## **Photoreceptor Mimetics**

## "Turn ON/OFF your LOV light": Borondipyrromethene–Flavin Dyads as Biomimetic Switches Derived from the LOV Domain\*\*

## Christian Trieflinger, Knut Rurack,\* and Jörg Daub\*

The induction and control of many important biological processes rely on light signals. Chromophores participate in actions such as charge separation, ion pumping, neural responses, or photostimulated movement as photoreceptors or photosensors.<sup>[1]</sup> In the course of evolution, Nature has created rather complex biochemical processes that are photoregulated or -mediated, for example, the visual process or light-activated plant defense. As light is a very convenient tool to be used as a trigger or control signal, chemists have been attracted by the potential that lies in photobiological systems to be adopted for the design of artificial supramolecular ensembles that show addressable photochemical activity. Primary research areas here include photoresponsive membranes, light-controlled host-guest chemistry, and photochemical switching.<sup>[2]</sup> Among the latter, however, biologically inspired systems are still scarce, and the examples reported so far have been mainly accomplished in conjunction with polymeric structures.<sup>[3]</sup>

Distinct molecular systems are even less abundant.<sup>[4]</sup> This is surprising as particular biochemical moieties are very attractive candidates for multimode supramolecular photoswitches, a field of research in which we have become increasingly interested over the years.<sup>[5]</sup> One such example is the functionalized isoalloxazine chromophore. Flavins are among the most important biological redox-active dye units, and their rich photo, redox, and (inter)molecular chemistry distinguish them as ideal composites for sophisticated chemical switches.<sup>[6]</sup> Furthermore, a flavin residue is at the heart of the active site of one of the most exciting photorecepting proteins, the LOV (light-, oxygen-, or voltage-sensing)

- [\*] Dr. C. Trieflinger, Prof. J. Daub
  Institut für Organische Chemie
  Universität Regensburg
  Universitätsstrasse 31, 93053 Regensburg (Germany)
  Fax: (+49) 941-943-4984
  E-mail: joerg.daub@chemie.uni-regensburg.de
  Dr. K. Rurack
  Div. I.3
  Bundesanstalt für Materialforschung und -prüfung (BAM)
  Richard-Willstätter-Strasse 11, 12489 Berlin (Germany)
  Fax: (+49) 30-8104-5005
  E-mail: knut.rurack@bam.de
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domains of phototropin.<sup>[7,8]</sup> Based on this background and owing to the exceptional complementarity of flavin and the borondipyrromethene (BDP) fluorophore, which itself exhibits several important features of efficient photoswitching,<sup>[9]</sup> we designed the BDP–flavin dyads **1–3** to realize our idea of a distinctly controllable and reversible fluorescent switch (for synthetic details, see Supporting Information).



The data in Table 1 and Figure 1 clearly reveal the composite nature of the dyads as the absorption spectra of 1-3 are a linear combination of the bands of the subchromo-

**Table 1:** Absorption (in CHCl<sub>3</sub>) and redox data (in MeCN) of 1–3 ( $E_{1/2}$  in V vs. Fc<sup>+</sup>/Fc).

	$\lambda_{ m abs}$ [nm] ([lg $arepsilon$ ]/[lg ( ${ m M}^{-1}  { m cm}^{-1}$ )])	$E_{1/2}^{\rm red}$ [V]	$E_{\rm p}^{\rm c,red}$ [V]	$E_{1/2}^{\rm red}$ [V]	<i>E</i> <sup>ox</sup> <sub>1/2</sub> [V]
1	529 (4.86), 448 (4.15)	-1.11	-	-1.60	0.65
2	531 (4.81), 449 (4.12)	-1.11	-	-1.63	0.66
3	531 (4.81), 448 (4.10)	-1.10	-0.92	-1.63	0.65



**Figure 1.** Absorption spectra of 1 (solid line) and twofold reduced  $1^{red}$  ( $c = 5 \times 10^{-6}$  m; dashed line) in MeCN/MeOH (1:1). Inset: Difference spectrum of the flavin region.

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phores, the main maxima being centered at 530 nm (BDP) and 449 nm (flavin). This indication of electronically decoupled ground states is further supported by cyclic voltammetry, in which the reversible reduction of flavin  $(-1.1 \text{ V vs. Fc}^+/\text{Fc})$ and the quasi-reversible oxidation and reduction of BDP  $(0.65 \text{ V}, -1.6 \text{ V} \text{ vs. Fc}^+/\text{Fc})$  are found at the expected potentials. The different electrochemical behavior of 3 compared with 1 and 2, deducible from the additional reoxidation peak at -0.92 V vs. Fc+/Fc (Table 1), is ascribed to the well-known ECE (electron transfer-chemical stepelectron transfer) mechanism of flavin which involves an intermolecular proton-assisted redox reaction.<sup>[10]</sup> The results obtained for the ground-state properties of 1-3 presented so far suggest that one important prerequisite for the facile control of a switch is fulfilled, that is, both switching modules are independent. However, to generate ON/OFF behavior as expressed by strong signal changes in light output, a welldefined OFF state is necessary, especially when a switch is constructed from two strongly emitting fluorophores such as flavin and BDP. In this respect, the suitability of our design rationale is evident from Table 2 (see also the theoretical considerations detailed in the Supporting Information).

Table 2:	Fluorescence	properties	of 1	-3.
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	Solvent	$arPhi_{ m f} imes$ 10 $^{-3[a]}$	$ au_{\rm f}  [{\rm ps}]^{[{\rm b}]}$	$ au_{ m f}  [ m ns]^{[ m c]}$		
1	MeCN/toluene <sup>[d]</sup>	1.3/2.3	10/17	-		
	EtOH	1.6	13	5.33		
2	MeCN/toluene <sup>[d]</sup>	0.7/1.3	6/11	-		
	EtOH	1.0	8	5.54		
3	MeCN/toluene <sup>[d]</sup>	0.8/1.5	6/10	-		
<b>3</b> <sup>-[e]</sup>	MeCN	8.8	96	-		

[a] Relative to fluorescein 27 in NaOH (0.1  $\times$ ; 0.90±0.03),<sup>[15]</sup> ±15%. [b] At 298 K, ±3 ps. [c] At 77 K, ±0.003 ns. [d] First value in MeCN, second in toluene. [e] Deprotonated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

All three dyads show only a weak fluorescence with typical BDP features, regardless of solvent polarity and proticity. No isoalloxazine emission could be detected, even with excitation at 449 nm. The fluorescence also decays rapidly, stressing the fact that an efficient nonradiative deactivation path exists in the title compounds. Furthermore, 1 and 2 show a very similar behavior, excluding any influence of a "meta" effect.<sup>[11]</sup> The important role of the directly fused phenylene spacer becomes apparent, especially from the emission data. This moiety acts simultaneously as an orbital insulator (by preventing a mixing of states that is often found in connection with the "meta" effect) and as an electronic mediator (by enabling efficient fluorescence quenching). At 77 K, the fluorescence increases dramatically, and decay times that are typical for BDP dyes and close to the value of the reference compound 4 (6.27 ns) are measured. Again, after excitation of the flavin, only BDP emission can be detected, thus suggesting that efficient energy transfer to the BDP occurs. At room temperature, photoinduced electron transfer (PET) from the BDP to the electron-poor flavin most probably accounts for the strong quenching of the fluorescence.

## Communications

In glassy ethanol at 77 K, PET is inhibited as a result of the freezing out of solvent reorganization and intramolecular movement. This interpretation is supported by the results obtained upon deprotonation of **3** at N3. Such an increase in electron density at the flavin entails a > 10-fold enhancement of the BDP emission (Table 2). Dyads **1** and **2**, which are methylated at N3, do not show any spectroscopic changes upon base addition.

Once the conventional OFF state of the switch was defined, the next step was to install a control function that allows this state to be "unlocked". The versatile redox chemistry of flavin<sup>[6]</sup> allows such an activation to be performed chemically. In this experiment and in analogy to thiolbased natural systems, the spectroscopic changes were monitored in situ during the chemical reduction of the flavin moiety with 1,3-propanedithiol<sup>[12]</sup> and DBU in MeCN/MeOH (1:1) under an argon atmosphere. The products were the twofold reduced dyads  $\mathbf{1}^{\text{red}}$  and  $\mathbf{2}^{\text{red}}$  and [1,2]dithiolane. As can be seen in Figure 1, the flavin absorption at 440 nm decreases, whereas the BDP absorption is not affected, which clearly indicates that the redox process occurs in the flavin. However, both  $\mathbf{1}^{\text{red}}$  and  $\mathbf{2}^{\text{red}}$  are still weakly fluorescent, with fluorescence lifetimes within the resolution of the instrument ( $\tau_f = 3-5$  ps). These observations now mark the chemically controlled OFF state and can be explained by a reversal of the ET process (Supporting Information). In the reduced dyads, the electron density at the flavin is so high that excitation of the BDP triggers a PET from the flavin to the BDP, reductive PET processes being well-known for donor-substituted BDPs (Figure 2).<sup>[9b]</sup>

Now that we were able to switch between an inactive and an activated OFF state, the last requirement concerned the input that allows reversible OFF/ON switching. Keeping in



**Figure 2.** Reaction scheme of the thermal and photochemical switching between the non-activated OFF states (1, 2), the activated OFF states (1<sup>red</sup>, 2<sup>red</sup>), and the ON state (reporter state). Inset: Reversible switching between the activated OFF (mauve) and the ON state (yellow) of 1<sup>red</sup> as reflected by the change in emission intensity in MeCN/MeOH (1:1) during several irradiation cycles with cycle times of 30 s irradiation and 300 s thermal reverse reaction ( $c_1 = 1 \times 10^{-6}$  M).

mind the discourse in the introductory paragraph, at this stage the photomediated reaction cycle of the LOV domain comes into play. If a solution with the chemically activated ensemble is irradiated with UV light of 254 nm, a strong increase in BDP fluorescence is noticed. The high efficiency of the switching process is strikingly evident from a several-100-fold enhancement of BDP emission and is manifested in  $\tau_{\rm f}$  values of 4.96 and 5.27 ns for the systems based on **1** and **2**. The two reduced dyads are fully and rapidly converted, and after turning off the UV-light source, the fluorescence decreases again to its initial value.

The reversibility of this reaction is shown in Figure 2, which indicates that irreversible side reactions do not play a major role. Most probably, UV irradiation initiates the formation of a thermally unstable but highly emissive species, which is accumulated upon continuous irradiation. Dyads  $1^{red}$  and  $2^{red}$  are then regenerated in a thermal reverse reaction. Although the real nature of the emitting species could not yet be analytically determined with methods available to us, the results strongly suggest that the electron density of the flavin is considerably lower than in the oxidized state, but higher than in the fully reduced state. A mono-reduced state can be excluded as such radicals would also quench the emission.

Thus, we tentatively assume that the light-induced formation of an emissive flavin–thiol adduct in analogy to the LOV domain photocycle occurs under the prevailing reaction conditions. The thermal recovery rates reported for LOV domains are commonly in the range of 0.02 to 0.003 s<sup>-1</sup>, depending on its type, the medium, and possible protein assistance.<sup>[7,13]</sup> These properties suggest that a C4a adduct or a C4a–C10a cycloadduct might be involved under the present, more-extreme conditions. Further experiments are currently underway to elucidate the mechanism in more detail.

In conclusion we have presented novel BDP–flavin dyads with distinctive electrochemical and optical properties, including energy- and electron-transfer-modulated emission. The direction and efficiency of the ET process can be influenced by various input signals, such as the addition of base, redox processes, and irradiation with light. These features distinguish 1 and 2 as potent biomimetic photochemical switches<sup>[14]</sup> in which the activation state can be independently controlled and the output emission reversibly switched.

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