

VUV photofragmentation of protonated leucine-enkephalin peptide dimer below ionization energy[★]

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Abstract. The experimental investigation of 5–8 eV photons induced dissociation of the leucine enkephalin (Leu-Enk) peptide dimer, performed by coupling a linear ion trap with a synchrotron beamline, in combination with the tandem mass spectrometry, has been reported. The present work extends the existing results on Leu-Enk VUV-induced dissociation to lower sub-ionization photon energy range. The measured tandem mass spectra show that even at the photon energies below the ionization threshold, VUV irradiation of the protonated Leu-Enk dimer precursor can lead to a rich fragmentation pattern, including peptide sequence ions and neutral losses. The photodissociation yields of selected ionic fragments reveal the absorption bands at about 6.7–7.1 eV (185–175 nm). The experimental results have been supported by theoretical description of the $[2\text{Leu-Enk} + \text{H}]^+$ precursors, optimized at B3LYP/6-31+G(*d,p*) level of DFT.

1 Introduction

There has been a long standing effort to understand details of radiation damage of biomaterials at the molecular level. Particularly, a large amount of results have been published in the recent years on electron, ion and photon interaction with isolated molecules representing building blocks or parts of large biological macromolecules such as DNA and RNA [1]. The investigation of an isolated molecular system under well-defined conditions allows for a more detailed insight into fundamental biological processes initiated by the impact of a high-energy particle. Furthermore, this research could help developing important applications in medicine, such as optimizing the type and dose of the radiation in cancer therapy, in order to maximize killing of the cancer cells, while minimizing the damage to surrounding healthy tissues [2].

Although DNA and RNA have been mostly in the focus of the radiation damage research so far, protein damage is a very important issue that must be also taken into account for realistic modeling. Along this line, most of the existing results have been reported on vacuum ultra-

violet (VUV) photon interaction with amino acids – the building blocks of proteins (see [3] and references therein). Nevertheless, the susceptibility of amino acids to energetic photon irradiation (VUV/X-ray) cannot necessarily be transferred directly to their polymers. For example, it has been shown recently that an isolated full protein was extremely resistant to fragmentation upon soft X-ray irradiation in comparison with amino acids [4]. Therefore, it is important to study the high-energy photon interaction with the isolated targets representing polymers of amino acids (peptides), as well as their non-covalent complexes. Unfortunately, this used to be experimentally very challenging – especially to bring the amino acids or their polymers intact into the gas phase [4]. The development of experimental techniques in recent years, particularly those coupling synchrotron radiation with ion traps, allowed for the use of novel ionization methods such as electrospray ionization (ESI) to produce and study gas-phase intact peptides, proteins and even non-covalent complexes [3–7].

In the present article, we report on the experimental investigation of 5–8 eV photon induced dissociation of the leucine enkephalin (Leu-Enk) peptide dimer, performed by coupling a linear ion trap with a synchrotron beamline, in combination with the tandem mass spectrometry (MS²) method [6]. The Leu-Enk pentapeptide (see Fig. 1) has been intensively studied previously and has become a

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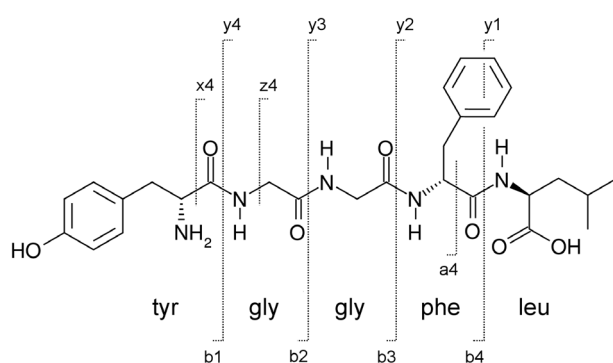


Fig. 1. Schematic drawing of leucine-enkephalin peptide (Leu-Enk) and the amino acid sequence.

standard model in mass spectrometry (see [3,8] and references therein). It has also been used as a model system for peptide-peptide interactions [9]. The existing results on high-energy photon interaction with gas phase Leu-Enk include the photodissociation study of protonated Leu-Enk monomer in the 8–40 eV VUV range [3,10] and soft X-ray [11], as well as the most recent study of both bare and nanosolvated Leu-Enk dimer in 7–10 eV range [9].

Therefore, the present work extends the existing results on Leu-Enk VUV-induced dissociation to lower sub-ionization photon energy range, which is particularly important regarding the intensive photoabsorption by peptides in the 4–7 eV region (300–180 nm) due to the strong electronic transitions associated with aromatics, disulfids, amides and the charge transfer [12]. Furthermore, the present work deals with the isolated gas phase Leu-Enk dimer, thus allowing an insight into the sub-ionization dissociation of non-covalent peptide complexes, a model system for the peptide-peptide interaction and ternary structure acquisition. In short, we could study the stability of fragile non-covalent peptide complexes against sub-ionization VUV irradiation by analyzing direct dissociation and backbone fragmentation as a function of the photon energy. Finally, extensive high-level molecular dynamics (MD) and density functional theory (DFT) calculations were performed in order to study the structure and physicochemical properties of the Leu-Enk dimer.

2 Experimental method

The experimental system [5,6] has been assembled by coupling a commercial linear quadrupole ion trap mass spectrometer (Thermo Finnigan LTQ XL) with the DESIRS VUV beamline [13] of the SOLEIL synchrotron radiation facility in France. Ions were produced from a solution by a detachable ESI source mounted on the mass spectrometer. The electrosprayed protonated Leu-Enk dimer ions were introduced from the front side of the mass spectrometer and guided through a transfer tube and a system of ion lenses into ion trap. Desired precursor ions $[2\text{Leu-Enk} + \text{H}]^+$ were isolated in the trap and irradiated during about 500 ms by a monochromatic VUV photon beam, which was introduced from the back side of the

mass spectrometer. The photon beam is produced by an undulator and monochromatized by a normal incidence monochromator [13], with a typical bandwidth of 12 meV and an absolute energy calibration of ± 10 meV. High harmonics of the undulator producing the photons are filtered out by using a gas filter [13], as well as MgF_2 or Suprasil windows, depending on the photon energy region. A vacuum manifold with turbo pumping stage has been used to accommodate the pressure difference between the beamline (10^{-8} mbar) and mass spectrometer (10^{-5} mbar). A fast digitally activated rotating mechanical shutter has been fitted in the vacuum manifold in order to define the irradiation time [14]. The mass spectrometer was mounted on a home-made supporting frame which allows for a fine tuning of the position of the trapped ions packet with respect to the VUV photon beam, in order to have a high ion activation efficiency and an optimized signal to noise ratio.

Trapped ions were activated and fragmented in a repeated sequence, during which MS^2 spectra were recorded as a function of a particular photon activation energy. The ion trapping, the opening of the mechanical shutter, the recording of the spectra and the photon energy scanning are synchronized by a home-made software, in order to perform automated long-period acquisition. The photodissociation ion yields were obtained from the recorded mass spectra according to the intensity of a desired m/z region, and normalized to both the precursor intensity and the photon flux which has been measured separately under the same experimental conditions. Leucine-enkephalin (Leu-Enk : Try-Gly-Gly-Phe-Leu) was provided by Sigma Aldrich, from bovine erythrocytes in powder form and solvated with water/acetonitrile (75:25) solution at 10 μM concentration.

3 Results and discussion

3.1 Tandem mass spectrometry

Figure 2 presents the tandem mass spectrum of Leu-Enk dimer upon VUV photon activation at 6.7 eV. Clearly, the most intensive dissociation channel corresponds to the cleavage of non-covalent bonds between the monomers and production of the protonated Leu-Enk monomer $[\text{M} + \text{H}]^+$ at m/z 556.

Nevertheless, a zoom-in in the tandem mass spectrum reveals that the photon absorption also leads to an intensive peptide backbone (BB) fragmentation, as well as neutral losses. Figure 2b shows that both N-terminal (a,b) and C-terminal (x,y,z) sequence ionic fragments are formed upon photoactivation of the Leu-Enk dimer precursor. Moreover, the dissociation of the dimer produces fragments from BB fragmentation of one peptide which can stay attached to the other peptide, as can be clearly seen in the rich fragmentation pattern presented in Figure 2c. Finally, the most intensive fragmentation channels, beside the production of the charged monomer, correspond to the loss of neutral molecules, dominantly tyrosine (m/z 107) and phenyl (m/z 91), while more intensive loss of H_2O ,

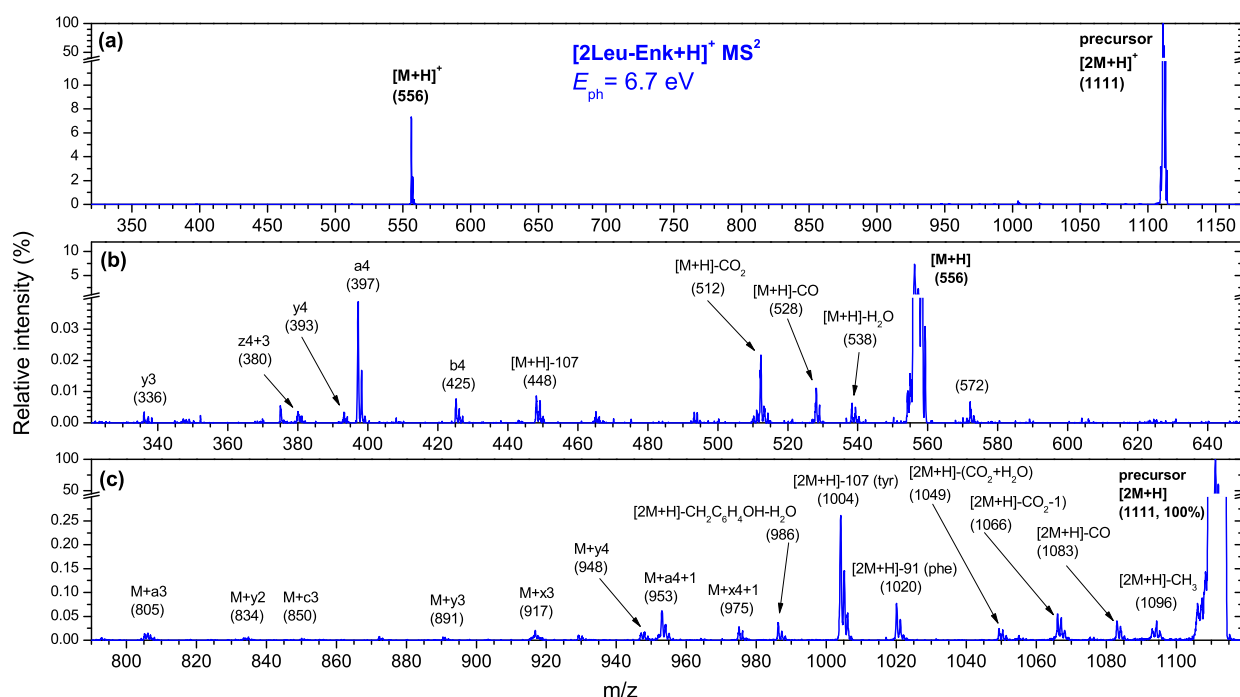


Fig. 2. Photo-activation tandem mass spectrum of leucine-enkephalin (Leu-Enk) peptide dimer recorded after irradiation of $[2\text{Leu-Enk} + \text{H}]^+$ precursors at 6.7 eV photon energy. The lower panels (b,c) show close up of the mass regions up to (m/z 650) (b) and down to (m/z 800) (c), respectively. The proposed assignments of the fragments are given in the figure, where notation “M” corresponds to “Leu-Enk”. The theoretical fragmentation of Leu-Enk peptide and the nomenclature of the fragments have been taken from reference [15].

1 m/z 28 and CO_2 accompanies the direct dissociation to the monomer units (Fig. 2b).

3 The measured tandem mass spectra show that even at the photon energies below the ionization threshold [9], VUV irradiation of the protonated Leu-Enk dimer precursor can lead to a rich fragmentation pattern, including peptide sequence ions and neutral losses. The mechanism should thus involve an electronic excitation of the precursor due to a resonant photon absorption, followed by a fast deexcitation to the hot ground state and subsequent fragmentation [9]. Therefore, the investigation of the energy dependence of the dissociation should allow for a direct mapping of the electronic transitions, as well as of the VUV photostability of this fragile non-covalent peptide complex.

3.2 Photodissociation ion yields

17 Figures 3–6 present photodissociation yields of the selected ionic fragments resolved in the tandem mass spectra, as indicated in Figure 2. The photodissociation intensity of the protonated Leu-Enk dimer is strongly energy dependent. The photodissociation yield of the dominant fragment – protonated monomer $[M + \text{H}]^+$ – reaches a maximum at about 7 eV (Fig. 3a). As indicated previously [9], this intensive sub-ionization photodissociation occurs from electronically excited states of the Leu-Enk dimer precursor. The relaxation of the electronically excited states and redistribution of energy among the vibra-

28 tional degrees of freedom leads to the dissociation of the non-covalent complex from the hot ground state. Therefore, the maximum dissociation intensity directly points to the strongest electronic transition (absorption band). Furthermore, the slow decrease of the $[M + \text{H}]^+$ ion yield above 7 eV also indicates that several different absorption bands in the 6.5–8 eV energy region contribute to the dissociation. The electronic spectroscopy of small peptides has been theoretically investigated by Serrano-Andre and Fülcher [12]. Briefly, electronic structure of the peptidic backbone may be approximated by a 4-level system including two π orbitals involving the amide bond, the oxygen lone pair and one virtual π orbital involving the amide [12]. The amide $\pi\pi^*$ and charge transfer $n\pi^*$ transitions have been tentatively assigned to the 6.5–8 eV spectral region. The contribution of different absorption bands is even more pronounced for the dissociation accompanied by loss of small neutral molecules H_2O and CO_2 (Figs. 3b and 3c, respectively).

Nevertheless, the loss of neutral amino acids phenyl and tyrosine is more resonant, as the corresponding photodissociation ion yields (Figs. 4a and 4b) reveal well defined absorption bands centered at about 6.9 eV. Therefore, sub-ionization site-specific covalent bond breaking and fragmentation of Leu-Enk dimer is conducted by a specific electronic transition from highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). The similar photodissociation ion yields have been measured for the peptide sequence

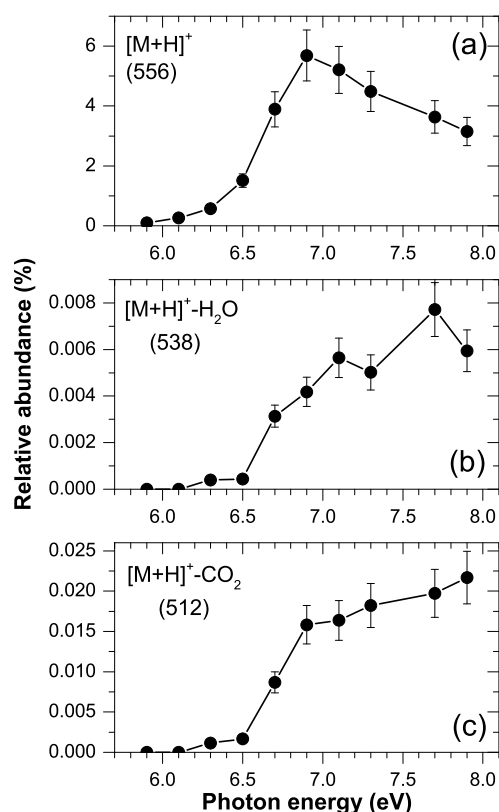


Fig. 3. Photodissociation yields of (a) $[M+H]^+$ (m/z 556.1–556.4), (b) $[M+H]^+-H_2O$ (m/z 538.08–538.38) and (c) $[M+H]^+-CO_2$ (m/z 512.1–512.4) fragments.

1 fragments, both isolated and attached to the monomer
 2 (Figs. 5 and 6). The maxima of the measured ion yields,
 3 corresponding to the maximum absorption, are at about
 4 6.8–7.1 eV. Still, the bands of the C-terminal fragments
 5 (x,y) seem to be slightly red-shifted in comparison to
 6 the N-terminal fragments (a,b), although a more detailed
 7 experimental study is needed for a definite conclusion.

8 3.3 Theoretical modeling of dimer electronic structure

9 Theoretical description of $[2\text{Leu-Enk}+H]^+$ was carried
 10 out in order to better understand the experimental
 11 results. The geometry of the lowest-energy conformer
 12 (CF) were obtained following reference [9], optimized at
 13 B3LYP/6-31+G(d,p) level of DFT after reference [16]
 14 using NWChem [17]. The calculated vertical ionization
 15 energy of this CF is 9.31 eV [9].

16 The geometry of the lowest-energy CF found of the
 17 bare Leu-Enk dimer is shown in Figure 7. Four HOMOs
 18 are all localized on aromatic groups, as shown in the figure
 19 (not shown HOMO-2 has the same localization as HOMO,
 20 on Phe(n)). LUMO, in contrast, is spread across the BB
 21 of the protonated monomer. LUMO+1 and LUMO+2, fur-
 22 thermore, also occupy about the same position as LUMO,
 23 with more weight on the Tyr(p) end of the monomer.

24 Given the obtained localization pattern, one could ex-
 25 pect that some excitations of the type $\text{HOMO} - n \rightarrow$

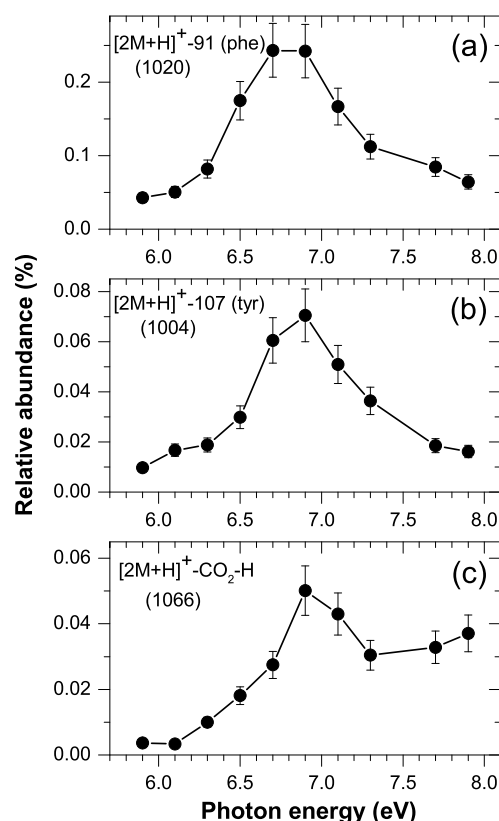


Fig. 4. Photodissociation yields of (a) $[2M+H]^+-91$ (phenyl) (m/z 1020.0–1020.3), (b) $[2M+H]^+-107$ (tyrosine) (m/z 1003.98–1004.28) and (c) $[2M+H]^+-CO_2$ (m/z 1066.0–1066.3) fragments.

LUMO + m for $n = 0, 1, 2, 3$ and $m = 0, 1, 2$ consist of
 26 transfer of charge from aromatic ring to the BB(p). Tak-
 27 ing into account the well-known electronic stability of
 28 the aromatic ring, the main effect of the transfer is weak-
 29 ening of the BB(p) due to the negative charge repulsion
 30 along the bone, leading to the conclusion that protonated
 31 monomer in the dimer is less stable than the neutral. Fur-
 32 thermore, it could be also argued that excitations involv-
 33 ing a charge transfer from the neutral to the protonated
 34 monomer lead to the loss of Tyr(p), since the hole created
 35 by the excitation of electron on Phe(n) is well localized
 36 on the aromatic ring preventing the loss of Phe fragment.
 37 On the other hand, excitations involving transitions that
 38 take place only on the protonated monomer could addi-
 39 tionally initiate loss of Phe fragment, due to the repulsion
 40 between positively charged Phe(p) and the NH^+ . Never-
 41 theless, a more detailed analysis and definite conclusions
 42 demand for time-dependent DFT theoretical calculations.
 43

44 4 Conclusion

45 The present paper reports a detailed experimental study
 46 on photo-induced dissociation of protonated Leu-Enk pep-
 47 tide dimer isolated in the gas phase, at photon energies be-
 48 low the ionization threshold. The results show that photo-
 49 induced electronic excitation of the non-covalent dimer

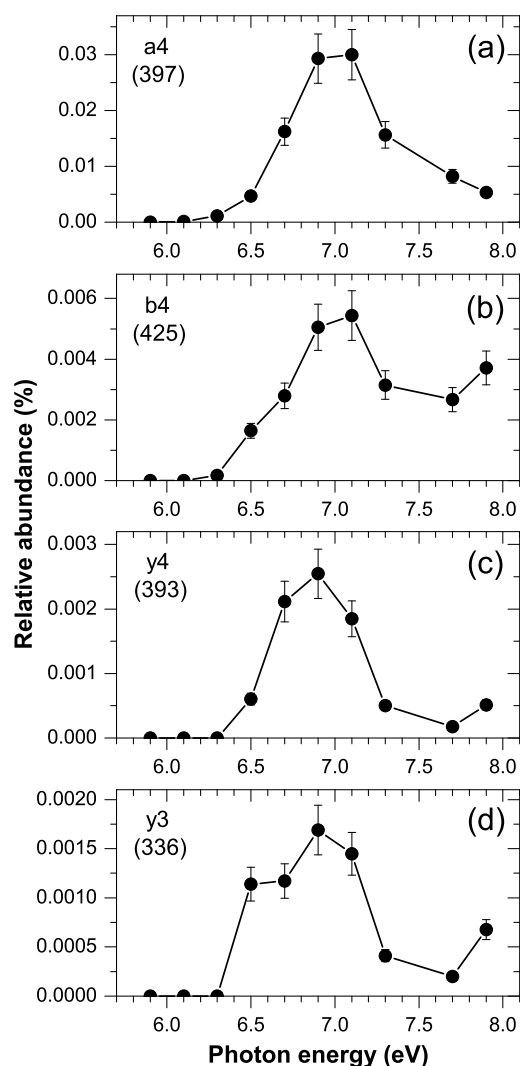


Fig. 5. Photodissociation yields of (a) a_4 (m/z 397.05–397.35), (b) b_4 (m/z 425.08–425.38), (c) y_4 (m/z 393.0–393.3) and (d) y_3 (m/z 336.0–336.3) fragments.

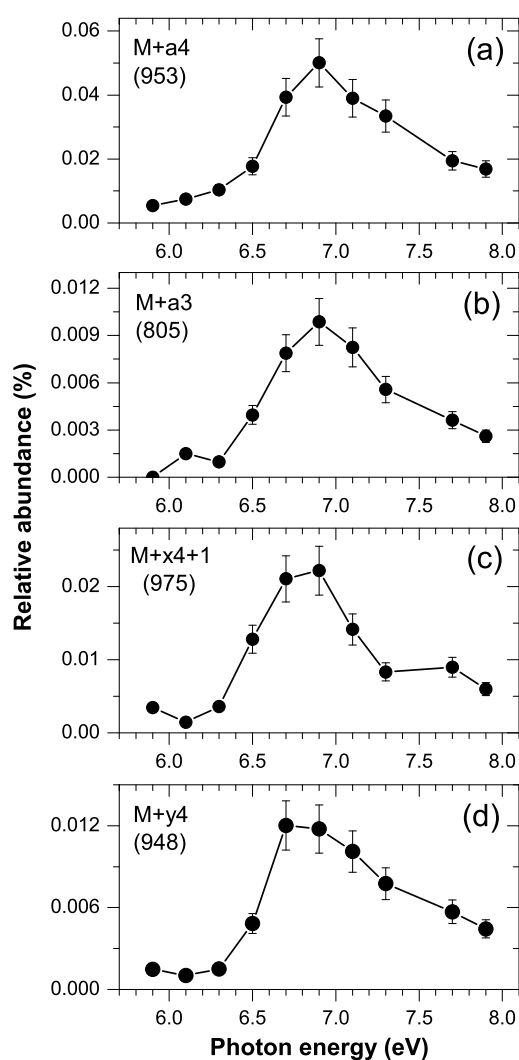


Fig. 6. Photodissociation yields of (a) $M+a_4$ (m/z 952.92–953.22), (b) $M+a_3$ (m/z 805.05–805.35), (c) $M+x_4+1$ (m/z 975.0–975.3) and (d) $M+y_4$ (m/z 948.0–948.3) fragments.

1 system leads to intensive dissociation to the monomer
 2 units (as expected), but also to backbone destruction and
 3 loss of neutral molecules. Furthermore, the fragmentation
 4 process can end up with the peptide sequence fragments
 5 both isolated and still attached to the monomer.

6 The intensity of the photo-induced fragmentation of
 7 the protonated Leu-Enk dimer is strongly dependent
 8 on the photon energy. The measured photodissociation
 9 yields of selected ionic fragments reveal the absorption
 10 bands at about 6.7–7.1 eV (185–175 nm). The exper-
 11 imental results have been supported by theoretical de-
 12 scription of the $[2\text{Leu-Enk} + \text{H}]^+$ precursors, optimized at
 13 B3LYP/6-31+G(*d,p*) level of DFT. According to the cal-
 14 culated distribution of HOMO and LUMO, the exper-
 15 imental results and measured fragmentation pattern could
 16 be tentatively rationalized. Finally, the present work is im-
 17 portant for the field of radiation damage and brings new
 18 results revealing the susceptibility of complex biopolymers
 19 to energetic photons.

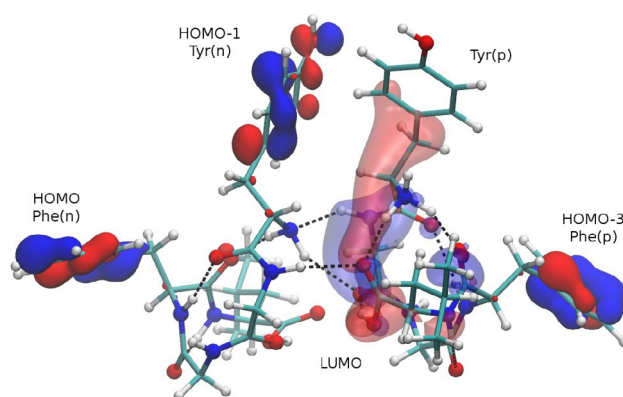


Fig. 7. Geometry of the lowest-energy CF found, shown together with several frontier orbitals, as indicated in the figure. HOMO-2 (not shown) is localized on Phe(n) just as HOMO. LUMO+1 and LUMO+2 (not shown) occupy about the same part of the molecule as LUMO. (n) and (p) next to the group name indicate whether the group belongs to the neutral or protonated monomer, respectively.

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