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Mass spectrometric diffusion parameters and 3D structural analysis of oligomeric associates of glycylhomopeptides and their complexes of silver(I) ion – a stochastic dynamics approach

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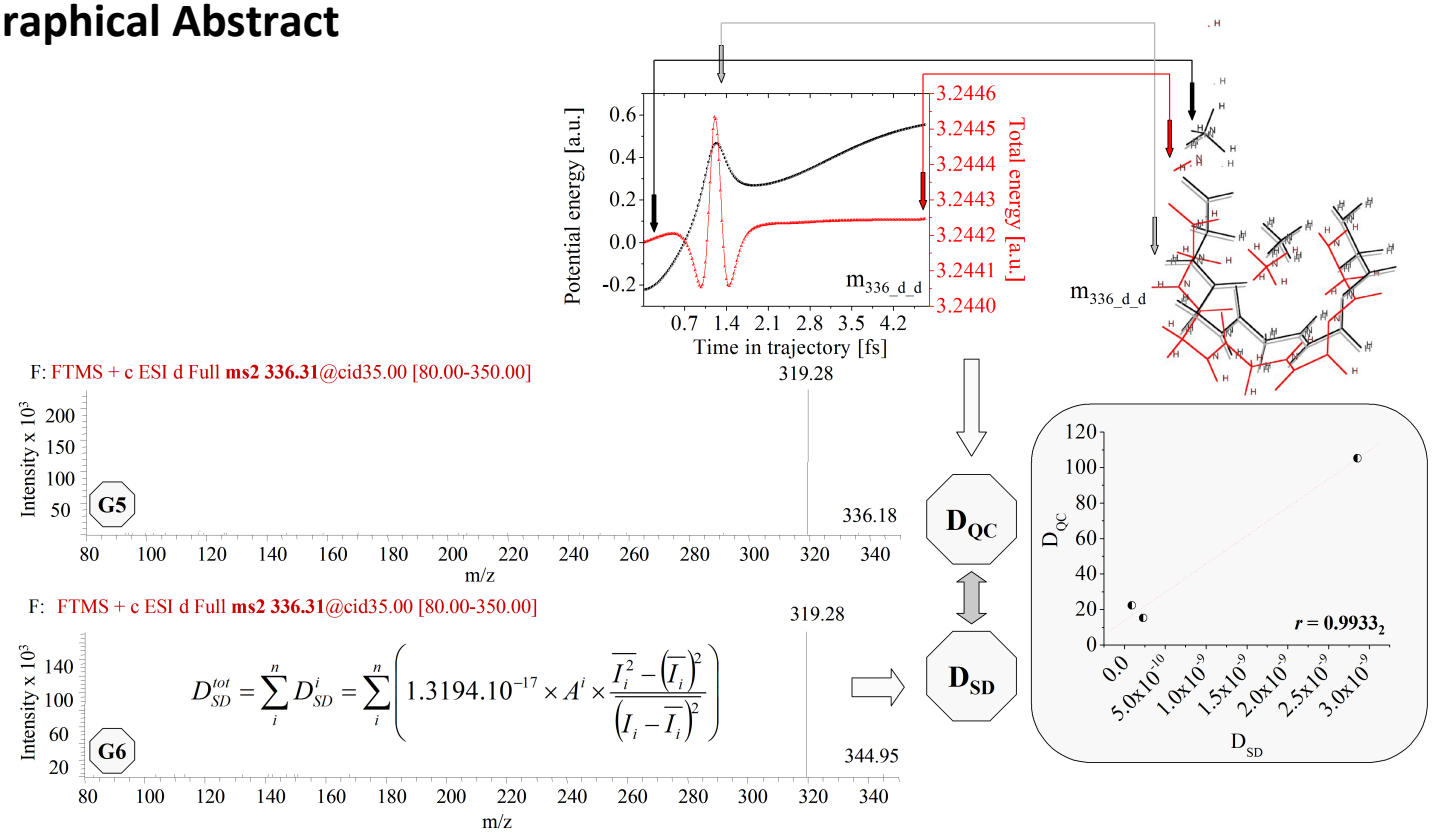
Graphical Abstract



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Abstract:

The topic to this study is determination of mass spectrometric **(MS)** diffusion parameters "D_{SD}" of oligomeric associates of glycylhomopeptides and their Ag^{I}–complexes according to our *"stochastic dynamic"* approach and model equations under electrospray **(ESI)** ionization condition. The problematic has recently gained attenion thanks to innovative formulas connecting among "D_{SD}" data; measurable outcome *"intensity"* of analyte ions; and the experimental parameter *"temperature,"* respectively. The equations are empirically testable and verifiable. In advancing this innovative view upon which models we will carry out analysis of ions of oligomeric associates of peptides, we should point out that it is crucial to further test our formulas on a larger set of chemical classes and experimental conditions, in order to, verify their universal applicability to different MS ionization methods. Because of, on the concept sketched above the D_{SD} parameters correlate excellent linearly with kinetic parameters of fragment reactions and quantum chemical diffusions according to the Arrhenius's approximation which reflects the 3D molecular structures of analytes. The most important point regarding our concept is that it extends crucially the capability of the mass spectrometry of multidimentional structural analysis when is applied to high accuracy quantum chemical static and molecular dynamic approaches.

Keywords: mass spectrometry; diffusion; quantum chemistry; oligomeric associates; peptides

Introduction

This work is an outcome of research effort devoted to development of theoretical concept and model equations connecting the ***mass spectrometric*** measurable outcome ***"intensity"*** with thermodynamic, kinetic and diffusion parameters of ions, respectively. Our main aim has been to develop protocols for 3D structural analysis, taking as a given view that there is correlation between these parameters and the molecular structure of the ions within the concept of the ***"free Gibbs energy"*** (hereafter ΔG.) Seen in the aforementioned terms and several different approximations to ΔG — it represents macroscopic quantity determining the most probable molecular conformation or 3D molecular and electronic structures with respect to the so-called intramolecular and environmental factors in energy terms — it provides link between microscopic state of a molecular system — in our description given 3D molecular or electronic structures or both of these — and macroscopic measurable kinetic and diffusion parameters. In order to, understand comprehensively the vast majority of real chemical reactions the research on methodological developments on reliable approximations to different macroscopic parameters in context derivation of corresponding parameter with respect to behavior of ΔG has expanded significantly over recent decades. Subsequent body of empirical research has evidenced that this strategy produces robust theoretical models fitting excellent to results from the chemical experiment. Therefore, it stands to reason that the concept of *"free Gibbs energy"* might have much wider real implications in describing MS phenomena. However, due to a significant complexity of desorption–ionization mechanisms under different MS methods and still not well understood phenomenology, there is a major research question: **"What are the real implications of the latter concept in a quantitative treatment of experimental MS parameters in context exact model relationships and equations providing directly a connection between experimental measurable quantities and thermodynamic, kinetic or diffusion parameters?"**

Because of, an in-depth review of available body of literature devoted to develop methods for calculations or computations of ΔG or both of these has shown that there are already determined a well-established links between energetics of molecular system and the discussed parameters, for instance, reaction kinetics and diffusion. (Consider the theoretical approximations by Iribarne, Thomson, Eyring and Arrhenius.) But establishing a link between MS kinetics, diffusion parameters and 3D structures of analyte ions is complex research task, because of, as mentioned before, MS operates with a large set of experimental methods based on different as phenomenology ionization–desorption mechanisms. It is significant research challenge to develop methodology producing straightforward outcomes of kinetic and diffusion parameters on the base on MS, which on the one hand, to express exactly the relationships under real experimental conditions; and, on the other hand, to be universally applicable with respect to all available soft ionization MS approaches.

As we have written still in the "Abstract" to this work, our more recent contributions to the latter problematic has resulted to model equations (1) and (2) connecting MS stochastic dynamic diffusion parameter "D_{SD}" with MS intensity "I" of analyte ions, accounting, as well as, for the experimental parameter *"temperature"* "T" [1].

$$D_{SD}^{tot} = \sum_{i}^{n} D_{SD}^{i} = \sum_{i}^{n} \left(1.3194.10^{-17} \times A^{i} \times \frac{\overline{I_i^2} - \left(\overline{I_i}\right)^2}{\left(I_i - \overline{I_i}\right)^2} \right) \quad \textbf{(1)}$$

$$\ln\left(\overline{\left(I - \overline{I}\right)^2}\right) = -\ln\left(-\left(\ln\left(\frac{k_B \times T}{m} \right) \right)^3 \times \frac{2 \times \Delta t \times T \times k_B}{m \times D_{SD}} \right) \quad \textbf{(2)}$$

[1] B. Ivanova, M. Spiteller, J. Mol. Liq. 292 (2019) 111307

The former relation has been exploited successfully within a small-scale research on organics and metal–organics [2]. There has been demonstrated its universal application to studied systems in quantitative terms, which represent a significant advantage of this approach, as far as, it is applicable to different molecular systems, experimental conditions and ionization methods, for instance, CID, APCI, ESI and MALDI methods, respectively. The D_{SDs} fit excellent, linearly to corresponding quantum chemical diffusions "D_{QC}" obtained on the base on Arrhenius' formalism. As is well-known, D_{QC} parameter reflects a concrete conformational or 3D molecular and electronic structures of analyte ion. Therefore, **the kernel idea and problematic for employment of MS for exact 3D structural determination of analyte ions has simplistic solution which has been offered by our model equation (1)**. For instance, the analysis of cyclodextrins has shown a coefficient of correlation $r = 0.99_{639}$ [2]. The analysis of other carbohydrates within m/z = 100–600 has resulted in $r = -0.99_{951}$. The coefficients of correlation reported, so far, of analyses of small organics such as amio acids and paptides or oligopeptides are $r = 0.9806_{8}$ (Cu^{II}–G5), 0.9901 (G6), 0.95575 (H-Trp-Trp-OH), 0.9806_{8}–0.9956 (Ag^{I}–containing metal–organics), and 0.9833 (Cu^{II}–Gly,) respectively [2]. The chemometrics of *repeatability* and *reproducibility* of D_{SD} parameters has yielded to r = 1 studying a representative set of eleven multiplications of carbolydrates. Their exploration together with the Arrhenius' formalism bridged between experimental MS and theoretical quantum chemical treatment of gas– and condense phase chemical reactions and phenomena; furthermore, this so-called *"bridging statistical model"* between two different formalisms has its own quantitative expression in chemometric terms showing a linear approximation with an excellent statistical significance [2].

[2] (a) B. Ivanova, M. Spiteller, Quantification by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Using An Approach Based On Stochastic Dynamics. Experimental And Theoretical Correspondences, GRIN Verlag, Muenchen, 2018, pp. 1–86, ISBN 9783668703179; (b) B.Ivanova, M. Spiteller, J. Mol. Struct. 1173 (2018) 848-864; (c) B.Ivanova, M.Spiteller, Experimental mass spectrometric and theoretical treatment of the effect of protonation on the 3D molecular and electronic structures of low molecular weight organics and metal–organics of silver(I) ion, In book: Protonation: Properties, Applications and Effects, A. Germogen (Ed.) (2019), Nova Science Publishers, N.Y., pp. 1–182, ISBN: 978-1-53614-886-2; (d) B.Ivanova, M.Spiteller, Bioorg. Chem. 93 (2019) 103308; (e) B. Ivanova, M. Spiteller, Mass Spectrometric Experimental and Theoretical Quantification of Reaction Kinetics, Thermodynamics and Diffusion of Piperazine Heterocyclics in Solution, In book: Advances in Chemistry Research, J. Taylor (Ed.), Publisher: NOVA Science Publishers Inc., N.Y., Volume 48, (2019) pp.1-82, ISBN: 978-1-53614-724-7; (f) B. Ivanova, M. Spiteller, J. Mol. Struct. 1179 (2019) 192–204; (g) B. Ivanova, M. Spiteller, J. Mol. Struct. 1199 (2020) 127022.

Equation (2) has been designed to provide a detail precise quantitative account of the environmental factor *"temperature"* for the experimental MS parameter *"intensity"* as one of the most important experimental parameters affecting on the MS ionization efficiency; the 3D molecular conformation of the analyte ions and the chemical reactivity, among others [1]. Its validity and universal applicability to differen MS methods has been tested, as well as, using a small-scale analytes. Again, excellent statistical correlation parameters *"r"* have been obtained [1,2d,g,3]. For instance, $r = 0.9977_4$ (Cu^{II}–Gly, ESI;) 0.9979_6 and 0.9920 (*m/z* 115, 247, 259 and 252 (G5), *m/z* 361, 246, 210, 190, 172, 133, 115 and 109 (G6), ESI;) **0.9990_{26}–0.9994_{27}** (*m/z* 387, 441, 450, 326 and 304 (Zn^{II}–G5,) ESI;) 0.99279 (*m/z* 106, 131, 171, 154, 137, 214, 184, 159, 199 and 137, nitroamine derivative of 3-aminomethyl-3,5,5-trimethyl-cyclohexylamine, ESI;) **0.9995** (*m/z* 606, 522, 454, 389 and 306, cytidine, APCI;) and 0.9969_8 (coupled products of interaction of substituted benzoic acids,) respectively [1,2d,g,3].

In particuar, talking about, application of the mass spectrometry to 3D structural analysis from the perspective of ***"medicinal chemistry"*** and ***"pharmacy"*** there are a number of additional points that call for further comments on. The quantitative description of drug-protein interaction remains a significant challenge in the drugs design and discovery of new efficacious therapeutics. There is a critical need to a comprehensive understanding of molecular level processes of bond formation of drug-candidate and target peptide/protein, in order to, detail chemical reactions governing the biological function of therapeutics in living cells. It is acknowledged that the task of characterization of interactions/bonds of small biologically active molecules and peptides/proteins remains a major technology gap in the *clinical trials*. **As is well-known, the methods of mass spectrometry, amongst others analytical tools, are acknowledged as powerful and irreplaceable approaches to quantify analytes in complex mixtures encompassing small molecules and biologically active macromolecules (molecular weights ∈ 10–100 kDa.) The major reasons for the latter fact are the superior instrumental features of the soft ionization MS methods**. Therefore, any methodological contribution to the field of mass spectrometry affects crucially on the development of the fields of the ***"medicinal chemistry"*** and ***"pharmacy."***

[3] B. Ivanova, M. Spiteller, On the temperature dependence on the stochastic dynamic mass spectrometric diffusion parameter, GRIN Verlag, Muenchen (2019), ISBN: 9783668985797, pp. 1–40.

However, the mass spectrometric analysis of peptides and proteins is very frequently complicated by abundance ions of adducts and complex oligomeric associates or complexes of ions of alkali metals and analytes. This fact, difficults the simultaneously determination of these analytes; furthermore, in complex biological or environmental matrixes or both of these using the currently implemented in the analytical practice methods for quantification. Owing to the polyproton accepting ability of oligopeptides and their larger biomacromolecular analogous; the heterogeneous distribution of dipoles and charges over the molecular skeleton; capability of an intramolecular cyclization; and multiple proton transfer effects, it seems reasonable to assume that the fact that the oligopeptides tend to stabilize adducts, rather appears a disadvantage, than an advantage in studying these systems quantitatively and structurally by mass spectrometry. Furthermore, in cases of a homology series of homooligopeptides, there are a set of common structural fragments and, thus, common MS ions to NH_4^+–adducts, in particular, NH_4^+–adducts. For that reason, so far, adducts of oligopeptides have been described mainly qualitatively or the individual structures have been characterized by semi–quantitative approaches. But, it has not been yet shown how these adducts, in particular, talking about common NH_4^+–adducts to homooligopeptides fit mutually in quantitative terms. The limitations of qualitative or semi–quantitative–descriptive strategies can be underlined by a comparative analysis between ESI– and CID–MS spectra of homology series of homooligopeptides, for instance, studying adducts of glycylhomopenta– (G5) and glycylhomohexapeptides (G6) (**Figures 1** and **2**) and; thus, from the perspective of qualitative and semi–quantitative ESI–MS analyses such analytes cannot be distinguished unambiguously; furthermore, in mixture, nevertheless, superior instrumental characteristics of the soft–ionization methods; and despite their ultra-high resolving power. It is easy to see from the large body of literature devoted to adducts of peptides either ammonium adducts or complex species of analytes with alkali metal ions, that the aforementioned point of view is, in general, adopted.

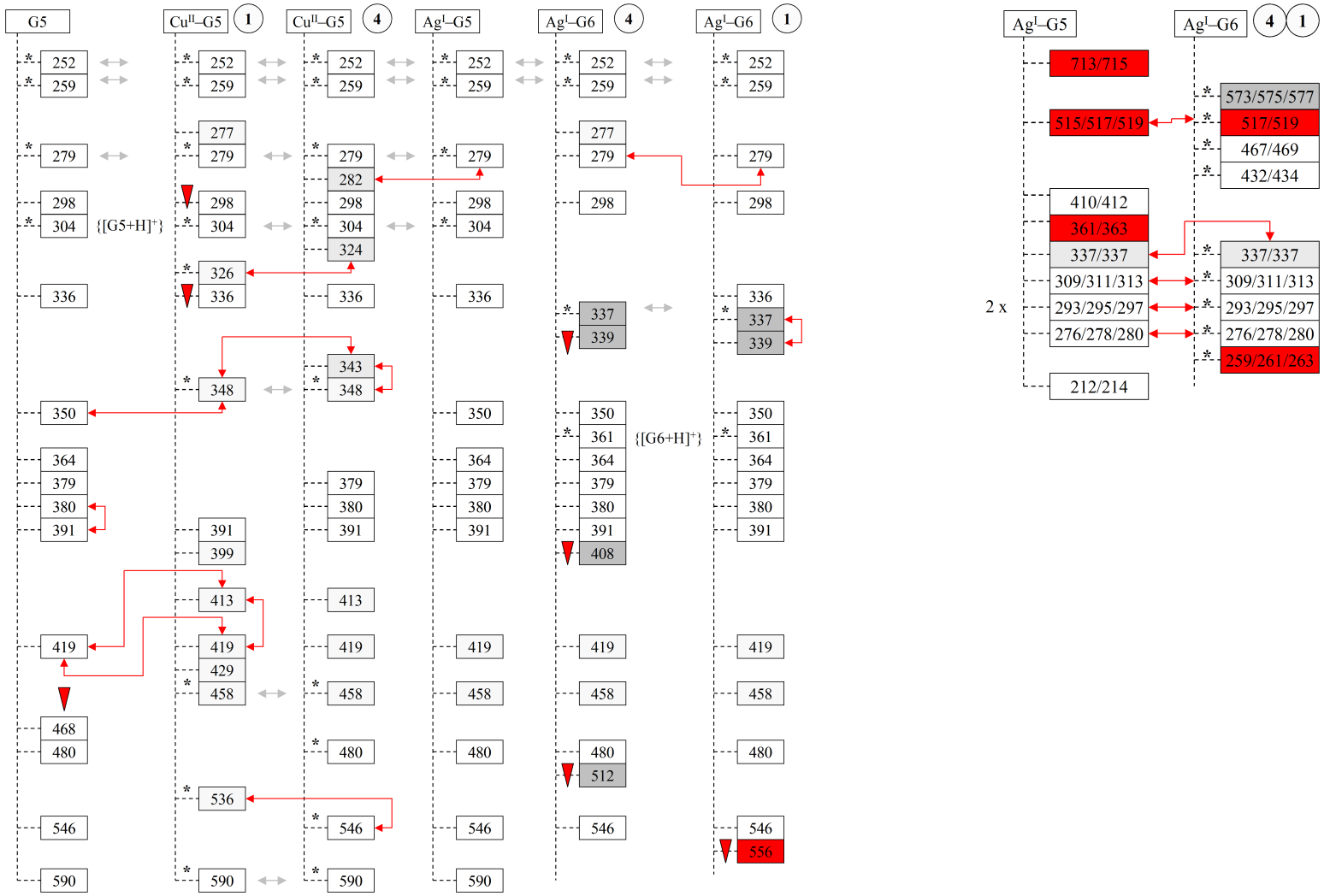

Figure 1. Fragment CID–MS reactions of G5, G6 and their Ag^{I}-complexes studied correlatively in this paper; there are detailed molecular level mechanistic aspects of reactions shows with arrows; *–fragment reactions, which have been already examined in works [2c]; reference [2c] contains only D_{SD} parameters of the isotope sub-components of Ag^{I}–G6.

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At this point, we should undeline one of the most obviouse contribution of our stochatic dynamic theory and model equations (1) and (2). They extremely successful are able to quantify precisely, accurately, sensitively, and explicitly, selectively even subtle changes of the 3D molecular and electronic structures of the analyte ions; thus, distinguishing quantitatively among even homology series of homopeptides. Our method; therefore, dramatically changes the capability of the mass spectrometry, because of, its application to the analytical practice goes far beyond the routine implementation for the analytical purposes. Conversely, by means of our innovative formulas (1) and (2), the methods of mass spectrometry appear powerful experimental approaches for multidimentional structural analysis.

Therefore, in this work, we take an opposite position against the common view mentioned before and consider quantitatively CID–MS fragment reactions of common adducts and their Ag^{I}–complexes of G5 and G6 by means of equations (1) and (2). Thus, our study aims at answering to the following questions: **What, in fact, insights *into* the molecular level interactions and quantitative parameters of these interactions have we actually gained from experimental soft–ionization ESI– and CID–MS mass spectra of glycylhomooligopeptides and their coordination species; and does mass spectrometry provide capability of quantitative distinguishing among structurally very similar analytes like the homology series of homopeptides?**

At this point of the introductory section it seems adequately to clarify briefly the latter line of though. Despite, the complexity of the molecular skeleton of homooligopeptides there are a set of common fragment paths, which yield to identical patterns of daughter MS ions. As can be expected these common fragment products to reactions produce a set of common adducts to these oligopeptides. The formal limit of though is that on the base on the *m/z*–values the fragment products of adducts are undistinguishable. Namely this point entails that common adducts to homooligopeptides cannot be employed for a precise determination of these analytes even within a single component mass spectrometric analysis. **Is this latter stance true, however? We have found that equations (1) and (2) *via* the D_{SD} parameter are adequate quantitative criteria of not only precise and sensitive, but also selective determination of analytes; furthermore, from the perspective of the chemometrics.**

Therefore, the main aims of the current research are manyfolds: *(i)* Quantification of MS kinetic and diffusion parameters according to our model equations (1) and (2) of Ag^{I}–complexes of associates of G5 and G6; (*ii)* correlative analysis among MS diffusions obtained within the framework of an independent method, for instance, *"current monitoring method;" (iii)* quantification of common CID–MS ions to NH_4^+–adducts of G5 and G6 aiming at establishing the quantitative relationships between the discussed parameters of the common reactions: *m/z* 380→363, *m/z* 379→363 + 250, *m/z* 364→347, *m/z* 350→333, *m/z* 339→321 and *m/z* 336→319, respectively, from the perspective of the stochastic dynamic concept discussed in this work; and *(iv)* 3D structural analysis of complex ions on the base on a correlative analysis between D_{SD} and D_{QC} parameters.

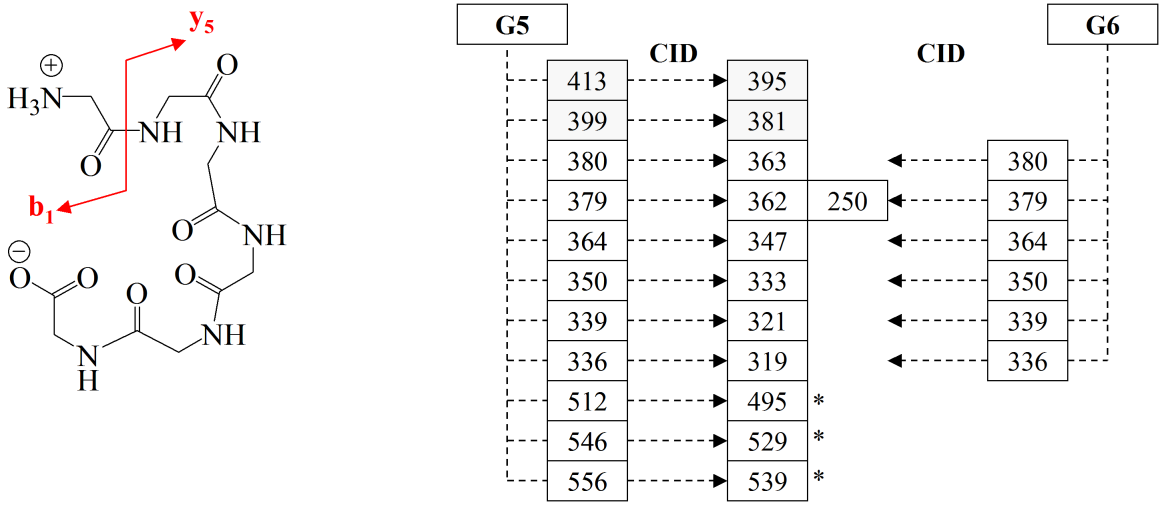


Figure 2. Chemical diagram of G6; major fragment path leading to b_1 ion; common to G6 and G5 oligopeptides fragment paths under CID–MS reactions; the fragment CID reactions of MS ions at *m/z* 399 and 413 of the free ligand G5 are typical at presence of transition metal ions; the highlighted MS ions (*) have been partially described in [2c] (the other fragment reactions are detailed, herein, for the first time in the literature.)

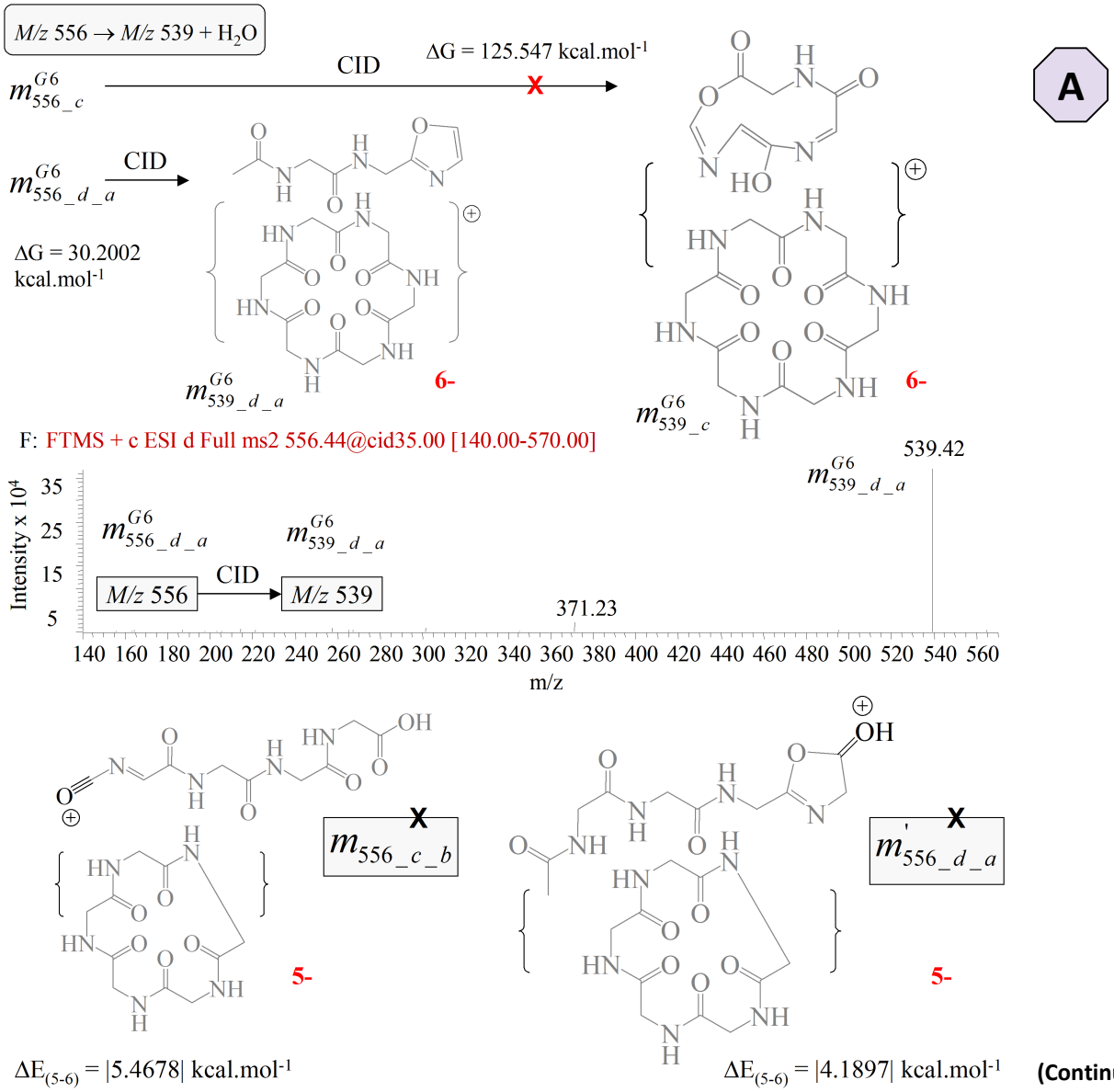
Results and discussion

Assignment of experimental mass spectrometric peaks to corresponding molecular ions

Throughout this werk we argue that the complementary application of our equation (1) provides highly accurate 3D structural information on analyte MS ions. The persistent challenge facing the determination of D_{QC} parameters with respect to the latter equation is to account for the correlation between 3D molecular and electronic structures of MS ions and their energetics. Because of with increasing in complexity of molecular and electronic structures of analyte MS ions a number of additional challenges are raised both to obtain information about the minimum of the potential energy surface **(PES)** of ionic species and to account for subtle electronic effects. The difficulty of determining accurately PESs is based on the fact that peptides exhibit heterogeneous distributions of dipoles and charges together with a capability of intramolecular cyclization and proton transfer effect. It follows; therefore, that the complexity of research tasks is increased in associates of peptides, their fragment ions and complexes with ions of transition metals. In order to capture this problematics in detail we carry out a comparative analysis of static and molecular dynamic quantum chemical analyses taking into consideration subtle electronic effects which as can be seen from the results presented and discussed below play crucial role at accurate distinguishing between most stable from the perspective of chemical thermodynamics complex MS ions of peptide associates and their metal–organic species. If we would like to understand comprehensively the governing forces driving fragment MS processes under MS experimental conditions and to assign accurately corresponding MS peaks to 3D structures of molecular ions, then we need to be able to distinguish precisely between energies of structural isomers of oligopeptide ions; furthermore talking about analysis of G5 and G6 which are structurally very similar analytes. Moreover, they exhibit a large set of identical fragment MS reactions in particular looking at the low *m/z* values.

Logically, first, we should address the following questions: ***(i)*** **Does the fragment behavior of dimeric associates of G5 and G6 follow common rules typically observed studying MS ions at low *m/z* values?; and *(ii)* if so, then, do these reactions produce fragment ions with one and the same 3D structures?** In the course of the next discussion we shall examine namely common fragment reactions in Ag^{I}–G5 and Ag^{I}–G6 in order to answer above questions, because of it is self–evident that from this analysis depends the accuracy of corresponding D_{QC} parameters which we correlate with experimental D_{SD} parameters. **Figure 3** depicts experimental MS spectra of peptides and their metal–organic complexes. As we have discussed [2] both G5 and G6 tend to form dimeric associates. The CID–MS fragment process of MS ion at *m/z* 556 of G6 causes for an observation of product ion at *m/z* 539. On the base on typically found in MS spectra of monomers of oligopeptides fragments [2c] we propose a series of acyclic and cyclic species of dimeric ions of G6 (**Figure 4.**) The MD computations of energetics of these species indicate as most preferred associates $m^{G6}_{556\_d\_a}$ and $m_{556\_c\_a}$. However, the isolated oligopeptides exhibit a common fragment reaction yielding to b_1 ion thus expecting a formation of cyclic associates of type $m^{G6}_{556\_d\_b}$ and $m_{556\_c\_b}$ as depicted in **Figure 4**. Despite the fact that associates $m^{G6}_{556\_d\_a}$ and $m_{556\_c\_a}$ appear highly probably complex ions belonging to MS peak at *m/z* 556 it is important to underline that differences in energies of associates $m^{G6}_{556\_d\_a}$ and $m^{G6}_{556\_d\_b}$, as well as, $m_{556\_c\_a}$ and $m_{556\_c\_b}$ are only ΔE = |5.5| and |4.2| kcal.mol^{-1} (or |0.00876| and |0.00669| a.u.)

The resulting insignificant difference raises a question about the importance of the knowledge on energetics of common competitive reactions of associates of G5 and G6, as well as, their complex species, because of as we could expect, the assignment of MS ions in mixtures of oligopeptides can be carried out accurately and precisely only if we operate with an accurate assignment of forms of peptides and their complexes in isolated systems; furthermore, the assignment should be carried out quantitatively as demonstrated in this paper. Same is true for the analysis of fragment MS ions produced as a result from CID–MS reaction of MS ions at *m/z* 546 and 458. Among the competitive reactions favoring stabilization of given ensemble of interacting oligopeptides and/or their fragment ions, in particular, focusing the attention on those associates containing cyclic G6 and G5 molecules are those associated with cyclization of G6 and G5 as well as the reaction G6 $\rightarrow$ G5 + b_1.



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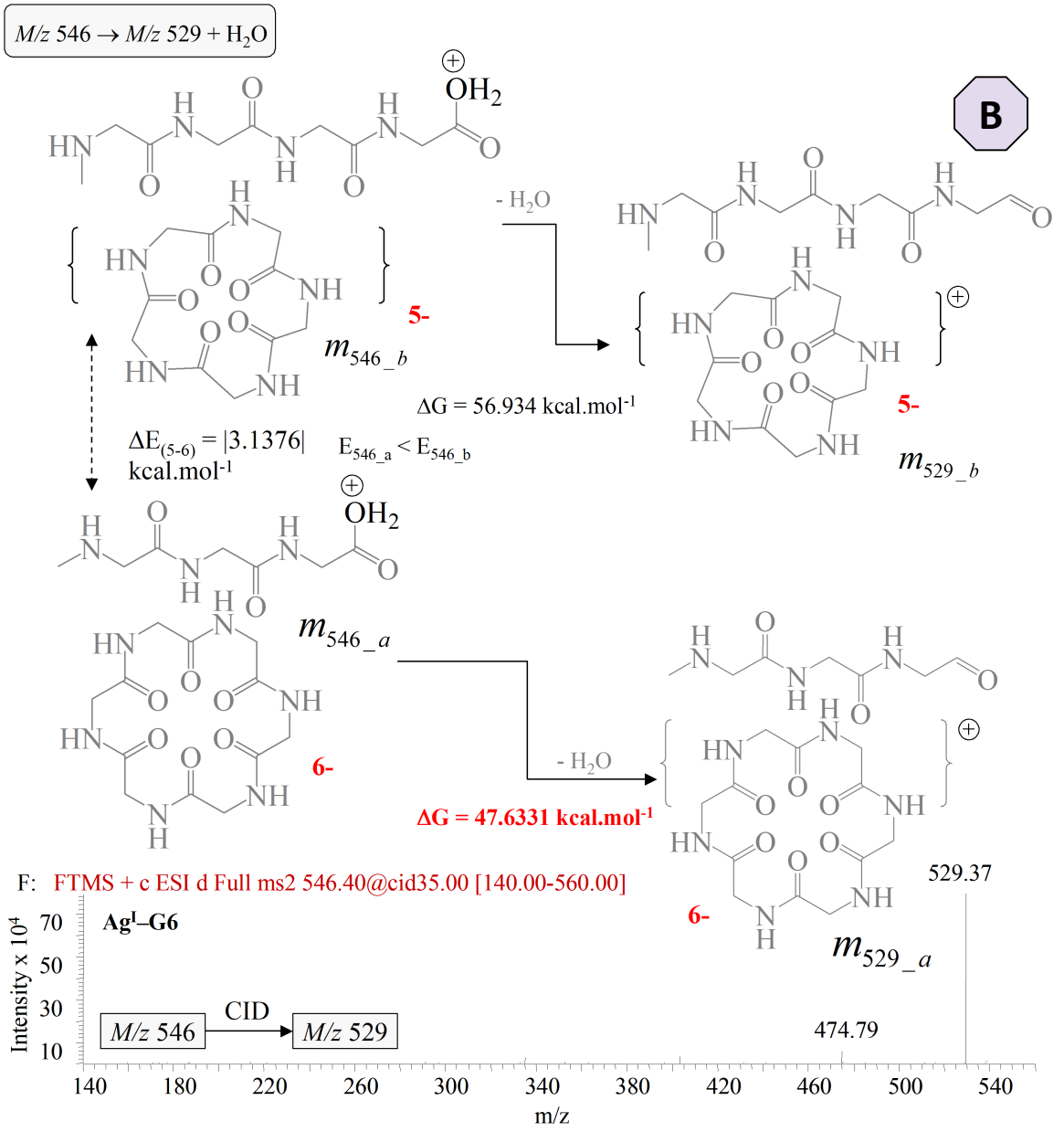

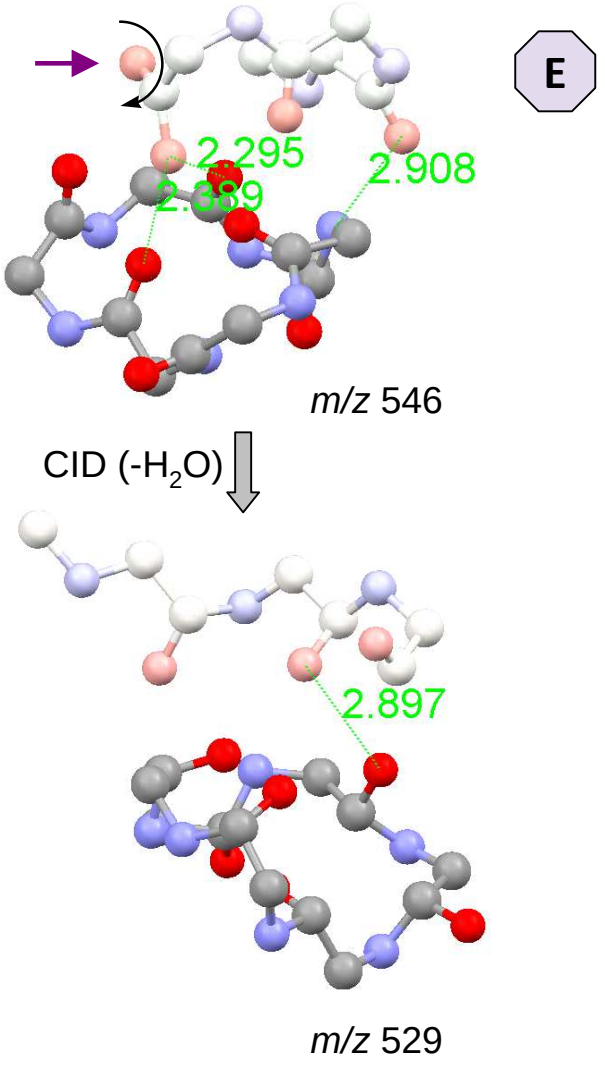

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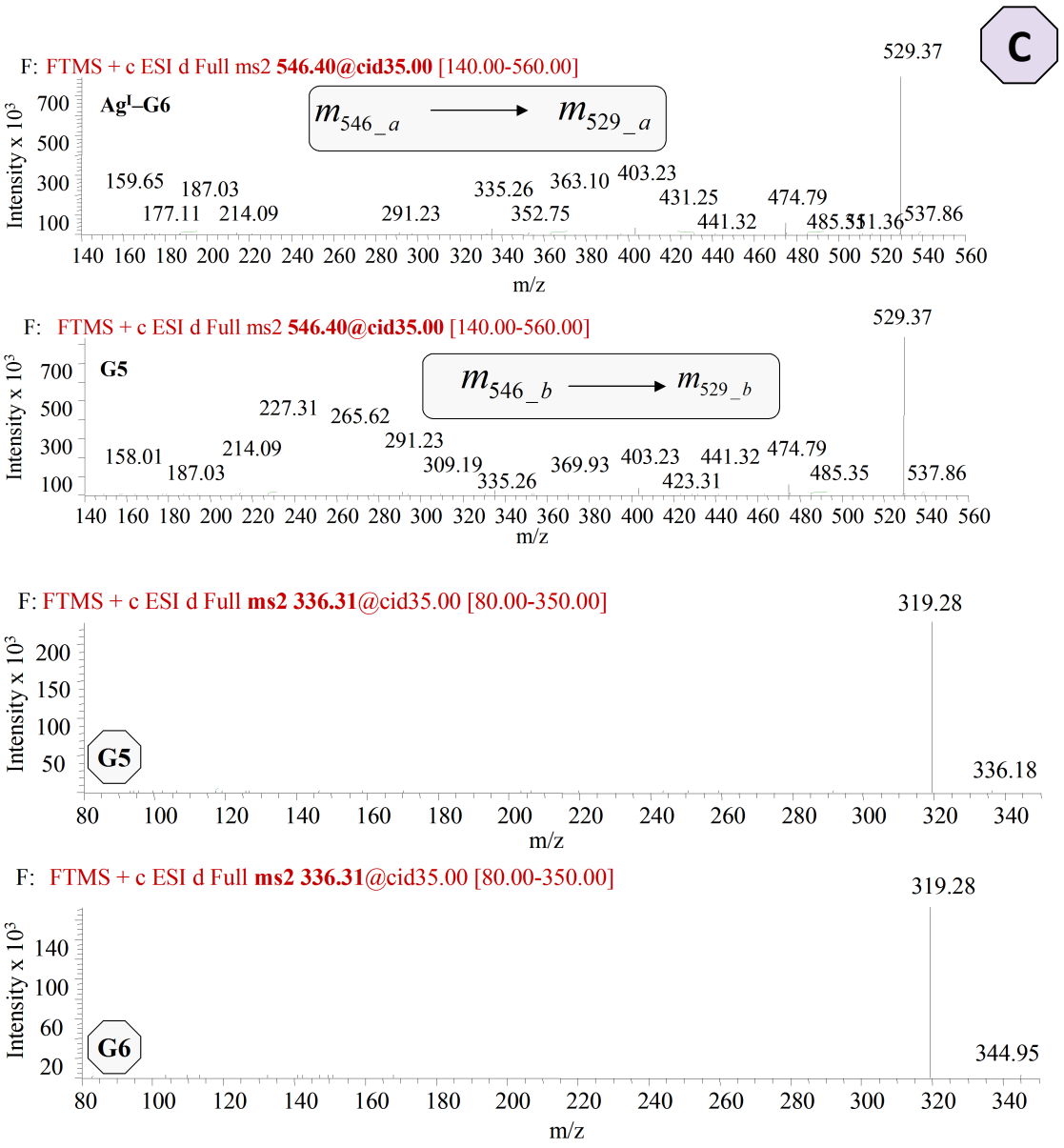

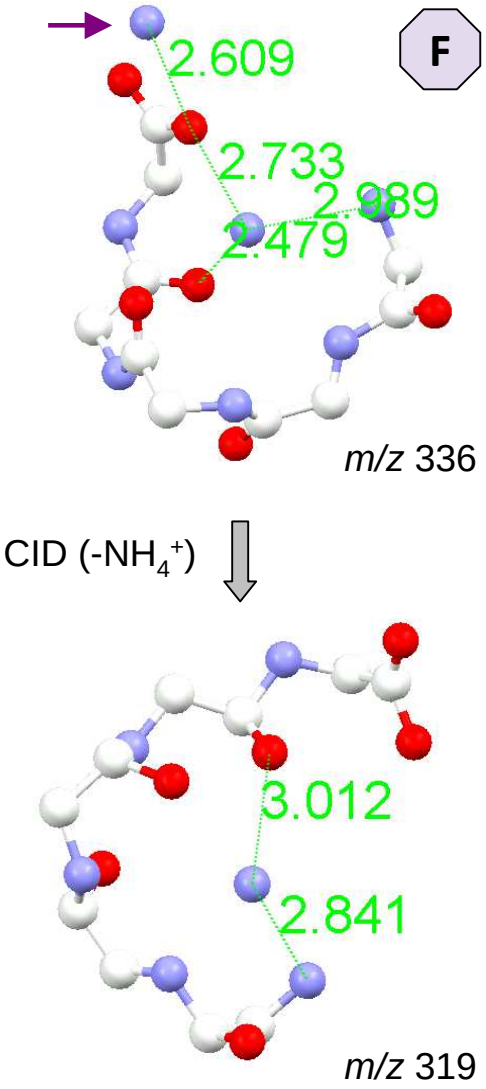

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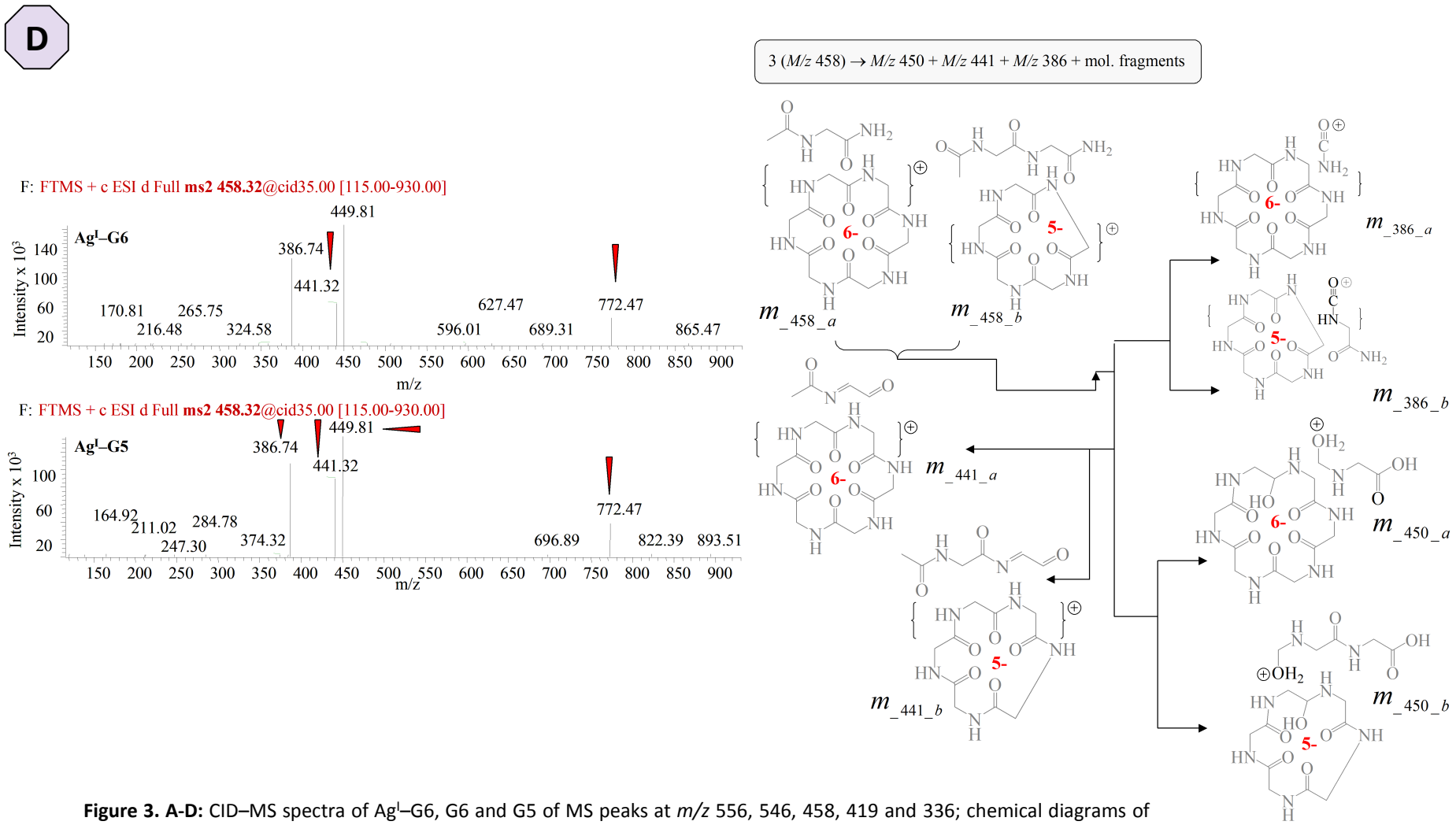

Figure 3. A-D: CID–MS spectra of Ag^{I}–G6, G6 and G5 of MS peaks at *m/z* 556, 546, 458, 419 and 336; chemical diagrams of the studied MS species; free Gibbs energy parameters of the fragment reactions $m^{G6}_{556\_c} \rightarrow m^{G6}_{539\_c}$ and $m^{G6}_{556\_d\_a} \rightarrow m^{G6}_{539\_d\_a}$; **E:** change of the 3D molecular structures of ions at *m/z* 546 and 529, due to, a cleavage of H_2O molecule under CID reaction; **F:** Change of the 3D molecular structures of ions at *m/z* 336 and 319, due to, loss of NH_4^+ ion under CID reaction; interionic hydrogen bonds [Å]; Hs are omitted.

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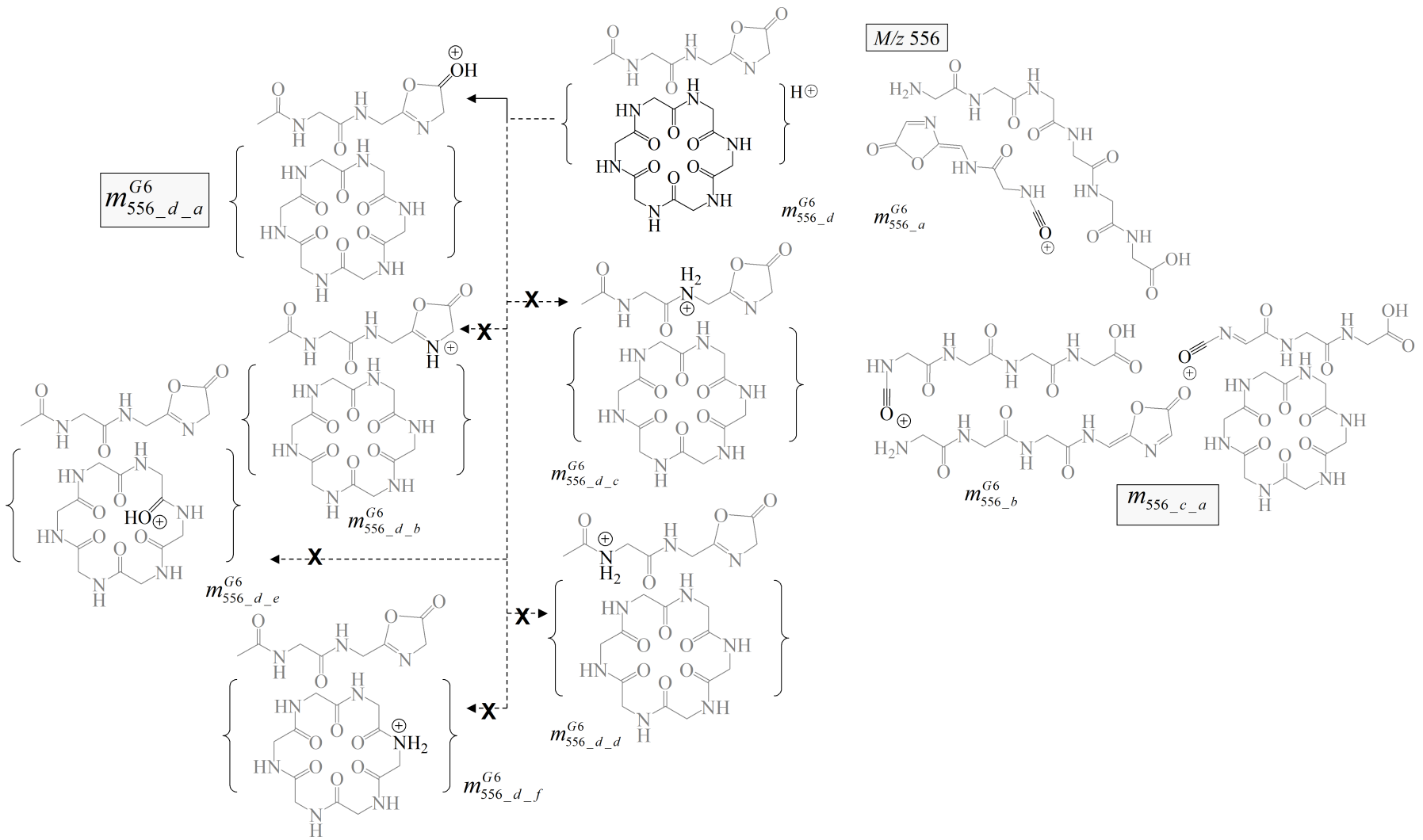

Figure 4. Chemical diagrams of ensembles of G6 molecules/ions within the framework of dimeric associates in assigning the MS peak at *m/z* 556.

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Next, we will argue this statement through a critical interpretation of the *ab initio* and DFT MD data of these processes comparing with the energetics of the presented above MS fragment reactions. However, it is important to mention that in this analysis we did not carried out examination of specific geometry parameters of the optimized ensembles of interacting ions according to the reaction stated above (**Figure 5**.) The main priority is examining of energetics of molecular ions with respect to the most stable state at PES (**Table 1**.) Reaction G5 → *c*G5 + H_2O appears less preferred from the perspective of chemical thermodynamics. On the contrary, the loss of b_1 ion appears preferred fragment reaction examining both cyclic and acyclic derivatives of the peptides. Nevertheless, the latter reaction of acyclic fragment species shows lower ΔG value.

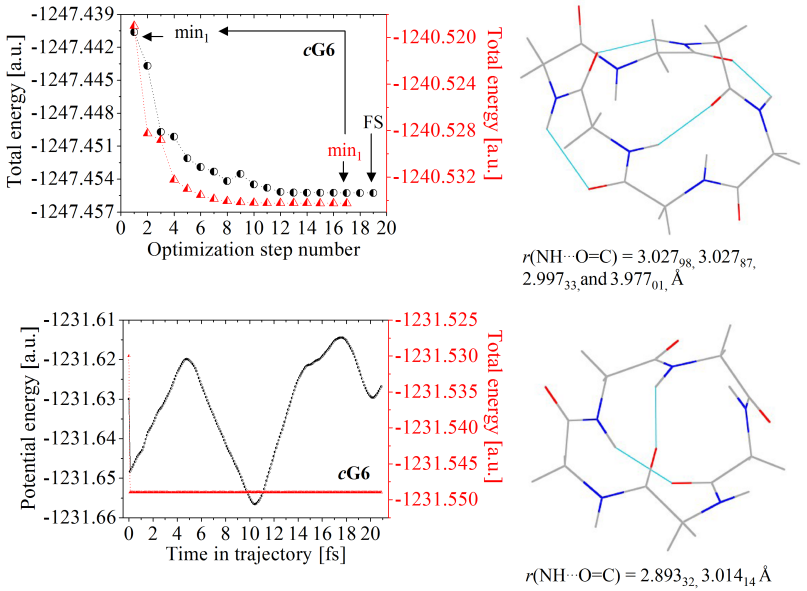


Figure 5. DFT optimization and molecular dynamics of cyclic G6 and G5 peptides; total energy [a.u.] with respect to the optimization step number; potential or total energies [a.u.] *versus* time in trajectory [fs]; optimized 3D molecular structures; intramolecular hydrogen bond lengths [Å].

Table 1. Gas–phase thermochemistry (M062X/SDD) of *c*G5 and *c*G6; their protonated species or interacting ensembles of fragment ions; the conformations correspond to most stable forms of MM/MD analysis.

	***c*G5**	***c*G6**	**G5H (G5_O^4H form)**	**G6H**
E_{ZPVE}	179.86686	229.32821	204.22922	252.01150
E_{corr}	0.301479	0.383411	0.343089	0.419371
H_{corr}	0.302344	0.384276	0.343954	0.420236
G_{corr}	0.247493	0.321314	0.281608	0.359754
ε_0	0.286636	0.365458	0.325460	0.401606
E	-1039.247966	-1240.150839	-1115.956933	-1316.507958
H	-1039.233122	-1240.149974	-1115.956068	-1316.490192
G	-1039.232257	-1240.212937	-1116.018414	-1316.489327
E^*	-1039.5346016	-1240.5342506	-1116.3000219	-1316.9095636

E_{ZPVE} – Zero-point vibrational energy [kcal.mol^{-1}]; ε_0 – Zero-point correction [Hartree.(partice)$^{-1}$]; E_{corr} – Thermal correction to energy [Hartree.(partice)$^{-1}$]; H_{corr} – Thermal correction to enthalpy [Hartree.(partice)$^{-1}$]; G_{corr} – Thermal correction to free energy [Hartree.(partice)$^{-1}$]; E – Sum of electronic and thermal energies [Hartree.(partice)$^{-1}$]; E – Sum of electronic and thermal enthalpies [Hartree.(partice)$^{-1}$]; G – Sum of electronic and thermal free energies [Hartree.(partice)$^{-1}$]; E^* – E_{SCF} [Hartree.(partice)$^{-1}$].

Central to our discussion is the capability of the MS of distinguishing, quantitatively, between glycylhomooligopeptides under CID–MS experimental conditions, owing to the similarity of the molecular skeleton. To put this concept differently, as shown above, our goal is to develop method which to determine quantitatively these species in complex mixtures. The interaction with Ag^{I}–ion appears prominent approach for such purposes because of as **Figure 3** shows the fragment patterns of the associates in presence of Ag^{I}–ion are different mutually, and comparing with the patterns of the isolated peptides themselves. Considering CID–MS data of the peak at *m/z* 419 can be seen that in the case of Ag^{I}–G6 complexes there are found fragment MS ions at *m/z* 316, while the CID–MS spectrum of Ag^{I}–G5 shows peaks at *m/z* 367 and 392. On the contrary, CID–MS spectrum of G5 of peak at *m/z* 419 reveals fragment species at *m/z* 410 and 341. It do not need to me underline that our approach leads to a very distinguishable fragment patterns both qualitatively and quantitatively, of G5 and G6 in presence of Ag^{I}–ion, looking at the data shown above. However, it should be kept in mind that the 3D structural determination represents challenge, as far as, the molecular ensembles corresponding to the parent ion at *m/z* 419 and the fragment ions at *m/z* 367 and 391 can be complex. Nevertheless, the energetic of these systems is unambiguous.

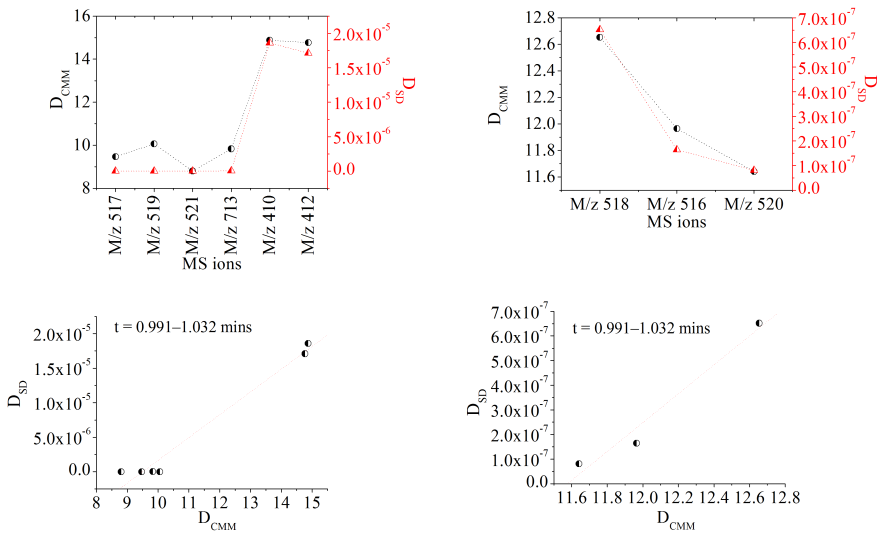
Quantitative analysis – determination of the stochastic dynamic mass spectrometric diffusion parameters

To begin with, the absolute MS intensity of analyte peaks of associates obtained experimentally with respect to different spans of time are determined. Perhaps, it would be useful to remind the reader that a detailed description of the computations according to the stochastic dynamic methodology and equation (1) can be found in [2]. In the course of our next discussion, we are looking at the D_{SD} parameters in **Table 2**. The analysis of metal-organics of the associates focuses on MS ions at *m/z* 515/517/519, 410/412, 337/339, 309/311/313, 293/295/297, 276/278/280 and 212/214, respectively.

Next, **Figure 6**, reflects the correlative analysis between D_{SD} and D_{CMM} parameters according to the independent experimental method called *'current monitoring method"* as mentioned before. There are obtained coefficients of correlations r = 0.98364–0.98736. Again, like in the case of Ag^{I}–G6 complex [2d] there is an excellent linear correlation. Furthermore, so far, our studies have shown that D_{SD} parameter appears very sensible quantity in distinguishing between diffusions of the components of isotope shape. Among the most important conclusion based on the data, herein, is that the analysis of MS ions of Ag^{I}–G5, in fact, supports for the same statement.

Table 2. Experimental ESI(+)– and CID–MS parameters of G5 and G6 of MS peaks at *m/z* 363, 319, 333 and 336; σ^2 and σ'^2 – variance parameters; RT = 0.83 mins.

G5	*m/z* 363	*m/z* 319	*m/z* 333	*m/z* 336
t [min]	1.307–1.336	1.116–1.143	0.087–0.130	0.087–0.130
$\bar{I}$	4105.33333	6933	269.75	374.75
$(\bar{I})^2$	$1.68538.10^7$	48066489	72765.0625	140437.5625
$\overline{(I)^2}$	$2.52019.10^7$	140942349.667	281457.25	548367.25
σ^2	8348096.667	92875860.667	208692.1875	407929.6875
$\overline{(I-\bar{I})^2}$	8348139.815	92875972	208692.1875	407929.6875
P1	***3.924***	***$3.92468.10^{-8}$***	***$3.92477.10^{-8}$***	***$3.92477.10^{-8}$***
lnP1	***-17.053_4***	***-17.053_{39}***	***-17.053_{373}***	***-17.053_{373}***
σ'^2	508344.155	6347508.407	101720.35	198833.319
D_{SD}	$2.288.10^{-10}$	$2.85637878.10^{-9}$	$4.57741581.10^{-11}$	$8.9475.10^{-11}$
G6	*m/z* 363	*m/z* 319	*m/z* 333	
t [min]	12.112–12.142	12.306–12.351	12.306–12.351	
$\bar{I}$	8163.667	2761	1380.5	
$(\bar{I})^2$	66645453.444	7623121	1905780.25	
$\overline{(I)^2}$	100080451	14968029.5	3779655	
σ^2	33434997.556	7344908.5	1873874.75	
$\overline{(I-\bar{I})^2}$	33435004.593	7344907.75	1873873.625	
P1	***$3.92476.10^{-8}$***	***$3.92477.10^{-8}$***	***$3.92481.10^{-8}$***	
lnP1	***-17.053_{376}***	***-17.053_{371}***	***-17.053_{362}***	
σ'^2	1933991.46	272696.363	163745.8135	
D_{SD}	$8.70296.10^{-10}$	$1.227134.10^{-10}$	$7.368562.10^{-11}$	



	Value	Error	*r*	SD	N	*p*
A	$-3.137.10^{-5}$	$3.0688.10^{-6}$	**0.98736**	$1.6349.10^{-6}$	6	$1.6349.10^{-6}$
B	$3.303.10^{-6}$	$2.651.10^{-7}$				

	Value	Error	*r*	SD	N	*p*
A	$-6.792.10^{-6}$	$1.2993.10^{-6}$	**0.98364**	$7.858.10^{-8}$	3	0.11531
B	$5.8666.10^{-7}$	$1.074.10^{-7}$				

Figure 6. Correlative analysis between the stochastic dynamic diffusion parameters "D_{SD}" according to equation (1) and corresponding diffusions "D_{CMM}" obtained according to *"current monitoring method"* of MS ions of complex Ag^I–G5; chemometric; D_{SD} parameters are tabulated; D_{CMM} parameters within the span of time t = 0.991–1.032 mins (Δt = 2.46 s) are: 11.964173 (*m/z* 516), 12.6535 (*m/z* 518), 11.64088 (*m/z* 520), 9.46666 (*m/z* 517), 10.061426 (*m/z* 519), 8.805158 (*m/z* 521), 9.83413 (*m/z* 713), 14.87042 (*m/z* 410) and 14.7654 (*m/z* 412), respectively.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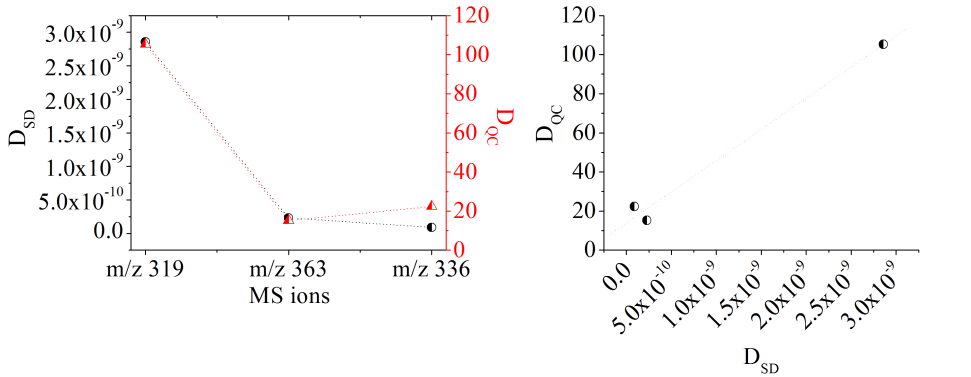
Correlative analysis between experiment and theory – stochastic dynamic and quantum chemical diffusion parameters

In the light of the concept of development of universally applicable model equation treating quantitatively the experimental mass spectrometric intensity presented in this work together with a small-scale of previous research on this topic [2], as well as, observed facts that the D_{SD} parameters according equation (1) provide direct 3D molecular and structural information about the analyte MS ions when they are correlated with the quantum chemical diffusion parameters according to Arrhenius's theory — consider detail in [2] — in this sub-section we correlate D_{SD} and D_{QC} parameters of adducts of the studied glycylhomooligopeptides. The corresponding data on other ESI– and CID–MS fragment ions of G5 and G6, as well as, their complexes with Ag^{I}-ion can be found [2d]. There have been examined the following MS species: *m/z* 87, 98, 109, 115, 133, 172, 190, 201, 229, 247, 252, 259, 286, 302, 304, 343, 361, 546 and 556, respectively. As reference [2d] clearly shows excellent coefficients of correlations have been found for the monomeric species and their cordination compounds.

Logically, there lies the question: Does D_{SD} and D_{QC} parameters of flexible noncovalently bonded associates fit mutual linearly with a statistical significance? This sub-section addresses namely this problem. **Figure 7** highlights a coefficient of correlation in this case $r = 0.9933_2$.

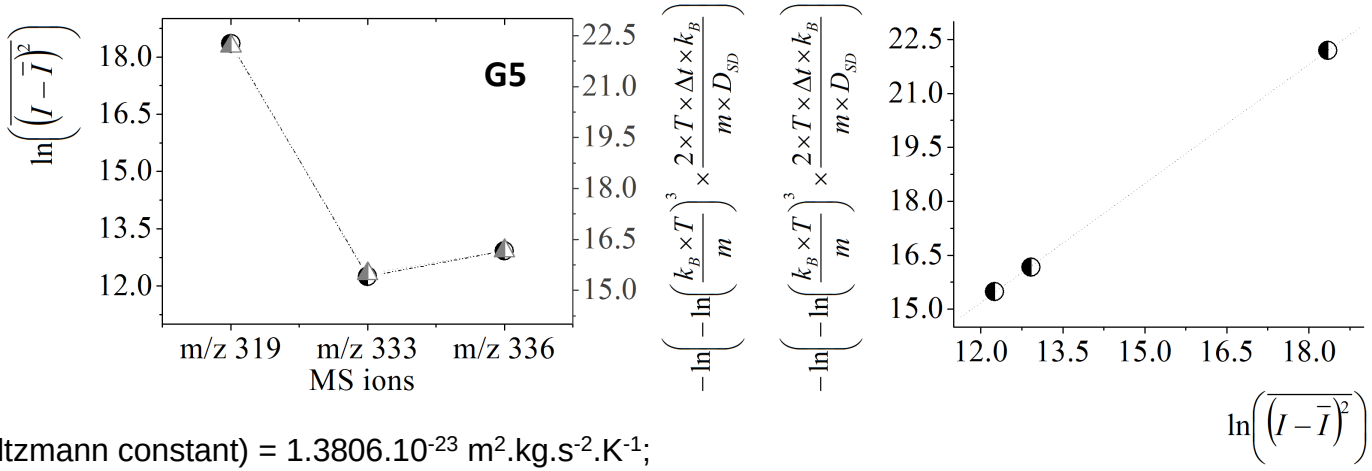


	Value	Error	*r*	SD	N	*p*
A	13.81546	6.13481	**0.99332**	8.1706	3	0.07362
B	$3.191.10^{10}$	$3.71.10^{9}$				

Figure 7. Correlative analysis between D_{SD} [$cm^2.s^{-1}$] and D_{QC} parameters; the thermo chemical data are obtained and used, together with, frequency analysis; the D_{QC} data are: 105.25_{09} (m_{319_e}), 22.9529 ($m_{336\_d\_d}$) and 15.19_{49} (m_{363_c}).

Temperature dependence on the stochastic dynamic diffusion parameter

In this sub-section equation (2) is exploited [1,3]. Its capability of accounting for the effect of the temperature on the 3D molecular and electronic structures of the analyte ions via the D_{SD} parameter is vindicated. **Figure 8** depicts the functional relationships of the MS intensity *per* span of the scan time of the experimental measurements. As can be seen from the data in Table 2 and the latter figure there is obtained, again, an excellent coefficient of correlation ***r* = 0.9999$_{66}$**.



k_B (Boltzmann constant) = 1.3806.10^{-23} m^2.kg.s^{-2}.K^{-1};
Δt (span of scan time) = $t - t_0$ = 1.74–2.58 s;
T = 299.15 K;
m – mass of the ion; and
D_{SD} – stochatic dynamic diffusion parameter [cm^2.s^{-1}] according to equation (2).

	Value	Error	
A	1.93665	0.1355	
B	1.10378	0.0092	
	r	SD	*p*
	0.9999$_{66}$	0.0434	0.0053

Figure 8. Relation between $\ln\left(\overline{\left(I-\bar{I}\right)^2}\right)$ and $-\ln\left(-\ln\left(\frac{k_B\times T}{m}\right)^3\times\frac{2\times T\times\Delta t\times k_B}{m\times D_{SD}}\right)$ of MS ions of G5; chemometrics; temperature and scan times (Δt) [s]; the $\overline{\left(I-\bar{I}\right)^2}$ and D_{SD} values [cm^2.s^{-1}] are taken from **Table 2**.

Remark

Drawing on our small-scale empirical research, so far, this paper defends our innovative stochastic dynamic concept and model equations (1) and (2) connecting experimental intensity values with D_{SD} with respect to different spans of measurement time and their applicability to 3D structural determination of analytes. According to this concept the experimentally determined D_{SD} parameters and those obtained theoretically correlate mutually and linearly with excelent coefficians of correlations from the perspective of the chemometrics.

In analysing the foregoing results in this work as we can expect the energy criterion ***"free Gibbs energy"*** used in our computations — the essential methodology behind the computation of the D_{QC} parameters — appears very sensitive and selective toward subtle electronic effects and differences of the charge redistribution within the framework of peptide molecular skeleton. However, the comparative analysis of ΔG parameters of the fragment reactions in gas– and polar continuum phases has shown that depending on the polarity of the environment the reaction paths are different (**Figures 9** and **10.**) Therefore, a reliable and adequate design of chemical reactions needs crucially a detail understanding of the mechanism of ESI–MS. The latter task requires a systematic correlative study between experiment and theory accounting for different molecular and environmental factors and parameters. Despite, enormous contributions to this field as was underlined still in the introductory section to this paper, a common concept is still debated [4]. All data in polar continuum, support the experimental observations, which correlate with recent report, as well [4].

[4] B. Marsh, K. Iyer, R. Cooks, J. Am. Soc. Mass Spectrom. (2019) DOI: 10.1007/s13361-019-02264-w.

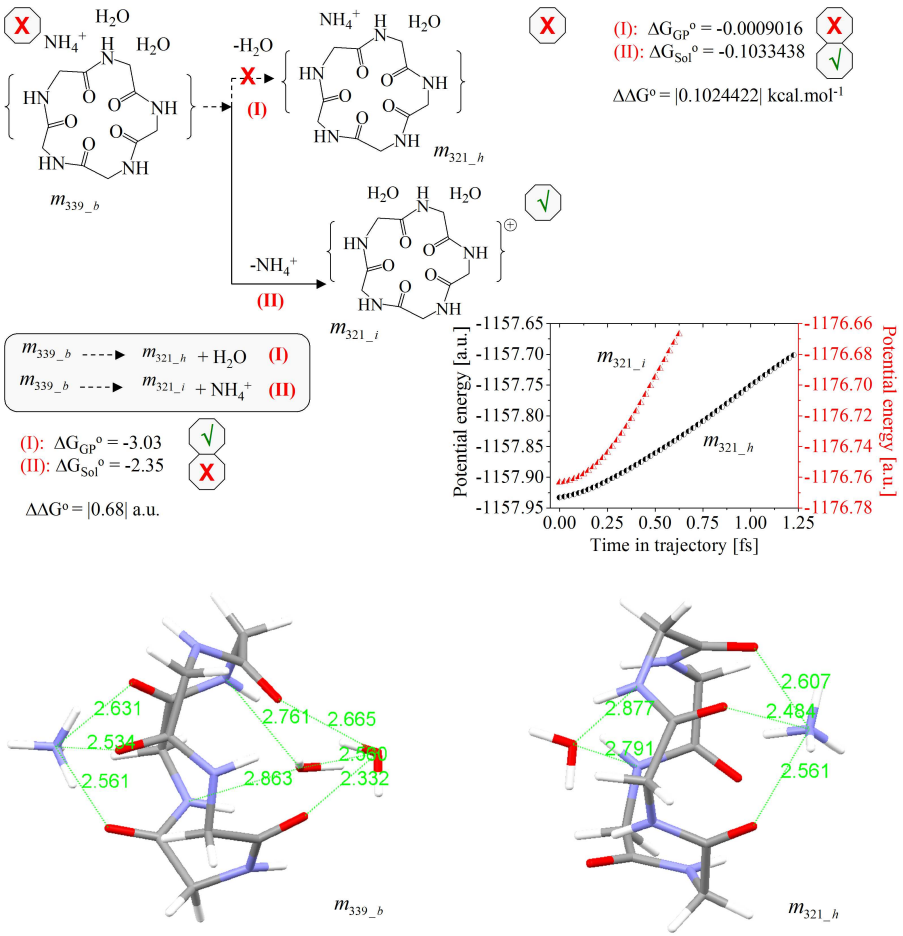


Figure 9. Energetics (classical and DFT molecular dynamics) of ion m_{321_b}, m_{321_h}, and m_{321_i}: total energy (E^{TOT}) [kcal.mol^{-1}] *versus* time [fs] or potential energy [a.u.] *versus* time [fs]; 3D structural conformation of ion m_{321_e}; interionic/molecular hydrogen bond network; selected bond lengths in [Å]; chemical diagrams of the ions; the discrimination among the ions m_{321_e}, m_{321_f} and m_{321_g} is carried out on the base on DFT–MD data; same is true for ions m_{321_h} and m_{321_i}, respectively.

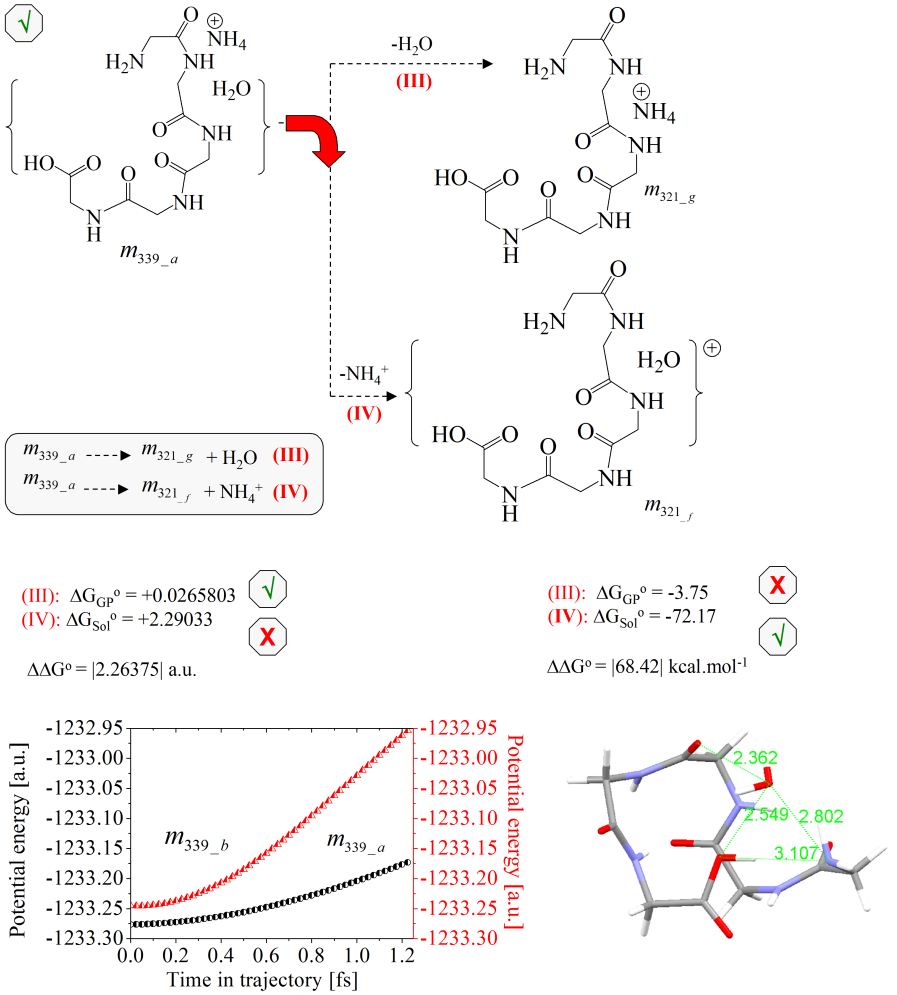


Figure 10. Energetics (classical and DFT molecular dynamics) of ion m_{321_f} and m_{321_g}: total energy (E^{TOT}) [kcal.mol^{-1}] *versus* time [fs] or potential energy [a.u.] *versus* time [fs]; 3D structural conformation of ion m_{321_e}; interionic/molecular hydrogen bond network; selected bond lengths in [Å].

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Conclusions

Contrary to the widespread view that the soft ionization mass spectrometric methods, for instance, the electrospray ionization approach used to this work, cannot be used for multidimensional structural analysis, we argue in this work that the complementary employment of our stochastic dynamic concept and model equations (1) and (2) treating quantitatively the experimental mass spectrometric outcome *"intensity"* together with the high accuracy quantum chemical methods are able to provide exact 3D molecular and electronic structure of the analyte and its fragment ions. Actually, the results in this work are further support for our innovative concept and the shown above formulas which have been testes, so far, within the framework of a small-scale research [1–3]. As far as the physical meaning of the experimental mass spectrometric parameter *"intensity"* is tackled by our theory, the above equation provides a direct link between experimental measurable parameter and the 3D structure of the analyte ion. It is obvious that the complementary application of the experimental mass spectrometry within our stochastic dynamic concept and the computational quantum chemistry extends crucially the capability of the mass spectrometry for multidimensional determination of the molecular structure; furthermore, highly accurately, precisely and selectively, as far as, the *ab initio* and DFT methods of the quantum chemistry are able to account for subtle electronic effects and conformational changes of the structure. The same is true for the experimental mass spectrometric data showing a ultrahigh accuracy and precision of the experimental measurements of the intensity-parameter. It must be stressed that: *(i)* the objects of analysis in this paper are very complex from the perspective of the molecular structural analysis. On the one hand, the peptides are characterized by flexible molecular skeleton allowing for a stabilization of a large number of closely disposed as energetics molecular conformations; *(ii)* the intermolecular/interionic interactions between the peptide ions tolerates a diversity of bonding fashions; and *(iii)* the involvement of solvent molecules, in addition complicated the intermolecular/interionic interactions, respectively. Despite, this complexity of the molecular systems examined in this work, the analysis of eleven adducts of glycyloligopeptides and their ESI–CID–MS fragment reactions, shows excellent chemometric parameters computing the stochastic dynamic D_{SD} coefficients according to our model relations shown above and the quantum chemical diffusion parameters "D_{QC}" according to the Arrhenius's approximation. The coefficient of correlation is $r = 0.9933_2$.

Acknowledgments

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