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Natural and artificial protein cages: design, structure and therapeutic applications

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Advanced electron microscopy techniques have been used to solve many viral capsid structures. The resulting detailed structural knowledge contributes to understanding of the mechanisms of self-assembly, maturation pathways and virion–host cell interactions. It also acts as inspiration for design and production of capsid-like artificial protein cages. Both natural and artificial cages have potential uses in medicine including as vaccines and in drug delivery. For vaccines, virus-like particles formed only from outer virion shells, lacking genetic material, offer the simplest basis for development, while encapsulation of target molecules inside protein cages is potentially more challenging. Here we review advances in cryo-electron microscopy with particular reference to viral capsid structures. We then consider why knowledge of these structures is useful, giving examples of their utilization as encapsulation and vaccine agents. Finally we look at the importance of structural techniques including cryo-EM in the rapidly progressing field of designed protein cages.

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Introduction

In nature, spherical protein cages fulfill a number of important roles. Perhaps the most well known cages are the virus capsids, macromolecular machines which act as protective containers and delivery vehicles for viral genetic material [1]. Capsid proteins are the main constituents of the outer shell of viruses (which may also include membrane components) forming the interface between viruses and host cells and playing a vital role in cell invasion. Therefore the study of virus protein cages, in particular their structures, is of importance for understanding pathogenicity and, possibly, for producing therapeutics that inhibit capsid–host interactions to limit virus entry. Cryo-electron microscopy (cryo-EM) has proved a vital tool for understanding capsid structures and continues to advance in terms of speed, capability and achievable resolution.

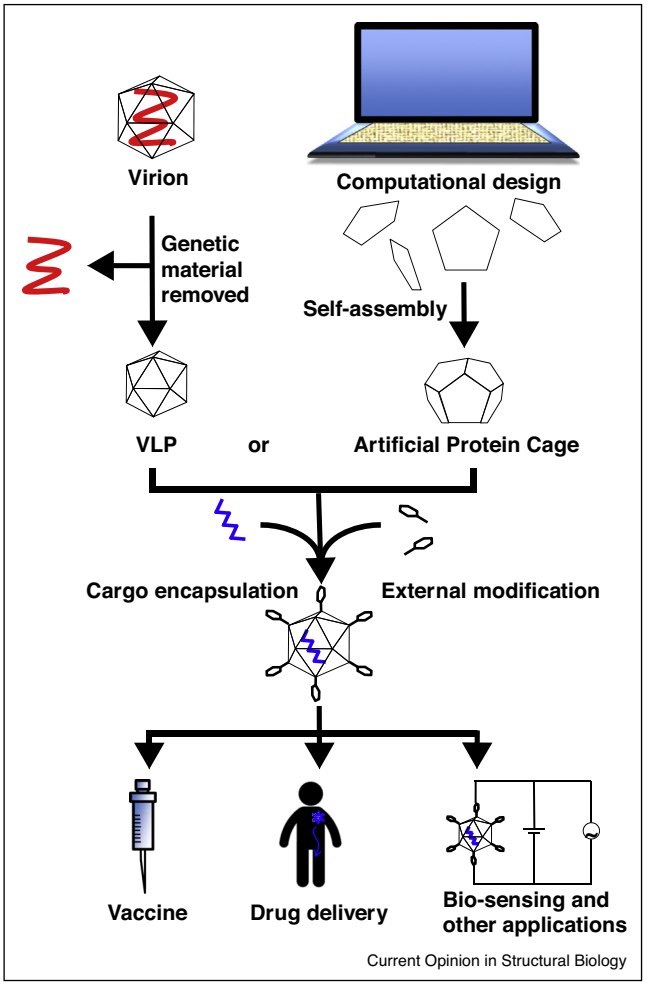
Virus cages also have applications apart from their natural role: Non-infectious particles can be constructed from viral structural proteins produced using recombinant technology to yield virus-like particles (VLPs). These can self-assemble to produce an exact reproduction of a viral capsid, devoid of genetic material. The outer surface of such VLPs can be modified either chemically or genetically to produce vaccines with a large number of attachment points to which antigens can be connected, resulting in high immunogenicity. VLPs have been utilized as vaccines both clinically and experimentally [2,3]. Clinically, HEPTAVAX-B (Merck) was the first commercial vaccine developed using VLPs, and the HPV vaccines Gardasil1 (Merck and Co., Inc.) and Cervarix1 (GlaxoSmithKline) have also been launched. VLP cavities can also be filled and their natural cell penetrating abilities taken advantage of in order to deliver cargoes. These may be genetic (gene therapy [4]) or a host of other substances including drugs, inorganic materials and even functional proteins [5].

Finally, synthetic biology approaches are increasingly allowing us to design bespoke protein cages with desired properties. This represents the frontier of protein cage research and offers the prospect of a widening of protein cage capabilities to include new structures with novel medical applications. A visual summary is shown in Figure 1.

Advanced cryo-EM for understanding protein cage structures

Producing symmetrical and spherical structures from multiple copies of the same protein is an efficient way to build a container to protect a genome if the genome itself is small and so can only encode a limited number of proteins. Many viruses adopt this idea to build a protein cage (known as a capsid) with the most common capsid

Figure 1



Production, modification and application of natural and artificial protein cages. Virus-like particles (VLPs) are generated by removing genetic material, making them non-infectious. However, they retain the antigenicity of the native virus, meaning that they remain useful as vaccines. Non-antigenic VLPs can be converted to useful vaccines by attachment of antigens from other viruses. Artificial protein cages can be designed from component proteins *in silico* and then assembled *in vitro*. Both VLPs and artificial cages can be used to encapsulate useful molecules (*e.g.*, therapeutics) and can be surface-modified with antigens, cell-targeting/penetrating groups or with groups that may provide a detectable signal depending on interaction with the environment. Such assembled VLPs and cages have applications as vaccines, drug delivery systems and bio-sensors, amongst others.

geometry being icosahedral. These icosahedral virions have been used as a target of single-particle reconstruction since the early days of the technique because the produced images are easy to process. In fact, the first near-atomic resolution structure exploiting single-particle reconstruction was achieved using icosahedral viruses [6–9]. The first *de novo* model building was also carried out using an icosahedral virus [6]. The advantages of using cryo-EM instead of X-ray crystallography is that a small amount of virus particles is often enough to achieve 3D structures. Rhinovirus C is a good example [10,11] where, in spite of a relatively low concentration of the virions, a 2.8 Å resolution structure was obtained by cryo-EM and single-particle reconstruction. As a result of not requiring a potentially time-consuming crystallization step, timely structural analysis of emerging viruses such as Zika virus becomes possible [12]. This will allow important viruses in terms of pathology to be studied in more detail. For instance, differences between genogroups can be obtained, leading to vaccine designs for viruses with various kinds of serotype.

Whereas the high-resolution structures of icosahedral and non-enveloped viruses have been successfully pursued, the next and more formidable challenges are the non-symmetrical virus particles such as enveloped viruses. For example, the whole structure of herpes simplex virus type 1 (HSV-1) was elucidated using cryo-electron tomography (cryo-ET) instead of single-particle reconstruction [13]. HSV is composed of three major components, a glycoprotein-containing envelope, a proteinaceous layer between the capsid and the envelope called the tegument, and a nucleocapsid. Cryo-ET was essential for understanding the outline structure of HSV because the components (excepting the icosahedral nucleocapsid shell [14]) are non-symmetrical.

Recent advanced cryo-ET has revealed very high resolution structures of non-symmetrical virus particles such as enveloped viruses. Among them, a historical event is that of a subtomogram averaging technique reaching a resolution of 3.9 Å, which was reported for the atomic model of the capsid domain and spacer peptide 1 of Gag polyprotein of HIV-1 in the immature state [15••] (Figure 2a). This is notable as most of the structures that had been obtained by subtomogram averaging up until this point were at a resolution of around 20 Å at best, whereas this work succeeded in building an atomic model. Here, a brand-new method to acquire tomographic tilt-series; a dose-symmetric tilt scheme developed by the authors, was used. With the development of direct electron detectors and appearance of well-controlled electron microscopes equipped with a stable stage, for example, Titan KRIOS (FEI), the method of acquiring images has improved. In fact, the implementation of the direct electron detector is a major factor in the recent dramatic increase in near atomic resolution structures accomplished using single-particle electron cryomicroscopy [16]. Before its appearance, charge-coupled device (CCD) sensors having phosphor screens with fiber-optic coupling were popular. These type of cameras are more convenient for the microscopist to check the examined specimen compared to photographic film. However, since the detective quantum efficiency (DQE) that represents the loss of input signal of a detector due to noise or the signal conversion systems in the detector, is worse than photographic film at high frequency, they are not