PROTAC Design Using Electrostatic Complementarity™

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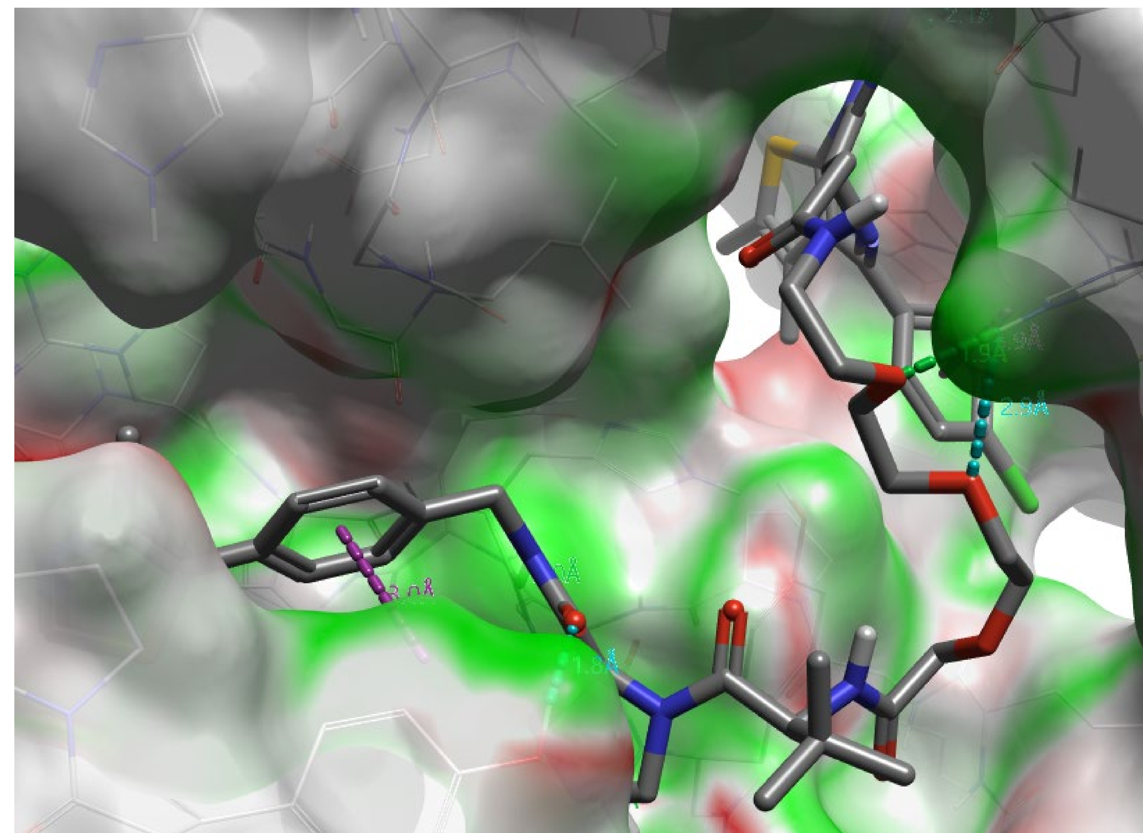
cresset CADD software

Introduction

With the recent resurgence of investigating targeted protein degradation as an approach in drug discovery, computational techniques provide a promising solution to the challenging task of designing protein-targeting chimeras, or PROTACs®. With Cresset's proprietary Electrostatic Complementarity (EC) analysis, available within the molecular modeling platform Flare™,[1] we are able to assess the complementarity of a PROTAC structure to a protein target of interest when the linker is involved in stabilization of the ternary complex.

In this poster, we illustrate how slight changes in the electrostatics of a PROTAC structure can affect its complementarity to the binding sites and investigate methods for modifying the linker moiety. Critical to our approach is the use of electrostatic and shape similarity to ensure that potential replacement linkers maintain favorable interactions with the target proteins.



Good EC Electrostatic Clash

Figure 1: PROTAC **MZ1** bound to Brd4^{BD2} and VHL. The protein surface is colored according to the EC.

Background

Flare's EC feature uses the electrostatics of the ligand and an associated protein to determine how well they match.[2] To illustrate the utility of this feature in designing PROTAC linkers, we will use PROTAC **MZ1** which targets Bromodomain and extra-terminal domain (BET) proteins, namely the bromodomain-containing protein 4 bromodomain 2 (Brd4^{BD2}), for degradation. In particular, **MZ1** exhibits tight binding to the Brd4^{BD2} and the E3 ubiquitin ligase von Hippel-Lindau tumor suppressor protein (VHL) (K_d = 3.7 nM). **MZ1** has been cocrystallized with Brd4^{BD2} and VHL (pdb 5T35),[3] shown in Figure 1.

In this experiment, we will replace the PEG linker with a fragment that yields a bioisosterically similar PROTAC but also simultaneously improves the EC to the binding site of the protein complex.

Method

To automate the design of potential new linkers, we utilized the Spark™ Scaffold Hopping wizard.[4] Figure 2 shows the Spark workflow.

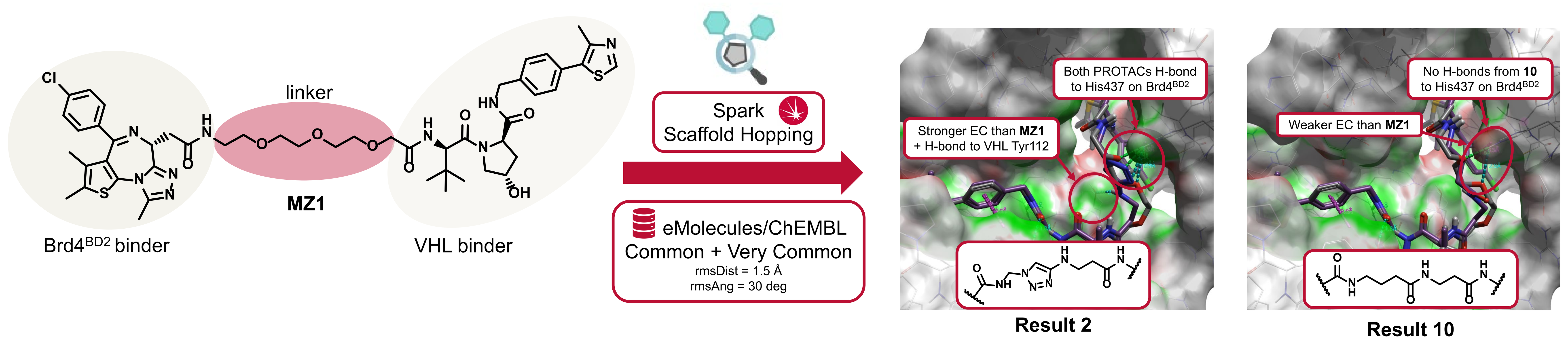


Figure 2. The Spark workflow to obtain new linkers for assessment in Flare. (Left) The structure of **MZ1** with the fragment to be replaced highlighted in red. (Right) Two of the top results (violet) visualized in Flare overlayed with **MZ1** (gray). The protein surface is colored according to the EC of each ligand to the Brd4^{BD2}-VHL complex (green = good EC; red = electrostatic clash)

The PEG chain on the PROTAC **MZ1** was identified as the 'scaffold' to be replaced. Spark was then setup to search for fragments that would give final PROTAC structures that (1) are bioisosterically similar to the starting **MZ1** and (2) would not clash with the surrounding proteins. The top results, sorted by similarity score, were exported to Flare. Figure 2 shows results **2** and **10** in Flare with their associated EC surfaces.

Results

The Spark ligand-based search results gave some interesting analogues of the PEG chain in **MZ1**. Two of the most notable were results **2** and **10** (Figure 2), both contain hydrogen bond donors in lieu of the hydrogen bond acceptors in the PEG chain. The EC surfaces (Figure 2 and 3) of the designed PROTACs were then calculated using Flare. The triazole **2** potentially makes the same interactions as the PEG chain to His437 on Brd4^{BD2}, with an additional improved EC compared to **MZ1** (See Figure 1).

This suggests that the designed linker, with its different electrostatics, would be an improvement over the PEG linker. The amide chain **10** on the other hand, does not hydrogen bond to Brd4^{BD2} as **MZ1**, and there is an additional clash with Brd4^{BD2} when compared to the EC of **MZ1** (See Figure 1).

If we look more closely and apply the electrostatic potential (ESP) and EC surfaces to the ligands, we can see the similarities and differences in the linkers (Figure 3). While we see only subtle difference when viewing the ESP surfaces, they are more-clearly identified with the EC surfaces. The PEG chain in **MZ1** has minor electrostatic clashes which seem to be resolved with the triazole **2**. The amide chain **10** has significant clashing with the proteins. When we calculate the EC score for each PROTAC, they match the observed EC surfaces: **MZ1** and **2** are similar, with **2** slightly better, and **10** is the poorest with the most clash.

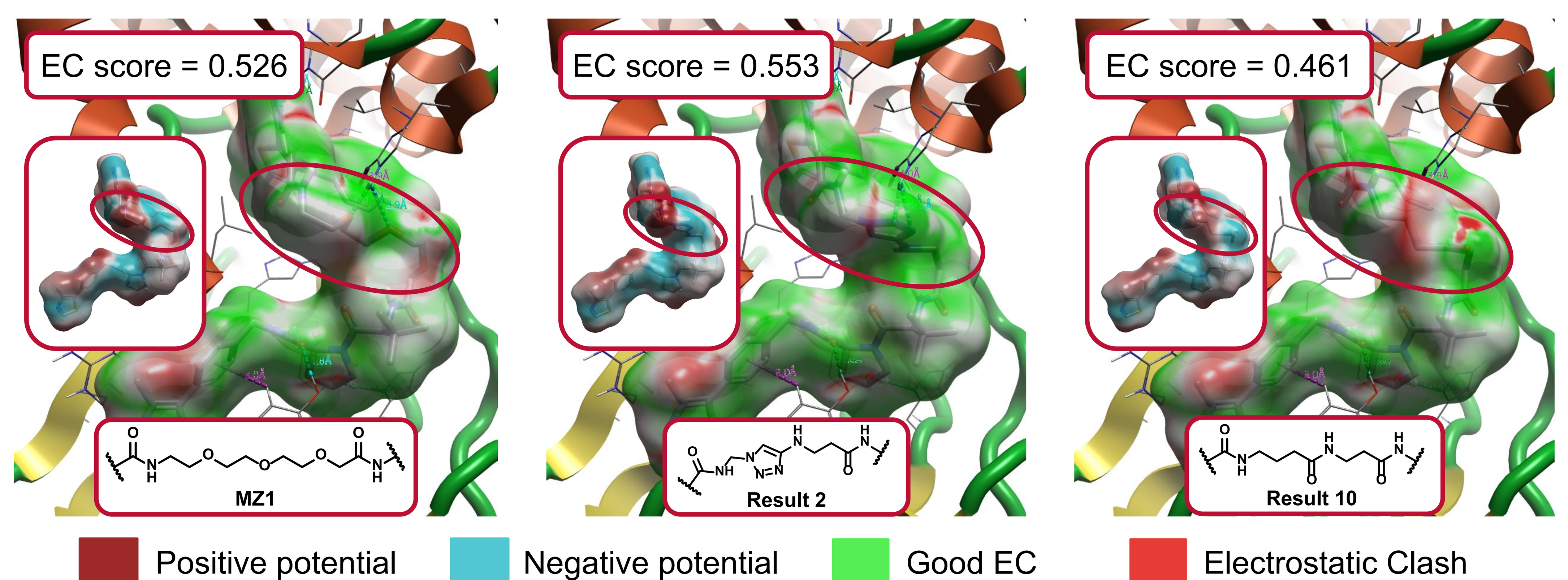


Figure 3. The ESP (red/blue) and EC surfaces (green/red) and scores of the co-crystallized **MZ1** (left) with the two selected Spark results, results **2** (middle) and **10** (right). Linkers are circled.

Conclusion

In this experiment, we have computationally evaluated new PROTAC linker designs with the EC feature in Flare. Using Spark, we have found a linker that has potentially better binding to the Brd4^{BD2}-VHL complex. While these results are not biologically evaluated, they do offer insight into the potential diversity of linkers that complement the binding site of the protein complex.
EC can also be used to study the protein-protein interactions at the binding site. Figure 4 shows the EC surfaces of Brd4^{BD2} and VHL.

Good EC Electrostatic Clash

References

1. https://www.cresset-group.com/software/flare/
2. Bauer, M. R. and Mackey, M. D. *J Med Chem* **2019**, 62, 3036 -3050.
3. Gadd, M. S. *et al. Nat Chem Biol* **2017**, 13, 514-524.
4. https://www.cresset-group.com/software/spark/

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