From Fragments to Inhibitors: an *in silico* approach to identifying new biomimetics

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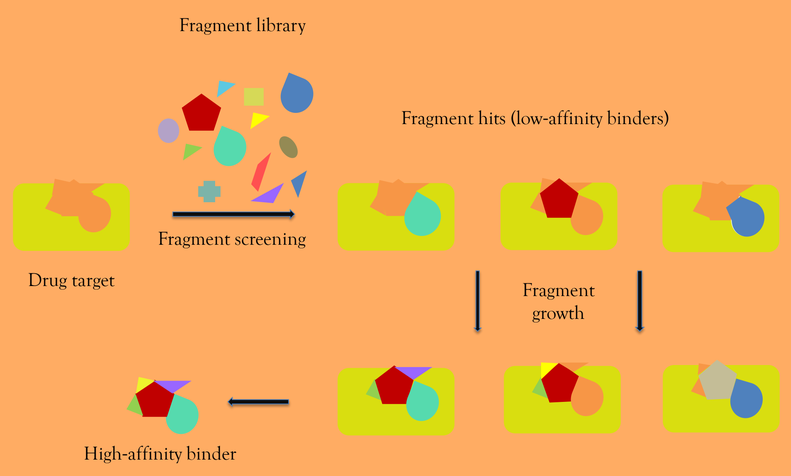
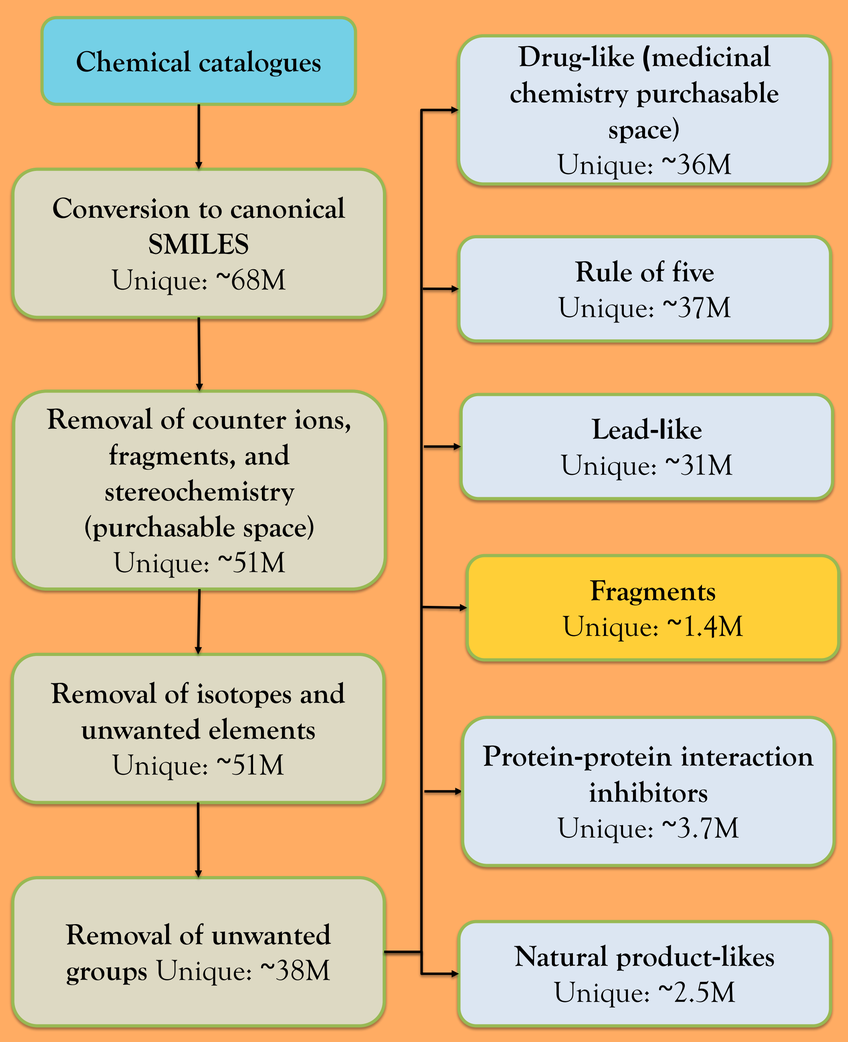
Fragment-based virtual screening

Fragment-based virtual screening (FBVS) utilizes low molecular weight (MW<300) compounds to target subpockets within a protein's binding site [1]. The identified fragments are generally weak binders which can be combined or optimized to produce high affinity binders. Compared to ligand-based virtual screening, FBVS allows for coverage of a much larger portion of chemical space by using a smaller library [2].



Preparation of focused libraries

Commercially available compounds were collected and filtered using an automated workflow designed within the ChemicalToolBoX [8, 9]. Fragments were selected using the Rule of Three [1]. The prepared, ready-to-dock library contained a total of ~7M fragments.



Targets: Bromodomains and NAD/NADP(H)-binding proteins

Bromodomains are emerging epigenetic targets in various types of cancer [3]. They recognize ε-*N*-acetylated lysine residues (K_{ac}) on the unstructured histone tails. The K_{ac} binding site of most bromodomains features a conserved asparagine residue responsible for substrate recognition [4]. This site was successfully targeted in our workgroup [5] using the screening workflow shown below. However, some bromodomains such as BRWD1, PHIP, and BRWD3 have a threonine residue in the same position (Figure 2). This threonine could act both as a hydrogen-bond donor and acceptor and is a good starting point for the identification of selective inhibitors. We are currently screening the target BRWD1 and would like to extend our method to the more difficult NAD/NADP(H)-binding proteins.

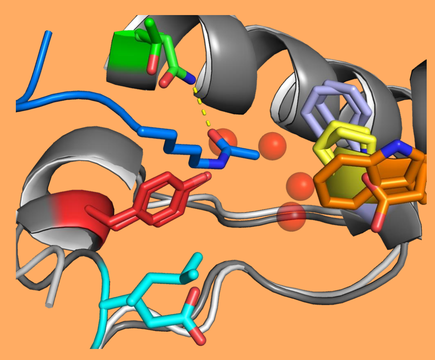


Figure 1. Superposition of Kac binding sites of BRD4(1) (PDB: 3UVW) and BRWD1(2) (PDB: 3Q2E). The acetylated lysine residue (shown in blue) is engaged in a hydrogen-bond with the conserved asparagine of BRD4(1).

Nicotinamide adenine dinucleotide (NAD) participates in a diverse range of cellular processes, including hydride transfer in enzymatic reactions, signal transduction, and DNA repair. These processes and the related proteins are involved in various diseases, thus molecules that affect NAD-binding proteins have a high therapeutic potential.

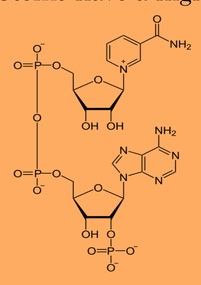
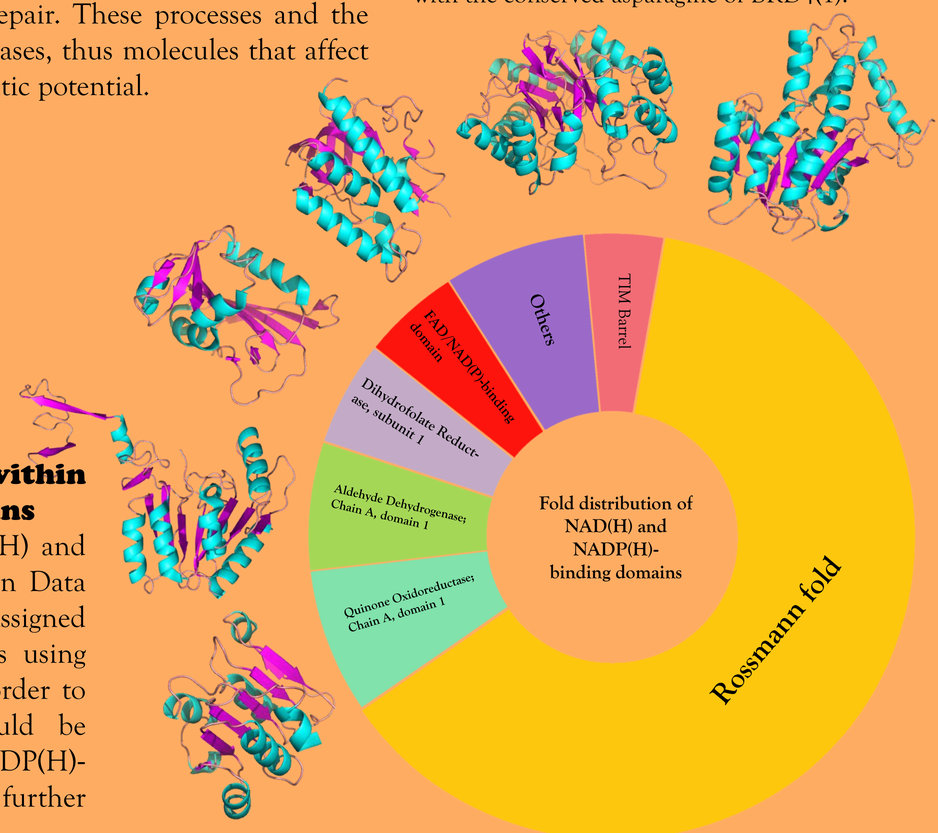


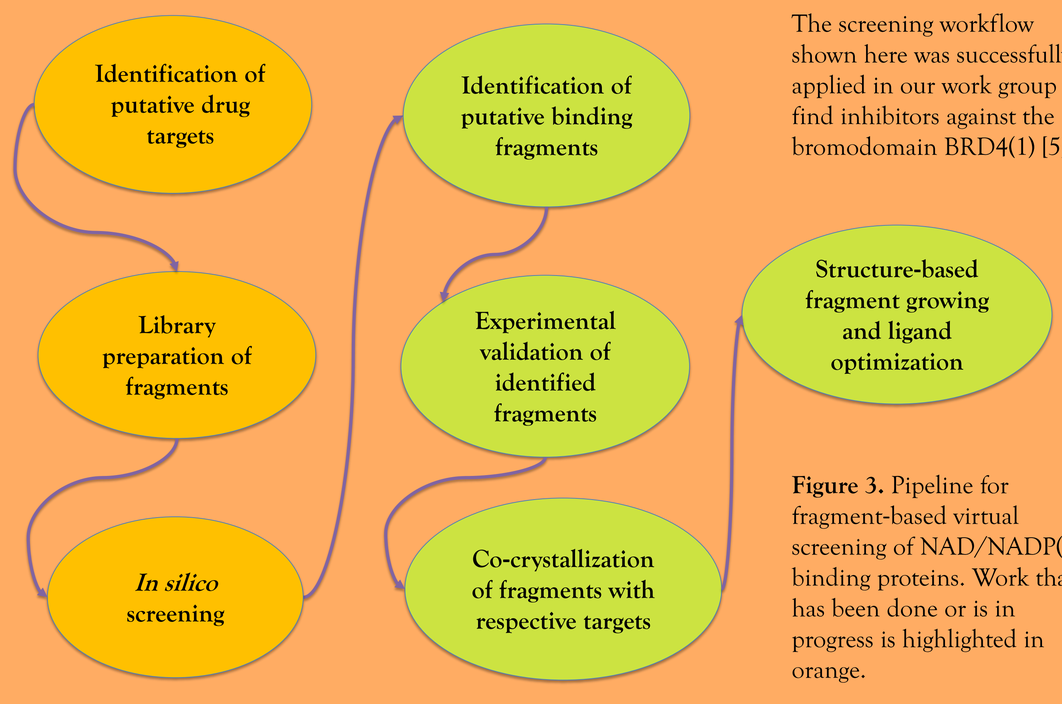
Figure 2. Chemical structure of the cofactor $NADP^+$.

Identification of drug targets within NAD/NADP(H)-dependent proteins

Protein structures complexed with NAD(H) and NADP(H) were retrieved from the Protein Data Bank (PDB) [6]. Folds/Topologies were assigned to the NAD/NADP(H)-binding domains using the CATH classification scheme [7]. In order to identify important residues which could be targeted, differences between NAD/NADP(H)-binding pockets within each fold will be further analyzed.



Screening workflow



The screening workflow shown here was successfully applied in our work group to find inhibitors against the bromodomain BRD4(1) [5].

Figure 3. Pipeline for fragment-based virtual screening of NAD/NADP(H)-binding proteins. Work that has been done or is in progress is highlighted in orange.

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References

1. Murray C.W., Rees D.C. (2009).The rise of fragment-based drug discovery. *Nature Chem.* 1:187-192.
2. Congreve M., Carr R., Murray C., Jhoti H. (2003).A 'Rule of Three' for fragment-based lead discovery? *Drug Discov Today.* 8(19):876-77.
3. Muller S., Fillippakopoulos P., Knapp S. (2011). Bromodomains as therapeutic targets. *Expert Rev Mol Med.* 13:e29.
4. Owen D.J., Ornaghi P., Yang J.C., Evans P.R., Ballario P., Neuhaus D., Filetici P., Travers A.A. (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. *EMBO J.* 19(22):6141-9.
5. Lucas X., Wohlwend D., Hügle M., Schmidtkunz K., Gerhardt S., Scühle R., Jung M., Einsle O., Günther S. (2013) 4-Acyl Pyrroles: Mimicking Acetylated Lysines in Histone Code Reading. *Angew Chem Int Ed Engl.* 52:14055-59.
6. BernsteinF.C., KoetzleT.F., WilliamsG.J., Meyer Jr. E.E., BriceM.D., RodgersJ.R., KennardO., ShimanouchiT., TasumiM. (1977). TheProtein Data Bank: A Computer-based Archival File For Macromolecular Structures. *J.of.Mol.Biol.*112:535.
7. Sillitoe I., Cuff A.L., Dessailly B.H., Dawson N.L., Furnham N., Lee D., Lees J.G., Lewis T.E., Studer R.A., Rentzsch R., Yeats C., Thornton J.M., Orengo C.A. (2013) New functional families (FunFams) in CATH to improve the mapping of conserved functional sites to 3D structures. *Nucleic Acids Res.* 41(Database issue):D490-8.
8. Grüning B.A., Lucas X., Von Kuster G., Patel H., Chbeib M., Bleher S., Telukunta K.K., Merfort I., Günther S. ChemicalToolBoX, a new Galaxy for cheminformatics; (*submitted*); http://www.ctb.pharmaceutical-bioinformatics.org.
9. Lucas X., Grüning B.A., Bleher S., Günther S. Navigating the purchasable chemical space to accelerate drug discovery (*manuscript in preparation*).

Collaborations

Institute of Biochemistry, Albert-Ludwigs University of Freiburg (experimental validation, X-ray crystallography):
Martin Hügle, Dr. Daniel Wohlwend, Prof. Dr. Oliver Einsle

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