Publisher version: https://doi.org/10.1016/j.trac.2023.117161

Advances in Analytical Technologies Detecting and Characterizing Noncovalent Interactions for Fragment-Based Drug Discovery

Clara Davoine[a,b], Lionel Pochet[b,†], Marianne Fillet[a,†,*]

[a]*Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, Avenue Hippocrate 15, B36 Tour 4 +3, 4000 Liège, Belgium.*
[b]*Namur Medicine & Drug Innovation Center (NAMEDIC – NARILIS), University of Namur, Rue de Bruxelles 61, 5000 Namur, Belgium.*

[†] equally contributed to the work

**Corresponding author. E-mail address:* marianne.fillet@uliege.be

Publisher version: https://doi.org/10.1016/j.trac.2023.117161

Abstract

Detection and characterization of biomolecular interactions are the backbone of the drug discovery process. One of the most widely used approaches to developing new drugs is the fragment-based drug discovery (FBDD) strategy. The FBDD approach begins with the discovery of low molecular weight chemical fragments that bind weakly to the target of interest. The identified fragments are then combined or optimized into potent drug-like compounds. Despite its advantages over the high-throughput screening approach, its execution can be challenging. The reason is that discovering weak binders and determining how to grow or bind them are difficult. Therefore, intensive research is still underway to develop analytical technologies to detect and characterize weak, non-covalent interactions. The purpose of this article is to comprehensively review the emerging analytical technologies used in FBDD compared with the conventional ones. Particularly, we summarize their principle, advantages, limitations, and potential artifacts. For each emerging technique, we provide practical examples. Accurate detection and characterization of weak interactions are critical for the success of a FBDD project. Hence, knowledge of the features of the different techniques can support the selection and implementation of the project's analytical platform.

Keywords

Biomolecular interaction; Weak affinity chromatography; Microscale Thermophoresis; Affinity capillary electrophoresis; DNA-encoded chemical libraries; Fragment-based drug discovery; Analytical techniques; Drug discovery.

Abbreviations

ACE, affinity capillary electrophoresis; BGE, background electrolyte; CPMG, Carr-Purcell-Meiboom-Gill; DEL, DNA-encoded chemical libraries; DSF, differential scanning fluorimetry; FBDD, fragment-based drug discovery; HSQC, heteronuclear single-quantum correlation; K_D, dissociation constant; MST, microscale thermophoresis; SAR, structure–activity relationship; SPR, surface plasmon resonance; STD, saturation transfer difference; TGFBIp, transforming growth factor beta induced protein; Tm, melting temperature; WAC, weak affinity chromatography.

Publisher version: https://doi.org/10.1016/j.trac.2023.117161

1. Introduction

Fragment-based drug discovery (FBDD) is a well-established approach to discovering small molecules that can bind to a protein or nucleic acid target [1–3]. A critical step in FBDD is the screening of a collection of fragments against a purified target of interest. A fragment is a compound with a molecular weight of less than 300 Da. The idea is that the small size of the fragments increases the probability of discovering functional motifs that are not hindered by another part of the molecule [1,2]. Therefore, it can explore an extensive protein chemical space with a small library containing less than a few thousand fragments. The following step after the screening is the generation of lead compounds. Lead compounds refer to drug-sized molecules that serve as starting points to develop clinical drug candidates. Turning a fragment with a weak affinity into lead compounds is challenging and often requires information on the fragment's interaction within the binding site [1,2,4]. Overall, the ideal screening methodology in FBDD (1) should have a high sensitivity, meaning that it can detect very weak binders with affinity up to several mM; (2) should rank the binders according to their affinity strengths; (3) should give information to generate the binding model; (4) should avoid false positive and negative results; (5) should consume a limited quantity of target protein, and (6) should require a fragment concentration compatible with its solubility during the screening.

Every year, there is around 30 successful fragment-to-lead programs that are reported in the literature. Conventional technologies are used in more than 90% of them [5–7]. There are several reasons that can explain this small number: (1) FBDD is a drug discovery strategy among others, (2) drug discovery programs require several years to achieve publishable results, (3) due to their cost, most screening campaigns are performed by industrials, leading to confidential results, and (4) drug discovery programs can fail or be aborted. The conventional technologies used in FBDD are biochemical assay, NMR, X-ray crystallography, thermal shift assay, and surface plasmon resonance (SPR). Each of them has its strengths and weaknesses [8,9]. For example, X-ray crystallography can produce the three-dimensional structure of the protein-fragment complex but cannot measure the affinity of the interaction. While many successful discovery programs used these conventional technologies [1,2,6,10], other approaches are still needed to overcome their limitations [11]. In consequence, several technologies were developed and emerged as valuable alternatives.

In this article, we comprehensively reviewed the analytical technologies used in FBDD. We focused our discussion on the emerging techniques in comparison with conventional ones. We detailed their principle, strengths, weaknesses, and potential artifacts. We also presented practical examples of their use reported in the literature.

2. *Conventional technologies used in fragment-based drug discovery*

Several technologies are well-established in FBDD [12]. Table 1 compares the main characteristics of conventional FBDD technologies. It is critical to assess the detectable affinity range and the concentration at which the fragment should be used and should therefore be soluble. When techniques can detect binding at a low level of site occupancy (i.e., below the dissociation constant (K_D)), they are usually more sensitive [2,9]. A good practice is to estimate the expected range of affinity that needs to be detected and then evaluate all available options before starting to develop a fragment assay [8].

The **biochemical assay** is the simplest and most widely used approach to identify fragments that affect the target activity [6,13]. Biochemical assay refers to an enzymatic assay that has a spectrophotometric readout. This approach only detects the biochemically active fragments, but not the ones that bind the target without affecting its activity. This aspect could be considered as an advantage since biologically active fragments are better starting points [9,13]. During the assay development, proper optimization of several parameters, such as substrate and ligand concentrations, is necessary to achieve a sensitivity enabling the detection of weakly active hits [2,13]. The typical sources of false-positive results are spectrophotometric interferences, precipitation or aggregation of fragments, and the presence of impurities [2,13,14]. Additives, such as nonionic detergents, can be spiked to reduce nonspecific inhibitions responsible for aggregation [15]. With appropriate precautions in assay development and eviction of artifacts, biochemical assays are an effective high-throughput screening technology for discovering fragments that affect the activity of the target [16].

Ligand-observed NMR is the most efficient and widely used approach for detecting mM binders [6,9]. Ligand-observed NMR regroups different binding experiments that monitor the changes in NMR parameters caused by a modification of the fragment's motion upon binding [8,9]. Indeed, bound ligands behave more like slowly tumbling macromolecules than rapidly tumbling small molecules [8]. Some NMR parameters, such as relaxation and transfer of magnetization, detect the population which has bound and then dissociated during the experiment. This key feature explains why binding can be observed at a low occupancy of the protein binding site and why false negatives arise with tight binders [8,9]. This technology has the advantages of following fragment binding in solution, without labeling, and at a concentration well below their K_D. Each measurement can provide information about the integrity of the fragment, which explains its low false-positive rate [1,2]. However, nonspecific binding can be an issue. A competition experiment with a well-identified ligand is generally beneficial to validate the binding specificity [2,8,10]. NMR data can also generate a binding model of the fragment to the target, albeit to a lesser extent than X-ray crystallography [2,10]. Still, the accessibility of this technique is limited by the cost of the instrument and its high protein consumption [2]. The following articles [1,8,14,17] give more information on the

different ligand-observed NMR experiments (e.g., saturation transfer difference (STD), water LOGSY, and CPMG) and their respective potential artifacts.

X-ray crystallography, one of the earliest approaches to detect weak binders, remains well-implanted in the FBDD field [1,6]. Its key advantage is that it generates the three-dimensional model of the fragment–protein complex [2,10,18,19]. However, the observed crystal static structure can be different from the structure of the complex adopted in solution. Moreover, no quantitative affinity information can be extracted, and it requires near full occupancy of the protein binding site [14,19]. Therefore, screenings are performed at very high fragment concentrations (50 mM or more), which is a limitation for poorly soluble fragments [9,19]. This technology is also not immune to artifacts and overinterpretation [14]. A resolution of around 2 Å is necessary to orient the fragments properly [10]. There are two methods to obtain structures of the complex: co-crystallization and crystal soaking. In co-crystallization, the protein–ligand complex crystallizes together. Thus, a crystallization step is necessary for each compound and can be affected by the presence of the ligand. This method is not optimal for FBDD because the high fragment concentrations used can interfere with the crystallization process [19]. In crystal soaking, an apoprotein crystal is immersed in a highly-concentrated solution of ligand. The ligand diffuses into the crystal through the solvent channels and binds to energetically favorable locations [19]. Crystal soaking is the method commonly used in FBDD. The main limitation of this technology is the generation of a protein crystal suitable for soaking. The crystals must be robust, with an open binding site accessible through solvent channels, and withstand binding-induced conformational changes [2,8,9,19]. Finally, only 20-30% of the soluble proteins are crystallizable [10,18]. The following review [19] reports the practical details.

Thermal shift, also known as Tm shift, differential scanning fluorimetry (DSF), or thermofluor, is another frequently used technique in FBDD [1,2,6,9]. This technique detects binding indirectly by monitoring the effect of a compound on the protein's thermal stability (melting temperature or Tm). Measurement of the thermal stability requires a fluorescent dye that binds to proteins as they unfold, such as the most commonly used Sypro Orange [1,2,9,18]. The attractive features of this technology include its high throughput, low protein consumption, and ease of setup on most real-time PCR instruments and data analysis [1]. However, fragments do not powerfully affect the protein's thermal stability. High occupancy of the protein binding site is required to observe a significant Tm shift. In consequence, this technology has a low sensitivity [1,2,18]. Thermograms should be checked to evict artifacts from fluorescence quenching, high initial fluorescence, or aggregation [1]. Contaminants and counterions in the samples may also affect thermal stability [9]. Finally, the user must also be aware that fragments reducing the protein's thermal stability should be discarded without further examination. Indeed, negative Tm shifts often arise from multiple

weak interactions between the fragment and the denatured or partially denatured conformation of the protein [1].

SPR is also commonly used in FBDD [6]. Generally, SPR uses a thin gold film with immobilized proteins at its surface. On one side of the metal film, a dielectric medium passes over it, creating a metal–dielectric interface. On the other side, a plane-polarized light is totally reflected and excites a surface plasmon wave propagating along the metal–dielectric interface [9,10,20,21]. The refractive index of the dielectric band near the metal surface has a strong influence on the surface plasmon resonance frequency and, consequently, on the critical angle of the total internal reflection. The analytes are added to the dielectric medium. When the analyte binds the immobilized protein, the amount of matter entering the region of the plasmon wave rises, increasing the local refractive index and thus changing the angle. The modification of this angle is real-time monitored in real time, which allows the determination of the binding kinetics [1,2,20]. The other advantage of this technology is the low amount of protein required [1,2]. However, protein immobilization is a critical step because the protein must retain its binding integrity after attachment to the surface [1,2,9,18,20]. Control of this integrity using a known ligand is necessary to optimize the immobilization process and evaluate the surface activity [1,2,20]. Nonspecific binding is a central problem in SPR, which limits the concentration of the fragments that can be used and thus hinders the sensitivity of this technology [1,2,9]. Like NMR, a competition SPR experiment is beneficial to validate the binding specificity [1,2,9]. Other artifacts can be generated by solvent effects, aggregators, and compounds that bind the chip surface instead of the immobilized protein [1,2,8,9]. Overall, SPR is prone to artifacts and overinterpretation [1,8,14]. The guide written by Giannetti et al. provides good background before starting a SPR screening [20].

Table 1. Characteristics of the conventional technologies used in fragment-based drug discovery.

	Biochemical assay	***Ligand-observed NMR***	***X-ray crystallography***	***Thermal shift***	***SPR***
Affinity range	Low nM to low mM[b]	100 nM to 5 mM	No lower limit to 1 M	1 nM to 100 μM	1 nM to 500 μM
Fragment concentration[a]	Around the K_D	Below the K_D	5–10 times the K_D	5–10 times the K_D	5–10 times the K_D
Total protein required	Low (<1 mg)	High (10s mg)	High (10s mg)	Low (a few mg)	Low (<1 mg)
Instrumentation (cost)	Spectrophotometer (low)	High-field NMR (high)	Synchrotron facilities (very high)	Thermal cycling instrument (low)	SPR system (low)
Throughput	High	Medium	Low – medium[e]	Medium – high	Medium
Rate of false results	High false-positive rate, mainly by interfering with the detection	False negatives when strong binders do not have fast binding kinetics[d]	False negatives, sometimes crystal structures with bound compounds are not formed at each attempt	High false-positive and false-negative rates	False negatives and positives, nonspecific binders and compounds that bind the chip surface instead of the protein
Target requirements	Biological activity	Soluble at around 10 μM	Crystallizable	Not suitable for disordered or hydrophobic protein	Immobilized

Information on the binding mode	Low[c]	Medium	Very high	None	Low[c]
References	[2,9,13,16]	[1,2,9,10]	[2,9]	[1,2,9,18]	[1,2,9,20]

[a]Due to the difference in the affinity range, the fragment concentration used during the screening is ranked by the lowest detectable K_D.
[b]The detectable affinity range of biochemical assay is dependent on the assay conditions.
[c]Some structural information can be extracted from (cross-)competition assay with a well-identified ligand.
[d]Other potential artifacts that are particular to a specific ligand-based NMR experiment exist [14].
[e]The progresses in synchrotron facilities and their automation increase the throughput [2].

3. *Emerging technologies in fragment-based drug discovery*

Besides conventional techniques, other technologies have emerged as valuable tools for FBDD [1,9]. In this review, an emerging technology is a nonconventional approach that is supported by at least 3 examples using different targets, including at least one of large-scale fragment screening (screened fragments > 500). Table 2 compares the characteristics of the four emerging technologies discussed in this review.

Table 2. Characteristics of the emerging technologies for fragment-based drug discovery.

	Weak affinity chromatography	***Microscale thermophoresis***	***Affinity capillary electrophoresis***	***Dual-pharmacophore DNA-encoded libraries***
Affinity range	1 μM to 10 mM[b]	1 pM to 1 mM	Low nM to low mM[d]	Low nM to low μM[f]
Fragment concentration[a]	Below the K_D	5–10 times the K_D	Below the K_D	Below the K_D
Total protein required	Medium (1–5 mg)	Low (<1 mg)	Low – medium[d]	Low (<1 mg)
Instrumentation (cost)	HPLC system often coupled with MS (medium)	Microscale thermophoresis instrument (low)	Capillary electrophoresis instrument (low)	PCR instrument and DNA sequencer (medium)
Throughput	High	Medium	Medium (– high[e])	High
Rate of false results	False positives when nonspecific binding occurs False negatives with poor ionizing compounds or when strong binders do not elute within a reasonable time	High false-positive and -negative rates	False positives when nonspecific binding occurs	High rate of false negatives
Target requirements	Immobilization	Fluorescent labeling	Soluble at around 10 μM	Immobilization
Information on the binding mode	Low[c]	Low[c]	Low[c]	Medium
References	[9,22–24]	[2,9,23,25]	[26–30]	[31–35]

[a]Due to the difference in the affinity range, we ranked the fragment concentration used during screening according to the lowest detectable K_D.
[b]The detectable affinity range of WAC is dependent on the amount of immobilized receptor per volume unit.
[c]Some structural information can be extracted from (cross-)competition assay with a well-identified ligand.
[d]Dependent on the methodology.
[e]The emergence of an automated capillary electrophoresis instrument adapted for 96-well plates increases the throughput [30].
[f]Dual-pharmacophore encoded libraries bypass this affinity limitation by evaluating pairs of fragments simultaneously.

Publisher version: https://doi.org/10.1016/j.trac.2023.117161

Table 3. The applications of the emerging FBDD techniques. ACE: affinity capillary electrophoresis, AGP: alpha-1-acid glycoprotein, BRD4: bromodomain 4, CAII: carbonic anhydrase II, CAIX: carbonic anhydrase IX, DEL: DNA-encoded chemical libraries, DMSO: dimethyl sulfoxide, EDTA: ethylenediaminetetraacetic acid, GAK: cyclin G-associated kinase, GPCR: G protein-coupled receptor, HEPES: N-(2-hydroxyethyl)piperazine-N′-ethanesulfonic acid, HRP: horseradish peroxidase, HSP90: heat shock protein 90, IDO1: Indoleamine 2,3-dioxygenase 1, MDH: malate dehydrogenase, PEG: polyethylene glycol, PPI: protein-protein interaction, SIRT: sirtuin, TCEP: tris(2-carboxyethyl)phosphine, TGFBIp: transforming growth factor beta induced protein, and Tris: tris(hydroxymethyl)aminomethane.

Entry (reference), by technology	*Biological target (type or class)*	*Fragment library size*	*Experimental conditions*
Weak affinity chromatography			
1 ([36])	Mutant TGFBIp (extracellular matrix protein)	2500	*Target modification*: immobilization on spherical porous diol silica particles by reductive amination reaction *Buffer*: 10 mM ammonium acetate, pH 6.6 *Temperature*: 18-19°C *Fragment*: 0.4 µL of a mixture of 50 fragments at 20 µM each (in 1% DMSO) *Detector*: Q-TOF *Blank*: same analysis performed on a column without immobilized target
2 ([37])	Thrombin (enzyme)	27[a]	*Target modification*: immobilization *in situ* on a capillary column containing diol-silica particles *Buffer*: 10 mM phosphate buffer saline, pH 7.4 *Temperature*: 22°C *Fragment*: 0.4 µL of 1 fragment at 100 µM each (in 1% DMSO) *Detector*: UV diode array detector at wavelength 214 nm and 254 nm *Blank*: same analysis performed on a column where the active site of thrombin is blocked by PPACK
3 ([38])	HSP90 (chaperone protein)	111	*Target modification*: immobilization *in situ* on a capillary column containing diol-silica particles *Buffer*: 20 mM ammonium acetate, pH 6.8 *Temperature*: 22°C *Fragment*: 0.4 µL of a mixture of fragments at 100 µM each (in <1% DMSO) *Detector*: diode array multiple wavelength detector and a single quadropole mass spectrometry detector *Blank*: same analysis performed on an ethanolamine reference column
4 ([39])	GAK (kinase)	170	*Target modification*: immobilization *in situ* on a capillary column containing diol-silica particles *Buffer*: 20 mM ammonium acetate, pH 6.8 *Temperature*: 22°C *Fragment*: 0.4 µL of a mixture of fragments at 5 µM each (in 4.6 or 4.7% DMSO) *Detector*: single quadropole mass spectrometry detector *Blank*: same analysis performed on an ethanolamine reference column
5 ([40])	Human aquaporin-1 (channel)	200	*Target modification*: insertion in lipodisks, immobilization of the proteolipodisks on spherical porous diol silica particles by reductive amination reaction

			Buffer: 20 mM ammonium acetate, pH 6.9 *Temperature*: 22°C *Fragment*: 0.4 µL of a mixture of 10 fragments at 7.5 µM each (in 5% DMSO) *Detector*: single quadropole mass spectrometry detector *Blank*: same analysis performed on a column with immobilized lipodisks without target
6 [41]	Adenosine A_{2A} receptor (GPCR)	6[a]	*Target modification*: insertion in nanodisks, immobilization of the proteonanodisks on a monolithic nano-column *Buffer*: 67 mM phosphate buffer, pH 7.4 *Temperature*: 25°C *Fragment*: 1 fragment percolated at 10 µM *Detector*: diode array detector operated in a multi-wavelength mode *Blank*: same fragment percolated on the same column at 1 mM
Microscale thermophoresis			
7 ([42])	KRAS (enzyme)	1800	*Target modification*: fluorescence labelling with NT647 dye *Buffer*: 20 mM HEPES, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM TCEP, 0.05% Tween-20, pH 7.4 *Fragment*: 1 fragment at 500 µM (in 2% DMSO) *Detector*: fluorescence detector *Blank*: same analysis without fragment (DMSO alone)
8 ([43])	IDO1 (enzyme)	NA[b]	*Target modification*: fluorescence labelling with NT647 dye *Buffer*: 50 mM Tris, 150 mM NaCl, 10 mM $MgCl_2$, 2 mM DTT, 0.05% Tween-20, pH 7.4 *Fragment*: 1 fragment at various concentrations (in 2% DMSO) *Detector*: fluorescence detector
9 ([44])	14-3-3/Amot-p130 interface (PPI)	NA[b]	*Target modification*: 13-mer Amot-p130 peptide labelled with Cy5 *Buffer*: phosphate-buffered saline, 0.05% Tween-20, pH 7.4 *Fragment*: 1 fragment at various concentrations (in 1% DMSO) *Detector*: fluorescence detector
10 ([45])	Zika virus NS2B-NS3 protease (enzyme)	NA[b]	*Target modification*: fluorescence labelling *Buffer*: not mentioned *Fragment*: 1 fragment at various concentrations (% DMSO not mentioned) *Detector*: fluorescence detector
11 ([46])	SARS-CoV-2 nsp10 (viral protein)	NA[b]	*Target modification*: fluorescence labelling *Buffer*: 50 mM $NaPO_4$, 150 mM NaCl and 0.05% Tween-20, pH 7.5 *Temperature*: 25°C *Fragment*: 1 fragment at various concentrations (% DMSO not mentioned)

			Detector: fluorescence detector
12 ([47])	MDH (enzyme)	NA[b]	*Target modification*: fluorescence labelling *Buffer*: 100 mM Na-phosphate buffer, 400 mM NaCl, 0.05% Tween-20, pH 7.4 *Fragment*: 1 fragment at various concentrations (% DMSO not mentioned) *Detector*: fluorescence detector
Affinity capillary electrophoresis			
13 ([48])	HSP90 (chaperone protein)	609	*Target modification*: none *Buffer*: 10 mM Tris-HCl, 0.0005% Tween-20, 5 mM $MgCl_2$, pH 7.5 *Fragment*: 1 fragment added in the buffer at 500 μM *Detector*: UV diode array detector at wavelength 254 nm *Blank*: same analysis without fragment in the buffer (DMSO alone) *ACE mode*: mobility shift ACE in indirect mode
14 ([49])	Thrombin (enzyme)	23[a]	*Target modification*: none *Buffer*: 10 mM Tris HCl, 10 mM HEPES-Na, 100 mM NaCl, and 0.1% PEG 6000, adjusted at pH 7.4 with 0.1 M H_3PO_4 *Temperature*: 25°C *Fragment*: 1 fragment at 10 μM or in mixture of 5 fragments at 10 μM each *Detector*: UV diode array detector *Blank*: same analysis without the target *ACE mode*: partial-filling ACE in direct mode
15 ([50])	Thrombin (enzyme)	40[a]	*Target modification*: none *Buffer*: 10 mM Tris HCl, 10 mM HEPES-Na, 100 mM NaCl, and 0.1% PEG 6000, adjusted at pH 7.4 with 0.1 M H_3PO_4 *Temperature*: 25°C *Fragment*: 1 fragment added in the buffer at 100 μM *Detector*: UV diode array detector *Blank*: same analysis without fragment in the buffer *ACE mode*: partial-filling ACE in indirect mode
16 ([51])	Factor XIIa (enzyme)	24[a]	*Target modification*: none *Buffer*: acetate buffer (4 mM acetate sodium, 150 mM NaCl, adjusted at pH 5.3 with 0.1 M H_3PO_4) and Tris-HEPES buffer (10 mM Tris HCl, 10 mM HEPES-Na, 100 mM NaCl, adjusted at pH 7.4 with 0.1 M H_3PO_4) in 1:2 (v/v) *Temperature*: 25°C *Fragment*: 1 fragment at 7.5 μM *Detector*: UV diode array detector *Blank*: same analysis without the target *ACE mode*: partial-filling ACE in direct mode
17 ([52])	Factor XIIa (enzyme)	23[a]	*Target modification*: none *Buffer*: 21 mM Tris, 21 mM HEPES, 81 mM NaCl, 1.6 mM sodium acetate, and 100 μM EDTA, pH 7.4 *Temperature*: 25°C

			Fragment: mixture of 5 fragments added in the buffer at 600 µM each *Detector*: UV diode array detector at wavelength 262 ± 2 nm (using a reference wavelength at 360 ± 50 nm) *Blank*: same analysis without fragment in the buffer *ACE mode*: partial-filling ACE in indirect mode
18 ([53])	Adenosine A_{2A} receptor (GPCR)	Not mentioned	*Target modification*: introduction of a small number of point mutations to thermostabilize the protein (StaR®). *Buffer*: not mentioned *Fragment*: 1 fragment added in the buffer *Detector*: UV diode array detector *Blank*: same analysis without fragment in the buffer *ACE mode*: mobility shift ACE in indirect mode
DNA-encoded chemical libraries			
19 ([54])	HRP CAIX (enzymes) AGP (transport protein)	550 × 202 fragments 111100 compounds	*Target modification*: immobilization on magnetic beads *Buffer*: 50 mM phosphate buffer saline, 0.05% Tween-20, pH 7.4 *Detector*: PCR and DNA sequencing *DEL mode*: dual-pharmacophore DEL
20 ([55])	Trypsin CAII matrix metalloprotease-2 and −9 (enzymes)	883 × 30 × 890 fragments 23.576 million compounds	*Target modification*: immobilization on magnetic beads *Buffer*: 50 mM phosphate buffer saline, 0.05% Tween-20, pH 7.4 *Detector*: PCR and DNA sequencing *DEL mode*: trio-pharmacophore DEL
21 ([56])	SIRT1, 2, 5 (enzyme) BD1 and BD2 domains of BRD4 (PPI)	10000	*Target modification*: none *Buffer*: phosphate buffer saline, pH 7.2 *Detector*: PCR and DNA sequencing *Blank*: same protocol without target selection *DEL mode*: DNA-encoded dynamic chemical library
22 ([57])	SIRT3 (enzyme)	10000	Idem entry 21

[a]Proof-of-concept studies.
[b]Non applicable, method used for K_D determination only.

3.1. Weak affinity chromatography (WAC)

In weak affinity chromatography (WAC), the biological target is immobilized on a stationary phase, and a mobile phase (often ammonium acetate buffer) is pumped isocratically. The analysis starts with the injection of a short plug containing a fragment or a mixture of 50-100 fragments. Due to binding with the immobilized target, the fragments are retained (see Figure 1). This retention increases with the fragment affinity and the number of active target sites on the column [22,23]. Fundamental aspects of WAC are detailed in the following paper [23]. WAC is usually coupled to mass spectrometry to identify the retained compounds [22,23]. Affinity and binding kinetics can be determined directly from one chromatogram at one fragment concentration. The equations

determining these constants are described in the following paper [22]. Potential competition between the compounds can occur in mixture. Thus, fragments must be analyzed separately to determine accurately their affinity or binding kinetics [22].

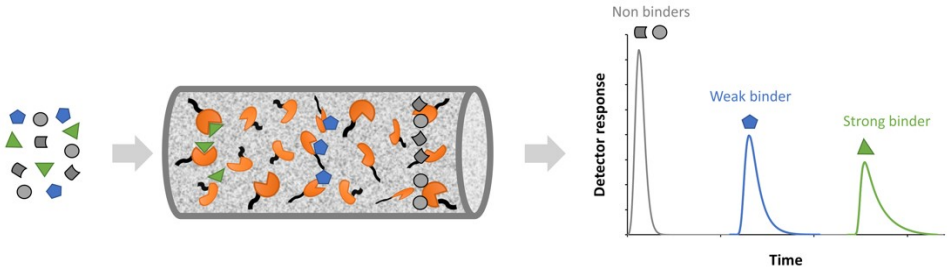

Figure 1: Principle of weak affinity chromatography (WAC). A mixture of compounds is injected into a chromatography column that exposes the immobilized protein target (in orange) on its stationary phase. Due to binding with the immobilized target, the compounds are retained on the column. Non-binders (in grey) elute at the solvent front. The retention of the binders increases with their affinity for the target, as depicted by the difference in retention times between the weak binder (in blue) and the strong binder (in green). Regarding packing materials, WAC requires sorbents, such as silica particles and monoliths.

Carbohydrate supports used for purification purposes have low mechanical stability and are unsuitable for WAC [22,23]. Porous, spherical silica microparticles with a high surface area are preferred as support for fragment screening by WAC because they can accommodate a large amount of proteins per volume [23]. However, silica materials must be derivatized with diol groups to reduce nonspecific binding to the silica surface [23]. These diols support the protein immobilization through an oxidation step followed by reductive amination. This step can be performed on prepacked columns (*in situ*) or silica particles (*in batch*). The immobilization in batch is only recommended in case of large proteins or lipodisks. Lipodisks are stabilized bilayer disks that can incorporate membrane proteins [22,23]. The protocols for protein immobilization are described elsewhere [24]. After preparation, the amount of active protein immobilized in the column must be frequently checked using a known ligand. This verification is generally achieved by frontal analysis [22–24].

WAC is robust and high-throughput. The main advantages are the low consumption of samples and the separative character. Indeed, WAC does not require high sample purity and can perform multiplexed analysis of mixtures, natural extracts, or stereoisomers [22,23]. However, the tethered proteins must retain their integrity, and a high protein load in the column is required for high sensitivity. Sensitivity can be hampered by large proteins and lipodisks, as steric constraints limit the amount that can be loaded onto the column [9,22,23]. The main issue of this technology is the significant contribution of nonspecific interactions between the fragments and the chromatographic system or the protein, generating false-positive results [8,9,22,23]. Fragments should be screened on one column without the target to evict a part of the nonspecific interactions. Yet the most efficient approach is to use a column where the active site of the target is blocked [22,23]. WAC can also generate false negatives. This could happen with high-affinity binders that do not elute

during the analysis time. Also, compounds that are poorly ionized in the MS source may not be detected [23].

Venkatraman et al. [36] recently used WAC to discover three modulators of the proteolytic processing of the mutant Transforming Growth Factor Beta induced protein (TGFBIp) to treat TGFBI-corneal dystrophy. WAC screened 2500 fragments, distributed in 50 mixtures of 50 compounds, against the wild-type and two mutants of TGFBIp. The 134 primary hits were then analyzed individually by WAC for confirmation and K_D determination. The measured K_D ranged from 10 μM to 2 mM. Three fragments of the top 10 were validated by ^{15}N heteronuclear single-quantum correlation (HSQC)-based NMR analysis. Finally, these three compounds were found to effectively modulate the proteolytic processing of mutant TGFBIp [36]. Several studies used WAC as a fragment screening technology with other targets, such as protease [37], chaperone protein [38], kinase [39], and channel [40]. The WAC technology for screening is still protected by the patent EP1941269B8 [58]. Its commercial rights are owned by SARomics Biostructures AB and Red Glead Discovery [59,60], which impede its use. A proof-of-concept study also used frontal affinity chromatography, a variant of the common WAC technology to identify fragments against adenosine A_{2A} receptor, a G-protein coupled receptor [41]. In frontal affinity chromatography, the tested fragment is continuously infused into the column. Once the fragment saturates the binding sites present in the column, the signal baseline jumps, leading to a sigmoid-like profile at the breakthrough time. Binders are detected by determining the breakthrough time of two extreme fragment concentration using the same column [41].

3.2. Microscale thermophoresis (MST)

Microscale thermophoresis (MST) tracks the movement of a fluorescently labeled macromolecule under a temperature gradient. A hot spot is created by an infrared laser in a narrow zone of a sample-loaded glass capillary (see Figure 2). Simultaneously, the fluorescence is recorded in this area, which allows the monitoring of the target movement. This movement induced by a temperature gradient is affected by modifications in the hydration shell, charge, and size of the protein. If at least one of these parameters is altered upon ligand binding, a change in the MST trace is observed [2,8,9,23]. This technology operates in near-physiological conditions and consumes a low amount of protein. MST is also applicable with solubilized membrane proteins and complex matrices, such as serum [9,23].

Publisher version: https://doi.org/10.1016/j.trac.2023.117161

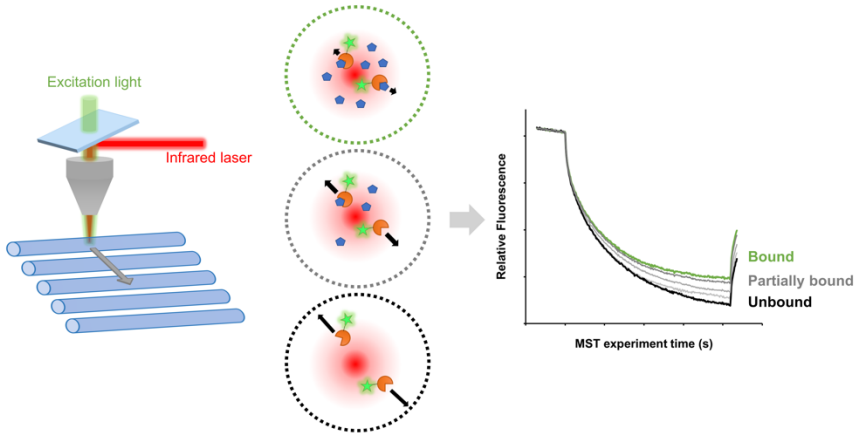

Figure 2. Principle of microscale thermophoresis (MST). An infrared laser creates a temperature gradient in a narrow zone of a glass capillary. Simultaneously, the fluorescence (excitation light) is recorded in this area to monitor the movement of the fluorescently-labeled target. Upon binding, this thermophoretic movement is altered, and a change in the MST trace is observed.

The challenge is to fluorescently label the protein without affecting its integrity or occluding the binding site [2,23]. MST also requires elevated ligand concentration to detect weak affinity, which limits this technology to highly soluble fragments [23]. Several effects can generate a false-positive result in MST. Some of them can be spotted with a proper inspection of the MST trace, such as photobleaching, photoenhancement, autofluorescence, or aggregation. Others are difficult to flag, such as compounds that bind the label or the protein His-tag [25].

Kessler et al. [42] used MST in combination with STD NMR to identify fragments that bind to KRAS, a GTPase of interest in many cancers. The two techniques screened 1800 fragments and identified 16 fragments displaying mM affinity (validated by HSQC NMR). Further chemical optimizations guided by a SAR-by-catalog, HSQC NMR, and X-ray crystallography led to the discovery of the cell-active KRAS inhibitor BI-2852 [42]. Other studies [43–47] used MST to determine fragments K_D.

3.3. Affinity capillary electrophoresis (ACE)

Electrophoretic techniques are not commonly used in drug discovery screening platforms. Only specialized groups reported its utility for FBDD [26–29,51,52,61]. Capillary electrophoresis is characterized by low consumption of samples and reagents, near-physiological conditions, easy or no sample preparation, and high resolution [26–28]. Affinity capillary electrophoresis (ACE) includes a plethora of methods. Each has its detectable affinity range, advantages, and limitations [26,27,62]. This review focuses on ACE approaches used in a FBDD context, namely mobility-shift ACE and partial-filling ACE. These two methods work with unmodified proteins in solution [26].

ACE is based on the modification of the mass-to-charge ratio of an analyte upon interaction with the target. When the analyte binds to the target, a shift in its migration time is observed if the mobility of the complex is different from the analyte mobility [26,27]. In mobility-shift ACE, the entire capillary is filled with the protein-spiked background electrolyte (BGE), and then a short sample plug is injected. After the voltage application, the analyte migrates and can interact with the target. Therefore, the observed electrophoretic mobility of the analyte becomes the weighted average of the mobility of the free analyte and the mobility of the complex [26,27,62]. However, the mobility shift method presents two substantial drawbacks. First, as the capillary, the inlet and outlet vials are filled with the target, it is highly target consuming (around 500 µL). Secondly, the presence of the protein during detection causes a high background UV absorbance [26–28,62].

A variant of mobility-shift ACE overcomes these limitations and is known as partial-filling ACE. In this approach, the target fills only a part of the capillary. After the voltage application, the analyte migrates through the target plug and is detected in the neat BGE. Its observed mobility is therefore a combination of its effective mobility in the target plug and its effective mobility in the neat BGE [26,27,63]. These two methods are separative and thus do not require highly pure analyte. Moreover, they only need low fragment concentration. Therefore, this technology can evaluate fragments with low solubility. Regarding the drawbacks of this technology, fragments with an electrophoretic mobility close to the one of the target or UV transparent cannot be screened. Also, the target needs to be soluble at µM concentrations.

It is important to note that the conductivity, field strength, and viscosity must be uniform throughout the capillary to generate accurate results. In addition, precautions must be taken to avoid the adsorption of the target on the silica surface. This technology also suffers from nonspecific binding [28]. Proof-of-concept studies used direct ACE to screen small sets of positively-charged fragments against thrombin [49] or factor XIIa [51]. The separative character of direct ACE allows multiplexed analyses [49] and the cross-validation of fragment hits [51]. However, the fragments in the two studies were limited to positively-charged compounds [49,51].

To overcome the nonspecific binding and screen negatively-charged and neutral compounds, we can use indirect methods, commonly known as competitive ACE (see Figure 3). In this configuration, a known binder is utilized as a reporter, and its migration time is recorded. When the target is present in the capillary, the reporter interacts with it, leading to a shift. Then, the tested fragment is added to the buffer filling the capillary and reservoirs. If the fragment displaces the reporter from the target, the reporter shift is reduced, and a shift-back can be observed.

Publisher version: https://doi.org/10.1016/j.trac.2023.117161

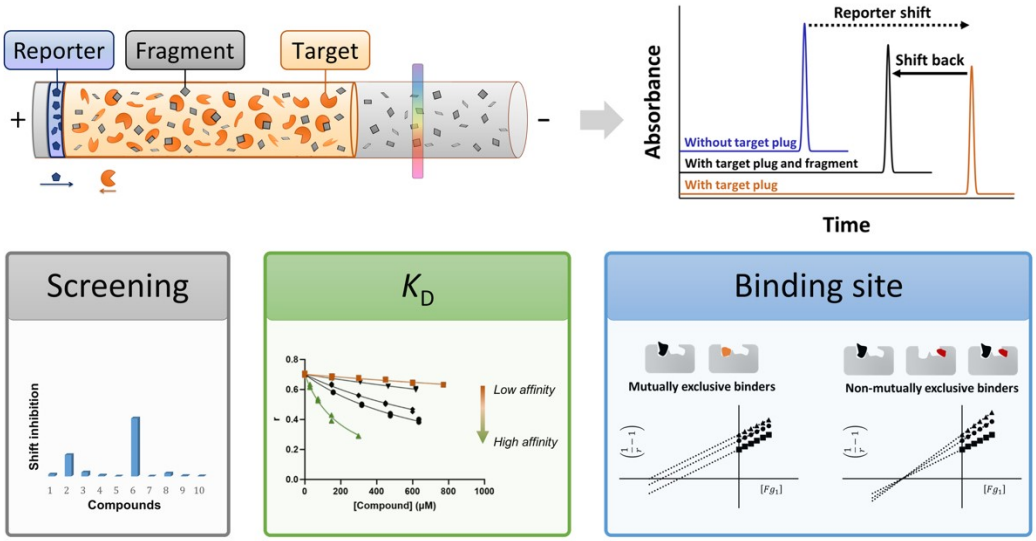

Figure 3. Principle and uses of affinity capillary electrophoresis (ACE) in indirect mode. The reporter plug is present at the beginning of the capillary. After the voltage application, the reporter migrates through the target plug. Because the reporter interacts with the target, its migration is slower and a shift in its migration time appears. When a binder is added to the buffer, it affects the binding equilibrium of the reporter with the target, leading to a shift-back of the reporter migration time. This strategy can perform screening, determine dissociation constants (K_D), and identify fragment pairs that bind simultaneously to the target [52].

This indirect mode can provide some insights into the fragment binding site. However, the advantages of the separative character are lost [26,28,29,52]. Austin et al. [48] used indirect ACE to screen 609 fragments against the human heat shock protein 90α. The hit rate was 6.9%, consistent with the results obtained from the NMR screening performed by another group (6.1%). Their method detected interactions in the μM-mM range and was more sensitive than the fluorescence polarization binding assay [48]. Additional studies reinforced these findings on other targets, such as proteases [50,52] and stabilized G-protein coupled receptors [53]. Besides its value for screening, indirect ACE also enables the determination of K_D in the μM-mM range and the identification of fragment pairs that bind simultaneously to the target [52] (see Figure 3). Compared with WAC, ACE for screening is not under patent's protection (EP2480879A1 was withdrawn [64]).

3.4. Dual-pharmacophore DNA-encoded chemical libraries (DEL) and associated

DNA-encoded chemical libraries (DEL) are constituted of compounds individually coupled to a specific DNA sequence that serves as an amplifiable barcode. After an affinity selection, PCR amplification followed by high-throughput DNA sequencing allow the identification of the binders (see Figure 4). Ideally, the enriched compounds are resynthesized, and their affinity is measured to confirm the hits [31–33,35]. More details on the encoding and affinity selection methods can be found in the following papers [31,34,35]. Dual-pharmacophore DEL uses two sets of partially

complementary oligonucleotides that hybridize to form stable DNA heteroduplex. These oligonucleotides are bonded individually to specific fragments. Consequently, the DNA heteroduplex displays pairs of fragments in close proximity and with some flexibility [33].

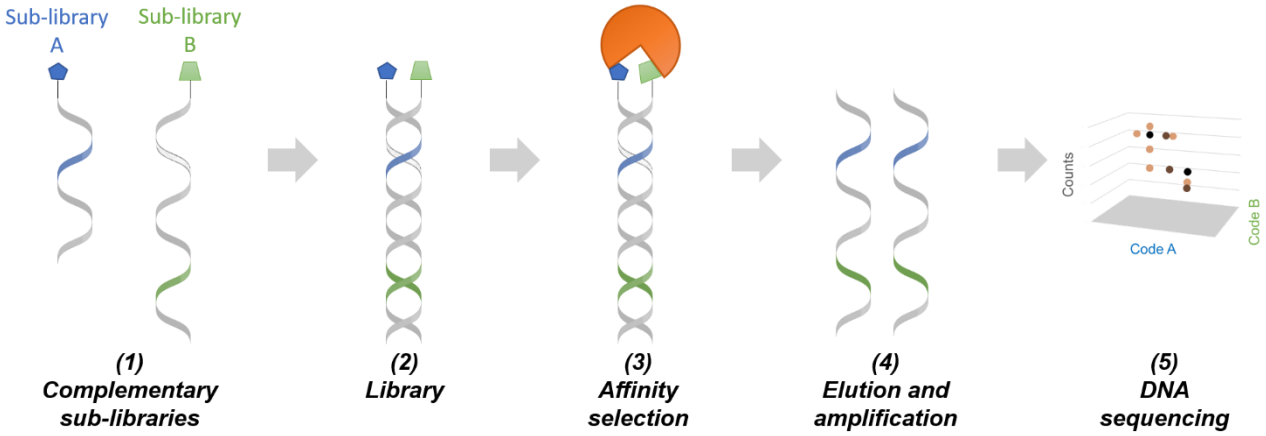

Figure 4. Principle of dual-pharmacophore DNA-encoded libraries (DEL). (1) Complementary sub-libraries are produced by coupling fragments to individual identifier oligonucleotides. The sub-library A possesses a coding region (in blue) and a shorter sequence compared with the sub-library B. The sub-library B possesses an abasic site (dotted part of the sequence) and a coding region (in green). (2) The library is formed by hybridizing the complementary sub-libraries and filling the A-strand using a DNA polymerase. Therefore, the A-strand bears the two coding regions and allows the identification of the fragment pair. Only the A-strand can be amplified by PCR, because the B-strand has an abasic site. (3) The library is mixed with the target protein to select the binding pairs. (4) The binders are eluted and PCR-amplified. (5) After decoding by high-throughput DNA sequencing, a count is attributed to each library member. The enrichment of library members due to the affinity selection is evaluated to identify the binding fragment pairs. To validate the hits, compounds containing the two fragments are synthesized. Different linkers must be tested due to the intrinsic flexibility of dual-pharmacophore chemical libraries [33].

This technology explores non-overlapping pockets of the target protein, allowing the discovery of a synergistic effect between two fragments [33]. The advantages of this technology are the simultaneous screening of a very large library, the low consumption of protein and fragments, the improvement of fragment solubility due to the polyelectrolyte nature of DNA, and the selection of binding pairs that can be joined to obtain a high-affinity binder [33]. The challenge is the synthesis of the library. The fragments need to be attached to their DNA strand with acceptable yields and without affecting the DNA integrity [31]. High purity of the library is a critical factor, and the conditions of the affinity selection can have a significant impact on the identified binders [31,33].

Wichert et al. [54] first validated their dual-pharmacophore DEL method on streptavidin and horseradish peroxidase by identifying ligands with previously known structural motifs. Then, they used their methodology to discover a low μM ligand of alpha-1-acid glycoprotein. They also used this technology to mature a nanomolar inhibitor of carbonic anhydrase IX into a subnanomolar one [54]. Recent developments led to trio-pharmacophore DEL, where three fragments are presented simultaneously (instead of two for dual-pharmacophore DEL) [55]. Its utility was demonstrated with four targets: trypsin, carbonic anhydrase II, and matrix metalloprotease-2 and -9 [55]. For ligand discovery, Zhou et al. [56,57] also used DNA-encoded dynamic chemical library, a variant of dual-pharmacophore DEL where the two libraries continuously exchange partners. The target stabilizes

duplexes where the two fragments bind. The stabilized duplexes are then photo-crosslinked and the ligated DNA barcodes are identified [57].

3.5. Other technologies

Some authors also investigated other techniques for FBDD that use different types of physical principles for detection, such as biolayer interferometry [65], grating-coupled interferometry [66], second-harmonic generation [67], and optical waveguide grating [68]. Examples are still sparse, and more reports are needed to establish their place in FBDD.

4. Conclusion

The success of a FBDD project strongly relies on the ability of the selected analytical technology to detect and gauge accurately weak interactions with the target. Several constraints must be considered during the selection of adequate analytical techniques. First, the target of the FBDD project can narrow the possibilities. Some targets are difficult to modify or produce in large quantities. In these cases, analytical techniques that require target modification (such as SPR, WAC, and MST) or a large protein quantity (such as ligand-observed NMR and X-ray crystallography), respectively, should be avoided. Secondly, easily accessible analytical technologies with high throughput are preferred to perform screening on fragment libraries. Costly instruments (such as high-field NMR and synchrotron facilities) have limited accessibility; they are generally not the first choice for small medicinal chemistry groups. Thirdly, the desired range of detectable affinities must be defined to select and develop the methodology appropriately. When the screening campaign must discover mM binders, highly sensitive techniques are required. The FBDD techniques compatible with a project can be determined by the decision tree depicted in Figure 5. Once the compatible technologies are determined, the users must select the most accessible one according to their facilities and identify its potential artifacts to set up appropriate orthogonal assays. Overall, the knowledge of the strengths and limitations linked to the different analytical technologies, briefly summarized in this review, is essential before starting an FBDD project.

Publisher version: https://doi.org/10.1016/j.trac.2023.117161

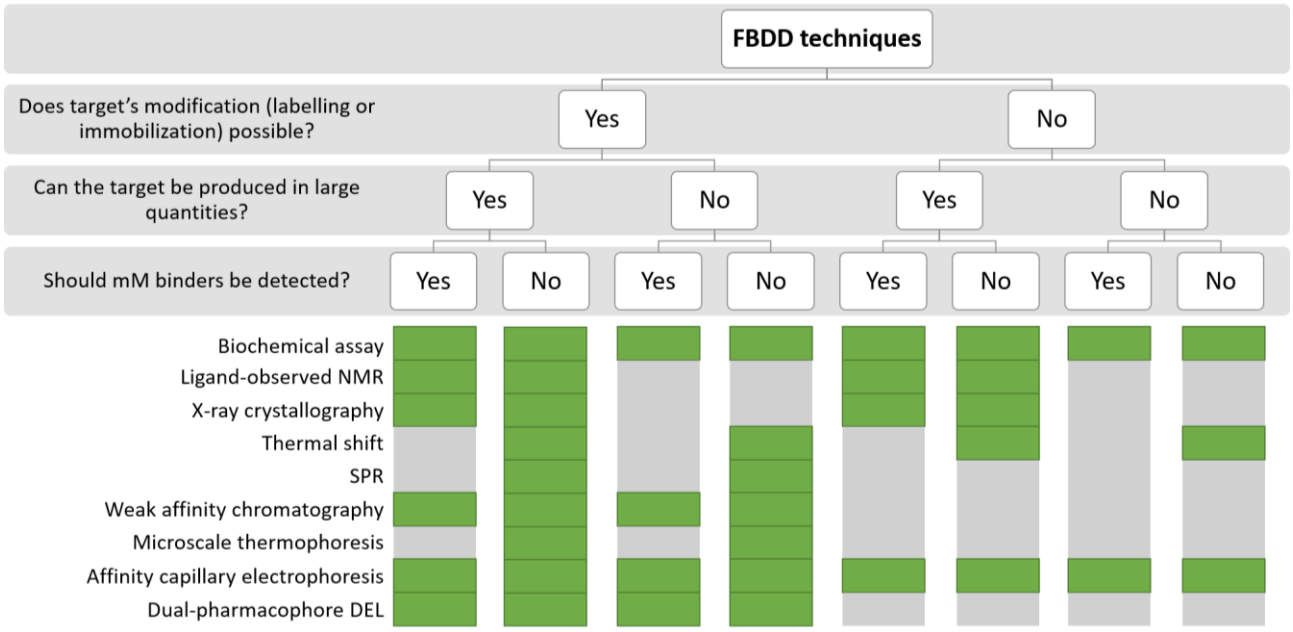

Figure 5. Decision tree to determine the FBDD techniques compatible with the target and the desired range of detectable affinities. After this first sorting, the users must select the most accessible technique depending on their facilities.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

C. D. received funding from the National Fund for Scientific Research (FNRS) [grant number 40000455]; and Fondation Léon Frédéricq [grant number 2020-2021-30-C.F.F]. L. P. received funding from the University of Namur [grant number L. POCHET-12/2021]. M. F. received funding from the University of Liège Fonds Spéciaux—Crédits facultaires [grant number 14758].

References

[1] J. Coyle, R. Walser, Applied Biophysical Methods in Fragment-Based Drug Discovery, SLAS Discov. Adv. Sci. Drug Discov. 25 (2020) 471–490. https://doi.org/10.1177/2472555220916168.

[2] B. Lamoree, R.E. Hubbard, Current perspectives in fragment-based lead discovery (FBLD), Essays Biochem. 61 (2017) 453–464. https://doi.org/10.1042/EBC20170028.

[3] W. Lu, M. Kostic, T. Zhang, J. Che, M.P. Patricelli, L.H. Jones, E.T. Chouchani, N.S. Gray, Fragment-based covalent ligand discovery, RSC Chem. Biol. 2 (2021) 354–367. https://doi.org/10.1039/D0CB00222D.

[4] D.A. Erlanson, B.J. Davis, W. Jahnke, Fragment-Based Drug Discovery: Advancing Fragments in the Absence of Crystal Structures, Cell Chem. Biol. 26 (2019) 9–15. https://doi.org/10.1016/j.chembiol.2018.10.001.

[5] L. Walsh, D.A. Erlanson, I.J.P. de Esch, W. Jahnke, A. Woodhead, E. Wren, Fragment-to-Lead Medicinal Chemistry Publications in 2021, J. Med. Chem. (2023).

https://doi.org/10.1021/acs.jmedchem.2c01827.

[6] I.J.P. de Esch, D.A. Erlanson, W. Jahnke, C.N. Johnson, L. Walsh, Fragment-to-Lead Medicinal Chemistry Publications in 2020, J. Med. Chem. 65 (2022) 84–99. https://doi.org/10.1021/acs.jmedchem.1c01803.

[7] D.A. Erlanson, I.J.P. de Esch, W. Jahnke, C.N. Johnson, P.N. Mortenson, Fragment-to-Lead Medicinal Chemistry Publications in 2018, J. Med. Chem. (2020). https://doi.org/10.1021/acs.jmedchem.9b01581.

[8] B.J. Davis, S.D. Roughley, Fragment-Based Lead Discovery, Annu. Rep. Med. Chem. 50 (2017) 371–439. https://doi.org/10.1016/BS.ARMC.2017.07.002.

[9] J.P. Renaud, C.W. Chung, U.H. Danielson, U. Egner, M. Hennig, R.E. Hubbard, H. Nar, Biophysics in drug discovery: Impact, challenges and opportunities, Nat. Rev. Drug Discov. 15 (2016) 679–698. https://doi.org/10.1038/nrd.2016.123.

[10] V. Velvadapu, B.T. Farmer, A.B. Reitz, Fragment-Based Drug Discovery, in: Pract. Med. Chem., Elsevier, 2015: pp. 161–180. https://doi.org/10.1016/B978-0-12-417205-0.00007-9.

[11] Z. Konteatis, What makes a good fragment in fragment-based drug discovery?, Expert Opin. Drug Discov. 00 (2021) 1–4. https://doi.org/10.1080/17460441.2021.1905629.

[12] W. Jahnke, D.A. Erlanson, I.J.P. de Esch, C.N. Johnson, P.N. Mortenson, Y. Ochi, T. Urushima, Fragment-to-Lead Medicinal Chemistry Publications in 2019, J. Med. Chem. 63 (2020) 15494–15507. https://doi.org/10.1021/acs.jmedchem.0c01608.

[13] J. Barker, S. Courtney, T. Hesterkamp, D. Ullmann, M. Whittaker, Fragment screening by biochemical assay, Expert Opin. Drug Discov. 1 (2006) 225–236. https://doi.org/10.1517/17460441.1.3.225.

[14] B.J. Davis, D.A. Erlanson, Learning from our mistakes : The ‘ unknown knowns ’ in fragment screening, Bioorg. Med. Chem. Lett. 23 (2013) 2844–2852. https://doi.org/10.1016/j.bmcl.2013.03.028.

[15] S.L. McGovern, B.T. Helfand, B. Feng, B.K. Shoichet, A Specific Mechanism of Nonspecific Inhibition, J. Med. Chem. 46 (2003) 4265–4272. https://doi.org/10.1021/jm030266r.

[16] D.A. Erlanson, Introduction to Fragment-Based Drug Discovery, Top Curr Chem. 310 (2012) 1–32. https://doi.org/10.1007/128.

[17] I. Krimm, Applications of Ligand and Protein-Observed NMR in Ligand Discovery, in: Appl. Biophys. Drug Discov., John Wiley & Sons, Ltd, Chichester, UK, 2017: pp. 175–195. https://doi.org/10.1002/9781119099512.ch10.

[18] Q. Li, Application of Fragment-Based Drug Discovery to Versatile Targets, Front. Mol. Biosci. 7 (2020). https://doi.org/10.3389/fmolb.2020.00180.

[19] T.G. Davies, I.J. Tickle, Fragment Screening Using X-Ray Crystallography, in: Top Curr Chem, Springer, Berlin, Heidelberg, 2011: pp. 33–59. https://doi.org/10.1007/128_2011_179.

[20] A.M. Giannetti, From Experimental Design to Validated Hits: A Comprehensive Walk-Through of Fragment Lead Identification Using Surface Plasmon Resonance, Methods Enzymol. 493 (2011) 169–218. https://doi.org/10.1016/B978-0-12-381274-2.00008-X.

[21] J. Homola, Present and future of surface plasmon resonance biosensors, Anal. Bioanal. Chem. 377 (2003) 528–539. https://doi.org/10.1007/s00216-003-2101-0.

[22] F. Tsopelas, A. Tsantili-Kakoulidou, Advances with weak affinity chromatography for fragment screening, Expert Opin. Drug Discov. 14 (2019) 1125–1135. https://doi.org/10.1080/17460441.2019.1648425.

[23] S. Ohlson, M.-D. Duong-Thi, Emerging Technologies for Fragment Screening, in: D.A.

Erlanson, W. Jahnke (Eds.), Fragm. Drug Discov. Lessons Outlook, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2016: pp. 173–196. https://doi.org/10.1002/9783527683604.ch08.

[24] S. Ohlson, M.-D. Duong-Thi, Fragment screening for drug leads by weak affinity chromatography (WAC-MS), Methods. 146 (2018) 26–38. https://doi.org/10.1016/j.ymeth.2018.01.011.

[25] J.M. Rainard, G.C. Pandarakalam, S.P. McElroy, Using Microscale Thermophoresis to Characterize Hits from High-Throughput Screening: A European Lead Factory Perspective, SLAS Discov. Adv. Sci. Drug Discov. 23 (2018) 225–241. https://doi.org/10.1177/2472555217744728.

[26] M. Olabi, M. Stein, H. Wätzig, Affinity capillary electrophoresis for studying interactions in life sciences, Methods. 146 (2018) 76–92. https://doi.org/10.1016/j.ymeth.2018.05.006.

[27] V.A. Galievsky, A.S. Stasheuski, S.N. Krylov, Capillary electrophoresis for quantitative studies of biomolecular interactions, Anal. Chem. 87 (2015) 157–171. https://doi.org/10.1021/ac504219r.

[28] E. Farcas, L. Pochet, J. Crommen, A. Servais, M. Fillet, Capillary electrophoresis in the context of drug discovery, J. Pharm. Biomed. Anal. 144 (2017) 195–212. https://doi.org/10.1016/j.jpba.2017.02.022.

[29] Y. Wang, D.I. Adeoye, E.O. Ogunkunle, I.-A. Wei, R.T. Filla, M.G. Roper, Affinity Capillary Electrophoresis: A Critical Review of the Literature from 2018 to 2020, Anal. Chem. 93 (2021) 295–310. https://doi.org/10.1021/acs.analchem.0c04526.

[30] C.M. Ouimet, C.I. D'amico, R.T. Kennedy, Advances in capillary electrophoresis and the implications for drug discovery, Expert Opin. Drug Discov. 12 (2017) 213–224. https://doi.org/10.1080/17460441.2017.1268121.

[31] N. Favalli, G. Bassi, J. Scheuermann, D. Neri, DNA-encoded chemical libraries - achievements and remaining challenges, FEBS Lett. 592 (2018) 2168–2180. https://doi.org/10.1002/1873-3468.13068.

[32] G. Zimmermann, D. Neri, DNA-encoded chemical libraries: foundations and applications in lead discovery, Drug Discov. Today. 21 (2016) 1828–1834. https://doi.org/10.1016/J.DRUDIS.2016.07.013.

[33] J. Scheuermann, D. Neri, Dual-pharmacophore DNA-encoded chemical libraries, Curr. Opin. Chem. Biol. 26 (2015) 99–103. https://doi.org/10.1016/j.cbpa.2015.02.021.

[34] B. Shi, Y. Zhou, Y. Huang, J. Zhang, X. Li, Recent advances on the encoding and selection methods of DNA-encoded chemical library, Bioorg. Med. Chem. Lett. 27 (2017) 361–369. https://doi.org/10.1016/j.bmcl.2016.12.025.

[35] B. Shi, Y. Zhou, X. Li, Recent advances in DNA-encoded dynamic libraries, RSC Chem. Biol. 3 (2022) 407–419. https://doi.org/10.1039/D2CB00007E.

[36] A. Venkatraman, M.-D. Duong-Thi, K. Pervushin, S. Ohlson, J.S. Mehta, Pharmaceutical modulation of the proteolytic profile of Transforming Growth Factor Beta induced protein (TGFBIp) offers a new avenue for treatment of TGFBI-corneal dystrophy, J. Adv. Res. 24 (2020) 529–543. https://doi.org/10.1016/j.jare.2020.05.012.

[37] M.-D. Duong-Thi, G. Bergström, C.-F. Mandenius, M. Bergström, T. Fex, S. Ohlson, Comparison of weak affinity chromatography and surface plasmon resonance in determining affinity of small molecules, Anal. Biochem. 461 (2014) 57–59. https://doi.org/10.1016/j.ab.2014.05.023.

[38] E. Meiby, H. Simmonite, L. le Strat, B. Davis, N. Matassova, J.D. Moore, M. Mrosek, J. Murray, R.E. Hubbard, S. Ohlson, Fragment Screening by Weak Affinity Chromatography: Comparison with Established Techniques for Screening against HSP90, Anal. Chem. 85

(2013) 6756–6766. https://doi.org/10.1021/ac400715t.

[39] E. Meiby, S. Knapp, J.M. Elkins, S. Ohlson, Fragment screening of cyclin G-associated kinase by weak affinity chromatography, Anal. Bioanal. Chem. 404 (2012) 2417–2425. https://doi.org/10.1007/s00216-012-6335-6.

[40] M.-D. Duong-Thi, M. Bergström, K. Edwards, J. Eriksson, S. Ohlson, J. To Yiu Ying, J. Torres, V. Agmo Hernández, Lipodisks integrated with weak affinity chromatography enable fragment screening of integral membrane proteins, Analyst. 141 (2016) 981–988. https://doi.org/10.1039/C5AN02105G.

[41] L. Lecas, L. Hartmann, L. Caro, S. Mohamed-Bouteben, C. Raingeval, I. Krimm, R. Wagner, V. Dugas, C. Demesmay, Miniaturized weak affinity chromatography for ligand identification of nanodiscs-embedded G-protein coupled receptors, Anal. Chim. Acta. 1113 (2020) 26–35. https://doi.org/10.1016/j.aca.2020.03.062.

[42] D. Kessler, M. Gmachl, A. Mantoulidis, L.J. Martin, A. Zoephel, M. Mayer, A. Gollner, D. Covini, S. Fischer, T. Gerstberger, T. Gmaschitz, C. Goodwin, P. Greb, D. Häring, W. Hela, J. Hoffmann, J. Karolyi-Oezguer, P. Knesl, S. Kornigg, M. Koegl, R. Kousek, L. Lamarre, F. Moser, S. Munico-Martinez, C. Peinsipp, J. Phan, J. Rinnenthal, J. Sai, C. Salamon, Y. Scherbantin, K. Schipany, R. Schnitzer, A. Schrenk, B. Sharps, G. Siszler, Q. Sun, A. Waterson, B. Wolkerstorfer, M. Zeeb, M. Pearson, S.W. Fesik, D.B. McConnell, Drugging an undruggable pocket on KRAS, Proc. Natl. Acad. Sci. 116 (2019) 15823–15829. https://doi.org/10.1073/pnas.1904529116.

[43] A. Coletti, F. Camponeschi, E. Albini, F.A. Greco, V. Maione, C. Custodi, F. Ianni, U. Grohmann, C. Orabona, F. Cantini, A. Macchiarulo, Fragment-based approach to identify IDO1 inhibitor building blocks, Eur. J. Med. Chem. 141 (2017) 169–177. https://doi.org/10.1016/j.ejmech.2017.09.044.

[44] F. Centorrino, B. Andlovic, P. Cossar, L. Brunsveld, C. Ottmann, Fragment-based exploration of the 14-3-3/Amot-p130 interface, Curr. Res. Struct. Biol. 4 (2022) 21–28. https://doi.org/10.1016/j.crstbi.2021.12.003.

[45] J. Miao, H. Yuan, J. Rao, J. Zou, K. Yang, G. Peng, S. Cao, H. Chen, Y. Song, Identification of a small compound that specifically inhibits Zika virus in vitro and in vivo by targeting the NS2B-NS3 protease, Antiviral Res. 199 (2022) 105255. https://doi.org/10.1016/j.antiviral.2022.105255.

[46] F. Kozielski, C. Sele, V.O. Talibov, J. Lou, D. Dong, Q. Wang, X. Shi, M. Nyblom, A. Rogstam, T. Krojer, Z. Fisher, W. Knecht, Identification of fragments binding to SARS-CoV-2 nsp10 reveals ligand-binding sites in conserved interfaces between nsp10 and nsp14/nsp16, RSC Chem. Biol. 3 (2022) 44–55. https://doi.org/10.1039/D1CB00135C.

[47] A. Reyes Romero, S. Lunev, G.M. Popowicz, V. Calderone, M. Gentili, M. Sattler, J. Plewka, M. Taube, M. Kozak, T.A. Holak, A.S.S. Dömling, M.R. Groves, A fragment-based approach identifies an allosteric pocket that impacts malate dehydrogenase activity, Commun. Biol. 4 (2021) 949. https://doi.org/10.1038/s42003-021-02442-1.

[48] C. Austin, S.N. Pettit, S.K. Magnolo, J. Sanvoisin, W. Chen, S.P. Wood, L.D. Freeman, R.J. Pengelly, D.E. Hughes, Fragment screening using capillary electrophoresis (CEfrag) for hit identification of heat shock protein 90 ATPase inhibitors, J. Biomol. Screen. 17 (2012) 868–876. https://doi.org/10.1177/1087057112445785.

[49] E. Farcaş, C. Bouckaert, A.C. Servais, J. Hanson, L. Pochet, M. Fillet, Partial filling affinity capillary electrophoresis as a useful tool for fragment-based drug discovery: A proof of concept on thrombin, Anal. Chim. Acta. 984 (2017) 211–222. https://doi.org/10.1016/j.aca.2017.06.035.

[50] E. Farcaş, J. Hanson, L. Pochet, M. Fillet, Capillary electrophoretic mobility shift displacement assay for the assessment of weak drug-protein interactions, Anal. Chim. Acta.

1034 (2018) 214–222. https://doi.org/10.1016/j.aca.2018.06.024.

[51] C. Davoine, M. Fillet, L. Pochet, Capillary electrophoresis as a fragment screening tool to cross-validate hits from chromogenic assay: Application to FXIIa, Talanta. 226 (2021) 122163. https://doi.org/10.1016/j.talanta.2021.122163.

[52] C. Davoine, A. Pardo, L. Pochet, M. Fillet, Fragment Hit Discovery and Binding Site Characterization by Indirect Affinity Capillary Electrophoresis: Application to Factor XIIa, Anal. Chem. 93 (2021) 14802–14809. https://doi.org/10.1021/acs.analchem.1c03611.

[53] S.P. Andrews, B. Tehan, Stabilised G protein-coupled receptors in structure-based drug design: a case study with adenosine A 2A receptor, Medchemcomm. 4 (2013) 52–67. https://doi.org/10.1039/C2MD20164J.

[54] M. Wichert, N. Krall, W. Decurtins, R.M. Franzini, F. Pretto, P. Schneider, D. Neri, J. Scheuermann, Dual-display of small molecules enables the discovery of ligand pairs and facilitates affinity maturation, Nat. Chem. 7 (2015) 241–249. https://doi.org/10.1038/nchem.2158.

[55] M. Cui, D. Nguyen, M.P. Gaillez, S. Heiden, W. Lin, M. Thompson, F. V. Reddavide, Q. Chen, Y. Zhang, Trio-pharmacophore DNA-encoded chemical library for simultaneous selection of fragments and linkers, Nat. Commun. 14 (2023) 1481. https://doi.org/10.1038/s41467-023-37071-1.

[56] Y. Zhou, W. Shen, J. Peng, Y. Deng, X. Li, Identification of isoform/domain-selective fragments from the selection of DNA-encoded dynamic library, Bioorg. Med. Chem. 45 (2021) 116328. https://doi.org/10.1016/j.bmc.2021.116328.

[57] Y. Zhou, C. Li, J. Peng, L. Xie, L. Meng, Q. Li, J. Zhang, X.D. Li, X. Li, X. Huang, X. Li, DNA-Encoded Dynamic Chemical Library and Its Applications in Ligand Discovery, J. Am. Chem. Soc. 140 (2018) 15859–15867. https://doi.org/10.1021/jacs.8b09277.

[58] S. Ohlson, S. Shoravi, T. Fex, R. Isaksson, A method of screening a biological target for weak interactions using weak affinity chromatography, EP1941269B8, 2006.

[59] SARomics Biostructures AB, Fragment Screening & Lead Discovery Services, (2023). https://www.saromics.com/services/drug-discovery/#wac-screen.

[60] M. Kvist Reimer, R&D Initiatives, (2023). https://www.redglead.com/rd-initiatives-and-science/#publications.

[61] M. Congreve, J.A. Christopher, Fragment Screening in Complex Systems, in: D.A. Erlanson, W. Jahnke (Eds.), Fragm. Drug Discov. Lessons Outlook, 2016: pp. 267–292. https://doi.org/10.1002/9783527683604.ch12.

[62] S. Štěpánová, V. Kašička, Application of Capillary Electromigration Methods for Physicochemical Measurements, in: Capill. Electromigr. Sep. Methods, Elsevier, 2018: pp. 547–591. https://doi.org/10.1016/B978-0-12-809375-7.00024-1.

[63] M. Ansorge, P. Dubský, K. Ušelová, Into the theory of the partial-filling affinity capillary electrophoresis and the determination of apparent stability constants of analyte-ligand complexes, Electrophoresis. 39 (2018) 742–751. https://doi.org/10.1002/elps.201700385.

[64] D.E. Hughes, Affinity capillary electrophoresis method for assessing a biological interraction of a ligand/receptor pair such as g protein coupled receptor and its targets as well as for drug screening, EP2480879A1, 2010.

[65] C.A. Wartchow, F. Podlaski, S. Li, K. Rowan, X. Zhang, D. Mark, K.-S. Huang, Biosensor-based small molecule fragment screening with biolayer interferometry, J. Comput. Aided. Mol. Des. 25 (2011) 669–676. https://doi.org/10.1007/s10822-011-9439-8.

[66] Ö. Kartal, F. Andres, M.P. Lai, R. Nehme, K. Cottier, waveRAPID—A Robust Assay for High-Throughput Kinetic Screens with the Creoptix WAVEsystem, SLAS Discov. Adv. Sci.

Drug Discov. 26 (2021) 995–1003. https://doi.org/10.1177/24725552211013827.

[67] E.A. FitzGerald, M.T. Butko, P. Boronat, D. Cederfelt, M. Abramsson, H. Ludviksdottir, J.E. van Muijlwijk-Koezen, I.J.P. de Esch, D. Dobritzsch, T. Young, U.H. Danielson, Discovery of fragments inducing conformational effects in dynamic proteins using a second-harmonic generation biosensor, RSC Adv. 11 (2021) 7527–7537. https://doi.org/10.1039/D0RA09844B.

[68] S. Geschwindner, J.F. Carlsson, W. Knecht, Application of Optical Biosensors in Small-Molecule Screening Activities, Sensors. 12 (2012) 4311–4323. https://doi.org/10.3390/s120404311.