Sensors

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A General Strategy To Construct Fluorogenic Probes from Charge-Generation Polymers (CGPs) and AIE-Active Fluorogens through Triggered Complexation**

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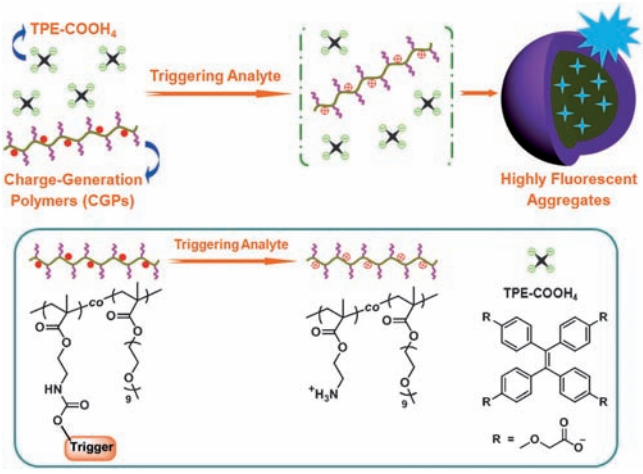
Fluorescent probes have been extensively used in chemical biology, clinical diagnosis, and biosensing because of their high sensitivity, fast response, and excellent spatial and temporal resolution.[1] On the basis of photo-induced electron transfer (PET), internal charge transfer (ICT), fluorescence resonance energy transfer (FRET), and excited-state intramolecular proton transfer (ESIPT) mechanisms, various fluorometric probes based on small molecule fluorophores, quantum dots, and conjugated polymers have been developed.[2] The concept of aggregation-induced emission (AIE)[3] or aggregation-induced enhanced emission (AIEE)[4] has emerged to be a powerful and versatile strategy for the design of novel types of fluorescent probes.[5] Typical AIE-active molecules, such as tetraphenylethene (TPE), are non-emissive in the molecularly dissolved state, whereas enhanced fluorescence emission was achieved when they are in the aggregated state.[6] In recent years, a great variety of AIE-based small-molecule chemosensors and biosensors for analytes ranging from heavy-metal ions, thiols, explosives, gas, carbohydrates, and DNA, to proteins and enzymes have been fabricated.[5–7] Current research trends in this field involve the development of general strategies towards water-soluble fluorogenic probes selective and sensitive for variable analytes.

Polyelectrolytes undergo aggregation and assembly in the presence of oppositely charged polyelectrolytes, multivalent small organic molecules, and inorganic nanoparticles owing to electrostatic interactions.[8] However, to the best of our knowledge, polyelectrolyte-induced aggregation of AIE-active charged fluorogens has not been utilized in sensing and detection systems, mainly because of the lack of suitable smart polyelectrolytes exhibiting specific analyte-triggerable charge generation or conversion characteristics. It can be envisaged that the intrinsic multivalent nature of polyelectrolytes should enable the design of a new-generation of fluorometric probes when integrated with water-soluble AIE-active fluorogens.

By taking advantage of the fact that small molecule amine moieties can be caged by carbamate functionalization and selectively decaged on demand,[9] we recently designed charge-generation polymers (CGPs) possessing pendent carbamate-masked amine functionalities, which can undergo stimuli-triggered transition from the initially neutral state to a charged one in the presence of a specific analyte of interest.[10a] The charge-generation process was then coupled with the induced aggregation of Au nanoparticles to design colorimetric probes. We hypothesize that the integration of appropriately designed CGPs possessing varying triggerable motifs with AIE-active charged fluorogens, such as the TPE derivative bearing four carboxyl acid moieties (TPE-$COOH_4$), should allow the construction of CGP-based fluorogenic probes with enhanced detection sensitivity and designing flexibility (Scheme 1). In the presence of triggering analyte of interest, electrostatic complexation between TPE-$COOH_4$ and the newly generated cationic polyelectrolytes can "turn on" the fluorescence emission through induced aggregation of TPE-$COOH_4$. Owing to the versatile design and facile synthesis of CGPs bearing different analyte-specific charge-generation moieties, this conceptual strategy is



Scheme 1. Construction of fluorogenic sensors through the integration of negatively charged AIE-active fluorogens (TPE-$COOH_4$) with water-soluble amine-caged charge-generation polymers (CGPs) exhibiting selective and specific analyte-triggered switching from the initially uncharged state to cationic polyelectrolytes.

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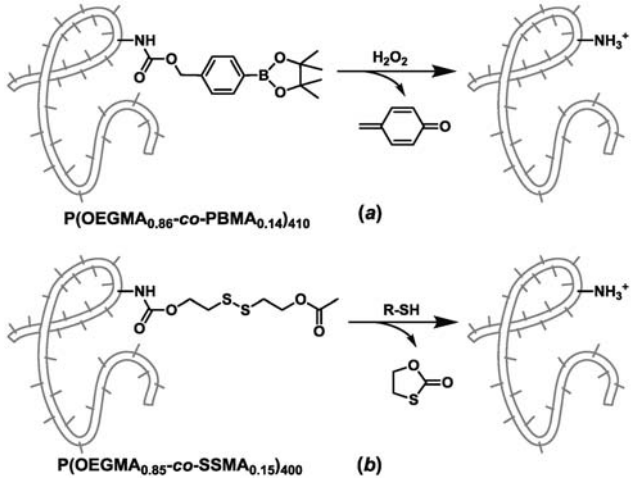
expected to provide a general approach toward engineering a broad range of fluorogenic probes with diverse sensing functions. Herein, we report proof-of-concept examples for the fabrication of fluorogenic probes for two types of biologically relevant species, namely, H_2O_2 and thiols, on the basis of newly designed amine-caged CGPs and negatively charged AIE-active fluorogens (Scheme 1 and Scheme 2). In addition, preliminary results concerning their application for the fluorogenic assay of glucose and D-glucose 6-phosphate (G6P) are also presented.



Scheme 2. Schematic illustration of the stimuli-triggered charge-generation process of amine-caged CGPs bearing a) H_2O_2- and b) thiol-reactive moieties.

According to the above design (Scheme 1), two polymerizable and analyte-triggerable carbamate-based monomers, H_2O_2-reactive PBMA (Scheme S1 in the Supporting Information) and thiol-reactive SSMA (Scheme S1 and Figure S1), were synthesized at first by the reaction of 2-isocyanatoethyl methacrylate with hydroxy-functionalized precursors bearing boronate and disulfide moieties, respectively. Since these two monomers lack sufficient water solubility, we then opted to prepare triggering moiety-loaded water-soluble polymers by reversible addition–fragmentation chain transfer (RAFT) copolymerization of PBMA or SSMA with oligo(ethylene glycol) methyl ether methacrylate (OEGMA) (Scheme S2). ^{1}H NMR spectroscopic analysis (Figures S2a and S3a) revealed overall degrees of polymerization (DPs) of 410 and 400 and functional comonomer contents of 14 and 15 mol%, respectively, for P(OEGMA-*co*-PBMA) and P(OEGMA-*co*-SSMA) copolymers. Moreover, relatively narrow-disperse copolymers were obtained (Figure S4).

P(OEGMA-*co*-PBMA) and P(OEGMA-*co*-SSMA) copolymers are water-soluble and remain in the uncharged state when dissolved in water. As both of them contain carbamate-caged amine moieties, we can visualize that they will undergo the transition from the initially uncharged state to positively charged one upon addition of H_2O_2[9b–e] and thiol compounds,[9f–h] respectively (Scheme 2). This is indeed the case, and it was found that the presence of H_2O_2 or thiol groups can selectively deprotect carbamate caging moieties, as evidenced by ^{1}H NMR spectroscopic analysis from the complete disappearance of signals characteristic of carbamate protecting groups (Figures S2 and S3). Since pendent aliphatic amine moieties typically possess a pK_a value of 8–9,[10,11] decaged amine residues are mainly in the protonated state under neutral conditions.

The negatively charged AIE-active TPE derivative bearing four carboxy acid moieties (TPE-$COOH_4$) was then synthesized (Scheme S3 and Figure S5).[12] TPE-$COOH_4$ has a pK_a value of 4–5 is soluble in water, and exists in its ionized form at pH 7.4, exhibiting essentially no fluorescence emission, whereas at pH 3.0 it forms aggregates as a result of decreased solubility upon protonation and emits intense blue emission (Figure S6). This result confirms that TPE-$COOH_4$ retains similar AIE features to that of TPE.[6] The colorless aqueous solution of TPE-$COOH_4$ at pH 7.4 remains nonfluorescent upon the sole addition of H_2O_2 or dithiothreitol (DTT), indicating that they do not induce the aggregation of TPE-$COOH_4$ (Figure S6).

The aqueous based fluorogenic sensing system for H_2O_2 was then optimized to consist of negatively charged TPE-$COOH_4$ (7.5 μM) and the CGP, $P(OEGMA_{0.86}\text{-}co\text{-}PBMA_{0.14})_{410}$ copolymer ([PBMA] = 30.0 μM) in buffer solution (PBS; pH 7.4). Initially, TPE-$COOH_4$ exhibits no fluorescence emission (Figure 1a–c), which is quite reasonable considering that negatively charged TPE-$COOH_4$ does not exhibit any specific interactions with the uncharged amine-caged copolymer.

Upon addition of H_2O_2 (30 equiv relative to PBMA residues) to the sensing mixture, the emission intensity increases gradually with time and then stabilizes after around 180 min (Figure 1a and Figure S7). Concomitantly, the solution gradually turns from clear to a bluish tinge (Figure 1b), indicating the formation of colloidal nanoparticles. In addition, the H_2O_2-induced fluorescence can also be visualized by the naked eye, as evidenced by the transition from nonfluorescent to the intense blue emission (Figure 1c). The reaction of the CGP with H_2O_2 can chemoselectively decage boronate-based carbamate protecting groups,[9b–e] leading to the generation of amine moieties and spontaneous transformation from the uncharged state into a cationic polyelectrolyte (Scheme 2a). Subsequently, the newly generated cationic polyelectrolyte electrostatically interacts with negatively charged TPE-$COOH_4$ molecules to form polyion complex (PIC) nanoparticles,[8] leading to fluorescence emission (Scheme 1). TEM and AFM analyses revealed the presence of robust spherical nanoparticles with diameters in the range 30–40 nm (Figures S8 and S9). Electrophoresis measurements further indicated that micellar nanoparticles possess a zeta potential of approximately −8 mV. Dynamic laser light scattering (LLS) revealed an intensity-average hydrodynamic radius $\langle R_h \rangle$ of 47 nm and polydispersity (μ_2/Γ^2) of 0.12 (Figure S10). Static LLS measurements revealed an apparent molar mass $M_{w,app}$ of 2.33×10^7 g mol^{-1} and average radius of gyration $\langle R_g \rangle$ of 38 nm (Figure S11). Note that the $\langle R_g \rangle / \langle R_h \rangle$ ratio of 0.81 is quite close to that theoretically predicted for hard spheres (0.774). Assuming that all charged species have participated in the formation of PIC micelles, we can roughly estimate that, on average, each micellar nano-

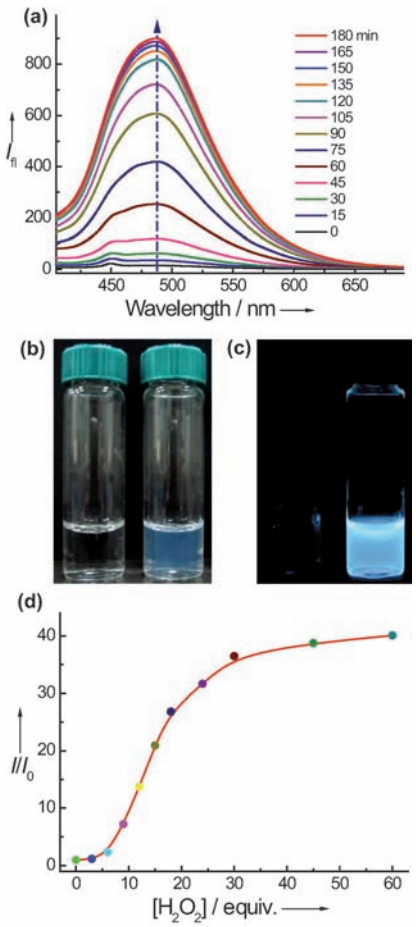


Figure 1. a) Time evolution of fluorescence emission spectra (λ_{ex} = 390 nm, slit widths for excitation and emission: 5 nm) of the fluorogenic sensing mixture (pH 7.4) consisting of TPE-$COOH_4$ (7.5 μM) and P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$ ([PBMA] = 30.0 μM) upon addition of 30.0 equiv H_2O_2 (relative to PBMA moieties) at 25 °C. Optical photographs recorded under b) visible and c) UV (365 nm) light for the aqueous fluorogenic sensing mixture (left) before and (right) 180 min after the addition of 30.0 equiv H_2O_2. d) Relative fluorescence intensity changes recorded for the aqueous fluorogenic sensing system at 180 min after the addition of varying amounts of H_2O_2 (0–60.0 equiv relative to PBMA moieties).

particle consists of 120 copolymer chains and 1710 TPE-$COOH_4$ moieties. In addition, the average chain density of micellar nanoparticles was estimated to be 0.089 g cm^{-3} based on $M_{w,app}$ and $\langle R_h \rangle$ values, indicating that these colloidal nanoparticles are highly hydrated. Note that the observed enhanced emission of TPE-$COOH_4$ within PIC micelles is due to the restriction of intramolecular rotation of phenyl rotors in complexed TPE-$COOH_4$, which is, in principle, independent of the presence of surrounding water molecules.[5,6a–d]

H_2O_2-triggered complexation between the CGP, P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$ copolymer, and TPE-$COOH_4$ can be further quantitatively determined by emission intensity changes. Spectrofluorometric analysis was then conducted to evaluate the H_2O_2 detection performance. The time duration needed for the fluorometric changes to reach the final stable state was estimated to be 180 min (Figure 1a and Figure S7). To maintain the consistency and repeatability of the sensing system, in subsequent experiments all aqueous mixtures were then incubated for 180 min upon addition of H_2O_2. Typical fluorescence emission intensity obtained for the aqueous sensing mixture upon addition of H_2O_2 (0–60.0 equiv relative to PBMA moieties) are shown in Figure 1c and Figure S12. The emission intensity dramatically increases with the H_2O_2 concentration and tends to stabilize out at more than 60.0 equiv H_2O_2, exhibiting approximately 40-fold cumulative enhancement in emission intensity.

The detection limit at which a 10% increase in emission intensity can be achieved was determined to be 30 μM (1.02 mg L^{-1}) (Figure 1d), which is quite comparable to those of fluorescent sensors based on small molecule aryl boronates.[9b,d] On the basis of the above results, we established that highly sensitive fluorogenic H_2O_2 detection system can be constructed by integrating negatively charged AIE-active fluorogen, TPE-$COOH_4$, with the novel type of CGP exhibiting H_2O_2-triggerable transformation from the initially uncharged state to a cationic polyelectrolyte.

To evaluate the generality of the proposed strategy (Scheme 1), we then replaced H_2O_2-reactive CGP, P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$ copolymer, with the thiol-reactive one, P($OEGMA_{0.85}$-*co*-$SSMA_{0.15}$)$_{400}$ copolymer. The latter contains disulfide moieties as the triggerable motif (Scheme 2b). Thus, it should allow for the construction of fluorogenic sensor for thiol compounds. The optimized thiol-sensing system consists of TPE-$COOH_4$ (7.5 μM) and P($OEGMA_{0.85}$-*co*-$SSMA_{0.15}$)$_{400}$ copolymer at a SSMA concentration of 30.0 μM in aqueous medium (pH 7.4). Previous studies have demonstrated that thiols can selectively trigger the cleavage of disulfide-based carbamate protecting groups and spontaneously release primary amine moieties.[9f–h]

Thus, the reaction of P($OEGMA_{0.85}$-*co*-$SSMA_{0.15}$)$_{400}$ copolymer with thiols can again chemoselectively transform uncharged P($OEGMA_{0.85}$-*co*-$SSMA_{0.15}$)$_{400}$ into a cationic polyelectrolyte (Scheme 2b and Figure S3). As shown in Figure 2a and Figure S13, upon addition of DTT (500 equiv relative to SSMA residues), the fluorescence emission intensity increases gradually with time and then stabilizes after 180 min. This result indicates that the above described fluorogenic H_2O_2-sensing strategy can be further adapted for fluorescent thiol detection (Scheme 1 and Scheme 2b).

Comparable time-dependent emission intensity changes (i.e., both accomplished within around 3 h) were observed for P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$ and P($OEGMA_{0.85}$-*co*-$SSMA_{0.15}$)$_{400}$ copolymers in the presence of TPE-$COOH_4$ upon addition of 30 equiv H_2O_2 (relative to PBMA) or 500 equiv DTT (relative to SSMA), respectively (Figures 1a and 2a). This result must be a coincidence because in both cases, the carbamate deprotection kinetics are quite dependent on the H_2O_2 or DTT concentration (Scheme 2).[9] Figure 2b and Figure S14 show that the emission intensity increases considerably with increasing amount of DTT and stabilizes at greater than 1000 equiv of DTT (relative to SSMA moieties), exhibiting 42-fold cumulative enhancement in the DTT range 0–1000 equiv and a detection limit of 0.5 mM. Thus, the integration of thiol-reactive CGP with TPE-

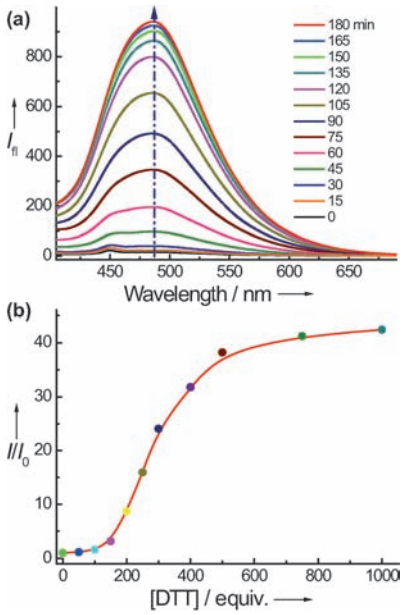


Figure 2. a) Time evolution of fluorescence emission spectra (λ_{ex} = 390 nm, slit widths for excitation and emission: 5 nm) of the fluorogenic sensing mixture (7.5 μM of TPE-$COOH_4$ and P($OEGMA_{0.85}$-*co*-$SSMA_{0.15}$)$_{400}$ with [SSMA] = 30.0 μM) at pH 7.4 upon addition of 500 equiv DTT (relative to SSMA moieties) at 25 °C. b) Relative fluorescence intensity changes recorded for the aqueous sensing mixture at 180 min after the addition of varying amounts of DTT (0–1000 equiv relative to SSMA moieties).

$COOH_4$ can further lead to the construction of highly sensitive fluorogenic thiol-sensing assay.

Finally, we explored the applications of P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$ copolymer/TPE-$COOH_4$ based sensing mixture for the quantitative assay of two additional types of analytes, glucose and D-glucose 6-phosphate (G6P), by taking advantage of glucose oxidase (GOx)-catalyzed[13a,b] and cascade acid phosphatase (AP)/GOx-catalyzed[13c,d] production of H_2O_2, respectively (Scheme S4). As shown in Figure S15, upon addition of 60.0 equiv glucose (relative to PBMA) to the mixture of P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$, TPE-$COOH_4$, and GOx, we observed a time-dependent increase of fluorescence emission, which essentially stabilizes after 210 min (Figure S16). This result is quite comparable to that observed upon H_2O_2 addition (Figure 1a) and confirms that GOx-assisted fluorogenic glucose sensing is feasible (Scheme S4). Figure S17 indicates that the emission intensity after 210 min incubation with glucose (0–120 equiv; 0–2.4 mM) exhibited 36-fold cumulative emission intensity increase. The two-enzyme cascade reaction of G6P in the presence of AP and GOx also results in the formation of H_2O_2, and this can be utilized for fluorogenic G6P sensing (Scheme S4). We can clearly tell from Figure S18 that the emission intensity at 487 nm dramatically increases with the G6P concentration in the range 0–120 equiv after 300 min incubation with the aqueous mixture containing P($OEGMA_{0.86}$-*co*-$PBMA_{0.14}$)$_{410}$, TPE-$COOH_4$, AP, and GOx. In principle, this fluorogenic assay system should also be applicable for the fluorometric quantification of phosphatase activities and the detection of AP-specific inhibitors such as pesticides (e.g., paraoxon) and organic pollutants (e.g., benzidine).[13c,d] Further work toward this end are currently underway.

In summary, we have presented a general and conceptual strategy for the construction of quantitative, selective, and sensitive fluorogenic sensors by integrating a novel type of stimuli-triggerable CGPs bearing caged amine moieties with negatively charged AIE-active fluorogens, TPE-$COOH_4$ (Scheme 1). The presence of specific biorelevant analytes, H_2O_2 or thiols, can trigger the transformation of CGPs from the initially uncharged state into a cationic polyelectrolyte through the chemoselective cleavage of carbamate protecting moieties. This transformation induces the aggregation of negatively charged TPE-$COOH_4$, leading to the dramatic enhancement of fluorescence emission. Further applications to the fluorogenic sensing of glucose and G6P were also demonstrated. A variety of analyte-specific carbamate-based amine-caging motifs could be incorporated into the CGP design.[9a] We envisage that these proof-of-concept examples can be further generalized to the design of more sophisticated sensing systems.

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