Tuning the molecular mechanism of Hsp70 via a new allosteric network

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The study of the correlations among protein structure, dynamics and function can be efficiently addressed by means of novel Molecular Dynamics (MD) based methods. In this scenario, the study of the impact of ligand-dependent conformational perturbations on functionally relevant protein motions provides new opportunities to regulate their biological activity. Herein, we address this question by advancing the mechanistic understanding on heat shock protein 70 (Hsp70) regulation at the atomic level and identifying molecules capable of selectively interfering with key structural and functional sub-states. Hsp70 system is a model for the next generation of difficult drug targets: it is composed of multiple protein components that show nucleotide-dependent allosteric regulation, it intersects many different biochemical and cellular activities and its dysregulation is implicated in several diseases, including neurodegenerative disorders and several types of cancer lines.[1,2,3] In this framework, the rational understanding of the best ways turning this system “on” or “off” with small molecules is particularly challenging, but highly promising. This idea has already proved its efficacy: the anticancer compound MKT-077 and its analogs have been shown to derive their activity by differentially interacting with Hsp70 allosteric states and impact multiple disease phenotypes.[4] Based on these findings, we combined the computational analysis of the main traits of internal dynamics differentially induced by MKT-077 on various Hsp70 nucleotide sub-states with *in vitro* mutagenesis experiments to identify the substructures that are most relevant in modulating the functional motions of the Hsp70. With this approach, we show that it is possible to rationally identify mutations that mimic the impact of MKT-077 on Hsp70 functions: in particular, we show that both the allosteric ligand and the mutations trap the chaperone in an ADP-like conformation where it can no longer carry out its physiological activity. The combination of our structural and biochemical studies provides new insights into possible ways to tune Hsp70’s functions to treat disease. While motivated by the challenges of Hsp70 as a drug target, the approach used is general and can be broadly extended to other difficult protein targets.

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