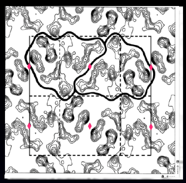
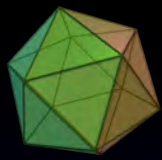
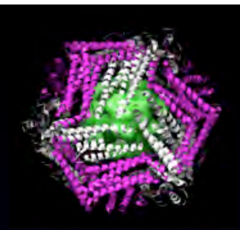
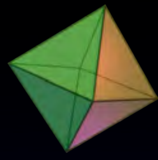
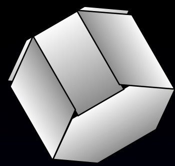










SPACE GROUPS AND SYMMETRY

Michael Landsberg

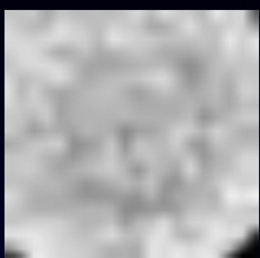
Electron Crystallography Workshop
C-CINA, Basel, 1-7 Aug 2010

m.landsberg@uq.edu.au

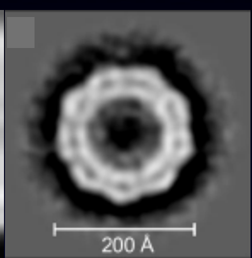
The University of Queensland Australia | IMB Institute for Molecular Bioscience | ammrf

Averaging

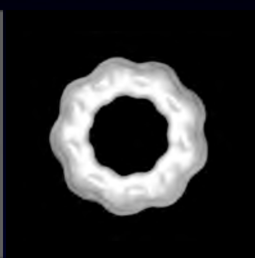
- Why single molecule EM techniques are far superior in resolution than electron tomography



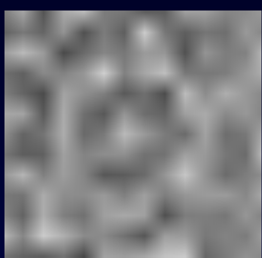
single image



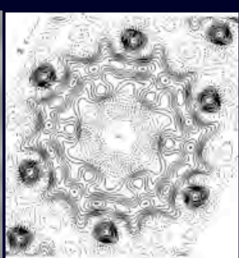
class average



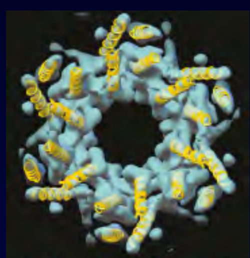
3d from 100s of class averages



one unit cell



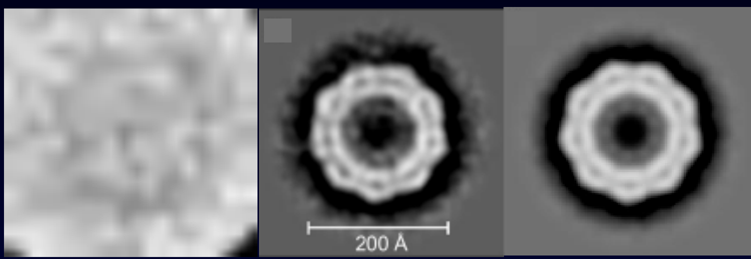
projection map from 1 image (10^2-10^3 unit cells)



3d from several images (10^4? unit cells)

What is symmetry?

- An object is symmetrical if, when an *operation* is applied, the result of the operation is indistinguishable
- Imposing symmetry is a form of averaging

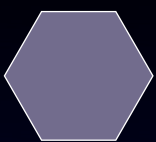


single image | unsymmetrized class sum | symmetrized class sum

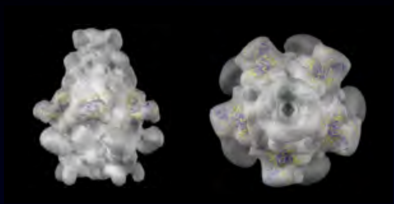
What symmetry is. How do we identify it. How do we take advantage of it.

Molecular symmetry

Cyclic symmetry

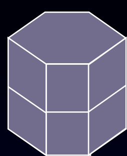


C6

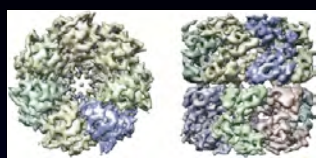


C5 supercomplex

Dihedral symmetry



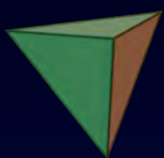
D6

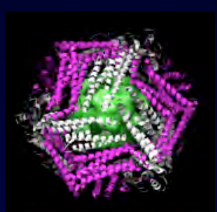


D7 - GroEL

Ludtke et al. *Structure* (2004) 12:1129-36

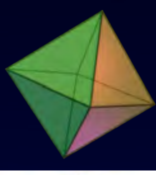
Tetrahedral symmetry

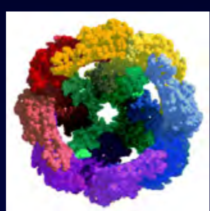




Insect Ferritin

Octahedral symmetry

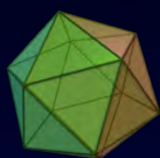


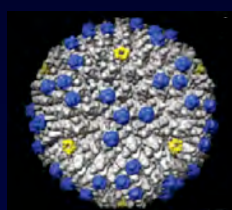


Hsp16.5

Kim et al. *Nature* (1998) 394:595-99

Icosahedral symmetry



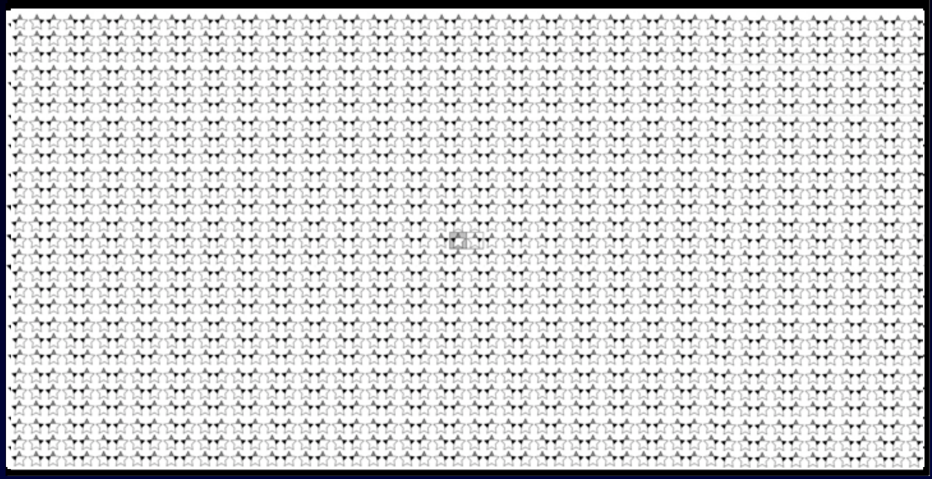


Virus

Crystallographic symmetry

- A 2D crystal is generated by consecutively shifting a unit cell, *ad infinitum*, along either of two vectors (*a* or *b*) separated by an included angle (γ)

- All crystals have translation symmetry



asymmetric unit unit cell 2D array crystal

Group theory

- A crystallographic ***space group*** is the mathematical group of symmetry operations which apply to both the given unit cell and the crystal array

- Finite number of crystal packing arrangments

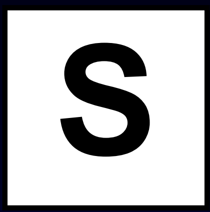
- There are 230 possible crystallographic space groups in 3D
  - 65 for proteins and chiral molecules

- 17 ***plane groups*** describe all the possible symmetry arrangements in projection images of 2D crystals

- These plane groups are different (but correlate somewhat trivially) to the 17 ***2D space groups*** which describe all possible 2D crystal arrangements

Rotation

***n*-fold rotational symmetry** dictates that rotation about a point by an angle of 360°/*n* generates an image indistinguishable from the original



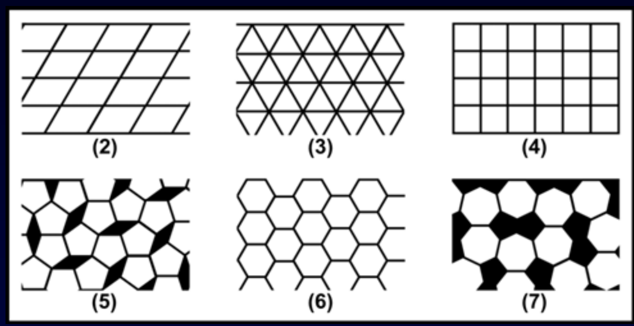
360/2 = 180°

360/3 = 120°

360/4 = 90°

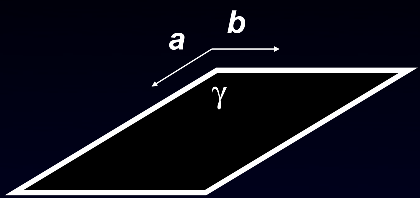
360/6 = 60°

why no 5 fold?

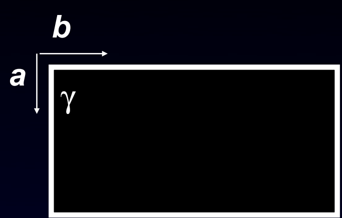


Unit cell geometry

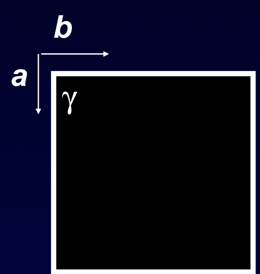
Unit cells in projection can be one of 4 different shapes



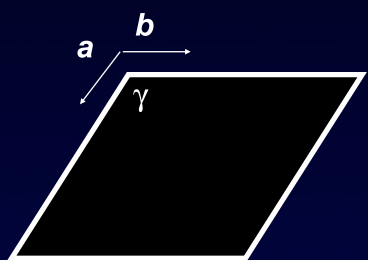
Rhomboid (oblique, monoclinic)
a ≠ b; $\gamma \geq 90$



Rectangle (rectangular)
a ≠ b; $\gamma = 90$



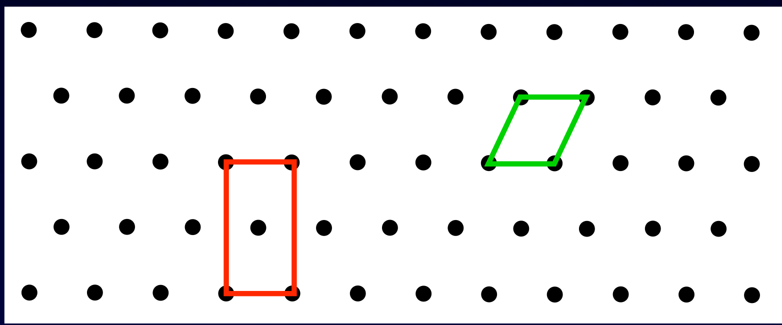
Square (tetragonal)
a = b; $\gamma = 90$



Rhombus (hexagonal)
a = b; $\gamma = 120$

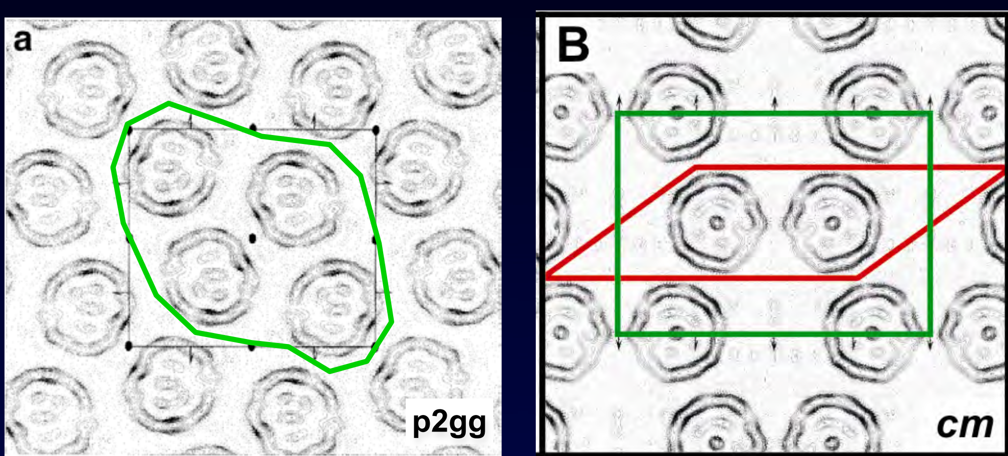
Rectangular unit cells are a special case

- 15 of the 17 possible 2D space groups are ***primitive*** cells. The remaining 2 are ***centered*** cells
  - A primitive cell describes a minimal motif repeate by lattice translations
  - A centered cell contains internal repetition



Rectangular unit cells are a special case

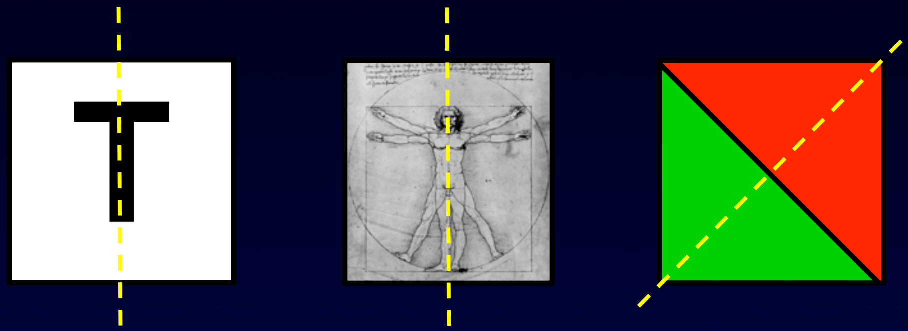
- 15 of the 17 possible 2D space groups are ***primitive*** cells. The remaining 2 are ***centered*** cells
  - A primitive cell describes a minimal motif repeate by lattice translations
  - A centered cell contains internal repetition
  - Either describes the crystal correctly, but the centered cell "buys you more symmetry"



Behlau et al. *J Mol Biol* (2001) 305:71-77

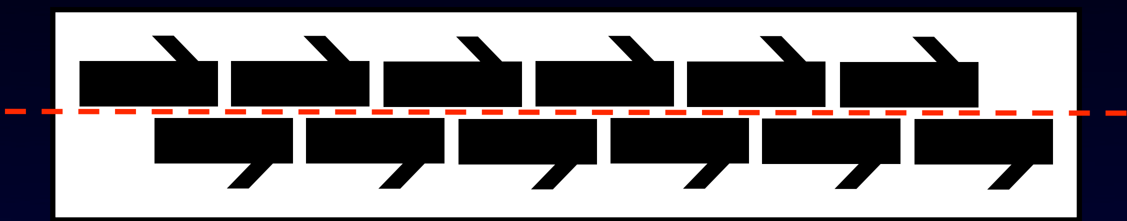
Reflection

- Also known as *mirror* or *bilateral* symmetry
- Any two points perpendicular to and equidistant from the line of reflection are identical



Translation + Reflection = Glide Reflection

Translation by ½ unit cell combined with a reflection about a line parallel to the direction of translation



The 17 2D plane groups

Unit cell geometry
rhomboid (oblique)
rectangle
square
rhombus (hexagonal)

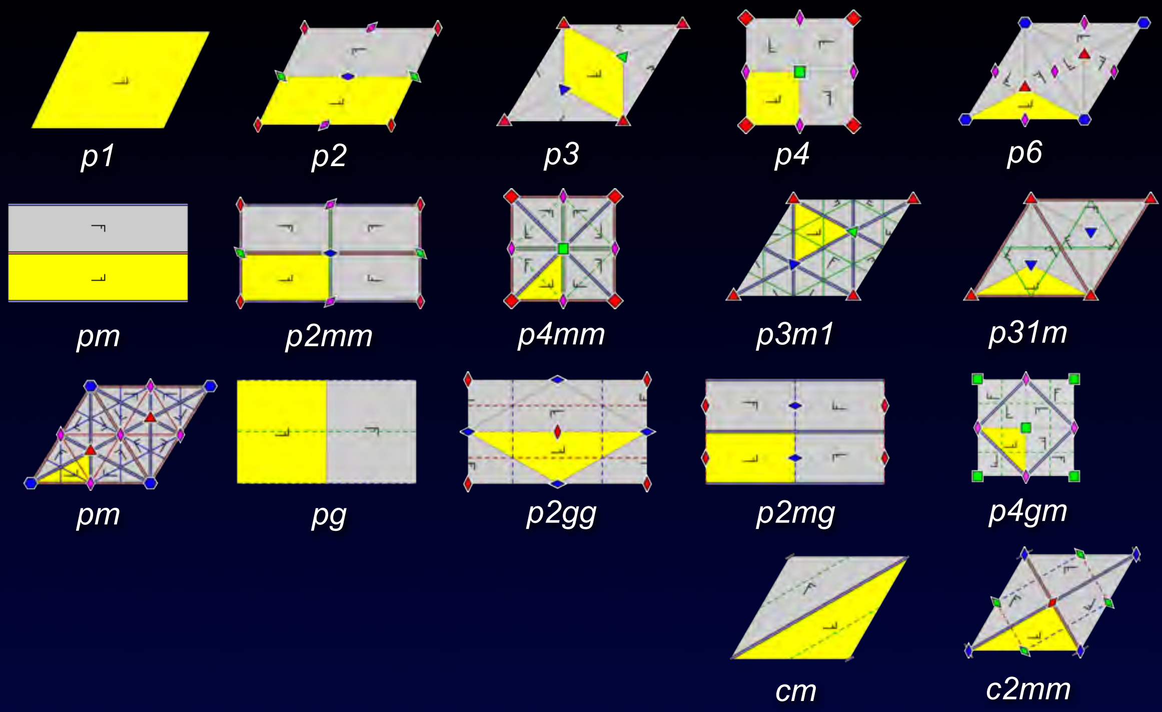
Plane group notation

Hermann-Mauguin style

- Begins with either ***p*** or ***c***, for a **p**rimitive cell or a face-**c**entered cell
- This is followed by a digit, *n*, indicating the highest order of rotational symmetry: 1-fold (none), 2-fold, 3-fold, 4-fold, or 6-fold
- The next two symbols indicate symmetries relative to the "main" translation axis of the pattern; if there is a mirror perpendicular to a translation axis this is the main one (or if there are two, one of them).
  - The symbols are either ***m***, ***g***, or ***1***, for mirror, glide reflection, or none.
  - The axis of the mirror or glide reflection is perpendicular to the main axis for the first letter…
  - …and either parallel or tilted 180°/*n* (when *n* > 2) for the second letter.

p2mg

Plane group definitions



2-fold rotation center 3-fold 4-fold 6-fold

asymmetric unit mirror ...... glide

Group theory

- A crystallographic ***space group*** is the mathematical group of symmetry operations which apply to both the given unit cell and the crystal array

- There are **230** possible crystallographic space groups in 3D
  - **65** for proteins and chiral molecules

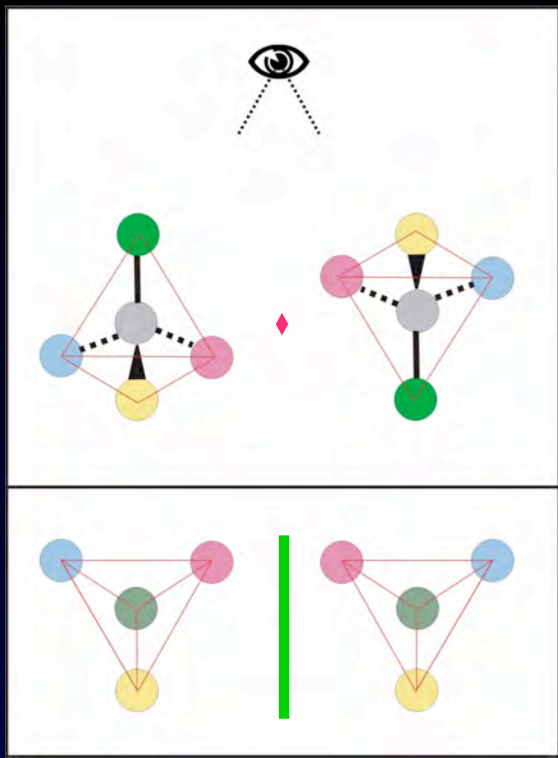
- **17 *plane groups*** describe all the possible symmetry arrangements in projection images of 2D crystals

- These plane groups are different (but correlate somewhat trivially) to the **17 *2D space groups*** which describe all possible 2D crystal arrangements

Symmetry operations in 2D crystals

In-plane center of rotation

- A rotation axis centered in the xz or yz plane
- A 2-fold in plane center of rotation is equivalent to a mirror in 2D projection space



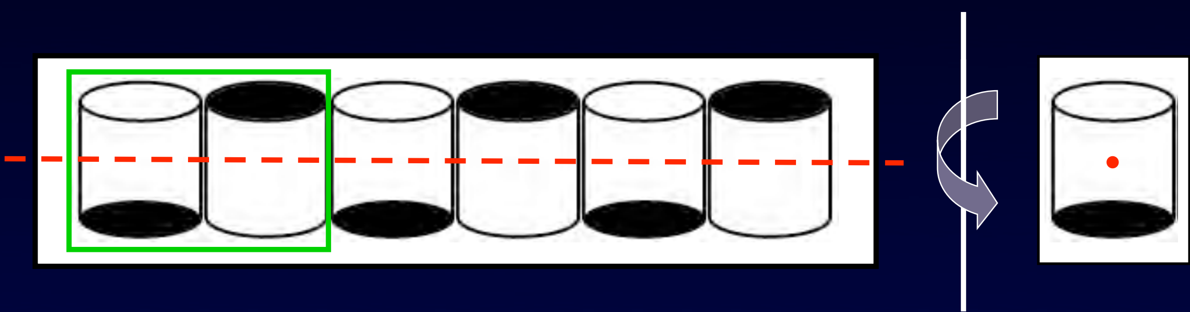
♦ 2-fold center of rotation

▬ mirror plane

Symmetry operations in 2D crystals

Screw axis

- Translation by $1/n$ unit cell combined with a rotation by $360°/n$ about the axis of translation
- A 2_1 screw axis is equivalent to a glide plane reflection in 2D



Plane groups and 2D space groups

Plane group	Unit cell geometry	Highest order	Point group	Glide/screw	2d space group
p1	rhomboid (oblique)	1	1	N	P1
p2	rhomboid (oblique)	2	2	N	P2
pm	rectangle	1	m	N	P12
pg	rectangle	1	m	Y	$P12_1$
cm	rectangle	1	m	N	C12
p2mm	rectangle	2	2mm	N	P222
p2mg	rectangle	2	2mm	Y	$P222_1$
p2gg	rectangle	2	2mm	Y	$P22_12_1$
c2mm	rectangle	2	2mm	N	C222
p4	square	4	4	N	P4
p4mm	square	4	4mm	N	P422
p4gm	square	4	4mm	Y	$P42_12$
p3	rhombus (hexagonal)	3	3	N	P3
p3m1	rhombus (hexagonal)	3	3m	N	P321
p31m	rhombus (hexagonal)	3	3m	N	P312
p6	rhombus (hexagonal)	6	6	N	P6
p6mm	rhombus (hexagonal)	6	6mm	N	P622

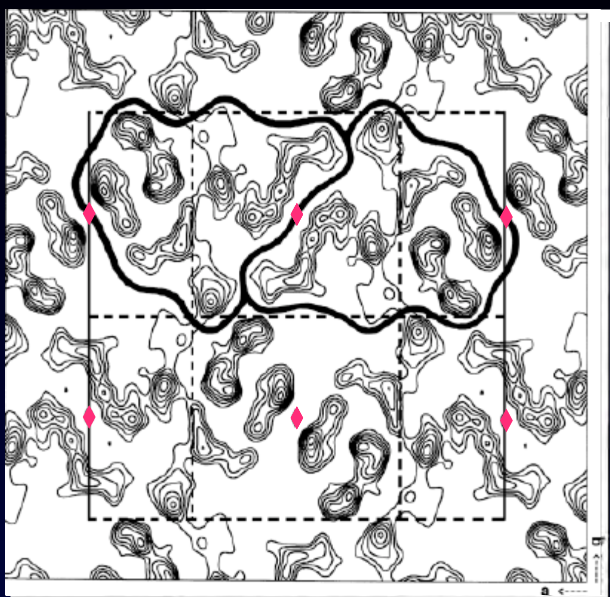
Real space example – RC47 crystal

p2gg ($P22_12_1$)

- **2 fold rotational symmetry**
- **2 x glide axes**

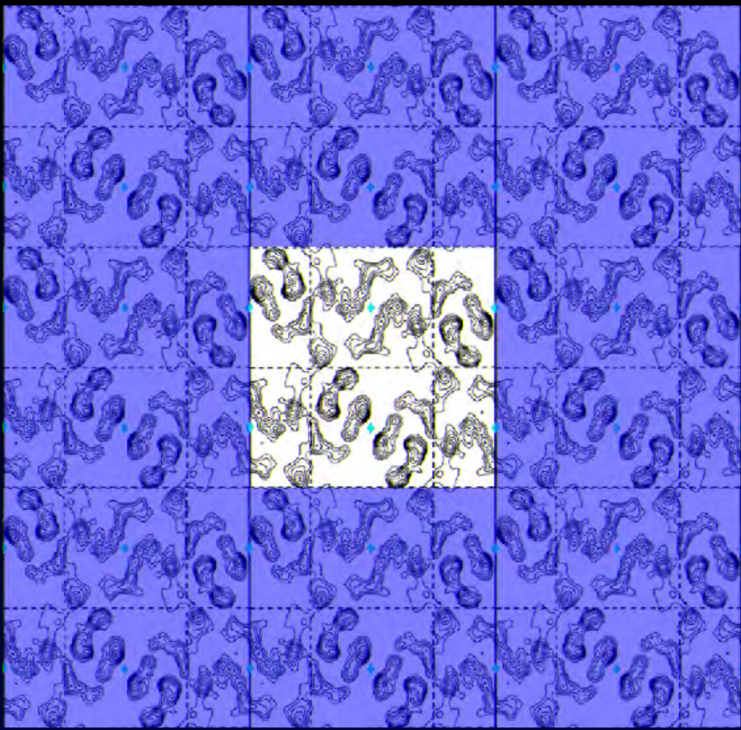
♦ **2-fold center of rotation**

– – – **glide axis**



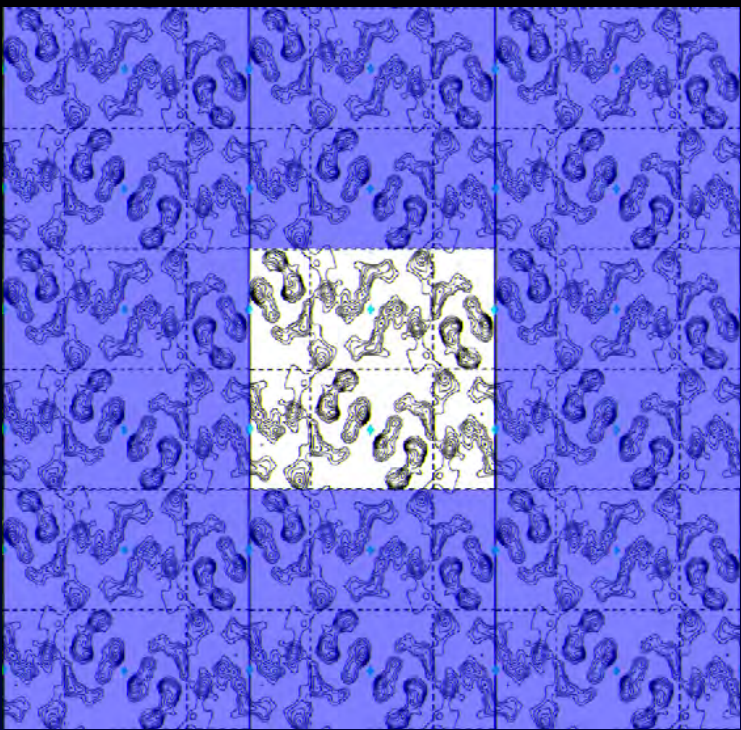
Rhee et al. *Nature* (1997) 389:622-26

Real space example – RC47 crystal



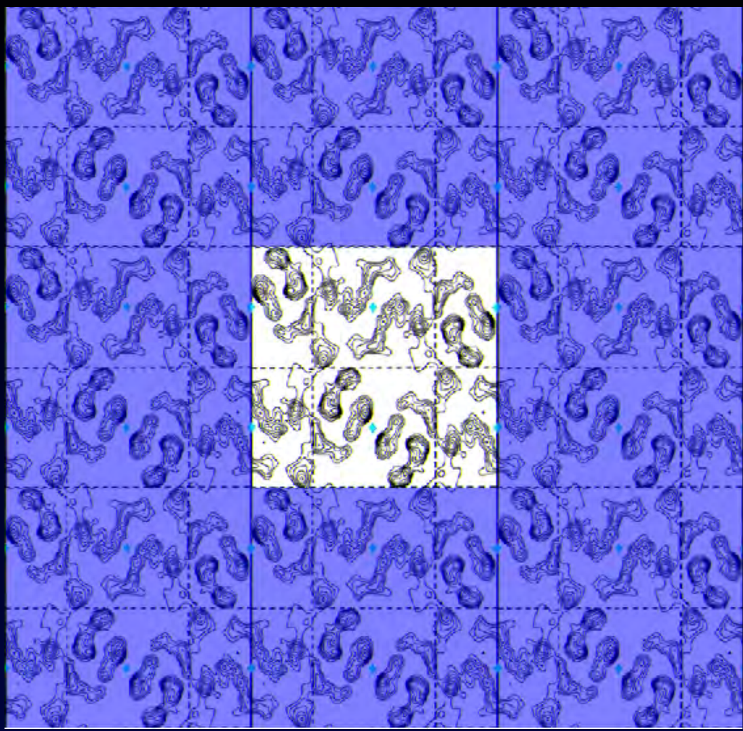
p2gg

Real space example – RC47 crystal



p2gg

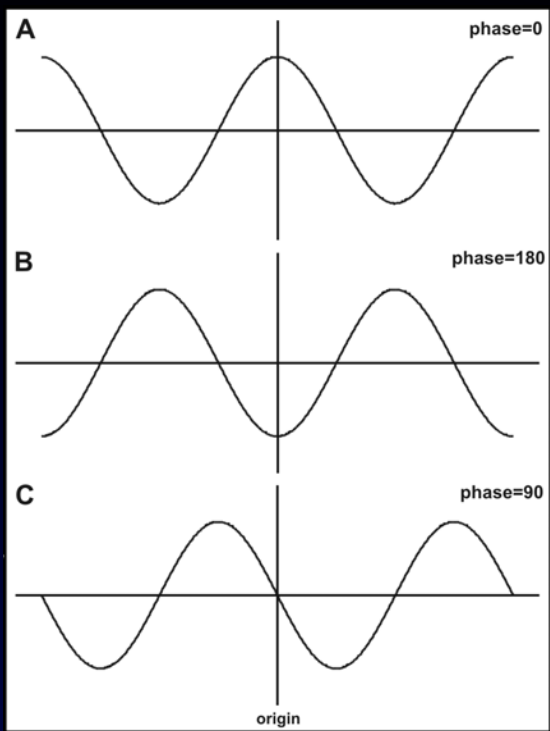
Real space example – RC47 crystal



p2gg

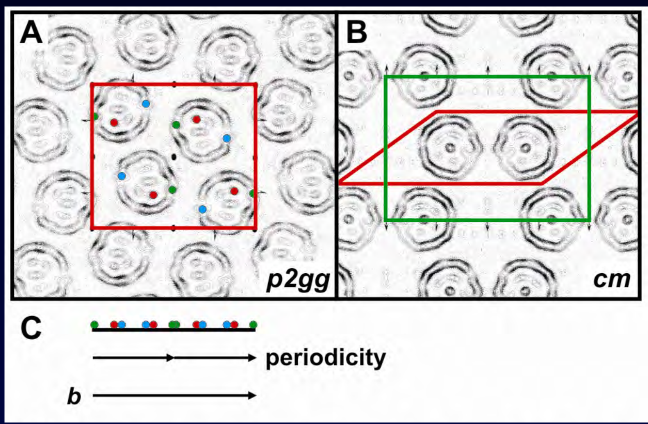
The centrosymmetric condition

- Symmetry in real space is preserved in reciprocal space
- All space groups with 2-fold symmetry must have phases universally equal to 0 or 180 degrees
- These are the only phases which satisfy the requirement that symmetry is conserved in Fourier space



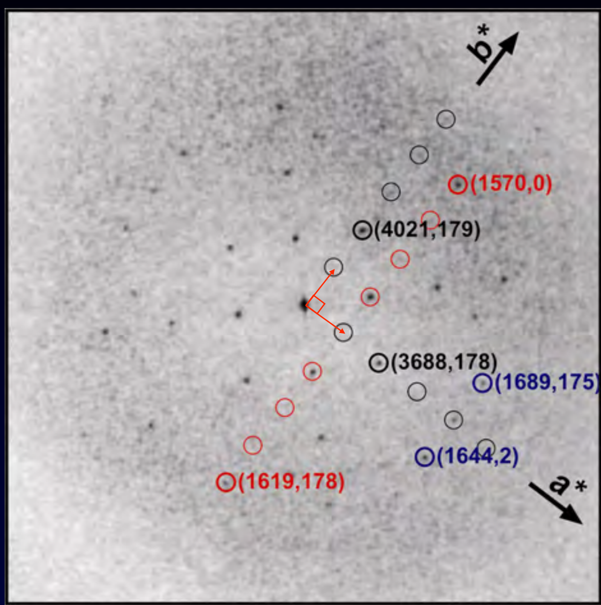
Systematic absences

- *Symmetry forbidden reflections* result when a crystal has periodicity over less than one unit cell
- Axial/zonal systematic absences arise from glides/screws
  - > Loss of odd reflections
- Integral systematic absences arise when a centered cell is chosen
  - > Twice as many reflections



Fourier space example – CHIP28 (Aqp1)

- *a=b, γ=90*
  - – square unit cell
- all phases = 0/180
  - – centrosymmetric space group
- points related by 4-fold rotation are equal
  - – base symmetry is p4
- odd reflections absent
- spots equidistant from *a** are out of phase by 180
  - – glide symmetry



CHIP28 (Aqp1) in real space

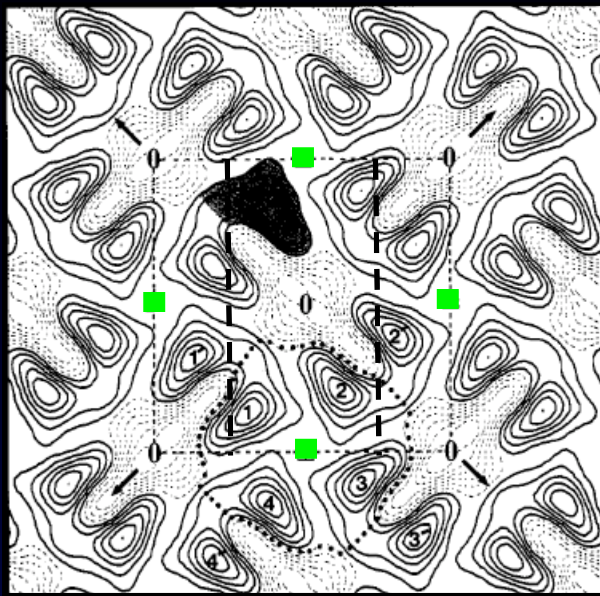
p4gm ($P42_12$)

- 4 fold rotational symmetry
- 1 pair of glide axes
- 1 pair of mirror lines

■ 4-fold center of rotation

– – – glide axis

⟶ mirror line



Mitra et al. *Biochem* (1994) 33:12735-40

Searching for symmetry – ALLSPACE & 2DX

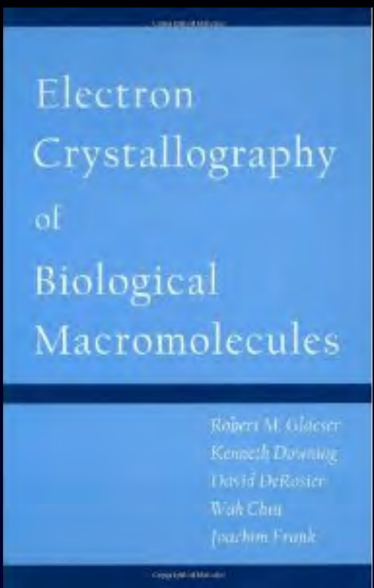
SPACEGROUP		Phase resid(No) v.other spots (90 random)		Phase resid(No) v.theoretical (45 random)		OX	OY
1	p1	29.8	176	22.0	176		
2	p2	62.6	88	31.3	176	-55.7	-17.6
3b	p12_b	76.7	38	32.7	12	-85.6	120.0
3a	p12_a	64.8	37	28.1	10	-170.0	54.8
4b	p121_b	66.6	38	35.1	12	89.6	-145.0
4a	p121_a	58.9	37	32.2	10	-185.0	-40.6
5b	c12_b	76.7	38	32.7	12	-85.6	120.0
5a	c12_a	64.8	37	28.1	10	-170.0	54.8
6	p222	72.0	163	31.2	176	122.4	160.9
7b	p2221b	72.4	163	38.0	176	5.3	139.3
7a	p2221a	71.4	163	38.0	176	89.2	140.5
8	p22121	75.5	163	38.8	176	-180.4	149.7
9	c222	72.0	163	31.2	176	122.4	160.9
10	p4	65.2	172	31.6	176	-58.7	161.0
11	p422	72.6	369	31.8	176	-58.9	160.6
12	p4212	73.2	369	31.9	176	-58.9	-19.6
13	p3	62.2	118	--	--	-158.7	-81.0
14	p312	72.3	298	23.5	20	-154.9	-69.8
15	p321	70.0	305	28.5	34	154.9	-165.9
16	p6	72.0	324	31.8	176	120.9	161.0
17	p622	73.5	691	39.5	176	-39.0	-170.8

Why 21 space groups?

Other considerations

- When might symmetry fall apart?
  - Plane group symmetry rules only hold for untilted specimens
  - Astigmatism causes a non-uniform effect of the CTF on symmetry related spots, potentially making symmetry evaluation unreliable
  - Symmetry rules only hold when data are shifted to phase origin
  - Stain exclusion patterns can cause over-estimation of symmetry
  - Low resolution data may also over-estimate symmetry
- Always check for the satisfaction of sub-symmetries to help
- *ALLSPACE* does not check for systematic absences
- Check that symmetry rules continue to hold when merging and moving up in resolution

Further Reading



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Symmetry: A guide to its application in 2D electron crystallography

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Available online 17 July 2007

…also: V. Unger *et al.* "Structure determination from electron micrographs of 2d crystals"

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