Dominant folding pathways of a peptide chain, from \textit{ab-initio} quantum-mechanical simulations

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Using the Dominant Reaction Pathways (DRP) method, we perform an \textit{ab-initio} quantum-mechanical simulation of a conformational transition of a peptide chain. The method we propose makes it possible to investigate the out-of-equilibrium dynamics of these systems, without resorting to an empirical representation of the molecular force field. It also allows to study rare transitions involving rearrangements in the electronic structure. By comparing the results of the \textit{ab-initio} simulation with those obtained employing a standard force field, we discuss its capability to describe the non-equilibrium dynamics of conformational transitions.

\section*{INTRODUCTION}

The theoretical investigation of conformational transitions of polypeptide chains is usually performed using techniques like molecular dynamics (MD), Monte Carlo, transition path sampling based on classical Molecular Mechanics (MM) approaches\cite{1}. MM methods are computationally very efficient, thus making simulations of molecules with thousands of atoms feasible on modern computer clusters\cite{2, 3}. In many cases the outcome of the simulations compares favorably with experimental results\cite{4}.

MM methods are based on an empirical representation of the potential energy function of molecules (the so-called force field) which relies on a chemical model of the bonding fitted on quantum calculations and experimental results. A force field is defined by the functional form of the different components that make it up, and by the values of a set of parameters appearing in the components. The parameters are usually determined based on the equilibrium configuration of molecules. This approach is acceptable when the focus is on small thermal oscillations, but may become inadequate when the system undergoes out-of-equilibrium transitions.

Another limitation of the MM approach arises when the transition involves a rearrangement of the electronic structure, as is the case for the cleavage or formation of chemical bonds, like for instance a sulphur bridge.

All these problems could in principle be solved by adopting a quantum mechanical approach to the dynamics of the molecule. Given the formidable complexity of a full quantum description, two approximations are usually invoked in \textit{ab-initio} simulations: the Born-Oppenheimer separation of the dynamics of nuclei and electrons\cite{5}, and a classical treatment of nuclear degrees of freedom.

However, for molecular systems the size of polypeptide chains, the quantum calculation of the molecular energy of a conformation is computationally quite expensive. As a result, the \textit{ab-initio} quantum-mechanical approach is usually adopted to infer static properties or to study the dynamics over very short time intervals, typically up to hundreds of picoseconds \cite{6}. On the other hand, the time scales involved in the conformational transitions of polypeptide chains range from nanoseconds —for the rotations around dihedral angles— to milliseconds or even seconds —for the formation of tertiary structures in proteins—.

In this work, we show that the DRP formalism \cite{7, 12} provides a rigorous and computationally efficient framework which makes it feasible to investigate \textit{ab-initio} the folding dynamics of peptide chains.

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The DRP approach is a method which yields the statistically most significant reaction pathways, in systems described by the over-damped Langevin equation. Its computational advantage resides in the fact that it does not waste CPU time in simulating thermal oscillations in (meta)-stable states visited during the reaction and that the sampling of the transition pathways is performed at constant spatial displacement steps, rather than at constant time steps. The reliability of the DRP approach when applied to investigating thermally activated conformational reactions of peptide chains within an MM framework has been tested in a series of works based on both atomistic[10] and coarse-grained models [11,12]. In particular, in [12] the folding trajectories obtained by means of Molecular Dynamics (MD) simulations were compared directly with those calculated in the DRP approach, and the two methods were found to give consistent results.

In [13] the DRP formalism was applied to perform an ab-initio calculation of the dominant pathways in the cyclobutene → butadiene transition, a thermally activated reaction which involves the breaking and formation of covalent bonds. In this approach, the electronic structure and the molecular energy of the chain was determined at each step of the calculation, by approximatively solving the Schrödinger equation.

In the present work, we use the same approach to compute ab-initio the dominant reaction pathway for a conformational transition of tetra-alanine, leading to the formation of the elementary unit of a helix — see Fig. 2—.

Our first goal is to show that by using the DRP formalism the ab-initio simulation of the reaction can be performed at a relatively low computational cost. Indeed, the calculation of the most probable pathway connecting a single initial and final configuration required about 23,000 CPU hours.

A second goal of the present work is to address the question whether force fields which are fitted on ab-initio calculations of equilibrium properties also agree with quantum mechanical calculations in non-equilibrium conditions. To this goal, we compare the dominant classical reaction pathways obtained ab-initio with those calculated with the AMBER–99 force field [4]. We find that the classical approximation describes with reasonable accuracy the dynamics also in the transition region, producing trajectories which are in semi-quantitative agreement with those obtained ab-initio.

Finally, as an example of an observable which cannot be computed from classical MM simulations, we analyze the evolution of the partial charges of the atoms which are involved in the hydrogen bonds in the helix configuration.

MODEL

The ab-initio dominant reaction pathways approach

We consider a generic molecule consisting of $N$ atoms with nuclear coordinates $X = (x_1, \ldots, x_N)$, in contact with a thermal-bath at temperature $T$. The atomic nuclei are assumed to be classical point-like particles, evolving according to Langevin dynamics. The electrons are coupled quantum mechanically to the nuclear positions, in the Born–Oppenheimer approximation, i.e. their wave-function is assumed to instantaneously relax to the ground state, for each nuclear configuration $X$.

On time-scales larger than a ps the dynamics of atoms in proteins is well described by the over-damped limit of the Langevin equation

$$
\dot{X} = -\frac{D}{k_B T} \nabla U(X) + \eta(t).
$$

In this Eq., $k_B$ is the Boltzmann constant, $T$ is the temperature and $D$ is the diffusion coefficient, which we shall assume to be the same for all atoms (the generalization to the case in which each atom has a different diffusion coefficient is straightforward). $U(X)$ is the molecular energy for system with the nuclei in configuration $X$. In MM simulations this quantity is given by the force field. In ab-initio simulations, $U(X)$ is the sum of the ground-state energy of the electron wave-function and of the electrostatic energy of the classical nuclei.

In Eq. (1), $\eta(t)$ is a random noise with Gaussian distribution, zero average and correlation given by

$$
\langle \eta_i(t)\eta_j(t') \rangle = 2D \delta_{ij} \delta(t - t'),
$$

where $i,j$ label all the nuclear degrees of freedom.

Let us now consider the probability for the molecule to be found in the configuration $X_f$ at time $t_f$, provided it was prepared in some initial configuration $X_i$ at time $t_i$. Such a conditional probability can be represented in the following path integral form —for the details of the derivation see e.g. [3]—:

$$
P(X_f,t_f|X_i,t_i) = e^{-\frac{U(X_f) - U(X_i)}{k_B T}} \int_{X_i}^{X_f} \mathcal{D}X(\tau) \ e^{-S_{eff}[X(\tau)]},
$$

where $S_{eff}$ is the effective action

$$
S_{eff}[X(\tau)] = \frac{1}{2} \int_{t_i}^{t_f} dt \left( D \left( \frac{\dot{X}}{k_B T} - \frac{\nabla U(X)}{k_B T} \right)^2 + k_B T \frac{\eta^2}{2} \right).
$$

Finally, as an example of an observable which cannot be computed from classical MM simulations, we analyze the evolution of the partial charges of the atoms which are involved in the hydrogen bonds in the helix configuration.
where $S_{ eff}[X(\tau)]$ is called the effective action functional calculated on the trajectory $X(\tau)$ and is given by

$$S_{ eff}[X(\tau)] = \int_{t_i}^{t_f} d\tau \left( \frac{1}{4D} \dot{X}^2(\tau) + V_{ eff}[X(\tau)] \right).$$

(3)

$V_{ eff}(X)$ is called the effective potential, and reads

$$V_{ eff}(X) = \frac{D}{4(k_BT)^2} \left[ \left| \nabla U(X) \right|^2 - 2 \ k_BT \ \nabla^2 U(X) \right].$$

(4)

Notice that, if $X_f$ and $X_i$ are product and reactant configurations respectively, then the factor $\exp(-S_{ eff}[X])$ inside the path integral expression \([2]\) represents the statistical weight of a given reactive trajectory $X(t)$.

The most probable (or dominant) reaction pathways are those which minimize the effective action functional $S_{ eff}[X]$. Hence, they are the solutions of the classical equations of motion generated by the effective action, i.e.

$$\ddot{X} = 2 \ D \ \nabla V_{ eff}(X),$$

(5)

with boundary conditions $X(t_i) = X_i$ and $X(t_f) = X_f$.

Note that the dynamics described by the equation of motion \([5]\) conserves the effective energy $E_{ eff} = \frac{1}{2D} \dot{X}^2(t) - V_{ eff}(X(t))$. This property allows to switch from the time-dependent Newtonian description to the equivalent energy-dependent Hamilton–Jacobi (HJ) description. In the HJ framework, the most probable pathways connecting the given initial and final configurations can be shown to be those which minimize the target HJ functional

$$S_{ HJ}[X] = \int_{X_i}^{X_f} dl \sqrt{\frac{1}{D} [E_{ eff} + V_{ eff}(X(l))]},$$

(6)

where $dl = \sqrt{d\mathbf{X}^2}$ is the elementary displacement in configuration space, along the dominant reaction path. Note that the dominant trajectory in Eq. \([6]\) is parametrized in terms of the curvilinear abscissa $l$, which plays the role of the reaction coordinate and measures the total distance covered along the reaction pathway.

The computational difficulty of investigating thermally activated transitions by ordinary MD simulations is related to the decoupling of the time scales characterizing the dynamics of the system. The computational advantage of the DRP approach with respect to MD simulations comes from the fact that, by switching to the HJ formulation, the time variable $t$ has been replaced by the curvilinear abscissa $l$. The key point is that molecular systems are not characterized by a decoupling of the intrinsic length scales. As a result, typically only $N_s = 10 - 100$ space displacement steps are sufficient to attain a realistic representation of the path. This number should be compared to the $10^9 - 10^{12}$ MD time steps required to simulate a single transition with mean-first-passage time in the $\mu s$ – ms range. Ultimately, such a huge computational gain originates from the fact that the DRP does not waste time to simulate the dynamics of the system when it is trapped in metastable states.

Although in the HJ formulation the time variable has been replaced by the curvilinear abscissa $l$, the DRP formalism retains information about the time evolution of the system. Indeed, the time $t \{X\}$ at which the configuration $X$ is visited, during the most probable reaction pathway is given by

$$t \{X\} = \int_{X_i}^{X_f} dl \sqrt{\frac{1}{4D} [E_{ eff} + V_{ eff}(X(l))]},$$

(7)

From this equation it follows that the choice of the effective energy parameter $E_{ eff}$ determines the total time of the transition. In particular, the longest possible transition path time is obtained by choosing $E_{ eff} = -V_{ eff}(X_f) \ [8, 11]$, which is a positive number if $X_f$ is an equilibrium configuration. We stress the fact that the total time $t_{tot} = t(\mathbf{X}_f)$ is much shorter than the mean-first-passage time, as it corresponds to the time it takes to reach the product, once the system has left the reactant state.

Once the dominant path has been determined, it is also possible to identify the configuration $X_{ts}$ which belongs to the transition state, defined in terms of commitment analysis. This is achieved by requiring that the probability in the saddle-point approximation to diffuse back to the initial configuration $X_i$, $P(X_i, t_f | X_{ts}, t_i)$ equates that of evolving toward the final configuration $X_f$, $P(X_f, t_f | X_{ts}, t_i)$. In the saddle-point approximation, this condition leads to the simple equation \([11]\):

$$\frac{U(X_f) - U(X_i)}{2k_BT} = S_{ HJ}(\{X(l)\} | X_{ts}, X_i) - S_{ HJ}(\{X(l)\} | X_{ts}, X_f).$$

(8)
Details of the simulations

We have studied the folding of tetra-alanine at a temperature $T = 300 K$. We discretized the path using $N_s = 16$ equal displacement slices. The effective energy parameter $E_{\text{eff}}$ was chosen to be slightly larger than the value corresponding to the longest possible transition time, i.e.

$$ E_{\text{eff}} = -\frac{3}{2} V_{\text{eff}}(X_f). \quad (9) $$

The molecular energy in the quantum simulations, $U(X)$, was evaluated by solving the Schrödinger Eq. in the Parameterized Model 3 (PM3) scheme, in the MOPAC implementation. The choice of a semi-empirical quantum mechanical method was made because it combines a very low computational cost with a reasonable description of the hydrogen bond energetics. In addition, parameterized model quantum mechanical calculations have been recently shown to reliably describe various types of non-covalent complexes. Since one of the purposes of this work is to compare classical and quantum descriptions of the inter-atomic interactions, we chose to neglect solvation terms in both the MM and \textit{ab-initio} simulations. However, the inclusion of such contributions at the implicit level in the quantum-mechanical simulations does not lead to a significant increase of the computational cost, as the bottleneck of the calculation is the solution of the Schrödinger Eq., at each step of the minimization.

Finding the dominant reaction pathway amounts to minimizing a discretized version of the effective HJ functional:

$$ S_{HJ}^{\text{eff}}[X] = \sum_{i=1}^{N_s-1} \sqrt{\frac{1}{D} \left[ E_{\text{eff}} + V_{\text{eff}}(X_i) \right] \Delta l_{i,i+1}} \quad (10) $$

where the effective potential $V_{\text{eff}}(X)$ is determined according to \cite{4} by numerically differentiating the molecular potential $U(X)$. $\Delta l_{i,i+1}$ is the Euclidean distance between the slices $i$ and $i+1$, i.e $\Delta l_{i,i+1} = \sqrt{(X_{i+1} - X_i)^2}$.

In the discretized representation of the HJ effective action, the width of the distribution of the Euclidean distances between consecutive path slices, $\Delta l_{i,i+1}$, should not be allowed to increase in an uncontrolled way, in order to prevent all frames to collapse into the reactant or product configurations. As discussed in \cite{13}, the most convenient way to achieve this is to introduce a Lagrange multiplier, which holds fixed at 0.2 the ratio between the mean-square deviation from the average of the inter-slice distances $\sigma^2$ of the average square inter-slice distance $(\Delta l^2)$.

The global minimization of the HJ effective action is in general a very challenging task. The main difficulties arise from the ruggedness of the effective potential and the high dimensionality of the system. Indeed, most commonly used global optimization algorithms — such as e.g. simulated annealing — tend to get stuck in secondary minima of the action functional. On the other hand, the results of a DRP calculation can be considered reliable only if the minimization algorithm explores a significant region of the path space. In fact, in the opposite scenario the calculated dominant paths would be strongly biased by the choice of the initial trial path.

In our previous work \cite{13} we tested the performances of several global minimization algorithms, and we found that the Fast Inertial Relaxation Engine (FIRE) \cite{18} method was performing best. The minimization protocol which was adopted in the present work was the following: we started from a high-temperature (800 K) MD trial unfolding path, from the helix configuration. We then relaxed the path by means of a Nudged Elastic Band (NEB) \cite{19} minimization, followed by a zero-temperature DRP minimization, and finally by a finite temperature DRP minimization.

RESULTS

A sequence of configurations which are visited by the dominant reaction pathway determined from \textit{ab-initio} calculations is shown in Fig. \ref{fig:results}. The dashed circle highlights the configuration along the path which is representative of the transition state, according to Eq. \ref{eq:transition}. Polyalanine chains form $\alpha$-helices, stabilized by a sequence of $i - i + 4$ hydrogen bonds. On the other hand, in the tetra-alanine molecule there are significant termination effects, and the minimum energy configuration is slightly distorted from that of an ideal $\alpha$-helix. In particular, the hydrogen bonds stabilizing the tetra-alanine system occur between the $O - 6$ and the $H - 28$ atoms and the $O - 16$ and the $H - 38$ atoms.

In order to perform a quantitative analysis of the transition, let us study the evolution along the dominant path of the following order parameters — see Fig. \ref{fig:parameters}:
• the distance $d_{6-28}$ between the $O - 6$ and the $H - 28$ atoms
• the distance $d_{16-38}$ between the $O - 16$ and the $H - 38$ atoms
• the dihedral angle $\phi_1$ between the atoms $C - 5, N - 7, C - 9, C - 15$
• the dihedral angle $\phi_2$ between the atoms $C - 25, N - 27, C - 29, C - 35$.

In Fig. 3, we compare the evolution of these quantities, as a function of the reaction coordinate $l$ obtained from classical and $ab$-initio simulations. We also plot the initial high-temperature MD path and the path obtained at the end of the preliminary relaxation based on the NEB algorithm — cfr. the discussion in the section “Model” —. In Fig. 4 we show the same dominant paths, projected onto the planes selected by the distances $d_{6-28}$ and $d_{16-38}$ involved in the formation of hydrogen bonds.

Some comments on these results are in order. First of all, these plots clearly show that the FIRE algorithm allows to sizeably move away from the initial trial path, irrespective of the order parameter used to characterize the transition. Secondly, we observe that the dominant paths obtained from MM and $ab$-initio simulations agree at an almost quantitative level. In particular, both calculations predict that the contact between the C – 6 and the H – 28 atoms is formed before the contact between the O – 16 and the H – 38 atoms. However, in the MM calculation, the formation of the second contact occurs at a slightly later stage of the reaction than in the quantum calculation. We emphasize that the observed good agreement between classical and quantum calculations is not due to the insufficient exploration of the path space. In fact, such dominant paths are qualitatively different from those obtained in the last stage of the preliminary NEB minimization. Thus, these results clearly imply that the AMBER–99 classical force field provides a rather accurate description of the dynamics, even in non-equilibrium conditions.

The quantum calculation provides additional physical information, not accessible by means of MM simulations. For example, in Fig. 5, we plot the evolution of the partial charges of $O - 6$ and $H - 28$ and of $O - 16$ and $H - 38$ during the reaction. We recall that these pairs of atoms form hydrogen bonds in the final helix state. By cross-correlating this information with the evolution of the interatomic distance — cfr. Fig. 3 — we can infer that a significant modification of the electronic structure of the $O - 6$ and $H - 28$ atoms sets in when they are separated by a distance of the order 3 Å. Interestingly, the electronic structure of the $O - 16$ and $H - 38$ begin to change when these two atoms are separated by a much larger distance, of the order of 4 Å. These results exhibit an example of the fact that the validity of some of the assumptions involved in MM calculations — such as the invariance of partial charge — may depend on the detail of the chemical environment in which the bond is formed and on the specific non-equilibrium dynamics of the reaction.

Using Eq. (7), it is in principle possible to obtain information about the dynamics, i.e. to compute the time at which each of the configurations of the dominant path is visited during the course of the transition. In particular, assuming a diffusion constant $D = 2 \cdot 10^{-2}$ Å$^2$ ps$^{-1}$ for all atoms, as in [10], the total transition path times in the classical and quantum calculations are found to be $t_{\text{classical}} = 1.4$ ps and $t_{\text{ab-initio}} = 2.3$ ps, respectively. We note, however, that these numbers should be taken with care, because in the present exploratory calculation we used only $N_s = 16$ path discretization steps, and Eq. (7) is known to be quite sensitive to discretization errors.

**DISCUSSION**

In this work, we have used the DRP method to perform the first simulation of the folding reaction of a peptide chain, based on a $ab$-initio quantum-mechanical approach. We have calculated the most statistically probable reaction pathway connecting an initial coil configuration and a final helix configuration, assuming the over-damped Langevin dynamics.

By comparing the results of the $ab$-initio simulation in the PM3 scheme with MM simulations with the AMBER–99 force field, we argue that MM approaches provide a quite reliable description even for out-of-equilibrium dynamics. We also studied the evolution of the partial charges involved in the formation of two hydrogen bonds stabilizing the helix, and found that the dynamics of these observable depends on the chemical environment.

The similarity of the quantum and classical paths suggests a “perturbative” approach to performing $ab$-initio DRP simulations at a much smaller computational cost. Indeed, our results show that the minimum of the classical HJ functional lies in the vicinity of the minimum of the $ab$-initio HJ functional. A first approximate dominant pathway can be calculated using the classical DRP approach, and used as a starting point for a further local minimization, in the $ab$-initio framework. We expect that a few quantum mechanical minimization steps should be enough to reach convergence.
We note that in order to characterize the folding mechanism, it should be taken into account that the structure of the folding pathways may significantly depend on the specific initial condition from which the transition is initiated. Hence, an exhaustive study of the folding dynamics of this system requires a statistical analysis of an ensemble of folding trajectories, corresponding to different initial conditions, as discussed in [11] 1.

We conclude this paper by discussing the computational cost of performing a similar calculation for a larger system, for instance a 15-residue $\beta$-hairpin in implicit solvent. The scaling of MOPAC energy calculation with the number of atoms can be made linear for large molecules by introducing a cut-off for matrix elements of the Hamiltonian between orbitals on atoms beyond a certain distance. Using a discretization of the path in $N_s=100$ slices, we estimate that a ab-initio DRP calculation could be carried out using approximately 500,000 total CPU hours per trajectory.

While this is a substantial amount of computing time, we point out that it can be achieved on existing large computing facilities.

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1 We thank R. Elber for an important discussion on this point.
FIG. 1: The definition of the order parameters $d_{6-28}$, $d_{16-38}$, $\phi_1$ and $\phi_2$ used to analyze the dominant reaction pathways.

FIG. 2: Configurations on the dominant reaction pathway calculated \textit{ab-initio}. The colors on the surface represent the projection of the molecular electro-static potential on the solvent accessible surface.
FIG. 3: Upper-left panel: Evolution of the order parameter $d_{6-28}$ as a function of the reaction coordinate $l$ for four different paths. Upper-right panel: Evolution of the order parameter $d_{16-38}$ as a function of the reaction coordinate $l$ for four different paths. Lower-left panel: Evolution of the order parameter $\phi_1$ as a function of the reaction coordinate $l$ for four different paths. Lower-right panel: Evolution of the order parameter $\phi_2$ as a function of the reaction coordinate $l$ for four different paths.

FIG. 4: Comparison of the dominant reaction pathways obtained from classical and ab-initio DRP simulations, projected onto the plane selected by the $d_{6-28}$ and $d_{16-38}$ order parameters.
FIG. 5: Evolution of the partial charges of the $O - 6$ and $H - 28$ atoms (upper panel) and of the $O - 16$ and $H - 38$ atoms (lower panel), along the dominant reaction pathway. In both figures, the partial charge of the $H$ atoms has been shifted by $-0.42$ atomic units for seek of graphical clarity.