Photoactivation of Aggregation-Induced Emission Molecules for Fast and Efficient Synthesis of Highly Fluorescent Single-Chain Nanoparticles

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ABSTRACT: Single-chain nanoparticles (SCNPs) are ultrasmall soft nanomaterials constructed via intrachain cross-linking of individual precursor polymer chains, with promising prospects for nanomedicine, catalysis, and sensing, among other different application fields. SCNPs are versatile building blocks for the construction of new fluorescent probes with ultrasmall size, higher brightness, and better photostability than previous particle-based systems. Herein, we report on a new, fast, and efficient method to produce SCNPs with intense fluorescence emission in solution which is based on the photoactivation of appropriate aggregation-induced emission (AIE) cross-linking molecules containing azide functional groups. Remarkably, the presence of the azide moiety— that can be transformed to highly reactive nitrone species upon UV irradiation—was found to be essential for the SCNPs to display intense fluorescence emission. We attribute the fluorescence properties of the SCNPs to the immobilization of the initially nonfluorescent AIE molecules via intrachain cross-linking upon photoactivation. Such cross-linking-induced immobilization process activates the AIE mechanism and, hence, leads to fluorescent SCNPs in both solution and solid state.

INTRODUCTION

Single-chain polymer nanoparticles (SCNPs) are soft nanomaterials constructed via intrachain cross-linking of individual precursor polymer chains, with promising prospects for nanomedicine, catalysis, and sensing, among other different application fields. The SCNPs local pockets formed upon chain collapse at high dilution provide a unique (and tunable) environment for attachment—either temporarily or permanently—of active species, such as drugs, catalytic metal ions, or dyes. Moreover, SCNPs are promising building blocks for the construction of new fluorescent probes with ultrasmall size, higher brightness, and better photostability than previous particle-based systems.

In general, fluorescent nanoprobes are receiving enthusiastic pursuits from medical scientists because of their promising potential applications in cell imaging, targeting, tracing, and therapeutic uses. Conventional luminescent nanoparticles are mainly based on inorganic quantum dots, lanthanide ion-doped nanomaterials, and metallic nanoclusters because of their excellent photostability with narrow emission, subnanometer size, and superior upconversion performance, although potential toxicity issues and poor biodegradability have largely hindered their common employment in biomedical applications.

Even if the field of SCNPs is still very incipient, a variety of fluorescent SCNPs have been synthesized through four different synthetic approaches based on common, traditional fluorophores: (i) functionalization of the SCNP precursor polymer with a conventional fluorophore molecule before intrachain cross-linking; (ii) physical entrapment of an external traditional fluorophore molecule into nonfluorescent SCNPs or in situ generation of this fluorophore molecule inside the SCNP; (iii) postfunctionalization of the SCNP surface via chemical reaction with appropriate, complementary reactive fluorophore molecules; and (iv) generation of fluorophore functional moieties through intramolecular cross-linking.

In spite of recent advances in this field, to the best of our knowledge, the use of aggregation-induced emission (AIE) cross-linking fluorophores to endow SCNPs with fluorescent properties has not been investigated yet. AIE compounds are...
opposite to conventional fluorescent probes which often suffer from the well-known (and deleterious) aggregation-caused quenching (ACQ) effect. On the contrary, AIE molecules—as first described by Tang and co-workers in 2001—are nearly nonemissive compounds in solution but become highly fluorescent upon restriction of their intramolecular rotations (the so-called RIR mechanism). In short, AIE fluorophores usually show very weak fluorescence or non emissivity when molecularly dissolved in a good solvent, but are highly emissive when aggregate—or after molecular immobilization—because of the restriction of the intramolecular rotation in the aggregate (immobilized) state leading the excitations to decay radiatively. For a more detailed description of the AIE effect, the RIR mechanism, and several practical applications of AIE compounds in biological sensors, chemical probes, optoelectronic devices, and intelligent materials, the reader is referred to recent, comprehensive review papers. We hypothesized that by generating a combination of both highly reactive nitrene species (from Silole-N3) and highly reactive carbene species (from precursor P) via UV irradiation, the probability of efficient immobilization of the AIE compound in the resulting SCNP will increase considerably, when compared to the use of an AIE compound without azide functional groups (hexaphenylsilole, HPS). To confirm this hypothesis, we performed two complementary experiments.

In a control experiment, we placed precursor P in the CH2Cl2 solvent at high dilution (0.5 mg/mL) in the presence of HPS (AIE compound without azide functional groups). We employed a molar ratio of HPS to α-diazo-β-ketoester units of 1/2. As illustrated in Figure 1, even if efficient SCNP formation was observed by size exclusion chromatography (SEC) due to an increase in retention time upon chain compaction (Figure 1B), the resulting SCNPs NP were found to be essentially nonfluorescent nanomaterials (Figure 1D). In this case, upon irradiation of the α-diazo-β-ketoester units of precursor P, highly reactive carbene species were generated (see below) accompanied by N2 formation and further N2 release from the solution. The resulting carbenes—which have high tendency to react through many different pathways—promoted intrachain cross-linking reactions leading to the observed chain compaction. Confirmation of successful photoinduced carbene generation from precursor P was obtained through complementary Fourier transform infrared (FTIR) spectroscopy and elemental analysis (EA) measurements, as illustrated in Figure 2 and summarized in Table 1. Consequently, even if carbene generation was proved to be successful (efficiency of carbene generation >90%), most of the subsequent carbene C–H insertion reactions were intrachain reactions without allowing immobilization of a significant amount of external HPS molecules. To the best of our knowledge, this is the first report of SCNPs (although without fluorescence properties) synthesized through photoactivated carbene generation.

On the other hand, when the synthesis was performed under exactly the same conditions but replacing HPS by Silole-N3, single-chain polymer nanoparticles endowed with highly fluorescent properties (FNP) were obtained (see Figure 1A,C,D). These complementary experiments confirmed that the presence of the azide moiety in the AIE compound—that generates highly reactive nitrene species upon UV irradiation—is essential for FNP to display intense fluorescence emission. The progressive compaction of precursor P in the presence of Silole-N3 upon increasing the UV irradiation time is shown in Figure 3, as determined by SEC experiments. The increase in retention time observed by SEC is a consequence of the reduction in hydrodynamic size of precursor P because of intramolecular cross-linking upon the simultaneous generation of highly reactive nitrene and carbene species. The unimolecular (single-chain) nature of FNP was confirmed through absolute Mn measurements, using multiance laser light scattering (MALLS) detection during the SEC experiments (see Table 2). Hence, upon SCNP formation, an increase in Mn was observed that can be attributed (within the experimental uncertainty of the MALLS technique) to the progressive immobilization of Silole-N3 molecules in the nanoparticles. Concomitantly, a reduction in both the average values of radius of gyration (Rg) and hydrodynamic radius (Rh) of precursor P was observed (Table 2), as a consequence of its

Scheme 1. Schematic Illustration of the Synthesis of Precursor P

RESULTS AND DISCUSSION

We investigate in this work a new strategy toward highly fluorescent SCNP s based on the combined photoactivation of an AIE cross-linking compound containing azide functional groups, Silole-N3, and a precursor P containing α-diazo-β-ketoester units (see Scheme 1). AIE compounds are a new kind of fluorophores that are nearly nonemissive molecules in solution but become highly emissive when aggregate—or are immobilized—because of the restriction of intramolecular rotations leading the excitations to decay radiatively. We employed a molar ratio of HPS to α-diazo-β-ketoester units of 1/2. As illustrated in Figure 1, even if efficient SCNP formation was observed by size exclusion chromatography (SEC) due to an increase in retention time upon chain compaction (Figure 1B), the resulting SCNPs NP were found to be essentially nonfluorescent nanomaterials (Figure 1D). In this case, upon irradiation of the α-diazo-β-ketoester units of precursor P, highly reactive carbene species were generated (see below) accompanied by N2 formation and further N2 release from the solution. The resulting carbenes—which have high tendency to react through many different pathways—promoted intrachain cross-linking reactions leading to the observed chain compaction. Confirmation of successful photoinduced carbene generation from precursor P was obtained through complementary Fourier transform infrared (FTIR) spectroscopy and elemental analysis (EA) measurements, as illustrated in Figure 2 and summarized in Table 1. Consequently, even if carbene generation was proved to be successful (efficiency of carbene generation >90%), most of the subsequent carbene C–H insertion reactions were intrachain reactions without allowing immobilization of a significant amount of external HPS molecules. To the best of our knowledge, this is the first report of SCNPs (although without fluorescence properties) synthesized through photoactivated carbene generation.

On the other hand, when the synthesis was performed under exactly the same conditions but replacing HPS by Silole-N3, single-chain polymer nanoparticles endowed with highly fluorescent properties (FNP) were obtained (see Figure 1A,C,D). These complementary experiments confirmed that the presence of the azide moiety in the AIE compound—that generates highly reactive nitrene species upon UV irradiation—is essential for FNP to display intense fluorescence emission. The progressive compaction of precursor P in the presence of Silole-N3 upon increasing the UV irradiation time is shown in Figure 3, as determined by SEC experiments. The increase in retention time observed by SEC is a consequence of the reduction in hydrodynamic size of precursor P because of intramolecular cross-linking upon the simultaneous generation of highly reactive nitrene and carbene species. The unimolecular (single-chain) nature of FNP was confirmed through absolute Mn measurements, using multiance laser light scattering (MALLS) detection during the SEC experiments (see Table 2). Hence, upon SCNP formation, an increase in Mn was observed that can be attributed (within the experimental uncertainty of the MALLS technique) to the progressive immobilization of Silole-N3 molecules in the nanoparticles. Concomitantly, a reduction in both the average values of radius of gyration (Rg) and hydrodynamic radius (Rh) of precursor P was observed (Table 2), as a consequence of its
collapse to SCNPs. This behavior is in good agreement with previous results reported in the literature.\textsuperscript{22}
morphological transitions\textsuperscript{59} or to photodegradation of the AIE compound via secondary reactions. To determine if a change in morphology was taking place at long UV irradiation times, SCNPs FNP obtained after 60 and 120 min of UV irradiation were visualized, after solvent removal, by transmission electron microscopy (TEM). It is worth of mention that characterization of SCNP shape was visible by TEM, so we can tentatively attribute the reduction in fluorescence emission intensity after prolonged UV irradiation to competitive photodegradation reactions. Nevertheless, more studies—out of the scope of the current work—are required to confirm this assumption.

It is worth mentioning that purification of FNP from potentially unreacted Silole-N\textsubscript{3} nanoparticles was highly facilitated by the excellent solubility of Silole-N\textsubscript{3} in Et\textsubscript{2}O (a precipitant for FNP). In addition to efficient nitrene generation from the azide—containing AIE compound, confirmation of successful carbene generation from α-diazo-β-ketoester units upon UV irradiation of precursor P was obtained from FTIR spectroscopy measurements, as illustrated in Figure 6. Hence, upon SCNP formation, the complete disappearance of the FTIR vibration band associated with the diazo moieties (\(\nu \approx 2200 \text{ cm}^{-1}\)) of precursor P (green trace) after the formation of SCNP FNP (blue trace).

### CONCLUSIONS

This work establishes a new strategy based on photoactivation of appropriate AIE molecules containing azide functional groups for the fast and efficient generation of SCNPs with fluorescence emission in both solution and solid state. We demonstrate that by the generation of a combination of both highly reactive nitrene species (from an azide-containing AIE cross-linking molecule, Silole-N\textsubscript{3}) and highly reactive carbene species (from an appropriate polymeric precursor containing α-diazo-β-ketoester units, P) via UV irradiation, the probability of efficient immobilization of the AIE compound in the resulting SCNP increases considerably, when compared to the use of an AIE compound without azide functional groups. Confirmation of successful single-chain compaction upon UV irradiation of precursor P in the presence of Silole-N\textsubscript{3} at high dilution is obtained by SEC with multidetection, including MALLS detector (for absolute \(M_w\) and \(R_g\) values) and VIS detector (for \(R_h\) data). The optimum UV irradiation time during the SCNP formation process is found to be 60 min. The resulting fluorescent SCNPs FNP (\(R_h = 9 \text{ nm}\)) show a maximum emission peak in the THF solvent centered at \(\lambda_{em} \approx 455 \text{ nm}\) and excellent stability of the fluorescence emission intensity over time, as determined by FS. Moreover, FNP retains its fluorescent properties in the solid state. To conclude, this work opens a new way to endow other soft nanomaterials (e.g., microgels, dendrimers, and micelles) with

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**Table 2. Absolute Weight Average Molecular Weight, Radius of Gyration, and Hydrodynamic Radius of Precursor P as a Function of UV Irradiation Time, as Determined by SEC with Triple Detection**

| UV irradiation time (min) | \(M_w\) (10\(^{3}\) Da)\textsuperscript{a} | \(R_g\) (nm)\textsuperscript{b} | \(R_h\) (nm)\textsuperscript{c} |
|--------------------------|-------------------------------|-----------------|-----------------|
| 0                        | 7.348 ± 0.1                  | 17.9 ± 1.0      | 11.4 ± 0.1      |
| 15                       | 7.381 ± 0.1                  | 11.4 ± 1.2      | 10.0 ± 0.2      |
| 30                       | 7.413 ± 0.1                  | 12.6 ± 1.0      | 10.6 ± 0.1      |
| 60                       | 7.561 ± 0.1                  | 6.2 ± 2.0       | 9.0 ± 0.3       |
| 120                      | 7.626 ± 0.1                  | 3.23 ± 0.1      | 7.8 ± 0.5       |

\textsuperscript{a}SEC retention time at peak maximum. \textsuperscript{b}As determined by SEC with MALLS detection. \textsuperscript{c}As determined by SEC with VIS detection.

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**Figure 4.** (A) Fluorescent properties of precursor P upon UV irradiation in the presence of Silole-N\textsubscript{3} for: 0 (black line), 15 (blue line), 30 (green line), 60 (yellow line), and 120 (red line) min. (B) Maximum fluorescence was observed for SCNPs FNP synthesized after 60 min of UV irradiation.

**Figure 5.** TEM images of isolated SCNPs FNP synthesized after 60 min (scale bar: 50 nm) and 120 min (scale bar: 100 nm) of UV irradiation time.

**Figure 6.** Illustration of the disappearance of the infrared vibration band associated with the diazo moieties (\(\nu \approx 2200 \text{ cm}^{-1}\)) of precursor P (green trace) after the formation of SCNP FNP (blue trace).
UV spot light source (LC8, Hg irradiation experiments were performed using a Hamamatsu W/cm²) equipped with an A9616-05 (anhydrous, >99.0%), dichloromethane (diphenylsilole (Silole-N3 HPS synthesized; blue curve is the PL spectrum of over storage time. (B) Fluorescence spectrum of FNP in the solid state (λex = 364 nm).

Fig. 7. (A) Stability of the fluorescence properties of purified FNP over storage time: red curve is the PL spectrum of FNP in THF as synthesized; blue curve is the PL spectrum of FNP in THF after one week of storage. (B) Fluorescence spectrum of FNP in the solid state (λex = 364 nm).

### EXPERIMENTAL SECTION

**Materials.** Methyl methacrylate (MMA) (99%), (2-acetoacetoyl)ethyl methacrylate (AEMA) (95%), 2,2'-azo(2-methylpropionitrile) (AIBN) (≥98%), p-carboxybenzenesulfonazide (p-CBSA) (97%), triethylamine (Et3N) (≥99%), HPS (98%), 1,1-dimethyl-2,5-bis(4-(azidomethyl)phenyl)-3,4-diphenylsilole (Silole-N3) (96%), ethyl acetate (EtOAc) (anhydrous, 99.8%), diethyl ether (Et2O) (anhydrous, >99.0%), and deuterated chloroform (CDCl3) (99.96 atom % D, containing 0.03% (v/v) tetramethylsilane) were purchased from Sigma-Aldrich and used, unless specified, as received. 2-Cyanoprop-2-yl-dithiobenzoate (≥97%) was purchased from Strem Chemicals. Methanol (MeOH) (synthesis grade) and THF (HPLC grade) were purchased from Scharlab. AIBN was recrystallized from methanol. MMA was purified by distillation before use. AEMA was purified by passing through alumina.

**Techniques.** *Experimental UV Irradiation Setup.* UV irradiation experiments were performed using a Hamamatsu UV spot light source (LC8, Hg–Xe Lamp, UV intensity: 3.5 W/cm²) equipped with an A9616-05 filter [transmittance wavelength: 300–400 nm (λmax = 365 nm); transmitted intensity: 80%] and the corresponding UV-light guide. The distance from the guide to the solution containing the SCNP precursor was 10 cm. The open area during irradiation was 2 cm². A picture of the experimental setup is available in the Supporting Information of ref 61.

**Size Exclusion Chromatography.** SEC measurements were performed at 30 °C on an Agilent 1200 system equipped with PLgel 5 µm Guard and PLgel 5 µm MIXED-C columns, and triple detection: a differential refractive index detector (Optilab Rex, Wyatt), a MALLS detector (MiniDawn Treos, Wyatt), and a viscosimetric (VIS) detector (ViscoStar-II, Wyatt). Data analysis was performed with ASTRA Software (version 6.1) from Wyatt. THF was used as an eluent at a flow rate of 1 mL/min. A value of dn/dc = 0.083 was used for precursors and SCNPs.

**1H Nuclear Magnetic Resonance.** 1H NMR spectra were recorded at r.t. on a Bruker spectrometer operating at 400 MHz, using CDCl₃ as a solvent. AEMA content was determined following the procedure described in ref 62.

**FTIR Spectroscopy.** FTIR spectra were recorded at r.t. on a JASCO FTIR spectrometer.

**Elemental Analysis.** EA measurements were performed in a Euro EA3000 elemental analyzer (CHNS).

**Fluorescence Spectroscopy.** PL spectra were recorded at r.t. on an Agilent Cary Eclipse spectrometer at an excitation wavelength of 364 nm.

**Transmission Electron Microscopy.** TEM measurements were performed using a high-resolution transmission electron microscope TECNAI G220 TWIN. The measurements were carried out using an accelerating voltage of 200 kV under low dose conditions.

**Procedures.** *Synthesis of the Precursor.* The whole synthesis of precursor P was performed protected from light. In a typical procedure, a random copolymer of MMA and AEMA (485 mg, 0.77 mmol) prepared according to the methodology reported in ref 41 (weight average molecular weight, Mw (SEC/MALLS) = 208 kDa; polydispersity, D = 1.25; molar fraction of AEMA units (1H NMR) (32% x = 0.32) was dissolved in CH₂Cl₂ (10 mL) at r.t. Then, p-CBSA (250 mg, 1.1 mmol) and Et₃N (0.4 mL, 2.87 mmol) were added, and the mixture was maintained under stirring for 24 h (see Scheme 1). After reaction, the solution was concentrated and precipitated in MeOH and the resulting precursor P was dried in a vacuum oven in the dark [P: Yield = 73%; Mw (SEC/MALLS) = 289 kDa; D = 1.24; molar fraction of azido-β-ketoester units (EA) = 23%, y = 0.23; radius of gyration, Rg (SEC/MALLS) = 17.9 ± 0.08 nm; hydrodynamic radius, Rh (SEC/VIS) = 11.4 ± 0.1 nm].

**Synthesis of Single-Chain Polymer Nanoparticles in the Presence of HPS.** In a typical reaction, precursor P (6 mg, 9.5 × 10⁻³ mmol) and HPS (2.6 mg, 4.75 × 10⁻³ mmol) were dissolved in CH₂Cl₂ (12 mL) at r.t. and the solution was subject to UV light irradiation through an open area of 2 cm² in the 300–400 wavelength range (λmax = 365 nm) for 60 min. The resulting (nonfluorescent) SCNP s were purified by precipitation in Et₂O and further drying in a vacuum oven [NP: Yield = 72%, Mw (SEC/MALLS) = 290 kDa; D = 1.25; Rh (SEC/MALLS) = 7.3 ± 1.1 nm, Rh (SEC/VIS) = 9.5 ± 1.1 nm].

**Synthesis of Single-Chain Polymer Nanoparticles in the Presence of Silole-N₃.** In a typical reaction, precursor P (6 mg, 9.5 × 10⁻³ mmol) and Silole-N₃ (2.5 mg, 4.75 × 10⁻³ mmol) were dissolved in CH₂Cl₂ (12 mL) at r.t. and the solution was subject to UV light irradiation (λmax = 365 nm) for 60 min. The resulting highly fluorescent SCNPs FNP were purified by precipitation in Et₂O and further drying in a vacuum oven [FNP: yield = 78%, Mw (SEC/MALLS) = 330 kDa; D = 1.26; Rh (SEC/MALLS) = 6.2 ± 2.0 nm, Rh (SEC/VIS) = 9.0 ± 0.3
Fluorescence quantum yield determination and additional data on the precursors of SCNPs (PDF)

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