

# UV-induced disulfide formation and reduction for dynamic photopatterning

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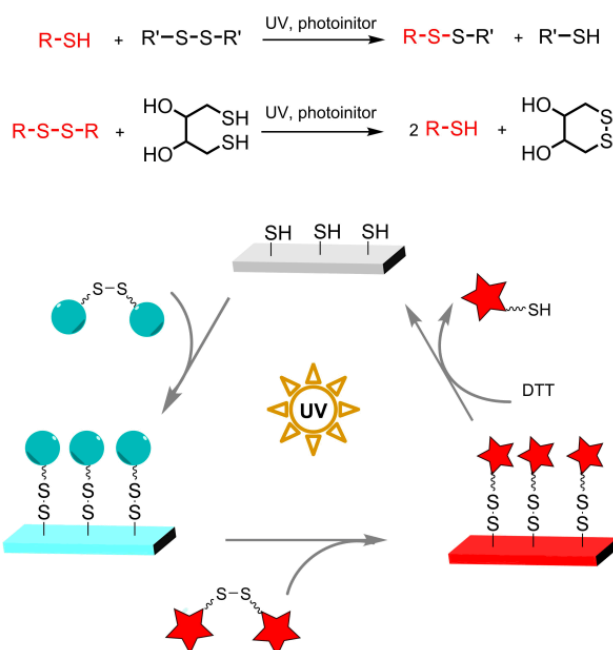
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**Abstract:** *Here we present the UV-induced disulfide formation (UV-DF) and disulfide reduction (UV-DR) reactions for surface functionalization and dynamic photopatterning. Both photochemical reactions allow for the spatially- and temporally-controlled, reversible transition between thiol-functionalized and disulfide-functionalized surfaces. The dynamic photopatterning strategy was demonstrated by the UV-induced attachment, exchange and detachment on thiol-modified substrates.*

Spatially resolved functionalization of surfaces is important in a variety of research fields ranging from microfluidics<sup>[1]</sup> and electronics<sup>[2]</sup> to biotechnology.<sup>[3]</sup> In this context, photochemical reactions attracted attention, because they offer both spatial and temporal control of surface modification.<sup>[4]</sup> To date, a variety of light-triggered reactions, for example, thiol–ene<sup>[5]</sup>/yne,<sup>[6]</sup> photoinitiated azide-yne,<sup>[7]</sup> tetrazole-ene,<sup>[8]</sup> tetrazole-thiol,<sup>[9]</sup> and phototriggered Diels–Alder reactions,<sup>[10]</sup> have been exploited for surface functionalization. However, the majority of the existing methods are restricted to irreversible surface functionalization because of the formation of relatively non-reactive covalent bonds. In contrast, reversible photochemical modifications provide more diverse possibilities for surface manipulation. These can be applied for example, for dynamic tuning of interfacial properties, stimuli-responsive activation and deactivation of specific functions, or reversible attachment/detachment of functional moieties. To the best of our knowledge, only three examples of reversible phototriggered surface functionalization methods based on thiol–quinone methide reaction,<sup>[11]</sup> addition fragmentation chain transfer reaction of allyl sulfides with thiols,<sup>[12]</sup> and photodynamic disulfide exchange reaction (PDDE),<sup>[13]</sup> have been reported. Here we demonstrate two photo-induced reactions allowing for the spatially and temporally controlled thiol-disulfide interconversions, namely UV-induced disulfide formation (UV-DF) and disulfide reduction (UV-DR) reactions. These reactions are used to demonstrate reversible UV-induced surface modification, patterning, attachment as well as detachment of functional molecules (Scheme 1).

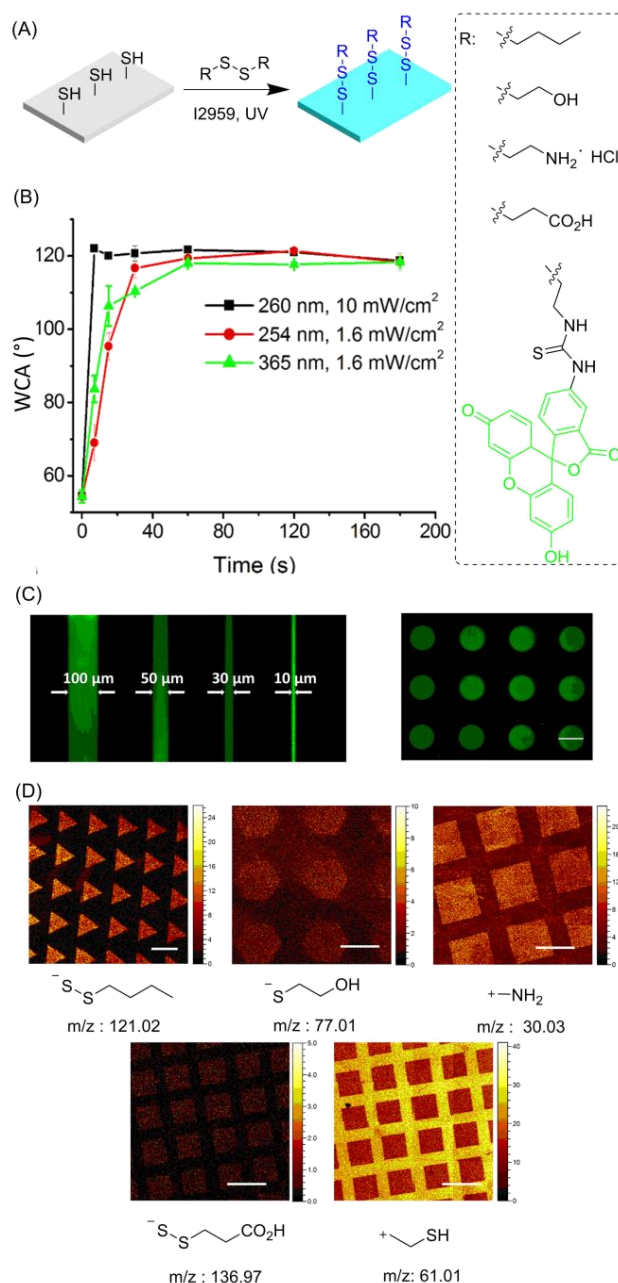
Thiol-disulfide interconversion is well-documented to proceed via the thiolate-centered mechanism;<sup>[14]</sup> however, it lacks the spatial control required for surface patterning. Recently,

several works have demonstrated that disulfide exchange reactions can be performed by photoirradiation.<sup>[13, 15]</sup> Inspired by this process, we envisioned that thiol-disulfide exchange reactions could also be initiated by light via the thiyl-centered mechanism. Apart from one example<sup>[16]</sup> of using this strategy as a gelation reaction, the potential of the UV-DF and UV-DR for surface modification, to the best of our knowledge, has not been explored.



**Scheme 1.** Schemes of the UV-DF and UV-DR reactions (above). Reversible attachment, exchange and detachment of disulfide-modified substrates via the dynamic UV-DF, UV-DR and PDDE photoreactions.

First we explored the UV-DF for functionalizing a thiol-modified surface (Figure 1A). A porous polymeric film was used as substrate to facilitate the monitoring of surface modification using static water contact angle (WCA) and fluorescence measurements. It was prepared via the esterification of a porous poly(hydroxyethyl methacrylate-co-ethylene dimethacrylate) film<sup>[17]</sup> with bis(2-carboxyethyl) disulfide (CDS) with subsequent reduction of the disulfides by 1,4-dithiothreitol (DTT) catalyzed by triethyl amine (Scheme S1). The thiol content on the surface, determined spectrophotometrically by titration with 2,2'-dipyridyl disulfide according to the literature procedure,<sup>[18]</sup> was  $1.94 \times 10^{-9}$  mol/mm<sup>2</sup>. The obtained film was hydrophilic with a static WCA of  $54 \pm 2^\circ$ .



**Figure 1.** (A) Schematic representation of the UV-DF reaction on thiol-modified surfaces. (B) Surface modification process of the UV-DF reaction in DMF. Static WCA on a thiol-functionalized surface after the UV-DF reaction with dibutyl disulfide as a function of irradiation time using different UV sources. (C) Fluorescence microscope images of a thiol-functionalized surface patterned with FITC-disulfide through different photomasks. Scale bar: 500 μm. (D) From left to right, ToF-SIMS images of a thiol-modified surface patterned with dibutyl disulfide, bis(2-hydroxyethyl) disulfide, cysteamine dihydrochloride, and below bis(2-carboxyethyl) disulfide, respectively. The last image shows a ToF-SIMS image of intact thiol groups after patterning with bis(2-carboxyethyl) disulfide.

To test the UV-DF reaction, dibutyl disulfide (BDS) was used as the model compound. The thiol-functionalized film was wetted with a solution of BDS (20 wt%) and the commercial photoinitiator I2959 (1 wt%) in DMF. After 2 min of UV irradiation (260 nm, 10 mW/cm<sup>2</sup>), the static WCA increased from  $54 \pm 2^\circ$  to  $118 \pm 2^\circ$ , demonstrating the successful immobilization of hydrophobic butyl groups. The conversion degree of thiol groups was determined to be 80 % (Figure S11 and Table S2). To follow the surface modification process, we measured the static

WCA of the surface exposed to different irradiation times. As shown in Figure 1B, the static WCA increased to  $118 \pm 2^\circ$  after only 7 s irradiation and remained stable afterwards, demonstrating the fast reaction kinetics, being important for creating patterns with high contrast and resolution. Figure S13 shows that the photoinitiator is necessary for the rapid kinetics. The UV-DF modifications are also efficient under UV irradiations both with lower intensity (254 nm,  $1.6 \text{ mW/cm}^2$ ) and at longer wavelength (365 nm,  $1.6 \text{ mW/cm}^2$ ), showing promise for patterning UV-sensitive biomolecules.

This photochemical reaction allows for a spatially controlled disulfide attachment on thiol-modified surfaces. Figure 1C demonstrates sharp fluorescent patterns with features as small as  $10 \mu\text{m}$  produced by photopatterning of FITC-disulfide on a thiol-functionalized surface. Considering the roughness and thickness ( $12.5 \mu\text{m}$ ) of the used polymeric surface, this is a remarkable result attributed to the fast kinetics of the UV-DF reaction. Wettability gradient on the thiol-functionalized surface could also be prepared after modification with BDS using a moving photomask (Figure S15).

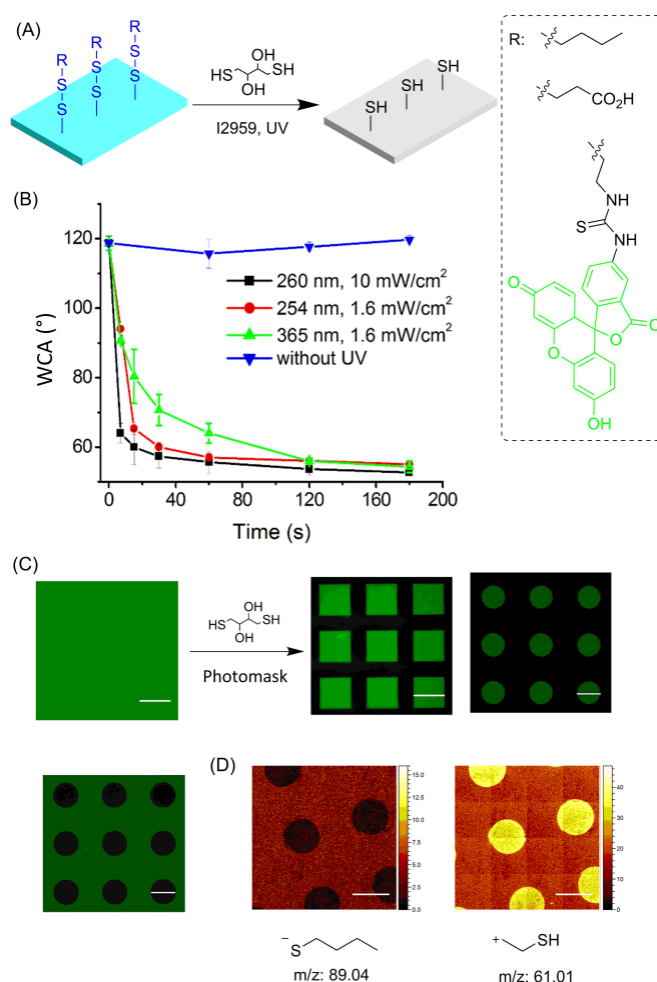
The chemical compositions of the surfaces were characterized with the time-of-flight secondary ion mass spectrometry (ToF-SIMS). As for the thiol-derivatized film patterned with BDS, Figure 1D shows the pattern of butyl moieties with a good resolution. We further evaluated this photopatterning with bis(2-hydroxyethyl) disulfide (HDS), cystamine dihydrochloride (ADS) and CDS. The high-contrast patterns in Figure 1D demonstrate the good tolerance of this reaction towards hydroxyl, amino, and carboxyl groups.

Next, we investigated the possibility of UV-induced disulfide reduction to cleave off previously grafted groups. It is well documented that disulfide bonds can be reduced by DTT via the thiolate-centered mechanism.<sup>[14, 19]</sup> Under UV light, the disulfide reduction will proceed via two processes, the thiolate-centered one and thiyl-centered one. To compare the rates of these two processes, we performed the model reaction of HDS (11 mM) with DTT (22 mM) in different solvents with or without the presence of a photoinitiator (I2959, 2.2 mM) and UV irradiation (254 nm,  $1.6 \text{ mW/cm}^2$ ). Figure S1–S4 and Table S1 show the  $^1\text{H}$  NMR results under different conditions. In  $\text{DMSO-d}_6$ , the conversion of HDS under UV is 3.4 times faster than without UV; in  $\text{CDCl}_3$  and  $\text{D}_2\text{O-DCl}$  (0.72 wt% DCl), the reduction reaction only occurred under UV irradiation. These results imply that this reaction is being dominated by the thiyl-centered process in less-polar solvents and/or acidic solutions.

These primary results prompted us to explore this UV-DR reaction on the surface. We tested the reduction of CED-modified films and determined spectrophotometrically the conversion of disulfides. The results are summarized in Table S2. In DMF, the conversion of disulfide was 34% within 2 min of UV (260 nm,  $10 \text{ mW/cm}^2$ ), almost ten times higher than that without UV.

In  $\text{CHCl}_3$ , 95% of disulfides have been reduced after 5 min of UV while the conversion is only 4.3% without UV. This high rate difference is important for achieving temporal and spatial control for surface modification. We further characterized this UV-DR reaction on the hydrophobic DBS-modified surface using contact angle measurements. As shown in Figure 2B, the static WCA decreased from  $118 \pm 2^\circ$  to  $54 \pm 2^\circ$  corresponding to the original thiol-modified surface after 2 min of UV irradiation (260 nm,  $10 \text{ mW/cm}^2$ ). This indicates that hydrophobic butyl groups have been removed by the UV-DR reaction. In contrast, no change of the static WCA is detected after 2 min without UV irradiation, which is consistent with the observed slow kinetics of this reaction in organic solvents without any catalyst (Table S1).

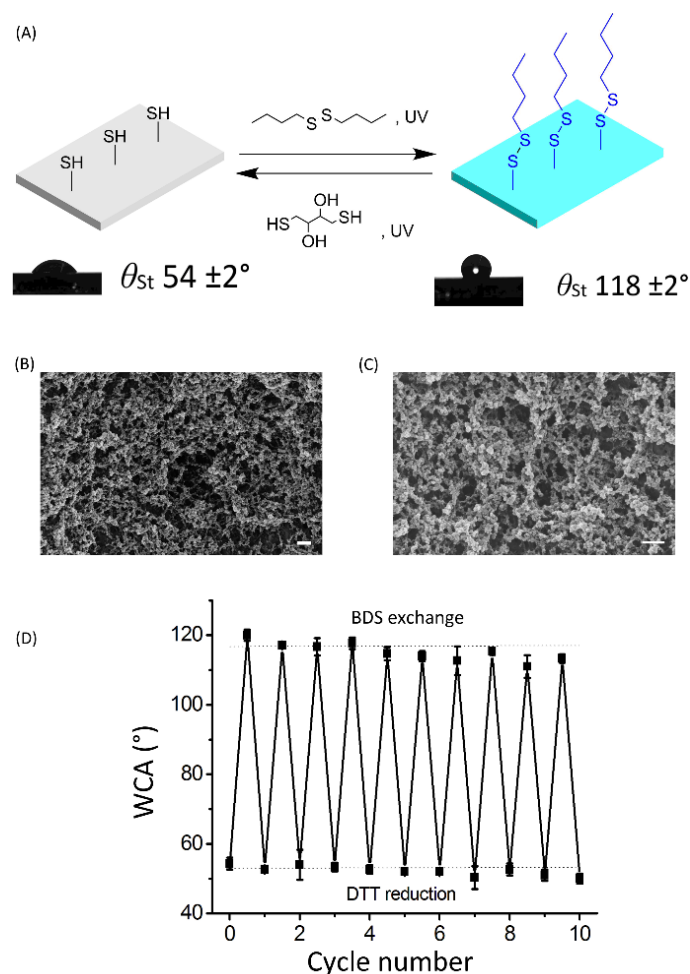
Further study of the surface modification process shows that the UV-DR reaction completes in less than 2 min and also proceeds effectively under 260 nm light ( $1.6 \text{ mW/cm}^2$ ) and 365 nm light ( $1.6 \text{ mW/cm}^2$ ) (Figure 2B).



**Figure 2.** (A) Schematic representation of the UV-DR reaction on disulfide-modified surfaces. (B) Surface modification process of the UV-DR reaction in DMF. The graph shows the static WCA on BDS-modified surface after the UV-DR reaction as a function of irradiation time under different UV sources. (C) Fluorescence microscope image of (FITC-disulfide)-modified surfaces before and after patterning via the UV-DR reaction through different photomasks. (D) ToF-SIMS images of (dibutyl disulfide)-modified surface patterned via the UV-DR reaction (scale bars 500  $\mu\text{m}$ ).

To demonstrate the possibility for photopatterning using the UV-DR, the (FITC-disulfide)-functionalized film was subjected to UV irradiation (260 nm, 10 mW/cm<sup>2</sup>) through a photomask in the presence of a solution of DTT (20 wt%) and I2959 (1 wt%) in CHCl<sub>3</sub>. Patterns with various shapes could be easily prepared by using different photomasks (Figure 2C). The photopatterning was also confirmed by the ToF-SIMS results of a BDS-functionalized film irradiated with UV through a photomask in the presence of DTT in CHCl<sub>3</sub>. Both the high-contrast pattern and the appearance of thiol groups support the spatially-controlled reduction of disulfides into thiols (Figure 2D). This strategy also enabled the fabrication of wettability gradient on the surface as shown in Figure S16.

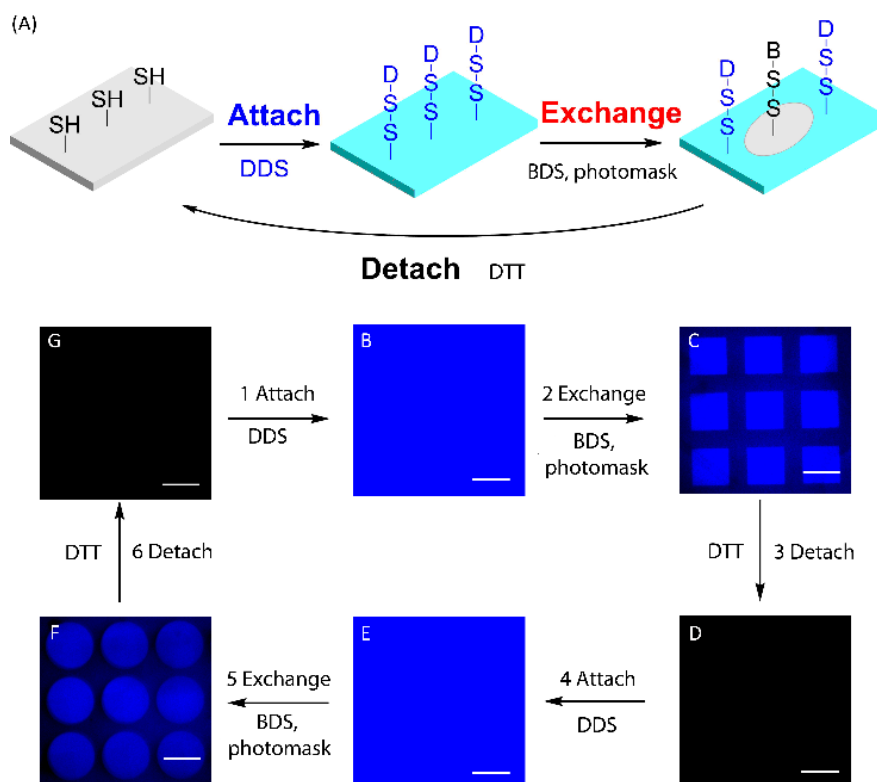
These two photochemical reactions, UV-DF and UV-DR, allow for the reversible transition between thiol-functionalized surfaces and disulfide-functionalized surfaces. To evaluate this reversibility, we sequentially modified a thiol-functionalized film via the UV-DF reaction with BDS, followed by the UV-DR reaction with DTT (Figure 3A). After exchange with BDS, this hydrophilic surface becomes hydrophobic with static WCA increasing from 54 ±2° to 118 ±2°; subsequent UV-DR reaction restores the original hydrophilic surface with the static WCA of 54 ±2°. The transformation between hydrophilic and hydrophobic surfaces is very quick as every step needs only 2 min of UV irradiation. Further SEM measurements reveal no change of surface topography after the UV-DR reaction (Figure 3B) and the UV-DF reaction (Figure 3C). This process can be repeated for at least ten cycles, demonstrating good reversibility (Figure 3D). The slight decrease of static WCA for both surfaces possibly results from oxidation of thiol groups or disulfide groups over time and under UV light. After ten cycles, the thiol concentration on this surface was determined to be 1.64×10<sup>-9</sup>mol/mm<sup>2</sup>, indicating that 85% of thiols remained intact.



**Figure 3.** Reversible surface modification based on the UV-DF reaction and the UV-DR reaction. (A) Schematic representation of the change of surface wettability via the UV-DF reaction with dibutyl disulfide and the UV-DR reaction in DMF. SEM images showing the porous structures of thiol-modified surface (B) and (dibutyl disulfide)-modified surface (C). Scale bar: 300 nm. (D) The reversible process was repeated ten times and the static WCA of the surface was measured after each modification.

Reversible UV-induced disulfide formation and reduction offer unique opportunities for dynamic photopatterning. In addition, as the formed disulfide-modified surface is photodynamic itself, it opens the possibility for the photodynamic disulfide exchange using other disulfides.<sup>[13]</sup> Therefore, we can use light to direct the attachment, exchange and detachment of moieties immobilized via the disulfide bond with spatial and temporal control (Scheme 1). This dynamic photopatterning has been demonstrated with dansyl disulfide (DDS) (Figure 4A). By the UV-DF, DDS was attached onto a thiol-functionalized film, producing the dansyl fluorescence image in Figure 4B. The PDDE reaction permits the exchange between immobilized DDS and the nonfluorescent BDS with spatial control, demonstrated by the disappearance of dansyl fluorescence in the exposed regions (Figure 4C). Finally, the detachment process was confirmed by the disappearance of dansyl pattern after the UV-DR reaction (Figure 4D). This strategy possesses the 'living' nature for surface photopatterning, allowing multiple and reversible manipulation of surface properties using light. This possibility has been

demonstrated by the second attachment, exchange and detachment of DDS on the regenerated thiol-modified surface (Figure 4E-4G).



**Figure 4.** Dynamic photopatterning allows reversible attachment, exchange and detachment of disulfide-bonded substrates on the surface. (A) Schematic representation of UV-induced attachment of dansyl disulfide (DDS), exchange between DDS and dibutyl disulfide (BDS) using a photomask, and detachment of disulfide molecules. Respective fluorescence microscope images of a thiol-modified surface after sequential treatments: flood irradiation in the presence of DDS (B), masked irradiation with BDS (C), flood irradiation with DTT (D), flood irradiation with DDS (E), masked irradiation with BDS (F), and flood irradiation with DTT (G). Scale bar: 500 μm.

This study demonstrates the UV-induced disulfide formation (UV-DF) and disulfide reduction (UV-DR) reactions for surface functionalization and dynamic photopatterning, which permits the light-induced attachment, exchange and detachment of functional groups on the surface. In this work, UV light was adapted to prove these possibilities; in fact, visual light and even NIR light are also possible to trigger these reactions via the rational selection of photoinitiators. We foresee that this dynamic photopatterning strategy will provide a robust tool for both the development of stimulus-responsive surfaces and the precise-manipulation of interfacial properties. Furthermore, this photodynamic thiol-disulfide exchanging process will also provide an excellent opportunity for the design of new smart materials.

**Keywords:** disulfide formation • disulfide reduction • dynamic photopatterning • photochemistry



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