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Virus-inspired nucleic acid delivery system: Linking virus and viral mimicry☆

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ABSTRACT

Targeted delivery of nucleic acids into disease sites of human body has been attempted for decades, but both viral and non-viral vectors are yet to meet our expectations. Safety concerns and low delivery efficiency are the main limitations of viral and non-viral vectors, respectively. The structure of viruses is both ordered and dynamic, and is believed to be the key for effective transfection. Detailed understanding of the physical properties of viruses, their interaction with cellular components, and responses towards cellular environments leading to transfection would inspire the development of safe and effective non-viral vectors. To this goal, this review systematically summarizes distinctive features of viruses that are implied for efficient nucleic acid delivery but not yet fully explored in current non-viral vectors. The assembly and disassembly of viral structures, presentation of viral ligands, and the subcellular targeting of viruses are emphasized. Moreover, we describe the current development of cationic material-based viral mimicry (CVM) and structural viral mimicry (SVM) in these aspects. In light of the discrepancy, we identify future opportunities for rational design of viral mimics for the efficient delivery of DNA and RNA.

Contents

Abbreviations: AAV, adeno-associated virus; AdV, adenovirus; ASFV, African swine fever virus; BLV, bovine leukemia virus; CCMV, Cowpea chlorotic mottle virus; CMV, cytomegalovirus; CP, capsid protein; CPP, cell-penetrating peptides; CVM, cationic material-based viral mimicry; DENV, Dengue virus; EBV, Epstein–Barr virus; ER, endoplasmic reticulum; EV, enteroviruses; FIV, feline immunodeficiency virus; HA-1, hemagglutinin-1; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HS, heparan sulfate; HSV, herpes simplex virus; MLV, murine leukemia viruses; MPyV, murine polyomavirus; NLS, nuclear localization signal; NPC, nuclear pore complex; PBCV-1, large paramecium bursaria chlorella virus 1; PSVM, protein/peptide-based structural viral mimicry; RVG, rabies virus glycoprotein; SA, sialic acid; SINV, Sindbis virus; SIV, Simian immunodeficiency virus; STMV, small satellite tobacco mosaic virus; SVM, structural viral mimicry; SV40, Simian vacuolating virus 40; TBEV, Tick-borne encephalitis virus; TMV, tobacco mosaic virus; VACV, vaccinia virus; Vpr, viral protein R; VSV, vesicular stomatitis virus; WNV, West Nile virus.

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1. Introduction

Since the discovery of gene therapy as a potential strategy to treat genetic diseases, the development of efficient delivery carriers of nucleic acids has been prompted. Gene-based delivery vectors are required to condense and protect nucleic acids (DNA and RNA) in the circulation system, deliver them into disease cells, facilitate their endosomal escape and their cytosolic transport to appropriate subcellular compartments. Viruses, as naturally evolved infectious agents, are efficient vectors with the highest transfection efficiency. Viral vectors have therefore been extensively investigated for gene therapy in recent years [1]; and a number of them have successfully progressed to clinical trials [1–3]. However, due to the size limitation of the payload, inherent immunogenicity and the difficulty of large-scale production [4], the translation from bench to bedside has been hampered. Non-viral vectors provide opportunities to overcome these limitations [5,6].

Numerous materials have been attempted for non-viral delivery, with examples from linear and branched polymers, dendrimers, lipids, to polypeptides and proteins [7–9]. The initial focus on viral mimicry is in the condensation and protection of genetic materials. This goal has been successfully achieved, mostly by nucleic acid condensation with polycations through charge complementarity [7,10]. To enhance cellular entry, and to confer more specific uptake by selected cells (such as tumor cells), the non-viral vectors are modified with various ligands [11,12]. Some of these ligands are derived from viral capsid proteins or envelope proteins, to mimic the first step of cell entry by viruses [13]. Depending on the type of nucleic acid therapy, the target location is in the cytoplasm or the nucleus. Inspiration is drawn from viruses for subcellular navigation. Fusogenic peptides, such as hemagglutinin (HA) from influenza virus, have been used to achieve pH-triggered endosomal escape [14–16]. Nuclear localization signal (NLS), derived from Simian vacuolating virus 40 (SV40), guides the nuclear localization of nanocarriers upon surface modification [17–19]. Even though certain success has been achieved and the gene transfection efficiency has been improved, the level of gene expression is still much lower than viruses and far away from clinical requirement. More understanding of the role of viral ligands and their orchestrated functions in viruses is required to enhance viral mimicking capability. With the expansion of nucleic acid medicine from DNA gene therapy to RNAi (siRNA and miRNA) and genome editing [12,20,21], the requirements of vectors vary due to the nature of cargoes and their intended destinations. This motivates us to examine a variety of viruses to draw insights for delivering different cargoes to different subcellular locations.

Recent advance on atomic level structure characterization techniques accelerates the identification of virus features, such as capsid architecture, capsid assembly and disassembly intermediates [22–24]. These achievements reveal more structure–function correlation about the packing and release of nucleic acids. To deliver gene-based theraputics, nucleic acids must be protected and condensed in the carriers to prevent degradation and allow cell entry. On the other hand, the same nucleic acids must be efficiently released from the carriers for biological function inside the cell in a timely manner. This dilemma remains one of the biggest challenges for the design of non-viral vectors. The viral structures have evolved to address this dilemma in elegant ways, which have not been fully explored in viral mimicry.

Therefore, in this review, we summarize the common features of viruses and highlight how biological functions are conferred by structural properties. We also summarize the current development of virus-inspired mimicry and compare with viruses in parallel, to shed light on the future directions for designing non-viral vectors. An emerging class of synthetic carriers based on bottom-up mimicry of viral architecture is described.

2. Essential features of viruses for efficient nucleic acid delivery

Viruses can be grouped into different categories according to shape, size, host type, genome composition, and morphology. In this review, we emphasize on their capability to deliver cargoes to different destinations, so they are categorized into two main groups: nuclear viruses and cytoplasmic viruses (see some representative viruses in Table 1). As the name suggests, nuclear viruses deliver their cargoes into the nucleus and naturally hijack host cell machinery for gene transcription. Examples include the DNA viruses (e.g. adenovirus (AdV), adeno-associated virus (AAV), Herpes simplex virus (HSV)) and RNA viruses (e.g. retrovirus Murine leukemia viruses (MLV) and lentivirus Human immunodeficiency virus (HIV)), which have been developed as delivery vectors for gene-transcription or silencing-based therapy [25,26]. According to the nature of these viral gene vectors, they can induce gene transfection of different duration [1,27]. For example, AAV, retrovirus and lentivirus can induce long-term gene transfection by integrating their viral genome into the host chromosome; while AdV, which does not have genome integration capability, provides transient transgene expression.

Besides the conventional DNA-based gene therapy, RNA interference has been extensively explored in the therapeutic application, since the discovery of small non-coding RNA (siRNA and miRNA) [28]. SiRNA/miRNA can be produced through an intrinsic biogenesis pathway by nuclear viruses: 1) small hairpin RNA (shRNA) or primary microRNA (pri-miRNA) and precursor microRNA (pre-miRNA) production followed by gene transcription of viral genome in the nucleus; 2) shRNA/pre-

Table 1
Summary of representative viruses and their cell binding receptors, ligands, and other properties.

Name of viruses	Virus type & genome type	Cell surface receptor and tissue/cell tropism	Viral ligands for tropism	Therapeutic agents delivered	References
Cytoplasmic virus (delivery and replication in cytoplasm)					
SINV	Enveloped ssRNA (+)	Heparan sulfate; Tropism unknown	Viral E glycoprotein	miRNA	[31]
WNV	Enveloped ssRNA (+)	Tropism: epithelial cells in the skin, kidney, intestine and testes	Viral envelope protein E	miRNA	[32]
Vesiculovirus	Enveloped ssRNA (−)	Tropism: mainly neurons	Viral G glycoproteins	shRNA, miRNA	[33]
DENV	Enveloped ssRNA(+)	Sulfated glycosaminoglycans (GAGs), lectins that recognize carbohydrates, glycosphingolipid (GSL) Tropism: Monocytes/macrophages, phagocytes, hepatocytes	Viral envelope protein E	NA	[34]
Ebola virus	Enveloped ssRNA(−)	Niemann–Pick C1 (NPC1), a cholesterol transporter protein; TIM-1 (aka HAVCR1) Tropism: Liver, skin, spleen, lymph nodes and gastrointestinal tract; fibroblasts	Glycoprotein, phosphatidyl serine	NA	[35,36]
HCV	Enveloped ssRNA(+)	CD81, SR-BI, claudin-1 (CLDN1), Heparan sulfate, LDL-R	Viral envelope protein E	NA	[37,38]
Rabies virus	Enveloped ssRNA(−)	Nicotinic acetylcholine receptor (nAChR), the neuronal cell adhesion molecule (NCAM), and the p75 neurotrophin receptor (p75NTR) Tropism: Primarily neuronal tissue	Viral G glycoproteins	NA	[39–41]
VACV	Enveloped dsDNA	Heparan sulfate Tropism: Dendritic cells, monocytes/macrophages, B lymphocytes, primary hematolymphoid cells	Surface (SU) and transmembrane (TM) glycoproteins	NA	[42]
Nuclear virus (delivery and replication in nucleus)					
AdV	Non-enveloped dsDNA	CAR, CD46, sialic acid, CD80/86, heparan sulfate, $\alpha_V\beta_3$- and $\alpha_V\beta_5$-integrins Tropism: Epithelial cells and lymphoid cells	Viral fiber glycoproteins	DNA, siRNA, shRNA	[43,44]
AAV	Non-enveloped ssDNA	HSPG, Human fibroblast growth factor receptor 1, $\alpha_V\beta_5$ integrin		DNA, siRNA, shRNA, miRNA	[45]
SV40	Non-enveloped dsDNA	MHC class I molecules		NA	[46]
CMV	Enveloped dsDNA	Epidermal growth factor receptor, heparan sulfate	Envelope glycoproteins	NA	[47]
HBV	Enveloped dsDNA(RT)	NTCP, HSPG Tropism: Hepatocytes	Major surface antigen	NA	[48]
HSV-1	Enveloped dsDNA	HSPG and glycoprotein Tropism: Epithelial cells and neutrons		DNA, miRNA	[49]
HIV	Enveloped ssRNA(RT)	CD4, chemokine receptors, glycosphingolipids Tropism: T cells, dendritic cells or macrophages, brain cells	Glycoprotein (gp120, gp41)	DNA, siRNA, shRNA, miRNA	[50]
SIV	Enveloped ssRNA(RT)	CD4, CXCR4, CCR5		shRNA	[51,52]
Influenza A virus	Enveloped ssRNA(−)	Sialic acids Tropism: Respiratory tract	Hemmaglutinin (HA) protein	RNA, miRNA	[53]

AAV: Adeno-associated viruses, AdV: Adenovirus, CMV: Cytomegalovirus, DENV: Dengue Virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HSV-1: Herpes simplex virus, type 1, HIV: Human immunodeficiency virus, MLV: Murine leukemia viruses, SINV: Sindbis virus, SIV: Simian Immunodeficiency Virus, SV40: Simian vacuolating virus 40, WNV: West Nile virus, VACV: Vaccinia virus. GAG: Glycosaminoglycans; CAR: coxsackie and adenovirus receptor; MHC: major histocompatibility complex; DAG1: Dystroglycan 1; CCR5: C-C chemokine receptor type 5; CXCR4: (C-X-C motif) receptor 4; HSPG: heparan sulfate proteoglycan; XPR1: xenotropic and polytropic retrovirus receptor.

miRNA exported into cytoplasm being cleaved by Dicer into siRNA/miRNA. This biogenesis process indicates that the cellular production of both siRNA and miRNA requires the microprocess machinery in the nucleus. Therefore, nuclear viruses serve as vectors for the delivery of DNA for gene transcription as well as shRNA and pre-miRNA for gene silencing.

Compared to the nuclear viruses, the use of cytoplasmic viruses as viral vectors for therapy is much less common. Cytoplasmic viruses are mostly RNA viruses; examples include Sindbis virus (SINV), West Nile virus (WNV), Tick-borne encephalitis virus (TBEV) and vesicular stomatitis virus (VSV). Recently, several short RNAs (such as virus-derived short RNA) were identified in the cytoplasmic virus-infected mosquito cells [29]. The discovery of cytoplasmic distribution of Drosha might explain non-conical pathway of miRNA production in cytoplasm. These findings suggest the possible application of cytoplasmic RNA viruses as vectors for miRNA delivery [30]. As cytoplasm is where both miRNA and siRNA function, studying cytoplasmic RNA viruses could lead to better design of non-viral delivery for RNA interference (RNAi).

In the following sections, we will focus on the structural properties of both kinds of viruses. We will discuss their capsid structure and formation, genome package and ligand presentation.

2.1. Packing of nucleic acids

2.1.1. Structure of viral capsids

All viruses have viral genome encapsulated within protein capsids, which are composed of repeats of one or more types of similar or identical capsid proteins. Understanding the architecture of viral capsids as well as the stabilization forces and elements are critical for us to decipher the correlation between virus structure and transfection. This section focuses on the structural aspect of virion, describing the secondary structure of their capsid protein monomer, the association of monomers into subunits, and the contribution of interfacial interaction between subunits for capsid formation. By discussion of several typical viruses, the common structural properties of general viruses will be deduced for the reference of further viral mimicking.

The viral capsids are mainly categorized into filamentous and spherical shapes, independent on virus genome types (RNA vs DNA) and assembly pathways. The architecture of filamentous virus capsids is relatively simple and the multiple repeats of one main capsid protein of stable secondary structure stack together through hydrophobic interaction, wrapping around viral genome [54]. For the widely studied filamentous virus - tobacco mosaic virus (TMV), its capsid protein

monomer adopts four main α-helices, linked by loops. The interaction between α-helices of the capsid proteins drives the assembly of the disc-like structures, which stack to form the capsids [54].

Protein capsids of icosahedral symmetry are most common for spherical viruses. They usually have 12 pentameric vertices and 20 faces, constructed with an ordered array of pentamers or hexamers. The number of subunits on each face correlates with the size of the viral capsid. X-ray studies of HIV capsid have elucidated that pentamers and hexamers are composed of the same capsid protein monomers. For all icosahedral viral capsids, the capsid protein monomers exhibit some common secondary structures with either β-strand or α-helical structures. The interaction of several β-sheets or α-helices within a capsid protein monomer results in a hydrophobic core, contributing to the stability of the capsid monomer [55]. Numerous types of viruses, such as small Satellite tobacco mosaic virus (STMV), AAV, dengue virus (DENV), and large Paramecium bursaria chlorella virus 1 (PBCV-1), belong to the first category and their capsid proteins are typically in jellyroll β-barrel structure, with dominant β-strand domain of eight-stranded β-sheets (Fig. 1A, B) [56]. Beside jellyroll β-barrel, α-helical structure is another dominant conformation in capsid protein of some viruses (e.g. HBV, HIV-1) (Fig. 1C) [57].

In viral capsids, protein monomers of β-barrel or α-helical structures associate with each other to form subunits. The capsids of viruses are composed of merely one kind of subunits or a combination of different subunits. For example, papillomavirus type 1 (PV-1), SV40 and Murine polyomavirus (MPyV) adopt pentamers as their capsid subunits, while HIV, AdV and AAV have a mixture of pentamers and hexamers [57,59]. Multiple structural studies have shown that amphipathic capsid protein monomer associates into subunits through hydrophobic interaction [55] and extensively buried area in the interface has been identified for HBV and SV40 [55,60]. X-ray studies of HIV capsid have identified that the hydrophobic interaction between N-terminal domains (NTD) and C-terminal domains (CTD) drives the formation of both hexamers and pentamers (Fig. 1C) [23,57]. Therefore, hydrophobic interaction is the major driving force to stabilize the capsid subunits.

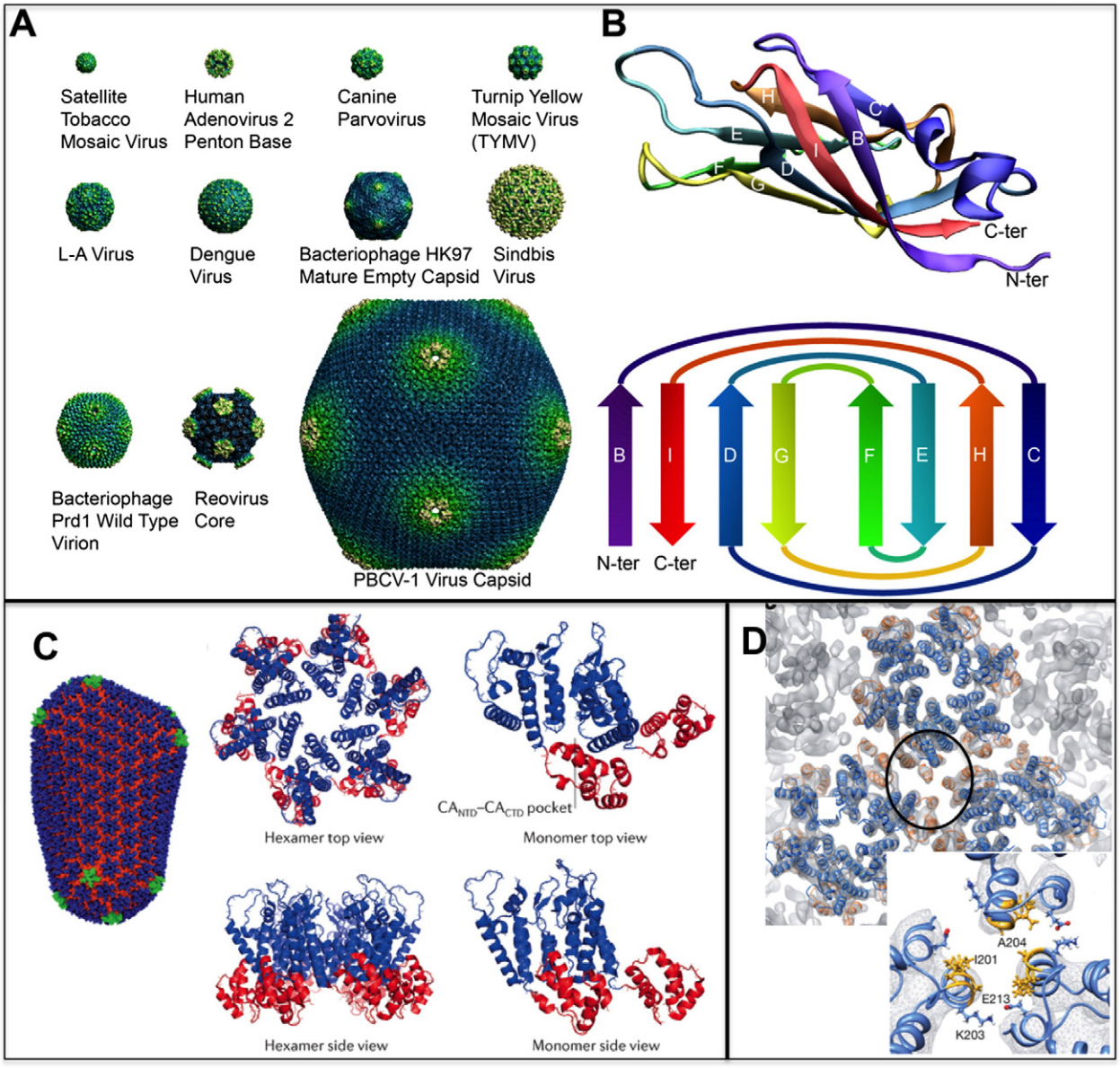

Fig. 1. Capsid architecture of spherical viruses. A) Representative viruses with icosahedral capsid. B) The characteristic jellyroll with 8 β-strands in viral capsid proteins [56]. (C). HIV capsid model [57]. The capsid protein monomer with α-helical structure contains N-terminal domain (blue color) and C-terminal domain (red color). Six monomers link together to form a hexon, driven by NTD–NTD and NTD–CTD interaction [58]. (D). Packing of hexons in the capsids, showing the trimer interface in the zoom-in image [57].
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The hierarchical association of these subunits leads to the viral capsids. For HIV, the hexamer or pentamer rings are linked by CTD–CTD interaction to form the capsid. Structural characterization has identified several hydrophobic residues (e.g. Trp and Met) at HIV C-terminal dimer interface with extensive hydrophobic contacts [22,23,57]. In addition, hydrophilic charged residues are also identified at the inter-subunit interface, which may counterbalance the inter-subunit hydrophobic interaction.

Therefore, it is concluded that general viruses share similar architecture of capsids, from the basic secondary structure of their capsid protein monomer to the organization of monomers in subunits and hierarchical association of subunits into capsids. In general, the virus capsids are formed through the hierarchy association of capsid monomer and subunits. The different degree of the intra- and inter-subunit interaction may contribute to the stability and uncoating of viral capsids. This will be discussed in Section 2.3.

2.1.2. Co-assembly of viral genome and their capsid proteins

Virus assembly is carried out by the association of capsid protein and genome. The viral genome is usually packaged into protein capsids through two strategies. In this section, we focus on the general non-covalent forces that initiate and drive the virus assembly. In addition, we discuss two main assembly pathways based on the nature of the genome. This discussion would be instructive for designing synthetic materials for viral mimicry.

Some viruses package their viral genome during the co-assembly process (Fig. 2A, B). The examples include most of ssRNA viruses (e.g. TMV, influenza virus and HIV), and some of the dsRNA viruses and dsDNA viruses (e.g. Totiviridae viruse, SV40) [61,62]. The bulky electrostatic interaction between capsid protein and RNAs initiates co-assembly [63–67]. The detailed structural studies have elucidated that the binding of ssRNAs with capsid proteins is via a positively charged cleft or flexible terminal arginine-rich motifs (ARM) [66–68]. This non-specific interaction drives the *in vitro* assembly of capsid protein around nucleic acids or polymers.

Simulation studies elucidate two possible RNA virus co-assembly mechanisms: nucleation-and-growth and *en masse* mechanism [69,71] (Fig. 2D, E). In the "nucleation-and-growth" process, the binding of capsid protein on RNA forms the nucleus, to which more subunits are added in the fast growth phase until equilibrium is reached [71]. In the *en masse* model, subunits randomly bind on RNA, followed by the cooperative rearrangement of these subunits to form the capsid. The underlying difference between both mechanisms is the different interaction intensity between protein–protein and protein–RNA. High pH and low salt concentration prefers protein–RNA interaction and low pH and high salt concentration strengthens the protein–protein interaction [71]. Therefore, assembly involving both conditions in sequence leads to the most productive assembly of Cowpea chlorotic mottle virus (CCMV) [66,72].

Another strategy for viral genome packaging is through a step-wise manner with the formation of immature empty capsid, followed by the insertion of viral genome assisted by motor proteins (Fig. 2C). Viruses that adopt this strategy include most of dsDNA and dsRNA viruses (e.g. AdV, HSV and baterialphage φ6) [62]. Their rigid double-stranded genome restricts the co-assembly with capsid protein. The formation of empty capsid usually requires the scaffolding protein or assembly-activating protein, which is removed later by protease digestion [73]. The mechanism study of empty capsid assembly has elucidated the sigmoidal "nucleation-and-growth" process [71], which is mainly driven by hydrophobic interaction between capsid proteins. The involvement of scaffold proteins and motor proteins during the virion assembly poses a big challenge for *in vitro* viral mimicking. More work is required to understand how these proteins are coordinated in this process.

These capsid protein and viral genome hybrid units are the virion of naked viruses or the nucleocapsid of enveloped viruses. The coating of

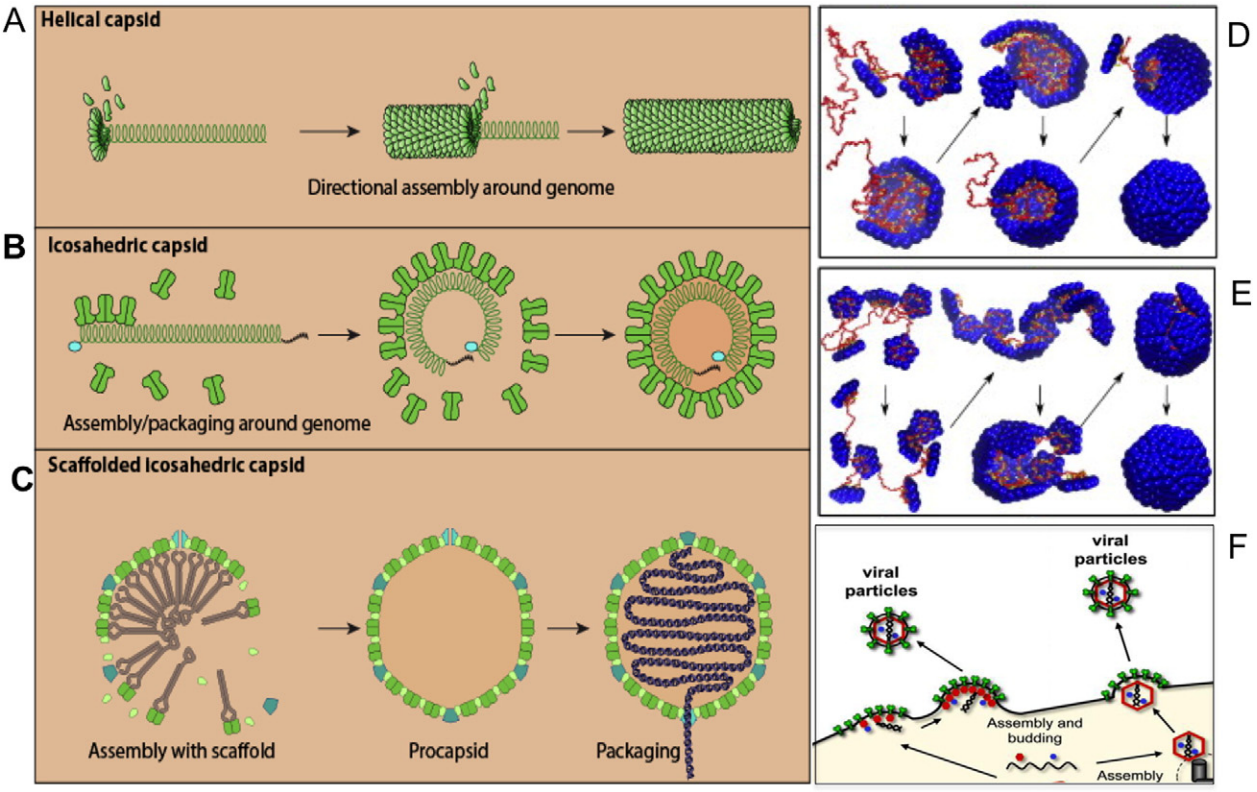

Fig. 2. Virus assembly model and mechanism. (A) Co-assembly model for filamentous viruses. (B) Co-assembly model for icosahedral capsids; (C) Step-wise assembly model for icosahedral capsids. The empty capsid is assembled first, followed by genome packaging. Virus co-assembly mechanisms: (D) nucleation-and-growth and (E) the *en masse* [69]. (F) Enveloped virus assembly model: concomitant assembly model (left), and step-wise assembly model (right) [70].
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the nucleocapsid with an extra layer of lipid results in enveloped viruses. Enveloped viruses can be constructed either through concomitantly formation of nucleocapsid, lipid envelop and budding, or through a step-wise manner (Fig. 2F). The assembly of influenza virus and alphaviruses follows the concomitant pathway and the step-wise manner, respectively [74]. Therefore, both enveloped virus assembly pathways provide flexibility and feasibility for viral mimicry.

Common features of *in vivo* virus assembly imply that the electrostatic interaction between protein and viral genome is critical to initiate the co-assembly, while hydrophobic interaction between capsid proteins plays an important role to stabilize the virus capsids. For some viruses, the step-wise assembly of empty capsid and further genome package has been identified. Even though the latter case poses a big challenge for *in vitro* viral mimicking, it also sheds light on capsid formation and capsid–genome interaction.

2.2. Cellular trafficking of viruses

Viruses exhibit high gene transfection efficiency, and this unique function is contributed by their sophisticated virion structure and the efficient interaction with host cells. The surface protein and polysaccharide ligands render cell targeting and internalization, efficient endosomal escape, cytoplasmic trafficking, as well as the active nuclear entry (for nuclear viruses). In addition, the meta-stability of the capsids renders efficient gene protection without compromising the uncoating process. In this section, we focus on the interaction of virions with host cell surface and the subcellular compartments and discuss the mechanisms used by viruses to reach the cellular destinations.

2.2.1. Cell targeting and entry

Some viruses can actively target certain types of tissues or cells, called tropism. Virus tropism is achieved by the binding of their surface ligands to specific receptors on the surface of certain cell types [75]. There are plenty examples of employing virus surface ligands as an active targeting strategy. For example, influenza virus specifically interacts with human tracheal and bronchial epithelia cells through the binding of their ligand hemagglutinin-1 (HA-1) with sialic acid of glycans with α2-6 linkage on the host cell surface [76]. HIV can bind to DC-SIGN on dendritic cells, which carry the virus to lymphoid tissue rich in CD4+ T cells [77,78]. Rabies virus is capable of neurotrophic infection through the binding of the surface rabies-G to p75NTR receptors [79]. To alter the target of viral vectors, pseudotyping is a proven technique, wherein the viral envelope-proteins are replaced by those from other viruses. Virus-originated protein ligands and shorter oligopeptide ligands have become useful tools for non-viral vectors to achieve specific cell and tissue targeting (Table 2).

To increase the avidity of binding, multivalent presentation is a common tactic found in viruses. The number of virus attachment proteins on the surface ranges from 12 for AdV to over 1000 for VSV [80]. Influenza virus exemplifies multivalent attachment on the surface of a bronchial epithelial cell. The attachment occurs by the interaction between multiple trimers of the HA at a density of 600–1200 per virus particle and sialic acid (SA) at a density of 50–200 per 100 nm^2 on the targeted cell surface [81]. This multivalent interaction ensures strong attachment on the cell surface and the subsequent internalization of influenza virus.

Multivalency is not only about quantity. Viruses mediate cell entry via spatial arrangement of multiple viral ligands at nano-scale. SV40 initiates cell entry by the binding of pentameric VP1 capsid proteins with GM1 gangliosides in the host cell membrane. The pentameric association is necessary to induce membrane curvature, which leads to membrane tubulation and promotes endocytosis [82]. Interestingly, pentameric arrangement of binding sites on capsid proteins also appears in other polyomaviruses. This property of multivalency has been explored in viral mimicry construction to facilitate efficient cell binding and entry.

Binding of viral ligands to receptors can be more than homing the viruses to the right cell types. Clustering of cell receptors is a prelude to intracellular signaling. Thus, the structural assembly of viral ligands with coordinated spatial arrangement is necessary to orchestrate the biological functions. Herpesvirus, hantavirus, picornavirus and reoviridae employ integrins as the receptor for cellular uptake [43]. Apart from being an attachment point, integrins (which comprise a group of integral membrane proteins with α and β subunits) play important roles in cellular signaling related to receptor-mediated endocytosis [83]. In addition, clustering of sialylated receptor tyrosine kinases by the binding of influenza A virus may activate tyrosine kinase and link to virus internalization [84].

For some viruses (e.g. AdV, HIVs and HCV), the binding with the host cells through multiple types of cell receptors is required for their internalization [38]. Initial attachment on cells through primary receptor binding is usually followed by secondary receptor binding to initiate fusion or receptor-mediated endocytosis. Correspondingly, coordinated display of different binding sites on the surface of viruses is demanded. Attachment and entry of HCV involve more than five attachment factors or receptors [85]. The involvement of multiple receptors suggests that viral ligands are not merely responsible for physical binding. More

Table 2
Representative examples of virus-derived peptides and synthetic ligands for virus-inspired gene delivery vectors.

Peptide name and origin	Sequence	Modified carriers	Reference
Tissue targeting			
RV Glycoprotein (RVG)	YTIWMPENPRPGTPCDIFTNSRGKRASNG	PEI, PLGA nanoparticles	[236,237,295,296]
AdV RGD peptides	Cyclic peptides c(RGDfK) and c(RGDyK), RGD4C, and RGD10	Lipoplex, dendriplex, polyplex	[239]
Attachment/entry ligands (cell-penetrating peptides)			
HIV-1 TAT (48–60)	GRKKRRQRRRPPQ	Polyplex, lipoplex	[297]
HSV-1 VP22 peptide	NAKTRRHERRRKLAIER	HPV DNA vaccine	[298]
Endosomal escape ligands (fusion and pore formation)			
IV HA-2	GLFGAIAGFIENGWEGMIDGWYG	Polylysine polyplex	[14,16]
IV HA-derived E5CA	GLFEAIAEFIEGGWEGLIEGCA	Polyplex	[299]
Glycoprotein H from HSV	GLASTLTRWAHYNALIRAF	Lipofectamine–DNA complex	[300]
HIV-1 gp41-derived HGP peptide	LLGRRGWEVLKYWWNLLQYWSQELC RGWEVLKYWWNLLQY	PEI-polyplex	[263,301]
IV diINF-7	GLFEAIEGFIENGWEGMIDGWYGC	Liposome	[262,302,303]
Subcellular targeting ligands (nuclear localization signal: NLS)			
SV40 large T antigen NLS	PKKKRKV	PEI polyplex	[17–19]
HBV core antigen	PRRRTPSPRRR	Virus-like particle; bio-nanocapsule	[243,304,305].
Nucleic acid condensing peptide			
AdV core peptide mu	MRRAHHRRRRASHRR MRGG	Lipoplex	[306]

Rabies virus (RV); Adenovirus (AdV); Herpes simplex virus (HSV); Human immunodeficiency virus-1 (HIV-1); Influenza virus (IV); and Hepatitis B virus (HBV).

sophisticated intracellular signaling to facilitate viral entry is orchestrated by the coordinated arrangement of multiple viral ligands. Human cytomegalovirus (HCMV) exemplifies this phenomenon. Glycoproteins gB and gH of HCMV bind to EGFR and integrin ($\alpha_V\beta_3$). The clustering of EGFR and integrin activates signals for stress-fiber assembly and nuclear trafficking [38].Therefore, combination of specific and non-specific ligands to achieve coordinate binding and internalization is a strategy to be explored for viral mimicry.

The presentation of viral ligands is not only coordinated spatially, but also temporally, to coordinate sequential events. This is made feasible as binding of a viral ligand can lead to the conformational change of another viral ligand. The entry of HIV to host cells provides an intriguing illustration of this smart feature of viruses [86–88]. Passing signals via conformational change of multiple viral ligands is observed in herpesvirus [89].

All these coordinated surface binding facilitates the internalization of viruses into cells. The virus internalization is usually achieved through two main mechanisms: direct fusion and endocytosis, depending on the surface property of viruses. Enveloped viruses, due to the presence of lipid membrane, can enter cells via direct fusion pathway. Direct fusion requires fusion proteins to interact and insert into plasma membrane under a pH-independent manner [90]. These fusion proteins, adopting either α-helical coiled-coil structure, β-sheet structure or the combination of both, can all induce apposition and merge of the two bilayers by their ligand-triggered conformational changes [91,92]. HSV-1, Epstein–Barr virus (EBV), vaccinia virus and alphaviruses are able to enter cells by direct fusion [93–95].

Most viruses (e.g. non-enveloped viruses and some enveloped viruses) enter cells by endocytosis, which is a complex process containing various pathways and unknowns. Endocytosis owns several unique advantages as an entry route for viruses: 1) Endosome provides fast shuttles to the perinuclear area through microtubule-mediated active trafficking. 2) The acidic environment of endosome can prime the function of viruses by inducing conformational alteration of capsid protein and gene release. 3) Cargos encapsulated by endosomal compartments can avoid inducing immune responses [96].

Three main well-characterized endocytic pathways are reviewed here. Clathrin-mediated endocytosis (CME), which is a fast and highly regulated pathway using clathrin-coated vesicles [97,98], is the entry route for numerous viruses, such as AAV, influenza virus and AdV 2/5 [99,100]. The second one is caveolae-mediated endocytosis (CvME) and the examples using this pathway include Echovirus 1, and SV40 [46,101,102]. After internalization, cargo-containing caveolae passes through early endosomes and late endosomes, and may further transport to the ER, where the viruses partially disassemble triggered by ER enzymes and release into the cytosol [96], providing another approach for endosomal escape. The third endocytosis pathway is macropinocytosis, which is characterized by several advantages including uptake enhancement of macromolecules, lower level of lysosomal degradation and inherently leaky property of macropinosomes [103,104]. Examples of viruses internalized via macropinocytosis include AdV 3, coxsackievirus B and HSV1 [44,105].

The binding of viral ligand(s) with receptor(s) also triggers the following endocytosis process. The ligand-receptor binding can strongly activate the cell signaling and might facilitate viral uptake and appropriate intracellular targeting [106]. The receptor-mediated signaling can further induce the physical uptake by the cells. For example, influenza viruses bind to receptors containing sialic acid on the cell surface, and are then endocytosed through the *de novo* formation of clathrin-coated pit at the virus binding sites using clathrin-mediated endocytosis [107,108].

Physical properties of viruses have a direct effect on their choice of endocytic pathways. Under most conditions, the virus size cannot exceed the vesicle size of each endocytic pathway [38]. For example, clathrin-coated vesicles have spherical shape in the size of 30–200 nm, and flask-shaped caveolae is only 60–80 nm in size, while macropinocytosis has vesicle as large as 10 μm, which can therefore mediate the uptake of large volumes of fluid and bulky cargoes. Indeed, the viruses we listed above and in other reference [109] showed that the virus size usually would not exceed the vesicle size in the specific endocytic pathway. Shape is another key physical property that can affect the endocytic pathway of viruses. For example, a recent study showed that influenza virus was uptaken in a shape-dependent manner: the spherical ones (100 nm in diameter) were internalized via clathrin-mediated endocytosis but the filamentous ones (100 nm by 20 μm) were internalized via macropinocytosis [110]. Another study revealed that different lengths of vesicular stomatitis virus (VSV) altered the endocytic pathways. Although both long (200 nm) and short (75 nm) VSV enter via clathrin-mediated endocytosis, only the longer VSV requires local actin polymerization for uptake [111]. Other experimental factors also influence the uptake pathways. A study showed that vaccinia virus entered cells in cell-type-dependent manner. Not only did the pathway vary in different cell types, but the rate of vaccinia virus entry also differed on different cell types [112].

Viruses of different physical properties can enter cells through different pathways or combination of several pathways. Independent on the pathways they choose, they all show the capability to reach their destinations and induce high gene transfection. These diversified entry pathways provide flexibility to tune the surface property of viral mimicry to achieve the desired physical property and biological function.

2.2.2. Endosomal escape

Most viruses enter cells through the endocytosis pathways. After endocytosis, these viruses usually experience pH drop during the intracellular trafficking process, from neutral to 6.0–6.5 in early endosomes, and then fuse with late endosomes accompanied by another pH drop to 5.0–6.0 [113]. To avoid lysosome degradation, viruses have to escape from the endosomes [114]. This is primarily achieved by the interaction between endosome membrane and viral capsid protein that causes either membrane fusion or pore formation [114–116].

In general, enveloped viruses escape from endosomes by the membrane fusion through their surface pH-sensitive fusogenic protein ligands [117,118]. Different from cytoplasmic fusogenic proteins, pH-sensitive fusogenic proteins are activated in an acidic environment. When pH drops in the endosomes, these pH-sensitive fusogenic proteins undergo a loop-to-helix transition, allowing them to penetrate into the endosomal membrane and eventually recoil and fuse with the endosomal membrane [119,120]. One of the well-characterized fusogenic proteins is HA from influenza virus [121]. Fusogenic short peptides have been identified from viral fusogenic proteins [92] (Table 2), which are useful for synthetic vectors to mimic the escape of viruses from endosomes.

Non-enveloped viruses usually escape from endosomes through pores induced by lytic proteins [114]. Similar to pH-sensitive fusogenic proteins, these lytic proteins undergo conformational change upon the trigger of low pH, allowing them to insert the hydrophobic part into the endosome membrane and expose the hydrophilic part in the lumen for pore formation [122,123]. For example, the N-terminal domain of AdV capsid protein and VP4 protein of hepatitis A virus are able to change their protein conformation in the endosome lumen, leading to a size-selective pore formation [124,125].

2.2.3. Cytoplasmic trafficking of nuclear viruses

The viscous and crowded cytosol limits the free diffusion of large biomolecules, including viral genome [126]. Therefore, active trafficking is required to achieve efficient nuclear transportation of nuclear viruses. From peripheral area to perinuclear region, endocytosed viruses experience vesicular trafficking, followed by direct virus trafficking after endosomal escape [127–131]. While for the directly fused viruses, they only experience direct virus trafficking. Therefore, in this section, the strategies utilized by viruses employing both vesicular trafficking and direct virus trafficking are discussed.

Majority of viruses enter cells through receptor-mediated endocytosis and endosome/lysosome pathway, posing a big challenge for the survival of their cargoes. However, endosome/lysosome provides a fast universal shuttle, actively transporting their contents along microtubule towards the nucleus [132]. During this process, cytoplasmic motor protein dynein plays an important role to load endosome/lysosome on microtubule [133], though the ligands on endosome/lysosome involved in microtubule loading remains to be identified. Then the strategy to get on this universal shuttle depends on the surface property of viruses and the correlated cellular entry pathways.

Similarly, dynein interaction-signal protein present on virion surface is required to facilitate nuclear viruses to catch on microtubule-assisted active track once they miss (direct fusion with plasma membrane) or get off the fast shuttle (after endosomal escape) [130,131,134]. For example, vaccinia virus (VACV), HSV and AdV in cytosol are able to hijack microtubule-based cellular transport machinery to traffic toward the nucleus [127–131]. Diversified dynein interaction-signal proteins have been identified from the capsid protein of these viruses, and the examples include the viral protein L4R from VACV [128], U34 protein from HSV [129], and the capsid hexon protein from AdV [130,131]. This indicates that catching on microtubule is beneficial to the nuclear active trafficking either by taking universal endosome/lysosome shuttle or by surface modification of dynein interaction-signal protein.

2.2.4. Nuclear targeting and entry

For nuclear viruses, they need to transport their genomic cargoes into the nucleus. The nuclear pore of limited size only allows small biomolecules (size up to 40 kDa) to pass through. Due to the big size of viruses (ranging from 20–400 nm), the nuclear membrane is another delivery barrier [135].

Passive entry into the nucleus occurs when the nuclear membrane is disassembled during cell division. Viruses that can only transfect dividing cells, such as simple retrovirus MLV, rely on this mechanism [136]. More interesting cases for viral mimicry are those viruses (e.g. Adv, AAV and lentivirus) that make use of nuclear pore complex (NPC) for active nuclear entry and therefore capable of transfecting non-dividing cells [137]. Examples include HIV, feline immunodeficiency virus (FIV) and Simian immunodeficiency virus (SIV) [138,139].

Nuclear localization signal (NLS) proteins have been well recognized as the key factor for active nuclear entry of viruses. The nuclear entry is initiated by the binding of their specific surface NLS with soluble transport factor importin α through electrostatic interaction and H-bonding [140–142]. Then importin α in this dimer binds with importin β, and the specific interaction between importin β with nuclear pore complex (NPC) docks the viruses on NPC, followed by nuclear entry through different mechanisms [142–144]. For example, a classical NLS was identified from influenza virus ribonucleocapsid, which is responsible for the nuclear targeting and import of influenza virus genome [145]. Multiple NLSs have been identified from the matrix protein, integrase and Vpr of HIV-1, which guide the nuclear entry of nucleoprotein complex [146–148]. In addition, *cis*-acting sequences and the central copy of 3′-polypurine tract (PPT) on HIV-1 also enhance the nuclear import of genome through NPC [149,150]. These suggest that the interaction with NPC is critical for active nuclear entry into non-dividing cells. Many short NLS peptides have been identified from nuclear viruses (Table 2). They are rich in basic residues (e.g. arginine (R) and lysine (K)), and most share a loose consensus sequence of K(K/R)X(K/R) (X = any amino acid). These short NLS ligands render the active nuclear entry feasible for viral mimicry.

Subsequent to docking on NPC, several different nuclear entry pathways have been identified (refer to reviews [151–155] for more details). These pathways depend on the state of viruses when they reach the perinuclear area [156], that is, intact capsid, partial-intact capsid or naked genome. For example, AdV gradually sheds during the cellular trafficking process. The remaining capsid interacts with NPC filament protein CAN/Nup214, inducing the complete capsid uncoating. The binding of nuclear histone H1 with the released AdV genome drives the irreversible nuclear entry [157]. For the nuclear entry of HSV-1 genome, the attachment of HSV-1 with NPC triggers the genome release and entry, with the intact-looking capsid left in the cytoplasm [158]. HIV-1 forms pre-integration complex (PIC) in cytosol and its MA, Vpr and IN proteins work together to facilitate the nuclear transportation of PIC [159]. Small viral capsids (e.g. AAV) or small ribonucleocapsid (e.g. Influenza A virus) can be imported intactly through the NPC [151,160,161].

Therefore, it is clear that interaction with NPC proteins is a prerequisite for active nuclear entry of viruses. There remain important questions: 1) what components in NPC trigger virus uncoating; 2) what is the mechanism of virus uncoating on NPC and inside the nucleus? The answers to these questions will help to develop better viral mimicry for overcoming the nuclear entry barrier.

2.3. Release of nucleic acids

Genome release is generally believed to be the prerequisite for gene transcription. Therefore, capsids of balanced stability are necessary to achieve both genome protection during transporation and efficient genome release at the intracellular destination. While there are common mechanisms for capsid uncoating, differences in the tactics and timing of events are found depending on the location for genome replication.

2.3.1. Genome release from cytoplasmic viruses

Cytoplasmic viruses include most RNA viruses and some DNA viruses (e.g. poxvirus). They replicate their viral genome in cytosol; so cytoplasmic genome release from their capsid is required. The common strategy employed by these cytoplasmic viruses is through receptor-mediated capsid uncoating. For some of the non-enveloped RNA viruses in this category, like enteroviruses (EV), polioviruse and human rhinovirus (HRV) [162,163], they achieve genome release without complete capsid uncoating [164]. Binding to the receptors on the host cell surface turns the capsid into an expanded state, which allows viral genome to move into the cytosol. This process highlights the dynamic nature of the viral capsid. For example, in EV71, the capsid allows the N-terminus of an internal protein to be partially and transiently exposed from the two-fold axes [164]. The binding with cellular receptor allows the insertion of the receptor into the canyon-like depression around the five-fold axis of the capsid. This interaction triggers the conformational change and “unscrews” the viral capsid. As a consequence, VP1 pocket is collapsed and VP2 helices are separated, leading to the opening of the major channel at the two-fold axis and small channel at the base of the canyon, which allows the egress of RNA. A similar receptor-mediated uncoating process has been adopted by HRV [165]. The binding of HRV with intercellular adhesion molecule ICAM-1 interrupts the inter-subunits interaction and shifts the virion into the expanded state, which promotes the egress of RNA genome without complete capsid uncoating.

Some cytoplasmic viruses utilize uncoating factor proteins to facilitate their capsid uncoating and genome release, For example, in poxviruses, depletion of the viral encoded uncoating factor helicase D5 from the cytoplasmic core leads to the capsid uncoating [166]. In summary, the cytoplasmic release of viral genome reveals the dynamic nature of the viral capsids. The exits for genome occur after the viral capsid structure are altered by the conformational switch of capsid proteins, triggered by external signals such as receptor-binding.

2.3.2. Genome release from nuclear viruses

Unlike cytoplasmic viruses, nuclear viruses replicate their viral genome in the nucleus. Therefore, complete or partial genome protection is required before they reach the perinuclear area. In these cases, capsid uncoating can be initiated upon cell-binding and triggered by endosomal acidic pH, but the completion of uncoating and genome release is around perinuclear region for large viruses or inside the nucleus

for small viruses. Due to the capsid structure complexity and surface property differences of the nuclear viruses, they employ different strategies to achieve capsid uncoating and genome release.

For example, the uncoating of AdV is a sequential process and it is triggered by multiple cellular cues, including receptor binding, acidic pH and cytosolic contact (Fig. 3A) [167]. Uncoating is initiated upon the binding of AdV with cell receptors. This triggers the conformational change of pentons and the release of some surface fibers [168]. Following endocytosis, low pH in the endosome induces virus uncoating and release of the internal proteins [169]. The exposure of the internal protein VI in the acidic pH induces the interaction with endosome membrane, leading to endosomal escape of AdV [170]. The remaining viruses inside cytosol continues to move towards the nucleus [171] and the interaction with nuclear pore complex (NPC) induces the complete uncoating of capsids and release of the DNA genome into the nucleus (Fig. 3A).

For influenza virus, uncoating of the protein layer is carried out through two steps with gradual pH decrease during the endocytosis pathway [172]. At the first step, from pH 7.5 to 6.0, the virus surface spike glycoprotein HA acquires conformational change and virus lumen is acidified, initiating the dissociation of M1 from viral ribonucleocapsid. At the second step, the decrease of pH to <6.0 drives the dissociation of M1 from viral envelope bilayer and the fusion of HA with endosomal membrane leads to the ribonucleocapsid release into cytosol. The cytosolic ribonucleocapsid further moves and enters nucleus through nuclear pore complex. The genome release from ribonucleocapsid upon nuclear entry is not clear yet.

Besides acidic pH-trigger, enzyme-dependent depletion of divalent cation and breakage of disulfide bond can induce capsid uncoating. For example, SV40 viruses enter cells through caveolae-mediated endocytosis and take a detour through ER before nuclear entry. Their capsid uncoating is managed by the coordination of ER resident isomerase and protein disulfide isomerase [173]. Specifically, ER resident isomerase depletes the calcium ions and opens the calcium bridge between capsid proteins. This allows the access of protein disulfide isomerase to break the inter-pentamer disulfide bonds, leading to capsid disassembly and genome release.

Capsid uncoating and genome release is a very complicated process, and the controversial results of HIV-1 uncoating lead to three different models (Fig. 3B). The first model indicates that viral capsids disassemble quickly under plasma membrane right after they enter into the cytoplasm by plasma membrane fusion [174–177]. Identification of cytoplasmic HIV-1 of diversified size and shape results in the second model. It emphasizes on the role of sequential capsid conformational changes triggered by different cellular factors along the way to the nucleus [178–180]. The third model based on recent studies highlights the cues from the viruses but not the cells that trigger capsid uncoating. It was proposed that the intact capsid moves toward the nucleus and binds on the nuclear membrane. Reverse transcription happens inside the intact capsid during cytoplasmic trafficking and the completion of reverse transcription at the nuclear pore triggers the capsid uncoating [181].

For some small DNA viruses (e.g. AAV and HBV), it is believed that they remain intact during the intracellular trafficking and the whole capsid can enter the nucleus directly through the nuclear pore. It is

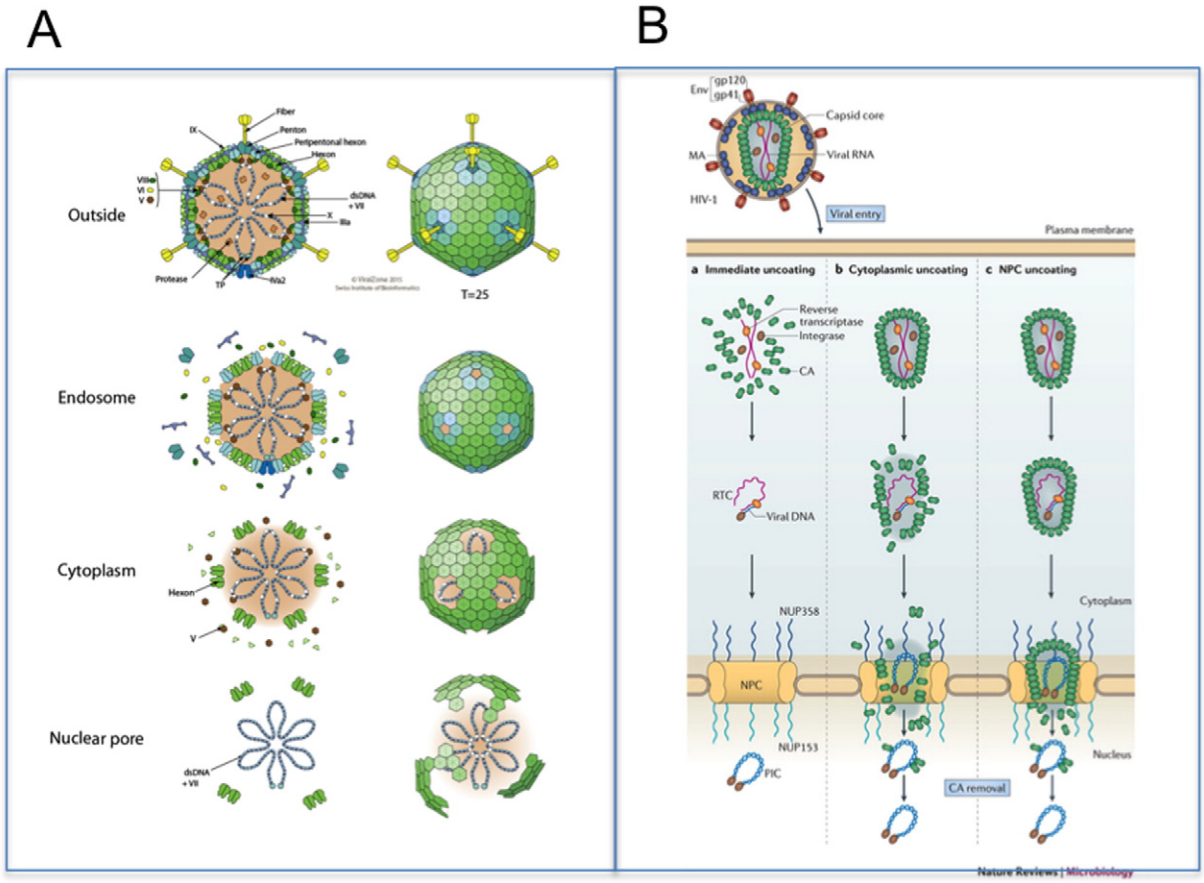

Fig. 3. Virus disassembly model. (A) Disassembly of viruses during the intracellular trafficking process, Human adenovirus type C virion as an example. (B) HIV-1 nucleocapsid core uncoating models: a. Immediate uncoating: the uncoating starts right after HIV-1 fusion with the plasma membrane and completes fast [134]. b. Cytoplasmic uncoating: partial disassembly occurs in the cytoplasm, and some remains associated with genome till nuclear entry [182,183]. c. NPC uncoating: the core is intact in the cytoplasm and starts to uncoat at the nuclear pore complex (NPC) [58,184,185].
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proposed that the anion exchange with host cell DNA or protein prompts capsid uncoating and genome release inside the nucleus, but the exact mechanism remains to be addressed.

Diversified types of viruses plus the complicated capsid uncoating process pose big challenges for the viral mimicking of gene release at the right time and right place. These different uncoating mechanisms all point out that the uncoating process is accompanied by the conformational change of the protein capsids. This can be triggered by the cellular cues such as pH, redox or protein factors. Therefore, introducing these trigger-sensitive units into the capsids will benefit the controlled gene release from the virus mimicry.

2.3.3. Balanced stability of viral capsids

Though different viruses may employ different strategies for intracellular uncoating, common themes are shared. 1) The timing and location of uncoating is critical for the infection of all viruses. 2) The uncoating of cytoplasmic RNA viruses is initiated upon cell binding and complete in cytosol. While for nuclear viruses, intact or partially uncoated viruses reach the nucleus and the complete uncoating happens around perinuclear region or inside the nucleus. 3) Metastable capsid structure is critical for viral infection. Both premature uncoating (unstable capsids) and failure of uncoating (hyperstable capsids) result in poor transfection [186]. For most viruses, maturation process accompanied by the cleavage of precursor proteins and the reorganization of the capsid proteins is required for the formation of the brittle and metastable capsid. 4) Viruses are dynamic entity and the competitive interaction between subunits maintains the stability of the capsids. The conformational changes induced by the cellular cues shift the force balance. Structures still remain for the intermediates during the uncoating process. This is an effective means to reduce the activation energy barrier for viral uncoating.

Therefore, viruses also evolve some structural "defects" that can counterbalance the stability of the capsids. For example, the polar or charged residues (such as Lys, Pro, Glu, Thr, and Gln) in HIV C-terminal trimer interface can balance the inter-subunit hydrophobic interaction [187,188]. Similarly, the charged residues at the center of pentameric ring of HIV and respiratory syncytial virus (RSV) capsid also reduce the capsid stability. Switching the charged residues into non-charged residue, such as E213A or E213Q and R18L or R18A, leads to hyper-stable capsid and reduced infectivity [188]. Therefore, charged residues play an important role to regulate the capsid stability by inducing repulsive electrostatic interaction [189].

In addition, balanced stability has been implicated by weak inter-subunit interaction. In the capsids of human hepatitis B virus (HBV), HIV-1 and SV40, the hydrophobic buried area between all these inter-subunit interfaces is smaller than that between intra-subunit interfaces [60,189–191]. This suggests that the hydrophobic interaction between subunits is weaker than that within subunits and this weaker local capsid subunit interaction may minimize the kinetic trap of capsid protein and offer the regulation of the assembly and also disassembly inside cells.

According to viral capsid assembly and uncoating, it is clear that the hierarchical assembly of subunits and subunit-association is critical for viruses to balance their capsid stability. Stable subunits, which are associated by strong monomer interaction, contribute to the stability of the whole capsids; while the weaker local inter-subunit association attributes to the capsid uncoating. Therefore, the hierarchical structure with different degrees of interaction strength is desirable for a tunable viral mimicked capsid.

3. Virus-inspired nucleic acid delivery systems

Several viruses have been extensively exploited as gene delivery vectors for gene therapy. Besides the recently developed cytoplasmic viral delivery of miRNA, gene transfection and silencing is usually achieved through nuclear delivery of DNA by nuclear viruses. Viruses contain highly ordered capsids and display sophisticated presentation of surface ligands. The structures are stable yet dynamic. These features enable viruses to cross biological barriers and release the genome to attain biological effects efficiently. Though non-viral vectors can perform gene transfection and silencing function to a certain extent, intracellular barriers more or less limit their success. There is still much room for viral mimicry to advance non-viral nucleic acid therapy. In Section 2, we have already discussed the viral features relevant for the design of delivery vectors in details, including 1) protein capsid for cargo encapsulation and protection, 2) hierarchically assembled capsids of dynamic stability, and 3) surface ligands targeting different cellular barriers. Some of these features have already been exploited for designing non-viral vectors but some others remain to be explored.

Common to all non-viral vectors, cargo protection, cellular uptake and endosomal escape are necessary. The vectors may share the same uptake pathways and mechanisms of endosomal escape. However, distinct physical properties such as length and flexibility of long DNA versus short synthetic RNA result in differences in interaction with packaging materials. In addition, the differences in the sites of action render different vector designs, with the underlying goals to vary the subcellular location to release the nucleic acid. Non-viral vectors are typically formulated by the selection of packaging materials and surface ligands.

Mimicking viruses for delivery of gene-based theraputics started decades ago with the construction of nanoparticles using polycations (polymers, dendrimers, polypeptides) and lipids [192–195]. More recently, rationally designed self-assembly protein/peptide-based structural viral mimicry (PSVM) has emerged as a new class of nucleic acid delivery vehicles. In this section, we focus on virus-inspired strategies in non-viral vectors for nucleic acid packaging, cytoplasmic trafficking and gene release.

3.1. Packing of nucleic acids

Viruses have evolved different strategies to encapsulate their genome during the process of virion assembly. Most of the viruses with single-stranded genome prefer to co-assemble the flexible genome with capsid proteins; while most of the viruses of double-stranded genome adopt a step-wise manner, starting with the formation of empty capsids followed by pumping the rigid genome into the capsids through motor proteins. Though some specific sequence recognition is required for the differentiation of viral genome from host genome during the packaging process, the initiation force for the binding of their genome to the protein capsids is electrostatic interaction. The positive charges on the protein capsids attract the negative-charged genome, leading to the genome condensation and encapsulation. Therefore, this nonspecific force has been extensively exploited in non-viral vectors that are based on cationic materials, including polymers, dendrimers, proteins, and lipids. To better mimic the layered structure of enveloped viruses, the condensed DNA/polycation complexes have been coated with a lipid bilayer membrane to form core–shell structure, which facilitates cellular uptake and exhibits high gene transfection. These polycations, generally lacking any specific interaction, build vectors without ordered architecture; while lipid-based vectors display lamellar lipid organization. Rationally designed peptides/proteins, that possess similar physical properties as viral capsid proteins, do not only achieve charge-driven gene packaging, but also mimic viral vectors on the structural aspects.

3.1.1. Co-assembly with single cationic-material

Non-viral delivery vectors have been developed to deliver different genetic cargoes, including pDNA, short synthetic siRNA, miRNA and oligonucleotide (ODN), for gene transcription and silencing. The polyanionic nature of nucleic acids prevents their translocation into cells. The initial goal of viral mimicry is to condense and to protect nucleic acids from enzymatic digestion. Relative to DNA, synthetic siRNA/miRNA/ODN are much shorter and the function of non-viral

vectors is more about encapsulation and protection, rather than condensation. Due to the shared anionic property of RNA and DNA, charge complementarity has been extensively exploited in the past decades to develop viral mimicry with cationic materials, including natural/synthetic polymers, dendrimers, proteins, and cationic lipids [196,197]. In addition, differences in size of siRNA/miRNA and DNA change the capability to complex with the same materials, such that reformulation is required for each specific cargo. On the other hand, variations in cationic materials, in terms of chemical structures and physical properties, produce different complexes with the same nucleic acid cargos.

For DNA-based viral mimicries, the assembly is simply driven by electrostatic interaction between the flexible polycation and pDNA (Fig. 4), resulting in efficient DNA condensation. Different from ordered viral capsids, polycation-based viral mimicry usually displays disordered structure. Polycations are hydrophilic polymers of diversified chain length and flexibility, and they are quite soluble in aqueous solution. The charge-neutralization of polycation and polyanion (DNA) leads to polyplex formation with efficient DNA condensation. The random entanglement of two kinds of polymers produces disordered complex architecture. For example, PEI and chitosan can efficiently condense DNA into nanometer-sized particles. The particle size is related to the N/P ratio and the molecular weight of the polymers. Generally, tighter interaction with DNA leads to the smaller-sized particles, achievable by increasing the length or the amount of the polymer. Dendrimers, polycations of well-defined size and macromolecular structure, are able to condense DNA through surface binding or inner core encapsulation, again via electrostatic interaction.

Lipids are popular vector materials because of their low toxicity and the conceived advantages similar to lipids on the virus envelope [198]. Several lipoplex-based gene transfection reagents have become commercially available, such as Lipofectamine® and TurboFect™. They are considered as the gold standards for *in vitro* gene transfection. The lipids employed are small amphipathic molecules with long hydrophobic alkyl tails and hydrophilic heads. They usually assemble into liposomes of ordered bilayer structure in aqueous solution. Nucleic acids are packaged inside lipoplex through two steps: 1) electrostatic interaction between preformed cationic liposome with DNA; 2) fusion and rearrangement of liposome [199]. Structural characterization identified assorted types of lipoplexes, ranging from the spherical lipoplex of multilamellar or hexagonal phase with hydrated DNA embedded in lipid layer to the filamentous rod-like structure with DNA being surrounded by lipids [200]. Functional studies indicate that the gene transfection efficiency is correlated with lipoplex architecture, transfection condition and lipoplex size [201]. The morphologies and structures of lipoplexes are determined by the type of lipids used (anionic, cationic or neutral) and the lipid/DNA ratio [200,202,203]. For example, cationic lipid dioleoyl trimethylammonium propane (DOTAP) and neutral "helper" lipid dioleoyl phosphatidylcholine (DOPC) complex with DNA to form lipoplex of lamellar architecture [200,203]. Replacement of DOPC with another neutral lipid dioleoyl phosphatidylethanolamine (DOPE) usually leads to lipoplex of inverse hexagonal architecture [203]. X-ray diffraction data supported that increasing the ratio of DOTAP to DOPE from 0.4 to 0.6 shifted the hexagonal architecture to lamellar structure [204,205].

Proteins and peptides rich in lysine and arginine have also been used to complex genes. Long polypeptide and large proteins, such as polylysine (PLL) and protamine have been extensively exploited to complex with DNA through charge complementarity. PLL efficiently condense DNA into nanosized particles, but this polyplex tends to be trapped inside endosomes without obvious endosomal escape [206]. Protamines are short proteins (50–110 amino acids) and they are able to condense DNA into nanosized particles. In these cases, they function as polycations and produce polyplexes lacking ordered structure [207] (Fig. 4). Some multi-domain peptides with different functional segments have been investigated. For example, a multi-domain peptide

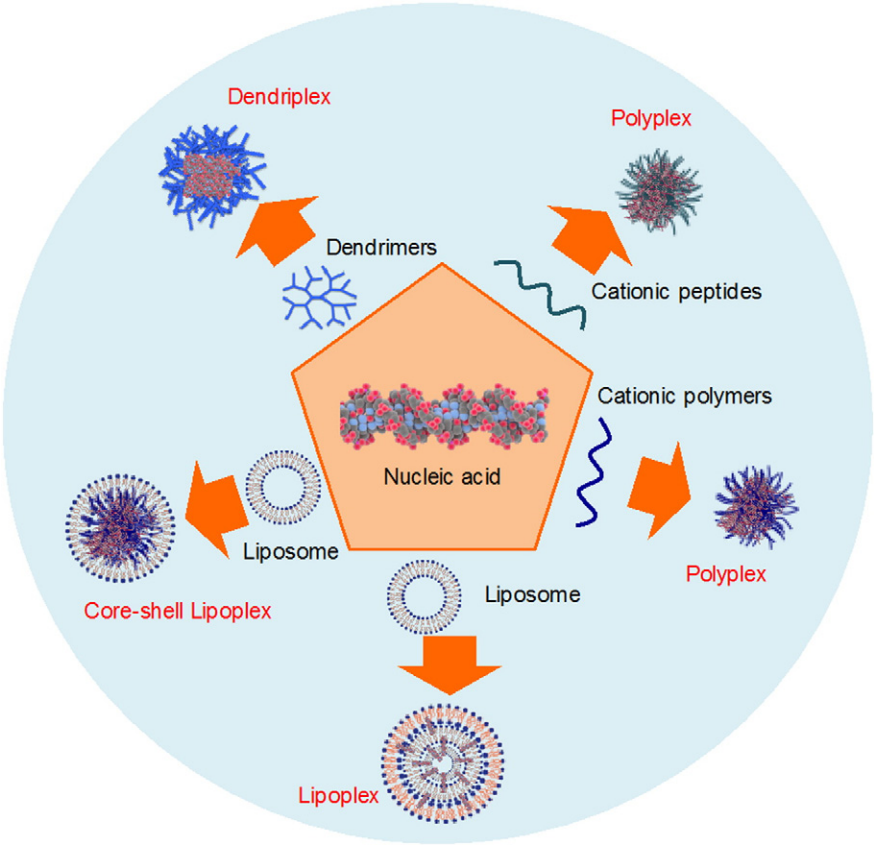

Fig. 4. Assembly and architecture of cationic material-based viral mimicries (CVMs): polyplex, dendriplex and lipoplex.

includes four functional segments: a truncated histone H1 segment, a fusogenic segment, a targeting ligand segment and a nuclear localization signal segment. This multi-domain peptide condenses DNA into small particles of around 200 nm in size through charge-complementarity [208]. They do not have ordered structure characteristic of viral capsids. Short cationic peptides, such as YKAK8WK and R8, were used to complex genes [209]. However, due to the short chain length, the complexes are unstable [209]. Stearylation of R8 increased the complex stability and also improved the gene transfer [210]. Overall, the complex of DNA with the aforementioned hydrophilic cationic proteins or peptides is driven by electrostatic interaction and no specific secondary peptide structure is involved.

Similar to DNA, electrostatic interaction is the main driving force for packaging of siRNA and miRNA. Some polycation-based vectors originally developed for DNA have been directly applied for RNA encapsulation, but not all are successful. The double-stranded siRNA and miRNA of shorter length (~20 bp) and more rigid backbone give rise to weaker interaction with these cationic materials (Fig. 4). The packing of siRNA/miRNA aims for encapsulation and protection, rather than condensation. The differences in physical properties of siRNA/miRNA from DNA demand further optimization of previous DNA delivery systems for RNA delivery. For example, linear PEI (LPEI) efficiently condensed DNA for gene transfection. While the same linear PEI (LPEI) encapsulated siRNA into nanosized particles of disordered inner architecture, but gene silencing had not been observed [211]. A possible reason is that the LPEI/siRNA complex is unstable. Branched PEI (BPEI) formed more stable nanoparticles with siRNA than linear PEI, and the intrathecal injection of BPEI/siRNA polyplex silenced mRNA and reduced the expression of targeted protein [212]. For dendrimer/siRNA complex, it is similarly driven by charge complementary and the acylation of dendrimer outer surface nitrogen group significantly increases the binding and encapsulation of siRNA [213].

Cell-penetrating peptides (CPP) rich in basic residues have been tested as siRNA/miRNA delivery vectors, due to their capability to transport cargoes across the cell membrane. This topic has been reviewed elsewhere [214] and we briefly discuss two representative examples here. Oligoarginine, such as R9, is able to complex with siRNA into nanoparticles. These vehicles delivered siRNA to perinuclear region and suppressed around 40% GFP expression [215]. A chimeric CPP MPG, composed of a fusion peptide derived from HIV-I gp41 protein and a mutant NLS from SV40 large T antigen, complexed siRNA and delivered it to cytosol, suppressing ~90% and 95% of luciferase activity in Cos-7 and HeLa cells, respectively [216].

In addition to polycations, cationic lipids have been extended for RNA delivery. siRNA/miRNA can easily bind and be encapsulated into lipoplex. Similar as DNA-based lipoplex, siRNA/miRNA lipoplex adopts diversified morphologies of unilamellar or multilamellar structure, depending on the lipids used as well as lipid/RNA ratio [217].

Even though these viral mimicries (polyplex, dendriplex and lipoplex) can mimic virus-like gene condensation and protection, their assembly is mainly driven by charge complementary. Strong multivalent charge interaction between polycation and DNA leads to stable nanoparticles. While for the shorter siRNA/miRNA, their weaker intrinsic interaction with polycations requires higher hydrophobicity of cationic materials to form more stable complex. It is worth noting that increasing the stability of the complex may in turn compensate for the intracellular gene release, so balancing gene encapsulation and release is necessary.

3.1.2. Co-assembly with multiple cationic-materials

A hybrid core–shell gene delivery system, called multifunctional envelope type nano device (MEND), has been developed [218–225]. It is composed of lipid, polymer or protein/peptide. As the name suggests, it aims to mimic enveloped virus. MEND combines the advantages of polycation and lipid to efficiently condense genes and shields the core with lipid membrane. In this system, genes are packaged in the core by electrostatic interaction-driven gene condensation with cationic materials such as PLL, PEI, protamine and CPP [218,220]. The core is polyplex or dendriplex with disordered architecture. Surrounding the core is lipid membrane, composed of anionic CHEMS (cholesteryl hemisuccinate), cationic DOTAP, or neural DOPE. The membrane protects the core and offers a platform for surface ligand modification. For example, cationic cell-penetrating peptide R8, pH-sensitive fusogenic peptide ligands GALA or IFN7 and nuclear localization signal (NLS) have been incorporated at the surface [219,221].

The components in MEND are organized in a topological order that facilitates their functions. For example, the core of siRNA/STR-R8 (stearylated R8) complex is surrounded by a lipid membrane decorated with R8 and GALA [221]. R8 facilitates cellular uptake and pH-sensitive GALA induces endosomal escape through membrane fusion. This multifunctional system successfully delivered siRNA into dendritic cells and suppressed the cytokine signaling 1 [221]. In addition, by incorporation of a new pH-responsive fusogenic cationic lipid YSK05, the MEND achieved a higher gene knockdown than Lipofectamine 2000 did [226]. In addition, MEND is also superior over lipoplex regarding pDNA delivery [222]. With the same lipid components in both pDNA complexes, MEND could achieve 5 times higher luciferase activity in the liver than simple lipoplex [222]. MEND represents a good example of viral mimicry by packaging nucleic acids in a layered structure. In this regard, it is one step closer to how viruses present the functional ligands, coordinating the ligands spatially and temporally with the intracellular trafficking process to overcome respective barriers.

3.1.3. Co-assembly with self-assembling protein/peptide

The polycation-based polyplexes/dendriplexes pack and encapsulate genetic materials in disordered “capsids”, which are different from the ordered viral protein capsids. Lipoplexes package genes in an ordered unilamellar or multilamellar “capsids”, and their stability is mainly contributed by the hydrophobic interaction between short alkyl tails, which may pose the challenge of easy disassembly.

The hierarchical assembly of the viral capsids is able to benefit both the gene protection and gene release. Therefore, virus-like ordered structures stabilized with α-helical or β-sheet secondary structure have gradually emerged as a new class of nucleic acid delivery vectors. We shall call it structural viral mimicry (SVM).

Viral-like particles (VLPs) are one kind of structural viral mimicry. They are nanoparticles assembled *in vitro* with virus-derived structural proteins. These VLPs have been exploited as gene carriers. For example, VLPs formed by major structural protein of John Cunningham virus (JCV) were used to deliver exogenous DNA into human colon carcinoma cells (COLO-320 HSR), and the cancer cell growth was inhibited [227]. However, the origin of protein-based VLPs eventually poses similar limitations as viral vectors, which include immunogenicity, limited cargo size, and difficulty for large scale production. Specifically, due to the presence of virus-specific antigens on the surface, they are prone to induce immune responses, so they are more frequently utilized as vaccines. VLPs derived from the assembly of virus-derived protein usually form cages with specific volume, limiting the cargo size. The relatively long protein sequence also increases the challenge to make predictable change of the final structure. These reasons have motivated the development of a safer system to mimic viral structures.

Based on the assembly property of viral capsid protein, multi-domained proteins have been rationally designed for gene delivery. It usually includes several domains: 1) ligand domain, 2) assembly domain and 3) cationic charge domain, which can cooperatively interact with DNA so that it is encapsulated within an ordered structure of protein assembly. For example, GFP protein of ordered beta-barrel structure is fused with His6 and TAT at both ends. The interaction of DNA with positively charged tail links GFP together to form ordered “capsid” with DNA encapsulated in the center [228]. This system provides a conceptual proof of designer materials for structural viral mimicry. Nevertheless, a simpler synthetic system is desirable.

Inspired by the properties of capsid proteins, several short peptides and proteins have been rationally designed. These designer peptides and proteins share a similar modular design concept. The sequence comprises a segment of oligocations for DNA/siRNA-binding, an α-helix or β-sheet segment for capsid formation and stability, and a hydrophilic segment for the dispersion of the nanostructures in aqueous phase (Fig. 5).

A filamentous artificial virus has been developed for siRNA delivery. It has been constructed by adsorption of siRNA on the preformed filamentous fibril surface [229]. The designer peptide contains three segments: an N-terminal hydrophilic glucose, an oligolysine, and a β-sheet forming segment. The preformed nanofibrils are composed of hydrophobic β-sheet core and disordered oligolysine and glucose surface. The electrostatic interaction between the fibril and siRNA drives the binding. The exposure of siRNA on the surface of this artificial virus raises questions about the capability for gene protection. On the other hand, the ready access may offer efficient release of siRNA for gene silencing.

Unlike siRNA, long chain DNA can be efficiently condensed. Co-assembly of rationally designed peptides and proteins with DNA mimics the assembly process of virions. In these structural viral mimicries, genes are encapsulated within a capsid-like "shell". Specifically, inspired by filamentous virus TMV, Stupp's group designed an α-helical peptide with an N-terminal spermine for DNA binding and C-terminal PEG for surface shielding. This hybrid monomer preformed into subunits, displaying peptide coiled-coil structure (Fig. 5A). The binding of DNA with spermine on the preformed coiled-coil subunits produced filamentous viral mimicry, with DNA surrounded by the peptide "capsid" [230]. This hierarchical assembly of filamentous viral mimicry in a step-wise manner provides opportunities for the regulation of gene release.

Inspired by the same filamentous virus TMV, Vries's group designed a simple viral capsid protein (Fig. 5B) [231]. It contains 12-lysine residues at the N-terminus for DNA binding, ten repeats of silk-like β-sheet forming segments for capsid formation, and a long hydrophilic protein exposed at the C-terminus for well aqueous dispersion [231]. In this case, ten β-sheet-repeats mimic viral capsid protein jellyroll β-barrel. The binding of lysine with DNA pre-organizes designer protein along DNA, and the interaction between β-sheets promotes the "capsid" formation (Fig. 5B).

The above two filamentous viral mimicries exploited large monomers with either long polymer (PEG) or protein. Based on the same design principle, we have demonstrated a simpler system through the co-assembly of DNA with short peptides (Fig. 5C) [232]. This peptide is composed of 16 amino acid residues with three main functional segments: an N-terminal three lysine residues for DNA binding, a central region of eight-hydrophobic residues (WLVFFAQQ) for β-sheet formation, and a C-terminal three hydrophilic residues (SPD) for particle dispersion. This short peptide itself self-assembles into nanofibrils in aqueous solution at neutral pH. The co-assembly with DNA produces cocoon-like nanoparticles of distinct surface stripes, morphologically similar to parapoxvirus [233]. Systematic structural characterization of peptide nanofibrils and peptide/DNA nanococoons reveals the virus-like architecture. The β-sheet peptide nanofiber organizes on the nanococoon surface to form a "capsid" around the plasmid DNA in the core [232]. This structure property led us to propose an assembly mechanism: the mixing of the short designer peptide (16 amino acids) with DNA leads to the preorganization of peptide strands along DNA by electrostatic interaction, mimicking the initial stage of genome-involved virus assembly [234] (Fig. 5C). The increase of local peptide concentration along DNA drives in the assembly of peptide to form β-sheet nanofibrils and further lateral association of nanofibrils leads to the encapsulation of DNA inside.

For structural viral mimicry (SVM), the "capsid" is formed by virus-like protein–gene and protein–protein interactions, which offer the potential intracellular dissociation through peptide/protein nanostructure disassembly. Filamentous artificial virus developed by Lee's group has the siRNA absorbed on the surface of the preformed nanofibril. The *in vitro* studies of this structural viral mimicry showed high gene silencing, indicating the efficient siRNA dissociation from nanofibrils [229]. In this case, siRNA was absorbed on the surface, so nucleic acid release could be achieved without nanofibril disassembly.

For the aforementioned structural viral mimicries (SVMs), DNA is encapsulated in the center of the "capsid" shell, so gene release requires complete or partial disassembly of the nanostructures. TMV-mimicking filamentous structure developed in Vries's group showed the capability to transfect Hela cells, but no improvement in transgene expression was found relative to other non-viral vectors [231]. This suggests that gene release from this structural viral mimicry may not be as efficient as

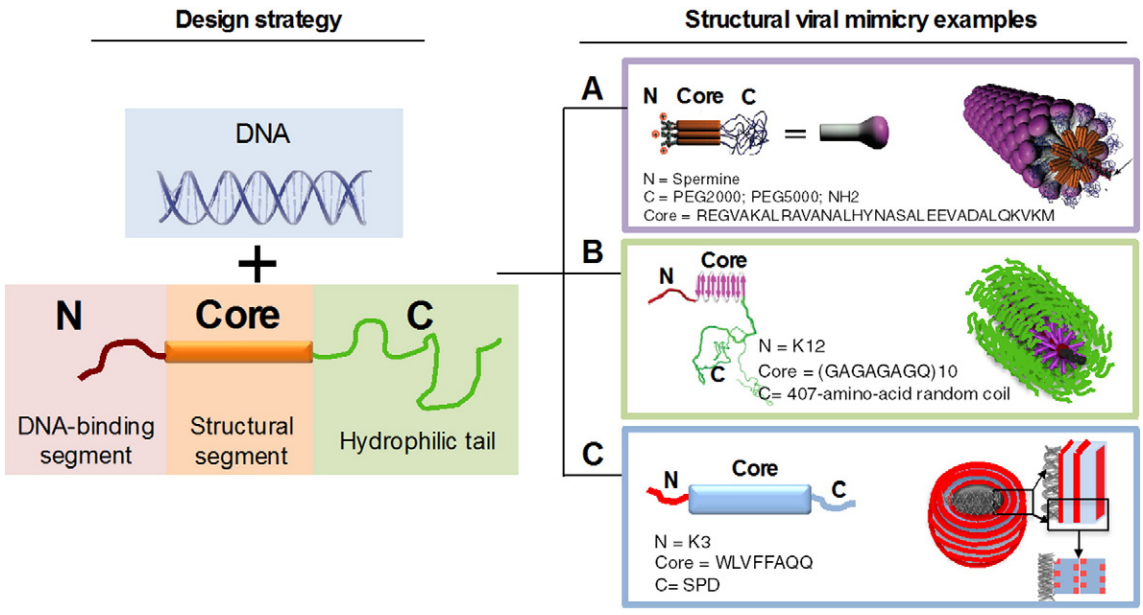

Fig. 5. Virus-inspired self-assembling protein/peptide-based structural viral mimicry (PSVM). (A) Representative strategy for synthetic filamentous viruses [230]; (B) scheme of artificial viruses by simple coat protein [231]; and (C) scheme of peptide-based structural viral mimicry [232].
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expected. For another filamentous viral mimicry from Stupp's group, transfection results have not been reported [230]. The nanococoons developed by our group are very stable and face the difficulty of efficient gene release [232]. The comparison between natural viruses and SVMs reveals a major difference. The natural viral capsid is formed by a limited number of subunits (from dozens to hundreds depending on the size of viral capsids) and the uncoating is achieved by disrupting the weak inter-subunit interaction instead of the strong inter-monomer interaction. Therefore, how to tune the stability of viral mimicry via controlling the inter-subunit force is critical for improving gene transfection.

3.2. Cellular trafficking of viral mimicry

For efficient intracellular trafficking, both cytoplasmic viruses and nuclear viruses must overcome barriers mounted by the cytoplasmic membrane and endosome. For nuclear viruses, the need to deliver viral genome into the nucleus means crossing extra barriers that involve moving in cytosol towards the nucleus and making nuclear entry. The protein ligands on the capsid surface play important roles for both cytoplasmic and nuclear delivery.

As discussed earlier, structural and functional studies have identified numerous short peptide ligands from the surface of viruses, which correspond to how viruses respond in each step during the intracellular trafficking process (Table 2). Therefore, nanoparticles have been functionalized with virus-derived ligands for cell targeting, cellular entry, endosomal escape and subcellular targeting [13,235]. A list of examples are summarized in Table 2.

3.2.1. Cell targeting and entry

In order to produce efficient gene delivery carriers, tissue targeting and cell targeting are required. Two main approaches of virus mimicry have been used in designing nanocarriers: 1) incorporating virus-derived or virus-inspired functional ligands; 2) optimizing the physiochemical properties of particles such as size and charge.

Protein/peptide ligands for virus tropism have been identified and applied to non-viral vectors to achieve tissue targeting. For example, rabies virus specifically transfects neurons, so the ligand derived from rabies virus glycoprotein (RVG) was conjugated to polymers for neuron targeting. The RVG-reducible PEI polyplex showed successful delivery of pDNA and miRNA into neutron cells. Targeted gene expression and gene silencing were achieved [236,237]. For tumor targeting, RGD motif derived from AdV penton base [238] specifically interacts with integrins overexpressed on tumor cells. Linear/cyclic RGD and the other derivatives were frequently conjugated with non-viral vectors, including liposomes, dendrimers, and polymeric nanoparticles, for gene delivery into tumor cells [239]. Virus targeted receptors inspire ligand selection too. For example, mannan/mannose interacts with C-type lectin/lectin-like receptors, which bind with many viruses (such as HIV) [240]. This molecule is now widely used as dendritic cell-targeting ligand for nanocarriers and vaccines.

Multivalency is a common tactics used by viruses to increase binding avidity. This approach is an effective way to enhance the targeting efficiency of non-viral carriers. Recent Monte Carlo simulation results showed multivalent ligands in nanoparticle targeting could be beneficial under certain conditions, especially for a high binding energy of ligand–receptor interactions and large receptor densities [241]. Also, nanoparticles with longer tether length or larger core size are more likely to deliver the same number of functional groups to a cell surface by using a smaller number of ligated tethers with multivalency. Multivalent ligands were able to enhance the selectivity of targeting nanoparticles [241]. A fifth-generation PAMAM dendrimer was conjugated with multivalent folate or riboflavin ligands for cell receptor targeting, and with 3,8-diamino-6-phenylphenanthridinium-derived ligands for DNA payload [242].

Additionally, the presentation of multiple ligands can either enhance specific targeting on one target, or achieve targeting at multiple levels. For example, hepatitis B virus L protein, the viral surface antigen (HBsAg), a lipid bilayer, and a Her2-selective antibody have been introduced together into a nanoparticle system, which showed enhanced *in vitro* selectivity for Her2-positive cells followed by successful gene silencing [243]. Multi-functional carriers were applied to gene delivery and mimic presentation of ligands in a sequential way [244]. For example, pH-sensitive biomaterials-coated polymer/DNA nanocomplexes were developed as an efficient non-viral gene delivery system. The system contains multiple ligands to cross multiple delivery barriers: folic acid for tumor targeting; pH-sensitive polymer PAMAM for endosomal escape; and finally NLS-containing endogenous molecules (a high mobility group box 1) for nuclear targeting [245].

Viruses are natural nanoparticles with size ranging from 20 nm to 400 nm [152]. Interestingly, this size range is also considered as the "ideal" size range for gene delivery [246]. Particles within this size range can prevent elimination by renal excretion (<20–30 nm) or by the mononuclear phagocytic system (MPS) in the liver, the spleen, and the bone marrow [246]. Also, particles within this size range take advantage of enhanced permeability and retention (EPR) effect, which passively targets tumor tissues due to the poorly operational lymph system of tumors and accumulation of macromolecules leaking from the blood [247].

After targeting specific tissue and anchoring on the cell surface, nanocarriers need to be internalized by cells, mostly via endocytosis. Similar to viruses, targeting ligands facilitate cellular uptake of nanoparticles via specific endocytic pathways. Several viral-derived peptides have been used in nano-systems to enhance cellular uptake and to target specific endocytic pathways [248,249]. Additionally, some non-virus derived ligands can target specific endocytic pathways, mimicking what viral ligands do. For example, transferrin-modified liposome was reported to undergo clathrin-mediated endocytosis [250], while folate is a typical ligand to channel cargoes to caveolae-mediated endocytosis [251]. However, it is worth noting that some experimental factors, such as ligand concentration, cell type, and temperature affect the endocytic pathways [252]. Hence experiments investigating endocytosis should be carefully designed to take these factors into consideration.

Except from introducing virus-derived ligands for specific cell targeting, non-viral vectors also learn from the non-specific attachment used in the early stage of virus-cell interaction. By binding with ubiquitous attachment factor (HSPG receptor) on the cell surface, arginine-rich cell-penetrating peptides significantly enhance the cellular uptake [253], which have been widely applied to various gene delivery systems [254]. Another recent study showed that phenylboronic acid (PBA)-grafted PEI/siRNA nanocomplex enhanced cellular uptake and transfection efficiency due to the interaction between PBA and sialic acid [255], which is an attachment factor for several viruses.

In addition, the preferred particle size differs among different endocytic pathways. Similar to what we discussed in the previous section on the study of viruses, the size preference is generally determined by the vesicle size of the specific pathway: CME usually uptakes nanoparticles in the size range of 100–200 nm, while CvME usually internalizes 60–80 nm sized nanoparticles, although some larger-sized nanoparticles (~250 nm) were found to undergo CvME as well [256]. On the other hand, the shape of nanoparticles also affects their cellular uptake and endocytic pathways. Though the shape does not alter the endocytic pathway as influenza viruses, a study did show that phagocytosis by macrophages was strongly dependent on shape. Interestingly, it is the local geometry of the particle at the very point of cell attachment rather than the overall particle shape that determines whether macrophages initiate cellular uptake or not [257]. It was found that sharp particles pierced the endosome membrane, escaping into the cytoplasm, resulting in reduced cellular excretion rate compared to their counterparts with the same surface chemistry, size, and composition [258]. Another study comparing quasi-ellipsoidal particles and spherical particles demonstrated that quasi-ellipsoidal particles had fewer uptake by cells than their spherical counterparts with a negative correlation between

aspect ratio and uptake rate. This is attributed to the larger average curvature radius of adsorbed non-spherical particles experienced by the cells [259].

3.2.2. Endosomal escape

After entering into the cells via endocytosis, delivery carriers usually experience pH drop from early endosomes (EE), late endosomes (LE) to lysosomes during the intracellular trafficking process. In order to avoid degradation by enzymes, siRNA carriers and most of DNA carriers must escape from these vesicular compartments before they are merged with lysosome. We have discussed in previous sections that viruses evolve efficient endosomal escape mechanism by pH-triggered endosome fusion or disruption, which have inspired the design of virus-mimetic carriers [260,261].

The peptides derived from viral fusogenic proteins display pH-triggered conformational change. They induce fusion with endosome membrane or pore formation, which are effective means to achieve endosomal escape. The incorporation of diINF-7 fusogenic peptide from influenza virus into Lipofectamine/siRNA lipoplex enhanced gene-silencing efficiency of epidermal growth factor receptor (EGFR) and K-ras oncogenes [262]. In another study, influenza virus-derived fusogenic peptide ligand HA2 was attached to PLL/DNA polyplex to achieve pH-triggered endosomal escape [14]. Similarly, fusogenic peptide named HGP, derived from HIV-1 gp41 protein, was utilized to facilitate endosomal escape of PEI/pDNA polyplex [263] and PEI/siRNA polyplex [264].

In addition to peptide ligands directly derived from viruses, synthetic peptides and fusion lipids inspired by viruses were applied on delivery carriers. The synthetic peptides usually contain hydrophobic residues, such as leucine or alanine, and protonating residues, such as glutamate or aspartate. A typical example is GALA, which is a 30 amino acid pH-sensitive amphiphilic peptide [265]. Inside the acidic endosomes, GALA turns into a helical conformation, causing liposomal leakage and delivering cargoes into the cytosolic space of cells [266]. Fusogenic lipids, such as DOPE, are used as a component in lipoplex for promoting endosomal release by fusion with the endosomal membrane [267].

To fully take advantage of the low-pH environment, some polycations were designed to facilitate endosomal escape by proton sponge effect [267]. It is proposed that molecules like PEI, which are rich in secondary or tertiary amines, can accumulate protons in the endosome, causing influx of water from the cytosol to balance the high osmotic pressure inside the endosome. Eventually, the endosome swells and bursts, releasing the cargoes into the cytosol [268]. Histidine-rich carriers are another example that escapes endosome via proton sponge effect. The histidine residue of pKa ~6 becomes protonated in endosomes and lysosomes, so that polyhistidine is able to disrupt membranes and enhance endosomal escape. Histidylated polylysine, for example, showed improved transfer efficiency of pDNA in cells [269].

Although the above strategies have undoubtedly enhanced gene transfection efficiency, the efficiency of endosomal escape of these non-viral vectors is still very low [270]. Based on image analysis, researchers showed that only 1–2% of lipoplex encapsulated siRNAs escaped from endosomes into the cytosol during a limited window of time when the lipoplexes reside in early and late endosomes [271]. Considering the endosomal escape efficiency of viruses reaches as high as 70% [272], further understanding of the endosomal escape mechanism will be beneficial for improving gene delivery by non-viral vectors.

It is worth noting that endosomal escape may not be the only choice for nucleic acid carriers. For example, SV40 bypasses endosome pathway and takes ER as an alternative route for nuclear entry. Recently, a histone-targeted polyplex has been developed, which was shown to circumvent issues of endosomal escape and enter the nucleus during postmitotic redistribution of ER membranes [273]. Interestingly, by incorporating H3-targeting peptides to PEI polyplexes, the endocytic pathway has been shifted to caveolar endocytic route and the gene transfer efficacy has been enhanced.

3.2.3. Cytoplasmic trafficking of nuclear viral mimicry

The viscous and crowded cytosol limits the free diffusion of macromolecules. Studies, performed to track a series of pDNAs by post cytoplasmic injection, revealed that pDNA of the length longer than 250 bp is immobilized in cytosol [274]. Therefore, to achieve nuclear delivery of DNA, active trafficking from peripheral area to perinuclear region is necessary. Two main active trafficking routes utilized by viruses—vesicular trafficking and direct virus trafficking—have been experimented in non-viral vectors. Endosome is a fast shuttle, allowing the endocytosed non-viral vectors to move towards the center of the cell along the microtubule track [275]. For example, R8-MEND took the vesicular trafficking via microtubules to transport towards the nucleus [275]. Fluorescence microscope imaging revealed that R8-MEND co-localized with both endosome and microtubule. Another study highlighted the important role of vesicular trafficking on gene transcription [276]. PEI/DNA polyplex, which was able to take microtubule-facilitated vesicular trafficking, led to significant transgene expression; while PEI-Arg/DNA polyplex, which did not show microtubule-facilitated trafficking, resulted in negligible gene transfection.

After the vectors escape into cytosol from endosomes, prematurely released pDNA risks being degraded. Previous studies have identified that the half-life of double-stranded pDNA in the cytoplasm of HeLa and COS cells is between 50–90 min [277] Therefore, gene protection for further trafficking toward nucleus is demanded for nuclear delivery. It is known that AdV in cytosol can move towards the nucleus by catching on microtubule through binding of their surface ligand with dynein [171,278,279]. The motor protein dynein plays an important role to load cellular vesicles or other cargoes on microtubule [280,281]. Therefore, incorporation of dynein-interaction ligands to non-viral vectors is able to mimic virus active trafficking in cytosol. For example, incorporation of dynein interaction ligands P79-98 from adenovirus E3 onto polyplexes promoted the colocalization of microtubule and polyplexes, and improved gene transfection efficiency by a factor of 2.5 [282]. Lipoplex modified with stearylated octaarginine (STR-R8) and dynein light chain (LC8)-associated peptide displayed higher transfection activity by one to two orders of magnitude [283].

For nuclear delivery of pDNA, microtubule-assisted active trafficking plays a significant role for nuclear accumulation. Vesicular trafficking mediated by endosomes is a general tool employed by current non-viral vectors, which is achieved by regulating cellular uptake pathway. Conjugation of dynein binding ligands on non-viral vectors will continue the active cytoplasmic trafficking toward the nucleus, which may further improve nuclear delivery.

3.2.4. Nuclear targeting and entry

For nuclear delivery of pDNA, the nuclear membrane is a major barrier for non-viral vectors. Microinjection of pDNA into the nucleus induced around 50–70% transfection, but cytoplasmic injection of pDNA did not result in any gene transfection [126]. Nuclear membrane is composed of a double bilayer structure, which limits the passive diffusion of large molecules up to 60 kDa [284]. Viruses usually achieve nuclear membrane accumulation by interacting with nuclear pore complex (NPC) directly or indirectly through the cellular adaptor protein importin [285,286].

A class of importin-interacting ligands rich in basic residues, called nuclear localization signal (NLS), are believed to play an important role in the active nuclear entry pathway through the interaction of importin with Phe-Gly (FG) repeats of NPC [287]. A library of NLSs derived from virus capsids have already been applied for non-viral vectors [284] (Table 2). NLSs can be introduced by three strategies: 1) directly conjugating NLS with DNA, followed by gene condensation with polycations such as PEI and PLL; 2) binding DNA with NLS modified PNA (peptide nucleic acid) through base pairing, followed by gene

condensation; 3) condensation of DNA with NLS or NLS modified cationic materials (such as PLL and PEI) [284,288]. For example, the NLS derived from SV40 large T antigen was conjugated with pDNA and compacted with cationic vectors (ExGen500, 25 kDa PEI, and Transfectam®). Significantly improved gene transfection (10 to 1000-fold) was achieved independent on the cationic vectors and cell types [289]. Non-covalent incorporation of NLS into DOTAP/protamine/pDNA vectors enhanced sustained gene expression in non-dividing cells [290]. A stearylated form of the SV40 large T antigen NLS (PKPKRKV) is able to decrease nuclear translocation capacity and enhance endosomal escape efficiency. The mixing with the peptide/siRNA complexes enhanced the siRNA transfection *in vitro* [291].

The basic nature of NLSs allows the sequences to form a complex with DNA, but then NLSs may be buried in the complex preventing them from binding to importin. Hence, strategies to expose NLS for importin binding will improve nuclear trafficking and entry. Some non-viral strategy is able to solve this problem and improve nuclear import. Some cellular protein receptors contain NLS segment, which is usually buried in the core. Upon binding with small molecules, these proteins switch their conformation and their NLS is exposed to the surface. For example, binding with dexamethasone is able to trigger the exposure of NLS from glucocorticoid. Therefore, incorporating dexamethasone to PAMAM or PEI polyplexes improved nuclear import in glucocorticoid-expressing H9C2 cells, eventually enhancing gene expression level by 20 to 40 folds [292].

Besides NLS, DNA nuclear targeting sequence (DTS) is able to significantly improve nuclear import of pDNA. Microinjection of the SV40 genome into cytoplasm resulted in nuclear localization within 6–8 h, while the cytoplasmic injection of other plasmid such as pUC19 led to no nuclear import [293]. A 72 bp SV40 enhancer has been identified as DTS, and the incorporation of this enhancer sequence into pDNA can induce nuclear entry [293]. Mechanism studies further have elucidated that the nuclear import capability of SV40 enhancer is because it can bind with a number of transcription factors containing NLSs [294].

In conclusion, the above examples eventually point out that the NLS is the main functional signal ligands to achieve nuclear targeting and entry. Therefore, viral mimicking nuclear entry can be enhanced by introducing NLS to non-viral vectors or pDNA through: 1) directly covalent conjugation or non-covalent binding; 2) small molecule triggered surface exposure; 3) DTS induced specific binding.

3.3. Release of nucleic acids

For both viral and non-viral gene vectors, it is generally assumed that gene release is necessary for their function of transfection or silencing. Viruses evolve diversified strategies to have well-controlled spatial and temporal gene release, depending on their functional location (cytosol vs nucleus). For some cytoplasmic viruses, binding with cell surface receptors is able to induce capsid protein conformational change and trigger gene release into cytosol. For nuclear viruses, nuclear delivery is demanded, so viral genome requires complete or partial protection during the whole cytoplasmic trafficking journey. Some of them start uncoating upon binding on the cell surface, and gradually loose the capsid integrity by acid-trigger in endosomes or redox trigger in ER, then complete uncoating upon docking on nuclear pore complex. On the other hand, small nuclear viruses keep the capsid intact during the whole cytoplasmic trafficking route and even nuclear entry process, only to release the gene inside the nucleus. It is assumed that the anionic exchange with host genome or protein is responsible for this last step, though the exact mechanism remains to be elucidated. Ideally, spatial and temporal control of nucleic acid release is engineered in viral mimicries for siRNA/miRNA and DNA delivery.

3.3.1. Release of DNA

For the nuclear delivery of DNA, the longer intracellular trafficking journey requires more stable carriers for gene protection. Lipids of amphipathic property self-assemble into multilamellar lipoplexes with DNA being embedded in lipid bilayers. The shorter length and limited charge number per lipid unit yields weaker charge interaction. The stability of lipoplex is modulated by the hydrophobic interaction between alkyl tails. Similar properties of lipids with subcellular organelle (e.g. endosome, ER and mitochondria) membrane components help to explain the mechanism of nucleic acid dissociation from lipoplexes [200]. Fluorescence resonance energy transfer (FRET) between fluorophore-labeled DNA and lipids was tracked to understand the intracellular distribution of genes carried by lipoplexes. The data showed that lipoplexes remained condensed after 3 h of incubation with cells. At 5–9 h, dissociation of DNA from lipoplexes happened while the lipid components were spread inside the whole cytoplasm [307]. Nuclear entry study of Lipofectamine® 2000/DNA lipoplex was investigated by quantifying the copy number of pDNA inside the nucleus. [308]. It was proposed that endosomal escape led to the complete or partial dissociation of pDNA from lipoplex and the remained lipoplex completely dissociated by fusion with intracellular organelles such as ER and mitochondria. Eventually, naked pDNA at the periphery nuclear area entered the nucleus [308,309]. Early endosomal escape and cytosol entry of pDNA present several challenges for DNA delivery: 1) concern of nuclease degradation of pDNA in cytosol; and 2) passive diffusion towards the nucleus and crossing of the nuclear membrane. While for nuclear delivery of DNA, late endosomal escape at the perinuclear region is preferred. However, another study discovered the lipoplex inside the nucleus and the limited gene release accounted for the much lower gene transfection level than AdV [310]. These contradictory results also complicate the application of lipoplex-based gene vectors for DNA delivery.

For DNA polyplexes, the formation of the complex is driven by the multivalent electrostatic interaction, which results in tight interaction and limits the gene release. Less compacted polyplex formed by shorter polymer favors the dissociation of nucleic acids and transfection [311]. The fluorescence study elucidated that the polyplex released into cytosol from endosomes was compacted, so they could protect genes from enzymatic digestion during further cytoplasmic trafficking. Therefore, polyplexes such as PEI and dendrimer have been frequently utilized for DNA delivery. However, fluorescence studies of pDNA/PEI or pDNA/histidylated PLL polyplexes showed that pDNA inside the nucleus was still packaged by polymers [312]. Currently, it is generally assumed that the gene release from the polyplex is realized by anionic exchange with surrounding polyanion (e.g. host mRNA, DNA, spermidine and spermine) [311,313]. Therefore, to balance gene protection and release is the next challenge to be addressed for viral mimicking.

Due to tight interaction with polycations and inefficient nucleic acid release from polyplexes, virus strategies such as acid and redox-triggered gene release has been employed to viral mimicry investigation. Similar to viruses, non-viral vectors are able to take advantage of endosome acidic pH to prompt gene release. To achieve this purpose in synthetic materials, various acid-labile linkers, such as imine linkage [314,315], ketals [315–318], acylhydrozone [319], vinyl ethers [320], ortho esters [321–324], have been used to construct high molecular weight polymers, by conjugating macromers of lower molecular weight or joining diblock polymers [314,325,326]. For example, pH-sensitive comb-like polymers, constructed by pH-sensitive ethyl acrylic acid monomer and acid-labile hydrazone linked cationic side chains, successfully encapsulated and protected anti-GAPDH-siRNA by forming stable nanoparticles [325]. Incubation of this polyplex in acidic condition (pH 5.8) led to the hydrolysis of hydrazone-linked side chain. *In vitro* transfection of MCF-7 cells by this pH-sensitive polyplex silenced GAPDH expression [325]. In addition, biodegradable PEI with acid-labile imine linkage of low molecular weight PEI showed shorter half-life and lower cytotoxicity relative the standard used PEI (25 kDa) [314]. The micelles formed by PLL and pH-sensitive siRNA/lactosylated PEG (Lac-PEG-siRNA) showed much higher gene silencing than siRNA/PLL polyplex, attributing to the acid-induced siRNA release from micelles [326].

Besides acid-trigger, redox-trigger is another strategy employed by viruses to achieve gene release. The isomerase in ER induces the genome release from SV40 through the breakage of the inter-pentamer disulfide [173]. The non-viral vector material is exemplified by the conjugation of low molecular weight polymer units with disulfide linkage. A stable polyplex with nucleic acids is formed which has the capability of redox-triggered dissociation, resulting in cargo release and improved transfection [311,327–329]. For example, the redox-sensitive thiopolymer polyaspartamide showed significant improvement in gene transfection [330]. Oligolysine (K18) with terminal Cys residues efficiently condensed pDNA and enhanced *in vitro* gene expression. Intracellular dissociation of complex by redox-trigger facilitated gene release [331]. Further shortening the oligolysine (K4) and the partial substitution of lysine by histidine led to further improvement in gene transfection in HepG2 cells, due to the redox-triggered gene release [331]. Conjugation of low molecular weight PEI with disulfide bond efficiently condensed pDNA, inducing significant gene transfection in Chinese hamster ovary (CHO) cells [329]. Another group explored the capability of reducible polycation (RPC) formed by conjugating peptides with cysteine ends (e.g. CHHHKKKHHHC and CHHHHHHKKKHHHHHHC). The vectors composed of RPC could deliver a broad range of nucleic acids, including pDNA, mRNA and siRNA [332]. These studies support the benefit of redox-triggered gene release and transfection.

3.3.2. Release of RNA

For siRNA/miRNA, the gene release in cytosol is preferred. Lipid-based lipoplexes or core–shell MEND are popular non-viral vectors for cytoplasmic delivery of siRNA/miRNA. Their unilamellar or multilamellar bilayer structure mimics the enveloped viruses, which can induce membrane fusion to achieve cytoplasmic delivery of their cargoes. The fusion with endosome membrane also facilitates the disruption of lipoplex for gene release. In addition, the lipid components in lipoplex benefit membrane fusion with intracellular organelles to achieve gene release in cytosol. Indeed, lipoplex-based delivery is among the first non-viral vectors to enter clinic trials for RNAi therapy [333]. While for polyplex and dendriplex, the short length of siRNA/miRNA leads to weak interaction with polycations, usually resulting in an unstable complex. This can lead to the easy uncoating, but at the expense of poor protection. For example, linear PEI of 22 kDa formed nanoparticles with siRNA, which gave rise to no gene silencing. Confocal studies revealed LPEI/siRNA polyplex was dissociated before cellular uptake. Branched PEI (BPEI) of 25 kDa could form more stable BPEI/siRNA polyplexes, but majority of these polyplex was transported into the nucleus [211].

Gene release is a critical step for the biological function of viral mimicries, and it is highly correlated with gene protection. How to balance both functions to achieve gene release at the right time and right place is a big challenge. Viruses evolve their strategies to gradually shed their coat during the intracellular trafficking process, cued by the subcellular conditions. These strategies have already been exploited for current viral mimicry, but there is a big room for improvement. Another important feature of viruses is the hierarchical architecture of the protein capsids, which offers the platform for the regulation of genome protection and release by cellular triggers. Therefore, structural viral mimicries (SVMs) of hierarchical "capsid" architecture may open new opportunities for better control of gene release.

3.4. New opportunities brought by structural viral mimicry (SVM)

Both cationic material-based viral mimicries (CVMs) (e.g. polyplex, dendriplex, lipoplex) and self-assembling protein/peptide-based structural viral mimicries (PSVMs) can effectively encapsulate and protect genes (Figs. 4, 5). Their particle architecture is very different (Table 3). Polyplexes and dendriplexes are formed by non-specific electrostatic interaction. The flexible nature of these polymers results in disordered organization within the complex and random surface presentation of the conjugated ligands. Lipoplexes, owing to the amphipathic property of lipid components, display some ordered unilamellar or multilamellar structure, but the surface ligand-presentation is not controllable. The

Table 3
The features of viruses, cationic material-based viral mimicries (CVMs: polyplex/dendriplex/lipoplex) and self-assembling protein/peptide-based structural viral mimicry (PSVM)

	Viruses	Cationic material-based viral mimicries (CVMs): polyplex, dendriplex and lipoplex	Protein/peptide-based structural viral mimicry (PSVM)
Monomer unit	Capsid protein	Polyplex: cationic polymer, polypeptide, Dendriplex: dendrimer Lipoplex: cationic lipid	Segmented peptide, protein
Assembly	1. Hydrophobic interaction, 2. Electrostatic interaction, 3. H-bonding	Electrostatic interaction Hydrophobic interaction	1. Hydrophobic interaction, 2. Electrostatic interaction, 3. H-bonding
Secondary structure	α-Helical structure or jelly-roll β-barrel	N/A	α-Helix or β-sheets
Stabilization force	1. Strong intra-subunit hydrophobic interaction and H-bonding; 2. Weak inter-subunit hydrophobic interaction; 3. Weak capsid-nucleic acid electrostatic interaction	Polyplex/dendriplex: strong electrostatic interaction; Lipoplex: weak electrostatic interaction, Hydrophobic interaction	1. Strong inter-peptide strand hydrophobic interaction and H-bonding; 2. Strong inter-nanofibril hydrophobic interaction; 3. Weak peptide–nucleic acid electrostatic interaction
Release of nucleic acid	1. Cell binding-induced conformational change; 2. Endosome acidic pH-triggered destabilization, 3. Nuclear pore complex interaction-induced release; 4. Gene release in cytosol and nucleus with unknown mechanism	Polyplex/dendriplex: Intracellular triggered chemical bond breakage Lipoplex: fusion with cellular suborganelles: endosome, RE or mitochondria	Inefficient if genes are encapsulated Note: Potential to achieve dissociation by sequence regulation or by introducing conformational switch protein/peptide units
Ligand display	Ordered ligand clustering and distribution	Disordered	Ordered ligand distribution possible. Note: Potential to mimic virus ligand spatial display and clustering through regulating peptide/protein assembly
Gene silencing, genome editing	Nuclear delivery of pDNA for cellular production of siRNA, miRNA, sgRNA	Cytoplasmic delivery of synthetic siRNA, miRNA, sgRNA	Cytoplasmic delivery of synthetic siRNA, miRNA, sgRNA
Transgene expression, genome editing	Nuclear delivery of pDNA	Nuclear delivery of pDNA	Nuclear delivery of pDNA

association of lipids by alkyl tail hydrophobic interaction is weak and there is concern of the stability of lipoplexes for *in vivo* applications [334]. In addition, the intrinsic lipid-serum protein interaction may alter the biophysical properties of lipoplexes and decrease the lipoplex *in vivo* transfection efficiency [334,335]. The self-assembling PSVMs form stable "capsids" by the combination of hydrophobic interaction, hydrogen-bonding and π–π aromatic stacking, etc. The intrinsic α-helical or β-sheet organization in the self-assembled nanostructures is able to organize the surface ligands in a controlled manner. The features of viral vectors are compared with conventional cationic systems and SVMs (Table 3), In addition to the assembly of vectors, strategies in overcoming cellular and intracellular barriers are contrasted (Fig. 6). The discrepancies between viral and non-viral vectors point to opportunities for future research.

Protein dimer/trimer or higher order multimeric cluster have been reported in biological events [336]. A sophisticated mechanism employed by viruses for cell entry and intracellular trafficking involves receptor clustering by viral ligands. This phenomenon is more than physical binding. It orchestrates a cascade of intracellular signals that assist uptake and trafficking of viruses [82]. Not only nano-scale spatial arrangement is essential, but also the timing of the events is well coordinated by sequential activation of viral ligands. Virus entry studies have showed that the synergetic interactions of multiple ligands with cell receptors and co-receptors are critical for cellular uptake [38,85]. Inherently disordered structure of polycation-based polyplexes/dendriplexes and lipid-based lipoplexes is not able to offer spatial control of surface ligand display at nano-scale. On the contrary, self-assembled α-helical or β-sheet protein/peptide structure provides a feasible platform to organize virus-derived ligands in an ordered manner. Therefore, self-assembling PSVM may resume the viral capsid surface property of controlled ligand organization and multiple-ligand clustering.

Moreover, the hierarchical capsid architecture of viruses is the key to the spatial and temporal control of gene protection and release. Polyplexes, dendriplexes and lipoplexes are formed by mass association of polymers/lipids with genetic cargoes, and no hierarchical assembly is involved. Gene release is either achieved by reverse process or chemical breakage of polymer chain by cellular triggers. In contrast, the assembly

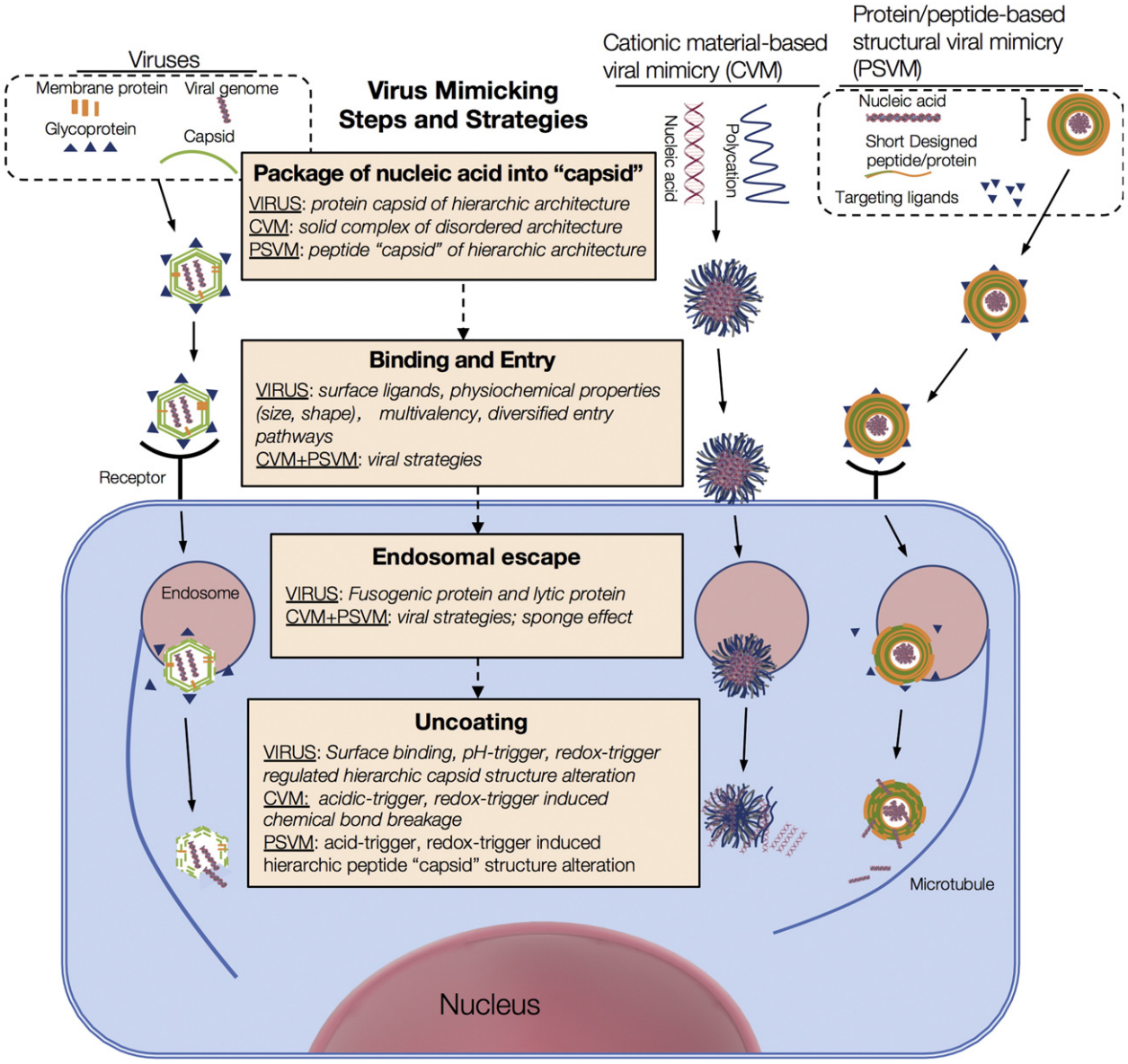

Fig. 6. Comparison of viral mimicry strategies of cationic material-based viral mimicry (CVM: polyplex/dendriplex/lipoplex) and self-assembly protein/peptide-based structural vial mimicry (PSVM) in different steps: 1) Package of nucleic acids into "capsids"; 2) Binding and entry; 3) Endosomal escape; 4) Uncoating. CVM and PSVM take similar virus-mimicry strategy in step 2) and 3). However, only PSVM is capable of mimicking the virus capsids in a hierarchical manner at the stage of package and uncoating.

of structural viral mimicries resembles the assembly of virion. The self-assembly of protein/peptide builds subunits, and subunits association and gene binding lead to the formation of SVMs. To a certain degree, non-covalent forces for intra-subunit and inter-subunit interaction can be engineered independently via peptide design. This offers new opportunities to balance the stability essential for gene protection and the disassembly ability essential for gene release. It is also conceptually possible to incorporate protein or peptide, which can switch the secondary structural upon cellular triggers, into SVMs. Therefore, SVMs open opportunities to adopt virus surface properties, and viral-like controllable gene release. To achieve these goals, further studies of structural regulation of the self-assembling peptide and protein are required.

4. Conclusion

Learning from viruses and incorporating virus-like properties into designing viral mimicries for the delivery of nucleic acids has been attempted for decades. Cationic systems are capable of condensing and protecting nucleic acids. Surface modification, including virus-derived sequences, imparts nanocarriers with the ability for cellular and subcellular targeting to a certain extent. However, there is still much room for improvement for viral mimicry. In all important steps leading to nucleic acid delivery: from packing of nucleic acid, cellular entry, endosomal escape, subcellular targeting, release of nucleic acid, to nuclear entry, the hierarchical ordered structure of viruses plays important functional roles. The comparison between viruses and different viral mimicries has been highlighted in Fig. 6. It is clear to see the fundamental differences in the structural aspects of viruses and conventional non-viral vectors based on cationic systems, as well as their corresponding intracellular trafficking (Fig. 6). Electrostatic force between cationic systems and nucleic acids is responsible for the assembly. This mode of interaction must sacrifice efficiency of nucleic acid release in exchange of the formation of stable and compact complex. On the other hand, viruses invoke a delicate balance of non-covalent forces (including electrostatic interaction, hydrophobic interaction and hydrogen bonding) to create metastable structure, maximizing both the efficiency of packing of nucleic acids and uncoating of virus capsids.

Recently, SVM has been constructed by the co-assembly of self-assembling protein/peptide and nucleic acids. The results represent a step forward for viral mimicking, with nanostructures adopting virus-like appearance and architecture (Fig. 5). However, this new class of nucleic acid carriers has not yet attained significant improvement in transfection efficiency. A critical issue to be addressed is to design building blocks to attain balanced stability for timely release of nucleic acids (Table 3).

Biofunctional ligands from viral capsids and envelopes have been discovered and virus-derived sequences have been used by non-viral vectors to target cells and subcellular compartments. However, ligands useful for active intracellular trafficking such as those involved in dynein interaction remain few. Spatial and temporal orchestration of multiple ligands is important for directing cell entry and intracellular trafficking of viruses. Such coordination of biofunctional ligands is absent in current non-viral vectors. Structural viral mimicry opens doors to exploit hierarchical and dynamic display of viral ligands (Fig. 6). Therefore, further understanding on the regulation of structural viral mimicry would lead to opportunities for functional viral mimicry and improvement in delivery of nucleic acid therapy.

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