Photochemistry

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Sequential Control over Thiol Click Chemistry by a Reversibly Photoactivated Thiol Mechanism of Spirothiopyran**

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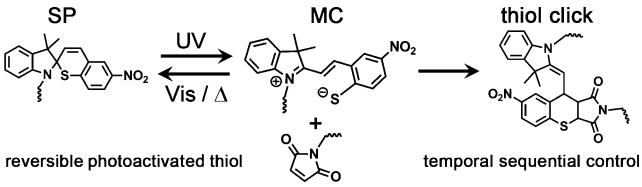
Abstract: *A novel photocontrolled thiol click chemistry based on spirothiopyran and maleimide is reported. Upon irradiation with $\lambda = 365$ nm light, the spirothiopyran can isomerize to the open merocyanine form, a thiophenolate group, which can rapidly react with maleimide. The unreacted MC will readily isomerize back to the starting spirothiopyran, which can be repeatedly photoactivated as needed. Thus, this reversible photoactivated thiol confers spatiotemporal sequential control on the thiol–maleimide reaction using only one type of photochemical reaction. Polymer post-functionalization and hydrogel building with subsequent multipatterning using different maleimide molecules in a temporal sequential manner indicate that this photocontrolled Michael addition reaction can modulate the specific chemical events in a sequence.*

Implementing chemical processes with high efficiency, easy handling, and precise control is one of the key goals worth pursuing in modern chemistry. Currently, highly efficient chemical methods are often referred to as "click chemistry".[1] Enabling spatiotemporal control to a click process is particularly interesting.[2] Spatial control is necessary in some applications which feature surface patterns or three-dimensional scaffolds,[3] while temporal control can initiate a reaction on demand.[2a] Ideally, light could be exploited as a valuable method to determine where, when, and to what extent a chemical process is started or stopped.[2b,4] Recently, a class of prominent photocontrolled click techniques was developed.[2b] These light-triggered methods are successful for spatial control and can determine when a chemical process is started.[2a,3b,5] However, the precise control of a reaction according to a temporal sequence by light remains a challenge, and such a process would be particularly important when a specific sequence of events is required. To achieve this effect, tedious approaches of multiple and orthogonal click reactions[6] or wavelength-selective photoactivation[7] have to be employed.

Similar to cycloaddition reactions,[8] some thiol–alkene/alkyne reactions also exhibit characteristics of click chemistry, hence they are also known as thiol click chemistry.[9] In particular, thiol Michael addition reactions proceed through a nonradical pathway in quantitative yields and with rapid reaction rates in a physiological medium even without any catalyst.[10] However, free thiols can react very easily with themselves to form disulfide bridges under air oxidation, and thus severely limit the expansion of thiol chemistry.[11] Recently, photoactivated thiol chemistry has been proposed, and free thiols which are caged by a phototrigger group are activated in situ to rapidly react with maleimide molecules upon light irradiation.[3a,7,10b,12] This strategy not only reduces oxidation of thiols but also confers the thiol Michael addition reaction with spatiotemporal control. However, to realize a thiol click process with temporal sequential control using irreversible photoactivation is unrealistic, as precise control over the dosage of photoreleased thiols so as to exactly match the stoichiometry of maleimide for each illumination is very difficult. Therefore, the excess released thiol is wasted. In addition, an equal amount of photolytic by-products will be generated during the photo-uncaging process.

Herein, we report a novel photocontrolled thiol click reaction in which spirothiopyran acts as a reversible photoactivatable thiol to optionally regulate the thiol-maleimide reaction using light. Spirothiopyran, a century-old molecule, is a sulfur-containing spiropyran photoswitch.[13] As shown in Scheme 1, under dark conditions or exposure to visible light, the spirothiopyran exists in a closed form (SP), which can isomerize to the open merocyanine form (MC) upon irradiation with UV light by cleavage of the spiro C–S bond in the heterocyclic ring. The MC isomer has a thiophenolate anion group which can rapidly react with maleimide in solution through a thiol Michael reaction. Simultaneously, the unreacted MC will readily isomerize back to the SP, which can be repeatedly photoactivated as needed, thus conferring spatial and temporal sequential control to the thiol–maleimide reaction using only one type of photochemical reaction.



Scheme 1. The reversibly photoactivated thiol in spirothiopyran sequentially regulates thiol–maleimide click reaction.

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Meanwhile, such a reversible photoactivated thiol click reaction offers additional salient features such as reduced thiol oxidation, no generation of photolytic by-products, and easy synthesis.

The reversible photoswitchable property of spirothiopyran was first verified using UV/Vis absorption spectroscopy. SP 1 (for structure see Figure 1) was synthesized because of its hydrophilic properties (see Figure S1 in the Supporting Information). Its solution shows almost no absorption at greater than $\lambda = 450$ nm in the dark and its spectrum does not change even when the sample is left for one week, thus indicating that SP 1 exists only as the SP form and exhibits excellent stability. Irradiation of a solution of SP 1 at $\lambda = 365$ nm (10 mW cm^{-2}, 0–50 s) creates a distinctive absorption at $\lambda = 550$ nm, which is assigned to the spirocycle-opened MC form (see Figure S2 a).[13a] Upon irradiation with light at $\lambda = 525$ nm, the MC form could be accelerated to revert to its SP form (Figure S2 b). As noted in a previous report, only about 20% of SP 1 could be converted into the MC form when this photoreversible reaction reached equilibrium.[13b] HPLC was used to detect the residual SP 1 after each repeated irradiation cycle [UV-365 nm (1 min, 10 mW cm^{-2}) $\leftrightarrow$ Vis-525 nm (5 min, 100 mW cm^{-2}); see Figure S3 a]. About 6% of the SP 1 molecules were lost after each irradiation cycle (Figure S3 b). This result indicates that the intermediate MC form could not fully isomerize back to the SP form because of the "fatigue effect" observed for most photoswitch molecules,[13c] and side reactions occur to some degree during irradiation with UV light.

When PEG_6-Mal was added to the solution of SP 1, no reaction was detected by HPLC. However, upon irradiation at $\lambda = 365$ nm, three distinct isoabsorptive points appear in the UV/Vis absorption spectrum (see Figure S5), and the color of the solution also changed [see Figure S4; yellow (SP 1 + PEG_6-Mal), brown (UV + SP 1, MC), yellow (UV + (SP 1 + PEG_6-Mal))]. As shown in Figure 1 a, a pure and stable photoproduct, SPP, was generated rapidly and the amount of SP 1 gradually decreased as the UV irradiation time increased. Finally, about 95% of SPP could be obtained, and the sum of formation value of SPP and consumption value of SP 1 always kept over 95% (Figure 1 b). This high conversion indicates the SP form will constantly convert into the MC form when the photoisomerization equilibrium of spirothiopyran is broken by maleimide. Meanwhile, the instantaneous reaction of the formed MC with maleimide could effectively inhibit the active MC from generating side reactions. Moreover, to determine the molecular structure of the product of the spirothiopyran–maleimide photoreaction, SP 2 was synthesized (Figure S1) and *N*-ethylmaleimide was chosen as the rigid structures easily induced single-crystal formation. The model photoproduct (M-SPP) was isolated and purified using column chromatography (yield: 95%) and its structure was confirmed by single-crystal X-ray diffraction (Figure 1 c; see Figure S7 and Table S1).[14] Finally, the reaction mechanism was proposed and is shown in Figure 1 d. The SP form first isomerizes to the MC form upon irradiation at $\lambda = 365$ nm. Simultaneously, the thiophenolate anion group contained in the MC form attacks the maleimide group, and a cyclic adduct is obtained after the 1, 4-addition reaction.

To demonstrate the temporal sequential controllability of this reversible photoactivation process, PEG_6-Mal was designed to be added stepwise into the solution of SP 1 by modulating the molar ratio of PEG_6-Mal to SP 1 from 0:1 to 1:1 ($\Delta = 0.2$ equiv). As shown in Figure 2 a, when fresh PEG_6-Mal (0.2 equiv) was added, the value of SPP was unchanged and only increased after irradiation at $\lambda = 365$ nm. The excess MC form was promoted to isomerize back to the SP form by irradiation with light at $\lambda = 525$ nm for each cycle. After five cycles, the conversion of SP 1 reaches about 87% when the total PEG_6-Mal was at 1.0 equivalent. The conversion of SP 1 was slightly less than that in the process when excess PEG_6-Mal (the molar ratio of PEG_6-Mal to SP 1 is 1.5:1) was added

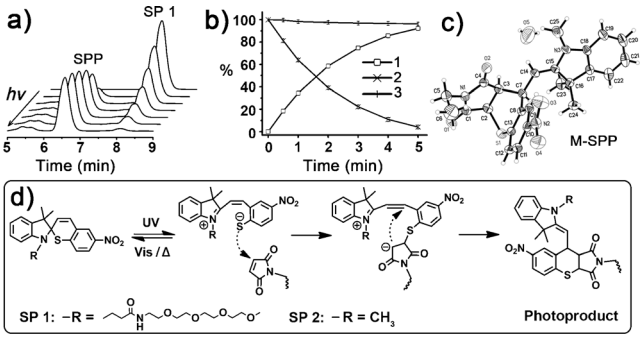

Figure 1. The spirothiopyran–maleimide photochemical reaction. a) HPLC profiles of the SP 1-PEG_6-Mal photochemical reaction upon irradiation at $\lambda = 365$ nm for 0, 0.5, 1, 2, 3, 4, 5 min (10 mW cm^{-2}, molar ratio of SP 1/PEG_6-Mal = 1:1.5, 1/1 v/v CH_3OH/tris-HCl, pH 7.4). b) HPLC analytic curve of the above photochemical reaction: 1) curve for SPP formation; 2) curve for consumption of SP 1; 3) summation curve of SPP and SP 1. c) Single-crystal XRD of the model photoproduct M-SPP generated from SP 2 and *N*-ethylmaleimide. Thermal ellipsoids shown at 30% probability. d) The proposed reaction mechanism of the photocontrolled spirothiopyran–maleimide reaction.

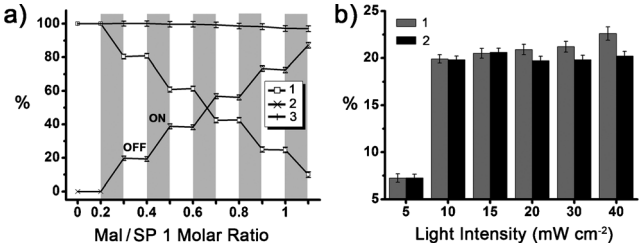

Figure 2. Temporal sequential control of the spirothiopyran–maleimide photochemical reaction. a) Determined by stepwise addition of PEG_6-Mal into a solution of SP 1 (Mal/SP 1 molar ratio from 0, 0.2, 0.4, 0.6, 0.8, to 1). "OFF" indicates a fresh portion of PEG_6-Mal (0.2 equiv) was added and reacted for 5 min without irradiation. "ON" indicates the above solution was first irradiated with light at $\lambda = 365$ nm for 1 min (10 mW cm^{-2}), then reacted for an additional 5 min, and finally irradiated with light at $\lambda = 525$ nm for 5 min (100 mW cm^{-2}) to promote isomerization of the excess MC form to the SP form. HPLC was used to monitor the progress of the reaction. 1) Curve for SP 1 consumption; 2) curve for SPP formation; 3) summation curve for SP 1 and SPP. b) Comparison of the percentage of SP 1 consumed (1) and SPP formed (2) by increasing the light intensity ($\lambda = 365$ nm for 1 min, molar ratio of Mal/SP 1 = 0.2:1).

as a single portion into the solution of SP 1 (Figure 1b). The results clearly demonstrate that the spirothiopyran–maleimide photochemistry shows excellent temporal and sequential control, as the excess MC form could readily convert back into the starting SP form, even though some of the spirothiopyran is lost during each irradiation cycle. In addition, this temporal sequential control has excellent tolerance. For example, when PEG_6-Mal (0.2 equiv) was added into SP 1, we intentionally increased the light intensity (from 5 to 40 $mW\,cm^{-2}$, irradiation time was fixed at 1 min; Figure 2b) or prolonged the irradiation time (from 1 to 3 min, light intensity was fixed at 10 $mW\,cm^{-2}$; see Figure S8) to compare the consumption of SP 1 and the formation of SPP. The consumption of SP 1 and the formation of SPP was kept reasonably consistent. These results indicate the rate-determining step of this thiol click process is the reversible opening of the spirothiopyran, and this photoregulated mechanism could facilely modulate the thiol–maleimide reaction, which is insensitive to irradiation conditions or stoichiometry ratio

To further demonstrate the temporal sequential control of the spirothiopyran–maleimide photoreaction, we translated this new photocontrolled thiol click reaction strategy into a polymer post-functionalization protocol. A series of desired photoresponsive polymers (PSP) were copolymerized by varying the molar ratio of SP 3 to PEG-MA (see Table S2), and PSP 4 [the molar ratio of SP 3 to PEG-MA is 1:4.2, as verified by ^{1}H NMR spectroscopy; see Figure S9; and GPC ($M_n = 24240$, PDI = 2.0)] was chosen as the test material because it contained caged thiols and had hydrophilic properties. In addition, three model maleimide derivatives were selected in this section: 1) PEG-Mal (5 K) as it is commercially available and easily detected by GPC; 2) RGDH-Mal as a model peptide, which could facilitate cell adhesion;[15] and 3) Fluorescein-Mal (Flu-Mal) or Rhodamine-Mal (Rh-Mal) because of their excellent fluorescence emission properties. Firstly, we explored this photoregulated conjugation of PSP 4 with PEG-Mal and RGDH-Mal independently under one irradiation mode (Figure 3a). The conjugation was performed in PBS (pH 7.4; the molar ratio of maleimide to spirothiopyran was 1.5:1) at ambient temperature with irradiation at $\lambda = 365$ nm (10 $mW\,cm^{-2}$). After the reaction was completed, the mixture was purified by dialysis to remove the unreacted PEG-Mal or RGDH-Mal. The final modified polymers (PSP 4-PEG, PSP 4-RGDH) were recovered by lyophilization, and analyzed by GPC and ^{1}H NMR spectroscopy. Evolution of the molecular weight of the polymer with irradiation time provides an excellent method to monitor the photochemical reaction. As shown in Figure 3b, the molecular weight increases as the irradiation time is increased. In addition, the grafted rate of spirothiopyran by PEG-Mal and RGDH-Mal could be quantified by ^{1}H NMR spectroscopy (see Figure S10b and Figure 3c). The SP signal at $\delta = 7.4$ ppm completely disappeared and shifted to $\delta = 7.6$ ppm, thus indicating the effective photolysis of spirothiopyran. In addition, by comparing the integration at $\delta = 7.4$ ppm with that of the PEG-Mal signal at $\delta = 3.5$ ppm and the RGDH-Mal signal at $\delta = 7.55$ ppm (the detailed calculation is shown in the Supporting Information), the graft rate of the spirothiopyran by PEG-Mal and RGDH-Mal was

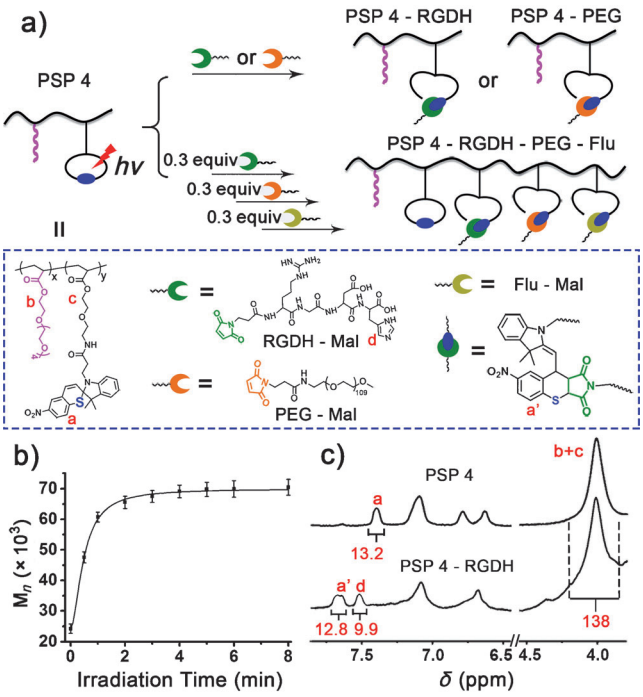

Figure 3. Post-functionalization of PSP 4 using the spirothiopyran-maleimide photochemical reaction. a) Post-functionalization with maleimide (Mal) molecules in two modes: independent conjugation with either PEG-Mal (5 K; orange) or RGDH-Mal (green), and temporal sequential conjugation with RGDH-Mal, PEG-Mal, and Flu-Mal (yellow). b) GPC was used to monitor the photoconjugation process between PSP 4 and PEG-Mal (5 K), at $\lambda = 365$ nm, 10 $mW\,cm^{-2}$. c) ^{1}H NMR spectroscopy was used to determine the successful conjugation of PSP 4-RGDH ([D_6]DMSO).

calculated at 70 and 75%, respectively. In comparison with small molecules, the steric hindrance of the polymer may decrease the reaction efficiency of spirothiopyran.

Subsequently, we investigated the sequential functionalization of a polymer with different maleimide molecules (Figure 3a). Specifically, upon irradiation with light at $\lambda = 365$ nm, 0.3 equivalents of RGDH-Mal (the molar ratio of maleimide to spirothiopyran was 0.3:1) was added to functionalize PSP 4 in the first step. After completion of the first step, PEG-Mal (0.3 equiv) and Flu-Mal (0.3 equiv) were added sequentially into reaction mixture and irradiated using the protocol used in the first step. After completion of the coupling reaction in each step, a small amount of sample was taken from the reaction mixture and analyzed. The modified polymer was recovered by lyophilization. Meanwhile, ^{1}H NMR spectroscopy was used to calculate the graft rate of spirothipyran by different maleimide molecules in each functionalization step according to the integration method described beforehand. The integration results revealed that the graft rate of the spirothiopyran by RGDH-Mal and PEG-Mal in the final PSP 4-RGDH-PEG-Flu copolymer was about 20 and 15.5%, respectively (see Figure S11b). In addition, the UV-vis absorption spectrum also confirms the grafted rate of spirothiopyran by Flu-Mal was about 16% (see Figure S12).

To provide a more visual effect to reflect the spatiotemporal sequential control of the photoregulated thiol click

reaction, we applied the process to the sequential photopatterning of three-dimensional (3D) hydrogels. 3D hydrogel scaffolds provide a more biomimetic environment for cell culture. Designing the 3D scaffold with appropriate chemical and physical properties is the first step to understanding the cues important to cell survival and stimulation.[3a,6c,16] So far, two techniques have been developed to control the sequential patterning of biological functionalities within a hydrogel, that is, use of two orthogonal click reactions[6c] or a two-photon technique.[3a] In this work, we only employ one type of photochemical reaction to go from hydrogel formation to sequential post-functionalization. PEG-4Mal (10 kDa) was chosen as the multi-maleimide crosslinker because it is commercially available and its excellent hydrophilic properties, while Flu-Mal and Rh-Mal were used as the model functionalities. As shown in Figure 4a, upon irradiation with

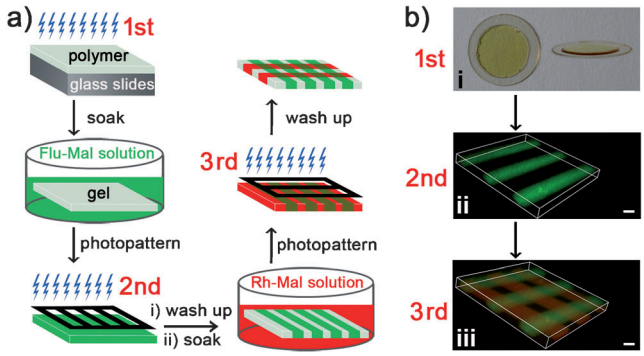

Figure 4. Sequential building and multipatterning of hydrogels using a single spirothiopyran–maleimide photochemical reaction. a) The photochemical reaction to initially form a hydrogel by photo-cross-linking, and successive patterning with the Flu-Mal and Rh-Mal dyes in a temporal sequence. b) i) Image of the initially formed hydrogel (10% wt, molar ratio of spirothiopyran/Mal = 4:1, irradiation at $\lambda = 365$ nm, 10 mW cm^{-2}, 1 min). Confocal fluorescence microscope images (Z-scan mode) of the patterned hydrogel using Flu-Mal alone (ii), and both Flu-Mal and Rh-Mal (iii). Scale bars: 100 μm.

light at $\lambda = 365$ nm, PSP 4 liberated thiophenolate anion groups which subsequently reacted with PEG-4Mal to generate a hydrogel network in the first step. Subsequently, Flu-Mal and Rh-Mal were patterned within the preformed hydrogel by conventional photolithographic methods based on the same photochemical reaction strategy in the second and third steps, respectively. The final dual-dye-patterned gels were imaged using confocal Z-stack scanning (Figure 4b). This distinct image effectively demonstrates the successful sequential occurrence of the spirothiopyran-maleimide photoreaction within one system. Thus, this photocontrolled click reaction provides an integrative strategy for hydrogel construction and subsequent functionalization.

As presented, we provide a novel photocontrolled thiol click reaction based on spirothiopyran and the maleimide group. This unique mechanism of reversible photoactivated thiols makes this method especially well-suited for applications which require spatiotemporal and sequential control. In addition to maleimide, the photouncaged thiol of spirothiopyran is anticipated to react with more Michael addition acceptors, such as acrylates and vinylsulfones. Considering its high efficiency, ease of use, and unique sequential control, this methodology provides numerous opportunities applications in various fields, especially the post-functionalization of polymers, surfaces, or hydrogels.

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