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Chirality

Photoelectron Circular Dichroism in the Photodetachment of Amino Acid Anions

*Peter Krüger and Karl-Michael Weitzel**

Abstract: *The chirality of chemical compounds is of undisputed importance in science and technology. In particular with respect to pharmacological application most molecules of interest cannot be accessed by the powerful techniques developed in recent years for gas phase analytes. Here, we demonstrate that the combination of electrospray ionization (ESI) with the detection of photoelectron circular dichroism (PECD) provides access to chirality information applicable to molecular materials with negligible vapor pressure, for example, amino acids. To this end, glutamic acid and 3,4-dihydroxyphenylalanine (DOPA) have been electrosprayed into the source of a chirality spectrometer, where photodetachment is enforced and the PECD is detected. The technique can be expected to be conceptually applicable to all chemical systems with chirality based on molecular properties.*

Enantiomers often exhibit different bioactivity (pharmacology, toxicology, pharmacokinetics, and metabolism) in the homochiral human body, despite having similar physical and chemical properties in achiral environments.[1] Therefore, the identification and quantification of non-identical mirror images is a crucial task for analytical methods. Among the chiroptical tools commonly used for the distinction of enantiomers and structural investigation of biopolymers are the Raman optical activity (ROA)[2] and the differential absorption of left (LCP) and right circularly polarized light (RCP)—the circular dichroism (CD), in general observed in one photon electronic (ECD) or vibrational (VCD) absorption.[2–4] Due to the limited selectivity of the CD the analysis of mixtures containing multiple components or structures poses a major challenge. To overcome this limitation, the combination with mass spectrometry (MS) has been implemented for volatile compounds by using circularly polarized light for multiphoton ionization of neutrals in the gas phase and measuring the CD in total ion yields Y (PICD) [Eq. (1)].[5,6]

$$\mathrm{PICD} = 2\left(\frac{Y_{\mathrm{LCP}} - Y_{\mathrm{RCP}}}{Y_{\mathrm{LCP}} + Y_{\mathrm{RCP}}}\right) \quad (1)$$

This was first demonstrated with nanosecond lasers[5,6] and later extended to the ultrafast time regime using femtosecond lasers.[7,8] Recently the first measurements of mass-selected biomolecular anions (DNA strands) via photodetachment were reported, extending the scope of the PICD method to structural analysis in the gas phase.[9]

Chiral information is not only contained in the total yields of ions (and electrons), but can be found in asymmetries of the angular photoelectron distributions along the laser propagation axis as well. The latter is known as photoelectron circular dichroism (PECD) and can be defined in terms of yields Y in forward (F) and backward direction (B) for different helicities of light (LCP/RCP) [Eq. (2)].[10]

$$\mathrm{PECD} = 2\left(\frac{Y_{\mathrm{LCP,F}} - Y_{\mathrm{LCP,B}}}{Y_{\mathrm{LCP,F}} + Y_{\mathrm{LCP,B}}} - \frac{Y_{\mathrm{RCP,F}} - Y_{\mathrm{RCP,B}}}{Y_{\mathrm{RCP,F}} + Y_{\mathrm{RCP,B}}}\right) \quad (2)$$

The PECD effect was first predicted theoretically[11] and subsequently observed experimentally in single photon ionization[12] and in multiphoton ionization.[10,13,14] While CD and PICD rely on magnetic and electrical transition dipole moments, the PECD is a pure electric dipole effect.[14]

PECD was shown to be sensitive not only to absolute configuration, but also to static[15–22] and dynamic[23] conformation due to scattering of the outgoing electron in the chiral molecular potential. The complementary nature of PECD and PICD was investigated by coincidence experiments on methyloxirane.[24]

In a recent review the PECD associated with the Lyman-α photoionization of amino acids was discussed as symmetry-breaking element possibly involved in the origin of homochirality.[25] Large asymmetries in the photoelectron angular distribution of L-Alanine were predicted theoretically[26] and observed experimentally[27,28] at a synchrotron by single photon PECD studies.

The development of PICD and PECD has brought tremendous progress in chiroptical experiments. However, they require the presence of isolated molecules in the gas phase limiting their application to rather small molecules. Here we present a new approach employing single photon detachment of anions generated by electrospray ionization (ESI) for measuring the PECD in a simple table-top experiment. ESI is a soft ionization technique developed by J. B. Fenn, which allows intact transfer of non-volatile analytes beyond 100 kDa into the gas phase.[29] Utilizing an ESI source for PECD measurements not only extends the mass range compared to the commonly used molecular beam or effusive inlets, but also opens up new analytic possibilities by

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providing charged instead of neutral precursors. In general, anions have considerably lower electron detachment thresholds than their neutral analogues, enabling detachment by single ultraviolet photons. This not only generalizes the excitation scheme by avoiding the prerequisite of resonances and reduces technical requirements for light sources, but also simplifies the theoretical description and interpretation of PECD measurements by eliminating intermediate state as well as related alignment dependencies, discussed recently for REMPI-PECD.[30]

This work demonstrates for the first time the measurement of PECD in the photodetachment from electrosprayed anions. To this end two amino acids were chosen as analytes. Glutamic acid (GLU) was investigated as a prototype for proteinogenic amino acids and 3,4-dihydroxyphenylalanine (DOPA) was selected due to its pharmaceutical relevance.[1]

The experimental setup is schematically shown in Figure 1, details are available in the Supporting Information. Gas phase anions were generated by ESI of 0.1 mmol L^{-1} amino acids solutions in a 1:3 (v/v) mixture of water and acetonitrile (ACN). In the case of glutamic acid two equivalents of sodium hydroxide were added. The ions are accumulated inside an octopole ion trap before they are intersected orthogonally with circularly polarized UV laser pulses and analyzed inside a time-of-flight mass spectrometer (TOF-MS).

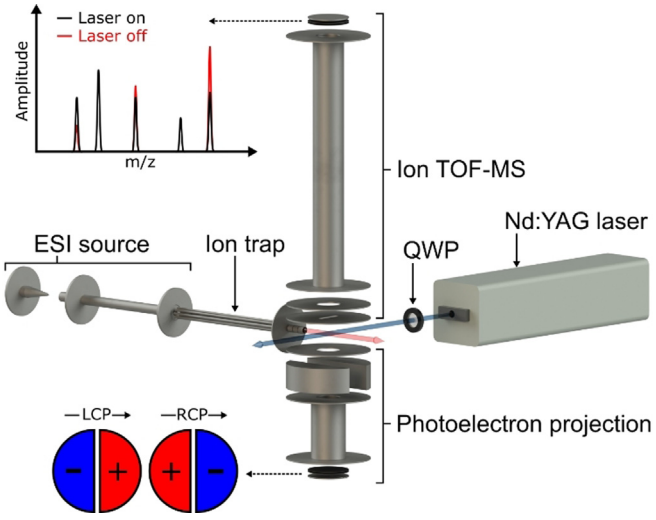

Figure 1. Schematic of the experimental setup: Anions are generated by electrospray ionization (ESI), accumulated inside an octopole ion trap and then guided into the extraction region of a linear time-of-flight mass spectrometer (TOF-MS), where they can interact with ns laser pulses. Circularly polarized light is generated from the third harmonic of a Nd:YAG laser (355 nm) by means of a quarter wave plate (QWP). Created photoelectrons and remaining anions are projected alternatively on one of the two detectors by a pulsed extraction field. The lower half of the spectrometer is used for quantification of forward–backward asymmetries of electrons relative to the laser propagation axis via a microchannel plate detector with two half circle anodes (schematic top view on the bottom left, different colors reflecting intensities relative to a mean value). The upper half offers a higher mass resolution suitable for acquiring ion mass spectra (top left).

Mass spectra of L-GLU and L-DOPA are shown in Figure 2 (additional data for the other enantiomers are given in the Supporting Information). The black trace, labeled as "laser on", shows the spectrum obtained after photodetachment by the laser, whereas in red the background mass spectrum of the ESI beam with the laser turned off (laser off) is displayed. The difference of these is depicted in blue. The ESI mass spectrum of DOPA features the deprotonated molecule $[M-H]^-$ with a mass-to-charge ratio (m/z) of 196 and multiple peaks at higher m/z corresponding to solvent adducts. The difference caused by photodetachment is clearly dominated by the unsolvated $[M-H]^-$ ion. Its background signal is depleted by 55%, which is 92% of the total difference observed using a pulse energy of 31 mJ.

In case of GLU the mass spectrum reveals multiple signals of solvated dianions $[(M-2H)(ACN)_x(H_2O)_y]^{2-}$ in addition to the monoanion $[M-H]^-$ ($m/z = 146$). While the latter exhibits

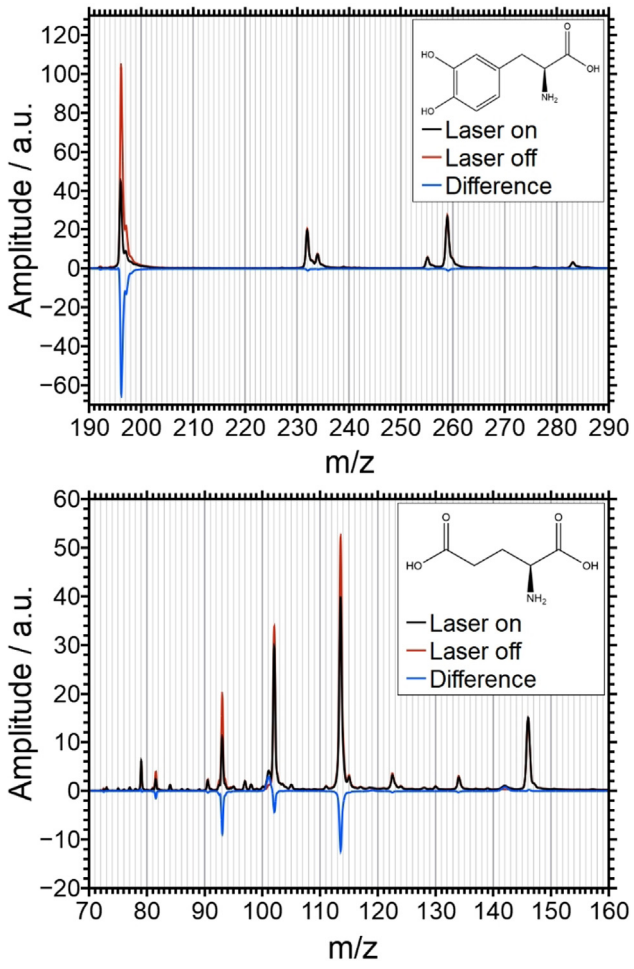

Figure 2. Typical electrospray mass spectra of L-DOPA (top) and L-GLU (bottom) in the negative ion mode. In black the spectrum with the laser on (mean of LCP & RCP) and in red with the laser off is displayed, respectively. The corresponding differences in blue illustrate the yield changes caused by photodetachment at 355 nm. For L-DOPA a depletion of the $[M-H]^-$ ion ($m/z = 196$) is apparent. The mass spectrum of L-GLU exhibits a decrease of signals corresponding to different solvated dianions $[(M-2H)(ACN)_x(H_2O)_y]^{2-}$ (e.g. $m/z = 93$, 102, and 113.5) and an increase of decarboxylated radical monoanions $[(M-2H-CO_2)(ACN)_x(H_2O)_y]^{\bullet-}$ (e.g. $m/z = 101$).

no significant yield difference, the dianions ($m/z = 81.5$, 90.5, 93, 102, 113.5, 122.5, and 134) are depleted to a certain degree by photodetachment. Simultaneously the appearance of new peaks ($m/z = 119$, $m/z = 142$) and increase of existing signals ($m/z = 101$) by formation of decarboxylated radical ions $[(M-2H-CO_2)(ACN)_x(H_2O)_y]^{\bullet-}$ is observable (see Table 1). The fact that the total signal increase does not match the observed amount of depletion suggests that a feasible reaction pathway exists which causes a second electron to be detached. Such dissociative autodetachment of carboxyl radical anions created from glutamate was theoretically predicted and experimentally verified in the Wang group by observation of near 0 eV kinetic energy electrons using photoelectron spectroscopy.[31]

By projection of the generated photoelectrons onto a microchannel plate detector with two half circle anodes oriented along the laser propagation axis the forward–backward asymmetry upon photodetachment by LCP and RCP can be observed. According to Equation (2) PECD values were obtained by integration of the electron peaks on the two detector halves for both polarization helicities and then averaging multiple measurements (details in Supporting Information). The mean PECD values for the L- and D-enantiomers of the two amino acids are summarized in Table 2 and illustrated in Figure 3 along with the results for racemic mixtures.

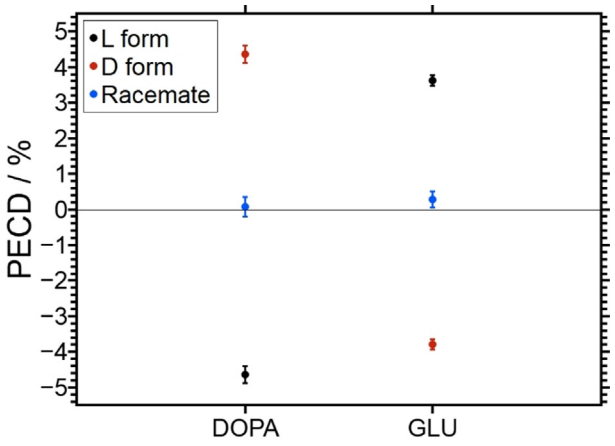

Figure 3. Mean PECD values of the L- and D-enantiomers as well as racemic mixtures of the amino acids 3,4-dihydroxyphenylalanine (DOPA) and glutamic acid (GLU) measured via photodetachment of electrosprayed anions as discussed in the text.

Table 1: Mass-to-charge ratios (m/z) of 3,4-dihydroxyphenylalanine (DOPA) and glutamic acid (GLU) anions created and depleted by photodetachment at a wavelength of 355 nm.

Analyte	m/z	Depleted ions	m/z	Created ions
DOPA	196	$[M-H]^-$		–
GLU	81.5	$[(M-2H)(H_2O)]^{2-}$	101	$[M-2H-CO_2]^{\bullet-}$
	90.5	$[(M-2H)(H_2O)_2]^{2-}$	119	$[(M-2H-CO_2)(H_2O)]^{\bullet-}$
	93	$[(M-2H)(ACN)]^{2-}$	142	$[(M-2H-CO_2)(ACN)]^{\bullet-}$
	102	$[(M-2H)(ACN)(H_2O)]^{2-}$		
	113.5	$[(M-2H)(ACN)_2]^{2-}$		
	122.5	$[(M-2H)(ACN)_2(H_2O)]^{2-}$		
	134	$[(M-2H)(ACN)_3]^{2-}$		

Both amino acids exhibit a significant forward–backward asymmetry in the photoelectron angular distribution. For the DOPA enantiomers a mean PECD effect of 4.5% was observed, while GLU on average exhibits a slightly lower effect of 3.7%. The expected sign inversion upon enantiomer exchange is apparent, considering the given standard errors. Furthermore, racemic mixtures prepared from the enantiomeric pure substances were measured resulting in PECD values close to zero, as required for randomly oriented net-achiral samples.[14] The slight deviation in case of *rac*-GLU could be due to experimental imperfections of the circular polarization or electron projection.

DOPA and GLU not only exhibit different magnitudes of the PECD, but also show opposite signs for the same absolute configuration. This could be due to the differing side chains or might be a consequence of the solvation and charge state of the precursor ions. For DOPA the PECD value corresponds almost exclusively to the $[M-H]^-$ anion, while in the case of GLU the mean PECD reported originates from a combination of the photodetachment of different solvent adducts of the dianion $[M-H]^{2-}$ and the autodetachment of resulting radical ions. The various precursor ions of GLU could feature different individual PECD values. This interesting question of charge and solvation shell dependence will be addressed in future studies. Future work will also include the determination of enantiomeric excess (*ee*) values for relevant pharmaceuticals.

Nevertheless, the presented data allow an unambiguous distinction between the investigated enantiomers and demonstrate the applicability of this technique for chirality analysis. The strength of the observed PECD effect is comparable to values of several percent reported previously for neutral amino acids (alanine,[27,28] proline,[25] serine[32]) and therefore magnitudes of order larger than anisotropy factors commonly encountered in conventional solution phase absorption CD.[13,14,25]

Daly et al. reported non-zero CD in total ion yields for photodetachment of electrosprayed anions.[9] For both amino acids investigated in this work no circular dichroism in either total electron or total ion yields was detectable within the error margins of the experiment ($\leq 0.2\%$). While the information contained in the total ion or electron yields (PICD) does not allow to distinguish between the investigated enantiomer pairs, the change in the

Table 2: PECD values for L/D-enantiomers and a racemic mixture of 3,4-dihydroxyphenylalanine (DOPA) and glutamic acid (GLU) measured via anion photodetachment at 355 nm. For DOPA the relevant precursor ion is the monoanion $[M-H]^-$, while for GLU a mean for electrons detached from solvated dianions $[(M-2H)(ACN)_x(H_2O)_y]^{2-}$ and subsequent radical ions is reported.

Substance	PECD(L) [%]	PECD(D) [%]	PECD(*rac*) [%]
3,4-Dihydroxyphenylalanine	-4.6 ± 0.2	4.4 ± 0.2	0.1 ± 0.3
Glutamic acid	3.6 ± 0.1	-3.8 ± 0.1	0.3 ± 0.2

forward–backward asymmetry of photoelectron angular distribution (PECD) allows clear chiral discrimination for the title molecules. This fact highlights the complementary nature of PICD and PECD for electrosprayed anions. The PECD approach is considered superior because it includes the total electron yield information as a subset, but offers additional information.

In conclusion, we have demonstrated the viability of anion photodetachment for investigation of the photoelectron circular dichroism for the first time. A large PECD effect on the order of several percent was observed for the two amino acids DOPA and GLU. This new anion single photon detachment approach offers various advantages over the PECD measurements reported so far, which employed photoionization of neutrals. In general, single UV photons are suitable for detaching anions in a bound to continuum transition. Consequently, no sharp resonances are needed. Only, the photon energy must exceed a certain threshold value and partial wave restrictions in the free electron must be fulfilled.[33] The absence of resonance conditions is an important prerequisite for chemical analysis, which is expected to be applicable to a wide range of compounds under conditions of a mixture. We note that this condition is also met in the photoionization of neutrals employing chirped femtosecond laser ionization.[8,34] Moreover, utilizing single photon detachment of anions rather than photoionization of neutrals reduces the technical requirements for light sources, because less photon energy compared to previous single photon experiments at synchrotron facilities and less intensity compared to multiphoton ionization approaches are necessary. In addition, using charged species as targets in PECD experiments enables precursor selection to be implemented by MS, overcoming the need to mass-tag electrons by coincidence techniques, which limit count rates and therefore cause longer measurement times. Today, many PECD studies employ a spatially resolved electron detector, which opens the possibility to energy-select electrons of interest. While such a device can be easily implemented into the current approach, the two half circle anodes employed in the current experiment have the clear advantage of simplicity and robustness.

Finally, the combination of an ESI source with the well-known, exceptionally conformation-sensitive PECD technique[15–22] opens the door for PECD investigations of non-volatile biopolymers like peptides and proteins in the gas phase. The wavelength-dependent PECD could be an additional, valuable tool to study the secondary and tertiary structure in the gas phase and also folding processes, for example, by comparison to quantum chemical or experimental values, analogous to conventional circular dichroism studies in solution phase.[3,4]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: amino acids · chirality · circular dichroism · mass spectrometry

[1] L. A. Nguyen, H. He, C. Pham-Huy, *Int. J. Biomed. Sci.* **2006**, *2*, 85.
[2] *Circular dichroism. Principles and applications* (Eds.: N. Berova, K. Nakanishi, R. W. Woody), Wiley-VCH, Weinheim, **2000**.
[3] S. M. Kelly, T. J. Jess, N. C. Price, *Biochim. Biophys. Acta Proteins Proteomics* **2005**, *1751*, 119.
[4] N. J. Greenfield, *Nat. Protoc.* **2006**, *1*, 2876.
[5] U. Boesl von Grafenstein, A. Bornschlegl, *ChemPhysChem* **2006**, *7*, 2085.
[6] R. Li, R. Sullivan, W. Al-Basheer, R. M. Pagni, R. N. Compton, *J. Chem. Phys.* **2006**, *125*, 144304.
[7] a) H. G. Breunig, G. Urbasch, P. Horsch, J. Cordes, U. Koert, K.-M. Weitzel, *ChemPhysChem* **2009**, *10*, 1199; b) P. Horsch, G. Urbasch, K.-M. Weitzel, *Z. Phys. Chem.* **2011**, *225*, 587; c) P. Horsch, G. Urbasch, K.-M. Weitzel, D. Kröner, *Phys. Chem. Chem. Phys.* **2011**, *13*, 2378.
[8] P. Horsch, G. Urbasch, K.-M. Weitzel, *Chirality* **2012**, *24*, 684.
[9] S. Daly, F. Rosu, V. Gabelica, *Science* **2020**, *368*, 1465.
[10] C. S. Lehmann, N. B. Ram, I. Powis, M. H. M. Janssen, *J. Chem. Phys.* **2013**, *139*, 234307.
[11] B. Ritchie, *Phys. Rev. A* **1976**, *13*, 1411.
[12] N. Böwering, T. Lischke, B. Schmidtke, N. Müller, T. Khalil, U. Heinzmann, *Phys. Rev. Lett.* **2001**, *86*, 1187.
[13] C. Lux, M. Wollenhaupt, T. Bolze, Q. Liang, J. Köhler, C. Sarpe, T. Baumert, *Angew. Chem. Int. Ed.* **2012**, *51*, 5001; *Angew. Chem.* **2012**, *124*, 5086.
[14] I. Powis in *Advances in Chemical Physics* (Ed.: S. A. Rice), Wiley, Hoboken, **2008**, pp. 267–329.
[15] D. Di Tommaso, M. Stener, G. Fronzoni, P. Decleva, *ChemPhysChem* **2006**, *7*, 924.
[16] C. J. Harding, I. Powis, *J. Chem. Phys.* **2006**, *125*, 234306.
[17] G. A. Garcia, L. Nahon, C. J. Harding, I. Powis, *Phys. Chem. Chem. Phys.* **2008**, *10*, 1628.
[18] S. Turchini, D. Catone, G. Contini, N. Zema, S. Irrera, M. Stener, D. Di Tommaso, P. Decleva, T. Prosperi, *ChemPhysChem* **2009**, *10*, 1839.
[19] G. A. Garcia, H. Soldi-Lose, L. Nahon, I. Powis, *J. Phys. Chem. A* **2010**, *114*, 847.
[20] S. Turchini, D. Catone, N. Zema, G. Contini, T. Prosperi, P. Decleva, M. Stener, F. Rondino, S. Piccirillo, K. C. Prince, et al., *ChemPhysChem* **2013**, *14*, 1723.
[21] S. Daly, M. Tia, G. A. Garcia, L. Nahon, I. Powis, *Angew. Chem. Int. Ed.* **2016**, *55*, 11054; *Angew. Chem.* **2016**, *128*, 11220.
[22] S. Turchini, *J. Phys. Condens. Matter* **2017**, *29*, 503001.
[23] a) G. A. Garcia, L. Nahon, S. Daly, I. Powis, *Nat. Commun.* **2013**, *4*, 2132; b) M. M. Rafiee Fanood, H. Ganjitabar, G. A. Garcia, L. Nahon, S. Turchini, I. Powis, *ChemPhysChem* **2018**, *19*, 921; c) G. A. Garcia, H. Dossmann, L. Nahon, S. Daly, I. Powis, *ChemPhysChem* **2017**, *18*, 500; d) H. Ganjitabar, R. Hadidi, G. A. Garcia, L. Nahon, I. Powis, *J. Mol. Spectrosc.* **2018**, *353*, 11; e) H. Ganjitabar, G. A. Garcia, L. Nahon, I. Powis, *J. Chem. Phys.* **2020**, *153*, 034302.
[24] C. S. Lehmann, K.-M. Weitzel, *Phys. Chem. Chem. Phys.* **2020**, *22*, 13707.
[25] R. Hadidi, D. K. Bozanic, G. A. Garcia, L. Nahon, *Adv. Phys. X* **2018**, *3*, 1477530.
[26] I. Powis, *J. Phys. Chem. A* **2000**, *104*, 878.
[27] M. Tia, B. Cunha de Miranda, S. Daly, F. Gaie-Levrel, G. A. Garcia, I. Powis, L. Nahon, *J. Phys. Chem. Lett.* **2013**, *4*, 2698.

[28] M. Tia, B. Cunha de Miranda, S. Daly, F. Gaie-Levrel, G. A. Garcia, L. Nahon, I. Powis, *J. Phys. Chem. A* **2014**, *118*, 2765.
[29] a) M. Yamashita, J. B. Fenn, *J. Phys. Chem.* **1984**, *88*, 4451; b) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Science* **1989**, *246*, 64; c) J. B. Fenn, *Angew. Chem. Int. Ed.* **2003**, *42*, 3871; *Angew. Chem.* **2003**, *115*, 3999.
[30] a) R. E. Goetz, T. A. Isaev, B. Nikoobakht, R. Berger, C. P. Koch, *J. Chem. Phys.* **2017**, *146*, 024306; b) A. Kastner, T. Ring, B. C. Krüger, G. B. Park, T. Schäfer, A. Senftleben, T. Baumert, *J. Chem. Phys.* **2017**, *147*, 013926; c) M. Tia, M. Pitzer, G. Kastirke, J. Gatzke, H.-K. Kim, F. Trinter, J. Rist, A. Hartung, D. Trabert, J. Siebert, et al., *J. Phys. Chem. Lett.* **2017**, *8*, 2780; d) S. Beaulieu, A. Comby, D. Descamps, S. Petit, F. Légaré, B. Fabre, V. Blanchet, Y. Mairesse, *J. Chem. Phys.* **2018**, *149*, 134301; e) A. Pier, K. Fehre, S. Grundmann, I. Vela-Perez, N. Strenger, M. Kircher, D. Tsitsonis, J. B. Williams, A. Senftleben, T. Baumert, et al., *Phys. Rev. Res.* **2020**, *2*, 033080.
[31] S. H. M. Deng, G.-L. Hou, X.-Y. Kong, M. Valiev, X.-B. Wang, *J. Phys. Chem. A* **2014**, *118*, 5256.
[32] S. Hartweg, G. A. Garcia, D. K. Božanić, L. Nahon, *J. Phys. Chem. Lett.* **2021**, *12*, 2385.
[33] A. Sanov, *Annu. Rev. Phys. Chem.* **2014**, *65*, 341.
[34] V. Schäfer, K.-M. Weitzel, *Anal. Chem.* **2020**, *92*, 5492.

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