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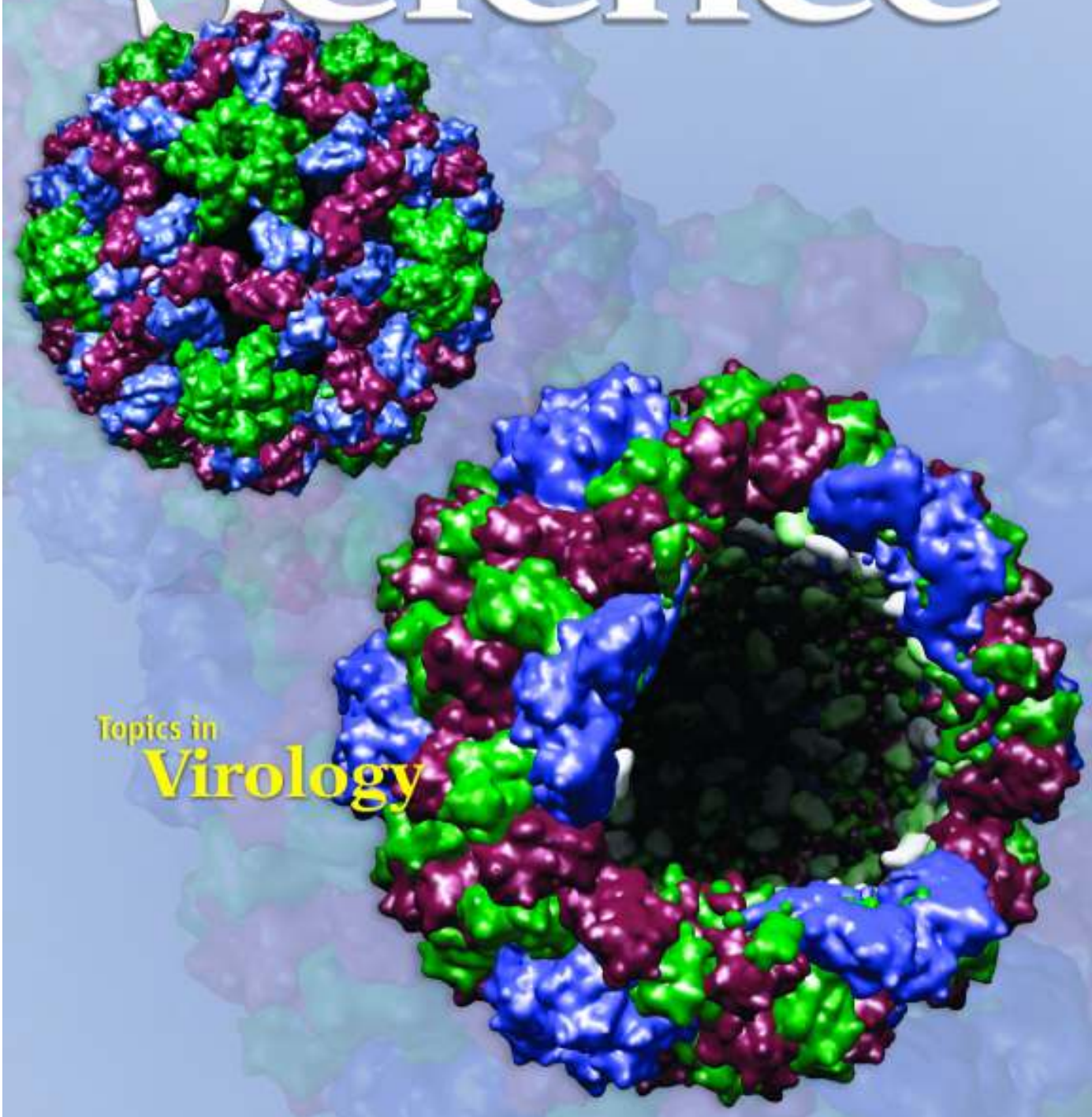
Science



Topics in Virology

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PERSPECTIVE

Viruses: Making Friends with Old Foes

Trevor Douglas and Mark Young

The study of viruses has traditionally focused on their roles as infectious agents and as tools for understanding cell biology. Viruses are now finding a new expanded role as nanoplatforms with applications in materials science and medicine. Viruses form highly symmetrical monodisperse architectures and are ideal templates for engineering multifunctionality, including multivalent display of surface ligands and encapsulation of inorganic and organic materials. These developments assure that viruses will find applications as versatile nanoscale materials.

The essential nature of all viruses is to infect a host cell, replicate, package its nucleic acid, and exit the cell. In the process, viruses have evolved to move through a broad range of chemical environments. In their journey, viruses demonstrate a remarkable plasticity in their metastable structure and dynamics, including coordinated assembly and disassembly and site-specific delivery of cargo molecules. Viruses have emerged as platforms for synthetic manipulation with a range of applications from materials to medicine. Chemical or genetic manipulation makes it possible to impart new functions to protein cage architectures, combining the best of evolution and truly intelligent design. Characterized viruses represent only a fraction of the predicted viral diversity present in the biosphere. Recent revelations suggest that viruses are the most abundant biological entities on the planet and are second only to prokaryotes in terms of biomass (*1*). It is thus an exciting time for virology and the export of this field to a wide range of scientific endeavors.

If we view viruses as molecular containers, there are three important interfaces that can be exploited (Fig. 1): the exterior, the interior, and the interface between protein subunits making up the container (or capsid). Typically, viruses are assembled from repeating subunits to form highly symmetrical and homogeneous architectures (Fig. 2) (*2*, *3*). Viruses occur in a range of shapes and sizes, from 18 to 500 nm for icosahedral structures and >2 μm in length for filamentous or rod-shaped viruses. This variety provides a library of platforms for tailored applications where size, shape, and stability are required. All viruses encode, package, and transport viral nucleic acid. However, many will assemble (either naturally or through genetic manipulation) into noninfectious containers devoid of genetic material. Conceptually, this allows one to replace the natural viral cargo with a wide range of synthetic cargos. The plasticity of the structural building blocks (subunits) to both chemical and genetic modifications, without affecting the overall architecture, gives rise to a rich resource for materials and pharmaceutical applications.

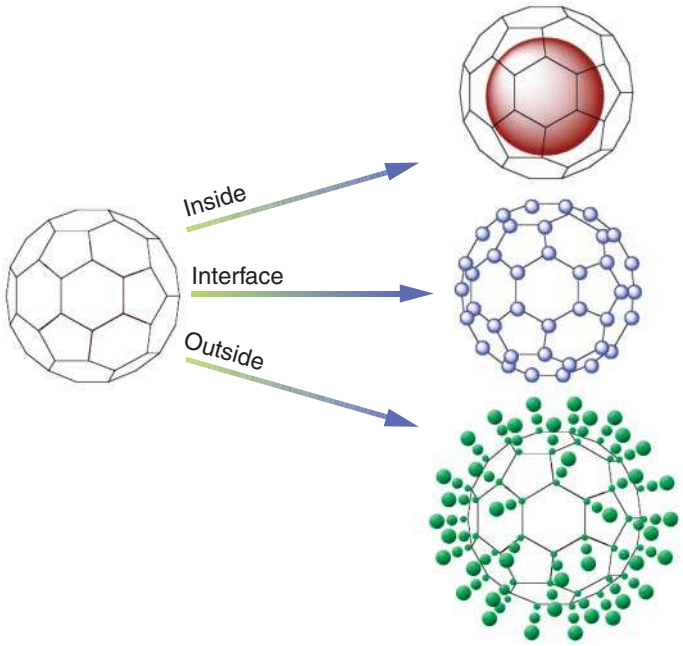

Fig. 1. A schematic of the three important interfaces available for chemical and genetic manipulation in an assembled viral protein cage architecture. The outer surface, the interior surface, and the interface between subunits have all been used for the construction of multivalent, multifunctional viral cage–based materials.

The interior interface of the viral capsid architecture has been used for directing encapsulation and synthesis of both organic and inorganic materials. All viruses package their viral nucleic acid within their capsid architecture, and the principles governing the packaging of this cargo have been exploited to package nonviral cargoes (*4*). For example, the native positive-charge density on the interior interface of empty (nucleic acid–free) cowpea chlorotic mottle virus (CCMV) capsid was used for nucleating inorganic mineralization reactions to form spatially constrained nanoparticles of polyoxometalate salts (tungstates $H_2W_{12}O_{42}^{10-}$, molybdates, and vanadates $V_{10}O_{28}^{6-}$) (*4*). In addition, through protein design and genetic engineering, the charge on the interior surface of the CCMV capsid has been altered, from positive to negative, without disrupting the ability to assemble. This negative-charge density was effective at directing the surface nucleation of transition metal oxides (Fe_2O_3, Fe_3O_4, and Co_2O_3), which proceed though cationic precursors stabilized at the highly anionic capsid interior interface (*5*). Spatially resolved elemental imaging of these materials provides a view of the hard-soft interface, an important aspect of biomaterials (Fig. 3) and one that is experimentally difficult to probe. The anisotropic rod-shaped tobacco mosaic virus (TMV) has also been used as a template for formation of metal nanowires using the interior cavity of the virus as a constraining environment (*6*). The interior interface of the capsid architectures also provides a rich, highly symmetric, and repetitive surface for encapsulation of cargo molecules through covalent attachment to site-specifically engineered residues on the interior surface. Thus, a cysteine residue genetically introduced into a subunit presents a reactive thiol group in the assembled protein cage architecture at all symmetry-related sites. Medically relevant small molecules such as therapeutics and imaging agents can be chemically attached to these reactive functional groups (*7*–*9*). The utility of this approach has been demonstrated with the use of a viruslike protein cage architecture to attach and selectively release the anticancer drug doxorubicin (*10*). This approach is medically advantageous because the protein cage acts to sequester its cargo (either natural or synthetic) until directed to be released. During administration and clearance, the encapsulated drug or imaging agent potentially remains invisible to the exterior environment and is therefore inert and biologically unavailable en route to its targeted cell.

Interactions at the subunit interface in the viral architecture provide an assembly-dependent

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surface for manipulation of cage architecture and stability. Many viral capsids exhibit pleomorphism, the ability to assemble into a range of different architectures, either naturally or through genetic and chemical manipulation (*11*). This can result in alteration of the structure from icosahedral cages through tubes to planar sheetlike architectures (*12*). This structural flexibility is an example of how subtle changes to the noncovalent intersubunit interactions can direct capsid assembly and architectures. These noncovalent interactions also direct a range of programmable structural transitions and control the underlying structural dynamics of viral capsids. In general, it is likely that most virus capsids are dynamic metastable structures whose transitions are only now being revealed (*13*). In the case of CCMV, 180 metal-binding sites are created at the interface between subunits in the assembled capsid. The metal ions play a key role in controlling a structural transition in which 60 separate 2-nm pores in the capsid structure are reversibly opened and closed (*14*). This allows molecular communication between the interior of the cage and the exterior environment through a controlled gating mechanism. In a synthetic approach, using the endogenous metal-binding sites of the CCMV architecture, Gd(III) ions have been incorporated into the capsid, and the activity of the construct as a magnetic resonance imaging contrast agent is being evaluated in vitro and in vivo. In vitro, these viral-based magnetic materials exhibit some of the highest relaxivities measured to date (*15*) and demonstrate the value of precise spatial control achieved with the intersubunit interface.

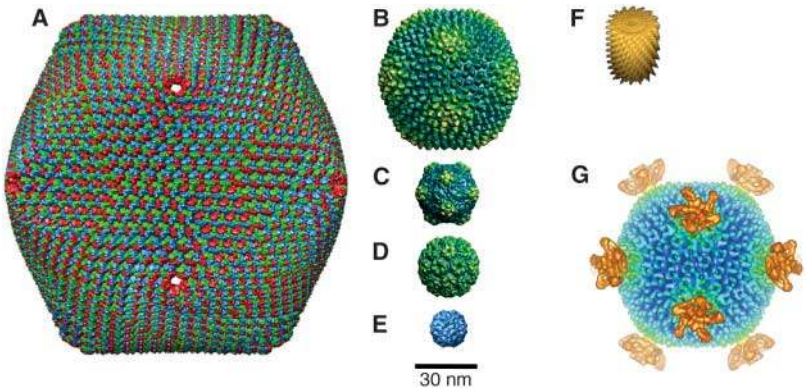

Fig. 2. Cryo–electron micrograph and image reconstructions of a library of viral capsids, including both icosahedral (*2*) and helical viruses (*3*). (**A**) *Paramecium bursaria* Chlorella virus type 1 (PCB-1), 170-nm diameter. (**B**) Murine polyoma virus, 51-nm diameter. (**C**) Cowpea mosaic virus, 31-nm diameter. (**D**) CCMV, 28-nm diameter. (**E**) Satellite tobacco mosaic virus, 18-nm diameter. (**F**) A small section of the rod-shaped TMV, which measures 18 by 300 nm. (**G**) Sulfolobus turreted icosahedral virus isolated from a boiling, acid environment in Yellowstone National Park (*30*).

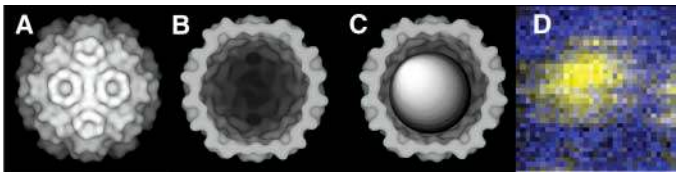

Fig. 3. (**A**) Cryo–electron micrograph reconstruction of CCMV. (**B**) Cut-away view of the CCMV cage showing the hollow interior cavity. (**C**) Schematic of a "guest" material encapsulated within the cage. (**D**) Spatially resolved spectral imaging by high-angle annular dark field scanning transmission electron microscopy of genetically modified CCMV with Fe_2O_3 synthesized within the cage [blue, N (from the protein); yellow, Fe (from the Fe_2O_3)], indicating the spatial relationship between the hard inorganic guest material (Fe_2O_3) and the soft viral protein cage (*5*).

The viral container interacts with its environment through the external surface and allows the cage architecture to be modified with small molecules or directed toward specific interactions to both biological and nonbiological surfaces. By design, the exterior surface of all viruses serves as a platform for multivalent presentation. Multivalent presentation allows for markedly enhanced interaction as compared with monovalent interactions. Viruses often display surface-exposed molecular apparatus for host cell–specific recognition and avoidance of host defense mechanisms. With a biomimetic approach to redirect virus cell targeting toward therapeutic applications, substantial progress has been made in genetically and synthetically incorporating ligands onto exterior viral surfaces. Incorporation of antibodies and targeting peptides to the exterior surfaces of viral capsids and other protein cage architectures has been shown to impart cell- and tissue-specific targeting, with clear implications for delivery of therapeutics and imaging agents (*16*, *17*). In a similar approach, through selective incorporation of surface-exposed thiol groups (cysteine), viral capsid monolayers have been selectively patterned onto Au surfaces (*18*). Molecular biology allows modification of amino acid residues at defined points within the cage structures. These reactive groups have been used for site-specific attachment of small molecules, including Au nanoparticles (*19*), fluorophores (*7*, *9*), carbohydrates (*20*), nucleic acids (*21*), and peptides (*16*, *17*). Contributions from synthetic chemistry will provide a broader palette of reaction chemistries for attachment of small molecules (*22*, *23*).

Viruses provide a platform for the surface display of an incredible range of biological ligands. This advantage has been used in phage display technology, which provides a powerful use of biological combinatorial engineering to generate sequence diversity for practical applications. Small, randomized peptides expressed on the surface of a virus population are a powerful tool for identifying specific protein interactions with both organic and inorganic materials. Phage display has been used to identify peptide-based ligands for targeting specific cell and tissue types (*24*). Phage display libraries have also been exploited for materials applications, including specific binding to material surfaces and the ability to use those peptides to direct nucleation with control over composition, polymorph, and morphology (*25*, *26*). Identification of these active peptides has led to the synthesis of materials under mild biomimetic conditions, a notable advantage to materials processing. The phages displaying these peptides have themselves been used as templates for materials synthesis, thus exploiting two very important aspects: the organic-inorganic molecular recognition from the peptides and the unique viral architecture upon which these peptides are displayed (*26*). The nucleation peptides identified by phage display can be engineered into other virus and viruslike protein cage architectures to direct nucleation and spatial control over mineral particle growth (*27*).

As with all new approaches, there are challenges to overcome. For biomedical applications, the interactions of the protein capsids with the immune system and evaluation of toxicity need to be fully addressed. The capsid is protein based and immune responses are anticipated. While these are important issues, it is likely that they are not insurmountable, given that viruses and their hosts have coexisted and coevolved. In addition, the systems currently under investigation are all nonreplicating viral capsids, thus avoiding issues of viral mutation, recombination, replication in nontargeted tissue, and infection. Similar to other approaches using biological molecules, the effects of the immune system can be mitigated, resulting in effective therapies. This is often achieved

through preventing host recognition, either by using humanized proteins or by masking the recognition surface through attachment of poly(ethylene glycol) [pegylation (*28*)]. One can even imagine taking advantage of the immune system to enhance the utility of viral capsids through the rapid clearance of cages not localized at the cell or tissue target. This potentially would reduce the deleterious effects of exposure to nontargeted therapeutic agents.

There are other attributes of viruses that have not yet been exploited but are well within the realm of possibility. Besides delivering nucleic acids, many viruses deliver other cargo, including catalysts and regulatory molecules. One can envision the targeted delivery of synthetic catalytic capacity or designer regulatory molecules. Engineered viral capsids can be envisioned as Trojan horses, remaining quiescent until a cellular signal causes release of their cargo. The ability to sequester a cargo within a protein architecture is not exclusive to viruses; similar architectures are common in the biological world, including ferritins (*29*) and heat shock proteins, and these have also been used for pharmaceutical and materials applications. Synthetic approaches in materials science often use extreme conditions, and the biological world has been traditionally viewed as a limited source of raw materials due to the relatively narrow temperature and chemical environments in which they exist. This perception is being challenged by the amazing array of environments that support life and their associated viruses. For example, the recent discovery of viruses from extreme thermal environments (Fig. 2G) potentially expands the synthetic window in which virus architectures may be used for materials applications (*30*). An extension of the principles exemplified by viruses to biomimetic approaches with the full range of protein templates will open the range of synthetic possibilities. We have turned a corner from viewing viruses only as hostile enemies to seeing and using them as a potentially vast and beneficial resource. Ultimately, the utility of viral capsids will be limited only by our own creativity.

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REVIEW

Aggresomes and Autophagy Generate Sites for Virus Replication

Thomas Wileman

The replication of many viruses is associated with specific intracellular compartments called virus factories or virioplasm. These are thought to provide a physical scaffold to concentrate viral components and thereby increase the efficiency of replication. The formation of virus replication sites often results in rearrangement of cellular membranes and reorganization of the cytoskeleton. Similar rearrangements are seen in cells in response to protein aggregation, where aggresomes and autophagosomes are produced to facilitate protein degradation. Here I review the evidence that some viruses induce aggresomes and autophagosomes to generate sites of replication.

Autophagy is a cellular response to starvation as well as a quality control system that can remove damaged organelles and long-lived proteins from the cytoplasm. Autophagy is involved in several developmental pathways and disease processes (*1*, *2*) and may provide defense against pathogens (*3*–*5*). In resting cells, autophagy is inhibited by the TOR (target of rapamycin) kinase and is triggered by events such as starvation or by the presence of rapamycin, either of which leads to dephosphorylation and inactivation of TOR. Autophagy begins with the sequestration of an area of the cytoplasm within a crescent-shaped isolation membrane (Fig. 1). Isolation membranes mature into large double-membraned vesicles (diameter 500 to 1000 nm) called autophagosomes, which eventually fuse with endosomes and lysosomes (*6*). Isolation membranes contain Atg5, Atg8 (also called LC3 in mammals), and Atg12 proteins. The Atg8 protein remains associated with the autophagosome and can be used to track the fusion of autophagosomes with endosomes and lysosomes.

Recent studies show that autophagy plays an important role in the removal of protein aggregates from cells. Protein aggregates—for example, those associated with neurodegenerative conditions such as Huntington's disease—are first delivered to the microtubule organizing center (MTOC) by dynein-dependent retrograde transport along microtubules (Fig. 2, step 1). When the degradative capacity of proteasomes is exceeded, protein aggregates accumulate in perinuclear inclusions called aggresomes (*7*). Aggresomes are surrounded by vimentin filaments and recruit chaperones, proteasomes, and mitochondria, suggesting a site specialized for protein folding and degradation. Many protein aggregates that cannot be refolded or degraded by proteasomes are eventually removed from aggresomes by autophagy, allowing delivery to lysosomes for degradation (*8*, *9*) (Fig. 2, step 3).

Large Cytoplasmic DNA Viruses Replicate in Factories that Resemble Aggresomes

The factories generated by large cytoplasmic DNA viruses such as vaccinia virus, irido-

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