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Anthracene-bridged “Texas-sized” box for the simultaneous detection and uptake of tryptophan

Dan Luo[a], Jinya Tian[a], Jianqiao Zhou[a], Xiaodong Chi[a,b,*]

[a] *State Key Laboratory of Materials Processing and Die & Mold Technology, School of Materials Science and Engineering, Huazhong University of Science and Technology, Wuhan 430074, China*

[b] *Shenzhen Huazhong University of Science and Technology Research Institute, Shenzhen 518057, China*

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ABSTRACT

Tryptophan (Trp) is an essential amino acid that plays a critical role in human physiology. The increasing demand for Trp has created a highly promising market, underscoring the urgent necessity for the development of efficient strategies for the simultaneous detection and uptake of tryptophan. Herein, we report an expanded “Texas-sized” molecular box (**An-TxSB**), which incorporates luminescent anthracene bridging subunits and molecular recognition motifs. This luminescent molecular box demonstrates exceptional sensitivity to Trp in water, permitting its precise quantification with a notably low limit of detection (LOD) of 0.42 µmol/L. Moreover**, An-TxSB** facilitates the proficient uptake of Trp from simulated water samples, thereby revealing an impressive Trp adsorption capacity of up to 226.0 µmol/g.

Tryptophan (Trp), an essential amino acid, holds a vital role in the human body as a crucial precursor to melatonin, serotonin, and niacin [1–3]. Its unique molecular structure and distinctive biological functions, along with the impact of its metabolic byproducts on organisms, highlight its significance in the growth and metabolism of animals and humans [4,5]. Tryptophan is crucial for physiological development and has extensive use in food, feed additives, medicine, and agriculture [6]. The expansion of the feed and pharmaceutical industries has resulted in an increased demand for tryptophan, signifying a promising market [7]. Microbial fermentation is the primary approach for large-scale Trp production, but the production costs remain prohibitive [8]. Downstream engineering processes, such as separation and purification, contribute significantly to these costs [9]. As a result, there is an urgent and critical need for methods that would allow for the rapid, sensitive detection and efficient uptake of Trp from water.

Various methods have been employed for Trp detection, including high-performance liquid chromatography [10,11], spectrophotometry [12], electrochemistry [13], and mass spectrometry [14]. However, these methods may have issues such as high cost, low sensitivity and specificity, and sophisticated sample preparation. Rapid, low-cost, and user-friendly detection technologies could thus provide a useful complement to traditional methods. To date, several potential Trp detection methods have been explored in this context, with a major emphasis on optical sensing approaches [15,16]. For example, Cao’s group reported novel tetraphenyl ethylene based octacationic cages, which could selectively binds biomolecules, especially for aromatic dipeptides and Trp [17,18]. Pillar[*n*]arene can be modified to achieve a wide range of applications [19–28], including detection ʟ-Trp [29]. Wei’s group construct a ‘trapezoid’ molecular boxes (**TBox**) that selectively recognize Trp in aqueous solution *via* donor-acceptor interactions [30]. However, to our knowledge, none of these detection systems have permitted the uptake of Trp *via* simple adsorption means. We believe that the ability to both detect and uptake Trp from water could abet remediation efforts. As demonstrated below, we found that the **An-TxSB** of this study not only detects Trp with high sensitivity but also enables efficient uptake from simulated water sources through absorption (Scheme 1).

The “Texas-sized” molecular boxes (TxSBs), a new kind of innovative macrocyclic hosts first reported by Sessler’s group, have shown excellent abilities to selectively complex various electron-rich guests and offered a good platform for the construction of functional materials [31–35]. For instance, Sessler’s group has successfully employed TxSBs to discern a wide range of acid compounds [36–38], leading to a breakthrough discovery with practical applications in hydrogel for anions removal from water and information encryption [39–41]. Moreover, TxSBs can be employed in amphiphilic copolymers for stimuli-responsive drug delivery [42]. Amino acids are a type of amphoteric compound that possess a carboxyl group. However, to our knowledge TxSBs have not been

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[*] Corresponding author.
E-mail address: xchi@hust.edu.cn (X. Chi).

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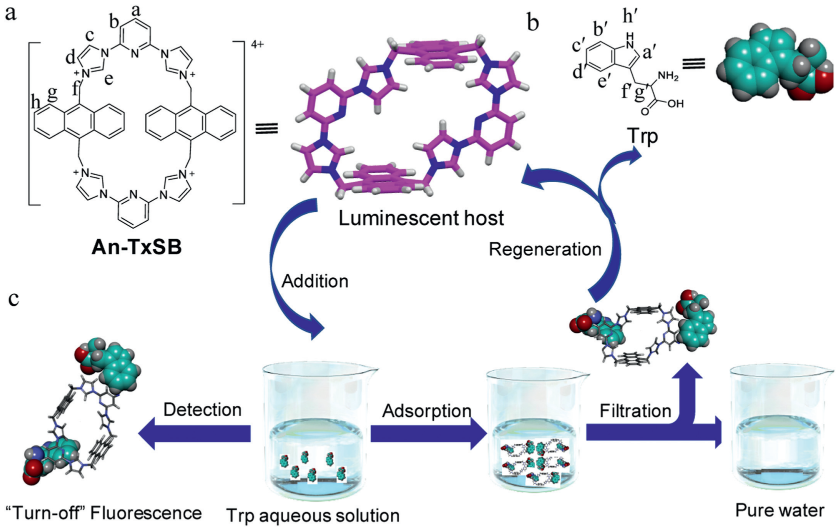

Scheme 1. Chemical structures of (a) **An-TxSB**; (b) Trp; and (c) cartoon illustration of the application of **An-TxSB** on selective detection and absorption of Trp.

employed to recognize amino acids. The present study was undertaken in an effort to explore the potential of utility of **An-TxSB** in the context of selective recognition and efficient uptake of Trp from water.

Taking inspiration from Davis' research on the use of bis-anthracenyl monocyclic for various biogenic heterocycles [43,44], we anticipate that anthracenyl-bridged TxSB may exhibit the ability to recognize Trp in water. The synthesis of **An-TxSB** is summarized in Scheme S1 (Supporting information). The key trimeric fragment, 2,6-di(1*H*-imidazol-1-yl)pyridine, and 9,10-bis(bromomethyl)anthracene were prepared according to previous reports. Cyclization then gave the corresponding macrocycle in the form of its tetrabromide salt. Exposure to aqueous NH_4PF_6 gave the corresponding tetrahexafluorophosphate salt, **An-TxSB-PF$_6$**, in 24.1% yield (based on cyclization and salt exchange). A single crystal of **An-TxSB-PF$_6$** was obtained by vaporizing a mixed solvent of acetonitrile and water. The analysis of the single crystal structure disclosed that the anthracene group assumes a perpendicular orientation relative to the pyridine plane, primarily attributable to steric hindrance effects (Fig. 1). Additionally, the solid-state structure unveiled the existence of π-π stacking interactions and C–H$\cdots\pi$ interactions between the anthracene group of **An-TxSB-PF$_6$** and its adjacent counterpart (as depicted in Fig. 1d). These intermolecular interactions play a pivotal role in bolstering the stability and overall arrangement of the crystal lattice.

We then focused on the host-guest complexation between **An-TxSB-Cl** and various amino acids. To facilitate this study, and considering the limited water solubility of **An-TxSB-PF$_6$**, we conducted an anion exchange procedure, converting PF_6^- to Cl^- to obtain **An-TxSB-Cl**. We initiated our investigation into the binding behavior of **An-TxSB-Cl** with various amino acids by employing ^{1}H NMR at 298 K. Interestingly, the addition of amino acids such as L-Phe, L-Asp, L-Ser, L-Cys, L-His, L-Gln, and L-Tyr did not elicit any noticeable chemical shifts, except for L-Trp (Fig. S12 in Supporting information). Upon introducing L-Trp to **An-TxSB-Cl**, significant chemical shift changes became apparent, particularly evident in the upfield shift of pyridine protons H_a and H_b on **An-TxSB-Cl**. The signal from anthracene protons H_g and H_h also exhibited an downfield shift. Imidazole protons H_c and H_e displayed a upfield shift, while H_d shifted to downfield. In contrast, all protons on L-Trp shifted upfield, indicating a robust shielding effect exerted by the host molecule (Fig. 2a). This compelling result offers substantial evidence of **An-TxSB-Cl**'s selective recognition ability towards Trp.

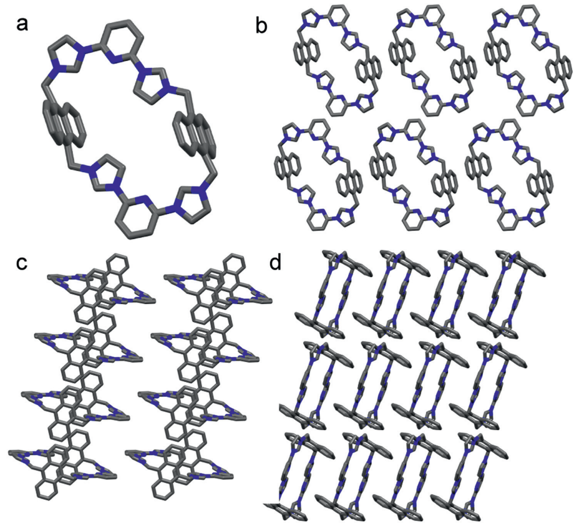

Fig. 1. (a) Single-crystal structure of **An-TxSB-PF$_6$**. Different views of the packing arrangements of **An-TxSB-PF$_6$** seen in the solid state. (b) Down the *a* axis, (c) down the *b* axis, (d) down the *c* axis. Solvent molecules, anions and hydrogen have been omitted for clarity.

In order to gain insight into the presumed molecular recognition modes, the NOESY study was carried. The interaction between pyridine group on **AnTxSB-Cl** and indole group on L-Trp can be observed through NOESY experiments, suggesting a π-π stacking interaction (Fig. S11 in Supporting information). Additionally, proton H_f on **An-TxSB** also exhibits a C–H$\cdots\pi$ interaction with indole group on L-Trp. We simulated the host-guest complex structure based on these findings. Fluorescence titration experiments and isothermal titration calorimetry (ITC) were subsequently conducted to elucidate the binding behavior of **An-TxSB-Cl** with L-Trp. The results unveiled a binding ratio of approximately 2, accompanied by association constants of $K_{a1} = 2.52 \times 10^4\,(\text{mol/L})^{-1}$. $K_{a2} = 1880.61\,(\text{mol/L})^{-1}$ (Fig. 2b and Fig. S16 in Supporting information). Possible structure for the Trp@**An-TxSB** was inferred from DFT calculations (Fig. 2c). Calculations performed on this structure revealed a notable energy decrease following the recognition of tryptophan, in comparison to the energy of the isolated **An-TxSB-Cl** (Table S2 in Supporting information).

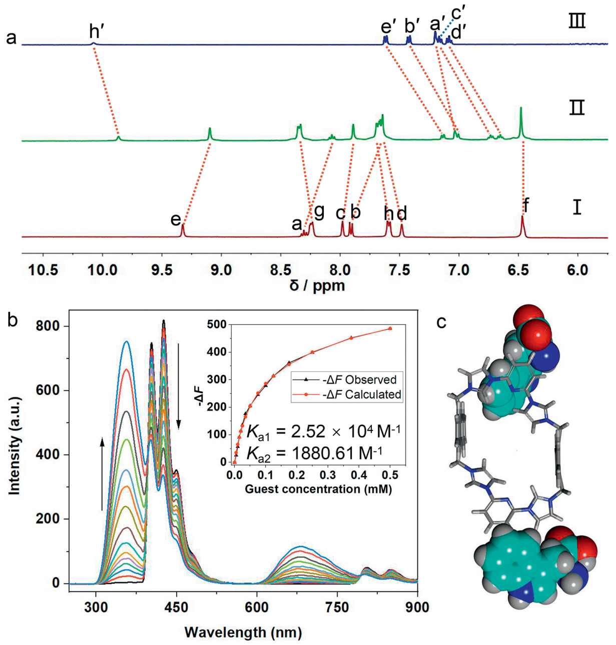

Fig. 2. (a) ^{1}H NMR spectra (400 MHz, H_2O:D_2O = 9:1, 298 K) of **An-TxSB-Cl** (I), **An-TxSB-Cl** + 2 equiv. L-Trp (II), and L-Trp (III). (b) Fluorescence titration of **An-TxSB-Cl** (2.5×10^{-6} mol/L) with the addition of L-Trp in water (λ_{ex} = 252 nm). Inset: binding data (423 nm) and fitting curve. (c) DFT optimized structure of Trp@**An-TxSB**. M^{-1} is $(mol/L)^{-1}$.

An-TxSB-Cl exhibited robust fluorescence in aqueous solution (Fig. 3), prompting us to explore its potential as a fluorescent sensor for Trp. Remarkably, upon the addition of various concentrations of Trp to **An-TxSB-Cl**, the fluorescence intensity exhibited a linear decrease with increasing Trp concentration (Fig. 3a). These results allowed us to calculate a corresponding limit of detection (LOD) of 0.42 μmol/L for L-Trp (Fig. 3b) using the 3σ/slope method. Moreover, **An-TxSB** demonstrated exceptional adsorption capabilities for Trp, as discussed further below.

We then proceeded to assess the detection selectivity of **An-TxSB-Cl** for Trp. We introduced 200 equiv. of L-Trp alongside various other amino acids including L-Gly, L-Ala, L-Val, L-Leu, L-Ile, L-Phe, L-Tyr, L-Asp, L-Asn, L-Glu, L-Gln, L-Met, L-Ser, L-Thr, L-Cys, L-Pro, L-His, and L-Arg to the aqueous solution of **An-TxSB-Cl**. Utilizing fluorescence spectroscopy and visual observations, it became evident that the fluorescence intensity of **An-TxSB-Cl** remained minimally affected upon the addition of other amino acids, with the exception of L/D-Trp (Figs. 3c and d). Competitive experiments were further conducted in the presence of 100 equiv. of L-Trp and 200 equiv. of various amino acids in water (Fig. 3c). These experiments demonstrated a direct decrease in fluorescence intensity. Based on these comprehensive studies, we deduce that **An-TxSB-Cl** exhibits selectivity for Trp, rendering it a promising candidate for Trp detection. Moreover, in contrast to the Davis' report [44], **An-TxSB-Cl** lacks the ability to identify uric acid. Consequently, it exhibits a higher capacity to resist interference when detecting Trp.

Given the robust host-guest interaction between **An-TxSB** and Trp, we proceeded to investigate whether **An-TxSB** could effectively extract Trp from water. **An-TxSB-PF$_6$** was employed as the adsorbent for this investigation. Our fluorescence analysis exhibited a conspicuous reduction in Trp concentration within the aqueous solution upon the introduction of **An-TxSB-PF$_6$** (Fig. 4a and Fig. S18a in Supporting information). By establishing a standard curve and conducting subsequent calculations, we ascertained a remarkable adsorption capacity of 226 μmol/g for L-Trp and 211 μmol/g for D-Trp, respectively (Fig. 4b and Fig. S18b in Supporting information). Notably, these values surpass those typically observed in most porous materials (Table S3 in Supporting information). Further analysis of adsorption kinetics unveiled a second-order kinetics model for absorption behavior, with absorption rates of 0.022 g^{-1} min^{-1} for L-Trp and 0.030 g^{-1} min^{-1} for D-Trp (Fig. S19 in Supporting information).

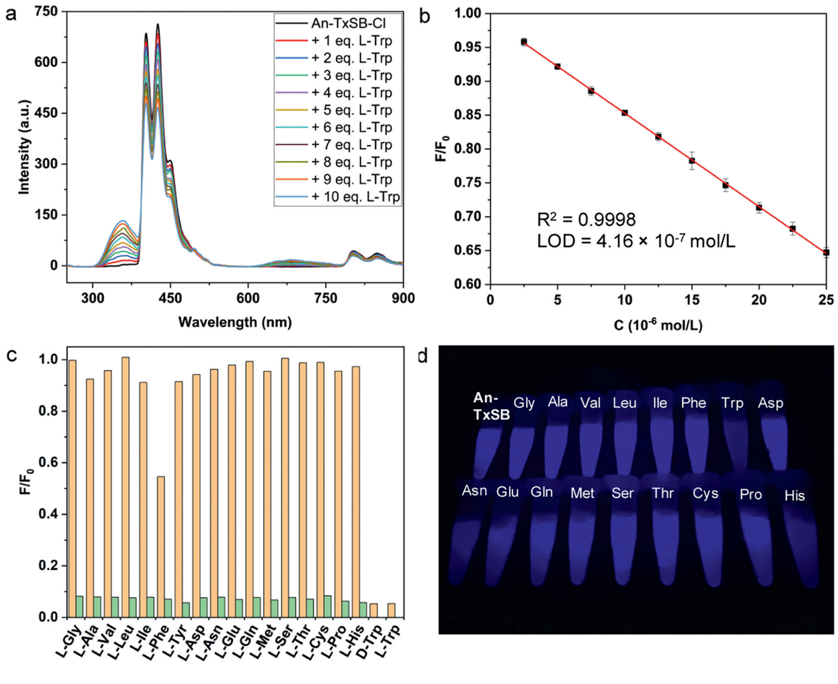

Fig. 3. (a) Fluorescence emission titration spectra of **An-TxSB-Cl** (2.5×10^{-6} mol/L) with various amounts of L-Trp in H_2O under excitation at 254 nm. (b) Fluorescence at 416.5 nm of compound **An-TxSB-Cl** as a function of L-Trp equivalent. (c) Fluorescence intensity of **An-TxSB-Cl** after adding different amino acids (200 equiv.) in H_2O (λ_{ex} = 295 nm, orange) and followed by adding 100 equiv. of L-Trp (green). (d) Photograph shown the color changes of **An-TxSB-Cl** with 200 equiv. various amino acid in H_2O at 365 nm.

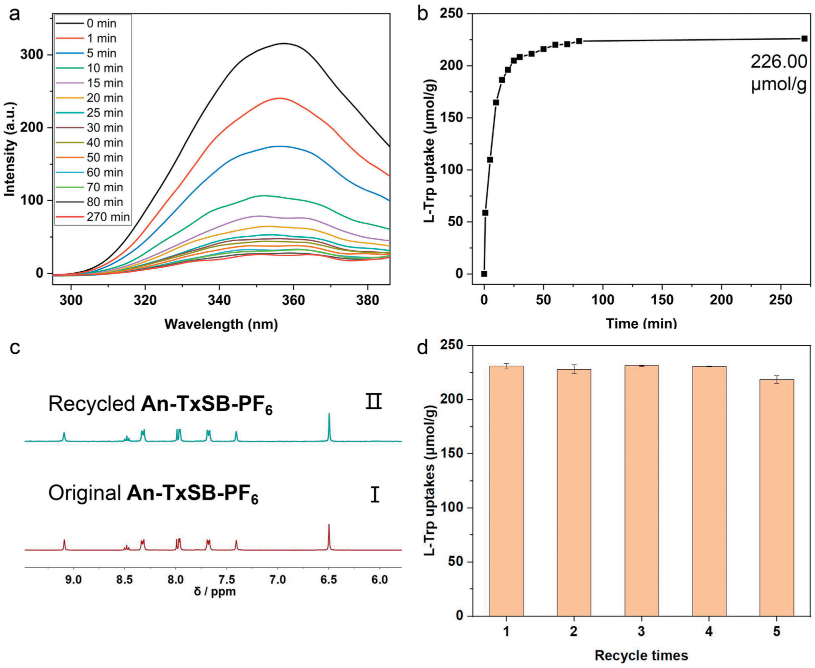

Fig. 4. (a) Fluorescence spectra of L-Trp (1×10^{-4} mol/L, 3 mL) with **An-TxSB-PF$_6$** (1 mg) in H_2O under excitation at 254 nm. (b) L-Trp uptake over time in **An-TxSB-PF$_6$**. (c) ^{1}H NMR spectrum (400 MHz, CD_3CN, 298 K) of **An-TxSB-PF$_6$** (I) and **An-TxSB-PF$_6$** after wash out the Trp (II). (d) Reuse of **An-TxSB-PF$_6$** for the extraction of L-Trp.

In addition, we delved into the potential reusability of **An-TxSB-PF$_6$**. Encouragingly, it was found that **An-TxSB-PF$_6$** could be readily reused through a straightforward water wash process, exhibiting no apparent loss of performance even after five adsorption cycles (Fig. 4d and Fig. S20 in Supporting information).

In summary, we have prepared a luminescent anthracene-bridged TxSB (**An-TxSB**) that exhibits a highly sensitive “turn-off” fluorescence response upon interaction with Trp. Impressively low limits of detection were achieved, with values as low as 0.42 μmol/L for Trp. Furthermore, the **An-TxSB** demonstrated remarkable efficacy in efficiently extracting Trp from simulated water samples, showcasing both high absorption efficiency and capacity. Collectively, we believe that the results presented in this study will pave the way for advancements in Trp detection and adsorption techniques. Additionally, our findings suggest that luminescent crystalline materials hold significant promise as an avenue for the detection and uptake of amino acids, offering a broader application potential.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109444.

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