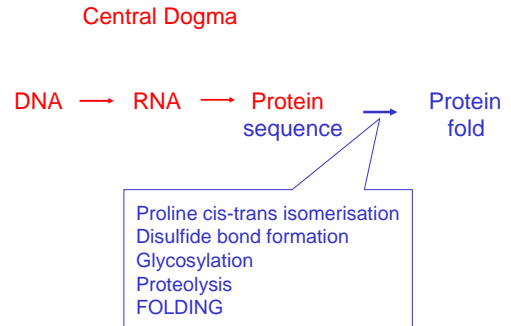


**C22: Molecular Chaperones**

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 people.cryst.bbk.ac.uk/~ubcg16z/hsplec.html



**MOLECULAR CHAPERONES: Definition**

A large group of unrelated protein families whose role is to stabilize unfolded proteins, unfold them for translocation across membranes or for degradation, and/ or to assist in their correct folding and assembly.

**Properties**

- Molecular chaperones interact with unfolded or partially folded protein subunits, e.g. nascent chains emerging from the ribosome, or extended chains being translocated across subcellular membranes.
- They stabilize non-native conformation and facilitate correct folding of protein subunits.
- They do not interact with native proteins, nor do they form part of the final folded structures.
- Some chaperones are non-specific, and interact with a wide variety of polypeptide chains, but others are restricted to specific targets.
- They often couple ATP binding/hydrolysis to the folding process.
- Essential for viability, their expression is often increased by cellular stress.

**Main role:** They prevent inappropriate association or aggregation of exposed hydrophobic surfaces and direct their substrates into productive folding, transport or degradation pathways.

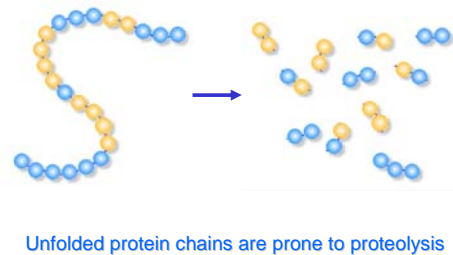
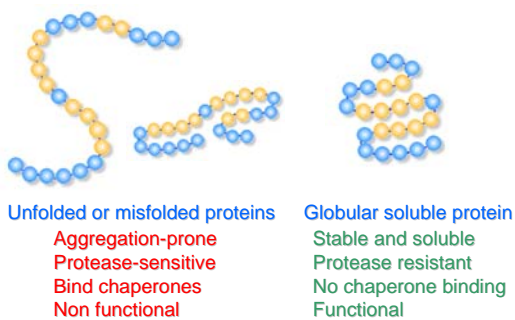
**Examples of molecular chaperones**

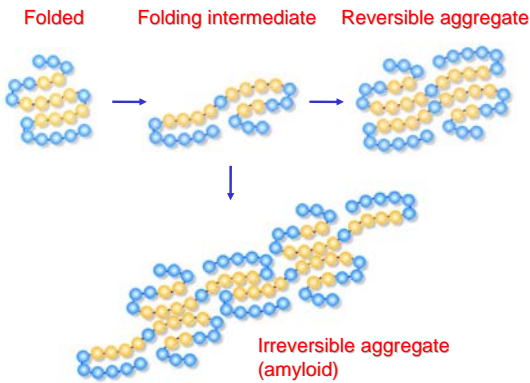
Heat shock proteins: hsp104, 90, 70, 60 and small hsps, including homologues of lens  $\alpha$ -crystallin.

Catalysts of folding: Protein disulfide isomerase, Peptidyl prolyl cis-trans isomerase

Nucleoplasmin: nucleosome assembly

Prosequences: subtilisin,  $\alpha$ -lytic protease (intramolecular chaperones)

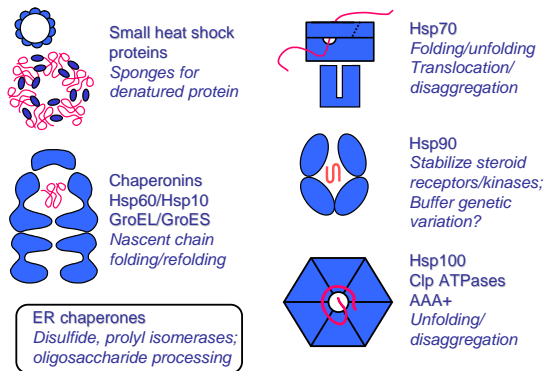




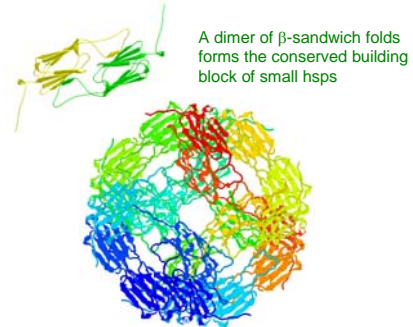
### FAMILIES OF MOLECULAR CHAPERONES

- Small heat shock proteins (hsp25)** [holders]
  - protect against cellular stress
  - prevent aggregation in the lens (cataract)
- Hsp60 system (cpn60, GroEL) ATPase** [(un)folders]
  - protein folding
- Hsp70 system (DnaK, BiP) ATPase** [(un)folders]
  - stabilization of extended chains
  - membrane translocation
  - regulation of the heat shock response
- Hsp90** [holder]
  - binding and stabilization/regulation of steroid receptors, protein kinases
  - Buffer for genetic variation?
- Hsp100 (Clp) ATPase** [unfolder]
  - thermotolerance, proteolysis, resolubilization of aggregates
- Calnexin, calreticulin**
  - glycoprotein maturation in the ER
  - quality control
- Folding catalysts:** PDI, PPI [folders]
- Prosequences:** alpha-lytic protease, subtilisin (intramolecular chaperones) [folders]

### Molecular chaperones



### Small heat shock proteins

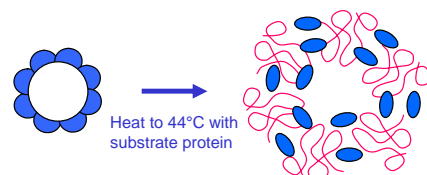


Crystal structure of sHsp from *Methanococcus jannaschii* (Kim et al, 1998)

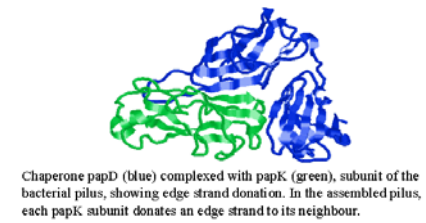
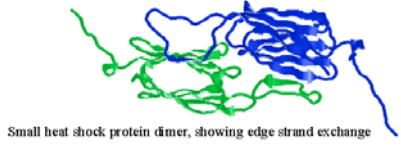
### Small Hsp function

- Superfamily includes the eye lens protein  $\alpha$ -crystallin
- Protective role in suppressing protein aggregation *in vivo* and *in vitro*
- Bind and stabilise denatured protein under conditions of cellular stress, ageing and degenerative disease
- Do not appear to have unfolding and refolding activity
- High capacity for protein binding: up to 2 denatured proteins per subunit

### Formation of substrate complexes

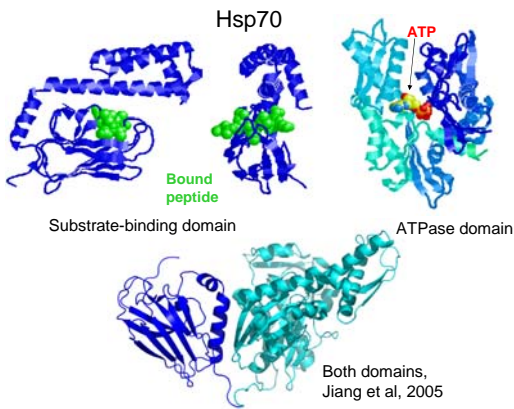


Immunoglobulin fold chaperones

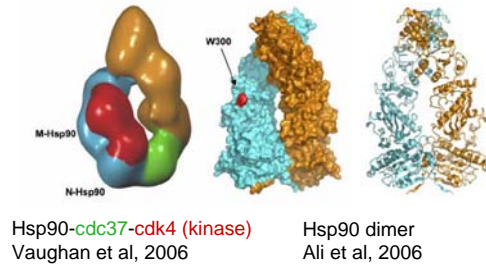


HSP70 FAMILY

Location	Chaperone	Roles
Prokaryotic cytosol	<b>DnaK</b> <b>DnaJ, GrpE</b>	Stabilizes newly synthesised polypeptides and preserves folding competence; reactivates heat-denatured proteins; controls heat-shock response
Eukaryotic cytosol	<b>SSA1</b> , <b>SSB1</b> (yeast) <b>Hsc/hsp70</b> , <b>hsp40</b> (mammalian)	Protein transport across organelle membranes; binds nascent polypeptides; dissociates clathrin from coated vesicles; promotes lysosomal degradation of cytosolic proteins
ER	<b>KAR2</b> , <b>BiP/Grp78</b>	Protein translocation into ER
Mitochondria/ Chloroplasts	<b>SSC1</b> <b>ctHsp70</b>	Protein translocation into mitochondria; Insertion of light-harvesting complex into thylakoid membrane



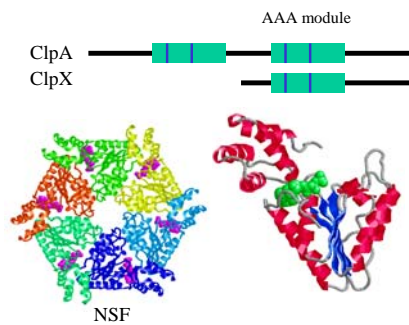
Asymmetric complex of Hsp90 dimer, cofactor and substrate protein



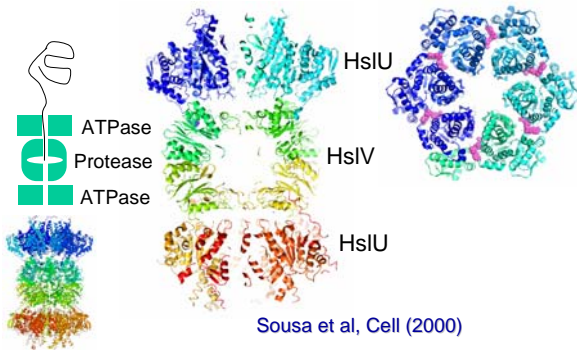
AAA Proteins

- A large and diverse family of ATPases associated with unfolding, unwinding, assembly and disassembly of protein and nucleic acid complexes (ATPases Associated with various cellular Activities)
- Includes the Hsp100/Clp family of chaperones/ proteases, components in DNA replication, recombination and restriction, the NSF protein in vesicle fusion, dynein motor proteins and many others
- Hsp100 proteins can totally unfold their substrate proteins, in order to deliver them to associated proteases, or they can dissolve large aggregates, in cooperation with the Hsp70 system

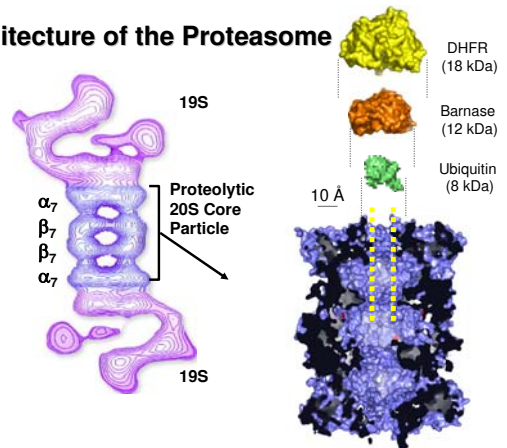
AAA ATPases



## Hsp100 chaperone-protease complexes



## Architecture of the Proteasome



## CHAPERONINS

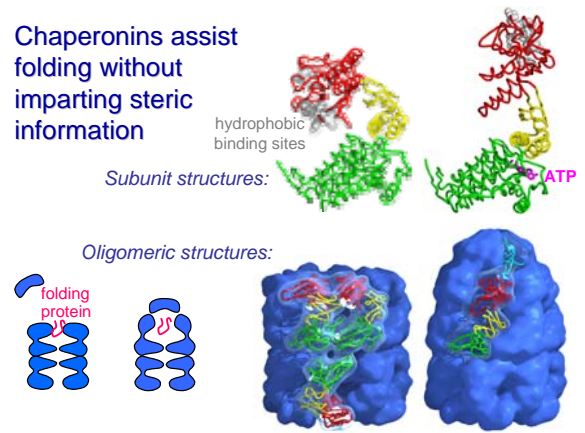
### Group I

- GroEL/Hsp60 - 14 x 60 kD subunits
- GroES/Hsp10 - 7 x 10 kD subunits
- found in eubacteria, mitochondria, chloroplasts
- very abundant and non-specific, will interact with most non-native proteins

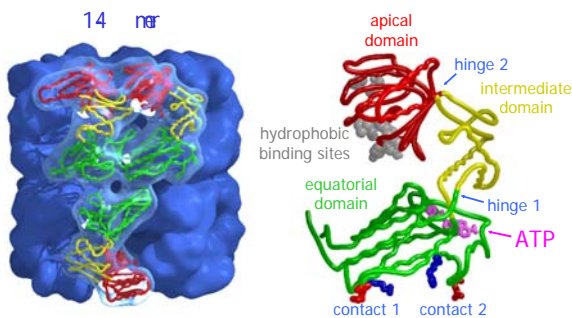
### Group II

- 16 or 18 x 55 kD subunits
- TF55 & thermosome in archaea
- TCP-1 in eukaryotic cytosol, >8 related gene products
- TCP-1 not very abundant, folds actin, tubulin, transducin, WD-40 domain proteins

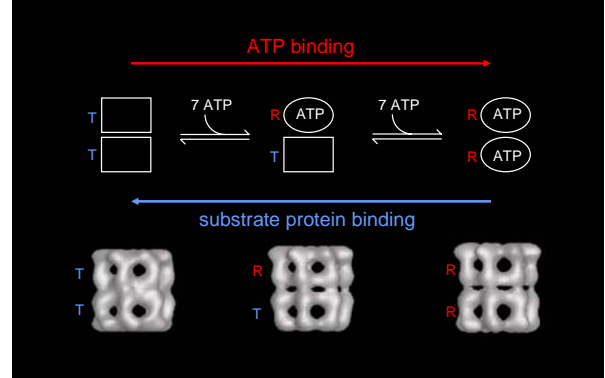
## Chaperonins assist folding without imparting steric information



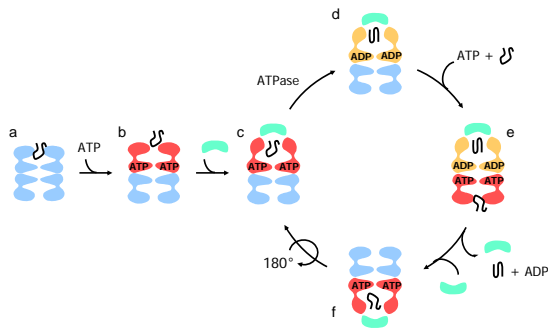
## *E. coli* GroEL



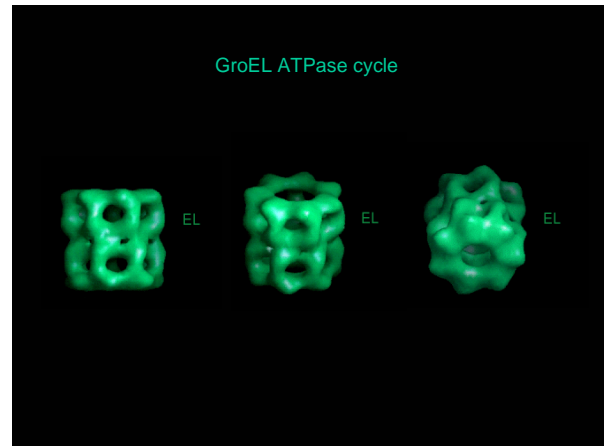
## Allosteric states in GroEL



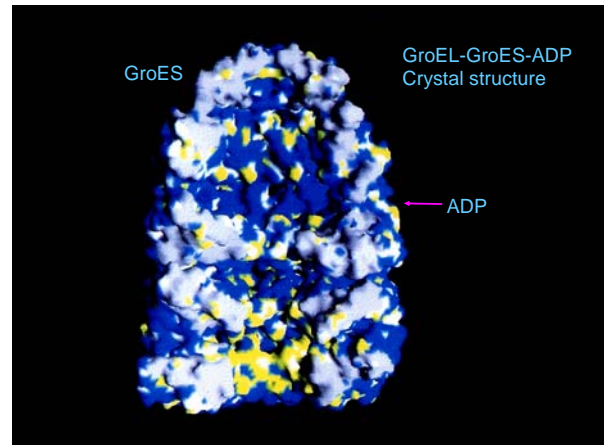
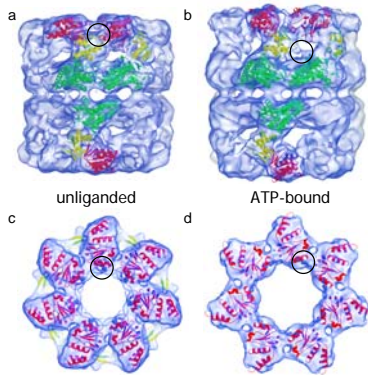
## The chaperonin functional cycle



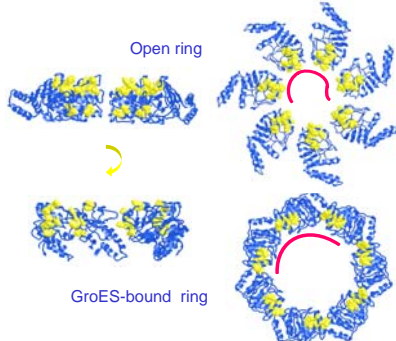
## GroEL ATPase cycle



## Conformational changes induced by ATP binding to GroEL

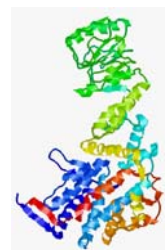


## Apical domain movements in GroEL



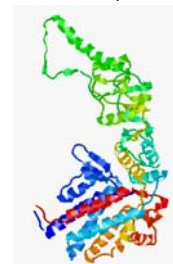
Rotation and separation of hydrophobic binding sites on GroEL could unfold a substrate protein bound to several sites (Shtilerman et al, 1999).

## Group I



GroEL subunit in GroES-bound conformation

## Group II

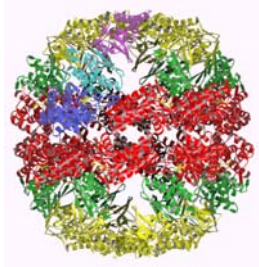


Thermosome subunit in closed conformation

GroEL GroES  
Group I



Thermosome  
Group II  
(conformation with  
both ends closed)



#### Molecular Chaperones: References

##### Reviews

Hartl, FU (1996) Molecular chaperones in cellular protein folding. *Nature* 381, 571-580.

Bukau & Horwich (1998) The Hsp70 and Hsp60 chaperone machines. *Cell* 92, 351-366.

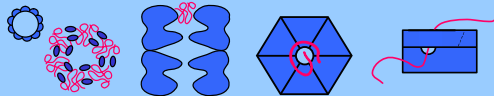
Sigler, PB, Xu, Z, Rye, H, Burston, SG, Fenton, WA & Horwich, AL (1998) Structure and function in GroEL-mediated protein folding. *Ann. Rev. Biochem.* 67, 581-608.

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Saibil, H & Ranson, N. (2002) The chaperonin molecular machine, *Trends in Biochem. Sci.* 27, 627-632 .

Bukau, B, Weissman, J, Horwich A (2006) Molecular chaperones and protein quality control. *Cell* 125, 443-451.

Research papers & additional references on lecture notes web site:  
[people.cryst.bbk.ac.uk/~ubcg16z/hsplec.html](http://people.cryst.bbk.ac.uk/~ubcg16z/hsplec.html)



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