Summary

Allosteric regulation is a ubiquitous and effective mechanism that controls protein activity and therefore, biological processes.
The main characteristic of allosteric proteins is the ability of transmitting signals within their structure to trigger specific responses at remote sites.
The current work focuses on an allosteric phosphatase, the human PTPN11, consisting of two Src homology-2 (SH2) and one Protein Tyrosine Phosphatase (PTP) domains. Its enzymatic activity is auto-inhibited by the interaction between the PTP domain and the N-terminal SH2 domain. When a bi-phosphorylated ligand binds both SH2 domains, the PTP domain is released and the enzyme becomes active. This binding event induces the large conformational change in the protein of releasing the catalytic domain from the N-SH2 domain.
Notwithstanding this large effect at the protein level, few conformational changes have been described in the regulatory domains upon tyrosyl-phosphorylated (pTyr) ligand binding. Indeed different studies (i.e. Src, PTPN11 N-SH2 and Fyn, among others) revealed little conformational effects at the level of SH2 domains. Therefore, protein dynamics must play a key role in transmitting the allosteric signal from the binding site of the SH2 domains to the PTP domain.
The main goal of this study is to shed light on the intra-domain communication mechanism inherent to the C-terminal SH2 domain of PTPN11, identifying experimentally how pTyr binding induces dynamic effects within the domain itself, potentially revealing for the first time how signals are transduced through its structure and how communication with the rest of the molecule could occur.
With that aim, we first determined the 3D solution structure of PTPN11 C-SH2 domain in its free form and bound to a pTyr peptide derived from the natural target EpoR. As previously described for other SH2 domains, ligand binding did not induce significant conformational changes in the structural core of PTPN11 C-SH2 domain.
Subsequently, we studied the dynamics of the backbone and methyl side-chains of the apo C-SH2 domain and upon binding of two natural PTPN11 ligands (i.e. EpoR and Gab1). The dynamical and chemical shift changes experienced upon both binding events were described on a per-residue basis and compared with each other and with the changes experienced by

other SH2 domains upon pTyr binding (i.e. PTPN11 N-SH2 binding PDGFR-1009 and Fyn binding Fyn's C-tail).

The changes in flexibility in C-SH2 domain of methyls measured in both conditions (EpoR and Gab1 binding) show a high degree of homology (86% of the methyls common to both conditions experience the same increase/decrease in flexibility). These common residues could be part of a dynamic pattern responsible for propagating the signal throughout the structure.

However, some differences between both binding events become evidentwhen considering the extent of these changes, 41% of the methyl groups analyzed in the case of EpoR show significant changes in flexibility, while in the case of Gab1 this is only 32%. Furthermore, only 5 of these significant changes overlap in both conditions, with one methyl group experiencing a significant increase in rigidity upon binding EpoR and a decrease upon Gab1 binding (i.e. Val-170 γ1).

Differences were also observed when comparing this data with dynamical data available for other SH2 domains (i.e. PTPN11 N-SH2 domain and Fyn SH2 domain). From the data available for this study, while 44% of the methyls studied in C-SH2 overlap with methyls in N-SH2, only 28% overlap in the case of Fyn SH2.

In the case of N-SH2, no explicit data of the differences between the methyls in the bound and free states of N-SH2 was available. We made a comparison based on interpreting the independent results, which showed 50% of the common methyls to experience different dynamical changes while 31% remained the same (the comparison of the remaining results did not lead to an obvious conclusion).

In the case of Fyn SH2, despite the low overlap, the data available show high degree of similarity, with 80% of the common methyls showing the same trend. However, once more, the extent of these changes did not overlap, with only one methyl experiencing a significant change in C-SH2 upon binding both peptides and in Fyn SH2 upon binding the C-tail (i.e. C-SH2's Leu125 δ1 aligning with Fyn's Leu-163 δ1).

In conclusion, despite the high degree of similarity in sequence and structure (including conserved protein-ligand contacts), the similarity in binding preferences and even affinities, the dynamics induced by binding might not be universal for all SH2 domains, with even potentially important differences within the same SH2 domain binding two different ligands. Further experiments will be required to validate the importance of the similarities and differences in relation to the functional role of the domain in its protein context.