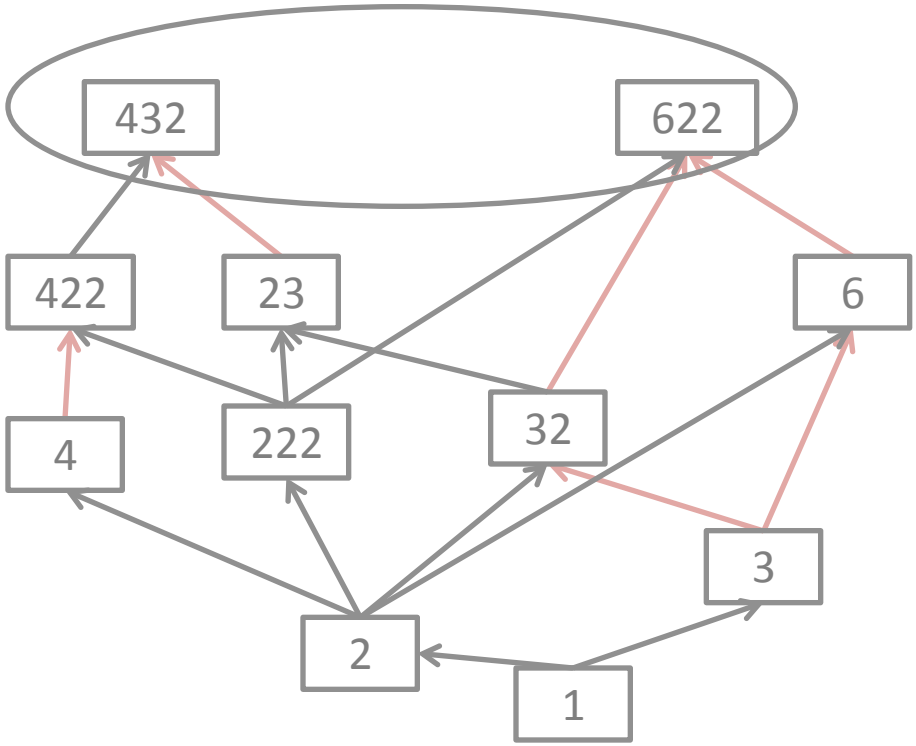
Twinning by merohedry

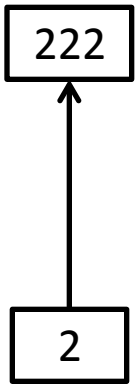
– higher lattice symmetry is determined by crystal symmetry

Twinning by pseudomerohedry

– specialised unit cell parameters

Twinning analysis





1/2 molecule per AU: Impossible!

One molecule per AU

Two reasons to show this slide

- Crystal content analysis may help recognising twinned crystal
- Deterministic case: structure cannot be "solved" in wrong space group

Not always that easy

Monoclinic OD-twin (twin by pseudomerohedry)

Au et al. (2006).
Acta Cryst. D**62**, 1267-1275.

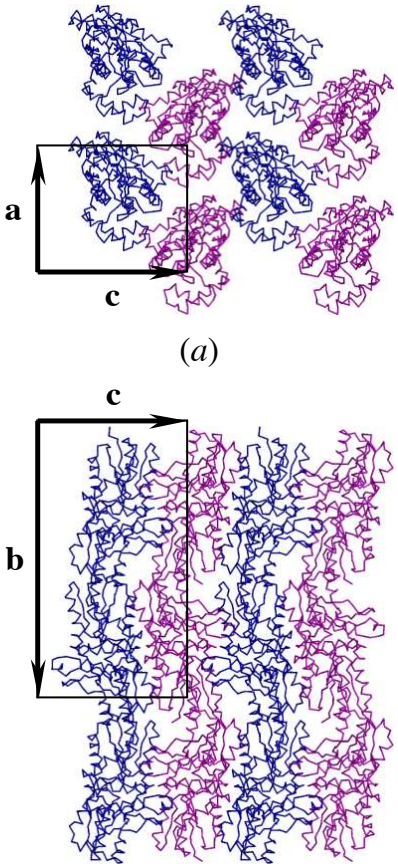
Ferrochelatase-1 from *B. anthracis*

PDB code 2c8j

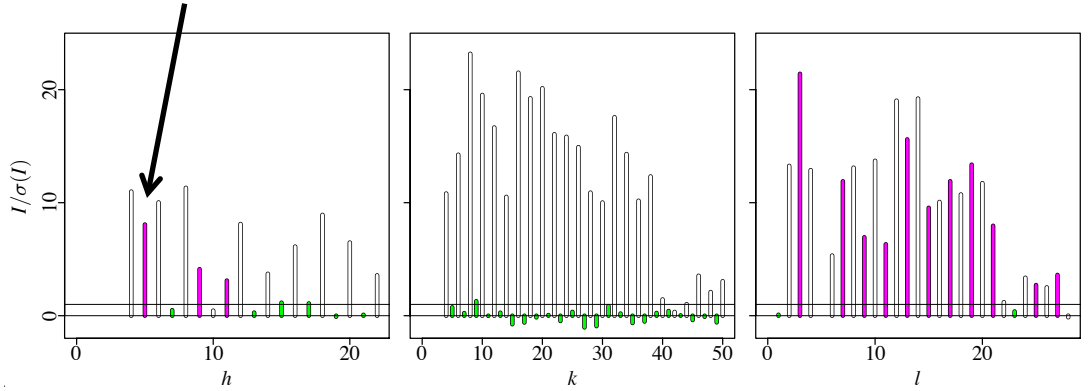
Space group: $P2_1$
Resolution 2.2Å

a = 49.9, b = 109.9, c = 59.4 Å
α = β = γ = 90°

OD layer: P2(1)1



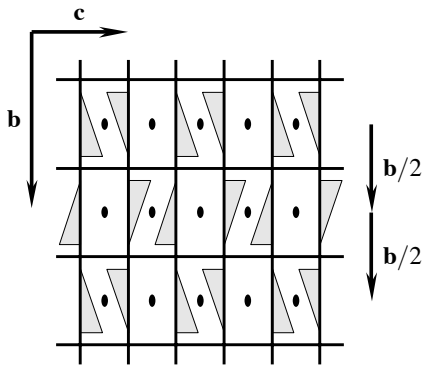
The only reflection with h = 2n, k=l=0 and I > 3 sig(I)



Monoclinic OD-twin (twin by pseudomerohedry)

$P2_12_12$ symmetrised structure

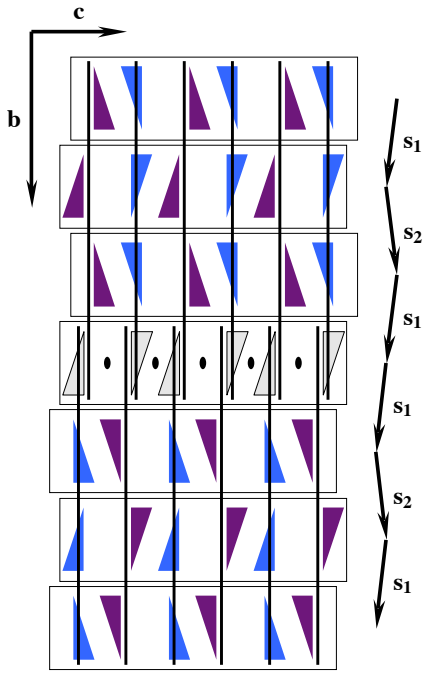
Molecules shifted along **c** by 2.5Å
R-free = 40%



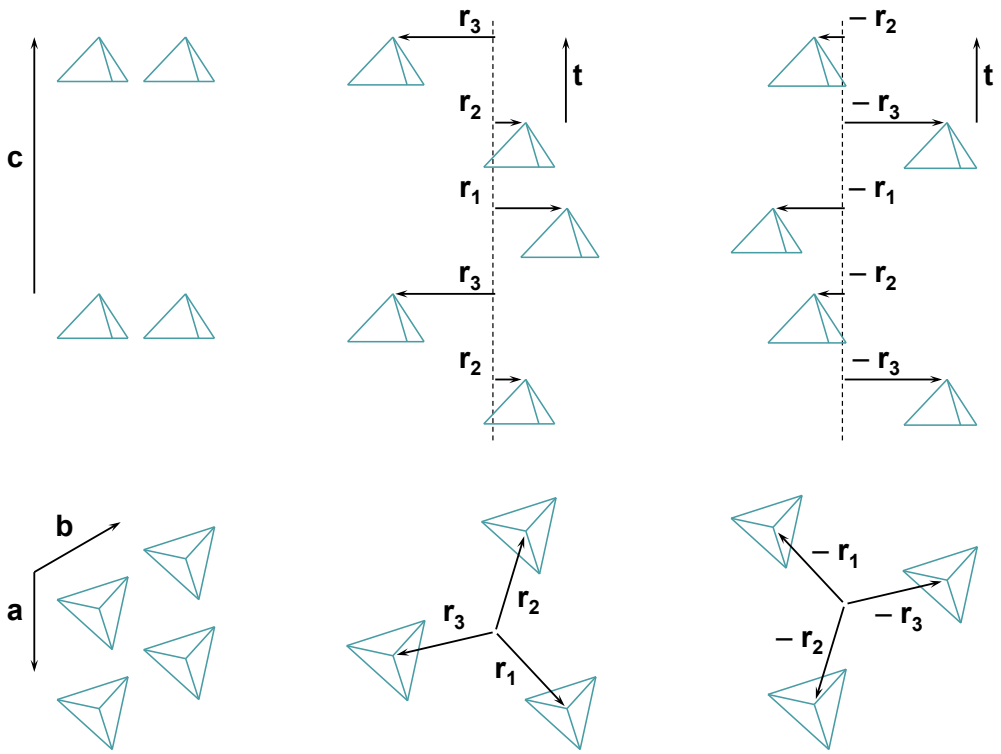
Twinning was suspected only after several unsuccessful attempts at solving structure in an orthorhombic space group

$P2_1$ true structure

The lattice is exactly orthorhombic
R-free = 27%



Enantiomorphic stacking vectors



(1) (2)

Structures (1) and (2)

- belong to different space groups:

  (1) $P3_1$ (2) $P3_2$

- are not necessarily related by inversion
- but have the same structure amplitudes:

  $F(1) = F(2)$

- and belong to the same OD family

Enantiomorphic stacking vectors

Gulbis et al. (1996). Structure of the C-terminal region of p21WAF1/CIP1 complexed with human PCNA. *Cell* **87**, 297–306.

Space group: $P3_221$
a = 83.5 Å, c = 233.9Å

OD layer: P(3)21

PDB code 1axc

Structure:	from PDB	generated
Space group:	$P3_221$	$P3_121$
R (%):	22.09	22.35
R-free (%):	29.15	30.02

Asymmetry of OD layer is within 0.2Å, but it helps choosing the right space group

Examples of crystal pathologies

Twinning by (pseudo)merohedry

Statistics of one observation

Statistics of two observations

Twinning tests summary

Space group validation

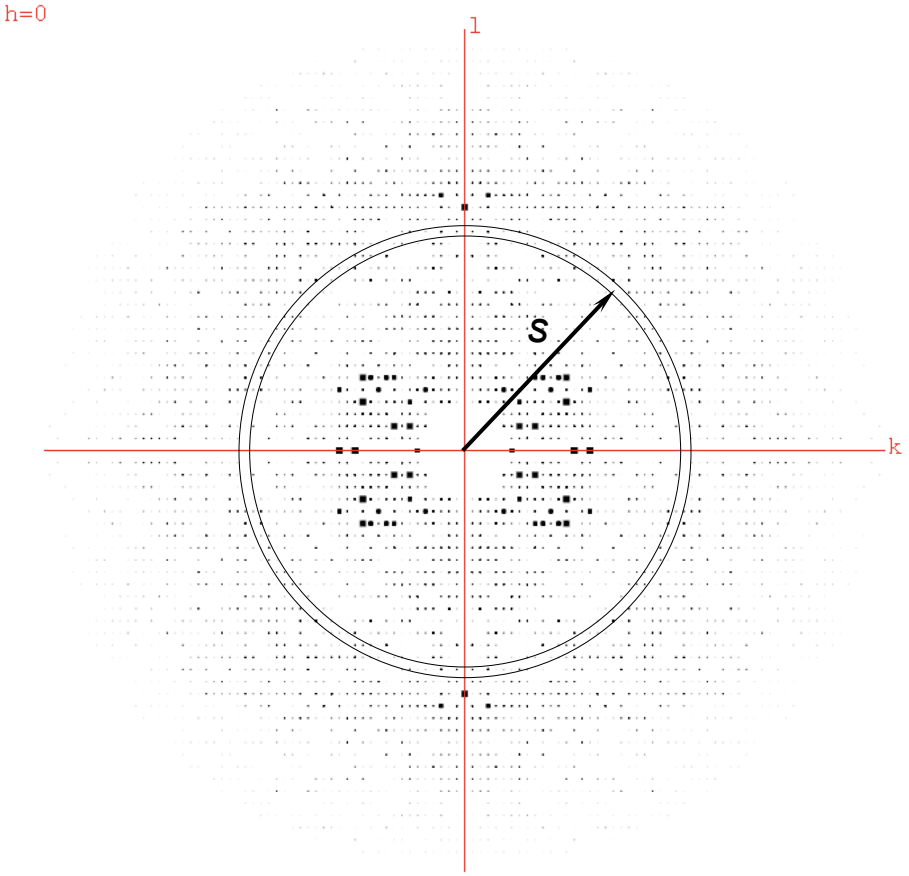
Resolution bins (resolution shells)

$s < s(h,k,l) < s + ds$

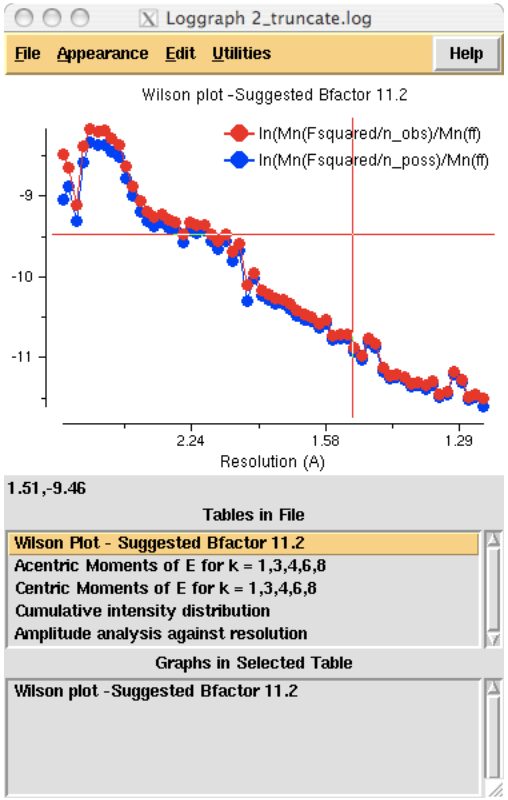
$\langle I \rangle(s) = \text{mean}(I(h,k,l))$

$\langle I \rangle(s) \approx C^* \exp(-2^* B^* s^2)$

B - Overall temperature factor



Wilson Plot:
log(<I>) against s

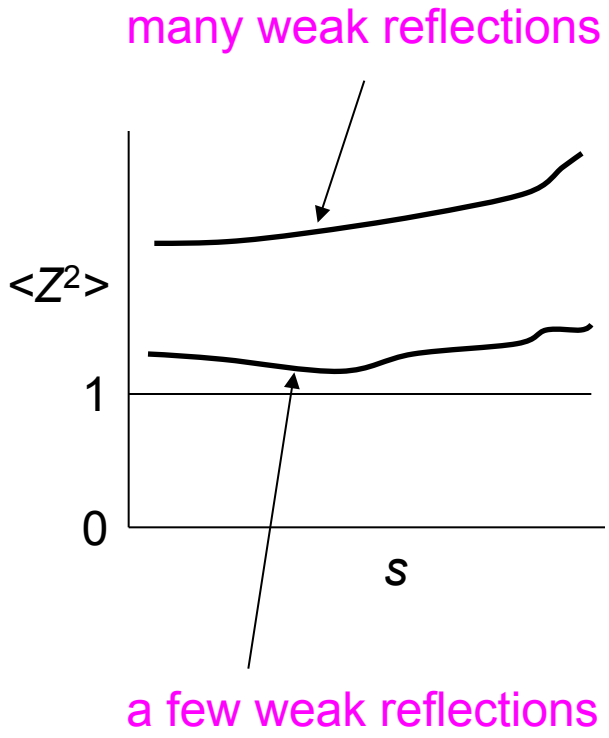


Normalised intensity: Second moment

$$Z(hkl) = \frac{I(hkl)}{\langle I \rangle(s)}$$

$\langle Z \rangle(s) = 1$ (by definition of Z)

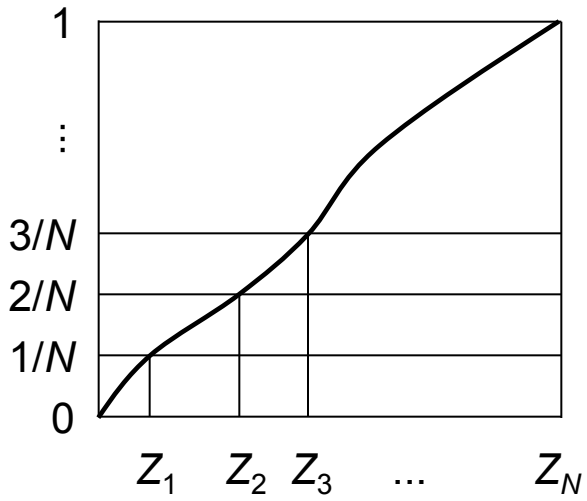
$Z(hkl)$:	1 1 1 1	$\langle Z \rangle(s) = 1$
$Z^2(hkl)$:	1 1 1 1	$\langle Z^2 \rangle(s) = 1$
$Z(hkl)$:	0 0 2 2	$\langle Z \rangle(s) = 1$
$Z^2(hkl)$:	0 0 4 4	$\langle Z^2 \rangle(s) = 2$
$Z(hkl)$:	0 0 0 4	$\langle Z \rangle(s) = 1$
$Z^2(hkl)$:	0 0 0 16	$\langle Z^2 \rangle(s) = 4$

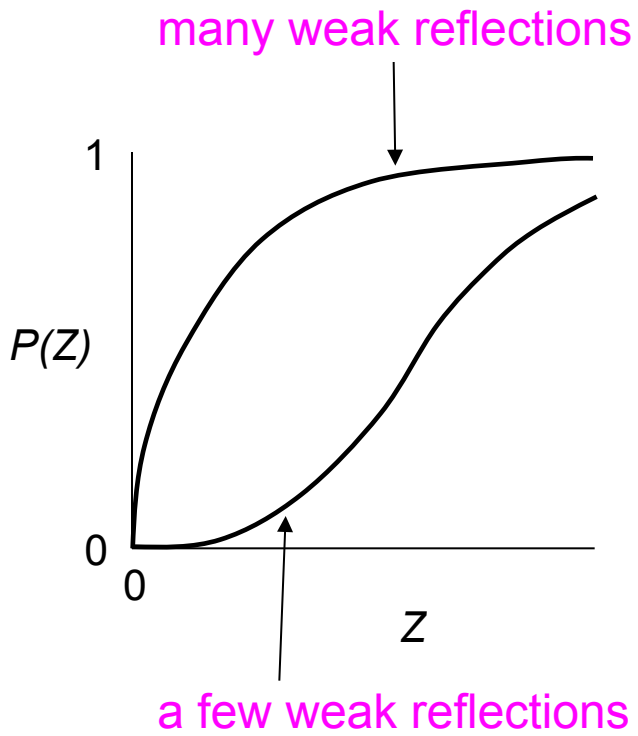


Normalised intensity: Cumulative distribution

{ $Z(hkl)$ } --> sort --> { Z_i }

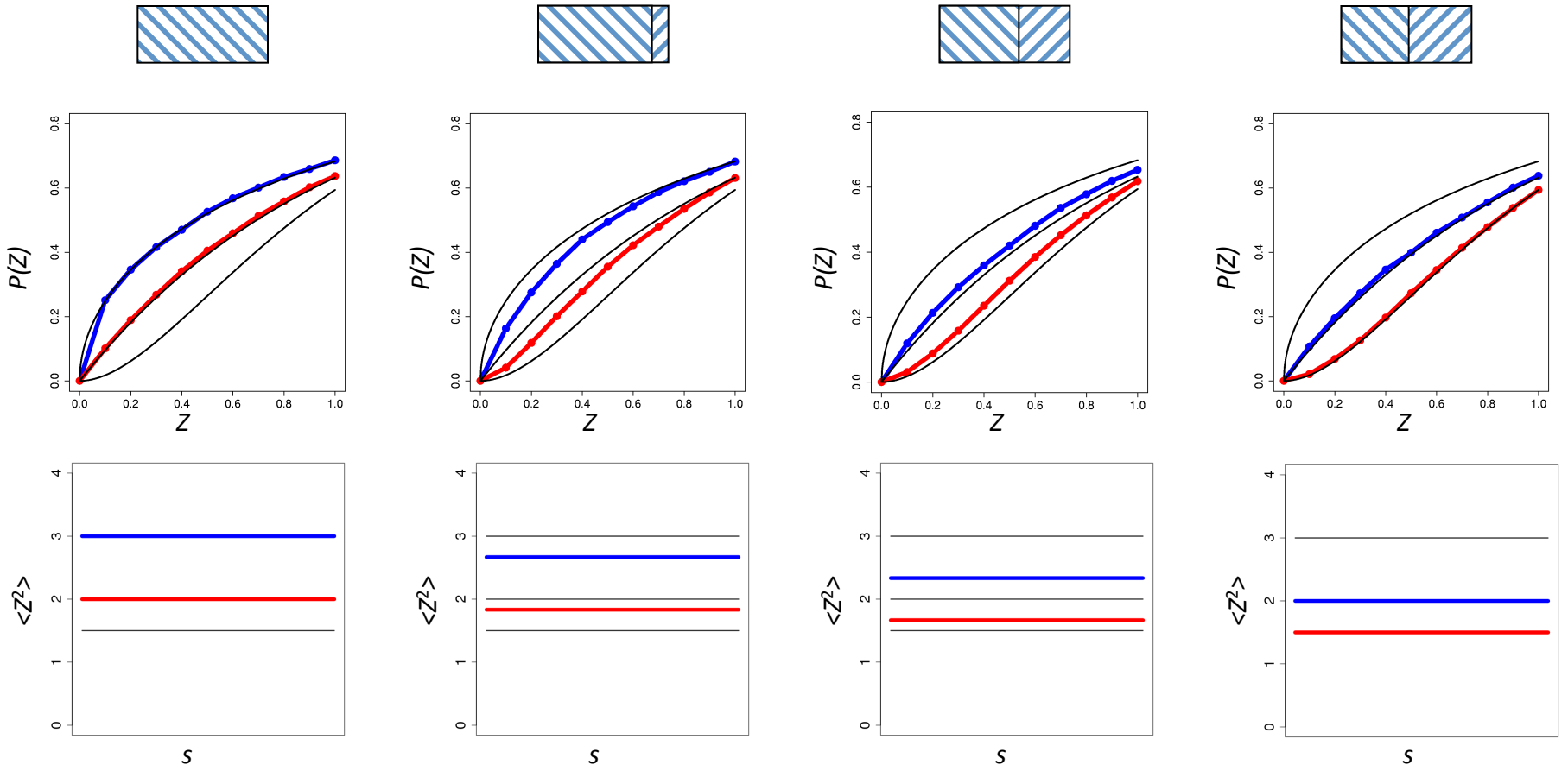
$$Z_1 < Z_2 < Z_3 < \ldots < Z_N$$





Theoretical distribution of intensities

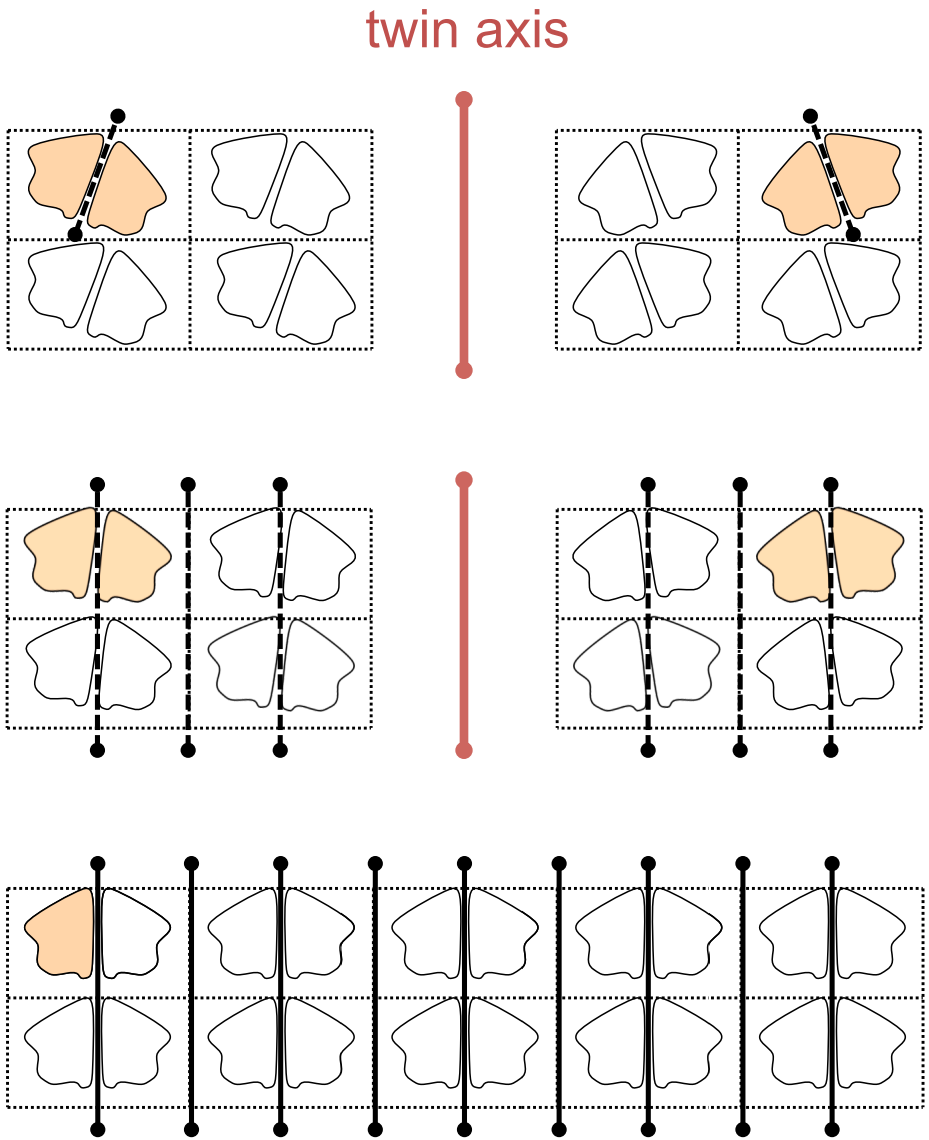
Single crystal | Partial twin | Partial twin | Perfect twin



Acentric reflections
Centric reflections

(works for incomplete data set)

Pseudosymmetry



Twinning + Generic NCS

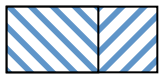
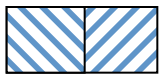
Twinning axis || NCS axis

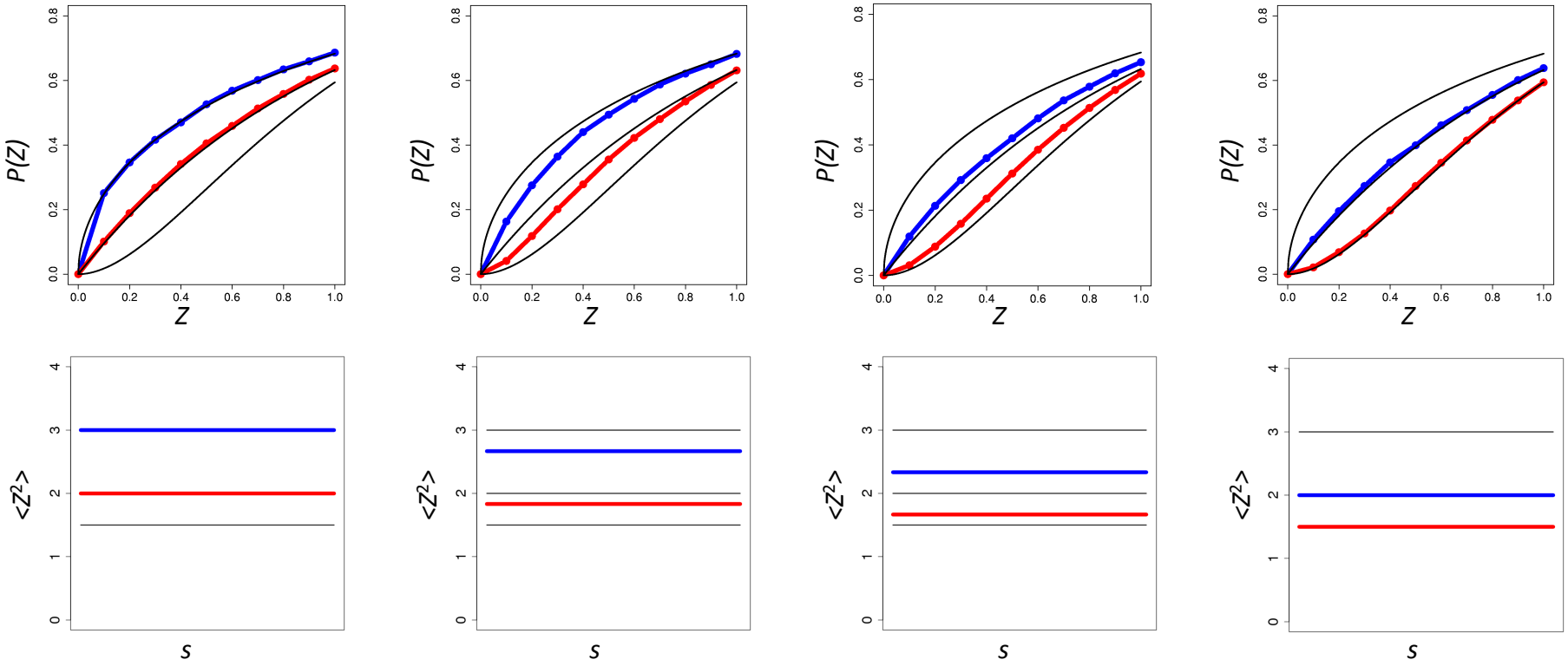
Single crystal

Theoretical distribution of intensities

Single crystal | Partial twin | Partial twin | Perfect twin





Acentric reflections
Centric reflections

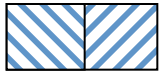
(works for incomplete data set)

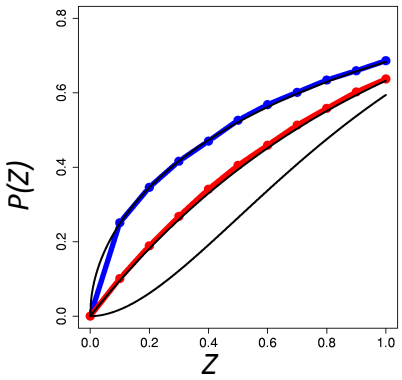
Theoretical distribution of intensities

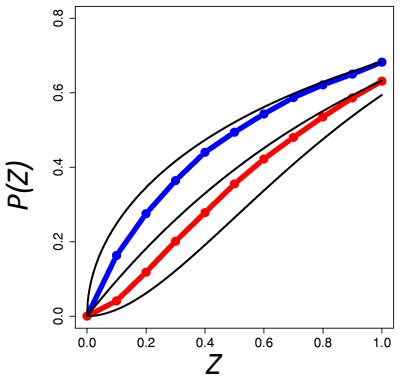
Single crystal

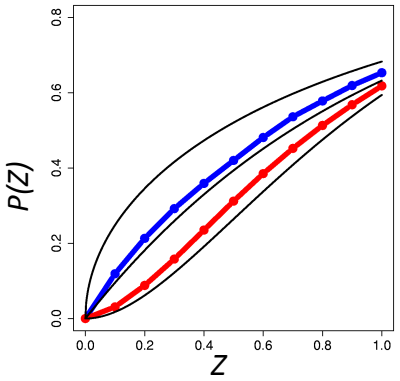
Partial twin
OR
Perfect twin + pseudosymmetry axis || twin axis

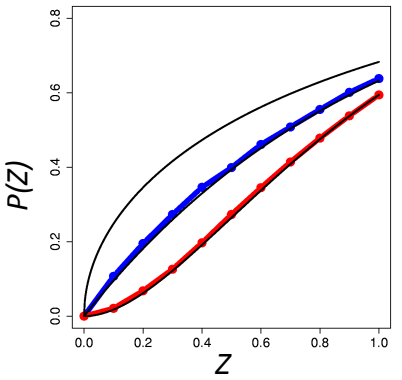
Perfect twin

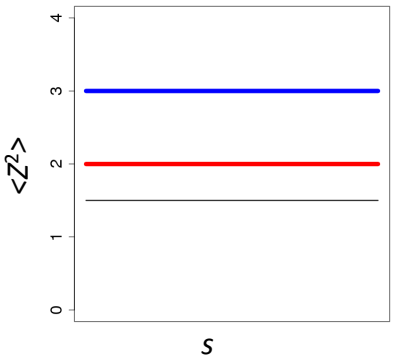


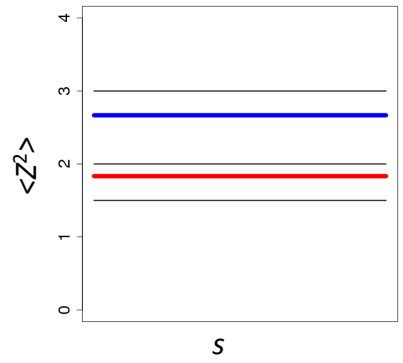


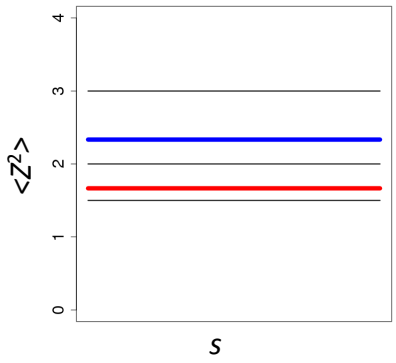


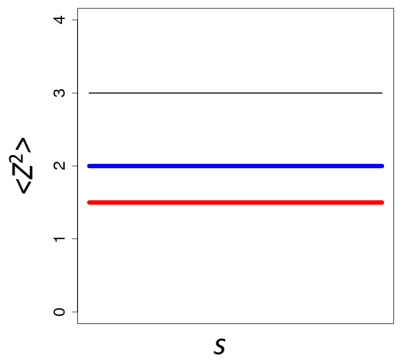










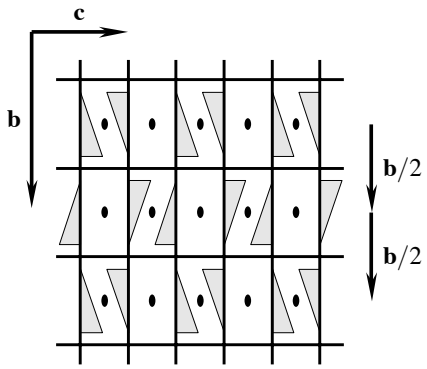


Acentric reflections
Centric reflections

Monoclinic OD-twin (twin by pseudomerohedry)

$P2_12_12$ symmetrised structure

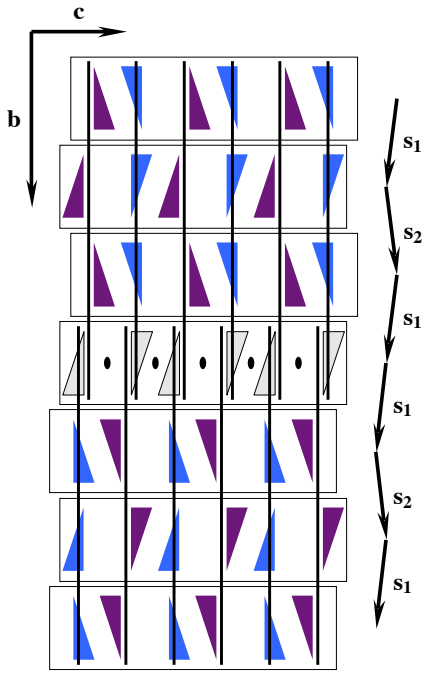
Molecules shifted along **c** by 2.5Å
R-free = 40%



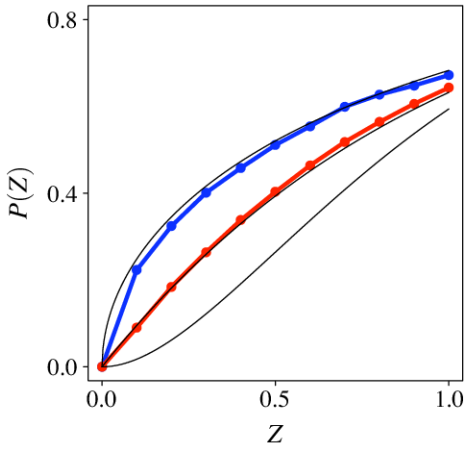
Twinning was suspected only after several unsuccessful attempts at solving structure in an orthorhombic space group

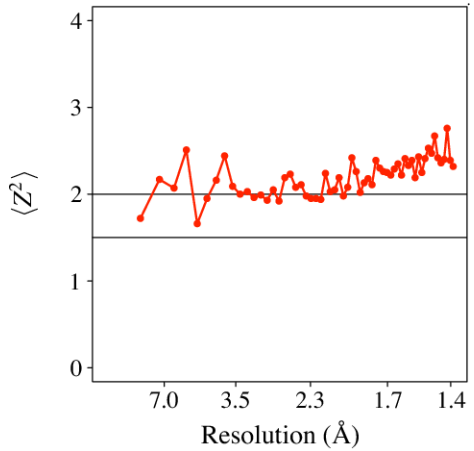
$P2_1$ true structure

The lattice is exactly orthorhombic
R-free = 27%

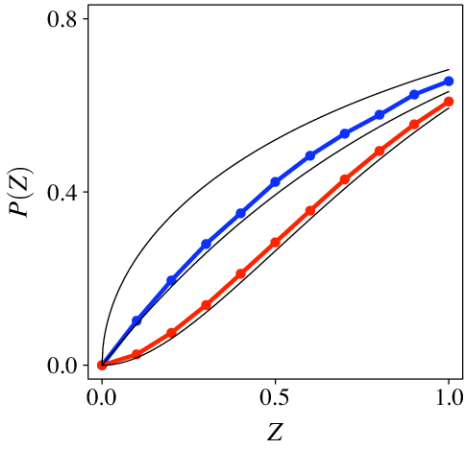


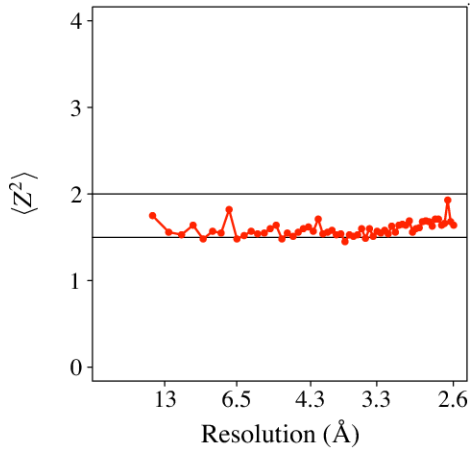
Two good, two bad





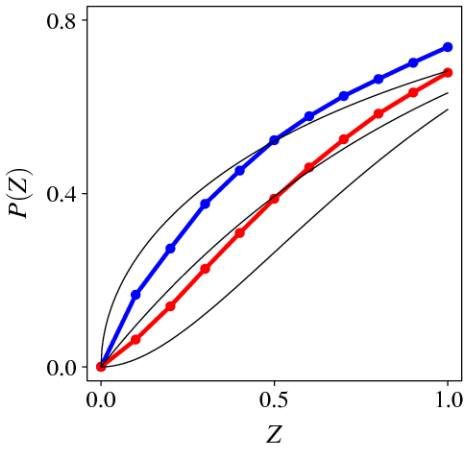
PDB entry 1i1j
single crystal



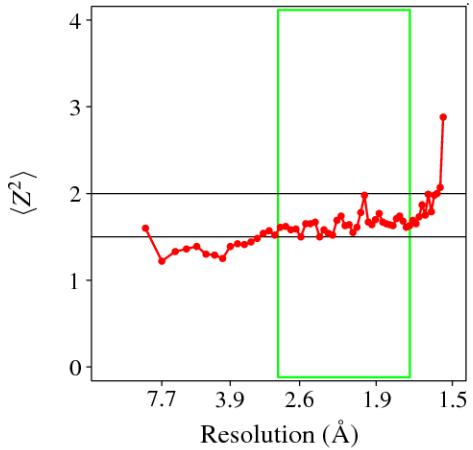


C-terminal domain of gp2 protein from phage SPP1
perfect twin

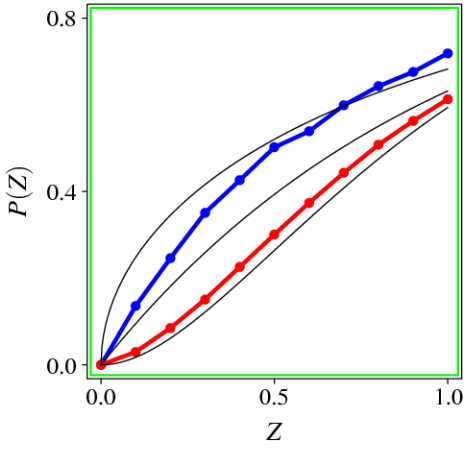
Bad example 1



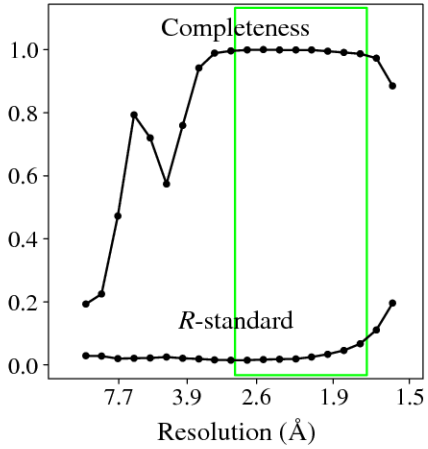
(a)



(b)



(c)

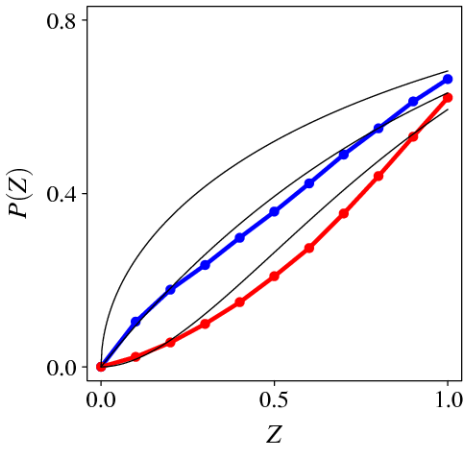


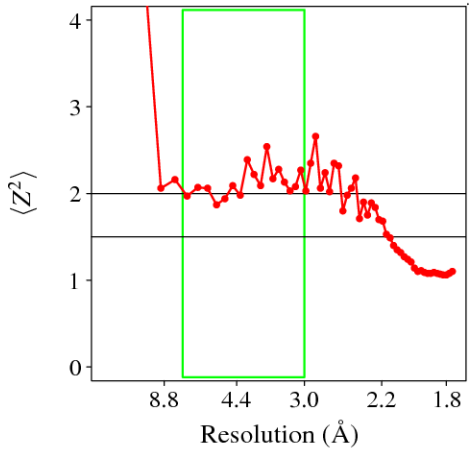
(d)

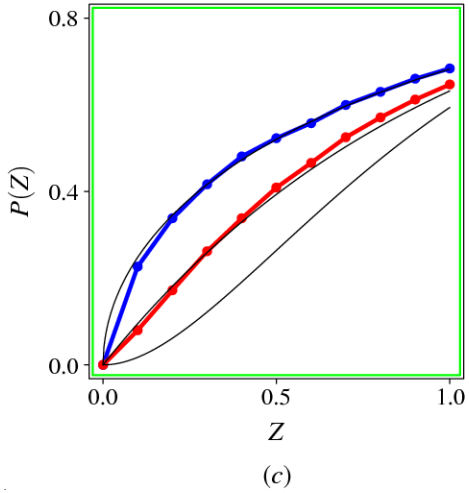
PDB code 1l2h

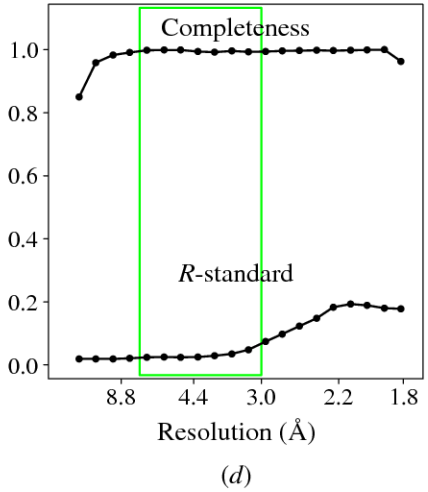
partial twin

Bad example 2



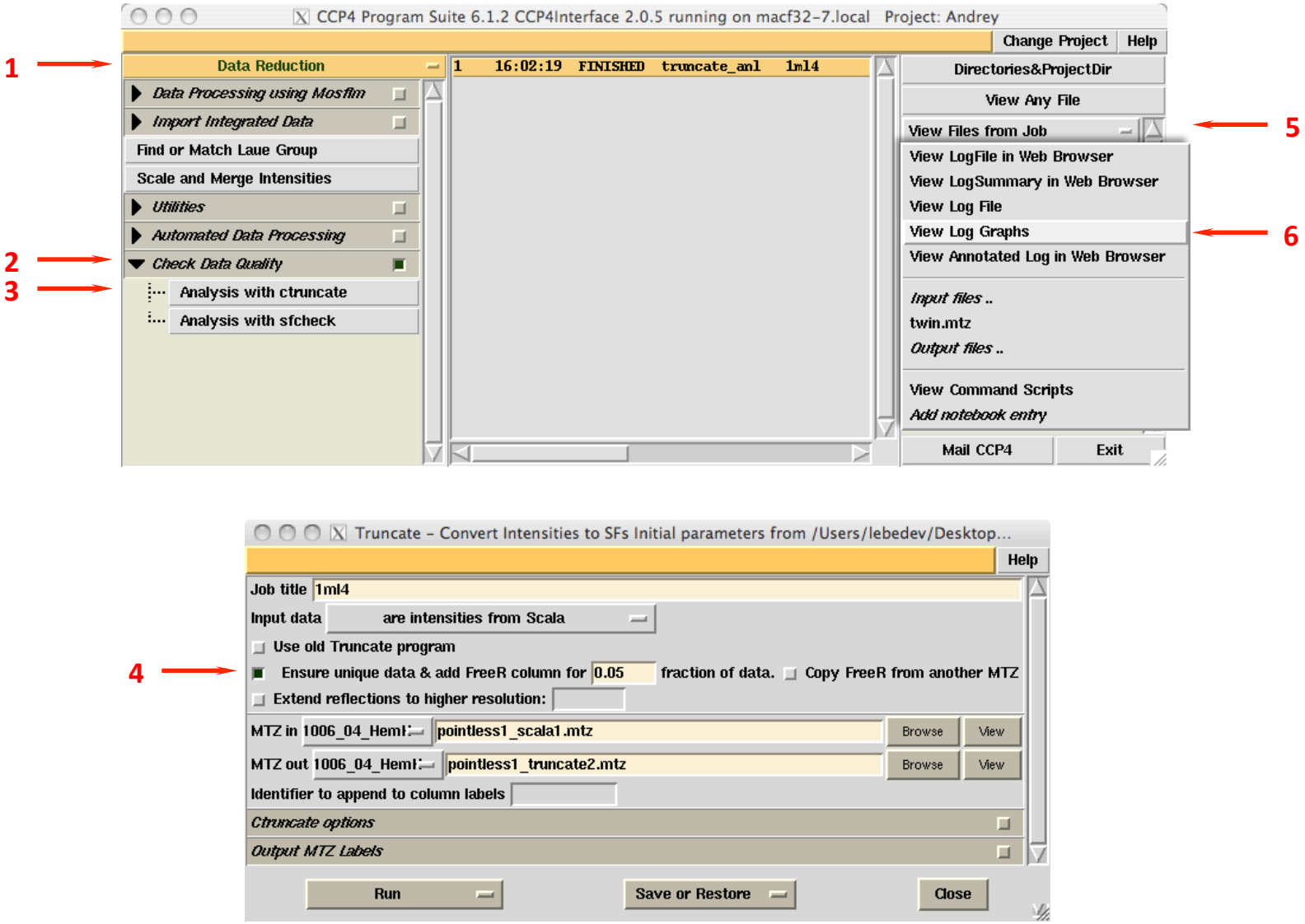






human deoxycytidine kinase single crystal

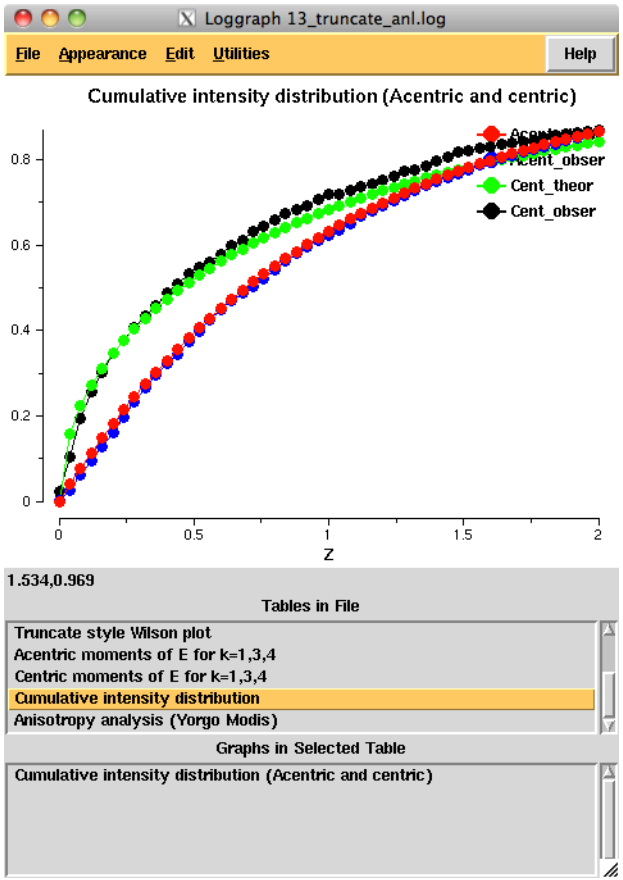
Twinning tests in CCP4I (ctruncate)



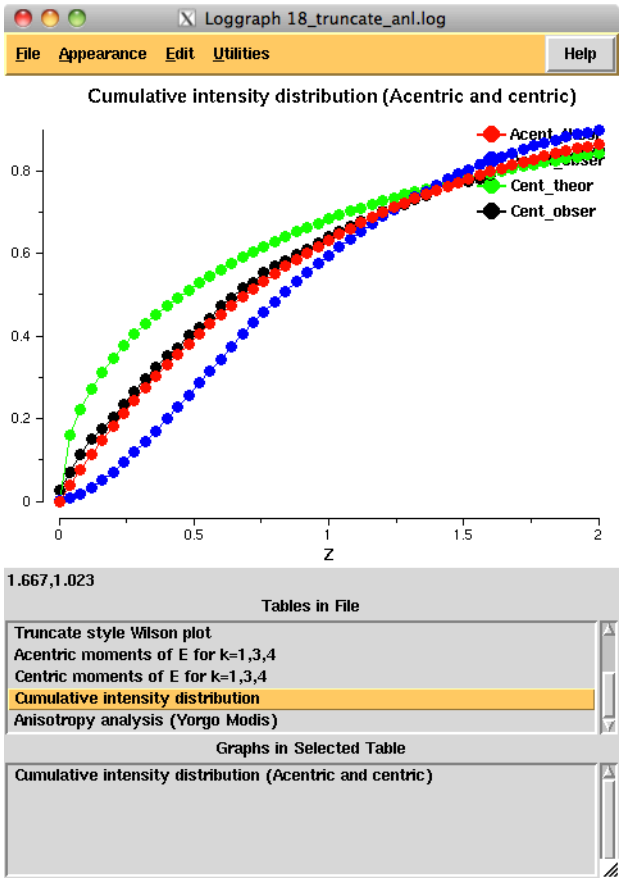
Cumulative intensity distribution

To compare: Red: Acentric theoretical, Blue: Acentric observed $Z \approx |E|^2$

Untwinned data



Twinned data

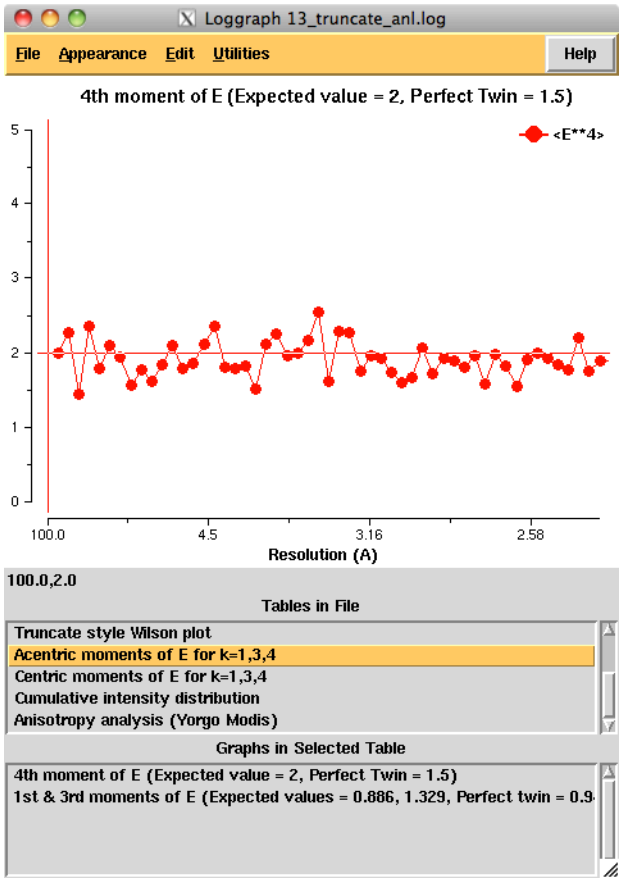


> Cumulative intensity distribution
> Cumulative ... (Centric and acentric)

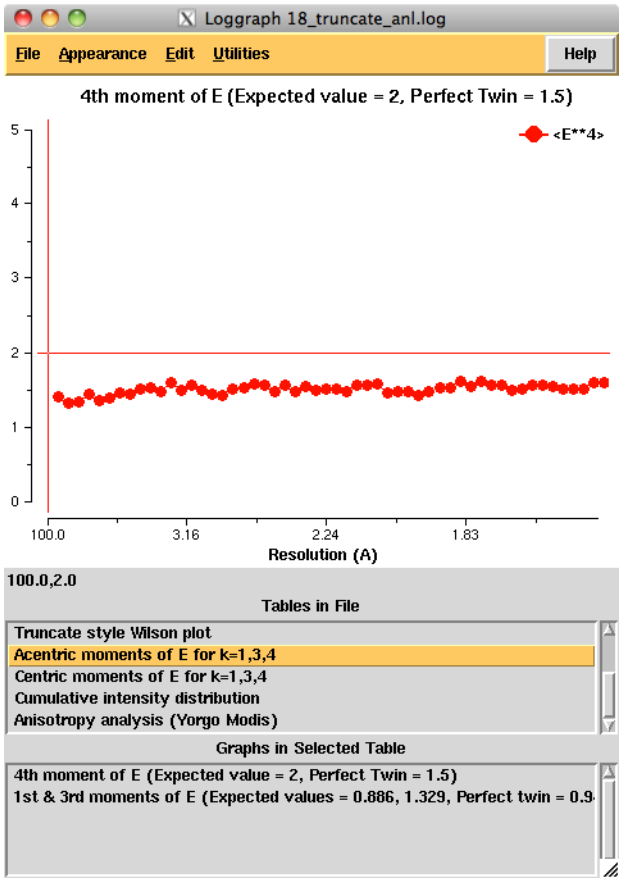
Second moments of Z (fourth moments of |E|)

Compare the experimental curve with the line $\langle E^4 \rangle = 2$

Untwinned data



Twinned data



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

> 4th moments of E ...

OD-structures

Twinning by (pseudo)merohedry

Statistics of one observation

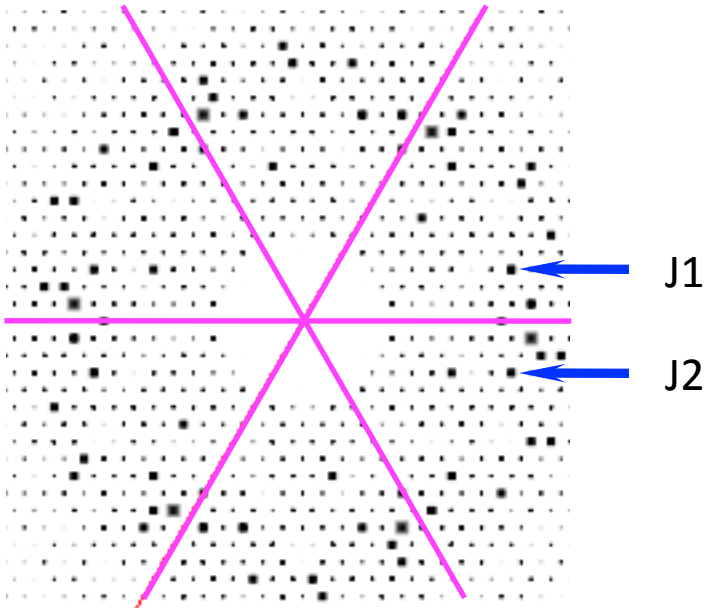
Statistics of two observations

Twinning tests summary

Space group validation

H-test and L-test

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



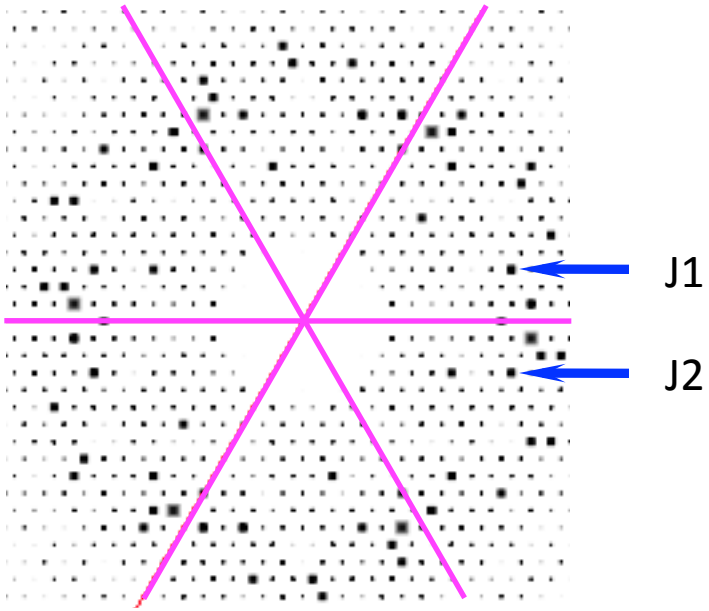
twin axes

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

sublattices with strong and weak reflections (pseudotranslation)

H-test and L-test

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

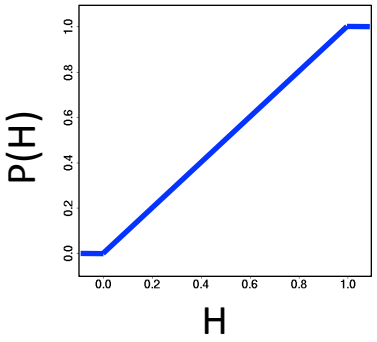


$L = | J1 - J2 | / (J1 + J2)$

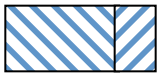
sublattices with strong and weak reflections (pseudotranslation)

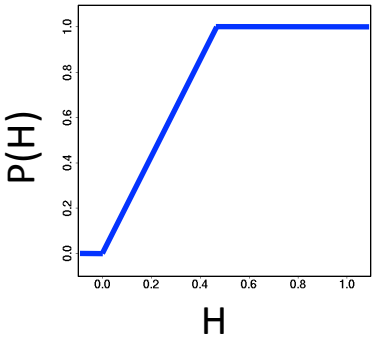
Theoretical distribution of H

Single crystal

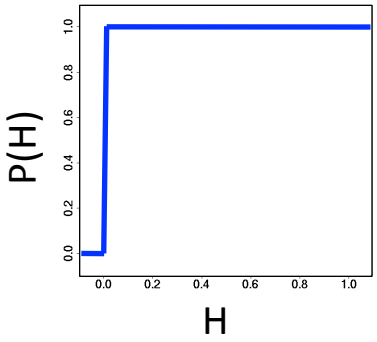


Partial twin

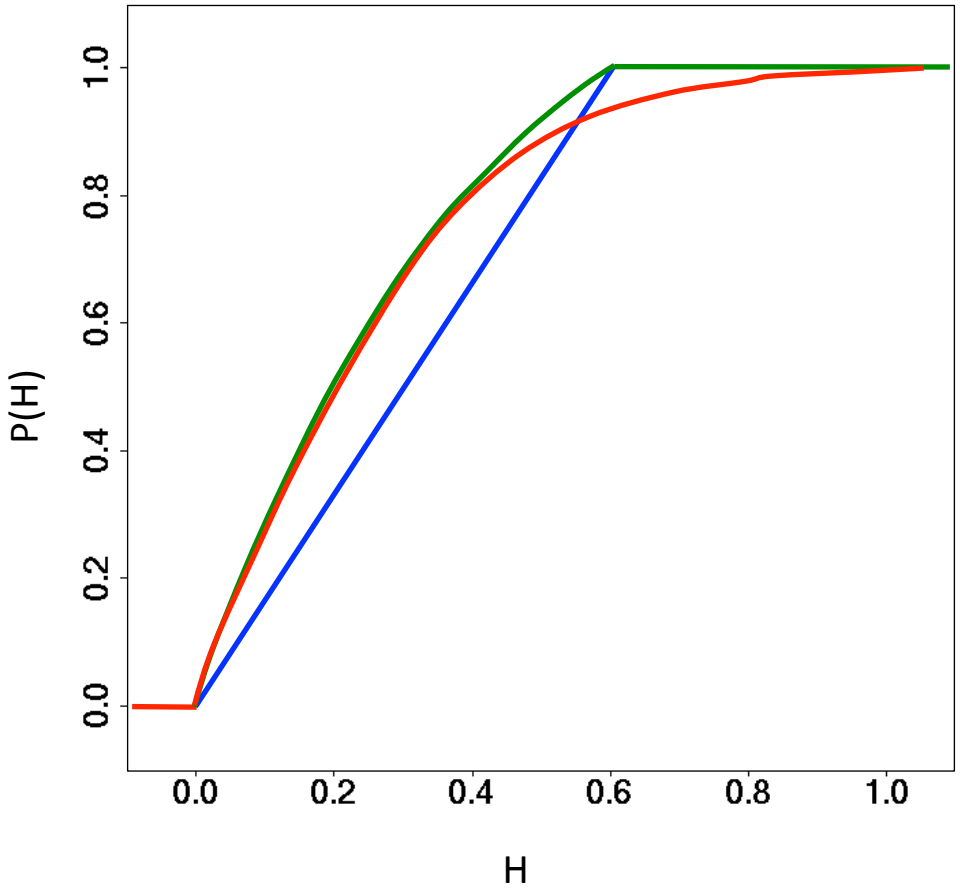




Perfect twin



Distribution of H can be perturbed by NCS and weak observations

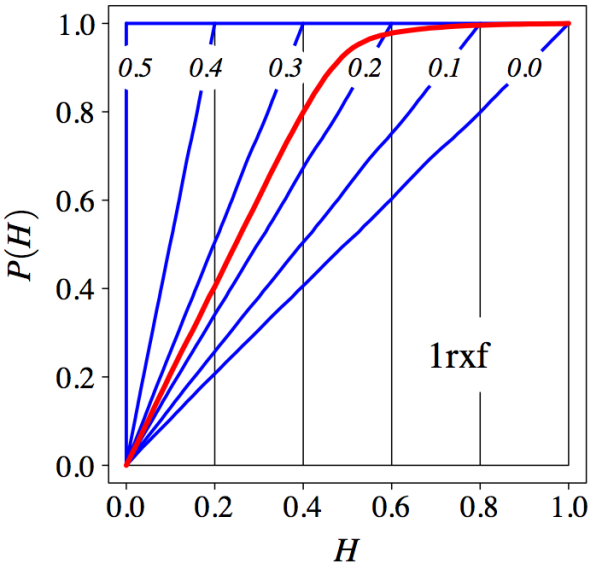


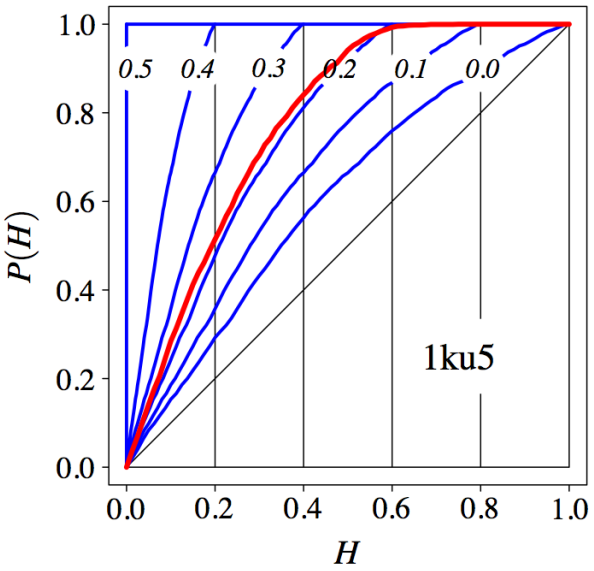
Blue:
ideal distribution for
partial twin

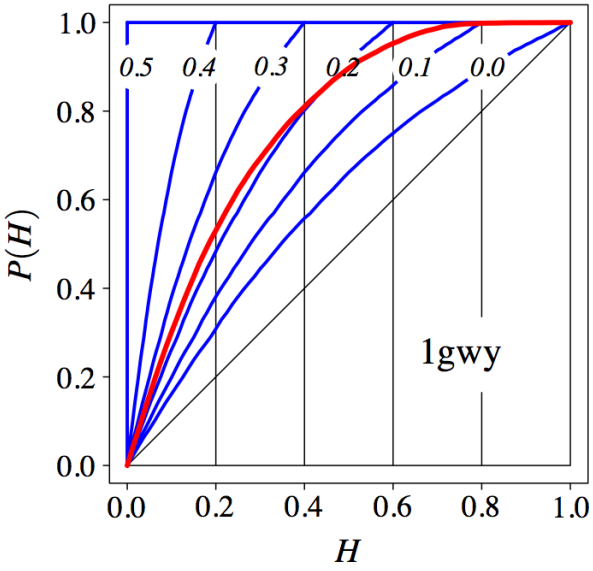
Green:
blue + effect of
NCS axis || twin axis

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

Examples of experimental P(H)







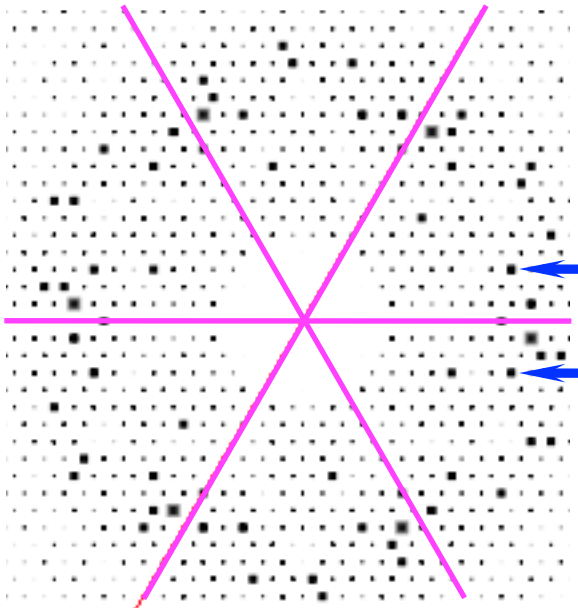
An almost ideal case

+ effect of
NCS axis || twin axis

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

H-test and L-test

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



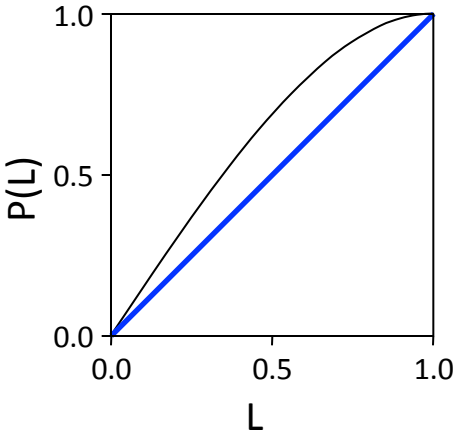
twin axes

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

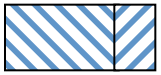
sublattices with strong and weak reflections (pseudotranslation)

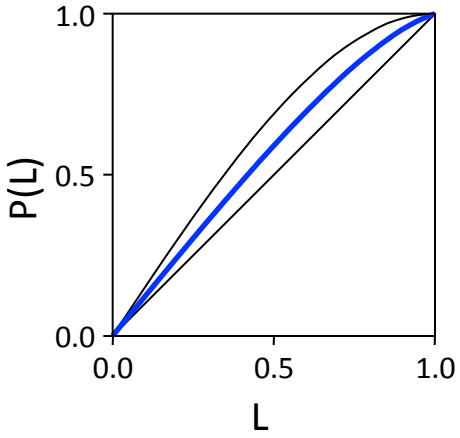
Theoretical distribution of L

Single crystal

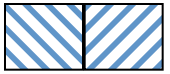


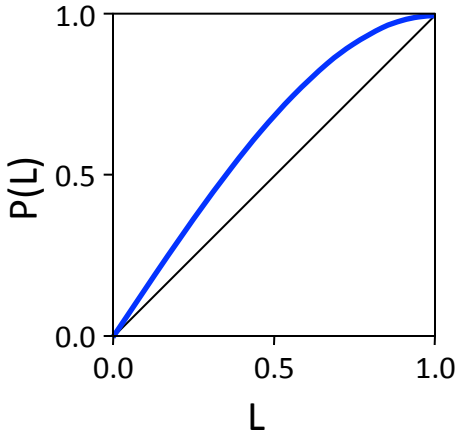
Partial twin





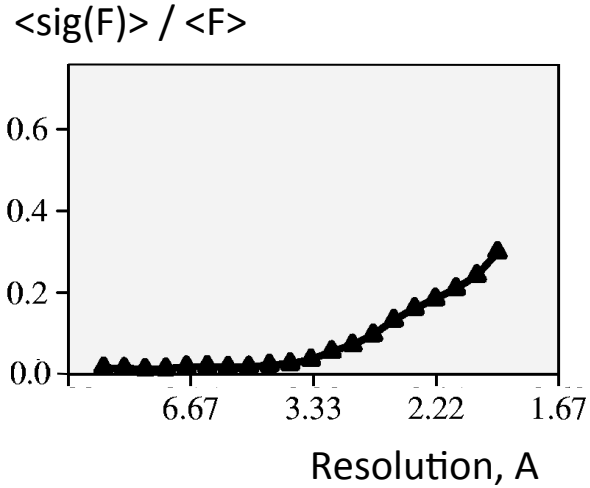
Perfect twin



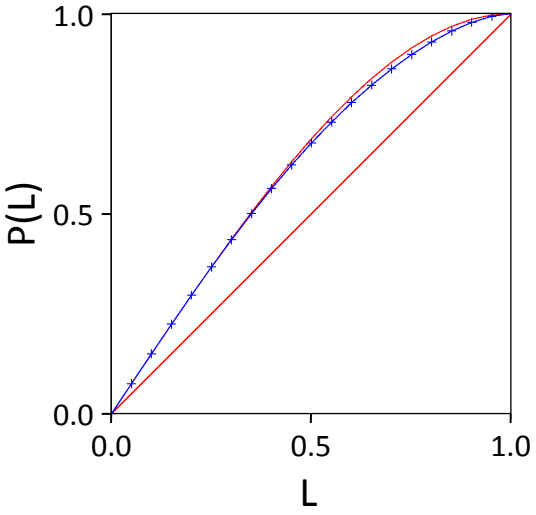


Distribution of L can be strongly perturbed by weak observations

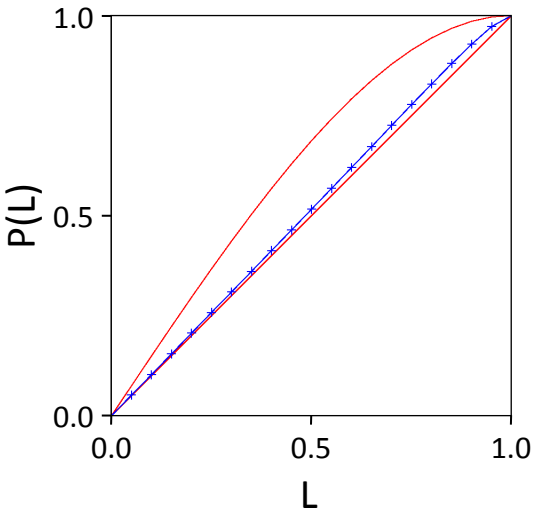
Cell: 64.2 109.2 100.2 90 93.8 90
Space group: $P2_1$
No pseudo symmetry
Pseudomerohedral twinning is impossible



All data:
as if a perfect twin



Data below 3A:
untwinned

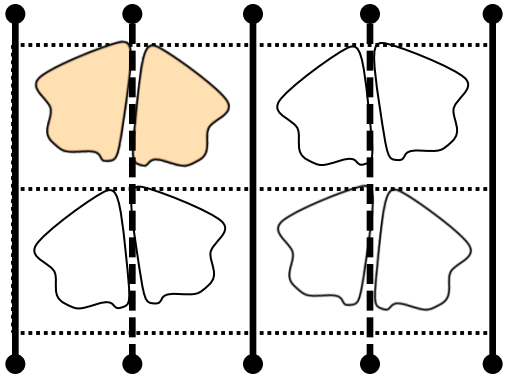


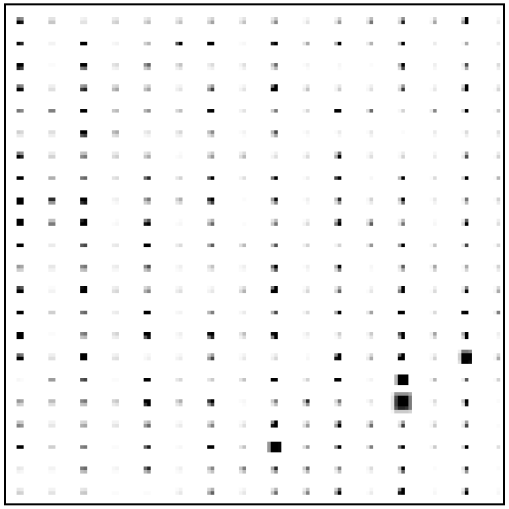
Nevertheless the L-test is very useful when performed with right resolution range (or with several ranges)

Pseudotranslation

Crystallographic translation

Pseudo-translation

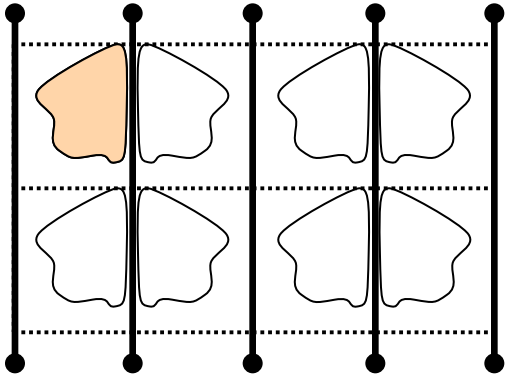


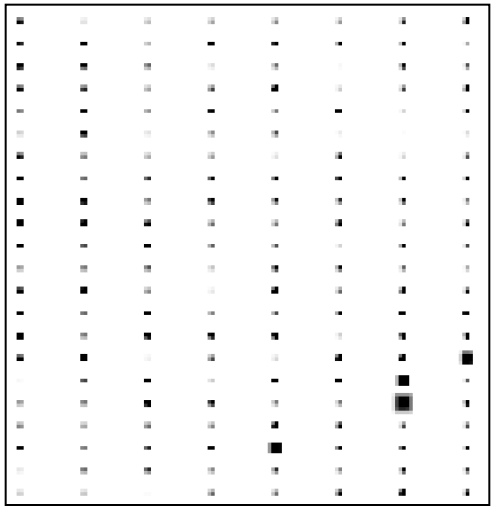


Pseudotranslation **C**/2

Planes 2L+1 contain weak reflections

Crystallographic translation





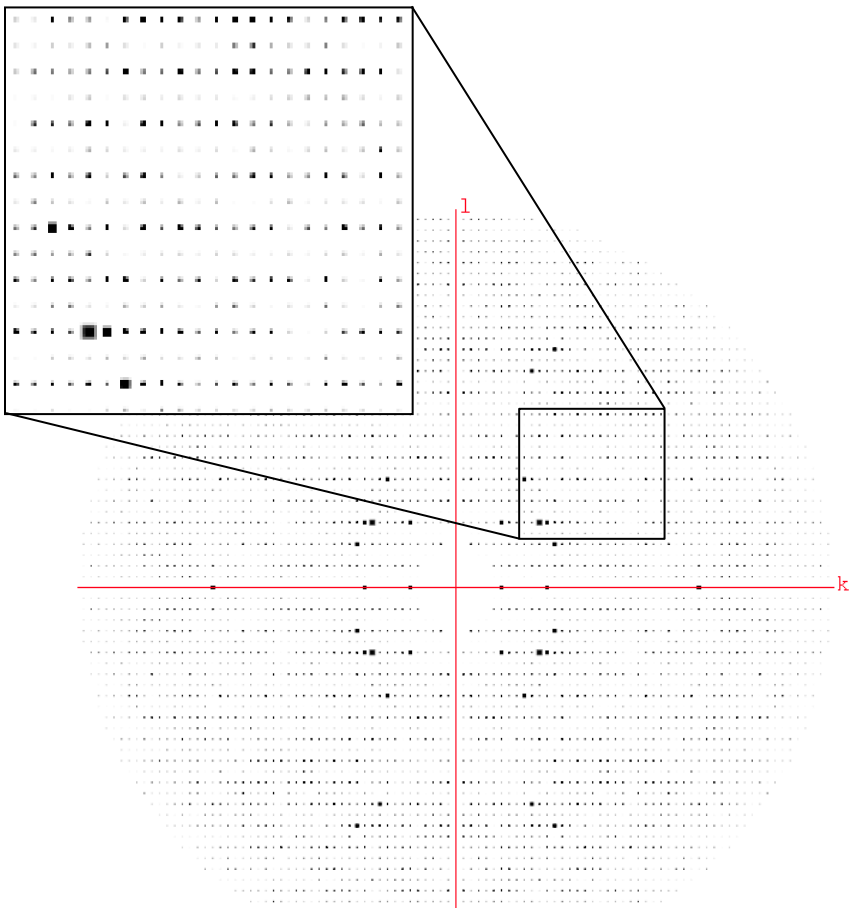
Limiting case, **C'** = **C**/2

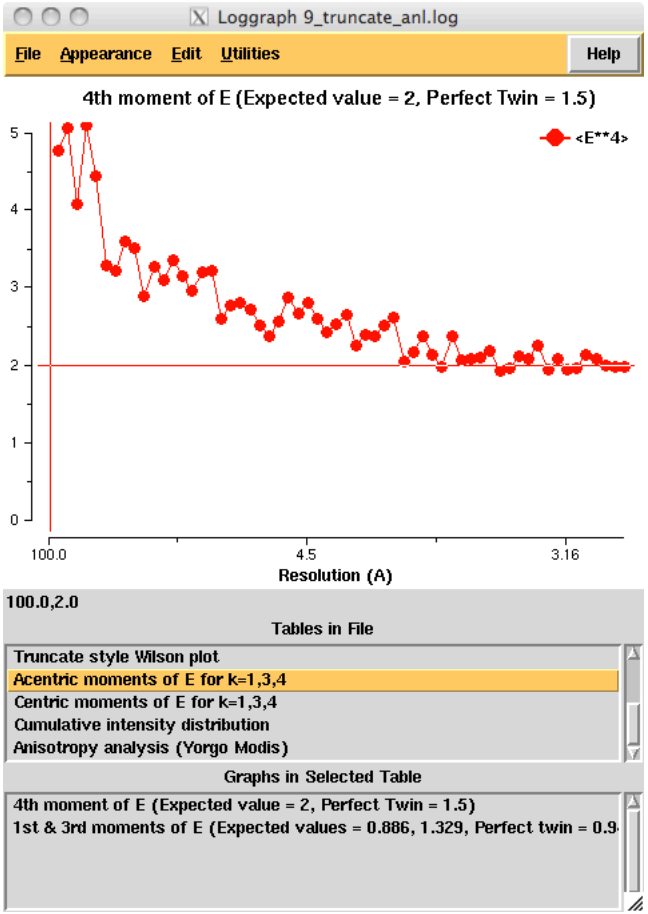
- Weak reflections vanish

Two times larger reciprocal lattice spacing

Statistics of one intensity are strongly affected by pseudotranslation

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

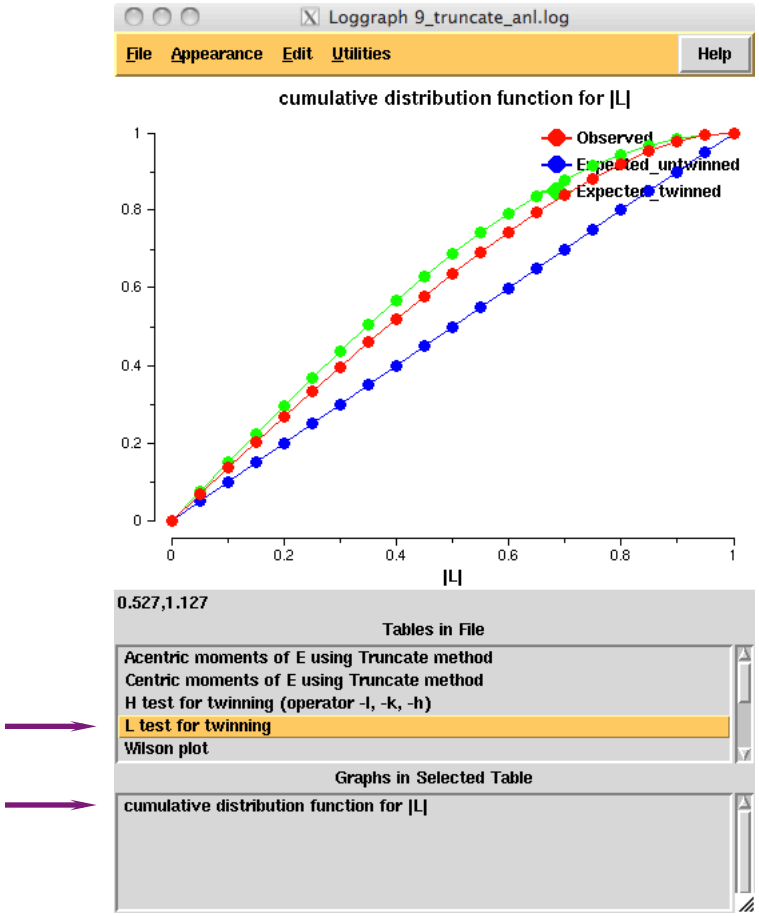


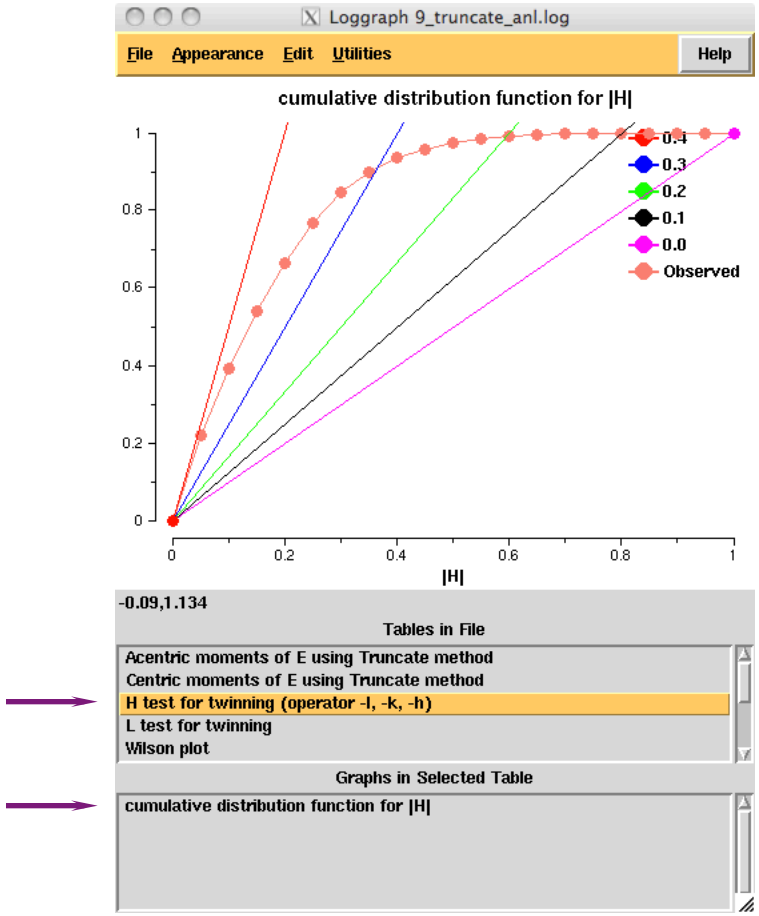


> Acentric moments of E for k=1,3,4

> 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

Twinning by (pseudo)merohedry

Statistics of one observation

Statistics of two observations

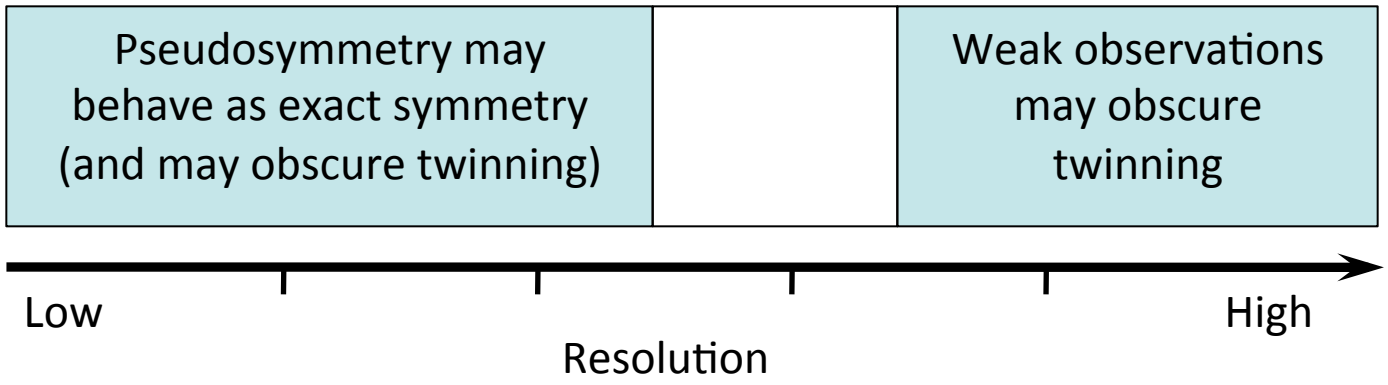
Twinning tests summary

Space group validation

Why so many tests?

	Statistics of one observation		Statistics of two observations	
	P(Z)	<Z^2>	H-test	L-test
Specific for a given resolution shell	No	Yes	No	No
Specific for a given twin operation	No	No	Yes	No
Can detect perfect twinning	+	+	–	+
Works for incomplete data	+	+	–	+
Insensitive to pseudotranslation	–	–	+ / –	+
Insensitive to anisotropy	–	–	+ / –	+
Insensitive to weak reflections at high resolution	–	(–)	–	–

Are these tests always sufficient?



How to handle the cases with strong pseudosymmetry?

Validation of crystallographic symmetry:

refinement in space groups compatible with

- unit cell
- current model (considered as at least approximately correct)

OD-structures

Twinning by (pseudo)merohedry

Statistics of one observation

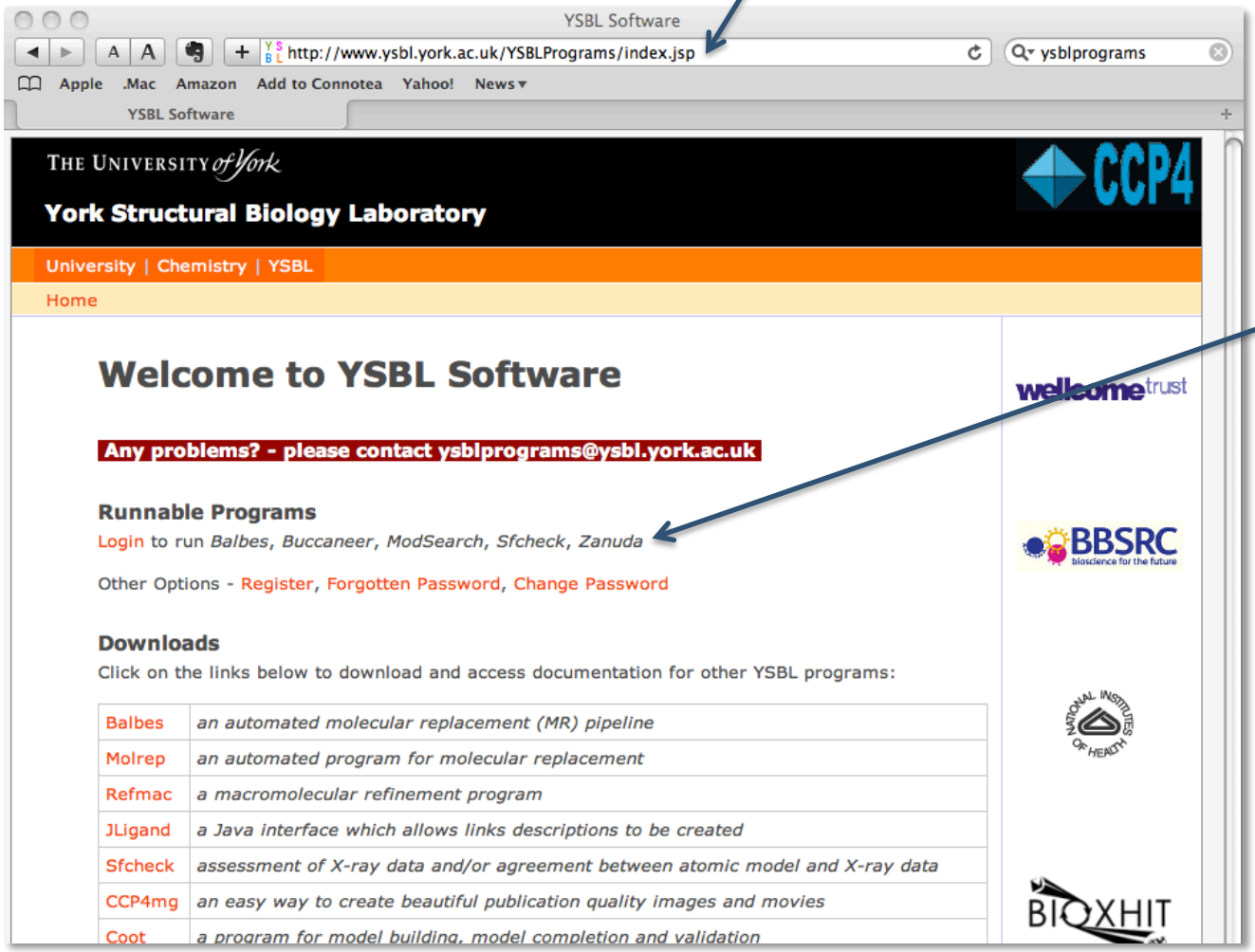
Statistics of two observations

Twinning tests summary

Space group validation

YSBL server

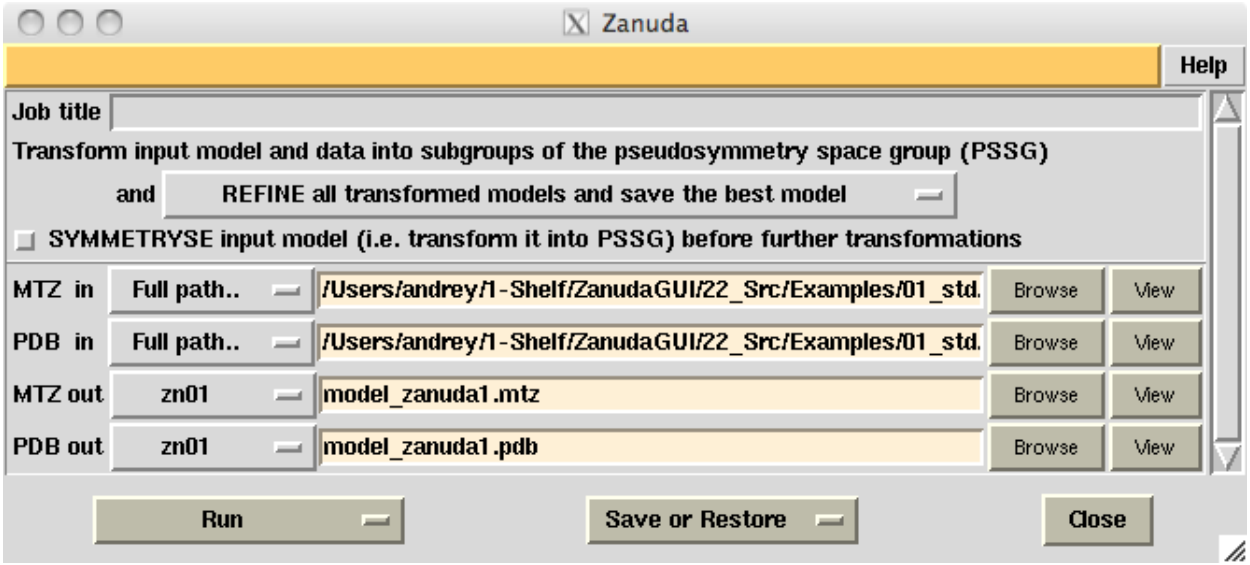
http://www.ysbl.york.ac.uk/YSBLPrograms/index.jsp



Zanuda

CCP4I interface

CCP4I > Validation & Deposition > Validate space group



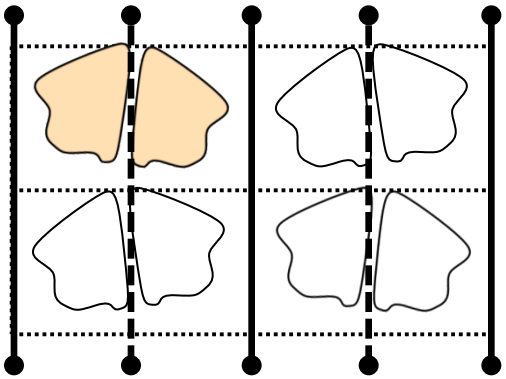
Starting from ccp4-6.3.0 (forthcoming release)

Pseudotranslation: what else can go wrong?

Cell and H-M symbol are the same

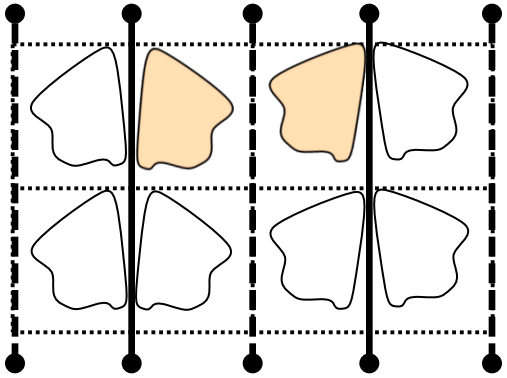
Crystallographic and pseudosymmetry axes are confused

P2 (a,b,c)



True structure

P2 (a,b,c)



False structure

Molecular Replacement:

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

An example of symmetry correction

PDB code:	1yup	
space group (PDB):	P1	8 molecules per a.u.
space group (true):	$P2_1$	4 molecules per a.u.
Pseudo-symmetry space group: (because of pseudo-translation)	C2	2 molecules per a.u.

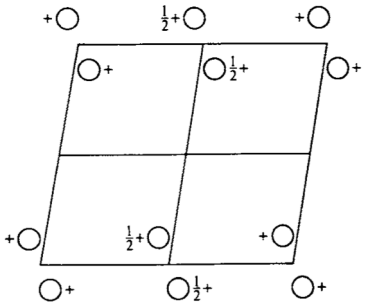
Monoclinic structures related to 1yup

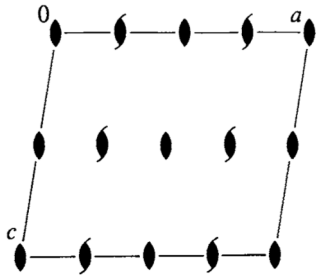
Positions of molecules

Crystallographic axes

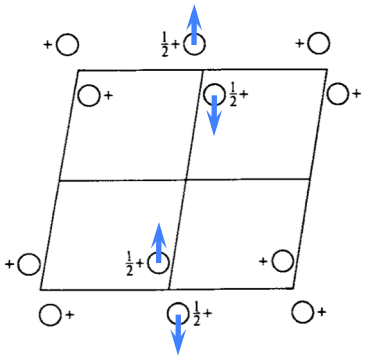
Pseudosymmetry axes

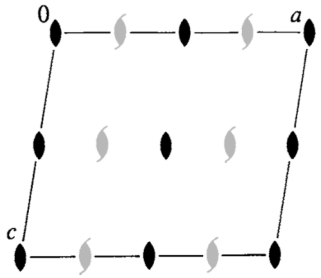
Space group and its relation to the structure 1yup



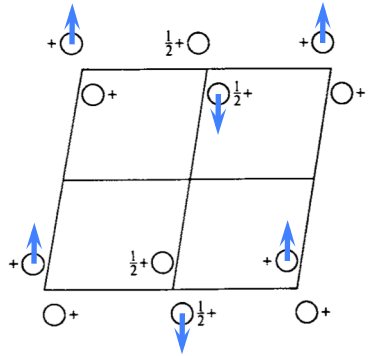


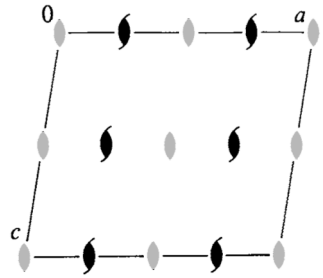
C2 Pseudo-symmetry space group





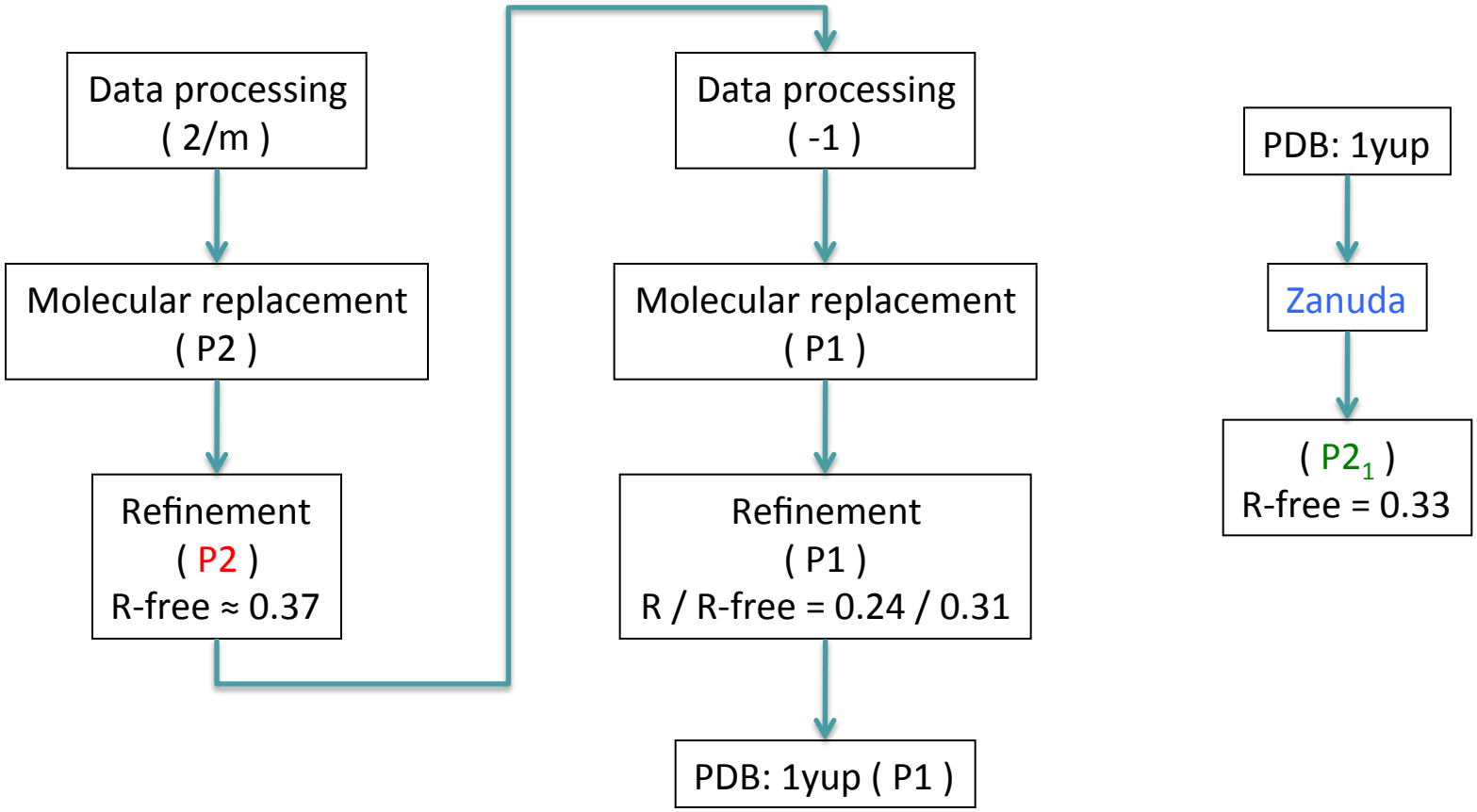
P2 False space group





$P2_1$ True space group

Structure solution and symmetry validation



Zanuda: limitations

Assumptions:

- The pseudosymmetry is very strong (r.m.s.d. from exact symmetry ≈ 1A)
- The structures of individual molecules are almost correct
  - although they might have been refined / rebuilt in an incorrect space group

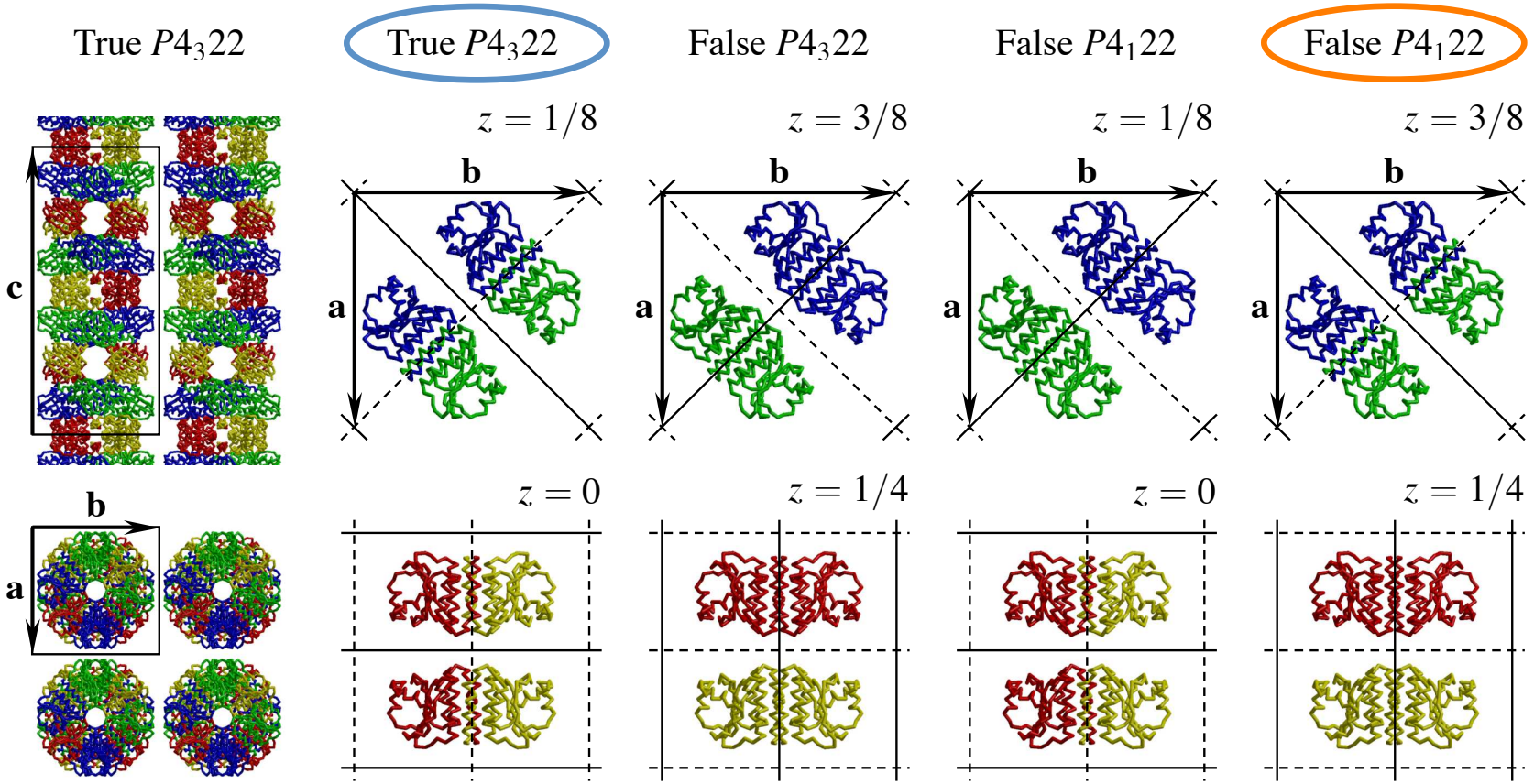
If assumptions are not satisfied, the results will likely to be wrong.

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.

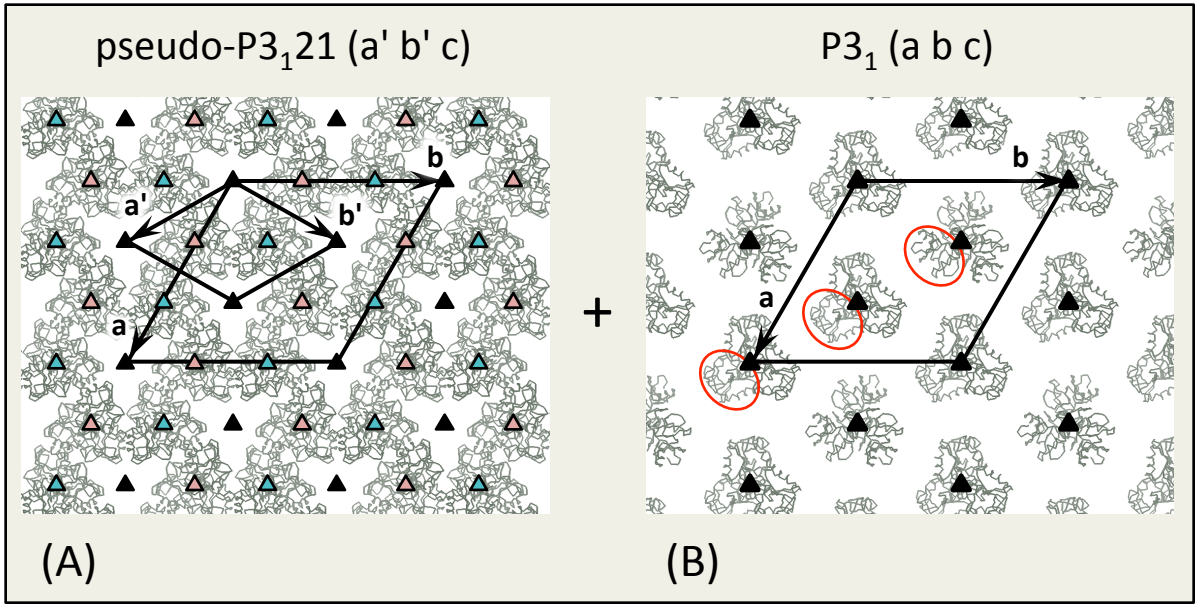
MR solution



Twinned crystal with pseudo-symmetric substructure

Human macrophage receptor CLEC5A for dengue virus

Watson, A. A. et al. (2011). J Biol Chem 286, 24208-18.



3-fold axes with respect to the true structure:

▲ crystallographic

△ △ pseudosymmetry for (A)

- Substructure (A) is common for twin individuals
- Substructure (B) is not even approximately symmetric relative to △ and △
  - The choice of correct origin was essential for structure completion

Acknowledgements

Eleanor Dodson	University of York
Roberto Steiner	King's College, London
Zbigniew Dauter	APS, Chicago
Gleb Bournkov	EMBL-Hamburg
Michail Isupov	University of Exeter
Garib Murshudov	MRC LMB Cambridge

All the authors of cited papers

Zanuda: space group validation

Algorithm:

- From input model: determine pseudosymmetry space group (PSSG)
- From PSSG: select subgroups with observed unit cell
- For each such subgroup:
  - Convert model and data into the subgroup
  - Restrained refinement
- Repeat refinements of the best (R-free) model
  - Starting from P1
  - Adding the best (r.m.s.d.) symmetry element at each refinement
    - » Terminate if there is no symmetry elements to be added
    - » Terminate and cancel the last symmetry element if R-free jumps

Tutorial

Ferrochelatase-1 Tutorial:

Space group assignment in the presence of pseudosymmetry and twinning

Data:

http://www.ysbl.york.ac.uk/mxstat/andrey/hemh.html

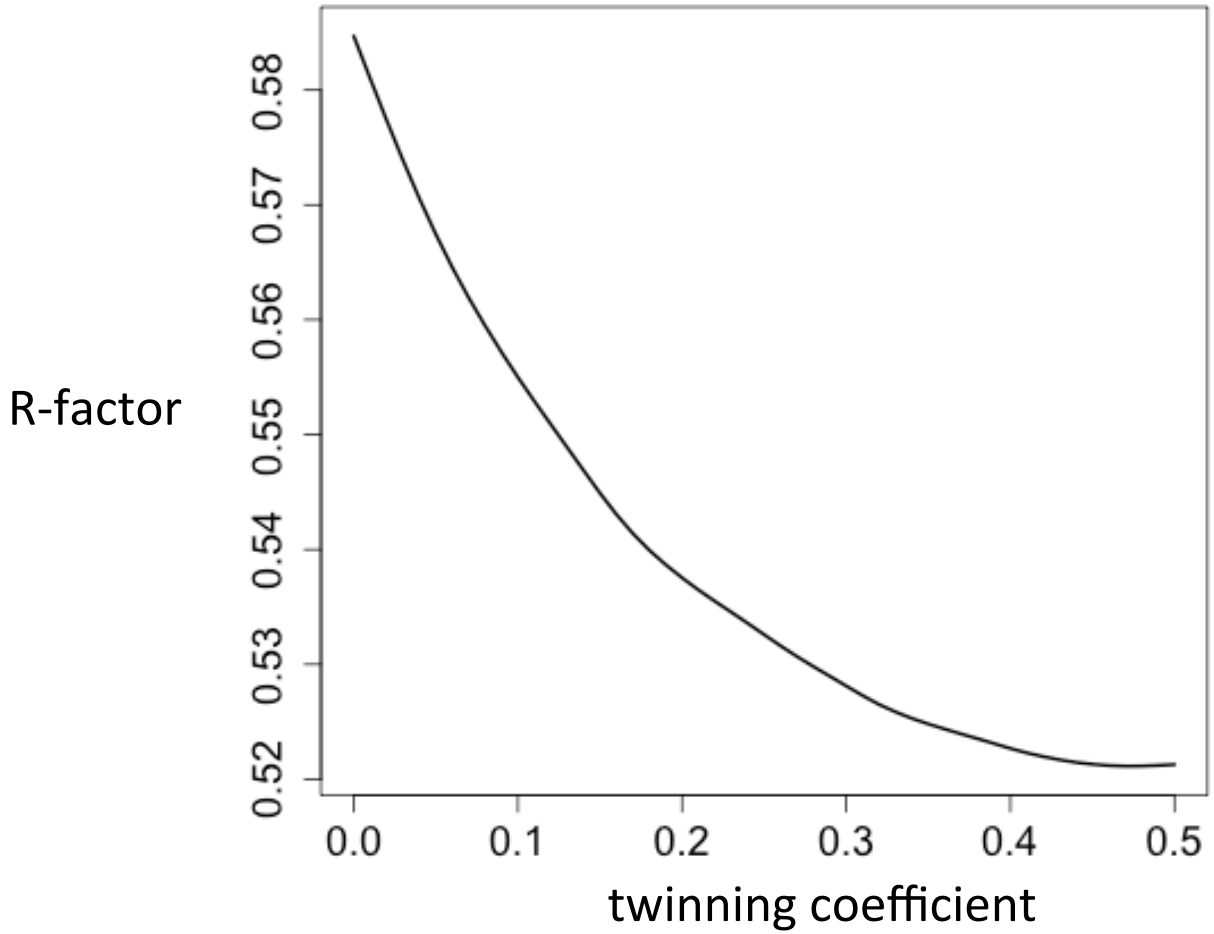
- OD-twin by pseudomerohedry
- use of pointless for point group detemination in a relatively difficult case
- use of molecular replacement

Twinned refinement against non-twinned data

Beginning of refinement:

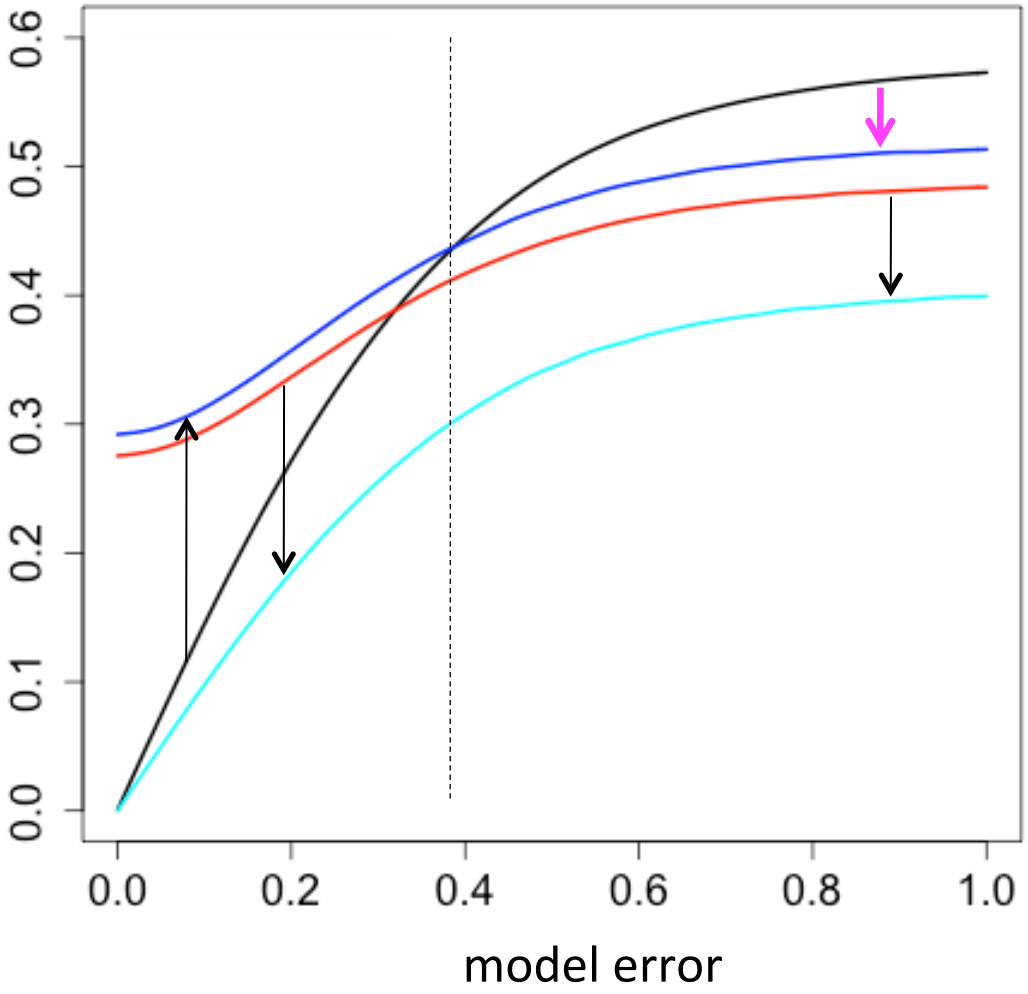
The structure and data are unrelated (data correspond to different structure)

Twinning coefficient would converge to 0.5



Switching to twin refinement

R-factor



obs	refinement
–	–
–	T
T	–
T	T