Infrared signatures of the peptide dynamical transition: A molecular dynamics simulation study

Maja Kobus,1 Phuong H. Nguyen,2 and Gerhard Stock1,a)
1Biomolecular Dynamics, Institute of Physics, Albert Ludwigs University, 79104 Freiburg, Germany
2Institute of Physical and Theoretical Chemistry, Goethe University, 60438 Frankfurt, Germany

(Received 25 May 2010; accepted 20 June 2010; published online 20 July 2010)

Recent two-dimensional infrared (2D-IR) experiments on a short peptide 310-helix in chloroform solvent [E. H. G. Backus et al., J. Phys. Chem. B 113, 13405 (2009)] revealed an intriguing temperature dependence of the homogeneous line width, which was interpreted in terms of a dynamical transition of the peptide. To explain these findings, extensive molecular dynamics simulations at various temperatures were performed in order to construct the free energy landscape of the system. The study recovers the familiar picture of a glass-forming system, which below the glass transition temperature \( T_g \) is trapped in various energy basins, while it diffuses freely between these basins above \( T_g \). In fact, one finds at \( T_g \approx 270 \) K a sharp rise of the fluctuations of the backbone dihedral angles, which reflects conformational transitions of the peptide. The corresponding C–O frequency fluctuations are found to be a sensitive probe of the peptide conformational dynamics from femtosecond to nanosecond time scales and lead to 2D-IR spectra that qualitatively match the experiment. The calculated homogeneous line width, however, does not show the biphasic temperature dependence observed in experiment. © 2010 American Institute of Physics. [doi:10.1063/1.3462961]

I. INTRODUCTION

Hydrated proteins and nucleic acids are known to exhibit a dynamical transition near 200 K, which is characterized by a rapid increase of their molecular flexibility above this temperature.1–4 The phenomenon resembles the behavior of glass-forming liquids, which below the glass transition temperature \( T_g \) are trapped in various, essentially harmonic basins of the system’s free energy, while they diffuse freely between these basins above \( T_g \).5–4 A number of experimental and computational works have indicated that the dynamical transition is closely related to the fluctuations of the solvent.9–16 Attempts to explain this finding include the existence of a liquid-liquid critical point of water17 and the effects of protein-water hydrogen bond dynamics.13 Nonetheless, the mechanism of the rapid change of the molecular flexibility still remains a matter of controversial discussions.

Recently it has been demonstrated that the dynamical transition also occurs in the absence of tertiary or secondary structure of the protein and, in fact, even occurs in small peptides.18–20 Employing terahertz spectroscopy to short chain alanines in aqueous solution, Markelz and co-workers18 showed that the characteristic temperature dependence can be monitored down to penta-alanine. Interestingly, a quite similar behavior was found in time-resolved vibrational experiments of Hamm and co-workers, who studied the photoinduced energy transport through a short peptide 310-helix in chloroform.19–22 In particular, it was shown that the overall transport efficiency stays approximately constant at 220–260 K, but rises steeply for higher temperatures. Nonequilibrium molecular dynamics (MD) simulations were able to qualitatively reproduce this effect19 and showed a prominent rise at 270 K of the atomic fluctuations of the peptide.19 Very recently, Backus et al.20 also presented equilibrium two-dimensional infrared (2D-IR) experiments on the same system, which showed a quite similar temperature behavior for the homogeneous dephasing rate 1/T2 of the C–O vibrations.

There are various intriguing aspects in the experimental findings of Hamm and co-workers.19,20 First the choice of a different solvent but water turns out to be quite instructive. Chloroform remains in its liquid state throughout the studied temperature range, hence there are no complications due to solvent phase transitions as has been discussed for aqueous solution.19 Due to its small polarity, chloroform hardly forms hydrogen bonds with the peptide. Hence, if the dynamic transition is caused by solvent-peptide interactions, mostly Lennard-Jones interactions should cause the effect. The relatively high transition temperature (\( T_g \approx 270 \) K) of the short 310-helix in chloroform is advantageous for the sampling in MD simulations, which are notoriously difficult to converge at low temperatures. Moreover, the homogeneous dephasing rate observed in the 2D-IR experiments is interesting, because it reports on dynamics that is faster than the intrinsic time scale of the experiment of about 1 ps. This is certainly shorter than typical time scales expected from a temperature-enhanced diffusion between energy basins.

To study the origin of the temperature dependence of the homogeneous dephasing, in this work we perform extensive (in total 2.2 \( \mu s \)) equilibrium MD simulations for seven temperatures from 220 to 300 K. We find a clear increase of the fluctuations around 270 K which are caused by conformational transitions of the peptide helix. However, these transi-
tions take place on a time scale much longer than 1 ps and therefore may contribute only indirectly to the increase of the homogeneous line width. The resulting temperature dependence of the homogeneous line width is in good agreement with the antidiagonal width of the calculated 2D-IR spectra but matches the experiment only qualitatively. Nonetheless, we show that the C==O frequency fluctuations are a sensitive probe that monitors the conformational dynamics of the peptide over multiple time scales.

II. THEORY AND METHODS

A. MD simulations

All simulations were performed with the GROMACS program suite, using the GROMOS96 force field to model the short peptide 310-helix and the rigid all-atom model of Ref. 24 to describe the chloroform solvent. The simulated molecular system, PAZ-Aib-OH, consists of a photoswitchable azobenzene unit that is attached to an α-aminoisobutyric acid (Aib) peptide. Test calculations have shown that this system is quite similar to the molecule considered in experiment, in which one Aib was replaced by an isotope labeled alanine residue. The equation of motion was integrated by using a leap-frog algorithm with time step of 2 fs. We employed the particle-mesh Ewald method to treat the long-range electrostatic interaction. The non-bonded interaction pair-list were updated every 10 fs, using a cutoff of 1.4 nm. Bonds containing a hydrogen atom were constrained via the SHAKE (Ref. 26) procedure with a relative geometric tolerance of 10−4.

Starting with a left-handed 310-helical conformation, the Aib peptide was placed in an octahedral box containing about 700 chloroform molecules. After energy minimization, the system was simulated at constant temperature and pressure (1 atm), using the Berendsen coupling procedure with a coupling time of 0.1 and 0.5 ps, respectively. For each of the temperatures 220, 250, 260, 270, 280, and 300 K, two production runs of ≈160 ns were performed. In the first set of runs, the C==O frequency shift due to the solvent and the peptide was calculated (see below) and all data were stored every 20 fs to monitor the subpicosecond characteristics of the C==O vibration. The second set of runs served as a check of the conformational sampling, hence the data were only stored every picosecond.

B. Modeling of the C==O frequency

To obtain the information of an isolated C==O vibration of the Aib peptide, Backus et al. employed 13C isotope labeling of the carbonyl group of one of the amino acids. As a consequence, one may neglect the coupling of the labeled mode to the remaining modes of the amide I band to a good approximation. To calculate the instantaneous frequency $\varepsilon(t)$ of the tagged C==O vibration for each snap shot of the MD simulation, we make the ansatz

$$\varepsilon(t) = \varepsilon_0 + \delta\varepsilon_M(t) + \delta\varepsilon_S(t) + \delta\varepsilon_P(t).$$

Here $\varepsilon_0 = 1717$ cm$^{-1}$ denotes the C==O frequency of isolated N-methylacetamide, and $\delta\varepsilon_M$, $\delta\varepsilon_P$, and $\delta\varepsilon_S$ represent the shift of this frequency due to the interaction with (i) the neighboring peptide units, (ii) the remaining peptide units, and (iii) the solvent, respectively. Adopting the building block model of Ref. 26, the next-neighbor contribution $\delta\varepsilon_{M}(t)$ to the C==O frequency of the $n$th peptide unit is given by

$$\delta\varepsilon_n = e_C^{GD}(\phi_{n-1},\psi_{n-1}) + e_N^{GD}(\phi_n,\psi_n) - 2e_0,$$

where $e_C^{GD}$ and $e_N^{GD}$ denote the frequencies of the C- and N-terminal local modes of isolated glycine dipeptide, respectively. Employing density functional theory calculations at the B3LYP/6-31+G(d) theoretical level, these frequencies were calculated for an equidistant map of the peptide backbone dihedral angels $\phi_n$ and $\psi_n$. The frequency shifts of the remaining peptide units ($\delta\varepsilon_P$) and due to the solvent atoms ($\delta\varepsilon_S$) are estimated by the method of Jansen and Knoester using the electric field and its gradient on the C, O, N, and D atoms.

Within semiclassical line shape theory, the fluctuations of the peptide and the surrounding solvent molecules result in a classical time-dependence of the vibrational frequency. As the C==O frequency distribution of the Aib peptide is well approximated by a Gaussian function (see Fig. 5 below), it is sufficient to describe this time evolution via a second-order cumulant expansion of the propagator. (Test calculations that directly solved the time-dependent Schrödinger equation of the vibrational system confirmed the validity of the cumulant approximation for this system.) The central quantity in this theory is the frequency fluctuation correlation function

$$\langle \delta\varepsilon(t)\delta\varepsilon(0) \rangle \approx \delta\varepsilon^2 e^{-\tau^2/\tau},$$

where $\delta\varepsilon(t) = \varepsilon(t) - \langle \varepsilon \rangle$ and the brackets $\langle \cdot \cdot \cdot \rangle$ denote the statistical average over the frequency fluctuations. In many cases, this function is well approximated by one or several exponential functions with the overall fluctuations $\delta\varepsilon^2 = \langle \delta\varepsilon(0)^2 \rangle$ and the decay time $\tau$. Within the cumulant approximation, the frequency fluctuation correlation function determines the line shape function

$$g(t) = \frac{1}{2} \int_0^t dt_1 \int_0^{t_1} dt_2 \langle \delta\varepsilon(t_2-t_1)\delta\varepsilon(0) \rangle,$$

which directly enters the calculation of all linear and nonlinear IR spectra below. In the fast modulation limit, $\sqrt{\delta\varepsilon^2} \tau \ll 1$, we obtain $g(t) = \Gamma t$ with the homogeneous line width

$$\Gamma = \delta\varepsilon^2 \tau,$$

which gives rise to Lorentzian broadening of the corresponding spectrum. This arises if the correlation time of the fluctuations is faster than the time scale of the experiment (here, typically 1 ps). In the opposite limit of slow frequency modulations, $\sqrt{\delta\varepsilon^2} \tau \gg 1$, we obtain inhomogeneous broadening via a Gaussian line shape function.

C. 2D-IR spectra

Since the linear infrared spectrum is dominated by inhomogeneous broadening, it is difficult to extract the homogeneous dephasing time $T_2$ from it. In contrast, the line shapes of the third-order response functions provide a direct mea-
fulfill the impulsive limit condition, since the width of the IR separated in time, resulting in a well-defined time ordering of 2D-IR photon-echo setup, where the three IR pulses are of the first and second excited state of the C—O vibration. In this limit, the measured photon-echo signal is directly proportional to the third-order response function

\[
S(t_3,t_2,t_1) = \left( \frac{i}{\hbar} \right)^3 \left\langle \mu(t_1 + t_2 + t_3) \right.
\times \left[ \mu(t_2 + t_1) \left[ \mu(t_1) \left[ \mu(0), \rho^{(0)} \right] \right] \right]\nonumber
\left. \right\rangle
\]

\[
= \left( \frac{i}{\hbar} \right)^3 \sum_{k=SE,GB,ESA} \left\{ R_k^+ (t_3,t_2,t_1) + R_k^- (t_3,t_2,t_1) \right\},
\]

(6)

where \( \mu(t) \) represent the transition dipole operators of the local C—O oscillator, \( \rho^{(0)} = |0\rangle \langle 0 | \) is the unperturbed initial density matrix that is composed of the vibrational ground state |0\rangle, and the outer brackets represent the ensemble average. Due to the time ordering of the pulses, the experimental phase matching condition, and in rotating wave approximation, the third-order response function is given by three contributions, that is, stimulated emission, ground state bleach, and excited state absorption signals, which radiate in the so-called rephasing (+) and the nonrephasing (−) directions. Fourier transform of the response function with respect to the two coherence times \( t_1 \) and \( t_3 \) yields the 2D-IR spectrum. Buckus et al.\(^{20}\) considered the purely absorptive spectrum, which is given as the real part of the sum of rephasing and nonrephasing signals

\[
I(\omega_3,\omega_2,\omega_1) \propto \Re \sum_k \int_0^\infty dt_3 \int_0^\infty dt_1 \left\{ e^{[i(\omega_1 t_1+\omega_3 t_3)]} R_k^+ + e^{-[i(\omega_1 t_1+\omega_3 t_3)]} R_k^- \right\},
\]

(7)

As in the experiment,\(^{20}\) the waiting time \( t_2 \) was set to 300 fs to ensure the second and the third laser field do not overlap in time.

As explained above, in the considered case the response functions can be evaluated within a second-order cumulant expansion, which yields

\[
R_{SE}^+ = R_{GB}^+ = |\mu_{01}|^2 e^{[i(\omega_1 t_1+\omega_3 t_3)]} e^{-(t_1+2t_2+t_3)/2T_1} e^{G^+},
\]

\[
R_{ESA}^+ = |\mu_{01}|^2 |\mu_{12}|^2 e^{[i(\omega_1 t_1+\omega_3 (t_1+2t_2+t_3))] \times e^{-(t_1+2t_2+3t_3)/2T_1} e^{G^+},
\]

\[
G^+ = g(t_1) \pm g(t_2) \pm g(t_3) \pm g(t_1 + t_2) \pm g(t_1 + t_3) \pm g(t_2 + t_3),
\]

where the line shape function \( g(t) \) is given in Eq. (4). We included phenomenological decay terms in order to account for the line broadening due to the life time \( T_1 = 1 \) and 0.5 ps of the first and second excited state of the C—O vibration.\(^{24}\) To account for excited state absorption, we have adopted the experimental anharmonicity \( \Delta = 16 \text{ cm}^{-1} \) (Ref. 44) and approximated the excited-state dipole moment in harmonic approximation, i.e., \( |\mu_{12}|^2 = |\mu_{01}|^2 \).

Within the semiclassical line shape theory outlined above, the homogeneous width of an isolated infrared vibration is given by

\[
1/T_2 = \Gamma + 1/2T_1,
\]

(9)

where \( \Gamma = \delta \epsilon^2 \tau \) represents the pure dephasing rate and the term \( 1/2T_1 \) accounts for the life time broadening of the first excited state. The homogeneous line width can be extracted from a cut of the 2D spectrum (7) along the antidiagonal axis defined by \( \omega_1 + \omega_3 = 2\epsilon \).\(^{43}\) The situation is similar to a hole-burning experiment, where the pump pulse burns a hole of homogeneous width in the absorption band. Since the antidiagonal spectrum is mainly caused by intrinsic homogeneous dephasing, it has a Lorentzian shape. As in experiment, the antidiagonal width was therefore estimated from the width (FWHM) of a fitted Lorentzian.

III. RESULTS

A. Energy landscape

As explained in the Introduction, the hallmark of the peptide dynamical transition is the rapid increase of the atomic fluctuations above the transition temperature. To illustrate this effect, Fig. 1 shows the root mean square (rms) fluctuations of the backbone dihedral angles of the Aib peptide. Interestingly, the dihedral angles of the inner residues (3–6) show indeed a clear and pronounced rise of the fluctuations at 270 K. The overall shape of these curves agrees nicely with the experimental behavior of the transport efficiency\(^{19}\) and the homogeneous line width\(^{20}\) of the system. The terminal residues, on the other hand, do not show this transition behavior but rather fluctuate relatively strongly at all temperatures. This finding is interesting because it is in
line with the result of Markelz and co-workers, who showed that polyalanine needs a minimum length of five residues to exhibit a dynamical transition. As the terminal residues typically show unspecific fluctuations of the ends, it takes apparently at least a few inner residues for the effect to occur.

It is peculiar that for the inner residues the fluctuations of the $\phi$ and $\psi$ dihedral angles are almost identical. To explain this finding, we calculate the free energy landscape

$$
\Delta G(\phi, \psi) = -k_B T\ln P(\phi, \psi) - \ln P_{\text{max}},
$$

where $P$ is the probability distribution along the dihedral angles of the Aib peptide and $P_{\text{max}}$ denotes its maximum, which is subtracted to ensure that $\Delta G=0$ for the lowest free energy minimum. Exploiting the fact that the fluctuations of the inner residues are quite similar, we averaged $P(\phi, \psi)$ over the residues $n=3, \ldots, 6$, i.e., $P(\phi, \psi)=\sum P(\phi_n, \psi_n)$.

Figure 2 shows the resulting free energy landscape of the Aib peptide as obtained from the MD simulations at 220 and 300 K. Starting with a left-handed $3_{10}$-helical conformation at $(\phi, \psi)\approx(-40^\circ, -40^\circ)$, at 220 K the system is essentially trapped in this conformation on our simulation time of several 100 ns. Only a very small part of the time, the peptide adopts a structure with positive values of $\phi$, which indicates a right-handed conformational state. At 300 K, on the other hand, we find that the peptide has sampled both left- and right-handed conformations with similar probability. This is because—similar to glycine with two hydrogen atoms at C$_a$—the Aib peptide with two CH$_3$ groups at C$_a$ is a non-chiral peptide. The symmetric shape of the energy landscape readily explains the similar behavior of the $\phi$ and $\psi$ fluctuations in Fig. 1. Figure 2 moreover reveals that the rise of the rms fluctuations in Fig. 1 is to a large extend caused by the coexistence of left- and right-handed conformational states.

Nonchiral peptides occur in experiment as a 50/50 mixture of left- and right-handed species, which for our considerations show identical properties. To improve our overall sampling of the system, we therefore average the probability distribution in Eq. (10) over both chiralities via $P_{RL} = P(\phi, \psi) + P(\phi, \psi)$ and restrict $\phi$, e.g., to the left-handed interval $[-180,0]$. Hence we concentrate on the transition between closed and opened structured peptide within one chirality subspace. As there hardly is interesting structure left along the $\phi$ coordinate, we furthermore may average the resulting distribution over $\phi$. This way we obtain a one-dimensional representation $\Delta G(\psi)$ of the energy landscape which is shown in Fig. 3(a) for various temperatures. The free energy profile clearly shows three conformational states of the system, the ground state 0 at $\psi=-40^\circ$ and two excited conformations 1 and 2 at $\psi=40^\circ$ and 100$^\circ$, respectively. From this representation, we can extract the (minimum) free energies $\Delta G_1$ and $\Delta G_2$ of the excited states 1 and 2 ($\Delta G_0=0$) as well as the barrier heights $\Delta G_{10}$ and $\Delta G_{12}$. Plotted as a function of temperature, Fig. 3(b) reveals that state energies as well as barrier heights decrease significantly from 220 to 260 K, but stay approximately constant for higher temperatures. Whatever interaction causes this effect, it seems to affect both, state energies and barriers.

Since the left- and right-handed species are not discriminated in the experiment of Backus et al., it is interesting to
consider the rms fluctuations of the $\phi$ and $\psi$ dihedral angles averaged over both chiral states, see Fig. 4(a). As the fluctuations due to the left-handed $\rightarrow$ right-handed transitions are eliminated by the averaging, we see only a very small increase of the fluctuations in $\phi$ along which these transitions occur. Reflecting transitions along $\psi$, on the other hand, we find a step-like increase of the $\psi$ fluctuations at 260 K, which is quite similar to the behavior of the nonaveraged fluctuations shown in Fig. 1. That is, the fluctuations raise by 60% at 220–260 K and by 150% at 260–300 K. Taken together, Figs. 1–4 strongly suggest that the experimentally observed dynamical transition of the peptide helix is closely related a change in the free energy landscape.

**B. C$\equiv$O frequency**

As explained in Sec. II B, the instantaneous C$\equiv$O frequency $\nu(t)$ of a single Aib unit consists (apart from a constant) of the contributions $\delta e_M$, $\delta e_P$, and $\delta e_S$, which represent the shift of the gas-phase C$\equiv$O frequency due to the interaction with (i) the neighboring peptide units, (ii) the remaining peptide units, and (iii) the solvent, respectively. The probability distributions $P(\delta e)$ of these frequency shifts at various temperatures are shown in Fig. 5, where—for the sake of better statistics—we again averaged over the inner residues 3–6 and over both chiral states. Given as a function of the backbone dihedral angles ($\phi, \psi$), the temperature dependence of the $\delta e_M$ distribution in Fig. 5(b) directly reflects the thermal population of the excited conformational states along $\psi$ [cf. Fig. 3(a)]. As the ground and excited states correspond to easily distinguishable frequencies of 12 and $\sim$7 cm$^{-1}$, respectively, the thermal population of the excited state above the transition temperature manifests itself in a significant increase of the peak at 1710 cm$^{-1}$. Moreover, the population of the excited conformational state causes an overall redshift of the distribution with increasing temperature. Hence the contribution $\delta e_M$ to the C$\equiv$O frequency represents a versatile reaction coordinate that monitors the conformational dynamics of the peptide.

The contribution $\delta e_P$ due to the non-next-neighbor peptide groups amounts to a large overall redshift of about 50 cm$^{-1}$ of the C$\equiv$O frequency [Fig. 5(c)]. The shift reflects the existence of $3_{10}$ helix-stabilizing hydrogen bonds between the O atom at residue $n$ and the H atom at residue $n+3$. With increasing temperature, this redshift becomes somewhat smaller due to the weakening of these hydrogen bonds. Compared to $\delta e_P$, the overall redshift $\delta e_S$ due to the chloroform solvent is significantly smaller [$\sim$10 cm$^{-1}$, see Fig. 5(d)]. This result is, however, at least in part an artifact of the applied frequency model, which was originally designed for polar solvents and therefore only considers electrostatic interactions rather than Lennard-Jones interactions, which are certainly important for the almost nonpolar solvent CHCl$_3$. Again, we find that this redshift becomes weaker with increasing temperature. Put together, the distribution of the instantaneous C$\equiv$O frequency $\nu(t)$ shown in Fig. 5(a) exhibits an overall width of about 50 cm$^{-1}$. In agreement with the experiments of Backus et al.,$^{20}$ the distribution...
shifts slightly to higher frequencies with increasing temperature, which reflects the weakening of the hydrogen bonds that stabilize the 3_{10}-helix.

To study to what extent the width of the C=O frequency distributions reflects the fluctuations of the temperature-averaged $\psi$ angles, Fig. 4 compares these fluctuations (a) to the rms fluctuations of the various frequency contributions (b). Overall, we find that the fluctuations of the frequency shifts $\delta \epsilon_m$ and $\delta \epsilon_p$ are quite similar to the $\psi$ fluctuations. At 240 K, though, we find enhanced frequency fluctuations, which are caused by fluctuations of the end groups (residues 1, 2, and 7) that may affect $\delta \epsilon_p$ and $\delta \epsilon_m$ [via Eq. (2)]. The fluctuations of the solvent shift, on the other hand, stays virtually constant with increasing temperature ($\delta \epsilon_S \approx 24$ cm$^{-1}$, data not shown), which again might be a consequence of the inappropriate frequency model. At all temperatures, we note that the total fluctuations $\delta \epsilon_{\text{MSP}} = (\delta \epsilon_m + \delta \epsilon_p + \delta \epsilon_S)^2$, are significantly smaller than the sum of the fluctuations $\delta \epsilon^2_m + \delta \epsilon^2_p + \delta \epsilon^2_s$, which indicates a strong cross-correlation between the various contributions. We note that this anticorrelation prevents a clear biphasic temperature behavior of the total fluctuations $\delta \epsilon_{\text{MSP}}$.

To study the time scales of the C=O frequency fluctuations, we next consider the (normalized) frequency fluctuation correlation function

$$C_i(t) = \langle \delta \epsilon_i(t) \delta \epsilon_i(0) \rangle / \delta \epsilon_i^2 = \sum_j w_j^{(k)} e^{-\tau_1 j(t)}$$

with $k = M, S, P$, and MSP. Figure 6 shows a logarithmic representation of these functions at 220 and 300 K, which reveals the existence of several time scales. A multiexponential fit of $C_{\text{MSP}}(t)$ at 220 K, for example, yields four decay times: a femtosecond time, $\tau_1 \approx 60$ fs, with a weight of $w_1 = 54\%$, 2 ps time scales, 8 ps (15%), and 70 ps (17%), and a nanosecond time $\tau_2 \approx 1$ ns (14%). Increase of the temperature to 300 K changes mainly the femtosecond decay time (see below). A closer analysis of the MD trajectory reveals that the femtosecond and picosecond time scales correspond to various motions of the Aib peptide in a single energy basin, see the free energy curve in Fig. 3(b). Transitions between various conformational states of this landscape occur on a nanosecond time scale. Due to the averaging over both chiral states, the analysis does not consider transitions between the left- and right-handed states, which occur on a time scale of 10–100 ns. Hence we find that the C=O frequency fluctuations are a sensitive probe that monitors the conformational dynamics of the peptide over multiple time scales.

Since the overall shift $\delta \epsilon_{\text{MSP}}$ is dominated by $\delta \epsilon_p$, the correlation function of the latter contribution exhibits similar decay times as $C_{\text{MSP}}(t)$. The remaining two contributions, $\delta \epsilon_m$ and $\delta \epsilon_S$, differ mainly in their femtosecond components. $C_m(t)$ shows a similar decay time $\tau_1$ but with a significantly lower weight (13%) than $C_{\text{MSP}}(t)$, and the remaining weight is mostly assigned to a 100 ps decay. The solvent correlation function, $C_s(t)$, on the other hand, exhibits a significantly slower initial decay, $\tau_1 \approx 0.5$ ps (20%) while the remaining decay components are similar to the ones of $C_{\text{MSP}}(t)$. The slow first time scale is a consequence of the relatively large mass of the CHCl$_3$ solvent molecules as well as of the fact that we use a rigid solvent model.

As explained in the Introduction, the homogeneous dephasing rate observed in the 2D-IR experiments reports on dynamics that is faster than the intrinsic time scale of the experimental of about 1 ps. Hence, only the femtosecond component of the frequency fluctuations is of interest for the calculation of the homogeneous dephasing. Figure 7 shows
the short-time evolution of this function at 220 and 300 K, which can be approximated by the monoexponential function $C(t) = w e^{-\gamma t} + (1 - w)$. The fit neglects an oscillatory transient of the correlation function, which reflects a normal mode vibration that includes a hydrogen bond that stabilizes the 310-helix. Interestingly, the resulting decay time $\tau$ rises with temperature from 0.06 to 0.09 ps, while the weight $w$ stays approximately constant. Assuming that the overall friction decreases with temperature, this indicates that high-frequency motions are weakly damped by the environment, since in the weakly damped regime the decrease of the friction leads to a slower decay of the vibration and the corresponding correlation function. This effect may be caused by the viscosity of the chloroform solvent which decreases by a factor of 3 (from 1.5 to 0.5 cP) when the temperature rises from 220 to 300 K.

Putting together the above decay times $\tau$ and the weights $w$ [Fig. 7(b)], the overall rms fluctuations $\delta e^2$ (Fig. 4) and the life time broadening of $1/2T_1 = 15$ cm$^{-1}$, we finally obtain the total homogeneous dephasing rate $1/T_2 = \Gamma^2 + 1/2T_1$ with $\Gamma = w \delta e^2 \tau$. Somewhat surprisingly, Fig. 7(c) reveals that this rate increases more or less linearly with temperature. In particular, there is no biphasic temperature behavior of $1/T_2$, although the structure fluctuations of the peptide [Figs. 1 and 4(a)] and—to a weaker extent—its frequency fluctuations [Fig. 4(b)] clearly show a rapid increase at 260 K. This is in contrast to experiment (Fig. 4 in Ref. 20), where the homogeneous broadening remains roughly constant (16 cm$^{-1}$) from 220 to 260 K, and then rises abruptly via $\approx 9$ cm$^{-1}$ at 270 and 280 K to $\approx 11$ cm$^{-1}$ at 300 K.

Several aspects might contribute to this finding. As already mentioned, the applied model for the solvent frequency shift neglects the van der Waals interactions of CHCl$_3$ with the C═O vibration, which results in a reduced redshift as well as in a too small line width of the amide I spectrum. At present, though, we are not aware of a better model for the C═O frequency shift of chloroform. While the models of the other two contributions to the frequency shift, $\delta \epsilon_M$ and $\delta \epsilon_P$, are well established and seem to yield reasonable results, it is nonetheless not obvious if these models are suited to account for the correct thermal increase of the fluctuations. Assuming, for example, that this increase is (at least partly) caused by thermally activated low-frequency modes that anharmonically couple to the C═O vibrations, it is unclear if our model for $\delta \epsilon_M$ [Eq. (2)], which is based on (zero-temperature) ab initio calculations, can account for this effect. Finally, we note that the too small increase of the homogeneous broadening may be also a consequence of the significant anticorrelation between the various contributions of the frequency fluctuations [Fig. 4(b)], which makes the total result very sensitive to even small changes of the various contributions.

C. 2D-IR spectra

In the experiment of Backus et al., the homogeneous dephasing rate was estimated from the antidiagonal width of the 2D-IR spectra. To test to what extent this approximation yields the true homogeneous dephasing, we have directly calculated the corresponding vibrational spectra as described in Sec. II C. The resulting 2D-IR spectra at 220 and 300 K are depicted in Fig. 8. Blue negative signals represent stimulated emission and bleaching signals, while the red positive signal is caused by excited state absorption. With increasing temperature the 2D signals become elongated along the diagonal and broader along the antidiagonal. Following the experiment, we took antidiagonal cuts at the maximum of the negative peaks of the 2D-IR spectra, which were normalized and shifted with respect to the antidiagonal at 300 K. Figure 8(c) shows the resulting antidiagonal cuts, which are indeed almost exclusively governed by the Lorentz-shaped peak of the homogeneous broadening. Plotting the resulting total homogeneous line width $1/T_2^{AD}$ together with the above calculated width $1/T_2 = \Gamma^2 + 1/2T_1$ versus temperature, Fig. 7 reveals that both measures of the homogeneous dephasing are apart from a small overall shift— in good agreement.
IV. CONCLUDING REMARKS

To elucidate the intriguing experimental findings of Backus et al.,20 we have performed extensive equilibrium MD simulations of a photoswitchable Aib peptide in chloroform solvent. By studying the free energy landscape of the Aib peptide as a function of temperature, we have recovered the familiar picture of glass-forming systems, which below the glass transition temperature \( T_g \) are trapped in various basins of the system’s energy landscape, while they diffuse freely between these basins above \( T_g \). As a consequence, at \( T_g \approx 270 \) K we find a sharp rise of the rms fluctuations of the \( \phi \) dihedral angles which reflects left-handed \( \rightarrow \rightarrow \) right-handed transitions of the nonchiral Aib peptide. At the same temperature we also obtain a significant increase of the fluctuations of the \( \psi \) dihedral angles which reflects conformational transitions within one chiral state. These results provide a structural basis for the experimentally observed dynamical transition of the Aib peptide, which is still missing in the case of the conventional dynamical transition in proteins such as myoglobin.

To model the experimental 2D-IR spectra, we have calculated the instantaneous frequency of an isotope labeled C––O mode. The frequency fluctuation correlation function of this vibration shows a complex relaxation behavior including a femtosecond, 2 ps, and a nanosecond decay time. Reflecting the conformational motion within and between the basins of the free energy landscape of the peptide, the C––O frequency fluctuations represent a sensitive probe of the peptide conformational dynamics. The homogeneous broadening of the C––O vibrational spectrum has been calculated (i) directly from the femtosecond frequency fluctuations and (ii) from the antiadjunct width of the calculated 2D-IR spectra (as in experiment). Apart from a small overall shift, both results are in good agreement, which suggests that the antiadjunct of the spectra is indeed a good measure of the homogeneous dephasing.

The main unresolved issue of the present work is certainly that the calculated homogeneous broadening does not follow the biphasic temperature behavior of the peptide’s conformational motion and therefore also does not match the experimental findings. Although this clearly points to some shortcoming of our description of the instantaneous C––O frequency [Eq. (1)], it is not obvious which part of the description fails, e.g., the \textit{ab initio}-based model for the next-neighbor contribution \( \delta \omega_{\text{II}}(\phi, \psi) \), the model of the frequency shift of the solvent, or—enhanced by the strong anticorrelation between the various contributions—some combination of it. Moreover, it is clear that the observed conformational transitions take place on a much longer time scale than the subpicosecond time scale of the homogeneous dephasing. The conformational transitions may therefore contribute only indirectly to the increase of the homogeneous line width, for example, due to the thermal activation of low-frequency vibrational modes that anharmonically couple to the C––O vibrations.23,50 Future work will be directed to identify these modes and also to understand the role of the solvent for the peptide dynamical transition.

ACKNOWLEDGMENTS

We thank Peter Hamm for numerous inspiring and helpful discussions. This work has been supported by the Frankfurter Center for Scientific Computing, the Fonds der Chemischen Industrie, and the Deutsche Forschungsgemeinschaft.


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at left- and right-handed conformations), we should also obtain at 220 K

a $\phi$-symmetric form of the free energy landscape. Hence, the rise of the

fluctuations along $\phi$ in Fig. 1 is to some extent a sampling artifact, which

disappears when we average over both chiralities as done in Fig. 4(a).