HUMAN HSP90 A CONTORTIONIST DRUG TARGET: MODULATION OF ITS ENERGY LANDSCAPE UPON LIGAND BINDING

F. HENOT [1], E. RIOUAL [1], A. FAVIER [1], P. MACEK [1, 2], E. CRUBLET [2], B. BRUTSCHER [1], M. FRECH [3], C. LOISON [4], P. GANS [1], J. BOISBOUVIER [1]

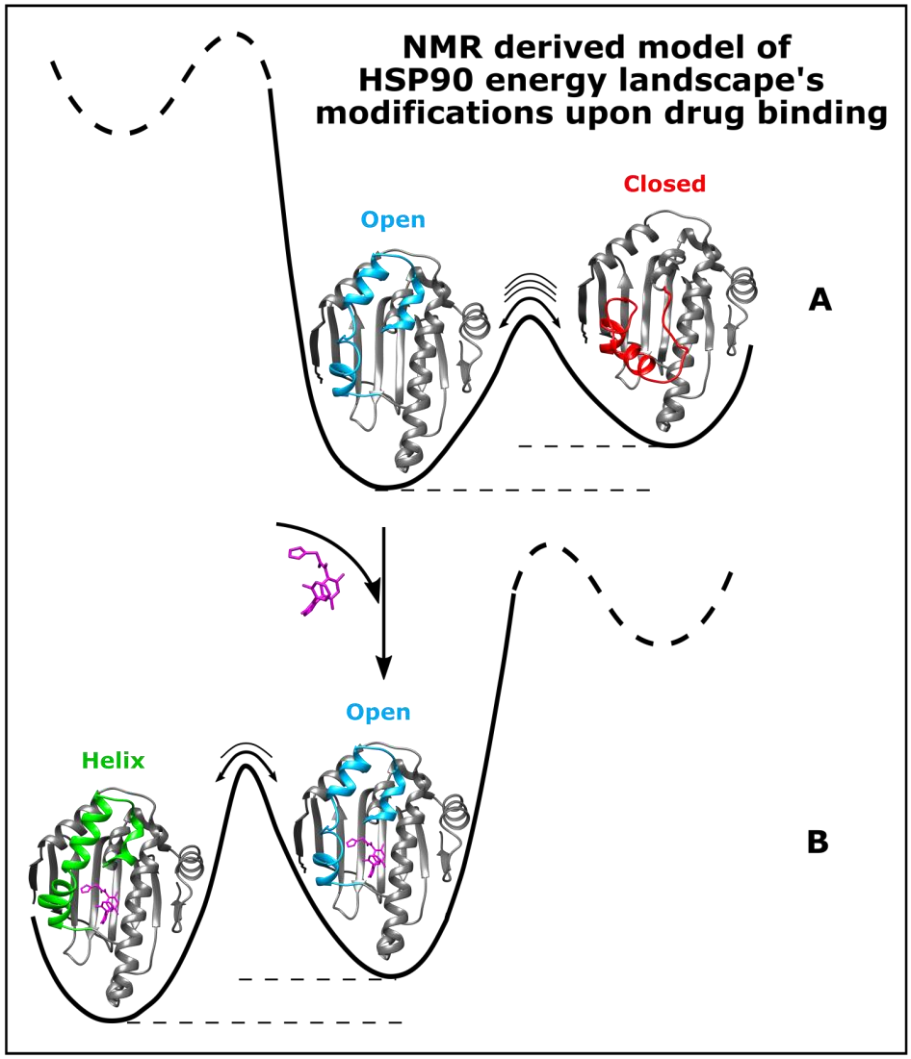
[1]IBS - Grenoble (France), [2]NMR-BIO - Grenoble (France), [3]MERCK KGAA - Darmstadt (Germany), [4]ILM - Lyon (France)

HSP90 is a major chaperone required for folding of various client proteins that has been reported as a therapeutic target for cancer[1]. Several ATP-competitive inhibitors have been developed by pharmaceutical companies. However, as of yet, none has been approved and HSP90 remains an important objective. HSP90 is a very flexible protein, thus a challenging target, showing structural variability, especially on its ATP-lid covering the nucleotide/drug binding site, as pointed out by more than 300 crystallographic structures of N-HSP90. For drug design studies, it is crucial to characterize the exact structure of the ATP-lid segment but also to investigate its dynamics as it was shown to play a role both in the kinetics and thermodynamics of the ligand binding.

We used NMR to characterize in solution the 26 kDa ATP-binding domain of human HSP90a[2]. We demonstrated that HSP90 ATP-lid exchanges between two conformations in the millisecond time scale, and we succeeded in elucidating the structures of both the major and minor states. While the major state is found with the ATP-lid in an open state as expected from previously determined X-ray structures of N-HSP90, we established that the ATP-lid also samples a closed conformation distant by up to 30 Å from the major state[3]. This is the first time that this important structural change, involved in HSP90 functional cycle, is observed in apo N-HSP90. Using NMR CPMG relaxation dispersion experiments, we characterized kinetically and thermodynamically the exchange between the open and closed states of apo HSP90 (Fig. A).

We next investigated how resorcinol derivatives modulate the energy landscape of HSP90. We demonstrated that most resorcinol ligands bind to pre-existing conformations, stabilize the closed state and speed up the structural rearrangement of the ATP-lid domain. Interestingly, few resorcinol derivatives, differing only by one substituent, are able to induce the formation of a new helix in the ATP-lid major state conformation and have a residence time on target increased by two orders of magnitude[4]. While those ligands induced a new major state conformation, we also demonstrated that they slow down the ATP-lid conformational exchange (Fig. B). Our results bring a structural basis to understand target protein conformational dynamics' role in binding of a potential drug candidate.

[1]Miyata et al. Curr Pharm Des.(2013) doi:10.2174/138161213804143725 [2]Henot F et al. J. Biomol NMR.(2021) doi:10.1007/s10858-021-00370-0. [3]Henot F et al. *Structural and Dynamics Characterization of Transiently Populated States of Human HSP90 ATP-lid.(submitted)* [4]Amaral et al. Nat Commun.(2017) doi:10.1038/s41467-017-02258-w


NMR derived model of
HSP90 energy landscape's
modifications upon drug binding
Closed
Open
A
Open
Helix
B