Abstract

Ferritin is present in almost all kingdoms of life and is responsible for storing and regulating iron homeostasis inside the cell. It forms a cage-like structure of 12 nm with a hollow cavity of 8 nm. This cavity can be utilized for various purposes, such as encapsulation of sensitive drug molecules, to display antigen (vaccine), MRI contrast agent, quantum dots etc. However there is a fundamental limitation with the human/traditional ferritins: they cannot be opened and closed easily, and usually require harsh chemical treatments that are not suitable for sensitive cargo molecules. In the first part of the thesis, I studied a recently discovered *Thermotoga maritima* ferritin (TmFtn), and engineered it for differential assembly and stability. The wild-type TmFtn dimer requires 50 mM $MgCl_2$ to assemble into a complete 24-mer cage-like structure. Through structural and bioinformatics analysis of wild-type TmFtn, a crucial position, E65 was identified between the dimeric interface. Changing a single residue at this position can change the entire cage assembly and stability behavior. Five variants were created and two of them (E65K and E65R) showed assembly without any external triggers (metals/salts/$MgCl_2$). While three mutations (E65A, E65D and E65Q) showed assembly at a lower $MgCl_2$ concentration (10 mM). These engineered cages are capable of encapsulating cargo molecules under mild conditions without disrupting the native function (i.e. iron mineralization). To understand the cage assembly mechanism these mutants were characterized using analytical SEC, native PAGE, X-ray crystallography, and TEM imaging. DNA origami, with its ability to design and construct a wide array of 2D and 3D structures, offers a highly programmable approach. However, its ability to encapsulate small molecules in a leakage-free manner has been limited. On the other hand, ferritin, a protein cage, can efficiently encapsulate a number of small therapeutic molecules with high density, but its modifiability is limited by factors such as its high symmetry. In the second part of the thesis, I have combined these two approaches of creating a hybrid structure that leverages the strengths of both. The open DNA origami container can specifically bind a single modified, cargo-carrying ferritin within its cavity. This is achieved through base pairing between single-stranded DNA sequences in the DNA origami and complementary sequences attached to the surface of the ferritin via copper-free click chemistry. The dimension of this DNA origami glove structure is 40 nm × 40 nm × 14 nm with a central 20 nm × 20 nm × 11 nm cavity. The produced DNA glove and conjugate structure was confirmed with gel based assay and with direct observation using atomic force microscopy (AFM) and transmission electron microscopy (TEM).