Conformer-dependent VUV photodynamics and chiral asymmetries in pure enantiomers of gas phase proline

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Abstract. Proline is a unique amino-acid, with a secondary amine fixed within a pyrrolidine ring providing specific structural properties to proline-rich biopolymers. Gas-phase proline possesses four main H-bond stabilized conformers differing by the ring puckering and carboxylic acid orientation. The latter defines two classes of conformation, whose large ionization energy difference allows a unique conformer-class tagging via electron spectroscopy. Photoelectron circular dichroism (PECD) is an intense chiroptical effect sensitive to molecular structures, hence theorized to be highly conformation-dependent. Here, besides a conformer-dependant cation fragmentation behaviour, we present experimental evidence of an intense and striking conformer-specific PECD, measured in the VUV photoionization of proline. This finding, combined with theoretical modelling, allows a refinement of the conformational landscape and energetic ordering, that proves inaccessible to current molecular electronic structure calculations. Additionally, astrochemical implications regarding a possible link of PECD to the origin of life’s homochirality are considered in terms of plausible temperature constraints.

Introduction

Proline (Pro) is the only proteic α-amino acid containing a secondary amine (N-H) fixed within a pyrrolidine ring, which makes it conformationally less flexible than most other amino acids. Due to its specific structure, Pro plays an important role in determining the structures of proteins and peptides. Pro is indeed strongly involved in their formation and alteration, as its cyclic nature constrains the peptide backbone, providing Pro-rich proteins with very specific and important structural properties, for example in Intrinsically Disordered Proteins (IDP) and in collagen.
In the gas phase, on which we focus from now, the study of elementary building blocks of life such as amino acids is at the basis of the so-called bottom-up approach of biomolecular complexity. The gas phase offers a solvent-free and substrate-free environment, so that all intermolecular interactions, including those with a solvent or substrate, can be neglected; only intramolecular interactions are to be taken into account, such as non-covalent bonds responsible for conformations. In such a context, molecules can be studied in detail within an optimized interplay between experiment and theory. Besides, dilute matter may be probed by photons over a wide “transparent” spectral range including the vacuum ultra-violet (VUV).

The molecular and electronic structures of gas phase neutral Pro have been the subject of a large number of experimental studies based upon IR (in a matrix), microwave and photoelectron spectroscopies, as well as theoretical modelling. In addition, the VUV and soft X-ray photoabsorption and photoionization of proline were studied in the gas phase by mass spectrometry, as well as its fragmentation by electron impact. But as far as we know, no photoelectron/photoion coincidence (PEPICO) studies have been done to measure state-selected fragmentation. Moreover, and most of all, no gas phase chiroptical study of any kind on this major amino acid is available in the literature.

Most of the previous works considered the presence of 4 main low energy conformers, divided into two groups (I and II), differing by their intramolecular hydrogen bond network. The strength of the H-bond interactions, especially in the neutral, leads to a surprisingly large difference in the ionization energy (IE) of the highest occupied molecular orbital (HOMO) of ~ 0.7 eV between group I and group II conformers. By using Photoelectron Spectroscopy (PES) as a conformer tag, the rich and unusual conformer landscape of Pro allows us to template various photon-induced processes in a conformer-specific way, such as the VUV photodynamics (state-selected ion fragmentation) and most of all chiroptical electron asymmetries induced by the so-called Photoelectron Circular Dichroism (PECD) effect.

PECD is an electric-dipole allowed, orbital-specific and photon-energy dependent effect, observed when pure enantiomers, randomly-oriented in the gas phase, are ionized by Circularly-Polarized Light (CPL), this leading to a forward/backward asymmetry (with respect to the photon axis) in the photoelectron angular distribution (PAD). More precisely, the normalized PADs for one-photon ionization takes the form

\[ I_p(\theta) = 1 + b_1^{(p)} P_1(\cos \theta) + b_2^{(p)} P_2(\cos \theta) \]

where \( P_j \) are the Legendre polynomials, \( \theta \) is the direction of the emitted electron and \( p \) the polarization of the ionizing radiation (\( p = 0, +1 \) and -1 for linear, left circular and right circular polarizations respectively). For CPL, \( \theta \) is measured from the photon propagation axis. The so-called dichroic parameter \( b_1 \) is non-zero only for chiral systems photoionized with CPL and is antisymmetric under swapping of either light helicity or enantiomers. PECD is defined as

\[ 2b_1^{(+1)} P_1(\cos \theta), \]

which is just the difference between the angular distributions obtained with left and right circular polarization radiations, corresponding to a maximum asymmetry of \( 2b_1^{(+1)} \) in the forward–backward direction. Therefore \( b_1^{(+1)} \) encapsulates the chiral contribution as well as the whole dynamics of the departing photoelectron scattering off an intrinsically asymmetric potential. The \( b_2^{(p)} \) parameter expresses the symmetric part of the angular distribution, and for linear polarization \( b_2^{(0)} \equiv \beta \), the familiar anisotropy parameter.
Since PECD is fully developed in the electric dipole approximation, it leads to very intense asymmetries, in the few %–few tens % range up to 37 % as recently measured, which makes it very well adapted to low density media such as the gas phase. Besides, PECD has been shown to be very sensitive to static molecular structures, such as conformers, isomers, clusters, chemical substitution and to dynamic molecular motions such as vibrations (for reviews see Refs.28,29).

Because of the specific sensitivity of PECD to conformations (for a review see also Ref.32), there is a considerable interest in applying PECD to study biomolecules, especially amino acids, which are known to possess a broad conformational landscape of crucial importance for the building up of larger biopolymers such as peptides and proteins. However, so far, all PECD studies of floppy systems possessing several conformers have been based upon a Boltzmann-averaged distribution of all conformers. In this context, the easily separable conformer-specific IE of Pro offers a unique opportunity to retrieve experimental conformer-specific PECD data to benchmark the theoretical modelling. Conversely, careful experiment/theory interplay may allow the use of PECD to refine the conformational landscape of Pro, especially in terms of energetics. Note that while conformer-specific CD in the ion yield has already been demonstrated via a resonant multi-photon ionization (REMPI) scheme, so far no corresponding conformer-specific REMPI-PECD has been reported although the needed high spectral resolution ns-REMPI PECD has recently been demonstrated.

Here we present a comprehensive valence-shell photoionization study over a broad VUV range (8.7–17.5 eV) on Pro brought into the gas phase via two complementary vaporization methods. This study includes PES, state-selected fragmentation patterns and chiral asymmetries (PECD), as measured by double imaging PEPICO (i²PEPICO) and supported by dedicated theoretical calculations. By varying the experimental temperature, and consequently changing the Boltzmann conformer population we attempt to refine the conformational landscape of Pro and to benchmark scattering models on individual conformers.

Finally, an astrophysical PECD-based scenario for the origin of life’s homochirality has been proposed on Ala and Pro, both relevant in terms of astrochemistry since they belong to the first five amino acids to have been recruited into the genetic code, and have also been detected in the Murchison meteorite in large quantities and with an excess of the L enantiomer. The above-mentioned thorough conformer-dependent analysis on Pro, is used here to examine possible temperature constraints and astrophysical implications regarding the relevance of this scenario to interstellar medium conditions.

Results and discussion

Conformational landscape. In the first micro-wave study of the proline rotational spectrum, only two conformers were detected, denoted IIa and IIb. A short distance between the nitrogen atom and the hydrogen of the carboxyl group supports the prediction of an N – H–O hydrogen bond interaction, similar to conformation II found for all aliphatic α-amino acids, with IIa and IIb differing by the pyrrolidine ring puckering. Then, a further MW experiment identified two other conformers Ia and Ib, which are NH–O = C hydrogen bonded, this second
pair again differing by the pyrrolidine ring puckering. Other works on proline confirm that energetics, H-bond strength (see Table 1) and population of both types of conformers supports the preference for the N···H-O interaction\textsuperscript{9,10,13,15} (Type II), as the most stable conformer type, due to geometric constraints imposed by the pyrrolidine ring.

In Figure 1, we show the geometric structure of four proline conformers, with the two alternative H-bonding arrangements (Type I, Type II), and the two alternative up-down ring puckering. Unfortunately, the labels Ia, Ilb ... etc... have not been used consistently in the literature. We therefore choose labels A – D for the individual conformers to avoid this ambiguity, and in Table S1 (supplementary Information) we provide a table showing the correspondence with the various labelling choices used by other authors.

![Proline conformer structure](image)

**Fig. 1. Proline conformer structure.** (The O atoms are coloured in red, the N atoms in blue).

In Table 1, we gather various calculations of Pro conformer geometry and energetics from previous studies and compare these with our present set of G3 calculations (see Methods). The conformer energetics are shown schematically in Figure 2. The calculations consistently show the N···H-O H-bonding interaction is the energetically most favourable (conformers Type II: A,B) Table 1 also shows that there is generally a very small energy difference between the calculated Type I conformers (C, D), with some corresponding controversy concerning the relative stability of C, D. This is to be expected since the predicted energy differences of 1 kJ mol\textsuperscript{-1} are at the limit of achievable computational accuracy.
Fig. 2. Calculated proline conformer energetics (see Table 1). The blue curve represents the electronic energies of the neutral and the red one the energies of the cation, with the difference being the estimated adiabatic (0K) ionization energy. The vertical ionization energies (IE_{vert}) appear as a dark blue curve. Also shown (broken green line) is the experimentally estimated 0 K appearance energy of the principal fragment m/z 70.
Table 1. Summary of calculated energetics/ ionization energies /geometry for the four main conformers of proline. Results obtained in this work are from G3 composite method calculations except for vertical ionization energies, which are OVGF/cc-pVTZ//MP2/cc-pVTZ calculations. The relative energies are evaluated at 415 K, everything else assumes a temperature of 0K

| Conformer Type II | Conformer Type I |
|------------------|------------------|
|                  |                  |
| **Vertical Ionization Energy (eV)** |                  |
| This work        | 9.581            | 9.717            | 9.022            | 9.054        |
| Lu et al. 18     | 9.49             |                  |                  | 8.91         |
| Tian et al. 15   | 9.41             | 9.52             | 8.71             | 8.83         |
| Fathi et al. 10  | 8.81             | 8.941            |                  |              |
| Dehareng et al. 13 | 9.36            |                  | 8.75             |              |

| **Adiabatic Ionization Energy (eV)** |                  |
| This work                  | 8.75             | 8.68             | 8.30             | 8.36         |
| Lu et al. 18              | 8.61             |                  | 8.16             |              |

| **H-bond length in neutral (Å)** |                  |
| This work                  | 1.828            | 1.864            | 2.404            | 2.237        |
| Czinki et al. 14           | 1.877            | 1.898            | 2.248            | 2.363        |
| Tian et al. 15             | 1.869            | 1.885            | 2.365            | 2.243        |
| Lesarri et al. 8           | 1.915            |                  |                  |              |

| **NCC=O Dihedral angle in neutral [°]** |
| This work                  | -177.4           | -165.3           | -1.1             | -9.8         |
| Lesarri et al. 8           | -180             |                  |                  |              |

| **Relative Energy (kJ.mol⁻¹)** |
| This work (a)               | 0.0              | 3.59             | 6.91             | 6.59         |
| Czinki et al. 14(b)         | 0.0              | 1.98             | 7.66             | 8.56         |
| Mata et al. 7(c)            | 0.0              | 3.21             | 8.91             | 9.35         |
| Fathi et al. 10(d)          | 0.0              | 1.13             | 4.22             | 3.81         |

(a) ΔG (G3) at 415 K (see Methods)
(b) ΔE (B3LYP level)
(c) ΔE MP2/6-311++G(d,p)
(d) ΔE MP2/6-311++G(d,p) at 403 K
**Photoelectron spectroscopy.** The first threshold electron spectroscopy and state-selected fragmentation measurements, using the TPEPICO technique, were performed for proline using the three vaporization conditions. These are presented in Figure 3. In the top panel, we show the TPES spectrum of Pro recorded between 8 and 11.6 eV (up to 10.1 eV only for the RH condition), obtained in coincidence with the total mass (*i.e.* sum of the parent and fragment masses).

Fig. 3. TPES and TPEPICO of Pro obtained for the 3 vaporization conditions TD$_{415}$ (blue), TD$_{493}$ (green) and RH (red). The relative signals have been normalized according to the maximum of the TPES first band centred around 9.5 eV. The shaded area correspond to the error bars. The dashed lines in the lower panel show the estimated apparent ionization energy obtained from the m/z 115 TPEPICO curve as the first point to rise above the baseline.
Two main bands are observed in our TPES in good agreement with the PES of the literature. The first broad band in the 8.1-10 eV energy range, corresponds to ionization from the HOMO orbital. As seen in Figure 4, (and the Mulliken population analysis provided as Table S2 in the Supplementary Information), for the Type I conformer pair this has the expected nitrogen lone pair character \( n_N \). But the Type II conformers are now seen to have HOMOs that are more mixed in character. Although not shown here, a similar distinction can be made for the HOMO-1 orbital characters of these conformers, so that the second PES band, centred around 10.7 eV corresponds to ionization of an oxygen lone pair orbital \( n_O \) from the carbonyl group \((C = O)\) of the Type I conformers, but more mixed and delocalised orbitals around the NH-C-COOH grouping in case of the Type II conformers.

With increasing temperature conditions \((TD_{415}, TD_{493} and then RH)\), three main effects are induced in the observed TPES (Fig. 3): (i) a clear shape change with the appearance of a broad shoulder around \( \sim 8.8 \) eV. This feature was already reported by Plekan et al., and attributed to the increased population of conformers C and D possessing the lowest \( I_{E_{\text{vert}}} \); (ii) an apparent shift (from 9.5 to 9.65 eV) of the maximum of the HOMO peak for the RH case relative to the TD cases. This behaviour could be due to, in such a hot condition (see below), a population increase of neutral conformer B with respect to the conformer A, which possesses a higher \( I_{P_{\text{vert}}} \) (by \( \sim 0.15 \) eV) than conformer A (see Table 1); (iii) a slight red shift of the spectrum probably due to hot bands in the neutral. This last feature is better appreciated on the lower panel of Figure 3, showing the parent-filtered TPES \((m/z 115)\) on which the apparent ionization thresholds under the three vaporization conditions are marked as the first point to rise above the baseline. These values are listed in Table 2 for each experimental condition. Note that the \( TD_{415} \) threshold value of \( 8.30\pm0.01 \) eV matches well the calculated adiabatic ionization energies of 8.30 eV and 8.36 eV for conformers C and D respectively (see Table 1). We thus concur with the conclusion of Plekan et al. that in the low energy side of the HOMO TPES band one selectively observes ionization of the thermodynamically less stable Type I conformer structures C and D. We also recorded the PES of Pro at different photon energies in \( TD_{415} \) condition (Figure S1 and S2). These show inner orbital ionizations with a peak centred around \( \sim 11.7 \) eV (shoulder) attributed to the HOMO-2 orbital, \( (\pi_{C=O}) \) of the carbonyl group, while the peak located around \( \sim 12 \) eV is attributed to the HOMO-3 orbital, \( (\sigma_{CC}) \) orbital.
Overall, our experimental results are in a good agreement with the calculated ionization energies of Pro, as reported in Table S3.

**Appearance energies and fragmentation behaviour.** In Figure 3 (lower two panels) are shown the parent-selected \((m/z\) 115) and fragment-selected \((m/z\) 70) TPES for the three conditions TD\(_{415}\), TD\(_{493}\) and RH. By correlating the fragmentation pattern with the electronic structure at different temperatures, a significant temperature effect is visible on these fragmentation diagrams. Quite generally, one may expect to observe increasing ion fragmentation (decreasing parent ion yield) with increasing internal vibrational excitation (temperature) in the neutral at the instant of its ionization. Correspondingly, there is an expected apparent reduction in the fragment appearance energy with increasing thermal excitation in the neutral. Indeed, when looking at the trend of the \(m/z\) 70 fragment curve, we note a clear red shift of the appearance energy of this fragment in the sequence TD\(_{415}\), TD\(_{493}\), RH from, respectively, 8.6 eV, 8.4 eV to 8.3 eV. Noting also an increasingly prominent TPES shoulder at \(~8.8\) eV, which as mentioned above is expected to show such an increase with increasing temperature, one deduces that although the oven was heated only to 468 K in the RH method, the RH source apparently produces much hotter neutrals than does the TD heated at 493 K.

To get a more precise knowledge of the actual temperatures of the neutrals, we modelled, for each vaporization condition, the breakdown diagram, *i.e.* the normalized fragment-to-parent abundance, as a function of photon energy (Figure S3). This can be described using a statistical model assuming that all internal degrees of freedom are thermalized and that the energy stored in these modes can flow freely to be used, for instance, to excite a particular vibrational mode leading to fragmentation. We assume that the fragmentation is faster than the typical residence time of the ion in the acceleration region (\(~\mu\)sec). The output of the model fit (Figure S3), internal temperatures and the corresponding 0K appearance energies (AE) that are deduced, are summarized in Table 2.

| Vaporisation condition | Internal Temperature deduced (K) | Fitted 0K fragment \((m/z\) 70) appearance energy (eV) | Expected Conf. I /Conf. II ratio | Ionization Energy Thresholds (eV) |
|------------------------|----------------------------------|---------------------------------------------------|---------------------------------|----------------------------------|
| TD\(_{415}\)           | 384                              | 9.03                                              | 0.17                            | 8.30(1)                          |
| TD\(_{493}\)           | 452                              | \(\ldots\)^a                                     | 0.24                            | 8.25(2)                          |
| RH                     | 600                              | 9.01                                              | 0.35                            | 8.10(7)                          |

^a For TD\(_{493}\) the AE\(_{0K}\) was held at 9.03 eV and only the slope was modelled.

For the two TD conditions, the observed internal energy corresponds to temperatures which are between 30 and 40 K colder than the TD tip, which is consistent with our past experience with alanine. This decrease of temperature could be due to the slight cooling of the TD tip due to the sublimation process, or to some mild expansion of the plume. Contrastingly, the apparent temperature for the RH source is found to be 600 K, considerably above the actual temperature read by the thermocouple of the oven (468 K). We attribute this mismatch to the non-equilibrium conditions in the supersonic expansion, rendering the use of a single
descriptive “temperature” rather questionable and/or poor location of the thermocouples on the oven body giving misleading temperature readings.

Finally, we note that the 0K AE of m/z 70 fragment is determined here for the first time, with a good precision as confirmed by the very close values obtained from two fits providing a mean value of 9.02 eV.

Returning to the TPES of Fig.3, we notice that the HOMO orbital has a double-band structure whose shape changes with the temperature. This we attribute to the varying population of four main conformers populated in our experimental conditions. As already mentioned, and shown in Table 1, the first shoulder of the first band centred around ~8.7 eV, corresponds to the ionization of Type I conformer (C and D), while the second peak centred around 9.5-9.7 eV would correspond predominantly to the ionization of conformer Type II (A and B).

It is clear that near their respective ionization thresholds, conformers Type I initially survive as a stable parent ion whereas Type II conformers undergo C-Cα bond breakup. This difference can be rationalized in more mechanistic terms by considering the much larger changes in geometry between the neutral and the cation, both in terms of H-bond length and NCC=0 dihedral angles, predicted for Conformer Type II (A, B) than Type I (C, D) (see Table S4). In the vertical Franck-Condon approximation, the possibility for significant excitation of the corresponding vibrational modes in the cations can be expected, capable of driving the fragmentation process at the origin of the m/z 70 fragment. This is especially the case for Type II conformers, since vertical excitation leaves the parent cation with excess energy above the fragment appearance energy as shown in Figure 2.

Furthermore, the different orbital characteristics (Fig. 4) may be considered. The N lone pair HOMOs of Type I conformers can be expected to be essentially non-bonding, with minimal consequences for the C-Cα bonding upon ionization. In contrast, the more delocalized Type II HOMOs, that include density around the C-Cα bond are likely to lead to its weakening in the ion. Hence, it is very reasonable that close to their respective ionization thresholds the dissociative ionization behaviour provides a signature of the two types of conformers: Type I conformers are partially stable while Type II ones are fully dissociative. The PEPICO scheme, by selecting parent ion production, can effectively isolate the specific conformer Type I (C/D), with a low vibrational energy content, which is exploited in the forthcoming PECD astrophysical implications.

Ionization of the HOMO-1 orbital, commencing well above the fragmentation threshold, is fully dissociative leading solely to the m/z 70 fragment. These features are also clearly visible on the mass-selected PES recorded at the 11.5 eV fixed photon energy, shown in Figure S2.

**PECD: experimental data and theoretical modelling.** Figure 5 shows photoelectron spectra (PES) and PECD curves as a function of the ionization energy (eV), measured for both L and D Proline enantiomers at different photon energies, in TD415 conditions.
Fig. 5. PECD and PES measurements on D- and L-proline enantiomers, produced in TD_{415} conditions, filtered on all masses, i.e. the sum of the parent and the fragment \((m/z\ 115 + m/z\ 70)\). a at 8.7 eV. b at 9.5 eV. c at 11.5 eV. The red and blue circles correspond to PECD \((2b_1)\) of L-Pro and D-Pro respectively. Inserts show raw difference images.

At 8.7 eV, the PECD of L and D enantiomers shows a clear and expected mirroring effect, with a magnitude of the order of 10\% for the low binding energy range that, as noted, corresponds to the pure conformer Type I (C/D) ionization. This value changes with the photon energy. At 9.5 eV photon energy, the PECD magnitude reaches 18\% in the low binding energy region, an asymmetry level unprecedented for a multi-conformer system. The higher binding energy part of the PES, corresponding predominantly to Type II (A/B) conformer ionization, exhibits a very different PECD level, close to zero. Therefore, the large IE difference between the two types of Pro conformers offers the opportunity to directly observe, for the first time experimentally, a clear conformer specificity of PECD. At a photon energy of 11.5 eV (now for D-Pro) one notices a sign change of PECD for the low binding energy side (conformer type II region) as compared the lower photon energy cases, as well as a different PECD level for the HOMO and HOMO-1 orbital. Such orbital and photon energy dependences are quite typical of PECD.

The PES and PECD of L/D-proline recorded by velocity map imaging at 10.2 eV photon energy in the three vaporization conditions (TD_{415}, TD_{393} and RH) are shown in Figure 6. The electron images used for these determinations have been mass-filtered on the parent \((m/z\ 115)\) only, the fragment \((m/z\ 70)\) only, and total mass (parent + fragment) ion coincidences, providing three alternative PECD curves for each sample condition. We note that the PES curve obtained by the RH method filtered on the parent has a maximum shifted by about 0.2-0.3 eV towards lower energies by comparison with the TD_{415}, a thermal effect already observed on the TPEPICO spectra of Figure 3.
Fig. 6. PECD (red markers L-Pro, blue D-Pro) and PES (black curve) of proline recorded at 10.2 eV for the 3 vaporization conditions (a), (b) and (c), with filtering on the parent (m/z 115) and the fragment (m/z 70) as well as the total mass. a TD$_{415}$ (L-Pro). b TD$_{493}$ (D-Pro). c RH (D-Pro). The total PES in panel (a) shows the deconvolution procedure too, with two Gaussian functions used to fit the PES (dashed cyan line), one for Type I centred at 8.75 eV (dashed green curve) and Type II centred at 9.65 eV (dashed orange curve) conformers. Note that data in panel (c) are displayed with a different vertical axis scale.

For the L-Pro TD$_{415}$ PECD on (panel (a)), the Type I/Type II conformer–specificity is very clear already from the total mass curve, with an intense PECD of $\sim$10% at ionization energies around 8.8 eV (Type I), switching sign to $\sim$+5% for ionization energies around 9.6 eV (Type II). This is even more pronounced when parent mass-filtered data are taken into account, with the selection of low vibrational energy content only (IE$\sim$8.2-8.7 eV as seen from Fig.3 bottom panel) for Type I conformers reaching about -12% asymmetry. Besides, the PECD of Pro appears to be very sensitive to the temperature, in particular for the parent, whose PECD curve exhibits a lower magnitude at higher temperature: 12% for TD$_{415}$ and 6% with the TD$_{493}$, and an average value of around 2-3% for the RH method. We attribute this change in magnitude to a relative change in the population of conformers C/D present in our experimental conditions, as we will further rationalize below.

Figure 7(a) shows the results of CMS-Xα calculations on the HOMO photoionization observables made for the four proline conformers A, B, C and D across a range of electron kinetic energy. It is clear that the conformer sensitivity displayed by the $b_1$ parameter is dramatic, but less so for the cross sections, $\sigma$. For low kinetic energy electrons, which are known to be the most sensitive to molecular structures, there are differences in the sign of $b_1$ predicted within both the Type I A/B and the Type II C/D conformer pairings, where the principal structural differences between those conformers of a given type are just the ring puckering. Equally, there are significant differences between the B/C pair and between the A/D pair. The latter, particularly, shows an enantiomer-like approximate mirroring of the A and D $b_1$ curves. Now, the principal structural change between A and D is a near 180° difference in the NCC=O dihedral angle (see Table S4), but retaining the same ring puckering.
It is interesting to compare with similar calculations made for the HOMO-1 ionization, as shown in Figure 7(b). The conformer-sensitivity of the $b_1^{(+1)}$ parameters is now much less pronounced despite the HOMO-1 orbitals retaining quite similar characteristics to the HOMOs — Type I localised lone pairs (however on O instead of N for the HOMO orbital), Type II delocalised around the N-C-COOH grouping — and clearly sharing the same nuclear geometries.

Focusing now on the HOMO ionization, each conformer $b_1$ parameter in Fig. 7(a) individually shows variations in sign and magnitude that are commensurate with the experimental variability noted above. But a more detailed comparison of these theoretical predictions and experimental results is essential. We do so by extracting characteristic PECD values for the Type I and Type II conformer regions across a range of experimental photon energies using a simple, approximate deconvolution procedure. First two Gaussian functions are fitted to the VMI-PES as illustrated in Fig. 6(a). Weighted averages of the corresponding PECD (=2$b_1$) are then formed across the FWHM of each Gaussian function as an attempt to isolate and reduce the data for Type I and Type II conformer regions.

Type I C+D mean $b_1^{(+1)}$ values that have been extracted from the low binding energy region using a gaussian weighting function FWHM of 0.6 eV and centred at ~8.75 eV ionization energy, recorded using photon energies ranging from 8.7 eV to 17.5 eV, are shown in Figure 8. Also included in this figure are the calculated PECD curves for the Type I conformers, taken from Fig. 7(a).
Fig. 8. Type I conformer (C+D) \( b_1^{(+1)} \) values for the HOMO orbital photoionization as a function of electron KE. The mean experimental \( b_1^{(+1)} \) data points, given by symbols, extracted from total (parent + fragment) mass-filtered TD\(_{415}, \) TD\(_{495}, \) RH data sets, are shown for L-Pro with any D-Pro data negated prior to plotting. Horizontal error bars are attached to the RH data only, representing the FWHM of the gaussian sampling function that isolates the Type I region; the same FWHM was also used for the TD data sets but for clarity has not been explicitly shown. Also included are solid curves showing the CMS-Xα calculations for conformers C and D. Best fits to the experimental data sets obtained by combining these predictions (see text) appear as broken lines.

Overall, the \( b_1 \) experimental data exhibits a very dynamical behavior with large variation and sign changes with the photon (equivalent electron kinetic) energy, reflecting the quantum nature of the scattering process from which PECD originates. On examination, it is readily seen that the experimental data are essentially bounded between the theoretical curves. Moreover, while the experimental data fall closer to the theoretical curve for conformer C, there is a clear temperature dependence with an increasing displacement of the TD\(_{493} \) and RH data sets towards the conformer D prediction. Qualitatively, this appears to argue strongly for the C conformer being the more stable of the two, with an increasing population of the D conformer with increasing temperatures.

To pursue this more quantitatively, we have taken an expression, \( b_1(\text{expt}) = x \times b_1(C) + (1 - x) \times b_1(D), \)

that blends the C and D conformer curves, where \( x \) and \( (1 - x) \) indicate the relative proportion of each conformer in the mix. By applying a least squares fit for \( x \) to each data set, estimates for the C conformer relative populations of 78%, 74%, and 58% for, respectively, the TD\(_{415}, \) TD\(_{493}, \) and RH data sets are extracted (Note that KE=1.5 eV point for the TD\(_{415} \) set has been excluded from the fitting as an outlier). Going further, the C:D population ratios may be equated to a Boltzmann factor, and using the inferred sample temperatures for each data set (Table 2) we deduce an energy difference, \( \Delta E (= E_D - E_C) \) of 4.02±0.07 kJ mol\(^{-1}\), 3.91±0.11 kJ mol\(^{-1}\), and 1.55±0.29 kJ mol\(^{-1}\) for, again, the TD\(_{415}, \) TD\(_{495}, \) and RH data sets.

The \( \Delta E \) value deduced from the RH data set appears unexpectedly lower than the TD derived values. The quoted error estimates reflect only the statistical uncertainty from the fitting procedure, but do not include more systematic errors. The small variations around 1 in the
relative conformer cross-sections (see Fig. 7(a)) imply variations in the relative sensitivity to 
these conformers across the energy range but this has not been incorporated into the fit. The 
shape and position of the gaussian sampling function may also inadvertently induce some bias 
against one or the other conformer — in particular the ~0.2 eV shift noted for the parent mass 
filtered RH TPES and PES (Figs. 3 and 6) probably indicates that a more sophisticated algorithm 
is required for consistent sampling of both RH and TD data sets.

Overall, however, Figure 8 demonstrates that a convincing agreement between theory and 
experiment is achieved for the Type I conformers, with the C:D population differing in an 
understandable manner with the various sample inlet conditions. Qualitatively, it clearly 
shows that conformer C is the more stable, being more strongly populated under cooler 
sample conditions. Various calculations of the C and D conformer energies, reported in Table 
1, are inconsistent in predicting the energy ordering of these two conformers — 
understandably since the calculated energy differences of \( \lesssim 1 \text{ kJ.mol}^{-1} \) are below the expected 
limit of achievable computational accuracy. While several caveats apply to our quantitative 
experimental estimates of the energy differences, they suggest the D conformer lies somewhat higher in energy, approximately 1–4 kJ.mol\(^{-1}\) above C. We are thus able to refine 
the conformer landscape and conclude unambiguously that conformer C is more stable than 
conformer D by a few kJ.mol\(^{-1}\).

![Fig. 9. Conformer Type II (A+B) mean experimental \( b_1^{(+1)} \) values for the HOMO orbital photoionization as a function of electron KE, extracted from total (parent + fragment) mass-filtered TD\(_{415}\), TD\(_{495}\), RH data sets using a gaussian sampling function centred at 9.65 eV binding energy, FWHM of 0.5 eV. The mean experimental \( b_1^{(+1)} \) data points are plotted at their central sampling energy for L-Pro, with any D-Pro data negated prior to plotting. Broken lines joining data points are to guide the eye only. CMS-X\(\alpha\) calculations for conformers A and B are shown as solid curves.](image-url)

A similar attempt was made to examine the Type II A,B conformer pair using a gaussian 
sampling function centred at 9.65 eV ionization energy to attempt to exclude Type I conformer 
ionizations. Figure 9 then provides an analogous plot to Fig. 8 for the A,B conformers, but with 
an evidently less satisfactory outcome. As already identified, the PECD asymmetries recorded 
in the Type II conformer region are of a lesser magnitude. While below 3 eV electron energy 
this could be a result of partial cancellation of the larger, but opposing PECD calculated for
conformers A and B, in the mid-range the experimental values remain small but positive, falling outside the range of negative values bounded by the theoretical predictions.

One possible explanation could be that the gaussian sampling method is unable to exclude contributions made by Type I conformers in this higher binding energy range. For example, we note that the conformer D PECDD in particular, is strongly positive across this central kinetic energy region, which could act to counter any negative A, B. However, a preponderant contribution by D conformer in this region is unlikely in consideration of predicted conformer populations. Moreover, given D conformer has a non-bonding n\textsubscript{H} HOMO its ionization is unlikely to provide a Franck-Condon envelope that extends much beyond its ionization threshold. Alternatively, we may consider possible limitations of the CMS-X\alpha method, particularly in relation to the A/B conformer calculations. Experience has shown that such PECDD calculations perform well for localized initial orbitals — such as 1s core orbitals\textsuperscript{43,44} and lone pair valence orbitals\textsuperscript{45,46} and now, evidently, including the C, D conformer HOMO orbitals of Pro. In contrast, less well localized valence orbitals, such as the HOMO orbitals of the A and B conformers (see Fig. 1), can sometimes pose a greater challenge. This follows from the manner in which the X\alpha potential is partitioned into spherical atomic regions. The spatial extent of core and lone pair orbitals is largely confined to one such spherical region, whereas an adequate description of more delocalized bonding orbitals would require additional modelling of the internuclear electron density. Overlapping adjacent spherical regions can partially assist in reliably modelling such non-local electron density, but specifically in the Type II conformers these challenges are compounded by the particularly strong H-bonds influencing electron density between N and H nuclear centres at intermediate ~1.8 Å separations. Permitting sphere overlap at this range would require over-extended atomic spheres, and cannot be readily accommodated. Such considerations do not appear to apply to the C/D conformers due to the much weaker N-H-O H-bonding.

Since there is no clear evidence of temperature dependent behaviour in the experimental data, this suggests that the A and B conformers may be nearly isoenergetic, with approximately 50:50 population. But as we are unable to judge whether the poor theory-experiment agreement evident in the 3.5 eV — 5.5 eV energy range of Fig. 9 is primarily experimental or theoretical in origin — or indeed is attributable to both causes — there are no more definite conclusions to be drawn concerning the Type II(A, B) conformer energetics.

**Astrochemical implications.** The origin of life’s homochirality, the fact that almost exclusively L-amino acids in proteins, and D-sugars in nucleic acids, are found in the biosphere, is still a puzzling and open question, a cornerstone in modern science.\textsuperscript{37} More than 170 years after Pasteur’s first intuition, who saw in this biomolecular asymmetry a signature of life, as opposed to symmetric inert matter, the origin of homochirality and of life itself appears strongly entangled. Most scenarios, so-called abiotic, imply that homochirality, or at least a significant enantiomeric excess (ee), existed prior to the emergence of life and was even a necessary condition for its development, since a racemic life appears unlikely, for structural reasons in particular.\textsuperscript{47} A very broad range of abiotic scenarios for the origin of biomolecular asymmetry have been proposed based either on random processes or on deterministic ones involving chemical and/or physical forces (for a review see Ref. \textsuperscript{48}). The deterministic branch can be subdivided into scenarios based upon parity-violation (PV) in the weak interaction possibly leading to tiny energy differences between enantiomers,\textsuperscript{49,50} and those based upon
the interaction with chiral fields such as magnetic fields\textsuperscript{51} or the ones associated to CPL\textsuperscript{52,53} on which we will focus from now.

Among the various media from which life might have originated, the circumstellar/interstellar medium (CSM/ISM) appear as very promising with the discovery of many proteic and non-proteic amino-acids, all with several \% L-ee, in various meteorites including the well-known Murchison one.\textsuperscript{39,54-56} These findings suggest an extra-terrestrial origin of life, more precisely that building blocks of life, such as amino-acids, would have been formed in the ISM and would have then been delivered to early Earth \textit{via} meteorites and comets. During their journey towards Earth, they could have been submitted to a chiral bias, such as CPL, so that the organic material delivered on Earth would be enantio-enriched, before reaching homochirality (100 \% ee) \textit{via} autocatalytical reactions on Earth.\textsuperscript{57} Such an astrophysical scenario is supported by the discovery of partially-polarized CPL in large portion of space in heavy star formation regions such as the Orion Nebulae.\textsuperscript{58,59} This major finding motivated several SR studies on UV/VUV asymmetric photochemistry in condensed matter\textsuperscript{60-62} leading to a few \% ee on amino-acids produced by asymmetric photochirogenesis from interstellar-analogue ices\textsuperscript{63,64} and by CD-driven asymmetric photolysis from racemic thin films.\textsuperscript{65,66}

In a previous study on alanine,\textsuperscript{33,34} we proposed PECD as an alternative asymmetric photophysical route in the gas phase for the production of ee in amino-acids in a given direction of space.\textsuperscript{29} Indeed, gas phase amino-acids produced by evaporation from hot cores\textsuperscript{67} or by desorption induced by photons or energetic particles from icy grains,\textsuperscript{68} \textit{a priori} as a racemic mixture, might be submitted in the ISM to CPL irradiation in the VUV range, and especially at the strongly dominant Lyman-\(\alpha\) radiation (10.2 eV).\textsuperscript{64,69,70} Because of the PECD effect, such an interaction would generate, for each enantiomer, asymmetric PADS of opposite forward/backward asymmetry with respect to the photon axis. Then, because of momentum conservation, the corresponding ions would be recoiling in an opposite direction exhibiting a reverse angular bias for each enantiomer, as compared to the photoelectrons. In other words, PECD would produce two enantio-enriched hemispherical clouds of parent ions, recoiling one from the other in a given line of sight corresponding to the photon axis, with opposite ee. One of these two parent ion clouds, enriched with enantiomers of a given handedness, would have then been captured and embedded into a shower of meteorites and comets which would have seeded early Earth with this enantio-enriched organic matter, triggering therefore life’s homochirality.

In the case of Ala photoionized at Lyman-\(\alpha\) radiation (10.2 eV), the filtered PECD (2\(b_1\)) on the parent mass (\(m/z\) 89) reaches a value of 4 \% with a negative value for the L-enantiomer.\textsuperscript{33} Because the calculated conformer-resolved PECD at 10.2 eV for the 3 main conformers of Ala have the same exact value,\textsuperscript{34} it was not possible to give any constraints in terms of temperature since the PECD at this energy appears totally insensitive to Boltzmann averaging whatever the temperature.

The case of Proline offers a possibility to refine this PECD-based scenario as a possible effect involved in the origin of homochirality. Indeed Pro, as well as Ala, are both relevant for the origin of life having been one of the first amino-acid to be recruited by life in the genetic code, Pro being n°5 while Ala is n°2.\textsuperscript{38} In a preliminary study, we showed that parent-filtered Pro (\(m/z\) 115), produced by TD (TD\textsubscript{415}) led to a PECD (2\(b_1\)) of 12 \%, a threefold increase as compared to Ala, and most of all of the same sign (negative for L-enantiomer) as Ala.\textsuperscript{29} This
sign matching is of course a crucial point for the validity of the scenario which should bring the same ee sign for all amino-acids.

The Pro full PECD study at the core of the present paper allows further understanding of the astrochemical implications in terms of ee and abundance as a function of temperature. This is an important issue because a wide range of temperature can be found in the ISM ranging from a few K to several 100’s K in hot cores.\(^{64}\)

![Figure 10](image.png)

**Fig.10. Parent mass filtered \( b_1^{(+1)} \) for L-pro HOMO ionization in the TD\(_{415}\), TD\(_{495}\), and RH inlet conditions.** These are plotted as mean values, formed by PES intensity weighted averaging across the PECD profiles extracted from m/z 115 filtered electron images. Broken lines are to guide the eye only.

Let us only consider here the data regarding conformer Type I (C and D) whose photoionization of the HOMO orbital is not dissociative, leading to intact parent ions, the only species to be considered for astrochemical implications. In Figure 10 we show PECD data for several photon energies, extracted from parent mass-filtered profiles, such as shown for \( h\nu = 10.2 \) eV in Fig 6. These data points display the same temperature dependence and follow the same trend with increasing energy as discussed for Fig. 8. However, the magnitude of the asymmetry at the lower energies studied is somehow increased compared to Fig. 8. This is attributable to the narrow electron energy selection implied by non-dissociative ionization subset included here, as opposed to the non-selective 0.6 eV FWHM gaussian used to obtain the Fig. 8 data. Such a KE effect, consequent on the skewing of the sampling function between these alternative selection strategies, is not surprising given the predicted rapid variation in conformer C’s \( b_1 \) parameter near threshold (Fig. 7(a)). Experimentally also, this is explicitly visible in Fig. 5(b) \( (h\nu = 9.5 \) eV) and Fig. 6 \( (h\nu = 10.2 \) eV) where the most intense asymmetries correspond to the lowest binding energy range.

It is clear that overall the magnitude of PECD at 10.2 eV (corresponding to 1.5 eV KE for conformer Type I) decreases with increasing temperature, while keeping the same sign for a given enantiomer (always negative for L-Pro). Since both conformers at 10.2 eV (1.5 eV KE) appear to have a very similar computed magnitude with opposite sign, this leads to a decreasing PECD magnitude at very high temperature, down to ~2 % at 600 K (RH case). At the opposite, the extrapolation towards very low temperatures should lead to intense
asymmetries, at least as high as the 12 % measured at 384 K (TD$_{415}$), only governed by the PECD of conformer C.

Of course, temperature might also have an impact on the abundance of parent cations, because of the temperature dependence of ion fragmentation processes. In this respect Pro provides a very peculiar example. Indeed, as it can be seen in Figure 11 showing the parent survival rate ($P/(P+F)$) as a function of the photon energy and temperature, the temperature effect is twofold: at low photon energy (8.7 eV) where mainly only the Type I conformers can be ionized, the higher the temperature, the lower is $(P/(P+F))$, an expected behaviour because of the increasing statistical dissociation in the hot ground state of the cation as observed for instance in Trp.$^{71,72}$

![Figure 11](image-url)  
*Fig. 11. Survival rate ($P/(P+F)$) of the Pro parent (P) ion (m/z 115) vs fragmentation (F) (m/z 70 + m/z 43), as a function of the photon energy and temperature: 384 K (TD$_{415}$), 452 K (TD$_{493}$) and 600 K (RH). The data have been obtained from the mass spectra recorded during the PECD PEPICO acquisition.*

Above the IE of Type II conformers, 8.7 eV, the P/F ratio is in addition governed by a Pro-specific competing conformer population effect, which at the opposite, tends to increase $(P/(P+F))$ with increasing temperature, by populating Type I (C/D) conformer in the neutral, which, as seen above, is partially non-dissociative. The combination of both processes leads for instance at 10.2 eV to an unexpected temperature ordering in which the highest survival rate (~5 %) is found for the intermediate 452 K case (TD$_{493}$). At 10.2 eV, in the high temperature limit, we found a survival rate of ~2 % at 600 K. Extrapolation to very low temperatures, by depopulating the stable conformer Type I (C/D) should lead, non-intuitively, to a decreasing survival of the parent cation.
Overall, as it was the case of Ala, Pro finally does not show any temperature constraints for the considered astrophysical scenario, with a constant sign of PECD, and therefore of the ee sign of Pro parent cation recoiling in a given line of sight along the direction of the propagation of the CPL, whatever the temperature. At high temperature (say 600 K) the magnitude of the ee is reduced (to a few %), while it should reach high values at very low temperature (at least 12 %), with however a decreasing abundance of parent ions.

Conclusions. By using two complementary vaporization methods, TD and RH, coupled to a i²PEPICO detection scheme, we have been able to carry out a complete VUV photodynamics study on gas phase pure enantiomers of the amino acid proline, mainly focused on processes mediated by ionization from the HOMO orbital. Proline possesses four main conformers, populated in our experimental conditions, which can be divided into two groups I (C/D) and II (A/B) differing by the carboxylic group orientation versus the pyrrolidine ring, i.e. stabilized by different intra-molecular H-bonds. Very interestingly, these two groups are associated with very different (by ~0.8 eV) IE offering a unique way to probe specific conformer dynamics when coupled to electron spectroscopy.

The TPES/TPEPICO analysis yielded an observed adiabatic IE of 8.30±0.01 eV, (at 384 K) and a 0K AE of the fragment m/z 70, corresponding to a C-C\alpha bond cleavage, of 9.02 eV. Most of all, the TPEPICO spectra unravelled an unusual conformer-dependent fragmentation behaviour of the state-selected cation, Type I conformer being partially stable, leading to a parent (m/z 115) cation, while Type II is fully dissociative upon ionization because of a very large geometry change between the neutral and the cation. Such a specific fragmentation pattern is also clearly visible on the fixed-photon energy mass-filtered PES shown in Figure S2. Moreover, the fitting of TPEPICO provided us with the internal temperatures corresponding to the three vaporization conditions we used (TD415, TD493 and RH).

The PECD data appear as very structured, reaching very high values of up to 18 % at a photon energy of 9.5 eV, which is quite unexpected for a multi-conformer molecule. Using the IE tagging to decipher conformers, it has been possible to observe directly, for the first time, a very strongly conformer-dependent PECD dynamics. Dedicated CMS-X\alpha calculations of the \( b_1 \) dichroic parameter indicate a striking conformer-dependence for the HOMO ionization in particular. These calculations for Type I(C,D) HOMO PECD clearly bound the corresponding experimental data, revealing also a clear C/D conformer temperature dependent population changes across our experimental temperature range. Most strikingly, the C conformer is unambiguously identified as the more stable of the two, something which is not established by existing electronic structure calculations (including ours), due to the small energy differences that must be at the expected limit of computational accuracies. By fitting the PECD results for the three sample vaporization methods as a weighted average of the two theoretical predictions, quantitative C:D population ratios were extracted. By further taking the internal temperatures inferred for these inlet conditions, an energetic difference favouring the C conformer by a few kJ.mol\(^{-1}\) was estimated. This demonstrates that PECD, via its strong sensitivity to subtle details of the molecular potential, may allow refining the conformational landscape of floppy systems.

An analogous comparison of theory and experiment for the A/B conformer pair proved less successful. While the reasons for this remain to be fully established, it may be pertinent to note that while the C and D conformers have HOMOs possessing N lone pair character, the A
and B conformer HOMOs have more delocalised character between and around the NH and COOH functional groups. Experience suggests that this non-local character may pose a greater challenge for the CMS-Xα theory. Experimentally also, the A/B PECD is weak, varying only slowly across the full energy range studied, and without any obvious temperature dependent variation.

As an extension of this first chiroptical study of proline, future experimental efforts for a direct study of conformer-specific PECD on floppy systems could take two avenues: (i) using methods such as electrostatic deflection to select a given conformer to be studied by one-photon PECD; (ii) using high spectral resolution REMPI-PECD, as recently-demonstrated to tag a given conformer via the first exciting photon.

Besides, PECD has been suggested to possibly be involved in the origin of life’s homochirality as an asymmetric photophysical process applied on gas phase amino-acids in the ISM, giving rise to enantiomeric excesses in a given line of sight of recoiling parent cations. Such a scenario proposed for Ala as well as for Pro is strengthened here. Indeed, the present temperature dependent PECD study, carried out at the astrophysically-relevant Lyman-α radiation energy, does not set any constraints in terms temperatures, i.e. in terms of ISM/CSM types of environment for the validity of this scenario leading in all situations to the same sign of the e for Ala and Pro, whatever the temperature. Such a thorough study should be extended in the future to other astrobiologically-relevant amino acids, as well as to the even more challenging case of nucleic acid sugars (ribose) for which, considering a given CPL helicity, the Lyman-α radiation PECD should exhibit a reverse asymmetry as compared to amino-acids, to account for the occurrence of only D-sugars in the biosphere.

Methods

Except for a few recent improvements, the experimental set-up based upon the SAPHIRS versatile molecular beam chamber, a permanent endstation of the DESIRS beamline at Synchrotron SOLEIL (St Aubin, France), is quite similar to the one that has been used for Ala. We used two complementary methods to bring the thermolabile Pro molecule into the gas phase: (i) resistive heating (RH) which might lead to severe decomposition/polymerization and (ii) aerosol thermodesorption (TD). The interplay of both methods allows us to benefit from their combined individual advantages and, in the present context, leads to different internal temperatures of gas phase Pro. In both cases, L- and D-Pro were purchased from Sigma-Aldrich (> 99 % purity).

Resistive heating vaporization method (RH). A few grams of Pro powder were nested into several layers of fiberglass wool and placed in the metallic reservoir of a new multipurpose high-temperature oven (able to reach 800 K), heated up by two collar-shaped heaters, one around the reservoir compartment set at 468 K and the other one around the nozzle assembly, slightly warmer at 490 K to maintain a temperature gradient and thus avoid nozzle clogging. The resulting vapor was seeded into 0.5 bar of He and expanded via a 70 μm nozzle to form a molecular beam, which was then collimated by two skimmers (1 and 2 mm) separated by a differential pumping stage, before entering the interaction region of the DELICIOUS3 double imaging Photoelectron/Photoion coincidence (i2PEPICO) spectrometer where it crossed the
VUV synchrotron radiation (SR) beam from the DESIRS beamline at a right angle. In the following, this method will be referred to as RH.

**Aerosol thermodesorption vaporization method (TD).** Intact gas phase enantiopure parent neutral Pro have been produced in situ from the corresponding homochiral Pro aerosol by thermodesorption on a hot tip inserted in the ionization region, following a method already used for Ala. These aerosols were produced by nebulization of a 1 g/L solution of Pro in He (2 bars) within an atomizer (TSI, model 3062) followed by a drying stage composed of two silica gel columns before being introduced into a new aerodynamic lens (ADL) that transmits and focuses sub-micron nanoparticles (30-300 nm with 100 % efficiency) into the ionization region. As compared to the previously-used ADL for Ala, the present one offers a much higher nanoparticle throughput, mechanical stability and reproducibility, and operational duty cycle. After traversing two 2-mm skimmers, the aerosols impinged onto a hot tip made of porous tungsten and heated up via a heating cartridge so that the tip temperature was set to 415 K and 493 K, the two temperatures used for the two sets of data obtained with the TD method, in conditions referred to as TD$_{415}$ and TD$_{493}$. The released plume of intact neutrals was then ionized by the SR at the centre of DELICIOUS3.

**Electron/ion spectrometer.** DELICIOUS3 combines a Velocity Map Imaging (VMI) spectrometer on the electron side with a modified Wiley-McLaren 3D momentum imaging mass spectrometer on the ion side operated in a multi-start/multi-stop coincidence scheme. This allows performing angle-resolved photoelectron spectroscopy (AR-PES) (with forward/backward capabilities) on mass-selected samples, with possible additional ion imaging / kinetic energy filtering with the RH method. This latter ion imaging filtering (also called Region Of Interest (ROI)) filtering increases the sensitivity of the experiment by selecting ionization events corresponding only to the spatially well-defined supersonic component of the molecular beam, discarding any thermal background contribution. Ultimate electron energy resolution on the detector edge is in the 3 % range for the RH method and ~5-10 % for the TD method (because of the electrostatic field distortion induced by the TD tip). DELICIOUS3 can also be operated in the threshold photoelectron spectroscopy mode (TPES) with sub-meV up to 10’s meV resolution according to an already-described method, allowing the study of state-selected cation fragmentation in the so-called TPEPICO scheme.

**Characterization of the vapor by mass spectra.** The performance of the RH and TD vaporization methods was checked by observing the ion ToF mass spectra across a range of photon energies (see supplementary information Fig. S4). Both methods produced relatively clean spectra with a strong Pro parent ion ($m/z$ 115) at $h\nu$=8.7 eV (see Fig. 12), switching to a clearly dominant $m/z = 70$ fragment channel with increasing photon energy. On close examination of the RH mass spectra, additional much narrower peaks (at $m/z$ 67, 69, 71, and 72) were noted adjacent to the $m/z$ 70 fragment peak. While the latter’s width remains consistent with broadening by translational energy release in dissociative ionization, the additional very narrow RH peaks are indicative of non-dissociative ionization from species that have been translationally cooled in the supersonic expansion. We therefore conclude that these narrow peaks evidence that some degree of thermal decomposition occurs in the RH oven prior to reaching the ionization source. These thermal impurities could, however, be discriminated against by mass filtering, giving confidence that when this is done all three sources yield results attributable to ‘cleanly’ vaporized proline.
Fig. 12. Time-of-flight mass spectra (TOF) of proline recorded at 8.7 eV. a TD\textsubscript{415} condition. b TD\textsubscript{493} condition. c RH condition (ROI filtered) with a zoom in the m/z 65-76 range. The fragments at m/z 67, 69, 71 and 72 are clearly decomposition products in the oven. The asymmetric shapes of the peaks in (a) and (b) are the product of off-axis ions due to the large interaction region and the inhomogeneous extraction field (see Ref. 80).

For both vaporization methods, PECD for a given enantiomer and photon energy was measured by recording mass-filtered (if needed) and ROI-filtered (for RH only) electron images obtained by alternating CPL helicities, switched every ~ 15 minutes. According to a previously detailed procedure,\textsuperscript{82} the corresponding left- and right-CPL obtained images were merged into two files and then used to provide the PES and the dichroic parameter $b_1$ from, respectively, the total (left-right) and difference (left-right) images after inversion via the pBasex algorithm.\textsuperscript{83} The statistical error bars on the dichroic parameter $b_1$ are given as the standard error on the principle that each image pixel acts as an independent counter that follows a Poisson distribution, with the associated error properly propagated through all subsequent operations.

**VUV photon source.** VUV photons with quasi-perfect circular polarization, above 97 % absolute circular polarization rate at the sample location as measured with a dedicated home-made polarimeter,\textsuperscript{84} were provided by the variable-polarization undulator-based beamline DESIRS.\textsuperscript{85} This beamline is equipped with a gas filter suppressing high harmonics of the undulator that could be transmitted by the grating’s high orders. The harmonic-free radiation was monochromatized with a 6.65 m-long normal incidence monochromator from which we chose to use the 200 grooves/mm grating providing high flux (in the $10^{12}$-$10^{13}$ photon.sec\textsuperscript{-1} range) and moderate resolution (typically in the few meV to 10’s meV range).

**Numerical methods.** Geometrical coordinates for the four neutral conformers of L-proline were obtained by Møller-Plesset (MP2) and density functional theory (DFT) calculations, made with the B3LYP functional, both using the aug-cc-pVTZ basis set. Electric dipole photoionization matrix elements were subsequently calculated at the DFT geometries by
static-exchange calculations using the continuum multiple scattering method with a Slater Xα exchange potential\textsuperscript{86} (CMS-Xα)\textsuperscript{87,88} following procedures as previously described.\textsuperscript{43,89-91} In this study the Xα model potential was constructed using overlapping spherical regions placed at the atomic centres. Radii of these atomic spherical regions were estimated by the Norman algorithm\textsuperscript{92} empirically scaled by a factor 0.85. A spherical harmonic angular basis truncated at \( l_{\text{max}} = 18, 7, 6 \) (referring, respectively, to the asymptotic region, the first row atomic regions, and the H atomic regions) was employed for the continuum electron calculations. For the initial neutral state a smaller basis \( (l_{\text{max}} = 7, 4, 3) \) sufficed, whilst retaining sufficient flexibility to model H atom polarization in the H-bonding interactions.\textsuperscript{31} Partial allowance for relaxation effects in the photoionized core were made by using experimentally estimated ionization energies rather than Koopmans approximations in calculating the dipole matrix elements. Subsequently, photoionization cross-sections, chiral asymmetry parameters, \( b_1 \), and PECD were obtained in an independent electron, fixed nuclear geometry approximation using these CMS-Xα photoionization dipole matrix elements.

A further set of neutral molecule calculations were made for thermochemically accurate conformer data by using the G3 composite method\textsuperscript{93} and these were repeated for the electronic ground state cation structures. Adiabatic ionization energies were thus estimated by a \( \Delta E \) approach using zero-point energy corrected, 0K energies for each conformer. Vertical ionization energy estimates were calculated using the outer valence Green's function (OVGF) method\textsuperscript{94,95} with a cc-pVTZ basis.

Our calculated energies and conformer structures are summarized in Table 1, where they can also be compared with literature values where these are available.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**References**

1. Kim, T. Y., Valentine, S. J., Clemmer, D. E. & Reilly, J. P. Gas-phase conformation-specific photofragmentation of proline-containing peptide ions. *J. Am. Soc. Mass Spectrom.* **21**, 1455-1465, (2010).
2. Mateos, B. *et al.* The Ambivalent Role of Proline Residues in an Intrinsically Disordered Protein: From Disorder Promoters to Compaction Facilitators. *J. Mol. Biol.* **432**, 3093-3111, (2020).
3. Schwob, L. *et al.* Radical-driven processes within a peptidic sequence of type I collagen upon single-photon ionisation in the gas phase. *Phys Chem Chem Phys* **19**, 22895-22904, (2017).
4. Schermann, J.-P. *Spectroscopy and Modelling of biomolecular building blocks.* (Elsevier, 2008).
5. Reva, I. D. *et al.* Infrared matrix isolation studies of amino acids. Molecular structure of proline. *J. Mol. Struct.* **318**, 1-13, (1994).
6. Stepanian, S. G., Reva, I. D., Radchenko, E. D. & Adamowicz, L. Conformers of Nonionized Proline. Matrix-Isolation Infrared and Post-Hartree–Fock ab Initio Study. *The Journal of Physical Chemistry A* **105**, 10664-10672, (2001).
Mata, S. et al. Observation of two new conformers of neutral proline. Phys Chem Chem Phys 11, 4141-4144, (2009).

Lesarri, A. et al. The structure of neutral proline. Angew. Chem. Int. Ed. Engl. 41, 4673-4676, (2002).

Plekan, O. et al. Investigation of the amino acids glycine, proline, and methionine by photoemission Spectroscopy. J. Phys. Chem. A 111, 10998, (2007).

Fathi, F. & Farrokhpour, H. Valence ionization of l-proline amino acid: Experimental and theoretical study. Chem. Phys. Lett. 565, 102-107, (2013).

Cannington, P. H. & Ham, N. S. He(I) and he(II) photoelectron-spectra of glycine and related molecules. J. Elec. Spec. Rel. Phen. 32, 139-151, (1983).

Ramek, M., Kelterer, A.-M. & Nikolić, S. Ab initio and molecular mechanics conformational analysis of neutral L-proline. Int. J. Quantum. Chem. 65, 1033-1045, (1997).

Dehareng, D. & Dive, G. Vertical ionization energies of alpha-L-amino acids as a function of their conformation: an ab initio study. International Journal of Molecular Sciences 5, 301-332, (2004).

Czinki, E. & Császár, A. G. Conformers of Gaseous Proline. Chemistry – A European Journal 9, 1008-1019, (2003).

Tian, S. X. & Yang, J. Effects of intramolecular hydrogen bonding on the ionization energies of proline. Angew. Chem. Int. Ed. Engl. 45, 2069-2072, (2006).

Ebrahimi, A., Roohi, H., Habibi, M., Mohammadi, M. & Vaziri, R. Characterization of conformers of non-ionized proline on the basis of topological and NBO analyses: Can nitrogen be a donor of hydrogen bond? Chem. Phys. 322, 289-297, (2006).

Close, D. M. Calculated Vertical Ionization Energies of the Common alpha-Amino Acids in the Gas Phase and in Solution. J. Phys. Chem. A 115, 2900-2912, (2011).

Lu, J., Meng, D., Li, F., Guo, M. & Li, Y. Theoretical Study of the Structure and Ionization Potentials of Proline. Russian Journal of Physical Chemistry A 94, 1427-1432, (2020).

Coutinho, L. et al. Photoabsorption and photoionization studies of the amino acid proline in the VUV region. Brazilian Journal of Physics 35, 940-944, (2005).

Lago, A. F., Coutinho, L. H., Marinho, R. R. T., de Brito, A. N. & de Souza, G. G. B. Ionic dissociation of glycine, alanine, valine and proline as induced by VUV (21.21 eV) photons. Chem. Phys. 307, 9-14, (2004).

Pilling, S. et al. Photostability of gas- and solid-phase biomolecules within dense molecular clouds due to soft X-rays. Monthly Notices of the Royal Astronomical Society 411, 2214-2222, (2011).

Marinho, R. R. T. et al. Gas phase photoabsorption and mass spectra of L-alanine and L-proline in the soft X-ray region. Chem. Phys. 324, 420-424, (2006).

Tamuliene, J. et al. On the influence of low-energy ionizing radiation on the amino acid molecule: proline. The European Physical Journal D 70, 143, (2016).

Abdoulcarime, H. & Illenberger, E. Fragmentation of proline induced by slow electrons. Chem. Phys. Lett. 397, 309-313, (2004).

Vukstich, V. S., Romanova, L. G., Megela, I. G., Papp, A. V. & Snegursky, A. V. Electron-impact-induced fragmentation of proline molecule. Technical Physics Letters 40, 901-904, (2014).

Powis, I. Photoelectron Circular Dichroism in Chiral Molecules. Adv. Chem. Phys. 138, 267-329, (2008).
Ganjitabar, H., Hadidi, R., Garcia, G. A., Nahon, L. & Powis, I. Vibrationally-resolved photoelectron spectroscopy and photoelectron circular dichroism of bicyclic monoterpene enantiomers. J. Mol. Spec. 353, 11-19, (2018).

Nahon, L., Garcia, G. A. & Powis, I. Valence shell one-photon photoelectron circular dichroism in chiral systems. J. Elec. Spec. Rel. Phen. 204, 322-334, (2015).

Hadidi, R., Bozanic, D., Garcia, G. & Nahon, L. Electron asymmetries in the photoionization of chiral molecules: possible astrophysical implications. Advances in Physics: X 3, 1477530, (2018).

Nahon, L., Garcia, G. A. & Powis, I. Valence shell one-photon photoelectron circular dichroism in chiral systems. J. Elec. Spec. Rel. Phen. 204, 322-334, (2015).

Turchini, S. et al. Conformational Effects in Photoelectron Circular Dichroism of Alaninol. ChemPhysChem 10, 1839-1846, (2009).

Stener, M., Tommaso, D. D., Fronzoni, G., Decleva, P. & Powis, I. Theoretical study on the circular dichroism in core and valence photoelectron angular distributions of camphor enantiomers. J. Chem. Phys. 124, 024326, (2006).

Ulrich, V. et al. Giant chiral asymmetry in the C 1s core level photoemission from randomly oriented fenchone enantiomers. J. Phys. Chem. A 112, 3544-3549, (2008).

Nahon, L. et al. Determination of accurate electron chiral asymmetries in fenchone and camphor in the VUV range: sensitivity to isomerism and enantiomeric purity. Phys Chem Chem Phys 18, 12696-12706, (2016).

Daly, S., Powis, I., Garcia, G. A., Soldi-Lose, H. & Nahon, L. Photoionization of epichlorohydrin enantiomers and clusters studied with circularly polarized vacuum ultraviolet radiation. J. Chem. Phys. 134, 064306, (2011).
Brack, A. From interstellar amino acids to prebiotic catalytic peptides: A review. *Chemistry & Biodiversity* **4**, 665-679, (2007).

Evans, A. C., Meinert, C., Giri, C., Goesmann, F. & Meierhenrich, U. J. Chirality, photochemistry and the detection of amino acids in interstellar ice analogues and comets. *Chem. Soc. Rev.* **41**, 5447-5458, (2012).

Quack, M., Stohner, J. & Willeke, M. High-Resolution Spectroscopic Studies and Theory of Parity Violation in Chiral Molecules. *Ann. Rev. Phys. Chem.* **59**, 741-769, (2008).

Darquie, B. et al. Progress toward the first observation of parity violation in chiral molecules by high-resolution laser spectroscopy. *Chirality* **22**, 870-884, (2010).

Rikken, G. & Raupach, E. Enantioselective magnetochiral photochemistry. *Nature* **405**, 932-935, (2000).

Engel, M. H. & Nagy, B. Distribution and enantiomeric composition of aminoacids in the murchinson meteorite. *Nature* **296**, 837-840, (1982).

Cronin, J. R. & Pizzarello, S. Enantiomeric excesses in meteoritic amino acids. *Science* **275**, 951-955, (1997).

Burton, A. S., Stern, J. C., Elsila, J. E., Glavin, D. P. & Dworkin, J. P. Understanding prebiotic chemistry through the analysis of extraterrestrial amino acids and nucleobases in meteorites. *Chem. Soc. Rev.* **41**, 5459-5472, (2012).

Soai, K., Shibata, T., Morioka, H. & Choji, K. Asymmetric autocatalysis and amplification of enantiomeric excess of a chiral molecule. *Nature* **378**, 767-768, (1995).

Bailey, J. et al. Circular polarization in star-formation regions: Implications for biomolecular homochirality. *Science* **281**, 672-674, (1998).

Kwon, J. et al. Near-Infrared Circular Polarization Images of Nhg 6334-V. *The Astrophysical Journal* **765**, L6, (2013).

Meinert, C. et al. Photochirogenesis: Photochemical models on the absolute asymmetric formation of amino acids in interstellar space. *Physics of Life Reviews* **8**, 307-330, (2011).

Myrgorodksa, L. et al. Light on Chirality: Absolute Asymmetric Formation of Chiral Molecules Relevant in Prebiotic Evolution. *ChemPlusChem* **82**, 74-87, (2017).

Sugahara, H. et al. d-Amino acids in molecular evolution in space - Absolute asymmetric photolysis and synthesis of amino acids by circularly polarized light. *Biochim. Biophys. Acta* **1866**, 743-758, (2018).

de Marcellus, P. et al. Photon-induced enantiomeric excesses in initially achiral solid-state interstellar molecules. *Astrophysical Journal letters* **727**, L27, (2011).

Modica, P. et al. Enantiomeric Excesses Induced in Amino Acids by Ultraviolet Circularly Polarized Light Irradiation of Extraterrestrial Ice Analogs: A Possible Source of Asymmetry for Prebiotic Chemistry. *The Astrophysical Journal* **788**, 79, (2014).

Meierhenrich, U. J. et al. Asymmetric vacuum UV photolysis of the amino acid leucine in the solid state. *Angew. Chem.-Int. Edit.* **44**, 5630-5634, (2005).

Meinert, C. et al. Photonenergy-controlled symmetry breaking with circularly polarized light. *Angew. Chem. Int. Ed. Engl.* **53**, 210-214, (2014).
Millar, T. J. in *Science with the Atacama Large Millimeter Array* Vol. 235 *Astronomical Society of the Pacific Conference Series* 45-58 (2001).

Kuan, Y. J., Charnley, S. B., Huang, H. C., Tseng, W. L. & Kisiel, Z. Interstellar glycine. *Astrophys. J.* **593**, 848-867, (2003).

Robitaille, T. P., Whitney, B. A., Indebetouw, R., Wood, K. & Denzmore, P. Interpreting spectral energy distributions from young stellar objects. I. A grid of 200,000 YSO model SEDs. *Astrophysical Journal Supplement Series* **167**, 256-285, (2006).

Pilling, S. *et al.* in *IAU: Organic Matter in Space* Vol. 251 *IAU Symposium and Colloquium Proceedings Series* (eds S. Kwok & S. Sandford) 371-375 (2008).

Wilson, K. R. *et al.* Thermal vaporization of biological nanoparticles: fragment-free vacuum ultraviolet photoionization mass spectra of tryptophan, phenylalanine-glycine-glycine, and, beta-carotene. *J. Phys. Chem. A* **110**, 2106-2113, (2006).

Gaie-Levrel, F., Garcia, G., Schwell, M. & Nahon, L. VUV state-selected photoionization of thermally-desorbed biomolecules by coupling an aerosol source to an imaging photoelectron/photoion coincidence spectrometer: case of the amino-acids tryptophan and phenylalanine. *Phys. Chem. Chem. Phys.* **13**, 7024-7036, (2011).

Teschmit, N., Horke, D. A. & Kupper, J. Spatially Separating the Conformers of a Dipeptide. *Angew. Chem. Int. Ed. Engl.* **57**, 13775-13779, (2018).

Tang, X., Garcia, G., Gil, J.-F. & Nahon, L. Vacuum upgrade and enhanced performances of the double imaging electron/ion coincidence end-station at the vacuum ultra-violet beamline DESIRS. *Rev. Sci. Inst.* **86**, 123108, (2015).

Božanić, D. K. *et al.* Interfacial Charge Transfer Transitions in Colloidal TiO2 Nanoparticles Functionalized with Salicylic acid and 5-Aminosalicylic acid: A Comparative Photoelectron Spectroscopy and DFT Study. *The Journal of Physical Chemistry C* **123**, 29057-29066, (2019).

Milosavljevic, A. R. *et al.* Electronic Properties of Free-Standing Surfactant-Capped Lead Halide Perovskite Nanocrystals Isolated in Vacuo. *J Phys Chem Lett* **9**, 3604-3611, (2018).

Bozanic, D. K. *et al.* Velocity Map Imaging VUV Angle-Resolved Photoemission on Isolated Nanosystems: Case of Gold Nanoparticles. *J. Phys. Chem. C*, 10.1021/acs.jpcc.0c08152 (2020).

Garcia, G., Cunha de Miranda, B., Tia, M., Daly, S. & Nahon, L. DELICIOUS III: a multipurpose double imaging particle coincidence spectrometer for gas phase vacuum ultra-violet photodynamics studies. *Rev. Sci. Inst.* **84**, 053112, (2013).

Garcia, G. A. *et al.* Synchrotron-based double imaging photoelectron/photoion coincidence spectroscopy of radicals produced in a flow tube: OH and OD. *J Chem Phys* **142**, 164201, (2015).

Garcia, G., Soldi-Lose, H. & Nahon, L. A versatile electron-ion coincidence spectrometer for photoelectron momentum imaging and threshold spectroscopy on mass selected ions using synchrotron radiation. *Rev. Sci. Inst.* **80**, 023102, (2009).

Baer, T. & Tuckett, R. P. Advances in threshold photoelectron spectroscopy (TPES) and threshold photoelectron photoion coincidence (TPEPICO). *Phys Chem Chem Phys* **19**, 9698-9723, (2017).

Nahon, L., Garcia, G. A., Harding, C. J., Mikajlo, E. A. & Powis, I. Determination of chiral asymmetries in the valence photoionization of camphor enantiomers by
photoelectron imaging using tunable circularly polarized light. *J. Chem. Phys.* **125**, 114309, (2006).

83 Garcia, G. A., Nahon, L. & Powis, I. Two-dimensional charged particle image inversion using a polar basis function expansion. *Rev. Sci. Inst.* **75**, 4989-4996, (2004).

84 Nahon, L. & Alcaraz, C. SU5: a calibrated variable-polarization synchrotron radiation beam line in the vacuum-ultraviolet range. *Applied Optics* **43**, 1024-1037, (2004).

85 Nahon, L. *et al.* DESIRS : a state-of-the-art VUV beamline featuring high resolution and variable polarization for spectroscopy and dichroism at SOLEIL *J. Synchrotron Rad.* **19**, 508-520, (2012).

86 Slater, J. C. *Quantum theory of molecules and solids*. Vol. 4 (Mc-Graw Hill, 1974).

87 Dill, D. & Dehmer, J. L. Electron-molecule scattering and molecular photoionization using the multiple scattering method. *J. Chem. Phys.* **61**, 692-699, (1974).

88 Davenport, J. W. Ultraviolet photoionization cross-sections for N{\text{-}}2 and CO. *Phys. Rev. Lett.* **36**, 945-948, (1976).

89 Powis, I. Photoelectron circular dichroism of the randomly oriented chiral molecules glyceraldehyde and lactic acid. *J. Chem. Phys.* **112**, 301-310, (2000).

90 Downie, P. & Powis, I. The 5a_{1}^{-1} photoionization of oriented CF_{3}I molecules: Angular distributions of the ka_{1} and ke photoelectron continua. *J. Chem. Phys.* **111**, 4535-4547, (1999).

91 Harding, C. J. & Powis, I. Sensitivity of photoelectron circular dichroism to structure and electron dynamics in the photoionization of carvone and related chiral monocyclic terpenone enantiomers. *J. Chem. Phys.* **125**, 234306, (2006).

92 Norman, J. G. SCF-Xa-SW calculations on PH{-}3 using a non-empirical scheme for choosing overlapping-sphere radii. *J. Chem. Phys.* **61**, 4630-4635, (1974).

93 Curtiss, L. A. & Raghavachari, K. Gaussian-3 and related methods for accurate thermochemistry. *Theoretical Chemistry Accounts: Theory, Computation, and Modeling (Theoretica Chimica Acta)* **108**, 61-70, (2002).

94 von Niessen, W., Schirmer, J. & Cederbaum, L. S. Computational methods for the one-particle green-function. *Comp. Phys. Rep.* **1**, 57-125, (1984).

95 Zakrzewski, V. G., Dolgounitcheva, O. & Ortiz, J. V. Ionization energies of anthracene, phenanthrene, and naphthalene. *J. Chem. Phys.* **105**, 8748-8753, (1996).

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Author contributions

LN designed the research project. DB conceived the aerodynamic lens. All authors conducted the experiment. RH treated the data. GAG performed the internal energy modelling of the breakdown diagrams. IP and RH performed the electronic structure and PECD theoretical modelling. LN, RH, GAG and IP wrote the manuscript with contributions from all authors.

Competing interests

The authors declare no competing interests.