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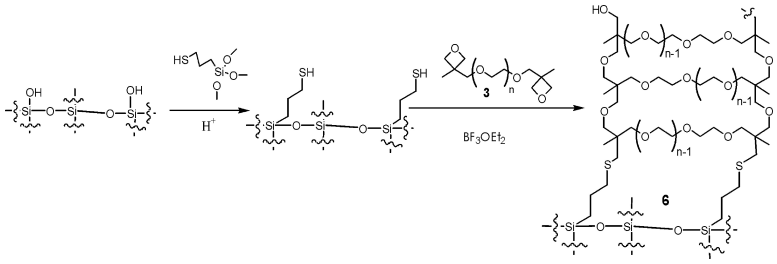

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Chemically Stable Films for Combinatorial Fluorosensor Arrays

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Sensor arrays are useful for many purposes. Our interests include quasidistributed intrinsic fiber optic arrays, those distributed along the length of an optical fiber. We have demonstrated an optical time-of-flight approach to distinguishing the fluorescence output of such arrays, as well as a synthesis of combinatorial libraries that takes advantage of a support of linear morphology to make numerous compounds in a simple manner without information loss in the synthesis. To unite these research areas, we needed an optical fiber cladding material that meets demanding synthetic and optical requirements. We have chosen the Meldal SPOCC polymer support as the best candidate for such a material and report here our initial results with this material.

Introduction

The Meldal SPOCC polymer support is made from poly(ethylene glycol) (PEG), a stable polymer distinguished by low toxicity and low protein adsorption.[1−3] Many PEG-based polymers are known, but the SPOCC polymer is distinct in that only 1° aliphatic ether linkages are present, and carbonyl and aromatic chromophores are absent. These structural attributes ensure polymer stability to most organic synthetic procedures and transparency compatible with many analytical measurements. In addition, polarity is appropriate for organic synthesis as well as aqueous assays. Meldal has demonstrated bulk[4−6] polymerization, and bead[7] preparation. Here, we describe synthesis of films of this polymer and demonstrate that it is appropriate for fiber-optic fluorosensor preparation.

Optical fibers provide an advantageous support for arrays of fluorescent chemosensor molecules. They allow optical spectroscopy to be performed on samples that are traditionally not accessible to spectroscopy, including those separated by long distances. Very active areas in research are clinical and environmental applications of indicator-based sensors.[8−19] Especially promising is fiber-based evanescent wave spectroscopy, which allows delivery of light to a very well-defined interface without dispersion throughout the sample. Light propagates along the fiber, being absorbed by chromophores only within a very small distance of the core-cladding boundary.[20−22] If the chromophore subsequently emits light, it may be coupled back into guided fiber modes.

Combinatorial Libraries as Sensor Arrays. We have recently reported combinatorial synthesis on a linear solid support so as to achieve fully parallel synthesis with full library spatial identification.[23] The library is spatially encoded: each compound is uniquely identified by its position along the solid support, and the synthesis fully parallel: all reactions for a given step are simultaneous, and each reagent used in a given step need be handled only once.

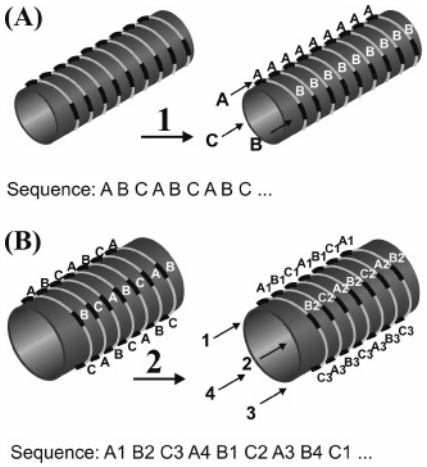

Figure 1. Combinatorial synthesis on linear fiber with discontinuous regions of synthetic support. A: Fiber is wrapped around a cylinder, aligning evenly spaced (dark) regions of synthetic support. Each row is exposed to a different soluble reagent. B: Fiber wrapped around a cylinder of different appropriate diameter forms rows, each with all species represented. Subsequent reaction leads to all combinations.

The solid support is wrapped around a cylinder and divided lengthwise into regions that constitute separate reaction vessels. After coupling, the thread is rewrapped around a cylinder of a different appropriate size and again divided into regions for subsequent reactions, each region bearing all previous structures, as shown in Figure 1. Repetition allows combinatorial library synthesis, with each member present at a known position. Initial work[23] described cotton thread as the solid support and wax as a barrier impermeable to peptide synthesis reagents. The SPOCC support should increase the versatility of our scheme.

Our synthetic method leads to a library of diverse substances distributed along a substrate of linear morphology.

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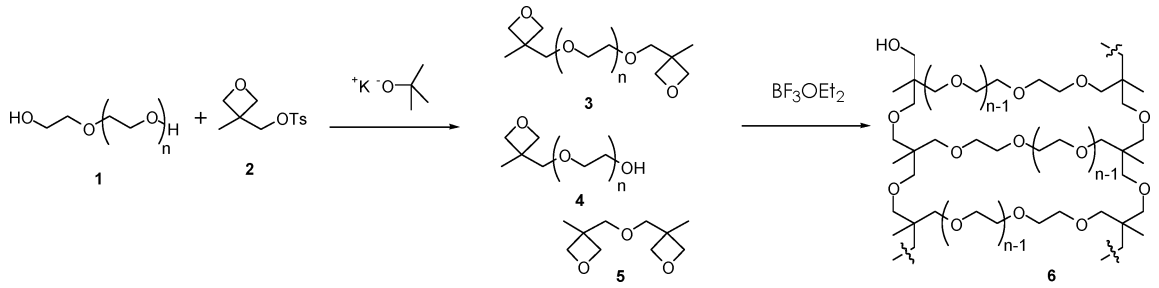
Scheme 1. Synthesis of Highly Cross-Linked SPOCC Polymer Support



In our investigations of fluorosensor molecules, the advantages of fiber-optic light delivery are attained by distribution along an optical fiber. Thus, our synthetic arrays could constitute functional sensor arrays if synthesis could take place in the cladding of an optical fiber. Optical requirements of a fiber cladding include transparency to the relevant wavelengths and a refractive index sufficiently lower than that of the fiber core to enable total internal reflection. Requirements for a synthetic support include compatibility with and permeability to various reagents and the presence of specific functional groups. Requirements for use as fluorosensors include permeability to analytes and a suitable environment for association and spectral response. We demonstrate preparation of films that meet these disparate requirements.

Results and Discussion

Desired Properties of the Polymer Support. A suitable candidate matrix is the PEG-based SPOCC polymer[4−6] **6**. It is stable to many reaction conditions, including acids strong enough to degrade other polymers, and swells well in both polar and nonpolar solvents for reactions and aqueous assays. It has low absorbance in the UV and visible regions and corresponding low fluorescence. Its refractive index of 1.38−1.49 is lower than the 1.55 index of quartz, making SPOCC appropriate as a cladding for quartz optical fibers. The refractive index of PEG and its aqueous solutions is appropriate for fiber cladding, so the polymer is likely to be appropriate in its dry and swollen states. Goals of this study include evaluation of absorbance, fluorescence, and index of refraction of this material. We have modified the original monomer synthesis and developed a method for film preparation.

Polymer Synthesis. Meldal Synthesis. The SPOCC polymer of Meldal et al.[6] is a poly(ethylene glycol) (PEG)-based polymer containing only primary ether linkages. Meldal's procedure, upon which our sequence of Scheme 1 is based, allowed control of cross-link density and functional group loading by choice of starting material **1** chain length and the ratio of cross-linker **3** to functional monomer **4** in polymer synthesis.

Synthesis of Monomer 3. Because we need low levels of functional groups, we prepare polymer from essentially pure bisoxetane cross-linker **3**, formed by carrying out the PEG alkylation to completion, forming very little of **4** (see Scheme 1). This has allowed us to use more prosaic reagents than originally used by Meldal et al., since the careful conditions required for the ratio of functional monomer **4** to cross-linker **3** to reflect that of the precursors is no longer needed. Alkylation of PEG-400 with oxetane tosylate **2** in the presence of potassium *tert*-butoxide in refluxing toluene is simple and effective; potassium hexamethyldisilazide in DMF is unneeded, as it would be for controlled incomplete alkylation.[6] Meldal et al. have also reported a modified procedure.[4] The bisoxetane **3** ($n \times 8$) that is derived from PEG-400 is termed BOP-400. An excess of tosylate **2** was employed to ensure complete alkylation of **1**: residual alcohol is <0.1%, as determined by integration of acetylated product as described.[6] Under our conditions, no remaining tosylate can be detected by TLC or ^{1}H NMR, but symmetrical ether **5** is present. Ether **5** is removed by stirring at 45 °C overnight in vacuo to yield 94% of crude **3**.

Purification of monomer **3** from traces of water or other species that can interfere with cationic polymerization is critical, as it was in previous work. In addition, for our studies, UV−vis absorbing and fluorescing impurities would be a significant problem. Crude BOP-400 is obtained by filtering the hot toluene solution through an alumina plug to remove polar impurities: the high temperature prevents substantial loss of monomer **3** by sticking to salts. Although the ^{1}H NMR spectrum of **3** shows no signals other than those expected, the resulting monomer has significant absorbance below 350 nm, with a tail into the visible region, as well as substantial fluorescence. Although we do not mean to suggest that **3** made by the Meldal procedures is contaminated in the same way as our material, those workers describe an alternative method of decolorizing **3** and photocleavage from the derived SPOCC polymer, suggesting that their method could also be appropriate.[4]

We have addressed the problem in two ways. Initial films prepared from highly absorbing monomer were photobleached as described below to convert them into a highly transparent material with low fluorescence. We now routinely remove absorbing contaminant from **3** using decolorizing charcoal, which is effective if acetonitrile solvent is used. Figure 2 shows the significant decrease in absorbance obtained by this method. Interestingly, other solvents were much less effective.

The polymerization process that leads to formation of SPOCC polymer **6** requires dry conditions, an even distribution of initiator to ensure consistent cross-linking, and a temperature appropriate for a convenient reaction time. For easy handling, the free-standing films are 700 μm, relatively thick compared to those affixed to a substrate. These thick films were made by placing a measured volume of neat **3** by syringe onto a clean glass slide and spreading it to a predetermined area. Exposure to BF_3 vapor now provides

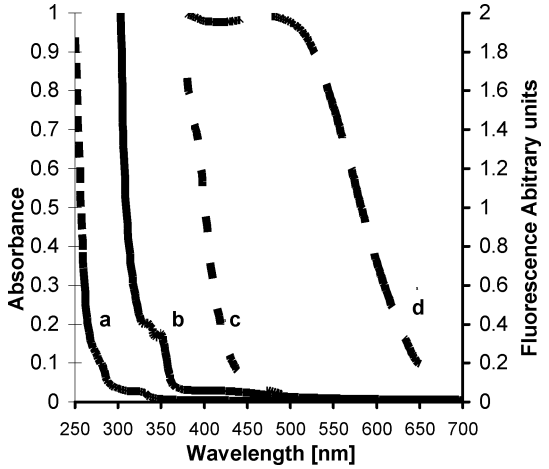


Figure 2. Absorbance and fluorescence spectra of monomer **3** in CH_3CN. UV/vis absorbance of monomer **3** at 0.2 M: (a) purified, (b) crude. Fluorescence of monomer **3** at 0.02 M: (c) purified, (d) crude.

initiator throughout the film without the difficulties inherent in spreading monomer−initiator mixtures. By carrying out polymerization in an Abderhalden drying pistol, we are able to conveniently control its temperature and exposure to vapor phase reagents. Thick films described here were polymerized at 82 °C for 14 h, considerably more than required, to ensure completion.

The polymer physical property that is simplest to measure is swelling. Because the swelling of polymer gels is dependent on cross-link density, it serves as a rapidly evaluated proxy. Our measurements on small quantities of polymers of various shapes led us to measure the mass of absorbed solvent, rather than the volume change accompanying such absorption. Consequently, we report "swelling" in units of mass of solvent contained by a given mass of dry polymer, and these numbers may not exactly parallel the volume change.

Films of polymer **6** prepared by our method swell in various solvents as follows: CH_2Cl_2, 5.18 ± 0.06; CH_3CN, 1.86 ± 0.02; and H_2O, 1.5 ± 0.2, expressed as the mass (g) of solvent included per dry mass (g) of polymer. These numbers are comparable to those reported on Meldal's bulk polymer.[6] Dimensional change on swelling in CH_2Cl_2 is large enough to shear the thick films of **6** from the glass surface, forming an intact free-standing film. Other solvents do not change the volume sufficiently to remove these films from the glass substrate.

Not surprisingly, the polymer film prepared from strongly UV−vis absorbing monomer had substantial absorbance and high fluorescence. These films could be rendered colorless by exposing them, while wet with water, to air and various UV−vis light sources. Water has an important role, but acid and base are not required, because inferior results were obtained at high and low pH values. Films prepared from decolorized monomer had low fluorescence and did not need photobleaching. Summarizing, we were able to obtain low fluorescence films from both colored and purified monomer.

The loading of hydroxyl groups in the polymer support films was determined by acylation with Fmoc-Gly (assumed to be quantitative[24]), followed by deprotection and quantitation of the resulting amino groups by the ninhydrin test,[25] to be 0.15 meq/g.

For sensor use, we need fluorophores present at sufficient concentration to enable a strong signal, but low enough for a linear response. To estimate this level, we measured the fluorescence of scraps of SPOCC film swollen in aqueous solutions of fluorescein at various concentrations. These rough measurements show a response roughly linear below 5×10^{-3} M fluorescein. As our polymer support loading of OH groups as 0.15 meq/g as synthesized, we used it without adjustment.

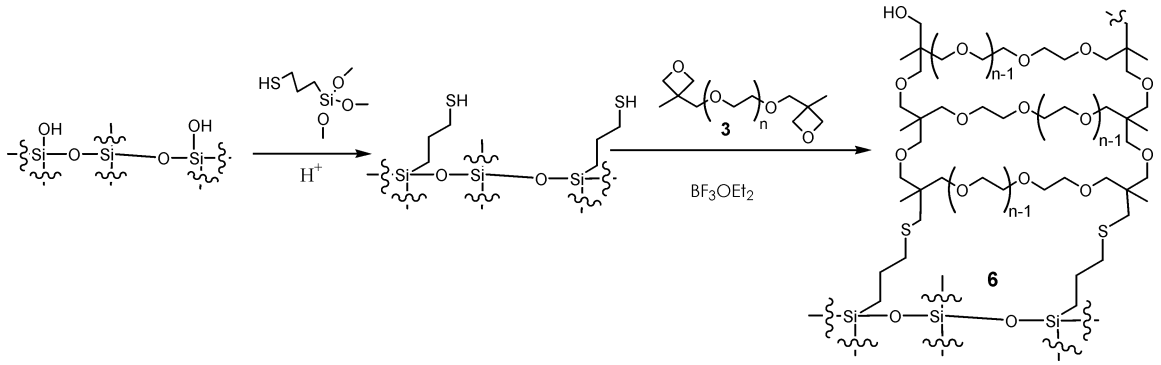
Synthesis of Glass-Tethered Polymer Films. For sensor use, we require thin films with robust attachment to the glass substrate. This is carried out as described above with modifications of the glass surface to allow covalent attachment and of procedure to obtain thinner films. Covalent attachment to the glass was enabled by mercaptopropyl groups on the glass surface, as shown in Scheme 2. Precleaned slides were exposed to a solution of 3-mercaptopropyltrimethoxysilane in ethanolic aqueous HCl, rinsed with ethanol, and heated at 115 °C.[26] The concentration of mercaptopropyl groups on large (1.9 cm^2) glass samples was measured using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)[27] to be 1×10^{-6} mol/cm^2, which corresponds roughly to 20% of a monolayer. The loading on smaller samples was below the measurement limit. Because thiols are easily oxidized to disulfides by air and disulfides would not be detected by DTNB or effective for polymer tethering, slides were routinely reduced with tributylphosphine just prior to either titration or polymerization.

Thin films of monomer were applied to the mercaptopropyl grafted slides by dipping them into CH_3CN solutions of monomer **3** rather than neat monomer; the lesser viscosity and concentration of these solutions ensured films substantially thinner and more consistent than those described above. Polymerization was carried out in an Abderhalden pistol as before. Rinsing of the polymer films was carried out more carefully than with free-standing films, because we wanted to prevent detachment of the film in any region. Washing with CH_2Cl_2 causes substantial rapid swelling: while these films do not shear off the glass substrate, wrinkling of the film is observed and probably indicates localized cleavage from the glass. By avoiding rapid changes in the rinsing solvent composition, and rinsing with no more than 70% CH_2Cl_2 in CH_3CN, wrinkles are prevented. We considered wrinkling occurring in this solvent to indicate poor polymerization and a low degree of cross-linking.

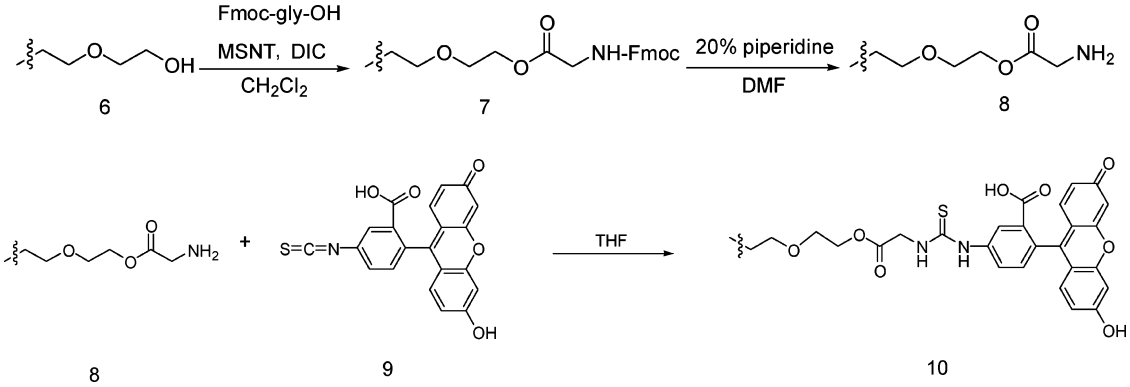
Fluorosensor Films. Our goal is to use SPOCC-400 polymer **6** as a solid support for preparation of libraries of optical fiber-based sensors. To evaluate our films for this purpose, we prepared pH sensors by coupling fluorescein to the polymer. Fluorescein is widely used as a fluorescent tag.[28−30] It exhibits multiple, pH-dependent ionic equilibria. Both monoanion and dianion are fluorescent, with high quantum yields of 0.37 and 0.93. With excitation close to its absorption maximum of 490 nm, the fluorescence emission spectrum is dominated by the dianion.

Synthesis of Fluorescein-Coupled Compounds. We coupled fluorescein isothiocyanate to glycine-functionalized

Scheme 2. Synthesis of Glass-Tethered Polymer Films



Scheme 3. Conversion of Polymer **6** to Fluorosensor Film **10**



polymer support films to use as an example of a fluorescent sensor for pH, as shown in Scheme 3.

Polymer was acylated with Fmoc glycine following a standard MSNT coupling procedure.[24] This procedure was chosen to ensure complete acylation for analytical purposes, whereas for preparative procedures, 1,3-diisopropylcarbodiimide (DIC) is used. After deprotection, fluorescein isothiocyanate treatment gave **10**, which was stored below 4 °C in the dark.

Characterization. Spectra of fluorescein-coupled SPOCC polymer taken at various pHs, were compared with solution spectra of free fluorescein. Film fluorescence measurements on fluorescein-coupled SPOCC polymer, equilibrated for 3 min in a solution of the appropriate pH, were performed with excitation at 450 nm and emission measured at 538 nm. Phosphate at 10 mM buffered those samples near pH 7; other pH values were measured, but essentially unbuffered.

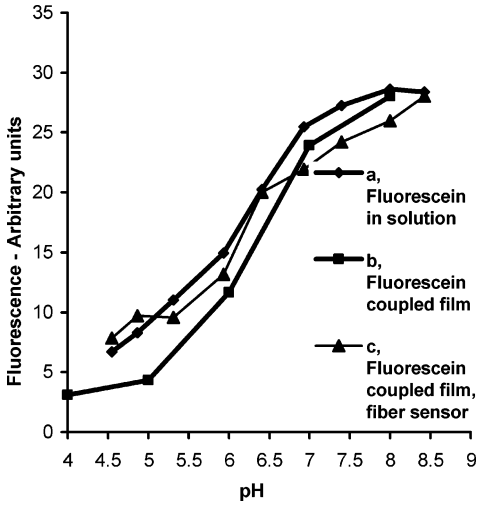


Figure 3. pH dependence of 538-nm emission. (a) Fluorescence of 1×10^{-6} M fluorescein isothiocyanate solution with excitation at 488 nm. (b) Fluorescence of free-standing fluorosensor films **10** with excitation at 450 nm, measured in fluorescence spectrometer. (c) Fluorescence of fluorosensor films **10** with excitation at 337 nm in fiber setup.[31,32]

Both excitation wavelengths gave pH intensity dependence for fluorescein-coupled films that were very similar to soluble fluorescein, providing evidence that our derivatized polymer is useful as a pH sensor. Curve c shows that laser measurements on these films, described below, give similar results.

Laser Measurements. As part of the development of a chemosensor array,[20] polymer films prepared with coupled fluorophores were tested to determine if evanescent excitation and detection could be achieved through the polymer using the two-fiber sensor[20,33] configuration shown in Figure 4. One of the fibers carries a 0.5-ns laser excitation pulse to the sensor region, while the second fiber crosses the first at each sensor region and carries captured fluorescence to the detector. Orthogonal geometry provides zero refractive coupling between the fibers. The sensor regions behave as cladding for the fibers so that coupling between the guided fiber modes and the sensor molecules is via the evanescent

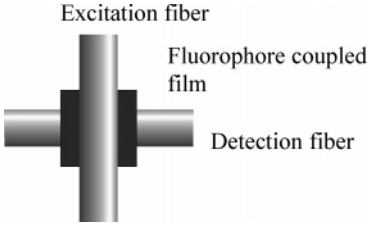


Figure 4. Two-fiber sensor setup.

field. Signal comes only from the small region very close to both fibers, presumably of nanoliter size, but yet to be measured.

A very short (0.5-ns) laser pulse that propagates down the exciting fiber leads to excitation at a precise instant. If a series of such sensor regions is arrayed along one fiber, each region will be excited at a slightly different time as the pulse speeds past. An emission pulse detected in the other fiber also appears at a specific time. Our previously described studies have addressed the way these times can be used to identify the location of the emitting region, discriminating sensor outputs by optical time-of-flight.[20,33] The key point is that the detection time depends on the distance the light has traveled, but that this travel can take place in either the excitation fiber or the emission capturing fiber. Closely spaced sensor regions on one fiber may still be resolved by optical time-of-flight, as long as the other fiber provides a sufficient distinction in overall distance.

Unlike a one-fiber scheme, the two-fiber sensor thus overcomes the spatial resolution limits imposed by the fluorescence lifetimes of the sensor molecules. In addition, the evanescent excitation of the sensors results in a low attenuation of the excitation pulse so that large arrays of sensors may be accommodated. These properties of the two-fiber design are conducive to use with our one-dimensional combinatorial technique.

Data shown in Figure 3c were obtained on our pH-sensing films using the experimental procedures previously described.[20] The two-fiber sensor regions were formed by sandwiching thin fragments of fluorescein-derivatized SPOCC film between optical fibers from which the original cladding had been removed.

Conclusions

We have prepared and demonstrated the applicability of SPOCC polymer films for fluorosensor presentation,. SPOCC polymer has already been shown to be an appropriate support for solid-phase synthesis, and we have previously demonstrated combinatorial synthesis in regions along a different support of linear morphology. If combinatorial synthesis of fluorosensors were carried out in regions of SPOCC films distributed along an optical fiber, the library as fabricated would constitute a readable sensor array. Our polymer preparation yields material of low absorbance and fluorescence, necessary for these purposes. Derivatization of this polymer allows fluorsensor film construction; pH-sensitive fluorescence has been demonstrated using conventional measurement techniques, as well as using subnanosecond optical pulses and time-of-flight discrimination of sensor regions. Thus, nanoliter-scale sensors are probed on a nanosecond time scale.

Experimental Section

Monomer Synthesis. 3-Methyl-3-tosyloxymethyl Oxetane 2. To a solution of tosyl chloride (21.5 g, 110 mmol) and 3-hydroxymethyl-3-methyl oxetane (10.3 g, 100 mmol) in freshly distilled CH_2Cl_2 (60 mL) on an ice−NaCl bath, pyridine was added below 0 °C dropwise over 10 min, and the colorless mixture was allowed to warm to room temp while stirring overnight. The precipitate was removed by filtration, rinsed with CH_2Cl_2 (2 × 20 mL), and the combined organic layers were washed three times with water (20 mL). The combined aqueous layers were back-extracted with 20 mL of dichloromethane. The combined organic layers were dried over Na_2SO_4, and solvents were removed at reduced pressure to yield 24.1 g (96%) of crude, solid product **1**. Recrystallization from ethyl ether (60 mL) gave 21.6 g (86%) of pure product spectroscopically identical to that reported.[6]

Bisoxetane 3 (BOP-400 Mixture of Chain Lengths). Poly(ethylene glycol), average MW = 400 (PEG 400, 10.0 g, 25.0 mmol) in 250 mL of toluene was azeotropically dried by reflux under N_2 with a Dean Stark trap overnight until no more water was removed. After cooling to 60 °C, oxetane tosylate **1** (16.7 g, 65.0 mmol) was added, followed by potassium *tert*-butoxide (8.400 g, 0.075 mol). The vigorously stirred reaction mixture was then heated at reflux for 4 h with toluene added if necessary to allow stirring. Once reaction completion was confirmed by acylation test (see below), the mixture was rapidly filtered while hot through a plug of ∼10 g of Celite, which was then washed with an additional 200 mL of hot toluene. The Celite was heated with 300 mL of toluene until the solids evenly dispersed, and the mixture was filtered hot again. The combined toluene layers were cooled to ambient temperature and passed through a column of basic alumina that had been activated at 275 °C for 20 h. The column was rinsed with 300 mL of toluene, and the combined toluene solutions were concentrated by rotary evaporation to yield 13.4 g (94.2%) of crude BOP-400 **3** as an oil, which was stirred at 45 °C, ≤ 0.3 mm Hg for 16 h to yield 12.9 g (90.7%). Decolorizing charcoal (26.6 g) and dry acetonitrile (150 mL) were added, the mixture was stirred for 1 h, filtered, and the solvent was removed by rotary evaporation to yield BOP-400 **3** as a colorless oil (10.7 g, 75.4%). ^{1}NMR($CDCl_3$): δ 4.44 (d, J = 50.7, 2H), 4.41 (d, J = 50.7, 2H), 3.65 (s, 2H), 3.54 (s, 2H), 1.31 (s, 6.5H). UV (CH_3CN): absorbance less than 0.01was found over the range 350 to 800 nm.

Acylation Test of Alkylation Completeness: A ∼200-μL aliquot of the well-stirred reaction mixture suspension was rotary-evaporated. The residue in pyridine (1 mL) and acetic anhydride (0.5 mL) was stirred at room temperature for 1 h and concentrated by rotary evaporation. The residue was analyzed by NMR in $CDCl_3$. Unreacted PEG alcohol was detected as its acetate: δ = 4.22 (t, J = 4.81 Hz), the absence of which indicates that alkylation reaction is complete and <2.3% of the alcohols groups remain.

Polymer 6 Synthesis. Free-Standing Polymer Films. On slides cleaned with Alconox and acetone, dry slides BOP400 (50 μL) was put as drops with a syringe and evenly distributed as a quite thick layer (area 6.5 cm^2). The slides were then inserted into an Abderhalden drying pistol, and a vacuum was applied overnight to remove the traces of water and solvent. The pistol was placed under N_2 pressure and the bulb placed in a dry ice cooling bath and left to equilibrate (∼30 min). The bulb was then sealed. BF_3OEt_2 (30 μL,) was dissolved in dichloromethane (300 μL) and injected into the cold bulb of the pistol (it is important to ensure the pistol is not under vacuum at this point, this can

be tested by adding a small aliquot of dichloromethane first). The initiator was allowed to equilibrate to the dry ice temperature over 15 min, and then a vacuum was applied for a couple minutes. The pistol was then sealed, the cold bath was removed from the bulb, and the vessel was allowed to warm to 20 °C in a water bath. The pistol was then heated with refluxing MeCN overnight (14 h). The reaction was cooled, and the polymerization was quenched by the addition of EtOH. The polymer was rinsed with water, 2 M HCl, water, 1 M NaOH, water, MeCN, dichloromethane, MeCN, and water (5 mL each). The polymer was stored under water.

Polymer support functional group loading was determined by acylation with Fmoc glycine and subsequent deprotection to determine the amount of amine via the ninhydrin test[21] (0.15 meq/g).

Swelling was determined by taking a known amount of polymer and measuring the change in mass of the polymer when swollen 10 min in the desired solvent in the absence of excess liquid. The measured swelling was 5.18 g of CH_2Cl_2, 1.86 g of CH_3CN, and 1.5 g of H_2O for 1 g of dry polymer.

Polymer Film Attached to Glass. Mercaptopropyl Derivatization of Slides. Slides were cleaned with Alconox, rinsed with water, and put into 100 mL of piranha solution[34] (30% H_2O_2/H_2SO_4) for 12 h. After that, the slides were rinsed with Nanopure water and placed into 100 mL of 3% solution of 3-mercaptopropyltrimetoxysilane in 5% (5 mL) HCl/(95 mL) ethanol for 1 h. Next, the slides were rinsed thoroughly with 95% EtOH then in 100% ethanol and dried in a vacuum for 20 min at room temperature and heated at 115 °C for 1 h. After cooling to room temperature, the slides were washed with 95% ethanol and left under an active vacuum for 12 h (the slides have to be kept under passive vacuum; otherwise, they oxidize).

SH Group Concentration Check by DTNB.[3] The slide was put into a Petri dish, and 1.6 mL of water, 4.00 μL of DTNB solution (10 mM), and 0.4 mL of pH 8 buffer were added. After 20 min, the UV spectra of the solution were taken, and the concentration was measured as absorbance at 412 nm.

Reduction of S−S Groups Back to Thiols. A low concentration of SH group indicated oxidization. To recover more SH groups, reduction with tributyl phosphine ($(Bu)_3P$) was performed.

The slide was put into a clean, round-bottomed flask. Ethanol was added so that it covered the slide surface (10 mL). $(Bu)_3P$ (4.05 g, 20.0 mmol) was added into the previously sealed flask and left for 1 h. After that, the solvent was removed, and the slide was washed several times with ethanol, a total of 300 mL in 20-mL portions. The remaining ethanol was evaporated with a vacuum. The DTNB test was performed to check the SH concentration after reduction.

Polymerization. Mercaptopropyl-derivatized slides were dipped in a solution of BOP400 in acetonitrile (1:3 v/v 1.13 g; 3 mL of acetonitrile).

The slides were then inserted into an Abderhalden drying pistol, and a vacuum was applied overnight to remove the traces of water and solvent. The pistol was filled with N_2, and BF_3OEt_2 (30.0 μL) in CH_2Cl_2 (300 μL) was added to the bulb, which was cooled in a dry ice/acetone bath. The initiator was allowed to equilibrate to the dry ice temperature over 15 min, and then a vacuum was applied for a couple minutes. The pistol was then sealed, the cold bath was removed from the bulb, and the vessel was allowed to warm to 20 °C in a water bath. The pistol was then heated with refluxing MeCN overnight (14 h). The reaction was then cooled, and the polymerization was quenched by the addition of EtOH. The polymer was then rinsed with water; 2 M HCl; water; 1 M NaOH; water; MeCN; and 10, 20, 30, 40, 50, 60, and 70% of dichloromethane in MeCN. The polymer was then dried under vacuum.

Polymer Derivatization. Preparation of Fmoc-Gly-Acylated SPOCC-400 Polymer Support 7. Fmoc-Gly-OH (145.5 mg, 0.5 mmol) was suspended in dichloromethane (5 mL), and methyl imidazole (40 μL) was added to initiate dissolution. This mixture was then added to MSNT (145.2 mg, 0.5 mmol) via a syringe and allowed to react for ∼5 min. The yellow, activated amino acid solution was then added to the hydroxy-functionalized SPOCC polymer support (49 mg), and the reaction was allowed to run at room temperature for 3 h. The polymer was then filtered and washed with DMF and dichloromethane (6 × 3 mL each) and then dried under vacuum. This procedure is generally carried out on a smaller scale for analytical purposes.

Deprotection of Fmoc-Gly-Acylated SPOCC-400 Polymer Support 7. The acylated polymer support 6 was suspended in 1 mL of 20% piperidine/DMF and allowed to react at room temperature for 1 h. The polymer was then filtered and washed with DMF, MeCN, and dichloromethane (6 × 3 mL each) and then dried under vacuum. The loading was measured by the ninhydrin test.

Ninhydrin Test. The ninhydrin test with five standard solutions of glycine in the range 1×10^{-8} M to 1×10^{-7} M and underivatized, piperidine-washed SPOCC-400 **5** film as control, was performed.[21] A standard solution of glycine 5.03×10^{-3}M in water was prepared. 0, 10, 30, 40, and 60 μL of this solution was put into vials. Acylated film was put into a vial. To all the vials EtOH was added to fill to 100 μL. A 100-μL portion of solution A (2.0 mL of 10^{-2} M KCN in H_2O diluted to 100 mL with pyridine), 50 μL of solution B (20.7 g of phenol in 5.0 mL of EtOH), and 25 μL of solution C (0.5 g ninhydrin in 10 mL of EtOH) was added. The mixtures were simultaneously heated in an oven at 105 °C for 10 min, and then the reaction was quenched by cooling in an ice bath. To each sample, 2.2 mL of EtOH and 1.5 mL of H_2O was added, and the UV−vis spectra of the solutions were taken at 570 nm. The linear regression was fitted, and the loading was determined as 0.15 meq/g.

Preparation of Fluorescein SPOCC-400 Polymer 10. Amino acylated SPOCC-400 polymer **8** (73 mg, 11 μmol of NH_2-group-based loading) was agitated on a vortex genie with fluorescein isothiocaynate (14 mg, 35 μmol) in THF (3.5 mL) at rom temperature for 2 h while protected from light. The SPOCC-400 film was rinsed with THF (until no fluorescence was detected in the washings), then rinsed with 10-mL aliquots of THF, CH_2Cl, CH_3CN, and H_2O and stored under H_2O at 4 °C in the dark to avoid photobleaching.

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