Dual-Stimuli Responsive 2D Supramolecular Organic Framework for the Detection of Azoreductase Activity

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DOI: 10.1002/chem.201904443
Abstract: A 2D supramolecular organic framework (SOF) based on synthetic macrocycles has been constructed in water by a self-assembly strategy. Two new organic monomers of this SOF, possessing viologen and azobenzene functional groups, form a stimuli-responsive host–guest system upon cooperatively binding with cucurbit[8]uril rings. The reversible formation and dissociation of 2D SOF can be realized by the isomerization of azobenzene under ultraviolet and visible light. The light-responsive property of the SOF is highly reversible and stable for up to four cycles. Moreover, azoreductase produced by Escherichia coli can reduce the N=N double bond of azobenzene entities, resulting in fluorescence recovery of the system. As an excellent and effective fluorescent probe, the SOF can detect azoreductase activity for real-time monitoring of the growth process of Escherichia coli. The dual-stimuli responsive 2D SOF is envisioned to drive the development of responsive devices with complex functions.

Introduction

2D materials with distinctive planar structures and excellent mechanical properties have received considerable attention in the fields of separation, sensing recognition, electronic components, biomaterials, etc.[1–4] To satisfy the growing demand of 2D materials, direct self-assembly has been introduced as a convenient and effective strategy for large-scale preparation.[5–10] 2D supramolecular organic frameworks (SOFs) are a typical and popular kind of materials based on the self-assembly strategy, which exhibit exceptional properties and enormous application potential.[11–13]

In the self-assembly process of 2D SOFs, disordered organic monomers are constructed orderly to form a planar structure driven by supramolecular interactions, which not only provides a powerful and highly directional driving force for self-assembly, but also further stabilizes the obtained 2D SOFs.[12–15] Different from covalent connections, supramolecular interactions show more versatile chemical properties in a self-assembly system. Manifold organic monomers have been introduced into 2D SOFs through inclusion-enhanced dimerization of supramolecular macrocycles, enriching the variety of self-assembly systems.[16–17] On the other hand, additional stimuli, such as pH, temperature, and redox, can reversibly break and restore the dynamic balance of supramolecular interactions.[14–21] The reversibility of stimuli-responsive supramolecular chemistry enables the attractive structures and prospective applications of 2D SOFs include sensing, detection, and bio-related fields.[22–25]

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Cucurbit[8]uril (CB[8]) has been confirmed as a common rigid macrocycle that can drive the organic monomers to self-assemble into supramolecular polymers.[26–30] Viologen and azobenzene derivatives can form ternary complexes with CB[8], which are well-documented supramolecular light-responsive systems based on the photoisomerism of azobenzene derivatives.[31–32] Furthermore, azobenzene derivatives are extensively applied as fluorescent probes as the system exhibits fluorescence recovery after reducing the nitrogen double bond.[31–33] CB[8] ternary complexes offer a general approach to construct highly complex supramolecular structures and materials.[36–39]

Herein, we have designed and synthesized two special guest compounds, that is, TPE-4MV and AZO (Figure 1, Schemes S1–S3 in the Supporting Information), which can form a dual-stimuli responsive 2D SOF with CB[8] in water through cooperative self-assembly. Through light-mediated isomerization of AZO, we have realized the reversible formation and dissociation of the 2D SOF. Significantly, the SOF can detect in real-time the azoreductase activity of Escherichia coli by monitoring the enzymatic reduction of AZO.

Results and Discussion

Compounds of TPE-4MV and AZO have multiple viologen and azobenzene functional groups, respectively. The detailed synthesis is described in the Supporting Information. To confirm the formation of the host–guest complex, TPE-4MV and AZO were mixed with CB[8] in D2O one after another. As shown in the 1H NMR spectra (Figure S1 in the Supporting Information), clear chemical shifts were observed after the addition of two guests, indicating the complex formation in water. Moreover, UV/Vis titration was performed, and Job’s plots (Figure 2a,b) showed that the stoichiometry of TPE-4MV, CB[8], and AZO was 1:4:2 in the host–guest complex, which proved the formation of these host–guest complexes constructed from TPE-4MV, CB[8], and AZO in water.

After the formation of the 2D SOF by mixing TPE-4MV, CB[8], and AZO at a molar ratio of 1:4:2 in water was confirmed, the properties of the SOF were explored by various testing methods including dynamic light scattering (DLS), small-angle X-ray scattering (SAXD), atomic force microscopy (AFM), and scanning electron microscopy (SEM). DLS results (Figure 2c) showed that the hydrodynamic diameter (Dh) of AZO and TPE-4MV was 0.63 and 1.29 nm, respectively. When
CB[8] was added, the $D_h$ of the complex increased rapidly to 190 nm, indicating that the organic monomers formed large assemblies in water. In addition, a scattering peak with d spacing of approximately 5.53 nm was clearly noticed in the SAXD profile (Figure 2d), suggesting the periodicity of the interior of the assemblies. All these results confirmed the SOF formation. In addition, the morphology of the SOF was investigated by SEM (Figure 3a) and AFM (Figure 3b). As expected, a large number of sheet-like structures were observed, which validated the 2D structure of the SOF. The thickness of the structures was approximately 32 nm, which was a result of the accumulation of the SOF during the drying process. The height of the structure was further calculated by AFM and the obtained values (Figure 3d) conformed to the outer diameter of CB[8] (1.75 nm), which strongly indicated the structure of the 2D SOF.

In a supramolecular self-assembly system, stimuli-responsive host–guest complexes are a research focus of great interest and significance, which are applied in structuring logical molecular devices and controlling material properties. Generally, the complexes will be dissociated when stronger guest or host molecules are added. Meanwhile, the introduced chemicals will cause system pollution. As a green and environmentally friendly method, light can provide a stable, accurate, and long-range external stimulus and has become a more appropriate and attractive option. AZO with azobenzene groups showed trans–cis isomerism under the irradiation of UV light (365 nm) and visible light (450 nm). Two isomers can be distinguished from the UV/Vis spectra (Figure S2 in the Supporting Information) and $^1$H NMR spectra (Figure S3 in the Supporting Information). Based on the photoinduced isomerization phenomenon, reversible formation and dissociation of the 2D SOF can be regulated by UV light and visible light. As shown in Figure 4a, the absorption peak at 345 nm in the near-UV region for the SOF solution decreased, whereas the absorption peak at 432 nm in the visible region increased, showing the rapid transition from trans-AZO to cis-AZO under UV light (365 nm) within 3 min (Figure S4 in the Supporting Information). It is geometrically unsuitable for the structurally altered AZO to enter the cavity of CB[8], resulting in the disintegration of CB[8].
The 2D SOF was further illustrated by SEM (Figure 4c), which showed the disorderly structure of the SOF after UV irradiation.

To prove the reassembly of the 2D SOF, the mixture was exposed to visible light (450 nm). The absorption peaks of the SOF showed slow recovery during 8 min (Figure S5 in the Supporting Information), as shown in the UV/Vis spectra (Figure 4a). Meanwhile, the chaotic mixture reshaped into smooth and large-area planes, testifying to the reformation of the 2D SOF (Figure 4d). In sharp contrast, the UV/Vis spectra exhibited...
no change (Figure S6 in the Supporting Information) when the mixture was kept in dark for 12 h, suggesting the reversible formation and dissociation of the 2D SOF was induced by light. In addition, the absorbance of the SOF at 345 nm showed periodic variations over four cycles, indicating the recyclable and stable photo-responsiveness of this system (Figure 4b). The successful preparation of the reversible light-responsive 2D SOF greatly promotes the development of stimuli-responsive 2D devices and lays a foundation for structuring advanced materials.

Interestingly, trans–cis isomerism of azobenzene derivatives can deplete the excited energy, resulting in no fluorescence emissions. On this basis, AZO could serve as an efficient quencher in this system. As shown in Figure 5a, trans-AZO and...
cis-AZO can both quench the fluorescence of TPE-4MV without CB[8]. The greater spectra overlap between the absorption of cis-AZO and the emission of TPE-4MV resulted in far stronger quenching ability of cis-AZO than that of trans-AZO (Figure 5b and d). On the other hand, after forming the host–guest complex, trans-AZO exhibited higher energy transfer efficiency and stronger quenching effect as it was much closer to TPE-4MV in the cavity of CB[8]. Therefore, during the reversible formation and dissociation of the SOF, no fluorescence was observed (Figure S7 in the Supporting Information), suggesting that the trans–cis isomerism of AZO does not interfere with the subsequent fluorescence recovery.

As an alternative to light-mediated isomerization, the reduction of the nitrogen double bond is another stimulus of the system to restore fluorescence. Azoreductase, widely found in most bacteria, can reduce azobenzene groups to anilines with high efficiency and selectivity. Hence, it is expected that the 2D SOF would be an excellent fluorescent probe to detect azoreductase activity in bacteria. To imitate the physiological environment of bacteria, the fluorescence variation of the 2D SOF was studied in phosphate buffered saline (PBS). In Figure 5c, the fluorescence quenching of the SOF was affirmed as expected. Then, sodium dithionite (SDT), as an effective and biocompatible chemical substitute for azoreductase, was introduced to reduce the nitrogen double bond.[51] The kinetics of the reduction reaction were measured by UV/Vis spectra (Figure 6a) and fluorescence spectra (Figure 6b). The steady absorbance of the SOF at 345 nm showed a sharp decline after the addition of SDT (20 equiv) and the light-yellow color of the SOF disappeared in 15 min, all of which indicate the reduction of AZO. Meanwhile, the reduction of AZO resulted in a fast fluorescence recovery of the system. This process proved that the change of fluorescence intensity of the 2D SOF was stimulated by the reduction of the nitrogen double bond.

Based on the above results, the 2D SOF, as the fluorescent probe to detect azoreductase activity, was mixed into Luria-Bertani culture media. After the growth of Escherichia coli, culture media showed a recovery of fluorescence as shown in the photos (Figure 6d), whereas the blank control group did not change. Azoreductase activity can not only track the proliferation of Escherichia coli, but also be used as a real-time monitor of the growth process. The fluorescence intensity of the 2D SOF with the growth of Escherichia coli is shown in Figure 6d. The extreme rise of fluorescence intensity was consistent with the logarithmic growth period of Escherichia coli from 1 h to 5 h. Then, the fluorescence intensity of the solution increased gently because the growth of Escherichia coli has become stable.[52–53] Certainly, potential interference factors, such as inorganic salts (NaCl, KCl, CaCl₂, and MgCl₂), biothiols (cysteine and dithiothreitol), glutamic acid, arginine, reactive oxygen...
species (H₂O₂), and bovine serum albumin (BSA), have all been estimated under the same conditions before actual testing (Figure 6c). The fluorescence intensity of 2D SOF hardly changed under these interferences, indicating the selectivity of the SOF to azoreductase. The 2D SOF exhibited an excellent performance of being a fluorescence probe, detecting azoreductase activity and real-time monitoring the growth process of Escherichia coli.

**Conclusion**

We have demonstrated that a 2D SOF can be constructed in aqueous solutions through the formation of supramolecular stimuli-responsive host–guest complexes of TPE-4MV, CB[8], and AZO. The reversible formation and dissociation of the 2D SOF was induced by light, a stable, accurate, and long-range external stimulus, through the trans–cis isomerization of AZO, rather than by harsh conditions and extra chemicals. Moreover, the 2D SOF showed a “turn-on” fluorescence response to the reduction of the nitrogen double bond. High selectivity to azoreductase against other bio-relevant interferences and the ability to real-time monitor the growth process of Escherichia coli made the SOF an excellent and effective fluorescent probe. The 2D SOF provides a new direction for the construction of chemical and biological devices with good stimulus responsiveness and easy manipulation.

**Experimental Section**

**General information**

All the reagents and solvents were purchased from commercial sources and used as received unless otherwise noted. Ultrapure water, purified by using an Experimental Water System (Lab-UV-20), was used in the relevant experiments. 1H NMR spectra were recorded with a Bruker AVANCE III 300 MHz NMR spectrometer at 298 K. 13C NMR spectra were measured with a Bruker Digital Avance III HD 400 WB NMR spectrometer at ambient temperature with a magic angle spinning rate of 7.0 kHz. SEM images were recorded with a Hitachi SU8020 electron microscope. SAXD patterns were recorded with a Rigaku Smartlab X-ray diffractometer. UV/Vis spectra were recorded with a Shimadzu UV-2550 instrument. Fluorescence spectra were recorded with a Shimadzu RF-5301PC spectrometer. AFS images were taken with a Keysight SPN6500. Precursors 1,1,2,2-tetrakis(4-(4-bromobutoxy)phenylethene (T2), (S)-1-(4-(4-bromobutoxy)phenyl)-2-phenyldiazene (A2), and CB[8] were synthesized according to literature procedures.⁵⁴–⁵⁸

**Synthesis of TPE-4MV**

Compound T2 (1.87 g, 2 mmol) and 1-methyl-[4,4'-bipyridin]-1-ium iodide (2.98 g, 10 mmol) were added into a flask. Then, MeCN (50 mL) was added at room temperature. Then, the mixture was heated at reflux for 72 h. After cooling to room temperature, the precipitate was filtered and washed with MeCN and CH₂Cl₂ to result in a red product (2.68 g, yield: 63%). 1H NMR (D₂)DMSO, 300 MHz): δ = 9.45–9.43 (d, J = 5.9 Hz, 8H), 9.32–9.30 (d, J = 6.7 Hz, 8H), 8.83–8.77 (m, 16H), 6.84–6.83 (d, J = 4.6 Hz, 8H), 6.71–6.69 (d, J = 7.7 Hz, 8H), 4.80–4.75 (m, 8H), 4.45 (s, 12H), 3.96–3.92 (m, 8H), 2.16–2.10 (m, 8H), 1.78–1.71 ppm (m, 8H); 13C NMR (126 MHz, CDCl₃): δ = 150.79, 149.78, 147.22, 146.32, 137.88, 133.06, 128.02, 127.95, 127.62, 127.51, 114.65, 67.97, 62.52, 37.53, 26.65, 26.22 ppm; HRMS (ESI): calculated for [M]+ [C₅₉H₄₆Br₂IN₃O₇]+: m/z = 852.4326, found: 852.4364.

**Real-time detection of azoreductase produced by Escherichia coli**

The Luria-Bertani (LB) culture medium was prepared by using bacto-trypthone (2 g), bacto-yeast extract (1 g), and NaCl (2 g) in water (200 mL), and the pH was adjusted to 7.4 by using NaOH (1 M). The Escherichia coli was first grown at 37°C in LB culture media. Then, the bacterial colonies were added into fresh LB culture media (200 mL) containing the 2D SOF material. The Escherichia coli was cultured in Luria-Bertani broth at 37°C and taken at different periods of time (0–12 h).⁵⁹

**Acknowledgments**

We thank the National Natural Science Foundation of China (51673084 and 21871108), the Jilin Province-University Cooperative Construction Project-Special Funds for New Materials (SXGJSF2017-3), and the Jilin University Talents Cultivation Program for financial support.

**Conflict of interest**

The authors declare no conflict of interest.

**Keywords:** cucurbit[n]uril · fluorescent probes · photosomization · stimuli-responsive systems · supramolecular organic frameworks

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