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AN ANALYSIS OF THE ENERGETIC ROUGHNESS OF PROMINENT WATER MODELS BY MOLECULAR DYNAIMCS SIMULATIONS

by

QUENTIN JOHNSON

Under the Direction of Dr. Donald Hamelberg

ABSTRACT

Water is the most common solvent for most biological reactions, therefore it is vital that we fully understand water and all its properties. The complex hydrogen bonding network that water forms can influence protein-protein and protein-substrate interactions and can slow protein conformational shifts. Here, I examine an important property of water known as energetic roughness. The network of interactions between individual water molecules affect the energy landscape of proteins by altering the underlying energetic roughness. I have attributed this roughness to the making and breaking of hydrogen bonds as the network of hydrogen bonds constantly adopts new conformations. Through a novel computational approach I have analyzed five prominent water models and have determined their inherent roughness to be between 0.43 and 0.62 kcal/mol.

INDEX WORDS: Abstract, Thesis, Energetic Roughness, Department of Chemistry, Quentin Johnson, Masters degree, Georgia State University

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by

QUENTIN JOHNSON

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in the College of Arts and Sciences

Georgia State University

2010

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Quentin Johnson

2010

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Electronic Version Approved

Office of Graduate Studies

College of Arts and Sciences

Georgia State University

May 2010

DEDICATION

I would like to take this opportunity to dedicate this manuscript to Dr. Donald Hamelberg, who introduced me to the field of computational chemistry and has been a constant inspiration for me. Dr. Hamelberg has not only been my advisor throughout this process but has also been a valued friend and mentor.

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I am very appreciative for the boundless friendship, advisement and acceptance that I have incurred throughout my stay here at Georgia State University. I would like to thank Dr. Donald Hamelberg for the direction he has given me and all the help he has afforded me over the years. Also, a thank you goes to Adam Velasquez for his friendship and pressure lifting attitude that helped me make it through the hard days. Urmi Doshi deserves my thanks for the help she gave me and also for her stern but constructive criticism. I would also like to thank Jennifer Kelley for providing assistance and experience in the lab. Thank you Yao Xin for being a soft spoken leader in the lab giving me an idea of how to roll with the punches. Thank you to Lauren McGowan for giving me someone to identify with in the lab and providing a few laughs in times of need.

I would also like to extend my thanks to Dr. Alfons Baumstark for his excellent advisement, for working hard to help me achieve this goal and for introducing me to Dr. Hamelberg. Thank you to Will Lovett for his help every semester and for making sure that I graduated. A special thank you goes to the entire department for helping me get to this point and for accepting me into the fold.

I would like to thank my mother Sylvia Johnson, sister Monique Johnson, and father George Johnson for believing in me and providing support throughout my college career. I can always count on my family to make a vacation a vacation. I appreciate all the holidays I spent with you all and am only sorry that in my pursuit of education I have not had more time for the most important part of life, family.

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LIST OF ABBREVEATIONS

fs femto seconds

ns nano seconds

ps pico second

MD molecular dynamics

AMBER Assisted Model Building with Energy Refinement

CHAPTER 1

INTRODUCTION

Purpose of Study

Water plays a very important role in the dynamics and function of proteins, and the network of interactions between individual water molecules affects the energy landscape of proteins by altering the underlying energetic roughness. The purpose of this study is to mathematically evaluate the amount that water contributes to this "roughness" in hopes to better understand the functions of proteins and assess the differences of the water models.

Expected Results

I expect that the effect of the interactions of water on the overall energy landscape by alteration of the energetic roughness will be substantial considering that that hydrogen bond network of water is intricate and vast. The energetic component of hydrogen bond network rearrangements has been estimated to be between 0.8 and 1.5 kcal/mol using x-ray absorption spectroscopy. This value represents the average thermal energy required to distort a hydrogen bond or to rearrange or change the fully coordinated configuration of water to a configuration with a broken hydrogen bond to the donor. I expect that the contribution of water's energetic roughness will fall somewhere in this range, approximately 1 kcal/mol.

Molecular Dynamics Background

Molecular dynamics (MD) is a type of computer simulation that combines chemistry, math and physics to simulate the motions and interactions of atoms, molecules, and biomolecules. Atoms and molecules are set up to interact for a period of time by approximations of known physics, in order to give a view of the motion of the molecules. This type of simulation

is frequently utilized in the study of proteins and other biomolecules. It is possible to take snapshots of crystal structures and probe features of the motion of molecules through nuclear magnetic resonance NMR, however no conventional experiment allows access to all the time scales of motion with atomic resolution. Molecular dynamics normally employs crystal structure from the Protein Data Bank or PDB as the starting structures of a multitude of biomolecules and adds velocities and coordinates through a combination of complex algorithms, physical chemistry and physics. Molecular dynamics allows scientists to visualize the motions of individual atoms in a way that is not possible in laboratory experiments. Molecular dynamics is based on statistical mechanics and uses a potential energy function with multiple components to define a system as well as the time-dependent behavior of the system.

$$E = E_{bondstretch} + E_{angle\text{-}bend} + E_{dihedral} + E_{other} + E_{nonbonded}$$

(Equation 1.1: General form of potential energy formula)

$$V(r^N) = \sum_{\text{bonds}} \frac{1}{2} k_b (l - l_0)^2 + \sum_{\text{angles}} k_a (\theta - \theta_0)^2$$
$$+ \sum_{\text{torsions}} \frac{1}{2} V_n [1 + \cos(n\omega - \gamma)]$$
$$+ \sum_{j=1}^{N-1} \sum_{i=j+1}^{N} \left\{ \epsilon_{i,j} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - 2 \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} \right\}$$

(Equation 1.2: Amber force field function-Note that despite the term force field, this equation defines the potential energy of the system; the force is the derivative of this potential with respect to position.)

The MD method relies on the assumption that statistical ensemble averages are equal to time averages of the system. This assumption is known as the Ergodic hypothesis. MD simulations are usually done over short time scales, pico to nano seconds, because longer simulations require extreme computational power and can be very computationally expensive.

Energetic Roughness Background

While the concept is relatively abstract, it is a physical anomaly and can be defined through mathematic means. Energetic roughness results in the slowing of bimolecular motion by restricting the biomolecule through bumps in the energy landscape, during conformational transitions for example. Somewhat like speed bumps in a road slowing a car from moving from place to place. Conformational transitions of biological molecules play a very important role in

sub-cellular processes. However, switching between different ensembles of protein conformations that are important for function can result in complex dynamics due to the complexity of the energy landscape . The energy landscape of proteins is very rugged and represents a huge number of conformational states, including the surrounding water molecules that are an integral part of biomolecular structure . The unevenness of the energy landscape could lead to kinetic traps if it is comparable or much greater than k_BT, where k_B is the Boltzmann constant and T is the temperature. Theories have been developed to highlight the nature of the inherent roughness of the energy landscape of biomolecules, and experiments have been proposed and carried out to measure this property . The ruggedness of the energy landscape is due mainly to intra-protein, protein-water, and water-water interactions that are formed and broken in different substates or as the protein undergo large conformational changes between two different substates. Characterizing the nature of the underlying landscape roughness and calculating the magnitude of the different contributions will provide a better insight into the dynamics and function of proteins.

Here, we have calculated the effect of water on the energy landscape of proteins using molecular dynamics simulations and by developing a model based on the position space analog of the Ornstein-Uhlenbeck process to extract the contribution of the energetic roughness. This approach has allowed us to study an important property of the widely used atomistic simulation water models that directly affects the dynamics of biomolecular systems. When the roughness, which is calculated to be around ~1 kcal/mol, is much greater than k_BT, the network of hydrogen bonds could increase the frustration on the landscape of proteins. Also, this aspect of protein dynamics could have much broader implications on the function of some classes of enzymes as the topology of the landscape is altered when the substrate changes environment.

Water Roughness

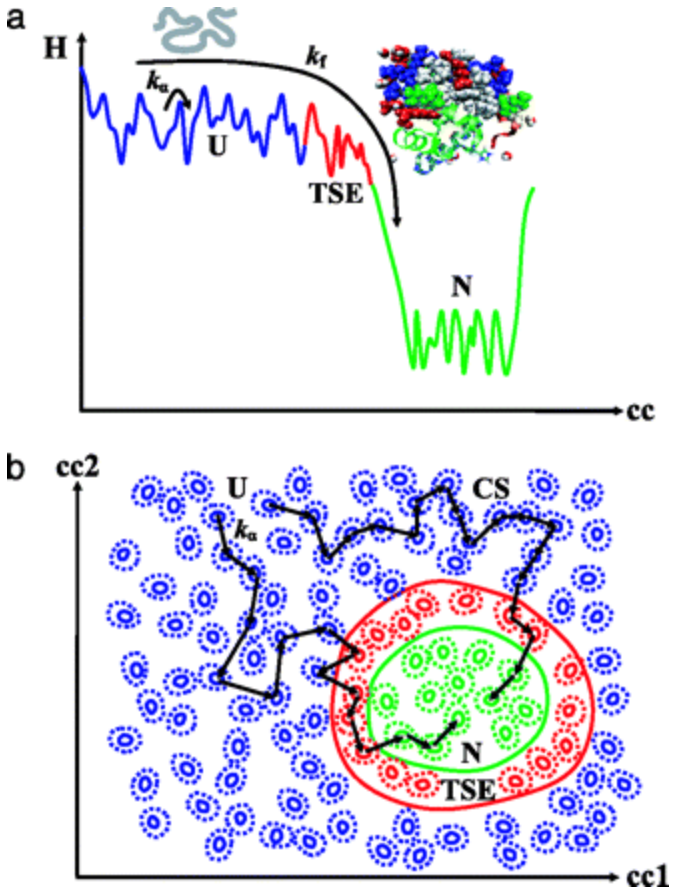
In addition to the ruggedness of energy landscape of protein, water also has a rugged potential energy landscape, with each point representing a different hydrogen bonding network. Therefore, water by itself has slow dynamics, since it has to form and break hydrogen bonds as the network of hydrogen bonds rearranges . The energetic component of hydrogen bond network rearrangements has been estimated to be between 0.8 and 1.5 kcal/mol using x-ray absorption spectroscopy. This value represents the average thermal energy required to distort a hydrogen bond or to rearrange or change the fully coordinated configuration of water to a configuration with a broken hydrogen bond to the donor. However, the exact structure of liquid water and the average thermal energy associated with hydrogen bond rearrangements are still unresolved and controversial. The quality of the data and the interpretation of the results have been questioned. Nonetheless, forming and breaking of the hydrogen bonds of water molecules around proteins will undoubtedly have an effect on the dynamics of proteins that will show up as an energetic component on the overall protein energy landscape. This process can lead to slaving of biomolecules by the solvent.

Slaving

Protein motions can be slaves to explicit solvent fluctuations. Basically, a surrounding solvent of a protein controls the rate of motion/folding. Frauenfelder and coworkers postulate that protein folding has the same temperature dependence as the α-fluctuations in the bulk solvent but is much slower. Large-scale protein motions follow the solvent fluctuations with rate coefficient $k\alpha$ but can be slower by a large factor. Slowing occurs because large-scale motions proceed in many small steps, each determined by $k\alpha$.

$$k_\alpha(T) = \frac{G_0(T)}{\eta(T)}, \qquad [3]$$

(Equation 1.3: Slaving equation. 'Frauenfelder')



(Figure 1.1: A schematic description of protein folding. 'Frauenfelder')

In Figure 1.1 the real space representation of protein folding, the unfolded polypeptide (U) folds into the working protein (N). In conformational space, the protein makes a random walk through the high-dimensional energy landscape. (*a*) A 1D cross-section through the energy landscape showing the U (blue), TSE (red), and N (green) conformational basins. The long arrow represents a folding path with an overall rate k_f, whereas the short arrow shows a single step, with a rate k_α, in the conformational diffusion during folding. (*b*) A 2D cross-section through the energy landscape illustrating two different paths for the folding motions of proteins. Starting

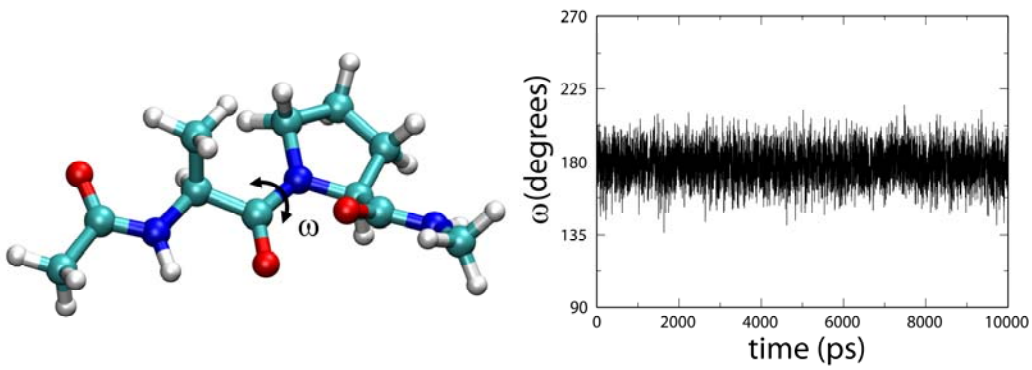
from a U conformation, proteins make a Brownian walk in the conformational space until they finally fall into the ensemble of N substates.

Importance of Study

The role of water on protein dynamics has been studies extensively . As proteins undergo constant thermal motions, they also displace the surrounding water molecules as they change conformational states. The effect of the viscosity of the aqueous medium on the motions of proteins is manifested, at the microscopic level, by water molecules that form a dynamic network of hydrogen bonds. This network of hydrogen bonds constantly rearranges as proteins move from one conformational state to another. Breaking and reforming of these hydrogen bonds contribute to the roughness on the overall energy landscape of the protein and could enslave the motion of proteins, especially at low temperature. The magnitude of the roughness due to hydration on the energy landscape is not well characterized. A full understanding of this aspect of protein dynamics has much broader implications, such as the dynamic effects of de-solvated molecules relative to that of solvated molecules, the low temperature behavior of solvated biomolecules, and the fundamental nature of water hydrogen bond network.

In order to quantitatively capture the energetic effect of water on the landscape of proteins, we have developed a model and presented a novel approach to tease out the extent to which the (energetic) roughness of water influences the energy landscape and the dynamics of proteins. We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme (Figure 1) at different temperatures in four widely used explicit simulation water models (TIP3P, SPC/E, TIP4P-EW, and TIP5P) using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at

the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen.



(Figure 1.2: Depicts the Ace-Ala-Pro-Nme, shows the omega angle used in dihedral data. Also gives a plot of ω versus time.)

CHAPTER 2

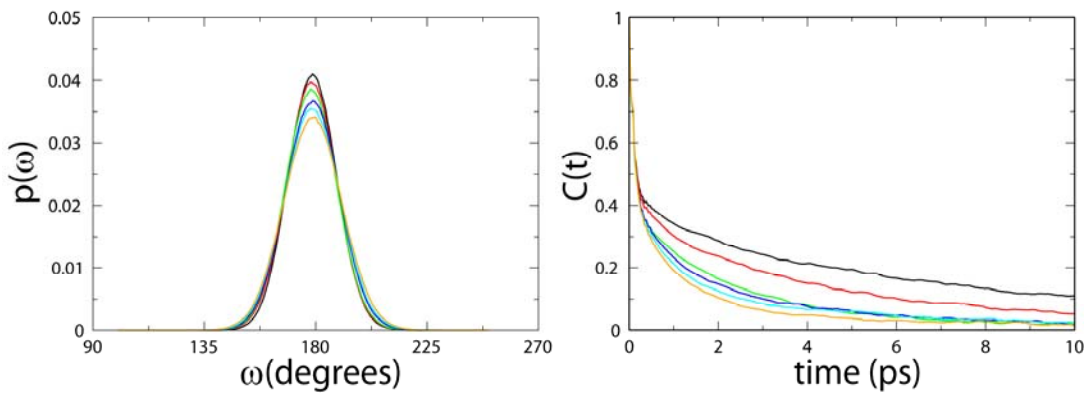
RESULTS

The dynamics of the ω-bond angle (CA-C-N'-CA') of Ace-Ala-Pro-Nme, shown in Figure 1.2, were monitored during the course of the simulation. If the velocity autocorrelation function along the ω-bond angle has a much shorter characteristic timescale compared to that of the displacement of ω, which is the case for biomolecular dynamics in general, we can use the diffusive motion of ω on an effective one dimensional energy profile, U(ω), to describe the actual complicated motion of the peptide along that degree of freedom. The diffusive motion of the peptide on this effective 1D energy profile is generally described by the Smoluchowski equation,

$$\frac{\partial p(\omega,t)}{\partial t} = D\frac{\partial}{\partial \omega}\left[\frac{\partial p(\omega,t)}{\partial \omega} + \frac{p(\omega,t)}{k_B T}\frac{\partial U(\omega)}{\partial \omega}\right]$$

(Equation 2.1: Smoluchowki equation)

where $p(\omega,t)$ is the time-dependent probability distribution of the peptide ω-bond angle, and the diffusion coefficient D is assumed to be independent of ω. The probability distribution of the ω-bond angle in the energy basin of the *trans* configuration is approximately Gaussian, as shown in Figure 2.1.



(Figure 2.1: The distribution of the peptide ω bond between Ala and Pro in Ace-Ala-Pro-Nme at six different temperatures (275, 300, 325, 350, 375, and 400 K) for SPC/E.)

Therefore, the effective 1D potential landscape, U(ω), of the motion of the peptide along the ω-bond angle in the *trans* basin is approximately described by a harmonic potential where C is the effective spring constant and $\gamma \approx 180^{o}$

$$U(\omega) = \frac{C}{2}(\omega - \gamma)^2$$

(Equation 2.2: harmonic potential)

Furthermore, the Brownian motion in a harmonic potential described as a position space analog of the Ornstein-Uhlenbeck (OU) process has been studied extensively, and the autocorrelation function of ω is given by

$$< \omega(0)\omega(t) > = < \omega^2 > \exp(-tDC/k_B T)$$

(Equation 2.3: autocorrelation function)

where

$$< \omega^2 >= k_B T/C$$

(Equation 2.4)

The autocorrelation functions of ω at six different temperatures (275, 300, 325, 350, 375, and 400 K) calculated from the simulation data in the SPC/E water model are also shown in Figure 1.3. Fitting the tail of the autocorrelation functions in Figure 1.3 using Equations 1.6 and 1.7 to a single exponential, we obtained D, the diffusion coefficient, of the displacement of the ω-bond angle on the effective 1D harmonic well at different temperatures.

Previously, Zwanzig showed by analytically solving the Smoluchowski equation (Equation1.4) that the diffusion coefficient, D, on an effective 1D landscape is related to the underlying energetic roughness, ε, by Equation 2.5.

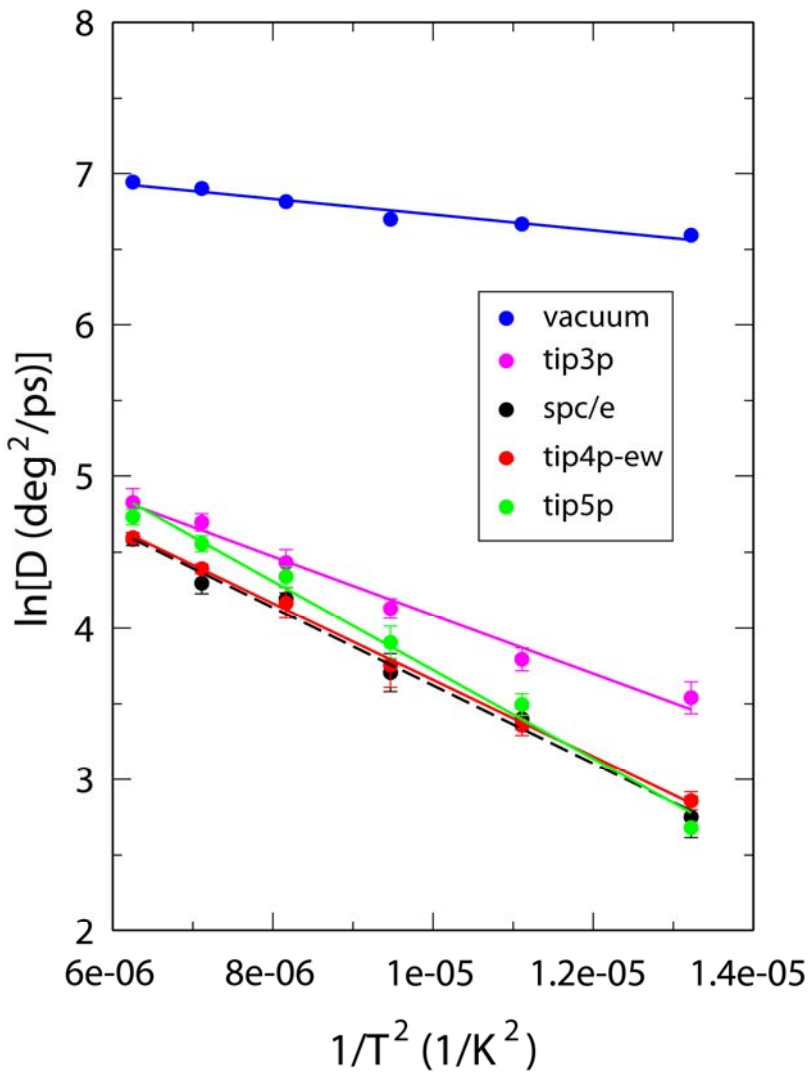
$$D = D_0 \,\mathbf{exp[-(}\varepsilon/k_B T\mathbf{)}^{\theta}\mathbf{]}$$

(Equation 2.5)

Where D_0 is the diffusion coefficient on the smooth potential energy surface. Subsequently, it was shown that for a protein system in an effective 1D energy profile θ = 2 , and the quadratic dependence in Equation 3 implies that the energetic roughness is random and has a Gaussian

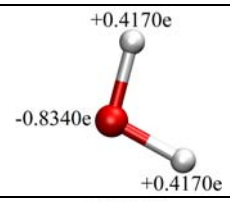
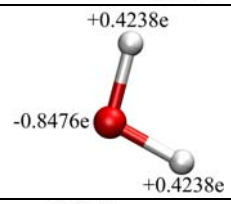
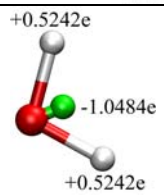
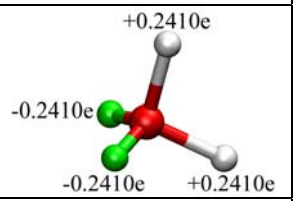
distribution. If the energetic roughness is θ = 1, then the roughness is uniform and evenly distributed.

From the diffusion coefficients, D, derived from fitting the autocorrelation function to an OU process at different temperatures, we obtain a plot of equation 2.5, as shown in Figure 2.2 for the simulations in the different water models, and also in vacuum.



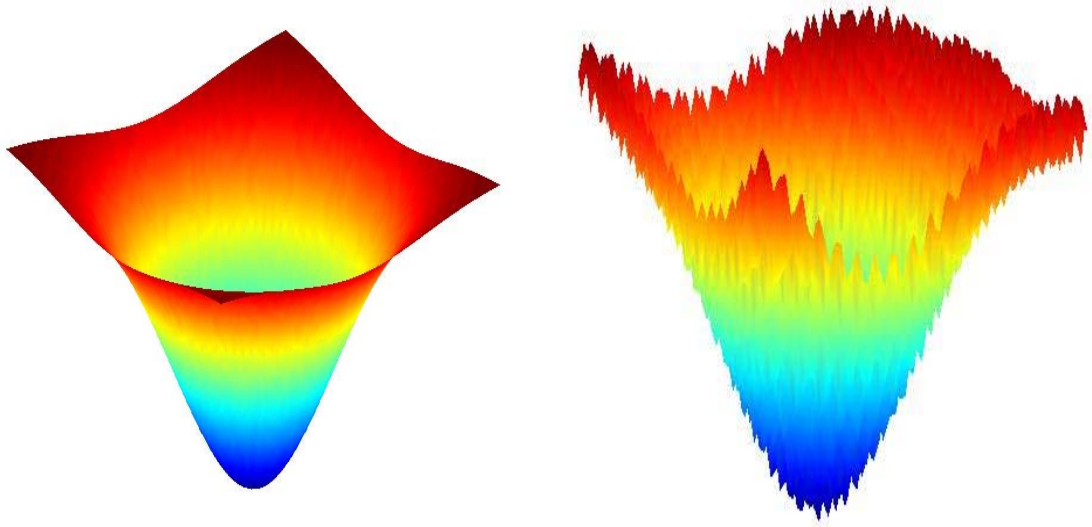
(Figure 2.2: Plot of equation 2.5 for vacuum, TIP3P, TIP4P, SPC/E and TIP5P)

The data fits very well when $\theta = 2$. We further confirmed that $\theta = 2$ is a better fit than $\theta = 1$ by increasing the temperature range and carrying out simulations in TIP4P-EW at 600 K. From Figure 2.4, we calculated the roughness for the different water models and vacuum (the baseline) as shown in Figure 2.3.

Water model	Roughness, ε (kcal/mol)	Self-diffusion coefficient ($\times 10^{-5}$ cm^2/s) at ~25°C and 1 atm	Structure of water model (A ball and stick model with partial charges on each charge center)
Vacuum	0.45	-	
TIP3P	0.87	5.06 5.65	
SPC/E	1.01	2.49 2.76	
TIP4P-Ew	1.00	2.4	
TIP5P	1.08	2.62	
Experimental	-	2.23 2.3	

(Table 2.1 and Figure 2.3: Table of raw energetic roughness of vacuum, TIP3P, TIP4P. SPC/E, and TIP5P, with figure of individual water molecules.)

If one considers the roughness of Ace-Ala-Pro-Nme in vacuum as the baseline, then TIP5P contribute ~0.63 kcal/mol of additional roughness to the energy landscape. TIP4P-EW and SPC/E contribute about ~0.56 kcal/mol. The roughness contributed by TIP3P (~0.42 kcal/mol) is the smallest. Therefore, SPC/E, TIP4P-EW, and TIP5P are slightly "rougher" than TIP3P. It is also important to note that the self diffusion coefficient of TIP3P is about twice that of SPC/E, TIP4P-EW, and TIP5P, and the difference in energetic roughness could be partly attributed to that as well.



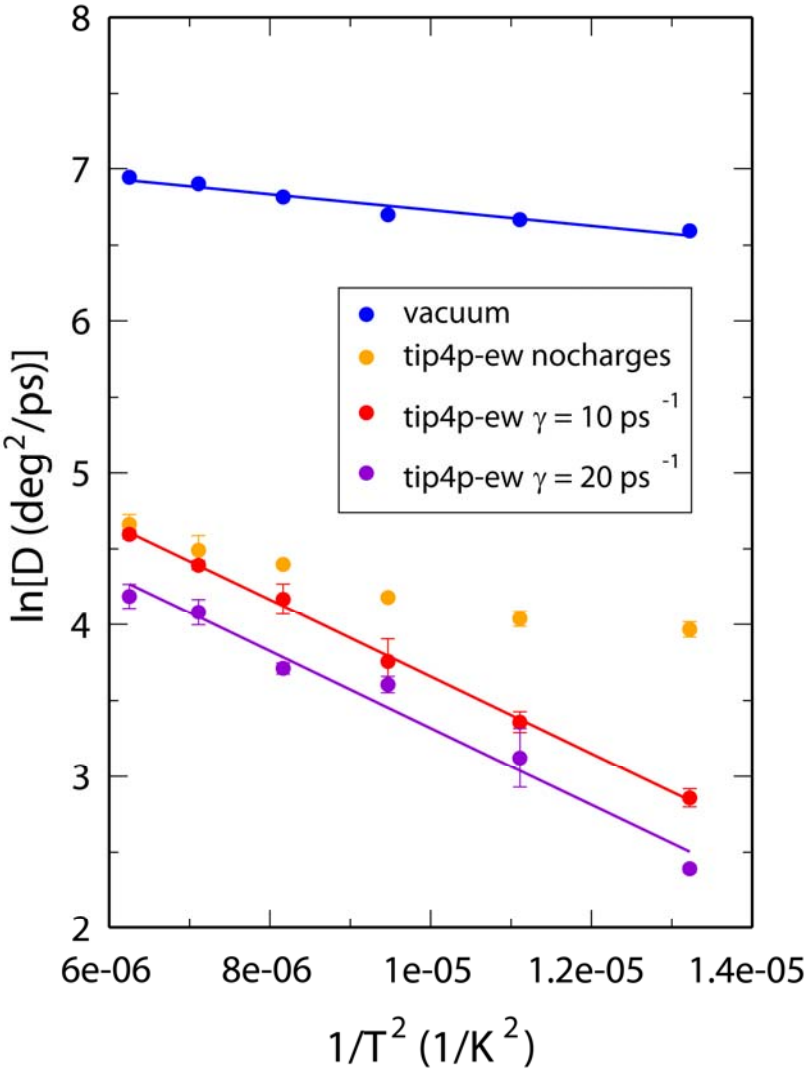
(Figure 2.4: Example of a smooth energy landscape versus a rough one.)

While Figure 2.4 is not a completely true to life representation of the energetic landscape of the peptide used in this experiment, it is useful in illustrating the difference that adding water to a system causes in the overall energetic landscape. The roughness of water are manifested as the bumps in the landscape where proteins must scale these hills to reach the next conformational state.

CHAPTER 3

DISCUSSION

The question still remains, what is the main contributing factor to the energetic roughness of water? By looking at the partial charges on the water models with three charged centers (SPC/E, TIP3P, and TIP4P-EW), one could see that the absolute magnitude of the partial charges is larger in SPC/E and TIP4P-EW than in TIP3P, and the increase correlates with a slight increase in the energetic roughness. This observation therefore raised the possibility that majority of the contribution to the energetic roughness is due to hydrogen bonding, which is electrostatic in nature in the classical definition of the water models. Alternatively, one could argue that the roughness may have artificial component due to the Langevin thermostat and the random noise associated with it. However, it is important to note that the roughness is distinctly different for the different water models under similar conditions and using the same thermostat. Nonetheless, in order to test the effect of the Langevin thermostat on the energetic roughness, we have doubled the collision frequency to 20/ps and repeated the simulation for TIP4P-EW water as shown in Figure 3.1 (magenta). We clearly see that overall the dynamics is slightly slower, as expected at higher friction, but the slope of the line and hence the roughness is almost identical to simulations with a smaller collision frequency (Figure 3.1, red line). Changing the collision frequency or fiction only affect D_0 in equation 2.5.



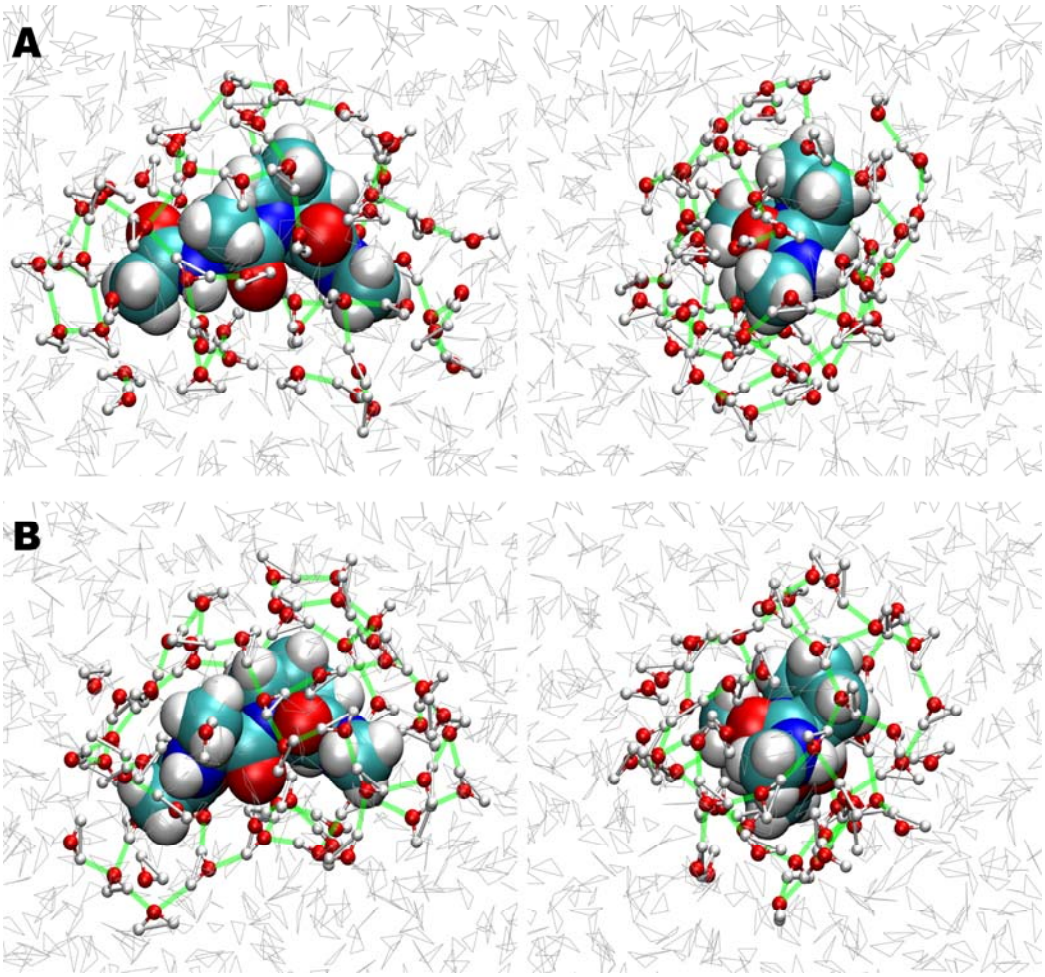
(Figure 3.1: Plot of equation 2.5 for TIP4P with collision frequency 10/ps and 20/ps, TIP4P with no charges, and vacuum)

Consequently, I have hypothesized that the main source of the energetic roughness is due to the forming and breaking of hydrogen bonding interactions between the water molecules, since the other non-bonded van der Waals interactions are far weaker. As noted above, the description of the hydrogen bonding interaction in the current classical model is purely electrostatic. Therefore, in order to test this hypothesis, we have repeated the simulation of the peptide in a modified version of TIP4P-EW water, by zeroing all the partial charges only on the water

molecules, thus eliminating the electrostatic interactions between the water molecules. The simulations were carried out with the same box sizes as those of the simulations with full partial charges at the different temperatures, in order to only capture the effect of eliminating the electrostatic interactions. For this model system of TIP4P-EW water with no partial charges, the energetic roughness is reduced considerably, as shown in Figure 3.1 (orange). We clearly see that by eliminating the partial charges on the water molecules, the slope of the line and hence the roughness also decreases considerably and is now comparable to that in vacuum. However, the data for TIP4P-EW with zero partial charges fits Equation 2.5 better with $\theta = 1$ than with $\theta = 2$, implying that without the electrostatic interactions the roughness is more uniform and evenly distributed. This change in the nature of the roughness could be attributed to the fact that van der Waals interactions are very short ranged and are due only to the oxygen, since the radius of hydrogen for these water models is zero. On the other hand, electrostatic interactions are longer-ranged, and a slight change could have implications far away, adding to the randomness of this interaction.

Using this approach, we have calculated the contribution to the roughness of proteins by the most widely used water models for atomistic simulations of biomolecules. This approach has allowed us to study one property of the solvent that directly affects the dynamics of biomolecular systems. It also presents a very important and an additional property of water that can be taken into account when optimizing simulation water models, if need be, to reproduce experimental results and its effects on protein dynamics. The network of hydrogen bonds formed by water molecules around proteins (as depicted in Figure 3.2) that is constantly rearranging as the protein changes conformation manifests itself as roughness on the underlying energy surface. The water molecules immediately around the peptide form transient, cage-like structure that is held together by the network of hydrogen bond (Figure 3.2). As the conformation of the peptide changes, the

hydrogen bonds break and re-form to alter the network of hydrogen bonds. Each arrangement of the network of water molecules is an energetic substate of the water that provides the additional roughness on the energy landscape of proteins.



(Figure 3.2: Depiction of Ace-Ala-Pro-Nme with the first shell of hydration enclosing Ace-Ala-Pro-Nme like a net. [bulk water appears in gray in the background])

Conclusion

The energetic roughness could have several implications for protein chemistry and motions. For example, when $\theta = 2$ in equation 3, the effect of the roughness on the dynamics of the protein will become very pronounced at low temperature and will enslave the motions of proteins. At higher temperature, when the roughness is much less than k_BT, the effect of water molecules on the dynamics will be predominately due to the internal frictional drag of the molecules and less on the roughness of water, since the energetic roughness is ~1 kcal/mol

Additionally, proteins that function as enzymes usually provide an environment in a cavity or binding site that is usually devoid of any appreciable amount of solvent. Taking away the specific interactions of the active site of proteins, the effect on the catalytic process of moving the substrate from the aqueous medium to the active site is still not fully understood. From the above results, it could be suggested that the change in environment of the substrate would change the frictional drag and the topological features of the energy landscape of the protein substrate or other ligand that is not limited to the reduction of the energetic roughness and thus "paving" the surface along the reactions coordinate for the conformational transition to occur. This change in the topology of the landscape would most likely manifest itself by altering the kinetic prefactor, in addition to the dominate effect due to transition state stabilization or other energetic effects that would be part of the exponential component of the kinetic equation.

CHAPTER 4

EXPERIMENTAL

TIP3P

We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme at different temperatures using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen. The system was first created using the xleap program by first constructing the Ace-Ala-Pro-Nme peptide. Next the peptide was solvated using the TIP3P water model from the xleap library. Xleap was then utilized to generate a topology file and an initial coordinate file. The topology and coordinate files were then combined with a minimization input file in the Amber10 program in the minimization step. This minimization step is used to bring the system to a global energy minimum while keeping the system in a periodic box. The minimization was carried out for 0.01 ns. A total of eighteen minimizations were done in sets of three for six different temperatures (275K, 300K, 325K, 350K, 375K, 400K).

-Minimization parameters: The minimization was carried out under the following conditions

imin- set to 1, so that a minimization will be performed

ntx-set to 1, so that X is read formatted with no initial velocity information.

npr- set to 10, so that every 10 steps energy information was printed in the output file.

ntwv- set to 0, so that all velocity output was inhibited.

ibelly- set to 0, so that no subset of atoms was allowed to move.

ntmin- set to 1, so that for NCYC cycles the steepest descent method is used then conjugate gradient is switched on.

nstlim- set to 10000, so that 10000 steps are performed.

nscm- set to 0, so that the position of the center-of-mass of the molecule is not reset.

dt- set to 0.001, so that each step takes 0.001 fs.

tempi- set to 100K, however this parameter is ignored due to this being a minimization

temp0- set to target temp, so that the minimization is carried out at this average temperature

ig- set to 71277, 11297, or 61267 depending on the run, this parameter affects the set of pseudo-random values used for Langevin dynamics. It was set to a different value for three seperate run in order to produce some variation in data for error sake.

ntp- set to 1, so that pressure is kept constant

ntb- set to 2, so that pressure is kept constant

comp- set to 44.6, compressibility that is appropriate for water

ntc- set to 1, so that SHAKE algorithm is not performed

pres0- set to 1 to keep the system a 1 bar of pressure

ntf- set to 1, so that forces between constrained bonds is calculated

cut- set to 9, so that all non bonded interactions will be cut off at 9 Angstoms

iwrap- set to 1, so that the coordinates written to the restart and trajectory files will be "wrapped"into a primary box. This means that for each molecule, the image closest to the middle of the "primary box" [with x coordinates between 0 and a, y coordinates between 0 and b, and z coordinates between 0 and c] will be the one written to the output file. This often makes the resulting structures look better visually, but has no effect on the energy or forces.

At the completion of the minimization step a new coordinate file was created (for each respective temperature). This coordinate file , deemed the restart file, was used in conjunction with the original topology file and a new input file (MD input) in the amber program to perform a molecular dynamics run to bring the system up to the target temperature. The target temperature varied from 275K to 400K. This MD run was carried out for 0.1 ns.

MD Parameters: The MD run was carried out under the following conditions

imin- set to 0, so that a minimization will not be performed

ntx-set to 5, so initial velocity information is read.

npr- set to 100, so that every 100 steps energy information was printed in the output file.

ntwv- set to 0, so that all velocity output was inhibited.

ibelly- set to 0, so that no subset of atoms was allowed to move.

ntmin- set to 1, so that for NCYC cycles the steepest descent method is used then conjugate gradient is switched on.

nstlim- set to 100000, so that 100000 steps are performed.

nscm- set to 0, so that the position of the center-of-mass of the molecule is not reset.

dt- set to 0.001, so that each step takes 0.001 fs.

tempi- set to 100K, so that the initial temperature is set to 100K

temp0- set to target temp, so that the simulation is carried out at this average temperature

ig- set to 71277, 11297, or 61267 depending on the run, this parameter affects the set of pseudo-random values used for Langevin dynamics. It was set to a different value for three seperate run in order to produce some variation in data for error sake.

ntp- set to 1, so that pressure is kept constant

ntb- set to 2, so that pressure is kept constant

comp- set to 44.6, compressibility that is appropriate for water

ntc- set to 2, so that SHAKE algorithm is performed

pres0- set to 1 to keep the system a 1 bar of pressure

ntf- set to 1, so that forces between constrained bonds is calculated

cut- set to 9, so that all non bonded interactions will be cut off at 9 Angstoms

iwrap- set to 1, so that the coordinates written to the restart and trajectory files will be "wrapped"into a primary box. This means that for each molecule, the image closest to the middle of the "primary box" [with x coordinates between 0 and a, y coordinates between 0 and b, and z coordinates between 0 and c] will be the one written to the output file. This often makes the resulting structures look better visually, but has no effect on the energy or forces.

After this step was completed another restart file was created. This restart file was used in the next step to specify where atoms should be found at the beginning of the next simulation.

So, the restart file, along with the original topology file and a new input file (equilibration input) were used to start the equilibration step in the amber program. The equilibration step was done for each set at the respective temperature. The equilibration step was carried out for 0.5 ns.

Equilibration Parameters: The equilibration step was performed under the following conditions.

imin- set to 0, so that a minimization will not be performed

ntx-set to 5, so initial velocity information is read.

npr- set to 100, so that every 100 steps energy information was printed in the output file.

ntwv- set to 0, so that all velocity output was inhibited.

ibelly- set to 0, so that no subset of atoms was allowed to move.

ntmin- set to 1, so that for NCYC cycles the steepest descent method is used then conjugate gradient is switched on.

nstlim- set to 100000, so that 100000 steps are performed.

nscm- set to 10000, so that every 1000 steps translational and rotational motion will be removed..

dt- set to 0.002, so that each step takes 0.002 fs.

tempi- set to 100K, so that the initial temperature is set to 100K

temp0- set to target temp, so that the simulation is carried out at this average temperature

ig- set to 71277, 11297, or 61267 depending on the run, this parameter affects the set of pseudo-random values used for Langevin dynamics. It was set to a different value for three seperate run in order to produce some variation in data for error sake.

ntp- set to 1, so that pressure is kept constant

ntb- set to 2, so that pressure is kept constant

comp- set to 44.6, compressibility that is appropriate for water

ntc- set to 2, so that SHAKE algorithm is performed

pres0- set to 1 to keep the system a 1 bar of pressure

ntf- set to 1, so that forces between constrained bonds is calculated

cut- set to 9, so that all non bonded interactions will be cut off at 9 Angstoms

iwrap- set to 1, so that the coordinates written to the restart and trajectory files will be "wrapped"into a primary box. This means that for each molecule, the image closest to the middle of the "primary box" [with x coordinates between 0 and a, y coordinates between 0 and b, and z coordinates between 0 and c] will be the one written to the output file. This often makes the resulting structures look better visually, but has no effect on the energy or forces.

Upon completion of the equilibration step another restart file was created. This restart file was used in the next step to specify where atoms should be found at the beginning of the next simulation. Next, the restart file along with the original topology file and a new input file (production input) were used to start the production step in the amber program. The production step was done for each set at the respective temperature. The production step was also set to output dihedral energy information for each step. This production step was done for 10 ns.

<u>Production Step:</u> The production step was performed under the following conditions.

imin- set to 0, so that a minimization will not be performed

ntx-set to 5, so initial velocity information is read.

npr- set to 100, so that every 100 steps energy information was printed in the output file.

ntwv- set to 0, so that all velocity output was inhibited.

ibelly- set to 0, so that no subset of atoms was allowed to move.

ntmin- set to 1, so that for NCYC cycles the steepest descent method is used then conjugate gradient is switched on.

nstlim- set to 5000000, so that 5000000 steps are performed.

nscm- set to 10000, so that every 1000 steps translational and rotational motion will be removed..

dt- set to 0.002, so that each step takes 0.002 fs.

tempi- set to 100K, so that the initial temperature is set to 100K

temp0- set to target temp, so that the simulation is carried out at this average temperature

ig- set to 71277, 11297, or 61267 depending on the run, this parameter affects the set of pseudo-random values used for Langevin dynamics. It was set to a different value for three seperate run in order to produce some variation in data for error sake.

ntp- set to 1, so that pressure is kept constant

ntb- set to 2, so that pressure is kept constant

comp- set to 44.6, compressibility that is appropriate for water

ntc- set to 2, so that SHAKE algorithm is performed

pres0- set to 1 to keep the system a 1 bar of pressure

ntf- set to 1, so that forces between constrained bonds is calculated

cut- set to 9, so that all non bonded interactions will be cut off at 9 Angstoms

iwrap- set to 1, so that the coordinates written to the restart and trajectory files will be "wrapped"into a primary box. This means that for each molecule, the image closest to the middle of the "primary box" [with x coordinates between 0 and a, y coordinates between 0 and b, and z coordinates between 0 and c] will be the one written to the output file. This often makes the resulting structures look better visually, but has no effect on the energy or forces.

TIP4P

We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme at different temperatures using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen. The system was first created using the xleap program by first constructing the Ace-Ala-Pro-Nme peptide. Next the peptide was solvated using the TIP4P water model from the xleap library. Xleap was then utilized to generate a topology file and an initial coordinate file. The topology and coordinate files were then combined with a minimization input file in the Amber10 program in the minimization step. This minimization step is used to bring the system to a global energy minimum while keeping the system in a periodic box. The minimization was carried out for 0.01 ns. A total of eighteen

minimizations were done in sets of three for six different temperatures (275K, 300K, 325K, 350K, 375K, 400K). Then a molecular dynamics run, equilibration and production run to calculate the dihedral data were all done on the system at the respective temperatures. All simulations in this sections were carried out under the same conditions as the conditions specified in the TIP3P section.

SPC/E

We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme at different temperatures using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen. The system was first created using the xleap program by first constructing the Ace-Ala-Pro-Nme peptide. Next the peptide was solvated using the SPC/E water model from the xleap library. Xleap was then utilized to generate a topology file and an initial coordinate file. The topology and coordinate files were then combined with a minimization input file in the Amber10 program in the minimization step. This minimization step is used to bring the system to a global energy minimum while keeping the system in a periodic box. The minimization was carried out for 0.01 ns. A total of eighteen minimizations were done in sets of three for six different temperatures (275K, 300K, 325K,

350K, 375K, 400K). Then a molecular dynamics run, equilibration and production run to calculate the dihedral data were all done on the system at the respective temperatures. All simulations in this sections were carried out under the same conditions as the conditions specified in the TIP3P section.

TIP5P

We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme at different temperatures using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen. The system was first created using the xleap program by first constructing the Ace-Ala-Pro-Nme peptide. Next the peptide was solvated using the TIP3P water model from the xleap library. Xleap was then utilized to generate a topology file and an initial coordinate file. The topology and coordinate files were then combined with a minimization input file in the Amber10 program in the minimization step. This minimization step is used to bring the system to a global energy minimum while keeping the system in a periodic box. The minimization was carried out for 0.01 ns. A total of eighteen minimizations were done in sets of three for six different temperatures (275K, 300K, 325K, 350K, 375K, 400K). Then a molecular dynamics run, equilibration and production run to calculate the dihedral data were all done on the system at the respective temperatures. All

simulations in this sections were carried out under the same conditions as the conditions specified in the TIP3P section.

TIP4P With no Charge

We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme at different temperatures using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen. The system was first created using the xleap program by first constructing the Ace-Ala-Pro-Nme peptide. Next the peptide was solvated using the TIP4P water model from the xleap library. The charges on the water molecules were then set to zero after the system was solvated. Xleap was then utilized to generate a topology file and an initial coordinate file. The topology and coordinate files were then combined with a minimization input file in the Amber10 program in the minimization step. This minimization step is used to bring the system to a global energy minimum while keeping the system in a periodic box. The minimization was carried out for 0.01 ns. A total of eighteen minimizations were done in sets of three for six different temperatures (275K, 300K, 325K, 350K, 375K, 400K). Then a molecular dynamics run, equilibration and production run to calculate the dihedral data were all done on the system at the respective temperatures. All simulations in this

sections were carried out under the same conditions as the conditions specified in the TIP3P section.

Vacuum

We carried out a series of molecular dynamics simulations using Ace-Ala-Pro-Nme at different temperatures using the pmemd module in the AMBER 10 suite of programs and the modified version of the Cornell et al force field. The Langevin thermostat was used to regulate the temperature, with a collision frequency of 10 ps^{-1} and the particle mesh Ewald method was used to treat long-ranged electrostatic interactions. The system was equilibrated at the set temperature and a constant pressure of 1 bar in a periodic cubic box with the edges of the box at least 10 Å away from the peptide. The simulation was then carried out for 10 ns at constant temperature and volume (NVT ensemble) using an integration time step of 2fs and applying the SHAKE algorithm to all bonds involving hydrogen. The system was first created using the xleap program by first constructing the Ace-Ala-Pro-Nme peptide. Xleap was then utilized to generate a topology file and an initial coordinate file. The topology and coordinate files were then combined with a minimization input file in the Amber10 program in the minimization step. This minimization step is used to bring the system to a global energy minimum while keeping the system in a periodic box. The minimization was carried out for 0.01 ns. A total of eighteen minimizations were done in sets of three for six different temperatures (275K, 300K, 325K, 350K, 375K, 400K). Then a molecular dynamics run, equilibration and production run to calculate the dihedral data were all done on the system at the respective temperatures. All simulations in this section were carried out under the same conditions as the conditions specified in the TIP3P section.

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