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Broad spectrum assessment of the epitope fluctuation—Immunogenicity hypothesis

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ABSTRACT

Prediction of immunogenicity is a substantial barrier in vaccine design. Here, a molecular dynamics approach to assessing the immunogenicity of nanoparticles based on structure is presented. Molecular properties of epitopes on nonenveloped viral particles are quantified via a set of metrics. One such metric, epitope fluctuation (and implied flexibility), is shown to be inversely correlated with immunogenicity for each of a broad spectrum of nonenveloped viruses. The molecular metrics and experimentally determined immunogenicities for these viruses are archived in the open-source vaccine computer-aided design database. Results indicate the promise of computer-aided vaccine design to bring greater efficiency to traditional lab-based vaccine discovery approaches.

1. Introduction

Nanoparticle-based vaccines are in widespread use or in various stages of development [1–3]. For example, human papillomavirus (HPV) [4,5] and hepatitis E virus (HEV) [6] vaccines are based on virus-like particles (VLPs) and are in clinical use. VLPs are noninfectious nanoparticles that display the key structural features of native target viruses and induce a high immune response [7]. Here, a correlation between properties of VLPs predicted by molecular dynamics computations and experimentally determined immunogenicity is presented.

A priori, it is challenging to predict whether a vaccine candidate can induce a neutralizing antibody response. Recently, there have been advances in systems biology to predict vaccine efficacy, but there does not seem to be methods that relate molecular properties to vaccine immunogenicity [8]. In experimental studies, a positive correlation between immunogenicity and increasing nanoparticle assembly size has been observed [9]. For instance, HPV VLPs exhibit higher immunogenicity than subunit assemblies, proteins, or peptides extracted from the same target virus (Fig. 1) [9]. These findings seem to suggest that larger viral protein assemblies elicit stronger immune responses, even after accounting for differences in total number of epitopes delivered (i.e. the dosage effect). However, Varsani et al. [10] showed that the immune response can vary with epitope position on the same VLP. In summary, these studies imply that there may be a correlation between VLP molecular-scale properties and immunogenicity that changes with VLP assembly size.

In computational studies of HPV VLPs, the intensity of fluctuations expressed by epitopes decreased across monomers, pentamers, and VLPs, respectively [11]. Moreover, the degree of epitope fluctuation was observed to have an inverse relationship with antibody binding energy in flaviviruses [12]. These results suggest that computationally predicted epitope fluctuation can serve as a molecular indicator of vaccine immunogenicity. Other molecular metrics that could be predictive of immunogenicity include epitope density (i.e. the number of epitopes presented per particle) [13]. It has been proposed that VLPs have a greater ability than lower-order assemblies (monomers, pentamers, etc.) to present epitopes in a packed array, which encourages B-cell receptor cross linking and a stronger immune response [14].

Computer-aided vaccine design holds promise for reducing the immense amount of time and cost of experimental vaccine discovery. Currently, most bioinformatics approaches to vaccine design focus on antigen prediction via sequence alignment algorithms [15–17]. Such methods can be effective for T-cell epitope discovery [15,16], and there has been some effort to link the number of predicted epitopes per particle to immunogenicity [8]. However, these methods are not effective at discovering B-cell epitopes [18–20], which is required for a strong neutralizing antibody response [21]. These methods provide information on epitope location, but not on vaccine efficacy.

The present study introduces a vaccine computer-aided design (VCAD) method to quantify the relationship between molecular properties and immunogenicity. Here, the epitope

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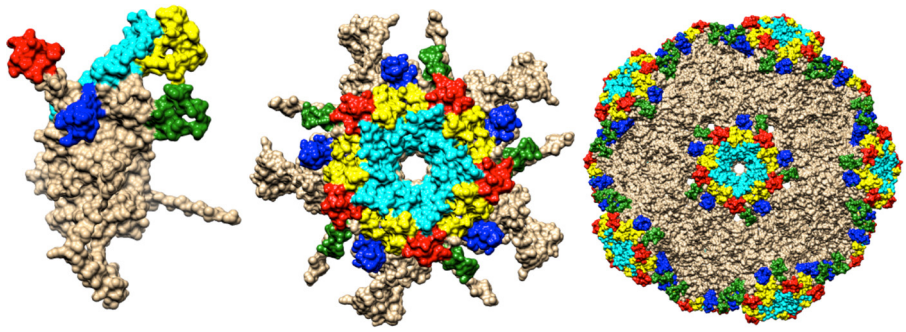

Fig. 1. The HPV VLP assembly hierarchy: (a) monomer, (b) pentamer, and (c) VLP. Epitopes are highlighted: BC (blue), DE (cyan), EF (green), FG (yellow), and HI (red).

fluctuation–immunogenicity hypothesis [11,22]—a proposed inverse correlation between immunogenicity and epitope fluctuation intensity—is assessed using a spectrum of nonenveloped viruses. This study represents the first attempt to link computationally predicted molecular properties and experimentally derived immunogenicity for a spectrum of viruses. Molecular dynamics (MD) was used to simulate VLPs of these nonenveloped viruses and correlations are made between intrinsic properties of their epitopes and experimentally derived immunogenicity.

2. Methods and materials

2.1. Nanoparticle assemblies and molecular dynamics simulations

Atomic coordinates for HPV 16 L1 coat protein [23], HEV VLP [24], human enterovirus (EV) A71 [25], poliovirus (PV) types 1 [26] and 3 [27] nanoparticle assemblies were obtained from crystal structures available in the RCSB Protein Data Bank [28]. A monomer and VLP were constructed for HEV. For EV A71, the following were constructed: peptides (residues 211–225 and 136–150), monomers (VP1 and VP2), pentamer, and VLP. For PV1, the following were constructed: peptides (residues 121–141, 182–201, and 270–287), monomers (VP2 and VP3), pentamer, and VLP. Only the VLP was constructed for PV3. A monomer, pentamer, and VLP were constructed for HPV 16. VLPs were constructed using monomer information from the RCSB Protein Data Bank and ViperDB [29].

HEV, EV, and PVs were solvated using TIP3P water and 0.3 M NaCl through the VMD 1.9.2 Autoionize 1.3 plugin [30,31]. Each one was placed in the center of the solution, and was separated by at least 1.0 nm from the box wall. Periodic boundary conditions were used, with at least 2.0 nm of water between images. System sizes varied in the range of $\sim 10^5$–10^6 atoms. The short-range cutoff was set to 1.2 nm with a switching function starting at 1.0 nm. The long-range electrostatics was handled using the particle mesh Ewald's method with a grid spacing of 1.0 Å. A constant temperature of 300 K was maintained using Langevin dynamics with a damping coefficient of 5 ps for nonhydrogen atoms. A constant pressure of 1 atm was maintained using the Nose-Hoover Langevin piston with a period of 100 fs and a damping time scale of 50 fs. 1 fs time steps were used and data was collected every 1 ps.[32] Using NAMD 2.9 [33] with the CHARMM27 force field [34], steepest decent energy minimization and equilibration were applied for each system prior to simulation. Each system was equilibrated until the RMSD relative to the initial structure was approximately constant and the potential energy was minimized. The RMSD was calculated as follows:

$$\sqrt{\frac{1}{N}\sum_{n=1}^{N}|r_n - r_{n,\mathrm{ref}}|^2} \tag{1}$$

Where r_n is the position of backbone atom n and $r_{n,\mathrm{ref}}$ is the position of the reference for atom n. Production runs were 5 ns in duration after the RMSD was constant.

To determine whether calculated molecular quantities (Section 2.2) were sensitive to computational conditions, HPV 16 was simulated under different conditions than the previous viruses. Each HPV 16 structure was solvated using SPC/E water [35] and 0.142 M NaCl using GROMACS 4.6.5 [36]. Each viral structure used the GROMOS 54A7 force field and was at least 1.4 nm from the box wall [37]. Steepest decent energy minimization and equilibration were performed for each system prior to each of the production runs, as above. A constant temperature of 300 K was maintained using the v-rescale thermostat [38] with a 0.1 ps coupling time and the Parrinello–Rahman barostat [39] with a 1 ps relaxation time. Hydrogen bonds were fixed using the LINCS algorithm [40], allowing for 2 fs time steps. Production runs were 5 ns in duration.

2.2. Selection of intrinsic metrics

Mean square fluctuations (MSF) and solvent accessible surface area (SASA) were calculated to correlate them with experimental immunogenicity. These metrics were chosen because MSF can measure epitope flexibility, which has been shown experimentally to mediate binding between epitopes and antibodies [41–43]. SASA was chosen because epitopes must be accessible to bind with antibodies. Therefore, it might be hypothesized that the most solvent accessible epitopes elicit the strongest immune responses.

2.2.1. Mean square fluctuations

An inverse correlation between MSF and immunogenicity was hypothesized for HPV VLPs and possibly for other systems [11,44]. Large MSF values indicate high epitope flexibility, resulting in conformational epitopes in improper orientations and unable to bind effectively to immune cell surface receptors [45–47]. MSF was calculated with center of mass translation and rotation removed via the following formula:

$$\frac{1}{N}\sum_{n=1}^{N}\frac{1}{T}\sum_{t=1}^{T}|\vec{r}_n(t) - \langle\vec{r}_n\rangle|^2 \tag{2}$$

where $\vec{r}_n(t)$ is the position of backbone atom n of a residue with N backbone atoms at time t and $\langle\vec{r}_n\rangle$ is the time average position of atom n and T is the number of discrete time steps. For a pentamer or larger assembly, each of its monomers were analyzed separately and averaged.

2.2.2. Solvent accessible surface area

High SASA of epitopes is hypothesized to be a necessary, though not sufficient, condition for epitopes to elicit a neutralizing antibody response. From this perspective, SASA would be expected to be positively correlated with immunogenicity. SASA is computed via the "rolling ball" algorithms implemented in GROMACS by g_sas,

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