Development of Virus-Like Particle Technology from Small Highly Symmetric to Large Complex Virus-Like Particle Structures

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Key Words

Virus-like particles · Three-dimensional structure · Vaccines · Drug delivery · Biotechnology

Abstract

Virus-like particle (VLP) technology is a promising approach for the construction of novel vaccines, diagnostic tools, and gene therapy vectors. Initially, VLPs were primarily derived from non-enveloped icosahedral or helical viruses and proved to be viable vaccine candidates due to their effective presentation of epitopes in a native conformation. VLP technology has also been used to prepare chimeric VLPs decorated with genetically fused or chemically coupled epitope stretches selected from immunologically defined target proteins. However, structural constraints associated with the rigid geometrical architecture of icosahedral or helical VLPs pose challenges for the expression and presentation of large epitopes. Complex VLPs derived from non-symmetric enveloped viruses are increasingly being used to incorporate large epitopes and even full-length foreign proteins. Pleomorphic VLPs derived from influenza or other enveloped viruses can accommodate multiple full-length and/or chimeric proteins that can be rationally designed for multifunctional purposes, including multivalent vaccines. Therefore, a

second generation of VLP carriers is represented by complex particles reconstructed from natural or chimeric structural proteins derived from complex enveloped viruses. Further development of safe and efficient VLP nanotechnology may require a rational combination of both approaches.

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VLP Concept and Its Evolution from Simple Icosahedral to Complex Enveloped Particles

Recombinant virus-like particles (VLPs) are nanodimensional structures that (1) are built from one or several viral structural constituents in the form of recombinant proteins synthesized in efficient homologous or primarily heterologous expression systems (bacteria, yeast, or eukaryotic cell culture); (2) are identical or closely related by their three-dimensional architecture and immunochemical characteristics to naturally occurring viral structures, and (3) lack genomes or infectivity [1–5]. VLPs can be formed from nucleocapsid or envelope proteins alone and the term VLP will be used here to describe any multimolecular structure formed by viral capsid and/or envelope proteins or by capsid and envelope combinations. The term *non-chimeric VLPs* is used for particles

that are constructed from the original native non-modified viral proteins, whereas VLPs are defined as *chimeric* when the original structural proteins are covalently modified by the addition or substitution of foreign polypeptide stretches with functional properties, such as immunological epitopes or cell-targeting or encapsidation signals [5]. Covalent integration into chimeric VLPs can be achieved either by the expression of VLP monomer genes containing the appropriate insertions encoding the desired protein stretches [1–3, 5] or by the chemical coupling of peptides, proteins, or other molecules to VLPs [6]. Complex VLPs carrying full-length foreign proteins are defined here as *hybrids*.

Chimeric and/or hybrid VLPs target three main functional applications: (1) presentation of foreign epitopes leading to novel immunological content and subsequently to the creation of novel vaccines; (2) encapsidation of various therapeutic or diagnostic agents, such as nucleic acids as adjuvants for vaccines or gene therapy, proteins or mRNAs for diagnostic or therapeutic purposes, or low-molecular-weight drugs to be delivered to specific cells, and (3) specific targeting of desired organs, tissues, or cells.

Historically, the first two VLPs were derived from hepatitis B virus (HBV) and expressed in *Escherichia coli* and *Saccharomyces cerevisiae* during the mid-1980s. These VLPs were hepatitis B core (HBc) [7–9] and surface (HBs) [10] particles, which possessed icosahedral and octahedral symmetry, respectively [for a detailed review, see 11]. Later, the first representatives of VLPs derived from small RNA bacteriophages, non-enveloped bacterial viruses, were described [12–16]. The first rod-shaped chimeric VLPs with helical symmetry were derived at the same time from tobacco mosaic virus [17]. Enveloped VLPs from complex enveloped viruses, such as influenza viruses or the Ebola virus, were only introduced after more than 20 years of research [18–20].

In this review, we illustrate the evolution of the VLP concept from highly ordered symmetric VLPs to large complex VLPs derived from enveloped viruses. In most cases, symmetric VLPs are structurally well characterized by high-resolution techniques (protein X-ray crystallography and cryo-electron microscopy), whereas complex enveloped VLPs are often less structurally characterized. Figure 1 presents images of crystal structures of non-enveloped icosahedral viruses and experimental photomicrographs of complex enveloped viruses that have been used for the preparation of VLPs. Table 1 summarizes data on the various VLPs and demonstrates that almost all viral families have contributed to the development of VLP technology.

Non-Enveloped Icosahedral and Helical VLPs

Native Non-Chimeric VLPs

Non-enveloped native VLPs composed of unmodified viral proteins have been used as vaccines and configured as epitope display carriers (table 1). Recombinant icosahedral and, to lesser extent, helical VLP structures provide exceptionally effective environments for a high-density symmetric display of foreign oligopeptides, whereas three-dimensional maps of VLPs allow for the rational design of sites for epitope insertion. The regular repetitive pattern and correct conformation of the initial and inserted epitopes of symmetric VLPs have been recognized as critical features for the induction of a strong immunological response and for their potential use as highly efficient vaccines [for reviews, see 5, 6, 11, 21–28].

The interest in rationally designed manipulations of chimeric VLPs has been reinforced by the determination of the structures of many viral capsids by protein X-ray crystallography. The upper part of figure 1 shows crystal structures of representative icosahedral VLPs. As is evident, VLPs have been derived from all classes of viral genomes, i.e., double- and single-stranded DNA and RNA. Thus far, most VLPs have been derived from viruses with single-stranded RNA genomes of positive polarity lacking a DNA intermediate. Complex VLPs were recently constructed from large enveloped negative-strand RNA viruses such as influenza viruses and Ebola virus (see the section ‘Complex VLPs derived from enveloped viruses’ below).

As indicated in table 1, some icosahedral and helical VLPs have been used as vaccines in their native, non-chimeric form without the introduction of foreign sequences (for references, see table 1). For example, the HBs antigen that represents 22-nm particles produced in yeast has been a successful HBV vaccine since 1986. Another structural HBV protein, a nucleocapsid or HBc particle, is being tested as a component of a therapeutic HBV vaccine (table 1). Yet another example of a native, non-chimeric VLP vaccine are human papillomavirus (HPV) VLPs produced in yeast or baculovirus expression systems, which went onto the market in 2006 and 2007, respectively, as vaccines against cervical cancer. Norwalk virus and rotavirus VLPs against the major etiologic agents of epidemic gastroenteritis in humans are currently in clinical trials.

Animal circovirus and parvovirus VLP vaccines have been designed against infections in pigs and dogs (table 1). Other animal vaccines that are based on non-modified native VLPs have been prepared against calicivirus

Fig. 1. Size-scaled presentation of the crystal structures of non-enveloped icosahedral viruses and experimental photomicrographs of the complex enveloped viruses that have been used for the preparation of VLPs. Three-dimensional models of structur-

ally resolved particles are from the VIPERdb database [400, 401]. The structure of the HBs particle with octagonal symmetry is from Gilbert et al. [402]. Photomicrographs of enveloped viruses are from publicly available resources. For abbreviations, see table 1.

(RHDV), papillomavirus (BPV and CRPV), reovirus (BTV), and birnavirus (AHSV) infections.

Chimeric VLPs

Table 1 lists the published chimeric VLPs (including hybrid VLPs) that have been prepared by either gene fusion or chemical coupling of epitope peptides to the VLP surface, with an emphasis on vaccine and drug delivery applications.

The earliest chimeric structures, which appeared during the mid-1980s, were either based on tobacco mosaic virus [17] or derived from HBs protein [29, 30]. The development of the HBs protein has recently resulted in the first successful phase III clinical trial of a chimeric VLP vaccine, specifically the RTS,S vaccine against malaria [31, 32]. Among icosahedral VLPs, which appeared at the same time in the 1980s, HBc and VLPs derived from RNA phages have often been used as scaffolds for the construction of both prophylactic and therapeutic vaccine candi-

dates, which are in clinical trials. For example, single-stranded RNA phages have been extensively used for the development of therapeutic vaccines by attaching genetically fused and chemically coupled epitopes (for references, see table 1).

Among other icosahedra that have advanced to chimeric VLPs, capsids from parvoviruses (B19, PPV, and CPV), comoviruses (CPMV), nodaviruses (FHV), polyomaviruses (MuPV and HaPV), and papillomaviruses (HPV and BPV) have proved to be sufficiently flexible to retain the ability to self-assemble after genetic fusion with foreign peptides. CPMV, a representative of cucumoviruses, has demonstrated the unique ability to remain replication-competent and to produce progeny of chimeric viruses carrying foreign insertions. Bromoviruses, along with the CPMV [for a review, see 33], have formed a niche of nanotechnological materials involved mostly in a variety of packaging protocols using BrMV and CCMV VLPs [for a review, see 34, 35].

Table 1. Summary of VLPs used for the development of vaccines, vectors/nanocontainers, and cell-targeting tools

Taxonomic description of the VLP sources: family, genus, species	VLPs as vaccines and/or vaccine candidates		VLPs as nanocages for the transportation of the following molecules	VLPs as delivery tools to the specified cell targets
	native non-chimeric VLPs	chimeric and/or hybrid VLPs carrying fused or chemically conjugated epitopes/fragments from the proteins indicated below		
Non-enveloped viruses: icosahedral symmetry				
<i>Circoviridae</i> <i>Circovirus</i> Porcine circovirus 2 (PCV2)	PCV2 [92] (yeast), [93] (<i>E. coli</i>)			
<i>Parvoviridae</i> <i>Erythrovirus</i> Human parvovirus B19	B19 [94, 95] (insect cells)	HSV-1, murine hepatitis virus (MHV) [96], dengue [97], anthrax [98] (all insect cells)		
<i>Parvoviridae</i> <i>Brevidensovirus</i> Infectious hypodermal and hematopoietic necrosis virus (IHNV)	IHNV [99] (<i>E. coli</i>)			shrimp hemocytes [99]
<i>Parvoviridae</i> <i>Dependovirus</i> Goose parvovirus (GPV)	GPV [100] (insect cells)			
<i>Parvoviridae</i> <i>Parvovirus</i> Porcine parvovirus (PPV)	PPV [101] (insect cells)	poliovirus [102], PCV2 [103] (all insect cells)		
<i>Canine parvovirus</i> (CPV)	CPV [104] (insect cells)	poliovirus [105], rabies [106]		transferrin receptors [107]
<i>Hepeviridae</i> <i>Hepevirus</i> Hepatitis E virus (HEV)	HEV [108] (plants)	herpes simplex virus [109] (insect cells)		
<i>Bromoviridae</i> <i>Alfavirus</i> Alfalfa mosaic virus (AIMV)		rabies [110], respiratory syncytial virus (RSV) [111], anthrax [112] (plants)	nanogold [113]	
<i>Bromoviridae</i> <i>Bromovirus</i> Brome mosaic virus (BrMV)			polystyrene [114], nanometal [115], RNA [116], phthalocyanine [117]	
<i>Cowpea chlorotic mottle virus</i> (CCMV)			DNA, a protein and a fluorophore [121]	
<i>Bromoviridae</i> <i>Cucumovirus</i> Cucumber mosaic virus (CMV)		HCV [118], Newcastle disease virus (NDV) [119], Alzheimer's disease [120] (plants)	RNA [125 – 130], ricin A [131], proteins and drugs [129]	leukaemia cells [126], HCC [129]
<i>Leviviridae</i> <i>Levivirus</i> Enterobacteria phage MS2		HIV-1 [122, 123], malaria [124] (all <i>E. coli</i>)	CpG (Renhofa R, unpubl.)	
Enterobacteria phage PP7		HBV [132], HPV [132, 133] (<i>E. coli</i>)	RNA [135], CpG, LMW drugs (Renhofa R, unpubl.)	CXCR4-overproducing cells (Renhofa R, unpubl.)
Enterobacteria phage φ			RNA, CpG, magnetic iron nanoparticles (Renhofa R, unpubl.)	HBV receptors (Renhofa R, unpubl.)
Enterobacteria phage GA				
Acinetobacter phage AP205		hypertension, GnRH, HIV-1, influenza A [136], WNV [137] (<i>E. coli</i>)	RNA [78], enzymes [152], proteins [153], drugs [154, 155]	targeting by ZZ domain [156], EGF targeting [157], heparin reversion [158]
<i>Leviviridae</i> <i>Allolevivirus</i> Enterobacteria phage Qβ	Allergy, therapeutic vaccine [80, 138] (<i>E. coli</i>)	HBV [139], influenza [140], therapeutic vaccines: smoking [141], hypertension [142], melanoma [143], arthritis [144, 145], Alzheimer's disease [146], obesity [147], osteoporosis [148], eosinophilia [149], HIV-1 [150], cat allergy [151] (all <i>E. coli</i>)		

Table 1 (continued)

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	native non-chimeric VLPs	chimeric and/or hybrid VLPs carrying fused or chemically conjugated epitopes/fragments from the proteins indicated below		
<i>Leviviridae</i> unclassified Caulobacter phage phiCb5			metal ion-dependent assembly [159] (<i>E. coli</i>)	
<i>Secoviridae</i> <i>Comovirus</i> Cowpea mosaic virus (CPMV)		<i>Pseudomonas aeruginosa</i> [160], mink enteritis (MEV) [161], CPV [162] (plants)	nanobuilding blocks (plants) [163–166]	tumour cells [167, 168], intravital imaging [169, 170]
<i>Picornaviridae</i> <i>Cardiovirus</i> Porcine encephalomyocarditis virus (EMCV)	EMCV [171] (insect cells)			
<i>Picornaviridae</i> <i>Enterovirus</i> Enterovirus type 71 (EV71)	EV71 [172] (insect cells)			
Coxsackievirus B (CVB3)	CVB3 [173] (insect cells)			
<i>Picornaviridae</i> <i>Aphthovirus</i> Foot-and-mouth disease virus (FDMV)	FDMV [174] (<i>E. coli</i>), [175] (insect cells)			
<i>Nodaviridae</i> <i>Alphanodavirus</i> Flock house virus (FHV)		anthrax [176] (insect cells)		
<i>Tombusviridae</i> <i>Tombusvirus</i> Tomato bushy stunt virus		ricin toxin [177] (insect cells)		
<i>Nodaviridae</i> <i>Betanodavirus</i> Malabaricus grouper nervous necrosis virus (MGNV)	viral nervous necrosis [178] (insect cells)			
Dragon grouper nervous necrosis virus (DGNNV)	viral nervous necrosis [179] (<i>E. coli</i>)			
<i>Caliciviridae</i> <i>Norovirus</i> Norwalk virus (NV)	NV [180–182] (plants), [183] (insect cells)			
<i>Caliciviridae</i> <i>Lagovirus</i> Rabbit hemorrhagic disease virus (RHDV)	RHDV [184] (insect cells), [185] (<i>S. cerevisiae</i>), [186] (<i>Pichia pastoris</i>)	FMDV [187, 188], feline calicivirus (FCV) [188], ovalbumin [189] (insect cells)		dendritic cells [190]
<i>Polyomaviridae</i> <i>Polyomavirus</i> Murine polyomavirus (MuPV)		HBV[191] (yeast), antitumour [192–194] (insect cells), influenza [195] (<i>E. coli</i>)		tumour cells [196]
Hamster polyomavirus (HaPV)		HBV [197], hanta [198], antitumour [199, 200], <i>Gardnerella vaginalis</i> [201] (all <i>S. cerevisiae</i>)	DNA [202]	
Simian virus 40 (SV40)			proteins [203], quantum dots [204]	RGD targeting [205], EGF receptor [206]
JC polyomavirus			RNAi [207], DNA [208]	
<i>Papillomaviridae</i> <i>Alphapapillomavirus</i> Human papillomavirus (HPV)	HPV: Gardasil [209] (yeast), Cervarix [210] (insect cells)	SIV/HIV [211], HPV: L1+E7 [212, 213] (insect cells), influenza [214], HPV E6, E7 [215] (plants)	DNA, packaging in GFP-decorated VLPs [216], HEV ORF [217]	DNA delivery to dendritic cells [218], B cells [219]

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<i>Papillomaviridae</i> <i>Deltapapillomavirus</i> Bovine papillomavirus (BPV)	BPV [220] (insect cells)	HPV [221], Alzheimer's disease [222], antitumour [223] (insect cells)	DNA [224]	
<i>Papillomaviridae</i> <i>Kappapapillomavirus</i> Cottontail rabbit papillomavirus (CRPV)	CRPV [225] (<i>S. cerevisiae</i>), [226, 227] (insect cells), [228] (plants)			
<i>Reoviridae</i> <i>Orbivirus</i> Bluetongue virus (BTV)	BTV [229–232] (insect cells)	HBV [233], rabies virus [234], influenza [235]		
African horse sickness virus (AHSV)	AHSV [236] (mammalian cells)	FMDV, AHSV 2 [237]		
<i>Reoviridae</i> <i>Rotavirus</i> Rotavirus (RT)	RT [238–240], RT + noro [241] (insect cells)	chimeric RT, VP8 fusion to VP2 [242] (insect cells)	GFP [243] (insect cells)	intestinal cells [243]
Yeast retrotransposon Ty1		malaria [244–246], HIV [247–249], influenza, Sendai virus, VSV [250] (all yeast)		
<i>Podoviridae</i> <i>T7-like viruses</i> Enterobacteria phage T7				HepG2 cells [251]
<i>Podoviridae</i> <i>P22-like viruses</i> Enterobacteria phage P22			small molecules, polymers, metals [252] (<i>E. coli</i>)	
<i>Siphoviridae</i> <i>Lambda-like viruses</i> Enterobacteria phage lambda		HIV-1 [253] (<i>E. coli</i> , conjugation)		
<i>Adenoviridae</i> <i>Mastadenovirus</i> Human adenovirus B (type 3) (Ad3)		cancer [254] (insect cells)	proteins [255], bleomycin [256]	
Non-enveloped viruses: helical symmetry				
<i>Alphavirus</i> <i>Potexvirus</i> Papaya mosaic virus (PapMV)		influenza A [257, 258] (plants)		
Potato virus X (PVX)		HIV-1 [259], beet necrotic yellow vein virus (BNYVV) [260], tuberculosis [261], influenza [262], HCV [263], HPV [264, 265] (all plants)		targeting model [266]
Bamboo mosaic virus (BaMV)		FMDV [267], IBDV [268] (all plants)		
<i>Potyviridae</i> <i>Potyvirus</i> Johnsongrass mosaic virus (JGMV)		<i>Plasmodium falciparum</i> [269], Japanese encephalitis virus (JEV) [270], gamete epitopes for immunoreception [271] (all <i>E. coli</i>)		
Plum pox virus (PPV)	Sharka disease [272] (plants)	CPV [273] (plants)		
Potato virus Y (PVY)		HBV [274] (<i>E. coli</i>)		
<i>Virgaviridae</i> <i>Tobamovirus</i> Tobacco mosaic virus (TMV)		poliovirus: [17] (<i>E. coli</i>), [275] (plants); malaria [276], influenza, HIV-1 [277], rabies virus (RV) [278], MHV [278, 279], <i>P. aeruginosa</i> [280], FMDV [281], CRPV, rabbit oral papillomavirus (ROPV) [282] (plants)	RNA [283]	

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Enveloped viruses: icosahedral symmetry			
<i>Hepadnaviridae Orthohepadnavirus</i>	therapeutic HBV [11, 284], combined with HBs [285] (<i>E. coli</i>)	FMDV: eukaryotic cells [286], <i>E. coli</i> [287], plants [288]; HPV: <i>E. coli</i> [289, 290], <i>Salmonella typhimurium</i> [291], HIV-1, HBV [11, 191], hepatocellular carcinoma [292], hanta [293, 294], HCV [295, 296], anthrax [297], Crohn's disease [298], tuberculosis [299], influenza [300–303] (<i>E. coli</i>), [304] (plants), malaria [305–307], <i>Borrelia burgdorferi</i> [308–310], cattle theileriosis [311], IBDV [312] (<i>E. coli</i>)	fibronectin targeting by <i>B. burgdorferi</i> BBK32 protein fragments [313]
Hepatitis B virus (HBV) surface (HBs) particles	HBV: Engerix-B [314], H-B-Vax II , or Recombivax HB [315] (<i>S. cerevisiae</i>), Hepilisav [316] (<i>Hansenula polymorpha</i>), combined with HBc [285] (yeast), edible vaccine [317–320] (plants)	HBV: preS-HBs therapeutic GenHevac B [321, 322], Bio-Hep-B [323], Hepacare (mammalian cells) [324] (mammalian cells), malaria RTS,S [31, 32] (<i>S. cerevisiae</i>), poliovirus 1 and 2 [30], SIV [325] (both mammalian cells), HIV-1: mammalian cells [326], yeast [327], plants [328], HPV [290] (<i>S. cerevisiae</i>), HEV [329], dengue [330] (both <i>P. pastoris</i>), HCV [331, 332], influenza [333], <i>Helicobacter pylori</i> [334] (all mammalian cells) influenza [335] (<i>S. typhimurium</i>)	
Woodchuck hepatitis virus (WHV) core (WHc) particles			
<i>Flaviviridae Flavivirus</i>	WNV [336] (mammalian cells)		
West Nile virus (WNV)			
Dengue virus	Dengue [337] (mammalian cells), [338] (<i>P. pastoris</i>), [339, 340] (<i>E. coli</i>)		
Hepatitis C virus (HCV)	HCV [341–343] (insect cells), [344, 345] (<i>P. pastoris</i>)		
Japanese encephalitis virus (JEV)	JEV [346–348] (mammalian cells)		
Tick-borne encephalitis virus (TEV)	TEV [345] (mammalian cells)		
<i>Togaviridae Alphavirus</i>	SIN: core-like particles [349, 350] (<i>E. coli</i>)	gold nanoparticles [352]	
Sindbis virus (SIN)	RRV: core-like particles [349, 350] (<i>E. coli</i>), VLPs [351] (<i>E. coli</i> ; mammalian cells)		
Ross river virus (RRV)			
Chikungunya virus (CHIKV)	CHIKV [353] (mammalian cells)		
Salmonid alphavirus (SAV)	SAV [354] (insect cells)		
<i>Coronaviridae Betacoronavirus</i>	SARS [355] (insect cells)		
Severe acute respiratory syndrome-related coronavirus (SARS-CoV)	SARS influenza [48] (insect cells)		

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	native non-chimeric VLPs	chimeric and/or hybrid VLPs carrying fused or chemically conjugated epitopes/fragments from the proteins indicated below		
<i>Bunyaviridae</i> <i>Nairovirus</i> Crimean-Congo hemorrhagic fever (CCHF) virus	CCHF [356] (insect cells)			
<i>Bunyaviridae</i> <i>Phlebovirus</i> Rift Valley fever virus (RVFV)	RVFV [357] (mammalian cells)			
<i>Bunyaviridae</i> <i>Hantavirus</i> Hantaan virus (HTNV)	HTNV [358] (mammalian cells)			
<i>Retroviridae</i> <i>Lentivirus</i> Human immunodeficiency virus 1 (HIV-1)	HIV-1 [41, 49, 65, 359] (mammalian cells), [360–362] (insect cells)	HIV-1 [363] (mammalian cells), [46, 364] (insect cells), equine herpesvirus type 1 (EHV-1) [365], pseudorabies virus [366] (both insect cells), HPV [367] (mammalian cells)	proteins [368–370]	
Human immunodeficiency virus 2 (HIV-2)	HIV-2 [371] (insect cells)	HIV-1 and HIV-2 [372, 373] (insect cells)	proteins [368]	
Simian immunodeficiency virus (SIV)	SIV [374] (mammalian cells), [375] (insect cells)	SHIV [376, 377] (insect cells)		
<i>Retroviridae</i> <i>Alpharetrovirus</i> Rous sarcoma virus (Rous SV)	Rous SV [378] (insect cells)	human protenin receptor (hPRR) [379] (insect cells)		
<i>Retroviridae</i> <i>Gammaretrovirus</i> Murine leukemia virus (MLV)		Alzheimer's disease [380], prion disease [381], VSV [382], cytokines [383], influenza [384] (all insect cells)		
<i>Herpesviridae</i> <i>Lymphocryptovirus</i> Human herpesvirus 4 (Epstein-Barr virus, EBV)	EBV [385] (mammalian cells)			
Enveloped complex viruses				
<i>Orthomyxoviridae</i> <i>Influenzavirus A</i> Influenza A virus	Influenza A [42–44, 54, 58, 386–388] (mammalian, insect cells)	multi-subtype influenza [70], influenza-pseudotyped Gag [52]	CAT RNA [389]	TLR [59, 390]
<i>Arenaviridae</i> <i>Arenavirus</i> Lassa virus	Lassa virus [45] (mammalian cells)			
Tacaribe and Junin viruses		Tacaribe and Junin arenaviruses [391]		
<i>Paramyxoviridae</i> <i>Avulavirus</i> Newcastle disease virus		RSV-NDV (avian) [47, 76]		
Respiratory syncytial virus (RSV)				
<i>Paramyxoviridae</i> <i>Henipavirus</i> Nipah virus (NiV)	NiV [392] (mammalian cells)			
<i>Paramyxoviridae</i> <i>Respirovirus</i> Sendai virus (SeV)	SeV [393] (mammalian cells), [87] (<i>S. cerevisiae</i>)			
Human parainfluenza virus 1 and 3	[89] (<i>S. cerevisiae</i>)			
<i>Paramyxoviridae</i> <i>Rubulavirus</i> Mumps virus (MuV)	MuV [394] (mammalian cells), [85] (<i>S. cerevisiae</i>)			

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	native non-chimeric VLPs	chimeric and/or hybrid VLPs carrying fused or chemically conjugated epitopes/fragments from the proteins indicated below		
Menangle virus (MeV)		[88] (<i>S. cerevisiae</i>)		
Tioman virus (TiOV)		[90] (<i>S. cerevisiae</i>)		
<i>Paramyxoviridae</i> <i>Metapneumovirus</i> Human metapneumovirus (hMPV)		[91] (<i>S. cerevisiae</i>)		
<i>Paramyxoviridae</i> <i>Morbillivirus</i> Measles virus (MV)		[86] (<i>S. cerevisiae</i>)		
<i>Filoviridae</i> <i>Marburgvirus</i> Marburg marburgvirus (MARV)	Marburg marburgvirus [395] (mammalian cells)	hybrid MARV and EBOV [396]		
<i>Filoviridae</i> <i>Ebolavirus</i> Zaire ebolavirus (EBOV)	Zaire ebolavirus [18, 397–399] (mammalian, insect cells)	hybrid MARV and EBOV [396]		

VLPs are listed in the order of size according to figure 1. Clinically and veterinary-approved vaccines are depicted in bold. Expression systems used for the production of VLPs are shown in parentheses.

The taxonomic orders of VLP sources are provided in accordance with the official ICTV 2011 virus taxonomy available at <http://ictvonline.org/virusTaxonomy.asp?version=2011>. LMW = Low-molecular-weight.

Rod-shaped plant viruses of helical symmetry provide a number of replication-competent examples allowing the production of chimeric viruses, with the exception of potexviruses (papaya and bamboo mosaic viruses and PVX) and some potyviruses (JGMV, PVA, and PVY), which accept foreign insertions in the VLPs after propagation in *E. coli* and baculovirus expression systems (for references, see table 1).

Complex VLPs Derived from Enveloped Viruses

Construction of Complex Enveloped VLPs

Enveloped viruses are widely used for the development of VLPs. The viral envelope represents a lipoprotein membrane that surrounds a nucleocapsid [36, 37]. The lipid bilayer is derived from host cell membranes and contains embedded viral proteins responsible for receptor recognition or other functions. It can also include carbohydrates and proteins derived from the host cell. Envelope proteins usually contain hydrophobic domains that allow their localization within the lipid bilayer. Similar to non-enveloped VLPs, the repeated regular patterns of epitopes in the native conformation make complex enveloped VLPs exceptionally effective immunogens [24, 38].

From the point of view of their spatial structure, the majority of viral envelopes are asymmetrical. However, for some enveloped viruses, viral proteins within the envelope reflect the symmetry of the inner nucleocapsid. For example, alphaviruses contain 240 heterodimers of E2-E1 proteins arranged in an icosahedral lattice according to the 240 subunits of the capsid protein within the inner icosahedral nucleocapsid [39, 40]. Other viruses, such as the orthomyxovirus influenza virus, contain envelopes that do not possess an apparent regular geometric symmetry, although they may contain elements of symmetry such as the trimers of hemagglutinin (HA) or tetramers of neuraminidase (NA) in influenza viruses.

The preparation of complex enveloped VLPs is challenging. Unlike simple icosahedral particles, enveloped VLPs often contain multiple species of lipid-associated structural proteins. In most cases, the protein-protein interactions and the roles of the proteins, lipids, and host cell factors in the assembly of VLPs are not fully understood. To achieve correct assembly of enveloped VLPs, proper interactions must occur between the nucleocapsid proteins, between the envelope proteins within the lipid bilayer, and between the envelope and the nucleocapsid proteins. The production must include an appropriate cell line and expression system, which should express suf-

ficient quantities of enveloped VLPs that are immunologically similar to their cognate virus, including the arrangement of the proteins within the envelope. Furthermore, purification of enveloped VLPs can be a difficult task. During production and purification of VLPs, various cellular lipids, carbohydrates, proteins, and nucleic acids co-purify with VLPs, which may cause difficulties in purification as well as safety and regulatory concerns if VLPs are intended for clinical applications.

Despite the challenges, enveloped VLPs of several viruses have been constructed, including VLPs for HIV, influenza viruses, Ebola virus, Lassa virus, and other viruses (table 1). VLPs have been shown to induce advantageous immune responses compared with soluble recombinant proteins. For example, membrane-embedded HIV-1 envelope proteins on the surface of VLPs elicited broader immune responses than did soluble envelope proteins [41]. Similarly, influenza VLPs containing HA protein within the envelope demonstrated greater immunogenicity than subunit HA [19, 42, 43]. In some studies, native enveloped VLPs could be made with unmodified viral proteins [19, 38, 44–46]. In other cases, extensive optimization and engineering of component proteins were required to generate VLPs [46–48].

Rational Design of Enveloped VLPs

Rational design of VLPs has been used extensively to improve the properties of enveloped VLPs, including expression levels, stability, immunogenicity, and other characteristics. First, attempts have been made to improve the expression of component proteins to improve the production of the assembled VLPs. For example, codon optimization of the expressed viral genes has improved the expression of HIV and HTLV-1 VLPs [49, 50]. Second, efforts have been made to optimize the composition of VLPs and the interactions between the individual VLP components. For example, in many cases, influenza VLPs were prepared using HA, NA, and M1 proteins derived from the same strain [19, 42–44]. Other configurations of VLPs have included a heterologous M1 derived from an unrelated influenza virus strain [51] or even a retrovirus Gag protein in place of M1 [52]. Furthermore, influenza VLPs have been made from HA and NA only [53], from HA and M1 proteins only [54], and from NA and M1 only [55]. VLPs have also been generated in the presence of additional influenza virus proteins, such as M2 [56–58], or in the presence of immunostimulatory antigens such as flagellin, a ligand for Toll-like receptor 5 [59]. These studies have helped elucidate the contributions of individual components to in-

fluenza VLP formation. For example, M1 protein is the major component of the virus and an important antigen due to the presence of subtype cross-reactive epitopes [60]. However, the role of M1 in the formation of VLPs or viral particles is not completely understood. According to one view, M1 is the driving force for influenza virus particle formation, penetrating through the cell membrane from inside the cell and interacting with the cytoplasmic tails (CT) of the structural glycoproteins HA and NA during budding [61–63]. An alternative model suggests that HA and NA partition into lipid raft microdomains and recruit the internal viral components, including M1, into the budding viral particle [53]. VLP studies have shown that VLPs can be formed either with or without M1, suggesting both M1-dependent and M1-independent mechanisms of VLP formation. This hypothesis is further supported by the fact that both M1-deficient and M1-containing types of the mature enveloped virions have been observed by cryo-electron microscopy [64].

Rational design has also been applied to HIV VLPs to improve immunogenicity and efficacy. One approach utilized consensus Env protein sequences [65]. To address the sequence diversity in Env sequences, consensus sequences were made for clade B and clade C envelope proteins. The rationale for using a consensus sequence is that the genetic difference between the vaccine strain and any given viral isolate is decreased, and, therefore, a consensus sequence will provide broader vaccine coverage. The consensus Env sequence elicited broader cell-mediated peripheral and mucosal immune responses than did polyvalent and monovalent Env protein vaccines [65]. In another study, the consensus approach was combined with protein engineering methods to increase the density of Env glycoprotein within the VLP envelopes [46]. Chimeric Env gene constructs have been engineered, in which the coding sequences for the signal peptide (SP), transmembrane (TM), and CT domains of HIV-1 Env were replaced with those of other viral or cellular proteins. Substitution of the SP from the honeybee mellitin protein resulted in 3-fold higher expression of recombinant HIV-1 Env on insect cell surfaces and increased Env incorporation into VLPs. Substitution of the HIV TM-CT with sequences derived from mouse mammary tumour virus (MMTV) envelope glycoprotein, influenza virus HA, or baculovirus (BV) gp64, but not Lassa fever virus glycoprotein, was shown to enhance Env incorporation into VLPs. The highest level of Env incorporation into VLPs was observed in constructs containing the MMTV and BV gp64 TM-CT domains in which the Gag/

Env molar ratios were estimated to be 4:1 and 5:1, respectively, compared with a 56:1 ratio for full-length consensus gp160 [46].

Additional examples of rational design include engineering of simian human immunodeficiency virus (SHIV) VLPs containing mutant HIV Env with reduced glycosylation, variable loop-deleted mutations, or combinations of both types of mutations [66].

Presentation of Foreign Proteins within Enveloped VLPs

Unlike VLPs from icosahedral viruses such as HPV, which are composed of a specific number of subunits symmetrically arranged in a geometric structure [67, 68], enveloped VLPs allow considerable structural flexibility. The lipid bilayer is relatively fluid, allowing greater flexibility than the rigid geometric lattice of icosahedral VLPs. This flexibility may provide considerable structural capacity and allow the expression of large epitopes or even entire foreign proteins as an integral part of enveloped VLPs.

In addition to accommodating larger foreign epitopes, the structural flexibility of enveloped VLPs makes possible the preparation of chimeric and hybrid VLPs that co-localize proteins from distinct strains or even from distinct viruses.

Influenza VLPs have been used as carriers for foreign antigenic epitopes or proteins. For example, it has been shown that influenza VLPs can incorporate vesicular stomatitis virus (VSV) G protein [56]. As mentioned previously, influenza VLPs can also incorporate flagellin [59]. In another study, *Bacillus anthracis* protective antigen (BPA) fragments 90 or 140 aa in length were inserted at the C-terminal flank of the HA SP and expressed as the HA1 subunit [69]. The chimeric proteins could be cleaved into the HA1 and HA2 subunits by trypsin and incorporated into influenza viruses, suggesting that the viral envelope can tolerate foreign insertions without precluding assembly. The inserted BPA domains were maintained in the HA gene segments following several passages in MDCK cells or embryonated chicken eggs. Immunization of mice with viruses that expressed the chimeric BPA/HA proteins induced antibody responses against both the HA and BPA components of the protein [69]. Although VLPs were not generated in this study, these experiments suggest that similar modifications of HA with foreign epitopes may be compatible with the formation of chimeric influenza VLPs.

A novel influenza VLP platform containing HA subtypes derived from three distinct strains has also been re-

ported [70]. This recombinant VLP design resulted in the expression of three HA subtypes co-localized within a VLP (fig. 2). Experimental triple-HA VLPs containing HA proteins derived from seasonal H1N1, H3N2, and type B influenza viruses were immunogenic and protected ferrets from challenge with all three seasonal viruses. Similarly, VLPs containing HA subtypes derived from H5N1, H7N2, and H2N2 viruses protected ferrets from three potentially pandemic influenza viruses [70]. This technology may represent a novel strategy for the rapid development of trivalent seasonal and pandemic influenza vaccines.

The possibility of co-localizing proteins from distinct viruses within the VLP is somewhat similar to the phenotypic mixing that has been observed with live viruses. Phenotypic mixing of proteins from different enveloped viruses has been described between SV5, a paramyxovirus, and VSV, a rhabdovirus, as well as between Sendai and VSV [71, 72]. Reassortant viruses have also been generated by co-infection of embryonated chicken eggs with influenza type A and type B viruses [73]. Recently, live-attenuated chimeric viruses containing HA from A/PR8 (H1), A/HK68 (H3), or A/VN (H5) strains in the backbone of the B/Yamagata/88 virus were reported [74]. Reassortment is routinely used in licensed influenza vaccines. In the reassortant vaccine virus, vaccine-relevant HA and NA glycoproteins are embedded into the envelope, whereas other viral proteins are derived from type-specific donor strains, such as the A/PR/8/34 (H1N1) or B/Ann Arbor/1/66 viruses [75].

In some cases, foreign antigens can only be expressed as VLPs by displaying them within chimeric/hybrid VLPs. For example, VLPs composed entirely of RSV proteins were produced at levels that were inadequate for their use as vaccines [47]. However, VLPs composed of the Newcastle disease virus (NDV) nucleocapsid and membrane proteins and chimeric proteins containing the ectodomains of RSV F and G proteins fused to the TM and cytoplasmic domains of NDV F and HN proteins, respectively, were quantitatively prepared from cultured avian cells. Immunization with a single dose of VLPs resulted in the complete protection of mice against RSV replication in lungs [47]. Another chimeric VLP was composed of the NP and M proteins of NDV and a chimeric protein containing the cytoplasmic and TM domains of the NDV HN protein and the ectodomain of the human RSV G protein (H/G) [76]. The VLP-H/G candidate vaccine was immunogenic in BALB/c mice and prevented replication of RSV in murine lungs with no evidence of immunopathology [76]. Chimeric VLPs consist-

Fig. 2. Preparation and electron microscopy of a triple-subtype VLP vaccine containing seasonal influenza A H1 and H3 subtypes and influenza B virus HA. Recombinant baculovirus (rBV) for the expression of the triple-HA VLPs in Sf9 cells contained influenza HA gene sequences derived from A/New Caledonia/20/1999 (H1N1), A/New York/55/2004 (H3N2) and B/Shanghai/361/2002. NA and M1 gene sequences were from the A/Indonesia/05/2005 (H5N1) virus [70]. Negative stain transmission electron microscopy (bottom middle) was performed by staining with 1% phosphotungstic acid. Immunoelectron microscopy (bottom right) was performed using sucrose gradient-purified VLPs. VLPs were probed with a mixture of primary antibodies specific for H1, H3, and type B influenza from rabbit, mouse, and guinea pigs, respectively. Secondary antibodies were donkey anti-rabbit labelled with 18-nm gold particles, anti-mouse labelled with 6-nm gold particles, and anti-guinea pig labelled with 12-nm gold particles. Bars: 100 nm.

Fig. 3. Preparation of hybrid/chimeric SARS-CoV VLPs using influenza M1 protein and chimeric SARS-CoV S protein (GenBank AAP13441), containing a carboxy-terminal TM sequence derived from influenza HA. TM helices were predicted using TransMem based upon a Hidden Markov Model. The TM domain was derived from influenza A/Indonesia/5/2005 HA (aa 531–568). Chimeric SARS-CoV S and influenza M1 proteins (ABI36004) were co-expressed from recombinant baculovirus in *Spodoptera frugiperda* Sf9 cells [48]. An electron photomicrograph of hybrid/chimeric SARS-CoV VLPs is shown with a schematic model.

ing of an influenza virus matrix (M1) protein core and RSV-F or RSV-G on the surface have also been produced [77]. These data support the further development of VLPs as vaccines for protection against RSV.

Similar to RSV VLPs, VLPs composed entirely of SARS-CoV proteins have been produced at low levels [48]. A method was developed to produce higher levels of SARS VLPs containing the SARS spike (S) protein and the influenza M1 protein (fig. 3). The SARS VLPs protected mice from death when administered via the intramuscular or intranasal routes [48].

Conclusions

It has been more than 25 years since the idea of using highly symmetric self-assembling VLPs for vaccine purposes attracted considerable attention (fig. 1). Applications of VLPs have included their use as carriers to improve the immunogenicity of poorly immunogenic epitopes. With time, over-optimistic expectations for simple and rapid generation of effective VLP-based vaccines have encountered numerous challenges. Nevertheless, as shown in this review, many promising vaccine candidates have been produced (table 1). Renewal of the

previous optimism is due at least in part to the recent development of VLPs of large complex viruses, such as orthomyxoviruses, filoviruses, and paramyxoviruses. Due to their larger size and versatile structure, complex VLPs demonstrate greater capacity and flexibility than highly symmetric icosahedral VLPs for the inclusion of full-length, properly folded foreign proteins. Furthermore, complex VLPs are capable of simultaneously accepting various foreign proteins, enabling construction of multivalent vaccines. Full-length foreign proteins or epitopes delivered by large complex VLPs offer many immunological advantages over short epitopes, which have been inserted onto the surface of icosahedral scaffolds. First, complex VLPs can enhance T helper and cytotoxic T cell responses by inducing highly specific immunological responses directed against conformational epitopes, which is difficult to achieve with chimeric symmetric VLPs. Second, due to their size and organization, complex VLPs may be stable in the blood and thereby obviate the need for adjuvants. Third, if necessary, complex VLPs can be configured to contain specific and efficient adjuvants, including immune stimulatory sequences such as CpGs [78–81]. The presence of a lipid envelope may contribute to the correct folding of certain vaccine antigens and thereby improve immunogenicity. For example, the immunogenicity of purified subunit HA could be improved by adding liposomes [19]. The immunogenicity of split influenza vaccines could also be improved by adding liposomes or lipid-based adjuvants, such as MF59, an oil-in-water emulsion containing the unsaturated aliphatic hydrocarbon squalene [82]. Interestingly, highly immunogenic influenza vaccines, such as whole virus vaccines, VLPs, and virosomes, all have a lipid component in common, suggesting that lipids may play an important role in influenza vaccine immunogenicity.

Growing interest in knowledge-based applications of complex VLPs is strengthened by the development of high-resolution methods [64, 83, 84].

The rapid development of therapeutic candidates based on complex VLPs is propelled by the constant progress of cGMP-compliant production systems, such as baculovirus system-driven production, and the recent approval of a baculovirus-derived HPV VLP vaccine by the US Food and Drug Administration and other regulatory agencies (table 1). However, purification of complex enveloped VLPs to a standard that meets regulatory and safety requirements can be a difficult task. Unlike icosahedral VLPs such as HPV, complex enveloped VLPs are difficult to purify by conventional methods such as re-

folding *in vitro*. Therefore, the icosahedral VLP platform remains a good option for many applications due to the advantages of purification as well as a clear regulatory pathway due to the availability of licensed vaccines. The introduction of yeast expression systems into the production of complex VLPs can potentially improve the safety and quality control of biotechnological processes [85–91].

One potential method for the development of VLP-based vaccines may involve the combination of both complex and icosahedral particles, from a simple mixing of potential vaccine candidates (e.g., the therapeutic hepatitis B vaccine DV-601, http://www.dynavax.com/hepatitis_bt.html) to the knowledge-based packaging of small icosahedral VLPs into large complex VLPs in the future.

Finally, both symmetric and complex VLPs, which were initially used primarily for vaccine development, are now evolving in the direction of broader applications, including gene and drug therapies. New applications for VLPs include the targeting of specific cells and encapsidation of new materials, from oligonucleotides, genes, proteins, and peptides to therapeutic drugs and inorganic compounds such as gold and iron nanoparticles. For each application, careful consideration of both non-enveloped and enveloped VLPs may be necessary.

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References

- 1 Gren EJ, Pumpen PP: Recombinant viral capsids as a new age of immunogenic proteins and vaccines (in Russian). *J All-Union Mendeleev's Chem Soc* 1988;33:531–536.
- 2 Kingsman SM, Kingsman AJ: Polyvalent recombinant antigens: a new vaccine strategy. *Vaccine* 1988;6:304–306.
- 3 Ulrich R, Gerlich WH, Krüger DH: Chimaera and its modern virus-like descendants. *Inter-virology* 1996;39:126–132.
- 4 Johnson JE, Chiu W: Structures of virus and virus-like particles. *Curr Opin Struct Biol* 2000;10:229–235.

- 5 Pumpens P, Grens E: Artificial genes for chimeric virus-like particles; in Khudyakov YE, Fields HA (eds): *Artificial DNA: Methods and Applications*. Boca Raton, CRC Press LLC, 2002, pp 249–327.
- 6 Bachmann MF, Dyer MR: Therapeutic vaccination for chronic diseases: a new class of drugs in sight. *Nat Rev Drug Discov* 2004;3: 81–88.
- 7 Pasek M, Goto T, Gilbert W, Zink B, Schaller H, MacKay P, Leadbetter G, Murray K: Hepatitis B virus genes and their expression in *E. coli*. *Nature* 1979;282:575–579.
- 8 Borisova GP, Pumpen PP, Bychko VV, Pushko PM, Kalis IV, Dishler AV, Gren EJ, Tsinogin VV, Kukaine RA: Structure and expression in *Escherichia coli* cells of the core antigen gene of the human hepatitis B virus (HBV) (in Russian). *Dokl Akad Nauk SSSR* 1984;279:1245–1249.
- 9 Nassal M: Total chemical synthesis of a gene for hepatitis B virus core protein and its functional characterisation. *Gene* 1988;66:279–294.
- 10 Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD: Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* 1982;298:347–350.
- 11 Pumpens P, Ulrich R, Sasnauskas K, Kazaks A, Ose V, Grens E: Construction of novel vaccines on the basis of the virus-like particles: hepatitis B virus proteins as vaccine carriers; in Khudyakov Y (ed): *Medicinal Protein Engineering*. Boca Raton, CRC Press, Taylor & Francis Group, 2008, pp 205–248.
- 12 Kastelein RA, Berkhout B, Overbeek GP, van Duin J: Effect of the sequences upstream from the ribosome-binding site on the yield of protein from the cloned gene for phage MS2 coat protein. *Gene* 1983;23:245–254.
- 13 Kozlovskaya TM, Pumpen PP, Dreilina DE, Tsimanis Alu, Ose VP, Tsinogin VV, Gren EJ: Formation of capsid-like structures as a result of expression of the cloned gene of the envelope protein of the RNA-containing bacteriophage fr (in Russian). *Dokl Akad Nauk SSSR* 1986;287:452–455.
- 14 Borisova G, Bundule M, Grinstein E, Dreilina D, Dreimane A, Kalis J, Kozlovskaya T, Loseva V, Ose V, Pumpen P, Pushko P, Snikere D, Stankevica E, Tsinogin V, Gren EJ: Recombinant capsid structures for exposure of protein antigenic epitopes. *Mol Gen (Life Sci Adv)* 1987;6:169–174.
- 15 Kozlovskaya TM, Pushko PM, Stankevich EI, Dreimane Alu, Sniker DI, Grinstein EE, Dreilina DE, Veinina AE, Ose VP, Pumpen PP, Gren EJ: Genetically engineered mutants of the envelope protein of the RNA-containing bacteriophage fr (in Russian). *Mol Biol (Mosk)* 1988;22:731–740.
- 16 Kozlovskaya TM, Cielens I, Dreilina D, Dislers A, Baumanis V, Ose V, Pumpens P: Recombinant RNA phage Q beta capsid particles synthesized and self-assembled in *Escherichia coli*. *Gene* 1993;137:133–137.
- 17 Haynes JR, Cunningham J, von Seefried A, Lennick M, Garvin RT, Shen SH: Development of a genetically-engineered, candidate polio vaccine employing the self-assembling properties of the tobacco mosaic virus coat protein. *Biotechnology (NY)* 1986;4:637–641.
- 18 Warfield KL, Bosio CM, Welcher BC, Deal EM, Mohamadizadeh M, Schmaljohn A, Aman MJ, Bavari S: Ebola virus-like particles protect from lethal Ebola virus infection. *Proc Natl Acad Sci USA* 2003;100:15889–15894.
- 19 Pushko P, Tumpey TM, Van Hoven N, Belser JA, Robinson R, Nathan M, Smith G, Wright DC, Bright RA: Evaluation of influenza virus-like particles and Novasome adjuvant as candidate vaccine for avian influenza. *Vaccine* 2007;25:4283–4290.
- 20 Pushko PM, Bright RA, Tumpey TM, Smith GE: Engineering better influenza vaccines: traditional and new approaches; in Khudyakov Y (ed): *Medicinal Protein Engineering*. Boca Raton, CRC Press, Taylor & Francis Group, 2008, pp 169–204.
- 21 Chackerian B: Virus-like particles: flexible platforms for vaccine development. *Expert Rev Vaccines* 2007;6:381–390.
- 22 Ramqvist T, Andreasson K, Dalianis T: Vaccination, immune and gene therapy based on virus-like particles against viral infections and cancer. *Expert Opin Biol Ther* 2007;7:997–1007.
- 23 Jennings GT, Bachmann MF: Immunodrugs: therapeutic VLP-based vaccines for chronic diseases. *Annu Rev Pharmacol Toxicol* 2009; 49:303–326.
- 24 Roy P, Noad R: Virus-like particles as a vaccine delivery system: myths and facts. *Adv Exp Med Biol* 2009;655:145–158.
- 25 Whitacre DC, Lee BO, Milich DR: Use of hepatitis B core proteins as vaccine platforms. *Expert Rev Vaccines* 2009;8:1565–1573.
- 26 Herbst-Kralovetz M, Mason HS, Chen Q: Norwalk virus-like particles as vaccines. *Expert Rev Vaccines* 2010;9:299–307.
- 27 Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM: Virus-like particles in vaccine development. *Expert Rev Vaccines* 2010; 9:1149–1176.
- 28 Schiller JT, Lowy DR: Vaccines to prevent infections by oncoviruses. *Annu Rev Microbiol* 2010;64:23–41.
- 29 Valenzuela P, Coit D, Medina-Selby MA, Kuo CH, Van Nest G, Burke RL, Bull P, Urdea MS, Graves PV: Antigen engineering in yeast: synthesis and assembly of hybrid hepatitis B surface antigen – herpes simplex 1 gD particles. *Biotechnology (NY)* 1985;3:323–326.
- 30 Delpeyroux F, Chenciner N, Lim A, Malpierre Y, Blondel B, Crainic R, van der Werf S, Strecker RE: A poliovirus neutralization epitope expressed on hybrid hepatitis B surface antigen particles. *Science* 1986;233:472–475.
- 31 Rutgers T, Cordon D, Gathoye AM, Hollingdale M, Hockmeyer W, Rosenberg M, De Wilde M: Hepatitis B surface antigen as carrier matrix for the repetitive epitope of the circumsporozoite protein of *Plasmodium falciparum*. *Biotechnology (NY)* 1988;6:1065–1070.
- 32 Asante KP, Abdulla S, Agnandji S, Lyimo J, Vekemans J, Soulanoudjingar S, Owusu R, Shomari M, Leach A, Jongert E, Salim N, Fernandes JF, Dosoo D, Chikawe M, Issifou S, Osei-Kwakye K, Lievens M, Paricek M, Möller T, Apanga S, Mwangoka G, Dubois MC, Madi T, Kwara E, Minja R, Hounkpatin AB, Boahen O, Kayan K, Adjei G, Chandramohan D, Carter T, Vansadia P, Sillman M, Savarese B, Loucq C, Lapierre D, Greenwood B, Cohen J, Kremsner P, Owusu-Agyei S, Tanner M, Lell B: Safety and efficacy of the RTS,S/AS01E candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. *Lancet Infect Dis* 2011;11: 741–749.
- 33 Steinmetz NF, Lin T, Lomonosoff GP, Johnson JE: Structure-based engineering of an icosahedral virus for nanomedicine and nanotechnology. *Curr Top Microbiol Immunol* 2009;327:23–58.
- 34 Kao CC, Ni P, Hema M, Huang X, Dragnea B: The coat protein leads the way: an update on basic and applied studies with the Brome mosaic virus coat protein. *Mol Plant Pathol* 2011; 12:403–412.
- 35 Soto CM, Ratna BR: Virus hybrids as nanomaterials for biotechnology. *Curr Opin Biotechnol* 2010;21:426–438.
- 36 Nayak DP, Balogun RA, Yamada H, Zhou ZH, Barman S: Influenza virus morphogenesis and budding. *Virus Res* 2009;143:147–161.
- 37 Vaney MC, Rey FA: Class II enveloped viruses. *Cell Microbiol* 2011;13:1451–1459.
- 38 Warfield KL, Posten NA, Swenson DL, Olinger GG, Esposito D, Gillette WK, Hopkins RF, Costantino J, Panchal RG, Hartley JL, Aman MJ, Bavari S: Filovirus-like particles produced in insect cells: immunogenicity and protection in rodents. *J Infect Dis* 2007; 196(suppl 2):S421–S429.
- 39 Strauss JH, Strauss EG: The alphaviruses: gene expression, replication, and evolution. *Microbiol Rev* 1994;58:491–562.
- 40 Strauss JH, Strauss EG: Virus evolution: how does an enveloped virus make a regular structure? *Cell* 2001;105:5–8.
- 41 McBurney SP, Young KR, Ross TM: Membrane embedded HIV-1 envelope on the surface of a virus-like particle elicits broader immune responses than soluble envelopes. *Virology* 2007;358:334–346.
- 42 Bright RA, Carter DM, Daniluk S, Toapanta FR, Ahmad A, Gavrilov V, Massare M, Pushko P, Mytle N, Rowe T, Smith G, Ross TM: Influenza virus-like particles elicit broader immune responses than whole virion inactivated influenza virus or recombinant hemagglutinin. *Vaccine* 2007;25:3871–3878.

- 43 Perrone LA, Ahmad A, Veguilla V, Lu X, Smith G, Katz JM, Pushko P, Tumpey TM: Intranasal vaccination with 1918 influenza virus-like particles protects mice and ferrets from lethal 1918 and H5N1 influenza virus challenge. *J Virol* 2009;83:5726–5734.
- 44 Pushko P, Tumpey TM, Bu F, Knell J, Robinson R, Smith G: Influenza virus-like particles comprised of the HA, NA, and M1 proteins of H9N2 influenza virus induce protective immune responses in BALB/c mice. *Vaccine* 2005;23:5751–5759.
- 45 Branco LM, Grove JN, Geske FJ, Boisen ML, Muncy IJ, Magliato SA, Henderson LA, Schoepp RJ, Cashman KA, Hensley LE, Garry RF: Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* 2010;7:279.
- 46 Wang BZ, Liu W, Kang SM, Alam M, Huang C, Ye L, Sun Y, Li Y, Kothe DL, Pushko P, Dokland T, Haynes BF, Smith G, Hahn BH, Compans RW: Incorporation of high levels of chimeric human immunodeficiency virus envelope glycoproteins into virus-like particles. *J Virol* 2007;81:10869–10878.
- 47 McGinnes LW, Gravel KA, Finberg RW, Kurt-Jones EA, Massare MJ, Smith G, Schmidt MR, Morrison TG: Assembly and immunological properties of Newcastle disease virus-like particles containing the respiratory syncytial virus F and G proteins. *J Virol* 2011;85:366–377.
- 48 Liu YV, Massare MJ, Barnard DL, Kort T, Nathan M, Wang L, Smith G: Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and influenza matrix 1 efficiently form virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. *Vaccine* 2011;29:6606–6613.
- 49 Diaz-Griffero F, Taube R, Muehlbauer SM, Brojatsch J: Efficient production of HIV-1 viral-like particles in mouse cells. *Biochem Biophys Res Commun* 2008;368:463–469.
- 50 Grigsby IF, Zhang W, Johnson JL, Fogarty KH, Chen Y, Rawson JM, Crosby AJ, Mueller JD, Mansky LM: Biophysical analysis of HTLV-1 particles reveals novel insights into particle morphology and Gag stoichiometry. *Retrovirology* 2010;7:75.
- 51 Prel A, Le Gall-Reculé G, Jestin V: Achievement of avian influenza virus-like particles that could be used as a subunit vaccine against low-pathogenic avian influenza strains in ducks. *Avian Pathol* 2008;37:513–520.
- 52 Haynes JR, Dokken L, Wiley JA, Cawthon AG, Bigger J, Harmsen AG, Richardson C: Influenza-pseudotyped Gag virus-like particle vaccines provide broad protection against highly pathogenic avian influenza challenge. *Vaccine* 2009;27:530–541.
- 53 Chen BJ, Leser GP, Morita E, Lamb RA: Influenza virus hemagglutinin and neuraminidase, but not the matrix protein, are required for assembly and budding of plasmid-derived virus-like particles. *J Virol* 2007;81:7111–7123.
- 54 Quan FS, Huang C, Compans RW, Kang SM: Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. *J Virol* 2007;81:3514–3524.
- 55 Quan FS, Kim MC, Lee BJ, Song JM, Compans RW, Kang SM: Influenza M1 VLPs containing neuraminidase induce heterosubtypic cross-protection. *Virology* 2012;430:127–135.
- 56 Latham T, Galarza JM: Formation of wild-type and chimeric influenza virus-like particles following simultaneous expression of only four structural proteins. *J Virol* 2001;75:6154–6165.
- 57 Matasov D, Cupo A, Galarza JM: A novel intranasal virus-like particle (VLP) vaccine designed to protect against the pandemic 1918 influenza A virus (H1N1). *Viral Immunol* 2007;20:441–452.
- 58 Watanabe T, Watanabe S, Neumann G, Kida H, Kawaoka Y: Immunogenicity and protective efficacy of replication-incompetent influenza virus-like particles. *J Virol* 2002;76:767–773.
- 59 Wang BZ, Quan FS, Kang SM, Bozja J, Skountzou I, Compans RW: Incorporation of membrane-anchored flagellin into influenza virus-like particles enhances the breadth of immune responses. *J Virol* 2008;82:11813–11823.
- 60 Terajima M, Cruz J, Leporati AM, Orphin L, Babon JA, Co MD, Pazoles P, Jameson J, Ennis FA: Influenza A virus matrix protein 1-specific human CD8+ T-cell response induced in trivalent inactivated vaccine recipients. *J Virol* 2008;82:9283–9287.
- 61 Gómez-Puertas P, Albo C, Pérez-Pastrana E, Vivo A, Portela A: Influenza virus matrix protein is the major driving force in virus budding. *J Virol* 2000;74:11538–11547.
- 62 Barman S, Ali A, Hui EK, Adhikary L, Nayak DP: Transport of viral proteins to the apical membranes and interaction of matrix protein with glycoproteins in the assembly of influenza viruses. *Virus Res* 2001;77:61–69.
- 63 Schmitt AP, Lamb RA: Influenza virus assembly and budding at the viral budzone. *Adv Virus Res* 2005;64:383–416.
- 64 Harris A, Cardone G, Winkler DC, Heymann JB, Brecher M, White JM, Steven AC: Influenza virus pleiomorphism characterized by cryoelectron tomography. *Proc Natl Acad Sci USA* 2006;103:19123–19127.
- 65 McBurney SP, Ross TM: Human immunodeficiency virus-like particles with consensus envelopes elicited broader cell-mediated peripheral and mucosal immune responses than polyvalent and monovalent Env vaccines. *Vaccine* 2009;27:4337–4349.
- 66 Quan FS, Sailaja G, Skountzou I, Huang C, Vzorov A, Compans RW, Kang SM: Immunogenicity of virus-like particles containing modified human immunodeficiency virus envelope proteins. *Vaccine* 2007;25:3841–3850.
- 67 Chen XS, Garcea RL, Goldberg I, Casini G, Harrison SC: Structure of small virus-like particles assembled from the L1 protein of human papillomavirus 16. *Mol Cell* 2000;5:557–567.
- 68 Sasagawa T, Pushko P, Steers G, Gschmeissner SE, Hajibagheri MA, Finch J, Crawford L, Tommasino M: Synthesis and assembly of virus-like particles of human papillomaviruses type 6 and type 16 in fission yeast *Schizosaccharomyces pombe*. *Virology* 1995;206:126–135.
- 69 Li ZN, Mueller SN, Ye L, Bu Z, Yang C, Ahmed R, Steinhauer DA: Chimeric influenza virus hemagglutinin proteins containing large domains of the *Bacillus anthracis* protective antigen: protein characterization, incorporation into infectious influenza viruses, and antigenicity. *J Virol* 2005;79:10003–10012.
- 70 Pushko P, Pearce MB, Ahmad A, Tretyakova I, Smith G, Belser JA, Tumpey TM: Influenza virus-like particle can accommodate multiple subtypes of hemagglutinin and protect from multiple influenza types and subtypes. *Vaccine* 2011;29:5911–5918.
- 71 Choppin PW, Compans RW: Phenotypic mixing of envelope proteins of the parainfluenza virus SV5 and vesicular stomatitis virus. *J Virol* 1970;5:609–616.
- 72 Metsikkö K, Garoff H: Role of heterologous and homologous glycoproteins in phenotypic mixing between Sendai virus and vesicular stomatitis virus. *J Virol* 1989;63:5111–5118.
- 73 Gotlieb T, Hirst GK: The experimental production of combination forms of virus. III. The formation of doubly antigenic particles from influenza A and B virus and a study of the ability of individual particles of X virus to yield two separate strains. *J Exp Med* 1954;99:307–320.
- 74 Hai R, García-Sastre A, Swayne DE, Palese P: A reassortment-incompetent live attenuated influenza virus vaccine for protection against pandemic virus strains. *J Virol* 2011;85:6832–6843.
- 75 Chen Z, Aspelund A, Kemble G, Jin H: Molecular studies of temperature-sensitive replication of the cold-adapted B/Ann Arbor/1/66, the master donor virus for live attenuated influenza FluMist vaccines. *Virology* 2008;380:354–362.
- 76 Murawski MR, McGinnes LW, Finberg RW, Kurt-Jones EA, Massare MJ, Smith G, Heaton PM, Fraire AE, Morrison TG: Newcastle disease virus-like particles containing respiratory syncytial virus G protein induced protection in BALB/c mice, with no evidence of immunopathology. *J Virol* 2010;84:1110–1123.
- 77 Quan FS, Kim Y, Lee S, Yi H, Kang SM, Bozja J, Moore ML, Compans RW: Viruslike particle vaccine induces protection against respiratory syncytial virus infection in mice. *J Infect Dis* 2011;204:987–995.

- 78 Storni T, Ruedl C, Schwarz K, Schwendener RA, Renner WA, Bachmann MF: Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects. *J Immunol* 2004;172:1777–1785.
- 79 Kazaks A, Balmaks R, Voronkova T, Ose V, Pumpens P: Melanoma vaccine candidates from chimeric hepatitis B core virus-like particles carrying a tumor-associated MAGE-3 epitope. *Biotechnol J* 2008;3:1429–1436.
- 80 Senti G, Johansen P, Haug S, Bull C, Gottschaller C, Müller P, Pfister T, Maurer P, Bachmann MF, Graf N, Kündig TM: Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial. *Clin Exp Allergy* 2009;39:562–570.
- 81 Krieg AM: CpG still rocks! Update on an accidental drug. *Nucleic Acid Ther* 2012;22:77–89.
- 82 Podda A: The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine. *Vaccine* 2001;19:2673–2680.
- 83 Terrier O, Rolland JP, Rosa-Calatrava M, Lina B, Thomas D, Moules V: Parainfluenza virus type 5 (PIV-5) morphology revealed by cryo-electron microscopy. *Virus Res* 2009;142:200–203.
- 84 Badia-Martinez D, Peralta B, Andrés G, Guerra M, Gil-Carton D, Abrescia NG: Three-dimensional visualization of forming hepatitis C virus-like particles by electron-tomography. *Virology* 2012;430:120–126.
- 85 Slibinskas R, Zvirbliene A, Gedvilaite A, Samuel D, Jin L, Beard S, Staniulis J, Sasnauskas K: Synthesis of mumps virus nucleocapsid protein in yeast *Pichia pastoris*. *J Biotechnol* 2003;103:43–49.
- 86 Slibinskas R, Samuel D, Gedvilaite A, Staniulis J, Sasnauskas K: Synthesis of the measles virus nucleoprotein in yeast *Pichia pastoris* and *Saccharomyces cerevisiae*. *J Biotechnol* 2004;107:115–124.
- 87 Juozapaitis M, Slibinskas R, Staniulis J, Sakaguchi T, Sasnauskas K: Generation of Sendai virus nucleocapsid-like particles in yeast. *Virus Res* 2005;108:221–224.
- 88 Juozapaitis M, Serva A, Kucinskaite I, Zvirbliene A, Slibinskas R, Staniulis J, Sasnauskas K, Shiell BJ, Bowden TR, Michalski WP: Generation of menangle virus nucleocapsid-like particles in yeast *Saccharomyces cerevisiae*. *J Biotechnol* 2007;130:441–447.
- 89 Juozapaitis M, Zvirbliene A, Kucinskaite I, Sezaite I, Slibinskas R, Coiras M, de Ory Manchon F, López-Huertas MR, Pérez-Breña P, Staniulis J, Narkeviciute I, Sasnauskas K: Synthesis of recombinant human parainfluenza virus 1 and 3 nucleocapsid proteins in yeast *Saccharomyces cerevisiae*. *Virus Res* 2008;133:178–186.
- 90 Petraityte R, Tamosiunas PL, Juozapaitis M, Zvirbliene A, Sasnauskas K, Shiell B, Russell G, Bingham J, Michalski WP: Generation of Tioman virus nucleocapsid-like particles in yeast *Saccharomyces cerevisiae*. *Virus Res* 2009;145:92–96.
- 91 Petraitytė-Burneikienė R, Nalivaiko K, Lasickienė R, Firantienė R, Emužytė R, Sasnauskas K, Zvirblinė A: Generation of recombinant metapneumovirus nucleocapsid protein as nucleocapsid-like particles and development of virus-specific monoclonal antibodies. *Virus Res* 2011;161:131–139.
- 92 Bucarey SA, Noriega J, Reyes P, Tapia C, Sáenz L, Zuñiga A, Tobar JA: The optimized capsid gene of porcine circovirus type 2 expressed in yeast forms virus-like particles and elicits antibody responses in mice fed with recombinant yeast extracts. *Vaccine* 2009;27:5781–5790.
- 93 Wu PC, Lin WL, Wu CM, Chi JN, Chien MS, Huang C: Characterization of porcine circovirus type 2 (PCV2) capsid particle assembly and its application to virus-like particle vaccine development. *Appl Microbiol Biotechnol* 2012;95:1501–1507.
- 94 Bansal GP, Hatfield JA, Dunn FE, Kramer AA, Brady F, Riggan CH, Collett MS, Yoshimoto K, Kajigaya S, Young NS: Candidate recombinant vaccine for human B19 parvovirus. *J Infect Dis* 1993;167:1034–1044.
- 95 Bernstein DI, El Sahly HM, Keitel WA, Wolff M, Simone G, Segawa C, Wong S, Shelly D, Young NS, Dempsey W: Safety and immunogenicity of a candidate parvovirus B19 vaccine. *Vaccine* 2011;29:7357–7363.
- 96 Brown CS, Welling-Wester S, Feijlbrief M, Van Lent JW, Spaan WJ: Chimeric parvovirus B19 capsids for the presentation of foreign epitopes. *Virology* 1994;198:477–488.
- 97 Amexis G, Young NS: Parvovirus B19 empty capsids as antigen carriers for presentation of antigenic determinants of dengue 2 virus. *J Infect Dis* 2006;194:790–794.
- 98 Ogasawara Y, Amexis G, Yamaguchi H, Kajigaya S, Leppla SH, Young NS: Recombinant viral-like particles of parvovirus B19 as antigen carriers of anthrax protective antigen. *In Vivo* 2006;20:319–324.
- 99 Hou L, Wu H, Xu L, Yang F: Expression and self-assembly of virus-like particles of infectious hypodermal and hematopoietic necrosis virus in *Escherichia coli*. *Arch Virol* 2009;154:547–553.
- 100 Ju H, Wei N, Wang Q, Wang C, Jing Z, Guo L, Liu D, Gao M, Ma B, Wang J: Goose parvovirus structural proteins expressed by recombinant baculoviruses self-assemble into virus-like particles with strong immunogenicity in goose. *Biochem Biophys Res Commun* 2011;409:131–136.
- 101 Antonis AF, Bruschke CJ, Rueda P, Maranga L, Casal JI, Vela C, Hilgers LA, Belt PB, Weerdmeester K, Carrondo MJ, Langeveld JP: A novel recombinant virus-like particle vaccine for prevention of porcine parvovirus-induced reproductive failure. *Vaccine* 2006;24:5481–5490.
- 102 Sedlik C, Sarraseca J, Rueda P, Leclerc C, Casal I: Immunogenicity of poliovirus B and T cell epitopes presented by hybrid porcine parvovirus particles. *J Gen Virol* 1995;76:2361–2368.
- 103 Pan Q, He K, Huang K: Development of recombinant porcine parvovirus-like particles as an antigen carrier formed by the hybrid VP2 protein carrying immunoreactive epitope of porcine circovirus type 2. *Vaccine* 2008;26:2119–2126.
- 104 López de Turiso JA, Cortés E, Martínez C, Ruiz de Ybáñez R, Simarro I, Vela C, Casal I: Recombinant vaccine for canine parvovirus in dogs. *J Virol* 1992;66:2748–2753.
- 105 Rueda P, Martínez-Torrecuadrada JL, Sarraseca J, Sedlik C, del Barrio M, Hurtado A, Leclerc C, Casal JI: Engineering parvovirus-like particles for the induction of B-cell, CD4(+) and CTL responses. *Vaccine* 1999;18:325–332.
- 106 Feng H, Liang M, Wang HL, Zhang T, Zhao PS, Shen XJ, Zhang RZ, Hu GQ, Gao YQ, Wang CY, Wang TC, Zhang W, Yang ST, Xia XZ: Recombinant canine parvovirus-like particles express foreign epitopes in silkworm pupae. *Vet Microbiol* 2011;154:49–57.
- 107 Singh P: Tumor targeting using canine parvovirus nanoparticles. *Curr Top Microbiol Immunol* 2009;327:123–141.
- 108 Maloney BJ, Takeda N, Suzaki Y, Ami Y, Li TC, Miyamura T, Arntzen CJ, Mason HS: Challenges in creating a vaccine to prevent hepatitis E. *Vaccine* 2005;23:1870–1874.
- 109 Niikura M, Takamura S, Kim G, Kawai S, Saijo M, Morikawa S, Kurane I, Li TC, Takeda N, Yasutomi Y: Chimeric recombinant hepatitis E virus-like particles as an oral vaccine vehicle presenting foreign epitopes. *Virology* 2002;293:273–280.
- 110 Yusibov V, Hooper DC, Spitsin SV, Fleish N, Kean RB, Mikheeva T, Deka D, Karasev A, Cox S, Randall J, Koprowski H: Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine* 2002;20:3155–3164.
- 111 Yusibov V, Mett V, Mett V, Davidson C, Musiychuk K, Gilliam S, Farese A, Macvittie T, Mann D: Peptide-based candidate vaccine against respiratory syncytial virus. *Vaccine* 2005;23:2261–2265.
- 112 Brodzik R, Bandurska K, Deka D, Golovkin M, Koprowski H: Advances in alfalfa mosaic virus-mediated expression of anthrax antigen in planta. *Biochem Biophys Res Commun* 2005;338:717–722.

- 113 Chen C, Daniel MC, Quinkert ZT, De M, Stein B, Bowman VD, Chipman PR, Rotello VM, Kao CC, Dragnea B: Nanoparticle-templated assembly of viral protein cages. *Nano Lett* 2006;6:611–615.
- 114 Hu Y, Zandi R, Anavitaré A, Knobler CM, Gelbart WM: Packaging of a polymer by a viral capsid: the interplay between polymer length and capsid size. *Biophys J* 2008;94:1428–1436.
- 115 Minten IJ, Wilke KD, Hendriks LJ, van Hest JC, Nolte RJ, Cornelissen JJ: Metal-ion-induced formation and stabilization of protein cages based on the cowpea chlorotic mottle virus. *Small* 2011;7:911–919.
- 116 Cadena-Nava RD, Comas-García M, Garman RF, Rao AL, Knobler CM, Gelbart WM: Self-assembly of viral capsid protein and RNA molecules of different sizes: requirement for a specific high protein/RNA mass ratio. *J Virol* 2012;86:3318–3326.
- 117 Brasch M, de la Escosura A, Ma Y, Uetrecht C, Heck AJ, Torres T, Cornelissen JJ: Encapsulation of phthalocyanine supramolecular stacks into virus-like particles. *J Am Chem Soc* 2011;133:6878–6881.
- 118 Nuzzaci M, Vitti A, Condelli V, Lanorte MT, Tortorella C, Boscia D, Piazzolla P, Piazzolla G: In vitro stability of Cucumber mosaic virus nanoparticles carrying a hepatitis C virus-derived epitope under simulated gastrointestinal conditions and in vivo efficacy of an edible vaccine. *J Virol Methods* 2010;165:211–215.
- 119 Natilla A, Hammond RW, Nemchinov LG: Epitope presentation system based on cucumber mosaic virus coat protein expressed from a potato virus X-based vector. *Arch Virol* 2006;151:1373–1386.
- 120 Vitti A, Piazzolla G, Condelli V, Nuzzaci M, Lanorte MT, Boscia D, De Stradis A, Antonaci S, Piazzolla P, Tortorella C: Cucumber mosaic virus as the expression system for a potential vaccine against Alzheimer's disease. *J Virol Methods* 2010;169:332–340.
- 121 Lu X, Thompson JR, Perry KL: Encapsulation of DNA, a protein and a fluorophore into virus-like particles by the capsid protein of cucumber mosaic virus. *J Gen Virol* 2012;93:1120–1126.
- 122 Mastico RA, Talbot SJ, Stockley PG: Multiple presentation of foreign peptides on the surface of an RNA-free spherical bacteriophage capsid. *J Gen Virol* 1993;74:541–548.
- 123 Peabody DS, Manifold-Wheeler B, Medford A, Jordan SK, do Carmo Caldeira J, Chackerian B: Immunogenic display of diverse peptides on virus-like particles of RNA phage MS2. *J Mol Biol* 2008;380:252–263.
- 124 Heal KG, Hill HR, Stockley PG, Hollingdale MR, Taylor-Robinson AW: Expression and immunogenicity of a liver stage malaria epitope presented as a foreign peptide on the surface of RNA-free MS2 bacteriophage capsids. *Vaccine* 1999;18:251–258.
- 125 Brown WL, Mastico RA, Wu M, Heal KG, Adams CJ, Murray JB, Simpson JC, Lord JM, Taylor-Robinson AW, Stockley PG: RNA bacteriophage capsid-mediated drug delivery and epitope presentation. *Intervirology* 2002;45:371–380.
- 126 Wu M, Sherwin T, Brown WL, Stockley PG: Delivery of antisense oligonucleotides to leukemia cells by RNA bacteriophage capsids. *Nanomedicine* 2005;1:67–76.
- 127 Wei B, Wei Y, Zhang K, Wang J, Xu R, Zhan S, Lin G, Wang W, Liu M, Wang L, Zhang R, Li J: Development of an antisense RNA delivery system using conjugates of the MS2 bacteriophage capsids and HIV-1 TAT cell-penetrating peptide. *Biomed Pharmacother* 2009;63:313–318.
- 128 Sun S, Li W, Sun Y, Pan Y, Li J: A new RNA vaccine platform based on MS2 virus-like particles produced in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 2011;407:124–128.
- 129 Ashley CE, Carnes EC, Phillips GK, Durfee PN, Buley MD, Lino CA, Padilla DP, Phillips B, Carter MB, Willman CL, Brinker CJ, Caldeira Jdo C, Chackerian B, Wharton W, Peabody DS: Cell-specific delivery of diverse cargos by bacteriophage MS2 virus-like particles. *ACS Nano* 2011;5:5729–5745.
- 130 Pan Y, Zhang Y, Jia T, Zhang K, Li J, Wang L: Development of a microRNA delivery system based on bacteriophage MS2 virus-like particles. *FEBS J* 2012;279:1198–1208.
- 131 Wu M, Brown WL, Stockley PG: Cell-specific delivery of bacteriophage-encapsidated ricin A chain. *Bioconjug Chem* 1995;6:587–595.
- 132 Caldeira Jdo C, Medford A, Kines RC, Lino CA, Schiller JT, Chackerian B, Peabody DS: Immunogenic display of diverse peptides, including a broadly cross-type neutralizing human papillomavirus L2 epitope, on virus-like particles of the RNA bacteriophage PP7. *Vaccine* 2010;28:4384–4393.
- 133 Tumban E, Peabody J, Peabody DS, Chackerian B: A pan-HPV vaccine based on bacteriophage PP7 VLPs displaying broadly cross-neutralizing epitopes from the HPV minor capsid protein, L2. *PLoS One* 2011;6:e23310.
- 134 Voronkova T, Grosch A, Kazaks A, Ose V, Skrastina D, Sasnauskas K, Jandrig B, Arnold W, Scherneck S, Pumpens P, Ulrich R: Chimeric bacteriophage fr virus-like particles harboring the immunodominant C-terminal region of hamster polyomavirus VP1 induce a strong VP1-specific antibody response in rabbits and mice. *Viral Immunol* 2002;15:627–643.
- 135 Rūmnieks J, Freivalds J, Cielēns I, Renhofa R: Packaging of specific mRNAs in bacteriophage GA virus-like particles in yeast *Saccharomyces cerevisiae*. *Acta Universitatis Latviensis Biol* 2008;745:145–154.
- 136 Tissot AC, Renhofa R, Schmitz N, Cielens I, Meijerink E, Ose V, Jennings GT, Saudan P, Pumpens P, Bachmann MF: Versatile virus-like particle carrier for epitope based vaccines. *PLoS One* 2010;5:e9809.
- 137 Spohn G, Jennings GT, Martina BE, Keller I, Beck M, Pumpens P, Osterhaus AD, Bachmann MF: A VLP-based vaccine targeting domain III of the West Nile virus E protein protects from lethal infection in mice. *Virol J* 2010;7:146.
- 138 Klimek L, Willers J, Hammann-Haenni A, Pfaar O, Stocker H, Mueller P, Renner WA, Bachmann MF: Assessment of clinical efficacy of CYT003-QbG10 in patients with allergic rhinoconjunctivitis: a phase IIb study. *Clin Exp Allergy* 2011;41:1305–1312.
- 139 Vasiljeva I, Kozlovskaya T, Cielens I, Strelnikova A, Kazaks A, Ose V, Pumpens P: Mosaic Qbeta coats as a new presentation model. *FEBS Lett* 1998;431:7–11.
- 140 Bessa J, Schmitz N, Hinton HJ, Schwarz K, Jegerlehner A, Bachmann MF: Efficient induction of mucosal and systemic immune responses by virus-like particles administered intranasally: implications for vaccine design. *Eur J Immunol* 2008;38:114–126.
- 141 Cornuz J, Zwahlen S, Jungi WF, Osterwalder J, Klingler K, van Melle G, Bangala Y, Guesous I, Müller P, Willers J, Maurer P, Bachmann MF, Cerny T: A vaccine against nicotine for smoking cessation: a randomized controlled trial. *PLoS One* 2008;3:e2547.
- 142 Tissot AC, Maurer P, Nussberger J, Sabat R, Pfister T, Ignatenko S, Volk HD, Stocker H, Müller P, Jennings GT, Wagner F, Bachmann MF: Effect of immunisation against angiotensin II with CYT006-AngQb on ambulatory blood pressure: a double-blind, randomised, placebo-controlled phase IIa study. *Lancet* 2008;371:821–827.
- 143 Braun M, Jandus C, Maurer P, Hammann-Haenni A, Schwarz K, Bachmann MF, Speiser DE, Romero P: Virus-like particles induce robust human T-helper cell responses. *Eur J Immunol* 2012;42:330–340.
- 144 Spohn G, Keller I, Beck M, Grest P, Jennings GT, Bachmann MF: Active immunization with IL-1 displayed on virus-like particles protects from autoimmune arthritis. *Eur J Immunol* 2008;38:877–887.
- 145 Röhn TA, Ralvenius WT, Paul J, Bortner P, Hernandez M, Witschi R, Grest P, Zeilhofer HU, Bachmann MF, Jennings GT: A virus-like particle-based anti-nerve growth factor vaccine reduces inflammatory hyperalgesia: potential long-term therapy for chronic pain. *J Immunol* 2011;186:1769–1780.
- 146 Wiessner C, Wiederhold KH, Tissot AC, Frey P, Danner S, Jacobson LH, Jennings GT, Lüönd R, Ortmann R, Reichwald J, Zurini M, Mir A, Bachmann MF, Staufenbiel M: The second-generation active Aβ immunotherapy CAD106 reduces amyloid accumulation in APP transgenic mice while minimizing potential side effects. *J Neurosci* 2011;31:9323–9331.

- 147 Fulurija A, Lutz TA, Sladko K, Osto M, Wielinga PY, Bachmann MF, Saudan P: Vaccination against GIP for the treatment of obesity. *PLoS One* 2008;3:e3163.
- 148 Spohn G, Schwarz K, Maurer P, Illges H, Rajasekaran N, Choi Y, Jennings GT, Bachmann MF: Protection against osteoporosis by active immunization with TRANCE/RANKL displayed on virus-like particles. *J Immunol* 2005;175:6211–6218.
- 149 Zou Y, Sonderegger I, Lipowsky G, Jennings GT, Schmitz N, Landi M, Kopf M, Bachmann MF: Combined vaccination against IL-5 and eotaxin blocks eosinophilia in mice. *Vaccine* 2010;28:3192–3200.
- 150 Hunter Z, Smyth HD, Durfee P, Chackerian B: Induction of mucosal and systemic antibody responses against the HIV coreceptor CCR5 upon intramuscular immunization and aerosol delivery of a virus-like particle based vaccine. *Vaccine* 2009;28:403–414.
- 151 Schmitz N, Dietmeier K, Bauer M, Maudrich M, Utzinger S, Muntwiler S, Saudan P, Bachmann MF: Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. *J Exp Med* 2009;206:1941–1955.
- 152 Fiedler JD, Brown SD, Lau JL, Finn MG: RNA-directed packaging of enzymes within virus-like particles. *Angew Chem Int Ed Engl* 2010;49:9648–9651.
- 153 Rhee JK, Hovlid M, Fiedler JD, Brown SD, Manzenrieder F, Kitagishi H, Nycholat C, Paulson JC, Finn MG: Colorful virus-like particles: fluorescent protein packaging by the Q β capsid. *Biomacromolecules* 2011;12:3977–3981.
- 154 Lau JL, Baksh MM, Fiedler JD, Brown SD, Kussrow A, Bornhop DJ, Ordoukhanian P, Finn MG: Evolution and protein packaging of small-molecule RNA aptamers. *ACS Nano* 2011;5:7722–7729.
- 155 Wu Z, Tang LJ, Zhang XB, Jiang JH, Tan W: Aptamer-modified nanodrug delivery systems. *ACS Nano* 2011;5:7696–7699.
- 156 Brown SD, Fiedler JD, Finn MG: Assembly of hybrid bacteriophage Q β virus-like particles. *Biochemistry* 2009;48:11155–11157.
- 157 Pokorski JK, Hovlid ML, Finn MG: Cell targeting with hybrid Q β virus-like particles displaying epidermal growth factor. *ChemBioChem* 2011;12:2441–2447.
- 158 Gale AJ, Elias DJ, Averell PM, Teirstein PS, Buck M, Brown SD, Polonskaya Z, Udit AK, Finn MG: Engineered virus-like nanoparticles reverse heparin anticoagulation more consistently than protamine in plasma from heparin-treated patients. *Thromb Res* 2011;128:e9–e13.
- 159 Plevka P, Kazaks A, Voronkova T, Kotelovica S, Dishlers A, Liljas L, Tars K: The structure of bacteriophage phiC β 5 reveals a role of the RNA genome and metal ions in particle stability and assembly. *J Mol Biol* 2009;391:635–647.
- 160 Brennan FR, Gilleland LB, Staczek J, Bendig MM, Hamilton WD, Gilleland HE Jr: A chimeric plant virus vaccine protects mice against a bacterial infection. *Microbiology* 1999;145:2061–2067.
- 161 Dalsgaard K, Uttenthal A, Jones TD, Xu F, Merryweather A, Hamilton WD, Langeveld JP, Boshuizen RS, Kamstrup S, Lomonosoff GP, Porta C, Vela C, Casal JJ, Meloen RH, Rodgers PB: Plant-derived vaccine protects target animals against a viral disease. *Nat Biotechnol* 1997;15:248–252.
- 162 Langeveld JP, Brennan FR, Martínez-Torrecuadrada JL, Jones TD, Boshuizen RS, Vela C, Casal JJ, Kamstrup S, Dalsgaard K, Meloen RH, Bendig MM, Hamilton WD: Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus. *Vaccine* 2001;19:3661–3670.
- 163 Aljabali AA, Shah SN, Evans-Gowing R, Lomonosoff GP, Evans DJ: Chemically-coupled-peptide-promoted virus nanoparticle templated mineralization. *Integr Biol (Camb)* 2011;3:119–125.
- 164 Blum AS, Soto CM, Sapsford KE, Wilson CD, Moore MH, Ratna BR: Molecular electronics based nanosensors on a viral scaffold. *Biosens Bioelectron* 2011;26:2852–2857.
- 165 Aljabali AA, Lomonosoff GP, Evans DJ: CPMV-polyelectrolyte-templated gold nanoparticles. *Biomacromolecules* 2011;12:2723–2728.
- 166 Sainsbury F, Saunders K, Aljabali AA, Evans DJ, Lomonosoff GP: Peptide-controlled access to the interior surface of empty virus nanoparticles. *ChemBioChem* 2011;12:2435–2440.
- 167 Destito G, Yeh R, Rae CS, Finn MG, Manchester M: Folic acid-mediated targeting of cowpea mosaic virus particles to tumor cells. *Chem Biol* 2007;14:1152–1162.
- 168 Steinmetz NF, Cho CF, Ablack A, Lewis JD, Manchester M: Cowpea mosaic virus nanoparticles target surface vimentin on cancer cells. *Nanomedicine (Lond)* 2011;6:351–364.
- 169 Lewis JD, Destito G, Zijlstra A, Gonzalez MJ, Quigley JP, Manchester M, Stuhlmann H: Viral nanoparticles as tools for intravital vascular imaging. *Nat Med* 2006;12:354–360.
- 170 Leong HS, Steinmetz NF, Ablack A, Destito G, Zijlstra A, Stuhlmann H, Manchester M, Lewis JD: Intravital imaging of embryonic and tumor neovasculature using viral nanoparticles. *Nat Protoc* 2010;5:1406–1417.
- 171 Jeoung HY, Lee WH, Jeong W, Shin BH, Choi HW, Lee HS, An DJ: Immunogenicity and safety of virus-like particle of the porcine encephalomyocarditis virus in pig. *Virology* 2011;53:170.
- 172 Chung YC, Ho MS, Wu JC, Chen WJ, Huang JH, Chou ST, Hu YC: Immunization with virus-like particles of enterovirus 71 elicits potent immune responses and protects mice against lethal challenge. *Vaccine* 2008;26:1855–1862.
- 173 Zhang L, Parham NJ, Zhang F, Aasa-Chapman M, Gould EA, Zhang H: Vaccination with coxsackievirus B3 virus-like particles elicits humoral immune response and protects mice against myocarditis. *Vaccine* 2012;30:2301–2308.
- 174 Lee CD, Yan YP, Liang SM, Wang TF: Production of FMDV virus-like particles by a SUMO fusion protein approach in *Escherichia coli*. *J Biomed Sci* 2009;16:69.
- 175 Cao Y, Sun P, Fu Y, Bai X, Tian F, Liu X, Lu Z, Liu Z: Formation of virus-like particles from O-type foot-and-mouth disease virus in insect cells using codon-optimized synthetic genes. *Biotechnol Lett* 2010;32:1223–1229.
- 176 Manayani DJ, Thomas D, Dryden KA, Reddy V, Siladi ME, Marlett JM, Rainey GJ, Pique ME, Scobie HM, Yeager M, Young JA, Manchester M, Schneemann A: A viral nanoparticle with dual function as an anthrax antitoxin and vaccine. *PLoS Pathog* 2007;3:1422–1431.
- 177 Kumar S, Ochoa W, Singh P, Hsu C, Schneemann A, Manchester M, Olson M, Reddy V: Tomato bushy stunt virus (TBSV), a versatile platform for polyvalent display of antigenic epitopes and vaccine design. *Virology* 2009;388:185–190.
- 178 Thiéry R, Cozien J, Cabon J, Lamour F, Baud M, Schneemann A: Induction of a protective immune response against viral nervous necrosis in the European sea bass *Dicentrarchus labrax* by using betanodavirus virus-like particles. *J Virol* 2006;80:10201–10207.
- 179 Lu MW, Liu W, Lin CS: Infection competition against grouper nervous necrosis virus by virus-like particles produced in *Escherichia coli*. *J Gen Virol* 2003;84:1577–1582.
- 180 Ball JM, Graham DY, Opekun AR, Gilger MA, Guerrero RA, Estes MK: Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. *Gastroenterology* 1999;117:40–48.
- 181 Zhang X, Buehner NA, Hutson AM, Estes MK, Mason HS: Tomato is a highly effective vehicle for expression and oral immunization with Norwalk virus capsid protein. *Plant Biotechnol J* 2006;4:419–432.
- 182 Santi L, Batchelor L, Huang Z, Hjelm B, Kilbourne J, Arntzen CJ, Chen Q, Mason HS: An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles. *Vaccine* 2008;26:1846–1854.
- 183 Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, Estes MK, Graham DY, Opekun AR, Richardson C, Mendelman PM: Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J Med* 2011;365:2178–2187.
- 184 Laurent S, Vautherot JF, Madelaine MF, Le Gall G, Rasschaert D: Recombinant rabbit hemorrhagic disease virus capsid protein expressed in baculovirus self-assembles into viruslike particles and induces protection. *J Virol* 1994;68:6794–6798.

- 185 Boga JA, Martín Alonso JM, Casais R, Parra F: A single dose immunization with rabbit haemorrhagic disease virus major capsid protein produced in *Saccharomyces cerevisiae* induces protection. *J Gen Virol* 1997;78: 2315–2318.
- 186 Farnós O, Fernández E, Chiong M, Parra F, Joglar M, Méndez L, Rodríguez E, Moya G, Rodríguez D, Leonart R, González EM, Alonso A, Alfonso P, Suárez M, Rodríguez MP, Toledo JR: Biochemical and structural characterization of RHDV capsid protein variants produced in *Pichia pastoris*: advantages for immunization strategies and vaccine implementation. *Antiviral Res* 2009;81: 25–36.
- 187 Crisci E, Fraile L, Moreno N, Blanco E, Cabezón R, Costa C, Mussá T, Baratelli M, Martínez-Orellana P, Ganges L, Martínez J, Bárcena J, Montoya M: Chimeric calicivirus-like particles elicit specific immune responses in pigs. *Vaccine* 2012;30:2427–2439.
- 188 Luque D, González JM, Gómez-Blanco J, Marabini R, Chichón J, Mena I, Angulo I, Carrascosa JL, Verdaguier N, Trus BL, Bárcena J, Castón JR: Epitope insertion at the N-terminal molecular switch of the rabbit hemorrhagic disease virus t = 3 capsid protein leads to larger t = 4 capsids. *J Virol* 2012; 86:6470–6480.
- 189 Peacey M, Wilson S, Perret R, Ronchese F, Ward VK, Young V, Young SL, Baird MA: Virus-like particles from rabbit hemorrhagic disease virus can induce an anti-tumor response. *Vaccine* 2008;26:5334–5337.
- 190 Win SJ, McMillan DG, Errington-Mais F, Ward VK, Young SL, Baird MA, Melcher AA: Enhancing the immunogenicity of tumour lysate-loaded dendritic cell vaccines by conjugation to virus-like particles. *Br J Cancer* 2012;106:92–98.
- 191 Skrastina D, Bulavaite A, Sominskaya I, Kovalevska L, Ose V, Priede D, Pumpens P, Sasnauskas K: High immunogenicity of a hydrophilic component of the hepatitis B virus preS1 sequence exposed on the surface of three virus-like particle carriers. *Vaccine* 2008;26:1972–1981.
- 192 Tegerstedt K, Lindencrona JA, Curcio C, Andreasson K, Tullus C, Forni G, Dalianis T, Kiessling R, Ramqvist T: A single vaccination with polyomavirus VP1/VP2Her2 virus-like particles prevents outgrowth of HER-2/neu-expressing tumors. *Cancer Res* 2005;65:5953–5957.
- 193 Andreasson K, Tegerstedt K, Eriksson M, Curcio C, Cavallo F, Forni G, Dalianis T, Ramqvist T: Murine pneumotropic virus chimeric Her2/neu virus-like particles as prophylactic and therapeutic vaccines against Her2/neu expressing tumors. *Int J Cancer* 2009;124:150–156.
- 194 Eriksson M, Andreasson K, Weidmann J, Lundberg K, Tegerstedt K, Dalianis T, Ramqvist T: Murine polyomavirus virus-like particles carrying full-length human PSA protect BALB/c mice from outgrowth of a PSA expressing tumor. *PLoS One* 2011; 6:e23828.
- 195 Middelberg AP, Rivera-Hernandez T, Wibowo N, Lua LH, Fan Y, Magor G, Chang C, Chuan YP, Good MF, Batzloff MR: A microbial platform for rapid and low-cost virus-like particle and capsomere vaccines. *Vaccine* 2011;29:7154–7162.
- 196 May T, Gleiter S, Lilie H: Assessment of cell type specific gene transfer of polyoma virus like particles presenting a tumor specific antibody Fv fragment. *J Virol Methods* 2002; 105:147–157.
- 197 Gedvilaite A, Frömmel C, Sasnauskas K, Micheel B, Ozel M, Behrsing O, Stanulius J, Jandrig B, Scherneck S, Ulrich R: Formation of immunogenic virus-like particles by inserting epitopes into surface-exposed regions of hamster polyomavirus major capsid protein. *Virology* 2000;273:21–35.
- 198 Gedvilaite A, Zvirbliene A, Stanulius J, Sasnauskas K, Krüger DH, Ulrich R: Segments of puumala hantavirus nucleocapsid protein inserted into chimeric polyomavirus-derived virus-like particles induce a strong immune response in mice. *Viral Immunol* 2004;17:51–68.
- 199 Dorn DC, Lawatscheck R, Zvirbliene A, Aleksaite E, Pecher G, Sasnauskas K, Ozel M, Raftery M, Schönrich G, Ulrich RG, Gedvilaite A: Cellular and humoral immunogenicity of hamster polyomavirus-derived virus-like particles harboring a mucin 1 cytotoxic T-cell epitope. *Viral Immunol* 2008;21: 12–27.
- 200 Mazeike E, Gedvilaite A, Blohm U: Induction of insert-specific immune response in mice by hamster polyomavirus VP1 derived virus-like particles carrying LCMV GP33 CTL epitope. *Virus Res* 2012;163:2–10.
- 201 Pleckaityte M, Zvirbliene A, Zezaite I, Gedvilaite A: Production in yeast of pseudotype virus-like particles harboring functionally active antibody fragments neutralizing the cytolytic activity of vaginolysin. *Microb Cell Fact* 2011;10:109.
- 202 Voronkova T, Kazaks A, Ose V, Ozel M, Scherneck S, Pumpens P, Ulrich R: Hamster polyomavirus-derived virus-like particles are able to transfer in vitro encapsidated plasmid DNA to mammalian cells. *Virus Genes* 2007;34:303–314.
- 203 Inoue T, Kawano MA, Takahashi RU, Tsukamoto H, Enomoto T, Imai T, Kataoka K, Handa H: Engineering of SV40-based nanocapsules for delivery of heterologous proteins as fusions with the minor capsid proteins VP2/3. *J Biotechnol* 2008;134:181–192.
- 204 Li F, Zhang ZP, Peng J, Cui ZQ, Pang DW, Li K, Wei HP, Zhou YF, Wen JK, Zhang XE: Imaging viral behavior in mammalian cells with self-assembled capsid-quantum-dot hybrid particles. *Small* 2009;5:718–726.
- 205 Takahashi RU, Kanesashi SN, Inoue T, Enomoto T, Kawano MA, Tsukamoto H, Takeshita F, Imai T, Ochiya T, Kataoka K, Yamaguchi Y, Handa H: Presentation of functional foreign peptides on the surface of SV40 virus-like particles. *J Biotechnol* 2008; 135:385–392.
- 206 Kitai Y, Fukuda H, Enomoto T, Asakawa Y, Suzuki T, Inouye S, Handa H: Cell selective targeting of a simian virus 40 virus-like particle conjugated to epidermal growth factor. *J Biotechnol* 2011;155:251–256.
- 207 Chou MI, Hsieh YF, Wang M, Chang JT, Chang D, Zouali M, Tsay GJ: In vitro and in vivo targeted delivery of IL-10 interfering RNA by JC virus-like particles. *J Biomed Sci* 2010;17:51.
- 208 Chang CF, Wang M, Ou WC, Chen PL, Shen CH, Lin PY, Fang CY, Chang D: Human JC virus-like particles as a gene delivery vector. *Expert Opin Biol Ther* 2011;11:1169–1175.
- 209 Centers for Disease Control and Prevention (CDC): FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;59:630–632.
- 210 Centers for Disease Control and Prevention (CDC): FDA licensure of bivalent human papillomavirus vaccine (HPV2, Cervarix) for use in females and updated HPV vaccination recommendations from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;59:626–629.
- 211 Dale CJ, Liu XS, De Rose R, Purcell DF, Anderson J, Xu Y, Leggatt GR, Frazer IH, Kent SJ: Chimeric human papilloma virus-simian/human immunodeficiency virus virus-like-particle vaccines: immunogenicity and protective efficacy in macaques. *Virology* 2002;301:176–187.
- 212 Greenstone HL, Nieland JD, de Visser KE, De Bruijn ML, Kirnbauer R, Roden RB, Lowy DR, Kast WM, Schiller JT: Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. *Proc Natl Acad Sci USA* 1998;95:1800–1805.
- 213 Kaufmann AM, Nieland JD, Jochmus I, Baur S, Friese K, Gabelsberger J, Gieseke F, Gissmann L, Glasschröder B, Grubert T, Hillemanns P, Höpfel R, Ikenberg H, Schwarz J, Karrasch M, Knoll A, Küppers V, Lechmann M, Lelle RJ, Meissner H, Müller RT, Pawlita M, Petry KU, Pilch H, Walek E, Schneider A: Vaccination trial with HPV16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). *Int J Cancer* 2007;121:2794–2800.

- 214 Matic S, Rinaldi R, Masenga V, Noris E: Efficient production of chimeric human papillomavirus 16 L1 protein bearing the M2e influenza epitope in *Nicotiana benthamiana* plants. *BMC Biotechnol* 2011;11:106.
- 215 Paz De la Rosa G, Monroy-García A, Mora-García Mde L, Peña CG, Hernández-Montes J, Weiss-Steider B, Gómez-Lim MA: An HPV 16 L1-based chimeric human papilloma virus-like particles containing a string of epitopes produced in plants is able to elicit humoral and cytotoxic T-cell activity in mice. *Virol J* 2009;6:2.
- 216 Windram OP, Weber B, Jaffer MA, Rybicki EP, Shepherd DN, Varsani A: An investigation into the use of human papillomavirus type 16 virus-like particles as a delivery vector system for foreign proteins: N- and C-terminal fusion of GFP to the L1 and L2 capsid proteins. *Arch Virol* 2008;153:585–589.
- 217 Renoux VM, Fleury MJ, Bousarghin L, Gaitan J, Sizaret PY, Touzé A, Coursaget P: Induction of antibody response against hepatitis E virus (HEV) with recombinant human papillomavirus pseudoviruses expressing truncated HEV capsid proteins in mice. *Vaccine* 2008;26:6602–6607.
- 218 Malboeuf CM, Simon DA, Lee YE, Lankes HA, Dewhurst S, Frelinger JG, Rose RC: Human papillomavirus-like particles mediate functional delivery of plasmid DNA to antigen presenting cells in vivo. *Vaccine* 2007;25:3270–3276.
- 219 Wang H, Yang Z, Li F, He Y, Wang S, Yang D, Wu Y: Insertion of a targeting peptide on capsid surface loops of human papillomavirus type-16 virus-like particles mediate elimination of anti-dsDNA Abs-producing B cells with high efficiency. *J Immunother* 2009;32:36–41.
- 220 Hainisch EK, Brandt S, Shafit-Keramat S, Van den Hoven R, Kirnbauer R: Safety and immunogenicity of BPV-1 L1 virus-like particles in a dose-escalation vaccination trial in horses. *Equine Vet J* 2012;44:107–111.
- 221 Slupetzky K, Gambhira R, Culp TD, Shafit-Keramat S, Schellenbacher C, Christensen ND, Roden RB, Kirnbauer R: A papilloma-virus-like particle (VLP) vaccine displaying HPV16 L2 epitopes induces cross-neutralizing antibodies to HPV11. *Vaccine* 2007;25:2001–2010.
- 222 Zamora E, Handisurya A, Shafit-Keramat S, Borchelt D, Rudow G, Conant K, Cox C, Troncoso JC, Kirnbauer R: Papillomavirus-like particles are an effective platform for amyloid-beta immunization in rabbits and transgenic mice. *J Immunol* 2006;177:2662–2670.
- 223 Pejavar-Gaddy S, Rajawat Y, Hilioti Z, Xue J, Gaddy DF, Finn OJ, Viscidi RP, Bossis I: Generation of a tumor vaccine candidate based on conjugation of a MUC1 peptide to polyionic papillomavirus virus-like particles. *Cancer Immunol Immunother* 2010;59:1685–1696.
- 224 Zhao KN, Hengst K, Liu WJ, Liu YH, Liu XS, McMillan NA, Frazer IH: BPV1 E2 protein enhances packaging of full-length plasmid DNA in BPV1 pseudovirions. *Virology* 2000;272:382–393.
- 225 Jansen KU, Rosolowsky M, Schultz LD, Markus HZ, Cook JC, Donnelly JJ, Martinez D, Ellis RW, Shaw AR: Vaccination with yeast-expressed cottontail rabbit papilloma-virus (CRPV) virus-like particles protects rabbits from CRPV-induced papilloma formation. *Vaccine* 1995;13:1509–1514.
- 226 Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, Schiller JT, Lowy DR: Immunization with virus-like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J Virol* 1995;69:3959–3963.
- 227 Govan VA, Rybicki EP, Williamson AL: Therapeutic immunisation of rabbits with cottontail rabbit papillomavirus (CRPV) virus-like particles (VLP) induces regression of established papillomas. *Virol J* 2008;5:45.
- 228 Kohl T, Hitzeroth II, Stewart D, Varsani A, Govan VA, Christensen ND, Williamson AL, Rybicki EP: Plant-produced cottontail rabbit papillomavirus L1 protein protects against tumor challenge: a proof-of-concept study. *Clin Vaccine Immunol* 2006;13:845–853.
- 229 Roy P, Urakawa T, Van Dijk AA, Erasmus BJ: Recombinant virus vaccine for bluetongue disease in sheep. *J Virol* 1990;64:1998–2003.
- 230 Stewart M, Bhatia Y, Athmaran TN, Noad R, Gastaldi C, Dubois E, Russo P, Thiéry R, Saillieu C, Bréard E, Zientara S, Roy P: Validation of a novel approach for the rapid production of immunogenic virus-like particles for bluetongue virus. *Vaccine* 2010;28:3047–3054.
- 231 Pérez de Diego AC, Athmaran TN, Stewart M, Rodríguez-Sánchez B, Sánchez-Vizcaino JM, Noad R, Roy P: Characterization of protection afforded by a bivalent virus-like particle vaccine against bluetongue virus serotypes 1 and 4 in sheep. *PLoS One* 2011;6:e26666.
- 232 Stewart M, Dovas CI, Chatzinasiou E, Athmaran TN, Papanastassopoulou M, Papadopoulos O, Roy P: Protective efficacy of Bluetongue virus-like and subvirus-like particles in sheep: presence of the serotype-specific VP2, independent of its geographic lineage, is essential for protection. *Vaccine* 2012;30:2131–2139.
- 233 Belyaev AS, Roy P: Presentation of hepatitis B virus preS2 epitope on bluetongue virus core-like particles. *Virology* 1992;190:840–844.
- 234 Le Blois H, Roy P: A single point mutation in the VP7 major core protein of bluetongue virus prevents the formation of core-like particles. *J Virol* 1993;67:353–359.
- 235 Adler S, Reay P, Roy P, Klenk HD: Induction of T cell response by bluetongue virus core-like particles expressing a T cell epitope of the M1 protein of influenza A virus. *Med Microbiol Immunol* 1998;187:91–96.
- 236 Guthrie AJ, Quan M, Lourens CW, Audonnet JC, Minke JM, Yao J, He L, Nordgren R, Gardner IA, Maclachlan NJ: Protective immunization of horses with a recombinant canarypox virus vectored vaccine co-expressing genes encoding the outer capsid proteins of African horse sickness virus. *Vaccine* 2009;27:4434–4438.
- 237 Rutkowska DA, Meyer QC, Maree F, Vosloo W, Fick W, Huismans H: The use of soluble African horse sickness viral protein 7 as an antigen delivery and presentation system. *Virus Res* 2011;156:35–48.
- 238 Crawford SE, Estes MK, Ciarlet M, Barone C, O'Neal CM, Cohen J, Conner ME: Heterotypic protection and induction of a broad heterotypic neutralization response by rotavirus-like particles. *J Virol* 1999;73:4813–4822.
- 239 González AM, Nguyen TV, Azevedo MS, Jeong K, Agarib F, Iosef C, Chang K, Lovgren-Bengtsson K, Morein B, Saif LJ: Antibody responses to human rotavirus (HRV) in gnotobiotic pigs following a new prime/boost vaccine strategy using oral attenuated HRV priming and intranasal VP2/6 rotavirus-like particle (VLP) boosting with ISCOM. *Clin Exp Immunol* 2004;135:361–372.
- 240 Azevedo MS, Gonzalez AM, Yuan L, Jeong KI, Iosef C, Van Nguyen T, Lovgren-Bengtsson K, Morein B, Saif LJ: An oral versus intranasal prime/boost regimen using attenuated human rotavirus or VP2 and VP6 virus-like particles with immunostimulating complexes influences protection and antibody-secreting cell responses to rotavirus in a neonatal gnotobiotic pig model. *Clin Vaccine Immunol* 2010;17:420–428.
- 241 Blazevic V, Lappalainen S, Nurminen K, Huhti L, Vesikari T: Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. *Vaccine* 2011;29:8126–8133.
- 242 Istrate C, Hinkula J, Charpilienne A, Poncet D, Cohen J, Svensson L, Johansen K: Parental administration of RF 8–2/6/7 rotavirus-like particles in a one-dose regimen induce protective immunity in mice. *Vaccine* 2008;26:4594–4601.
- 243 Cortes-Perez NG, Sapin C, Jaffredo L, Daou S, Grill JP, Langella P, Seksik P, Beaugier L, Chwetzoff S, Trugnan G: Rotavirus-like particles: a novel nanocarrier for the gut. *J Biomed Biotechnol* 2010;2010:317545.
- 244 Gilbert SC, Plebanski M, Harris SJ, Allsopp CE, Thomas R, Layton GT, Hill AV: A protein particle vaccine containing multiple malaria epitopes. *Nat Biotechnol* 1997;15:1280–1284.

- 245 Plebanski M, Gilbert SC, Schneider J, Hannan CM, Layton G, Blanchard T, Becker M, Smith G, Butcher G, Sinden RE, Hill AV: Protection from *Plasmodium berghei* infection by priming and boosting T cells to a single class I-restricted epitope with recombinant carriers suitable for human use. *Eur J Immunol* 1998;28:4345–4355.
- 246 Oliveira-Ferreira J, Miyahira Y, Layton GT, Savage N, Esteban M, Rodriguez D, Rodriguez JR, Nussenzweig RS, Zavala F: Immunogenicity of Ty-VLP bearing a CD8(+) T cell epitope of the CS protein of *P. yoelii*: enhanced memory response by boosting with recombinant vaccinia virus. *Vaccine* 2000;18:1863–1869.
- 247 Adams SE, Dawson KM, Gull K, Kingsman SM, Kingsman AJ: The expression of hybrid HIV:Ty virus-like particles in yeast. *Nature* 1987;329:68–70.
- 248 Martin SJ, Vyakarnam A, Cheingsong-Popov R, Callow D, Jones KL, Senior JM, Adams SE, Kingsman AJ, Matear P, Gotch FM, et al: Immunization of human HIV-seronegative volunteers with recombinant p17/p24:Ty virus-like particles elicits HIV-1 p24-specific cellular and humoral immune responses. *AIDS* 1993;7:1315–1323.
- 249 Peters BS, Cheingsong-Popov R, Callow D, Foxall R, Patou G, Hodgkin K, Weber JN: A pilot phase II study of the safety and immunogenicity of HIV p17/p24:VLP (p24-VLP) in asymptomatic HIV seropositive subjects. *J Infect* 1997;35:231–235.
- 250 Layton GT, Harris SJ, Myhan J, West D, Gotch F, Hill-Perkins M, Cole JS, Meyers N, Woodrow S, French TJ, Adams SE, Kingsman AJ: Induction of single and dual cytotoxic T-lymphocyte responses to viral proteins in mice using recombinant hybrid Ty-virus-like particles. *Immunology* 1996;87:171–178.
- 251 Tang KH, Yusoff K, Tan WS: Display of hepatitis B virus PreS1 peptide on bacteriophage T7 and its potential in gene delivery into HepG2 cells. *J Virol Methods* 2009;159:194–199.
- 252 Uchida M, Morris DS, Kang S, Jolley CC, Luccon J, Liepold LO, LaFrance B, Prevelige PE Jr, Douglas T: Site-directed coordination chemistry with P22 virus-like particles. *Langmuir* 2012;28:1998–2006.
- 253 Mattiaccio J, Walter S, Brewer M, Domm W, Friedman AE, Dewhurst S: Dense display of HIV-1 envelope spikes on the lambda phage scaffold does not result in the generation of improved antibody responses to HIV-1 Env. *Vaccine* 2011;29:2637–2647.
- 254 Villegas-Mendez A, Garin MI, Pineda-Molina E, Veratti E, Bueren JA, Fender P, Lenormand JL: In vivo delivery of antigens by adenovirus dodecahedron induces cellular and humoral immune responses to elicit antitumor immunity. *Mol Ther* 2010;18:1046–1053.
- 255 Garcel A, Gout E, Timmins J, Chroboczek J, Fender P: Protein transduction into human cells by adenovirus dodecahedron using WW domains as universal adaptors. *J Gene Med* 2006;8:524–531.
- 256 Zochowska M, Paca A, Schoehn G, Andrieu JP, Chroboczek J, Dublet B, Szolajska E: Adenovirus dodecahedron, as a drug delivery vector. *PLoS One* 2009;4:e5569.
- 257 Denis J, Acosta-Ramirez E, Zhao Y, Hameelin ME, Koukavica I, Baz M, Abed Y, Savard C, Pare C, Lopez Macias C, Boivin G, Leclerc D: Development of a universal influenza A vaccine based on the M2e peptide fused to the papaya mosaic virus (PapMV) vaccine platform. *Vaccine* 2008;26:3395–3403.
- 258 Savard C, Laliberté-Gagné MÈ, Babin C, Bolduc M, Guérin A, Drouin K, Forget MA, Majeau N, Lapointe R, Leclerc D: Improvement of the PapMV nanoparticle adjuvant property through an increased of its avidity for the antigen [influenza NP]. *Vaccine* 2012;30:2535–2542.
- 259 Marusic C, Rizza P, Lattanzi L, Mancini C, Spada M, Belardelli F, Benvenuto E, Capone I: Chimeric plant virus particles as immunogens for inducing murine and human immune responses against human immunodeficiency virus type 1. *J Virol* 2001;75:8434–8439.
- 260 Uhde K, Fischer R, Commandeur U: Expression of multiple foreign epitopes presented as synthetic antigens on the surface of Potato virus X particles. *Arch Virol* 2005;150:327–340.
- 261 Zelada AM, Calamante G, de la Paz Santangelo M, Bigi F, Verna F, Mentaberry A, Cataldi A: Expression of tuberculosis antigen ESAT-6 in *Nicotiana tabacum* using a potato virus X-based vector. *Tuberculosis (Edinb)* 2006;86:263–267.
- 262 Lico C, Mancini C, Italiani P, Betti C, Borschi D, Benvenuto E, Baschieri S: Plant-produced potato virus X chimeric particles displaying an influenza virus-derived peptide activate specific CD8+ T cells in mice. *Vaccine* 2009;27:5069–5076.
- 263 Uhde-Holzem K, Schlösser V, Viazov S, Fischer R, Commandeur U: Immunogenic properties of chimeric potato virus X particles displaying the hepatitis C virus hypervariable region I peptide R9. *J Virol Methods* 2010;166:12–20.
- 264 Plchova H, Moravec T, Hoffmeisterova H, Folwarczna J, Cerovska N: Expression of Human papillomavirus 16 E7ggg oncoprotein on N- and C-terminus of Potato virus X coat protein in bacterial and plant cells. *Protein Expr Purif* 2011;77:146–152.
- 265 Cerovska N, Hoffmeisterova H, Moravec T, Plchova H, Folwarczna J, Synkova H, Ryslava H, Ludvikova V, Smahel M: Transient expression of human papillomavirus type 16 L2 epitope fused to N- and C-terminus of coat protein of Potato virus X in plants. *J Biosci* 2012;37:125–133.
- 266 Steinmetz NF, Mertens ME, Taurog RE, Johnson JE, Commandeur U, Fischer R, Manchester M: Potato virus X as a novel platform for potential biomedical applications. *Nano Lett* 2010;10:305–312.
- 267 Yang CD, Liao JT, Lai CY, Jong MH, Liang CM, Lin YL, Lin NS, Hsu YH, Liang SM: Induction of protective immunity in swine by recombinant bamboo mosaic virus expressing foot-and-mouth disease virus epitopes. *BMC Biotechnol* 2007;7:62.
- 268 Chen TH, Chen TH, Hu CC, Liao JT, Lee CW, Liao JW, Lin MY, Liu HJ, Wang MY, Lin NS, Hsu YH: Induction of protective immunity in chickens immunized with plant-made chimeric Bamboo mosaic virus particles expressing very virulent infectious bursal disease virus antigen. *Virus Res* 2012;166:109–115.
- 269 Jagadish MN, Hamilton RC, Fernandez CS, Schoofs P, Davern KM, Kalnins H, Ward CW, Nisbet IT: High level production of hybrid potyvirus-like particles carrying repetitive copies of foreign antigens in *Escherichia coli*. *Biotechnology (NY)* 1993;11:1166–1170.
- 270 Saini M, Vrati S: A Japanese encephalitis virus peptide present on Johnson grass mosaic virus-like particles induces virus-neutralizing antibodies and protects mice against lethal challenge. *J Virol* 2003;77:3487–3494.
- 271 Choudhury S, Kakkar V, Suman P, Chakrabarti K, Vrati S, Gupta SK: Immunogenicity of zona pellucida glycoprotein-3 and spermatozoa YLP(12) peptides presented on Johnson grass mosaic virus-like particles. *Vaccine* 2009;27:2948–2953.
- 272 Jacquet C, Ravelonandro M, Dunez J: High resistance and control of biological risks in transgenic plants expressing modified plum poxvirus coat protein. *Acta Virol* 1998;42:235–237.
- 273 Fernández-Fernández MR, Martínez-Torrecuadrada JL, Casal JL, García JA: Development of an antigen presentation system based on plum pox potyvirus. *FEBS Lett* 1998;427:229–235.
- 274 Kalnciema I, Skrastina D, Ose V, Pumpens P, Zeltins A: Potato virus Y-like particles as a new carrier for the presentation of foreign protein stretches. *Mol Biotechnol* 2012;52:129–139.
- 275 Fujiyama K, Saejung W, Yanagihara I, Nakado J, Misaki R, Honda T, Watanabe Y, Seki T: In planta production of immunogenic poliovirus peptide using tobacco mosaic virus-based vector system. *J Biosci Bioeng* 2006;101:398–402.
- 276 Turpen TH, Reinl SJ, Charoenvit Y, Hoffman SL, Fallarme V, Grill LK: Malarial epitopes expressed on the surface of recombinant tobacco mosaic virus. *Biotechnology (NY)* 1995;13:53–57.
- 277 Sugiyama Y, Hamamoto H, Takemoto S, Watanabe Y, Okada Y: Systemic production of foreign peptides on the particle surface of tobacco mosaic virus. *FEBS Lett* 1995;359:247–250.

- 278 Bendahmane M, Koo M, Karrer E, Beachy RN: Display of epitopes on the surface of tobacco mosaic virus: impact of charge and isoelectric point of the epitope on virus-host interactions. *J Mol Biol* 1999;290:9–20.
- 279 Koo M, Bendahmane M, Lettieri GA, Paoletti AD, Lane TE, Fitchen JH, Buchmeier MJ, Beachy RN: Protective immunity against murine hepatitis virus (MHV) induced by intranasal or subcutaneous administration of hybrids of tobacco mosaic virus that carries an MHV epitope. *Proc Natl Acad Sci USA* 1999;96:7774–7779.
- 280 Staczek J, Bendahmane M, Gilleland LB, Beachy RN, Gilleland HE Jr: Immunization with a chimeric tobacco mosaic virus containing an epitope of outer membrane protein F of *Pseudomonas aeruginosa* provides protection against challenge with *P. aeruginosa*. *Vaccine* 2000;18:2266–2274.
- 281 Wu L, Jiang L, Zhou Z, Fan J, Zhang Q, Zhu H, Han Q, Xu Z: Expression of foot-and-mouth disease virus epitopes in tobacco by a tobacco mosaic virus-based vector. *Vaccine* 2003;21:4390–4398.
- 282 Palmer KE, Benko A, Doucette SA, Cameron TI, Foster T, Hanley KM, McCormick AA, McCulloch M, Pogue GP, Smith ML, Christensen ND: Protection of rabbits against cutaneous papillomavirus infection using recombinant tobacco mosaic virus containing L2 capsid epitopes. *Vaccine* 2006;24:5516–5525.
- 283 Mueller A, Kadri A, Jeske H, Wege C: In vitro assembly of Tobacco mosaic virus coat protein variants derived from fission yeast expression clones or plants. *J Virol Methods* 2010;166:77–85.
- 284 Kutscher S, Bauer T, Dembek C, Sprinzl M, Protzer U: Design of therapeutic vaccines: hepatitis B as an example. *Microb Biotechnol* 2012;5:270–282.
- 285 Betancourt AA, Delgado CA, Estévez ZC, Martínez JC, Ríos GV, Aureoles-Roselló SR, Zaldívar RA, Guzmán MA, Baile NF, Reyes PA, Ruano LO, Fernández AC, Lobaina-Matos Y, Fernández AD, Madrazo AI, Martínez MI, Baños ML, Alvarez NP, Baldo MD, Mestre RE, Pérez MV, Martínez ME, Escobar DA, Guanche MJ, Cáceres LM, Betancourt RS, Rando EH, Nieto GE, González VL, Rubido JC: Phase I clinical trial in healthy adults of a nasal vaccine candidate containing recombinant hepatitis B surface and core antigens. *Int J Infect Dis* 2007;11:394–401.
- 286 Clarke BE, Newton SE, Carroll AR, Francis MJ, Appleyard G, Syred AD, Highfield PE, Rowlands DJ, Brown F: Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein. *Nature* 1987;330:381–384.
- 287 Zhang YL, Guo YJ, Wang KY, Lu K, Li K, Zhu Y, Sun SH: Enhanced immunogenicity of modified hepatitis B virus core particle fused with multi-epitopes of foot-and-mouth disease virus. *Scand J Immunol* 2007;65:320–328.
- 288 Huang Y, Liang W, Wang Y, Zhou Z, Pan A, Yang X, Huang C, Chen J, Zhang D: Immunogenicity of the epitope of the foot-and-mouth disease virus fused with a hepatitis B core protein as expressed in transgenic tobacco. *Viral Immunol* 2005;18:668–677.
- 289 Tindle RW, Herd K, Londoño P, Fernando GJ, Chatfield SN, Malcolm K, Dougan G: Chimeric hepatitis B core antigen particles containing B- and Th-epitopes of human papillomavirus type 16 E7 protein induce specific antibody and T-helper responses in immunised mice. *Virology* 1994;200:547–557.
- 290 Pumpens P, Razanskas R, Pushko P, Renhof R, Gusars I, Skrastina D, Ose V, Borisova G, Sominskaya I, Petrovskis I, Jansons J, Sasnauskas K: Evaluation of HBs, HBc, and fCP virus-like particles for expression of human papillomavirus 16 E7 oncoprotein epitopes. *Intervirology* 2002;45:24–32.
- 291 Londoño LP, Chatfield S, Tindle RW, Herd K, Gao XM, Frazer I, Dougan G: Immunisation of mice using *Salmonella typhimurium* expressing human papillomavirus type 16 E7 epitopes inserted into hepatitis B virus core antigen. *Vaccine* 1996;14:545–552.
- 292 Ding FX, Wang F, Lu YM, Li K, Wang KH, He XW, Sun SH: Multi-epitope peptide-loaded virus-like particles as a vaccine against hepatitis B virus-related hepatocellular carcinoma. *Hepatology* 2009;49:1492–1502.
- 293 Ulrich R, Lundkvist A, Meisel H, Koletzki D, Sjölander KB, Gelderblom HR, Borisova G, Schnitzler P, Darai G, Krüger DH: Chimeraic HBV core particles carrying a defined segment of Puumala hantavirus nucleocapsid protein evoke protective immunity in an animal model. *Vaccine* 1998;16:272–280.
- 294 Geldmacher A, Skrastina D, Borisova G, Petrovskis I, Krüger DH, Pumpens P, Ulrich R: A hantavirus nucleocapsid protein segment exposed on hepatitis B virus core particles is highly immunogenic in mice when applied without adjuvants or in the presence of pre-existing anti-core antibodies. *Vaccine* 2005;23:3973–3983.
- 295 Mihailova M, Boos M, Petrovskis I, Ose V, Skrastina D, Fiedler M, Sominskaya I, Ross S, Pumpens P, Roggendorf M, Viazov S: Recombinant virus-like particles as a carrier of B- and T-cell epitopes of hepatitis C virus (HCV). *Vaccine* 2006;24:4369–4377.
- 296 Sominskaya I, Skrastina D, Dislers A, Vasiljev D, Mihailova M, Ose V, Dreilina D, Pumpens P: Construction and immunological evaluation of multivalent hepatitis B virus (HBV) core virus-like particles carrying HBV and HCV epitopes. *Clin Vaccine Immunol* 2010;17:1027–1033.
- 297 Yin Y, Zhang J, Dong D, Liu S, Guo Q, Song X, Li G, Fu L, Xu J, Chen W: Chimeric hepatitis B virus core particles carrying an epitope of anthrax protective antigen induce protective immunity against *Bacillus anthracis*. *Vaccine* 2008;26:5814–5821.
- 298 Guan Q, Ma Y, Hillman CL, Ma A, Zhou G, Qing G, Peng Z: Development of recombinant vaccines against IL-12/IL-23 p40 and in vivo evaluation of their effects in the down-regulation of intestinal inflammation in murine colitis. *Vaccine* 2009;27:7096–7104.
- 299 Yin Y, Li H, Wu S, Dong D, Zhang J, Fu L, Xu J, Chen W: Hepatitis B virus core particles displaying *Mycobacterium tuberculosis* antigen ESAT-6 enhance ESAT-6-specific immune responses. *Vaccine* 2011;29:5645–5651.
- 300 Neirynck S, Deroo T, Saelens X, Vanland-schoot P, Jou WM, Fiers W: A universal influenza A vaccine based on the extracellular domain of the M2 protein. *Nat Med* 1999;5:1157–1163.
- 301 Jegerlehner A, Schmitz N, Storni T, Bachmann MF: Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. *J Immunol* 2004;172:5598–5605.
- 302 De Filette M, Martens W, Smet A, Schotsaert M, Birkett A, Londoño-Arcila P, Fiers W, Saelens X: Universal influenza A M2e-HBc vaccine protects against disease even in the presence of pre-existing anti-HBc antibodies. *Vaccine* 2008;26:6503–6507.
- 303 Fiers W, De Filette M, El Bakkouri K, Schepens B, Roose K, Schotsaert M, Birkett A, Saelens X: M2e-based universal influenza A vaccine. *Vaccine* 2009;27:6280–6283.
- 304 Ravin NV, Kotlyarov RY, Mardanov ES, Kuprianov VV, Migunov AI, Stepanova LA, Tsybalova LM, Kiselev OI, Skryabin KG: Plant-produced recombinant influenza vaccine based on virus-like HBc particles carrying an extracellular domain of M2 protein. *Biochemistry (Mosc)* 2012;77:33–40.
- 305 Schödel F, Wirtz R, Peterson D, Hughes J, Warren R, Sadoff J, Milich D: Immunity to malaria elicited by hybrid hepatitis B virus core particles carrying circumsporozoite protein epitopes. *J Exp Med* 1994;180:1037–1046.
- 306 Sallberg M, Hughes J, Jones J, Phillips TR, Milich DR: A malaria vaccine candidate based on a hepatitis B virus core platform. *Intervirology* 2002;45:350–361.
- 307 Nardin EH, Oliveira GA, Calvo-Calle JM, Wetzel K, Maier C, Birkett AJ, Sarpotdar P, Corado ML, Thornton GB, Schmidt A: Phase I testing of a malaria vaccine composed of hepatitis B virus core particles expressing *Plasmodium falciparum* circumsporozoite epitopes. *Infect Immun* 2004;72:6519–6527.
- 308 Nassal M, Skamel C, Kratz PA, Wallich R, Stehle T, Simon MM: A fusion product of the complete *Borrelia burgdorferi* outer surface protein A (OspA) and the hepatitis B virus capsid protein is highly immunogenic and induces protective immunity similar to that seen with an effective lipidated OspA vaccine formula. *Eur J Immunol* 2005;35:655–665.

- 309 Skamel C, Ploss M, Böttcher B, Stehle T, Wallich R, Simon MM, Nassal M: Hepatitis B virus capsid-like particles can display the complete, dimeric outer surface protein C and stimulate production of protective antibody responses against *Borrelia burgdorferi* infection. *J Biol Chem* 2006;281:17474–17481.
- 310 Nassal M, Skamel C, Vogel M, Kratz PA, Stehle T, Wallich R, Simon MM: Development of hepatitis B virus capsids into a whole-chain protein antigen display platform: new particulate Lyme disease vaccines. *Int J Med Microbiol* 2008;298:135–142.
- 311 Boulter N, Brown D, Wilkie G, Williamson S, Kirvar E, Knight P, Glass E, Campbell J, Morzaria S, Nene V, Musoke A, d'Oliveira C, Gubbels MJ, Jongejan F, Hall R: Evaluation of recombinant sporozoite antigen SPAG-1 as a vaccine candidate against *Theileria annulata* by the use of different delivery systems. *Trop Med Int Health* 1999;4:A71–A77.
- 312 Wang YS, Ouyang W, Liu XJ, He KW, Yu SQ, Zhang HB, Fan HJ, Lu CP: Virus-like particles of hepatitis B virus core protein containing five mimotopes of infectious bursal disease virus (IBDV) protect chickens against IBDV. *Vaccine* 2012;30:2125–2130.
- 313 Ranka R, Petrovskis I, Sominskaya I, Bogans J, Bruvere R, Akopjana I, Ose V, Timofejeva I, Brangulis K, Pumpens P, Baumanis V: Fibronectin binding nanoparticles for intracellular targeting addressed by *B. burgdorferi* BBK32 protein fragments. *Nanomedicine* 2012, E-pub ahead of print.
- 314 Engerix B: Summary for basis of approval. FDA, 1988. <http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM110155.pdf>.
- 315 Recombivax HB: Summary for basis of approval. FDA, 1987. <http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM244544.pdf>.
- 316 Cooper C, Mackie D: Hepatitis B surface antigen-1018 ISS adjuvant-containing vaccine: a review of HEPLISAV™ safety and efficacy. *Expert Rev Vaccines* 2011;10:417–427.
- 317 Kapusta J, Modelska A, Figlerowicz M, Pniewski T, Letellier M, Lisowa O, Yusibov V, Koprowski H, Plucienniczak A, Legocki AB: A plant-derived edible vaccine against hepatitis B virus. *FASEB J* 1999;13:1796–1799.
- 318 Richter LJ, Thanavala Y, Arntzen CJ, Mason HS: Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nat Biotechnol* 2000;18:1167–1171.
- 319 Thanavala Y, Mahoney M, Pal S, Scott A, Richter L, Natarajan N, Goodwin P, Arntzen CJ, Mason HS: Immunogenicity in humans of an edible vaccine for hepatitis B. *Proc Natl Acad Sci USA* 2005;102:3378–3382.
- 320 Hayden CA, Streatfield SJ, Lamphear BJ, Fake GM, Keener TK, Walker JH, Clements JD, Turner DD, Tizard IR, Howard JA: Bioencapsulation of the hepatitis B surface antigen and its use as an effective oral immunogen. *Vaccine* 2012;30:2937–2942.
- 321 Senturk H, Tabak F, Akdogan M, Erdem L, Mert A, Ozaras R, Sander E, Ozbay G, Badur S: Therapeutic vaccination in chronic hepatitis B. *J Gastroenterol Hepatol* 2002;17:72–76.
- 322 Senturk H, Tabak F, Ozaras R, Erdem L, Canbakan B, Mert A, Yurdakul I: Efficacy of pre-S-containing HBV vaccine combined with lamivudine in the treatment of chronic HBV infection. *Dig Dis Sci* 2009;54:2026–2030.
- 323 Weinstein T, Chagnac A, Boaz M, Ori Y, Herman M, Zevin D, Schmilovitz-Weiss H, Gafer U: Improved immunogenicity of a novel third-generation recombinant hepatitis B vaccine in patients with end-stage renal disease. *Nephron Clin Pract* 2004;97:c67–c72.
- 324 Page M, Jones CD, Bailey C: A novel, recombinant triple antigen hepatitis B vaccine (Hepacare). *Intervirology* 2001;44:88–97.
- 325 Schlienger K, Montefiori DC, Mancini M, Riviere Y, Tiollais P, Michel ML: Vaccine-induced neutralizing antibodies directed in part to the simian immunodeficiency virus (SIV) V2 domain were unable to protect rhesus monkeys from SIV experimental challenge. *J Virol* 1994;68:6578–6588.
- 326 Michel M, Lone YC, Centlivre M, Roux P, Wain-Hobson S, Sala M: Optimisation of secretion of recombinant HBsAg virus-like particles: impact on the development of HIV-1/HBV bivalent vaccines. *Vaccine* 2007;25:1901–1911.
- 327 Eckhart L, Raffelsberger W, Ferko B, Klima A, Purtscher M, Katinger H, Rüker F: Immunogenic presentation of a conserved gp41 epitope of human immunodeficiency virus type 1 on recombinant surface antigen of hepatitis B virus. *J Gen Virol* 1996;77:2001–2008.
- 328 Shchelkunov SN, Salyaev RK, Pozdnyakov SG, Rekoslavskaya NI, Nesterov AE, Ryzhova TS, Sumtsova VM, Pakova NV, Mishutina UO, Kopytina TV, Hammond RW: Immunogenicity of a novel, bivalent, plant-based oral vaccine against hepatitis B and human immunodeficiency viruses. *Biotechnol Lett* 2006;28:959–967.
- 329 Li HZ, Gang HY, Sun QM, Liu X, Ma YB, Sun MS, Dai CB: Production in *Pichia pastoris* and characterization of genetic engineered chimeric HBV/HEV virus-like particles. *Chin Med Sci J* 2004;19:78–83.
- 330 Bisht H, Chugh DA, Swaminathan S, Khanna N: Expression and purification of Dengue virus type 2 envelope protein as a fusion with hepatitis B surface antigen in *Pichia pastoris*. *Protein Expr Purif* 2001;23:84–96.
- 331 Lee IH, Kim CH, Ryu WS: Presentation of the hydrophilic domains of hepatitis C viral E2 envelope glycoprotein on hepatitis B surface antigen particles. *J Med Virol* 1996;50:145–151.
- 332 Cheong WS, Drummer HE, Netter HJ: Delivery of a foreign epitope by sharing amino acid residues with the carrier matrix. *J Virol Methods* 2009;158:35–40.
- 333 Cheong WS, Reiseger J, Turner SJ, Boyd R, Netter HJ: Chimeric virus-like particles for the delivery of an inserted conserved influenza A-specific CTL epitope. *Antiviral Res* 2009;81:113–122.
- 334 Kotiw M, Johnson M, Pandey M, Fry S, Hazell SL, Netter HJ, Good MF, Olive C: Immunological response to parenteral vaccination with recombinant hepatitis B virus surface antigen virus-like particles expressing *Helicobacter pylori* KatA epitopes in a murine *H. pylori* challenge model. *Clin Vaccine Immunol* 2012;19:268–276.
- 335 Ameiss K, Ashraf S, Kong W, Pekosz A, Wu WH, Milich D, Billaud JN, Curtiss R 3rd: Delivery of woodchuck hepatitis virus-like particle presented influenza M2e by recombinant attenuated *Salmonella* displaying a delayed lysis phenotype. *Vaccine* 2010;28:6704–6713.
- 336 Ohtaki N, Takahashi H, Kaneko K, Gomi Y, Ishikawa T, Higashi Y, Kurata T, Sata T, Kojima A: Immunogenicity and efficacy of two types of West Nile virus-like particles different in size and maturation as a second-generation vaccine candidate. *Vaccine* 2010;28:6588–6596.
- 337 Zhang S, Liang M, Gu W, Li C, Miao F, Wang X, Jin C, Zhang L, Zhang F, Zhang Q, Jiang L, Li M, Li D: Vaccination with dengue virus-like particles induces humoral and cellular immune responses in mice. *Virol J* 2011;8:333.
- 338 Liu W, Jiang H, Zhou J, Yang X, Tang Y, Fang D, Jiang L: Recombinant dengue virus-like particles from *Pichia pastoris*: efficient production and immunological properties. *Virus Genes* 2010;40:53–59.
- 339 Lazo L, Hermida L, Zulueta A, Sánchez J, López C, Silva R, Guillén G, Guzmán MG: A recombinant capsid protein from Dengue-2 induces protection in mice against homologous virus. *Vaccine* 2007;25:1064–1070.
- 340 Lazo L, Gil L, Lopez C, Valdes I, Marcos E, Alvarez M, Blanco A, Romero Y, Falcon V, Guzmán MG, Guillén G, Hermida L: Nucleocapsid-like particles of dengue-2 virus enhance the immune response against a recombinant protein of dengue-4 virus. *Arch Virol* 2010;155:1587–1595.
- 341 Baumert TF, Ito S, Wong DT, Liang TJ: Hepatitis C virus structural proteins assemble into virus-like particles in insect cells. *J Virol* 1998;72:3827–3836.
- 342 Lechmann M, Murata K, Sato J, Vergalla J, Baumert TF, Liang TJ: Hepatitis C virus-like particles induce virus-specific humoral and cellular immune responses in mice. *Hepatology* 2001;34:417–423.
- 343 Elmowalid GA, Qiao M, Jeong SH, Borg BB, Baumert TF, Sapp RK, Hu Z, Murthy K, Liang TJ: Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. *Proc Natl Acad Sci USA* 2007;104:8427–8432.

- 344 Falcón V, García C, de la Rosa MC, Menéndez I, Seoane J, Grillo JM: Ultrastructural and immunocytochemical evidences of core-particle formation in the methylotrophic *Pichia pastoris* yeast when expressing HCV structural proteins (core-E1). *Tissue Cell* 1999;31:117–125.
- 345 Acosta-Rivero N, Rodríguez A, Musacchio A, Falcón V, Suarez VM, Martínez G, Guerra I, Paz-Lago D, Morera Y, de la Rosa MC, Morales-Grillo J, Dueñas-Carrera S: In vitro assembly into virus-like particles is an intrinsic quality of *Pichia pastoris* derived HCV core protein. *Biochem Biophys Res Commun* 2004;325:68–74.
- 346 Kojima A, Yasuda A, Asanuma H, Ishikawa T, Takamizawa A, Yasui K, Kurata T: Stable high-producer cell clone expressing virus-like particles of the Japanese encephalitis virus e protein for a second-generation subunit vaccine. *J Virol* 2003;77:8745–8755.
- 347 Yoshii K, Goto A, Kawakami K, Kariwa H, Takashima I: Construction and application of chimeric virus-like particles of tick-borne encephalitis virus and mosquito-borne Japanese encephalitis virus. *J Gen Virol* 2008;89:200–211.
- 348 Okamoto S, Yoshii H, Matsuura M, Kojima A, Ishikawa T, Akagi T, Akashi M, Takahashi M, Yamanishi K, Mori Y: Poly-γ-glutamic acid nanoparticles and aluminum adjuvant used as an adjuvant with a single dose of Japanese encephalitis virus-like particles provide effective protection from Japanese encephalitis virus. *Clin Vaccine Immunol* 2012;19:17–22.
- 349 Tellinghuisen TL, Hamburger AE, Fisher BR, Ostendorp R, Kuhn RJ: In vitro assembly of alphavirus cores by using nucleocapsid protein expressed in *Escherichia coli*. *J Virol* 1999;73:5309–5319.
- 350 Mukhopadhyay S, Chipman PR, Hong EM, Kuhn RJ, Rossmann MG: In vitro-assembled alphavirus core-like particles maintain a structure similar to that of nucleocapsid cores in mature virus. *J Virol* 2002;76:11128–11132.
- 351 Cheng F, Mukhopadhyay S: Generating enveloped virus-like particles with in vitro assembled cores. *Virology* 2011;413:153–160.
- 352 Goicochea NL, De M, Rotello VM, Mukhopadhyay S, Dragnea B: Core-like particles of an enveloped animal virus can self-assemble efficiently on artificial templates. *Nano Lett* 2007;7:2281–2290.
- 353 Akahata W, Yang ZY, Andersen H, Sun S, Holdaway HA, Kong WP, Lewis MG, Higgs S, Rossmann MG, Rao S, Nabel GJ: A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. *Nat Med* 2010;16:334–338.
- 354 Metz SW, Feenstra F, Villoing S, van Hulten MC, van Lent JW, Koumans J, Vlak JM, Pijlman GP: Low temperature-dependent salmonid alphavirus glycoprotein processing and recombinant virus-like particle formation. *PLoS One* 2011;6:e25816.
- 355 Bai B, Hu Q, Hu H, Zhou P, Shi Z, Meng J, Lu B, Huang Y, Mao P, Wang H: Virus-like particles of SARS-like coronavirus formed by membrane proteins from different origins demonstrate stimulating activity in human dendritic cells. *PLoS One* 2008;3:e2685.
- 356 Zhou ZR, Wang ML, Deng F, Li TX, Hu ZH, Wang HL: Production of CCHF virus-like particle by a baculovirus-insect cell expression system. *Viol Sin* 2011;26:338–346.
- 357 Habjan M, Penski N, Wagner V, Spiegel M, Overby AK, Kochs G, Huiskenon JT, Weber F: Efficient production of Rift Valley fever virus-like particles: the antiviral protein MxA can inhibit primary transcription of bunyaviruses. *Virology* 2009;385:400–408.
- 358 Li C, Liu F, Liang M, Zhang Q, Wang X, Wang T, Li J, Li D: Hantavirus-like particles generated in CHO cells induce specific immune responses in C57BL/6 mice. *Vaccine* 2010;28:4294–4300.
- 359 Tong T, Crooks ET, Osawa K, Binley JM: HIV-1 virus-like particles bearing pure env trimers expose neutralizing epitopes but occlude nonneutralizing epitopes. *J Virol* 2012;86:3574–3587.
- 360 Visciano ML, Diomede L, Tagliamonte M, Tornesello ML, Asti V, Bomsel M, Buonaguro FM, Lopalco L, Buonaguro L: Generation of HIV-1 virus-like particles expressing different HIV-1 glycoproteins. *Vaccine* 2011;29:4903–4912.
- 361 Tobin GJ, Nagashima K, Gonda MA: Immunologic and ultrastructural characterization of HIV pseudovirions containing Gag and Env precursor proteins engineered in insect cells. *Methods* 1996;10:208–218.
- 362 Tagliamonte M, Visciano ML, Tornesello ML, De Stradis A, Buonaguro FM, Buonaguro L: HIV-Gag VLPs presenting trimeric HIV-1 gp140 spikes constitutively expressed in stable double transfected insect cell line. *Vaccine* 2011;29:4913–4922.
- 363 Rovinski B, Haynes JR, Cao SX, James O, Sia C, Zolla-Pazner S, Matthews TJ, Klein MH: Expression and characterization of genetically engineered human immunodeficiency virus-like particles containing modified envelope glycoproteins: implications for development of a cross-protective AIDS vaccine. *J Virol* 1992;66:4003–4012.
- 364 Deml L, Kratochwil G, Osterrieder N, Knüchel R, Wolf H, Wagner R: Increased incorporation of chimeric human immunodeficiency virus type 1 gp120 proteins into Pr55gag virus-like particles by an Epstein-Barr virus gp220/350-derived transmembrane domain. *Virology* 1997;235:10–25.
- 365 Osterrieder N, Wagner R, Brandmüller C, Schmidt P, Wolf H, Kaaden OR: Protection against EHV-1 challenge infection in the murine model after vaccination with various formulations of recombinant glycoprotein gp14 (gB). *Virology* 1995;208:500–510.
- 366 Garnier L, Ravallec M, Blanchard P, Chaabihi H, Bossy JP, Devauchelle G, Jestin A, Cerutti M: Incorporation of pseudorabies virus gD into human immunodeficiency virus type 1 Gag particles produced in baculovirus-infected cells. *J Virol* 1995;69:4060–4068.
- 367 Di Bonito P, Grasso F, Mochi S, Petrone L, Fanale-Belasio E, Mei A, Cesolini A, Laconi G, Conrad H, Bernhard H, Dembek CJ, Cosma A, Santini SM, Lapenta C, Donati S, Muratori C, Giorgi C, Federico M: Anti-tumor CD8+ T cell immunity elicited by HIV-1-based virus-like particles incorporating HPV-16 E7 protein. *Virology* 2009;395:45–55.
- 368 Wu X, Liu H, Xiao H, Kim J, Seshiah P, Nat-soulis G, Boeke JD, Hahn BH, Kappes JC: Targeting foreign proteins to human immunodeficiency virus particles via fusion with Vpr and Vpx. *J Virol* 1995;69:3389–3398.
- 369 Kaczmarczyk SJ, Sitaraman K, Young HA, Hughes SH, Chatterjee DK: Protein delivery using engineered virus-like particles. *Proc Natl Acad Sci USA* 2011;108:16998–17003.
- 370 Sistigu A, Bracci L, Valentini M, Proietti E, Bona R, Negri DR, Ciccaglione AR, Tritarelli E, Nisini R, Equestre M, Costantino A, Marcantonio C, Santini SM, Lapenta C, Donati S, Tataseo P, Miceli M, Cara A, Federico M: Strong CD8+ T cell antigenicity and immunogenicity of large foreign proteins incorporated in HIV-1 VLPs able to induce a Nef-dependent activation/maturation of dendritic cells. *Vaccine* 2011;29:3465–3475.
- 371 Luo L, Li Y, Kang CY: Expression of gag precursor protein and secretion of virus-like gag particles of HIV-2 from recombinant baculovirus-infected insect cells. *Virology* 1990;179:874–880.
- 372 Luo L, Li Y, Cannon PM, Kim S, Kang CY: Chimeric gag-V3 virus-like particles of human immunodeficiency virus induce virus-neutralizing antibodies. *Proc Natl Acad Sci USA* 1992;89:10527–10531.
- 373 Kang CY, Luo L, Wainberg MA, Li Y: Development of HIV/AIDS vaccine using chimeric gag-env virus-like particles. *Biol Chem* 1999;380:353–364.
- 374 Shen L, Mazzara GP, DiSciullo SO, Panicali DL, Letvin NL: Immunization with lentivirus-like particles elicits a potent SIV-specific recall cytotoxic T-lymphocyte response in rhesus monkeys. *AIDS Res Hum Retroviruses* 1993;9:129–132.
- 375 Yamshchikov GV, Ritter GD, Vey M, Compans RW: Assembly of SIV virus-like particles containing envelope proteins using a baculovirus expression system. *Virology* 1995;214:50–58.
- 376 Yao Q, Kuhlmann FM, Eller R, Compans RW, Chen C: Production and characterization of simian-human immunodeficiency virus-like particles. *AIDS Res Hum Retroviruses* 2000;16:227–236.
- 377 Zhang R, Zhang S, Li M, Chen C, Yao Q: Incorporation of CD40 ligand into SHIV virus-like particles (VLP) enhances SHIV-VLP-induced dendritic cell activation and boosts immune responses against HIV. *Vaccine* 2010;28:5114–5127.

- 378 Deo VK, Tsuji Y, Yasuda T, Kato T, Sakamoto N, Suzuki H, Park EY: Expression of an RSV-gag virus-like particle in insect cell lines and silkworm larvae. *J Virol Methods* 2011;177:147–152.
- 379 Tsuji Y, Deo VK, Kato T, Park EY: Production of Rous sarcoma virus-like particles displaying human transmembrane protein in silkworm larvae and its application to ligand-receptor binding assay. *J Biotechnol* 2011;155:185–192.
- 380 Leb VM, Jahn-Schmid B, Kueng HJ, Schmetterer KG, Haiderer D, Neunkirchner A, Fischer GF, Hartl A, Thalhamer J, Steinberger P, Bohle B, Seed B, Pickl WF: Modulation of allergen-specific T-lymphocyte function by virus-like particles decorated with HLA class II molecules. *J Allergy Clin Immunol* 2009;124:121–128.
- 381 Nikles D, Bach P, Boller K, Merten CA, Montrasio F, Heppner FL, Aguzzi A, Cichutek K, Kalinke U, Buchholz CJ: Circumventing tolerance to the prion protein (PrP): vaccination with PrP-displaying retrovirus-particles induces humoral immune responses against the native form of cellular PrP. *J Virol* 2005;79:4033–4042.
- 382 Bach P, Kamphuis E, Odermatt B, Sutter G, Buchholz CJ, Kalinke U: Vesicular stomatitis virus glycoprotein displaying retrovirus-like particles induce a type I IFN receptor-dependent switch to neutralizing IgG antibodies. *J Immunol* 2007;178:5839–5847.
- 383 Kueng HJ, Leb VM, Haiderer D, Raposo G, Thery C, Derdak SV, Schmetterer KG, Neunkirchner A, Sillaber C, Seed B, Pickl WF: General strategy for decoration of enveloped viruses with functionally active lipid-modified cytokines. *J Virol* 2007;81:8666–8676.
- 384 Haynes JR, Dokken L, Wiley JA, Cawthon AG, Bigger J, Harmsen AG, Richardson C: Influenza-pseudotyped Gag virus-like particle vaccines provide broad protection against highly pathogenic avian influenza challenge. *Vaccine* 2009;27:530–541.
- 385 Ruiss R, Jochum S, Wanner G, Reisbach G, Hammerschmidt W, Zeidler R: A virus-like particle-based Epstein-Barr virus vaccine. *J Virol* 2011;85:13105–13113.
- 386 Bright RA, Carter DM, Crevar CJ, Toapanta FR, Steckbeck JD, Cole KS, Kumar NM, Pushko P, Smith G, Tumpey TM, Ross TM: Cross-clade protective immune responses to influenza viruses with H5N1 HA and NA elicited by an influenza virus-like particle. *PLoS One* 2008;3:e1501.
- 387 Galarza JM, Latham T, Cupo A: Virus-like particle (VLP) vaccine conferred complete protection against a lethal influenza virus challenge. *Viral Immunol* 2005;18:244–251.
- 388 Kang SM, Pushko P, Bright RA, Smith G, Compans RW: Influenza virus-like particles as pandemic vaccines. *Curr Top Microbiol Immunol* 2009;333:269–289.
- 389 Mena I, Vivo A, Pérez E, Portela A: Rescue of a synthetic chloramphenicol acetyltransferase RNA into influenza virus-like particles obtained from recombinant plasmids. *J Virol* 1996;70:5016–5024.
- 390 Wang BZ, Gill HS, Kang SM, Wang L, Wang YC, Vassilieva EV, Compans RW: Enhanced influenza virus-like particle vaccines containing the extracellular domain of matrix protein 2 and a Toll-like receptor ligand. *Clin Vaccine Immunol* 2012;19:1119–1125.
- 391 Casabona JC, Livingston Macleod JM, Loureiro ME, Gomez GA, Lopez N: The RING domain and the L79 residue of Z protein are involved in both the rescue of nucleocapsids and the incorporation of glycoproteins into infectious chimeric arenavirus-like particles. *J Virol* 2009;83:7029–7039.
- 392 Walpita P, Barr J, Sherman M, Basler CF, Wang L: Vaccine potential of Nipah virus-like particles. *PLoS One* 2011;6:e18437.
- 393 Sugahara F, Uchiyama T, Watanabe H, Shimazu Y, Kuwayama M, Fujii Y, Kiyotani K, Adachi A, Kohno N, Yoshida T, Sakaguchi T: Paramyxovirus Sendai virus-like particle formation by expression of multiple viral proteins and acceleration of its release by C protein. *Virology* 2004;325:1–10.
- 394 Li M, Schmitt PT, Li Z, McCrory TS, He B, Schmitt AP: Mumps virus matrix, fusion, and nucleocapsid proteins cooperate for efficient production of virus-like particles. *J Virol* 2009;83:7261–7272.
- 395 Warfield KL, Swenson DL, Negley DL, Schmaljohn AL, Aman MJ, Bavari S: Marburg virus-like particles protect guinea pigs from lethal Marburg virus infection. *Vaccine* 2004;22:3495–3502.
- 396 Swenson DL, Warfield KL, Negley DL, Schmaljohn A, Aman MJ, Bavari S: Virus-like particles exhibit potential as a pan-filovirus vaccine for both Ebola and Marburg viral infections. *Vaccine* 2005;23:3033–3042.
- 397 Warfield KL, Aman MJ: Advances in virus-like particle vaccines for filoviruses. *J Infect Dis* 2011;204(suppl 3):S1053–S1059.
- 398 Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S: Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J Infect Dis* 2007;196(suppl 2):S430–S437.
- 399 Ye L, Lin J, Sun Y, Bennouna S, Lo M, Wu Q, Bu Z, Pulendran B, Compans RW, Yang C: Ebola virus-like particles produced in insect cells exhibit dendritic cell stimulating activity and induce neutralizing antibodies. *Virology* 2006;351:260–270.
- 400 VIPERdb (Virus Particle ExploreR). <http://viperdb.scripps.edu/>.
- 401 Carrillo-Tripp M, Shepherd CM, Borelli IA, Venkataraman S, Lander G, Natarajan P, Johnson JE, Brooks CL 3rd, Reddy VS: VIPERdb2: an enhanced and web API enabled relational database for structural virology. *Nucleic Acids Res* 2009;37:D436–D442.
- 402 Gilbert RJ, Beales L, Blond D, Simon MN, Lin BY, Chisari FV, Stuart DI, Rowlands DJ: Hepatitis B small surface antigen particles are octahedral. *Proc Natl Acad Sci USA* 2005;102:14783–14788.