Advanced Drug Delivery Reviews 156 (2020) 214–235

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Viral nanoparticles for drug delivery, imaging, immunotherapy, and theranostic applications

Young Hun Chung [a,1], Hui Cai [b,1], Nicole F. Steinmetz [a,b,c,d,e,*]

[a] Department of Bioengineering, University of California-San Diego, La Jolla, CA 92093, United States
[b] Department of NanoEngineering, University of California-San Diego, La Jolla, CA 92093, United States
[c] Department of Radiology, University of California-San Diego, La Jolla, CA 92093, United States
[d] Moores Cancer Center, University of California-San Diego, La Jolla, CA 92093, United States
[e] Center for Nano-ImmunoEngineering, University of California-San Diego, La Jolla, CA 92093, United States

ARTICLE INFO

Article history:
Received 4 May 2020
Received in revised form 19 June 2020
Accepted 21 June 2020
Available online 27 June 2020

Keywords:
Plant viruses
Bacteriophages
Drug delivery systems
Chemotherapy
Vaccine
Cancer
Infectious disease
Contrast agents
Theranostics

ABSTRACT

Viral nanoparticles (VNPs) encompass a diverse array of naturally occurring nanomaterials derived from plant viruses, bacteriophages, and mammalian viruses. The application and development of VNPs and their genome-free versions, the virus-like particles (VLPs), for nanomedicine is a rapidly growing. VLPs can encapsulate a wide range of active ingredients as well as be genetically or chemically conjugated to targeting ligands to achieve tissue specificity. VLPs are manufactured through scalable fermentation or molecular farming, and the materials are biocompatible and biodegradable. These properties have led to a wide range of applications, including cancer therapies, immunotherapies, vaccines, antimicrobial therapies, cardiovascular therapies, gene therapies, as well as imaging and theranostics. The use of VLPs as drug delivery agents is evolving, and sufficient research must continuously be undertaken to translate these therapies to the clinic. This review highlights some of the novel research efforts currently underway in the VNP drug delivery field in achieving this greater goal.

Contents

Abbreviations: VLP, virus-like particle; CPMV, cowpea mosaic virus; CCMV, cowpea chlorotic mottle virus; TMV, tobacco mosaic virus; PVX, potato virus X; RCNMV, red clover necrotic mosaic virus; PapMV, papaya mosaic virus; HBc, hepatitis B core; HPV, human papillomavirus; HIV, human immunodeficiency virus; TVEC, talimogene laherparepvec; GFP, green fluorescent protein; DC, dendritic cell; APC, antigen presenting cell; CTL, cytotoxic T-cell; NAbs, neutralizing antibodies; TLR, toll-like receptor; TAA, tumor-associated antigens; TACA, tumor-associated carbohydrate antigens; CD, cardiovascular disease; CT, computed tomography; MRI, magnetic resonance imaging; PET, position emission tomography; NIR, near-infrared; PTT, photothermal therapy.

* Corresponding author at: Department of Bioengineering, University of California-San Diego, La Jolla, CA 92093, United States.
E-mail address: nsteinmetz@ucsd.edu (N.F. Steinmetz).

[1] These authors contributed equally

https://doi.org/10.1016/j.addr.2020.06.024
0169-409X/

1. Introduction

1.1. Viral nanocarrier platforms

Over the past decades, the progress of nanotechnology has opened up new avenues for medical science, especially in the field of drug delivery. Traditional drug carriers have been developed based on liposomes/lipids [1], polymers of synthetic and natural origin [2], and inorganic nanoparticles [3]. To make a clinical impact, nanoparticles for drug delivery must be biocompatible and biodegradable with minimal toxicity. However, some synthetic carriers are restricted by toxicity and/or low delivery efficiency necessitating the development and application of alternate systems [4]. There is no "perfect solution", thus there is a need to fuel the development pipeline with novel drug delivery systems. An emerging class of novel drug carriers are protein-based nanoparticles such as protein cages and viruses [5]. Protein cages are self-assembled supramolecular structures arranged from their individual protein monomers. Their subunits are not viral by nature. VLPs are similar, but are assembled specifically from the coat proteins of the native virus. Viruses are stable structures that can withstand environmental pressures and escape degradation, but at the same time are sensitized to detect signals in the cellular environment thereby releasing their genome when instructed. Within the class of viruses, there are two subsets: virus-like particles (VLPs) and viral nanoparticles (VNPs). VLPs are the genome-free versions of their VNP counterparts and are considered noninfectious. They may also impart different immunostimulatory profiles due to the presence/absence of the viral genomes [6]. In our review, we mention VLP and VNP plant viruses and bacteriophages; we mention application of some protein cages in some examples and also highlight a few mammalian virus nanotechnologies. Virus capsids are formed through the self-assembly of repeating protein subunits, therefore providing a high degree of multivalency. Plant viruses in general are non-enveloped structures and they can assume a spherical/icosahedral or filamentous/tubular shape (Fig. 1). Viruses are regarded as naturally occurring nucleic acid carriers as they protect and carry their cargo, and that is the main property exploited for drug delivery [7]. Drug cargo can be infused, encapsulated, absorbed, or conjugated to the interior and exterior surfaces of the coat protein interfaces using a combination of chemistries and through attachment to various functional groups offered by the protein structure [7]. This flexibility offers a variety of possibilities including reversible binding of active molecules, protection within proteinaceous matrices, as well as specific targeting to the site of action.

Natural delivery carriers, particularly VNP-based carriers, offer some unique advantages due to their morphological uniformity, biocompatibility, water solubility, easy functionalization and high uptake efficiency [8]. Further, ideal nanomedical approaches for drug delivery or imaging aim to utilize biological behaviors to develop smart nanosized cages with high stability, appropriate pharmacokinetics, cell-targeting, and efficient cell penetrability. Plant viruses generally do not exhibit tissue tropisms, therefore these properties can be engineered as an added function to give the nanoparticle formulation, whether of synthetic or natural origin, properties such as cell surface receptor binding, targeting abilities, membrane crossing, and nuclear penetration [9].

While there is no clinically approved plant or bacteriophage-based nanomedicine, several are undergoing preclinical development while some systems are poised to enter translational development. The plant VLP and VNP-based nanotechnology platforms undergoing development for various nanomedical applications are tobacco mosaic virus (TMV), cowpea mosaic virus (CPMV), cowpea chlorotic mottle virus (CCMV), physalis mottle virus (PhMV), and potato virus X (PVX) (not figured) amongst others. Notable are also the following bacteriophages: MS2, P22, Qβ and M13 (not figured). These viruses range in sizes from ~30 nm to over a micron and can be found in a variety of distinctive shapes (Fig. 1). With advances in biochemistry and directed evolution techniques, continuous progress has been made in the development of viral nanocarriers for drug delivery, imaging, and theranostic applications. In this review, we focus on the use of VNPs and VLPs as therapeutics and diagnostics in biomedical applications including antimicrobial, cancer, protein/peptide, and gene therapies; monotherapy and combination therapies against cancer; vaccines against infectious diseases, cancer and other diseases; nanocarriers for imaging modalities; and theranostics with photothermal therapy (PTT).

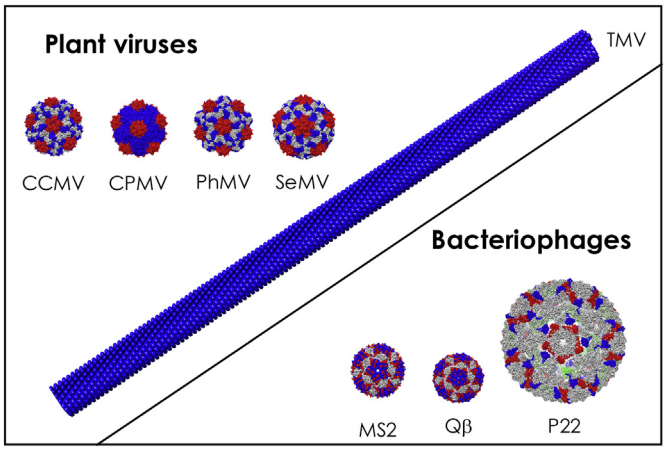

Fig. 1. Different plant VLPs and bacteriophages to scale. CCMV (PDB ID: 1ZA7), CPMV (PDB ID: 1NY7), PhMV (PDB ID: 1QJZ), SeMV (PDB ID: 1X33), TMV (PDB ID: 2TMV), MS2 (1AQ3), Qβ (PDB ID: 5KIP), and P22 (PDB ID: 5UU5) and images were reconstructed using UCSF Chimera software.

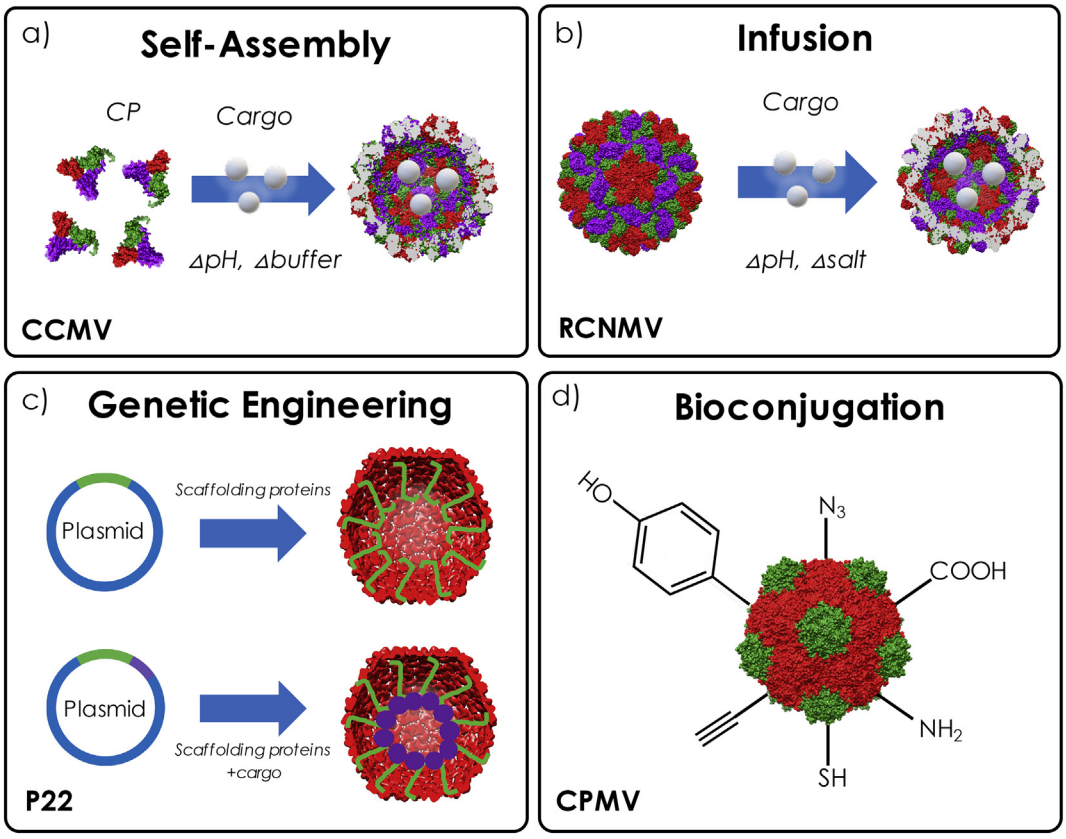

Fig. 2. Strategies to carry cargo using VLPs include: a) self-assembly around cargo by altering pH and buffer conditions with CCMV, b) infusion of cargo within RCNMV due to changes in pH and salt concentrations, c) genetic engineering techniques utilizing genetically conjugated scaffolding proteins to encapsulate drugs within P22, d) bioconjugation onto CPMV using exterior surface-exposed residues.

2. Delivery of drug cargo with VLPs

2.1. Encapsulation and conjugation techniques for VLPs

While their natural cargo is nucleic acids, VLPs are flexible and versatile tools and amenable for incorporating and delivering a wide range of small or large molecule cargo, both of biologic (e.g. nucleic acids and proteins) and synthetic origin (e.g. chemotherapy). Different strategies have been developed to carry the payloads either inside or outside the capsid (Fig. 2). Viruses generally package their genetic material inside the capsid through a simple supramolecular self-assembly process, and the principles governing the packaging can be used to load functional payloads within the viruses. VLPs that have been programmed to encapsulate cargo during reassembly include CCMV, brome mosaic virus, red clover necrotic mosaic virus (RCNMV), and Hibiscus chlorotic virus (reviewed in [8]). Changing the buffer conditions, pH, and ionic strength *in vitro* disassembles the capsid and releases the viral genome. Following disassembly, viral capsids are reassembled to encapsulate the desired cargo using buffer exchange methods (Fig. 2a) [10,11]. For instance, at physiological pH or high ionic strength (I ~ 1M), CCMV disassembles releasing its inner RNA; changing the buffer to a pH between 3 and 6 with low ionic strength (I ~0.1M) causes reassembly of the capsid [12]. Using this method, our group has demonstrated CCMV encapsulation of CpG oligodeoxynucleotides (ODNs) for use in cancer immunotherapy [13]. Reassembly can also be triggered by mixing the cargo with coat proteins above the critical assembly concentration of the VLPs in a similar fashion to the formation of micelles [14]. The successful encapsulation of cargo depends on the size and surface charge of the cargo, electrostatic interactions, hydrophobicity/hydrophilicity, and other unique binding interactions that occur during nanoassembly [15–17]. In some cases, the reassembly is based on electrostatic interactions between the positively charged interior surfaces of the capsid proteins and a negatively charged cargo that mimics the negatively charged nucleic acids [18]. Encapsulation of positively charged payloads can be accomplished by mixing with another negatively charged molecule providing sufficient net negative charge and thereby catalyzing capsid assembly [19].

While often depicted as solid nanoparticles (e.g. Fig. 1), one should keep in mind that many VLPs are porous and the materials "breeze" in that they change conformation under various bathing conditions allowing the infusion of small molecules into the capsid's cavity (Fig. 2b). Lommel et al. demonstrated this property with RCNMV by adding chelators to remove calcium and magnesium ions from the solvent leading to the formation of 11–13 Å-diameter channels; the re-addition of the ions closes the formed channels [20]. Building off of this work, RCNMV was conjugated to a CD46-targeting peptide and loaded with doxorubicin (DOX) through passive diffusion of DOX through the formed channels during the capsid's "open" conformation [21]. The targeted and DOX-loaded RCNMV nanoparticles showed significant cytotoxicity *in vitro* in HeLa cells. Another example is CCMV; changes in pH and salt concentration can affect CCMV capsid stability. Careful adjustment of buffer conditions enables CCMV production in the open and expanded conformation forming pores of ~2 nm allowing for cargo loading [22]. Reversal to the closed and condensed conformation traps the cargo inside in a mechanism referred to as gating [23].

In addition to passive or non-specific encapsulation, cargo can be tethered and stabilized to the interior of the VLPs making use of biospecific interactions. For example, Peabody et al. encapsulated cargo into MS2 VLPs by utilizing RNA stem loops fused to the desired cargo. These RNA stem loops are derived from the MS2 genome and bind to

the interior of the capsid proteins with high affinity using electrostatic interactions. During infection, these interactions drive assembly and viral genome packing [24]. Additionally, the RNA stem loops can be used to guide cargo to the interior of the MS2 capsids. MS2 particles were packaged with quantum dots, chemotherapeutics including doxorubicin (DOX), cisplatin, and 5-fluorouracil (5-FU), as well as ricin toxin A-chains by conjugating the molecules to these RNA stem loops. In another example by Finn et al., a positively charged Rev peptide, which binds to α-Rev aptamers within the Qβ RNA sequence, was genetically conjugated to the prodrug-modifying enzymes cytosine deaminase and purine-nucleoside phosphorylase. Encapsulation of these enzymes was accomplished via noncovalent attachment of Qβ aptamer sequences within the RNA to the Qβ coat proteins and the Rev peptides on the enzymes [25].

Genetically enabled directed loading during *in vivo* assembly is another technique for cargo encapsulation into VLPs. The cargo and capsid protein can be expressed simultaneously or separately by heterogeneous expression systems followed by self-assembly *in vivo* or *in vitro* (Fig. 2c). Genetic techniques will need to be utilized to insert the cargo-encoding genetic sequences into the genes encoding the capsid protein [26]. Douglas et al. employed this technique using scaffolding proteins that help direct the assembly of P22 procapsids with their helix-turn-helix domains within the C-terminus [27]. The scaffolding proteins were genetically fused to streptavidin, which serves as a molecular linker to entrap biotin-labeled cargo. The technique was also used to display biotinylated green fluorescent protein (GFP) on the surface of the P22 procapsid by tethering the scaffold proteins to the interior streptavidin. While the GFP was too large to traverse the viral capsid, the scaffolding proteins could penetrate through thus allowing for exterior functionalization. Genetically enabled loading is particularly powerful, as it improves the quantitative control of the introduced cargos leading to increased homogeneity between samples. The aforementioned methods are not an exhaustive list of all the methods utilized to load cargo into VLPs and other techniques may be applied.

Besides encapsulation techniques, VLPs can also deliver payloads through covalent attachments making use of reactive amino acid side chains of the protein structure. Functional addressable groups are principally amines (Lys), carboxylates (Asp, Glu), thiols (Cys), and aromatic groups (Tyr, Trp), all of which can be utilized for bioconjugation chemistries (Fig. 2d) [28,29]. Some powerful chemical reactions used for bioconjugation on these moieties include NHS esters, carbodiimides, maleimides, and click chemistries respectively. Moreover, residues such as lysines can be site-specifically engineered into the capsid protein to provide non-native reactive sites [30,31].

To increase drug loading capacity, structures of higher order have been introduced. For instance, layer-by-layer techniques to deliver biomolecules has been widely accomplished with additional compartmentalization even allowing for multiple-drug delivery [32]. Hierarchical assembly using virus capsids on DNA origami templates has also been exploited [30].

2.2. Delivery of small molecule drugs for cancer therapy

Currently, chemotherapy remains the first choice for cancer treatments in the clinic [31]. However, high resistance and recurrence rates, together with rapid drug clearance and non-targeted administration, require the use of maximum-tolerated drug dose in cancer therapy, which may result in high-grade toxicities and limited clinical applicability [33]. Drug delivery systems that can provide targeted and intracellular delivery, promote active drug accumulation in tumor tissues, and minimize required doses would ameliorate these issues and therefore improve therapeutic outcomes.

VLPs possess numerous features that make them highly suitable for targeted delivery of therapeutic agents. In an early study, MS2 VLPs were modified with a targeting peptide (SP94) and were used to selectively deliver chemotherapeutic drugs (DOX, cisplatin and 5-FU) to human hepatocellular carcinoma cells (HCC) [24]. These modified VLPs exhibited high avidity and specificity towards HCC with minimal uptake in healthy cells and induced selective cytotoxicity *in vitro* even at very low doses. For instance, the DOX-loaded VLPs killed HCC cells at IC_{50} values of 10–15 nM, 20 times better than free DOX.

In fact, various VNP and VLP systems have been utilized for DOX delivery. The loading and release mechanisms of DOX were extensively studied using the aforementioned RCNMV in which the breezing properties of RCNMV were used to encapsulate DOX [34]. Additionally, DOX was electrostatically bound to the exterior of the viral capsid using both phosphate and Tris buffer with the phosphate buffer increasing levels of surface-bound DOX (Fig. 3a). Different ratios of encapsulated and conjugated DOX tuned the release kinetics of the chemotherapeutic. The RCNMV particles demonstrated Fickian diffusion kinetics attributed to the rapid release of the exterior DOX followed by the slower release of the encapsulated DOX. Qβ VLPs have additionally been investigated with DOX for cancer cell killing. DOX was conjugated using a photocleavable nitroveratryl linker to the exterior of Qβ VLPs that had been modified via a dibromomaleimide chemistry [35]. Light exposure for 15 min caused dose-dependent cytotoxic killing in MCF-7 cells *in vitro*.

To study the impact of nanoparticle morphology on drug delivery, Francis et al. loaded DOX on three morphologically distinct VLPs: MS2, TMV disks (Fig. 3b), and nanophage filamentous rods [36]. MS2 is spherical in shape with a diameter around 27 nm, the TMV disks are flat and round measuring 18x5 nm, and the nanophages are short filaments measuring 50x6 nm. Increased survival rates were only observed in intracranial U87-Luc glioblastoma-bearing mice that were treated via convection-enhanced delivery using the TMV disks and MS2 VLPs, with the TMV disk-treated mice showing the greatest efficacy. The nanophage filamentous rods showed no efficacy compared to free DOX and phosphate buffered saline controls indicating that the carrier itself can also affect drug delivery. Other studies have demonstrated that high aspect ratio materials tend to improve drug delivery – these materials marginate more towards the vessel walls increasing the probability of binding to diseased areas, retain and accumulate in tumor tissue, and transport better through membranes and tissues compared to their spherical counterparts [37]. A review by Muzykantov et al., discusses how nanoparticle shape plays distinct roles in areas of adhesion and biodistribution, cell internalization, and drug loading and release [38]. Several plant viruses form filamentous and tubular structures and therefore make intriguing platforms for drug delivery. Our group has used 300x18 nm-sized nanotubes of TMV [39] as well as 515x13 nm filamentous PVX [40] (Fig. 3c) to deliver DOX.

In the treatment of athymic mice bearing human MDA-MB-231 breast cancer xenografts injected subcutaneously (s.c.) into the right flank, DOX loaded onto PVX through simple adsorption to the exterior surface significantly reduced tumor growth showing superior efficacy over free DOX. Similar observations were made using albumin-coated TMV loaded with DOX [41]. It should be noted that in this example, VNPs (rather than VLPs) of TMV and PVX were used. The reason for the use of VNPs is that the RNA-cargo acts as a ruler defining the length of the nucleoprotein complex. Therefore, while TMV VNPs measure 300x18 nm; VLPs thereof are variable in length [42]. The same is true for other filamentous viral nanoparticles such as PVX. Therefore, from here on out any instances of TMV and PVX will be utilizing the VNP form.

Although not the focus of this review, it is noteworthy to mention that mammalian viruses have also been repurposed to deliver DOX. Some examples include the influenza virus, foot-and-mouth disease virus, the hepatitis B core protein (HBc), and the VLP of *Macrobrachium rosenbergii nodavirus* [43–46].

To increase drug loading potential, polymerization chemistries can be used to increase the functional handles and thus drug loading. An example of this technique has been illustrated using Qβ formulated as a

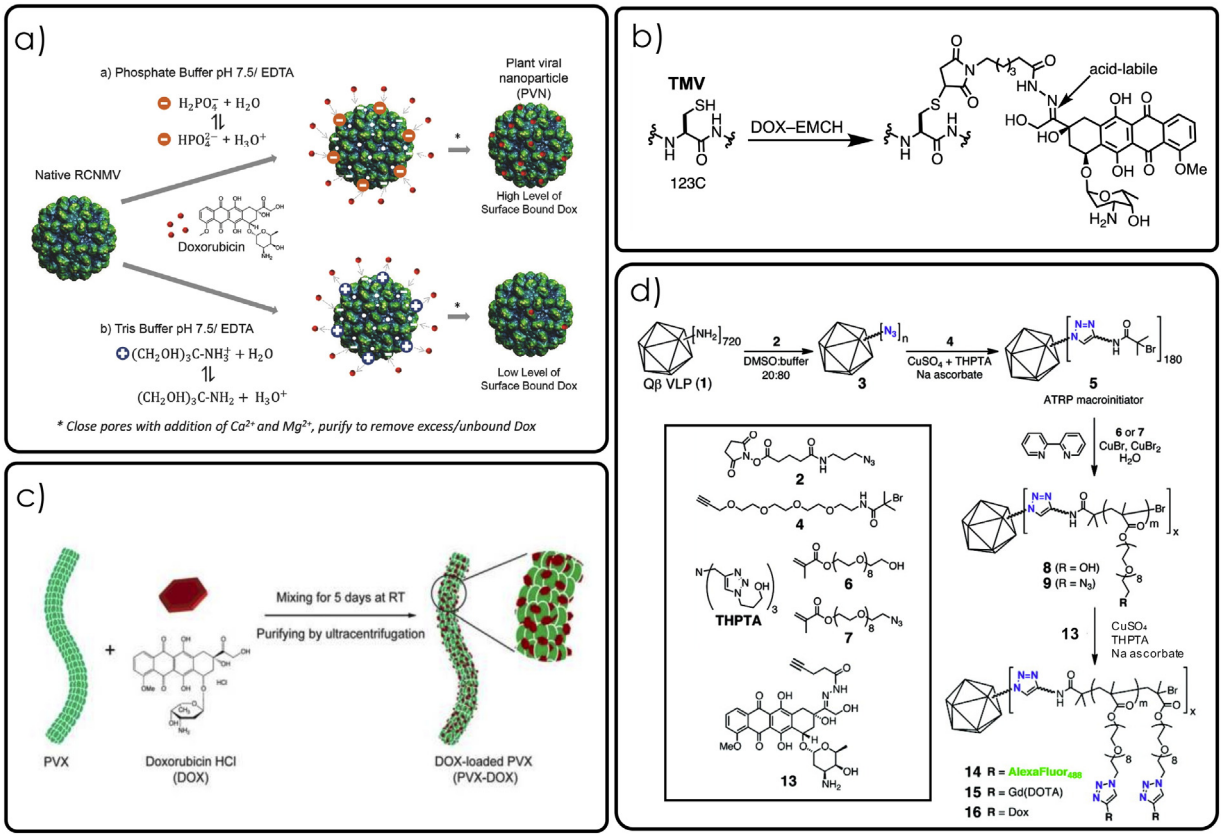

Fig. 3. Various VLPs and their incorporation of DOX through infusion, bioconjugation, adsorption, and using polymerization chemistries a) Infusion and surface binding of DOX onto RCNMV VLPs in different buffers to study the binding and release characteristics of DOX from the nanoparticles (reproduced with permission from ref [34]) b) Bioconjugation of DOX-*N*-ε-maleimidocaproic acid hydrazides onto TMV disk VLPs through maleimide linkages to the cysteine residues (reproduced with permission from ref [36]) c) Adsorption of DOX onto the exterior surface of PVX (reproduced with permission from ref [40]) d) Copper-catalyzed azide-alkyne cycloaddition of DOX onto OEGMA-N_3-polymerized Qβ (reproduced with permission from ref [47]).

polymer-protein hybrid nanomaterial [47]. Qβ was polymerized with an azido-functionalized oligo(ethylene glycol)-methacrylate (OEGMA-N_3) via atom transfer radical polymerization (ATRP), which was then functionalized to DOX through copper-catalyzed azide-alkyne cycloaddition (Fig. 3d). The DOX-Qβ particles showed comparable cytotoxicity to free DOX in HeLa cells *in vitro*.

Plant virus-based nanoparticles have been widely used for targeted delivery of platinum-based anticancer drugs, which are used in the treatment of nearly 50% of cancer patients undergoing chemotherapy [48]. TMV makes an intriguing platform for efficient delivery of platinum-based drugs such as cisplatin [49] and drug candidate phenanthriplatin (phenPt) [50] as they can be efficiently loaded into the TMV cavity via a charge-driven reaction or by forming stable covalent adducts (Fig. 4a,b). TMV-cisplatin VNP complexes were efficiently taken up by cancer cells and exhibited superior cytotoxicity compared to free cisplatin [49]. For targeted drug delivery, cisplatin-loaded TMV VNPs were modified with mannose and lactose moieties on the exterior surface of the TMV [51]. Through specific recognition between mannose and galectin and lactose and the asialoglycoprotein receptor on the cell membranes, the targeted and drug-loaded TMV showed increased cytotoxicity in MCF-7 and HepG2 cancer cell lines. In a more recent study by the same group, the coat protein of TMV was modified with a small molecular fluorous ponytail at specific sites precipitating self-assembly into spherical nanoparticles (Fig. 4c) [52]. Cisplatin was loaded through metal-ligand coordination into this spherical assembly with high stability.

Other examples of plant VNP drug delivery applications include the functionalization of TMV and CPMV with mitoxantrone (MTO). MTO is a topoisomerase II inhibitor with potent activity against most cancers, but like most anti-neoplastic agents, MTO produces severe cardiac effects. We encapsulated MTO into TMV VNPs by a charge-driven strategy and demonstrated superior efficacy with the MTO-TMVs compared to controls in a s.c.-injected MDA-MB-231 triple negative breast cancer (TNBC) nude mouse model [53]. The MTO-TMV was injected intratumorally (i.t.) when the tumors reached 100 mm^3. We also loaded MTO into the interior cavity of CPMV through passive diffusion and found that MTO-CPMV showed enhanced cytotoxic effects *in vitro* in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [54]. Lastly, the antimitotic drug valine-citrulline monomethyl auristatin E (vcMMAE) was loaded onto the exterior surface of TMV VNPs to target non-Hodgkin's lymphoma. Particle uptake studies confirmed internalization into endolysosomal components with likely protease-mediated drug release. *In vitro* studies confirmed cytotoxicity toward the non-Hodgkin's lymphoma cell line, Karpas 299, exhibiting an IC_{50} of ~250 nM [55].

2.3. Delivery of small molecule drugs for anti-microbial therapy

Bacterial populations continue to evolve and escape current antibacterial treatments requiring improved antimicrobial approaches [56]. One therapeutic option is the use of drug delivery approaches, and VLPs have been engineered toward that goal. Bacteriophages, especially, can be designed to specifically target microbes making use of phage display techniques to select microbe-specific peptide binders [57]. Benhar et al. utilized phage display on filamentous fd phages to isolate and then conjugate and display peptides specific towards the gram-positive bacterium *Staphylococcus aureus;* co-delivery of the

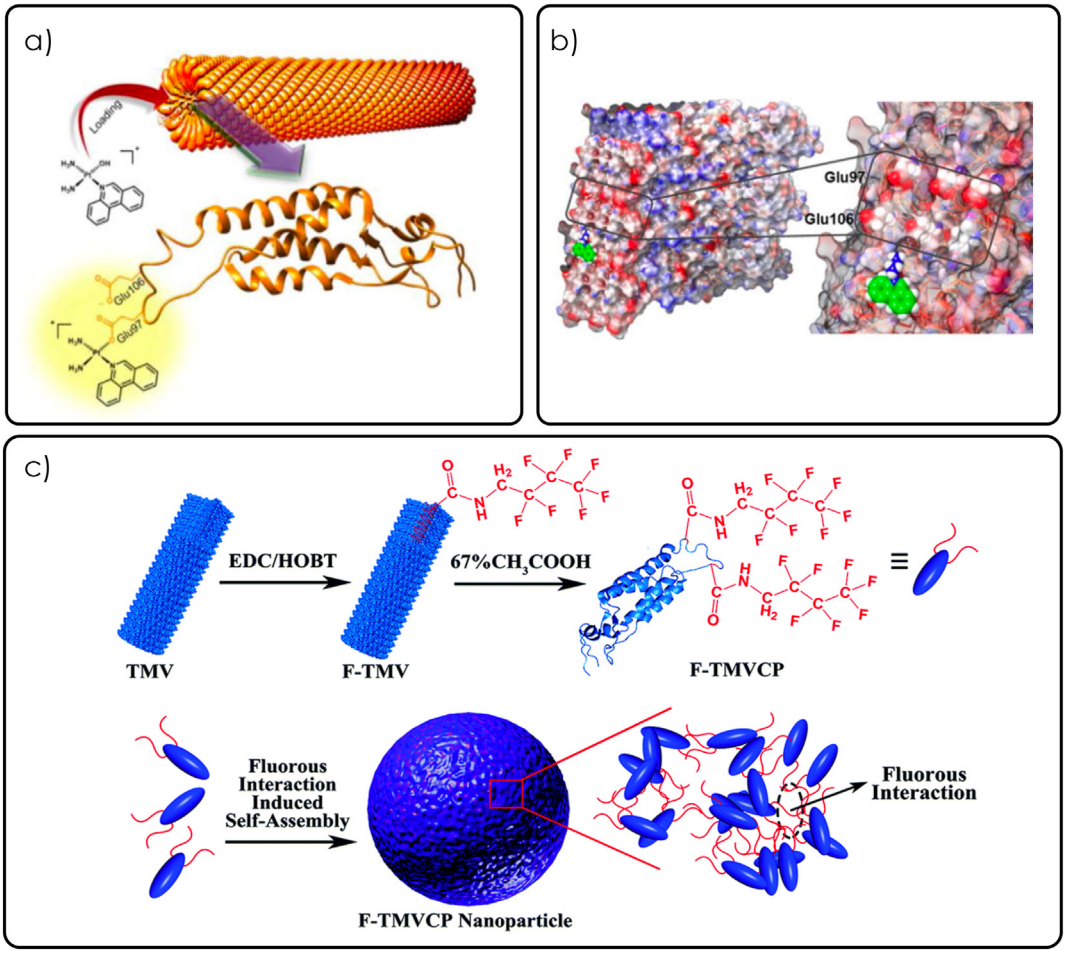

Fig. 4. Different mechanisms of loading platinum-based drugs such as phenPt and cisplatin into TMV a) Graphical abstract from Lippard, S.J., et al. showing the loading schematic for phenPt loading into wild-type TMV particles (reproduced with permission from ref [50]) b) Graphical illustration of the phenPt docking onto the Glu97 and Glu106 residues of TMV as discovered through matrix-assisted laser desorption/ionization – mass spectrometry and nuclear magnetic resonance spectroscopy c) Graphical schematic of the assembly of TMV spherical nanoparticles using a fluorous ponytail interaction (F-TMVCP). Cisplatin was loaded into the F-TMVCP using the same glutamic residues as in b (not shown) (reproduced with permission from ref [52]).

antibiotic, chloramphenicol, enabled targeted drug delivery [57]. The chloramphenicol-conjugated fd phages retarded bacterial growth by a factor of 20 compared to chloramphenicol alone. Recently, Finn et al. demonstrated the conjugation of variants of macrolide antibiotics (e.g. azithromycin, clarithromycin) onto Qβ particles for the potential treatment of microbes such as *Mycobacterium tuberculosis* and *Legionella pneumophilia* that reside in pulmonary macrophages [58]. The VLPs were assessed for uptake both *in vitro* in RAW264.7 macrophages as well as *in vivo* in lung tissue from mice and were found to traffic into the RAW264.7 cells as well as to the lungs significantly greater than the negative control tolyl-labeled Qβ particles. As of yet, no efficacy studies have been reported.

2.4. Delivery of genes and nucleic acids

Viruses are natural vehicles for delivery of genes. Because VLPs are devoid of their own genome, they can encapsulate nucleic acids easily and therefore have been broadly used for the delivery of genes as well as therapeutic nucleic acids. In fact, about 67% of gene therapy clinical trials world-wide are using viruses as vectors to transport genetic material [59]. However, traditional gene delivery strategies are based on mammalian viral vectors, and the application of plant viruses and phages is a newer iteration. While some reports indicate that cell targeting or cell penetrating peptides are not required to achieve gene delivery with plant VLPs as they are proteinaceous in nature imparting better cell penetration and endolysosomal escape than some synthetic nanoparticle systems [13], a common strategy to enhance the delivery efficiency is the display of peptides that improve cell uptake and trafficking [60]. Plant viruses and phages did not evolve to circumvent mammalian cellular compartments and therefore to some degree become trapped within intracellular vesicles.

To engineer cell trafficking properties, bacteriophages such as PP7 and MS2 have been modified with cell penetrating ligands such as the transacting activation transduction (TAT) peptide for microRNA delivery [61,62]. The TAT peptide has also been used by Niu et al., who conjugated the TAT peptide onto the exterior surface of TMV [63]. This engineered TMV-TAT system exhibited enhanced internalization, acquired endo/lysosomal escape capacity, and were successfully used to intravenously (i.v.) and i.t. deliver GFP silencing RNA (siRNA) into GFP-expressing hepatocellular carcinoma tumors (MHCC97-H/GFP) *in vivo* in Balb/c-nude mice. To aid VLPs in crossing the blood-brain barrier and targeting malignant brain tumors, RNAi loaded Qβ VLPs were modified with cell-penetrating peptides and apolipoprotein E (ApoE) [64]. The engineered Qβ VLPs delivered i.v. acted synergistically with temozolomide to eradicate intracranial U87 brain tumors in nude mice. Our group has used CCMV VLPs carrying a cell penetrating peptide (M-lycotoxin peptide L17E) to deliver siRNA to mammalian cells [65].

Plant VLPs have also been used for the delivery of mRNA. In one study, CCMV was examined for the delivery of heterologous mRNA encoding enhanced yellow fluorescent protein (EYFP) to mammalian

BHK-21 cells. It was shown that the RNA cargo was stabilized through encapsulation into the plant viral capsid and when cells were transfected using lipofectamine, the nucleic acid was successfully delivered and released from the VLPs into the cytoplasm of the BHK-21 cells facilitating EYFP expression [66]. In clinical settings, mRNA delivery for vaccination purposes can be ineffective due to low antigen production. To overcome this predicament, CCMV has been demonstrated to successfully deliver replicons enabling amplification and expression of model antigens containing a variety of reporter genes [67]. Preliminary *in vitro* studies show that compared to controls of naked mRNA, naked replicon mRNA, and VLPs with non-replicon mRNA, the VLPs with replicon mRNA produced one to two orders of magnitude greater activation. In another example, TMV was utilized to deliver mRNA for possible vaccine development; proof-of-concept was demonstrated by encapsulating mRNA encoding GFP into TMV coat proteins. The TMV vector was administered s.c. into Balb/c mice and immune responses against the target gene, which in this case was GFP, were demonstrated [68].

Most VLPs are immunogenic and are taken up efficiently by immune cells, such as macrophages and dendritic cells (DCs). This function can be utilized to deliver therapeutic nucleic acids to innate immune cells in the treatment of cancer. Toward this goal, Bachmann et al. have showcased CpG oligodeoxynucleotide (ODN) loading into Qβ, HBc antigen (HBcAg), and liposomes [69]. CpG ODNs are ligands of the toll-like receptor 9 (TLR9), and when activated, TLR9 has the capability to activate macrophages. The CpG loaded Qβ particles were able to induce greater cytotoxic T lymphocyte responses than CpG alone. Liposomes are one of the most commonly studied nanoparticles in clinical trials, but the HBcAg VLPs induced greater responses and outperformed dose-matched liposomes with encapsulated CpG. The HbcAg particles (100 μg dose) were able to completely eradicate s.c. administered fibrosarcoma tumors (MC57G-GP) in 13 of 18 C57BL/6 mice. The aforementioned Qβ formulation administered concurrently with pembrolizumab has advanced into clinical trials in immunotherapy-resistant patients with advanced melanomas [70]. Similarly, we used the dis- and reassembly of CCMV, as mentioned in Section 2.1, to encapsulate CpG ODNs. The CpG loaded CCMV VLPs showed significantly enhanced uptake by tumor associated macrophages (TAM), but not by cancer cells. When delivered i.t., the CpG-loaded CCMV VLPs significantly inhibited the growth of solid CT26 colon cancer and B16F10 melanoma tumors in Balb/c mice through the activation of these macrophages [13].

2.5. Delivery of peptides and protein drugs

Delivery of polypeptides and proteins requires different strategies as they can be more difficult to encapsulate. One strategy is chemical or genetic fusion of the peptide/protein to a targeting agent or capsid component that directs localization of the cargo to the VLP interior during capsid synthesis and assembly *in vivo* [71]. Spontaneous self-assembly of the viral capsid protein around the peptide/protein *in vitro* is also feasible with or without a targeting element [72]. These strategies were discussed in Section 2.1.

In an early study, Tullman-Ercek et al. reported two improved methods for the encapsulation of enzymes inside MS2. First, DNA stem loops initiated reassembly of the viral coat proteins similarly to the work by Peabody et al., who instead used RNA stem loops to encapsulate cargo into MS2 capsids [24,73]. Second, the protein cargo was expressed with a genetically encoded negatively charged peptide tag [73]. Black et al. further demonstrated packaging of protein and DNA together within phage T4 [74]. The T4 phage was genetically engineered to encapsulate Cre together with linearized double-stranded DNA (encoding a gene of interest), which is packaged within the procapsid via an ATP-driven terminase motor protein (gp17). The encapsulated Cre re-circularizes the DNA within the T4 and subsequent delivery to lung cancer epithelial A549 cells *in vitro* leads to expression of the gene of interest. The aforementioned study encapsulated DNA and protein, but at separate stages during the assembly process. A self-sorting and self-assembling system based on murine polyomavirus (MPyV) was developed by Sainsbury et al. with the capability of programming *in vitro* co-encapsidation of multiple guest proteins simultaneously [75]. Simultaneous co-encapsidation allows for precise control of the stoichiometric ratios between the proteins and may translate better for therapies requiring multiple components. MPyV capsomeres, which form the VLP capsids, were genetically conjugated to GFP and mRuby3 and isolated and self-assembled *in vitro*. Co-encapsidation was verified through Förster resonance energy transfer of GFP to the mRuby3.

A lot of recent work has been accomplished with encapsulating drug-activating enzymes within VLPs for therapeutic purposes [76]. Enzymes within the cytochrome P450 family are responsible for catalyzing chemotherapeutic pro-drugs into their active forms, and encapsulating these enzymes within VLPs can reduce side effects, increase targeting to the tumor site, and boost retention within tumors via the enhanced permeability and retention effect [77,78]. Initial results show that a bacterial cytochrome, CYP_{BM3}, encapsulated within CCMV, could efficiently activate the pro-drugs tamoxifen and resveratrol [77]. An additional study with CYP_{BM3}-encapsulated bacteriophage P22 indicated that the enzyme-VLP therapy could enhance the activity of the CYP_{BM3} up to 10x that of endogenous levels when delivered with lipofectamine [79]. In addition to the delivery function, the VLP can offer protection against degradation by proteases. P22-CYP_{BM3} and free CYP_{BM3} were incubated in trypsin for 1 h; the CYP_{BM3} within the P22 retained up to 90.3% of its activity compared to 59.5% for the free CYP_{BM3} indicating protection from the protease.

In a different example, M13 bacteriophages were bioconjugated to horseradish peroxidase (on coat protein p9) and a peptide sequence called Ypep, which has targeting and penetration capability in PC-3 prostate cancer cells (on coat protein p3) [80]. Horseradish peroxidase converts the prodrug indole-3-acetic acid (IAA) into a peroxyl radical, and concurrent addition of the M13 phages with IAA led to near complete cell death *in vitro* against the PC-3 cells. Lastly, protein display strategies have been developed for mammalian viruses: Handa et al. showed that cytosine deamidase encapsulated within simian virus 40 (SV40) VLPs could sensitize CV-1 cells to 5-fluorocytosine, a prodrug to 5-FU, *in vitro* better than cytosine deamidase alone [81].

In addition to encapsulating proteins, VLPs can tether and display proteins on their surfaces with the proteins serving cell targeting or therapeutic functions. For example, for cell targeting applications, Francis et al. covalently conjugated anti-epidermal growth factor receptor (EGFR) antibodies to MS2 VLPs to target EGFR on breast cancer cells. Antibody conjugation significantly increased uptake of VLPs *in vitro* in MDA-MB-231 and HCC1954 cell lines; although this effect was not observed *in vivo* [82]. Another similar study tested the targeting capabilities of MS2 by conjugating four different functional single-chain variable fragments to the surface of MS2 VLPs; the engineered VLPs demonstrated specificity and binding to their cognate antigens [83]. In these aforementioned examples, protein/peptide display was accomplished via bioconjugate chemistries. With genetic fusion however, typically only short peptide fragments can be fused to avoid disrupting capsid assembly. Finn et al. overcame this shortcoming with a PP7 phage-derived VLP platform with C-terminal fused peptides [84]. PP7 is amenable to C-terminus display as it is signficantly less crowded around its threefold axes increasing its tolerance to peptide and protein display. PP7 capsid dimers were engineered with peptides of up to 14.2 kDa, and cryo-electron microscopy of the formed VLPs revealed surprising structural transformations from original $T = 3$ to $T = 4$ capsids.

Several examples have demonstrated the utility and efficacy of bacteriophages and plant viruses to display and deliver therapeutic peptides and proteins. Li et al. used P22 bacteriophages as the vehicle for intracellular delivery of two cytotoxic and synergistic peptides to MDA-MB-231 breast cancer cells [85]. The peptides were released in a

controllable manner through Cathepsin B, an overexpressed protease in many cancer cells. As mentioned in Section 2.2, high aspect ratio platforms such as TMV and PVX make intriguing platforms for drug delivery. Based on their shape, these particles have enhanced cellular interactions, and therefore conjugated cargo can increasingly interact with cell surfaces. Indeed, PVX has been engineered in various ways for biomedical applications: in early work, a PVX mutant with a deleted 5' terminus was modified by encoding exogenous peptide sequences within the genome for attachment to the deleted 5' end [86]. The study elucidated that amino acid content, mainly tryptophan, and isoelectric point could drastically alter the trafficking of the PVX through plants. For biosensing and diagnostic applications, PVX was genetically altered to display protein A fragments from *Staphylococcus aureus* [87]. The protein A coated PVX was then readily functionalized with IgG and was applied in biosensing of plant viruses [88]. PVX can further be conjugated to fluorescent reporters to be used for *in vivo* imaging applications and theranostics [89]. PVX was engineered as a fluorescent probe through genetic manipulation to encode a small fluorescent protein called iLOV. Of note, iLOV display has also been applied to TMV [90]. To be used as a therapeutic, PVX was used as a display platform for TNF-related apoptosis inducing ligand (TRAIL); multivalent display enhanced its engagement and activation of death receptors expressed on MDA-MB-231 breast cancer, HCC-38 primary ductal carcinoma, and BT-549 ductal carcinoma cell lines. Indeed, potent efficacy was demonstrated *in vivo* using the PVX-TRAIL formulation to treat athymic nude mice with MDA-MB-231 triple negative breast cancer xenografts injected s.c. into the right flank [91]. The PVX-TRAIL was administered i.t. Compared to initial tumor volume, the PVX-TRAIL showed essentially no signs of tumor growth even by day 30. Chatterjee et al. showed similar efficacy *in vitro* against prostate cancer (PC-3) cells via bioconjugation of TRAIL to influenza neuraminidase VLPs reducing cell viability by 70–80% [92].

Our lab has also explored the application of TMV for cardiovascular therapy. In one example, we conjugated tissue plasminogen activator (TMV-tPA) to TMV; tPA is a thrombolytic used in acute myocardial infarctions to break down thrombus obstructions [93]. Illumination of the right carotid artery of C57BL/6 mice with a 540 nm green laser produced thrombi. TMV-tPA was delivered via tail vein injection and maintained its efficacy and performed similarly to free tPA; more importantly, the TMV-tPA formulations displayed an improved safety profile indicated by a decreased average bleeding time of 429 s to 858 by the free tPA [94]. The decreased bleeding time is of noteworthy importance as tPA does not specifically congregate at the diseased area, and systemic administration can lead to life-threatening systemic hemorrhaging and/or angioedema [95].

3. Vaccines and immunotherapy

VLPs are powerful platforms for vaccines. VLPs mimic the conformation of native viruses harnessing their inherent immunogenicity but do not compromise on safety, as VLPs cannot infect nor replicate due to the absence of the viral genome [96]. VLPs have been developed as subunit vaccines while plant viruses and bacteriophages have been developed as heterologous nanoparticle display platforms. Plant viruses and bacteriophages, with or without their viral genome, are also unable to replicate within mammalian cells conferring a secondary level of safety. When presented to a host immune system, VLPs – whether of mammalian origin or not – are taken up by antigen presenting cells (APCs) evoking effective immune responses. Therefore, VLPs are strong candidates for use as carriers to deliver and present epitopes for vaccine design. Another emerging area is the use of VLPs in cancer immunotherapy – here, the inherent immune-stimulatory properties are utilized to reprogram the tumor microenvironment (TME) to launch antitumor immunity. This section will discuss the recent progress within these fields.

3.1. Cancer immunotherapy with VLPs

3.1.1. VLPs as monotherapy

The TME is highly immunosuppressive and favors tumor immune escape by suppressing the production, activation and function of antitumor T-cells [97]. As some VLPs are highly immunogenic, i.t. administration of VLPs into TMEs can trigger innate immune activation capable of instigating the transition to adaptive antitumor immunity [98]. Mammalian viruses have long been used as therapies in oncology. Oncolytic viruses directly lyse tumor cells leading to the release of tumor-associated antigens (TAAs). At the same time, the viral infection causes release of danger signals and type I interferon (IFN), and the combination of events can elicit antitumor immunity [99]. Recently, Nair et al. showed the immunotherapy potential using a recombinant poliovirus/rhinovirus chimera (PVSRIPO), which is currently in clinical trials against recurrent grade IV malignant glioma [100]. PVSRIPO infection of primary human dendritic cells (DCs) and macrophages produced persistent IFN-dominant activation and increased resistance to cancer cell-mediated immunosuppression. A single i.t. injection of PVSRIPO elicited systemic antitumor cytotoxic T-cell (CTL) responses in B16F10.0-OVA-CD155 implanted C57BL/6 mice.

In 2015, the first oncolytic viral immunotherapy named Imlygic, otherwise known as talimogene laherparepvec (TVEC), was approved by the Food and Drug Administration [101]. TVEC is derived from type I herpes virus, but has been genetically modified to be unable to create the fever blisters seen in the infectious virus. As an immunotherapy, TVEC works in two main ways: 1) by propagating only in tumor cells through the dysregulation of the protein kinase R (PKR) pathway, and 2) activation of the innate immune system response through expression of proinflammatory molecules such as human granulocyte-macrophage colony-stimulating factor (GM-CSF) [102]. Further, TVEC itself acts on the innate immune system, similarly to any virus or VLP, and is recognized by pathogen-associated molecular pattern (PAMP) receptors. Viral infiltration into the tumor cells leads to cell lysis releasing TAAs extracellularly for DCs to process and activate CTLs, which go on to attack additional tumor cells, in an expanding cyclic fashion. TVEC immunogenicity is amplified by GM-CSF, which has been shown to further augment DC recruitment and maturation and CTL activation [103]. TVEC is currently undergoing further clinical trials in combination therapies and in monotherapies for other cancers such as non-melanoma skin cancers, pancreatic cancer, and angiosarcomas [104–107]. The success of TVEC through clinical trials warrants the continued research and development of new and exciting oncolytic viruses and sets the stage as an important stepping stone for future viral therapies.

We and others have shown that plant viruses and their VLPs have potential to be utilized for cancer therapeutics. Our early study indicated that inhalation of CPMV VLP nanoparticles reduced i.v. administered B16F10 lung melanomas in C57BL/6J mice and generated potent systemic antitumor immunity [108]. In the same study, *in situ* vaccination of CPMV demonstrated efficacy in the treatment of ovarian (ID8-*Defb*29/*Vegf*-A), colon (CT26), and breast tumor (4T1) models. Mechanistic studies indicated that activated neutrophils triggered systemic antitumor responses in the TME, which rapidly took up the administered CPMV VLPs. In follow-up studies, we delineated the different mechanisms of the CPMV-triggered anti-tumor immunity: CPMV reprograms the TME recruiting neutrophils and natural killer cells, and macrophages are reprogrammed from M2 to M1 anti-tumor macrophages. The innate immune cells then attack the tumor, and subsequent cell lysis allows APCs to process and present TAAs triggering tumor-specific T-cell responses. T-cell activation not only treats the injected tumor, but also induces systemic anti-tumor immunity [109]. The overall mechanism is outlined in Fig. 5. We further verified the immunotherapeutic efficacy of CPMV in separate studies in the treatment of murine dermal melanoma [110], intracranial glioma [111] and ovarian cancer [109]. In addition to the mouse studies, we have begun testing the immunotherapy in canine patients, and our data indicate similar efficacy

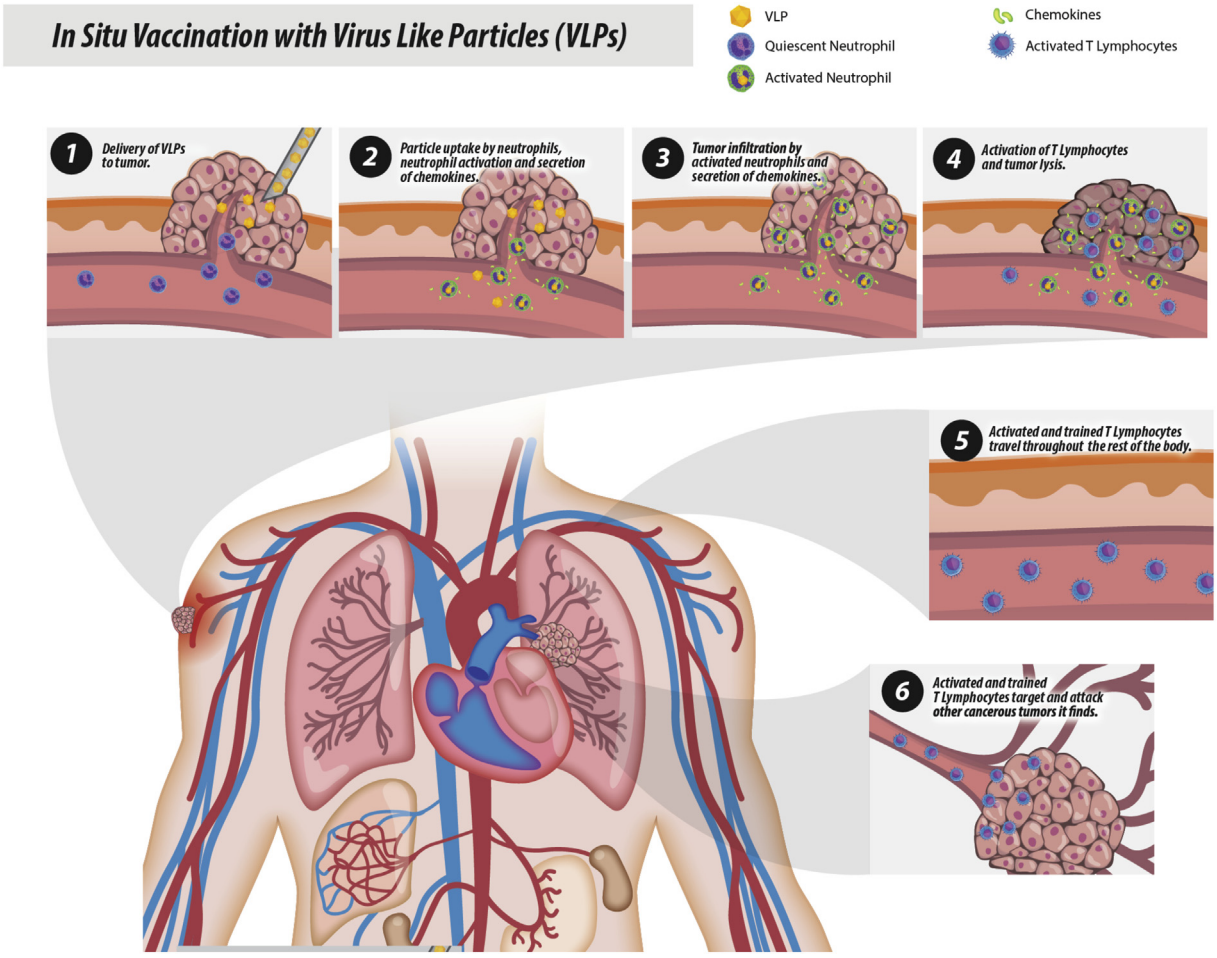

Fig. 5. Overall mechanism of VLP *in situ* vaccination in treating solid tumors: 1) the VLP is delivered directly into the tumor, 2) the VLP is taken up by neutrophils, which become activated and release chemokines, 3) neutrophils activated by the released chemokines infiltrate the tumor and release more chemokines, 4) T-lymphocytes become activated leading to tumor lysis, 5) activated T-lymphocytes travel systemically throughout the body, 6) T-lymphocytes attack metastatic tumors throughout the body.

in these patients [112]. In the aforementioned CPMV studies, the immunotherapeutic vaccines are administered multiple times; however, multiple dosing regimens can lead to low patient compliance and increased hospital burden. Therefore, the VNPs of CPMV have also been applied as a slow-release formulation by forming aggregates with polyamidoamine generation 4 dendrimers (CPMV-G4) [113]. The CPMV-G4 formulation can be tailored to contain different CPMV release profiles by altering either salt or CPMV concentrations. The release of CPMV was measured by the decrease in the hydrodynamic diameters of the CPMV-G4 aggregates and through ultraviolet-visible spectrophotometry. When compared to multiple weekly intraperitoneal (i,p.) injections of CPMV, the single i.p. administration of CPMV-G4 treated ID8-*Def*29/*Vegf*-A ovarian cancer cells with similar efficacy in C57BL/6 mice. As plant virus-based VLPs are stable, nontoxic, modifiable with drugs and antigens, and can be mass-manufactured [15], this study has pioneered a new strategy of using plant virus-based VLPs for cancer immunotherapy.

We also tested the efficacy of other plant viruses as *in situ* vaccines. We found that while TMV showed some degree of efficacy, it could not match that reported for CPMV [110]. On the contrary, Lamarre et al. showed that i.t. administration of papaya mosaic virus (PapMV) could slow down B16-OVA melanoma progression and prolong survival [114], through TLR7 activation induced by PapMV [98]. Similar observations of therapeutic efficacy have been made using bacteriophages. The M13 bacteriophage has been used for monotherapy against B16F10 melanoma cells triggering the immune response through TLR signaling [115]. MyD88$^{-/-}$ mice, which are unable to propagate TLR signals, showed lack of tumor regression or neutrophil infiltration while the tumors in wild type (WT) mice necrosed following M13 s.c. peritumoral administration. M13 phages polarized tissue-associated macrophages from the protumor M2 phenotype into the antitumor M1 phenotype and increased levels of the proinflammatory cytokines tissue necrosis factor-α and interleukin (IL)-6 by 246 and 314 times controls respectively. The increased cytokine expression led to significantly greater recruitment of neutrophils for enhanced tumor killing. Taken together, data indicate the potential of plant viruses and bacteriophages in tumor therapy, and one such formulation has already advanced to clinical testing. The aforementioned Qβ formulation packaged with CpGs is currently in clinical trials for melanoma immunotherapy [70].

3.1.2. VLPs in combination therapy

Although i.t. administration of VLPs into the TME has shown potential to induce antitumor immune responses, cancer is inherently a heterogenous disease and even cells within the same tumor can showcase dissimilar responses to treatments [116]. Therefore, the therapeutic efficacy of any immunotherapeutic monotherapy is limited, and combination therapies between chemotherapeutics and checkpoint blockades have been heavily investigated in the clinic for improving therapeutic outcomes.

Plant VLPs are no exception and have been investigated concurrently with other drugs. Our lab has developed several strategies using plant virus-based immunotherapies. In some early work, we compared the

coadministration of PVX + DOX to the two monotherapies alone [117]. Compared to singular i.t. administration with either PVX or DOX, PVX + DOX significantly increased C57BL/6J mice survival in the treatment of intradermal B16F10 melanoma, and the antitumor cytokine/chemokine profile stimulated by the PVX + DOX was far superior to the other tested regimes. Cyclophosphamide (CPA) is another chemotherapeutic utilized alongside our plant VLPs; CPA is the most widely used alkylating agent for chemotherapy and has extensive immunomodulatory activity including the capability to induce pro-immunogenic activity in tumor cells. When we combined CPA with CPMV VNPs, CPA induced cell death to provide a burst of extracellular TAAs while CPMV induced immune cell infiltration and promoted the recognition and processing of the TAAs [118]. The combination therapy induced systemic antitumor responses and achieved synergistic efficacy in the treatment of 4T1 murine models.

Aside from chemotherapeutics, monoclonal antibody therapies have become a widely utilized therapeutic option in clinical trials. To investigate concurrent *in situ* administration of antibodies and our VNPs, we used CPMV in combination with CD47-blocking antibodies in the treatment of murine ovarian cancer (ID8-*Def*29/*Vegf*-A) and TNBC (4T-1) in Balb/c mice [119]. Vaccination led to recruitment and activation of phagocytes while the anti-CD47 inhibited antiphagocytic signals. Thus, CD47 blockade with CPMV synergized to promote phagocytosis of cancer cells and help to activate APCs thus priming the adaptive arm of the immune system. The anti-CD47 and CPMV acted synergistically to combat tumor growth. And just recently, we demonstrated the synergistic effects of combining CPMV with checkpoint inhibitors, such as anti-programmed cell death-1 [120].

CPMV has also been investigated in combination with radiation therapy (RT). RT leads to tumor debulking and tumor cell apoptosis, leaking a wide array of TAAs [121]. As mentioned above with the CPA studies, the CPMV promotes APC recruitment; the APCs phagocytose the extracellular TAAs leading to a sustained and improved response. Indeed, combining radiation therapy with CPMV *in situ* vaccination significantly improved tumor growth delay in ID8-*Def*29/*Vegf*-A-Luc tumors compared to either therapy alone [121]. The potent efficacy of CPMV + radiation therapy has also been translated to canine patients with melanoma [112]; therefore, this approach demonstrates high translational potential.

Outside of plant viruses and bacteriophages, mammalian viruses have been utilized in combination therapies. The inactivated modified vaccinia virus Ankara, Maraba rhabdovirus, oncolytic human *Orthoreovirus*, and TVEC have been utilized in conjunction with the checkpoint blockades, anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4 and anti-programmed death ligand (PD-L)-1 [122–125]. As with the plant viruses, combination therapies improved outcomes compared to monotherapy controls.

3.2. Vaccine development: epitope presentation using VLPs

As discussed in Section 3.1, VLPs are immunogenic and are taken up by APCs; recognition through PRRs leads to activation of the APCs and antigen processing. VLPs comprised of multiple copies of their capsid proteins represent multivalent, repetitive scaffolds, which further benefit the multivalent antigen presentation. These features make VLPs ideal platforms for epitope delivery and presentation to the immune system, and therefore VLP vaccines usually induce stronger immunity than antigens in their soluble forms. At the same time, the VLPs have adjuvant properties and many epitope display platforms do not require use of additional adjuvant to elicit potent immunity. We discuss the recent progress in using VLPs as carriers in vaccine design highlighting various application areas.

3.2.1. VLP-based vaccines against infectious diseases

Several approaches of VLP-based vaccines are being considered: VLP subunit vaccines, viral vector vaccines, and VLP-based display platforms. Their mechanism of action is outlined in Fig. 6. Several viral vaccines are commercially available, these include human papillomavirus (HPV) and hepatitis B vaccines (HBV) and well as potential vaccines for influenza, Epstein-Barr virus, and malaria [126–130].

Using the traditional methods of genetic insertion and chemical conjugation it can be difficult to obtain site-specific and highly efficient display of epitopes on VLPs. To address this issue, an improved VLP display strategy via chemoenzymatic site-specific tailoring of antigens on VLPs with high efficiency was recently reported [131]. HBc VLPs were tagged through a sortase A-mediated transpeptidation reaction with an enterovirus 71 (EV71) SP70 epitope, which elicited effective antibodies against EV71 and protected Balb/c mice from lethal challenge. This chemoenzymatic site-specific approach showed great potential in VLP-based vaccine design for its simplicity, site specificity, high efficiency, and versatility.

Besides displaying antigens on the surface, VLPs can also encapsulate antigens within their interior and deliver antigens to immune cells for enhanced T-cell response (Fig. 6). Douglas et al. developed a P22-based platform delivering both matrix and matrix 2 proteins derived from respiratory syncytial virus within the same particle [132]. Following intranasal administration in CB6F1/J mice, the vaccine stimulated CD8+ T-cell memory responses against both antigens, and the T-cells maintained tissue-resident memory phenotypes. Tissue-resident memory T-cells are critical in forming quick antiviral responses against re-exposure rapidly triggering both the innate and adaptive responses [133].

Of the infectious disease vaccines being developed, human immunodeficiency virus (HIV) remains one of the major milestones to be achieved for vaccine development as there is still no vaccine that can induce broadly neutralizing antibodies (bNAbs) to prevent infection [134]. The only existing target for bNAbs is the envelope glycoprotein (Env), a trimer of heterodimers consisting of gp120 and gp41 subunits; however, subunit vaccines are oftentimes immunologically deficient. To improve the immunogenicity, Überla et al. generated Env VLPs containing helper T-cell epitopes derived from tetanus toxoid [135]. Pre-immunization of Balb/c and C57BL/6J mice with the tetanus toxoid via intramuscular injection into the gastrocnemius muscle of the hind legs significantly increased Env-specific IgG titers mainly aided by the intrastructrual help of the helper T-cell epitopes.

Also, protein nanotechnology can enable next-generation vaccine development; for HIV, several protein cages have been utilized for subunit display. While the following examples are not VLPs, these protein cages – like VLPs – are self-assembled from repeating protein units. In an early study, Env was genetically fused to a ferritin protein nanocage yielding a 24-subunit nanoparticle of 30 nm presenting 8 trimers [136]. Two following efficacy studies showed that Env trimers presented on ferritin elicited significantly higher binding of antibodies and therefore autologous neutralizing antibody responses than single trimers after immunization in rabbits [137,138]. To help delineate how these vaccine nanoparticle candidates traffic *in vivo*, Irvine et al. generated a ferritin-Env presenting eight copies of a gp140 envelope trimer (MD39). They found that the ferritin-based Env immunogens were rapidly shuttled to a follicular DC network and then concentrated in germinal centers via a complement-, mannose-binding lectic-, and immunogen glycan-dependent manner [139].

The aforementioned examples are from naturally occurring protein cages; recently, computational protein structure prediction research has fueled the design and implementation of new protein nanostructures for vaccine development. Sanders et al. fused HIV Env trimers to a computationally designed, synthetic icosahedral protein cage, I53-50, generating monodisperse, well-ordered VLPs presenting 20 trimers [140]. Immunization via i.m. injections into the quadriceps of naïve New Zealand White rabbits with I53-50-Env significantly increased the immunogenicity, inducing NAb responses, which were 40-fold higher compared to single Env immunizations. Furthermore, I53-50-Env VLPs exhibited superior efficacy to ferritin-Env VLPs in enhancing autologous NAb responses, most likely due to the high density of presented Env trimers on the I53-50.

As we are writing this article, a novel coronavirus (SARS-CoV-2) has spread throughout the world triggering globally mandated lockdowns

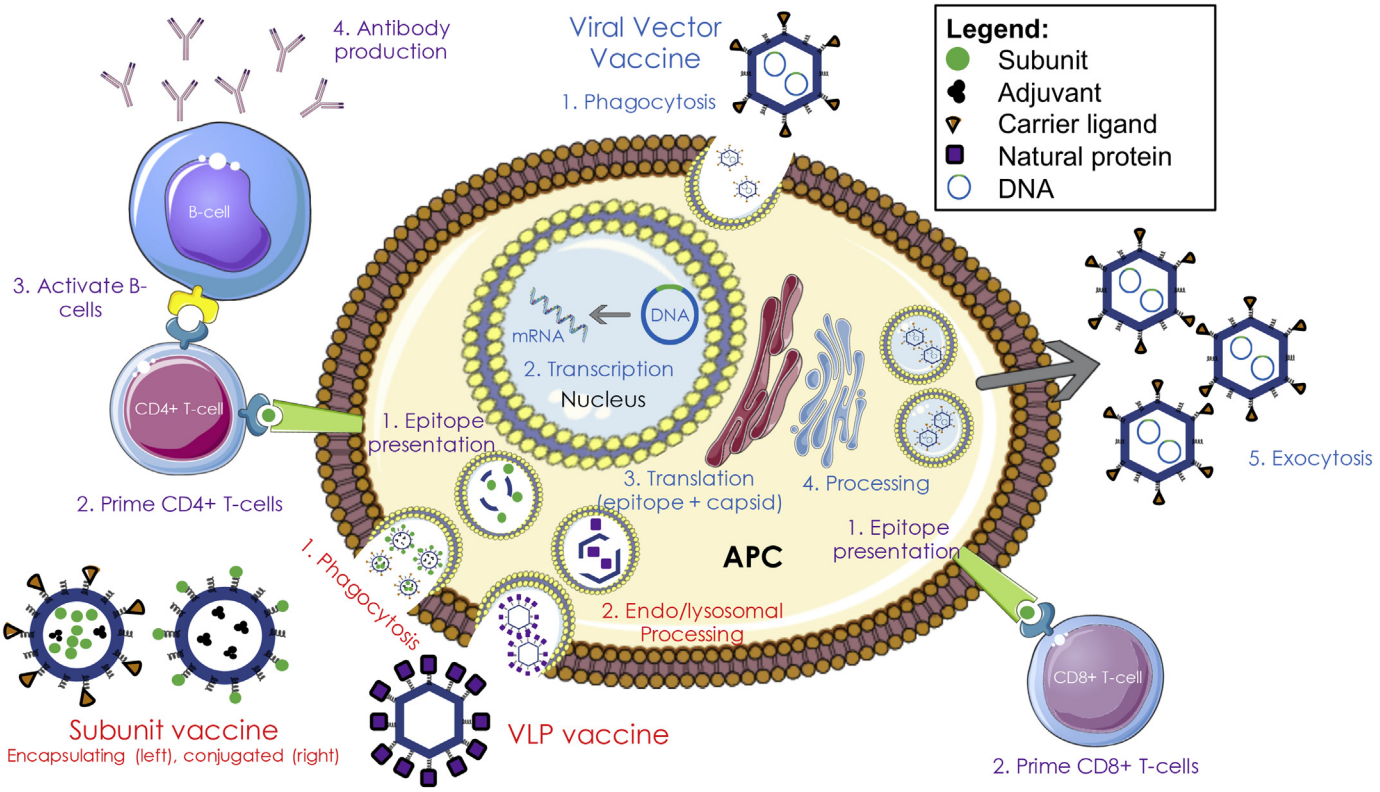

Fig. 6. Mechanism of action of different viral vaccine candidates. Subunit vaccines (bottom left) can be of two different types: 1) encapsulating the antigenic subunit and 2) displaying the subunit on the exterior. VLP vaccines (bottom center) mimic the natural virus, but are devoid of their genome thus disabling replication. Their capsids are still decorated with the antigens displayed by the APC. Viral vector vaccines can be replicating (top center) or non-replicating (not shown). Viral vector vaccines contain the genomic information for the necessary antigens within their own genomes. Cell entry leads to genomic processing leading to the display of the encoded antigens on the cell surface. The replication machinery remains intact for replicating viral vector vaccines producing more of the viral vaccines, which goes on to infect other APCs. The steps highlighted in red are those taken by the subunit and VLP vaccines. The steps highlighted in blue are those taken by the viral vector vaccine. Those in purple are steps taken by the subunit, VLP, and viral vector vaccines. The antigens are displayed on the cell surfaces priming both CD4+ and CD8+ T-cells. The CD4+ T-cells go on to activate B-cells, which secrete antibodies. Activated CD8+ T-cells induce a CTL response.

and quarantines while institutions and pharmaceutical companies work towards the discovery of a novel and efficacious vaccine [141]. Of the vaccines under development, VLP, attenuated/inactivated, and viral vector vaccines are strong candidates. In fact, of the 90+ vaccines being developed, 5 vaccines are strictly VLP-based, 8 are inactivated/attenuated, and 25+ are viral vectors [142]. Medicago is one of the companies producing a VLP-based vaccine in plants using *N. benthamiana* for in-planta production of empty SARS-CoV-2 viral particles [143]. The University of Wisconsin-Madison/Flugen/Bharat Biotech's live-attenuated influenza vaccine [144] or Sinovac's formalin-inactivated SARS-CoV-2 viral vaccine [145], which has recently moved into Phase1/2 clinical trials [146], also remain strong candidates.

3.2.2. VLP-based vaccines against cancer

VLPs have also been widely explored as a platform to deliver TAAs for use as cancer vaccines. Genetic fusion, chemical conjugation techniques, as well as enzyme-mediated ligation strategies have been utilized to attach TAAs onto VLPs. For cancer vaccines, a particular challenge is to break tolerance for self-antigens. Based on their propensity to boost immune responses, VLPs are an attractive platform for the delivery of self-antigens and otherwise poorly immunogenic antigens. The geometry and multivalency of VLPs boosts immunity based on recognition through PAMPs, most commonly TLRs [147]. These immunestimulatory properties in combination with their size make VLPs ideal candidates for vaccine delivery to the draining lymph nodes and priming interactions with APCs.

Chemical conjugation is the most commonly utilized method for attachment of TAAs to VLPs, and our group has been utilizing diverse plant virus-based nanotechnology platforms for the delivery of TAAs. The comparison of icosahedral CPMV and filamentous VNPs for human epidermal growth factor receptor 2 (HER2) epitope delivery revealed that the icosahedral CPMV was more efficient in terms of lymphatic drainage, and uptake by and activation of APCs, leading to enhanced anti-HER2 immunity compared to filamentous PVX [148,149]. The CPMV-HER2 vaccine candidate delayed the progression of DDHER2 primary tumors and metastasis in Balb/c mice thereby prolonging survival [150]. The displayed epitope plays an integral part in the immunotherapeutic response, but the nanocarrier itself can also affect the effectiveness of the therapy. In a recent study, we compared three icosahedral plant VNPs, CPMV, CCMV and sesbania mosaic virus (SeMV), displaying a HER2 epitope as a cancer vaccine [151]. Although the three viruses are similar in shape and structure, there were significant differences between the immune profiles elicited by the viruses. CPMV-HER2 induced a Th1 predominant immune response while CCMV- and SeMV-HER2 induced a Th2 predominant immune response thus indicating that the carrier itself can play a key role in the regulation of Th1/Th2 bias. The differences in the immunogenic profiles are attributed to the epitope displayed on the VNP surface and capsid itself. To overcome this drawback and focus the immune responses on solely the displayed epitope, we developed a heterologous prime-boost strategy utilizing different HER2 displaying carriers (CCMV, CPMV and SeMV) at 2 week intervals [151]. Indeed, the heterologous prime-boost strategy significantly enhanced the HER2-specific immune responses and induced an efficacious Th1-predominant response delaying s.c. inoculated DDHER2 tumor progression and prolonging Balb/c mice survival.

Although chemical conjugation remains the most widely used methodology for attachment of antigens to VLP surfaces, specific ligation strategies such as the previously mentioned Spy-tag/Spy-catcher

conjugation can confer additional spatial regulation and provide increased stoichiometric control of antigen presentation. *Acinetobacter* bacteriophage AP205 derived VLPs were engineered in this fashion to display multivalent HER2 epitopes on the surface [152]. Immunization studies indicated that the vaccine overcame B-cell tolerance and induced strong polyclonal anti-HER2 response.

Aside from breast cancer, cervical cancer is responsible for the most cancer-related deaths in women worldwide [153]. Cervical cancer is mainly caused by HPV, and recent studies have utilized plant VLPs and bacteriophage as potential vaccines. Chackerian et al. genetically engineered MS2 bacteriophages to display the L2 coat protein of HPV at the N-terminal (MS2-L2) [154]. Compared to the lab's previously engineered PP7 bacteriophage vaccines, the MS2-L2 elicited much greater cross-reactivity and cross-protectivity against 10 heterologous HPV types in Balb/c mice. HPV-GFP challenge in the vaccinated mice led to 80–7190 – fold reduction in radiance when compared against VLP-only controls.

Preclinical studies may show strong efficacy, but oftentimes subunit vaccines may require the use of adjuvants. Plant viruses are beneficial in this aspect as their genome may act as an inherent adjuvant thereby augmenting the immune response. PVX conjugated to a recombinant idiotypic (Id) TAA via a biotin/streptavidin linker showed 7x greater anti-Id IgG response compared to Id alone in a murine B-cell lymphoma model (BCL1) [155]. When compared to a gold-standard Id vaccine, the PVX produced 3x the anti-Id antibodies. The study found that TLR7 was vital for the recognition of the viral RNA, and cytokine profiling demonstrated production of IL-12 and IFN-α.

Another variation of cancer vaccines are those targeting tumor associated carbohydrate antigens (TACAs). TACAs can promote tumor progression and are useful to target as they are overexpressed on a wide range of malignant cells; however, the carbohydrates by themselves do not elicit strong immunogenic responses as they are weak T-cell activators and oftentimes require novel approaches to boost effectiveness. One emerging method to combat the weak immunogenicity is the use of plant viruses and bacteriophages as carriers of TACAs. For example, Qβ has been engineered to present TACAs such as the poorly immunogenic monomeric Tn antigen and the ganglioside antigen GM2 [156,157]. Furthermore, tumor associated MUC1 glycopeptide antigens were conjugated to Qβ, and the obtained Qβ-MUC1 vaccine induced high titers of anti-MUC1 IgG antibodies in both MUC1 transgenic and WT mice, conferring protection against primary and metastatic breast cancers [158–160]. Likewise, Huang et al. developed CPMV-TACA conjugates, specifically targeting the Tn antigen (GalNAc-α-O-Ser/Thr) [161]. Immunological studies showed that by day 35, C57BL/6 mice given the vaccine via s.c. injection produced high IgG titers indicating T-cell mediated responses and induced antibody isotype switching. Addition of the mice sera in both MCF-7 and multidrug resistant NCI-ADR RES breast cancer cell lines led to the binding of IgG antibodies to the Tn antigens. The addition of free GalNAc led to marked decrease of antibody binding indicating high antibody specificity to the Tn. In a follow-up study, TMV was used to display Tn antigens [162]. Unlike with CPMV, the location of the Tn conjugation was an important factor in IgG production; Tn linked to the N-terminus of TMV was immunosilent, but conjugation to Tyr 139 elicited strong immune responses.

Alternatively, tumor associated neoantigens can produce more specific antitumor immune responses and have the potential to be used in personalized vaccines [163]. A multi-target vaccine strategy using Qβ VLPs containing TLR ligands was evaluated against B16F10 murine melanoma. The study compared vaccines containing germline epitopes identified through immunopeptidomics, mutated epitopes predicted by whole exome sequencing, and a third combining both germline and mutated epitopes [164]. Pre-clinical evaluation indicated that the Qβ-based vaccines presenting both the germline and mutated epitopes achieved the greatest therapeutic efficacy.

Lastly, nucleic acid vaccines have garnered strong interest as next-generation cancer vaccines, and VLPs have been explored as potential carriers. RNA is instable *in vivo* due to the presence of RNases; thus, nanocarriers – such as VLPs – can help protect and extend the biodistribution and half-lives of these therapies. Wang et al. developed a messenger RNA (mRNA) vaccine encoding for GM-CSF loaded on recombinant MS2 VLPs against prostate cancer [165]. Following administration, GM-CSF production was measured as early as 12 h after APC phagocytosis *in vitro*. Fluorescence activated cell sorting of DC markers confirmed DC activation and maturation leading to strong humoral and cellular immune responses. The vaccine was administered i.v., which protected C57BL/6 mice against TRAMP-C2 prostate cancer prophylactically, and as a therapeutic vaccine, retarded tumor growth compared to soluble GM-CSF and VLP-only controls.

3.2.3. VLP-based vaccines targeting various inflammatory and autoimmune diseases

VLP vaccines can also be used to treat a range of other disorders, such as neurological, cardiovascular and autoimmune diseases. Currently, there are no approved vaccines for tauopathies such as frontotemporal dementia (FTD) and Alzheimer's disease (AD). These diseases cause unrecoverable cognitive decline and lower quality of life extremely for patients. Data suggests that tauopathies occur due to aggregation of amyloid beta (Aβ) peptides as well as the accumulation of microtubule-associated protein tau (pTau) within neurons forming neurofibrillary tangles [166]. Therefore, tauopathy vaccines should target either Aβ or pTau and produce long-lived antibody responses against these proteins. AD vaccines against Aβ fail in the clinic due to strong T-cell responses from the vaccine and the simultaneously administered adjuvant leading to meningoencephalitis and self-tolerance to the anti-Aβ antibodies [167]. To counteract these deficiencies, Morgan et al. developed Qβ-derived VLPs conjugated on their N- and C-termini to Aβ peptide sequences [167]. The N-terminal vaccine mainly elicited an IgM response while the C-terminal vaccine elicited an IgG response – highlighting an inherent need in increasing understanding in design rules that govern the engineering of effective vaccines. Regardless, both vaccines elicited strong antibody titers without the need for an adjuvant in an amyloid precursor protein mouse model and reduced amyloid plaques in the cortex significantly. In a later study, Bhaskar et al. engineered Qβ-derived VLPs to deliver multivalent tau peptide with phosphorylation at threonine 181 (pT181) [168]. Bi-weekly i.m. vaccination in rTg4510 mice models indicated that the vaccine candidate induced a robust and long-lived anti-pT181 antibody response leading to reduction of phosphorylated pathological tau in the hippocampus and cortex, prevention of atrophy in the hippocampus and corpus callosum, and rescue of cognitive dysfunction. It is also worth mentioning that mammalian VLPs such as HBc [169], murine leukemia virus [170], and HPV [171] have been explored with relative success as tauopathy vaccines.

VLPs have also been investigated as vaccines for inflammatory and autoimmune diseases. IL-17 mediates the release of pro-inflammatory cytokines in many cells, and IL-17 producing T helper cells (Th17) are implicated in several autoimmune disorders, such as rheumatoid arthritis and multiple sclerosis [172]. Monoclonal IL-17 antibody blockade has shown previous treatment efficacy, and thus one alternative strategy is the development of vaccines that can produce anti-IL-17 antibodies [173]. For this purpose, Qβ-based VLPs carrying IL-17 were developed; the VLPs overcame self-tolerance against IL-17 and generated high titers of anti-IL-17 antibodies to slow the progression of autoimmune arthritis and encephalomyelitis [174]. Another inflammatory disease being considered for vaccine development is allergic asthma; the disease is induced by chronic inflammation in the lungs, which is mainly mediated by the recruitment and activity of eosinophils [175]. IL-5 and eotaxin are chemokines vital for the recruitment and maturation of eosinophils from the bone marrow [176]. Qβ particles were chemically crosslinked to either IL-5 or eotaxin, and immunization with

these particles overcame self-tolerance and stimulated high antibody response against the two particles. Co-immunization with Qβ-IL-5 and Qβ-eoxtaxin lowered eosinophil-induced inflammation in Balb/c mice. Allergic airway inflammation in the mice was induced through the administration of ovalbumin and alum [176].

Another target application of engineered VLP vaccines lies in the treatment of cardiovascular diseases (CD). Mutations that increase the activity of proprotein convertase subtilisin/kexin type 9 (PCSK9), a secreted protein that controls cholesterol homeostasis by enhancing the degradation of the low-density lipoprotein receptor (LDL-R), can lead to hypercholesterolemia, atherosclerosis, and early-onset CD [177]. Qβ-based VLPs were engineered to display PCSK9-derived peptides, and subsequent vaccination in mice and macaques elicited high titers of PCSK9-specific antibodies thereby inhibiting the enzyme and lowering the levels of cholesterol, phospholipids and triglycerides [177]. The VLP also worked synergistically with simvastatin in lowering LDL levels. The pathophysiology of atherosclerosis can also be attributed to overproduction of the proinflammatory cytokine IL-1α [178]. Vaccines targeting IL-1α should therefore produce NAbs against the cytokine, effectively disrupting its function. Bachmann et al. developed a Qβ-based vaccine with conjugated full-length IL-1α, and vaccination in ApoE-deficient mice fed a Western diet led to significant anti-IL-1α titers in 21 days [179]. Immunization further led to a 50 and 37% decrease of plaque progression in the aorta and aortic root respectively, 22% decrease in macrophage infiltration, and decreased levels of the proinflammatory molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1).

Lastly, VLPs have been investigated as vaccines against pain and as contraceptives. Nerve growth factor (NGF) is a key cause underlying the chronic pain associated with osteoarthritis [180]. Cucumber mosaic virus (CuMV) was used as a scaffold for conjugation of NGF to its exterior surface. The CuMV-NGF VLPs induced high titers of anti-NGF antibodies in mice that had undergone partial meniscectomy-induced osteoarthritis, leading to the reversal of pain behavior [181]. As a contraceptive, Johnson grass mosaic VLPs displaying the spermatozoa peptide YLP12 and zona pellucida epitope ZP3 elicited antibodies that block the ability of the sperm to interact with the zona pellucida of the egg [182]. s.c. immunization in FVB/cJ mice with the contraceptive VLPs decreased litters born compared to controls. The far-ranging applicability of VLPs in neurological, cardiovascular, and autoimmune diseases as well as in pain and contraception highlight the flexibility of VLPs as vaccination agents and evidences the justified advancement in the field for future vaccine strategies.

4. VLPs in theranostic applications: imaging and therapy

4.1. Delivery of contrast agents using VLPs

Molecular imaging is a growing biomedical research discipline that enables the visualization, characterization and quantification of biological processes *in vivo*. Various imaging technologies including optical imaging, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) have been widely used to depict cellular and molecular pathways and mechanisms of disease present in the context of living subjects (Fig. 7). Ideal molecular imaging techniques should quickly provide optimal signal-to-noise ratios at the target site with minimal toxicity. Compared to synthetic nanoparticles, VLPs can be more advantageous as imaging delivery agents because of their short circulation and retention time decreasing possible side effects [183]. Moreover, VLPs can be tailored to carry a wide range of fluorescent labels and/or contrast agents while being modified with aptamers, peptides or antibodies for improved targeting to particular cells and tissues. In this section, we will summarize the VLP-based platforms for delivery of imaging agents.

4.1.1. Optical imaging using VLPs

Fluorescent VLPs are used in optical imaging for specific labeling of cells and tissues in addition to analyzing particular biodistributions, pharmacokinetics and biological interactions [148,184]. The plant virus, CPMV, has been extensively used for optical imaging applications.

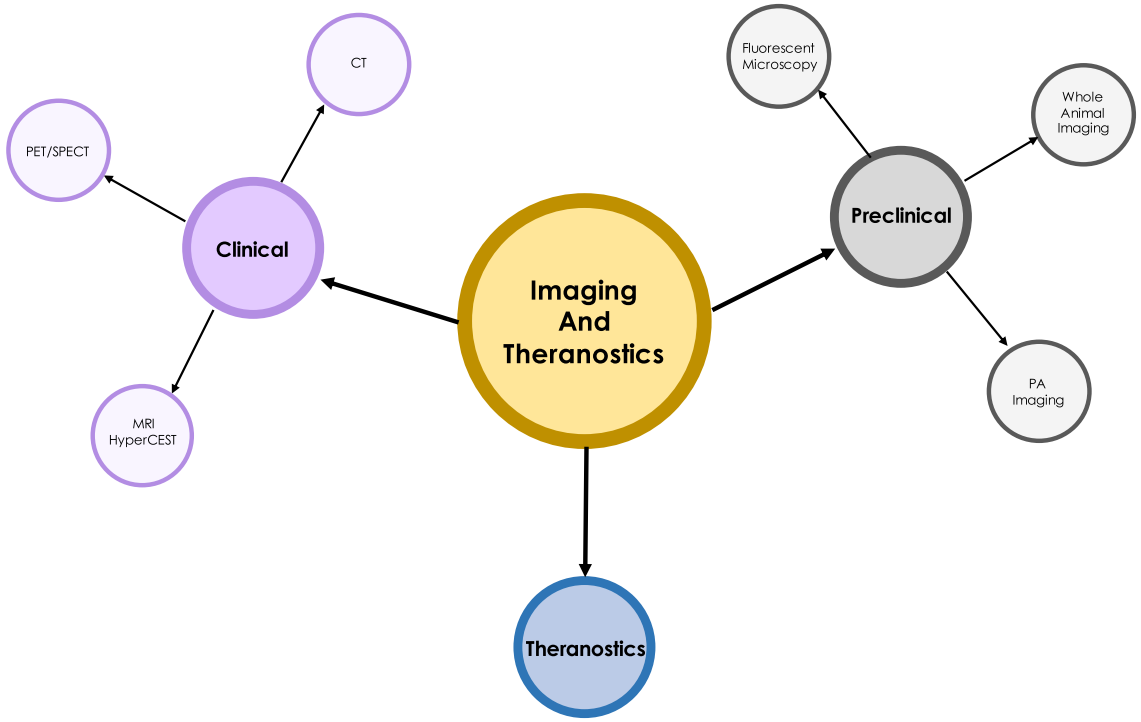

Fig. 7. Different modalities that VLPs can be used in for imaging and theranostics. The gray circles represent imaging modalities mainly used in preclinical settings (e.g. animal studies) or those that are in development for the clinic. The circles in purple represent imaging modalities utilized in the clinic.

A unique feature of CPMV is that it naturally interacts with the mammalian protein surface vimentin and this enables imaging of the endothelium [185], and in particular the tumor endothelium [186]. In an early study, the tumor neovasculature was imaged with fluorescent CPMV VNPs using intravital two-photon laser scanning microscopy (TPLSM) [185]. Future studies explored engineered VNPs with added receptor specificity: for example, CPMV was modified with a peptide ligand, E7p72, which has high affinity for epidermal growth factor-like domain 7 (EGFL7) [187]. The E7p72-CPMV particles showed high specificity to EGFL7 and demonstrated binding to tumor neovasculature within 90 min as seen through real-time intravital imaging. While native CPMV also showed interaction with the endothelium – this was much less profound due to non-specific macrophage clearance. In the CPMV studies, fluorescence was introduced through chemical coupling of near-infrared (NIR) fluorophores. However, genetic engineering approaches have also been exploited to develop fluorescent VLPs. In Section 2.5, we already discussed PVX and TMV engineered to express the small fluorescent protein called iLOV [90] amongst other examples. Further, our lab has constructed PVX VNPs produced in *N. benthamiana* plants that have been genetically engineered to display GFP or mCherry at their N-termini [188]. The PVX displayed 1 fluorescently-modified coat protein for every 3 wild-type coat proteins. The fluorescent PVX was used to track the viral infection process in plants, to optically image HT-29 cells *in vitro*, and for particle tracking *in vivo* using a HT-29 murine model in C57BL/6 mice.

Besides cancer imaging, VLPs have been engineered and studies in the context of CD imaging. One application has focused on the design of VLPs loaded with contrast agents to stratify atherosclerotic plaques, thus improving the accuracy of prognoses and reducing the frequency of heart attacks and strokes. MS2 VLPs have been utilized to carry NIR fluorescent dyes (to enable imaging) and anti-VCAM-1 antibodies (to enable targeting of the sites of inflammation) [189]. The study demonstrated that the engineered VLPs were effective in targeting and detecting atherosclerotic plaques of different sizes in the descending aorta and aortic arch in ApoE knockout mice while untargeted controls showed limited signs of fluorescence in the plaques. Our lab has focused on the study and application of TMV for CD imaging applications targeting both atherosclerosis (early stage disease) and thrombosis (late stage disease). For example, TMV was conjugated to both an NIR dye and a peptide targeting S100A9 (aka myeloid-related protein 14) [190]. S100A9 is a molecular marker found in atherosclerotic lesions that are prone to rupture and was identified as one of the most promising potential markers for acute myocardial infarctions [191,192]. The targeted TMV particles were injected i.v. and detected the presence of macrophage-rich atherosclerotic lesions in $ApoE^{-/-}$ mice while untargeted TMV controls showed insignificant detection capabilities enabling the platform's possible use in the future as a marker for at-risk lesions. The two studies above solely targeted single proteins (e.g. VCAM-1 and S100A9), but targeting different molecules may provide additional information such as the extent of progression of CD. Toward this goal, a trifunctional VLP platform derived from SV40 carrying NIR quantum dots, a targeting peptide, and an anticoagulant drug (Hirulog) was developed for *in vivo* targeting, imaging and treatment of atherosclerotic plaques [193]. Depending on the targeting peptide used (against VCAM-1, macrophages, or fibrin), the VLP could detect atherosclerosis at different stages of CD (early, developmental, and late respectively). Furthermore, the delivery of the Hirulog to aortic atherosclerotic plaques led to increased antithrombotic activity compared to nontargeted VLPs.

Lastly, fluorescent reporters can oftentimes be easily degraded within the body leading to diminished imaging capabilities. To improve imaging times, VLPs can be incorporated into long-lasting formulations using slow-release polymers, scaffolds, and metal-organic frameworks (MOFs). A recent paper by Gassensmith et al. details a TMV-MOF hybrid nanoparticle that increases TMV VNP retention when delivered s.c. into Balb/c mice [194]. Cy5-encpsulated TMV is coated with zeolitic imidazolate framework-8 (Cy5-TMV@ZIF), and when compared to native Cy5-TMV, the Cy5-TMV@ZIF retained its fluorescence for ~2.5x the time of Cy5-TMV (288 h vs. 120 h). Histology studies showed no signs of toxicity due to the MOF. The TMV nanoparticles were also stressed in harsh conditions through heating at 100°C for 20 min or soaking in methanol or 6M guanidinium chloride overnight. While WT TMV showcased significant deterioration, the TMV@ZIF remained stable.

4.1.2. VLPs as MRI contrast agent carriers

Optical imaging techniques are considered safe and do not expose patients to harmful radiation, but they are usually limited to 1–2 mm of tissue penetration and are restricted translationally [195]. On the contrary, MRI, PET, and CT are used extensively in the clinic and can provide extremely accurate images deep within tissues. VLPs can be loaded with MRI, PET or CT contrast agents and these approaches are discussed in the following sections. VLPs can be loaded with hundreds-to-thousands of copies of contrast agents; this increases the local concentration and therefore the signal-to-noise ratio particularly when these nanoparticles are engineered to target specific tissues and cells. When it comes to MRI application, the protein-based contrast agents also confer advantages in reducing residence time of the contrast agents within tissues, therefore lowering the risk of toxicity. For example, contrast agents such as gadolinium (Gd) can be quite toxic; but VLPs are generally cleared rapidly from circulation and tissues therefore reducing systemic toxicity [196]. In early work, we demonstrated that TMV loaded with Gd-dodecane tetraacetic acid (Gd-DOTA) and modified to target VCAM-1 enabled sensitive detection and delineation of atherosclerotic plaques in $ApoE^{-/-}$ mice using submicromolar doses of the contrast agent [197]. The high aspect ratio of TMV promoted ligand-receptor interactions to enhance margination to the diseased vessel wall, and the slower tumbling and enhanced relaxivity of the Gd-DOTA coupled to the macromolecular carrier increased the signal-to-noise ratio. The TMV platform increased the imaging sensitivity considerably allowing for a 400x reduction in Gd dose compared to standard clinical concentrations. Given their high degree of surface functionality offering distinct amino acid residues on both their internal and external surfaces, VLPs make excellent platforms for development of multi-modal imaging probes. In one example from our lab, we demonstrated the loading of a dysprosium (Dy^{3+}) complex in the internal cavity of TMV to increase T2 relaxivity for MRI; and the simultaneous delivery of NIR fluorescent dyes enabled dual optical-MR imaging. Target specificity was achieved by labeling the external surface of TMV with an Asp-Gly-Glu-Ala peptide to target integrin $\alpha_2\beta_1$ on prostate cancer cells. Feasibility of this dual-modal imaging approach was demonstrated in athymic nude mice with PC-3 prostate cancer xenografts [198]. While most VLPs exhibit short circulation times and rapid tissue clearance (~3 days); we discovered that the VLPs from PhMV allow imaging of tumors over extended periods of time (~10 days). In this study, PhMV was modified identically to the aforementioned TMV probe: PhMV was loaded with a NIR dye for optical imaging, Gd-DOTA for MRI, and the Asp-Gly-Glu-Ala targeting peptide; and longitudinal imaging of prostate tumors *in vivo* was demonstrated [184]. The long residence time within tumors may be utilized to follow disease progression and therapeutic responses.

Outside of plant virus nanotechnology, Kang et al. has utilized P22 bacteriophages for intravascular MRI imaging [199]. Here, paramagnetic Gd ions complexed to chelating agent diethylenetriamine pentaacetic acid (DTPA) were conjugated to the interior and exterior of the P22 capsids (P22-Gd-DTPA) using maleimide chemistry. When administered into Balb/c nude mice, the P22-Gd-DTPA contrast agent enabled imaging of the carotid, mammary arteries, jugular vein, and superficial vessels at a resolution of 250 μm (it should be noted that non-targeted P22 was utilized). Using the P22 platform, work has also been done to increase the contrast agent loading capacity through use of polymerization chemistry [200]. Here instead of conjugating the contrast agent to the surface of the capsid; a polymer network is formed within the capsid and the contrast agent is incorporated within, therefore enhancing

loading capacity. Specifically, a 2-aminoethyl methacrylate (AEMA) polymer was synthesized within P22 capsids using ATRP, enabling loading with up to 3646 paramagnetic manganese (III) protoporphyrin IX (MnPP) sites [201]. The resulting ionic relaxivities were comparable to free MnPP, but initial studies suggest the relaxivity could be improved by reducing the intramolecular interactions between MnPP molecules.

Contrast agents in the clinic have been mainly restricted to paramagnetic metal complexes such as Gd and Mn; however, newer generation contrast agents can improve resolution and additionally provide functional information. These newer contrast agents utilize mechanisms such as chemical exchange saturation transfer (CEST) to improve upon current standards [202]. As a proof-of-principle with VLPs, filamentous fd bacteriophages expressing anti-EGFR were transaminated to introduce ketone groups followed by oxime ligation of a cage-like xenon-binding molecule (CryA) [203]. ^{129}Xe NMR spectroscopy of live EGFR-expressing MDA-MB-231 cells showed strong saturation at 70 ppm, indicating successful CEST of Xe@CryA. Previous studies show that this system can be detected at concentrations as low as 230 fM, which is near the forefront of current standards. In another study, TMV loaded with a metal-free paramagnetic nitroxide organic radical contrast agent (ORCA) for MRI and electron paramagnetic resonance probes for superoxide detection showed enhanced *in vitro* r1 and r2 relaxivities [204]. The probes functioned as both T1 and T2 contrast agents making this platform suitable for pre-clinical and clinical MRI scanners. Furthermore, by conjugating fluorescent dyes on the internal surface and ORCA on the outer surface, the TMV probes could be used as a concentration marker regardless of the ORCA oxidation state. In a more recent study by the same group, TMV was conjugated to a derivative of the aminoxyl radical TEMPO (coined Compound 6) by a copper-catalyzed azide-alkyne cycloaddition reaction (TMV-6) [205]. Subsequent binding to cucurbit[8]uril (CB[8]) forms an ORCA inclusion complex called a semirotaxane that helps shield the aminoxyl radicals from reduction into hydroxylamines – a process which makes aminoxyl-based ORCAs MRI-silent. Measurements for r_1 values of TMV-6 rivaled that of Gd-DOTA while the semirotaxane also showed an order of magnitude increase in r_1 compared to CB[8] and 6 alone. Most importantly, in the presence of sodium ascorbate, TMV-6 was reduced at a half-life of 7.2 min while the semirotaxane showcased half-lives of 577.6 min, an increase in two orders of magnitude.

4.1.3. VLPs in PET and CT imaging

While MRI works on the structural level, PET can image at the metabolic level for refined imaging of pathophysiological changes within the human body [206]. PET is mainly used in the clinic for oncological imaging as cancerous cells undergo metabolic changes that PET scans can detect much earlier than CT or MRI. PET is oftentimes combined with CT imaging to provide both structural and metabolic information of tissues within the patient. One example by Francis et al. was the engineering of DOTA-MS2 bacteriophages to deliver ^{64}Cu for microPET/CT imaging [207]. Compared to free ^{64}Cu, which was cleared rapidly, the ^{64}Cu-labeled DOTA-MS2 phages lasted in circulation for over 24 h at > 20% injected dose (ID)/gram although specific tumor homing was not evidenced. To improve the specificity of the PET imaging modalities and improve tumor homing, one can attach targeting ligands. This was demonstrated with RGD peptide-conjugated T7 [208] and anti-EGFR conjugated MS2 bacteriophages [82]. The T7 phages were genetically engineered to express solvent-exposed RGD sequences followed by the chemical addition of ^{64}Cu-DOTA. These phages were able to specifically extravasate into U87MG tumor xenografts displaying up to 10% ID/gram. When cold RGD peptide was added before T7 administration (for receptor blocking), tumor uptake was significantly reduced verifying the specificity of the PET imaging. Also, the EGFR-conjugated MS2 bacteriophages showed up to 10–15% ID/gram 24 h after injection in plasma and up to 2–5% ID/gram within HCC1954 orthotopic breast cancer tumors [82].

PET is not only utilized in solid tumor imaging, but also can be used to track micrometastases in sentinel lymph nodes [209]. NV1023 is an oncolytic herpes simplex virus with preferential targeting towards tumor cells. The virus platform technology was engineered to catalyze the production of [^{18}F]-2′-fluoro-2′-deoxy-1-β-D-β-arabinofuranosyl-5-ethyluracil ([^{18}F]FEAU) upon entering of tumor cells. [^{18}F]FEAU cannot diffuse extracellularly and thus becomes entrapped within the tumor cell – [^{18}F]FEAU is then imaged using PET. In a mouse B16F10 melanoma model, [^{18}F]FEAU PET imaging successfully classified 8 out of 8 tumor-positive nodes with two standard deviations difference of radioactivity levels between tumor-free nodes.

Although CT is oftentimes used concurrently with PET, it can also be employed as a stand-alone imaging modality. Recently, gold-coated VNPs were developed using CPMV. These materials were engineered for CT imaging using scattered X-ray for 3D visual reconstruction and tissue segmentation [210]. The gold-coated CPMV improved the sensitivity of the technique by reducing the scan time to less than 2 min and achieved a resolution of nearly 150 Hounsfield units.

4.2. Photothermal therapy and other theranostic approaches

In newer generation imaging applications, VLPs can be simultaneously loaded with imaging reagents and drugs; and therefore, be used for both diagnosis and therapy – a concept known as theranostics. One of the most promising strategies is PTT, which employs NIR laser photoabsorbers to generate heat for thermal ablation of cancer cells upon laser irradiation [211]. Compared with conventional therapeutic modalities, PTT exhibits unique advantages in cancer therapy such as high specificity, minimal invasiveness, and precise spatial-temporal selectivity [212,213]. Qβ-based VLPs were engineered for PTT through the attachment of sub 7-nm sized gold nanoparticles on their surface; the inner cavity was loaded with upwards of 500 DOX molecules [214]. Upon 6-nanosecond laser irradiation, DOX was rapidly released from the capsid without heating the bulk solution, allowing for highly targeted cell killing of RAW264.7 macrophage and A549 lung cancer cells *in vitro*. Live-cell imaging indicated that only cells inside the irradiated area showed morphological alterations and detachment signifying highly localized treatment capabilities. Another study with Qβ conjugated to metalloporphyrin derivatives and a CD22 receptor targeting glycan was utilized in photodynamic therapy (PDT) [215]. *In vitro* studies with CD22 positive Chinese hamster ovary cells indicated dose-responsive phototoxicity and greater cell killing in targeted vs. non-targeted viral particles. PDT has additionally been utilized with P22 VLPs encapsulating CYP (which converts the prodrug tamoxifen) and decorated with the photosensitizer protoporphyrin IX against estrogen receptor positive breast cancer cells [216]. To confer MRI and photoacoustic imaging capabilities to the PTT treatment for use in theranostics, the nanorods of TMV were used to load Gd and coated with polydopamine (PDA); the PDA coating enhances the MRI properties and confers PDA contrast while enabling PTT [217]. MRI imaging of Gd-TMV nanoparticles without PDA produced r_1 values of up to ~13 $mM^{-1}s^{-1}$, better than the r_1 values of commercial MRI contrast agents (3–5 $mM^{-1}s^{-1}$). PDA coating onto the TMV (Gd-TMV-PDA) led to far greater r_1 values, reaching up to ~80 $mM^{-1}s^{-1}$. This increase in relaxivity may be explained by the fact that the PDA coating traps bulk water in the structure of TMV therefore further enhancing the magnetic properties and interaction of the Gd centers with water. Furthermore, the Gd-TMV-PDA particles showed strong NIR absorption *in vitro* with high photothermal conversion efficiency (28.9%). In comparison, other commonly used photothermal agents report photothermal conversion efficiencies of 24.4% ($Fe@Fe_3O_4$) [218], 13% (gold nanocages) [219], 21% (gold nanorods) [220], and 22% ($Cu_{2-x}Se$) [221]. The nanoparticles showed strong efficacy *in vitro* in treating PC-3 prostate and 4T1 breast cancer cells while the controls of cells + irradiation and cells + Gd-TMV-PDA without irradiation showed minimal cytotoxicity.

Mammalian viruses have also been utilized in PTT. HBc has been used to encapsulate copper sulfide for PTT only [222], conjugated to a RGD peptide for tumor targeting and loaded with indocyanine green for image-guided PTT [223], and loaded with iron oxide and methotrexate for MRI and PTT [224]. Lastly, ferritin based VLPs have been used to load copper sulfate [225] and gold nanoparticles [226] for cancer theranostics.

In some more advanced theranostic modalities, the imaging reagents and drugs can be activated simultaneously. The bacteriophage M13 was conjugated with DOX, a fluorophore, and a targeting ligand to secreted protein, acidic, and rich in cysteine (SPARC), which is overexpressed in melanoma, breast, brain, prostate, colon, and lung cancers [227]. The multifunctional M13 nanoparticles were tested using two prostate cancer lines: one with high SPARC expression (C4-2B) and one with low SPARC expression (DU145). The IC_{50} curves indicated strong cytotoxicity towards the C4-2B cell line, but not the DU145 cells, demonstrating specificity towards SPARC. Fluorescence images showed greater cell adherence of the VLPs towards the C4-2B cell line.

5. Challenges moving forward and future directions

The field of viral drug delivery has taken tremendous steps forward in recent years; this includes the application of plant viruses for treatment of canine cancer patients [112], clinical trials using CpG-laden bacteriophages [70], as well as adenoviral COVID-19 vaccine candidates that are currently in phase 2 clinical testing [228,229]. These examples highlight that the field has grown out of its infancy. However, there remain challenges in further advancing the viral drug delivery field. The most obvious hurdle is whether pre-existing or *de novo* acquired adaptive immunity against the viral carrier would lead to adverse effects or reduce efficacy upon repeat administration [230]. This may be a challenge for traditional drug delivery approaches targeting diseased cells and tissues – however, surface passivation or immune editing methods have shown promise [231–233]. When it comes to immunotherapy applications, the immunogenicity of the carrier is less likely to be a barrier. Rather, opsonization of the carrier primes the VNP for immune cell uptake, therefore boosting efficacy rather than interfering with it. This was validated for both *in situ* vaccine applications and mRNA vaccines delivered by the plant viruses CPMV and CCMV respectively [67,234].

As the field matures and more products enter clinical testing and development, efforts must focus on improving process development and large-scale manufacturing techniques. Lab protocols often involve centrifugation/ultracentrifugation for purification of the viruses or VLPs, but these processes are generally not scalable and require adaptation to industry standards (e.g. chromatography). Also, attention should be paid toward formulation quality control and assurance – when VLPs are produced by (heterologous) expression they are devoid of their genome; however, they can oftentimes package host RNA causing unwanted side effects [235]. Mammalian viruses may also cause horizontal genetic transfer events evolving both humans and viruses in unimaginable ways [236]. These possibilities must be carefully examined and studied as well as regulated.

Lastly, from a regulatory perspective, while mammalian viral vectors and VLP subunit vaccines have long been tested for clinical application, with several formulations already approved and licensed, bacteriophage- and plant virus-based therapeutic approaches are just at the horizon to enter clinical development stages. This is an exciting time for the field, and we hope to see emerging viral nanotechnologies soon make the bench-to-bed transition.

6. Conclusion

As highlighted throughout this article, plant viruses and bacteriophages are recognized nanotechnologies, which have been engineered for diverse applications as nanomachines (Fig. 8). VLPs have evolved to carry and deliver cargo making them natural experts at drug delivery.

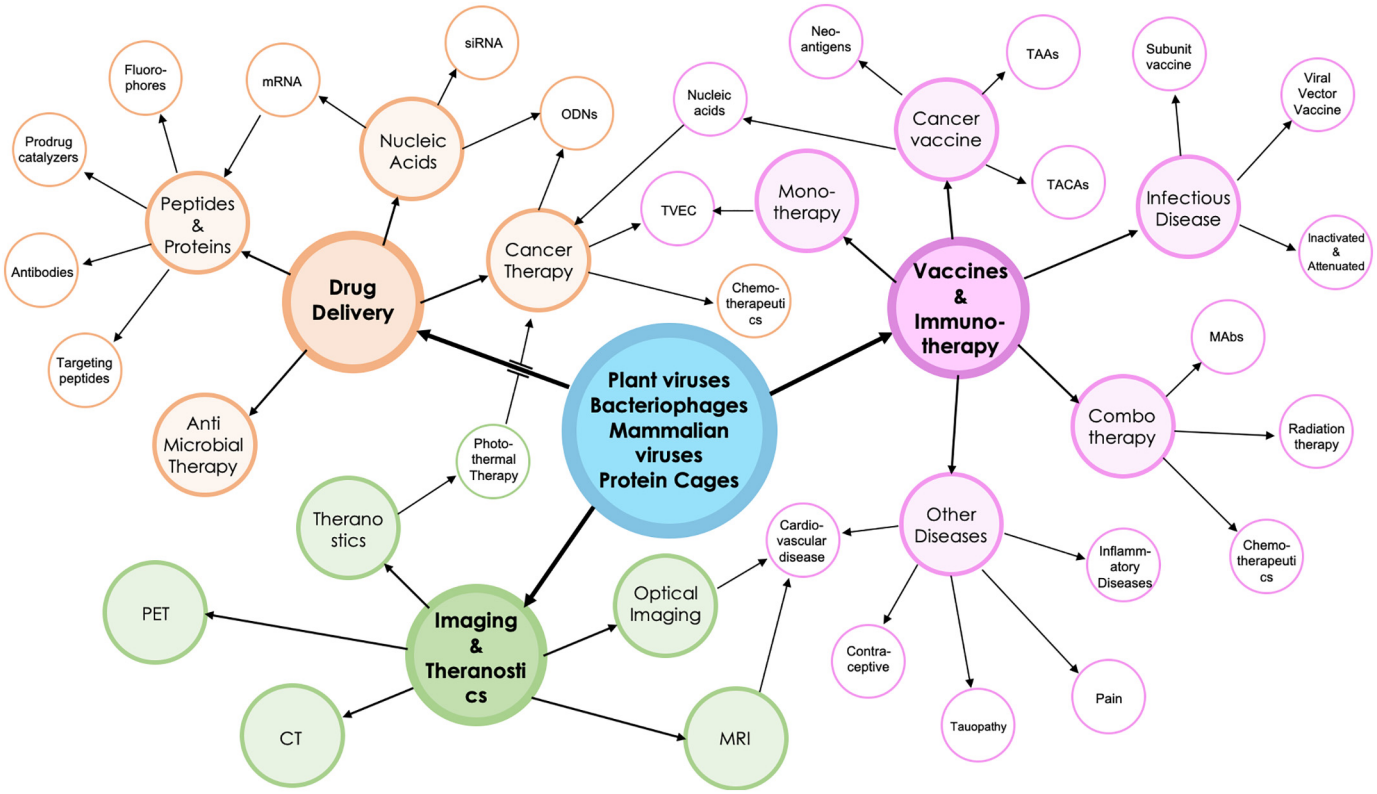

Fig. 8. Bubble diagram of the proposed drug delivery and imaging/theranostic platforms discussed in this review by plant viruses, bacteriophages, mammalian viruses, and protein cages.

Most VLPs can withstand harsh environments, which allows their functionalization and engineering in the test tube; yet, VLPs are biocompatible and biodegradable. The engineering design space is unparalleled enabling genetic programming, self-assembly and chemical biology approaches to be utilized yielding functionalized hybrid VLP nanomaterials. Furthermore, the structures of many viruses are understood at atomic resolution allowing modifications with precise spatial selectivity. The shape and size of VLPs facilitate their vascular transport, cellular uptake and interactions. Large doses of VLPs are usually well tolerated, but proteolytic degradation ensures their rapid and complete clearance for reduced side effects. Moreover, VLPs can be easily engineered to generate new structures that may interact predictably with biological systems; VLPs can display functional groups that include targeting ligands, imaging dyes, epitopes, or they can carry a payload of drugs or dyes to specific cells and tissues. The field of VLPs for drug delivery applications has come a long way since its introduction. As the field continues to grow and flourish, the number of virus-based therapies in clinical trials will continue to propagate and hopefully lead to advanced therapeutics in the clinic in the near future.

Declaration of competing interests

There are no competing interests to declare.

Funding

This work was supported in part by the following grants to N.F.S.: CAREER DMR-1841848 and RAPID CMMI-2027668 from the National Science Foundation; R01 CA202814, R01 HL137674, R01CA224605, U01CA218292 from the National Institute of Health; and 128319-RSG-15-144-01-CDD from the American Cancer Society.

Acknowledgement

Sourabh Shukla is thanked for help with the graphics shown in Fig. 6.

References

[1] L. Sercombe, T. Veerati, F. Moheimani, S.Y. Wu, A.K. Sood, S. Hua, Advances and challenges of liposome assisted drug delivery, Front. Pharmacol. 6 (2015) https://doi.org/10.3389/fphar.2015.00286.
[2] A. Puiggalí-Jou, L.J. del Valle, C. Alemán, Drug delivery systems based on intrinsically conducting polymers, J. Control. Release 309 (2019) 244–264, https://doi.org/10.1016/j.jconrel.2019.07.035.
[3] F. Wang, C. Li, J. Cheng, Z. Yuan, Recent advances on inorganic nanoparticle-based cancer therapeutic agents, Int. J. Environ. Res. Public Health 13 (2016) https://doi.org/10.3390/ijerph13121182.
[4] M. Yan, J. Du, Z. Gu, M. Liang, Y. Hu, W. Zhang, S. Priceman, L. Wu, Z.H. Zhou, Z. Liu, T. Segura, Y. Tang, Y. Lu, A novel intracellular protein delivery platform based on single-protein nanocapsules, Nat. Nanotechnol. 5 (2010) 48–53, https://doi.org/10.1038/nnano.2009.341.
[5] N.F. Steinmetz, Biological and evolutionary concepts for nanoscale engineering: viruses as natural nanoparticles have great potential for a wide range of nanoscale products, EMBO Rep. 20 (2019) https://doi.org/10.15252/embr.201948806e48806.
[6] C. Wang, V. Beiss, N.F. Steinmetz, Cowpea mosaic virus nanoparticles and empty virus-like particles show distinct but overlapping immunostimulatory properties, J. Virol. 93 (2019) https://doi.org/10.1128/JVI.00129-19.
[7] A.O. Elzoghby, W.M. Samy, N.A. Elgindy, Protein-based nanocarriers as promising drug and gene delivery systems, J. Control. Release 161 (2012) 38–49, https://doi.org/10.1016/j.jconrel.2012.04.036.
[8] Virus-based nanocarriers for drug delivery, ScienceDirect 64 (2012) 811–825, https://doi.org/10.1016/j.addr.2012.01.005. https://www.sciencedirect.com/science/article/abs/pii/S0169409X12000087 accessed April 30, 2020.
[9] N. Ferrer-Miralles, E. Rodríguez-Carmona, J.L. Corchero, E. García-Fruitós, E. Vázquez, A. Villaverde, Engineering protein self-assembling in protein-based nanomedicines for drug delivery and gene therapy, Crit. Rev. Biotechnol. 35 (2015) 209–221, https://doi.org/10.3109/07388551.2013.833163.
[10] J. Zhang, K. Zhou, Q. Wang, Tailoring the self-assembly behaviors of recombinant tobacco mosaic virus by rationally introducing covalent bonding at the protein–protein interface, Small 12 (2016) 4955–4959, https://doi.org/10.1002/smll.201503487.
[11] J.F.C. Steele, H. Peyret, K. Saunders, R. Castells-Graells, J. Marsian, Y. Meshcheriakova, G.P. Lomonossoff, Synthetic plant virology for nanobiotechnology and nanomedicine, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 9 (2017) https://doi.org/10.1002/wnan.1447.
[12] L. Lavelle, J.-P. Michel, M. Gingery, The disassembly, reassembly and stability of CCMV protein capsids, J. Virol. Methods 146 (2007) 311–316, https://doi.org/10.1016/j.jviromet.2007.07.020.
[13] H. Cai, S. Shukla, N.F. Steinmetz, The antitumor efficacy of CpG oligonucleotides is improved by encapsulation in plant virus-like particles, Adv. Funct. Mater. 30 (2020) 1908743, https://doi.org/10.1002/adfm.201908743.
[14] L. Li, C. Xu, W. Zhang, F. Secundo, C. Li, Z.-P. Zhang, X.-E. Zhang, F. Li, Cargo-compatible encapsulation in virus-based nanoparticles, Nano Lett. 19 (2019) 2700–2706, https://doi.org/10.1021/acs.nanolett.9b00679.
[15] A.M. Wen, N.F. Steinmetz, Design of virus-based nanomaterials for medicine, biotechnology, and energy, Chem. Soc. Rev. 45 (2016) 4074–4126, https://doi.org/10.1039/C5CS00287G.
[16] K.J. Koudelka, A.S. Pitek, M. Manchester, N.F. Steinmetz, Virus-based nanoparticles as versatile nanomachines, Annu. Rev. Virol. 2 (2015) 379–401, https://doi.org/10.1146/annurev-virology-100114-055141.
[17] M.V. de Ruiter, R.M. van der Hee, A.J.M. Driessen, E.D. Keurhorst, M. Hamid, J.J.L.M. Cornelissen, Polymorphic assembly of virus-capsid proteins around DNA and the cellular uptake of the resulting particles, J. Control. Release 307 (2019) 342–354, https://doi.org/10.1016/j.jconrel.2019.06.019.
[18] I.L. Aanei, J.E. Glasgow, S.L. Capehart, M.B. Francis, Encapsulation of negatively charged cargo in MS2 viral capsids, Methods Mol. Biol. 1776 (2018) 303–317, https://doi.org/10.1007/978-1-4939-7808-3_21.
[19] Y. Ren, S.M. Wong, L.-Y. Lim, Folic acid-conjugated protein cages of a plant virus: a novel delivery platform for doxorubicin, Bioconjug. Chem. 18 (2007) 836–843, https://doi.org/10.1021/bc060361p.
[20] M.B. Sherman, R.H. Guenther, F. Tama, T.L. Sit, C.L. Brooks, A.M. Mikhailov, E.V. Orlova, T.S. Baker, S.A. Lommel, Removal of divalent cations induces structural transitions in red clover necrotic mosaic virus, revealing a potential mechanism for RNA release, J. Virol. 80 (2006) 10395–10406, https://doi.org/10.1128/JVI.01137-06.
[21] D.M. Lockney, R.N. Guenther, L. Loo, W. Overton, R. Antonelli, J. Clark, M. Hu, C. Luft, S.A. Lommel, S. Franzen, The red clover necrotic mosaic virus capsid as a multifunctional cell targeting plant viral nanoparticle, Bioconjug. Chem. 22 (2011) 67–73, https://doi.org/10.1021/bc100361z.
[22] Y. Zhang, Y. Dong, J. Zhou, X. Li, F. Wang, Application of plant viruses as a biotemplate for nanomaterial fabrication, Molecules 23 (2018) https://doi.org/10.3390/molecules23092311.
[23] T. Douglas, M. Young, Host-guest encapsulation of materials by assembled virus protein cages, Nature 393 (1998) 152–155, https://doi.org/10.1038/30211.
[24] C.E. Ashley, E.C. Carnes, G.K. Phillips, P.N. Durfee, M.D. Buley, C.A. Lino, D.P. Padilla, B. Phillips, M.B. Carter, C.L. Willman, C.J. Brinker, J.do.C. Caldeira, B. Chackerian, W. Wharton, D.S. Peabody, Cell-specific delivery of diverse cargos by bacteriophage MS2 virus-like particles, ACS Nano 5 (2011) 5729–5745, https://doi.org/10.1021/nn201397z.
[25] J.D. Fiedler, M.R. Fishman, S.D. Brown, J. Lau, M.G. Finn, Multifunctional enzyme packaging and catalysis in the Qβ protein nanoparticle, Biomacromolecules 19 (2018) 3945–3957, https://doi.org/10.1021/acs.biomac.8b00885.
[26] I. Balke, A. Zeltins, Use of plant viruses and virus-like particles for the creation of novel vaccines, Adv. Drug Deliv. Rev. 145 (2019) 119–129, https://doi.org/10.1016/j.addr.2018.08.007.
[27] J. Sharma, M. Uchida, H.M. Miettinen, T. Douglas, Modular interior loading and exterior decoration of a virus-like particle, Nanoscale 9 (2017) 10420–10430, https://doi.org/10.1039/C7NR03018E.
[28] K. Mohan, G.A. Weiss, Chemically modifying viruses for diverse applications, ACS Chem. Biol. 11 (2016) 1167–1179, https://doi.org/10.1021/acschembio.6b00060.
[29] J.N. de Gruyter, L.R. Malins, P.S. Baran, Residue-specific peptide modification: a chemist's guide, Biochemistry 56 (2017) 3863–3873, https://doi.org/10.1021/acs.biochem.7b00536.
[30] D. Wang, S.L. Capehart, S. Pal, M. Liu, L. Zhang, P.J. Schuck, Y. Liu, H. Yan, M.B. Francis, J.J. De Yoreo, Hierarchical assembly of plasmonic nanostructures using virus capsid scaffolds on DNA origami templates, ACS Nano 8 (2014) 7896–7904, https://doi.org/10.1021/nn5015819.
[31] Q. Hu, W. Sun, C. Wang, Z. Gu, Recent advances of cocktail chemotherapy by combination drug delivery systems, Adv. Drug Deliv. Rev. 98 (2016) 19–34, https://doi.org/10.1016/j.addr.2015.10.022.
[32] M. Keeney, X.Y. Jiang, M. Yamane, M. Lee, S. Goodman, F. Yang, Nanocoating for biomolecule delivery using layer-by-layer self-assembly, J. Mater. Chem. B 3 (2015) 8757–8770, https://doi.org/10.1039/C5TB00450K.
[33] U. Unzueta, M.V. Céspedes, E. Vázquez, N. Ferrer-Miralles, R. Mangues, A. Villaverde, Towards protein-based viral mimetics for cancer therapies, Trends Biotechnol. 33 (2015) 253–258, https://doi.org/10.1016/j.tibtech.2015.02.007.
[34] J. Cao, R.H. Guenther, T.L. Sit, C.H. Opperman, S.A. Lommel, J.A. Willoughby, Loading and release mechanism of red clover necrotic mosaic virus derived plant viral nanoparticles for drug delivery of doxorubicin, Small 10 (2014) 5126–5136, https://doi.org/10.1002/smll.201400558.
[35] Z. Chen, N. Li, L. Chen, J. Lee, J.J. Gassensmith, Dual functionalized bacteriophage Qβ as a photocaged drug carrier, Small 12 (2016) 4563–4571, https://doi.org/10.1002/smll.201601053.
[36] J.A. Finbloom, I.L. Aanei, J.M. Bernard, S.H. Klass, S.K. Elledge, K. Han, T. Ozawa, T.P. Nicolaides, M.S. Berger, M.B. Francis, Evaluation of three morphologically distinct virus-like particles as nanocarriers for convection-enhanced drug delivery to glioblastoma, Nanomaterials (Basel) 8 (2018) https://doi.org/10.3390/nano8121007.
[37] K.L. Lee, L.C. Hubbard, S. Hern, I. Yildiz, M. Gratzl, N.F. Steinmetz, Shape matters: the diffusion rates of TMV rods and CPMV icosahedrons in a spheroid model of extracellular matrix are distinct, Biomater. Sci. 1 (2013) https://doi.org/10.1039/C3BM00191A.

[38] Y. Liu, J. Tan, A. Thomas, D. Ou-Yang, V.R. Muzykantov, The shape of things to come: importance of design in nanotechnology for drug delivery, Ther. Deliv. 3 (2012) 181–194.

[39] M.A. Bruckman, A.E. Czapar, A. VanMeter, L.N. Randolph, N.F. Steinmetz, Tobacco mosaic virus-based protein nanoparticles and nanorods for chemotherapy delivery targeting breast cancer, J. Control. Release 231 (2016) 103–113, https://doi.org/10.1016/j.jconrel.2016.02.045.

[40] D.H.T. Le, K.L. Lee, S. Shukla, U. Commandeur, N.F. Steinmetz, Potato virus X, a filamentous plant viral nanoparticle for doxorubicin delivery in cancer therapy, Nanoscale 9 (2017) 2348–2357, https://doi.org/10.1039/c6nr09099k.

[41] A.S. Pitek, H. Hu, S. Shukla, N.F. Steinmetz, Cancer theranostic applications of albumin-coated tobacco mosaic virus nanoparticles, ACS Appl. Mater. Interfaces 10 (2018) 39468–39477, https://doi.org/10.1021/acsami.8b12499.

[42] R.A. Miller, A.D. Presley, M.B. Francis, Self-assembling light-harvesting systems from synthetically modified tobacco mosaic virus coat proteins, J. Am. Chem. Soc. 129 (2007) 3104–3109, https://doi.org/10.1021/ja063887t.

[43] V.K. Deo, T. Kato, E.Y. Park, Chimeric virus-like particles made using GAG and M1 capsid proteins providing dual drug delivery and vaccination platform, Mol. Pharm. 12 (2015) 839–845, https://doi.org/10.1021/mp500860x.

[44] D. Yan, Z. Teng, S. Sun, S. Jiang, H. Dong, Y. Gao, Y. Wei, W. Qin, X. Liu, H. Yin, H. Guo, Foot-and-mouth disease virus-like particles as integrin-based drug delivery system achieve targeting anti-tumor efficacy, Nanomedicine 13 (2017) 1061–1070, https://doi.org/10.1016/j.nano.2016.12.007.

[45] W. Shan, D. Zhang, Y. Wu, X. Lv, B. Hu, X. Zhou, S. Ye, S. Bi, L. Ren, X. Zhang, Modularized peptides modified HBc virus-like particles for encapsulation and tumor-targeted delivery of doxorubicin, Nanomedicine 14 (2018) 725–734, https://doi.org/10.1016/j.nano.2017.12.002.

[46] Q.X. Thong, R. Biabanikhankahdani, K.L. Ho, N.B. Alitheen, W.S. Tan, Thermally-responsive virus-like particle for targeted delivery of cancer drug, Sci. Rep. 9 (2019) 1–14, https://doi.org/10.1038/s41598-019-40388-x.

[47] J.K. Pokorski, K. Breitenkamp, L.O. Liepold, S. Qazi, M.G. Finn, Functional virus-based polymer–protein nanoparticles by atom transfer radical polymerization, J. Am. Chem. Soc. 133 (2011) 9242–9245, https://doi.org/10.1021/ja203286n.

[48] T.C. Johnstone, K. Suntharalingam, S.J. Lippard, The next generation of platinum drugs: targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs, Chem. Rev. 116 (2016) 3436–3486, https://doi.org/10.1021/acs.chemrev.5b00597.

[49] C.E. Franke, A.E. Czapar, R.B. Patel, N.F. Steinmetz, Tobacco mosaic virus-delivered cisplatin restores efficacy in platinum-resistant ovarian cancer cells, Mol. Pharm. 15 (2018) 2922–2931, https://doi.org/10.1021/acs.molpharmaceut.7b00466.

[50] A.A. Vernekar, G. Berger, A.E. Czapar, F.A. Veliz, D.I. Wang, N.F. Steinmetz, S.J. Lippard, Speciation of phenanthriplatin and its analogs in the core of tobacco mosaic virus, J. Am. Chem. Soc. 140 (2018) 4279–4287, https://doi.org/10.1021/jacs.7b12697.

[51] X. Liu, B. Liu, S. Gao, Z. Wang, Y. Tian, M. Wu, S. Jiang, Z. Niu, Glyco-decorated tobacco mosaic virus as a vector for cisplatin delivery, J. Mater. Chem. B 5 (2017) 2078–2085, https://doi.org/10.1039/C7TB00100B.

[52] S. Gao, X. Liu, Z. Wang, S. Jiang, M. Wu, Y. Tian, Z. Niu, Fluorous interaction induced self-assembly of tobacco mosaic virus coat protein for cisplatin delivery, Nanoscale 10 (2018) 11732–11736, https://doi.org/10.1039/C8NR03748E.

[53] R.D. Lin, N.F. Steinmetz, Tobacco mosaic virus delivery of mitoxantrone for cancer therapy, Nanoscale 10 (2018) 16307–16313, https://doi.org/10.1039/c8nr04142c.

[54] P. Lam, R. Lin, N. Steinmetz, Delivery of mitoxantrone using a plant virus-based nanoparticle for the treatment of glioblastomas, J. Mater. Chem. B 6 (2018) https://doi.org/10.1039/C8TB01191E.

[55] D.L. Kernan, A.M. Wen, A.S. Pitek, N.F. Steinmetz, Featured article: delivery of chemotherapeutic vcMMAE using tobacco mosaic virus nanoparticles, Exp. Biol. Med. (Maywood) 242 (2017) 1405–1411, https://doi.org/10.1177/1535370217719222.

[56] C.L. Ventola, The antibiotic resistance crisis, Pharm. Ther. 40 (2015) 277–283.

[57] I. Yacoby, M. Shamis, H. Bar, D. Shabat, I. Benhar, Targeting antibacterial agents by using drug-carrying filamentous bacteriophages, Antimicrob. Agents Chemother. 50 (2006) 2087–2097, https://doi.org/10.1128/AAC.00169-06.

[58] S.N. Crooke, J. Schimer, I. Raji, B. Wu, A.K. Oyelere, M.G. Finn, Lung tissue delivery of virus-like particles mediated by macrolide antibiotics, Mol. Pharm. 16 (2019) 2947–2955, https://doi.org/10.1021/acs.molpharmaceut.9b00180.

[59] Gene Therapy Clinical Trials Worldwide, http://www.abedia.com/wiley/ 2019 accessed April 30, 2020.

[60] B. Bakhshinejad, Phage display and targeting peptides: surface functionalization of nanocarriers for delivery of small non-coding RNAs, Front. Genet. 6 (2015) https://doi.org/10.3389/fgene.2015.00178.

[61] Y. Sun, Y. Sun, R. Zhao, Establishment of MicroRNA delivery system by PP7 bacteriophage-like particles carrying cell-penetrating peptide, J. Biosci. Bioeng. 124 (2017) 242–249, https://doi.org/10.1016/j.jbiosc.2017.03.012.

[62] G. Wang, T. Jia, X. Xu, L. Chang, R. Zhang, Y. Fu, Y. Li, X. Yang, K. Zhang, G. Lin, Y. Han, J. Li, Novel miR-122 delivery system based on MS2 virus like particle surface displaying cell-penetrating peptide TAT for hepatocellular carcinoma, Oncotarget 7 (2016) 59402–59416, https://doi.org/10.18632/oncotarget.10681.

[63] Y. Tian, M. Zhou, H. Shi, S. Gao, G. Xie, M. Zhu, M. Wu, J. Chen, Z. Niu, Integration of cell-penetrating peptides with rod-like bionanoparticles: virus-inspired gene-silencing technology, Nano Lett. 18 (2018) 5453–5460, https://doi.org/10.1021/acs.nanolett.8b01805.

[64] H.-H. Pang, C.-Y. Huang, Y.-W. Chou, C.-J. Lin, Z.-L. Zhou, Y.-L. Shiue, K.-C. Wei, H.-W. Yang, Bioengineering fluorescent virus-like particle/RNAi nanocomplexes act synergistically with temozolomide to eradicate brain tumors, Nanoscale 11 (2019) 8102–8109, https://doi.org/10.1039/c9nr01247h.

[65] P. Lam, N.F. Steinmetz, Delivery of siRNA therapeutics using cowpea chlorotic mottle virus-like particles, Biomater. Sci. 7 (2019) 3138–3142, https://doi.org/10.1039/C9BM00785G.

[66] O. Azizgolshani, R.F. Garmann, R. Cadena-Nava, C.M. Knobler, W.M. Gelbart, Reconstituted plant viral capsids can release genes to mammalian cells, Virology 441 (2013) 12–17, https://doi.org/10.1016/j.virol.2013.03.001.

[67] A. Biddlecome, H.H. Habte, K.M. McGrath, S. Sambanthamoorthy, M. Wurm, M.M. Sykora, C.M. Knobler, I.C. Lorenz, M. Lasaro, K. Elbers, W.M. Gelbart, Delivery of self-amplifying RNA vaccines in in vitro reconstituted virus-like particles, PLoS One 14 (2019), e0215031. https://doi.org/10.1371/journal.pone.0215031.

[68] Y. Zhou, P.D. Maharaj, J.K. Mallajosyula, A.A. McCormick, C.M. Kearney, In planta production of flock house virus transencapsidated RNA and its potential use as a vaccine, Mol. Biotechnol. 57 (2015) 325–336, https://doi.org/10.1007/s12033-014-9826-1.

[69] T. Storni, C. Ruedl, K. Schwarz, R.A. Schwendener, W.A. Renner, M.F. Bachmann, Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects, J. Immunol. 172 (2004) 1777–1785, https://doi.org/10.4049/jimmunol.172.3.1777.

[70] Phase 1B Study Evaluating Alternative Routes of Administration of CMP-001 in Combination With Pembrolizumab in Participants With Advanced Melanoma - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03084640 2017 accessed April 30, 2020.

[71] A. O'Neil, C. Reichhardt, B. Johnson, P.E. Prevelige, T. Douglas, Genetically programmed in vivo packaging of protein cargo and its controlled release from bacteriophage P22, Angew. Chem. Int. Ed. Eng. 50 (2011) 7425–7428, https://doi.org/10.1002/anie.201102036.

[72] M. Comellas-Aragonès, H. Engelkamp, V.I. Claessen, N.A.J.M. Sommerdijk, A.E. Rowan, P.C.M. Christianen, J.C. Maan, B.J.M. Verduin, J.J.L.M. Cornelissen, R.J.M. Nolte, A virus-based single-enzyme nanoreactor, Nat. Nanotechnol. 2 (2007) 635–639, https://doi.org/10.1038/nnano.2007.299.

[73] Jeff E. Glasgow, Stacy L. Capehart, Matthew B. Francis, Danielle Tullman-Ercek, Osmolyte-mediated encapsulation of proteins inside MS2 viral capsids, ACS Nano 6 (2012) 8658–8664, https://doi.org/10.1021/nn302183h.

[74] J.L. Liu, A.B. Dixit, K.L. Robertson, E. Qiao, L.W. Black, Viral nanoparticle-encapsidated enzyme and restructured DNA for cell delivery and gene expression, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 13319–13324, https://doi.org/10.1073/pnas.1321940111.

[75] N.H. Dashti, R.S. Abidin, F. Sainsbury, Programmable in vitro coencapsidation of guest proteins for intracellular delivery by virus-like particles, ACS Nano 12 (2018) 4615–4623, https://doi.org/10.1021/acsnano.8b01059.

[76] R. Koyani, J. Pérez-Robles, R.D. Cadena-Nava, R. Vazquez-Duhalt, Biomaterial-based nanoreactors, an alternative for enzyme delivery, Nanotechnol. Rev. 6 (2017) 405–419, https://doi.org/10.1515/ntrev-2016-0071.

[77] L. Sánchez-Sánchez, R.D. Cadena-Nava, L.A. Palomares, J. Ruiz-Garcia, M.S.T. Koay, J.J.M.T. Cornelissen, R. Vazquez-Duhalt, Chemotherapy pro-drug activation by biocatalytic virus-like nanoparticles containing cytochrome P450, Enzym. Microb. Technol. 60 (2014) 24–31, https://doi.org/10.1016/j.enzmictec.2014.04.003.

[78] E. Strable, M.G. Finn, Chemical modification of viruses and virus-like particles, in: M. Manchester, N.F. Steinmetz (Eds.), Viruses and Nanotechnology, Springer, Berlin, Heidelberg 2009, pp. 1–21, https://doi.org/10.1007/978-3-540-69379-6_1.

[79] L. Sánchez-Sánchez, A. Tapia-Moreno, K. Juarez-Moreno, D.P. Patterson, R.D. Cadena-Nava, T. Douglas, R. Vazquez-Duhalt, Design of a VLP-nanovehicle for CYP450 enzymatic activity delivery, J. Nanobiotechnol. 13 (2015) 66, https://doi.org/10.1186/s12951-015-0127-z.

[80] S.M. DePorter, B.R. McNaughton, Engineered M13 bacteriophage nanocarriers for intracellular delivery of exogenous proteins to human prostate cancer cells, Bioconjug. Chem. 25 (2014) 1620–1625, https://doi.org/10.1021/bc500339k.

[81] T. Inoue, M. Kawano, R. Takahashi, H. Tsukamoto, T. Enomoto, T. Imai, K. Kataoka, H. Handa, Engineering of SV40-based nano-capsules for delivery of heterologous proteins as fusions with the minor capsid proteins VP2/3, J. Biotechnol. 134 (2008) 181–192, https://doi.org/10.1016/j.jbiotec.2007.12.006.

[82] I.L. Aanei, A.M. ElSohly, M.E. Farkas, C. Netirojjanakul, M. Regan, S. Taylor Murphy, J.P. O'Neil, Y. Seo, M.B. Francis, Biodistribution of antibody-MS2 viral capsid conjugates in breast cancer models, Mol. Pharm. 13 (2016) 3764–3772, https://doi.org/10.1021/acs.molpharmaceut.6b00566.

[83] C.A. Lino, J.C. Caldeira, D.S. Peabody, Display of single-chain variable fragments on bacteriophage MS2 virus-like particles, J. Nanobiotechnology 15 (2017) 13, https://doi.org/10.1186/s12951-016-0240-7.

[84] L. Zhao, M. Kopylov, C.S. Potter, B. Carragher, M.G. Finn, Engineering the PP7 virus capsid as a peptide display platform, ACS Nano 13 (2019) 4443–4454, https://doi.org/10.1021/acsnano.8b09683.

[85] J. Wang, T. Fang, M. Li, W. Zhang, Z.-P. Zhang, X.-E. Zhang, F. Li, Intracellular delivery of peptide drugs using viral nanoparticles of bacteriophage P22: covalent loading and cleavable release, J. Mater. Chem. B 6 (2018) 3716–3726, https://doi.org/10.1039/C8TB00186C.

[86] C. Lico, F. Capuano, G. Renzone, M. Donini, C. Marusic, A. Scaloni, E. Benvenuto, S. Baschieri, Peptide display on Potato virus X: molecular features of the coat protein-fused peptide affecting cell-to-cell and phloem movement of chimeric virus particles, J. Gen. Virol. 87 (2006) 3103–3112, https://doi.org/10.1099/vir.0.82097-0.

[87] K. Uhde-Holzem, M. McBurney, B.D.B. Tiu, R.C. Advincula, R. Fischer, U. Commandeur, N.F. Steinmetz, Production of immunoabsorbent nanoparticles by displaying single-domain protein A on potato virus X, Macromol. Biosci. 16 (2016) 231–241, https://doi.org/10.1002/mabi.201500280.

[88] L. Yang, M.E. Biswas, P. Chen, Study of binding between protein A and immunoglobulin G using a surface tension probe, Biophys. J. 84 (2003) 509–522, https://doi.org/10.1016/S0006-3495(03)74870-X.
[89] J. Röder, C. Dickmeis, R. Fischer, U. Commandeur, Systemic infection of nicotiana benthamiana with potato virus X nanoparticles presenting a fluorescent iLOV polypeptide fused directly to the coat protein, Biomed. Res. Int. (2018), e9328671. https://doi.org/10.1155/2018/9328671.
[90] Sean Chapman, Christine Faulkner, Eirini Kaiserli, Carlos Garcia-Mata, Eugene I. Savenkov, Alison G. Roberts, Karl J. Oparka, John M. Christie, The photoreversible fluorescent protein iLOV outperforms GFP as a reporter of plant virus infection, PNAS 105 (2008) 20038–20043, https://doi.org/10.1073/pnas.0807551105.
[91] D.H.T. Le, U. Commandeur, N.F. Steinmetz, Presentation and delivery of tumor necrosis factor-related apoptosis-inducing ligand via elongated plant viral nanoparticle enhances antitumor efficacy, ACS Nano 13 (2019) 2501–2510, https://doi.org/10.1021/acsnano.8b09462.
[92] S.J. Kaczmarczyk, K. Sitaraman, H.A. Young, S.H. Hughes, D.K. Chatterjee, Protein delivery using engineered virus-like particles, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 16998–17003, https://doi.org/10.1073/pnas.1101874108.
[93] S.J. McRae, J.S. Ginsberg, Initial treatment of venous thromboembolism, Circulation. 110 (2004) I3–I9, https://doi.org/10.1161/01.CIR.0000140904.52752.0c.
[94] A.S. Pitek, J. Park, Y. Wang, H. Gao, H. Hu, D.I. Simon, N.F. Steinmetz, Delivery of thrombolytic therapy using rod-shaped plant viral nanoparticles decreases the risk of hemorrhage, Nanoscale 10 (2018) 16547–16555, https://doi.org/10.1039/c8nr02861c.
[95] D.J. Miller, J.R. Simpson, B. Silver, Safety of thrombolysis in acute ischemic stroke: a review of complications, risk factors, and newer technologies, Neurohospitalist 1 (2011) 138–147, https://doi.org/10.1177/1941875211408731.
[96] J. Chroboczek, I. Szurgot, E. Szolajska, Virus-like particles as vaccine, Acta Biochim. Pol. 61 (2014) 531–539.
[97] T.F. Gajewski, H. Schreiber, Y.-X. Fu, Innate and adaptive immune cells in the tumor microenvironment, Nat. Immunol. 14 (2013) 1014–1022, https://doi.org/10.1038/ni.2703.
[98] M.-È. Lebel, M.-P. Langlois, J.-F. Daudelin, E. Tarrab, P. Savard, D. Leclerc, A. Lamarre, Complement component 3 regulates IFN-α production by plasmacytoid dendritic cells following TLR7 activation by a plant virus–like nanoparticle, J. Immunol. 198 (2017) 292–299, https://doi.org/10.4049/jimmunol.1601271.
[99] P.K. Bommareddy, M. Shettigar, H.L. Kaufman, Integrating oncolytic viruses in combination cancer immunotherapy, Nat. Rev. Immunol. 18 (2018) 498–513, https://doi.org/10.1038/s41577-018-0014-6.
[100] M.C. Brown, E.K. Holl, D. Boczkowski, E. Dobrikova, M. Mosaheb, V. Chandramohan, D.D. Bigner, M. Gromeier, S.K. Nair, Cancer immunotherapy with recombinant poliovirus induces IFN-dominant activation of dendritic cells and tumor antigen-specific CTLs, Sci. Transl. Med. 9 (2017) https://doi.org/10.1126/scitranslmed.aan4220.
[101] R.M. Conry, B. Westbrook, S. McKee, T.G. Norwood, Talimogene laherparepvec: first in class oncolytic virotherapy, Hum. Vaccin. Immunother. 14 (2018) 839–846, https://doi.org/10.1080/21645515.2017.1412896.
[102] P.K. Bommareddy, A. Patel, S. Hossain, H.L. Kaufman, Talimogene laherparepvec (T-VEC) and other oncolytic viruses for the treatment of melanoma, Am. J. Clin. Dermatol. 18 (2017) 1–15, https://doi.org/10.1007/s40257-016-0238-9.
[103] H.L. Kaufman, C.E. Ruby, T. Hughes, C.L. Slingluff, Current status of granulocyte-macrophage colony-stimulating factor in the immunotherapy of melanoma, J. Immunother. Cancer 2 (2014) 11, https://doi.org/10.1186/2051-1426-2-11.
[104] Combining Talimogene Laherparepvec With BRAF and MEK Inhibitors in BRAF Mutated Advanced Melanoma - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03088176 2017 accessed March 26, 2020.
[105] T-VEC in Non-melanoma Skin Cancer - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03458117 2018 accessed March 26, 2020.
[106] Study of Talimogene Laherparepvec (T-VEC) in Pancreatic Cancer - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03086642 2017 accessed March 26, 2020.
[107] Study of T-VEC in Locally Advanced Cutaneous Angiosarcoma - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03921073 2019 accessed March 26, 2020.
[108] P.H. Lizotte, A.M. Wen, M.R. Sheen, J. Fields, P. Rojanasopondist, N.F. Steinmetz, S. Fiering, In situ vaccination with cowpea mosaic virus nanoparticles suppresses metastatic cancer, Nat. Nanotechnol. 11 (2016) 295–303, https://doi.org/10.1038/nnano.2015.292.
[109] C. Wang, S.N. Fiering, N.F. Steinmetz, Cowpea mosaic virus promotes anti-tumor activity and immune memory in a mouse ovarian tumor model, Adv. Ther. 2 (2019) 1900003, https://doi.org/10.1002/adtp.201900003.
[110] A.A. Murray, C. Wang, S. Fiering, N.F. Steinmetz, In situ vaccination with cowpea vs tobacco mosaic virus against melanoma, Mol. Pharm. 15 (2018) 3700–3716, https://doi.org/10.1021/acs.molpharmaceut.8b00316.
[111] A. Kerstetter-Fogle, S. Shukla, C. Wang, V. Beiss, P.L.R. Harris, A.E. Sloan, N.F. Steinmetz, Plant virus-like particle in situ vaccine for intracranial glioma immunotherapy, Cancers (Basel) 11 (2019) https://doi.org/10.3390/cancers11040515.
[112] P.J. Hoopes, R.J. Wagner, K. Duval, K. Kang, D.J. Gladstone, K.L. Moodie, M. Crary-Burney, H. Ariaspulido, F.A. Veliz, N.F. Steinmetz, S.N. Fiering, Treatment of canine oral melanoma with nanotechnology-based immunotherapy and radiation, Mol. Pharm. 15 (2018) 3717–3722, https://doi.org/10.1021/acs.molpharmaceut.8b00126.
[113] A.E. Czapar, B.D.B. Tiu, F.A. Veliz, J.K. Pokorski, N.F. Steinmetz, Slow-release formulation of cowpea mosaic virus for in situ vaccine delivery to treat ovarian cancer, Adv. Sci. 5 (2018) 1700991, https://doi.org/10.1002/advs.201700991.
[114] M.-È. Lebel, K. Chartrand, E. Tarrab, P. Savard, D. Leclerc, A. Lamarre, Potentiating cancer immunotherapy using papaya mosaic virus-derived nanoparticles, Nano Lett. 16 (2016) 1826–1832, https://doi.org/10.1021/acs.nanolett.5b04877.
[115] F. Eriksson, P. Tsagozis, K. Lundberg, R. Parsa, S.M. Mangsbo, M.A.A. Persson, R.A. Harris, P. Pisa, Tumor-specific bacteriophages induce tumor destruction through activation of tumor-associated macrophages, J. Immunol. 182 (2009) 3105–3111, https://doi.org/10.4049/jimmunol.0800224.
[116] I. Dagogo-Jack, A.T. Shaw, Tumour heterogeneity and resistance to cancer therapies, Nat. Rev. Clin. Oncol. 15 (2018) 81–94, https://doi.org/10.1038/nrclinonc.2017.166.
[117] K.L. Lee, A.A. Murray, D.H.T. Le, M.R. Sheen, S. Shukla, U. Commandeur, S. Fiering, N.F. Steinmetz, Combination of plant virus nanoparticle-based in situ vaccination with chemotherapy potentiates antitumor response, Nano Lett. 17 (2017) 4019–4028, https://doi.org/10.1021/acs.nanolett.7b00107.
[118] H. Cai, C. Wang, S. Shukla, N.F. Steinmetz, Cowpea mosaic virus immunotherapy combined with cyclophosphamide reduces breast cancer tumor burden and inhibits lung metastasis, Adv. Sci. (Weinh) 6 (2019) https://doi.org/10.1002/advs.2018022811802281.
[119] C. Wang, N.F. Steinmetz, CD47 blockade and cowpea mosaic virus nanoparticle in situ vaccination triggers phagocytosis and tumor killing, Adv. Healthc. Mater. 8 (2019) 1801288, https://doi.org/10.1002/adhm.201801288.
[120] C. Song, H. Phuengkham, Y.S. Kim, V.V. Dinh, I. Lee, I.W. Shin, H.S. Shin, S.M. Jin, S.H. Um, H. Lee, K.S. Hong, S.-M. Jin, E. Lee, T.H. Kang, Y.-M. Park, Y.T. Lim, Syringeable immunotherapeutic nanogel reshapes tumor microenvironment and prevents tumor metastasis and recurrence, Nat. Commun. 10 (2019) 1–15, https://doi.org/10.1038/s41467-019-11730-8.
[121] R. Patel, A.E. Czapar, S. Fiering, N.L. Oleinick, N.F. Steinmetz, Radiation therapy combined with cowpea mosaic virus nanoparticle in situ vaccination initiates immune-mediated tumor regression, ACS Omega 3 (2018) 3702–3707, https://doi.org/10.1021/acsomega.8b00227.
[122] P. Dai, W. Wang, N. Yang, C. Serna-Tamayo, J.M. Ricca, D. Zamarin, S. Shuman, T. Merghoub, J.D. Wolchok, L. Deng, Intratumoral delivery of inactivated modified vaccinia virus Ankara (iMVA) induces systemic antitumor immunity via STING and Batf3-dependent dendritic cells, Sci. Immunol. 2 (2017) https://doi.org/10.1126/sciimmunol.aal1713.
[123] M.-C. Bourgeois-Daigneault, D.G. Roy, A.S. Aitken, N. El Sayes, N.T. Martin, O. Varette, T. Falls, L.E. St-Germain, A. Pelin, B.D. Lichty, D.F. Stojdl, G. Ungerechts, J.-S. Diallo, J.C. Bell, Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy, Sci. Transl. Med. 10 (2018) https://doi.org/10.1126/scitranslmed.aao1641.
[124] A. Samson, K.J. Scott, D. Taggart, E.J. West, E. Wilson, G.J. Nuovo, S. Thomson, R. Corns, R.K. Mathew, M.J. Fuller, T.J. Kottke, J.M. Thompson, E.J. Ilett, J.V. Cockle, P. van Hille, G. Sivakumar, E.S. Polson, S.J. Turnbull, E.S. Appleton, G. Migneco, A.S. Rose, M.C. Coffey, D.A. Beirne, F.J. Collinson, C. Ralph, D. Alan Anthoney, C.J. Twelves, A.J. Furness, S.A. Quezada, H. Wurdak, F. Errington-Mais, H. Pandha, K.J. Harrington, P.J. Selby, R.G. Vile, S.D. Griffin, L.F. Stead, S.C. Short, A.A. Melcher, Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade, Sci. Transl. Med. 10 (2018) https://doi.org/10.1126/scitranslmed.aam7577.
[125] P.K. Bommareddy, S. Aspromonte, A. Zloza, S.D. Rabkin, H.L. Kaufman, MEK inhibition enhances oncolytic virus immunotherapy through increased tumor cell killing and T cell activation, Sci. Transl. Med. 10 (2018) https://doi.org/10.1126/scitranslmed.aau0417.
[126] J. Schiller, D. Lowy, Explanations for the high potency of HPV prophylactic vaccines, Vaccine 36 (2018) 4768–4773, https://doi.org/10.1016/j.vaccine.2017.12.079.
[127] M.O. Mohsen, L. Zha, G. Cabral-Miranda, M.F. Bachmann, Major findings and recent advances in virus-like particle (VLP)-based vaccines, Semin. Immunol. 34 (2017) 123–132, https://doi.org/10.1016/j.smim.2017.08.014.
[128] M. Kanekiyo, C.-J. Wei, H.M. Yassine, P.M. McTamney, J.C. Boyington, J.R.R. Whittle, S.S. Rao, W.-P. Kong, L. Wang, G.J. Nabel, Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies, Nature. 499 (2013) 102–106, https://doi.org/10.1038/nature12202.
[129] M. Kanekiyo, W. Bu, M.G. Joyce, G. Meng, J.R.R. Whittle, U. Baxa, T. Yamamoto, S. Narpala, J.-P. Todd, S.S. Rao, A.B. McDermott, R.A. Koup, M.G. Rossmann, J.R. Mascola, B.S. Graham, J.I. Cohen, G.J. Nabel, Rational design of an epstein-barr virus vaccine targeting the receptor-binding site, Cell 162 (2015) 1090–1100, https://doi.org/10.1016/j.cell.2015.07.043.
[130] Y. Wu, D.L. Narum, S. Fleury, G. Jennings, A. Yadava, Particle-based platforms for malaria vaccines, Vaccine 33 (2015) 7518–7524, https://doi.org/10.1016/j.vaccine.2015.09.097.
[131] S. Tang, B. Xuan, X. Ye, Z. Huang, Z. Qian, A modular vaccine development platform based on sortase-mediated site-specific tagging of antigens onto virus-like particles, Sci. Rep. 6 (2016) 25741, https://doi.org/10.1038/srep25741.
[132] B. Schwarz, K.M. Morabito, T.J. Ruckwardt, D.P. Patterson, J. Avera, H.M. Miettinen, B.S. Graham, T. Douglas, Viruslike particles encapsidating respiratory syncytial virus M and M2 proteins induce robust T cell responses, ACS Biomater. Sci. Eng. 2 (2016) 2324–2332, https://doi.org/10.1021/acsbiomaterials.6b00532.
[133] J.M. Schenkel, K.A. Fraser, L.K. Beura, K.E. Pauken, V. Vezys, D. Masopust, Resident memory CD8 T cells trigger protective innate and adaptive immune responses, Science 346 (2014) 98–101, https://doi.org/10.1126/science.1254536.
[134] D.R. Burton, L. Hangartner, Broadly neutralizing antibodies to HIV and their role in vaccine design, Annu. Rev. Immunol. 34 (2016) 635–659, https://doi.org/10.1146/annurev-immunol-041015-055515.
[135] H. Elsayed, G. Nabi, W.J. McKinstry, K.K. Khoo, J. Mak, A.M. Salazar, M. Tenbusch, V. Temchura, K. Überla, Intrastructural help: harnessing t helper cells induced by

licensed vaccines for improvement of HIV Env antibody responses to virus-like particle vaccines, J. Virol. 92 (2018) https://doi.org/10.1128/JVI.00141-18.
[136] K. Sliepen, G. Ozorowski, J.A. Burger, T. van Montfort, M. Stunnenberg, C. LaBranche, D.C. Montefiori, J.P. Moore, A.B. Ward, R.W. Sanders, Presenting native-like HIV-1 envelope trimers on ferritin nanoparticles improves their immunogenicity, Retrovirology 12 (2015) 82, https://doi.org/10.1186/s12977-015-0210-4.
[137] L. He, S. Kumar, J.D. Allen, D. Huang, X. Lin, C.J. Mann, K.L. Saye-Francisco, J. Copps, A. Sarkar, G.S. Blizard, G. Ozorowski, D. Sok, M. Crispin, A.B. Ward, D. Nemazee, D.R. Burton, I.A. Wilson, J. Zhu, HIV-1 vaccine design through minimizing envelope metastability, Sci. Adv. 4 (2018) https://doi.org/10.1126/sciadv.aau6769eaau6769.
[138] Kwinten Sliepen, Byung Woo Han, et al., Structure and immunogenicity of a stabilized HIV-1 envelope trimer based on a group-M consensus sequence, Nat. Commun. 10 (2019) 2355, https://doi.org/10.1038/s41467-019-10262-5.
[139] Talar Tokatlian, Benjamin J. Read, Christopher A. Jones, Daniel W. Kulp, Sergey Menis, Jason Y.H. Chang, et al., Innate immune recognition of glycans targets HIV nanoparticle immunogens to germinal centers, Science 363 (2019) 649–654, https://doi.org/10.1126/science.aat9120.
[140] P.J.M. Brouwer, A. Antanasijevic, Z. Berndsen, A. Yasmeen, B. Fiala, T.P.L. Bijl, I. Bontjer, J.B. Bale, W. Sheffler, J.D. Allen, A. Schorcht, J.A. Burger, M. Camacho, D. Ellis, C.A. Cottrell, A.-J. Behrens, M. Catalano, I. del Moral-Sánchez, T.J. Ketas, C. LaBranche, M.J. van Gils, K. Sliepen, L.J. Stewart, M. Crispin, D.C. Montefiori, D. Baker, J.P. Moore, P.J. Klasse, A.B. Ward, N.P. King, R.W. Sanders, Enhancing and shaping the immunogenicity of native-like HIV-1 envelope trimers with a two-component protein nanoparticle, Nat. Commun. 10 (2019) 1–17, https://doi.org/10.1038/s41467-019-12080-1.
[141] T. Lupia, S. Scabini, S. Mornese Pinna, G. Di Perri, F.G. De Rosa, S. Corcione, 2019 novel coronavirus (2019-nCoV) outbreak: a new challenge, J. Glob. Antimicrob. Resist. 21 (2020) 22–27, https://doi.org/10.1016/j.jgar.2020.02.021.
[142] E. Callaway, The race for coronavirus vaccines: a graphical guide, Nature 580 (2020) 576–577, https://doi.org/10.1038/d41586-020-01221-y.
[143] Medicago Develops Plant-Based Coronavirus Vaccine Candidate. , BioSpace, 2020. https://www.biospace.com/article/medicago-successfully-produces-a-viable-vaccine-candidate-for-covid-19/ accessed April 7, 2020.
[144] UW–Madison, FluGen, Bharat Biotech to Develop CoroFlu, a Coronavirus Vaccine, https://www.businesswire.com/news/home/20200402005666/en/UW%E2%80%93Madison-FluGen-Bharat-Biotech-develop-CoroFlu-coronavirus 2020 accessed April 14, 2020.
[145] Sinovac Biotech to Move Ahead with COVID-19 Vaccine Trial in China, https://www.pharmaceutical-business-review.com/news/sinovac-biotech-covid-19-vaccine-trial/ 2020 accessed April 20, 2020.
[146] Safety and Immunogenicity Study of Inactivated Vaccine for Prophylaxis of SARS CoV-2 Infection (COVID-19) - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT04352608 2020 accessed April 30, 2020.
[147] M.F. Bachmann, G.T. Jennings, Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns, Nat. Rev. Immunol. 10 (2010) 787–796, https://doi.org/10.1038/nri2868.
[148] S. Shukla, R.D. Dorand, J.T. Myers, S.E. Woods, N.M. Gulati, P.L. Stewart, U. Commandeur, A.Y. Huang, N.F. Steinmetz, Multiple administrations of viral nanoparticles alter in vivo behavior—insights from intravital microscopy, ACS Biomater. Sci. Eng. 2 (2016) 829–837, https://doi.org/10.1021/acsbiomaterials.6b00060.
[149] S. Shukla, J.T. Myers, S.E. Woods, X. Gong, A.E. Czapar, U. Commandeur, A.Y. Huang, A.D. Levine, N.F. Steinmetz, Plant viral nanoparticles-based HER2 vaccine: immune response influenced by differential transport, localization and cellular interactions of particulate carriers, Biomaterials 121 (2017) 15–27, https://doi.org/10.1016/j.biomaterials.2016.12.030.
[150] S. Shukla, M. Jandzinski, C. Wang, X. Gong, K.W. Bonk, R.A. Keri, N.F. Steinmetz, A viral nanoparticle cancer vaccine delays tumor progression and prolongs survival in a HER2+ tumor mouse model, Adv. Ther. 2 (2019) 1800139, https://doi.org/10.1002/adtp.201800139.
[151] H. Cai, S. Shukla, C. Wang, H. Masarapu, N.F. Steinmetz, Heterologous prime-boost enhances the antitumor immune response elicited by plant-virus-based cancer vaccine, J. Am. Chem. Soc. 141 (2019) 6509–6518, https://doi.org/10.1021/jacs.9b01523.
[152] A. Palladini, S. Thrane, C.M. Janitzek, J. Pihl, S.B. Clemmensen, W.A. de Jongh, T.M. Clausen, G. Nicoletti, L. Landuzzi, M.L. Penichet, T. Balboni, M.L. Ianzano, V. Giusti, T.G. Theander, M.A. Nielsen, A. Salanti, P.-L. Lollini, P. Nanni, A.F. Sander, Virus-like particle display of HER2 induces potent anti-cancer responses, Oncoimmunology 7 (2018) https://doi.org/10.1080/2162402X.2017.1408749.
[153] M. Daniyal, N. Akhtar, S. Ahmad, U. Fatima, M. Akram, H.M. Asif, Update knowledge on cervical cancer incidence and prevalence in Asia, Asian Pac. J. Cancer Prev. 16 (2015) 3617–3620, https://doi.org/10.7314/apjcp.2015.16.9.3617.
[154] E. Tumban, J. Peabody, M. Tyler, D.S. Peabody, B. Chackerian, VLPs displaying a single L2 epitope induce broadly cross-neutralizing antibodies against human papillomavirus, PLoS One 7 (2012) https://doi.org/10.1371/journal.pone.0049751.
[155] J. Jobsri, A. Allen, D. Rajagopal, M. Shipton, K. Kanyuka, G.P. Lomonossoff, C. Ottensmeier, S.S. Diebold, F.K. Stevenson, N. Savelyeva, Plant virus particles carrying tumour antigen activate TLR7 and Induce high levels of protective antibody, PLoS One 10 (2015), e0118096. https://doi.org/10.1371/journal.pone.0118096.
[156] Z. Yin, M. Comellas-Aragones, S. Chowdhury, P. Bentley, K. Kaczanowska, L. BenMohamed, J.C. Gildersleeve, M.G. Finn, X. Huang, Boosting immunity to small tumor-associated carbohydrates with bacteriophage Qβ capsids, ACS Chem. Biol. 8 (2013) 1253–1262, https://doi.org/10.1021/cb400060x.
[157] Z. Yin, S. Dulaney, C.S. McKay, C. Baniel, K. Kaczanowska, S. Ramadan, M.G. Finn, X. Huang, Chemical synthesis of GM2 Glycans, bioconjugation with bacteriophage Qβ, and the induction of anticancer antibodies, Chembiochem 17 (2016) 174–180, https://doi.org/10.1002/cbic.201500499.
[158] X. Wu, Z. Yin, C. McKay, C. Pett, J. Yu, M. Schorlemer, T. Gohl, S. Sungsuwan, S. Ramadan, C. Baniel, A. Allmon, R. Das, U. Westerlind, M.G. Finn, X. Huang, Protective epitope discovery and design of MUC1-based vaccine for effective tumor protections in immunotolerant mice, J. Am. Chem. Soc. 140 (2018) 16596–16609, https://doi.org/10.1021/jacs.8b08473.
[159] X. Wu, C. McKay, C. Pett, J. Yu, M. Schorlemer, S. Ramadan, S. Lang, S. Behren, U. Westerlind, M.G. Finn, X. Huang, Synthesis and immunological evaluation of disaccharide bearing MUC-1 glycopeptide conjugates with virus-like particles, ACS Chem. Biol. 14 (2019) 2176–2184, https://doi.org/10.1021/acschembio.9b00381.
[160] Z. Yin, X. Wu, K. Kaczanowska, S. Sungsuwan, M.C. Aragones, C. Pett, J. Yu, C. Baniel, U. Westerlind, M.G. Finn, X. Huang, Antitumor humoral and T cell responses by mucin-1 conjugates of bacteriophage Qβ in wild-type mice, ACS Chem. Biol. 13 (2018) 1668–1676, https://doi.org/10.1021/acschembio.8b00313.
[161] A. Miermont, H. Barnhill, E. Strable, X. Lu, K.A. Wall, Q. Wang, M.G. Finn, X. Huang, Cowpea mosaic virus capsid: a promising carrier for the development of carbohydrate based antitumor vaccines, Chemistry 14 (2008) 4939–4947, https://doi.org/10.1002/chem.200800203.
[162] Z. Yin, H.G. Nguyen, S. Chowdhury, P. Bentley, M.A. Bruckman, A. Miermont, J.C. Gildersleeve, Q. Wang, X. Huang, Tobacco mosaic virus as a new carrier for tumor associated carbohydrate antigens, Bioconjug. Chem. 23 (2012) 1694–1703, https://doi.org/10.1021/bc300244a.
[163] U. Sahin, Ö. Türeci, Personalized vaccines for cancer immunotherapy, Science 359 (2018) 1355–1360, https://doi.org/10.1126/science.aar7112.
[164] M.O. Mohsen, M. Vogel, C. Riether, J. Muller, S. Salatino, N. Ternette, A.C. Gomes, G. Cabral-Miranda, A. El-Turabi, C. Ruedl, T.M. Kundig, S. Dermime, A. Knuth, D.E. Speiser, M.F. Bachmann, Targeting mutated plus germline epitopes confers preclinical efficacy of an instantly formulated cancer nano-vaccine, Front. Immunol. 10 (2019) https://doi.org/10.3389/fimmu.2019.01015.
[165] J. Li, Y. Sun, T. Jia, R. Zhang, K. Zhang, L. Wang, Messenger RNA vaccine based on recombinant MS2 virus-like particles against prostate cancer, Int. J. Cancer 134 (2014) 1683–1694, https://doi.org/10.1002/ijc.28482.
[166] B.T. Hyman, C.H. Phelps, T.G. Beach, E.H. Bigio, N.J. Cairns, M.C. Carrillo, D.W. Dickson, C. Duyckaerts, M.P. Frosch, E. Masliah, S.S. Mirra, P.T. Nelson, J.A. Schneider, D.R. Thal, B. Thies, J.Q. Trojanowski, H.V. Vinters, T.J. Montine, National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease, Alzheimers Dement. 8 (2012) 1–13, https://doi.org/10.1016/j.jalz.2011.10.007.
[167] Q. Li, M.N. Gordon, B. Chackerian, J. Alamed, K.E. Ugen, D. Morgan, Virus-like peptide vaccines against Aß N-terminal or C-terminal domains reduce amyloid deposition in APP transgenic mice without addition of adjuvant, J. NeuroImmune Pharmacol. 5 (2010) 133–142, https://doi.org/10.1007/s11481-009-9183-1.
[168] N.M. Maphis, J. Peabody, E. Crossey, S. Jiang, F.A. Jamaleddin Ahmad, M. Alvarez, S.K. Mansoor, A. Yaney, Y. Yang, L.O. Sillerud, C.M. Wilson, R. Selwyn, J.L. Brigman, J.L. Cannon, D.S. Peabody, B. Chackerian, K. Bhaskar, Qß Virus-like particle-based vaccine induces robust immunity and protects against tauopathy, NPJ Vaccines 4 (2019) 26, https://doi.org/10.1038/s41541-019-0118-4.
[169] M. Ji, X. Xie, D. Liu, X. Yu, Y. Zhang, L.-X. Zhang, S. Wang, Y. Huang, R. Liu, Hepatitis B core VLP-based mis-disordered tau vaccine elicits strong immune response and alleviates cognitive deficits and neuropathology progression in Tau.P301S mouse model of Alzheimer's disease and frontotemporal dementia, Alzheimers Res. Ther. 10 (2018) https://doi.org/10.1186/s13195-018-0378-7.
[170] P. Bach, J.-A. Tschäpe, F. Kopietz, G. Braun, J.K. Baade, K.-H. Wiederhold, M. Staufenbiel, M. Prinz, T. Deller, U. Kalinke, C.J. Buchholz, U.C. Müller, Vaccination with Abeta-displaying virus-like particles reduces soluble and insoluble cerebral Abeta and lowers plaque burden in APP transgenic mice, J. Immunol. 182 (2009) 7613–7624, https://doi.org/10.4049/jimmunol.0803366.
[171] Q. Li, C. Cao, B. Chackerian, J. Schiller, M. Gordon, K.E. Ugen, D. Morgan, Overcoming antigen masking of anti-amyloidbeta antibodies reveals breaking of B cell tolerance by virus-like particles in amyloidbeta immunized amyloid precursor protein transgenic mice, BMC Neurosci. 5 (2004) 21, https://doi.org/10.1186/1471-2202-5-21.
[172] D. Khan, S. Ansar Ahmed, Regulation of IL-17 in autoimmune diseases by transcriptional factors and microRNAs, Front. Genet. 6 (2015) https://doi.org/10.3389/fgene.2015.00236.
[173] D. Wendling, F. Verhoeven, C. Prati, Anti-IL-17 monoclonal antibodies for the treatment of ankylosing spondylitis, Expert. Opin. Biol. Ther. 19 (2019) 55–64, https://doi.org/10.1080/14712598.2019.1554053.
[174] T.A. Röhn, G.T. Jennings, M. Hernandez, P. Grest, M. Beck, Y. Zou, M. Kopf, M.F. Bachmann, Vaccination against IL-17 suppresses autoimmune arthritis and encephalomyelitis, Eur. J. Immunol. 36 (2006) 2857–2867, https://doi.org/10.1002/eji.200636658.
[175] M.F. Bachmann, A. El-Turabi, A. Fettelschoss-Gabriel, M. Vogel, The prospects of an active vaccine against asthma targeting IL-5, Front. Microbiol. 9 (2018) https://doi.org/10.3389/fmicb.2018.02522.
[176] Y. Zou, I. Sonderegger, G. Lipowsky, G.T. Jennings, N. Schmitz, M. Landi, M. Kopf, M.F. Bachmann, Combined vaccination against IL-5 and eotaxin blocks eosinophilia in mice, Vaccine 28 (2010) 3192–3200, https://doi.org/10.1016/j.vaccine.2010.02.048.
[177] E. Crossey, M.J.A. Amar, M. Sampson, J. Peabody, J.T. Schiller, B. Chackerian, A.T. Remaley, A cholesterol-lowering VLP vaccine that targets PCSK9, Vaccine 33 (2015) 5747–5755, https://doi.org/10.1016/j.vaccine.2015.09.044.
[178] D.J. Rader, IL-1 and atherosclerosis: a murine twist to an evolving human story, J. Clin. Invest. 122 (2012) 27–30, https://doi.org/10.1172/JCI61163.
[179] A.C. Tissot, G. Spohn, G.T. Jennings, A. Shamshiev, M.O. Kurrer, R. Windak, M. Meier, M. Viesti, M. Hersberger, T.M. Kündig, R. Ricci, M.F. Bachmann, A VLP-based vaccine

against interleukin-1α protects mice from atherosclerosis, Eur. J. Immunol. 43 (2013) 716–722, https://doi.org/10.1002/eji.201242687.
[180] R.E. Miller, J.A. Block, A.M. Malfait, Nerve growth factor (NGF) blockade for the management of osteoarthritis pain: what can we learn from clinical trials and preclinical models? Curr. Opin. Rheumatol. 29 (2017) 110–118, https://doi.org/10.1097/BOR.0000000000000354.
[181] I.S. von Loga, A. El-Turabi, L. Jostins, J. Miotla-Zarebska, J. Mackay-Alderson, A. Zeltins, I. Parisi, M.F. Bachmann, T.L. Vincent, Active immunisation targeting nerve growth factor attenuates chronic pain behaviour in murine osteoarthritis, Ann. Rheum. Dis. 78 (2019) 672–675, https://doi.org/10.1136/annrheumdis-2018-214489.
[182] S. Choudhury, V. Kakkar, P. Suman, K. Chakrabarti, S. Vrati, S.K. Gupta, Immunogenicity of zona pellucida glycoprotein-3 and spermatozoa YLP(12) peptides presented on Johnson grass mosaic virus-like particles, Vaccine 27 (2009) 2948–2953, https://doi.org/10.1016/j.vaccine.2009.03.002.
[183] N.F. Steinmetz, Viral nanoparticles as platforms for next-generation therapeutics and imaging devices, Nanomedicine 6 (2010) 634–641, https://doi.org/10.1016/j.nano.2010.04.005.
[184] H. Hu, H. Masarapu, Y. Gu, Y. Zhang, X. Yu, N.F. Steinmetz, Physalis mottle virus-like nanoparticles for targeted cancer imaging, ACS Appl. Mater. Interfaces 11 (2019) 18213–18223, https://doi.org/10.1021/acsami.9b03956.
[185] H.S. Leong, N.F. Steinmetz, A. Ablack, G. Destito, A. Zijlstra, H. Stuhlmann, M. Manchester, J.D. Lewis, Intravital imaging of embryonic and tumor neovasculature using viral nanoparticles, Nat. Protoc. 5 (2010) 1406–1417, https://doi.org/10.1038/nprot.2010.103.
[186] P.H. Beatty, J.D. Lewis, Cowpea mosaic virus nanoparticles for cancer imaging and therapy, Adv. Drug Deliv. Rev. 145 (2019) 130–144, https://doi.org/10.1016/j.addr.2019.04.005.
[187] C.-F. Cho, L. Yu, T.K. Nsiama, A.N. Kadam, A. Raturi, S. Shukla, G.A. Amadei, N.F. Steinmetz, L.G. Luyt, J.D. Lewis, Viral nanoparticles decorated with novel EGFL7 ligands enable intravital imaging of tumor neovasculature, Nanoscale 9 (2017) 12096–12109, https://doi.org/10.1039/c7nr02558k.
[188] S. Shukla, C. Dickmeis, R. Fischer, U. Commandeur, N.F. Steinmetz, In planta production of fluorescent filamentous plant virus-based nanoparticles, Methods Mol. Biol. 1776 (2018) 61–84, https://doi.org/10.1007/978-1-4939-7808-3_5.
[189] I.L. Aanei, T. Huynh, Y. Seo, M.B. Francis, Vascular cell adhesion molecule-targeted MS2 viral capsids for the detection of early-stage atherosclerotic plaques, Bioconjug. Chem. 29 (2018) 2526–2530, https://doi.org/10.1021/acs.bioconjchem.8b00453.
[190] J. Park, H. Gao, Y. Wang, H. Hu, D.I. Simon, N.F. Steinmetz, S100A9-targeted tobacco mosaic virus nanoparticles exhibit high specificity toward atherosclerotic lesions in ApoE $^{-/-}$ mice, J. Mater. Chem. B 7 (2019) 1842–1846, https://doi.org/10.1039/C8TB02276C.
[191] Aileen M. Healy, Michael D. Pickard, Aruna D. Pradhan, Wang Yunmei, Chen Zhiping, Croce Kevin, Sakuma Masashi, Shi Can, C. Zago Alexandre, Garasic Joseph, I. Damokosh Andrew, L. Dowie Tracy, Poisson Louis, Lillie James, Libby Peter, M. Ridker Paul, I. Simon Daniel, Platelet expression profiling and clinical validation of myeloid-related protein-14 as a novel determinant of cardiovascular events, Circulation 113 (2006) 2278–2284, https://doi.org/10.1161/CIRCULATIONAHA.105.607333.
[192] M.G. Ionita, P. van den Borne, L.M. Catanzariti, F.L. Moll, J.-P.P.M. de Vries, G. Pasterkamp, A. Vink, D.P.V. de Kleijn, High neutrophil numbers in human carotid atherosclerotic plaques are associated with characteristics of rupture-prone lesions, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1842–1848, https://doi.org/10.1161/ATVBAHA.110.209296.
[193] X. Sun, W. Li, X. Zhang, M. Qi, Z. Zhang, X.-E. Zhang, Z. Cui, In vivo targeting and imaging of atherosclerosis using multifunctional virus-like particles of simian virus 40, Nano Lett. 16 (2016) 6164–6171, https://doi.org/10.1021/acs.nanolett.6b02386.
[194] M.A. Luzuriaga, R.P. Welch, M. Dharmarwardana, C.E. Benjamin, S. Li, A. Shahrivarkevishahi, S. Popal, L.H. Tuong, C.T. Creswell, J.J. Gassensmith, Enhanced stability and controlled delivery of MOF-encapsulated vaccines and their immunogenic response in vivo, ACS Appl. Mater. Interfaces (2019) https://doi.org/10.1021/acsami.8b20504.
[195] S.M. Erturk, C. Johnston, C. Tempany-Afdhal, A.D. Van den Abbeele, Chapter 6 – imaging tools in human research, in: D. Robertson, G.H. Williams (Eds.), Clinical and Translational Science, Academic Press, San Diego 2009, pp. 87–104, https://doi.org/10.1016/B978-0-12-373639-0.00006-6.
[196] M. Rogosnitzky, S. Branch, Gadolinium-based contrast agent toxicity: a review of known and proposed mechanisms, Biometals 29 (2016) 365–376, https://doi.org/10.1007/s10534-016-9931-7.
[197] M.A. Bruckman, K. Jiang, E.J. Simpson, L.N. Randolph, L.G. Luyt, X. Yu, N.F. Steinmetz, Dual-modal magnetic resonance and fluorescence imaging of atherosclerotic plaques in vivo using VCAM-1 targeted tobacco mosaic virus, Nano Lett. 14 (2014) 1551–1558, https://doi.org/10.1021/nl404816m.
[198] H. Hu, Y. Zhang, S. Shukla, Y. Gu, X. Yu, N.F. Steinmetz, Dysprosium-modified tobacco mosaic virus nanoparticles for ultra-high-field magnetic resonance and near-infrared fluorescence imaging of prostate cancer, ACS Nano 11 (2017) 9249–9258, https://doi.org/10.1021/acsnano.7b04472.
[199] J. Min, H. Jung, H.-H. Shin, G. Cho, H. Cho, S. Kang, Implementation of P22 viral capsids as intravascular magnetic resonance T1 contrast conjugates via site-selective attachment of Gd(III)-chelating agents, Biomacromolecules 14 (2013) 2332–2339, https://doi.org/10.1021/bm400461j.
[200] S. Qazi, M. Uchida, R. Usselman, R. Shearer, E. Edwards, T. Douglas, Manganese(III) porphyrins complexed with P22 virus-like particles as T1-enhanced contrast agents for magnetic resonance imaging, J. Biol. Inorg. Chem. 19 (2014) 237–246, https://doi.org/10.1007/s00775-013-1075-4.
[201] J. Lucon, S. Qazi, M. Uchida, G.J. Bedwel, B. LaFrance, P.E. Prevelige, T. Douglas, Using the interior cavity of the P22 capsid for site specific initiation of atom transfer radical polymerization with tremendously increased cargo loading, Nat. Chem. 4 (2012) 781–788, https://doi.org/10.1038/nchem.1442.
[202] M. Woods, D.E. Woessner, A.D. Sherry, Paramagnetic lanthanide complexes as PARACEST agents for medical imaging, Chem. Soc. Rev. 35 (2006) 500–511, https://doi.org/10.1039/b509907m.
[203] K.K. Palaniappan, R.M. Ramirez, V.S. Bajaj, D.E. Wemmer, A. Pines, M.B. Francis, Molecular imaging of cancer cells using a bacteriophage-based 129Xe NMR biosensor, Angew. Chem. Int. Ed. Eng. 52 (2013) 4849–4853, https://doi.org/10.1002/anie.201300170.
[204] M. Dharmarwardana, A.F. Martins, Z. Chen, P.M. Palacios, C.M. Nowak, R.P. Welch, S. Li, M.A. Luzuriaga, L. Bleris, B.S. Pierce, A.D. Sherry, J.J. Gassensmith, Nitroxyl modified tobacco mosaic virus as a metal-free high-relaxivity MRI and EPR active superoxide sensor, Mol. Pharm. 15 (2018) 2973–2983, https://doi.org/10.1021/acs.molpharmaceut.8b00262.
[205] H. Lee, A. Shahrivarkevishahi, J.L. Lumata, M.A. Luzuriaga, L.M. Hagge, C.E. Benjamin, O.R. Brohlin, C.R. Parish, H.R. Firouzi, S.O. Nielsen, L.L. Lumata, J.J. Gassensmith, Supramolecular and biomacromolecular enhancement of metal-free magnetic resonance imaging contrast agents, Chem. Sci. 11 (2020) 2045–2050, https://doi.org/10.1039/C9SC05510J.
[206] S. Anand, H. Singh, A. Dash, Clinical applications of PET and PET-CT, Med. J. Armed Forces India 65 (2009) 353–358, https://doi.org/10.1016/S0377-1237(09)80099-3.
[207] M.E. Farkas, I.L. Aanei, C.R. Behrens, G.J. Tong, S.T. Murphy, J.P. O'Neil, M.B. Francis, PET Imaging and biodistribution of chemically modified bacteriophage MS2, Mol. Pharm. 10 (2013) 69–76, https://doi.org/10.1021/mp3003754.
[208] Z. Li, Q. Jin, C. Huang, S. Dasa, L. Chen, L. Yap, S. Liu, H. Cai, R. Park, P.S. Conti, Trackable and targeted phage as positron emission tomography (PET) agent for cancer imaging, Theranostics 1 (2011) 371–380.
[209] P. Brader, K. Kelly, S. Gang, J.P. Shah, R.J. Wong, H. Hricak, R.G. Blasberg, Y. Fong, Z. Gil, Imaging of lymph node micrometastases using an oncolytic herpes virus and [F]FEAU PET, PLoS One 4 (2009), e4789. https://doi.org/10.1371/journal.pone.0004789.
[210] A.A.A. Aljabali, M.S.A. Zoubi, K.M. Al-Batanyeh, A. Al-Radaideh, M.A. Obeid, A.A. Sharabi, W. Alshaer, B. AbuFares, T. Al-Zanati, M.M. Tambuwala, N. Akbar, D.J. Evans, Gold-coated plant virus as computed tomography imaging contrast agent, Beilstein J. Nanotechnol. 10 (2019) 1983–1993, https://doi.org/10.3762/bjnano.10.195.
[211] V. Shanmugam, S. Selvakumar, C.-S. Yeh, Near-infrared light-responsive nanomaterials in cancer therapeutics, Chem. Soc. Rev. 43 (2014) 6254–6287, https://doi.org/10.1039/c4cs00011k.
[212] A.M. Alkilany, L.B. Thompson, S.P. Boulos, P.N. Sisco, C.J. Murphy, Gold nanorods: their potential for photothermal therapeutics and drug delivery, tempered by the complexity of their biological interactions, Adv. Drug Deliv. Rev. 64 (2012) 190–199, https://doi.org/10.1016/j.addr.2011.03.005.
[213] Y. Liu, P. Bhattarai, Z. Dai, X. Chen, Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer, Chem. Soc. Rev. 48 (2019) 2053–2108, https://doi.org/10.1039/c8cs00618k.
[214] C.E. Benjamin, Z. Chen, P. Kang, B.A. Wilson, N. Li, S.O. Nielsen, Z. Qin, J.J. Gassensmith, Site-selective nucleation and size control of gold nanoparticle photothermal antennae on the pore structures of a virus, J. Am. Chem. Soc. 140 (2018) 17226–17233, https://doi.org/10.1021/jacs.8b10446.
[215] J.-K. Rhee, M. Baksh, C. Nycholat, J.C. Paulson, H. Kitagishi, M.G. Finn, Glycan-targeted virus-like nanoparticles for photodynamic therapy, Biomacromolecules 13 (2012) 2333–2338, https://doi.org/10.1021/bm300578p.
[216] K. Chauhan, J.M. Hernandez-Meza, A.G. Rodríguez-Hernández, K. Juarez-Moreno, P. Sengar, R. Vazquez-Duhalt, Multifunctionalized biocatalytic P22 nanoreactor for combinatory treatment of ER+ breast cancer, J. Nanobiotechnology 16 (2018) 17, https://doi.org/10.1186/s12951-018-0345-2.
[217] H. Hu, Q. Yang, S. Baroni, H. Yang, S. Aime, N.F. Steinmetz, Polydopamine-decorated tobacco mosaic virus for photoacoustic/magnetic resonance bimodal imaging and photothermal cancer therapy, Nanoscale 11 (2019) 9760–9768, https://doi.org/10.1039/C9NR02065A.
[218] Z. Zhou, Y. Sun, J. Shen, J. Wei, C. Yu, B. Kong, W. Liu, H. Yang, S. Yang, W. Wang, Iron/iron oxide core/shell nanoparticles for magnetic targeting MRI and near-infrared photothermal therapy, Biomaterials 35 (2014) 7470–7478, https://doi.org/10.1016/j.biomaterials.2014.04.063.
[219] Y. Wang, K.C.L. Black, H. Luehmann, W. Li, Y. Zhang, X. Cai, D. Wan, S.-Y. Liu, M. Li, P. Kim, Z.-Y. Li, L.V. Wang, Y. Liu, Y. Xia, Comparison study of gold nanohexapods, nanorods, and nanocages for photothermal cancer treatment, ACS Nano 7 (2013) 2068–2077, https://doi.org/10.1021/nn304332s.
[220] R. Vankayala, Y.-K. Huang, P. Kalluru, C.-S. Chiang, K.C. Hwang, First demonstration of gold nanorods-mediated photodynamic therapeutic destruction of tumors via near infra-red light activation, Small 10 (2014) 1612–1622, https://doi.org/10.1002/smll.201302719.
[221] C.M. Hessel, V.P. Pattani, M. Rasch, M.G. Panthani, B. Koo, J.W. Tunnell, B.A. Korgel, Copper selenide nanocrystals for photothermal therapy, Nano Lett. 11 (2011) 2560–2566, https://doi.org/10.1021/nl201400z.
[222] Q. Jia, D. Li, Q. Zhang, S. Ye, Z. Xi, X. Wang, W. Shan, L. Ren, Biomineralization synthesis of HBc-CuS nanoparticles for near-infrared light-guided photothermal therapy, J. Mater. Sci. 54 (2019) 13255–13264, https://doi.org/10.1007/s10853-019-03613-6.
[223] W. Shan, R. Chen, Q. Zhang, J. Zhao, B. Chen, X. Zhou, S. Ye, S. Bi, L. Nie, L. Ren, Improved stable indocyanine green (ICG)-mediated cancer optotheranostics with

naturalized hepatitis B core particles, Adv. Mater. Weinheim. 30 (2018), e1707567. https://doi.org/10.1002/adma.201707567.

[224] Q. Zhang, W. Shan, C. Ai, Z. Chen, T. Zhou, X. Lv, X. Zhou, S. Ye, L. Ren, X. Wang, Construction of multifunctional Fe3O4-MTX@HBc nanoparticles for MR imaging and photothermal therapy/chemotherapy, Nanotheranostics 2 (2018) 87–95, https://doi.org/10.7150/ntno.21942.

[225] Z. Wang, P. Huang, O. Jacobson, Z. Wang, Y. Liu, L. Lin, J. Lin, N. Lu, H. Zhang, R. Tian, G. Niu, G. Liu, X. Chen, Biomineralization-inspired synthesis of copper sulfide–ferritin nanocages as cancer theranostics, ACS Nano 10 (2016) 3453–3460, https://doi.org/10.1021/acsnano.5b07521.

[226] A. Espinosa, A. Curcio, S. Cabana, G. Radtke, M. Bugnet, J. Kolosnjaj-Tabi, C. Péchoux, C. Alvarez-Lorenzo, G.A. Botton, A.K.A. Silva, A. Abou-Hassan, C. Wilhelm, Intracellular biodegradation of Ag nanoparticles, storage in ferritin, and protection by a Au shell for enhanced photothermal therapy, ACS Nano 12 (2018) 6523–6535, https://doi.org/10.1021/acsnano.8b00482.

[227] D. Ghosh, A.G. Kohli, F. Moser, D. Endy, A.M. Belcher, Refactored M13 bacteriophage as a platform for tumor cell imaging and drug delivery, ACS Synth. Biol. 1 (2012) 576–582, https://doi.org/10.1021/sb300052u.

[228] A Phase II Clinical Trial to Evaluate the Recombinant Vaccine for COVID-19 (Adenovirus Vector) - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT04341389 2020 accessed May 27, 2020.

[229] University of Oxford, A Phase 2/3 Study to Determine the Efficacy, Safety and Immunogenicity of the Candidate Coronavirus Disease (COVID-19) Vaccine ChAdOx1 nCoV-19, clinicaltrials.gov, https://clinicaltrials.gov/ct2/show/NCT04400838 2020 accessed May 26, 2020.

[230] B. Schwarz, T. Douglas, Development of virus-like particles for diagnostic and prophylactic biomedical applications, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 7 (2015) 722–735, https://doi.org/10.1002/wnan.1336.

[231] N.M. Gulati, P.L. Stewart, N.F. Steinmetz, Bio inspired shielding strategies for nanoparticle drug delivery applications, Mol. Pharm. 15 (2018) 2900–2909, https://doi.org/10.1021/acs.molpharmaceut.8b00292.

[232] J.K. Smith, M. Agbandje-McKenna, Creating an arsenal of Adeno-associated virus (AAV) gene delivery stealth vehicles, PLoS Pathog. 14 (2018) https://doi.org/10.1371/journal.ppat.1006929.

[233] J.V. Jokerst, T. Lobovkina, R.N. Zare, S.S. Gambhir, Nanoparticle PEGylation for imaging and therapy, Nanomedicine (London) 6 (2011) 715–728, https://doi.org/10.2217/nnm.11.19.

[234] S. Shukla, C. Wang, V. Beiss, N.F. Steinmetz, Antibody response against Cowpea mosaic viral nanoparticles improves in situ vaccine efficacy in ovarian cancer, ACS Nano (2020) https://doi.org/10.1021/acsnano.9b07865.

[235] M.O. Mohsen, A.C. Gomes, M. Vogel, M.F. Bachmann, Interaction of viral capsid-derived virus-like particles (VLPs) with the innate immune system, Vaccines (Basel) 6 (2018) https://doi.org/10.3390/vaccines6030037.

[236] D.-S. Chen, Y.-Q. Wu, W. Zhang, S.-J. Jiang, S.-Z. Chen, Horizontal gene transfer events reshape the global landscape of arm race between viruses and homo sapiens, Sci. Rep. 6 (2016) 26934, https://doi.org/10.1038/srep26934.