# Journal of Materials Chemistry C

# PAPER

Cite this: J. Mater. Chem. C, 2013, 1, 6120

Received 21st January 2013 Accepted 4th March 2013

DOI: 10.1039/c3tc30127c

www.rsc.org/MaterialsC

# Introduction

Inverse opal, as a kind of three-dimensional ordered (3Dordered) macroporous photonic structure, has attracted considerable attention due to its periodic porous structure, high surface-to-volume ratio of skeleton, and unique optical properties of Bragg diffraction.<sup>1-3</sup> The optical properties of opal are closely related to lattice spacing, and effective refraction index, which can be described by the Bragg equation.<sup>4-6</sup> Due to these attractive features, various functional systems based on inverse opal have been developed as chemical sensors, molecule gating and chromotropic devices.7-12 In particular, if made from special active polymers, the photonic structures exhibit morphological and optical changes in response to external stimuli, usually resulting in a naked-eye-observable and distinct color change by the Bragg diffraction of these ordered macroporous structures. Thus, inverse opal is an ideal sensitive selfreporting functional material suitable for numerous practical

### applications. However, to achieve the desired function as well as a sufficient mechanical strength, each fabrication is strongly dependent on the screening and arduous synthesis of functional monomers as well as the optimization of polymerization conditions. Especially in the case of sensitive functional moieties (e.g. bioactive units) incompatible with the conventional thermal- or photo-induced polymerization processes, the traditional polymerization method is inappropriate or difficult for achieving the corresponding functional inverse opals. In our previous work, in order to solve these problems, we developed multifunctional inverse opals based on the counterion exchange properties of ionic liquids11 and the azide-alkyne click reaction.12 However, there were still several shortages restricting their applications such as incomplete counterion exchange for the ionic liquid and the utilization of environmentally hazardous cuprous ions for the azide-alkyne click reaction. Therefore, it is highly desirable to develop a new kind of inverse opal platform with great extendibility to face different applications.

"Click chemistry" is a set of versatile reactions and is widely used to develop functional materials on account of the benefit of high yields, good aqueous competency, as well as regio- and

# Maleimide-containing polymer inverse opals: a new kind of reactive photonic structure with significant extendibility

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In this work, maleimide-containing polymer inverse opals have been successfully prepared by utilization of an acrylate monomer bearing a masked (protected) maleimide unit. As an ideal clickable functional group, maleimide can promote the Michael addition of thiol-containing molecules and the thermoreversible Diels-Alder reaction of furan derivatives, providing tremendous opportunities to produce various functional materials from one maleimide-containing polymer. As a demonstration, four chemical systems were facilely evolved from the prepared maleimide-containing inverse opal. By exploiting the nucleophilic thiol-ene reaction of maleimide, the zwitterionic pH-responsive inverse opal and electroactive inverse opal were first fabricated by reacting with cysteine and thiol-containing ferrocene derivatives, respectively. Based on the same reaction, it was also found that the maleimide-containing inverse opal could serve as a self-reporting sensing platform to sensitively detect the reduction of oxidized glutathione by specific enzymes. On the other hand, the thermoreversible nature of the Diels-Alder reaction of the maleimide groups made it possible to construct a dynamic molecule gating system by reacting with alkyl-chain-substituted furan derivatives from the prepared interconnected macroporous film. In fact, due to the unlimited variety provided by the two kinds of reactions mentioned above, the described photonic material exhibits a significant extendibility and could be easily post-modified for special purposes with maintenance of the opaline structure. Thus, this novel clickable maleimide-containing polymer inverse opal could serve as a reactive platform for producing a variety of functional photonic materials.

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stereospecificity.<sup>13</sup> As a starting functional unit in "click chemistry", the maleimide group has been extensively exploited in the biomolecular immobilization on metallic and glass surfaces, due to its distinguishing biocompatibility.<sup>13</sup> More importantly, the maleimide functional group can promote the Michael addition of thiol-containing molecules and the thermoreversible Diels–Alder reaction of furan derivatives. In most cases, the reactions require no additional reagents and generate no byproducts.

Maleimide-based click reactions have a broad and very powerful application potential.<sup>14</sup> For example, Hartinger *et al.* synthesized organoruthenium anticancer agents bearing maleimide groups used as reactive sites to immobilize biomolecules by Michael addition reaction.<sup>15</sup> McElhanon's group took advantage of the dynamic covalent nature of the Diels–Alder reaction of maleimide groups to fabricate a new kind of thermoreversible dendronized polymer.<sup>16</sup> More recently, Sanyal and other groups constructed maleimide-containing reactive hydrogels and polymers based on maleimide chemistry, demonstrating the tremendous potential of maleimide in postfunctionalization, immobilization, and drug-delivery.<sup>17–22</sup>

Thus, on account of the discussion above, it is conceivable that the introduction of maleimide groups into the inverse opal could afford a new kind of reactive inverse opal, which could serve as an efficient platform with significant extendibility for producing functional photonic materials and chemical systems. Under this consideration, polymeric inverse opals bearing maleimide units have been successfully prepared in this work by utilization of an acrylate monomer bearing a masked (protected) maleimide unit. As a demonstration, the prepared reactive inverse opal served as a clickable framework, and four different kinds of photonic materials were constructed based on the unique maleimide chemistry (Scheme 1). First, simple treatment of the prepared inverse opal with cysteine afforded zwitterionic inverse opals, which showed rapid response to both acid and alkali. Secondly, a ferrocene unit was introduced into the maleimide-containing film through a thiol-ene reaction, producing an electroactive opaline structure. Based on the same reaction, it was also found that the maleimide-containing photonic film could serve as a self-reporting sensing platform to sensitively detect the reduction of oxidized glutathione by specific enzymes. More interestingly, by exploiting the



**Scheme 1** Schematic illustration of the preparation of maleimide-containing polymer inverse opal and its use as a reactive platform for fabricating functional systems including zwitterionic pH-response inverse opal, electroactive inverse opal, enzyme sensor, and dynamic molecule gating.

thermoreversible nature of the Diels–Alder reaction of maleimide groups, dynamic molecule gating systems were developed through the formation of dynamic covalent bonding between maleimide groups and furan derivatives within the prepared inverse opal. All performed experiments obviously confirmed that the maleimide-containing polymer inverse opal could be utilized as a useful platform with great potential and extendibility to efficiently produce functional photonic materials and systems.

### Experimental

#### Chemicals

exo-3,6-Epoxy-1,2,3,6-tetrahydrophthalic anhydride, 3-amino-1propanol, cysteine, ethylene glycol dimethylacrylate (EGDMA), triethylamine, oxidized glutathione (oxidized GSH), 2-ethylfuran, 2-n-butylfuran and 2-n-hexylfuran were purchased from Alfa Aesar. Methyl methacrylate (MMA), 2,2'-azobisisobutyronitrile (AIBN), anhydrous methanol, acrylic acid, tetraethoxyammonia, silane (TEOS), ethanol, dichloromethane, magnesium sulfate (MgSO<sub>4</sub>) and PBS buffer solution were purchased from Beijing Chemical Company. Glutathione reductase (GSHR) from baker's yeast (S. cerevisiae), poly-(ethylene glycol) methacrylate (average  $M_n$  360) and 1,1'-ferrocene dimethanol were obtained from Sigma-Aldrich. The ferrocene derivative bearing thiol group (Fc-SH) was synthesized according to a published procedure.23 All other chemicals were purchased from local commercial sources. All solvents and chemicals are of reagent quality and were used without further purification unless otherwise specified.

#### Synthesis of masked maleimide acrylate monomer

The acrylate monomer bearing masked maleimide with furan was synthesized according to a published report.<sup>17</sup> Briefly, *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (3.32 g, 20 mmol) and 3-amino-1-propanol (1.65 g, 22 mmol) were dissolved in 100 mL anhydrous methanol. The reaction mixture was heated to 56 °C with vigorous stirring for 3 days under N<sub>2</sub> atmosphere. After cooling, the mixture was washed with diluted HCl and saturated brine, dried with MgSO<sub>4</sub>, and then purified by column chromatography. The product 2-(3-hydroxypropyl)-3a,4,7,7*a*-tetrahydro-1*H*-4,7-epoxyisoindole-1,3(2*H*)-dione was obtained. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  6.53 (s, 2H), 5.28 (s, 2H), 3.65 (t, 2H), 3.53 (s, 2H), 2.88 (s, 2H), 2.50 (s, 1H), 1.77 (m, 2H).

The obtained compound described above (2.00 g, 8.86 mmol) and triethylamine (1.05 mL, 10.63 mmol) were dissolved in dichloromethane (120 mL). The resulting mixture was placed in a salt-ice bath and then methacryloyl chloride (0.92 mL, 9.50 mmol) was slowly added. The reaction was allowed to proceed for 12 hours. The mixture was washed three times with brine and water, and then dried with anhydrous MgSO<sub>4</sub> overnight. After purification by flash chromatographic column, the desired monomer was obtained as a white waxy solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.97 (m, 5H), 2.84 (s, 2H), 3.61 (t, 3H), 4.12 (t, 3H), 5.26 (s, 2H), 5.57 (s, 1H), 6.13 (s, 1H), 6.51 (s, 2H).

#### Preparation of silica colloidal crystal templates

The highly uniform silica colloidal particles were synthesized by using the Stöber method. In a typical preparation, 8.80 g TEOS and 180 mL anhydrous ethanol were mixed in a 250 mL flask under intensive stirring with a magnetic beater. Then, 15.5 mL ammonia and 4.50 mL deionized water were slowly added and the resulting reaction mixture was stirred overnight. After alternate centrifugation and dispersion using anhydrous ethanol for 5 or 6 times to expunge residues, monodisperse silica particles with a diameter of 200 nm were obtained. The formation of the colloidal crystal templates was carried out by vertical deposition of the fully dispersed silica particles in anhydrous ethanol in glass vials. Circa 7 mL of the dispersion were added to each vial. A clean glass slide was put into each vial vertically for silica colloidal crystal growth. After complete volatilization of the ethanol, silica colloidal crystal templates were formed. Before deposition, the glass slides and vials were cleaned with a  $H_2SO_4-H_2O_2$  mixture (7:3), washed with deionized water, and then dried in argon flow.

#### Formation of maleimide-containing inverse opaline film

0.3 g (1 mmol) masked maleimide monomer, 0.1 g (1 mmol) MMA, 0.1 g (0.5 mmol) EGDMA and 0.18 g (0.5 mmol) poly-(ethylene glycol) methacrylate were dissolved in dichloromethane. Afterwards, 0.05 g (0.3 mmol) AIBN were added, and the resulting mixture was degassed with N2 for 10 minutes to provide the precursor solution. A colloidal crystal template deposited on a glass substrate was coated with glass slices and held together to absorb the precursor mixture by capillary force. Once the formed sandwich structure became transparent, a successful infiltration process was completed. After the removal of excess precursors, thermopolymerization was performed in an oven at 70 °C for about 8 h. Then, the sandwich was immersed in 1% hydrofluoric acid for 2 h to separate the double slides and fully etch the silica template. After rinsing with deionized water several times, free-standing masked maleimide films were obtained. The activation process (removal of the the maleimide protection group) was carried out by boiling the masked maleimide film in toluene at 110 °C for over 4 h. After washing with a mixture of alcohol and water, the maleimide containing inverse opal films (the activated maleimide films) were acquired and stored in deionized water.

# Preparation of functional inverse opal using maleimide film as a platform

**Zwitterionic pH-responsive inverse opal film.** The activated maleimide film was immersed in a 10 mM cysteine PBS buffer solution (pH = 7.0) containing ethanol (v/v = 10 : 1) in a vial, and then shaken for 24 hours. After washing with ethanol and deionized water, the desired pH-responsive photonic film was fabricated.

**Electroactive inverse opal film.** The activated maleimide film was soaked in a 10 mM Fc–SH ethanol solution in a vial, and then was shaken for *ca.* 24 hours. After washing with ethanol

and deionized water, electroactive ferrocene units were attached onto the maleimide-containing inverse opal film.

Detection of the reduction of oxidized glutathione under GSHR. The maleimide-containing inverse opal served as a selfreporting sensing platform to sensitively detect the reduction of oxidized glutathione by GSHR enzyme. All UV-vis curves were obtained in pH = 7.4 PBS buffer solution. To explore the single GSHR activity and the reaction time, the concentration of oxidized glutathione substrate buffer solution was maintained at 3 mM. Upon addition of GSHR (10 U) into the substrate solution, the activated maleimide photonic film was immediately immersed in the solution. Then, the UV responses of the photonic film were collected at specific time intervals. To study the dependence of the response of the photonic film on GSHR activity, different amounts of GSHR (from 0 to 20 U) were added to the oxidized glutathione substrate buffer solution (3 mM) with the activated maleimide photonic film in. After 30 min of incubation, the UV spectra of the film were collected.

**Dynamic molecule gating system.** By exploiting the thermoreversible nature of the Diels–Alder reaction of maleimide groups, dynamic molecule gating systems were developed through the formation of dynamic covalent bonding between maleimide groups and furan derivatives within the prepared inverse opal.<sup>24–26</sup> Briefly, the activated maleimide film was immersed in a dioxane solution containing a furan derivative (10 mM). After bubbling of N<sub>2</sub> for 20 min, the reaction medium was kept at 80 °C for 24 hours. The modified film was obtained after washing with ethanol and deionized water.

#### Instrumentation and characterization

<sup>1</sup>H NMR spectra were recorded at 300 MHz on a JOEL JNM-ECA300 spectrometer. The morphology and microstructure of silica colloidal crystals and photonic films were observed by a Hitachi S-6700 field emission scanning electron microscope (SEM). Water contact angles were measured by a Dataphysics contact angle system by dropping 2  $\mu$ L water droplets onto the surfaces. Thermogravimetric analysis (TGA) was carried out by TA Instruments TGA2050 with a heating rate of 10 °C min<sup>-1</sup> in N<sub>2</sub> atmosphere. Fourier Transform Infrared Spectroscopy (FT-IR) spectra were collected with an IFS-66v/S FTIR spectrometer (Bruker) in vacuum at around 1 to 2 mbar of pressure. Optical spectra were acquired with an Ocean Optics USB2000 fibre optic spectrophotometer coupled to an optical microscope. Electrochemical experiments were carried out by a HEKA PG310 potentiostat (Dr Schulz GmbH, Germany).

#### **Electrochemical measurements**

Electrochemical measurements (cyclic voltammogram, CV) were performed using a traditional three-electrode glass cell. A saturated Ag/AgCl electrode was employed as reference electrode in the aqueous phase, while in the organic phase the silver electrode was used instead. The counter electrode was a platinum wire. The Pt electrode used as the working electrode was mechanically polished with 0.05  $\mu$ m  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> and washed ultrasonically with deionized water. Afterwards, the film was mounted on the Pt electrode with a Teflon shell. The supporting

electrolytes used in the aqueous phase and the organic phase were  $K_2SO_4$  (2 mM in water) and tetrabutylammonium perchlorate (2 mM in acetonitrile), respectively. The electrolytes were purged with Ar gas for 10 min before testing. In the molecule gating measurement, 1,1'-ferrocene dimethanol served as the neutral redox probe molecule in the aqueous phase.

### **Results and discussion**

#### Preparation of the maleimide-containing inverse opaline film

The procedure for fabricating the maleimide-containing inverse opal film is displayed in Scheme 1. Maleimide groups might be unstable under the free radical polymerization conditions of the fabrication of polymer inverse opals, thus protection (masking) of the maleimide containing monomer is necessary. Following the reported literature,17 the methacrylate monomer containing a protected maleimide group was successfully prepared. The traditional approach to fabricate polymer inverse opals was carried out, and is detailed in the Experimental section. To be specific, the masked maleimide monomer acted as the aimfunctional monomer; MMA acted as the co-monomer to improve the film-formation; poly(ethylene glycol) methacrylate acted as the co-monomer to increase the integral hydrophilicity; and EGDMA acted as the cross-linker to maintain the rigidity of the resulting inverse opal film. However, during the polymerization process, even protected maleimide groups can be partially crosslinked within the polymer. Thus, the choice and optimization of the preparation conditions, especially the polymerization temperature, are critical. The observed side effects could be alleviated by adjusting the polymerization temperature.17 In addition, the amount of EGDMA added should be controlled to avoid forming a rigid film by excessive cross-linking. The prepared 3D-ordered silica colloidal crystal served as the sacrifice template and skeleton to support the precursor solution (Fig. 1A).



**Fig. 1** SEM images of (A) the silica colloidal crystal template, (B) the protected maleimide-containing polymer inverse opal, (C) the activated maleimide inverse opal after thermotreatment in toluene and (D) its section.

After the polymerization, the preparation of the masked maleimide inverse opal film was easily achieved by etching the silica template with 1% HF aqueous solution. Under SEM observation, the resulting polymer film displayed a perfect 3Dordered interconnected macroporous photonic structure (Fig. 1B). The optical appearance and UV absorption spectrum provided further evidence of the formation of a well-defined inverse opaline structure. As mentioned above, a portion of masked maleimide groups could be consumed in the polymerization process. In order to determine the remaining maleimide amount and monitor the process of thermotreatment, a TGA analysis was performed. From the TGA curve (black line in Fig. 2A), a distinct weight loss was detected between 100 and 300 °C. This detected weight loss was ascribed to the release of furan protection groups due to the retro-Diels-Alder reaction. The calculated weight loss of this process was about 9.2 wt%.



**Fig. 2** (A) TGA analysis of the furan-protected photonic films before (black line) and after (blue line) the thermotreatment; (B) FT-IR spectra of the protected (black line), activated (red line), modified with cysteine (blue line), modified with Fc–SH (green line) and reacted with glutathione (pink line) maleimide-containing polymer inverse opal.

Although the value was lower than expected (10.3 wt%), it still confirmed that most of masked maleimide units remained unaffected during the polymerization process. The FT-IR spectrum of the masked film was depicted in Fig. 2B (black line) as reference for subsequent modification experiments.

The activating (deprotection) process of the inverse opal film described above was carried out at 110 °C in anhydrous toluene solution. The masked maleimide film was required to be dried first in strict accordance with the reported procedure.17 During the experiment, stirring was replaced by adding zeolite to prevent breaking the polymer film. After 4 hours of boiling, the activated (deprotected) inverse opal film was obtained with perfect maintenance of its original morphology and structure (Fig. 1C and D), indicating that the photonic crystal structure was preserved even after a harsh treatment. The optical properties and the UV-absorption of the film supported the above conclusion. TGA was also applied for confirming the successful activation treatment (blue line in Fig. 2A). Comparing the TGA curve of the masked maleimide film, almost no weight loss below 300 °C could be detected for the thermally treated sample, verifying the successful deprotection of the marked maleimide groups. Moreover, the IR data provided evidence for furan release (red line in Fig. 2B). The obvious decrease of the vibration peak at 1772 cm<sup>-1</sup> was due to the reduction of the furan-maleimide adduct.18 The residual intensity could be explained by noting that some of the masked maleimide groups were embedded in the inner film, making the protection groups hard to release due to the steric effect. In our case, because of the high reactivity of the maleimide groups, the prepared polymer film was only activated prior to use.

#### Zwitterionic pH-responsive inverse opal film

In order to fabricate an ideal naked-eye pH-detector, different kinds of responsive inverse opals have been developed.<sup>10,27,28</sup> However, none of them is zwitterionic pH-responsive, probably due to the difficulty of synthesising zwitterionic monomers and implementing the corresponding polymerization. However, zwitterionic compounds have the ability to respond to both acids and alkali, which provides great potential for developing a series of diverse functional materials.<sup>29-31</sup> In this context, amino acids and their derivatives are the most used zwitterionic compounds. A simple and convenient method was developed to produce zwitterionic pH-responsive inverse opals based on the reactive film described above.

In practice, the prepared maleimide film was soaked into the nucleophilic thiol solution, normally at neutral pH, which triggered the radical-mediated thiol–ene Michael addition reaction. In our case, cysteine was chosen to fabricate the desired pH-responsive film, because of its remarkable zwitter-ionic character and the presence of the thiol group.<sup>30,31</sup> After exposure of the maleimide film to the cysteine solution for *ca.* 24 h,<sup>32</sup> the successful grafting of the cysteine was proved by IR spectroscopy with the appearance of a new peak at 1642 cm<sup>-1</sup>, which was ascribed to the asymmetric stretching of –COOH in cysteine (blue line in Fig. 2B).<sup>31</sup> Additionally, the hydrophilicity of the film also changed after treatment due to the introduction

of carboxyl and amino groups (Fig. 3A and B). After washing carefully with deionized water, the modified film was stored in a pH 6 buffer solution in order to keep it in a neutral state as the pI value of anchored cysteine is near  $6.3^{30}$ 

The pH response of the zwitterionic inverse opal was easily detected by UV spectrometry. As shown in Fig. 3C, a rapid and efficient response of the cysteine-modified inverse opal film could be observed when exposed to an alkaline solution. Gradually adjusting the pH from 6 to 11 led to a continuous shift of the UV absorption peak to longer wavelengths. The red shift reached its maximum when the pH was regulated to 11, and no further shift was observed, even in more alkaline conditions. Similar phenomena could be detected in the acid case, as shown in Fig. 3D. The electrically neutral state of cysteine at pH = 6 served as the turning point for acid and alkali response. Fig. 3E plots the change in lambda-max from the UVvis spectra. Obviously, the pH response is reversible. Accordingly, the opal film exhibited a dynamic change in its color in response to acid and alkali conditions (inset in Fig. 3E). As a control experiment, no pH response was detected in the unmodified maleimide film (Fig. 3F). The presence of the amino and carboxyl groups of the immobilized cysteine in the opal film should be responsible for the observed pH response. In the case of pH = 6, the anchored cysteine is electrically neutral and the polymer film showed a green color. When the pH shifted to



**Fig. 3** Water contact angles (CA) of the film before (A) and after (B) modification with cysteine; UV-absorption spectra of the cysteine-modified maleimide inverse opal with (C) alkali and (D) acid response, and (E) the cycle data of response to both acid and alkali by adjusting the film in the neutral state (inset: the optical images of the film in pH 1, pH 6 and pH 12 solution); (F) UV-absorption spectra of the unmodified maleimide inverse opal with pH response.

alkaline (pH > pI), both  $-COO^-$  and  $-NH_3^+$  groups in the zwitterionic state converted to  $-COO^-$  and  $-NH_2$ , causing an increase of the osmotic pressure within the film due to the electrostatic interactions and thus a red shift of the Bragg diffraction peak. When the pH was altered to acid (pH < pI), the pH-responsive groups became protonated and similar results were obtained. Therefore, the prepared zwitterionic inverse opal demonstrates an outstanding stimuli response as a naked-eye pH detector.

#### Electroactive inverse opal

As another example to testify the excellent thiol–ene addition reaction of the activated maleimide film, an electroactive molecule was introduced into the prepared inverse opal film. Ferrocene, as one of the most studied electroactive molecules, has frequently been utilized in performance examinations.<sup>12,33</sup> Therefore, a ferrocene derivative with a mercapto moiety was synthesized in this work. Recently, a similar experiment was carried out by Brozik and co-workers who modified an electrode surface with a maleimide containing coating and then introduced ferrocene moieties onto the surface. The modified electrode surface exhibited a good electro-triggered reversible redox reaction.<sup>34</sup>

In this case, the functionalization procedure is identical to that described in the last section with only slight modifications due to the insolubility of the synthesized ferrocene compound. After the thiol-ene reaction, the inverse opal film changed its appearance from green to the color of ferrocene (orange) without any obvious shift of the Bragg diffraction peak. The IR measurements showed an efficient modification (green line in Fig. 2B). The emergence of the characteristic absorptions of amide I (1642 cm<sup>-1</sup>) and amide II (1537 cm<sup>-1</sup>)<sup>35</sup> as well as ferrocene (847 cm<sup>-1</sup>)<sup>12</sup> indicated the successful immobilization of the ferrocene moieties. The cyclic voltammogram (CV) further confirmed the presence of ferrocene moieties in the modified inverse opal film. When the modified inverse opal film was mounted on a platinum electrode, the typical electrochemical signature of ferrocene was detected with a similar peak position as the molecule in solution (Fig. 4A). As expected, the maleimide containing film showed no response. By gradually increasing the scan rate, the intensity of the redox current peak increased linearly, indicating that the ferrocene moieties were covalently immobilized (not adsorbed) on the film (Fig. 4B).<sup>36</sup> The redox peaks, especially at high scanning rates, showed relatively poor signal which could be attributed to the poor conductivity between the immobilized redox-active molecules and the underlying electrode surface compared with those directly modified on the electrode or the free electroactive probes in solution. To sum up, a novel kind of electroactive responsive inverse opal was formed by a simple modification.

# Detection of the reduction of oxidized glutathione under GSHR as self-reporting sensing platform

In recent years, developing an enzyme-responsive smart system has become one of the most popular research topics for chemical sensing, controlled release and so forth.<sup>37-39</sup> However,



**Fig. 4** (A) Electrochemical characterization of the maleimide film (black line), Fcmodified film (red line) and the free Fc–SH molecule in solution (blue line); (B) repeated CV sweeping of the modified film from 50 mV s<sup>-1</sup> to 500 mV s<sup>-1</sup> (inset is the linear relationship of the intensity of redox current peaks *versus* the scanning rate).

plenty of works were based on fluorescence or other inconvenient detection methods. Benefiting from their unique and naked-eye detectable optical properties, stimuli-responsive photonic crystal materials are considered as a novel and convenient detection platform. In this context, maleimidecontaining inverse opal polymer films represent a powerful tool for label-free detection of thiol-containing analytes. Obviously, the maleimide inverse opal as a self-reporting sensing platform is particularly useful for screening those enzymes for which the product of the enzyme catalysis contains a mercapto group.

In the present work, an activated maleimide film was soaked into oxidized glutathione PBS buffer solution (pH = 7.4), which was employed as a substrate and could be reduced by GSHR. After adding a certain amount of enzyme (GSHR) to the buffer solution, oxidized glutathione was reduced immediately, and the resulting product subsequently reacted with the maleimide moieties of the inverse opal film. The occurrence of the cascade reaction was confirmed by FT-IR measurement (pink line in Fig. 2B). The appearance of the new peaks of the amide bonds at 1646 cm<sup>-1</sup> and 1535 cm<sup>-1</sup> indicates the attachment of the produced glutathione. The anchored glutathione has a similar structure as glutamic acid and its pI value is about 3. It can be deduced that the anchored glutathione is negatively charged in

the buffer solution, leading to a swelling of the inverse opal due to the increase in the osmotic pressure. The optical response of the maleimide inverse opal to the proceeding of the enzyme catalysis was first measured. In Fig. 5A, it can be observed that, with prolonged reaction times, the Bragg diffraction peak of the inverse opal shifts gradually to longer wavelength ranges. Even when the reaction time is as short as 0.2 h, a rapid response of 7 nm shift was also detected. The inset in Fig. 5A displays the evolution of the diffraction peak with the enzyme reaction. Additionally, the response of the maleimide film to the concentration of the used enzyme was also studied. All UV spectra of the maleimide films were collected after exposure to the reaction medium for 30 min. The more enzyme is added to the reaction solution, the more the diffraction peak shifts (Fig. 5B). This result indicates that, as a self-reporting sensing platform, the response of the maleimide inverse opal is very sensitive. The detection limit for GSHR was at least 5 U.

#### Dynamic molecular gating system

Molecular gating plays a vital role in a wide realm of applications including biosimulation, separation and other domains.<sup>29,40-42</sup> However, most reported systems are hardly able to dynamically control the gating effects. As a typical dynamic covalent chemistry reaction, the Diels–Alder/retro-Diels–Alder reaction can be employed to realize a switch which reversibly



**Fig. 5** (A) Shifts of the Bragg diffraction peak with prolonged reaction times with 10 U GSHR enzyme in reaction solution (inset is the plot of diffraction peak location *versus* reaction time); (B) UV response of the maleimide film to different concentrations of the used enzyme in reaction solution after exposure for 30 min.

alters the physicochemical properties of a nanochannel.<sup>22,43,44</sup> In this work, furan and furan derivatives with different long alkyl side chains were used to dynamically modify the nanochannels of inverse opal films *via* Diels–Alder/retro-Diels–Alder reaction. As a result, a dynamic molecular gating system was realized, as demonstrated by using 1,1'-ferrocene dimethanol as a redox probe molecule.

The inset in Fig. 6A displays the TGA analyses, which support the successful implementation of the Diels-Alder reaction between the maleimide films and the furan derivatives. The relatively smaller weight loss of the film modified with 2-nhexylfuran is probably due to a low reaction efficiency resulting from steric hindrance. The measurement of the water contact angle on the modified films further confirmed the occurrence of the Diels-Alder reaction (Fig. 6C). As expected, with the use of furan derivatives modified with longer alkyl side chains, the modified film became more hydrophobic. The dynamic molecular gating properties of the furan-marked inverse opal film were exemplified by using ferrocene dimethanol as a redox probe molecule. As shown in Fig. 6A, the intensity of the redox peak of the probe molecule decreased gradually with the increasing length of the alkyl chain in the used furan derivatives. The inverse opal had a 3D interconnected macroporous structure like a huge interlaced "nanochannel". When these channels exhibit certain hydrophilicity, water molecules fill the porous film and the molecular probe can easily pass through these channels as if the gate is "open". In contrast, when relatively more hydrophobic groups are introduced into the channels of the film, water is driven out and flow drops immediately as if the gate is "closed". Interestingly, the gating effect could be



**Fig. 6** (A) Electrochemical characterization of the furan- (black line), ethylfuran-(red line), butylfuran- (blue line) and hexylfuran- (green line) masked inverse opals using 1,1'-ferrocene dimethanol as a redox probe (inset is the TGA analysis of the corresponding inverse opal films); (B) reversible change of the current peak of the redox probe between the masked films with furan and hexylfuran molecules; (C) water contact angles of the films protected by furan, ethylfuran, butylfuran and hexylfuran, from the top down.

dynamically adjusted by using a Diels–Alder/retro-Diels–Alder reaction. For example, the channels showed "open" and "closed" states successively when furan and hexylfuran were alternately used as protecting groups for the maleimide units in the prepared inverse opal film (Fig. 6B).

# Conclusions

In this work, a clickable maleimide-containing polymer inverse opal was successfully developed for fabricating multifunctional photonic structure materials by thiol-based Michael addition reaction or furan-based Diels-Alder reaction. As a demonstration, four chemical systems were facilely evolved from the prepared maleimide inverse opaline film. First, zwitterionic cysteine was introduced into the maleimide inverse opal which displayed a rapid response to both acid and alkali. Additionally, an electroactive inverse opal was constructed by using ferrocene derivatives bearing thiol groups based on the thiol-ene reaction. Thirdly, the maleimide-containing inverse opal also served as a self-reporting sensing platform to sensitively detect the reduction of oxidized glutathione with specific enzymes. Finally, a dynamically tunable molecule gating system was fabricated via the thermoreversible nature of the Diels-Alder reaction through the formation of dynamic covalent bonding between maleimide groups and furan derivatives within the prepared inverse opal. Considering the unlimited options of both thiol- and furan-containing molecules, the described clickable platform showed a significant extendibility. Thus, we believe this maleimide-containing polymer inverse opal could act as a very useful platform for designing novel functional photonic materials with great potential and extendibility.

# Acknowledgements

We gratefully acknowledge the financial support from the National Science Foundation of China (no. 21025311, 21121004, 21261130581 and 91027016), Ministry of Education (2011Z01014), MOST Program (2011CB808403, 2013CB834502) and the transregional project (TRR61).

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