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HSP90 - KINASE RECOGNITION
Role of kinase domain stability in interaction strenght

Abstract

Using bioinformatic tools to model the conformational and thermodynamic properties of proteins we address the hypothesis launched by Susan Lindquist et al. in 2012 in the work *"Quantitative Analysis of HSP90-Client Interactions Reveal Principles of Substrate Recognition"*, suggesting that the destabilization of the kinase domain in a subset of protein kinases makes them substrates of HSP90 protein in a reaction mediated by de CDC37 cochaperone.

-- ¿Do thermodynamic parameters determine HSP90-cient binding within the kinase superfamily? --

Background

After their syntesis at the ribosomes, proteins need to fold into specific threedimensional structures in order to exert their correct cell fuction. Therefore folding tends to be a highly regulated pathway. The primary sequence encodes both the structure and folding reaction[1].

If polypeptide chains should fold in a random way, the stablishment of native interactions would take an astronomic lapse of time [2]. Thus the formation of energetically favorable contacts shapes the folding reaction towards the attainement of the native conformation. They accelerate the pathway by decreasing the energetic barrier of folding. Protein missfolding has a huge impact in cell healthness.

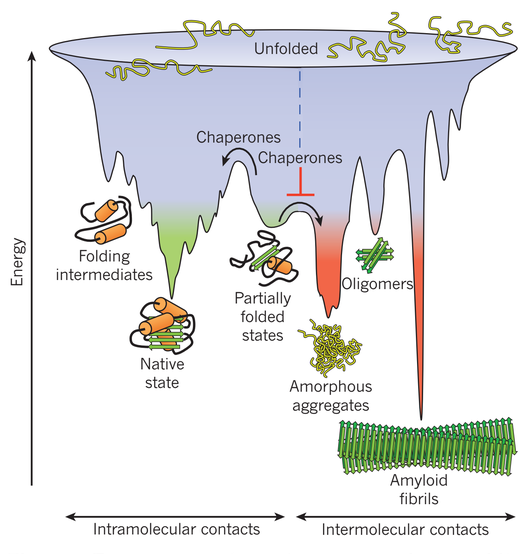

Figure 1: Energy states of protein structures during folding[3]

Accordingly, cell has evolved a molecular machinery in order to give the missfolded species a second opportunity to get the native fold. Sometimes proteins escape the correct folding way, so cell has many molecules in order to give them a second opportunity to fold monitoring it.
The best characterized proteins are named **molecular chaperones,** and have a critical role in proper function of proteins and as an anti-aggregation agent[5].

HSP90

The HSP90 chaperone performs its function as a dimer consisting of two subunits ensembled by their C-terminal domains. The N-terminal domain (ND) binds and hydrolyses ATP and it is connected to the C-terminal domain (CD) through a middle domain[3].

When ATP and client proteins join HSP90, the two ND domains interact with each other promoting a conformational tortion of the complex. This change is transmited to the peptide giving it a new chance to fold. At ATP hidrolisis the two ND domains split up.

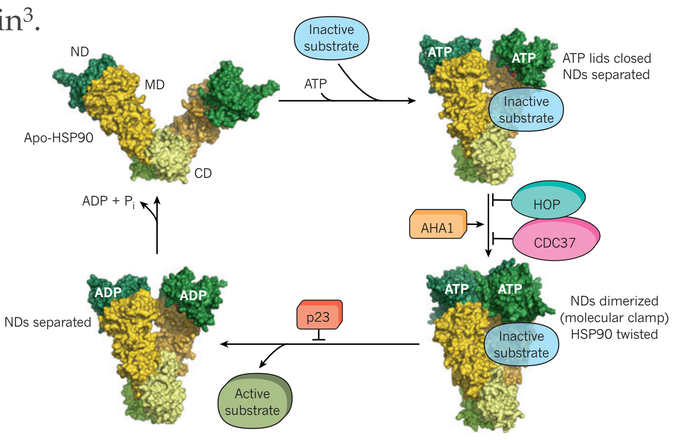

Figure 2: HSP90 machinery function[3]

Materials and Methods

- ***HSP90:Kinase interaction score***

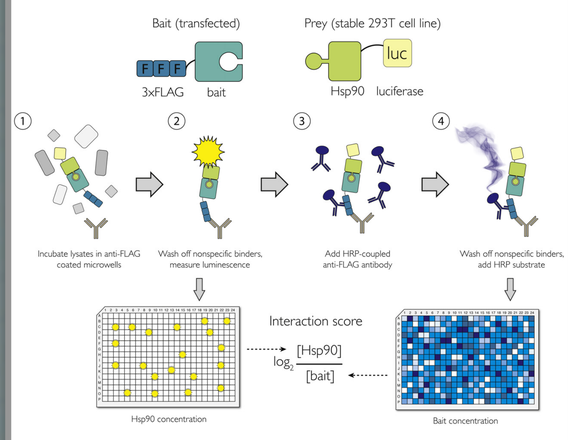

Figure 3: LUMIER with BACON assay scheme. Used in HSP90:client interaction determination[1]

- ***Bioinformatic Stability estimation***

- Selection of 84 kinase structures determined without gaps. Source: protein data bank (pdb).
- KobaMIN energy minimization (figure 4)
- FoldX stability determination

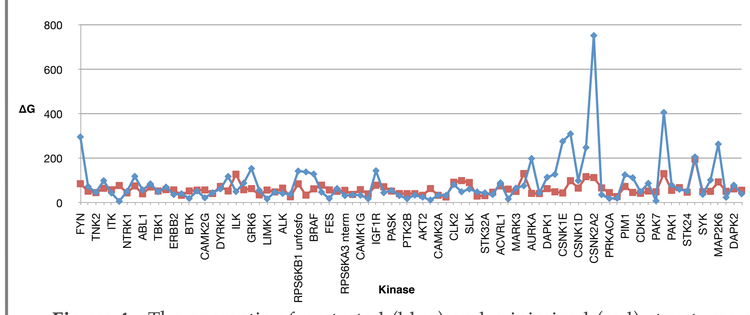

Figure 4: The energetic of pretrated (blue) and minimized (red) structures are shown in absolute ΔG values. Trated ones have a more homogeneous thermodynamic stability,

Results

- Stability prediction of minimized structures

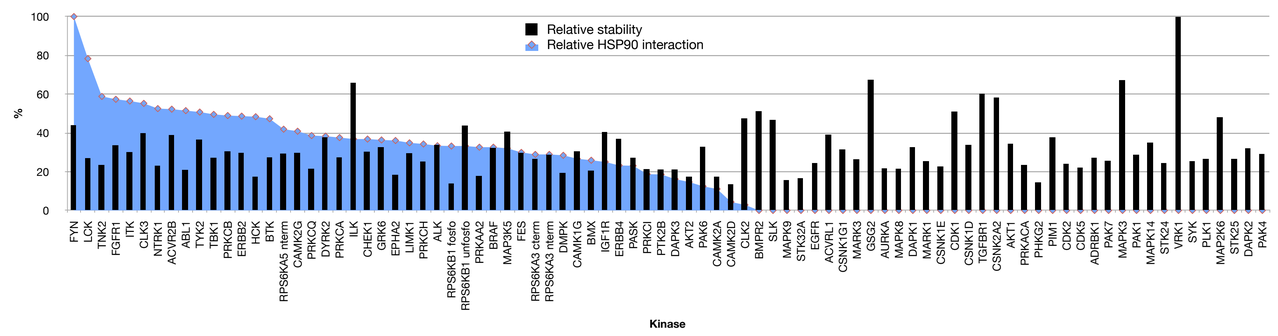

Figure 5: HSP90 interaction score (blue area) against kinase domain stability (black bars), both are shown in relative ΔG values.

Discussion and Conclusions

According to our analysis, the global kinase domain stability in protein kinases does not seem to account for the recognition and binding to the HSP90 chaperone, since the correlation between interaction strenght and predicted stability is not significant with a r^2=0,04.
It is clear that kinases should comprise determinants in their structure responsible for their differential binding ability to HSP90, but it is not obvious that this propery is directly linked to thermodynamic stability. It is likely that instead, specific sequence stretches, which differ among clients, would comprise the binding sites to the chaperone. This process would imply at least partial desestructuration and the exposition of hydrophobic residues to solvent.

Here it is suggested that hydrophobic solvent-exposed residues could be implicated in the recognition process. Conformational fluctuations may uncover them or be permenently exposed.

To extend this work, the studies should focus on these regions using molecular dynamics and prediction of exposed aggregation propensity. In addition, the involvement of other structural and regulatory domains in the binding to the chaperone should be explored.

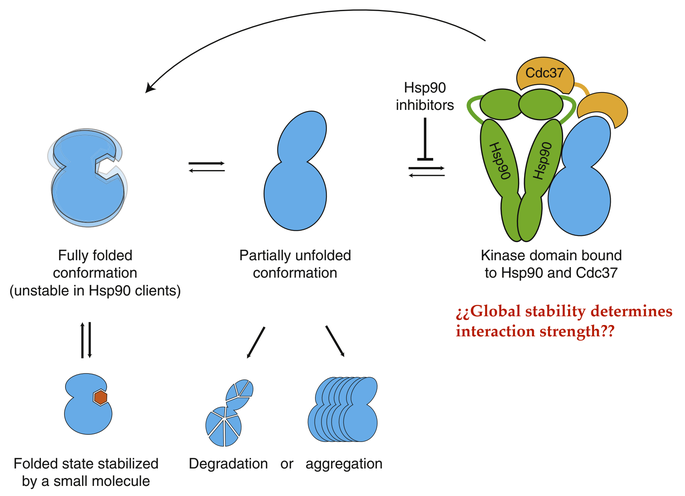

Figure 6: Involucration of HSP90 in correct structure-function of partially folded proteins. Modified scheme of S. Lindquist et al. 2012.

References

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2. **Alba Espargaró Colomé:** *Modelos proteicos para el estudio de la agregación in vitro e in vivo [doctoral thesis].* Universitat Autònoma de Barcelona, 2012.

3. **F. Ulrich Hartl, Andreas Bracher & Manajit Hayer-Hartl:** *Molecular chaperones in protein folding and proteostasis.* Nature 475, 324-332 (2011).

4. **Mikko Taipale, Irina Krykbaeva, Martina Koeva, Can Kayatekin, Kenneth D. Westover, Georgios I. Karras, and Susan Lindquist:** *Quantitative Analysis of Hsp90-Client Interactions Reveals Principles of Substrate Recognition*. Cell *150*, 987–1001, August 31, 2012