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Effects of peptide labeling on fluorescence binding assays to Src homology 2 domains: the case of protein-protein interactions inhibitors of the oncogenic phosphatase SHP2

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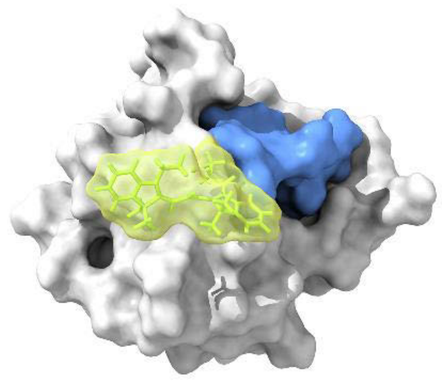
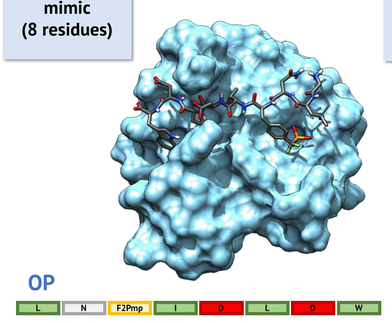
Introduction

Due to their role in numerous signaling pathways, Src homology 2 (SH2) domains have garnered significant attention as potential targets for pharmaceutical intervention. Their primary biochemical function is to recognize polypeptide sequences containing phosphotyrosine (pTyr). Tyrosine phosphorylation plays a critical role in eukaryotic cell regulation; hence, mutations in the genes for at least 23 distinct SH2 domain proteins contribute to various human disorders, including cancers, leukemias, developmental disorders, diabetes, and immunodeficiencies. Peptide-based drugs targeted to SH2 domains are a powerful tool to inhibit therapeutically relevant protein-protein interactions [1]. Fluorescence-based assays, such as anisotropy/polarization measurements [2], or quantitative microarrays [3] are commonly used to determine the affinity and selectivity of these molecules for their target domain. We recently developed protein-protein interaction (PPI) inhibitors targeted at the N-terminal Src homology 2 (SH2) domain of the oncogenic phosphatase SHP2 [1].



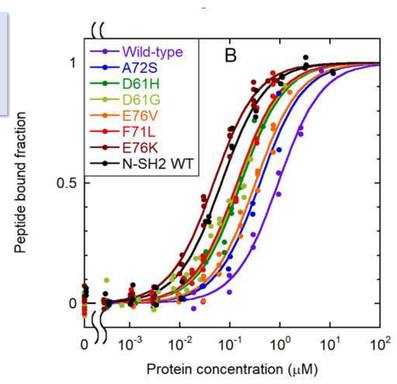
Our PPI inhibitors

Short peptide mimic (8 residues)

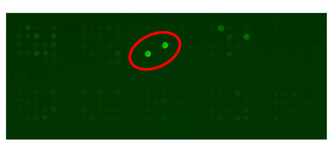


Patent 102020000004849, 2020 PCT/EP2021/055624, 2021
L. Stella, B. Biondi, G. Bocchinfuso, S. Martinelli, M. Tartaglia

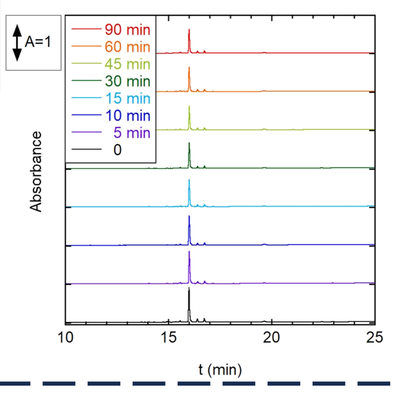
nM activity Selectivity for oncogenic variants



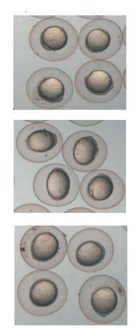
High specificity



Resistance to degradation

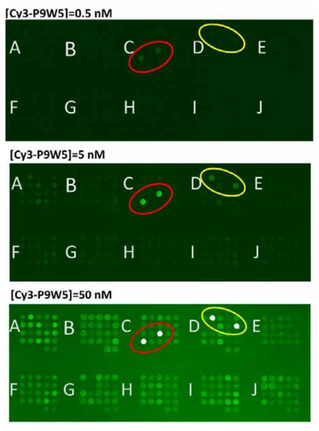


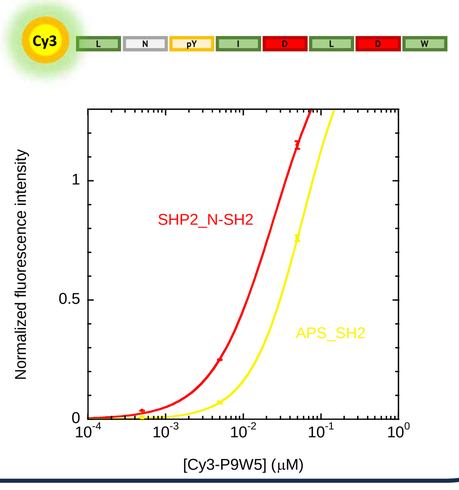
In vivo activity



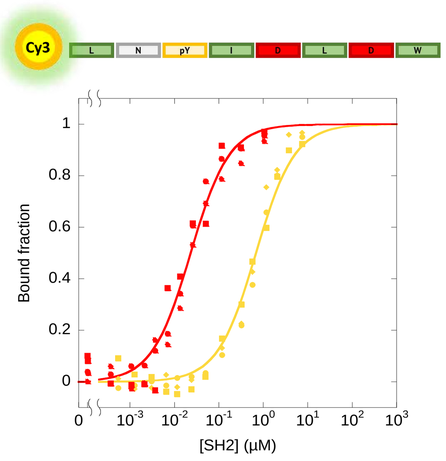
1. Quantitative analysis of the microarray data

While highly active and selective, these molecules showed a weaker unwanted interaction with the SH2 domain of another protein, called APS, in a fluorescence microarray assay [1].

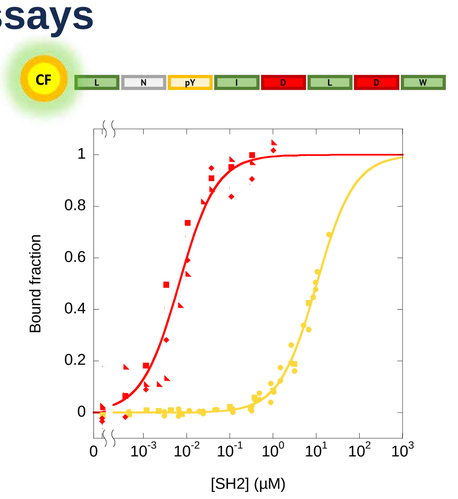




2. Fluorescence anisotropy binding assays



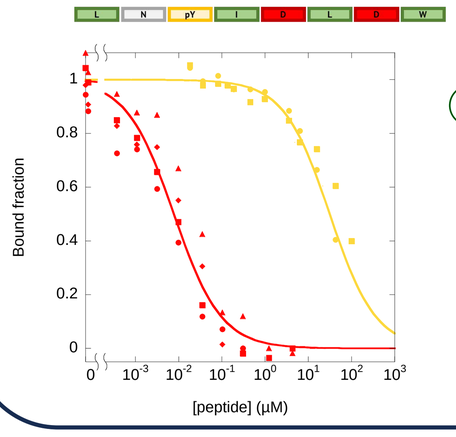
$$\frac{K_d\ \mathrm{APS}}{K_d\ NSH2} = 30 \pm 3$$



$$\frac{K_d\ \mathrm{APS}}{K_d\ NSH2} = 1700 \pm 100$$

Labeling can enhance or inhibit binding by more than one order of magnitude.

3. Displacement binding assays



Competition displacement assays can be used to determine the dissociation constants of unlabeled peptides

$$\frac{K_d\ \mathrm{APS}}{K_d\ NSH2} = 10000 \pm 2000$$

Molecular modeling

The fluorophore interacts significantly with the protein surface of both domains, thus providing a structural rationale for the observed effects of labeling on the binding affinity.

Conclusions

Protein-protein association can be perturbed by any chemical modification (ex. the insertion of a **fluorescent probe**), to one of the binding partner. This is particularly true when one of the two molecules is relatively small, as in the case of peptides.

Peptide	K_d (APS_SH2) (μM)	K_d (SHP2_N-SH2) (μM)	Selectivity (K_d APS/K_d SHP2)
Cy3-P9W5	$(6.9 \pm 0.3)\ 10^{-1}$	$(2.3 \pm 0.2)\ 10^{-2}$	30 ± 3
CF-P9W5	5.8 ± 0.2	$(3.3 \pm 0.2)\ 10^{-3}$	1700 ± 100
P8W5	$(1.6 \pm 0.2)\ 10^{1}$	$(1.6 \pm 0.2)\ 10^{-3}$	10000 ± 2000
OP	$(7.0 \pm 1.0)\ 10^{1}$	$(1.5 \pm 0.3)\ 10^{-2}$	5000 ± 1000

- ✓ The perturbation of the dissociation constant caused by labeling can be significant (more than an order of magnitude).
- ✓ The effect can depend strongly on the specific fluorophore used, and even on the protein domain.
- ✓ Our findings suggest that displacement binding assays should always be employed to obtain binding data free from labeling artifacts.

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