PEPTIDE INHIBITORS OF PROTEIN-PROTEIN INTERACTIONS TARGETED TO SRC HOMOLOGY 2 DOMAINS: A COMBINED COMPUTATIONAL AND SPECTROSCOPIC DESIGN APPROACH

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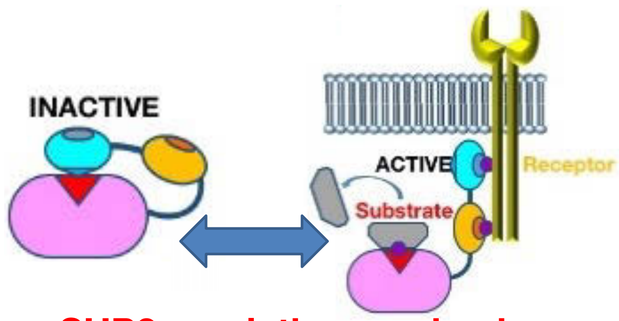
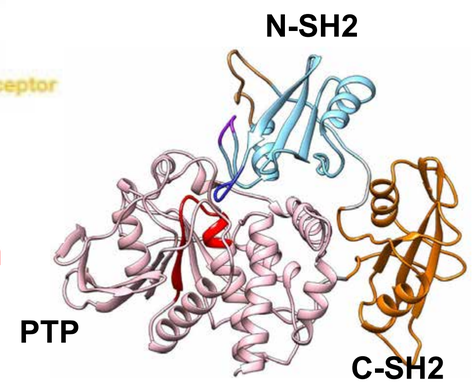
INTRODUCTION

SHP2 is an important molecular target for therapies against cancer and rare diseases, like RASopathies [1,2]. Mutations of the gene coding for SHP2, PTPN11, have been associated with cancer and developmental disorders. The structure of SHP2 includes two SH2 domains (N-SH2 and C-SH2) followed by the catalytic domain PTP. SH2 domains recognize and bind phosphopeptide [3]. They also have an important role in modulating the catalytic activity of the protein. Under basal conditions, the N-SH2 domain blocks the catalytic site and SHP2 is inactive. The association to binding partners favors a conformational transition from this autoinhibited conformation to an active state. Most of the SHP2 pathological amino acid substitutions perturb the autoinhibitory interaction in favor of open conformations, resulting in a hyperactivated protein [4]. SHP2 can be efficiently inhibited by targeting the binding of the N-SH2 domain to cognate proteins [5], furthermore the development of inhibitors of SHP2 protein-protein interactions can take advantage of the role of the C-SH2 domain for increasing both specificity and affinity.

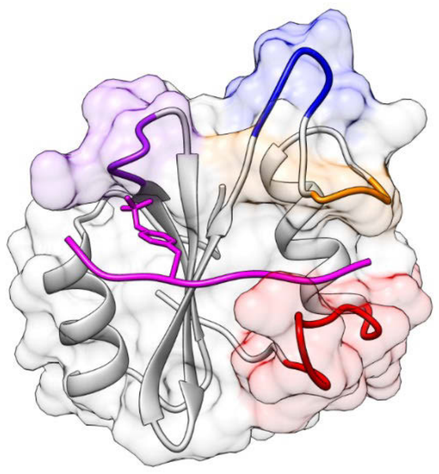


SHP2 regulation mechanism



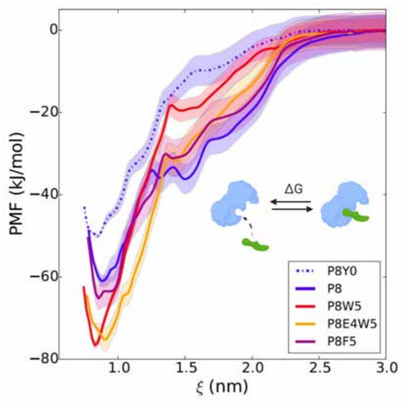
N-SH2

Current SHP2 inhibitors, which target either the catalytic site or an allosteric pocket, often lack specificity and are ineffective against disease-associated SHP2 mutants.
In response to the signaling hyperactivation caused by pathogenic lesions, we developed peptide-based molecules that exhibit nanomolar affinity for the N-SH2 domain of SHP2.
These peptides offer good selectivity, stability to degradation, and display 2–20 times higher affinity for pathogenic SHP2 variants compared to the wild-type protein.

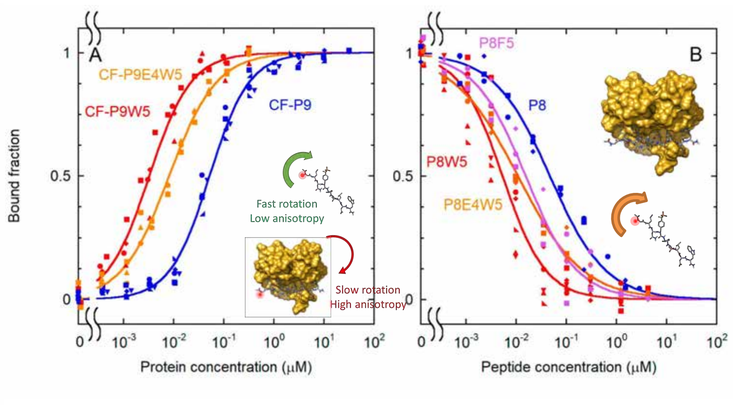


Binding affinity

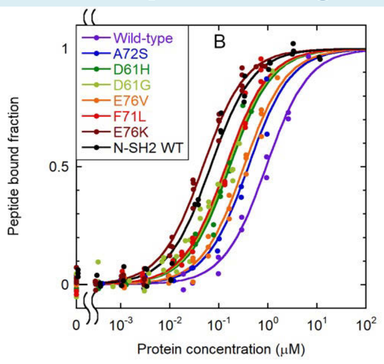
Umbrella sampling simulations



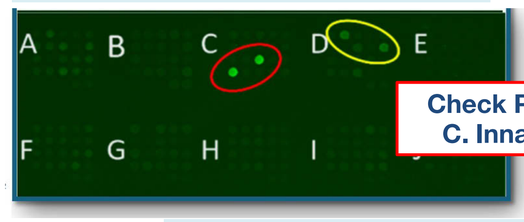
Fluorescence polarization assay



Selectivity for oncogenic variants

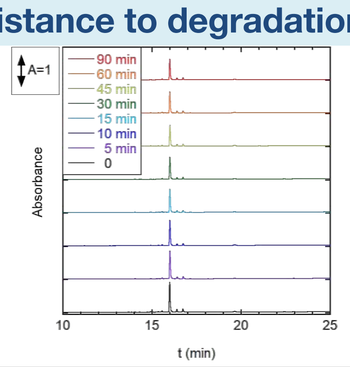


High specificity

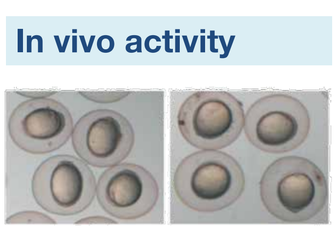


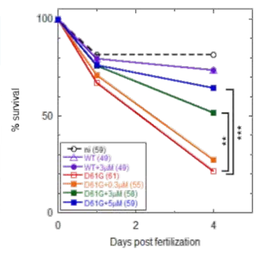
Check P2.128 !!!
C. Innamorati

Resistance to degradation



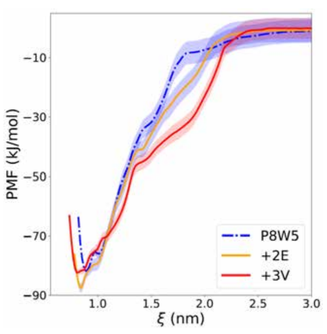
In vivo activity



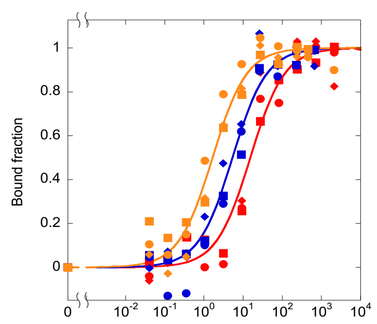


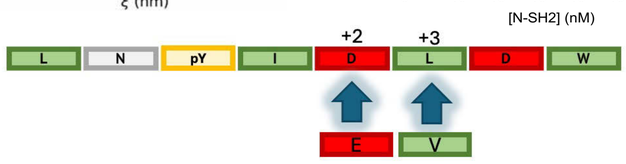
Further developments

US simulations



Fluorescence anisotropy assay





Affinity improvement

CF-GLN-pY-IDLDW K_d=(5.3±0.6) Nm

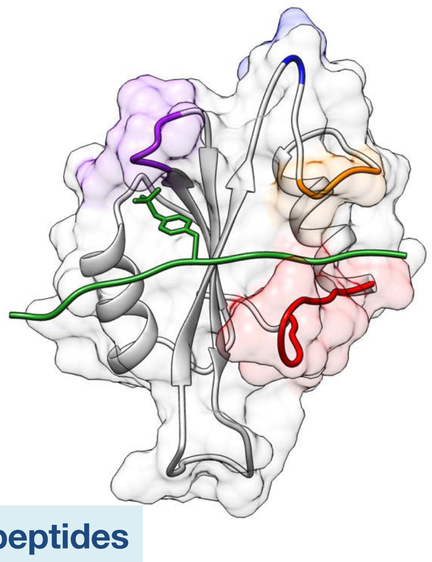
CF-GLN-pY-IDVDW K_d=(15.3±0.2) nM

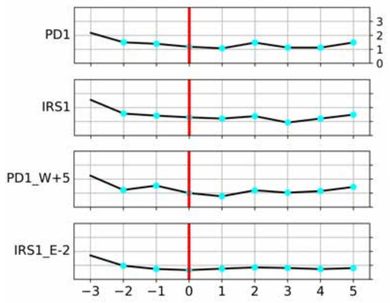
CF-GLN-pY-IELDW K_d=(1.66±0.03) nM

C-SH2

To evaluate the binding specificity of different peptide sequences for the C-SH2 domain, we conducted MD simulations of the domain complexed with high-affinity natural partners (PD1, GAB1, and IRS1) and their mutants, starting from the PD1 NMR structure (PDB ID: 6R5G). We also performed a similar analysis using crystallographic structures (PDB IDs: 5DF6, 5X7B, 5X94).

- GROMACS software package
- Force field AMBER99SB-ildn
- 1.2 μs





Structure and Dynamics of bound peptides

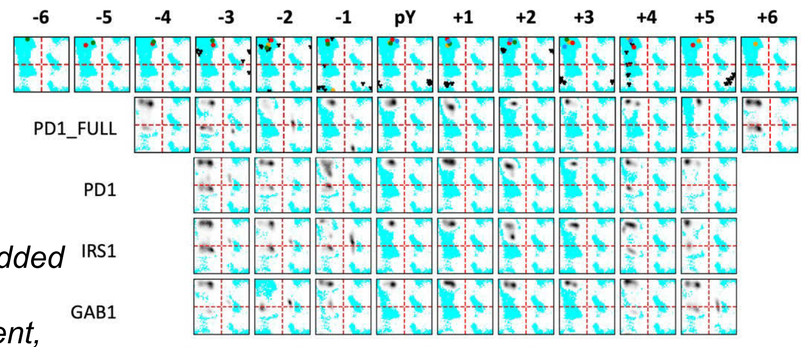
RMSF between peptide backbone and C-SH2 domain

Peptides stay within the binding cleft for the entire trajectory. The N-terminal portion exhibited higher mobility compared to the C-terminal side.

Ramachandran Plots of peptide backbone

Peptide maintain an extended conformation in the crystallographic structure. The central region (+1 to +3 residues) remains extended, occasionally extending to residue +4.

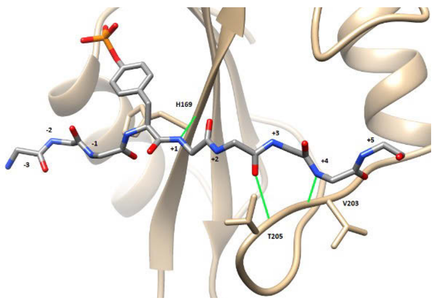
Residues +1 and +3 remained stably embedded in the hydrophobic groove.
Residues +2 and +4 point towards the solvent, so it is better to introduce polar residue in these positions.



X-Ray and NMR structures (first line), MD simulations (other lines).

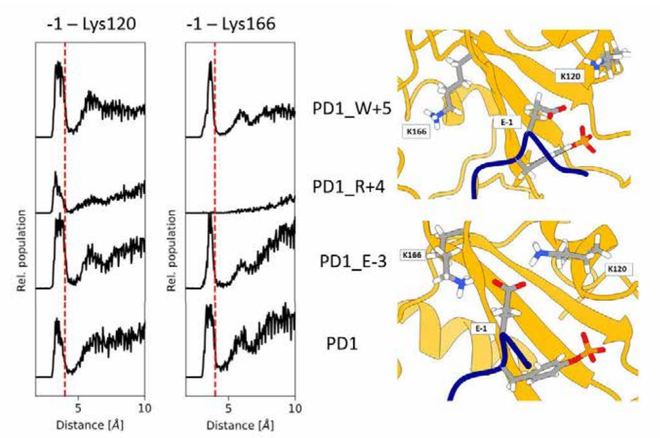
Hydrogen bond interactions

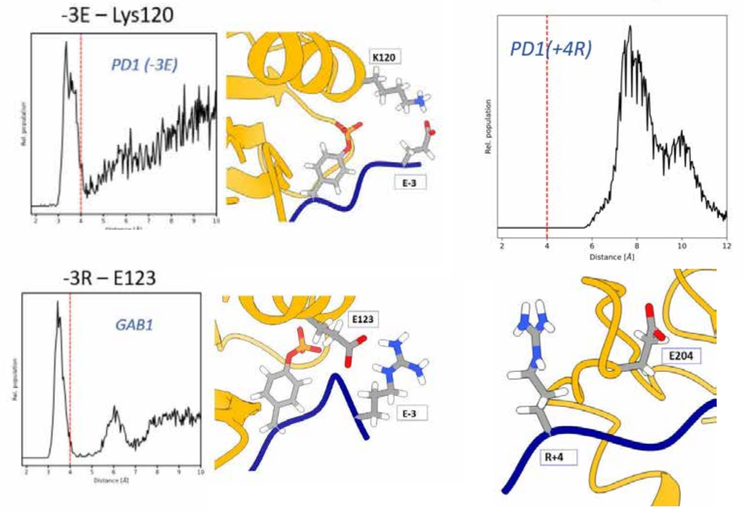
Backbone of the phosphopeptide residues +1, +2 and +4 forms HB respectively with the 169H^0, 205$T^N$and 203V^0 of C-SH2 domain.



Inter-molecular ion-pair interactions

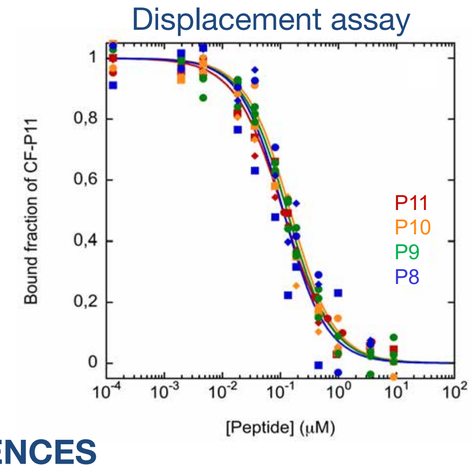
N-terminal and C-term portions of phosphopeptides can form different ion-pair interactions with the residue of the C-SH2 domain.





Further developments

Displacement assay



Minimal high affinity sequence length

	Sequence	Kd (nM)
P11	GETEpYATIVFP	77±4 (CF-P11, fluorescence anisotropy assay) 28±4 (displacement assay)
P10	ETEpYATIVFP	46±7 (displacement assay)
P9	TEpYATIVFP	38±4 (displacement assay)
P8	TEpYATIVF	29±5 (displacement assay)

All peptides showed comparable affinities toward C-SH2 domain.

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