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Amplifying the reactivity of BODIPY photoremovable protecting groups†

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Conjugated polymer nanoparticles (CPNs or Pdots) are used to sensitize the photorelease reaction of a BODIPY photoremovable protecting group. Sensitization yields effective values of $\varepsilon_\lambda\Phi_{pr}$ – the product of the extinction coefficient at the irradiation wavelength and the photorelease quantum yield – that are more than 60-fold greater than those measured upon direct excitation.

Photoremovable protecting groups (PPGs) are prized for their ability to release a specific chemical species with the high spatiotemporal resolution afforded by light stimulation.[1] The damaging effects and poor penetration depth of ultraviolet (UV) light have made development of PPGs that undergo bond cleavage in response to visible or infrared light an urgent priority.[2,3] PPGs based on visible-absorbing *meso*-methyl BODIPY dyes initially developed by the groups of Winter[4] and Weinstain[5] are among the most promising of the new generation of PPGs. The first reported BODIPY PPGs exhibited reactivity similar to that of well-known UV-responsive PPGs due to large extinction coefficient values (ε) that offset low photorelease quantum yields (Φ_{pr}).[5] These BODIPY PPGs typically require long irradiation times (minutes to hours),[4,5] which are impractical in many cases. Furthermore, many BODIPY PPGs have limited water solubility and are studied in methanol or in buffer with an organic cosolvent. Here, we present a strategy for amplifying the reactivity of BODIPY PPGs in a completely aqueous environment using conjugated polymer nanoparticles (CPNs or Pdots) as sensitizers.

CPNs are brightly fluorescent organic materials that are stably suspended in water.[6] High extinction coefficients and good quantum yields make the multichromophoric CPNs outstanding light absorbers and emitters.[7,8] CPNs can be doped or covalently functionalized with small molecule dyes and other polymers to tune properties or impart function. In particular, CPNs doped with dyes that act as fluorescence resonance energy transfer (FRET) acceptors have been employed in applications from singlet oxygen generation[9] to sensing and imaging.[10]

CPNs are remarkable FRET donors because exciton diffusion can deliver excited state energy from conjugated polymer chromophores to acceptor dyes from well beyond the Förster radius. Jiang and McNeill have shown that an acceptor at the center of a CPN of 24 nm diameter has an effective quenching radius that is 63% larger than that predicted in the absence of exciton diffusion.[11] The observed energy transfer efficiency in CPN FRET systems is thus higher than expected for a simple donor–acceptor system due to the combined action of exciton diffusion and energy transfer processes. We have used this phenomenon – sometimes called "amplified energy transfer"[11] – to enhance the reactivity of dyes that undergo low-quantum-yield photochemical reactions.[12,13] Pairing CPNs' highly efficient excitation and FRET processes with a dye's inefficient photochemical reaction facilitates a fast dye reaction without inadvertent ambient light reaction. In systems with highly efficient CPN-to-dye FRET, we determined that the dye's quantum yield of reaction must be $\sim$0.05 or lower to achieve "controlled amplification," where the reaction is facile but not so much so that it is initiated by ambient light.[12]

We seek to develop PPG-doped CPNs that undergo efficient photorelease on the timescale of seconds, without unwanted ambient light reactivity, in a completely aqueous environment. The *meso*-methyl BODIPY PPGs are ideal candidates for this effort. They absorb in the visible with good extinction coefficients, which makes them well-suited to act as FRET acceptors for CPN chromophores. Their relatively poor water solubility makes them highly suitable for doping onto the CPN surface. Finally, their low photorelease quantum yields enable us to amplify PPG reactivity while maintaining control. With Φ_{pr} values on the order of 10^{-4}, the first-generation BODIPY PPGs are an excellent match for this design.[4] The key figure of merit in PPG studies is $\varepsilon_\lambda\Phi_{pr}$, which is the product of the PPG extinction coefficient at the irradiation wavelength and the photorelease quantum yield. Here, we will use photokinetic

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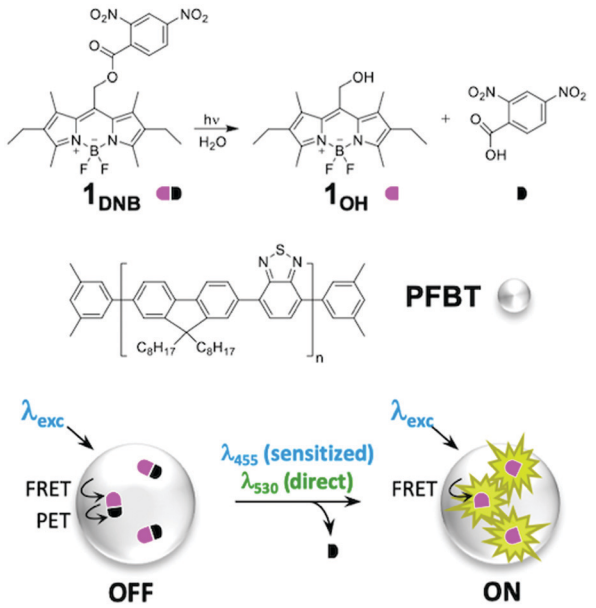

Scheme 1 Components and operation of doped CPNs.

methodology to demonstrate that the effective $\varepsilon_\lambda \Phi_{pr}$ value increases more than 60-fold when we use the CPNs to sensitize BODIPY PPGs in water.

Our goal of extracting $\varepsilon_\lambda \Phi_{pr}$ values from photokinetic data requires tracking the PPG reactant or product concentration as a function of time during continuous irradiation. The absorbance of many BODIPY PPGs does not change significantly during the photorelease reaction so cannot be used to obtain kinetic data here. Alternatively, we targeted a BODIPY PPG functionalized with a fluorescence quencher so that we could track the appearance of a reaction product *via* fluorescence. Analogous to a construct reported by Winter,[4] $\mathbf{1_{DNB}}$ (Scheme 1) is initially non-fluorescent due to photoinduced electron transfer (PET) from the BODIPY core to a quencher derived from 2,4-dinitrobenzoic acid. Upon photorelease of the quencher, the PPG forms a carbocation that reacts with the surrounding medium[14] to yield the fluorescent product $\mathbf{1_{OH}}$.

We doped $\mathbf{1_{DNB}}$ onto the surface of CPNs prepared *via* reprecipitation from the conjugated polymer poly[(9,9-dioctylfluorenyl 2,7-diyl)-*co*-1,4-benzo-{2,1′-3}-thiadiazole] (PFBT, Scheme 1). The fluorescence spectrum of PFBT CPNs overlaps significantly with the absorbance spectrum of $\mathbf{1_{DNB}}$ (Fig. S1A, ESI†), which is a requirement for efficient FRET. CPNs doped with $\mathbf{1_{DNB}}$ are expected to be non-fluorescent in their as-prepared form: excited CPN chromophores are quenched by FRET to the BODIPY core, which in turn is quenched by PET. Based on our previous findings on sensitization of low-quantum-yield photoreactions,[12] we expect that photorelease should not be stimulated by ambient light or low intensity excitation of the CPNs. When higher intensity LED irradiation is delivered to the top of the sample cuvette, $\mathbf{1_{DNB}}$ will undergo photorelease, and fluorescence from the uncaged BODIPY fluorophore will be observed (Scheme 1). The photorelease reaction kinetics will be monitored by tracking the increase in fluorescence as the fluorescent $\mathbf{1_{OH}}$ product forms.

CPNs doped with $\mathbf{1_{DNB}}$ have an average diameter of 15 ± 7 nm in aqueous suspension as measured by dynamic light scattering (Fig. S1B, ESI†). As described in the ESI,† the particle size, polymer molecular weight, and absorbance values can be used to estimate that each CPN is comprised of an average of *ca.* 7 polymer chains and 30 dyes. The absorbance spectrum (Fig. 1A) of the dye-doped CPNs is dominated by the CPN absorbance (λ_{max} ∼ 461 nm) with a much smaller contribution from $\mathbf{1_{DNB}}$ (λ_{max} ∼ 555 nm). The $\mathbf{1_{DNB}}$ absorbance can be more easily visualized in a difference spectrum from which the CPN absorbance has been subtracted (Fig. 1A inset). The corresponding 555 nm λ_{max} value represents a ∼5 nm bathochromic shift from the dye's spectrum in THF, consistent with weak positive solvatochromism and the more polar environment of the water-exposed CPN surface. Blue light selectively excites the CPNs due to $\mathbf{1_{DNB}}$'s minimal absorbance at this wavelength (ε_{455} = 1300 M^{-1} cm^{-1}). Green light is used to directly excite $\mathbf{1_{DNB}}$ (ε_{530} = 21 400 M^{-1} cm^{-1}). Compared to a control sample of undoped CPNs, as-prepared $\mathbf{1_{DNB}}$-doped CPNs exhibit highly quenched fluorescence (Fig. 1B). The nearly complete quenching of CPN fluorescence ($\lambda_{max,fl}$ = 537 nm) is consistent with CPN-to-BODIPY FRET. Algar and coworkers recently determined that FRET is the dominant quenching mechanism in similar doped CPNs.[15] Slightly higher fluorescence intensity is observed in the BODIPY ($\lambda_{max,fl}$ = 568 nm) channel due to incomplete quenching of BODIPY fluorescence by the DNB moiety, which has been observed previously.[4]

To initiate photorelease, we excited $\mathbf{1_{DNB}}$ dyes directly (530 nm) and *via* the CPN-mediated FRET pathway (455 nm) by irradiating samples for 60 s at a moderate photon flux. Steady-state fluorescence spectra recorded before and after irradiation (Fig. 2A) demonstrate that FRET-sensitization is far more effective at initiating photorelease than direct excitation of the dye. Direct excitation with 530 nm yields a 21% increase in fluorescence intensity at 568 nm over the residual fluorescence in the as-prepared CPNs. In contrast, FRET-sensitization with 455 nm irradiation yields a 320% increase in fluorescence intensity. The dramatic increase in fluorescence activation is accompanied by a 7 nm hypsochromic shift in

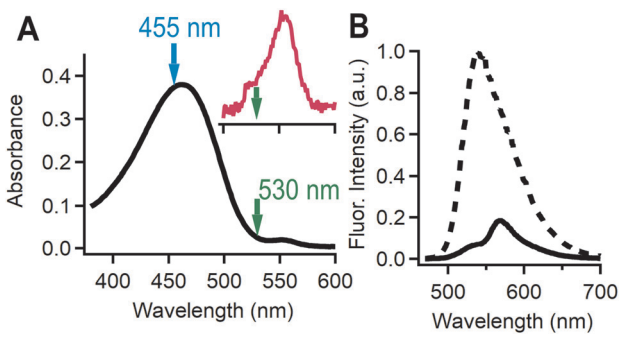

Fig. 1 (A) Absorbance of $\mathbf{1_{DNB}}$-doped CPNs with irradiation wavelengths marked and (inset) difference spectrum of $\mathbf{1_{DNB}}$ absorbance in the CPN environment. (B) Fluorescence spectrum of identically-concentrated suspensions of undoped (dashed) and $\mathbf{1_{DNB}}$-doped (solid) CPNs.

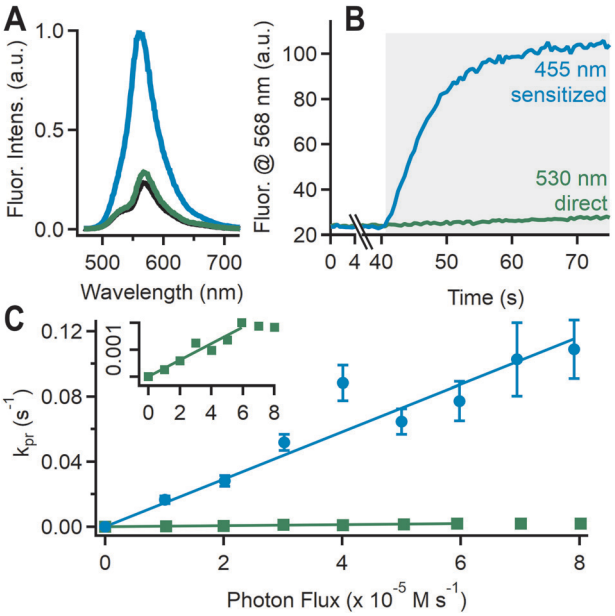

Fig. 2 (A) Fluorescence spectra of $\mathbf{1_{DNB}}$-doped CPNs as-prepared (black) and after 1 min of irradiation at a photon flux of 4×10^{-5} M s^{-1} from 455 nm (blue) and 530 nm (green) LEDs. (B) Fluorescence intensity of $\mathbf{1_{DNB}}$-doped CPNs at 568 nm as a function of time at the same photon flux and LED colors as part A. The shaded area indicates the irradiation period. (C) k_{pr} *vs.* input photon flux for FRET-sensitized (blue) and direct (green) reaction in $\mathbf{1_{DNB}}$-doped CPNs. Inset: Expansion of direct reaction trace. Error bars for direct excitation are within the height of the symbols.

$\lambda_{max,fl}$ that reflects the conversion of $\mathbf{1_{DNB}}$ to $\mathbf{1_{OH}}$, which fluoresces at a lower wavelength. The dramatic difference between the two post-irradiation fluorescence spectra suggests that far more $\mathbf{1_{DNB}}$ undergoes photorelease when excited *via* the FRET-sensitization than directly.

We can obtain a more detailed evaluation of photorelease in the CPN environment by studying the reaction kinetics under continuous irradiation. The fluorescence intensity of the doped CPNs increases only when quenched $\mathbf{1_{DNB}}$ is converted to fluorescent $\mathbf{1_{OH}}$, so we can use this fluorescence signal to track the progress of the photorelease reaction. Fig. 2B shows representative kinetic traces of two different aliquots of the same sample, one irradiated at 530 nm (direct) and the other at 455 nm (FRET-sensitized). For both aliquots, the initial fluorescence intensity remains stable upon exposure to the fluorimeter lamp demonstrating that photorelease is not stimulated by low-intensity light. Upon exposure to the higher intensity LED irradiation, the fluorescence intensity increases very slowly over time with direct excitation but much more rapidly with FRET-sensitization. This behavior is consistent with the fluorescence spectra in Fig. 2A that showed dramatically higher fluorescence intensity for the FRET-sensitized sample.

Following standard photokinetics methodology,[16,17] traces like those in Fig. 2B can be fit to obtain rate constants from which we can ultimately determine values of $\varepsilon_{\lambda}\Phi_{pr}$. The rate constant for the photorelease reaction upon direct excitation with 530 nm irradiation, $k_{pr,direct}$, is given by eqn (1)

$$k_{pr,direct} = I_0 l F_{530}\, \varepsilon_{530} \Phi_{pr} \quad (1)$$

where I_0 is the incident photon flux, l is the path length, F_{530} is the photokinetic factor at the irradiation wavelength ($F_{530} = [1 - 10^{-Abs_{530}}]/Abs_{530}$), ε_{530} is the extinction coefficient of $\mathbf{1_{DNB}}$ at the irradiation wavelength, and Φ_{pr} is the quantum yield for photorelease. Upon 455 nm irradiation, photorelease proceeds only *via* sensitization of $\mathbf{1_{DNB}}$ by the CPNs; no direct excitation is detected upon 455 nm irradiation due to $\mathbf{1_{DNB}}$'s extremely poor absorbance at this wavelength. The rate constant for photorelease upon FRET-sensitization, $k_{pr,sens}$, is given by eqn (2)

$$k_{pr,sens} = I_0 l F_{455} \varepsilon_{455} \Phi_{pr} E_{obs} \frac{[CPN]}{[\mathbf{1_{DNB}}]} = I_0 l F_{455} (\varepsilon_{455} \Phi_{pr})_{eff} \quad (2)$$

where the relevant extinction coefficient is now that of the CPNs, E_{obs} is the observed efficiency of the combined exciton diffusion and FRET processes, and the concentration ratio $[CPN]/[\mathbf{1_{DNB}}]$ accounts for the composition of the system. Eqn (1) and (2) predict a linear relationship between the rate constants and the input photon flux. Slopes of plots of k_{pr} *vs.* I_0 can be used to calculate $\varepsilon_{\lambda}\Phi_{pr}$ for both directly excited and FRET-sensitized reactions. For direct excitation, the $\varepsilon_{530}\Phi_{pr}$ value expresses the ability of $\mathbf{1_{DNB}}$ to be excited and undergo the photorelease reaction. The parallel term for the FRET-sensitized reaction is defined as $(\varepsilon_{455}\Phi_{pr})_{eff}$ in eqn (2), where this effective value incorporates all terms related to CPN excitation, energy transfer to $\mathbf{1_{DNB}}$, and photorelease.

We obtained photorelease rate constants for direct and sensitized excitation of $\mathbf{1_{DNB}}$ in doped CPNs (Fig. 2C) as described in the ESI.† The rate constants depict a stark difference between direct excitation and FRET-sensitization: k_{pr} values are more than an order of magnitude higher for the sensitized reaction. Values of $k_{pr,sens}$ increase linearly with photon flux over the entire range studied. In contrast, values of $k_{pr,direct}$ initially exhibit a linear increase but plateau at the high end of the photon flux range (Fig. 2C inset). If the plateauing were due to photobleaching at higher photon fluxes, we would expect to observe the same behavior in the sensitized data as well since both direct and sensitized pathways produce excited BODIPY chromophores. While the origin of this phenomenon is not yet known, it represents another possible advantage of sensitization in this system.

Values of $\varepsilon_{\lambda}\Phi_{pr}$ were calculated by dividing the slopes of the k_{pr} *vs.* I_0 plots by the path length, which is known, and the photokinetic factor, which is calculated from absorbance data. Direct excitation of $\mathbf{1_{DNB}}$ in the CPNs at 530 nm yields an $\varepsilon_{530}\Phi_{pr}$ value of 14 M^{-1} cm^{-1}, which is slightly lower than reported values for many other first-generation BODIPY PPGs.[4] This result is expected due to the unusually low extinction coefficient of $\mathbf{1_{DNB}}$ (ε_{530} = 21 400 M^{-1} cm^{-1}), which is similar to the ε value observed for another nitro-containing BODIPY PPG.[14] From these data we obtain a Φ_{pr} value of 6.5×10^{-4} for direct excitation in CPNs. This value is comparable to those reported for other first-generation BODIPY PPGs.[4] Here, it likely represents an upper limit due to a small amount of 530 nm light absorbed by

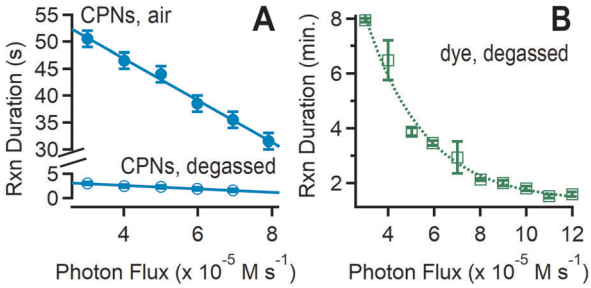

Fig. 3 Duration of photorelease reaction for (A) $\mathbf{1_{DNB}}$-doped CPNs in air-equilibrated (closed circles) and degassed (open circles) aqueous suspension and (B) $\mathbf{1_{DNB}}$ in degassed methanol. Error bars for degassed CPNs are within the height of the symbols.

the CPNs. For the sensitized reaction, the value of $(\varepsilon_{455}\Phi_{pr})_{eff}$ as defined in eqn (2) is 860 M^{-1} cm^{-1}, which is more than 60 times greater than the $\varepsilon_{530}\Phi_{pr}$ value for direct excitation in CPNs.

In practical terms, the amplified $\varepsilon_\lambda\Phi_{pr}$ value observed for the sensitized reaction translates to a drastic reduction in the irradiation time and intensity required to stimulate photorelease. To quantify this effect, we measured the total duration of the photorelease reaction from the onset of irradiation until the fluorescence intensity leveled off. We attempted this measurement for direct excitation of $\mathbf{1_{DNB}}$ in CPNs and in methanol to furnish context for the sensitized reactions. The reaction in both CPN and solution environments was too slow for a complete measurement. However, the duration was clearly in the range of hours for the photon flux range used in these studies. Sensitized photorelease is dramatically faster, with reactions complete within 35.5–50.5 s in the aqueous CPN suspensions (Fig. 3A). The remarkable decrease in reaction duration upon going from direct excitation to sensitization demonstrates the practical utility of CPN sensitization.

Mechanistic work by Slanina *et al.* has shown that the photorelease reaction occurs from the singlet excited state (S_1) but can also proceed from the triplet excited state (T_1) in degassed environments where oxygen is not available to quench T_1.[14] We degassed aqueous suspensions of doped CPNs to assess how the combination of FRET-sensitization and triplet state reactivity affects photorelease. Reaction durations in degassed CPNs are significantly shorter than their air-equilibrated counterparts: 1.6–3.0 s (Fig. 3A). Decreased reaction durations are expected as Φ_{pr} values in degassed solutions are generally double those in aerated environments due to the additional triplet reaction pathway.[14] For both air-equilibrated and degassed CPN suspensions, the reaction duration decreases linearly as the photon flux is increased. As a reference, we also studied direct excitation of $\mathbf{1_{DNB}}$ in degassed methanol. Reaction duration decreases from an estimate of hours in air-equilibrated solution to minutes in the degassed environment (Fig. 3B). This change represents a dramatic decrease in the irradiation period required to complete the reaction under direct excitation conditions. However, the reaction duration for $\mathbf{1_{DNB}}$ in degassed methanol solution at the highest flux studied (1.5 min at 1.2 $\times$ 10^{-4} M s^{-1}) is still longer than the sensitized reaction in air-equilibrated conditions at the lowest flux studied (50.5 s at 3 $\times$ 10^{-5} M s^{-1}). Unlike the sensitized reactions, the directly excited reactions in degassed methanol solution exhibit a curved dependence of reaction duration on photon flux, with durations leveling out at higher fluxes (Fig. 3B). This result – which may be related to the plateauing of $k_{pr,direct}$ values (Fig. 2C inset) – indicates that reaction duration is more tunable when the reaction is sensitized by CPNs.

In conclusion, CPNs are powerful sensitizers for the photorelease reaction of a first-generation BODIPY PPG in a completely aqueous environment. The sensitized reaction has an effective $\varepsilon_\lambda\Phi_{pr}$ value that is more than 60 times greater than that measured under direct excitation conditions. In practical terms, a photorelease reaction that requires hours of irradiation when directly excited goes to completion in under a minute when sensitized by the CPNs. BODIPY PPGs with higher photorelease quantum yields have been developed but sometimes react so efficiently with light that they are difficult to control.[14] CPN-sensitization of a low-quantum-yield PPG enables a much faster reaction without unwanted ambient light reactivity.

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Conflicts of interest

There are no conflicts to declare.

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