The peculiar asymmetry of the Hsp90 dimer

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Picture taken from:

Thomson. M., (2014) Asymmetric butterflies *Heliconius Homepage*. http://www.heliconius.org/2014/asymmetric-butterflies/

Contents

Summary

Since its discovery in 1996, the Hsp90 chaperone has been studied in its role of protein stabilisation in cell stress conditions and in its role of maintaining proteostasis under normal conditions. Abnormalities in its wide variety of clients have been linked to several diseases like cancer, Alzheimer's disease, and cystic fibrosis. Because of this, the Hsp90 dimer is a prime target for the development of treatments against these diseases. Hsp90 is aided in its functioning by several co-chaperones, but the homodimer is observed to form multiple asymmetric stoichiometries and configurations with its co-chaperones and client proteins. In this thesis three causes of asymmetry in the Hsp90 dimer are explained through the interaction of Hsp90 with three of its co-chaperones: p23, Hop/Sti1, and Aha1. Despite its many client proteins, it should be possible to design treatments with high specificity when using the many ways Hsp90 has developed to interact with its clients.

Introduction

Heat shock protein 90 (Hsp90) is a family of proteins well preserved across most prokaryotes and all branches of eukaryotes (Chen et al., 2006). The protein was first discovered in 1965 when exposing *Drosophila melanogaster* cells to heat stress and because of the 90kDa size of the protein it was named accordingly. This already abundant protein accounting for 1 – 2% of all protein in a cell, is upregulated in response to cellular stress to an expression level of 4 – 6% (Crevel et al., 2001; Csermely et al., 1998). While the bacterial homologue HtpG is only essential under stress conditions (Thomas & Baneyx, 1998), most eukaryotes require cytoplasmic Hsp90 for proteostasis and thus normal cell functioning. Cytoplasmic Hsp90 is not the only isoform of Hsp90 present in the human cell, in fact there are 2 different cytosolic Hsp90 isoforms, Hsp90α and Hsp90β, as well as an endoplasmic reticulum specific analogue called Grp94, and a mitochondrial analogue named Trap1. Despite the differences in localisation, expression, and function, they do share a high degree of sequence and structural homology.(Girstmair et al., 2019; Sanchez et al., 2020; Subbarao Sreedhar et al., 2004). As a chaperone protein Hsp90 is involved in the folding, stabilising, activation and degeneration of many and a wide variety of client proteins (Echeverría et al., 2011), and it is thus a target in the search of new treatments of cancer, neurodegenerative diseases, viral and fungal infections (Sanchez et al., 2020; Taldone et al., 2014; VERI & COWEN, 2014; Whitesell & Lindquist, 2005).

Hsp90 exists as a homodimer with each monomer consisting of 3 main domains linked by 2 flexible hinges (Southworth & Agard, 2008): the N-terminal domain (NTD), middle domain (MD) and C-terminal domain (CTD). The NTD has nucleotide binding and ATPase activity and via a charged linker is linked to the MD. The MD is also involved in the hydrolysis of ATP through the Arg380 residue. The CTD is responsible for the dimerization of the two monomers and contains a tetratricopeptide repeat (TPR) binding motif that enables the binding of co-chaperones, with TPR-motifs. However, the TPR-motif is not a necessary factor for association with Hsp90. In fact, there is no reliable way to predict Hsp90 association as all three domains have shown to interact with clients and co-chaperones (Hagn et al., 2011; Vaughan et al., 2006).

Despite the multiple binding sites and evidence of Hsp90 being able to bind two clients at a time, the Hsp90 dimer has only been observed remodelling one client per cycle (Flynn et al., 2015). This leads to a model of rare asymmetry of the Hsp90 homodimer. But is this

behaviour caused by asymmetrical binding of other proteins? Or does the Hsp90 dimer have its own catalytic or structural asymmetry? And could it be a possible target for future clinical studies?

Results

Homodimers and homo-oligomers in general are often observed to exhibit a high degree of symmetry. (Blundell & Srinivasan, 1996) This frequently occurs through the association of copies of the same protein to form complexes, this leads to symmetries within the complex caused by the repetition of amino acid sequences. Examples of this are: 2-fold symmetry in immunoglobulin and triosephosphate isomerase, or 3-fold symmetry in glycogen for instance. Higher degrees of symmetry are less common but do occur like the 5-fold symmetry in the pentraxin serum amyloid P-component and 32-fold symmetry in zinc insulin hexamer, or even an icosahedral 532-fold symmetry used by viruses to create a capsule to deliver its content to hosts. Hsp90 is also an example of this type of symmetry. Symmetry in contrast to asymmetry has various biologically relevant advantages as it provides cooperativity and multivalent binding while leading to highly stable complexes. (Blundell & Srinivasan, 1996) Another benefit is that building large proteins out of copies of smaller subunits requires less genetic code and thus shorter genes, this results in fewer mutations as the chance of copying errors or mutagen induced errors increases with gene length.

Asymmetry in contrast to symmetry is much rarer and thus is less studied, but conformational asymmetric homodimers (homodimers in which both monomers have the same amino acid sequence but when dimerised they have been observed to exhibit different conformations) have been observed to bind asymmetric ligands like heme activator protein 1 (HAP1) binding to DNA (Marmorstein et al., 1999), or to bind ligands in an asymmetrical stoichiometry like NSP3 binding to the 3'-end of a single RNA strand (Deo et al., 2002).

The first resolved full length structure of Hsp90 in complex with the p23 co-chaperone reveals a symmetrical complex of two Hsp90 monomers dimerised through the CTD and NTD, and two p23 cochaperones bound to each side the NTD of the hsp90 dimer (Ali et al., 2006). Later studies discovered the mechanism of nucleotide binding and hydrolysis of Hsp90

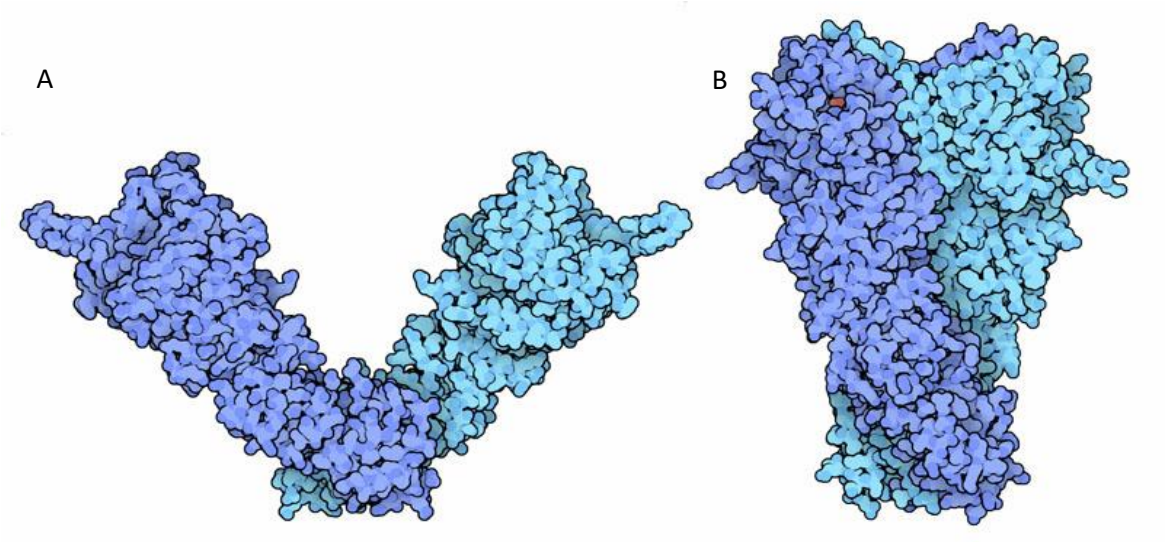

Figure 1: Conformational change of Hsp90 **A**. The 'open' configuration of the Hsp90 dimer, with each monomer its own shade of blue. The CTD is dimerised, but the NTD are far apart, and its nucleotide binding sites are exposed. **B.** The 'closed' configuration of the Hsp90 dimer. After binding ATP (red) the NTD dimerise and exchange a β-sheet.
picture by: (Goodsell, 2008)

and its conformal changes during this process. In Its Apo state the Hsp90 dimer mainly adopts a V-shaped confirmation, the monomers are dimerised through the CTD, but the NTD are apart from each other, and the nucleotide binding pockets are exposed (figure 1A). This conformation is usually called the 'open'-state. Upon ATP binding the Hsp90 dimer slowly adopts a different conformation: the nucleotide binding pocket closes by a conserved 'lid'. Furthermore, NTD dimerization through the exchange of a β-stand is observed to be essential for ATP hydrolysis (Cunningham et al., 2008). The new conformation is often referred to as the 'closed 1'-state.

The dimer then further transitions by MD-NTD dimerization, this leads to Arg380 residue of the MD finishing the catalytic site in preparation of hydrolysis of the bound ATP (Wolf et al., 2021). This confirmation is usually called the 'closed 2-state. Upon ATP hydrolysis the dimer transitions to the 'open'-state releasing the ADP and inorganic phosphate now bound in its binding pocket.

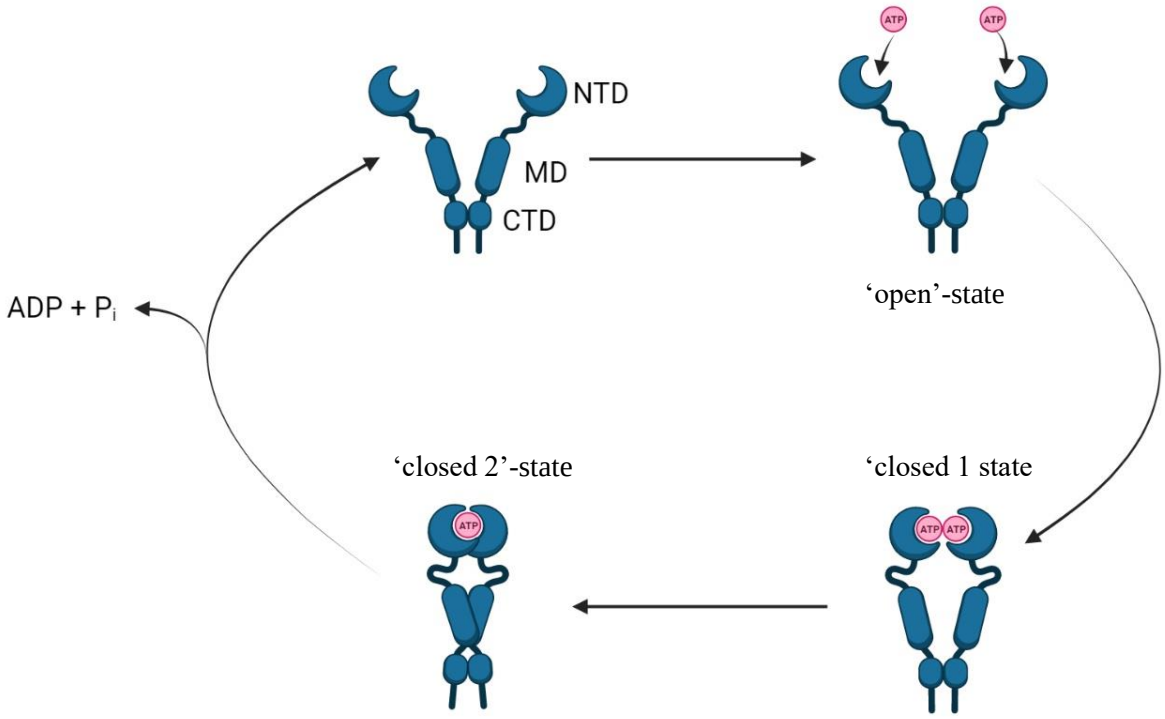

Figure 2: The catalytic cycle of the Hsp90 dimer. In the 'open' configuration of the dimer the CTD are dimerised while the nucleotide binding pocket, located in the NTD, is exposed. After binding ATP moves to the 'closed 1'-state, where the binding pocket is closed and the NTD dimerised. The Hsp90 dimer further transitions by NTD and MD dimerization within each monomer. This completes the catalytic site, creating the 'closed 2'-state . After hydrolysis the configuration reopens releasing ADP and inorganic phosphate (P_i).

Different co-chaperones interact at all stages to induce various effects.
Hsc70/Hsp90-organizing protein (Hop or Sti1 in *Saccharomyces cerevisiae*) binds to the open conformation and stabilizes it, thus preventing the Hsp90 dimer from reaching the closed state in turn inhibiting nucleotide hydrolysis (Prodromou, 2012; Sima & Richter, 2018). Co-chaperone p23 also inhibits ATPase activity of Hsp90, but it does so by binding and stabilising the NTD during the 'closed 2'-state preventing the release of ADP and inorganic phosphate, reducing the recycling rate of Hsp90 in its cycle(Ali et al., 2006). Activator of Hsp90 ATPase homologue 1 (Aha1) binds both monomers after ATP is bound and promotes NTD dimerization, thus accelerating the chaperone cycle (Flynn et al., 2015; Prodromou, 2012). Protein inhibition and promotion is very common, especially in eukaryotes, to control the specificity and rate of almost any process. The binding asymmetry of these proteins however is far less common (Swapna et al., 2012) and is the result of a few different factors.

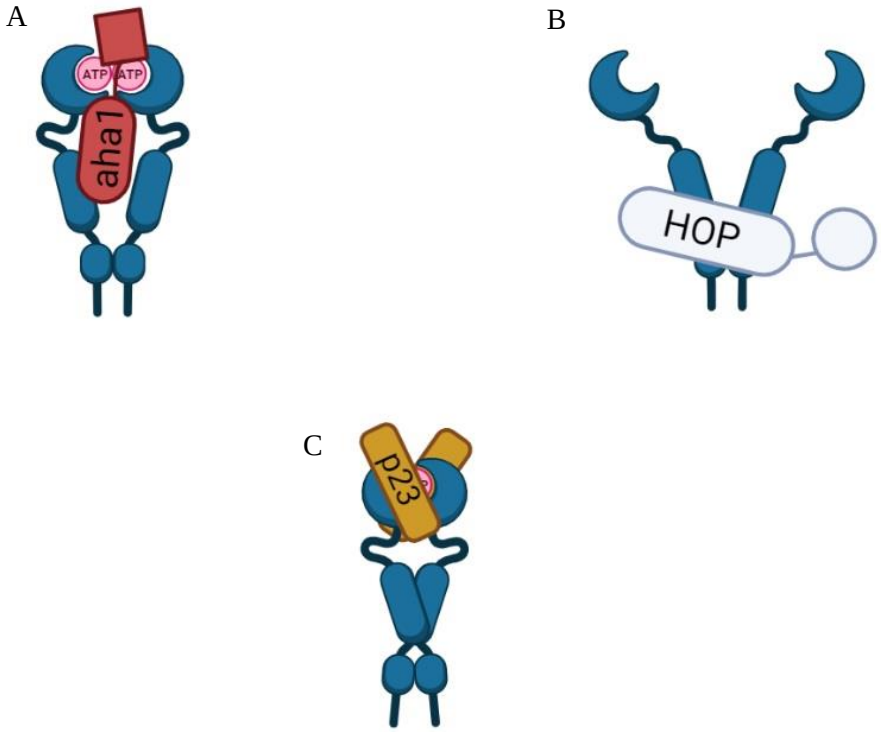

Figure 3: binding configurations of Hsp90 dimer and its co-chaperones. **A**. The NTD of Aha1 binds to the MD and NTD of one of the Hsp90 monomers. Aha1 is thought to promotes the change from the 'closed 1'-state to the 'closed 2'-state increasing the catalytic activity of the Hsp90 dimer. A single Aha1 protein binding to the Hsp90 dimer is enough to induce its stimulation, despite two, presumed identical, binding sites (Wolmarans et al., 2016). **B**. Using 2 TPR motifs, Hop/Sti1 binds to the CTD of the Hsp90 dimer. Hop/Sti1 stabilises the 'open' configuration of Hsp90 inhibiting the catalytic cycle, this inhibition allows for transfer of a client protein form Hsp40 and Hsp70 to the Hsp90 dimer. **C**. p23 is an inhibitory co-chaperone of Hsp90 that binds to the 'closed 2'-state, stabilising it and thus preventing the hydrolysis of the bound nucleotide. Although two p23 proteins can bind to the dimer, only one is enough to inhibit the hydrolysis.

There are multiple possible causes of asymmetry in Hsp90. One of the theories for asymmetrical binding of co-chaperones to the Hsp90 dimer as mentioned by Schopf *et al.* (2017) is that the asymmetry is due to the abundance of the chaperone in comparison to its co-chaperones and simple equilibrium mechanics. In their 2017 review they state: "For many co-chaperones, the binding of two co-chaperone molecules per Hsp90 dimer is possible; however, given that all co-chaperones are notably less abundant than Hsp90 in cells, it is more likely that asymmetric complexes that contain the Hsp90 dimer and one molecule of a cochaperone will be relevant in vivo" (Schopf et al., 2017). As an example, the p23 inhibiting co-chaperone has 2 binding sites at the NTD of the Hsp90 dimer (Ali et al., 2006). It is safe to assume that Hsp90 is far more abundant than p23 (Ghaemmaghami et al., 2003). This means that there will be a high chance of a p23 protein to bind to an empty hsp90 dimer. Despite this skewed equilibrium the unbound Hsp90 dimer is still relatively abundant compared to Hsp90 dimer in complex with p23, so the chance of a p23 protein binding to an Hsp90-dimer:p23 complex is much lower than it is binding an unbound Hsp90 dimer, and even more so when taking into account that the Hsp90-dimer:p23 complex only has one open binding site while the Hsp90 dimer has two leading to more probable binding. It is, however, important to note that Hsp90 without p23 bound can be at any stage in its chaperone cycle, while the already complexed dimer is most likely to be in its 'closed 2'-state which is the only state p23 can bind to. Furthermore, the binding site of p23 overlaps with the binding sites of other co-chaperones (Schopf et al., 2017). This could lead to more symmetrical complexes, however the exact exchange rates and affinity (differences) of the binding of p23 to the Hsp90 dimer are not yet known, thus the ratio of stoichiometries *in vivo* is still to be determined. This theory of asymmetry also applies to client proteins that can bind symmetrically like the glucocorticoid receptor (GR), one of the client chaperone interactions that is well studied. Although GR can bind symmetrically (Lorenz et al., 2014) to the Hsp90 dimer this interaction is barely biologically relevant not only because of the relative abundance but also because of co-chaperone interactions. Apo GR first binds to Hsp40 and Hsp70 and through Hop/Sti1 in recruited to Hsp90 (Lorenz et al., 2014; Sima & Richter, 2018). However, Hop also causes unfavourable binding between GR and one of the monomers of Hsp90 resulting only one GR protein being chaperoned at a time. This type of interaction also occurs in other instances, as with the co-chaperone Cdc37. Cdc37 binds its client kinase and proceeds to recruit the Hsp90 dimer. Cdc37 binds asymmetrically in between both monomers allowing only one kinase to interact with the Hsp90 dimer at a time (Vaughan et al., 2006; Verba et al., 2016).

In contrast to Cdc37, Aha1 only binds to a single monomer of Hsp90 but is thought to do so in an asymmetrical stoichiometry *in vivo* (Wolmarans et al., 2016). When bound, Aha1 increases ATPase activity in Hsp90. It does so despite only being bound to a single monomer, suggesting the idea of asymmetrical enzyme activity instead of only asymmetrical binding. This hypothesis was proven by (Mishra & Bolon, 2014). To prove this, they engineered Hsp90 in yeast in such a way that heterodimers were formed preferentially. The dimer was then engineered to consist of a functional wildtype monomer and a non-functional monomer. This study concluded that ATP binding is essential for both subunits, however ATP hydrolysis was only required in one of the two monomers. This discovery leads to the model that one of the monomers of the Hsp90 dimer is the first to hydrolyse its bound ATP starting its conformational change. This change then induces the conformational change in the other monomer. This mechanism has been demonstrated in TRAP1 (Lavery et al., 2014) but not yet

in cytosolic Hsp90 or ER Grp94. The moment of asymmetry in between the two catalytic reactions could be used by co-chaperones and clients to bind to the observed conformations.

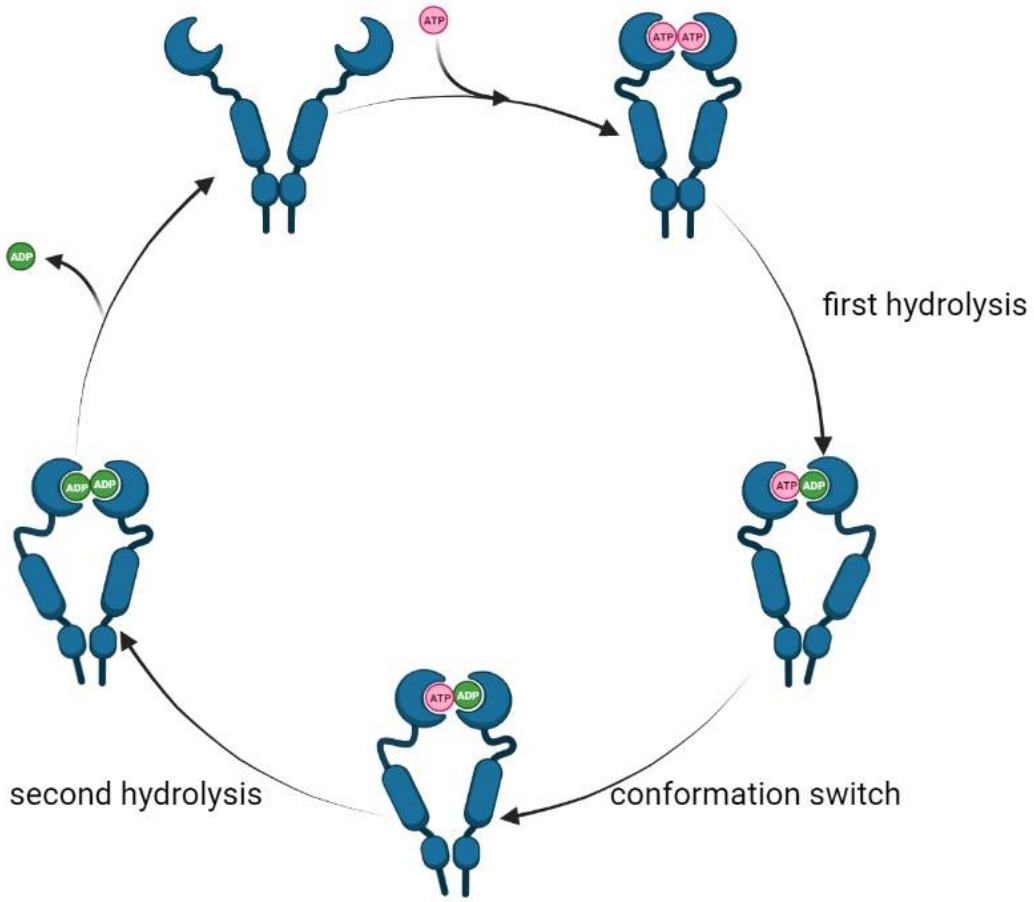

Figure 4: The asymmetrical catalytic cycle of TRAP1. Like cytosolic Hsp90, TRAP1, in its Apo state, adopts in an open configuration. The binding of ATP causes the binding pocket to close and results in NTD dimerization. Then the first hydrolysis occurs in one of the monomers causing an asymmetrical conformational change. This conformational change induces tension which can lead to a switch conformation. This then induces the second hydrolysis. After hydrolysis the TRAP1 dimer transitions into the 'open'-state resulting in the release of ADP from the binding pocket.

To analyse the process of ATP hydrolysis and the conformational change that occurs within the Hsp90 dimer, Wolf and colleagues (Wolf et al., 2021) performed a series of experiments, both empirical and computational, which they published in March 2021. In this paper, they simulated the Hsp90 dimer using molecular dynamic simulations and used this data to design single-molecule Förster resonance energy transfer (smFRET) experiments, the data collected from the smFRET experiments was then incorporated into the molecular dynamic simulations. Using this data, the Wolf and colleagues identified MD residue Arg380, its connected MD-helix (sequence positions 376–408) and the MD-loop (sequence positions 323–340) to be critical for the conformational change after nucleotide hydrolysis and for then

newly predicted asymmetrical ‘closed’ configuration. They identify Arg380 to be a ‘piston’, entering the nucleotide binding pocket during MD/MTD dimerization forming a salt-bridge with the Glu33 residue and the P_γ of the bound ATP. This interaction causes stain to be applied to the MD-helix of which the Arg380 residue is a part at the N-terminal side. The helix, identified to be a ‘crossbeam’, is located across the length of the MD, and has a large influence on the configuration of this domain and thus the MD-loop. The MD-loop is located in between the monomers and is believed to be involved in client binding (Verba et al., 2016) After ATP hydrolysis the salt bridge between Arg380 and Glu33 breaks. This causes the strain of the MD-helix to be lifted resulting in the relaxation of the protein into an entropically more favourable conformation. This new more constricted conformation results in the MDs moving closer to each other and rotating causing static hindrance of the MD-loops, resulting in one of the two monomers contracting into an asymmetrical state. The timescale of several hundreds of microseconds calculated in this paper suggests a rugged free energy landscape with multiple semi-stable configurations and small total free energy differences (Buchenberg et al., 2015).

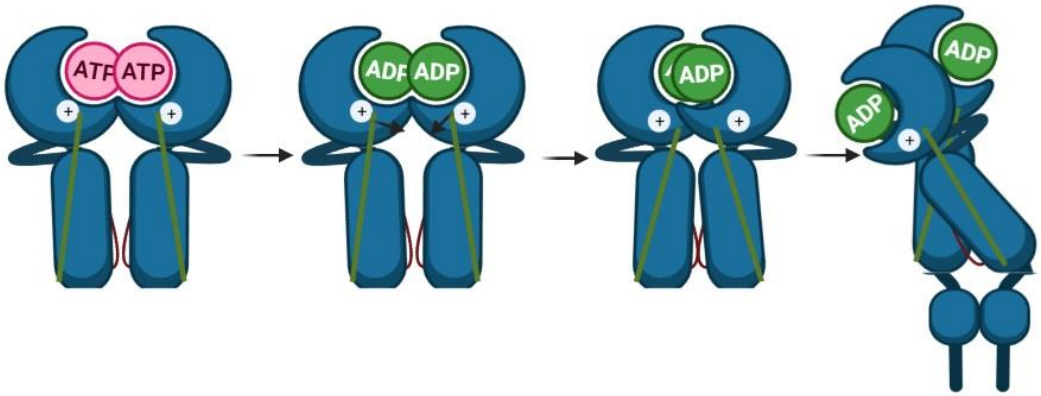

Figure 5: Asymmetry in the configuration of the Hsp90 dimer during the catalytic cycle. Through interactions with ATP the Arg380 residue (white) causes restrain on the MD-helix (green). When ATP is hydrolysed, this interaction is disrupted causing conformational change in the Hsp90 dimer. The MD-loops end up statically hindering each other leading the dimer to a more energetically stable but asymmetrical configuration.

Conclusion

The full extent and cause of the various types of asymmetry in Hsp90 are not fully know yet, but great efforts in uncovering these mechanisms have been and are still being made. Like with most biological systems it seems that the Hsp90 dimer is a product of extensive evolutionary pressure to form a chaperone that by association with co-chaperones can fold, activate, and stabilise a large variety of clients. Through several ways of influencing the ATPase activity and client binding affinity of the Hsp90 dimer, a cell can very precisely fine tune the functioning and binding specificity of the dimer and its many clients in the endless pursuits of creating the highest fitness. Due to its many energetically similar states, the Hsp90 dimer can take on many conformations, both symmetrical and asymmetrical. Whether these conformations are induced by co-chaperone and client binding or catalytic and structural and catalytic processes by the dimer seems to vary between each reaction and is yet to be fully characterised. This brings the strenuous task of studying each individual clients and its way of interacting with Hsp90, but perhaps with time and knowledge high throughput screening methods of analysing chaperone client interactions will be developed. On the other hand, the many different ways that Hsp90 interacts with client proteins and co-chaperones provides opportunities, for the creation of targeted inhibitors with high specificity, potentially allowing the use of these inhibitors for the treatment of some of the many diseases and disorders Hsp90 plays a critical role in, while not disturbing the many vital tasks the dimer performs within healthy cells.

Afterword

I would like to thank Kasia Tych for guiding me through this project and for the clear, fast, and kind interactions despite her busy schedule. And I would like to thank both Kasia Tych and Giovanni Maglia for making time during the holidays so I can finish my bachelors' program in time. To conclude I want to credit Biorender for the creation of the schemes in this paper

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