Protein-Chromophore Interaction and its Influence on the Photophysics of Chromophore: Insights from Solvatochromic Analysis

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Artificial photosynthesis inspired by nature merits its place in the holy grail of chemistry.[1] Photosynthesis is the highly efficient sustainable process accessible for converting light to chemical energy. The initial process of photosynthesis involves light absorption by chlorophyll pigment present in the light-harvesting complex II (LHII). The subsequent process consists of relaying the absorbed energy to the reaction center through light-harvesting complex I (LHI) in a few hundreds of picoseconds (10^{-12} s) with almost 100% efficiency.[2]

The funneling of energy from the antenna complexes (LHII) to the reaction center through LHI has been proposed by Fö rster resonance energy transfer (FRET). Thus, understanding the energy transfer mechanism in photosynthetic complexes has been an active area of research. The assemblies of this multichromophoric system in the protein scaffold improves the energy transfer efficiency in the manifold, like modulating the spatial and energy landscape of this chromophore system.[2] For example, for the typical concentrations of chlorophyll found in photosynthetic complexes, the fluorescence of the donor chromophore will be quenched in solution. Furthermore, for an efficient FRET, the donor and acceptor chromophore absorption and emission spectral overlap are quintessential. Thus protein's heterogeneous environment interacting with the chromophore provides necessary spectral overlap and facilitates efficient long-range energy transfer.

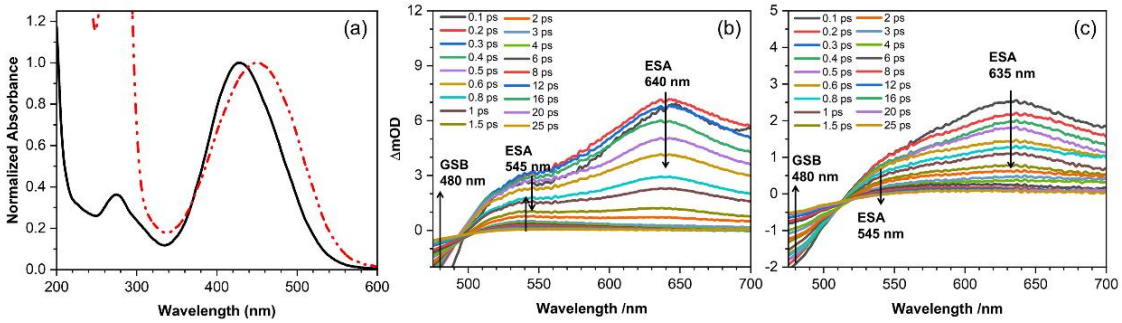

Figure 1. (a) UV-visible absorption spectra of Disperse Orange-3 in acetonitrile solution (dashed-dotted red curve) and 10% v/v of acetonitrile HEPES buffer (pH - 7) solution with α-helical heptamer barrel (solid black curve). Time-resolved electronic absorption spectra (450 nm pump excitation) of DO3 in (a) neat acetonitrile solution and (b) 10% v/v of acetonitrile HEPES buffer (pH - 7) solution containing α-helical heptamer barrel. The insets display spectra at various pump-probe time delays. The absorption band at 640 nm is assigned to the first singlet excited state (S_1), the band at 545 nm at later time-delays corresponds to the cis-stilbene in the ground electronic state, and the negative band below 500 nm is due to the ground state bleach.

A novel approach to artificial photosynthesis is to use antennas comprising different classes of organic chromophores, which can undergo photoinduced energy, electron transfers, and isomerization assembled in the synthetic α-helical protein barrels (α-HPBs). This presentation will discuss the binding interaction of the chromophore, Disperse Orange-3 (DO3), with α-HPBs and how binding modulates its photochemistry. The UV-visible spectroscopy studies (Figure 1a) confirmed the binding of DO3 with α-HPBs and corroborated by UV-circular dichroic absorption spectroscopy and molecular docking studies. A detailed solvatochromic analysis[3-4] of DO-3 and its dimethyl derivative (DM-DO3) indicates that the amino group of DO3 binds with the protein barrel.

The time-resolved electronic absorption spectroscopy investigations of DO3 in different solvents of diverse polarity revealed ultrafast photo-isomerization within sub-picosecond timescale (See Figure 1b). The ultrafast photoisomerization of DO3 through conical intersection leads to formation of both *cis* and *trans* configurations of DO3 in the ground electronic states but vibraionaly hot. The relaxation of this non-equilibrium product mixture of *cis* and *trans* form of DO3 occurs in tens of picosecond timescale depending on the solvent polarity and viscosity. The ground state bleach remains unrecovered over the timescales of our experimental time window of 8 ns due to slow thermal equilibriation of the *cis-trans* forms of DO3 (typically in milliseconds). Time-resolved studies of DM-DO3 in different solvents also yielded similar results indicating that the dimethyl derivative had altered the static electronic absorption spectra but the ultrafast dynamics remains the same.
Interestingly, the ultrafast dynamics of DO3 in α-HPBs also showed similar kinetics (see Figure 1c) and photoisomerization mechanism despite the fact that DO3 is constrained in the α-HPBs relative to the neat solvents. This indicate that the *trans-cis* isomerization can occur through N=N inversion rather than conventional rotation of the N=N bond. The kinetics and mechanism of photoisomerization of DO3 in α-HPBs of different barrel size will be discussed in the presentation.

References:

1. Allen J. Bard, George M. Whitesides, Richard N. Zare & Fred W. McLafferty, *Acc. Chem. Res.* **28**, 91 (1995).
2. Scholes, G. D., Graham R. Fleming, Alexandra Olaya-Castro & Rienk van Grondelle, *Nat. Chem.* **3**, 763 (2011).
3. R. K.Venkatraman & A. J. Orr-Ewing, *J. Am. Chem. Soc.* **141**, 15222 (2019).
4. R. K.Venkatraman & A. J. Orr-Ewing, *Acc. Chem. Res.* **54**, 4383 (2021).