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On-Demand Acid-Gated Fluorescence Switch-On in Photo-Generated Nanospheres

Received 00th January 20xx, Accepted 00th January 20xx Jordan P. Hooker,^a Florian Feist,^{ab} Laura Delafresnaye,^a Federica Cavalli,^c Leonie Barner^{*a} and Christopher Barner-Kowollik^{*a}

DOI: 10.1039/x0xx00000x

Herein, we introduce a fast, additive-free, ambient temperature photochemical approach – utilising the novel Diels-Alder cycloaddition of a photo-active *ortho*-methylbenzaldehyde (oMBA) with an asymmetric alkyne – for preparing functional acid-sensitive profluorescent nano-/micro-spheres in one step. Never previously reported, the possibility of applying such a reaction in the context of particle synthesis provides new possibilities for particle design, where multi-step reactivity can be gated into distinct steps. First, a photochemically-gated particle formation step yields a material possessing a reactive, spring-loaded intermediate at every cross-linking point. A second, on-demand step to initiate fluorescence generation subsequently imparts the novel properties of the chemical transformation to the material itself. The synthesised particles are narrow-disperse with an average diameter ranging from 170 - 380 nm.

Polymer particles are an important and ubiquitous class of materials possessing an array of characteristics attracting wideranging application and continuous development.¹⁻⁶ Such applications include toners, chromatography, instrument calibration, photonic crystals, coatings and composite materials.⁷⁻¹⁰ More specifically, fluorescent nano- and microspheres have been developed as powerful biochemical and medicinal tools, for instance in *in vivo* imaging, for drug delivery, biological tracers and flow cytometry.¹¹⁻¹⁵ However, such broad application obviously necessitates control over the properties of these particles. Considering this, traditional methods of particle synthesis – often requiring elevated temperatures, radical reactions, additives, extended reaction times and restricted functionality – certainly become limiting. 6,16,17

Importantly, new synthetic methods for polymer particle synthesis with advantages over classical methods have developed into powerful tools in both research and industry. Some important examples include polymerisation-induced self-assembly (PISA) – utilising the power of modern reversible-deactivation radical polymerisation techniques – and the extensive development of particle synthesis *via* thiol-ene step-growth polymerisation into functional particles with complex backbone structures.¹⁸⁻²⁴

Synthetic photochemistry is key developmental strategy, and a powerful enabling technology for the construction of advanced (macro)-molecular architectures. One recently reviewed aspect of this is its ability to achieve precise spatiotemporal control over a chemical reaction, potentially opening the door to a plethora of applications.²⁵ Indeed, although light has been extensively employed for the fabrication of sophisticated materials, the use of modern synthetic photo-reactions in particle synthesis has largely remained unexplored. Particularly noting the difficulty of translating new chemistries to particle synthesis, we have recently highlighted the potential of utilising such reactions by developing a new platform for particle synthesis. Specifically, we reported how well-defined polymers functionalised with photo-active moieties (tetrazole or oMBA) were rapidly crosslinked into functional microspheres at ambient temperature under UV (λ = 320 nm) or visible (λ = 415 nm) irradiation without the need for stabilisers, bases or initiators.^{26,27} Further, control over the concentration, solvent combination and irradiation intensity led to a broad and tunabe size range (0.2 – 5 μ m). Importantly, the novel properties of the resulting moieties were translated to the particles, for instance to prepare inherently fluorescent particles, multi-functional particles with useful acrylate and hydroxyl groups for further functionalisation, and even a novel visible-light [4+4] cycloaddition facilitating a single polymer system.

^{a.} Centre for Materials Science, School of Chemistry and Physics, Institute for Future Environments, Queensland University of Technology (QUT), 2 George Street, QLD 4000, Brisbane, Australia. Email: christopher.barnerkowollik@qut.edu.au; leonie.barner@qut.edu.au

^{b.} Max Planck Institute for Polymer Research, Ackermannweg 10, Mainz 55128, Germany.

^{c.} Macromolecular Architectures, Institut für Technische Chemie und Polymerchemie, Karlsruhe Institute of Technology (KIT), Engesserstr. 18, 76131 Karlsruhe, Germany.

⁺ Electronic Supplementary Information (ESI) available: Experimental details, characterisation methods and supplemental data is included. See DOI: 10.1039/x0xx00000x

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Scheme 1. (A) Preparation of cross-linkable polymers via functionalisation with oMBA 1 and propiolic acid. (B) Synthesis of fluorescent particles from functionalised cross-linkable polymer precursors P2 and P3.

Of interest due to their unprecedented reactivity and orthogonality, light-driven reactions of oMBAs and omethylbenzophenones have been extensively developed over the past decades, although previously never for use in particle synthesis.^{25,28,29} Furthermore, although a select few examples report the reaction of photochemically generated oquinodimethanes (oQDM) with electron-deficient alkynes, the use of either an asymmetric or terminal alkyne has never been reported. More importantly, the subsequent E1-elimination (dehydration) of the generated 1,4-dihydronaphthalen-1-ol to the naphthalene was either overlooked or required undesirable conditions such as elevated temperatures, extended reaction times in combination with low yields and side products.³⁰⁻³⁵ Unfortunately, despite significant application potential, these factors have thus far limited the reaction from realizing any utility in materials synthesis and applications, such as in photoinduced macromolecular ligation.

Herein, we report a method for the synthesis of nano- and micro-spheres using a novel Diels-Alder cycloaddition involving the reaction of an oMBA with an asymmetric terminal alkyne, yielding an acid-triggered re-aromatisation to a fluorescent naphthalene (Scheme 1). Re-aromatisation is achieved via the generation of a highly sensitive hydroxyl containing 1,4dihydronaphthalene moiety at each cross-linking point, which upon exposure to acid, subsequently undergoes a rapid E-1 elimination and dehydration to yield a fluorescent naphthalene moiety. Moreover, the catalytic nature of the re-aromatisation makes the particles highly sensitive to even very low concentrations of acid. Importantly, the intermediate is nonfluorescent and does not absorb light at the employed wavelength (λ = 365 nm) (Fig. S6, ESI⁺). The advantages of this are two-fold: first, a post-synthesis, acid-gated generation of fluorescence brings novel and interesting properties for use and application. Second, fluorescent species can absorb photons during a reaction, which can lead to undesirable side reactions, reduced reaction rates and less overall control. Thus, this lack of initial fluorescence ensures that the reaction is not hindered as nucleation begins to generate higher local concentrations of photoreactive moieties, which is particularly important in stabiliser-free methods of particle synthesis, where the nucleation phase is highly sensitive and adverse events can lead to loss of colloidal stability.

First, a well-defined, low molecular weight *poly*(styrene-*co*chloromethylstyrene) p(St-co-CMS) copolymer **P1** was synthesised using nitroxide mediated polymerisation (NMP). The percentage of functional monomer (32%) and average molecular weight (M_n) (2100 g mol⁻¹) were determined by ¹H NMR and size exclusion chromatography (SEC), respectively (Fig. S7 – S8, ESI⁺). These values were calculated to correspond to approximately 5 functional units per polymer chain (Section 6, ESI⁺). Subsequent functionalisation with *o*MBA (**3**, ESI⁺) and commercially available propiolic acid yielded p(St-co-oMBA) **P2** (2800 g mol⁻¹, Fig. S10 ESI⁺) and p(St-co-propiolate) **P3** (1600 g mol⁻¹, Fig. S12 ESI⁺), respectively. For copolymer **P3**, molecular weights determined *via* SEC were found to be shifted to lower molecular weights, presumably due to either chemical interaction of the attached alkyne with the column material, or

Table 1 Synthesis of nano- and micro-spheres from functional prepolymers P2 and P3where C represents the combined polymer concentration (P2 and P3), D_n represents thenumber average particle diameter, D_w represents the weight-average particle diameter,D represents particle dispersity (D_w/D_n) and σ represents the standard deviation

C [mg mL⁻¹]	ACN:THF [%]	D n ^a [μm]	D _w ª [μm]	Đ [<i>D</i> _w / <i>D</i> _n]	σ [μm]	
2	100:0	0.172	0.177	1.03	0.02	
3	90:10	0.304	0.314	1.03	0.03	
3	80:20	0.384	0.409	1.07	0.06	
3	70:30	0.236	0.335	1.42	0.09	

^a Determined by SEM.

Published on 07 April 2020. Downloaded by University of Reading on 4/8/2020 1:53:57 PM

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Fig. 1 Analysis of particles resulting from reactions of P(St-*co*-photoenol) P2 and P(St-*co*-propiolate) P3 (A) SEM image of particles from 100:0 ACN:THF; (B) SEM image of particles from 90:10 ACN:THF; (C) DLS of particles from 90:10 ACN:THF. Scale bars are 1 µm and 5 µm, respectively.

a decrease in hydrodynamic volume in the SEC solvent (tetrahydrofuran -THF) after functionalisation.

Given that the rate of particle formation in this case would be expected to correlate with the reactivity of the photo-active moiety employed as a cross-linker, the high quantum efficiency of the employed oMBA (P2) facilitates fast reaction times. Particle synthesis reactions were performed with 1:1 mass ratios of P2 and P3 dissolved in varying ratios of acetonitrile (ACN):tetrahydrofuran (THF) (Table 1; Fig. S13, ESI⁺), without need for stabilisers, bases or initiators. Upon irradiation (λ_{max} = 365 nm), nucleation began almost instantly, and colloidally stable, spherical, narrow-disperse particles could be yielded in only 2 h as supported by scanning electron microscopy (SEM) analyses (Fig. 1a and b). Subsequent particle counting showed average diameters (D_n) ranging from 170 nm up to 380 nm and a narrow size dispersity (*D*) from 1.03 – 1.07 for solvent systems containing up to 20% THF. Dynamic light scattering (DLS) additionally showed a narrow size-dispersity, although with a slightly larger D_n (compared to SEM) due to the swellability of the particles in THF.

Precipitation polymerisation - being a stabiliser-free method of particle synthesis - necessitates lower loading concentrations compared to other methods. However, another strategy for tuning particle size is the addition of a solvent which solubilises the growing nuclei. Thus, by increasing the ratio of THF, nucleation is expected to be delayed, with more crosslinking and aggregation occurring prior to precipitation, leading to fewer and larger growing nuclei. Although small size increases are observed in reactions containing up to 20% THF, increasing to 30% THF lead to a comparatively polydisperse sample, with particle diameters ranging from below 200 nm and up to 500 nm (Fig. S13, ESI⁺). Traditionally, precipitation polymerisation proceeds via an initial nucleation phase, followed by a growth phase. In our case, it appears that nuclei continue to form even after this initial nucleation phase, leading to polydisperse particles.

Next, the generation of the fluorescent naphthalene adduct was analysed. The excitation ($\lambda_{max} = 350 \text{ nm}$) and emission ($\lambda_{max} = 400 \text{ nm}$) spectra of the particles were measured *via* fluorescence spectroscopy (Fig. S14, ESI⁺). In order to explore the control over the catalytic elimination, fluorescence was

measured at ten minute intervals both before and after addition of acid (Fig. 2a). Initially, a small amount of fluorescence was observed, probably from re-aromatisation during the initial reaction. However, in the absence of acid (while still open to air), only a negligible increase in fluorescence was observed over the next 3 h, confirming the stability of the non-fluorescent dihydronaphthalene moiety in the absence of acid. Upon subsequent addition of a catalytic amount of *p*-toluenesulfonic acid, fluorescence increased greater than four-fold over the next hour, and almost ten-fold over the next 12 h. Given that nucleation leads to high local concentrations of photo-active moieties, and the aforementioned disadvantages of having



Fig. 2. Analysis of the fluorescent properties of the particles (A) kinetic analysis of the fluorescence generation before and after addition of acid, where the black line represents the fluorescence at 400 nm, before and after addition of acid; (B) particles after addition of acid, observed under ambient light (left) and under 365 nm light (right).

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fluorescent species absorbing during the reaction, we expect that in the photochemical synthesis of fluorescent particles, this reaction is likely ideal. The appearance of the particles (after addition of acid) under both ambient and UV (λ_{max} = 365 nm) light is also shown in Fig. 2b.

Finally, in terms of functionality, we have previously shown that residual functional groups are both present on the surface and can be further reacted.²⁷ Herein, residual alkyne functionalities – a readily reactive functional group for further particle functionalisation – were expected. IR analysis of the synthesised particles shows a residual -C=C- stretching peak at 2150 cm⁻¹ (Fig. S15, ESI⁺). In addition, the characteristic absorption peak at 3300 cm⁻¹ for the terminal alkyne C-H stretch is also present, although absorption from the newly formed hydroxyl groups from 3100 – 3700 cm⁻¹ overlaps and prevents this peak from resolving.

In conclusion, we introduce a simple, mild and fast method of particle synthesis, highlighting the power of exploiting modern synthetic photochemistry to impart novel properties and functionality to materials. Utilising the novel cycloaddition of a terminal, asymmetric alkyne with a photo-excitable oMBA for cross-linking and particle formation, fluorescent particles can subsequently be prepared via a catalytic dehydrative elimination. The main advantages are two-fold; first, the presence of fluorescent species during particle formation could lead to undesirable absorption of photons, leading to side reactions and reduced control over the reaction, impacting important factors such as reproducibility and particle dispersities. Second, the subsequent acid-triggered generation of fluorescence was shown to be both controllable and highly sensitive, maximising the potential use and application of the particles; the non-fluorescent 1,4-dihydronapthalene moiety is stable in the absence of acid, but rapidly undergoes rearomatisation to generate a fluorescence naphthalene moiety upon exposure to acid.

C.B.-K. acknowledges the Australian Research Council (ARC) for funding in the form of a Laureate Fellowship underpinning his photochemical research program as well as the Queensland University of Technology (QUT) for key continued support. C.B.-K. and L. B. acknowledge additional funding in the context of an ARC Linkage grant on advanced particle synthesis. The authors acknowledge the facilities and the scientific and technical assistance from the Australian Microscopy & Microanalysis Research Facility (AMMRF) at the Central Analytical Research Facility (CARF) operated by the Institute for Future Environments (IFE).

Conflicts of interest

There are no conflicts to declare.

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