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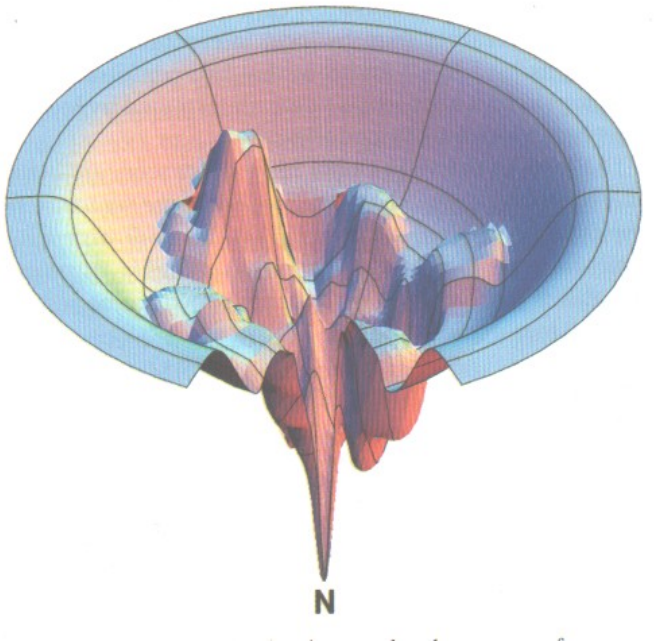
Chapter 9: Protein Folding, Dynamics and Structural Evolution

Voet & Voet: Pages 276-299



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Folding Accessory Proteins

In vitro

Not all protein refold efficiently

In vivo

Virtually all proteins fold efficiently

Why ?

All cells contain three types of folding accessory proteins that improve the efficiency of protein folding

(1) protein disulfide isomerase (PDI)

- alters disulfide bonding pattern

(2) peptidyl prolyl isomerase (PPI)

- catalyzes *trans*- to *cis*- isomerization of peptide bonds preceding proline residues

(3) molecular chaperones (Hsp70s, chaperonins, Hsp90s)

- minimize misfolding

Folding Accessory Proteins: (1) Protein Disulfide Isomerase (PDI)

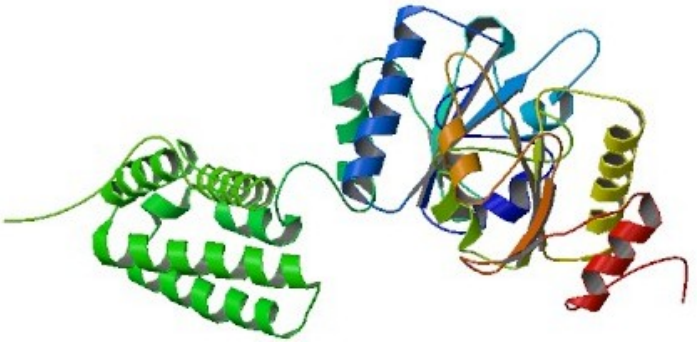
Catalyzes disulfide interchange reactions

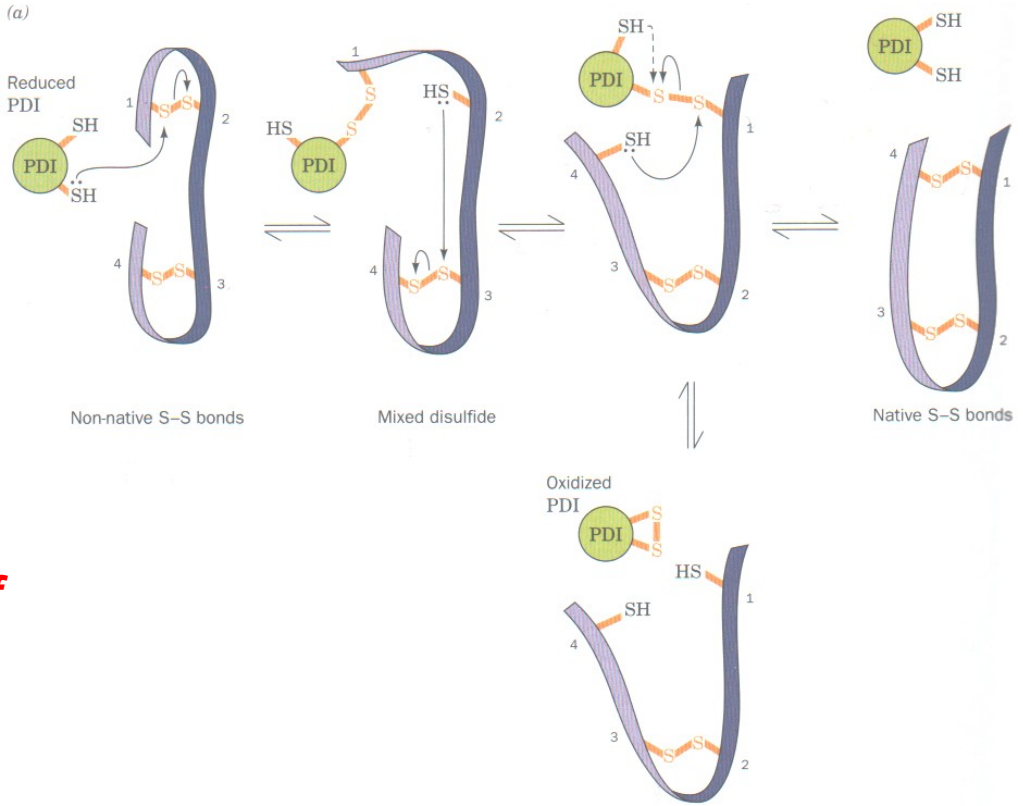
Correct disulfide bonds are typically resistant to PDI

- **Tend to be buried in hydrophobic core (inaccessible)**

Incorrect disulfide bonds are the preferred substrate

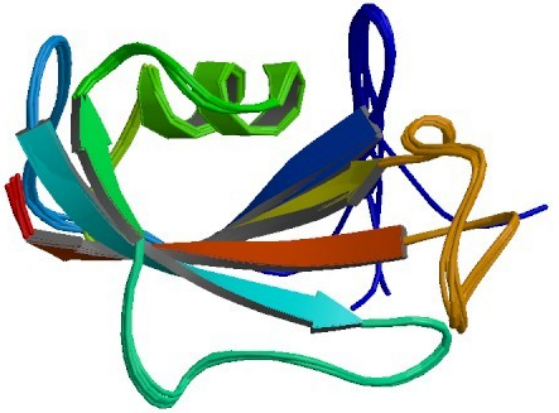
- **Tend to be exposed on the protein surface**





PDI assists folding of proteins that remain denatured in the absence of correct disulfide bond formation

Folding Accessory Proteins:
(2) Peptidyl Prolyl Isomerase (PPI)



PPIs catalyzes *trans* to *cis* conformational changes in selected Xaa-Pro peptide bonds
(Xaa = any amino acid)

All peptide bonds are *trans* in newly translated proteins

Speeds up 'slow' *trans* to *cis* isomerization

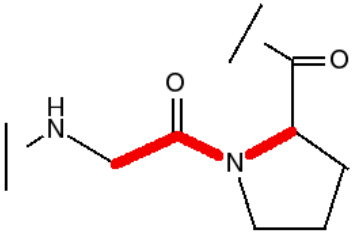
PPI active site is specific (more complementary) for *cis* proline peptide bonds

Indicates interconversion will be *trans* to *cis*

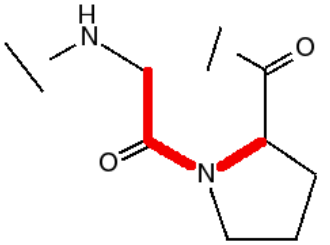
***trans* peptide bonds bind and are distorted into a *cis* like conformation**

Two unrelated families (primary sequence) of PPIs that share catalytic properties

trans



cis



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Folding Accessory Proteins: (3) Molecular Chaperones

Proteins (folded or unfolded) have a tendency to form aggregates (intra- or intermolecular)

Newly synthesized (and unfolded) proteins have:

(1) solvent exposed hydrophobic patches and

(2) must fold in the presence of extremely high protein concentrations (up to 300 mg/mL)

Molecular Chaperones prevent or reverse improper associations during folding

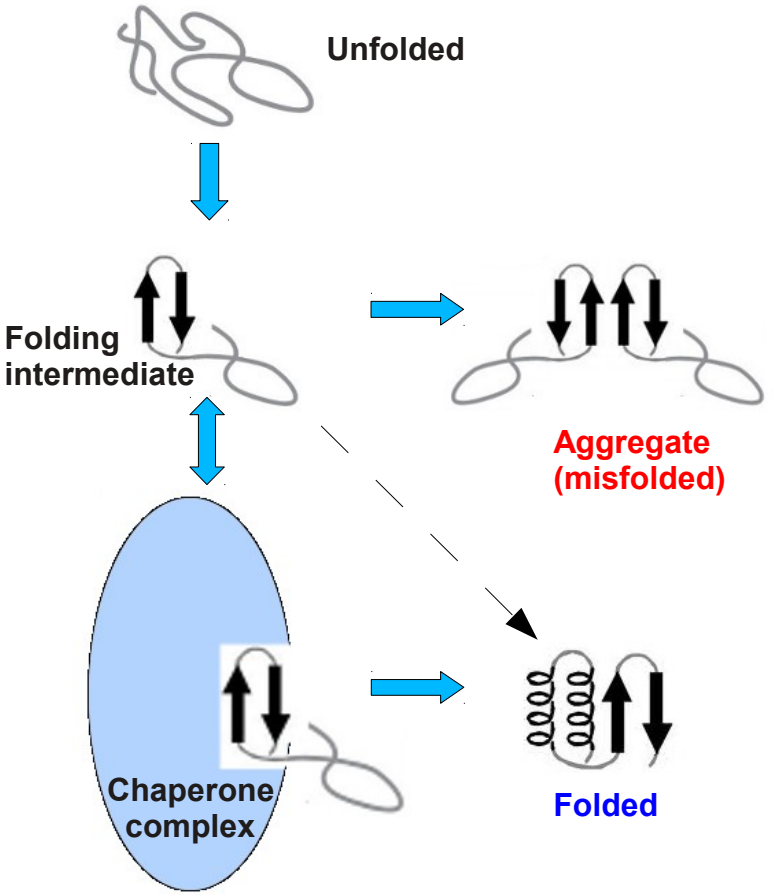
Particularly in multisubunit and multidomain proteins (ie. large proteins)

Folding Accessory Proteins:
(3) Molecular Chaperones (cont.)

Chaperones bind solvent exposed, hydrophobic regions of improperly folding proteins

Binding followed by release improves folding efficiency

Binding & release cycle can be repeated many times



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Chaperones

Unrelated classes of chaperones

(1) Heat shock proteins (Hsp70)

- ATP requiring enzymes that bind denatured and misfolded substrates and utilize the energy of ATP hydrolysis to reverse the folded state of these aggregates
- Unfold proteins prior to translocation across plasma membrane

(2) Chaperonins

- Large, cage like structures that bind improperly folded globular proteins
- Utilize ATP to induce proper folding within a protected, internal cavity

(3) Heat shock proteins (Hsp90)

- Involved in folding of signal transduction proteins

(4) Nucleoplasmins

- Involved in folding of nucleosomes within nucleous

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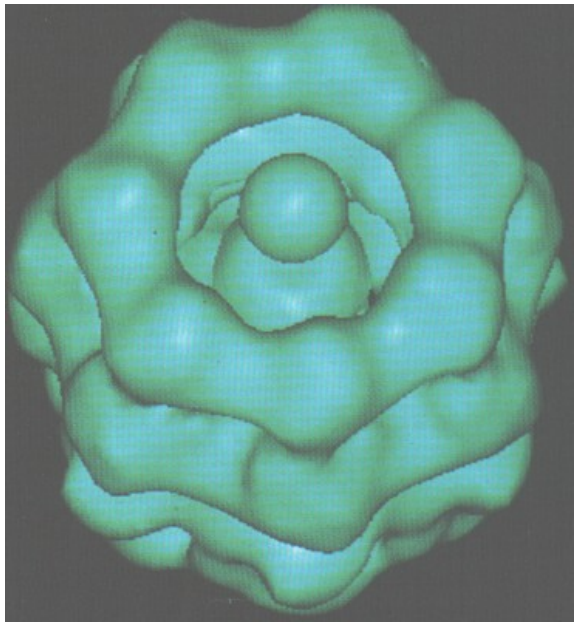
Chaperonin: GroEL/ES

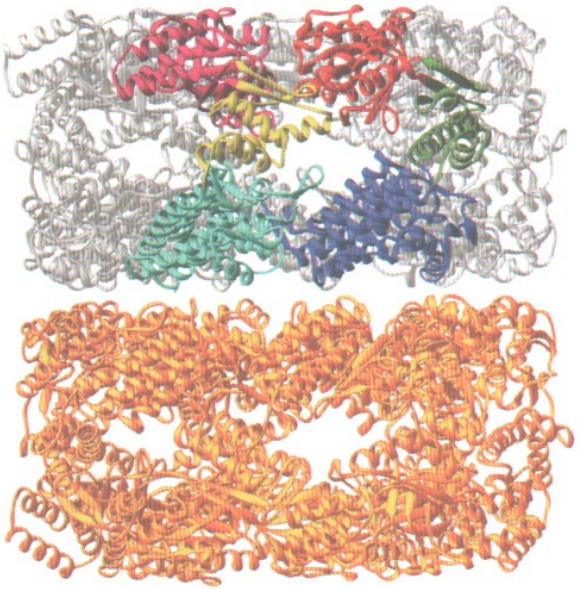
Chaperonin function requires two proteins that work in concert (transiently associate)

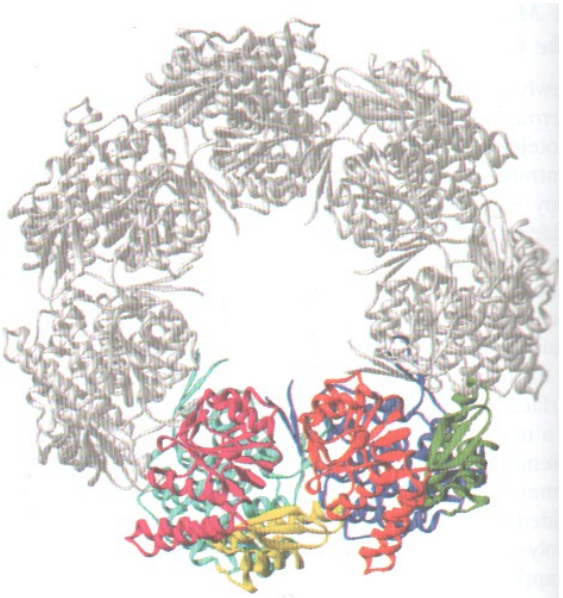
(1) GroEL – A multisubunit structure composed of 14 protomers (60 kDa each) that form a pair of 7 subunit rings

(2) GroES – A multisubunit structure composed of 7 protomer (10 kDa each) that form a single ring

Human Hsp60 / Hsp 10 are homologs of GrolEL / ES







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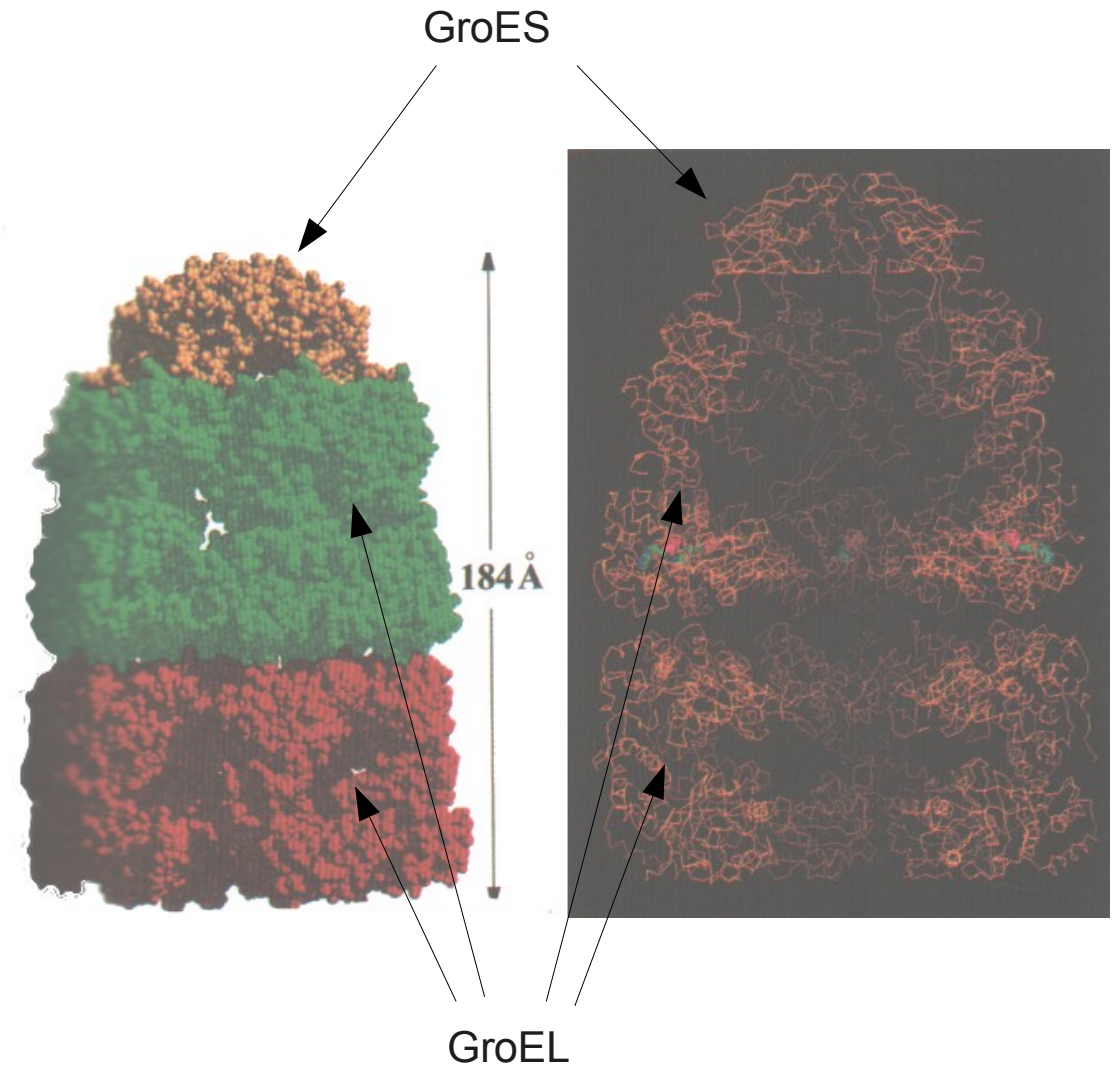
Chaperonin: GroEL/ES

Cage-like structure defines a 45 Å diameter central channel (1 per GroEL heptamer)

- **Cavity is blocked in the center and does not form a tunnel between GroEL heptamers**
- **Misfolded proteins bind to the entrance of the central channel**
- **GroES binds to the GroEL:misfolded protein complex**
- **GroES binding 'traps' misfolded protein in the central channel**



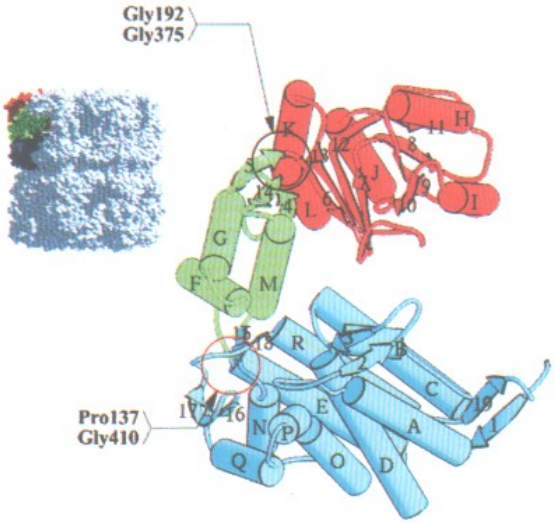
GroEL/ES

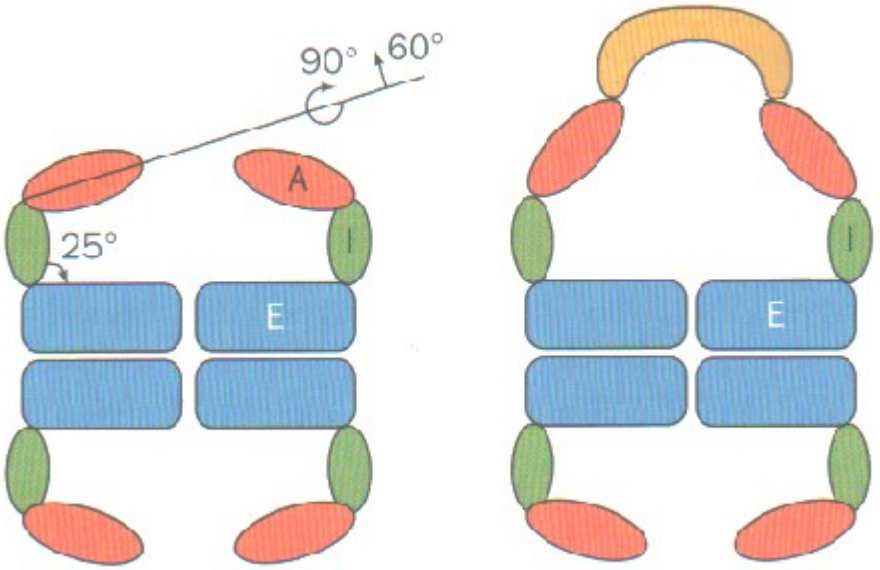
Misfolded proteins bind to the apical (A or red) domain

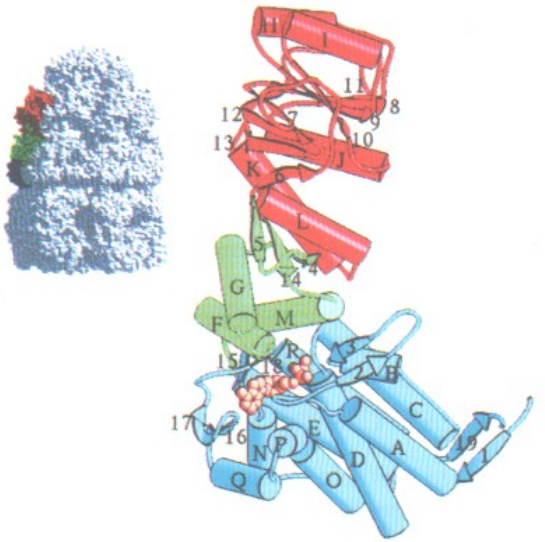
- **Binding induces conformational change in GroEL**

GroEL (or GroES) mutants that fail to bind misfolded proteins map near the tip of the apical (A or red) domain

- **implies misfolded proteins bind to the tip of the apical domain near the GroES heptamer**







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GroEL/ES

GroEL/ES only forms in the presence of ATP and misfolded proteins

- ATPase activity (GroEL) is stimulated by the conformational change that

  (1) results from binding misfolded protein (and ATP)

  (2) and facilitates GroES binding

- Conformational change is concerted and requires all subunit to simultaneously change conformation

  - Functional groups required for ATP hydrolysis (*eg* Asp398) move into the vicinity of the ATP in the GroEL/ES complex

Misfolded proteins bind primarily to the apical domain on the inside of the cavity (based upon mutagenesis studies)

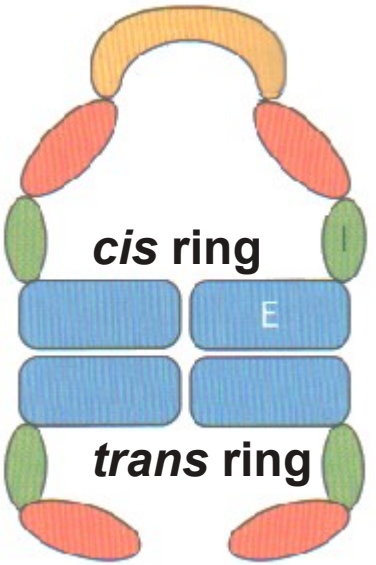
- ATP hydrolysis changes the conformation and accessibility of the apical domain and facilitates the rearrangement of the misfolded protein

  - Recent studies suggest "cavity" becomes more hydrophilic following ATP hydrolysis and initiates folding

GroEL/ES – second active site

Only one of the two GroEL rings is active (contains misfolded protein) at a given time

- Active sites from each ring alternate roles
  - Active sites of one ring are active, then from the second ring, then from the first ring ....
  - Active sites (within and between rings) communicate with one another through conformational rearrangements



Unused ring has an important role releasing "refolded" protein

- Binding of ATP to the unused (trans) ring releases GroES, ADP and the correctly folded protein
- ATP and misfolded protein binding to the *trans* ring requires hydrolysis of the ATP bound to the *cis* ring

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GroEL/ES – function

GroEL/ES catalysis is cyclic

(0) GroEL binds 7 ATP and a misfolded protein (top left).

(1) GroES then binds resulting in an enlarged cavity encapsulating misfolded protein (top right)

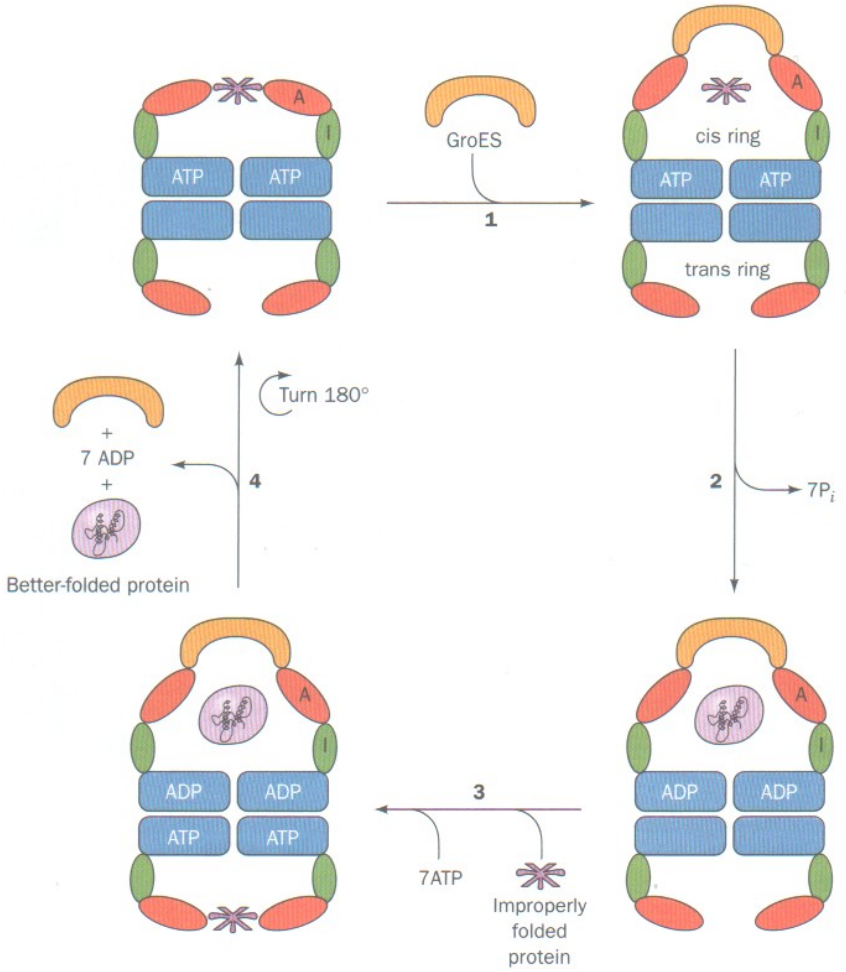
(2) Within 15 s, all ATP (*cis* ring) are hydrolyzed to ADP commencing refolding (bottom right)

(3) Second misfolded protein and 7 ATP bind to the GroEL *trans* ring (bottom left)

(4) GroES, ADP and the folded protein are released from the *cis* ring

(...) repeat cycle ...

Recall: the *cis* and *trans* ring alternate being active and inactive



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How is folding improved?

Two models (non exclusive) have been proposed to explain improved refolding

(1) **Anfinsen Cage Model** – Refolding occurs in a protected microenvironment that shields folding intermediates from nonspecific aggregation with other misfolded proteins (limits intermolecular aggregation)

(2) **Iterative Annealing Model** – Refolding is a second chance to fold after a stably misfolded protein is unfolded.

- Conformational change due to ATP hydrolysis stretches or otherwise alters interactions in the misfolded intermediate and allows a second chance at refolding

Regardless of the model that allows for refolding, chaperonin assisted folding is substantially faster than free in solution

- GroEL interacts strongly with over 300 *E. coli* proteins

  (these protein require GroEL/ES to efficiently fold)