A PLATFORM FOR EFFICIENT X-RAY CRYSTALLOGRAPHY-BASED FRAGMENT SCREENING AND HIT EVOLUTION OF KINASE TARGETS

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Library design

Here, we present an X-ray crystallography fragment screening (XFS) library designed for kinases, where fragments were selected from the Enamine REAL Space to enable rapid hit evolution through "SAR by catalogue". High solubility in DMSO is required for XFS, while a good aqueous solubility is required for screening and hit evolution of weakly binding fragments. Based on these requirements, fragments with a heavy atom count (HAC) of 9-16 from the Enamine
Water-soluble library were selected and further refined using substructures from KinFragLib (1) representing the adenosine pocket scaffolds (Figure 1). The resulting compound set was filtered based on physicochemical properties, ranked based on the number of commercially available analogues, followed by final manual curation.

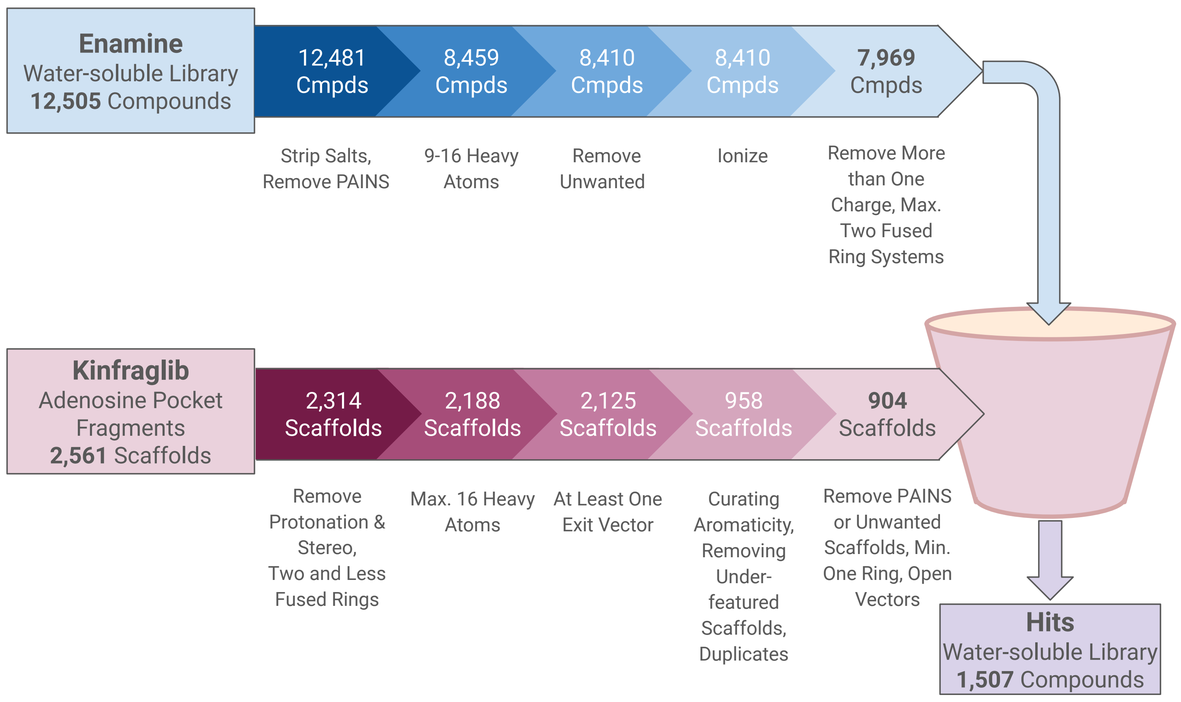


Figure 1: Design process for a kinase-focused XFS library

Fragment screening results

The final fragment library consists of 300 fragments, where the number of commercially available analogues from Enamine Real Space is 12 million, allowing for rapid hit evolution. The kinase-focused fragment library was experimentally verified using two different kinases, CDK2 (Figure 2) and one internal target (Figure 3), where the data was collected using the FragMax platform at the MAXIV Laboratory. A hit rate of ~13-16% was achieved versus the two kinases (Table 1), where almost all fragments bound to the kinases as classical hinge binders.

Table 1: Statistics for XFS screen using kinase focused fragment library

Protein	Hits	Fragments	Non-hinge binding	Resolution range	Hit rate
CDK2	26	208	1	1.1-1.85	13%
SBX2301	47	299	11	1.28-2.3	16%

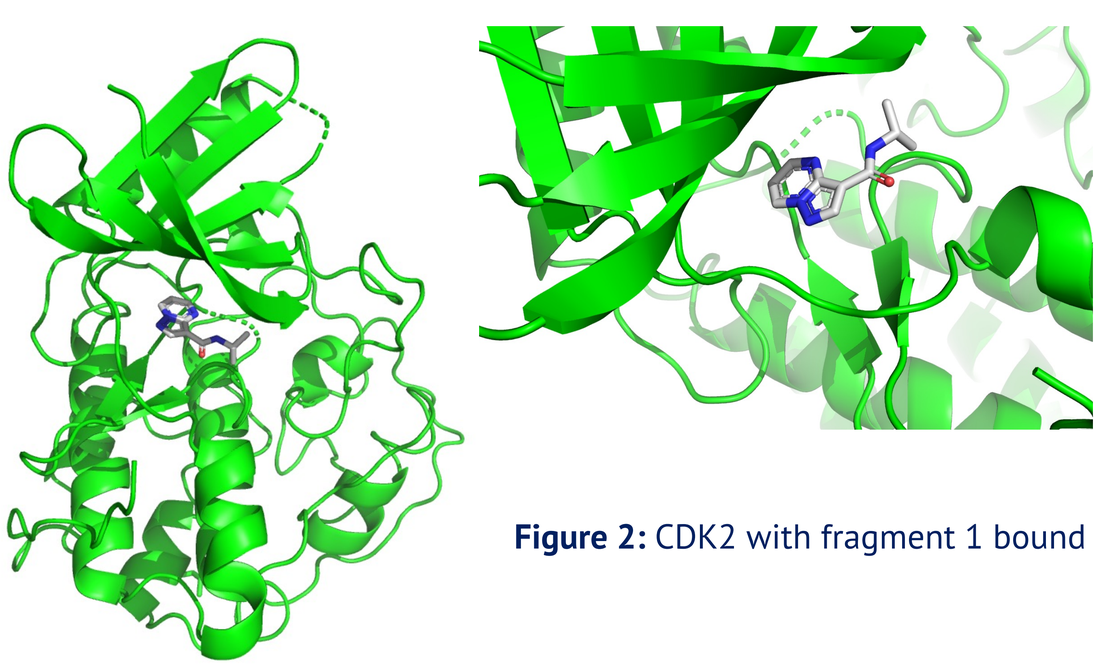


Figure 2: CDK2 with fragment 1 bound

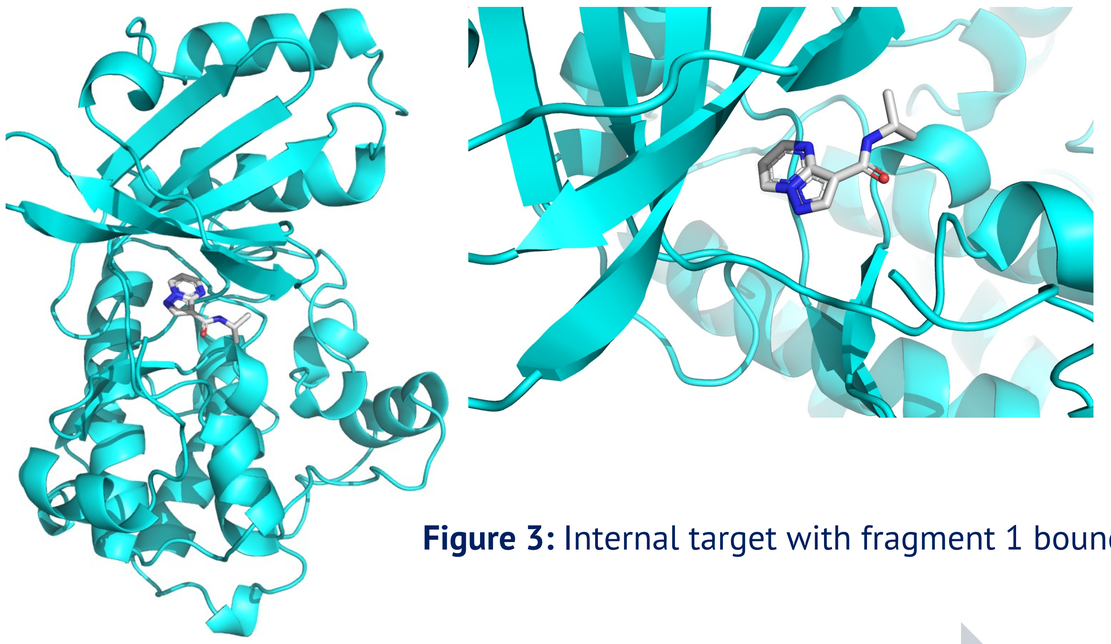
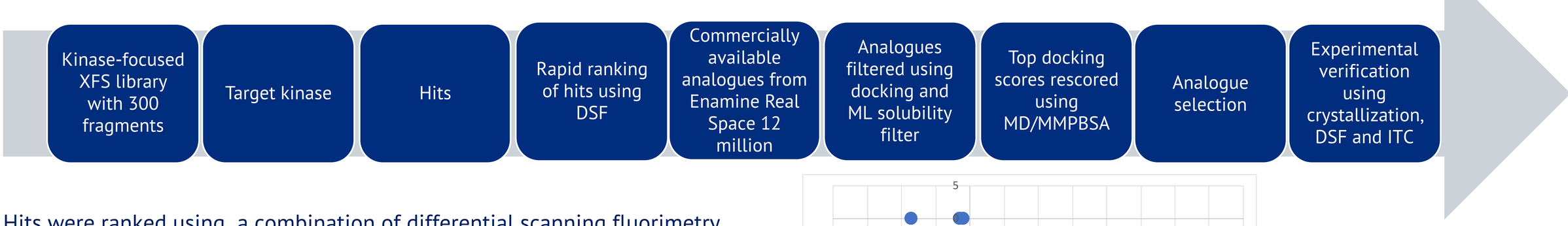


Figure 3: Internal target with fragment 1 bound



Hits were ranked using a combination of differential scanning fluorimetry (DSF) and free energy calculations using molecular dynamics (MD) simulations (Figure 4), for easy ranking of fragment hits.

Rapid SAR identification and hit expansion

Based on these XFS results, we have set up a hit expansion and evolution pipeline where fragment hits from XFS can be rapidly ranked using a combination of DSF and MD simulations, followed by hit expansion and filtering of commercially available analogues using a combination of docking, ML and MD to select analogues for experimental verification using crystallization, DSF and ITC.

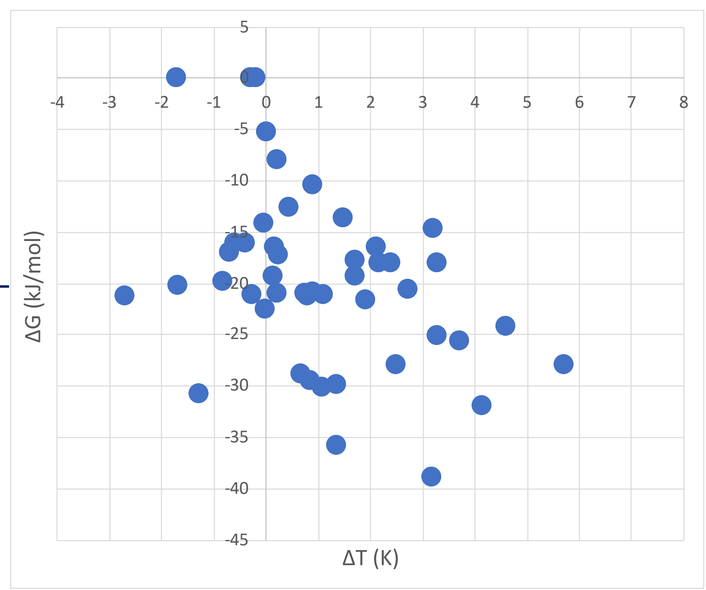


Figure 4: Correlation plot of ΔG from MD/MMPBSA versus ΔT from DSF

References

1. Sydow et al., (2020), "KinFragLib: Exploring the Kinase Inhibitor Space Using Subpocket-Focused Fragmentation and Recombination", J. Chem. Inf. Model., 60, 12, 6081–6094.

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