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Harnessing physicochemical properties of virus capsids for designing enzyme confined nanocompartments

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Abstract

Viruses have drawn significant scientific interest from a wide variety of disciplines beyond virology because of their elegant architectures and delicately balanced activities. A virus-like particle (VLP), a noninfectious protein cage derived from viruses or other cage-forming proteins, has been exploited as a nano-scale platform for bioinspired engineering and synthetic manipulation with a range of applications. Encapsulation of functional proteins, especially enzymes, is an emerging use of VLPs that is promising not only for developing efficient and robust catalytic materials, but also for providing fundamental insights into the effects of enzyme compartmentalization commonly observed in cells. This review highlights recent advances in employing VLPs as a container for confining enzymes. To accomplish larger and more controlled enzyme loading, various different enzyme encapsulation strategies have been developed; many of these strategies are inspired from assembly and genome loading mechanisms of viral capsids. Characterization of VLPs' physicochemical properties, such as porosity, could lead to rational manipulation and a better understanding of the catalytic behavior of the materials.

Introduction

Virus capsids are typically constructed from multiple copies of a few structural proteins via a self-assembly process. The capsids as a system are delicately balanced to enable multiple and potentially conflicting functions including encapsulating viral genomes, protecting them from the external environments, delivering their genomes to the host cells, and releasing them for amplification. The sophisticated mechanism of capsid assembly and their delicate functionalities have garnered interest among scientists beyond virology and cell biology [1]. Scientists are repurposing the functionalities of capsids to design gene vectors and vaccines, and are also expanding their use as nanoplatforms for synthetic manipulation [2•,3-6]. In this regard, they have exploited a virus-like particle (VLP), which is a noninfectious protein cage derived from viruses or other cage-forming proteins, to encapsulate non-nucleic acid cargos such as proteins, polymers, and inorganic nanoparticles, for a range of applications from medicine to nanotechnology.

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Among the many cargo molecules that have been encapsulated inside of VLPs, the confinement of enzymes has recently drawn particular interest [7,8]. The interior of a viral capsid is a unique environment, spatially separated from the bulk exterior environment by the capsid shell, in which an enzyme can be sequestered to create well-defined nanoreactors that are capable of catalyzing a wide range of chemical transformations, conceptually resembling bacterial microcompartments [9,10]. Enzyme compartmentalization into a confined environment could confer the ability to control fluxes of substrates, intermediates, and products across compartments allowing for control over enzymatic reaction efficiency [7,11••,12,13•]. VLPs are typically comprised of a single type of coat protein (CP) and could serve as a simplified model system to study the effect of enzyme confinement in a CP microcompartment in contrast to bacterial microcompartments whose shells are generally composed of multiple homologous proteins [11••,12,13•,14••,15•,16,17]. Additionally, virus capsids serve as a shield for cargos from protease digestion, thermal degradation, and other external disturbances [18-20]. Furthermore, enzyme encapsulated VLPs have been used as building blocks for construction of catalytically active protein arrays [21-24]. Altogether, encapsulation of enzymes within VLPs is a promising approach towards creating efficient catalytic materials and aiding in the understanding of the effects of enzyme confinement and crowding on their activities.

Viruses have evolved a variety of different mechanisms for their genome encapsulation and capsid assembly. Scientists have harnessed these mechanisms to explore encapsulation of enzymes and other cargos inside of VLPs. The first part of this review gives an account of several different approaches developed to encapsulate enzymes and other proteinaceous cargos. The second part of this review discusses some recent studies on physicochemical properties of virus capsids relevant to designing VLP-based nanoreactors. Characterization and rational manipulation of physicochemical properties of VLPs, such as molecular diffusion across the capsid shell, are critical components for understanding the catalytic behavior of enzymes confined inside of VLPs. Rational manipulation of these properties could lead to designing more efficient nanoreactors.

Passive encapsulation

The CP of many single-stranded RNA viruses can assemble into capsids *in vitro* even in the absence of their genomic RNA [25,26]. For instance, cowpea chlorotic mottle virus (CCMV) can be disassembled into subunit dimers at a near neutral pH (pH 7.5), and upon lowering the pH (pH 5.0), it subsequently assembles into a T = 3 capsid [27,28]. One of the initial demonstrations of enzyme encapsulation inside of a VLP exploited this *in vitro* assembly capability of a CCMV capsid. Cornelissen *et al.* reconstituted CCMV subunits into a capsid in the presence of a horseradish peroxidase (HRP) enzyme and achieved encapsulation of a single HRP per CCMV VLP (Figure 1a) [16]. While this encapsulation is stochastic in nature and not particularly efficient, this study demonstrates an important concept that paves the way for further studies. The enzyme entrapped within the capsid was still highly active, indicating that the capsid shell is permeable for small molecule substrates and products, whereas the enzyme itself remains trapped inside of the VLP (Figure 1b).

Electrostatic interaction mediated encapsulation

Although many single-stranded RNA viruses can assemble into capsids *in vitro*, even in the absence of their genomic RNA, capsid formation is typically more efficient in the presence of their genomic RNA [26,29•]. Strong electrostatic interactions between the CP and the RNA provide the initial driving force for capsid assembly and genomic cargo encapsulation [30]. Similarly, by introducing a complementary charge interaction between cargo molecules and the CPs of capsids, the loading efficiency of cargo molecules into capsids can be enhanced [31,32•,33-35]. For example, Hilvert *et al.* genetically engineered a mutant of the lumazine synthase protein cage derived from *Aquifex aeolicus*, which has a negatively charged internal surface (AaLS-neg), and a green fluorescence protein tagged with a positively charged decaarginine tag (GFP-R10). Co-expression of AaLS-neg and GFP-R10 in *Escherichia coli* led to the formation of an AaLS-neg cage loaded with GFP-R10 (Figure 2a) [34]. Electrostatic interactions between CPs and cargos have also been applied to encapsulate non-proteinaceous cargos, such as pre-formed inorganic nanoparticles inside of VLPs derived from several different viruses [36-38].

RNA packing signal mediated encapsulation

Some single-stranded RNA viruses such as MS2 and Qβ are known to use specific sequences in their genomic RNA, which have stem-loop structures, to bind to the coat proteins and thereby direct both capsid assembly and RNA encapsulation [26,39,40]. For example, the genomic RNA of MS2 has a 19-nucleotide translational repressor (TR) fragment, which is known to serve as a packaging signal due to specific interactions with the CP [26,39]. Stockley *et al.* pioneered a method to exploit this assembly mechanism to encapsulate a cargo protein inside of an MS2 capsid [41]. They covalently attached a protein, ricin A chain (RAC), to the TR oligonucleotides and successfully directed RAC to the inside of the capsid while the CP assembled into a capsid *in vitro*.

Finn *et al.* employed a similar strategy to achieve *in vivo* encapsulation of enzyme cargos inside of a Qβ capsid while co-expressing these components in *E. coli* (Figure 2b) [18,42,43]. A chimeric single-stranded RNA was used as a linker to mediate the encapsulation of the cargo proteins. This RNA linker is composed of three segments, a hairpin (hp) that associates with interior-facing residues of the Qβ CP, an RNA aptamer sequence (α-Rev apt) that binds to an arginine-rich peptide (Rev-tag), and an mRNA for the Qβ CP that additionally serves as the spacer between the other two segments. A cargo protein was genetically fused with the Rev-tag so that the α-Rev apt segment of the RNA linker interacts with the cargo protein, whereas the hp segment associates with the CP and directs the cargo into the Qβ capsid. Thus, the transcribed mRNA can simultaneously serve as the CP transcript and as the cargo encapsulation mediator. Finn *et al.* demonstrated that co-expression of the CP/RNA mediator with the tagged protein cargo is a versatile approach to encapsulate a number of enzyme cargos (10–36 cargos per capsid) within the Qβ capsid [42].

Scaffolding protein (SP) mediated encapsulation

Large double-stranded RNA or DNA viruses, including some bacteriophage, typically require scaffolding proteins (SP) or an inner core to template proper capsid assembly [44••,45,46]. Bacteriophage P22 is one of the most well-studied model viruses whose capsid is formed through SP mediated assembly of CPs [47]. VLPs derived from P22, which is assembled from 420 copies of CPs into a T = 7 icosahedral capsid with the aid of approximately 100–300 copies of SPs, can be produced by a heterologous expression system in *E. coli*. Douglas *et al*. have harnessed the SP mediated capsid assembly process to direct the encapsulation of enzymes inside of P22 VLPs in a highly efficient manner [17,20,21,48-52]. The 303-residue-long SP can be severely truncated to an essential helix-turn-helix scaffolding domain located near the C-terminus without losing the ability to direct the assembly of CPs into the capsid [53,54]. Genetic fusion of cargo proteins to either the N-terminus or C-terminus of the truncated SP has been demonstrated to be a powerful and versatile approach for encapsulating a wide variety of functional proteins including enzymes [51,55]. In one of the initial studies of this approach, Douglas *et al*. demonstrated encapsulation of approximately 250 alcohol dehydrogenase (AdhD) per P22 capsid (Figure 2c) [49]. Based on the volume of the P22 interior cavity, this gives a local enzyme concentration of 7.16 mM or ~300 mg/mL, which is comparable to the reported value of macromolecular concentration inside cells [56]. Dense packing of enzymes within the cavities of the capsid achieved by this approach has allowed them to study enzyme behaviors in a crowded and confined environment such as enzymatic kinetics and protein stability.

Significant work has been done to establish the assembly of P22 VLPs *in vitro* from individually purified CPs and SPs [57,58]. Douglas *et al*. have adopted the *in vitro* assembly of P22 for encapsulating multiple populations of cargo proteins inside of the same capsid [14••,59,60]. This *in vitro* assembly approach allows for control over the packaging stoichiometry of multiple cargos which is difficult to attain by the *in vivo* encapsulation approaches. For example, co-encapsulation of two different cargos, SP tagged AdhD (AdhD-SP) and wild-type SP (wtSP), with controlled stoichiometry, has been achieved by modulating the input ratio of these two components (Figure 3a, b) [14••]. The turnover number of the AdhD enzyme decreased while increasing the number of co-encapsulated wtSP (Figure 3c). This result suggests that co-encapsulated wtSP can act as a molecular crowding agent to AdhD resulting in a reduction of activity of AdhD. This approach also has the capability to control the packing density of cargo enzymes, because wtSP can be selectively removed from the co-assembled capsids using 0.5 M guanidine hydrochloride [61], providing more space for other cargos in a confined VLP environment (Figure 3a). Selective removal of wtSP from the P22 VLP resulted in a similar turnover of AdhD regardless of the packing density of AdhD, suggesting that the encapsulated AdhD does not experience self-crowding even at a high packing density. The *in vitro* assembly approach would allow for further investigation of the effect of confinement and crowding on the activities of enzymes encapsulated inside of a microcompartment.

Other non-covalent interactions mediated encapsulation

Other types of specific interactions, which are not involved in viral genome packing, have also been adopted to direct cargo into a capsid [62-64]. For example, a complementary pair of coiled-coil sequences was used to achieve higher cargo loading [62]. Cornelissen *et al.* demonstrated that a positively charged K-coil peptide was genetically fused to the CCMV CP and the complementary E-coil peptide was introduced to lipase B (PalB) from *Pseudozyma antarctica*. Introduction of the coiled-coil peptide allowed non-covalent interaction between the cargo and the CP before pH-mediated capsid assembly, resulting in encapsulation of one to four PalB per capsid.

A new class of cage-like proteins called encapsulins has recently been identified in bacteria and archaea [15•,65-67]. Encapsulins exhibit an icosahedral symmetry and three different architectures corresponding to triangulation numbers of one, three, and four, have been found to date [68••]. Interestingly, all encapsulins have an HK97-like fold despite sharing little sequence homology. As the name suggests, encapsulins involve packing cargo proteins within their interior cavity. For example, the encapsulin in *Thermotoga maritima* encapsulates ferritin-like proteins, which possess ferroxidase activity, and is likely to mitigate oxidative stress [66,69]. The protein encapsulation is typically guided by a short targeting peptide (TP) which is located at the C-terminus of a cargo protein and interacts with the interior side of the encapsulin protomer. This specific interaction between the TP and encapsulin has been utilized to direct encapsulation of heterologous proteins as cargos [15•,70,71]. For example, Savage *et al.* demonstrated that a non-native cargo protein can be loaded into the encapsulin from *T. maritima* both *in vivo* and *in vitro* by the fusion of the 30 amino acids TP or the 15 amino acids truncated TP to the cargo protein [15•].

Covalent conjugation of cargo to VLPs

Covalent conjugation of a cargo protein to a CP is another approach to encapsulate the cargo inside of VLPs. The covalent conjugation can be achieved not only through genetic fusion [72-74] but also through post-translational protein ligation methods such as Sortase A [75•,76], SpyTag/SpyCatcher [77•], and split intein [78•]. In a recent study, Giessen and Silver demonstrated *in vivo* encapsulation of various enzymes in MS2 capsid by using the SpyTag/SpyCatcher mediated protein fusion technique (Figure 2d) [77•]. The SpyTag sequence (16 aa) was inserted into the CP of MS2 (MS2-Spy), which is still able to assemble into a capsid. Meanwhile, enzyme cargos were genetically fused with the SpyCatcher (116 aa) that recognizes the SpyTag and forms an isopeptide bond with it. Co-expression of MS2-Spy and a SpyCatcher fused enzyme led to covalent ligation of enzyme-SpyCatcher with the interior surface of the capsid *in vivo*.

Addressing physicochemical properties of enzyme encapsulated VLPs

The physicochemical properties of VLPs, such as possible diffusion limitations across the capsid shell, electrostatic potential around the capsid pores, and the pH inside the capsid are critical for understanding and predicting catalytic behavior for enzymes encapsulated in VLPs. However, many aspects of these properties have not yet been well characterized.

For example, although high resolution structures obtained from X-ray crystallography or cryo-electron microscopy are available for many viral capsids, this structural information may not accurately predict the permeability of molecules across the capsid shell because of the dynamic nature of viral capsids in solution. To address this issue, Douglas *et al.* recently developed a methodology for studying diffusion across the protein shells of VLPs (Figure 4a) [11••]. In this study, AdhD encapsulating P22 VLPs with two different morphologies, procapsid form and expanded form, were used as a model system. In both the infectious phage and the VLP, the procapsid is the initial structure with 56 nm in diameter formed via SP templated assembly [79]. The infectious phage undergoes a structural transformation by DNA packing, resulting in expansion of the procapsid to a more angular particle of 62 nm in diameter [80]. This morphogenesis can be recapitulated in the P22 VLPs with either heat [81,82] or chemical [83] treatment and the resulting particles are referred to as the expanded form. To investigate diffusion across procapsid and expanded forms of P22 VLPs, Douglas *et al.* conjugated NADH, a cofactor for the AdhD enzyme, to six different sized PAMAM dendrimers ranging from 1.3 to 7.8 nm in diameter. These conjugated cofactors were used as a molecular probe to investigate their diffusion across the porous protein shell layer of P22 VLPs by monitoring NADH oxidation to NAD^+ catalyzed by AdhD. The pore size of the procapsid and expanded forms were revealed to be 4.4 nm and 2.7 nm, respectively. This study provides a generalizable method to analyze diffusion limitation across a viral capsid and many other porous nanoparticles.

Several recent studies have illustrated that electrostatic interactions between substrates and protein cages have a significant effect on the transportation of substrates and products across protein cages and hence the overall catalytic reaction [11••,12,13•,84]. For example, Tullman-Ercek *et al.* demonstrated that for alkaline phosphatase encapsulated inside of MS2 capsid, the k_{cat} and K_M could be varied by modulating the surface charge around pores at the fivefold and quasi-sixfold axes of the capsid through point mutation of the CP. As enzyme activity itself should not be altered by the mutation to the CP, the change in the catalytic parameters is likely due to changes in substrate and product fluxes through the pores. This result supports the hypothesis that the shell layer of protein compartments could modulate the transport of small molecules into and out of the compartments and thus influence catalytic reactions.

The pH inside of a viral capsid could be significantly different from that of the bulk solution outside of the capsid because of the high concentration of charged residues exposed on the interior cavity surface [74,85,86]. As pH is a critical parameter in the role of enzymatic reactions, it is important to assess the pH condition inside of a virus capsid. Cornelissen *et al.* have addressed this issue by encapsulating a negatively charged pH-responsive polymer probe within the CCMV VLP [85]. The study indicated that the pH inside the CCMV VLP is around 0.5 pH units more acidic than the outside environment.

It is well studied that genetic materials affect the mechanical stability of viral shells [87]. In some cases, the viral genome structurally reinforces the shell through structural interactions with the shell [88], whereas in other cases, it destabilizes the shell by increasing internal pressure [89]. Similarly, heterologous cargos encapsulated inside of VLPs could affect the stability of the capsids. Caston and Pablo *et al.* investigated mechanical properties of

proteinaceous cargo-loaded P22 with two different morphologies, procapsid and expanded forms, by using cryo-electron microscopy and atomic force microscopy (Figure 4b) [90]. Their study indicated that the presence of cargos stiffens the P22 capsid, but the underlying mechanism could be different between the two capsid morphologies. As discussed in the previous section, in the case of the procapsid form, the cargo is constrained to the interior of the capsid lumen via the C-terminus of SP. Therefore, the procapsid is structurally reinforced by a cargo-SP fusion protein (Figure 4b left). On the other hand, in the case of the expanded form, cargo-shell interactions are lost, thus the cargo-SP is detached from the shell. The cargos are still trapped but become free inside the capsid (Figure 4b right). The different concentration of cargo proteins between the inside and the outside of the shell creates osmotic pressure and this pressurization increases the rigidity of the capsid. The estimated osmotic pressure could be up to 3 MPa, which is comparable to the DNA induced pressurization in natural viruses. A better understanding of the stabilization mechanism of cargo-loaded VLPs could lead to the development of a method to optimize the mechanical properties of VLP-based materials.

Conclusion

This review followed recent studies regarding the encapsulation of enzymes within VLPs from a simple passive stochastic approach to actively guided approaches. Studies on virus assembly have provided scientists with inspiration and means to develop more efficient and better controlled enzyme encapsulation methods. Sequestration of enzymes and other proteins within microcompartments has been observed in many biological systems and is believed to confer enhancement of specific metabolic processes. Enzyme encapsulation within VLPs could be used as a model system for better understanding the effects of enzyme confinement on its activity as well as designing more efficient catalytic materials.

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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. Douglas T, Young M: Viruses: making friends with old foes. Science 2006, 312:873–875. [PubMed: 16690856]

2•. Aumiller WM, Uchida M, Douglas T: Protein cage assembly across multiple length scales. Chem Soc Rev 2018, 47:3433–3469 [PubMed: 29497713] This article not only provides an overview of how virus capsids have been exploited as a platform for developing functional nanomaterials, but also covers recent advances in how they have been utilized as building blocks to fabricate three-dimensional protein array materials.

3. Wen AM, Steinmetz NF: Design of virus-based nanomaterials for medicine, biotechnology, and energy. Chem Soc Rev 2016, 45:4074–4126. [PubMed: 27152673]

4. Schwarz B, Uchida M, Douglas T: Biomedical and catalytic opportunities of virus-like particles in nanotechnology. In Advances in Virus Research, , vol 97. Edited by Kielian M, Mettenleiter TC, Roossinck MJ. 2017:1–60. [PubMed: 28057256]

5. Rother M, Nussbaumer MG, Renggli K, Bruns N: Protein cages and synthetic polymers: a fruitful symbiosis for drug delivery applications, bionanotechnology and materials science. Chem Soc Rev 2016, 45:1–37.

6. Mateu MG: Assembly, engineering and applications of virus-based protein nanoparticles. Adv Exp Med Biol 2016, 940:83–120. [PubMed: 27677510]

7. Kuchler A, Yoshimoto M, Luginbuhl S, Mavelli F, Walde P:Enzymatic reactions in confined environments. Nat Nanotechnol 2016, 11:409–420. [PubMed: 27146955]

8. Chakraborti S, Lin T-Y, Glatt S, Heddle JG: Enzyme encapsulation by protein cages. RSC Adv 2020, 10:13293–13301.

9. Kerfeld CA, Heinhorst S, Cannon GC: Bacterial microcompartments. Annu Rev Microbiol 2010, 64:391–408. [PubMed: 20825353]

10. Yeates TO, Thompson MC, Bobik TA: The protein shells of bacterial microcompartment organelles. Curr Opin Struct Biol 2011, 21:223–231. [PubMed: 21315581]

11••. Selivanovitch E, LaFrance B, Douglas T: Molecular exclusion limits for diffusion across a porous capsid. Nat Commun 2021, 12:2903 [PubMed: 34006828] This article develops a method to investigate the molecular porosity and permeability of a model capsid and determines the diffusion limitations across the capsid shell in terms of size and charge.

12. Azuma Y, Bader DLV, Hilvert D: Substrate sorting by a supercharged nanoreactor. J Am Chem Soc 2018, 140:860–863. [PubMed: 29278496]

13•. Glasgow JE, Asensio MA, Jakobson CM, Francis MB, Tullman-Ercek D: Influence of electrostatics on small molecule flux through a protein nanoreactor. ACS Synth Biol 2015, 4:1011–1019 [PubMed: 25893987] By introducing point mutations around pores of the MS2 capsid, this article demonstrates that a protein compartment could modulate transportation of small molecules across the shell of the compartment.

14••. Sharma J, Douglas T: Tuning the catalytic properties of P22 nanoreactors through compositional control. Nanoscale 2020, 12:336–346 [PubMed: 31825057] This article demonstrates control over the composition and packing density of enzymes encapsulated in the P22 VLP by using an *in vivo* assembly approach and exploits this to interrogate the effect of molecular crowding on the functional properties of enzymes confined in an enclosed system.

15•. Cassidy-Amstutz C, Oltrogge L, Going CC, Lee A, Teng P, Quintanilla D, East-Seletsky A, Williams ER, Savage DF: Identification of a minimal peptide tag for in vivo and in vitro loading of encapsulin. Biochemistry 2016, 55:3461–3468 [PubMed: 27224728] This article demonstrates encapsulation of foreign protein cargos inside of an encapsulin cage via both *in vivo* and *in vitro* assembly approaches.

16. Comellas-Aragonès M, Engelkamp H, Claessen VI, Sommerdijk NAJM, Rowan AE, Christianen PCM, Maan JC, Verduin BJM, Cornelissen JJLM, Nolte RJM: A virus-based single-enzyme nanoreactor. Nature Nanotechnol 2007, 2:635–639. [PubMed: 18654389]

17. Patterson DP, Schwarz B, Waters RS, Gedeon T, Douglas T: Encapsulation of an enzyme cascade within the bacteriophage P22 virus-like particle. ACS Chem Biol 2014, 9:359–365. [PubMed: 24308573]

18. Fiedler JD, Brown SD, Lau JL, Finn MG: RNA-directed packaging of enzymes within virus-like particles. Angew Chem Int Ed 2010, 49:9648–9651.

19. O'Neil A, Prevelige PE, Douglas T: Stabilizing viral nano-reactors for nerve-agent degradation. Biomater Sci 2013, 1:881–886. [PubMed: 32481934]

20. Jordan PC, Patterson DP, Saboda KN, Edwards EJ, Miettinen HM, Basu G, Thielges MC, Douglas T: Self-assembling biomolecular catalysts for hydrogen production. Nature Chem 2016, 8:179–185. [PubMed: 26791902]

21. Uchida M, McCoy K, Fukuto M, Yang L, Yoshimura H, Miettinen HM, Lafrance B, Patterson DP, Schwarz B, Karty JA et al. : Modular self-assembly of protein cage lattices for multistep catalysis. ACS Nano 2018, 12:942–953. [PubMed: 29131580]

22. McCoy K, Uchida M, Lee B, Douglas T: Templated assembly of a functional ordered protein macromolecular framework from P22 virus-like particles. ACS Nano 2018, 12:3541–3550. [PubMed: 29558117]

23. Chakraborti S, Korpi A, Kumar M, Stepien P, Kostiainen MA, Heddle JG: Three-dimensional protein cage array capable of active enzyme capture and artificial chaperone activity. Nano Lett 2019, 19:3918–3924. [PubMed: 31117758]

24. Liljestrom V, Mikkila J, Kostiainen MA: Self-assembly and modular functionalization of three-dimensional crystals from oppositely charged proteins. Nat Commun 2014, 5:4445. [PubMed: 25033911]

25. Lavelle L, Gingery M, Phillips M, Gelbart WM, Knobler CM, Cadena-Nava RD, Vega-Acosta JR, Pinedo-Torres LA, Ruiz-Garcia J: Phase diagram of self-assembled viral capsid protein polymorphs. J Phys Chem B 2009, 113:3813–3819. [PubMed: 19673134]

26. Twarock R, Bingham RJ, Dykeman EC, Stockley PG: A modelling paradigm for RNA virus assembly. Curr Opin Virol 2018, 31:74–81. [PubMed: 30078702]

27. Zlotnick A, Aldrich R, Johnson JM, Ceres P, Young MJ: Mechanism of capsid assembly for an icosahedral plant virus. Virology 2000, 277:450–456. [PubMed: 11080492]

28. Bancroft JB, Wagner GW, Bracker CE: The self-assembly of a nucleic-acid free pseudo-top component for a small spherical virus. Virology 1968, 36:146–149. [PubMed: 4970235]

29•. Hagan MF, Zandi R: Recent advances in coarse-grained modeling of virus assembly. Curr Opin Virol 2016, 18:36–43 [PubMed: 27016708] This is an excellent review that discusses recent advances in the use of coarse-grained modeling approaches to study the underlying mechanisms of capsid assembly.

30. Garmann RF, Comas-Garcia M, Knobler CM, Gelbart WM: Physical principles in the self-assembly of a simple spherical virus. Acc Chem Res 2016, 49:48–55. [PubMed: 26653769]

31. Glasgow JE, Capehart SL, Francis MB, Tullman-Ercek D: Osmolyte-mediated encapsulation of proteins inside MS2 viral capsids. ACS Nano 2012, 6:8658–8664. [PubMed: 22953696]

32•. Azuma Y, Edwardson TGW, Hilvert D: Tailoring lumazine synthase assemblies for bionanotechnology. Chem Soc Rev 2018, 47:3543–3557 [PubMed: 29714396] This is a great review that discusses engineering of lumazine synthase for applications in biomaterials and synthetic biology.

33. Worsdorfer B, Woycechowsky KJ, Hilvert D: Directed evolution of a protein container. Science 2011, 331:589–592. [PubMed: 21292977]

34. Seebeck FP, Woycechowsky KJ, Zhuang W, Rabe JP, Hilvert D: A simple tagging system for protein encapsulation. J Am Chem Soc 2006, 128:4516–4517. [PubMed: 16594656]

35. Brasch M, Putri RM, de Ruiter MV, Luque D, Koay MS, Caston JR, Cornelissen JJ: Assembling enzymatic cascade pathways inside virus-based nanocages using dual-tasking nucleic acid tags. J Am Chem Soc 2017, 139:1512–1519. [PubMed: 28055188]

36. Sun J, DuFort C, Daniel M-C, Murali A, Chen C, Gopinath K, Stein B, De M, Rotello VM, Holzenburg A et al. : Core-controlled polymorphism in virus-like particles. Proc Natl Acad Sci U S A 2007, 104:1354–1359. [PubMed: 17227841]

37. Chen C, Daniel M-C, Quinkert ZT, De M, Stein B, Bowman VD, Chipman PR, Rotello VM, Kao CC, Dragnea B: Nanoparticle-templated assembly of viral protein cages. Nano Lett 2006, 6:611–615. [PubMed: 16608253]

38. Vieweger SE, Tsvetkova IB, Dragnea BG: In vitro assembly of virus-derived designer shells around inorganic nanoparticles. In Virus-Derived Nanoparticles for Advanced Technologies: Methods and Protocols. Edited by Wege C, Lomonossoff GP. New York: Springer; 2018:279–294.

39. Witherell GW, Gott JM, Uhlenbeck OC: Specific interaction between RNA phage coat proteins and RNA. Prog Nucleic Acid Res Mol Biol 1991, 40:185–220 [PubMed: 2031083]

40. Lim F, Spingola M, Peabody DS: The RNA-binding site of bacteriophage Q beta coat protein. J Biol Chem 1996, 271:31839–31845. [PubMed: 8943226]

41. Wu M, Brown WL, Stockley PG: Cell-specific delivery of bacteriophage-encapsidated ricin A chain. Bioconjug Chem 1995, 6:587–595. [PubMed: 8974458]

42. Fiedler JD, Fishman MR, Brown SD, Lau J, Finn MG: Multifunctional enzyme packaging and catalysis in the Qbeta protein nanoparticle. Biomacromolecules 2018, 19:3945–3957. [PubMed: 30160482]

43. Rhee JK, Hovlid M, Fiedler JD, Brown SD, Manzenrieder F, Kitagishi H, Nycholat C, Paulson JC, Finn MG: Colorful virus-like particles: fluorescent protein packaging by the Qbeta capsid. Biomacromolecules 2011, 12:3977–3981. [PubMed: 21995513]

44••. Li S, Roy P, Travesset A, Zandi R: Why large icosahedral viruses need scaffolding proteins. Proc Natl Acad Sci U S A 2018,115:10971–10976 [PubMed: 30301797] Using a computational approach, this paper provides an insight into the role of scaffolding proteins on assembly of large icosahedral viruses.

45. Lander GC, Tang L, Casjens SR, Gilcrease EB, Prevelige P, Poliakov A, Potter CS, Carragher B, Johnson JE: The structure of an infectious P22 virion shows the signal for headful DNA packaging. Science 2006, 312:1791–1795. [PubMed: 16709746]

46. Saugar I, Irigoyen N, Luque D, Carrascosa JL, Rodriguez JF, Caston JR: Electrostatic interactions between capsid and scaffolding proteins mediate the structural polymorphism of a double-stranded RNA virus. J Biol Chem 2010, 285:3643–3650. [PubMed: 19933276]

47. Thuman-Commike PA, Greene B, Malinski JA, Burbea M, McGough A, Chiu W, Prevelige PE Jr: Mechanism of scaffolding-directed virus assembly suggested by comparison of scaffolding-containing and scaffolding-lacking P22 procapsids. Biophys J 1999, 76:3267–3277. [PubMed: 10354452]

48. Patterson DP, Schwarz B, El-Boubbou K, van der Oost J, Prevelige PE, Douglas T: Virus-like particle nanoreactors: programmed encapsulation of the thermostable CelB glycosidase inside the P22 capsid. Soft Matter 2012, 8:10158.

49. Patterson DP, Prevelige PE, Douglas T: Nanoreactors by programmed enzyme encapsulation inside the capsid of the bacteriophage P22. ACS Nano 2012, 6:5000–5009. [PubMed: 22624576]

50. Patterson DP, LaFrance B, Douglas T: Rescuing recombinant proteins by sequestration into the P22 VLP. Chem Commun 2013, 49:10412.

51. Qazi S, Miettinen HM, Wilkinson RA, McCoy K, Douglas T, Wiedenheft B: Programmed self-assembly of an active P22-Cas9 nanocarrier system. Mol Pharm 2016, 13:1191–1196. [PubMed: 26894836]

52. Wang Y, Uchida M, Waghwani HK, Douglas T: Synthetic virus-like particles for glutathione biosynthesis. ACS Synth Biol 2020, 9:3298–3310. [PubMed: 33232156]

53. Weigele PR, Sampson L, Winn-Stapley D, Casjens SR: Molecular genetics of bacteriophage P22 scaffolding protein's functional domains. J Mol Biol 2005, 348:831–844. [PubMed: 15843016]

54. Parker MH, Casjens S, Prevelige PE: Functional domains of bacteriophage P22 scaffolding protein. J Mol Biol 1998,281:69–79. [PubMed: 9680476]

55. O'Neil A, Reichhardt C, Johnson B, Prevelige PE, Douglas T: Genetically programmed in vivo packaging of protein cargo and its controlled release from bacteriophage P22. Angew Chem Int Ed 2011, 50:7425–7428.

56. Ellis RJ: Macromolecular crowding: an important but neglected aspect of the intracellular environment. Curr Opin Struct Biol 2001, 11:114–119. [PubMed: 11179900]

57. Fuller MT, King J: Regulation of coat protein polymerization by the scaffolding protein of bacteriophage P22. Biophys J 1980, 32:381–401. [PubMed: 7018607]

58. Prevelige PE, Thomas D, King J: Scaffolding protein regulates the polymerization of P22 coat subunits into icosahedral shells in vitro. J Mol Biol 1988, 202:743–757. [PubMed: 3262767]

59. 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. [PubMed: 32319761]

60. Sharma J, Uchida M, Miettinen HM, Douglas T: Modular interior loading and exterior decoration of a virus-like particle. Nanoscale 2017, 9:10420–10430. [PubMed: 28702648]

61. Greene B, King J: Binding of scaffolding subunits within the P22 procapsid lattice. Virology 1994, 205:188–197. [PubMed: 7975215]

62. Minten IJ, Hendriks LJA, Nolte RJM, Cornelissen JJLM: Controlled encapsulation of multiple proteins in virus capsids. J Am Chem Soc 2009, 131:17771–17773. [PubMed: 19995072]

63. Lizatovic R, Assent M, Barendregt A, Dahlin J, Bille A, Satzinger K, Tupina D, Heck AJR, Wennmalm S, Andre I: A protein-based encapsulation system with calcium-controlled cargo loading and detachment. Angew Chem Int Ed Engl 2018, 57:11334–11338. [PubMed: 29975817]

64. Li T, Jiang Q, Huang J, Aitchison CM, Huang F, Yang M, Dykes GF, He HL, Wang Q, Sprick RS et al. : Reprogramming bacterial protein organelles as a nanoreactor for hydrogen production. Nat Commun 2020, 11:5448. [PubMed: 33116131]

65. Akita F, Chong KT, Tanaka H, Yamashita E, Miyazaki N, Nakaishi Y, Suzuki M, Namba K, Ono Y, Tsukihara T et al. : The crystal structure of a virus-like particle from the hyperthermophilic archaeon *Pyrococcus furiosus* provides insight into the evolution of viruses. J Mol Biol 2007, 368:1469–1483. [PubMed: 17397865]

66. Sutter M, Boehringer D, Gutmann S, Gunther S, Prangishvili D, Loessner MJ, Stetter kO, Weber-Ban E, Ban N: Structural basis of enzyme encapsulation into a bacterial nanocompartment. Nat Struct Mol Biol 2008, 15:939–947. [PubMed: 19172747]

67. Giessen TW, Orlando BJ, Verdegaal AA, Chambers MG, Gardener J, Bell DC, Birrane G, Liao M, Silver PA: Large protein organelles form a new iron sequestration system with high storage capacity. eLife 2019, 8.

68••. Jones JA, Giessen TW: Advances in encapsulin nanocompartment biology and engineering. Biotechnol Bioeng 2021, 118:491–505 [PubMed: 32918485] This is an excellent source for an overview of the discovery of various encapsulins, their characterization, and recent advances in their applications as platforms for biomaterials.

69. LaFrance BJ, Cassidy-Amstutz C, Nichols RJ, Oltrogge LM, Nogales E, Savage DF: The encapsulin from *Thermotoga maritima* is a flavoprotein with a symmetry matched ferritin-like cargo protein. Sci Rep 2021, 11:22810. [PubMed: 34815415]

70. Putri RM, Allende-Ballestero C, Luque D, Klem R, Rousou KA, Liu A, Traulsen CH, Rurup WF, Koay MST, Caston JR et al. : Structural characterization of native and modified encapsulins as nanoplatforms for in vitro catalysis and cellular uptake. ACS Nano 2017, 11:12796–12804. [PubMed: 29166561]

71. Lau YH, Giessen TW, Altenburg WJ, Silver PA: Prokaryotic nanocompartments form synthetic organelles in a eukaryote. Nat Commun 2018, 9:1311. [PubMed: 29615617]

72. Inoue T, Kawano MA, Takahashi RU, Tsukamoto H, Enomoto T, Imai T, Kataoka K, Handa H: Engineering of SV40-based nano-capsules for delivery of heterologous proteins as fusions with the minor capsid proteins VP2/3. J Biotechnol 2008, 134:181–192. [PubMed: 18243389]

73. Deshpande S, Masurkar ND, Girish VM, Desai M, Chakraborty G, Chan JM, Drum CL: Thermostable exoshells fold and stabilize recombinant proteins. Nat Commun 2017, 8:1442. [PubMed: 29129910]

74. Waghwani HK, Douglas T: Cytochrome C with peroxidase-like activity encapsulated inside the small DPS protein nanocage. J Mater Chem B 2021, 9:3168–3179. [PubMed: 33885621]

75•. Schoonen L, Maassen S, Nolte RJM, van Hest JCM: Stabilization of a virus-like particle and its application as a nanoreactor at physiological conditions. Biomacromolecules 2017, 18:3492–3497 [PubMed: 28631927] This article demonstrates not only encapsulation of enzymes inside of the CCMV VLP, but also the enhancement of the stability of the VLP at physiological conditions by fusing the elastin-like polypeptide and the hexahistidine tag to the CCMV coat protein.

76. Schoonen L, Nolte RJ, van Hest JC: Highly efficient enzyme encapsulation in a protein nanocage: towards enzyme catalysis in a cellular nanocompartment mimic. Nanoscale 2016, 8:14467–14472. [PubMed: 27407020]

77•. Giessen TW, Silver PA: A catalytic nanoreactor based on in vivo encapsulation of multiple enzymes in an engineered protein nanocompartment. Chembiochem 2016, 17:1931–1935 [PubMed: 27504846] This article shows *in vivo* encapsulation of enzyme cargos inside of the MS2 VLP by using SpyTag/SpyCatcher protein fusion technology.

78•. Choi H, Eom S, Kim HU, Bae Y, Jung HS, Kang S: Load and display: engineering encapsulin as a modular nanoplatform for protein-cargo encapsulation and protein-ligand decoration using split intein and SpyTag/SpyCatcher. Biomacromolecules 2021, 22:3028–3039 [PubMed: 34142815]

This article adopts two protein fusion technologies, split intein to achieve the encapsulation of protein cargos inside of an encapsulin, as well as SpyTag/SpyCatcher for decorating its exterior surface.

79. Thuman-Commike PA, Greene B, Jakana J, Prasad BV, King J, Prevelige PE Jr, Chiu W: Three-dimensional structure of scaffolding-containing phage P22 procapsids by electron cryo-microscopy. J Mol Biol 1996, 260:85–98. [PubMed: 8676394]
80. Tuma R, Prevelige PE, Thomas GJ: Mechanism of capsid maturation in a double-stranded DNA virus. Proc Natl Acad Sci U S A 1998, 95:9885–9890. [PubMed: 9707570]
81. Galisteo ML, King J: Conformational transformations in the protein lattice of phage P22 procapsids. Biophys J 1993, 65:227–235. [PubMed: 8369433]
82. Teschke CM, McGough A, Thuman-Commike PA: Penton release from P22 heat-expanded capsids suggests importance of stabilizing penton-hexon interactions during capsid maturation. Biophys J 2003, 84:2585–2592. [PubMed: 12668466]
83. Selivanovitch E, Koliyatt R, Douglas T: Chemically induced morphogenesis of P22 virus-like particles by the surfactant sodium dodecyl sulfate. Biomacromolecules 2019, 20:389–400. [PubMed: 30462501]
84. Kraj P, Selivanovitch E, Lee B, Douglas T: Polymer coatings on virus-like particle nanoreactors at low ionic strength-charge reversal and substrate access. Biomacromolecules 2021, 22:2107–2118. [PubMed: 33877799]
85. Maassen SJ, van der Schoot P, Cornelissen J: Experimental and theoretical determination of the pH inside the confinement of a virus-like particle. Small 2018, 14:e1802081. [PubMed: 30102454]
86. Nap RJ, Bozic AL, Szleifer I, Podgornik R: The role of solution conditions in the bacteriophage PP7 capsid charge regulation. Biophys J 2014, 107:1970–1979. [PubMed: 25418178]
87. Roos WH, Ivanovska IL, Evilevitch A, Wuite GJ: Viral capsids: mechanical characteristics, genome packaging and delivery mechanisms. Cell Mol Life Sci 2007, 64:1484–1497. [PubMed: 17440680]
88. Carrasco C, Carreira A, Schaap IA, Serena PA, Gomez-Herrero J, Mateu MG, de Pablo PJ: DNA-mediated anisotropic mechanical reinforcement of a virus. Proc Natl Acad Sci U S A 2006, 103:13706–13711. [PubMed: 16945903]
89. Ortega-Esteban A, Condezo GN, Perez-Berna AJ, Chillon M, Flint SJ, Reguera D, San Martin C, de Pablo PJ: Mechanics of viral chromatin reveals the pressurization of human adenovirus. ACS Nano 2015, 9:10826–10833. [PubMed: 26491879]
90. Llauro A, Luque D, Edwards E, Trus BL, Avera J, Reguera D, Douglas T, Pablo PJ, Caston JR: Cargo-shell and cargo-cargo couplings govern the mechanics of artificially loaded virus-derived cages. Nanoscale 2016, 8:9328–9336. [PubMed: 27091107]

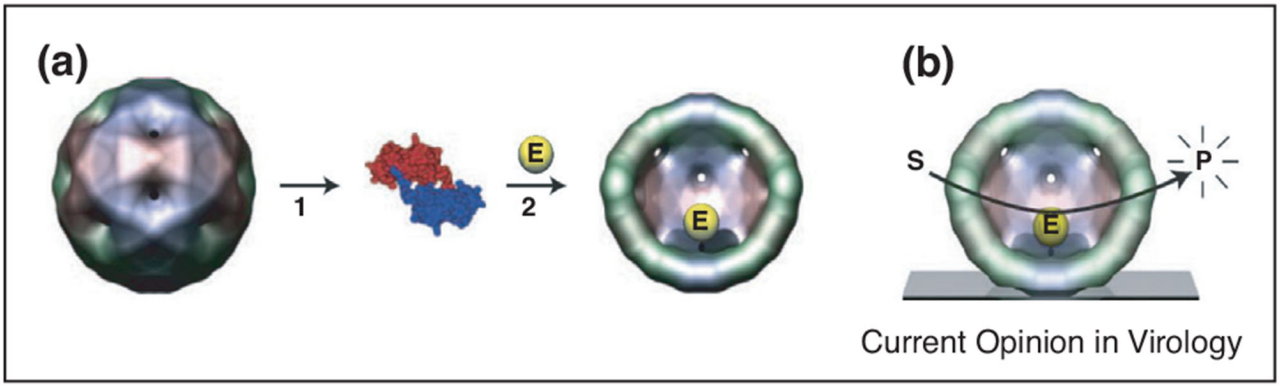

Figure 1.

(a) Schematic illustration of a passive enzyme encapsulation process in a CCMV capsid. Step 1: A CCMV capsid is disassembled into dimers at pH 7.5. Step 2: A guest enzyme (E) added to the sample is encapsulated when the CCMV dimers are reassembled into the capsid by lowering the pH to 5. **(b)** A substrate molecule (S) diffuses into the capsid and are subsequently converted to a product molecule (P) by the enzyme. The images were reproduced from Ref. [16] with permission from Springer Nature.

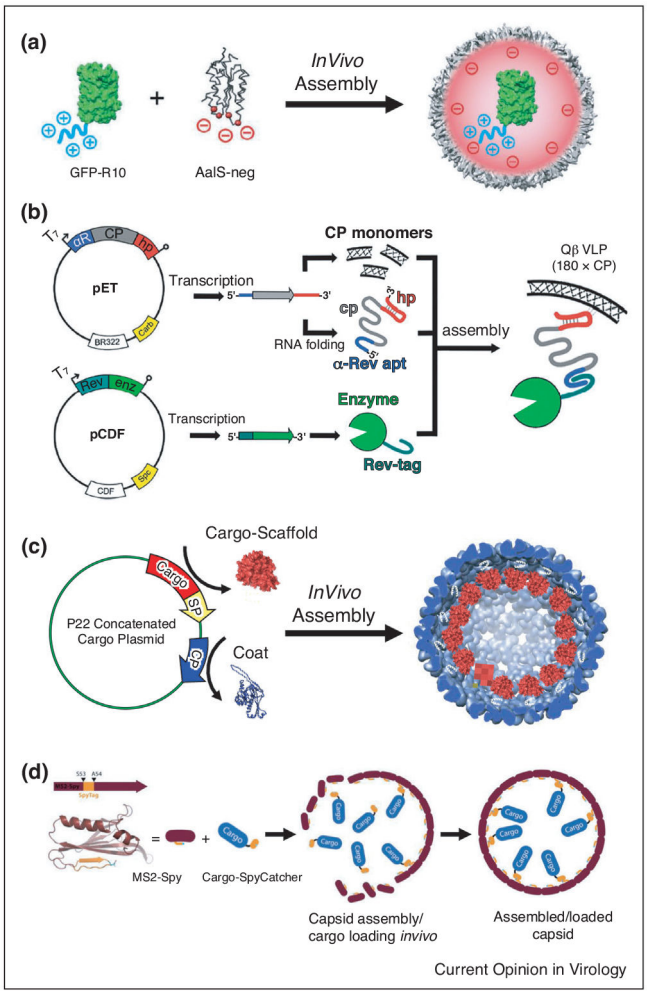

Figure 2.

Examples of directed encapsulation of cargo proteins inside of capsids *in vivo*. **(a)** GFP fused with positively charged tag is directed to inside of the AaLS-neg cage via electrostatic attraction. The image was reproduced from Ref. [32•] with permission from the Royal Society of Chemistry. **(b)** A Rev-tagged enzyme is directed to bind to the interior of the Qβ capsid via an RNA linker which possesses a Rev aptamer and a Qβ genome packaging hairpin domain. The image was reproduced from Ref. [18] with permission from Wiley. **(c)** Fusing a cargo protein to the N-terminus of a truncated P22 SP leads to directed encapsulation of the cargo to the P22 capsid. The image was reproduced from Ref. [49] with permission from the American Chemical Society. **(d)** A cargo protein fused with the SpyCatcher is covalently conjugated to the SpyTag inserted MS2 CP via isopeptide bond formed between the SpyCatcher and the SpyTag, while the CP assembles into the capsid. The image was reproduced from Ref. [77•] with permission from Wiley.

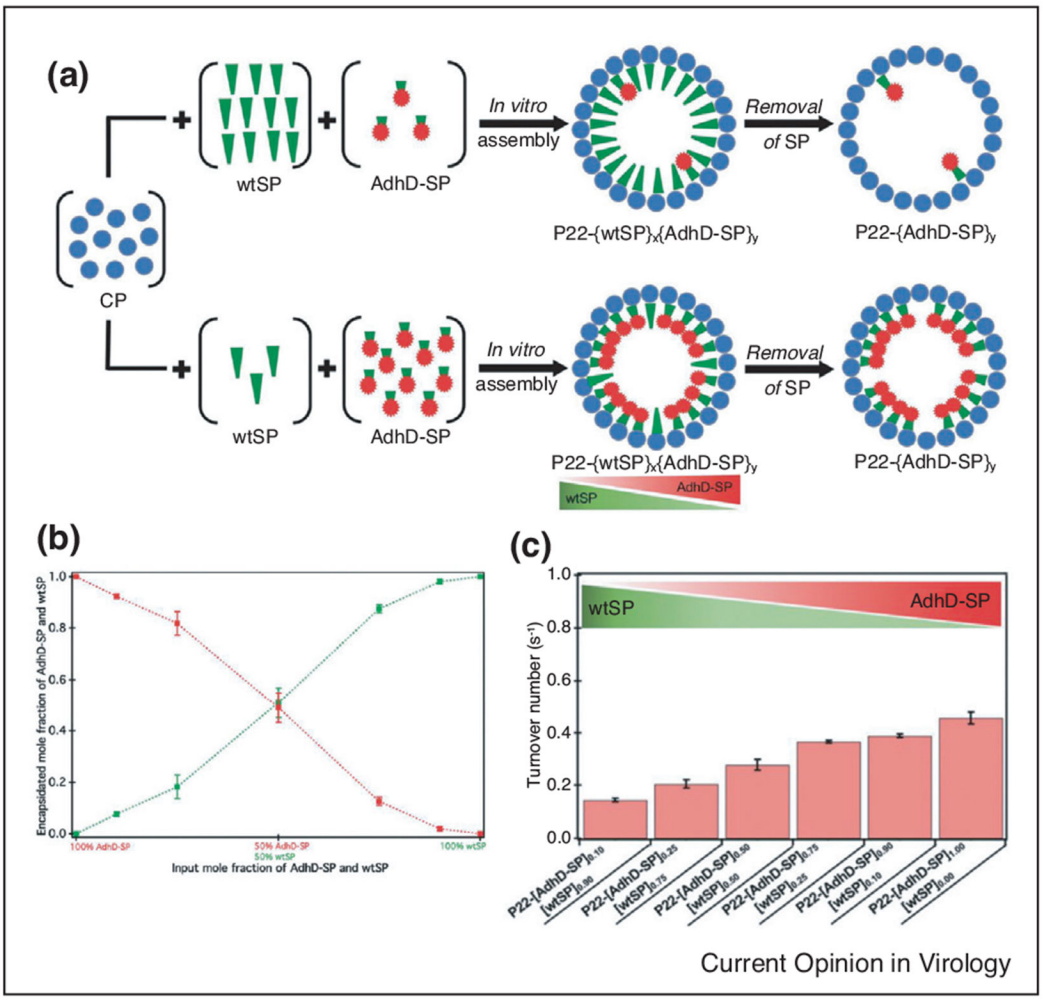

Figure 3.

Controlled packing of multiple cargo proteins using *in vitro* assembly of P22 CP and SP into a capsid. **(a)** Schematic representation of co-encapsulation of AdhD-SP and wtSP. To control the stoichiometry of the two cargos, a constant amount of CP was added to the mixture of AdhD-SP and wtSP with a range of two-component ratios while keeping the total SP amount constant. **(b)** Plots of the encapsulation ratio of AdhD-SP and wtSP over a range of input stoichiometry ratios of the two components. **(c)** The turnover number (k_{cat}) of AdhD within P22 VLP co-encapsulated with wtSP. Error bars represent standard deviations of triplicate measurements. The images were reproduced from Ref. [14••] with permission from the Royal Society of Chemistry.

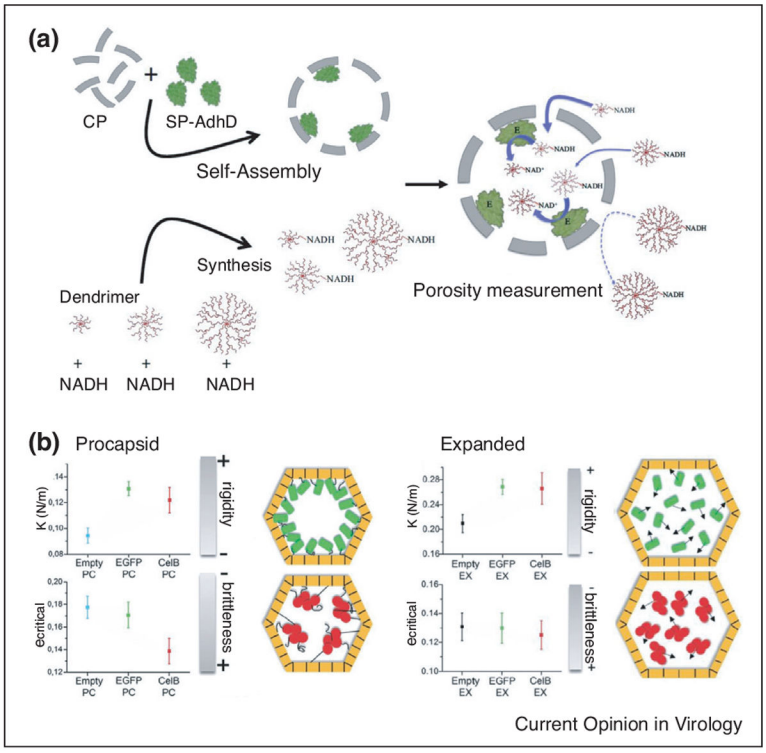

Figure 4.

(a) Schematic illustration of an experiment for investigating molecular diffusion across a capsid shell. P22 VLP self-assembled with CP and SP-AdhD fusion proteins was used as a model capsid. NADH, a cofactor of AdhD enzyme, was conjugated to various sizes of dendrimers and used as a probe to assess the porosity of the P22 capsid. If the probe accessed the interior of the capsid, the redox reaction of NADH to NAD^+ was observed. The images were reproduced from Ref. [11••]. **(b)** Mechanical properties of empty and cargo proteins loaded P22 capsids. The average rigidity and brittleness of procapsid (left) and expanded (right) forms of P22 capsids loaded with GFP (green) or CelB (red) was compared with those of empty capsids. The cartoons present that GFP and CelB were bound to the interior surface of the procapsid shell, whereas those were detached from the surface and free in the expanded shell. The images were reproduced from Ref. [90] with permission from the Royal Society of Chemistry.