POINTGROUP SYMMETRY OF OLIGOMERIC MACROMOLECULES

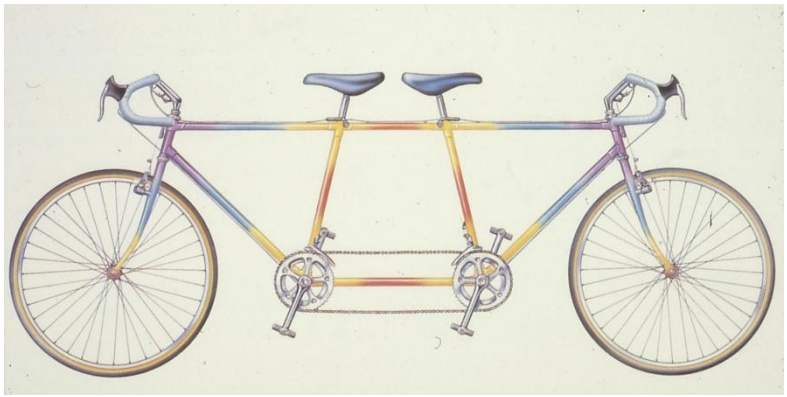
Marin van Heel

Leiden University / Imperial College London

First version 21st October 1986 / Last updated: August 2012

Regular assemblies of proteins come only in certain flavours since there are only so many ways to assemble oligomers. Oligomeric assemblies obey specific symmetry rules and exhibit characteristic symmetry axes. A basic understanding of these symmetry rules may prevent one from adding (yet) another impossible oligomeric structure to the literature. For example, oligomers of a biological macromolecule cannot contain *mirror symmetry* planes since that would imply that the protein structure would for example contain both right-handed and left-handed α-helices. Mirror-symmetric pointgroups are possible for small metal clusters, since metal atoms themselves can have internal (mirror) symmetry. Similarly, the chain of a biopolymer in oligomeric assembly cannot cross a symmetry axis of the assembly: there necessarily must be "empty" space around every symmetry axis. Information about pointgroup symmetries of biological molecules may be found in various handbooks in X-ray crystallography.

Note that two different nomenclature systems exist for describing pointgroup symmetries: the "**international**" and the "**Schönflies**" notation (named after **Arthur Moritz Schönflies,** but mostly cited as "Schoenvlies" see Wikipedia: "Schönflies notation**"**). Both notations are in widespread use and both will be listed below. Although this is a complete list of all possible pointgroup symmetries for biological macromolecules, the reader is referred, for a complete list of all *possible* pointgroup symmetries including those with *mirror symmetry*, to the International Tables of Crystallography, and to Wikipedia: "List of spherical symmetry groups".

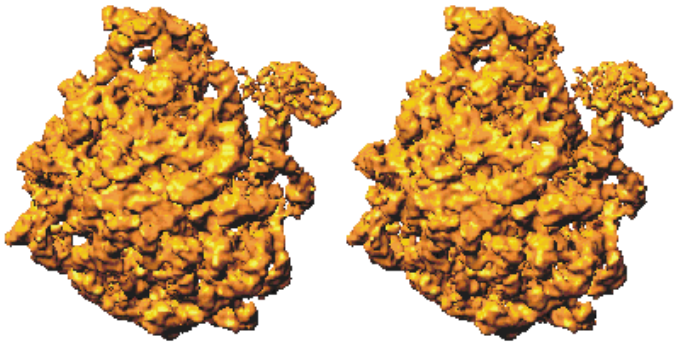


There is no mirror symmetry in Proteins!

CYCLIC POINTGROUPS (SCHOENVLIES NOTATION: "Cn")

C1

The simplest possible pointgroup symmetry has no symmetry at all. This is called pointgroup "**1**" in "international" notation or "**C1**" in the Schönflies notation ("**C**" stands for cyclic, which nomenclature will become clear below). Examples of such asymmetric oligomers are the 70S *E. coli* ribosome, as well as the 30S and 50S *E. coli* ribosomal subunits. Because of the asymmetry of **C1** molecules, every electron microscopical projection through their structure is unique, that is, does not reappear at a different combination of Euler angles. The only form of symmetry that is seen in the projections is a mirror symmetry between projections in a given Euler angle orientation, and the corresponding projection in the opposite Euler direction. Note that hetero-oligomers may have **C1** symmetry. The "**asymmetric triangle**" of a **C1** structure covers the whole unit sphere.



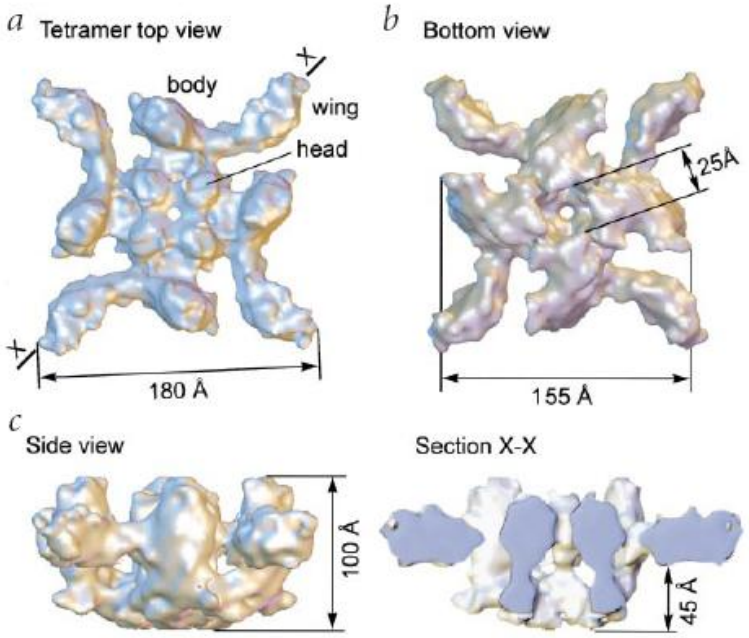
Stereographic Surface representation of the 50S E. coli ribosome (Rishi Matadeen, Ardan Patwardhan, Brent Gowen, Elena V. Orlova, Tillmann Pape, Florian Mueller, Richard Brimacombe, & Marin van Heel, **The *E. coli* 50S ribosomal subunit at 7.5Å resolution**. *Structure* **7** (1999) 1575-1583)

C2

The pointgroup "**2**" (international) or "**C2**" (Schoenvlies) contains one two-fold axis. For simplicity we will always assume the main symmetry axis to be along the "Z-"direction. A 180° rotation along the dyad (two-fold) axis along the Z-direction superimposes the dimeric molecule upon itself. Any normal projection of the dimer appears twice: there always is a second combination of Euler angle orientations that leads to an identical projection of the dimer. The only unique projection is the projection along the two-fold axis: the two-fold rotation axis rotates this projection direction back upon itself. An example of a pointgroup 2 (**C2**) protein is human $\alpha_2ß_2$ hemoglobin. The molecule consists of two **αß** hetero-dimers. Another **C2** molecule is the 2x6 subunit of *Limulus polyphemus* hemocyanin, that we have studied in the past in the group. Note that projections perpendicular to the 2-fold axis will exhibit mirror symmetry. Any projection perpendicular to an even-fold symmetry axis will exhibit mirror symmetry. The "**asymmetric triangle**" of a **C2** structure covers one half of the unit sphere covering, for example, everywhere from the equator to the North Pole.

Cn

All other cyclical pointgroups: **C3**, **C4**, **Cn**. The 3, 4 or n subunits are organised in a circular arrangement in a head-to-tail fashion; a 3- 4- or n-fold rotational axis is placed perpendicular to the plane of the circular arrangement of molecules. In our convention that symmetry axis is oriented along the Z-direction. The 13-fold symmetric SPP1 portal protein studied in our group is a **C13** structure. The F1-ATPase molecule consisting of three alpha and three beta subunits may be a pointgroup 3 (**C3**) molecule but this symmetry cannot really be exact since there is also one gamma and one delta (and epsilon) subunit in the hetero-oligomer. Electron microscopists tend to call a molecule **C4** if an arrangement of four blobs in a plane is seen in negatively stained EM preparations. However, it is actually difficult to visually distinguish **C4** from some of the other pointgroups (like **222**, see below) without proper symmetry analysis. Head-to-tail types of inter-subunit links often lead to helical polymers rather than to closed **Cn** pointgroup assemblies with relatively high "n" values. To form a **Cn** structure requires "n" identical units, typically "n" identical polypeptide chains.



The 3D reconstruction of the LTX oligomers. ***a***, The top and ***b***, bottom views of the ***C4*** tetramer. ***c***, The side and cut-open views of the tetramer. The final structure was calculated from 128 class averages that included ~1,900 original molecular images. (E.V. Orlova, M.A. Rahman, B. Gowen, K.E. Volynski, F. Meunier, M. van Heel, and Y.A. Ushkaryov: **Structure of α-Latrotoxin: Dimers Assemble into Tetramers and Form Large, Gated Membrane Pore**s. *Nature Struct. Biol.* **7** (2000) 48-53.)

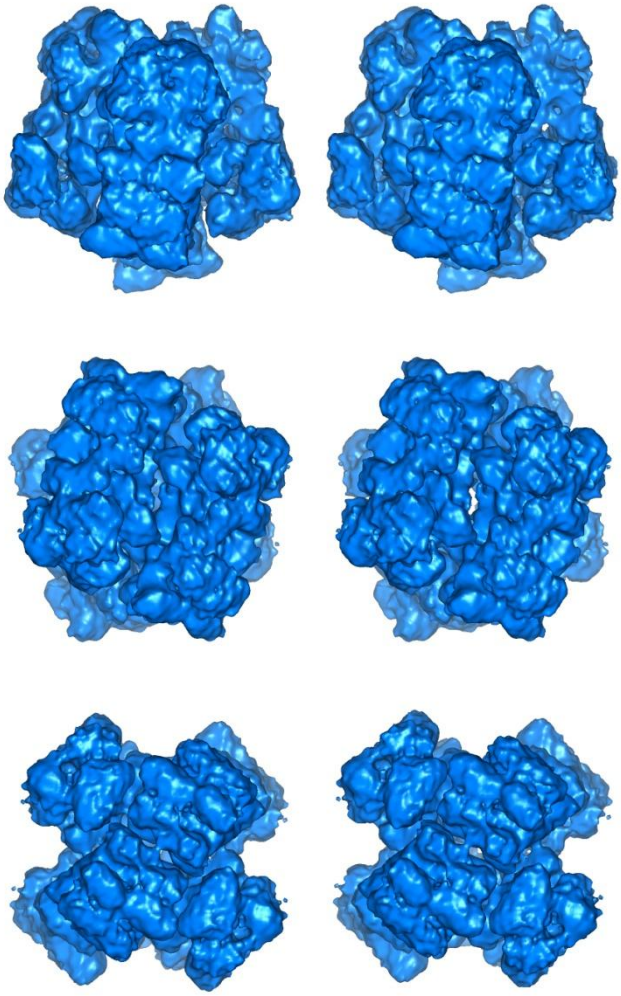
Portal protein oligomer of SSP1 bacteriophage: the first 13-fold (**C13**) structure found. (P Dube, P Tavares, R Lurz, and M van Heel, **Bacteriophage SPP1 portal protein: a DNA pump with 13-fold symmetry**, *EMBO J.* **12** (1993) 1303-1309)

DIHEDRAL POINTGROUP SYMMETRIES ("Dn" : SCHOENVLIES)

The dihedral pointgroups "Dn", are a combination of an n-fold rotational axis and a two-fold axis perpendicular to it. (From this definition it follows that a "**D1**" pointgroup is equivalent to a **C2** group discussed above.) A "Dn" structure thus consists of two Cn structures stacked top-to-top or bottom-to-bottom. Top-to-bottom connections of Cn structures do occur but those typically lead to long linear polymers. In the international notation, the dihedral groups are designated as "n22" for n is even (say, 622 for the worm hemoglobin) and as "n2" for n is uneven (say, "32" for the *Panulirus interruptus* hemocyanin).

D2 (or 222)

The pointgroup 222 (or D2 in Schoenvlies notation) has three two-fold axes that are all perpendicular to each other. The minimum number of identical subunits needed to fulfil 222 (D2) symmetry is four. A projection of a 222 oligomeric molecule will normally represent 4 different projection directions. The projections along the two-fold axes are "degenerated" in that they appear only once. Since these projections are projections perpendicular to the two other two-fold axes, these projections have a double mirror-symmetry. Moreover, the projection in the "positive" Euler direction is the same as the projection in the "negative" direction along the same two-fold axis. Normally such projection pairs are only *each other's* mirror versions. An example of a 222-pointgroup molecule is the *Limulus polyphemus* 8x6 hemocyanin. The "**asymmetric triangle**" of a D2 structure covers one quarter of the unit sphere.



Stereo views of the arthropod *Limulus Polyphemus* 8x6 hemocyanin viewed along its three different 2-fold axes (Structure by Tim Grant *et al.* 2007 unpublished).

D3 (or 32)

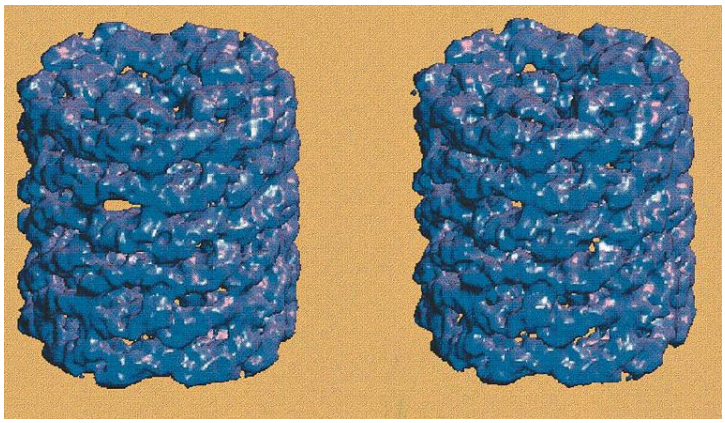
For the pointgroup 32 (D3), six equivalent monomers are required. The 32 pointgroup has a threefold axis and three two-fold axes perpendicular to that 3-fold axis (Z-direction). An example of such a molecule is the *Panulirus interruptus* hemocyanin (Volbeda & Ho1: **Crystal structure of hexameric hemocyanin from *Panulirus interruptus* refined at 3.2Å resolution**. *J. Mol. Biol.* **209** (1989) 249-279). Projections along the two-fold axes of a 32-pointgroup structure do not show mirror symmetry; they do, of course, show two-fold rotational symmetry. Projections along the threefold axis are projections perpendicular to three two-fold axes and will thus exhibit three mirror planes (apart from the 3-fold rotational symmetry). The asymmetric triangle of a D3 structure covers one sixth of the unit sphere. This can be, for example, from the equator to the North pole and spanning 120° along the equator, say from the 0° meridian (Greenwich) to the 120°meridian (passing close to Peking).

D4 (422)

Pointgroup 422 (D4) contains a minimum of 8 equivalent monomers. The rubisco molecule is thought to have this symmetry. Like with the 622 pointgroup (below), two different types of projections along two-fold axes are possible. The projections along the two two-fold axes or along the four-fold axis show two perpendicular mirror planes (projections along the four-fold axis also show two perpendicular mirror planes in the 45º and 135º directions). These mirror planes are the result of projecting the structure in directions perpendicular to even-fold symmetry axes. Eight identical monomers are required to form a D4 structure, four for each layer of the bi-layered structure. The asymmetric triangle of a D4 structure covers one eighth of the unit sphere, for example, the area of $X > 0$, $Y > 0$, and $Z > 0$ in a Cartesian 3D coordinate space.

D5 (or 52)

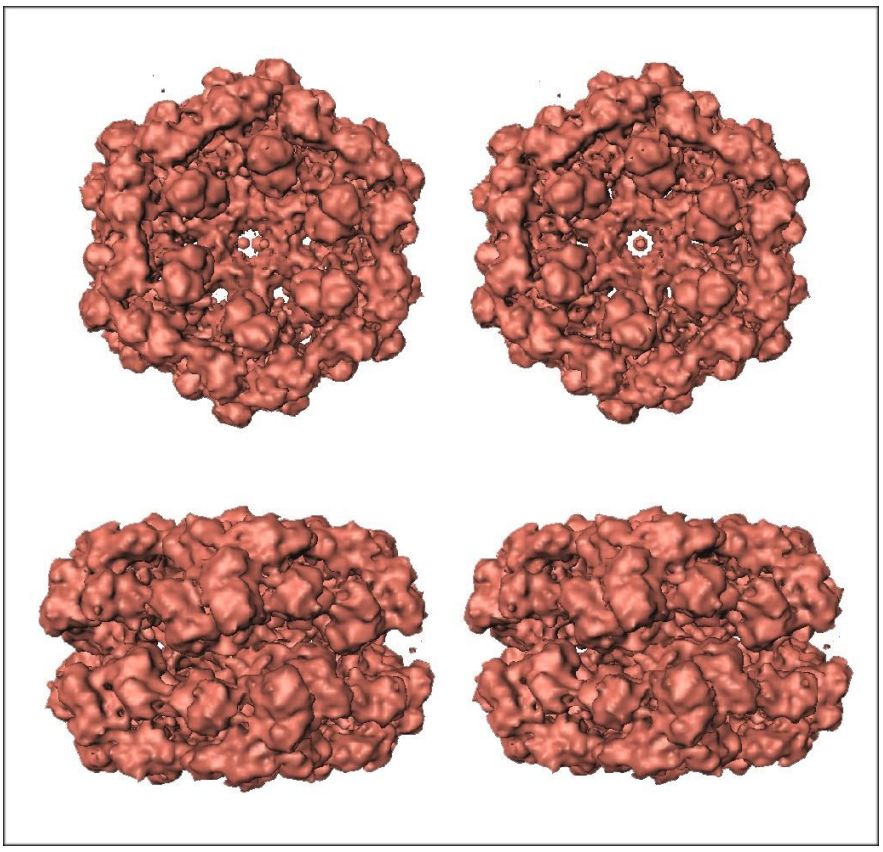
Pointgroup 52 (D5) consists of ten equivalent subunits. Each projection in a general direction through this structure corresponds to a total of ten different combinations of Euler angles. The projection along the 5-fold axis contains a total of five mirror planes. The projections along the two-fold axes have no mirror symmetry because that projection direction is NOT perpendicular to an EVEN-fold axis. An example of a 52 structure is the Keyhole Limpet Hemocyanin (KLH). The asymmetric triangle of a D5 structure covers one tenth of the unit sphere: from the equator to the North Pole and spanning 72° (=360°/5), say, from 0° to 72° on the equator.



Stereo pair of the structure of the D5 pointgroup symmetry KHL hemocyanin (E. V. Orlova, P. Dube, J. R Harris, E Beckman, F Zemlin, J Markl, and M van Heel, **Structure of Keyhole Limpet Hemocyanin Type 1 (KLH1) at 15Å resolution by electron cryomicroscopy and angular reconstitution**, *J. Mol. Biol.* **271** (1997) 417-437.) Apart from the overall D5 symmetry the structure exhibit many local symmetries (see paper).

D6 (or 622)

Pointgroup 622 (**D6**) contains twelve equivalent subunits. Each projection in a general direction through this structure corresponds to a total of twelve different combinations of Euler angles. The projection along the 6-fold axis contains a total of six mirror planes which are three-by-three equivalent. There are two different types of two-fold axes and therefore there are two different projections along two-fold axes. The projections along the two-fold axes have two perpendicular planes of mirror symmetry because that projection direction is perpendicular to one of the other two-fold axes and is perpendicular to the 6-fold axis itself. The 6-fold is also an even-fold axis and thus the projections along the two-fold axes exhibit a total of two mirror planes, which are perpendicular to each other. An example of a 622 structure is the giant hemoglobin from earthworms.



Stereo pairs of *Lumbricus terrestris* hemoglobin at ~13Å resolution. The 144 myoglobin domains are organised as 72 dimers. The $1/12^{th}$ subunit – the asymmetric unit of this D6 structure - contains six such dimers, organised around the local 3-fold axis of the $1/12^{th}$ subunit. (M van Heel, B Gowen, R Matadeen, E.V. Orlova, R. Finn, T. Pape, D. Cohen, H. Stark, R. Schmidt, M. Schatz, and A. Patwardhan, **Single-particle cryo electron microscopy: towards atomic resolution**, *Quart. Rev. Biophys*.**33** (2000) 307-369)

Dn …

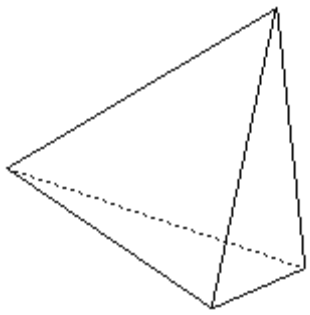
We could go on and on... However, there may be better ways to pack a large number of monomers. I am aware of the GroEL **D7** structure which 14-mer consists of two **C7** layers but often, however, functions as a **C7** structure rather than a **D7** one. Chaperonins with a **D9** structure have been reported.

THE THREE CUBIC POINTGROUP SYMMETRIES

The cubic pointgroup symmetries are closely related to the "Platonic polyhedrons" such as tetrahedron, cube, octahedron, dodecahedron, and icosahedron. (See: Wikipedia, Platonic solid)

T (or 23)

A tetrahedron has four triangular faces, and has four corners in which three of the triangular planes meet around a three-fold axis. The 23 or "T" pointgroup (tetrahedral) symmetry requires a minimum of 12 identical subunits. Whereas in the original write-up of this document it was stated in this at this position that no 23 structures were known, that is no longer true since 1998 when 23-pointgroup-symmetry structure of a DNA Binding Protein was published. (R.A. Grant, D.J. Filman, S.E. Finkel, R. Kolter & J.M. Hogle: **The crystal structure of Dps, a ferritin homolog that binds and protects DNA**, *Nature Structural Biology* **5** (1998) 294 - 303)



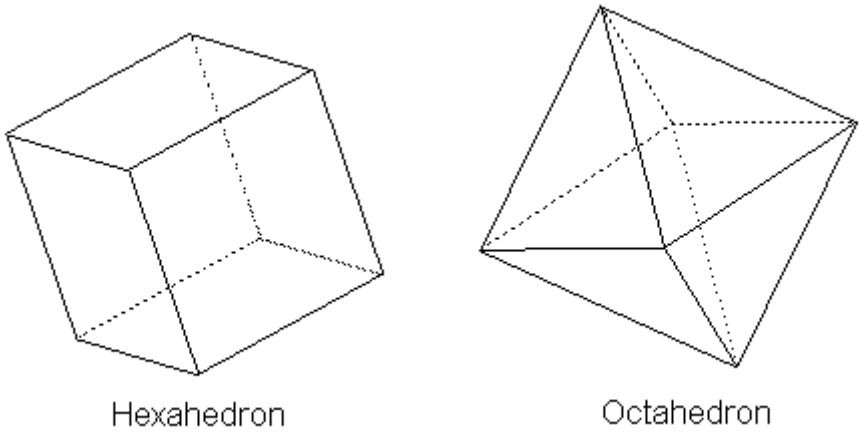
Tetrahedron

Tetrahedrons have 23 pointgroup symmetry: four 3-fold axes (in the middle of each triangular face), and six 2-fold axes intersecting all vertices.

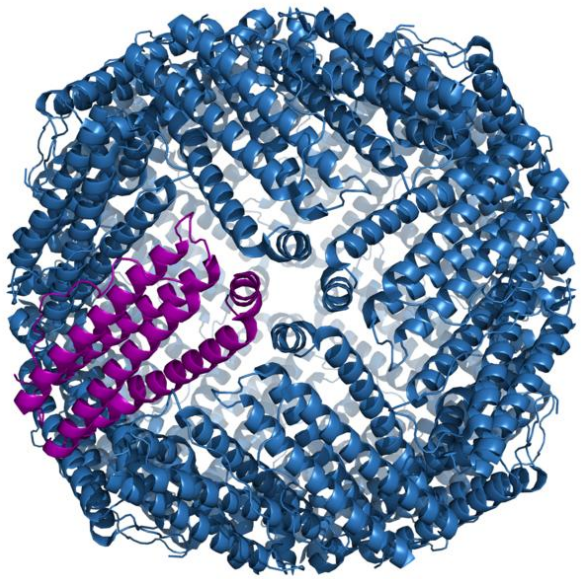
O (or 432)

Although the 432-pointgroup symmetry is known as the octahedral pointgroup symmetry, I prefer to think of this structure as the cubic structure. A cube has 6 square faces with a 4-fold symmetry axis in the middle of each face. The cube has eight corners with a 3-fold axis in each one. Also, the cube has twelve edges which are all intersected by a 2-fold axis. One monomer can be thought placed on one of the

squares: the 4-fold axis then implies that each square contains four monomers. Since there are six faces to the cube, a total of 24 identical subunits are required to build a cubic (octahedral) structure. Alternatively, we can think of a monomer placed close to the 3-fold axes in the corners of the cube. Since the cube has eight corners, this though path also leads to 24 monomers in the full structure. The classical example of a 432-pointgroup structure is ferritin. Very few other biological structures are known to have this symmetry.



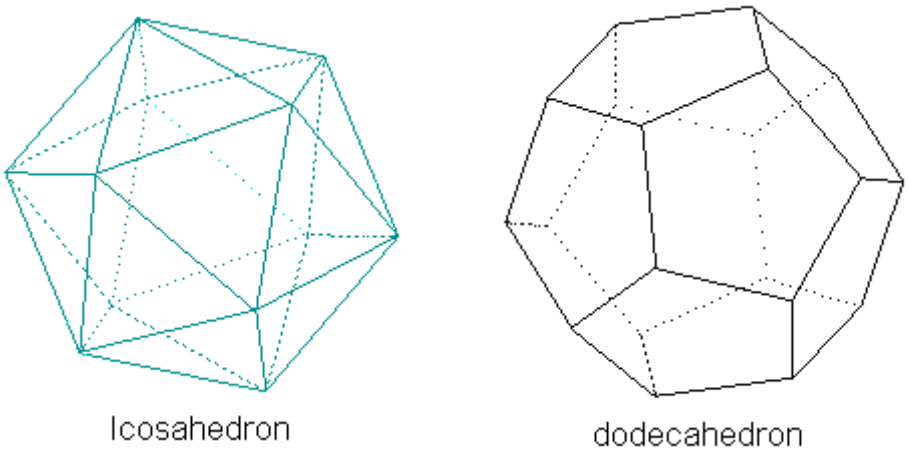
Cubes or "hexahedrons" (left) and octahedrons have a 432 pointgroup symmetry with six 4-fold axes, eight 3-fold axes (corners of the cube or centres of the octahedral triangles), and twelve 2-fold axes (intersecting all edges).



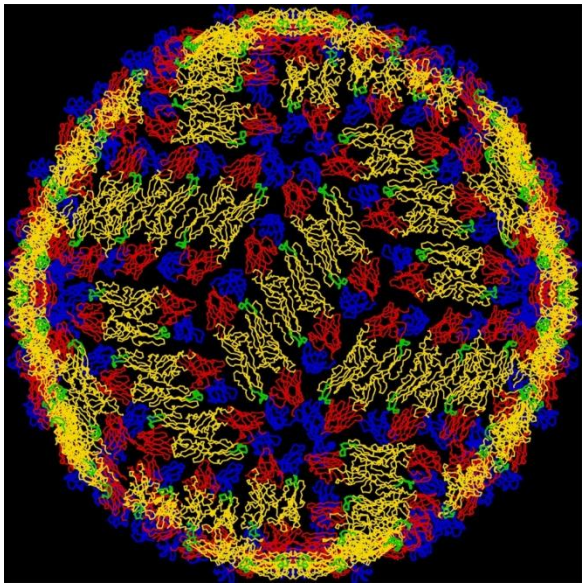
The structure of ferritin has "432" Pointgroup symmetry. (Granier, T., Langlois D`Estaintot, B., Gallois, B., Chevalier, J-M., Precigoux, G., Santambrogio, P., Arosio, P. ***Structural description of the active sites of mouse L-chain ferritin at 1.2 A resolution*** *J. Biol. Inorg. Chem*. 8 (2003) 105-111.

I (or 532)

The queen of all pointgroup symmetries contains 12 5-fold axes, 20 3-fold axes, and 30 2-fold axes. Many virus capsids have icosahedral symmetry. Scores of icosahedral viruses have been studied by 3D cryo-EM. One of the standard forms of the football ("soccer" ball) has icosahedral symmetry. Buckyballs (C60) have icosahedral symmetry. Many stories have been written about icosahedral symmetry so I can be brief here. One example of 532 structure solved by cryo-EM is the capsid structure of the dengue virus (Michael Rossmann group, 2002).



Icosahedrons (left) and dodecahedrons (right) both have a 532 pointgroup symmetry with twelve 5-fold axes, twenty 3-fold axes, and thirty 2-fold axes.

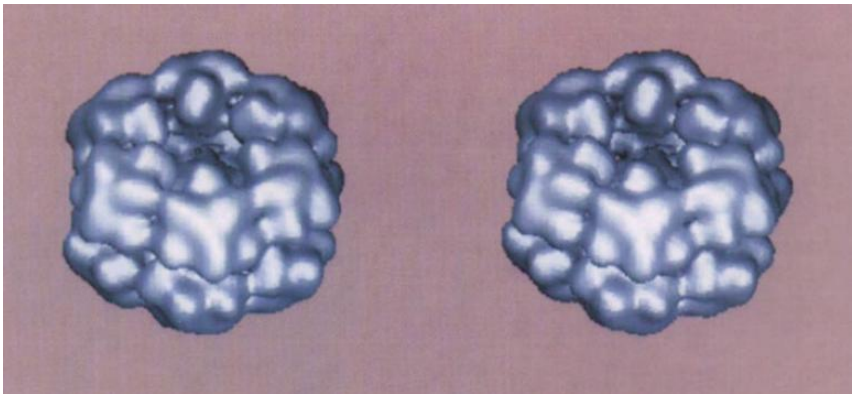


Structure of the dengue virus capsid solved by cryo-EM.(R.J. Kuhn, W.. Zhang, M.G. Rossmann, *et al*., and J.H. Strauss: **Structure of Dengue Virus: Implications for Flavivirus Organization, Maturation, and Fusion.** *Cell* 108 (**2002**) 717-725.)

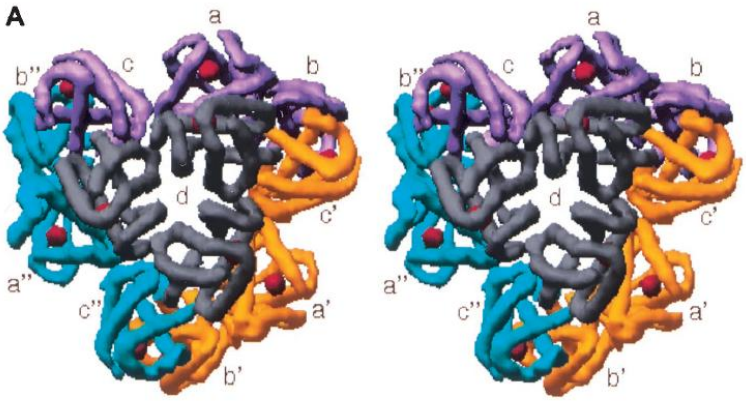
Nature playing with pointgroup symmetries

Nature has a way of playing with (pointgroup) symmetries. Local pointgroup symmetries (different for the overall pointgroup symmetry of the complex) are often found in large oligomeric structures. For example, instead of using 60 copies of a monomer to form an icosahedral virus capsid, nature may use 60 copies of a *trimer* to construct a capsid. Such viruses thus have 180 copies of a monomer in the capsid. This mechanism appears to have been used in evolution to create larger icosahedral viruses able to pack a larger genome.

The worm hemoglobin discussed above has local three-fold symmetry axis creating a sub organisation in this huge structure containing no less than 144 heme groups (see illustrations). The **D5** (52) symmetric Keyhole Limpet Hemocyanin discussed above consists of 6 tiers, each of which have and an approximate D5 symmetry.



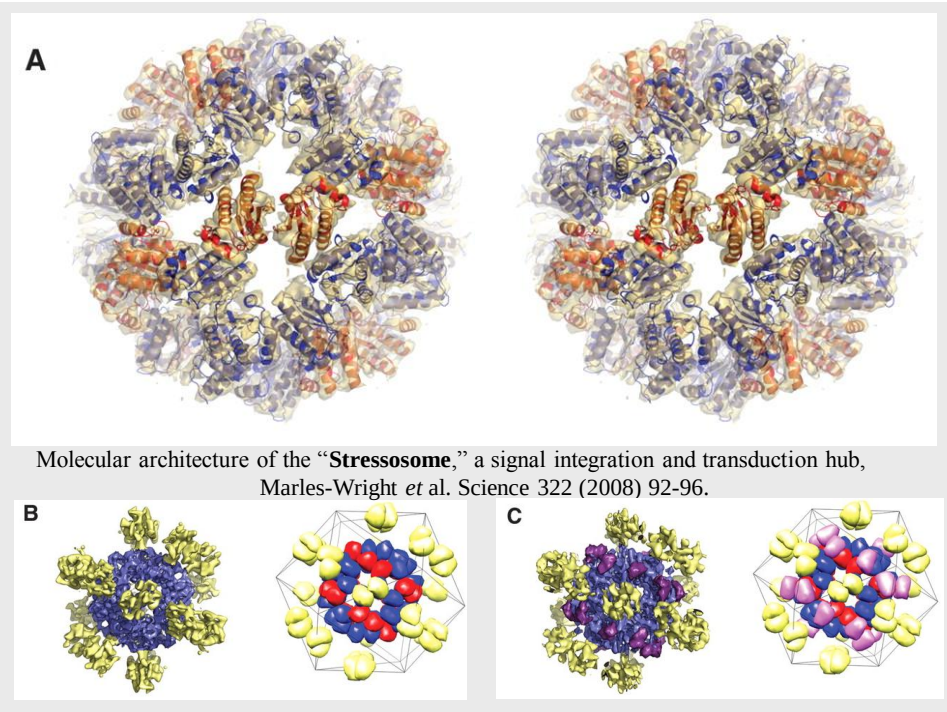
Stereo pair (look at the left image with you left eye and simultaneously at the right image with your right eye) of an early cryo-EM reconstruction of the *Lumbricus Terrestris* hemoglobin (Schatz *et a,l* Ultramicroscopy 1995). Protruding towards the observer in this stereo image is the 12th part of the overall structure. This 1/12th subunit has a *local* three-fold axis pointing directly at the observer.



Stereo pair of an X-ray crystallography reconstruction of the *Lumbricus Terrestris* hemoglobin . In this stereo image the 12th part of the overall structure is depicted. The higher-resolution X-ray map confirmed the presence of the *local* three-fold axis. (Royer et al.: **"Structural hierarchy in erythrocruorin, the giant respiratory assemblage of annelids"**, *PNAS* 97 (2000) 7107-7111).

Symmetry mismatches may be very important for the functioning of biological machinery. For example, the portal protein (discussed above) in vivo has a **C12** pointgroup symmetry yet is embedded in the 5-symmetric environment of one of the icosahedral vertices. The symmetry mismatch the two structures is thought to facilitate the free rotation of structure with respect to the other.

With the recently solved cryo-EM structure of the stressosome (see below), the structure appears to reflect subtle balance in exploiting an effectively icosahedral core as a scaffold for an apparently D5 structure of protrusions, which upon closer inspection only has an exact D2 pointgroup symmetry.



Molecular architecture of the "**Stressosome**," a signal integration and transduction hub, Marles-Wright *et* al. Science 322 (2008) 92-96.

Notes:

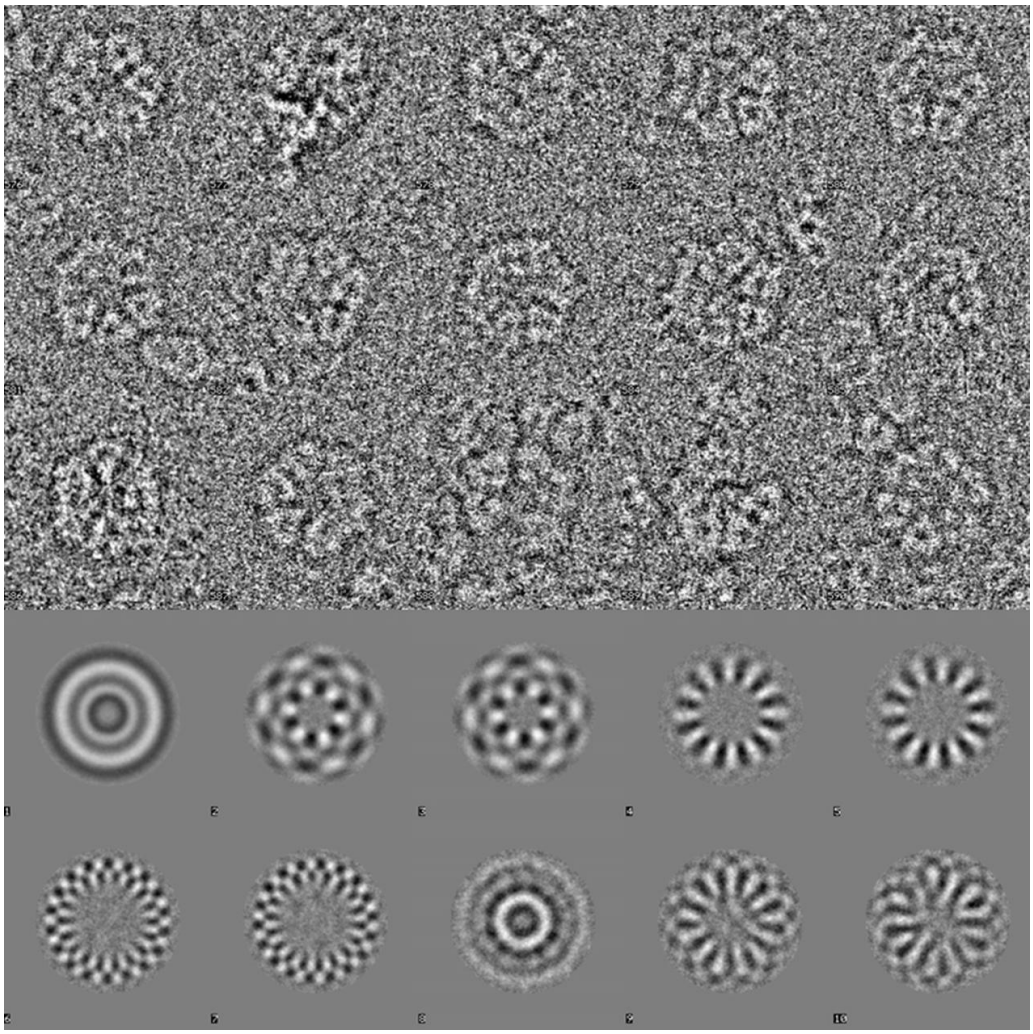
1) There are conventions as to how to place structures with pointgroup symmetry into a three-dimensional (3D) volume. No restrictions exist on, for example, how to put a C1 structure in a 3D reconstruction volume. Typically one will place the centre of mass of the "centre of modulation" (centre of mass of the absolute densities) into the centre of the 3D map.

2) For a structure with real symmetry, however, the convention is to place the axis of the highest rotational symmetry along the "Z" direction. Even then significant freedom can exist in how to place the structure within the volume. For example, **C13** structure can be placed anywhere along the Z direction. Also, the rotational orientation (in IMAGIC: the "Gamma" direction) of a **C13** structure is arbitrary as is the up-or-down orientation along the Z-axis. It is important to think about such inherent freedom of orientations in the data when comparing independently conducted experiments.

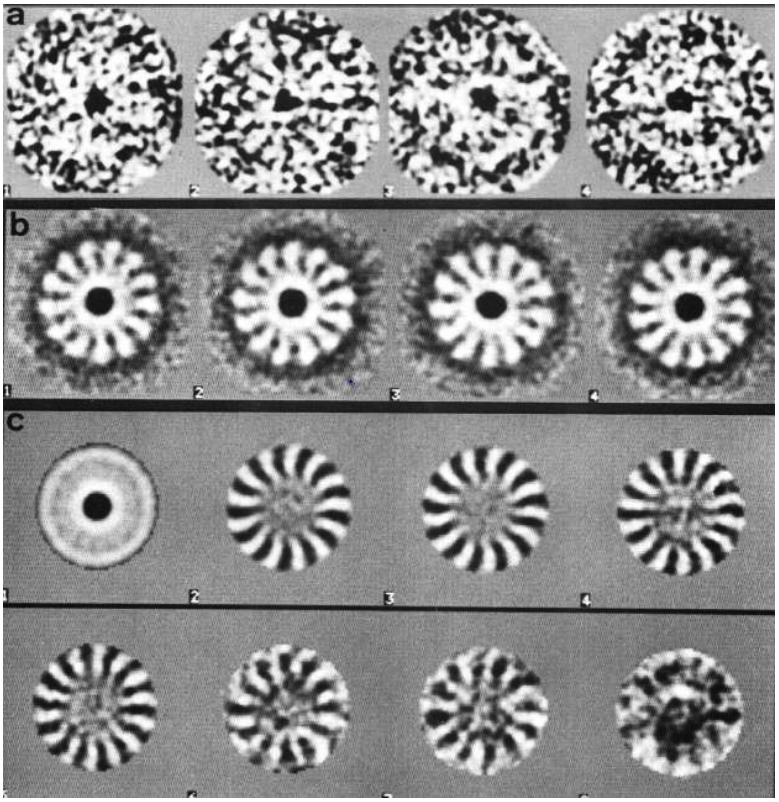
3) More Tricky Stuff: there may be multiple ways of placing the same structure in a 3D co-ordinate system! For example, a 222 pointgroup symmetry structure is fixed absolutely in a 3D Cartesian co-ordinate system with a two-fold axis along X, a two-fold axis along Y, and a two-fold axis along Z. However, there are no restrictions as to which of the three two-fold axes of the 222 structure must point along the Z-, the Y-, or the X-axis. Similarly, there are multiple ways of placing an icosahedral structure in a Cartesian 3D volume. The 532 (icosahedral) pointgroup symmetry contains the **222** (**D2**) and the **52** (**D5**) symmetries as sub-symmetries; each of these sub-symmetries can, in turn, be placed in different ways in the volume.
.

How do I find the pointgroup symmetry of a complex?

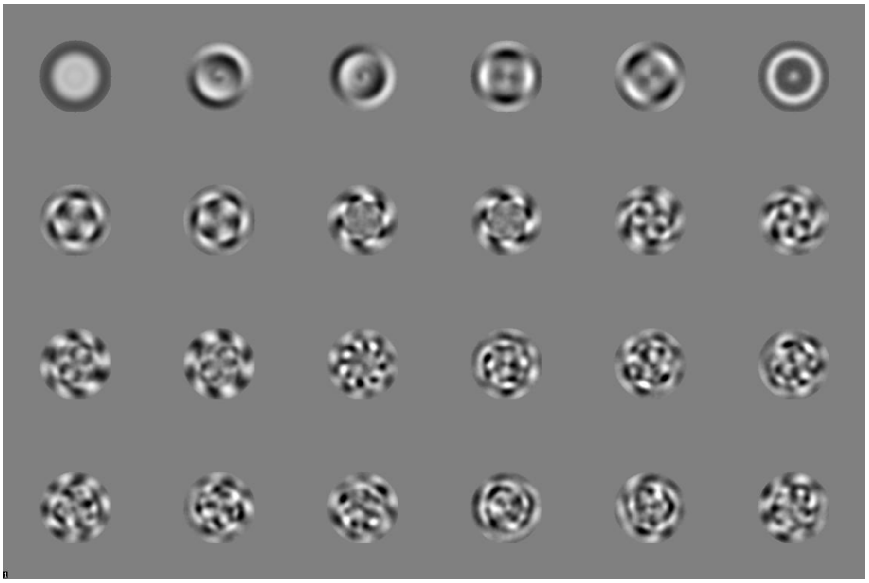
An eigenvector procedure proposed by Dube *et al.* (1993) is the main tool for a "reference-free" determination of the symmetry components of a data set. (See: Dube P, Tavares P, Lurz R, van Heel M. **Bacteriophage SPP1 portal protein: a DNA pump with 13-fold symmetry**. *EMBO J.* **15** (1993) 1303-1309). In that original analysis the symmetry was found to be C13.



After centring all *Lumbricus terrestris* hemoglobin images with respect to the total average of the data set, one uses an MSA eigenvector procedure to find the main symmetry components of the data. This hemoglobin exhibits D6 pointgroup symmetry as can be seen from eigenvectors #2 and #3 which are identical apart from a 30º rotation with respect to each other. These eigenvectors are also mirror symmetric which discriminate between a D6 and a C6 pointgroup symmetry.



After centring all *SPP1* portal protein images with respect to the total average of the data set, one uses an MSA eigenvector procedure to find the main symmetry components of the data. This oligomer exhibits C13 pointgroup symmetry as can be seen from eigenvectors #2 and #3 which are identical apart from a small rotation with respect to each other. These eigenvectors are NOT mirror symmetric which discriminates between a C13 and a D13 pointgroup symmetry.



The symmetry story is not always straightforward. After centring a data set of the asymmetric 50S ribosomal subunits, the MSA eigenvector procedure also yields pairs of symmetric eigenimages. The lower ones (#2 and #3) show C1 symmetry, the higher eigenvectors come in pairs with 2-fold, 3-fold, etc symmetry.