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Quasiperiodic oscillation and possible Second Law violation in a nanosystem

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ABSTRACT

Simulation of a virus-like particle reveals persistent oscillation about a free-energy minimizing structure. For an icosahedral structure of 12 human papillomavirus (HPV) L1 protein pentamers, the period is about 70 picoseconds and has amplitude of about 4 Å at 300 K and pH 7. The pentamers move radially and out-of-phase with their neighbors. As temperature increases the amplitude and period decrease. Since the dynamics are shown to be friction-dominated and free-energy driven, the oscillations are noninertial. These anomalous oscillations are an apparent violation of the Second Law mediated by fluctuations accompanying nanosystem behavior.

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1. Introduction

Pentamers of the L1 protein of human papillomavirus assembles into $T = 1$ or $T = 7$ icosahedral structures, depending on conditions and mutation [1]. Computer simulation of the $T = 1$ structure shows that the pentamers oscillate radially in time with a specific spatial pattern (Figure 1). The oscillations are nearly periodic in time despite the omnipresent stochastic environment existing at the nanoscale. The objective of this study is to delineate the properties of these anomalous oscillations and their implications for the Second Law at the nanoscale.

The Second Law of thermodynamics is formulated for macroscopic systems, i.e. containing many atoms evolving over long time periods. However, it is observed for small systems (e.g. spherical latex particles of radius 6.3 microns [2]) that Second Law violations do occur. The question emerges regarding whether the observed anomalous oscillations about equilibrium (Figure 2) could be a manifestation of Second Law violation.

Periodic or near-periodic oscillatory behavior is of several types:

- Type I: conservative oscillations such as in a spring-weight system wherein the energy is constant and the amplitude depends strongly on initial data [3].
- Type II: limit cycle dynamics occur in far-from-equilibrium systems, and have amplitude that is independent of initial data [4,5].
- Type III: slow oscillations in systems near, but out of, equilibrium, and with multiple free energy-minimizing states [6].
- Type IV: oscillatory switching between multiple far-from-equilibrium steady states due to the influence of thermal fluctuations [7].

None of the above mechanisms seem to explain the oscillations as in Figure 1. Such a nanoscale system is strongly frictional so that oscillations of Type I are not expressed. The system is at equilibrium so that Type II oscillations cannot be supported. The period of oscillation is of magnitude on the timescale of structural transitions in nanoscale assemblies [8]; hence, since the system appears to be at equilibrium oscillations of Type III are not likely. While fluctuations are significant at the nanoscale, the system is at equilibrium, suggesting that Type IV oscillations are also not expressed.

A possible mechanism for the oscillations observed here is that in the 36 dimensional space of the centers of mass (CMs) of the twelve pentamers there is a pathway to which the system is attracted, and along which there is a barrier. After fluctuations drive the system over the barrier, it revisits the free-energy driven long excursion to ultimately again arrive at the barrier, resetting the system to begin a fluctuation-induced jump to the slow, more coherent phase of the cycle. With this two-phase cycle, quasiperiodicity is achieved if the slow, coherent phase of the cycle takes much longer than the typical time to surmount the barrier.

In this study, the above hypothesis is tested via numerical simulation. Since the simulations are relatively long for traditional molecular dynamics, an approach is used that addresses the multiscale character of large assemblies. This Deductive Multiscale Simulator (DMS) is built on the classical many-atom Newtonian Liouville equation and multiscale theoretical and computational techniques [9]. While DMS has an established record of agreement

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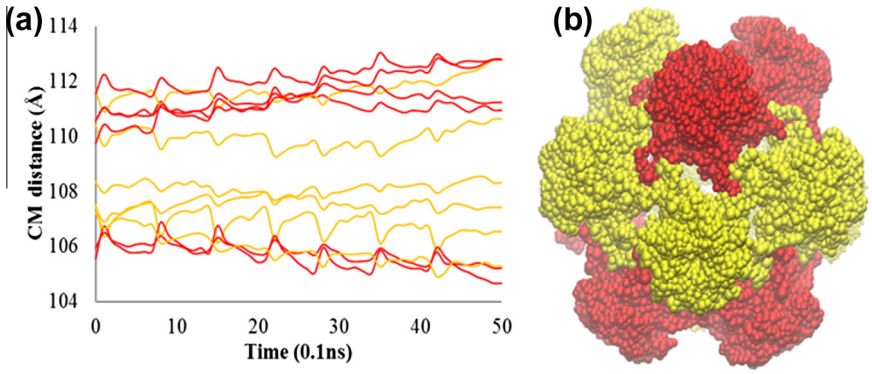

Figure 1. (a) DMS results at equilibrium shows pentamer CM distance oscillates radially in time. Pentamers oscillate in-phase and out-of-phase with one another represented by the colors red and yellow, respectively. (b) Spatial representation of the 12 pentamers in the $T = 1$ VLP structure showing in-phase (red) and out-of-phase (yellow) pentamers.

with traditional molecular dynamics (MD), some MD runs were used to validate our conclusion.

2. Materials and methods

The system considered was the $T = 1$ icosahedral structure of the L1 protein of HPV type 16 [10]. This structure consists of 12 L1 pentamers. This structure was simulated in an aqueous medium with Na^+ and Cl^- ions at 0.25 M concentrations, and at constant volume. The latter was adopted to eliminate the possibility that the oscillations were due to a numerical artifact associated with the constant altering of system volume to otherwise maintain constant pressure if the runs were run in the isobaric mode. Simulations of two types were undertaken. In both cases the simulations were based on the all-atom Newtonian model with the CHARMM27 force field [11]. They were run for a set of temperatures between 100 and 350 K to probe the effects of variation on the intensity of fluctuations. These all-atom simulations were run to insure that all thermal fluctuations were accounted for since one of the questions of interest is to assess the coherence of the oscillations, i.e., how does periodicity emerge despite omnipresent atomistic noise and environmental conditions.

The system, i.e. the $T = 1$ structure plus the electrolyte, consisted of 3,295,892 atoms. Thus traditional MD would take considerable computational resources. However, with the availability of the DMS software, the all-atom simulations were achieved with much greater efficiency. DMS increases efficiency via techniques that capture the coupled dynamics of coarse-grained (CG) and micro variables. The latter describe the overall structure of a system and evolve much more slowly in time than do individual atomic vibrations. The CG variables used have the important property that they facilitate the construction of a quasi-equilibrium ensemble of all-atom structures that coevolve with the CG state. These ensembles are used to construct the diffusions and thermal forces needed to evolve the CG variables. The DMS software yields results in agreement with traditional MD as validated for a number of systems [8,12,13]. The latter include viral capsids and RNA. DMS was shown to accurately capture the MD results but with orders of magnitude greater efficiency. However, it should also be noted that DMS is an ensemble MD run so DMS is even more efficient than a single MD run, and its predictions are therefore more statistically significant than a single MD run.

3. Results and discussion

A study was carried out via sets of isothermal runs to test the role of temperature on the characteristics of the oscillation and the loss of features in the free energy landscape. The characteristics of the oscillation were probed by monitoring the motion of each of the 12 pentamers in the structure. These motions tend to be out-of-phase for neighboring pentamers (Figure 1a), but the spatial pattern (Figure 1b), though checkerboard like, is more complex due to icosahedral geometric constraints.

The spatial pattern of the oscillation (i.e. selected nearest-neighbors are out-of-phase) as shown in Figure 1 is preserved across the range of temperature studied. To assess the overall amplitude of oscillation, the average amplitude of oscillation for each pentamer in the VLP was measured for a range of tempera-

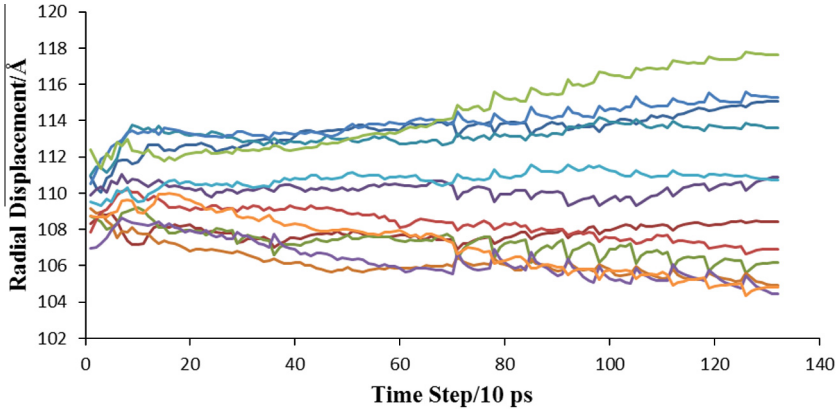

Figure 2. DMS results for all 12 pentamers of the VLP show that the observed oscillation evolves around equilibrium. This is confirmed by MD simulation.

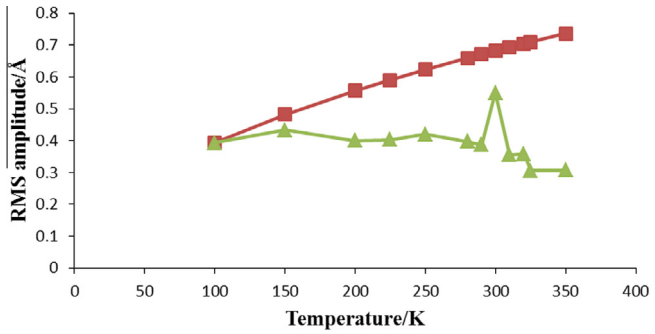

Figure 3. Comparison of the expected radial displacement of an elastic mode (red) and the observed oscillation (green). RMS CM displacement for the observed oscillaiton generally decreases with an increase in temperature, whereas the expected harmonic oscillator result should increase as $T^{1/2}$.

tures. These data were used to calculate RMS amplitude of oscillation for the VLP. The variation of amplitude shows a small anomalous rise around 300 K, but generally decreases as temperature increases (Figure 3). It should be noted that at every temperature the CM displacement of the most active pentamers are radially displaced at least twice as far from the center of the VLP than the average. The largest pentamer radial displacement occurs at 300 K and is 1.2 Å. Generally, polar opposite pentamer displacements are in the same sense (outward versus inward). Thus, at one phase of the dynamic, the maximum extension is around 2 Å and the maximum shortening is as well, implying a deviation of about 4 Å from spherical. Figure 3 suggests that the phenomenon is not a simple elastic mode. If it was, the amplitude should increase roughly as $T^{1/2}$, as suggested by the classical harmonic oscillator. Furthermore, a supramolecular assembly as in Figure 1b expresses many such modes so that the spectrum should increase in complexity as T increases. Hence, the oscillatory dynamics of Figure 1 would not be observed. This suggests that the oscillation is not a simple elastic, or even an inertial nonelastic, mode.

The period of oscillation varies slightly with temperature. The period varies between 75 and 63 ps for the temperature range of 100–350 K simulated and shows the expected decrease with temperature (Figure 4).The additional thermal noise reduces the waiting time for the system to overcome a barrier and reenter the slow, free-energy driven phase of oscillation. As the temperature increases, the free energy landscape tends to smoothen, with consequent reduction in the height of the barrier, which the fluctuations overcome. With this and the increase in fluctuation amplitude with temperature, one expects the period should decrease with increasing temperature, as observed. Finally, the oscillation does not correspond to a noise induced transition between two energy wells, which would not likely express the quasi-periodicity observed. This is not a simple example of the VLP transitioning between two free energy wells in one dimension (here 1 CG variable).

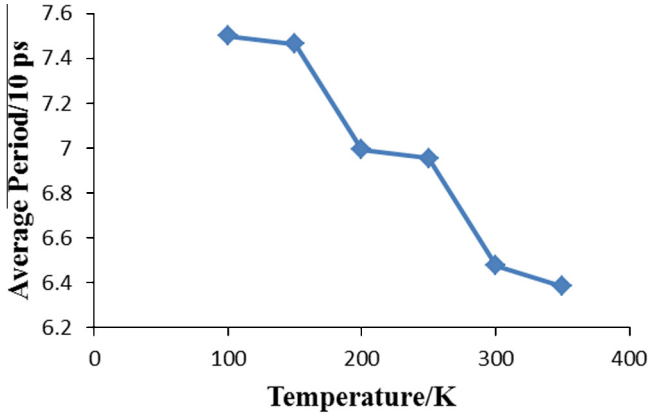

Figure 4. Average period of oscillation for pentamers in the $T = 1$ VLP structure decreases as temperature increases.

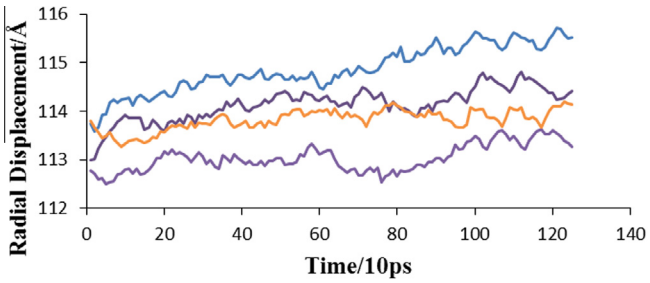

Figure 5. MD results for selected pentamers of the VLP shows that the observed oscillation appears when the system reaches equilibrium.

It was also observed that across the temperature range the in-phase and out-of-phase pentamers (Figure 1b) remain conserved. This helps confirm this mode of oscillation. The imposed icosahedral pattern of the VLP restricts the motions of pentamers to movements like those observed in this simulation. The possibility that the oscillation is a non-equilibrium phenomenon was addressed as follows. A traditional MD run (Figure 5) shows oscillation is expressed as the system approaches equilibrium, and does not disappear at equilibrium.

One of the implications of the Second Law is that a macroscopic isothermal system free from external forcing will evolve to decrease Helmholtz free energy, and ultimately reside in a time-independent equilibrium state. A corollary of this is that a macroscopic system in contact with a single bath of constant temperature cannot display perpetual motion. However, microscopic variables (e.g., the length of a covalent bond) can oscillate, i.e., experience thermal fluctuations. Such oscillations are continuously excited and annihilated by the thermal environment, and therefore are not readily considered as perpetual motion. The $T = 1$ structure simulated here is sufficiently large that it is not viewable as a microscopic (few atom) system subject to a randomly fluctuating environment. In fact, a number of studies have shown viruses and VLPs can be studied macroscopically [14] (and references therein). Thus, the present example, though large, does exhibit quasiperiodic, long-time oscillations and not dominantly random fluctuations. Oscillations in such a nearly macroscopic system are forbidden by the Second Law. These oscillations are unlike damped elastic modes that are omnipresent features of a supramolecular assembly and are part of their fluctuating dynamic near equilibrium (Figure 3).

To sustain oscillation in the present friction dominated VLP-aqueous medium system, work must be performed on the VLP. The origin of this work lies in thermal fluctuations as follows. One period of oscillation is divided into two Phases (Figure 6a). In Phase I, the system brought to a barrier by coherent free energy minimizing forces, while in the shorter Phase II fluctuations drive the system over the barrier and Phase I is reentered. This two Phase scenario is realized through the balance of fluctuations and coherent behaviors operating at the nanoscale. In Phase I the coherent dynamics seem to dominate the behavior, while in the short Phase II it is the fluctuations (Figure 6b). This suggests that the free energy landscape provides a zone of coherent free energy minimizing forces underlying the slow return to a barrier, which is then surmounted in the fluctuation dominated, and short, Phase II. Although noise plays a critical role, the overall dynamic is nearly periodic because Phase II is much briefer than Phase I. While the random forces shown in Figure 6b as an RMS average are small,

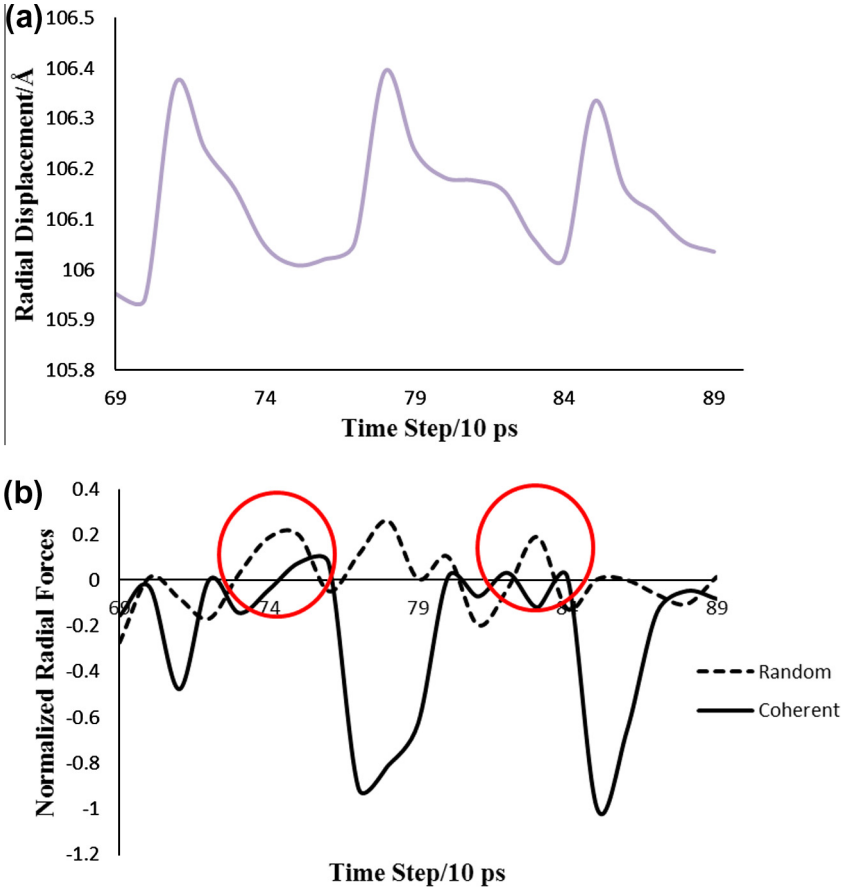

Figure 6. (a) Radial displacement of a selected pentamer over 200 ps showing repetitive Phase I and II; color code is the same as Figure 2. (b) Coherent and fluctuating forces during the two phases of oscillation shown via the evolution of their radial components. While the Phase II (encircled) is fluctuation driven, the Phase I dominated by the coherent free energy minimizing forces.

it is the occasional anomalous fluctuation that appears to bring the system over a barrier, resetting the system for entry into Phase I. Finally, the dynamics of this 12 pentamer system must be understood as a high dimensional non-linear dynamical system. The above narrative for the cycle is therefore too simplistic, and a deeper understanding awaits a more complete analysis wherein the free energy landscape and the role of fluctuations are considered in a space of 12 or more dimensions. A preliminary insight into the complexity of the dynamic is suggested in the correlation diagram of Figure 7.

Second Law violations have been observed experimentally for micron sized latex particles [2], fluid under a shear flow [15], and the stretching of biopolymers [16]. Such violations imply microscopic systems are not rescaled versions of their macroscopic counter parts. Using concepts of microscopic time reversibility and macroscopic irreversibility, fluctuation theorems (Evans–Searles [17–19] and Crook's [20]) were developed that suggest a finite probability of entropy decrease as the system size decreases. For larger systems the probability of entropy increase becomes dominant thereby respecting the Second Law. Thus, Second Law violations for smaller systems have been reconciled theoretically [21–25].

In DMS, fluctuations follow the Green–Kubo relationship, e.g., properties of the fluctuation are related to diffusion factors [26,27]. The related correlation functions are used to estimate correlation times; the latter are used as an indicator of the self-consistency of our approach when these times are short relative to the characteristic time of CG evolution [28,29]. It has been shown that this relationship is consistent with Evans–Searles as well as Crook's fluctuation theorems [27,30]. Thus, these fluctuation theorems and

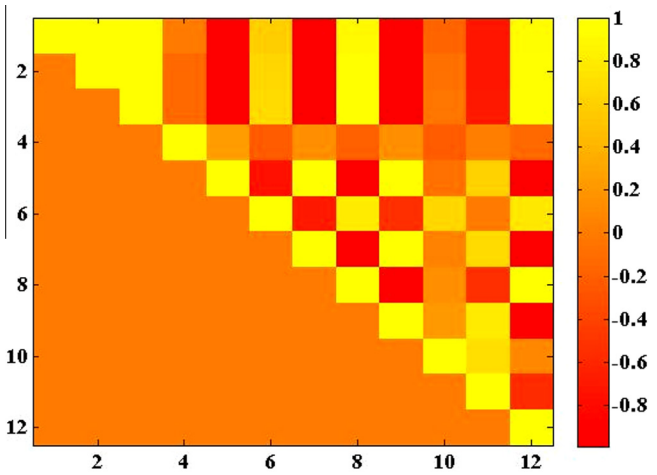

Figure 7. Correlation plot showing in-phase and out-of-phase pentamers in the same color code as in Figure 1. With minor exceptions, alternate red and yellow blocks indicate opposite phase of nearest neighbors. This reflects the complexity of the 12-dimensional oscillatory dynamics.

associated Second Law violations are possible within the DMS framework, as illustrated in the present example.

4. Conclusions

The temperature studies suggest that the oscillation consists of a fluctuation induced transition between structures followed by a

relatively long residence time in a given structure. At low temperatures one expects that free energy barriers are larger, i.e., elevated temperature tends to flatten the free energy landscapes. In this view, as temperature increases, one expects higher frequency oscillation and ultimately, at high temperature, a random migration across a relatively flat landscape.

The observed oscillation in pentamer CM distances from the center of the $T = 1$ assembly is small for the conditions studied. However, this does not preclude larger amplitude oscillation in metrics that reflect other structural changes. For example, stress, nearest neighbor distance, and capsid permeability that is strongly affected by small changes in transcapsid escape routes. Furthermore, it is likely that larger amplitudes may emerge for various mutations or deletions of L1 HPV protein constituting the $T = 1$ structure, similarly for capsids of other viruses.

The possibility exists that this phenomenon is not limited to the case of HPV $T = 1$ VLP. Other VLPs may also display the fluctuation-dependent oscillations observed in this study. The online Virus Particle Explorer database (VIPERdb) [31] lists almost 70 $T = 1$ structures for viruses including Hepatitis E, Satellite Tobacco Mosaic Virus, Brome Mosaic Virus, Parvovirus, and Adeno-associated virus. The list comprises about 20 different virus genera and at least 5 have a similar capsid radius to that of the HPV 16 L1 $T = 1$ VLP. Future studies will attempt to show if these viruses are also capable of the type of oscillation shown here.

Recent advances in terahertz spectroscopy [32] include a frequency range that overlaps with that of the present oscillations. This suggests that the oscillations predicted here could be observed as resonances in the terahertz spectrum. The effect could be enhanced by including small supraparamagnetic particles within the cavity of the VLP.

The question arises as to the role of capsid oscillation in the virus life cycle. These structural oscillations could provide the encapsulated RNA or DNA brief exposure to the microenvironment that may make the virus more responsive, e.g., to the proximity of a host cell.

In conclusion, we believe that the results presented here provide evidence for sustained oscillation about equilibrium for some nanosystems, and that these anomalous oscillations may reflect a violation of the Second Law for nanoscale systems. In this Letter, we elucidate this idea via the interplay of free energy minimizing forces and thermal fluctuations. In ongoing research, we are revisiting this phenomenon to more explicitly understand the interplay of fluctuations and the free energy landscape which underlies sustained oscillations.

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