

# Overview

- Much, if not most, protein in the cell is not monomeric
  - Many proteins exist as part of macromolecular complexes (*e.g.*, ribosome), or as polymers (*e.g.*, actin, microtubules)
- History is important: 1950's to 60's, realized that small viral genome encoded large viral capsids
  - polymeric structures containing many copies of an identical gene product
- Closed shells - point group symmetries

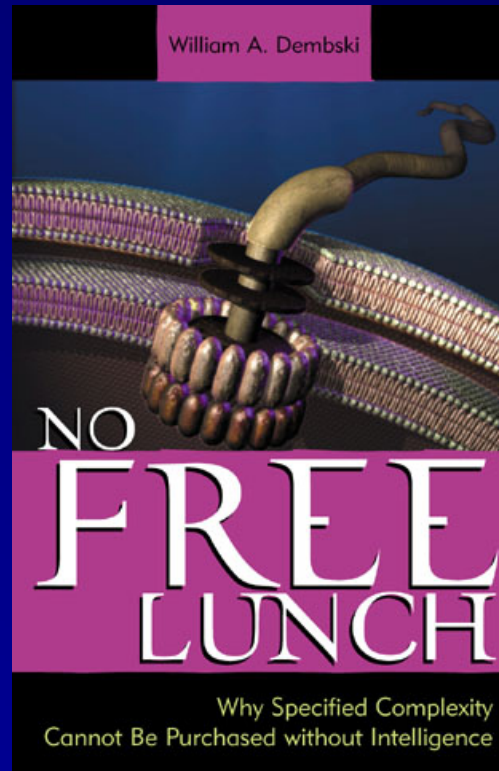
## Looking at Macromolecular Complexes

- Previously: EM as a tool for looking at macromolecular assemblies
- Today: Protein multimers and polymers, general features
- Monday, 6 Oct: What can kinetics of assembly tell us?

# Flagellum has been icon of "ID"

- "Intelligent Design" is being promoted as a scientific alternative to evolution

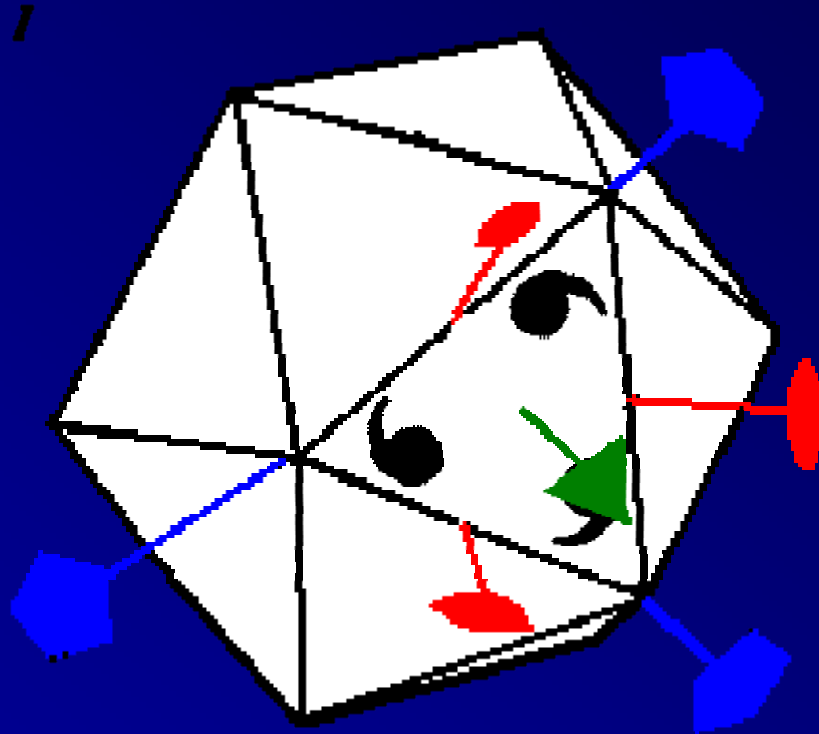
bacterial flagellum is example of "irreducible complexity", establishing that evolution could not have happened



# Symmetry and "irreducible complexity"

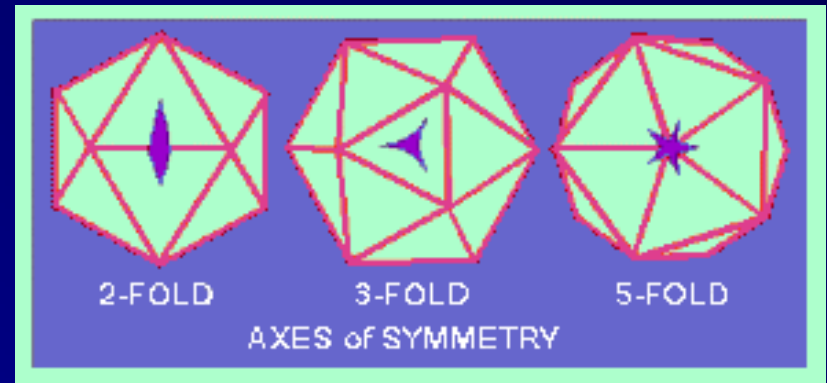
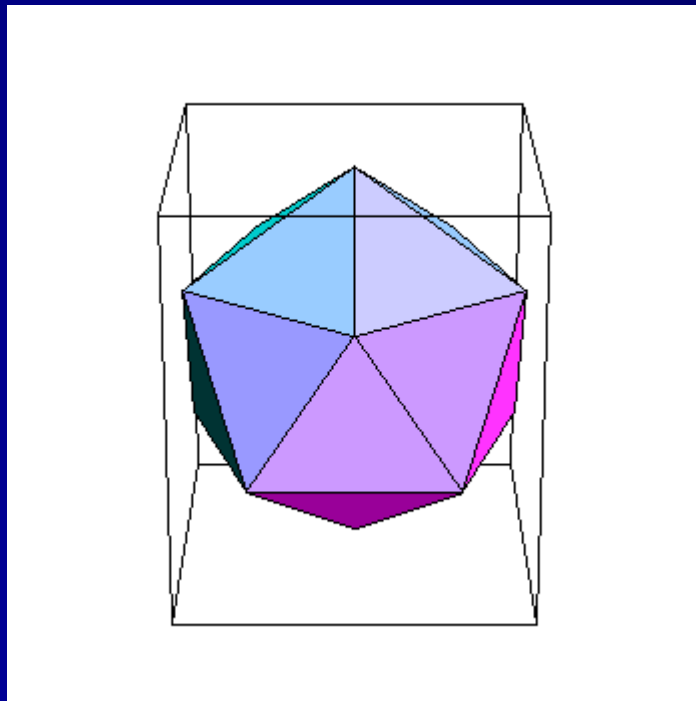


Icosahedron has 532 symmetry: 20 identical faces

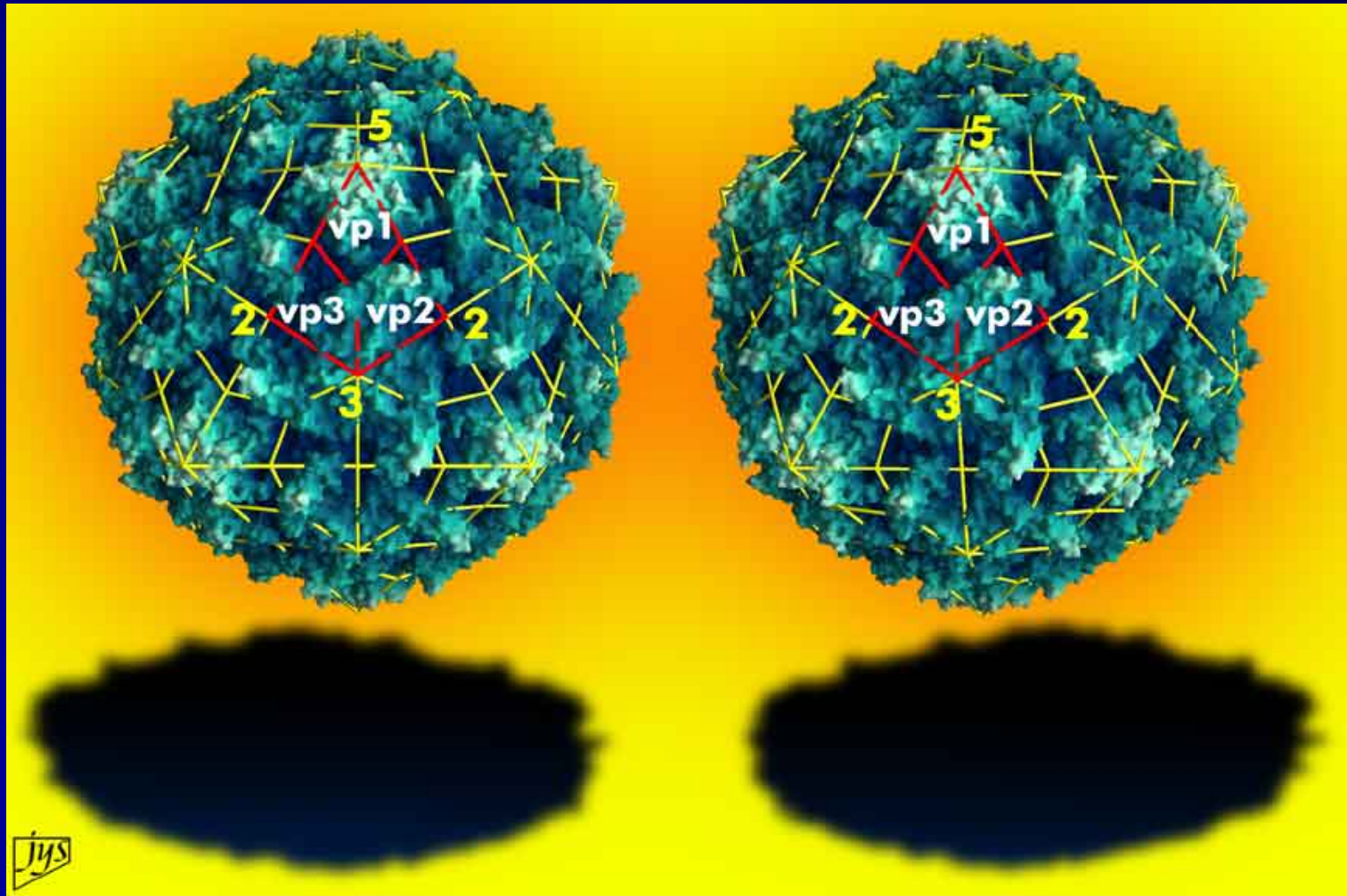


- Can be formed from 60 identical subunits, each in an identical environment
- But larger capsids cannot have strict equivalence

# Different views of icosahedron show symmetry

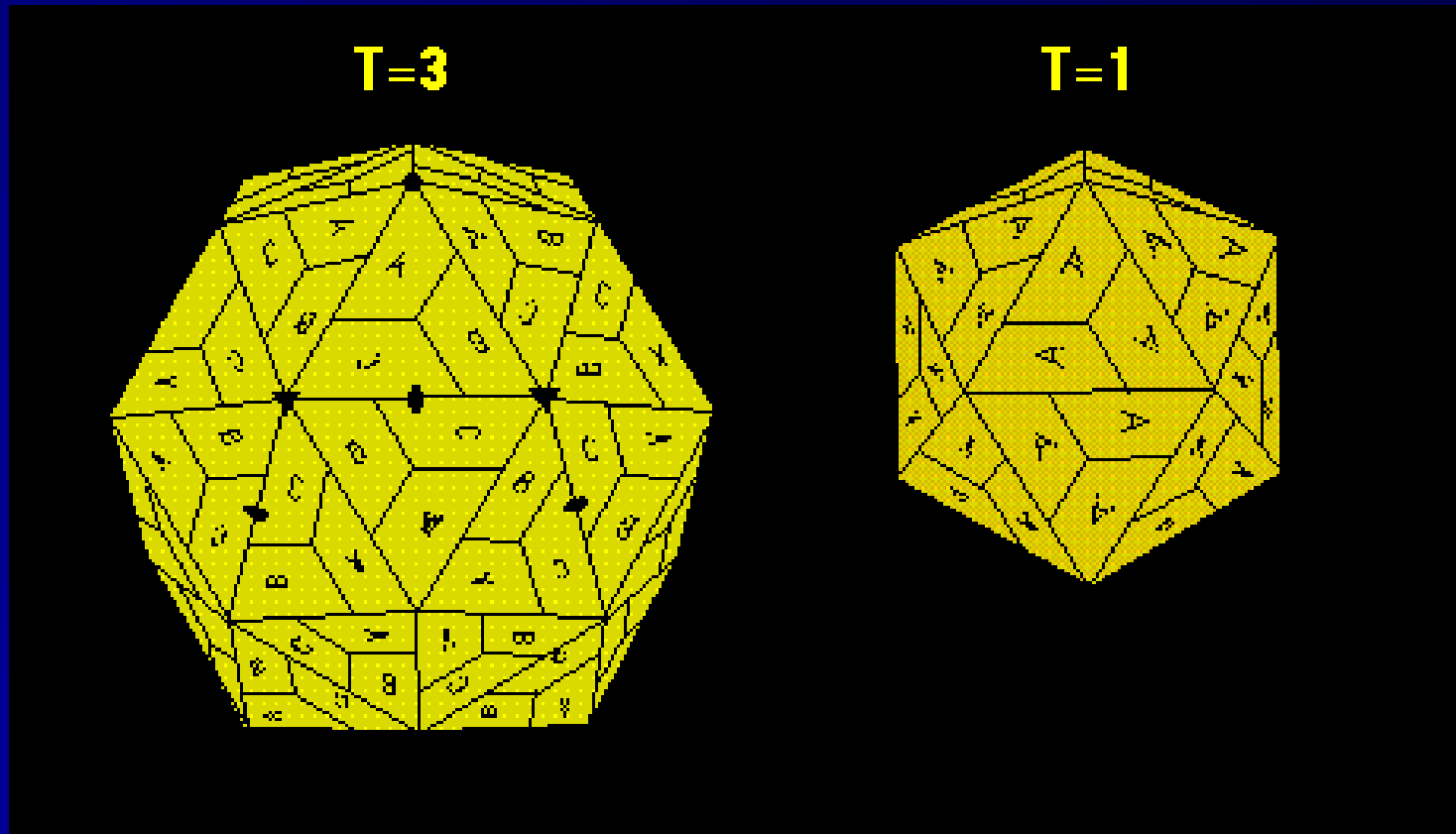


# T numbers and capsids



Poliovirus Type 1 Mahoney (PDB: 2PLV) STEREOVIEW. X-Ray crystallography data by J.M. Hogle, M.Chow, D.J. Filman, Science 229: 1358,1985. GRASP Molecular surface rendering showing surface topography. Lighter blue surface colors depict prominent surface features, darker colors indicate surface crevices and canyons. Red lines outline one biological protomer (vp1, vp2 and vp3) as indicated, vp4 is inside and not visible from the surface. Surrounding yellow numbers mark the icosahedral 5, 3, and 2- fold axes. The large protrusion just above the vp2 sign is the C-terminal arm of vp3. For more virus Xray coordinates see [www.rcsb.org](http://www.rcsb.org) or [mmtsb.scripps.edu/viper](http://mmtsb.scripps.edu/viper). For downloadable images see also [www.bocklabs.wisc.edu](http://www.bocklabs.wisc.edu). Illustration by Dr. Jean-Yves Sgro, Institute for Molecular Virology, UW-Madison.

# T numbers and capsids



For larger viral capsids, number of subunits is  $3 \cdot T$

# How does this relate to cells?

REPORTS

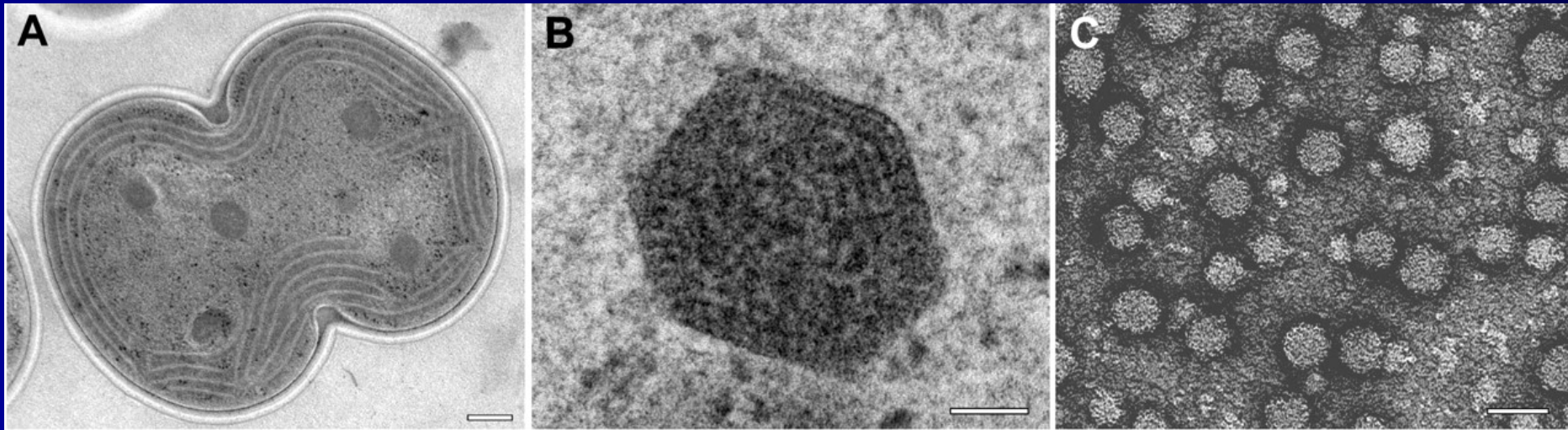
## Protein Structures Forming the Shell of Primitive Bacterial Organelles

Cheryl A. Kerfeld,<sup>1,2,4</sup> Michael R. Sawaya,<sup>1,3,4</sup> Shiho Tanaka,<sup>1</sup>  
Chau V. Nguyen,<sup>1</sup> Martin Phillips,<sup>1,3</sup> Morgan Beeby,<sup>1,3</sup>  
Todd O. Yeates<sup>1,3,4\*</sup>

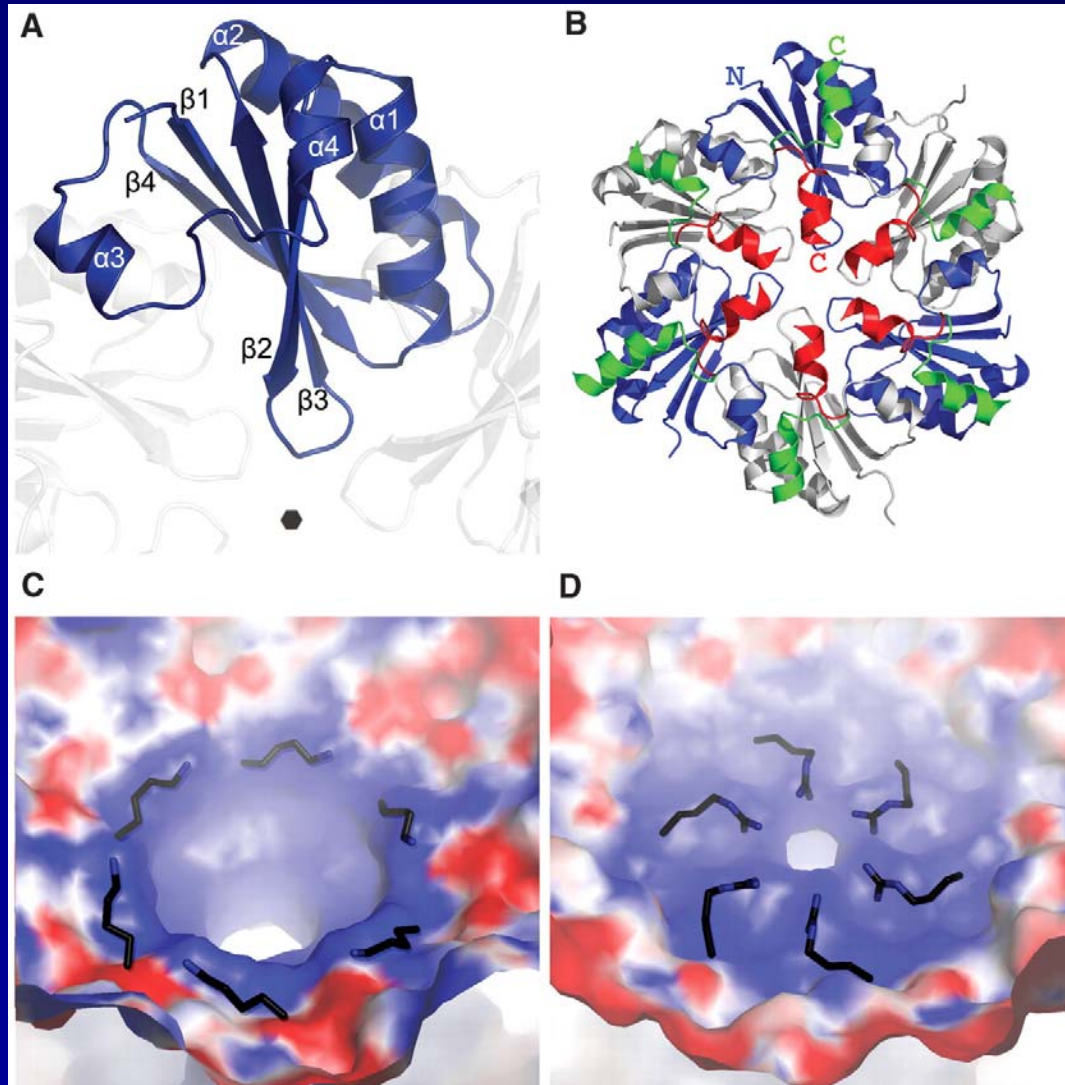
Bacterial microcompartments are primitive organelles composed entirely of protein subunits. Genomic sequence databases reveal the widespread occurrence of microcompartments across diverse microbes. The prototypical bacterial microcompartment is the carboxysome, a protein shell for sequestering carbon fixation reactions. We report three-dimensional crystal structures of multiple carboxysome shell proteins, revealing a hexameric unit as the basic microcompartment building block and showing how these hexamers assemble to form flat facets of the polyhedral shell. The structures suggest how molecular transport across the shell may be controlled and how structural variations might govern the assembly and architecture of these subcellular compartments.

**Science, 5 August 2005**

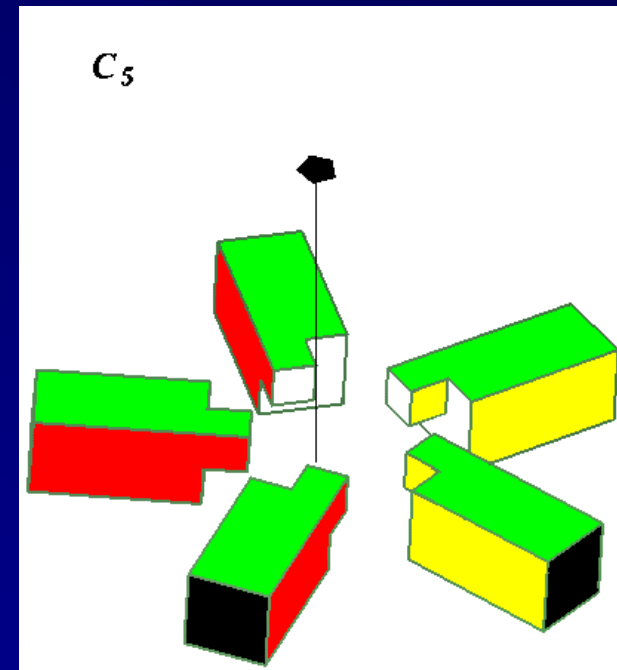
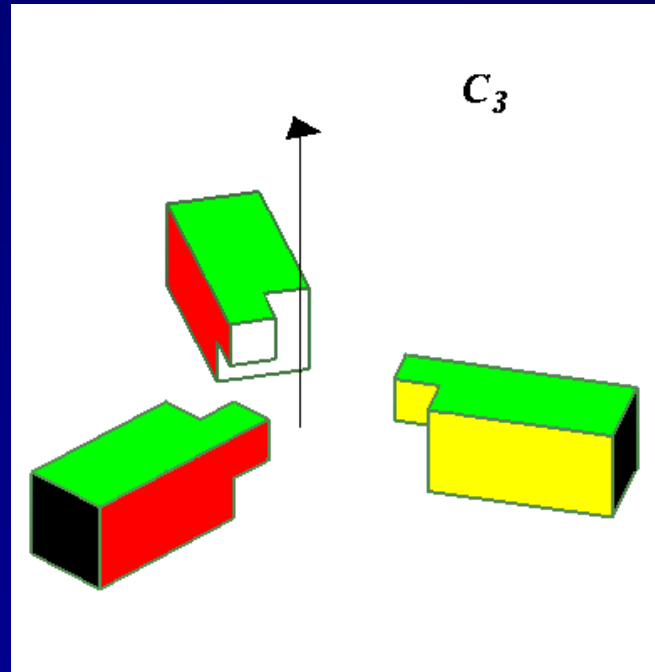
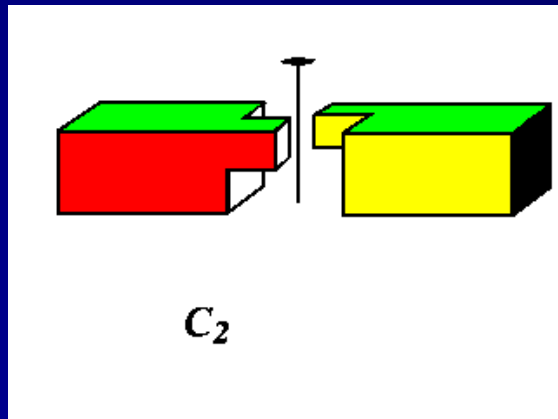
How does this relate to cells?



# Symmetrical arrangements of protein



# Cyclic point group symmetry

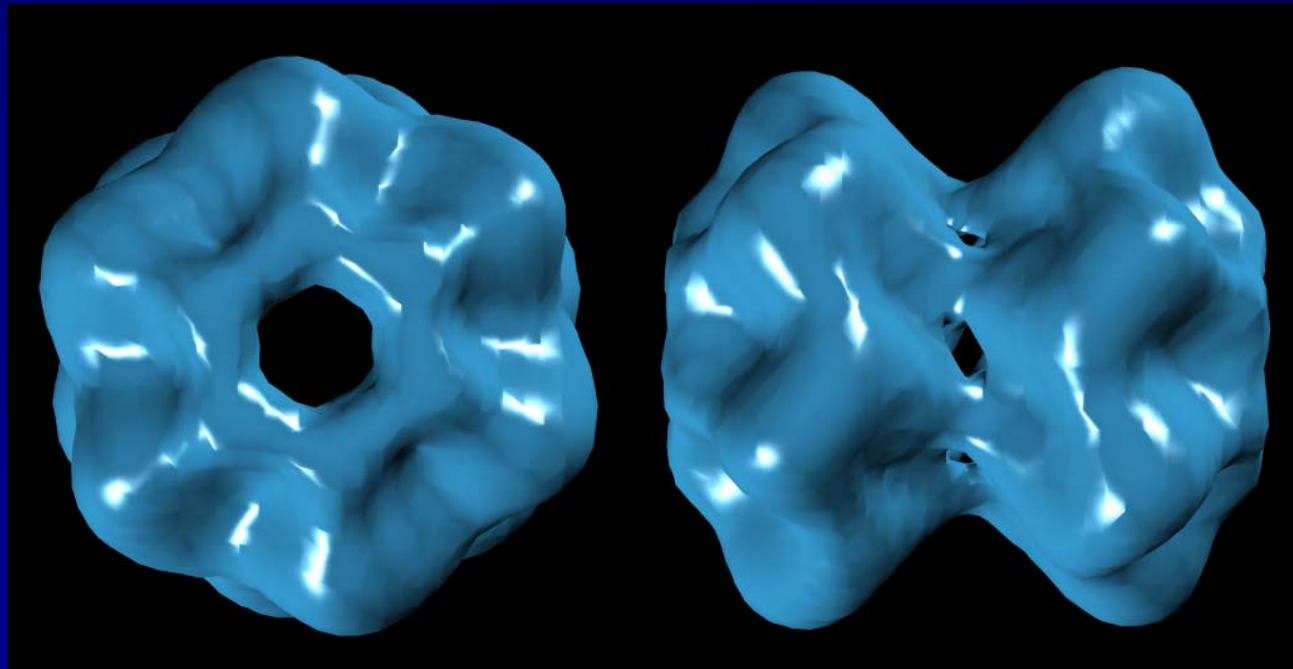


In general,  $C_n$

## Dihedral point group symmetry

2-fold axis perpendicular to  $C_n$

RuvB from *E. coli*  
D<sub>6</sub>



In general, D<sub>n</sub>

While there are a few examples of icosahedral enzyme complexes, many examples of  $C_n$  rings and helical polymers

A few examples of such helical polymers:

Tobacco Mosaic Virus

bacteriophage tails

microtubules

bacterial flagella

bacterial pili

**globular subunits**

myosin thick filaments

collagen

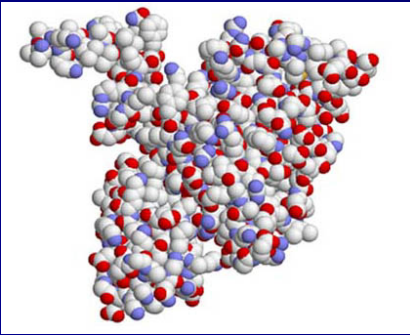
keratin

neurofilaments

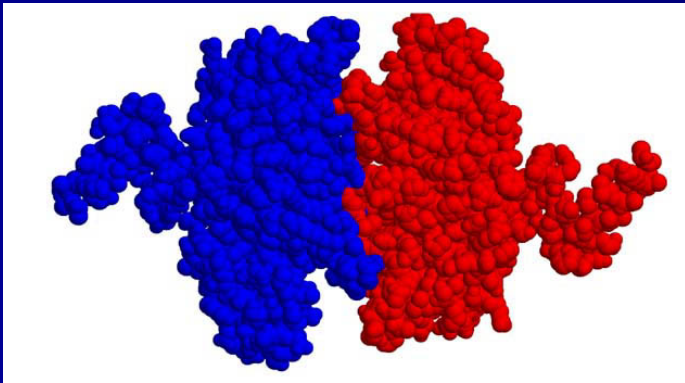
tropomyosin

**coiled-coils**

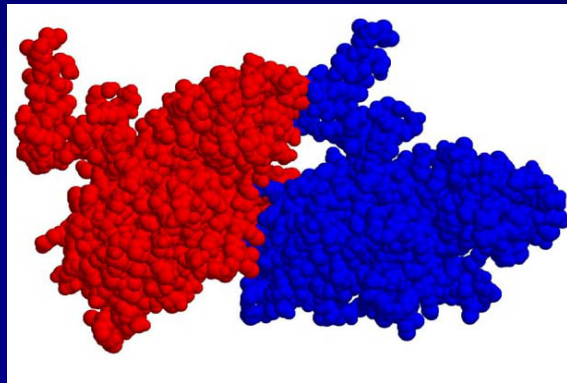
## Helical Symmetry Reflects Simplest Bonding Rule



Asymmetric subunit

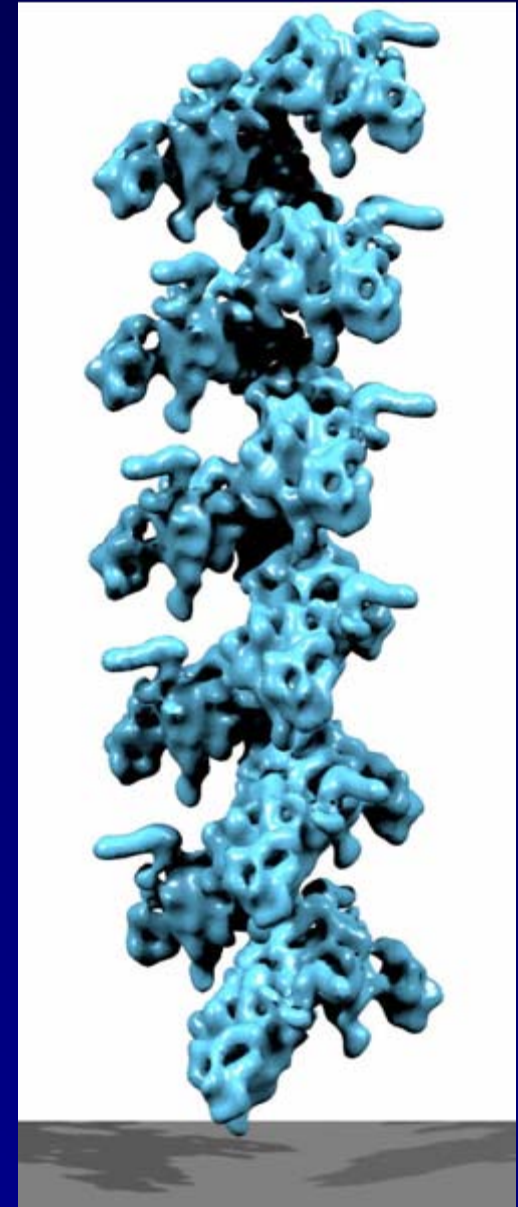


Symmetric dimer



Asymmetric bond

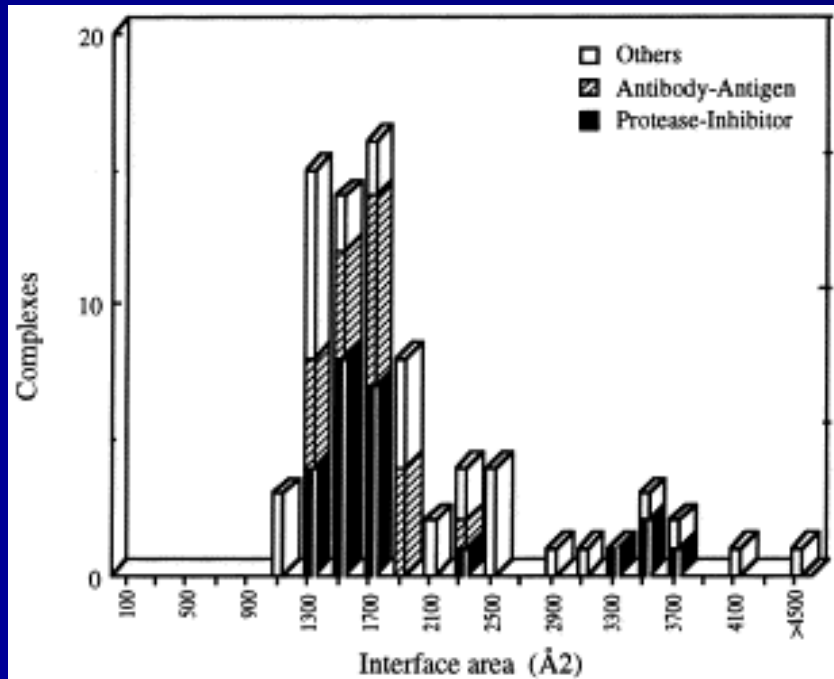
Symmetrical helix



What holds viral capsid, protein polymer, or ribosome together?

- Interface largely hydrophobic, similar to interior of protein itself!
- Chothia and Janin (Nature, 1975):

$$\Delta G \sim 0.025 \text{ kcal/mol/\AA}^2$$

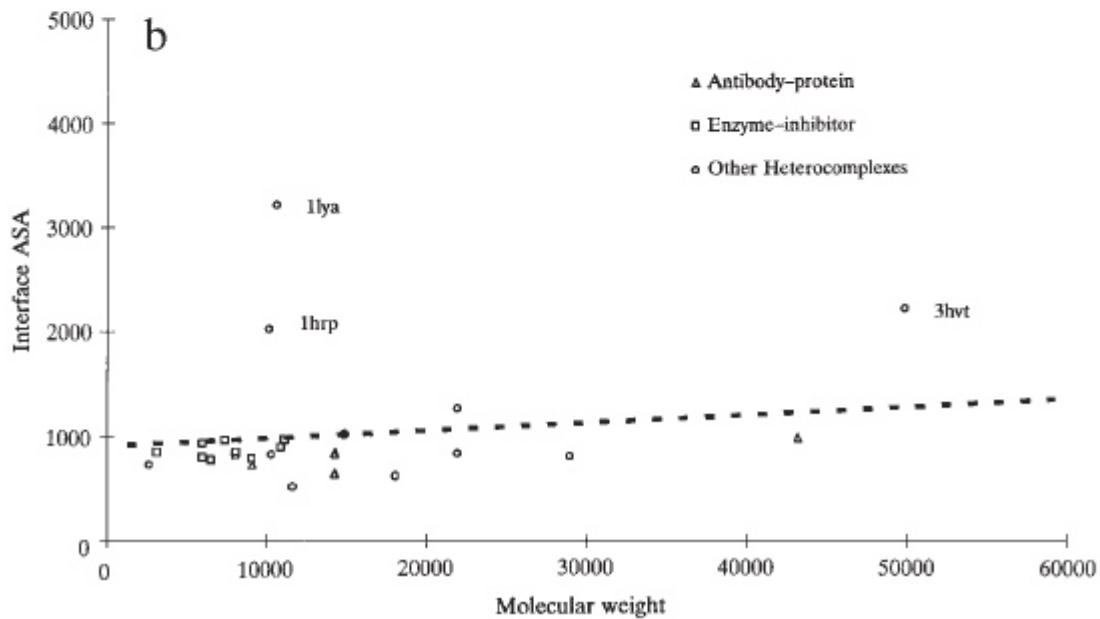
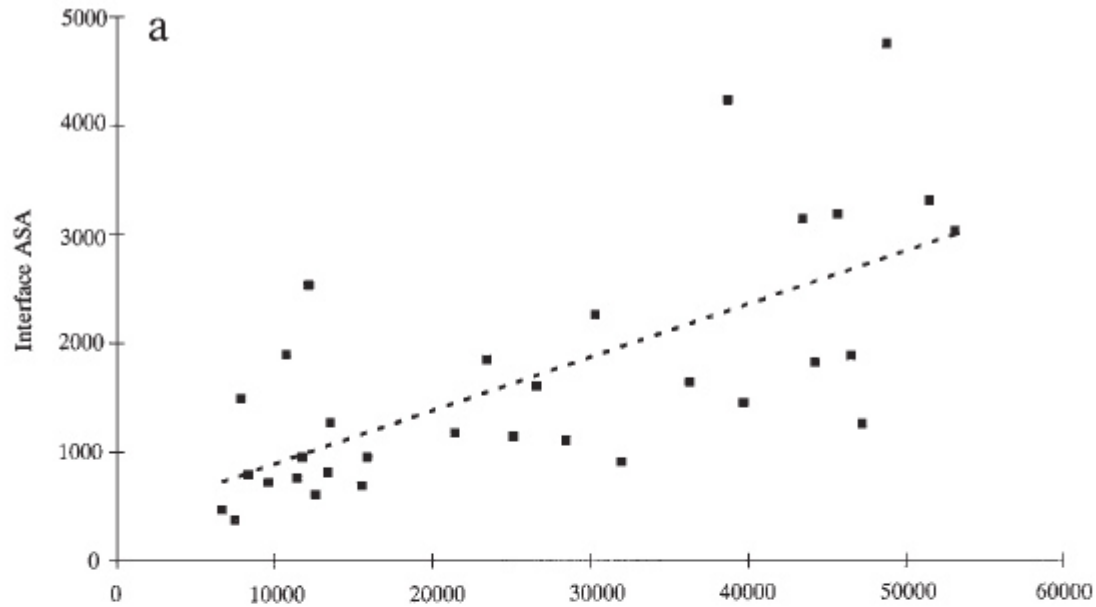


Analysis of 75 high-resolution protein-protein interfaces (Lo Conte *et al.*, JMB 1999)

For insulin dimer: ~28 kcal/mol

For 1,700 Å², ~ 42 kcal/mol

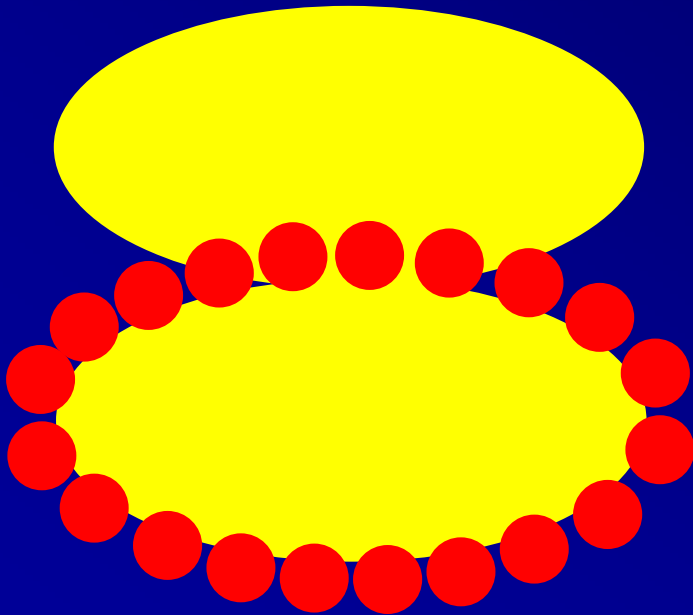
# Size of interface correlates with MW



from Jones and Thornton, PNAS 1996

## What will oppose this?

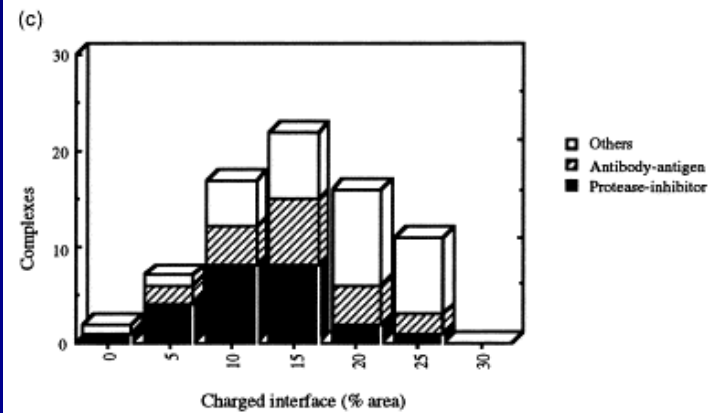
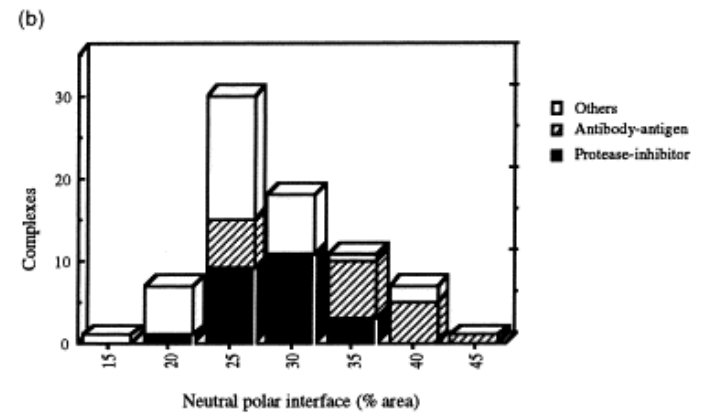
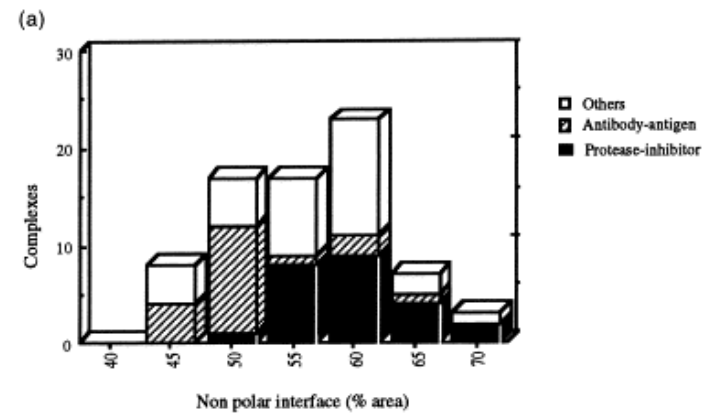
- Loss of entropy of protein subunits!
- This can make assembly unfavorable
- But entropy of water needs to be considered...



What can we say about residues at the protein-protein interface?

- For solvent accessible surface of small globular monomeric proteins, residues are:
  - 57% non-polar
  - 24% neutral polar
  - 19% charged
- For 75 interfaces studied, 53% non-polar!
- Thus, the "patches of protein surface that form interfaces are no more hydrophobic than the accessible surface of small globular proteins."

# What residues are found at protein-protein interface:



# Small changes in sequence can produce dramatic changes in quaternary structure

articles

## Core mutations switch monomeric protein GB1 into an intertwined tetramer

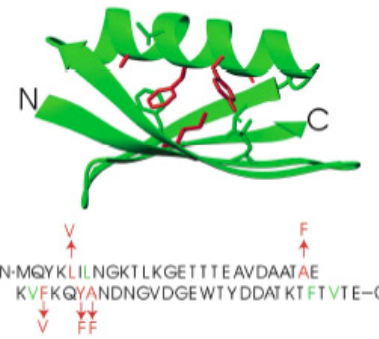
M. Kirsten Frank<sup>1</sup>, Fred Dyda<sup>2</sup>, Anatoliy Dobrodurnov<sup>1</sup> and Angela M. Gronenborn<sup>1</sup>

Published online 15 October 2002; doi:10.1038/nsb854

The structure of a mutant immunoglobulin-binding B1 domain of streptococcal protein G (GB1), which comprises five conservative changes in hydrophobic core residues, was determined by NMR spectroscopy and X-ray crystallography. The oligomeric state and quaternary structure of the mutant protein are drastically changed from the wild type protein. The mutant structure consists of a symmetric tetramer, with intermolecular strand exchange involving all four units. Four of the five secondary structure elements present in the monomeric wild type GB1 structure are retained in the tetrameric structure, although their intra- and intermolecular interactions are altered. Our results demonstrate that through the acquisition of a moderate number of pivotal point mutations, proteins such as GB1 are able to undergo drastic structural changes, overcoming reduced stability of the monomeric unit by multimerization. The present structure is an illustrative example of how proteins exploit the breadth of conformational space.

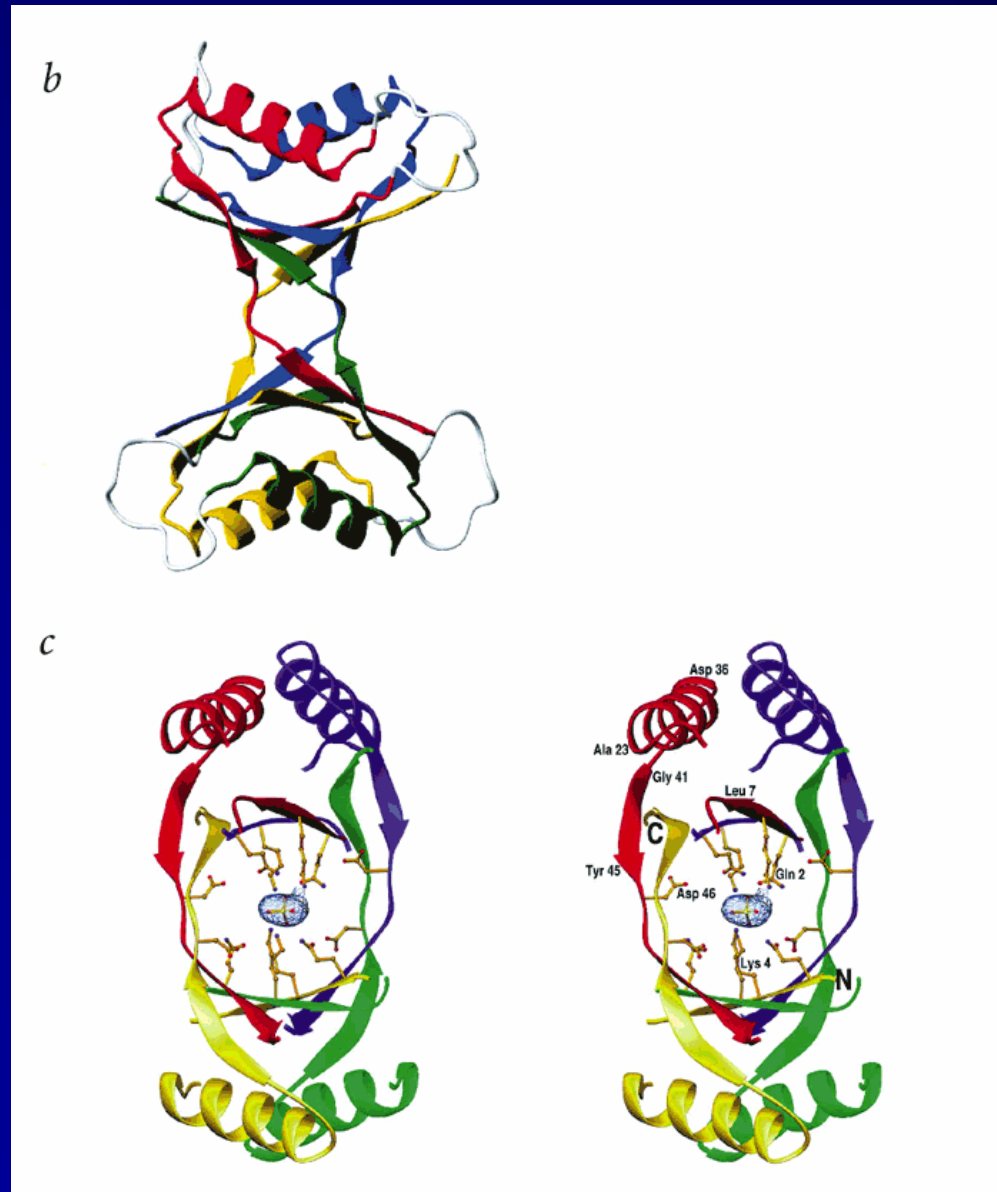
Several model systems for protein folding and design have found widespread use over the last several years. One such model protein is the immunoglobulin binding domain B1 of streptococcal protein G (GB1), which has been used in numerous studies from both an experimental and a theoretical perspective. GB1 is a small (56 residues), stable single-domain protein with one  $\alpha$ -helix, a four-stranded  $\beta$ -sheet and two hairpins. The structure of GB1 has been determined by NMR spectroscopy<sup>1</sup> and X-ray crystallography<sup>2</sup> (Fig. 1). The central part of the hydrophobic core of GB1 comprises residues Leu 5, Leu 7, Ala 26, Phe 30, Ala 34, Phe 52 and Val 54, whose side chains are completely solvent inaccessible. A single Trp residue (Trp 43) is located on  $\beta$ -strand 3. Its side chain is in van der Waals contact ( $<5 \text{ \AA}$ ) with two aromatic (Phe 30 and Phe 52) and three aliphatic residues (Leu 5, Ala 34 and Val 54) at the helix-sheet interface, therefore constituting an ideal probe for studying the protein by fluorescence spectroscopy<sup>3</sup>.

In the past, extensive mutagenesis studies have been carried out on GB1, using computational redesign<sup>4</sup> or random sequence libraries<sup>5</sup>. The vast body of GB1 mutants confirmed the general notion that proteins are remarkably tolerant to substitutions within their cores, easily accommodating 1–4 changes. Indeed, most computational protein redesign strategies are based on the assumption that a substantial number of core residues can be

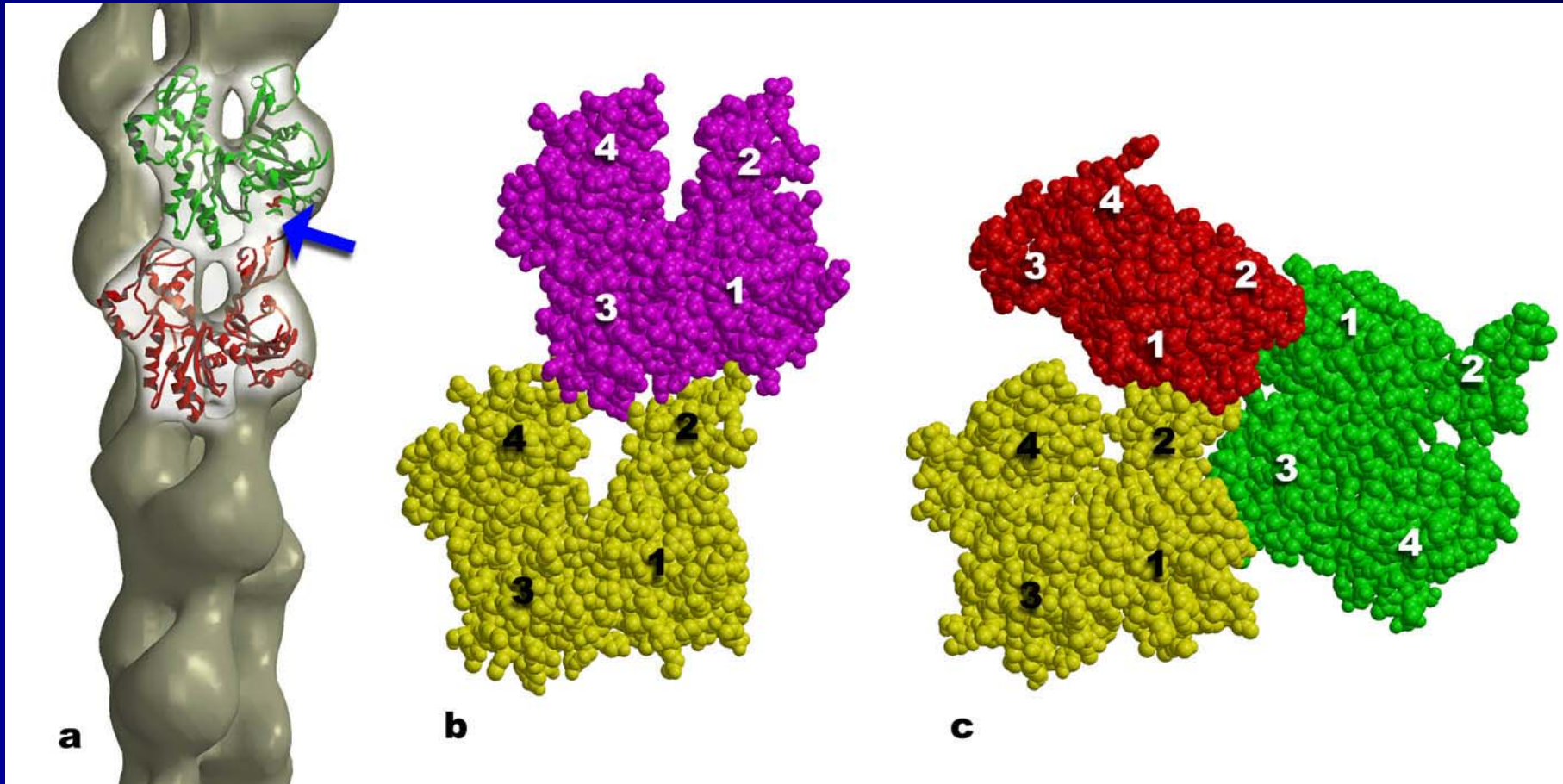


**Fig. 1** Structure and sequence of the B1 domain of streptococcal protein G. Top, ribbon diagram of GB1. The side chains of the positions targeted in the mutagenesis are shown. Altered side chains in the L5V/A26F/F30V/Y33F/A34F mutant (H5#124) are red. Bottom, the amino acid sequence of GB1. Sites targeted for mutagenesis are marked green and red, sites that were changed in the H5#124 mutant are red and the actual mutations are indicated by arrows.

# GB1 - intertwined secondary structure



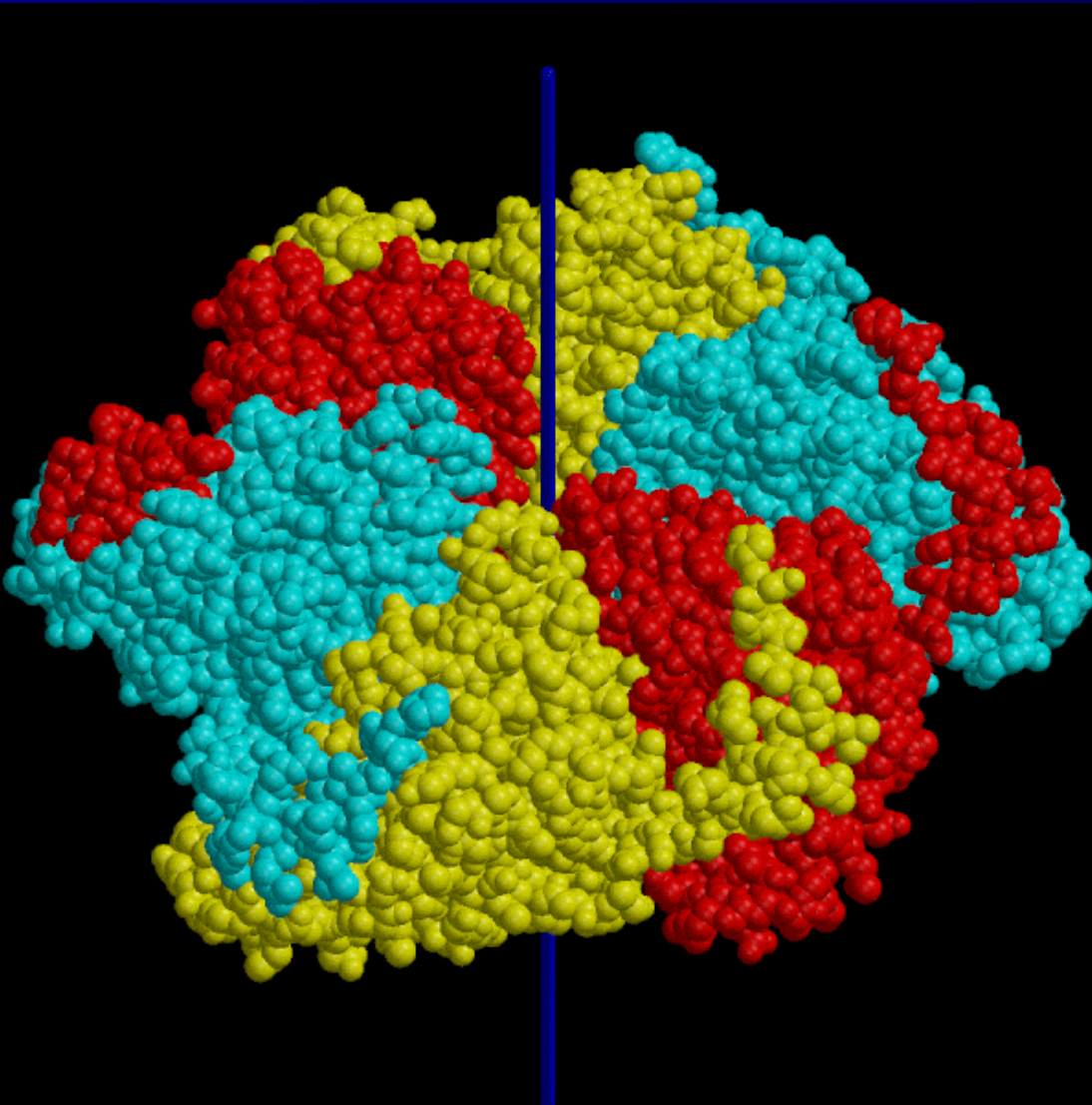
# Complications in studying protein-protein interfaces: polymorphisms



F-Actin model

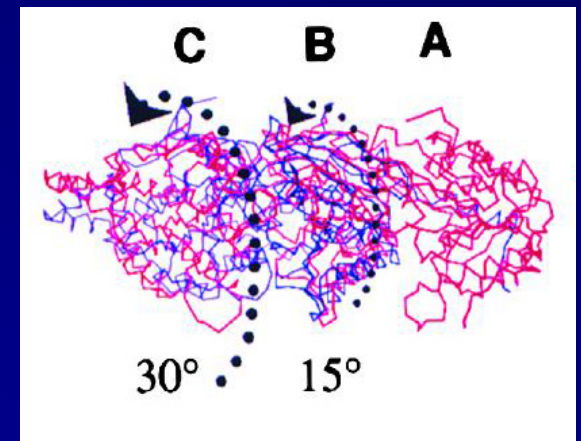
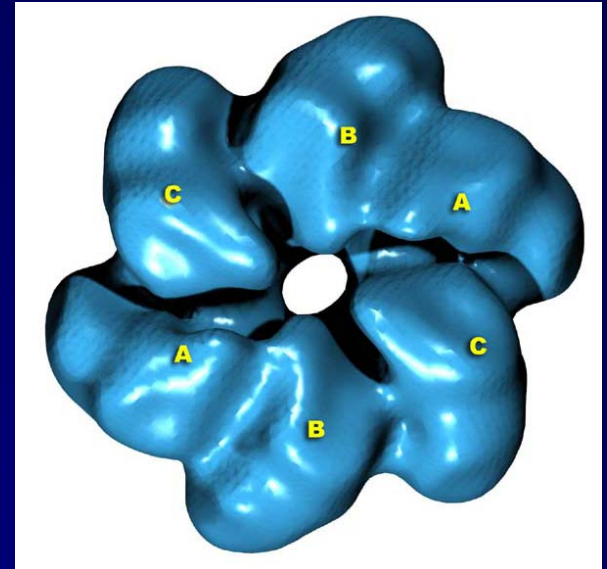
crystal

# Polymorphism in T7 gp4 helicase crystal structure

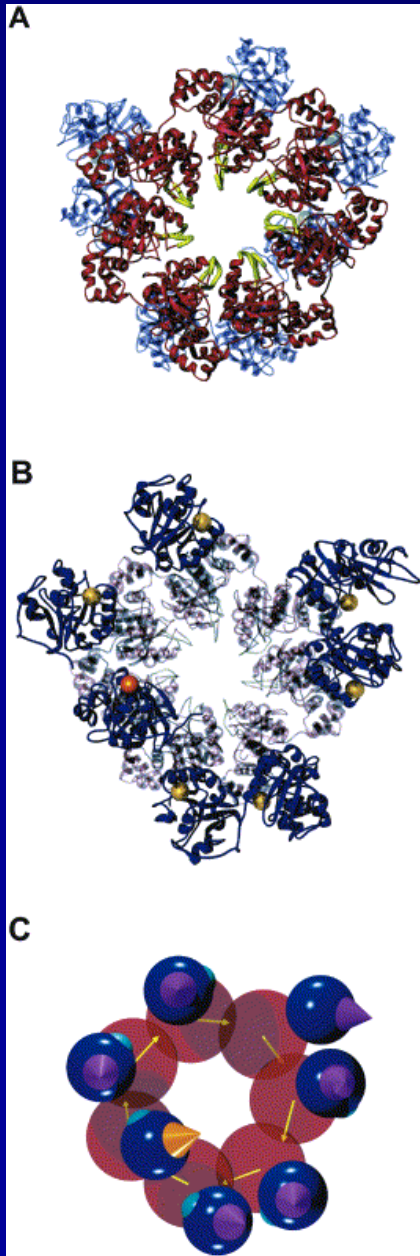


Singleton *et al.*, 2000

Three different subunit-subunit interfaces!



# Polymorphism in T7 gp4 helicase crystal structure



Seven different subunit-subunit interfaces!

Toth *et al.*, 2003

# Octameric membrane transporter shows large degree of polymorphism

## The RCK Domain of the KtrAB K<sup>+</sup> Transporter: Multiple Conformations of an Octameric Ring

Ronald A. Albright,<sup>1</sup> José-Luís Vazquez Ibar,<sup>1</sup> Chae Un Kim,<sup>2</sup> Sol M. Gruner,<sup>2,3</sup> and João Henrique Morais-Cabral<sup>1,\*</sup>

<sup>1</sup>Department of Molecular Biophysics and Biochemistry, Yale University, 266 Whitney Avenue, New Haven, CT 06520, USA

<sup>2</sup>Cornell High Energy Synchrotron Source

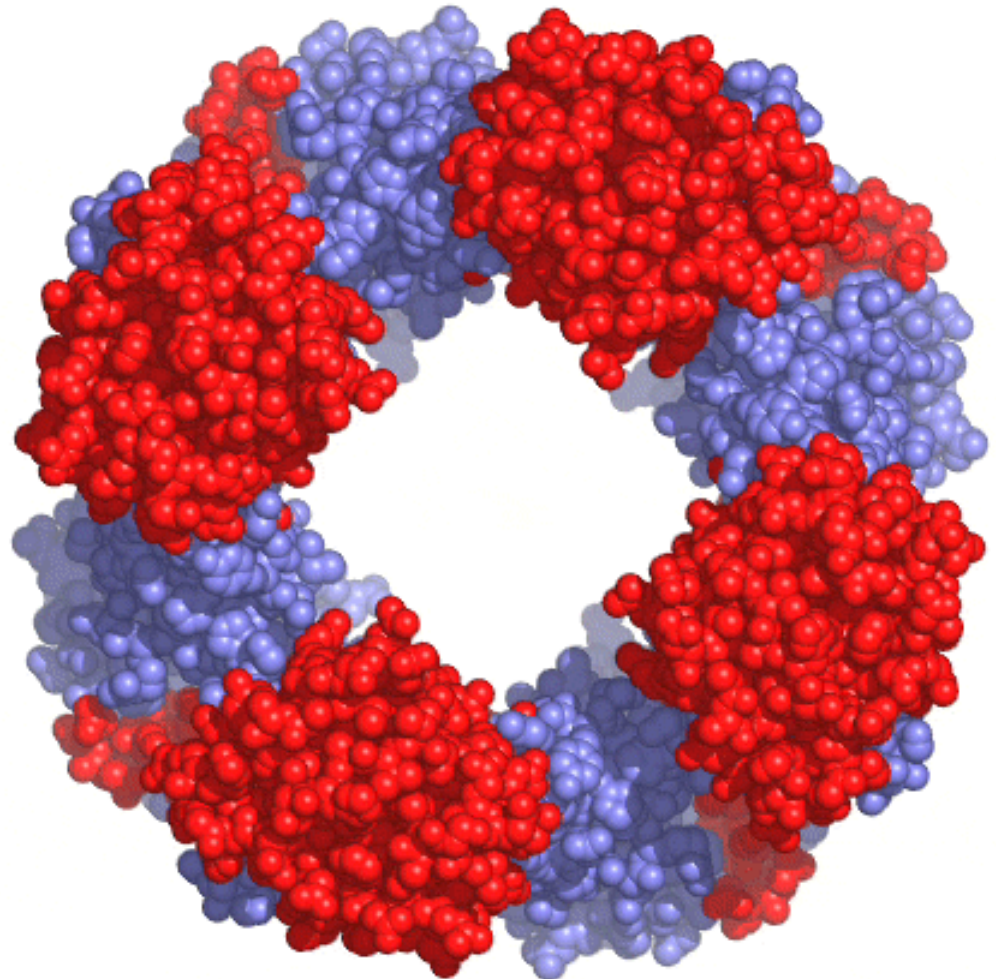
<sup>3</sup>Physics Department

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\*Contact: joao.cabral@yale.edu

DOI 10.1016/j.cell.2006.08.028

Three states observed in crystals, with relative domain angles of 35°, 46° and 80°



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