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REVIEW ARTICLE

Biomolecular Engineering of Virus-Like Particles Aided by Computational Chemistry Methods

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Virus-like particles (VLPs) are repetitive organizations of viral proteins assembled in an appropriate physicochemical environment. VLPs can stimulate both innate and adaptive immune responses, due to their particulate structure enabling uptake by antigen presenting cells. These characteristics have led to successful development of VLP-vaccine products, and will ensure their vast potential in years to come. Future success of VLP therapeutic products will be determined by advances in their bioengineering, and also by the development of tools to design for their stability, function and application. This review focuses on approaches for VLP assembly in controlled chemical environments *in vivo* and *in vitro*, and the application of computational tools for improved chemical sequence design, and fundamental understanding of assembly.

1. Introduction

Virus-like particles (VLPs) are highly organized multimeric protein complexes[1] that self-assemble from viral structural proteins.[2-4] The beautifully simple architecture of viruses enables assembly from few structural elements;[5] VLPs assemble from copies of one or more structural proteins to form native viral conformation whilst containing no genetic material and are therefore incapable of spreading infection.[6] Their repetitive antigenic structure efficiently stimulates beneficial cellular and humoral immune responses,[7,8] accelerating research into their potential for new vaccine technology. VLP vaccines[4,9-13] have proven success with currently licensed VLP-based human vaccines on the market including those for Hepatitis B (HBV), Human Papillomavirus (HPV) (Gardasil®, Merck & Co; Cervarix®, GlaxoSmithKline), and Hepatitis E (HEV) (Hecolin®, Xiamen Innovax Biotech Co. Ltd.). Whilst their native architecture can be exploited for antigenic display, VLPs also show great potential in gene therapy,[12] drug delivery,[14,15] diagnostics, materials science[16,17] and catalysis.[18-20] Using the VLP directly as an empty shell holds potential for packaging a payload, such as DNA or other therapeutics.[2] The increasing need for more complex biological products and new therapeutic technologies ensures that the advancement of VLPs will continue for many years to come. Success of future VLP application is dependent on the advances in their bioengineering, along with the tools available to understand and design for their stability, function and application. This review focuses on approaches for VLP assembly in controlled chemical environments *in vivo* and *in vitro*, and the application of computational tools for improved understanding of VLP architecture and stability, and for improved chemical sequence design.

2. Self-assembly

The self-assembly of VLPs is dependent on the native architecture of the virus and its complexity. The viral structural proteins can either self-assemble to form the particle 'shell' or can assemble through intermediate steps, with or without scaffolds or chaperones; regardless of the route, the VLP usually forms multimeric subunit structures, such as capsomeres, in the process of assembly.[2] These subunits can be expressed in a range of recombinant expression systems to produce VLPs. VLP bioprocessing with *in vivo* assembly is widely accepted, though has many challenges, including contamination from host proteins and/or DNA.[21] *In vitro* assembly provides a welcome alternative to cell-orchestrated assembly enabling more sophisticated control, and has addressed many of the challenges faced with *in vivo* contamination of VLPs.[22] Advances in the biopharmaceutical industry have enabled production of viral subunit protein at increasingly large scale, such that cell-free VLP assembly becomes one of the final steps in VLP production.

Assembly of the purified subunit protein is somewhat controlled by tailoring the surrounding physicochemical environment, such that the forces that drive self-assembly are triggered to a desired on or off state.[23] Previous work has shown that solution conditions such as pH, ionic strength, salt concentration, and protein concentration each have an effect on VLP assembly, such that they can trigger or suppress assembly and/or aggregation of protein subunits.[23,24] Despite the observed effects of solution conditions on self-assembly,

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the exact mechanisms are not entirely understood. This is in part due to the inability to observe assembly in real-time due to the rapid transition, and the microscopic scale, as well as the inability to precisely measure the macromolecular forces in play. Probing the self-assembly mechanisms at the micro- and macro-scale, can be performed with the use of computational methods.

3. Computational Methods

As mentioned above, VLP self-assembly is a complicated process[25] as a result of its multilevel structure. For instance, murine polyomavirus (MPV) VLP consists of 72 capsomeres comprising five VP1 structural proteins.[2,22] Two stages are involved in the formation of VLP, one is from VP1 to capsomere, and the other is from capsomere to VLP. Herein, this review will focus on the formation of non-enveloped VLPs,[26] and the application of computational methods on non-enveloped capsids and their subunits. Most well-studied viral capsids form spherical shells with icosahedral symmetry, or rod-shaped structures with helical symmetry,[12,25,27,28] or other shapes such as conical capsid,[29] and this review will focus on spherical capsids because they are widely utilized in practical applications. There are already more than 450 completed icosahedral virus capsid structures within the VIPERdb database[30] (http://viperdb.scripps.edu). Most of these structures are determined using X-ray crystallography,[31] while the others are determined using nuclear magnetic resonance (NMR)[32] or cryo-electron microscopy (cryo-EM),[33-36] or by a combination of these methods.[37] There are many other experimental approaches used to visualize the shape and morphology of VLP in a direct manner, such as scanning electron microscopy (SEM),[38-40] transmission electron microscopy (TEM),[41,42] and atomic force microscopy (AFM).[43,44] Structure determination approaches have enabled understanding of VLP and characterization of both protein subunit and VLP.[22] However, a more difficult question is the multilevel self-assembly from subunit to VLP. Examination of kinetic process and extracting mechanistic information using experimental approaches[45] is still challenging.[46] Crystallized structures are limited to a static conformation and may not represent the proteins in their native solution conditions. NMR should be helpful to examine the protein structure in solution, but it is usually not practical for large molecular assemblies such as VLP. Asymmetrical flow field-flow fractionation (AF4)[40] is powerful for online evaluation of VLP self-assembly, but it focuses more on the self-assembly process or colloidal outcome rather than the microscopic structure such as the conformational transition of protein subunits. From the thermodynamic point of view, self-assembly is limited by unfavorable intra-entropy change, although this can be compensated by favorable contribution gained from both the intra-enthalpy and the support of surroundings. Then dynamic control such as careful regulation of the molecular interactions within VLP or between the VLP and surroundings has been proposed crucial to facilitate or improve the self-assembly process. A modeling investigation of Ding *et al.*[24] indicated that the initial contacts between two capsomeres are crucial for the correct self-assembly. However, self-assembly of protein subunits to VLP is still a poorly understood phenomenon of which elucidation in molecular scale may aid the exploration of beneficial applications of VLPs.

Computational approaches, such as bioinformatics, homology modeling, and molecular simulation,[47-49] have been extensively utilized and almost universally accepted as complementary to empirical structural analysis methods, and in combination to provide molecular details, especially the molecular interactions involved in the self-assembly.[22,25,50] Computer modeling of VLPs has largely focused on the self-assembly kinetics of VLPs, directed at minimizing protein aggregation during processing.[22] A limiting factor for computational modeling of VLPs is their large size, as it requires extensive computational resources and long time periods for simulation. Multi-scale models[51-53] have thus been used to reduce the required computing resources for various VLPs,[22] as illustrated in Fig. 1.

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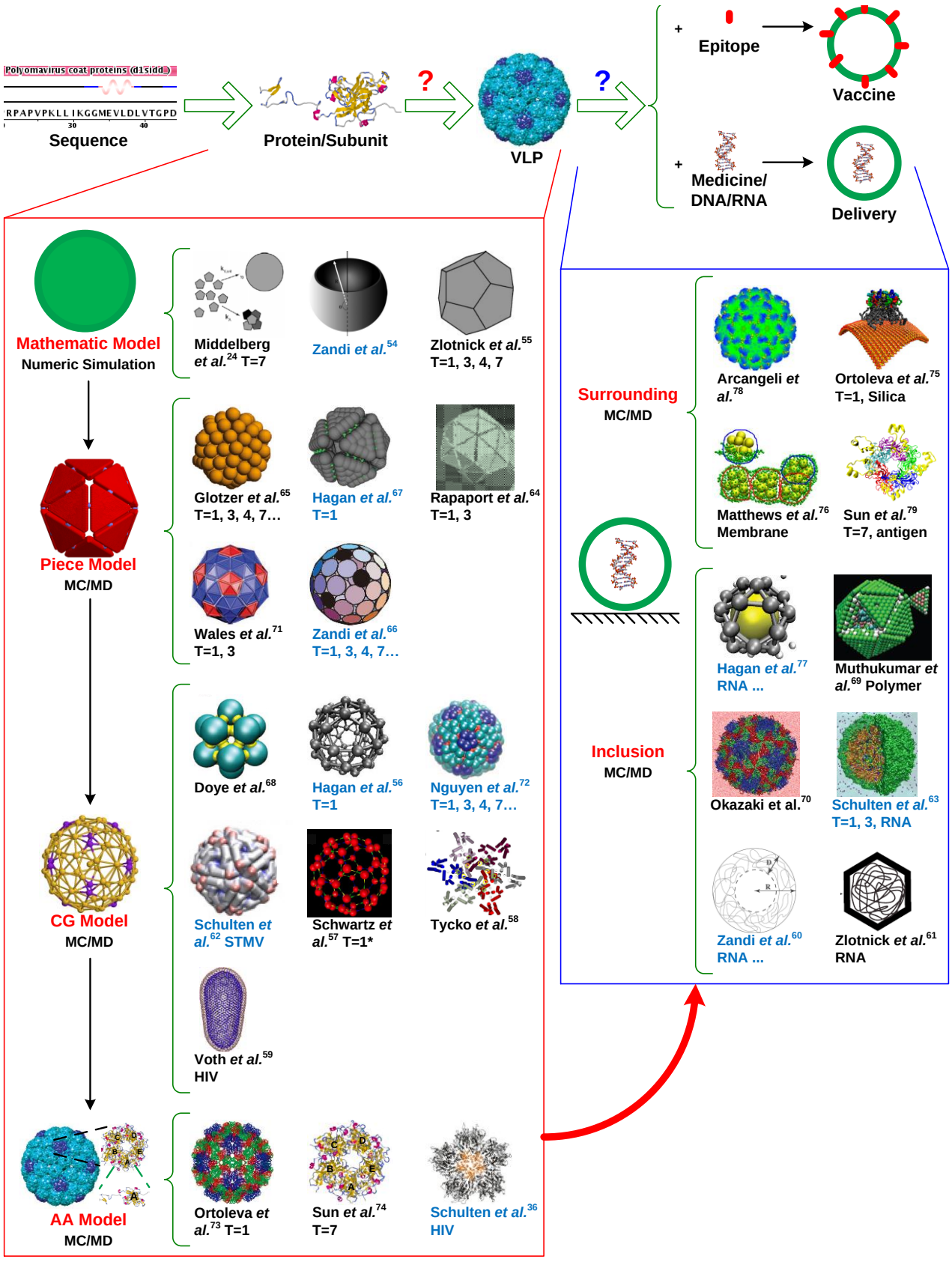
Polyomavirus coat proteins (d1sidd)
RPAPVPKLLIKGGMEVLDLVTGPD
Sequence
Protein/Subunit
VLP
+ Epitope
Vaccine
+ Medicine/ DNA/RNA
Delivery
Mathematic Model
Numeric Simulation
Middelberg et al.[24] T=7
Zandi et al.[54]
Zlotnick et al.[55] T=1, 3, 4, 7
Piece Model
MC/MD
Glotzer et al.[65] T=1, 3, 4, 7…
Hagan et al.[67] T=1
Rapaport et al.[64] T=1, 3
Wales et al.[71] T=1, 3
Zandi et al.[66] T=1, 3, 4, 7…
CG Model
MC/MD
Doye et al.[68]
Hagan et al.[56] T=1
Nguyen et al.[72] T=1, 3, 4, 7…
Schulten et al.[62] STMV
Schwartz et al.[57] T=1*
Tycko et al.[58]
Voth et al.[59] HIV
AA Model
MC/MD
Ortoleva et al.[73] T=1
Sun et al.[74] T=7
Schulten et al.[36] HIV
Surrounding
MC/MD
Arcangeli et al.[78]
Ortoleva et al.[75] T=1, Silica
Matthews et al.[76] Membrane
Sun et al.[79] T=7, antigen
Hagan et al.[77] RNA ...
Muthukumar et al.[69] Polymer
Inclusion
MC/MD
Okazaki et al.[70]
Schulten et al.[63] T=1, 3, RNA
Zandi et al.[60] RNA ...
Zlotnick et al.[61] RNA

Fig. 1 Computational studies on VLP's structure and self-assembly. The computational method commonly used with each model, such as Monte Carlo (MC) or molecular dynamics (MD) simulation, is listed below the model names. The T number is listed below the author names if available. The studies involving VLP and other materials such as DNA/RNA, membrane, surface are marked by blue rectangle, while studies focusing on the hollow VLP are marked by red rectangle. Author names marked blue represent multi-scale simulation studies, while others use various methods. *indicates study was performed using stochastic discrete event simulations. Adapted with permission from Ref. 24 Copyright 2010, Wiley Periodicals, Inc; adapted with permission from Ref. 54 Copyright 2006, Ref. 55 Copyright 2002, Ref. 56 Copyright 2006, Ref. 57 Copyright 2008, Ref. 58 Copyright 2011, Ref. 59 Copyright 2010, Ref. 60 Copyright 2009, and Ref. 61 Copyright 2013, the Biophysical Society; adapted with permission from Ref. 62 Copyright 2006, Ref. 63 Copyright 2006, Ref. 64 Copyright 2004, Elsevier Ltd; adapted with permission from Ref. 65 Copyright (2007) National Academy of Sciences, U.S.A., and Ref. 66 Copyright (2004) National Academy of Sciences, U.S.A.; adapted with permission from Ref. 67 Copyright 2011, Ref. 68 Copyright 2007, and Ref. 69 Copyright 2012, American Institute of Physics; adapted with permission from Ref. 70 Copyright 2014, AIP Publishing LLC; adapted with permission from Ref. 71 Copyright 2009, the PCCP Owner Societies; adapted with permission from Ref. 72 Copyright 2008, Ref. 73 Copyright 2010, Ref. 74 Copyright 2013, Ref. 75 Copyright 2013, Ref. 76 (http://pubs.acs.org/doi/pdf/10.1021/jp4037099, Fig. 8a) Copyright 2013, and Ref. 77 Copyright 2008, American Chemical Society; adapted with permission from Ref. 36 Copyright 2013, Macmillan Publishers Limited; adapted with permission from Ref. 78 Copyright 2013 Taylor & Francis; adapted from Ref. 79, PLoS ONE.

3.1 Computational study of VLP self-assembly using multi-scale models

The computational study of multilevel structure of VLP started from mathematical modeling[80] (Fig. 1). Icosahedral Symmetry,[27] Caspar-Klug Theory,[28] and Tiling Theory[81-84] have been proposed successively to characterize VLP structure.[80] Using these mathematical models, the structures of various spherical VLPs can be well described and characterized using triangulation number T. The following attempts are then focused on the dynamics of self-assembly, as well as the regulation, with an objective to improve the production of VLPs.

The initial step, forming correct contacts between two capsomeres, was proven crucial for the formation of correct VLP using a mathematical model.[24] The competition between correct VLP formation and incorrect aggregation of capsomere was then investigated. The following kinetics of self-assembly was examined by Zandi *et al.*[54,85,86] based on a combination of the theoretical methods of the physics of equilibrium polymerization with those of the classical nucleation. The effects of the ambient conditions on capsid nucleation were explored by these studies. The kinetics of assembly was confirmed strongly concentration-dependent and that the late-stage relaxation time varied as the inverse of the square of the concentration. Furthermore, extracting robust estimates of such assembly parameters from accessible experimental data was proposed by Zlotnick *et al.*[55,87-93] using a model of capsid assembly based on a cascade of low-order reactions. The parameters, including nucleus size, average nucleation rate, and average free energy of association, could be determined from measurement of subunit and capsid as time and concentration varied.

Using these theoretical models, the enhanced understanding of assembly allows a more quantitative analysis of virus stability and biological or antiviral factors that affect assembly, which provides fundamental hints for the following tune and regulation of self-assembly process. More questions emerged about the molecular details, especially the determinants on molecular interactions between capsomeres or protein subunits. Then a more refined model of VLP was proposed as an icosahedral sphere composed of small pieces, usually triangle pieces or pentagon pieces, which is named as 'piece model' herein.

For the piece model, molecular simulation was raised as a common computational tool instead of theoretical calculation. Theoretical calculation or numerical simulation is usually related to mathematical models to explore the macroscopic thermodynamic or dynamic parameters for evaluating the self-assembly process. Molecular simulations,[47,94,95] usually referring to Monte Carlo (MC) simulation or molecular dynamics (MD) simulation,[96,97] focus more on the microscopic evolution of such processes. Originating during the middle of the twentieth century, molecular simulation[94] has significantly progressed as facilitated by the fast development of computer science. It has become a versatile research tool capable of providing clear microscopic information in a direct manner.[98-100] So, it has been widely used to understand protein conformational transition at molecular level,[98,101] as a complementary technique to experimental and theoretical studies.[99] Molecular simulation in combination with the piece model has been used to examine the thermodynamic and dynamics parameters in VLP self-assembly.

For thermodynamic parameters, equilibrated structure of VLP was examined by Zandi *et al.*[66,102,103] using a minimal model and MC simulations. Large chiral clusters and a cluster that may correspond to several nonicosahedral spherical virus capsids were observed by Chen *et al.*[65,104] using MC simulations and a model consisting of cone-shaped particles with specific, attractive interactions. The energy landscape for VLP self-assembly was examined by Wales *et al.*[105] using the piece model consisting of pentagonal and hexagonal pyramids. Molecular interactions involved in this process such as hydrophobic–hydrophilic repulsion were studied by introduction of repulsive sites in pyramids. For dynamic parameters, self-assembly of pyramids into icosahedral shells was examined over a wide range of temperature.[71] Using Wales's model, Johnston *et al.*[106] further examined the reversible and monodisperse self-assembly of simple icosahedral virus capsid structures using MC simulations. The self-assembly dynamics underlying protein shell formation in spherical viruses was also examined by Rapaport *et al.*[64,107-111] using MD simulations and reduced models consisting of simplified trapezoidal capsomere representation, usually a pyramidal shape with several interaction sites. A similar model to Rapaport's model but consisting of triangle pieces was constructed by Hagan *et al.*[67] to examine the kinetic trapping effects in self-assembly processes.

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Therefore, with the piece model, exploration of molecular details involved in VLP self-assembly was conducted. However, in the piece model, each triangle or pentagon piece was generated according to the icosahedral symmetry spherical structure rather than the shape or geometry of capsomeres. Therefore, various coarse-grained (CG) models[112-114] were proposed at the capsomere level to evaluate the self-assembly process. Usually, a capsomere was simplified to a bead or several beads. The force field proposed was usually only appropriate for a specific VLP, although universal CG force field such as Martini force field[115,116] has been used to investigate VLP self-assembly.

Using CG models several determinants of successful assembly, especially the dynamics of self-assembly were proposed, such as the interaction between units, the shape of units, the insertion of final subunits, protein concentration, and temperature. First of all, the interactions between units should be specific enough to prevent the assembly of malformed shells, but while maintaining kinetic accessibility.[68,117] Arkhipov *et al.*[62] found that interlocking between coat proteins of satellite tobacco mosaic virus (STMV) was a key factor determining the stability of the capsids. For HIV-1, Chen and Tycko[58] found that a simple representation of N-terminal domain/N-terminal domain and N-terminal domain/C-terminal domain interactions, coupled with the correct protein shape, was sufficient to drive formation of an ordered lattice with the correct hexagonal symmetry in two dimensions. Voth *et al.*[59,118] found that the interaction between carboxyl and hexameric amino terminal domains in HIV-1 was important to generate the curvature of the capsid shell. Variation of the strength of this interaction for different subunits caused the formation of asymmetric, conical-shaped closed capsid shells. Variations in the structure of the additional carboxyl-amino terminal binding interface during self-assembly were found important for capsid cone formation. So, specific interaction and shape of units are confirmed necessary for the self-assembly. Besides these, the insertion of final subunits is found important for the dynamics of self-assembly. Nguyen *et al.*[72,119,120] found that self-assembly occurred kinetically as a cascade of elementary reactions in which free monomers were added to the growing oligomers on a downhill free-energy landscape. The insertion of the final subunits was the rate-limiting, energetically unfavorable step in assembly. Chen and Tycko[58] found that introduction of a preformed hexamer at the beginning of self-assembly of the HIV-1 capsid protein did not directly seed lattice formation, but did facilitate the formation of large clusters. Furthermore, ordered clusters were found to be formed[68,117] through a number of different dynamic pathways, including direct nucleation and indirect pathways involving large disordered intermediates. Binding of intermediates of various sizes[56,121-123] and requirement of a high level of assembly fidelity[72,119,120] were concluded important for the self-assembly of icosahedral capsids. The critical nucleus size could be determined from the concentration dependence of the assembly half-life and that the elongation time was revealed by the length of the lag phase. A kinetically trapped system was observed when nucleation is fast compared with elongation. The competition between the formation of full capsids and non-idealized structures was found strongly dependent upon the protein concentration and temperature.[72,119,120] A phase diagram was proposed[72,119,120] to show the regions where capsids or non-idealized structures were stable at each concentration and temperature. The dependence of assembly kinetics on protein concentration was also observed by the simulation results of Schwartz *et al.*[57,124-131] The effect of solution conditions on self-assembly was further examined using CG models. Modest changes in assembly conditions, consistent with expected differences between *in vitro* and *in vivo* assembly environments, could produce substantial shifts in assembly pathways.[57,124-131] Molecular crowding, like the densely crowded environment of the cell, often enhanced assembly efficiency at high crowding levels.

Therefore, with CG models, the determinants of successful assembly have been illustrated. However, simulations with higher resolution and more molecular details are still pursued. Computational power available now from supercomputers enables simulations of multi-million atom systems using all-atom (AA) MD simulations with the finest resolution atomic details,[46,67,120] thus allowing *in silico* investigation of macromolecular structures including viruses,[51] such as the whole poliovirus.[36,132] More refined examination, such as the molecular mechanics-Poisson-Boltzmann surface area (MM-PBSA) method,[38,133,134] has been combined into AA simulations for the evaluation of free energies and the contribution of each residue.

The binding free energy in the capsomere of a murine polyomavirus VLP was examined by Zhang *et al.*[74,79] using MD simulations in combination of the MM-PBSA method. Hydrophobic interaction was found favorable for the formation of a capsomere, and the key residues involved were identified through the evaluation of the contribution of each residue. However, this work was more focused on the capsomere level. A complete cowpea chlorotic mottle virus (CCMV) capsid was examined by Miao *et al.*[73] using an AA computational modeling approach, molecular dynamics/order parameter extrapolation (MD/OPX),[46,135,136] focusing on its swelling in a host medium. The capsid swelling was found as a symmetry-breaking process involving local initiation and front propagation. Although multiscale, microscopic features were captured in these studies to facilitate the computer-aided design of capsids; a major challenge was the compromise between the large size of VLP and available computational resource.[74,79] A native capsid could be generated by reorienting a certain number of copies of the protein unit according to the icosahedral symmetry if the structure of protein unit was available.[73] However, a large simulation system was necessary to include the complete capsid, leading to the high requirement on the development of both hardware and software for simulation.

3.2 Design/redesign or modification of VLP

The elucidation on self-assembly facilitates the VLP-based application, such as the development of vaccines. However, a common problem is the design/redesign or modification of VLP according to the specific requirement for the application. For example, insertion of foreign epitope is necessary for the design of novel vaccine, leading to an evaluation of the self-assembly after the insertion of foreign fragments. Experimental approaches are capable of providing direct evidence for the modification of VLP at the macro scale, however, they do not enable examination of

microscopic information. Experimental approaches are capable of providing direct evidence for the applications of VLP, such as in vaccination and drug delivery. With the fast development of computer science, computational approaches have been used for the design/redesign or modification of VLP to adapt various functions, to reduce the cost, to simplify the protocol, and to provide information about modification, especially at the micro scale. Using computational approaches, more detail about the surroundings or inclusions has been considered and investigated explicitly.

Epitopes have been extensively investigated for the rational design of vaccine.[22] Bioinformatics analyses such as homology modeling have been used to predict the atomic structures and to optimize epitopes for presentation on VLPs. Three-dimensional structure of influenza epitopes for presentation on Flock House Virus (FHV) VLPs was employed to predict and understand experimental immunological results.[137] The atomic structures of unmodified and chimeric VLPs were obtained by Arcangeli *et al.*[78,138] using homology modeling and then refined using MD simulations. Conformational changes of surface exposed loops between subunits and capsids of HPV VLPs were also obtained[139] using MD simulations. MD simulations on HIV epitopes presented on rhinovirus capsid subunits were performed to predict epitope native-like conformation for antibody binding.[140,141]

The effect of the insertion of epitopes on the self-assembly or stabilities of VLPs was also examined[75,142] with detailed analysis on the molecular energetics.[79] The importance of native structure to the desired immunological result was recently evidenced in a study on modular polyomavirus VLPs presenting a hypervariable helix epitope.[39] MD simulations on variant epitope peptides supported the immunological animal results, where the peptide design with less structural deviation from the native helical epitope achieved a higher quality of immune response. This further validated the use of computational analyses for both the design of modular VLPs and as a complementary approach to experimentation. Hence, various computer-aided vaccine design strategies have been proposed for the rational design of immunologically active modular VLPs carrying immunogenic peptides[78] and to accelerate the discovery of vaccines with high immunogenicity and thermal stability,[75,142] usually combining computational methods and experimental virology.[143] The prediction of functional epitopes and the ability to present the native 3D structure of the epitope on the carrier molecule are current challenges in epitope-based VLP vaccine design.[22]

Besides the epitopes, the effect of a fluctuating fluid membrane on the dynamics of patchy-particle assembly was examined by Matthews *et al.*[76,144] using a CG model, referring to the formation of enveloped viruses additionally surrounded by a lipid bilayer. Attraction to a membrane may promote assembly, including for subunit interaction strengths for which it does not occur in the bulk, and may also decrease single-core assembly time.

Besides the examination of epitopes for the rational design of vaccines, the examination of the inclusions has also been investigated with the help of computational methods,[25] with a major objective for rational design of drug/gene delivery, or templated self-assembly.[145] The dynamic encapsulation of RNA,[25] functionalized nanoparticles, polymers,[69,146] and electrostatic cores by viral capsid proteins was extensively examined, such as the simulation results of Hagen *et al.*[77,147-153] using CG models and the numerical studies of Zandi *et al.*[60,154-160] Different assembly pathways from the ones for empty capsid formation were observed. Cooperative interactions between protein subunits and nanoparticles were found, which can dramatically enhance the rates and robustness of assembly, as compared to the spontaneous assembly of subunits into empty capsids.[77,147-153] Electrostatic interactions between the negatively charged RNA and the positively charged inner capsid wall were found as driving force for the encapsulation,[60,154-160] indicating the dominant importance of molecular interactions between inclusions and VLP, especially the electrostatic interaction.[70] For large core-subunit interactions, subunits could adsorb onto core surfaces in a disordered manner, and then undergo a cooperative rearrangement into an ordered capsid structure. However, adaptive cargo encapsulation required moderate cargo-subunit interaction strengths. Stronger interactions frustrated assembly by stabilizing intermediates with incommensurate curvature.[77,147-153] In contrast, capsid without RNA exhibits a pronounced instability, as indicated by the AA MD simulation results of Freddolino *et al.*[63] using a complete STMV capsid. The properties of inclusions were also found to affect the encapsulation, where more compact structures were more easily encapsulated in the cavity of the virus capsid. The morphology of included nucleic acid could favor or impede assembly.[61] The inherently branched RNA secondary structure allowed viruses to maximize the amount of encapsulated genome and make assembly more efficient.[60,154-160]

In summary, computational studies with multi-scale models have explored not only the general description of VLPs, but also the molecular insights into the self-assembly process. Further evaluation on the surrounds or inclusions significantly facilitates the design, redesign, and modification of VLPs. Herein, multi-scale investigation is highlighted, where the common approach is constructing CG models based on AA MD simulations[112,113,161-163] or calibrated against AA simulations.[62] The attempt to construct multi-scale models is still ongoing not only with an objective to provide or explore the whole picture of self-assembly, but also to achieve a clear description of the involved micro details. Another driving force for multi-scale models, or specifically simplified models, is the limitation on computational resources, although computational power available now from supercomputers enables simulations of whole poliovirus.[36,132] Through acceleration of computing technology, including the development of both hardware (supercomputers) and software, simulations of whole modular subunits and their VLPs can be expected, along with enhanced ability to design VLP-based vaccine or drug/gene delivery systems in the near future.

4. VLP-based applications

As a result of the highly organized nanostructure, possible modification of structure and regulation of self-assembly, VLP-based applications have exploded in popularity,[164] such as in vaccine,[4,9-13] drug/gene delivery,[12,14,15,19] diagnostics, templated synthesis,[16,17] and catalysis,[18-20] as shown in recent reviews.[165,166] These applications focus on two main categories. One is modification of exterior surface (via surrounds), while the other is utilization via inclusions, as illustrated in Fig. 1.

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Modification of VLP surface has been extensively investigated to develop novel vaccines, imaging agents, conjugates of enzymes, and nanomaterials using VLP as templates. The major challenge is the influence of structure modification on the self-assembly of VLPs. Many preferred sites for the modification of outer surface without large sacrifice of self-assembly have been proposed, such as HI and FG loops of HPV VLP.[167,168] Modification using these surface exposed sites is usually accomplished by genetic engineering, which is now easily performed due to the rapid development of genetic engineering. Meanwhile, chemical modification on assembled VLPs is extensively used, having benefited from the development of various chemical modification methods.[169,170] Thereafter, self-assembly of modified VLP subunits can be evaluated using experimental or simulation approaches. For instance, the effect of insertion of antigen fragment on the outer surface of MPV VLP has been examined using MD simulation in combination with DSC analysis.[79] Moreover, the molecular mechanism involved has been explored for the guidance of further modification. The relationship between the structural modification and the self-assembly of VLPs is still ongoing, accompanied by the rational design and development of novel self-assembling protein nanomaterials.[171,172]

The other utilization of VLPs is a result of its special structure as a hollow spherical shell. VLPs can be used as a carrier to load drug, gene, or enzymes. Loading efficiency is an important parameter. Moreover, the risk of leaking of inclusions, and the mass transfer through the VLP shells should be considered, especially the permselective transfer of water molecules as compared to ions across the capsid,[70] which could be related to the controlled release or the regulation of reaction using VLP conjugate as a catalyst. For instance, for drug delivery, the loading or encapsulation of inclusions is crucial for the performance. However, at the target sites, the release of inclusions, maybe via disassembly of VLP shells, should be easily realized and in a regulated controlled manner. In the contrary, for enzymes loaded on the interior of VLPs, keeping the enzymes inside without leaking is required for its performance and reusability. However, the transfer of reactants or substrates into the shell as well as the transfer of products out of the shell is of major concerned. The regulation of VLP shells to realize the control of flow in/out may have potential use for the control of reaction processes.

5. Conclusions and Prospects

The success on VLP-based human vaccines for HPV brings great encouragement for the modification and utilization of VLPs. The emergence of nanomaterials using VLPs as templates furthers the investigation of VLPs. Due to their natural highly ordered and biocompatible structure, large broadening of VLP based applications can be expected in the future. However, the fundamentals related to VLPs should be clarified, especially the nature of self-assembly and its regulation, the correlation between its function or performance with the structure, and the mechanical behavior of assembly and disassembly of VLPs at various environments. Investigation of these fundamentals requires the use of computational methods, due to their appropriate focus at the macro-scale and ability to examine the microscopic phenomena, quantitatively. Multi-scale investigation is sought to provide the whole picture of self-assembly with clear description of the involved molecular details. Utilization of computational methods has already emerged in recent years to explore the molecular mechanism of VLP self-assembly, and structural redesign for various applications. An increasing role of computational methods would provide great contribution to the investigation of VLPs, with an objective of exact control of VLPs. While computational tools have largely been focused on interactions within the VLP and on its self-assembly mechanism, future simulation prospects are likely to involve multiple VLP particles in atomic detail as computing technology advances. Until this time, multi-scale methods may enable multi-particle simulation for understanding VLP-VLP interactions. Although there is still a long way for computational methods to realize exact control of VLP assembly, the continued advancement in algorithm development and increases in storage and processing capacities will ensure an increase in the use of computational methods to design functionalized VLPs in the future.

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