Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Virus-like particles (VLPs) can play important roles in prevention and therapy for infectious diseases and cancer. Here we describe recent advances in rational construction of VLP assemblies, as well as new approaches to enhance long-lasting antibody and CD8+ T cell responses. DNA origami and computational protein design identified optimal spacing of antigens. Chemical biology advances enabled simple and irreversible VLP decoration with protein or polysaccharide antigens. Mosaic VLPs co-displayed antigens to generate cross-reactive antibodies against different influenza strains and coronaviruses. The mode of action of adjuvants inside VLPs was established through knock-outs and repackaging of innate immune stimuli. VLPs themselves showed their power as adjuvants in cancer models. Finally, landmark clinical results were obtained against malaria and the SARS-CoV-2 pandemic.

Address
Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

Corresponding author: Howarth, Mark (mark.howarth@bioch.ox.ac.uk)

Current Opinion in Biotechnology 2022, 73:346–354
This review comes from a themed issue on Nanobiotechnology
Edited by Peter M Tessier and Ravi S Kane
For complete overview of the section, please refer to the article collection, “Nanobiotechnology”
Available online 29th October 2021
https://doi.org/10.1016/j.copbio.2021.09.012
0958-1669/© 2021 Elsevier Ltd. All rights reserved.

Introduction
The study of virus-like particles (VLPs) represents an exciting nexus of nanotechnology, immunology and synthetic biology. VLPs have potential to protect against some of the most important medical challenges, but are also powerful tools for basic understanding of the immune system. Initial work on VLPs hijacked proteins from viral capsids, but it was soon found that similar behavior was achieved with non-viral self-assembling nanocages [1]. Here we focus principally on non-enveloped VLPs and their recent application against infectious disease and cancer. We highlight insights on building different VLP architectures through directed evolution and computational design. The effect of different architectures for induction of the immune response is considered, including the impact of surface glycosylation and interior cargo. There have been striking advances in the use of VLPs to protect against highly variable pathogens, through the use of mosaic VLPs. We also consider how VLPs have been employed against SARS-CoV-2 and momentous advances against malaria.

How different protein architectures affect the immune response
It has long been known that clustering an antigen greatly enhances induction of an immune response [1]. For deeper understanding of the effect of antigen multivalency, an HIV immunogen was injected as a monomer, 4-mer, 8-mer or 60-mer [2]. High multimerization facilitated strong induction of transcription factors interferon regulatory factor 4 and Bcl6 in B cells. High multimerization also led to activation and division of B cells with a broader range of antigen binding affinities. Activating a diverse B cell pool may induce broadly protective immune responses to combat the viral diversity in HIV [2].

An important question for VLP architecture is what is the optimal spacing between antigens (Figure 1) [1]. DNA origami allows excellent spatial control: icosahedral or 6 helical bundle DNA nanoparticles were used for precision display of an HIV antigen. Antigen pairs held rigidly on DNA bundles with 28 nm spacing gave the strongest activation of calcium flux in a B cell line (Figure 2a). Antigen pairs on flexible single-stranded DNA or polyethylene glycol (PEG) chains were less stimulatory [3**].

The caveat at this stage is that these studies used a B-cell receptor with subnanomolar antigen affinity, not typical during immune priming. Also, the study did not look at immune induction in an animal, where DNases and innate immune activation by DNA may cause further complexity.

Enhanced coupling to VLPs
Modular VLP assembly describes when the antigen and scaffold are expressed separately and coupled post-purification. This approach avoids many of the challenges posed by genetic fusion (e.g. misfolding of complex antigens, Figure 1) [4]. Modular coupling can also facilitate coupling of personalized neoantigens for cancer therapy. We previously developed SpyTag/SpyCatcher for plug-and-display VLP coupling. More recently we generated the SpyTag003/SpyCatcher003 system, giving 400-fold faster reaction than the original pair [5]. It was
unclear whether antigens of different symmetries could couple efficiently to the VLPs or would provoke catastrophic aggregation (Figure 2b). We found that a panel of antigens of different sizes and symmetries could effectively couple to the SpyCatcher@03-mi3 platform, including trimeric influenza hemagglutinin or tetrmeric neuraminidase (Figure 2b) [5]. Another emerging conjunction approach is the incorporation of cobalt porphyrin–phospholipid (CoPoP) into a liposome, which facilitates stable interaction with His-tagged proteins (Figure 2c) [6]. The malaria antigen PfS230 has been coupled to liposomes in this manner and elicited antibodies that inhibit parasite transmission [7].

Polysaccharides are important antibody targets for protection against a range of bacterial diseases. Polysaccharides on their own do not efficiently induce helper T cells and so are often chemically conjugated to protein antigens, to generate conjugate vaccines. Efficient display of polysaccharides on VLPs could further enhance vaccine potency. The ‘Glycoli platform’ expresses glycosyltransferases in the Escherichia coli cytoplasm, for generation of protein-linked oligosaccharide chains. Glycans could be enzymatically coupled to protein cages that were fused with an acceptor sequence and co-expressed in the cytoplasm [10]. Synthesis of some bacterial oligosaccharides may be more tractable in the periplasm. Li Zhu’s group expressed PgL in the inner membrane of Shigella flexneri. PgL allowed Shigella oligosaccharides to be coupled onto transfected SpyCatcher bearing a glycosylation sequence in the periplasm. The purified Spy-Catcher-oligosaccharide could then react in vitro with SpyTag-fused AP205 or QB VLPs. Multimerization of the protein-oligosaccharide conjugate greatly increased the antibody response and protection to Shigella infection [11].

**Building new architectures**

In the era of synthetic biology, we should not just rely on nature to furnish us with VLP architectures. The Hilvert group used directed evolution, starting from the lumazine synthase 60-mer that does not bind nucleic acids. Rounds of selection for RNA encapsulation led to an icosaheiral 240-mer packaging its encoding mRNA, with spontaneous appearance of stem-loop packaging motifs in the mRNA [12].

Computational design optimized the assembly of tetrahedral, octahedral, and icosahedral nanoparticles for efficient display of trimeric antigens from HIV, influenza and Respiratory Syncytial Virus (RSV). These scaffolds matched the natural spacing of the antigen termini to maximize antigen stability. In addition, designed scaffolds concealed regions of the antigen, to favor induction of antibodies against the tips of the antigen (Figure 3a) [13].

Assembly of cages within a cage was shown by packing ferritin nanoparticles within the 56 nm diameter bacteriophage P22 capsid [14]. Simultaneous control of the interior and exterior decorations of a VLP was shown by Sebyung Kang’s group. Split intein reaction coupled reporter proteins on the inside of encapsulin and SpyTag/SpyCatcher allowed outside functionalization with a cancer-directed affibody [15].

Metagenomic sequences were mined for new single-stranded RNA bacteriophage coat proteins. From E. coli expression of 110 different sequences, including 11 new classes, 80 led to VLP assembly [16].
How VLPs provide adjuvant effects

Injecting antigen alone does not usually induce a strong immune response — it is important to activate the innate immune system. Vaccines are nearly always given with an adjuvant to increase innate stimulation [17]. Beyond multimeric presentation on VLPs, other internal and external VLP features affect the size and quality of the immune response. VLPs from phage capsids would naturally package nucleic acid from the expressing organism, which stimulates the innate immune system. The coat protein of Qβ phage was expressed in E. coli and disassembled with reducing agent, before different RNAs

Advances in antigen display technologies. (a) DNA origami testing of antigen spacing. 6-helix DNA bundles were prepared with two copies of antigen anchored at different spacing, before testing of B cell activation. Also, DNA icosahedra were constructed with 5 copies of antigen at different spacing. Based on Ref. [39]. (b) Challenges in matching of symmetry between the nanoparticle and antigen. For modular coupling, display of multimeric antigen (in orange) on the VLP (purple) may lead to different densities of display. There is potential for coupling to destabilize the antigen (misfolding shown in magenta) and for bridging of different VLPs by the antigen. Taken from Ref. [5]. (c) Cobalt porphyrin phospholipid (CoPoP) for anchoring His-tagged antigen to the bilayer of liposomes, taken from Ref. [6]. (d) SnoopLigase (dark gray) directs formation of an isopeptide bond (red) from a lysine of SnoopTagJr peptide (blue) to an asparagine of DogTag peptide (yellow), for linking antigen to nanoparticles. Adapted from Ref. [8].
(from *E. coli*, HEK 293T, or yeast tRNA) were packaged inside. Packaging of bacterial RNA in VLPs induced the greatest protection in an influenza model, altering antibody isotypes. This difference depended on innate immune activation by Toll-like receptor (TLR) 7 and not mitochondrial antiviral signaling protein (MAVS) or retinoic acid-inducible gene-I-like receptor (RIG-I) [18**].

TLR agonists can also facilitate anti-cancer immune responses. A small molecule TLR7/8 agonist linked to neoantigen peptides enhanced CD8+ T cell-mediated responses in different mouse tumor models [19]. TLR5 and the Naip5 inflamasome can be stimulated by flagellin, the building block of the bacterial flagellum. Flagellin attachment to a ferritin-based nano-cage enhanced Th1 immune responses [20].

VLPs can be a potent adjuvant even without antigen loading. Human Papillomavirus-16 VLPs, conjugated with a photoactivatable drug, were injected intravenously. Then the tumor was illuminated with near infra-red light to induce necrotic cell death and stimulate long-lasting CD8+ T cell-mediated anti-tumor responses [21]. Different VLP types were compared for their adjuvant effects following intratumoral injection. Cowpea mosaic virus nanoparticles outperformed other plant, phage or mammalian-derived nanoparticles [22]. TLR reporter assays and knock-out mice indicated that Cowpea mosaic virus nanoparticles activate TLR2, 4 and 7 and promote secretion of type I interferons [23]. The value of recruiting other arms of innate immunity was shown by packaging 2',3'-cyclic GMP-AMP (cGAMP) in VLPs, which increased CD4+ and CD8+ T cell responses and enhanced influenza protection [24].
To supercharge anti-tumor efficacy, VLPs have even been equipped with micro-motors. Qβ VLPs were coupled onto 25 μm particles containing magnesium metal, which reacts with water to generate hydrogen bubbles and drive propulsion. These motorized assemblies for treatment of ovarian cancer were injected into the mouse peritoneum, where micro-motors increased the Qβ in the tumor [25].

VLPs certainly can induce good CD8+ T cell responses, but have rarely reached the potency of viral vectors. Recent priming and boosting with different arenavirus vectors induced anti-tumor responses up to 50% of the circulating CD8+ T cell pool [26].

We note that animal studies often focus on inducing the strongest immune response without worrying much about inflammatory side-effects. Clinical translation, at least for infectious disease vaccines, requires a balance of mild side-effects with strong induction of a specific immune response [17].

**Fundamental understanding of the immune system and its interaction with VLPs**

There has been extensive work on how the localization and biological activity of non-biological nanoparticles (e.g. liposomes, quantum dots) depends on the protein corona — the set of proteins that spontaneously associate with a nanoparticle surface exposed to biological fluids. A protein corona also forms on viruses and influences viral infectivity (Figure 3b) [27**], so this surface complexity is an important concept for VLP engineers.

VLP glycosylation can also be an important signature for the immune system (Figure 1). Increasing the glycosylation on Hepatitis B surface antigen (HBsAg) enhanced immunogenicity, when particles were expressed in a human cell-line and ~40% of glycans were oligomannose [28]. Mannose-binding lectin recognition of oligomannose-rich surfaces on VLPs activated deposition of complement C3 and enhanced transport to follicular dendritic cells, promoting a strong immune response [29**]. Fluorescent imaging of ferritin-based nanoparticles indicated the involvement of a dendritic cell population expressing SIGN-R1 (specific intercellular adhesion molecule-3-grabbing nonintegrin-related 1) in VLP delivery to follicular dendritic cells. These particles generated activity in a model for therapy of chronic Hepatitis B Virus [30].

The route of immunization is also important. To understand the correlates for induction of a strong anti-cancer CD8+ T cell response, intravenous VLP vaccination led to a superior response to subcutaneous delivery. Here neoantigen peptides were induced to self-assemble by conjugation to a hydrophobic polymer linked to a TLR7/8 agonist. T-cell specific transcription factor 1 (TCF1) was identified as an important feature of the arising CD8+ T cells, maintaining a stem cell-like state where cells keep self-renewing [31].

**Improved analysis of VLP assembly and composition**

In nanotechnology, understanding what has been built is often as much of a challenge as building new structures. Precise antigen conformation is crucial to eliciting protective antibodies. It is now relatively standard to obtain high resolution structures of protein antigens in isolation. However, when antigens are attached to a nanoparticle, it is much harder to use structural analysis. Advanced solid-state nuclear magnetic resonance (NMR) established that the structure of an influenza hemagglutinin variant was retained, following coupling to Qβ particles via thiol-based cross-linking [32]. AFM now enables real-time visualization at the single molecule level of nucleation and reversibility in capsid formation [33]. Many expect perfection in the assembly of a protein nanoparticle. However, cryo-electron microscopy, along with statistical mechanics modeling, showed that mistakes in VLP assembly are normal and hard to avoid, with >15% particles mis-shapen from Hepatitis B Virus capsid [34]. Nonetheless, assembly of computationally designed two component nanoparticles could be uniform and complete, as assessed by dynamic light scattering and native mass spectrometry [35*].

**How to improve the antigens displayed on VLPs**

Recent innovations provide strategies to tackle a central challenge — how to generate a vaccine against divergent and evolving pathogens. Mosaic nanoparticles have been produced with several different but related antigens displayed on a single particle. B-cell receptors that bind regions conserved between the different antigens on the VLP should receive the strongest stimulus. Thus mosaic particles encourage the production of antibodies binding a wider variety of pathogen strains [36**]. Mosaic nanoparticles displayed a panel of up to eight different sarbecovirus receptor-binding domains (RBDs) (Figure 4a). These VLPs elicited a good response against evolutionarily related viruses not represented on the particle, suggesting potential against new SARS-CoV-2 variants and emergent zoonotic coronaviruses [36**]. Mosaic nanoparticles have also been developed for a more broadly neutralizing influenza response, with different hemagglutinin trimers displayed through genetic fusion [37**] or isopeptide coupling [38].

Antigen has been modified to focus the antibody response, using an innovative strategy called nanopatterning. Zika is an especially difficult vaccine target because non-neutralizing antibodies cause antibody-dependent enhancement (ADE), increasing viral entry into cells. The unnatural amino acid p-azido-L-phenylalanine was introduced at specific sites of Domain III of Zika
VLP assemblies against coronavirus and malaria. (a) Mosaic vaccine for broad coronavirus protection. RBD variants from SARS-CoV-2 alone or other sarbecoviruses were expressed bearing SpyTag, allowing covalent coupling onto the SpyCatcher003-mi3 nanoparticle. Display of different RBDs on the same VLP elicited antibodies with broad reactivity to sarbecoviruses. Taken from Ref. [36*]. (b) Two component SARS-CoV-2 VLP vaccine. Model of the SARS-CoV-2 RBD (light blue) fused to the trimeric IS3-50A component (gray) and the pentameric IS3-50B component (orange). These components are expressed separately and then mixed, to yield a nanoparticle bearing 60 copies of RBD for immunization, taken from Ref. [47]. (c) Malaria VLP vaccine trials results. Incidence of malaria is plotted over time following the third vaccination. Group 1: 5 μg R21/25 μg Matrix M. Group 2: 5 μg R21/50 μg Matrix M. Group 3, rabies control vaccination. The organization of the R21 fusion protein is indicated. Adapted from Ref. [50*].

envelope glycoprotein, allowing click coupling to PEG. When displayed on VLPs, the antibody response was targeted away from the PEGylated region, to favor induction of effective Zika-neutralizing antibodies [39].

Important clinical results from VLPs
The first vaccines approved against SARS-CoV-2 were adenoviral vectors, mRNA vaccines, or inactivated SARS-CoV-2 [40]. A huge range of nanoparticle
strategies have been tested towards a COVID-19 vaccine, since VLPs are effective at inducing neutralizing antibodies and may have advantages in stability and scalability [41**,42,43]. NVX-CoV2373 from Novavax is a synthetic lipid nanoparticle displaying SARS-CoV-2 spike, stabilized in pre-fusion conformation, and has successfully completed Phase III trials [41**]. Another lipid nanoparticle candidate employs CoPoP coupling [44], undergoing Phase I/II [45] (Trial ID: NCT04783311).

Genetic fusions to VLPs have produced promising results against COVID-19. Stabilized spike on ferritin is undergoing Phase I [46] (Trial ID: NCT04784767). RBDb was fused to IS3-50A and mixed with IS3-50B, to assemble into a two-component nanoparticle (Figure 4b) [47] which has entered Phase I/II (Trial ID: NCT04742738). COVID-19 vaccine candidates have been developed using Tag/Catcher ligation, coupling to the protein nanoparticles m13 [42], AP205 [48] (Trial ID: NCT04839146), or HBsAg [43] (Trial ID: ACTRN12620000817943). Also, VLPs displaying spike trimer, budded from intracellular membranes of N. benthamiana, entered Phase II/III (Trial ID: NCT04636697) [49].

Against malaria, rational design of VLPs has led to a major advance. R21 is a genetic fusion between a portion of Plasmodium falciparum Circumsporozoite protein and HBsAg, adjuvanted with Matrix-M (Figure 4c). Phase IIb trials demonstrated good safety and a landmark 77% efficacy after 1 year (Figure 4c) [50**]. A Phase III trial is now testing a larger population over a longer time-period (Trial ID: NCT04704830). The antibody response to the NANP repeats correlated to vaccine efficacy [50**]. R21/MATRIX-M was previously shown to induce substantial CD4+T cells but very low CD8+T cell responses against Circumsporozoite protein [51]. Antibody responses to the HBsAg scaffold (which would provide some protection to Hepatitis B Virus) were very low in response to R21/MATRIX-M [51]. Unlike for viral vectors which need to infect cells, the immune response to antigens displayed on VLPs is not blocked by pre-existing antibodies against the VLP scaffolds [4,52].

Conclusions
Advances now make it routine to array complex antigens on VLPs, which are scalable for clinical development and induce protective antibody responses for infectious disease vaccination. Data are now accumulating to compare the effect of specific VLP features on the immune response in pre-clinical models. Multiple trials against SARS-CoV-2 will help to uncover how VLP features impact human immunity. Persistent infectious diseases and cancer provide the most challenging targets and it is here that testing of new VLP designs may achieve the greatest benefit.

Conflict of interest statement
M.H. is an author on patents or patent applications for Tag/Catcher/Ligase technologies (UK Intellectual Property Office 1002362.0, 1509782.7, 1705750.6, 1706430.4, 1903479.2, 2104999.4.) and a SpyBiotech co-founder, shareholder and consultant. R.A.H. has no conflicts of interest.

Acknowledgement
R.A.H. was funded by the Rhodes Trust.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Bachmann MF, Jennings GT: Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol 2010, 10:787-796.

2. Kato Y, Abbott RK, Freeman BL, Hapt, S, Groeschel B, Silva M, Menis S, Irvine DJ, Schief WR, Crotty S: Multifaceted effects of antigen valency on B cell response composition and differentiation in vivo. Immunity 2020, 3:548-563.e8.

3. Veneziano R, Meyer TJ, Stone MB, Wanhoff E-C, Read BJ: Role of nanoscale antigen organization on B-cell activation probed using DNA origami. Nat Nanotechnol 2020, 15:716-723

This study achieved a new precision in controlling the number and spacing of antigens, presenting the engineered HIV antigen on DNA origami nanoparticles.

4. Brune KD, Howarth M: New routes and opportunities for modular construction of particulate vaccines: stick, click, and glue. Front Immunol 2018, 9:1432.

5. Rahikainen R, Rijal P, Tan TK, Wu H, Andersson AC, Barrett JR, Bowden TA, Draper SJ, Townsend AR, Howarth M: Overcoming symmetry mismatch in vaccine nanossembly through spontaneous amimation. Angew Chem Int Ed Engl 2021, 60:321-330.

6. Federison J, Feugmo CGT, Huang W-C, He X, Miura K, Razi A, Ortega J, Karttunen M, Lovell JF: Experimental and computational observations of immunogenic covalent porphyrin lipid bilayers: nanodomain-enhanced antigen association. Pharmaceutics 2021, 13:38.

7. Huang W-C, Deng B, Selfouh A, Ortega J, Long CA, Suresh RV, He X, Miura K, Lee S-M, Wu Y et al.: Antibody response of a particle-inducing, liposome vaccine adjuvant admixed with a Pfs230 fragment. NPJ Vaccines 2020, 5:23.

8. Andersson A-MC, Buldun CM, Pattinson DJ, Draper SJ, Howarth M: SnoopLigase peptide-peptide conjugation enables modular vaccine assembly. Sci Rep 2019, 9:4625.

9. Keeble AH, Yadav VK, Flera MP, Bauer CC, Chuntharsurat-Bon E, Huang J, Bon RS, Howarth M: DogCatcher allows loop-friendly protein-protein ligation. Cell Chem Biol 2021, 28:451-9456(21):00315-9.

10. Tygat HLP, Lin C, Lavasseur MD, Tomek MB, Rutschmann C, Mock J, Liebscher N, Terasaka N, Azuma Y, Wetter M et al.: Cytoplasmic glycoengineering enables biosynthesis of nanoscale glycoprotein assemblies. Nat Commun 2019, 10:5403

This study introduced the ‘Glycoli platform’, which works in the E. coli cytoplasm to synthesize a defined glycan at a specific motif on recombinant proteins.

11. Li X, Pan C, Sun P, Peng Z, Feng E, Wu J, Wang H, Zhu L: Orthogonal modular biosynthesis of nanoscale conjugate vaccines for vaccination against infection. Nano Res 2021 http://dx.doi.org/10.1007/s12274-021-3713-4
This study established a genetically encodable system for oligosaccharide antigen display on the surface of a VLP, leading to a strong anti-bacterial immune response.

12. Tetter S, Terasaka N, Steinuera A, Bingham RJ, Clark S, Scott AJP, Patel N, Leibundgut M, Wroblewski E, Ban N et al.: Evolution of a virus-like architecture and packaging mechanism in a repurposed bacterial protein. Science 2021, 372:1220-1224

Through directed evolution, a bacterial enzyme incapable of binding nucleic acid became a 240-subunit icosahedral VLP that encapsulated its own encoding mRNA.

13. Ueda G, Antanasievic A, Fallas JA, Sheffler W, Copps J, Ellis D, Hutchinson GB, Moyer A, Yaseinna A, Tsybovsky Y et al.: Tailored design of protein nanoparticle scaffolds for multivalvent presentation of viral glycoprotein antigens. eLife 2020, 9: e57659

This paper introduces a method for computational design of VLP scaffolds, tuned for the display of specific viral glycoproteins and modifying antigen accessibility.

14. Waghwani HK, Uchida M, Fu C-Y, LaFrance B, Sharma J, McCoy K, Douglas T: Virus-like particles (VLPs) as a platform for hierarchical compartmentalization. Biomacromolecules 2020, 21:2060-2072.

15. Choi H, Eom S, Kim H, Bae Y, Jung HS, Kang S: Load and display; engineering encapsulins as a modular nanoflatform for protein-cargo encapsulation and protein-ligand decoration using split intein and SpyTag/SpyCatcher. Biomacromolecules 2021, 22:3028-3039.

16. Leknia I, Kalniņš G, Akopiana I, Bogans J, Šīšovs M, Jansons J, Patrniko J, Tors K: Production and characterization of novel ssRNA bacteriophage virus-like particles from metagenomic sequencing data. J Nanobiotechnol 2019, 17:61.

17. Moser BA, Steinhardt RC, Escalante-Buendia Y, Boltz DA, Barker KM, Cassadby BJ, Rosenberger MG, Yoo S, McGonnigal BG, Esser-Kahn AP: Increased vaccine tolerability and protection via NF-κB modulation. Sci Adv 2020, 6: eaaz2870.

18. Gomes AC, Roesti ES, El-Turabi A, Bachmann MF: Type of RNA packed in VLPs impacts IgG class switching—implications for an influenza vaccine design. Vaccines 2019, 7:47

Repackaging of different RNAs in a VLP shows the importance for the production of different IgG subclasses and the overall immune response.

19. Lynn GM, Sedlick C, Baharom F, Zhu Y, Ramirez-Valdez RA, Coble VL, Tobin K, Nichols SR, Itzkovitz Y, Zaid N et al.: Peptide–TLLR-7/8a conjugate vaccines chemically programmed for nanoparticle self-assembly enhance CD8 T-cell immunity to tumor antigens. Nat Biotechnol 2020, 38:320-332.

20. Lee EB, Jeon H-M, Kim C-U, Park SM, Cho G, Kim H-J, Kim Y, Kim D-J, Lim E, Lee H et al.: Attachment of flagellin enhances the immunostimulatory activity of a hemagglutinin-ferritin nano-cage. Nanomedicine 2019, 17:233-235.

21. Kines RC, Thompson CD, Spring S, Li Z, de los Pinos E, Monks S, Schiller JT: Virus-like particle-drug conjugates induce protective, long-lasting adaptive antitumor immunity in the absence of specifically targeted tumor antigens. Cancer Immunol Res 2021, 9:693-706.

22. Shukla S, Wang C, Beiss V, Cai H, Washington T, Murray AA, Gong X, Zhao Z, Masarapu H, Zlotnick A et al.: The unique potency of Cowpea mosaic virus (CPMV) in situ cancer vaccine. Biomater Sci 2020, 8:5489-5503.

23. Mao C, Beiss V, Fields J, Steinmetz NF, Fiering S: Cowpea mosaic virus stimulates antitumor immunity through recognition by multiple MLYDSB-dependent toll-like receptors. Biomaterials 2021, 275:120914.

24. Chauveau L, Bridgeham A, Tan TK, Beveridge R, Frost JN, Rijal P, Pedroza-Pacheco I, Partridge T, Gilbert-Jaramillo J, Knight ML et al.: Inclusion of cGAMP within virus-like particle vaccines enhances their immunogenicity. EMBO Rep 2021, 22:e52447.

25. Wang C, Fernández de Ávila BE, Mundaca-Uribe R, Lopez-Ramirez MA, Ramirez-Herrera DE, Shukla S, Steinmetz NF, Wang J: Active delivery of VLPs promotes anti-tumor activity in a mouse ovarian tumor model. Small 2020, 16:e1907150.

This study describes the production of magnesium-based micromotors loaded with Qβ VLPs, where autonomous propulsion enhanced their immunostimulatory effects.

26. Bonilla WV, Kirchhammer N, Marx A-F, Kallert SM, Krzyzanika MA, Lu M, Darbe S, Schmidt S, Raguz J, Berka U et al.: Heterologous arenavirus vector prime-boost overrules self-tolerance for efficient tumor-specific CD8 T cell attack. Cell Rep Med 2021, 2:100209.

27. Ezzat K, Pernemalm M, Pålsson S, Roberts TC, Järver P, Dentatska A, Bestas B, Sobkowiak MJ, Leváén B, Skóld M et al.: The viral protein corona directs viral pathogenesis and amyloid aggregation. Nat Commun 2019, 10:2331

This study demonstrated that viruses form a protein corona dependent on the nature of both virus and the biological media, influencing immune cell stimulation and viral infectivity.

28. Joe CCD, Chatterjee S, Lovrecz G, Adams TE, Thaysen-Andersen M, Walsh R, Locarnini SA, Smooker P, Netter HJ: Glycoengineered hepatitis B virus-like particles with enhanced immunogenicity. Vaccine 2020, 38:3892-3901.

29. Tokatlian T, Read BJ, Jones CA, Kulp DW, Menis S, Chang JYH, Steinchen JM, Kumari S, Allen JD, Dane EL et al.: Innate immune recognition of glycans targets HIV nanoparticle immunogens to germinal centers. Science 2019, 363:649-654

This study illuminates how immunogen glycosylation engages the innate immune system and affects lymph node responses.

30. Wang W, Zhou X, Bian Y, Wang S, Chai Q, Guo Z, Wang Z, Zhu P, Peng H, Yan X et al.: Dual-targeting nanoparticle vaccine elicits a tumour-specific antibody response against chronic hepatitis B. Nat Nanotechnol 2020, 15:406-416.

31. Baharom F, Ramirez-Valdez RA, Tobin KKS, Yamane H, Dutertre C-A, Khallinezhad A, Reynoso GV, Coble VL, Lynn GM, Mulé JM et al.: Intravenous nanoparticle vaccination generates stem-like TC1+ neontigen-specific CD8+ T cells. Nat Immunol 2021, 22:41-52.

32. Jaudzems K, Kirsteina T, Schubels T, Casano G, Ourai O, Bogans J, Kazaks A, Tars K, Lesage A, Pintacuda G: Structural analysis of an antigen chemically coupled on virus-like particles in vaccine formulation. Angew Chem Int Ed 2021, 60:12847-12851.

33. Valbuena A, Maity S, Mateu MG, Roos WH: Visualization of single molecules building a viral capsid protein lattice through stochastic pathways. ACS Nano 2020, 14:8724-8734.

34. Spiriti J, Conway JF, Zuckerman DM: Should virus capsids assemble perfectly? Theory and observation of defects. Biophys J 2020, 119:1781-1790.

35. Waragacki AJ, Wörner TP, van de Waterbeemd M, Ellis D, Heck AJR, King NP: Complete and cooperative in vitro assembly of computationally designed self-assembling protein nanomaterials. Nat Commun 2021, 12:883

This study provided deep investigation of the assembly of computationally designed two-component nanoparticles, achieving complete VLP formation in a process that is normally error-prone.

36. Cohen AA, Gnanapragasam PNP, Lee YE, Hoffman PR, Ou S, Kakatuni LM, Keefe JR, Wu H-J, Howarth M, West AP et al.: Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. Science 2021, 371:735-741

Mosaic display of multiple coronavirus RBDs on a single nanoparticle induced cross-reactive antibodies, with potential for protective immunization across multiple coronaviruses.

37. Boyoglu-Barmum S, Ellis D, Gillespie RA, Hutchinson GB, Park Y-J, Moin SM, Acton OJ, Ravichandran R, Murphy M, Petitie D et al.: Quadrivalent influenza nanoparticle vaccines induce broad protection. Nature 2021, 592:623-628

Mosaic display of different hemagglutinin trimers on a computationally designed nanoparticle elicited broadly protective antibodies to move beyond seasonal influenza vaccination.

38. Cohen AA, Yang Z, Gnanapragasam PNP, Ou S, Dam K-MA, Wang H, Bjorkman PJ: Construction, characterization, and immunization of nanoparticles that display a diverse array of influenza HA trimers. PLoS One 2021, 16:e0247963.

39. Castro A, Carreño JM, Duehr J, Krammer F, Kane RS: Refocusing the immune response to selected epitopes on a Zika virus
Cobalt-porphyrin-phospholipid coupling allowed facile display of the SARS-CoV-2 RBD on a liposome for immunization.

45. EuBiologics Co., Ltd: A Phase 1/2 Dose-exploration, Randomized, Observer-blind, Placebo-controlled Study to Determine Safety, Tolerance and Immunogenicity of EuCorVac-19, a Recombinant Protein Vaccine, for the Prevention of COVID-19 in Healthy Adults. 2021clinicaltrials.gov.

46. Joyce MG, Chen W-H, Sankhala RS, Hajduczki A, Thomas PV, Choe M, Chang W, Peterson CE, Martinez E, Morrison EB et al.: SARS-CoV-2 ferritin nanoparticle vaccines elicit broad SARS coronavirus immunogenicity. bioRxiv 2021 http://dx.doi.org/10.1101/2021.05.09.443331.

47. Walls AC, Fiala B, Schäfer A, Wrenn S, Pham MN, Murphy M, Tse LV, Shehata L, O'Connor MA, Chen C et al.: Elicitation of potent neutralizing antibody responses by designed protein nanoparticle vaccines for SARS-CoV-2. Cell 2020, 183:1367-1382.e17.

48. Fougeroux C, Goksöyr L, Idorn M, Soroka V, Myeni SK, Dagil R, Jantzek CM, Sogaard M, Aves K-L, Horsted EW et al.: Capsid-like particles decorated with the SARS-CoV-2 receptor-binding domain elicit strong virus neutralization activity. Nat Commun 2021, 12:324.

49. Gobeil P, Pillet S, Séquin A, Boulay I, Mahmood A, Vinya DC, Charland N, Boutet P, Roman F, Most RVD et al.: Interim report of a phase 2 randomized trial of a plant-produced virus-like particle vaccine for Covid-19 in healthy adults aged 18-64 and older adults aged 65 and older. medRxiv 2021 http://dx.doi.org/10.1101/2021.05.14.21257248.

50. Datoo MS, Natama MH, Somé A, Traoré O, Rouamba T, Bellamy D, Yameogo P, Valia D, Tegneri M, Ouedraogo F et al.: Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. Lancet 2021, 397:1809-1818

A phase II clinical trial for a VLP vaccine showed strong protection against malaria.

51. Collins KA, Snaith R, Cottingham MG, Gilbert SC, Hill AVS: Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. Sci Rep 2017, 19:46621.

52. Marini A, Zhou Y, Li Y, Taylor IJ, Leneghan DB, Jin J, Zaric M, Meikael D, Long CA, Miura K et al.: A universal plug-and-display vaccine carrier based on HBsAg VLP to maximize effective antibody response. Front Immunol 2019, 10:2931.