# Elasticity of peptide omega bonds

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We calculated the changes of the free energy profile of the peptidyl-prolyl torsional angle of the dipeptide valine-proline under pulling forces by simulations. Using a dynamic model built on the equilibrium properties of this system and previously studied dynamic properties of *cis-trans* isomerization of other dipeptides, we calculated the dynamic viscoelasticity of this degree of freedom. The results show significant differences between how thermal and mechanical forces alter the equilibrium and the dynamics of the isomerization transition. The former does not change the barrier heights but changes the prefactor of the kinetics owing to temperature effects, while the latter changes minima and thus the population. The force that is required to "excite" this degree of freedom is small. Compared to other systems, we found that this degree of freedom becomes already quite rigid at several hertz, which is a much lower value due to the high barrier of the *cis-trans* isomerization. We also found that the tensile elastic modulus of densely packed omega bonds is at the order of GPa, which is comparable to that of polymer materials. These results give mechanical properties of polyproline elasticity of a local nature and provide guidance for future experimental designs.

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## I. INTRODUCTION

Interesting questions such as "where does the elasticity of an elastic protein come from?" and "why can spider silk outperform many man-made fibers?" have been intensively pursued for decades [1,2]. For many years researchers have studied the conformations of proteins subjected to mechanical forces. It turns out that many proteins having major roles in elasticity are somewhat different from the familiar structural or catalytic proteins that have relatively stable and well-defined domains and tertiary structures. Although there are many results obtained on the sequence information of these proteins, the structures of these mechanical elastomeric proteins are hotly debated [3–5]. Many researchers believe the structures of such proteins are largely disordered *in vivo* based on many experiments and theories of a disordered coiled state [4,5]. But a few researchers think that there is an ordered local structure such as a beta-spiral structure, based on some recent experiments [3]. Nevertheless, the consensus is that the sequences of many short peptide repeats such as poly(VPGVG) (elastin), poly(VPGG) (elastin), poly(PGVGVA) (elastin), poly(PGGGG) (byssus), poly(PGGXG) (flagelliform silks), poly(PGQQG) (dragline silks), and poly(PGGYG) (dragline silks) are present in elastomers and are important for functions [2,6]. Here poly(x) $\equiv$(-x-)$_n$ and $n$ is on the order of ten or more. Due to the unusual nature of proline residue, many researchers believe that proline increases backbone disorder and thus is favored by nature for the design of biological springs. Moreover, another celebrated example of the use of proline for a mechanical protein is the PEVK region of the giant muscle protein titin. The PEVK region is believed to be largely disordered [7,8]. It is named as such because it has high percentages of four residues: proline (P), glutamate (E), valine (V), and lysine (K). Researchers suggested in this case that proline might be directly responsible for the elastic resistance of muscle contractions.

Since there is no clear structural information about the aforementioned elastic proteins, it is therefore unknown as to whether the elasticity of these proteins comes from the breaking of secondary and tertiary structures. Although there are many pulling experiments [9–11], simulations [12–16], and theoretical studies [17] that exercise the tertiary contact degrees of freedom for well-structured proteins, these are, with few exceptions, rather generic studies designed to understand the fundamental underlying protein internal interactions rather than for studying bio-elasticity properties under physiological conditions. Putting the issues of secondary and tertiary contacts aside, we still have local conformational changes left. Hence, if proline is important according to the analyses of the sequence information, what is unique about this residue compared to others? One guess is the *cis-trans* isomerization degree of freedom of the omega bond, which exhibits a higher *cis* content than other residues. Indeed, the *cis-trans* degree of freedom is a focus of many researchers in the field of protein structure-function relationship ranging from (mis)folding kinetics [18] to chemomechanical ratchets [19].

In light of this local-conformational-switch hypothesis, we limit the current study to this degree of freedom alone, i.e., we assume that the response of this bond under tension is additive to the responses of the remaining degrees of freedom. Recently, this idea was proposed and tested by Sakar, Caamano, and Fernandez using several exon repeats of the PEVK [20]. But due to the procedures and methods, the results turn out to be inconclusive [20]. This might relate to the highly heterogeneous systems used and the conditions (such as pulling speed) at which the experiments were conducted. However, another more recent experiment by Nagy *et al.* does show encouraging results that the elasticity of the PEVK region may be linked to its proline content [21].

 

We think that the fundamentally critical missing piece is a microscopic theory of the elasticity of the proline omega bond ensemble. Owing to the high barriers of the *cis-trans* isomerization, we expect to see dynamical effects such as hysteresis among others.

## II. SINGLE OMEGA BOND UNDER TENSION

The protein backbone has three types of atoms repeated in sequence: C, N, and $C_\alpha$. Therefore, it has three types of backbone torsional angles. Compared to the other two backbone torsional angles $\phi$ (-C-N-C$\alpha$-C-) and $\psi$ (-N-C$\alpha$-C-N-), the $\omega$ angle (-C$\alpha$-C-N-C$\alpha$-) has the highest barriers among different isomers and is the least flexible. The conformation is locked in the *trans* isomer the majority of the time as observed in protein structures. However, the omega angle preceding the proline residue has much less steric penalty to be in the *cis* conformation. Still, there is a high barrier between these two conformations. Here we adopted the accelerated MD method [22] developed by Hamelberg, Mongan, and McCammon in order to sample this transition under the influence of the stretching forces applied on two atoms at both ends of the peptide. This accelerated dynamics method allows the system to evolve on a modified energy landscape and accelerates the escape of conformations trapped in potential energy wells. The accelerated MD method allows for the sampling of the *trans* to *cis* transition, which is on the order of seconds and cannot be observed using normal molecular dynamics simulation. This method has been previously applied to study several other aspects of *cis-trans* isomerization such as the effects of phosphorylation [23], the dynamics [24], and the effects of hydration [25].

We have used the blocked dipeptide $CH_3$(O)C-valine-proline-N(H)$CH_3$ as our model system. The blocking by the chemical groups acetyl and methylamide at the two ends is routinely performed in order to eliminate the electrostatic effects of the terminals and thus to represent longer peptides better. From our analysis of the protein sequential information, valine is the most common residue that precedes proline in many of the elastomeric proteins as well as the PEVK region of titin.

In the current study, all-atom molecular dynamics simulations were carried out using the AMBER all-atom force field and by solving the Langevin dynamics equation [26]. Since conformational changes in proteins directly reflect changes in torsions, we have applied the accelerated MD boost to only the dihedral torsions as previously described [22–24]. More specifically, we performed the simulation using an altered potential with $\Delta U = U_{\rm sim} - U_{\rm ori} = \Theta(E-U)(E-U)^2/(\alpha + E - U)$. Here the Heaviside function $\Theta(x)=1$ if $x>0$ and zero otherwise. The boost energy $E$ was set at 55.0 kcal/mol for the total torsional energy, and the simulations were carried out with $\alpha$ set to 10.0 kcal/mol. By a post-simulation process, we recovered the equilibrium results for the original potential. The constant pulling forces were applied at opposite ends of the peptide: on the nitrogen of valine and the backbone carbon of proline, as shown in Fig. 1. The pair of forces creates an internal tension in the molecule while they

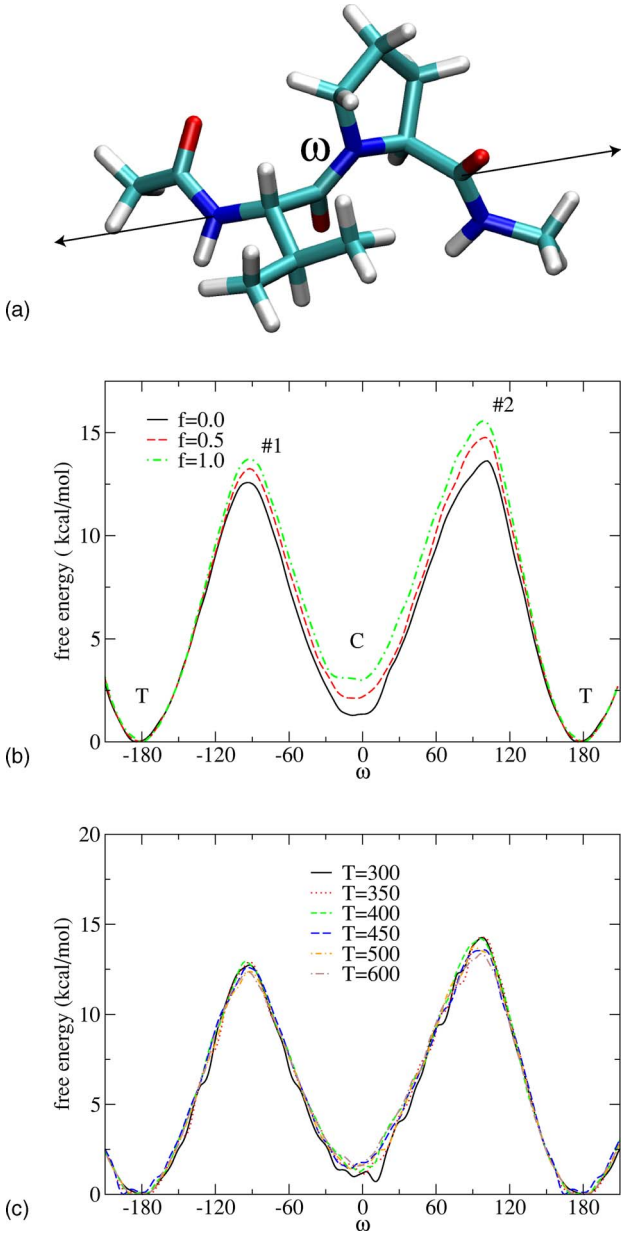


FIG. 1. (Color online) The blocked dipeptide Val-Pro and the forces applied on the system are illustrated in the upper panel. The free energy profiles of the omega angle of the dipeptide Val-Pro at forces 0.0, 0.5, and 1.0 unit in the middle panel. As a comparison, we show the results of dipeptide Ser-Pro at different temperatures in the lower panel.

are balanced out by each other and leave the motion of the center of mass unchanged.

The simulations were carried out using the Sander module in the AMBER 7 suite of programs that was modified to perform the accelerated molecular dynamics simulation with the applied force. The electrostatic interaction was treated using the generalized Born solvation method as implemented in AMBER 7, and the apolar solvation term was also included in the potential function with the surface tension parameter set to the default value of 0.005 kcal/mol/Å$^2$. The temperature of the simulations is 350 K. The SHAKE algorithm was applied to all bonds involving hydrogen atoms, and an integra-

tion time step of 2.0 fs was used for the integration of the Langevin equation of motion. A value of 2.0 $ps^{-1}$ was used for the collision frequency.

We present the free energy profiles under different constant pulling forces in Fig. 1. Three simulations with forces of 0, 0.5, and 1 kcal/mol/Å were carried out with $10^8$ steps each. The convergence is checked by examining the differences between the statistics of split trajectories. Throughout the current study, the unit of force is kcal/mol/Å. One kcal/mol/Å is equivalent to 69.5 pico-Newton. From Fig. 1, we can see that the free energy of the *cis* minimum rises quite quickly with increasing forces. The population of the *cis* is thus depleted to about 2.6% from the inital 23% in a force-free simulation. Meanwhile, the height of the transition state No. 1 (the lower of the two) is increased slightly. The effect of the force is relatively stronger on the equilibrium population between *cis* and *trans* conformation than that on the dynamics. This is *opposite* to the effect of temperature. Based on the earlier study [24] of a similar tetrapeptide system, blocked Thr-Ser-Pro-Ile, carried out over a range of temperatures from 300 to 600 K, the stability $\Delta G_{ct}$ and the barriers changed very little, while a much faster rate of isomerization is observed due to the increase of the temperature. The free energy profiles of omega are not very sensitive to the temperature change since the barrier is a high energetic barrier, which overshadows the entropic contribution.

It is convenient to reduce the complicated multidimensional conformational changes between *cis* and *trans* to just the torsional angle degree of freedom. However, the end-to-end length is more directly related to the elastic property and forms a conjugate pair with the external pulling force in thermodynamics. We therefore also study the length distribution. The actual distance we measure aligns with the force we applied, i.e., we measure the distance between the nitrogen of the residue valine and the backbone carbon of the residue proline.

As shown in Fig. 2, the overall density of the length shows a bimodal distribution at zero force. When we calculate the normalized conditional distribution when the omega bond is *cis* or *trans*, we see that each peak basically corresponds to the *cis* or the *trans* conformation as classified by the omega angle distribution from Fig. 1. Generally the length distribution of *trans* is narrower than that of *cis*. With increasing forces, the peak of *cis* is decreased and the peak of *trans* is increasingly populated. It is interesting to point out also that though the overall peak heights are changed by force, the shape of the conditional densities, when renormalized, are similar. This means that the major effect of the force is changing the population between the *cis* and *trans* states. It does not change the length much within the *cis* or the *trans* ensemble; i.e., we did not see the significant lengthening of the *trans* conformation by increasing forces. Admittedly, this is less clearly shown in the case of *cis*. The *cis*, especially under relatively large pulling forces, is difficult to sample due to its diminishing population. We did not observe any trend as to how force changes the internal distribution of *cis* conformers.

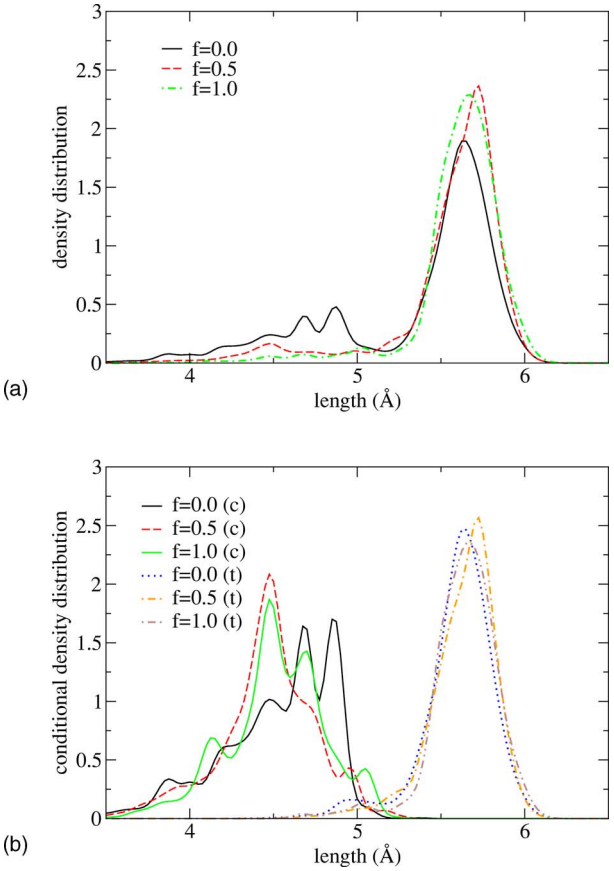


FIG. 2. (Color online) The length distributions at constant pulling forces 0, 0.5, and 1 unit. Top panel: The probability densities of the length between the nitrogen of the residue valine and the backbone carbon of the residue proline at different forces. Bottom panel: The conditional probability densities of the length *normalized* under the *cis(c)* or the *trans(t)* conformation.

## III. THE DYNAMIC RESPONSES OF OMEGA BONDS

In this case, elasticity is the lengthening response of the system to the pulling force. Due to the high barrier nature of the system, the response of the system to the mechanical disturbance depends on how fast (or time-dependently) the perturbation is applied. Thus viscoelasticity, not just elasticity, is the focus of the current section. To fully characterize the dynamically elastic properties of this torsional degree of freedom, we study the dynamic responses of the omega bonds. We probed the response of the system to small amplitude time-dependent oscillating forces with various frequencies. The examination of the dynamic response under a sinusoidal load is widely used to characterize the linear viscoelasticity of materials. To calculate the distribution of the conformations under the time-dependent force, we started by obtaining the free energy profile of $G(\omega,f)$ as a function of the $\omega$ angle and the pulling force $f$ using the interpolation method, based on the results of the previous section $G(\omega,0)$, $G(\omega,0.5)$, and $G(\omega,1)$. Ideally, we will then solve the general dynamic equation that describes the ensemble of omega bonds, which can be approximated by a dynamic Fokker-Planck equation

$$\partial_t p(\omega,t) = D\partial_\omega[\partial_\omega + \beta\partial_\omega G(\omega,f(t))]p(\omega,t) \qquad (1)$$

with the input force as $f(t)=(A/2)(1+\sin \nu t)$. However, we found out that practically it is quite slow to solve the Fokker-

TABLE I. Parameters (300 K).

| $i$ | init $\rightarrow \ddagger \rightarrow$ final | $a$ ($s^{-1}$) | $b$ | $c$[Å/(kcal/mol)] |
|---|---|---|---|---|
| 1 | *cis* $\rightarrow$ No.1 $\rightarrow$ *trans* | $3.60\times10^7$ | 19.5 | −1.72 |
| 2 | *cis* $\rightarrow$ No.2 $\rightarrow$ *trans* | $4.51\times10^7$ | 21.4 | 0.11 |
| 3 | *trans* $\rightarrow$ No.1 $\rightarrow$ *cis* | $5.72\times10^7$ | 21.0 | 1.35 |
| 4 | *trans* $\rightarrow$ No.2 $\rightarrow$ *cis* | $6.93\times10^7$ | 23.0 | 3.20 |

Planck equation over the long (however required) time. The desired time should be a duration of time much longer than the relaxation time scale from *cis* to *trans* or from *trans* to *cis* and the time scale of $1/\nu$. It is difficult to scan a large range of frequencies and solve the Fokker-Planck equation for a very long time using the method we previously adopted [24] (in which case only a single relaxation time is required). With the discretization of the angle at $d\omega=2\pi/180=2$ deg, we use a reasonable $dt=O(1)\times D^{-1}$. Here the angular diffusional constant $D$ is evaluated from previous all-atom simulations to give 0.15 $(\text{deg})^2/\text{ps}$. It takes quite a long time for even a single relaxation over the *cis*/*trans* barrier due to the high transition state.

To avoid this problem, we use a simple two-state discrete model of *cis*-*trans* isomerization, i.e., we assume that the system is either in the *cis* or the *trans* state with probability $P_c$ and $P_t$, respectively. A similar method has been applied to the two-level system of a single domain unfolding previously [27]. Note that here we have obtained all the parameters from the direct measurements of simulations. The transition rates between these two states are modeled using Kramers kinetic theory. We have the dynamic equations describing the transitions,

$$dP_c(t)/dt = -k_l P_c + k_s P_t,$$

$$dP_t(t)/dt = -k_s P_t + k_l P_c. \quad (2)$$

Here $P_c$ and $P_t=1-P_c$ are the percentages of the omega bond in the *cis* or the *trans* state, respectively.

We set the rates as functions of force $f$ as follows: the rate from *cis* to *trans* as $k_l=a_1\exp(-b_1-c_1\times f)+a_2\exp(-b_2-c_2\times f)$ and the rate from *trans* to *cis* as $k_s=a_3\exp(-b_3-c_3\times f)+a_4\exp(-b_4-c_4\times f)$. We assume that the small force perturbs the barriers only linearly in the exponential term. Physically, $a_i$ are the prefactors, while $b_i=\beta\Delta G_i(f=0)$ are the heights of free energy barriers in units of $k_BT$, i.e., $\Delta G_{1C}$, $\Delta G_{2C}$, $\Delta G_{1T}$, and $\Delta G_{2T}$ for $i=1$, 2, 3, and 4, respectively, $c_i=\beta\times\partial_f\Delta G_i|_{f=0}$. From the relation of force and potential landscape in the previous section, the previous studies of kinetics, the effect of temperature on kinetics[24], and the effect of the explicit hydration [25], we obtained the parameters as shown in Table I. Although for reasons of better sampling we have obtained the free energy profiles under pulling forces at 350 K, we have shown that the free energy profile is not sensitive to temperature. The essential effect of the temperature for this system is the dynamics. We used this assumption to set up all the following calculations at 300 K, which is a more biologically relevant temperature than 350 K.

After supplying the dynamic information of the forces as a function of the time $f(t)=\frac{A}{2}(1+\sin\nu t)$, we solve the above differential equations numerically. The results are shown in Fig. 3. At the zero frequency limit, the stiffness (defined as $\Delta f/\Delta l$) is on the order of 1 kcal/mol/Å$^2$. The dimensionality of the stiffness is the same as that of the spring constant. The conversion from our unit to the SI unit is 1 kcal/mol/Å$^2$ = 0.695 N/m. When supplying the cross section area $S$ with 10 Å$^2$ and length of the system $l_o=5$ Å, the elastic modulus is estimated as $\Delta f/S/(\Delta l/l_o)=\text{stiffness}\times l_o/S\sim\text{GPa}$, which can be compared to the tensile elastic modulus of polymer materials.

Figure 3(a) shows several samples of the population dynamics of *cis* under modulating forces with amplitude $A=1.0$ at different frequencies. All trajectories start with *cis* and *trans* being 50% each. For a comparison, we also show trajectories of the system under zero frequency [i.e., constant force $f(t)=1$] and zero force conditions. They serve as the lower and upper bounds for the oscillating cases. It is generally seen that the amplitude of the response is decreasing with increasing frequency. We also observed that the response of the system shows a phase delay compared to the input modulation.

To make these observations more quantitative, we use the analogy with the stress-strain relation. We know that (at the linear level) the response (strain) is $\varepsilon(t)=\varepsilon_o\sin(\nu t-\phi)$, given the stress $\sigma(t)=\sigma_o\sin(\nu t)$. The dynamic stiffness is therefore defined by $\sigma(t)/\varepsilon(t)=E'+iE''=|\sigma_o/\varepsilon_o|e^{i\phi}$, where $\phi$ is the loss angle. Similarly, we study the relationship between the length $l(t)$ and the force $f(t)$. We show in (b) that the system becomes more "stiff" at high frequency and in (c) that the loss angle increases with the increasing frequency. We tested three different amplitudes of oscillating force, i.e., with $A=1$ [shown in (a)] and $A=0.5$ and 0.25. It should be noted that for a truly linear system, the three curves will coincide. The small deviations we see among the three curves are due to the effect of nonlinearity.

Despite the small nonlinearity, the dynamic results are quite clear. With increasing frequency, the "spring" becomes very stiff. This happens at a frequency around 2 Hz. It makes sense, since the period is getting small compared to the time scale of the barrier crossing, which is on the order of a second. The force alternates too fast at these high frequencies for the system to respond, thus resulting in a rigid system.

It is very interesting to discuss the pulling speed used in the AFM experiments. The speed of stretching molecules should be zero in the ideal case that a truly equilibrium measurement is desired. However, in real situations, a finite speed is used, ranging from $10^{-2}$ to 1 $\mu$m/s. To properly measure the elasticity calculated here in a designed experiment, we need to estimate the upper speed limit that will allow the system to respond to the perturbation. This limit is about 1 Å/s for a single proline, since the length scale is about 1 Å for the transition and the time scale is a second. This is lower than the ability of the current experiments. However, one could use a polyproline chain to measure an ensemble of prolines. For the simple two-state dynamics we are considering here, the reaction ratio (one minus the surviving probability) of a single particle in one of the two

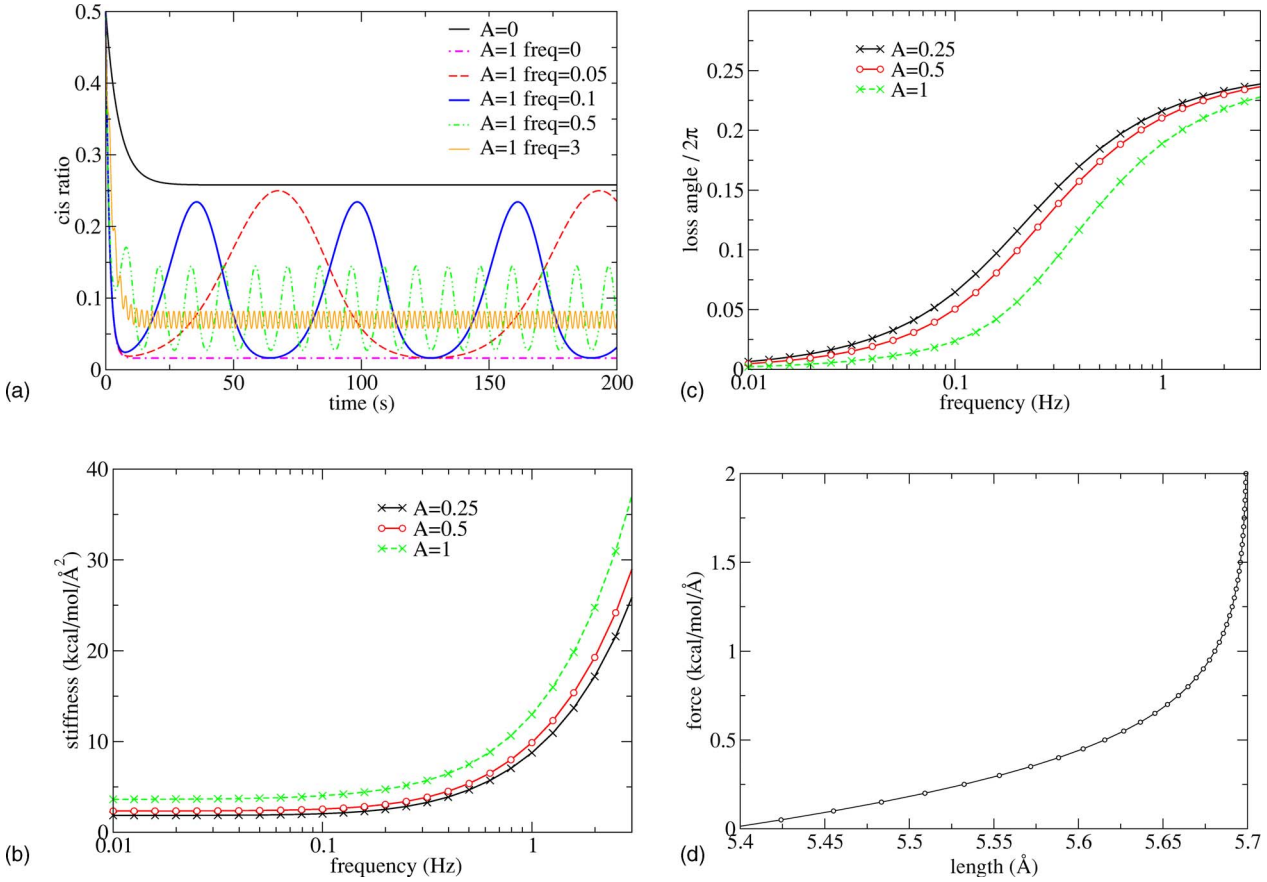


FIG. 3. (Color online) The trajectories of dynamic responses (*cis* percentage) of an ensemble of omega bonds are shown in (a). The amplitude and the loss angle of the stiffness of the omega bond with several different inputs are shown in (b) and (c), respectively. The force-length curve at zero frequency is shown in (d).

states is $P_1(t)=P(t)=1-\exp(-\lambda t)$. Here $\lambda$ is a rate constant with the characteristic time of $\lambda^{-1}$ at one second. Thus a total number of $N$ such particles will have a simple reaction ratio (if at least one has reacted) $P_N(t)=1-[1-P(t)]^N$. It means that the characteristic time of the multiparticle system will be linearly scaled and will have a value of $\lambda^{-1}/N$.

Thus to make it experimentally visible, at least an ensemble of 100 prolines is required for a pulling speed of $10^{-2}$ $\mu$m/s or slower. It is also interesting to compare the pulling speed needed for this study and those conducted for systems breaking secondary and tertiary contacts. If the barrier heights for those contacts are smaller and/or the effective diffusion constants along the reaction coordinates are faster, there will be fewer restrictions on the pulling speed. Still, generally it is always observed and it also makes sense that a system becomes more rigid at high pulling speeds.

## IV. CONCLUSIONS

To summarize, we found that the omega bond of the blocked dipeptide, valine-proline, has a barrier of 12 kcal/mol at zero force and $T=350$ K. The barrier of *cis* to *trans* decreases with increasing forces, and there are larger changes in the relative populations of *cis* and *trans* isomers. At 1 kcal/mol/Å, the force already raises the minimum of the *cis* isomer enough to deplete the *cis* population according to the Boltzmann probability distribution. More specifically, we note that at the simulation temperature 350 K, the *cis* population changes from 23% at zero force to 2.6% at $f=1$ kcal/mol/Å. The effect of force on the free energy profile is opposite that of the temperature. The force modulates the equilibrium distribution. On the other hand, the change in temperature modulates the prefactor of the kinetics but leaves the equilibrium distribution almost untouched.

One may want to compare the forces needed to excite this omega angle to the forces needed to unfold a protein domain. We want to stress that what is meant by force required to excite the transition is the following: even without any forces, the transition of *cis*/*trans* in the current study, or the unfolding of single domain proteins, can happen spontaneously. The *equilibrium* tensile force required to unlock a folded structure can be defined as the critical value of the constant pulling force that tilted the free energy landscape until the native basin has the same height of free energy as the unfolded basin (analogous to the folding temperature of a thermal folding transition). In contrast, the force seen in experiments to unlock a folded domain often results in quite a discontinuous jump in the (sawtooth shaped) force-length plot. It has quite a different meaning: a measurement of the combination of the nonequilibrium effects and the equilibrium critical force. That value is often as high as several

hundred pico-Newton. But here in the free energy profiles, the zero force populations already favor the *trans*, and *trans* is even more favored with increasing pulling force. Thus we cannot define a critical force as the case of unfolding in the current situation. What we can quantify is how force modulates the energy landscape and thus alters the length distribution that results in elasticity.

It is a different story for thermal excitation, in which case the switching of the population is not easily controllable by temperature. This may contribute to the difference of the mechanical unfolding and thermal unfolding observed for some proteins.

From the dynamic study we estimated the buckling frequency for this elasticity. It is quite low (at around several hertz) due to the high barrier for the *cis-trans* isomerization. That is, above this frequency the system is quite rigid and this omega degree of freedom is frozen. We estimate that a minimum of an ensemble of 100 prolines is required to show the elasticity at the pulling speed of $10^{-2}$ $\mu$m/s for experiments.

Even though we have focused on the omega degree of freedom alone, we think that carefully designed experiments can be carried out to compare with the results from our theoretical calculations. Ideally one would perform AFM experiments on engineered polyproline sequences, such as $(\mathrm{VPA})_n$, and use another sequence, such as $(\mathrm{VAA})_n$, in order to calibrate and correct for other degrees of freedom.

## ACKNOWLEDGMENTS

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