Design and evaluation of new linkers in PROTACs

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Introduction

Scaffold hopping in drug discovery is a useful approach that has been successfully used in hit and lead optimization to improve the activity and/or properties of ligands, as well as to generate new ideas that overcome novelty issues associated with the ligand structure. With the recent resurgence of targeted protein degradation, scaffold hopping and bioisostere replacement techniques have been applied to the design of protein-targeting chimeras, or PROTACs, either to design ligands that bind to the protein of interest or the attached ligand linkers. In this poster, we have used our scaffold hopping and bioisostere replacement application, Spark™,[1] along with a recently curated linker database[2] to design and evaluate new linkers for a chimera complex. Central to the approach is the detailed description of the electrostatic interactions of the linker moiety with the proteins of interest and use of these descriptions to search for and rank replacements. This approach provides both novel and known linkers that maintain the protein-protein interactions in the ternary complex.

Background

Spark's scaffold hopping tool, though initially designed for small molecules, can also be applied to PROTACs and PROTAC linkers. The product-centric scoring function uses the electrostatics and shape of the starting PROTAC as a reference to determine the similarity of the final PROTACs. To illustrate the utility of this bioisostere replacement feature, we will use PROTAC **48** from Krieger *et al.*, which targets Bromodomain and extra-terminal domain proteins, namely the bromodomain-containing protein 4 (Brd4) bromodomains 1 and 2 (Brd4$^{BD1/2}$), for degradation. In particular, PROTAC **48** exhibits tight binding to the Brd4$^{BD1/2}$ and the E3 ubiquitin ligase von Hippel-Lindau tumor suppressor protein (VHL) (K_D = 4.3 nM). PROTAC **48** has been cocrystallized with Brd4^{BD2} and the VHL-elongin complex (pdb: 8BDX),[3] shown in Figure 1.

In this experiment, we will replace the PEG linker with a fragment that yields a bioisosterically similar PROTACs but moves away from the sometimes-problematic PEG linker.

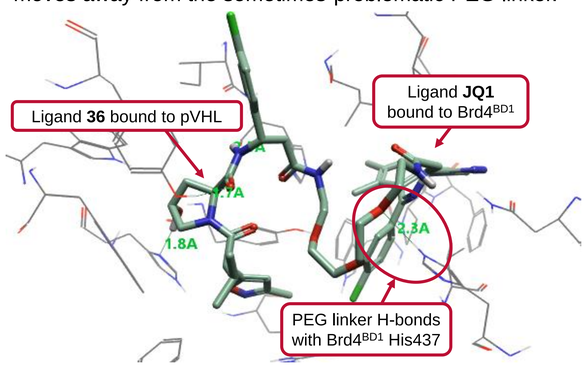
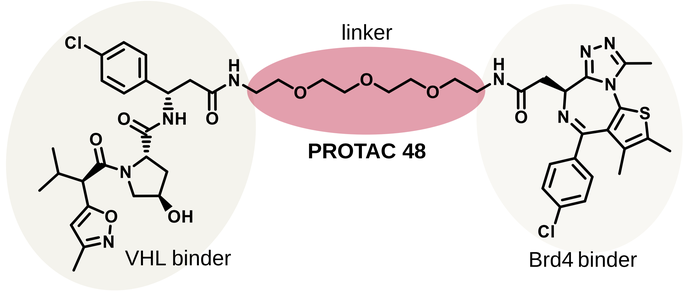
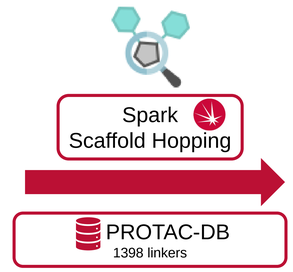


Figure 1: PROTAC **48** bound to Brd4^{BD2} and VHL. The hydrogen bonds are shown in green.

Method

To automate the design of potential new linkers, we utilized the Spark™ Scaffold Hopping wizard.[1] Figure 2 shows the Spark workflow.





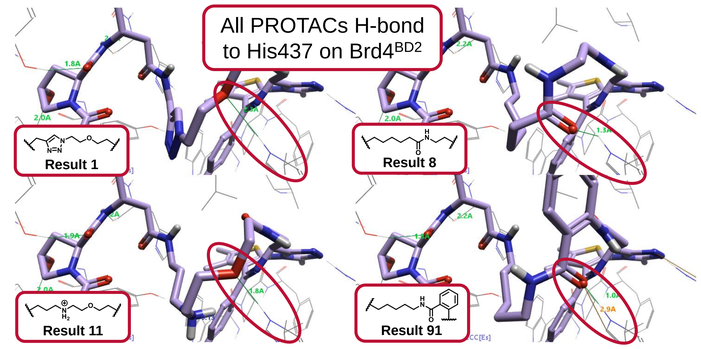


Figure 2. The Spark workflow used to obtain new linker designs. (Left) The structure of PROTAC **48** with the linker fragment to be replaced highlighted in red. (Right) Four of the top results (violet) visualized in Spark. The hydrogen bonds between each ligand to the Brd4^{BD2}-VHL complex are shown in green.

The PEG chain on the PROTAC **48** was identified as the 'scaffold' to be replaced. Spark was then set up to search for linkers from the Tingjun Hou group database[2] to give final PROTAC structures that (1) are bioisosterically similar to **48** and (2) would not clash with the surrounding proteins. Figure 2 shows results **1**, **8**, **11**, and **91** in Spark.

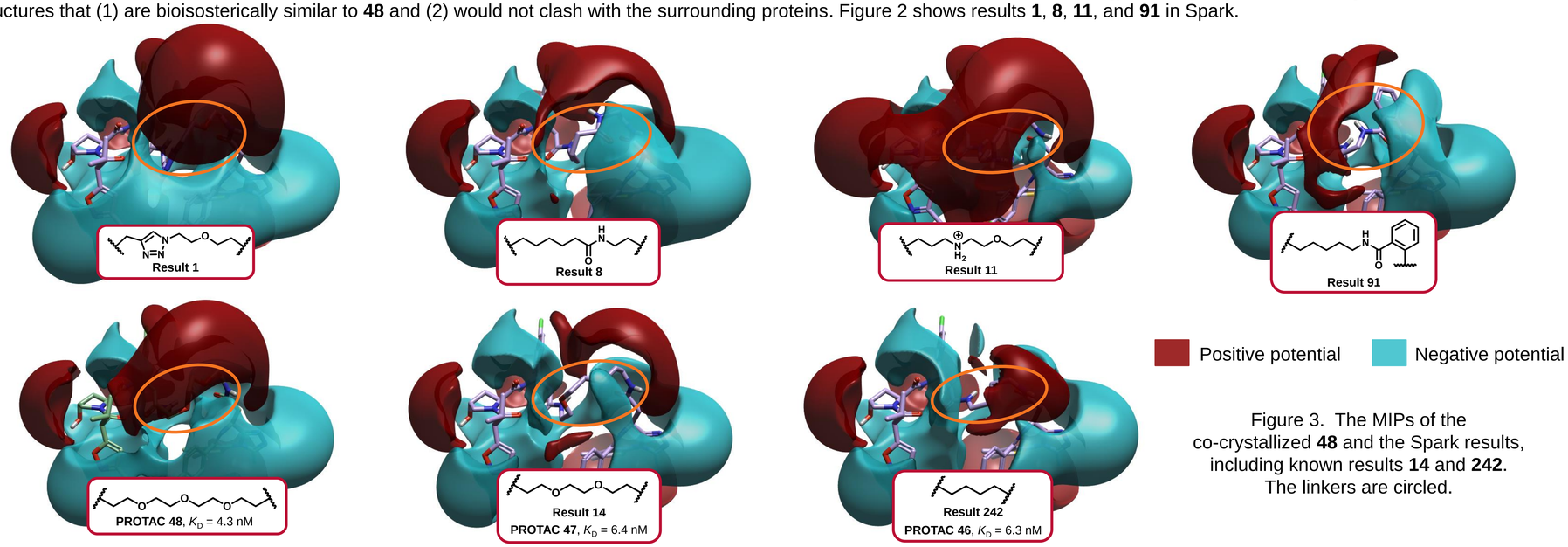


Figure 3. The MIPs of the co-crystallized **48** and the Spark results, including known results **14** and **242**. The linkers are circled.

Results

The Spark ligand-based search results not only included both analogs of PROTAC **48** from the study within the top 250 results, but also gave some structurally diverse non-PEG linkers. Although most of the top results were PEG chains or similar, many of them retained their electrostatic and shape similarities but lost interactions with the protein. However, four of the most notably diverse designs that also retained hydrogen bonding with His437 on Brd4^{BD2} were **1**, **8**, **11**, and **91** (Figure 2). Figure 3 shows each PROTAC with their associated molecular electrostatic interaction potentials (MIPs), as per the unique Cresset extended electron distribution forcefield.[4] Despite the known PROTACs having similar K_D values and similar structures, their MIP surfaces are quite different. PROTAC **48** appears to have more positive electrostatics around the linker than PROTACs **46** and **47**. The unknown results, on the other hand, share more positive electrostatics with a higher similarity to PROTAC **48** despite their different 2D structures, especially triazole **1** and positively charged amine **11**. This similarity in electrostatics suggests that the new linkers could be a promising alternatives to the original linkers, especially since the highlighted results scored higher than PROTAC **46**, appearing as result **242**.

Conclusion

In this experiment, we conducted a bioisostere replacement of a known PROTAC PEG linker to search for new and interesting linkers. Using Spark, we have found linkers that have potentially similar binding to the Brd4^{BD2}-VHL complex, as well as similar electrostatics. 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.

References

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2. http://cadd.zju.edu.cn/protacdb/
3. J. Krieger, F. J. Sorrell *et al. ChemMedChem* **2023**, 18, e202200615
4. https://www.cresset-group.com/science/overview/