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Epitope engineering and molecular metrics of immunogenicity: A computational approach to VLP-based vaccine design

Harshad Joshi [a,1], Kristen Lewis [a,b,1], Abhishek Singharoy [a], Peter J. Ortoleva [a,*]

[a] Center for Cell and Virus Theory, Department of Chemistry, Indiana University, Bloomington, IN 47405, USA
[b] Interdisciplinary Center for Nanotoxicity, Department of Chemistry, Jackson State University, Jackson, MS 39217, USA

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ABSTRACT

Developing antiviral vaccines is increasingly challenging due to associated time and cost of production as well as emerging drug-resistant strains. A computer-aided vaccine design strategy is presented that could greatly accelerate the discovery process and yield vaccines with high immunogenicity and thermal stability. Our strategy is based on foreign viral epitopes engineered onto well-established virus-like particles (VLPs) and demonstrates that such constructs present similar affinity for antibodies as does a native virus. This binding affinity serves as one molecular metric of immunogenicity. As a demonstration, we engineered a preS1 epitope of hepatitis B virus (HBV) onto the EF loop of human papillomavirus VLP (HPV-VLP). HBV-associated HzKR127 antibody displayed binding affinity for this structure at distances and strengths similar to those for the complex of the antibody with the full HBV (PDBID: 2EH8). This antibody binding affinity assessment, along with other molecular immunogenicity metrics, could be a key component of a computer-aided vaccine design strategy.

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1. Introduction

A number of virus-like particle (VLP)-based vaccines against high priority viruses are either FDA approved, under development, or in clinical trials [1–5]. Examples include Cervarix and Gardasil [6,7] against human papillomavirus (HPV), and vaccines against Chikungunya virus (CHIK) [8] and hepatitis E virus (HEV) [9]. The challenge in developing such vaccines is to create VLPs with surface and other properties that mimic the target virus, and thereby evoke a neutralizing antibody response. The objective of this article is to investigate computationally the interaction of antibodies with VLPs as one way to assess their potential immunogenicity, and thereby take one step toward achieving computer-aided vaccine design.

Traditional laboratory-based vaccine discovery methods are costly and time-consuming. To design a viable vaccine, the VLP must self-assemble, be thermally stable, and display surface protein structures (epitopes) in a manner similar to those of the target virus [10]. For example, capsid proteins self-assemble into VLP structures under specific conditions either *in vitro* or in a cell-based system (e.g., yeast or bacteria) [11]. The microenviromental conditions favoring self-assembly usually reside in a narrow window.

To illustrate the subtleties in designing highly immunogenic VLPs, consider nanoparticles constituted of the L1 protein of HPV (i.e. Cervarix and Gardasil). The VLP's outer surface features (i.e. epitopes) are read by the immune system to generate a specific response [12]. Several characteristics of the epitopes may influence a vaccine's immunogenicity. These include peptide sequence, loop conformation, or proximity and relative conformation of neighboring loops simultaneously [12]. Immunogenicity of a candidate vaccine may be a delicate and dynamic interplay between local epitope characteristics and a more VLP-wide effect [13]. However, experimental information on these characteristics is often not available. If such information was available, it would greatly assist in assessing the likely immunogenicity of a VLP. We suggest this information could be developed computationally. If the aforementioned epitope-localized factors are the only relevant ones, then one might expect that a L1 protein monomer or L1 pentamer could serve as a vaccine. However, the monomer is essentially not immunogenic and the pentamer is only weakly so, in contrast to the highly immunogenic whole $T = 7$ or $T = 1$ L1 VLP structures [14]. This is not explicable by the fact that a VLP contains more epitopes than a pentamer or monomer as demonstrated experimentally by increasing the dosage (and hence the number of epitopes). This is also despite the fact that epitope geometry is similar across all three structures [15]. Thus, to understand such VLP-wide effects, a local simulation approach is not likely to be sufficient. Therefore, a computational methodology is needed that accounts for epitope-scale properties within a whole VLP modeling framework.

* Corresponding author. Tel.: +1 812 856 6000.
E-mail address: ortoleva@indiana.edu (P.J. Ortoleva).
[1] These authors contributed equally to this work and should be considered co-first authors.

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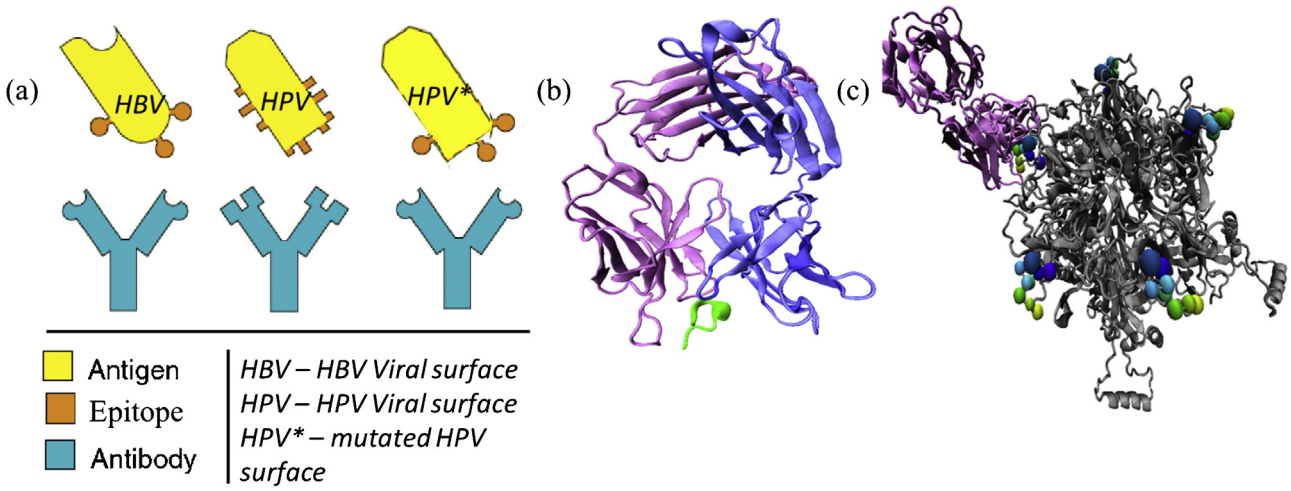

Fig. 1. Schema of the structures simulated in the present work. (a) Cartoon representation of epitope engineering of VLP concept. Epitopes (orange) presented on the viral surface (yellow) evoke specific immune response to generate the antibody (blue). In HPV* epitope structures for HBV is mutated onto the HPV viral surface to generate the antibodies specific to HBV. (b) Hepatitis B (HBV) epitope (green) complexed with the antibody HzKr127 (purple and magenta). This structure serves as the benchmark system for assessing viability of novel vaccine constructs. (c) human papilloma virus (HPV) 16 L1 pentamer (gray) engineered with HBV epitope (sphere representation) complexed with hepatitis B antibody HzKR127 (purple). This construct is demonstrated to yield similar binding affinity as in (b) and therefore presenting likely vaccine efficacy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Computer simulations to assess likely immunogenicity of putative vaccine candidates is taken here as a first step in computer-aided vaccine design. Thus, computational methods (and notably molecular dynamics (MD)) are used to score nanoparticles. MD has shown to be successful in the design of drugs and materials [16–19]. However, there is a large gap between predicting molecular-scale properties and the neutralizing antibody response of the immune system. In our approach, we consider a variety of molecular metrics to bridge this gap. The ultimate goal of introducing these multiple metrics will be to arrive at a predictive model of a given VLP immunogenicity, and, in particular, here we focus on the strength of antibody–epitope interaction as one of these metrics.

Though it is commonly thought that epitope structure plays a predominant role in VLP immunogenicity [20,21], other factors may also be crucial and add additional reliability in predicting VLP immunogenicity. Studies investigating relationship between identification of neutralizing and protective epitopes from a virus and high affinity interaction with antibodies demonstrate potential of this criterion as one of the factors [22,23]. Potential molecular metrics of immunogenicity include the following:

(1) thermal stability in blood or other relevant fluids;
(2) epitope peptide sequence;
(3) overall VLP size and shape;
(4) epitope 3-dimensional conformation;
(5) structures of a set of closely lying epitopes relative to each other;
(6) epitope structure fluctuations and its root mean-square deviation;
(7) strength of epitope binding to an antibody for the target virus.

We have studied 3–6 in the context of HPV-VLP stability and immunogenicity [13,24,25]. Here we introduce the 7th as another molecular metric of immunogenicity.

We propose to computationally engineer well-established, stable VLPs originally designed for one virus, by inserting epitopes of a target virus. We hypothesize that the presence of a target epitope sequence in the major epitope region of a VLP will elicit an immune response against the target. With this approach, we preserve the cost of synthesis by keeping the stable VLP as the platform for each application. At the same time, computational insertion/replacement of epitopes of the target virus enables testing different positions on the VLP surface to obtain maximum exposure for the "new" epitope, a procedure that otherwise would require a costly experimental study. That the knowledge of epitope structure or sequence is available for many target viruses makes the proposed approach feasible. Another advantage of our approach is that the complete structure of the target virus is not needed for simulating a VLP candidate.

An experimental epitope engineering approach has been explored by insertion of a hepatitis B virus (HBV) amino acid sequence into HPV epitope loops [26]. Here, we take HPV VLPs of type 16 ($T=1$ symmetry) as the stable delivery platform and use HBV as the target. One of the highly immunogenic epitopes of HPV, the EF loop (residues 170–189) (Fig. 1), is computationally replaced with a preS1 epitope of HBV; the latter was recently found to be responsible for eliciting an immune response against HBV [27] (Fig. 1). The resulting mutated HPV-pentamer is simulated here in the presence of the antibody HzKR127 [27] (Fig. 1). To test the validity of our approach, we use MD simulations to compare the interaction of antibody to three structures: (a) wtAB, for which the native complex of HBV epitope and antibody (HzKR127) are taken from the X-ray crystal structure (PDB ID: 2EH8); (b) mutAB, in which HBV epitope was inserted within the EF loop of HPV pentamer, thus presenting HBV epitope on the stable HPV pentamer platform; and (c) HPV-AB, wherein unmodified HPV EF loop is simulated with the antibody as a control. Comparison of these structures via their biophysical characteristics (such as RMSD and energetics) reflects how effectively the HzKR127 antibody binds to mutAB as opposed to HPV-AB. We demonstrate that mutAB exhibits similar binding affinity as wtAB, thereby suggesting that it could be used as an effective vaccine against HBV. We conclude by proposing a generalized workflow that uses molecular simulations of VLP candidates combined with informatics and wet-lab experiments (Fig. 2).

2. Methods

2.1. Epitope selection and placement

The epitope sequence of the major preS1 HBV peptide (residues 37–45; PDB ID: 2EH8 [27]) was engineered on the pentameric

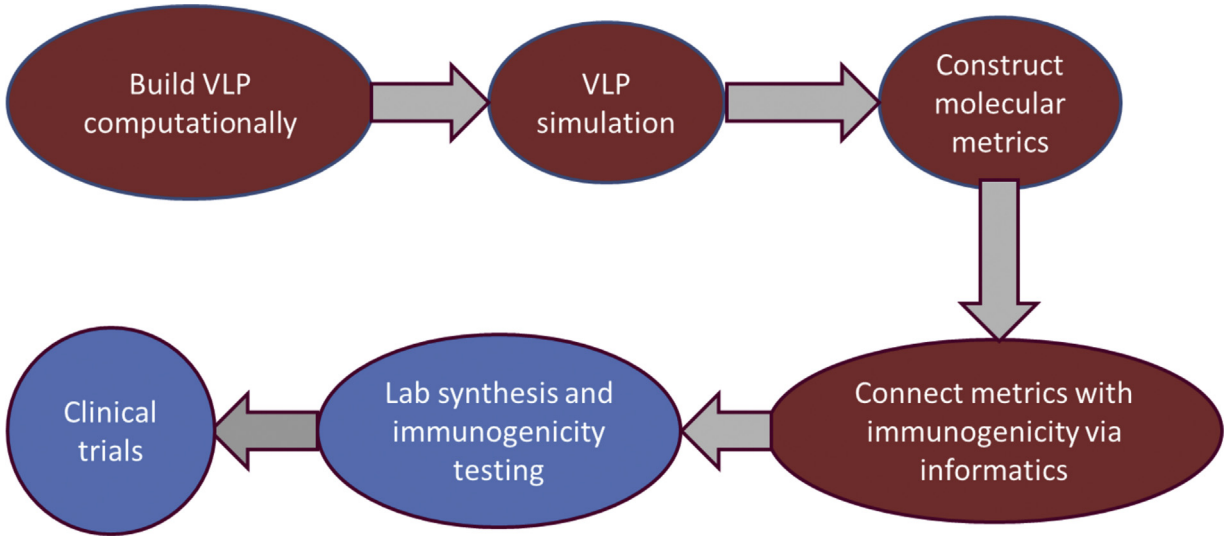

Fig. 2. Proposed workflow of combined computational/laboratory procedure for novel vaccine discovery based on virus-like particles (VLPs).

substructure of HPV 16 L1 protein $T = 1$ VLP (PDB ID: 2R5H [28]). The epitope comprised of the following amino acid sequence: Asn-Ser-Asn-Asn-Pro-Asp-Trp-Asp-Phe [29]. Through sequence alignment using EMBOSS utility,[2] appropriate positioning of the EF loop (residues 170–189; Fig. 1) of the HPV 16 L1 monomer was identified.

Starting from residue 170 the residues of EF loop were replaced and a short minimization was carried out to see whether the mutated epitope region faced outward to facilitate antibody docking. Finally, residues 177–185 on the EF loop of the HPV VLP were identified as optimal on basis of maximum surface exposure. Epitope engineering was carried out via VMD mutator plugin, version 1.3 [30]. The HzKR127 antibody was then placed near the mutated epitope of the HPV pentamer. This new system along with the crystal structure 2EH8 (denoted as wtAB in the main text) as well as HPV pentamer complexed with the antibody (denoted as HPV-AB) were then subjected to molecular dynamics (MD) simulation of 10 ns using NAMD [31] in explicit solvent. Systems simulated are summarized in Table S1.

2.2. Molecular dynamics simulation details

All simulations were performed with the MD software NAMD [31] using the CHARMM27 force field [32] for proteins and the TIP3P model for water [33]. The simulated systems were kept at constant temperature using Langevin dynamics for all non-hydrogen atoms, with a Langevin damping coefficient of 5 picosecond^{-1}. A constant pressure of 1 atm was maintained using the Nosé-Hoover Langevin piston [34] with a period of 100 femtosecond (fs) and damping timescale of 50 fs.

Simulations were performed with an integration time step of 1 fs under a multiple time stepping scheme [35]; bonded interactions were computed every time step, short-range non-bonded interactions every two time steps, and long-range electrostatic interactions every four time steps. A cutoff of 12 Å was used for van der Waals and short-range electrostatic interactions; a switching function was started at 10 Å for van der Waals interactions to ensure a smooth cutoff. The simulations were performed under periodic boundary conditions, with full-system, long-range electrostatics calculated by using the particle-mesh Ewald method with a grid point density of 1 Å. The unit cell was large enough so that adjacent copies of the system did not interact via short-range interactions. Prior to simulation, each system was subjected to 1000 steps of conjugate gradient energy minimization, followed by 100 picoseconds (ps) of equilibration. We then performed all-atom molecular dynamics as described above, at 300 K for 10 ns for each system to equilibrate the whole system while the root mean square deviation was monitored. Finally, the production run were continued for 10 ns after the equilibration was achieved, storing coordinates at every 2 ps. To increase statistical significance 5 different simulations were performed with slightly different conditions in initial coordinates.

2.3. Critical distances between residue-pairs

Distances between the epitope and key antibody residues [27] were examined in order to compare the locale similarities of the three structures. These 5 antibody residues consisted of Asn35H in CDR H1, Arg50H in CDR H2, Tyr32L in CDR L1, and Gly91L and His93L in CDR L3 [27]. The following epitope–antibody pairs were evaluated: Asn2P (residue 177) with hotspot Gly91L, Asn4P (residue 179) with Arg50H, Asn5P (residue 180) with hotspot Gly91L, Asn5P (residue 180) with His93L, Asn5P (residue 180) with Arg50H, Asp7P (residue 182) with Asn35H, and Asp9P (residue 184) with Tyr32L. For HPV-AB, the aforementioned epitope residues were not present since its EF loop remained unchanged. However the same epitope position was used to gauge distances between that specific epitope residue and its associated AB hotspot.

2.4. Binding affinity

The binding affinity of the aforementioned pairs was also evaluated using VMD and NAMD [31]. The following equation was used in order to calculate the average binding affinity of each system over 10 ns:

$$(E_{\mathrm{TOTAL}} - E_{\mathrm{WATER+ION}}) - (E_{\mathrm{ANTIBODY+WATER+ION}} + E_{\mathrm{PENTAMER+WATER+ION}}) = \text{Binding Affinity}.$$

[2] EMBOSS utilty – European Bioinformatics Institute. *EMBOSS Stretcher – Pairwise Sequence Alignment*. 2012. http://www.ebi.ac.uk/Tools/psa/emboss_stretcher.

3. Results and discussions

3.1. Overview

We present a computer-aided VLP-based vaccine discovery workflow based on all-atom computer simulations. Using molecular metrics constructed by these simulations, we seek a method to achieve reliable predictions of immunogenicity of a candidate vaccine nanoparticle. The present study focuses on establishing binding affinity of the antibody to the epitope–antibody complexes as one of the molecular metrics to assess likely immunogenicity of a vaccine candidate. When combined with traditional laboratory approaches, this strategy will yield significant time and cost-efficiencies for vaccine discovery and production (see Fig. 2). Furthermore to reduce calibration for simulation parameters, our strategy uses well-established interatomic force fields so that range of viruses and their microenvironmental conditions can be simulated [24,36–38].

3.2. Equilibrium distances between key residue-pairs

The time course of distances between key residues on the antibody versus on the epitope were obtained from the MD simulations, and time–average distances were calculated. These residue pairs were postulated to play a key role in immunogenic response through strong binding affinity with the antibody [27]. wtAB simulation shows that evolution of distances between specific residue epitope–antibody pairs agreed with those observed in the crystal structure and remained stable for the course of the simulation. As hypothesized, the time evolution of distances for the mutAB complex was similar to that of wtAB complex in almost all cases (see Fig. S1: 2, 3, 5, 7). It was noted that these distances fluctuate minimally even after epitope engineering onto the HPV pentamer. In general, the distances for most residue pairs in HPV-AB system were observed to be much larger than those in the wtAB. This supported the hypothesis that the HPV-AB complex is not as stable as the other two systems (also see Fig. S2 in supplementary information).

3.3. RMSD measurements

RMSD measurements were performed to assess the overall stability as following. The epitope and the region of the antibody that binds to the epitope (heavy chain: residue 1 to 110; light chain: residue 1–107; denoted AB + EP henceforth) showed overall higher stability in mutAB and HPV–AB complexes as compared to that in wtAB complex. However, the distance evolution for the key epitope–antibody residue pairs in AB + EP for wtAB was seen to be stable, thereby showing that the epitope is strongly bound throughout the course of the simulation. Thus, the unexpected higher structural stability in mutAB and HPV–AB complexes was initially attributed to the presence of the stable HPV pentamer platform. To test this reasoning and to probe fluctuations of individual components, RMSD was measured for (a) epitope (EP), and (b) the region of the antibody that binds to the epitope (AB).

This decomposition (Table 1, also see Fig. S3) shows that antibody structure and dynamics is greatly affected by the presence of the pentamer. It appears that the epitope binding region of the antibody is stabilized in the presence of the pentamer platform. In wtAB such a stabilizing platform is absent, and antibody fluctuations are significantly larger than those in the other two systems. This high level of antibody fluctuation adds to the overall RMSD of AB + EP system for wtAB. Fluctuations for the epitope (EP) in mutAB are also lower than those in wtAB. The former is expected because in wtAB, the epitope-containing loop is unsupported by any stabilizing platform thus yielding larger fluctuations. In mutAB, these fluctuations are significantly reduced because the epitope-containing loop is now supported by the HPV pentamer. However, despite the similar stable platform in the HPV–AB, epitope fluctuations in HPV–AB are the largest. This observation supports our earlier hypothesis that for neutralizing antibody response, the epitope should present low fluctuations [13]. Since, the epitope-containing loop here corresponds to HPV epitope, it fluctuates more in the presence of the HBV antibody and therefore the HBV antibody would not readily bind to it.

Table 1
Root mean square deviation (RMSD) values for individual structural components of the three simulated systems. AB + EP denotes RMSD for epitope and its binding region in antibody, EP denotes RMSD for epitope only while AB denotes RMSD for epitope binding region of the antibody.

System	wtAB			HPV-AB			mutAB		
Subsystem for RMSD	AB + EP	AB	EP	AB + EP	AB	EP	AB + EP	AB	EP
Mean RMSD [Å]	2.28	2.26	2.62	2.19	2.09	3.98	1.95	1.97	1.08
Std dev.	0.74	0.72	1.12	0.45	0.42	1.26	0.46	0.47	0.33

To further validate our conclusions, another set of simulations was carried out wherein the end residues of the epitope were frozen in position. RMSD calculations displayed wtAB as the most stable structure followed by mutAB, while HPV-AB is the least stable system (see Table S2, and also Fig. S4). This set of observations confirmed that the HPV pentameric platform suppresses the fluctuations of the bound antibody. These observations also provide a plausible molecular mechanism of immunogenicity. In this reasoning, for strong binding, both epitope and antibody should exhibit lowest possible fluctuations. Therefore, since antibody HzKR127 in the presence of HPV pentamer (HPV-AB) exhibits the largest fluctuations, its binding to the HPV pentamer is inhibited as expected (Table 2).

3.4. Binding affinity

Affinities for antibody–epitope binding were calculated for each system. Favorable binding of antibody occurs for both wtAB and mutAB, whereas the binding affinity for antibody for the HPV-AB system was three times weaker than for the other two systems. This observation further qualifies mutAB to be a promising vaccine candidate for HBV. The above observation also demonstrates that binding affinity between antibody and an epitope-containing loop can serve as one of the molecular metrics to predict likely immunogenicity.

3.5. Identification of key epitope–antibody residue pairs

Site directed alanine-scanning mutagenesis studies [27] suggest that antigen–antibody binding activity was completely disrupted upon mutations in antibody residues Arg50H (bound to EP residues 4 and 5), Gly91L (bound to EP residue 5), or Tyr32L (bound to EP residue 9). Similar alanine mutations of EP residues 5–9 completely abrogate antigen binding, thus suggesting that these residues are also important in binding with the antibody HzKR127 [27]. Our distance analysis (Section 3.1) showed that the distance between the

Table 2
Binding affinities between the antibody and the epitope region for three simulated systems. mutAB and wtAB showed similar affinities while unmutated epitope region showed lower affinity toward HBV antibody. The results shown are summarized for 5 simulations.

Binding affinity [kCal/mol]			
System	wtAB	mutAB	HPV-AB
Mean	−264.8	−246.07	−72.82
Std. dev.	29.15	24.93	22.93

aforementioned pairs is stable in wtAB as well as mutAB indicating that the engineered pentamer responded to the antibody in a similar ways as did native HBV. Thus, our analyses show that with computer-aided design, residues that play a critical role in antibody binding can be distinguished.

4. Discussions

The *in-silico* vaccine design approach proposed here based on such all-atom multiscale simulations, combined with wet-lab experiments will accelerate novel vaccine discoveries by many folds. Efficiencies enabled by the present computer-aided vaccine design approach could greatly reduce mortality due to infectious diseases.

Results presented here are based on traditional MD simulations. However, present all-atom whole VLP simulation via traditional MD approach faces difficulties arising mainly from the sheer number of atoms (10^6–10^9 for the VLP-microenvironment system), that must be simulated for long times (10^{-6}–10^{-4} s). Considering the many simulations that are required for a computer-aided vaccine design strategy, a more efficient approach which, like traditional MD, preserves all-atom details is needed. The all-atom multiscale approach developed earlier can be utilized to achieve this efficiency [13,24,36].

To demonstrate our epitope engineering approach we chose pentameric substructure of $T = 1$ HPV-VLP system rather than whole VLP for following reasons. Recent studies showed that pentameric substructures of the whole HPV-VLP also exhibit at least mild immunogenicity, while monomers show little to none [39]. Thus even though it is less immunogenic than a whole VLP (for HPV), a pentameric substructure may prove to be a viable stable platform to present epitopes of other viruses reliably (so that they can be read by the immune system). Second, we have previously studied HPV-VLP and its sub-constituent structures in detail with all-atom and our multiscale simulation methods [13,24,40–46]. Thus, for a hybrid system based on a HPV platform, benchmarking against non-mutated HPV systems can readily delineate differences arising from mutations in the HPV platform. For example, we have performed full $T = 1$ HPV-VLP simulations as long as 100 ns which show remarkable stability of the $T = 1$ structure as observed experimentally [24]. Finally, pentamer-based structures can be prepared efficiently in laboratories in cell-free environment, thus reducing cost and time compared to full-VLP based vaccine candidates. With this we have also shown that pentameric HPV-VLP can be tethered to curved silica nanoparticle to mimic most of the behavior of a complete HPV-VLP. Such synthetic nanostructures can be efficiently designed via our proposed *in-silico*/wet-lab approach.

There is currently a large gap between predicting VLP configuration and its evolution in time, versus predicting the antibody response of the immune system. In our on-going work we aim to achieve by using molecular simulation of a proposed vaccine nanoparticle to predict the proposed vaccine nanoparticle's overall and molecular scale properties, and applying bioinformatics to the thus-predicted properties to predict the likely immunogenicity of the proposed vaccine nanoparticle. Earlier we defined epitope fluctuations as a molecular property affecting likely immunogenicity [13]. In the present work a next step toward computer-aided vaccine design approach is attained. The thus-predicted properties include overall size, geometry and thermal stability of the proposed vaccine nanoparticle as well as epitope conformation, the geometric relation between multiple closely-lying epitopes, and the statistics of the variations of epitope structure over time as predicted by the molecular simulation. These properties measured on molecular or atomic scale are termed as molecular metrics of immunogenicity. They together define the extent of immunogenicity of the proposed vaccine nanoparticle thus bridging the gap between system wide immune response and the molecular scale behavior. Since verifying viability of each vaccine nanoparticle experimentally is time and cost consuming, this *in-silico* route helps to delineate the short-listed highly immunogenic (predicted) vaccine nanoparticles which then can be synthesized and tested time-efficiently.

To quantify immunogenicity of the vaccine nanoparticle, we involve multiple molecular metrics and one of these, binding affinity between an epitope and antibody, is assessed here. Having multiple metrics yields more reliability for the prediction. Reliability from multiple metrics is also supported from the RMSD measurements in Section 3.2. Here the structural considerations play an important role when comparing the fluctuations. In our earlier works [13], we proposed fluctuations as one of the molecular indices of immunogenicity. In this case, inertial mass of the scaffold dictate the fluctuations. Thus, HPV-AB system shows less fluctuation than wtAB. We hypothesize that fluctuations in wtAB also arise from the fact the structure of wtAB only holds epitope segment which is unsupported as against in HPV-AB case. Larger inertia arising due to pentameric scaffold suppresses fluctuations in HPV-AB system. When the ends of epitope were frozen in wtAB to mimic the pentameric scaffold, the fluctuations were more reliably seen (Supplementary Information). Thus instead of relying on only one metric we combine multiple molecular metrics to yield a single quantified measure of immunogenicity.

Since our atomistic simulations have 3D structure as the starting point, it is also possible that all of the input information may not always be available. For example, the epitope-antibody binding affinity is only assessed if structure of the antibody is known. Similarly, ever present threat of virus mutation is not dealt with in the present work. It is possible that a virus changes and thus the antibody is no longer protective. Our molecular metrics approach deals with assessing the potential immunogenicity of a nanoparticle given knowledge of antibody and epitope for a particular virus. This approach does not address broader issue of design of new vaccine particle. However, as stated earlier, silica based nanoparticles tethered with multiple epitopes can be readily synthesized. Thus, with a library of known epitope sequence/structure information and stable silica based nanoparticles designing a vaccine may become as easy as plug-and-play. With such constructs made available, this will also eventually address the issue of mutating viruses. This current study is a natural proof of concept toward this goal.

5. Conclusions

Recent computational engineering approaches have shown promise to guide the modification of proteins for therapeutic objectives [19,48]. However, to our knowledge, there is still a gap between simulated molecular characteristics of viral proteins and predicted antibody response of the immune system. Our strategy is a natural step toward bridging this gap to arrive at immunogenicity prediction.

The present study is based on computational engineering of a thermochemically stable platform that could serve as a vaccine. If these platforms can be dressed with epitopes of a target virus, the resulting hybrid structure could serve as a vaccine against the target virus. In our demonstration, an HBV epitope was inserted into an HPV pentameric platform to evoke response to HBV antibody. We demonstrate that epitope–antibody binding affinity may be treated as one of multiple molecular metrics to assess likely immunogenicity of such structures as putative vaccines. The strategy we suggest starts with constructing putative vaccine design computationally, simulating these constructs to arrive at quantitative metrics, and then using the latter to predict immunogenicity reliably. The

designs scored to be highly immunogenic can then be synthesized and tested by e.g., pseudovirion neutralization assays. The resulting agile vaccine discovery workflow integrating computation schemes and laboratory techniques (Fig. 2) could considerably reduce time and costs associated with wet-lab-only procedures. It is prudent and timely that such an *in-silico/in vitro* procedure be adapted for efficient vaccine discovery warranted amidst a pandemic. Stable platforms that will be dressed in such strategy can be independently refined for more thermochemical stability so that minimal cold-chain is required to transport them to remote and difficult places.

Current HBV vaccinations successfully suppressed HBV infection. Concerns are recently growing due to the appearance of HBV variants/mutants in immunized children, and due to the scarcity in the vaccination supply chain [49,50]. These concerns have also been expressed regarding other pathogens [49,51], thereby suggesting a need for a more efficient vaccine discovery workflow. Recently, several successful vaccines highlight the potency of antibody responses induced by VLPs [12]. However, laboratory methods to prepare VLPs and test them for stability and immunogenicity are time-consuming, raising a concern regarding addressing an unfolding pandemic. In this study, we demonstrate how computer-aided design could serve as a first step in a highly efficient vaccine discovery workflow.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.07.075.

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