Photoresponsive porphyrin-imprinted polymers prepared using a novel functional monomer having diaminopyridine and azobenzene moieties†

Toshifumi Takeuchi,* Kana Akeda, Shinya Murakami, Hideyuki Shinmori, Satoru Inoue, Woo-Sang Lee and Takayuki Hishiya

Received 30th March 2007, Accepted 31st May 2007
First published as an Advance Article on the web 21st June 2007
DOI: 10.1039/b704830k

A novel photoresponsive functional monomer bearing diaminopyridine and azobenzene moieties was synthesized and applied to the preparation of photo-regulated molecularly imprinted polymers, which can recognize porphyrin derivatives through hydrogen bonding. The binding affinity of the imprinted cavities was regulated by UV irradiation, suggesting that azobenzene groups located inside the binding sites worked as photosensitizers and the *trans–cis* isomerization could regulate the affinity for the target compounds. Repetitive binding of the target compound to *trans*-IP and *cis*-IP was directly monitored by slab optical waveguide spectroscopy and the photo-mediated regulation of binding affinity was successfully confirmed.

Introduction

Molecular imprinting has been known as a useful technique for the preparation of molecular recognition materials, which have tailor-made binding sites for target molecules.[1] Molecularly imprinted polymers (MIPs) have been often compared to enzymes and antibodies, and applied to various fields ranging from analysis to catalysis.[2] In MIP syntheses, functional monomers are assembled around a template molecule by covalent or non-covalent interaction, and co-polymerized with cross-linking monomer(s). The template is then removed from the resulting polymer to generate selective binding sites, which are complementary to the template in size, shape and functional groups.

External stimuli-responsive polymers have been intensively developed in modern polymer science. To date, polymers controlled by external stimuli such as electric fields,[3] magnetic fields,[4] light,[5] *etc.* have been reported. The stimuli-responses have been utilized to control chemical functions by on–off switching.

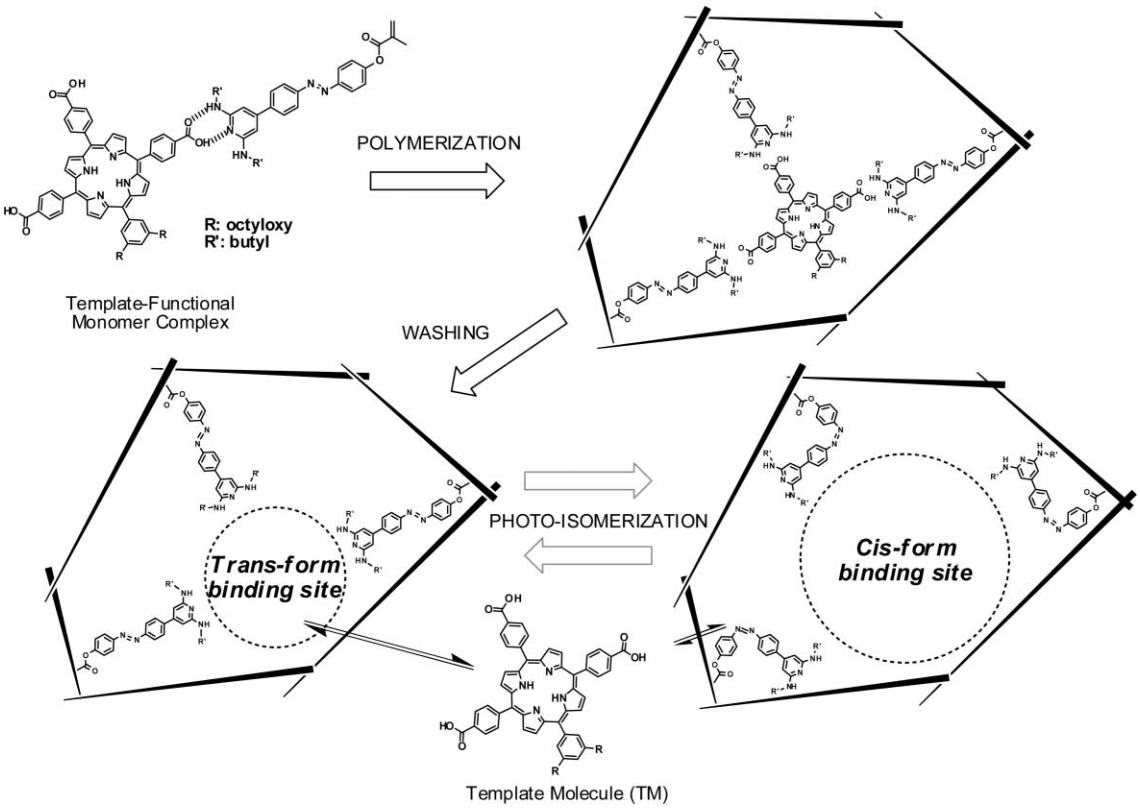
Photoirradiation is one of the external stimuli for stimuli-responsive materials. Recently, many photoresponsive molecular systems have been reported.[6] It is well known that azobenzene derivatives show photoreversible isomerization. The azobenzene chromophore group exists in two isomeric states, a thermodynamically more stable *trans* and a metastable *cis*. When irradiated with light of appropriate wavelength, azobenzene and its derivatives undergo photoisomerization: the *trans*-form is converted into the *cis*-isomer by UV light irradiation, and the *cis*-isomer can return to the *trans*-form photochemically under visible light irradiation or thermally in the dark. Quantum yields of the process are generally high and there are no significant competing reactions. Also, *trans–cis* photoisomerization of azobenzene brings about large changes in the geometry and dipole moment to the chromophore.[7] Minoura *et al.* reported that dansylamide-imprinted polymer membranes containing azobenzene chromophore and their binding properties were regulated by light,[8] where *p*-phenylazoacrylanilide was used as a functional monomer. In this system, the binding activity and the selectivity were not high, since the functional monomer may not form strong hydrogen bonds with the target molecule. Recently, Gong *et al.*[9] reported photoresponsive imprinted polymer materials capable of photoregulated uptake and release of caffeine, where the adsorption selectivity in each case of the *trans*-form and the *cis*-form was not fully studied.

Herein we report a newly designed photoresponsive functional monomer having diaminopyridine and azobenzene moieties, 4-{4-[2,6-bis(*n*-butylamino)pyridine-4-yl]-phenylazo}-phenyl methacrylate (FM) for preparing photoresponsive imprinted polymers for porphyrin derivatives with carboxylic acids (Scheme 1). Multiple hydrogen bonds could be formed between the template and FM, facilitating the assembly of FM with the template in appropriate positions by polymerization, yielding selective imprinted cavities complementary to the target molecule.

The reversible binding of target molecules to the imprinted polymer is directly investigated by using optical waveguide spectroscopy,[10] which is internal reflection spectroscopy based on absorption of an evanescent wave emerging from the outer surface of a slab-type waveguide where incident light is propagated by repeated total reflection. It is known that molecular interactions at a liquid–solid interface can be directly detected by this technique, therefore, in the present work, the target molecule bound to a thin film on the waveguide can be directly detected with or without external light (365 nm), meaning that the effect of light irradiation on the binding of the target molecule to the prepared polymer thin film can be directly investigated.

Graduate School of Science and Technology, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan. E-mail: takeuchi@gold.kobe-u.ac.jp; Fax: +81-78-803-6158; Tel: +81-78-803-6158

† Electronic supplementary information (ESI) available: ^{1}H NMR spectral changes of **1** by photoirradiation in toluene-d_8–DMSO-d_6 and Scatchard plot from binding experiments in toluene–DMSO containing polymer particles and various amounts of TM. See DOI: 10.1039/b704830k

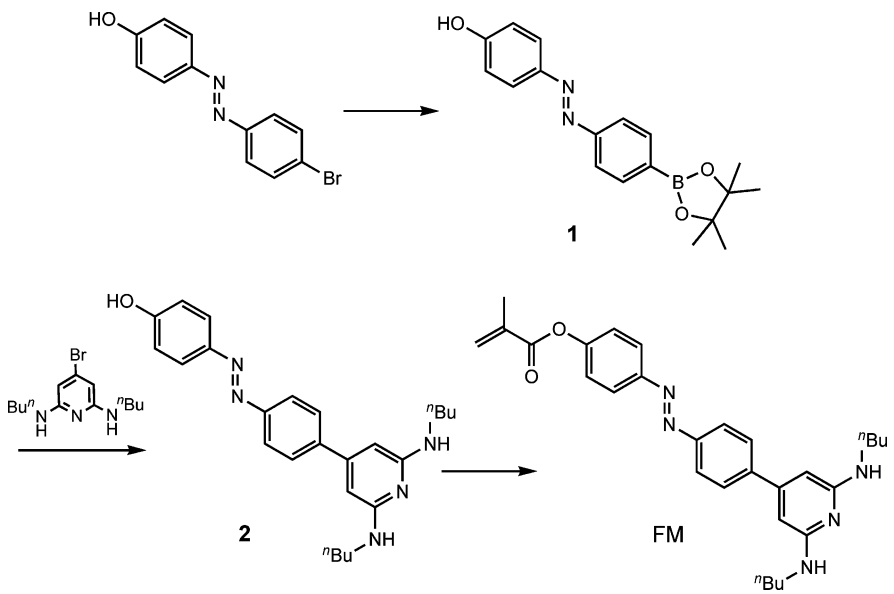


Scheme 1 Schematic illustration of photoresponsive binding site generation in the imprinted polymer.

Results and discussion

Synthesis and photoisomerization properties of FM

To develop photoresponsive polymer materials possessing molecular recognition ability, we synthesized a polymerizable azobenzene derivative as a designed functional monomer (FM). FM has a polymerizable methacrylate moiety, a photoresponsive azobenzene moiety, and a diamino pyridine group capable of forming hydrogen bonding with a carboxylic acid. The synthesis of FM is shown in Scheme 2. We prepared FM by Suzuki coupling reaction and the condensation of methacryloyl chloride. First, a boronate azobenzene derivative (**1**) was prepared by a borylation reaction catalyzed by $PdCl_2(PPh_3)_2$. **2** was obtained by the coupling of **1** with 4-bromro-*N*,*N*′-dibutylpyridine-2,6-diamine under standard Suzuki coupling conditions in 61% yield. In the final step, FM was



Scheme 2 Synthesis of FM.

afforded from the reaction of **2** with methacryloyl chloride in 60% yield.

Absorption spectra of the *trans*-FM in toluene–DMSO (9 : 1, v/v) solution showed two bands at 349 and 440 nm, related respectively to the π–π* and n–π* electronic transitions of the azobenzene chromophore (Fig. 1). *trans*-FM was photoisomerized to *cis*-FM by irradiation with UV light. The *cis*-form yield in the photostationary state was estimated to be 74%, which was measured by ^{1}H-NMR spectroscopy of FM after the irradiation of UV light for 20 min in toluene-d_8–DMSO-d_6 (9 : 1, v/v).† In contrast, the photoisomerization to the *trans*-form from the *cis*-form was induced by Vis light, and both *trans–cis* and *cis–trans* transformations were reversible.

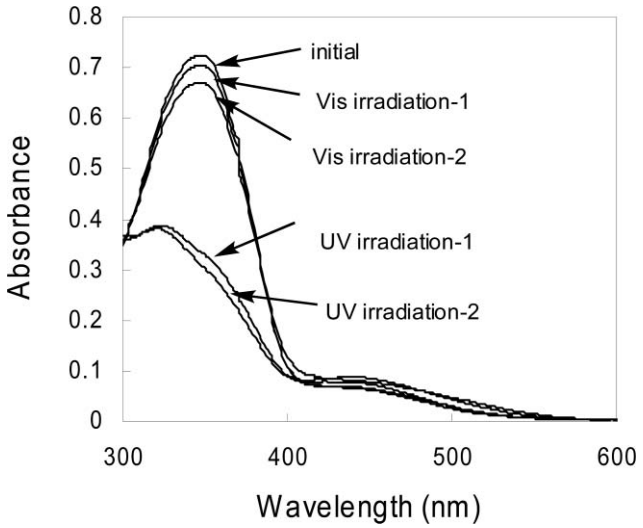


Fig. 1 Absorption spectral changes of FM by photoirradiation.

Preparation and characterization of the photoresponsive polymers

The template molecule (TM): 5-(3,5-dioctyloxyphenyl)-10,15,20-tri-4-carboxyphenyl-porphyrin has a porphyrin structure with three *p*-benzoic acid and one dioctyl benzene moieties at *meso* positions. The carboxylic acids can interact with the diamino pyridine group of FM and the alkyl groups can improve TM's solubility in organic solvents. The imprinted polymer (*trans*-IP) was prepared by thermally initiated polymerization in the dark, where the pre-polymerization mixture contained TM, FM (*trans*-form), divinylbenzene and styrene in toluene–DMSO (9 : 1, v/v) (Table 1). After the polymerization, *trans*-IP was obtained by washing out the template with aqueous $CsCO_3$ solution, THF–MeOH (1 : 1, v/v), and toluene–THF (3 : 1, v/v), successively. After the washing step, 21% of the template was removed from the polymer. This low template recovery may be due to the large size of the template molecule and the rigid polymer network formed by styrene and divinylbenzene. Blank polymer (*trans*-BP) was prepared in the same manner without adding TM in the pre-polymerization mixture.

Table 1 Polymer recipe for *trans*-IP

FM	21.5 mg
TM	15 mg
Divinylbenzene	210 μL
Styrene	85 μL
ADVN	22.4 mg
Toluene	450 μL
DMSO	50 μL

For examining the photoisomerization of azobenzene residues in the polymers, we used an integrating sphere-equipped UV–vis spectrophotometer. The absorption bands arising from the azobenzene residues in the *trans*-IP and *trans*-BP in toluene–DMSO (9 : 1, v/v) appeared at 334 and 340 nm, respectively. The absorbance was decreased by the irradiation of UV light, confirming that the *trans*-azobenzene moieties in the polymers were photoisomerized to the *cis*-form (Fig. 2). The reverse photoisomerization to the *trans*-form was induced by the irradiation of visible light. These phenomena mean that *trans–cis* isomerization of the azobenzene residues also occurs even in the rigid crosslinked polymer networks.

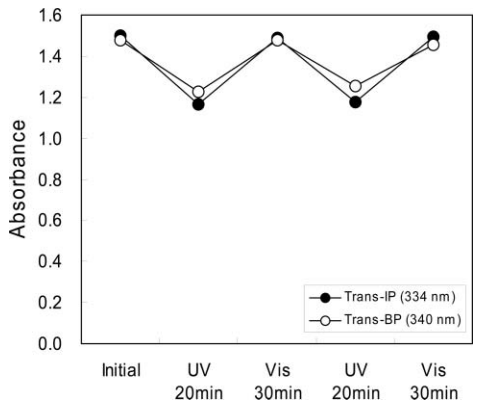


Fig. 2 Reversibility of the photoisomerization process in the imprinted polymer and the blank polymer.

Binding activities of TM and structurally related compounds to *trans*-IP and *trans*-BP

The binding selectivity of the polymers (*trans*-IP and *trans*-BP, 3.0 mg) was evaluated by incubation with TM and related compounds (150 μM in toluene–DMSO, 7 : 3 v/v, 2.0 ml), as shown in Fig. 3. TM showed the strongest binding to the *trans*-IP. In the *trans*-IP, the diaminopyridine groups of the binding cavities could be assembled in positions suitable for hydrogen bond formation with three carboxylic groups at the *meso* positions in the porphyrin ring of TM. Compared with TM, structurally related analogues with two or one *p*-benzoic acid group(s) at *meso* position(s), namely DCPP and MCPP, were weakly adsorbed, and they were more strongly bound to *trans*-BP than *trans*-IP. Strong hydrogen bond formation cannot be achieved and the apparent binding may be attributed to weak π–π stacking, resulting in non-specific binding. THPP, which has four *p*-hydroxyphenyl groups at *meso* positions, and TM(Me), trimethylesters of TM, were hardly adsorbed. These results indicate that carboxylic acid groups capable of hydrogen bond formation are necessary for binding to the *trans*-IP. TCPP was strongly adsorbed to the *trans*-IP, because it has four carboxylic acid groups. However, its binding activity was inferior to that of TM. This means that the polymer could recognize the differences between TM and TCPP in their substitution patterns at one of the *meso* positions. MCPP was more strongly bound to *trans*-BP than *trans*-IP.

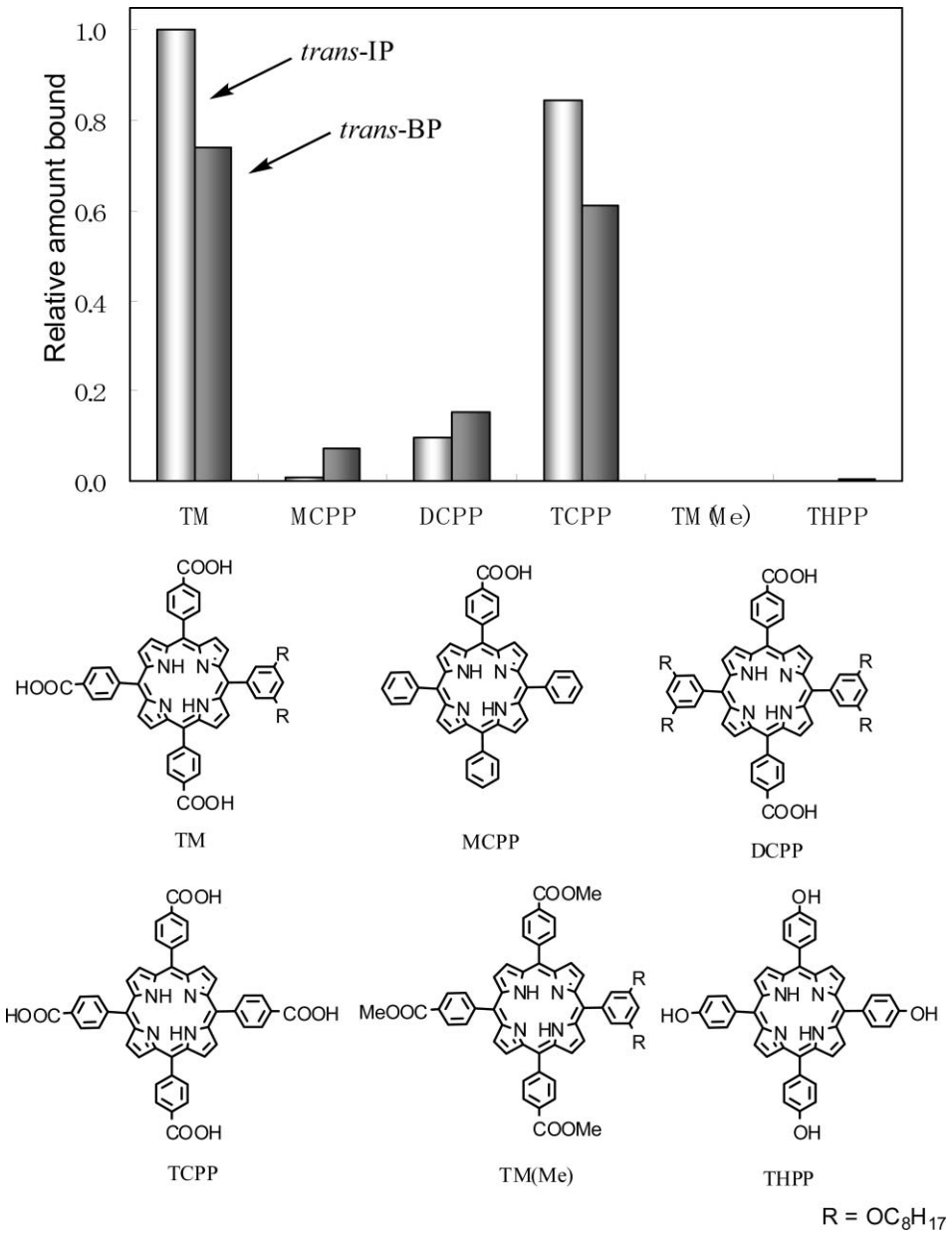


Fig. 3 Binding activities of the polymer for TM and related compounds.

Photoresponsive adsorption of TM to the imprinted polymer

In order to evaluate the photoresponsive binding of TM to the imprinted polymer, batch rebinding tests were carried out in the dark (*trans*-IP) and under the irradiation of UV light (*cis*-IP). The binding isotherm depicted a saturation curve, meaning that a finite number of binding sites exist in the IP. The binding activities of the polymers for TM were quantitatively studied by Scatchard analysis from the binding data (Fig. 4). The association constants (K_a) and the maximum numbers of binding sites (B_{max}) in *trans*-IP and *cis*-IP are listed in Table 2. The values of K_a and B_{max} in the *trans*-IP were higher than those in *cis*-IP. At a lower concentration range, the slope of the Scatchard plot appeared to be steeper,† meaning that the binding sites constructed by the imprinting process are not homogeneous as is often the case with non-covalent MIPs. For this heterogeneity, we estimated the association constants using a near concentration range employed in this work.

Table 2 Association constants (K_a) and maximum numbers of binding sites (B_{max})

Polymers	K_a/M^{-1}	B_{max}/μmol g^{-1}
trans-IP	1.8×10^5	3.82
cis-IP	7.9×10^4	3.19

Since it is difficult to see differences directly in the binding of TM during reciprocal switching between *trans*-IP and *cis*-IP by common transmittance spectroscopy, we employed slab optical waveguide spectroscopy for the measurement of absorbance change on the surfaces of *trans*-IP and *cis*-IP thin films, allowing

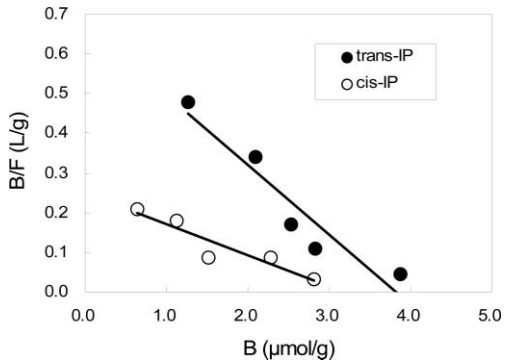


Fig. 4 Scatchard plots of TM binding to *trans*-IP and *cis*-IP.

the direct observation of TM binding on thin films. The *trans*-IP thin film was prepared on a slab-type high reflection optical waveguide and TM binding tests were conducted, and the binding was monitored by measuring absorbance at 423 nm. Fig. 5 shows the continuous measurements of TM binding for the repetitive photo-switching of *trans*-IP and *cis*-IP for 20 h. Although the template was not completely extracted in this work and it made the spectroscopic background high, the results clearly show that the affinity of *trans*-IP for TM was higher than that of *cis*-IP. In previous imprinted polymer studies, binding behaviors of samples to the polymers have been investigated indirectly by the determination of unbound samples in bulk solutions. In contrast, waveguide spectroscopy can provide direct information on the bound TM, therefore it is confirmed that the specific binding sites are photo-responsive and the binding affinity can be switched by photoirradiation. These direct analyses on the films could make possible the detailed study of binding modes in the imprinted polymer and help develop highly specific materials with molecular recognition ability.

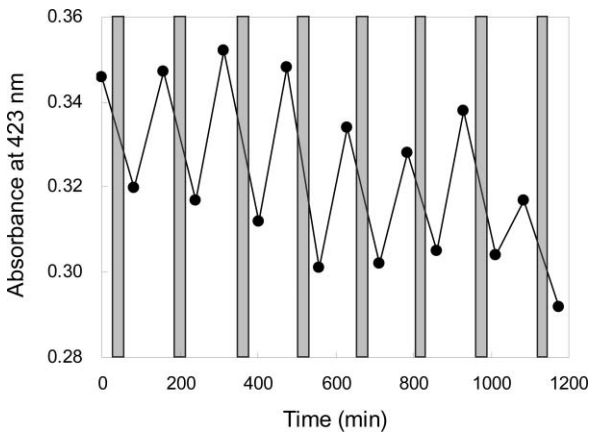


Fig. 5 Binding behaviors of TM for the repetitive photo-swiching of *trans*-IP and *cis*-IP. The UV light was irradiated during the grey period (25 min).

Conclusions

The present study demonstrates photoresponsive imprinted polymers prepared using the newly developed photoresponsive functional monomer. The photoisomerization of azobenzene residues in the imprinted binding cavities capable of multiple hydrogen bond formation successfully regulated binding affinity. In the present work, *trans*-FM was used for TM imprinting. When the azobenzene residues in a recognition cavity of the imprinted polymer is transformed from *trans* to *cis* configuration by UV light irradiation, the arrangement of diaminopyridine groups in the cavities may not be preserved, therefore, the difference in the binding affinity between *trans*-IP and *cis*-IP can be explained by this photoisomerization. We believe that further elaboration of the present system would lead to the design of novel molecularly imprinted materials with photoswitching functions such as light-triggered release and capture, light-mediated selective extraction and separation, and so on.

Experimental

Synthesis of FM and TM

4-[4-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenylazo]-phenol (1). To a toluene (5.6 mL) solution of 4-(4-bromophenylazo)phenol (551 mg, 2.00 mmol), $Pd(PPh_3)_2Cl_2$ (84.5 mg, 0.12 mmol) and triethylamine (0.89 mL, 6.40 mmol) was added pinacolborane (0.59 mL, 3.00 mmol). The reaction mixture was stirred at 90 °C for 17 h under a nitrogen atmosphere. After evaporation, the residue was dissolved in ethyl acetate. The organic layer was washed with aqueous $NaHCO_3$ and NaCl solution. The crude product was purified by silica gel column chromatography (dichloromethane–methanol = 40 : 1, v/v) to yield **1** (310 mg, 48%); δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.37 (12H, s, CH_3), 6.95 (2H, d, J = 7.1 Hz, ArH), 7.83–7.95 (6H, m, ArH).

4-{4-[2,6-Bis(butylamino)pyridine-4-yl]-phenylazo}-phenol (2). To a solution of **1** (589 mg, 1.80 mmol), $Pd(PPh_3)_4$ (205 mg, 0.18 mmol) and $CsCO_3$ (405 mg, 5.40 mmol) was added 4-bromo-*N*,*N*′-dibutylpyridine-2,6-diamine (530 mg, 1.80 mmol) in DMF (30 mL). The reaction mixture was stirred at 100 °C for 17 h under a nitrogen atmosphere. After evaporation, the residue was dissolved in ethyl acetate. The organic layer was washed with aqueous NaCl solution. The crude product was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 2 : 1, v/v) to yield **2** (460 mg, 61%); δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.97 (6H, t, J = 6.9 Hz, CH_3), 1.40–1.47 (4H, m, CH_2), 1.60–1.65 (4H, m, CH_2), 3.25–3.27 (4H, m, NCH_2), 4.48 (2H, s, NH), 5.96 (2H, s, ArH), 6.96 (2H, d, J = 8.4 Hz, ArH), 7.72 (2H, d, J = 8.4 Hz, ArH), 7.87–7.94 (4H, m, ArH).

4-{4-[2,6-Bis(butylamino)pyridine-4-yl]-phenylazo}-phenyl methacrylate (FM). A solution of **2** (144 mg, 0.35 mmol), methacryloyl chloride (0.027 mL, 0.28 mmol) and triethylamine (0.058 mL, 0.41 mmol) in dichloromethane (6.0 mL) was stirred at room temperature for 2 h. The resulting mixture was washed with aqueous NaCl solution. The mixture was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 3 : 1, v/v) to yield FM (100 mg, 60%); δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.96 (6H, t, J = 7.1 Hz, CH_3), 1.38–1.51 (4H, m, CH_2), 1.58–1.65 (4H, m, CH_2), 2.35 (3H, s, CH_3), 3.24–3.30 (4H, m, NCH_2), 4.35 (2H, s, NH), 5.80 and 6.39 (2H, d, $=CH_2$), 5.95 (2H, s, ArH), 6.96 (2H, d, J = 8.4 Hz, ArH), 7.31 (2H, d, J = 8.3 Hz, ArH), 7.73 (2H, d, J = 8.3 Hz, ArH), 7.90–8.01 (4H, m, ArH).

5-(3,5-Dioctyloxyphenyl)-10,15,20-tri-4-methoxycarbonylphenyl-porphyrin (TM(Me)). 3,5-Dioctyloxybenzaldehyde (1.09 g, 3.00 mmol), 4-formyl benzoic acid methyl ester (1.48 g, 9.00 mmol) and pyrrole (0.81 g, 9.00 mmol) were dissolved in propionic acid (45 mL) under a nitrogen atmosphere. The reaction mixture was refluxed for 3 h. Then the mixture was poured into methanol and filtered. The resulting solid was purified by silica gel column chromatography (dichloromethane–methanol = 100 : 1, v/v) to yield TM(Me) (283 mg, 9%); δ_H (300 MHz; $CDCl_3$; Me_4Si) −2.82 (2H, s, NH), 0.85 (9H, t, $J = 6.8$ Hz, CH_3), 1.26–1.90 (24H, m, CH_2), 4.07–4.16 (13H, m, CO_2CH_3 and OCH_2), 6.89 (1H, s, ArH), 7.37 (2H, s, ArH), 8.30 (6H, d, $J = 7.9$ Hz, ArH), 8.46 (6H, d, $J = 7.9$ Hz, ArH), 8.78 (2H, d, $J = 4.8$ Hz, β-H), 8.80 (4H, s, β-H), 9.00 (2H, d, $J = 4.8$ Hz, β-H).

5-(3,5-Dioctyloxyphenyl)-10,15,20-tri-4-carboxyphenyl-porphyrin (TM). TM(Me) (130 mg, 0.12 mmol) was treated with 0.5 M NaOH solution (10 mL) in THF (50 mL)–H_2O (5.0 mL) at room temperature for 4 h. The resulting mixture was neutralized by 1 M HCl solution and extracted with chloroform. The crude product was purified by recrystallization from dichloromethane–*n*-hexane to yield TM (115 mg, 96%); δ_H (300 MHz; DMSO-d_6; Me_4Si) −2.95 (2H, s, NH), 0.81 (9H, t, $J = 6.8$ Hz, CH_3), 1.23–1.80 (24H, m, CH_2), 4.14 (4H, t, $J = 6.6$ Hz, OCH_2), 6.94 (1H, s, ArH), 7.36 (2H, s, ArH), 8.30 (6H, d, $J = 7.9$ Hz, ArH), 8.46 (6H, d, $J = 7.9$ Hz, ArH), 8.82 (6H, bs, β-H), 9.00 (2H, d, $J = 3.8$ Hz, β-H).

Preparation of *trans*-IP and *trans*-BP

A pre-polymerization mixture was prepared according to Table 1. The mixture was purged with nitrogen for 1 min and was polymerized at 60 °C for 18 h. The resulting polymer was ground to obtain polymer particles. The polymer particles were suspended in 2 mM $CsCO_3$ THF–methanol (20 mL, 1 : 1, v/v) solution and stirred at room temperature for 48 h, then were washed with toluene–THF (3 : 1, v/v) and methanol. Corresponding blank polymer was prepared with the same recipe without TM.

Absorption measurements for FM and the prepared polymers with and without photoirradiation

Absorption spectra of FM (25 μM) in toluene–DMSO (9 : 1, v/v) were measured with a Jasco V-560 spectrophotometer. *trans*-FM was photoisomerized to *cis*-FM by irradiation with a high-pressure Hg-lamp (500 W, USHIO, USH-500SC) through a filter (Sigma, UTAF-33U; $230 < \lambda < 430$ nm). In contrast, photoisomerization to the *trans*-form from the *cis*-form was induced by irradiation with a 500 W high-pressure Hg-lamp through a filter (Sigma, SCF-42L; $\lambda > 410$ nm).

Absorption spectra of the suspensions of polymer particles (2.0 mg) in toluene–DMSO (2.7 mL, 9 : 1, v/v) were measured with a Jasco V-560 spectrophotometer equipped with an integrating sphere (ISV-469). The photoisomerization of polymers was carried out in the same manner as that for FM.

Binding tests of TM and structurally related compounds to *trans*-IP and *trans*-BP

The polymer particles (3.0 mg) were incubated with 5-(3,5-dioctyloxyphenyl)-10,15,20-tri-4-carboxyphenyl-porphyrin (TM), 5-(4-carboxyphenyl)-10,15,20-triphenyl-porphyrin (MCPP), 5,10-di-(3,5-dioctyloxyphenyl)-15,20-di-4-carboxyphenyl-porphyrin (DCPP), 5,10,15,20-tetra-4-carboxyphenyl-porphyrin (TCPP), 5-(3,5-dioctyloxyphenyl)-10,15,20-tri-4-methoxycarbonylphenyl-porphyrin (TM(Me)) and 5,10,15,20-tetra-4-hydroxyphenyl-porphyrin (THPP) (150 μM at 25 °C) in toluene–DMSO (2.0 mL, 7 : 3, v/v) for 5 h. After incubation in the dark, the suspensions were filtered, and the filtrates were analyzed by the UV–vis spectrophotometer.

Scatchard analysis

The polymer particles (3.0 mg) were suspended in toluene–DMSO (2.0 mL, 9 : 1, v/v) containing various amounts of TM (5.0 to 90 μM). After incubation for 17 h at 25 °C in the dark and under the photoirradiation, the suspensions were filtered, and the concentrations of free TM (F) were determined with the UV–vis spectrophotometer. The amount of TM bound to polymers (B) was calculated by subtraction from the initial TM concentration. Scatchard analysis was based on the equation $B/F = (B_{max} - B)K_a$, where K_a is an association constant and B_{max} is a maximum number of binding sites, and was performed from the data of the binding tests.

Preparation of *trans*-IP thin films and optical waveguide spectroscopy

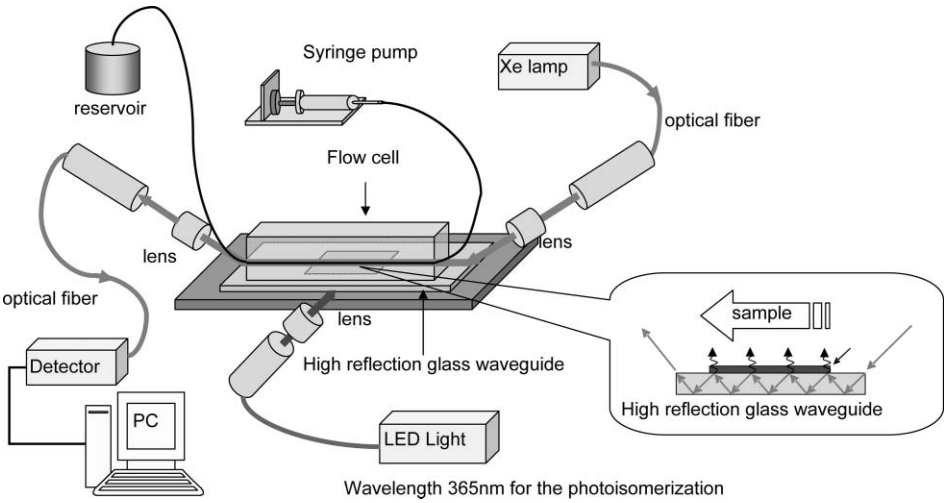
Vinylation of the surface of slab-type optical waveguides. In order to keep the prepared film sticking on a glass waveguide (20 mm × 65mm, thickness: 0.2 mm) stably, vinyl groups were introduced on the waveguide. Acetic acid solution (5% (v/v), 20 μL) containing 42 mM 3-methacryloxypropyltrimethoxysilane was dropped on the glass waveguide and a cover glass (18mm × 18mm) was put on it. After 24 h, the cover glass was peeled from the glass waveguide and the prepared film was washed with acetone.

Preparation of *trans*-IP thin films. The pre-polymerization mixture was prepared according to Table 3. The mixture (2 μL) was dropped on the vinylated glass waveguide and a cover glass (9mm × 9mm) was put on it. The polymerization was carried out for 2 h at 60 °C. The cover glass was peeled from the glass waveguide and the *trans*-IP film formed on the waveguide was washed with methanol. For preparing the film, the pre-polymerization mixture was diluted with toluene to obtain homogeneous films on the waveguide.

Optical waveguide spectroscopy (Scheme 3). Optical waveguide spectroscopy was carried out with an S-SPR6000 (System Instruments Co., Ltd., Japan). At first, the polymer-grafted glass waveguide was mounted on the pedestal of S-SPR6000 and the

Table 3 Polymer recipe for the thin films

FM	2.13 mg
TM	1.54 mg
Divinylbenzene	21 μL
Styrene	8.5 μL
ADVN	2.20 mg
Toluene	363 μL
DMSO	5 μL



Scheme 3 Schematic illustration of the optical waveguide spectrometer setup.

flow cell was attached, then DMSO solution containing 1% (v/v) acetic acid was pumped into the flow cell at 20 μL min^{-1} for 20 min to wash the template molecule out, and finally the solvent was replaced by dichloromethane.

For the target binding to *trans*-IP film step, the target solution (1 mM in DMSO–toluene, 1 : 9, v/v) was poured into the flow cell and kept for 20 min, then washed with dichloromethane for 20 min at 20 μL min^{-1}, and absorbance at 423 nn of the target molecule bound to *trans*-IP was measured. After the measurement, the *trans*-IP film was washed with DMSO containing 1% (v/v) acetic acid at 20 μL min^{-1} for 20 min and dichloromethane at 20 μL min^{-1} for 5 min, successively.

For the target binding to *cis*-IP film step, the film was irradiated with LED light (365 nm, ZUV-C10, OMRON Corporation, Japan) for 5 min to photoisomerize *trans*-IP into *cis*-IP. The target solution (1 mM in DMSO–toluene 1 : 9, v/v) was poured into the flow cell and kept for 20 min under UV irradiation, then washed with dichloromethane for 20 min at 20 μL min^{-1}, and the absorbance at 423 nn of the target molecule bound to the *cis*-IP was measured. After the measurement, the *cis*-IP film was washed with DMSO containing 1% (v/v) acetic acid at 20 μL min^{-1} for 20 min and dichloromethane at 20 μL min^{-1} for 5 min, successively. During the washing period, *cis*-IP was transformed into *trans*-IP. The measurements were carried out continuously for 20 h. The blank value was measured on the waveguide without the thin film and subtracted from the sample values. DMSO–toluene (1 : 9, v/v) was employed during the binding due to the solubility of TM, and optical waveguide spectroscopy was conducted in dichloromethane because DMSO–toluene (1 : 9, v/v) did not give clear spectra.

Notes and references

1 (*a*) K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495; (*b*) *Molecularly Imprinted Polymers*, ed. B. Sellergren, Elsevier, Amsterdam, 2001; (*c*) M. Komiyama, T. Takeuchi, T. Mukawa, H. Asanuma, *Molecular Imprinting*, Wiley-VCH, Weinheim, 2002.

2 (*a*) G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812; (*b*) S. Vidyasankar and F. H. Arnold, *Curr. Opin. Biotechnol.*, 1995, **6**, 218; (*c*) O. Ramström and K. Mosbach, *Curr. Opin. Chem. Biol.*, 1999, **3**, 759; (*d*) G. Wulff, *Chem. Rev.*, 2002, **102**, 1.

3 (*a*) P. Burgmayer and R. W. Murray, *J. Phys. Chem.*, 1984, **88**, 2515; (*b*) T. Shiga, *Adv. Polym. Sci.*, 1997, **134**, 131.

4 M. Zrinyi, *Trends Polym. Sci.*, 1997, **5**, 280.

5 (*a*) S. R. Lustig, G. J. Everlof and G. D. Jaycox, *Macromolecules*, 2001, **34**, 2364; (*b*) A. Izumi, R. Nomura and T. Masuda, *Macromolecules*, 2001, **34**, 4342; (*c*) A. Rachkov, N. Minoura and T. Shimizu, *Opt. Mater.*, 2002, **21**, 307; (*d*) Y. Takashima, T. Nakayama, M. Miyauchi, Y. Kawaguchi, H. Yamaguchi and A. Harada, *Chem. Lett.*, 2004, **33**, 890; (*e*) G. D. Jaycox, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 566.

6 (*a*) M. Inouye, *Coord. Chem. Rev.*, 1996, **148**, 265; (*b*) B. L. Feringa, R. A. van Delden, N. Koumura and E. M. Geertsema, *Chem. Rev.*, 2000, **100**, 1789; (*c*) S. Yagai, T. Karatsu and A. Kitamura, *Chem.–Eur. J.*, 2005, **11**, 4054.

7 T. Asano and T. Okada, *J. Org. Chem.*, 1984, **49**, 4387.

8 (*a*) N. Minoura, K. Idei, A. Rachkov, H. Uzawa and K. Matsuda, *Chem. Mater.*, 2003, **15**, 4703; (*b*) N. Minoura, K. Idei, A. Rachkov, Y.-W. Choi, M. Ogiso and K. Matsuda, *Macromolecules*, 2004, **37**, 9571.

9 C. Gong, M. H.-W. Lam and H. Yu, *Adv. Funct. Mater.*, 2006, **16**, 1759.

10 (*a*) D. A. Stephens and P. W. Bohn, *Anal. Chem.*, 1989, **61**, 386; (*b*) N. Matsuda, A. Takatsu and K. Kato, *Chem. Lett.*, 1996, 105; (*c*) S. B. Mendes, L. Li and J. J. Burke, *Langmuir*, 1996, **12**, 3374; (*d*) M. Mitsuishi, T. Tanuma, J. Matsui, J. Chen and T. Miyashita, *Langmuir*, 2001, **17**, 7449; (*e*) J. T. Bradshaw, S. B. Mendes, N. R. Armstrong and S. S. Saavedra, *Anal. Chem.*, 2003, **75**, 1080; (*f*) K. Ogawa, J. Harada, T. Fujiwara and H. Takahashi, *Chem. Lett.*, 2004, **33**, 1446; (*g*) K. Fujita, K. Taniguchi and H. Ohno, *Talanta*, 2005, **65**, 1066.

