Ligand Binding, Unbinding and Allosteric Effects:

Deciphering Small-Molecule Modulation of HSP90

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The molecular chaperone HSP90 oversees the functional activation of a large number of client proteins[1]. Because of its role in multiple pathways linked to cancer and neurodegeneration, drug discovery targeting HSP90 has been actively pursued[2]. Yet, a number of inhibitors failed to meet expectations due to induced toxicity problems. In this context, allosteric perturbation has emerged as an alternative strategy for the pharmacological modulation of HSP90 functions. Specifically, novel allosteric stimulators showed the interesting capability of accelerating HSP90 closure dynamics and ATPase activities, while inducing tumor cell death. Here we gain atomistic insight into the mechanisms of allosteric ligand recognition and their consequences on the functional dynamics of HSP90, starting from the fully unbound state. We integrate advanced computational sampling methods based on Funnel-Metadynamics[3], with the analysis of internal dynamics of the structural ensembles visited during the simulations. We observe several binding/unbinding events and from these we derive an accurate estimation of the absolute binding free energy. Importantly, we show that different binding poses induce different dynamics states. Our work for the first time explicitly correlates HSP90 responses to binding/unbinding of an allosteric ligand to the modulation of functionally oriented protein motions.

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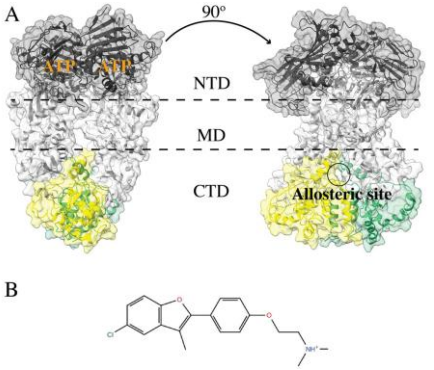

Figure 1. Representation of the molecules involved in the study. (A) Two views of the structure of the yeast HSP90 isoform. NTDs are shown in dark grey, MDs and CTDs are shown in light grey. The allosteric site, formed by the MDs:CTDs borders is depicted by yellow and green colors for protomer A and B, respectively. The ATP binding pockets are also evidenced in the figure. (B) Molecular structure of compound 18.

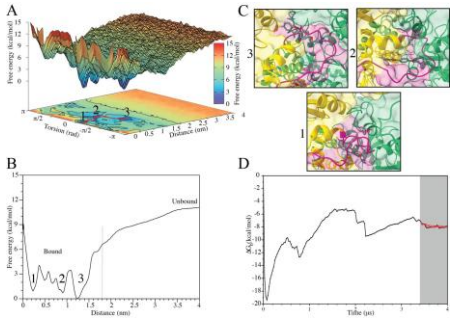

Figure 2. Analysis of Free Energy Surface (FES) obtained through the FM simulation. (A) 2D and (B) 1D FES calculated as a function of the two CVs (i.e. distance and torsion). (C) Representative poses extracted from the ensemble of structures collected in minima 1, 2 and 3 detected by FM. In the three panels the protomers A and B are shown in yellow and green, respectively. The "gating loop" (aa 597-611) from protomer A is depicted in magenta. (D) Time evolution of the ΔG_b^0, showing the convergence in the last 600 ns of trajectory (i.e. grey region). We reported in red the weighted average with respect to time and the relative error bars.