Nonequilibrium molecular dynamics simulation of a photoswitchable peptide

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Abstract

Femtosecond time-resolved experiments on photoswitchable peptides provide a new and promising way to study the folding and unfolding of biomolecules in real time and unprecedented detail. To obtain an appropriate theoretical description of these experiments, a computational strategy is presented that aims to extend well-established molecular dynamics simulation techniques to the description of photoinduced conformational dynamics in peptides. Adopting a bicyclic azobenzene octapeptide as a representative example for a photoswitchable biomolecule, detailed nonequilibrium molecular dynamics studies are performed in which (i) the laser-induced initial state of the molecule is represented by a suitable nonequilibrium phase-space distribution that is sampled by an ensemble of many trajectories and (ii) the time-dependent mean values of the system are calculated from these trajectories by an ensemble average. To establish the applicability and the accuracy of the methodology, it is investigated to what extent the photoinduced conformational dynamics depends on the details of the nonequilibrium method, including the sampling of the initial state, the initially assumed excess energy, and the coupling of the system to a temperature bath. Furthermore, the photoinduced conformational dynamics is analyzed and the results are discussed in the light of recent time-resolved infrared experiments.

1. Introduction

In recent times, a number of experimental techniques have been developed which study biomolecular processes such as protein folding directly and in a time-resolved manner. Beautiful examples include atomic force microscopy techniques [1], which allow us, for example, to study the molecular recognition in binding processes, as well as laser-induced pH and temperature-jump experiments [2], which may induce the folding or unfolding of a biosystem. Furthermore, there have been various suggestions to include a molecular photoswitch into biomolecules [2–9]. For example, Moroder and co-workers [5–7] have synthesized various peptides, in which an azobenzene unit was incorporated directly in the backbone. This guarantees that the light-induced structural changes of the chromophore upon photoisomerization around the central N=N double bond are directly transferred into the peptide chain. Femtosecond time-resolved pump–probe experiments with optical [10,11] and infrared [12] detection indicate that the main conformational changes of the peptide backbone are completed after only 20 ps, although the subsequent structural equilibration of the peptide continues for about 20 ns. These types of experiments, especially in combination with two-dimensional infrared probing [13], provide a new and promising way to study the folding and unfolding of peptides in unprecedented detail.

On the theoretical side, the large majority of molecular dynamics (MD) simulations of biomolecules are performed under equilibrium conditions to calculate thermodynamic mean values. Recently, nonequilibrium MD simulations have been reported that apply an external force to a biomolecule in order to simulate single-molecule force microscopy experiments [14,15]. In a similar way, nonequilibrium calculations have been employed to calculate the potential of mean force along a reaction coordinate defined by, e.g., pulling a ligand out of a binding pocket [16–20]. So far,
however, there are only very few attempts to describe laser-induced conformational dynamics of biomolecules starting from the experimentally achieved nonequilibrium preparation of the system [10,21–29].

In this work, we present a simple computational strategy that allows us to extend well-established MD techniques to the description of photoinduced nonequilibrium dynamics. As an experimentally well characterized molecular system [6,10–13], we consider the octapeptide fragment H-Ala-Cys-Ala-Thr-Cys-Asp-Gly-Phe-OH which was connected head to tail via (4-aminomethyl)-phenylazobenzoic acid as well as by a disulfide bridge, see Fig. 1. We have recently performed extensive replica-exchange MD simulations [30] of this bicyclic azobenzene peptide (bcAMPB) in its cis and trans equilibrium states [31]. In direct agreement with nuclear magnetic resonance (NMR) results [5], it was found that the trans azopeptide is predominantly in a single conformational state, while there are many conformations of similar energy in the cis state of the peptide.

Adopting bcAMPB as a representative example, we perform detailed nonequilibrium MD studies in which (i) the laser-induced initial state of the molecule is represented by a suitable nonequilibrium phase-space distribution that is sampled by an ensemble of many trajectories and (ii) the time-dependent mean values of the system are calculated from these trajectories by an ensemble average. We investigate to what extent the photoinduced conformational dynamics depends on the details of the initial conditions, including the sampling of the initial state, the initially assumed excess energy, and the coupling of the system to a temperature bath. Furthermore, the photoinduced conformational dynamics is analyzed and the results are discussed in the light of recent time-resolved infrared experiments [12].

2. Modeling of the laser-induced initial state

The excitation of a molecular system by an optical laser pulse prepares the molecule in an excited electronic state. In the case of an effective molecular photoswitch, the system rapidly decays via a conical intersection of the potential-energy surfaces in the electronic ground state [32]. Various mixed quantum-classical and semiclassical approaches have been proposed [33], which allow us to extend standard MD simulations on the electronic ground-state potential-energy surface to the description of nonadiabatic processes on coupled potential-energy surfaces. Although the direct ab initio MD description of photochemical reactions has recently become possible, calculations employing a level of theory that is appropriate for excited electronic states are quite tedious and still restricted to relatively small systems and short times. Alternatively, a suitable model of the photoinduced dynamics may be employed in order to gain an understanding of the essential physics involved [34]. Time-dependent quantum-mechanical [34–37] and semiclassical [38,39] studies have shown that nonadiabatic cis–trans photoisomerization dynamics via a conical intersection occurs on a subpicosecond time scale and results in a highly delocalized vibrational wave packet on the electronic ground-state potential-energy surface. By calculating the phase-space distribution of this quantum state (using, e.g., the Wigner representation), we can generate initial atom positions and velocities which may be employed as initial conditions for subsequent nonequilibrium classical trajectory calculations.

In the case of the azobenzene peptide bcAMPB, the cis–trans photoisomerization dynamics occurs within \( \approx 250 \) fs, while the subsequent conformational dynamics of the peptide backbone takes place on a time scale of pico- to nanoseconds [10–13]. Hence it may be expected that the conformational dynamics of interest does not sensitively depend on the details of the laser-induced initial state. Rather than explicitly calculating the laser-induced phase-space distribution [29], we therefore construct a simple model of the photoexcitation process, which allows us to employ a standard MD program package. To motivate such a model, Fig. 2 shows a schematic view of the adiabatic potential-energy surfaces of the electronic ground-state \( S_0 \) and the first \( n\pi^* \) electronic excited state \( S_1 \) of bcAMPB. Recent high-level ab initio calculations on azobenzene [40–42] agree that

![Fig. 2. Scheme of the \( S_0 \) and the \( S_1 \) potential-energy curves of bcAMPB as a function of the N=N cis–trans isomerization coordinate \( \phi \). The solid lines represent the adiabatic potentials of a model which is designed to reproduce the experimental cis and trans absorption bands and the ground-state cis–trans energy barrier. The dashed line corresponds to the GROMOS force field potential of the N=N torsion, the dotted line shows a model of the cis–trans photoisomerization potential, which simply connects the \( S_1 \) cis state and the \( S_0 \) trans state of bcAMPB. Within this model, the photoexcitation of the system by an ultrashort laser pulse is simulated by instantly switching from the ground-state to the excited-state potential, which causes the isomerization of the system within \( \approx 250 \) fs.](image)
the torsion around the N=N double bond is the isomerization coordinate, and it is expected that this is also the case for the azobenzene peptide bcAMPB [11]. The potential-energy curves shown in Fig. 2 were obtained by assuming cosine functions for the corresponding diabatic potentials [34,35], the energy gaps of which are designed to reproduce the maxima of the experimental cis and the trans absorption spectra and the ground-state cis–trans energy barrier [6].

Also shown in Fig. 2 is the N=N torsional potential-energy function as assumed in the GROMOS96 force field, \( V'(\phi) = {\frac {K_1}{2}}(1 - \cos 2\phi) \) with \( K_1 = 84 \text{ kJ/mol} \). The force field model clearly underestimates the cis–trans energy barrier and also neglects energy difference of the cis and the trans configurations. At thermal energies \( (k_B T \approx 2.5 \text{ kJ/mol at 300 K}) \) and short time scales, however, these differences are hardly relevant. As a minimal model of the cis–trans photoisomerization process, the dotted line in Fig. 2 shows a potential-energy curve which is designed to connect \( S_1 \) cis state and the \( S_0 \) trans state by using a cosine function, \( V(\phi) = {\frac {K_1}{2}}(1 + \cos \phi) \) with \( K_1 = 320 \text{ kJ/mol} \). In the absence of an ab initio description of bcAMPB and since we do not know the detailed phase-space distribution of the system after passing the conical intersection, the simple model by construction yields the correct experimental excess energy that is initially put in the N=N torsional degree of freedom. A similar potential-energy model of a photoswitchable peptide was suggested in [10].

Adopting the model developed above, a nonequilibrium MD description of the cis–trans photoisomerization in bcAMPB can be rationalized as follows. Starting point is the calculation of the equilibrium structure of solute and solvent, which generates a number of (typically some hundred) statistically independent equilibrium initial conditions. In the study shown below, these data were obtained from a previously performed replica-exchange MD simulation of cis–trans bcAMPB [31]. Next, we mimic the photoexcitation of the system by an ultrashort laser pulse by instantly switching from the ground-state N=N torsional potential \( V(\phi) \) to the excited-state potential \( V'(\phi) \), see Fig. 2. Following this nonequilibrium preparation at time \( t = 0 \), the system isomerizes along excited-state N=N potential within 250 fs, see Fig. 3. After the isomerization (i.e., for times \( \geq 500 \text{ fs} \)), the N=N torsional potential is switched back to its ground state form, and a standard MD simulation is performed up to 1 ns.

Following the simulations, the time-dependent observables of interest, for example the energy \( E \), are obtained via an ensemble average over the initial distribution

\[
\langle E(t) \rangle = \frac{1}{N_{\text{traj}}} \sum_{i=1}^{N_{\text{traj}}} E^{(i)}(t),
\]

where \( E^{(i)} \) denotes the energy pertaining to an individual trajectory and \( N_{\text{traj}} \) is the number of trajectories. As a first example, Fig. 3 shows the time evolution of the potential energy of the N=N cis–trans isomerization coordinate. In agreement with the femtosecond time-resolved experiments of [10,11], the simple model of bcAMPB undergoes photoisomerization within 250 fs, as is monitored by the decay of the potential energy on this time scale.

Another issue that deserves some attention is the question whether nonequilibrium MD simulations should be run at constant energy or at constant temperature. The latter is the standard for equilibrium MD simulations which aim to mimic a canonical ensemble typical for macroscopic systems. In practice, the coupling to the temperature bath is achieved by rescaling the velocities of all atoms at every time step, such that the desired temperature is maintained [43]. In a photoinduced nonequilibrium experiment, on the other hand, the photoexcitation results in a vibrationally hot molecule, which is cooled via vibrational energy transfer to the surrounding solvent molecules. Only at longer times, it can be expected that the excess energy has been distributed among so many solvent molecules that the temperature of the peptide and the solvent is constant again. In this work, the first 500 fs (i.e., during the photoisomerization) were always simulated at constant total energy, while the subsequent MD simulations were performed either at constant energy (NVE ensemble) or at a constant temperature of 300 K (NVT ensemble), using the Berendsen coupling method [43] with a temperature coupling constant of 0.1 ps.

### 3. Simulation details

We used the GROMOS96 force field 43a1 [44] to model the bcAMPB peptide and the united-atom model of [45] to describe the DMSO solvent. Additional force field parameters for the azobenzene unit were derived from density functional theory [31]. In all simulations, the GROMACS program suite [46,47] was employed. The bcAMPB peptide was placed in an octahedral box containing \( \approx 700 \) DMSO molecules. The equation of motion was integrated by using a leap-frog algorithm with a time step of 2 fs. Covalent bond lengths were constrained by the procedure SHAKE [48] with a relative geometric tolerance of 0.0001. We employed the particle-mesh Ewald
method to treat the long-range electrostatic interactions \[49\]. The nonbonded interaction pair-list were updated every 5 fs, using a cutoff of 1.4 nm.

As explained above, we used the results of a previously performed replica-exchange MD simulation [31] of the cis-isomer of bcAMPB to generate statistically independent equilibrium initial conditions. Because the cis-isomer represents an energetically frustrated system, there are many conformations of similar energy in the cis state of the peptide. To characterize this conformational heterogeneity, a principal component analysis \([50–52]\) has been performed in [31], which provides an efficient and well-established way to represent the conformational distribution of a high-dimensional system in terms of a few “principal” coordinates. Restricting ourselves to the first two (i.e., largest) principal components (say, \(v_1\) and \(v_2\)), the probability distribution \(P(v_1,v_2)\) obtained from the replica-exchange MD simulation of cis-bcAMPB is shown in Fig. 4(a). We find a rather broad distribution with numerous peaks, reflecting the large conformational heterogeneity of the frustrated cis-isomer. To generate representative equilibrium initial conditions from this distribution, two strategies were adopted. First, four main conformational states (labeled I, II, III, and IV in Fig. 4(a)) were identified and each one was sampled using 50 representative conformations. As a second option, we simply selected 200 statistically independent conformations from the complete trajectory. The resulting distribution of these selected conformations is shown in Fig. 4(b).

### 4. Computational results

Adopting the computational strategy described above, we have performed a detailed nonequilibrium MD study of the cis-trans-photoisomerization and the subsequent conformational transitions of bcAMPB. To obtain a first impression on the conformational dynamics initiated by the photoexcitation, Fig. 5 shows the time-dependent mean values of the backbone dihedral angles \(\langle \phi_i(t) \rangle\) and \(\langle \psi_i(t) \rangle\) \((n = 1 . . 8)\) pertaining to the eight amino acids of bcAMPB. In the equilibrium simulations of [31], the dihedral angles for the residues Cys2, Ala3, Thr4, and Cys5 were found to be quite similar for the cis and trans isomers. As therefore expected, there are only minor changes observed for these angles after photoexcitation. The most significant conformational changes are observed for Ala1, which is directly connected to the photoswitch, and for Gly7, which is the most flexible residue found in the NMR [6] and MD [31] studies. While the width of the fluctuations is quite small for residues occurring in a single conformation (e.g., Ala3), the pronounced fluctuations of the backbone angles of Phe8 and the two cystines indicate that there are several conformational states contributing to their averages. In the case of Gly7, in particular, we find conformations for \(\phi \approx -50^\circ\) and \(\phi \approx 50^\circ\), where \(\psi\) can take on almost any value. For clarity, we have therefore calculated separate mean values for the cases \(\phi < 0\) and \(\phi > 0\) (and similarly for \(\psi\)), which, in fact, exhibit different time evolutions.

To establish the applicability and the accuracy of the methodology, we next investigate to what extent the photoinduced conformational dynamics depends on the details of the nonequilibrium method. The model introduced in Fig. 2 to represent the nonequilibrium initial state is quite simple, as it virtually accounts only for the excess energy put into the system via photoexcitation. To study the consequences of this approximation, Fig. 5 compares the time evolution of the backbone dihedral angles obtained for \(E_{\text{ex}} = 285\ \text{kJ/mol}\) (upper panels) and \(320\ \text{kJ/mol}\) (lower panels). Interestingly, the results are quite similar for both energies, which indicates that the nonequilibrium conformational dynamics of the peptide does not depend on the details of the photoisomerization potential-energy curve.

Next, we wish to study the effect of performing the nonequilibrium MD simulations at either constant energy or at constant temperature. To this end, Fig. 6(a) compares the time evolution of the mean kinetic energy of bcAMPB obtained for the two cases. As mentioned above, the first 500 fs (i.e., during the photoisomerization) were always simulated at constant total energy, i.e., the time evolution is identical for both simulations. At \(t = 0.5\ \text{ps}\), the coupling to the temperature bath is turned on, and the kinetic energy of the constant-temperature run is seen to decay to its equilibrium value on the time scale of the temperature coupling constant \(\tau = 0.1\ \text{ps}\). In the constant-energy run, on the other hand, the kinetic energy of bcAMPB decays on the much slower time scale of 10 ps, which describes the cooling of the molecule in the solvent.

The results suggest that nonequilibrium MD simulations should be performed at constant energy to correctly describe the cooling of the photoexcited system via vibrational energy transfer to the surrounding solvent molecules. However, after 100 ps, the kinetic energy (and thus the temperature) of the constant-energy run is seen to drop below the equilibrium value of the constant-temperature run. This artifact is a consequence of the insufficient accuracy of the integration method, which causes within 100 ps a \(\approx 2\ \text{kJ/mol}\) drift of the total energy of the

![Fig. 4. (a) Conformational probability distribution of the cis-isomer of bcAMPB as obtained from an equilibrium MD simulation at 295 K, represented as a function of the first two principal components \(v_1\) and \(v_2\). (b) The probability distribution of (a) sampled by 200 statistically independent conformations.](http://dx.doi.org/10.1016/j.chemphys.2005.08.047)
peptide. Although the energy conservation can be improved by increasing the pair list cutoff and by using a smaller time step, this computationally expensive solution can be avoided by turning on the coupling to the temperature bath, once the cooling of the hot photoproducts by the solvent molecules is completed. This way, the initial energy transfer is described correctly (the above mentioned energy drift is negligible for short times), and it is assured that the simulation remains at equilibrium at long times. Alternatively, the temperature bath may be coupled to the solvent molecules only [10]. This strategy is found to yield quite similar results as for the constant energy run (data not shown), including the artificial energy drift at long times. Since the solvent molecules surrounding the peptide are not only cooled by the other solvent molecules but also by the bath, however, this method results in a cooling time of the peptide that is somewhat too short.

By partitioning the kinetic energy of the complete molecule (a) into the kinetic energy of the azobenzene photoswitch (b) and the octapeptide (c), respectively, Fig. 6 also shows that the excitation of the photoswitch occurs within less than 100 fs, and the subsequent transfer of energy to the peptide occurs within the next 200 fs. Furthermore, panel (d) shows the time evolution of the potential
energy of the complete bcAMPB molecule. Similar to the kinetic energy, the potential energy rapidly decays to its equilibrium value, once the coupling to the temperature bath is turned on. This finding reveals that the simple picture of the virial theorem for the harmonic oscillator, stating that the expectation values of the kinetic and potential energy are equivalent, holds surprisingly well for a MD simulation of a small peptide.

From the comparison of the time-dependent energies of the constant-energy and constant-temperature simulations one might also expect significant differences for the associated conformational dynamics in the two cases. Comparing the time evolution of the backbone dihedral angles obtained for the constant-temperature run (Fig. 5) and the corresponding constant-energy run (Fig. 7), however, we find that the conformational dynamics of the peptide is surprisingly similar in the two cases. Although there are significantly different amounts of energy available to the peptide, the resulting conformational transitions seem to occur in the same way and on the same time scale. To summarize, the similarity of the conformational transitions shown in Figs. 5 and 7 appears to justify the neglect of finer details of the initial phase-space distribution, including the quantum-mechanical zero-point energy in high-frequency modes [29] or effects due to the constraint of high-frequency modes.

The convergence of an equilibrium MD calculation with respect to the conformational sampling is a crucial and often quite cumbersome aspect of the simulation. To consider this issue in the case of a nonequilibrium simulation, one may study the convergence by either varying the number of trajectories in the ensemble average (Eq. (1)) or by the choice of initial conformations sampled from the equilibrium trajectory. As explained in Section 3, two strategies were employed for the latter task. The results discussed so far were obtained by identifying four main conformational states from the principal component analysis (Fig. 4(a)) and sampling each one by using 50 representative conformations. Alternatively, one may select 200 statistically independent conformations (Fig. 4(b)) from the complete equilibrium trajectory. Fig. 8 shows the time evolution of the backbone dihedral angles for the latter sampling.
strategy, which looks surprisingly different compared to the results of Fig. 7. While the residues Cys2, Ala3, Thr4 that are in a similar conformation in the cis and the trans isomers are sampled equally well by both methods, we notice significant differences for the residues Ala1 and Gly7, which undergo a conformational transition. Interestingly, we also note considerable changes for the residues Cys5 and Phe8, which otherwise appear almost constant in time. This indicates that several conformations contribute to the average of these angles.

The comparison shows that for a conformationally heterogeneous system like cis-bcAMPB it can be misleading to restrict the discussion to the conformational states identified by only the first two principal components. To achieve an appropriate conformational sampling of the initial state, one either needs to take into account more than two principal components (five in the case of cis-bcAMPB [31]), or – much simpler – one directly samples statistically independent conformations from the complete equilibrium trajectory.

To assess the convergence of the nonequilibrium simulation with respect to the number of trajectories contributing to the ensemble average (Eq. (1)), we choose the ϕ-angle of Phe8 as a representative example. Selecting again the initial conditions directly from the complete equilibrium trajectory, Fig. 9 shows that it takes at least 200 trajectories to obtain a qualitatively correct ensemble average for this observable. In the present case, for example, less than ≈50 trajectories reveal conformational changes of individual conformational states, which do not show up in the converged ensemble average. For longer times, it is expected that even more trajectories are necessary for converged results.

Finally, it is interesting to compare the nonequilibrium simulation results discussed above to the time-resolved femtosecond optical-pump infrared-probe experiments of Bredenbeck et al. [12]. Recording the time-dependent infrared cis–trans difference spectra of bcAMPB, these authors discussed two main features of the amide I spectra: (i) A red-shifted transient absorption band, which occurs immediately after photoswitching and decays on a time scale of 4 ps. This finding was interpreted as a consequence of the heating or nonthermal excitation of low-frequency modes that are anharmonically coupled to the amide I vibrations [53]. (ii) A transient blue-shifted signature which is formed on a time scale of 6 ps. After 20 ps, the intensity and peak position of this feature are almost equivalent to the stationary FTIR difference spectrum. Since the blue shift of the amide I band can be directly related to the change of
backbone structure [54,55], it was concluded that the main conformational changes associated with the stretching of the peptide are completed after only 20 ps.

In order to explain the red-shifted hot band of the amide I peptide vibrations, we reconsider the time evolution of the peptide kinetic energy shown in Fig. 6(c), which directly reflects the laser-induced heating (for $t \leq 1$ ps) and subsequent cooling (for $1 \text{ ps} \leq t \leq 100$ ps) of the peptide. An exponential fit of the decay of the kinetic energy between 1 and 100 ps yields a decay time of 16 ps, which is longer than the experimental value of 4 ps. Taking into account the sum of all uncertainties in experiment (e.g., deconvolution and interpretation of the spectrum) and computation (e.g., force field and sampling), the agreement appears reasonable.

Although the blue-shifted signature of the infrared difference spectrum qualitatively reflects the conformational changes of the backbone structure, it is not straightforward to estimate this spectral change from the time evolution of the MD trajectories. This is, because the infrared spectrum of a peptide depends in a complex way on the individual conformations of the residues [54,55]. Hence a small conformational change of a peptide depends in a complex way on the individual conformations of the residues, however, it is not straightforward to estimate small spectral changes from the time evolution of MD trajectories. Work in this direction is in progress.

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