EXPLORING THE ALLOSTERIC MECHANISM OF ONCOGENIC TYROSINE PHOSPHATASE SHP2 FOR THE DESIGN OF PEPTIDE INHIBITORS

V. Santucci,[a] P. Calligari,[a] S.Bobone, [a] G. Bocchinfuso,[a,*] and L. Stella[a]

[a] Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", Rome, 00133, Italy.

[*] *e-mail: gianfranco.bocchinfuso@uniroma2.it*

PTPN11 was the first proto-oncogene encoding a phosphatase to be identified [1]: gain of function mutations in this gene cause >30% of cases of juvenile myelomonocytic leukemia and its encoded tyrosine phosphatase (SHP2) is known to play a key role in several malignancies [2].

The structure of SHP2 (Figure 1) is composed by two SH2 domains (N-SH2 and C-SH2), which bind signaling protein partners containing phosphorylated motifs, followed by a tyrosine phosphatase domain (PTP). In basal conditions, the N-SH2 domain blocks the catalytic site and SHP2 is inactive. Conversely, the association to binding partners favors a conformational transition from this autoinhibited conformation to an active state of the protein. Interestingly, most of the amino acid substitutions in oncogenic mutants of SHP2 are located at the interface between PTP and N-SH2 and perturb the autoinhibitory interaction in favor of open conformations, resulting in a hyperactivated protein [3].

Very recently, two different structures (obtained from X-ray [4] and SAXS/NMR [5] experiments, respectively) have been proposed as representative of the open state conformation for the SHP2 oncogenic mutant E76K. Interestingly, in both structures, the C-SH2 domain is oriented differently with respect to the closed state, thus suggesting a peculiar and previously unappreciated role of this domain in the open/closed transition. Alternatively, the N-SH2 domain assumes slightly different positions in the two structures. Furthermore, the interface between the PTP and N-SH2 domains in the proposed open state structures seem to be affected by pathological mutations much less than the corresponding interface in the closed state. These evidences rise the question whether the proposed structures are truly representative of the SHP2 active state open. As an alternative, an "ensemble" of open state configurations could characterize the active state.
Beyond the reliability of the proposed open-structures, the open/close transition pathway followed by SHP2 remains unknown. To provide clues on both of these aspects and more in general on the mechanism of allosteric regulation of SHP2 and on the effects of pathogenic mutations, the active/inactive transition was studied by means of molecular dynamics simulations (MD), for both the wild-type protein and mutants linked to leukemia.

To thoroughly explore the conformational changes that are involved in the transition, high energy barriers need to be crossed during the MD: therefore, enhanced sampling techniques are needed to achieve this task in a reasonable computational time. In this study, different simulative techniques (Metadynamics (metaD), Replica Exchange Molecular Dynamics (REMD) and Steered Molecular Dynamics (SMD) simulations) were used to elucidate both the SHP2 active state and the mechanism of activation.

In the REMD approach, different replicas of the system are simulated in parallel at different temperatures and exchanges between conformations at adjacent temperatures are attempted with a given acceptance probability. REMD is an "unbiased" enhanced sampling technique that allows the system to cross moderately high free-energy barriers on all degrees of freedom.

REMD trajectories starting from either the closed or the open experimental structures have been performed. Simulation staring form the closed state explored conformations where the PTP catalytic site was fully accessible, but remained far from the experimental proposed open state. On the other hand, simulation starting from the open state. This ensemble comprises accessible conformations quite different from the proposed single active conformation. A comparison with the available experimental data will be presented

As opposed to REMD, metaD and SMD are "biased" techniques that are used to guide the system in crossing high energy barriers along selected collective variables and allow the estimation of free-energy differences. In our study, these simulations were used to estimate the activation free energy associated to a possible path leading to the opening of SHP2. In the SMD simulations, an external pulling force is added to the system and forces the NSH2 domain to leave the position adopted in either the closed or the open state. A number of independent pulling dynamics simulations can be used to estimate the activation free-energy.
These techniques neglect the evolution of those degrees of freedom that are not directly related to the chosen collective variable. For this reason, only a combined use of these techniques and REMD may provide a detailed picture of the structural properties of the large conformational changes involved in the open/closed transition.

These simulations could shed light on the general mechanism of opening of SHP2. Furthermore, the knowledge of the conformational changes involved in the allosteric regulation could provide new bases for the development of novel pharmacological strategies.

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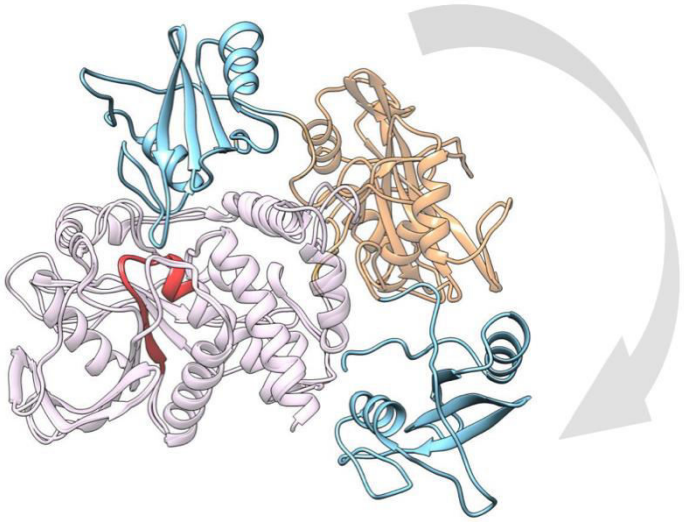

Figure 1. Crystallographic structures of SHP2 close state (PDB ID: 4DGP) and SHP2 open state (PDB ID: 6CRF). The PTP and CSH2 domains are represented in transparent pink and transparent orange, respectively. NSH2 domains are represented in sky blue.