Experiment title:
" SAXS studies of complexes of Hsp90, Rar1 and Sgt1 proteins from *Hordeum vulgare*"

Experiment number:
Experiment MX-1388

Beamline:
BM 29

Date of experiment:
from: 15/07/2012 to: 16/07/2012

Date of report:

Shifts:
3 shift(s)

Local contact(s):
Adam Round

Received at ESRF:

Names and affiliations of applicants (* indicates experimentalists):
KOZAK Maciej*, Faculty of Physics, Adam Mickiewicz University, Poznan, Poland
TAUBE Michał*, Faculty of Physics, Adam Mickiewicz University, Poznan, Poland
PIEŃKOWSKA Joanna R., Faculty of Biology, Adam Mickiewicz University, Poznan, Poland
JARMOŁOWSKI Artur, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

REPORT:

Heat shock protein 90kDa (Hsp90) is a molecular chaperon that assist in proper protein folding and helps maintain metastable conformation in the absence of proper stimuli in both normal and heat stressed conditions [1,2]. Many important cellular factors, like p53, mutated forms of oncogenic kinase c-Src, glucocorticoid receptor belong to the Hsp90 clients. Hsp90 in widely studied anticancer therapy drug target. It is formed by two identical subunits both of 90kDa. Each monomer has modular arrangement of three domains: N-terminal ATPase domain, Middle domain, and C-terminal domain that is responsible for dimerization. All domains participate in co-chaperon binding that may modulate Hsp90 conformation and ATPase activity. In solution, Hsp90 proteins exist in equilibrium of open and closed conformations observed in crystal structures of bacterial and yeast homologs of Hsp90 proteins. In ATP bound state Hsp90 is in the closed conformation with dimerized N-terminal domains and middle domains in close proximity. In the apo- state Hsp90 forms the open conformation with N-terminal domains apart in the crystal structure of bacterial Hsp90 HtpG. In solution, HtpG takes a more open conformation with a greater angle between monomers. Many co-chaperons bind Hsp90 and modulate its function. Aha1 increases the ATP hydrolysis rate and yeast homologs of human p23 and Hop1 proteins inhibit the ATPase activity. In plants Suppressor of Skp1 G2 allele (Sgt1) and Required for Mla12 resistance (Rar1) proteins interact with Hsp90 and together participate in the plant immune response to various pathogenic organisms [2,3]. To investigate the role of Sgt1 and Rar1 in the context of Hsp90 function, we study the solution structure of Hsp90:Rar1:Sgt1 complex with ADP. In solution, Hsp90, Rar1, Sgt1 form tertiary complexes as we concluded from an increased radius of gyration in comparison to that of Hsp90 in complex with ADP. Preliminary DAMMIN *ab-initio* modeling reveals that the former complex has an asymmetric elongated shape in contrast to the asymmetric Hsp90 dimer in complex with ADP.

Richter et al. [4] have observed for human Hsp90α and Hsp90β and yeasts Hsp90 that a higher salt concentration increases specific activity about 1.5 fold in case of yeasts homolog and 3-4 times in case of human homologs. They have also established conformational changes taking place

prior to hydrolysis as a rate limiting step in ATP turnover by Hsp90. We investigated the impact of ionic strength on the conformation of wheat Hsp90 in the hope to explain these data. We investigated apo-Hsp90 and in complexes with ADP and AMPPNP upon increasing KCl concentration from 0mM to 800mM. With increased ionic strength we observed gradual opening of the Hsp90 dimer by changes in P(r) function and value of Rg in apo and nucleotides bound states as shown in Figures 1 and 2.

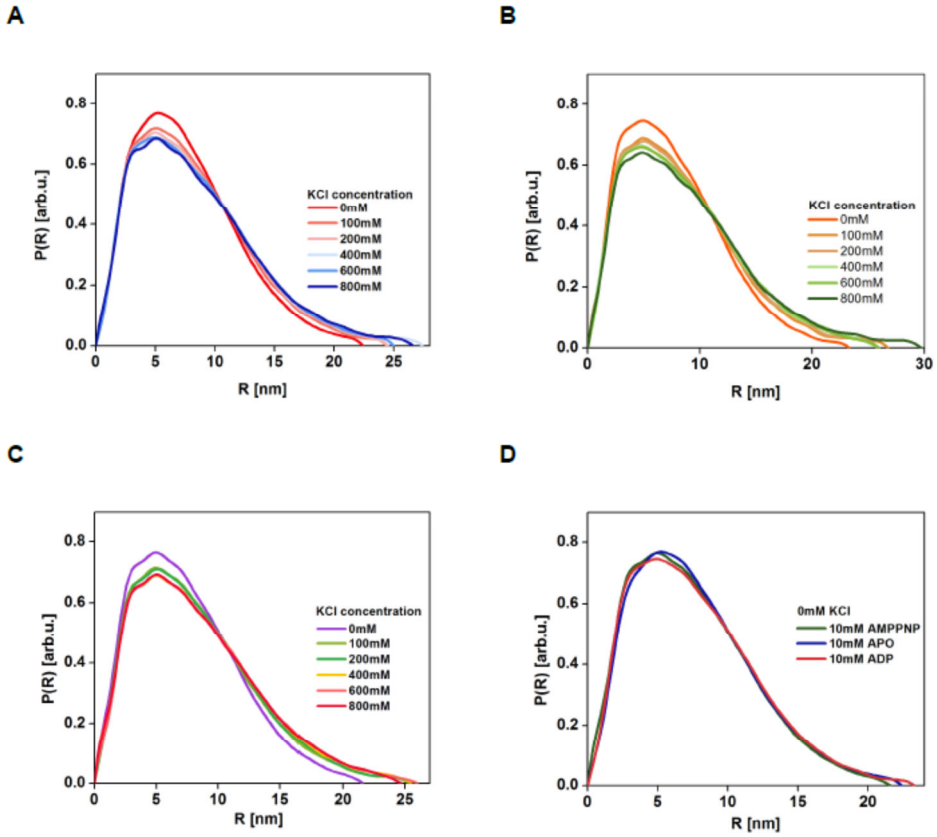

Figure 1. Pair distance distribution function P(r): A. TaHsp90 APO. B. TaHsp90+10mM ADP Mg2+. C. TaHsp90+10mM AMPPNP Mg2+. D. Comparison of P(r) function for Hsp90 complexed with various nucleotides at 0mM KCl.

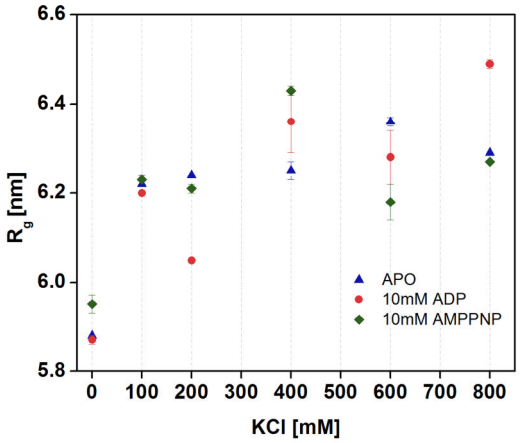

Figure 2. Radius of gyration value for TaHsp90 APO complexed with ADP Mg2+and AMPPNP Mg2+ with increasing salt concentration.

Preliminary results from low resolution *ab-initio* modeling using DAMMIN program for AMPPNP bound Ta Hsp90 are presented in Figure 3.

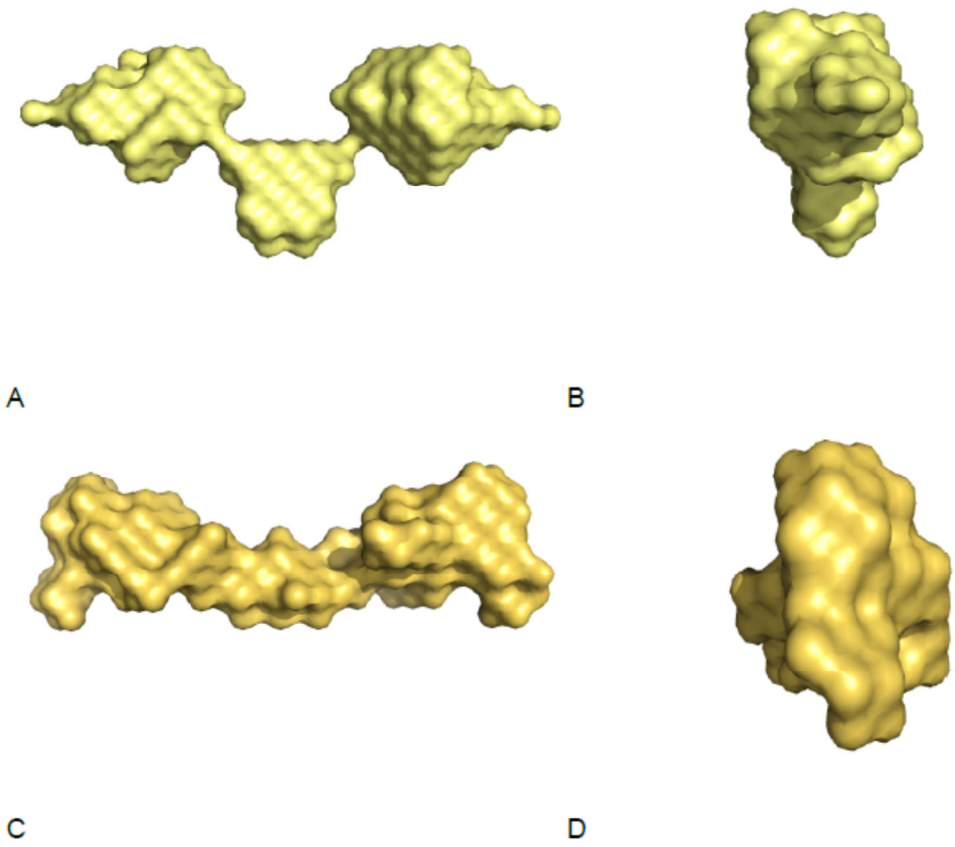

Figure 3. Low resolution *ab-initio* averaged models of TaHsp90 bound to AMPPNP at 0mM and 800mM KCl concentration. A. Model of TaHsp90:AMPPNP complex at 0mM KCl. B. Model rotated about 90 degrees. C. Model of TaHsp90:AMPPNP complex at 800mM KCl. D. Model rotated about 90 degrees.

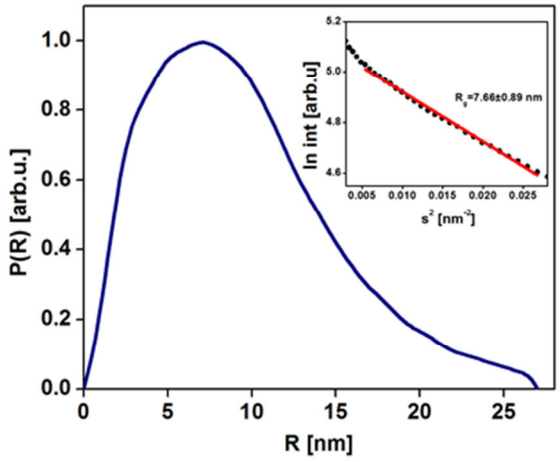

Figure 4. Pair distance distribution function P(r) for TaHsp90 HvRar1 HvSgt1 10mM ADP Mg2+ and Guinier fit to the experimental data.

Hsp90 at low ionic strength condition assumes a more bent conformation in comparison to the almost linear conformation of the molecule at high ionic strengths.

We also measured TaHsp90:HvSgt1:HvRar1:ADP complex. The calculated P(r) function together with Guinier plot with the calculated radius of gyration are presented in Figure 4. A comparison of P(r) functions of the complex and Hsp90 alone permits concluding that the complex has been formed. The calculated radius of gyration is larger than for TaHsp90 even at high KCl concentration. The calculated low resolution model of complex is presented in Figure 5.

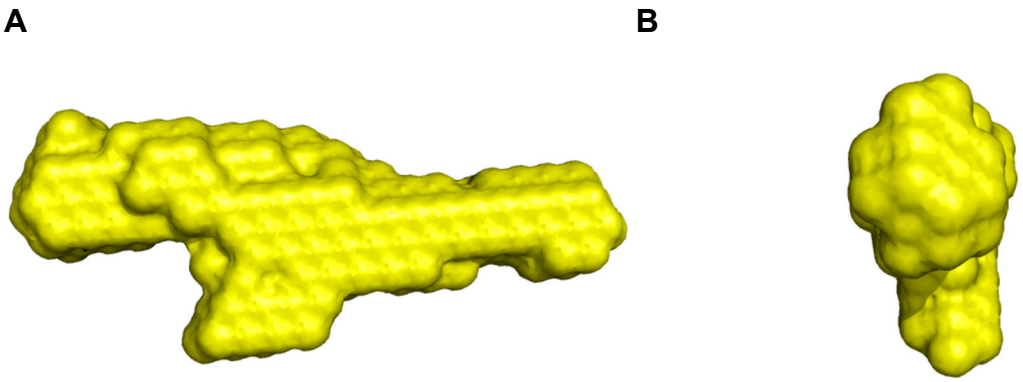

Figure 5. Low resolution averaged *ab-initio* model of TaHsp90-Rar1-Sgt1-ADP complex obtained by DAMMIN program [5].

References

1. Queitsch C, Sangster TA, Lindquist S. (2002). HSP 90 as a capacitor of phenotypic variation. *Nature* **417**, 618-624.
2. Seo YCS, Lee SCK, Song MCY, Suh JCP., Hahn TCR, Ronald P, Jeon JCS. (2008). The RAR1 SGT1 HSP90 Molecular Chaperone Complex: a Core modulator in Plant Immunity. *J. Plant Biol.* **51**, 1-10.
3. Takahashi A, Casais C, Ichimura K, Shirasu K. (2003). HSP 90 interacts with RAR1 and SGT1 and is essential for disease resistance RPS2mediated in Arabidopsis. *Proc Natl Acad Sci USA*. **100** 11777-82.
4. Wandinger SK, Richter K, Buchner J (2008). The hsp90 chaperone machinery. *J. Biol. Chem.* **283**, 18473–7.
5. Svergun, D. I., (1999) Restoring Low Resolution Structure of Biological Macromolecules from Solution Scattering Using Simulated Annealing. *Biophys. J.* **76**, 2879-2886.