Exploring the conformational dynamics of alanine dipeptide in solution subjected to an external electric field: A nonequilibrium molecular dynamics simulation

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Abstract

In this paper, we investigate the conformational dynamics of alanine dipeptide under an external electric field by nonequilibrium molecular dynamics simulation. We consider the case of a constant and of an oscillatory field. In this context we propose a procedure to implement the temperature control, which removes the irrelevant thermal effects of the field. For the constant field different time-scales are identified in the conformational, dipole moment, and orientational dynamics. Moreover, we prove that the solvent structure only marginally changes when the external field is switched on. In the case of oscillatory field, the conformational changes are shown to be as strong as in the previous case, and non-trivial nonequilibrium circular paths in the conformation space are revealed by calculating the integrated net probability fluxes.
I. INTRODUCTION

The possible effect of the electric field (EF) on the conformation of proteins is a subject of increasing attention since it is linked to the problem of exposing human tissues to electromagnetic radiation. Despite its major relevance, a satisfactory understanding of how the EF influences the behaviour of proteins has not been reached so far although a considerable number of experimental e.g. [1–5] and theoretical e.g. [6–15] studies have been devoted to this topic. In this perspective, molecular dynamics (MD) has been proven useful in understanding the behaviour of a protein in both static and oscillatory EF [6–15]. In these studies, the initial configurations are usually taken from the Protein Data Bank (PDB), and then temperature controlled simulations are performed for a maximum time range of the order of 10-60 nanoseconds. During these simulations time relevant conformational changes characterizing the solvated molecule can be observed. Along the trajectories, various observable, such as the secondary structure, root mean square displacement (RMSD), dipole moment, and shape parameters are calculated as a function of time, or in stationary condition, taking time averages. This approach releases useful information on how the secondary structure of the molecule is changed by external EF. However, the behaviour of observables in time is characterized by strong fluctuations. The entity of the fluctuations is such that a quantitative analysis becomes difficult and does not allow for a clear understanding of the rationale behind the conformational changes. A time average can reduce the statistical uncertainty, however in non-stationary conditions this would be a not-well-founded procedure. In fact, we are studying non-stationary nonequilibrium process (e.g. relaxing process to the new conformations under a static EF, or the dynamics from one conformation to the other under an oscillatory EF). In this perspective, if one wants to use MD, the proper way to do it would be that of treating a situation of nonequilibrium with algorithms that can explicitly describe it. This is the approach that will be followed in this work, using the dynamical nonequilibrium molecular dynamics simulation (D-NEMD). The method, firstly developed by one of us and coworkers [16–19], has been recently applied, to the study of hydrodynamics [20, 21]. The essential feature of D-NEMD is to provide a way of evaluating time-dependent nonequilibrium averages starting from a properly determined initial ensemble. In this context, the method can be used to analyze the conformational changes of a solvated molecule, subject to a time-dependent EF.
In the present paper, we study a small peptide, alanine dipeptide, as a model for showing how the method can be used. The choice of a small peptide, rather than a larger one, is done firstly because it is easier to draw clear conclusions on the nonequilibrium properties of a single peptide by excluding the slow-going interplay between different peptide segments along a chain. Secondly, the computational cost required is rather massive and thus testing the various methodology proposed here would be hampered for large molecules, although they remain our goal. In any case, alanine dipeptide is large enough so that the conformational changes of its internal degrees of freedom represent valid observables for a test of validity and utility of our approach. The paper is organized as follows: We firstly describe the idea of D-NEMD, and propose a technical improvement consisting in the definition and application of a local thermostating process. Next, after introducing the physical observables chosen to analyze the nonequilibrium response, the numerical results for the case of a static and oscillatory EF, respectively, are discussed. By calculating the nonequilibrium properties from D-NEMD simulations, we are able to address a few relevant questions: (i) How the conformation of the peptide changes in an EF (conformational space); (ii) When these changes happen (time scale), and how likely they are (probability). One may wonder whether the information extracted from the D-NEMD simulation could have been extracted just as well from conventional equilibrium simulations. In the appendix we show that in this case the D-NEMD approach shall be preferred to the equilibrium approach. The argument is provided by the comparison between the results of the nonequilibrium method and those from equilibrium simulations, within the linear response theory, based on the analysis of correlation functions. To our knowledge, this study is one of the few (if not the first) where time-dependent nonequilibrium w.r.t. conformation is explicitly considered for a (relatively large) molecule in solution subject to an external time-dependent EF.

II. METHODOLOGY

A. Dynamical nonequilibrium molecular dynamics simulation (D-NEMD)

In this section we briefly review the dynamical approach to nonequilibrium molecular dynamics (D-NEMD) [16,17,20,21]. In the following we denote the macroscopic observable by $O(t)$. If at $t$ the configurational probability distribution is $\rho(x,t)$, where $x$ is the phase
space variable, then the observable can be expressed by

\[ O(t) = \int d\mathbf{x} \hat{O}(\mathbf{x}) \rho(\mathbf{x}, t) = \langle \hat{O}(\mathbf{x}), \rho(\mathbf{x}, t) \rangle, \]

we refer to \( \hat{O}(\mathbf{x}) \) as the microscopic observable, which is measured at each point in the phase space \( \mathbf{x} \). Here we always assume that the initial probability distribution \( \rho(\mathbf{x}, 0) \) is known. In particular, in our case, it is identical to the equilibrium distribution of the system without EF. The bracket on the right hand side of Eq. (1) denotes, as usual, the inner product in the phase space. We assume that the dynamics of the system is governed by the Hamiltonian equation, i.e. \( \mathbf{\dot{x}} = J \cdot \nabla_x \mathcal{H}(\mathbf{x}, t) \), where \( \mathcal{H} \) is the time-dependent Hamiltonian, and \( J \) is the symplectic matrix

\[ J = \begin{pmatrix} 0 & I \\ -I & 0 \end{pmatrix}. \]

The Liouville equation for the probability distribution writes:

\[ \frac{\partial \rho(\mathbf{x}, t)}{\partial t} = -iL(t)\rho(\mathbf{x}, t), \]

where \( iL(t) = \{\cdot, \mathcal{H}\} \) is the Liouville operator. Eq. (3) can be formally solved by \( \rho(\mathbf{x}, t) = U^\dagger(t, 0)\rho(\mathbf{x}, 0) \), where \( U^\dagger(t, 0) = T \exp\{ -i \int_0^t dt' L(t') \} \), and \( T \) is the time ordering operator. On the other hand,

\[ \frac{d\hat{O}(\mathbf{x}(t))}{dt} = \nabla_x \hat{O} \cdot \mathbf{\dot{x}} = \nabla_x \hat{O} \cdot J \cdot \nabla_x \mathcal{H} = iL(t)\hat{O}(\mathbf{x}(t)). \]

This equation can be formally solved by \( \hat{O}(\mathbf{x}(t)) = U(t, 0)\hat{O}(\mathbf{x}(0)) \), therefore,

\[ O(t) = \langle \hat{O}(\mathbf{x}), \rho(\mathbf{x}, t) \rangle = \langle \hat{O}(\mathbf{x}), U^\dagger(t, 0) \rho(\mathbf{x}, 0) \rangle = \langle U(t, 0)\hat{O}(\mathbf{x}), \rho(\mathbf{x}, 0) \rangle = \langle \hat{O}(\mathbf{x}(t)), \rho(\mathbf{x}, 0) \rangle. \]

Since we assume that the system starts from the equilibrium distribution (without EF) Eq. (5) expresses the fact that the observable \( O(t) \) calculated in a situation of nonequilibrium is equal to the ensemble average of the microscopic observable computed along trajectories, starting from initial configurations sampled from an initial or, sometimes, an equilibrium distribution. In practical terms we proceed by first running an equilibrium MD simulation to generate a sample of configurations. Next we employ these configurations as initial configurations and, for each, the full Hamiltonian dynamics is integrated until time.
FIG. 1. Schematic plot of the nonequilibrium temperature control scheme. The white area is the region characterized by pure dynamics without any direct action by the external thermostat. The gray area is the region where the thermostat is used to keep the target temperature. $R_{ex}$ denotes the size of the purely dynamical region, which is a sphere around the molecule of interest (in our case it is the alanine dipeptide). The whole system is subject to periodic boundary conditions.

In this paper, these trajectories are called *branching trajectories*. Finally, the value of the macroscopic observable at time $t$ is calculated by averaging the microscopic observable measured at each time $t$ along each branching trajectory. Stochastic dynamics (e.g. Langevin dynamics) can be handled analogously.

**B. Control of temperature in nonequilibrium MD**

For this study, we are interested in nonthermal [22] effects of the EF on the configuration of the solvated alanine dipeptide. For this reason a proper control of the temperature in a nonequilibrium system becomes an important issue; in fact it was argued that an accurate control of the internal reaction temperature is essential to perform reproducible experiments [12]. A straightforward (and realistic) implementation of the temperature control consists in embedding the alanine into an infinitely large solvent environment, and the EF is only applied to the neighborhood of the alanine. In this case any extra heat generated by the EF can be effectively dispersed in the large solvent environment. In this way one can identify the effect of the EF on the conformational space of the solvated molecule, without the artifact that the surrounding solvent cannot release the additional energy of the EF. However, in practice, in most of the cases the computational cost of having large solvent
environment is rather massive if not prohibitive. Therefore, it is of interest to substitute
the infinitely large solvent environment with a smaller affordable system and at the same
time accurately reproduce the nonequilibrium physical properties of interest. In MD, for
equilibrium situations, the solution is to couple the system to an external thermostat, under
which the desired ensemble (canonical in this case) can be sampled by simulating a finite size
system with periodic boundary conditions. However, coupling the system to a thermostat
for nonequilibrium situations is a more delicate issue. In fact, since a thermostat is explicitly
designed to bring the system into thermal equilibrium, the perturbation produced by the EF
would be mostly adsorbed by the thermostat. This means that the physics of the original
dynamics in presence of the EF would be lost and the response to the perturbation would
be unphysical.

In this section we propose a procedure to solve the problem: we start observing that
the dynamics without thermostat of the alanine, and the water molecules in the closer
solvation shells, is crucial to determine the response of the observable of interest under the
EF perturbation (in this work, the observable is the conformational change of alanine).
Instead the detailed dynamics of the water molecules far away is not of primary importance:
the region beyond the first solvation shells plays mainly the role of a thermodynamic bath.
Therefore, instead of simulating an infinitely large system, we simulate our finite size system
with periodic boundary condition, and with the following characteristics: (i) The simulating
region is divided in two subregions, a spherical region around the alanine dipeptide of radius
$R_{ex}$, centered at the alpha-carbon. Here the dynamics is not subject to a thermostat. We
refer to such subregion as the dynamical region. (ii) Beyond $R_{ex}$, the dynamics of the water
molecules is coupled to a Langevin thermostat. This region is called the thermostated region.
For technical convenience we fix the position of the alpha-carbon in space, so that the alanine
is always located at the center of the simulation region (see Fig. [1]). In these conditions,
the dynamics is preserved in the dynamical region (where the properties of interest are
observed), while possible artificial effects of the thermostating process in the outside region
are negligible due to the finite correlation range of liquid water. At the same time, the
thermostated region works as a infinitely large environment that effectively absorbs the extra
heat produced by the EF. The validity of the above statements will be checked by a series
of numerical tests where it is shown that the response of observables in nonequilibrium (i.e.
under the effect of the EF) does not depend on the size of the system and on the size of the
dynamical subregion, provided that they are reasonably large. The proposed temperature control method has similarities with the stochastic boundary condition proposed by Brooks and Karplus [23]. However, differently from their approach, we do not explicitly consider a boundary region: The system is instead divided into a dynamical region (corresponding to the reaction region of [23]) and a thermostated region (corresponding to the stochastic buffer region in [23]) in a 3-D cubic periodic simulation box.

III. CASE I: ALANINE DIPEPTIDE UNDER A UNIFORM CONSTANT EF

A. System settings and simulation protocol

The system is set up in a $2.7 \times 2.7 \times 2.7$ nm$^3$ periodic simulation region, with one alanine dipeptide described by the CHARMM27 force field [24], and dissolved in 644 TIP3P [25] water molecules. The grid-based energy correction map (CMAP) [26] for the backbone dihedral angles is also used. The size of the dynamical region is $R_{ex} = 1.0$ nm. All simulations are performed by a home-modified GROMACS 4.5 [27] together with the CHARMM force field [28]. First, an equilibrium NVT simulation at 300 K of 100 ns was performed with a Langevin thermostat (time-scale $\tau_T = 0.5$ ps). Along the trajectory, configurations were taken every 50 ps and we used 2000 initial configurations for each nonequilibrium MD simulation (if not stated otherwise for specific cases). The branching trajectories were integrated by the Leap-frog scheme (standard Gromacs implementation) with the aforementioned nonequilibrium temperature control technique. The time step was 0.002 ps. The short-range interaction (van der Waals interaction) have a cut-off radius of 1.0 nm, and has been smoothed from 0.8 to 1.0 nm by the “switch” method provided by the GROMACS code. A energy conserving Particle Mesh Ewald (PME) [29, 30] method was applied to calculate the electrostatic interaction in this periodic system. For the direct space part of PME the cut-off and smoothing follow the same principles as those applied to the van der Waals interaction. All hydrogen involving bonds are constrained by LINCS [31], except the TIP3P water molecules which are constrained by the SETTLE algorithm [32]. In the thermostated region, the original dynamics was coupled to a Langevin thermostat with $\tau_T = 0.1$ ps. In all testing cases, this local thermostat is able to control the system at the desired temperature, i.e. 300 K (the results are not presented in this paper). The whole system is also coupled to
a Parrinello-Rahman barostat $^{33}$ (in standard Gromacs implementation) with $\tau_P = 2.0$ ps to keep the pressure at ambient condition (1 Bar). Since the change of the system size is small and slow, the pressure control does not have an sizable effect on the dynamics of the system. At time $t = 0$ ps, the system has been fully equilibrated without any EF. From $t = 0$ to the warm-up time $t = t_{\text{warm}}$, the EF is switched on linearly, while after $t = t_{\text{warm}}$, the field is kept constant in time at $E_\infty$. In this work we consider $t_{\text{warm}} = 10$ ps, and $E_\infty = 1$ V/nm. The direction of the field is arbitrarily chosen along the $x$ direction. In this paper we denote the EF as a function of time $E(t)$. Therefore, for the case of constant EF, we have $E(t) = (E_\infty \cdot t / t_{\text{warm}}, 0, 0)$ for $0 \leq t < t_{\text{warm}}$, and $E(t) = (E_\infty, 0, 0)$ for $t \geq t_{\text{warm}}$.

**B. Molecular conformation and net probability flux**

The change of conformation in time of the alanine dipeptide is investigated by analyzing the probability density $p(\phi, \psi, t)$ on the Ramachandran plot at time $t$. The definition of the
dihedral angles $\phi$ and $\psi$ is given in Fig. 2 (a). The probability density of conformations of equilibrium in absence of external field is shown in Fig. 2 (b), while the same quantity, resulting from the molecular relaxation to the action of $E_\infty = 1 \text{ V/nm}$ is given in Fig. 2 (c). In the plot one can identify several clusters in which conformations are grouped. Therefore, in order to simplify the analysis, we divide the Ramachandran plot into 5 subregions \{\(\alpha_R, \alpha'_R, C_{7\text{eq}}, C_5, \alpha_L\)\} (see caption of Fig. 2 for the corresponding definition). It is worth to notice that these conformations are found in different secondary structures of a peptide chain: $\alpha_R$ and $\alpha'_R$ correspond to the $\alpha$-helix. $C_{7\text{eq}}$ and $C_5$ correspond to the $\beta$-sheet. $\alpha_L$ corresponds to the left-handed $\alpha$-helix.

From Fig. 2 it is evident that the probability with which a conformation occurs changes when the external EF is applied. The most evident case is conformation $\alpha_L$; in fact it is almost not present before the external field is applied but appears in a clear way as a response to the action of the EF. Following this line of thought, we are interested to study the change of conformation under the action of EF. Specifically, for each conformational change, we will analyze the relation between the structural relaxation of the molecule and its corresponding time scale. The starting point is the calculation of the probability of each conformation:

\[
P_I(t) = P((\phi_t, \psi_t) \in I), \quad I \text{ being one of the five regions } \{\alpha_R, \alpha'_R, C_{7\text{eq}}, C_5, \alpha_L\},
\]

Notice that the probability is time dependent, being an observable in nonequilibrium situation. Next, we consider the net probability flux from conformation $J$ to $I$, defined by:

\[
F_{J,I}(t) = \frac{1}{\Delta t} \left[ P((\phi_{t-\Delta t}, \psi_{t-\Delta t}) \in J, (\phi_t, \psi_t) \in I) - P((\phi_{t-\Delta t}, \psi_{t-\Delta t}) \in I, (\phi_t, \psi_t) \in J) \right],
\]

\[
J, I \in \{\alpha_R, \alpha'_R, C_{7\text{eq}}, C_5, \alpha_L\}.
\]

Where $P((\phi_{t-\Delta t}, \psi_{t-\Delta t}) \in J, (\phi_t, \psi_t) \in I)$ is the joint probability of the alanine being in conformation $J$ at time $(t - \Delta t)$ and being in conformation $I$ at time $t$. The same definition applies to $P((\phi_{t-\Delta t}, \psi_{t-\Delta t}) \in I, (\phi_t, \psi_t) \in J)$. Here the time interval $\Delta t$ should be small enough compared to the time scale of conformational dynamics, so that the changes in the conformation probabilities can be treated in linear approximation. At the same time, $\Delta t$ should be also large enough compared with the time step of the MD integrator, so that the quantities of Eq. (7) are well-defined, and can be estimated with sufficient numerical accuracy (see also Ref. [34]). In all the numerical examples of this work, $\Delta t = 1 \text{ ps}$ is used.
A positive value of $F_{J,I}(t)$ indicates that the net flux goes from $J$ to $I$, while a negative value indicates a net flux from $I$ to $J$. Through $F_{J,I}(t)$, the analysis of the nonequilibrium process is projected onto the analysis of the probabilistic link between discretized conformations \{α_R, α'_R, C7_{eq}, C5, α_L\}. The concept of net probability flux employed by us is very close to the concept of kinetic rate. However, we prefer to stick to our definition which is mathematically simpler and univocal. In the case of oscillatory EF, the behaviour of the net probability flux is also highly oscillatory, thus, in order to clearly identify the essential features of the molecular conformational changes, it is convenient to study the integrated net probability flux, defined as:

$$Q_{J,I}(t) = \int_0^t F_{J,I}(\tau)d\tau,$$

which expresses the cumulative effects due to the action of EF on the conformations of the molecule.

C. Conformational dynamics

The time-dependent probability density of the system to stay in a certain conformation is given in Fig. 3. In order to show that our simulation results are robust with respect to the randomness introduced by the (local) Langevin thermostat, we have tested the effects of changing random seeds for the thermostat in the thermostated region. We compared simulation results obtained by starting a branching trajectory from each initial conformation with those obtained from four different branching trajectories from each initial conformation (each of four with different random seed for the Langevin thermostat in the thermostated region). Results are consistent within the statistical uncertainty (see Fig. 3). The β-sheet conformations C7_{eq} and C5, which are present at the initial stage after EF is switched on, fade away as the system relaxes. The probability of the α-helix conformation α_R, grows from 0.45 to 0.69. The α-helix α'_R does not change significantly under the action of EF. The left-hand helix conformation α_L noticeably grows from 0 to 0.22. We plot the net probability fluxes of the four-branching-trajectories case in Fig. 4. Results show that for short $t$, the net fluxes are generally non-zero, however, as $t$ goes to infinity, all fluxes converge to zero. This indicates that as the EF is switched on, the system is driven away from the initial equilibrium (at zero EF), starting a dynamical nonequilibrium process. After sufficiently
FIG. 3. The time-dependent probability of conformations $P_I(t)$ when a constant EF is present, where $\sum_I P_I(t) = 1$, $\forall t$. The warm-up time is $t_{\text{warm}} = 10$ ps. The red line refers to conformation $I = \alpha_R$, green to $\alpha'_R$, dark blue to $C_7_{\text{eq}}$, purple to $C_5$ and light blue to $\alpha_L$. The solid lines denote the results of one branching trajectory starting from each initial conformation, while the dashed lines denote the average of the four branching trajectories (different random seeds for the Langevin thermostat, see text) starting from each initial conformation. The shadowed region with each solid line denotes the statistical uncertainty of the result at 95% confidence level.

long time, the system is fully relaxed to the new stationary state, in which it remains as long as the EF is switched on. Of course we cannot exclude very slow nonequilibrium processes, which cannot be captured by the duration of our nonequilibrium simulation (1 ns), since it is obviously that the proposed nonequilibrium approach can only study time-dependent behaviours that are shorter than the total time of the branching trajectories. An important point is that despite the system is finally relaxed to the new conformation, the relaxation timescales corresponding to each conformation are rather different. From Fig. 3 and 4 we observe mainly three different timescales 10 ps, 100 ps and 500 ps, whose corresponding conformations are summarized in Tab. 1. This is a relevant result because it shows the possibility of employing an external EF as a tool to identify relevant time scales in the conformational behaviour of a molecule in solution.
FIG. 4. The net probability flux for the constant EF case. The warm-up time is $t_{\text{warm}} = 10$ ps. The probability flux $F_{J,I}(t)$ (defined by Eqn. (7)) is reported in units of ps$^{-1}$. From top, left to right we report $I = \alpha_R, \alpha'_R, C7_{eq}$; bottom, left to right $C5$ and $\alpha_L$, respectively. In each plot, the red line stands for $J = \alpha_R$, green for $J = \alpha'_R$, dark blue for $J = C7_{eq}$, purple for $J = C5$ and light blue for $J = \alpha_L$. This plot is drawn from the four-branching-trajectories case (see the text).

| Direction | time scale [ps] |
|-----------|-----------------|
| $\alpha_R \rightarrow \alpha'_R$ | $\sim 10$ |
| $C5 \rightarrow \alpha_R, \alpha'_R, \alpha_L$ | $\sim 50$ |
| $\alpha'_R \rightarrow \alpha_R$ | $\sim 100$ |
| $C5 \rightarrow C7_{eq}$ | $\sim 100$ |
| $C7_{eq} \rightarrow \alpha_R, \alpha_L$ | $\sim 500$ |

TABLE I. A list of the main probability fluxes and the corresponding time scales observed in the constant EF case.

D. Molecular dipole response

The fact that our external perturbation corresponds to the action of an electric field, naturally leads to the question of the alignment of the molecular dipole vector along the direction of external EF, and, in turn, of how this is related to the overall conformational change of the molecule as reported in the previous section.
The dipole moment of the alanine dipeptide is defined by

$$\mu_{\text{alanine}}(t) = \left\langle \sum_{i \in \{\text{point charges of alanine}\}} q_i \mathbf{r}_i(t) \right\rangle,$$

(9)

where $q_i$ denotes the partial charge of any point charge $i$ defining our model of alanine, the molecule being neutral, i.e. $\sum q_i = 0$. $\mathbf{r}_i$ denotes the position of the point $i$. Fig. 5 shows that the $x$-component of the dipole moment reaches 85% of its maximum value in only 20 ps, which is comparable to the warm-up time $t_{\text{warm}}$, and is 25 times smaller than the slowest time scale of the conformational relaxation. Then in the following 400 ps, the dipole slowly relaxes to its maximum value, that is, 6.8 Debye, and corresponds to an energy $(\mu_{\text{alanine}} \cdot E)$ of c.a. $-13.6$ kJ/mol. We calculated the averaged dipole moment of different conformations (defined by taking averages in Eqn. (9) only for given conformations) under constant EF, and found 6.8, 6.0, 5.1, 3.1 and 7.1 Debye for $\alpha_R$, $\alpha'_R$, $C_7_{\text{eq}}$, C5 and $\alpha_L$, respectively. The dipole energy difference between 3.1 and 7.1 Debye is roughly 8.0 kJ/mol. Under a constant EF, the system will be likely to be driven towards those conformations with higher dipole moment, because the energy of the system will be lowered. We suggest that this energy difference may be the reason why we observe that the $\beta$-sheet conformations with lower dipole moment are driven towards $\alpha$-helix conformations $\alpha_R$ and $\alpha_L$, whose dipole moments are the highest among all conformations. Moreover, the C5 conformation vanishes because its dipole is significantly lower than other conformations. Comparing Fig. 5 with 3, the sharp
increment of dipole moment before 20 ps is due to the quick vanishing of conformation C5, while the slow increment of dipole moment until 400 ps is due to the slow migration from conformation C7_{eq} to \( \alpha_{R} \) and \( \alpha_{L} \).

E. Response of the orientational order parameter

As a complementary information to the behaviour of the molecular dipole moment it is of interest to describe the overall orientational behaviour of the molecules w.r.t. the EF. In fact while the dipole moment specifically expresses the rearrangement of charges within the molecule as a response to the EF, the overall direction of the molecule tells us about the interplay between the internal positional rearrangement of the atoms and their alignment w.r.t. the EF. To this aim, let us define the geometric direction of the alanine by the red vector in the inset in Fig. 5, i.e. by the angle bisector of the two black vectors, which connect the \( \alpha \)-carbon and the carbons on the methyl groups. We considered the angle \( \theta \) made by the orientation of the alanine dipeptide in space w.r.t. the direction of EF as a function of time. Then we defined an orientational order parameter

\[
S_{\theta} = \langle 3 \sin^{2} \theta - 2 \rangle. \tag{10}
\]

The ensemble average is made along the branching trajectories, so it is a time dependent observable. The order parameter indicates the (time dependent) average orientation of the molecule w.r.t. the direction of the EF. If the molecule is perfectly perpendicular to the EF, then \( S_{\theta} = 1 \), if is perfectly parallel then \( S_{\theta} = -2 \), if it has no directional preference, then \( S_{\theta} = 0 \). From \( t = 0 \) to roughly 10 ps, the orientational order parameter rapidly decreases from 0 to \(-0.15\), which means a weak alignment of the molecule to the external field. Then from \( t = 10 \) to 500 ps, the molecule slowly changes to the orientation that is almost perpendicular to the EF. In fact, the vector of the geometric direction tends to be perpendicular to the dipole moment, if one observes the correlation between the value of the dipole moment (which tends to be more parallel to the EF as its value increases) and the angle of the vector of the geometric direction, the result above can be easily explained. This orientational change is linked to the observed slowest time scale of the molecular conformational change.
FIG. 6. The solvent structure around the alanine dipeptide. (a): the radial distribution function (RDF) between the α-carbon and the center-of-mass (COM) of the water molecules at $t = 0, 10, 100$ and $1000$ ps. (b): the number of hydrogen bonds formed between the alanine and water, and between two water molecules. The number of hydrogen bonds between two water molecules is normalized by the number of water molecules in the system.

F. Effects of EF on the solvent around the molecule

So far we have focused our attention on the behaviour of the alanine under the action of the EF. However, it is also important to understand the effects of the EF on the solvating water molecules. Being water a polar molecule the electrostatic interaction with an EF can dramatically change its solvation structure around the alanine and thus, in turn, influence its conformational behaviour. For this reason, in this section we analyze the behaviour of the solvent around the alanine under the action of the EF by investigating the radial distribution function (RDF) between α-carbon and the center-of-mass (COM) of the water molecules (see Fig. 6 (a)). The average RDF does not change significantly as a function of time (within the statistical error). Fig. 6 (b) presents the number of hydrogen bonds between the alanine and water molecules, and between two neighboring water molecules. The number of hydrogen bonds are calculated by standard GROMACS routine g hbond, with donor-acceptor distance cut-off 0.3 nm, and hydrogen-donor-acceptor angle cut-off 20°. We observe that the number of hydrogen bonds remains almost the same when the EF is turned on, although molecular dipoles tend to align along the direction of the EF (result not shown). The EF-induced solvent effect on the alanine can be also accounted by calculating the difference of the water-alanine electrostatic interaction without and with the EF. The
energy difference is found to be $-19.1 \text{ kJ/mol}$ that is comparable to the electrostatic energy associated to the dipole of the alanine itself. Therefore, we conclude that the solvation structure around the alanine does not change or contribute significantly in determining the conformational dynamics of the alanine under the action of EF.

G. The finite-size effect on the dynamical and thermostated regions

Just as described in Sec. II B, we performed nonequilibrium MD simulations in a finite size periodic system, further divided into a dynamical and a thermostated region. Since the periodic boundary conditions and the division of the system are technical approximations to the real system with dynamics-preserving thermal control, the effect of the finite-size in the system settings should be carefully checked. Therefore, we perform two additional simulations: one with box dimensions $L = 4.0 \text{ nm}$ (the box is cubic) and a Hamiltonian dynamical region of radius $R_{ex} = 1.0 \text{ nm}$. A second simulation is done with box size $L = 4.0 \text{ nm}$ and a dynamical region of radius $R_{ex} = 1.5 \text{ nm}$. We compare the results of these two simulations to those of the system we have used so far, i.e. $L = 2.7 \text{ nm}$ and $R_{ex} = 1.0 \text{ nm}$. Fig. 7 shows essential consistency in the simulation results for the three systems. The finite-size effects are negligible.
FIG. 8. Illustration of the effect of the oscillatory EF on the system: (a) the dipole moment and (b) the orientational order parameter of alanine dipeptide as a function of time. In (a), only the $x$-component of the dipole moment is shown. The black line refers to the static EF which is turned on at time $t_{\text{warm}} = 10$ ps. The red line refers to the oscillatory field with period of 10 ps. The green line is of 40 ps; and the blue of 200 ps.

IV. CASE II: PERIODICALLY OSCILLATORY EF

A step forward in our study is to consider a periodically oscillatory EF which has a sin-wave shape:

$$E(t) = (E_0 \sin(2\pi t/T_P), 0, 0),$$

(11)

where $E_0$ is the intensity of the field, which is chosen to be 1.0 V/nm. $T_P$ is the oscillating period. Here we tested three different periods 10, 40 and 200 ps. Fig. 8 (a) presents the $x$-component of the molecular dipole moment as a function of time. The red, green and blue lines report the results of $T_P = 10$ ps, 40 ps and 200 ps, respectively. The black line shows the dipole moment under constant EF for reference. It shows that the oscillating period of the molecular dipole moment is the same as the period of the oscillatory EF. Therefore, the molecular dipole moment is able to respond to the external EF almost immediately. At $T_P = 200$ ps, the maximum magnitude of the molecular dipole moment is close to the value obtained in the case of constant EF, which means that the variation of the oscillatory EF is so slow that the alanine has enough time to relax its dipole. However, for $T_P = 10$ and 40 ps, the EF oscillates faster and the molecule does not have enough time to fully relax the dipole, as a consequence the maximum dipole moment is smaller than the previous case. Fig. 8 (b)
FIG. 9. The probability of being in a given of conformation for alanine in a periodically oscillatory EF. Different periods, i.e. $T_P = 10$ ps, 40 ps and 200 ps, are considered here. The probability of being in a certain conformation $I$, which is denoted by $P_I$ in this paper, is plotted by colored lines against time. Red line: $I = \alpha_R$, green line: $I = \alpha'_R$, dark blue line: $I = C7_{eq}$, pink line: $I = C5$ and light blue line: $I = \alpha_L$. For $T_P = 10$ ps and 40 ps, the nonequilibrium simulations last 1000 ps, while for $T_P = 200$ ps, they last 6400 ps.

presents the orientational order parameter. The notations are the same as Fig 8 (a). For all cases the order parameter is much smaller than in the case of constant EF. One possible reason is that the relaxation of the order parameter is very slow, and the molecule in the case of oscillatory field is not exposed to a strong enough EF for a time long enough. For $T_P = 10$ and 40 ps, the orientation of the alanine shows a very weak tendency to be parallel to the EF. It is worth to notice that for constant EF, we also observe a quick alignment of the molecular orientation vector to the EF on the time scale of 10 ps. For $T_P = 200$ ps, the molecule tends periodically to be more perpendicular to the EF, but this directional preference is much weaker than in the case of constant EF.

Fig. 9 shows the probability of the conformations against time for periodically oscillatory EF, while Fig. 10 presents the integrated net probability fluxes (defined by Eqn. (8)) between conformations. Here the probability fluxes are not reported because the profiles are highly oscillating and they would not offer a better information than the one that can be obtained from the integrated probability flux. Coming back to Fig. 9, for all periods investigated, the observed time-dependent probabilities in $\alpha_R$, $\alpha'_R$, $C7_{eq}$ and $C5$ are highly oscillating and the average value over time cycles does not change considerably w.r.t. time. However, the probability in conformation $\alpha_L$ significantly increases to approximately 0.17 for $T_P = 10$ ps, 0.27 for $T_P = 40$ ps, and 0.26 for $T_P = 200$ ps. In the case of $T_P = 10$ and 40 ps, the probability of conformation $\alpha_L$ reaches the steady value in around 300 ps, while it requires
FIG. 10. Integrated probability flux $Q_{J,I}(t)$ for the periodically oscillatory EF, is plotted against time (horizontal axis, in picoseconds). The integrated probability flux is defined by $Q_{J,I}(t) = \int_0^t F_{J,I}(\tau) d\tau$, where $F_{J,I}(t)$ is the net probability flux from conformation $J$ to $I$. See also Eqns. (7) and (8) for definitions of $F_{J,I}(t)$ and $Q_{J,I}(t)$, respectively. From top to bottom, rows report results with period 10, 40 and 200 ps, respectively. From left to right, the five columns show the integrated flux $Q_{J,\alpha_R}$, $Q_{J,\alpha'_R}$, $Q_{J,C7_{eq}}$, $Q_{J,C5}$ and $Q_{J,\alpha_L}$, respectively. In each plot, the red line stands for $J = \alpha_R$, green for $J = \alpha'_R$, dark blue for $J = C7_{eq}$, purple for $J = C5$ and light blue for $J = \alpha_L$. An increasing value of $Q_{J,I}$ indicates a net probability flux from conformation $J$ to $I$, while a decreasing value of $Q_{J,I}$ indicates a net probability flux from $I$ to $J$.

about 1200 ps for the $T_P = 200$ ps case. The notable increment of probability of $\alpha_R$ and the vanishing of $C7_{eq}$ in the constant EF case (see Fig. 3) are not observed in the oscillatory EF case. In comparison, the periodic EF case, where the probability flux vanishes when the system reaches steady state (Fig. 4), Fig. 10 shows increasing integrated probability net fluxes, which imply some lasting and directional fluxes among the conformations in steady state. Fig. 11 presents a schematic plot of the main probability fluxes for $T_P = 10$ ps. The thickness of the arrows and the numbers nearby indicate the steady value of average net fluxes over periods. The relevance of Fig. 11 is in the fact that it suggests, in perspective, a general scenario common to all molecules characterized by $\beta$-sheet-like or $\alpha$-helix-like conformations and how an electric field is likely to modify it. Although the network presented in Fig. 11 is similar to those discovered in the equilibrium studies in the sense of the steady conformations and connections among them (e.g. in Ref. 35, 36), the physical meaning
FIG. 11. Schematic plot of the main probability fluxes between conformations for oscillatory EF, $T_P = 10$ ps. The thickness of the arrows approximately presents the strength of the flux. The numbers near the arrows indicates the strength of the averaged probability flux, the unit of which is $10^{-3} \times \text{ps}^{-1}$.

is quite different in our study: The system is under nonequilibrium conditions due to a controlled physical external perturbation, and the related net probability fluxes are based on trajectories generated by such a perturbation. In contrast, in the equilibrium studies, such net probability fluxes do not explicitly exist. The probability flux of $T_P = 40$ ps is quantitatively comparable to the case $T_P = 10$ ps, except that the probability fluxes going into conformation $\alpha_L$ is stronger, which actually results in a higher steady probability in $\alpha_L$. The probability flux of case $T_P = 200$ ps is qualitatively similar to $T_P = 10$ ps and $T_P = 40$ ps, however, the strength is much lower than the latter two cases. Moreover, we see that the integrated fluxes reach a steadily increasing stage after about 3200 ps, which is even longer than the time scale at which the probability of $\alpha_L$ reaches its steady value. This indicates a longer intrinsic time scale in the $T_P = 200$ ps case.

We have not studied periods longer than $T_P = 200$ ps, because the trend suggests that a longer period indicates even longer intrinsic time scales. However, the long-period-limit case can be safely guessed: When the EF changes so slowly that at each time the system can be viewed as in equilibrium, the process falls in the category of a quasi-equilibrium process.
V. DISCUSSION AND CONCLUSION

We have investigated the conformational dynamics of a solvated alanine dipeptide under the action of a constant and oscillatory electric field (EF). We have employed the dynamical nonequilibrium molecular dynamics (D-NEMD) method. This allowed us to analyze the conformational changes of the molecule in terms of a response to an external perturbation which drives the system away from equilibrium. From the technical point of view, we have proposed a local thermostating procedure which does not introduce invalidating artifacts and at the same time avoids the necessity of considering large water bulk systems to solvate the molecule. The conformations of the alanine dipeptide are firstly projected onto the Ramachandran plot, and then grouped in 5 conformations. These conformations correspond to different secondary structures in larger molecules (i.e. proteins). Next, the time-dependent probability of being in a certain conformation and the net probability flux between them are calculated. We have compared the case of constant EF and that of oscillatory EF and reported the main differences. Worth to notice is the possibility of employing an EF in order to identify several time scales related to conformational changes, this would not be straightforward for standard equilibrium MD simulations and thus suggest that D-NEMD proposed here is a powerful tools to investigate the conformational properties of a large molecule in solution.

The intensity of the EF used in this paper is 1 V/nm for both constant and oscillatory cases. This intensity is 4-5 orders of magnitude higher than that reachable in a standard laboratory microwave instrument [12]. However, this intensity is achievable by a sharp electric field emitter tip [37], near the surface of mica [13], or by a modern laser equipment [35]. The minimal frequency investigated here (5.00 GHz, i.e. $T_P = 200$ ps) is of the same order of magnitude as the microwave radiation available in laboratory (2.45 GHz) [12]. The highest frequency (100 GHz, i.e. $T_P = 10$ ps) is one order of magnitude lower than the tetrahertz spectroscopy experiments [39, 40], so our results cannot be directly connected to these experimental results. The authors are fully aware that the EFs investigated in the present paper are, at this stage, still ideal, above all regarding their spatially homogeneity and the fact that the time-dependency is always well defined (as pointed out by previous simulation studies [6-15]). However, the present work is an attempt to understand how the use of D-NEMD as a simulation tool can be applied to the important case of an EF and which
information one may extract that is not easily accessible to other MD procedures (e.g. EF induced conformational changes and its related time scales which opens the possibility of conformational manipulation of the protein by applying an external EF.)

In order to sharply define the advantages and limitations of the approach used we must also clarify that in order to describe the system, we use a classical force field with TIP3P water model. The EF is well known to be able to protonate the water molecule at the intensity we used in the paper. However, this happens at the time scale of femtoseconds, which is much shorter than the slowest time scale resolved by the current research, so on average it should not play an essential role on the conformational dynamics. The polarization w.r.t. the external EF also does not play an important role, because under the EF of 1 V/nm, the induced dipole of water is only c.a. 0.05 Debye that is negligible comparing with the dipole moment response observed in our simulations. Therefore, it is reasonable to use a non-polarizable model in this study. The reason we prefer a rigid water model is that it is computationally more efficient than any polarizable water model. We also want to remind the reader that it has been reported recently that the physical scale of the slowest dynamics differs in a considerable way between different force fields [41]. However, the main qualitative conclusions, for example those concerning the determination of time scales or the map of conformational changes, are not likely to change significantly as a function of the force field.

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Appendix A: Comparison with the linear response theory

A commonly used equilibrium approach that can describe the nonequilibrium response is the linear response theory (Green-Kubo relation [42, 43]). We employed such a method to
test the conformational dynamics of alanine dipeptide under the EF. The response function, for example the time-dependent probability of left-handed $\alpha$-helix, is defined as

$$P_{\alpha L}(t) = \langle \chi_{\alpha L}(t) \rangle = \langle \chi_{\alpha L} \rangle_0 - \beta \int_0^t ds \ M(t-s) \langle j(0) \cdot \chi_{\alpha L}(t) \rangle_0, \quad (A1)$$

where $\chi_{\alpha L}$ is the characteristic function of set $\alpha_L$ that takes the value of 1 for $(\phi, \psi) \in \alpha_L$, and takes value 0 otherwise. $M(t)$ is the relative magnitude of the EF. For the case of constant EF, for example, $M(t) = t/t_{\text{warm}}$ for $0 \leq t < t_{\text{warm}}$, and $M(t) = 1$ for $t \geq t_{\text{warm}}$. $j$ is the dissipative flux that is defined by

$$j = - \sum_{i=1}^N E_{\infty} q_i v_{i,x}, \quad (A2)$$

where $q_i$ is the partial charge of the $i$th atom, and $v_{i,x}$ is the $x$ component of the velocity of the $i$th atom. The notation $\langle \cdot \rangle_0$ denotes the equilibrium ensemble average. We report the correlation function $\langle j(0) \cdot \chi_{\alpha L}(t) \rangle_0$ as a function of correlation time $t$ in Fig. 12(a). The value of the function is estimated from two independent 1 $\mu$s equilibrium simulations. It must be noticed that the total length of the equilibrium trajectories is the same as the nonequilibrium simulation (2000 trajectories, each 1000 ps long). Results show that the statistical error is much larger than the value of correlation function itself. In Fig. 12(b), the probability of conformation $\alpha_L$ calculated from the linear response theory (A1) is compared with that computed from D-NEMD (1). The linear response result follows the D-NEMD result only in the very first 15 ps, then it diverges. Since it is meaningless for a probability being larger than 1, the linear response result is qualitatively wrong. Therefore, the overwhelming statistical uncertainty suggest to prefer D-NEMD Eq. (1) to calculate the nonequilibrium averages. In fact, since the total computational effort of the equilibrium simulation is the same as the nonequilibrium simulation, the nonequilibrium simulation is more accurate than the conventional equilibrium approach (linear response) at the same computational cost. A similar observation has already been reported in Ref. [16].

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FIG. 12. The numerical result of the linear response theory. (a): The correlation function <j(0)·χαLT>0 against time t. The error bars in the figure denote the statistical uncertainty at 95% confidence level. (b): The probability of conformation αL computed from the linear response theory (A1) versus that computed from D-NEMD (I).

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