

Factors affecting light energy conversion in dual fluorophore–nitroxide molecules in solution and a protein*

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Abstract: Donor–acceptor structures capable of retaining the charge-photo-separated state during the time long enough for secondary chemical reactions of these charges to occur attract special interest from the viewpoint of the problem of light energy utilization. We proposed dual fluorophore–nitroxide compounds (**FNO**[•]) as systems for the conversion of light energy to chemical energy. In these systems, the fluorophore segment in the excited singlet state serves as an electron donor, and the nitroxide segment is an electron acceptor. In **FNO**[•], the photo- and chemical reduction of nitroxide results in the drastic decay of the electron spin resonance (ESR) signal from the nitroxide and the parallel enhancement of fluorescence. The same groups allow one to measure the factors affecting the electron transfer, namely, molecular dynamics and micropolarity of the medium in the vicinity of the donor (by fluorescence technique) and acceptor (by ESR) moieties. We demonstrate that in the dual probes the nitroxide segment is photoreduced to hydroxylamine in solution and in such nanoscale structures as serum albumins. The photoreduction occurs by very weak reducing agents (glycerol, ethanol, ethylene glycol, etc.) without a violation of the fluorophore structure. Therefore, photochemical reactions in the dual compounds with the formation of a reducing agent as hydroxylamine can be considered as processes of light energy transfer. The nitroxide segment tethered to the donor–bridge–acceptor triad affects the photo-separated charge recombination via the mechanism of spin catalysis. Proficiency of the dual compounds for developing energy conversion systems can be extended by an optimal choice of the participants of the photochemical and -physical processes.

Keywords: light energy conversion; fluorophore–nitroxide; reaction center; electron transfer; protein dynamics.

INTRODUCTION

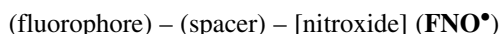
The creation of the life-giving system of light energy utilization, photosynthesis, appears to be one of the most imposing physicochemical achievements in Nature. Mimicking of this process and construction of molecular structures for the efficient conversion of light energy to chemical energy is a challenging problem of the 21st century, which unites the fundamental importance for human welfare and intellectual fascination for investigation. As it was well established, the primary photocharge separation between the donor, a chromophore in the excited state (D^{*}), and the acceptor (A) centers with the for-

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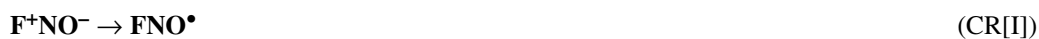
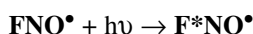
mation of the pair (D^+A^-) is a key stage in any biological and model photosynthetic systems [1–12]. Donor–acceptor structures, which are able to retain the photoseparated state long enough for secondary chemical reactions of these charges to occur, attract the special interest of researchers working in the area of light energy utilization.

Dual **FNO**[•] as model systems for conversion of light energy to chemical energy were proposed and developed. An idea to combine a chromophore and a nitroxide in one molecule for the study of molecular dynamics of media, intramolecular fluorescence quenching (IFQ), and nitroxide fragment photoreduction was designed in the early 1980s [13,14]. Two types of photoseparation systems with different roles of the nitroxide fragment were developed.

In a system 1 [14]



the fluorophore (**F**) in an excited state is an electron donor and nitroxide fragment (**NO**[•]) serves as an electron acceptor. In this system, photochemical and photophysical processes follow a scheme



where RH is a solvent or an ingredient; Q[I], CS[I], CR[I], and ChR designate fluorescence quenching, charge separation, charge recombination, and chemical reaction, correspondingly.

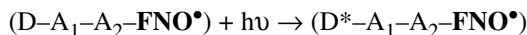
We first demonstrated that nitroxide photoreduction in the dual probes occurs without a violation of the fluorophore structure [14]. This process is, in fact, the photoinduced electron transfer (ET) from molecules of the medium, which are very weak reducing agents (glycerol, ethanol, ethylene glycol, etc.) to nitroxide with the formation of hydroxylamine derivatives (**FNOH**) with a moderate reducing power. Therefore, the photochemical reactions of the dual molecules in system 1 can be considered as processes of light energy transfer.

The ability of the stable nitroxide radical segment of (**FNO**[•]) to act as a quencher of the excited state of the chromophore segment was intensively exploited as the basis of several methodologies including molecular probing, modeling intramolecular photochemical and photophysical processes, analysis of antioxidants and nitric oxide, and construction of new magnetic materials [4,7,12, refs. cited therein).

Fluorophore–nitroxide compounds of system 2 consist of triad tethered with nitroxide [2,4]:



In these systems, the nitroxide segment effects on the separated charge recombination by mechanism of spin catalysis. The following intramolecular photochemical processes take place in compounds of system 2 with two acceptors A_1 and A_2





The presence of nitroxide allows one to control the lifetimes of photoinduced radical ion pairs, which is important for developing an energy conversion system, as well as molecular materials for electronics, photonics, and spintronics [2,4,7]. Owing to the spin catalysis effect, the charge recombination can be locked up and the ability of system 2 to retain the photoseparated state long enough for secondary chemical reactions of these charges to occur enhances.

In this work, we review the theoretical principles and experimental data on the photochemical and photophysical processes in the dual fluorophore–nitroxide molecules of systems 1 and 2. In building up the systems of light energy conversion, we followed the principles of this process in the photosynthetic reaction centers (PRCs).

PRINCIPLES OF LIGHT ENERGY CONVERSION IN PHOTOSYNTHETIC REACTION CENTERS

Light energy conversion in the PRCs is characterized by the high energetic efficiency and the quantum yield close to 100 % [1]. Such a result was achieved by the fulfillment of several principal conditions.

- As predicted in [9,10], the donor and acceptor groups should be disposed at a certain optimum distance relative to each other. In other words, the process of ET from the donor to acceptor should be non-adiabatic and the value of the coupling factor or resonance integral (V) should be optimum as well. This requirement is necessary to provide a sufficiently fast direct ET with the formation of the pair D^+A^- , on the one hand, and to prevent the fast pair recombination (CR), on the another hand. The best way for the efficient charge separation is to arrange a cascade of the donor and acceptor centers, which are separated in space at a distance of 7–10 Å [15–17].
- The ET driving force (ΔG_0) and redox potentials of D^* and A should be optimal. A combination of a strong donor and strong acceptor is favorable for the fast photoseparation but unfavorable for secondary reactions, since in this case the chemical reactivity of the photoseparated charges D^+A^- would be low. Weak donors and acceptors cannot provide efficient photoseparation. In biological PRC, the optimization is achieved by a “choice” of specific components and local micropolarity. The latter, in turn, depends on the molecular mobility in the vicinity of the donor and acceptor groups. Figure 1 demonstrates the effect of the protein dynamics in the nanoscale correlation time (τ_c) on the ET between the secondary quinone acceptors in the PRC, which has been detected by a series of molecular probes [18–19].
- Charge-separated pair D^+A^- keeping strong chemical reactivity should be isolated from side reactions. In Nature, this problem has been solved by the immersion of donors and acceptors into the protein globule and its specific arrangement relative to the donors and acceptors out of the PRC [17].

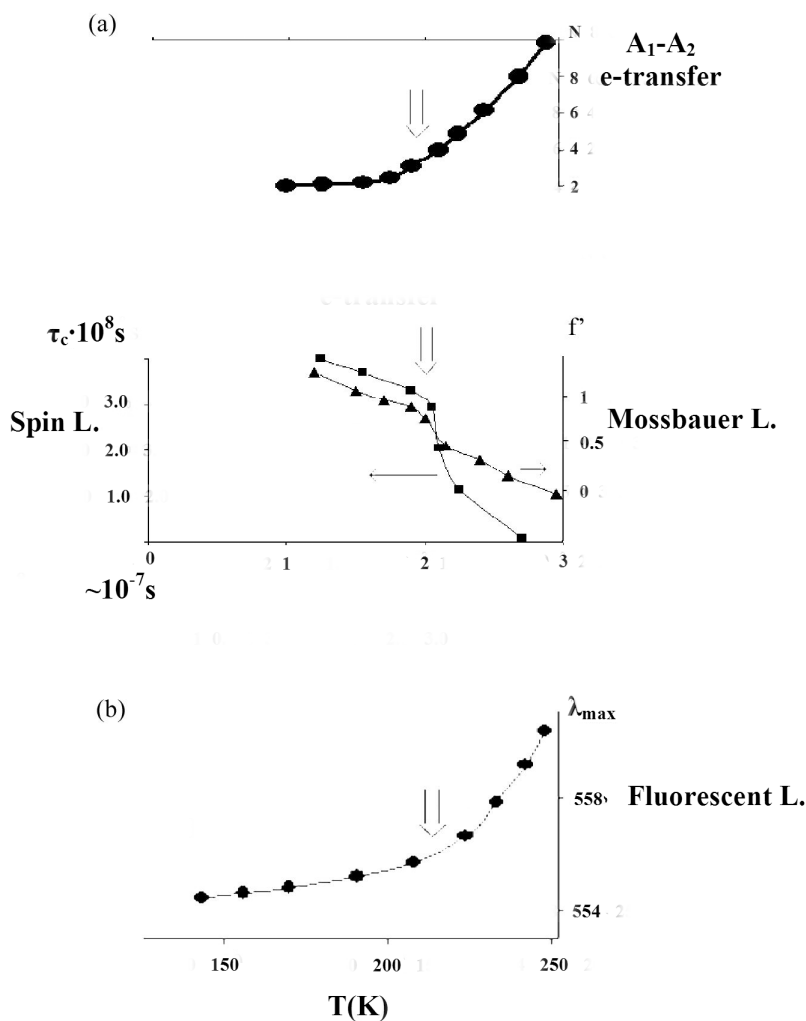


Fig. 1 Temperature dependence of dynamic parameters of physical labels on chromatophore from *R. rubrum*: (a) schematic diagram of the location of spin, fluorescent, and Mössbauer labels; (b) experimental data: N is the efficiency of ET from the primary (A_1) to secondary (A_2) acceptors. Arrows indicate the beginning of the dynamic process with $\tau_c < 10^{-7}$ s [21].

THEORETICAL GROUNDS

Mechanisms of fluorescence quenching by nitroxides in FNO*

The efficiency of the intramolecular light energy conversion in the dual compounds depends strongly on a competition between ET and fluorescence quenching. Three mechanisms of the fluorescence quenching have been considered: (1) the energy transfer via the Förster dipole–dipole and/or Dexter exchange mechanism; (2) the electron exchange-induced intersystem crossing (ISC) and internal conversion; and (3) the ET between an excited chromophore and nitroxide.

Previous investigations [6,20–22] have ruled out the energy-transfer mechanisms. The overlap between the fluorophore emission and nitroxide absorption spectra is very small, and the shift of the overlap integral in a series of solvents did not correspond to experimental data on the rate constant of fluorescence quenching. Therefore, the quenching probably occurs by mechanisms 1 or 2, or both.

ISC quenching mechanism of the excited chromophore by a nitroxide radical

Electron-exchange interactions with paramagnetic species have been known to generate the excited-state relaxation of chromophores. The quenching mechanism originates from changes in the spin multiplicity of the electronic states [7 and refs. therein]. The singlet ground (S_0) state and the lowest excited singlet (S_1) state of the chromophore become the doublet (D_0 and D_n , respectively) states because of the unpaired electron spin of the doublet nitroxide radical (FNO^\bullet). On the other hand, the lowest excited doublet (D_1) and quartet (QA_1) states are generated by an interaction between the FNO^\bullet and the T_1 chromophore. Thus, the spin-forbidden transitions of the chromophore, i.e., $S_1 \rightarrow T_1$ and $T_1 \rightarrow S_0$, partially transform into the $D_n \rightarrow D_1$ and $D_1 \rightarrow D_0$ transitions, respectively. Since these doublet states (D_n , D_1 , and D_0) have the same spin multiplicity, the lifetimes of the excited state appear to be very short as comparable to those of the $S_1 \rightarrow S_0$ transition. Figure 2 shows an example of such a process in dual phthalocyanine–nitroxide compound.

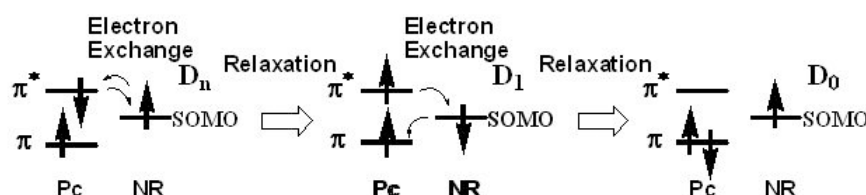


Fig. 2 The $D_n \rightarrow D_1$ and $D_1 \rightarrow D_0$ transitions via electron exchange processes [7].

Mechanism of non-adiabatic electron transfer

The theory of non-adiabatic ET was developed in works [23–25]. The work authors, utilizing the Landau–Zener theory for the intersection area crossing and suggesting harmonic one-dimensional potential surface, proposed a Marcus–Levich formula for non-adiabatic ET

$$k_{ET} = \frac{2\pi V^2}{h\sqrt{4\pi\lambda k_B T}} \exp\left[-\frac{(\lambda + \Delta G_0)^2}{4\lambda k_B T}\right] \quad (1)$$

where ΔG_0 is the driving force of the value of the process, standard Gibbs free energy, λ is the reorganization energy defined as energy for the vertical ET without replacement of the nuclear frame, V is the electronic coupling or the resonance integral.

Equation 1 predicts the $\log k_{ET} - \Delta G_0$ relationships depending on the relative magnitudes of λ and ΔG_0 : (1) $\lambda > \Delta G_0$, when $\log k_{ET}$ increases if ΔG_0 decreases (normal Marcus region), (2) $\lambda = \Delta G_0$, the reaction becomes barrierless with a maximum rate, and (3) $\lambda < \Delta G_0$, when $\log k_{ET}$ decreases with increasing driving force.

According to the theory of exchange interactions [26], the value of the rate constant of all exchange processes is affected by the orbital overlap of the interacting particles, which are characterized quantitatively by the values of the exchange integral J . The values of V and J are related in turn to the orbital overlap integral S . The theory of exchange processes predicts strong but different dependence of the ET rate constant (k_{ET}) and rate constants of triplet–triplet energy transfer (k_{TT}), ISC, and spin exchange (SE) on the distance between the donor and acceptor groups. The dependence of these exchange processes on the edge–edge distance between centers (ΔR) is approximated by the following empirical equation [21,22,27]:

$$k_{ET}, k_{SE}, k_{TT}, k_{ISC}, k_{SE} = A \exp(-\beta \Delta R) \quad (2)$$

For systems in which the centers are separated by a “nonconductive” medium (molecules or groups with saturated chemical bonds) $\beta_{\text{TT}} = 2.6 \text{ \AA}^{-1}$, $\beta_{\text{ISC}} = 2.0 \text{ \AA}^{-1}$, and $\beta_{\text{SE}} = \beta_{\text{ET}} = 1.3 \text{ \AA}^{-1}$. For systems in which the radical centers are linked by “conducting” conjugated bonds, $\beta_{\text{SE}} = 0.3 \text{ \AA}^{-1}$. The β_{SE} values for specific chemical bonds, the van der Waals contacts, and the hydrogen bond have been tabulated in [21,22,27]. It should be taken into consideration that the predicted values of pre-exponential factor A in eq. 2 for the TT, ISC, and SE processes at the van der Waals contacts equal about 10^{14} s^{-1} while for the k_{ET} in the Marcus maximum rate area $A_{\text{ET}} \approx 10^{12} \text{ s}^{-1}$. Thus, the exchange processes are predicted to prevail over ET at short distances ($\Delta R < 6 \text{ \AA}$) while the ET is preferable at longer distances ($\Delta R > 6 \text{ \AA}$) (Fig. 3).

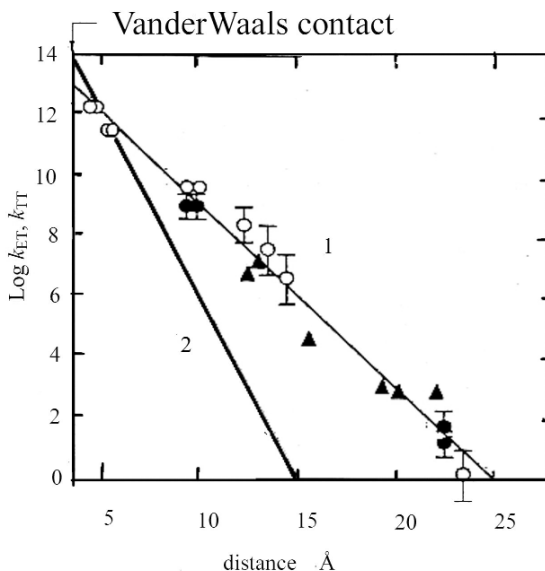


Fig. 3 Dependence of logarithm of rate constant of exchange processes on the edge–edge distances between centers: (1) rate constant of ET in the Marcus maximum rate area; (2) experimental rate constant of triplet–triplet energy transfer (obtained by averaging experimental data on the phosphorescence quenching). Straight line **1** is related to theoretical dependence of the spin-exchange integral J on the distance [21,22].

Spin catalysis

Chemical reactions are known to be controlled by two fundamental parameters, energy (both free and activation energy) and angular momentum (spin) of reactants [28,29]. The latter results in electron and nuclear spin selectivity of reactions: only those spin states of reactants are chemically active whose total spin is identical to that of products.

For example, for the triplet radical pair (R_1, R_2) prepared by photolysis, radiolysis, or encounter of freely diffusing radicals to recombine and produce diamagnetic, zero-spin molecule R_1R_2 , triplet–singlet spin conversion of the radical pair is required (Fig. 4).

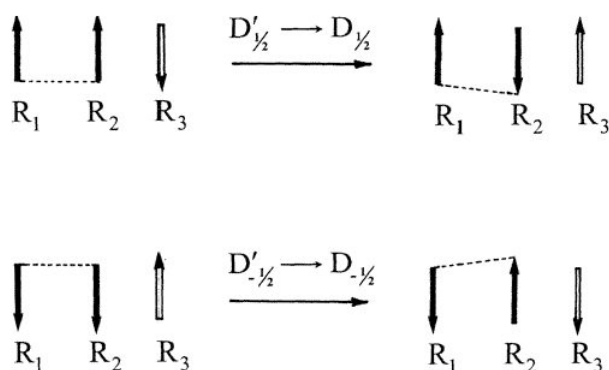


Fig. 4 Visual presentation of spins in radical triad (R_1, R_2, R_3). Exchange interaction between catalyst (open arrow) and radical R_2 produces reorientation of their spins and transforms the triplet state of the pair (R_1, R_2) into the singlet state. Black arrows denote spins of the partners of radical pair (R_1, R_2) [28].

In a static model of spin catalysis, for example, in PRC or model systems, if the starting spin state of the pair is triplet (it corresponds to D' state of the triad), then the probability of finding this pair in the singlet state (it corresponds to D state of the triad) [28] is

$$\rho_S(t) = (\Delta J/2\Omega)\sin^2\Omega t$$

where

$$\Omega = 2^{-1/2}[(J_{12} - J_{13})^2 + (J_{12} - J_{23})^2 + (J_{13} - J_{23})^2]^{1/2}$$

and

$$\Delta J = |J_{13} - J_{23}|$$

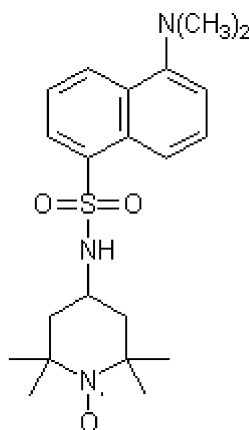
J_{ij} are the pairwise exchange energies for pairs R_i and R_j ($i \neq j$), ($i, j = 1, 2, 3$). Both conjugated processes, triplet–singlet conversion of the pair and doublet–doublet evolution of the triad, oscillate in time with a period $\tau = (2\Omega)^{-1}$.

Photogenerated radical pairs are capable of exhibiting coherent spin motion over microsecond time scales, which is considerably longer than coherent phenomena involving photogenerated excited states. The rate of radical pair ISC between photogenerated singlet and triplet radical pairs has been shown to increase in the presence of stable free radicals and triplet state molecules. Spin catalysis was proved to operate in radical recombination (termination reaction in chain processes), biradical decay, *cis–trans* isomerization of molecules, primary light-harvesting reactions in photosynthetic centers, charge separation and water oxidation by photosystem II, in particular, paramagnetic quenching of excited molecules, etc. [28 and refs. therein].

Another way for controlling the lifetime of the photoseparated charges by the spin chemistry mechanism appears to be the introduction to donor or acceptor molecules an isotope bearing a nuclear spin [29]. As an example, the catalytic activities of ATPase, creatine kinase, and glycerophosphate kinase containing $^{25}\text{Mg}^{2+}$ were found to be two to four times higher than those of the enzymes with spinless, nonmagnetic magnesium cation isotopes (^{24}Mg or ^{26}Mg) [29]. This demonstrates that ATP synthesis can be a spin-selective process involving Mg^{2+} as the electron-accepting reagent. The authors of [29] suggested that ATP synthesis proceeds in an ion-radical pair consisting of an ADP oxyradical and Mg^{2+} .

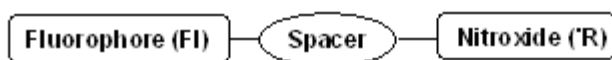
System 1 as a model for intermolecular light energy conversion

As it has first been shown in our work [14], the FNO^\bullet of system 1, keeping all properties of the fluorescence and spin probes, possess a new advantage: in (FNO^\bullet) the nitroxide segment is a strong fluorescent quencher. Thus, irradiation of the chromophore segment of the dual compound, dansyl-TEMPO (FN1) in a glassy liquid (glycerol, 75 %, water 20 %, ethanol 5 %) invoked the production of the hydroxylamine derivative accompanying the decay of the electron spin resonance (ESR) signal from nitroxide and a parallel eightfold increase in fluorescence. Both processes occur with the same rate constant k_{red} under identical conditions. The photoreduction rate constant k_{red} increased drastically when the temperature increased starting from 210 K. The k_{red} change correlated with an animation of the nanosecond relaxation dynamics in medium monitored by the fluorescence and ESR techniques, while the IFQ rate constant k_q was found to be temperature-independent. At ambient temperature, the k_{red} value was strongly dependent on media nature increasing while the media dielectric constant increased [14].

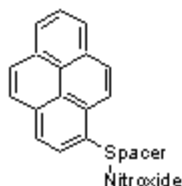


(FN1)

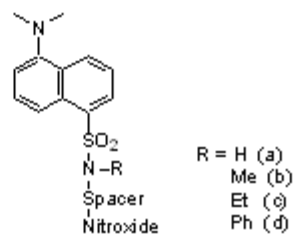
In order to establish the mechanism of IFQ and photoreduction of the nitroxide segment in the dual molecules, a series of 17 dansyl-nitroxides of different structures and flexibilities of the spacer group and different redox potentials of nitroxide was synthesized and investigated in different media (Scheme 1) [6,7].



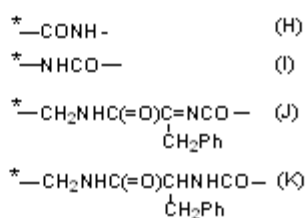
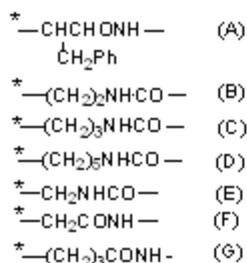
Pyrene Derivatives (FI = PYR)



Dansyl Derivatives (FI = DAN = NR-Dns)

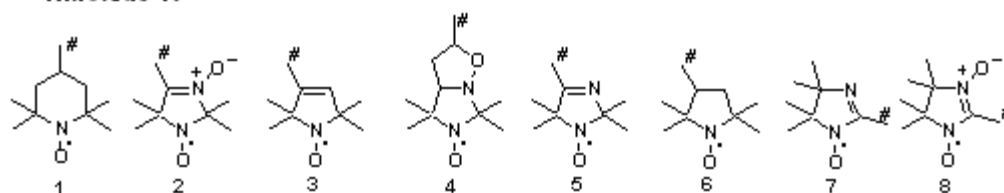


Spacer =



*-Linked to FI

Nitroxide R =



Scheme 1 Structure of pyren- and dansyl nitroxides [7].

Figure 5 shows the positive correlation between the rate constant of nitroxide fragment photoreduction k_{red} and the equilibrium constant K_{eq} for the chemical exchange reaction between nitroxides of different redox potential [6,7]. The chemical structure of the medium, dielectric constants (ϵ_0), and capability of proton donating drastically effected on k_{red} . Nevertheless, the k_q values were found to be independent of K_{eq} and the solvent nature [6,7,14].

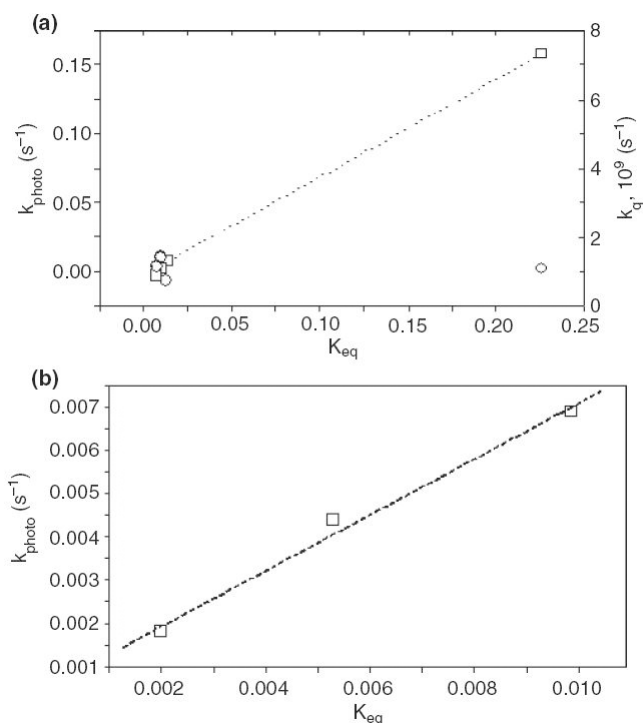
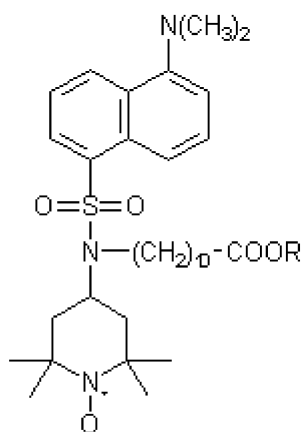


Fig. 5a,b Dependence of rate constant of photoreduction, k_{red} , of fluorophore–nitroxides on redox power of nitroxide fragment, K_{eq} , (squares) contrary to independence of rate constant of IFQ, k_q , (circles) [7].

Based on these and other available data, two mechanisms of IFQ were proposed: the major mechanism, ISC, and the minor mechanism, intramolecular electron transfer (IET) from the excited singlet of the fluorophore (donor D^*) to nitroxide (acceptor A) followed by fluorophore segment regeneration and hydroxylamine formation [3,6,7,14]. The latter mechanism is responsible for the photoreduction of nitroxide to hydroxylamine during light energy conversion.



(FN2)

In order to model effects of protein on the ET in FNO^\bullet , two dual probes FN1 and FN2 were incorporated in bovine (BSA) and human serum albumin (HSA) [3,19]. The photoreduction of the nitroxide fragment was monitored by ESR, and fluorescence quenching was measured by steady-state and picosecond time-resolved techniques. The same groups allow us to investigate the factors affecting the ET to be measured, namely, the molecular dynamics and micropolarity of the medium in the vicinity of the donor (by fluorescence technique) and acceptor (by ESR) moieties.

It has been shown [3] that beneath 240 K the photoreduction rate constant (k_{red}) for FN1 incorporated into BSA was close to zero. Above this temperature, the photoreduction rate constant k_{red} drastically increased with temperature. This increase is accompanied by a decrease in the apparent correlation time of probe nitroxide fragment rotation and the apparent correlation time of the media polar relaxation in the vicinity of the excited dansyl segment. At ambient temperature, both values reach the nanosecond scale. This conclusion was supported by the measurements of fluorescence polarization and ESR at ambient temperature, and by direct monitoring of relaxation dynamics of the protein binding site around the dansyl moiety of the dual fluorophore–nitroxide probe FN2 using picosecond fluorescence time-resolved technique (Fig. 6).

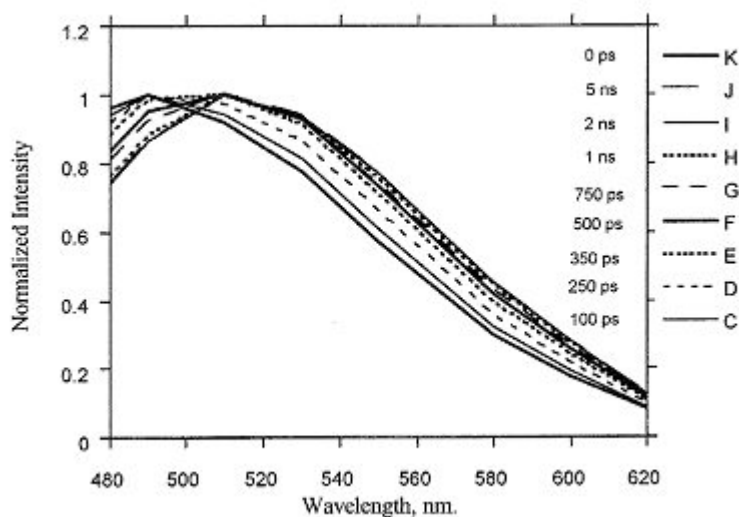


Fig. 6 Time-resolved fluorescence spectra of dansyl-TEMPO dual probe in HSA [19].

Hence, the nanosecond dynamics of the protein medium is one of the decisive factors affecting the photoreduction and the light energy conversion in biological and model systems. Such an intramolecular flexibility of the protein in a nanosecond range makes it possible to stabilize products of the reactions of the oxidized donor D^+ with reducing agents due to interactions with dipoles surrounding the protein, thus providing favorable thermodynamics for these reactions.

Based on our experimental data on the local apparent dielectric constant ϵ_0 in the vicinity of the donor dansyl groups (fluorescence technique) and around the acceptor nitroxide segments (ESR), the following parameters of the Marcus–Levich theory were estimated for the ET at $T = 300$ K [3]: Gibbs energy $\Delta G_0 = -1.7$ eV, reorganization energy $\lambda = 0.9$ eV, and activation energy $\Delta G^\ddagger \approx 0.25$ eV. Such a set of parameters is closely related to the inverted Marcus region and the ET under thermodynamically equilibrium conditions should occur with the rate substantially lower ($k_{\text{ET}} \approx 10^5 \text{ s}^{-1}$) than the experimental value $k_q = 1 \times 10^9 \text{ s}^{-1}$. We suggested that the fast primary ET in the system under investigation occurs at the expense of vibrational stabilization of the photoseparated ionic pair (D^+A^-) and at the expense of partial stabilization due to the fast polar relaxation modes [30,31].

PHOTOPHYSICAL AND -CHEMICAL PROCESSES IN DUAL MOLECULES OF SYSTEM 2

In reference [8], time-resolved picosecond optical and EPR (nanosecond) spectroscopy were used to study the influence of stable free radical 2,2,6,6-tetramethylpiperidinoxyl (TEMPO, T^\bullet) on photophysical and photochemical properties of a donor–chromophore–acceptor ($D^*–C–A$) system, $\text{MeOAn}–6\text{ANI}–\text{Ph}_n–\text{A}–T^\bullet$. The distances between each component [$\text{MeOAn} = p$ -methoxyaniline, $6\text{ANI} = 4$ -(*N*-piperidinylnaphthalene-1,8-dicarboximide, $\text{Ph} = 2,5$ -dimethylphenyl ($n = 0,1$), and $\text{A} =$ naphthalene-1,8:4,5-bis(dicarboximide) (NI) or pyromellitimide (PI)] was well defined by their chemical structure. The several principle results were obtained: (1) T^\bullet modulates the charge recombination rate within the triradical compared with the corresponding biradical lacking T^\bullet . For example, for the system 2 triad the following values of time constants for the charge separation CS1, CS2, and the charge recombination CR in toluene have been reported for triad without tethered nitroxide (a) and with nitroxide (b) (in brackets): CS1 = 9.8 ± 0.2 ps (7.0 ± 0.2 ps), CS2 = 430 ± 20 ps (400 ± 20 ps), CR = 210 ± 5 ns (506 ± 10 ns). The following values of magnitudes of the resonance integral $2J$ were determined: 1 ± 0.5 mT (a) and <1 mT (b). Therefore, the nitroxide tethering does not affect markedly the charge separation and the resonance integral but increases the charge recombination time by factor 2.5. The energy diagram in triad is shown in Fig. 7.

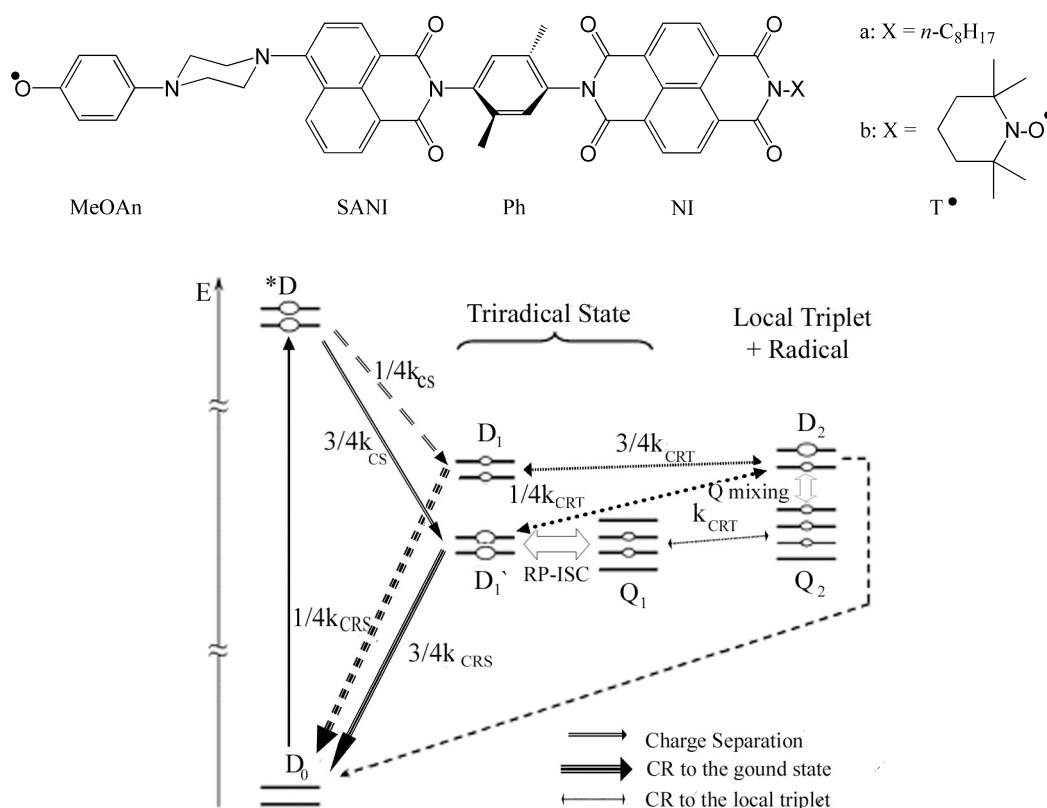


Fig. 7 Energy level diagram showing the spin manifolds triad **b** after charge separation and charge recombination, in a magnetic field of ~ 0.35 T. The size of the ellipse on each spin level represents its population qualitatively [8].

In Fig. 7, arrows denote charge separation forming the triradical state, charge recombination to the ground doublet state; reversible D–D and Q–Q charge recombination steps leading to the local triplet $^3^*\text{NI}$. D_1 , D_1' , and Q_1 form a complete spin basis set of the triradical state. Dashed lines mean less probable transitions.

Similar photochemical, photophysical, and spin catalysis effects were observed in triad system also having well-defined distances between the components: MeOAn-6ANI-Ph(*t*-butylphenylnitroxide, BPNO)-NI, where MeOAn = *p*-methoxyaniline, 6ANI = 4-(*N*-piperidinylnaphthalene-1,8-dicarboximide, Ph = phenyl, and NI = naphthalene-1,8:4,5-bis(dicarboximide) [2]. MeOAn-6ANI, BPNO, and NI are attached to the 1, 3, and 5 positions of the Ph bridge, respectively, show that BPNO influences the spin dynamics of the photogenerated triradical states $^{2,4}(\text{MeOAn}^+-6\text{ANI-Ph(BPNO)-NI}^-)$. As a result, the charge recombination within the triradical is slower, as compared to the corresponding biradical lacking BPNO.

The above-mentioned results clearly demonstrated the possibility of controlling the charge recombination process in triads.

CONCLUSIONS AND OUTLOOK

The use of the dual fluorophore–nitroxide molecules of system 1, where the nitroxide segment is the electron acceptor, opens unique opportunities to investigate the molecular dynamics and local polarity of the medium in the vicinity of the donor and acceptor groups and its role in IFQ, IET, and nitroxide photoreduction. We demonstrated that the photoreduction reaction of the nitroxide radicals in the dual probes of system 1 is a nondestructive process preserving the chemical structure of the fluorophore group. In fact, this process is the photoinduced ET from the surrounding molecules of the medium (glycerol, ethanol, ethylene glycol, etc.), which are very weak reducing agents, to the nitroxide moiety following the formation of the hydroxylamine derivatives of a moderate reducing power. So, the discussed photochemical reactions in the dual molecules can be considered as the light energy transfer processes, which meets the main requirements for any efficient light energy conversion system: favorable thermodynamics, optimum orbital overlap of the donor and acceptor groups, and nanosecond molecular dynamics of the medium. The role of the intramolecular dynamics process in protein function should be stressed [3,18,19,21,22,27,32–34].

Controlling the lifetimes of photoinduced radical ion pairs is important for developing molecular systems of light energy conversion. A method for controlling the charge transport in system 2, organic donor–chromophore–acceptor (D–C–A) triads tethered to the stable nitroxide radicals has been developed [2,8]. The method is based on the effect of spin catalysis [28], which is attributed to the anti-ferromagnetic coupling between the nitroxide segment and paramagnetic charge-separated pair. Under certain conditions, this exchange interaction can lock in and somewhat extend the pair recombination increasing the lifetime of the pair and, therefore, a possibility of secondary reactions leading to stable products.

Future progress in the use of the FNO^\bullet for solving problems of light energy conversion can be associated with the developments in the following directions.

- Choosing an optimum set of the donor and acceptor components of ET in system 1: donors, fluorophores with long lifetime in the excited (e.g., triplet) state and nitroxides of different redox potential, and composing a cascade systems of acceptors between the primary donor D^* and nitroxide. For example, using D^* in the millisecond scale of lifetimes and increasing the distance between D and A to several angstroms to shift the competition in favor of ET as compared to ISC (see Fig. 3) would prolong the D^+A^- lifetime and, therefore, efficiency of the light energy conversion.
- Controlling the chemical reactivity of D^+A^- in compounds of system 2 by optimum choice of D, A and a compound bearing electron or nuclear spin, and by playing with the capacity of mole-

cules of the medium or ingredients to donate an electron for the reaction with D^+ and a proton for A^- .

- Incorporation of dual molecules into a nanoscale object of optimum polarity and molecular dynamics, which would be able to provide specific secondary reactions and to prevent the system from side reactions. Building such systems appears to be the most challenging problem in the 21st century.

The above-mentioned new structures would allow one to prolong the lifetime of the photo-separated charged pairs and improve their specific reactivity. Construction and investigation of these structures would pave a way to the creation of efficient systems of light energy conversion that can be used for solar energy utilization.

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