III.C CRYSTALS, SYMMETRY AND DIFFRACTION

KEY CONCEPTS:

- Biomacromolecules quite often occur naturally or *in vitro* as organized structures composed of subunits arranged in a **symmetrical** way

- Such structures readily studied by **diffraction** (*i.e.* Fourier-based) methods

- Fundamental concepts concerning **crystalline** matter, **symmetry** relationships, and **diffraction theory** form a *basic framework* for understanding the *principles and practice of image processing* and interpretation of structural results

III.C CRYSTALS, SYMMETRY AND DIFFRACTION

III.C.1 Definition of Terms

Read pp.178-179 of lecture notes very carefully so you have a good grasp of the terminology

III.C CRYSTALS, SYMMETRY AND DIFFRACTION

III.C.2 Crystals

DEFINITION: Crystal

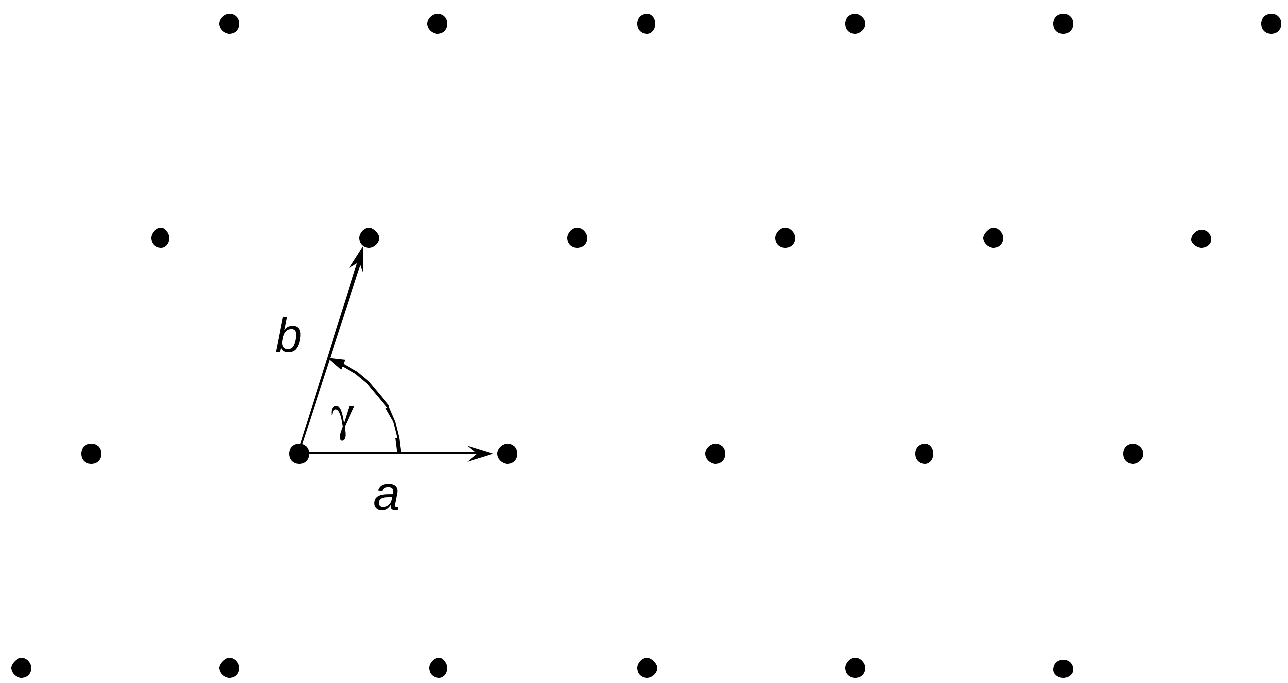
Regular arrangement of atoms, ions, or molecules

- Conceptually built up by **continuing translational repetition** of some structural pattern

- Pattern (**unit cell**) may contain one or more molecules or a complex assembly of molecules

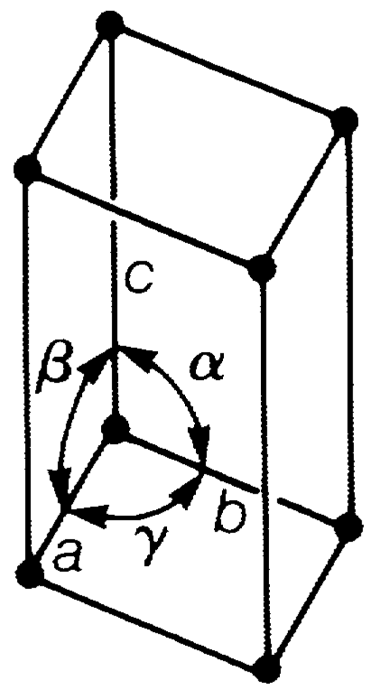
III.C.2 Crystals

Unit cell (in **2D**) defined by two edge lengths **(*a,b*)** and one interaxial angle **(g)**



III.C.2 Crystals

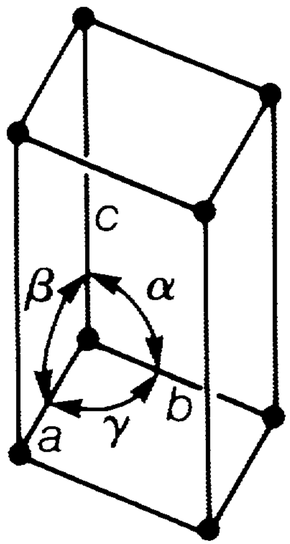
Unit cell (in **3D**) defined by three edge lengths **(*a,b,c*)** and three interaxial angles **(**a**,ß,**g**)**



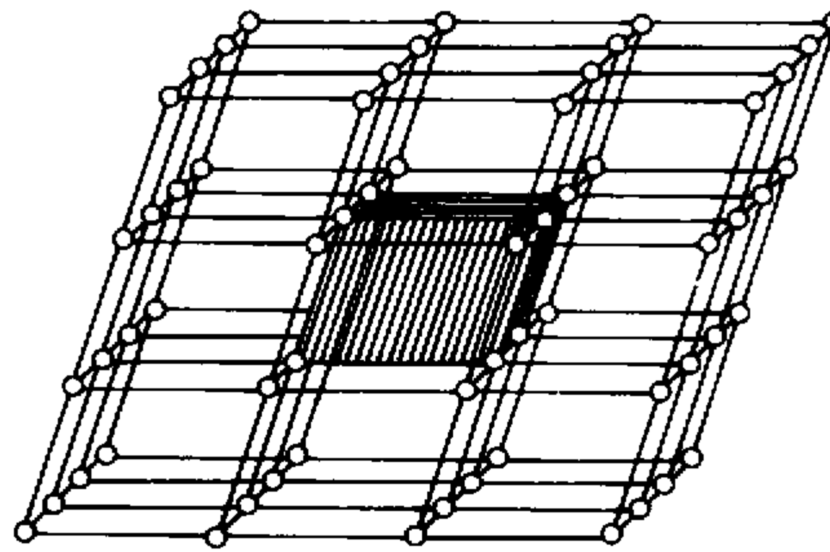
From Eisenberg & Crothers, Fig. 16-16, p.790

III.C.2 Crystals

Unit cell (in **3D**) defined by three edge lengths **(*a,b,c*)** and three interaxial angles **(a,ß,g)**



From Eisenberg & Crothers, Fig. 16-16, p.790



From Glusker & Trueblood, Fig. 2b, p.10

III.C CRYSTALS, SYMMETRY AND DIFFRACTION

III.C.3 Lattices

DEFINITION: Lattice

A **rule for translation**

A **mathematical formalism** - defines an **<u>infinite array</u>** of **<u>imaginary points</u>**

- Each point in the lattice is **identical** to every other point

View from each point is identical with the view in the same direction from any other point (condition not obeyed at the boundary of a finite, but otherwise perfect crystal)

III.C.3 Lattices

Crystal **structure** and crystal **lattice** NOT equivalent

- Structure is an array of **objects**

- Lattice is an array of **imaginary**, infinitely small **points**

2D lattice:

Defined by two translations, *a,b*, and two axes at an angle α to each other

3D lattice:

Defined by three translations, *a,b,c,* and three axes at angles α,β,γ to each other

III.C.3 Lattices

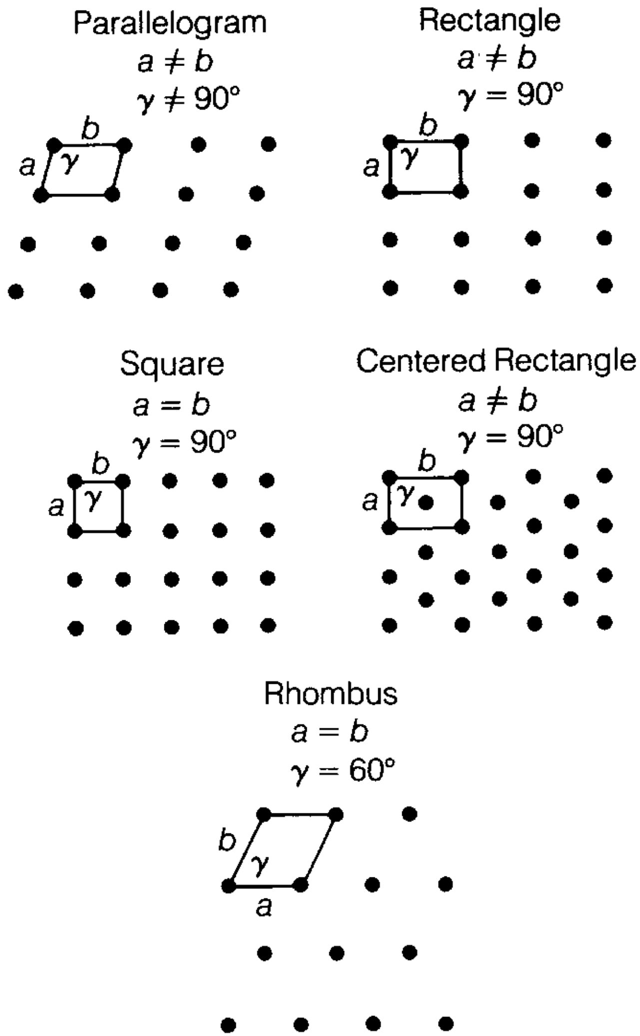
2D or 3D crystal lattices may be:

- **Primitive** (P) - one lattice point per unit cell
- **Body-centered** (I) - two lattice points per cell
- **Face-centered** (F) - four lattice points per cell

Four 2D lattice systems subdivided into five 2D lattices

III.C.3 Lattices

The 5 2D Lattices



From Eisenberg & Crothers, Fig. 16-14, p.786

III.C.3 Lattices

Four **2D** lattice systems subdivided into five 2D lattices

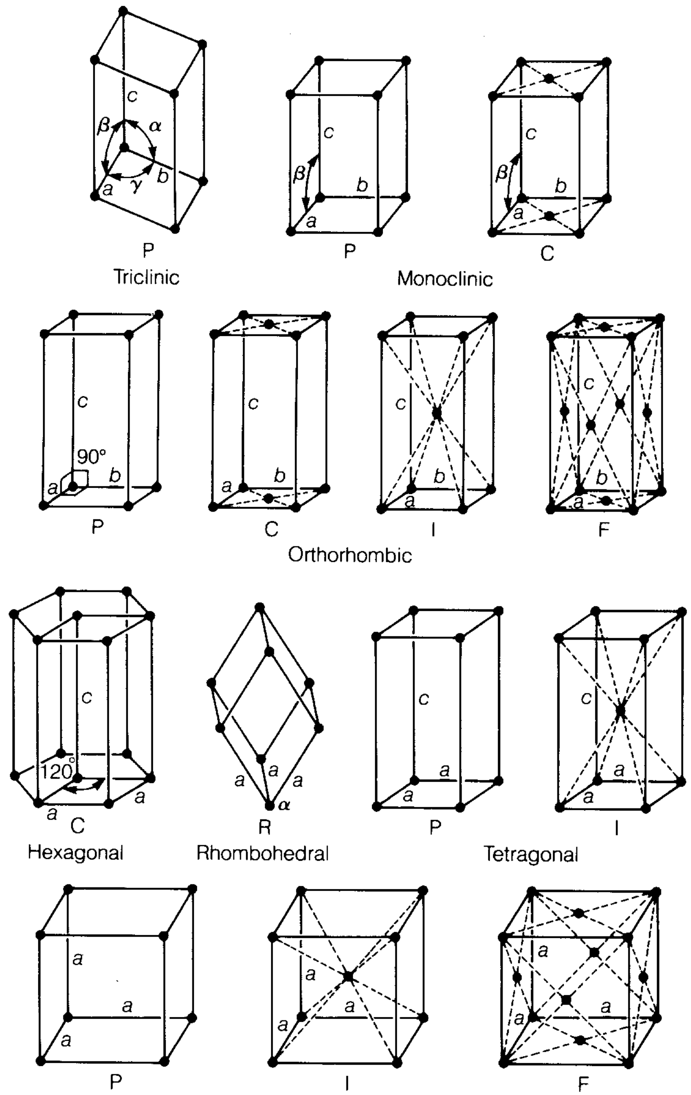
Seven 3D crystal systems correspond to the seven basic space-filling shapes that unit cells can adopt

- Subdivided into 14 Bravais lattices

- Cubic crystal system *e.g.* includes three Bravais lattices: P, I, and F

III.C.3 Lattices

The 14 3D Bravais Lattices



From Eisenberg & Crothers, Fig. 16-16, p.790

III.C CRYSTALS, SYMMETRY AND DIFFRACTION

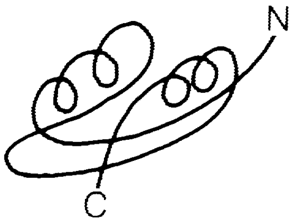
III.C.4 Crystal Structure

Crystal structure: built by placing a **motif** at every lattice point

DEFINITION: Motif

The **object** that is **translated**

- May be **asymmetric** (*e.g.* a single polypeptide chain)

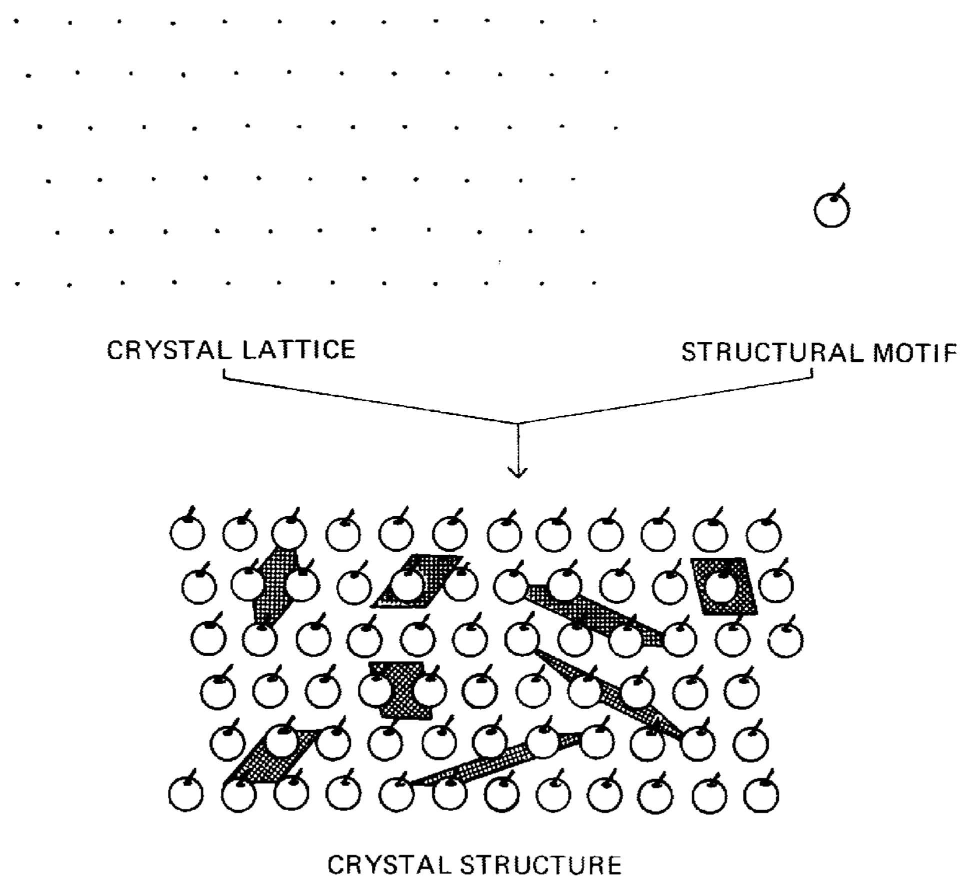


- May be **symmetric** (*i.e.* containing two or more symmetrically arranged subunits)

From Eisenberg & Crothers, Table 16-1, p.756

III.C.4 Crystal Structure

Crystal structure - built by placing a **motif** at every lattice point



From Glusker & Trueblood, Fig. 2, p.10

Crystal Structure, Crystal Lattice and Motif

All are **restricted** in the symmetries they can *display*

But: biomacromolecular assemblies themselves are **not restricted**

- They may display additional internal (**non-crystallographic**) symmetry

From this emerges the following corollary:

Asymmetric unit of the **crystal structure** may itself contain a **symmetrical arrangement** of identical, **asymmetric** molecules

III.C.4 Crystal Structure

DEFINITION: Asymmetric Unit

Part of the symmetric object from which the whole is built up by repeats

The **smallest unit** from which the object can be generated by the symmetry operations of its **point group**

III.C CRYSTALS, SYMMETRY AND DIFFRACTION

III.C.5 Symmetry

Biological objects may display symmetry about a **point** or along a **line**

DEFINITION:

An object is **symmetrical** if it is **indistinguishable** from its initial appearance when spatially manipulated (ignore boundary effects)

III.C.5 Symmetry

III.C.5.a Symmetry Operators

Four types of **symmetry operations** which lead to superimposition of an object on itself:

Rotation

Translation

Reflection

Inversion

DEFINITION: Symmetry Element

Geometrical entity such as a point, line, or plane about which a symmetry operation is performed

III.C.5 Symmetry

III.C.5.a Symmetry Operators

Symmetry of any object is described by some **combination** of the symmetry operations

Biological aggregates or crystals:

- Symmetry **only** described by **rotation** and/or **translation** operations

Why?

Example: Protein molecules mainly consist of *l*-amino acids, hence, **reflection or inversion** symmetries are **not allowed**

III.C.5 Symmetry

III.C.5.b Asymmetric Unit

DEFINITION: Asymmetric Unit

Part of the symmetric object from which the **whole is built up by repeats**

The **smallest unit** from which the object can be generated by the symmetry operations of its point group

III.C.5 Symmetry

III.C.5.b Asymmetric Unit

of ASUs may be <, =, or > # of molecules in unit cell

If # of ASUs = # molecules in unit cell:

- Molecule either **contains no symmetry or** it contains **non-crystallographic symmetry** (symmetry not contained within the allowed lattice symmetries)

If # of ASUs > # molecules in unit cell:

- Molecules **must occupy special positions** and possess the appropriate symmetry element of the space group

III.C.5 Symmetry

III.C.5.c Point Groups

DEFINITION: Point Group

Collection of symmetry operations that define the **symmetry about a point**

Notation Systems:

S or Schoenflies (capital letters; mainly used by spectroscopists)

H-M or Hermann-Mauguin (explicit list of symmetry elements; preferred by crystallographers).

III.C.5 Symmetry

III.C.5.c Point Groups

Types of Symmetry about a Point:

Rotational (*n*)

Mirror or Reflection (*m*)

Inversion (*i*)

Improper Rotations

III.C.5 Symmetry

III.C.5.c Point Groups

Rotational Symmetry (*n*)

Object appears identical if rotated about an axis by $\alpha = 360/n$ degrees ($= 2\pi/n$ radians)

Only allowed *n*-fold axes for crystal **lattices** are:

$n = 1,2,3,4,$ and 6

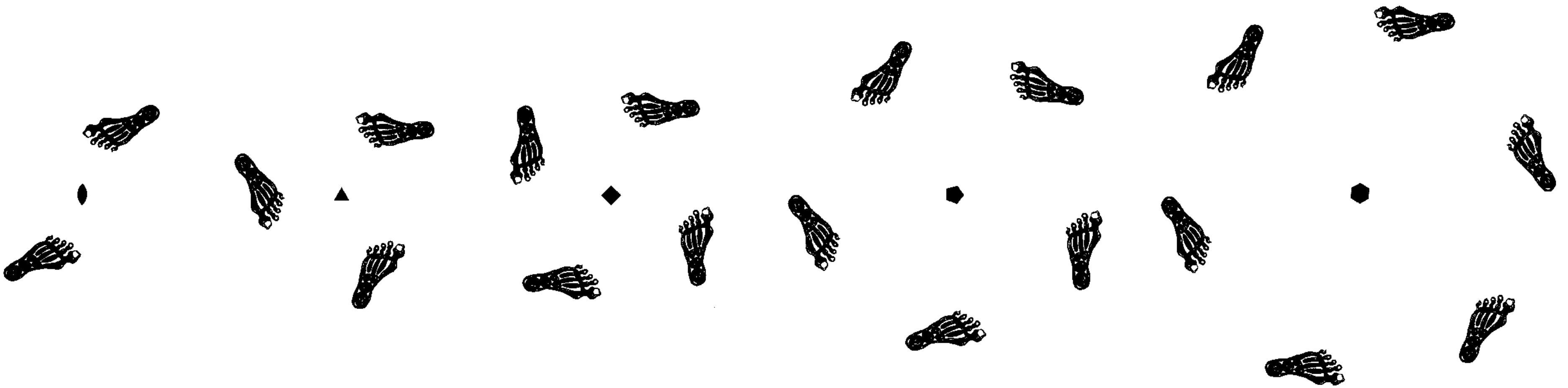
Why the restriction?

Lattices must be **space filling**

III.C.5 Symmetry

III.C.5.c Point Groups

Rotational Symmetry (*n*)



n=2 *n*=3 *n*=4 *n*=5 *n*=6

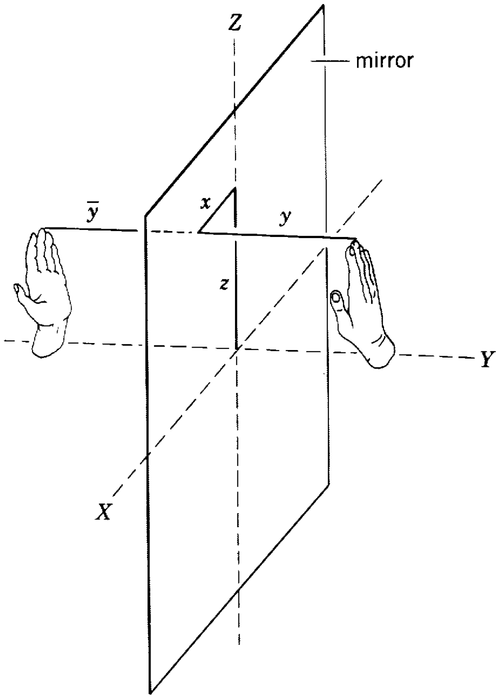
From Bernal, pp.35,45,48,50 and 52

III.C.5 Symmetry

III.C.5.c Point Groups

Mirror (Reflection) Symmetry (*m*)

Each point in the object is converted to an identical point by projecting through a **mirror plane** and extending an equal distance beyond this plane



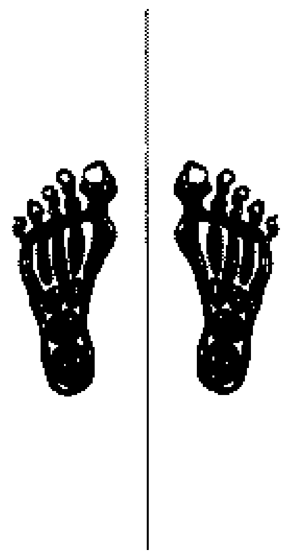
From Burger, Fig. 4, p.8

III.C.5 Symmetry

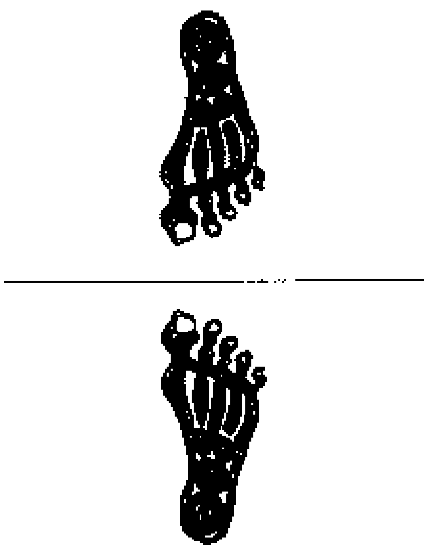
III.C.5.c Point Groups

Mirror (Reflection) Symmetry (*m*)

m_y: $x,y \text{ ---> } -x,y$



m_x: $x,y \text{ ---> } x,-y$



From Bernal, pp.26,27 and 34

III.C.5 Symmetry

III.C.5.c Point Groups

Inversion Symmetry (*i*)

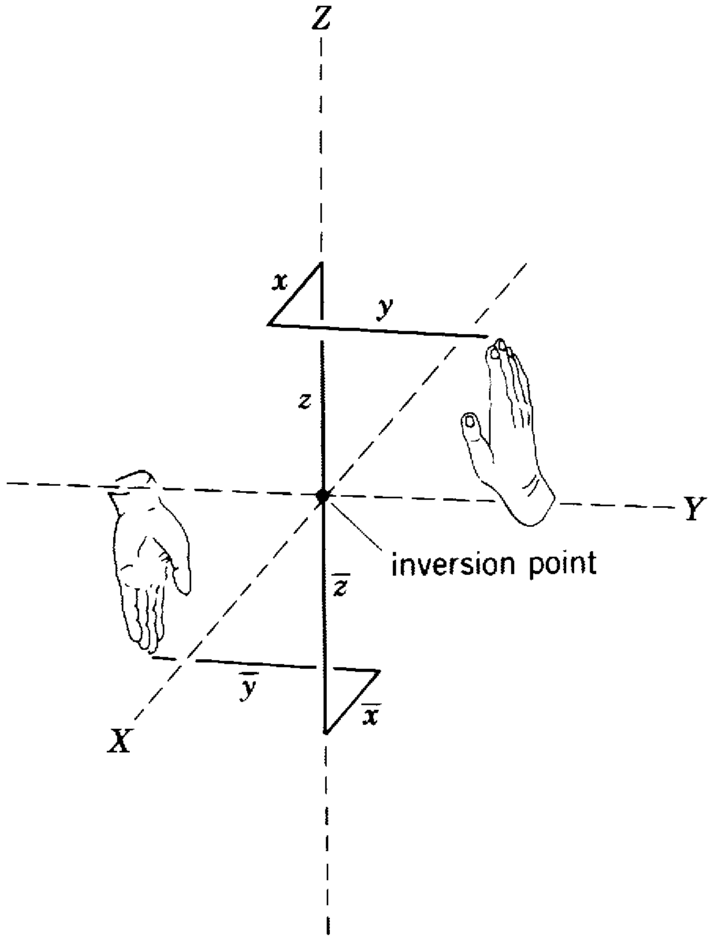
Each point in the object is converted to an identical point by projecting through a **common center** and extending an equal distance beyond this center

Objects with *i* symmetry said to be **centrosymmetric**

III.C.5 Symmetry

III.C.5.c Point Groups

Inversion Symmetry (*i*)



From Burger, Fig. 5, p.8

III.C.5 Symmetry

III.C.5.c Point Groups

Improper Rotations

Rotations followed by *m* or *i*

Include:

- **Rotoinversion:** *n* followed by *i*
- **Rotoreflection:** *n* followed by *m*

Only inversion axes for crystal **lattices** are:

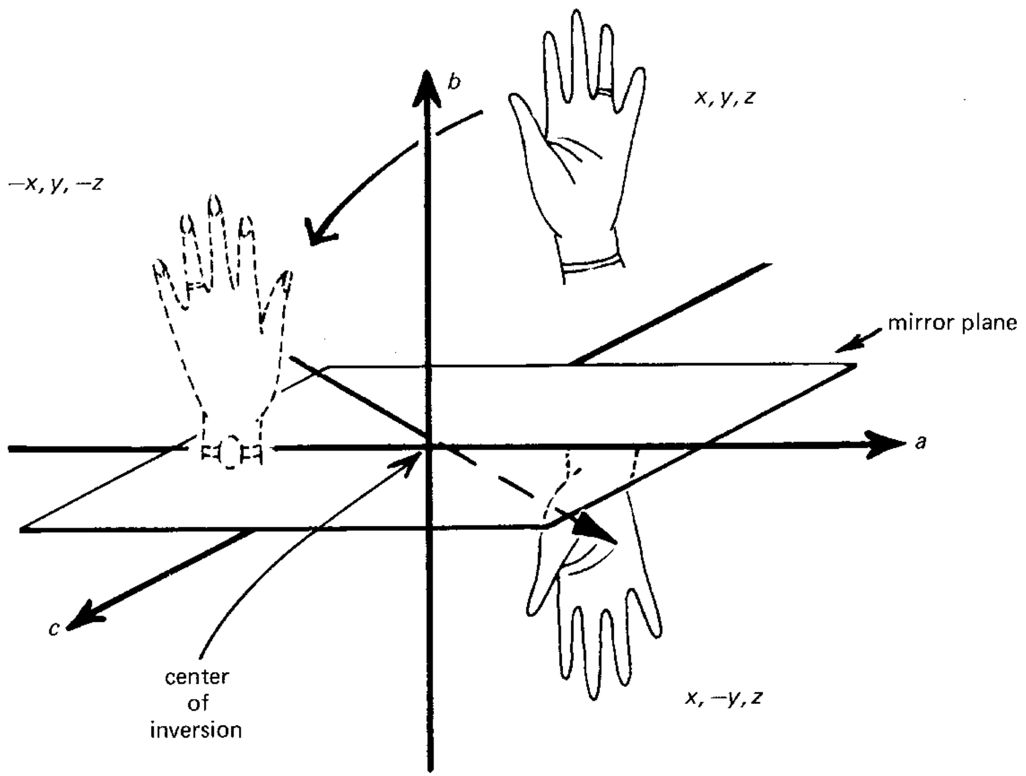
$$\bar{1}, \bar{2}, \bar{3}, \bar{4}, \bar{6}$$

III.C.5 Symmetry

III.C.5.c Point Groups

Improper Rotations

Two-fold Rotary Inversion ($\bar{2}$)



(b) two-fold rotatory-inversion axis *or* mirror plane

From Glusker & Trueblood, Fig. 18, p.72

Types of Point Groups

The collection of symmetry operations about a point are defined by three point groups:

Cyclic

Dihedral

Cubic

III.C.5 Symmetry

III.C.5.c Point Groups

Types of Point Groups

Cyclic Point Groups:

- Single *n*-fold axis of rotation
- *n* can be any positive integer
- Notations:

  H-M system: $\boldsymbol{n}$
  S system: $\boldsymbol{C_n}$ (*C* stands for cyclic)

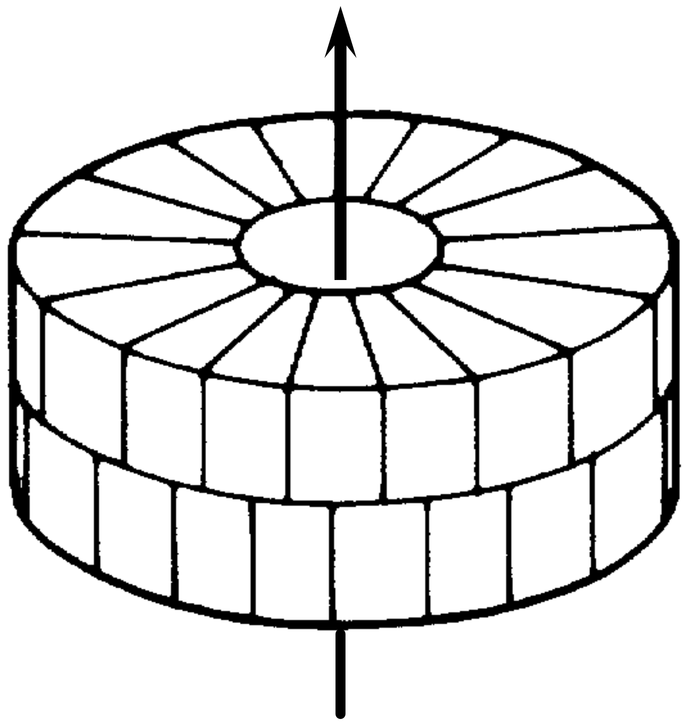
Example:

34 subunit TMV stacked disk aggregate = C_{17}

III.C.5 Symmetry

III.C.5.c Point Groups

Cyclic Point Group Symmetry



TMV stacked disk (C_{17})

From Eisenberg & Crothers, Table 16-1, p.757

III.C.5 Symmetry

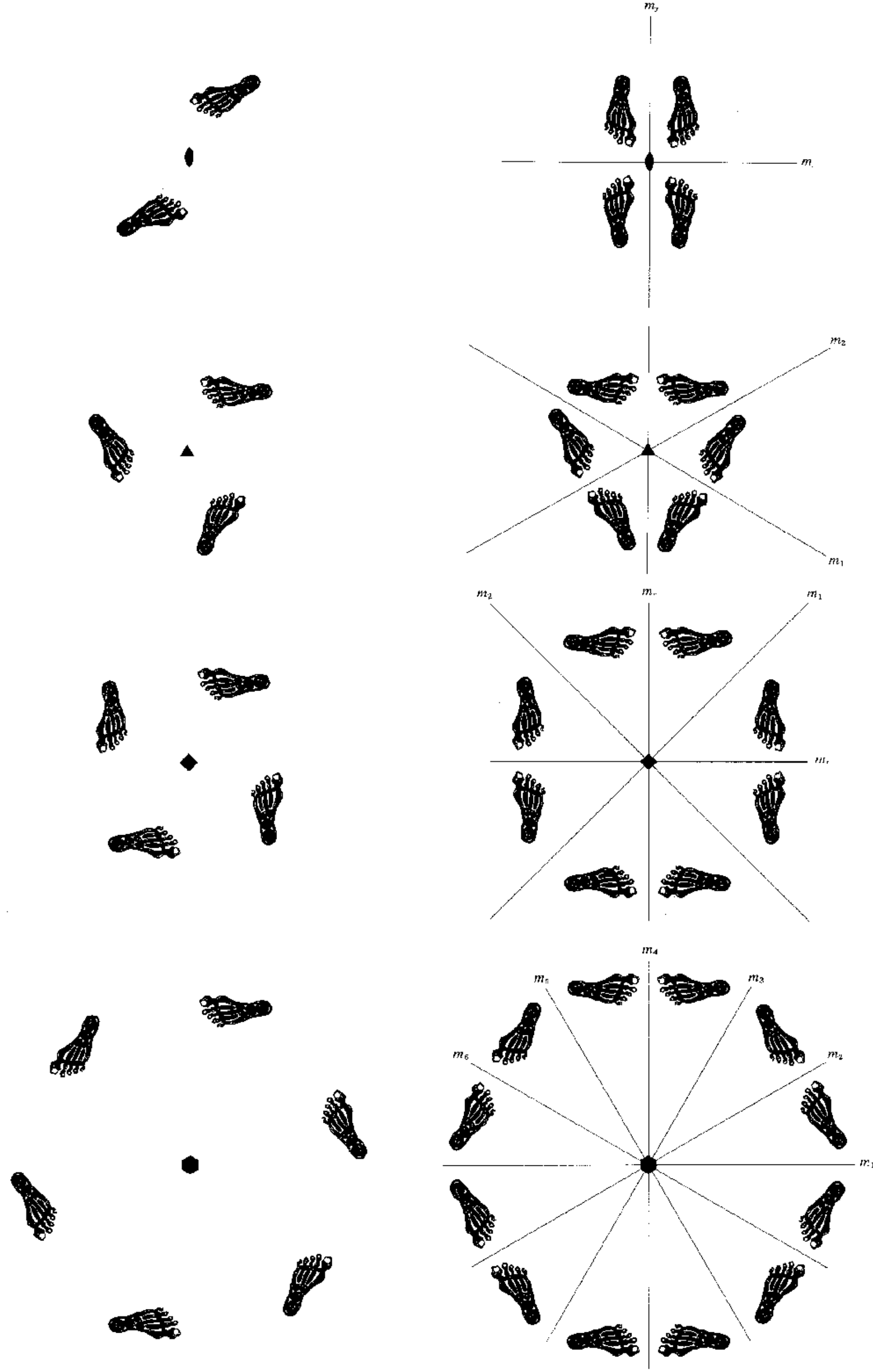
III.C.5.c Point Groups

Types of Point Groups

Cyclic Point Groups (*n* or C_n):

Non-biological molecules can also have **mirror planes** of symmetry either parallel or perpendicular to the *n*-fold axis of symmetry

Cyclic Point Groups



2 2mm

3 3m

4 4mm

6 6mm

From Bernal, pp.45 and 47-53

III.C.5 Symmetry

III.C.5.c Point Groups

Types of Point Groups

Dihedral Point Groups:

- Axes of rotation at right angles to each other
- *n*-fold axis perpendicular to *n* 2-fold axes
- Notations:

    H-M system: ***n2*** (odd *n*) or ***n22*** (even *n*)
    S system: $\boldsymbol{D_n}$ (*D* stands for dihedral)

- # ASUs for $D_n = 2n$

III.C.5 Symmetry

III.C.5.c Point Groups

Types of Point Groups

Dihedral Point Groups (*n2* or *n22* or D_n):

- Most oligomeric enzymes display dihedral symmetry

Example:

Ribulose bisphosphate carboxylase/oxygenase (RuBisCO) has D_4 symmetry (422 in H-M notation)

ASUs in the point group D_n is $2n$, thus RuBisCO has eight asymmetric units

III.C.5 Symmetry

III.C.5.c Point Groups

Types of Point Groups

Cubic Point Groups:

- Essential characteristic:

  Four 3-fold axes arranged as the four body diagonals (lines connecting opposite corners) of a cube

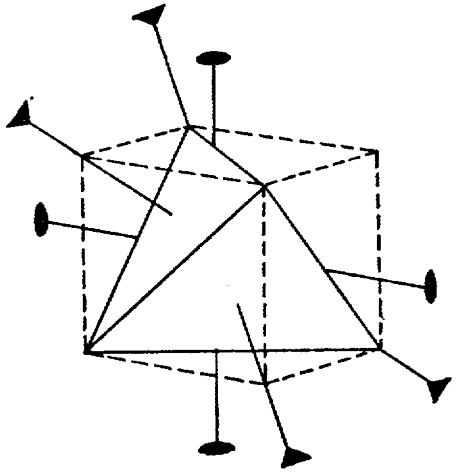
- Three cubic point groups:

Group name	S notation	HM notation	# ASU
Tetrahedral	T	23	12
Octahedral	O	432	24
Icosahedral	I	532	60

III.C.5 Symmetry

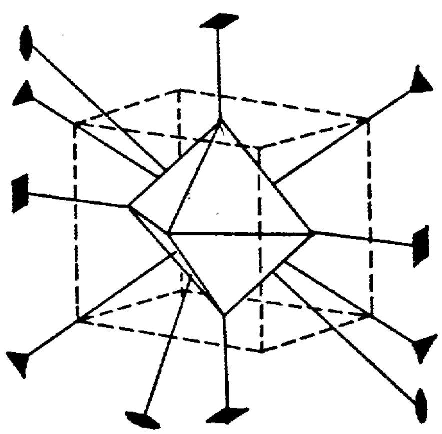
III.C.5.c Point Groups

Cubic Point Groups



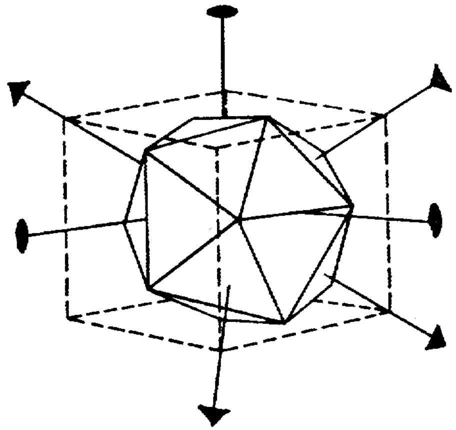
Tetrahedral (T, 23)

3 two-fold
4 three-fold



Octahedral (O, 432)

6 two-fold
4 three-fold
3 four-fold



Icosahedral (I, 532)

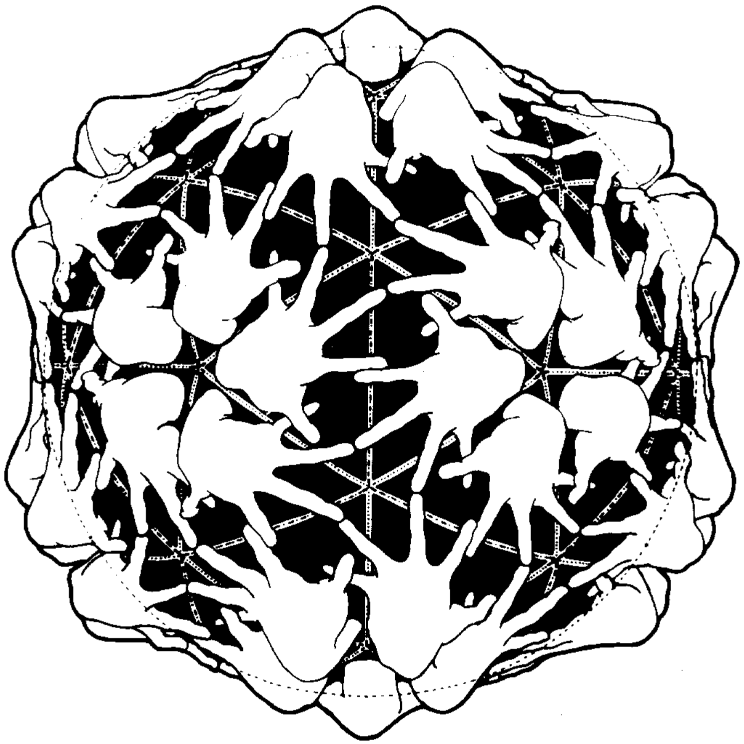
15 two-fold
10 three-fold
6 five-fold

From Wilson, Fig. 5.7, p.126

III.C.5 Symmetry

III.C.5.c Point Groups

Icosahaedral Cubic Point Group

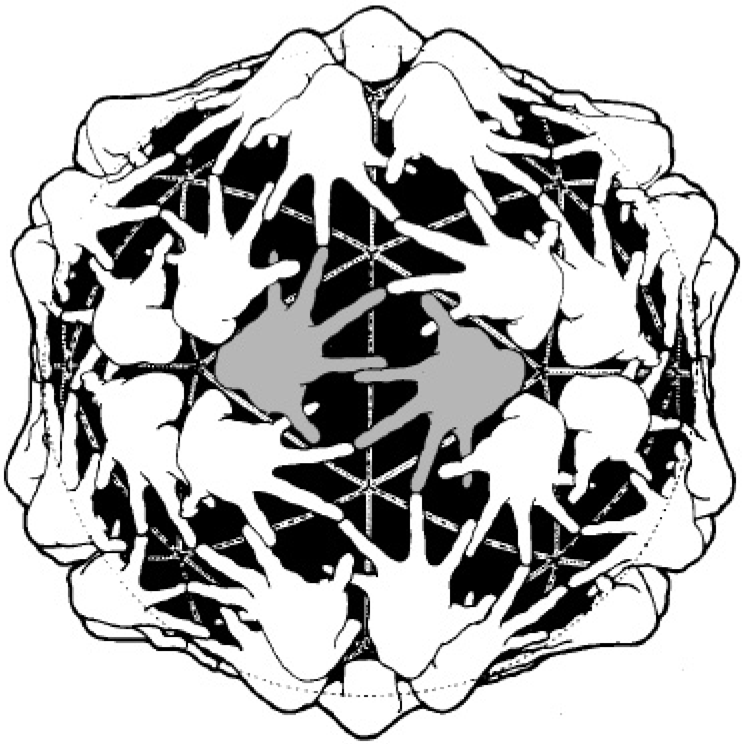


From Eisenberg & Crothers, Table 16-3, p.767

III.C.5 Symmetry

III.C.5.c Point Groups

Icosahaedral Cubic Point Group



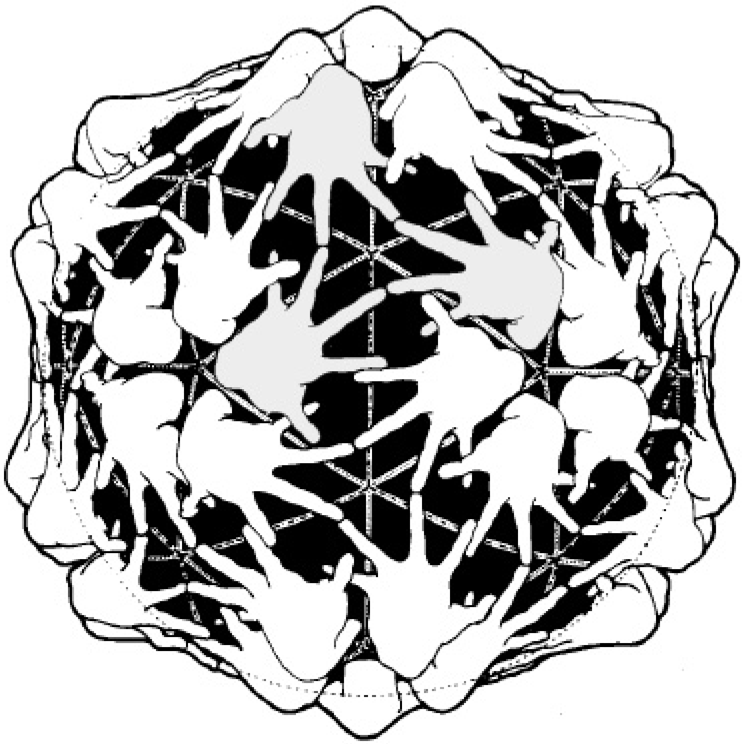
30 dimers

From Eisenberg & Crothers, Table 16-3, p.767

III.C.5 Symmetry

III.C.5.c Point Groups

Icosahaedral Cubic Point Group



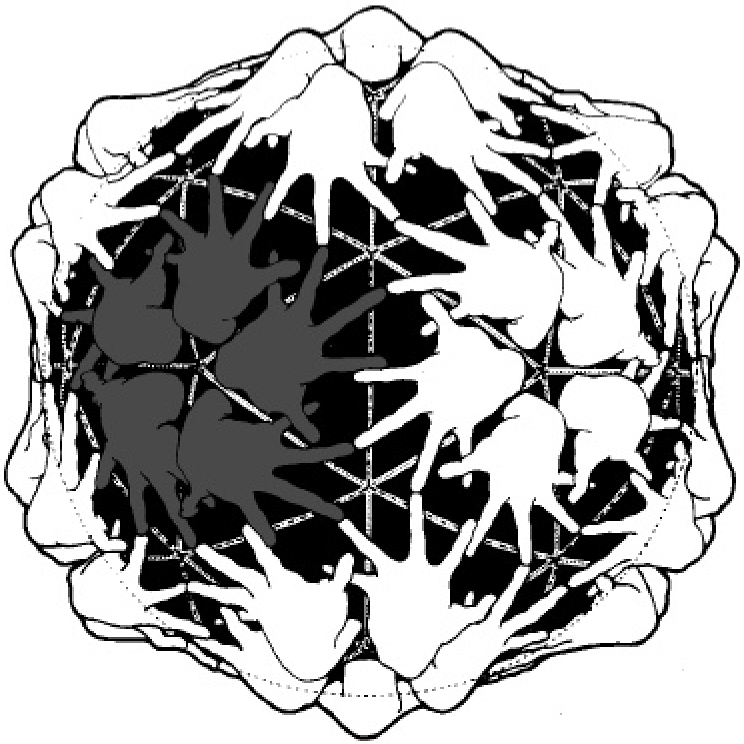
20 trimers

From Eisenberg & Crothers, Table 16-3, p.767

III.C.5 Symmetry

III.C.5.c Point Groups

Icosahaedral Cubic Point Group



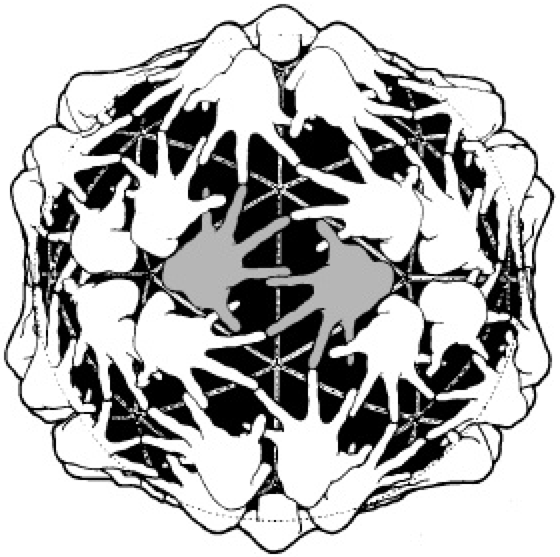
12 pentamers

From Eisenberg & Crothers, Table 16-3, p.767

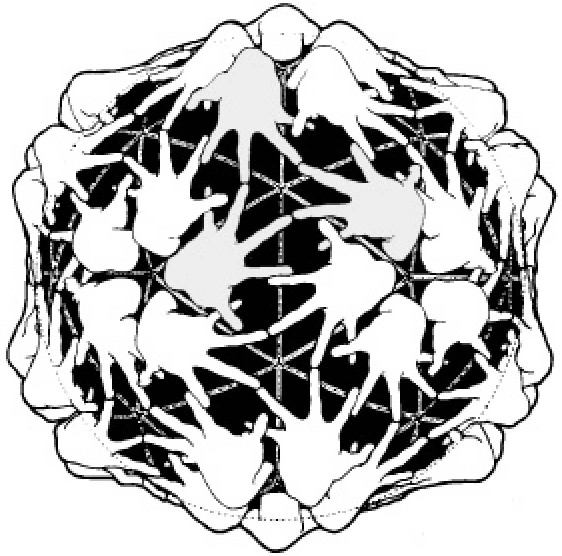
III.C.5 Symmetry

III.C.5.c Point Groups

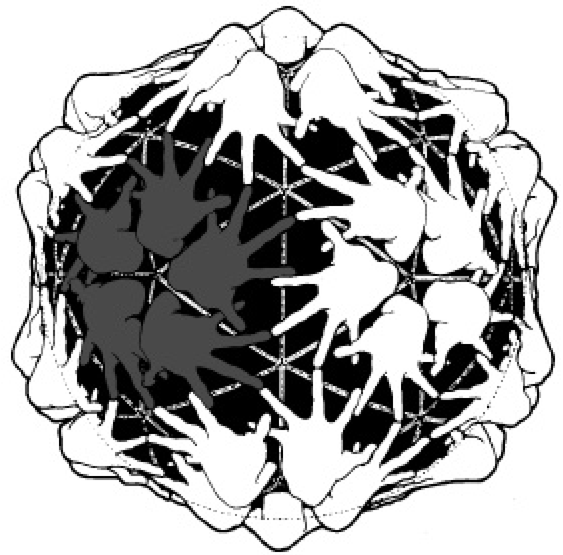
Icosahaedral Cubic Point Group



30 dimers



20 trimers



12 pentamers

From Eisenberg & Crothers, Table 16-3, p.767

III.C.5 Symmetry

III.C.5.c Point Groups

Lattice Restrictions and Non-Crystallographic Symmetry

- Crystal structure and crystal lattice may **only** contain 1-, 2-, 3-, 4-, or 6-fold rotational symmetry axes (crystal **lattice** must be space filling) though the motif can have additional symmetries

Example: 34 subunits in the TMV disc aggregate are arranged about a 17-fold axis of rotation (C_{17})

- TMV disc forms true 3D crystals
- Has been studied by X-ray crystallography
- Disc occupies a **general position** in the crystal unit cell, and therefore displays **non-crystallographic** symmetry

Many spherical viruses are icosahedral (cubic point group) and hence contain some symmetry elements compatible with allowed lattice symmetries, and crystallize and **display crystallographic as well as non-crystallographic** symmetry

III.C.5 Symmetry

III.C.5.d Translational Symmetry

1) Repetition in One Dimension

Translational symmetry is symmetry **along a line**

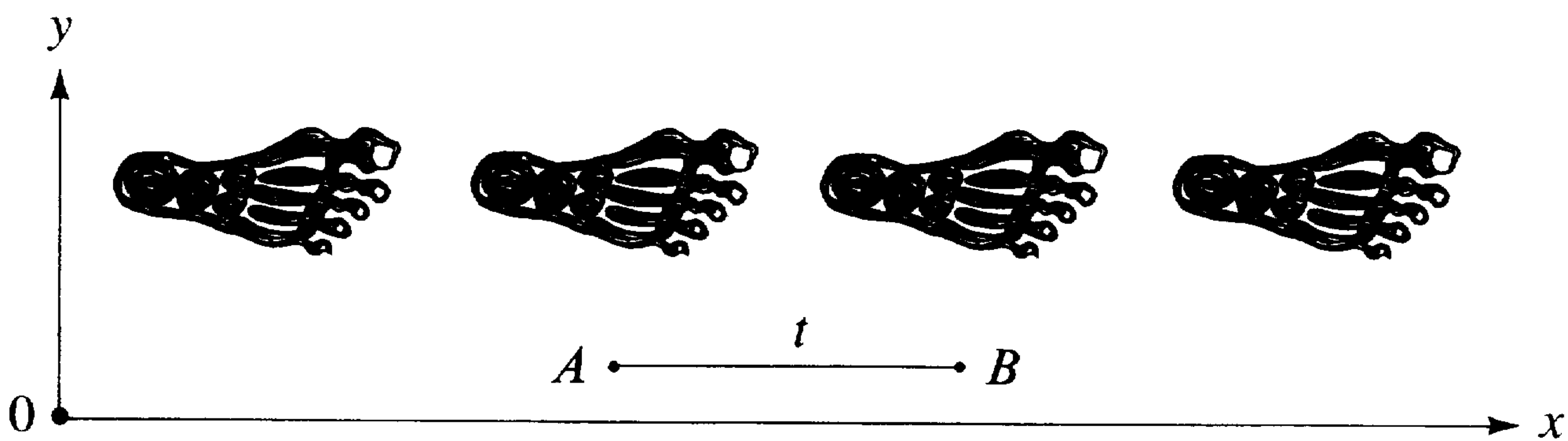
DEFINITION:

Translation is symmetry operation of shifting object a given distance in a given direction

III.C.5 Symmetry

III.C.5.d Translational Symmetry

1) Repetition in One Dimension



1-D crystal of right feet

From Bernal, p. 27

III.C.5 Symmetry

III.C.5.d Translational Symmetry

2) Screw Axes

A screw axis **combines translation and rotation** operations to produce a structure with **helical** symmetry

Screw axes are symmetry elements of **crystals** that are helices with an **integral** # of ASUs per turn of the helix

DEFINITION: n_m screw axis

- **Rotation** of **2**p***/n*** radians about an axis followed by:
- **Translation** of ***m/n*** of the repeat distance (unit cell edge)

III.C.5 Symmetry

III.C.5.d Translational Symmetry

2) Screw Axes

Screw axes found in **crystals**:

2_1, 3_1, 3_2, 4_1, 4_2, 4_3, 6_1, 6_2, 6_3, 6_4, and 6_5

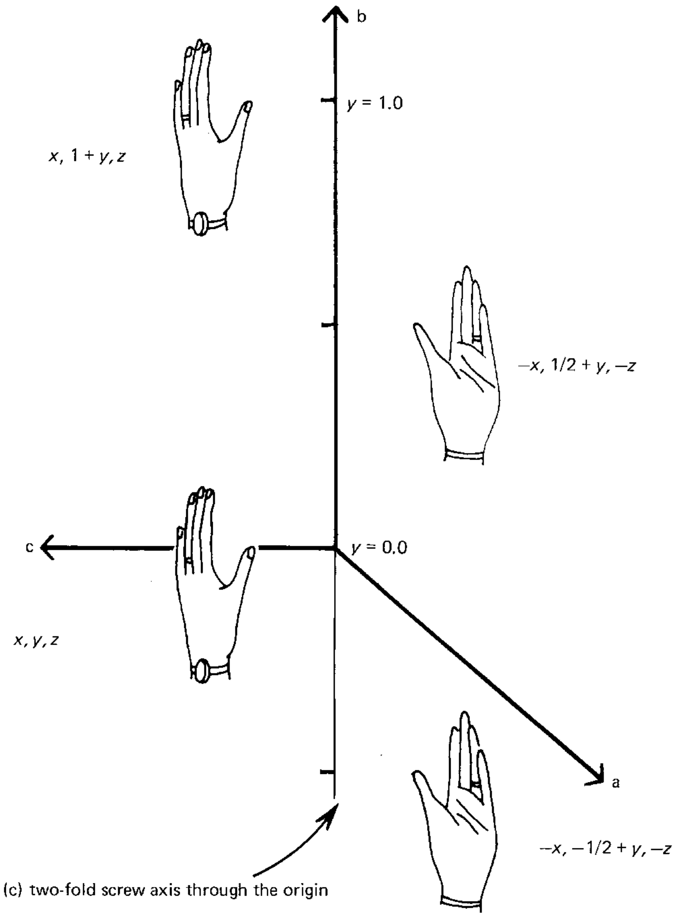
Crystal lattice only accommodates an **integral** # of ASUs per turn of the helix

NOTE: above rule need **not** apply to helices in general

III.C.5 Symmetry

III.C.5.d Translational Symmetry

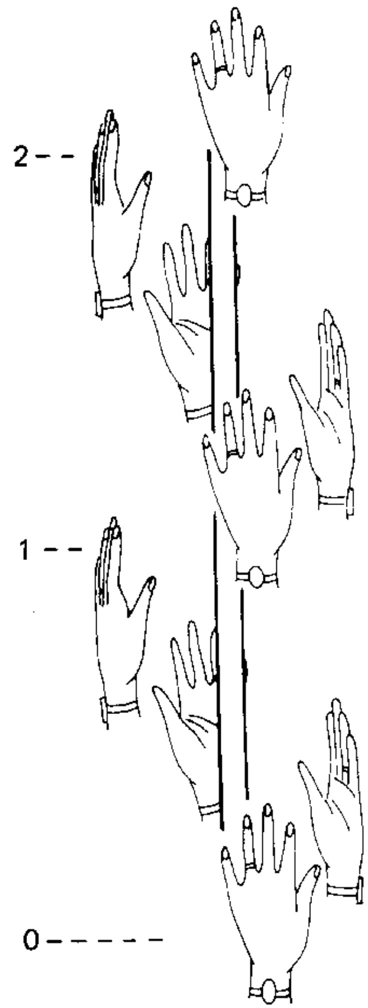
2_1 Screw Axis Parallel to b



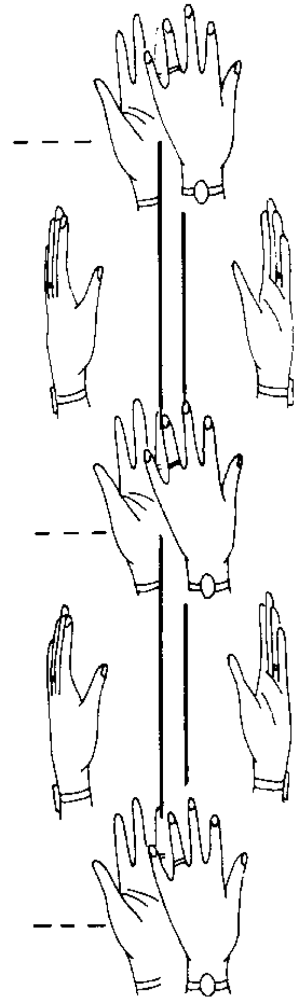
(c) two-fold screw axis through the origin

From Glusker & Trueblood, Fig. 18, p.73

Screw Axis Symmetries



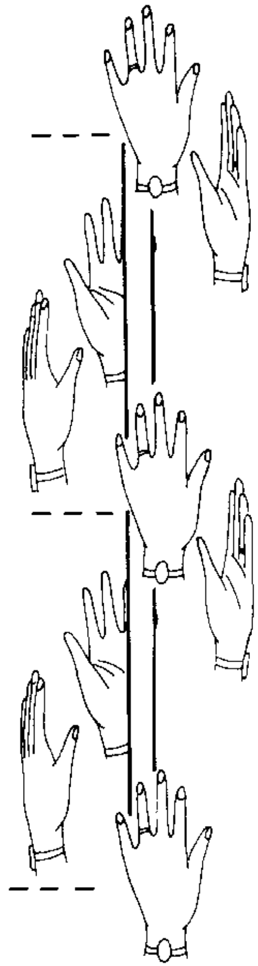
4_1 on left hand



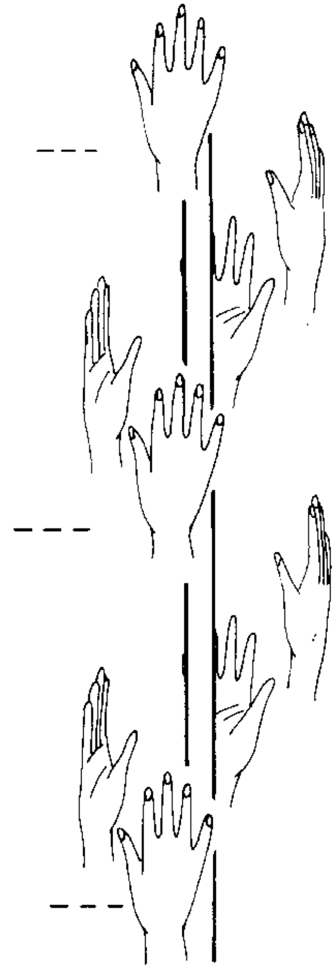
4_2 on left hand

From Glusker & Trueblood, Fig. 18, p.74

Screw Axis Symmetries



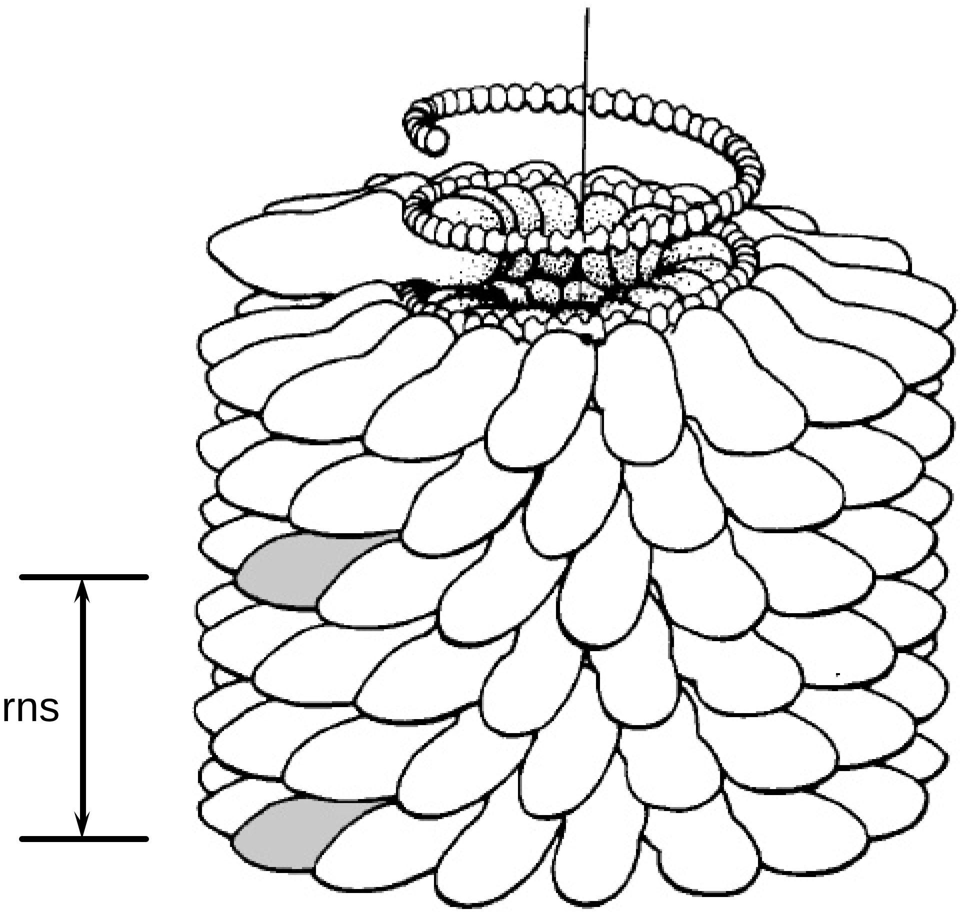
4_3 on left hand



4_3 on right hand

From Glusker & Trueblood, Fig. 18, p.74

Helical Symmetry (n_m)



TMV virion

$n = 16.33$; $m = 1/3$

16.33 subunits per turn of basic helix

From Eisenberg & Crothers, Fig. 16-12, p.782

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Symmetry of a structure is described by:

Plane group if it is **2D**

Space group if it is **3D**

All possible **crystal** symmetries are generated by combining all types of **lattice** symmetries with all types of **motif** symmetries

If **internal** structure of crystal is considered, **additional symmetry exists** due to the presence of **screw axis** and **glide plane** symmetries

Leads to:

17 possible 2D plane groups

230 possible 3D space groups

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

17 possible 2D plane groups
230 possible 3D space groups

With enantiomorphic biological structures:
5 possible plane groups
65 possible space groups

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Periodic Structure

Generate by placing a **motif** at every point of a **lattice**

Lattice = rule for translation

Motif = object that is translated

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Periodic Structure

Thought of as being built up in two steps:

1. A **motif** is generated from the ASU by the symmetry operations of the point group

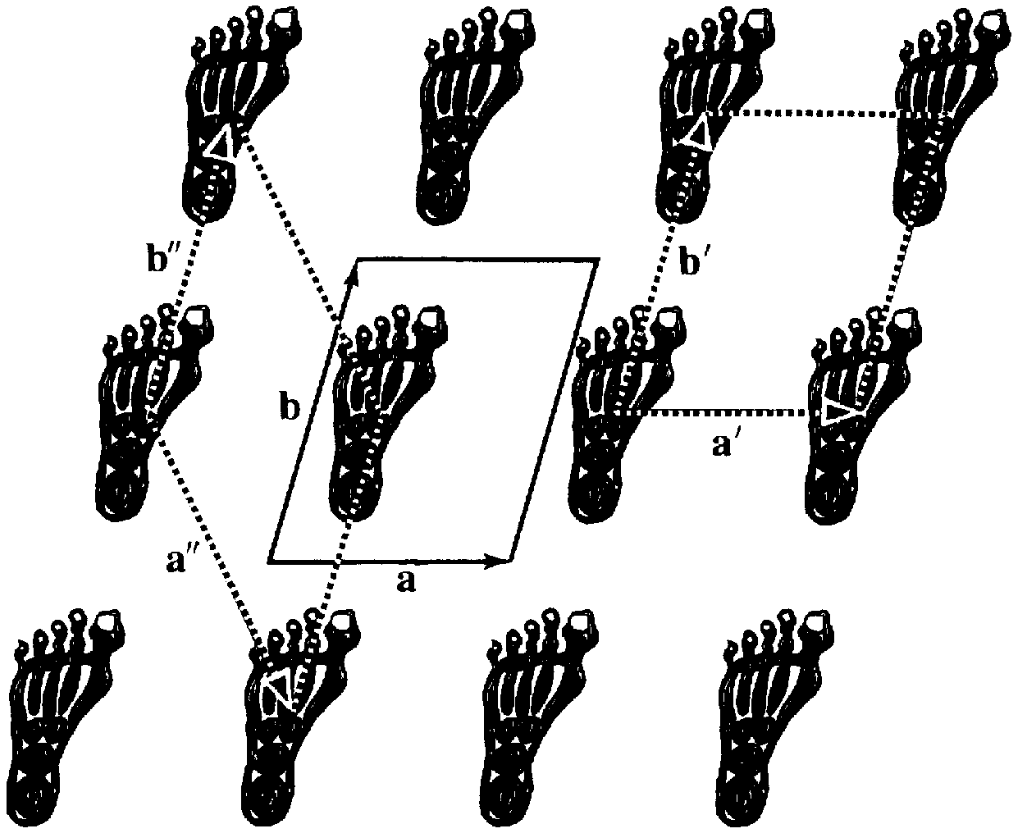
2. The **structure** is generated from the motif by the translational symmetry operations of the lattice

$$\textbf{Asymmetric unit} \xrightarrow[\text{symmetry}]{\text{point-group}} \textbf{motif} \xrightarrow[\text{symmetry}]{\text{lattice}} \textbf{structure}$$

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Plane Group Symmetry P1

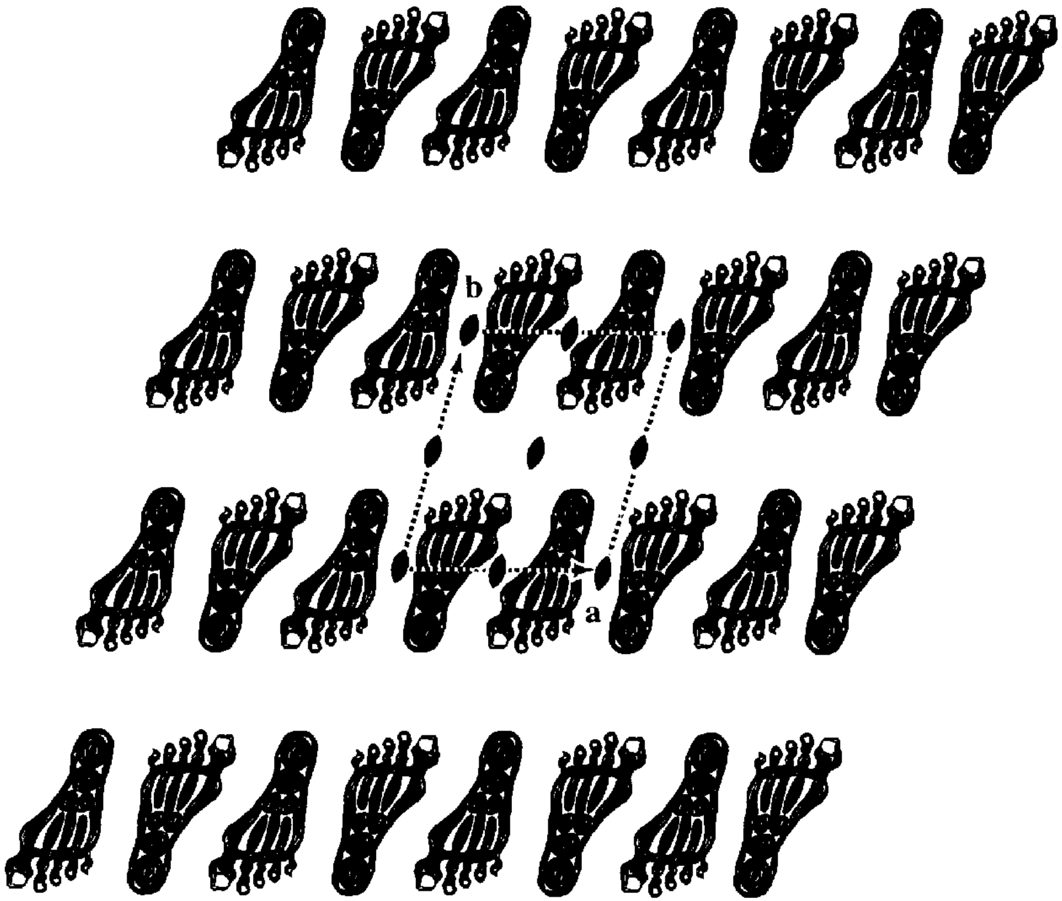


From Bernal, p.58

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Plane Group Symmetry P2



From Bernal, p.59

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Glide Plane Symmetry

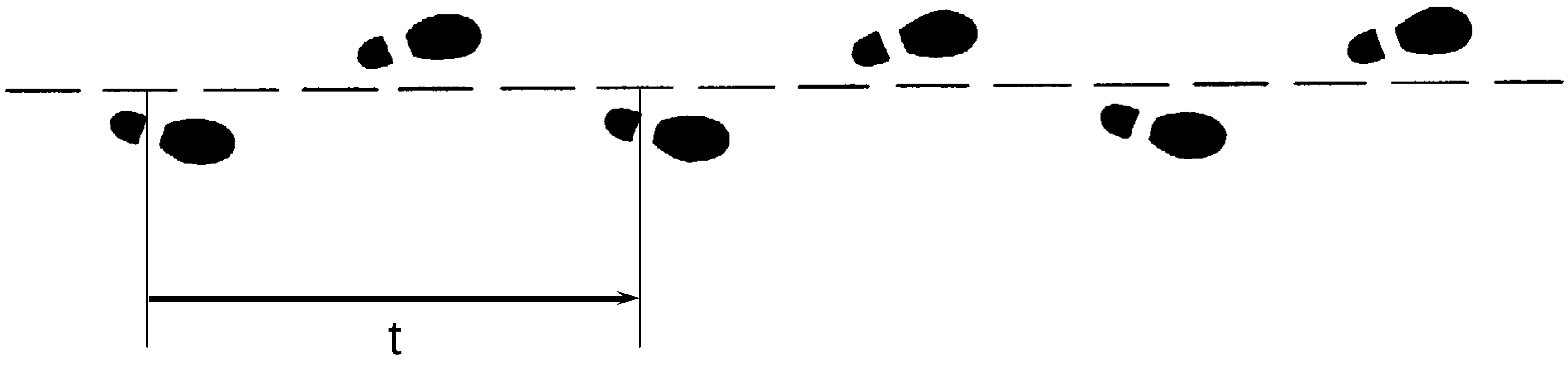
Translation followed by a **mirror** operation (or vice versa)

- Biological molecules generally do **not** display glide plane symmetries because they do not exist in enantiomorphic pairs
- **However**, biological molecules (or crystals) when viewed in two-dimensions (*i.e.* in projection) **can** display mirror symmetry

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Glide Symmetry Operation

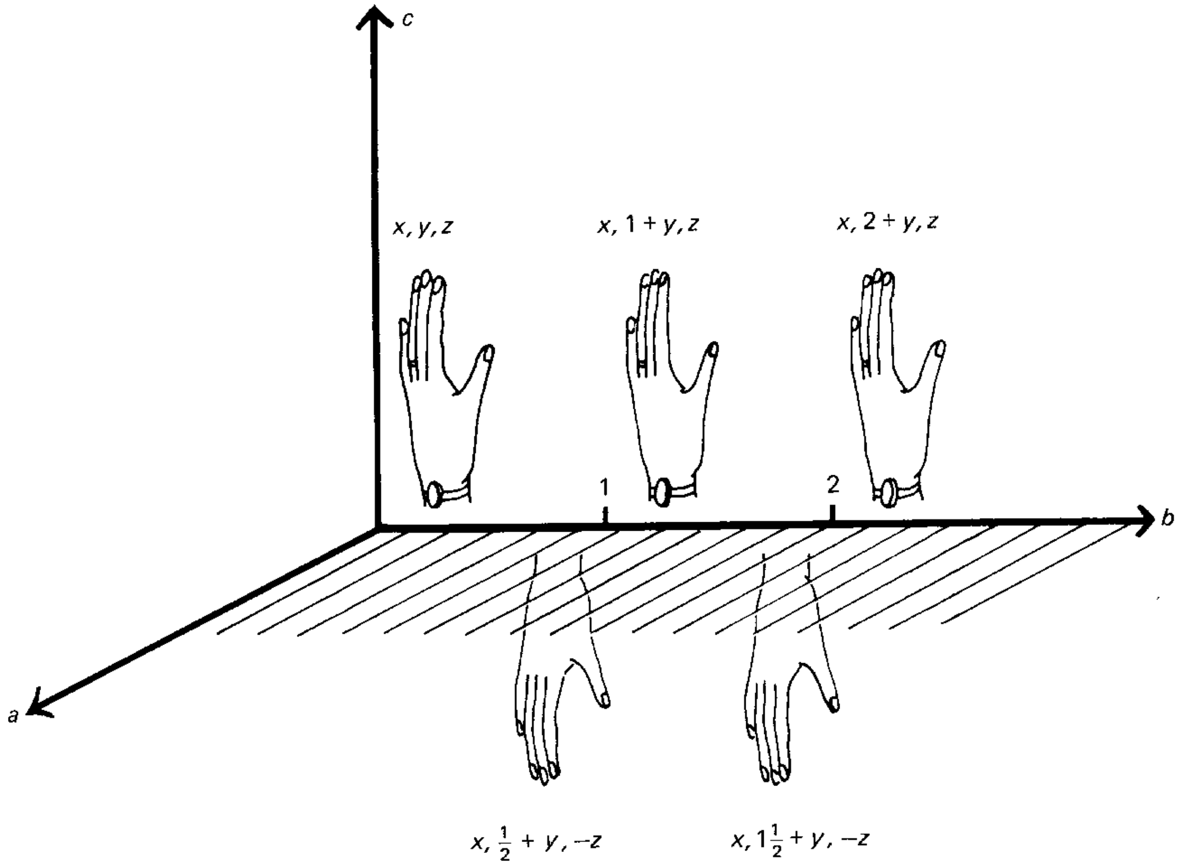


From Buerger, Fig. 6, p.8

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

b-glide plane normal to c



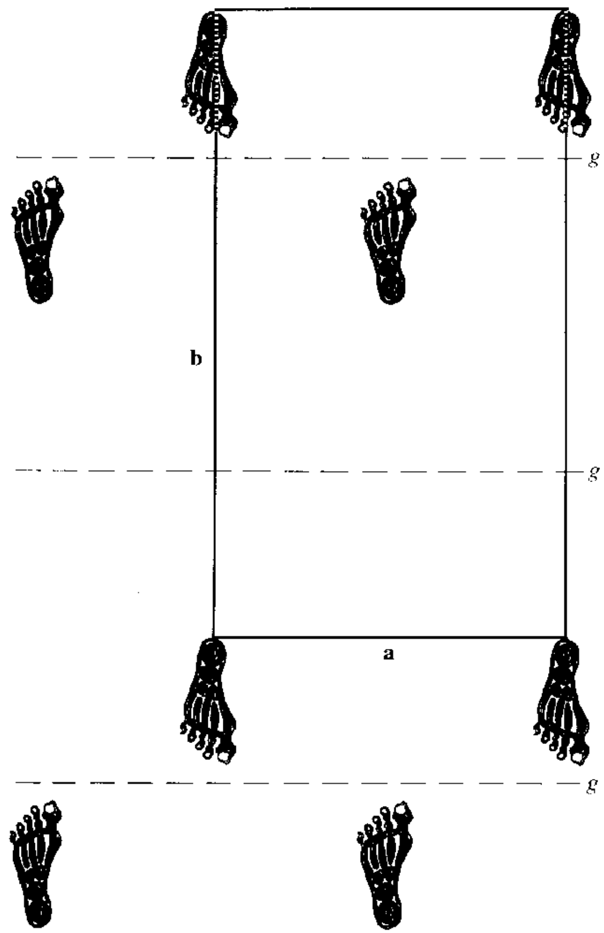
(e) *b*-glide plane through the origin and normal to **c**

From Glusker & Trueblood, Fig. 18, p.74

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Plane Group Symmetry Pg

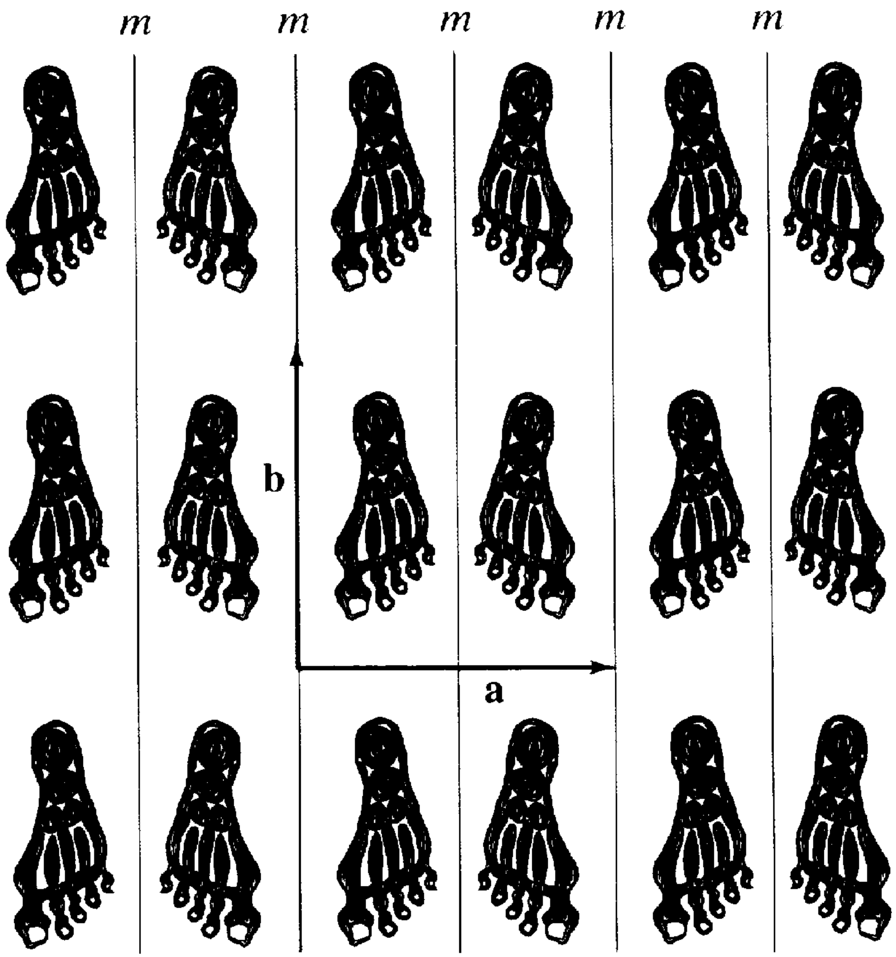


From Bernal, p.61

III.C.5 Symmetry

III.C.5.e Plane Groups and Space Groups

Plane Group Symmetry Pm



From Bernal, p.60

III.C.5 Symmetry

III.C.5.f Examples of Symmetrical Biological Molecules

Helical Symmetry

Actin, actin-myosin filaments

Bacterial flagella

Bacterial pili

Chromatin fibers

Enzyme aggregates (*e.g.* catalase tubes)

Neurotubules

Sickle cell hemoglobin fibers

Tobacco mosaic virus (and many others)

T4 bacteriophage sheath (extended or contracted configuration)

III.C.5 Symmetry

III.C.5.f Examples of Symmetrical Biological Molecules

Point Group Symmetry

MOLECULE/AGGREGATE	S	H-M	# ASU
Asymmetric aggregates: *e.g.* ribosome	C_1	1	1
Fibrous molecules: *e.g.* fibrinogen	C_2	2	2
Enzymes:			
lactate dehydrogenase	D_2	222	4
catalase	D_2	222	4
aspartate transcarbamylase	D_3	32	6
ribulose bisphosphate carboxylase/oxygenase	D_4	422	8
glutamine synthetase	D_6	622	12
asparate-b-decarboxylase	T	23	12
dihydrolipoyl transsuccinylase	O	432	24
Spherical viruses: *e.g.* polyoma, polio, rhino, tomato bushy stunt, human wart, etc.	I	532	60

III.C.5 Symmetry

III.C.5.f Examples of Symmetrical Biological Molecules

Plane Group Symmetry (2-D Crystals)

Aquaporin

Bacterial cell walls (*e.g. Bacillus brevis* T layer)

Bladder luminal membrane

Gap junctions

Light harvesting complex

Purple membrane

Space Group Symmetry (3-D Crystals)

Various intracellular inclusions

Various *in vitro* grown crystals suitable for X-ray crystallography

End of Sec.III.C