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## Mechanisms of ionization and of chemical reactions in charged microdroplets

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Over the past decade the use of electrospray ionization mass spectrometry (ESI-MS) has been extended from the domain of chemical analysis to that of chemical synthesis. This development was spurred by the observation of reaction acceleration (by as much as  $10^6$  times compared to analogous bulk reactions) occurring in microdroplets, entities once thought merely to deliver ionized compounds to the mass analyzer. Interest in microdroplet synthesis is fueled by the fact that many reactions proceed rapidly without catalysts or pH adjustment. In this Perspective, we explore the basis for these features and propose that field ionization (FI) followed by chemical ionization (CI) occurs in water-containing microdroplets and generates reactive intermediates that account for their unique chemistry. The implications of this proposal extend to the mechanism of ESI itself and to applications of accelerated reactions in drug discovery, green synthesis, and prebiotic chemistry.

### Introduction

Microdroplet reactions are of intense interest, driven by the phenomenon of interfacial reaction acceleration. The topic has been thoroughly reviewed.<sup>1–3</sup> This field started with the observation of enhanced rates for ‘on water’ reactions<sup>4,5</sup> and was propelled by the observation of orders of magnitude increases in reaction rates for simple derivatizations in sprayed reaction

mixtures in organic solvents.<sup>6</sup> The synthetic potential of the experiment was underlined by the collection of products of Claisen–Schmidt and Pomeranz–Fritsch reactions, amongst others.<sup>7–9</sup>

Features widely agreed to be essential for reaction acceleration in microdroplets are the following: (i) reaction acceleration is an interfacial phenomenon,<sup>1–3,10,11</sup> as shown by the effects of microdroplet size and by direct experiments on single levitated droplets.<sup>12,13</sup> (ii) The earliest microdroplet experiments used organic solvents, but completely dry solvents do not exhibit acceleration.<sup>12,14–17</sup> Water thus appears to be essential for reaction acceleration. (iii) Reaction acceleration is driven by a strong interfacial electric field at the water/air interface.<sup>18–20</sup> (iv)

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Incomplete solvation of the reagents at the interface provides a significant energy advantage.<sup>2,21–26</sup>

Other features of accelerated reactions on which there is general agreement in the literature are these: (i) except in rate, the reaction products almost always parallel those of the corresponding bulk-phase reactions.<sup>27</sup> Correspondingly, many reactions that are slow in bulk occur more rapidly and under much milder conditions (e.g. ambient temperature, no catalyst) in microdroplets. (ii) The observed reactions have an extraordinarily wide scope. They include redox (in which interfacial water drives electron transfer), acid-base, condensation, hydrolysis, and various types of substitution reactions, amongst others.<sup>2</sup> (iii) Chemistry in water-containing microdroplets is remarkably 'green': there is no need for extraneous catalyst and reactions typically occur under ambient conditions. (iv) While often integrated with MS for real-time reaction monitoring, increasingly some investigators<sup>28,29</sup> are foregoing MS and simply collecting sprayed microdroplets, obtaining product in

quantities sufficient for characterization by NMR.<sup>16,30,31</sup> (v) Some features of the reactions are explained by interfacial superacidic or superbasic properties of microdroplets, for example the capture of CO<sub>2</sub> by amines.<sup>14</sup>

What is still needed is a coherent explanation for this assembly of empirical data. Such a model must account for enhanced reaction rates and the extreme diversity of the reactions observed. These features are best explained by the hypothesis that interfacial environments generate initial reactive chemical species that are responsible for this rich chemistry. The nature and formation of the initial reactive species is one subject of this Perspective.

## Proposed mechanism

In this section, we consider the question "what is the mechanism of microdroplet reaction acceleration?" We answer that it is based on two factors, partial solvation and strong interfacial electric fields, both previously suggested. Then we tackle the key remaining question concerning the nature of the primary reactive species. In our view, the primary oxidizing agent is H<sub>2</sub>O<sup>+</sup> with HO<sup>•</sup> being a secondary oxidizing agent. Similarly, the primary reducing agent is H<sub>2</sub>O<sup>•−</sup> or the solvated form, e<sup>−</sup>(aq.). These redox species react further to generate water-derived acid/base forms. This novel proposal<sup>132,33</sup> incorporates known properties of the electric fields in microdroplets to explain the diverse types of droplet reactivity.

### Key concepts

- (i) Reaction rate constants at the microdroplet interface are increased due to the destabilization of interfacial species by partial solvation. This effect is more important in the reagents than in the transition state, so increasing rate constants.<sup>2,21–23</sup>
- (ii) Microdroplet and related interfacial chemistry depends on the existence of a strong electric field at the interfaces of



Myles Quinn Edwards

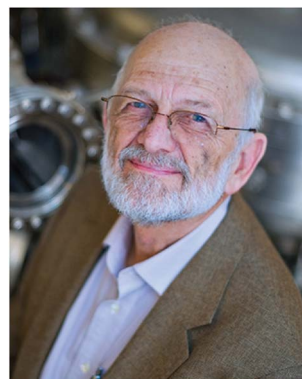
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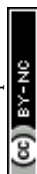
discovery. His work has resulted in several honors including the Eastman Fellowship in Analytical Chemistry, the American Chemical Society Division of Analytical Chemistry Fellowship, the Tomas B. Hirschfeld Scholar Award, and the Journal of Mass Spectrometry postgraduate award.



R. Graham Cooks

R. Graham Cooks is the Henry Bohn Hass Distinguished Professor of Chemistry at Purdue University. He is a pioneer in the field of mass spectrometry, and his research spans fundamental phenomena, instrumentation, and analytical applications. He is an elected member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the National Academy of Inventors. His work

has been recognized with numerous honors including the Mass Spectrometry and Analytical Chemistry awards of the American Chemical Society, the Boyle Medal and Centennial Prize of the Royal Society of Chemistry, and the Dreyfus Prize in Chemical Sciences.



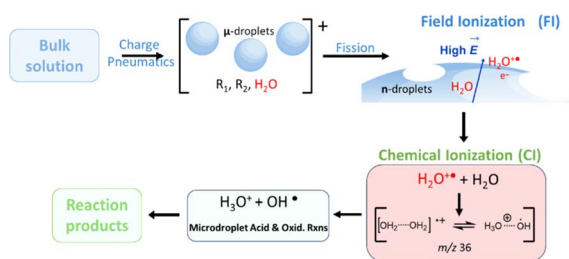


Fig. 1 Proposed combination of FI and CI processes that operate at the solution/gas interface of water-containing microdroplets.

microdroplets that contain water, as originally noted by Zare, Min, and Head-Gordon.<sup>18–20</sup> This electric field is intrinsic to the interface.

(iii) Field ionization (FI)<sup>34</sup> of water to create  $\text{H}_2\text{O}^{+\bullet}$  is suggested to be the primary step in the generation of reactive species that drive droplet reactions. The strong electric fields associated with atomic-scale structures ionizes adjacent molecules by electron tunnelling as in the FI technique of Beckey.<sup>34</sup> This technique was later modified, under the name field desorption, to include ionization of low-volatility compounds on the surfaces of nanomaterials.<sup>35</sup> FI of molecules gives molecular radical cations. These species then undergo ion/molecule reactions to give the observed protonated molecules.<sup>36,37</sup> We suggest that FI of water itself,<sup>38</sup> followed by secondary ‘self-protonation’ reactions, is the key initial step in microdroplet chemistry (Fig. 1).

‘Field-assisted’ ionization has been suggested to facilitate hydroxyl radical formation from hydroxide.<sup>39,40</sup> It has even been suggested<sup>41,42</sup> that the strong electric field is responsible for unpairing the water radical cation/anion pair ( $\text{H}_2\text{O}^{+\bullet}/\text{H}_2\text{O}^{-\bullet}$ ). However, the view that strong electric fields cause ionization by electron tunnelling at droplet interfaces is novel (presented at the spring 2025 ACS meeting and in the PhD thesis of one of the authors<sup>32,33</sup>).

### Field ionization explains microdroplet reactivity

The FI step generates the initial reactive species, the strong oxidizing reagent  $\text{H}_2\text{O}^{+\bullet}$ , that initiates the chemical transformations that are accelerated in microdroplets. FI is rapid and requires effectively zero activation energy, given that electron tunnelling is involved. In fact, the ready tunnelling of electrons in water is evident in surface science experiments,<sup>43</sup> such as scanning tunnelling microscopy in aqueous environments.<sup>44</sup> It has also been extensively modelled across thin water layers and empirically observed in water clusters, both cases with clear implications for water microdroplet interfaces.<sup>44–49</sup> These studies also show that electron tunnelling has a lower effective energy barrier in water than in vacuum (the latter being the scenario for Beckey’s early work with FI)<sup>47</sup> with the tunnelling probabilities heavily depending on the orientation of the water molecules as well as the magnitude of the electric field across the water layer.<sup>48</sup> These observations align with the known properties of the water-containing microdroplet surface.

Note that the electron released by the FI mechanism is captured by water at the interface and is the likely initial reducing agent in subsequent chemistry.<sup>50</sup> These two entities,  $\text{H}_2\text{O}^{+\bullet}$  and  $\text{e}^{-}(\text{aq.})$ , ensure the creation of a redox active region, primed for chemical reactions and for molecular ionization. Note that the water molecule that is field ionized might be in the vapor phase near the interface or be part of a small cluster or occur in a bubble within a microdroplet (recent computational efforts show that electrosprayed droplets take time to adopt a simple spherical morphology<sup>51</sup>).

The water radical cation can be stabilized by solvation<sup>52</sup> (if not generated directly as a solvated species), to give the water radical cation dimer ( $m/z$  36) and higher oligomers.<sup>36,38</sup> The water radical cation dimer is easily dissociated to give hydronium cation and hydroxy radical (eqn (1)). The second step in this reaction can be described as ‘self-chemical ionization’ or ‘self CI’, following early suggestions by Harrison and others in the chemical ionization literature.<sup>53</sup>



Ionization and reactivity in microdroplets are suggested to involve a combination of two of the oldest and best-known of the mass spectrometry ionization methods, FI and CI.<sup>54</sup> This statement is conveniently summarized: the electric field at the interfaces of aqueous microdroplets causes field ionization of water, followed by chemical ionization, and this results in (i) ionization of molecules for subsequent MS measurements as their gas phase ions and (ii) accelerated reactions between partially solvated ions and molecules for subsequent product collection or online MS product analysis.

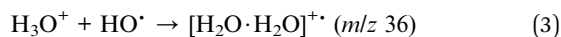
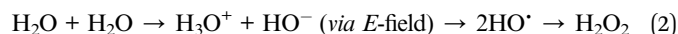
Not only does the FI mechanism explain how acid/base<sup>55</sup> and redox<sup>42,56</sup> chemistry can be performed in a single population<sup>57</sup> of microdroplets, but the products can produce the commonly detected oxidant  $\text{H}_2\text{O}_2$ , unifying seemingly disparate chemistry.<sup>58,59</sup> The water radical cation/anion pair has been invoked in microdroplet redox chemistry<sup>41,60,61</sup> and theorized based on fleeting charge transfer and quantum mechanical delocalization of electrons between water molecules.<sup>62</sup> FI produces the water radical cation directly.<sup>36,38,63</sup> Indeed, the electric field strengths determined computationally<sup>20,57</sup> and measured experimentally<sup>18,19</sup> at the air/water interface of nebulized—not electrosprayed—microdroplets are  $10^7$ – $10^8$   $\text{V cm}^{-1}$ , a sufficient electric field to drive FI.

### How this view differs from current explanations of reaction acceleration in microdroplets

The most significant point of distinction lies in the nature and origin of the oxidizing agent. The oxidation of the hydroxyl anion to create the hydroxy radical is often suggested in a process that is *ca.* 3 eV endothermic (eqn (2)). A related view is that water disproportionation is followed by electron transfer from interfacial  $\text{OH}^{-}$  to an acceptor molecule (in one case pyridine) to form the molecular anion radical and  $\text{OH}^{\bullet}$ , mediated by the inherent peripheral electric field.<sup>64</sup> Further, electron transfer between surface  $\text{H}^{+}$  and  $\text{OH}^{-}$  to form  $\text{H}^{\bullet}$  and  $\text{OH}^{\bullet}$  ( $2\text{OH}^{\bullet}$  then recombining to form  $\text{H}_2\text{O}_2$ )<sup>40</sup> becomes thermodynamically



favorable due to limited interfacial hydration<sup>65</sup> and electric field fluctuations.<sup>39</sup>



In our view, the water radical cation (and its hydrate, *m/z* 36) is the product of the primary chemistry shown in eqn (1), rather than being generated as a secondary product (eqn (3)) as suggested in papers by Zhang *et al.*<sup>66,67</sup> (note the opposite direction of the reactions shown in eqn (1) and (3)). Our suggestions in no way negate the importance of the hydroxyl radical or hydrogen peroxide as oxidizing agents, they do suggest a different route to their production. The most recent idea in this area, from Zare *et al.*, proposes photoionization—‘microlightning’ (via a spark)—instead of field-assisted oxidation (eqn (2)) as a route to hydroxyl radical formation.<sup>68</sup> Nevertheless, the energy requirements for FI (with electron tunnelling) is much less than that for any form of photoionization. Still, we do not dispute the contribution of a variety of processes to generate ions seen in ESI, including among others, electron ionization resulting from emission from negatively charged microdroplets.<sup>69</sup>

### Consequences for understanding of the ESI mechanism

The suggested FI model for accelerated reactions in microdroplets has consequences for the ionization step of ESI and related spray ionization methods, as well as for the subsequent reactions that may occur (and be deliberately enhanced by measures such as extending the droplet flight length). It would be illogical to argue the FI mechanism for reactions in microdroplets generated *via* spray ionization without implicating this process in conventional ESI ionization as well as newer ionization methods. For example, Stein and co-workers have constructed a nanopore source which also has appropriate field strength for FI.<sup>70–74</sup> In these cases, FI may exert as a yet unaccounted influence on coulomb explosion<sup>75</sup> and ion formation by ion evaporation.<sup>51,76–81</sup>

Beyond the initial ionization event, an interesting modification to current understanding of the ESI mechanism<sup>82</sup> is the contribution of FI to charge accumulation. Each FI event creates individual charge and, correspondingly, coulomb explosions with the formation of smaller droplets increasing with the number of these FI events. Provided charge losses do not offset this process, a feedback loop will rapidly generate the smallest possible units (very small droplets by some accounts<sup>83–85</sup>), leading to rapid and irreversible generation of the ions observed in MS through ion emission or the charge residue process.<sup>70</sup> We also speculate that the initial charge supplied to the earliest droplets might direct the electric field polarity (see below). The major source of charge supplied to the large and increasing surface area of the progeny droplets is provided by FI together with water autoionization.

### Access to microdroplet reactions

The choice of MS conditions, especially initial microdroplet size and the distance the droplets travel, determines whether an

‘analytical mass spectrum’ will be recorded. This spectrum shows ionized analytes but alternative MS conditions can be chosen to operate as a tool for synthesis. In the latter case, the recorded spectrum will not show the reagents but instead the products of accelerated reactions. For multistep transformations like the Hantzsch reaction,<sup>86</sup> increasing the distance the microdroplets travel increases the extent to which reagents are transformed into early then later intermediates and then into products. The occurrence of multistep reaction sequences indicates that reacting molecules have substantial lifetimes at the interface, while the common occurrence of condensation reactions means that the interface must be relatively dry under normal conditions, as in Fig. 1.

This sequence of processes continues with the distance of droplet travel until one of two limits is reached: either the process completes and final products are fully formed (for droplets of a given original size) or the ion source is reached and the reaction is quenched by rapid desolvation.

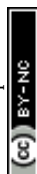
### *m/z* 36 and the extraordinary oxidizing power of pure water

The extraordinary oxidizing power of water—in the form of microdroplets—is responsible for oxidation products. Some of these results have been confirmed by tandem mass spectrometry and by comparison to commercial standards in the positive and, more rarely, negative ion modes. Examples include phosphonates,<sup>42</sup> sulfonates,<sup>41</sup> dinitrogen,<sup>87</sup> and common redox-active organic molecules.<sup>56,88–90</sup>

A report in 2021 (ref. 91) noted the presence of an ion at *m/z* 36, observed when pure water was ionized gently. These authors used both high resolution MS (HRMS) and characteristic chemical reactivity to argue that the responsible species was  $[\text{H}_2\text{O} \cdot \text{H}_2\text{O}]^{+\bullet}$  (*m/z* 36) and that it was present in two isomeric forms, the O–O bound dimer and the hydronium/hydroxy radical complex (a subject studied at length more than a decade earlier<sup>92–95</sup>). Zhang and Zare later made similar arguments.<sup>64,66</sup>

These and related conclusions regarding  $[\text{M} + 18]^+$  ions in mass spectra have been questioned by Williams<sup>96</sup> whose HRMS data indicate that the ions observed at *m/z* 36 correspond to hydrated ammonium cations,  $(\text{H}_2\text{O}) \cdot \text{NH}_4^+$ . Huanwen Chen *et al.*, however, have contested this argument with EPR<sup>97</sup> and HRMS of their own.<sup>98</sup> The issue remains unresolved,<sup>99</sup> although the possibility that the extraordinary reactivity of water microdroplets might involve a species of *m/z* 36 (and other hydrated forms of the water radical cation),<sup>93,95</sup> which is reactive and relatively unstable, is not nullified by the observation of an isobaric ion in similar—not identical—experiments.

To the general question of whether there is a water-derived species responsible for the unusual reactivity of water-containing microdroplets, the answer is unquestionably yes. If the examples of oxidation above are insufficient, then consider the striking reactivity with  $\text{N}_2$ . Song and Zare reported ammonia synthesis from  $\text{N}_2$  and water,<sup>100</sup> which was extended in catalyst-free experiments by the group of Huanwen Chen,<sup>87</sup> and then Fan *et al.*<sup>101</sup> Both Chen and Fan report hydrolysis processes with



intermediate *N*-hydroxide ions. Similarly, reactions implicating  $\text{H}_2\text{O}^{+}$  and  $\text{H}_2\text{O}^{-}$  have been proposed for nitrogen oxidation (to  $\text{N}_2\text{O}$ ) by Francisco and colleagues.<sup>102</sup>

### Microdroplet reaction scope: features of the initial reactants that allow diverse reactions

The range of microdroplet reactions is extraordinary.<sup>103</sup> Consider the synthesis of benzimidazoles from *o*-phenylenediamine and substituted carboxylic acids without a metal catalyst, strong base, or high temperature.<sup>104</sup> The observation of protonated carboxylic acids in their mass spectra suggest intrinsic droplet superacidity (*i.e.*, interfacial hydronium is a stronger acid than hydronium in bulk).<sup>14</sup> Superacidity of the solvent primes the carboxylic acid electrophile for nucleophilic attack by the diamine, regardless of the lack of an extrinsic catalyst. Analogous reactivity is observed in negatively charged droplets, implying a complementary superbasic surface. These and other examples<sup>105–108</sup> point to the superacid/superbase properties of microdroplets.

This presents a conundrum. How is it possible for one 'reagent' to be both a strong acid and a strong base, an oxidizing reagent and a reducing agent? How can such a reagent be an excellent condensation reagent (linking amino acids to form peptides,<sup>109–111</sup> sugars to form oligosaccharides,<sup>55</sup> *etc.*) but also a powerful hydrolysis reagent (hydrolyzing microparticles of quartz to make nanoparticles,<sup>112</sup> for example)? The answer, in part, is simply that reactivity depends on the choice of the other reagent(s). There is a second factor, however, the fact that large numbers of microdroplets are used in a given experiment and they need not have the same charge state (consider that when no potential is applied, reactions proceed in spontaneously charged microdroplets). A third factor is the polarity selected for reaction which controls the initial droplet charge. A positive potential is usually chosen to observe acid-catalyzed reactions and this applied potential likely determines the polarity of the very strong electric field ( $10^8 \text{ V cm}^{-1}$ ) at the droplet interface that produces reactive species.

The competition between condensation (one of the most common of all microdroplet reactions) and hydrolysis is particularly interesting. Condensation reactions do not readily occur in bulk water under equilibrium conditions, suggesting that the microdroplet surface must be effectively dry.<sup>109,113,114</sup> This raises interesting speculations regarding the fine structure of a droplet surface in which water is present but not available, for chemical or topological reasons.<sup>1,19,20,39,85</sup> At the molecular level, partial solvation has a strong effect on reducing the activation energy required for bimolecular *vs.* unimolecular reactions, in principle, it therefore favors condensation over hydrolysis (entropic considerations go in the opposite direction). Here, there is also the effect of seeing what one is looking for—in examining organic polymers or minerals,<sup>112</sup> condensation lies outside the detector capability, and one expects and can observe hydrolysis.

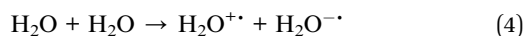
In summary, the FI event gives two primary species,  $\text{H}_2\text{O}^{+}$  and  $\text{e}^{-}(\text{aq.})$ , which are responsible for oxidation and reduction, as well as acid- or base-catalyzed reactions of great variety.

### Equilibria

When microdroplets are created using an applied potential (often with pneumatic assistance)<sup>115,116</sup> the polarity of the applied voltage will control the direction of the electric field at the surface of the microdroplets. An excess of positive charge will favor hydronium ions at the interface with hydroxide immediately below. Therefore, it is expected that a relatively small initial excess of positive ions may control the large interfacial charge population, much of which is supplied by autoionization as a fundamental property of aqueous droplet interfaces.<sup>1,57,62</sup> This argument is supported by the fact that droplets generated without purposely introducing charge nevertheless behave identically in type, if not in the extent of reaction, to droplets generated with charge.<sup>1,20</sup>

This control of field direction answers the question posed above of how droplets can be both acidic and basic. On the other hand, the magnitude of the field will depend on the charges and the distance between them, as well as the radius of the droplet. Thus, field orientation is set by the initial small excess charge, but field strength depends on microdroplet size, and a population of droplets can include individual droplets of either net polarity (and hence interfacial field orientation). A referee has asked how fields strong enough for FI can be generated when the excess charge in the droplet is minimal. The answer is that the excess charge of the microdroplet determines the direction of the interfacial field, but it does not account for its existence, which is intrinsic to the air/water interface.

The qualitative description of the microdroplet surface as 'superacidic' (the outer layer is hydronium) is simply a reference to a chemical property of the droplet.<sup>117</sup> The partial solvation of hydronium ions provides their uniquely high protonating power. Beyond the Brønsted equilibrium, Ben-Amotz<sup>62</sup> has suggested a parallel equilibrium involving interfacial redox, *i.e.*, involving electrons, not protons (eqn (4)). This description<sup>61</sup> is intriguing but unsubstantiated.



## Extensions and applications

### Scaled-up microdroplet reactions

Much research naturally focuses on the speed of reaction and the fact that small quantities of reagent can convert quickly into products. Yet it is often necessary to generate larger amounts of material than the nano- to microgram amounts accessed using MS spray ionization sources. Incorporating multiplexing and recycling into the standard droplet regimen facilitates this. Using multiple sprayers to process and reprocess larger quantities of reaction solution repeatedly through the microdroplet phase increases yields. These methods are described in the literature.<sup>16,30,31,118</sup>

### Interfaces other than aqueous/air microdroplets

Aqueous solution/oil interfaces are common in microfluidics and it was in multi-phase systems that the first on-water



accelerated reactions were encountered.<sup>5,119</sup> There are few reports of large acceleration factors at the aqueous/oil interfaces, but droplets generated from emulsions have shown significant acceleration.<sup>120</sup> Planar surfaces have also been used in the study of reactivity of thin films,<sup>121</sup> and in a few cases, the same solutions studied in sprayed microdroplet form have been allowed to react after deposition on a surface. Reactions are accelerated in these films, although much less so than in the droplet medium.<sup>122</sup> It is not clear if this difference is an electric field or a morphological effect. Even when organic solvents are used, traces of water are essential for reaction acceleration as shown in several studies.<sup>12,14–17</sup>

### Implications for origin of life chemistry

Microdroplet chemistry in environmental/atmospheric science has been considered elsewhere,<sup>1,11,24,123</sup> yet progress has intensified in the consideration of accelerated reactions in the area of origin of life research. Microdroplet processes may have played a central role in overcoming the challenges in the production of the first biomolecules.

The dilution constraint of prebiotic Earth's vast oceans, where low concentration reduces the probability of productive chemical interactions, is alleviated in microdroplets. As discussed in Tuck and Vaida's seminal work,<sup>124,125</sup> interfaces increase the local concentration of otherwise dilute organic molecules by surface access mechanisms,<sup>76</sup> in combination with the later discovery that they drive otherwise unfavorable reactions, these aerosols offer a solution to challenges in this area.<sup>126</sup> Abiotic microdroplets could concentrate organic molecules at the dry air/water interface, where condensation reactions yield biomolecules like peptides, nucleotides, and oligosaccharides, overcoming the water paradox.<sup>110,127,128</sup> Curiously, microdroplets may have additional, not yet fully understood properties that make them a significant feature in origins of life chemistry: for example, in microdroplet dipeptide formation, a unique peptide isomer with interesting chiral properties is formed.<sup>109,110</sup>

Adding to the interest of this medium for origin of life chemistry, microdroplets can promote condensation reactions that constitute a kinetic trap, addressing the susceptibility of molecules like ribose to hydrolysis. Disaccharide products spontaneously formed in microdroplets are orders of magnitude more stable<sup>129</sup> and so persist in water-containing environments, resolving a problem which had been previously held to thwart RNA-based abiogenesis theories.<sup>130</sup>

Condensation is critical for biological oligomer and biopolymer formation, as well as being a favored reaction in aqueous microdroplets. This was shown in 2017 by Zare and colleagues, who published a study in which sugar phosphates and uridine ribonucleoside are formed in water droplets.<sup>131</sup> The enthalpically forbidden bulk solution reaction ( $\Delta G = +5.4 \text{ kcal mol}^{-1}$ ) of formation of  $\beta$ -ribose-1-phosphate in microdroplets returned a  $\Delta G$  of  $-1.1 \text{ kcal mol}^{-1}$  at room temperature.<sup>113,132,133</sup> Later studies on peptide formation with loss of water also impinged on the energetic unfavourability of water loss in water. This led to the suggestion that the dry

surfaces of microdroplets have bare hydronium ions,<sup>109</sup> which readily accept the eliminated water.<sup>134,135</sup>

The simple monomeric chemical precursors to larger biological molecules might also have been generated by reactions between vapor-phase species in air, as the Miller-Urey experiments posited.<sup>136</sup> The use of discharges in these enormously influential experiments achieved the purpose of making the compounds studied (dinitrogen, oxygen, methane, and ammonia) highly reactive. However, the microdroplet environment is an alternative that also achieves this purpose. So, it is reasonable to search for putative prebiotic reactions leading to the key units (amino acids, monosaccharides<sup>55</sup> and nucleic acid bases) in water microdroplets.

### High-throughput experiments, chemical space exploration and drug discovery

Desorption electrospray ionization (DESI) is an MS ionization technique.<sup>137</sup> Like select other methods, notably MALDI, it is capable of operation in a high-throughput array-based format and provides high-quality MS analytical data.<sup>103</sup> However, unlike other MS methods, it also allows chemical synthesis in solution<sup>138</sup> and, in turn, allows collection of the synthetic product(s). Moreover, as an imaging method, it can be used to examine arrays of collected products.<sup>139</sup>

Microdroplet reaction acceleration allows DESI to be used to (i) optimize conditions for generating particular compounds by droplet reactions, (ii) perform those reactions with online MS analysis (iii) deposit the product-containing droplets on a suitable surface as arrays (up to 6144 samples) with correlation to the source array and (iv) confirm the product assignment in the product array. Arrays of reaction mixtures have been converted at rates of 1 reaction/second into arrays of products, which can then be subjected to (v) further chemical (*e.g.*, NMR) or bioactivity characterization in the context of drug discovery.<sup>140,141</sup> The automated integration of these synthetic and analytical capabilities represents a unique alternative for rapid, large-scale drug candidate generation and testing, consolidating the early drug discovery pipeline in a single-platform workflow of unprecedented speed (Fig. 2).<sup>142</sup>

With this technology, robotically prepared reaction mixture arrays can be rapidly screened without incubation for synthetic route optimization towards selected drug candidates,<sup>143</sup> combinatorial synthesis of large compound libraries for testing,<sup>144,145</sup> or chemical space exploration to provide analogs of already complex bioactive molecules *via* late-stage functionalization for lead optimization.<sup>138,146</sup> Importantly, online structural confirmation of microdroplet products generated on the fly is readily available *via* tandem MS.<sup>138,142,146</sup> After screening, drug candidates can be synthesized at tens of nanograms per minute by simply collecting the secondary DESI droplets instead of sampling them for MS analysis.

This collection process has been extended to provide an array-to-array format in a configuration analogous to a type-writer: DESI microdroplets type arrays of new products from arrays of deposited reaction mixtures.<sup>139</sup> The newly synthesized



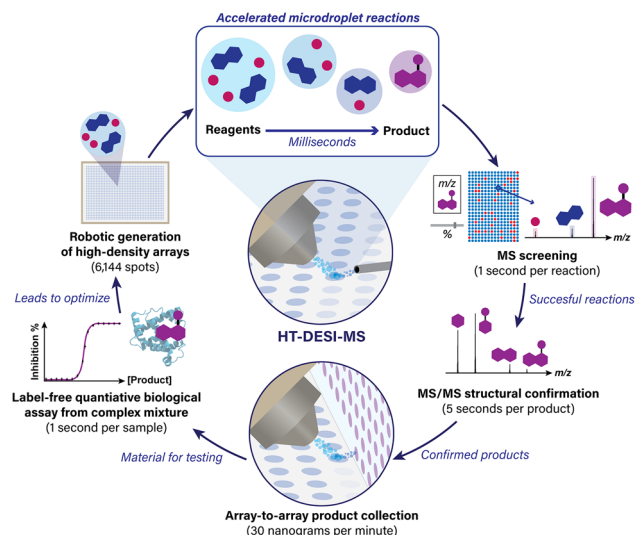


Fig. 2 Closed-loop drug discovery workflow enabled by DESI-MS and associated accelerated reactions in microdroplets.

products are collected and are sufficient for testing their activities against a biological target (*e.g.*, enzymes, receptors) using the same instrumentation and with the same throughput (>1 Hz).<sup>140,141,147–149</sup> These high-throughput DESI-MS bioassays are carried out in a workup and label-free fashion, exploiting the unique capabilities of DESI for direct analysis of complex samples,<sup>137</sup> including those not available in solution (*e.g.* bacteria, tissue)<sup>150,151</sup> or those rich in non-volatile matrices (such as typical biological buffers),<sup>140,141</sup> as well as the inherent features of MS, including high chemical specificity and broad molecular coverage. Iteration on these, together with machine learning (ML) algorithms trained by vast amounts of single-platform high-quality data, could efficiently yield lead drug candidates, with a demonstrated current technological capacity of approximately 100+ compounds synthesized and tested per hour—while consuming <1 mL of solvent.

A recent illustration of these capabilities is the characterization of the so-called ‘Novichok’ chemical warfare agents and the discovery of countermeasures.<sup>152</sup> In this case, microdroplet reactions enable an ultrafast workflow for response to emerging threats. Numerous derivatives of promising oxime scaffolds (*e.g.*, bispyridinium) have been generated and assayed with label-free efficacy monitoring using a ML-controlled single-platform optimization loop aimed towards the discovery of new antidotes.

### Systematic organic synthesis: scope, yield, mechanism and green chemistry

Applications of accelerated reactions in organic synthesis are facilitated by the high speed of reactions and the fact that one reaction channel is often dominant. This allows, for example, green chemical synthesis<sup>153</sup> (mild conditions, no metal catalysts, water as solvent) of many classes of heterocyclic compounds, *e.g.*, imidazoles,<sup>104</sup> pyrazoles,<sup>154</sup> and borazines.<sup>155</sup> Nitrogen heterocycles have been of particular interest given their potential importance in the discovery of new small molecule drugs.<sup>154</sup> Given that one can generate reaction products very quickly on a small scale but still obtain information on yield and purity, a number of groups, including that of Heyong Cheng, have applied accelerated reactions to systematically synthesize large numbers of members of particular compound classes.<sup>16,30,122</sup>

In these experiments, online MS can provide information on the nature of the reaction products and some idea of purity and yield. Automatically acquired data can be used to determine when tandem mass spectrometry should be implemented to confirm product identities. Scaled-up experiments allow NMR data to be obtained. Systematic synthesis of some 10 ring systems (Table 1) shows all the above features. They also show that yields are good to excellent, even in the shortest time scale experiments.

Note that the fact that water is essential for reaction acceleration means that water<sup>156,157</sup> or an aqueous mixture with

Table 1 Heterocyclic synthesis using accelerated reactions in microdroplets & thin films<sup>a</sup>

| Heterocycle                                          | Reaction                                   | Solvent <sup>b</sup>            | Ionization      |
|------------------------------------------------------|--------------------------------------------|---------------------------------|-----------------|
| Dihydropyridine <sup>86</sup>                        | Hantzsch (enamine + ester)                 | Ethanol                         | ESSI; thin film |
| Quinoline <sup>161</sup>                             | Friedlander (2-aminoarylaldehyde + ketone) | Methanol                        | ESSI            |
| Fischer indole synthesis <sup>162</sup>              | Phenylhydrazine + acetone                  | Methanol                        | nESI            |
| Pyrazole <sup>163</sup>                              | Phenylhydrazine + ethyl acetoacetate       | Methanol                        | nESI            |
| Diazine <sup>164</sup>                               | Phenylhydrazine + phthalic acid            | Dimethylformamide, acetonitrile | ESSI            |
| Benzimidazole <sup>104</sup>                         | Phenyldiamine + formic acid                | Methanol                        | nESI            |
| Polynitro compound <sup>165</sup>                    | Amine + glyoxal                            | Acetonitrile                    | nESI; ESSI; PSI |
| Spiro-pyrrolidine <sup>166</sup>                     | Multi-component synthesis                  | Ethanol                         | SSI + thin film |
| Zymonic acid <sup>114,167,168</sup>                  | Condensation of pyruvic acid               | Water                           | Aerosol         |
| Thioisindolinone and $\gamma$ -Lactam <sup>169</sup> | 2-Acylbenzaldehyde + amine or thiol        | Ethyl acetate                   | SSI + thin film |
| Tricyclic quinolone <sup>170</sup>                   | Phenylacetylene + indoline                 | Methanol                        | Sub-AP-ESI      |
| Borazole <sup>155</sup>                              | Amine + boronic acids                      | Acetonitrile                    | nESI            |
| Pyrrole <sup>16</sup>                                | Amine + acetonylacetone                    | Isopropanol                     | SSI + thin film |
| Pyrazole <sup>154</sup>                              | Hydrazine + 1,3-diketone                   | Water                           | nESI            |

<sup>a</sup> ESSI electrosonic spray, a variant on ESI; SSI sonic spray; PSI paper spray ionization. <sup>b</sup> Solvents are not rigorously dried and kept dry.



a solvent like acetonitrile can be used. Acceleration also means that harsh conditions are typically not used: most reactions are performed under ambient conditions with no heating. Many reactions can proceed uninhibited in droplets without supplemental reagents or catalysts.<sup>104,158</sup> Given the gentle nature of the reaction conditions, products are often pure as seen by NMR spectra recorded on the crude material.<sup>106,154,155</sup> Given these considerations, microdroplet reactions may find substantial utility as a sustainable means for accelerated environmental pollutant degradation (*e.g.*, drugs,<sup>159</sup> PFAS,<sup>160</sup> *etc.*), as well as a strategy to reduce the laboratory environmental footprint.

## Conclusions

Two principal matters have been considered: (i) the mechanism of acceleration to which we contribute a simple insight regarding FI as a route to highly reactive intermediates that, we suggest, drive the rich chemistry of microdroplets. (ii) The application of reaction acceleration in origin of life chemistry and synthesis, both for scale-up ( $\text{g h}^{-1}$ ) and in conjunction with small-scale high-throughput reactions (1 Hz). This drug discovery platform is capable of rapid screening, collection, and bioactivity testing of nanogram amounts of drug analogs based on reactions occurring during flight of secondary DESI droplets from the primary array of reaction mixtures to a secondary product collection array.

We draw attention to some limitations in our understanding of accelerated reaction mechanisms. First, interfacial phenomena are difficult to characterize comprehensively, so quantitative information on reaction rates remains limited. Second, questions remain as to why some reactions are accelerated more than others. The use of NMR and other alternatives to MS for product analysis will likely help avoid an over-emphasis on acceleration mechanisms that involve ionic intermediates. Third, there is little detail available from experiment on the nature of the interfacial electric field.

Two topics not detailed here, but of growing significance, are the role of microdroplets in enzymatic reactions (such as protein hydrolysis<sup>171–173</sup>) and their emerging role in materials degradation, as evidenced by the silica micro- to nanoparticle breakdown.<sup>112</sup> Also noteworthy is the build-up of nanoparticles by droplet deposition,<sup>174–176</sup> including one instance where nanoparticles are implicated in nitrogen fixation.<sup>177</sup>

Because of their small scale, convenience of implementation, low sample consumption, and high duty cycle, the fundamental insights provided by microdroplet acceleration are already being harnessed: we have discussed how new capabilities facilitate faster and more robust chemical analysis and chemical synthesis.

## Author contributions

RGC conceived the key concepts and directed the project. DTH, BAS, MQE, and NMM provided data and ideas. RGC and DTH wrote the manuscript and all authors contributed to finalizing it.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

No primary research results, software or code have been included, and no new data were generated or analysed as part of this review.

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## Notes and references

- 1 R. A. LaCour, J. P. Heindel, R. Zhao and T. Head-Gordon, The Role of Interfaces and Charge for Chemical Reactivity in Microdroplets, *J. Am. Chem. Soc.*, 2025, **147**, 6299–6317.
- 2 Z. Wei, Y. Li, R. G. Cooks and X. Yan, Accelerated Reaction Kinetics in Microdroplets: Overview and Recent Developments, *Annu. Rev. Phys. Chem.*, 2020, **71**, 31–51.
- 3 X. Yan, R. M. Bain and R. G. Cooks, Organic Reactions in Microdroplets: Reaction Acceleration Revealed by Mass Spectrometry, *Angew. Chem., Int. Ed.*, 2016, **55**, 12960–12972.
- 4 S. Narayan, J. Muldoon, M. G. Finn, V. V. Fokin, H. C. Kolb and K. B. Sharpless, “On Water”: Unique Reactivity of Organic Compounds in Aqueous Suspension, *Angew. Chem., Int. Ed.*, 2005, **44**, 3275–3279.
- 5 A. Chanda and V. V. Fokin, Organic Synthesis “On Water”, *Chem. Rev.*, 2009, **109**, 725–748.
- 6 M. Girod, E. Moyano, D. I. Campbell and R. G. Cooks, Accelerated bimolecular reactions in microdroplets studied by desorption electrospray ionization mass spectrometry, *Chem. Sci.*, 2011, **2**, 501–510.
- 7 A. K. Badu-Tawiah, D. I. Campbell and R. G. Cooks, Reactions of Microsolvated Organic Compounds at Ambient Surfaces: Droplet Velocity, Charge State, and Solvent Effects, *J. Am. Soc. Mass Spectrom.*, 2012, **23**, 1077–1084.
- 8 T. Müller, A. Badu-Tawiah and R. G. Cooks, Accelerated Carbon-Carbon Bond-Forming Reactions in Preparative Electrospray, *Angew. Chem., Int. Ed.*, 2012, **51**, 11832–11835.
- 9 A. K. Badu-Tawiah, D. I. Campbell and R. G. Cooks, Accelerated C–N Bond Formation in Dropcast Thin Films on Ambient Surfaces, *J. Am. Soc. Mass Spectrom.*, 2012, **23**, 1461–1468.



- 10 R. G. Cooks and D. T. Holden, Breaking down microdroplet chemistry, *Science*, 2024, **384**, 958–959.
- 11 M. F. Ruiz-Lopez, J. S. Francisco, M. T. C. Martins-Costa and J. M. Anglada, Molecular reactions at aqueous interfaces, *Nat. Rev. Chem.*, 2020, **4**, 459–475.
- 12 L. Qiu, X. Li, D. T. Holden and R. G. Cooks, Reaction acceleration at the surface of a levitated droplet by vapor dosing from a partner droplet, *Chem. Sci.*, 2024, **15**, 12277–12283.
- 13 J. N. Smith, R. C. Flagan and J. L. Beauchamp, Droplet Evaporation and Discharge Dynamics in Electrospray Ionization, *J. Phys. Chem. A*, 2002, **106**, 9957–9967.
- 14 K.-H. Huang, Z. Wei and R. G. Cooks, Accelerated reactions of amines with carbon dioxide driven by superacid at the microdroplet interface, *Chem. Sci.*, 2021, **12**, 2242–2250.
- 15 Z. Song, C. Zhu, K. Gong, R. Wang, J. Zhang, S. Zhao, Z. Li, X. Zhang and J. Xie, Deciphering the Microdroplet Acceleration Factors of Aza-Michael Addition Reactions, *J. Am. Chem. Soc.*, 2024, **146**, 10963–10972.
- 16 J. Li, J. Sun, Y. Wang, J. Liu and H. Cheng, Spontaneous Catalytic Paal-Knorr Reactions for N-Substituted Pyrrole Synthesis by Microdroplet Chemistry, *ACS Sustain. Chem. Eng.*, 2025, **13**, 571–582.
- 17 T. Khuu, K. Takematsu and J. Dawlaty, Probing Proton Delivery in Microheterogeneous Water Structures Using a Proton-Dependent Isomerization Reaction of a Merocyanine Photoacid, *J. Phys. Chem. A*, 2025, **129**, 5450–5457.
- 18 H. Xiong, J. K. Lee, R. N. Zare and W. Min, Strong Electric Field Observed at the Interface of Aqueous Microdroplets, *J. Phys. Chem. Lett.*, 2020, **11**, 7423–7428.
- 19 L. Shi, R. A. LaCour, N. Qian, J. P. Heindel, X. Lang, R. Zhao, T. Head-Gordon and W. Min, Water structure and electric fields at the interface of oil droplets, *Nature*, 2025, **640**, 87–93.
- 20 H. Hao, I. Leven and T. Head-Gordon, Can electric fields drive chemistry for an aqueous microdroplet?, *Nat. Commun.*, 2022, **13**, 280.
- 21 N. Narendra, X. Chen, J. Wang, J. Charles, R. G. Cooks and T. Kubis, Quantum Mechanical Modeling of Reaction Rate Acceleration in Microdroplets, *J. Phys. Chem. A*, 2020, **124**, 4984–4989.
- 22 K. D. Judd, S. W. Parsons, T. Majumder and J. M. Dawlaty, Electrostatics, Hydration, and Chemical Equilibria at Charged Monolayers on Water, *Chem. Rev.*, 2025, **125**, 2440–2473.
- 23 K. D. Judd, S. W. Parsons, D. B. Eremin, V. V. Fokin and J. M. Dawlaty, Visualizing partial solvation at the air-water interface, *Chem. Sci.*, 2024, **15**, 8346–8354.
- 24 J. Zhong, M. Kumar, J. S. Francisco and X. C. Zeng, Insight into Chemistry on Cloud/Aerosol Water Surfaces, *Acc. Chem. Res.*, 2018, **51**, 1229–1237.
- 25 J. Zhong, M. Kumar, C. Q. Zhu, J. S. Francisco and X. C. Zeng, Surprising Stability of Larger Criegee Intermediates on Aqueous Interfaces, *Angew. Chem., Int. Ed.*, 2017, **56**, 7740–7744.
- 26 D. J. Nesbitt, A. M. Zolot, J. R. Roscioli and M. Ryazanov, Nonequilibrium Scattering/Evaporation Dynamics at the Gas-Liquid Interface: Wetted Wheels, Self-Assembled Monolayers, and Liquid Microjets, *Acc. Chem. Res.*, 2023, **56**, 700–711.
- 27 L. Qiu, Z. Wei, H. Nie and R. G. Cooks, Reaction Acceleration Promoted by Partial Solvation at the Gas/Solution Interface, *ChemPlusChem*, 2021, **86**, 1362–1365.
- 28 K. J. Vannoy, M. Q. Edwards, C. Renault and J. E. Dick, An Electrochemical Perspective on Reaction Acceleration in Microdroplets, *Annu. Rev. Anal. Chem.*, 2024, **17**, 149–171.
- 29 L. E. Krushinski, L. Qiu and J. E. Dick, Levitating Droplet Electroanalysis, *Anal. Chem.*, 2024, **96**, 2764–2766.
- 30 S. Chen, K. Dong, J. Sun, M. Ye, J. Liu and H. Cheng, Mild Diarylmethylation of Indoles through Regioselective 1,6-Hydroarylation of para-Quinone Methides in Microdroplets/Thin Film, *ACS Sustain. Chem. Eng.*, 2024, **12**, 9522–9533.
- 31 H. M. Brown, J. E. Estevez, J. C. Bottaro, B. G. Harvey and P. W. Fedick, Accelerated formation of trioximes through confined volume reactors and scale-up using thin film methods, *React. Chem. Eng.*, 2023, **8**, 1576–1582.
- 32 R. G. Cooks, D. T. Holden, N. Morato, L. Qiu, K.-H. Huang, K. Chen, Y. Feng, J. Ghosh, B. Shira and M. Q. Edwards, *High-throughput chemical synthesis using microdroplet reactions*, San Diego, 2025.
- 33 D. T. Holden, PhD, Purdue University, 2025.
- 34 H. D. Beckey, *Massenspektrometrische Untersuchungen mit Hilfe einer Feldemissions-Ionenquelle*, 1959, **14**, 712–721.
- 35 R. P. Lattimer and H. R. Schulten, Field ionization and field desorption mass spectrometry: past, present, and future, *Anal. Chem.*, 1989, **61**, 1201A–1215A.
- 36 W. T. Huntress Jr and R. F. Pinizzotto Jr, Product distributions and rate constants for ion-molecule reactions in water, hydrogen sulfide, ammonia, and methane, *J. Chem. Phys.*, 1973, **59**, 4742–4756.
- 37 P. J. Bryant and J. M. Phillips, *Development of Field Ionization for the Improvement of Mass Spectrometry*, NASA-CR-86223, 1969.
- 38 A. R. Anway, Field Ionization of Water, *J. Chem. Phys.*, 1969, **50**, 2012–2021.
- 39 J. P. Heindel, R. A. LaCour and T. Head-Gordon, The role of charge in microdroplet redox chemistry, *Nat. Commun.*, 2024, **15**, 3670.
- 40 J. P. Heindel, H. Hao, R. A. LaCour and T. Head-Gordon, Spontaneous Formation of Hydrogen Peroxide in Water Microdroplets, *J. Phys. Chem. Lett.*, 2022, **13**, 10035–10041.
- 41 L. Qiu, M. D. Psimos and R. G. Cooks, Spontaneous Oxidation of Aromatic Sulfones to Sulfonic Acids in Microdroplets, *J. Am. Soc. Mass Spectrom.*, 2022, **33**, 1362–1367.
- 42 L. Qiu and R. G. Cooks, Simultaneous and Spontaneous Oxidation and Reduction in Microdroplets by the Water Radical Cation/Anion Pair, *Angew. Chem., Int. Ed.*, 2022, **61**, e202210765.
- 43 T. M. Schwarz, E. M. Weikum, K. Meng, E. Hadjixenophontos, C. A. Dietrich, J. Kästner, P. Stender



- and G. Schmitz, Field evaporation and atom probe tomography of pure water tips, *Sci. Rep.*, 2020, **10**, 20271.
- 44 E. Guo and D. R. McKenzie, A post Gurney quantum mechanical perspective on the electrolysis of water: ion neutralization in solution, *Proc. R. Soc. A*, 2017, **473**, 20170371.
  - 45 W. Schmickler, Tunneling of electrons through thin layers of water, *Surf. Sci.*, 1995, **335**, 416–421.
  - 46 I. Benjamin, D. Evans and A. Nitzan, Electron tunneling through water layers: Effect of layer structure and thickness, *J. Chem. Phys.*, 1997, **106**, 6647–6654.
  - 47 A. Mosyak, P. Graf, I. Benjamin and A. Nitzan, Electron Tunneling through Water Layers: Effect of Polarizability, *J. Phys. Chem. A*, 1997, **101**, 429–433.
  - 48 A. Mosyak, A. Nitzan and R. Kosloff, Numerical simulations of electron tunneling in water, *J. Chem. Phys.*, 1996, **104**, 1549–1559.
  - 49 J. O. Richardson, C. Pérez, S. Lobsiger, A. A. Reid, B. Temelso, G. C. Shields, Z. Kisiel, D. J. Wales, B. H. Pate and S. C. Althorpe, Concerted hydrogen-bond breaking by quantum tunneling in the water hexamer prism, *Science*, 2016, **351**, 1310–1313.
  - 50 M. L. Hill, B. R. Layman and J. E. Dick, Real-Time Visualization of an Elusive, Strong Reducing Agent during Tris(2,2'-bipyridyl)ruthenium(II) Electro-Oxidation in Water, *J. Am. Chem. Soc.*, 2025, **147**, 17701–17709.
  - 51 M. Dolatkhah Ouch Bolagh and L. Konermann, Modeling a Continuously Operating Electrospray Ionization Emitter Using Molecular Dynamics Simulations: From Bulk Solution to Gaseous Ions, *J. Phys. Chem. B*, 2025, **129**, 4096–4109.
  - 52 S. Aloisio and J. S. Francisco, Radical–Water Complexes in Earth's Atmosphere, *Acc. Chem. Res.*, 2000, **33**, 825–830.
  - 53 A. G. Harrison, *Chemical ionization mass spectrometry*, Routledge, 2018.
  - 54 W. J. Richter and H. Schwarz, Chemical Ionization—A Mass-Spectrometric Analytical Procedure of Rapidly Increasing Importance, *Angew. Chem. Int. Ed. Engl.*, 1978, **17**, 424–439.
  - 55 M. Q. Edwards, D. T. Holden and R. G. Cooks, Abiotic formation of hexoses and disaccharides in aqueous microdroplets, *Chem. Sci.*, 2025, **16**, 7057–7065.
  - 56 J. K. Lee, D. Samanta, H. G. Nam and R. N. Zare, Micrometer-sized water droplets induce spontaneous reduction, *J. Am. Chem. Soc.*, 2019, **141**, 10585–10589.
  - 57 S. Lin, L. N. Y. Cao, Z. Tang and Z. L. Wang, Size-dependent charge transfer between water microdroplets, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**, e2307977120.
  - 58 J. K. Lee, K. L. Walker, H. S. Han, J. Kang, F. B. Prinz, R. M. Waymouth, H. G. Nam and R. N. Zare, Spontaneous generation of hydrogen peroxide from aqueous microdroplets, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 19294–19298.
  - 59 M. A. Eatoo and H. Mishra, Busting the myth of spontaneous formation of H<sub>2</sub>O<sub>2</sub> at the air–water interface: contributions of the liquid–solid interface and dissolved oxygen exposed, *Chem. Sci.*, 2024, **15**, 3093–3103.
  - 60 Q. Liang, C. Zhu and J. Yang, Water Charge Transfer Accelerates Criegee Intermediate Reaction with H<sub>2</sub>O–Radical Anion at the Aqueous Interface, *J. Am. Chem. Soc.*, 2023, **145**, 10159–10166.
  - 61 L. Qiu and R. G. Cooks, Spontaneous Oxidation in Aqueous Microdroplets: Water Radical Cation as Primary Oxidizing Agent, *Angew. Chem., Int. Ed.*, 2024, **63**, e202400118.
  - 62 D. Ben-Amotz, Electric buzz in a glass of pure water, *Science*, 2022, **376**, 800–801.
  - 63 P. J. Bryant and J. M. Phillips, *Development of Field Ionization for the Improvement of Mass Spectrometry*, NASA-CR-86223, 1969.
  - 64 L. Zhao, X. Song, C. Gong, D. Zhang, R. Wang, R. N. Zare and X. Zhang, Sprayed water microdroplets containing dissolved pyridine spontaneously generate pyridyl anions, *Proc. Natl. Acad. Sci. U. S. A.*, 2022, **119**, e2200991119.
  - 65 A. J. Colussi, Mechanism of Hydrogen Peroxide Formation on Sprayed Water Microdroplets, *J. Am. Chem. Soc.*, 2023, **145**, 16315–16317.
  - 66 D. Xing, Y. Meng, X. Yuan, S. Jin, X. Song, R. N. Zare and X. Zhang, Capture of Hydroxyl Radicals by Hydronium Cations in Water Microdroplets, *Angew. Chem., Int. Ed.*, 2022, **61**, e202207587.
  - 67 H. Chen, X. Li, B. Li, Y. Chen, H. Ouyang, Y. Li and X. Zhang, Microdroplet Chemistry with Unactivated Droplets, *J. Am. Chem. Soc.*, 2025, **147**, 11399–11406.
  - 68 Y. Meng, Y. Xia, J. Xu and R. N. Zare, Spraying of water microdroplets forms luminescence and causes chemical reactions in surrounding gas, *Sci. Adv.*, 2025, **11**, eadt8979.
  - 69 C. J. Chen, V. S. Avadhani and E. R. Williams, Electronic Excitation and High-Energy Reactions Originate From Anionic Microdroplets Formed by Electrospray or Pneumatic Nebulization, *Angew. Chem., Int. Ed.*, 2025, **64**, e202424662.
  - 70 N. Drachman, M. Lepoitevin, H. Szapary, B. Wiener, W. Maulbetsch and D. Stein, Nanopore ion sources deliver individual ions of amino acids and peptides directly into high vacuum, *Nat. Commun.*, 2024, **15**, 7709.
  - 71 I. G. Loscertales and J. Fernández de la Mora, Experiments on the kinetics of field evaporation of small ions from droplets, *J. Chem. Phys.*, 1995, **103**, 5041–5060.
  - 72 M. Magnani and M. Gamero-Castaño, Energy barrier for ion field emission from a dielectric liquid sphere, *Phys. Rev. E*, 2022, **105**, 054802.
  - 73 W. D. Luedtke, U. Landman, Y. H. Chiu, D. J. Levandier, R. A. Dressler, S. Sok and M. S. Gordon, Nanojets, Electrospray, and Ion Field Evaporation: Molecular Dynamics Simulations and Laboratory Experiments, *J. Phys. Chem. A*, 2008, **112**, 9628–9649.
  - 74 N. Segreto, T. M. Schwarz, C. A. Dietrich, P. Stender, R. Schuldt, G. Schmitz and J. Kästner, Understanding the Underlying Field Evaporation Mechanism of Pure Water Tips in High Electrical Fields, *J. Phys. Chem. A*, 2022, **126**, 5663–5671.
  - 75 P. Kebarle, A brief overview of the present status of the mechanisms involved in electrospray mass spectrometry, *J. Mass Spectrom.*, 2000, **35**, 804–817.



- 76 L. Konermann, E. Ahadi, A. D. Rodriguez and S. Vahidi, Unraveling the Mechanism of Electrospray Ionization, *Anal. Chem.*, 2013, **85**, 2–9.
- 77 E. Ahadi and L. Konermann, Ejection of Solvated Ions from Electrosprayed Methanol/Water Nanodroplets Studied by Molecular Dynamics Simulations, *J. Am. Chem. Soc.*, 2011, **133**, 9354–9363.
- 78 H. Metwally, Q. Duez and L. Konermann, Chain Ejection Model for Electrospray Ionization of Unfolded Proteins: Evidence from Atomistic Simulations and Ion Mobility Spectrometry, *Anal. Chem.*, 2018, **90**, 10069–10077.
- 79 L. Konermann, R. G. McAllister and H. Metwally, Molecular Dynamics Simulations of the Electrospray Process: Formation of NaCl Clusters via the Charged Residue Mechanism, *J. Phys. Chem. B*, 2014, **118**, 12025–12033.
- 80 E. Aliyari and L. Konermann, Formation of Gaseous Peptide Ions from Electrospray Droplets: Competition between the Ion Evaporation Mechanism and Charged Residue Mechanism, *Anal. Chem.*, 2022, **94**, 7713–7721.
- 81 E. M. Stuve, Ionization of water in interfacial electric fields: An electrochemical view, *Chem. Phys. Lett.*, 2012, **519**–520, 1–17.
- 82 D. Duft, T. Achtzehn, R. Müller, B. A. Huber and T. Leisner, Rayleigh jets from levitated microdroplets, *Nature*, 2003, **421**, 128.
- 83 S. W. Devlin, F. Bernal, E. J. Riffe, K. R. Wilson and R. J. Saykally, Spiers Memorial Lecture: Water at interfaces, *Faraday Discuss.*, 2024, **249**, 9–37.
- 84 K. R. Wilson and A. M. Prophet, Chemical Kinetics in Microdroplets, *Annu. Rev. Phys. Chem.*, 2024, **75**, 185–208.
- 85 M. Ahmed, M. Blum, E. J. Crumlin, P. L. Geissler, T. Head-Gordon, D. T. Limmer, K. K. Mandadapu, R. J. Saykally and K. R. Wilson, Molecular Properties and Chemical Transformations Near Interfaces, *J. Phys. Chem. B*, 2021, **125**, 9037–9051.
- 86 R. M. Bain, C. J. Pulliam and R. G. Cooks, Accelerated Hantzsch electrospray synthesis with temporal control of reaction intermediates, *Chem. Sci.*, 2015, **6**, 397–401.
- 87 X. Zhang, R. Su, J. Li, L. Huang, W. Yang, K. Chingin, R. Balabin, J. Wang, X. Zhang, W. Zhu, K. Huang, S. Feng and H. Chen, Efficient catalyst-free N<sub>2</sub> fixation by water radical cations under ambient conditions, *Nat. Commun.*, 2024, **15**, 1535.
- 88 S. Jin, H. Chen, X. Yuan, D. Xing, R. Wang, L. Zhao, D. Zhang, C. Gong, C. Zhu, X. Gao, Y. Chen and X. Zhang, The Spontaneous Electron-Mediated Redox Processes on Sprayed Water Microdroplets, *JACS Au*, 2023, **3**, 1563–1571.
- 89 T. Majumder, D. B. Eremin, B. Delibas, A. Sarkar, V. Fokin and J. M. Dawlaty, Calibrating the Oxidative Capacity of Microdroplets, *Angew. Chem., Int. Ed.*, 2025, **64**, e202414746.
- 90 M. A. Mehrgardi, M. Mofidfar and R. N. Zare, Sprayed Water Microdroplets Are Able to Generate Hydrogen Peroxide Spontaneously, *J. Am. Chem. Soc.*, 2022, **144**, 7606–7609.
- 91 M. Wang, X.-F. Gao, R. Su, P. He, Y.-Y. Cheng, K. Li, D. Mi, X. Zhang, X. Zhang, H. Chen and R. G. Cooks, Abundant Production of Reactive Water Radical Cations under Ambient Conditions, *CCS Chem.*, 2021, **4**, 1224–1231.
- 92 Q. Cheng, F. A. Evangelista, A. C. Simmonett, Y. Yamaguchi and H. F. Schaefer III, Water Dimer Radical Cation: Structures, Vibrational Frequencies, and Energetics, *J. Phys. Chem. A*, 2009, **113**, 13779–13789.
- 93 G. H. Gardenier, M. A. Johnson and A. B. McCoy, Spectroscopic Study of the Ion–Radical H-Bond in H<sub>4</sub>O<sub>2</sub><sup>+</sup>, *J. Phys. Chem. A*, 2009, **113**, 4772–4779.
- 94 J. M. Headrick, E. G. Diken, R. S. Walters, N. I. Hammer, R. A. Christie, J. Cui, E. M. Myshakin, M. A. Duncan, M. A. Johnson and K. D. Jordan, Spectral Signatures of Hydrated Proton Vibrations in Water Clusters, *Science*, 2005, **308**, 1765–1769.
- 95 J. W. Shin, N. I. Hammer, E. G. Diken, M. A. Johnson, R. S. Walters, T. D. Jaeger, M. A. Duncan, R. A. Christie and K. D. Jordan, Infrared Signature of Structures Associated with the H+(H<sub>2</sub>O)<sub>n</sub> (n = 6 to 27) Clusters, *Science*, 2004, **304**, 1137–1140.
- 96 C. J. Chen and E. R. Williams, A Source of the Mysterious m/z 36 Ions Identified: Implications for the Stability of Water and Unusual Chemistry in Microdroplets, *ACS Cent. Sci.*, 2025, **11**, 622–628.
- 97 L. Li, Q. Wu, S.-k. Xiang, S. Mu, R. Zhao, M. Xiao, C. Long, X. Zheng and C. Cui, Electron Paramagnetic Resonance Tracks Condition-Sensitive Water Radical Cation, *J. Phys. Chem. Lett.*, 2023, **14**, 9183–9191.
- 98 X. Zhang, M. Duan, K. Chingin, R. Balabin, L. Liu, X. Zhang and H. Chen, Detection and Characterization of Water Radical Cations by Mass Spectrometry and Electron Paramagnetic Resonance Spectroscopy, *Anal. Chem.*, 2025, **97**(29), 16004–16010.
- 99 D. Xing, X. Gao, H. Chen, J. Zhang, M. E. Edwards, C. Liang, C. Zhu, Y. Meng, R. N. Zare, Y. Xia and X. Zhang, Challenges in Detecting Hydroxyl Radicals Generated in Water Droplets with Mass Spectrometry, *Anal. Chem.*, 2025, **97**, 7995–8000.
- 100 X. Song, C. Basheer, J. Xu and R. N. Zare, Onsite ammonia synthesis from water vapor and nitrogen in the air, *Sci. Adv.*, 2024, **10**, eads4443.
- 101 Y. Wang, J. Luo, Y.-G. Fang, Z.-A. Nan, X. Cui, T. Chen, X. Zeng, X. Wang, X. Song, J. Zhao, W. Li, C. Zeng, D. Chen, C. Zhu, Z. Wei, Z.-Q. Tian and F. R. Fan, Catalyst-Free Nitrogen Fixation by Microdroplets through a Radical-Mediated Disproportionation Mechanism under Ambient Conditions, *J. Am. Chem. Soc.*, 2025, **147**, 2756–2765.
- 102 Q. Jiang, D. Xia, X. Li, H. Zhang, R. Yin, H. Xie, H.-b. Xie, J. Jiang, J. Chen and J. S. Francisco, Rapid N<sub>2</sub>O Formation from N<sub>2</sub> on Water Droplet Surfaces, *Angew. Chem., Int. Ed.*, 2025, **64**, e202421002.
- 103 N. M. Morato, M. T. Le, D. T. Holden and R. Graham Cooks, Automated High-Throughput System Combining Small-Scale Synthesis with Bioassays and Reaction Screening, *SLAS Technol.*, 2021, **26**, 555–571.
- 104 P. Basuri, L. E. Gonzalez, N. M. Morato, T. Pradeep and R. G. Cooks, Accelerated microdroplet synthesis of



- benzimidazoles by nucleophilic addition to protonated carboxylic acids, *Chem. Sci.*, 2020, **11**, 12686–12694.
- 105 S. Enami, L. A. Stewart, M. R. Hoffmann and A. J. Colussi, Superacid Chemistry on Mildly Acidic Water, *J. Phys. Chem. Lett.*, 2010, **1**, 3488–3493.
- 106 J. Ghosh, J. Mendoza and R. G. Cooks, Accelerated and Concerted Aza-Michael Addition and SuFEx Reaction in Microdroplets in Unitary and High-Throughput Formats, *Angew. Chem., Int. Ed.*, 2022, **61**, e202214090.
- 107 T. Khuu, T. Schleif, A. Mohamed, S. Mitra, M. A. Johnson, J. Valdiviezo, J. P. Heindel and T. Head-Gordon, Intra-cluster Charge Migration upon Hydration of Protonated Formic Acid Revealed by Anharmonic Analysis of Cold Ion Vibrational Spectra, *J. Phys. Chem. A*, 2023, **127**, 7501–7509.
- 108 Y. Wang, Y. Ju, J. He, J. Zhao, Z. Zhou, J. Jiang and H. Zhang, Harnessing the Interface of Water Microdroplets to Accelerate Energy Substance Adenosine Triphosphate Formation, *J. Phys. Chem. Lett.*, 2025, **16**, 2621–2626.
- 109 D. T. Holden, N. M. Morato and R. G. Cooks, Aqueous microdroplets enable abiotic synthesis and chain extension of unique peptide isomers from free amino acids, *Proc. Natl. Acad. Sci. U. S. A.*, 2022, **119**, e2212642119.
- 110 L. Qiu and R. G. Cooks, Oxazolone mediated peptide chain extension and homochirality in aqueous microdroplets, *Proc. Natl. Acad. Sci. U. S. A.*, 2024, **121**, e2309360120.
- 111 B. A. Shira and R. G. Cooks, Serine Octamer Clusters Direct the Chirality of Peptides Formed in Water Microdroplets, *J. Phys. Chem. A*, 2025, **129**, 4535–4542.
- 112 B. K. Spoorthi, K. Debnath, P. Basuri, A. Nagar, U. V. Waghmare and T. Pradeep, Spontaneous weathering of natural minerals in charged water microdroplets forms nanomaterials, *Science*, 2024, **384**, 1012–1017.
- 113 V. Vaida and A. M. Deal, Peptide synthesis in aqueous microdroplets, *Proc. Natl. Acad. Sci. U. S. A.*, 2022, **119**, e2216015119.
- 114 M. Li, C. Boothby, R. E. Continetti and V. H. Grassian, Size-Dependent Sigmoidal Reaction Kinetics for Pyruvic Acid Condensation at the Air–Water Interface in Aqueous Microdroplets, *J. Am. Chem. Soc.*, 2023, **145**, 22317–22321.
- 115 L. W. Zilch, J. T. Maze, J. W. Smith, G. E. Ewing and M. F. Jarrold, Charge Separation in the Aerodynamic Breakup of Micrometer-Sized Water Droplets, *J. Phys. Chem. A*, 2008, **112**, 13352–13363.
- 116 Y. Xia, J. Xu, J. Li, B. Chen, Y. Dai and R. N. Zare, Visualization of the Charging of Water Droplets Sprayed into Air, *J. Phys. Chem. A*, 2024, **128**, 5684–5690.
- 117 K. Gong, J. Ao, K. Li, L. Liu, Y. Liu, G. Xu, T. Wang, H. Cheng, Z. Wang, X. Zhang, H. Wei, C. George, A. Mellouki, H. Herrmann, L. Wang, J. Chen, M. Ji, L. Zhang and J. S. Francisco, Imaging of pH distribution inside individual microdroplet by stimulated Raman microscopy, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**, e2219588120.
- 118 H. Nie, Z. Wei, L. Qiu, X. Chen, D. T. Holden and R. G. Cooks, High-yield gram-scale organic synthesis using accelerated microdroplet/thin film reactions with solvent recycling, *Chem. Sci.*, 2020, **11**, 2356–2361.
- 119 D. B. Eremin and V. V. Fokin, On-Water Selectivity Switch in Microdroplets in the 1,2,3-Triazole Synthesis from Bromoethenesulfonyl Fluoride, *J. Am. Chem. Soc.*, 2021, **143**, 18374–18379.
- 120 X. Yan, H. Cheng and R. N. Zare, Two-Phase Reactions in Microdroplets without the Use of Phase-Transfer Catalysts, *Angew. Chem., Int. Ed.*, 2017, **56**, 3562–3565.
- 121 E. C. Griffith and V. Vaida, In situ observation of peptide bond formation at the water–air interface, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 15697–15701.
- 122 Y. Wu, J. Li, J. Sun, Y. Wang, J. Liu and H. Cheng, Synthesis of 3-Substituted Indoles by Yonemitsu Three-Component Reactions Accelerated in Microdroplet/Thin Film, *J. Org. Chem.*, 2024, **89**, 18493–18503.
- 123 G. Rubasinghe and V. H. Grassian, Role(s) of adsorbed water in the surface chemistry of environmental interfaces, *Chem. Commun.*, 2013, **49**, 3071–3094.
- 124 C. M. Dobson, G. B. Ellison, A. F. Tuck and V. Vaida, Atmospheric aerosols as prebiotic chemical reactors, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 11864–11868.
- 125 A. Tuck, The Role of Atmospheric Aerosols in the Origin Of Life, *Surv. Geophys.*, 2002, **23**, 379–409.
- 126 A. M. Deal, R. J. Rapf and V. Vaida, Water–Air Interfaces as Environments to Address the Water Paradox in Prebiotic Chemistry: A Physical Chemistry Perspective, *J. Phys. Chem. A*, 2021, **125**, 4929–4942.
- 127 S. Nader, A. Baccouche, F. Connolly, M. Abou-Ghanem, S. A. Styler, J. D. Lewis, D. Pink and S. S. Mansy, Model Atmospheric Aerosols Convert to Vesicles upon Entry into Aqueous Solution, *ACS Earth Space Chem.*, 2023, **7**, 252–259.
- 128 H. J. Cleaves, Prebiotic Chemistry: What We Know, What We Don't, *Evol.: Educ. Outreach*, 2012, **5**, 342–360.
- 129 R. Wolfenden and Y. Yuan, Rates of Spontaneous Cleavage of Glucose, Fructose, Sucrose, and Trehalose in Water, and the Catalytic Proficiencies of Invertase and Trehalase, *J. Am. Chem. Soc.*, 2008, **130**, 7548–7549.
- 130 R. Larralde, M. P. Robertson and S. L. Miller, Rates of decomposition of ribose and other sugars: implications for chemical evolution, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 8158–8160.
- 131 I. Nam, J. K. Lee, H. G. Nam and R. N. Zare, Abiotic production of sugar phosphates and uridine ribonucleoside in aqueous microdroplets, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 12396–12400.
- 132 V. Vaida, Prebiotic phosphorylation enabled by microdroplets, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 12359–12361.
- 133 A. D. Castañeda, Z. Li, T. Joo, K. Benham, B. T. Burcar, R. Krishnamurthy, C. L. Liotta, N. L. Ng and T. M. Orlando, Prebiotic Phosphorylation of Uridine using Diamidophosphate in Aerosols, *Sci. Rep.*, 2019, **9**, 13527.
- 134 A. Bodi, J. Csontos, M. Kállay, S. Borkar and B. Sztáray, On the protonation of water, *Chem. Sci.*, 2014, **5**, 3057–3063.



- 135 A. N. Hayhurst and N. R. Telford, Proton affinity of water and the mechanism and kinetics of production of  $\text{H}_3\text{O}^+$  in flames of  $\text{H}_2$ ,  $\text{O}_2$  and  $\text{N}_2$ , *J. Chem. Soc., Faraday Trans. 1*, 1975, **71**, 1352–1362.
- 136 S. L. Miller, A Production of Amino Acids Under Possible Primitive Earth Conditions, *Science*, 1953, **117**, 528–529.
- 137 N. M. Morato and R. G. Cooks, Desorption Electrospray Ionization Mass Spectrometry: 20 Years, *Acc. Chem. Res.*, 2023, **56**, 2526–2536.
- 138 K.-H. Huang, N. M. Morato, Y. Feng, A. Toney and R. G. Cooks, Rapid Exploration of Chemical Space by High-Throughput Desorption Electrospray Ionization Mass Spectrometry, *J. Am. Chem. Soc.*, 2024, **146**, 33112–33120.
- 139 K.-H. Huang, K. Chen, N. M. Morato, T. C. Sams, E. T. Dziekonski and R. G. Cooks, High-throughput microdroplet-based synthesis using automated array-to-array transfer, *Chem. Sci.*, 2025, **16**, 7544–7550.
- 140 S. C. Kulathunga, N. M. Morato, Q. Zhou, R. G. Cooks and A. D. Mesecar, Desorption Electrospray Ionization Mass Spectrometry Assay for Label-Free Characterization of SULT2B1b Enzyme Kinetics, *ChemMedChem*, 2022, **17**, e202200043.
- 141 N. M. Morato, D. T. Holden and R. G. Cooks, High-Throughput Label-Free Enzymatic Assays Using Desorption Electrospray-Ionization Mass Spectrometry, *Angew. Chem., Int. Ed.*, 2020, **59**, 20459–20464.
- 142 R. G. Cooks, Y. Feng, K.-H. Huang, N. M. Morato and L. Qiu, Re-Imagining Drug Discovery using Mass Spectrometry, *Isr. J. Chem.*, 2023, **63**, e202300034.
- 143 Z. Jaman, T. J. P. Sobreira, A. Mufti, C. R. Ferreira, R. G. Cooks and D. H. Thompson, Rapid On-Demand Synthesis of Lomustine under Continuous Flow Conditions, *Org. Process Res. Dev.*, 2019, **23**, 334–341.
- 144 D. L. Logsdon, Y. Li, T. J. Paschoal Sobreira, C. R. Ferreira, D. H. Thompson and R. G. Cooks, High-Throughput Screening of Reductive Amination Reactions Using Desorption Electrospray Ionization Mass Spectrometry, *Org. Process Res. Dev.*, 2020, **24**, 1647–1657.
- 145 Z. Jaman, D. L. Logsdon, B. Szilágyi, T. J. P. Sobreira, D. Aremu, L. Avramova, R. G. Cooks and D. H. Thompson, High-Throughput Experimentation and Continuous Flow Evaluation of Nucleophilic Aromatic Substitution Reactions, *ACS Comb. Sci.*, 2020, **22**, 184–196.
- 146 K.-H. Huang, N. M. Morato, Y. Feng and R. G. Cooks, High-Throughput Diversification of Complex Bioactive Molecules by Accelerated Synthesis in Microdroplets, *Angew. Chem., Int. Ed.*, 2023, **62**, e202300956.
- 147 J. Li, N. M. Morato, L. S. Westover, P. Abeywickrema, J. Geng, M. Piassek, D. Harden, I. Strambeanu, Z. Shi, R. G. Cooks and J. Meng, High-Throughput Assessment of Bile Salt Export Pump Inhibition Using RapidFire-MS and DESI-MS, *ACS Med. Chem. Lett.*, 2024, **15**, 1584–1590.
- 148 Y. Feng, N. M. Morato, K.-H. Huang, M. Lin and R. G. Cooks, High-throughput label-free opioid receptor binding assays using an automated desorption electrospray ionization mass spectrometry platform, *Chem. Commun.*, 2024, **60**, 8224–8227.
- 149 J. Ghosh, N. M. Morato, Y. Feng and R. G. Cooks, High-Throughput Drug Derivatization and Bioassay by Desorption Electrospray Ionization Mass Spectrometry, *ChemPlusChem*, 2025, **90**, e202500164.
- 150 L. J. Szalwinski, L. E. Gonzalez, N. M. Morato, B. M. Marsh and R. G. Cooks, Bacterial growth monitored by two-dimensional tandem mass spectrometry, *Analyst*, 2022, **147**, 940–946.
- 151 N. M. Morato, H. M. Brown, D. Garcia, E. H. Middlebrooks, M. Jentoft, K. Chaichana, A. Quiñones-Hinojosa and R. G. Cooks, High-throughput analysis of tissue microarrays using automated desorption electrospray ionization mass spectrometry, *Sci. Rep.*, 2022, **12**, 18851.
- 152 N. M. Morato, K. E. Mason, T. H. Corzett, C. A. Valdez, T. M. Alfaro, R. G. Cooks and B. P. Mayer, Accelerating Countermeasure Candidate Discovery for A-Series Chemical Warfare Agent Exposure, *Proc. Natl. Acad. Sci. U. S. A.*, 2025, **122**(29), e2512471122.
- 153 P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, 2000.
- 154 M. Jana, K. Unni, T. Pradeep and R. G. Cooks, Accelerated Synthesis of Pyrazoles Mediated by Water Microdroplets, *ACS Sustain. Chem. Eng.*, 2025, **13**, 7645–7654.
- 155 J. Ghosh and R. G. Cooks, Accelerated click reactions using boronic acids for heterocyclic synthesis in microdroplets, *Chem. Sci.*, 2025, **16**, 8800–8806.
- 156 Y. Meng, E. Gnanamani and R. N. Zare, Catalyst-Free Decarboxylative Amination of Carboxylic Acids in Water Microdroplets, *J. Am. Chem. Soc.*, 2023, **145**, 32–36.
- 157 T. Wang, Z. Li, H. Gao, J. Hu, H.-Y. Chen and J.-J. Xu, Ultrafast C–C and C–N bond formation reactions in water microdroplets facilitated by the spontaneous generation of carbocations, *Chem. Sci.*, 2023, **14**, 11515–11520.
- 158 X. Jin, Y. Wu, J. Sun, J. Liu and H. Cheng, Catalyst-Free Three-Component Pétasis Reactions Accelerated in Microdroplets: Reaction Optimization and Sensitive Detection by Mass Spectrometry, *Analysis Sensing*, 2023, **3**, e202300031.
- 159 X. Liu, Y. Pan, Y. Yao, S. Chen, B. Chen and C. Chu, Accelerated Pollutant Degradation by UV/ $\text{H}_2\text{O}_2$  at the Air–Water Interface of Microdroplets, *Environ. Sci. Technol.*, 2025, **59**, 5406–5414.
- 160 D. Xia, H. Zhang, Y. Ju, H.-b. Xie, L. Su, F. Ma, J. Jiang, J. Chen and J. S. Francisco, Spontaneous Degradation of the “Forever Chemicals” Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) on Water Droplet Surfaces, *J. Am. Chem. Soc.*, 2024, **146**, 11266–11271.
- 161 S. Banerjee and R. N. Zare, Syntheses of Isoquinoline and Substituted Quinolines in Charged Microdroplets, *Angew. Chem., Int. Ed.*, 2015, **54**, 14795–14799.
- 162 R. M. Bain, S. T. Ayrton and R. G. Cooks, Fischer Indole Synthesis in the Gas Phase, the Solution Phase, and at the Electrospray Droplet Interface, *J. Am. Soc. Mass Spectrom.*, 2017, **28**, 1359–1364.



- 163 S. Banerjee, F. Liu, D. M. Sanchez, T. J. Martínez and R. N. Zare, Pomeranz–Fritsch Synthesis of Isoquinoline: Gas-Phase Collisional Activation Opens Additional Reaction Pathways, *J. Am. Chem. Soc.*, 2017, **139**, 14352–14355.
- 164 D. Gao, F. Jin, X. Yan and R. N. Zare, Selective Synthesis in Microdroplets of 2-Phenyl-2,3-dihydrophthalazine-1,4-dione from Phenyl Hydrazine with Phthalic Anhydride or Phthalic Acid, *Chem.–Eur. J.*, 2019, **25**, 1466–1471.
- 165 H. M. Brown, K. R. Doppalapudi and P. W. Fedick, Accelerated synthesis of energetic precursor cage compounds using confined volume systems, *Sci. Rep.*, 2021, **11**, 24093.
- 166 D. Kuai, H. Cheng, K.-Y. Kuan and X. Yan, Accelerated five-component spiro-pyrrolidine construction at the air–liquid interface, *Chem. Commun.*, 2021, **57**, 3757–3760.
- 167 M. Li, S. Yang, M. Rath, S. Kumar, C. S. Dutcher and V. H. Grassian, Enhanced condensation kinetics in aqueous microdroplets driven by coupled surface reactions and gas-phase partitioning, *Chem. Sci.*, 2024, **15**, 13429–13441.
- 168 P. Kim, R. S. Reynolds, A. M. Deal, V. Vaida, M. Ahmed and K. R. Wilson, Accelerated Zymonic Acid Formation from Pyruvic Acid at the Interface of Aqueous Nanodroplets, *J. Phys. Chem. Lett.*, 2024, **15**, 11131–11138.
- 169 C. Dai, C. Huang, M. Ye, J. Liu and H. Cheng, Mild Catalyst- and Additive-Free Three-Component Synthesis of 3-Thioisoindolinones and Tricyclic  $\gamma$ -Lactams Accelerated by Microdroplet Chemistry, *J. Org. Chem.*, 2024, **89**, 14818–14830.
- 170 S. Sun, Q. Shi, X. Li, X. Wang and Q. Yu, Microdroplet-accelerated cycloaddition of gaseous phenylacetylene and indoline derivatives to form tricyclic quinolines, *Chem. Eng. J.*, 2024, **495**, 153179.
- 171 X. Zhong, H. Chen and R. N. Zare, Ultrafast enzymatic digestion of proteins by microdroplet mass spectrometry, *Nat. Commun.*, 2020, **11**, 1049.
- 172 Y. Ai, J. Xu, H. P. Gunawardena, R. N. Zare and H. Chen, Investigation of Tryptic Protein Digestion in Microdroplets and in Bulk Solution, *J. Am. Soc. Mass Spectrom.*, 2022, **33**, 1238–1249.
- 173 T. Rainer, R. Eidelpes, M. Tollinger and T. Müller, Microdroplet Mass Spectrometry Enables Extremely Accelerated Pepsin Digestion of Proteins, *J. Am. Soc. Mass Spectrom.*, 2021, **32**, 1841–1845.
- 174 A. Li, Q. Luo, S.-J. Park and R. G. Cooks, Synthesis and Catalytic Reactions of Nanoparticles formed by Electrospray Ionization of Coinage Metals, *Angew. Chem., Int. Ed.*, 2014, **53**, 3147–3150.
- 175 J. K. Lee, D. Samanta, H. G. Nam and R. N. Zare, Spontaneous formation of gold nanostructures in aqueous microdroplets, *Nat. Commun.*, 2018, **9**, 1562.
- 176 D. Schröder, J. Roithová and H. Schwarz, Electrospray ionization as a convenient new method for the generation of catalytically active iron-oxide ions in the gas phase, *Int. J. Mass Spectrom.*, 2006, **254**, 197–201.
- 177 D. T. Holden, M. Q. Edwards, Z. Shang and R. G. Cooks, Nitrogen fixation using metallic lithium nanoparticles formed by electrospray deposition, *Chem. Sci.*, 2025, **16**, 13048–13056.

