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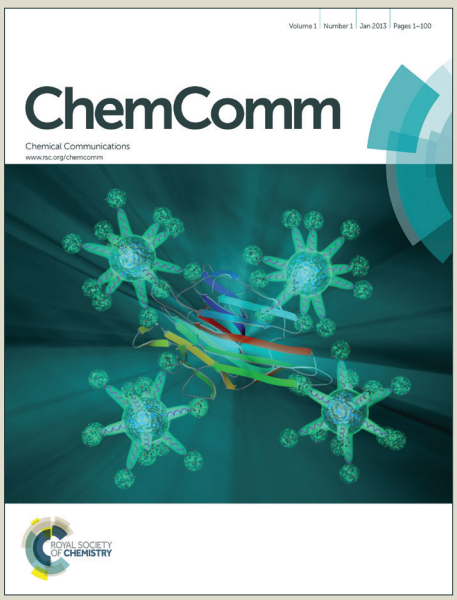

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ARTICLE TYPE

Photoinduced Heterodisulfide Metathesis for Reagent-free Synthesis of Polymer Nanoparticles

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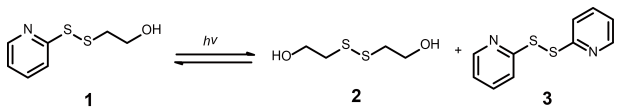
Reagent-free synthetic methods are of great interest, because of their simplicity and implications in green chemistry. We have taken advantage of photoinduced heterodisulfide metathesis to generate crosslinked polymer nanoparticles. The method development and the mechanistic basis for the synthetic approach are outlined in this manuscript.

Metathesis reactions have found utility in many synthetic and supramolecular chemistry strategies. For example, diene metathesis has found use in the syntheses of polymers and small molecules,[1] while strategies such as imine[2] and hydrazone[3] metathesis reactions have found use in supramolecular chemistry and dynamic combinatorial chemistry, among others. All these reactions are reversible under the reaction conditions and this provides the opportunity to obtain the thermodynamically favorable structure, under a given set of conditions. For example, the host identification process for a guest molecule in dynamic combinatorial chemistry reaction is driven by the templation by the guest molecule to select for a specific host structure among the myriad possibilities. Disulfide metathesis reactions have also been used in this context both by Mother Nature for stabilizing the desired protein secondary and tertiary structures[4] and for identifying optimal host geometries in supramolecular chemistry.[5] More recently, photolabile nature of disulfides have been exploited to produce bulk hydrogels from oligomeric disulfide molecules and polymeric disulfides.[5c] Although this has provided very interesting materials in their own respect, this observation also indicates a rather uncontrolled nature of photo-induced disulfide metathesis reactions. We sought to develop a photo-induced disulfide metathesis reaction that can provide greater control such that we can achieve well-defined, crosslinked polymeric nanostructures and we outline our findings in this manuscript.

Photo-chemically driven reactions are interesting, because these are reagentless reactions. Photoreactions based on the dimerization of thymine[6] and coumarin[7] or that of benzophenone moieties[8] have been used quite extensively. The dimerization reactions are reverted by a higher energy photo-chemical irradiation, while the reactive radicals generated from benzophenone often provides irreversible products. The photo-induced disulfide formation reaction has the potential of being generated by a photochemical reaction, but being reverted by a biologically relevant stimulus, the redox potential of the intracellular environment.[9]

Photochemical reactivity of disulfides has been known for several decades.[10] Disulfides are thought to undergo hemolytic cleavage, giving thiol radicals, which can attack nearby disulfide bonds, resulting in disulfide exchange under photoirradiation.[5c, 10b, 10e] We were interested in exploring this photo-induced metathesis of disulfide molecules containing pyridyl disulfide (PDS) units. PDS units are well known for their unreactive byproduct, pyridothione, during disulfide exchange reactions, which provides the opportunity for reliably generating unsymmetrical disulfides.[11] However, it is not clear whether such a possibility exists in a photochemical disulfide metathesis reaction. In fact, ultraviolet (uv) irradiation of 2,2'-dipyridyldisulfide (DPDS) results in the formation of pyridine-2-sulfonic acid, presumably due to oxidation (scheme S1).[12] This suggests that hydrogen abstraction by the thiyl radical is much slower than the oxidation reaction. Considering that pyridyl groups are relatively electron poor compared to the alkyl thiols, we were also concerned that the homolytic cleavage of the disulfide bond between an alkyl group and a pyridyl group will result in the formation of two sulfonic acids.



Scheme 1. Hypothesized reaction scheme of photo-induced disulfide metathesis of PDS-OH

To investigate the possibility, we first used photochemical reaction of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH, **1**) as a model system. A solution of 2.5 mg/mL of 1 in CD_3OD was irradiated with a UV lamp (15 W) with a 350 nm light source. If this reaction were to be a purely disulfide exchange reaction due to thiyl radical formation and then recombination, molecule **1** will be in equilibrium with bis(2-hydroxyethyl) disulfide (**2**) and DPDS (**3**). Ideally, a statistical ratio of 2:1:1 of the products **1-3** would be obtained (Scheme 1). However, the starting material **1** was being continuously consumed with a concurrent increase in the concentration of **2**. As shown in the ^1H NMR spectra over irradiation time (Fig. 1), the integrated signal intensity of the triplet at 2.94 ppm, arising from the CH_2 protons attached to sulfur in **1**, decreased with the corresponding increase in the intensity of the triplet at 2.83 ppm (the same CH_2 protons in **2**). Finally, the disappearance of peak at 2.94 ppm showed that no starting material **1** remained in the reaction mixture. Also, there is

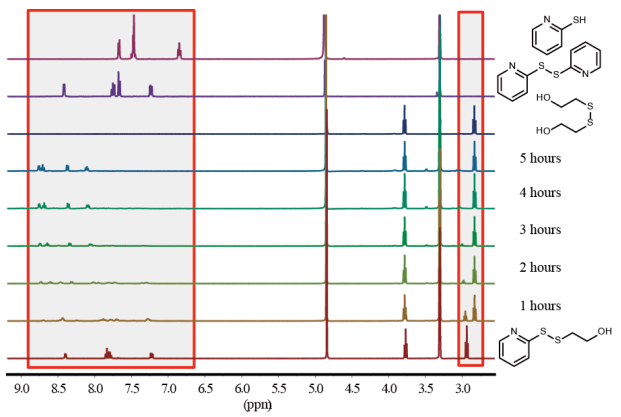
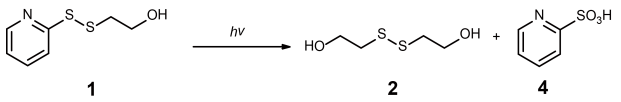


Fig. 1 Time-dependent 1H NMR spectra of the photo-induced reaction of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH, **1**). Control spectra of bis(2-hydroxyethyl) disulfide (DPDS, **2**), 2, 2'-dipyridyl disulfide (**3**) and 2-thiopyridone were also included. The concentration was 2.5 mg/mL. The final product after photoirradiation was determined to be pyridine-2-sulfonic acid (**4**).



Scheme 2. Scheme of photo-induced chemistry reaction of PDS-OH

no evidence of the formation of DPDS (**3**) in the reaction mixture. However, we did find that the formation of **2** was accompanied by the formation of pyridine-2-sulfonic acid (**4**) (Scheme 2). Finally, we found that the percent conversion in this reaction was lower at higher concentrations (Fig. S4). This is typical of photochemical reactions as the fluence of light was kept the same in all these reactions. Two features are readily discerned from these observations: (*i*) no equilibrium is observed in this photo-induced disulfide metathesis; (*ii*) while the pyridothiyl radical is readily oxidized, the alkylthiyl radical undergoes a radical recombination reaction to produce **2**. These initial observations

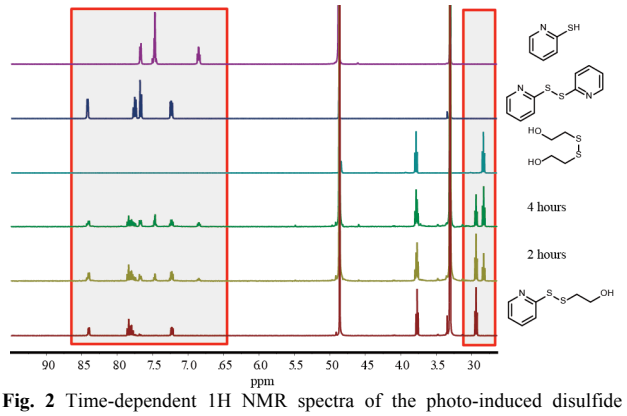
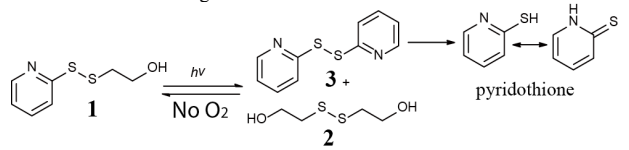


Fig. 2 Time-dependent 1H NMR spectra of the photo-induced disulfide metathesis of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH, **1**) under anaerobic condition. Control spectra of bis(2-hydroxyethyl) disulfide (**2**), 2, 2'-dipyridyl disulfide (DPDS, **3**) and 2-thiopyridone were also included. The concentration was 2.5 mg/mL.

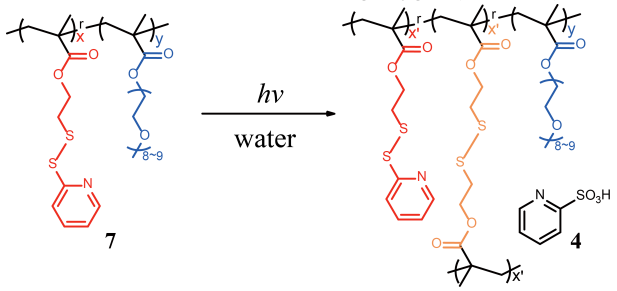


Scheme 3. Photo-induced reaction of PDS-OH under unaerobic condition.

are encouraging for the use of heterodisulfides containing PDS units for controlled polymer nanoparticle synthesis.

Prior to utilizing this in nanoparticle synthesis however, we were interested in investigating the reasons for the observed oxidation of pyridine thiyl radical. Presumably, this oxidation is caused by the oxygen in the reaction mixture. To check this hypothesis, we excluded oxygen from the reaction by performing three freeze-pump-thaw cycles. Analysis of the PDS-OH (**1**) photochemical reaction under these conditions shows that the product generation has become seemingly slower than the reaction where the oxygen was not rigorously removed (Fig. 2). Even after 4 hours of irradiation, there was still 50 % of starting material **1** left in the reaction, consistent with a reaction in equilibrium. Note that the starting material **1** was completely consumed in about 2 hours without oxygen exclusion. In addition to the expected DPDS (**3**) product, there are additional peaks in the aromatic region in this reaction mixture, as shown in Fig. 2. Analysis of this NMR indicates that the byproduct of this reaction is indeed due to hydrogen abstraction by the thiopyridyl radical to generate pyridothione (Scheme 3). In fact, we have shown that direct irradiation of DPDS (**3**), after the freeze-pump-thaw cycle, cleanly produces pyridothione rather than **4** (Fig. S6). Several observations are noteworthy here: (*i*) the thiyl radical from pyridine undergoes oxidation to pyridine-2-sulfonic acid (**4**) under ambient conditions; (*ii*) this reaction can be mitigated by rigorously excluding oxygen from the reaction mixture; (*iii*) in the absence of oxygen, the thiopyridyl radical undergoes both radical recombination (evidenced by the formation of DPDS from **1**) and hydrogen abstraction (evidenced by the formation of pyridothione from **1** and from DPDS irradiations).

Overall, photochemical irradiation of PDS-OH (**1**) results in the homolytic cleavage of the disulfide bond to generate a pyridyl and an aliphatic thiyl radical. The aliphatic radical undergoes rapid recombination to generate the corresponding disulfide. However, the stability provided by resonance in thiopyridyl radical seems to be sufficient to slow down radical recombination or hydrogen abstraction, but is not sufficient to inhibit oxidation to the corresponding sulfonic acid **4**. In the absence of oxidative pathway, this radical does ultimately undergo radical recombination and hydrogen abstraction. These latter processes also provide the opportunity for the photochemical reaction of **1** to be under equilibrium and thus slow down the conversion of starting material to the product. In other words, the oxidized byproduct serves to drive the equilibrium towards the right in the reaction of **1**. Therefore, in reactions where we need the thiopyridyl byproduct to be continuously removed, the reaction should be carried out without excluding oxygen (vide infra).



Scheme 4. Representation of photo-induced crosslinking reaction of random copolymers containing PDS groups.

Encouraged by these observations, we then envisaged the possibility of utilizing this methodology for preparing a crosslinked polymer nanoparticle. We wanted to test this for a well-characterized polymer and polymer nanoparticle so that the validation of this methodology will be robust. A 3:7 random copolymer of the oligoethyleneglycol methacrylate monomer (**5**) and the PDS-ethyl methacrylate monomer (**6**), shown as structure **7** in Scheme 4, has been shown to form micelle-like assemblies in water.[9c, 11b, 13] This assembly has been previously shown to be also crosslinked to form a polymer nanogel, triggered by the thiol exchange reaction with dithiothreitol (DTT).

A solution of polymer **7** (1 mg/mL) in water was found to aggregate to form an assembly of 9 nm in hydrodynamic diameter, as discerned by dynamic light scattering (Fig. 3a). This solution was subjected to irradiation in photochemical reaction chamber at 350 nm for 2 hours. At this time, the size of the assembly assessed by DLS again and the size of the assembly was found to be 13 nm, which is close to the size of the aggregate prior to crosslinking (Fig. 3b). This observation suggests that the disulfide metathesis occurs sufficiently fast such that the crosslinking reaction is mostly intra-aggregate. The slight size increase could be the result of a small amount of inter-aggregate crosslinking or the swelling that is likely to occur in a crosslinked nanoparticle, when the hydrophobic components are reduced. Alternately, this size increase could also be attributed to the adventitious heating that occurs during photochemical irradiation of the sample. This latter possibility is consistent with the size increase in PEG-based assembles at higher temperatures.

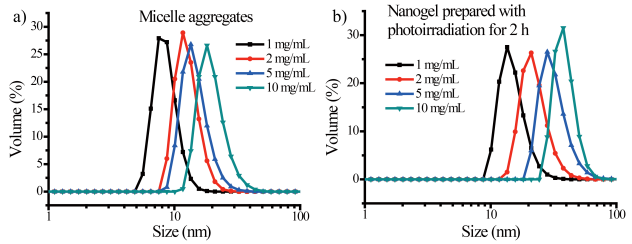


Fig. 3 DLS sizes in hydrodynamic diameter, a) micelle aggregates prepared with polymer solutions with different concentrations, b) nanogels prepared with polymer solutions with different concentrations, the photoirradiation time was 2 h for all samples.

If it is indeed due to temperature variations and not due to inter-aggregate reaction, we should be able to systematically tune the size of the nanoparticle by varying the concentration of the polymer. Indeed, when we varied the sample concentrations from 1 mg/mL to 10 mg/mL, the size of the micelle aggregates increased from 9 nm to 20 nm with the increasing concentration (Fig. 3a). Upon irradiation, the size of the nanogel only slightly increased even at 10 mg/mL concentration from 20 nm to 38 nm (Fig. 3b). If the observed size increase at 1 mg/mL were due to inter-aggregate crosslinking, when the concentration is increased by an order of magnitude the size of the nanoparticle should have substantially increased or even formed a bulk gel. Instead, the size has only slightly increased. This suggests that the observed size increase upon crosslinking is most likely due to temperature increase in solution, and the photo-induced crosslinking is predominantly intra-aggregate.

Next, we were interested in estimating the crosslink density in the nanoparticle. We utilized the residual PDS units present in the polymer nanoparticle to estimate this. According to our

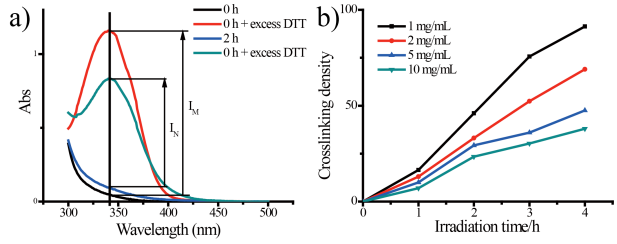


Fig. 4 a) Absorption spectra of pyridothione in UV-vis. Pyridothione, which is a byproduct by the reaction between PDS with DTT and shows characteristic absorption at 343 nm wavelength, is monitored in each nanogel (2 mg/mL) prepared. b) Time dependent crosslinking density of the nanogels with different concentrations of polymer solution, from 1 mg/mL to 10 mg/mL.

analysis using the small molecule model system above, the thiopyridyl radical gets converted to pyridine-2-sulfonic acid **4**. The unreacted PDS in the polymer can be treated with a thiol to reliably generate pyridothione, which can be readily monitored spectroscopically due to its distinct absorption at 343 nm.[11a] The concentration of pyridothione generated then gives us the amount of unreacted PDS in the mixture. As shown in Fig. 4a, there was very little difference between the absorption spectra before and after the photoirradiation. However, the addition of excess DTT into both samples results in significant difference between the absorbance of the peak at 343 nm due to the difference in extent of pyridothione generated. The crosslinking density was then calculated using ratio of the intensities. If I_N is the increased intensity at 343 nm of the irradiated sample by adding excess DTT, while I_M is that of the unirradiated sample, then the crosslinking density was calculated from I_N/I_M. Thus, the crosslinking density of the nanoparticle was found to be 33% after 2 hours photoirradiation, when the concentration of the polymer was 2 mg/mL. The crosslinking density could be systematically varied using different irradiation times, as shown in Fig. 4b. Interestingly, the crosslink density scales much slower with irradiation time at higher concentrations (Fig. 4b). This might seem counter-intuitive at first, because a reaction seems slower at higher concentration. This is indeed consistent with

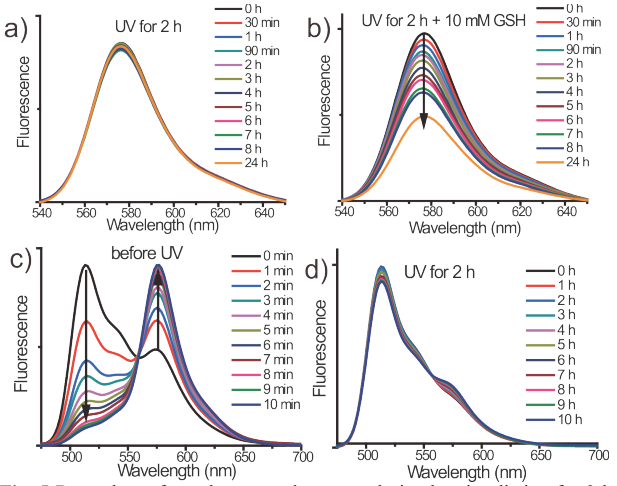


Fig. 5 Dye release from the nanogels prepared via photoirradiation for 2 h in response to varied GSH concentrations, a) 0 mM, b) 10 mM. Sample concentration was 0.1 mg/mL. FRET experiment based DiI/DiO exchange, c) micelle aggregates, d) nanogels prepared via photoirradiation for 2 h. Sample concentration was 0.1 mg/mL.

photochemical reactions between small molecules (Fig. S4), because we are measuring the percent conversion of starting material and the reagent here is light, which is constant at all different concentrations.

To test if the difference in the extent of PDS generation is indeed due to crosslinking, we incorporated hydrophobic guest molecules inside these nanogels prior to the photoirradiation. With the dye molecule, DiI, incorporated into the nanoparticle, the guest molecule was found to be stably encapsulated in the nanoparticle over a 24 hours time period, even after a 10-fold dilution (Fig. 5a). However, when glutathione (GSH) was added to this solution at 10 mM concentration, the guest molecule seems to be releasing over time (Fig. 5b).

To further confirm that the encapsulation stability could be easily tuned by simply varying the irradiation time, fluorescence resonance energy transfer (FRET) based encapsulation stability assay was carried out.[14] In this experiment, a faster FRET evolution suggests leaky nanoparticle, while an unchanged FRET over time suggests stably encapsulated guests inside the nanoparticle. Indeed, we observed that there was a rapid evolution of FRET with time in the micelle aggregates without any photoirradiation (Fig. 5c), compared to the nanoparticle prepared using a 1 hour irradiation (Fig. S8). The evolution of FRET was even slower after 2 hours of irradiation (Fig. 5d), suggesting that the encapsulation stability can indeed be tuned by varying the irradiation time.

Conclusions

In summary, we have demonstrated that: (*i*) PDS-containing asymmetrically substituted disulfides can be homolytically cleaved using photochemical irradiation; (*ii*) although the alkyl substituted thiyl radical consistently undergoes radical recombination, the reactivity of the thiopyridyl radical is dependent on the presence of oxygen; (*iii*) in the presence of oxygen, the thiopyridyl radical gets converted to pyridine-2-sulfonic acid, while it undergoes hydrogen abstraction or radical recombination in deoxygenated solutions; (*iv*) the oxidation reaction also provides a pathway for driving the equilibrium towards the desired product; (*v*) this feature was used for the reagentless synthesis of crosslinked polymer nanoparticles; (*vi*) the size of the nanoparticle can be conveniently varied by tuning the size of the nanoaggregates, because the photo-induced crosslinking was predominantly intra-aggregate; and (*vii*) the crosslink density and encapsulation stability of this nanoparticle can be simply varied by altering the irradiation time. In addition to the typical advantages of a reagentless reaction, the fact that this reaction is carried out in water and that the byproduct of this reaction is highly water-soluble sulfonic acid that can be easily removed by dialysis suggest that this methodology offers a promising new approach for nanoparticle synthesis. Since the crosslinks generated here are biologically relevant, this method also could have implications in generating new materials of interest in biology and medicine.

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Notes and references

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† Electronic Supplementary Information (ESI) available: See the Supporting Information for the synthesis and characterization of PDS-OH, random copolymer and nanoparticles. See DOI: 10.1039/b000000x/

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Entry for the Table of Contents