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Modulating molecular transport across peptide-modified nanoporous alumina membranes with light

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ABSTRACT

We designed and fabricated a smart and stimuli responsive membrane to cater on demand molecular transporting applications. A novel photoswitchable peptide (PSP) was synthesized and attached inside nanoporous anodic alumina membranes (NAAMs) pores. The PSP specifically switched between its cis and trans photostationary states on exposure to 364 nm and 440 nm wavelength lights respectively, which not only provided the ability to control its pore diameter but also the surface chemistry. The switchable molecular transport properties of the PSP-NAAMs have been shown as a function of the light exposure. Most importantly, the molecular transport across PSP-NAAMs could be repeatedly switched between on and off state, which is highly significant for on-demand triggered drug release systems.

Keywords: Nanoporous anodic alumina, molecular transport, photoswitchable peptides, stimuli responsive systems, nanoporous membranes

1. INTRODUCTION

The controlled transport of molecules across membranes is a challenging problem for many highly-valuable applications such as desalination, on-demand drug delivery, chromatography, and others. Artificial nanoporous membranes are important tools for studying the mechanisms and dynamics of controlled molecular transporting, which is already mastered by Nature (i.e. protein channels and ion pumps). In particular, artificial membranes are key for studying membrane surface interactions, size-exclusion effects, and other key determinants of transmembrane molecular transport; while also providing an opportunity to control or regulate transport on demand[1-3]. A number of strategies have been developed to fabricate artificial membranes with tunable and controlled molecular transport properties[1,4]. Incorporation of responsive polymeric brushes or hydrogels onto porous membranes (i.e. nanoporous anodic alumina, porous polymers, porous silicon, and carbon nanotube membranes) seems to be a popular choice for preparation of smart membranes with stimuli responsive molecular transport properties[4]. It is noteworthy that polymer brushes respond to a broad range of possible external stimuli (i.e. thermal, electrical, ionic strength, and pH) with fast stimuli response and do not completely block the pores (i.e. under optimized grafting conditions)[5].

Stimuli responsive polymers have been grafted on to nanoporous anodic alumina membranes (NAAMs) for imparting it with controlled and responsive molecular transporting abilities with a focus on on-demand and actuated release of model drugs to target chronic diseases that require daily dosing (i.e. angina pectoris, migraine or other hormone-related diseases)[6]. However, grafting of stimuli responsive polymer brushes is mostly envisaged to modify the top surface or the pore mouth of the NAAMs resulting in minimal chemical interactions between transporting molecules and the grafted polymer (as the polymer brushes are not present throughout the nanochannels). Furthermore, the polymerization process used in their preparation often results in non-uniform polymer chains, leading to non-uniform distribution of pore diameter across the membrane and bulk material with inefficient swelling and de-swelling properties. Also, this leads to randomly organized functional groups, consequently resulting in poor immobilization efficiency for biosensing, filtration, and chromatography applications[7]. NAAMs have been prepared by electrochemical anodization of aluminum

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and offer straight cylindrical nanopores with minimal tortuosity and controllable geometry and tunable surface chemistry[8]. An alternative approach to overcome the inherent limitations of polymer-based stimuli-responsive membranes, while providing the practical and functional requirements for selective on-demand molecular transport applications, is synthetic nanoporous membranes modified with optically switchable molecules. This study presents a photo stimuli-responsive membrane system based on NAAMs functionalized with an azobenzene-containing photoswitchable peptide (PSP). PSP molecules were selectively immobilized along the internal surface of the NAAM pores, in order to manipulate the effective pore diameter of the NAAMs, depending on their isomeric form. This pore diameter modulation then regulates the transport of model dye molecule (Rose Bengal (RosB)) across the PSP modified NAA membranes. Photo-mediated switching of the PSP is experimentally confirmed by UV/Visible spectroscopy, contact angle measurements, atomic force microscopy (AFM), and molecular transport characterization including reversible On/Off switching of RosB transport across PSP/NAAMs as a function of PSP isomeric state (i.e. cis = On and trans = Off).

2. EXPERIMENTAL

2.1 Photostationary states of light-responsive peptide

The photoswitchable peptide used in the study (Figure 1a) consists of an azobenzene-derivative reversible light switch (4-aminomethyl phenylazobenzoic acid[9]), which is flanked by two Aib (α-aminoisobutyric acid) oligomers (Aib3 and Aib6). It was synthesized using the solid phase peptide synthesis (SPPS) procedure. This particular azobenzene derivative was chosen since it undergoes fast photo-controlled reversible isomerism while remaining chemically stable[10]. An additional advantage of optically actuated switches is that they are non-responsive to electromagnetic interference[11]. Aib oligomers were used specifically, since they are known to form predictable and stable helical structures, which are crucial for modulating the 'effective pore diameter' in the NAAMs. The resulting PSP compound was purified using reverse-phase HPLC and characterized using ^{1}H NMR spectroscopy.
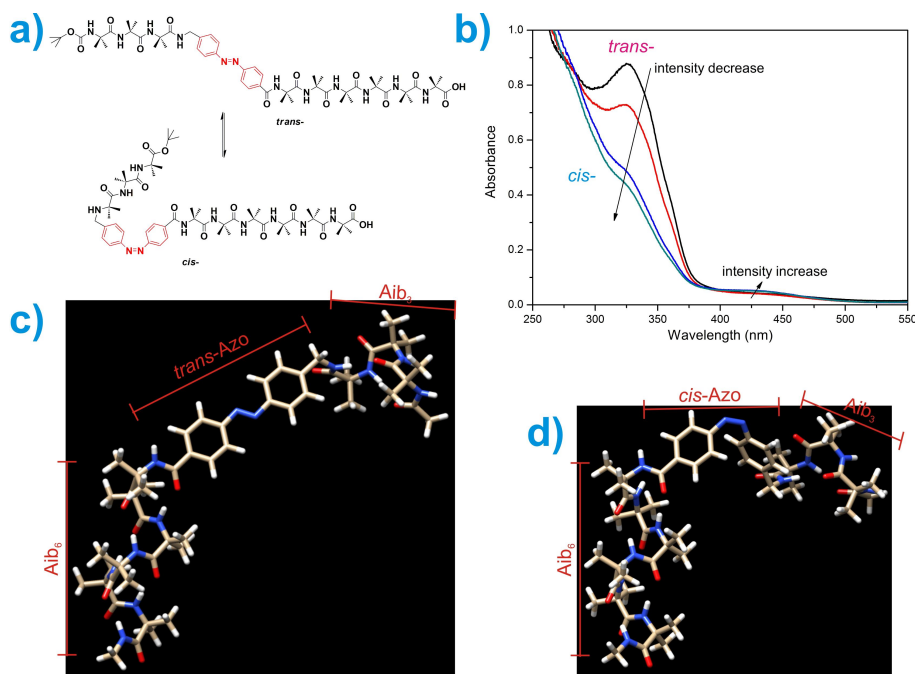

Figure 1. a) Chemical structures of trans- and cis- states of photoswitchable peptide. b) UV-Vis absorption of trans-photoswitchable peptide in methanol solution upon increasing time of irradiation at the wavelength of 360 nm light. From the top at the wavelength of 335 nm, t = 0, 5, 10 and 30 min. Computational models of photo-switchable peptide in its b) trans state, and c) cis state.

The photo-isomerization of the PSP molecules was initially characterized by recording the UV/Vis spectrum after exposure to 440 and 364 nm wavelengths of light. For this, first the PSP molecules were exposed to 440 nm wavelength of light (i.e. more PSP molecules in trans isomer state) for 10 min prior to measuring their absorption spectrum, which

showed a strong band at 325 nm and a weak band at 430 nm (Figure 1b). Then, the PSP solution was illuminated at 364 nm to enforce the transition from trans to cis state. A noticeable decrease in the peak at 325 nm was observed on exposing the PSP to 364 nm for 10 min (Figure 1b), which can be attributed to the formation of the cis isomer and the subsequent disappearance of the trans isomer. The switching between trans to cis form or vice-versa was found to be reversible, which could be repeated for several cycles proven by complete recovery of the absorption signal. In order to further define the backbone conformations of the peptide, the lowest energy conformers for both the trans and cis states of the PSP were determined in Gaussian 09, with tight convergence criteria using a hybrid B3LYP method with 6-31G** basis set (Figure 1c and d). The molecular model predicts that the length of a PSP molecule in the trans state is approximately 2.41 nm and 1.93 nm in the cis state.

2.2 Fabrication of peptide-modified nanoporous anodic alumina membranes

Nanoporous anodic alumina membranes used in this work were fabricated through a two-step electrochemical anodization in 0.3 M sulfuric acid at 6°C and 25 V, to obtain membranes with a nominal pore-channel diameter of 20 ± 3 nm[7]. Figure 2a shows the top view of a NAAM, while Figure 2b presents a cross-sectional view, with the inset showing perfectly straight and vertically aligned cylindrical nanopores. Note that the thickness of NAAMs used for this study was fixed to 50 μm by adjusting the anodization time. Photoswitchable NAAMs were fabricated by covalent immobilization of PSP molecules onto 3-aminopropyl triethoxysilane (APTES) modified NAAMs. DIPEA/HATU coupling reagents were used to form an amide linkage between carboxyl group on PSP molecules as well as acetic acid molecular spacer and amine group on APTES. This not only covalently couples PSP molecules to NAAMs inner surface but also incorporates acetic acid spacers to ensure efficient and homogeneous switching PSP molecules between the two isomeric states (i.e. trans and cis)[12]. The FTIR spectrum for the APTES-NAAM and PSP/NAAM are shown in Figure 2c, confirming successful functionalization of NAAMs with APTES and PSP molecules, respectively. The presence of carbon peak for only the top surface of PSP/NAAMs in EDX spectra presented in Figure 2d confirms the selective attachment of PSP molecules on NAAMs.
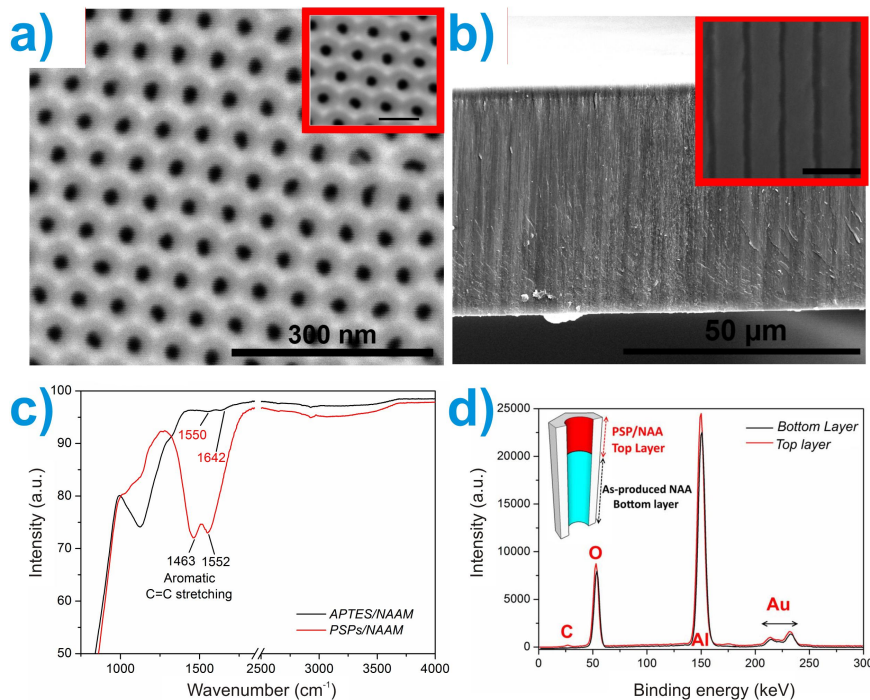

Figure 2. Typical SEM images of NAAM prepared in sulfuric acid electrolyte, while (a) top view with self-organized pores and (b) cross-sectional view showing entire thickness of the NAAM with a magnified view inset (scale bar: 100 nm). (c) FTIR spectra of APTES/NAAM and PSP-modified NAAM. (d) EDAX spectrum of NAAM with layered APTES chemistry, having APTES and PSP only in top layer while as-produced NAAM on the bottom layer.

3. RESULTS AND DISCUSSION

3.1 Contact angle measurements

The cis/trans switching capabilities of PSP molecules on a solid surface were examined by contact angle measurements after covalently attaching PSP molecules to silicon wafer surface using the aforementioned process. For this, the PSP-modified Si wafers were first exposed to 364 nm light for 20 min to enrich the number of PSP molecules on Si surface is cis isomer state and subjected to water contact angle (WCA) measurements. Similarly, subsequent exposure to 440 nm light to enrich the number of PSP molecules on Si surface were followed by WCA measurements. The results shown in Figure 3, reveal an average WCA of 64° and 81° for PSP molecules in trans and cis states, respectively. The lower WCA for PSP molecules in trans states is due to hydrophilic terminal group on the free end of PSP, whereas higher WCA for cis state is due to carbon backbone of the PSP molecules. This process was repeated three times to confirm the reversibility of the photoswitchable isomeric states of PSP molecules. The WCA measurements on NAAMs functionalized with PSP displayed similar results to WCA measurements on Si wafer with an average WCA of 44° and 53° for trans and cis states of PSP molecules, respectively.
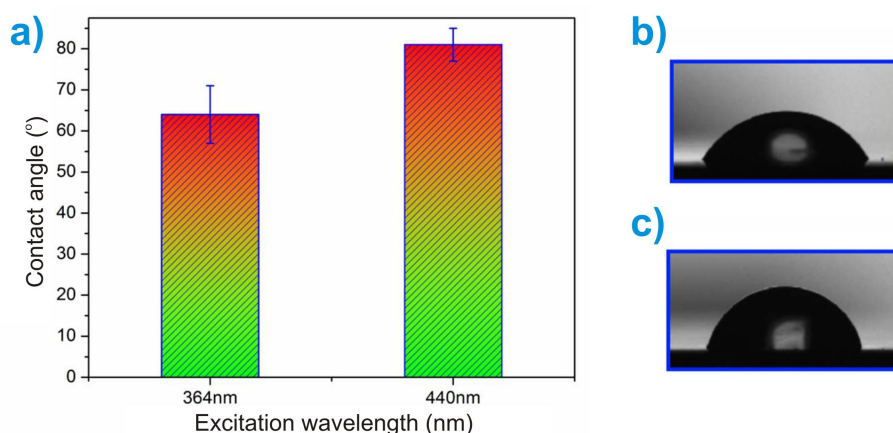

Figure 3. Water contact angle measurements of PSP in trans or cis conformational states (a) WCA of PSP on Si chip. Photograph of water droplet on Si surface with PSP in (b) trans state, and (c) cis state.

3.2 Molecular transport properties

We assessed the molecular transport performance of PSP modified NAAMs after exposure to 364 and 440 nm wavelength of light (i.e. PSP under cis and trans isomeric state) using RosB as model molecule. This was examined by clamping PSP-modified membranes between two halves of a U-tube permeation unit composed of a feed and a permeate chamber (Figure 4a). The amount of RosB dye molecules transported was measured in real-time by following the changes in absorbance in the permeate chamber at 552 nm using a miniature fiber optical spectrometer[13]. In this experiment, a PSP/NAAM was first exposed to 364 nm for 20 min in order to provide a photostationary state enriched in the cis azobenzene isomer (cis-PSP/NAAM). Subsequently, this cis-PSP/NAAM was then packed in the U-tube permeation cell and changes in absorbance of the RosB dye molecules in the feed chamber were measured for 5 h. This was repeated with a PSP-modified NAAM, which was exposed to 440 nm for 20 min in order to provide a photostationary state enriched in the trans azobenzene isomer (trans-PSP/NAAM) prior to transport analysis. Figure 4b shows changes in concentration of RosB in the permeate chamber for cis-PSP/NAAM and trans-PSP/NAAM systems. A linear increase in the concentration of RosB in the permeate chamber occurred for the cis-PSP/NAAM system. In contrast, the transport of RosB from the feed to the permeate chamber was greatly reduced for the trans-PSP/NAAM system. RosB concentrations of 0.194 ± 0.0098 μM and 2.134 ± 0.056 μM were obtained in the permeate chamber after 5 h, which corresponds to a permeation flux of 3.48×10^{-4} μmol h^{-1} cm^{-3} and 3.79×10^{-2} μmol h^{-1} cm^{-3} for trans-PSP/NAAM and cis-PSP/NAAM, respectively. Figures 4c and d show AFM images of this system in the cis-PSP/NAAM and trans-PSP/NAAM configurations. The average pore diameter of cis-PSP/NAAM and trans-PSP/NAAM was 20 ± 3 nm and 13 ± 5 nm, respectively. These clearly show a significant change in the diameter of pores on exposure to 364 nm and 440 nm light, respectively, which are in good agreement with the obtained permeation flux data.
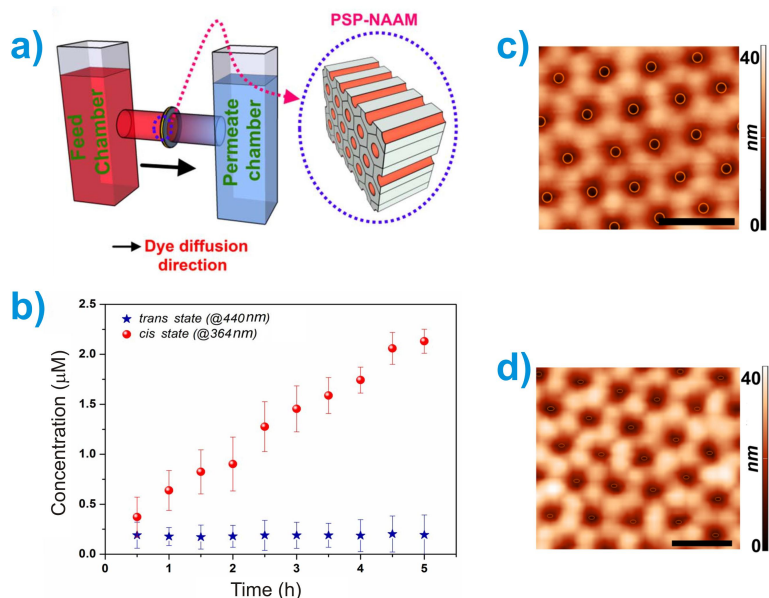
Figure 4. (a) U-tube transporting setup with two half chambers for RosB transporting through PSP/NAAMs. (b) Molecular transport of dye (RosB) through PSP/NAAM under different isomeric states of PSP. AFM height images of PSP/NAAMs corresponding to its (c) cis state and (d) trans state of the PSP, respectively (scale bar: 100 nm).

3.3 Reversible On/Off switching

The ability of this system to perform on-demand transport of dye molecules was then demonstrated by cyclic switching between the two photostationary states (i.e. cis and trans) of azobenzene group of PSP molecules grafted onto NAAMs surface. This was carried out by cyclic exposure of PSP/NAAM to 440 and 364 nm wavelength of light and transport of RosB was monitored in real-time to measure the molecular transport rate. Figure 5 shows transport of RosB across PSP/NAAM as a function of exposure to 364 and 440 nm wavelength of light establishing the on-demand transport of dye molecules from the feed to the permeate chamber as a function of the exposure wavelength. The PSP/NAAM system was first exposed to 440 nm for 60 min, resulting in very low molecular transport. This was followed by exposure to 364 nm light, which resulted in a sharp increase in the concentration of dye in the permeate chamber (Figure 5a) presumably due to isomerism of trans to cis with an associated change in pore size. The RosB transport results (Figure 5b) demonstrate that the proposed system is able to switch between On and Off states, thus allowing for on-demand transport of dye molecules. This system, thus mimics a cell membranes ability to control the transport the nutrients and molecules at will, and clearly demonstrates its suitability for on-demand transport applications. The trend for the flux data and is presented in Figure 5b showing the average flux for PSP/NAAM at 440 nm is 9.24×10^{-4} µmol h^{-1} cm^{-2} and 2.04×10^{-2} µmol h^{-1} cm^{-2} for 364 nm.
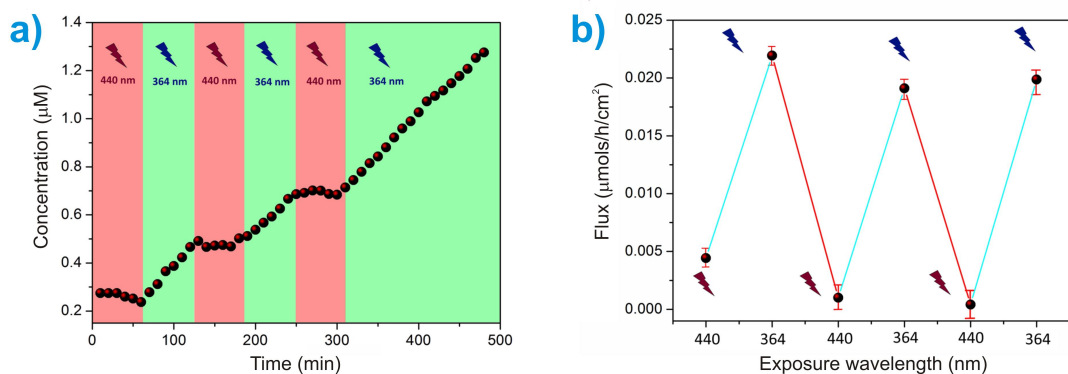

Figure 5. (a) Molecular transport of dye (RosB) through PSP/NAAM after alternative exposure to 440 nm and 364 nm light. (b) flux of dye transport through PSP/NAAM as a function of different wavelengths of light.

4. CONCLUSIONS

Novel functional membranes with capabilities of controlled and on-demand molecular transport were developed using photoswitchable peptides and nanoporous anodic alumina membranes. Their photo-regulated molecular transport properties were assessed using model molecule (Rose Bengal dye). The molecular transport across photoswitchable peptide-modified nanoporous anodic alumina membranes (PSP-NAAMs) system can be reversibly and cyclically actuated by switching the photostationary state of the azobenzene unit on PSP molecules. The controlled transport of RosB molecules is based on synergy between the size-exclusion effects and affinity between dye molecules and surface chemistry on PSP/NAAMs. The on-demand photostationary state switching response time of PSP molecules was found to be less than 10 min, and thus on-demand regulation in this system is possible by optical stimulation. Considering these promising results, this system could be applied for water purification and various biotechnological applications.

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