Analysis of Dynamic Behavior of Aromatic Interactions using Protein Cages

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Aromatic clusters formed by multiple aromatic residues are important in biological systems such as folding and channel gating.[1] Recent spectroscopic studies have implied that aromatic residues exhibit cooperative dynamic behavior when clustered;[2] however, it is still difficult to assess physical properties of aromatic clusters and relevance to their structural correlation at the atomic level. To address these issues, we performed a) systematic construction and structural determination of aromatic clusters using protein cages, and b) analysis of aromatic interactions during the unfolding process from a dynamic perspective.

Aromatic ferritin cages (**Fr-F4in**, **Fr-F4ex** and **Fr-F6**) were designed by introducing phenylalanine residues at the two-fold symmetric interface of the cage (Fig 1) by following our previous design.[3] The X-ray crystal structural analysis showed aromatic cluster formation at inter and intra-subunit. The results of Circular Dichroism (CD) and Differential Scanning Calorimetry (DSC) showed that **Fr-F4ex** has lower thermal stability than **Fr-F4in**, which highlights the influence of geometry and location of the aromatic clusters. Furthermore, the thermal measurements suggested that the thermal disassembly and unfolding process of the ferritin cages undergoes multiple transient states. This study would provide insights into the unique physical properties of aromatic interactions and guidelines for designing aromatic interactions in a protein cage.

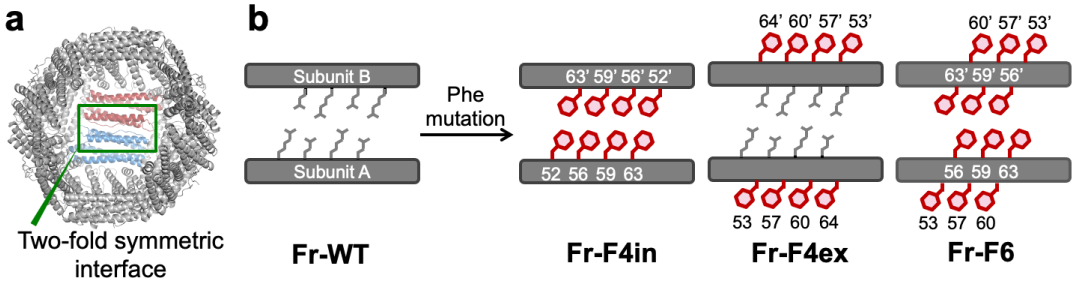

Fig 1. (a) The structure of a ferritin cage and the two-fold symmetric interface (PDB ID: 1DAT). (b) Design of aromatic ferritin mutants by introducing phenylalanine residues.

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