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Competition between secondary structures in gas phase polyalanines

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Abstract – The temperature-dependent conformations of alanine-rich polypeptides are investigated using generalized ensemble Monte Carlo simulations. Pure polyalanines form α helices at low temperature, but exhibit an intermediate β-sheet structure below the coil transition. For the substituted peptide WA_{13}, the simulation predicts the β conformation to be more stable than helices already at low temperatures, and the β motif is further favored by entropy. Measurements of the electric dipole of this peptide do not provide evidence for helical structures even at room temperature. These experimental observations are thus compatible with our suggestion of β conformations, even though random-coil structures cannot be ruled out. Finally, we show how to stabilize α helices by an intense electric field, possibly leading to electrofreezing behavior.

Introduction. – Proteins are involved in a large variety of functions, such as chemical reaction catalysis, oxygen transport, or muscle contraction. These functions are associated with specific protein structures. Conversely, misfolding can cause malfunction, as in the case of Alzheimer's neurodegenerative disease where some soluble proteins with high α-helix content are converted into β-sheet–based insoluble structures, leading to a wrong global shape and a pathogenic behavior. The relative stabilities of secondary structures are thus of crucial importance due to their early appearance in the folding process [1]. Here we discuss structural transitions in gas phase polyalanines and show how small changes in the sequence or the environment can trigger $\alpha \leftrightarrow \beta$ conversion.

Even though isolated proteins are far away from their biological medium, some major problems and fundamental issues of solvated proteins, including folding itself, are preserved. Therefore, studying *in vacuo* polypeptides helps in understanding the interplay between intermolecular and intramolecular interactions [2]. Experimentally, IR spectroscopy [3] provides useful conformational information, but is currently limited to small peptides. Ion mobility [4], electron capture [5] or collision-induced dissociations [6], proton/deuterium exchange reactions [6] as well as electric dipole measurements [7] have been used to determine secondary and even tertiary structures of peptides containing up to tens of amino acids. A recent paper by Jarrold [8] reviews the various influences of the charge, the extent of hydration, and the sequence on the preferential secondary structures of small isolated peptides. From the theoretical side, the problem is only tractable through some approximations, starting with the use of classical force fields such as Charmm [9], Amber [10], Gromos [11], or ECEPP [12]. Once an appropriate model is chosen, the conformational exploration of the potential energy landscape is commonly achieved by molecular dynamics or Monte Carlo algorithms.

Alanine is the simplest amino acid that adopts specific secondary structures, essentially α helices. In particular, polyalanines are often considered as prototypes for the helix/coil phase transition [13,14]. Due to their relative simplicity, they also provide convenient models for assessing the influences of the solvent [15] or the force field itself [16] on the conformation. So far, most simulation studies on polyalanines have predicted a single-step transition between α helix and random coil [14,15,17]. Recently, it was suggested from several theoretical investigations

on solvated polyalanines that α helices could indeed be disfavored in gas phase polyalanines, and that the β conformation may be stabilized under several circumstances below the folding temperature T_f [18–20], the α/β transition being driven by entropy, while α helices are energetically more stable.

In the present work, we study the secondary structure of neutral and isolated polyalanines using advanced Monte Carlo sampling methods [21,22], comparing with electric dipole measurements [23] as a powerful conformational probe. For the first time, we provide some numerical evidence that β-sheet conformations are stabilized over helices in gas phase polyalanines, in agreement with experimental measurements [23]. In the following, we briefly describe the model and methods, before presenting and discussing our results. A summary and conclusion end the paper.

Methods. – The peptides are modelled using the Amber force field [10] with *ff96* parameters [24] chosen for their good performances in reproducing gas phase measurements of electric dipoles [25]. As will be discussed below, additional calculations have also been performed with ECEPP/2 for the experimentally studied peptide. The Amber *ff96* force field was originally parameterized for hydrated biomolecules. In particular, the charges were increased over their gas phase value to account for polarization effects in solvent. To correct for this effect, we have used a dielectric constant $\varepsilon = 2$ to reduce the charges. Thus, we try to alleviate the intrinsic overstabilization of β-sheet structures with respect to helices that has been reported in simulations of hydrated peptides using the *ff96* parameter set [26].

The energy landscape is sampled by means of the Wang-Landau (WL) method [21] for joint densities of states Ω, improved with the recent annealing scheme [22] for the WL modification factor. Monte Carlo moves are performed with torsional angles as the only internal coordinates. Here we have chosen the electric dipole μ as the additional order parameter to the potential energy E, to build the function $\Omega(E, \mu)$ iteratively. The parameter μ is very sensitive to the conformation of helices, and is expected to facilitate folding significantly in the simulation.

From the joint density of states, the heat capacity C and the thermal average $\langle\mu\rangle$ were calculated by standard Laplace transformation. By performing an extra multicanonical simulation based on Ω, we also calculated the end-to-end distance d between the nitrogen atom in N-ter position and the hydrogen from the hydroxyl group in C-ter position, accumulating the histogram $h(E, \mu, d)$ and the thermal averages $\langle d\rangle$ after proper reweighting. This reweighting procedure also provides the probability distribution $p(T, \mu, d)$ at finite temperature T, or the Landau free energy $F(T, \mu, d) = -k_{\mathrm{B}}T \ln p(T, \mu, d)$. In the present case, the ranges of energy and dipole values were discretized into 400 and 200 bins, respectively, and the simulation details are essentially the same as those of ref. [22]. In particular, the results for each system have been checked by performing independent runs and, in the case of the smaller peptides, by carrying out extra parallel tempering Monte Carlo simulations in the temperature range $50 \leqslant T \leqslant 1100\,\mathrm{K}$ with a geometric allocation of 23 canonical temperatures (see below).

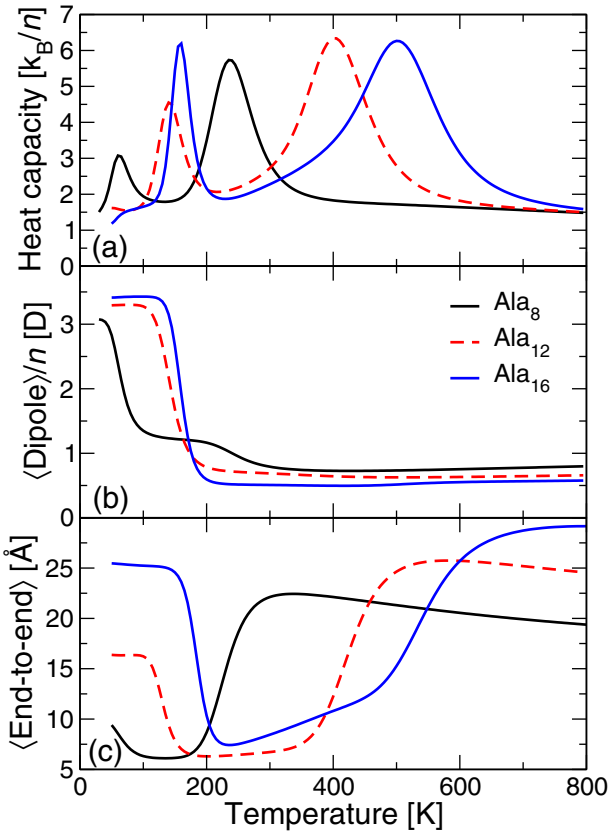

Fig. 1: (a) Heat capacity; (b) electric dipole; (c) end-to-end distance calculated, calculated as a function of temperature for the Ala_8, Ala_{12}, and Ala_{16} peptides.

Results and discussion. – Figure 1 shows the variations of the heat capacity, electric dipole and end-to-end distance for Ala_8, Ala_{12} and Ala_{16} at increasing temperature. All heat capacity curves exhibit two peaks, revealing three states. The high dipole values at low temperature indicate α helices, whereas low end-to-end distances at intermediate temperatures suggest structures folded in β sheets. At high temperatures, the peptides are preferentially found as extended random coil. One also observes strong but monotonic finite-size effects. As the number of monomers increases, both heat capacity peaks are shifted towards higher temperatures. This behavior has already been reported by Peng and coworkers for the α/coil transition [17], and is attributed to the energetic stability of α helices, which increases with the number of hydrogen bonds.

In fig. 2, the Landau free-energy maps $F(T, \mu, d)$ are represented for Ala_{12} at the two temperatures where the

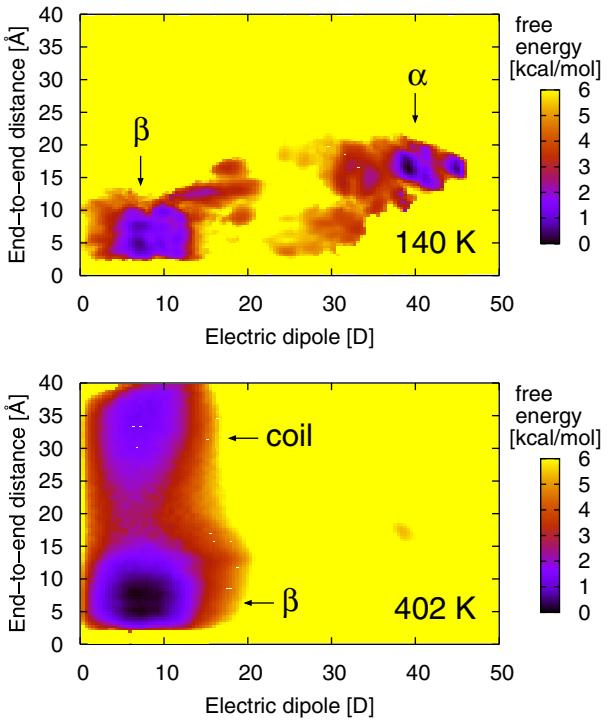

Fig. 2: Landau free-energy maps as a function of the electric dipole and end-to-end distance for the peptide Ala_{12} at 140 K (upper panel) and 402 K (lower panel).

heat capacity is maximum, namely 140 K for the α/β transition, and 402 K for the β/coil transition. The two maps display two regions of stability characterizing the α and β secondary structures, as well as the coil state. At 140 K the α and β regions are distinctively separated by a barrier exceeding 6 kcal/mol. At 402 K, the β and coil regions are broader and shallower, and the free-energy barrier is also lower, around 3 kcal/mol. These free-energy maps illustrate the competition between α helices, β sheets and random-coil structures. At very low temperatures, and at the present level of calculation, the most stable structures are pure α helices. β-sheet conformations, despite being higher in energy, are much more flexible hence more likely to be visited at higher temperature. The structural transition into β-sheet structures at moderate temperatures thus reflects a gain in entropy. The present results for gas phase polyalanines are consistent with previous simulation results on solvated peptides [18–20].

The experimental set-up built for measuring electric dipoles [7] cannot probe pure polyalanines, as they lack a chromophore required for the detection. A suitable amino acid is provided by tryptophan, which contains an indole group. We have thus investigated the $AceTrpAla_{13}NH_2$ (WA_{13}) polypeptide, protected by the acetyl and amino groups at the N-ter and C-ter extremities, respectively. This polypeptide will be compared to Ala_{16} (A_{16}), which has the same number of peptide bonds. Our measurements consist of a molecular beam deflection in a static and inhomogeneous electric field, as described in refs. [7] and [23].

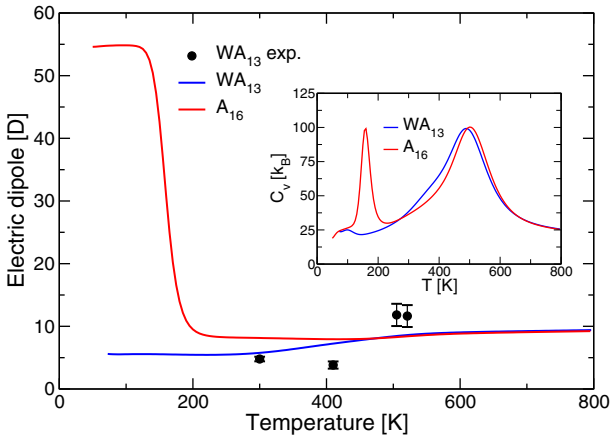

Fig. 3: Experimental (ref. [23]) and simulated electric dipole against temperature for $AceTrpAla_{13}NH_2$ (WA_{13}). The variations of the dipole of Ala_{16} are shown for comparison. Inset: canonical heat capacities of WA_{13} and A_{16} as a function of temperature.

The electric dipole measured at different temperatures is represented in fig. 3.

The results of Wang-Landau simulations for WA_{13} and A_{16} are superimposed in this figure, and the variations of the canonical heat capacities of these polypeptides are shown as an inset. For this peptide, the measured and calculated values of the dipole moment are in good agreement with each other in the range of sampled temperatures. In particular, and quite surprisingly for an alanine-rich polypeptide, neither in the experiment nor in the simulation any evidence is found for stable α helices. The heat capacities of the two molecules exhibit nearly similar variations, and a main broad peak near 500 K. The width of this folding peak is mainly due to the size of the peptide, which is comparable for WA_{13} and A_{16}. However, the additional α/β peak displayed by A_{16} is a strong manifestation of the influence of sequence on folding in such small systems.

Because the electric dipoles of extended coil and β-strand conformations lie in similar ranges, those cannot be distinguished in the experiment of ref. [23], hence the presence of extended coil structures at temperatures as low as 300 K should not be excluded. Unfortunately, no other experimental data are available at temperatures below 300 K, and it would be very interesting to extend the electric dipole measurement down to 150 K to determine whether helices become eventually stabilized. Yet, the present simulation results shed some light on a possible mechanism that is responsible for the loss of helical conformations, namely a structural transition.

The entropic stabilization of the β conformation in WA_{13} has been further investigated by performing Monte Carlo simulations of the slow cooling process from a hot, disordered coil state. More precisely, each simulation consisted in a series of MC trajectories with 10^4 cycles, the temperature being decreased linearly from 1000 K down to

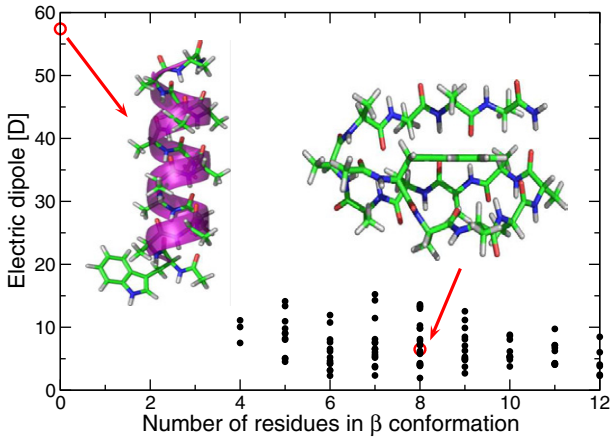

Fig. 4: Electric dipole moment and number of residues in β conformation in the 100 structures obtained after slowly cooling the WA_{13} peptide in Monte Carlo simulations from 1000 K down to 10 K. The stable conformations of the peptide $AceTrpAla_{13}NH_2$ in the helix and three-strand structure (global minimum) are also shown.

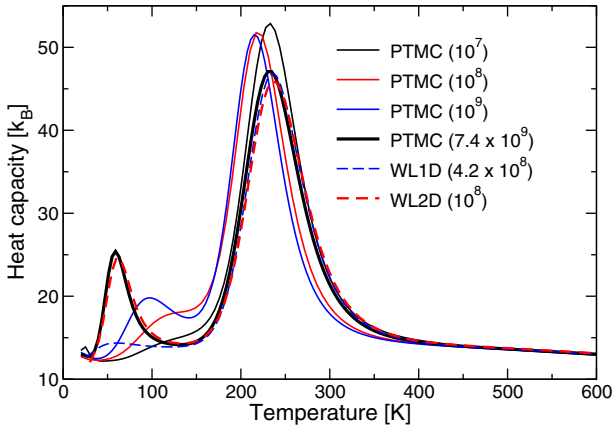

Fig. 5: Heat capacity of Ala_8 obtained with various simulation methods, including parallel tempering Monte Carlo (PTMC) simulations, Wang-Landau simulations using energy only (WL1D) or energy and the electric dipole (WL2D) as order parameters. Several PTMC curves are shown for increasing long simulations. For each curve, the method and the total number of MC cycles are indicated.

10 K with 10 K decrement (hence a total of 10^6 cycles per cooling trajectory). These simulations were repeated 100 times from independent, random starting conformations. The resulting structures are characterized by their values of the electric dipole, as well as the number N_β of residues in β contact. Here a β contact is defined according to the values of the torsion angles ϕ and ψ, which must lie in the ranges $[-180°, -110°]$ and $[+100°, +180°]$, respectively, given that they normally span the range $[-180°, 180°]$. The results of these cooling simulations are represented in fig. 4, with the value taken by the most stable helix structure ($N_\beta = 0, \mu = 57.4$ D) being highlighted.

As clearly seen in fig. 4, 100% of our cooling simulations have ended in conformations with a very low electric dipole, and a significant number of β contacts. Numerous trajectories have found a double-strand, full β structure with N_β close to its maximum possible value of 12. On the contrary, even though the helix conformation is quite close in energy to the global minimum, it was never found during the simulations. These observations provide further support for the entropic stabilization of β conformations over helices in the $AceTrpAla_{13}NH_2$ peptide.

By efficiently sampling the energy landscape of the peptides, the Wang-Landau method also acts as a global optimization method. The lowest-energy structure obtained here for WA_{13} with the Amber *ff96* force field is made of three strands with the indole group in-between, as shown in fig. 4. For this conformation, $N_\beta = 8$ and $\mu = 6.5$ D. The most stable α-helix conformation, also shown in fig. 4, lies 1.3 kcal/mol above this structure. The greater stability of the 3-strand isomer results from the favorable interactions between the backbone and side chains with the indole group. It would be interesting to check this stability at a more sophisticated computational level.

Additional calculations performed for the protected Ala_{16} peptide do not show any significantly different behavior with respect to the unprotected peptide. Therefore, by substituting a single alanine amino acid into tryptophan at the N-ter extremity, the entire energy landscape has been modified, which is reflected on the stable secondary structures inferred from both experiment and simulation.

Contrasted results can be obtained using different force fields, or different parameters sets for a given force field [27]. On the basis of generalized ensemble simulations with the ECEPP/2 force field [28], Peng and Hansmann found that Ala_{10} in gas phase undergoes a single helix/coil transition at 435 K [15]. For the present $AceTrpAla_{13}NH_2$ peptide, the α-helix structure of fig. 4 is found to be about 29 kcal/mol *lower* than the three-strand conformation when minimized with ECEPP/2, making it more stable up to high temperatures, in contradiction with the present experimental results. The more stable structure found here with Amber suggests that the tryptophan amino acid acts as an anchor around which the three β strands are stabilized. On the other hand, the parameterization of ECEPP/2 to reproduce crystal structures of biomolecules may excessively favor helices.

Beyond the model itself, the way of sampling the conformation space can be crucial as well. A basic, Metropolis-type Monte Carlo algorithm will fail in reproducing the competition between α and β structures and the associated, low-temperature structural transition. It will most often miss the intermediate β state completely, leading to a single α/coil transition. In order to illustrate the difficulties in reaching convergence, we have represented in fig. 5 the typical caloric curves obtained for Ala_8 using a variety of simulation methods, all based on the

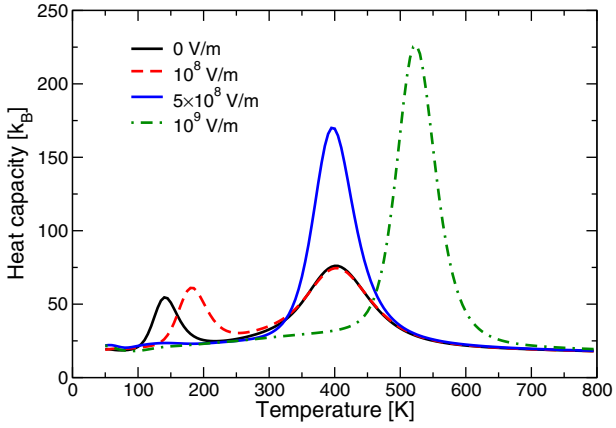

Fig. 6: Heat capacity variations of the Ala_{12} peptide against temperature, for several intensities of the electric field.

same force fields and the same individual Monte Carlo moves involving torsional angles only. In addition to the present 2D Wang-Landau method, we also show the heat capacity obtained with a conventional (1D) Wang-Landau calculation using energy only [22], as well as several curves obtained by parallel tempering Monte Carlo simulations using between 10^7 cycles and 7.4×10^9 cycles, with 23 replicas in the temperature range $50\,\mathrm{K} \leqslant T \leqslant 1100\,\mathrm{K}$.

From this figure, the standard 1D Wang-Landau simulation does not seem to reproduce the low-temperature $\alpha \leftrightarrow \beta$ transition, even with a significant number of MC steps. More surprisingly, the parallel tempering method also seems to have difficulties in converging the caloric curve of the present system. In particular, the low-temperature peak distinctly emerges only above 10^8 cycles. As more statistics are included, the peak gradually shifts to lower temperatures, a behavior which is also found in atomic clusters [29]. Eventually, after a sufficiently long simulation has been performed, we get a very satisfactory agreement between parallel tempering Monte Carlo and 2D Wang-Landau simulations. This indicates that the Wang-Landau annealing method for joint densities of states [22] with the electric dipole as order parameter is the most efficient of all the methods we tried, including parallel tempering.

Based on the above results, the stabilization of the β-sheet structures in simulation thus seems to result from a balance between an appropriate force field, which does not excessively favor α helices, and an appropriate simulation method, which is able to sample an entropy-induced structural transition. This also explains why the α/β competition has somewhat been overlooked in the past.

The high electric dipole of the helix secondary structures suggests a way to enhance their stability, by placing the peptides into an intense electric field. We have repeated the calculation of the heat capacity for the Ala_{12} peptide for various field intensities in the range 0–$10^9\,\mathrm{V/m}$. The results are shown in fig. 6. At low field, the α/β transition is slightly shifted towards higher temperatures. The β/coil peak, which only involves structures with low electric dipoles, is unaffected. As the field reaches $5 \cdot 10^8\,\mathrm{V/m}$, the β state is practically no longer stable, and the peptide undergoes a single α/coil transition. Above $5 \cdot 10^8\,\mathrm{V/m}$, the helix state is further stabilized and the coil transition itself is shifted to higher temperatures. These simulation results also provide evidence for possible field-induced conformational transitions at finite temperature. For instance, at 150–200 K, crossing $10^8\,\mathrm{V/m}$ leads to a $\beta \to \alpha$ transition, and at 450 K crossing about $7 \cdot 10^8\,\mathrm{V/m}$ leads to a coil $\to \alpha$ electrofreezing transition. Such values of the electric field are higher than those used in the experimental apparatus [7], but they are comparable to the field intensities which prevail in the inner parts of biological molecules [30].

Conclusion. – In summary, we have performed Monte Carlo simulations and electric dipole measurements to determine the stable conformations and relative stability of secondary structures in alanine-rich peptides. The Wang-Landau annealing scheme with the electric dipole as an extra order parameter was found to be particularly efficient for sampling the configuration space of such molecules, especially when compared with the standard one-dimensional Wang-Landau method, and even with parallel tempering. Pure polyalanines, in the frame of the Amber *ff96* force field with a dielectric constant chosen to correct for the large charges of the naturally hydrated peptides, are energetically lower as α helices but undergo an entropy-driven structural transition toward β-sheet conformations at intermediate temperatures, before eventually melting to the coil state. The temperature range where the β conformation is the free-energy minimum decreases with size, suggesting that the present α/β and β/coil transitions, rounded by size effects, merge into a single α/coil, first-order–like transition in large peptides. The absence of any experimental signature for α helices was interpreted as the consequence of substituting an alanine amino acid into tryptophan, consistently with the most stable conformation found in the simulation, made of three strands but with no helix content. For the experimentally studied peptide $AceTrpAla_{13}NH_2$, the Wang-Landau and additional cooling MC simulations indicate that β-strand conformations are more stable than helices not only entropically, but also energetically. Yet it should be recognized that, lacking any experimental measurement for $T < 300\,\mathrm{K}$, the possible formation of an helix at low temperatures cannot be excluded. However, according to the present work, the absence of helices does not imply that folding occurs below room temperature. The structural transition involving β conformations is compatible with a folding temperature significantly above room temperature, and our simulation predicts $T_f \simeq 500\,\mathrm{K}$.

Finally, by performing simulations of the bare polyalanines into an intense electrostatic field, we have found

helices to be stabilized thanks to their high electric dipole moment. The α/β transition is first shifted to higher temperatures, but above some value of the field the α/β and β/coil transitions merge. The remaining single α/coil transition is then further shifted to higher temperatures as the field is increased again. At intermediate temperatures, our simulations predict that changing the field may lead to electrofreezing transitions where the α-helix conformations are stabilized. These transitions will generally depend on the magnitude of the dipole, hence on the size of the peptide, and it would be useful to construct a (temperature, field) phase diagram which includes size as a variable.

The competition between the α-helix and β-sheet secondary structures could be affected by other factors, such as a solvent, of course, but also the kinetics of formation and the possible trapping of the peptide into metastable states in the energy landscape. However, we believe that the use of computer simulation will be invaluable for designing polypeptides with a strong propensity for α helices, taking experimental constraints into account.

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