Membranes

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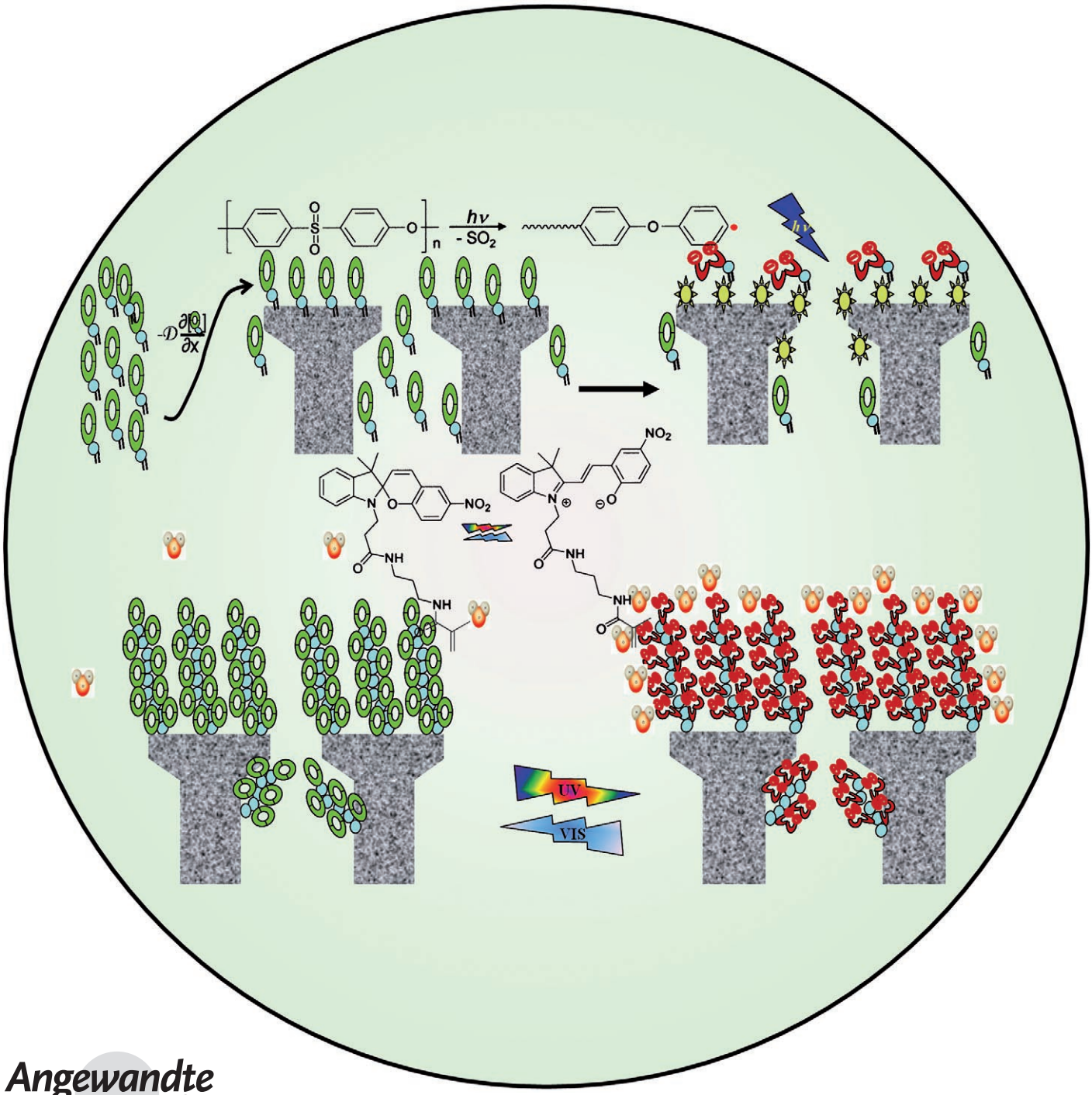
An Optically Reversible Switching Membrane Surface**

*Arpan Nayak, Hongwei Liu, and Georges Belfort**



The surface chemistry of synthetic membranes is of great interest to the bioprocessing of protein-containing solutions, to the filtration of medically relevant solutions, and to the performance of membrane-based sensors.[1] For biosensors, controlling the polarity of the membrane and reducing nonspecific protein adsorption would significantly improve signal to noise ratios. This may also be useful as a switch to control diffusive transport to biosensor elements (enzymes, chemoreceptors, etc) by changing the polarity of the protective membrane when desired. For bioprocessing, it has direct bearing on the efficacy of producing and delivering desirable proteins and other biologically derived molecules at high yield and purity.[2]

The performance of synthetic polymer membranes when filtering bioprocessing and biomedical fluids is severely limited by the interaction of proteins with membrane surfaces. Currently, expensive and labor-intensive methods such as controlling the pH and ionic strength of the feed solution, periodic cleaning, and back flushing are used to deal with the loss of filtration performance resulting from protein adhesion. It would therefore be extremely attractive to have an in situ cleaning procedure in which filtration performance could be re-established with minimum need for expensive cleaning chemicals and down time owing to back flushing. Synthesizing membranes with polymers that reduce such interactions has resulted in improved filtration performance.[3,4]

We suggest herein that grafting environmentally responsive polymers onto synthetic membranes by using simple inexpensive methods such as photograft-induced polymerization, which can be easily incorporated into the membrane synthesis process, may offer an alternative method to the approaches that use traditional protein-adhesion-resistant monomers such as polyethylene glycol and other hydrophilic monomers.[3,5] Specifically, we combine two known technologies, a patented UV-grafting process with the well-known photoresponsive properties of spiropyran molecules, to produce an optically reversible switching membrane surface.

Vinyl monomers with desirable functionality are easily grafted and polymerized onto UV-sensitive polymers such as poly(ether sulfone) (PES) membranes by using a UV-induced graft polymerization method (Figure 1 A).[6] Exposure of a PES synthetic membrane to 300-nm UV radiation produces radical sites onto which vinyl monomers such as vinyl spiropyran can graft and polymerize. The spiropyrans are comprised of a group of light-switchable photochromic organic molecules that are colorless, nonpolar, and in a "closed" form in the visible light.[7,8] When exposed to UV and visible light, spiropyrans isomerize and produce a colored polar "open" merocyanine form and a white nonpolar "closed" form, respectively (Figure 1 B–E, Figure 2).

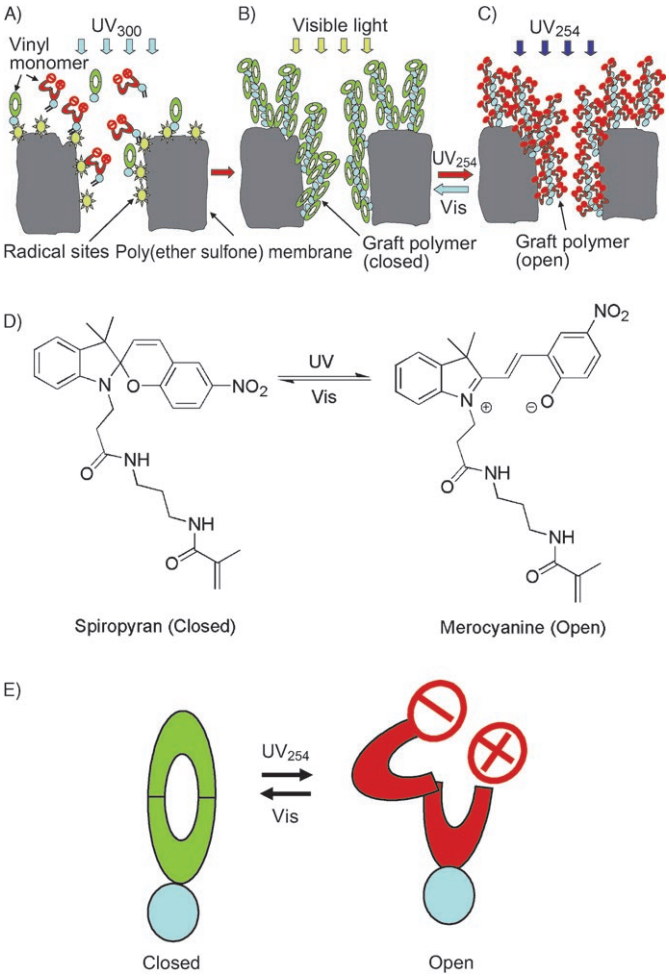


Figure 1. Schematic of graft polymerization and switching: A) Rensselaer's patented photografting process in which a synthetic ultrafiltration membrane formed from PES (photo sensitive polymer) is first dipped into a vinyl (spiropyran) monomer solution (1% w/v) in ethyl acetate for 1 h and then exposed to 300-nm UV irradiation to induce grating and polymerization. B) Exposure of the grafted PES membrane to visible light for 1 h resulting in the formation of the "closed" apolar white form of spiropyran. C) Exposure of the grafted "closed" form of the PES membrane to UV irradiation at 254 nm for 1 h formed the "open" polar red form of spiropyran. D) The chemical structure of the vinyl spiropyrans in two configurations as a function of UV and Vis irradiation. E) A schematic of the chemical structure of the vinyl spiropyrans in two configurations as a function of UV and visible (Vis) irradiation.

Light-switching of monolayers of azobenzene[9–11] and spiropyran[12] has been studied on solid substrates. Previously, binding of photochromic moieties to solid substrates required complicated and multi-step procedures,[13,14] whereas herein we use a relatively simple two-step dip and UV-irradiation process that could be easily incorporated into a commercial manufacturing process.[15] The challenge was to synthesize optically active vinyl spiropyran (1′-(2-propylcarbamylmethacrylamide)ethyl)-3′,3′-dimethyl-6-nitrospiro[2*H*-1]benzo-

[*] A. Nayak, H. Liu, Prof. G. Belfort
Howard P. Isermann Department of Chemical and Biological Engineering
Rensselaer Polytechnic Institute
Troy, New York 12180 (USA)
Fax: (+1) 518-276-4030
E-mail: belfog@rpi.edu

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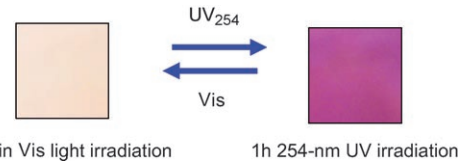


Figure 2. Color change of spiropyran-grafted PES membrane after exposure to UV irradiation at 254 nm for 1 h and visible light for 5 min in air.

pyran-2,2′-indoline) and determine whether the photografting process that uses UV light at 300 nm would allow the vinyl spiropyran molecules to graft and retain their switchable properties. A commercial PES 30-kDa synthetic membrane filter was chosen as a model membrane surface because of its high sensitivity to 300-nm UV radiation and because it is one of the most widely used polymeric materials that is used to produce commercial ultrafiltration membranes and support layers for reverse osmosis and nanofiltration membranes.[16]

The synthesized vinyl spiropyran was photografted onto the 30-kDa PES ultrafiltration membranes by using our standard grafting method. Attenuated total reflection Fourier transform infrared spectroscopy (ATR/FTIR) was used to demonstrate that the vinyl monomers were grafted onto the PES membranes. In Figure 3, ATR/FTIR spectra are compared for the unmodified PES membrane with those of the grafted-spiropyran-modified PES membranes. The peaks at 1410, 1487, 1579, and 1663 cm^{-1} are owing to the -SO_2 group, the aromatic-ring stretch of C=C, the aromatic C–H stretch, and the aromatic C=O stretch, respectively. The last peak may shift depending on the chemical and charge environment. Under exposed visible light for 5 min and on a dry membrane, the peak at $\approx$1663 cm^{-1} is maximized whereas the peak at $\approx$1720–5 cm^{-1} is reduced. Similarly, under 254-nm UV radiation for 1 h on a dry membrane, the peak at $\approx$1663 cm^{-1} is reduced whereas that at $\approx$1720–5 cm^{-1} is

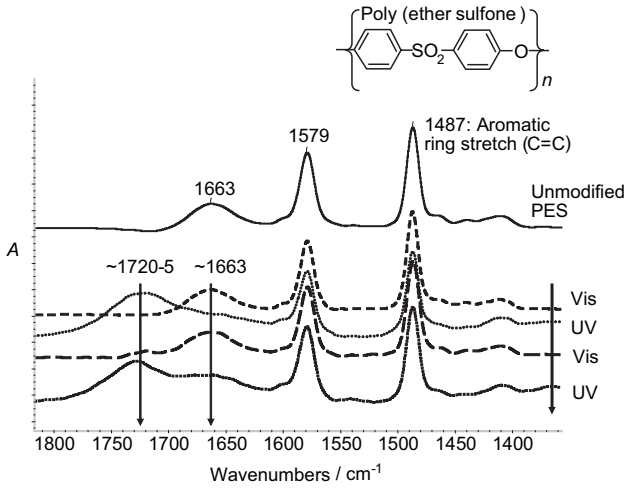


Figure 3. Five ATR/FTIR spectra of the unmodified (as received) PES ultrafiltration membrane and the spiropyran-grafted PES membrane after two cycles of exposure to UV irradiation at 254 nm for 1 h and visible light for 5 min. Note that the peak at $\approx$1663 cm^{-1} decreases whereas the peak at $\approx$1720–1725 cm^{-1} increases in intensity during exposure to UV at 254 nm.

increased. If the ratio of peak heights, $R = (h_{1720}/h_{1487})/(h_{1663}/h_{1487})$, is defined as a measure of the ratio of the relative chemical surface condition under ultraviolet radiation versus that under visible-light exposure, we can follow this ratio with changing irradiation exposure in Figure 4A. The reference peak at 1487 cm^{-1} is a measure of the carbon–carbon aromatic-ring stretch and is used as an internal standard as it is independent of the grafting process.

Similarly, measuring the sessile contact angle of the grafted dry PES membrane with a water droplet after alternating exposure to 254-nm UV (1 h) and visible light (5 min) demonstrates changes in surface wettability (Figure 4B). About a 16° drop in contact angle was obtained through this process. This is similar in magnitude to that reported by Lahann and co-workers who used electrical potential to induce a polarity change in a self-assembled polar molecule on a gold surface.[17] As protein–membrane interactions can determine success or failure for many processes including those mentioned above, we demonstrate, through two related experiments, that the switchable hydrophilic (on exposure to 254-nm UV light) and hydrophobic (with visible light) surfaces 1) exhibit low and high protein adsorbabilities, respectively, and 2) display high and low buffer-permeations rates after protein adsorption, respectively. Results for these two experiments are shown in Figure 4C,D. Bovine serum albumin (BSA; 67 kDa, pI 4.7) was used as a model protein for the adsorption experiments. Clearly, from the data shown in Figure 4C, the as-received PES membrane exhibited the highest adsorbed amount of BSA in 10 mM phosphate-buffered saline (PBS) buffer solution at pH 7.4 and 22 ± 1 °C followed by the grafted vinyl spiropyran in the "closed" (visible light) and "open" (254-nm UV light) configuration. The "closed" configuration of the vinyl spiropyran surface adsorbed about 26% more protein than the "open" configuration of the surface. These surfaces, with adsorbed protein on them, were then tested to see if this difference in protein adsorption translated into higher permeation flux. The modified membranes were then placed into a filtration test cell and the permeation rate of freshly prepared PBS at pH 7.4 and 22 ± 1 °C measured (Figure 4D). As expected, the "closed" configuration of the vinyl spiropyran gave about a 17% lower permeation flux as compared with the "open" configuration of the vinyl spiropyran surface. The contact-angle measurements of the base PES membrane, of the base PES membrane after washing with ethyl acetate solvent, and of the base PES membrane irradiated with 254-nm UV light for 1 h were also obtained (see the Supporting Information). No noticeable change in contact angle was observed in this control experiment. However, irradiation with UV light under wet rather than dry conditions reduces the switching time from "closed" to "open" conformation by about one third (data not shown).

The results presented herein demonstrate the reversible conversion of a photografted, photoresponsive, polymeric synthetic membrane surface from polar to nonpolar character. We have clearly shown that a UV/Vis-switchable moiety such as spiropyran can be grafted onto a commercial photoactive polymer such as PES by using a very simple photograft polymerization method. Both molecular (ATR/FTIR) and

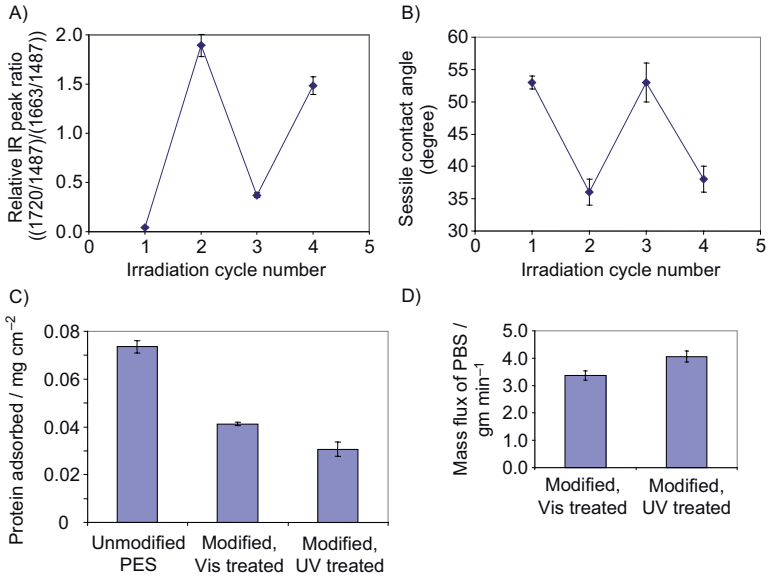


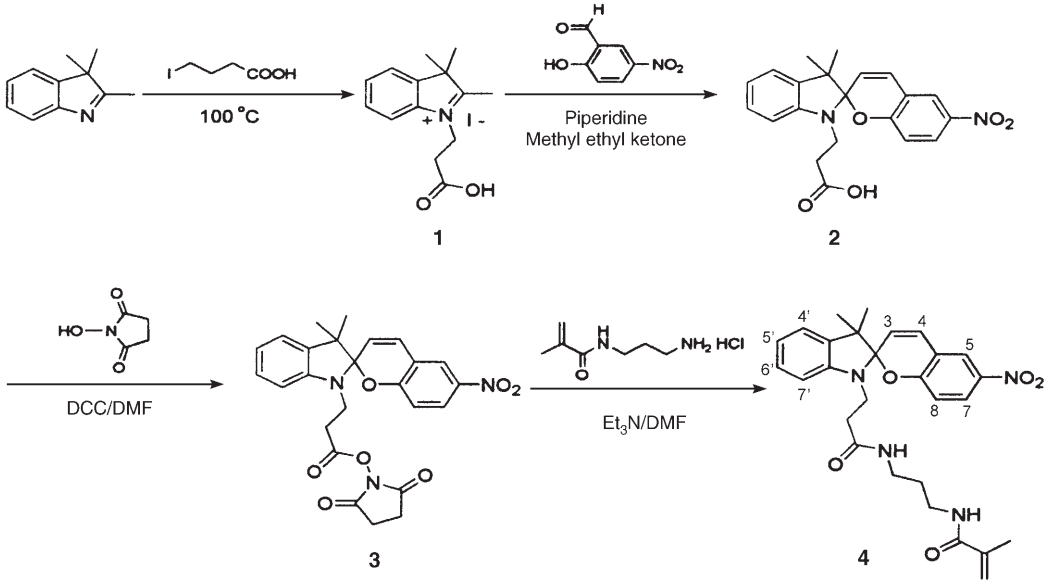
Figure 4. Summary data demonstrating the optically reversible switching of the wettability of the spiropyran grafted PES membrane surface after exposure to 254-nm UV light and visible light. A) Ratio of peak heights, $R=(h_{1720}/h_{1487})/(h_{1663}/h_{1487})$, from ATR/FTIR spectra from Figure 3 as a function of alternating exposure to 254-nm UV light (1 h) and visible light (5 min) or cycle number. B) Surface wettability changes as measured by the sessile contact angle of the grafted PES membrane with a water droplet after alternating exposure to 254-nm UV light (1 h) and visible light (5 min) or cycle number. C) Adsorption of BSA in PBS (10 mM; pH 7.4) at 22±1°C for 1 h on the as-received PES membrane and on modified PES membranes with grafted vinyl spiropyran in the "closed" (visible light) and "open" (254-nm UV light) configuration. D) Mass flux of PBS at pH 7.4 and 22±1°C permeating through modified PES membranes with grafted vinyl spiropyran in the "closed" (visible light) and "open" (254-nm UV light) configuration after exposure to BSA adsorption from (C).

classical (color, contact angle, protein adsorption, and subsequent buffer ultrafiltration) measurements were used to confirm the reversible polar–apolar reaction of grafted spiropyran owing to exposure of UV/Vis radiation. Rather than inducing a physical movement (conformational change) of long-chain SAMs toward an electrode by changing the surface potential of a gold substrate,[17,18] we have synthesized and grafted a photoresponsive molecule (vinyl spiropyran) onto a widely used industrial polymer. This was accomplished by combining two technologies, our patented UV grafting process with the well-known photoresponsive properties of spiropyran molecules, to produce an optically reversible switching membrane surface. The relative ease of this grafting and switching process should suggest many industrial opportunities including those dependent on surface wettability and molecular adhesion.

Future work will involve further optimization of the approach presented herein. Further research with triggers other than UV/Vis radiation, such as temperature, pH, and ionic strength, can be pursued with vinyl functional groups and photosensitive PES. Besides membrane filtration and sensors, there is a need to consider surface properties of materials for catheters, surgical instruments, and pulmonary breathing tubes that are exposed to body fluids, and for spot detection for genomic analysis and nanoprocessing in micro-fluidic devices.

Experimental Section

Vinyl spiropyran (1′-(2-(propylcarbamylmethacrylamide)ethyl)-3′,3′-dimethyl-6-nitrospiro[2H-1]benzo- pyran-2,2′-indoline): 3-aminopropyl methacrylamide hydrochloride was obtained from Polysciences Inc. All other chemicals were purchased from Aldrich Chemical Co. and used without further purification. 1-(2-carboxyethyl)-2,3,3-trimethylindolenine iodide (**1**), 1′-(2-carboxyethyl) −3′,3′-dimethyl −6- nitro spiro-[2H-1]benzopyran-2,2′-indoline (**2**), and 1′-(2-(carbosuccinimidyloxy)ethyl)-3′,3′-dimethyl-6-nitrospiro[2*H*-1]benzopyran-2,2′-indoline (**3**) were synthesized according to literature methods[19] (Scheme 1).



Scheme 1. Synthesis of the spiropyran-containing monomer compound **4**. DCC = dicyclohexyl carbodiimide.

NMR spectra were measured on a Varian spectrometer by using tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded by using electrospray ionization.

1′-(2-(Propylcarbamylmethacrylamide)ethyl)-3′,3′-dimethyl-6-nitrospiro[2H-1]benzo- pyran-2,2′-indoline (**4**): The mixture of **3** (3.26 g, 6.83 mmol), 3-aminopropyl methacrylamide hydrochloride (1.34 g, 7.50 mmol), and triethyl amine (1 mL, 7.19 mmol) was stirred in *N*,*N*-dimethylformamide (DMF; 50 mL) at room temperature for 20 h. After the evaporation of DMF, the residue was dissolved in chloroform, washed with water and then purified by silica-gel column chromatography (eluent, ethyl acetate/hexane; 1:1 v/v) to give **4** as a yellow solid (2.6 g, 76%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.99$ (m, 2H; 5-H and 7-H), 7.18 (t, 1H; 6′-H), 7.08 (d, 1H; 4′-H), 6.89 (m, 2H; 4-H and 5′-H), 6.75 (d, 1H; 8-H), 6.67 (d, 1H; 7′-H), 6.51 (m, 1H; NH), 6.39 (m, 1H; NH), 5.87 (d, 1H; 3-H), 5.74 (s, 1H; CH_2), 5.35 (s, 1H; CH_2), 3.68 (m, 1H; CH_2N), 3.51 (m, 1H; CH_2N), 3.20 (m, 4H; CH_2), 2.56 (m, 1H; CH_2CO), 2.44 (m, 1H; CH_2CO), 1.93 (s, 3H; CH_3), 1.58 (m, 2H; CH_2), 1.27 (s, 3H; CH_3), 1.17 ppm (s, 3H; CH_3); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 18.9$, 20.1, 26.0, 29.8, 35.9, 36.3, 40.2, 53.1, 107.0, 115.7, 118.9, 120.0, 120.2, 122.0, 122.2, 123.0, 126.1, 128.0, 128.5, 136.1, 139.9, 141.2, 146.7, 159.7, 169.2, 171.9 ppm.

MS calcd $C_{28}H_{32}N_4O_5$: 504.24. Found $[M+H]^+$: 505.2, $[M+Na]^+$: 527.1.

Preparation of modified membranes: 30-kDa PES membranes were modified by using a UV-induced graft polymerization method as described in detail in Taniguchi, Belfort and co-workers.[3,6,15,16] A Rayonet photochemical chamber reactor system (Model RPR-100, Southern New England, Ultraviolet Co., Branford, CT) containing 300-nm UV lamps ($\approx$15% of the energy was at $<$280 nm) was used. The membranes were dipped in spyropyran monomer solution (1% *w*/*v* in ethyl acetate) for 1 h with stirring at 22 ± 1 °C, removed from the monomer solution, purged with N_2 for 10 min, and irradiated with 300-nm UV light in water-saturated N_2 for 4 min. After photografting, the membranes were again cleaned with ethyl acetate by shaking overnight to remove homopolymer and unreacted monomer from the membrane. Then, the modified membranes were vacuum dried for use.

ATR/FTIR: ATR/IR (Magna-IR 550 Series II, Thermo Nicolet Instruments Corp., Madison, WI) was used to obtain a measure of the degree of grafting. By using an incident angle of 45°, the penetration or sampling depth was approximately 0.1–1.0 μm. Spectra were collected at a gain of 8 and resolution of 2 cm^{-1} with 512 scans for each sample.

Synthetic membrane: The PES membrane with a 30-kDa molecular-weight cut off from lot 9140E was obtained from Pall Corp. (East Hills, NY). These Omega series have been slightly hydrophilized by the manufacturer by an undisclosed process as evidenced by the small carbonyl peak at $\approx$1663 cm^{-1}.

Contact angle: The sessile contact angle of water in air on the membrane substrates was measured by using an optical system (SIT camera, SIT66, Dage-MTI, Michigan, IN) converted to a video display. Water droplets of 2.5 μL were placed on the membrane substrates at different positions and the contact angles were measured. At least five measurements were made and the average reported.

BSA adsorption: BSA was dissolved in PBS buffer solution (10 mM; pH 7.4) to prepare a 1 mg mL^{-1} protein solution. Membrane swatches (3 cm^2) were immersed in the BSA solution for 2 h at 22 ± 1 °C. The amount of adsorbed protein was determined by staining with Ponceau S solution (Ponceau S (2%), trichloroacetic acid (30%), and sulfosalicylic acid (30%)). Membranes with adsorbed protein were immersed for 1 h into a solution of Ponceau S, washed thoroughly with deionized water, immersed for 1 h in acetic acid (5% v/v), and again washed with deionized water. Then the protein–dye complex was quantitatively eluted with NaOH solution (3 mL, 100 mM) for 1 h. Next, the membranes were removed, the solutions neutralized with HCl (50 μL, 6 M), and the absorbance of the red-colored solutions was measured at 515 nm. Protein amounts were calculated based on a calibration which was performed with known BSA amounts ($m_{BSA} = 0.001$–1 mg) onto clean unmodified PES membranes ($A_{515\,nm} = 0.6799\,m_{BSA} + 0.0754$; $R^2 = 0.9944$).

PBS filtration: PBS buffer solution (10 mM; pH 7.4) was used as the feed. The PBS buffer solution was composed of NaCl (137 mM) and KCl (2.7 mM) in deionized water. Membranes were immersed in the BSA solution (1 mg mL^{-1} in PBS buffer) for 5 min to induce protein adsorption. Then, PBS permeation flux through the membranes with adsorbed BSA was measured. A dead-end stirred cell (Model 8010, Millipore Corp., Bedford, MA) filtration system was used for PBS flux measurements through the membranes. The active membrane area was 3.8 cm^2. All filtration experiments were conducted at a constant transmembrane pressure of 69 kPa, a stirring rate of 500 rpm, and a system temperature of 22 ± 1 °C.

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