

## Star-shaped Supramolecular Assembly Formed by the Conjugation of Hexameric Tyrosine-coordinated Hemoprotein with Cytochrome *b*<sub>562</sub>

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Various artificial supramolecular protein assemblies have emerged within the past decade due to its variety in structures and bio-functionalities which can be used for applications.<sup>1</sup> Biological and chemical strategies are used to create such artificial assembly systems driven by the naturally existing protein–protein, protein–ligand/cofactor, protein–compound, and receptor–ligand interactions.<sup>2</sup> In this context, we have successfully reported a number of protein-assembling system using hemoproteins such as cytochrome *b*<sub>562</sub> (Cyt *b*<sub>562</sub>), and hexameric tyrosine-coordinated hemoprotein (HTHP) with a highly symmetric structure.

In our recent studies, the attachment of an artificial heme onto the surface of the Cyt *b*<sub>562</sub> mutant, followed by removal of the prosthetic heme provided a rigid linear assembly via heme–heme pocket interaction and secondary hydrogen bond interaction.<sup>3</sup> In this work, we focused on the conjugation of this Cyt *b*<sub>562</sub> assembly with the apo-form of HTHP towards a star-shaped protein assembly (Figure 1). Initially, the transfer of the heme cofactors from Cyt *b*<sub>562</sub> into the apo-form of HTHP was observed by the mixing of these two proteins, showing the possibility of this strategy. Sequentially, the reaction mixture of the heme-mediated linear Cyt *b*<sub>562</sub> assembly and apo-HTHP at 45 °C forms a new assembly, which is confirmed by size exclusion chromatography. The further evaluation and analysis of the resulting assembly were carried out by SDS PAGE, UV-Vis spectroscopy, and dynamic light scattering.

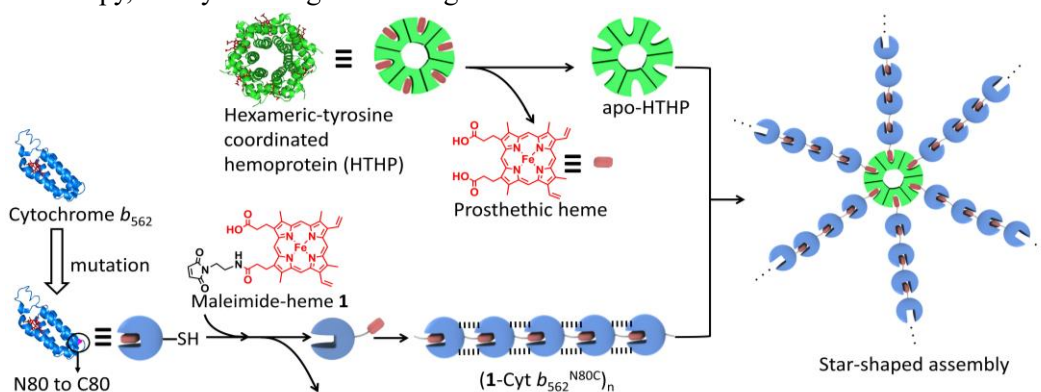


Figure 1. Schematic representation of star-shaped assembly of hemoproteins.

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