Sun *et al. Animal Diseases* (2024) 4:14
https://doi.org/10.1186/s44149-024-00119-w

Animal Diseases

REVIEW

Open Access

# Toward innovative veterinary nanoparticle vaccines

Meiqi Sun[1], Aldryan Cristianto Pratama[1], He Qiu[1], Zehui Liu[1,4*] and Fang He[1,2,3*]

## Abstract

Nanoparticles are significant for veterinary vaccine development because they are safer and more effective than conventional formulations. One promising area of research involves self-assembled protein nanoparticles (SAPNs), which have shown potential for enhancing antigen-presenting cell uptake, B-cell activation, and lymph node trafficking. Numerous nanovaccines have been utilized in veterinary medicine, including natural self-assembled protein nanoparticles, rationally designed self-assembled protein nanoparticles, animal virus-derived nanoparticles, bacteriophage-derived nanoparticles, and plant-derived nanoparticles, which will be discussed in this review. SAPN vaccines can produce robust cellular and humoral immune responses and have been shown to protect against various animal infectious diseases. This article attempts to summarize these diverse nanovaccine types and their recent research progress in the field of veterinary medicine. Furthermore, this paper highlights their disadvantages and methods for improving their immunogenicity.

**Keywords** Nanoparticles, Veterinary vaccine, Self-assembling protein nanoparticles (SAPNs), Virus-like nanoparticles (VLPs), Immune responses, Animal infectious diseases, Optimization strategies

## Introduction

Currently, one of the most effective methods for limiting the spread of several infectious illnesses affecting animal husbandry is to establish prophylactic vaccination policies whenever possible. Nevertheless, several current vaccines for animals still depend on conventional vaccination technologies, such as attenuated and inactivated vaccines, which might not offer the best safety and immunogenicity under certain conditions. Innovative vaccines comprising isolated, completely purified antigenic protein subunits have become the safest option, although they frequently fail to produce significant protective immunity (Vartak and Sucheck 2016). One option for improving the poor immunogenicity of epitope-based vaccines is to incorporate nanotechnology into vaccine research, which is a promising and quickly growing field.

Nanoparticles (NPs) are defined as any particulate material with a size of 1 to 100 nm or up to 1000 nm (Nguyen and Tolia 2021). Nanoscale materials consist of a variety of substances, including polymeric, inorganic, and biological building blocks (Doll et al. 2013). The use of nanotechnology in vaccine development is a particularly hot topic. They have been shown to be beneficial in the production of vaccines for infectious diseases and have been investigated for a variety of fungal, bacterial, viral and parasitic diseases (Tiwari, et al. 2023; Makabenta, et al. 2021; Kischkel, et al. 2020). Studies have been

Communicated by Wentao Li.

*Correspondence:
Zehui Liu
zehuiliu@zju.edu.cn
Fang He
hefangzj@zju.edu.cn
[1] Institute of Preventive Veterinary Medicine, College of Animal Sciences of Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China
[2] Zhejiang Provincial Key Laboratory of Preventive Veterinary Medicine, Hangzhou 310058, China
[3] ZJU-Xinchang Joint Innovation Centre (TianMu Laboratory), Gaochuang Hi-Tech Park, Xinchang 312500, Zhejiang, China
[4] Department of Cardiology, Cardiovascular Key Lab of Zhejiang Province, The Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China

carried out using a variety of nanomaterials to generate promising vaccine candidates, among which self-assembled protein nanoparticles (SAPNs) and virus-like nanoparticles (VLPs) seem very promising.

SAPNs are secondary structures generated by oligomerizing a monomeric protein, including helical or β-sheet secondary structures, endogenous peptides, and de novo structures (Fujita and Taguchi 2011). These multicopy protein building blocks can immediately integrate into well-ordered nanoparticle structures (Indelicato, et al. 2016). They allow the inclusion of antigens in their component/subunit structures, displaying repeating antigen molecules on the surface of the nanoparticle, thereby building chimeric SAPNs and serving as a redundant antigen delivery platform. Virus-like nanoparticles (VLPs) are self-assembled proteins; however, they tend to be investigated separately because of their viral origin and distinctive shape. Viral capsid proteins self-assemble into multiprotein complexes known as VLPs. These viruses are unable to infect host cells because they do not have a viral genome despite having an appearance and structure similar to that of native viruses (Nooraei, et al. 2021).

SAPN/VLPs, or antigens attached to the NP surface, can interact with pattern recognition receptors on innate immune cells, activating the adaptive immune system. As summarized in Fig. 1, NPs (SAPN/VLP), with a size range of 20–200 nm, are able to traffic into lymph nodes freely and are recognized by dendritic cells (DCs), which are antigen-presenting cells (APCs) (Manolova, et al. 2008; Cubas, et al. 2009). Recognition and uptake of NPs initiate the DC maturation process, leading to lysosomal proteolysis and degradation of the NPs into peptides, allowing their presentation to $CD4^+$ helper T cells as an MHC-peptide complex, which subsequently generates humoral immune responses (Look et al. 2010; Zabel et al. 2013; Win et al. 2011). The interaction between B cells and $CD4^+$ helper T cells can elicit antigen-specific antibodies by creating plasma cells and B memory cells to destroy the infectious pathogen (Zabel et al. 2013). B cells can detect NPs and activate humoral immunity directly in some circumstances. In addition, DCs can activate immature $CD8^+$ cytotoxic T lymphocytes (CTLs), which differentiate into effector and memory CTLs to initiate immunological responses and directly kill infected cells (Buonaguro, et al. 2006; McFall-Boegeman and Huang 2022).

On the other hand, viral and bacterial pathogens trigger the production of interferon gamma (IFN-γ) and pro-inflammatory cytokines by activating the helper T1 cell (TH1), which stimulate the activation of APCs such as dendritic cells, natural killer (NK) cells, and macrophages (Pulendran and Ahmed 2011). Activation of TH1 cells and the production of IFN-γ have significant effects on viral elimination. Specifically, IFN-γ is a predominant cytokine in the TH1 response. IFN-γ has several biological functions related to viral infection, including antigen presentation by the MHC pathway, the stimulation of autophagy and apoptosis, the induction of antiviral mediator proteins by countering certain viral replication stages, viral RNA editing that leads to lethal virus mutations, and the facilitation of pathogen degradation by lysosome-mediated enzymatic processes (Kak et al. 2018). The broad functions of the TH1 response and IFN-γ due to pathogen stimulation will be beneficial for providing a deep understanding of the immunomodulatory mechanism of vaccination. Activating Th1-IFNγ responses is crucial for improving vaccine effectiveness (Ivashkiv 2018).

What are the advantages of the SAPN and VLP vaccines? As shown in Fig. 2, the recurring array of antigens on SAPNs/VLPs replicates the recognition patterns on pathogens, allowing effective adhesion and stimulation of many B-cell receptors (BCRs) (Bachmann and Jennings 2010; Irvine and Read 2020; López-Sagaseta et al. 2016). Compared to single recombinant antigens that provide a 1:1 interaction with BCRs, SAPNs/VLPs enable the clustering of BCRs for multiple engagements (López-Sagaseta et al. 2016), which is a crucial phase in eliciting a robust immune response. Furthermore, the cumulative particle size of antigens and NPs is an ideal range for facilitating efficient uptake by APCs, promoting antigen presentation by APCs to stimulate helper T cells (Jia, et al. 2018; Oyewumi et al. 2010). NPs also efficiently trigger complement activation, which helps in binding to FDCs (follicular dendritic cells). In addition, the size of nanoparticles can prolong retention in lymph follicles and improve interactions with immune cells (Irvine and Read 2020). Naturally, adjuvants are used in combination with poorly immunogenic vaccines to improve their immunogenicity. Recent adjuvants offer a wide range of chemical substances with diverse mechanisms of action and potential side effects and hazards (Spickler and Roth 2003). Incomplete Freund's adjuvant (IFA) has been widely used due to its low toxicity and has been shown to increase the immune response in combination with conventional vaccines. However, IFA has one limitation regarding its inability to trigger a cellular immune response, which is important for viral infection and tumors (Jensen et al. 1998). Therefore, SAPNs/VLPs are more promising than the combination of adjuvants and commercial vaccines due to their high immunogenicity, adjuvant-like effects, safety benefits because of a lack of genetic material, and absence of the risk of pathogenicity and virulence.

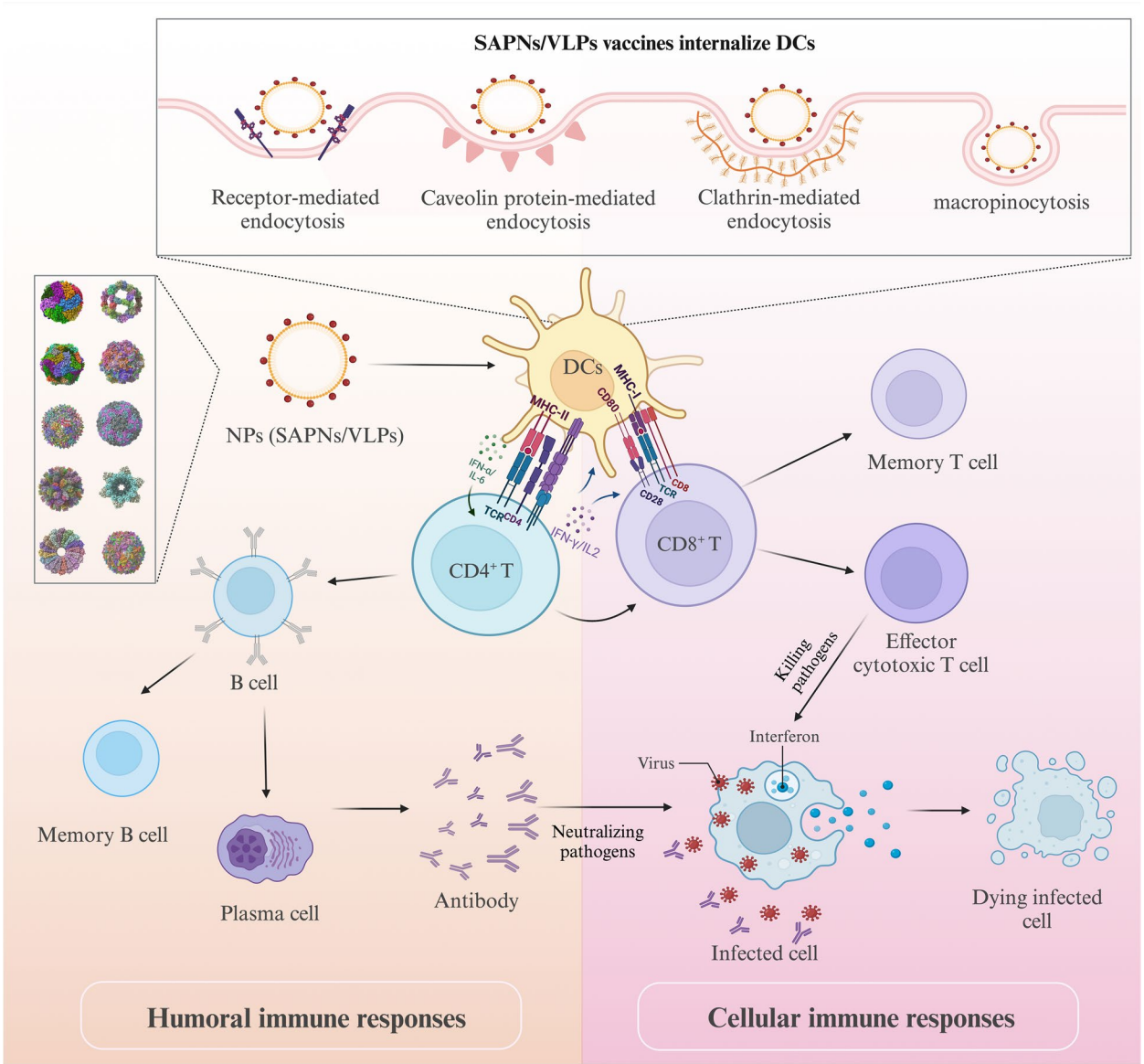


**Fig. 1** Adaptive immune activation induced by NP (SAPN/VLPS) vaccines. APCs recognize and take up NP-based vaccines when they are administered. After injection, NP-based vaccines are detected and taken up by APCs, which initiates DC maturation. Furthermore, DC maturation triggers the production of TNF-α (a proinflammatory factor) and the recruitment of more APCs to boost lysosomal proteolysis in the cell. Dendritic cells (DCs) process NP-based vaccines into small peptides, to form an MHC-peptide complex with T cell receptor (TCR) on $CD8^+$ and $CD4^+$ T cells. $CD4^+$ T cells interact with B cells, resulting in B cells being activated and then differentiating into plasma cells, which can secrete antibodies and neutralize pathogens. $CD4^+$ T-cell activation can also promote the development of B cells into memory B cells. $CD8^+$ cytotoxic T lymphocytes (CTLs) activated by APCs proliferate and differentiate into effector and specific memory CTLs. Effector CTLs are capable of causing apoptosis via the release of cytotoxic mediators to infected cells. Image created with BioRender.com

This review provides a current overview of the use of SAPNs and various types of VLPs as vaccination platforms for a variety of animal infections. Figure 3 shows the NP vaccine nanostructures. Furthermore, we discuss the adjuvants used, the route of administration, the approval status, the optimization strategy, and other information on these NP vaccines.

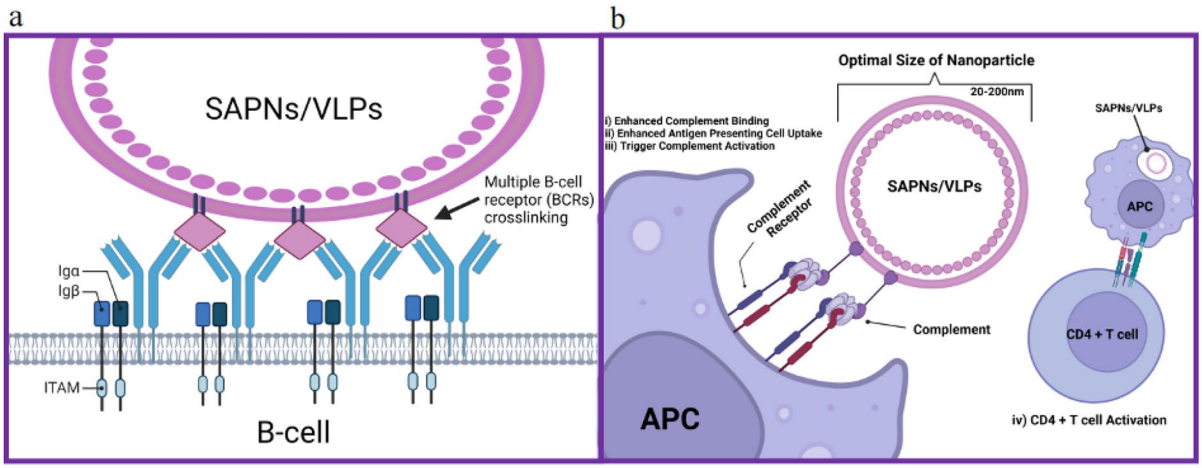


**Fig. 2** Advantages of SAPN/VLP vaccines. **a.** SAPN/VLP vaccines with a repetitive array of antigens provide a molecular scenario in which antigens and B-cell receptors (BCRs) interact multiple times, increasing B-cell activation. **b**. Antigen attachment on particles creates an optimum range of sizes for interactions with APCs and other immune cells. NPs can improve the binding of complement to FDCs (complement receptors), and the bound complement promotes the retention of NPs in the lymph nodes and further enhances interactions with immune cells. SAPN/VLP vaccines are of the appropriate size for uptake by APCs, allowing for enhanced antigen presentation to stimulate T-helper cells. Image created with BioRender.com

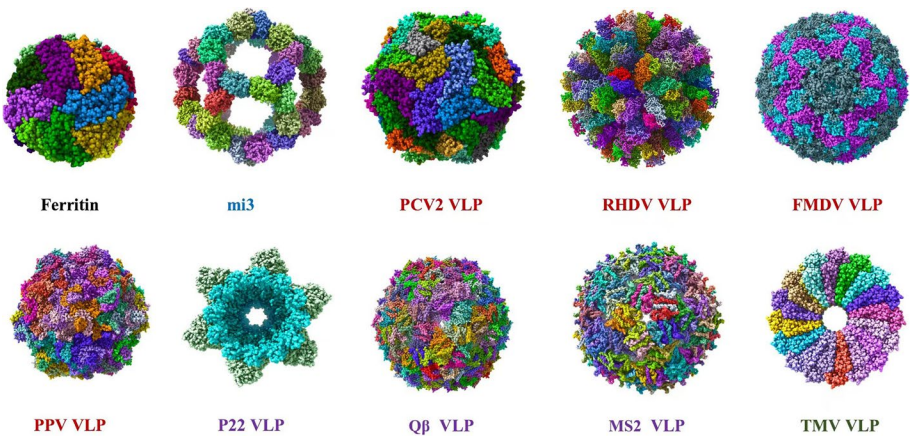


**Fig. 3** Crystal structures of SAPN/VLPS scaffolds. Natural self-assembled protein nanoparticles, rationally designed self-assembled protein nanoparticles, animal virus-derived VLPs, bacteriophage-derived VLPs, and plant virus-derived VLPs are labeled in black, blue, red, purple, and green, respectively. NP structures were constructed in ChimeraX software by using the Protein Data Bank ID codes Ferritin (1mfr), mi3 (7b3y), PCV2 VLP (5zbo), RHDV VLP (3zue), FMDV VLP (7eno), PPV VLP (1k3v), P22 bacteriophage VLP (8eb7), Qβ bacteriophage VLP (7lge), MS2 bacteriophage VLP (6rrs), and TMV VLP (6r7m). Image created with ChimeraX software

## Natural self-assembled peptide nanoparticles

This section will discuss the use of natural SAPNs as animal vaccination scaffolds for infectious illnesses. We will discuss the size, structure, self-assembly, antigen display, expression system, advantages and disadvantages, and immune effects of ferritin. A synopsis of these data is shown in Table 1.

Ferritin is a self-assembling iron storage protein nanoparticle found in practically every organism, including bacteria, fungi, plants, and animals (Munro and Linder 1978). It consists of 24 self-assembled subunits, each containing a four-alpha-helix bundle, which forms a spherical cage-like shape with octahedral symmetry (Cho, et al. 2009; Ford, et al. 1984; Harrison and Arosio 1275). This structure is characterized as a ferritin cage, with inner and outer diameters of 8 and 12 nm, respectively (Theil 2013). Because of their spontaneous self-assembly, petite uniform size, biocompatibility, biodegradability, affordability,

**Table 1** Overview of natural self-assembled peptide nanoparticles

| Scaffold platform | Structure | Size | Development stage | Antigenic components displayed | Expression system | Reference |
|---|---|---|---|---|---|---|
| Ferritin | Octahedral symmetry (24 subunits) | 12 nm | Phase 1 clinical trials | CSFV E2 | BEVS | Zhao et al. 2021; Chen et al. 2020; Kanekiyo et al. 2013; Yassine et al. 2015; Ma et al. 2021) |
| | | | | FMDV VP1 | BEVS | |
| | | | | H1N1 HA | HEK293 mammalian cells | |
| | | | | PRRSV GP5 | BEVS | |

*Abbreviations*: *CSFV* classical swine fever virus, *BEVS* Baculovirus expression vector system, *PRRSV* Porcine reproductive and respiratory syndrome virus, *FMDV* Foot-and-mouth disease virus

large-scale production, and ability to surface conjugate using chemical or genetic techniques, they are effective platforms for vaccine design (Silva et al. 2013; Khoshnejad et al. 2018). Antigens can be genetically fused to the N-terminus of a single ferritin subunit or directly adjacent to the surface of ferritin nanoparticles by modular assembly (Rodrigues et al. 2021). Ferritin synthesis using genetically fused antigens has been performed mainly in insect cells and baculovirus expression vector system (IC-BEVS) or HEK293 mammalian cells (Zhao, et al. 2021; Chen et al. 2020; Kanekiyo, et al. 2013; Yassine, et al. 2015; Ma, et al. 2021; Qu, et al. 2020). Furthermore, although there are few examples, *Escherichia coli* (*E. coli*) and CHO cells provide an alternative platform for manufacturing ferritin-based vaccines (Li, et al. 2019).

Ferritin-based nanoparticle vaccines are safe and can stimulate immune responses against a wide range of infections. Because of their remarkable thermal and chemical stability, these materials are less reliant on cold chains for transportation and storage. In addition, ferritin-based vaccines are inexpensive and easily produced (Weidenbacher, et al. 2149). Recently, a ferritin-hemagglutinin (HA) vaccine for influenza in phase 1 clinical trials (Clinical-Trials.gov ID: NCT03186781) was developed in 2019, demonstrating the increasing interest in this platform (Kanekiyo, et al. 2013; Yassine, et al. 2015). Modular assembly allows the conjugation of several types of antigens to ferritin, leading to the production of broad-spectrum vaccines. However, whether its structure can be preserved or elicit an immunological response is a significant challenge (Ma, et al. 2021).

Ferritin-based veterinary vaccines have been shown to stimulate humoral and cellular immune responses. For instance, vaccines containing classical swine fever virus (CSFV) E2 glycoproteins displayed on the exterior of ferritin NPs produced greater E2-specific antibody titers, neutralizing antibody titers, and innate immune cytokines in vivo than traditional subunit vaccines (Zhao, et al. 2021). Another investigation established a vaccine for foot and mouth disease virus (FMDV) by conjugating ferritin to the FMDV viral antigen VP1 through genetic fusion using a baculovirus expression system. NPs increase FMDV-specific IgG and IgG subclass antibody titers, IL-4 and IFN-γ production, and splenocyte proliferation and decrease the survival rate of mice (Chen et al. 2020). Ferritin has been evaluated in preclinical studies as a vaccine scaffold for influenza. Kanekiyo genetically attached the ectodomain of influenza virus hemagglutinin (HA) to ferritin, causing it to autonomously assemble and form eight trimeric viral spikes on its surface (Kanekiyo, et al. 2013). Immunization with this HA-nanoparticle vaccine produced antibody titers for hemagglutination inhibition (HAI) that were more than tenfold greater than those for the licensed inactivated vaccine, as well as broadly neutralizing antibodies against H1N1. Yassine reported that ferritin-based vaccines containing the stem region of the H1 HA glycoprotein elicited extensive cross-reactive antibodies, completely protecting mice from lethal dosages of heterosubtypic H5N1 virus and partially protecting ferrets (Yassine, et al. 2015). The ferritin platform has also been used for vaccine development for porcine reproductive and respiratory syndrome virus (PRRSV). In a recent investigation, multiple copies of the PRRSV envelope glycoprotein GP5 on ferritin were detected (Ma, et al. 2021). Compared with inactivated vaccines, it produced stronger antibody responses and neutralizing antibody titers against PRRSV in pigs at 28 and 35 days postimmunization (dpi). Immunization with the GP5m-ferritin (GP5m-Ft) nanoparticle vaccine encouraged all vaccinated groups to develop a TH1-dominant cellular immune response and to boost particular T lymphocyte immune responses. Vaccinating pigs with the GP5m-Ft vaccine dramatically reduced PRRSV viremia and the number of macroscopic and microscopic lung lesions.

In conclusion, the development of ferritin-based vaccines has enormous promise for treating a wide range of animal diseases. However, it has to address accompanying obstacles.

## Rationally designed self-assembled peptide nanoparticles

Although nanoscale assemblies might serve as effective candidate platforms for vaccines, they are limited by the number of accessible scaffolds and the presence of specific physicochemical features. Another method for developing nanovaccines is to use rationally constructed SAPNs. This section reviews current breakthroughs in computationally created artificial nanoparticles used in infectious disease vaccinations, as summarized in Table 2.

Recently, computational approaches for constructing protein nanocages with atomic-level accuracy have been promoted as a burgeoning topic, with the potential to create self-assembling proteins with customizable architectures. In terms of design methods, they typically apply symmetric principles to assemble naturally occurring or newly created cyclic oligomers into protein nanoparticles. This rationally- design based method is considered a bottom-up approach, using minor pieces and producing a more complicated assembly, considerably increasing the protein nanoparticle design space (Papapostolou and Howorka 2009). The main benefit is that rationally designed proteins can display a wider range of antigens, not limited by fixed configurations, and can induce strong immune responses by the addition of new antigenic domains. Kanekiyo performed antigen domain optimization by molecular design and presented trimeric HA spikes on HA-NPs to obtain the maximum potential for inducing neutralizing antibodies (Kanekiyo, et al. 2013). However, one important barrier to rational vaccination antigen design is that protein sequences may only sometimes align precisely with structure and function (Bromley et al. 2008). Many groups have investigated how to design and construct self-assembled protein nanoparticle components based on nonnative polyproteins. For example, Hsia and coworkers developed a self-assembling protein known as i301 based on 2-keto-3-deoxy-phosphogluconate (KDPG) aldolase from the hyperthermophilic bacterium *Thermotoga maritima*, which is composed of 60 subunits with a porous dodecahedral architecture (Hsia, et al. 2016). Brunn et al. reported that a mi3 platform, an i301 mutant, can display antigens of interest by fusing SpyCatcher and SpyTag (Bruun et al. 2018). Antigens can be genetically fused to either the N- or C-terminus of mi3 and produced in eukaryotic or bacterial cells.

Mi3 offers numerous benefits as a multimerization platform for presenting antigens. mi3 NPs can accommodate large target antigens or proteins of up to 354 amino acids while maintaining nanoparticle formation and stability (Liu, et al. 2021b) and are larger than many other self-assembling nanoparticle platforms. In addition, miR3 has been shown to promote antigen uptake and maturation in dendritic cells and to induce potent neutralizing antibody responses and cytotoxic T lymphocyte responses (Liu, et al. 2021a, 2021b; Tan, et al. 2021). However, in comparison to other platforms, mi3 is relatively new and has received less attention in terms of safety and efficacy; further validation is needed.

The mi3 platform was utilized to create a classical CSFV vaccine. Liu employed the mi3 platform to stimulate a stronger immune response. CSFV E2 was fused to mi3 (SP-E2-mi3), which was subsequently expressed and purified from the Bac-to-Bac system. The vaccination of pigs with SP-E2-mi3 NPs greatly enhanced humoral and cellular immune responses. Compared to monomeric E2, SP-E2-mi3 NPs can elicit CSFV-specific IFN-γ-cellular immunity and neutralize more than tenfold more antibodies. This formulation protected the pigs against deadly pathogen assaults (Liu, et al. 2021a). The mi3 platform has also been utilized to generate influenza vaccines. One study developed a vaccine for the influenza virus by purifying and expressing mi3 NPs that were genetically fused to homotypic and heterotypic HA antigens. The results revealed that mi3-HA NPs could produce powerful immune responses in mice but did not cause obvious cross-reactivity compared to immunization with combinations of homotypic particles (Cohen, et al. 2021).

Based on the studies presented above, mi3 is a potential platform for veterinary vaccine development. However, more research must be done to fully characterize the immune responses generated by mi3 nanoparticles and their ability to provide protective immunity against different pathogens.

**Table 2** Overview of the designed self-assembled peptide nanoparticles

| Scaffold platform | Structure | Development stage | Antigenic components displayed | Expression system | Reference |
|---|---|---|---|---|---|
| mi3 | dodecahedral symmetry (60 subunits) | Preclinical | CSFV E2<br>H1N1 HA | BEVS<br>*E. Coli* | Liu et al. 2021a<br>Bruun et al. 2018 |

*Abbreviations*: *CSFV* classical swine fever virus, *BEVS* batculovirus expression vector system, *E. coli Escherichia coli*

## Virus-like particles (VLPs): Animal virus-derived

VLPs resemble natural viruses in size and structure. They are supramolecular complexes consisting of several protein subunits. They can utilize genetic fusion to bind antigenic peptides from multiple pathogens and have been investigated as vaccine platforms for a variety of infectious diseases. Table 3 shows a summary of this information. This section will concentrate on immunization research using animal viral-derived VLPs, including porcine circovirus type 2 (PCV2), rabbit hemorrhagic disease virus (RHDV), FMDV, porcine parvovirus (PPV), and influenza A viruses (IAVs), as vaccine platforms.

### Porcine circovirus type 2 (PCV2)

PCV2 is a pig virus with a major economic impact. The lone structural protein of PCV2, the capsid (Cap) protein, can be assembled into an icosahedral spherical cage-like VLP with T = 1, making it a suitable vehicle for displaying foreign sequences. PCV2 VLPs consist of 60 Cap subunits, each with eight antiparallel β-sheets and seven loops produced between folded sheets (Khayat, et al. 2011). The loop CD region allows for the insertion or substitution of exogenous peptides and may not influence VLP assembly. In addition to the core region of the loop CD, the carboxyl terminus of the Cap also plays critical roles in immune recognition and can be fused to foreign peptides and assembled into chimeric virus-like particles (Wang, et al. 2016). PCV2 VLPs or chimeric PCV2 VLPs can be efficiently expressed in *E. coli*, yeast, and insect expression systems (Yin, et al. 2010; Bucarey, et al. 2009; López-Vidal, et al. 2015). PCV2 VLPs have been approved and are commercially available, and two Cap-based vaccines are now available on the market (Porcilis® PCV and CircoFLEX®) (Pagot, et al. 2017).

PCV2 VLPs can be adorned with immunostimulatory peptides or epitopes from other pathogens, which allows the production of bivalent vaccines (Mo, et al. 2019; Liu et al. 2020). In general, Cap can be modularly assembled with foreign antigens, allowing for the delivery of full-length proteins. PCV2 VLPs are not infectious because they lack a viral genome, making them safe for use as vaccines. In addition, PCV2 VLPs can elicit both cell-mediated and humoral immune responses, leading to a robust immune reaction against the target virus (Jung et al. 2020b; Li, et al. 2018; Hu, et al. 2016). It has shown broad cross-neutralizing activities, meaning that it can protect

**Table 3** An overview of animal virus-derived VLPs

| Scaffold platform | Structure | Size | Development stage | Antigenic components displayed | Expression system | Reference |
|---|---|---|---|---|---|---|
| PCV2 VLP | Icosahedral symmetry (60 subunits) | 25 nm | Licensed (Porcilis PCV® and Ingelvac CircoFLEX1®) | CSFV E2 | BEVS/*E. Coli* | Liu et al. 2022; Li et al. 2021; Jung et al. 2020a; Ding et al. 2019 |
| | | | | PRRSV GP5 (epitope B and epitope 7) | *E. Coli* | |
| | | | | PRRSV GP3/GP5 | BEVS | |
| | | | | H1N1 M2e | *E. Coli* | |
| RHDV VLP | Icosahedral symmetry (180 subunits) | 40 nm | Preclinical | RHDV VP60 | *E. Coli* | Guo et al. 2016; Dalton et al. 2021; Rangel et al. 2021; Crisci et al. 2012 |
| | | | | Chimeric two RHDV VP60 | BEVS | |
| | | | | FMDV VP1 and 3A | BEVS | |
| | | | | FMDV 3A | BEVS | |
| FMDV VLP | Icosahedral symmetry (60 subunits) | 30 nm | Preclinical | FMDV VP0, VP1 and VP3 | *E. Coli* | Xiao et al. 2021; Liu et al. 2017 |
| | | | | Chimeric two FMDV Cap | BEVS | |
| PPV VLP | Icosahedral symmetry (60 subunits) | 25 nm | Preclinical | PPV VP2 | *E. Coli* | Hua et al. 2020a; Liu et al. 2020; Anwar et al. 2021 |
| | | | | PPV VP2 and PCV2 Cap | *E. Coli* | |
| | | | | JEV E | *E. Coli* | |
| SIV VLP | spherical symmetry | Depend on type | Clinical trials | H1N1 and H3N2 HA-M1 | BEVS | Cai et al. 2022a; Mai et al. 2023a; Pliasas et al. 2022 |
| | | | | H1N1 and H3N2 HA-M1 | BEVS | |
| | | | | H3N2 NA and H1N1 M1 | BEVS | |

*Abbreviations*: *CSFV* classical swine fever virus, *RHDV* rabbit hemorrhagic disease virus, *PPV* porcine parvovirus, *FMDV* foot-and-mouth disease virus, *PCV2* porcine circovirus type 2, *PRRSV* porcine reproductive and respiratory syndrome virus, *BEVS* bactulovirus expression vector system, *E.* coli. Escherichia coli

against different genotypes or strains of PCV. However, current strategies for integrating exogenous antigens into PCV2 Cap VLPs are limited to the introduction of small epitopes/peptides (Lei et al. 2020). Steric hindrance can hinder strategies such as tandem core, split core, and mosaic particle technologies. Furthermore, PCV Cap VLPs may exhibit antigenic diversity, compromising the accuracy of immunological evaluation and diagnostic performance of commercial ELISAs (Kang, et al. 2021a).

The PCV2 VLP platform was tested for its ability to prevent various swine viral infections. In one study, investigators developed dual nanoparticle vaccination based on SpyTag/SpyCatcher technology to avoid PCV2 and CSFV coinfection. In this study, CSFV E2 was coupled to SpyTag and surface-displayed on SpyCatcher-decorated PCV2 Cap *via* in vitro conjugation based on isopeptide bonds generated between SpyCatcher and SpyTag. Compared to unconjugated vaccines (Cap + E2 and E2 alone), high-density E2 on Cap resulted in significantly greater antibody levels and neutralizing antibody responses. Compared with Cap VLPs, Cap-E2 NPs elicited equal quantities of PCV2-specific and neutralizing antibodies. Furthermore, Cap-E2 NPs significantly enhanced CSFV E2-specific cellular immunity. Cap-E2 nanoparticles stimulated lymphoproliferative responses and increased TH1-type cytokine production (IL-2 and IFN-γ) (Liu et al. 2022). Li isolated two epitopes, epitopes B and 7, from the highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) Gp5 for use in PRRSV vaccination research. These epitopes were selected for display on PCV2 VLPs. Research on animals has shown that the VLP vaccine may promote the development of neutralizing and specific antibodies against epitopes B and 7 and lower viral loads following HP-PRRSV challenge (Li, et al. 2021). In a similar study, Cap VLPs were altered by replacing the decoy epitope of the Cap protein with the PRRSV GP3, GP5 or GP3-GP5 epitope. Cap-GP3, Cap-GP5 and Cap-GP35 produced high GP3/GP5 and Cap antibody titers, while animals immunized with Cap-GP3 VLPs had high levels of both PCV2- and PRRSV-neutralizing antibodies (Jung et al. 2020b). PCV2 VLPs have also been used to enhance influenza vaccines. In one study, various copies of theIAV matrix protein 2 (M2e) were inserted into PCV2 Cap and expressed in *E. coli* to produce VLPs. The Cap-3M2e VLP nanomicroline produced the greatest levels of M2e-specific and neutralizing antibodies, and it entirely prevented deadly infection by H1N1 and H3N2. Furthermore, Cap-3M2e VLPs produce enormous levels of PCV2-specific neutralizing antibodies in pigs (Ding, et al. 2019).

These findings suggest that the PCV2 VLP platform can be used for dual nanoparticle vaccination to stimulate a strong immune response to PCV2 and other veterinary illnesses.

**Rabbit hemorrhagic disease virus (RDHV)**

RHDV usually causes highly contagious and fatal sickness in rabbits. It is a nonenveloped RNA virus with a capsid (about 40 nm diameter) composed of 180 monomeric units of VP60 (also termed VP1), which integrate into 90 dimers to create a T = 3 icosahedral form (Valícek et al. 1990; Wang, et al. 2013). The VP60 capsid protein can be expressed in heterologous recombinant systems such as recombinant baculovirus, plants, mammalian cell cultures, or yeasts, and this often leads to the formation of VLPs that resemble natural virions both morphologically and antigenically (Boga, et al. 1994; Fernández-Fernández, et al. 2001; Bertagnoli, et al. 1996a, 1996b; Pérez-Filgueira, et al. 2007; Laurent et al. 1994; Escribano, et al. 2020). The VP60 VLP is composed of an internally positioned N-terminal arm (NTA), a shell domain (S), which creates an unbroken scaffold, and a flexible protrusion domain (P) on the capsid surface. The latter can be further divided into P1 (aa 238–286, 450–466, 484–579) and P2 (aa 287–449 and 467–483) subdomains, with the P2 subdomain residing in the most accessible region of the VP60 VLP and possibly containing determinants of cell attachment and antigenic diversity (Wang, et al. 2013; Bárcena, et al. 2004, 2015; Leuthold et al. 2015). RHDV VLPs have been shown to be both an efficient preventive vaccine in rabbits and a vehicle for the delivery of heterologous antigens. Structural analysis indicated that the VP60 protein can accommodate the introduction of foreign antigenic sequences (chimeric VLPs) to the N-terminus without disrupting VLP assembly, yet the C-terminus is not suitable for introducing antigens due to the disruption of VLP assembly and antigen presentation (Leuthold et al. 2015).

RHDV VLPs are noninfectious and lack the genetic material required for replication, which reduces the likelihood of disease transmission in vaccinated patients. It closely resembles the native virus in structure, allowing for better recognition by the immune system and potentially enhancing the effectiveness of the vaccine (Laurent et al. 1994). Moreover, the use of Trichoplusia ni insect pupae as natural bioreactors for VLP production simplifies vaccine manufacturing and reduces downstream production-associated costs (Dalton, et al. 2021; Escribano, et al. 2020). Unfortunately, although numerous candidates for RHDV VLP vaccines are currently being studied, none have been effectively commercialized.

The RHDV VLP vaccine has been shown to produce widespread immunity in inoculated rabbits. VP60 VLP vaccination for RHDV was developed using *E. coli* expression systems according to one study. Following a two-dose schedule, vaccinated rabbits showed greater specific antibody titers and cell-mediated immune responses (Guo, et al. 2016). A different study used

recombinant baculovirus vectors to coexpress VP60 proteins from the two RHDV prevalent serotypes, resulting in chimeric VLPs that included both proteins. Rabbits were vaccinated with chimeric RHDV VLPs and subsequently challenged with two RHDV serotypes, resulting in total protection against the deadly challenge of both serotypes of infection (Dalton, et al. 2021). RHDV VLPs have also been tested as a vaccine scaffold for FMDV infections. To address FMDV, the coat protein epitopes of FMDV (T-cell epitope and neutralizing B-cell epitope) are genetically fused into two different locations of RHDV VP60. Extensive in vivo studies have shown that this chimeric VLP vaccine elicits potent FMDV-specific antibodies and strong neutralizing immune reactions in mice and pigs but is insufficient to induce complete protection at the level of cellular immune responses. Consequently, it provides partial clinical protection against FMDV infection (Rangel, et al. 2021). In another similar study, Crisci demonstrated that the FMDV-RHDV chimeric VLP vaccine can induce specific cellular immunity. The chimeric VLP vaccine can produce specific IFN-γ-secreting cells and lymphoproliferative T cells against FMDV and RHDV (Crisci, et al. 2012).

In recent years, major advances in tumor treatment involving RHDV VLPs have been achieved. However, its application in veterinary medicine is limited; it is mainly used as a carrier for FMDV. Therefore, further exploration of the application of RHDV in other veterinary diseases is urgently needed.

#### Foot and mouth disease virus (FMDV)

FMDV is a highly contagious and lethal disease in agricultural animals. The FMDV genome has one large open reading frame that codes for the P1-2A precursor polyprotein, which is processed by the 3C protease into mature VP0, VP1 and VP3 and assembled to form the icosahedral capsid (Ryan et al. 1989; Abrams et al. 1995). FMDV VLPs consist of 60 copies of capsid protein monomers (VP0, VP1 and VP3) arranged into 12 pentameric subunits that function as intermediates in capsid construction and disassembly (Han et al. 2015). There are currently two forms of FMDV VLP vaccines: one composed of FMDV capsid proteins and one composed of chimeric FMDV VLPs created by incorporating critical epitopes from multiple serotypes. FMDV VLPs or chimeric VLPs can be efficiently expressed in *E. coli*, mammalian cells, and insect expression systems (Silva, et al. 2013; Xiao, et al. 2016; Oem, et al. 2007; Felberbaum 2015; Ruiz, et al. 2014; Gullberg, et al. 2013). Previous research has shown that FMDV VLPs, as FMDV vaccines, can induce long-lasting humoral and cellular immune responses. Unfortunately, FMDV VLPs have been less frequently used as a carrier model for other pathogens.

2021; 2017; 2013). Similar to other VLPs, FMDV VLPs are not contagious due to their lack of infectious viral genetic material, which limits the possibility of infection. However, 3C-protease, which is typically used in the production of VLPs, can be harmful when present in large quantities, suggesting a potential disadvantage of its use in production systems (Veerapen et al. 2018).

FMDV VLPs have been studied as a vaccine platform for managing FMDV infection. In one study, an enhanced SUMO fusion protein system in *E. coli* produced the VP0, VP1 and VP3 proteins; removing the SUMO moiety from the fusion proteins resulted in the assembly of FMDV VLPs. The results revealed that FMDV VLP vaccination can promote specific antibodies, neutralizing antibodies, T-cell proliferation, and IFN-γ secretion in guinea pigs, swine and cattle. Vaccination with one dose of the VLP may elicit a high level of immunological response, which is adequate to protect against virulent viral infection (Guo, et al. 2013). In a similar study, researchers coexpressed FMDV capsid proteins (VP0, VP1and VP3) in *E. coli*. FMDV VLP vaccination successfully induced FMDV-specific cellular and humoral immune responses in pigs (Xiao, et al. 2021). Liu created a chimeric FMDV VLP vaccine using the baculovirus system, combining the antigenic structural protein VP1 from serotype O with viral capsid protein segments (VP2, VP3 and VP4) from serotype A. Animal tests demonstrated that the chimeric VLP vaccine increased the levels of anti-FMDV antibodies and cytokines (IFN-γ, IL-4, and IL-6) and strongly protected guinea pigs from FMDV challenge (Liu, et al. 2017). As a result, FMDV VLPs have the potential to be good vaccine candidates for treating FMDV infection.

#### Porcine parvovirus (PPV)

The primary cause of sow reproductive failure syndrome is PPV. The PPV capsid is an icosahedral, nonenveloped, and spherical shell with a diameter of approximately 20 ~ 25 nm. It is composed of 60 copies of a combination of VPs, VP1, VP2 and VP3 (Molitor et al. 1983). The primary capsid protein, VP2, can self-assemble into VLPs, a key antigen that triggers neutralizing antibodies (Ridpath and Mengeling 1988). It can successfully self-assemble in a variety of expression systems, including baculovirus/insect, mammalian cell, yeast and *E. coli* expression systems (Antonis, et al. 2006; Guo et al. 2014; Yang, et al. 2021; Wang, et al. 2020). According to previous studies, adding or deleting amino acids at the N-terminus of the VP2 protein does not affect VLP assembly (Wang, et al. 2021), opening up the prospect of using PPV VLPs as a vaccine carrier model for multiple diseases. Nonetheless, the processes controlling PPV VP2 self-assembly remain unknown.

PPV VLPs are very biologically safe and have been proven to induce high hemagglutination inhibition antibodies and neutralizing antibody responses in animals, indicating their usefulness in triggering immunological responses (Hua, et al. 2020a, 2020b; Liu, et al. 2020; Anwar, et al. 2021). PPV VLPs have demonstrated the ability to act as a vaccine carrier model for multiple pathogens that provide protection against PPV and other pathogen infections. However, the expression of the PPV VP2 protein was relatively low under the current expression conditions, which may require further optimization in high-density fermenters to increase yield (Hua, et al. 2020a; Zhou et al. 2010). Furthermore, the underlying processes of PPV VP2 protein synthesis in prokaryotic systems and its ability to generate VLPs in vitro still need to be fully characterized and may require further investigation and development (Liu, et al. 2020).

VP2-based VLP is the most commonly used for PPV infection. In one work, VLPs were generated by effectively expressing the PPV VP2 protein in *E. coli*, which had a structure and hemagglutination capabilities identical to those of natural PPV. Guinea pigs, weaned piglets, and primiparous gilts were vaccinated with VLPs, which elicited strong hemagglutination inhibition and neutralizing antibodies. In guinea pigs, inoculation with 20 μg or 10 μg of VLPs resulted in neutralizing antibody and HI antibody titers comparable to those of 200 μL of the commercial inactivated vaccine 28 dpi. Immunization with the VLP vaccine protected against reproductive failure following pathogenic PPV challenges in primiparous gilts (Hua, et al. 2020a). In another study, Liu described a combination vaccine that addresses PCV2 and PPV coinfections. The PPV-VP2 and PCV2-Cap proteins, which self-assemble into VLPs, were expressed in *E. coli*. The combined VLP vaccine generated robust cellular and humoral immune responses against PPV and PCV2, reduced the viral load in the tissue and serum, and eliminated clinical disease in pigs (Liu, et al. 2020). PPV VLP-based technology has also been utilized to prevent zoonotic diseases. In a single investigation, VLPs were created by introducing six JEV E protein epitopes into various loop areas of the pig parvovirus (PPV) VP2 protein. Mice and guinea pigs immunized with VLP(VP2-JEVe) vaccines developed impressive cell-mediated and humoral immune responses, providing complete protection against lethal JEV challenge in mice. It demonstrated effective hemagglutination inhibition (HI) and neutralizing reactions as well as a reduction in the amount of virus in guinea pig tissues (Anwar, et al. 2021).

For example, the VP2-based VLP platform has been effectively used to generate vaccines against PPV and other veterinary illnesses, eliciting robust cellular and humoral immune responses and providing defense against pathogenic challenges.

#### Influenza A viruses (IAVs)

IAVs are highly contagious viral diseases isolated from people, birds, horses, pigs, cats, dogs and marine mammals. They pose a substantial hazard to human and animal health (Sandrock and Kelly 2007). The IAV genome encodes a variety of polypeptides, including two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), as well as two matrix proteins (M1 and M2) that are necessary for protective immunity. A self-assembly method can produce VLPs from influenza proteins (HA, NA and M1) (Haynes 2009). The choice of a core component determines its size and shape. The creation of influenza VLPs has been achieved mostly in insect expression systems that involve insect cells infected with recombinant baculovirus expressing the HA, NA and M1 influenza genes, which leads to protein expression and VLP formation (Cox 2008). SIV, an acute and highly contagious IAV (subtypes H1N1, H1N2 or H3N2), poses a significant hazard to pig and human health. Thus, the VLP from the SIV will be emphasized.

SIV VLPs mimic the internal structure and conformation of the parent virus and display surface proteins in a highly immunogenic state. It does not require chicken embryos for production and can be mass-produced in cells, allowing for rapid updates in response to emerging antigens (Cai, et al. 2022b). Furthermore, SIV VLP vaccines can induce potent immune responses and elicit cross-protective immunity against different influenza strains (Cai, et al. 2022b; Mai, et al. 2023b). However, baculovirus virions and influenza VLPs have highly comparable densities, making it challenging to eradicate baculoviruses during purification (Margine et al. 2012). Furthermore, contradictory data have been reported regarding the protective efficacy of VLPs in various animal models, indicating potential differences in their effectiveness.

Influenza virus vaccination has been routinely utilized to prevent influenza virus infections. In one study, Cai created HA-M1 VLPs from H1N1 and H3N2 SIVs and tested their immunogenicity and protective qualities in a mouse model. The results demonstrated that bivalent VLP vaccines can induce higher levels of HI antibodies, neutralizing antibodies, specific IgG antibodies, and cytokines than can inactivated vaccines and provide sufficient protection against lethal challenges from homologous and heterologous H3N2 and H1N1 influenza viruses (Cai, et al. 2022a). Furthermore, he also investigated the immunological protective effects of the bivalent VLP vaccines H1 and H3 in piglets. After immunization with the SIV VLP vaccine, piglets produced high HI titers

of antibodies, induced more neutralizing antibodies than did piglets immunized with inactivated vaccines, and were protected against H1 and H3 influenza virus challenge (Mai, et al. 2023a). Pliasas constructed an NA2 VLP vaccine using the matrix 1 (M1) protein from H1N1 and the NA protein from H3N2. Pigs vaccinated with the NA2 VLP vaccine developed high levels of anti-NA antibodies and NAI titers. Compared to the commercially available QWIV, NA2 VLPs provided comparable protection against clinical IAV challenges, reduced virus replication in lung diseases, and decreased lung inflammation (Pliasas, et al. 2022). These findings revealed that vaccine design based on SIV VLPs provides excellent protection in piglets against H1N1 or other relevant influenza viruses. As a result, more emphasis should be given to its use in the creation of influenza virus vaccines.

## Bacteriophage-derived VLPs

Bacteriophages are viruses that attack bacteria. There are several varieties of bacteriophages. VLPs from Qβ, P22, MS2, λ, T4 and filamentous phages have been created to prevent animal diseases. This section examines the current developments in these platforms (Table 4).

### P22 bacteriophage

The P22 bacteriophage is a short-tailed phage with a 60-nm icosahedral capsid bearing double-stranded DNA (dsDNA) (Wang et al. 2019). The P22 phage capsid was constructed using 415 copies of coat protein (CP) and approximately 300 scaffolding proteins (SPs) (Kang, et al. 2010). VLPs from the Salmonella typhimurium bacteriophage P22 can be produced by coexpressing CP and SP, resulting in a T = 7 icosahedral structure with an outside diameter of 56 nm (Prevelige et al. 1988). Target antigens can be directed by the fusion of the target gene to the scaffolding domain of SP and subsequent coexpression with CP (O'Neil et al. 2011). P22 VLPs were found to be a suitable platform for delivering protein cargo.

P22 viral capsids are active macromolecules that can self-assemble. This structural flexibility allows for the genetic manipulation and modification of their interior surfaces, making them versatile nanoplatforms. As a result, P22 VLPs have been exploited as molecular structures for rapid modular high-density bioconjugation of antigens, including complete protein antigens, increasing the immunogenicity of the linked target antigen (Kang, et al. 2010).

The P22 platform was used to develop a vaccine against the iIAV. One study employed an enzymatic conjugation approach (SpyTag/SpyCatcher) to combine P22 VLPs with multiple copies of the HA protein's globular do-main head. Mice vaccinated with this formulation and challenged with the PR8 strain of IAV demonstrated 100% survival relative to unvaccinated animals. Furthermore, this formulation increased antigen-specific IgG antibody levels by twofold relative to controls (Sharma, et al. 2020). P22 is one of the most promising models for creating VLP nanocages. The utilization of the influenza virus prompted further investigations into the use of P22 VLPs for a variety of infectious illnesses.

### Qβ bacteriophage

Qβ is a positive single-stranded RNA bacteriophage that infects *E. coli* (Singleton, et al. 2018). The capsid of Qβ is composed of 180 monomeric proteins that self-assemble into an icosahedral VLP with a diameter of 28 nm (Golmohammadi et al. 1996; Machida and Imataka 2015). Qβ VLPs have been studied as a model for veterinary infectious disease vaccine applications. This modularity allows for greater freedom in the size and structure of the recombinant target antigen since the folding restrictions of the VLP monomer and subsequent self-assembly do not limit it. This scalability is beneficial for vaccine development and production since it enables the manufacture of large volumes of vaccines with short production timeframes (Skamel et al. 2014). Furthermore, antigens coupled to Qβ VLPs have been shown to induce highly

**Table 4** Overview of bacteriophage-derived VLPs

| Scaffold platform | Structure | Size | Development stage | Antigenic components displayed | Expression system | Reference |
|---|---|---|---|---|---|---|
| P22 bacteriophage | Icosahedral symmetry (415 CP and 300 SP) | 60 nm | Preclinical | H1N1 HA | *E. Coli* | Sharma et al. 2020 |
| Qβ bacteriophage | Icosahedral symmetry (180 subunits) | 28 nm | Phase 1 clinical trials | H1N1 HA<br>FMDV VP1 | *E. Coli*<br>*E. Coli* | Jegerlehner et al. 2013; Skamel et al. 2014 |
| MS2 bacteriophage | Icosahedral symmetry (180 subunits) | 30 nm | Preclinical | FMDV VP1 | *E. Coli* | Dong et al. 2015; Wang et al. 2018 |

*Abbreviations*: *FMDV* foot-and-mouth disease virus, *CP* (coat protein, *SP* scaffolding protein, *E. coli Escherichia coli*

enhanced antibody responses in both mice and humans compared to immunization with free antigens (Jegerlehner, et al. 2013; Alam, et al. 2021). However, the current size of Qβ limits the display of large protein domains (Skamel et al. 2014).

The Qβ platform was used to develop influenza vaccines. A study revealed a new influenza vaccine that uses Qβ phages coupled with the H1N1 virus antigen. Compared with those immunized with free antigens, vaccinated mice have shown increased antibody titers and survival rates (Jegerlehner, et al. 2013). The Qβ platform has also been used for preventing FMDV infection. For example, the VP1 G-H loop peptide of FMDV was attached to the Qβ VLP, resulting in significant antibody affinity for complete FMDV (Skamel et al. 2014). The Qβ phage platform is versatile and has shown promising results in developing vaccines for several animal illnesses.

#### MS2 bacteriophage

MS2, an icosahedral RNA bacteriophage, has a triangulation of T = 3 (Fu and Li 2016). Its genome encodes four proteins: the main coat protein (CP), the maturation protein (A-protein), the lysis protein, and the replicase (Bleckley and Schroeder 2012). A total of 180 copies of MS2 CP can join to form VLPs, monodisperse icosahedral capsids with a diameter of 22–29 nm (Mastico et al. 1993). The AB loop of MS2 phage CP can accept exogenous peptide insertion without compromising the self-assembly capabilities of CP. As a result, MS2-mediated chimeric nanoparticles provide an excellent platform for displaying foreign epitopes. Furthermore, MS2 VLP vaccines are known for their good stability and correct size, which is beneficial for vaccine development (Dong et al. 2015; Wang, et al. 2018). However, the limited tolerance of MS2 phage to long amino acid insertions may result in the aggregation, misfolding, or degradation of proteins (Caldeira and Peabody 2011; Peabody 1997). As a result, chimeric MS2 VLPs require additional optimization procedures.

MS2 VLP platforms have been evaluated for their ability to prevent FMDV infection. Dong YM and colleagues created an effective MS2-based FMDV vaccine by genetically modifying MS2 VLPs to express an epitope peptide of VP1. Vaccinating mice, guinea pigs, and swine with MS2 VLPs containing VP1 peptides not only elicited high-titer neutralizing antibodies but also protected the majority of the animals from FMDV challenge (Dong et al. 2015). In a similar study, mice were immunized with the FMDV-MS2 chimeric vaccine, which elicited more specific antibodies and stronger humoral immune responses (specific IFN-γ responses and lymphocyte proliferation) than were elicited by repeat peptide epitopes (Wang, et al. 2018). These findings show that MS2 VLP vaccination could provide possible preventative options for more widespread animal disease.

### Plant virus-derived VLPs

Currently, plant virus-derived VLPs have been investigated as vaccine platforms for a variety of animal diseases (see Table 5). In this section, we will focus on the immunization research of these platforms.

#### Tobacco mosaic virus (TMV)

TMV is a plant virus with a 300 nm × 20 nm rod-shaped capsid (Butler 1984). VLP derived from TMV is a high-surface nanotube structure with an 18 nm diameter constructed from helical arrangements of modified capsid coat proteins (CPs) (Lomonossoff and Wege 2018). As discussed, TMV has been studied as an antigen display scaffold. Most TMV formulations use conjugation or genetic fusion to load linear peptide epitopes and tiny immunological domains onto their capsid proteins.

TMV VLPs have many advantages (Mansour, et al. 1195; Liu et al. 2013; Jennings and Bachmann 2008). First, TMV VLPs provide a flexible backbone for the attachment of any subunit protein antigen of interest from any source, making TMV VLPs versatile carriers for a variety of antigens. Second, TMV VLPs have been stable at

**Table 5** Overview of plant virus-derived VLPs

| Scaffold platform | Structure | Size | Development stage | Antigenic components displayed | Expression system | Reference |
|---|---|---|---|---|---|---|
| TMV | rod-shaped | 300-nm × 20-nm | Preclinical | H1N1 HA | Plant | Mallajosyula et al. 2014; Jiang et al. 2006; Mansour et al. 1195 |
| | | | | FMDV VP1 | Plant | |
| | | | | Francisella OmpA, DnaK, Tul4, and SucB | Plant | |
| PapMV | rod-shaped | 500-nm × 14-nm | Preclinical | H1N1 and H3N2 M2e | Plant | Carignan et al. 2015; Bolduc et al. 2018 |

*Abbreviations*: *FMDV* foot-and-mouth disease virus, *TMV* tobacco mosaic virus, *PapMV* papaya mosaic virus

ambient temperature for decades, raising the possibility that vaccination formulations containing TMV do not require refrigeration. Finally, the TMV structure and size allow for quick uptake by antigen-presenting cells, and the positive sense RNA core of TMV provides an extra adjuvant effect. However, unlike plant-produced TMV, bacterial TMV VLPs have limited self-assembly properties and can only form nanorods under specific conditions (Lee, et al. 2021), which may limit their suitability for particular applications.

TMV VLP-based vaccines have been shown to protect against veterinary viral infections. In attempts to construct an influenza vaccine, TMV combined with peptides from the influenza HA protein has been demonstrated to elicit not only antigen-specific antibodies but also better protection against H1N1 influenza virus challenge in mice than the commercially available vaccine (Mallajosyula, et al. 2014). In another study, the defined epitope peptide F11 of VP1 from FMDV was genetically fused into a novel TMV VLP vector, which protected guinea pigs and swine from FMDV challenge. The TMV platform has also been used to develop tularemia vaccines (Jiang, et al. 2006). The TMV platform has also been used to develop tularemia vaccines. Utilizing genetic fusions, the anti-gens OmpA, DnaK, Tul4 and SucB proteins of Francisella have been conjugated to TMV to improve the immune response against Francisella tularensis. Immunization studies in mice revealed a significant humoral immune response and protected the majority of animals against deadly pathogen exposure (Mansour, et al. 1195).

#### Papaya mosaic virus (PapMV)

PapMV is a filamentous plant virus. It is composed of 1400 coat protein (CP) subunits that may self-assemble into a flexible rod-shaped VLP with a diameter of 14 nm and a length of 500 nm (Sit et al. 1989). PapMV VLPs have many features, such as stability. PapMV VLPs are highly stable even at temperatures exceeding 37°C. This stability ensures that VLPs can maintain their structural integrity and immunogenicity. Second, PapMV VLPs are highly immunogenic, meaning that they can effectively stimulate an immune response in the body. They cause the development of antibodies, specifically IgG2a antibodies, which are linked to a TH1-biased immune response. While PapMV VLPs can generate a robust immune response against the fused peptide, their ability to provide broad protection against other antigens may be limited. To widen the spectrum of protection, more antigens need to be added to VLPs.

PapMV VLPs have been demonstrated to be an effective adjuvant and vaccine platform for the development and enhancement of flu vaccines. One study fused influenza M2e peptides to PapMV particles. Mice vaccinated with PapMV-M2e generated a strongly specific antibody and protected them from H1N1 challenge (Carignan, et al. 2015). To improve the protection efficacy, another study designed a multimerized nucleoprotein PapMV nanoparticle vaccine combining M2e and nucleoprotein antigens of influenza strains, which protected mice from challenges by both H1N1 and H3N2 (Bolduc, et al. 2018). As a result, PapMV VLPs are an efficient adjuvant and vaccination platform for influenza vaccines. This is an encouraging alternative that warrants further examination.

## Optimization strategies for SAPN/VLPS vaccines

As discussed above, the NP vaccine platform can improve the immune response to subunit vaccines. However, compared with live attenuated vaccines, SAPN and VLP vaccines, which are made of pure protein or viral protein components, may not adequately elicit the innate immune response. Therefore, further optimization of NP vaccines is necessary. This section will concentrate on the optimal strategy for SAPN and VLP vaccination.

#### Adjuvant formulations

Adjuvants are functional excipients that are added to vaccines to boost immunogenicity and produce protection against infection. An adjuvant can enhance the quality, duration, and magnitude of an antigen-specific immune response through APC recruitment at the injection or administration site (Gregorio et al. 2013; Coffman et al. 2010). According to chemical composition, adjuvants are classified into mineral salt-based adjuvants, emulsion-based adjuvants, and liposome-based adjuvants (Firdaus et al. 2412). Mineral salt-based adjuvants such as aluminum salts, calcium phosphate, and AS04 are commonly utilized. What adjuvants are suitable for SAPN and VLPs? As mentioned above, SAPNs and VLPs are beneficial for APC recognition and uptake, as well as BCR crosslinking. Thus, aluminum salts, an adjuvant that is beneficial for the delivery of subunit vaccine antigens to APCs, may not necessitate the use of SAPN and VLP vaccines. Furthermore, because VLPs have a shape and surface antigenic configuration comparable to those of natural virions, the best adjuvant formulation should avoid interfering with complex structures.

One promising adjuvant formulation is cytosine phosphate guanosine (CpG) oligodeoxynucleotides (ODNs), a class of DNA analogs that trigger the innate immune system by binding to Toll-like receptor 9 (TLR9). CpG-ODN binding to TLR9 increases immunostimulatory activity due to the activation and maturation of APCs, including NK cells and dendritic cells. APC activation leads to a TH1 response that increases the production

of IFN-γ and other cytokines, such as TNF-α and IL-6. Therefore, proinflammatory cytokines directly activate and transform B cells into plasma cells that produce antigen-specific antibodies with high affinity. The expression of MHC, CD40 and CD86 due to the TLR9 signaling pathway also leads to increased antigen presentation and processing, as well as IFN-γ production and CTL activation (Dongye et al. 2022).

In one study, FMDV VLPs vaccinated with CpG demonstrated stronger cell-mediated immunity in guinea pigs than did those vaccinated with ISA206 and the poly I:C adjuvant (Terhuja et al. 2015). In another similar study, Shi used a squalene adjuvant containing CpG. The immunization of BALB/c mice and guinea pigs with FMDV VLPs and this adjuvant elicited specific antibodies, including higher levels of IgG1 and IgG2a, and increased the production of IFN-γ and IL-1β and the survival rate when the adjuvant was used (Shi, et al. 2022). In another study, *Toxoplasma gondii* VLP vaccination with a CpG adjuvant improved IgG and IgA antibody responses and elicited greater $CD4^+$ and $CD8^+$ T-cell responses than unadjuvanted immunization (Kang, et al. 2021b).

Flagellin, a potent TLR5 agonist, is a known immunostimulator that causes APC maturation and TH1- and TH2-mediated immunological responses, which are followed by B-cell activation and high antibody titers (Mizel and Bates 2010). Several investigations revealed that flagellin augmented the efficacy of H1N1 VLP vaccination by increasing humoral and cellular immunity (Wang, et al. 2008, 2010). Ren reported that H5N1 VLP membrane-anchored heat-labile enterotoxin B and flagellin produced stronger cellular and humoral immune responses than unadjuvanted H5N1 VLPs. It protected mice against lethal H5N1 challenge and showed tenfold higher IgG titers than unadjuvanted groups (Ren, et al. 2018). Furthermore, a flagellin-based HA vaccine and an M2e vaccine against influenza are being tested in clinical studies (Taylor, et al. 2012; Turley, et al. 2011). In another study, a truncated flagellin was inserted into the PCV2 Cap and displayed on VLPs. Compared with those of mice injected with wild-type Cap VLPs, Cap-flagellin-treated vaccinated mice produced more neutralizing antibodies and Cap-specific antibodies (Lu, et al. 2022a). The ability of flagellin to stimulate the immune response and improve the efficacy of influenza vaccines suggests that flagellin could play an important role as an adjuvant in the development of future vaccines.

ISCOMATRIX (CSL), a particulate complex of saponin, cholesterol, and phospholipids, is another adjuvant formulation that can deliver antigens to APCs and induce NALP3-, inflammasome-, TH1- and TH2-mediated responses and antibody formation (Baz Morelli, et al. 2012; Wilson, et al. 2014). In a phase I clinical trial of the H7N9 VLP flu vaccine, a researcher demonstrated the role of the ISCOMATRIX adjuvant in enhancing seroconversion; 80.6% of subjects receiving the H7N9 VLP vaccine containing HA with the ISCO-MATRIX™ adjuvant achieved HI responses, yet those receiving the unadjuvanted H7N9 VLP vaccine responded poorly (only 15.6% seroconversion rates) (Chung, et al. 2015). Overall, ISCOMATRIX (CSL) is a promising adjuvant that improves vaccine effectiveness by boosting immune system responses.

#### Targeting peptides

Another technique for improving vaccination efficacy is to include tailored peptides and ligands on the surface of protein cage nanoparticles, which aids in the transport of SAPN and VLPs to specific receptors. The primary target cells are DCs, which have many specialized receptors called pattern recognition receptors (PRRs), including TLRs, NOD-like receptors (NLRs), C-lectin type receptors (CLRs), helicases, and RIG-1-like receptors (RLRs) (Mazzoni and Segal 2004; Desmet and Ishii 2012; Figdor et al. 2002). SAPN and VLPs can be delivered to DCs *via* endocytic receptors on their surface.

Targeted APC strategies have been applied to various NP vaccines. In one study, multiple DC-binding peptides (DCbps) were added to PCV2 Cap to create chimeric VLPs. Mice vaccinated with Cap-DCbp VLPs exhibited improved cellular and humoral immune responses. Compared to wild-type Cap VLPs, Cap-DCbp VLPs exhibited higher levels of Cap protein-specific antibodies, intracellular cytokines, and neutralizing antibodies and an enhanced proliferation index in lymphocytes (Lu, et al. 2022b).

Lectin, such as DC-SIGN or CD209, dendritic cell-associated lectin-1 (dectin-1), dectin-2, macrophage-inducible C-type lectin receptor (MINCLE), and mannose receptor, is an appealing DC-targeting receptor for which VLP vaccines are used (Johannssen and Lepenies 2017; García-Vallejo, et al. 2013). Studies have demonstrated that mannosylation can result in enhanced antigen presentation on MHC class I and II through mannose receptors, leading to improved cell-mediated and humoral immunity. In another study, Al-Barwani conjugated a monoman-noside and novel dimanoside to the RHDV VLP capsid protein. This mannosylation greatly improved RHDV VLP adhesion and internalization by human dendritic cells, macrophages, B cells, and murine dendritic cells (Al-Barwani et al. 2014). Antigens that connect to the C-type lectin receptor DC-SIGN can be internalized and trafficked to endolysosomal regions before being processed and presented on MHC class II molecules, resulting in $CD4^+$ T-cell activation. In another study, Man (a natural DC-SIGN ligand) was conjugated

to the bacteriophage Qβ-VLPs, which can enhance $CD4^+$ TH1 responses and promote inflammatory and TH1-type cytokine production (Alam, et al. 2021). In conclusion, targeted APC methods for VLP vaccines increase both humoral and cellular immune responses.

## Conclusions and future perspectives

Vaccination has long been one of the most common strategies for preventing infectious diseases and minimizing the occurrence of global pandemics. Since the COVID-19 pandemic, nanoparticle-based vaccines have provided numerous candidates for clinical trials and other disease models. Because of their biodegradability, multivalency, molecular specificity, and biocompatibility, protein-based nanoparticles can be employed in vaccine manufacturing to improve vaccine immunogenicity and durability without requiring a cold chain. SAPN and VLP platforms, in particular, may carry antigens on their surfaces, resulting in an orderly repeated array of antigens on nanoparticles with small diameters that resemble PAMPs, leading to delivery, presentation, and robust immune reactions. Overall, the use of SAPNs and VLPs in vaccine development appears promising.

Several VLP-based vaccines, including those for malaria, hepatitis B virus (HBV), and human papillomavirus, are commercially marketed. However, SAPN- and VLP-based veterinary vaccines are still in their infancy and face a few challenges. The first challenge is the need for an in-depth understanding of the antigen release properties, internal distribution, or prophylactic mechanism of each nanoparticle-based platform in vivo. We still need to find an optimum way to achieve precise targeting and biodistribution of nanoparticles, which is critical for advancing this field. The second limitation is determining the synthesis and large-scale production of commercial nanoparticle vaccines. The current majority of SAPN and VLP-based veterinary vaccines are products of experimental studies and may take a considerable amount of time to complete clinical trials and licensing. However, we believe that it is only a matter of time. Recently, the development of low-cost expression systems, including the *E. coli* expression system, has facilitated easy manipulation, rapid growth, and cost efficiency. Compared to mammalian cell expression systems and insect cell expression systems, both have high yield and good safety but require longer expression times and higher costs. Additionally, the yeast expression system has several similarities to the *E. coli* expression system regarding production time and cost-effectiveness. However, the yeast expression system has potential disadvantages because of the thick and dense yeast cell wall, which interferes with cell disruption during protein extraction. The creation of nanoparticle vaccines using the *E. coli* expression system will enable large-scale manufacture and commercialization of nanoparticle vaccines.

Furthermore, we should focus more on enhancing immunological efficacy through a variety of approaches. In addition to CpG, flagellin, and CSL adjuvants, which are currently used in nanoparticle vaccine development research, additional novel adjuvants, such as liposomes, AS04, chitosan and PRR agonist adjuvants, which have achieved phased results in the field of human vaccines, should also be actively explored. Furthermore, we can combine several adjuvant compositions to achieve optimal targeting and distribution.

Despite these challenges, this sector anticipates commercial uses for veterinary vaccines based on the SAPN and VLP platforms described in this research, which can prevent and treat a wide range of illnesses. As vaccine research advances and numerous malignant and infectious diseases spread, SAPN and VLP nanoparticle-based platforms have demonstrated enormous promise and should be considered promising techniques in veterinary vaccine development.

**Acknowledgements**
Not applicable.

**Institutional review board statement**
Not applicable.

**Informed consent statement**
Not applicable.

**Authors' contributions**
F.H., M.-Q.S., A. C. P., Z.-H.L., and H.Q. conceived the study; M.-Q.S. and A. C. P. performed the literature search; M.-Q.S. and H.Q. wrote the manuscript; F.H. critically reviewed it. All authors have reviewed the published version of the manuscript and given their approval.

**Funding**
No external funds were used to support this research.

**Availability of data and materials**
Not applicable.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Competing of interests**
Figures 1 and 2 were created with software from BioRender. Figure 3 was created with ChimeraX software. The authors have no conflicts of interest to declare. Author Fang He was not involved in the journal's review or decisions related to this manuscript.

Received: 5 February 2024 Accepted: 29 March 2024
Published online: 10 May 2024

**References**


Abrams, C.C., A.M. King, and G.J. Belsham. 1995. Assembly of foot-and-mouth disease virus empty capsids synthesized by a vaccinia virus expression system. *J Gen Virol* 76 (Pt 12): 3089–3098. https://doi.org/10.1099/0022-1317-76-12-3089.

Al-Barwani, F., S.L. Young, M.A. Baird, D.S. Larsen, and V.K. Ward. 2014. Mannosylation of virus-like particles enhances internalization by antigen presenting cells. *PLoS One* 9: e104523. https://doi.org/10.1371/journal.pone.0104523.

Alam, M.M., Jarvis, C.M., Hincapie, R., McKay, C.S., Schimer, J., Sanhueza, C.A., Xu, K., Diehl, R.C., Finn, M.G., Kiessling, L.L. 2021. Glycan-modified virus-like particles evoke T helper type 1-like immune responses. *ACS Nano* 15: 309–321. https://doi.org/10.1021/acsnano.0c03023.

Antonis, A.F., Bruschke, C.J., Rueda, P., Maranga, L., Casal, J.I., Vela, C., Hilgers, L.A., Belt, P.B., Weerdmeester, K., Carrondo, M.J., Langeveld, JP. 2006. A novel recombinant virus-like particle vaccine for prevention of porcine parvovirus-induced reproductive failure. *Vaccine* 24: 5481–5490. https://doi.org/10.1016/j.vaccine.2006.03.089.

Anwar, M.N., Jiang, C., Di D., Zhang, J., Guo, S., Wang, X., Hameed, M., Wahaab, A., Shao, D., Li, Z., Liu, K., Li, B., Qiu, Y., Ma, Z., Wei, J. 2021. A Novel recombinant virus-Like particles displaying B and T cell epitopes of Japanese encephalitis virus offers protective immunity in mice and guinea pigs. *Vaccines (Basel)* 9. https://doi.org/10.3390/vaccines9090980.

Bachmann, M.F., and G.T. Jennings. 2010. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol* 10: 787–796. https://doi.org/10.1038/nri2868.

Bárcena, J., Verdaguer, N., Roca, R., Morales, M., Angulo, I., Risco, C., Carrascosa, J.L., Torres, J.M., Castón, J.R. 2004. The coat protein of rabbit hemorrhagic disease virus contains a molecular switch at the N-terminal region facing the inner surface of the capsid. *Virology* 322: 118–134. https://doi.org/10.1016/j.virol.2004.01.021.

Bárcena, J., Guerra, B., Angulo, I., González, J., Valcárcel, F., Mata, C.P., Castón, J.R., Blanco, E., Alejo, A. 2015. Comparative analysis of rabbit hemorrhagic disease virus (RHDV) and new RHDV2 virus antigenicity, using specific virus-like particles. *Vet Res* 46: 106. https://doi.org/10.1186/s13567-015-0245-5.

Baz, M.A., Becher, D., Koernig, S., Silva, A., Drane, D., Maraskovsky, E. 2012. ISCOMATRIX: a novel adjuvant for use in prophylactic and therapeutic vaccines against infectious diseases. *J Med Microbiol* 61: 935–943. https://doi.org/10.1099/jmm.0.040857-0.

Bertagnoli, S., Gelfi, J., Petit, F., Vautherot, J.F., Rasschaert, D., Laurent ,S., Le Gall, G., Boilletot, E., Chantal, J., Boucraut-Baralon, C. 1996. Protection of rabbits against rabbit viral haemorrhagic disease with a vaccinia-RHDV recombinant virus. *Vaccine* 14: 506–510. https://doi.org/10.1016/0264-410x(95)00232-p.

Bertagnoli S., Gelfi J., Le Gall G., Boilletot E., Vautherot J.F., Rasschaert D., Laurent S., Petit F., Boucraut-Baralon C., Milon A. 1996. Protection against myxomatosis and rabbit viral hemorrhagic disease with recombinant myxoma viruses expressing rabbit hemorrhagic disease virus capsid protein. *J Virol* 70: 5061–5066. https://doi.org/10.1128/jvi.70.8.5061-5066.1996.

Bleckley, S., and S.J. Schroeder. 2012. Incorporating global features of RNA motifs in predictions for an ensemble of secondary structures for encapsidated MS2 bacteriophage RNA. *Rna* 18: 1309–1318. https://doi.org/10.1261/rna.032326.112.

Boga, J.A., Casais, R., Marin, M.S., Martin-Alonso, J.M., Carmenes, R.S., Prieto, M., Parra, F. 1994. Molecular cloning, sequencing and expression in Escherichia coli of the capsid protein gene from rabbit haemorrhagic disease virus (Spanish isolate AST/89). *J Gen Virol* 75 (Pt 9): 2409–2413. https://doi.org/10.1099/0022-1317-75-9-2409.

Bolduc, M., Baz, M., Laliberté-Gagné ,M.È., Carignan, D., Garneau, C., Russel, A., Boivin, G., Savard, P., Leclerc ,D. 2018. The quest for a nanoparticle-based vaccine inducing broad protection to influenza viruses. *Nanomedicine* 14: 2563–2574. https://doi.org/10.1016/j.nano.2018.08.010.

Bromley, E.H., K. Channon, E. Moutevelis, and D.N. Woolfson. 2008. Peptide and protein building blocks for synthetic biology: from programming biomolecules to self-organized biomolecular systems. *ACS Chem Biol* 3: 38–50. https://doi.org/10.1021/cb700249v.

Bruun, T.U.J., A.C. Andersson, S.J. Draper, and M. Howarth. 2018. Engineering a Rugged Nanoscaffold To Enhance Plug-and-Display Vaccination. *ACS Nano* 12: 8855–8866. https://doi.org/10.1021/acsnano.8b02805.

Bucarey, S.A., Noriega, J., Reyes, P., Tapia, C., Sáenz, L., Zuñiga, A., Tobar, J.A. 2009. 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* 27: 5781–5790. https://doi.org/10.1016/j.vaccine.2009.07.061.

Buonaguro, L., Tornesello, M.L., Tagliamonte, M., Gallo, R.C., Wang, L.X., Kamin-Lewis, R., Abdelwahab, S., Lewis, G.K., Buonaguro, F.M. 2006. Baculovirus-derived human immunodeficiency virus type 1 virus-like particles activate dendritic cells and induce ex vivo T-cell responses. *J Virol* 80: 9134–9143. https://doi.org/10.1128/jvi.00050-06.

Butler, P.J. 1984. The current picture of the structure and assembly of tobacco mosaic virus. *J Gen Virol* 65 (Pt 2): 253–279. https://doi.org/10.1099/0022-1317-65-2-253.

Cai, M., Gan, P., Hu, X., Mai, Z., Ji, C., Yi, H., Li, M., Li, S., Ji, Y., Huang, J., Zhang, G., Gong, L. 2022. Protective effect of bivalent H1N1 and H3N2 VLP vaccines against Eurasian avian-like H1N1 and recent human-like H3N2 influenza viruses in a mouse model. Veterinary *Microbiology* 266: 109370. https://doi.org/10.1016/j.vetmic.2022.109370.

Cai, M., Gan, P., Hu, X., Mai, Z., Ji, C., Yi, H., Li, M., Li, S., Ji ,Y., Huang, J., Zhang, G., Gong, L. 2022. Protective effect of bivalent H1N1 and H3N2 VLP vaccines against Eurasian avian-like H1N1 and recent human-like H3N2 influenza viruses in a mouse model. *Vet Microbiol* 266: 109370. https://doi.org/10.1016/j.vetmic.2022.109370.

Caldeira, J.C., and D.S. Peabody. 2011. Thermal stability of RNA phage virus-like particles displaying foreign peptides. *J Nanobiotechnology* 9: 22. https://doi.org/10.1186/1477-3155-9-22.

Carignan, D., Thérien, A., Rioux, G., Paquet, G., Gagné, M.L., Bolduc, M., Savard, P., Leclerc, D. 2015. Engineering of the PapMV vaccine platform with a shortened M2e peptide leads to an effective one dose influenza vaccine. *Vaccine* 33: 7245–7253. https://doi.org/10.1016/j.vaccine.2015.10.123.

Chen, Y., Y. Hu, H. Chen, X. Li, and P. Qian. 2020. A ferritin nanoparticle vaccine for foot-and-mouth disease virus elicited partial protection in mice. *Vaccine* 38: 5647–5652. https://doi.org/10.1016/j.vaccine.2020.06.063.

Cho, K.J., Shin, H.J., Lee, J.H., Kim, K.J., Park, S.S., Lee, Y., Lee, C., Park, S.S., Kim, K.H. 2009. The crystal structure of ferritin from Helicobacter pylori reveals unusual conformational changes for iron uptake. *J Mol Biol* 390: 83–98. https://doi.org/10.1016/j.jmb.2009.04.078.

Chung, K.Y., Coyle, E.M., Jani, D., King, L.R., Bhardwaj, R., Fries, L., Smith, G., Glenn, G., Golding, H., Khurana, S. 2015. ISCOMATRIX™ adjuvant promotes epitope spreading and antibody affinity maturation of influenza A H7N9 virus like particle vaccine that correlate with virus neutralization in humans. *Vaccine* 33: 3953–3962. https://doi.org/10.1016/j.vaccine.2015.06.047.

Coffman, R.L., A. Sher, and R.A. Seder. 2010. Vaccine adjuvants: putting innate immunity to work. *Immunity* 33: 492–503. https://doi.org/10.1016/j.immuni.2010.10.002.

Cohen, A.A., Yang, Z., Gnanapragasam, P.N.P., Ou, S., Dam, K.A., Wang, H., Bjorkman, P.J. 2021. Construction, characterization, and immunization of nanoparticles that display a diverse array of influenza HA trimers. *PLoS One* 16: e0247963. https://doi.org/10.1371/journal.pone.0247963.

Cox, M.M., Hashimoto, Y. A fast track influenza virus vaccine produced in insect cells. *J Invertebr Pathol* 2011;107(Suppl:S31-41). https://doi.org/10.1016/j.jip.2011.05.003.

Crisci, E., Fraile, L., Moreno, N., Blanco, E., Cabezón, R., Costa, C., Mussá, T., Baratelli, M., Martinez-Orellana, P., Ganges, L., Martínez, J., Bárcena, J., Montoya, M. 2012. Chimeric calicivirus-like particles elicit specific immune responses in pigs. *Vaccine* 30: 2427–2439. https://doi.org/10.1016/j.vaccine.2012.01.069.

Cubas, R., Zhang, S., Kwon, S., Sevick-Muraca, E.M., Li, M., Chen, C., Yao, Q. 2009. Virus-like particle (VLP) lymphatic trafficking and immune response generation after immunization by different routes. *J Immunother* 32: 118–128. https://doi.org/10.1097/CJI.0b013e31818f13c4.

Dalton, K.P., Alvarado, C., Reytor, E., Del Carmen Nuñez, M., Podadera, A., Martínez-Alonso, D., Alonso, J.M.M., Nicieza, I., Gómez-Sebastián, S., Dalton, R.M., Parra, F. et al. 2021. Chimeric VLPs bearing VP60 from two serotypes of rabbit haemorrhagic disease virus are protective against both viruses. *Vaccines (Basel)* 9. https://doi.org/10.3390/vaccines9091005.

De Gregorio, E., E. Caproni, and J.B. Ulmer. 2013. Vaccine adjuvants: mode of action. *Front Immunol* 4: 214. https://doi.org/10.3389/fimmu.2013.00214.

Desmet, C.J., and K.J. Ishii. 2012. Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination. *Nat Rev Immunol* 12: 479–491. https://doi.org/10.1038/nri3247.

Ding, P., Jin, Q., Chen, X., Yang, S., Guo, J., Xing, G., Deng, R., Wang, A., Zhang, G. 2019. Nanovaccine confers dual protection against influenza a virus and porcine circovirus type 2. *Int J Nanomedicine* 14: 7533–7548. https://doi.org/10.2147/ijn.S218057.

Doll, T.A., S. Raman, R. Dey, and P. Burkhard. 2013. Nanoscale assemblies and their biomedical applications. *J R Soc Interface* 10: 20120740. https://doi.org/10.1098/rsif.2012.0740.

Dong, Y.M., G.G. Zhang, X.J. Huang, L. Chen, and H.T. Chen. 2015. Promising MS2 mediated virus-like particle vaccine against foot-and-mouth disease. *Antiviral Res* 117: 39–43. https://doi.org/10.1016/j.antiviral.2015.01.005.

Dongye, Z., J. Li, and Y. Wu. 2022. Toll-like receptor 9 agonists and combination therapies: strategies to modulate the tumour immune microenvironment for systemic anti-tumour immunity. *Br J Cancer* 127: 1584–1594. https://doi.org/10.1038/s41416-022-01876-6.

Escribano, J.M., Cid, M., Reytor, E., Alvarado, C., Nuñez, M.C., Martínez-Pulgarín, S., Dalton, R.M. 2020. Chrysalises as natural production units for recombinant subunit vaccines. *J Biotechnol* 324s: 100019. https://doi.org/10.1016/j.btecx.2020.100019.

Felberbaum, R.S. 2015. The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. *Biotechnol J* 10: 702–714. https://doi.org/10.1002/biot.201400438.

Fernández-Fernández, M.R., Mouriño, M., Rivera, J., Rodríguez, F., Plana-Durán, J., García, J.A. 2001. Protection of rabbits against rabbit hemorrhagic disease virus by immunization with the VP60 protein expressed in plants with a potyvirus-based vector. *Virology* 280: 283–291. https://doi.org/10.1006/viro.2000.0762.

Figdor, C.G., Y. van Kooyk, and G.J. Adema. 2002. C-type lectin receptors on dendritic cells and Langerhans cells. *Nat Rev Immunol* 2: 77–84. https://doi.org/10.1038/nri723.

Firdaus, F.Z., M. Skwarczynski, and I. Toth. 2022. Developments in Vaccine Adjuvants. *Methods Mol Biol* 2412: 145–178. https://doi.org/10.1007/978-1-0716-1892-9_8.

Ford, G.C., Harrison, P.M., Rice, D.W., Smith, J.M., Treffry, A., White, J.L., Yariv, J. 1984. Ferritin: design and formation of an iron-storage molecule. *Philos Trans R Soc Lond B Biol Sci* 304: 551–565. https://doi.org/10.1098/rstb.1984.0046.

Fu, Y., and J. Li. 2016. A novel delivery platform based on Bacteriophage MS2 virus-like particles. *Virus Res* 211: 9–16. https://doi.org/10.1016/j.virusres.2015.08.022.

Fujita, Y., and H. Taguchi. 2011. Current status of multiple antigen-presenting peptide vaccine systems: Application of organic and inorganic nanoparticles. *Chem Cent J* 5: 48. https://doi.org/10.1186/1752-153x-5-48.

García-Vallejo, J.J., Ambrosini, M., Overbeek, A., van Riel, W.E., Bloem, K., Unger, W.W., Chiodo, F., Bolscher, J.G., Nazmi, K., Kalay, H., et al. 2013. Multivalent glycopeptide dendrimers for the targeted delivery of antigens to dendritic cells. *Mol Immunol* 53: 387–397. https://doi.org/10.1016/j.molimm.2012.09.012.

Golmohammadi, R., K. Fridborg, M. Bundule, K. Valegård, and L. Liljas. 1996. The crystal structure of bacteriophage Q beta at 3.5 A resolution. *Structure* 4: 543–554. https://doi.org/10.1016/s0969-2126(96)00060-3.

Gullberg, M., Muszynski, B., Organtini, L.J., Ashley, R.E., Hafenstein, S.L., Belsham, G.J., Polacek, C. 2013. Assembly and characterization of foot-and-mouth disease virus empty capsid particles expressed within mammalian cells. *J Gen Virol* 94: 1769–1779. https://doi.org/10.1099/vir.0.054122-0.

Guo, H., Zhu, J., Tan, Y., Li, C., Chen, Z., Sun, S., Liu, G. 2016. Self-assembly of virus-like particles of rabbit hemorrhagic disease virus capsid protein expressed in *Escherichia coli* and their immunogenicity in rabbits. *Antiviral Res* 131: 85–91. https://doi.org/10.1016/j.antiviral.2016.04.011.

Guo, H.C., Sun, S.Q., Jin, Y., Yang, S.L., Wei, Y.Q., Sun, D.H., Yin, S.H., Ma, J.W., Liu, Z.X., Guo, J.H., et al. 2013. Foot-and-mouth disease virus-like particles produced by a SUMO fusion protein system in *Escherichia coli* induce potent protective immune responses in guinea pigs, swine and cattle. *Vet Res* 44: 48. https://doi.org/10.1186/1297-9716-44-48.

Guo, C., Z. Zhong, and Y. Huang. 2014. Production and immunogenicity of VP2 protein of porcine parvovirus expressed in Pichia pastoris. *Arch Virol* 159: 963–970. https://doi.org/10.1007/s00705-013-1907-0.

Han, S.C., H.C. Guo, and S.Q. Sun. 2015. Three-dimensional structure of foot-and-mouth disease virus and its biological functions. *Arch Virol* 160: 1–16. https://doi.org/10.1007/s00705-014-2278-x.

Harrison, P.M., and P. Arosio. 1996. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1275: 161–203. https://doi.org/10.1016/0005-2728(96)00022-9.

Haynes, J.R. 2009. Influenza virus-like particle vaccines. *Expert Rev Vaccines* 8: 435–445. https://doi.org/10.1586/erv.09.8.

Hsia, Y., Bale, J.B., Gonen, S., Shi, D., Sheffler, W., Fong, K.K., Nattermann, U., Xu, C., Huang, P.S., Ravichandran, R., et al. 2016. Design of a hyperstable 60-subunit protein dodecahedron. [corrected]. *Nature* 535: 136–139. https://doi.org/10.1038/nature18010.

Hu, G., Wang, N., Yu, W., Wang, Z., Zou, Y., Zhang, Y., Wang, A., Deng, Z., Yang, Y. 2016. Generation and immunogenicity of porcine circovirus type 2 chimeric virus-like particles displaying porcine reproductive and respiratory syndrome virus GP5 epitope B. *Vaccine* 34: 1896–1903. https://doi.org/10.1016/j.vaccine.2016.02.047.

Hua, T., Zhang, D., Tang, B., Chang, C., Liu, G., Zhang, X. The immunogenicity of the virus-like particles derived from the VP2 protein of porcine parvovirus. Veterinary Microbiology 248, 108795 (2020). https://doi.org/10.1016/j.vetmic.2020.108795.

Hua T., Zhang D., Tang B., Chang C., Liu G., Zhang X. 2020. The immunogenicity of the virus-like particles derived from the VP2 protein of porcine parvovirus. *Vet Microbiol* 248: 108795. https://doi.org/10.1016/j.vetmic.2020.108795.

Indelicato, G., Wahome, N., Ringler, P., Müller, S.A., Nieh, M.P., Burkhard, P., Twarock, R. 2016. Principles governing the self-assembly of coiled-coil protein nanoparticles. *Biophys J* 110: 646–660. https://doi.org/10.1016/j.bpj.2015.10.057.

Irvine, D.J., and B.J. Read. 2020. Shaping humoral immunity to vaccines through antigen-displaying nanoparticles. *Curr Opin Immunol* 65: 1–6. https://doi.org/10.1016/j.coi.2020.01.007.

Ivashkiv, L.B. 2018. IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. *Nat Rev Immunol* 18: 545–558. https://doi.org/10.1038/s41577-018-0029-z.

Jegerlehner, A., Zabel, F., Langer, A., Dietmeier, K., Jennings, G.T., Saudan, P., Bachmann, M.F. 2013. Bacterially produced recombinant influenza vaccines based on virus-like particles. *PLoS One* 8: e78947. https://doi.org/10.1371/journal.pone.0078947.

Jennings, G.T., and M.F. Bachmann. 2008. The coming of age of virus-like particle vaccines. *Biol Chem* 389: 521–536. https://doi.org/10.1515/bc.2008.064.

Jensen, F.C., J.R. Savary, J.P. Diveley, and J.C. Chang. 1998. Adjuvant activity of incomplete Freund's adjuvant. *Adv Drug Deliv Rev* 32: 173–186. https://doi.org/10.1016/s0169-409x(98)00009-x.

Jia, J., Zhang, Y., Xin, Y., Jiang, C., Yan, B., Zhai, S. 2018. Interactions between nanoparticles and dendritic cells: from the perspective of cancer immunotherapy. *Front Oncol* 8: 404. https://doi.org/10.3389/fonc.2018.00404.

Jiang, L., Li, Q., Li, M., Zhou, Z., Wu, L., Fan, J., Zhang, Q., Zhu, H., Xu, Z. 2006. A modified TMV-based vector facilitates the expression of longer foreign epitopes in tobacco. *Vaccine* 24: 109–115. https://doi.org/10.1016/j.vaccine.2005.09.060.

Johannssen, T., and B. Lepenies. 2017. Glycan-Based Cell Targeting To Modulate Immune Responses. *Trends Biotechnol* 35: 334–346. https://doi.org/10.1016/j.tibtech.2016.10.002.

Jung, B.-K., H.-R. Kim, H. Jang, and K.-S. Chang. 2020. Replacing the decoy epitope of PCV2 capsid protein with epitopes of GP3 and/or GP5 of PRRSV enhances the immunogenicity of bivalent vaccines in mice. *Journal of Virological Methods* 284: 113928. https://doi.org/10.1016/j.jviromet.2020.113928.

Jung, B.K., H.R. Kim, H. Jang, and K.S. Chang. 2020. Replacing the decoy epitope of PCV2 capsid protein with epitopes of GP3 and/or GP5 of PRRSV enhances the immunogenicity of bivalent vaccines in mice. *J Virol Methods* 284: 113928. https://doi.org/10.1016/j.jviromet.2020.113928.

Kak, G., M. Raza, and B.K. Tiwari. 2018. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. *Biomol Concepts* 9: 64–79. https://doi.org/10.1515/bmc-2018-0007.

Kanekiyo, M., Wei, C.J., Yassine, H.M., McTamney, P.M., Boyington, J.C., Whittle, J.R., Rao, S.S., Kong, W.P., Wang, L., Nabel, G.J. 2013. Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. *Nature* 499: 102–106. https://doi.org/10.1038/nature12202.

Kang, H.J., Chu, K.B., Kim, M.J., Lee, S.H., Park, H., Jin, H., Moon, E.K., Quan, F.S. 2021. Protective immunity induced by CpG ODN-adjuvanted virus-like particles containing *Toxoplasma gondii* proteins. *Parasite Immunol* 43: e12799. https://doi.org/10.1111/pim.12799.

Kang, S., Uchida, M., O'Neil, A., Li, R., Prevelige, P.E., Douglas, T. 2010. Implementation of p22 viral capsids as nanoplatforms. *Biomacromolecules* 11: 2804–2809. https://doi.org/10.1021/bm100877q.

Kang, S.J., Bae, S.M., Lee, H.J., Jeong, Y.J., Lee, M.A., You, S.H., Lee, H.S., Hyun, B.H., Lee, N., Cha, S.H.I. 2021. Porcine circovirus (PCV) genotype 2d-based virus-like particles (VLPs) induced broad cross-neutralizing antibodies against diverse genotypes and provided protection in dual-challenge infection of a PCV2d virus and a type 1 porcine reproductive and respiratory syndrome virus (PRRSV). *Pathogens* 10. https://doi.org/10.3390/pathogens10091145.

Khayat, R., Brunn, N, Speir, J.A., Hardham, J.M., Ankenbauer, R.G., Schneemann, A., Johnson, J.E. 2011. The 2.3-angstrom structure of porcine circovirus 2. *J Virol* 85: 7856–7862. https://doi.org/10.1128/jvi.00737-11.

Khoshnejad, M., H. Parhiz, V.V. Shuvaev, I.J. Dmochowski, and V.R. Muzykantov. 2018. Ferritin-based drug delivery systems: Hybrid nanocarriers for vascular immunotargeting. *J Control Release* 282: 13–24. https://doi.org/10.1016/j.jconrel.2018.02.042.

Kischkel, B., Rossi, S.A., Santos, S.R., Nosanchuk, J.D., Travassos, L.R., Taborda, C.P. 2020. Therapies and vaccines based on nanoparticles for the treatment of systemic fungal infections. *Front Cell Infect Microbiol* 10: 463. https://doi.org/10.3389/fcimb.2020.00463.

Laurent, S., J.F. Vautherot, M.F. Madelaine, G. Le Gall, and D. Rasschaert. 1994. Recombinant rabbit hemorrhagic disease virus capsid protein expressed in baculovirus self-assembles into viruslike particles and induces protection. *J Virol* 68: 6794–6798. https://doi.org/10.1128/jvi.68.10.6794-6798.1994.

Lee, K.Z., Basnayake, Pussepitiyalage, V., Lee, Y.H., Loesch-Fries, L.S., Harris, M.T., Hemmati, S., Solomon, K.V. 2021. Engineering tobacco mosaic virus and its virus-like-particles for synthesis of biotemplated nanomaterials. *Biotechnol J* 16: e2000311. https://doi.org/10.1002/biot.202000311.

Lei, X., X. Cai, and Y. Yang. 2020. Genetic engineering strategies for construction of multivalent chimeric VLPs vaccines. *Expert Rev Vaccines* 19: 235–246. https://doi.org/10.1080/14760584.2020.1738227.

Leuthold, M.M., K.P. Dalton, and G.S. Hansman. 2015. Structural analysis of a rabbit hemorrhagic disease virus binding to histo-blood group antigens. *J Virol* 89: 2378–2387. https://doi.org/10.1128/jvi.02832-14.

Li, Chester Q., Elizabeth Soistman, and Daniel C. Carter. 2006. Ferritin nanoparticle technology... A new platform for antigen presentation and vaccine development. *Industrial Biotechnology* 2: 143–147. https://doi.org/10.1089/ind.2006.2.143.

Li, G., Liu, L., Xu, B., Hu, J., Kuang, H., Wang, X., Wang, L., Cui, X., Sun, H., Rong, J. 2021. Displaying epitope B and epitope 7 of porcine reproductive and respiratory syndrome virus on virus like particles of porcine circovirus type 2 provides partial protection to pigs. *J Vet Med Sci* 83: 1263–1272. https://doi.org/10.1292/jvms.20-0543.

Li, X., Meng, X., Wang, S., Li, Z., Yang, L., Tu, L., Diao, W., Yu, C., Yu, Y., Yan, C., et al. 2018. Virus-like particles of recombinant PCV2b carrying FMDV-VP1 epitopes induce both anti-PCV and anti-FMDV antibody responses. *Appl Microbiol Biotechnol* 102: 10541–10550. https://doi.org/10.1007/s00253-018-9361-2.

Li, Z., Cui, K., Wang, H., Liu, F., Huang, K., Duan, Z., Wang, F., Shi, D., Liu, Q. 2019. A milk-based self-assemble rotavirus VP6-ferritin nanoparticle vaccine elicited protection against the viral infection. *J Nanobiotechnology* 17: 13. https://doi.org/10.1186/s12951-019-0446-6.

Liu, G., Qiao, X., Chang, C., Hua, T., Wang, J., Tang, B., Zhang, D. 2020. Reduction of postweaning multisystemic wasting syndrome-associated clinical symptoms by virus-Like particle vaccine against porcine parvovirus and porcine circovirus type 2. *Viral Immunol* 33: 444–456. https://doi.org/10.1089/vim.2019.0201.

Liu, R., R.A. Vaishnav, A.M. Roberts, and R.P. Friedland. 2013. Humans have antibodies against a plant virus: evidence from tobacco mosaic virus. *PLoS One* 8: e60621. https://doi.org/10.1371/journal.pone.0060621.

Liu, X., Fang, Y., Zhou, P., Lu, Y., Zhang, Q., Xiao, S., Dong, Z., Pan, L., Lv, J., Zhang, Z., et al. 2017. Chimeric virus-like particles elicit protective immunity against serotype O foot-and-mouth disease virus in guinea pigs. *Appl Microbiol Biotechnol* 101: 4905–4914. https://doi.org/10.1007/s00253-017-8246-0.

Liu, X., Y. Liu, Y. Zhang, F. Zhang, and E. Du. 2020. Incorporation of a truncated form of flagellin (TFlg) into porcine circovirus type 2 virus-like particles enhances immune responses in mice. *BMC Vet Res* 16: 45. https://doi.org/10.1186/s12917-020-2253-6.

Liu, Z.H., Xu, H.L., Han, G.W., Tao, L.N., Lu, Y., Zheng, S.Y., Fang, W.H., He, F. 2021. Self-assembling nanovaccine enhances protective efficacy against CSFV in pigs. *Front Immunol* 12:689187. https://doi.org/10.3389/fimmu.2021.689187.

Liu, Z.H., Xu, H.L., Han, G.W., Tao, L.N., Lu, Y., Zheng, S.Y., Fang, W.H., He, F. 2021. A self-assembling nanoparticle: Implications for the development of thermostable vaccine candidates. *Int J Biol Macromol* 183: 2162–2173. https://doi.org/10.1016/j.ijbiomac.2021.06.024.

Liu, Z.H., Z.F. Deng, Y. Lu, W.H. Fang, and F. He. 2022. A modular and self-adjuvanted multivalent vaccine platform based on porcine circovirus virus-like nanoparticles. *J Nanobiotechnology* 20: 493. https://doi.org/10.1186/s12951-022-01710-4.

Lomonossoff, G.P., and C. Wege. 2018. TMV Particles: The Journey From Fundamental Studies to Bionanotechnology Applications. *Adv Virus Res* 102: 149–176. https://doi.org/10.1016/bs.aivir.2018.06.003.

Look, M., A. Bandyopadhyay, J.S. Blum, and T.M. Fahmy. 2010. Application of nanotechnologies for improved immune response against infectious diseases in the developing world. *Adv Drug Deliv Rev* 62: 378–393. https://doi.org/10.1016/j.addr.2009.11.011.

López-Sagaseta, J., E. Malito, R. Rappuoli, and M.J. Bottomley. 2016. Self-assembling protein nanoparticles in the design of vaccines. *Comput Struct Biotechnol J* 14: 58–68. https://doi.org/10.1016/j.csbj.2015.11.001.

López-Vidal, J., Gómez-Sebastián, S., Bárcena, J., Nuñez Mdel, C., Martínez-Alonso, D., Dudognon, B., Guijarro, E., Escribano, J.M. 2015. Improved production efficiency of virus-Like particles by the baculovirus expression vector system. *PLoS One* 10: e0140039. https://doi.org/10.1371/journal.pone.0140039.

Lu, Y., Liu, Z., Li, Y., Deng, Z., Fang, W., He, F. 2022. The truncated form of flagellin (tFlic) provides the 2dCap subunit vaccine with better immunogenicity and protective effects in mice. *Anim Dis* 2: 11. https://doi.org/10.1186/s44149-022-00043-x.

Lu, Y., Liu, Z.H., Li, Y.X., Xu, H.L., Fang, W.H., He, F. 2022. Targeted delivery of nanovaccine to dendritic cells via DC-binding peptides induces potent antiviral immunity in vivo. *Int J Nanomedicine* 17: 1593–1608. https://doi.org/10.2147/ijn.S357462.

Ma, H., Li, X., Li, J., Zhao, Z., Zhang, H., Hao, G., Chen, H., Qian, P. 2021. Immunization with a recombinant fusion of porcine reproductive and respiratory syndrome virus modified GP5 and ferritin elicits enhanced protective immunity in pigs. *Virology* 552: 112–120. https://doi.org/10.1016/j.virol.2020.10.007.

Machida, K., and H. Imataka. 2015. Production methods for viral particles. *Biotechnol Lett* 37: 753–760. https://doi.org/10.1007/s10529-014-1741-9.

Mai, Z., Cai, M., Hu, X., Li, M., Ji, Y., Li, S., Huang, J., Liang, Q., Ji, C., Yi,H., et al. 2023. Protection efficacy of the H1 and H3 bivalent virus-like particle vaccine against swine influenza virus infection. *Veterinary Microbiology* 280: 109719. https://doi.org/10.1016/j.vetmic.2023.109719.

Mai, Z., Cai, M., Hu, X., Li, M., Ji, Y., Li, S., Huang, J., Liang, Q., Ji, C., Yi, H., et al. 2023. Protection efficacy of the H1 and H3 bivalent virus-like particle vaccine against swine influenza virus infection. *Vet Microbiol* 280: 109719. https://doi.org/10.1016/j.vetmic.2023.109719.

Mallajosyula, J.K., Hiatt, E., Hume, S., Johnson, A., Jeevan, T., Chikwamba, R., Pogue, G.P., Bratcher, B., Haydon, H., Webby, R.J., et al. 2014. Single-dose monomeric HA subunit vaccine generates full protection from influenza challenge. *Hum Vaccin Immunother* 10: 586–595. https://doi.org/10.4161/hv.27567.

Makabenta, J.M.V., Nabawy, A., Li, C.H., Schmidt-Malan, S., Patel, R., Rotello, V.M. 2021. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat Rev Microbiol* 19: 23–36. https://doi.org/10.1038/s41579-020-0420-1.

Manolova, V., Flace, A., Bauer, M., Schwarz, K., Saudan, P., Bachmann, M.F. 2008. Nanoparticles target distinct dendritic cell populations according to their size. *Eur J Immunol* 38: 1404–1413. https://doi.org/10.1002/eji.200737984.

Mansour, A.A., Banik, S., Suresh, R.V., Kaur, H., Malik, M., McCormick, A.A., Bakshi, C.S. 2018. An improved Tobacco mosaic virus (TMV)-conjugated multi-antigen subunit vaccine against respiratory tularemia. *Front Microbiol* 9: 1195. https://doi.org/10.3389/fmicb.2018.01195.

Margine, I., L. Martinez-Gil, Y.Y. Chou, and F. Krammer. 2012. Residual baculovirus in insect cell-derived influenza virus-like particle preparations enhances immunogenicity. *PLoS One* 7. https://doi.org/10.1371/journal.pone.0051559.

Mastico, R.A., S.J. Talbot, and P.G. Stockley. 1993. Multiple presentation of foreign peptides on the surface of an RNA-free spherical bacteriophage capsid. *J Gen Virol* 74 (Pt 4): 541–548. https://doi.org/10.1099/0022-1317-74-4-541.

Mazzoni, A., and D.M. Segal. 2004. Controlling the Toll road to dendritic cell polarization. *J Leukoc Biol* 75: 721–730. https://doi.org/10.1189/jlb.1003482.

McFall-Boegeman, H., and X. Huang. 2022. Mechanisms of cellular and humoral immunity through the lens of VLP-based vaccines. *Expert Rev Vaccines* 21: 453–469. https://doi.org/10.1080/14760584.2022.2029415.

Mizel, S.B., and J.T. Bates. 2010. Flagellin as an adjuvant: cellular mechanisms and potential. *J Immunol* 185: 5677–5682. https://doi.org/10.4049/jimmunol.1002156.

Mo, X., Li, X., Yin, B., Deng, J., Tian, K., Yuan, A. 2019. Structural roles of PCV2 capsid protein N-terminus in PCV2 particle assembly and identification of PCV2 type-specific neutralizing epitope. *PLoS Pathog* 15: e1007562. https://doi.org/10.1371/journal.ppat.1007562.

Molitor, T.W., H.S. Joo, and M.S. Collett. 1983. Porcine parvovirus: virus purification and structural and antigenic properties of virion polypeptides. *J Virol* 45: 842–854. https://doi.org/10.1128/jvi.45.2.842-854.1983.

Munro, H.N., and M.C. Linder. 1978. Ferritin: structure, biosynthesis, and role in iron metabolism. *Physiol Rev* 58: 317–396. https://doi.org/10.1152/physrev.1978.58.2.317.

Nguyen, B. & Tolia, N. H. 2021. Protein-based antigen presentation platforms for nanoparticle vaccines. *NPJ Vaccines* 6. https://doi.org/10.1038/s41541-021-00330-7.

Nooraei, S., Bahrulolum, H., Hoseini, ZS., Katalani, C., Hajizade, A., Easton, A.J., Ahmadian, G. 2021. Virus-like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. *J Nanobiotechnology* 19: 59. https://doi.org/10.1186/s12951-021-00806-7.

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

Oem, J.K., Park, J.H., Lee, K.N., Kim, Y.J., Kye, S.J., Park, J.Y., Song, H.J. 2007. Characterization of recombinant foot-and-mouth disease virus pentamer-like structures expressed by baculovirus and their use as diagnostic antigens in a blocking ELISA. *Vaccine* 25: 4112–4121. https://doi.org/10.1016/j.vaccine.2006.08.046.

Oyewumi, M.O., A. Kumar, and Z. Cui. 2010. Nano-microparticles as immune adjuvants: correlating particle sizes and the resultant immune responses. *Expert Rev Vaccines* 9: 1095–1107. https://doi.org/10.1586/erv.10.89.

Pagot, E., Rigaut, M., Roudaut, D., Panzavolta, L., Jolie, R., Duivon, D. 2017. Field efficacy of Porcilis® PCV M Hyo versus a licensed commercially available vaccine and placebo in the prevention of PRDC in pigs on a French farm: a randomized controlled trial. *Porcine Health Manag* 3: 3. https://doi.org/10.1186/s40813-016-0051-0.

Papapostolou, D., and S. Howorka. 2009. Engineering and exploiting protein assemblies in synthetic biology. *Mol Biosyst* 5: 723–732. https://doi.org/10.1039/b902440a.

Peabody, D.S. 1997. Subunit fusion confers tolerance to peptide insertions in a virus coat protein. *Arch Biochem Biophys* 347: 85–92. https://doi.org/10.1006/abbi.1997.0312.

Pérez-Filgueira, D.M., Resino-Talaván, P., Cubillos, C., Angulo, I., Barderas, M.G., Barcena, J., Escribano, J.M. 2007. Development of a low-cost, insect larvae-derived recombinant subunit vaccine against RHDV. *Virology* 364: 422–430. https://doi.org/10.1016/j.virol.2007.03.016.

Pliasas, V.C., Menne, Z., Aida, V., Yin, J.H., Naskou, M.C., Neasham, P.J., North, J.F., Wilson, D., Horzmann, K.A., Jacob, J., et al. 2022. A novel neuraminidase virus-like particle vaccine offers protection against heterologous H3N2 influenza virus infection in the porcine model. *Front Immunol* 13: 915364. https://doi.org/10.3389/fimmu.2022.915364.

Prevelige, P.E., Jr., D. Thomas, and J. King. 1988. Scaffolding protein regulates the polymerization of P22 coat subunits into icosahedral shells in vitro. *J Mol Biol* 202: 743–757. https://doi.org/10.1016/0022-2836(88)90555-4.

Pulendran, B., and R. Ahmed. 2011. Immunological mechanisms of vaccination. *Nat Immunol* 12: 509–517. https://doi.org/10.1038/ni.2039.

Qu, Z., Li, M., Guo, Y., Liu, Y., Wang, J., Gao, M. 2020. Expression, purification, and characterisation of recombinant ferritin in insect cells using the baculovirus expression system. *Biotechnol Lett* 42: 57–65. https://doi.org/10.1007/s10529-019-02755-6.

Rangel, G., Bárcena, J., Moreno, N., Mata, C.P., Castón, J.R., Alejo, A., Blanco, E. 2021. Chimeric RHDV virus-Like particles displaying Foot-and-Mouth disease virus epitopes elicit neutralizing antibodies and confer partial protection in Pigs. *Vaccines (Basel)* 9. https://doi.org/10.3390/vaccines9050470.

Ren, Z., Zhao, Y., Liu, J., Ji, X., Meng, L., Wang, T., Sun, W., Zhang, K., Sang, X., Yu, Z., et al. 2018. Inclusion of membrane-anchored LTB or flagellin protein in H5N1 virus-like particles enhances protective responses following intramuscular and oral immunization of mice. *Vaccine* 36: 5990–5998. https://doi.org/10.1016/j.vaccine.2018.08.053.

Ridpath, J.F., and W.L. Mengeling. 1988. Uptake of porcine parvovirus into host and nonhost cells suggests host specificity is determined by intracellular factors. *Virus Res* 10: 17–27. https://doi.org/10.1016/0168-1702(88)90054-8.

Rodrigues, M. Q., Alves, P. M. & Roldão, A. 2021. Functionalizing Ferritin Nanoparticles for Vaccine Development. *Pharmaceutics* 13. https://doi.org/10.3390/pharmaceutics13101621.

Ruiz, V., Mignaqui, A.C., Nuñez, M.C., Reytor, E., Escribano, J.M., Wigdorovitz, A. 2014. Comparison of strategies for the production of FMDV empty capsids using the baculovirus vector system. *Mol Biotechnol* 56: 963–970. https://doi.org/10.1007/s12033-014-9775-8.

Ryan, M.D., G.J. Belsham, and A.M. King. 1989. Specificity of enzyme-substrate interactions in foot-and-mouth disease virus polyprotein processing. *Virology* 173: 35–45. https://doi.org/10.1016/0042-6822(89)90219-5.

Sandrock, C., and T. Kelly. 2007. Clinical review: update of avian influenza A infections in humans. *Crit Care* 11: 209. https://doi.org/10.1186/cc5675.

Sharma, J., Shepardson, K., Johns, L.L., Wellham, J., Avera, J., Schwarz, B., Rynda-Apple, A., Douglas, T. 2020. A self-adjuvanted, modular, antigenic VLP for rapid response to influenza virus variability. *ACS Appl Mater Interfaces* 12: 18211–18224. https://doi.org/10.1021/acsami.9b21776.

Shi, X., Yang, K., Song, H., Teng, Z., Zhang, Y., Ding, W., Wang, A., Tan, S., Dong, H., Sun, S., et al. 2022. Development and efficacy evaluation of a novel nano-emulsion adjuvant for a Foot-and-Mouth disease virus-like particles vaccine based on squalane. *Nanomaterials (Basel)* 12. https://doi.org/10.3390/nano12223934.

Silva, T.M., Olinda, R.G., Rodrigues, C.M., Câmara, A.C., Lopes, F.C., Coelho, W.A., Ribeiro, M.F., Freitas, C.I., Teixeira, M.M., Batista, J.S. 2013. Pathogenesis of reproductive failure induced by Trypanosoma vivax in experimentally infected pregnant ewes. *Vet Res* 44: 1. https://doi.org/10.1186/1297-9716-44-1.

Singleton R.L., Sanders C.A., Jones K., Thorington B., Egbo T., Coats M.T., Waffo A.B. 2018. Function of the RNA coliphage Qβ proteins in medical In vitro evolution. *Methods Protoc* 1. https://doi.org/10.3390/mps1020018.

Sit, T.L., M.G. Abouhaidar, and S. Holy. 1989. Nucleotide sequence of papaya mosaic virus RNA. *J Gen Virol* 70 (Pt 9): 2325–2331. https://doi.org/10.1099/0022-1317-70-9-2325.

Skamel, C., S.G. Aller, and Waffo A. Bopda. 2014. In vitro evolution and affinity-maturation with Coliphage qβ display. *PLoS One* 9: e113069. https://doi.org/10.1371/journal.pone.0113069.

Spickler, A.R., and J.A. Roth. 2003. Adjuvants in veterinary vaccines: modes of action and adverse effects. *J Vet Intern Med* 17: 273–281. https://doi.org/10.1111/j.1939-1676.2003.tb02448.x.

Tan, T.K., Rijal, P., Rahikainen, R., Keeble, A.H., Schimanski, L., Hussain, S., Harvey, R., Hayes, J.W.P., Edwards, J.C., McLean, R.K., et al. 2021. A COVID-19 vaccine candidate using SpyCatcher multimerization of the SARS-CoV-2 spike protein receptor-binding domain induces potent neutralising antibody responses. *Nat Commun* 12: 542. https://doi.org/10.1038/s41467-020-20654-7.

Taylor, D.N., Treanor, J.J., Sheldon, E.A., Johnson, C., Umlauf, S., Song, L., Kavita, U., Liu, G., Tussey, L., Ozer, K., et al. 2012. Development of VAX128, a recombinant hemagglutinin (HA) influenza-flagellin fusion vaccine with improved safety and immune response. *Vaccine* 30: 5761–5769. https://doi.org/10.1016/j.vaccine.2012.06.086.

Terhuja, M., P. Saravanan, and R.P. Tamilselvan. 2015. Comparative efficacy of virus like particle (VLP) vaccine of foot-and-mouth-disease virus (FMDV) type O adjuvanted with poly I: C or CpG in guinea pigs. *Biologicals* 43: 437–443. https://doi.org/10.1016/j.biologicals.2015.09.004.

Tiwari, R., Gupta, R.P., Singh, V.K., Kumar, A., Rajneesh, Madhukar, P., Sundar, S., Gautam, V., Kumar, R. 2023. Nanotechnology-based strategies in parasitic disease management: from prevention to diagnosis and treatment. *ACS Omega* 8: 42014–42027. https://doi.org/10.1021/acsomega.3c04587.

Theil, E.C. 2013. Ferritin: the protein nanocage and iron biomineral in health and in disease. *Inorg Chem* 52: 12223–12233. https://doi.org/10.1021/ic400484n.

Turley, C.B., Rupp, R.E., Johnson, C., Taylor, D.N., Wolfson, J., Tussey, L., Kavita, U., Stanberry, L., Shaw, A. 2011. Safety and immunogenicity of a recombinant M2e-flagellin influenza vaccine (STF2.4xM2e) in healthy adults. *Vaccine* 29: 5145–5152. https://doi.org/10.1016/j.vaccine.2011.05.041.

Valícek, L., B. Smíd, L. Rodák, and J. Kudrna. 1990. Electron and immunoelectron microscopy of rabbit haemorrhagic disease virus (RHDV). *Arch Virol* 112: 271–275. https://doi.org/10.1007/bf01323171.

Vartak, A. & Sucheck, S. J. 2016. Recent Advances in Subunit Vaccine Carriers. *Vaccines (Basel)* 4. https://doi.org/10.3390/vaccines4020012

Veerapen, V.P., A.R. van Zyl, A. Wigdorovitz, E.P. Rybicki, and A.E. Meyers. 2018. Novel expression of immunogenic foot-and-mouth disease virus-like particles in Nicotiana benthamiana. *Virus Res* 244: 213–217. https://doi.org/10.1016/j.virusres.2017.11.027.

Wang, B.Z., Quan, F.S., Kang, S.M., Bozja, J., Skountzou, I., Compans, R.W. 2008. Incorporation of membrane-anchored flagellin into influenza virus-like particles enhances the breadth of immune responses. *J Virol* 82: 11813–11823. https://doi.org/10.1128/jvi.01076-08.

Wang, B.Z., Xu, R., Quan, F.S., Kang, S.M., Wang, L., Compans, R.W. 2010. Intranasal immunization with influenza VLPs incorporating membrane-anchored flagellin induces strong heterosubtypic protection. *PLoS One* 5: e13972. https://doi.org/10.1371/journal.pone.0013972.

Wang, C., J. Tu, J. Liu, and I.J. Molineux. 2019. Structural dynamics of bacteriophage P22 infection initiation revealed by cryo-electron tomography. *Nat Microbiol* 4: 1049–1056. https://doi.org/10.1038/s41564-019-0403-z.

Wang, G., Liu, Y., Feng, H., Chen, Y., Yang, S., Wei, Q., Wang, J., Liu, D., Zhang, G. 2018. Immunogenicity evaluation of MS2 phage-mediated chimeric nanoparticle displaying an immunodominant B cell epitope of foot-and-mouth disease virus. *PeerJ* 6: e4823. https://doi.org/10.7717/peerj.4823.

Wang, J., Liu, Y., Chen, Y., Wang, A., Wei, Q., Liu, D., Zhang, G. 2020. Large-scale manufacture of VP2 VLP vaccine against porcine parvovirus in *Escherichia coli* with high-density fermentation. *Appl Microbiol Biotechnol* 104: 3847–3857. https://doi.org/10.1007/s00253-020-10483-5.

Wang, J., Liu, Y., Chen, Y., Zhang, T., Wang, A., Wei, Q., Liu, D., Wang, F., Zhang, G. 2021. Capsid assembly is regulated by amino acid residues asparagine 47 and 48 in the VP2 protein of porcine parvovirus. *Veterinary Microbiology* 253: 108974. https://doi.org/10.1016/j.vetmic.2020.108974.

Wang, N., Zhan, Y., Wang, A., Zhang, L., Khayat, R., Yang, Y. 2016. In silico analysis of surface structure variation of PCV2 capsid resulting from loop mutations of its capsid protein (Cap). *J Gen Virol* 97: 3331–3344. https://doi.org/10.1099/jgv.0.000634.

Wang, X., Xu, F., Liu, J., Gao, B., Liu, Y., Zhai, Y., Ma, J., Zhang, K., Baker, T.S., Schulten, K. 2013. Atomic model of rabbit hemorrhagic disease virus by cryo-electron microscopy and crystallography. *PLoS Pathog* 9: e1003132. https://doi.org/10.1371/journal.ppat.1003132.

Weidenbacher, P.A., Sanyal, M., Friedland, N., Tang, S., Arunachalam, P.S., Hu, M., Kumru, O.S., Morris, M.K., Fontenot ,J., Shirreff, L., et al. 2023. A ferritin-based COVID-19 nanoparticle vaccine that elicits robust, durable, broad-spectrum neutralizing antisera in non-human primates. *Nat Commun* 14: 2149. https://doi.org/10.1038/s41467-023-37417-9.

Wilson, N.S., Duewell, P., Yang, B., Li Y., Marsters, S., Koernig, S., Latz, E., Maraskovsky, E., Morelli, A.B., Schnurr, M., et al. 2014. Inflammasome-dependent and -independent IL-18 production mediates immunity to the ISCOMATRIX adjuvant. *J Immunol* 192: 3259–3268. https://doi.org/10.4049/jimmunol.1302011.

Win, S.J., V.K. Ward, P.R. Dunbar, S.L. Young, and M.A. Baird. 2011. Cross-presentation of epitopes on virus-like particles via the MHC I receptor recycling pathway. *Immunol Cell Biol* 89: 681–688. https://doi.org/10.1038/icb.2010.161.

Xiao, Y., Chen, H.Y., Wang, Y., Yin, B., Lv, C., Mo, X., Yan, H., Xuan, Y., Huang, Y., Pang, W. 2016. Large-scale production of foot-and-mouth disease virus (serotype Asia1) VLP vaccine in *Escherichia coli* and protection potency evaluation in cattle. *BMC Biotechnol* 16: 56. https://doi.org/10.1186/s12896-016-0285-6.

Xiao, Y., Zhang, S., Yan, H., Geng, X., Wang, Y., Xu, X., Wang, M., Zhang, H., Huang, B., Pang, W., et al. 2021. The high immunity induced by the virus-like particles of foot-and-mouth disease virus serotype O. *Front Vet Sci* 8: 633706. https://doi.org/10.3389/fvets.2021.633706.

Yang, D., Chen, L., Duan, J., Yu, Y., Zhou, J., Lu, H. 2021. Investigation of kluyveromyces marxianus as a novel host for large-scale production of porcine parvovirus virus-like particles. *Microb Cell Fact* 20: 24. https://doi.org/10.1186/s12934-021-01514-5.

Yin, S., Sun, S., Yang, S., Shang, Y., Cai, X., Liu, X. 2010. Self-assembly of virus-like particles of porcine circovirus type 2 capsid protein expressed from *Escherichia coli*. *Virol J* 7: 166. https://doi.org/10.1186/1743-422x-7-166.

Yassine, H.M., Boyington, J.C., McTamney, P.M., Wei, C.J., Kanekiyo, M., Kong, W.P., Gallagher, J.R., Wang, L., Zhang, Y., Joyce, M.G., et al. 2015. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat Med* 21: 1065–1070. https://doi.org/10.1038/nm.3927.

Zabel, F., T.M. Kündig, and M.F. Bachmann. 2013. Virus-induced humoral immunity: on how B cell responses are initiated. *Curr Opin Virol* 3: 357–362. https://doi.org/10.1016/j.coviro.2013.05.004.

Zhao, Z., Chen, X., Chen, Y., Li, H., Fang, K., Chen, H., Li, X., Qian, P. 2021. A Self-assembling ferritin nanoplatform for designing classical swine fever vaccine: Elicitation of potent neutralizing antibody. *Vaccines (Basel)* 9. https://doi.org/10.3390/vaccines9010045.

Zhou, H., G. Yao, and S. Cui. 2010. Production and purification of VP2 protein of porcine parvovirus expressed in an insect-baculovirus cell system. *Virology Journal* 7: 366. https://doi.org/10.1186/1743-422X-7-366.

## Publisher's Note