Fragment screening by native state mass spectrometry

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Abstract

Native state mass spectrometry (MS) has been recognised as a rapid, sensitive and high throughput method to directly investigate protein-ligand interactions for some time, however there are few examples reporting this approach as a screening method to identify relevant protein–fragment interactions in fragment based drug discovery (FBDD). In this paper an overview of native state MS will be presented, highlighting the attractive properties of this method within the context of fragment screening applications. A summary of published examples using MS for fragment screening will be described and reflection on the outlook for the future adoption and implementation of native state MS as a complementary fragment screening method will be presented.

Introduction

Fragment based drug discovery (FBDD) is a recently validated approach that has confronted the challenge to identify more suitable small molecules as chemical starting points for drug discovery.^[1,2] The core elements of FBDD that are needed to enable success include (i) high quality fragment libraries and targets; (ii) a rapid and reliable method for screening fragments; and (iii) a strategy for evolving fragment hits into leads.^[3] Fragment screening is the focus of this review. The robust detection of fragment hits against a broad range of potential therapeutic targets presents a significant hurdle to the wider implementation and continued success of FBDD approaches. Fragment screening contrasts with high throughput screening (HTS) in almost every

aspect including screening library size and physicochemical profile, screening method and the close downstream association with structural chemistry.^[3,4] In contrast to HTS, where hit compounds are relatively potent (K_{d} s in the nM to μM range), the binding interactions of hit fragments tend to be considerably weaker (K_{d} s in the μM to mM range), so that fragment hits may not be as readily identified by classical biochemical screens as for HTS hits.^[5] A range of well established biophysical platforms for fragment based screening to detect and characterise fragment hits are however now in common use,^[6-8] most notably methods based on NMR,^[9] protein X-ray crystallography,^[10] surface plasmon resonance (SPR)^[11] and isothermal titration calorimetry (ITC). Technological advances in the hardware and software for these and other orthogonal techniques have considerably extended their capabilities as fragment screening options, however irrespective of each techniques particular advantages, each is applicable to a fraction of the potential therapeutic targets to which FBDD could conceivably contribute. This situation has arisen largely owing to the technical demands of the protein target requirements for running a fragment screening campaign.^[7] For example where there is insufficient protein quantity, where a protein fails to crystallise or fails to produce enough robust crystals or where a protein is not suitable for covalent modification and subsequent surface attachment, these methods are necessarily voided as viable options for the fragment screening campaign. There is consensus that for FBDD to further progress a suite of complementary fragment screening platforms that can comprehensively accommodate the diversity and demands of protein targets in future screening campaigns is required.^[6-8] Native state mass spectrometry (MS) has been recognised as a rapid, sensitive and high throughput method to directly investigate protein-ligand interactions for some time, including application to drug discovery,^[12-15] however there are few examples reporting this approach as a screening method to identify relevant protein-fragment interactions and articles describing FBDD generally give this method a cursory mention. In this paper an overview of the application of native state MS for small molecule screening will be presented, highlighting the key inherent attributes that are now sought after in fragment based screening to detect protein-fragment interactions. A summary of the published examples using MS for fragment screening will be described and reflection on the outlook for the future adoption and implementation of native state MS as a complementary fragment screening method will be presented.

Native state mass spectrometry to investigate protein-ligand noncovalent interactions

Electrospray ionisation mass spectrometry (ESI-MS) may be used to study native state proteins, protein-protein interactions and complexes of proteins with small molecules.^[13,16] ESI-MS analysis necessitates that the constituents of interest in a solution be transferred to the gas phase as fully desolvated ions. When screening for small molecules binding to a target protein the experimental parameters of the ESI-MS process need to be adjusted so that any specific noncovalent interactions between the small molecule and protein are maintained during the desolvation process and in the gas phase. The interested reader is directed to reviews that consolidate the extensive supporting literature on this application of native state MS.^[13,15,16]

The mass analyser of the mass spectrometer measures the mass-to-charge ratio (m/z) of the gas phase ions. When using ESI-MS for straightforward molecular weight determination of proteins, samples are usually prepared in a denaturing solvent (acetonitrile or methanol) that contains a few percent acetic or formic acid so that a very stable electrospray can be produced.^[17] Under these conditions the protein is denatured and many of the amino acid side chains are no longer concealed from the bulk solvent. The trace acid added to the sample ensures protonation of the exposed basic residues which in turn maximises the signal obtained from the protein (when running the mass spectrometer in positive ion mode). The ESI mass spectrum for such a denatured protein (carbonic anhydrase II, MW ~29 kDa) is shown in Figure 1a. This spectrum comprises an envelope of charge states, from approximately +35 to +20, that correspond to the broad family of differently protonated molecular species present in solution. In contrast, when using ESI-MS for native state protein studies, samples are prepared in aqueous solution with a volatile buffer (eg. ammonium carbonate or ammonium acetate) where the ionic strength is capable of maintaining the native conformation as well as specific noncovalent interactions thereof. The importance of these sample preparation considerations cannot be overstated and are probably the single most common reason why in the hands of the inexperienced MS operator experiments with native state proteins fail.^[17] Native state ESI-MS can be performed using either positive ion mode or negative ion mode. The negative ion mode ESI mass spectrum for carbonic anhydrase II acquired from native state conditions is shown in Figure 1b. Compared with the extensive charge state distribution that is observed under denaturing conditions just three charge

states are now observed (-10, -9, and -8). This narrow charge state envelope (low charge states and few charge states) indicates that the protein is compact and tightly folded with many of the acidic and basic side chains involved either in strong hydrogen bonding interactions (that help maintain the native state protein structure)^[17-20] or otherwise concealed from charging by their position in the protein structure. As reduced charging of proteins using native state ESI-MS results in ions with higher m/z values this imposes a requirement for mass analysers with extended m/z ranges.^[16] Time-of-flight (TOF) mass analysers theoretically have an unlimited m/z range, with quadrupole-TOF hybrid instruments also offering high sensitivity, specificity and rapid analysis.^[16] Fourier transform ion cyclotron resonance (FT-ICR) MS offers high mass range and ultra-high resolution. Together, TOF and FT-ICR mass spectrometers when coupled with ESI are the instruments of choice for the analysis of high mass proteins under native state conditions.

Protein function is very closely associated with protein structure and conformation, hence when using ESI-MS for the direct detection of fragment hits the experiment should be acquired under native state conditions. The ESI mass spectrum for carbonic anhydrase II acquired from native state solution conditions in the presence of a set of five small molecules that bind specifically to this protein is shown in Figure 1c. The noncovalent protein-small molecule complexes are observed in this spectrum together with the unbound protein. The noncovalent complexes are at a higher m/z values than the m/z value for the unbound protein, the difference between the complex and the unbound protein m/z values (i.e. $\Delta m/z$) when multiplied by the charge state (z) gives directly the molecular weight (MW) of the small molecule that is bound to the protein i.e. $MW_{\text{small molecule}} = \Delta m/z \times z$. In figure 1c the $\Delta m/z$ values for the five noncovalent complexes when multiplied by z ($= 9$) provides the five individual $MW_{\text{small molecule}}$ values (404, 418, 446, 460 and 493 Da). If screening fragments as cocktails the experimental protocol and data analysis is as just described. The hit fragment(s) may be identified against any background of non-hits, provided that the fragments in the cocktail each have a different molecular weight, with the mass of every fragment then serving as a unique detection 'label'.^[13] The determination of MW_{fragment} is thus an information rich parameter derived from the protein-fragment hit combination, as it reveals not just the presence of a fragment hit but also the identity of the fragment. If screening fragment libraries as singletons the experimental protocol and data analysis are even more straightforward.

Figure 1. ESI-MS mass spectra of carbonic anhydrase II (MW ~29 kDa); (a) under acidic denaturing conditions, (b) under native state conditions (10 mM NH₄OAc solution, 1% DMSO), and (c) under native state conditions (10 mM NH₄OAc solution, 1% DMSO) in the presence of five specific ligands. The MW for each ligand (= ligand identity) can be determined using the relationship $MW_{\text{ligand}} = \Delta m/z \times z$.

(a)

(b)

(c)

The binding affinity between a protein and small molecule is a combined function of the binding enthalpy and the binding entropy.^[21] A knowledge of the differing contribution of enthalpic and entropic interactions upon transfer of noncovalent protein-ligand complexes into the gas phase using ESI-MS is continuing to build, although it is not yet fully understood.^[22,23] Enthalpic interactions, dependent on electrostatic and polar interplay, include hydrogen bonds and Van der Waals interactions and are maximised by an optimal geometric fit between the small molecule and target protein. Enthalpic interactions survive transfer from solution to the gas phase, and are even strengthened in the gas phase.^[22,23] In contrast protein-ligand interactions, dependent primarily on the hydrophobic effect, are weakened in the gas phase to the extent that hydrophobic interactions may not survive transfer from solution to the gas phase.^[23] When a mixture of polar and hydrophobic interactions define the solution phase protein-ligand binding recognition, the noncovalent complex is likely to be retained and observed in the gas phase owing to the polar component of the interaction.^[22,23] It has been demonstrated that for improved outcomes in drug discovery that it is an advantage to start from enthalpically-driven leads, as compounds formed by optimising entropy or hydrophobic interactions often result in poorer drug development prospects and increased likelihood of attrition.^[21] When studying noncovalent complexes by ESI-MS, it is known that modulating the ionic strength or the addition of co-solvents to the aqueous buffer will affect the hydrophobic effect in solution.^[24] As the screening of fragments using ESI-MS may have a bias towards detecting fragments that bind via electrostatic interactions, and bias against those that are predominantly stabilised by hydrophobic interactions it is conceivable that the implications of native state ESI-MS, by favouring the detection of enthalpically driven binding fragments, may be the identification of preferred fragment candidates for follow up development efforts via medicinal chemistry.

Fragment libraries are often prepared and stored as high concentration stock solutions in dimethylsulfoxide (DMSO). For fragment screening by ESI-MS an understanding of the impact of the presence of DMSO as co-solvent on the electrospray process is needed. DMSO has a higher standard boiling point than water (189 °C compared to 100 °C), a lower vapour pressure than water and does not form an azeotrope with water.^[25] During transfer to the gas phase the addition of DMSO to the aqueous protein solution results in (i) preferential evaporation of water from the ESI droplets and enrichment in DMSO, and (ii) an increase in the lifetime of the ESI

droplets causing droplet heating.^[25,26] As both high DMSO concentration and heating act to denature a protein it is possible that in the presence of DMSO as a co-solvent proteins could undergo conformational changes during the ESI process resulting in increased charging of the protein.^[26] It has been shown that DMSO mediated protein unfolding occurs at around a 3-fold to 5-fold lower DMSO concentration during ESI than it does for protein unfolding in solution.^[26] At a level of DMSO that is more likely in fragment screening however (eg. <2% DMSO) a decrease in the average charge of proteins has been observed, indicative of minor compaction of the native protein structure rather than denaturation. Figure 2 shows the nanoESI mass spectra for hen egg white lysozyme (MW = 14.3 kDa) in water at 0%, 2% and 50% DMSO as well as the relationship between protein structure and %DMSO over the full range of 0-100 %DMSO.^[26] The ESI-MS findings with DMSO in Figure 2 were consistent with protein structural information provided from the same samples using near UV-CD spectroscopy and solution hydrogen/deuterium exchange MS.^[26] While protein unfolding during ESI-MS likely occurs at concentrations of DMSO well above those used for fragment screening, control experiments with the target protein that titrate against any employed co-solvent (DMSO or other) should be carried out to establish a baseline for any impact the co-solvent may have on the electrospray process prior to eventual fragment screening using native state ESI-MS.

Figure 2. NanoESI mass spectra of hen egg white lysozyme (MW 14.3 kDa) in water solutions containing (a) 0%, (b) 2%, and (c) 50% DMSO; (d) the average charge, calculated as the abundance-weighted average of the observed charge states, as a function of DMSO concentration. Reprinted with permission from Sterling, H.J. Protein Conformation and Supercharging with DMSO from Aqueous Solution. *J. Am. Soc. Mass Spectrom.* **2011**, 22, 1178. Copyright 2011 Springer.

With regard to detection, mass spectrometry has both very high sensitivity and a wide dynamic range. Previous studies have shown when using native state ESI-MS that it is possible to determine K_d s for noncovalent complexes of proteins in the range 10 nM to 1 mM,^[13,27] this encompasses the K_d s that are typical for weak binding fragments (K_d s of μ M to mM) so in principle the specific, yet weak, protein-fragment complexes are amenable to observation using native state MS. Modern ESI-MS instruments have sufficient sensitivity to detect proteins present at sub- μ M concentrations. The most common type of ESI sources use a pressurised pneumatic assist gas to assist in the formation of fine droplets by pneumatic nebulisation, and operate with flow rates around 1-2 μ L/min. Thus, a screen may be carried out with as little as 1 μ L of a solution at a protein concentration of 0.1 μ M, meaning that less than nanomole amounts of protein are sufficient to screen 1,000 fragments as singletons. If automated sample handling is used the screening throughput is further enhanced, and if also coupled to automated data analysis that provides a simple yes/no output then 1,000 fragments may be screened as singletons and the data processed in less than 24 hours. The identified hit fragments may be followed up by serial dilution experiments to measure a K_d value and determine the binding affinity if desired.^[16]

Nanoelectrospray (nanoESI) sources operate at lower sample flow rates (10-200 nL/min) than standard ESI sources. When using nanoESI smaller sample droplets are generated which improves the signal intensity of the protein-ligand noncovalent complexes while also reducing protein consumption up to 100-fold compared to standard ESI flow rates. NanoESI is historically associated with extensive manual work involving fine spray needles that produced poor signal stability and so was avoided by many MS operators. Recently a fabricated microfluidics chip-based nanoelectrospray source has been developed. The chip comprises an array of single use nanoelectrospray nozzles. This design eliminates sample-to-sample carryover effects as the spray is produced directly from a nozzle on each sample well of the chip.^[28,29] This automated device has greatly improved the ease-of-use and overall performance of nanoESI. There is a software option that allows operators the control to mix protein and ligand quantitatively prior to infusion to the chip to facilitate fully automated K_d determinations. The application of nanoESI to screening fragments appears in many ways ideal to support rapid analysis compared to standard ESI, with improved preservation and detection of noncovalent interactions and calculation of K_d s while allowing screens to be carried out with sub-picomole quantities of target protein.^[30] The use of ESI-MS to measure femtomole quantities of proteins (MW 1 to >100 kDa) in individual pico- to nanoliter droplets generated in microfluidic devices has very recently been described.^[31] The method provides a further reduction in protein quantity and significant increase in the rate of sample acquisition. The prospect of fragment screening from individual droplets may become possible with continued development of the integration of microfluidics with ESI-MS.

High resolution FT-ICR ESI-MS has been used to simultaneously screen multiple compounds against a protein target.^[18,19,32] ESI-MS has been extended to structure activity relationships (SAR), with SAR by MS successfully undertaken with RNA targets and small molecules.^[33,34] ESI-MS has also been used as a highly effective screen for in situ fragment based approaches, including dynamic combinatorial chemistry (DCC) and tethering, where fragments are linked in the presence of the protein target (effectively selected and amplified = molecular evolution) and the resulting 'best' linked fragment combination are identified owing to their unique mass.^[19,35-37] Membrane proteins are important therapeutic targets that are difficult to study in the context of FBDD. ESI-MS has undergone recent developments allowing for the transmission and study of

membrane proteins and their noncovalent complexes in the gas phase,^[38] although in its infancy, this may in the future address a key limitation of current FBDD screening methods.

Recent applications of fragment screening using native state mass spectrometry

To date there are few applications reporting the use of native state mass spectrometry as a direct approach for fragment screening against protein targets. An overview of the reported applications identified from a literature search for the period 2009 onwards is provided below.

Example 1. A detailed study reporting the development, reproducibility and validation of a fully automated fragment/small molecule screening protocol using nanoESI-MS was published by Crump, Crosby and co-workers in 2012.^[39] This study emphasised the practical considerations needed to routinely detect ligand binding using nanoESI-MS such as test protein sample preparation, spectrometer parameters and automation. Using hen egg white lysozyme (HEWL, MW = 14.3 kDa) as a test protein the ammonium acetate buffer concentration was optimised and the nanoESI conditions (voltages, temperatures, and pressures) iteratively tuned to maximise ion transmission of intact noncovalent complexes from solution in to the mass spectrometer. K_d values were determined with a series of known ligands for HEWL that represented a range of molecular weights (180, 221, 424 and 627 Da) and binding constants from μM to mM. A protein to ligand ratio of 2:1 proved optimum for K_d determination, limiting nonspecific binding while providing K_d values in good agreement with those obtained from the solution phase techniques of ITC, saturation transfer difference NMR (STD-NMR) as well as previously published values. Next, to evaluate the reproducibility of automation with both strong and weak binders a TriVersa NanoMate (Advion) robotic nanospray source was employed with samples of protein-ligand mixtures (5 μL) sprayed in to the mass spectrometer for 30 seconds. A low failure rate (~5%) to spray was observed, with failure attributed to blockage of the NanoMate chip nozzles. A fully automated analysis of 94 repeat experiments for each of two ligands (one strong and one weak binder) required 100 seconds per sample and took 6 h, this includes ~1 h for automated post-acquisition data processing. For each individual experiment both the signal intensity and the measured K_d value were recorded and although there was variation the values remained uniform and consistent with the earlier non-automated ESI-MS analysis.

With optimisation established on HEWL, nanoESI-MS screening was then carried out for a library of 157 phenylpyrazole-derived compounds (MW range 240-500 Da) against the anti-apoptotic Bcl-X_L protein. The phenylpyrazole scaffold is known to bind to Bcl-X_L so a high hit rate was expected and was observed (90%) when screening this targeted library. Of the 157 compounds tested, 66% were identified with K_d s <150 μ M, 18% with K_d s 150-300 μ M, 2% K_d s >300 μ M, while 9% were non-binders and 4% failed to spray. The full nanoESI-MS screen of 157 compounds was acquired at a rate of 100 seconds per sample, consumed 668 μ g of protein and ~44 μ g of ligand (2:1 ratio of ligand:protein), the speed and consumption values are competitive with techniques such as STD-NMR and ITC but greater than for other MS methods such as FT-ICR. The extraction of approximate K_d values (30–400 μ M) was shown to be viable using a rapid protocol that is much shorter than the full titration protocol used for K_d determination and allowed preliminary SAR to be determined for the targeted library. The nanoESI-MS screen was validated on a subset of 20 library compounds using ITC, STD-NMR and chemical shift perturbation by ¹H/¹⁵N heteronuclear single quantum coherence (HSQC) NMR, while there were some differences, the overall agreement in terms of the identification of hits and non-hits was good. The study confirmed that hits can both be confidently identified and distinguished nonbinding compounds and binding affinities ranked.

Example 2. Fragment screening by X-ray crystallography, native state mass spectrometry and ITC to identify fragment inhibitors of human phenylethanolamine *N*-methyltransferase (hPNMT; MW = 30.7 kDa) from a 384-member fragment library (MW_{average} = 142 Da) was described by Drinkwater, Martin and colleagues in 2010.^[40] hPNMT catalyses the final step of adrenaline biosynthesis, producing *S*-adenosyl-L-homocysteine (AdoHyc) as byproduct. This enzyme has a potential role in a wide range of disease-relevant processes. The commercial fragment library was provided as both singletons (200 mM in DMSO) and as cocktails of four fragments each (50 mM in DMSO). Library screening using X-ray crystallography was carried out blind by conducting a 15 minute soak of the 96 fragment cocktails with crystals of hPNMT containing AdoHyc bound to the cofactor binding site. A total of 16 soaks gave electron density suggestive of hits, the chemical identity of two of these hits was obvious while a deconvolution step was required on the remaining 14 due to ambiguous electron density present in the active site. For deconvolution 51 follow up datasets were measured in addition to the first round of cocktail soaks. Ten of the

initially identified hits were confirmed and characterised, while the remaining four were found to be false positives. In summary the protein X-ray crystallography primary screen identified 12 fragment hits (3.2% hit rate) as binding in the active site of hPNMT. Nine of these hits could be characterised by ITC. This is the first study to compare fragment based screening by X-ray crystallography and ESI-MS to evaluate if ESI-MS could identify the same hits as protein X-ray crystallography. High sensitivity FT-ICR ESI-MS was used on a subset of the fragment library and was able to identify the same hits as X-ray crystallography, as well as additional hit fragments. If automated the MS approach could have processed the 384-member fragment library in 24 h with 0.4 mg of hPNMT. In comparison the X-ray crystallography campaign took 2-3 months and used ~20 mg of protein. These findings point to significant advantages in combining ESI-MS as a durable, first-pass screen prior to X-ray crystallography. In this example the ESI-MS shortlist of fragments for subsequent characterisation by X-ray crystallography could in theory have reduced the number of crystal structures to be measured from ~150 to ~20, saving time and resources and increasing the overall accessibility of fragment screening, particularly for academic research labs. The paper reports very detailed experimental conditions for all three fragment screening methods, and the interested reader is directed to the original manuscript for further details.^[40]

Example 3. NovAliX, a company that supports the pharmaceutical industry outsourcing demands, has utilised an automated native state nanoESI-MS platform for fragment screening using two different approaches.^[41] In the initial approach a direct screen of a 350-member fragment library ($MW_{\text{average}} = 200$ Da) against heat shock protein 90 (Hsp90) using nanoESI-MS identified 40 hit fragments (12.5% hit rate). The best binding fragments were followed up using protein X-ray crystallography and binding interactions with Hsp90 ATP binding site characterised. In a second approach in silico docking of a virtual fragment library (22,000 fragments; $MW < 250$ Da) in to the Hsp90 ATP binding site was conducted. From the docking results 60 fragments were selected for follow up native state ESI-MS screening. A hit rate of 50% was obtained with the hits similarly moved forward to protein X-ray crystallography as a secondary screen. In both campaigns the fragment binding constants for hit fragments were reported to be in the high μM range and were able to prioritise the fragments that progressed to protein X-ray crystallography.

Example 4. A fragment-based drug discovery strategy that targeted discovery of non-peptide antagonists of protein-protein interactions involved in apoptosis was reported in 2009 by Moore and colleagues from Pfizer.^[42] The X-linked inhibitor of apoptosis protein (XIAP) is the most potent inhibitor of protein-protein interactions between caspase enzymes involved in apoptosis, and a small molecule that has this capability was sought after in this screening campaign. The Pfizer approach utilised a screening cascade against a ~9,000 member fragment library (150–300 Da) commencing with STD-NMR as the primary screen. A total of 830 weak binding fragments were identified and native state ESI-MS (with automated data processing) employed as the secondary screen against the separate component proteins rather than the protein-protein complex. This approach contrasts with those described above in that mass spectrometry was implemented for a different purpose, specifically to localise the fragment binding to a particular protein partner of the protein-protein complex. Both ITC and SPR were employed for follow up binding studies. A main outcome of this study was the ability to define and identify nonspecific binding interactions from specific interactions using mass spectrometry while again highlighting the advantage of minimal protein consumption alongside the ability to conserve weaker fragment-protein complexes.

Summary and outlook

Some general comparisons of the features of popular fragment screening methods employing NMR, X-ray crystallography, SPR and ITC and to a lesser extent MS have been described.^[2,6,7] There is as yet no definitive study that has systematically compared the attributes of the many different fragment screening methods when using an identical protein and fragment library combination. All fragment screening methods have distinct advantages and limitations associated with sensitivity, throughput, compatibility with screening fragment mixtures and the quantity of protein or fragment consumed and there is not an established consensus as to the best way to implement a fragment screening campaign. There is a clear distinction between methods that provide structural detail (X-ray crystallography and protein-observed NMR) and those that provide a ‘yes/no’ output of fragment binding. Structural methods require larger protein quantities and greater experimental time such that throughput for these methods is relatively low compared to orthogonal ‘yes/no’ output screening methods. The use of small quantities of material may be important owing to technical feasibility or prohibitive cost, while the advantages

of higher throughput are numerous. A primary screen that can provide a shortlist of prioritised fragment candidates for detailed structural interrogation with a structural method operating as a secondary screen is one way to minimise the analysis of non-hit fragments to save time and preserve reagents, without compromising findings.

The detection of specific noncovalent complexes between proteins and fragments by native state MS is in general possible, although caveats around maintenance of a stable complex in the gas phase do exist as described herein. The inherent strengths of this label-free method include high throughput, high sensitivity, wide dynamic range, compatibility with screening either individual fragments or fragment mixtures and applicability to a large footprint of therapeutic proteins making it a potentially excellent choice for a primary screen. Nonspecific binding during the electrospray process may lead to false positives and control experiments to identify these have been developed.^[43,44] The workflow of a fragment screening campaign that employs ESI-MS as a primary screen followed by an orthogonal secondary screen has many advantages that outweigh the impact of false positives. The recent examples of ESI-MS based fragment screening presented here provide some evidence to support confidence in the implementation of MS as a complementary fragment screening method. The future adoption of ESI-MS as a valued screening method for FBDD remains to be seen as it is yet to be put in to large scale practice, however the successes so far, together with a healthy respect for the potential limitations of the method suggest that it is arguably an area that both the private sector and academia are unlikely to ignore given the tremendous potential and the wide availability of increasingly high performance easy to use mass spectrometers. It is expected that continued development and increased uptake of the nanoESI-MS and droplet ESI-MS methods will also see the costs associated with microfluidic devices become less prohibitive to wider spread implementation, particularly in an academic setting.

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