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# Prospective on multiscale simulation of virus-like particles: Application to computer-aided vaccine design

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ABSTRACT

Simulations of virus-like particles needed for computer-aided vaccine design highlight the need for new algorithms that accelerate molecular dynamics. Such simulations via conventional molecular dynamics present a practical challenge due to the millions of atoms involved and the long timescales of the phenomena of interest. These phenomena include structural transitions, self-assembly, and interaction with a cell surface. A promising approach for addressing this challenge is multiscale factorization. The approach is distinct from coarse-graining techniques in that it (1) avoids the need for conjecturing phenomenological governing equations for coarse-grained variables, (2) provides simulations with atomic resolution, (3) captures the cross-talk between disturbances at the atomic and the whole virus-like particle scale, and (4) achieves significant speedup over molecular dynamics. A brief review of multiscale factorization method is provided, as is a prospective on its development.



## 1. Introduction

Vaccine computer-aided design (VCAD) computationally constructs candidates for virus-like particles (VLPs), performs molecular dynamics (MD) simulations to screen putative candidates, and therefore holds great promise for the efficient discovery of vaccines.

Key elements of the VCAD approach considered here include revealing self-assembly pathways, assessing thermal stability, and predicting likely immunogenicity for a target pathogen. However, VLP MD simulation places impractical demands on computational resources, particularly in light of the many simulations needed in the course of a VCAD project (e.g., to survey the effects of a range of host medium conditions, epitope sequences, and overall VLP structures considered). Multiscale MD methods that (1) avoid the need for calibrating [1] coarse-grained (CG) models (such as force matching [2], iterative Boltzmann inversion fitting [3], elastic network models [4], etc), (2) provide atomic resolution based on interatomic force fields, and (3) provide great efficiency over conventional MD, are now making this VLP VCAD possible.

MD is a method to simulate the evolution in time of a many-atom system (e.g., a VLP and its aqueous environment, other nanoscale objects, or a cell surface). However, conventional MD proceeds via timesteps of femtoseconds (as needed to capture atomic fluctuations), while phenomena such as structural transition and nanoparticle self-assembly take place over microseconds or longer. In addition, the systems of interest involve millions of atoms and express structures on a range of space scales from few atoms to a whole VLP and its microenvironment (Angstrom to microns). To answer key questions regarding structure, stability, and self-assembly of VLPs, one must account for all these space-time scales. This makes VLP simulation a multiscale challenge, and therefore requires new multiscale algorithms that provide efficiency over MD and also preserve atomic resolution. This is important for VCAD for the following reasons:

- The sensitivity of the immune system underlies its selectivity, e.g., an L1 protein-based vaccine against HPV is type-sensitive, and therefore simulation results are required that capture atomic scale features [5].
- The above sensitivity implies that criteria needed to assess the immunogenicity of a vaccine nanoparticle must provide details on epitope structure at the peptide or even the atomic level; the model should therefore capture VLP and solvent structure with atomic resolution.
- Simulations must typically run for more than 100 ns since transient effects can alter the statistics of fluctuation of epitope structure, known to be a key metric of immunogenicity [6].

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- Long time simulations are needed to predict thermal stability, which necessitates the use of well-calibrated interatomic force fields and thereby an atom-resolved method.
- Allosteric effects can mediate epitope fluctuations by structure at large distances from the epitopes [6c] (i.e., simulation of the isolated epitope may not provide reliable information for immunogenicity prediction).
- Phenomenological coarse-grained models require recalibration with each sequence, host medium, etc. with accompanying uncertainties in the form of the governing equations, and time delays in generating experimental data.

The above characteristics required for a vaccine candidate simulator imply that purely CG models are not sufficient for VCAD. These considerations are even more important due to the many simulations needed during the course of vaccine discovery.

Recently, multiscale coevolution algorithms that capture atomic resolution have been developed and are here proposed as a way to overcome the aforementioned challenges. The basic idea is to use the separation of scales that underlies the VLP simulation problem to overcome this challenge. Although multiscale MD algorithms are still evolving, results to date are encouraging.

A key element of the multiscale MD approach is the identification of CG variables which characterize the overall VLP and change slowly in time [7]. These CG variables are used to guide the evolution of the atom-resolved state over long timescales. In particular, the large-scale motions of all atoms in the system are advanced in time via changes in the CG variables, while atomic fluctuations are captured via short MD runs. This is all integrated in a manner which preserves the cross-talks between short and long scales. Namely, the CG variables and the atom-resolved structures coevolve in time via a fully coupled dynamic. This coevolution is achieved via multiscale factorization (MF) [8], an algorithm based on Lie-Trotter Factorization [9] as reformulated for multiscale systems. MF brings great efficiency to an MD simulation as needed for VCAD. At all stages, the MF algorithm is based directly on Newtonian mechanics and well-tested interatomic force fields (e.g., CHARMM [10], AMBER [11], GROMOS [12].) In this way, the following are avoided: conjectured CG governing equations, and recalibration of these governing equations with each new study. To date, efficiency of MF over conventional MD has been by an order of magnitude [8,13]. Work in progress promises a further increase in acceleration that will make vaccine CAD even more efficient. These advances include the use of the earlier history of a computation to increase stability and accuracy [14], and Padé approximants (PAs) to increase stability and provide an estimate of the optimal timesteps for time advancement scheme. The final element needed for a VCAD approach is to assess the likely immunogenicity of a VLP that is predicted to be stable based on MD (see the companion article of this volume).

The paper is organized as follows. A promising methodology for VLP simulation, MF, is reviewed in Section 2, illustrative examples and demonstrations on the application of MF to several VLPs are covered in Section 3, and a prospective for future developments is provided in Section 4.

## 2. The multiscale factorization approach

MF is a computational MD method that seeks efficiencies and conceptual advantage in separating the dynamics of a many-atom system into large-scale (i.e., whole VLP) and small-scale (i.e., few-atom) alternating phases. This factorization must be done in a way that preserves the cross-talk among large and small scales. A classic procedure for achieving such a separation in mathematics and theoretical physics is Lie-Trotter factorization [9]. Its formulation to nanoscience, and to VLP simulation specifically, is as follows.

The MF computational algorithm evolves the VLP over a time $t$ that is divided into finite intervals of duration $\Delta$. Evolving the system over each $\Delta$ interval proceeds in two phases: the microstate advancement and the CG (i.e., whole VLP) advancement. The microstate advancement is achieved by a short MD run over an interval $\delta \ll \Delta$; this evolution is representative of the atom-scale dynamics across entire $\Delta$, according to the "stationarity assumption" [8,13]. In the next phase, the overall VLP structure described by a set of CG variables is advanced over $\Delta$ using information from the short MD simulation of the first phase. This approach therefore achieves theoretical acceleration over MD by a factor of $\Delta/\delta$. The algorithm provides accurate and efficient simulations when a set of variables describing the overall VLP structure change slowly relative to individual atomic fluctuations. Preliminary results have shown this method can be an order of magnitude faster than conventional MD [8,13]. A more detailed description, and a rigorous justification in terms of the mathematics of evolution operators is presented elsewhere [8].

## 3. Illustrative examples

Several types of VLP simulations can play an important role in a vaccine CAD project. Illustrative examples are presented below. These simulations were performed using implementation of MF in our Deductive Multiscale Simulator (DMS) [8]. All multiscale simulations were done under NVT conditions. The Particle Mesh Ewald (PME) method was used to compute long range forces, the velocity rescaling algorithm was used to constrain the temperature to 310 K, and the salinity (NaCl in explicit solvent) was set to 0.15 M.

### 3.1. VLP stability and equilibrium structure

VLP candidates with icosahedral structure can be developed geometrically, e.g., by using icosahedral symmetry and X-ray structural data for the capsid protein. However, the question regarding the stabilities of such structures under thermal and chemical conditions of interest, and therefore their clinical relevance, must be addressed. Simulations to determine stability can be a burden on computational resources because of (1) the large number of atoms involved and (2) possible high energy barriers to disassembly. In the latter case, the VLP can be trapped in a potential energy well although after some time the system could escape this well due to thermal fluctuation in the structure. This requires achieving long simulation times not accessible to conventional MD. As an example, consider the $T = 1$ icosahedral structures of human papillomavirus (HPV) 16 and P22 (Fig. 1). Both systems form stable $T = 7$ structures. If the L1 coat protein of HPV 16 is appropriately truncated, it self-assembles into a stable $T = 1$ structure [15]. Longtime DMS simulation confirms the experimentally observed stability (Fig. 1a). However, if a similar truncation of a coat protein of P22 is assembled using the same symmetry argument, DMS simulation shows it to be unstable (Fig. 1b) in the absence of the scaffold protein (SP). Although experimental studies indicated that different sizes of VLPs were formed during self-assembly of P22 coat protein in the absence of SP [16], VLPs with $T = 1$ symmetry were not found. In addition, Fig. 1c shows that the potential energy of the P22 VLP decreases in time. This demonstrates that DMS can be used in conjunction with geometric constructions to visually screen putative VLPs for stability.

Next, consider the $T = 4$ VLP of Nudaurelia capensis omega virus (NωV). After constructing this VLP computationally using icosahedral symmetry, the evolution to the equilibrium occurs on a long time scale. This difficulty is addressed using MF as implemented in

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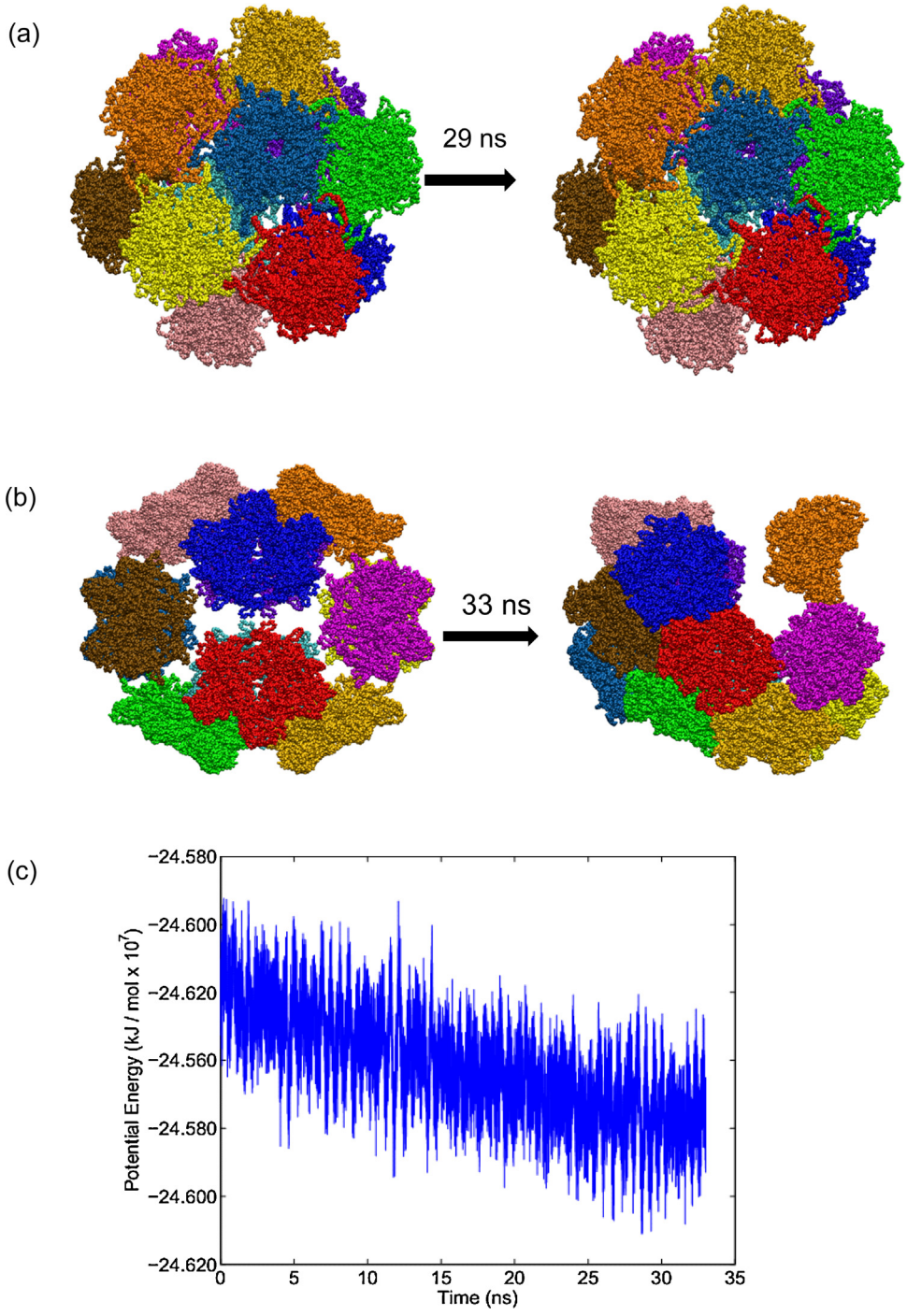


**Fig. 1.** Snapshots of the initial and final configurations of (a) HPV $T = 1$ VLP undergoing thermal fluctuations. After 29 ns, the system has slightly changed signifying the VLP is stable and has reached equilibrium, and (b) P22 $T = 1$ VLP significantly evolving from its initial symmetrical icosahedral form, which implies the VLP is unstable. (c) The potential energy of the P22 VLP and its surrounding water plus ions slightly decreases over 30 ns, which implies the simulation is stable although the VLP loses its icosahedral symmetry. The MD length $\delta$ was set to 50 fs, the selected CG timestep $\Delta$ was 1 ps in (a) and 0.5 ps in (b), and a total of 30 space-warping CG variables [7a,b,f–i,24] were used to capture both linear and quadratic deformations in both structures. The speedup over MD was approximately 9 in (a) and 4.5 in (b).

DMS. Compared to conventional MD, DMS is 5 times faster for this system containing ~14 million atoms (including water). The multiscale simulation suggests the system is approaching equilibrium after 10 ns (Fig. 2).

### 3.2. Self-assembly and design of synthesis pathways

Synthetic VLPs are generated via self-assembly methods by non-covalent bonding processes. The initial state is a cloud of disconnected components (e.g., protein pentamers or hexamers). The VLP emerges under the influence of Brownian random translation/rotation, and the forces between the VLP component and the host fluid. Such simulations are demanding on computational resources.

The GlaxoSmithKline HPV16 truncated L1 protein assembled into a $T = 1$ icosahedron is the active ingredient in the Cervarix vaccine [17]. The pentamer was shown to be the starting component in HPV16 self-assembly [18]. The time delays in the assembly due

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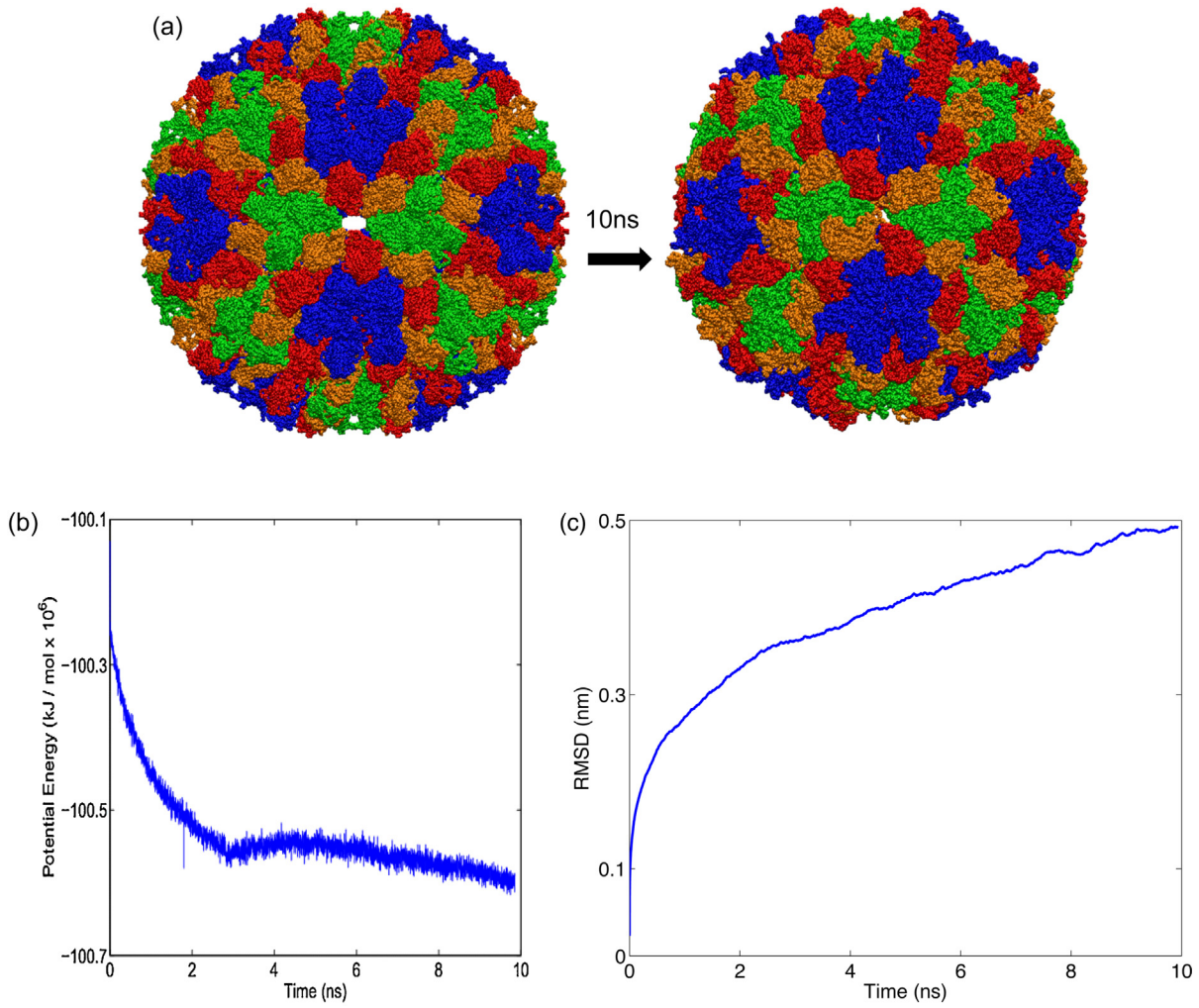


**Fig. 2.** During 10 ns (a) the diameter of NωV capsid decreases by 5.5 nm, (b) the total potential energy of the system initially decreases rapidly in time, and then it begins to form a plateau, suggesting the system is approaching equilibrium, (c) the RMSD of the protein (reference set to initial frame) increases almost monotonically to 0.5 nm. The MD length $\delta$ was set to 50 fs, the CG timestep $\Delta$ to 0.5 ps, and a total of 12 space-warping CG variables were used to capture the contraction of the capsid. The speedup over MD was approximately 5.

to translational and rotational diffusion of the pentamers presents a challenge for conventional MD. To assess the use of DMS in this context, an initial configuration of 12 disconnected HPV16 protein pentamers (with full, non-truncated peptide sequence (Fig. 1a)) was evolved in time with DMS. The ensuing evolution over 3 ns is shown in Fig. 3.

Each of the 12 pentamers in the T = 1 HPV16 structure was radially displaced outwards from an equilibrium icosahedral configuration by ∼10% of its radius to create an initial structure (Fig. 3a). The question to address is weather pentamers can self-assemble in an icosahedral structure. After a 3 ns simulation using DMS, clusters of three or two pentamers seem to form (Fig. 3b). Although longer simulations should be carried out, DMS provided an average speedup close to 12 over conventional MD in simulating self-assembly. The prospects for addressing the grand challenge of self-assembly will likely be realized when methods such as those outlined in the next section are implemented in DMS.

## 4. Prospective

A protocol for the VLP-based vaccines starts with predictions of stability and self-assembly (e.g., using DMS), and is followed by an assessment of immunogenicity using bioinformatics approach based on MD (companion paper). First, a VLP is assembled geometrically using a computational method (e.g., using VMD [19] and employing icosahedral symmetry), or alternatively placing VLP components at random in a host medium and preparing them to self-assemble computationally. Second, MD-type simulations are carried out using the result from the first step as input. Third, analysis of the result from the second step to delineate conditions for VLP stability and self-assembly. Fourth, a bioinformatics approach (Grosch et al. [20]) is used to assess the immunogenicity of the VLP. When integrated with an experimental and clinical effort for validation, the result of this protocol is to accelerate the discovery of thermally stable, highly immunogenic, and cross effective vaccines.

In the protocol addressed here, the first step is running long-time VLP simulations needed in the course of exploring for optimal VLP structure and self-assembly conditions. Considering the enormity of the task of carrying out MD-type simulations required, we suggest that the multiscale methods proposed here are promising. The MF methodology (implemented in the DMS software package) presented avoids the need to conjecture phenomenological governing equations (i.e., uses the formulas from standard quantum computations used in interatomic force field development). Avoiding use of conjectured governing equations for VLP dynamics brings efficiency and minimizes uncertainty.

DMS is presently faster by an order of magnitude than conventional MD [1]. However, due to the extensive simulations required

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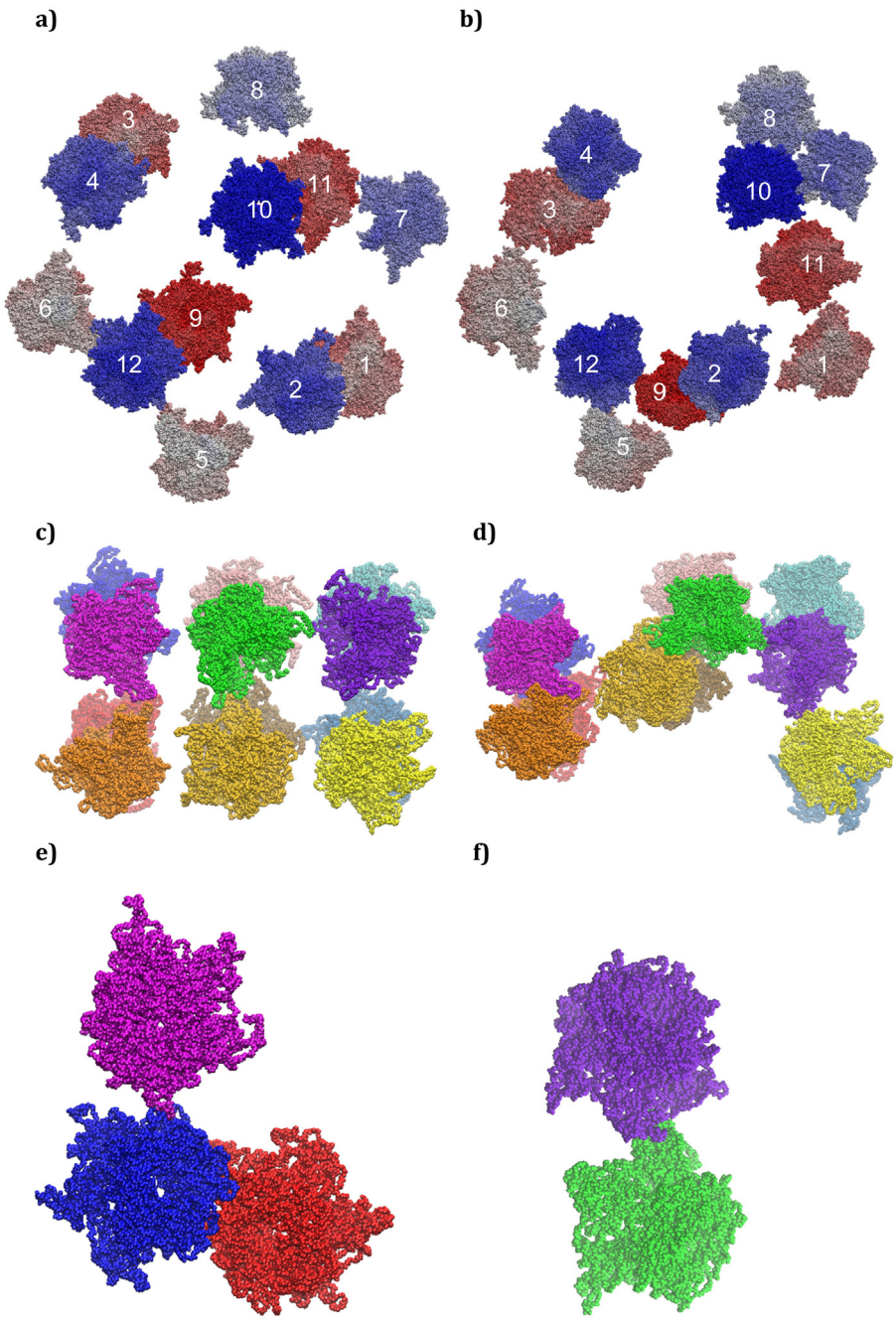


**Fig. 3.** DMS simulation of 12 HPV16 full-sequence pentamers. a) Initial configuration of pentamers radially displaced outwards, and b) after 3 ns. The MD duration δ was 50 fs, the selected CG timestep varied between 1-1.5 ps, and a total of 12 space-warping CG variables were used to capture the overall structure of the cloud of pentamers. The average speedup over MD was approximately 12. Coloring is according to out-of-planeatom coordinate (*blue* for top, *red* for bottom). The radius of gyration decreases from 211 to 201Å. The pairwise distance between the backbone center-of-mass of one pentamer and a neighboring one was calculated for all pairs. Pentamers 7, 8 and 10 tend to self-assemble into a trimer: the initial pairwise distance between pentamers7 and 8, 7 and 10, and 8 and 10 was 219Å, 220Å, and 220Å, respectively. After 3 ns, this distance decreases to 104Å, 136Å, and 140Å, respectively. Pentamers 1 and 11, 5 and 12, and 3 and 6 tend to self-assemble into three dimers: the respective pairwise distance decreases from 218Å, 223Å, and 214Å to 124Å, 115Å, and 132Å, respectively. c) Initial configuration of randomly oriented pentamers with centers of mass placed on $2\times2\times3$ grid, and d) after 7 ns. The overall curvature suggests the pentamers form a capsid larger than that of a T = 1. Pairs of pentamers appear in relative orientation which is appropriate for an icosahedral structure, e.g., pentamers in magenta, blue, and red form a trimer (e), and pentamers in green and violet form a dimer (f).

to achieve vaccine CAD, DMS is being accelerated further via the following advances and optimizations: (1) using a Padé approximant (PA) scheme to advance the CG variables, allowing greater CG timestep ($\Delta$) and stability, (2) automatically computing the CG timestep ($\Delta$) and the duration ($\delta$) of the MD run to optimize the balance between speed and accuracy, and (3) implementing different types of CG variables that facilitate the simulation of more sophisticated phenomena such as drug delivery to cell membranes and self-assembly of VLP monomers into pentamers and hexamers. These optimizations and extensions are outlined below as follows.

### 4.1. Pade approximant

The next advancement of the MF method in the context of VLP simulation should be to achieve even greater efficiency. Methods are needed that allow for larger $\Delta$ and smaller $\delta$ since the overall speedup is by $\Delta/\delta$. A promising way to achieve this is PAs [21].

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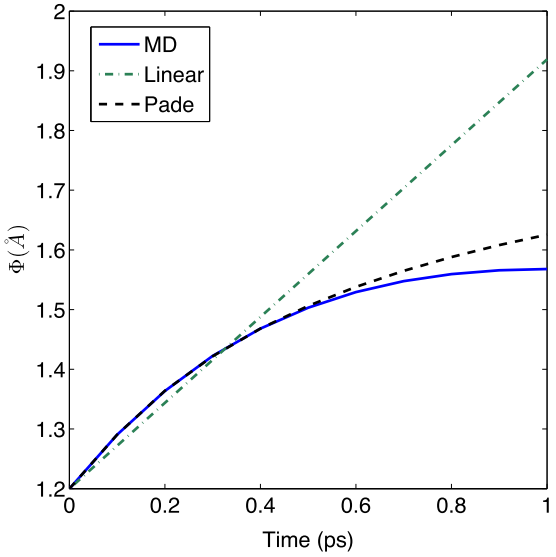


**Fig. 4.** Time variation in one space-warping CG variable obtained from MD (in blue line), linear extrapolation for MF (in teal dashes), and Pade extrapolation for MF (in black dashes) for a single, large CG timestep. This PA-enhanced extrapolation in time increases accuracy and allows for increasing the CG timestep $\Delta$, and therefore computational efficiency.

PAs are used in many fields to extend the range of an approximation [22]. Here, they are used to extend the interval of time over which a set of CG variables $\varphi$ is updated, i.e., to increase $\Delta$. Omnipresent random influences on the CG variables by the atomic fluctuations must be accounted for due to the cross-talk between atomic and CG processes. In a preliminary study (Fig. 4), the following PA is adopted to advance the CG variables over the next interval $[t, t+\Delta]$:

$$\varphi^{PA}(t+\tau) = \varphi(t) + \frac{a_{1/2}\tau^{1/2} + a_1\tau}{1 + b_1\tau}. \quad (1)$$

where $\tau$ is time relative to $t$-$\Delta$; $a_0 = \varphi(t\text{-}\Delta)$, and $a_{1/2}$, $a_1$, and $b_1$ coefficients are computed using $\varphi$ at $t$-$\Delta$, $t$-$\Delta$+$\delta$, and $t$+$\delta$. The result is a time advancement approximation, i.e., to $\varphi(t+\Delta)$ (when $\tau = 2\Delta$).

The $\tau^{1/2}$ term in Eq. (1) accounts for the formula of Ito derived in the context of analyzing Langevin equation [23]. The denominator is introduced to regularize the PA, i.e., to make $\varphi$ finite even for large $\tau$. More complex PAs can be constructed using additional data from the past.

### 4.2. Automation

For multiscale MD simulation of VLP to be practical, much of the modeling protocol must be automated, since the multiscale MD simulation methodology requires setting control parameters that are not familiar to the conventional MD. While there are many contemporary research groups that are familiar with traditional MD, application researchers may not use it. Thus, methods are being developed that require minimum user input by automating the multiscale control parameters.

The ideal values of the CG timestep $\Delta$, and the MD run $\delta$, can be automated as follows. The choice of $\delta$ is provided by the "stationarity" hypothesis [8,13] which suggests the existence of a "stationarity time" $t_s$ over which a system expresses a representative ensemble of CG velocities $\Pi$. This means that the ensemble is mostly the same for any $t$ within $[t_s, \Delta]$ and, as the result, the changes in the time integral of $\Pi$ (the "stationarity integral" [8,13]) become small after $t_s$. This notion has been used in the MF evolution algorithm to approximate the change in $\varphi$ over $[t, t+\Delta]$ interval as

$$\Delta\varphi^{PA} \cong \frac{\Delta}{\delta}\int_{t}^{t+\delta} \Pi(\tau)\mathrm{d}\tau. \quad (2)$$

Then the MD run time $\delta$ could be chosen as a minimum allowed value of $t_s$. The optimal $\Delta$ value is roughly the characteristic time of CG evolution, $t_{CG}$, which can be estimated as the ratio of the first to the second time derivatives of the $\varphi^{PA}$ (1). If $t_s \ll t_{CG}$ then the expected efficiency of the coevolution algorithm over a traditional MD one is $t_{CG}/t_s$, and thus the computational efficiency is $\Delta/\delta$.

### 4.3. Different types of CG variables

A critical issue in multiscale MD theory is the identification of CG variables that express a maximum of coherent behavior and a minimum of short timescale fluctuations. This is because such variables can be reliably extrapolated over long times based on information at an earlier time. Several types of CG variables have been investigated in this context: (1) space-warping parameters [7a,b,f–i,24], density fields [7d] scaled atom positions [25] and curvilinear coordinates [7e]. Their schematic definition and reason for their coherent evolution are as follows.

- *Scaled molecular position* [25]: scaled center-of-mass position of a cluster of covalently bonded atoms (i.e., molecules) is slow due to the large mass of the cluster. This variable tracks the long scale migration of a molecule as it meanders across an intracellular medium or across the blood stream.
- *Curvilinear coordinates* [7e]: these coordinates capture the conformation and twisting of a non-branched polymer and express minimal fluctuations due to the simultaneous movement of many atoms that they describe.
- *Population variables* [7d]: are density profiles of a molecular type in the vicinity of a spatial point and with orientation in a specified window of values; these variable evolve slowly due to the averaging effect over a large population of similar molecules.

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