

Protein Quaternary Structure

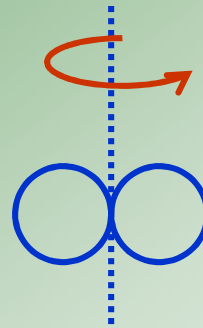
Protein Quaternary Structure

Non-covalent spatial arrangement of polypeptide subunits. This assembly takes place through highly **SPECIFIC** interactions.


Quite common for MWs > 100 kDa


Degrees of Complexity:

1. Dimer of identical subunits:



Two-fold symmetric axis

 = repeating subunit: “protomer”

 = “oligomer”

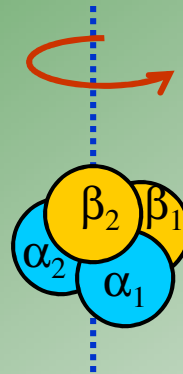
Examples:

Prealbumin

Alcohol Dehydrogenase

Protein Quaternary Structure

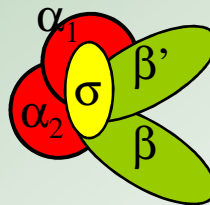
2. Molecules made up of 1 or 2 copies of several subunits



Hemoglobin

Can think of 2 $\alpha\beta$ protomers

Each protomer made up of 2 different polypeptide chains: one α and one β . Hb: dimer of $\alpha\beta$ protomers



E. Coli RNA polymerase

MW = 480,000

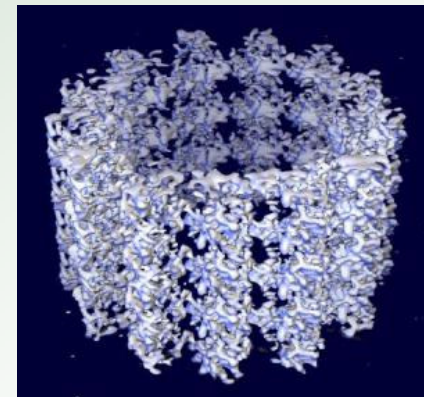
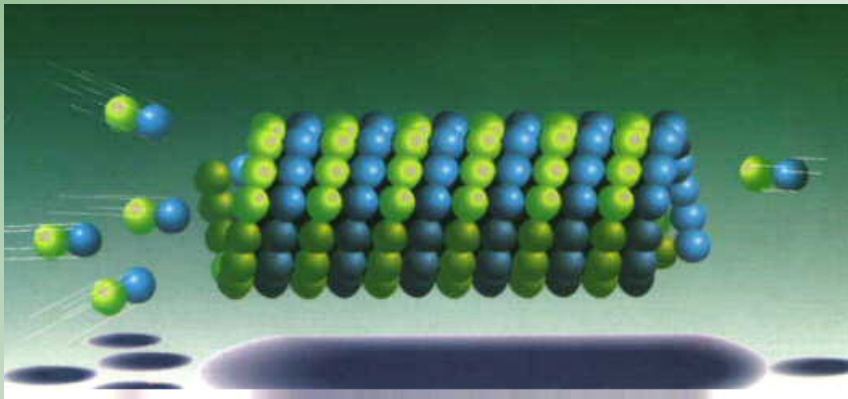
Protein Quaternary Structure

3. Multi-subunit with fixed total size and stoichiometry

Pyruvate Dehydrogenase (E. coli):



4. Multi-subunit with fixed stoichiometry, but varying size



Protein Quaternary Structure

Advantages of 4^o structure:

Enzymes

- Specificity
- Accuracy (selectivity of function)
- Catalytic Efficiency: improve with protein size

A multi-subunit design:

1. Defects are **local**, not global: easier to repair
2. Site of synthesis is not the same as site of assembly
3. Avoid increased complexity of folding huge polypeptides
4. Each chain can have a catalytic center: multiple sites
5. Provides a structure basis for **separation of function** (regulation)
6. Easier to attain by evolution (**modular design**)

Protein Quaternary Structure

Symmetry Considerations:

Most oligomeric protein protomers are arranged symmetrically (see table 2-8). Notice that number of copies of identical subunits appear in multiples of 2 or 3. This is a consequence of the **ways solid objects can be packed symmetrically in 3D space.**

Each polypeptide chain has these properties:

- Asymmetric
- α -carbon are asymmetric
- No mirror-reflection symmetry
- No inversion symmetry

Quaternary structures can only have rotational symmetry

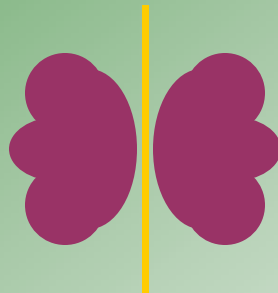
Protein Quaternary Structure

Structures with Cyclic Symmetry:

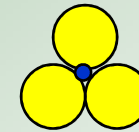
- These are structures with a single rotation axis (C_n axis)
- Structures repeat after $(360/n)^\circ$ rotation through the axis

• C_2 symmetry:

hemoglobin and pre-albumin



• C_3 symmetry:

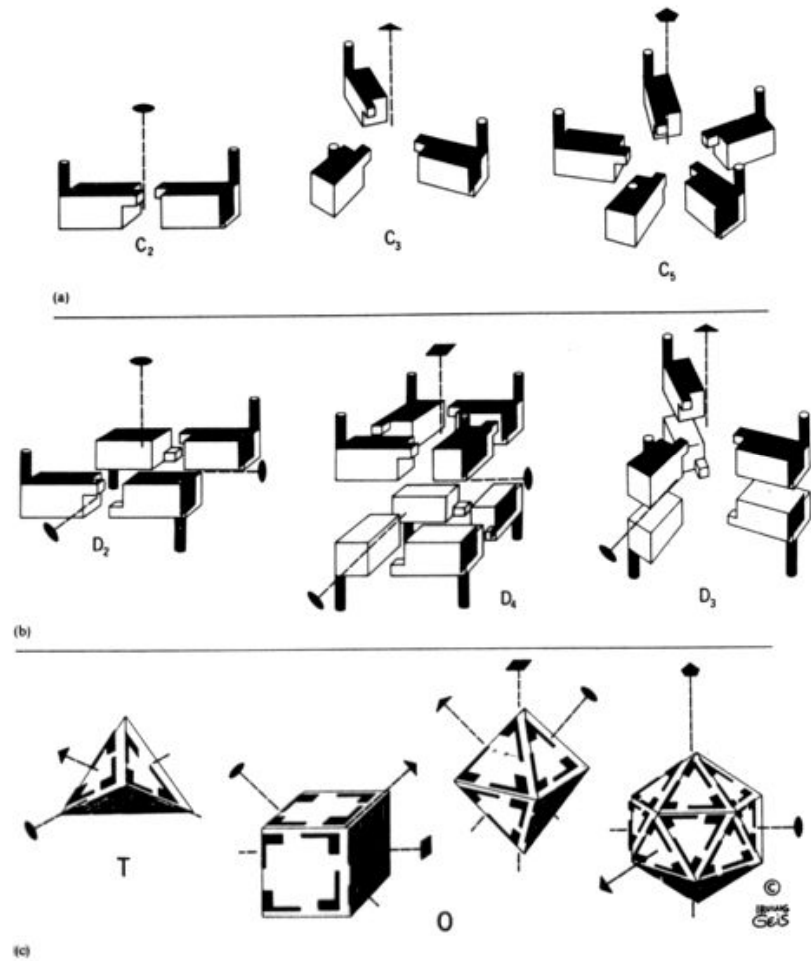


Dihedral Symmetry:

- Structures with one or more C_2 -axes perpendicular to another C_n -axis $\implies D_n$
- A D_n symmetric molecule needs $2n$ protomers
- D_2 is the most common: Concanavalin A; D_3 : insulin

Subunit composition of proteins with more than one subunit

Protein	Source	Molecular weight of protein	Number of subunits	Molecular weight of subunits
Nerve growth factor	Mouse	26,518	2	13,259
Luteinizing hormone	Sheep	27,322	1	12,500
			1	14,830
Chymotrypsin inhibitor	Potato	39,000	4	9,800
Superoxide dismutase	<i>E. coli</i>	39,500	2	21,600
Hemerythrin	<i>Phascolosoma</i>	40,600	3	12,700
Galactokinase	Human	53,000	2	27,000
Hemoglobin	Mammals	64,500	2	16,000
			2	16,000
Tu·Ts complex	<i>E. coli</i>	65,000	1	41,500
			1	28,500
Malate dehydrogenase	Rat	66,300	2	37,500
Avidin	Chicken	68,300	4	18,000
Troponin	Rabbit	80,000	1	37,000
			1	24,000
			1	20,000
Alkaline phosphatase	<i>E. coli</i>	86,000	2	43,000
Procarboxypeptidase A	Bovidae	88,000	1	40,000
			2	23,000
Seryl tRNA synthetase	<i>E. coli</i>	100,000	2	50,000
Nucleoside diphosphokinase	Yeast	102,000	6	17,000
Tubulin	Pig	110,000	1	56,000
			1	53,000
Lactate dehydrogenase	Pig	140,000	4	35,000
Tryptophan synthetase	<i>E. coli</i>	148,000	2	45,000
			2	28,700
<i>lac</i> Repressor	<i>E. coli</i>	160,000	4	40,000
Methionine tRNA synthetase	<i>E. coli</i>	170,000	2	85,000
Q β replicase	<i>E. coli</i>	205,000	1	70,000
			1	65,000
			1	45,000
			1	35,000
Arylamidase	Human	223,500	6	38,100
Leucine aminopeptidase	Swine	255,000	4	63,500
Isocitrate dehydrogenase	Yeast	300,000	8	39,000
Aspartate transcarbamoylase	<i>E. coli</i>	310,000	6	33,000
			6	17,000
Nitrogenase	<i>Clostridium</i>	330,000	2	59,500
			4	27,500
			2	50,700
Enolase	<i>T. aquaticus</i>	355,000	8	44,000
Glutamine synthetase	<i>Neurospora</i>	360,000	4	90,000
RNA polymerase core	<i>E. coli</i>	400,000	2	39,000
			1	155,000
			1	165,000
Apo ferritin	Horse	443,000	24	18,500
Glutamine synthetase	<i>E. coli</i>	592,000	12	48,500
Ovomacroglobulin	Chicken	650,000	2	325,000
Isocitrate dehydrogenase	Bovidae	670,000	16	41,000
Hemoglobin	<i>Arenicola</i>	2,850,000	48	54,000
Pyruvate dehydrogenase complex	<i>E. coli</i>	5,000,000	24	91,000
			24	65,000
			24	56,000



SOURCE: Condensed from D. W. Darnall and I. M. Klotz, *Arch. Biochem. Biophys.* 166:651 (1975).

Protein Quaternary Structure

Higher Order or Cubic Symmetry:

Tetrahedral (T):

- Needs at least 12 identical monomers
- If not, there can be no $C_3 \rightarrow D_2$

Octahedral or Cube (O):

- Needs 24 identical monomers or no $C_4 \rightarrow D_3$

Icosahedral (I):

- Needs at least 60 identical monomers
- Example: viruses

There are few examples of other types of symmetry arrangements:

Hexokinase from yeast has a screw-axis symmetry

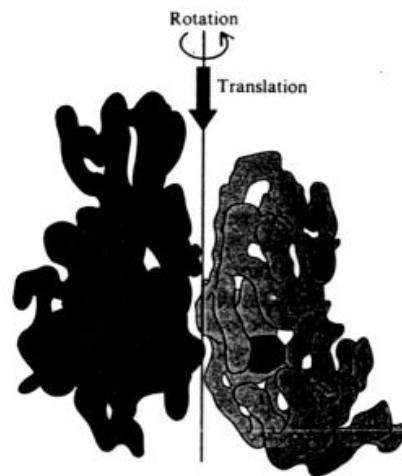


Figure 2-46

Structure of yeast hexokinase at low resolution, showing two subunits related by a screw axis. The polyhedron in the right-hand subunit represents a bound glucose. [After a drawing provided by Thomas Steitz.]

Table 2-9
Nature of subunit interfaces

Protein	Symmetry	Regions in contact	Van der Waals contacts	Hydrogen bonds	Ion pairs
α -Chymotrypsin	C_2	A	443	9	1
		B	57	6	—
Concanavalin A	D_2	to A	—	2	—
		to B	142	14	6
		to C	174	14	—
Hemoglobin Oxyhemoglobin	C_2	$\alpha_1\beta_1$	110	5	—
		$\alpha_1\beta_2$	80	1	—
		$\alpha_1\alpha_2$	—	—	—
		$\beta_1\beta_2$	—	—	—
Deoxyhemoglobin	C_2	$\alpha_1\beta_1$	98	5	—
		$\alpha_1\beta_2$	69	1	1
		$\alpha_1\alpha_2$	—	—	2
		$\beta_1\beta_2$	—	—	1
Insulin	D_3	OP	111	8	—
		OQ	99	2	1

SOURCE: Adapted from A. Liljas and M. Rossman, *Ann. Rev. Biochem.* 43:485 (1974).

Protein Quaternary Structure

Symmetry of Contact Interactions:

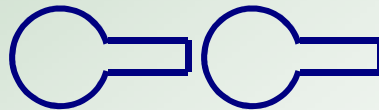
1. Symmetric contact:

- Two identical contacts
- C_2 -symmetry between subunits



2. Asymmetric Contacts:

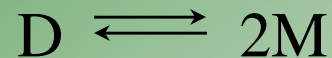
- Two different contacts
- Each subunit retains a surface for one more contact
- Can generate lines or helices or higher C_n s



Protein Quaternary Structure

What contacts stabilizes the quaternary structure? (Table 2-9)

Van der Waals non-bonded contacts predominate



$$K_{\text{diss}} = \frac{(M)^2}{D} \approx 10^{-8} - 10^{-16} \text{ Moles l}^{-1}$$

ΔG° for association = - 10 to -20 Kcal mol⁻¹ at 25°C

Association: loss of 3 translational degrees of freedom, as well as the loss of 3 rotational degrees of freedom

$$\Delta S = -70 \text{ to } -100 \frac{\text{cal}}{\text{mol K}} \quad \Rightarrow \quad -T\Delta S = 20 \text{ to } 30 \frac{\text{Kcal}}{\text{mol}}$$

Requires good steric fitting between the surfaces in contact: packing density calculations show that residues in these surfaces have densities as high as in aa crystals

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Stability seems to come from burying various hydrophobic groups present in these contact surfaces.

Estimation: Area of contact = 1000 – 2000 Å²

$$\Delta H^\circ = -10 \text{ cal mol}^{-1} \text{ \AA}^{-2}$$

$$\Delta H^\circ = -25 \text{ Kcal mol}^{-1} \text{ to } -50 \text{ Kcal mol}^{-1}$$

So its enough to take into account “hydrophobia” to account for the stability observed in quaternary interactions.

Additionally...

Protein Quaternary Structure

- β -sheet continuation at interface:

- Concanavalin A
- Insulin
- Alcohol dehydrogenase
- Pre-albumin

} Several H-bonds across boundary

- β -sheet stacking
- H-bond contribution
- Polar or salt bridges
- Inter-subunit S – S bonds

Other smaller contributors are H-bonding, and electrostatic bridges.