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ORIGINAL RESEARCH

Tailoring peptide conformational space with organic gas modifiers in TIMS-MS

Alyssa Garabedian[1] · Fenfei Leng[1,2] · Mark E. Ridgeway[3] · Melvin A. Park[3] · Francisco Fernandez-Lima[1,2]

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Abstract

Recently, we showed the advantages of Trapped Ion Mobility Spectrometry for the study of kinetic intermediates of biomolecules as a function of the starting solvent composition (e.g., organic content and pH) and collisional induced activation. In the present work, we further characterize the influence of the bath composition (e.g., organic content) on the conformational space of an intrinsically disordered, DNA binding peptide: AT-hook 3 (Lys-Arg-Pro-Arg-Gly-Arg-Pro-Arg-Lys-Trp). Results show the dependence of the charge state distribution and mobility profiles by doping the solution and the bath gas with organic modifiers (e.g., methanol and acetone). The high resolving power of the TIMS analyzer allowed the separation of multiple IMS band per charge state, and their relative abundances are described as a function of the experimental conditions. The use of gas modifiers resulted in larger ion-neutral collision cross sections, with a direct correlation between the size of the modifier and the CCS differences. Conformational isomer inter-conversion rates were observed as a function of the trapping time. Different from solution experiments, a larger variety of organic gas modifiers can be used to tailor the peptide conformational space, since peptide precipitation is not a problem.

Keywords Trapped ion mobility mass spectrometry · Intrinsically disordered protein · HMGA2 · ATHP

Introduction

Mass spectrometry-based methods have increasingly become a complementary or alternative research tool for investigating the conformational space of biomolecules under a variety of conditions, including biologically relevant conditions. [6, 16, 19, 37, 40] Specifically, ion mobility spectrometry combined with mass spectrometry (IMS-MS) has the capability to perform separation and selection of gas-phase ions, from heterogeneous solutions. It provides insight into both stable and intermediate structures, allowing for a more dynamic view and native-like folding information, [21] while resembling solution structures (memory effect). [5, 15, 18, 22, 25, 33, 35, 36, 38] Previous studies from our group showed the advantages of ESI-TIMS-MS for the study of kinetically trapped intermediates of biomolecules. [1–4, 10, 11, 15, 20, 24, 27, 29, 31] Relevant to this study, we presented the folding pathways between local, free energy minima of AT-hook peptide 3 (ATHP3) leading to multiple, stabilized conformations. [26] Protonation site, backbone relaxation and side-chain orientations were implicated in defining each structure. We have shown that the conformational space can be altered by introducing dopants into the TIMS cell for the case of flavin adenine dinucleotide.[20] Different from other experiments where gas modifiers are used to increase the analytical power of IMS by increasing the size of the collision partner or inducing higher order multi-pole interactions, [9, 13, 14, 23, 32, 39] in this project we focused on the influence of the microenvironment on the stabilization of the conformational space of biomolecules.

In the present work, a ten amino acid intrinsically disordered peptide, Lys-Arg-Pro-Arg-Gly-Arg-Pro-Arg-Lys-Trp, was studied using nanoESI-TIMS-MS as a function of starting solvent (e.g. organic content and pH), bath gas collision partner and time after desolvation. This study is the first to report on the use of TIMS gas modifiers to tailor the peptide conformational space.

✉ Francisco Fernandez-Lima
fernandf@fiu.edu

[1] Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA

[2] Biomolecular Sciences Institute, Florida International University, Miami, FL 33199, USA

[3] Bruker Daltonics Inc., Billerica, MA 01821, USA

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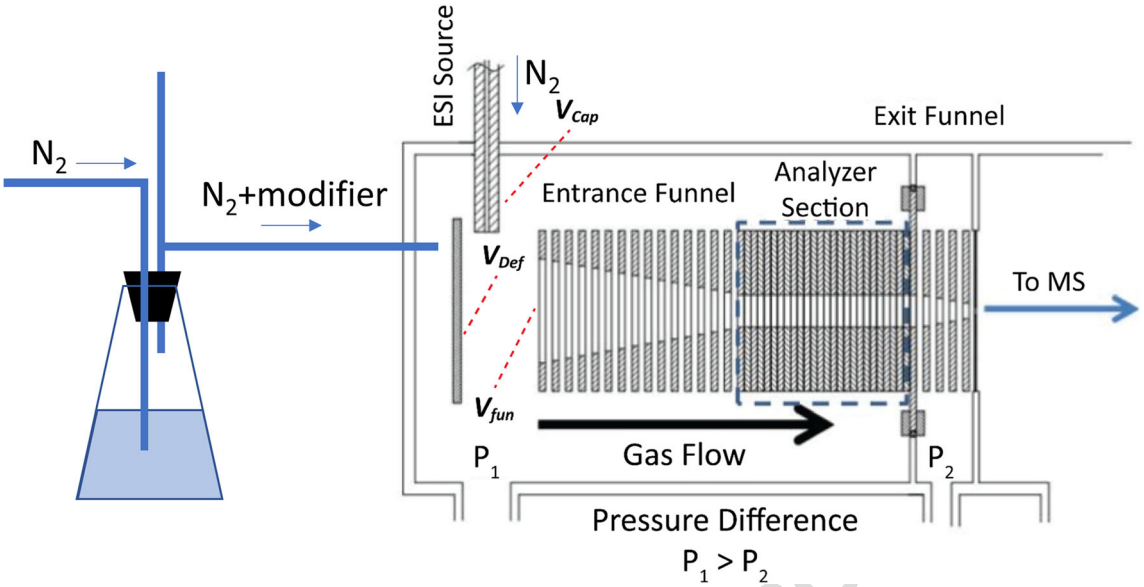


Fig. 1 Scheme utilized for the nESI-TIMS-MS experiments with organic gas modifiers. Notice that the gas velocity in the TIMS analyzer is kept constant

Experimental methods

Materials and reagents AT-hook peptides 3 (Lys-Arg-Pro-Arg-Gly-Arg-Pro-Arg-Lys-Trp) were purchased from Advanced ChemTech Inc. (Louisville, KY) and used as received. Methanol and acetone solvents, and ammonium acetate salts utilized in this study were analytical grade or better and purchased from Fisher Scientific (Pittsburgh, PA). A Tuning Mix calibration standard (G24221A) was obtained from Agilent Technologies (Santa Clara, CA) and used as received.

Trapped ion mobility spectrometry – Mass spectrometry analysis (TIMS-MS) Details regarding the TIMS operation and specifics compared to traditional IMS can be found elsewhere. [7, 8, 12, 28, 30] Briefly, mobility separation in TIMS is based on holding the ions stationary against a moving gas using an electric field. The separation in a TIMS device can be described in the center of the mass reference frame using the same principles as in a conventional IMS drift tube. [17] Since mobility separation is related to the number of ion-neutral collisions (or drift time in traditional drift tube cells), the mobility separation in a TIMS device depends on the bath gas drift velocity, ion confinement and ion elution parameters. The reduced mobility, K, of an ion in a TIMS cell is described by:

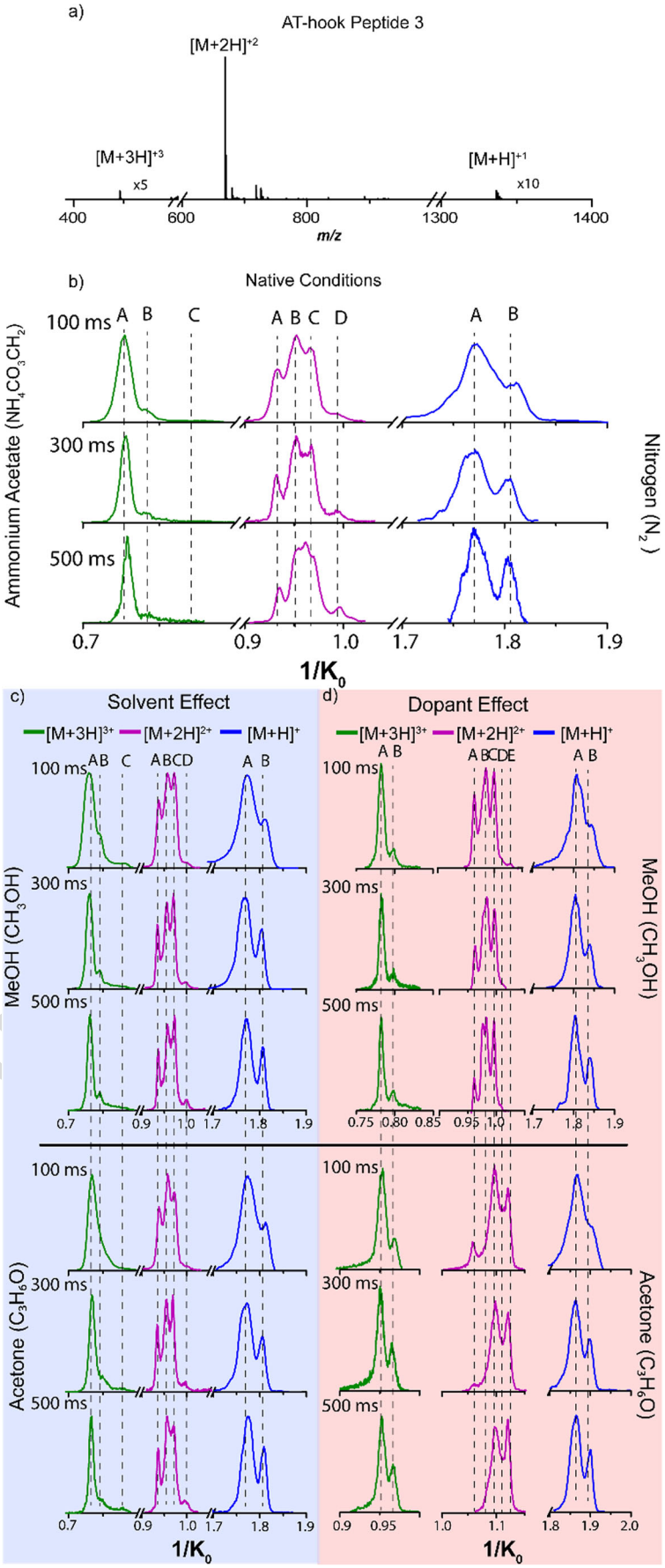
$$K = \frac{V_g}{E} \approx \frac{A}{(V_{elution} - V_{out})}$$

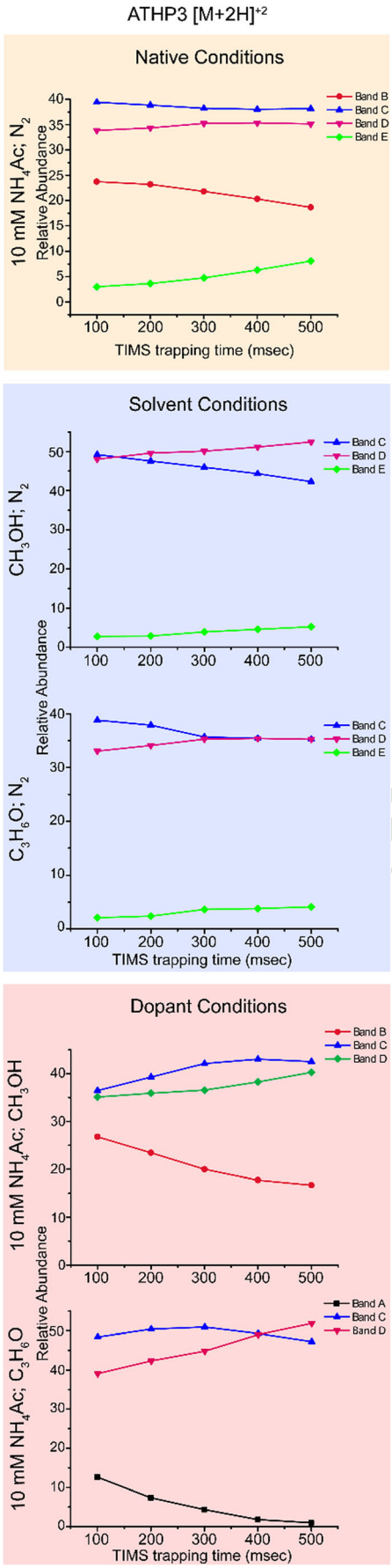
where v_g and E are the velocity of the gas and the applied electric field across the TIMS analyzer region. $V_{elution}$ is the voltage when the ions elute in the V_{ramp} sweep and V_{out} is the voltage applied at the end of the TIMS analyzer region. A is a constant that relates to the velocity of the bath gas and electric field axial distribution and can be calculated using mobility standards. Notice that, once A is calculated for a given bath gas (e.g., Tuning Mix as calibrants for N_2 bath gas), it will not change when using gas modifiers since the pressure difference between P1 and P2 are kept the same (see details in Fig. 1).

A custom-built, pulled capillary nanoESI source was utilized for all the experiments. Quartz glass capillaries (O.D.: 1.0 mm and I.D.: 0.70 mm) were pulled utilizing a P-2000 micropipette laser puller (Sutter Instruments, Novato, CA) and loaded with 10 μL aliquot of the sample solution. A typical nanoESI source voltage of +/− 600–1200 V was applied between the pulled capillary tips and the TIMS-MS instrument inlet. Ions were introduced via a stainless steel tube (1/16 × 0.020″, IDEX Health Science, Oak Harbor, WA) held at room temperature into the TIMS cell. It should be noted that all solvent studies were performed with nitrogen as the bath gas, and that all dopant experiments were conducted with peptides sprayed from 10 mM NH_4AC.

Mobility calibration was performed using the Tuning Mix calibration standard (G24221A, Agilent Technologies, Santa Clara, CA) in positive ion mode (e.g., $m/z = 322$, $K_0 = 1.376$ cm^2 V^{-1} s^{-1} and $m/z = 622$, $K_0 = 1.013$ cm^2 V^{-1} s^{-1}). [12] The TIMS operation was controlled using in-house software, written in National Instruments Lab VIEW, and synchronized with the maXis Impact Q-ToF acquisition program. [7, 8] Gas modifiers were introduced at the entrance of the

Fig. 2 **a** Typical mass spectra and (**b**) native IMS spectra of ATHP3 as a ▶ function of (**c**) starting solvent (methanol:H_2O or acetone:H_2O) or (**d**) dopant bath gas (methanol or acetone)





◀ **Fig. 3** The relative abundances of ATHP 3 $[M + 2H]^{+2}$ conformers as a function of the trapping time, stating solvent conditions and bath gas composition. Starting solvent and bath gas are listed to the left of the graphs

TIMS cell via vaporization of the respective solvents (e.g., methanol or acetone) at a ratio of 2:1 air:air modified mix (scheme shown in Fig. 1). For simplified mobility calibration, the gas velocity was kept constant in all experiments (P1 and P2 values).

Results and discussion

The analysis of ATHP3 peptide using nES-TIMS-MS resulted in a charge state distribution of $[M + H]^+$ to $[M + 3H]^{+3}$. The ATHP3 motif is mainly comprised of basic residues with seven potential locations for protonation (e.g., N-terminus, four arginines and two lysines), however, the most abundant ion under all experimental conditions was the $[M + 2H]^{2+}$ charge state (Fig. 2a). The mobility distributions observed from our previous study of ATHP 3 $[M + 2H]^{+2}$ using ESI-TIMS-MS are consistent with the current analysis by nanoESI-TIMS-MS. [26]

At native conditions (Fig. 2b, pink mobility bands) ATHP3 $[M + 2H]^{2+}$ populates four conformers (A-D). The mobility bands are also conserved across the different organic solvent conditions (Fig. 2c, pink mobility bands in blue panel). At higher trapping times (e.g., 500 ms), a kinetically trapped structure, and what we consider a more stable "desolvated" conformer, appears between bands B and C of the native. The presence of methanol in the TIMS cell did not alter the $[M + 2H]^{2+}$ conformers, while acetone significantly changed the relative abundance and distribution of structures (Fig. 2d, pink mobility band in pink panel). Three structures (A, B and C) were observed for ATHP3 $[M + 3H]^{3+}$ over the range of starting solvent solutions (Fig. 2b and c, green mobility bands). Conformer A was the major structure present in all experiments, followed by conformer B and C. The abundance of conformer B, however, increased in the presence of acetone in the TIMS cell. The mobility profiles of ATHP3 $[M + H]^+$ showed the presence of two structures (A and B) which were observed over the range of experimental conditions (Fig. 2b, c and d, blue mobility bands). The distribution of ATHP 3 structures using acetone solvent cannot be recreated using acetone in the TIMS cell. One possible explanation is that the differences in conformational space is due to interaction with the ketone functional group of acetone. Unlike methanol's alcohol group, acetone's ketone group can form various interactions with the peptide, including 1) hydrogen bonding with the amide of the peptide backbone, 2) disruption of hydrogen bonding networks or, more likely, 3) dipole-dipole interactions with

the charged residues of the peptide. Confirming this explanation will be the subject of future studies.

Changes in the conformational space as a function of the trapping showed stabilization towards more energetically favored structures as a function of the trapping time for $[M+H]^{+2}$ charge state (Fig. 3). While our measurements are only sensitive to the 50–500 ms time scale, potential rearrangements are possible in the first 50 ms after desolvation [34]. In the case of varying the starting solution (10 mM ammonium acetate, and with methanol and acetone), a common trend in the gas phase kinetics in nitrogen is the increase of the band E, which corresponds to the largest CCS (and largest $1/K_0$) for this charge state. However, in the case of gas modifiers, band E is not observed, and the trends are best characterized by a decrease of band B and band A for methanol and acetone, respectively, which correspond to the smallest CCSs. We interpret these results as the most stable gas-phase structures tending to have larger CCSs that those initially observed in solution. These effects may be a consequence of the absence of the solvent, since in the gas-phase the lack of water molecules promotes long range interactions. In the case of the $[M+H]^{+}$ and $[M+H]^{+3}$ charge states, similar trends were observed regarding the increase of larger CCS bands as a function of the trapping time (see Fig. 2).

Conclusions

The results presented here displayed the utility of gas modifiers in TIMS-MS for investigating and monitoring solution versus gas-phase microenvironment contribution to the peptide conformational space. When ionized from native conditions (10 mM NH_4Ac and nitrogen bath gas), the mobility profiles of ATHP 3 show an ensemble of conformers, which were preserved as a function of increasing organic content (methanol and acetone). Although the overall IMS profiles were maintained, changes in the relative abundance of conformers (e.g., conformational isomerization to the more stable gas-phase structure) were observed and recorded. The interconversion of structures, however, was small and often did not exceed growth or decay abundances of ~10%. Comparison between starting solvent and bath gas with the same organic modifier showed that acetone as a dopant consistently changed the original IMS profiles. Overall, we find evidence for multiple stable conformers of these "disordered" motifs as a function of starting solvent (e.g. organic content), bath gas collision partner and time after desolvation. The sensitivity of TIMS-MS allows for the observation of many low abundant conformers, separation of closely related structures and tracking of gas-phase stable structures via isomerization kinetics. This methodology opens new avenues for the study of biomolecules in the presence of gas modifiers that are not accessible during solution experiments, due to the typical precipitation of biomolecules during non-native conditions.

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References

1. Adams KJ, Montero D, Aga D, Fernandez-Lima F (2016) Isomer separation of polybrominated diphenyl ether metabolites using nanoESI-TIMS-MS. Int J Ion Mobil Spectrom 19(2-3):69–76. https://doi.org/10.1007/s12127-016-0198-z
2. Benigni P, Fernandez-Lima F (2016) Oversampling Selective Accumulation Trapped Ion Mobility Spectrometry Coupled to FT-ICR MS: Fundamentals and Applications. Anal Chem 88(14): 7404–7412. https://doi.org/10.1021/acs.analchem.6b01946
3. Benigni P, Marin R, Fernandez-Lima F (2015) Towards unsupervised polyaromatic hydrocarbons structural assignment from SA-TIMS –FTMS data. Int J Ion Mobil Spectrom:1–7. https://doi.org/10.1007/s12127-015-0175-y
4. Benigni P et al (2016) Towards the analysis of high molecular weight proteins and protein complexes using TIMS-MS. Int J Ion Mobil Spec 19:95–104. https://doi.org/10.1007/s12127-016-0201-8
5. Chen S-H, Russell DH (2015) How closely related are conformations of protein ions sampled by IM-MS to native solution structures? J Am Soc Mass Spectrom 26(9):1433–1443
6. Feng X, Liu X, Luo Q, Liu B-F (2008) Mass spectrometry in systems biology: an overview. Mass Spectrom Rev 27(6):635–660. https://doi.org/10.1002/mas.20182
7. Fernandez-Lima FA, Kaplan DA, Park MA (2011a) Note: Integration of trapped ion mobility spectrometry with mass spectrometry. Rev Sci Instrum 82:126106
8. Fernandez-Lima FA, Kaplan DA, Suetering J, Park MA (2011b) Gas-phase separation using a Trapped Ion Mobility Spectrometer. Int J Ion Mobil Spec 14:93–98
9. Fernández-Maestre R, Wu C, Hill HH (2012) Buffer gas modifiers effect resolution in ion mobility spectrometry through selective ion-molecule clustering reactions. Rapid Commun Mass Spectrom 26(19):2211–2223. https://doi.org/10.1002/rcm.6335
10. Frost L, Baez MAM, Harrilal C, Garabedian A, Fernandez-Lima F, Leng F (2015) The dimerization state of the mammalian high mobility group protein AT-hook 2 (HMGA2). PLoS One 10(6): e0130478. https://doi.org/10.1371/journal.pone.0130478
11. Gonzalez WG, Ramos V, Diaz M, Garabedian A, Molano-Arevalo JC, Fernandez-Lima F, Miksovska J (2016) Characterization of the Photophysical, thermodynamic, and structural properties of the terbium (III)–DREAM complex. Biochemistry 55(12):1873–1886
12. Hernandez DR, DeBord JD, Ridgeway ME, Kaplan DA, Park MA, Fernandez-Lima FA (2014) Ion dynamics in a trapped ion mobility spectrometer. Analyst 139(8):1913–1921. https://doi.org/10.1039/C3AN02174B
13. Kafle A, Coy SL, Wong BM, Fornace AJ Jr, Glick JJ, Vouros P (2014) Understanding gas phase modifier interactions in rapid analysis by differential mobility-tandem mass spectrometry. J Am Soc Mass Spectrom 25(7):1098–1113. https://doi.org/10.1007/s13361-013-0808-5

14. Levin DS, Vouros P, Miller RA, Nazarov EG, Morris JC (2006) Characterization of gas-phase molecular interactions on differential mobility ion behavior utilizing an electrospray ionization-differential mobility-mass spectrometer system. Anal Chem 78(1): 96–106. https://doi.org/10.1021/ac051217k
15. Liu FC, Kirk SR, Bleiholder C (2016) On the structural denaturation of biological analytes in trapped ion mobility spectrometry - mass spectrometry. Analyst 141(12):3722–3730. https://doi.org/10.1039/C5AN02399H
16. Loo JA (1997) Studying noncovalent protein complexes by electrospray ionization mass spectrometry. Mass Spectrom Rev 16(1):1–23. https://doi.org/10.1002/(sici)1098-2787(1997)16:1<1::aid-mas1>3.0.co;2-l
17. McDaniel EW, Mason EA (1973) Mobility and diffusion of ions in gases. Wiley series in plasma physics. John Wiley and Sons, Inc., New York
18. Meyer T, Gabelica V, Grubmüller H, Orozco M (2013) Proteins in the gas phase. Wiley Interdiscip Rev: Comput Mol Sci 3:408–425
19. Miranker A, Robinson CV, Radford SE, Aplin RT, Dobson CM (1993) Detection of transient protein folding populations by mass spectrometry. Science 262(5135):896–900. https://doi.org/10.1126/science.8235611
20. Molano-Arevalo JC, Hernandez DR, Gonzalez WG, Miksovska J, Ridgeway ME, Park MA, Fernandez-Lima F (2014) Flavin adenine dinucleotide structural motifs: from solution to gas phase. Anal Chem 86(20):10223–10230. https://doi.org/10.1021/ac5023666
21. Pi J, Sael L (2013) Mass spectrometry coupled experiments and protein structure modeling methods. Int J Mol Sci 14:20635–20657. https://doi.org/10.3390/ijms141020635
22. Pierson NA, Chen L, Valentine SJ, Russell DH, Clemmer DE (2011) Number of solution states of bradykinin from ion mobility and mass spectrometry measurements. J Am Chem Soc 133(35): 13810–13813. https://doi.org/10.1021/ja203895j
23. Porta T, Varesio E, Hopfgartner G (2013) Gas-phase separation of drugs and metabolites using modifier-assisted differential ion mobility spectrometry hyphenated to liquid extraction surface analysis and mass spectrometry. Anal Chem 85(24):11771–11779. https://doi.org/10.1021/ac4020353
24. Ridgeway ME, Silveira JA, Meier JE, Park MA (2015) Microheterogeneity within conformational states of ubiquitin revealed by high resolution trapped ion mobility spectrometry. Analyst 140(20):6964–6972
25. Rosu F, Gabelica V, Joly L, Gregoire G, De Pauw E (2010) Zwitterionic i-motif structures are preserved in DNA negatively charged ions produced by electrospray mass spectrometry. Phys Chem Chem Phys 12(41):13448–13454
26. Schenk ER, Ridgeway ME, Park MA, Leng F, Fernandez-Lima F (2013) Isomerization kinetics of AT hook Decapeptide solution structures. Anal Chem 86(2):1210–1214. https://doi.org/10.1021/ac403386q
27. Schenk ER, Mendez V, Landrum JT, Ridgeway ME, Park MA, Fernandez-Lima F (2014a) Direct observation of differences of carotenoid polyene chain cis/trans isomers resulting from structural topology. Anal Chem 86(4):2019–2024. https://doi.org/10.1021/ac403153m
28. Schenk ER, Mendez V, Landrum JT, Ridgeway ME, Park MA, Fernandez-Lima FA (2014b) Direct observation of differences of carotenoid polyene chain cis/trans isomers resulting from structural topology. Anal Chem 86(4):2019–2024. https://doi.org/10.1021/ac403153m
29. Schenk ER, Ridgeway ME, Park MA, Leng F, Fernandez-Lima F (2014c) Isomerization kinetics of AT hook decapeptide solution structures. Anal Chem 86(2):1210–1214. https://doi.org/10.1021/ac403386q
30. Schenk ER, Ridgeway ME, Park MA, Leng F, Fernandez-Lima FA (2014d) Isomerization kinetics of AT hook Decapeptide solution structures. Anal Chem 86(2):1210–1214. https://doi.org/10.1021/ac403386q
31. Schenk ER, Almeida R, Miksovska J, Ridgeway ME, Park MA, Fernandez-Lima F (2015) Kinetic intermediates of holo- and apo-myoglobin studied using HDX-TIMS-MS and molecular dynamic simulations. J Am Soc Mass Spectrom 26(4):555–563. https://doi.org/10.1007/s13361-014-1067-9
32. Schneider B, Covey T, Nazarov E (2013) DMS-MS separations with different transport gas modifiers vol 16. https://doi.org/10.1007/s12127-013-0130-8
33. Seo J, Hoffmann W, Warnke S, Bowers MT, Pagel K, von Helden G (2016) Retention of native protein structures in the absence of solvent: a coupled ion mobility and spectroscopic study. Angew Chem Int Ed 55(45):14173–14176. https://doi.org/10.1002/anie.201606029
34. Shelimov KB, Jarrold MF (1997) Conformations, unfolding, and refolding of Apomyoglobin in vacuum: an activation barrier for gas-phase protein folding. J Am Chem Soc 119(13):2987–2994. https://doi.org/10.1021/ja962914k
35. Shi L, Holliday AE, Glover MS, Ewing MA, Russell DH, Clemmer DE (2016) Ion mobility-mass spectrometry reveals the energetics of intermediates that guide Polyproline folding. J Am Soc Mass Spectrom 27(1):22–30
36. Silveira JA, Fort KL, Kim D, Servage KA, Pierson NA, Clemmer DE, Russell DH (2013) From solution to the gas phase: stepwise dehydration and kinetic trapping of substance P reveals the origin of peptide conformations. J Am Chem Soc 135(51):19147–19153. https://doi.org/10.1021/ja4114193
37. Simoneit BRT (2005) A review of current applications of mass spectrometry for biomarker/molecular tracer elucidations. Mass Spectrom Rev 24(5):719–765. https://doi.org/10.1002/mas.20036
38. Voronina L, Masson A, Kamrath M, Schubert F, Clemmer D, Baldauf C, Rizzo T (2016) Conformations of prolyl–peptide bonds in the bradykinin 1–5 fragment in solution and in the gas phase. J Am Chem Soc 138(29):9224–9233. https://doi.org/10.1021/jacs.6b04550
39. Waraksa E, Gaik U, Namieśnik J, Sillanpää M, Dymerski T, Wójtowicz M, Puton J (2016) Dopants and gas modifiers in ion mobility spectrometry vol 82. https://doi.org/10.1016/j.trac.2016.06.009
40. Winston RL, Fitzgerald MC (1997) Mass spectrometry as a readout of protein structure and function. Mass Spectrom Rev 16(4):165–179. https://doi.org/10.1002/(sici)1098-2787(1997)16:4<165::aid-mas1>3.0.co;2-f

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