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Optical Sensors 2013 / F. Baldini, J. Homola, R. A. Lieberman (eds.): Paper 877403

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3 March 2015

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Nanoliter-scale, Regenerable Ion Sensor: Sensing with Surface Functionalized Microstructured Optical Fiber

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ABSTRACT

The first nanoliter-scale regenerable ion sensor based on microstructured optical fiber (MOF) is reported. The air holes of the MOF are functionalized with a monoazacrown bearing spiropyran to give a switchable sensor that detects lithium ions down to 100 nM in nanoliter-scale volumes. Ion binding is turned on and off on upon irradiation with light, with the sensor being unaffected by multiple rounds of photoswitching. Unbound ions are flushed from the fiber in the ‘off’ state to allow the sensor to be reused. The integration of an ionophore into the sensor paves the way for the development of highly specific light-based sensing platforms that are readily adaptable to sense a particular ion simply by altering the ionophore design.

1. INTRODUCTION

With the advent of advanced medicine, early disease diagnosis often translates into better prognosis and treatment plans.^[1-4] Given the intricate relationship between metal ions and human health,^[5,6] there is a real need to develop new metal ion sensors that are user friendly, easily deployed, give rapid response and are affordable.^[7] One important advance in this context would be to develop sensors that are reusable and/or capable of continuous or repeated measurements. Such a reusable sensor would maintain the sensing surface in a passive state, in-between measurements and *switch* to an active state under an external stimulus only when a measurement is required. When the measurement is completed, the target species would be expelled by a selected external stimulus, with the surface once again returning to its inactive form. This ‘switchable’ property makes it possible to return the sensing surface to its original state after a measurement is made, thereby potentially extending the sensor’s useful lifetime.^[8] Such sensors would allow for multiple measurements to be made on a single sample without the need to change the sensor. This may offer some advantage in remote applications and also in biochemical studies. Despite these needs and associated opportunity, little work has been done in this area.

The most basic switchable metal ion sensor of this type would consist of a molecular switch combined with a sensing platform that would ideally allow detection within nanoliter-samples. A suitable external stimulus (e.g. light) would change or switch the structure and properties of the sensing platform in a non-invasive and reversible manner.

The use of an optical fiber as the sensing platform in this context offers attractive characteristics such as low loss, high bandwidth, immunity to electromagnetic interference, small size, light weight, safety, relatively low cost, low maintenance, that make them very suitable and, in some cases, the only viable sensing solution.^[9] Microstructured optical fibers (MOFs) offer the potential to improve performance relative to more traditional spectroscopic and fluorescence-based fiber sensors.^[10] MOFs have air holes incorporated within their cross-section and these holes can be used to control the interactions between guided light and matter located within the holes while simultaneously acting as

tiny sample chambers.^[11,12] The amount of guided light that is available to interact with the material located within the holes of MOFs can be increased by manipulating the geometry of the fiber cross-section.^[11,12] With appropriate design, the cross-sectional structure of a MOF provides the broad range of optical properties demanded by different sensors. These characteristics have allowed MOFs to be used as sensing platforms for a variety of chemical and biological substrates.^[13-15] In addition, integrating a photoswitchable molecule with MOFs as the sensor surface also means that light can also be used to quantify the binding of analytes via absorption, fluorescence or label-free techniques.

From the plethora of photoswitchable molecules that are known, spiropyrans stand out as attractive candidates for sensor development for several reasons. Spiropyrans are characterized by two spectrally well-separated states that are thermally stable, high switching reliability, low fatigue to maximize the number of switching cycles the molecules can survive, and tunable switching rates.^[16,17] Importantly, spiropyran undergoes a reversible structural isomerization between a colorless spiro (SP) form and a colored open form (merocyanine or MC) on irradiation with UV light and vice versa with visible light or heat (**Figure 1**).^[18,19] This substantial change in color between the two forms is due to the unavailability of the electron lone pair of the phenolate oxygen in MC, which contributes to the charge delocalization in the zwitterionic MC species.

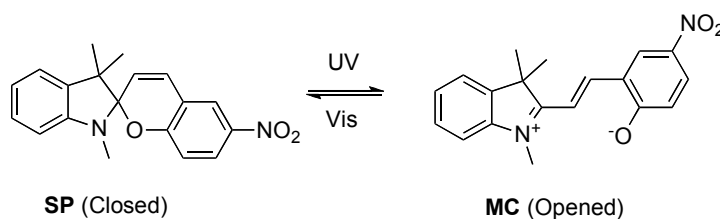


Figure 1. Structures of spiropyran (SP, closed) and merocyanine (MC, opened).

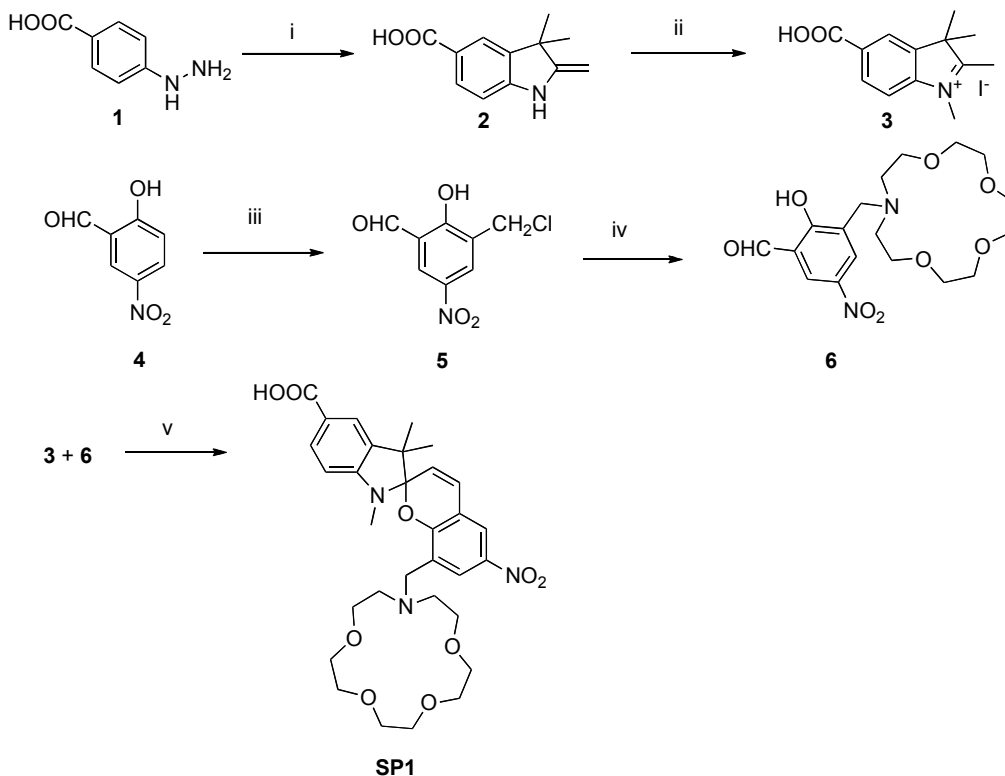
A basic spiropyran structure can be readily functionalized with a suitable metal-binding site to provide an opportunity to affect light-induced release of the metal ion to generate a re-generable sensing system. Indeed spiropyran-based chemosensory systems for the detection of alkali and alkaline earth, lanthanides, transition and non-transition metals have been reported.^{[20],[21]} In particular, crowned spiropyran have been developed as chemosensors for alkali metal ions, in which the metal ion binding in the crown ether moiety induces a large spectral change accompanied by isomerization to the merocyanine form.^[22,23]

In this work we report the design, synthesis and operation of a novel photo-responsive, regenerable ion sensor for the detection of lithium ions (**Sensor-1**). Here, we chemically modified a known monoazacrown bearing spiropyran (**SP1**, Scheme 1) to enable it to be attached covalently to the glass surfaces inside the air holes of MOFs. When the optical fibers have been surface functionalized with **SP1**, the operation of the sensor was investigated through several key characteristics such as the minimum concentration of lithium ions detectable, photoswitching and sensor re-use. While various surface functionalized spiropyran have been reported,^[24-27] this study is the first demonstration of a surface functionalized spiropyran derivative with an ionophore attached to the molecular switch. Prior to this work, the ion sensing ability of surface functionalized spiropyran mainly relied on the metal chelating ability of the molecular switch during the SP to MC transition.^[24,25,28] The integration of an ionophore to the sensor paves the way for the development of highly specific light-based sensing platforms that can be readily adapted to sense specific ions just by altering the ionophore design. In addition, this also represents the first reversible molecular switch that is functionalized within an optical fiber system. This integration of **SP1** and MOF results in a nanoliter-scale, light-driven ion sensor that can be turned on and off on demand in order to overcome the problem of a one-time use sensor.

2. RESULTS AND DISCUSSION

2.1 Synthesis of SP1

The synthesis of **SP1** with a monoazacrown moiety at the 8-position is based on the condensation of compounds **3** and **6** as shown in Scheme 1. The key indole **3** was synthesized in two steps according to literature.^[29] In particular, reaction of 4-hydrazinobenzoic acid (**1**) with isopropylmethylketone, followed by methylation with methyl iodide gave the desired product. The crowned nitrosalicylaldehyde (**6**) was obtained in two steps by the chloromethylation of 5-nitrosalicylaldehyde (**4**), in the presence of aluminum chloride, followed by reaction with 1-aza-15-crown-5 gave (**6**). Subsequent condensation of (**3**) with (**6**) in ethanol under reflux gave **SP1**.



Scheme 1 Reagents and conditions: i, 2-methyl-2-butanone, conc. H_2SO_4 , EtOH, reflux, 12 h; ii, methyl iodide, toluene:acetonitrile 2:1, reflux, 14 h; iii, chloromethyl methyl ether, aluminum chloride, rt, 1 h then reflux, 2 h; iv, 1-aza-15-crown-5, Et_3N , THF, 0 °C - rt, 14 h then reflux 3 h; v, EtOH, reflux, 3 h.

2.2 Microstructured Optical Fiber Experiments

The MOF used for this study was fabricated in-house from undoped high purity fused silica, using the cane and jacket method^[30] in combination with the process described by Kostecki et al.^[31] Silica is ideal for this work as it has high transmission properties in the UV-Vis-NIR spectral range.^[32,33]

A single 80 m length of polymer coated ($n = 1.54$) fiber was fabricated and the dimensions of this fiber were measured using cross-sectional images from a scanning electron microscope (SEM). Figure 2a shows the overall MOF structure with the coating removed, having an outside diameter of 270 μm . An enlargement of the core and holes region in the center of the fiber (with structure known as a wagon wheel fiber) is shown in Figure 2b, where the core of the fiber is the

small triangular element in the center of the image (highlighted by the green box), suspended on three thin struts. The 3 black voids are the holes within the fiber cross-section, which form the cladding region used to provide the refractive index contrast needed for light confinement, and are used for this study as sample chambers, with each hole diameter being $12.4\ \mu\text{m}$, defined as the diameter of a circle whose area is equal to the cross sectional area of the hole. The web thicknesses are $0.16\ \mu\text{m}$ minimum, while the core has an effective diameter of $1.7\ \mu\text{m}$, defined as the diameter of a circle whose area is equal to a triangle that fits wholly within the core area.^[30]

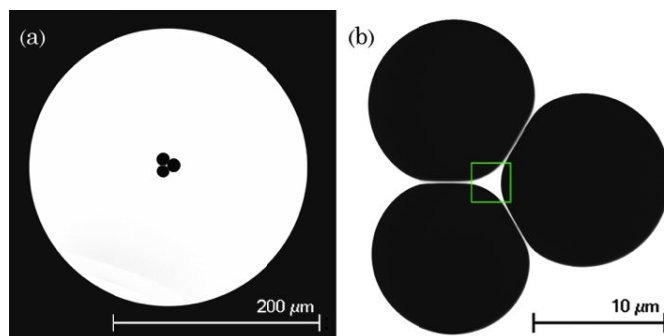


Figure 2: Scanning electron microscope images of (a) the silica microstructured optical fiber with a outside diameter of $270\ \mu\text{m}$; and, (b) an enlarged image of the core and hole region, where the core is highlighted by the green box, having an effective diameter of $1.7\ \mu\text{m}$.

The power fraction of guided light protruding into the holes of this silica fiber was theoretically investigated,^[34] using an air suspended rod approximation with a circular $1.7\ \mu\text{m}$ core diameter. For the cladding region, the refractive index used was $n = 1.34$, being the refractive index of the analyte used in this study. It was found that at the excitation wavelength of $532\ \text{nm}$, this fiber can support 12 propagating modes, and with a coupled Gaussian beam waist of $1.7\ \mu\text{m}$, 2.1% of the total optical power of all 12 modes is located outside of the core, which is available to interact with a fluorophore functionalized on the surface.

2.3 Photostability Studies on unfuntionalized MOF

SP1 must possess high chemical stability and photostability under the conditions of the experiments if the sensor is to be useful. Any photochemical destruction of SP1 can potentially impair the function of the sensor; therefore experiments were carried out to determine the intensity or time-span of light exposure that would cause significant photobleaching of SP1.

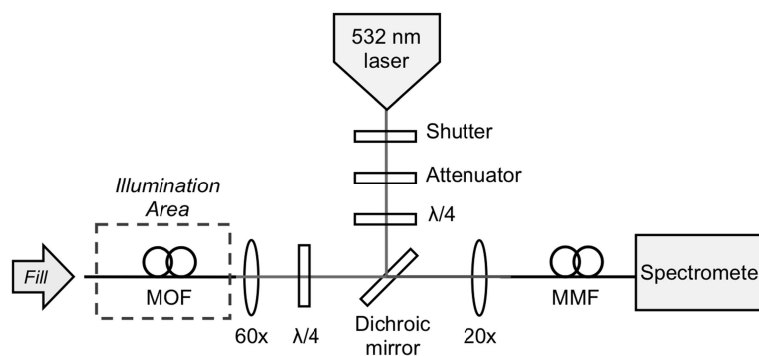


Figure 3. Optical setup used to measure the fluorescence from MOF. MMF is a multimode fiber.

The experimental set-up used for the in-fiber fluorescence measurements is showed in Figure 3. The MOF was filled by

immersing the left-hand tip into the solutions that were then left to fill via capillary forces. A UV lamp and a halogen light source were used to switch the molecules and were placed over the MOF. The area of illumination is shown by the dotted rectangle (Figure 3). An empty fiber was thus filled with a solution of **SP1** (1 μM) in acetonitrile by capillary action. Once filled, the fiber was exposed to UV light for 7 min to convert all the absorbed **SP1** from its closed, non-fluorescent form to the opened, fluorescent MC form.

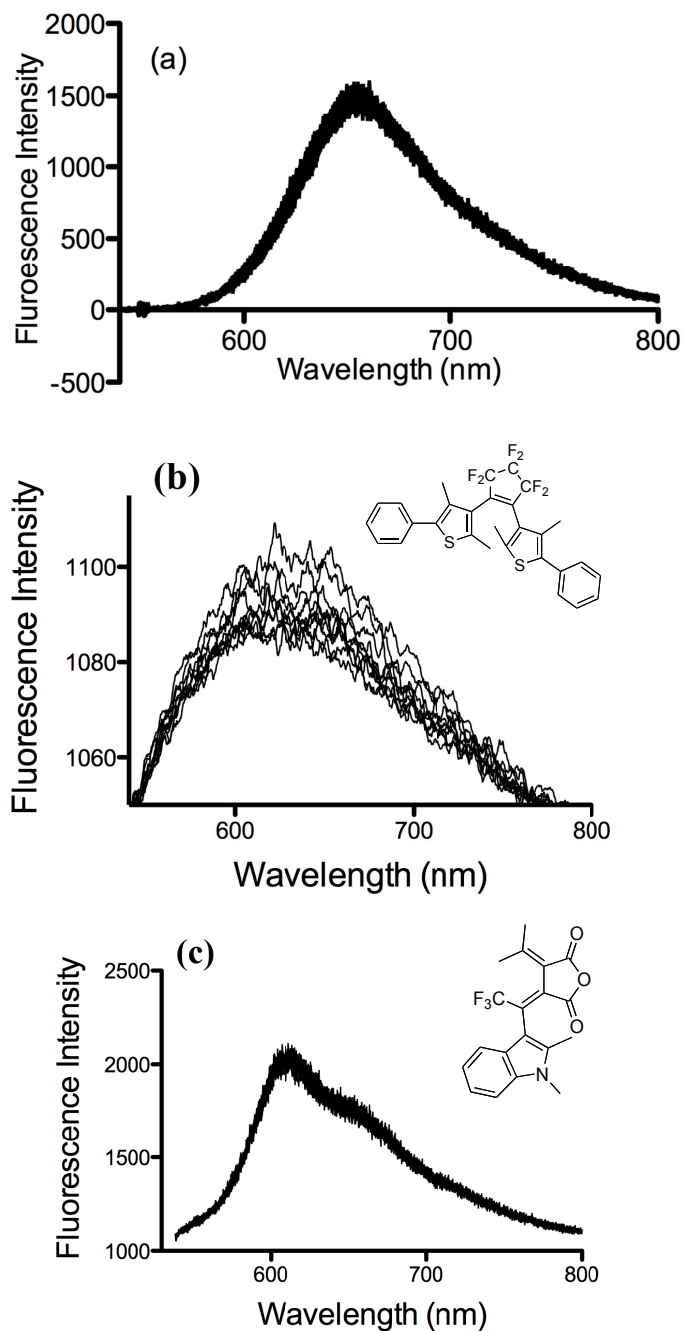


Figure 4. Photobleaching experiments where the respective photoswitches are absorbed onto MOF using capillary action and exposed to the 532 nm laser for 10 x 16 ms respectively (a) **SP1** (1 μM in acetonitrile) (b) diarylalkene (c) indolefulgide respectively.

Photobleaching experiments were carried out by subjecting the **SP1** absorbed within the holes of the MOF to multiple exposures of the 532 nm laser (10 x 16 ms, Figure 6a, 10 x 32 ms and 10 x 1 s, not shown). As apparent in Figure 4a, photobleaching was not observed for 10 x 16 ms of exposure, however photobleaching to approximately 60 % fluorescence intensity was observed after 10 cycles of exposure to the laser at 1 s per exposure. Given that **SP1** was found to be robust and less prone to bleaching under these conditions, the time span of light-exposure to the 532 nm laser for all subsequent experiments were maintained at 10 x 16 ms.

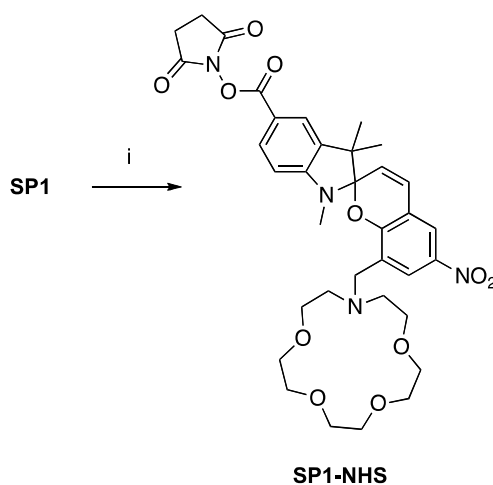
Photobleaching experiments were also performed on two other common photoswitches namely the diarylalkenes and indolefulgides. As shown in Figure 4b and c, the diarylalkene analog showed photobleaching under the same experimental conditions while the indolefulgide remained stable, making latter class of compounds potentially useful for creating reusable sensors.

2.4 Surface functionalization of MOF with SP1

As mentioned previously, for the sensor to function as a regenerable ion sensor the molecular switch (**SP1**) must be covalently attached to the internal surface of the MOF. This then renders the sensing surface photo-responsive.

It is important that this methodology is reliable, repeatable, and capable of withstanding the conditions employed during ion binding and photoswitching. To do this, fibers were sealed into a metal chamber and the solutions were forced through the fiber via positive pressure using nitrogen gas. The fibers were coated with 5% APTES in toluene (w/w) for 2 h at 100 psi.^[35,36] The next step usually involves pre-mixing the COOH-bearing molecule with excess coupling reagents (EDC/NHS) before applying the entire mixture to the amine-terminated silane surface. However, this approach is problematic in that it is hard to quantify the number of molecules that have been activated and are available to react with the surface. Secondly, long washing times are usually required to ensure that excess coupling reagents and by-products are completely flushed from the fiber. To eliminate having to perform the coupling reaction on the surface, **SP1** bearing a reactive succinimidyl ester moiety, for subsequent surface attachment, was first synthesized according to Scheme 2. **SP1** was reacted with *N*-hydroxysuccinimide (NHS)^[37] in the presence of *N,N'*-diisopropylcarbodiimide (DIC) at room temperature to give **SP1-NHS** in 70 % yield.

The fibers were then rinsed in preparation for coating with synthetic **SP1-NHS**. The fibers were prepared for coating by first flushing with toluene for 20 min, followed by drying with nitrogen for 20 min, a further flush with millipore water for 20 min and a final flush with nitrogen for 30 min. The fibers were then coated with a solution of **SP1-NHS** in acetonitrile (2 mM) for 2 h, and were flushed with acetonitrile, air and water for 20 min respectively. In all of the above steps, 100 psi of pressure was used, and the ends of the fibers were visually checked to ensure that the liquid/gas was flowing through.



Scheme 2 Reagents and conditions: i, *N*-hydroxysuccinimide, *N,N'*-Diisopropylcarbodiimide, THF, rt, 18 h.

The results in Figure 5 reveal that, unlike an unfunctionalized fiber, the **SP1** functionalized fiber (**Sensor-1**) has an inherent background fluorescence ($\lambda_{em} \sim 640$ nm). This is consistent with **SP1** having been successfully immobilized within the fiber's internal surface. Next, using only capillary forces, sections of **Sensor-1** were filled with lithium perchlorate solutions of concentration ranging from 50 nM to 10 μ M respectively. The respective sections were first exposed to white light for 2 min to ensure that all fluorescent **SP1-MC** form was converted to the non-fluorescent **SP1-SP** form. This was followed by exposure to UV light for 7 min with fluorescence measurements taken at regular intervals. This revealed that maximum fluorescence was achieved after 7 min of UV exposure. Figure 8 shows an increase in fluorescent intensity when the fiber was filled with solutions of 100 nM and 1 μ M of Li^+ respectively. The signal-to-background ratio obtained for the 50 nM sample was approximately 1 (not shown in Figure 8). This compared to values of 1.9 and 2.5 signal-to-background ratios for the exposure to 100 nM and 1 μ M of Li^+ respectively. The relatively poor signal-to-background ratio for concentrations lower than 100 nM is likely attributable to the high surface density of **SP1** on the fiber, which is common in surface-attachment experiments. This is not associated with the glass itself, as recently demonstrated by us in the sensing of CdSe quantum dots down to 10 pM in soft-glass microstructured optical fibers.^[38] This was achieved using the same fiber concept. Thus, further optimization of the coating procedure can be done to reduce this background signal, but was beyond the scope of the proof-of-concept work presented in this paper.

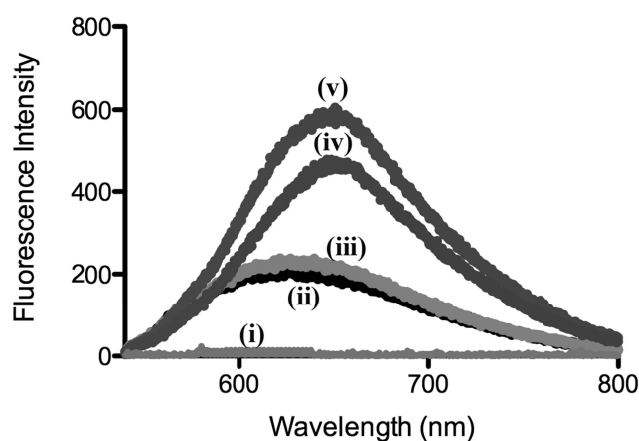


Figure 5. (i) Empty fiber (ii) Functionalized fiber (FF), not filled with Li^+ solution after photo-irradiation with UV for 7 min and exposed to white light 2 min respectively. (iii) FF, filled with 100 nM and 1 μ M Li^+ in ACN respectively, exposed to white light for 2 min. (iv) FF, filled with 100 nM Li^+ in ACN, exposed to UV ($\lambda = 360$ nm) for 7 min. (v) FF, filled with 1 μ M Li^+ in ACN, exposed to UV ($\lambda = 360$ nm) for 7 min.

2.5 MC \leftrightarrow SP transition in the presence of Li^+

It has been previously reported that the rate by which MC isomerizes back to SP is greatly influenced by exposure to metal ions.^[7] In order to investigate light-induced reversion of the metal-complexed MC form to SP, a section of **Sensor-1**, filled with acetonitrile only, was exposed to white light and emission spectra were recorded at regular intervals. The same piece of fiber was then irradiated with UV light to regenerate the fluorescent **SP1-Li** complex and this was again exposed to white light. Figure 6b reveals that fluorescence decreased much more rapidly when **Sensor-1** is not chelated to Li ions, with the intensity falling below 50 % in less than 2 min. In contrast, **Sensor 1** chelated to Li^+ demonstrated much slower photodecoloration (Figure 6a). There was little change in fluorescence intensity after 8 min of white light exposure. Although pseudo first order decolouration can be obtained for **Sensor 1** in the absence of any ions, we were unable to observe first-order decolouration for the metal bound complex. This observation is consistent with the work of Kimura et al⁷ where the authors were able to obtain the first order decolouration rates in THF at 40 °C but not in acetonitrile where the decolouration of the aza-crown spiropyran analogs was too slow to follow. It has been proposed that these anomalies in the decolouration kinetics could be due complexing between the opened, metal-bound MC form

and the closed SP forms of the spiropyran fragments in the different solvents. However this interaction between solvent and photodecoloration of the metal-bound MC species is still far from a complete understanding^[39] and is beyond the scope of this work.

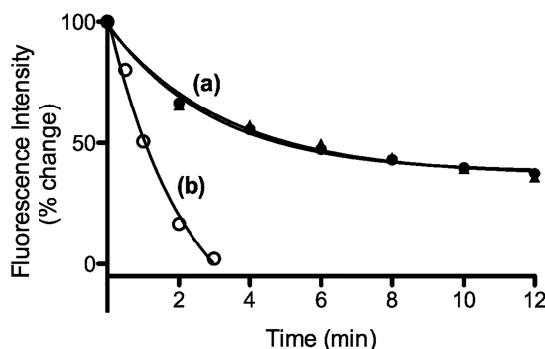


Figure 6. Photo-decolouration of **Sensor-1** in the absence and presence of Li ions. The points on the graphs are fitted using non-linear regression on GraphPad Prism version 5 (<http://graphpad.com/help/prism5/prism5help.html>). (a) (●) Cycle1. **Sensor 1** filled with 1 μM of Li^+ in acetonitrile, irradiated with UV for 7 min, followed by exposure to white light for 12 min. Pseudo $R^2 = 0.9943$ (▲) Cycle 2. Second exposure to UV for 7 min, followed by second round of exposure to white light for 12 min. Pseudo $R^2 = 0.9839$. (b) (○) **Sensor 1** filled with acetonitrile only, exposed to white light. Pseudo $R^2 = 0.9913$.

It is well documented that the isomerization of spiropyrans is known to be highly dependent on solvent effects, with polar solvents stabilizing the opened MC form.^[40] To investigate whether sensing can be performed in the presence of aqueous media, fluorescence data for **SP1** in varying percentages of water in acetonitrile was obtained (cuvette experiment). Figure 7 shows the effect of 5 % H_2O on fluorescence intensity. In the absence of any Li^+ , a slight increase in fluorescence intensity for **SP1** in **Solution 1** (5:95 H_2O :acetonitrile) (–) relative to that of **SP1** in acetonitrile alone (–) was observed. This increase in fluorescence agrees with the general observation that water is stabilizing the open MC form. However, when **Solution 1** was exposed to white light, the $\text{MC} \rightarrow \text{SP}$ conversion occurred almost instantly which is consistent with the data in Figure 9b and the fluorescence intensity for both solutions are now similar.

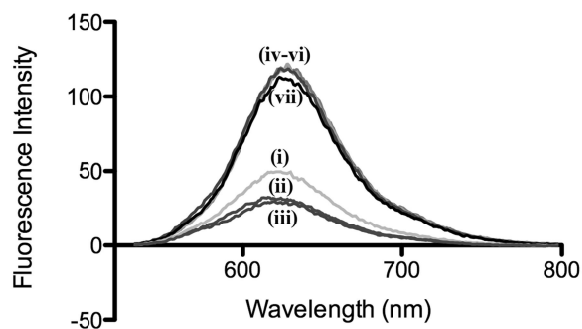


Figure 7. Comparison of the fluorescence intensity of **SP1** in 5:95 H_2O :acetonitrile (**Solution 1**) and in acetonitrile (**Solution 2**) with and without of Li^+ . (i) **Solution 1** prior to exposure to white light, (ii) **Solution 1** exposed to white light for 2 min, (iii) **Solution 2** exposed to white light for 2 min, (iv) **Solution 1** with 1 μM Li^+ exposed to UV for 7 min, (v) **Solution 2** with 1 μM Li^+ exposed to UV for 7 min, (vi) **Solution 1** with 1 μM Li^+ exposed to white light for 2 min, (vii) **Solution 2** with 1 μM Li^+ exposed to white light for 2 min.

Li^+ was then added to **Solutions 1** and **2** respectively and both solutions were exposed to UV for 7 min. Figure 7 shows that fluorescence intensity for the Li^+ bound **SP1** in **Solutions 1** and **2** is identical. Both also demonstrated a much slower rate of photodiscoloration that is consistent with the model proposed for a metal bound complex (Figure 6a). Finally, there is a subtle shift in λ_{max} from approximately 619 nm (**SP1**) to 625 nm for the **SP1-Li** complex. This wavelength shift is not observed for **Solution 1** in the absence of Li^+ . These experimental results are reproducible up to solutions containing 30 % water, above which **SP1** becomes non-dissolvable. Although acetonitrile was used in all fluorescence experiments in this work to minimize solvent related background fluorescence, it is clear that ion sensing experiments can still be performed the presence of aqueous media. While polar solvents do produce the opened MC form, these studies demonstrate that the compound can be readily converted to the closed SP form by exposure to white light prior to the addition of any Li^+ .

2.6 Photoswitching

The photoswitching process was next measured in the presence of $1 \mu\text{M}$ of Li^+ to show that ion binding and release could be optically controlled and measured by state-dependent fluorescence. Each “on-cycle” involved switching to the active merocyanine surface (fluorescent) on exposure to UV light to allow the formation of the **SP1-Li** complex. Each off-cycle was generated on exposure to white light to expel the Li ions back into solution to regenerate the passive spiropyran surface (non-fluorescent). The entire process occurred within the micron-scale holes of the MOFs. The resulting fluorescence spectra show that the binding process with **Sensor-1** can be repeated at least five times without problem (Figure 8).

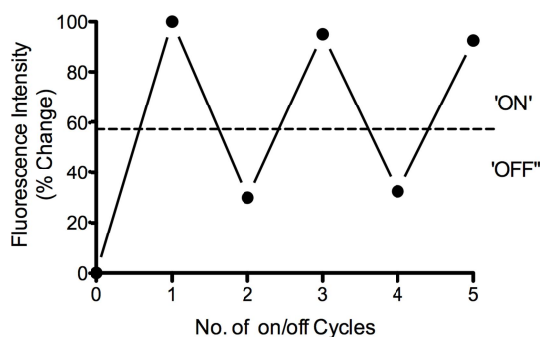


Figure 8. Photoswitching of the surface functionalized MOF. Cycle 0 represents the state before **Sensor-1** was filled with $1 \mu\text{M}$ Li^+ solution. Cycles 1, 3, 5 represent each ‘On-cycle’ where **Sensor-1** was exposed to UV ($\lambda = 365 \text{ nm}$) for 7 min respectively. Cycles 2 and 4 represents each ‘Off-cycle’ where **Sensor-1** was exposed to white light for 12 min respectively.

2.7 Sensor Re-use

The potential reusability of the sensor after each measurement was investigated. A section of **Sensor-1** already filled with $1 \mu\text{M}$ of Li^+ solution was removed from the optical set-up and placed back onto the fiber-filling chamber. The solution $1 \mu\text{M}$ of Li^+ was then expelled by successive flushing with air, acetonitrile and air. The fluorescence of the now ‘empty’ **Sensor-1** was then obtained as shown in Figure 9. The emission spectrum obtained for this ‘empty’ **Sensor-1** is identical to that of the fiber, which has not been surface functionalized with **SP1** (**empty fiber**, Figure 9). This strongly indicated that Li ions were completely removed from the sensor during flushing. **Sensor-1** was then refilled with a fresh solution of $1 \mu\text{M}$ of Li^+ ions, exposed to UV (7 min) and fluorescence spectra obtained. The resultant emission spectrum (Figure 9 blue) is comparable to that obtained in the initial experiment (Figure 9). This clearly shows that it is possible to empty the fiber of the ion solution and re-fill with a fresh solution with negligible effect on the function of the sensor or the surface attached **SP1** molecules. Future work will involve constructing a more sophisticated process that would include the likes of a syringe pump to the optical set-up shown in Figure 3 so that the fiber remains undisturbed.

Importantly in practical devices, the interaction between ion binding and light can be further improved by guiding white light through the fiber instead. This will enable the sensor system to be kept in a light-tight enclosure to avoid unintended light-molecular switch interactions.

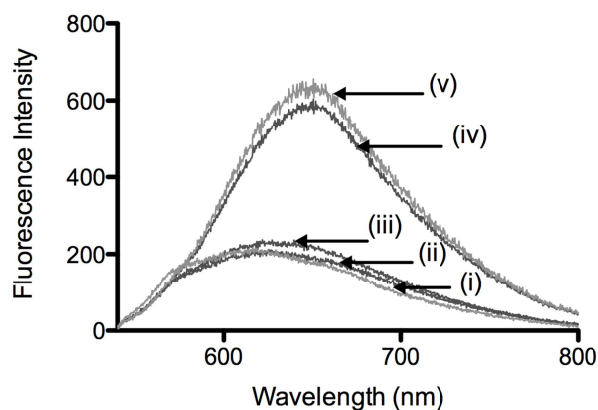


Figure 9. Fluorescence emission to demonstrate sensor re-use. (ii) Empty fiber. (iii) First round of filling with 1 μM Li^+ in acetonitrile. (iv) First round, after irradiation with UV for 7 min. (i) After removing Li ions from **Sensor-1**. (v) Second round of filling with 1 μM Li^+ , after irradiation with UV for 7 min.

3. CONCLUSION

The ubiquitous nature of metal ions in the environment and biology presents a real need to develop new methodology for their rapid and efficient detection. Such sensors would have applications in disease diagnosis and study, as well as environmental sensing. In an effort to meet this need, we have developed the first nanoliter-scale, regenerable ion sensor based on microstructured optical fiber (MOF). The air holes of the MOF were functionalized with a monoazacrown bearing spiropyran (**SP1**) to give a photo-switchable sensor that detects lithium ions down to 100 nM in nanoliter-scale volumes. The original compound reported by Kimura et al⁷ was not functionalized for surface attachment although the authors have recently reported the functionalization of silica with a similar crowned spirobenzopyran.^[41] Using the original compound as a model, we thus designed and prepared a modified compound containing a carboxyl group to allow attachment to the MOF and hence surface-based sensing. This enables the MOF's capacity to sense within small sample volumes (<10 nL) to be exploited.

The integration of a photoswitchable molecule bearing an ionophore within the MOF is new and we wished to understand the outcome of this integration and to then examine the operation of this novel sensing platform. We have demonstrated that ion binding is turned on and off on upon irradiation with light, with the sensor being unaffected by multiple rounds of photoswitching. More importantly, we have showed that unbound ions are flushed from the fiber in the 'off' state to allow the sensor to be reused. The integration of an ionophore into the sensor paves the way for the development of highly specific light-based sensing platforms that are readily adaptable to sense a particular ion simply by altering the ionophore design. This work presents advances in both fiber sensing technology and in developing fast, sensitive "point of care" ion sensing methods that can be easily deployed, are disposable and affordable, particularly in the areas of disease diagnosis. Ongoing work is concerned with designing novel ionophores for binding specific ions and decreasing the detection limit of the sensor, with the aim of sensing down to the pM levels as recently demonstrated by our group where the detection of CdSe quantum dots down to 10 pM levels has been demonstrated.^[38]

ACKNOWLEDGMENTS

S. Heng acknowledges the support of an ARC Super Science Fellowship. R. Kostecki acknowledges the Australian Defence Science and Technology Organisation (under the Signatures, Materials and Energy Corporate Enabling Research Program) for support of the suspended core Si fibre development. T. M. Monro acknowledges the support of an

ARC Federation Fellowship and A. D. Abell an ARC discovery grant. We acknowledge Tze (Herbert) Foo for useful discussions on surface functionalization, Daniel Stubing for the photostability data for diarylalkene and indolefulgide, Peter Henry for his contribution to the silica fiber drawing, and Stephen Warren-Smith for his contribution to the fiber optical power calculations.

REFERENCES

- [1] Costante, G.; Filetti, S., "Early diagnosis of medullary thyroid carcinoma: is systematic calcitonin screening appropriate in patients with nodular thyroid disease?", *Oncologist*, 16, 49 (2011)
- [2] Cymet-Ramirez, J.; Alvarez-Martinez, M. M.; Garcia-Pinto, G.; Frias-Austria, R.; Meza-Vernis, A.; Rosales-Munoz, M. E.; Isunza-Ramirez, A.; Isunza-Alonso, O. D.; Brito-Ramirez, J. A.; Anaya-Garcia, M.; Lizalde-Yanez, A., "Early diagnosis of hip dysplasia. Crippling disease for life. Consensus of the Mexican College of Orthopedics and Traumatology", *Acta Ortop Mex*, 25, 313 (2011)
- [3] Dartigues, J. F., "Alzheimer's disease: early diagnosis", *Rev Prat*, 61, 926 (2011)
- [4] Genoni, G.; Demarchi, I.; Bellone, S.; Petri, A.; Settanni, F.; Dondi, E.; Negro, M.; Cortese, L.; Prodram, F.; Bona, G., "Early diagnosis of Fabry disease in children", *Minerva Pediatr*, 63, 425 (2011)
- [5] Glusker, J. P., Katz, A. K., Bock, C. W., "Metal Ions in Biological Systems", *The Rigaku Journal* 16, 8 (1999)
- [6] Thompson, K. H.; Orvig, C., "Boon and bane of metal ions in medicine", *Science*, 300, 936 (2003)
- [7] Kimura, K.; Yamashita, T.; Yokoyama, M., "Syntheses, Cation Complexation, Isomerization and Photochemical Cation-Binding Control of Spirobenzopyrans Carrying a Monoazacrown Moiety at the 8-Position", *J. Chem. Soc., Perkin Trans. 2*, 613 (1992)
- [8] Ruhland, B.; Becker, T. H.; Muller, G., "Gas-kinetic interactions of nitrous oxides with SnO₂ surfaces", *Sens Actuators B Chem.*, 50, 85 (1998)
- [9] Lee, B.; Roh, S.; Park, J., "Current status of micro- and nano-structured optical fiber sensors", *Opt. Fiber Technol.*, 15, 209 (2009)
- [10] Afshar, S. V.; Ruan, Y. L.; Warren-Smith, S. C.; Monroe, T. M., "Enhanced fluorescence sensing using microstructured optical fibers: a comparison of forward and backward collection modes", *Opt. Lett.*, 33, 1473 (2008)
- [11] Monroe, T. M.; Belardi, W.; Furusawa, K.; Baggett, J. C.; Broderick, N. G. R.; Richardson, D. J., "Sensing with microstructured optical fibres", *Measurement Science & Technology*, 12, 854 (2001)
- [12] Monroe, T. M.; Warren-Smith, S.; Schartner, E. P.; Francois, A.; Heng, S.; Ebendorff-Heidepriem, H.; Afshar, S., "Sensing with suspended-core optical fibers", *Optical Fiber Technology*, 16, 343 (2010)
- [13] English, F. V.; Foo, T. C.; Richardson, A. C.; Ebendorff-Heidepriem, H.; Sumby, C. J.; Monroe, T. M., "Photoinduced Electron Transfer Based Ion Sensing within an Optical Fiber", *Sensors*, 11, 9560 (2011)
- [14] Warren-Smith, S. C.; Heng, S.; Ebendorff-Heidepriem, H.; Abell, A. D.; Monroe, T. M., "Fluorescence-Based Aluminum Ion Sensing Using a Surface-Functionalized Microstructured Optical Fiber", *Langmuir*, 27, 5680 (2011)
- [15] Ruan, Y.; Foo, T. C.; Warren-Smith, S.; Hoffmann, P.; Moore, R. C.; Ebendorff-Heidepriem, H.; Monroe, T. M., "Antibody immobilization within glass microstructured fibers: a route to sensitive and selective biosensors", *Opt. Express*, 16, 18514 (2008)
- [16] Wiedemann, U.; Alt, W.; Meschede, D., "Switching photochromic molecules adsorbed on optical microfibres", *Opt. Express*, 20, 12710 (2012)
- [17] Berkovic, G.; Krongauz, V.; Weiss, V., "Spiropyran and Spirooxazines for Memories and Switches", *Chem. Rev.*, 100, 1741 (2000)
- [18] Shen, K. H.; Li, X. L.; Choi, D. H., "Synthesis and photochromic behavior of spiropyran dyes", *Chemical Journal of Chinese Universities-Chinese*, 26, 935 (2005)
- [19] Lukyanov, B. S.; Lukyanova, M. B., "Spiropyran: synthesis, properties, and applications", *Chem. Heterocycl. Compd.*, 41, 281 (2005)
- [20] Phillips, J. P.; Mueller, A.; Przystal, F., *J. Am. Chem. Soc.*, 87, 4020 (1965)

- [21] Natali, M.; Giordani, S., "Molecular switches as photocontrollable "smart" receptors", *Chem. Soc. Rev.*, 41, 4010 (2012)
- [22] Yagi, S.; Nakamura, S.; Watanabe, D.; Nakazumi, H., "Colorimetric sensing of metal ions by bis(spiropyran) podands: Towards naked-eye detection of alkaline earth metal ions", *Dyes Pigm.*, 80, 98 (2009)
- [23] Kruttwig, K.; Yankelevich, D. R.; Brueggemann, C.; Tu, C.; L'Etoile, N.; Knoesen, A.; Louie, A. Y., "Reversible low-light induced photoswitching of crowned spiropyran-DO3A complexed with gadolinium(III) ions", *Molecules (Basel, Switzerland)*, 17, 6605 (2012)
- [24] Fries, K. H.; Driskell, J. D.; Sheppard, G. R.; Locklin, J., "Fabrication of Spiropyran-Containing Thin Film Sensors Used for the Simultaneous Identification of Multiple Metal Ions", *Langmuir*, 27, 12253 (2011)
- [25] Byrne, R. J.; Stitzel, S. E.; Diamond, D., "Photo-regenerable surface with potential for optical sensing", *J Mater Chem*, 16, 1332 (2006)
- [26] Scarmagnani, S.; Walsh, Z.; Lopez, F. B., "Photoswitchable Stationary Phase Based on Packed Spiropyran Functionalized Silica Microbeads", *e-J. Surf. Sci. Nanotech.*, 7, 649 (2009)
- [27] Katz, E.; Willner, B.; Willner, I., "Light-controlled electron transfer reactions at photoisomerizable monolayer electrodes by means of electrostatic interactions: active interfaces for the amperometric transduction of recorded optical signals", *Biosens Bioelectron*, 12, 703 (1997)
- [28] Shiraishi, Y.; Sumiya, S.; Manabe, K.; Hirai, T., "Thermoresponsive copolymer containing a coumarin-spiropyran conjugate: reusable fluorescent sensor for cyanide anion detection in water", *ACS Appl. Mater. Interfaces*, 3, 4649 (2011)
- [29] Tomasulo, M.; Kaanumal, S. L.; Sortino, S.; Raymo, F. M., "Synthesis and properties of benzophenone-spiropyran and naphthalene-spiropyran conjugates", *J. Org. Chem.*, 72, 595 (2007)
- [30] Ebdorff-Heidepriem, H.; Warren-Smith, S. C.; Monro, T. M., "Suspended nanowires: fabrication, design and characterization of fibers with nanoscale cores", *Opt. Express*, 17, 2646 (2009)
- [31] Kostecki, R.; Ebdorff-Heidepriem, H.; Davis, C.; McAdam, G.; Warren-Smith, S. C.; Monro, T. M., "Silica exposed-core microstructured optical fiber", *Opt. mater. express*, 2, 1538 (2012)
- [32] Kubodera, S.; Wisoff, P. J.; Tittel, F. K.; Sauerbrey, R. A., "Coupling of high-power UV laser radiation into fused silica fibers using pressurized gases", *Appl. Opt.*, 27, 1638 (1988)
- [33] Kawamura, K., "In situ UV-VIS detection of hydrothermal reactions using fused-silica capillary tubing within 0.08-3.2 s at high temperatures", *Anal. Sci.*, 18, 715 (2002)
- [34] Warren-Smith, S. C.; Afshar, S.; Monro, T. M., "Fluorescence-based sensing with optical nanowires: a generalized model and experimental validation", *Opt. Express*, 18, 9474 (2010)
- [35] Shimada, T.; Aoki, K.; Shinoda, Y.; Nakamura, T.; Tokunaga, N.; Inagaki, S.; Hayashi, T., "Functionalization on silica gel with allylsilanes. A new method of covalent attachment of organic functional groups on silica gel", *J. Am. Chem. Soc.*, 125, 4688 (2003)
- [36] Wen, K.; Maoz, R.; Cohen, H.; Sagiv, J.; Gibaud, A.; Desert, A.; Ocko, B. M., "Postassembly chemical modification of a highly ordered organosilane multilayer: new insights into the structure, bonding, and dynamics of self-assembling silane monolayers", *ACS nano*, 2, 579 (2008)
- [37] Zhang, P.; Meng, J. B.; Li, X. L.; Matsuura, T.; Wang, Y. M., "Synthesis and photochromism of photochromic spiro compounds having a reactive pendant group", *J. Heterocycl. Chem.*, 39, 179 (2002)
- [38] Schartner, E. P.; Ebdorff-Heidepriem, H.; Warren-Smith, S. C.; White, R. T.; Monro, T. M., "Driving down the detection limit in microstructured fiber-based chemical dip sensors", *Sensors (Basel, Switzerland)*, 11, 2961 (2011)
- [39] Zhang, S.; Zhang, Q.; Ye, B.; Li, X.; Zhang, X.; Deng, Y., "Photochromism of spiropyran in ionic liquids: enhanced fluorescence and delayed thermal reversion", *J. Phys. Chem. B*, 113, 6012 (2009)
- [40] Salhin, A. M. A.; Tanaka, M.; Kamada, M.; Ando, H.; Ikeda, T.; Shibutani, Y.; Yajima, S.; Nakamura, M.; Kimura, K., "Decisive Factors in the Photoisomerization Behavior of Crowned Spirobenzopyrans: Metal Ion Interaction with Crown Ether and Phenolate Anion Moieties", *Eur. J. Org. Chem.*, 655 (2002)
- [41] Nakahara, Y.; Yamaguchi, Y.; Iwamoto, H.; Sakamoto, H.; Kimura, K., "Effect of photoirradiation on chromatographic separation of alkali metal ions using crowned-spirobenzopyran-immobilized silica", *Anal Methods-Uk*, 4, 4025 (2012)