DOE Award # DE-SC0016155

Indiana University

Title: Self-Assembly of Virus Particle Based Materials for Hydrogen Catalysis

Principal Investigator: Professor Trevor Douglas
Indiana University

Co-Investigators: Dr. Masaki Uchida
Indiana University

Period Covered: 07/01/2016 - 07/31/2020

Report Date: 11/22/2020

This report has no patentable or protected data

Executive Summary

Viruses are sophisticated protein-based containers and their self-assembly from a small number of subunits into symmetrical, monodisperse architectures has inspired the work performed under this award. We have taken advantage of the robust self-assembly of the bacteriophage P22 for the encapsulation of enzymatic cargo materials and studied their activity in energy harvesting systems. The large internal cavity of the P22 is ideal for high-capacity loading of enzymes resulting in very high local catalyst concentrations. We have exploited the self-assembly properties of the bacteriophage P22 system for the packaging of a number of different enzymes, including a [NiFe] hydrogenase as well as enzymes capable of butanol biosynthesis, to create active and stable individual "nano-reactors".

Individual viral capsid-based nanoreactors are also ideal building blocks for the construction of higher order assemblies. We have demonstrated the construction of ordered 3-D arrays from active nanoparticle building blocks to form materials with collective behavior and properties beyond those of the individual particles and capable of catalyzing multi-step reactions. The successful implementation of the work performed under this DOE grant provides us with a great deal of knowledge about directed self-assembly at multiple lengthscales for the development of bioinspired catalytic materials for energy harvesting.

Comparison of the actual accomplishments with the goals and objectives of the project.

The project has been successful in the development of a new class of active materials, and in laying out the 'ground-rules' for directed assembly across multiple lengthscales. We have demonstrated that the aims originally proposed could be accomplished. The Aims of the Project were 1) Directed assembly of hydrogenase (Hyd-1) encapsulated within P22 virus-like particles (P22-Hyd) 2) Directed assembly of P22 VLPs into 3-D superlattice arrays 3) Directed assembly of P22-Hyd nanoreactors: probing the relationship between the structure of 3-D superlattice arrays and activity.

We have demonstrated the directed encapsulation of multiple active enzymes (including the Hyd-1 hydrogenase) within the P22 cage. We identified domains on the P22 capsid which are exposed to the exterior environment and demonstrated that we could use this to functionally manipulate the capsid. Additionally, we demonstrated the assembly of P22 capsids into ordered long range arrays that was achieved through the use of a charged polymer molecules that effectively crosslink the individual P22 capsids into an ordered bulk material. These materials retain the catalytic activity of the individual particles, and the coupled catalytic activity of multiple nanoreactors within the lattice. Additional approaches, which took advantage of a spider silk derived protein attached to the P22 VLPs, demonstrated the programmed assembly of VLP superlattices in response to environmental cues such as changes in pH and ionic strength.

The work demonstrating the encapsulation of a coupled electron transfer system, from a cytochrome b_5 to the hydrogenase enzyme, is not quite complete – the enforced interruption to the experimental work, by COVID-19, meant that the project was shut down for approximately 4 months. We anticipate completing the necessary experiments and submitting this work for publication in the near future.

ACCOMPLISHMENTS

1) Directed assembly of hydrogenase (Hyd-1) encapsulated within P22 virus-like particles (P22-Hyd) (Aim 1)

We have demonstrated the directed encapsulation of a range of enzymes with relevance to energy conversion and utilization inside the capsid of P22. The C-terminal region of the scaffold protein is required for the templated assembly of the P22 capsid and the N-terminal region of the scaffold protein can be truncated or fused to other proteins without loss of assembly. This results in sequestration of the fused cargo on the interior of the capsid at high packing density.

We have demonstrated the encapsulation of two enzymes that share a substrate-product relationship (ie the product of the first enzyme is the substrate for the second enzyme). Thus, we encapsulated a decarboxylase enzyme (KivD), which decarboxylates keto acids to aldehydes , and an alcohol dehydrogenase (AdhD), which reduces the aldehyde to alcohol using NADH and results in the formation of iso-butanol. This coupled series of reactions represents a portion of a synthetic metabolic pathway for the formation of iso-butanol and highlights the potential of this approach for directed synthesis of designed cascade reactions for energy applications. Synthesis was performed at the level of molecular biology and these unique P22 nanoreactors self-assemble in E. coli in a highly reproducible manner. Importantly this work demonstrates that it is not necessary to co-encapsulate all enzymes in a synthetic pathway into a single capsid and this suggested a way forward for the construction of complex coupled enzymatic systems in materials applications.

A similar approach using the SP fusion has been successfully applied to the co-encapsulation of the two subunits of the E. coli [NiFe] hydrogenase (Hyda and HydB). The resulting P22 nanoreactors self-assemble in E. coli, package up to 200 copies of the heterodimeric enzyme in each P22 capsid and are air stable. The enzyme very efficiently catalyzes the formation of H_2 (measured by gas chromatography) using reduced methyl viologen as a redox mediator.

As an extension of this P22-encapsulated hydrogenase design, we functionally integrated a biological electron mediator (cytochrome b_5; Cb_5) and its associated reductase (NADH-dependent cytochrome b_5 reductase; Cb_5r) with the EcHyd-1 hydrogenase - all encapsulated within the P22 capsid. The Cb_5 contains a heme center that is reduced by its natural redox partner, Cb_5r. The monomeric Cb_5r is an NADH-dependent flavoprotein that naturally catalyzes the two-electron transfer from NADH to the heme containing Cb_5. Inspired by the orientation and proximity of the cytochrome b on the C-terminal extension of the EcHyd-1 small subunit, we used molecular biology to attach the Cb_5 to the EcHyd-1 small subunit, by placing the gene for the Cb_5 in between the scaffold protein (SP) and the small subunit of the hydrogenase with a flexible linker. In the EcHyd-1 dimer, the hyaB N-terminus points away from the C-terminus. Therefore, the redox partner of the Cb5, the NADH-dependent cytochrome b_5 reductase, was cloned onto the C-terminus of hyaB, with a small flexible linker. The resultant material catalyzes the overall production of H_2 from NADH. This approach exemplifies the advantages of co-localizing a multi-component system using a bacterial expression system.

2) Directed assembly of P22 VLPs into 3-D superlattice arrays (Aim 2)
The assembly of the P22 VLPs into an ordered superlattice having a face-centered cubic (FCC) lattice structure, can be mediated by charged macromolecules, poly(amido amine) (PAMAM) dendrimers. We investigated dendrimer mediated assembly of P22 VLPs into large arrays (a few μm in size) in solution, under a range of ionic strength (I) conditions upon addition of a positively charged dendrimer (G6). The surface of the P22 has a net negative charge (measured zeta potential for wild type P22 is –30.7 ± 2.3 mV) and thus the positively charged PAMAM dendrimers electrostatically bridge the P22 VLPs, mediating the assembly. While extended arrays of wild type P22 (wtP22) and G6 dendrimer formed below the threshold ionic strength of 247 mM, (we define an ionic strength above which P22 VLPs are no longer assembled as a threshold ionic strength, I_t), broad peaks observed in the small angle x-ray scattering (SAXS) profiles of these wtP22-derived samples suggest that they do not have long-range order; strong electrostatic attractions alone leads to kinetically trapped amorphous aggregates, which do not allow rearrangement of particles (annealing) within the assembly.

An extension of this work has allowed us to successfully construct hierarchically organized protein arrays with a core-shell architecture by exploiting two different populations of P22 VLP mutants, P22-E2 and P22-K2, which each possess different surface charges. We analyzed the structure of the VLP arrays using SAXS and transmission electron microscopy. SAXS measurements revealed that when the lattice was formed at ionic strength (I) close to the threshold ionic strength, it had a face centered cubic (FCC) structure with the lattice parameter of 88.6 nm. When the ionic strength of the sample was reduced to 41 mM by dialysis, the array contracted and lattice parameter decreased to 86.3 nm (Fig 1c). This expansion and contraction of the lattice was reversible and increasing the ionic strength restored the original lattice constant.

Furthermore, we have established a collaboration with Dr Vikram Jadhao (at IU) to develop a coarse-grain computational method to model the hierarchically organized assembly of these VLPs. In this method, we modeled the assembly of two types of spheres representing P22 VLPs and the G6 dendrimer as a function of changing ionic strength. At high ionic strength ($I = 300\ mM$), no prominent assembly was observed. Exclusive assembly of E2 occurs at its ionic strength at 225 mM. Pair correlation function of an assembled cluster indicate that it is well ordered. Layered co-assembly occurred upon further dialysis to the lower ionic strength to 100mM. The formation of core (E2)-shell (K2) cluster with ordered structure agree well with experimental observations. In addition, it should be noted that tightening of the order of the E2 core due to strengthening of the E2-dendrimer bonds was observed in the computational model, that is also consistent with the results of SAXS analysis.

3) Directed assembly of nanoreactors: probing the relationship between the structure of 3-D superlattice arrays and activity (Aim 3)
The electrostatic mediated assembly of ordered P22 VLP superlattices described above was a consequence of interparticle interaction between exterior surfaces of individual particles, and thus independent of the enzyme cargos encapsulated within the VLPs. These superlattice materials, composed of two populations of enzyme packaged VLP modules, retained coupled catalytic activity in a two-step reaction. Two different enzymes, Ketoisovalerate Decarboxylase (KivD) and Alcohol Dehydrogenase A (AdhA), which perform a consecutive two-step reaction, were separately encapsulated inside of P22 VLPs (P22-KivD and P22-AdhA, respectively). KivD catalyzes the decarboxylation of keto acids to aldehydes, which are subsequently reduced to alcohols by AdhA using NADH. We demonstrated modular self-assembly of two populations of enzyme encapsulated P22 VLP, i.e. P22-KivD and P22-AdhA, into an ordered superlattice materials having an ordered FCC structure (analyzed by SAXS). The two separately

encapsulated enzymes retained their catalytic communication (i.e. the product of the first reaction is the substrate for the second enzyme) and together catalyze a coupled two-step reaction resulting in the production of isobutanol. Two interesting insights came out of the kinetic analysis; a) the formation of the superlattice did not result in enhanced overall turnover of the two step reaction- likely because small molecule diffusion is fast relative to the turnover rate of the enzymes and b) that the superlattice material did not inhibit the diffusion of small molecules i.e. was porous to small molecule diffusion. Because the superlattices are bulk materials, they are readily condensed and the bulk water of solvation can be removed without changing the local environment of the enzymes encapsulated inside the VLPs; the catalytic conversion is significantly accelerated in the condensed phase. The superlattices can be reused at least three times without loss of activity. We also demonstrated selective and reversible assembly and disassembly of two populations of VLP nanoreactors by modulating the surface charge of the VLPs and the ionic strength of the solution. This study demonstrates a significant step toward the bottom-up fabrication of functional superlattice materials using a self-assembly process, across multiple length scales, and exhibits properties and function that arise from the interaction between individual building blocks.

In a different approach, we demonstrated controlled assembly (and disassembly) of arrayed P22 VLPs into 3D materials responsive to two environmental stimuli, pH and ionic strength of the solution. We showed P22 VLPs functionalized with a spider silk protein derivative, on the capsid exterior, assemble into a hierarchical structure due to spider silk/spider silk interactions at low pH and reversibly dissembled by raising the pH. We also showed that the capsid arrays can be assembled through electrostatic interaction of the negatively charged capsids with a positively supercharged GFP mutant, which can be reversed by raising the ionic strength. Most notably, we found that the supercharged GFP could bind to the hierarchically assembled material under high salt conditions, but not to the individual capsids at high salt conditions. The binding of the charged macromolecule under high salt conditions demonstrates a collective behavior of a hierarchically assembled system not present with the unassembled, individual components of the system. The formation of 3D assemblies of functional P22 VLP, mediated by the spider silk protein has been successfully achieved allowing us to form highly condensed, connected and ordered materials upon change in the solution pH from 7 to 5. However the P22 morphology required for this is an expanded version of P22, which requires heat to initiate. The heat treatment damages the Hyd-1 so we adopted a more gentle approach by fusing spyTag to the c-terminus of P22 (which projects to exterior of the capsid) and spyCatcher to the Spider silk protein. Thus, the Spider silk protein can be covalently attached to the exterior of P22 using the spyTag-spyCatcher system and exhibits similar pH dependent assembly of individual capsids into a bulk material.

4) Directed assembly of nanoreactors within nanoreactors – hierarchical nested structures.

Our efforts to construct hierarchically assembled structures have also been directed to engineering individual VLPs which have multiple levels of compartmentalization. We have developed two different types of P22 VLPs possessing hierarchically organized structures inside of VLPs. First, we demonstrated the fabrication of a nested “cage-within-a-cage” structure as a synthetic organelle mimic. This was accomplished through the construction of a nested protein cage system in which multiple copies of smaller ferritin (Fn) cage (12 nm) were encapsulated within a larger (56 nm) P22 VLP. Sequential, temporal expression from two vectors was adopted to ensure assembly of Fn cage into its cage-like architecture prior to encapsulation inside the P22 VLP. A Fn mutant with the P22 scaffolding protein (SP) on its exterior, which templates the assembly of P22 coat protein (CP) into a VLP, and the P22 CP were sequentially expressed. SDS-PAGE analysis on purified VLPs confirmed the presence of two protein bands, with the molecular weights of Fn-SP and CP subunits. P22-Fn particles, indistinguishable from wtP22 VLP, were observed by TEM and molecular weight assessment by size-exclusion chromatography coupled with multiangle light scattering (SEC-MALS) suggested that approximately 14 Fn cages were encapsulated inside of P22 VLP. We further analyzed the VLP construct by single particle cryo-electron tomography (cryo-ET) to confirm the formation of the P22-Fn nested structure and verify encapsulation of the smaller Fn-cage inside P22. Cryo-ET reconstruction of P22-Fn VLPs clearly showed the presence of small cage-like densities inside the approximately 57 nm P22 VLP and confirmed the formation of hierarchically organized nested structure. Furthermore, we demonstrated the co-encapsulation of multiple (active) copies of the CelB an enzyme (cellobiose-hydrolyzing β-glycosidase) together with Fn sub-compartments inside of the P22 VLP, mimicking the macromolecular complexity within a cell-like environment.

These approaches of constructing hierarchically organized VLP could lead to develop synthetic protein-based protocells. Furthermore, by combining our ability of engineering catalytically active VLPs and controlling higher order assembly of VLP building blocks into hierarchically organized arrays would lead to fabrication of protein-based catalytic array materials which can catalyze a multi-step reaction with optimized efficiency.

PRODUCTS

Publications

1. William M. Aumiller Jr, Masaki Uchida, Daniel W Biner, Heini M Miettinen, Byeongdu Lee, Trevor Douglas "Stimuli Responsive Hierarchical Assembly of P22 Virus-Like Particles" Chem Materials (2018) 30, 2262-2273. DOI: 10.1021/acs.chemmater.7b04964.
2. William M. Aumiller, Jr., Masaki Uchida, and Trevor Douglas "Protein cage assembly across multiple length scales" Chemical Society Reviews, (2018), 47,3433-3469. DOI: 10.1039/C7CS00818J
3. Masaki Uchida, Kimberly McCoy, Masafumi Fukuto, Lin Yang, Hideyuki Yoshimura, Heini M. Miettinen, Ben LaFrance, Dustin Patterson, Benjamin Schwarz, Jonathan A. Karty, Peter E. Prevelige Jr., Byeongdu Lee, Trevor Douglas "Modular self-assembly of protein cage superlattices for multistep catalysis and material regeneration" ACS Nano (2018) 12, 942-953. doi: 10.1021/acsnano.7b06049
4. Kimberly McCoy, Masaki Uchida, Byeongdu Lee, Trevor Douglas "Templated Assembly of a Functional Ordered Protein Macromolecular Framework from P22 Virus-Like Particles" ACS Nano (2018) 12, 3541-3550. doi: 10.1021/acsnano.8b00528.
5. 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. doi: 10.1016/j.coviro.2019.02.010. PMID: 31071601; PMCID: PMC6729134.
6. Nicholas E. 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 Applied Bio Materials (2019). DOI: 10.1021/acsabm.9b00166
7. Waghwani, H. K., Uchida, M., Fu, C., LaFrance, B., Sharma, J., McCoy, K., Douglas, T. Virus-Like Particles (VLPs) as a Platform for Hierarchical Compartmentalization. Biomacromolecules (2020) 21, 2060-2072. DOI: 10.1021/acs.biomac.0c00030

Manuscripts in preparation:

Uchida, M., Nicholas E. Brunk, N. E., Lee, B., Jadhao, V., and Douglas, T "Multilayered Core-Shell Assembly of VLPs into Ordered 3D Lattices"

Hewagama, N, Jordan, P., Douglas, T. "VLP-based Encapsulation of Biological Electron Transfer Mediator Together with Hydrogenase for efficient Bio-Synthesis of H_2"

Patents

Nothing to report

3. SUPPORTED PERSONNEL

	Calendar Months of Support			
	Year 1	Year 2	Year 3	Year 4 (NCE)
Trevor Douglas (PI)	0.69	0.77	0.62	0.38
Masaki Uchida (co-PI)	3.0	6.0	6.0	1.0
Hitesh Waghwani (graduate student)	-	-	0.5	6.0
Nathasha Hewagama (graduate student)	-	-	0.5	6.0
Morgan Elkins (technician, hourly)				7.0
Cheri Goodall (technician)				3.0
Kelsey Godsey (undergraduate student, hourly)				2.0