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Mimicking dye-based functions of natural blue-light photoreceptors by studying photoinduced energy and electron transfer in a pyrene–isoalloxazine(flavin)–phenothiazine triad†

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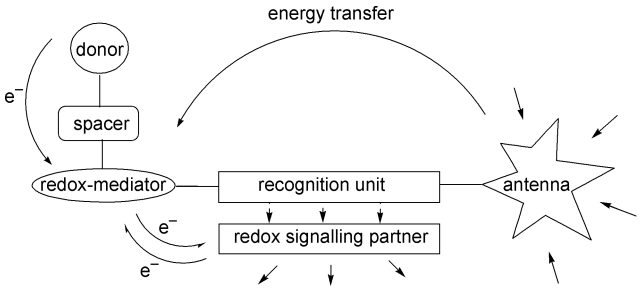
An artificial system containing phenothiazine as electron donor, isoalloxazine as flavinoid redox-mediator and pyrene as antenna has been built up in order to model photoinduced energy and electron transfer processes of natural blue-light photoreceptors.

Physiology and behaviour of plants, bacteria and mammals are stimulated by light as an abiotic factor regulating such processes as plant growth and development, photomovement, colour vision, and circadian rhythms.[1] Incoming light signals are transformed into chemical functionality by sensory photoreceptors which can be subclassified into a) blue (390–500 nm) and UV-A (320–390 nm) absorbing receptors and b) receptors responding toward the red (600–700 nm) and far red (700–750 nm) regions of the solar spectrum. In group-A type photoreceptors, among which are phototropin and cryptochrome blue-light receptors, either photoinduced thiolate addition to flavin in its oxidized state (phototropin) or electron transfer processes based on flavins (cryptochromes) are engaged.[2] The excited state of reduced flavin in its anionic form ($FADH^-$ in cryptochromes) seems to be crucial for stimulating the electron ejection. Although flavin itself is able to absorb and emit blue/UV-A light with high quantum yield, it is inefficient to absorb UV-light at its reduced state due to a low absorbance.[3] Therefore extra antenna are necessary (Scheme 1). In the present study a molecular array has been designed and studied with the objective to imitate the photoreceptor functions of blue-light photoreceptors.

Dye conjugate **1** employs the following subunits a) a phenothiazine as electron donor[4] covalently linked to an isoalloxazine ring, and b) a pyrene as antenna to absorb the UV light and transfer the excitation energy to the phenothiazine–isoalloxazine dyad which subsequently may generate reduced isoalloxazine as a reduced-flavin-prototype. Pyrene was chosen as a molecular antenna because of its high extinction coefficient at 340 nm ($\varepsilon \approx 3 \times 10^4$ M^{-1} cm^{-1}) and its emission peak at 380–400 nm ($\Phi_f = 0.06$, CH_3CN). In order to trace the energy flux from pyrene to isoalloxazine and the putative photoinduced electron transfer from phenothiazine to isoalloxazine in **1**, the reference compounds **2** and **3** were also studied (Scheme 2).

The isoalloxazine ring was constructed by using a strategy of classical flavin synthesis.[5] Compounds **1** and **2** were obtained by liquid-phase peptide synthesis.[6] Compound **3** was assembled using the Stille coupling reaction.



Scheme 1 Architecture and function of an artificial blue-light photoreceptor.

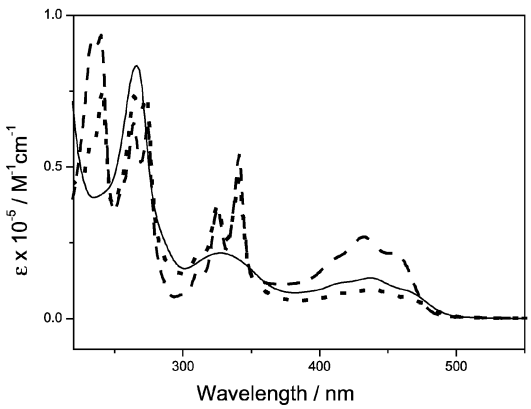
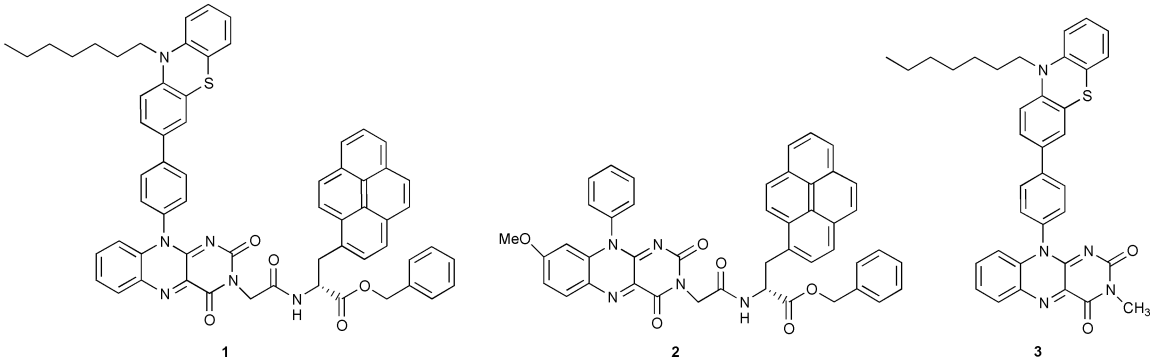


Fig. 1 Absorption spectra in CH_3CN, ··· **1** (3×10^{-5} M), **- - - 2** (3.5×10^{-5} M), **— 3** (2.7×10^{-5} M).

† Electronic supplementary information (ESI) available: spectroelectrochemistry of compound **1**, protocol for the peptide synthesis and analytical data. See http://www.rsc.org/suppdata/cc/b1/b109151d/



Scheme 2 Chemically synthesized model compounds.

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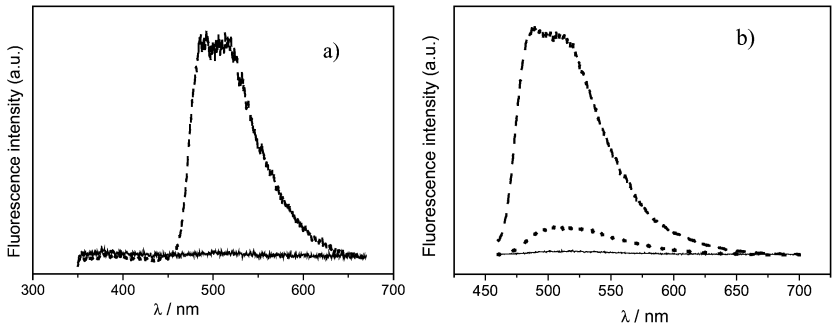

Fig. 2 Fluorescence spectra in CH_3CN: a) λ_{ex} = 340 nm; b) λ_{ex} = 450 nm: — **1** (3×10^{-5} M), - - - **2** (3.5×10^{-5} M), ··· **3** (2.7×10^{-5} M).

Table 1 Half-wave redox potentials $E_{1/2}$ of compounds **1**–**3** and of three reference compounds for comparison[a]

Compound	Oxidation: $E_{1/2}$(ox)/V	Reduction: $E_{1/2}$(red)/V
N-Methylphenothiazine[bc]	0.32	
(*S*)-α-Amino-1-pyrenepropanoic acid benzyl ester[d]		−2.45
10-Phenyl-3-methylisoalloxazine[e]		−1.08
1[d]	0.28	−1.07, −2.44
2[d]		−1.18
3[e]	0.29	−1.18

[a] Referenced *vs*.Fc^+/Fc. [b] H. Spreitzer, M. Scholz, G. Gescheidt and J. Daub, *Liebigs Ann*., 1996, 2069–2077. [c] In CH_3CN. [d] In DMSO. [e] In CH_2Cl_2.

The absorption spectra of the three model compounds **1**–**3** are given in Fig. 1. The absorption band in the region of 400–500 nm is typical for the oxidized form of isoalloxazine, the bands at about 340 nm belong to the intense long-wavelength absorption of pyrene and the broad band at 310 nm of compound **3** is due to the absorption of the phenothiazine unit. According to the absorption spectra, one can almost selectively excite one of the subunits by choosing appropriate excitation wavelengths. The absorption spectrum of **2** coincides with the sum of the absorption bands of the isoalloxazine and pyrene subunits.

Illumination of the pyrene unit at 340 nm in **2** results in the isoalloxazine fluorescence (Φ_f = 0.047) at $\lambda_{em} \approx$ 485 nm whereas the emission of the pyrene group ($\lambda_{em} \approx$ 380 nm) is nearly completely quenched. Illumination of the pyrene unit in **1** results in nearly no emission from both the pyrene and the isoalloxazine units (Fig. 2a). Illumination of the isoalloxazine at 450 nm results in isoalloxazine fluorescence (λ_{em} 485 nm, Φ_f = 0.076) in **2**, very weak isoalloxazine fluorescence (Φ_f = 0.006) in **3** and nearly no emission in **1** (Fig. 2b). We interprete these findings as indication of excited-state energy transfer from pyrene to the isoalloxazine in **1** and **2**. We also assume that the quenching of the isoalloxazine fluorescence in **1** may be caused by relaxation to a charge separated state containing isoalloxazine radical anion and phenothiazine radical cation. The efficiency of energy transfer from pyrene to isoalloxazine/phenothiazine is estimated to be >90%, based on the almost complete quenching of the pyrene emission and on the close matching of the fluorescence excitation spectrum and the absorption spectrum.[7]

Electrochemical studies were undertaken in order to estimate the energetics of the charge separated state (Table 1). The dyads **2** and **3** as well as triad **1** have similar redox potentials as found for the reference compounds *N*-methylphenothiazine, pyrenyl amino acid benzyl ester and 10-phenyl-3-methylisoalloxazine indicating almost no coupling of the subunits. An approximate evaluation[8] of the driving force G_{ET} for the charge separated state of **1** using equation $-\Delta G_{ET} = \Delta E_{oo} - (E_{ox} - E_{red})$ predicts a strongly exothermic process, G_{ET} = −1.9 eV (≈ −44 kcal mol^{-1}), with a supposedly short-lived charge separated state as intermediate.‡

From the electrochemical and spectroscopic studies, we conclude that triad **1** fulfils basic properties of the light-function of sensory blue-light receptors. Although one has to consider that the protein environment is of crucial importance and secondly the sensory behaviour very much depends also on the dark processes subsequent to light adaption. The system reported here has several advantages: first, the energy transfer efficiency from the pyrene antenna to the isoalloxazine can be tuned by the length of the peptide chain; second, the electron transfer from the light-excited, reduced isoalloxazine to a presumptive redox signalling partner (dark process) would not be affected by the pyrene antenna, due to the high negative reduction potential of the pyrene group of about −2.5 V. Thus an efficient energy and electron transfer may be achieved in the model system. Third, different peptide chains or recognition sites can be designed for the intermolecular assembling of substrates for signal transduction. Investigations are under way using time-resolved spectroscopy for the direct observation of the photogenerated intermediates and to incorporate the dye-conjugates into supramolecular architectures to investigate the switching-sensitivity of triad **1** toward external chemical and physical stimuli.

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Notes and references

‡ Quantum yields are calculated by using riboflavin as a reference (Φ_f = 0.26 in aqueous solution).

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