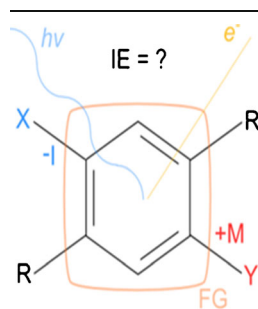**RESEARCH ARTICLE**

A Functional Group Approach for Prediction of APPI Response of Organic Synthetic Targets

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Abstract. Atmospheric pressure photoionization (APPI) is a technique of choice for ionization of non-polar molecules in mass spectrometry (MS). Reported APPI-based studies tend to focus on a selected compound class, which may contain a variety of functional groups. These studies demonstrate that APPI response frequently differs substantially, indicating a certain dependence on the functional group present. Although this dependence could be employed for APPI response prediction, its systematic use is currently absent. Here, we apply APPI MS to a judiciously-compiled set of 63 compounds containing a number of diverse functional groups commonly utilized in synthesis, reactive functional groups, as well as those containing boron and silicon. Based on the outcome of APPI MS of these compounds, we propose and evaluate a simple guideline to estimate the APPI response for a novel compound, the key properties of which have not been characterized in the gas phase. Briefly, we first identify key functional groups in the compound and gather knowledge on the known ionization energies from the smallest analogues containing said functional groups. We then consider local inductive and resonance effects on said ionization energies for the compounds of interest to estimate the APPI response. Finally, application of APPI MS to compounds of interest considered herein demonstrated extended upper mass ionization limit of 3.5 kDa for non-polymeric compounds.

Keywords: Atmospheric pressure photoionization, Mass spectrometry, Functional group, Ionization

Introduction

Atmospheric pressure photoionization (APPI) enables ionization of analytes extracted from the solvent at atmospheric pressure via a heated nebulizer by irradiating them with photons in the UV range, most commonly generated by a Kr lamp (other noble gases may also be used) [1–3]. The APPI-formed positively or negatively charged ions are analyzed with mass spectrometry (MS). To facilitate analyte ionization, the use of dopant (or have solvent acting as a dopant), such as toluene or acetone, is widely practised in APPI MS [1, 4–7]. Oftentimes, APPI compares favorably or is complementary to other commonly employed atmospheric pressure ionization techniques [8–12], such as electrospray ionization (ESI) [13] and atmospheric pressure chemical ionization (APCI) [14].

However, there are several important aspects that remain to be addressed for further progress in APPI MS applications.

First, mechanistic studies into the APPI process, for both direct and dopant-assisted positive and negative ionization modes, revealed that multiple mechanisms lead to formation of radical, protonated, and adducted species, thus voiding straightforward discussion on APPI response prediction [10, 15–19]. Secondly, quantitation studies or studies comparing atmospheric pressure ionization techniques tend to focus on a selected class of compounds, such a crude oil [20], lipids [21, 22], explosives [23], and drugs [11, 24], and evaluate the APPI response for the whole compound set within this class. Yet these compounds may represent chemically a highly heterogeneous group, resulting in varied and, at times, unexpected, APPI response [25]. Importantly, certain simple mechanistic models, based on various gas-phase ion energetics data (e.g., ionization energy values [15, 17, 18]), cannot always be employed for APPI response prediction; such data is generally not available for newly synthesized compounds and, oftentimes, for commonly utilized compounds as well [17, 18, 26]. Although one may try to use computational methods for estimation of these parameters, this may require a significant amount of computational power and time, and may result in values of low accuracy, especially if the complex is large and contains heavy elements.

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Hence, gauging the viability of successful ionization of a newly synthesized analyte seems like a daunting task, especially if said analyte is not readily ionizable via ESI.

A reference library of mass spectra with APPI response classification by compound type could be a useful approach towards APPI response prediction. However, what happens if the synthetic target is different from previously characterized compounds? The ability to extrapolate APPI response to analogous compounds, or compounds containing similar structural motifs, would be of great benefit, especially if rationalization can be done using basic chemistry principles without resorting to in silico simulations.

Here, we propose a simple guideline to rationalize and predict APPI response, despite the knowledge that inherently, multiple post-ionization chemical processes are occurring at atmospheric pressure prior to analyte entrance into the mass spectrometer. The guideline is based on a simple functional group model for estimation of APPI response of various synthetic complexes and qualitatively extrapolating gas-phase ion energetics data to molecules lacking thermodynamic characterization in the gas phase.

To ensure quasi-universal relevance for analysis of small to medium organic compounds of varied functionality and application, including drugs, energy, nanostructures, and macromolecular chemistry, the guideline is based on analysis of over 350 APPI mass spectra of current synthetic targets of interest from the Institute of Chemical Sciences and Engineering (ISIC) at École Polytechnique Fédérale de Lausanne (EPFL), with 63 representative cases selected here to highlight the guideline's application. Specific focus is placed on limits of ionization in terms of functional group presence, on in-source fragmentation of reactive functional groups and on determination of upper mass ionization limit. The guideline is additionally extended to include analytes with commonly employed heteroatoms, specifically boron and silicon. Examples including analytes containing transition metals and lanthanides are also presented.

It should be stressed that our rationalization is based on assumption of direct analyte–photon interactions. However, for relevance, most of the analytes were dissolved in toluene because of the widespread use of dopant-assisted APPI. Importantly, as shall be seen from the results and discussion below, this assumption does not detract from the approach, as APPI response (or lack of one) is consistent with expectations derived from the proposed guideline. Ultimately, this work is similar in spirit to the studies done on mass spectra interpretation from other atmospheric pressure ionization sources [27]. We hope that the discussion of APPI response estimation based on fundamental principles of organic chemistry, aided by significantly expanded diversity of molecular targets considered here, shall be of use both to synthetic chemists and mass spectrometrists.

Experimental

Sample Preparation

Most samples were acquired via collaboration with research laboratories of the ISIC section of EPFL (Lausanne, Switzerland). The samples represent current synthetic targets of interest and ionized with very low efficiency, if at all, with ESI. Particularly, new or first time synthesis has recently been reported in literature for compounds **5** [28], **6** [28], **9** [29], **28** [30], **29** [31], **36** [31], **42** [32], **43** [33], **48** [32], **49** [34], **B** [35], **D**[36], **F** [37], and analogues of **G** [38]. Compound **32** was synthesised by the group of Professor P. Dyson; compound **34** – by the group of Professor K. Sivula; compound **H** – by the group of Professor K. Severin, all of EPFL. Saponin immuno-modulator QS21, compound **E**, was isolated from *Quillaja saponaria* Molina bark extract by Vaccine Formulation Laboratory, University of Lausanne. A subset of small organic molecules with limited functional group presence was synthesized specifically for this study by the group of Professor X. Hu of EPFL. Additionally, 14 compounds were acquired from commercial vendors: compounds **2**, **12**, **13**, **21**, **26**, **38**, **50**, **53**, and **54** were obtained from Sigma-Aldrich (Steinheim, Germany); compounds **25** and **55** were obtained from Acros (Geel, Belgium); compound **37** was obtained from Alfa Aesar (Karlsruhe, Germany); compound **41** was obtained from Fluka (Buchs, Switzerland); and compound **14** was obtained from Fluorochem (Hadfield, UK). Samples were typically dissolved in tetrahydrofuran (THF) or dichloromethane (DCM), (both are >99.9% purity, Sigma-Aldrich), followed by further dilution in toluene (Chromasolv 99.9% purity, Sigma-Aldrich) for dopant-assisted APPI experiments. For direct APPI ionization, samples were diluted in DCM only; such cases are specifically highlighted in the text.

ESI and APPI Mass Spectrometry

MS experiments were performed using two hybrid Fourier transform mass spectrometers (10 T linear trap quadrupole Fourier transform ion cyclotron resonance mass spectrometer, LTQ FT-ICR MS [39], and LTQ Orbitrap Elite FTMS [40], both Thermo Scientific, Bremen, Germany) and two quadrupole time-of-flight mass spectrometers (6530 Accurate-Mass qTOF LC/MS from Agilent Technologies, Santa Clara, CA, USA and Xevo G2-S qTOF MS from Waters Corporation, Milford, MA, USA). For FTMS instruments, resolution was set to ~100,000 at 400 m/z. For Orbitrap Elite FTMS, absorption-type mode FT (enhanced FT or eFT) [41] was employed and mass spectra were acquired in reduced profile (i.e., with baseline noise removed) mode [42]. For FT-ICR MS, mass spectra were acquired in reduced profile magnitude FT mode. For TOF MS instruments, resolution was circa 20,000 at 400 m/z. ESI ionization sources included the standard ESI and heated (H)ESI source in Ion Max housing by Thermo Scientific, as well as the standard Z-spray ion source equipped with the Lock-Spray interface by Waters. APPI ionization sources with a VUV Kr lamp (Syagen, Tustin, CA, USA) were used with vendor-specific bodies: Thermo Scientific Ion Max APCI/APPI source, Agilent G1971A source, and Waters APPI source. Typical nebulizer temperature was 350°C for FTMS systems and ~650°C with desolvation gas at 500°C for TOF MS systems; typical infusion rate was 10 μL/min.

Due to the noncommercial nature of the samples provided and novelty of synthesis involved, wherein APPI did not produce a detectable signal, the solution containing the purported analyte was ionized via electron impact ionization, EI, and analyzed using a triple quadrupole instrument (Varian 1200 L MS/MSl Varian Inc., Palo Alto, CA, USA) interfaced with a gas chromatography (GC) system (Varian CP-3800) to verify analyte presence and avoid potential false negatives. Note also that for the same reasons, neither analyte concentration nor sample purity could be reliably verified in all cases, thus precluding a reliable discussion on sensitivity.

Data Processing and Data Analysis

APPI and ESI mass spectra were obtained via data acquisition and analysis software from the respective vendors; m/z values and analyte isotopic distributions were compared with theoretical values calculated using ChemCalc (http://www.chemcalc.org/) web tool [43]. To verify peak assignment when the analyte signal strength was significantly lower than the base peak (<0.1%) in reduced profile mass spectra, a noise thresholding procedure from Zhurov et al. [42] using a log plot was implemented to validate statistical significance of the signal of interest. Owing to the use of high-resolution FTMS mass spectrometers, the species of interest have been identified with high confidence levels, with mean error of 1.93 ppm for monoisotopic peak assignment (external calibration) for the 63 analytes reported here. Note that for the mass spectrometers employed in this study, it is not unusual to acquire broadband mass spectra with spectral dynamic range exceeding three orders of magnitude under typical experimental conditions.

All analytes were classified by their elemental compositions into organic compounds (i.e., composed only of H, C, N, O, or S) and those containing inorganic atoms. Within these selections, the former set was further classified into subsets based on functional groups present in the molecule. For the latter, the analytes were split into subsets of single heteroatom containing organic compounds (e.g., halogens, silicon, and boron) and large (usually) metal-containing complexes. Additionally, each analyte's mass spectrum was evaluated with respect to the strength of the APPI response and, if positive, precursor ion type was noted (e.g., radical or/and de-/protonated species, as well as prominent neutral losses, analyte adduction, fragmentation, etc. For certain analytes lacking response in positive mode, negative mode APPI mass spectra were further acquired.

Results

Use of the Toluene as a Dopant

Although addition of a dopant complicates the overall mechanistic picture by paving the way for numerous additional potential interactions in the gas phase, the use of dopant generally facilitates ionization for most compounds. Moreover, very few cases where an analyte is ionized by direct APPI but is not ionized or not ionized as well with dopant-assisted APPI, have been reported [24]. One notable drawback of dopant use is that oftentimes and especially with compounds containing aromatic systems, mass spectra show signals of dopant addition to analyte molecules (e.g., THF, methanol, toluene), as well as multiple background signals from solvent aggregation. Being able to differentiate between analyte and dopant-related signals in a mass spectrum may be important, especially in cases where the analyte signal does not dominate the mass spectrum and is of a similar order of magnitude as dopant-related peaks.

Specifically, for the case of direct infusion (via PEEK tubing) of analyte with toluene as solvent (with initial solvation in THF, DCM, or acetonitrile), a certain amount of chemical background noise is generated. If analyte ionization is the dominant channel, those background peaks tend to be below 10% of the analyte peak with a couple of notable exceptions (e.g., m/z 108.0576 and 199.113, $C_7H_8O^{+\bullet}$ and $C_{14}H_{15}O^+$, respectively). When analyte peaks are not prominent, it may be particularly difficult to detect them, especially if the chemical composition of solvent peaks is not fully known, and there is a possibility that the analyte is fragmenting. To this end, we examined the mass spectrum of one of the least well ionized analytes (see Supplementary Figure S1 in Supporting Information), for the dopant-assisted APPI mass spectrum. Specifically, circa 3500 peaks attributable to solvent were identified (note that most of those were of low abundance, less than 1% of base peak; few of these had intensity above 10%) within 100–500 m/z range. Nearly all of these were classified as hydrocarbons with 0 to 7 oxygens. Kendrick plots [40, 44] of each oxygen class can be found in Supplementary Figure S1. As such, if the analyte contains elements other than C, H, or O, the search for the analyte via elemental composition matching should be greatly simplified. However, if the analyte is composed solely of C, H, and O, such as compounds **21** and **25**, care must be taken to ensure that the assigned species is the analyte, rather than a solvent isomer. To that end, comparison of relative abundances of solvent peaks within a ~10 m/z window to those from a reference mass spectrum obtained under similar experimental conditions is recommended. Additionally, in situ fragmentation of the peak of interest may be used to verify the presence of the correct isomer.

Detection of [M – H]$^+$ species

Generally, analyte ion species reported for mass spectra acquired using APPI ionization include the radical cation, $M^{+\bullet}$, and the protonated analyte, $[M+H]^+$. However, we have additionally observed on multiple occasions formation of $[M – H]^+$ species clearly attributable to the analyte. Said analytes typically contain a triple bond, e.g., Figure 1 compounds **4-6**, or ethers, esters, and amides, e.g., Figure 1 compounds **11**, **19**, **22**, **27**, **32**, and **33** (see Supplementary Figure S2 for the associated mass spectra). In certain cases, such as compound **36**, the $[M – H]^+$ is the only analyte ion species detected. Given the nature of the analytes involved, it is likely that the formation of a radical is followed by proximate hydrogen loss.
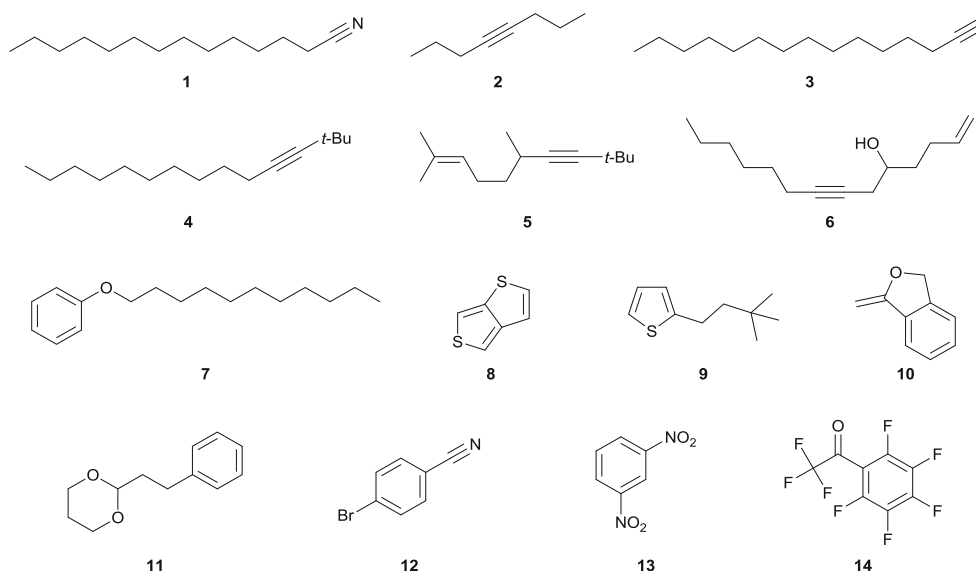
Figure 1.　Nitriles, alkynes, aromatic heterocycles, and an analyte with long alkyl chain bearing an amide and an amine (~1.3 kDa): 2D structures of compounds **1–14**. Associated APPI mass spectra may be found under assigned compound number in Figure S2, Supporting Information

APPI Mass Spectrometry of Selected Compounds

Figure 1 shows representative cases of analytes containing nitriles and substituted alkynes, compounds **1–6**. All of these analytes were successfully ionized via APPI, with compounds **1-3** ionized via direct APPI and **4–6** [28] ionized via dopant-assisted APPI. In all cases, a prominent signal from the analyte ion species was detected (see Supplementary Figure S2 for the associated mass spectra). Compounds **7**–**13** [29] in Figure 1 include a selection of unsaturated homo- and heterocycles commonly encountered in synthetic targets. Except for compounds **12** and **13**, all analytes were dissolved in toluene. In all cases, the analyte ion peaks are prominent in the mass spectra, frequently being the base peaks (Supplementary Figure S2).

Perfluoroactophenone, compound **14**, did not produce a detectable signal in APPI MS (Figure 1 and Supplementary Figure S2); its presence was verified by EI MS (data not shown).

Figure 2, compounds **15**–**33** [30], compiles analytes with various common carbonyl-based functional groups, including amides, esters, aldehydes, lactones, cyclic ketones, α,β-unsaturated carbonyls, as well as cyclic ethers. These analytes were mostly selected to highlight the limits of ionization in terms of functional group presence. In all cases, the analyte ion was detected, albeit in several instances, such as for compounds **17** or **23**, the mass spectra were dominated by ionized dopant derivatives (Supplementary Figure S2). Nevertheless, in all cases, the analyte ion could be isolated for further interrogation
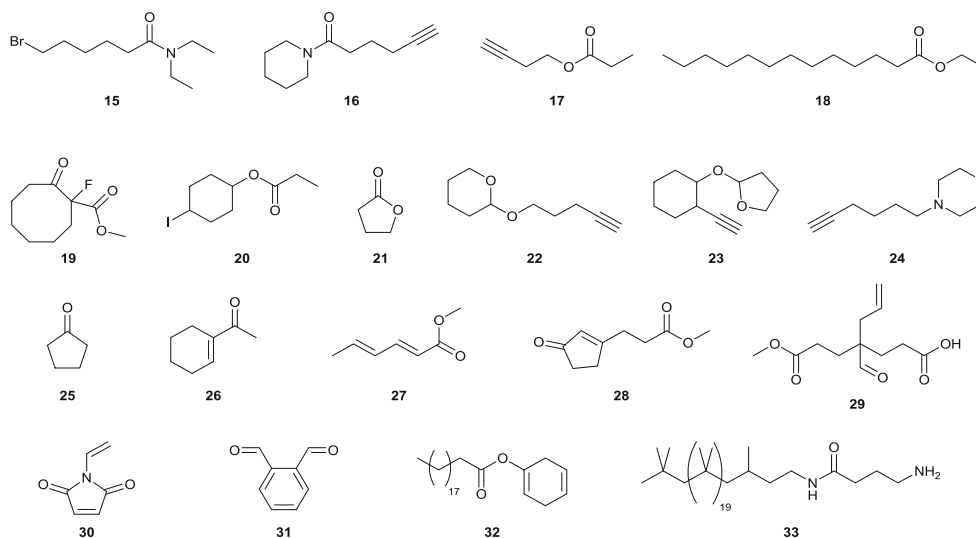

Figure 2.　Selection of analytes bearing typical functional groups: 2D structures of compounds **15–33**. Associated APPI mass spectra may be found under assigned compound number in Figure S2, Supporting Information

via tandem mass spectrometry (MS/MS) experiments. Compound **33**, a 1.3 kDa analyte with an amide and amine moiety (Figure 2), produced multiple signals in addition to that of the analyte ion, most of them coming from different degrees of polymerization as well as the oxidized form of the polymer (Supplementary Figure S2). Table 1 summarizes the key functional groups, the presence of which generally results in positive APPI response along with the dominant analyte ion type.

Compounds **34–41** [31], Figure 3, are analytes containing various reactive functional groups the presence of which usually impedes detection of the analyte ion: azide [45], nitro [23], epoxy [46], and peroxy acids (Supplementary Figure S2). Specifically, compounds **34–36** contain an azide group. For compound **35**, the analyte ion was not detected and strong loss of N_2 and N_3 was observed (presence of intact species was confirmed via EI MS, data not shown). Despite azide presence, analyte ion of compound **34** was the base peak in the APPI mass spectrum, whereas for compound **36**, strong loss of N_2 upon analyte ionization was observed (Supplementary Figure S2). Epoxy group containing compounds **37** and **38** were detected, albeit the mass spectra are dominated by the dopant derivatives. Compound **39**, with a nitro group, ionized well, with the analyte ion being the base peak. On the other hand, compounds **40** and **41**, containing a nitro and a peroxy group, respectively, did not ionize well in positive APPI mode, and the mass spectra were obtained in negative APPI mode.

Compounds **42–49** [32–34], Figure 4, all include trialkylsilyl moieties and ionized well, with analyte-related peaks being dominant in all mass spectra (Supplementary Figure S2). Note that analyte ionization was accompanied by various degrees of fragmentation by formation of an alkyl radical from the trialkylsilyl moiety.

Compounds **50–55**, Figure 4, represent a set of boron containing analytes. All the boronic esters ionized in positive APPI mode, albeit in most cases the mass spectra were dominated by dopant derivatives (Supplementary Figure S2). Boronic acids, compounds **53** and **54**, did not ionize in positive mode APPI and, instead, analyte ion signals were detected by using negative mode APPI (Supplementary Figure S2).

Compounds **A–H**, Figure 5, with the exception of compound **E**, were all detected in positive mode APPI (Supplementary Figure S2). Compound **E** was detected in negative mode APPI instead. Of particular interest is the mass of the analytes, ranging from 0.8 to 3.5 kDa, thereby extending the previously reported m/z values for non-polymeric analytes successfully ionized by APPI. In all cases, the isotopic envelope attributable to the analyte ion was identified (Figure 6 and Supplementary Figure S2).

Discussion

APPI Response Rationalization

Consider the primary ionization process in direct APPI ionization (i.e., without dopant assistance), $M + hv -> M^{+\bullet} + e^-$. Clearly, the APPI response will be primarily determined by the energy of the incipient photons (which in commercially available APPI sources is either 10 or 10.6 eV, for a Kr discharge lamp) and the ionization energy, IE, of the analyte. Disregarding any solvent–analyte interactions, it is evident that the analyte IE must be less than 10.6 eV for efficient analyte ionization. Simple as this process is, one caveat is that solution phase IE values differ from gas-phase IE values [47–49] and only a limited number of analytes (generally limited to small organic molecules) have gas-phase ion data available from experimental studies or from computations [26, 50, 51]. However, as gas-phase ion energetics data is usually available for the smallest molecules for a given common functional group [26], it is possible to use these IE values and apply them to larger analogues by considering the additional inductive and mesomeric effects from neighbouring groups.

For instance, acetonitrile, one of the most common MS solvents, has IE value of ~12.2 eV and, as such, is not ionized when irradiated with UV photons, thus allowing the latter to interact directly with the analyte. Note that solvent aggregation may enable additional analyte–solvent interactions, but this is outside the scope of current discussion [52, 53]. If the alkyl chain of a nitrile is extended, the positive inductive effect from the growing alkyl group should result in lower IE values. Indeed, such effect is observed for calculated IE values for propanenitrile (IE ~11.9 eV) and butanenitrile (IE ~11.6 eV) [26], albeit IE values are not readily available for pentanenitrile and nitriles with longer alkyl chains. Nevertheless, as may be expected, tetradecanenitrile (Figure 1, compound **1**) [54], is detected by APPI without dopant use (Supplementary Figure S2), indicating that the inductive effect is strong enough to lower its IE value below 10.6 eV.

Table 1. List of Functional Groups Present in Analytes That Were Successfully Ionized Via Positive Mode APPI and Their Corresponding Typical Ion Types Observed in APPI Mass Spectra

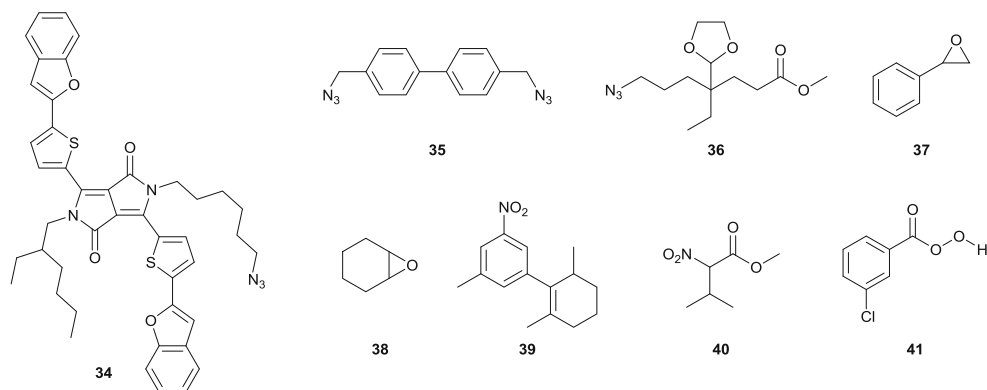| Functional group		Ion	Compound
Nitrile	R-C≡N	[M+H]$^+$	**1**
Alkyne	R-C≡C-R	M$^{+\bullet}$	**2, 3, 4, 5, 6**
Aromatic rings		M$^{+\bullet}$	**7, 8, 9, 10, 11, 12, 13, 14, 32**
Amide	R(C=O)NR$_2$	[M+H]$^+$	**15, 16**
Ester	R(C=O)OR	[M+H]$^+$	**17, 18, 19, 20, 32**
Cyclic ether	R-O-R	M$^{+\bullet}$	**21, 22, 23, 24**
Cyclic ketone	R(C=O)R	[M+H]$^+$	**25, 26**
α,β-unsaturated carbonyl	RC=C-(C=O)	[M+H]$^+$	**27, 28, 31**

Figure 3. Analytes bearing reactive functional groups: 2D structures of compounds **34–41**. Associated APPI mass spectra may be found under assigned compound number in Figure S2, Supporting Information

Another example can be alkynes, since alkyne bonds are commonly encountered and present borderline cases: small terminal alkynes have IE values above 11 eV, e.g., IE for 1-hexyne is estimated at ~10–10.5 eV [26]. For 3-hexyne and other more substituted alkynes, the IE values are circa 0.6–0.7 eV lower than for 1-hexyne, thus making them more amenable to ionization. For 4-octyne, compound **2**, the IE value is estimated to be ~9.2 eV [26] and, indeed, the (dopant-free) APPI mass spectrum reveals the analyte signal (Supplementary Figure S2). IE value of 1-octyne is estimated to be below 10 eV [26], also resulting in a detectable pseudomolecular ion signal in the APPI mass spectrum (data not shown). For 1-pentyne, no IE value is available, yet one may expect that the inductive effect will be stronger and the compound should be ionizable: it is, indeed, the case, with the analyte ion present in the mass spectrum (Supplementary Figure S2). Compounds **4–6** also represent analytes lacking gas-phase ion energetics data, yet from considerations above, are expected to produce a positive APPI response. Indeed, the APPI mass spectra of compounds **4–6** contain analyte and analyte-related peaks dominating the mass spectra (Supplementary Figure S2). These three mass spectra were acquired with dopant use; however, the odd electron precursor

dominates in ratio of at least 10:1 w.r.t. the protonated species. Other prominent peaks include solvent adduction to the analyte for compound **4** and formation of $[M+O]^+$ species for compounds **4** and **5**. Lastly, compound **6** contains a hydroxyl group that is typically prone to H_2O loss (via protonation), as has also been reported in other studies [22, 25].

The next step is to include resonance or mesomeric effects, especially on aromatic systems because of their ubiquitous presence. Successful ionization of compounds with unsaturated heterocycles by APPI is well documented [18] (e.g., the widespread use of APPI for analysis of nonpolar components of crude oils, where a significant percentage of analytes have polyaromatic hydrocarbon cores) [20, 55]. As may be expected, given that IE of benzene is 9.25 eV and of unsaturated heterocycles is even lower, representative compounds **7–11** (Figure 1) ionized well in APPI, producing very abundant radical cation $M^{+\bullet}$ peaks in the mass spectra, save for compound **10**, which produces mainly $[M+H]+$ species (Supplementary Figure S2). However, what happens if a benzene ring is substituted with multiple electron withdrawing groups? Work by Robb et al. [18] nicely summarizes the effects of a single electron donating/withdrawing substituent on IE value of benzene where electron withdrawing groups increase
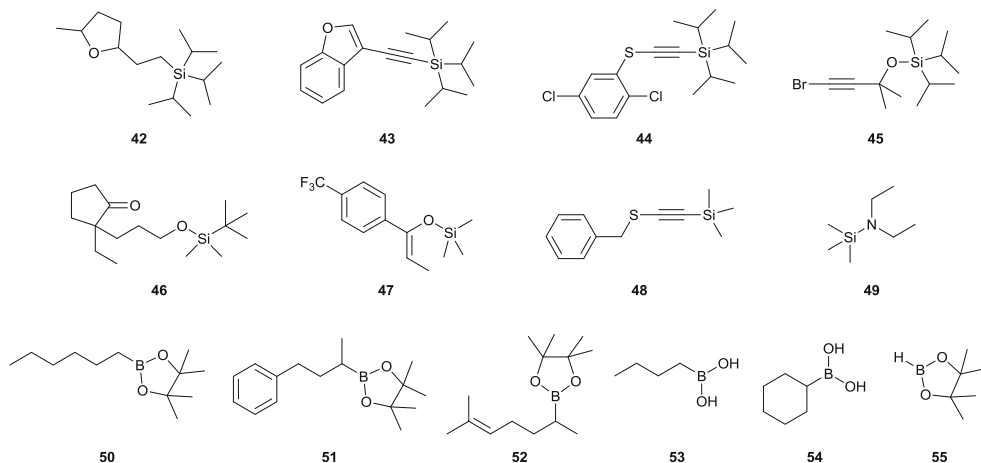

Figure 4. Silicon and boron-containing analytes: 2D structures of compounds **42–55**. Associated APPI mass spectra may be found under assigned compound number in Figure S2, Supporting Information
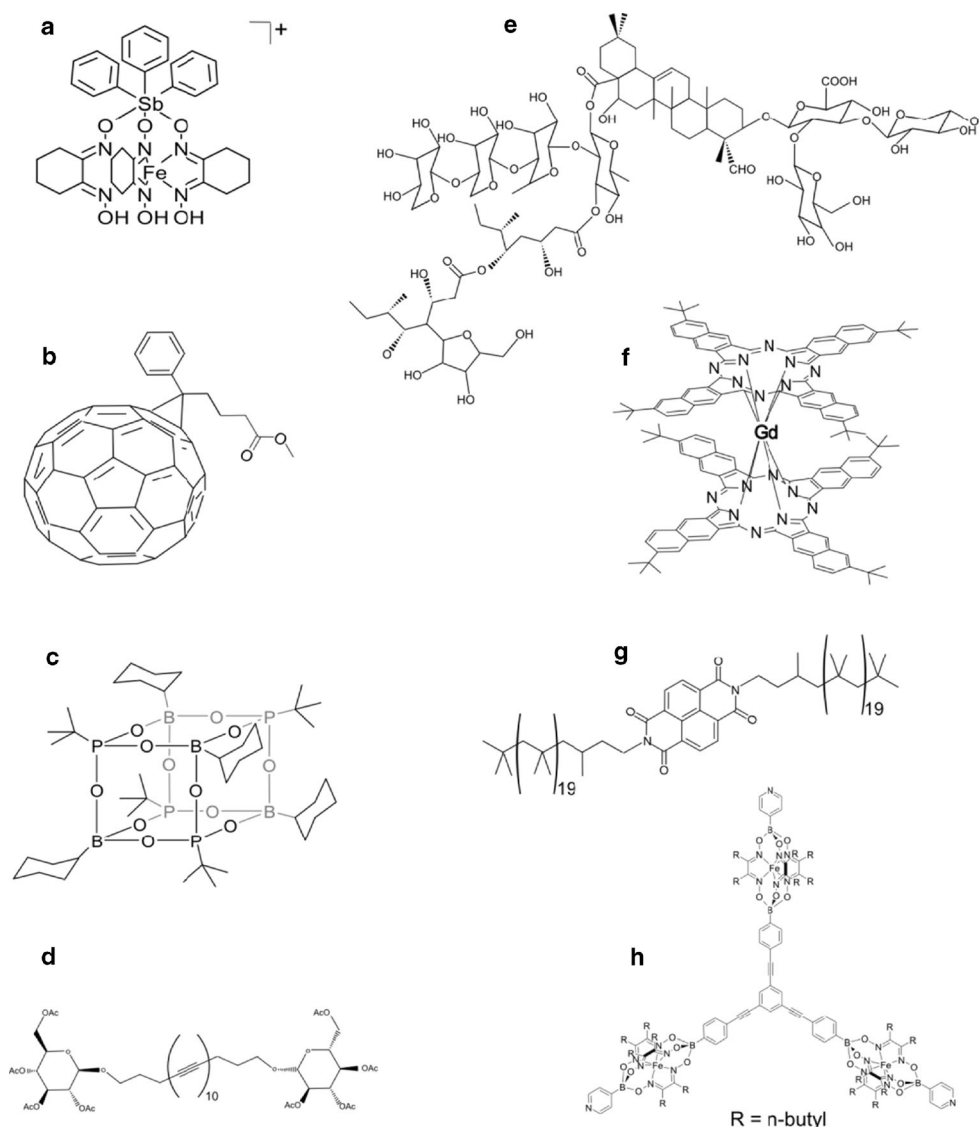
Figure 5. A selection of recent synthetic targets of interest with mass near 1 kDa and above. The compounds have been arranged by increasing nominal mass: **A**=830 Da; **B**=910 Da; **C**=921 Da; **D**=1018 Da; **E**=1989 Da; **F**=2031 Da; **G**=2649 Da; **H**=3534 Da
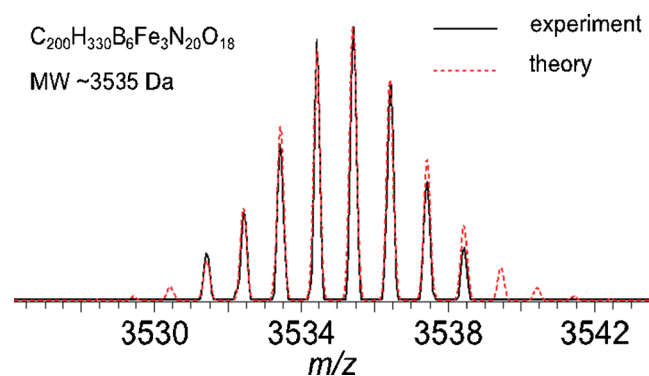

$C_{200}H_{330}B_6Fe_3N_{20}O_{18}$

MW ~3535 Da

Figure 6. An expanded view of a broadband APPI mass spectrum acquired on 10 T FT-ICR MS (black solid line) of the 3535 Da complex **H** from Figure 5. Resolution at 3500 m/z is circa 17,000 and the theoretical isotopic distribution (red dotted line) is calculated for this resolution setting

the IE. It has also been reported that two analytes with multiple electron withdrawing substituents on phenyl rings did not produce a detectable signal in the APPI mass spectra, presumably due to IE values being above 10.6 eV [25]. Compounds **12** and **13** represent two disubstituted benzene rings: 4-bromonitrile (IE~9.9 eV) [26] and 1,3-dinitrobenzene (IE~ 10.4-10.6 eV), respectively [26]. In both cases, in the (dopant-free) APPI mass spectra, a $[M+H]^+$ signal is detected, albeit multiple non-analyte peaks are also present (Supplementary Figure S2). The two compounds represent an upper limit in terms of ionization energy via direct photon–analyte interactions. Other analytes with disubstituted benzene rings with nitro, nitrile, and halo groups were all successfully ionized (data not shown; expected IE values less than 10.6 eV). Thus, generally one may expect to ionize any disubstituted benzene rings and related aromatic systems with commonly encountered functional groups. However, it is possible to

deactivate the ring towards ionization via more "exotic" substitutions, as is the case for compound **14** (Figure 1), which does not render a signal in the APPI mass spectrum (Supplementary Figure S2). Note that for triply substituted benzenes, such as trinitrobenzene, IE values are expected to be above 10.6 eV [26] and, indeed, studies of explosives with similar analytes employ APPI in negative ionization mode instead (see below for more detailed discussion of negative ionization mode) [23].

Figure 2 (compounds **15**–**32**) illustrates a selection of analytes with ubiquitous functional groups which may, from the considerations above, constitute borderline cases and which, with dopant-assisted APPI, have produced detectable signals in the mass spectra (Supplementary Figure S2). Note that while isolated aldehydes tend not to ionize, there are cases where the aldehydes are likely involved in primary ionization channels. The benzene ring in compound **31** is deactivated by the two aldehydes. However, the aldehydes are, in turn, activated, resulting in the presence of $[M+H]^+$ species in the mass spectrum (Supplementary Figure S2), as is typical for other carbonyl based compounds. Other benzene-based compounds mainly result in $M^{+\bullet}$ ions when lacking carbonyl substituents. A molecule with an ester and a cyclodiene in proximity, but not conjugated, such as compound **32**, produced near equi-abundant $M^{+\bullet}$ and $[M+H]^+$ peaks in the APPI mass spectrum (Supplementary Figure S2).

Functional Group Approach to APPI Response Prediction

From the discussion above, one can define key functional groups directing APPI response (see, for example, Table 1). These functional groups can be identified, akin to chromophore identification for UV-vis spectroscopy, in a molecule of interest. In the absence of such functional groups, one may identify other potential functional groups of interest based on high degree of electron delocalization (which stabilizes the resultant radical upon ionization) or regions of high electron density (which increases the energy of the neutral analyte). One then may consider vicinal atoms to determine any strong inductive or mesomeric effects, thus gauging potential APPI response. As an example, consider compound **33** (Figure 2), a 1.3 kDa analyte with an extensive alkyl chain terminating in an amine with a nearby amide group. The mass spectrum contains signals coming from the analyte, along with a small signal from the oxidized species, and multiple signals from other *n*-mers present in the sample (Supplementary Figure S2). The model can then be extended to analytes containing other important common functional groups [56–59]. The presence of these functional groups may indicate positive APPI response as long as strongly electron withdrawing moieties are not present nearby.

Reactive Functional Groups

Additional consideration was given to molecules containing reactive functional groups (Figure 3, compounds **34**–**41**). This set of compounds is of particular importance given that these reactive functional groups can fragment during ionization and, hence, quantitation attempts are affected by partially or fully depleted intact analyte ion signals (Supplementary Figure S2). On the other hand, partial fragmentation of the precursor ion may indicate presence of various functional groups within the molecule. For instance, alcohols and carboxylic acids typically lead to water loss (and COOH loss in some cases for the latter), whereas aldehydes tend not to decompose. Yet if the group itself is not directly ionized and is not in proximity of the suspected site of ionization, it may be preserved. Azide-containing analytes (compounds **34**–**36**), are a good illustration of this hypothesis. Typically, azides show strong $[M+H-N_2]^+$ loss [45], as well as, occasionally, loss of N_3 species (see APPI mass spectrum of compound **35** in Supplementary Figure S2). However, for compound **34**, the azide is far from the polyaromatic core and without nearby acidic hydrogens. This resulted in only minor $[M+H-N_2]^+$ fragment ion (14% of the base peak) with no N_3 loss. It is thus expected that APPI mass spectra of azide compounds with easily protonated functional groups in the vicinity of the azide will reveal strong signals associated with the $[M+H-N_2]^+$ ion. This hypothesis was further tested on five azide-containing compounds from Vita and Waser [60] (see Table 3, entries 1, 5, 6, 8, and 9 in the cited reference). Indeed, under typical experimental conditions (toluene as solvent, nebulizer temperature set at 350°C), none of the APPI mass spectra contained the intact analyte ion species and the most prominent analyte signal was that of the $[M+H-N_2]^+$ ion. However, by changing the solvent to DCM and lowering the nebulizer temperature to 200°C, the intact analyte ion was detected in all cases. The $[M+H-N_2]^+$ ion was detected in ratios varying from 10:1 to 1:100 w.r.t. the analyte ion for different analytes (data not shown).

Alternatively, the analyte may undergo extensive adduction and other reactions in the gas phase (e.g., Meerwein reaction of epoxides in presence of acetonitrile [46]). For the two epoxides considered here, compounds **37** and **38**, $M^{+\bullet}$ and $[M+H]^+$ ions were observed, respectively (note presence of toluene, rather than acetonitrile, as used by Wu et al. [46]). This principle can be applied to derivatization of other functional groups for their identification, such as gas-phase hydroboration reactions, see below.

Analytes with nitro groups are generally stable, such as compound **39**, albeit they may present a different challenge, as they are strongly deactivating. Thus, if the likely ionization site is in the proximity of one or more nitro groups, it may be difficult to generate the desired cation, although in case of compound **39** the nitro group is not sufficiently deactivating to increase the IE value above 10 eV.

So far, the discussion has been limited to generation of cations via APPI. However, the benefit of APPI lies in the fact that numerous processes occurring during ionization generate not only cations but anions as well (e.g., via capture of low-energy electrons ejected from other analyte/solvent molecules or via charge-exchange) [8, 17]. Importantly, those factors that contribute to the increase of IE may also contribute to the

likelihood of the analyte accepting an electron since extensive delocalization is more conductive to electron placement into the lowest unoccupied molecular orbital. Negative inductive effects create a local region of reduced electron density/electron deficiency, which, again, is favorable for electron attachment and increases electron affinity of the molecule. Indeed, successful use of negative mode APPI on explosives with benzene rings multiply substituted with nitro groups has recently been reported: these strongly electron withdrawing groups allowed for successful analyte ionization in negative mode APPI [23]. By extension, compound **40**, which was barely detected in positive mode APPI (less than 0.4% of base peak), showed a prominent signal (80% of solvent base peak) in negative mode. The proximity of the nitro group presumably deactivated the typical ester protonation pathway. Another example is benzoic acid, which ionized significantly better in negative mode than in positive mode (data not shown) [1]. Compound **41**, meta-chloroperoxybenzoic acid, a common oxidant in organic synthesis [61], was of greater interest: the precursor ion was present (2% of base peak), but there was a strong fragmentation via loss of OH (base peak in mass spectrum). Finally, negative mode APPI may be applied to ionization of other electron deficient analytes, including those containing heteroatoms, see below.

APPI Response: Boron and Silicon Containing Compounds

The next step is to consider other heteroatoms. Among the most commonly encountered heteroatoms in organic synthesis are phosphorous [62], silicon [63, 64], and boron [65, 66]. Since a number of APPI studies concerning phosphorous-containing molecules have already been published [16, 21, 67], we focus the discussion on analytes containing silicon and boron.

Silicon, as a more electropositive element than carbon (1.9 versus 2.55 on Pauling scale), should have a stronger positive inductive effect when present near functional groups. The vast majority of Si-containing synthetic targets of interest included a trialkylsilyl group, with few notable exceptions (Figure 4, compounds **42** to **49**). All of these produced prominent signals in APPI mass spectra, except for compound **49** (see Supplementary Figure S3 for the log plot), with various degrees of fragmentation of the analyte (Supplementary Figure S2). A loss of C_3H_7 was commonly observed for molecules containing triisopropylsilyl moiety (e.g., in compounds **42**–**45**). For compound **42**, the analyte contains only the tetraalkylsilyl moiety and a cyclic ether—note that the base peak is attributed to C_3H_7 loss, indicative of analyte ionization at the silicon, and a small signal attributable to the analyte ion (0.5% of base peak), presumably via protonation of the ether. Note that for compound **44**, C_3H_7 was coupled to addition of H_2O, likely a result of sulphur presence in the analyte. For compounds **45** to **47**, which contained Si–O bonds, the mass spectrum of compound **45** shows strong loss of isopropyl group, likely due to the siloxy group being most probable site of electron detachment, whereas compound **46** showed strong loss of the *t*-butyl group

and compound **47**, with only trimethylsilyl group, contained mainly the radical precursor species. Trimethylsilyl-containing compounds, such as compounds **47**, **48**, and **49**, produced no significant fragmentation (mass spectrum of compound **48** additionally includes a peak attributable to solvent adduction). This is easily rationalized in terms of stability of the forming radical, with *t*-butyl and isopropyl radicals being significantly more stable than the methyl radical. Note that compound **49** contains a Si–N bond, presence of which was sufficient to ionize the molecule, albeit the mass spectrum was dominated by dopant-related species.

Boron, with its empty *p* orbital, is a good electron acceptor, and boron-based compounds are a well-known class of Lewis acids. Indeed, among the selection of boronic acids and boronic esters (Figure 4, compounds **50**–**55**), only boronic esters, compounds **50**–**52** and **55**, ionized successfully in positive mode APPI (Supplementary Figure S2). Oxygen lone pairs can better conjugate with the boron *p* orbital because of inductive effect from the carbon reducing the Lewis acidity [65]. Boronic acids **53** and **54**, which are useful synthetic intermediates in medicinal chemistry and chemical biology [68], were successfully ionized by negative mode APPI instead (Supplementary Figure S2).

Finally, note the lack of B–H bonds in the examples above, which effectively preclude hydroboration reactions. Compound **55** is a boronic ester with a B–H bond and, as may be expected in presence of double bonds, extensive adduction throughout the mass spectrum was observed (see Supplementary Figure S3 for the log plot) [69]. In this case, it may be preferable to opt for a solvent such as DCM to preclude any extensive in situ hydroboration. Alternatively, as with Meerwein derivatization of epoxides [46], one may wish to take advantage of this reaction and thereby mark boron-containing adducts (since the resultant signals are intense and isotopic envelopes of analyte derivatives are readily recognizable by the presence of the ^{10}B isotopologue).

Application of the APPI Predictive Approach

The following stepwise procedure is recommended for positive mode APPI response estimation; see Scheme 1 for illustration of key steps. For the analyte of interest, locate key functional groups and determine whether gas phase ion energetics data is available for the smallest molecular analogues containing said functional groups. Consider whether the ionization energy value is larger than 10–10.6 eV and whether local inductive and mesomeric effects are likely to reduce or increase the IE value of the analyte of interest. If the resultant value is less than 10.6 eV, it is likely that the analyte will be ionized via positive mode APPI. If an analyte includes two or more functional groups with antagonist responses in APPI, the modus operandi is to focus on a functional group keyed towards positive APPI response and evaluate the proximate structure accordingly. Table 1 summarizes typical functional groups present in molecules that produced detectable pseudomolecular ion signals in positive mode APPI mass spectra considered in this work.
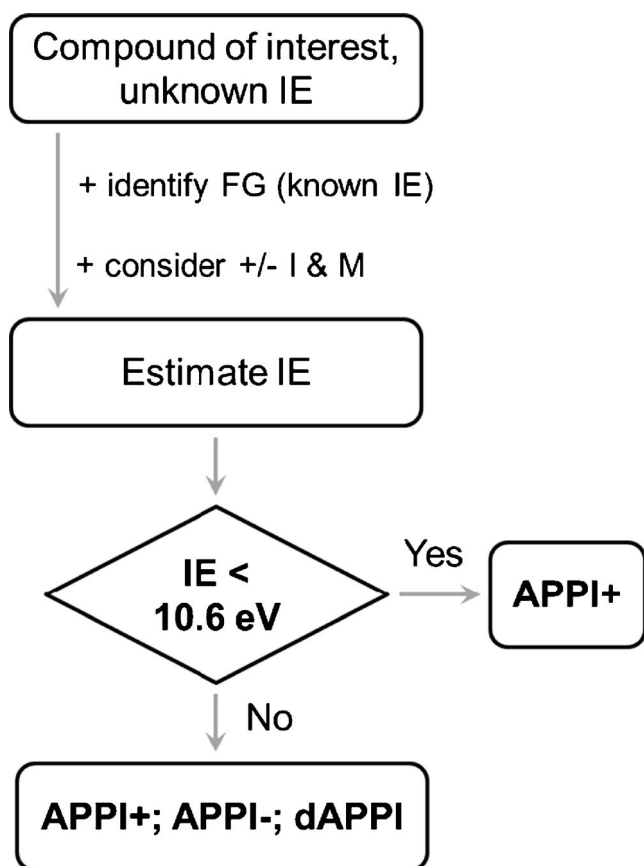
Scheme 1. A flow chart showing the key steps in the APPI predictive approach. Positive and negative inductive and mesomeric effects are labelled as "+/– I, M". Notations: IE= ionization energy; FG=functional group(s); I=inductive; M= mesomeric; and d=dopant

Those include substituted benzenes, various carbonyl groups, nitriles, and alkynes. The predictive approach outlined above has also been successfully applied to organic compounds containing functional groups commonly utilized in organic synthesis along with various heteroatom-containing analytes.

Contrastingly, if strong electron withdrawing effects create a region of low electron density, it is worth considering using APPI in negative mode, especially if electron affinity values are available for similar compounds. Owing to the low number of analytes ionized in negative mode in this work, no general conclusions can be made with a high degree of certainty. However, it may be noted that the presence of multiple deactivating groups (e.g., nitro) or high degree of resonance (e.g., C_{60}), is conductive towards electron attachment to the analyte and any functional groups that typically form stable anions, such as carboxylic acids, tend to enable formation of analyte anions.

Upper Mass Limit on APPI Ionization

The upper mass ionization limit for APPI, although briefly mentioned in various articles and vendor manuals [27], has never been systematically evaluated. Generally, it is assumed that it is

similar to the APCI limit, and some suggest that compounds below 3 kDa may be ionized [27]. With the sole exception of polymers, where signals exceeding 5 kDa were recently reported [70], to our knowledge no study to date explicitly demonstrated the ability of APPI to successfully ionize non-polymeric analytes above 2.5 kDa. However, this is of particular interest to researchers in areas such as supramolecular chemistry [71, 72], drug delivery via nanomaterials [73, 74], solar cells development [75–77], or vaccine adjuvants [78, 79], as oftentimes compounds of interest lack basic/acidic sites, may be non-polar, and do not ionize well with ESI. Here, a selection of such analytes investigated in our institute is given, compiled from recently published research articles [35, 37, 80–83]. Notably, APPI was able to ionize analytes near or above 3 kDa, with several selected complexes showcasing the variation in underlying chemical diversity demonstrated in compounds **A**–**H**, which also include metal complexes (see Figure 5 for compound structure and Supplementary Figure S2 for associated APPI mass spectra). Figure 6 shows the precursor ion isotopic cluster for the largest ionized complex **H**. Although for small coordination complexes, singly bound ligands were found to be partially substituted by the solvent (e.g., toluene) [84], and although it is generally the case for the considered analytes, the analyte precursor ion species is still detected in all cases, with varying degree of accompanying fragmentation. Another 30 complexes or so, of similar structural composition and in the mass range of 1–3.5 kDa have also been successfully ionized with APPI (data not shown). Note that 3.5 kDa is not necessarily the upper mass limit of ionization per se, and only represents the largest successfully ionized complex to date via positive mode APPI in this work. For negative mode APPI, compound **E**, circa 2 kDa, has been successfully ionized and represents the largest detected anionic species in this study.

Conclusions

In this work, a comprehensive set of compounds with diverse functional groups was analyzed with APPI mass spectrometry. The APPI response was evaluated by considering the functional groups present in analyzed compounds. A simple protocol was proposed to both rationalize the results and be used as a predictive tool for determining whether a newly synthesized compound, which lacks gas-phase characterization and which does not ionize well via ESI, is likely to have a positive APPI response. By identifying key functional groups in the analyte, and using gas-phase ion energetics data for the analyte analogues with similar functional groups, one can consider the local inductive and resonance effects to gauge the net effect upon IE values and thus qualitatively estimate the likelihood of positive APPI response. Despite the complexity of multiple mechanisms involved in dopant-assisted APPI ionization, the proposed functional group approach correlates well with the acquired data. We hope it will serve as a useful guide to analyte characterization by mass spectrometry. Additionally, upper ionization limits were considered for various complexes. It

was established that species beyond 3 kDa may be ionized with positive mode APPI and beyond 2 kDa with negative mode APPI.

Acknowledgments

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