

C22: Molecular Chaperones

Helen Saibil

Department of Crystallography

Birkbeck College

h.saibil@mail.cryst.bbk.ac.uk

Lecture notes at

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

Central Dogma

DNA → RNA → Protein sequence → Protein fold

Proline cis-trans isomerisation
Disulfide bond formation
Glycosylation
Proteolysis
FOLDING

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.

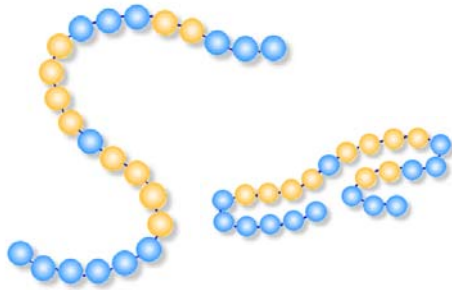
Examples of molecular chaperones

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

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

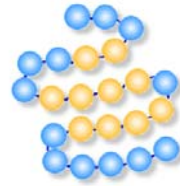
Nucleoplasmin: nucleosome assembly

Prosequences: subtilisin, α -lytic protease (intramolecular chaperones)



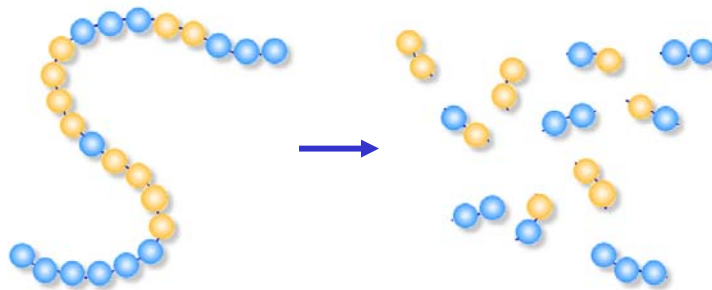
Unfolded or misfolded proteins

Aggregation-prone
Protease-sensitive
Bind chaperones
Non functional

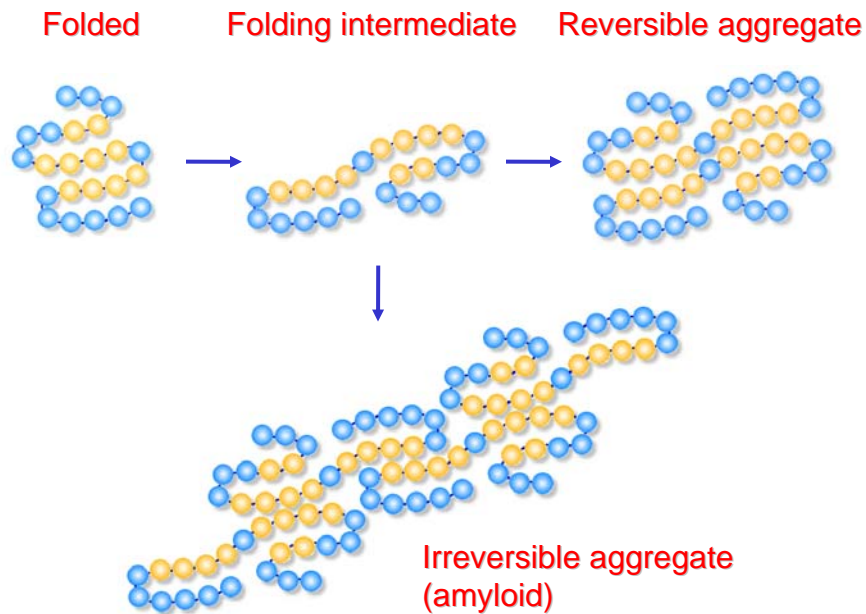


Globular soluble protein

Stable and soluble
Protease resistant
No chaperone binding
Functional



Unfolded protein chains are prone to proteolysis



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

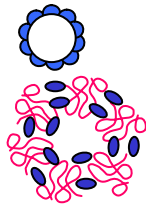
Calnexin, calreticulin

- glycoprotein maturation in the ER
- quality control

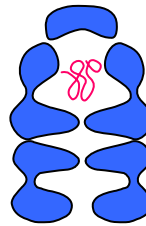
Folding catalysts: PDI, PPI [folders]

Prosequences: alpha-lytic protease, subtilisin (intramolecular chaperones) [folders]

Molecular chaperones

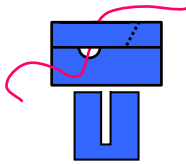


Small heat shock proteins
Sponges for denatured protein



Chaperonins
Hsp60/Hsp10
GroEL/GroES
Nascent chain folding/refolding

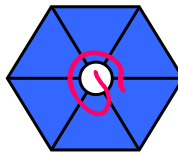
ER chaperones
Disulfide, prolyl isomerases;
oligosaccharide processing



Hsp70
Folding/unfolding
Translocation/
disaggregation



Hsp90
Stabilize steroid receptors/kinases;
Buffer genetic variation?

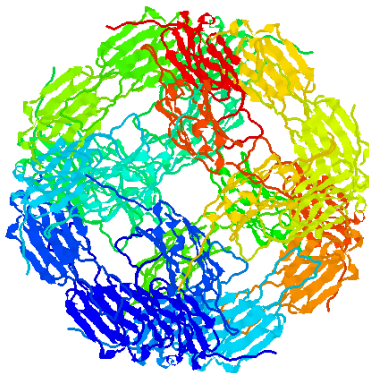


Hsp100
Clp ATPases
AAA+
Unfolding/
disaggregation

Small heat shock proteins



A dimer of β -sandwich folds
forms the conserved building
block of small hsps

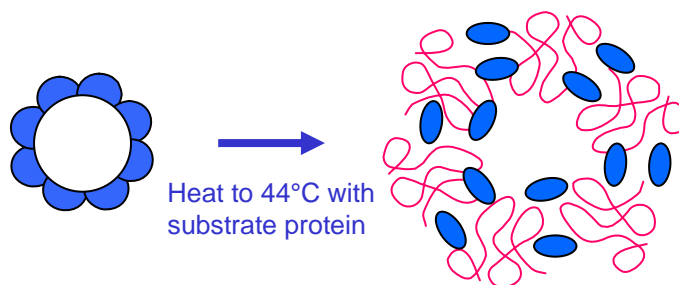


Crystal structure of sHsp from *Methanococcus jannaschi* (Kim et al, 1998)

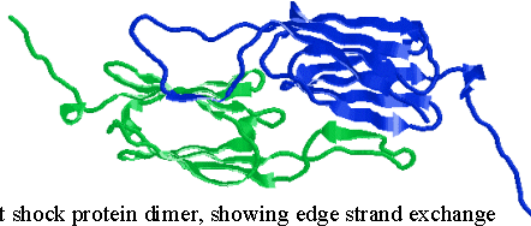
Small Hsp function

- Superfamily includes the eye lens protein α -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

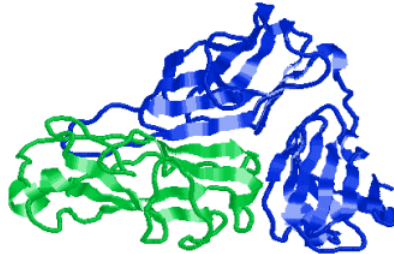
Formation of substrate complexes



Immunoglobulin fold chaperones



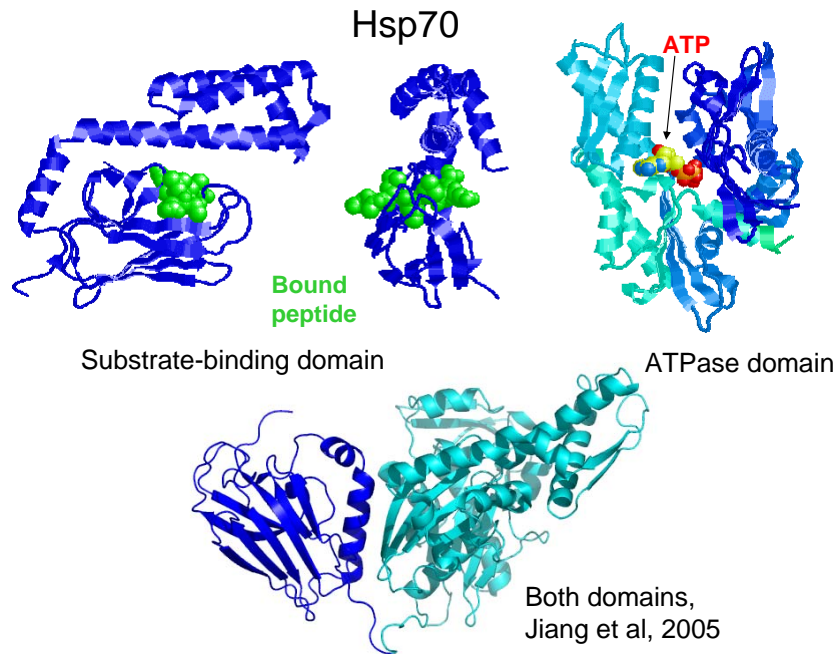
Small heat shock protein dimer, showing edge strand exchange



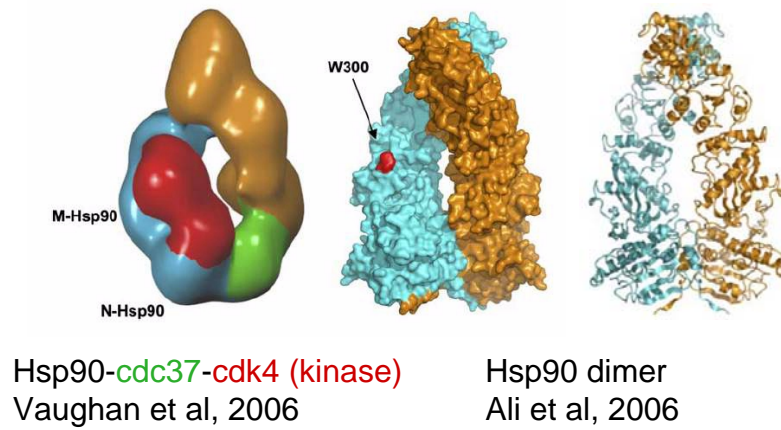
Chaperone papD (blue) complexed with papK (green), subunit of the bacterial pilus, showing edge strand donation. In the assembled pilus, each papK subunit donates an edge strand to its neighbour.

HSP70 FAMILY

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



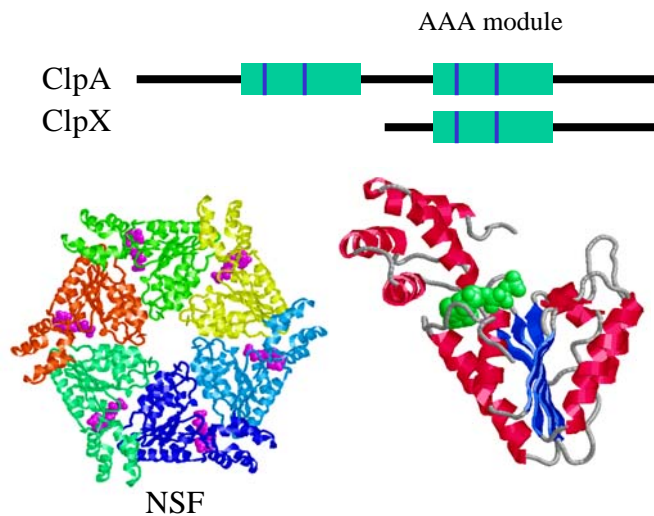
Asymmetric complex of Hsp90 dimer,
cofactor and substrate protein



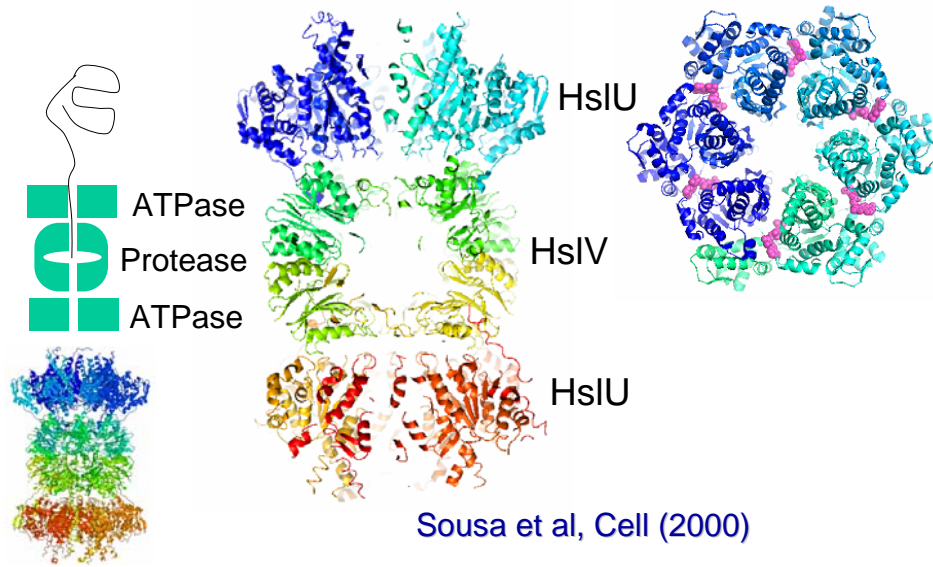
AAA Proteins

- A large and diverse family of ATPases associated with unfolding, unwinding, assembly and disassembly of protein and nucleic acid complexes (**A**T**P**ases **A**ssociated with various cellular **A**ctivities)
- 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

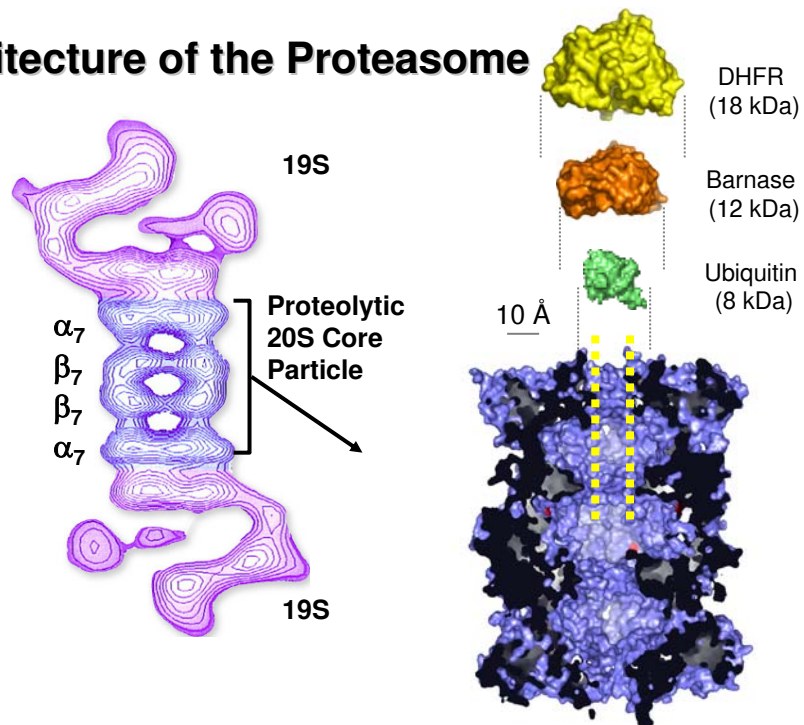
AAA ATPases



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

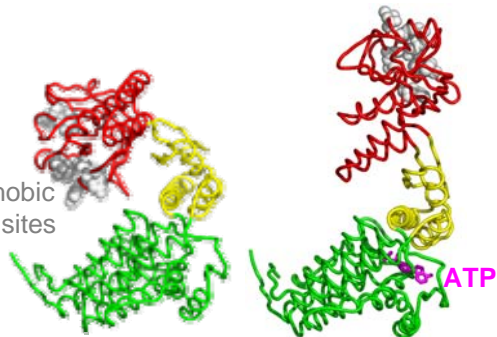
Group II

- 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

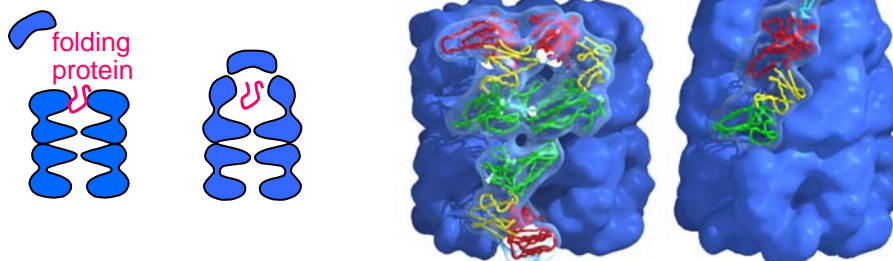
Chaperonins assist folding without imparting steric information

hydrophobic binding sites

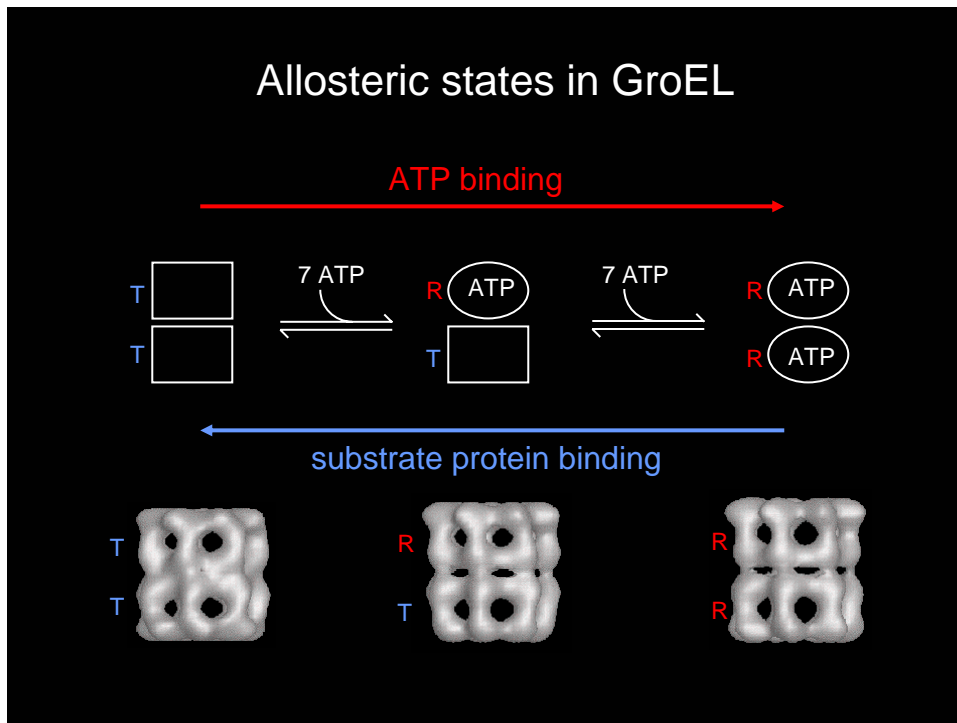
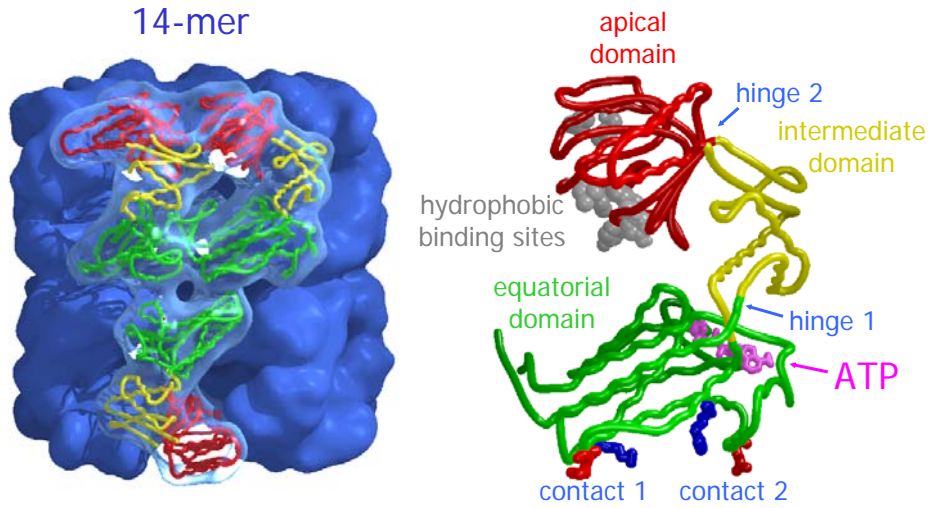
Subunit structures:



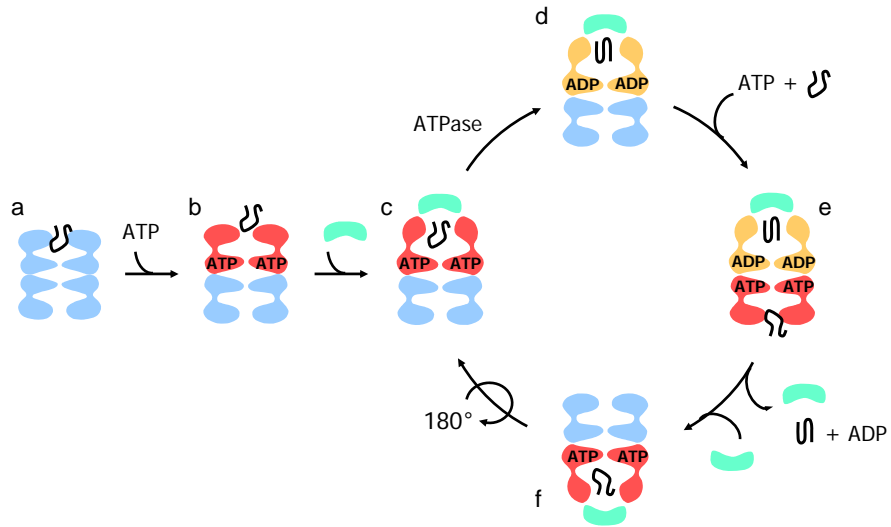
Oligomeric structures:



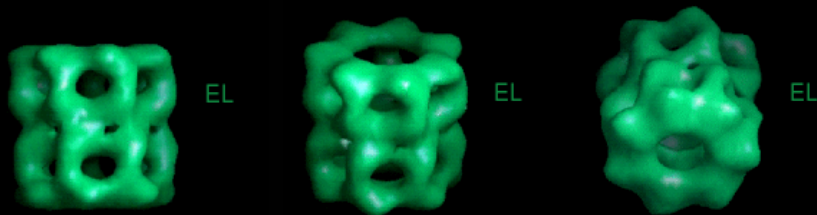
E. coli GroEL



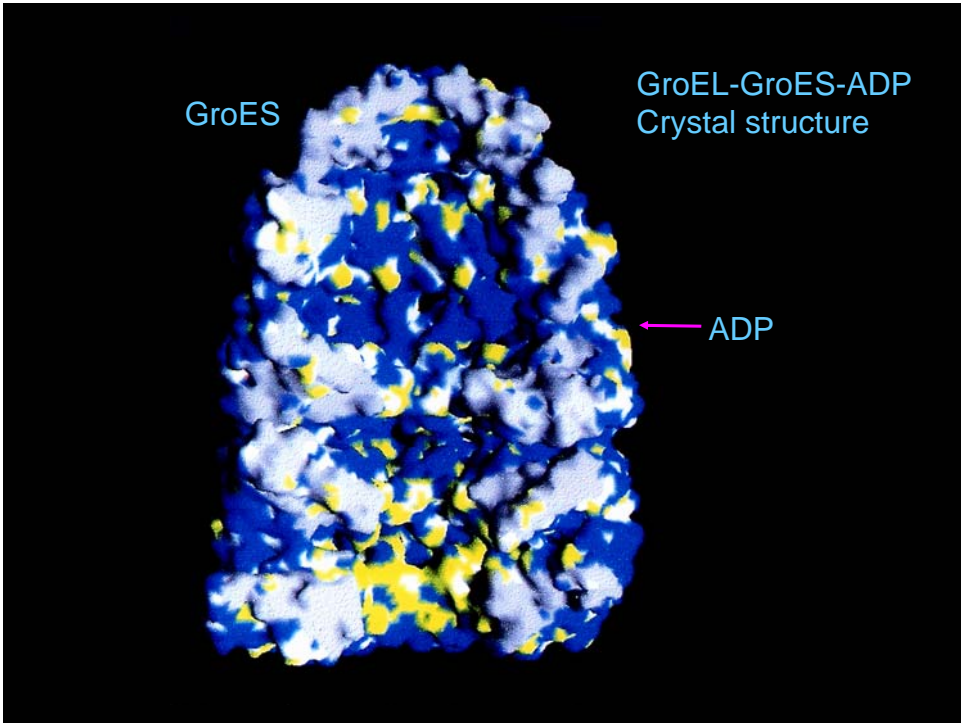
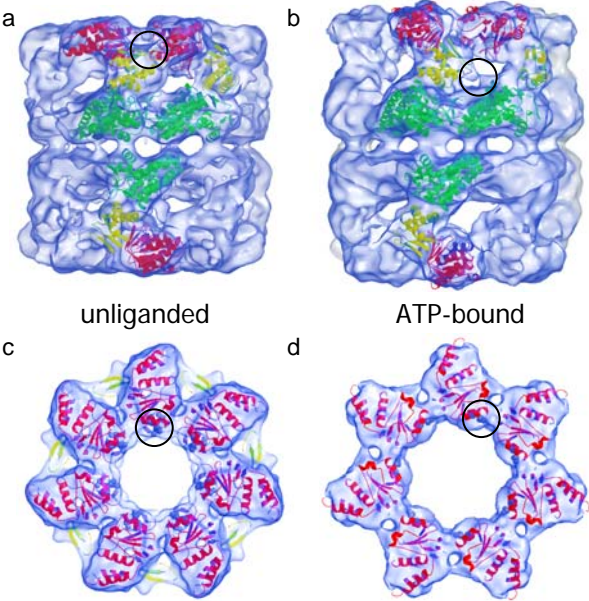
The chaperonin functional cycle



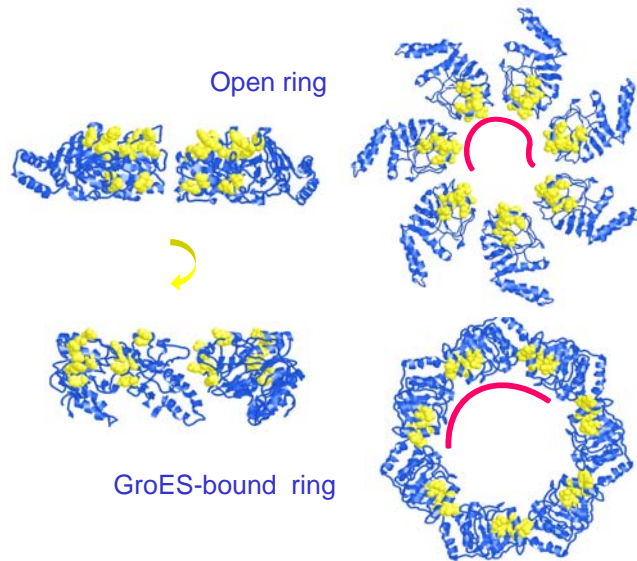
GroEL ATPase cycle



Conformational changes induced by ATP binding to GroEL

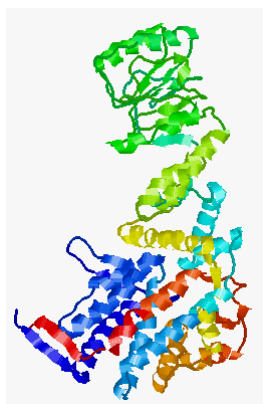


Apical domain movements in GroEL



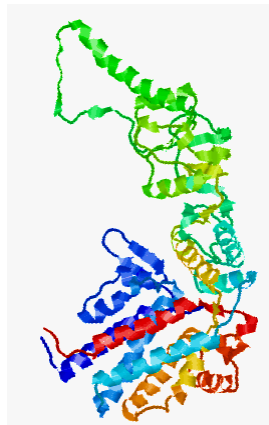
Rotation and separation of hydrophobic binding sites on GroEL could unfold a substrate protein bound to several sites (Shtilerman et al, 1999).

Group I



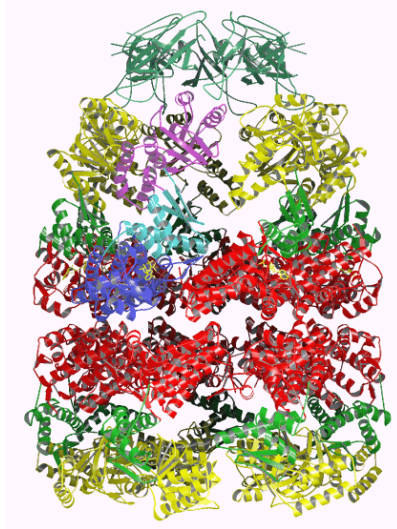
GroEL subunit in GroES-bound conformation

Group II

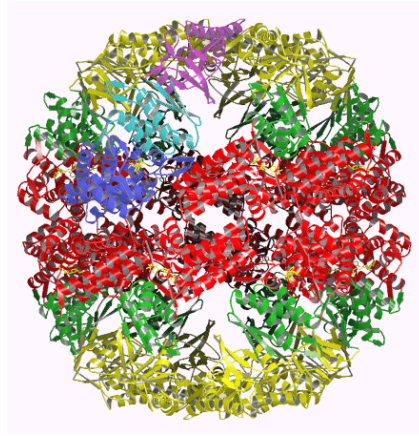


Thermosome subunit in closed conformation

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.

Saibil, H. (2000) Molecular chaperones: containers and surfaces for folding, stabilising or unfolding proteins. *Current Opinion in Struct. Biol.* 10, 251-258.

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