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Structure–property relations in crystalline L-leucine obtained from calorimetry, X-rays, neutron and Raman scattering

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We have studied the amino acid L-leucine (LEU) using inelastic neutron scattering, X-rays and neutron diffraction, calorimetry and Raman scattering as a function of temperature, focusing on the relationship between the local dynamics of the NH2, CH3, CH2 and CO2 moieties and the molecular structure of LEU. Calorimetric and diffraction data evidenced two novel phase transitions at about 150 K (T1) and 275 K (T2). The dynamical susceptibility function, obtained from the inelastic neutron scattering results, shows a re-distribution of the intensity of the vibrational bands that can be directly correlated with the phase transitions observed at T1 and T2, as well as with the already reported phase transition at T3 = 353 K. Through the analysis of the Raman modes, the new structural arrangement observed below T1 was related to conformational modifications of the CH and CH3 groups, while the behavior of the N–H stretching vibration, v(NH2), gave insight into the intermolecular N–H...O interactions. The observation of changes in the translational symmetry in the crystalline lattice, as well as anharmonic dynamics, allows for localized motions in LEU.

1. Introduction

Polymorphism—the ability of molecules to exist in two or more crystalline forms possessing different arrangements or conformations—is a long existing mystery.1 Polymorphism can potentially be found in any crystalline material such as polymers, minerals, metals, and organic compounds.2 Polymorphic structures display different physical characteristics, such as distinct thermodynamic, spectroscopic, kinetic and mechanical properties. Understanding why this phenomenon occurs and how it can be predicted or controlled is an interesting problem in chemistry, physics, biology and pharmacy. A possible origin points towards the idea that some molecules can adopt multiple conformations with almost the same ground-state energy, or that they can exist in a multi-well low barrier potential at which the molecules can quickly transform from one conformation to another.

In the case of α-amino acids, Bernal3 concluded that the observation of polymorphism can result from changes in molecular conformations as well as from changes in the arrangement patterns (without changes in molecular conformations). Understanding polymorphism of amino acids and how to control their structures and properties is critical for practical purposes, such as piezoelectric and non-linear optical devices,4 expression of specific physiological conditions5 as well as gene mutation.6,7 In addition, their three-dimensional crystalline structures can also be used as biomimetics for modeling interactions in biopolymers8 as well to better understand biomolecular homochirality.9 Moreover, it is important to mention that crystallization is a thermodynamic as well as a kinetically determined process and the precipitation of various polymorphs can be induced under different conditions, such as through the use of different solvents or additives.10–12 Interestingly, amino acids with hydrophobic side chains (L-valine, L-leucine and L-isoleucine) obtained by sublimation or from aqueous solution seem to preserve the same crystal structure,13 while hydrophilic amino acids form different assembling patterns depending on the crystallizing media.12,14,15

Here we focus on the amino acid L-leucine (C6H13NO2, hereafter LEU), which crystallizes in the P21 monoclinic...
structure \((a = 9.61(8), b = 5.31(8), c = 14.72(8) \text{ Å}, \beta = 86.2^\circ)\). LEU is a pure aliphatic amino acid that possesses a \(\gamma\)-branched side chain, strongly hydrophobic, formed by four carbon atoms, as illustrated in Fig. 1. Usually, hydrophobic \(x\)-amino acids are packed in hydrogen bonded layers and are considered to be the strongest \(\alpha\)-helix forming residue in proteins. In the chiral crystals, molecules within the layer are related by translation and interlinked by N–H…O bonds. The neighboring layers are interlinked by N–H…O bonds forming bilayers generated by (pseudo-) twofold symmetry. As a consequence of the monoclinic (pseudo)twofold axis parallel to the plane of the bilayer, LEU crystals are polar.\(^{18,19}\) Generally, in a protein molecule, LEU is attracted to hydrophobic side chains (e.g. valine, tryptophan and phenylalanine) and has the tendency to be located inside the protein. It is important to realize that in the gaseous phase amino acids are found to be neutral, although both in solution and in the solid state they are commonly found in a so-called zwitterionic form,\(^{20}\) whereby the amino group gains a hydrogen atom donated by the carboxyl group.

Our interest in LEU lies in the fact that its structural isomer\(^{1}\), \(\beta\)-isoleucine, exhibits polymorphism, crystallizing either in the same monoclinic \(P_2_1\) structure as LEU \((a = 9.57, b = 5.32, c = 14.12(3) \text{ Å}, \beta = 95.8^\circ)\)\(^{21}\) or in an orthorhombic \(P2_2_2_1\) structure \((a = 13.89(6), b = 20.17(5), c = 5.53(3)\text{ Å})\).\(^{22}\) Additionally, no structural changes are reported in \(\beta\)-isoleucine\(^{23}\) as a function of temperature whereas differential scanning calorimetric and Raman measurements\(^{24,25}\) show a phase transition around 353 K in LEU.

In this article, we show through INS and Raman spectroscopy that the dynamics of LEU are anharmonic, resulting in the generation of a soliton-like excitation in the lattice.\(^{26}\) In order to obtain such localized modes, translational symmetry has to be broken. Calorimetry and diffraction show phase transitions that are consistent with this picture.

\(^{1}\) Structural isomers are defined as compounds that have the same molecular formula, similar chemical and physical properties but different structures.

2. Experimental details

1-Leucine samples (anhydrous forms) were obtained from Sigma Aldrich. The presence of traces of water was checked by means of differential thermal analysis (TG-DTA) using a Netzsch TG 449 F3 up to 620 K. No water was found to be present. Colourless and elongated single crystal plates were grown from aqueous solution by the slow evaporation technique at 295 K. All the data reported in this study were collected on warming the sample unless explicitly stated otherwise.

2.1 Calorimetric measurements

Differential scanning calorimetry (DSC) measurements were performed using a Netzsch DSC 204F1, Phoenix model on a powder sample, with a mass of 4.3 mg, under a nitrogen atmosphere.

2.2 X-Ray and neutron powder diffraction

X-Ray powder diffraction (XPD) patterns of LEU were collected as a function of temperature using a Philips diffractometer operating in the Bragg–Brentano geometry, using Cu K\(\alpha\) radiation \((\lambda = 1.5418 \text{ Å})\) with a secondary monochromator and equipped with an Anton PAAR TTK 450 cryofurnace. Data were collected between 120 and 400 K over a 20 range of 4–40° with a step size of 0.02° and a step time of 2 s. The heating rate between data points was 0.02 K s\(^{-1}\) with 5 min of stabilization time. The powder diffraction data were analyzed using the program GSAS.\(^{27}\) Neutron powder diffraction (NPD) data were collected between 70 K and 300 K using the high resolution powder diffractometer E9 at the Helmholtz-Zentrum Berlin (HZB, Germany) with a neutron wavelength of \(\lambda = 1.797 \text{ Å}\).

2.3 Inelastic neutron scattering

Inelastic neutron scattering (INS) measurements on polycrystalline samples were undertaken between 20 and 300 K using the time-of-flight (ToF) spectrometer NEAT \((E_i = 3.15 \text{ meV})\), with an elastic resolution full width at half maximum \((\text{FWHM}) \Delta E = 98 \mu\text{eV}\) located at the HZB. Using this configuration, the characteristic time longer than 10 ps can be probed by analyzing the elastic and quasi-elastic part of the spectra. To minimize the effects of multiple scattering during the INS measurements, the sample transmission was kept at 0.9. The data were corrected for sample holder and background contributions using programs available at the neutron facility.

2.4 Raman scattering

Raman spectra between 50 and 300 K were recorded on a single crystal of LEU with a Jobin Yvon T64000 spectrometer equipped with a liquid nitrogen cooled charge-coupled device detection system. The 514.5 nm line of an argon ion laser, operating at less than 4 mW, was used for excitation. An Olympus microscope lens with a focal distance of \(f = 20.5 \text{ mm}\) and a numerical aperture of N.A. = 0.35 was used to focus the laser beam on the sample surface. The slits were set for a spectral resolution of 2 cm\(^{-1}\). The sample was held in a helium flux cryostat where the temperature could be stabilized.
within ±0.1 K. No damage to the crystal was observed during the whole experiment.

3. Results and discussion

3.1 Thermal characterization

DSC measurements obtained for a powder sample of LEU, heated at 10 °C min⁻¹, are shown in Fig. 2. Two different peaks, the first an exothermic event at $T_1 \approx 150$ K and the second a weak endothermic transition at $T_2 \approx 275$ K, were observed indicating two phase transitions at $T_1$ and $T_2$. Interestingly, a very similar endothermic peak has been reported for l-norvaline (C₅H₁₁NO₂) at about 273 K.²⁸ Additionally, it was observed that LEU starts melting at $T_{	ext{melting}} \approx 573$ K.

3.2 Structural characterization

Fig. 3(a) shows the XPD patterns for LEU between 10° and 25° in 2θ as a function of temperature. The vertical lines below the data indicate the expected positions of the Bragg reflections for the published $P\bar{2}_1$ structure calculated using the structural parameters at 120 K from Görbitz and Dallus.²⁹ The whole range collected is not shown in order to increase clarity of the following observations. A number of changes can be seen that indicate structural phase transitions. For instance, the previously reported²⁴,²⁵ structural phase transition at $T_3 = 353$ K can be related to the observation of the growth of the Bragg peak near 2θ = 18° (marked with *) between 370 and 300 K although the peak is allowed in the $P\bar{2}_1$ structure at high temperature. In contrast, the emergence of the new Bragg peak around 2θ = 13° (marked with an arrow) below 260 K (at or around $T_2$) and the noticeable splitting of the Bragg peak occurring near 2θ = 24° (also marked with an arrow) below 210 K that disappears between 160 and 120 K (at or around $T_1$) indicate further structural phase transitions that are in good agreement with the calorimetric results.

Moreover, the first peak could not be indexed using the reported unit cell, even in the lowest symmetry space group possible $P_2$, but could be indexed as the (-1 0 2) by doubling the $a$-axis. The limited range of XPD data did not allow full structural refinement using the Rietveld method but it was possible to determine the evolution of the unit cell parameters as a function of temperature using the Le Bail intensity extraction technique,³⁰ which consists of estimating the integrated intensities of an unknown structure without using the least squares optimization. This approach also allows investigation of the possible space groups for the doubled cell.

Le Bail extractions were performed in a variety of space groups across the whole temperature range using the original cell and the doubled cell with $d' = 2a$. For the high temperature data (260 K and above) the patterns were fitted equally well in all of the space groups with either no systematic absences or those with the condition that 0k0 = 2n and so the published space group $P\bar{2}_1$ was used. The doubled cell was required for all temperatures below 260 K and all possible space groups were investigated that were consistent with the observed reflections. Identical fit statistics in the limited scattering angle range available were obtained with space groups either having no systematic absences or the presence of the 2₁ screw axis in the standard setting (i.e. 0k0 = 2n). All other monoclinic space groups either fitted one, other or
neither of the observed extra reflections. Therefore the compatible space groups for the low temperature structures are $P2_1$, $P2_1$, $Pm$, $P2/m$ and $P2_1/m$.

The temperature dependence of the volume and $\beta$-angle (corrected to take into account the necessity to double the $a$-lattice parameter below 260 K) are presented in Fig. 3(b) and (c) respectively. The plots are from Le Bail intensity extractions in the same space group as for the original published model $P2_1$, using the standard setting, where all variable parameters (peak shape, wavelength, background and zero point) except the lattice parameters were kept constant for each temperature in order to minimize systematic errors. No obvious peak broadening effects or background steps were seen across the entire temperature range allowing the fixing of these parameters.

It is clear from both the $\beta$-angle and volume that there are discontinuities in their behavior with temperature. The significant step in the refined $\beta$-angle below 250 K accounts for the observed splitting of the (0 0 4) reflection near $2\theta = 24^\circ$ and also accounts for its behavior between 200 and 150 K, where the splitting disappears again, as the $\beta$-angle refines to a similar value as that at 300 K where no splitting of this reflection is observed. The volume shows two discontinuities that agree well with the phase transitions observed by DSC, marked as dashed lines in both plots.

The NPD data were collected on the fully hydrogenated materials but, due to the very high incoherent scattering from hydrogen, these data could not be analyzed beyond verifying the space group assignments from the XPD data.

Finally, while the changes in the $\beta$-angle are noticeable in the intermediate temperature range $[T_f - T_c]$, around 120 and 300 K, the values are very similar; confirming that investigation of the structure and dynamics at intermediate temperatures is extremely significant.\(^{31}\) As will be verified in the following sections, correlating the structural data to the dynamical responses probed by INS and Raman spectroscopy (RS), a picture of the driving mechanism of the observed solid–solid phase transitions in LEU could be obtained.

### 3.3 Hydrogen dynamics in a wide time range

To identify if a non-harmonic dynamical regime involving barrier crossing processes is related to the structural changes observed in LEU, we carried out INS measurements.

Depending on the temperature and energy resolution (time) range, the measured scattering function ($S(Q,\omega)$), where $Q$ is the magnitude of the scattering wave vector and $\omega$ is the energy transfer, will express different contributions allowing for distinguishing different types of motions. $S(Q,\omega)$ can be decomposed into three components: elastic ($S_{el}(Q,\omega = 0)$), quasi-elastic ($S_{QE}(Q,\omega \approx 0)$) and inelastic ($S_{IN}(Q,\omega > 0)$).\(^{32}\)

Thus the analysis of the measured elastic intensity, $I_{el}$, that is proportional to $S_{el}(Q,\omega = 0)$, allows us to estimate the mean squared displacement of motions, $\langle u^2 \rangle$, according to:\(^{33}\)

$$I_{el} \propto \exp(-Q^2\langle u^2 \rangle/3) \quad (1)$$

Besides, the temperature dependence of the elastic intensity, $S_{el}(Q,\omega = 0)$, binned over the total $Q$ range explored, allows us to identify relevant transitions as sharp changes in the elastic scattering decay and has the advantage of being model-independent.

In practice, the evolution of $S_{el}(Q,\omega = 0)$ vs. $T$, presented in Fig. 4(a), corresponds to the H atoms, whose scattering cross section is strongly dominant. As shown in Fig. 4(a), within the time scale given by the resolution of the spectrometer ($\sim 10$ ps) and the statistical accuracy of our data, the curves for LEU seem to point towards contributions of CH$_3$-groups in the transition at $\sim 150$ K ($T_f$). It is important to consider that similar behavior\(^{34}\) was recently observed at about the same temperature in poly-alanine, which has only methyl groups as its side chains, but absent for poly-glycine, which does not possess side chains. Moreover, in LEU this temperature agrees well with changes observed in the diffraction patterns and in the DSC curve. Finally, as shown in monoclinic l-cysteine, such behavior can also be related to changes in the lattice parameters and volume.\(^{35}\)

In order to get a larger view of the different motions that can be activated in LEU and promote structural responses on
varying the temperature, detailed analyses of the quasi-elastic (QE) and inelastic (IN) parts of the spectra were undertaken. As depicted in Fig. 4(b), the broadening of the QE response, summed over the total Q range studied, seems to disappear between 250 and 220 K. Simultaneously, the peak height decreases in agreement with the evolution of the analysis of $S_0(Q, \omega = 0)$ presented above. As the QE-signal is very weak, all attempts to evaluate its dependence as a function of $Q$ failed, jeopardizing a detailed analysis of the geometry of the observed motion. However, we were able to obtain a qualitative description by fitting $S_{QE}(Q, \omega \approx 0)$ assuming a Dirac function and a single Lorentzian line width, which describe the elastic and QE signals, respectively. Using this approach, it is possible to relate the QE broadening ($\Gamma$) to the activation energy by an Arrhenius relation, $\Gamma = \Gamma_0 e^{E_{act}/kT}$, with $\Gamma_0$ and $E_{act}$ being the attempt frequency and the activation energy, in that order. In this model it is inferred that the molecule oscillates about the equilibrium orientations and then moves to a new equilibrium orientation. The discontinuity observed in the Arrhenius plot between 360 and 345 K (see Fig. 4(c)) is related to the phase transformation observed using X-rays diffraction.

The dynamical susceptibility function $\chi''(\omega)$ for LEU calculated from the inelastic part of the NEAT data at selected temperatures is shown in Fig. 5. Excitations due to harmonic vibrations should have a susceptibility that follows in the same temperature is shown in Fig. 5. Excitations due to harmonic vibrations should have a susceptibility that follows in the same way and 250 K. A re-distribution of the intensity of $\chi''(\omega)$ is still obvious down to 130 K.

At this point we can summarize our experimental observations as follows. A deviation from linearity of the elastic intensity, $S_0(Q, \omega = 0)$, at around 150 K ($T_1$) suggests that the exothermic transition observed in the DSC data is related to activation of the CH$_2$-groups movement, while the endothermic peak at around 275 K ($T_2$) can be related to changes of the proton motions in the CH$_2$-group as indicated by the dynamical susceptibility results. To further verify this idea we turn to the discussion of the Raman results.

### 3.4 Evidence of the structural changes by Raman spectroscopy

Recently, RS has been successfully used to confirm conformational and structural phase transitions undergone by crystalline amino acids as well as to give insights on the dynamics relating intramolecular motions and the distortions of the intermolecular hydrogen-bond networks. Through the use of this technique, it was possible to realize that the high-temperature phase transition undergone by LEU at $T_3 = 353$ K is probably related to symmetry change from $C_2$ to $C_1$. Here we extended this approach to lower temperatures.

Fig. 6 presents Raman spectra of a LEU single crystal in the spectral region 20–600 cm$^{-1}$, for the Z(YY)Z scattering geometry in the temperature range 50–295 K. In all Raman spectra presented in this paper we used the conventional Porto notation $a(bcd)$, where $a$ and $d$ represent the directions of the incident and the scattered light and $b$ and $c$ represent the directions of polarizations of the incident and the scattered light. The large face (001) of the crystal is perpendicular to the $c$-axis and this was defined as the $z$-axis through X-ray diffraction measurements. The smaller faces have indices (100) and (110); the axis perpendicular to the (100) face is the $x$-axis in our experiments. The bands observed between 170 and 210 cm$^{-1}$ assigned as torsion of CO$_2$, $\tau$(CO$_2$), and torsion of CH, $\tau$(CH), initially have similar intensities, however the intensity of the $\tau$(CO$_2$) increases during cooling and at 55 K it is twice the intensity of the $\tau$(CH). Moreover, for $T < 150$ K, a new band appears close to the intense band at 536 cm$^{-1}$, that was previously assigned as rocking vibration of CO$_2$, $\tau$(CO$_2$). Additionally, at low temperatures, change in intensities of the bands between 440 and 470 cm$^{-1}$, assigned as skeletal deformation, $\delta$(skel.), was noticeable. Moreover, in the lattice modes (spectral range, $\omega < 200$ cm$^{-1}$), a region very responsive to structural changes, notable modifications can be distinguished: (i) the intensity of the band at 68 cm$^{-1}$ (indicated by an arrow) increases at low temperatures; (ii) a change in the frequency of the band at $\sim 52$ cm$^{-1}$ is observed around 250 K, as illustrated in the inset of Fig. 6.

As previously reported for DL-serine, the slight change in the intensity of a band at the low-wave-number region can be associated with a small molecular reorientation of the molecules in the unit cell. On the other hand, frequency changes of a band in the lattice mode region can be interpreted as a phase transition. Consequently, the variable temperature Raman

![Fig. 5 Dynamical susceptibility $\chi''(\omega)$ of the H-atoms in LEU at selected temperatures measured using NEAT in the energy range 1–30 meV between 130 and 375 K. Note the changes in the out of plane vibrations of the CH$_2$-group at 30 meV.](image-url)
spectra of LEU in the Z(YY)Z scattering geometry point towards a reorientation of the molecules in the unit cell as well as to a phase transition in LEU at \( T \approx 250 \) K. It is interesting to note that when room temperature is reached again (spectrum 295\(^*\)), LEU presents qualitatively the same original spectrum. This reversibility occurs for all spectral regions analyzed here. Moreover, as shown in Fig. 7, while there is no evidence of significant change in the lattice modes down to \( T \approx 150 \) K for the Z(YY)Z scattering geometry, this picture is different for the Z(YX)Z orientation. The most significant point is that the band at 45 cm\(^{-1}\) (marked by an arrow in Fig. 7), visible at room temperature and associated with a lattice mode, has lost intensity during a large interval of temperature and completely disappears on cooling at 150 K. Here again, we come across another indication that LEU undergoes a second structural phase transition at this temperature. We remember that changes in lattice modes were associated with phase transitions in other crystalline amino acids, such as \( \alpha \)-valine,\(^{38a}\) deuterated \( \alpha \)-alanine\(^{38b}\) and \( \alpha \)-alanine.\(^{38c}\)

We can gain further insight on the origin of the two phase transitions by analyzing other regions of the Raman spectrum. For instance, Fig. 8 represents the temperature evolution of the Raman spectrum of LEU in the region 2800–3200 cm\(^{-1}\), characterized by the stretching vibrations of CH, CH\(_2\) and CH\(_3\), on cooling. Furthermore, this region is known to be very sensitive to conformational rearrangements, especially those
related to CH and CH\textsubscript{3} groups. Calculations on \textalpha-methionine showed that different conformers present different Raman spectra in this spectral region.\textsuperscript{41} Also, for crystalline \textalpha-cysteine strong changes in the Raman spectral profile in this region were associated with changes in the orientation of the molecules in the unit cell.\textsuperscript{42}

The most remarkable point in the temperature evolution of the Raman spectra of LEU shown in Fig. 8 is the appearance of a band (marked with an arrow) at approximately 3020 cm\textsuperscript{-1}, for \( T \approx 150 \) K, and the increase in intensity of the poorly visible band located at 3075 cm\textsuperscript{-1} at room temperature. In other polycrystalline amino acids,\textsuperscript{43,44} analogous bands have been assigned to the N–H stretching vibration, \( \nu(NH_2) \), which involves intermolecular N–H…O bonds related to the NH-groups in the crystal unit cell.

Finally, it is worthwhile to note that such a conformational change, realized through the modifications of modes in the high wave number bands of the Raman spectrum, was reported for LEU itself under high pressure conditions.\textsuperscript{45} In ref. 45, in a similar way to our present work, such a conformational change together with changes in the lattice mode region was verified at a pressure value of \( \sim 0.46 \) GPa.

In conclusion, we confirm the INS results. Namely, that the new structural arrangement observed in LEU below 150 K is related to conformational modifications of the CH and CH\textsubscript{3} groups, which is in perfect agreement with the deviation of the \( S_1^0(Q,\omega = 0) \) from the harmonic behavior as shown in Section 3.3. In contrast, the phase transition at \( 250 \) K is not accompanied by changes in the high wave number region of the Raman spectra; and therefore not related to the CH\textsubscript{3} moieties. In fact, as shown by the analysis of the QE response and by the variation of the density of states, this change involves characteristic relaxation times between 4 and 12 ps, which are much slower than characteristic times of CH\textsubscript{3} rotations,\textsuperscript{46} but can be related to fast local reorientations of the CH\textsubscript{2}-group.

4. Conclusions

The interest in amino acids lies in the fact that a common property of biomolecular crystals, namely the extensive hydrogen bonding network throughout the lattice, can be simply reproduced. The final aim of such studies is to relate small changes on this intricate network with polymorphism. For LEU, such a hydrogen bond network extends between the zwitterionic end groups (NH\textsubscript{3}\textsuperscript{+} and CO\textsubscript{2}\textsuperscript{-}) and a side chain formed by a hydrocarbon group.

Although LEU is found to crystallize in only one type of polymorph a number of structural phase transitions as a function of temperature were revealed during this study. Other than the already known high temperature phase transition at \( \sim 350 \) K (\( T_3 \)), related to symmetry change from C\textsubscript{2} to C\textsubscript{4v}, a second transition between 250 K–275 K (\( T_2 \)) was observed. Moreover for \( T_3 < T < T_2 \), QE broadening is observed in the INS data, and a clear change is noticed in the lattice mode located at 52 cm\textsuperscript{-1}. These observations point towards fast local reorientations of the hydrogen in the backbone of the molecules occurring in the tenths of picoseconds time scale. On further cooling, around 150 K (\( T_1 \)), a third transition related to modification of the Debye-behavior and changes in the frequencies of the methyl groups and CH-vibrations was detected. Here it is worth emphasizing that, similar to the case of \textalpha-alanine,\textsuperscript{47} the structural changes in LEU associated with a doubling of the unit cell along the \( \alpha \)-direction are apparently not observed in the single crystal diffraction studies. Clearly, a combination of high-resolution synchrotron and neutron diffraction of high quality single crystals and powder samples with modern instrumentation is required to solve the structure of LEU as a function of temperature and explain the apparent inconsistencies between powder and single crystal results.\textsuperscript{48}

The present study provides us a new example of the richness of the property–structure relation in crystalline amino acids. In these systems, conformational dynamics allow reorientation of the various molecular groups and, in the particular case of LEU, such modifications are followed by a rearrangement of the structure.

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Notes and references

18. The notion of polar crystals is fully related to fundamental symmetry concepts. Thus, as a consequence of the (pseudo-) twofold axis parallel to the plane of the bilayer and the absence of another symmetry element that would negate polarity, chiral crystals are polar; the racemic crystals are centrosymmetric and therefore non-polar.

48 All the experimental data discussed in this work can be supplied on request to bordallo@helmholtz-berlin.de.