

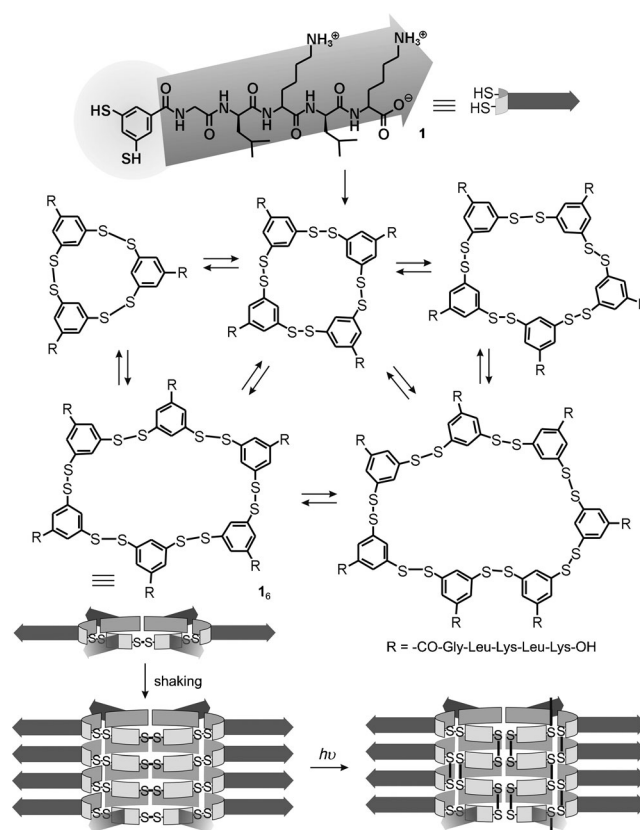
Dynamic Combinatorial Chemistry

Hydrogel Formation upon Photoinduced Covalent Capture of Macrocyclic Stacks from Dynamic Combinatorial Libraries**

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Dynamic combinatorial chemistry^[1] was originally conceived as a method for developing synthetic receptors and ligands for biomolecules. Dynamic combinatorial libraries (DCLs) are produced by linking building blocks together using a reversible reaction, resulting in a thermodynamically controlled product distribution. Addition of a guest or a biomolecule shifts the distribution to those library members that bind best to the external target; a process referred to as templating. Recently, the first examples have appeared of DCLs in which molecular recognition does not involve an externally added template, but takes place between library members.^[2] Such self-templated DCLs constitute a promising approach for the development of new self-assembling materials. Self-assembly provides the driving force to shift the equilibrium in favor of the very molecules that self-assemble, so that these materials are in effect self-synthesizing. We now report that a self-assembled material produced by dynamic combinatorial chemistry can be further stabilized by rearranging the dynamic covalent disulfide bonds that were underlying the dynamic combinatorial process. We show how photoinitiated disulfide exchange converts fibrous stacks of macrocycles into polymeric products, enhancing the stability of the fibers and causing gelation of the aqueous solution.

We recently reported how hexameric disulfide macrocycles emerge upon shaking a DCL made from dithiol **1** (Scheme 1).^[2e] This building block is equipped with a short peptide sequence, predisposed to β -sheet formation by alternating hydrophobic and hydrophilic amino acid residues. While the peptide is too short to self-assemble on its own, the DCL made upon oxidizing dithiol **1** in aqueous solution contains a number of macrocycles of different ring sizes that display a different number of peptides. We reasoned that self-assembly becomes feasible for a critical size of the macrocycle that displays a sufficient number of peptides. Indeed, for a DCL made from **1** that is agitated by shaking, self-assembly of the hexameric macrocycles (**1₆**) occurs, resulting in the formation of fibers and shifting the product distribution in favor of the cyclic hexamer. The solution remains free flowing



Scheme 1. Shaking a dynamic combinatorial library made from dithiol building block **1** in aqueous borate buffer (50 mM, pH 8.1) gives rise to stacks of disulfide macrocycles **1₆**, which are covalently captured upon photoirradiation (365 nm) to produce polymers/oligomers of **1**.

as the fibers are fragile and break when subjected to moderate shear forces.^[2e]

We have discovered that photoirradiation (three days, using an 8 W UV lamp, 365 nm) of a nonagitated solution containing hexamer fibers ($[\mathbf{1}_6]$ is around 0.6 mM) results in the formation of a hydrogel^[3] (Figure 1a). Oscillatory rheology^[4] experiments showed that a relatively rigid gel is formed with a ratio between storage (G') and loss (G'') moduli of 12 at low oscillatory frequencies (see Figure S1 in the Supporting Information).

Photoirradiation of disulfides can induce their homolytic cleavage^[5] giving thiol radicals that can attack nearby disulfide bonds, resulting in disulfide exchange.^[6] This underutilized radical-mediated exchange mechanism is different from the ionic (thiolate-anion-mediated) mechanism^[7] that is typically used in dynamic covalent disulfide chemistry. We believe that this photoinduced disulfide exchange causes a

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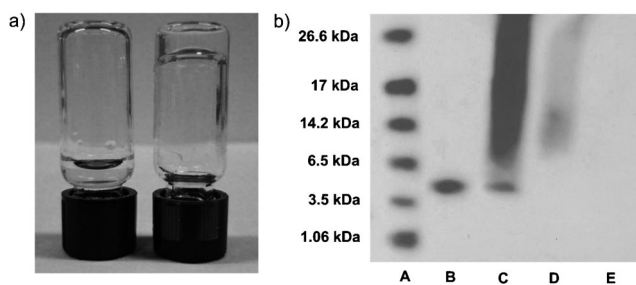


Figure 1. a) Photograph of samples containing predominantly $\mathbf{1}_6$ prior to (left) and after (right) three days of photoirradiation using an 8 W UV lamp. b) SDS-PAGE analysis of a sample of $\mathbf{1}_6$ prior to photoirradiation (lane B) and after 1 day (lane C), 2 days (lane D) and 3 days (lane E) of irradiation. Lane A shows the standard molecular weight ladder.

rearrangement of the disulfide bonds within the fibers, as shown in Scheme 1, without altering the global architecture of the fibers.^[8]

We analyzed the composition of the solution of fibrous $\mathbf{1}_6$ by HPLC/MS (see Figures S2–S3 in the Supporting Information) during photoirradiation and observed that the peak associated with $\mathbf{1}_6$ decreased gradually until, after three days, it was no longer detectable. As we were unable to detect by HPLC most of the compounds that were produced upon irradiation, we switched to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Figure 1b shows that, in the course of irradiation, species of large molecular weight are produced which subsequently also decrease in concentration (lanes C–E). After three days of irradiation we fail to detect any peptide material by SDS-PAGE (lane E), suggesting that compounds of molecular weight (MW) in excess of 30000 (42 units of $\mathbf{1}$) are present. These results suggest that photoirradiation induces the conversion of $\mathbf{1}_6$ (MW 4343.68) into polymers of $\mathbf{1}$ (Scheme 1). Attempts to analyze the length distribution of these polymers by gel permeation chromatography failed because of the gelatinous consistence of the samples. Analysis by MALDI-TOF mass spectrometry was hampered by extensive fragmentation of the disulfide linkages (see Figure S4 in the Supporting Information).

While the internal molecular structure of the fibers changed dramatically, their global appearance and organization into β -sheets were unaffected. Figure 2 shows cryo-TEM micrographs of the fibers of $\mathbf{1}_6$ prior to photoirradiation and the same sample after three days of irradiation. The appearances of the two samples are remarkably similar, despite one being a gel and the other a free-flowing solution.

We analyzed the effect of photoirradiation on a dilute sample of $\mathbf{1}_6$ by circular dichroism (CD), but could not detect any change in the CD spectrum. Both samples showed the typical signature of β -sheet assembly (see Figure S5 in the Supporting Information). We also performed thioflavin T fluorescence assays,^[9] which indicated that the amyloid-like β -sheet interactions that were present prior to photoirradiation persist (see Figure S6 in the Supporting Information).

To confirm that photoirradiation acted only on the disulfide bonds we have reduced the gel sample obtained

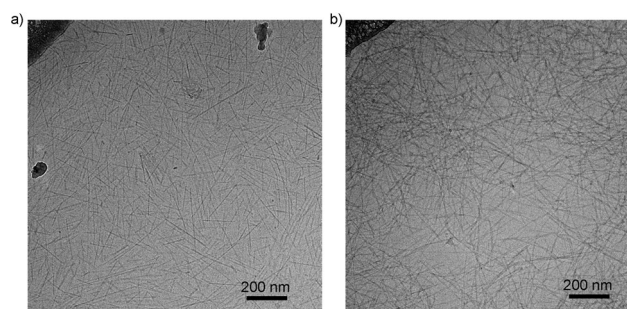


Figure 2. Cryo-TEM images of a sample containing predominantly $\mathbf{1}_6$ a) prior to photoirradiation and b) after three days of photoirradiation using an 8 W UV lamp.

after three days of photoirradiation by adding 10 equivalents of dithiothreitol, resulting in the quantitative recovery of building block $\mathbf{1}$ (see Figure S7 in the Supporting Information which shows the HPLC analysis). This experiment also demonstrates that the peptide-derived hydrogels are not only photoresponsive but also redoxresponsive.^[10,11]

The organization of the peptides into β -sheets prior to photoirradiation is essential for gel formation as apparent from the following control experiment: We irradiated a sample consisting of mainly trimer and tetramer macrocycles, that do not self-assemble,^[2c] and did not observe any gelation, nor any evidence for fibers in the cryo-TEM analyses, nor any evidence for β -sheet formation in the CD analysis (see Figure S8 in the Supporting Information). Yet disulfide polymerization did occur as evident from HPLC and SDS-PAGE analyses (see Figures S9 and S10 in the Supporting Information).

In conclusion, our results indicate that dynamic covalent disulfide linkages are not only instrumental in dynamic combinatorial discovery of self-assembling materials, but can also be used for further stabilization of the resulting self-assembled structures in a process akin to covalent capture.^[12] This strategy adds an element of kinetic control to the assembly process, thereby expanding the variety of structures that can be accessed using reversible covalent bond formation beyond those that represent directly accessible thermodynamic minima. The self-assembly by macrocyclization followed by a ring-opening polymerization route provides access to polymers with well-defined folded structures.

Experimental Section

Building block $\mathbf{1}$ was synthesized as reported previously.^[2c] The libraries were prepared by dissolving building block $\mathbf{1}$ in borate buffer (50 mM, pH 8.1) to obtain a 3.8 mM solution. The pH of the solution was adjusted to 8.1 by addition of 1.0 M KOH solution. The final volume of each library was 500 μ L. Shaken samples were placed in an Eppendorf Thermomixer Comfort (orbital shaker) and shaken at 1200 rpm with an orbital radius of 1.5 mm. Standing samples were placed in a dark environment giving a mixture of $\mathbf{1}_3$ and $\mathbf{1}_4$. All library experiments were carried out at ambient temperature. For the HPLC-MS, MALDI-TOF, and SDS-PAGE experiments, the concentration of the samples was 3.8 mM (with respect to the building block). For CD and fluorescent analysis, we used 300-times-diluted samples (with

respect to the building block). The samples were irradiated using a TLC desk lamp (8 W, 365 nm, 220 V) at a distance of 3 cm.

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