Fragment-Based Drug Design: From Then till Now, and Toward the Future

Weijun Xu* & CongBao Kang*

Experimental Drug Development Centre (EDDC), Agency for Science, Technology and Research (A*STAR), 10 Biopolis Road, #05-01, Chromos, Singapore, 138670

Correspondence: WX (xu_weijun@eddc.sg) and CK (kang_congbao@eddc.sg)

Abstract

Fragment-based drug design (FBDD) has emerged as a powerful strategy in drug discovery, offering a complementary approach to traditional high-throughput screening (HTS)-based drug discovery. Over almost half a century, FBDD has undergone significant evolution, leading to the discovery of multiple approved drugs in the market. The integration of structural and computational tools into FBDD has significantly enhanced its efficiency, facilitating rational drug design. As the field of drug discovery expands beyond traditionally druggable targets and explore novel modalities, FBDD is poised to play a pivotal role in targeting a wide range of biomolecules, including challenging and undruggable targets such as proteins and RNAs. The continued advancement of FBDD, particularly through the incorporation of cutting-edge computational and screening methods, will pave the way for future success in medicinal chemistry.

Since the first introduction in 1981, fragment-based drug design has proven its utility as a promising approach for the identification of hit molecules during early stage of a drug discovery campaign [1]. Fragment-based drug discovery involved screening low molecular weight compounds, which initially bind weakly to target proteins, however, offer potential for further modification into potent drugs through medicinal chemistry effort. Later, the "SAR by NMR" approach was developed in the 1990s, highlighting the application of NMR spectroscopy to identify small molecule fragment binders[2]. This strategy lays the groundwork for FBDD as a drug discovery strategy (**Figure 1**). Since then, new methods including X-ray crystallography, surface plasmon resonance (SPR), and thermal shift assays in fragment screening were developed, making fragment-based screening more accessible to drug discovery workflow. Today, FBDD has become a mainstream screening approach widely used in pharma, biotech companies and academic institutions. Historically, FBDD has led to several milestone successes in drug development (**Figure 1**)[3], with eight FDA-approved drugs (**Figure 2**) stemming from the approach and over 50 compounds advancing to clinical stages [4]. The first FDA-approved drug derived from FBDD was Zelboraf (vemurafenib, PLX4032), a BRAF inhibitor for melanoma developed by Plexxikon. Initiated in 2005, Zelboraf received approval in 2011, demonstrating the efficiency of FBDD in accelerating drug discovery timelines. Subsequent FDA-approved drugs benefiting from FBDD include: Pexidartinib (2015) – CSF-1R inhibitor; Venetoclax (ABT-199, 2016) – A Bcl-2 inhibitor disrupting protein-protein interactions; Erdafitinib (2019) – an FGFR inhibitor for urothelial carcinoma; Berotralstat (BCX7353, 2020) – a serine protease inhibitor for hereditary angioedema; Sotorasib (AMG 510, 2021) – a KRAS-G12C inhibitor targeting a notorious "undruggable" protein; Asciminib (ABL001, 2021) – a novel BCR-ABL1 allosteric inhibitor; and Capivasertib (AZD5363, 2023) – an AKT kinase inhibitor. Notably, the majority of drugs belong to kinase inhibitors. However, the success of Venetoclaxand and Sotorasib underscores the potential of FBDD in drugging a wide range of intractable targets. In addition to approved drugs, a significant number of hit-to-lead successes have been achieved via FBDD [5], highlighting its broad applicability to diverse target families.

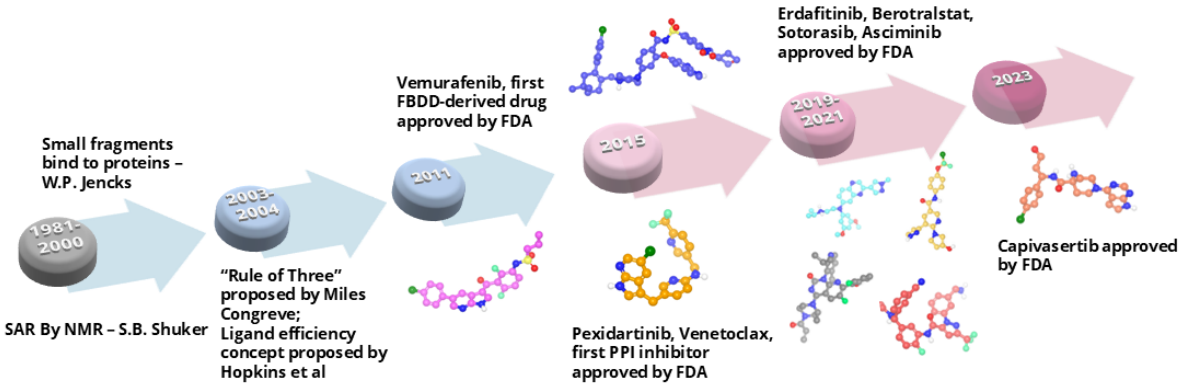

Figure 1. Milestones of fragment-based drug design over the past four decades.

Among different screening methods for hit discovery, FBDD offers several advantages: It requires fewer compounds from a diverse chemical space to be screened with a satisfied hit rate, making it suitable for a diverse range of targets including proteins and RNAs, providing versatility across targets. Fragment libraries consist of low-molecular-weight compounds (~150-300 Da), typically adhering to the "rule of three" [4, 6], allowing for greater potential for optimization. FBDD uses screening methods for the sensitive detection of weak binders that can be often missed out from high throughput screening [7]. Attractively, fragment screening can be completed within days, significantly reducing the time and cost associated with hit identification. In addition to obtaining hits, fragment screening also provides preliminary indication for the druggability of a target [8], serving as a risk assessment for project strategic planning. The user-friendly assay setup and the elimination of the need for a reference compound makes it easier for the approach to be implemented in a target-based drug discovery project. Upon the validation of a hit scaffold, fragment growth enables systematic SAR exploration, facilitating the development of novel lead compounds. Beyond the traditional "rule of five" territory, FBDD is also starting to show its promising application in new therapeutic modalities, such as proteolysis-targeting chimeras (PROTACs). These molecules typically consist of two functional components: one binding to the target protein of interest and the other engaging an E3 ligase, connected via a chemical linker. The strategy for designing PROTACs follows the fragment-linking principle, a key concept in FBDD. Interestingly, recent studies have shown that the binding moieties of PROTACs often exhibit characteristics in reminiscence of fragments, including low molecular weight weak intrinsic binding affinities to the protein of interest or E3 ligases. This similarity suggests that FBDD may play an extraordinary role in

optimizing and expanding the chemical space for the emerging drug modalities, further enhancing its impact on future drug discovery.

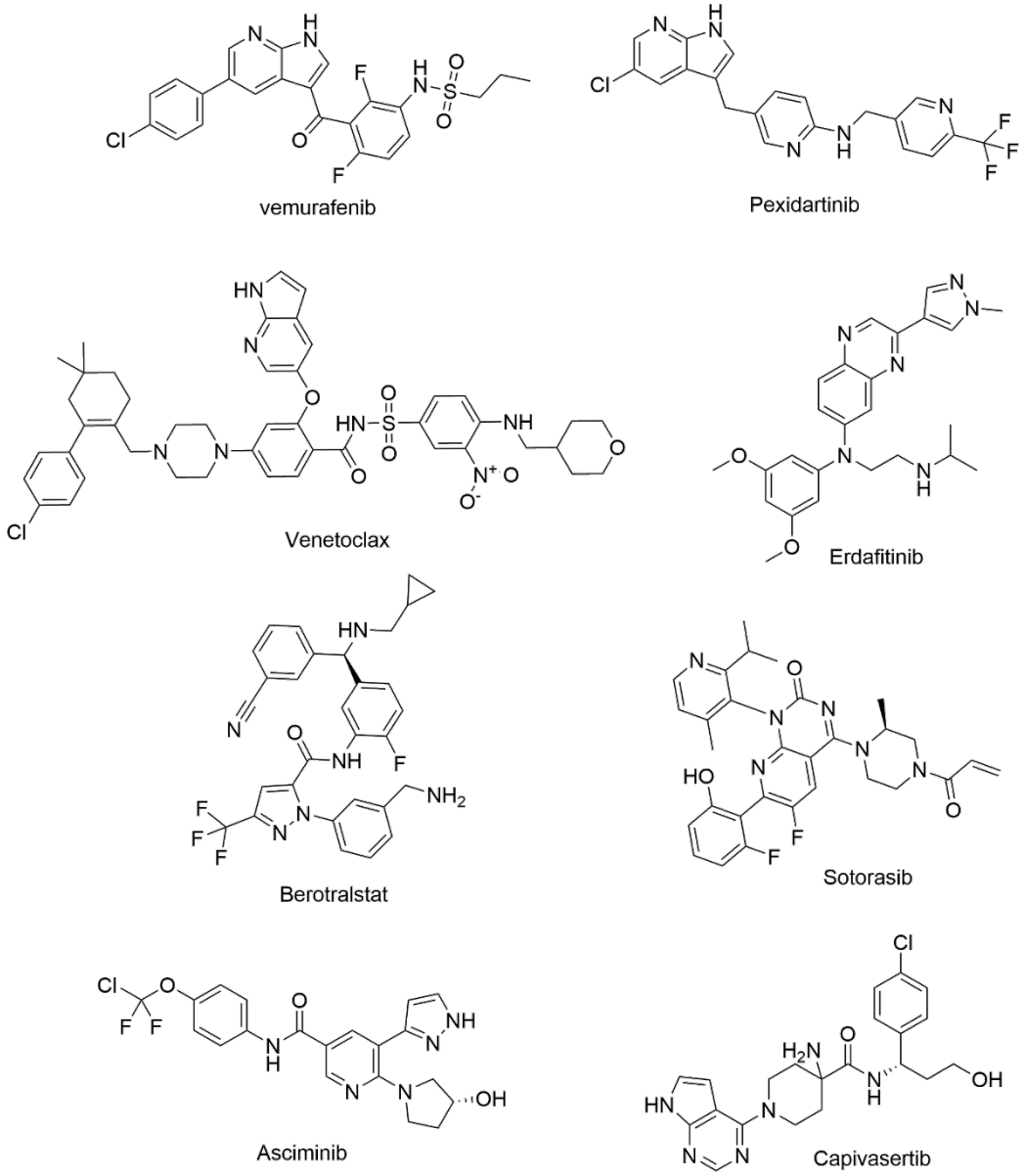

Figure 2. FDA-approved drugs derived from FBDD. Chemical structures of the FDA approved drugs derived from FBDD are shown. Please refer to the reference [4] for more detailed information.

Despite several successful stories, FBDD faces several challenges. First, the selection of a high-quality fragment library is complicated by a balanced consideration on factors including library diversity, coverage of chemical space, physicochemical properties and synthetic tractability. Second, the sensitivity of the screening methods is crucial in FBDD as fragments usually bind weakly to the targets [9]. Nearly a dozen screening methods are available

[9] (**Table 1**), among all, NMR represents a powerful tool in fragment screening and hit confirmation.[10]. Ligand and protein-observed NMR methods enable fragment screening with high sensitivity and satisfying hit rates for diverse targets. Third, hit selection for fragment growth is critical in FBDD. Fragment screening usually results in high hit rates across diverse targets. Ligand efficiency (LE) usually represents a "rule-of-thumb" practice during hit selection[11] to reflect the fragment-target interaction is specific but not promiscuous. In addition, understanding the binding modes of the hit is also an important criterion for prioritization. Thus, structural biology tools such as NMR, X-ray crystallography, and Cryo-EM play pivotal roles in determining the structures of the binary or ternary complexes[12], confirming the target engagement at a molecular level. Fourth, the rapidly expanding size of fragment libraries poses a pressure on the sheer volume of the screening effort. Today, in-stock libraries with millions of fragments have been developed, making it impractical to shortlist high-quality hits without substantial resource investment in both time and cost. To overcome the hurdle, computational methods such as virtual screening [13] offers a complementary solution screen ultra-large fragment libraries before wet lab validation. On the other hand, hits obtained from fragment-based screening can inform further rounds of virtual hit prioritization, allowing an iterative "computation-experiment" cycle to boost the success rate of hit identification. Lastly, the fragment growth and linker design require medicinal chemistry expertise to ensure the newly synthesized compounds occupy the protein binding site effectively. However, the human-driven process is inherently hampered by the number of increasing hits and the complexity of predicting their interactions with target proteins. Hence, one may consider leveraging artificial intelligence to analyse large datasets, predict protein-ligand interactions, and design compounds via automated fragment linking and expansion [14].

Table 1. Methods used in fragment screening*

Screening method	Sample requirement	Sensitivity (affinity)	Advantages	Disadvantages
Ligand-observed NMR (STD-NMR, Water-LOGSY, CMPG, T1pho)	Low (<50 μM sample)	mM- nM	No isotopic labeling is required. No limitation to the size of the target. Sensitive to detect weak binders. Mixture of fragments can be used in screening.	It cannot detect tight binders.
^{19}F-NMR	Low (<50 μM sample)	mM-μM	A sensitive screening method. A high throughput screening method. Suitable for both protein and RNA targets. No labeling of the target is required.	Fragments need to be labeled with fluorine. It can only detect weak binders.
Protein-observed NMR (HSQC, ^{19}F-NMR)	High (10-100 mg)	mM-nM	Detect both tight and weak binders Provide structural information. Identify binding sites.	Labeled protein is required. Longer time required for screening.
X-ray crystallography	High (10-50 mg)	mM-nM	Provides structural information. Detects binding mode.	Can not detect binding affinity and specificity. Require high-quality crystals for soaking.
Surface Plasmon Resonance (SPR)	Low (<1 mg)	μM-nM	A high throughput screening method. Provides binding kinetic data during screening.	Target needs to be immobilized. A reference compound is needed for the assay setup. Not suitable for all targets. Not suitable for detecting weak binders.
Isothermal Titration Calorimetry	High (>50 μM sample)	μM-nM	Provides binding curve for measuring binding affinity. Determine enthalpy or entropy-driven binding.	A low throughput assay. High concentration of sample is needed. Not suitable for every target. A reference compound is needed for setting up the assay. Not suitable for detecting weak binders.
Thermal shift assay (TSA)	Low (~μM)	μM-nM	A high throughput screening assay	High false positive hit rates. Not suitable for every target. A reference compound is needed for setting up the assay.
Native mass spectrometry	Low (μg-1 mg)	μM-nM	A high throughput screening assay Only μg of samples is needed.	Require careful condition optimization. Not suitable for weak binders
Microscale Thermophoresis (MST)	Low (< μM)	μM-nM	Can measure binding affinity.	A low throughput screening method Not suitable for weak binders
Biolayer interferometry (BLI)	Low (~μM)	μM-nM	A high throughput method	Not suitable for weak binders Suitable for confirming hits.
Fluorescence polarization (FP)	Low (< μM)	μM-nM	A high throughput screening method.	Not suitable for weak binders. Not suitable for every target. A reference compound is needed for setting up the assay.
Phenotypic screening	NA	μM-nM	Screens compounds with good cell permeability. Suitable for screening covalent fragments Does not require purified targets.	Low hit rates and requires confirming target-hit interactions. Only suitable for tight binders. Targets need to be confirmed.
Biochemical assays	Low (< μM)	μM-nM	A high throughput screening assay The effect of fragments on the target can be directly measured.	Target needs to harbor enzymatic activity or is well characterized. Not suitable for weak binders, resulting in low hit rate. High concentration of fragments is needed during screening.
Virtual screening	NA	NA	A high throughput method. No compounds and samples are needed during screening. Can screen million to billion compounds. Considers dynamics of targets. Can explore multiple pockets. Predicts chemical properties of fragments.	High false positive rate. Requires hit confirmation using experimental assays

*N.A.: not required. Concentration (μM) refers to the concentration of the target during screening.

Moving ahead, fragment-based drug design has the potential to be a key driver for future drug discovery. Recent advancements in molecular biology and bioinformatics have expanded the target space to RNAs and RNA-binding proteins (RBPs), offering untapped potential for therapeutic intervention beyond traditional protein targets [15]. For example, the FDA approved RNA splicing modulator: Risdiplam, works by altering the splicing of SMN2 pre-mRNA, promoting the of exon 7 in the final mRNA transcript, thereby acting as an enhancer for small nuclear binding protein to effectively splice its RNA target [16]. However, RNAs and RBPs often present highly polar and flexible binding sites that are difficult to target with conventional methods. FBDD, with its ability to identify small andweakly binding fragments, as well as the wider coverage of potential binding sites, offers an efficient approach to explore these complex targets. Moreover, the high-resolution cryo-EM structures are elucidating complex protein/RNA architectures, revealing potential binding sites for rational drug design [17]. Artificial intelligence based structural prediction tools are complementing experimental methods to gain an understanding of tertiary structures of RNAs and the complex between RNA and proteins [18]. Advanced molecular modelling techniques provide insights into conformational flexibility and ligand-induced structural changes, as well as the putative cryptic pockets that may offer opportunity for fragment growth or linking. Diverse fragment libraries, enriched with covalent warheads and specialized moieties, are expanding the chemical space for screening. The combination of experimental and computational screening approaches augments hit identification by uncovering new druggable sites and improving hit rates. Eventually, the synergy between FBDD and emerging computational technologies enables a deeper understanding of mechanisms of action, making fragment-based approaches increasingly robust and versatile. As the field continues to evolve, FBDD is posed to tackle historically intractable targets, such as allosteric regulatory sites, protein-protein interactions, RNAs, and RNA-binding proteins. The integration of machine learning and AI into FBDD workflows is expected to expand the scale and accelerate hit identification, prioritization optimization, ensuring a more efficient drug discovery process to address unmet medical needs.

References

(1) Jencks, W. P. On the attribution and additivity of binding energies. *Proceedings of the National Academy of Sciences* **1981**, *78* (7), 4046-4050. DOI: 10.1073/pnas.78.7.4046.
(2) Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **1996**, *274* (5292), 1531-1534.
(3) Erlanson, D. A.; Fesik, S. W.; Hubbard, R. E.; Jahnke, W.; Jhoti, H. Twenty years on: the impact of fragments on drug discovery. *Nat Rev Drug Discov* **2016**, *15* (9), 605-619, Review. DOI: 10.1038/nrd.2016.109.
(4) blog. Practical Fragments. DOI: https://practicalfragments.blogspot.com/.
(5) Zak, K. M.; Waterson, A. G.; Geist, L.; Braun, N.; Hauer, K.; Rumpel, K.; Ramharter, J.; Stadtmueller, H.; Wolkerstorfer, B.; Schoenbauer, D.; et al. Discovery of Small Molecules that Bind to Son of Sevenless 2 (SOS2). *Journal of medicinal chemistry* **2025**. DOI: 10.1021/acs.jmedchem.4c02007. Yao, Y.; Simes, M. L.; Ying, W.; Zhao, Q.; Winkler, A.; Shukla, S.; Gray, F.; Nikolaidis, C.; Hewett, G.; Grembecka, J.; et al. Development of PRC1 Inhibitors Employing Fragment-Based Approach and NMR-Guided Optimization. *Journal of medicinal chemistry* **2025**, *68* (2), 1382-1396. DOI: 10.1021/acs.jmedchem.4c01955. Lagiakos, H. R.; Zou, Y.; Igawa, H.; Therrien, E.; Lawrenz, M.; Kato, M.; Svensson, M.; Gray, F.; Jensen, K.; Dahlgren, M. K.; et al. In Silico Enabled Discovery of KAI-11101, a Preclinical DLK Inhibitor for the Treatment of Neurodegenerative Disease and Neuronal Injury. *Journal of medicinal chemistry* **2024**. DOI: 10.1021/acs.jmedchem.4c02074.
(6) Congreve, M.; Carr, R.; Murray, C.; Jhoti, H. A 'Rule of Three' for fragment-based lead discovery? *Drug Discovery Today* **2003**, *8* (19), 876-877. DOI: https://doi.org/10.1016/S1359-6446(03)02831-9.
(7) Li, Q. Application of Fragment-Based Drug Discovery to Versatile Targets. *Front Mol Biosci* **2020**, *7*, 180, Review. DOI: 10.3389/fmolb.2020.00180.
(8) Hajduk, P. J.; Huth, J. R.; Fesik, S. W. Druggability indices for protein targets derived from NMR-based screening data. *Journal of medicinal chemistry* **2005**, *48* (7), 2518-2525. DOI: 10.1021/jm049131r.
(9) Kirkman, T.; dos Santos Silva, C.; Tosin, M.; Bertacine Dias, M. V. How to Find a Fragment: Methods for Screening and Validation in Fragment-Based Drug Discovery. *ChemMedChem* **2024**, *19* (24), e202400342. DOI: https://doi.org/10.1002/cmdc.202400342.
(10) Holvey, R. S.; Erlanson, D. A.; de Esch, I. J. P.; Farkaš, B.; Jahnke, W.; Nishiyama, T.; Woodhead, A. J. Fragment-to-Lead Medicinal Chemistry Publications in 2023. *Journal of medicinal chemistry* **2025**, *68* (2), 986-1001. DOI: 10.1021/acs.jmedchem.4c02593.
(11) Hopkins, A. L.; Keserü, G. M.; Leeson, P. D.; Rees, D. C.; Reynolds, C. H. The role of ligand efficiency metrics in drug discovery. *Nature Reviews Drug Discovery* **2014**, *13* (2), 105-121. DOI: 10.1038/nrd4163.
(12) Kang, C.; Xu, W. Leveraging Structural and Computational Biology for Molecular Glue Discovery. *J Med Chem* **2025**. DOI: 10.1021/acs.jmedchem.5c00076.
(13) Chandraghatgi, R.; Ji, H.-F.; Rosen, G. L.; Sokhansanj, B. A. Streamlining Computational Fragment-Based Drug Discovery through Evolutionary Optimization Informed by Ligand-Based Virtual Prescreening. *Journal of Chemical Information and Modeling* **2024**, *64* (9), 3826-3840. DOI: 10.1021/acs.jcim.4c00234.
(14) Jinsong, S.; Qifeng, J.; Xing, C.; Hao, Y.; Wang, L. Molecular fragmentation as a crucial step in the AI-based drug development pathway. *Communications Chemistry* **2024**, *7* (1), 20. DOI: 10.1038/s42004-024-01109-2.
(15) Li, Q.; Kang, C. Targeting RNA-binding proteins with small molecules: Perspectives, pitfalls and bifunctional molecules. *FEBS Lett* **2023**, *597* (16), 2031-2047. DOI: 10.1002/1873-3468.14710.

(16) Kovachka, S.; Panosetti, M.; Grimaldi, B.; Azoulay, S.; Di Giorgio, A.; Duca, M. Small molecule approaches to targeting RNA. *Nature Reviews Chemistry* **2024**, *8* (2), 120-135. DOI: 10.1038/s41570-023-00569-9.
(17) Saur, M.; Hartshorn, M. J.; Dong, J.; Reeks, J.; Bunkoczi, G.; Jhoti, H.; Williams, P. A. Fragment-based drug discovery using cryo-EM. *Drug Discovery Today* **2020**, *25* (3), 485-490. DOI: https://doi.org/10.1016/j.drudis.2019.12.006.
(18) Xu, W. Current Status of Computational Approaches for Small Molecule Drug Discovery. *Journal of medicinal chemistry* **2024**, *67* (21), 18633-18636. DOI: 10.1021/acs.jmedchem.4c02462.