Computational Modeling of ERK2, DUSP6, and BCI

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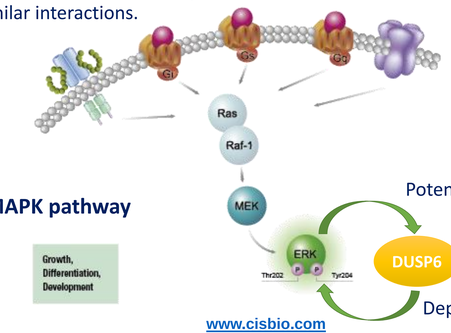
Abstract

Extracellular signal-regulated kinase 2 (ERK2) and dual specificity phosphatase 6 (DUSP6) are proteins involved in the mitogen-activated protein kinase (MAPK) pathway which triggers cell proliferation. DUSP6 dephosphorylates ERK2 and thus prevents continuous signaling of cell growth. BCI is a DUSP6 inhibitor first identified in a zebrafish screen that prevents the ERK2 mediated activation of DUSP6. BCI has been shown to reduce cell proliferation in cancer cells, but the BCI binding site and mechanism of action are not known. Determining how BCI regulates DUSP6 and the MAPK pathway would further our understanding of the role of DUSP6 in cancer and potentially aid in the development of new cancer drugs.

In order to create computational models of BCI binding, Amber, a program that creates dynamic simulations of proteins, was utilized to generate simulations of ERK2 and DUSP6 and obtain representative conformations. BCI was docked to the selected conformations using SwissDock to identify the best binding sites. The BCI ligands docked closest to the sites implicated in the DUSP6/ERK2 interaction were selected and their binding affinities were calculated using Smina.

BCI binding sites were identified near the Tyrosine 185 residue on ERK2, near the KIM domain on the DUSP6 N terminal, and close to the catalytic site on the DUSP6 C terminal. We have generated 3 computational models that suggest possible mechanisms of action of BCI inhibition of DUSP6. We used these models to perform a virtual screen for compounds with similar interactions.

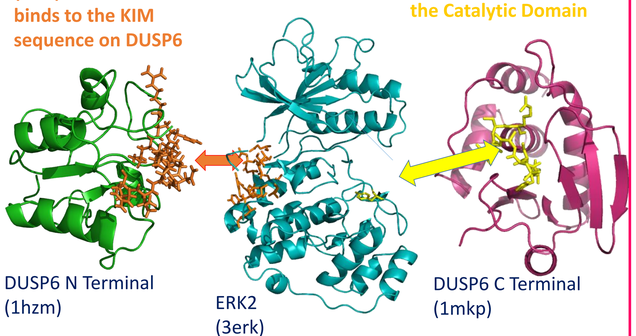


Introduction

Models of ERK2 and DUSP6's terminals

Kinase interaction Motif (KIM) Domain on ERK2 binds to the KIM sequence on DUSP6

Tyrosine 185 binds to the Catalytic Domain



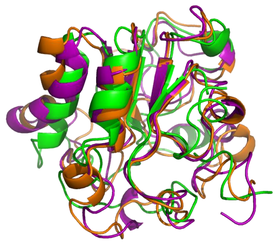
DUSP6 N Terminal (1hzm)

ERK2 (3erk)

DUSP6 C Terminal (1mkp)

Methods

1.Created molecular dynamic simulations of ERK2 and DUSP6 using Amber to understand their movement over time and selected the most representative conformations of each protein

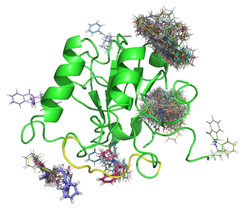


DUSP6 N terminal's overlaid frames

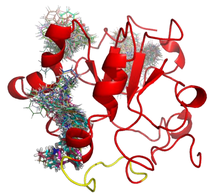
ERK2's overlaid frames

DUSP6 C terminal's overlaid frames

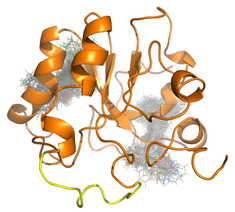
2. Docked BCI to several binding pockets in the ERK2 and DUSP6 conformations using SwissDock.



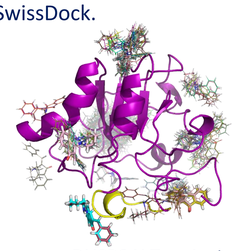
DUSP6 N terminal Original Crystal



DUSP6 N Terminal conformation 2



DUSP6 N terminal conformation 3

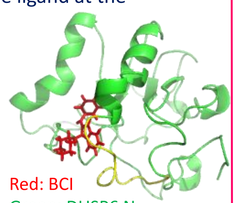


DUSP6 N Terminal conformation 4

3.Selected the BCI ligands that have the highest binding affinity at several binding pockets using Smina, which calculates the binding affinity of the ligand at the binding site.

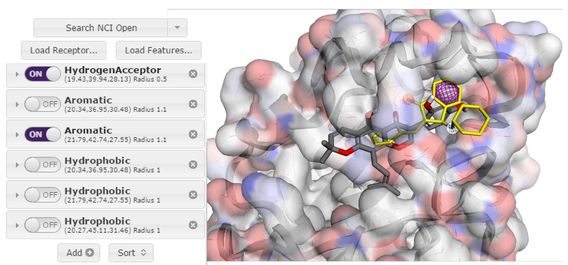
Site1-clusters.338_0149

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-9.1	0.000	0.000
2	-8.6	3.012	6.458
3	-8.5	4.161	7.086
4	-8.3	2.228	6.500
5	-8.3	1.901	6.172
6	-8.1	2.201	6.384



Red: BCI
Green: DUSP6 N terminal
Yellow: KIM sequence

4. Virtual screens for compounds with interactions similar to BCI was performed using Pharmit



Conclusion and Future direction

Based on the highest binding affinity scores generated by Smina and the location at which BCI binds on the proteins, it is most likely that the BCI structure binds near the KIM sequence on the DUSP6 N Terminal.

We plan to test the compounds identified in the virtual screen to see which binding modes result in DUSP6 inhibition

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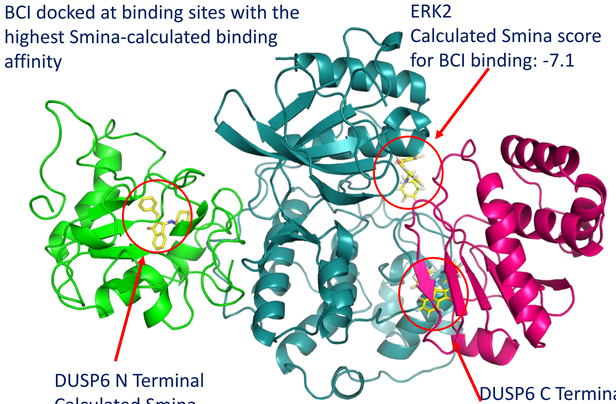
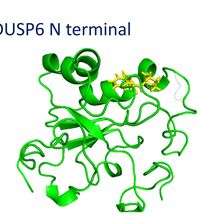
Gengkon Lum

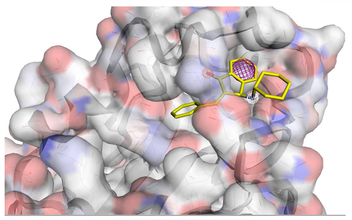
Results

ERK2/DUSP6 binding complex with BCI docked at binding sites with the highest Smina-calculated binding affinity

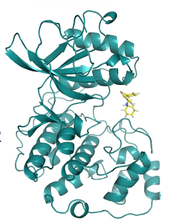


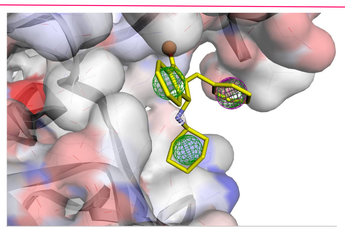
DUSP6 N terminal



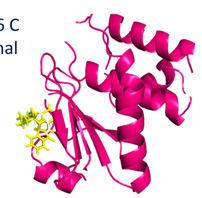


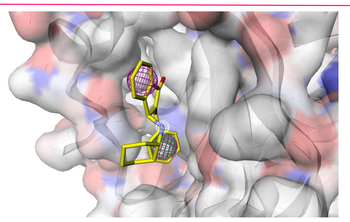
ERK2





DUSP6 C terminal





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