Energy Transfer in a Disulfide Bond-Mediated Heterodimer Consisting of a Fluorescent Protein and a Hemoprotein

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Inter-protein resonance energy transfer between the green fluorescent protein and its color variants has been widely reported toward practical applications such as biomarkers and biosensors.[1] Genetic fusion of multiple proteins has been a common approach in these applications, because this method allows the donor component to be in close proximity towards its acceptor to enable energy transfer.[2] In this work, the green fluorescent protein (GFP) is used as a donor protein, and cytochrome b_{562} (Cyt b_{562}), a simple electron transfer hemoprotein, as an acceptor protein.

A disulfide bond is employed for the covalent linkage of the donor and acceptor proteins. In general, selective heterodimerization via disulfide bond is difficult. However, the rapid thiol–pyridyl disulfide exchange reaction[3] allows the selective heterodimerization. First, site direct mutagenesis was carried out for the insertion of cysteine residues on GFP and Cyt b_{562} at K26 and N80 positions, respectively, as illustrated in Fig. 1, resulting in GFP^{K26C} and Cyt b_{562}^{N80C} mutants. Next, the 2,2'-dipyridyl disulfide was reacted with Cyt b_{562}^{N80C} providing an attached pyridyl disulfide moiety, and then the obtained protein selectively conjugated with GFP^{K26C}. The heterodimer was purified and characterized by SDS-PAGE, size exclusion chromatography, and UV-vis spectroscopy. The fluorescence quenching efficiency in the heterodimer was determined to be 87%. Furthermore, a much shorter fluorescence lifetime of the heterodimer was observed relative to the GFP^{K26C} monomeric protein, suggesting rapid energy transfer.

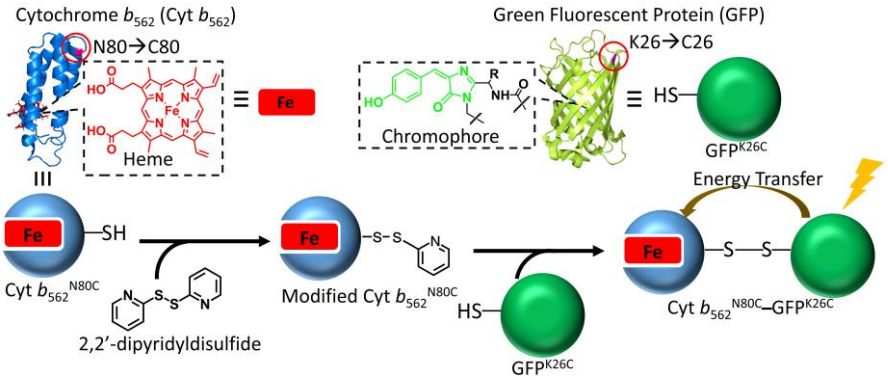

Figure 1. Schematic representation for heterodimerization of Cyt b_{562}^{N80C} and GFP^{K26C}.

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