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Bioinspired Approaches to Self-Assembly of Virus-like Particles: From Molecules to Materials

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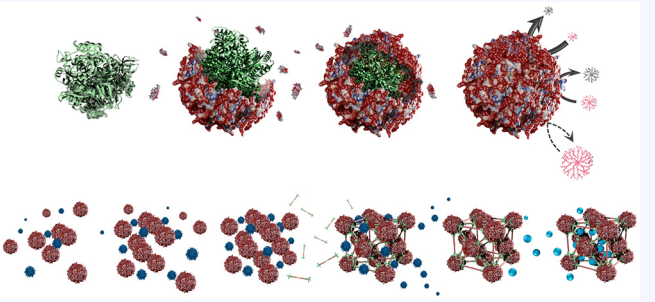
CONSPECTUS: When viewed through the lens of materials science, nature provides a vast library of hierarchically organized structures that serve as inspiration and raw materials for new synthetic materials. The structural organization of complex bioarchitectures with advanced functions arises from the association of building blocks and is strongly supported by ubiquitous mechanisms of self-assembly, where interactions among components result in spontaneous assembly into defined structures. Viruses are exemplary, where a capsid structure, often formed from the self-assembly of many individual protein subunits, serves as a vehicle for the transport and protection of the viral genome. Higher-order assemblies of viral particles are also found in nature with unexpected collective behaviors. When the infectious aspect of viruses is removed, the self-assembly of viral particles and their potential for hierarchical assembly become an inspiration for the design and construction of a new class of functional materials at a range of different length scales.



Salmonella typhimurium bacteriophage P22 is a well-studied model for understanding viral self-assembly and the construction of virus-like particle (VLP)-based materials. The formation of cage-like P22 VLP structures results from scaffold protein (SP)-directed self-assembly of coat protein (CP) subunits into icosahedral capsids with encapsulation of SP inside the capsid. Employing the CP−SP interaction during self-assembly, the encapsulation of guest protein cargos inside P22 VLPs can be achieved with control over the composition and the number of guest cargos. The morphology of cargo-loaded VLPs can be altered, along with changes in both the physical properties of the capsid and the cargo−capsid interactions, by mimicking aspects of the infectious P22 viral maturation. The structure of the capsid differentiates the inside cavity from the outside environment and serves as a protecting layer for the encapsulated cargos. Pores in the capsid shell regulate molecular exchange between inside and outside, where small molecules can traverse the capsid freely while the diffusion of larger molecules is limited by the pores. The interior cavity of the P22 capsid can be packed with hundreds of copies of cargo proteins (especially enzymes), enforcing intermolecular proximity, making this an ideal model system in which to study enzymatic catalysis in crowded and confined environments. These aspects highlight the development of functional nanomaterials from individual P22 VLPs, through biomimetic design and self-assembly, resulting in fabrication of nanoreactors with controlled catalytic behaviors.

Individual P22 VLPs have been used as building blocks for the self-assembly of higher-order structures. This relies on a balance between the intrinsic interparticle repulsion and a tunable interparticle attraction. The ordering of VLPs within three-dimensional assemblies is dependent on the balance between repulsive and attractive interactions: too strong an attraction results in kinetically trapped disordered structures, while decreasing the attraction can lead to more ordered arrays. These higher-order assemblies display collective behavior of high charge density beyond those of the individual VLPs.

The development of synthetic nanomaterials based on P22 VLPs demonstrates how the potential for hierarchical self-assembly can be applied to other self-assembling capsid structures across multiple length scales toward future bioinspired functional materials.

KEY REFERENCES

- Selivanovitch, E.; LaFrance, B.; Douglas, T. Molecular exclusion limits for diffusion across a porous capsid. *Nat. Commun.* **2021**, *12* (1), 2903.[1] *The effective sizes of the pores on P22 capsids were probed, elucidating the mechanism of molecular diffusion between the cavity and outside environment regulated by the porosity of the capsids.*

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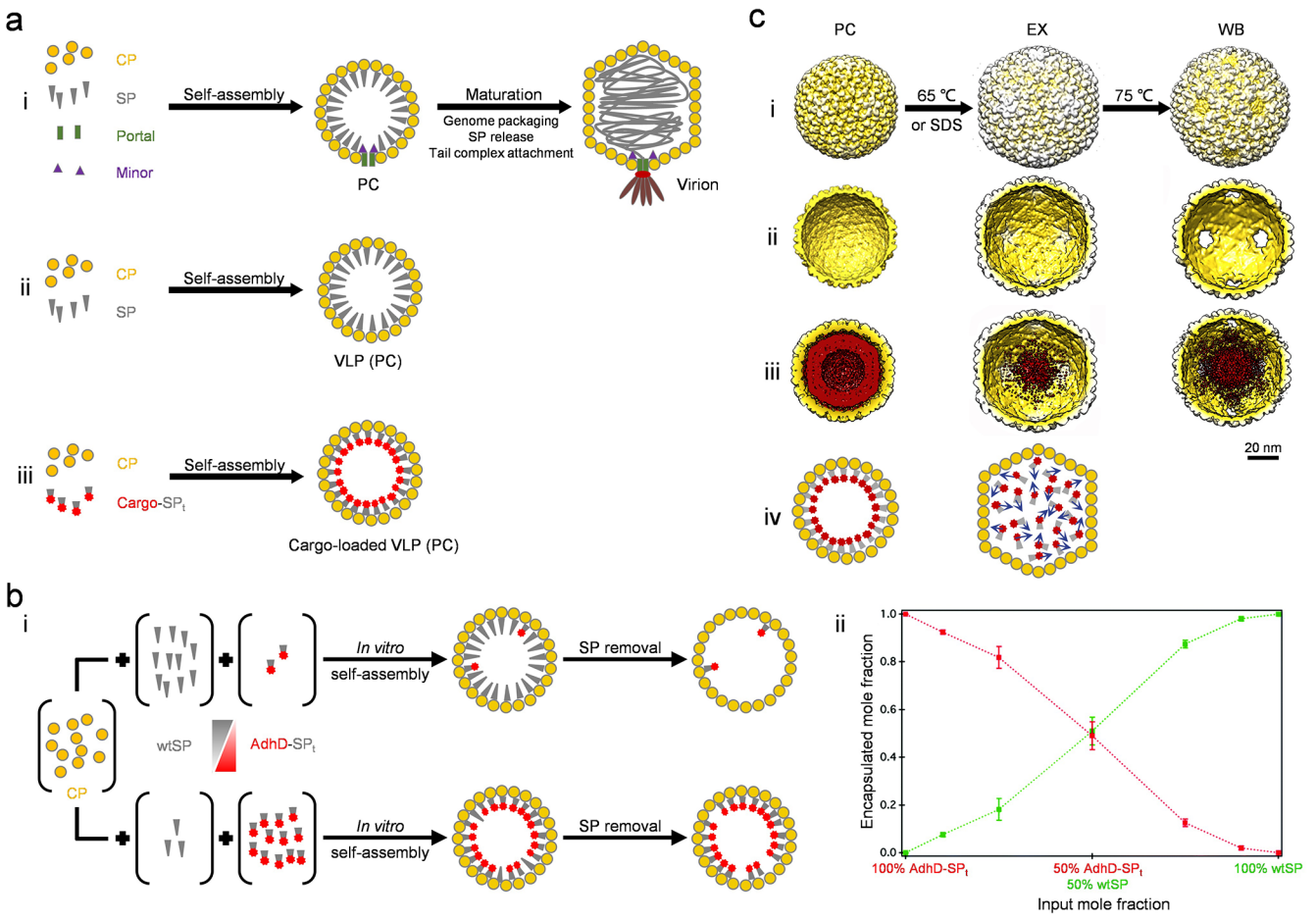

Figure 1. P22 self-assembly. (a) Assembly pathway of the infectious P22 virus (i), SP-directed self-assembly of P22 VLPs (ii), and self-assembly of cargo-loaded VLPs through genetic fusion of cargo and SP (iii). (b) *In vitro* assembly of P22 where the composition of encapsulated cargos, alcohol dehydrogenase D (AdhD-SP_t), and wtSP can be tuned (i) by input stoichiometry (results in ii) and subsequent removal of wtSP. Adapted with permission from ref 34. Copyright 2020 Royal Society of Chemistry. (c) Morphology changes of P22 VLPs, showing the outer (i) and inner (ii and iii) surfaces, based on 3D cryo-EM reconstructions. The encapsulated cargos (iii) form an inner shell in PC, but can diffuse freely in EX and WB morphologies (cartoon representation in iv). Adapted with permission from ref 38. Copyright 2016 Royal Society of Chemistry.

- Wang, Y.; Uchida, M.; Waghwani, H. K.; Douglas, T. Synthetic Virus-like Particles for Glutathione Biosynthesis. *ACS Synth. Biol.* **2020**, *9* (12), 3298−3310.[2] *This work provides experimental evidence and plausible explanations to show that the enforced intermolecular proximity between coupled biocatalytic entities does not necessarily induce kinetic advantages of multistep reactions.*
- Uchida, M.; McCoy, K.; Fukuto, M.; Yang, L.; Yoshimura, H.; Miettinen, H. M.; LaFrance, B.; Patterson, D. P.; Schwarz, B.; Karty, J. A.; Prevelige, P. E.; Lee, B.; Douglas, T.Modular Self-Assembly of Protein Cage Lattices for Multistep Catalysis. *ACS Nano* **2018**, *12* (2), 942−953.[3] *Controlled self-assembly of P22 VLPs results in three-dimensional ordered arrays, which was used to develop heterogeneous biocatalytic materials capable of multistep reactions.*
- Selivanovitch, E.; Uchida, M.; Lee, B.; Douglas, T.Substrate Partitioning into Protein Macromolecular Frameworks for Enhanced Catalytic Turnover. *ACS Nano* **2021**, *15* (10), 15687−15699.[4] *A catalytic material with enhanced catalytic efficiency and substrate selectivity was developed based on the collective behavior of a higher-order assembly of P22 VLPs.*

1. INTRODUCTION

Biomimetic approaches are powerful tools in materials design and construction. The properties and function of naturally occurring biological architectures offer us inspiration for new materials.[5] Hierarchical assembly is a theme that connects the formation of these architectures across length scales from the molecular to macroscopic levels.[6] The functioning of a complex organism requires the assembly and the subsequent interaction between organs and tissues with specialized function, which results from the organization of multiple types of cells.[7] Cells, the basic unit of life, also possess complex structures with a collection of subcellular compartments, comprising organized assemblies of molecules such as proteins and lipids.[7] This hierarchy results in unique properties and function (such as spatial segregation, molecular exchange, and biochemical transformations) at different levels of the assembly, and also endows higher-order structures with collective behaviors that are lacking in the individual components.[6−8]

Self-assembly is a crucial mechanism in the formation of hierarchical bioarchitectures, where small building blocks spontaneously assemble into large, well-defined structures.[9,10] For example, molecules can self-assemble with weak non-

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covalent interactions to create cellular or subcellular compartments (such as lipid bilayer-bounded cellular organelles and protein-based microcompartments), which spatially isolate subcellular activities and enhance the overall cell function. New properties that emerge from hierarchical self-assembly inspire us to mimic these behaviors in the design and construction of new functional materials.[5,10]

Hierarchical self-assembly also plays an essential role in the formation and resultant properties of viruses,[11,12] which are ubiquitous with life.[13] Viruses with proteinaceous shells self-assemble into cage-like structures (capsids) from their structural gene products, which act as vehicles for the storage, protection, and transportation of the viral genomes.[11,12] The capsid structures share similarity with protein-based subcellular compartments including bacterial microcompartments, ferritins, encapsulins, vault proteins, and heat shock proteins.[8,11,14] Removing the role of viruses as infectious agents and focusing instead on the synthetic utility of viral capsids has resulted in the development of a new class of functional nanomaterials.[11,12,14,15] Additionally, some infectious viruses show hierarchical assemblies and are observed to form higher-order structures with properties distinct from individual viral particles, such as the iridescence of crystalline close-packed iridoviruses in infected cells.[11,12,16] This too can be mimicked synthetically by modulating particle−particle interactions to form higher-order assemblies of virus-like particles (VLPs) to make nano- to microscale materials, in which the collective behavior of the VLP building blocks endows the materials with additional functionality.[11,12,17]

Although many viruses have been used as platforms for the development of bioinspired nanomaterials,[11] *Salmonella typhimurium* bacteriophage P22 is one of the most studied. P22 is a double-stranded DNA virus, whose assembly pathway (Figure 1a.i) starts with the self-assembly of 415 copies of a coat protein (CP) along with a dodecameric portal protein complex, resulting in a spherical $T = 7$ capsid with icosahedral symmetry: the immature viral shell called procapsid (PC).[11,18] Importantly, this process is directed by 60−300 copies of a scaffolding protein (SP), which are themselves encapsulated inside the PC.[11] The structure of the PC comprises 71 CP capsomers, including 60 hexamers and 11 pentamers, and a portal complex occupying one pentameric site.[18] The viral maturation occurs by the injection of the genome DNA into the PC through the portal complex, resulting in SP release and an expansion of the capsid.[11,19] The assembly pathway results in a virion composed of a capsid head with genome enclosed and a tail machinery.[18,19] This mature capsid shows an expanded and angular morphology (EX) compared to PC, with an increase in diameter from ∼56 to 64 nm and an increase in volume of ∼10%.[11,18,20]

This Account covers the development of functional P22-derived materials based on the self-assembly of its cage-like architecture and subsequent hierarchical assembly into three-dimensional materials. We will outline how the properties of these materials assist us to understand fundamental questions around self-assembly, biochemical function, and their use as building blocks for the development of complex and responsive materials.

2. SELF-ASSEMBLY OF P22 VIRUS-LIKE PARTICLE (VLP)

2.1. Scaffold Protein (SP) Directs Self-Assembly of Coat Protein (CP)

P22 VLPs without the portal complex can be assembled from CP and SP (Figure 1a.ii), both *in vivo* and *in vitro*.[11,21] Like the virus assembly, SP directs the self-assembly of 420 CP into a VLP, a consequence of the balance of interactions among the components.[21,22] In the absence of SP, weak CP−CP interactions drive the formation of aberrant particles.[23] However, when SP is present, the CP−SP interaction can drive a conformational switch of CP, facilitating capsid self-assembly with the correct geometry.[21,24] The oligomerization state of the SP (SP−SP interactions) influences CP−SP interactions and therefore the kinetics of capsid self-assembly.[21] The SP dimer binds to CP more tightly and accelerates the nucleation and initiation of the assembly, while SP monomer association is more transient and dynamic in making CP assembly competent, resulting in a faster elongation into complete particles. Computational simulation demonstrates that scaffolding proteins play a crucial role in the self-assembly of large icosahedral viruses: they decrease the energy barrier for CP aggregation and preserve the correct geometry of CP self-assembly.[25] Once the self-assembly of P22 VLPs is complete, SP can be removed without disrupting the capsid by treatment with chaotropes (such as guanidine) at low concentration,[11,21] suggesting the strong capsid structure results from many relatively weak directional interactions.

Only the C-terminus of the 303-amino acid P22 SP is critical for CP binding.[26] NMR data indicate that only the residue 264−303 fragment of SP has a defined helix-turn-helix structure,[27] with a high density of positive charges that can form salt bridges with CP to direct self-assembly.[28] Mutagenesis work demonstrates that if the minimal CP binding site remains, truncated SP (SP_t) is still able to direct the assembly of P22 VLPs *in vitro* and *in vivo*,[26] which paves the way for making P22 VLP-based self-assembled nanomaterials.

2.2. Guest Cargo Incorporation and the Composition Control

The SP-dependent self-assembly and resulting SP encapsulation are the inspiration for incorporation of guest cargos, especially proteins, inside the VLPs to make functional nanomaterials. A genetic fusion to SP_t containing the CP binding domain is an effective strategy for protein encapsulation (Figure 1a.iii). Among the very first examples are fluorescent proteins, which were genetically fused to the N-terminus of a SP_t (residue 141−303 fragment, $SP_{141-303}$) and then coexpressed with CP in *Escherichia coli*, resulting in fluorescent VLPs with a size and shape similar to those of wild-type (wt) VLPs.[29,30] The same strategy has been applied to enzyme encapsulation to make catalytic P22 VLP nanoreactors.[31] The average cargo loading density can be determined by the difference in the molecular masses between empty shell and the cargo-loaded VLPs, measured using multiangle light scattering.[29,31] These functional VLPs encapsulate up to 300 copies of SP-fused cargos. Cargo incorporation can also be achieved when fused to shorter versions of SP_t (such as $SP_{239-303}$ and $SP_{257-303}$)[2] or via the C-terminus of the SP_t:[32] a flexibility that allows accommodation of cargo proteins with buried termini.

Efforts to control the composition of the cargo proteins provides a means to study intermolecular interactions and

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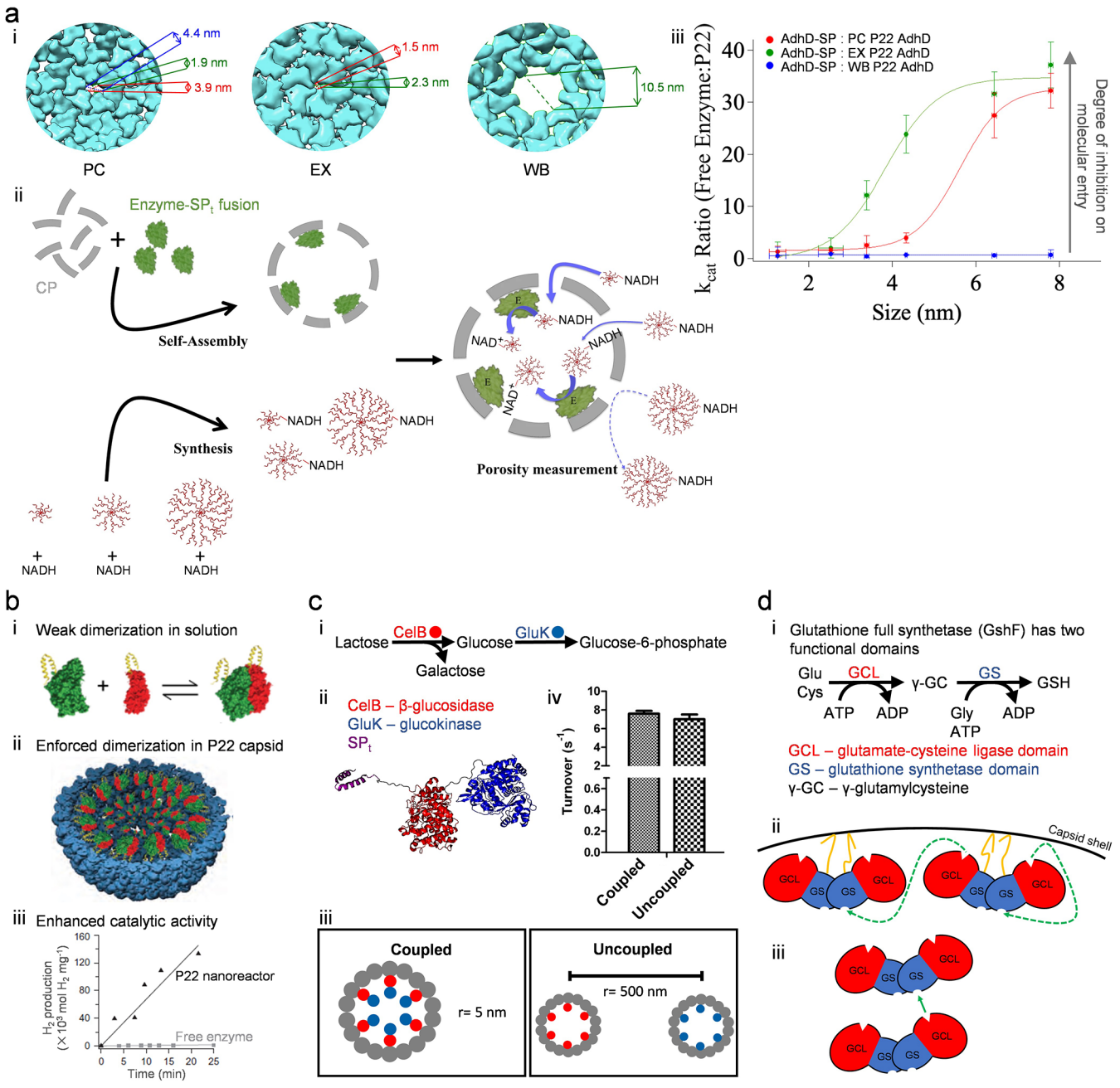

Figure 2. P22 VLP-based nanoreactors. (a) The porosity of P22 capsids can be assessed by the pore sizes from cryo-EM structures (i) and by probing effective pore sizes using P22 nanoreactors (ii and iii) with differently sized substrates. Reproduced with permission from ref 1. Copyright 2021 The Authors. Published by Springer Nature under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). (b) The catalytic activity of Hyd-1 requires intersubunit interactions, which are weak in solution (i) but enhanced by enforced proximity inside P22 (ii). Encapsulated Hyd-1 showed higher catalytic activity than it did when free in solution (iii). Adapted with permission from ref 40. Copyright 2016 Nature Publishing Group. (c) A metabolon comprising a multistep enzymatic pathway (i) can be constructed using P22 VLPs by the coencapsulation of two functionally coupled enzymes through fusion with SP_t (ii). The coencapsulation of the two enzymes, compared to the uncoupled, separate encapsulation (iii), does not exhibit an overall kinetic advantage (iv). Data from ref 33. (d) GshF-catalyzed glutathione (GSH) biosynthesis (i) is not enhanced by enforced intermolecular proximity of dimeric GshF molecules inside P22 upon encapsulation (ii), likely because sufficient proximity and optimal orientation between the two coupled active sites (iii) are not achieved to induce channeling of the intermediate γ-GC. Data from ref 2.

crowding effects (see section 3). By engineering a multiple-protein fusion that links several cargo proteins to SP_t within a single polyprotein (Figure 2c.ii), different cargos can be packaged.[30,33] This allows control of the cargo stoichiometry (usually 1:1) but requires design of the fusions. To address encapsulation of a more complicated stoichiometry, we have developed an *in vitro* assembly system, where CP is incubated with a mixture of purified SP variants (such as wtSP and cargo-SP_t) under a slightly denatured conditions and self-assembly is triggered by removing the denaturant (Figure 1b).[34] By modulating the input ratio of different SP variants, various cargo stoichiometries can be achieved inside the VLPs. Using

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this approach, we have coencapsulated multiple copies of a protein, which itself assembles into a smaller protein cage, together with multiple copies of an enzyme inside P22, demonstrating hierarchical compartmentalization in a cellular-mimetic structure.[35] An advantage of this system lies in the ability to manipulate the cargo loading density (usually 20−40% of the capsid volume) by coencapsulating wtSP, which can subsequently be selectively removed by mild treatment with a chaotrope after assembly; the cargo-SP_t fusion protein is retained due to large size (Figure 1b.i).[34,36] Taking advantage of this, we have studied how the packing density of an encapsulated enzyme is affected separately by crowding and confinement to the overall catalytic efficiency of the P22 nanoreactor, giving us insight into the design of biocatalytic nanomaterials.[34]

2.3. Morphology Change and Cargo−Shell Relations

The morphology change of P22 virion induced by genome packaging can be mimicked by applying external stimuli to VLPs (Figure 1c.i,ii).[20,37] Heat treatment at 65 °C transforms PC to EX, optimally for empty VLPs but only partially for cargo-loaded VLPs.[37] However, treatment with sodium dodecyl sulfate (SDS) effectively induces this morphology change with dense cargo loaded VLPs.[37] Interestingly, heating VLPs at 75 °C induces a morphology, undiscovered in the infectious virus, called wiffle ball (WB), where pentamers are lost from EX leaving large 10 nm pores through the icosahedral capsid.[20]

The structures of P22, determined by cyro-electron microscopy (EM), demonstrate the structural and spatial relationship among the protein components in different P22 morphologies (Figure 1c.iii,iv). Although the general fold of CP subunits remains unchanged, the intersubunit contacts are altered and minor structural shifts take place during the morphology change,[18,24] influencing SP binding and capsid porosity. Due to SP−CP binding in PC, the cargo-SP_t is localized to the interior surface of the capsid, forming an inner shell and leaving the cavity center largely empty; the cargo shell mechanically reinforces the capsid.[38] After expansion, the SP binding sites on CP disappear. The cargo-SP_t can then diffuse freely inside the cavity, and exerts an osmotic pressure on the shell, which increases the shell rigidity.[38] These basic insights into P22 and its VLPs have guided the design and synthesis of functional P22 VLP nanoparticles.

3. UTILIZATION OF THE SELF-ASSEMBLED STRUCTURE OF P22 VLPs

Compared to other types of proteins such as enzymes, the functionality of individual CP or SP subunits is limited, but the self-assembly into a cage-like structure endows a new collective behavior to these proteins. The cage-like architecture of the self-assembled capsid discriminates between the interior and exterior environments. The high symmetry of the capsid gives rise to a high degree of multivalency that has been used for molecular presentation on the exterior,[11,39] while the limited space enclosed by the capsid has been used for encapsulation of guest cargos.

3.1. Capsid Shell Is a Barrier Isolating the Inside Cavity from the Outside Environment

Genetic fusions to SP can direct the confinement of hundreds of protein cargos to the capsid cavity, with up to 40% of the cavity volume occupied: a densely packed environment. The local concentration of the cargo proteins can reach over 300 mg/mL, which is comparable to the estimated intracellular concentration of biomacromolecules and concentration of protein crystals.[2,31] This has allowed P22 to be used as a model to study the role of crowding on the behavior of biomacromolecules (see section 3.3).

The capsid, although porous (see section 3.2), physically shields the internal cargos from some external stimuli. Some enzymes become less susceptible to denaturation and degradation after encapsulation inside P22 VLPs, enhancing the efficacy of P22 nanoreactors as catalytic biomaterials with extended lifetime.[2,40]

The finite space of the capsid cavity can also constrain the processing of chemical reactions. We previously studied an atom-transfer radical polymerization inside empty P22 VLPs, which was initiated site-specifically at the interior shell surface, generated a huge number of labeling sites for molecules of interest, and got confined inside the capsid.[41] The mechanism of this constrained polymer growth has yet to be revealed, but it echoes the "head-full" mechanism of P22 genome packaging, stating the genome packaging is terminated when the length of DNA reaches the packing capacity of the cavity (~43.5 kbp, longer than the 41.7-kbp genome).[19]

3.2. Capsid Porosity Affects Molecular Exchange across the Barrier

Structural pores are important for control of diffusion across a barrier: they provide the means for the system to select and discriminate between diffusants and therefore control access.[42] Cyro-EM structures indicate there are pores in the P22 capsids in all three morphological states (Figure 2a.i).[18,24] In both PC and EX, the largest pores are located at the center of the hexamers and are about 4 and 2 nm in diameter, respectively; the loss of pentamers leaves 10 nm holes in the WB structure.[1]

We have probed the porosity of P22 by investigating the access of synthetic substrates to an enzyme encapsulated inside capsids, reflected by changes in the catalytic turnover (Figure 2a.ii-iii).[1] The results suggest relatively dynamic pores on P22 capsids, whose effective sizes, determined from the experimental data, match with cyro-EM structures. The pores regulate molecular entry primarily via size: small molecules freely diffuse into and out of the capsids, while the diffusion of molecules with sizes close to or larger than the pores is inhibited. In addition, the electrostatics of the pore affects the molecular diffusion when the molecular size is comparable to the pore size. Charged residues surrounding the pores create an electric field which facilitates diffusion of negatively charged molecules into the capsids. Knowledge of the pore selectivity allows us to design catalytic material systems with substrate selectivity.

The capsid porosity is also reflected in the cargo retention inside the capsids, which is governed by the cargo size and cargo−shell interactions.[36] The capsid pores completely block the release of encapsulated cargos that are larger than the pores (such as folded proteins). Small cargos (such as the poorly folded SP) can exit the capsids through the pores but this can be inhibited by cargo-shell interactions.

3.3. Enforced Proximity of Encapsulated Cargo

Confinement of a large number of cargo proteins inside the capsid cavity leads to enforced proximity, resulting in intermolecular communication as well as interdomain interactions. Encapsulation of a protein pair capable of Förster resonance energy transfer (FRET) demonstrates the FRET efficiency is substantially increased when the pair is

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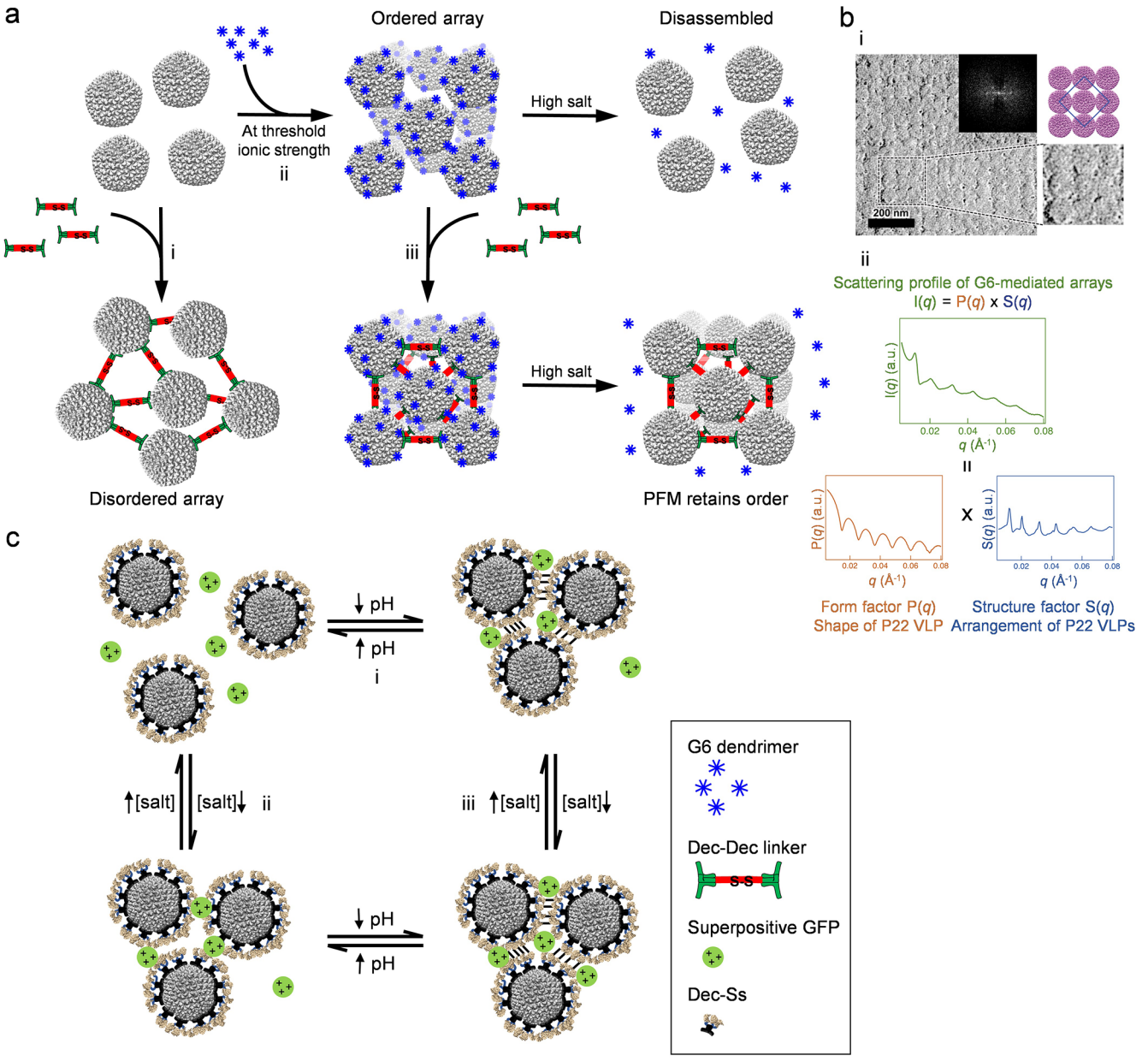

Figure 3. Higher-order assemblies of P22 VLPs. (a) Disordered arrays (i) and ordered arrays (ii) can be formed by the interaction between P22 VLPs mediated by Dec-Dec linkers and G6 dendrimers, respectively. High salt conditions disassemble G6-mediated arrays. Addition of the Dec-Dec linker to the ordered array locks its structure, so exposure to high salt conditions removes G6 dendrimers and forms PMF but does not lead to disassembly of the 3D structure. (b) G6-mediated arrays show close packed arrangement by TEM (i) and FCC ordering by SAXS measurements (ii). Data from ref 3. (c) Dec-Ss mediates pH-responsive higher-order assemblies (i). Charged macromolecules, such as superpositive GFP, induce ionic strength-dependent higher-order assembly (ii). The Dec-Ss mediated assembly shows affinity for superpositive GFP binding at high ionic strength (iii), demonstrating a collective behavior which is distinct from individual particles. Adapted from ref 46. Copyright 2018 American Chemical Society.

encapsulated inside P22.[30] Confinement of enzymes, where intersubunit interactions are important, can be reinforced to boost enzymatic activity. The activity of the Hyd-1 hydrogenase requires intersubunit interactions that are weak in solution but enforced by their encapsulation inside P22, and results in enhanced activity of the encapsulated enzyme compared to the free enzyme (Figure 2b).[40]

We have encapsulated metabolic enzyme pairs and multifunctional enzymes inside P22 to investigate coupled reactions of biochemical pathways in an environment that mimics the confined, crowded intracellular space (Figure 2c,d).[2,33] Although the packing density of enzymes inside the cavity leads to a high degree of crowding and enforces intermolecular proximity, this does not necessarily lead to direct channeling of small molecule intermediates in a multistep reaction.[2,33] When a multistep reaction is not diffusion-limited (*i.e.*, the diffusion constant of small molecules far exceeds the catalytic efficiency of the enzymes), the extremely dense packing of enzymes does not sufficiently reduce the distance between the coupled active sites so that local concentration of the intermediates is not high

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enough to make the turnover rate comparable to the diffusion rate of the intermediate species.[2,43] Consequently, the diffusion of intermediates into the bulk solution is so rapid that there is no change in the overall pathway efficiency. Therefore, a kinetic advantage for a multistep pathway is not supported by simple colocalization of the coupled catalytic entities, but rather their spatial arrangement (especially the relative locations of the active sites) likely plays an important role in proximity-induced substrate channeling.[2,43]

4. HIGHER-ORDER ASSEMBLY

A system that is thermodynamically poised for self-assembly with balanced attractive and repulsive interactions has the exquisite possibility to create an ordered structure through the dynamics of sampling many interaction geometries, akin to an annealing process in materials.[9,17] We have explored this approach toward the assembly of VLPs into higher order 2D[44] and 3D[3,45−47] materials using a number of different modalities to tune the attractive interaction between particles and relying on the high, and fairly uniform, charge density on VLPs to provide repulsive interactions between particles. In cases where the attractive interaction between particles is too large (and/or the dissociation rate is too slow), we have made disordered amorphous materials. However, when the attractive interaction is less strong (or the unbinding/dissociation rate is relatively fast) we can produce large highly ordered arrays of the VLPs that form quickly and reproducibly in solution.

4.1. Protein Mediated Formation of Disordered Arrays

Decoration proteins are common in double stranded DNA bacteriophages, serving as structural reinforcement for the capsid architecture.[11] While bacteriophage P22 does not have a native decoration protein, the trimeric decoration protein (Dec) from the homologous bacteriophage L binds with high affinity (nanomolar) to the mature P22 capsid (EX and WB).[48] Cryo-EM studies revealed that 80 Dec trimers bind at symmetry specific sites on the mature P22 capsid (60 at the quasi 3-fold axes and 20 at the true icosahedral 3-fold axes).[49] Dec binds to P22 via its N-terminal domain while the C-terminal domain extends away from the capsid surface.[50] A linear ditopic Dec structure, of a head-to-head dimer of trimers, was created by incorporation of a cysteine at the Dec C-terminus and oxidation to form disulfide linked proteins.[45] This ditopic Dec-Dec protein linker can bind and bridge between P22 VLPs to mediate their assembly into higher order structures (Figure 3a.i), which was demonstrated by both bulk assembly and layer-by-layer deposition.[45]

The hierarchically assembled P22-(Dec-Dec) materials were very stable, in part because of the high binding affinity of Dec for the P22 and multiple binding of the Dec-Dec linker proteins to each P22 (up to 80 Dec per P22), which contribute to high interparticle connectivity within the material.[45] However, these materials showed no long-range ordering, likely due to the very tight binding of Dec to the P22, as measured by the fast on rate for binding and very slow off rate,[48] which would kinetically trap the initially formed structure and not allow it to anneal into an ordered lattice.

Mediation of interparticle interactions by means of coiled-coil partners is another strategy used to make P22 VLP assemblies.[51] The coiled-coil partners are a pair of charged α-helix peptides that can dimerize due to electrostatic and hydrophobic association. Fusion of one of these peptides to the CP C-terminus, in two different VLP variants, results in multivalent display of either one of the coiled-coil partners on the exterior of the capsid. Once the two variants are mixed, formation of the coiled-coil mediates strong interparticle attractions and higher order VLP assembly.

4.2. Spider Silk Protein Mediated Partially Ordered Assembly

To affect controlled, pH responsive, interparticle interactions between P22 capsids, their exterior was modified with a small protein derived from spider silk.[46] This was accomplished by genetically fusing the N-terminal domain of the spider silk protein (Ss) to Dec with a flexible linker between them. This Dec-Ss protein assembled into a trimer and the icosahedral symmetry of each capsid provided binding sites for up to 80 Dec-Ss trimers on the exterior surface.

The Ss protein undergoes a monomer−dimer transition in response to pH, forming a dimer below ∼pH 6.4. P22 capsids, decorated with the Dec-Ss, undergo reversible hierarchical assembly in response to lowering the solution pH, mediated by spider silk protein interactions between capsids (Figure 3b.i). Raising the pH causes the dissociation of the Ss proteins and disassembly of the hierarchical P22 assembly.

Dec-Ss mediated hierarchical assembly of P22 capsids showed some long-range order with some face centered cubic (FCC) close packed domains, but the majority of the structure had only short-range ordering as determined by small-angle X-ray scattering (SAXS): the positions of particles have translational symmetry with uniform interparticle distances but show a poorly developed packing structure.

4.3. Generation 6 Polyamidoamine (G6 PAMAM) Dendrimer Mediated Ordered Assembly

The assembly of P22 VLP building blocks into ordered 3D arrays can be accomplished through complementary electrostatic interactions with charged macromolecules, such as polymers and supercharged green fluorescent protein (GFP, Figure 3b.ii).[3,46] The highly negatively charged P22 VLPs (ζ-potential ∼30 mV at pH 7), when mixed with positively charged amine terminated G6 PAMAM dendrimers, results in a 3D assembly that is highly ionic strength dependent (Figure 3a.ii).[3] This assembly has a specific threshold ionic strength, above which no assembly occurs due to charge screening by ions in solution. Assembly under conditions close to the ionic strength threshold results in formation of highly ordered arrays that show a largely FCC structure by SAXS (with some hexagonal close-packed structure observed), which can also be imaged directly using transmission electron microscopy (TEM, Figure 2b). This ordering suggests that under these conditions a balance is established between VLP−dendrimer attraction and VLP−VLP repulsion, where the dendrimers can bridge dynamically between P22 VLPs allowing the structure to anneal into a thermodynamically favorable (ordered) arrangement. This process has been successfully simulated with a computational model that highlights structural ordering, the close connection between the strength and the kinetics of dendrimer−VLP association, and the role of bridging dendrimers in the self-assembly and stabilization of the P22 arrayed materials.[52] Under ionic strength conditions much lower than the threshold the VLPs and G6 dendrimers still interact strongly to form aggregates but their long-range order is significantly reduced. The assembly behavior and resultant lattice structure are a consequence of interparticle interactions between the exterior surfaces of individual particles and bridging G6 dendrimers, independent of any cargos encapsu-

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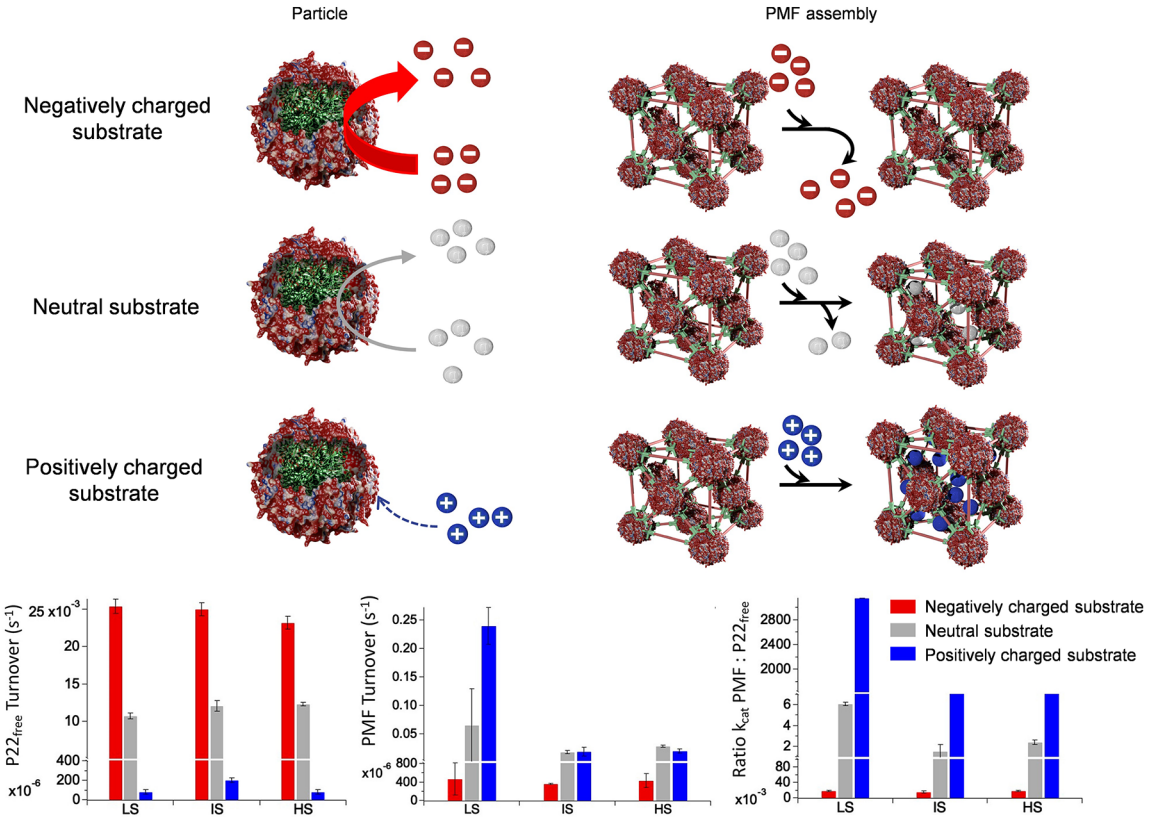

Figure 4. Enhanced catalytic efficiency and substrate selectivity of P22 nanoreactors using the collective behavior of PMFs. The catalytic rate of individual particles is fastest with negatively charged substrates and slowest with positively charged substrates. The opposite is observed for PMFs, which carry a high negative charge density, due to partitioning of positively charged substrates and exclusion of negatively charged substrates. This electrostatic-based property is most significant under low salt (LS) conditions and less significant when the charges are screened under intermediate (IS) and high salt (HS) conditions. Adapted from ref 4. Copyright 2021 American Chemical Society.

lated (such as enzymatic catalysts) within the VLPs.[3] The charge complementarity between P22 VLPs and G6 dendrimers leads to the near-neutral charge of the assembled material.

We have made a range of P22 VLP variants by incorporation of charged peptides on the exterior of each subunit to alter the particle surface charge.[52] This alteration in VLP surface charge significantly changes the threshold ionic strength for the assembly with G6 PAMAM dendrimers. Using this property, the assembled VLP lattice has been manipulated to control particle adjacency and the spatial arrangement of individual VLPs within core−shell layered assemblies.[53]

4.4. Ordered Protein Macromolecular Frameworks

The controlled ordered assembly of protein cages using dendrimers and other polymers (described in section 4.3) as templating agents can be followed by the symmetry specific binding of the ditopic Dec-Dec protein linker and results in the formation of protein macromolecular frameworks (PMFs), in which P22 VLPs are positionally maintained in an ordered 3D array but exhibit an increased interparticle spacing and associated expanded lattice parameter due to the size of the Dec-Dec linker protein (Figure 3a.iii).[47] The templating G6 dendrimer can subsequently be removed, under high ionic strength conditions, leaving the overall charge on the framework substantially negative.[4] The resultant material maintains the ordered FCC lattice structure of the P22 VLPs and is highly robust due to the ditopic Dec-Dec interparticle connectivity. These PMFs remain assembled after heating, freezing, drying, and transfer into organic solvents. These densely packed condensed-phases achieve exceptionally high protein concentrations, while still being porous to small molecules,[47] making them valuable as functional material.

5. COLLECTIVE BEHAVIOR OF P22 VLP ASSEMBLIES AND ITS UTILITY

Higher-order assembly of P22 VLPs results in porous framework materials which show some collective behaviors that are distinct from individual P22 particles. The VLP assemblies, held together by only protein−protein interactions without neutralizing the surface charges of the VLPs, results in materials with a higher charge density than the individual VLPs. This was first demonstrated in the Spider-silk mediated assembly of P22 where the assembled material accumulated and bound superpositive GFP under high ionic strength conditions, in stark contrast to the observed lack of interaction between superpositive GFP and the individual capsids at this ionic strength (Figure 3c.iii).[46] This demonstrates a collective behavior of the hierarchically assembled system not present with the individual components of the system.

Small molecules diffuse into the porous 3D assemblies as fast as into individual particles.[3] G6 dendrimer mediated assembly of P22 VLPs, encapsulating enzymatic cargos capable of carrying out multistep reactions, revealed no kinetic differences compared to the same VLPs free in solution.[3] This highlights that the proximity of cocatalysts within the arrayed material

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does not automatically provide an advantage for multistep reactions, likely because these assemblies are highly porous and small molecules can freely diffuse into and out of the 3D lattice, and thus the diffusion of intermediate species is much more rapid than the turnover rates of the enzymes.

We have constructed functional biomaterials with enhanced and selective catalytic activities based on a higher-order assembly of P22 VLPs. This is realized by the modulation of molecular diffusion into the lattice by means of the high charge density resulting from the collective behavior of the VLPs within the PMF (Figure 4).[4] The PMFs were able to discriminate between charged enzyme substrates through a process that excluded like-charged substrates and partitioned complementary charged substrates, similar to the selective binding of supercharged GFP in assembled Dec-Ss P22 materials. Due to the large interparticle distances and high charge density of the porous PMFs, multiply charged cationic macromolecules are selectively partitioned into the negatively charged PMF material creating a high local concentration of these species. When the PMF is assembled from enzyme loaded P22 VLPs, this high local concentration of the substrate can be used to drive enzymatic turnover at near maximal rates under conditions when the bulk concentration of these substrates is small. Additionally, oppositely charged substrates are actively excluded from entering the framework, resulting in a highly selective process where one substrate is massively preferred over another, based solely on charge. Furthermore, the design and construction of these materials has now reached a stage where the charge-based partitioning into the frameworks can be used to localize not only substrates but also catalysts themselves within the interstitial spaces to create complex multistep catalyst materials.

6. CONCLUSION

An appreciation of viruses, not as infectious agents but as remarkable templates for materials synthesis is highlighted by their robust self-assembly into precise well-defined architectures. We have presented our work on P22 here as an exemplary VLP that can be synthetically manipulated to package active cargo and assemble into functional materials with both short-range and long-range order. Most typically, we have reengineered the self-assembly behavior of P22 VLPs to incorporate biocatalysts (especially enzymes) and developed catalytic P22 VLP-based biomaterials across multiple length scales using biomimetic approaches, endowing the biocatalysts with emerging properties such as enhanced efficiency, substrate selectivity, heterogeneous catalysis, tunable spatial arrangement, and environmentally responsive behaviors.

Given the growing need for renewable green chemistry and environmentally friendly approaches, the biocompatible nature of VLPs makes them ideal for the development of new catalytic materials. This will continue to be a major theme of development of VLP-based functional materials, which can be achieved by tuning the hierarchical self-assembly of VLPs and mimicking naturally occurring structures. In addition, the biomedical utility of viruses and VLPs has also caught the attention of researchers and shown great potential. This field highlights approaches employing the infectious nature of viruses to battle pathogens (phage therapy),[54] and also using self-assembled VLPs as molecular nanocarriers for delivery of therapeutics (including small molecules, macromolecules, and molecular complexes), immunogenic proteins (antigens), imaging agents, as well as gene therapy.[14,55] Future challenges such as targeting, bioavailability, pharmacologic half-life, and unwanted immune responses will need to be addressed.

Even the modest number of VLPs currently used and characterized, by a growing number of researchers globally, have provided a library of possibilities each with different attributes that can be exploited. New approaches for *de novo* designed protein cages hold the promise of designer materials with controlled size, mechanical stability, cargo loading, and porosity.[11,14,56] The use of VLP-based materials in the assembly of higher-order structures is an emerging area where collective properties emerge at longer length scales. Thus, virology has provided us with inspiration as well as a framework for using virus-based building blocks for a new generation of functional biomaterials. Understanding self-assembly across multiple length scales has guided the development of these systems, together with the ability to control the properties of individual particles as well as new approaches for long-range assembly of functional materials.

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REFERENCES

(1) Selivanovitch, E.; LaFrance, B.; Douglas, T. Molecular exclusion limits for diffusion across a porous capsid. *Nat. Commun.* **2021**, *12* (1), 2903.

(2) Wang, Y.; Uchida, M.; Waghwani, H. K.; Douglas, T. Synthetic Virus-like Particles for Glutathione Biosynthesis. *ACS Synth. Biol.* **2020**, *9* (12), 3298−3310.

(3) Uchida, M.; McCoy, K.; Fukuto, M.; Yang, L.; Yoshimura, H.; Miettinen, H. M.; LaFrance, B.; Patterson, D. P.; Schwarz, B.; Karty, J. A.; Prevelige, P. E.; Lee, B.; Douglas, T. Modular Self-Assembly of Protein Cage Lattices for Multistep Catalysis. *ACS Nano* **2018**, *12* (2), 942−953.
(4) Selivanovitch, E.; Uchida, M.; Lee, B.; Douglas, T. Substrate Partitioning into Protein Macromolecular Frameworks for Enhanced Catalytic Turnover. *ACS Nano* **2021**, *15* (10), 15687−15699.
(5) Wegst, U. G.; Bai, H.; Saiz, E.; Tomsia, A. P.; Ritchie, R. O. Bioinspired structural materials. *Nat. Mater.* **2015**, *14* (1), 23−36.
(6) National Research Council *Hierarchical Structures in Biology as a Guide for New Materials Technology*; The National Academies Press, 1994. DOI: 10.17226/2215.
(7) Brooks, D. S. A New Look at 'Levels of Organization' in Biology. *Erkenn.* **2021**, *86*, 1483−1508.
(8) Diekmann, Y.; Pereira-Leal, J. B. Evolution of intracellular compartmentalization. *Biochem. J.* **2013**, *449* (2), 319−331.
(9) Whitesides, G. M.; Boncheva, M. Beyond molecules: self-assembly of mesoscopic and macroscopic components. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99* (8), 4769−4774.
(10) Mendes, A. C.; Baran, E. T.; Reis, R. L.; Azevedo, H. S. Self-assembly in nature: using the principles of nature to create complex nanobiomaterials. *Wiley Interdiscip Rev. Nanomed Nanobiotechnol* **2013**, *5* (6), 582−612.
(11) Aumiller, W. M.; Uchida, M.; Douglas, T. Protein cage assembly across multiple length scales. *Chem. Soc. Rev.* **2018**, *47* (10), 3433−3469.
(12) Selivanovitch, E.; Douglas, T. Virus capsid assembly across different length scales inspire the development of virus-based biomaterials. *Curr. Opin Virol* **2019**, *36*, 38−46.
(13) (a) Koonin, E. V.; Dolja, V. V. A virocentric perspective on the evolution of life. *Curr. Opin Virol* **2013**, *3* (5), 546−557. (b) Suttle, C. A. Marine viruses—major players in the global ecosystem. *Nat. Rev. Microbiol.* **2007**, *5* (10), 801−812.
(14) Bhaskar, S.; Lim, S. Engineering protein nanocages as carriers for biomedical applications. *NPG Asia Mater.* **2017**, *9* (4), e371.
(15) Douglas, T.; Young, M. Viruses: making friends with old foes. *Science* **2006**, *312* (5775), 873−875.
(16) Juhl, S. B.; Chan, E. P.; Ha, Y. H.; Maldovan, M.; Brunton, J.; Ward, V.; Dokland, T.; Kalmakoff, J.; Farmer, B.; Thomas, E. L.; Vaia, R. A. Assembly of Wiseana iridovirus: viruses for colloidal photonic crystals. *Adv. Funct Mater.* **2006**, *16* (8), 1086−1094.
(17) Kostiainen, M. A.; Hiekkataipale, P.; Laiho, A.; Lemieux, V.; Seitsonen, J.; Ruokolainen, J.; Ceci, P. Electrostatic assembly of binary nanoparticle superlattices using protein cages. *Nat. Nanotechnol* **2013**, *8* (1), 52−56.
(18) Parent, K. N.; Khayat, R.; Tu, L. H.; Suhanovsky, M. M.; Cortines, J. R.; Teschke, C. M.; Johnson, J. E.; Baker, T. S. P22 coat protein structures reveal a novel mechanism for capsid maturation: stability without auxiliary proteins or chemical crosslinks. *Structure* **2010**, *18* (3), 390−401.
(19) Lander, G. C.; Tang, L.; Casjens, S. R.; Gilcrease, E. B.; Prevelige, P.; Poliakov, A.; Potter, C. S.; Carragher, B.; Johnson, J. E. The structure of an infectious P22 virion shows the signal for headful DNA packaging. *Science* **2006**, *312* (5781), 1791−1795.
(20) Kang, S.; Uchida, M.; O'Neil, A.; Li, R.; Prevelige, P. E.; Douglas, T. Implementation of p22 viral capsids as nanoplatforms. *Biomacromolecules* **2010**, *11* (10), 2804−2809.
(21) Tuma, R.; Tsuruta, H.; French, K. H.; Prevelige, P. E. Detection of intermediates and kinetic control during assembly of bacteriophage P22 procapsid. *J. Mol. Biol.* **2008**, *381* (5), 1395−1406.
(22) Dokland, T. Scaffolding proteins and their role in viral assembly. *Cell. Mol. Life Sci.* **1999**, *56* (7−8), 580−603.
(23) Parker, M. H.; Brouillette, C. G.; Prevelige, P. E. Kinetic and calorimetric evidence for two distinct scaffolding protein binding populations within the bacteriophage P22 procapsid. *Biochemistry* **2001**, *40* (30), 8962−8970.
(24) Chen, D. H.; Baker, M. L.; Hryc, C. F.; DiMaio, F.; Jakana, J.; Wu, W.; Dougherty, M.; Haase-Pettingell, C.; Schmid, M. F.; Jiang, W.; Baker, D.; King, J. A.; Chiu, W. Structural basis for scaffolding-mediated assembly and maturation of a dsDNA virus. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (4), 1355−1360.
(25) Li, S.; Roy, P.; Travesset, A.; Zandi, R. Why large icosahedral viruses need scaffolding proteins. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (43), 10971−10976.
(26) Weigele, P. R.; Sampson, L.; Winn-Stapley, D.; Casjens, S. R. Molecular genetics of bacteriophage P22 scaffolding protein's functional domains. *J. Mol. Biol.* **2005**, *348* (4), 831−844.
(27) Sun, Y.; Parker, M. H.; Weigele, P.; Casjens, S.; Prevelige, P. E.; Krishna, N. R. Structure of the coat protein-binding domain of the scaffolding protein from a double-stranded DNA virus. *J. Mol. Biol.* **2000**, *297* (5), 1195−1202.
(28) Cortines, J. R.; Motwani, T.; Vyas, A. A.; Teschke, C. M. Highly specific salt bridges govern bacteriophage P22 icosahedral capsid assembly: identification of the site in coat protein responsible for interaction with scaffolding protein. *J. Virol* **2014**, *88* (10), 5287−5297.
(29) O'Neil, A.; Reichhardt, C.; Johnson, B.; Prevelige, P. E.; Douglas, T. Genetically programmed in vivo packaging of protein cargo and its controlled release from bacteriophage P22. *Angew. Chem., Int. Ed. Engl.* **2011**, *50* (32), 7425−7428.
(30) O'Neil, A.; Prevelige, P. E.; Basu, G.; Douglas, T. Coconfinement of fluorescent proteins: spatially enforced communication of GFP and mCherry encapsulated within the P22 capsid. *Biomacromolecules* **2012**, *13* (12), 3902−3907.
(31) Patterson, D. P.; Prevelige, P. E.; Douglas, T. Nanoreactors by programmed enzyme encapsulation inside the capsid of the bacteriophage P22. *ACS Nano* **2012**, *6* (6), 5000−5009.
(32) Qazi, S.; Miettinen, H. M.; Wilkinson, R. A.; McCoy, K.; Douglas, T.; Wiedenheft, B. Programmed Self-Assembly of an Active P22-Cas9 Nanocarrier System. *Mol. Pharmaceutics* **2016**, *13* (3), 1191−1196.
(33) Patterson, D. P.; Schwarz, B.; Waters, R. S.; Gedeon, T.; Douglas, T. Encapsulation of an enzyme cascade within the bacteriophage P22 virus-like particle. *ACS Chem. Biol.* **2014**, *9* (2), 359−365.
(34) Sharma, J.; Douglas, T. Tuning the catalytic properties of P22 nanoreactors through compositional control. *Nanoscale* **2020**, *12* (1), 336−346.
(35) Waghwani, H. K.; 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* (6), 2060−2072.
(36) McCoy, K.; Selivanovitch, E.; Luque, D.; Lee, B.; Edwards, E.; Castón, J. R.; Douglas, T. Cargo Retention inside P22 Virus-Like Particles. *Biomacromolecules* **2018**, *19* (9), 3738−3746.
(37) Selivanovitch, E.; Koliyatt, R.; Douglas, T. Chemically Induced Morphogenesis of P22 Virus-like Particles by the Surfactant Sodium Dodecyl Sulfate. *Biomacromolecules* **2019**, *20* (1), 389−400.
(38) Llauró, A.; Luque, D.; Edwards, E.; Trus, B. L.; Avera, J.; Reguera, D.; Douglas, T.; Pablo, P. J.; Castón, J. R. Cargo-shell and cargo-cargo couplings govern the mechanics of artificially loaded virus-derived cages. *Nanoscale* **2016**, *8* (17), 9328−9336.
(39) (a) Sharma, J.; Shepardson, K.; Johns, L. L.; Wellham, J.; Avera, J.; Schwarz, B.; Rynda-Apple, A.; Douglas, T. A Self-Adjuvanted, Modular, Antigenic VLP for Rapid Response to Influenza Virus Variability. *ACS Appl. Mater. Interfaces* **2020**, *12* (16), 18211−18224. (b) Goodall, C. P.; Schwarz, B.; Selivanovitch, E.; Avera, J.; Wang, J.; Miettinen, H.; Douglas, T. Controlled Modular Multivalent Presentation of the CD40 Ligand on P22 Virus-like Particles Leads to Tunable Amplification of CD40 Signaling. *ACS Appl. Bio Mater.* **2021**, *4* (12), 8205−8214.
(40) Jordan, P. C.; Patterson, D. P.; Saboda, K. N.; Edwards, E. J.; Miettinen, H. M.; Basu, G.; Thielges, M. C.; Douglas, T. Self-assembling biomolecular catalysts for hydrogen production. *Nat. Chem.* **2016**, *8* (2), 179−185.
(41) Lucon, J.; Qazi, S.; Uchida, M.; Bedwell, G. J.; LaFrance, B.; Prevelige, P. E.; Douglas, T. Use of the interior cavity of the P22

capsid for site-specific initiation of atom-transfer radical polymerization with high-density cargo loading. *Nat. Chem.* **2012**, *4* (10), 781−788.
(42) Kumar, M.; Altan-Bonnet, N. Viral pores are everywhere. *Mol. Cell* **2021**, *81* (10), 2061−2063.
(43) Wheeldon, I.; Minteer, S. D.; Banta, S.; Barton, S. C.; Atanassov, P.; Sigman, M. Substrate channelling as an approach to cascade reactions. *Nat. Chem.* **2016**, *8* (4), 299−309.
(44) (a) Suci, P. A.; Klem, M. T.; Arce, F. T.; Douglas, T.; Young, M. Assembly of multilayer films incorporating a viral protein cage architecture. *Langmuir* **2006**, *22* (21), 8891−8896. (b) Klem, M. T.; Suci, P.; Britt, D. W.; Young, M.; Douglas, T. In-Plane Ordering of a Genetically Engineered Viral Protein Cage. *J. Adhes* **2009**, *85* (2−3), 69−77. (c) Yoshimura, H.; Edwards, E.; Uchida, M.; McCoy, K.; Roychoudhury, R.; Schwarz, B.; Patterson, D.; Douglas, T. Two-Dimensional Crystallization of P22 Virus-Like Particles. *J. Phys. Chem. B* **2016**, *120* (26), 5938−5944.
(45) Uchida, M.; LaFrance, B.; Broomell, C. C.; Prevelige, P. E.; Douglas, T. Higher order assembly of virus-like particles (VLPs) mediated by multi-valent protein linkers. *Small* **2015**, *11* (13), 1562−1570.
(46) Aumiller, W. M., Jr; Uchida, M.; Biner, D. W.; Miettinen, H. M.; Lee, B.; Douglas, T. Stimuli Responsive Hierarchical Assembly of P22 Virus-like Particles. *Chem. Mater.* **2018**, *30* (7), 2262−2273.
(47) 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* (4), 3541−3550.
(48) Schwarz, B.; Madden, P.; Avera, J.; Gordon, B.; Larson, K.; Miettinen, H. M.; Uchida, M.; LaFrance, B.; Basu, G.; Rynda-Apple, A.; Douglas, T. Symmetry Controlled, Genetic Presentation of Bioactive Proteins on the P22 Virus-like Particle Using an External Decoration Protein. *ACS Nano* **2015**, *9* (9), 9134−9147.
(49) Tang, L.; Gilcrease, E. B.; Casjens, S. R.; Johnson, J. E. Highly discriminatory binding of capsid-cementing proteins in bacteriophage L. *Structure* **2006**, *14* (5), 837−845.
(50) Parent, K. N.; Deedas, C. T.; Egelman, E. H.; Casjens, S. R.; Baker, T. S.; Teschke, C. M. Stepwise molecular display utilizing icosahedral and helical complexes of phage coat and decoration proteins in the development of robust nanoscale display vehicles. *Biomaterials* **2012**, *33* (22), 5628−5637.
(51) Servid, A.; Jordan, P.; O'Neil, A.; Prevelige, P.; Douglas, T. Location of the bacteriophage P22 coat protein C-terminus provides opportunities for the design of capsid-based materials. *Biomacromolecules* **2013**, *14* (9), 2989−2995.
(52) Brunk, N. E.; Uchida, M.; Lee, B.; Fukuto, M.; Yang, L.; Douglas, T.; Jadhao, V. Linker-Mediated Assembly of Virus-Like Particles into Ordered Arrays via Electrostatic Control. *ACS Appl. Bio Mater.* **2019**, *2* (5), 2192−2201.
(53) Uchida, M.; Brunk, N. E.; Hewagama, N. D.; Prevelige, P. E.; Jadhao, V.; Douglas, T. Multilayered Ordered Protein Arrays Self-Assembled from a Mixed Population of Virus-like Particles. *ACS Nano* **2022**, DOI: 10.1021/acsnano.1c11272.
(54) Gordillo Altamirano, F. L.; Barr, J. J. Phage Therapy in the Postantibiotic Era. *Clin. Microbiol. Rev.* **2019**, *32* (2), e00066-18.
(55) (a) Schwarz, B.; Uchida, M.; Douglas, T. Biomedical and Catalytic Opportunities of Virus-Like Particles in Nanotechnology. *Adv. Virus Res.* **2017**, *97*, 1−60. (b) Steinmetz, N. F.; Lim, S.; Sainsbury, F. Protein cages and virus-like particles: from fundamental insight to biomimetic therapeutics. *Biomater Sci.* **2020**, *8* (10), 2771−2777. (c) Wang, Y.; Douglas, T. Protein nanocage architectures for the delivery of therapeutic proteins. *Curr. Opin. Colloid Interface Sci.* **2021**, *51*, 101395.
(56) Diaz, D.; Care, A.; Sunna, A. Bioengineering Strategies for Protein-Based Nanoparticles. *Genes* **2018**, *9* (7), 370.

https://doi.org/10.1021/acs.accounts.2c00056
Acc. Chem. Res. 2022, 55, 1349−1359