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Engineering the Self-Assembly of Bacterial Microcompartment Shell Proteins via Charged Mutations

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Protein self-assembly is a fundamental biological process of great importance for the design and synthesis of biomaterials. Developing the ability to precisely manip ulate protein assembly would greatly expand both our understanding of the process and our biotechnological capabilities. We demonstrate that altering hexamer charge offers a systematic strategy for modulating the higher-order assembly of hexameric proteins PduA and PduJ ,within Pdu bacterial microcompartments (MCPs), across multiple contexts by integrating molecular dynamics simulations with heterologous overexpression, cell-free, and in vivo *Salmonella enterica* serovar Typhimurium LT2 experiments. By integrating heterologous and cell-free expression studies with native co-assembly experiments, we demonstrate that charge-altering variants provide a systematic framework for modulating the morphology of hexameric proteins within MCPs. Molecular dynamics simulations elucidate the molecular-level interactions governing these assemblies. Specifically, in tubular structures, our results reveal a robust correlation between overall stability ,hydrogen bonding, and salt-bridge persistencey, highlighting the role of curvature in modulating these stabilizing interactions. Our results collectively reveal that both electrostatic interactions and fields generated by charges on proteins can be leveraged to control protein-based nanostructures. This work lays the groundwork for designing and controlling protein-based nanostructures to expand the versatility of bio-based materials.

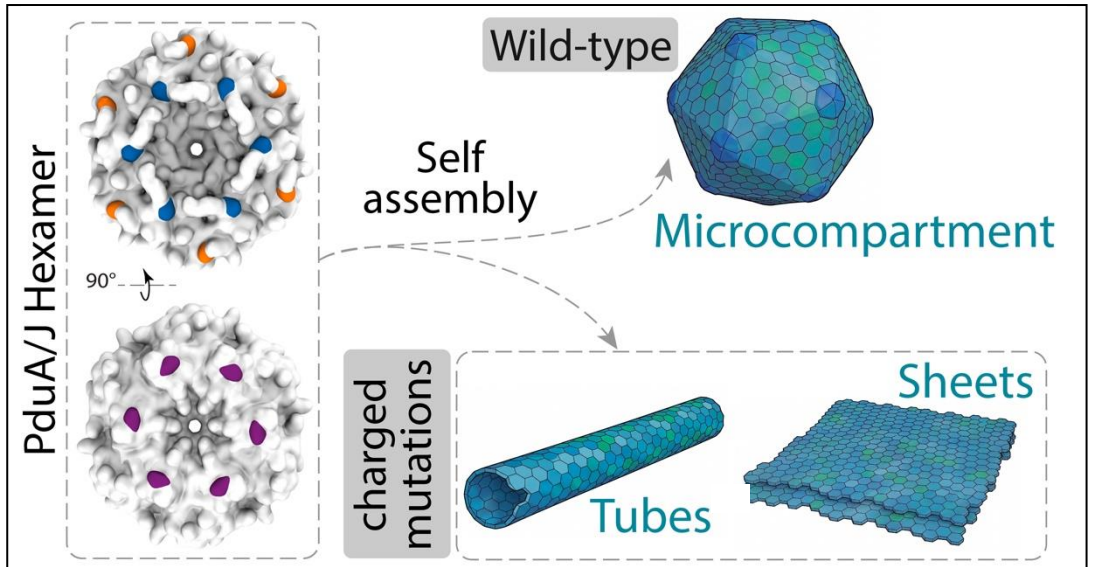

Figure: ***Concave-out and convex-in faces of the PduA/J hexamer.*** *These hexamers oligomerize with other shell and core proteins to build the polyhedral shell of the bacterial microcompartment. When PduA/J are overexpressed, they self-assemble into tubes and sheets of varying chirality and diameter.*