



# The Detection of Food Additives Using a Fluorescence Indicator Based on 6-*p*-Toluidinylnaphthalence-2-sulfonate and Cationic Pillar[6]arene

Qunpeng Duan\*, Yibo Xing and Kainan Guo

School of Chemical and Printing-dyeing Engineering, Henan University of Engineering, Zhengzhou, China

The current study investigated host-guest complexation in 6-*p*-toluidinylnaphthalene-2-sulfonate (**TNS**), a fluorescence probe used to investigate hydrophobic regions that contain the water-soluble cationic pillar[6]arene (**CP6**). After complexation with **CP6**, the fluorescence intensity of **TNS** was significantly increased. The decreases in the fluorescence intensity of the **TNS•CP6** complex when phenolic food-additives are added have been used in indicator displacement assays to detect food additives in the water.

## OPEN ACCESS

### Edited by:

Yong Yao,  
Nantong University, China

### Reviewed by:

Yincheng Chang,  
Beijing University of Chemical  
Technology, China  
Xie Han,  
Wuhan University of Science and  
Technology, China

### \*Correspondence:

Qunpeng Duan  
qpduan@haue.edu.cn

### Specialty section:

This article was submitted to  
Supramolecular Chemistry,  
a section of the journal  
Frontiers in Chemistry

**Received:** 22 April 2022

**Accepted:** 12 May 2022

**Published:** 31 May 2022

### Citation:

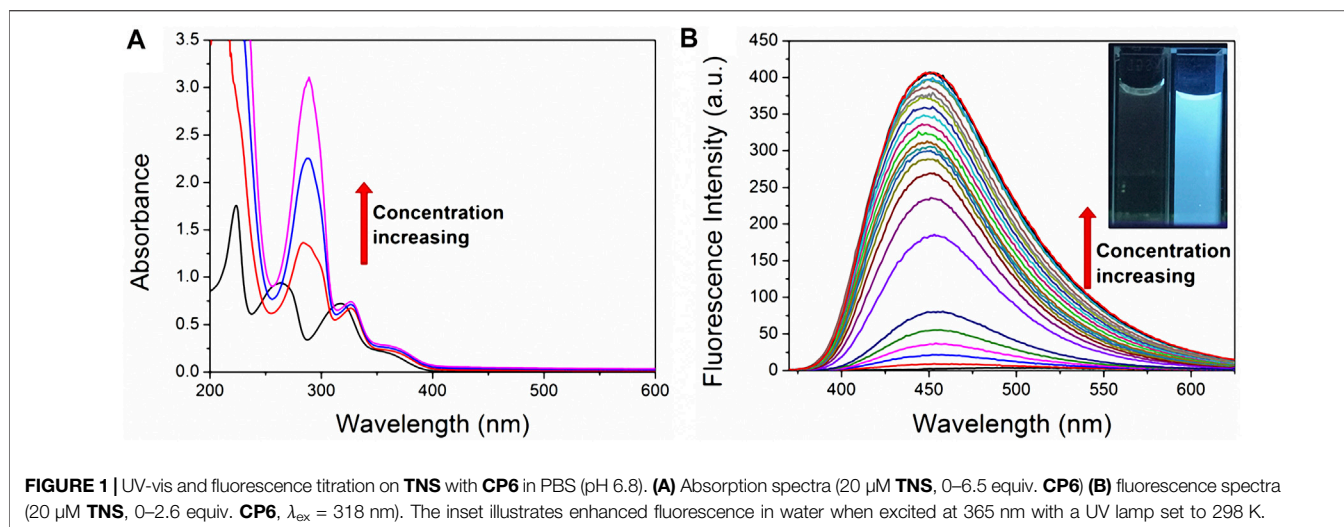
Duan Q, Xing Y and Guo K (2022) The  
Detection of Food Additives Using a  
Fluorescence Indicator Based on 6-*p*-  
Toluidinylnaphthalence-2-sulfonate  
and Cationic Pillar[6]arene.  
Front. Chem. 10:925881.  
doi: 10.3389/fchem.2022.925881

**Keywords:** pillararene, host-guest complex, fluorescence, indicator displacement assay, food additives

## INTRODUCTION

Fluorescent indicator displacement (FID) assays make use of fluorescent indicators and emission phenomena to detect important analytes by transiting different receptors to universal optical sensors. FID assays can bind to a wide variety of target molecules (Rather et al., 2021; Sedgwick et al., 2021). With the advancement of host-guest chemistry, macrocyclic host-based FID assays have garnered widespread attention for their potential application in the field of analytical testing, and several significant research results have been generated in recent years (Dsouza et al., 2011; Ghale et al., 2014; Cao et al., 2019; Jiang et al., 2020). Macrocyclic hosts, primarily cyclodextrins (Crini, et al., 2014; Pal, et al., 2015), calixarenes (Koh, et al., 1996; Hennig, et al., 2007; Guo, et al., 2014; Zheng, et al., 2018), cucurbiturils (Praetorius, et al., 2008; Florea, et al., 2011; Barrow, et al., 2015; Sonzini, et al., 2017), and pillararenes (Wang, et al., 2014; Bojtár, et al., 2015; Bojtár, et al., 2016; Hua, et al., 2016; Bojtár, et al., 2017; Hua, et al., 2018; Cai, et al., 2021; Wu, et al., 2021), are widely used as fluorescent probes in the majority of the FID-based sensing systems.

Phenolic food additives have been widely used in the food industry for their significant antioxidant, antimicrobial, and flavor-enhancing properties (Vinson, et al., 2012; Zhang, et al., 2014). Whereas the insolubility of food additives in water and their long-term stability contribute to their excessive use, ultimately resulting in their accumulation and negative effects on the biosphere (Tobacman, et al., 2001; Savjani, et al., 2012). Encapsulating small-molecule food-additives in non-toxic, water-soluble macrocyclic hosts improves their bioavailability and solubility by regulating their physical and chemical properties (Munin, et al., 2011). 2-Hydroxypropyl  $\beta$ -cyclodextrin (HP- $\beta$ -CD) is one representative example, with a binding affinity of  $\sim 10^2 \text{ M}^{-1}$  to food-additives (Pal, et al., 2016). It is critical to investigate artificial receptors with extremely high affinity for food additives to improve sensitivity and detection efficiency in compound detection.



We developed a new FID assay with a water-soluble cationic pillar[6]arene (**CP6**) for the detection of three important phenolic food additives, namely *p*-coumaric acid (**CA**), trans-ferulic acid (**FA**), and gallic acid (**GA**). Because of its enhanced fluorescence in non-polar environments, the widely used fluorescent probe, 6-*p*-toluidinylnaphthalene-2-sulfonate (Dotsikas, et al., 2000) (**TNS** in Scheme 1), was used as the fluorescent indicator in our FID system. Due to the complexation of **TNS** and **CP6**, we use an FID strategy to perform sensitive fluorescence detection on **CA**, **FA**, and **GA**.

## MATERIALS AND METHODS

The reagents used were marketable and applied directly without further purification. **CP6** (Duan et al., 2019) was synthesized by following the known procedures. Nuclear magnetic resonance (NMR) spectra were obtained using the Bruker Avance III HD 400 spectrometer with the deuterated solvent as the lock and the residual solvent as the internal reference. Fluorescence spectra were obtained by using the Agilent Cary Eclipse fluorescence spectrophotometer. To prevent the dilution effect during titration, **CP6** stock solutions were produced using the same **TNS** solution. The measurement was repeated three times for each experiment. Displacement assays for **CA**, **FA**, and **GA** were performed at pH 6.8 with **CP6** at varying concentrations of **CA**, **FA**, and **GA**, respectively. All the experiments were conducted at room temperature (298 K).

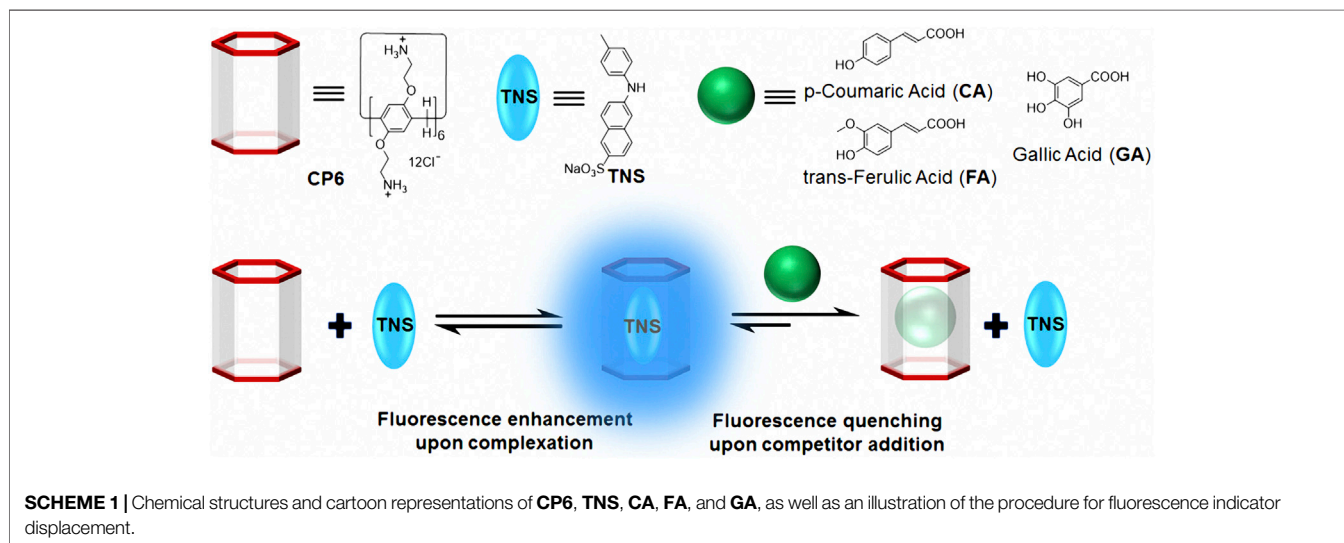
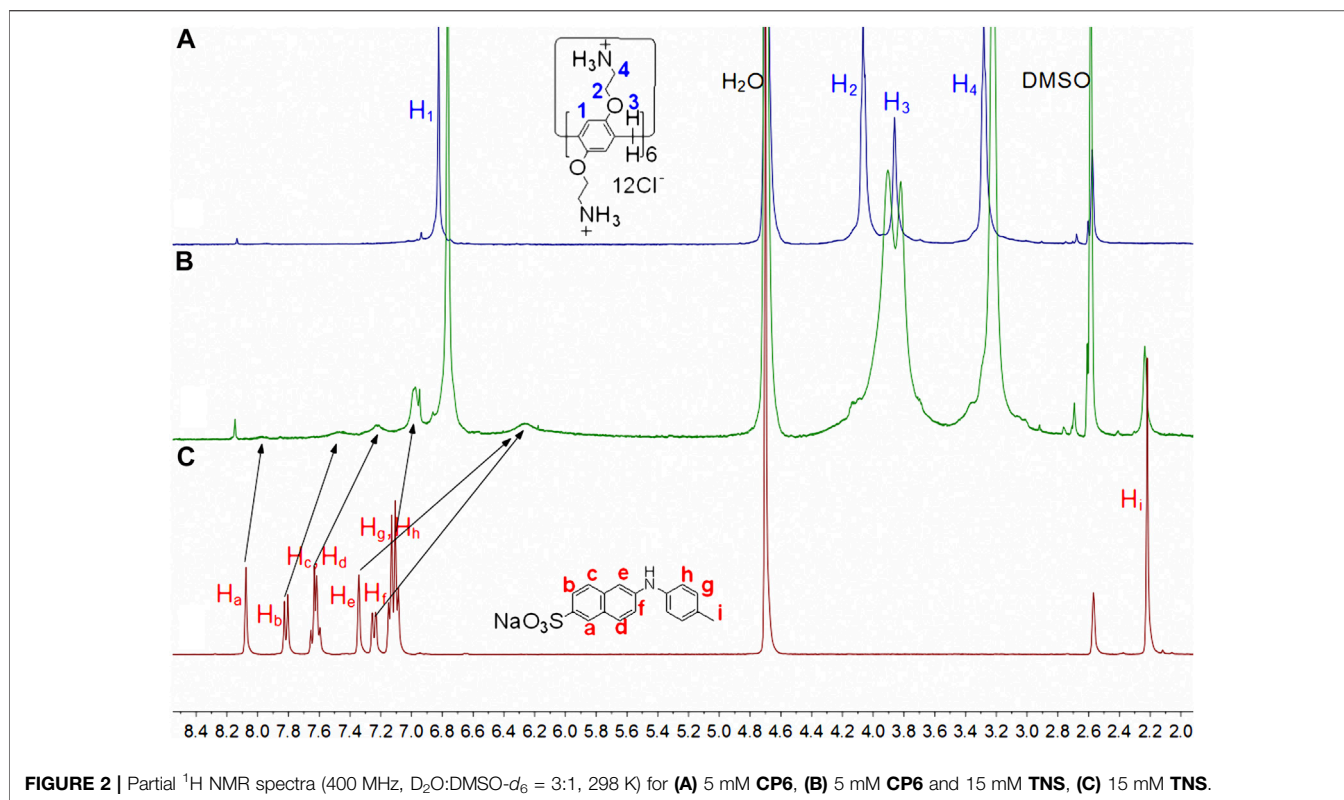
## RESULTS AND DISCUSSION

### Fluorescent Probe 6-*p*-Toluidinylnaphthalene-2-Sulfonate Complexed With Cationic Pillar[6]Arene

UV-vis absorption spectroscopy was used to confirm the host-guest complexation of fluorescent probe **TNS** with **CP6**.

Following successive additions of **CP6** to the phosphate-buffered solution (PBS) of **TNS** at pH 6.8, hyperchromic effects at the maximum absorption wavelengths of 223, 263, and 318 nm occurred with a significant bathochromic shift (Figure 1A). The variations appeared to be greater than those when  $\alpha$ -CD (Nishijo et al., 1992) or  $\beta$ -CD (Nishijo et al., 1995; Dotsikas et al., 2000) addition was used. The results indicate that **TNS** can form a stable complex with **CP6**. Additionally, a fluorescence titration on **TNS** with an increased **CP6** concentration was performed in PBS with a pH of 6.8 at room temperature. According to Figure 1B, as the concentration of **CP6** increased, a significant increase in fluorescence intensity was observed, along with a shift in the fluorescence maximum to shorter wavelengths. The results indicate that **TNS** molecules exist in a hydrophobic environment. Encapsulating **TNS** in **CP6** protects it from solvent collisions while also providing a distinct local environment for **TNS** in terms of polarity, which significantly enhances (approximately 400 times) fluorescence intensity. Additionally, the significant pale blue fluorescence was evident in UV light (the inset of Figure 1B).

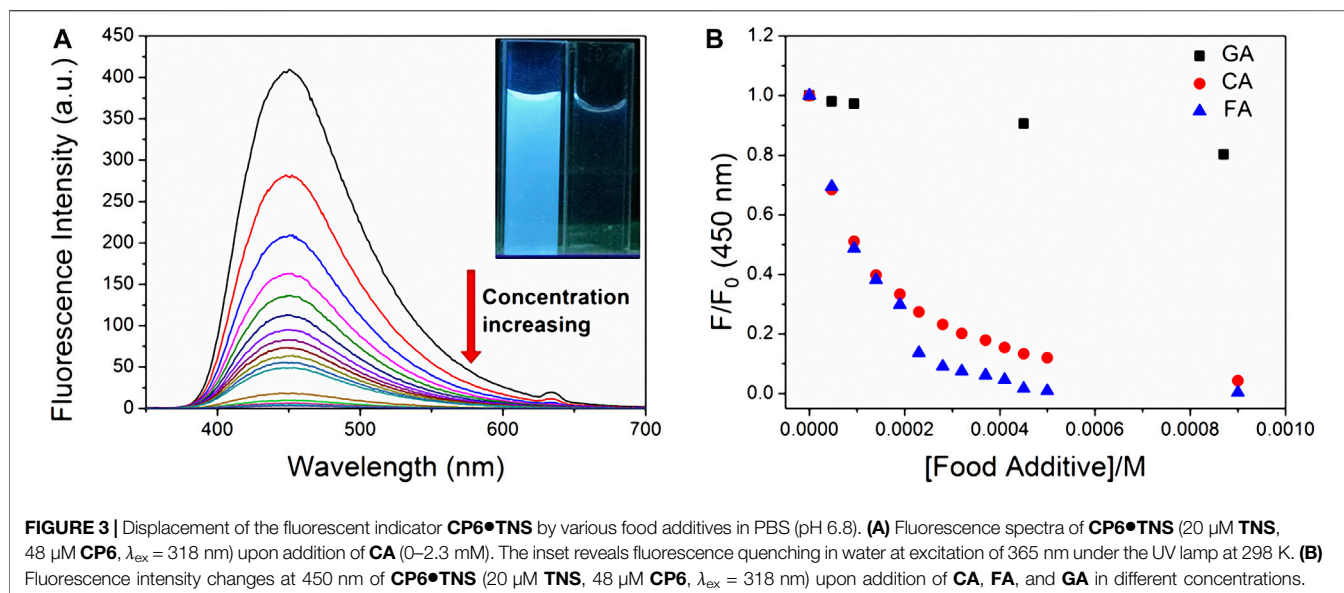
$^1\text{H}$  NMR tests were used to investigate the host-guest complexation. Because the solubility of the complex in neat  $\text{D}_2\text{O}$  was insufficient to reach the mM level,  $\text{DMSO-}d_6$  cosolvents were added. As illustrated in Figure 2, the naphthyl proton signals of **TNS** in the inclusion complex underwent varying degrees of upward shifts. The largest shift occurred in the direction away from the sulfonate group, whereas the smallest shift occurred in the direction toward the sulfonate group. Proton signal variations in the methylphenyl group are insignificant, indicating that this group may be located outside the cavity. When combined with the protons' shift and broadening in the sulfonate-naphthyl group, it is concluded that the fluorescence probe molecule is partially in the **CP6** cavity, where the shielding effects of the aromatic host produce the characteristic signal broadening (Li et al., 2010). Additionally, the 2D ROESY data



(Supplementary Figure S1) establish a correlation between the naphthyl protons ( $\text{H}_{a-f}$ ) in the entrapped TNS and the aromatic proton  $\text{H}_1$  in CP6, revealing the interpenetrated geometry.

To quantify the binding of TNS to CP6, the association constant ( $K_a$ ) was determined to be  $(4.51 \pm 0.90) \times 10^5 \text{ M}^{-1}$  using titration tests and a non-linear curve-fitting to the fluorescence spectra (Supplementary Figure S4). The complex formed by CP6 and TNS had a 1:1 binding

stoichiometry (Supplementary Figure S3A). We deduced that the complex formed between CP6 and TNS in aqueous solution as a result of multiple electrostatic interactions between the cationic ammonium groups on CP6 and the sulfonate anion on TNS, hydrophobic interactions, and  $\pi$ - $\pi$  stacking interactions between the benzene rings on host CP6 and naphthalene ring on guest TNS. The cooperativity of these non-covalent interactions is attributed to the binding affinity in the host-guest system.



## Detection of Food-Additives Using Fluorescent Indicator

**TNS** complexed with **CP6** exhibits a significant fluorescence response, allowing it to be used for FID detection. The binding affinities of **CP6** to **CA**, **FA**, and **GA** were determined in this study using FID detection (Scheme 1), and the fluorescent indicator **TNS** was first reversibly bound to the receptor **CP6**. The solution was then added with a weakly fluorescent or non-fluorescent analyte, which competitively displaced the highly fluorescent **TNS** from the indicator **CP6** cavity, altering the optical signal (You et al., 2015). Notably, titration of a preformed **TNS•CP6** complex with increasing concentrations of competitor food additive molecules results in a reversal of the fluorescence intensity, which is used to determine the competitor molecules' binding affinity. Competitive displacement was used to investigate the binding of three major phenolic food-additives, namely **CA**, **FA**, and **GA**, to **CP6**. Figure 3A illustrates a typical fluorescence displacement titration with **CA** as a strong competitor. The quenching of fluorescence in the presence of **CA** was easily observed with the naked eye using a simple UV-lamp (the inset of Figure 3A).

To avoid the effect of changes in pH and dilution on the displacement assay, the pH of complex and food-additive solutions was set to 6.8, and the concentrations of **TNS** and **CP6** in food-additive solutions were kept constant. We used that previously reported competitive binding formula to fit the reduced fluorescence intensities at the band maximum against the concentration of competitor food additives (Bakirci et al., 2006). Using **TNS•CP6** as the reporter pair, we determined the association constants ( $K_a$ ) for **CA**, **FA**, and **GA** to be  $(1.24 \pm 0.29) \times 10^4 \text{ M}^{-1}$ ,  $(1.19 \pm 0.16) \times 10^4 \text{ M}^{-1}$ , and  $(2.78 \pm 0.18) \times 10^2 \text{ M}^{-1}$  (Supplementary Figures S5–S7), respectively. Except for **GA**, the binding affinities are approximately two orders of magnitude greater than those of previously studied HP- $\beta$ -CD to the other two food additives, which are around  $10^2 \text{ M}^{-1}$  (Pal et al., 2016).

Additionally, the observed fluorescence response can also be used to quantify **CA**, **FA**, and **GA**. The fluorescence intensity

plots increase linearly as the **CA**, **FA**, and **GA** concentrations increase (Supplementary Figure S8), respectively. 0.047–2.3 mM, 0.047–0.14 mM, and 0.047–2.5 mM were the linear ranges. The results indicate that the limit of detection (LOD) values was 0.012, 0.08, and 0.17  $\mu\text{M}$ , respectively, using a  $3\sigma/\text{slope}$  method (MacDougall et al., 1980).

NMR research with **GA**, **CA**, and **FA** was used to determine the complexation of food additives. Supplementary Figure S9 illustrates the  $^1\text{H}$  NMR spectra for **GA** in the presence of **CP6**. As illustrated in the figure, shielding caused a shift in the benzene proton signal of **GA**, conclusively confirming the inclusion complex between **CP6** and **GA**. Additionally, the 2D NOESY data (Supplementary Figure S2) show NOE cross-peaks between the benzene proton ( $\text{H}_a$ ) in entrapped **GA** and the protons  $\text{H}_{1-4}$  in **CP6**, indicating the inclusion of a benzene ring in the **CP6** cavity. The signals in the NMR spectra of **CA** and **FA** changed similarly upon the addition of **CP6** (Supplementary Figures S10,S11).

## CONCLUSIONS

To summarize, we demonstrated a new fluorescence activation switch based on host-guest complexation between the fluorescent indicator probe **TNS** and cationic pillar[6]arene **CP6**. In **TNS** solution, the complexation significantly enhanced the fluorescence. A fluorescence switch-off displacement assay was used to detect three commonly used non-fluorescence phenolic food additives in the water. The study used molecular recognition and fluorescence indicator displacement assays to develop a prospective strategy for phenolic food additive detection.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary



**Material**, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

QD designed the work. YX and KG made contributions to the experiments and collective data. The paper was written by QD. All authors extensively discussed the results, reviewed the manuscript, and approved the final version of the manuscript to be submitted.

## REFERENCES

- Bakirci, H., and Nau, W. M. (2006). Fluorescence Regeneration as a Signaling Principle for Choline and Carnitine Binding: A Refined Supramolecular Sensor System Based on a Fluorescent Azoalkane. *Adv. Funct. Mat.* 16, 237–242. doi:10.1002/adfm.200500219
- Barrow, S. J., Kasera, S., Rowland, M. J., Del Barrio, J., and Scherman, O. A. (2015). Cucurbituril-Based Molecular Recognition. *Chem. Rev.* 115, 12320–12406. doi:10.1021/acs.chemrev.5b00341
- Bojtár, M., Kozma, J., Szakács, Z., Hessz, D., Kubinyi, M., and Bitter, I. (2017). Pillararene-Based Fluorescent Indicator Displacement Assay for the Selective Recognition of ATP. *Sensors Actuators B Chem.* 248, 305–310. doi:10.1016/j.snb.2017.03.163
- Bojtár, M., Paudics, A., Hessz, D., Kubinyi, M., and Bitter, I. (2016). Amino Acid Recognition by Fine Tuning the Association Constants: Tailored Naphthalimides in Pillar[5]arene-Based Indicator Displacement Assays. *RSC Adv.* 6, 86269–86275. doi:10.1039/C6RA15003A
- Bojtár, M., Szakács, Z., Hessz, D., Kubinyi, M., and Bitter, I. (2015). Optical Spectroscopic Studies on the Complexation of Stilbazolium Dyes with a Water Soluble Pillar[5]arene. *RSC Adv.* 5, 26504–26508. doi:10.1039/C4RA14809F
- Cai, Y., Zhang, Z., Ding, Y., Hu, L., Wang, J., Chen, T., et al. (2021). Recent Development of Pillar[n]arene-Based Amphiphiles. *Chin. Chem. Lett.* 32, 1267–1279. doi:10.1016/j.ccl.2020.10.036
- Cao, D., and Meier, H. (2019). Pillararene-Based Fluorescent Sensors for the Tracking of Organic Compounds. *Chin. Chem. Lett.* 30, 1758–1766. doi:10.1016/j.ccl.2019.06.026
- Crini, G. (2014). Review: A History of Cyclodextrins. *Chem. Rev.* 114, 10940–10975. doi:10.1021/cr500081p
- Dotsikas, Y., Kontopanou, E., Allagiannis, C., and Loukas, Y. L. (2000). Interaction of 6-P-Toluidinylnaphthalene-2-Sulphonate with  $\beta$ -cyclodextrin. *J. Pharm. Biomed. Analysis* 23, 997–1003. doi:10.1016/s0731-7085(00)00392-7
- Dsouza, R. N., Pischel, U., and Nau, W. M. (2011). Fluorescent Dyes and Their Supramolecular Host/Guest Complexes with Macrocycles in Aqueous Solution. *Chem. Rev.* 111, 7941–7980. doi:10.1021/cr200213s
- Duan, Q., Zhang, H., Mai, W., Wang, F., and Lu, K. (2019). Acid/Base- and Base/Acid-Switchable Complexation between Anionic-/Cationic-Pillar[6]arenes and A Viologen Ditosylate Salt. *Org. Biomol. Chem.* 17, 4430–4434. doi:10.1039/c9ob00398c
- Florea, M., and Nau, W. M. (2011). Strong Binding of Hydrocarbons to Cucurbituril Probed by Fluorescent Dye Displacement: A Supramolecular Gas-Sensing Ensemble. *Angew. Chem. Int. Ed.* 50, 9338–9342. doi:10.1002/anie.201104119
- Ghale, G., and Nau, W. M. (2014). Dynamically Analyte-Responsive Macrocyclic Host-Fluorophore Systems. *Acc. Chem. Res.* 47, 2150–2159. doi:10.1021/ar500116d
- Guo, D.-S., and Liu, Y. (2014). Supramolecular Chemistry of P-Sulfonatocalix[n]arenes and its Biological Applications. *Acc. Chem. Res.* 47, 1925–1934. doi:10.1021/ar500009g
- Hennig, A., Bakirci, H., and Nau, W. M. (2007). Label-Free Continuous Enzyme Assays with Macrocyclic-Fluorescent Dye Complexes. *Nat. Methods* 4, 629–632. doi:10.1038/nmeth1064

## FUNDING

This work was supported by Henan Province Science and Technology Research Program (No. 222102320067).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2022.925881/full#supplementary-material>

- Hua, B., Shao, L., Yu, G., and Huang, F. (2016). Fluorescence Indicator Displacement Detection Based on Pillar[5]arene-Assisted Dye Deprotonation. *Chem. Commun.* 52, 10016–10019. doi:10.1039/c6cc04919b
- Hua, B., Shao, L., Zhang, Z., Sun, J., and Yang, J. (2018). Pillar[6]arene/Acridine Orange Host-Guest Complexes as Colorimetric and Fluorescence Sensors for Choline Compounds and Further Application in Monitoring Enzymatic Reactions. *Sensors Actuators B Chem.* 255, 1430–1435. doi:10.1016/j.snb.2017.08.141
- Jiang, C., Song, Z., Yu, L., Ye, S., and He, H. (2020). Fluorescent Probes Based on Macrocyclic Hosts: Construction, Mechanism and Analytical. *TrAC Trends Anal. Chem.* 133, 116086. doi:10.1016/j.trac.2020.116086
- Koh, K. N., Araki, K., Ikeda, A., Otsuka, H., and Shinkai, S. (1996). Reinvestigation of Calixarene-Based Artificial-Signaling Acetylcholine Receptors Useful in Neutral Aqueous (Water/Methanol) Solution. *J. Am. Chem. Soc.* 118, 755–758. doi:10.1021/ja951488k
- Li, C., Zhao, L., Li, J., Ding, X., Chen, S., Zhang, Q., et al. (2010). Self-assembly of [2] Pseudorotaxanes Based on Pillar[5]arene and Bis(imidazolium) Cations. *Chem. Commun.* 46, 9016–9018. doi:10.1039/C0CC03575K
- MacDougall, D., Crummett, W. B., Amore, F. J., Cox, G. V., Crosby, D. G., and Estes, F. L. (1980). Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry. *Anal. Chem.* 52, 2242–2249. doi:10.1021/ac50064a004
- Munin, A., and Edwards-Lévy, F. (2011). Encapsulation of Natural Polyphenolic Compounds; a Review. *Pharmaceutics* 3 (4), 793–829. doi:10.3390/pharmaceutics3040793
- Nishijo, J., Nagai, M., Yasuda, M., Ohno, E., and Ushiroda, Y. (1995). Interaction of 6-P-Toluidinylnaphthalene-2-Sulfonate with  $\beta$ -Cyclodextrin. *J. Pharm. Sci.* 84, 1420–1426. doi:10.1002/jps.2600841207
- Nishijo, J., Yasuda, M., Nagai, M., and Ohno, E. (1992). Interaction of 6-P-Toluidinylnaphthalene-2-Sulfonate with .ALPHA.-Cyclodextrin. *Chem. Pharm. Bull.* 40 (10), 2591–2596. doi:10.1248/cpb.39.510.1248/cpb.40.2591
- Pal, K., Chandra, F., Mallick, S., and Koner, A. L. (2016). pH Dependent Supramolecular Recognition of Dapoxyl Sodium Sulfonate with 2-hydroxypropyl  $\beta$ -cyclodextrin: an Application towards Food-Additive Formulation. *New J. Chem.* 40, 6093–6100. doi:10.1039/c5nj03415a
- Pal, K., Mallick, S., and Koner, A. L. (2015). Complexation Induced Fluorescence and Acid-Base Properties of Dapoxyl Dye with  $\gamma$ -cyclodextrin: a Drug-Binding Application Using Displacement Assays. *Phys. Chem. Chem. Phys.* 17, 16015–16022. doi:10.1039/C5CP01696G
- Praetorius, A., Bailey, D. M., Schwarzlose, T., and Nau, W. M. (2008). Design of a Fluorescent Dye for Indicator Displacement from Cucurbiturils: A Macrocyclic-Responsive Fluorescent Switch Operating through a pKa Shift. *Org. Lett.* 10, 4089–4092. doi:10.1021/ol8016275
- Rather, I. A., and Ali, R. (2021). Indicator Displacement Assays: From Concept to Recent Developments. *Org. Biomol. Chem.* 19, 5926–5981. doi:10.1039/d1ob00518a
- Savjani, K. T., Gajjar, A. K., and Savjani, J. K. (2012). Drug Solubility: Importance and Enhancement Techniques. *ISRN Pharm.* 2012, 1–10. doi:10.5402/2012/195727
- Sedgwick, A. C., Brewster, J. T., II, Wu, T., Feng, X., Bull, S. D., Qian, X., et al. (2021). Indicator Displacement Assays (IDAs): the Past, Present and Future. *Chem. Soc. Rev.* 50, 9–38. doi:10.1039/c9cs00538b

- Sonzini, S., McCune, J. A., Ravn, P., Scherman, O. A., and van der Walle, C. F. (2017). A Simple Supramolecular Assay for Drug Detection in Urine. *Chem. Commun.* 53, 8842–8845. doi:10.1039/C7CC04081D
- Tobacman, J. K., Wallace, R. B., and Zimmerman, M. B. (2001). Consumption of Carrageenan and Other Water-Soluble Polymers Used as Food Additives and Incidence of Mammary Carcinoma. *Med. Hypotheses* 56, 589–598. doi:10.1054/mehy.2000.1208
- Vinson, J. A., and Cai, Y. (2012). Nuts, Especially Walnuts, Have Both Antioxidant Quantity and Efficacy and Exhibit Significant Potential Health Benefits. *Food Funct.* 3, 134–140. doi:10.1039/C2FO10152A
- Wang, P., Yao, Y., and Xue, M. (2014). A Novel Fluorescent Probe for Detecting Paraquat and Cyanide in Water Based on Pillar[5]arene/10-Methylacridinium Iodide Molecular Recognition. *Chem. Commun.* 50, 5064–5067. doi:10.1039/C4CC01403K
- Wu, L., Han, C., Jing, X., and Yao, Y. (2021). Rim-Differentiated Pillar[5]arenes. *Chin. Chem. Lett.* 32, 3322–3330. doi:10.1016/j.ccl.2021.04.046
- You, L., Zha, D., and Anslyn, E. V. (2015). Recent Advances in Supramolecular Analytical Chemistry Using Optical Sensing. *Chem. Rev.* 115, 7840–7892. doi:10.1021/cr5005524
- Zhang, X., Chen, F., and Wang, M. (2014). Antioxidant and Antiglycation Activity of Selected Dietary Polyphenols in a Cookie Model. *J. Agric. Food Chem.* 62, 1643–1648. doi:10.1021/jf4045827
- Zheng, Z., Geng, W.-C., Gao, J., Wang, Y.-Y., Sun, H., and Guo, D.-S. (2018). Ultrasensitive and Specific Fluorescence Detection of a Cancer Biomarker via Nanomolar Binding to a Guanidinium-Modified Calixarene. *Chem. Sci.* 9, 2087–2091. doi:10.1039/C7SC04989G

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Duan, Xing and Guo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.