Virus Capsids: A Starting Point for Precisely Tunable Nanoscale Containers

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The encapsulation of biological molecules at the nanoscale has important applications from drug delivery to the scaffolding of engineered metabolic pathways. There are many options for the nano-sized container, but protein-based containers such as viral capsids and bacterial microcompartments are extremely promising for the precise control over production and assembly that these systems offer. Great strides have been made in controlling the encapsulation of enzymes within such systems, yet much is still unknown about how small molecules can move into and out of these protein shells. Moreover, the choice of the viral capsid or microcompartment currently dictates the physical size and shape of the container, as such assemblies are not easily designed de novo. Here we demonstrate that protein engineering can be used to alter MS2 viral capsid permeability and size. We first show that charge interactions enable encapsulation of enzymes such as the reporter alkaline phosphatase [1], which is in turn used to explore the impact of altering the residues that line pores within the capsid shell. We find that manipulating the charge of the pores indeed affects the diffusion-limited reaction kinetics, supporting the hypothesis that such containers can be used to concentrate or sequester intermediates [2]. We next applied directed evolution to develop an MS2-based virus-like particle with a decreased size. Surprisingly, just a single amino acid change is sufficient to alter capsid geometry from a T=3 icosahedron to a T=1 icosahedron, and decrease the diameter of this protein cage from 27 nm to 18 nm. The altered geometry is confirmed by the crystal structure of the mutated VLP. To our knowledge, this is the first time such a remarkable change in geometry and size of a VLP was achieved with a single mutation. Coupled with our study of the pores, these results are quite encouraging: despite the tightly coordinated assembly of protein-based containers, they are highly permissive of changes to the compartment-forming protein and are tunable structures with respect to both size and permeability. This presentation will detail the methods used to engineer the MS2 capsid and discuss the implications of the malleability of these structures.

[1] Glasgow J.E., Capehart S.L., Francis M.B., Tullman-Ercek D. (2012) “Osmolyte-mediated encapsulation of proteins inside MS2 viral capsids.” ACS Nano **6**(10):8658-64.

[2] Glasgow J.E., Jakobson C.M., Tullman-Ercek D. (2015) “The Influence of electrostatics on small molecule flux through a protein nanoreactor.” ACS Synthetic Biology **4**(9):1011-9.