

Targeting hard-to-attack protein-protein interaction surfaces using UV-reactive surface fragments

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Aims: 80% of the human proteome consists of hard-to-attack proteins without targetable binding pocket. Efficient strategies like fragment-based drug development are not available for these proteins. Our goal was to map the surface of difficult-to-attack targets with peptidomimetic fragments and then increase the affinity with linking the foldamer fragments, thus extending fragment-based drug development to targets without a binding pocket.

Methods: Experiments were performed on one of the most difficult target proteins, KRas G12D, which has a high contribution to many malignant tumours. We tested a 100-membered foldamer library, containing a UV-reactive group, so that the bound foldamers covalently labelled the target that could be identified by HPLC-MS. The binding site of the most effective fragments were determined by proteomic MS-MS analysis and ¹⁵N HSQC NMR titration. In dynamic covalent library experiments, foldamer dimers with higher affinity were created.

Results: The surface fragments were able to recognize the protein surface and bind to the target. We found numerous foldamers with a side chain-dependent binding pattern with KRas G12D. The UV-reactive marker made the screening efficient, and we were able to localize the binding site of the fragments. The foldamers found the known protein-protein interaction surfaces of the target, but also discovered new, potentially attackable regions. In protein-templated dynamic covalent library experiments, dimers with higher affinity were created.

Conclusion: Using the protein surface mimetic foldamer fragment library and the connecting the building blocks can be a viable general hit generation strategy for challenging protein targets.

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