Water drives peptide conformational transitions

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Transitions between metastable conformations of a dipeptide are investigated using classical molecular dynamics simulation with explicit water molecules. The distribution of the surrounding water at different moments before the transitions and the dynamical correlations of water with the peptide’s configurational motions indicate that water is the main driving force of the conformational changes.

Investigations of protein dynamics have recently led to believe that water plays the major role in protein motion. There is a large body of experimental and simulation evidences [1–4] showing a close connection between the water dynamics and the protein conformations. Frauenfelder and colleagues have experimentally shown that protein dominant conformational motions are slaved by the hydration shell and the bulk solvent [5], while the protein molecule itself provides an ‘active matrix’ necessary for guiding the water’s dynamics towards biologically relevant conformational changes. The change in water dynamics at the shell of up to almost a dozen water molecule diameters around proteins is found in [6].

Despite extensive research on protein dynamics the investigations of elementary conformational motions are rare. Specific molecular mechanisms, including the involvement of water molecules, that drive the conformational moves are highly demanded as they ultimately define all rearrangements of proteins as a whole.

In this work we analyse molecular dynamics (MD) simulated peptide focusing on the moments of elementary conformational changes including explicit water molecules. We show that water indeed drives the changes, we elucidate the specific mechanisms of this phenomenon.

We study a zwitterion L-alanyl-L-alanine, Fig. 1, a very convenient model because i) the conformation of the molecule is completely defined by the two dihedral angles $\psi$ and $\phi$, ii) in water the conformation $\psi \approx 2.5$, $\phi \approx -2.2$ radians is prevalent, however very rare transitions to two other metastable conformations take place, and iii) the transitions only happen in water because of the molecule’s charged ends.

The three well separated metastable states, clearly visible on the density of states, Fig. 1, allow to introduce a simple natural discretisation of the conformational states. By also discretising time with a step $\Delta t$ the continuous MD trajectory can be converted into a string of symbols $\{s_i\}, i = 0 \ldots N$, where $s_i$ equals to ‘A’, ‘B’, or ‘C’ depending on where the trajectory point falls at the time moment $t_i$, $N$ is the number of time steps.

An important problem is how to identify the moments of transition. The described discretisation of the conformational states defines boundaries of the states. However, they delineate the density of conformations averaged over the whole trajectory. As we are interested in the dynamically metastable states, that is the configurations in which the trajectory spends significantly more time compared to the time it spends in transitions between the configurations, a dynamical model capable of identifying the transitions is required. Also, the trajectory does not go directly from one state to another, instead it winds in a complicated manner, often crossing the states boundaries many times.

One of the most popular description of protein dynamics lately is the Markov State Model (MSM) [7]. The model specifies the probabilities of each of the discrete states as well as the probabilities of the transitions between them. The MSM transition matrix can be calculated from the MD trajectory by counting the state changes and for the studied molecule it is

\[
\begin{array}{ccc}
A & B & C \\
A & 0.997 & 0.002 & 0.001 \\
B & 0.261 & 0.737 & 0.002 \\
C & 0.097 & 0.001 & 0.901 \\
\end{array}
\]
where the value at row $i$ and column $j$ gives the probability of going to state $j$ being currently at state $i$. MSM provides many useful quantities describing the system [7]. In particular, it tells that the transition $A \rightarrow B$ happens once in 2.8 ns on average. We will concentrate on this specific transition for the rest of the paper.

The moments of the $A \rightarrow B$ transitions within the MSM framework are the values of $t_i$ for which $s_i$ is equal to $B$ while being equal to $A$ at the previous time moment $t_{i-1}$. The time precision of identifying the transitions is $\Delta t$, which is insufficient for studying the transitions themselves. The model is valid only for relatively large time steps, that follows from the requirement for the transitions to be history independent (statistically uncorrelated). For this peptide the minimal valid time step is $\approx 6 \text{ps}$. This value is of the same order as the period of fluctuations within each conformational state and, most importantly, this is approximately the duration of the process of the trajectory passing from state to state. Therefore, the MSM has to be augmented in order to be able to describe the dynamics at significantly shorter time steps.

For this purpose we build a variant of the hidden Markov model using the same configurational states of the peptide. Specifically, we use the ‘$c$-machine’ by Crutchfield et al [8, 9]. Instead of the conformational states, $s_l$, themselves $(A,B,C)$ we consider the $l$-long sequences of states $\Sigma_l = \{s_{l-t+l}, \ldots, s_{l-2}, s_{l-1}, s_l\}$. The advantage of such description is that for a small time step, even if the original states are correlated over several steps, for long enough sequences $\Sigma_l$, these new states (the sequences) are uncorrelated. We, therefore, can build a Markov model on these new states.

The Markov property on the original states is fulfilled for $\Delta t > 6 \text{ps}$. For $\Delta t = 5 \text{ps}$ the sequences of at least two time steps are required to build the hidden Markov model. The model is shown in Fig. 2. Here the states ‘0’, ‘2’, and ‘1’ correspond to the conformational states $A$, $B$, and $C$ since they mostly consist of the sequences $0 \equiv AA$, $2 \equiv BB$, and $1 \equiv CC$ respectively. An additional state ‘2’ describes the transition process from $A$ to $B$. It consists of the sequence $AB$ and has two main transitions from it. With the probability 0.643 the following symbol is $B$ and the next state is 3 which means that the system is transferred to the conformational state $B$. There is, however, a significant probability of 0.351 for the next symbol to be $A$, which describes the return to state 0 or the original conformation $A$.

This analysis illustrates the advantage of the hidden Markov model: it elucidates the mechanism of the $A \rightarrow B$ transition. It also explains where the non-Markov property comes from and gives the time scale limit at which different pathways of the conformational transition start to differentiate from each other.

Using the hidden Markov model the time step can be reduced to 0.3 ps, the model is described in Fig. 3. The transitions are the moments when the system enters state 9 being before in any other state. The model predicts that the transitions last on average for $\approx 1 \text{ps}$, Fig. 3, right. Thus, the 0.3 ps precision in identifying them is satisfactory. This gives us a tool to investigate what happens at different moments before the transition. In particular, we can study the behaviour of water. For this we collect the time frames at specific times before the transitions, Fig. 4.

We calculate the density of oxygen (hydrogen) atoms by averaging over the selected time frames. The obtained field $\rho(\mathbf{x}, t)$ gives the probability of finding water atoms at various locations $\mathbf{x}$ around the peptide at times $t$ in advance of the transition.

The transition from conformation $A$ to conformation $B$ corresponds to a $\approx 180^\circ$ flip of the $NH_3$ group (the right hand side of the molecule in Fig. 5). Both ends of the
FIG. 3: Left: the $\epsilon$-machine for the time step 0.3 ps, the length of the sequences is $l = 4$, state '0' corresponds to the original state 'A' (mostly consists of the sequences $AAAA$), similarly, state '9' corresponds to 'B'; state '9' can only be reached from state '0' via the states '2', '7', '4', '8' that describe the mechanism (pathways) of the $A \rightarrow B$ transition; right: three typical cases during the transition: direct transition (top, probability 0.61), transition with several recrossings (middle, probability 0.04), failed attempt of transition (bottom, probability 0.07), the probabilities of these cases are given assuming that the probability of going from state '0' to state '9' by any possible route is 1.

FIG. 4: Collecting the time frames for the ‘time before transition’ statistics; the dots on the ‘time’ axes are the transition moments.

molecule possesses charges, negative on the $CO_2$ and positive on the $NH_3$ sites, which leads to relatively strong attachment of water molecules at the ends. Hydrogen bonded water molecules to the oxygens and to the hydrogens form the dense areas of water corresponding to more rigid hydrogen bonds network, that is more stable structures.

This is an intuitively clear result. More interesting is the moment just before the transition, Fig. 5, middle. Even though the overall structure of the dense areas remains similar to the $A$ state, evidently, the total area of the dense water is significantly reduced. We quantify this decrease by measuring the volume of the dense areas depicted in Fig. 5. The result as the function of time before the transition is given in Fig. 6. During the time period when the rotation of the $NH_3$ group is most significant, from $\approx 9$ps to $\approx 1$ps, the size of the dense areas of water is more than 2 times for oxygen and 3 times for hydrogen smaller compared to those during the stable periods of conformation $A$. This corresponds to more diffuse character of the hydrogen bonded network of water molecules as they tend to appear at different, less concerted locations for different transitions.

However, the most surprising effect following from this analysis is that the density of water starts reducing as early as $\approx 50$ps in advance of the transition. This is almost 10 times earlier than the actual conformational change of the peptide!

The above analysis reveals the changes in water densities but it does not show which part of it is directly correlated with the changes in the peptide angles. We analyse the dynamical correlations between the dihedral angles and the water density using the Linear Stochastic Estimation (LSE) model (originally developed for visualising coherent structures in turbulent flows [10] and the identification of noise sources in turbulent jets [11]).
The density field $\rho(x, t)$ is converted to a time fluctuating field by subtracting the time average $\bar{\rho}(x)$: $\rho'(x, t) = \rho(x, t) - \bar{\rho}(x)$. In the LSE framework an approximation $\hat{\rho}(x, t) = \alpha \hat{\phi}(t) + \beta \hat{\psi}(t)$ to $\rho'(x, t)$ is found that minimises the residual error $\langle \rho' - \hat{\rho} \rangle_t$ [12], where $\hat{\phi}(t), \hat{\psi}(t)$ are the angles averaged over all the frames at time $t$ and $\alpha, \beta$ are constants. $\hat{\rho}(x, t)$ represents the density time fluctuations correlated with the angles. It is calculated by solving for $\alpha, \beta$ the system of linear equations, obtained from $\langle \hat{\rho}'(x, t) \hat{\phi}(t) \rangle = \langle \{ \alpha \hat{\phi}(t) + \beta \hat{\psi}(t) \} \hat{\phi}(t) \rangle$ (and similarly for $\hat{\psi}(t)$) in the assumption of the zero mean of the uncorrelated part of the density fluctuations:

$$\begin{align*}
\alpha \langle \hat{\phi}(t) \hat{\phi}(t) \rangle + \beta \langle \hat{\psi}(t) \hat{\phi}(t) \rangle &= \langle \rho'(x, t) \hat{\phi}(t) \rangle \\
\alpha \langle \hat{\phi}(t) \hat{\psi}(t) \rangle + \beta \langle \hat{\psi}(t) \hat{\psi}(t) \rangle &= \langle \rho'(x, t) \hat{\psi}(t) \rangle.
\end{align*}$$

The density fluctuations field $\rho'(x, t)$ and its part correlated with the peptide’s conformation $\hat{\rho}(x, t)$ are shown in Fig. 7 for several representative time moments.

The water fluctuations (right column) are significantly stronger just before the transition (2.4ps) compared to the stable period ($\approx 100$ps). Very surprisingly the conditionally averaged water fluctuations (left column) are virtually uncorrelated with the peptide at all times except for the short period immediately before the transition (some fluctuations are also noticeable as early as $\approx 20$ps before the transition, which agrees with the onset of the density decrease in Fig. 6). Interestingly, at 0ps, when the transition process is complete, the water density becomes uncorrelated with the peptide, similar to the stable periods. However, the fluctuations of it remain strong, only slightly weaker than at 2.4ps. We explain this effect by the large inertia of the water shell, that needs relatively long time for the fluctuations to settle down. The fact that these post-transition water fluctuations are decoupled from the peptide emphasizes the discovered phenomenon of strong water-peptide interactions precisely during the transition process.

Summarising, we have found that (i) $\approx 5$ps before the transition, when the dihedral angles change the most, the water density significantly reduces; (ii) the change of water density begins at $\approx 50$ps before the transition, 10 times earlier than the changes in the angles (iii) during the transition the dynamics of water density becomes highly correlated with the dynamics of the angles; and (iv) these correlations are completely absent during the stable conformation periods.

We conclude that water and the peptide behave as an integral dynamical system. During the conformational transition the peptide and the surrounding water undergo transitions together. This is in contrast to the metastable periods when their dynamics is essentially decoupled. The transition is characterised by a more diffuse hydrogen bonds network of water. The changes in the peptide are substantially delayed in time. Thus, it is likely that water drives the whole process of conformational transitions.

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FIG. 7: The $xy$ cross-section (the value of the $z$ coordinate is chosen such that the cutting plane passes through the centre of mass of the peptide) of the density fluctuation field $\rho'(x, t)$ (right), and its part correlated with the peptide’s conformation $\hat{\rho}(x, t)$ (left) for oxygen; the time before transition are, from top to bottom, 94.8ps, 21ps, 2.4ps, and 0ps; the density scale is restricted to the $-0.2 \ldots 0.2$ interval for clarity, the maxima of the peaks reach the values of 0.78 and -0.42.