Lysine is able to avoid water crystallization up to a proportion 1:5.4. However the microscopic mechanism underlying the cryoprotective effect of lysine and the maximum proportion of water that can be added to lysine before it crystallizes has not been investigated. The concurrent use of simulations and neutron diffraction has allowed us to perform a very specific analysis of the hydration of lysine. By comparing two different lysine-water concentrations (one crystallizing, the other being able to glassify) we have been able to associate its cryoprotective properties to the water molecules tightly bonded in the first hydration shell. Moreover, we did not obtain any evidence about the formation of dimers or bigger clusters structures, concluding thus that an homogeneous scenario in which the interstitial water molecules are avoided to form crystallization seeds due to their attachment to Lysine molecules.

PACS numbers: 33.15.Fm,61.20.Ja,61.20.-p,61.25.-f

INTRODUCTION

Lysine (Lys) is an α-amino acid which contains an α-amino group, an α-carboxylic acid group, a side chain which consists of a long flexible backbone of four CH₂ groups and a terminal ε-amino group (see Fig. 1). Lys is classified as a basic, charged (at physiological pH), hydrophilic and essential amino acid since it cannot be synthesized in the body and it has to be supplied by nutrition [1].

In addition, Lys has an important role in many biological processes: it is the first limiting amino acid needed to digest food proteins; it aids on the production of collagen to build bone, cartilage and connective tissues and the strength and elasticity of ligaments and tendons depend on lysine. It can enhance the secretion of thyroid hormones in the elderly, which helps to prevent senile immunodeficiency and plays a key role in several neurodegenerative human diseases (such as Alzheimer’s disease). Finally, Lys participates in a combination of hydrophobic and electrostatic interactions that lead to the formation of protein aggregates: amyloid fibrils [2, 3]. By linking the α-carboxyl group of one lysine to the α-amino group of another lysine a peptide bond is formed (also called an amide bond) and, in fact, lysine can form long peptides (n-lysine). In addition, by linking the ε-amino to the α-carboxyl group of two lysine molecules (see Fig. 1), lysine can form a polyamide. This term covers a large variety of polymer compounds with their constituents linked by amide bonds [4]. Polyamides are divided in two categories: those consisting of a single type of monomers (homo-poly (amides) or homo-poly (amino acids) if the building blocks are amino acids) and those consisting of different monomers (for instance proteins). The homo-
polyamide acid of lysine is called e-Poly-lysine (e-PLL) and it consists of 20 or 30 residues of L-lysine. On the other hand, in aqueous solutions Lys shows very rich and interesting behavior since it is the only amino acid (from the common twenty AAs that make up proteins) which can be fully dissolved in water, and, at the same time, the water content is low enough to avoid crystallization of ice at low temperatures [5, 6]. This unusual characteristic, which is not possible to reach in other biomolecules such as proteins or DNA, opens a route to analyze the properties of super-cooled water in a suitable biological solution without interference of ice. In this sense, the dynamics of water in lysine solutions was previously studied by means of dielectric spectroscopy [6]. For the first time, we have observed a decoupling of the water relaxation into two components: one faster with identical characteristics of the main relaxation of water in hard confinements (i.e., a local relaxation) [7] and one slower with characteristics of a cooperative (α-like) water relaxation. This behavior was also observed in n-lysine peptides as well in e-PLL molecules [8].

In this work, we analyze the structure of lysine-water solutions at two concentrations: 1:5.4 and 1:11 lysine/water molecules. In the first case, the solution classifies for all temperatures whereas in the second case, water crystallizes when cooled down. We are specifically interested in analyzing if there is a tendency of the molecules to organize (to form clusters, phase separation, etc.) or by contrary lysine-water solutions are fully homogeneous. Interaction of water molecules with lysine or lysine-lysine interactions are also of interest in this work. In order to reveal the structure in these solutions, a combination of neutron diffraction measurements (on samples with different isotopic substitution) and MD simulations have been used to examine the structuring of solvent around lysine aqueous solution. To do that a combination of neutron diffraction measurements (on samples with different isotopic substitution) and MD simulations have been used to examine the structuring of lysine in aqueous solution. Additionally, we also analyze the probability of lysine to form e-PLL instead proteins.

II. METHODS

A. Experimental

Neutron diffraction measurements were performed in hydrated lysine samples of concentrations N=5.4, 11, 15, 20 and 25 (N stands for number of water molecules per lysine molecule) with the SANDALS diffractometer at ISIS in the UK. In the case of N=5.4 and N=11, 5 samples were studied with different ratios of H and D: a full deuterated, a full hydrogenated, and three partially deuterated (50% D / 50% H, 66% D / 44% H and 44% D / 66% H). For N= 15, 20 and 25, only full deuterated samples were studied. All samples were measured at ambient pressure and temperature conditions, as so were the vanadium and corresponding empty cans for background corrections.

B. Molecular Dynamics

MD simulations were performed for the 1:5.4 and 1:11 samples, L1W5.4 and L1W11 from now on. Systems of 80 lysine molecules and 432 or 880 water molecules were employed respectively. Two types of lysine molecules were used in order to account for the correct protonation states: a neutral charged lysine (LYS0) and a negatively charged lysine (LYS-). L1W5.4 consisted of 29 LYS0, 51 LYS-, 51 hydronium (H3O+) and 381 water molecules, hydronium is needed to maintain a zero total charge system. L1W11 consisted of 40 LYS0, 40 LYS-, 40 hydronium and 840 water molecules. The simulations were performed in an NPT ensemble (\( T = 300 \) K, \( P = 1 \) bar) with periodic boundary conditions using the Gromacs 4.5.4 program [9]. The lengths of the simulation boxes fluctuated around 30.45Å (L1W5.4) and 34.46Å (L1W11). Lysine molecules were modeled using the CHARMM27 forcefield while waters were modeled using the specific CHARMM TIP3P variant TIPS3P model [10]. Hydronium was modeled using the parameters from Sagnella and Voth [11], they used ab-initio calculations to modify the TIP3P parameters. For each simulation, the time step was set to 2 fs and the Lennard-Jones interactions were treated using a switch cut-off from 9Å to 11Å. The real part of the electrostatic interactions were cut-off at 12Å and the long range part treated using the Ewald summation technique. In addition, the nose-hoover thermostat [12] and parrinello-rahman barostat [13] with a coupling time of 0.2 ps and 2.0 ps, respectively, were used. The lincs algorithm [14] was used to keep hydrogen bonds rigid. After 1 ns of equilibration, 4 ns of the trajectories were used for analysis.

C. ANGULA analysis

Characterizing the molecular ordering in disordered phases is not an easy task. One of the most widely used ways to quantify the short range order in liquids are partial radial distribution functions \( g_{\alpha,i;\beta,j}(r) \). These magnitudes are calculated by promediating the distance of an atom \( \beta \) of a neighbour molecule \( j \) from an \( \alpha \) atom of a given molecule \( i \) taken as central. Although this quantity can give us a hint about some local ordered about a given atom of a certain molecule, the extrapolation to a 3D configuration from the information obtained by this unidimensional magnitude is always risky, and some times leads to wrong or incomplete conclusions. Moreover, since each partial radial distribution function
is calculated from each atom of each molecule to every atom of all molecular types, the number of partial radial distribution functions needed to have a picture of the molecular ordering rapidly increases with the complexity of the system.

On the other hand, more sophisticated calculations can be done to fully characterize molecular relative orientation. Spatial density functions (SDF) are directly related to the distribution function $g_{\alpha,i;\beta,j}(r,\phi_{pos},\theta_{pos})$ that give us the probability of finding a $\beta$ atom of a $j$ molecule from a reference system centered at the $\alpha$ atom of an $i$ molecule. The maxima of this function shows the points in space where can we locate a maximum probability to find certain atoms of interest [15, 16]. To complete this kind of analysis we can, in addition, study the orientation of certain parts of a molecule centered at the $\beta$ atom of a $j$ molecule with relation to an $\alpha$ atom of a molecule $i$ by performing a calculation of a Probability Density Function of the euler angles that define the relative orientation $g_{\alpha,i;\beta,j;\theta_{ori},\phi_{ori},\psi_{ori}}$. In the case of molecules with a simple geometry such as water or carbon tetrachloride these two functions lead to a complete and non-ambiguous analysis of the molecular ordering [17].

For complex molecules the situation drastically changes: Although the aforementioned method is able to describe molecular ordering of some groups of a molecule around a specific site of another molecule, it is of no practical use when the goal is to perform a general, though quantitative, analysis of the whole molecule. The only exception for which this method is useful is when molecules are rigid, such as in the case of Indole [18]. In such cases an SDF can be defined using an appropriate atom selection (see [18] for more details), however this method is again useless for flexible molecules: SDF would show a convolution of the molecular ordering and the molecular geometry. It is therefore desirable to establish a general method to study complex molecules in an intuitive and quantitative way. We propose in the following three complementary quantities that yield to a general description of molecular ordering for complex and flexible molecules. This method is useful to locate the groups or parts of the molecule that are of interest.

**Surface-surface distance distribution function (SS-ddf)**

Choosing a characteristic the distance between molecules is non-trivial for large and flexible molecules. One of the most common option is to use the distance between centers of mass. However, as it happens with SDF the results will be the result of intra- and inter-molecular effects. Different characteristic distances purely intermolecular might be chosen (as explained in detail in [18]) depending on the prior information of the system. From these options the most aseptic is the one obtained as the minimum distance between all atoms of the central and the neighbour molecules. This distance can be seen as a sort of minimum distance between molecular surfaces. This choose has three advantages: it is not necessary to choose any specific site for hand, the distance is univocally defined for each pair of molecules and the meaning of the PDF has an exact and quantitative meaning. We will call to this correlation function Surface Distance Distribution Function $g_{\alpha,i;\beta,j}^{SS}(d)$. Since the minimum distance between molecules is chosen the subindex $\alpha$ and $\beta$ are not needed anymore. It should be pointed out that this correlation function is not radial since it is not taken from a single point.

**Distance-Energy map**  Distance distribution functions have the drawback that all the information about intermolecular distances are compressed in one dimension. This implies that molecular arrangements that share a very close distance might be seen as a single peak in the distribution function, or as a peak with more or less subtle shoulders. In order to be able to separate close peaks coming from different molecular arrangements we propose to plot the SDDF as a function of the contact energy of the two molecules from which the distance is calculated. We will call this correlation function $g_{\alpha,i;\beta,j}^{SS}(d,E)$. This bivariate analysis helps to elucidate differences in molecular ordering seen as different spots in impossible to be seen when a 1D distance distribution function is calculated. Of course it might happen that two different molecular orderings share the same distance and energy ranges. For this reason, and to univocally determine the molecular origin of a spot in $g_{\alpha,i;\beta,j}^{SS}(d,E)$ we propose a third tool called the contact matrix.

**Contact Matrix**  The SSDDF is based on the selection of the closest contacts between two molecules. In order to get additional information concerning intermolecular correlations the atoms through which the closest distance is obtained might be recorded. We can then calculate a contact matrix between molecules if we represent a bivariate probability distribution function of the atoms of a molecule (chosen arbitrarily as central) as a function of the atoms of a neighbor molecule used in calculating the minimum distance between molecules. We can do that as a function of distance, or selecting regions of the 2-D correlation function $g_{\alpha,i;\beta,j}^{SS}(d,E)$. In this way the spots on this function can be univocally related to a certain molecular interaction. It must be pointed out that contact matrix is not only an excellent tool to summarize in a graph molecular interactions between molecules but also it can be used to select molecules interacting through certain chemical groups to perform a further geometric analysis like determining the full correlation function $g_{\alpha,i;\beta,j;\theta_{ori},\phi_{ori},\psi_{ori}}$ as described in our previous works.

All the calculations presented in this work were done using ANGULA software [19].
FIG. 2: (color online) Total radial distribution function for the mixture of 11 water molecules per Lysine molecule (L1W11) with the deuterium/hydrogen proportions described in the methods section. Circles are the experimental results from neutron diffraction experiments and lines the MD simulation results.

III. RESULTS

Figures 2 and 3 show the results of the total radial distribution obtained from neutron diffraction together with that calculated from the configurations obtained by Molecular Dynamics Simulation. As it can be seen in that figures the results obtained by the simulations are compatible with those of the experiment for all isotopic substitutions. It must be pointed out that the differences between both the experiment and the simulation are related to the intensity of the peaks, but not to their position. This differences show that the atomic vibrations in the simulation are underestimated, giving as a result a less flexible molecule, and thus more marked peaks. However the shape and peak positions are the same both in the experiment and the simulation, therefore, although the mean square displacement between atoms is slightly underestimated by the Molecular Dynamics calculation, the molecular conformations and the short range order determined by the calculation are compatible with the experimental results. Information concerning both intra- and inter- molecular ordering will be thus extracted from the calculated configurations.

Molecular conformation

In order to investigate the differences between the molecular ordering in both mixtures L1W5.4 and L1W11 we must first investigate how the molecular conformation changes among them. This is necessary to elucidate if differences are coming from differences in intra- or in intermolecular arrangement. To do that we have calculated a distance distribution function for both LYS0 and LYS- in the two mixtures between the nitrogen atoms in the side chain and that linked to the α-carbon, i.e., the atoms at the two molecular ends. As it can be seen in figure 4 the N1-N18 distances (see fig. 1 for atom definition)
of LYS0 and LYS- show almost no change between the two solutions. This result yields to the conclusion that changes between both mixtures must be searched in the differences of molecule-molecule arrangement, and not in a different molecular conformation. In order to support this conclusion we have also calculated the change in conformational entropy for LYS0 and LYS- when dissolved in L1W5.4 and L1W11 mixtures (see [20] for details). The relative differences for LYS0 and LYS- are 7% and 0.3%, thus supporting our conclusion based on the distance-based criterion.

Molecular Ordering of water around Lysine

The key of the antifreezing properties of lysine cannot be searched in its molecular conformation as proved in the previous section. Therefore we change our focus now to the search of any particular hydration of lysine in the L1W5.4 mixture that makes it the limit case for its antifreezing properties. In order to study the arrangement of water molecules around lysine we show in figure 5 the SS-DDF between an uncharged lysine molecule (LYS0) and water molecules \( \langle g_{SS}^{LYS0-W}(d) \rangle \) (panel a) together with the Molecular Coordination Number (MCN) (panel b) calculated via the minimum distance criteria explained in the previous section. The vertical line on the plot shows the distance for which the number of water molecules around Lysine equals the macroscopic Lysine-Water concentration \( d_{MCN=5.4} = 2.2\AA \) for the mixture L1W5.4 and \( d_{MCN=11} = 2.4\AA \) for the L1W11 mixture. As it can readily be seen in the figure 5, while the the distance \( d_{MCN=5.4} = 2.2\AA \) coincides with the minimum \( g_{SS}^{LYS0-W} \), this is not the case for L1W11. In other words: all the molecules added to lysine in the mixture L1W5.4 are tightly bonded to lysine, probably due to hydrogen bonding. On the other hand, in the case of L1W11, not all the water molecules were able to be bonded to lysine, being some of them either not so tightly bonded or linked to lysine via another water molecule.

In order to investigate how these contacts are made in both concentrations we show in figure 6 the contact matrix of uncharged lysine and water for both concentrations L1W5.4 and L1W11. In the case of L1W5.4 (panel a) water molecules are mainly bonded to the charged ends of the molecule via a Hydrogen Bond: as donors linked to the two oxygens of the molecule, and as acceptors to the positively charged amine group of the side chain. For the mixture L1W11, water molecules are also linked to the same groups (see the contact matrix in fig. 6) as before, but a bond between the hydrogens of the Lysine backbone and water molecules is also appreciated: either as donor or as acceptors (both \( H_{BB} - H_{W} \) and \( H_{BB} - O_{W} \) matrix elements have a non-zero probability). Since the binding energy of the water molecules linked to the protein backbone is almost zero (see \( g_{SS}^{LYS0,W}(d,E) \) in the appendix) this supports the idea that the bond is not energetically very favourable, thus being that water molecules free to eventually form interstitial crystals between lysine molecules.
Molecular Ordering of Lysine around Lysine

Water is surrounding lysine mainly bonded to both the carboxylic group and the amine group of the amino acid side-chain. However, lysine itself is also probably linked to the same groups thus competing to be bonded to the same sides. This bonding can even result on some kind of supra-molecular structure. Finally, such a molecular arrangement could also depend on water concentration thus being different for L1W5.4 and L1W11. Since the main goal of this work is to investigate the differences in molecular ordering between L1W5.4 and L1W11 we have calculated the SS-DDF for all the combinations of the molecules taking part of the mixture for both concentrations. This results on the need of calculating the twenty SS-DDFs $g_{i,j}^{SS}(d)$, being $i,j=\text{LYS0, LYS-}, \text{W}, \text{and H}$, for both L1W5.4 and L1W11 that are shown in the appendix. From that figure it is evident that the main differences between both mixtures are those involving LYS0- LYS0 and LYS0-LYS- contacts (see upper panels of figures 7 and 8).

In order to investigate the molecular ordering in both cases we show in the lower panels of figures 7 and 8 the bivariate distribution function $g_{i,j}^{SS,LY S0,LY S0}(d,E)$ between two uncharged lysine molecules (LYS0-LYS0) and between an uncharged and a charged one (LYS0-LYS-) together with their projection $g_{i,j}^{SS,LY S0,LY S0}(d)$ in the upper panels. Spots appearing on $g_{i,j}^{SS}(d,E)$ indicate that some special bonding with a certain energy signature is happening between two molecules. We have named with latin letters those spots that we will prove to be originated by the same type of molecular contact, and by a greek letter those arising from a different type of molecular bonding.

Lysine0-Lysine0: In the first case (LYS0-LYS0) we can see two spots located in the same (d,E) region, that can tentatively be associated to a similar molecular bonding. On the other hand a spot in the L1W11 ($\alpha^*$ in fig. 7) not to be seen in the L1W5.4 mixture evidences that some of LYS0 molecules are differently bonded in both mixtures.

Lysine0-Lysine-: In the second case (LYS0-LYS-) the two distance-energy maps $g_{i,j}^{SS,LY S0,LY S-}(d,E)$ are very similar, therefore we can tentatively conclude that in both mixtures the same kind of molecular contacts occur. Therefore, the differences between the SS-DDFs is coming simply from a different proportion of the contacts associated to each spot in $g_{i,j}^{SS,LY S0,LY S-}(d,E)$.

In both cases (LYS0-LYS0 and LYS0-LYS-) it is convenient to calculate the contact matrices associated to each spot in order to univocally associate a type of molecular contact to each spot in the distance-energy map. For the sake of clarity we have classified the spots appearing in the energy-distance maps depending on the binding energy: we will differentiate between direct contacts as those with a clear negative energy (i.e. associated to tightly bonded molecules) and those with a negative or even positive binding energy.

![FIG. 6: (color online). Contact matrix of water molecules around uncharged lysine for both mixtures L1W5.4 and L1W11.](image)

![FIG. 7: (color online). Probability distribution function of the distance between molecular surfaces of LYS0 as a function of the contact energy $g_{i,j}^{SS,LY S0,LY S0}(d,E)$ .](image)

![FIG. 8: (color online). Probability distribution function of the distance between molecular surfaces of LYS0 as a function of the contact energy $g_{i,j}^{SS,LY S0,LY S-}(d,E)$ .](image)
**Direct contacts**

Lysine0-Lysine0: Contact matrices for both mixtures have been generated by selecting the spot “a” appearing in figure 7. It must be pointed out that since the matrices are generated between the same molecular species they must be diagonal. As expected, for LYS0-LYS0 direct contacts are coming from the charged parts of the molecule: the oxygen of the carboxilic group and the Hydrogens from the amine group in the side chain. From figure 9 we also conclude that the strongest contact between LYS0 is coming from the same contact in both mixtures L1W5.4 (i=1) and L1W11 (i=2).

Lysine0-Lysine-: Mediated contacts, as in the previous case, the contact matrices for both mixtures between L0 and L- shown in figure 9 will help us to investigate the origin of the strong molecular contacts labeled as “a” in figure 8. For both mixtures the most frequent strong contact is that between the amino group in the side chain of LYS0 and the carboxilic group of LYS-. This is easily explained by the fact that the former is positively charged only for LYS0, being the carboxilic group negatively charged for both LYS0 and LYS-. However, contrary to what happened to LYS0-LYS0, some differences arise between both L1W5.4 and L1W11 mixtures: while LYS0 can be also bonded to LYS- through the amine groups in the first case, this bonding is almost nonexistent in the second case, most probably to the competition with water molecules linked to that group.

**Mediated contacts**

Lysine0-Lysine0: The contact matrices calculated for spots α and α’ in the distance energy map (fig. 7) are shown in figure 11. Already at a first glance of the figure it is evident that the differences in the hydration previously described do have an effect on the relative ordering of neutral lysine molecules: for the L1W5.4 most of the contacts are done between the amine groups of the side chains and for L1W11 the most probable binding is between the amine and carboxilic groups linked to the α-carbon, i.e. those responsible for the peptidic bond.

In figure 7 we can also appreciate a clear spot for positive energies (\(E \approx 40k\text{J/mol}\)) and distances of about 3.2Å designed as b in that figure. The contact matrix calculated for this spot both in L1W5.4 and L1W11 mixtures can be found in the appendix and is clearly related to the link between two carboxilic groups of lysine mediated by a water molecule.

Lysine0-Lysine-: As in the previous case, the differences on lysine hydration have a critical effect on the LYS0-LYS mediated contacts. For the L1W5.4 mixture the most probable contact is between the amine group of LYS0 linked to the α-carbon of Lysine and that of the side chain. For the L1W11 mixture there are several options for a mediated contact: the amino functional groups of LYS- are linked either to the hydrogens of the backbone of the side chain or to the amino group located close to the α-carbon of LYS0.

Concerning the spot named as b in the distance-energy map shown in fig. 7, it origin is the same than that de-
scribed in the previous paragraph for LYS0-LYS0: it is related to an Oxygen-Oxygen water mediated contact between both Lysine molecules (the charged one and the uncharged one)

On the other hand (as shown in figure 5) the addition of water beyond this concentration makes additional water molecules not to be directly linked to Lysine, and in any case, if they are linked is through a bond less intense than a Hydrogen bond (see fig. 6). We therefore have tentatively associated the antifreeze effect of Lysine to its strong bonding with water molecules that are not free to move and, eventually, form an ordered crystal. The mechanism of water sequestration of water molecules to avoid freezing has also been seen for trehalose [21] and...

Homogeneous vs heterogeneous mixture. Silvina: aqu me has de echar una mano An important issue concerning the antifreeze properties of lysine is to find out if the mixture of Lysine with water is homogeneous, or it is formed by clusters of lysine surrounded by water molecules. While it is evident that Lysine is able to form strong bonds between their carboxilic group and the amino group in the side chain, we have demonstrated that such contacts are not due to a tightly bonded dimerization of Lysine molecules (see fig. 10). However a question still holds: would it be possible for lysine to form more or less long polymeric-like chains that would lead to any kind of supra-molecular structure. To study this issue we have calculated the number of molecules that are united through an hydrogen bond. The results, show in figure 13 show that the most probable number of lysine molecules forming a chain is two. We thus conclude that no polymeric-like chains are formed between Lysine molecules. This result agrees with recent Small Angle Neutron Scattering experiments CITAR[SILVINA-OJO], where no signal at low momentum transfer was observed.

IV. DISCUSSION

Lysine antifreeze effect. One of the focuses of this work is to investigate if there is some special arrangement of water around lysine that avoid water crystallization for concentrations of water less than 5.4 water molecules per one of lysine. As seen in figure 5 for the limit concentration L1W5.4 all water molecules are found to be linked via a Hydrogen Bond to Lysine (see fig. 6.

$\epsilon$ Polylysine vs peptidic bond. (Silvina, aqu tambin me has de echar un cable) Lysine is an amino acid, and thus, it is able to form a peptidic bond with others amino acids to form a protein. However, lysine is also able to form a polymer linking its $\epsilon$ carbon atom with the carboxilic

FIG. 11: (color online). Contact matrix of closest mediated contacts (spots a in figures 7 and 8) between LYS0 and LYS0 molecules (panels $a_i$) and between LYS0 and LYS- (panels $b_i$) for both concentrations L1W5.4 ($i=1$) and L1W11 ($i=2$).

FIG. 12: (color online). Contact matrix of closest mediated contacts (spots a in figures 7 and 8) between LYS0 and LYS0 molecules (panels $a_i$) and between LYS0 and LYS- (panels $b_i$) for both concentrations L1W5.4 ($i=1$) and L1W11 ($i=2$).

FIG. 13: (color online). Number of bonded lysine molecules (black LYS0-LYS- and blue LYS0-LYS0) both in L1W5.4 (solid line) and L1W11 (dashed line).
group of another lysine molecule. As it can be seen from the contact matrices related to the direct binding of lysine molecules (see fig. 9), the most probable contact between lysine molecules is through their carboxilic and the amino acid linked to the ε carbon atom. This kind of liaison is thus favored by the local ordering of molecules thus helping the formation of polylisine. On the other hand a molecular arrangement favoring the peptidic bond is only found when it is mediated by water.

V. CONCLUSIONS

Studying the molecular ordering of flexible molecules is a demanding task that needs of new computational tools, as those showed in this work. Radial distribution functions or more sophisticated tools such as Spatial Density Maps are useful only for simple and rigid molecules. In this work we have shown that the concurrent use of distance energy maps together with contact matrices helps to determine the short range order of complex and flexible molecules. Moreover, the tools shown in this paper are able to draw a general picture of relative molecular ordering of complex and flexible molecules without using sophisticated analysis such as determining the six-dimensional \( g_{α,i;β,j;r,φ_{pos},θ_{pos},φ_{ori},ψ_{ori}} \) function, only useful for simple cases such as for water [17]. Of course the computations performed in this work, apart from allowing us to draw a quite detailed picture of molecular ordering, help also to discriminate the groups or atoms of the molecules worth to be further investigated through \( g_{α,i;β,j;r,φ_{pos},θ_{pos},φ_{ori},ψ_{ori}} \) as done before by some of the authors [16, 18].

Concerning the lysine investigation we have shown first that most probable contacts between lysine molecules and water-lysine molecules showed us the possibility that molecular ordering might help the formation of polylisine. Secondly, and most important, we have shown that the antifreeze properties of lysine in a water solution are originated by localized contacts of lysine with water molecules, and not by the formation of any supramolecular structure. More into specifics, water molecules are sequestrated by lysine through a strong Hydrogen Bonding that do not allow them to form any crystalline seed. This is possible only for concentration of water less that 5.3 per lysine molecule. If the water concentration is increased water is not able to be tightly bonded to lysine, being thus able to form crystals.

VI. APPENDIX

All Surface-Surface Distance Distribution Functions:

This work was supported by the Spanish Ministry of Science and Innovation through the project FIS2014-54734-P and by the Generalitat de Catalunya under the project 2014 SGR-581. The UK Science and Technology Facilities Council is gratefully acknowledged for partial financial support and beam time access at the ISIS Facility (RB1220294).