The HSP90 chaperone machinery

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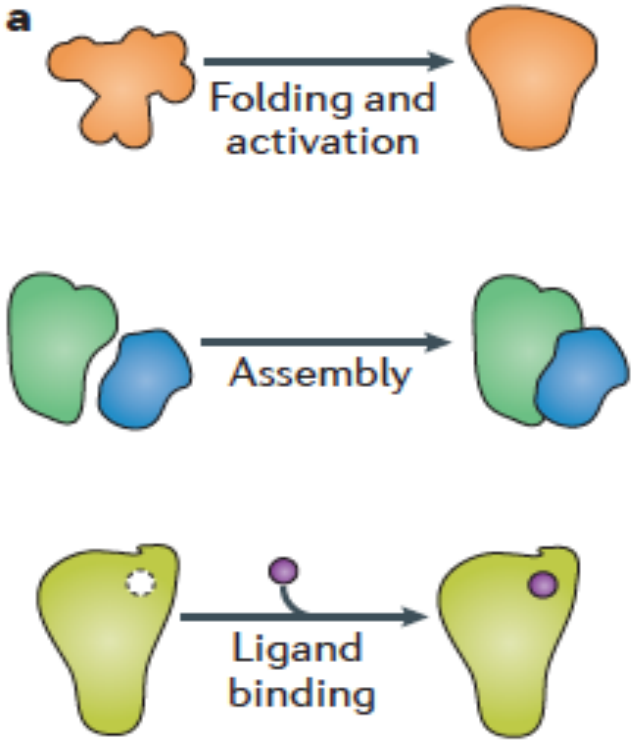
Key concepts we already know:

- ✓ Molecular chaperones are essential, as they guarantee proteostasis and thus cellular homeostasis;
  This is especially important under stress conditions such as a sudden increase in temperature, which is termed 'heat shock';
- ✓ Molecular chaperones are defined as proteins that interact with other proteins —**substrates or clients** — and help them to acquire a functionally active form, and that dissociate from the client once the final active structure is formed.
- ✓ They were also found to be important for various cellular processes under physiological conditions: an example is the signalling of steroid hormone receptors, which are eukaryotic transcription factors

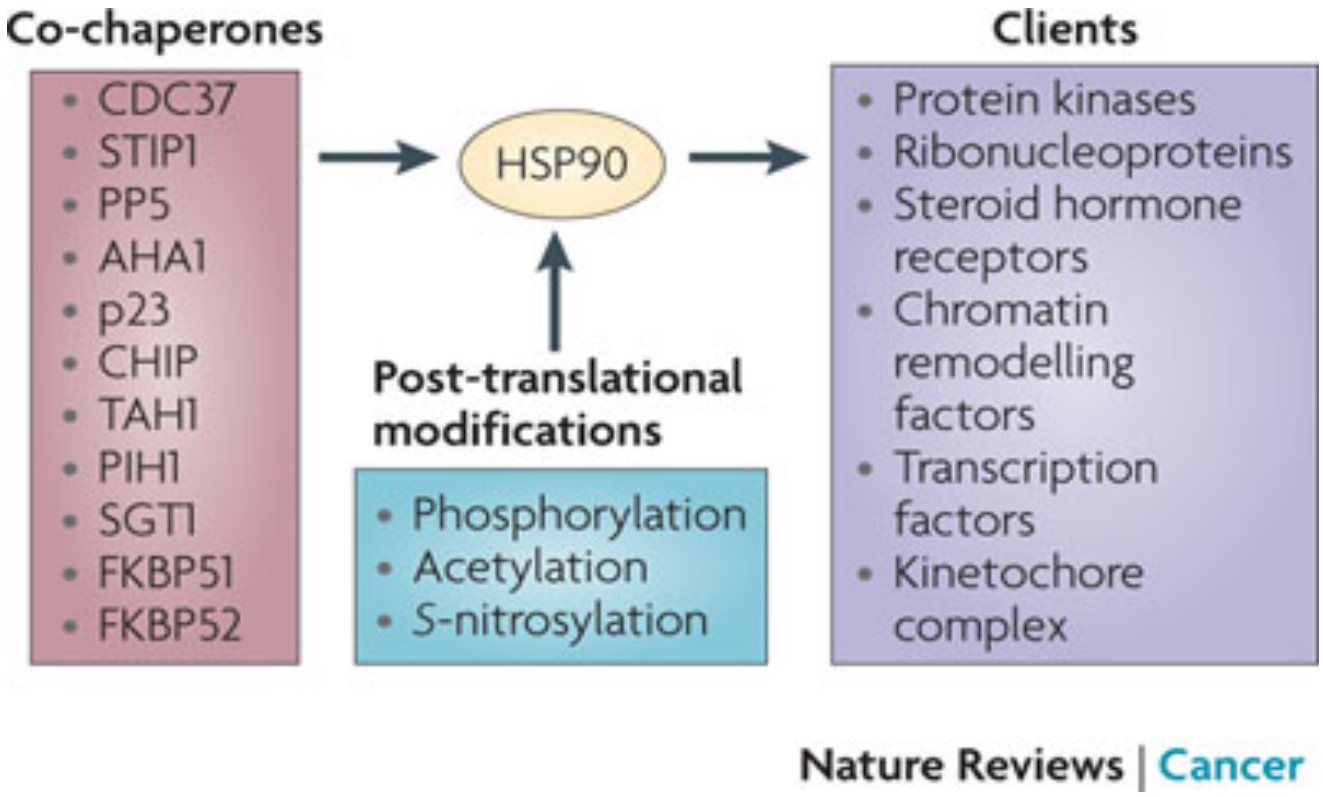
HSP90 is unique because in eukaryotes it is the central component of a machinery that comprises a large number of cofactors, which bind reversibly to HSP90, thus establishing a flexible unit for the conformational control of many different cellular proteins.

The HSP90 machinery **evolved to control protein function and activity** by facilitating protein folding, the binding of ligands to their receptors or targets, or the assembly of multiprotein complexes



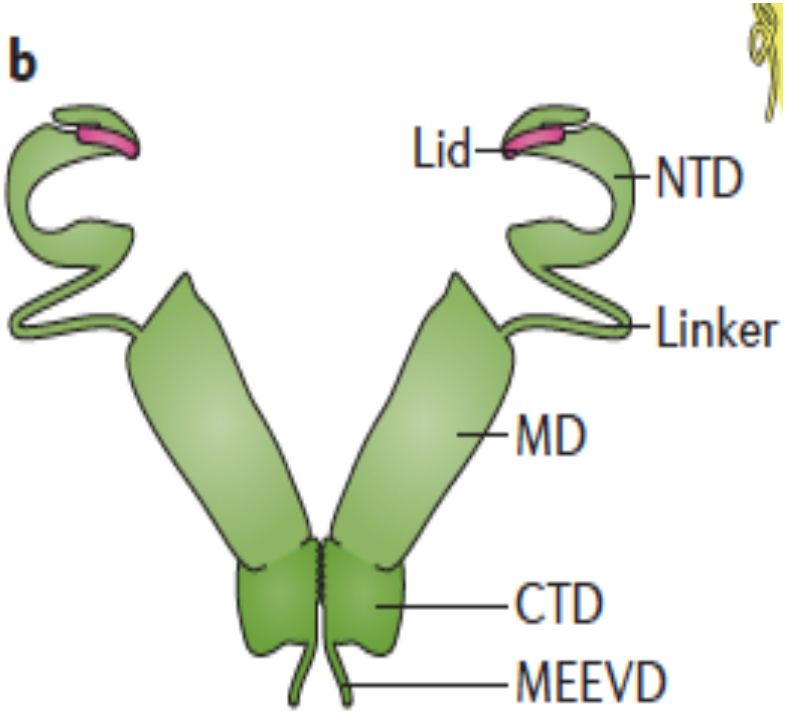
Schematic representation of the ways in which heat shock protein 90 (HSP90) can affect 'clients' (that is, substrates). HSP90 can facilitate **protein folding** (top panel), the **assembly** of multiprotein complexes (middle panel) or the **binding of a ligand** to its target or receptor (bottom panel).

In this lecture, we outline the **current understanding** of the structure and function of the **HSP90 machinery**, including its **co-chaperones**, the post-translational modifications (**PTMs**) that regulate its activity, its **interactions** with and **processing** of clients, its **involvement** in disease and its **prospects** as a target for therapeutic intervention


Co-chaperones
CDC37
STIP1
PP5
AHA1
p23
CHIP
TAH1
PIH1
SGT1
FKBP51
FKBP52
HSP90
Post-translational modifications
Phosphorylation
Acetylation
S-nitrosylation
Clients
Protein kinases
Ribonucleoproteins
Steroid hormone receptors
Chromatin remodelling factors
Transcription factors
Kinetochore complex
Nature Reviews | Cancer

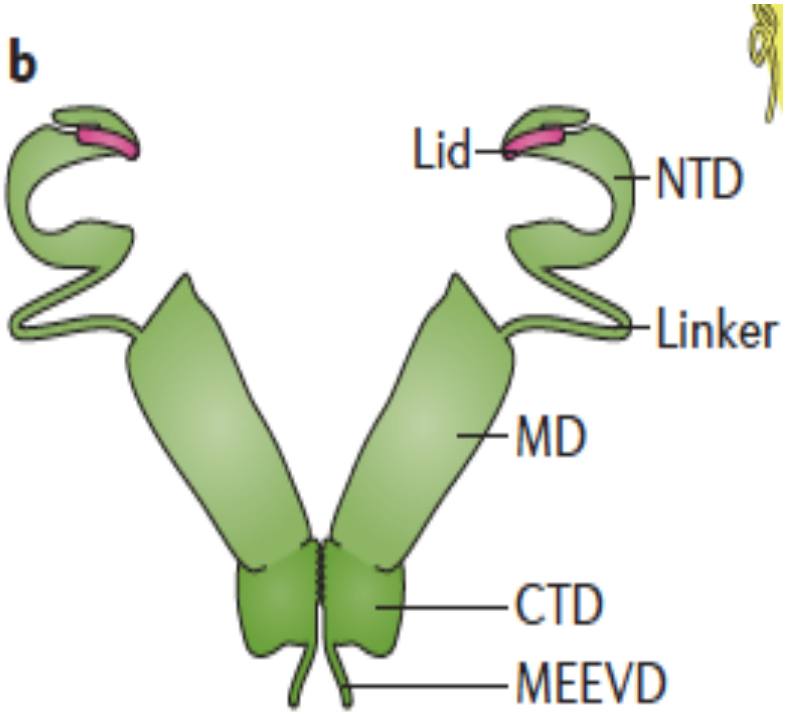
The structure and conformational cycle of HSP90

HSP90 functions as a **homodimer**, and dimerization is essential for its function in vivo. An HSP90 monomer consists of three highly conserved domains: the amino-terminal domain **(NTD)**, which mediates binding to ATP; the middle domain **(MD)**, which is important for ATP hydrolysis and the binding of HSP90 to clients; and the carboxy-terminal domain **(CTD)**, which is responsible for HSP90 dimerization



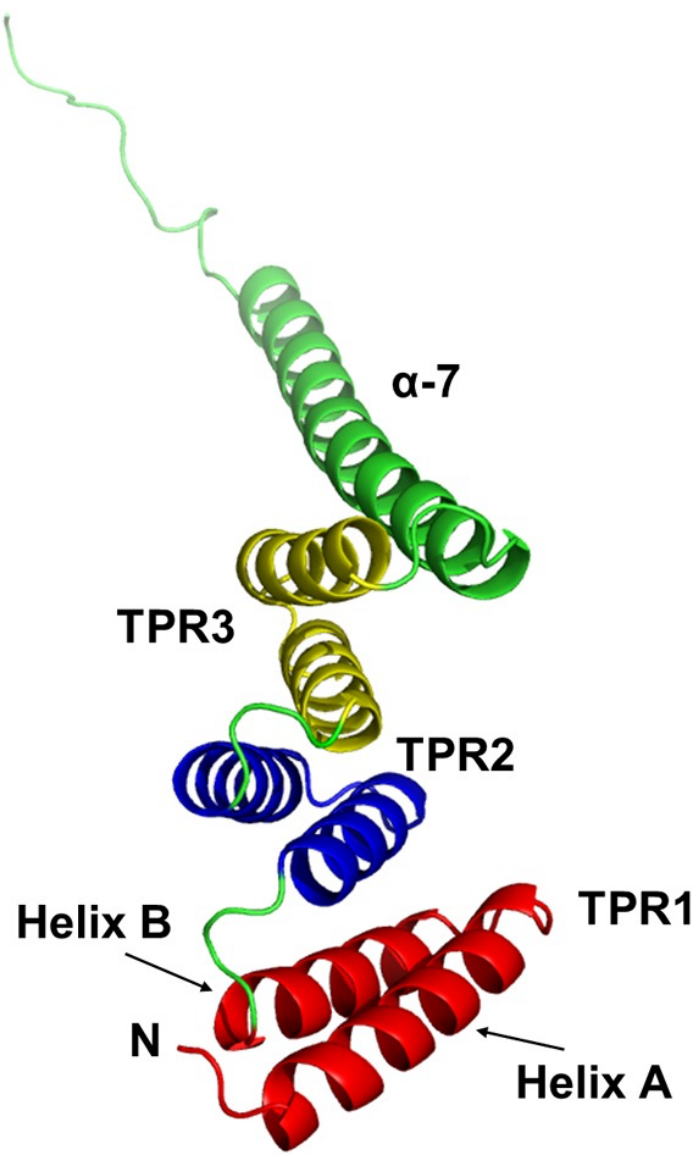
It also contains a C-terminal Met-Glu-Glu-Val-Asp **(MEEVD)** motif, which is important for the interaction with co-chaperones that contain tetratricopeptide repeat **(TPR)** domains**

The NTD and the MD are connected by a long, flexible, charged linker that modulates NTD–MD contacts and affects HSP90 function



TPR motifs are **minimally conserved (*degenerate and variable*) **34-residue-long regions.** The TPR sequence is centered around the **consensus residues W4-L7-G8-Y11-A20-F24-A27-P32.**

Although no positions are completely invariant, a consensus sequence pattern of small and large hydrophobic residues has been defined; small hydrophobic residues are commonly observed at positions 8, 20, and 27, while large ones are at 4, 7, and 24



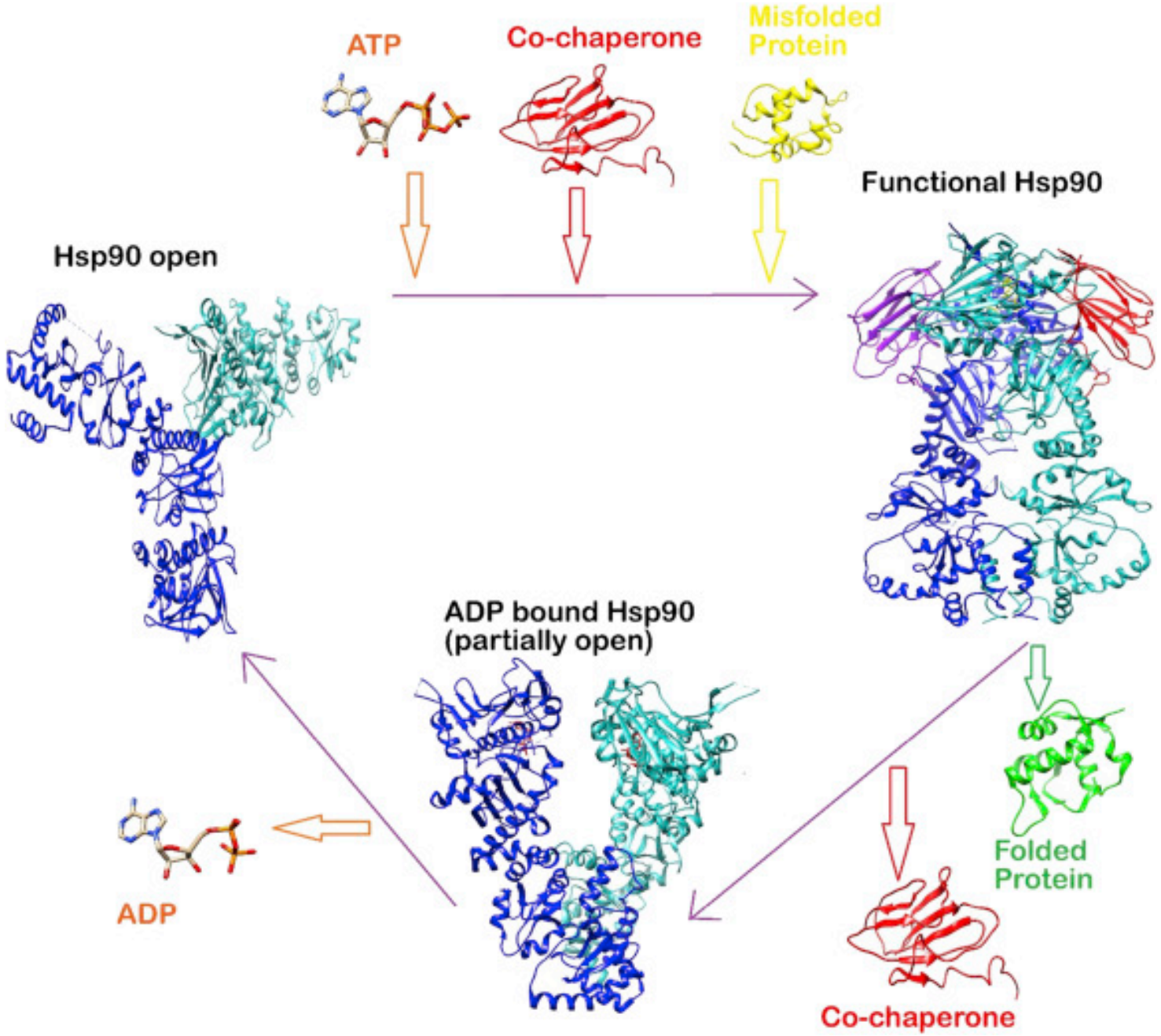
In the **absence of ATP**, HSP90 adopts mainly a V-shaped open conformation. It undergoes large, ATP-regulated conformational rearrangements that lead to an **N-terminally closed state via intermediate steps.**

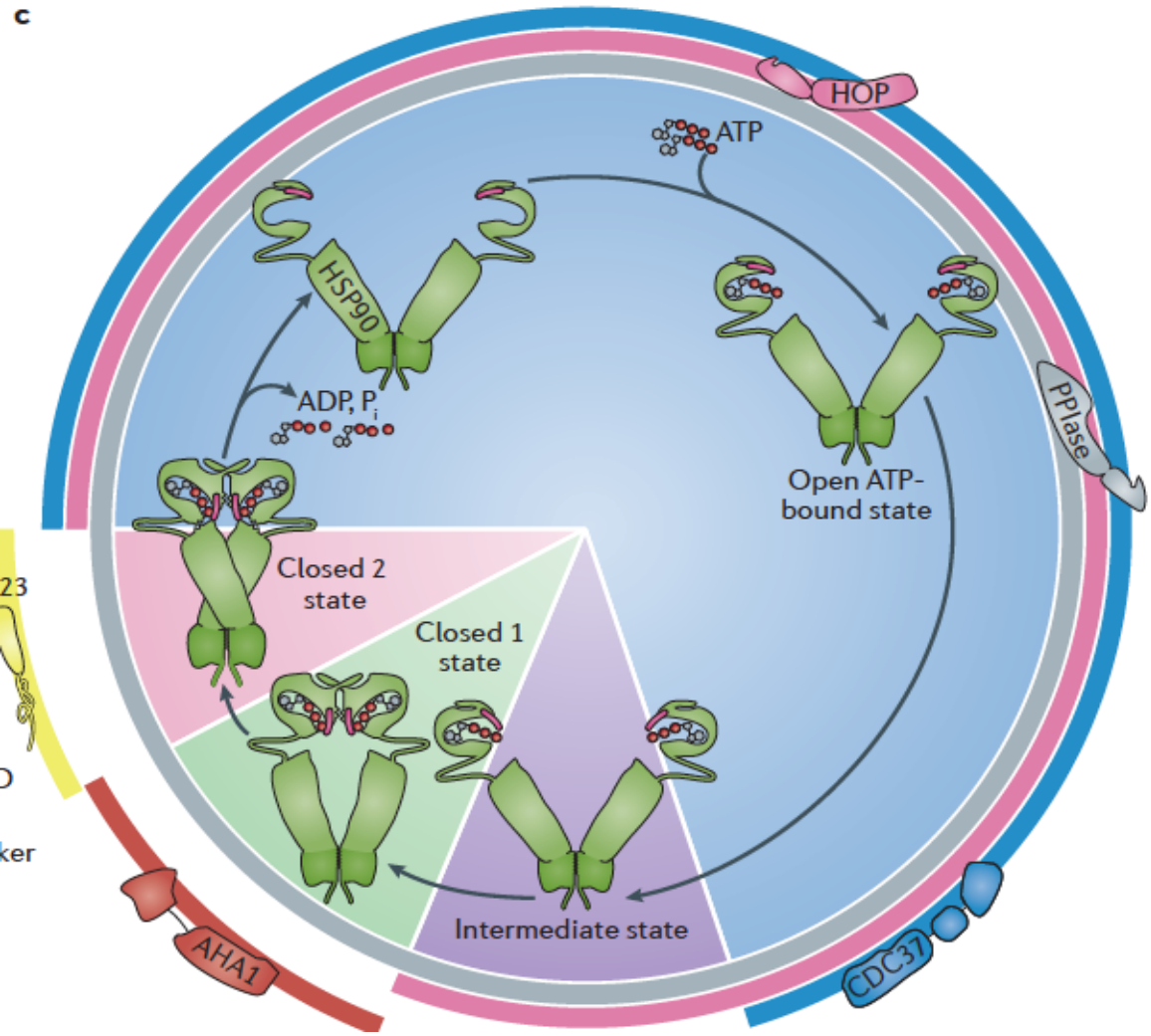
HSP90 is a member of the gyrase, HSP90, His kinase and MutL (GHKL) superfamily of '**split ATPases**'.

The ATP-binding site in the NTD must interact with the MD in order for ATP hydrolysis to occur. HSP90 has inherently low enzymatic activity: its affinity for ATP is very low (with a dissociation constant (Kd) of approximately 400 μM), and it only hydrolyses 0.1 ATP min–1 in humans and 1 ATP min–1 in yeast.

The conformational changes that lead to the closed state, in which formation of the split ATPase domain is completed, are the rate-limiting steps of this slow reaction

These large and dynamic conformational rearrangements affect all three domains and also involve interactions between the NTDs of the two monomers.

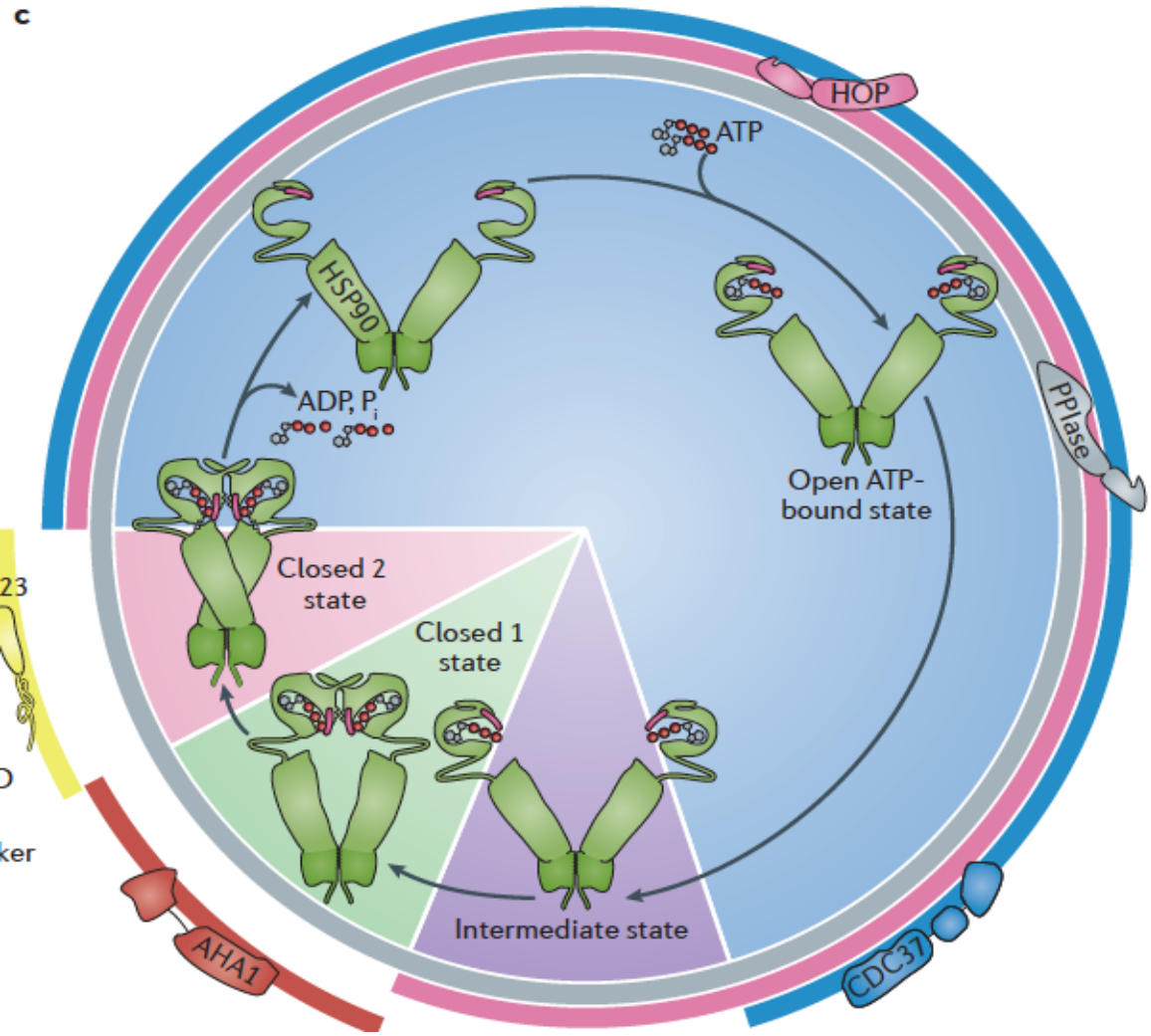

ATP
Co-chaperone
Misfolded Protein
Functional Hsp90
Hsp90 open
ADP bound Hsp90 (partially open)
ADP
Folded Protein
Co-chaperone



The HSP90 conformational cycle and the action of co-chaperones in different parts of the cycle.

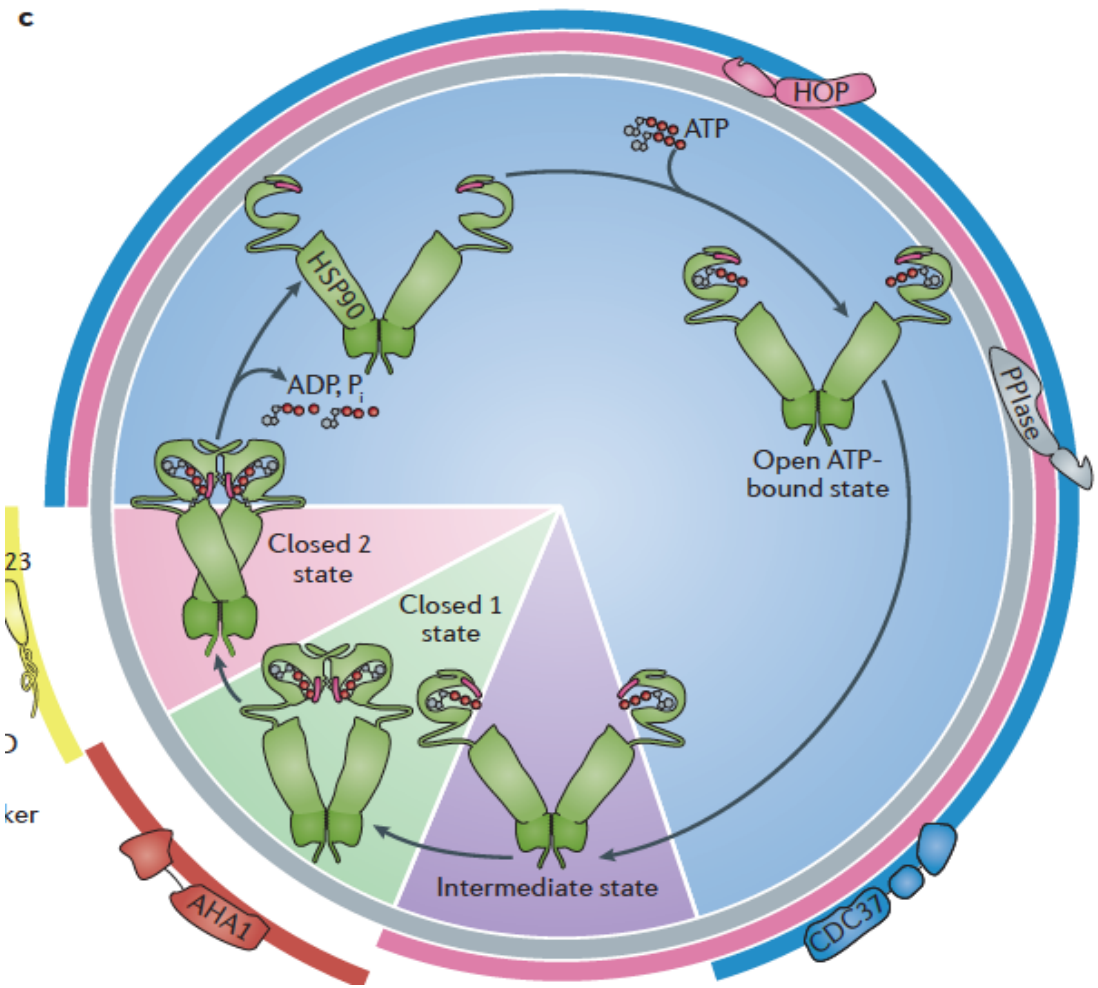
HSP90 remains in different states for different amounts of time.

HSP90 transitions from the open ATP-bound state to the intermediate state after ATP binding and closure of the 'lid', which is followed by an interaction between the NTDs of the monomers (closed 1 state) and twisting of the HSP90 monomers (closed 2 state).



Upon ATP binding, rearrangements take place in the NTD, and a loop containing several conserved amino acids, termed the 'lid' region, closes over the ATP-bound state, leading to the intermediate state.

Further structural rearrangements induce a closed state in which the NTDs dimerize (closed 1 state) and then associate with the middle domains (closed 2 state).



NTD dimerization and formation of the closed states are crucial for ATP hydrolysis, including the repositioning of a catalytic loop in the MD.
After ATP hydrolysis, the NTDs dissociate, ADP and inorganic phosphate (Pi) are released and HSP90 returns to the open conformation.

The conformational cycle appears to occur in a similar way in the presence or absence of client proteins

Regulation of HSP90 function

HSP90 function is regulated in many ways, which include **expression, PTMs, co-chaperones and clients.**

Transcriptional regulation.

The expression of HSP90 is induced by the stress-related transcription factor **heat shock factor 1** (HSF1), which is also an HSP90 client.

The current model suggests that, together with HSP70, Hsp90 binds to Hsf1 to keep it in an inactive state. When chaperone proteins are required for other functions and are no longer available for Hsf1 inhibition, the transcription of heat shock genes increases. **Thus, HSP90 links the stress status of the cell directly to the expression of heat shock proteins.**

Post-translational modifications.

Consistent with its function as a hub for integrating diverse signals, HSP90 function is modulated by numerous PTMs, which include **phosphorylation, sumoylation, acetylation and S-nitrosylation**

In general, phosphorylation was reported to slow down the HSP90 conformational cycle and to affect client maturation and the interaction with co-chaperones.

The picture emerging from the analysis of PTMs at different sites in HSP90 is that, in addition to local effects such as regulating the accessibility of binding sites, several of the modified sites function as allosteric switch points that regulate interdomain communication across the HSP90 dimer

HSP90 co-chaperones

Co-chaperones are the **most important regulators** of HSP90

Co-chaperone	Interacting domain in co-chaperone	Binding site in HSP90	Function
HOP (Sti1 in yeast)	TPR2A, TPR2B	Mainly MEEVD; also MD and CTD	Stabilizes the HSP90 open conformation; transfers clients from HSP70–HSP40 to HSP90
CDC37	MD, CTD	NTD, MD	Prevents closure of the 'lid' in HSP90; specific for maturation of kinases
SGTA (Sgt1 in yeast)	CS	NTD	Involved in NLR maturation in plants and kinetochore assembly in yeast
Tah1 (Spaghetti in *Drosophila melanogaster*)	TPR	MEEVD	Component of the Rvb1–Rvb2–Tah1–Pih1 (R2TP) complex
AHA1	NTD, CTD	NTD, MD	Stimulates ATPase activity of HSP90
PP5 (Ppt1 in yeast)	TPR	MEEVD	Phosphatase that dephosphorylates HSP90; maturation of kinases
FKBP51 and/or FKBP52	TPR	MEEVD	General co-chaperones
CHIP	TPR	MEEVD	E3 ubiquitin ligase
CYP40 (Cpr6 and Cpr7 in yeast)	TPR	MEEVD	PPIase; maturation of the glucocorticoid receptor
p23 (Sba1 in yeast)	CS	NTD, MD	Inhibition of HSP90 ATPase activity; stabilization of the HSP90 closed 2 state
TTC4 (Cns1 in yeast)	TPR	MEEVD	Interacts with CDC6
Unc45	TPR	MEEVD	Myosin-dependent processes

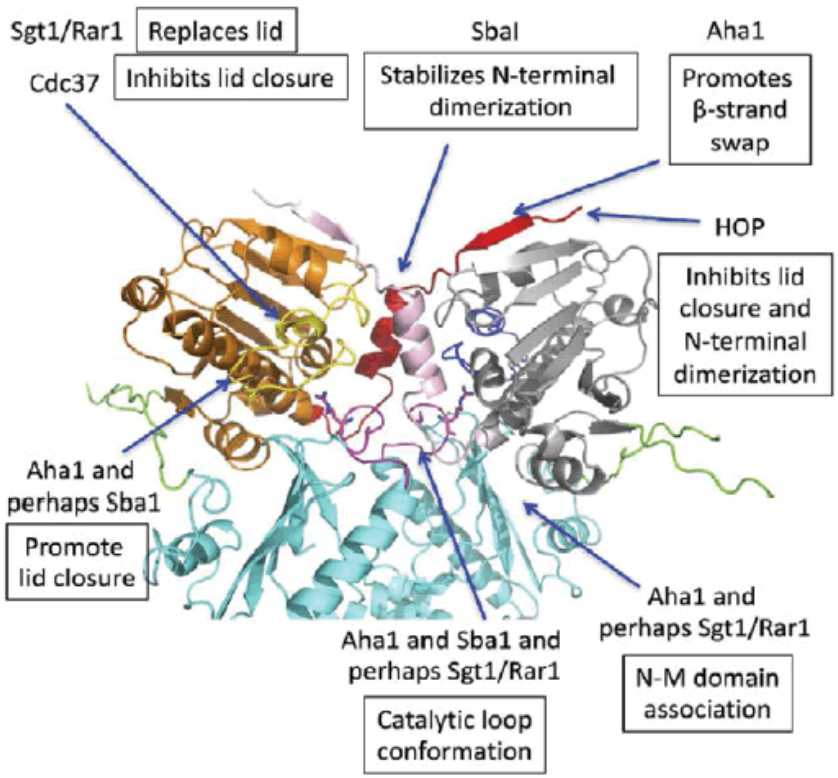
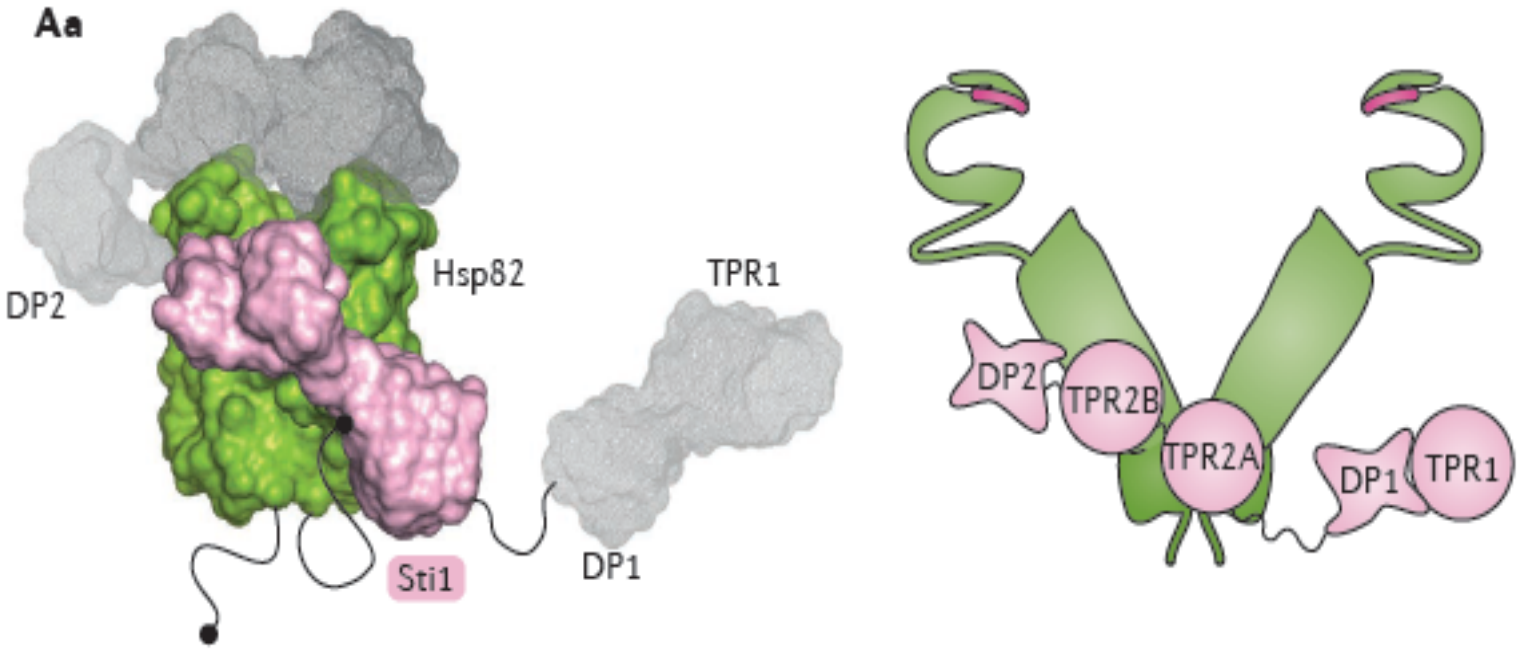


Figure 3 Points of co-chaperone action on structural elements of Hsp90 essential for its ATPase activity. Cdc37^{p50} prevents molecular rearrangement of the lids of Hsp90. HOP may prevent lid closure and N-terminal dimerization probably by interacting with the N-terminal segments of Hsp90. Aha1 appears to interact with all the structural elements leading to a co-operative N-terminally dimerized state of Hsp90. Sba1 can stabilize Hsp90 complexes by reducing the ATPase activity of Hsp90 and it appears to interact with both the lid and N-terminal domains of Hsp90. Sba1 may also modulate the middle domain catalytic loop. Sgt1-Rar1 complex, appear to activate Hsp90 in an open state and convert it to a stable ADP-bound state.

Binding sites for co-chaperones have been identified in all three domains of HSP90 and cover a significant part of its surface



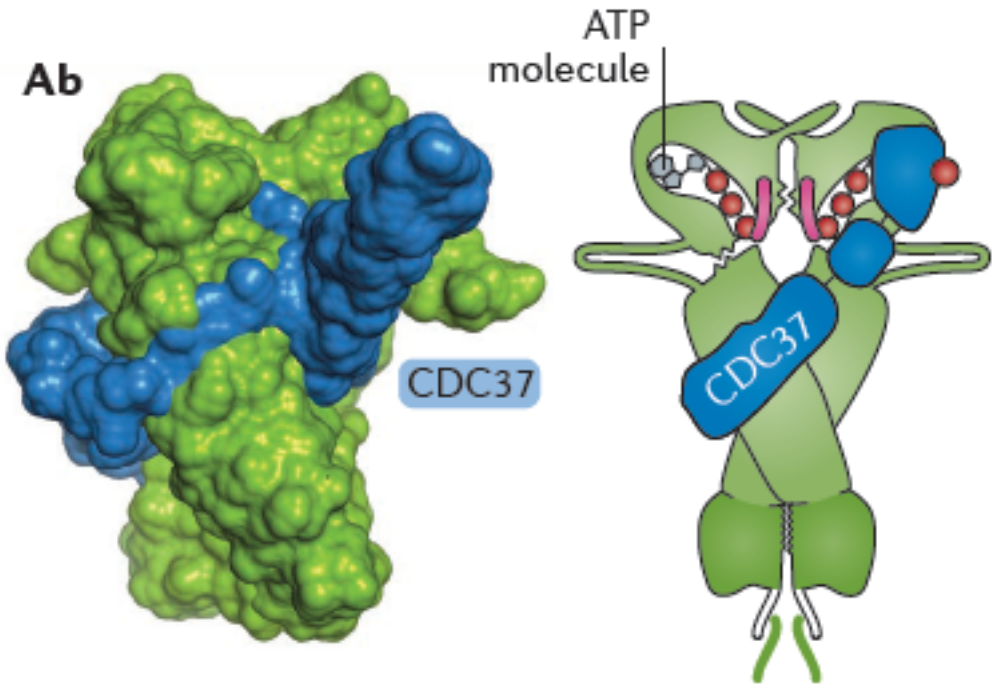
co-chaperones containing Tetratricopeptide repeat domain

Several co-chaperones that contain a TPR domain bind to the MEEVD peptide in the CTD of HSP90.

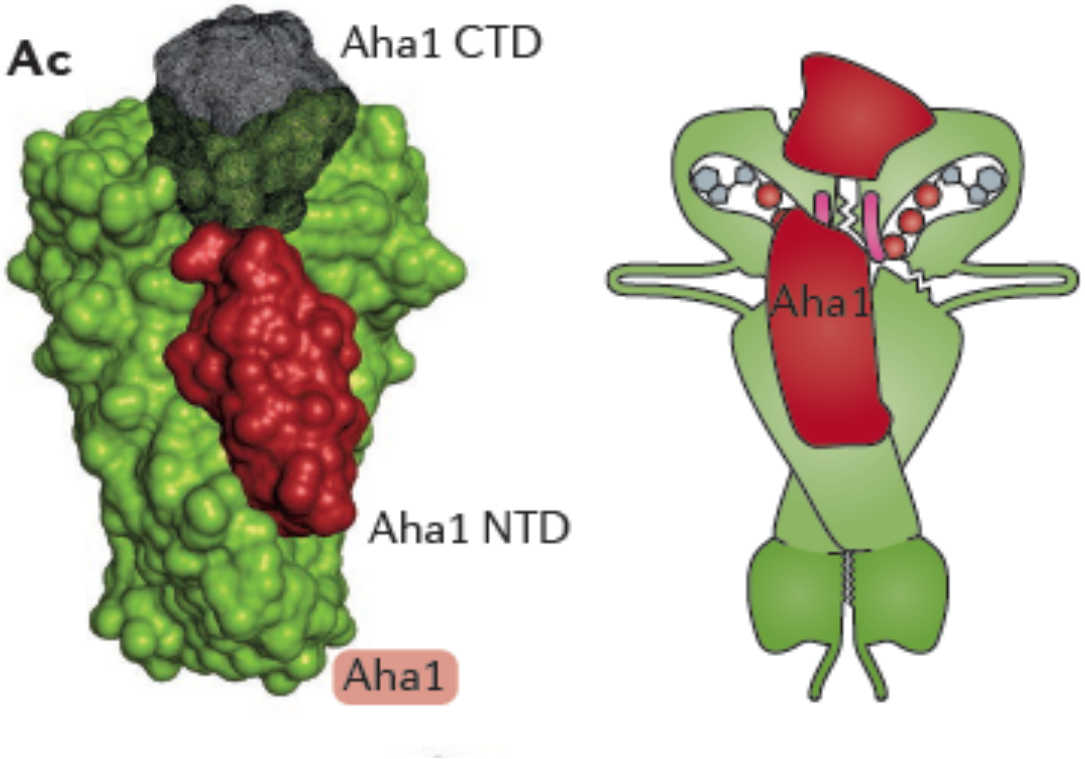
TPR domains consist of repeats of a 34-residue TPR motif, which forms two antiparallel α-helices that are separated by a turn. Three helix-turn-helix motifs stack on each other to form a super-helical groove for peptide binding.
One of the best-characterized TPR domain-containing co-chaperones is **HSC70/ HSP90-organizing protein (HOP**; Sti1 in yeast), which binds to both HSP70 and HSP90.

Co-chaperones without tetratricopeptide repeat domains

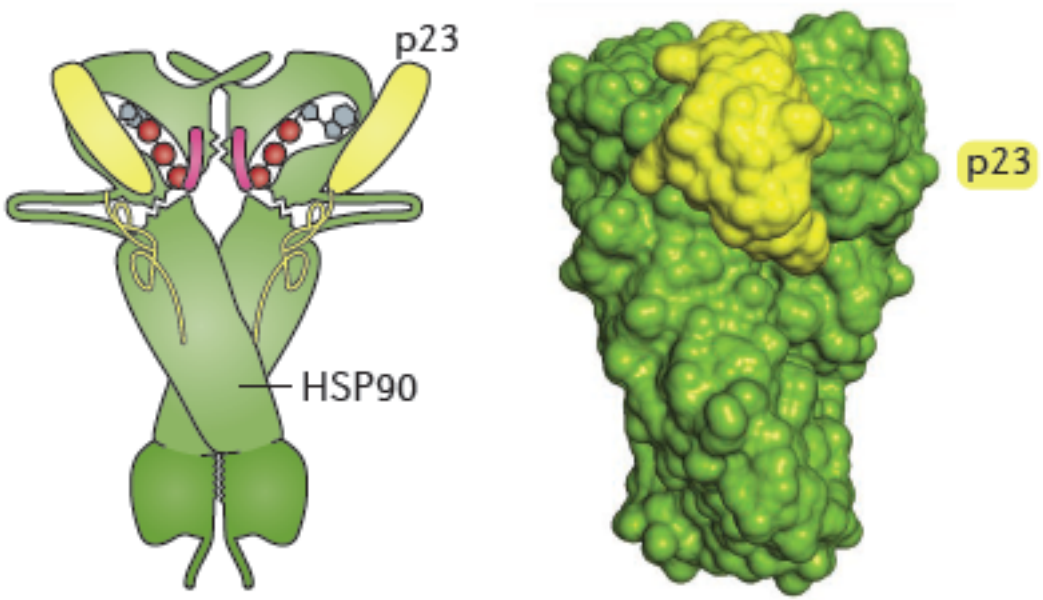
In contrast to the co-chaperones mentioned above, **CDC37** seems to be dedicated to the maturation of kinases. It binds to kinases and also to the NTD of HSP90, which leads to partial inhibition of the HSP90 ATPase activity



In contrast to HOP/Sti1 and CDC37, **AHA1** is a **strong activator** of the HSP90 ATPase activity. It promotes the formation of the closed 1 state of Hsp90. AHA1 binds to HSP90 in the MD as well as in the NTD in an asymmetric manner, and one AHA1 molecule per HSP90 dimer is sufficient to stimulate the ATPase activity of HSP90. This may modulate the amount of time that client proteins spend interacting with HSP90



p23/Sba1 is a co-chaperone that acts at a late stage of the chaperone cycle. It stabilizes the closed 2 conformation of HSP90 by binding in a groove that is formed between the dimerized NTDs of HSP90. p23/Sba1 reduces the ATPase activity of HSP90 and thus regulates the progression of the reaction cycle, which is beneficial for the maturation of steroid hormone receptors (SHRs)



In general, co-chaperones seem to broaden the functional range of HSP90.

The range of HSP90 clients

Several hundred proteins are clients of HSP90, which makes HSP90 a central modulator of important processes that range from stress regulation and protein folding to DNA repair, development, the immune response, neuronal signalling and many other processes (TABLE)

Table 2 | **The range of HSP90 clients**

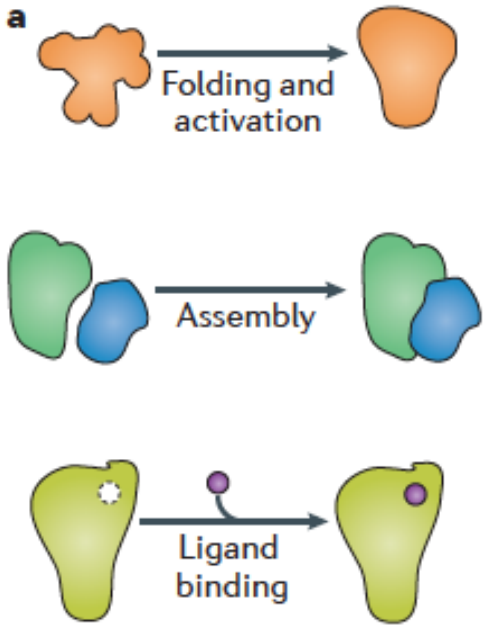
Client	Function	Diseases
Steroid hormone receptors		
Glucocorticoid receptor	Response to glucocorticoids	Cushing syndrome
Mineralocorticoid receptor	Response to mineralocorticoids	Chronic kidney disease
Progesterone receptor	Response to progesterone	Cancer
Androgen receptor	Response to androgens	Spinobulbar muscular atrophy
Oestrogen receptor	Response to oestrogens	Cancer
Kinases		
AKT (also known as PKB)	Mitogen signalling	Cancer
CDK4	Cell cycle control	Cancer
ERBB2	EGF receptor	Cancer
HCK	Immune response	Cancer
JAK1 and/or JAK2	Cytokine signalling	Cancer
SRC	Constitutively active Tyr kinase	Cancer
BRAF	Mitogen signalling	Cancer
BCR–ABL	Constitutively active Tyr kinase	Cancer
E3 ubiquitin ligases		
MDM2	p53 degradation	Cancer
UHRF1	DNA methylation	Cancer

Transcription factors		
p53	Tumour suppressor protein	Cancer
OCT4	Embryonic development	Cancer
HIF1α	Angiogenesis	Cancer
PPARα, PPARβ, PPARγ	Fat and glucose metabolism	Diabetes mellitus
STAT2, STAT3, STAT5	Cytokine signalling	Cancer
Others		
Argonaute 1, Argonaute 2	RNA interference	
Calcineurin	Immune response	Rheumatoid arthritis
Calmodulin	Various signalling pathways	
CFTR	Chloride channel	Cystic fibrosis
HSF1	Regulation of heat shock response	
NLR proteins	Innate immune response	Inflammation
TERT	Telomere maintenance	Cancer
eNOS	Nitric oxide synthesis	
RAD51 and/or RAD52	DNA repair	Cancer
Tau	Microtubule stabilization	Alzheimer disease

Historically, **protein kinases and Steroid Hormone Receptors** have been the most extensively studied HSP90 clients.
Recent quantitative analysis of HSP90 clients revealed that, whereas approximately 60% of the human kinome associates with HSP90, only a small fraction (approximately 7%) of transcription factors are dependent on HSP90.
Unexpectedly, about 30% of human E3 ubiquitin ligases were found to bind to HSP90

Despite apparent promiscuity, the number of HSP90 clients is limited compared with the general chaperones HSP70 or HSP60, which appear to interact with **all** unfolded proteins. In the case of HSP90, several categories of client interactions are apparent, and **clients can be classified into three broad groups**

First, HSP90 facilitates the formation of a specific, active conformation of a protein, as in the case of kinases; **second**, HSP90 aids the assembly of multiprotein complexes, such as the kinetochore or the Rvb1–Rvb2–Tah1–Pih1 (R2TP) complex; and **third**, HSP90 promotes the binding of ligands to proteins by stabilizing the binding-competent open conformation.



Examples of the third category include the binding of a steroid hormone to a steroid hormone receptors SHR, the insertion of haem into soluble guanylyl cyclase, and promoting the association of telomerase with DNA

This categorization summarizes a large body of work on HSP90–client interactions, but it is not comprehensive — other functions of these interactions, such as the **regulation of protein dynamics and ensembles of conformational states,** have been described.

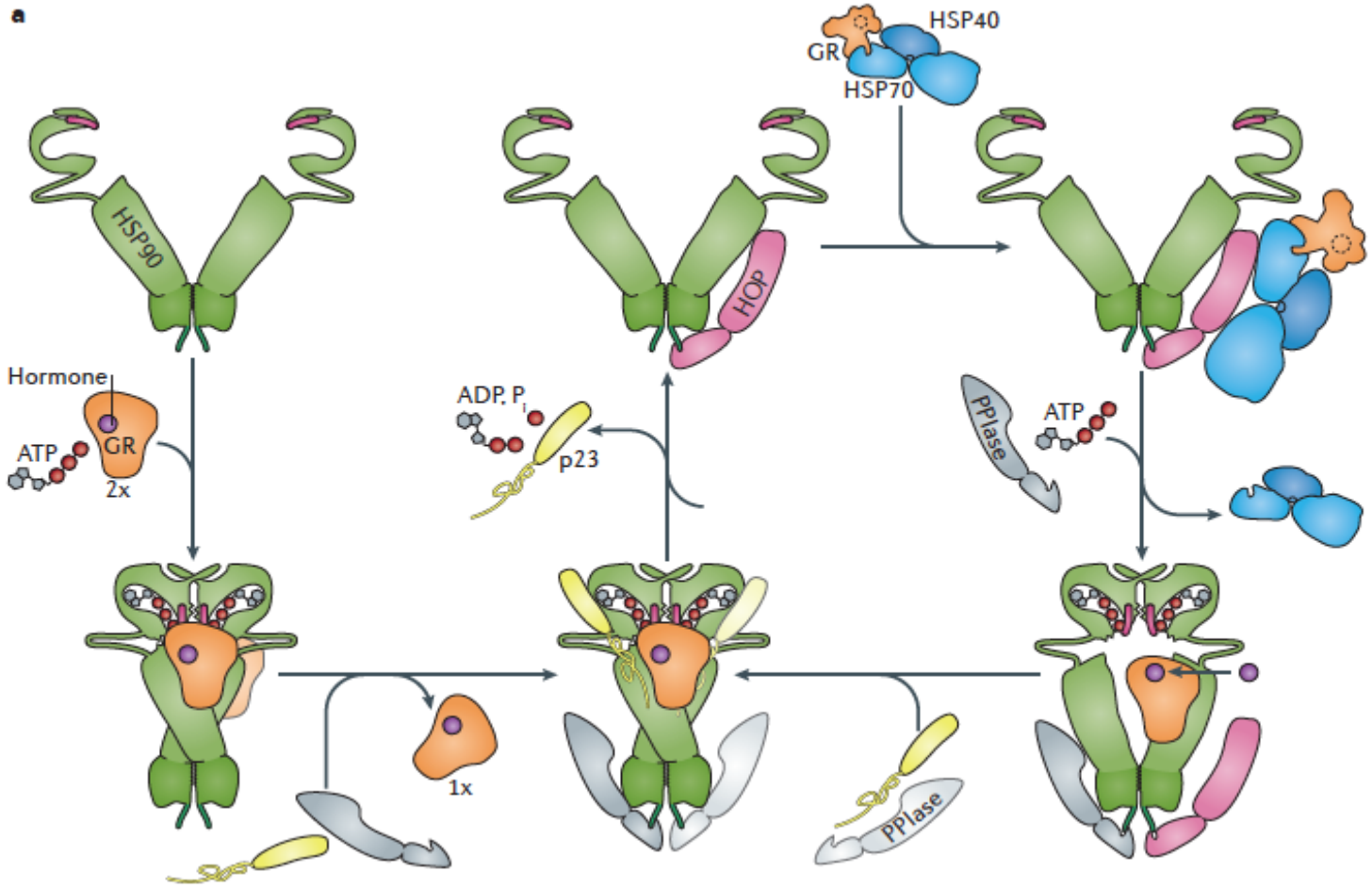
In conclusion, owing to its effect on proteins that represent ‘hubs’ in cellular networks and signalling cascades, HSP90 is a conformational regulator of a subset of the proteome, which integrates a plethora of signalling pathways.

Client maturation cycles

Research in the past few years has provided a clearer picture of how clients and co-chaperones can be integrated into the HSP90 conformational cycle . How client proteins **are recognized** by HSP90 is of particular interest.

Maturation of the glucocorticoid receptor

For GR, the interaction with HSP70 that precedes binding of GR to HSP90 seems to play an important part in maturation. After HSP70 binds to GR, the GR ligand-binding domain is transferred from HSP70 to HSP90 by the adaptor co-chaperone HOP/Sti1. Interestingly, it was shown that HSP70 partially unfolds the GR LBD, which is subsequently reversed by the action of HSP90 and HOP/ Sti1.

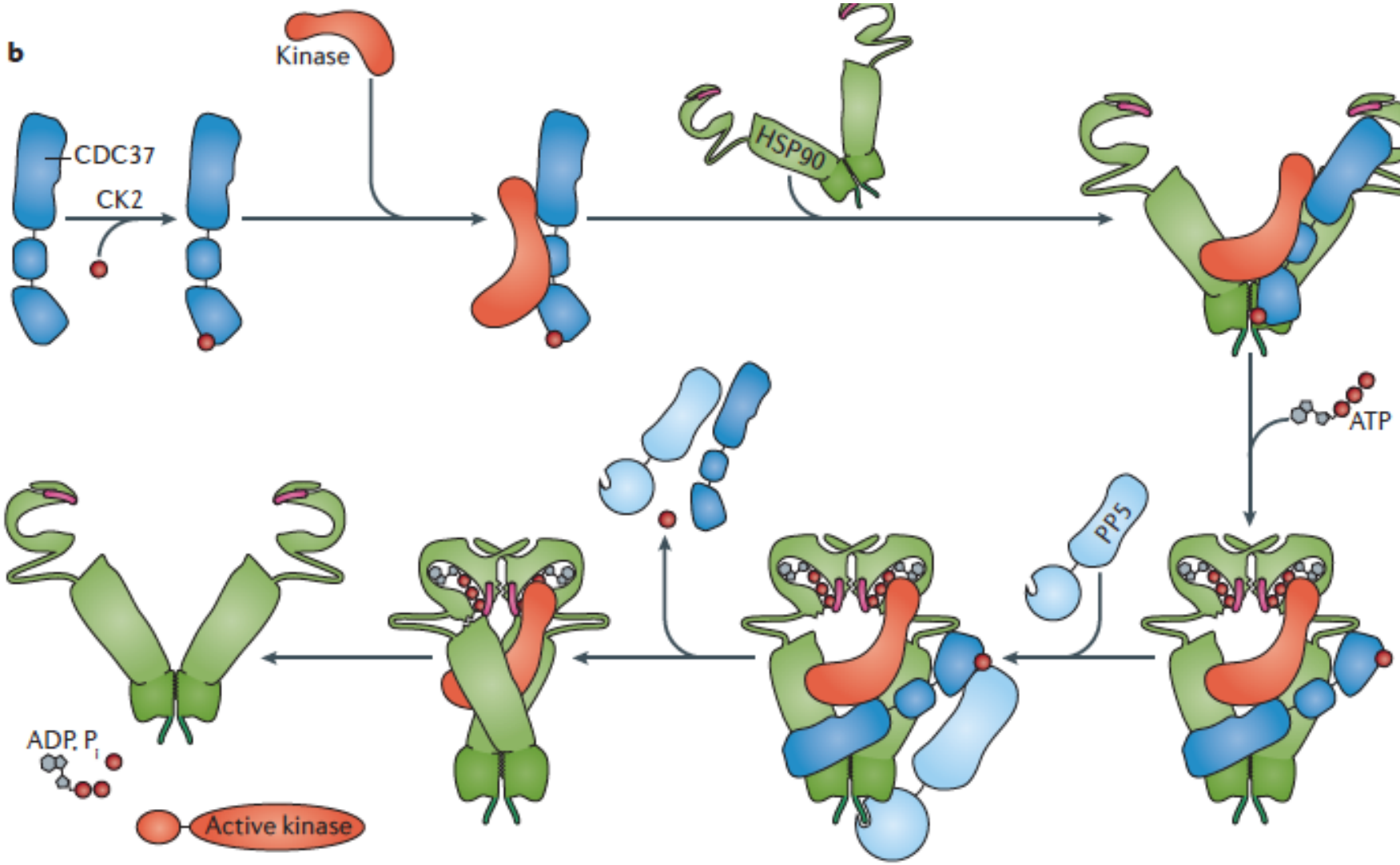


The HSP70–HSP40 complex associates with partially folded GR LBD and transfers it to HSP90 via the adaptor co-chaperone HSC70/HSP90-organizing protein (HOP; Sti1 in yeast). The subsequent binding of ATP and a large peptidyl-prolyl cis–trans isomerase (PPIase) results in the formation of the intermediate state of HSP90, which proceeds to the fully closed ATP-competent closed 2 state after the displacement of HOP/Sti1 and binding of p23

It has also been shown that the GR LBD can associate with HSP90 directly (**left side of Figure**)
After complex formation, binding of a large PPIase and p23to GR LBD–HSP90 yields the closed HSP90 structure that is competent for ATP hydrolysis, which leads to the release of active GR.

Maturation of kinases

As mentioned earlier, CDC37 has been established both as a recruiter of kinases to HSP90 and as a sensor that distinguishes between client and non-client kinases.
To achieve this, CDC37 assesses the stability of kinases using its NTD and, if an appropriate substrate is detected, a stable association between the kinase and the C-terminal region of CDC37 is established.
A model has been proposed in which the CDC37–kinase complex first binds to the NTD of HSP90 in its open conformation.
Subsequently, this ATPase-inhibited HSP90–CDC37– kinase complex is thought to rearrange to form a closed, compact structure with a closed ATP lid. ATP hydrolysis is followed by the opening of the HSP90 complex.


b
Kinase
CDC37
CK2
HSP90
ATP
PP5
ADP. P$_i$
Active kinase

HSP90 in disease

HSP90 has been reported to be involved in many human diseases, which include cancer, neurodegenerative diseases and infectious diseases

Table 3 | **Involvement of HSP90 in disease***

Class of disease	Type of disease
Infectious diseases	
Viral infections	• *Herpesviridae* • *Paramyxoviridae* • *Orthomyxoviridae* • *Reoviridae* • *Retroviridae* • *Hepadnaviridae*
Protozoan infections	• *Leishmania* spp. • *Plasmodium* spp. • *Toxoplasma* spp. • *Eimeria* spp. • *Trypanosoma* spp.
Non-infectious diseases	
Neurodegenerative diseases	• Alzheimer disease • Parkinson disease • Huntington disease • Spinobulbar muscular atrophy • Frontotemporal dementia and parkinsonism linked to chromosome 17
Cancer	• Li–Fraumeni syndrome • Breast cancer • Melanoma • Leukaemia • Colon carcinoma • Non-small-cell lung cancer • Prostate cancer

HSP90 in cancer

Tumor cells seem to be in a ‘stressed’ state, owing to the presence of mutant proteins and rapid proliferation, which puts additional pressure on controlling proteostasis.
Thus, HSP90 plays an important part in the survival of cancer cells, also because of their extensive dependence on HSP90-assisted signalling pathways.

Aside from the tumor suppressor p53 and the oncoprotein SRC, a number of HSP90 clients such as protein kinases, telomerase, hypoxia-inducible factor 1α (HIF1α) and UHRF1 are substantially involved in tumour growth

In addition, destabilizing oncogenic mutations could increase the dependence of tumor cells on HSP90.
Indeed, HSP90 levels are markedly increased in tumors and high HSP90 expression is associated with a negative prognosis in breast cancer. In this context, mutant p53 is thought to remain bound to HSP90, which leads to an accumulation of dysfunctional p53 in the cell.
Abrogation of this interaction prolongs survival and enhances tumor regression in mouse models. Cancer cells are generally more sensitive to chemical inhibitors of HSP90 than normal somatic cells. This seems to correlate with the presence of HSP90 in multimolecular complexes in tumor cells, which may enhance its affinity for inhibitors.

HSP90 in neurodegenerative diseases

HSP90 has been associated with a number of neurodegenerative diseases including Alzheimer disease and Parkinson disease, which are both characterized by protein aggregation. Protein amyloid-β (Aβ) and the hyperphosphorylated tau protein, are HSP90 clients.
It has been suggested that aberrant folds in both Aβ and tau can be targeted by HSP90 to prevent aggregation.

In Parkinson disease, α-synuclein, is also an HSP90 client. Thus, in these cases, **upregulation of HSP90 function may prove to be useful to treat these neurodegenerative diseases**. However, inhibition of HSP90 could result in the aberrant disease-associated proteins being targeted for degradation. In summary, we are only beginning to understand how HSP90 affects the different aspects of neurodegenerative diseases and how it can be targeted for the treatment of these conditions.

HSP90 in cystic fibrosis

Cystic fibrosis is caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR), which encodes a chloride channel.
The most frequent disease mutation — a deletion of Phe508 **(ΔF508)** — largely inactivates the protein and leads to its degradation.
HSP90 has been found to interact with both wild-type and mutant CFTR. **Intriguingly, the interaction of the mutant or wild-type CFTR with co-chaperones differs**. Co-chaperone AHA1 is of special interest in this context, as a modest knockdown of this co-chaperone results in increased transport of mutant CFTR channels to the plasma membrane. Therefore, AHA1 may have a specific role in sequestering the mutant protein in the HSP90 chaperone cycle, thus promoting its eventual degradation. **Disrupting the AHA1–HSP90 interaction may allow the mutant CFTR to escape this regulation and reach the plasma membrane**.

HSP90 in viral and protozoan infections

Recent studies suggest that **host HSP90** also has a major role in viral infections. A requirement for chaperones in viral infections seems obvious — viral proteins have high translation rates, and they are often multifunctional and therefore involve conformational flexibility as well as proteolytic processing.
In addition, the high mutation rates in viruses cause the accumulation of potentially unstable proteins, which could be buffered by HSP90 — in agreement with its proposed role in phenotypic evolution.

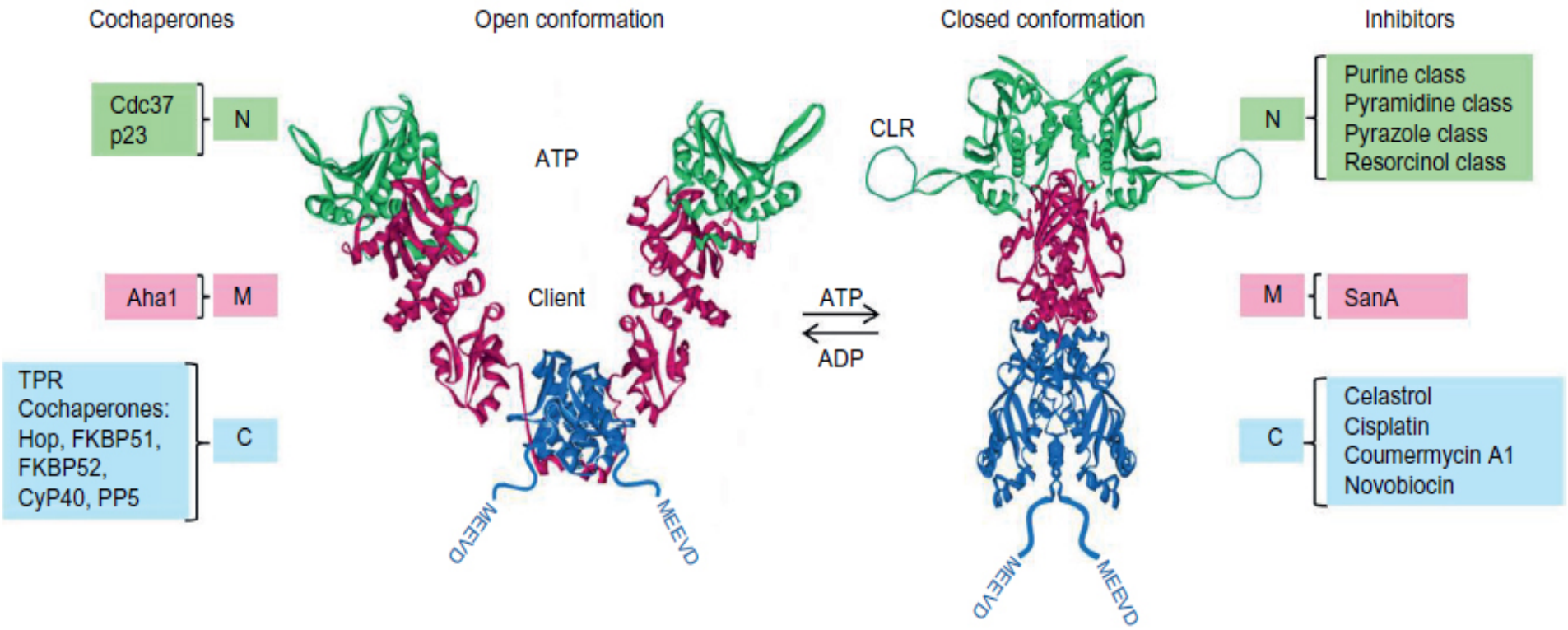
Owing to these traits, virus-infected cells are more sensitive to HSP90 inhibitors than non-infected cells.

In protozoans, the role of HSP90 has been investigated in various organisms including *Leishmania donovani* and *Plasmodium falciparum*, which cause **leishmaniasis and malaria**, respectively. As these organisms often experience a shift in temperature and pH during their life cycle, parasite heat shock proteins — in particular, the HSP90 system — are important for differentiation and development. As a consequence, treatment with **species-specific** inhibitors of parasite HSP90 can block their proliferation.

In summary, in the context of disease, the involvement of HSP90 in stabilizing a broad range of clients translates into generally increased buffering of stress during pathogenesis, which explains the role of HSP90 in protozoan infections and tumorigenesis.
Furthermore, the stabilization of specific proteins may promote the development of neurodegenerative disease and cancer, but it may also interfere with specific functions, as in the case of mutant CFTR, and thus drive disease.

HSP90 inhibitors

The mode of ATP binding to HSP90 is unusual, as the binding pocket in the NTD accommodates the base and the sugar, but the phosphates point outwards and the γ-phosphate only becomes buried once the MD associates with the NTD.
Furthermore, the ATP is bound in a '**kinked' conformation**.
These features allow specific inhibition of HSP90 chaperone activity by chemical compounds.
Known inhibitors of ATP binding to HSP90 include natural compounds such as the **ansamycin, geldanamycin and radicicol**, and designed synthetic inhibitors



Regulation of Hsp90 conformation by nucleotide binding, its association with co-chaperones and the competing modulating influences of inhibitory ligands.

Inhibitors with an entirely different mode of action bind to the C-terminal part of HSP90, thus disrupting interactions with the large group of TPR-containing co-chaperones.

One of these, **novobiocin**, binds to the CTD of HSP90 and does not trigger a heat shock response, which is one of the major drawbacks of inhibitors that target the N terminus of HSP90.

In addition, a large number of inhibitors that bind directly to HSP90, such as silybin, taxol, **cisplatin**, epigallocatechin-3-gallate and sansalvamide A-amide, have shown promising effects.

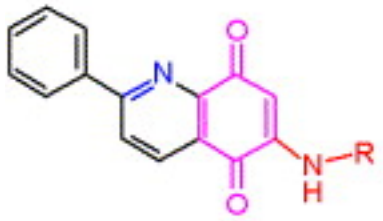
More-specific inhibitors such as compounds that disrupt HSP90–co-chaperone binding are currently being developed; for example, derrubone, withaferin A and **celastrol** block CDC37 binding to HSP90

The availability of specific inhibitors of HSP90, which is implicated in many different aspects of cancer biology, has prompted their development for cancer treatment.

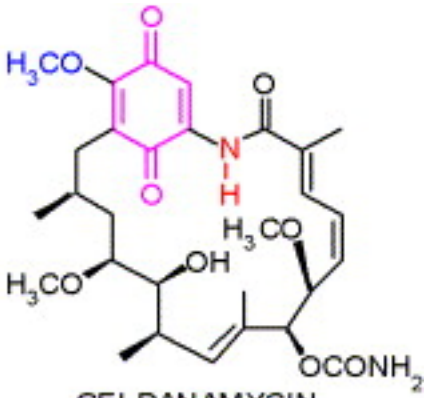
At first, this approach was met with scepticism, as HSP90 is an essential protein.

However, **non-cancer cells seem to require a much smaller pool of HSP90 molecules than cancer cells**. Therefore, a sufficient therapeutic window exists. Approximately 50 clinical trials have been performed or are in progress, using at least 15 different HSP90 inhibitors, many of which are **geldanamycin analogues** that exhibit improved pharmacological properties

Design of quinolinedione-based geldanamycin analogues



QUINOLINEDIONE



GELDANAMYCIN

Finally, the **simultaneous inhibition of HSP90 and protein kinases**, which was found to reduce oncogene switching in cancer models, appears to be a promising avenue to reduce drug resistance in both cancer and infectious diseases. Thus, the opportunities available to treat disease by targeting HSP90 have not yet been fully exploited.

Conclusions and perspectives

Different approaches that combine different techniques and model systems have recently revealed key structural and mechanistic aspects of the HSP90 machinery.
The crystal structures of several HSP90 family members have been solved, including proteins from the cytosol of bacteria and yeast, from the mitochondrial matrix and from the endoplasmic reticulum. Furthermore, the (partial) structures of many co-chaperones have been resolved, and the first atomic resolution structure of human HSP90 in a complex with the co-chaperone CDC37 and a protein kinase has been obtained.

This is an important landmark, for in this structure lies the answers to a number of questions about key aspects of the function of the interaction of HSP90 with kinases — however, it has raised many new questions.

At present, we also have a rough picture of the interactions of a core set of co-chaperones with HSP90, which includes binding sites and affinities.
We know **how they affect the HSP90 conformational cycle** and, for some of these co-chaperones, we have an idea of how they affect the interaction of HSP90 with clients.
Client interaction cycles for both kinases and SHRs were reconstituted in vitro starting with purified components.

However, many important aspects are enigmatic; for example, the **network of allosteric interactions** that we know exists throughout the large HSP90 molecule and also across the dimer.
It is unclear exactly how ATP is hydrolysed by HSP90 and what the direct conformational consequences are of ATP hydrolysis.