Emerging Chromatography-Free High-Throughput Mass Spectrometry Technologies for Generating Hits and Leads

Fan Pu, Nathaniel L. Elsen,* and Jon D. Williams*

Cite This: *ACS Med. Chem. Lett.* 2020, 11, 2108–2113 Read Online

ACCESS |

Metrics & More Article Recommendations

ABSTRACT: Mass spectrometry (MS) detection can offer unmatched selectivity and sensitivity. The use of MS without chromatography greatly increases the throughput, making it suitable for high throughput screening. However, the trade-offs of direct MS detection need to be carefully evaluated along with the development of novel strategies to ensure successful implementation. In this review, we will discuss the pros and cons of chromatography-free MS and discuss some of the currently used and future technologies being investigated to enable high-throughput MS.

KEYWORDS: Mass spectrometry, ionization methods, high-throughput screening, drug discovery

Most high-throughput screening (HTS) campaigns for lead identification use optical detection methods such as fluorescence or luminescence as an assay readout. Optical detection technologies are suitably fast methods for assay readout to enable the completion of an HTS campaign of a one to three million compound set in 1–3 months. However, these widely adopted technologies are not without limitations. Assay development can be challenging, as the biological targets of interest rarely contain the proper physicochemical properties for direct optical detection. Fluorescence or luminescence generating/quenching mechanisms need to be artificially generated for each assay by addition of the appropriate labels or tags. In rare cases, this is simply not possible, making the target unscreenable. However, in the majority of cases that are successful, this still leads to some significant disadvantages including potential for labels and tags to interfere with target biology,^{1,2} introduction of coupling reactions that have to be optimized and controlled for, and optical interference from screening compounds. Each of these disadvantages could lead to detection-based false positives or negatives.³ If false readouts are not filtered out, significant time and wet chemistry resources can be lost during the lead generation phase. Mass spectrometry (MS), on the other hand, directly measures the mass-to-charge ratio of ions, thus providing unprecedented selectivity and in most cases high sensitivity. Assay development is usually straightforward for MS-based assays, as one does not rely on any labels or coupling reactions to generate an optical signal. This reduces time to begin screening, eliminates all detection reagent cost, and mostly avoids all detection-based false positives and negatives.⁴ Versatility is another important feature of MS-based assays because multiple analytes can be monitored simultaneously, enabling multiparametric quantitative analysis.

Historically the main drawback of using MS methods for HTS was throughput. Conventional hyphenated MS technologies such as liquid chromatography-MS (LC-MS) or gas chromatography-MS (GC-MS) usually take at least 30 s to 60 min to run a sample. This may not be an issue for relatively low throughput assays such as *in vitro* ADME/toxicology, DMPK, and biomarker translational efforts,⁵ but screening of large (1M+) compound libraries with chromatography-MS is impractical. To complete a 1 M compound screen in one month, the analysis speed per sample should be at least faster than 2.6 s/sample running continuously for 24 h, 7 days a week without instrument downtime. It is important to point out that MS analysis alone is fast on most commercial mass spectrometers. Depending on the type of mass analyzer and scan setting, the time required to collect a MS-spectrum could be anywhere from milliseconds to a second. A chromatography-free MS assay thus has the potential to rival fluorescence-based assays. We must point out that there are trade-offs when running MS without chromatography. Chromatography separates analytes based on their physical properties, resulting in greatly simplified mass spectra. A high throughput screening sample is a complex sample, consisting of analyte and matrix components such as salts, detergents, lipids, protein, small molecule compounds, *etc.*, some of which can severely suppress analyte ionization and detection. Even for chromatography-MS runs, these matrix effects occur, and proper sample preparation

Received: June 6, 2020

Accepted: September 21, 2020

Published: September 21, 2020

Table 1. Comparison of the High-Throughput MS Technologies

Technology	Direct Analysis	Theoretical Throughput (samples/day)	Limitations	Commercialized/Vendor
MALDI	No	$\sim 10^5$	Requires matrix treatment; incompatible with salt and detergent; ionization in vacuum	Yes/Bruker
SAMDI	No	$\sim 10^5$	Requires chemical modification to capture analytes and matrix treatment; ionization in vacuum	Yes/SAMDI Tech
RapidFire	No	$\sim 10^4$	Sample preparation may be necessary; lower throughput compared with other technologies	Yes/Agilent
DESI	Yes	$\sim 10^5$	Subject to matrix effect; sensitivity and reproducibility for quantitation	Yes/Waters ^a
IR-MALDESI	Yes	$\sim 10^5$	Subject to matrix effect; sensitivity and reproducibility for quantitation; requires water in sample	No
ADE	Yes	$\sim 10^5$	Subject to matrix effect; requires homogeneous liquid sample, may not be suitable for direct cell/tissue analysis	Yes/Sciex
Droplet Microfluidics	Yes	$>10^5$	Complex workflow, need encoding to track compound information	No

^aWaters DESI system is designed for MS imaging.

is usually necessary to optimize sensitivity. However, sample preparation can also become tedious and time-consuming and is preferably avoided.

The goal of this review is to provide the readers with an overall picture of emerging chromatography-free MS technologies and their use in hit and lead discovery. Matrix-assisted desorption/ionization (MALDI) MS and RapidFire MS are well-established methods for HTS and have been demonstrated by numerous publications. Ambient MS technologies such as desorption electrospray ionization (DESI), infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI), and acoustic droplet ejection (ADE) have been increasingly reported for high-throughput assays. Droplet microfluidics as a promising direction for ultrahigh-throughput MS will be discussed. Key features of the technologies are summarized in Table 1.

MATRIX-ASSISTED LASER DESORPTION/IONIZATION

MALDI is an ionization method commonly used for MS imaging.⁶ In MALDI, a laser is used to deposit energy to a matrix that cocrystallized with the sample, which helps the desorption and ionization of the analytes. MALDI-TOF (time-of-flight) is a well-established platform for performing high throughput MALDI analysis; the throughput can reach <1 s/sample.^{7,8} Fully automated execution of MALDI biochemical assays on three distinct workstations could yield a throughput of ca. 60 000 samples per day.⁹ On the downside, MALDI-TOF experiments are often very sensitive to ion suppression from common buffer components and require an extra step to spot samples as well as matrix onto specialized target plates, which means biochemical reactions have to be performed off-line in buffers that may be selected more for MALDI than biological compatibility. This target plate preparation process is not an easy task as it requires multiple rounds of dispensing, washing, and drying. Automation of the spotting process could help bridge the gap, as demonstrated by Winter and co-workers using an in-house-built spotting station.⁸

To simplify sample preparation, while maintaining high-throughput, a novel strategy called self-assembled monolayer desorption ionization (SAMDI) was coupled with MALDI analysis. Substrates are chemically immobilized onto self-assembled monolayers so sample cleanup and matrix treatment can be performed without losing analytes. The analytes are released upon laser ablation and ionized for MS analysis. SAMDI has been reported for multiple HTS applications and

has been demonstrated for cell-based assays.^{10–14} This coupling effectively overcomes suppression from non-MALDI compatible buffers but does not avoid the inherent drawbacks of conventional MALDI, such as matrix application and ionization under vacuum.

RAPIDFIRE

As an alternative to conventional chromatography, solid phase extraction (SPE) was explored to realize rapid enrichment and cleanup of samples. SPE works on a similar principle to that of column chromatography: sorbent materials are packed in a syringe and several washes pass through the sorbent bed to load analytes, cleanup sample, and elute analytes. The successful implementation is an Agilent automated sampling system, the RapidFire 365 High-Throughput Mass Spectrometry System (RapidFire). In this system, samples are aspirated from 96- or 384-well plates, injected into SPE for analyte enrichment and sample cleanup, and then eluted with organic solvents for direct injection into the mass spectrometer. Analytes are ionized via electrospray ionization (ESI, sometimes referred to as direct injection/infusion when it is not coupled with chromatography). ESI is more suitable for small molecules than MALDI, and most LC-MS experiments utilize ESI as an ion source. RapidFire can handle up to 63 plates unattended (or >20 000 injections) and has a typical throughput of 8 s/sample. This system is well suited for medium-throughput screens, where typical screen sizes are on the order of ~ 100 000 compounds.^{15–17} Nonconventional use of the system is possible with hardware modifications, entirely bypassing the time limiting SPE separation step and direct injecting samples into the MS. This modification boosts the cycle time to 2.5 s/sample.¹⁸ A detailed review of the RapidFire can be found elsewhere.¹⁹ It should be noted that even with the rapid SPE, sample cleanup may still be necessary to prevent system clogging. Also, careful method development is required to ensure good data quality. RapidFire throughput is more suited for a lower throughput scenario such as hit confirmation, rather than a primary full deck screen.

DESORPTION-BASED AMBIENT IONIZATION

DESI,²⁰ together with direct analysis in real time,²¹ initiated a new field of MS research called ambient MS. These unconventional ionization methods require no sample preparation and are carried out in an ambient environment,²² both highly desired features for high throughput MS analysis.

In a typical DESI experiment, a nitrogen gas assisted electrospray jet is sprayed onto the sample surface at an optimized angle, which picks up and ionizes the sample that then gets aspirated into the MS.^{20,23} The noncontact feature plus ionization of samples in their native form makes it a popular platform for MS imaging of biological samples.²⁴ DESI has been implemented to accelerate chemical reactions²³ and reaction monitoring.²⁵ Wlekinski and co-workers performed accelerated droplet reactions on a PTFE membrane in a 6144 array, where analysis speed up to ca. 6000 reactions per hour was achieved.²³ Sawicki et al. have recently reported implementing DESI for high throughput reaction screening, providing assessment of such technology from an industry perspective. A speed of below 3 s/sample was readily achieved, representing a 25-fold improvement compared to UPLC-MS.²⁵ Recently, Cooks and co-workers have reported an enzymatic assay proof-of-concept study using DESI. The data suggest an effective analysis time of 0.3 s/sample could be achieved.²⁶ Waters acquired the exclusive rights for DESI from Prosolia in 2018, and it will be interesting to see future progress with this technology.

IR-MALDESI is another emerging technology suitable for high throughput analysis. The name suggests it is a combination of MALDI and ESI, but the ionization mechanism is closer to that of ESI.²⁷ In IR-MALDESI, the laser does not ionize the sample, rather it serves as a sampling method prior to the ionization event (Figure 1). The IR laser

Figure 1. Infrared matrix-assisted desorption electrospray ionization.²⁹ Reprinted with permission from Nazari, M.; Ekelof, M.; Khodjaniyazova, S.; Elsen, N. L.; Williams, J. D.; Muddiman, D. C. Direct screening of enzyme activity using infrared matrix-assisted laser desorption electrospray ionization. *Rapid Commun Mass Spectrom.* 2017, 31 (22), 1868–1874. Copyright 2017 Wiley.

deposits energy to the water-containing sample, where water acts as the matrix that absorbs this energy and generates a neutral plume. These neutral species collide with the charged ESI plume, incorporate into the droplets, and are then ionized through the typical ESI mechanism. Because only a small portion of sample is sampled each time, this effectively dilutes the sample and ion suppression is less severe compared to direct injection. Initially, charged ions such as salts and detergents are also excluded from the evaporating droplets by charge repulsion. Ion mobility separation could also be integrated in the IR-MALDESI MS system to further reduce ion suppression and increase molecular coverage.²⁸ The

obvious limitation is that this ionization mechanism requires water or ice in the sample to absorb IR energy. However, this may not be a significant issue for biological screenings since water is typically present. Our team and collaborators have previously demonstrated the potential of IR-MALDESI for biochemical HTS using the isocitrate dehydrogenase 1 enzymatic reaction as an example.²⁹ A rate of 0.5 s/sample with multiple scans per well was reported.

A common drawback for ambient ionization methods (DESI and IR-MALDESI) is their susceptibility to matrix effects. Unfortunately, this usually means that the sensitivity of an ambient ionization method is inferior to that of a well-developed LC-MS method, which could render applications that require measurement of low concentration analytes in complex samples challenging for ambient MS. Another complication is the higher level of fluctuation in detected signals, which could be especially problematic for a quantitative experiment. Quantification can be improved in ambient MS by the incorporation of an internal standard (preferably a stable isotope labeled internal standard).³⁰ This strategy is commonly deployed to improve quantitative performance for MS-based assays.

■ DROPLET-BASED AMBIENT IONIZATION

ADE is commonly used in HTS laboratories for plate preparation. In such devices (i.e., Labcyte Echo 555), an acoustic wave is applied to the bottom of a well to eject 2.5 nL of solution from a source plate to a target plate. In recent years, there has been a trend in coupling this sampling technology to a mass spectrometer. This new development was partially driven by the increased popularity of ambient MS, which enables the direct ionization of the ejected droplets. An early attempt in 2015 by Haarhoff and co-workers demonstrated using ADE to prepare a plate that was then subject to laser diode thermal desorption (LDTD) with atmospheric pressure chemical ionization.³¹ Shortly after, acoustic mist ionization (AMI) was reported by Sinclair and co-workers.³² AMI no longer requires transferring sample from sample plate to a target plate for ionization. Instead, ejected “mist” was directly ionized in a high voltage electric field and injected into the MS. The system was later improved and implemented in a high throughput biochemical screen of human histone deacetylase inhibitors. The authors were able to achieve a throughput of 100 000 samples per day and accomplished a screen campaign of ca. two million compounds in 7 weeks.³³

Continued efforts in coupling ADE to MS shifted the direction to the adoption of open-port interface (OPI) as a sample introduction method to MS (Figure 2).^{34,35} This technology is now commercially available from SCIEX under the name Echo-MS. A coaxial tube was aligned with the trajectory of the ejected droplet, where a vortex is formed at the tip of the OPI to admit droplets into the inner tubing. ESI occurs at the end of the transfer capillary, sending ions into MS. Over 1000-fold dilution during the transfer of ejected droplets was readily achieved, and theoretically this should significantly reduce ion suppression. The system is capable of a throughput as high as three samples per second. Applications of Echo-MS for high throughput biochemical screening and high-throughput reaction screening have recently been reported.^{34,35}

Droplet microfluidics is the ultimate liquid handling technology where tiny volumes (sub-nL to μ L) of liquid can be manipulated at high precision. The use of droplet

Figure 2. Acoustic droplet ejection-open port interface.³⁴ Reprinted with permission from DiRico, K. J.; Hua, W.; Liu, C.; Tucker, J. W.; Ratnayake, A. S.; Flanagan, M. E.; Troutman, M. D.; Noe, M. C.; Zhang, H. Ultra-High-Throughput Acoustic Droplet Ejection-Open Port Interface-Mass Spectrometry for Parallel Medicinal Chemistry. *ACS Med. Chem. Lett.* 2020. Copyright 2020 American Chemical Society.

microfluidics for high throughput analysis has been well-studied with fluorescence readout.³⁵ Interfacing droplet microfluidic devices with MS is an emerging direction with the benefit of dramatically increasing detectable molecular species without labeling. Successful coupling of droplet microfluidics to MS using both ESI and MALDI has been reported.^{37,38} Whereas MALDI still requires the deposition of droplets to a target plate, ESI is more popular due to it being amenable for online ionization. Sun and Kennedy demonstrated using simple direct droplet generation from a 384-well plate to form segmented flow for direct injection.³⁷ By multiplexing eight parallel droplet generation channels, the effective speed was *ca.* 1.9 s/sample, or 0.6 s/droplet. However, this implementation did not fully exploit the benefit of microfluidics HTS; only 70 nL was necessary to generate each droplet, yet the reaction volume was 10 μ L due to the use of a conventional microtiter plate. NanoESI, where the flow rate of ESI is reduced to 20–160 nL/m, was used for analysis of droplets to partially address this issue.³⁹ This coupling improved the signal stability, reduced the minimum analyzable droplet volume, and promoted the analysis speed up to 10 droplets/s. Feasibility of in-droplet incubation of an enzymatic reaction was also reported in this work. In a more sophisticated setup, MS measurement was used to trigger mass activated droplet sorting (MADS).⁴⁰ Different from fluorescence activated droplet sorting, droplets have to be split into two portions in MADS: one for MS detection and one for sorting and collection. The use of MS expands the detectable features to virtually any molecules that can be ionized with ESI.

To utilize unique properties of droplet microfluidics (*i.e.*, high speed, low reaction volume) for HTS, the ideal microfluidics platform for high throughput analysis will be fundamentally different/revolutionary in that it does not require the use of microtiter plates at all. Reactions should be incubated in the microchannels in isolated droplets rather than individual wells, and analysis of the droplets can be performed on demand. However, this could also be challenging to integrate into current HTS facilities, since all liquid handling robotics are designed for microtiter plates. One potential strategy is to reformat a conventional microtiter plate format library into a droplet library, although a compound encoding strategy, such as fluorescence coding, is required to track compounds.⁴¹ Successful implementation of such a droplet

library with a MS-based assay is yet to be seen, nor has a viable MS-compatible encoding strategy been developed. Microfluidics HTS experiments are more complicated than traditional HTS assays, but the potential is promising—screening up to 10⁸ samples per day (non-MS detection) is theoretically possible.³⁶

FUTURE DIRECTIONS

With robust and higher-performance mass spectrometers becoming more readily available, some of the challenges associated with complex matrices are now more manageable. Improved sensitivity of MS instrumentation means satisfactory performance can be achieved even in the presence of interfering species. Better mass resolution and mass measurement accuracy can improve identifying analyte peaks in a complex mass spectrum. Highly efficient tandem MS systems can select and fragment ions to further simplify mass spectra for analyte ions. Coupling ion mobility separation with MS could also provide extra separation power and an extra dimension of information, all without sacrificing analysis speed.⁴² With these innovations, novel sampling and ionization strategies have become the focus of recent research efforts in high throughput MS.

Researchers are addressing matrix effects in ambient MS with innovative, but simple, strategies. For example, coated blade spray could improve sensitivity by integration of rapid solid phase microextraction onto a stainless-steel spray device that holds and ionizes samples; with automation the analysis speed could reach under 15 s/sample.⁴³ In a recent study, a combination of multiplexed nanoESI and microelectrophoresis has enabled online cleanup of the sample, resulting in an improved signal-to-noise ratio with a throughput of *ca.* 2 s/sample.⁴⁴

DESI and IR-MALDESI share a common feature of the ability to sample directly from surfaces, which enables their utility for MS imaging, alongside MALDI. However, in contrast to MALDI, the ambient ionization of DESI and IR-MALDESI also allows them to directly sample from microtiter plates without addition of matrix. Coupling the inherent features of these ionization methods that allow ambient tissue imaging and direct sampling from microtiter plates presents intriguing possibilities of direct cell based HTS campaigns from either adherent or suspension cultures with no sample manipulations. Phenotypic assays that rely on gross changes to the lipid and metabolite composition should be feasible at a throughput that would allow a full deck screen.

In summary, we have discussed the pros and cons of chromatography-free MS as a powerful addition to the arsenal of high throughput analysis. ESI-based RapidFire and MALDI have been established as mature platforms over the years, whereas the advancement in MS instrumentation and invention of novel ionization methods have enabled the emergence of a variety of new platforms. In these new platforms, high throughput sampling and/or ionization could be achieved either by pneumatically assisted electrospray jet (DESI), acoustic droplet ejection, laser ablation (IR-MALDESI), or droplet microfluidics. All these emerging platforms have been demonstrated at analysis speeds below one second per sample, with droplet microfluidics having the potential to reach ultrahigh-throughput. We believe this highly innovative field has unlimited potential and will have a profound impact on modern drug discovery.

■ AUTHOR INFORMATION

Corresponding Authors

Nathaniel L. Elsen – Drug Discovery Science and Technology, AbbVie Inc., North Chicago, Illinois 60064, United States; Email: nathaniel.elsen@abbvie.com

Jon D. Williams – Drug Discovery Science and Technology, AbbVie Inc., North Chicago, Illinois 60064, United States; orcid.org/0000-0001-9873-4165; Email: jon.williams@abbvie.com

Author

Fan Pu – Drug Discovery Science and Technology, AbbVie Inc., North Chicago, Illinois 60064, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsmmedchemlett.0c00314>

Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge Sujatha M. Gopalakrishnan, Laura Miesbauer, and Nari Talaty and our reviewers for their critical insights. All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

■ REFERENCES

- (1) Beher, D.; Wu, J.; Cumine, S.; Kim, K. W.; Lu, S.-C.; Atangan, L.; Wang, M. Resveratrol is Not a Direct Activator of SIRT1 Enzyme Activity. *Chem. Biol. Drug Des.* **2009**, *74* (6), 619–624.
- (2) Pacholec, M.; Bleasdale, J. E.; Chruncyk, B.; Cunningham, D.; Flynn, D.; Garofalo, R. S.; Griffith, D.; Griffor, M.; Loulakis, P.; Pabst, B.; Qiu, X.; Stockman, B.; Thanabal, V.; Varghese, A.; Ward, J.; Withka, J.; Ahn, K. SRT1720, SRT2183, SRT1460, and Resveratrol Are Not Direct Activators of SIRT1. *J. Biol. Chem.* **2010**, *285* (11), 8340–8351.
- (3) Thorne, N.; Auld, D. S.; Inglese, J. Apparent activity in high-throughput screening: origins of compound-dependent assay interference. *Curr. Opin. Chem. Biol.* **2010**, *14* (3), 315–24.
- (4) Adam, G. C.; Meng, J.; Rizzo, J. M.; Amoss, A.; Lusen, J. W.; Patel, A.; Riley, D.; Hunt, R.; Zuck, P.; Johnson, E. N.; Uebele, V. N.; Hermes, J. D. Use of high-throughput mass spectrometry to reduce false positives in protease uHTS screens. *J. Biomol. Screening* **2015**, *20* (2), 212–22.
- (5) Kassel, D. B. Applications of high-throughput ADME in drug discovery. *Curr. Opin. Chem. Biol.* **2004**, *8* (3), 339–45.
- (6) Cornett, D. S.; Reyzer, M. L.; Chaurand, P.; Caprioli, R. M. MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. *Nat. Methods* **2007**, *4* (10), 828–33.
- (7) Haslam, C.; Hellicar, J.; Dunn, A.; Fuetterer, A.; Hardy, N.; Marshall, P.; Paape, R.; Pemberton, M.; Resemannand, A.; Leveridge, M. The Evolution of MALDI-TOF Mass Spectrometry toward Ultra-High-Throughput Screening: 1536-Well Format and Beyond. *J. Biomol. Screening* **2016**, *21* (2), 176–86.
- (8) Winter, M.; Ries, R.; Kleiner, C.; Bischoff, D.; Luippold, A. H.; Bretschneider, T.; Buttner, F. H. Automated MALDI Target Preparation Concept: Providing Ultra-High-Throughput Mass Spectrometry-Based Screening for Drug Discovery. *SLAS Technol.* **2019**, *24* (2), 209–221.
- (9) Simon, R. P.; Winter, M.; Kleiner, C.; Ries, R.; Schnapp, G.; Heimann, A.; Li, J.; Zuvela-Jelaska, L.; Bretschneider, T.; Luippold, A. H.; Reindl, W.; Bischoff, D.; Buttner, F. H. MALDI-TOF Mass Spectrometry-Based High-Throughput Screening for Inhibitors of the Cytosolic DNA Sensor cGAS. *SLAS Discov.* **2020**, *25* (4), 372–383.
- (10) Gurard-Levin, Z. A.; Scholle, M. D.; Eisenberg, A. H.; Mrksich, M. High-throughput screening of small molecule libraries using SAMDI mass spectrometry. *ACS Comb. Sci.* **2011**, *13* (4), 347–50.
- (11) Anderson, S. E.; Fahey, N. S.; Park, J.; O’Kane, P. T.; Mirkin, C. A.; Mrksich, M. A high-throughput SAMDI-mass spectrometry assay for isocitrate dehydrogenase 1. *Analyst* **2020**, *145* (11), 3899–3908.
- (12) Patel, K.; Sherrill, J.; Mrksich, M.; Scholle, M. D. Discovery of SIRT3 Inhibitors Using SAMDI Mass Spectrometry. *J. Biomol. Screening* **2015**, *20* (7), 842–8.
- (13) Berns, E. J.; Cabezas, M. D.; Mrksich, M. Cellular Assays with a Molecular Endpoint Measured by SAMDI Mass Spectrometry. *Small* **2016**, *12* (28), 3811–8.
- (14) Cabezas, M. D.; Mirkin, C. A.; Mrksich, M. Nanopatterned Extracellular Matrices Enable Cell-Based Assays with a Mass Spectrometric Readout. *Nano Lett.* **2017**, *17* (3), 1373–1377.
- (15) Langsdorf, E. F.; Malikzay, A.; Lamarr, W. A.; Daubaras, D.; Kravec, C.; Zhang, R.; Hart, R.; Monsma, F.; Black, T.; Ozbal, C. C.; Miesel, L.; Lunn, C. A. Screening for antibacterial inhibitors of the UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC) using a high-throughput mass spectrometry assay. *J. Biomol. Screening* **2010**, *15* (1), 52–61.
- (16) Hutchinson, S. E.; Leveridge, M. V.; Heathcote, M. L.; Francis, P.; Williams, L.; Gee, M.; Munoz-Muriedas, J.; Leavens, B.; Shillings, A.; Jones, E.; Homes, P.; Baddeley, S.; Chung, C. W.; Bridges, A.; Argyrou, A. Enabling lead discovery for histone lysine demethylases by high-throughput Rapid Fire mass spectrometry. *J. Biomol. Screening* **2012**, *17* (1), 39–48.
- (17) Leveridge, M.; Collier, L.; Edge, C.; Hardwicke, P.; Leavens, B.; Ratcliffe, S.; Rees, M.; Stasi, L. P.; Nadin, A.; Reith, A. D. A High-Throughput Screen to Identify LRRK2 Kinase Inhibitors for the Treatment of Parkinson’s Disease Using Rapid Fire Mass Spectrometry. *J. Biomol. Screening* **2016**, *21* (2), 145–55.
- (18) Bretschneider, T.; Ozbal, C.; Holstein, M.; Winter, M.; Buettner, F. H.; Thamm, S.; Bischoff, D.; Luippold, A. H. Rapid Fire BLAZE-Mode Is Boosting ESI-MS Toward High-Throughput-Screening. *SLAS Technol.* **2019**, *24* (4), 386–393.
- (19) Kempa, E. E.; Hollywood, K. A.; Smith, C. A.; Barran, P. E. High throughput screening of complex biological samples with mass spectrometry - from bulk measurements to single cell analysis. *Analyst* **2019**, *144* (3), 872–891.
- (20) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306* (5695), 471–3.
- (21) Cody, R. B.; Laramée, J. A.; Durst, H. D. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **2005**, *77* (8), 2297–302.
- (22) Cooks, R. G.; Ouyang, Z.; Takats, Z.; Wiseman, J. M. Detection Technologies. Ambient mass spectrometry. *Science* **2006**, *311* (5767), 1566–70.
- (23) Wlekinski, M.; Loren, B. P.; Ferreira, C. R.; Jaman, Z.; Avramova, L.; Sobreira, T. J. P.; Thompson, D. H.; Cooks, R. G. High throughput reaction screening using desorption electrospray ionization mass spectrometry. *Chem. Sci.* **2018**, *9* (6), 1647–1653.
- (24) Wu, C.; Dill, A. L.; Eberlin, L. S.; Cooks, R. G.; Ifa, D. R. Mass spectrometry imaging under ambient conditions. *Mass Spectrom. Rev.* **2013**, *32* (3), 218–43.
- (25) Sawicki, J. W.; Bogdan, A. R.; Searle, P. A.; Talaty, N.; Djuric, S. W. Rapid analytical characterization of high-throughput chemistry screens utilizing desorption electrospray ionization mass spectrometry. *Reaction Chemistry & Engineering* **2019**, *4* (9), 1589–1594.
- (26) Cooks, R. G.; Morato, N. M.; Holden, D. T. High Throughput Label-Free Enzymatic Assays using Desorption Electrospray Ionization Mass Spectrometry. *Angew. Chem., Int. Ed.* **2020**, DOI: 10.1002/anie.202009598.
- (27) Sampson, J. S.; Hawkrige, A. M.; Muddiman, D. C. Generation and detection of multiply-charged peptides and proteins

by matrix-assisted laser desorption electrospray ionization (MALDESI) Fourier transform ion cyclotron resonance mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2006**, *17* (12), 1712–6.

(28) Ekelof, M.; Dodds, J.; Khodjaniyazova, S.; Garrard, K. P.; Baker, E. S.; Muddiman, D. C. Coupling IR-MALDESI with Drift Tube Ion Mobility-Mass Spectrometry for High-Throughput Screening and Imaging Applications. *J. Am. Soc. Mass Spectrom.* **2020**, *31* (3), 642–650.

(29) Nazari, M.; Ekelof, M.; Khodjaniyazova, S.; Elsen, N. L.; Williams, J. D.; Muddiman, D. C. Direct screening of enzyme activity using infrared matrix-assisted laser desorption electrospray ionization. *Rapid Commun. Mass Spectrom.* **2017**, *31* (22), 1868–1874.

(30) Pu, F.; Chiang, S.; Zhang, W.; Ouyang, Z. Direct sampling mass spectrometry for clinical analysis. *Analyst* **2019**, *144* (4), 1034–1051.

(31) Haarhoff, Z.; Wagner, A.; Picard, P.; Drexler, D. M.; Zvyaga, T.; Shou, W. Coupling Laser Diode Thermal Desorption with Acoustic Sample Deposition to Improve Throughput of Mass Spectrometry-Based Screening. *J. Biomol. Screening* **2016**, *21* (2), 165–75.

(32) Sinclair, I.; Stearns, R.; Pringle, S.; Wingfield, J.; Datwani, S.; Hall, E.; Ghislain, L.; Majlof, L.; Bachman, M. Novel Acoustic Loading of a Mass Spectrometer: Toward Next-Generation High-Throughput MS Screening. *J. Lab Autom* **2016**, *21* (1), 19–26.

(33) Sinclair, I.; Bachman, M.; Addison, D.; Rohman, M.; Murray, D. C.; Davies, G.; Mouchet, E.; Tonge, M. E.; Stearns, R. G.; Ghislain, L.; Datwani, S. S.; Majlof, L.; Hall, E.; Jones, G. R.; Hoyes, E.; Olechno, J.; Ellson, R. N.; Barran, P. E.; Pringle, S. D.; Morris, M. R.; Wingfield, J. Acoustic Mist Ionization Platform for Direct and Contactless Ultrahigh-Throughput Mass Spectrometry Analysis of Liquid Samples. *Anal. Chem.* **2019**, *91* (6), 3790–3794.

(34) DiRico, K. J.; Hua, W.; Liu, C.; Tucker, J. W.; Ratnayake, A. S.; Flanagan, M. E.; Troutman, M. D.; Noe, M. C.; Zhang, H. Ultra-High-Throughput Acoustic Droplet Ejection-Open Port Interface-Mass Spectrometry for Parallel Medicinal Chemistry. *ACS Med. Chem. Lett.* **2020**, *11* (6), 1101–1110.

(35) Zhang, H.; Liu, C.; Hua, W.; Ghislain, L. P.; Liu, J.; Aschenbrenner, L.; Noell, S.; Dirico, K.; Lanyon, L. F.; Steppan, C. M.; Arnold, D. W.; Covey, T. R.; Datwani, S. S.; Troutman, M. D. Acoustic Ejection Mass Spectrometry for High-Throughput Analysis. *bioRxiv* **2020**, DOI: 10.1101/2020.01.28.923938.

(36) Guo, M. T.; Rotem, A.; Heyman, J. A.; Weitz, D. A. Droplet microfluidics for high-throughput biological assays. *Lab Chip* **2012**, *12* (12), 2146–55.

(37) Sun, S.; Kennedy, R. T. Droplet electrospray ionization mass spectrometry for high throughput screening for enzyme inhibitors. *Anal. Chem.* **2014**, *86* (18), 9309–14.

(38) Haidas, D.; Bachler, S.; Kohler, M.; Blank, L. M.; Zenobi, R.; Dittrich, P. S. Microfluidic Platform for Multimodal Analysis of Enzyme Secretion in Nanoliter Droplet Arrays. *Anal. Chem.* **2019**, *91* (3), 2066–2073.

(39) Steyer, D. J.; Kennedy, R. T. High-Throughput Nano-electrospray Ionization-Mass Spectrometry Analysis of Microfluidic Droplet Samples. *Anal. Chem.* **2019**, *91* (10), 6645–6651.

(40) Holland-Moritz, D. A.; Wismer, M. K.; Mann, B. F.; Farasat, I.; Devine, P.; Guetschow, E. D.; Mangion, I.; Welch, C. J.; Moore, J. C.; Sun, S.; Kennedy, R. T. Mass Activated Droplet Sorting (MADS) Enables High-Throughput Screening of Enzymatic Reactions at Nanoliter Scale. *Angew. Chem., Int. Ed.* **2020**, *59* (11), 4470–4477.

(41) Brouzes, E.; Medkova, M.; Savenelli, N.; Marran, D.; Twardowski, M.; Hutchison, J. B.; Rothberg, J. M.; Link, D. R.; Perrimon, N.; Samuels, M. L. Droplet microfluidic technology for single-cell high-throughput screening. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (34), 14195–200.

(42) Zhang, X.; Romm, M.; Zheng, X.; Zink, E. M.; Kim, Y. M.; Burnum-Johnson, K. E.; Orton, D. J.; Apffel, A.; Ibrahim, Y. M.; Monroe, M. E.; Moore, R. J.; Smith, J. N.; Ma, J.; Renslow, R. S.; Thomas, D. G.; Blackwell, A. E.; Swinford, G.; Sausen, J.; Kurulugama, R. T.; Eno, N.; Darland, E.; Stafford, G.; Fjeldsted, J.; Metz, T. O.; Teeguarden, J. G.; Smith, R. D.; Baker, E. S. SPE-IMS-MS: An automated platform for sub-sixty second surveillance of

endogenous metabolites and xenobiotics in biofluids. *Clin. Mass Spectrom* **2016**, *2*, 1–10.

(43) Kasperkiewicz, A.; Gomez-Rios, G. A.; Hein, D.; Pawliszyn, J. Breaching the 10 Second Barrier of Total Analysis Time for Complex Matrices via Automated Coated Blade Spray. *Anal. Chem.* **2019**, *91* (20), 13039–13046.

(44) Wei, Z.; Xie, Z.; Kuvelkar, R.; Shah, V.; Bateman, K.; McLaren, D. G.; Cooks, R. G. High-Throughput Bioassays using “Dip-and-Go” Multiplexed Electrospray Mass Spectrometry. *Angew. Chem., Int. Ed.* **2019**, *58* (49), 17594–17598.