Trapping Dynamic Conformational States and Cochaperone Interactions of the Hsp90 Molecular Chaperone

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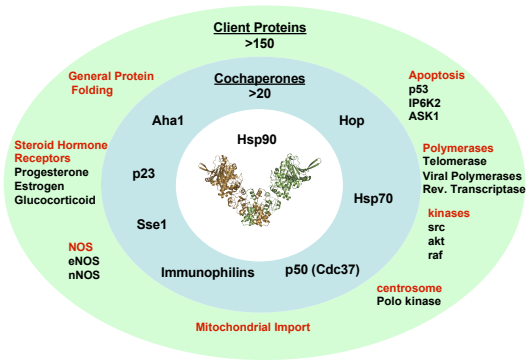
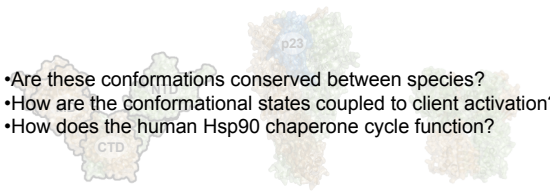
Hsp90: A molecular chaperone that activates specific substrate client proteins



Full-Length Crystal Structures of 180 kDa Hsp90 Dimer Identify Different Conformational States



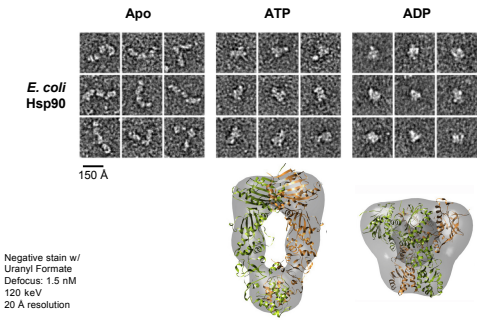
- Are these conformations conserved between species?
- How are the conformational states coupled to client activation?
- How does the human Hsp90 chaperone cycle function?

Apo Extended
E. coli Hsp90
Shiau, et al 2006

Closed, ATP conformation
Yeast Hsp90:AMPPNP:p23
-Ali MM, *et al.* 2006

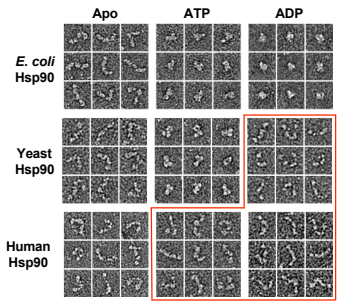
Compact ADP Dimer
Modeled from E. coli Hsp90:ADP tetramer
Shiau, et al 2006

Three Nucleotide-Stabilized Conformational States in E. coli Hsp90



Negative stain w/ Uranyl Formate
Defocus: 1.5 nM
120 keV
20 Å resolution

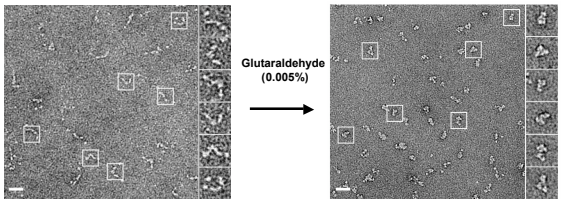
Hsp90 nucleotide-dependent conformations appear to vary between species



Are the mechanisms fundamentally different?

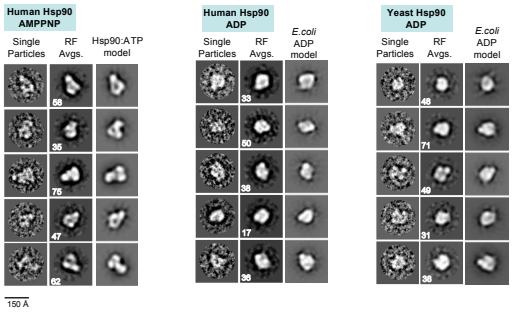
Using glutaraldehyde to crosslink and trap transiently sampled states

Apo human Hsp90 + AMPPNP

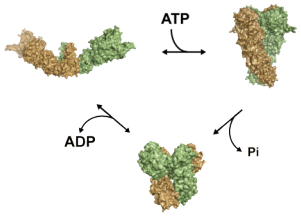


Glut. (0.005%) + protein (200 nM), 37° for 15 minutes. Stopped w/ 20 mM Tris.

Crosslinked States are Nucleotide Dependent and Match EM Structures

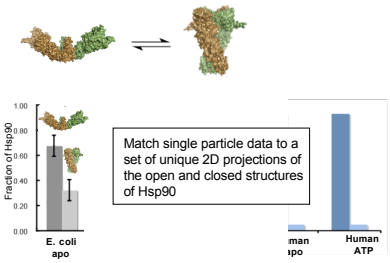


Hsp90 Chaperone Cycle Involves Three Universally Conserved Conformational States



•Hsp90 chaperone cycle involves a unique conformational equilibrium that is different between species.

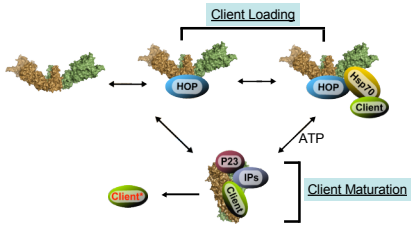
Quantitation of the open and closed state conformational equilibrium



Match single particle data to a set of unique 2D projections of the open and closed structures of Hsp90

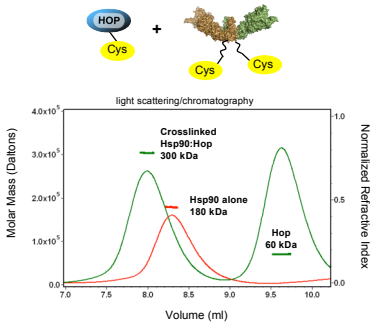
- Conformational states are isoenergetic.
- Nucleotide binding shifts inherent conformational equilibrium.

The Human Hsp90 Chaperone Cycle Involves Coordinated Interactions with Specific Co-Chaperones

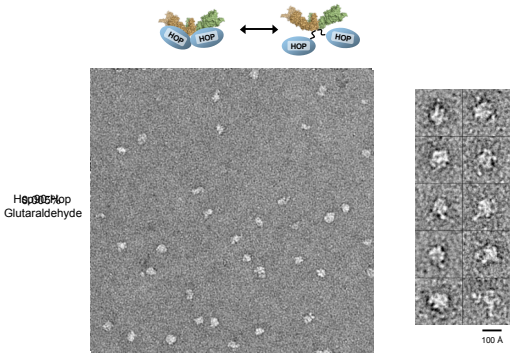


Complexes Dissociate: Low affinity and transient

Stable 300 kDa Hsp90:Hop tetramer complex formed following disulfide crosslinking



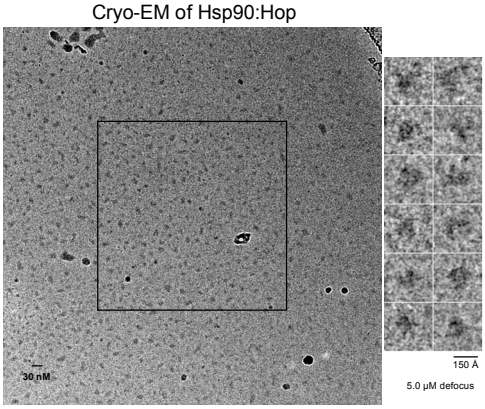
Negative-stain EM of 300 kDa Hsp90:Hop



Cryo-EM of Hsp90:Hop

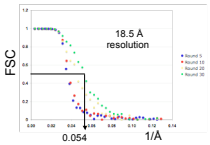
8k x 8k Image
1.19 Å/pixel

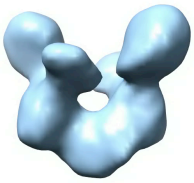
Technai TF20
120 Kev
Tietz 8k Camera



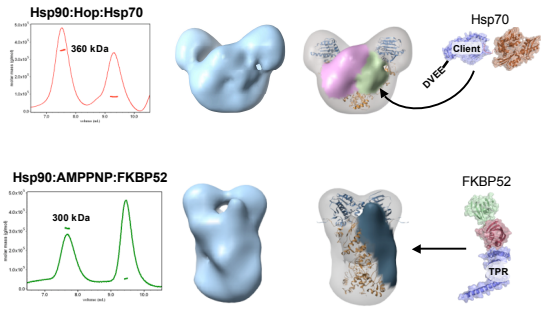
18.5 Å Cryo-EM Reconstruction of Hsp90:Hop

•Data collected at Scripps (Leginon).
•15,000 particles (10,000 in final model).
•Defocus: 1.5 to 3.5 µM.
•CTF correction (CTFFIND).
•Refinement using SPIDER, C2 symmetry.





Disulfide Crosslinking Stabilizes Multiple Hsp90 Complexes



20 Å negative-stain reconstructions.

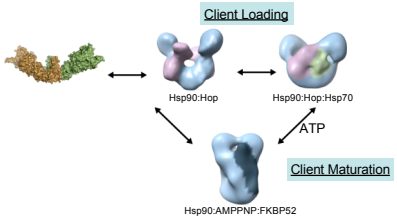
Conclusions

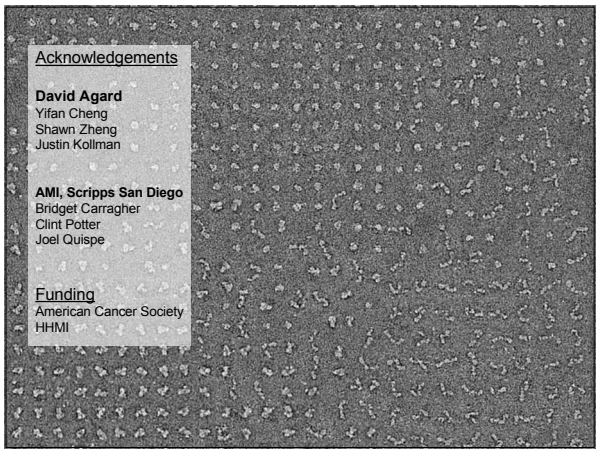
•Significant information can be gained from low-resolution structures

•Glutaraldehyde crosslinking at low concentrations for short times improves homogeneity and traps transient states.

•Disulfide crosslinking in known binding sites improves stability.

•Information about biological function gained from sorting heterogeneos data.




Acknowledgements
David Agard
Yifan Cheng
Shawn Zheng
Justin Kollman
AMI, Scripps San Diego
Bridget Carragher
Clint Potter
Joel Quispe
Funding
American Cancer Society
HHMI