

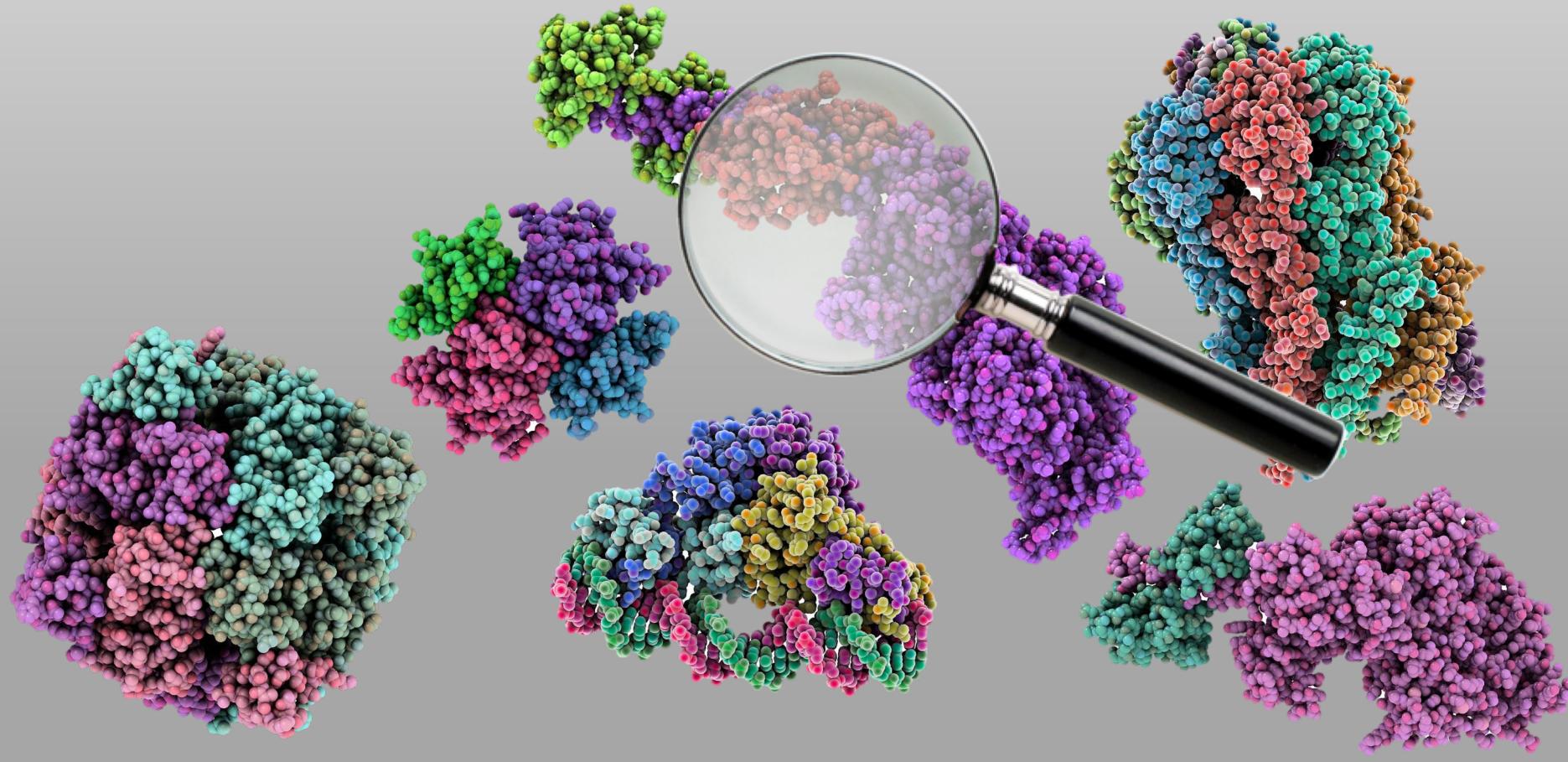
Molecular and Structural Biology 1 (Fall Semester 2019)

# Large Cellular Complexes in Protein Quality Control: Molecular Chaperones

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# Lecture Schedule

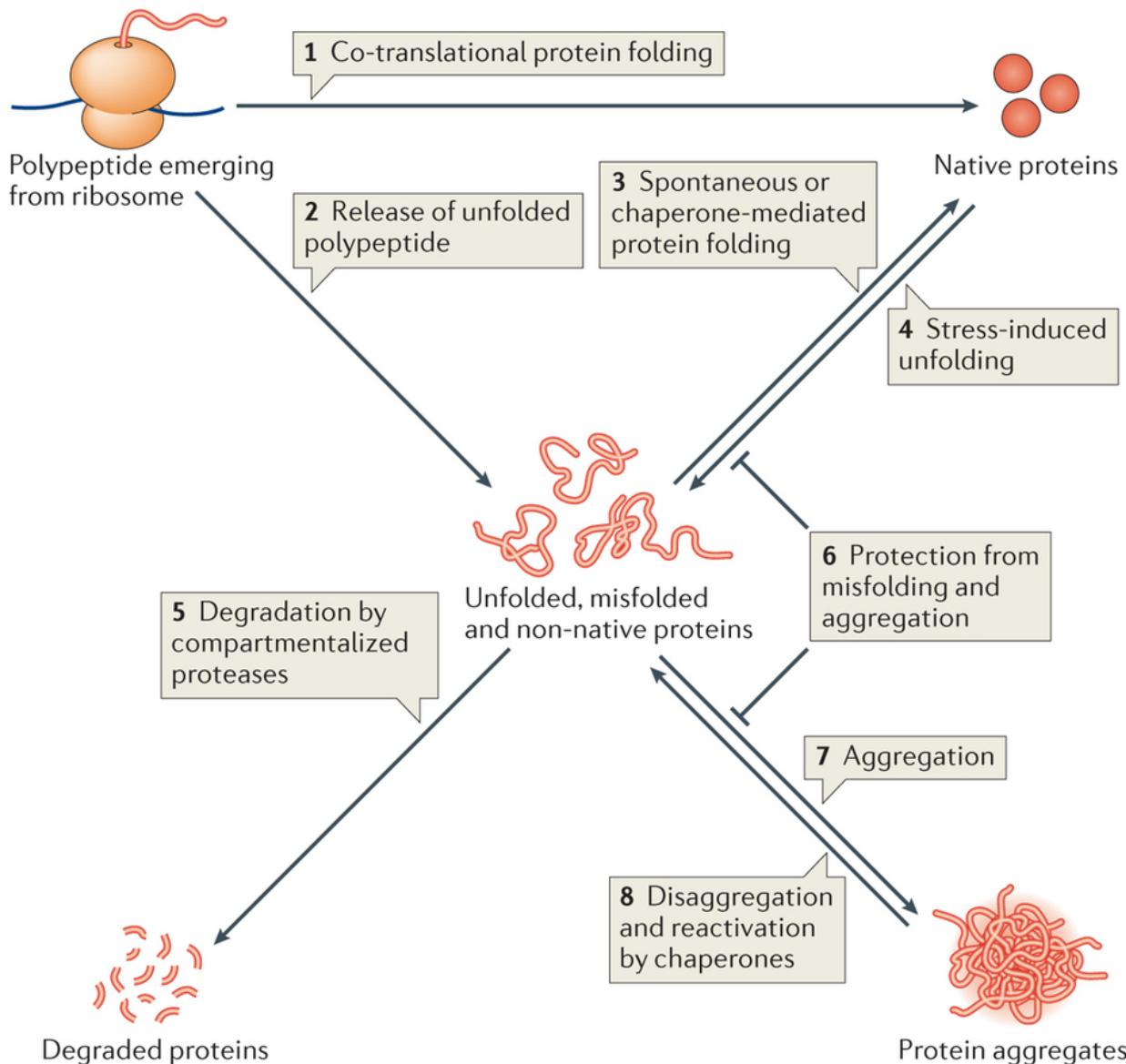
23 September 2018	R. Glockshuber
30 September 2018	R. Glockshuber
07 October 2018	R. Glockshuber
14 October 2018	R. Glockshuber
21 October 2018	R. Glockshuber
28 October 2018	E. Weber-Ban
04 November 2018	E. Weber-Ban
11 November 2018	E. Weber-Ban
18 November 2018	K. Locher
25 November 2018	K. Locher
02 December 2018	K. Locher
09 December 2018	K. Locher
16 December 2018	K. Locher

Protein folding: Thermodynamics and kinetics  
Basic physical principles, spectroscopic techniques,  
exercises with specific examples

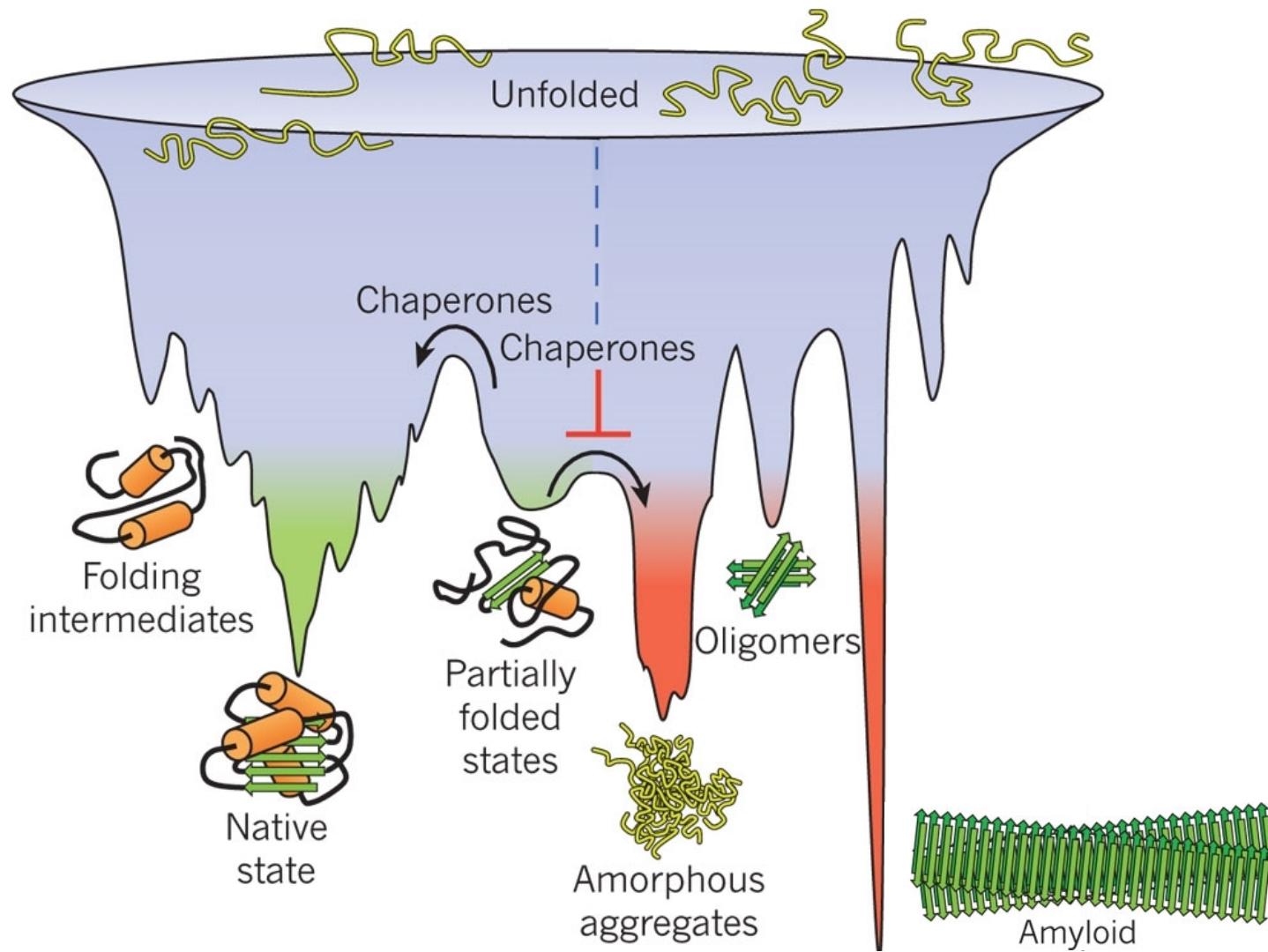
## **Large Cellular Complexes in Protein Quality Control: Molecular chaperones & Protein degradation**

Membrane channels and transporters

# Protein Quality Control



# Molecular Chaperones



# Molecular Chaperones - What is in the name?

The American Heritage Dictionary of the English Language

**Chaperone:** *A guide or companion whose purpose is to ensure propriety or restrict activity.*

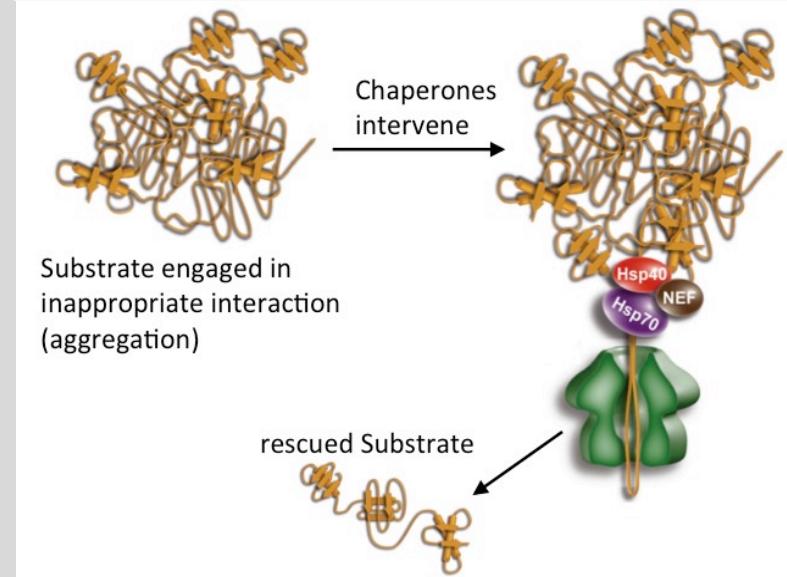
old French “chaperon = hood, head covering”.



## Molecular Chaperones

In the molecular world there are certain proteins that ensure the propriety in the interaction of macromolecules with other macromolecules:

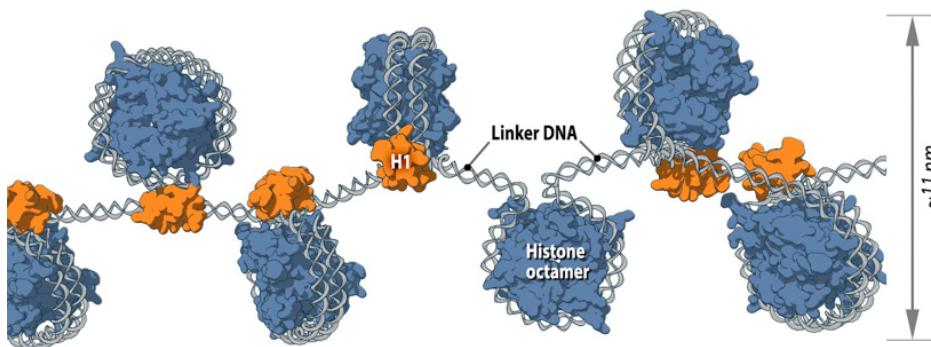
These **molecular chaperones** make certain that their substrates **don't interact with unwanted (inappropriate) partners** which would corrupt their destined role inside the cell.



# Chaperone: history of the terminology

The term “molecular chaperone” was first used in 1978 by Ron Laskey to describe the function of the nuclear protein nucleoplasmin: it assists in the formation of nucleosomes by preventing aggregation of histones with DNA

A typical mammalian nucleus contains ~2 m of DNA that is packaged with histones to form chromatin. The fundamental building blocks of chromatin are nucleosomes.



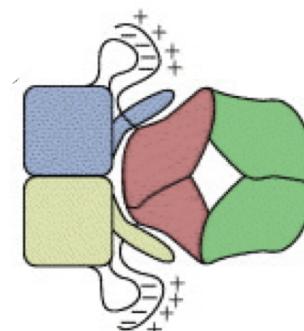
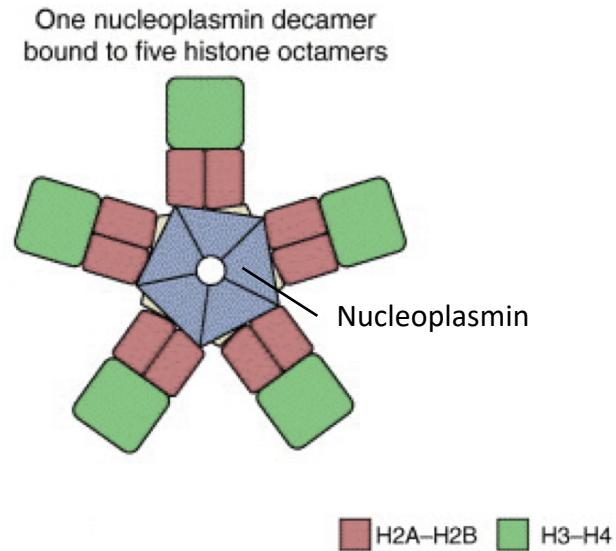
Nucleosome core particle:  
~147 bp DNA  
histone octamer (made of the four core histones)

When negatively charged DNA and positively charged histones are mixed in solution, irreversible precipitation occurs.  
Nuclear chaperones prevent this aggregation and help the nucleosome assembly.

The nucleus of frog egg cells contains enough histones to assemble chromatin in 10'000 cells. Nucleoplasmin is the most abundant protein in the frog egg cell nucleus.

Nucleoplasmin: nuclear chaperone specialized for egg cells

**nucleoplasmin decamer bound to five histone octamers:**



Ellis, *TiBS* (2006) Vol 31, 395.  
Dutta et al, *Molecular Cell* (2001) Vol 8, 841.

# Molecular Chaperones

Definition:

Molecular chaperones are proteins that assist other macromolecules in folding/unfolding and in assembly/disassembly of higher order structures without being components of these final structures.

For a protein to be called a molecular chaperone, two criteria have to be met:

1. It must assist the noncovalent assembly/disassembly of another protein-containing structure. The mechanism by which it does this is irrelevant.
2. It must not be a component of these structures when they carry out their biological function in the cell.

Number of protein families regarded as “chaperones” > 20.

# Families of Chaperones

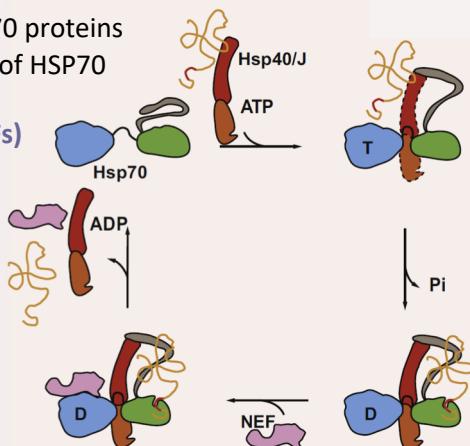
## HSP70, HSP40, (GrpE-like proteins) - Chaperone System

### HSP70

highly conserved, found in all species examined  
 many species have multiple types in various compartments  
 action: bind short stretches of extended polypeptide  
 function: prevention of aggregation of unfolded proteins  
     disassembly of multimeric complexes  
 role in: protein trafficking, protein folding, heat shock  
 example: DnaK in *E. Coli*

### HSP40

cochaperones that regulate HSP70 proteins  
 action: stimulate ATPase activity of HSP70  
 example: DnaJ in *E. coli*

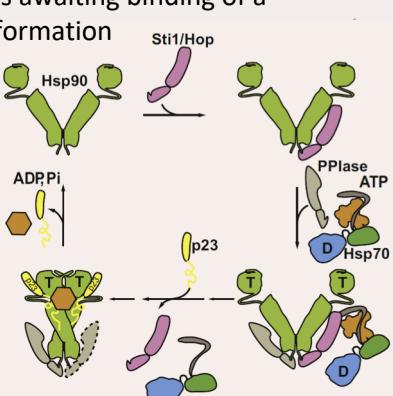


### Nucleotide exchange factors (NEFs)

cochaperones for HSP70  
 action: ADP/ATP exchange  
 example: GrpE in *E. Coli*

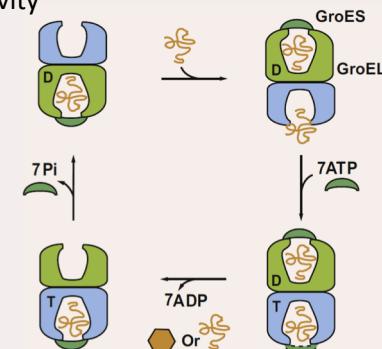
### HSP90

highly conserved  
 found in pro- and eukaryotic cytosol and ER  
 involved in regulating signal transduction pathways by assisting conformational changes in kinases and steroid receptors  
 act on near-native proteins awaiting binding of a ligand to reach active conformation  
 example: human Hsp90



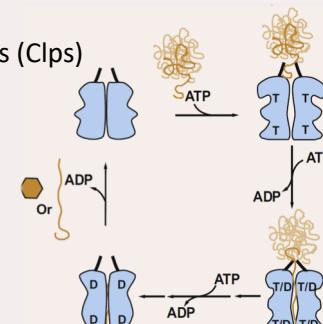
## Chaperonins or HSP60/10-Chaperone System

found in all prokaryotes and in mitochondria and chloroplasts  
 assist folding of many proteins in ATP-dependent manner  
 (prokaryotes: newly synthesized; mitochondria: newly imported)  
 recognize hydrophobic surfaces of globular, non-native conformations  
 barrel-like structure, bind substrates in their cavity  
 substrates fold in sequestered space  
 CPN60/10 constitutively expressed, further induced by stress  
 example: GroEL/ES in *E. Coli*



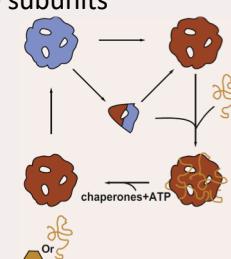
## HSP100 Proteins

ring-shaped complexes with nucleotide binding domains  
 found in pro- and eukaryotic cytosol, mitochondria, chloroplasts  
 act on substrates by influencing their conformation  
 two major roles:  
 1. Unfoldase component of barrel-shaped proteases (Clps)  
 2. Disassembly of protein aggregates (Hsp104)  
 examples: yeast Hsp104, *E. coli* ClpA



## Small HSPs

low molecular mass (15-30 kDa)  
 diverse group with only short sequence motifs conserved  
 quaternary structure: oligomers ranging from 9-50 subunits  
 mask hydrophobic patches on substrate proteins  
 example: mammalian α-crystallin



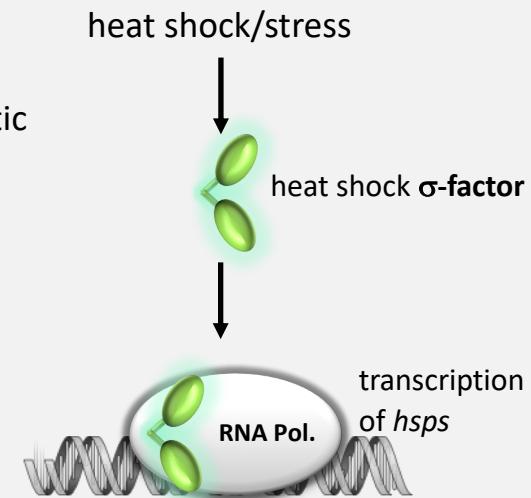
# The Heat Shock Response

In response to stress (heat, oxidizing conditions, toxic compounds) pro- and eukaryotic cells produce a set of well-conserved proteins called „heat shock proteins“ (Hsps). These proteins can protect cells against stress-induced damage.

## Heat shock response in *E. coli*.

Rapid increase in production of more than 20 Hsps (up to 20-fold).

After the first burst of production, the Hsp levels slowly decrease again to almost normal levels.



## Most heat shock proteins are molecular chaperones.

During stress, damaged proteins display partial loss of structure that could lead to aggregation or malfunction. Chaperones protect the damaged proteins by binding to their unraveled or misfolded parts thereby preventing them from interaction with each other or with other proteins in non-productive or damaging ways. They also assist their refolding to the fully native conformation or help degrade unrecoverable proteins.

## Hsps are present not only during stress.

Hsps were identified through their induction during heat stress, but many are also expressed constitutively and their activities are required under normal conditions.

## Hsp nomenclature.

Different Hsps are named according to their approximate molecular weight, for example Hsp70, Hsp100, Hsp90.

just in low level

# Role of Chaperones in Protein Folding

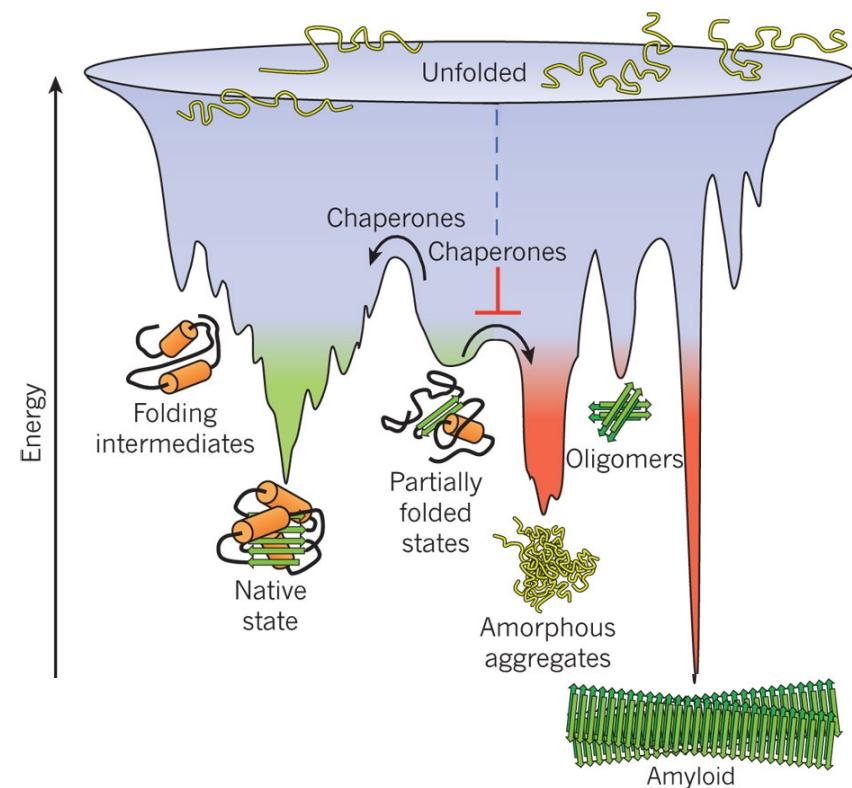
The most notorious role of chaperones is their **role as assistants to the protein folding process**.

They carry out this function by **binding hydrophobic surfaces exposed in non-native proteins**.

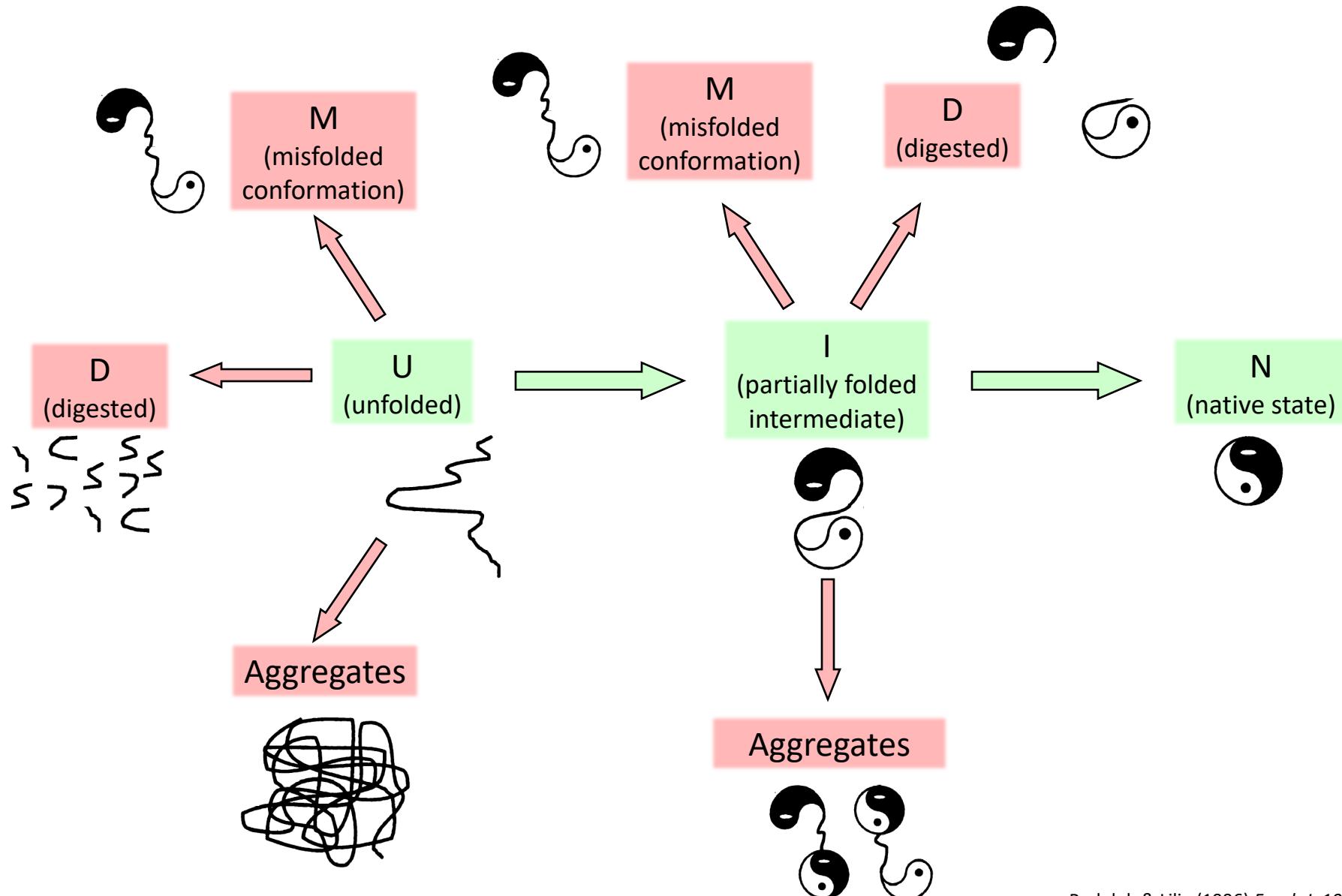
These surfaces will ultimately be buried in the interior of the native state and are exposed only while the protein still shows some non-native conformation.

Non-native states that expose hydrophobic patches occur in the cell:

1. During the process of **translation** the synthesized polypeptide chains emerge from the ribosome in an extended non-native conformation.
2. During the process of **translocation** across organelle membranes proteins have to be unfolded and then have to fold again in the new compartment.
3. After the synthesis of individual **components of multisubunit assemblies**, it can happen that the destined binding partner is not yet available and hydrophobic patches that are later buried in the protein/protein interface are still exposed.
4. When the cell is under **stress**, for example heat stress, native structures can get damaged. In addition, aggregation is a bigger problem at higher temperatures.



# Side Reactions Competing with Productive Protein Folding



# Protein Folding Inside the Cell

## Anfinsens's Dogma of Protein Folding

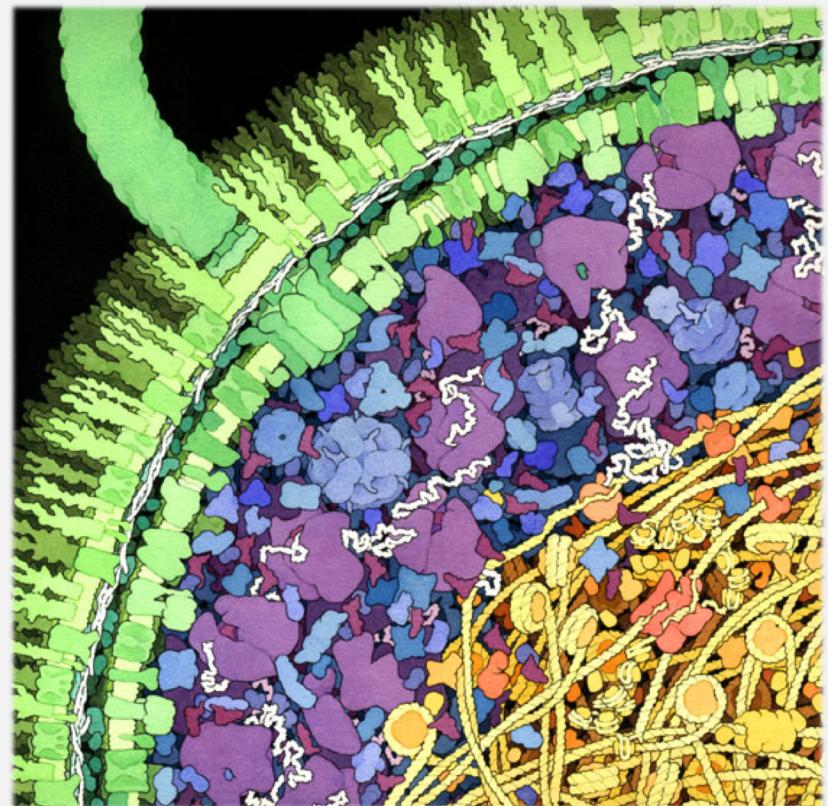
All the information necessary to direct the three-dimensional structure of a protein resides in its amino acid sequence.

Most denatured proteins refold spontaneously *in vitro*, but the situation inside the cell provides a greater challenge due to the high total concentration of macromolecules.

Folding under such conditions can become „inefficient“, that is, result in a larger fraction of misfolded or aggregated species.

Chaperones act to prevent or reverse these competing „side reactions“.

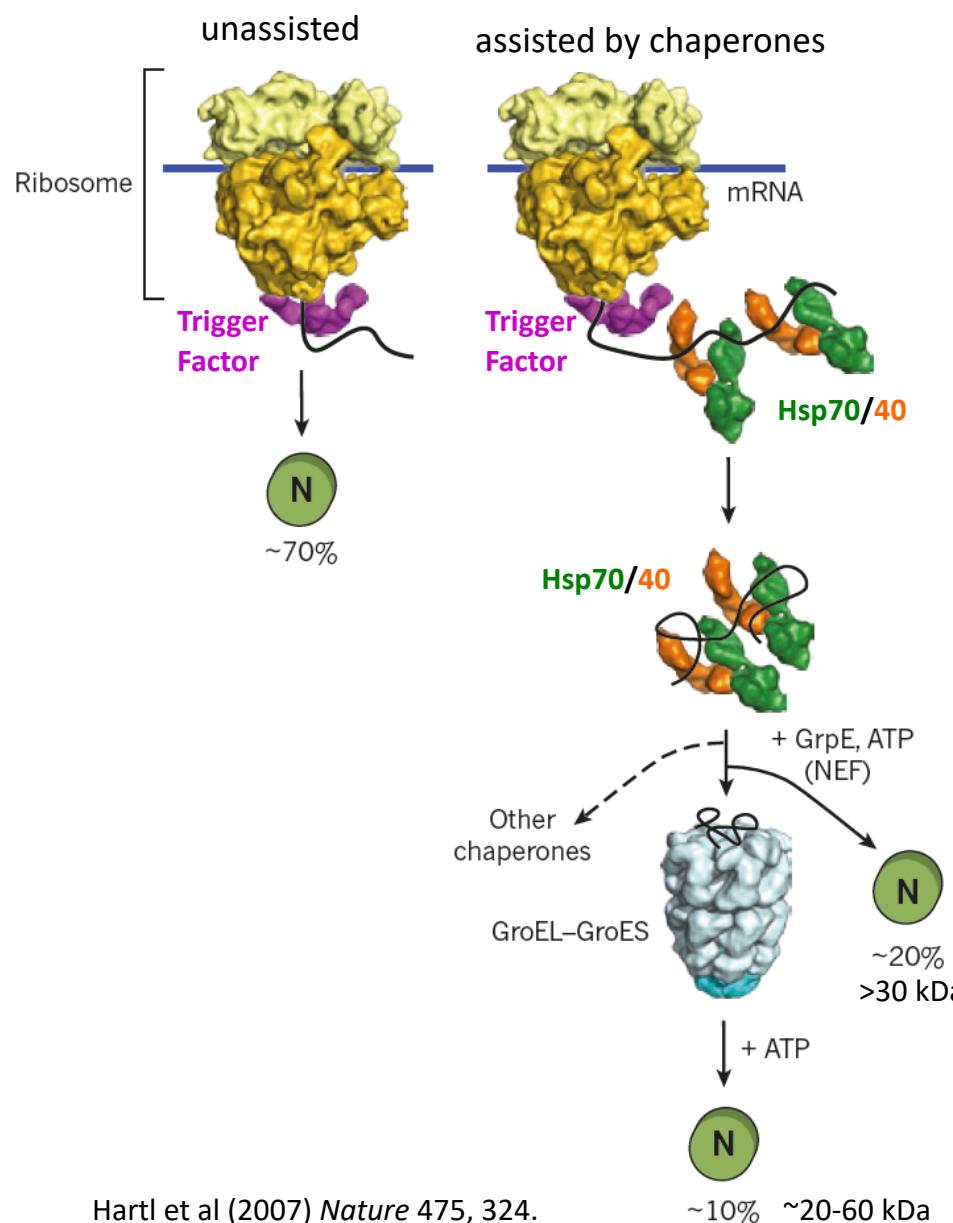
Chaperones do not provide steric information required for proteins to fold correctly, but either prevent or reverse aggregation and misfolding processes that would otherwise reduce the yield of functional molecules.



in *E. coli*: 300-400 mg/ml protein+RNA

The role of chaperones in protein folding does not contradict Anfinsens's Dogma.  
Anfinsens's Dogma still holds.

# Folding of Newly Synthesized Proteins in the Prokaryotic Cytosol



Chaperones that can interact with newly synthesized chains:

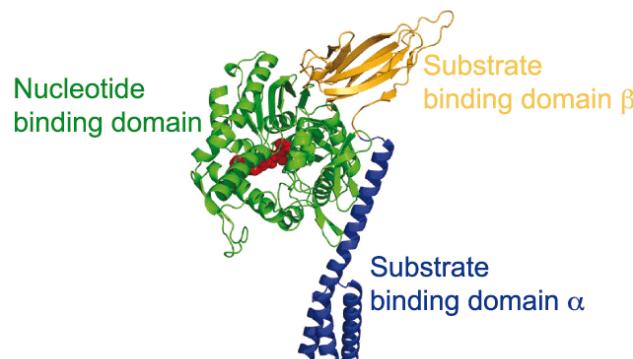
1. Chaperones bound at the exit site on the ribosome.  
example: trigger factor
2. Chaperones binding to nascent chain itself while it is attached to the ribosome: cotranslational interaction  
example: Hsp70/40/GrpE
3. Chaperones binding to polypeptide chain after release from the ribosome: posttranslational interaction  
examples: GroEL/ES and also Hsp70/40/GrpE

Potential Function:

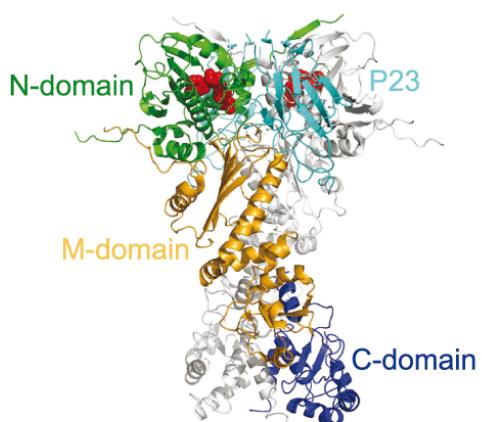
1. to physically separate non-native polypeptide from surface of ribosome
2. to postpone folding until complete sequence of autonomously folding domain has emerged
3. prevention of aggregation

# Examples of Individual Chaperone Systems

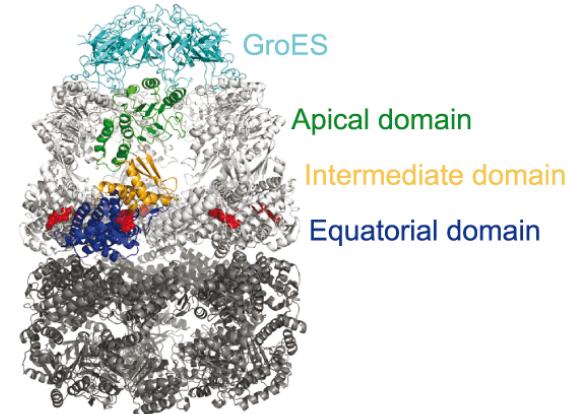
Hsp70/40 System



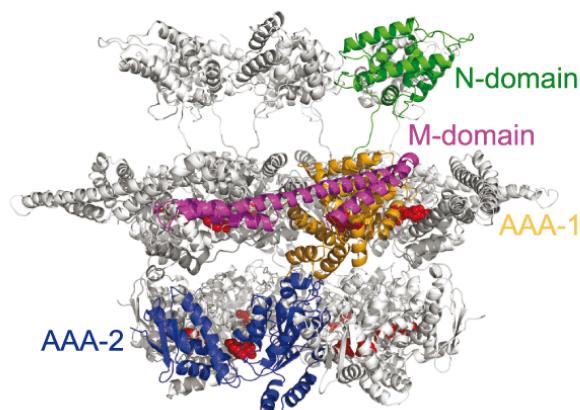
Hsp90



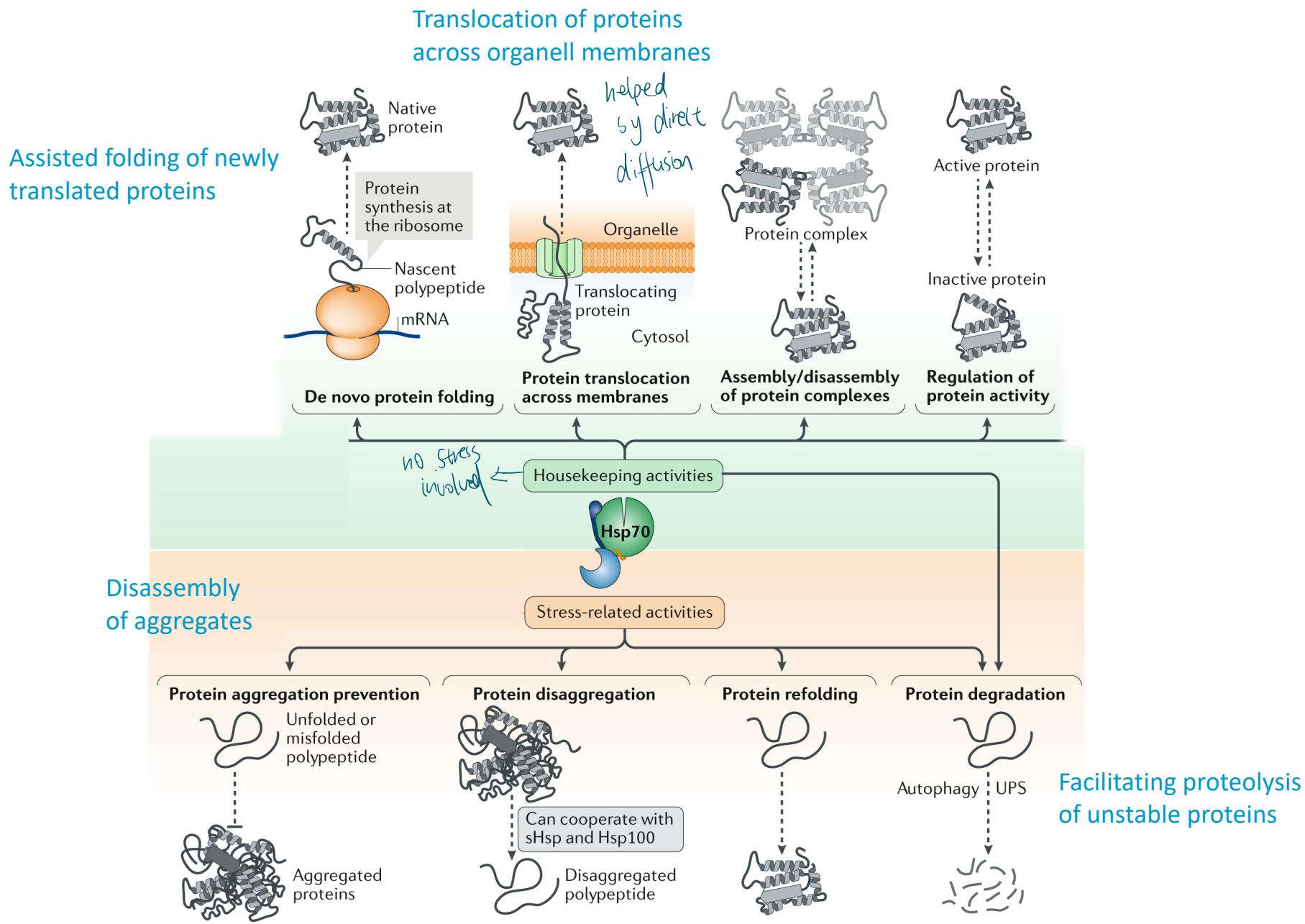
Chaperonin GroEL/ES



Hsp100 Proteins



# Hsp70/40 Chaperone System



# Structure of Hsp70



The **N-terminal domain** (~44 kDa) is highly conserved and contains the ATP binding site.

## Overall structure:

Two large lobes separated by a deep cleft.

## ATP binding pocket:

At the bottom of the cleft is the ATP binding pocket.

It is made of two phosphate binding loops and a hydrophobic adenosine binding pocket.

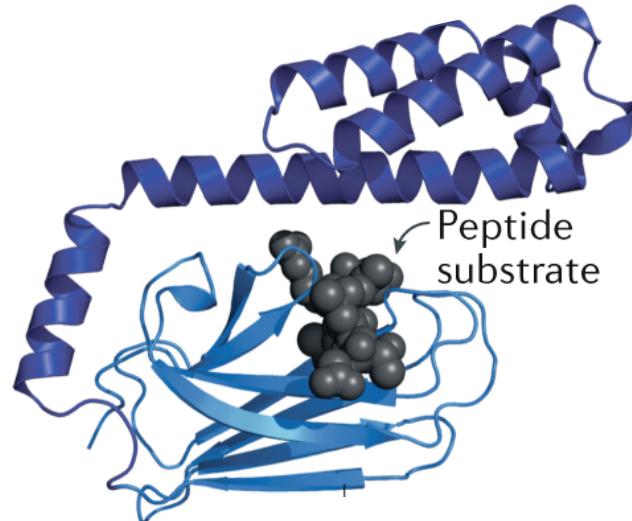
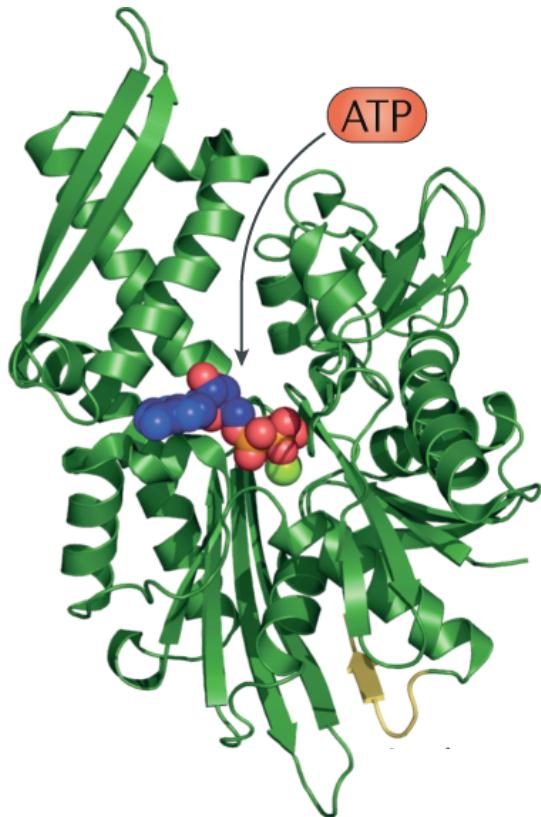
The **C-terminal domain** contains the substrate binding site.

## Overall structure:

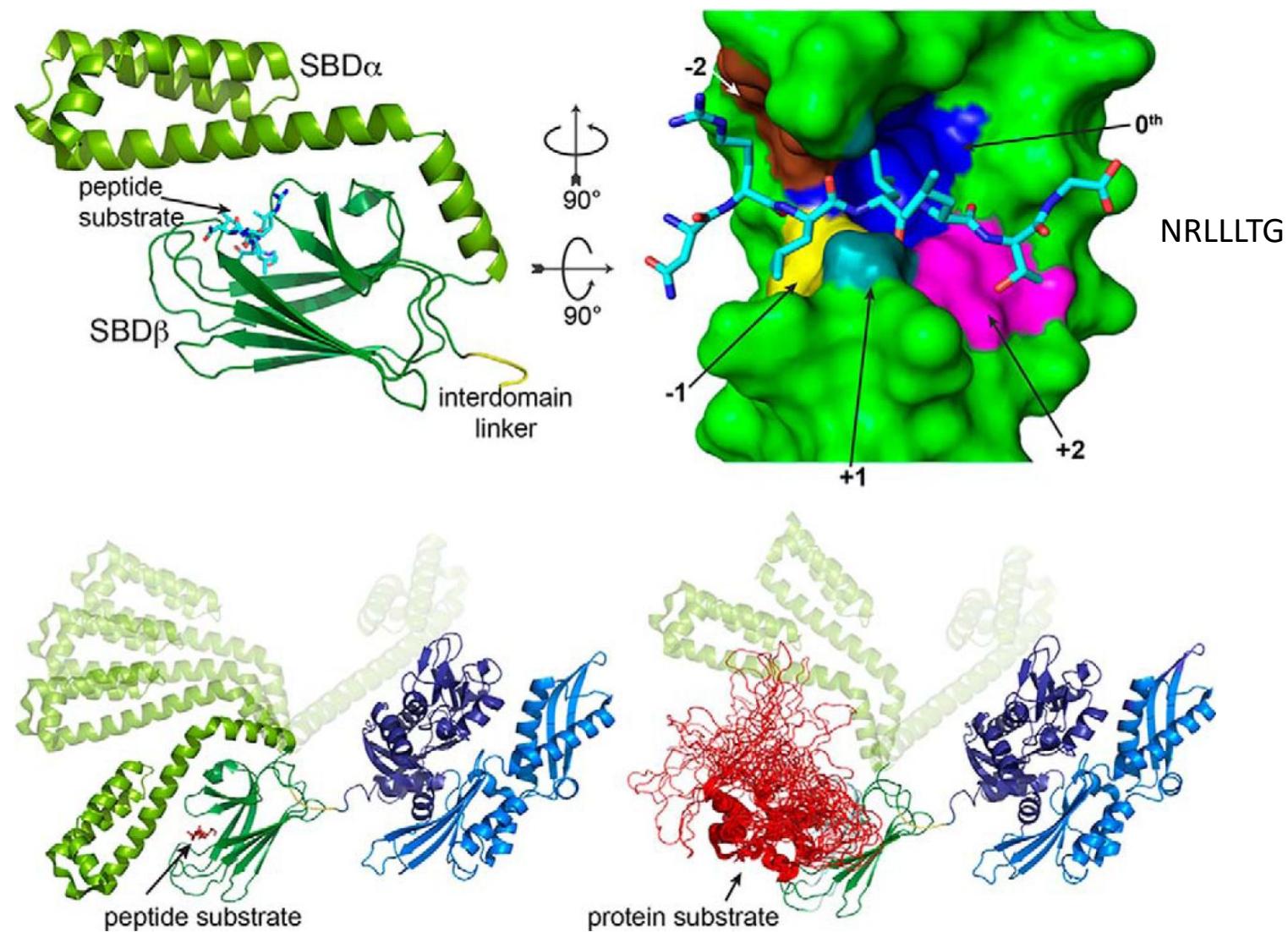
$\beta$ -sandwich of two 4-stranded sheets followed by two helices that span back over the sheet.

## Substrate binding pocket:

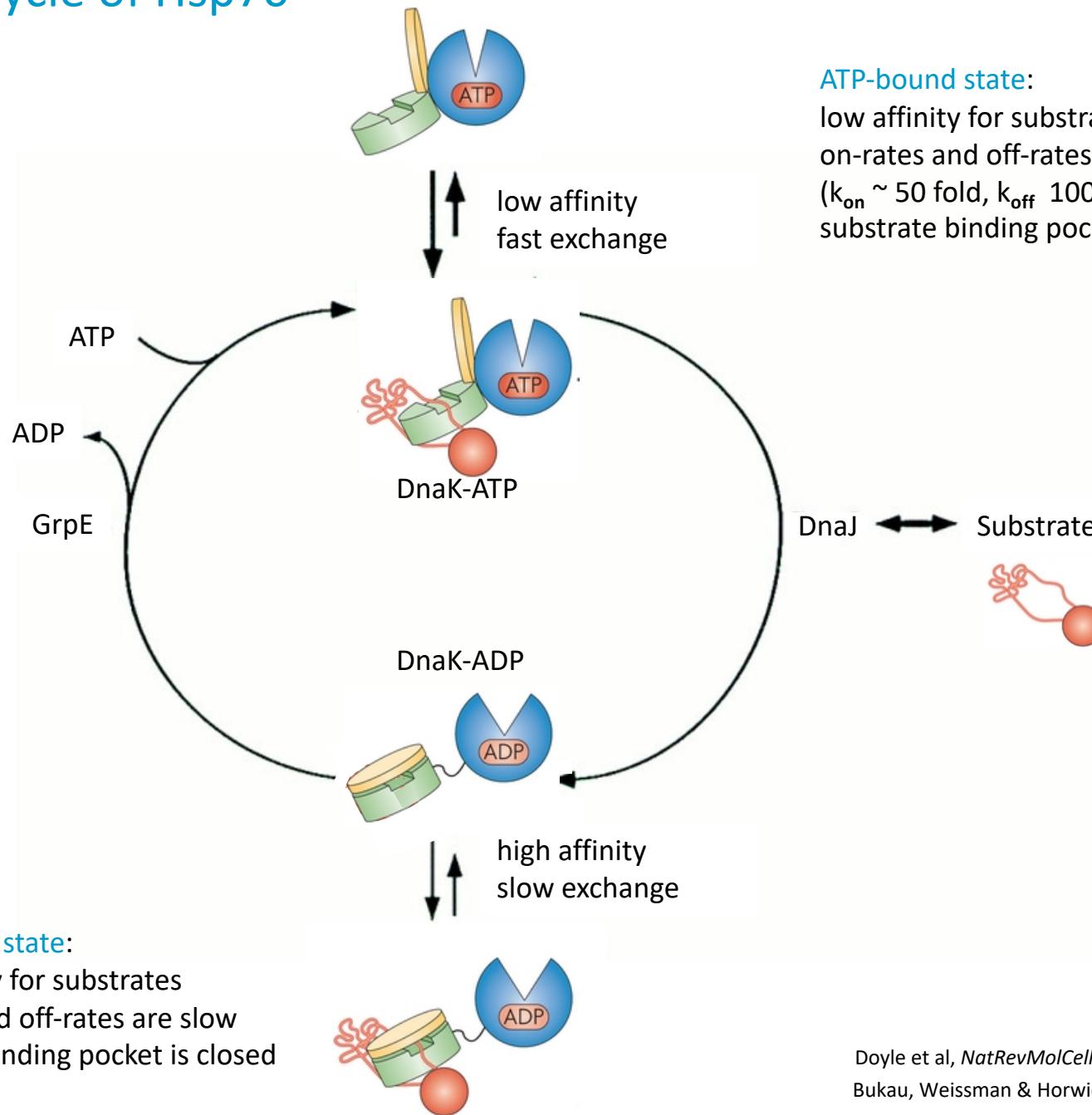
Top  $\beta$ -sheet emanates 4 loops that make contacts with the inner helix. The inner two loops form a channel with a cross section of ~5 X 7 Å, the substrate binding pocket. The helix functions as a lid allowing entry/release of substrate.



# Substrate Binding Domain Dynamics



# ATPase Cycle of Hsp70



Doyle et al, *NatRevMolCellBiol* (2013) Vol 14, 617.  
Bukau, Weissman & Horwich, *Cell* (2006)

# Role of Co-Chaperones in the Hsp70 Reaction Cycle

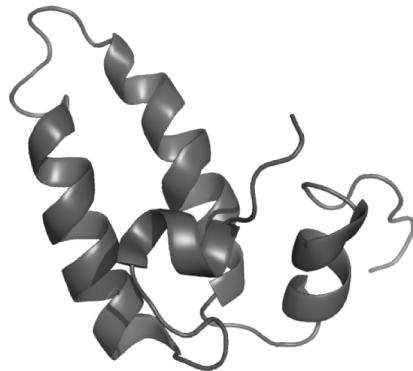
## Hsp40

Hsp40 has the role to stimulate the ATPase activity of Hsp70.

Substrate and Hsp40 have to interact with Hsp70 to stimulate the ATPase activity.

(In some cases, Hsp40 binds substrate first and then binds to Hsp70 thereby „delivering“ the substrate.  
In other cases, Hsp40 binds to Hsp70 that has just accepted substrate into its binding pocket.)

After hydrolysis of ATP, the affinity of Hsp70 for Hsp40 is reduced and it is released again.



DnaJ, J-domain (1-104 out of 376)  
Hunag et al (1999) Protein Science 8, 203.

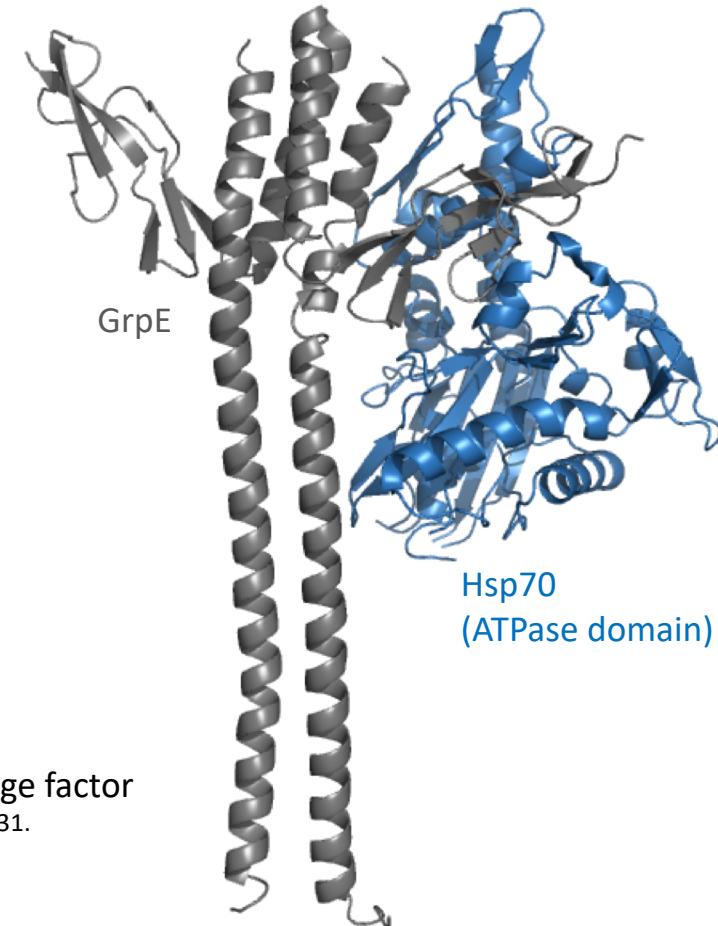
## Nucleotide Exchange Factors

GrpE-like chaperones trigger the release of ADP by opening up the ATP-binding cleft.

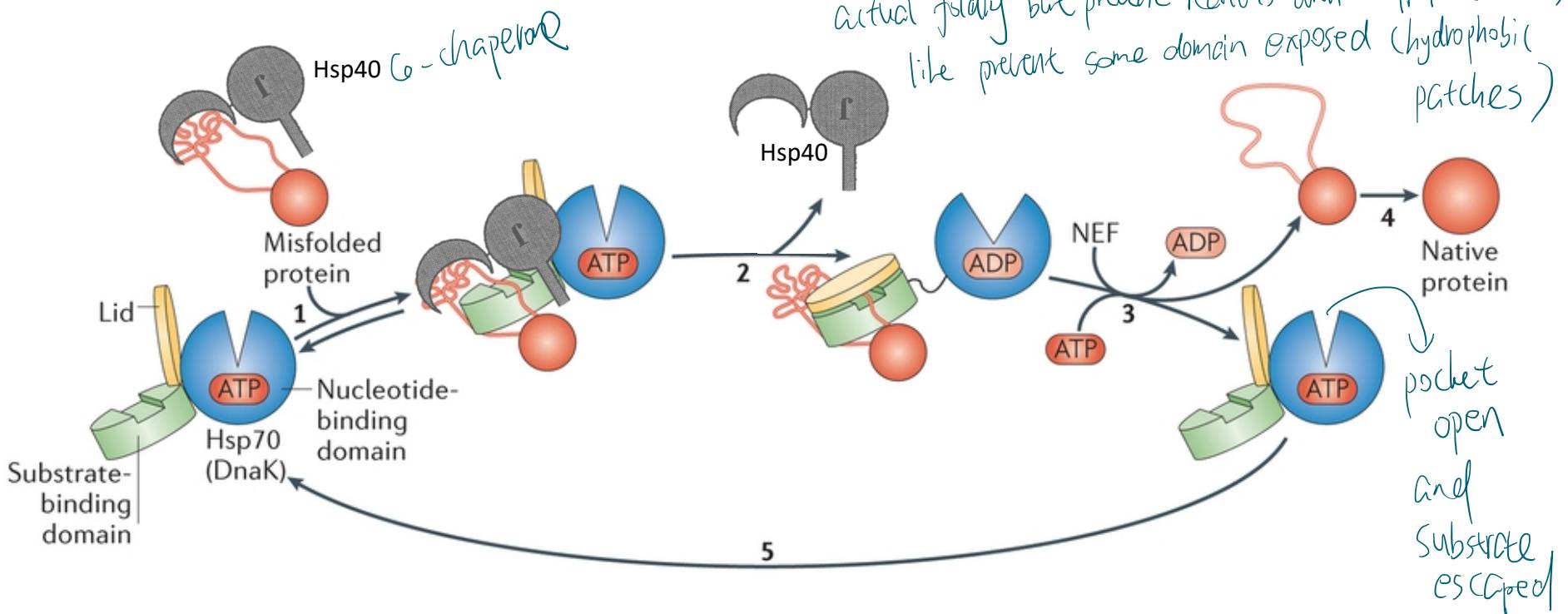
GrpE-like chaperones are only required for some Hsp70s, others can do the job alone.

After release of ADP, ATP rapidly rebinds and the substrate gets released.

GrpE – nucleotide exchange factor  
Harrison et al (1997) Science 276, 431.



# Chaperone Cycle of the DnaK System

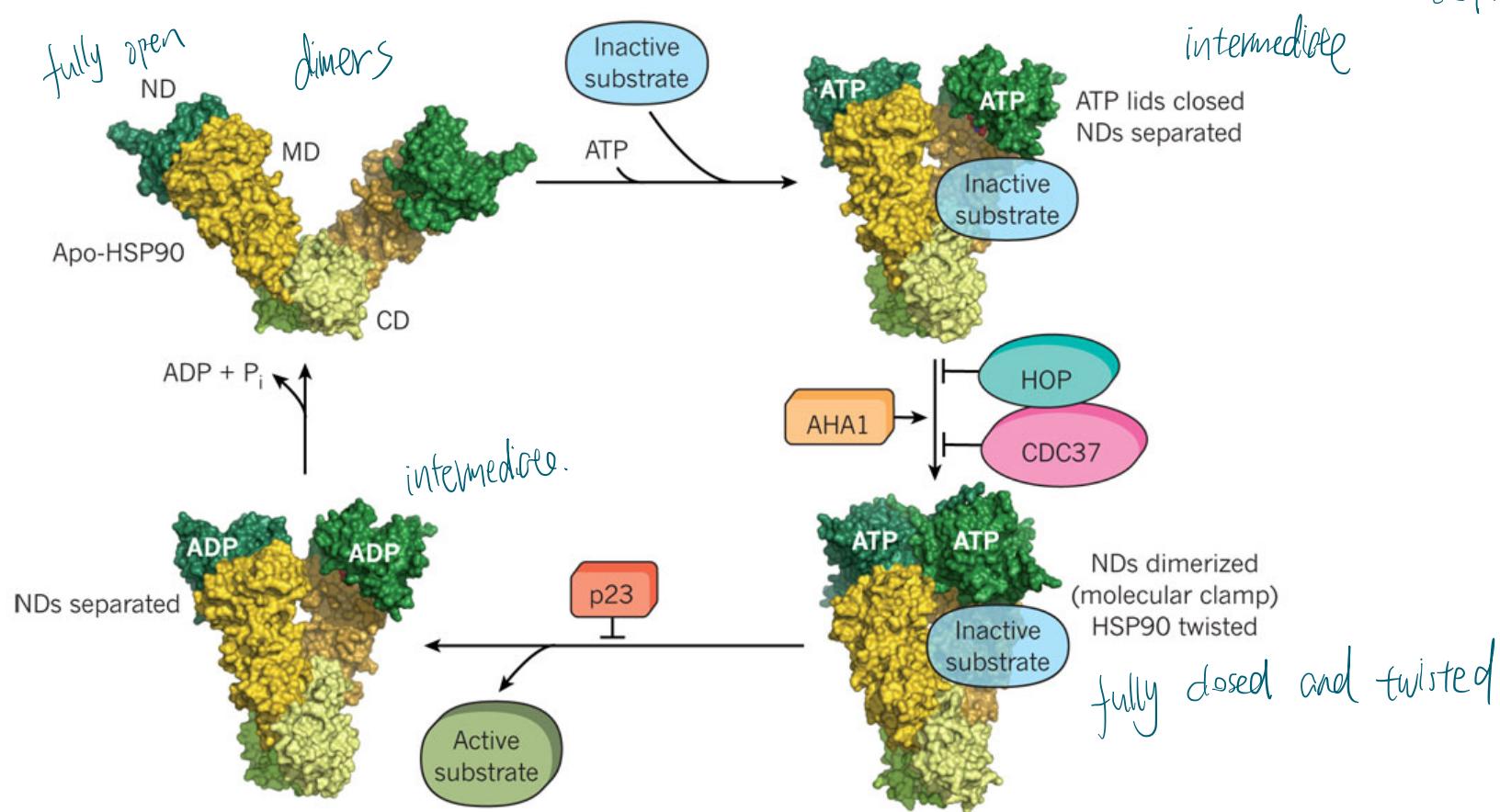


# Hsp 90 Chaperone



ATP-ADP cycle drives chaperones' pocket open / close. But which state is closed / open

- exists in bacteria, eukaryotes (cytosol, ER, mitochondria)
- flexible homodimer
- exists in open and closed conformation
  - open: ATP can bind, exposed hydrophobic patches for substrate binding
  - closed: lid is on ATP pocket, N- and middle domains dimerize and clamp down on substrate
- Substrates: e.g. transcription factors, signaling kinases



# The *E.coli* Chaperonin GroEL/ES

The GroEL/ES chaperonin system assists the folding of a variety of proteins in the *E. coli* cytosol. It accomplishes this by **cyclic binding and release of substrate polypeptides**.

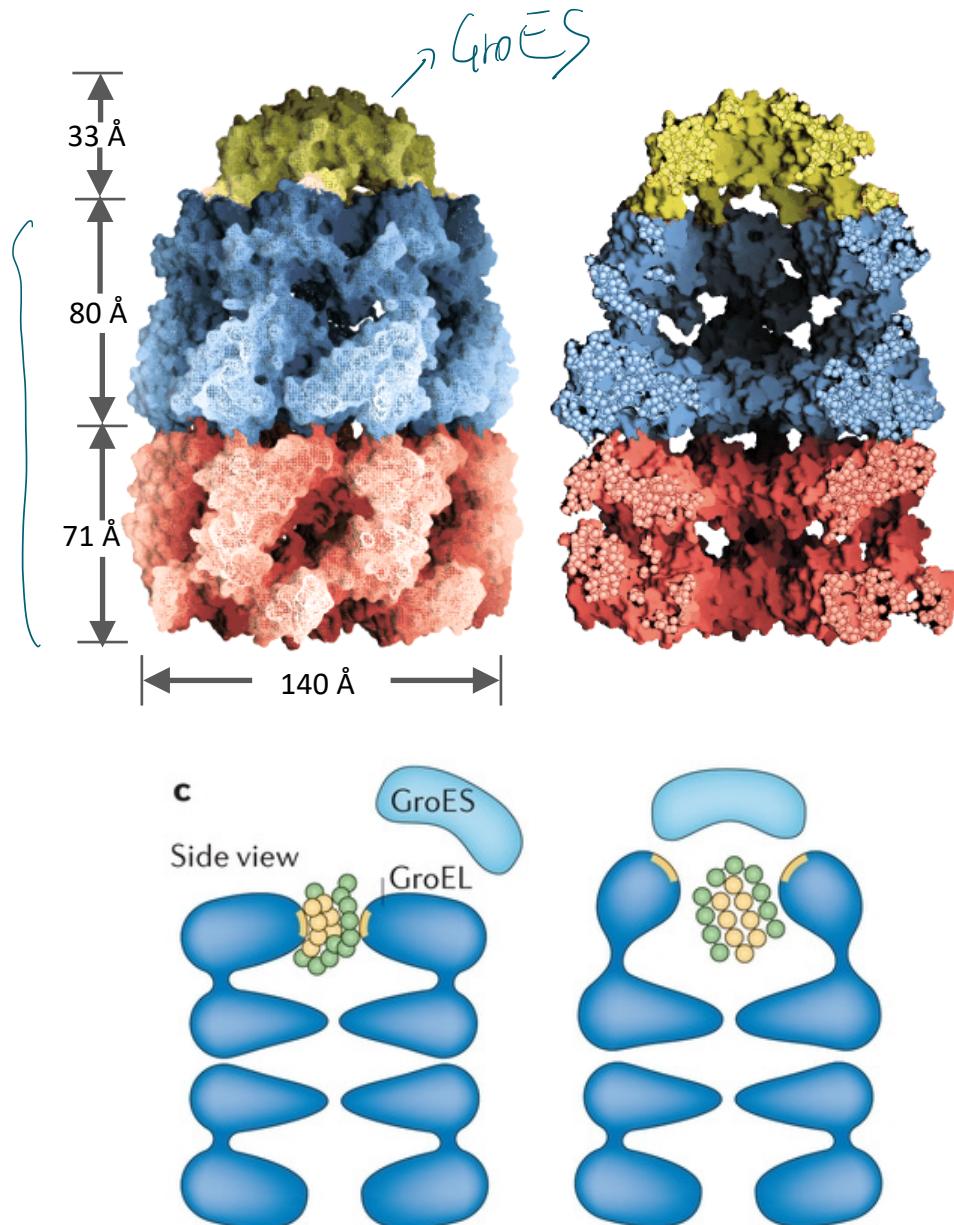
Overall architecture of GroEL and GroEL/ES:

## GroEL

GroEL alone is a double-ring of two back to back **7-membered rings** (57 kDa/subunit). It has the shape of a cylinder that is as high as it is wide and it contains a large cavity inside. Each subunit is made of three domains, equatorial, intermediate and apical.

## GroES

The co-chaperonin GroES alone is a single 7-membered ring (10 kDa/subunit). It has the shape of a half-dome. GroES binds to one end of the GroEL cylinder thereby changing the shape of the GroEL subunits of that ring considerably. When GroES caps GroEL, a closed chamber is formed underneath its half-dome.



# Subunit Structures of GroEL and GroES

## GroES:

- made of core β-barrel and two hairpin loops
- smaller loop forms the top of the dome
- larger loop called the „mobile loop“ is involved in binding to GroEL apical domains

## GroEL: 3 domains

### Apical

grey

- surrounds opening at the ends of the cylinder
- shows local flexibility
- hydrophobic patches lining the cavity for substrate binding
- hinge connecting it to intermediate domain
- upon ATP and GroES binding entire apical domain moves around the hinge

### Intermediate

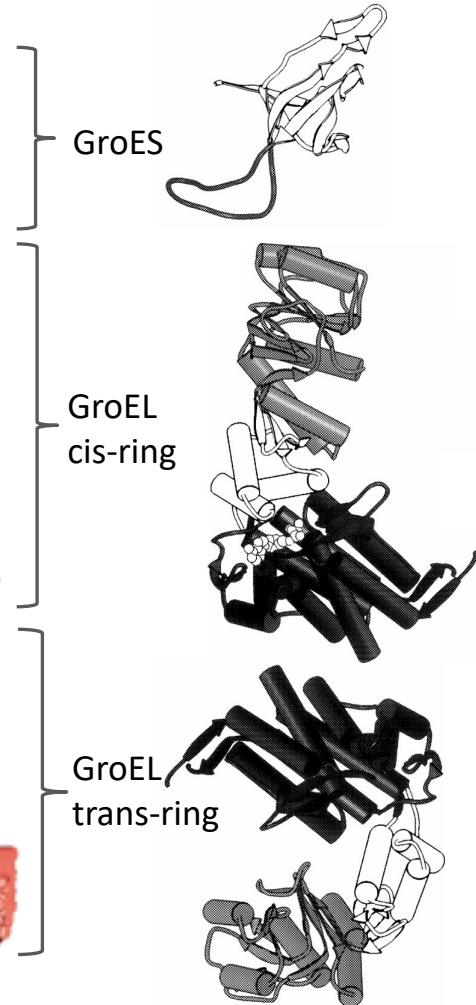
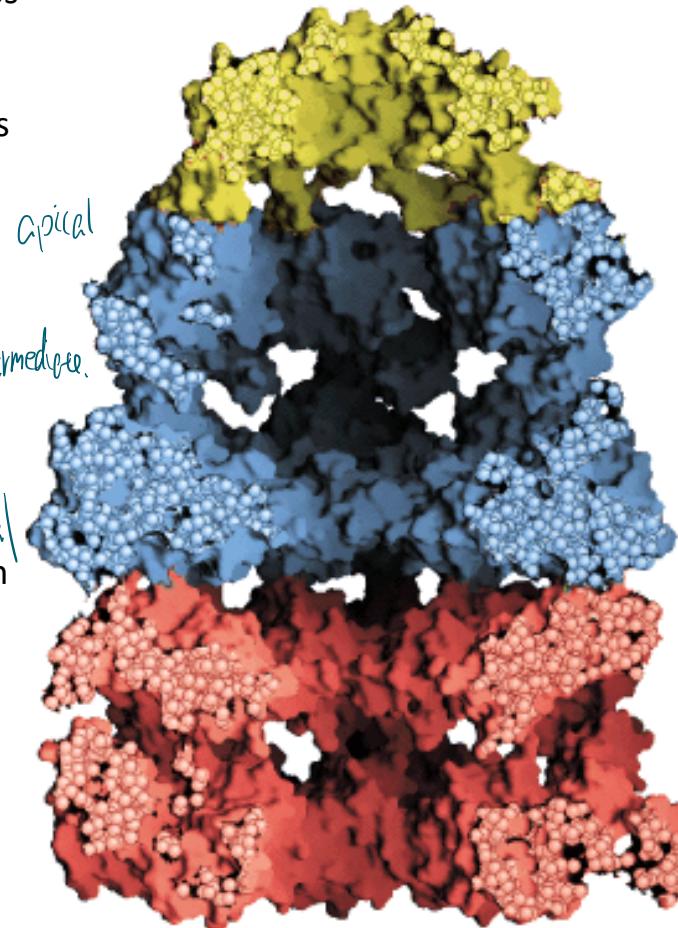
white

- slender domain linking equatorial and apical domains

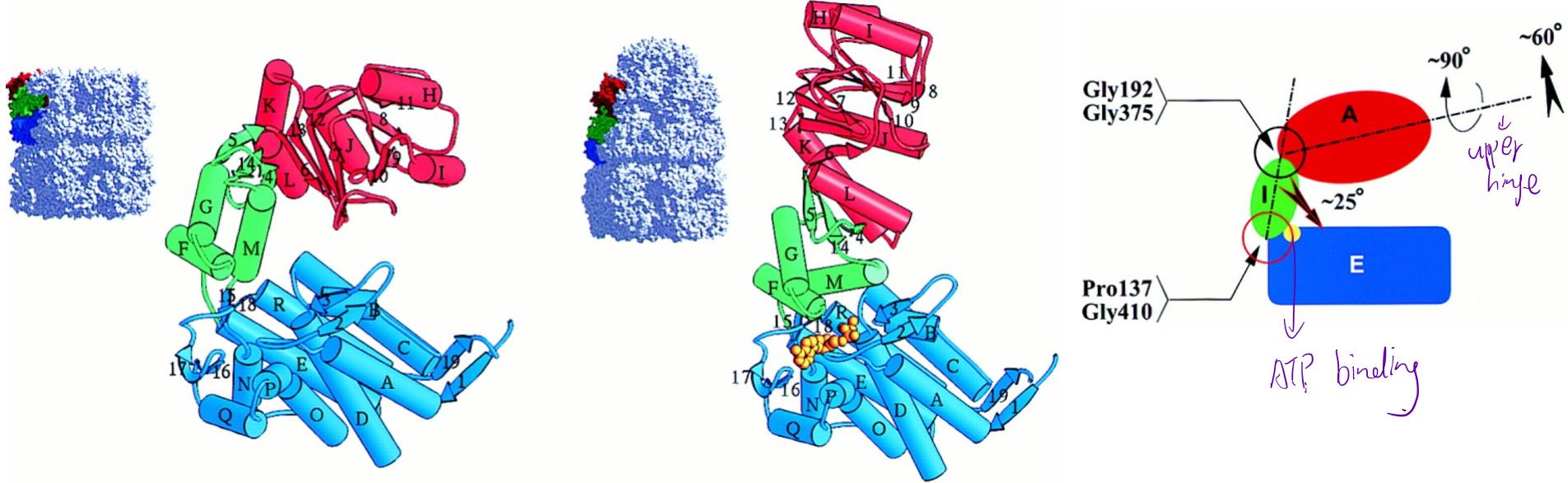
### Equatorial

dark

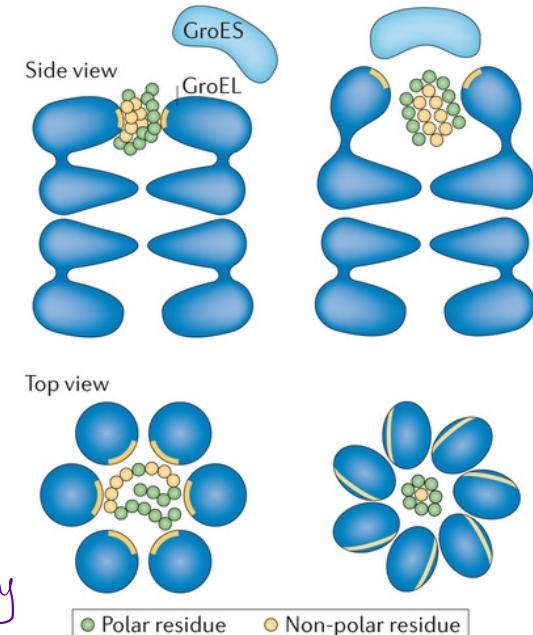
- well-ordered, highly α-helical
- provides the back-to-back ring contacts
- contains most residues of the ATPase site



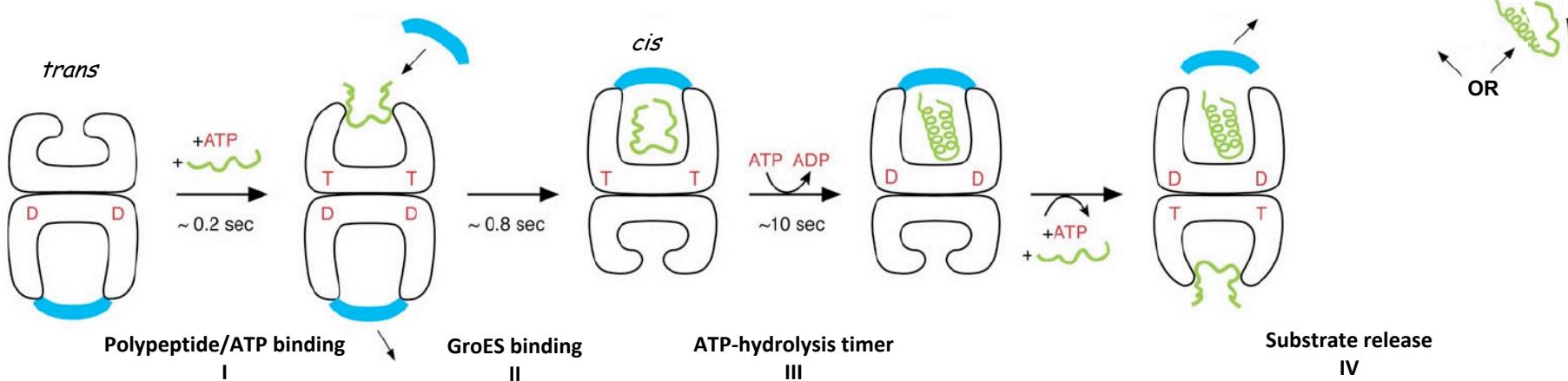
# Domain Movements in GroEL Subunits upon Binding of ATP and GroES



1. 25° downward rotation brings intermediate domain down on equatorial domain. → ATP gets locked in
2. dramatic 60° upward rotation of apical domain about the upper hinge
3. 90° twist of the apical domain causes the hydrophobic patches that before faced into the cylinder, i.e. lined its walls, to move 90° away to a position where the hydrophobic patches are buried in the binding interface with GroES and with other apical domains.



# GroEL-GroES Reaction Cycle

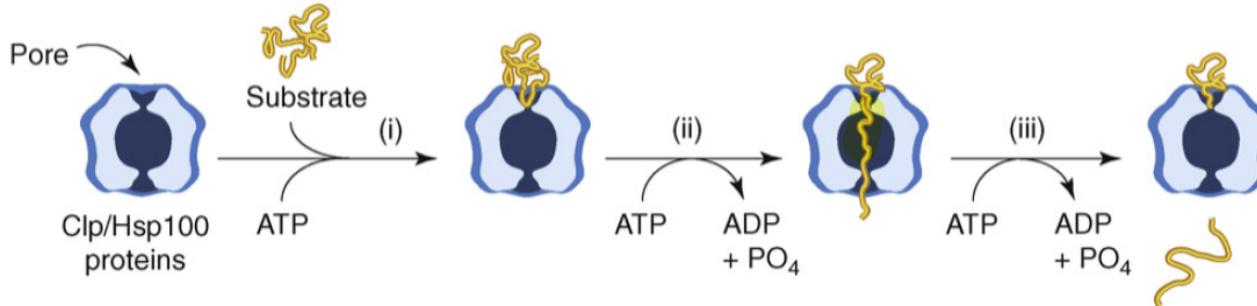


- I) The nonnative substrate and ATP bind to the open *trans* ring of the GroEL-GroES asymmetric complex.
- II) GroES binds to the same ring occupied by the substrate, forming a *cis* ternary complex in which the polypeptide is encapsulated within the GroEL-GroES structure. Binding of GroES induces a large conformational change in GroEL that leads to an approximate doubling of the volume of the central cavity and obscures GroEL's hydrophobic polypeptide recognition regions. As a consequence, the substrate polypeptide in the *cis* complex is encapsulated within a relatively polar environment that favors folding.
- (III) ATP bound to the *cis* complex acts as a timer, giving the substrate at least 8–10 s to fold inside the cavity after which ATP hydrolysis primes GroES for release from the *cis* ring. ATP hydrolysis in the *cis* ring also induces a conformational change in the *trans* (bottom) ring allowing it to bind ATP and a nonnative polypeptide.
- (IV) Binding of ATP to the *trans* ring induces dissociation of the *cis* ligands (GroES and polypeptide). This dissociation is accelerated 30- to 50-fold by binding of nonnative substrates to the *trans* ring. Note that polypeptide can be released in either a native form, a form committed to reaching the native state in the bulk solution, or an uncommitted nonnative state, which is either recaptured by another chaperone or eventually targeted for proteolysis.

# Hsp100/Clp Proteins

Hsp100 proteins are ring-shaped homohexamers with ATPase activity.

All Hsp100 proteins change the conformational state of their protein substrates under expense of ATP.

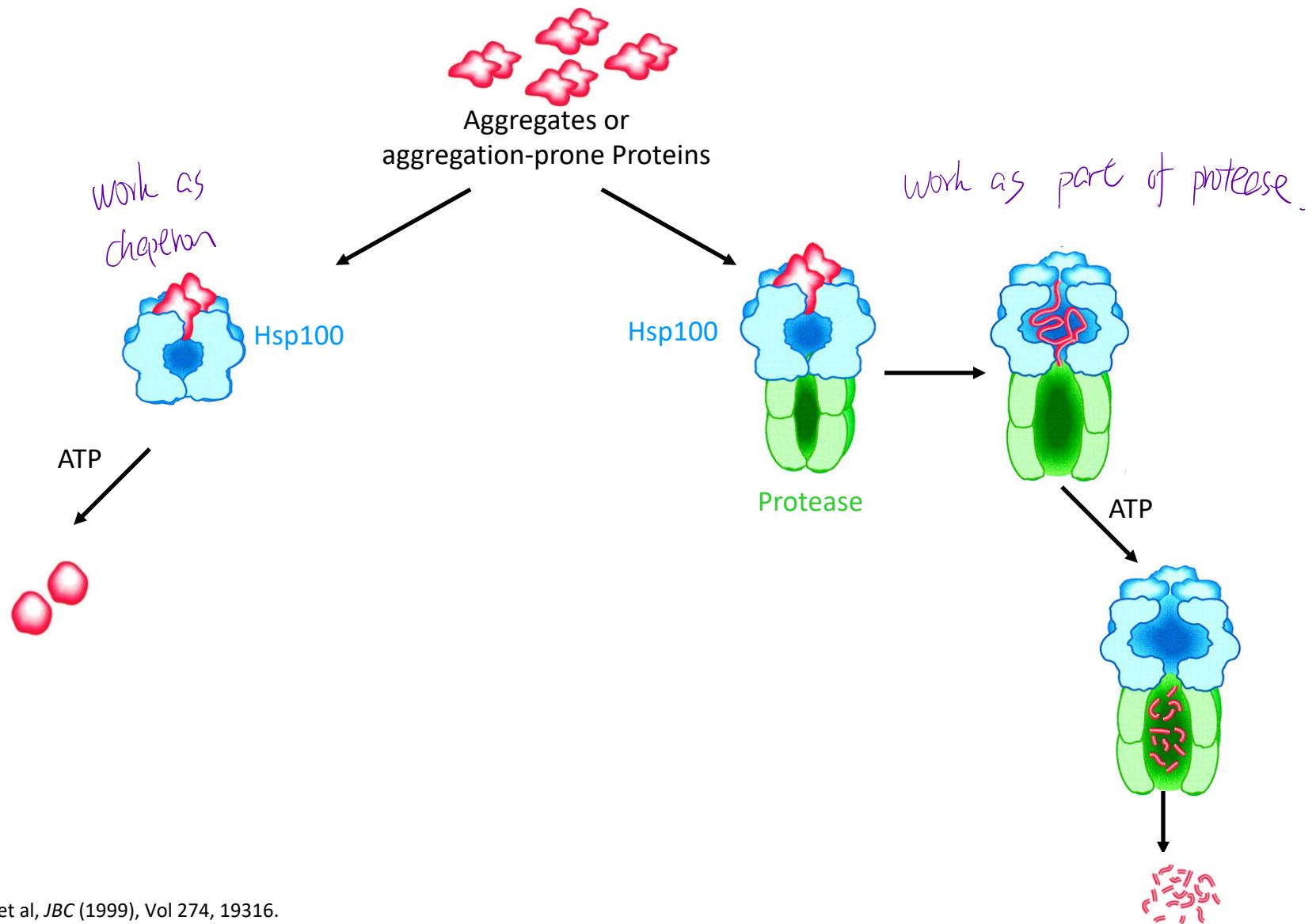


Hsp100 proteins are a recent addition to the community of „chaperones“ for the following reason: While some Hsp100 proteins (Hsp104 and ClpB) act like the classical chaperones by preventing or reversing aggregation of their substrate proteins, others act as components of large ring-shaped protease complexes. Their function there is the unfolding and destruction of their substrate proteins.

Despite this radically different outcome of their action on substrates, Clp proteins show many attributes of other chaperones:

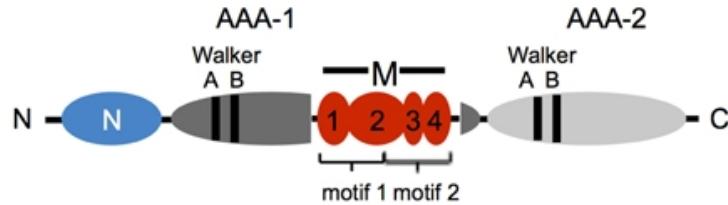
1. Ability to **change the conformational state** of their substrates.
2. **Consumption of ATP** to support this activity.
3. **Induction during stress** conditions.
4. **Universality** and high degree of **conservation**.
5. **High concentration** in the cell.
6. Multiple forms or subfamilies within the same cell.

# Different Functions of Hsp100 Proteins

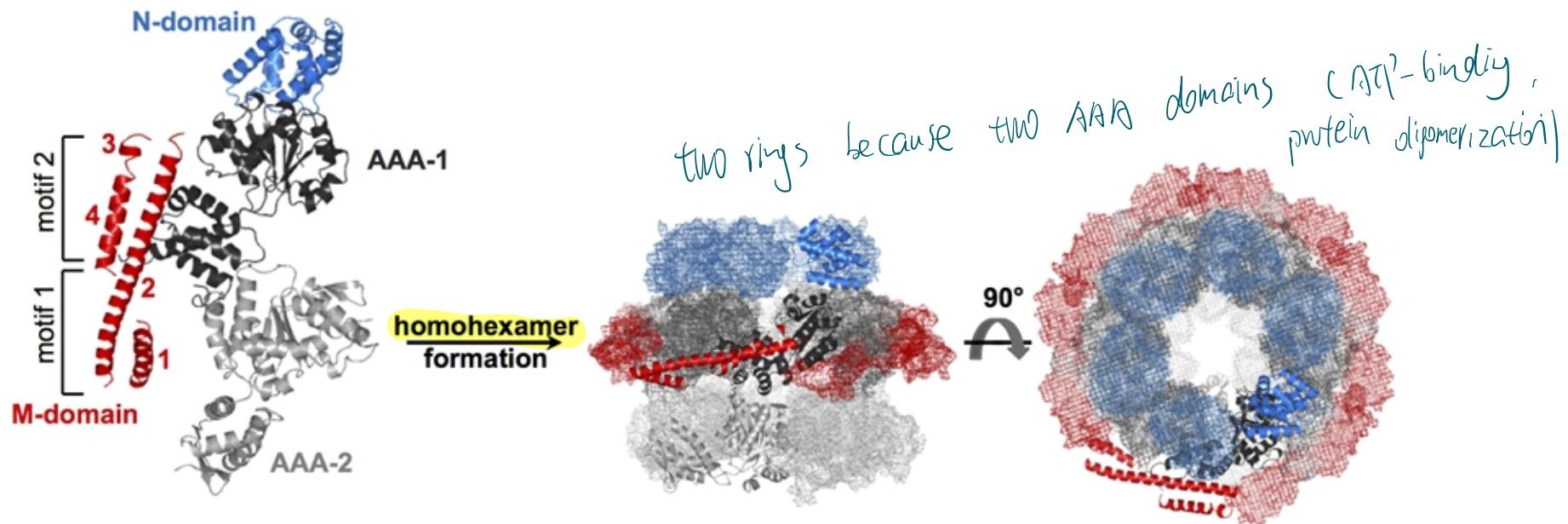


# ClpB/Hsp104 Chaperones: Protein Disaggregation

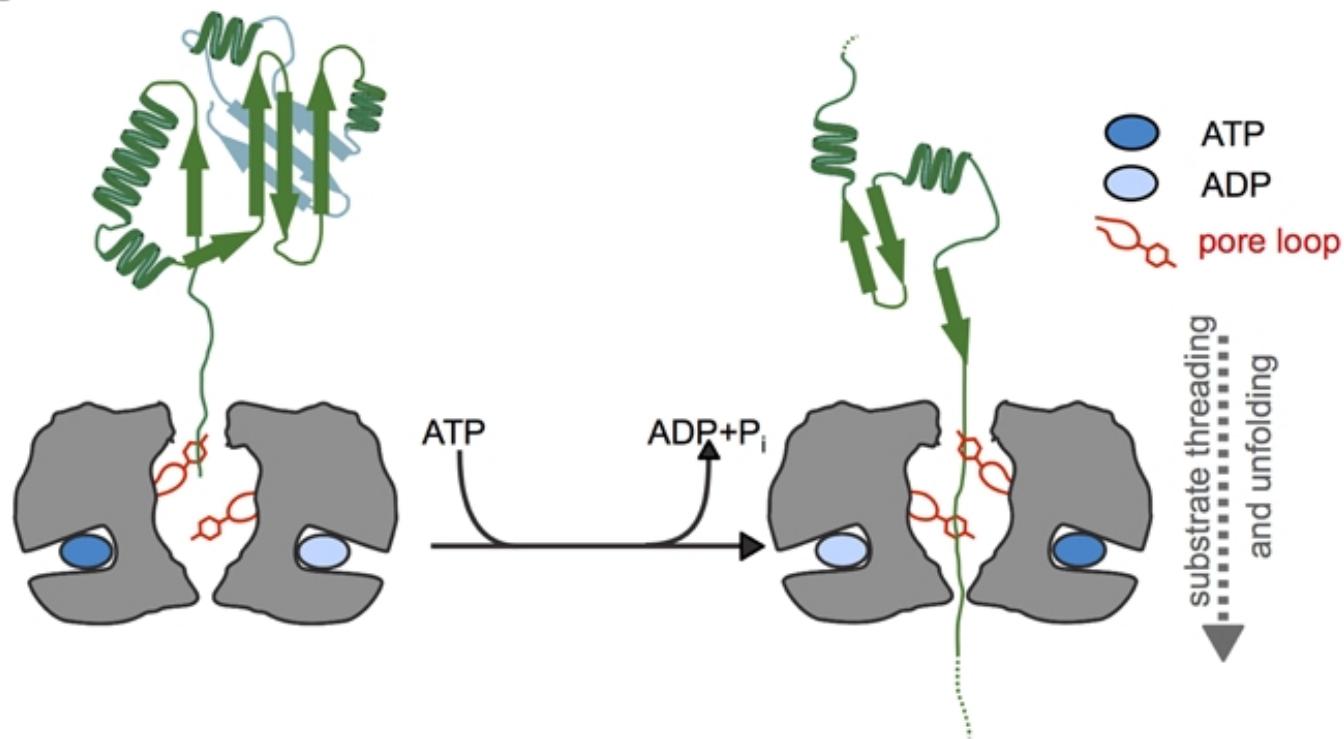
- Bacterial ClpB and the eukaryotic homolog Hsp104 are a subgroup of the Hsp100 protein family, characterized by a large linker between the two nucleotide binding domains.
- ClpB/Hsp100 proteins have the ability to disaggregate protein aggregates.
- Unlike other members of the Hsp100 family, they do not interact with proteases.

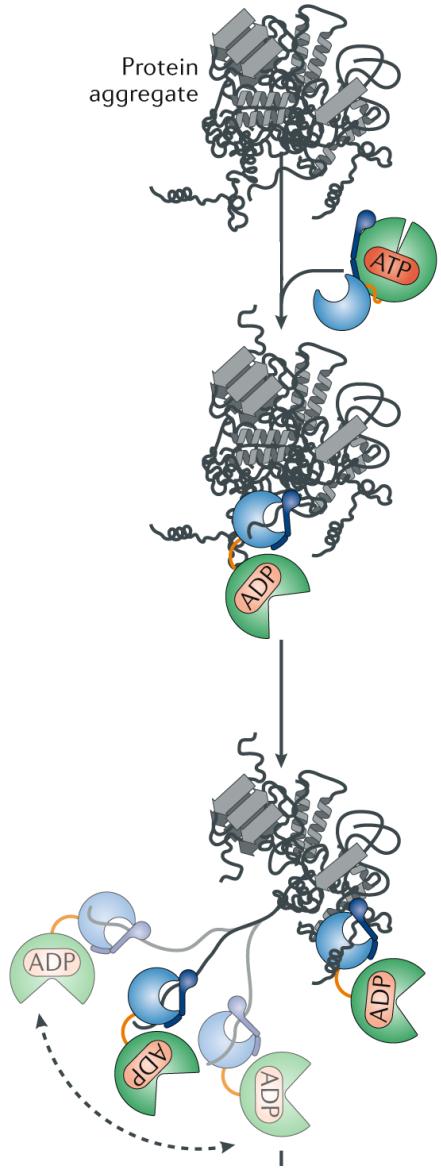


ClpA also contains 2 AAA module.

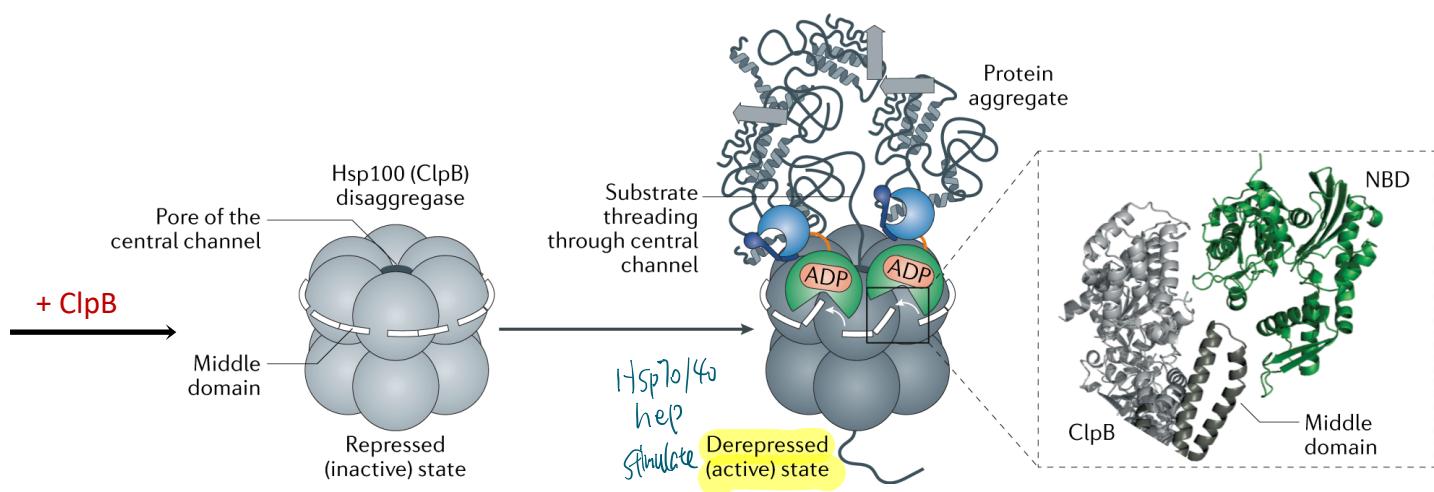


## Mechanistic Model for Protein Disaggregation by ClpB





## Hsp100-Hsp70/40-Chaperone Network



# Chaperones in the ER

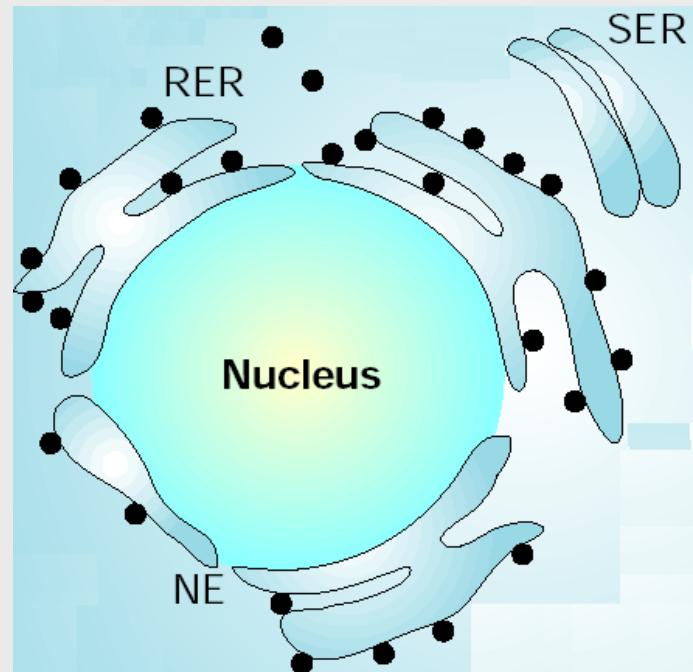
In eukaryotes there are additional compartments and this requires the presence of multiple folding and degradation systems.

The majority of eukaryotic proteins fold either in the cytosol (cytosolic proteins) or in the ER (secretory proteins).

Table 1 Personnel of the ER protein factory in mammalian cells

## Ubiquitous ER-resident proteins

Family	Main members	Functions
Hsp70	BiP/grp78	Chaperone
Hsp90	Endoplasmic/grp94	Chaperone
Hsp40	ERdj1–ERdj5, Sec63	Co-chaperones regulating BiP?
Lectins	Calnexin and calreticulin	Glycoprotein quality control
	EDEM	Glycoprotein degradation <sup>22</sup>
Glycan-processing enzymes	UGT	Folding sensor. Adds glucoses to misfolded glycoproteins (enters substrates into the calnexin cycle) <sup>5,6</sup>
	ER glucosidases I and II	Remove glucoses from N-glycans (on/off/calnexin cycle) <sup>5,6</sup>
	ER mannosidases I and II	Remove terminal mannoses — ERAD? <sup>22</sup>
Peptidyl-prolyl isomerase	Cyclophilins, FK506-binding proteins	Isomerize <i>cis-trans</i> peptidyl-prolyl bonds?
Ero1	Ero1 $\alpha$ , Ero1 $\beta$	Disulphide-bond formation <sup>7,8</sup>
Oxidoreductases	PDI, ERp72, ERp57, p5, and many others	Disulphide-bond formation, isomerization and reduction



# Protein Folding in the ER

Structural maturation of proteins in the ER requires post-translational modifications:

signal sequence cleavage

N-linked glycosylation

disulphide bond formation and reshuffling

## UPR – Unfolded protein response

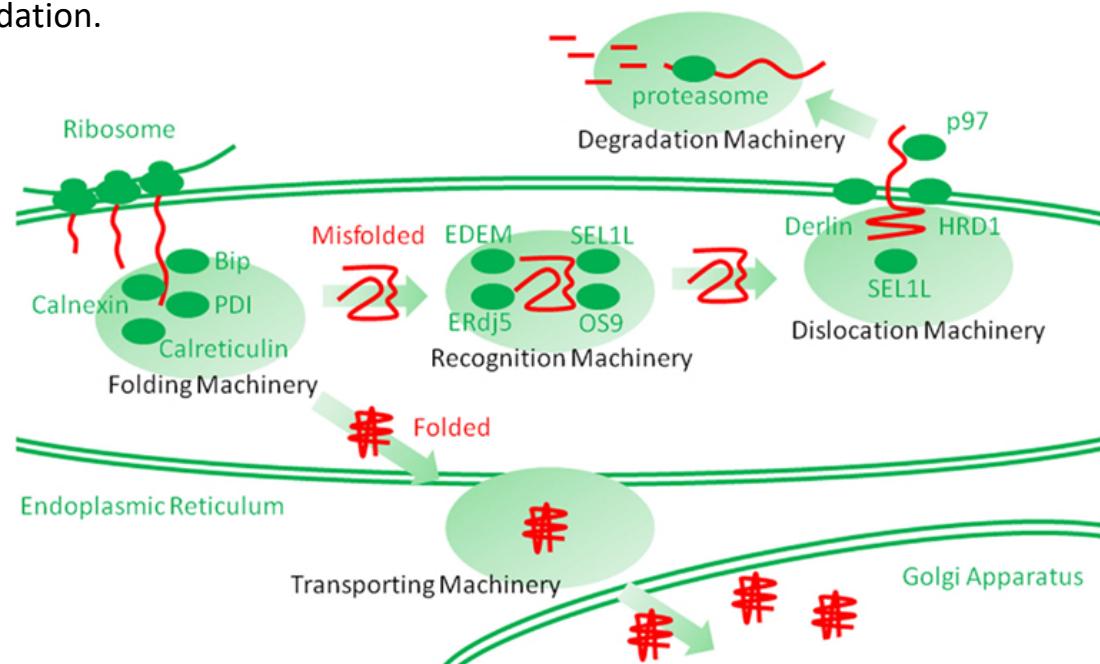
Under conditions of stress a range of genes gets activated for molecular chaperones and proteins involved in the secretory pathway to allow for damaged protein-refolding or retrotranslocation and subsequent degradation.

## Folding

Generally folding starts co-translationally, is completed post-translationally and is then followed by assembly and oligomerization. The process requires the general chaperones Hsp70/40 and the lectin chaperones.

## ERAD – ER-associated degradation

Misfolded proteins that are beyond repair will be degraded by this pathway:  
retrotranslocation to the cytosol  
ubiquitination  
degradation by proteasomes



# The main chaperone families of the endoplasmic reticulum

The endoplasmic reticulum (ER) contains chaperones that belong to several of the classical chaperone families, such as heat shock protein (Hsp)40, Hsp70 and Hsp90, with the two main exceptions being the Hsp104 and Hsp60/Hsp10 proteins. The ER also contains chaperones and folding enzymes that are unique, such as calnexin and calreticulin and the family of thiol-disulphide oxidoreductases.

## Hsp70s

The main protein of this family is BiP, which takes part in many aspects of ER quality control (QC). It binds to various nascent and newly synthesized proteins and assists their folding. In addition, it is involved in the processes of ER-associated degradation and the unfolded protein response.

## Hsp40s

Five ER proteins of the Hsp40 family (ERdj1–5) are known. They contain a luminally exposed J-domain and can stimulate BiP ATPase activity *in vitro*.

## Hsp90

The only known Hsp90 family member is GRP94. Despite being abundant in the ER, it is not essential for cell viability and seems to limit its interactions to a small set of substrates.

## Peptidyl-prolyl isomerases

Peptidyl-prolyl isomerases (PPIases) from both of the two main PPIase families — the cyclophilins and the FK506-binding proteins — are found in the ER. Catalysis of *cis/trans* isomerization of peptidyl-prolyl bonds *in vivo* remains to be shown conclusively for these proteins.

## Calnexin and calreticulin

These two lectin chaperones interact with and assist the folding of proteins that carry monoglycosylated N-linked glycans.

## Thiol-disulphide oxidoreductases

This large family of enzymes, of which protein disulphide isomerase (PDI) is the best known, catalyses the oxidation, isomerization and reduction of disulphide bonds.

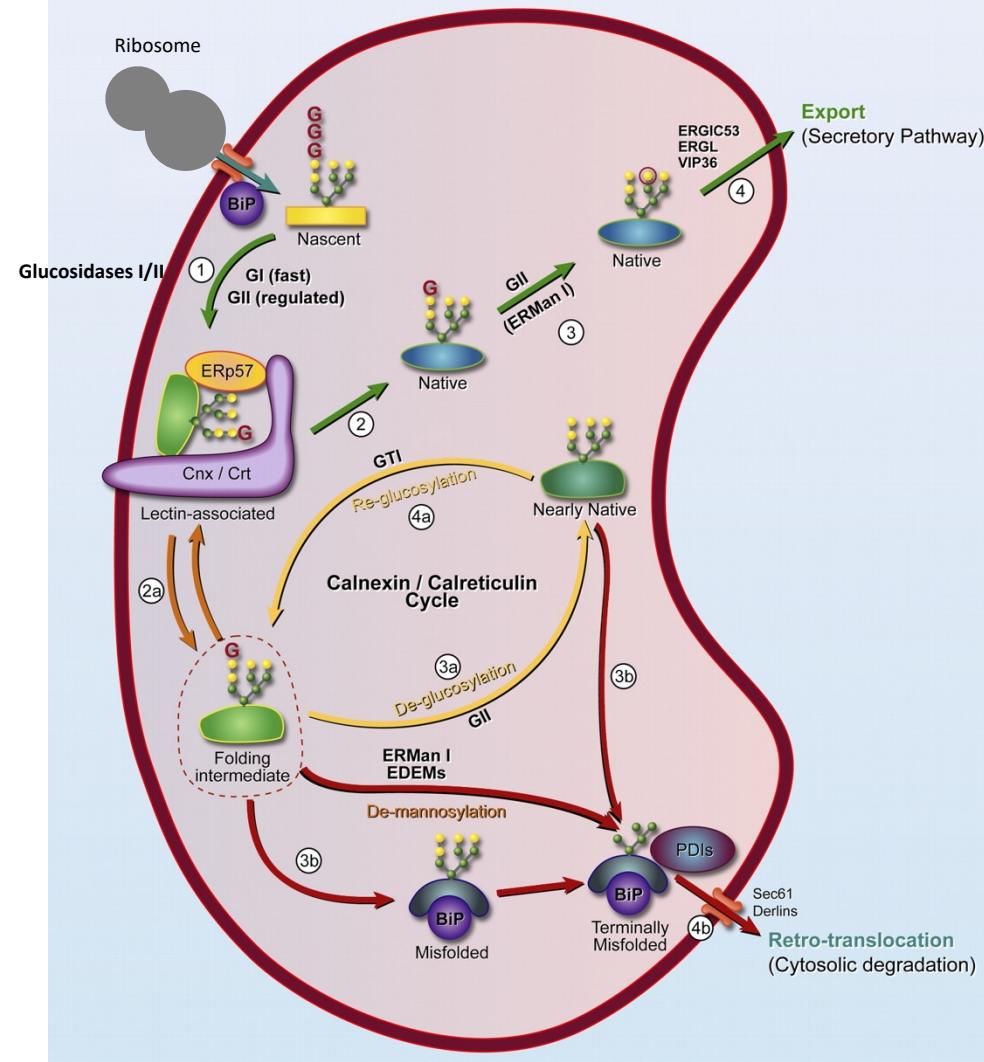
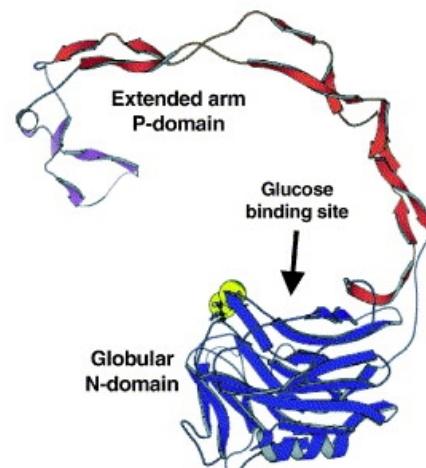
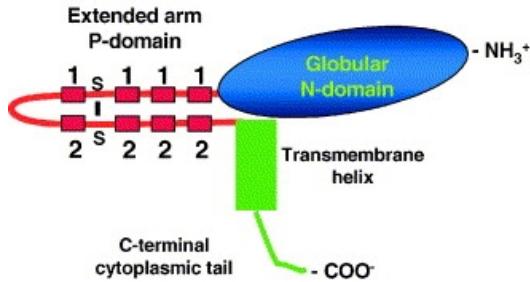
# Calreticulin and Calnexin

The two lectin chaperones CRT and CNX interact with and assist the folding of proteins that carry monoglycosylated N-linked glycans.

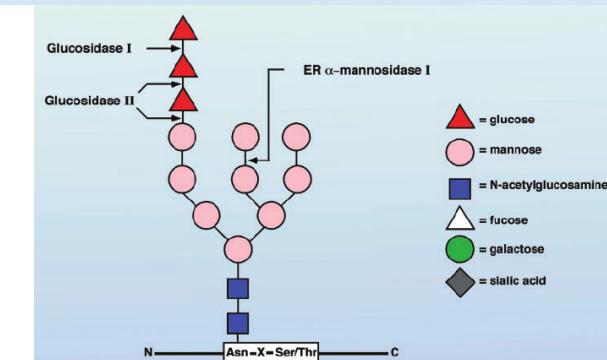
CRT and the membrane-bound CNX have a lectin domain (interacts with sugar moiety of substrate) and a peptide binding domain (likely interacts with cochaperone and might also interact with unfolded polypeptide).

CRT and CNX interact 1:1 with the cochaperone ERp57, a thiol oxidoreductase of the PDI family.

Calnexin



Herbert & Molinare (2007) Physiol. Reviews.



# Protein Homeostasis

