Photocycloadditions in Disparate Chemical Environments

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We elucidate the wavelength dependence of a photocycloaddition by (*i*) accessing action plots dependent on the reactivity relative to the number of absorbed photons, (*ii*) establishing the effect on substrate concentration on photochemical reactivity and (*iii*) determining wavelength-dependent reactivity as a function of the solvent environment, comparing acetonitrile with dimethyl sulfoxide.

The spatiotemporal control of photochemical reactions is their defining feature in both fundamental research and industrial applications.[1] Critically, light-gated reactions further enable the selective initiation of reactions based on the employed wavelength or intensity.[2–7] However, to address different reaction channels selectively, it is paramount to understand the wavelength-dependent reactivity of the employed chromophores to avoid reactivity overlaps, where multiple reactions are concomitantly initiated.

While classically absorption spectra are used to estimate at which wavelength a photo-responsive molecule may be triggered,[8,9] we have recently reported that the wavelength-dependent reactivity cannot be readily predicted from absorption spectra.[10–13] In contrast, we demonstrated that reactivity maxima were in many cases significantly shifted from the absorption maximum and high reactivities were observed at wavelengths of low extinction molar attenuation coefficients. These findings are captured in our action plot concept, in which a photoreactive molecule is irradiated at specific wavelengths using a monochromatic tunable nanosecond pulsed laser system, with the output energy adjusted so that a constant number of photons is emitted at each wavelength. Plotting the conversion of a photochemical reaction versus the irradiation wavelength to obtain an action plot provides access to the wavelength-dependent reactivity of a photo-induced reaction system experimentally – without the need for advanced computational or spectroscopic methods.

These action plots thus serve as reactivity maps and enabled the design of the first wavelength-orthogonal photoligation systems, implying that one molecule can be triggered using a specific wavelength, while the other molecule remains unreacted until it is addressed with a disparate wavelength.[14,15] Whereas monochromatic light is key to record an action plot, orthogonal initiation of complex photoreaction systems can be achieved even by using LED's with a rather broad spectral range – provided that detailed action plots are available.[14,16]

[2+2] cycloadditions are particularly suitable for wavelength-orthogonal ligation systems, where multiple photoreactions are independently initiated as a function of irradiation wavelength. While the cycloaddition is often triggered at higher wavelengths, shorter wavelengths favour the cycloreversion reaction. Thus, light of a shorter wavelength can be employed to actively suppress the [2+2] cycloaddition, while simultaneously triggering a second photoreactive molecule. Moieties such as cinnamic acid, coumarin, thymine and stilbene have been employed for [2+2] cycloaddition ligation reactions. However, the light used to trigger the reactions was mostly within the UV-A or even UV-B regime.[17–21]

A particularly powerful photochemical moiety for remote controlled ligation reactions is styrylpyrene, since it is able to undergo a [2+2] cycloaddition in the visible light regime, which can be supressed by mild UV light (**Scheme 1**).[11,22–24] Over the last years, styrylpyrene was used for the photocrosslinking of DNA[23] or polymers,[11,25] photopolymerizations[16] and hydrogelations.[24] Due to its reversible nature, styrylpyrene is

uniquely suitable for wavelength-orthogonal reactions, for example in combination with *o*-methyl benzaldehyde,[15] or anthracene[14]. Whereas the wavelength-dependent reactivity of styrylpyrene was previously investigated with an action plot,[11] critical reaction parameters remain unexplored, severely affecting the universal applicability of this photoreactive group.
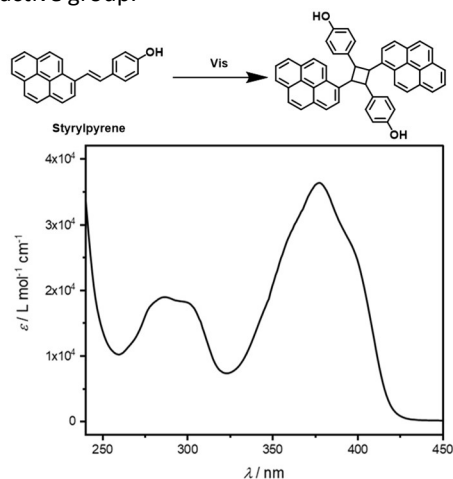
Scheme 1 Visible-light-triggered [2+2] cycloaddition of styrylpyrene including its UV/Vis spectrum in acetonitrile.

Herein, we investigate the wavelength-dependent reactivity of the powerful photoreactive styrylpyrene moiety on three unprecedented levels: **(i)** by developing a new action plot technique that takes into account the light absorption of the chromophore, which is critical where light attenuation is limiting applications (such as photocuring); **(ii)** by investing the effect of concentrations and **(iii)** solvent on wavelength-dependent and absorbance-dependent reactivity.

Since action plots are recorded with an identical number of photons at each wavelength,[10–13] the absorbance of the chromophore is not accounted for. In the case of styrylpyrene, the absorbance is more than 180 times lower at the determined most suitable wavelength to trigger the dimerization (λ = 435 nm) when compared to the absorption maximum (λ_{max} = 375 nm). As stated above,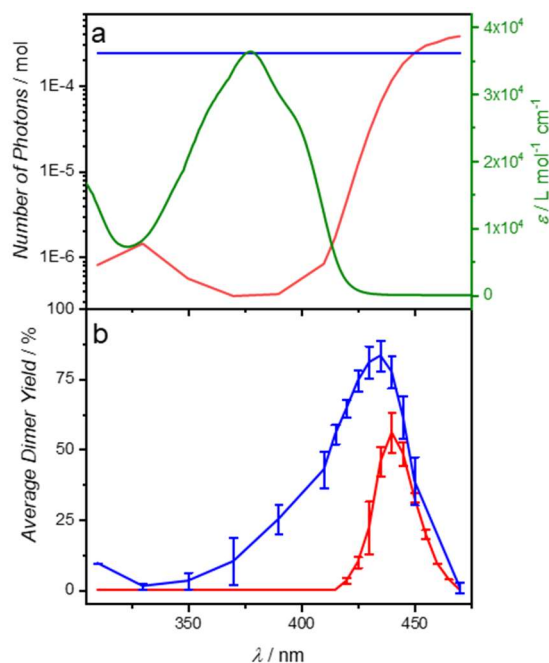 employing a constant number of photons has the effect that at long wavelengths (especially above 400 nm) less photons will be absorbed to trigger a reaction. While determining the exact quantum yield at different wavelengths is highly challenging, since absorbance of the solution changes drastically over time, we herein develop a facile method to reveal reactivity relative to the absorbed photons. By adjusting the number of photons to the absorption spectrum of styrylpyrene, an action plot is obtained that allows to approximate the reactivity of the absorbed photons without calculating exact quantum yields (refer to the Supporting Information for a detailed description of the tuneable laser system and the calculation of the photon count).

The resulting wavelength dependence of relative reactivity (**Figure 1**, red line) is on the one hand considerably narrower in shape, but also slightly red-shifted compared to the conventional action plot (**Figure 1**, blue line). The wavelength for the most efficient reaction when irradiating styrylpyrene relative to its absorbance is at 440 nm and, thus, shifted even

further into the visible light with a narrower action plot. These results underpin the importance of thoroughly investigating a chromophore with regard to its reactivity before employing it. The absence of reactivity in the lower wavelength regime validates the suitability of styrylpyrene for wavelength-orthogonal systems, where a limited reactivity window is key to selectively excited photoreactive species.

Figure 1 Comparison of classical action plot (constant number of photons) and absorbance referenced action plot. (a) Employed number of photons; *blue* (constant) and *red* (referenced to the absorption spectrum of styrylpyrene (*green*)). (b) When the number of incident photons is referenced to the absorbance, the action plot (*red*) is narrower (415–470 nm) and slightly red-shifted, peaking at 440 nm, as opposed to the classical action plot (*blue*), which is broader (330-470 nm) and peaks at 435 nm.

When irradiating a chromophore in solution at different concentrations, yet identical number of photons, at least two competing effects can be expected: A lower concentration may lead to a more efficient reaction, since the number of absorbing molecules is smaller and, thus, more photons are available per photoreactive moiety. On the other hand, the intermolecular photocycloaddition has to compete with radiative and nonradiative decay pathways that depopulate the excited state. At lower concentrations, excited molecules are more likely to undergo relaxation pathways before they encounter a reaction partner. To investigate which effect is dominant, styrylpyrene was irradiated at two concentrations (5 mM and 10 mM, in acetonitrile, **Figure 2**). Overall, at the most efficient wavelength (440 nm), the dimerization is 14% less efficient at lower concentrations, even though more photons are available per molecule. The dimerization yield is 56 % at the higher concentration, while at lower concentration it is only 42 %. In a previous study, we observed that the reaction of styrylpyrene as a polymer chain side group has a higher quantum yield than in free solution.[25] Within the confined environment of a polymer chain, the local concentration is significantly higher and therefore the reaction more favoured than at lower concentrations. The observed concentration-dependent

behaviour likely results from the fact that at lower concentrations the possibility of an excited-state molecule to encounter a ground-state species for a successful dimerization is less probable compared to other pathways for the molecule to relax back to its ground state (e.g. via fluorescence or internal conversion).
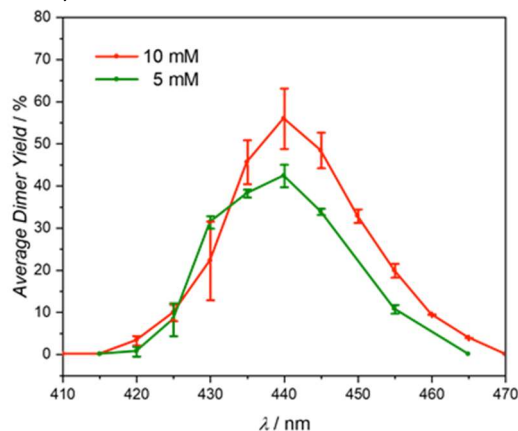

Figure 2 Comparison of the reactivity of styrylpyrene when irradiated at different concentrations with a constant number of absorbed photons (solvent: acetonitrile, *red*: 10 mM, *green*: 5 mM).

If the action plots at both concentrations (**Figure 2**) are compared to investigate the effect on the wavelength dependence, it can be seen that the wavelength-dependent reactivity does not significantly change with decreasing concentration. Attenuation effects, resulting from a thinner layer in which all photons are absorbed in the case of the more concentrated solution or vice versa, thus do not appear to affect the wavelength-dependent reactivity significantly.

In addition to the concentration dependence of the dimerization of styrylpyrene, the cycloaddition was investigated with regard to the employed solvent. Therefore, acetonitrile was exchanged with dimethyl sulfoxide and the respective action plots were compared with each other. DMSO was chosen as an alternative solvent due to the significant red-shift displayed in the absorption spectrum of styrylpyrene, compared to MeCN. Furthermore, DMSO shows an increased ability to dissolve styrylpyrene.

Inspection of **Figure 3** indicates that the map of wavelength-dependent reactivity is red-shifted by close to 10 nm when DMSO is used instead of acetonitrile. While the fact that reactivity does not necessarily follow absorptivity has been reported in earlier studies, it is remarkable that this non-congruence follows the solvent-dependent absorption shift. It has to be noted that the relative dimerization quantum yield of the reaction at the respective most effective wavelength is more than three-fold lower in dimethyl sulfoxide (450 nm, 15%) than in acetonitrile (440 nm, 56%). The decreased overall reactivity is particularly interesting, as the respective molar attenuation coefficients in both solvents are very similar (36,300 and 37,200 M^{-1} cm^{-1}, respectively; **Figure 3**).

Notably, increased reactivity in acetonitrile when compared to DMSO cannot readily be attributed to different branching of reaction channels. While radiative relaxation is a prominent competing pathway to photochemical reactions, the fluorescence quantum yield in acetonitrile is 1.6 times higher
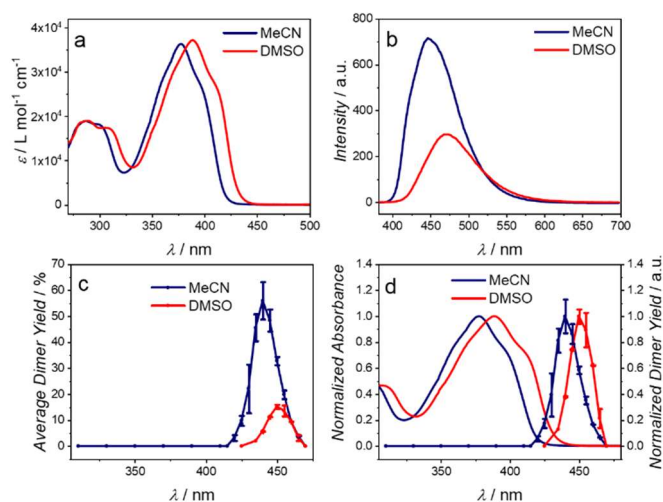
Figure 3 Influence of the solvent on the photophysical and -chemical properties of styrylpyrene; (a) Absorption spectra; (b) Fluorescence spectra, $\lambda_{exc.}$ = 370 nm; (c) Action plots (note that the action plot in DMSO was obtained employing the same photon numbers as in acetonitrile); (d) Normalized absorption spectra and action plots.

than in DMSO and can therefore not serve as an explanation for the difference in reactivity. In case of the solvent dependence of the well-known [2+2] photocycloadditions of coumarin and stilbene, both fluorescence increase[26] and decrease[27] with increasing reactivity are reported. However, styrylpyrene molecules are known to undergo stacking[28] and the degree of the latter can differ in dependence on the employed solvent, which has been thoroughly investigated in the field of DNA bases.[29] The differences in stacking can result in varying degrees of prearrangement of the styrylpyrene molecules, influencing the efficiency of the dimerization.[30] The propensity for a reactivity enhancing prearrangement is generally attributed to the solvent polarity, with higher solvent polarity favouring the solvophobic stacking of apolar reactive molecules.[26,27,31] However, DMSO is slightly more polar than acetonitrile, which can thus not account for the enhanced reactivity in acetonitrile (DMSO: ε_r = 47.24, μ = 3.96 D; acetonitrile: ε_r = 36.64, μ = 3.93 D).[32] In contrast, the viscosity of DMSO (1.99 cP) is close to six times higher compared to acetonitrile (0.37 cP).[32] The difference in solvent viscosity can have a significant influence on a dimerization reaction since in a more viscous environment an excited-state molecule is less likely to encounter a ground-state molecule before relaxing back to the ground state.[33] It is noteworthy that viscosity heavily depends on temperature, which is why it is highly important to keep a constant temperature during all photoreactions. We observed that, when elevating temperature from ambient to biological conditions (37 °C), the reactivity is decreased by a significant margin, despite the absorbance being only slightly affected (refer to the ESI). As previously shown, the absorption spectra of a chromophore are not necessarily congruent to its reactivity under irradiation and the current findings suggest that multiple effects are contributing to the reactivity of a photoreactive moiety, including viscosity, dipole moment and permittivity of the solvent, as well as concentration, underpinning the importance of experimental techniques to survey photoreactivity.

In summary, we introduce a new type of action plot to investigate wavelength-dependent reactivity by taking into account a chromophore's absorption spectrum to adjust the number of incident photons, resulting in a constant number of absorbed photons over all wavelengths. The constant absorption action plot technique is especially powerful for photocycloadditions, where determination of exact quantum yields is highly challenging due to concentration dependence of the reaction and overlapping absorption bands with the cycloadduct and photoisomers. Comparable to conventional quantum yields, it becomes possible to visualize how efficiently absorbed photons initiate photoreactions – a fundamental property especially important for materials applications such as hydrogels or photoresists, where light attenuation critically limits curing depth.

We applied this method to evaluate the effect of concentration and chemical environment on the [2+2] photocycloaddition of styrylpyrene. While the lower concentration decreased the overall reactivity, it did not affect the wavelength dependence of the investigated photocycloaddition. However, by replacing acetonitrile with DMSO, we show that the bathochromic shift of the absorption spectrum can translate into its wavelength-dependent reactivity, yielding a 10 nm red shift of the reactivity maximum. This solvent-dependent reactivity shift is not limited to styrylpyrene but can likely be translated to a variety of photoreactive groups, offering an interesting pathway for tuning wavelength-dependent reactivity. The results also highlight that experimental studies of wavelength-dependent reactivity are paramount to enable control over complex photoreactive systems through precisely controlled photonic fields.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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