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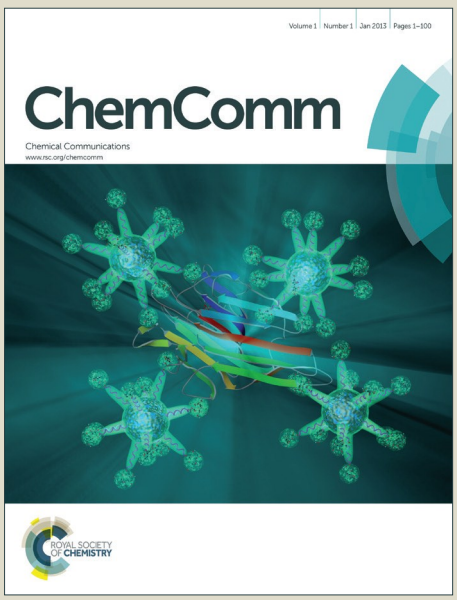

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Visible light-triggered disruption of micelles of an amphiphilic block copolymer with BODIPY at the junction†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x
www.rsc.org/

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A visible light-cleavable polymer is synthesised to overcome the limitations of UV-sensitive polymers. Photocleavable BODIPY functionalized with ATRP initiator and alkyne was used to obtain amphiphilic block copolymer by conducting click reaction and polymerization simultaneously. Micellar assembly of the polymer was disintegrated under visible light irradiation with controlled release of cargo.

Stimuli-responsive polymers have gained attention in the past decade because of their huge potential for applications in various areas.[1-6] For example, self-assembled polymers can be used for creating functional nanoporous membranes[7] as well as in targeted drug delivery to tumour cells by virtue of functionalities sensitive to specific stimulus.[8] Among the functional moieties, cleavable groups are of great interest due to possibility of complete removal of one of the blocks or change in polarity of the polymer.[3,9] Photocleavable groups, in particular, hold lot of promise because light is a stimulus that can be applied with spatio-temporal precision without need for any reagents.[10] For example, photo-removable caging of biomolecules is used for studying control over biological processes.[11] Photocleavable groups such as *o*-nitrobenzyl (ONB), pyrenemethyl esters and coumarin derivatives have been incorporated as side chain or in the main chain of amphiphilic polymers. Upon irradiation with light, micellar disassembly accompanied with release of cargo was achieved due to loss of hydrophilic-lipophilic balance.[12,13] Dissociation of polymer micelles assembled from block copolymers has been studied by introduction of ONB[14] or truxilic acid[15] as a single unit at the junction of two blocks.

The photocleavable groups employed in polymeric systems so far operate in the UV region (250-400 nm) or via two-photon absorption in the NIR region. UV radiation has less penetration into tissues and long exposure may result in damage to cells surrounding the target in drug delivery applications whereas NIR radiation is inefficient due to low two-photon absorption by the chromophores.[16,17] Moreover, UV light sources are expensive compared to visible light sources. Hence, it is highly desirable to introduce photocleavable groups that absorb in the visible or NIR region by single photon absorption into polymer architectures. Boron dipyrromethene (BODIPY) is a fluorescent dye molecule widely used in materials chemistry and for cell imaging due to its intense absorption in the visible region by single photon absorption.[18,19] Further, it is possible to tune its absorption profile from visible to NIR region (470-850 nm) by simple structural modification.[20] Very recently, BODIPY dyes that exhibit photocleavage in response to blue-green or green light were reported.[18,21,22] We hypothesised that incorporating a visible light-cleavable BODIPY unit in a polymer chain will open new avenues for application of polymeric assemblies in bulk as well as in solution. This will be particularly useful in case of block copolymers that are known for the ability to self-assemble into well-defined morphologies. Towards this goal, we report here a simple strategy for synthesis of an amphiphilic block copolymer with BODIPY at the junction and also demonstrate visible light-induced disruption of the block copolymer micelles.

We designed a BODIPY molecule that is functionalised with an alkyne as well as atom transfer radical polymerization (ATRP) initiator moiety. The goal was to achieve azide-alkyne cycloaddition and polymerization, both catalysed by copper complex, in a single step to afford the targeted block copolymer. Synthesis of the dual functional BODIPY and the block copolymer is shown in Scheme 1. A BODIPY derivative bearing phenoxy group at boron atom that shows photolysis via cleavage of B-O bond upon visible light irradiation was selected for ease of synthesis.[18] Compound 1 containing an alkyne on the phenyl ring at meso position was synthesised following reported procedure.[23,24] Then, a phenoxy group with an aldehyde at *para* position was installed on the boron atom using stoichiometric amount of $AlCl_3$ to yield compound 2. Reduction of the aldehyde with $NaBH_4$ and reaction of resulting benzyl alcohol with 2-bromoisobutyryl bromide afforded 4, which is the dual functional BODIPY with alkyne moiety and ATRP initiator. Compound 4 was fully characterized

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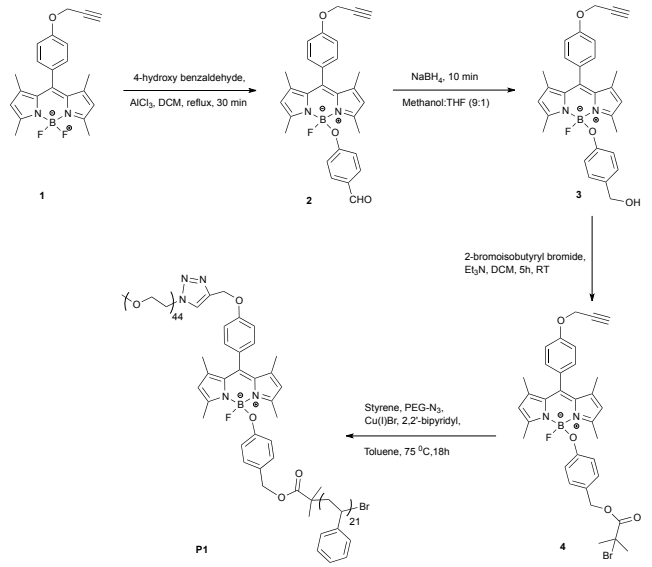
† Electronic Supplementary Information (ESI) available: [Synthetic procedures, NMR, IR, UV-vis and fluorescence spectra]. See DOI: 10.1039/x0xx00000x

by ^{1}H, ^{13}C NMR spectroscopy and HRMS technique (Fig. S9-S11 ESI†).



Scheme 1 Synthesis of alkyne-containing BODIPY ATRP initiator and P1.

The susceptibility of compound 4 to visible light was tested by irradiation of its solution in $CDCl_3$ with blue light (470 nm, 150 $mW.cm^{-2}$). A drop of D_2O was added as a nucleophile for reaction with unstable products formed after photolysis. The ^{1}H NMR spectrum (Fig. S12, ESI†) showed new peaks at 1.93 ppm corresponding to methyl groups in 2-bromoisobutyric acid, and at 5.13 and 6.83 ppm corresponding to aromatic protons of deuterated 4-hydroxybenzyl alcohol[25] formed by reaction of quinone methide with D_2O. Formation of 4-hydroxybenzyl alcohol in presence of water was also confirmed by HPLC analysis (Fig. S19, ESI†). Thin layer chromatography (TLC) analysis also showed new spots when compared to that of compound 4. Moreover, after photoirradiation, the sample appeared to be more fluorescent than 4 due to the absence of fluorescence quenching by photoinduced electron transfer (PET) from the phenyl ring to BODIPY ring[18] suggesting complete cleavage of the B-O bond. Fluorescence spectra of 4 before and after photoirradiation (Fig. S20, ESI†) confirmed this observation.

Poly(ethylene glycol)-*b*-polystyrene with a BODIPY at the junction was then targeted since PEG-*b*-PS block copolymers have been well studied for self-assembly.[26] To obtain the targeted PEG-BODIPY-PS, copper-catalysed click reaction of methoxy-poly(ethylene glycol) functionalised with an azide at one end (PEG_{2000}-N_3) and ATRP of styrene were carried out in a single step from compound 4, which has functional groups for both the transformations. The product P1 could be easily purified by precipitation and showed a monomodal peak in GPC with M_n of 4800 g/mol (PDI = 1.21) against polystyrene standards. Degree of polymerization (DP) for polystyrene block calculated from ^{1}H NMR spectra compared well with the value obtained from GPC analysis. The block copolymer structure was ascertained from ^{1}H NMR spectra and IR spectroscopy provided additional evidence of absence of azide group in the polymer (Fig. S13-S14, ESI†).

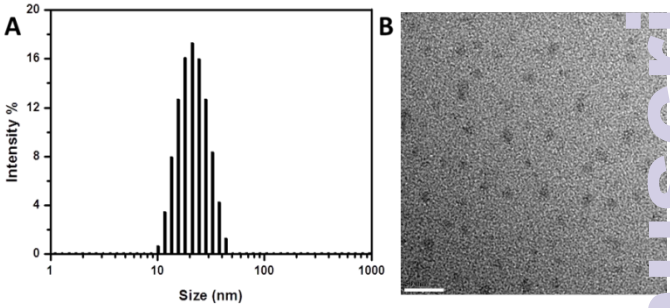
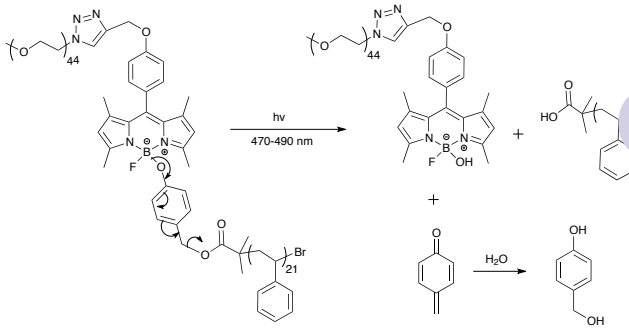


Fig. 1 A) DLS size distribution curve and B) TEM image (scale bar = 50 nm) for 0.1 wt % aqueous solution of P1.

Aqueous solution of PEG-BODIPY-PS (0.1 wt%) was prepared by cosolvent method. Deionised (DI) water was added dropwise to a solution of the polymer in tetrahydrofuran (THF) to induce micellization and the mixture was dialysed against water for 3 days to remove THF. Dynamic light scattering (DLS) measurement showed formation of aggregates with average hydrodynamic diameter (D_h) of 21 nm (Fig. 1A) and transmission electron microscopy (TEM) analysis revealed spherical micelles with almost uniform size (Fig. 1B). Micellar disassembly induced by visible light irradiation was then investigated using blue light. The block copolymer is likely to be fragmented into three components after photoirradiation: PEG attached to BODIPY, PS with -COOH group at one end and 4-hydroxybenzyl alcohol (Scheme 2). This mechanism is proposed on the basis of products observed after the photocleavage of initiator 4. In order to provide evidence for this mechanism, the polymer solution was analyzed by HPLC after photoirradiation. A peak corresponding to 4-hydroxybenzyl alcohol was observed in the chromatogram (Fig. S19, ESI†) suggesting that the mechanism is indeed possible. Precipitation was observed in the aqueous solution after photoirradiation (Fig. 2A, inset) as expected. DLS measurements on the polymer suspension after irradiation showed a bimodal distribution from 100 nm to 600 nm and complete disappearance of the peak for 21 nm (D_h) (Fig. 2A) that is probably due to suspended particles of precipitated polystyrene block along with intact polymer. TEM analysis also supported disruption of micelles (Fig. 2B).



Scheme 2 Proposed mechanism of cleavage of P1 under visible light irradiation.

Photocleavage of the self-assembled polymer in water was then monitored by recording fluorescence emission spectra of BODIPY (λ_{ex} = 506 nm and λ_{em} = 506-650 nm). The emission intensity was found to increase with time, reached a maximum and then decreased (Fig. 2C). We surmised that the decrease in intensity was probably due to precipitation of the intact polymer from micelles that become unstable after removal of most of the hydrophilic chains from the corona. To check this possibility, the polymer solution in THF, wherein self-assembly is non-existent, was irradiated. It was observed that emission intensity reached a maximum and remained constant (Fig. 2D).

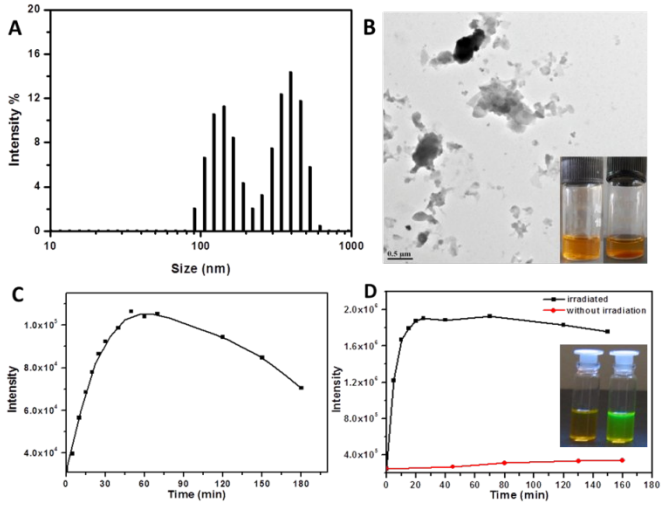


Fig. 2 Data after irradiation with visible light: A) DLS size distribution curve for 0.1wt% aqueous solution of P1; B) TEM image (scale bar = 500 nm) of 0.1wt% aqueous solution of P1, inset: photographs of polymer solution before (left) and after irradiation; Plot of fluorescence emission intensity versus time for 0.001wt% aqueous solution of P1 in C) aqueous solution and D) THF with one drop of water, inset: photographs of polymer solution before (left) and after irradiation.

This observation supports the hypothesis that decrease in emission intensity in fluorescence spectra of the aqueous micellar solution is due to precipitation of non-cleaved polymer. The polymer solution in THF was found to turn visibly fluorescent (Fig. 2D, inset) similar to the solution of compound 4 after the photo-induced cleavage.

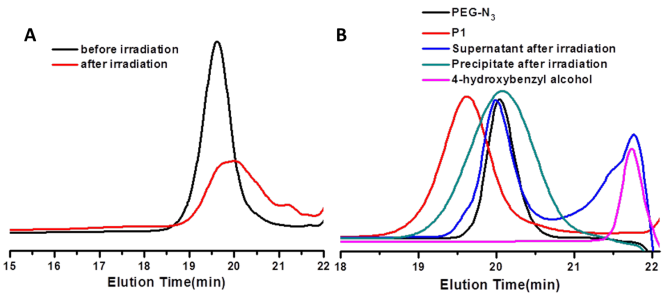


Fig. 3 GPC chromatograms for 0.1 wt% of P1 after visible light irradiation A) in aqueous solution B) in THF:water (1:1 v/v).

The precipitate from the irradiated aqueous solution along with supernatant was lyophilised, re-dissolved in THF and analysed by GPC, which showed a broad peak shifted towards lower molecular weight that most likely comprises the curves for PS, PEG fragments and non-cleaved polymer (Fig. 3A). To ensure complete cleavage of polymer in the micelles, a solution of P1 in THF:water (1:1 v/v) mixture was prepared. Presence of aggregates was confirmed by DLS analysis that showed a hydrodynamic size of 40 nm suggesting presence of micelles swollen with solvent (Fig. S17, ESI†). This solution was irradiated with blue light for 210 min and the precipitate and supernatant were analyzed by GPC (Fig. 3B). Chromatogram of the supernatant matched closely with that of PEG-N_3 with an extra peak at lower molecular weight that matched with that of 4-hydroxybenzyl alcohol providing another evidence in support of the mechanism proposed in Scheme 2. The shoulder to this peak is probably due to formation of dimer from the unstable quinone methide intermediate. GPC curve of the precipitate had also shifted to lower molecular weight compared to that of P1 suggesting near-quantitative fragmentation of the block copolymer.

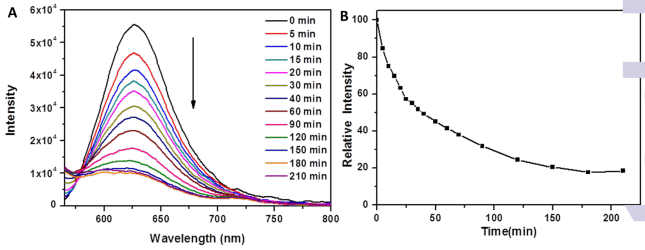


Fig. 4 A) Fluorescence emission spectra and B) decrease in fluorescence intensity of Nile red during irradiation of 0.1wt% aqueous solution of P1 with visible light.

Photolysis of P1 leading to disintegration of micelles can also be monitored by change in fluorescence emission of a hydrophobic dye encapsulated in the micellar core. Nile red, a poorly water-soluble dye, was used for this purpose. The dye encapsulation solution of P1 was excited at 565 nm to avoid absorption by BODIPY and fluorescence emission of Nile red was monitored while irradiating the solution with blue light. Emission intensity of Nile red was found to decrease as expected due to its release from micellar core into aqueous environment (Fig. 4A). The decrease in fluorescence intensity was about 80% in 210 min of light irradiation (Fig. 4B) whereas less than 1% decrease was observed over a period of 5h when the solution was stored in dark (Fig. S18, ESI†). To confirm that the decrease in fluorescence intensity was not due to photobleaching,[17] solution of the dye encapsulated polymeric micelles in THF:water (2:1 v/v) mixture, wherein the released dye is soluble, was irradiated with blue light. Fluorescence intensity of the dye was found to be constant with time (Fig. S21, ESI†) suggesting that decrease in intensity in pure aqueous solution is indeed due to change in its microenvironment caused by photocleavage of the polymer.

Release of the dye was also confirmed by an alternative technique, that is, by HPLC analysis. A solution of the dye encapsulated polymer in methanol:water (1:1) was prepared and dialysed against the same solvent to remove any un-encapsulated dye molecules. The solution was then irradiated with visible light and dialysed to separate the released dye

from the polymer. Chromatogram from HPLC analysis of the outer solution showed a peak at the same elution time as that of pure Nile red confirming the release of dye due to photocleavage of the polymer (Fig. S22, ESI†). To investigate the factors affecting rate of dye release, the polymer was assembled in THF:water (1:9) and dye was encapsulated. Upon photoirradiation the decrease in fluorescence intensity of the dye was found to be 23% more than in pure water during the same period (Fig. S23, ESI†). This suggests that slow release of the dye in aqueous solution is likely due to the compact PS core that gets swollen in THF-water mixture.

The visible light sensitive polymer was found to be relatively stable under ambient light conditions. Fluorescence intensity of BODIPY was found to increase slowly over a period of six days without any visible precipitation while the fluorescence intensity of encapsulated Nile red decreased by about 50% during the same period (Fig. S24, ESI†).

In summary, we designed and synthesized an amphiphilic block copolymer with a visible light-cleavable moiety to overcome the limitations of UV active chromophores in photoresponsive polymeric assemblies. The block copolymer was synthesised by carrying out click reaction and ATRP in a single step from a BODIPY compound. Photolysis of B-O bond in BODIPY on irradiation with blue light led to cleavage of block copolymer and subsequently to dissociation of micelles in aqueous solution as evidenced by release of encapsulated Nile red. Our design of block copolymer with a visible light-cleavable group can be easily extended to other polymer backbones and architectures thereby potentially advancing the application of polymeric assemblies in wide ranging areas.

This work is supported by CSIR-National Chemical Laboratory via project code MLP028826 and by a research fellowship to N.G.P. by UGC, New Delhi.

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