Combining Light-Gated and pH-Responsive Nanopore Based on PEG-Spiropyran Functionalization

Tianji Ma, Martin Walko, Mathilde Lepoitevin, Jean-Marc Janot, Emmanuel Balanzat, Armagan Kocer, and Sebastien Balme*

To mimic the selectivity and properties of biological channels and pumps, adaptive systems can be designed using artificial membranes. One big advantage of those membranes is that they are able to respond to several stimuli at the same time. Here, it is proposed to tailor conical nanopores by grafting polyethyleneglycol (PEG)-spiropyran in order to combine both light- and pH-responsive properties. The study of the ionic transport reveals that after UV irradiation the nanopore is open while after visible irradiation, it is closed. This gating property is due to the self-assembly of PEG-spiropyran. The reversibility of the assembly inside the nanopore strongly depends on the solvent. Indeed, water blocks the photoswitchable molecule in its zwitterionic form, while ethanol favors the conformational switching. Under UV irradiation, the pH can control the nanopore selectivity. It is demonstrated that at acidic pH, the nanopore is selective to anion while at neutral pH the cation transport is favored.

1. Introduction

The ion channels and pumps are involved in diverse biological processes such as neuron communication, regulation processes, water transport, energy production. Most of them exhibit a preferential transport of ions, which is characterized by a current rectification as well as reversible gating properties in response to external stimuli. One example of stimuli responsive channels is the light responsive ones which are involved in vision processes. The gating is regulated by a photoswitch molecule: the retinal, which isomerizes when absorbing a photon. Inspired by this process, many synthetic photoswitch molecules were synthetized to develop new types of materials or to trigger the nanopore aperture. Among them, spiropyran (SP) is particularly interesting because its photoinduced change is not only a simple stereo-isomerization but induces a modification of both its chemical structure and physical properties (Figure 1). Under visible light (VIS), the spiropyrans are uncharged and not soluble in water. Under UV irradiation, spiropyrans changes to a merocyanine (MC) form, which is zwitterionic and soluble in water. These exceptional properties have been used to functionalize both inorganic and polymer materials or to trigger the nanopore aperture. Among them, spiropyran (SP) is particularly interesting because its photoinduced change is not only a simple stereo-isomerization but induces a modification of both its chemical structure and physical properties (Figure 1). Under visible light (VIS), the spiropyrans are uncharged and not soluble in water. Under UV irradiation, spiropyrans changes to a merocyanine (MC) form, which is zwitterionic and soluble in water. These exceptional properties have been used to functionalize both inorganic and polymer materials. Spiropyrans were immobilized on inorganic surfaces to tune their wettability, the binding of metal ions, or DNP antibody and their electrochemical properties. They allow controlling the aggregation and the solubility of nanoparticles with light. When grafted to polymers, they form reversible micelles. Spiropyrans were also successfully used to functionalize biomacromolecules to control their enzymatic activity, DNA hybridation, and folding. To mimic light responsive channels, spiropyrans were linked to biological mechanical channel such as MscL.

In membrane science, spiropyrans are particularly interesting because ionic transport across the membrane can be tuned by simply using light. It can be done with polymeric as well as inorganic membranes. In the case of the polymeric membranes, the light-gating is mainly due to the reorganization of polymers and/or their swelling. In the case of inorganic membranes, the properties are due to the modification of the wettability of the surface. Using this concept, spiropyrans were grafted inside nanoporous membranes. Vlassiouk et al. have functionalized alumina membranes with an alkoxysilane ended by amine groups. Following a similar idea, Zhang et al. have grafted spiropyrans inside single conical nanopores. These nanopores were obtained by the track-etched technique in polyethylene terephthalate (PET) films and exhibited unique
properties of an ionic diode. In this case, depending on the isomer of the spiropyrans, a modification of the current rectification was observed. Since the spiropyrans are directly grafted onto the nanopore surface wall, only their charge (uncharged or zwitterionic or positively charged) plays a role in the ionic transport. However, the characterization of such effect required a high voltage rate (typically ±5 V). In addition to the light responsiveness, spiropyrans also react to the pH and can modify the ionic transport accordingly. This is of particular interest in the design of multifunctional membranes.[22]

In this work, we generated a nanopore that is responsive to both light and pH. To do that, conical nanopores obtained by the track-etched technique were functionalized with spiropyrans. In order to amplify the gating properties of the conical nanopore, the spiropyran was linked to a long polymer chain of polyethylene glycol (PEG). According to previous works on copolymer micelles, such a system facilitates a self-assembly inside the nanopore.[13,18] Then, we studied the ionic transport properties of NaCl and KCl through these nanopores. We intended to determine the conformation adopted by the PEG-spiropyran inside the nanopore depending on the irradiation type. We discuss also the impact of PEG chain on the reversibility of this photoswitch. The power generated by the nanopore under salt gradients was also evaluated. Beside the influence of light irradiation, the pH was also investigated mainly to drive and determine the ionic selectivity of the nanopore.

2. Results and Discussion

2.1. Nanopore Functionalization

For this work, we selected conical nanopores with tip diameters from 5 to 9 nm (Table 1) obtained by track-etching under disymmetrical conditions (Figure 2). The nanopore functionalization by PEG-spiropyran was done following the three steps procedure. First, the ortho-pyridyldisulfide-polyethylene glycolamine (OPSS-PEG-NH2) was attached onto the carboxylate groups present at the surface of the inner wall of the nanopore, by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) activation (Figure 3). Then, the ortho-pyridylsulfide-polyethylene was reduced by dithiothreitol. The last step was a nucleophilic substitution of the iodine moieties of spiropyrans by thiol groups terminated by PEG chain (Figure 2a). The result was the attachment of the spiropyran to the PEG via a thioether bond. Each step of the nanopore functionalization was evidenced by the modification of the $I-V$ response in 1 m KCl as shown in Figure 2b,c. The PEGylation induced a decrease of the conductance, likely due to the reduction of the nanopore diameter. The rectification factor is defined as $rf = |I_{UV}/I_{VIS}|$. Because, its value depends on the surface charge density, it is a simple and efficient method to confirm the functionalisation of the conical nanopore after each step (Figure 2d). For the raw nanopore, $rf < 1$ due to the carboxylate groups which induce selectivity to cation. After grafting of the ortho-pyridyldisulfide-polyethylene, we observed an inversion of rectification factor ($rf > 1$). The latter is assigned to the protonation of pyridyl moieties which confers a global positive surface charge of the nanopore inner wall. The reduction of ortho-pyridyldisulfide groups induces an $rf < 1$ which evidences the loss of pyridyl moieties. Finally, after the grafting of spiropyran, we observe a lower current and a rectification factor close to 1 confirming the grafting of an uncharged molecule. We attempted to confirm the nanopore functionalization by an alternative method. To do so, the PEG-SP was grafted inside a multipore membrane (density 106 pores cm$^{-2}$) using the same method. The absorbance spectrum recorded with the functionalized membrane after UV irradiation reveals a peak around 550 nm which is characteristic of the MC form Figure 2e. This confirms that our method allows we are able to graft PEG-SP inside nanopore with our method.

2.2. Light-Responsive Ionic Diode

After the functionalization was completed, the influence of light irradiation on ionic diode properties was characterized by the $I-V$ dependence. In Figure 4a,b the $I-V$ responses of the nanopore after the PEG-spiropyran grafting in 0.1 m and 0.01 m KCl at pH 7 are reported. The first experiment was performed under visible light directly after functionalization. Then, the nanopore was irradiated with UV light for 30 min and the $I-V$ curve was measured in the same solution (Figure 4a,b). We observed that $I-V$ responses were different, confirming the presence of photochromic molecule inside the nanopore. In the first experiment, the $I-V$ curves were almost linear. Under VIS irradiation, the spiropyran is neutral explaining the loss of current rectification. After irradiation by UV light, an increase of current and a nonlinear $I-V$ response is observed. This can be explained by the transformation of spiropyran molecule into merocyanine, which is a zwitterionic molecule. In this case, the current rectification was induced by the presence of charges on

![Figure 1. Chemical structure of spiropyran.](image)

Table 1. Properties of the nanopores used in this study.

<table>
<thead>
<tr>
<th>Pore</th>
<th>$d_{UV}$ [nm]</th>
<th>$d_{VIS}$ [nm]</th>
<th>$D$ [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>5.3</td>
<td>1.1</td>
<td>1200</td>
</tr>
<tr>
<td>NP-2</td>
<td>12.7</td>
<td>1.3</td>
<td>555</td>
</tr>
<tr>
<td>NP-3</td>
<td>10.3</td>
<td>1.9</td>
<td>290</td>
</tr>
</tbody>
</table>
the nanopore inner wall. At this stage, our results were in good agreement with the one previously reported by Zhang et al.\cite{21} However, two points have to be discussed (i) the reversibility of the photoswitch and (ii) the influence of the PEG chains.

First, the switching from the SP to the MC form took a couple of seconds in water solution while it takes more than 30 min inside the pore (Figure 4c,d). This should be assigned to the low transmittance of PET in UV. We focused then on the reversibility of this photoswitch. After UV switching, the irradiation by visible light did not show a significant modification of the \( I-V \) response. This could be because the molecules are blocked in their merocyanine configuration. In previous works,
authors have also reported the difficulty to switch from MC to SP form in water. Vlassiouk et al. reported that the photoswitching occurs in water when the spiropyrans are attached directly to the inner surface of the nanopore.\cite{20} However, the photoswitching was only monitored in ethanol. Zhang et al. reported that the nanopore has to be dried to switch from MC to SP form. The authors assigned this nonreversibility in water to a high activation barrier, which is accompanied by the

![Figure 3. SEM image of base side of conical nanopore after etching time close to the ones for a) NP-3 and b) NP-2.](image)

![Figure 4. Typical I–V response obtained after VIS irradiation (open black circle) and after UV irradiation (full violet circle) for a) NP-1 (d_{UV} = 5.3 nm/d_{VIS} = 1.1 nm) and b) NP-2 (d_{UV} = 12.7 nm/d_{VIS} = 1.3 nm) at different KCl concentrations, i.e., 0.1 and 0.01 M. Kinetics of the nanopore (NP-2) opening under UV irradiation: c) evolution of the I–V response regarding the time of the visible irradiation; d) evolution of the nanopore conductance as a function of the irradiation time (here the I–V curve was recorded under light).](image)
dewetting process inside a nanopore.[21] We propose another mechanism which takes into account the PEG chain. The spiropyran form of the molecule is not soluble in water. Thus, when it is grafted at the surface of the inner wall of the nanopore, it will minimize its interaction with water molecule by increasing its interaction with the surface, especially if the latter is hydrophobic. In our case, the functionalization involves a PEG chain between the PET and the photoswitchable molecule. These PEG could stabilize the molecule in its merocyanine form because they are highly hydrophilic. In this case, we suppose that the merocyanine form is favored in this environment and cannot switch back to its SP form because they are surrounded by too many water molecules.

In order to confirm this hypothesis, we modified the polarity of the solvent, to facilitate switching from the MC to the SP form. With a mixture EtOH/water at different ratios, the photoswitching did not occur. However, by using pure EtOH, we could observe that after VIS irradiation, the nanopore conductance dramatically decreased, confirming the switching from MC to SP form. This experiment was repeated several times (Figure 5a). Thus, the reversible gating of the nanopore requires the use of ethanol to switch from the MC to SP form (Figure 5b).

As previously mentioned, single conical nanopores behave as an ionic diode due to a preferential ionic transport,[23] which is commonly characterized by the current rectification. The origin of this phenomenon has been extensively studied by Poisson-Nernst-Plank (PNP) simulations.[24] It appears that the nanopore dissymmetry induces a depletion of ionic concentration of co-ions compared to counter-ions. It has been shown that in the case of conical nanopores, the current rectification is more pronounced with the increase of surface charges.[25] Thus, the analysis of current rectification should emphasize the configuration of the photo switch molecule. In Figure 6b are reported the rectification factor as a function of the salt concentration after VIS and UV irradiation at pH 7. According to our experimental protocol, a rectification lower than 1 means that the transport of cations is favored. This is the case when the photoswitchable molecule is in its merocyanine configuration. With the increase of the salt concentration, the rectification tends to approach 1, as usually observed for charged nanopore. After switching to spiropyran isomer, since the surface is uncharged, it is not surprising that the rectification factor is around 1 for both NaCl and KCl independently of the salt concentration.

To go further in the investigation of the ionic transport inside nanofluidic diode functionalized by PEG-spiropyran, we studied the I–V response under different salt concentrations (0.001, 0.01, 0.1, and 1 M) for KCl and NaCl. From the linear part of I–V response typically between ~60 and 60 mV, we calculated the conductance G and plotted it as a function of the salt concentration as shown in Figure 6a. Regardless of the type of salt, the conductance under VIS was lower than the conductance after UV irradiation (Table 1). This difference could be explained by the wetting/dewetting of the nanopore depending on the photoswitch molecule isomerization state. Interestingly, for spiropyran isomer, the nanopores exhibit a similar apparent tip diameter around 1.3 nm. The decrease of the amplitude of the conductance and the similar diameter after VIS irradiation suggest that other mechanisms occurred, such as self-assembly.[26] We have shown previously that the PEG chains play a role in the photoswitch reversibility and should be taken into account. In our case, it is likely due to self-assembly of spiropyrans inside the nanopore, which induce a modification of the PEG conformation. This scenario presumes that PEG can adopt a mushroom and a brush-like conformation, which depends on the density of the polymer chain on the nanopore inner walls. The surface charge of PET conical nanopore is commonly estimated about 0.10 C m⁻² corresponding to a distance of 1.48 nm between two carboxylate moieties. The mushroom radius of PEG estimated by Flory law is 3.8 nm. Since these two values are very close and that
it is likely that all carboxylate moieties are not functionalized, we can assume that a mushroom conformation of PEG is possible. In this case, under VIS irradiation the self-assembly of spiropyranes stretches the PEG chain to a brush-like conformation; while merocyanine is well solvated in water and thus the PEG chains adopt a mushroom conformation to minimize the energy of the system. This interpretation reinforces the fact that the transformation of merocyanine into spiropyran does not occur in water.

Usually, at the nanoscale, the conductance follows two regimes. At high concentration, it increases with the salt concentration in order to reach a bulk-like ionic transport. At low salt concentration, the conductance reaches a plateau. However, this plateau was reported also in the case of hydrophobic and uncharged nanopores. In this case, it was partially assigned to the slip length and the water organization close to the surface. After VIS irradiation, the spiropyran is uncharged, we can extrapolate a “plateau-like” behavior at very low salt concentrations, likely at concentrations lower than 1 × 10⁻³ M. More interestingly, the plateau was not clearly observed after UV irradiation when the photoswitch molecule is in the zwitterionic state. This suggested that the surface charges induced by merocyanine are not shielded by the counter-ions in solution. The charge compensation is between ammonium and phenolate from the same molecule or two neighbor molecules. It should be noticed that a similar behavior is observed for both KCl and NaCl even at high concentrations when the potassium should chelate the PEG.

To investigate the nanopore charges after VIS and UV irradiation, we measured the power generated by a single nanopore under a salt concentration gradient from the tip side to the base side (Figure 7a). Indeed, it has been shown that the maximum power \( P_{\text{max}} \) generated by a nanopore is strongly dependent on its surface charge density and its shape. This power is obtained from the zero-volt current \( i_0 \) and the open circuit potential \( V_{\text{rev}} \). These values were obtained from the \( I-V \) dependence measured under dissymmetrical salt concentrations (Figure 7b)

\[
P_{\text{max}} = i_0 \cdot V_{\text{rev}}
\]

The current \( i_0 \) is extracted from the current at \( V = 0 \) when the concentration \( c_{\text{tip}} \) in the tip and the base side \( c_{\text{base}} \) of the nanopore were 1 M and between 1 × 10⁻³ and 100 × 10⁻⁵ M, respectively. As expected, under the SP form, the current was lower (between 50 and 90 pA) than under the MC form (500–620 pA). These values are in the same range as the one reported for a conical nanopore. The difference of current is assigned to the increase of surface charges due to the zwitterionic character of MC and the fact that the nanopore is open. The \( P_{\text{max}} \) after UV and VIS irradiation follows a linear dependence with the \( \log(C_{\text{c tip}}/C_{\text{c base}}) \) (Figure 7c). To compare the efficiency in terms of energy harvesting of both isomers of the photoswitch molecule, we can assume that \( P_{\text{max}} = k \log(C_{\text{c tip}}/C_{\text{c base}}) \) where, \( k \) is a coefficient of efficiency. After UV irradiation, this coefficient was five times larger than after VIS irradiation (45.48 pW⁻¹ against 5.97 pW⁻¹).

### 2.3. pH Controlled Ionic Diode and Nanopore Selectivity

In the MC form the photoswitchable molecule has a phenol group that can be protonated at low pH. Thus, at low pH, we should expect an inversion of nanopore selectivity. To confirm it, the ion transport was studied at pH 3 and 7 (Figure 8a). The results show an inversion of the current rectification at pH 3. In terms of selectivity, the current rectification is greater than 1 showing that the anion transport is favored. This is easily explained by the modification of surface charge due to the protonation of the phenolate moieties of the merocyanine. It can be noticed that at pH 7 the rectification factor tends to be 1 when the salt concentration increases. In order to evaluate the selectivity of the nanopore, we measured the reverse voltage under dissymmetrical concentration (Figure 6). At pH 7, the \( P_{\text{Kt}}/P_{\text{Cl}} \) are 3 and 10 for nanopores with tip diameters 5 and 9 nm, respectively, while at pH 3 these are 0.5 and 0.05. This result confirms the preferential transport of ions.

At pH 3, the dependence of the conductance to the salt concentration exhibits a plateau of conductance at low concentrations as opposed to pH 7 (Figure 8c,d). The lack of plateau at pH 7 was interpreted by the internal charge compensation as the merocyanine is zwitterionic. At pH 3, the protonation of phenolate moieties breaks the internal charge compensation.
The positive charges of ammonium moieties are shielded by chlorines, which are the mobile charges inside the nanopore explaining the plateau of conductance observed at low concentrations (Figure 8e).

3. Conclusion

To sum up, we have successfully designed a light responsive nanopore by grafting PEG-SP chains inside conical nanopores. We have studied the ionic transport as a function of both the pH and light irradiation. After UV irradiation, the nanopore is open, while after VIS irradiation, the nanopore is closed. The reversible switching from MC to SP isomer cannot be done in water and requires a solvent with a lower polarity such as ethanol. Our results provide a direct evidence of the ability of PEG to block the photoswitch under its zwitterionic form. Besides the light gating properties, it is possible to modulate the nanopore selectivity with pH. This work shows the possibility to design multifunctional nanopores that combine both diode and gating behavior. Such nanopores could be interesting for many applications from nanovalves to sensing properties using a single pore. Because the nanopores obtained by track-etched method can be upscaled to multipore membranes, these properties could also bring new opportunities to develop adaptive membranes for separation or energy conversion.

4. Experimental Section

Material: 13 µm thick PET films, with biaxial orientations were purchased from Goodfellow (ref E5301061). EDC (03449), HCl (ref 30721), NaOH (30620), MES (M8250), NaCl (S7653), dithiothreitol (D9163), dimethyl sulfoxide (DMSO) (41640), and phosphate-buffered saline (PBS, Sigma-Aldrich ref P4417) were purchased from Sigma-Aldrich. OPSS-PEG-NH2 (Mw 3400 D, ref PG2-AMOS-3K) was purchased from Nanocs. KCl (POCL-00A) was purchased from Labkem. 3′,3′-dimethyl-1′-[(2-iodoacetyloxyethyl)-6-nitrospiro[2H-1-benzopyran-2,2′-indoline] (I-SP) was synthetized following the procedure detailed in Kocer et al.[17a]

Track-Etching Nanopores: Single conical nanopores were obtained by track-etched methods. The single tracks were produced by Xe irradiation (8.98 MeV u⁻¹) of PET film at GANIL, SME line (Caen, France). The tracks were activated by UV exposition (Fisher bioblock; VL215.MC, λ = 312 nm) for 12 h for the base side and 6 h for the tip side. Shortly after the UV activation, the PET film was mounted between the two chambers of a Teflon cell. The conical shape was obtained using the electro-stopping method. One chamber was filled with the etchant solution (9 m NaOH) and the other chamber was filled with the stopping solution (1 m KCl and 1 m acetic acid). The reference and working electrodes were placed in the stopping and the etchant solution, respectively. Then, 1 V potential was applied. The nanopore opening was characterized by the current as a function of time recorded by a patch-clamp amplifier (HEKA EPC-10).

The etching process was stopped by replacing the etchant solution with the stopping solution when the current was raised to several hundreds of pAs. The nanopore was then rinsed in ultrapure water for 24 h. The tip diameter \(d\) of the conical nanopore was determined from the dependence of the conductance \(G\) measured in the linear zone of the \(I-V\) curve

\[
G = \frac{\kappa D d \Delta t}{4L}
\]

where, \(\kappa\) is the ionic conductivity of the solution assuming a bulk like transport, \(L\) is the nanopore length (13 µm), and \(D\) is the base diameter which was calculated from the total etching time \(t\) using the relationship \(D = 2.5t\) (the factor 2.5 was determined in the experimental setup using multipore track-etched membranes Figure 3).

Functionalization of the Nanopore: PEGylation: A reactive solution of Ortho-tryptidylisulfide-polyethylene glycol-amine was prepared with...
0.1 M KCl, 0.1 M MES, pH 4.7. The pH was adjusted with HCl and NaOH solutions using a pH meter (Hanna HI 221 pH meter, pH electrode HI 1131). This solution was added inside each chamber of the cell. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide solution was added to reach $50 \times 10^{-3}$ M and let equilibrate for 2 h.

**Functionalization of the Nanopore: Reduction by Dithiothreitol:** 16.8 mg of dithiothreitol was dissolved in 5 mL of ultrapure water. 200 µL of that solution was added to the tip side of the cell in an 800 µL phosphate-buffered saline solution and the base side was filled with only 800 µL of PBS solution. The reaction ran for 2 h.

**Functionalization of the Nanopore: Spiropyran Attachment:** 40 µL of stock solution of I-SP (1 mg mL$^{-1}$) in DMSO was added in the tip side of the nanopore into 800 µL DMSO. The other side was filled with 800 µL DMSO. The incubation lasted 2 h.

**Current–Voltage Measurement:** Current–voltage measurements were performed with a patch-clamp amplifier (EPC10 HEKA electronics, Germany). The current was measured by Ag/AgCl, 1 M KCl electrodes. The working and ground electrodes were connected to the trans (base aperture of nanopore) and the cis side (tip aperture of the nanopore) of the cell, respectively (Figure 3). The two chambers of the cell were filled with the same electrolyte solution for the symmetric measurements. For asymmetric measurements, the cis side had the same concentration while the concentration varied on the trans side. Current traces were recorded as a function of time from 1 to $-1$ V by 100 mV steps for 2 s and from 100 to $-100$ mV by 10 mV steps for 2 s. Except when it is specifically mentioned, the $I$–$V$ curves were recorded in darkness.

The nanopore irradiation was performed by focusing the light provided by an arc lamp (HXP R 120 W/45C). UV and visible light were selected by a single-band bandpass filter 375/110 nm (Semrock BrightLine FF01) and long-pass filter 500 nm (Thorlabs FELH), respectively.

**Absorbance Spectra:** The absorbance spectra were performed on multipore membrane (density $10^6$ pore cm$^{-2}$) using a spectrophotometer JASCO equipped with an integration sphere.

**Acknowledgements**

This work was supported by a PHC bilateral project (Van Gogh/Nuffic). Single tracks were produced at GANIL (Caen, France) in the framework of an EMIR project.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

ionic diode, nanopore, photoswitch, spiropyran

Received: August 23, 2017
Revised: September 28, 2017
Published online: December 1, 2017


