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Review

# Viruses as vesicular carriers of the viral genome: A functional module perspective

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ABSTRACT

Enveloped viruses and cellular transport vesicles share obvious morphological and functional properties. Both are composed of a closed membrane, which is lined with coat proteins and encases cargo. Transmembrane proteins inserted into the membrane define the target membrane area with which the vesicle or virus is destined to fuse. Here we discuss recent insight into the functioning of enveloped viruses in the framework of the "functional module" concept. Vesicular transport is an exemplary case of a functional module, as defined as a part of the proteome that assembles to perform a specific autonomous function in a living cell. Cellular vesicles serve to transport cargo between membranous organelles inside the cell, while enveloped viruses can be seen as carriers of the viral genome delivering their cargo from an infected to an uninfected cell. The turnover of both vesicles and viruses involves an analogous series of submodular events. This comprises assembly of elements, budding from the donor compartment, uncoating and/or maturation, docking to and finally fusion with the target membrane to release the cargo. This modular perception enables us to define submodular building blocks so that mechanisms and elements can be directly compared. It will be analyzed where viruses have developed their own specific strategy, where they share functional schemes with vesicles, and also where they even have "hijacked" complete submodular schemes from the cell. Such a perspective may also include new and more specific approaches to pharmacological interference with virus function, which could avoid some of the most severe side effects.



## 1. Introduction to functional modules

In this contribution we want to compare viruses and cellular vesicles on the level of "functional modules". This term arose from the notion that major reaction pathways in the living cell are carried out by specific subsets of the proteome. Time-ordered interaction and complex formation within these ensembles of macromolecules facilitate an autonomous function. Functional modules include the important group of macromolecular machines organised as a compact structure, such as the ribosome or the proteasome. However, there are also ensembles that are more dynamic by changing their composition and/or organisation during function. To include the latter group as well, the term "functional module" is used. Common to all modules are a characteristic time domain at which the respective functional cycle proceeds and a certain sequestration from the rest of the cell by spatial limitations, by chemical specificity, and/or by the time domain at which function proceeds.

In terms of systems analysis [1], functional modules mean ensembles of molecular elements that are integral parts of a network but can be separated from its other parts. The separation is not only a method to facilitate the quantitative analysis of interaction data. It is rather supposed to reflect biological reality in the sense of the general definition of module as "a self-contained unit of a system that performs a specific task in support of its major function". Specific network motifs such as "hubs" or "cliques" can now be assigned to stable or dynamic protein complexes [2]. An alternative to such a "holistic" systems approach is a "bottom up" approach that starts from known properties of the single macromolecules and their interactions and attempts to reconstruct the behaviour of macromolecular assemblies from the behaviour of their elements. This approach is based on the methods and concepts of molecular biology, biochemistry and biophysics. It is naturally limited to small ensembles but has the potential to elucidate how the system's performance arises from the combination of its microscopic molecular properties. This becomes most important when the biological function of the module depends crucially on a specific molecular detail and/or a specific time window. Examples include the γ-secretase module, in which specific key amino acids determine the kinetics and extent of monomer assembly and eventually the toxicity of the whole polymer [3]. Another example are signal transduction modules, where the functional cycle comprises sequential interactions, which are – often with GTPases as timers – only activated in certain time windows. Also mechano-chemical GTPases of the dynamin superfamily employ nucleotide binding and hydrolysis to set a sophisticated time window in which their self-assembly, their membrane remodelling activity

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and their disassembly are coordinated (Oliver Daumke, personal communication).

Generally, functional modules may show either a stable organisation or dynamic assembly and disassembly during function. The first case includes the macromolecular machines with compact structure, such as the ribosome or the proteasome, while the latter case includes signal transducers and intracellular transport vesicles. Oriented on the machines, we assume that modules go through functional cycles. On receiving an input, only one (or a few) elements are initially activated. Subsequently the elements involved increase in their number and may form transient structures of higher hierarchy, each performing a well-defined subtask. This is the submodular level, which performs regulatory functions such as checking the input, signal amplification or negative feedback, which cannot be performed by single elements. On the modular level the submodular contributions are integrated and the output is generated. Towards the end of the functional cycle more and more elements are deactivated until eventually all elements of the module are back in place again [4].

Vesicular transport of proteins and other molecules in eukaryotic cells fits well the functional module scheme. The different steps are accomplished by submodular entities, which work together in a coordinated process. These include the sorting of cargo, the budding and scission of the vesicle from the donor membrane, the uncoating, and finally the tethering, docking and membrane fusion at the acceptor compartment [4,5]. Each transport step, e.g. between the endoplasmic reticulum and the Golgi or between the Golgi and the plasma membrane, depends on its own specific protein repertoire [6,7]. This does not exclude that, as will be discussed below, certain elements such as the Bet3 and p115 proteins can be associated with more than one submodular (or even modular) function [8]. One concept to explain the occurrence of multifunctional proteins might be called “protein parsimony”. In this view, there are simply not enough protein species (only 25,000 genes in humans) to serve all the different functions in a complicated eukaryotic cell under a variety of physiological states. Protein parsimony is especially important for replication of viruses, which is a race against time until the immune system of the host has acquired the ability to stop further spread and transmission of the virus. Thus, there is constant selection pressure for viruses to speed up their replication. One factor to achieve this goal are multifunctional proteins (and thus fewer genes), which allow faster amplification of the viral genome.

The functional cycle of transport vesicles starts with the uptake of cargo that carries an appropriate transport signal and ends when the same cargo is delivered to its acceptor compartment. A functional cycle of the transport module is closed when the cargo has been delivered and the molecules executing the function have been recycled to their starting positions. Analogously, we define the inclusion of the viral genome into virus particles preassembled at membranes of infected cells as the input into the viral module and the release of the genome into the cytoplasm of an uninfected cell as the modular output. In spite of their quite different donor and acceptor compartments, the individual steps of vesicle transport and virus replication can be divided into clearly defined and functionally conserved submodules and follow basically the same series of events: 1. assembly of elements, 2. vesicle budding from the donor membrane, 3. uncoating or maturation, 4. tethering and docking of the vesicle or virus to the acceptor compartment and finally 5. membrane fusion and cargo release. The general buildup of vesicles and viruses is schematically depicted in Fig. 1 and described further below, the processes of the respective functional module are shown in Fig. 2 and covered in-depth through the remainder of the article.

Using the concept of functional modules, we will depict the notion that enveloped viruses can be regarded as “vesicular carriers of the viral genome” which transport their cargo, the viral genome and (in the case of negative-stranded RNA and retroviruses) accessory proteins required for its replication, by budding from the donor membrane in the infected cell and fusing with an acceptor membrane in the target cell to be infected. In the following, we will focus on small, enveloped viruses, mainly on influenza virus, but also mention other well-characterized pathogens such as the retrovirus human immunodeficiency virus (HIV; see glossary for a list of abbreviations) or model viruses such as the alphavirus Semliki Forest Virus (SFV) and the vesicular stomatitis virus (VSV). It is our hope that the comparison with vesicular transport will provide insight into molecular mechanisms at the border between cell biology and virology.


**Fig. 1.** Cellular coated vesicles and enveloped viruses: basic composition. Both vesicles and viruses contain a membrane bilayer (thin black circle) derived from the donor membrane, which is lined by a coat (red circle) assembled from soluble, monomeric subunits. Inserted into the bilayer are transmembrane proteins (blue) required for targeting of the vesicle or virus. The interior contains cargo (grey ellipse), either protein or the viral genome. Insets: EM-pictures of COP I vesicles (left, by courtesy of Christoph Rutz and Britta Brügger, Biochemiezentrum, Heidelberg) and of influenza viruses (right, recreated 1918 influenza virus particles, taken from the Centers for Disease Control and Prevention's Public Health Image Library, identification number #8160). Note that the scheme is a drastic simplification to compare analogue structures in viruses and vesicles. Especially the term “coat” often comprises a multitude of different proteins, which might also have additional functions.

## 2. Similarities and differences between vesicles and enveloped viruses

Morphology is the most obvious level on which enveloped viruses and cellular transport vesicles resemble each other (Fig. 1). Both contain a membrane bilayer with a lipid composition identical (or very similar) to that of the membrane from which the vesicle or virus is derived. Transmembrane proteins are inserted into the bilayer and mediate attachment of the vesicle or virus to and fusion with the acceptor membrane. The bilayer is lined, either on the inside or on the outside, with coat proteins. Cellular vesicles involved in intra-Golgi transport contain a COP I coat, those that promote transport from the ER to the Golgi a COP II coat and for transport from the trans-Golgi-network to the endosome as well as for trafficking within the endocytic pathway a clathrin-coat is required. In the case of viruses the term “coat” comprises a variety of proteins, which might also have other functions. In the simple alphaviruses, such as SFV, the coat is an icosahedral capsid, which has also the function to encase the genome. In HIV, both functions are encoded by the gag-gene, whose product is proteolytically cleaved during virus budding to yield the coat and the capsid. Para- and orthomyxoviruses as well as VSV contain separate genes for the matrix protein, that builds the coat, and for the capsid protein. The large herpes viruses contain an amorphous structure beneath the envelope, called the tegument, which contains a multitude of proteins with a wide variety of functions. These coat proteins often drive the formation of the vesicle or virus by assembly and oligomerization at the budding site, and/or they define the shape of the particle. The interior of the particle contains the cargo, either cellular proteins or the viral genome encased in an icosahedral or helical capsid. Furthermore, cellular transport vesicles and enveloped viruses have almost the same size (50–100 nm and 40–300 nm, respectively), indicating that the curvature of their membranes is similar. A summary of the functional elements of the vesicle module and two representative viruses, influenza virus and HIV, is given in Table 1.

Since the high protein concentration in the cell's cytosol severely restricts diffusion of large size particles, such as vesicles or viruses (or submodular structures, such as capsids), an active mechanism is required for their transport over long distances. For that purpose both vesicles and viruses rely on interactions with the cytoskeleton. In