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Synthetic Virology: Engineering Viruses for Gene Delivery

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Abstract

The success of gene therapy relies heavily on the performance of vectors that can effectively deliver transgenes to desired cell populations. As viruses have evolved to deliver genetic material into cells, a prolific area of research has emerged over the last several decades to leverage the innate properties of viruses as well as to engineer new features into them. Specifically, the field of synthetic virology aims to capitalize on knowledge accrued from fundamental virology research in order to design functionally enhanced gene delivery vectors. The enhanced viral vectors, or “bionic” viruses, feature engineered components, or “parts”, that are natural (intrinsic to viruses or from other organisms) and synthetic (such as man-made polymers or inorganic nanoparticles). Various design strategies – rational, combinatorial, and pseudo-rational – have been pursued to create the hybrid viruses. The gene delivery vectors of the future will likely criss-cross the boundaries between natural and synthetic domains to harness the unique strengths afforded by the various functional parts that can be grafted onto virus capsids. Such research endeavours will further expand and enable enhanced control over the functional capacity of these nanoscale devices for biomedicine.

Introduction

The first gene therapy product on the Western market was approved in the fall of 2012,¹ and a number of other viral gene delivery vectors are in the pipeline towards clinical translation.² A majority of the viral vectors that have made it to clinical testing are naturally occurring viral variants, whose innate properties may be sufficient to treat certain diseases. For example, in the treatment of hemophilia B, the viral vector can be injected intravenously leading to transduction of liver cells.³ The resulting production and secretion of the delivered coagulation factor IX (FIX) into the patient’s blood is sufficient to ameliorate the clinical phenotype. To effectively treat other diseases, however, the gene therapies may need to be delivered specifically to diseased cells, which may require additional vector engineering.

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Synthetic virology aims to reprogram naturally occurring viruses into controllable and predictable devices. The field can broadly be divided into two main endeavours: 1) engineering of the virus capsid and 2) engineering of the genetic programs encoded by the viral genome. This review will focus on virus capsid engineering as applied to gene therapy, and readers are directed elsewhere for reviews about engineering viral genomes^{4, 5} Viruses have evolved to deliver genetic information into host cells, which means that molecular programs that dictate how the viruses behave *in vivo* have already been written into their capsid structure. A goal of synthetic virology, therefore, is to rewrite the details of which biomolecular features a virus uses during its infectious process (e.g. cellular receptors).

Engineering targeted viral gene delivery vectors has been a vibrant area of synthetic virology research. Researchers in this field have been working collectively towards the vision of a “bionic” virus, where new functionalities, which can be foreign to viruses, are imparted to the virus through genetic and/or chemical modification (Figure 1). Borrowing from the language of electrical/computer engineering, we describe functional motifs as “parts”, whose properties can be characterized independent of the virus capsid. The parts (e.g. targeting peptide) can be incorporated into viruses to enable them to carry out a new function (e.g. binding to a target cell). In this review, we will cover some of the work done on 1) mixing pre-existing viral parts, 2) inserting genetically encoded parts foreign to natural viruses, 3) tweaking viruses through point mutations, 4) incorporating small molecular parts, and 5) attaching completely synthetic parts, such as man-made polymers.

MIXING VIRAL PARTS

Mother Nature has already provided a palette of viral variants whose differential properties can be exploited to engineer viral vectors with improved properties. For instance, a number of naturally occurring adeno-associated virus (AAV) serotypes have been isolated, each with altered capsid phenotypes.⁶ These diverse capsid properties can be mixed together into one viral vector in order to create hybrid viruses with new functionalities. Below, we discuss both rational and combinatorial design strategies to mix virally derived parts.

Chimeric viruses

Chimeric viruses are created by genetically splicing together capsid genes of two or more viruses, resulting in a new virus with a hybrid capsid. Chimeras have been generated to retarget vectors by swapping receptor binding domains between viral serotypes.^{7, 8} For example, chimeric adenoviral (Ad) vectors have been engineered to alter their tropism. Adenovirus serotype 5 (Ad5) natively relies on interaction between its knob domain and coxsackievirus-adenovirus receptor (CAR) to bind and infect cells. Unfortunately, CAR is often not expressed on target cells, such as tumor cells. To overcome this limitation, the Ad5 fiber knob domain was replaced with that of porcine adenovirus type 4 NADC-1 strain to produce a chimera that binds to cell-surface glycans containing lactose and N-acetyl-lactosamine units.⁸ This chimera represents a step toward developing vectors targeting aberrantly glycosylated cancer cells. Beyond altering tropism, chimeric viruses have been generated as an approach to circumvent pre-existing anti-viral immunity by exchanging potential antibody epitopes on the capsid.⁹

In general, the chimeric virus approach is a rational design strategy, because it is helpful to know what functional capsid domains (e.g. cell binding domain, immunogenic epitope, etc.) are present and amenable to exchanging with analogous domains from other closely related virus strains. The chimera approach, however, can also be a useful tool for investigating the underlying capsid biology. For example, in Ho et al., generation of a panel of chimeric AAV revealed that this virus capsid's ability to assemble and package genomes is relatively insensitive to structural disruptions introduced by this type of mutagenesis.¹⁰

Mosaic viruses

Virus capsids are self-assemblies of multiple individual protein subunits. Mosaic viruses capitalize on this property by mixing subunits from different, but related, viruses into one capsid. In general, the mosaic approach appears to be an effective method for combining phenotypic traits of two or more serotypes. For example, adenovirus mosaics displaying both Ad3 and Ad5 fiber knobs are able to use both CD40 (Ad3 tropism) and CAR (Ad5 tropism) as cellular receptors and are able to transduce cells even when one of the two receptors are blocked, thereby expanding the tropism of adenoviral vectors.¹¹ Most recently, the mosaic capsid approach was used by Judd et al.¹² to design AAV vectors that require cleavage by two different extracellular proteases to mediate transgene delivery. The protease-activatable viruses (PAVs) are built by genetically inserting peptide “locks” into the cellular receptor binding domains of the capsid. When the locks are in place, the AAV vector is unable to bind and transduce cells. In the presence of target extracellular proteases, such as matrix metalloproteinases, the locks are cleaved off of the capsid, “unlocking” the virus and enabling gene delivery to occur. The efficiency of PAVs can reach wild-type (wt) levels by generating mosaic capsids composed of wt and protease-responsive subunits.¹³ By creating mosaic capsids, where each subunit displays one of two different peptide locks, the PAV can behave as an AND logic gate, requiring cleavage by two different proteases for gene delivery to occur.

Pseudotyped viruses

Pseudotyping is another rational design strategy to mix viral parts from different viruses and is most often referred to in the context of modifying enveloped viruses, such as lentivirus, although non-enveloped viruses can also be pseudotyped.¹⁴ In this approach, viral tropism is altered by exchanging the envelope protein of one virus with another. Lentiviral vectors are popularly pseudotyped with glycoprotein G from vesicular stomatitis virus (VSV-G), expanding vector tropism to a broad host-cell range (reviewed in¹⁵). Other glycoproteins have also been explored, including truncated forms of the measles virus glycoproteins hemagglutinin (H) and fusion protein (F).¹⁶ The truncated H protein is amenable to further modification through ablation of native binding and the fusion of ligands such as EGF or single-chain antibodies to its ectodomain, allowing the virus to bind to new targets such as EGFR-expressing cells and CD20-positive lymphocytes.

DNA shuffling for combinatorial chimeragenesis

In addition to the rational design strategies discussed above, viral parts can be mixed in a combinatorial fashion. Through DNA shuffling, chimeric virus libraries are constructed by random fragmentation, assembly, and amplification of capsid genes from different virus

variants. Beyond identifying the region of the viral genome targeted for mutagenesis, this method does not take into account other prior knowledge of functional domains embedded in the capsid genes. Thus, this approach is useful for exploring unknown protein sequence space and uncovering critical design rules of capsid assembly and function.

DNA shuffling has been used to modify viral capsids through both *in vitro* and *in vivo* selection.¹⁷ For example, Yang et al.¹⁸ shuffled the capsid genes of AAV serotypes 1 to 9 and conducted *in vivo* selection for capsids with high transduction in striated muscles and low transduction in liver following tail vein injection in mice. The optimal virus chimera was composed of capsid gene fragments from serotypes 1, 6, 7, and 8. DNA shuffling of AAV has also yielded chimeric vectors with enhanced delivery to glial cells¹⁹ and hepatocytes,²⁰ crossing of the blood brain barrier in seizure-compromised patients,²¹ and resistance to human intravenous immunoglobulin.²² DNA shuffling has been applied to retroviruses²³ where the envelope gene of murine leukemia virus was shuffled, and the virus library was subjected to multiple rounds of ultracentrifugal stress to isolate viruses with improved stability.

Overall, DNA shuffling is a useful combinatorial design strategy to generate large chimeric virus libraries. By performing selection *in vivo*, viral variants can be identified with abilities to overcome complex physiological barriers to reach target sites. However, applying DNA shuffling on viral genes with embedded complexity, for example in the form of overlapping open reading frames, may pose difficulties. Another downside of DNA shuffling and other annealing-based recombination methods is the lack of control over crossover sites, which only occur in regions of high sequence identity.^{24, 25} While amino acid substitutions created by DNA shuffling are more conservative than those created by random mutation,²⁶ random recombination approaches do not avoid amino acid substitutions that disrupt protein structure and, therefore, yield low fitness viral mutants. This weakness may be overcome by generating libraries using structure-guided protein recombination,^{27, 28} which uses the three-dimensional coordinates of a protein to identify domains that can be swapped among different homologous proteins without disrupting protein structure.

In summary, by mixing existing viral parts, the genetic and structural solutions that have been achieved evolutionarily by viruses to carry out productive infections can be fully harnessed for engineering the next generation of improved viral vectors. However, these new hybrid vectors may be limited to exhibiting functionalities already found natively in pre-existing viruses. Through the incorporation of exogenous peptides and proteins with capabilities foreign to natural viruses, as discussed in the following section, the functional capacity of viral vectors can increase greatly.

GENETICALLY ENCODED FOREIGN PARTS: INSERTION OF PEPTIDES AND PROTEINS

Viruses can be engineered to exhibit altered functionalities through the insertion of bioactive peptides and proteins into the capsid. The peptides and proteins can be completely foreign to viruses in general. This genetic modification strategy necessitates the identification of a capsid site that fulfills the following criteria: 1) tolerance to the insertion, resulting in

maintenance of important virus properties, such as capsid assembly, genome packaging, and infectivity; 2) maintenance of desired functional properties of the inserted domain; and 3) if appropriate, overwriting of any undesired innate virus properties (e.g. natural tropism). Such ideal capsid insertion sites can be identified through systematic mutagenesis efforts^{29, 30} and structural studies.^{31, 32} Going forward, molecular modelling strategies may be a promising approach to identifying optimal insertion sites *in silico*. For example, the AAV2 capsid residue 453 was identified as an alternative site for inserting a targeting peptide, instead of the commonly used residue 587, due to its prominence on the three-fold axis of symmetry as visualized through modeling.³³ Below, we discuss ongoing efforts to insert peptides/proteins into rationally chosen capsid sites as well as into random locations throughout the capsid in an attempt to identify optimal insertion sites.

Rational insertion of defined peptide/protein

For adenoviral vectors, the fiber knob region continues to be the insertion site of choice, for both small and large peptides. The HI loop is highly variable across serotypes and has been shown to tolerate insertions, and by inserting the A20FMDV2 peptide into this region, Coughlan et al. were able to retarget Ad5 to $\alpha v \beta 6$ integrin, an epithelial integrin significantly upregulated in many carcinomas.³⁴ After systemic *in vivo* delivery, the mutated virus demonstrated increased transduction of tumor cells and decreased off-target transduction of the liver. The NGR peptide has also been used to retarget Ad5 from CAR to aminopeptidase N, expressed by endothelial cells and upregulated in some cancers.³⁵ However, both retargeted vectors continue to demonstrate high liver uptake and other off-target biodistribution, indicating that small peptides may not be sufficient for complete retargeting. Beyond retargeting efforts, small peptide insertions have been used to reduce the immune response against adenovirus. Ad vectors that displayed the decay-accelerating factor (DAF), a human complement inhibitor, through fusion to the C terminus of the pIX capsid protein exhibited dramatically reduced immune responses in mice.³⁶

The adenovirus HI loop is also amenable to larger peptide insertions, such as the insertion of two different Affibody molecules into the same fiber knob, leading to dual binding specificity.³⁷ Antibodies have also been inserted into the adenovirus capsid; however, mutants with single-chain antibody fragment insertions are unable to retarget due to improper folding of the antibody. However, by fusing the single-domain antibody AFAI to the pIX capsid protein, Poulin et al. were able to successfully incorporate the antibody into adenovirus and retarget the vector to cells expressing CD66c, a marker upregulated in lung carcinomas.³⁸

For AAV vectors, the N-terminus of the VP2 capsid subunit has been identified as a site amenable for peptide/protein insertions.³⁹ Designed ankyrin repeat proteins (DARPs) have been incorporated into this location in order to retarget AAV2 to cells expressing HER2/neu.⁴⁰ Promisingly, the AAV-DARPin vectors demonstrated tumor-targeting capabilities with decreased transgene expression in off-target organs, such as the liver.

Lentiviruses have also been modified with proteins not endogenous to viruses. For example, antibodies were modified to become membrane-bound and incorporated into the lentiviral

envelope with pH-dependent fusogenic proteins, creating a viral vector that targets CD20-expressing cells both *in vitro* and *in vivo*.⁴¹

Rational insertion of random peptide

Over the last several decades, many targeting peptides have been identified through phage display⁴²—viral libraries that display some number of random amino acid residues in a specific site on a coat protein. It can be difficult, however, to predict if the peptide (identified in the context of the bacteriophage) will function similarly once placed in the framework of a different virus capsid. Additionally, peptides selected from phage display may mediate cell binding, but may not necessarily support downstream steps of the gene delivery process. To overcome these limitations, viral gene delivery vectors, rather than bacteriophage, have been used to create peptide display libraries that can be selected/ screened *in vitro* or *in vivo* to identify new variants with desired properties.

A number of viruses have been subjected to random peptide display and directed evolution. Bupp et al.⁴³ generated a random peptide display library on the feline leukemia virus subgroup A and demonstrated that applying appropriate selection pressures on this library can result in identification of fully functional viral vectors with altered cell specificity. For the adenovirus platform, Miura et al.⁴⁴ developed an improved method for high efficiency peptide display library generation. This method uses a fiberless adenovirus and relies on intracellular CRE-lox-mediated recombination between a library of fiber-encoding shuttle plasmids and the fiberless adenovirus genome. The library was used to identify a virus variant with selective infectivity for mesothelin-positive cells both *in vitro* and *in vivo*. Nishimoto et al.⁴⁵ used *in vivo* selection to obtain peritoneal tumor-targeting Ad vectors from random peptide display libraries. The peptide display approach was also applied to AAV serotype 2 (AAV2) by inserting a randomized seven-residue peptide into the receptor binding domain of the virus.⁴⁶ Upon selection, an AAV mutant was isolated with preferential transduction of coronary artery endothelial cells, which are weakly transduced by wt capsid AAV2 vectors. *In vivo* selection of an AAV2 peptide display library also led to identification of heart-targeting vectors in mice.⁴⁷ Overall, peptide display has been carried out successfully in the context of several different viruses.

Although conceptually easy to understand, directed evolution approaches to generating improved viral vectors have many challenges in practice. Producing naïve viral libraries (pre-selection) free of wt vectors can be difficult; therefore, selection may be overwhelmed if performed on cells susceptible to the wt vector. Additionally, native tropism may not be ablated by peptide display, leading to the expansion of, rather than a replacement of, tropism.

Random insertion of defined peptide/protein

In certain instances, the challenge may not be identification of the targeting peptide but rather identification of the optimal capsid insertion site. In this case, the defined peptide/protein can be inserted randomly throughout the capsid, and application of the appropriate selection pressure will help isolate the desired mutants.

One way to carry out random insertion is through the use of DNase I. The nuclease digestion is optimized to create single breaks in double-stranded DNA at random locations. The peptide/protein is then ligated into the cut site, yielding a library of viral genes with peptides/proteins inserted randomly throughout. The approach was first applied to viral vectors in the context of AAV serotype 5 (AAV5)⁴⁸ to identify capsid regions tolerant to deletions and duplications. This method was taken a step further to generate a platform library, named RaPID (random peptide insertion by DNase),⁴⁹ which could be used to insert desired peptides/proteins randomly throughout the AAV2 capsid gene in a facile manner. Selection of the virus library can then identify capsid insertion sites that fulfill the design criteria set forth in the beginning of this section. Using this library, an AAV2 capsid insertion site was identified that is tolerant to mCherry insertion. Notably, the insertion site was in the VP3 region of the *cap* gene – part of the capsid previously thought to be intolerant to the insertion of relatively large proteins. The isolated mutant was not only fluorescent but also exhibited similar infectivity to wt capsid.

Alternatively, transposase-mediated random insertion can be used to insert peptides/proteins into viral capsids. For example, this approach was used to insert hexahistidine tags into the AAV2 capsid, yielding viral mutants able to be captured on nickel affinity columns.⁵⁰ A commercially available kit is available for transposase-mediated random insertion, rendering the mutagenesis process to be more streamlined. The DNase-based method, although lacking a commercial kit to our knowledge, introduces additional crossover diversity in the form of deletions and duplications.

In summary, viral vectors can be designed to exhibit properties foreign to natural viruses by genetically incorporating bioactive peptides/proteins into the capsid. Known peptides/proteins can be inserted into the capsid at rationally chosen sites, or the optimal insertion site can be identified through combinatorial random domain insertion approaches. Alternatively, random peptides can be inserted into rationally chosen capsid locations in order to identify a bioactive peptide motif that would bestow upon the virus the desired phenotype.

TWEAKING VIRUSES THROUGH POINT MUTATIONS

In certain instances, introducing point mutations to a pre-existing virus capsid, either nature-derived or human-engineered, may be all that is needed to obtain the optimal vector. The point mutations can be scattered throughout a gene, or clustered in a rationally chosen capsid domain.

Random point mutations

Error-prone polymerase chain reaction (EP-PCR) has been used to create libraries containing AAV capsid genes with point mutations throughout.^{51, 52} Application of appropriate selection pressures resulted in vectors capable of evading neutralizing antibodies. Using EP-PCR on AAV, Asuri et al.⁵³ generated a virus variant that was able to more efficiently deliver a genetic payload to human stem cells. They further enhanced this vector by coupling it with the delivery of a zinc-finger nuclease to promote homologous recombination of the delivered transgene through induced double-strand breaks, thus enabling gene targeting. EP-PCR coupled with *in vivo* selection also led to the generation of

a novel AAV vector capable of transducing outer retinal cells after injection into the vitreous humor.⁵⁴ The *in vivo* selection was key in isolating vectors that can migrate through complex extracellular environments to reach the desired cell population. Lastly, rather than creating mutations throughout the capsid, Pulicherla et al.⁵⁵ took advantage of structure-function knowledge and generated an AAV library with random mutations focused in the VP3 GH loop, a region that is highly variable between AAV serotypes and previously identified to impact tropism in AAV serotype 9. Through directed evolution, they were able to identify an AAV9 variant with decreased tropism for the liver, but retained wt transduction levels in cardiac and skeletal muscle.

In summary, modification of viral properties may only require a small number of point mutations, identified in a completely random fashion (i.e. mutations scattered throughout capsid), or in a pseudo-rational manner (i.e. mutations localized to rationally chosen capsid region). These mutations can be layered on top of naturally occurring capsids or those engineered previously through other means.

MOLECULAR PARTS: INCORPORATION OF SMALL MOLECULES

Virus capsids are chemically addressable entities, often with surface-exposed amine groups amenable for chemical conjugation of bioactive moieties. Additional chemical groups, such as thiols, can be genetically incorporated into the capsid at specific sites. Furthermore, genetic insertion of certain peptide motifs, such as biotin acceptor peptide, can enable downstream attachment of other moieties onto the capsid.

Attachment of small molecule adaptors

One of the most popular molecular adaptor systems is biotin-avidin.⁵⁶ Binding between avidin and biotin has a dissociation constant K_D of 10^{-15} M, which has been recognized as the highest binding affinity between biological molecules. Thus, this adaptor system may be a promising approach for the attachment of bioactive entities (e.g. targeting peptides or antibodies) to virus capsids. Arnold et al.⁵⁷ developed a method that involves the genetic insertion of the biotin acceptor peptide (BAP) into the AAV capsid. The biotin molecule is then attached to the BAP via biotin ligase, a bacterial enzyme that carries out the ligation. Functional moieties, such as targeting peptides, can be attached to the biotinylated virus via avidin linkages. In a different study, Morizono et al.⁵⁸ developed a magnetofection strategy by first inserting BAP into the envelope proteins of lentivirus. Then, using streptavidin-coated magnetic beads, the viral vectors were more efficiently associated with cells, resulting in greater gene delivery.

The inserted BAP motif can also accept ligation of a ketone isotope of biotin, which could be used to conjugate other kinds of functional motifs, such as fluorophores and targeting peptides.⁵⁹ Similarly, genetic insertion of a small peptide sequence recognized by cellular formylglycine generating enzyme can lead to display of aldehyde groups, which can mediate site-selective conjugation of hydrazide-functionalized motifs to virus capsids.⁶⁰

Conjugation of small molecule drugs

The co-delivery of genes and small molecule drugs may be a promising approach for the treatment of certain diseases. By attaching drugs to the multivalent capsid, the payload of delivery can be greatly increased. Furthermore, some drugs may need to be freely diffusible to carry out their action; therefore, attaching them to the capsid creates a “pro-drug” that requires the appropriate environment (e.g. cellular endosome of a target cell) to convert into a potent drug. This pro-drug approach could potentially decrease undesired side-effects. To this end, Wei et al.⁶¹ conjugated the chemotherapeutic drug taxol to the AAV capsid via surface-exposed lysine residues. Promisingly, the conjugation did not negatively affect AAV gene delivery efficiency. However, the virus-taxol nanoparticle was not able to kill cancer cells effectively. Future improvements that may advance this delivery vector include use of linkers that degrade in the low pH environment of endosomes, and enhanced virus production procedures to ensure large amounts of starting material. A virus system that has demonstrated success in delivering drugs is the MS2 bacteriophage.⁶² Genome-free viral capsids were modified with cysteine alkylation chemistry to develop a platform to deliver chemotherapeutics. A water-soluble derivative of taxol was conjugated to newly introduced cysteine residues on the interior face of the MS2 capsid. When incubated with breast cancer cells, the water-soluble taxol derivative was released and caused a significant decrease in cell viability. The MS2 capsid, however, has not been engineered to deliver genes into mammalian cells so it cannot be used for co-delivery of genes and drugs as of yet.

In summary, small molecules can be attached to virus capsids to impart new properties. The molecules can act as adaptors to mediate the attachment of other bioactive moieties, such as targeting peptides, or the molecules themselves may possess desired bioactivities, such as drugs.

SYNTHETIC PARTS: INCORPORATION OF POLYMERS AND NANOPARTICLES

The functional properties of viruses can be further altered or enhanced through the incorporation of components that are non-biological in nature, such as synthetic polymers and nanoparticles. Inclusion of such man-made parts into the nature-derived capsids may ultimately lead to the generation of hybrid viruses that can synergistically leverage the unique strengths afforded by both natural and synthetic domains. Polymers are the most common synthetic parts that have been incorporated into viruses. Some polymers can prevent undesired interactions of viruses with biological components, reducing untargeted delivery and/or immunogenicity. Other polymers are used to broaden the tropism of viruses to expand their utility in more applications. In regards to nanoparticles, those that enable tracking of the viruses through imaging have been most studied.

Chemical conjugation of “stealth” polymers

The conjugation of polyethylene glycol (PEG) chains to proteins or nanomaterials, termed PEGylation, is a widely used approach to prevent unwanted biological interactions of nanomaterials to biological components,⁶³ such as serum proteins and cell surfaces. Steinmetz et al.⁶⁴ PEGylated cowpea mosaic virus (CPMV) to investigate if the shielding

afforded by the polymer can prevent the virus' interaction with endothelial cells. The PEG molecules were conjugated to the surface-exposed lysine residues on the CPMV capsid through *N*-hydroxysuccinimide (NHS) coupling. It was estimated only ~22 molecules of PEG-1000 and ~27 molecules of PEG-2000 need to be attached per viral nanoparticle to eliminate undesired cellular interactions. AAV vectors have also been modified via chemical conjugation of PEG-2000 to surface-exposed lysines on the capsid.⁶⁵ PEGylated AAV particles with a conjugation ratio 10³:1 (PEG:lysine) were able to evade neutralizing antibodies in serum. Hofherr et al.⁶⁶ attached PEG-5000 to adenoviral vectors, and studies with mammalian blood revealed the PEGylation inhibited virus activation and binding to platelets and endothelial cells.

Complexation with synthetic polymers

Viruses can be further modified by coating them with polymers, resulting in the formation of virus-polymer complexes. In this case, the polymers are usually not covalently attached to the capsid surface. This method normally enables viruses to broaden their tropism, allowing for the transduction of cells lacking the virus' natural cellular receptor. For example, Park et al.⁶⁷ synthesized a PEG grafted poly-L-lysine (PLL) copolymer (PLL-g-PEG) and complexed it with adenovirus. This simple capsid modification approach led to enhanced gene transduction efficiency in bone marrow-derived human mesenchymal stem cells. In another study, Han et al.⁶⁸ synthesized biodegradable polyethylenimine (PEI) and complexed it with adenovirus. The resulting adenovirus/PEI complexes demonstrated increased transduction of cells lacking the adenoviral cellular receptor. Adenoviruses have also been coated with polyamidoamine (PAMAM) dendrimers, leading to increased levels of viral uptake and transduction in tumour cells.^{69, 70}

Incorporation of nanoparticles

In addition to synthetic polymers, inorganic nanoparticles can be incorporated into viruses, either on the capsid surface or within the capsid interior. For example, quantum dots (QD) were chemically attached to surface-exposed lysine residues on AAV capsids.⁷¹ The resulting AAV-QD enabled real-time live-cell imaging of the virus-nanoparticle entities as they travelled intracellularly. In another study, iron oxide nanoparticles were encapsulated within the capsid of brome mosaic virus (BMV).⁷² Since the iron oxide nanoparticles are magnetic, these virus-nanoparticle systems can be detected using magnetic resonance imaging (MRI). BMV is a plant virus and, therefore, cannot be used for gene therapy in humans but may be useful for drug delivery.

In summary, synthetic components, such as polymers, can be incorporated into the design of viral vectors to minimize undesirable properties (e.g. delivery to nontarget cells) or to improve others (e.g. broaden tropism to previously resistant cells). Moreover, incorporation of synthetic nanoparticles, either to the outside or inside of virus capsids, can enable viral tracking. Integration of more synthetic parts, with other functionalities foreign to nature-derived systems, may further expand the capability of viruses designed for gene therapy.

Conclusion

With the approval of the first gene therapy drug on the Western market in 2012,¹ new momentum has been infused into the field of gene therapy. A number of other promising gene therapies are in the clinical pipeline.² Many of the vectors are natural variants; however, several engineered viruses with improved properties, especially in regards to tissue targeting, are working their way through preclinical and clinical testing. The field of synthetic virology, and the developed tools and techniques within, will be instrumental to realizing the full potential of gene therapy. The gene delivery vectors of the future will likely be “bionic” viruses – hybrid nanoscale devices with well-characterized components from nature (viral and nonviral) as well as those that are man-made. Many advances have already been made on mixing pre-existing viral parts, genetically incorporating parts foreign to viruses, tweaking viruses by point mutations, and incorporating small molecules as well as completely synthetic parts, such as polymers and inorganic nanoparticles. These design strategies can be rational, combinatorial, or a combination thereof (i.e. pseudo-rational). The field will benefit greatly from additional efforts to identify quantitative design rules that can guide the rational construction of new and improved vectors. We believe the future of synthetic virology, and its promising application outlets in gene therapy, will be full of hybrid nanodevices engineered to carry out functions currently unimagined.

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References

1. Yla-Herttuala S. Endgame: Glybera Finally Recommended for Approval as the First Gene Therapy Drug in the European Union. *Molecular Therapy*. 2012; 20:1831–1832. [PubMed: 23023051]
2. Asokan A, Schaffer DV, Samulski RJ. The AAV Vector Toolkit: Poised at the Clinical Crossroads. *Molecular Therapy*. 2012; 20:699–708. [PubMed: 22273577]
3. Nathwani AC, Tuddenham EGD, Rangarajan S, Rosales C, McIntosh J, Linch DC, Chowdary P, Riddell A, Pie AJ, Harrington C, et al. Adenovirus-Associated Virus Vector-Mediated Gene Transfer in Hemophilia B. *New England Journal of Medicine*. 2011; 365:2357–2365. [PubMed: 22149959]
4. Wimmer E, Mueller S, Tumpey TM, Taubenberger JK. Synthetic viruses: a new opportunity to understand and prevent viral disease. *Nature Biotechnology*. 2009; 27:1163–1172.
5. Mueller S, Coleman JR, Wimmer E. Putting Synthesis into Biology: A Viral View of Genetic Engineering through De Novo Gene and Genome Synthesis. *Chemistry & Biology*. 2009; 16:337–347. [PubMed: 19318214]
6. Wu Z, Asokan A, Samulski RJ. Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol Ther*. 2006; 14:316–327. [PubMed: 16824801]
7. Asokan A, Conway JC, Phillips JL, Li C, Hegge J, Sinnott R, Yadav S, DiPrimio N, Nam HJ, Agbandje-McKenna M, et al. Reengineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle. *Nat Biotechnol*. 2010; 28:79–82. [PubMed: 20037580]
8. Kim JW, Glasgow JN, Nakayama M, Ak F, Ugai H, Curiel DT. An adenovirus vector incorporating carbohydrate binding domains utilizes glycans for gene transfer. *PLoS One*. 2013; 8:e55533. [PubMed: 23383334]

9. Roberts DM, Nanda A, Havenga MJ, Abbink P, Lynch DM, Ewald BA, Liu J, Thorner AR, Swanson PE, Gorgone DA, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature*. 2006; 441:239–243. [PubMed: 16625206]
10. Ho ML, Adler BA, Torre ML, Silberg JJ, Suh J. SCHEMA Computational Design of Virus Capsid Chimeras: Calibrating How Genome Packaging, Protection, and Transduction Correlate with Calculated Structural Disruption. *Acs Synthetic Biology*. 2013; 2:724–733. [PubMed: 23899192]
11. Murakami M, Ugai H, Wang MH, Belousova N, Dent P, Fisher PB, Glasgow JN, Everts M, Curiel DT. An adenoviral vector expressing human adenovirus 5 and 3 fiber proteins for targeting heterogeneous cell populations. *Virology*. 2010; 407:196–205. [PubMed: 20828776]
12. Judd J, Ho ML, Tiwari A, Gomez EJ, Dempsey C, Van Vliet K, Igoshin OA, Silberg JJ, Agbandje-McKenna M, Suh J. Tunable protease-activatable virus nanonodes. *ACS Nano*. 2014
13. Ho ML, Judd J, Kuypers BE, Yamagami M, Wong FF, Suh J. Efficiency of protease-activatable virus nanonodes tuned through incorporation of stimulus-responsive capsid subunits. *Cellular and Molecular Bioengineering*. 2014 Special Issue on Young Innovators.
14. Halbert CL, Rutledge EA, Allen JM, Russell DW, Miller AD. Repeat transduction in the mouse lung by using adeno-associated virus vectors with different serotypes. *J Virol*. 2000; 74:1524–1532. [PubMed: 10627564]
15. Cronin J, Zhang XY, Reiser J. Altering the tropism of lentiviral vectors through pseudotyping. *Current Gene Therapy*. 2005; 5:387–398. [PubMed: 16101513]
16. Funke S, Maisner A, Muhlebach MD, Koehl U, Grez M, Cattaneo R, Cichutek K, Buchholz CJ. Targeted cell entry of lentiviral vectors. *Molecular Therapy*. 2008; 16:1427–1436. [PubMed: 18578012]
17. Li W, Asokan A, Wu Z, Van Dyke T, DiPrimio N, Johnson JS, Govindaswamy L, Agbandje-McKenna M, Leight S, Redmond DE Jr, et al. Engineering and selection of shuffled AAV genomes: a new strategy for producing targeted biological nanoparticles. *Mol Ther*. 2008; 16:1252–1260. [PubMed: 18500254]
18. Yang L, Jiang J, Drouin LM, Agbandje-McKenna M, Chen C, Qiao C, Pu D, Hu X, Wang DZ, Li J, et al. A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and in vivo selection. *Proc Natl Acad Sci U S A*. 2009; 106:3946–3951. [PubMed: 19234115]
19. Koerber JT, Klimczak R, Jang JH, Dalkara D, Flannery JG, Schaffer DV. Molecular evolution of adeno-associated virus for enhanced glial gene delivery. *Mol Ther*. 2009; 17:2088–2095. [PubMed: 19672246]
20. Grimm D, Lee JS, Wang L, Desai T, Akache B, Storm TA, Kay MA. In vitro and in vivo gene therapy vector evolution via multispecies interbreeding and retargeting of adeno-associated viruses. *J Virol*. 2008; 82:5887–5911. [PubMed: 18400866]
21. Gray SJ, Blake BL, Criswell HE, Nicolson SC, Samulski RJ, McCown TJ. Directed evolution of a novel adeno-associated virus (AAV) vector that crosses the seizure-compromised blood-brain barrier (BBB). *Mol Ther*. 2010; 18:570–578. [PubMed: 20040913]
22. Koerber JT, Jang JH, Schaffer DV. DNA shuffling of adeno-associated virus yields functionally diverse viral progeny. *Mol Ther*. 2008; 16:1703–1709. [PubMed: 18728640]
23. Powell SK, Kaloss MA, Pinkstaff A, McKee R, Burimski I, Pensiero M, Otto E, Stemmer WPC, Soong NW. Breeding of retroviruses by DNA shuffling for improved stability and processing yields. *Nature Biotechnology*. 2000; 18:1279–1282.
24. Stemmer WPC. Rapid Evolution of a Protein in-Vitro by DNA Shuffling. *Nature*. 1994; 370:389–391. [PubMed: 8047147]
25. Zhao HM, Giver L, Shao ZX, Affholter JA, Arnold FH. Molecular evolution by staggered extension process (StEP) in vitro recombination. *Nature Biotechnology*. 1998; 16:258–261.
26. Drummond DA, Silberg JJ, Meyer MM, Wilke CO, Arnold FH. On the conservative nature of intragenic recombination. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:5380–5385. [PubMed: 15809422]
27. Voigt CA, Martinez C, Wang ZG, Mayo SL, Arnold FH. Protein building blocks preserved by recombination. *Nature Structural Biology*. 2002; 9:553–558.

28. Meyer MM, Silberg JJ, Voigt CA, Endelman JB, Mayo SL, Wang ZG, Arnold FH. Library analysis of SCHEMA-guided protein recombination. *Protein Science*. 2003; 12:1686–1693. [PubMed: 12876318]
29. Wu P, Xiao W, Conlon T, Hughes J, Agbandje-McKenna M, Ferkol T, Flotte T, Muzyczka N. Mutational analysis of the adeno-associated virus type 2 (AAV2) capsid gene and construction of AAV2 vectors with altered tropism. *J Virol*. 2000; 74:8635–8647. [PubMed: 10954565]
30. Rabinowitz JE, Xiao W, Samulski RJ. Insertional mutagenesis of AAV2 capsid and the production of recombinant virus. *Virology*. 1999; 265:274–285. [PubMed: 10600599]
31. O'Donnell J, Taylor KA, Chapman MS. Adeno-associated virus-2 and its primary cellular receptor- Cryo-EM structure of a heparin complex. *Virology*. 2009; 385:434–443. [PubMed: 19144372]
32. Levy HC, Bowman VD, Govindasamy L, McKenna R, Nash K, Warrington K, Chen W, Muzyczka N, Yan X, Baker TS, et al. Heparin binding induces conformational changes in Adeno-associated virus serotype 2. *Journal of Structural Biology*. 2009; 165:146–156. [PubMed: 19121398]
33. Boucas J, Lux K, Huber A, Schievenbusch S, von Freyend MJ, Perabo L, Quadt-Humme S, Odenthal M, Hallek M, Buning H. Engineering adeno-associated virus serotype 2-based targeting vectors using a new insertion site-position 453-and single point mutations. *Journal of Gene Medicine*. 2009; 11:1103–1113. [PubMed: 19777441]
34. Coughlan L, Vallath S, Saha A, Flak M, McNeish IA, Vassaux G, Marshall JF, Hart IR, Thomas GJ. In Vivo Retargeting of Adenovirus Type 5 to alpha v beta 6 Integrin Results in Reduced Hepatotoxicity and Improved Tumor Uptake following Systemic Delivery. *Journal of Virology*. 2009; 83:6416–6428. [PubMed: 19369326]
35. Jullienne B, Vigant F, Muth E, Chaligne R, Bouquet C, Giraudier S, Perricaudet M, Benihoud K. Efficient delivery of angiostatin K1-5 into tumors following insertion of an NGR peptide into adenovirus capsid. *Gene Therapy*. 2009; 16:1405–1415. [PubMed: 19641530]
36. Seregin SS, Aldhamen YA, Appledorn DM, Hartman ZC, Schuldt NJ, Scott J, Godbehere S, Jiang HX, Frank MM, Amalfitano A. Adenovirus capsid-display of the retro-oriented human complement inhibitor DAF reduces Ad vector-triggered immune responses in vitro and in vivo. *Blood*. 2010; 116:1669–1677. [PubMed: 20511542]
37. Myhre S, Henning P, Friedman M, Stahl S, Lindholm L, Magnusson MK. Re-targeted adenovirus vectors with dual specificity; binding specificities conferred by two different Affibody molecules in the fiber. *Gene Therapy*. 2009; 16:252–261. [PubMed: 18946496]
38. Poulin KL, Lanthier RM, Smith AC, Christou C, Quiroz MR, Powell KL, O'Meara RW, Kothary R, Lorimer IA, Parks RJ. Retargeting of Adenovirus Vectors through Genetic Fusion of a Single-Chain or Single-Domain Antibody to Capsid Protein IX. *Journal of Virology*. 2010; 84:10074–10086. [PubMed: 20631131]
39. Warrington KH Jr, Gorbatyuk OS, Harrison JK, Opie SR, Zolotukhin S, Muzyczka N. Adeno-associated virus type 2 VP2 capsid protein is nonessential and can tolerate large peptide insertions at its N terminus. *J Virol*. 2004; 78:6595–6609. [PubMed: 15163751]
40. Munch RC, Janicki H, Volker I, Rasbach A, Hallek M, Buning H, Buchholz CJ. Displaying High-affinity Ligands on Adeno-associated Viral Vectors Enables Tumor Cell-specific and Safe Gene Transfer. *Molecular Therapy*. 2013; 21:109–118. [PubMed: 22968478]
41. Yang L, Bailey L, Baltimore D, Wang P. Targeting lentiviral vectors to specific cell types in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:11479–11484. [PubMed: 16864770]
42. Bratkovic T. Progress in phage display: evolution of the technique and its applications. *Cellular and Molecular Life Sciences*. 2010; 67:749–767. [PubMed: 20196239]
43. Bupp K, Roth MJ. Altering retroviral tropism using a random-display envelope library. *Molecular Therapy*. 2002; 5:329–335. [PubMed: 11863424]
44. Miura Y, Davydova J, Yamamoto M. Transductionally-Retargeted Infectivity Selective Oncolytic Adenovirus with Redesigned AB-Loop Targeting Cell Surface Mesothelin. *Molecular Therapy*. 2012; 20:S210–S211.

45. Nishimoto T, Yamamoto Y, Yoshida K, Goto N, Ohnami S, Aoki K. Development of Peritoneal Tumor-Targeting Vector by In Vivo Screening with a Random Peptide-Displaying Adenovirus Library. *Plos One*. 2012;7.
46. Muller OJ, Kaul F, Weitzman MD, Pasqualini R, Arap W, Kleinschmidt JA, Trepel M. Random peptide libraries displayed on adeno-associated virus to select for targeted gene therapy vectors. *Nat Biotechnol*. 2003; 21:1040–1046. [PubMed: 12897791]
47. Ying Y, Muller OJ, Goehring C, Leuchs B, Trepel M, Katus HA, Kleinschmidt JA. Heart-targeted adeno-associated viral vectors selected by in vivo biopanning of a random viral display peptide library. *Gene Ther*. 2010
48. Hida K, Won SY, Di Pasquale G, Hanes J, Chiorini JA, Ostermeier M. Sites in the AAV5 capsid tolerant to deletions and tandem duplications. *Arch Biochem Biophys*. 2010; 496:1–8. [PubMed: 20102698]
49. Judd J, Wei F, Nguyen PQ, Tartaglia LJ, Agbandje-McKenna M, Silberg JJ, Suh J. Random Insertion of mCherry Into VP3 Domain of Adeno-associated Virus Yields Fluorescent Capsids With no Loss of Infectivity. *Mol Ther Nucleic Acids*. 2012; 1:e54. [PubMed: 23629029]
50. Koerber JT, Schaffer DV. Transposon-based mutagenesis generates diverse adeno-associated viral libraries with novel gene delivery properties. *Methods Mol Biol*. 2008; 434:161–170. [PubMed: 18470644]
51. Maheshri N, Koerber JT, Kaspar BK, Schaffer DV. Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. *Nat Biotechnol*. 2006; 24:198–204. [PubMed: 16429148]
52. Perabo L, Endell J, King S, Lux K, Goldnau D, Hallek M, Buning H. Combinatorial engineering of a gene therapy vector: directed evolution of adeno-associated virus. *J Gene Med*. 2006; 8:155–162. [PubMed: 16285001]
53. Asuri P, Bartel MA, Vazin T, Jang JH, Wong TB, Schaffer DV. Directed Evolution of Adeno-associated Virus for Enhanced Gene Delivery and Gene Targeting in Human Pluripotent Stem Cells. *Molecular Therapy*. 2012; 20:329–338. [PubMed: 22108859]
54. Dalkara D, Byrne LC, Klimczak RR, Visel M, Yin L, Merigan WH, Flannery JG, Schaffer DV. In Vivo-Directed Evolution of a New Adeno-Associated Virus for Therapeutic Outer Retinal Gene Delivery from the Vitreous. *Science Translational Medicine*. 2013;5.
55. Pulicherla N, Shen S, Yadav S, Debbink K, Govindasamy L, Agbandje-McKenna M, Asokan A. Engineering Liver-detargeted AAV9 Vectors for Cardiac and Musculoskeletal Gene Transfer. *Molecular Therapy*. 2011; 19:1070–1078. [PubMed: 21364538]
56. Lesch HP, Kaikkonen MU, Pikkarainen JT, Yla-Herttuala S. Avidin-biotin technology in targeted therapy. *Expert Opinion on Drug Delivery*. 2010; 7:551–564. [PubMed: 20233034]
57. Arnold GS, Sasser AK, Stachler MD, Bartlett JS. Metabolic biotinylation provides a unique platform for the purification and targeting of multiple AAV vector serotypes. *Molecular Therapy*. 2006; 14:97–106. [PubMed: 16624620]
58. Morizono K, Xie YM, Helguera G, Daniels TR, Lane TF, Penichet ML, Chen ISY. A versatile targeting system with lentiviral vectors bearing the biotin-adaptor peptide. *Journal of Gene Medicine*. 2009; 11:655–663. [PubMed: 19455593]
59. Stachler MD, Chen I, Ting AY, Bartlett JS. Site-specific modification of AAV vector particles with biophysical probes and targeting ligands using biotin ligase. *Molecular Therapy*. 2008; 16:1467–1473. [PubMed: 18560418]
60. Liu YR, Fang Y, Zhou Y, Zandi E, Lee CL, Joo KI, Wang P. Site-Specific Modification of Adeno-Associated Viruses via a Genetically Engineered Aldehyde Tag. *Small*. 2013; 9:421–429. [PubMed: 23038676]
61. Wei F, McConnell KI, Yu TK, Suh J. Conjugation of paclitaxel on adeno-associated virus (AAV) nanoparticles for co-delivery of genes and drugs. *Eur J Pharm Sci*. 2012; 46:167–172. [PubMed: 22406091]
62. Wu W, Hsiao SC, Carrico ZM, Francis MB. Genome-Free Viral Capsids as Multivalent Carriers for Taxol Delivery. *Angewandte Chemie-International Edition*. 2009; 48:9493–9497.
63. Knop K, Hoogenboom R, Fischer D, Schubert US. Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angewandte Chemie-International Edition*. 2010; 49:6288–6308.

64. Steinmetz NF, Manchester M. PEGylated Viral Nanoparticles for Biomedicine: The Impact of PEG Chain Length on VNP Cell Interactions In Vitro and Ex Vivo. *Biomacromolecules*. 2009; 10:784–792. [PubMed: 19281149]
65. Lee GK, Maheshri N, Kaspar B, Schaffer DV. PEG conjugation moderately protects adeno-associated viral vectors against antibody neutralization. *Biotechnol Bioeng*. 2005; 92:24–34. [PubMed: 15937953]
66. Hofherr SE, Mok H, Gushiken FC, Lopez JA, Barry MA. Polyethylene glycol modification of adenovirus reduces platelet activation, endothelial cell activation, and thrombocytopenia. *Human Gene Therapy*. 2007; 18:837–848. [PubMed: 17767399]
67. Park JW, Mok H, Park TG. Physical adsorption of PEG grafted and blocked poly-L-lysine copolymers on adenovirus surface for enhanced gene transduction. *Journal of Controlled Release*. 2010; 142:238–244. [PubMed: 19913577]
68. Han JF, Zhao D, Zhong ZR, Zhang ZR, Gong T, Sun X. Combination of adenovirus and cross-linked low molecular weight PEI improves efficiency of gene transduction. *Nanotechnology*. 2010:21.
69. Vetter A, Viridi KS, Espenlaub S, Rodl W, Wagner E, Holm PS, Scheu C, Kreppel F, Spitzweg C, Ogris M. Adenoviral Vectors Coated with PAMAM Dendrimer Conjugates Allow CAR Independent Virus Uptake and Targeting to the EGF Receptor. *Molecular Pharmaceutics*. 2013; 10:606–618. [PubMed: 23281933]
70. Grunwald GK, Vetter A, Klutz K, Willhauck MJ, Schwenk N, Senekowitsch-Schmidtke R, Schwaiger M, Zach C, Wagner E, Goke B, et al. Systemic Image-Guided Liver Cancer Radiotherapy Using Dendrimer-Coated Adenovirus Encoding the Sodium Iodide Symporter as Theranostic Gene. *Journal of Nuclear Medicine*. 2013; 54:1450–1457. [PubMed: 23843567]
71. Joo KI, Fang Y, Liu YR, Xiao L, Gu Z, Tai A, Lee CL, Tang Y, Wang P. Enhanced Real-Time Monitoring of Adeno-Associated Virus Trafficking by Virus-Quantum Dot Conjugates. *ACS Nano*. 2011; 5:3523–3535. [PubMed: 21473596]
72. Huang XL, Bronstein LM, Retrum J, Dufort C, Tsvetkova I, Anagyei S, Stein B, Stucky G, McKenna B, Remmes N, et al. Self-assembled virus-like particles with magnetic cores. *Nano Letters*. 2007; 7:2407–2416. [PubMed: 17630812]

Figure 1. Synthetic virology aims to engineer viruses for gene delivery through the incorporation of natural or synthetic parts

Multiple facets of viruses may need to be enhanced or altered in order to become effective delivery agents for gene therapy applications. Depending on the biomedical application, desired functions of viral vectors may include cell targeting or co-delivery of small molecule drugs. Synthetic viruses can be created by mixing pre-existing viral parts, resulting in formation of chimeric or mosaic capsids. Molecular parts, such as biotin or small molecule drugs, can be attached to virus capsids to act as adaptors or to carry out therapeutic action, respectively. Synthetic parts, such as man-made polymers and inorganic nanoparticles, can be incorporated into viruses to endow functionalities new to viruses in general. Genetically encoded peptides and proteins can be inserted into viruses, either in rationally chosen sites or randomly throughout the capsid, to impart new functions. Finally, viral properties can be altered through introduction of point mutations scattered throughout the capsid or concentrated in specific capsid domains.