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# Photoinduced Electron Transfer between Non-Native Donor–Acceptor Moieties Incorporated in Synthetic Polypeptide Aggregates

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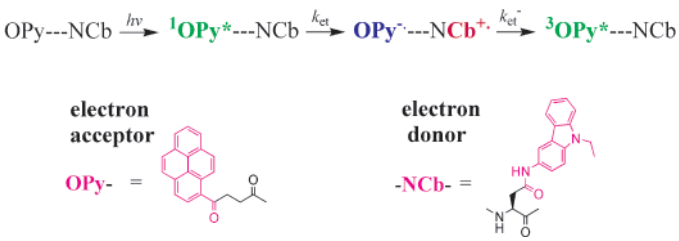
## ABSTRACT




**A series of analogous photoactive polypeptides that form helical homo and hetero aggregates in aqueous media were prepared. A pyrenyl ketone (the principal chromophore and an electron acceptor) was attached to the N-termini. An electron donor, amidoethylcarbazole, was introduced as a side chain of a non-native amino acid, NCb, 14 residues away. Photoinduced electron-transfer rate constants of the order of $10^8$ $s^{-1}$ between remote pyrene and carbazole were measured.**

Investigation of synthetic photoactive polypeptides is important to the understanding of the mechanism of initial charge separation in photosynthesis and of other redox processes in natural systems.[1] Here, we describe the design and photophysical properties of a 32-residue amphipathic polypeptide, TT2o (Scheme 1).[2] The principal chromophore, a pyrenyl ketone (OPy), was placed at the N-terminus. An electron donor, 3-amido-9-ethylcarbazole, was introduced as a side chain of a non-native asparagine derivative, $N_4$-(9-ethyl-9*H*-carbazol-3-yl)-L-asparagine (NCb), 14 residues (49 $\sigma$-bonds) away from the pyrenyl ketone (see Supporting Information for synthetic details). For comparative electron-transfer studies, polypeptide analogues lacking an electron donor (containing asparagine (Asn), instead of NCb) were prepared (e.g., TT2oX15N).

At physiological pH, the 32-residue TT2o contains nine positive and nine negative charges. Three of the positively charged residues (two Orn and a Lys) are situated at the N-terminus, while the rest of the charged amino acids, are distributed throughout the polypeptide sequence (Scheme 1). Although such a surface charge distribution creates a field co-directional with the helix dipole and destabilizes the expected secondary structure, concomitantly, it will reinforce the role of the helix dipole moment in propelling photoinduced electron transfer toward the N-terminus.[3]

The leucine zipper (the hydrophobic residues, located at positions $a/a'$ and $d/d'$ in Scheme 1) contains isoleucines and

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(1) For reviews see: Vullev, V. I.; Jones, G, II. *Res. Chem. Interm.* In press. Ogawa, M. Y. *Mol. Supramol. Photochem.* **1999**, *4*, 113. Mutz, M. W.; Wishart, J. F.; McLendon, G. L. *Adv. Chem. Ser.* **1998**, *254*, 145−159. Rabanal, F.; Gibney, B. R.; DeGrado, W. F.; Moser, C. C.; Dutton, P. L. *Inorg. Chim. Acta* **1996**, *243*, 213.

(2) The name TT (i.e., *Two Towers*) is assigned to series of polypeptides designed to form co-directional helix dimers, e.g., TT1, TT2, etc. The lowercase letter corresponds to the moiety that caps the N-terminus, e.g., "p" for pyrene, "o" for oxopyrene, "b" for butyrate, etc.

(3) Fox, M. A.; Galoppini, E. *J. Am. Chem. Soc.* **1997**, *119*, 5277. Galoppini, E.; Fox, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 2299.

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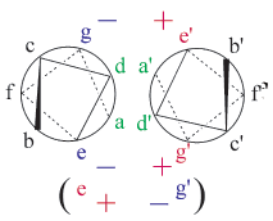
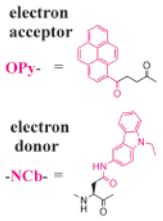
**Scheme 1.** Wheel Diagram,[4−6] Amino Acid Sequences and Formal Charges for TT2o and Analogous Polypeptides




| | e/e | f | g/g | a | b | c | d |
|---|---|---|---|---|---|---|---|
| OPy | Orn | Orn | Gly | Gly | Lys | Ala | Leu |
| | Phe | Glu | Gln | Ile | Ala | Glu | Leu |
| | NCb | Lys | *E/K* | Ile | Glu | Glu | Leu |
| | *E/K* | Lys | *E/K* | Ile | Glu | Glu | Leu |
| | *E/K* | Lys | Lys | Ala OH | | | |

**TT2o :** 9+, 9−, e = Lys; g = Glu
**TT2o+ :** 11+, 7−, e = g = Lys
**TT2o− :** 7+, 11−, e = g = Glu
**TT2o± :** TT2o+ and TT2o− (1:1)

leucines arranged to favor the formation of a parallel helix dimer.[4] Indeed, size-exclusion chromatographic analysis showed the presence of only dimers, at micromolar polypeptide concentrations (see Supporting Information).

Furthermore, the charges of the residues located next to the helical hydrophobic interface, i.e., positions *e*/*e*′ and *g*/*g*′ on the wheel diagram (Scheme 1), were used for electrostatic reinforcement of helix dimer formation.[5] In addition to the homo-dimers of TT2o and TT2oX15N (formed when *e*/*e*′ and *g*/*g*′ are positively and negatively charged, respectively) hetero-dimers, TT2o± and TT2o±X15N, were expected to be favored structures for equimolar mixtures of oppositely charged polypeptide analogues (Scheme 1).[6]

Comparison between the absorption spectra of *γ-oxo*-1-pyrenebutanoic acid (OPBA) and TT2oX15N indicated that for the polypeptides dissolved in water, ground-state aggregation of the principal chromophore occurs, characterized by band broadening, a 5−10 nm red shift, and a decrease in molar extinction coefficient (Figure 1). Concurrently, upon aggregation of pyrenyl ketone groups, the monomer fluorescence band (∼450 nm) is quenched and a broader, significantly weaker band appears at ∼545 nm (Figure 1).[7] Larger extinction coefficient values obtained for TT2o in comparison to those of TT2oX15N at wavelengths shorter than ∼370 nm are due to absorption of the carbazole moiety. The aggregation patterns of the principal chromophores of the polypeptides suggest that their N-termini are in proximity to each other.[8−10]

The two minima (∼207 and 220 nm) observed in the circular dichroism (CD) spectra of TT2o and its analogues are characteristic of the high helical content of the polypeptides (Figure 2a). The higher molar ellipticity obtained for TT2o, in comparison with that of TT2oX15N, is ascribed to an induced CD signal originating from the carbazole moiety, suggesting that the electron donor is uniquely oriented in proximity to the polypeptide helices as opposed to a more loose arrangement of the normally flexible NCb side chain.

CD spectroscopy was employed for examination of the formation of hetero-aggregates from oppositely charged polypeptide analogues (Figure 2b). Despite the low helical content of TT2oX15N, the mixture of the two polypeptides exhibits a strong CD signal at ∼222 nm. Similar evidence for aggregation between oppositely charged TT2o analogues was gathered from fluorescence data (see Supporting Information for details).

Alkylpyrene derivatives have a high aggregation propensity in aqueous media[10,11] and have been favorite units for photo labeling.[3,9,12] The selected pyrenyl ketone, the principal chromophore, shows a red-shifted absorption relative to the parent pyrene and serves as a better electron acceptor ($E^0_{OPy/OPy-\bullet} = -1.65$ V vs SCE) (see Supporting Information for electrochemical data).[13,14] The driving force for the photoinduced electron transfer involving the electron donor,

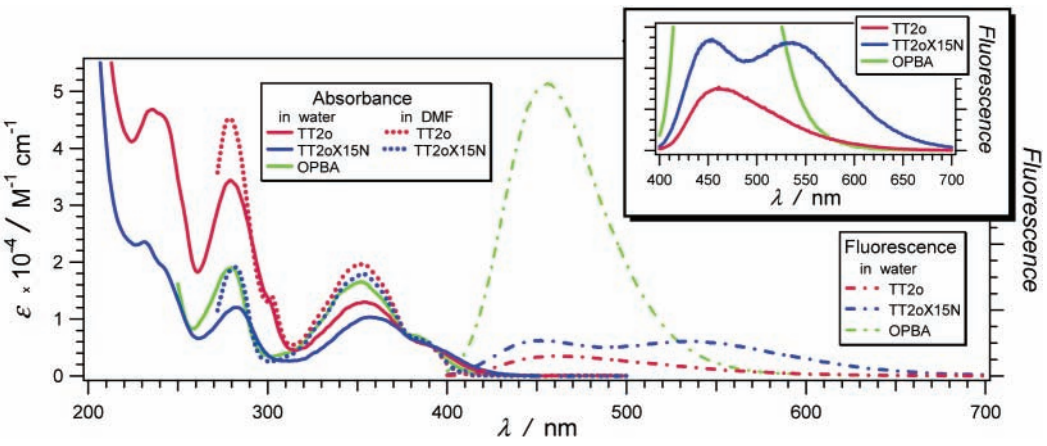



**Figure 1.** Absorption and emission properties of TT2o, TT2oX15N, and OPBA in water and DMF. The aqueous samples contained 100 mM phosphate buffer, pH 7, and the DMF samples contained 1 M guanidinium chloride. Inset: fluorescence spectra of TT2o and TT2oX15N, $\lambda_{ex} = 390$ nm. OPBA = *γ-oxo*-1-pyrene butanoic acid.

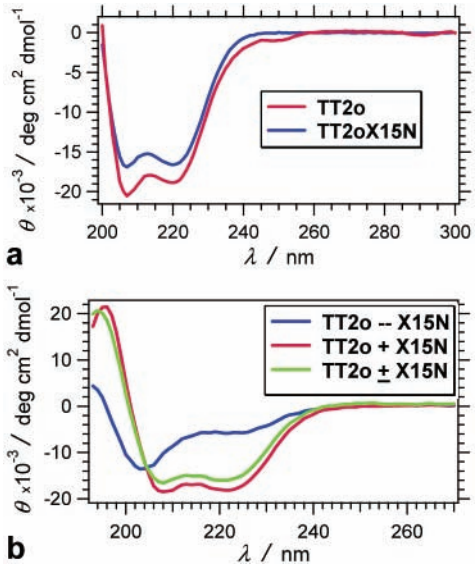



**Figure 2.** CD spectra of TT2o and its analogues in aqueous media: (a) TT2o and TT2oX15N, 8 $\mu$M, in the presence of 100 mM phosphate buffer, pH 7; (b) TT2o−X15N (10 $\mu$M), TT2o+X15N (10 $\mu$M), and TT2±X15N (i.e., 5 $\mu$M TT2o−X15N and 5 $\mu$M TT2o+X15N), in the presence of 1 mM phosphate buffer, pH 6.

amidoethylcarbazole, and the principal chromophore is $\Delta G_{et} = -0.54$ eV.[13−15]

Significant quenching of the red-shifted fluorescence (ascribed to the pyrenyl ketone aggregate) was observed for TT2o analogues in water, when the carbazole moiety is present (Figure 1, inset). For organic solvents, where the polypeptides exist only as monomers, this quenching phenomenon was not detected. The fluorescence quantum yields[16−18] and lifetimes[19,20] for the various polypeptide analogues and OPBA in trifluoroethanol (TFE) and buffered water are summarized in Table 1. The calculated values for nonradiative decay rate constants, $k_{nr}$, indicate that the

**Table 1.** Fluorescence Properties of OPBA and TT2o Analogues in Trifluoroethanol (TFE) and Aqueous Media

| | | $\Phi_{fl}{}^a$ ($\varphi_a$)$^b$ | $\tau^c$ (ns) | $k_{nr}{}^d \times 10^8$ ($s^{-1}$) | $k_{et}{}^e \times 10^8$ ($s^{-1}$) |
|---|---|---|---|---|---|
| OPBA | TFE | 0.32 | 1.93$^h$ | 3.5 | |
| | water$^f$ | 0.20 | 0.956$^h$ | 8.3 | |
| TT2o | TFE | 0.61 | 3.30$^h$ | 1.2 | |
| | water$^f$ | 0.017 | 2.54$^h$ | 1.4 | 0.70 |
| | | (0.59) | 6.90$^i$ | | |
| TT2oX15N | TFE | 0.57 | 3.46$^h$ | 1.2 | |
| | water$^f$ | 0.048 | 2.43$^h$ | 0.70 | |
| | | (0.62) | 13.8$^i$ | | |
| TT2o± | water$^g$ | 0.058 | 2.68$^h$ | 3.5 | 2.1 |
| | | (0.50) | 2.81$^i$ | | |
| TT2o±X15N | water$^g$ | 0.11 | 2.66$^h$ | 1.4 | |
| | | (0.56) | 6.89$^i$ | | |

[a] Total fluorescence quantum yield, $\lambda_{ex} = 390$ nm. [b] The portion of the total emission assigned to the aggregate fluorescence, $\varphi_a = (S_{aggregate}/S_{total})(A_{total}/A_{aggregate})$, obtained from deconvolution of the corresponding emission spectra, where $A$ is the absorption at $\lambda_{ex}$ and $S$ is the integrated fluorescence intensity (see Supporting Information). [c] $\lambda_{ex} = 337$ nm. [d] $k_{nr} = (1 - \Phi_{fl})/\tau$, or for aggregate, $k_{nr} = (1 - \varphi_a\Phi_{fl})/\tau$. [e] $k_{et} = k_{nr}$(TT2o) − $k_{nr}$(TT2oX15N) [f] 100 mM phosphate buffer, pH 7. [g] 1 mM phosphate buffer, pH 6. [h] $\lambda_{em} = 450$ nm. [i] $\lambda_{em} = 580$ nm.

pyrenyl ketone moiety behaves identically in TT2o and TT2oX15N, regardless of the presence of the carbazole.[21]

For polypeptides aggregates, however, (i.e., in aqueous media) a more than 2-fold increase in the value of the nonradiative rate constants was observed when the electron donor is present.[22] We ascribe this alteration of $k_{nr}$ to long-range electron entrainment from NCb to OPy (Scheme 1); the corresponding charge-transfer rate constants, $k_{et}$, were calculated from the difference between $k_{nr}$ obtained for polypeptides with and without an electron donor (Table 1).

In polypeptide media (i.e., $\beta \approx 1.4$ Å$^{-1}$)[23] the observed electron-transfer rates (i.e., $k_{et} \sim 10^8$ s$^{-1}$) can be achieved when the donor−acceptor distance is about 8−10 Å. However, if through-space electronic coupling is excluded as a possibility, the charge-transfer pathways should involve at least four hydrogen bonds,[24] suggesting a highly organized polypeptide structure in the region of the redox moieties. (see Supporting Information for details).[25]

To test if electron transfer, yielding radical-ion intermediates, is indeed responsible for the increase in nonradiative

(4) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401.
(5) McLachlan, A. D.; Stewart, M. *J. Mol. Biol.* **1975**, *98*, 293. Lumb, K. J.; Kim, P. S. *Science* **1995**, *268*, 436.
(6) O'Shea, E. K.; Lumb, K. J.; Kim, P. S. *Curr. Biol.* **1993**, *3*, 658.
(7) Armbruster, C.; Knapp, M.; Rechthaler, K.; Schamschule, R.; Parusel, A. B. J.; Köhler, G.; Wehrmann, W. *J. Photochem. Photobiol., A* **1999**, *125*, 29.
(8) Daugherty, D. L.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 4325.
(9) Jones, G., II.; Vullev, V. I.; Braswell, E.; Zhu, D. *J. Am. Chem. Soc.* **2000**, *122*, 388.
(10) Jones, G., II.; Vullev, V. I. *Org. Lett.* **2001**, *3*, 2457.
(11) Jones, G., II.; Vullev, V. I. *J. Phys. Chem. A* **2001**, *105*, 6402.
(12) García-Echeverría, C. *J. Am. Chem. Soc.* **1994**, *116*, 6031.
(13) The Rehm Weller equation[14] was used to estimate $\Delta G_{et}$: $E_{00}$(OPy) ≈ 3.1 eV.; the oxidation potential of the donor, $E^0(Cb^{+\bullet}/Cb) = 0.91$ V vs SCE.[15]
(14) Rehm, D.; Weller, A. *Israel J. Chem.* **1970**, *8*, 259.
(15) Ambrose, J. F.; Carpenter, L. L.; Nelson, R. F. *J. Electrochem. Soc.* **1975**, *122*, 876.
(16) The fluorescence quantum yields were calculated from emission data of samples with known optical density at the excitation wavelength, $\lambda_{ex} = 390$ nm.[17] Coumarin 102 in ethanol, $\Phi_{fl} = 0.95$,[18] was applied as a standard.
(17) Demas, J. N.; Crosby, D. A. *J. Phys. Chem.* **1971**, *75*, 991.
(18) Jones, G., II.; Rahman, M. A. *J. Phys. Chem.* **1994**, *98*, 13028.
(19) Although for TT2o and TT2o± at 337 and 355 nm up to ∼20% of the excitation energy is absorbed by the electron donor, i.e., the carbazole (Cb), we have a reason to believe that the energy (or an electron) is transferred from Cb to the pyrenyl ketone in less than a nanosecond since no carbazole emission or triplet formation has been detected.
(20) James, D. R.; Siemiarczuk, A. *Rev. Sci. Instrum.* **1992**, *63*, 1710.
(21) The emission of OPBA appears to be quenched, probably because of the carboxylate that is 3 $\sigma$-bonds away from the chromophore.
(22) From emission data it was deduced that in aqueous medium more than ∼85% of the principal chromophore of the polypeptides is in aggregated form and, hence, almost all of the excitation energy is absorbed by aggregated pyrenyl ketone. Therefore, the analysis of the fluorescence quenching is concentrated solely on the red-shifted band of the emission spectra (see Supporting Information for details).
(23) Moser, C. C.; Dutton, P. L. *Biochim. Biophys. Acta* **1992**, *1101*, 171.
(24) Beratan, D. N.; Skourtis, S. S. *Curr. Opin. Chem. Biol.* **1998**, *2*, 235.

decay rates, transient absorption studies were conducted using nanosecond laser flash photolysis.[26] When TT2o±X15N was flash-photolyzed, only the OPy triplet (at ~440 nm) was observed (Figure 3a and c). However, identical photoexcitation of TT2o± yielded, at early times, a strong transient ($\lambda_{max}$ ~500 nm) and a broad band at ~650 nm, which are ascribed to the pyrenyl ketone radical-anion and carbazole radical-cation, respectively (Figure 3b).[19] (See Supporting Information for assignments of the transient spectra.) The radical transients decayed in ~20 $\mu$s, with a simultaneous rise in the OPy triplet absorption at 440 nm (Figure 3d). Then both signals peaking at 440 and 500 nm decayed with rates comparable to that of the loss of the OPy triplet signature (Figure 3c and d).[27] These observations suggest that the back electron transfer in TT2o systems leads to pyrenyl ketone triplet formation, a finding that is energetically feasible since the OPy triplet state lies about 2.2 eV[7] above the ground state and 0.36 eV below the charge-separated state.

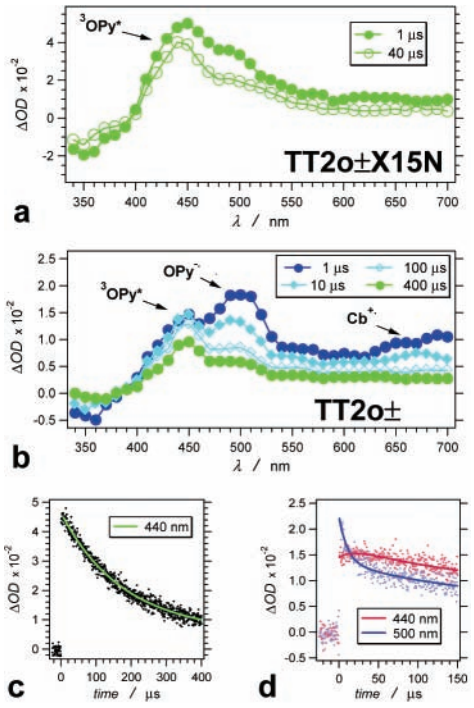



**Figure 3.** Transient absorption data for TT2o± and TT2o±X15N in argon-purged aqueous medium. Transient spectra of (a) TT2o±X15N, 12 $\mu$M; and (b) TT2o±, 8 $\mu$M. The corresponding decays were fit to biexponential functions: (c) for TT2o±X15N, $\tau_1 = 150$ $\mu$s, $\tau_2 = 830$ $\mu$s; (d) for TT2o±, at 440 nm, $\tau_{rise} = 19$ $\mu$s and $\tau_{fall} = 820$ $\mu$s, and at 500 nm $\tau_1 = 23$ $\mu$s and $\tau_2 = 780$ $\mu$s. (1 mM phosphate buffer, pH 6; $\lambda_{ex} = 355$ nm, 8 ns pulse, 10 mJ/pulse).

In summary, we have demonstrated that the medium provided by a polypeptide helical aggregate can mediate an efficient electron transfer between redox moieties that are well separated along a 32-residue polypeptide chain. Charge recombination that leads to a triplet-excited state is observed. Included also is the first report of a convenient pyrenyl ketone macromolecular photoprobe, as well as a straightforward procedure for preparation and introduction of a chromphoric amino acid, NCb, in a polypeptide via solid-phase synthesis (see Supporting Information).

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**Supporting Information Available:** Experimental section and additional photochemical and electrochemical data on various pyrene and carbazole derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) In such systems, the electronic coupling and the nature of the chromophore aggregates (e.g., geometry and hydrogen bonding) can be strongly influenced by even slight conformational changes explaining 2-fold difference in emission lifetimes and the 3-fold difference in $k_{et}$ observed for analogous homo and hetero polypeptide aggregates (Table 1).

(26) Jones, G., II.; Vullev, V. I. *Photochem. Photobiol. Sci.* In press. Vullev, V. I. PhD Dissertation, Boston University, 2001, pp 205−209 and 571−582.

(27) Note that the absorbances of OPy triplet and radical-anion overlap (Figure 3). Hence, the fast component at 500 nm is ascribed to the radical decay, and the slow component to the triplet (Figure 3b and d).

(28) This is article No. 13 in the series Photoactive Polypeptides from Boston University.