

# Pillararene-based fluorescent indicator displacement assay for the selective recognition of ATP

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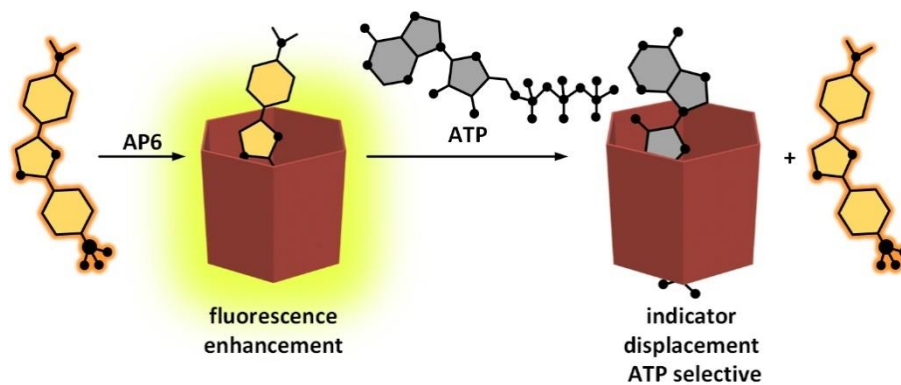
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## Graphical Abstract



## Abstract

The complexation of the cationic, water soluble ammonium pillar[6]arene was investigated with Dapoxyl sodium sulfonate. Large fluorescence enhancement was observed upon partial inclusion and the resulting complex was used as the first pillararene-based indicator displacement system for the selective recognition of adenosine-5'-triphosphate.

## Keywords

pillararene, indicator displacement, nucleotide sensing, ATP chemosensor

## Highlights

- The supramolecular analytical potential of water soluble pillararenes was further examined
- The complexation of Dapoxyl sodium sulfonate was investigated with ammonium pillar[6]arene
- The selective recognition of adenosine-5'-triphosphate by this macrocycle was exploited in the first indicator displacement system for nucleotides based on a pillararene platform
- An order of magnitude of selectivity over ADP and GTP was achieved in this model system

## 1. Introduction

Fluorescent indicator displacement (FID) assays exploit the principle of competitive binding: a fluorescent indicator is bound to the receptor, which is then displaced upon addition of a competing analyte [1–4]. Macrocycles are often ideal receptors for this purpose due to their ability to form inclusion complexes with various dyes that is followed by remarkable changes in their optical properties. These changes are usually more significant than the effects observed in direct sensing that makes FID assays more desirable. The macrocyclic components in the majority of these systems are cyclodextrins [5], calixarenes [6–8] and cucurbiturils [9–12].

Although pillar[*n*]arenes are a relatively new class of macrocycles[13], numerous applications were discovered due to their easy synthesis, versatile functionalization and diverse host-guest chemistry [14–22]. In particular, water-soluble pillararenes are of significance as prosperous hosts for various guests of biological importance [23–28]. With their ease of synthetic manipulation, completely different host-guest systems can be realized through the pillararene scaffold [29,30]. Amongst the most studied, carboxylato-pillar[5]arene [23] forms complexes with paraquat, various amines and basic amino acids [31] that can be exploited to compile FID assays for these analytes [32–36]. Cationic, ammonium-containing pillar[5]arene was used as receptor in a FID sensor for various phenols [37].

The target of our present work has been to construct a FID assay with a water soluble pillararene for the detection of ATP. Since the nucleotides, especially ATP is pivotal in living systems [38], numerous fluorescent chemosensors have been developed for their detection [39–43], involving direct sensing [44,45], cation-based recognition units [46–52] and also some indicator displacement assays [53–57].

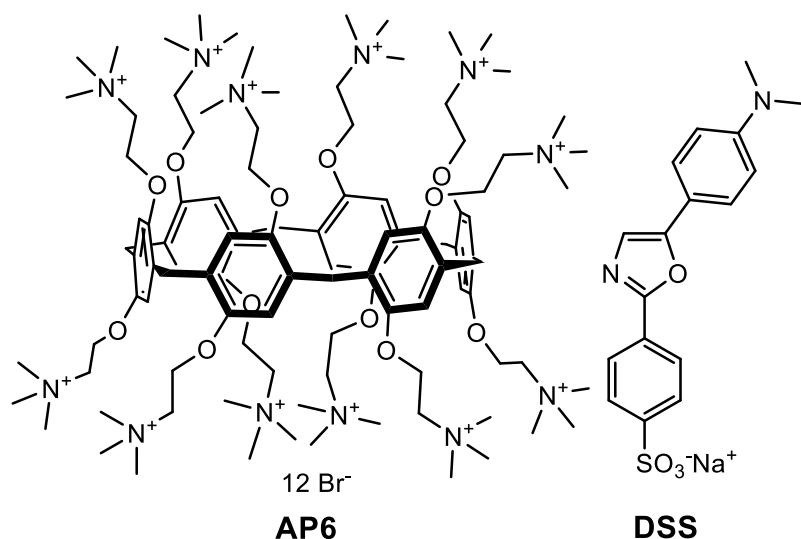
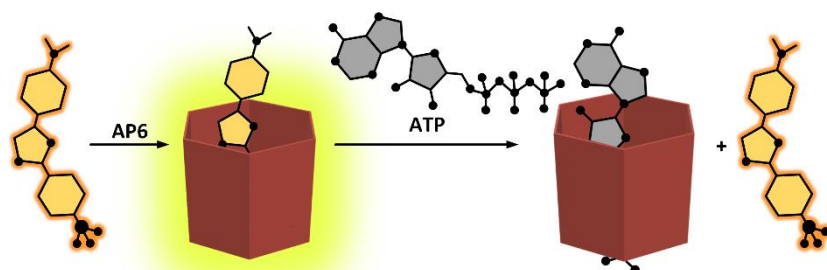


Fig. 1. Structures of **AP6** and **DSS**

We chose ammonium-pillar[6]arene (**AP6** in Fig. 1) as receptor, inspired by recent results on the complexation of nucleotides with **AP6** [58]. NMR spectroscopic and isothermal calorimetry experiments proved that **AP6** forms a surprisingly strong complex with adenosine-5'-triphosphate (ATP), whereas the complexation with adenosine-5'-diphosphate (ADP) and adenosine-5'-monophosphate (AMP) was found much weaker. The popular solvatochromic dye, Dapoxyl sodium sulfonate [59] (**DSS** in Fig. 1) was chosen, as fluorescent indicator for our FID system. For its strongly solvato- and fluorochromic character, a large signal modulation was expected upon binding to the **AP6** receptor or releasing from the complex. In addition, due to the cationic nature of the **AP6** host, only anionic dye guests could be considered.

In this paper, we report the complexation of **AP6** with **DSS**, and demonstrate the operation of the complex formed as a FID assay for the detection of ATP (see Scheme 1). As will be shown, the assay proved to be selective over ADP, AMP and even guanosine-5'-triphosphate (GTP).



Scheme 1 Pillar[6]arene-based indicator displacement system for ATP detection

## 2. Experimental

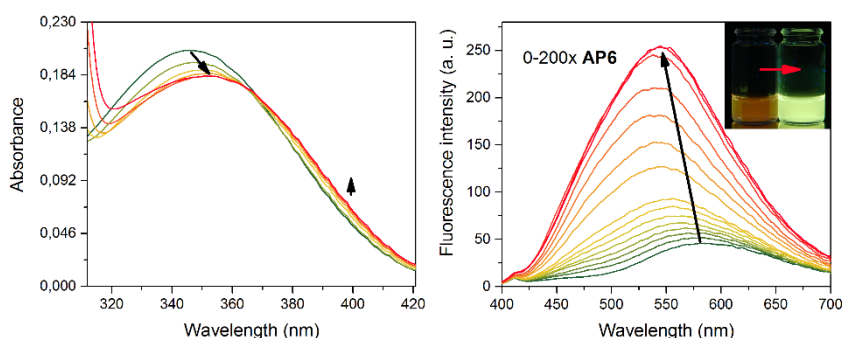
Solvents, reagents and starting materials were obtained from commercial supplier and used without further purification. **AP6** was prepared as described [58] with some modifications, using the recently introduced deep eutectic solvent method [60]. The synthetic procedures are described in the Supporting Information. **DSS** was synthesized as described [59].

All the spectroscopic experiments were carried out at 25°C. The UV–vis absorption spectra were recorded on an Agilent 8453 diode array spectrometer. The fluorescence spectra were measured on an Edinburgh Instruments FLSP 920 fluorescence spectrometer. The  $^1\text{H}$  NMR spectra were taken on a Bruker Avance DRX-500 spectrometer with chemical shifts reported in ppm (TMS in the case of  $\text{CDCl}_3$  and the residual HDO in the case of  $\text{D}_2\text{O}$  was used as internal standard). The exact mass measurements were performed using a Q-TOF Premier massspectrometer (Waters Corporation, 34 Maple St, Milford, MA, USA) using Electrospray ionization in positive mode. The methods for association constant determination and the details of the computational results are described in the Supporting Information.

## 3. Results and Discussion

### 3.1 Complexation of **DSS** with **AP6**

The fluorescence spectra of **DSS** changed considerably upon the addition of **AP6** in HEPES-buffered water. Strong fluorescence enhancement was observed as well as a hypsochromic shift in the emission spectra (Fig. 2.).



*Fig. 2. Variation of the absorption (left) and fluorescence (right) spectra of **DSS** upon addition of **AP6** in HEPES buffer (0.02 M, pH 7.4). Absorption spectra: 10  $\mu\text{M}$  **DSS**, 0-50 equiv. of **AP6**; fluorescence spectra: 1  $\mu\text{M}$  **DSS**, 0-200 equiv. of **AP6**, excitation wavelength: 364 nm. The photographs in the inset*

show a **DSS** solution (5  $\mu$ M, left vial) and a **DSS + AP6** mixture (25 equiv. of **AP6**, right vial) under UV lamp (365 nm).

The association constants and the optical spectroscopic data for **DSS** and its **AP6** complex are collected in Table 1, and compared to the respective data for the complexes of **DSS** with  $\beta$  cyclodextrin,  $\beta$ -**CD**·**DSS** [61],  $\gamma$ -cyclodextrin,  $\gamma$ -**CD**·**DSS**<sub>2</sub> [62] and cucurbit[7]uril, **CB7**·**DSS** [6]. An association constant of  $K_a = 7.62 \cdot 10^4 \text{ M}^{-1}$  was obtained by a least square fitting to the fluorescence spectra of **DSS**·**AP6** mixtures, presuming 1:1 complexation. The 1:1 stoichiometry was confirmed by the excellent fit (Fig. S6 in the Supplementary Information) of the regression curve to the experimental data [63].

	$K_a$	$\lambda_{\text{abs}}$ (nm)	$\lambda_{\text{em}}$ (nm)	$\Phi$
<b>DSS</b>	-	348	582	0.04 <sup>a</sup>
<b>AP6</b> · <b>DSS</b>	$7.62 \cdot 10^4 \text{ M}^{-1}$	353	545	0.21
$\beta$ - <b>CD</b> · <b>DSS</b> <sup>b</sup>	$5.49 \cdot 10^3 \text{ M}^{-1}$	353	560	0.31
$\gamma$ - <b>CD</b> · <b>DSS</b> <sub>2</sub> <sup>c</sup>	$4.63 \cdot 10^3 \text{ M}^{-2}$	369	488	0.68
<b>CB7</b> · <b>DSS</b> <sup>d</sup>	$2.0 \cdot 10^4 \text{ M}^{-1}$	336	380	0.50

Table 1. Association constants and optical spectroscopic data for the complexes of **DSS** with **AP6** and other macrocycles. <sup>a</sup>From ref. [61] <sup>b</sup>From ref. [61], 1:1 complex <sup>c</sup>From ref. [62], 1:2 (macrocycle-dye) complex <sup>d</sup>From refs. [6,64], 1:1 complex

The large signal modulation and the optimal association constant indicated that this system is suitable for FID purposes. The spectroscopic data of the complex **AP6**·**DSS** strongly resemble to those of the complex of the dye with  $\beta$ -cyclodextrin,  $\beta$ -**CD**·**DSS** [61], indicating the relatively similar polarity inside the two macrocycles. However, due to the weaker interactions, the association constant with the uncharged cyclodextrin is lower by an order of magnitude.

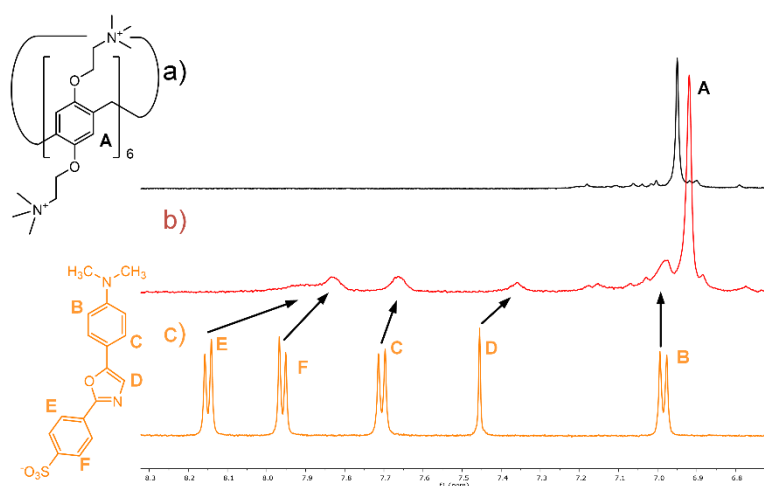


Fig. 3. Partial  $^1\text{H-NMR}$  spectra of **a)** AP6 (1.0 mM) **b)** AP6 + DSS (0.25 mM) **c)** DSS (1 mM) in  $\text{D}_2\text{O-CD}_3\text{OD}$  3:1

The complexation was further evaluated using NMR spectroscopy. As the solubility of the complex in neat  $\text{D}_2\text{O}$  did not reach the mM scale, methanol- $d_4$  or DMSO- $d_6$  cosolvents were added (see Section 3 in the ESI). As can be seen in Fig. 3., the aromatic protons of DSS in the complex shifted upfield in different extents. The largest deviation was observed near the sulfonato-group, while smaller shift was recorded on the oxazole proton. The changes in the signals of the dimethylaminophenyl group are insignificant (Fig. S8), indicating that this group is probably located outside the cavity. Adding this to the shifts and broadening of the sulfonato-aryl group, we can conclude that the dye molecule is only partially included in the cavity of the macrocycle, where the shielding effects of the aromatic host produce the characteristic signal broadening.

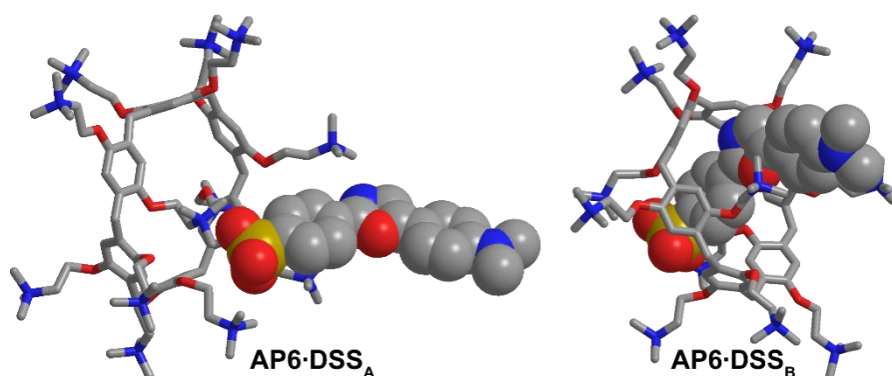
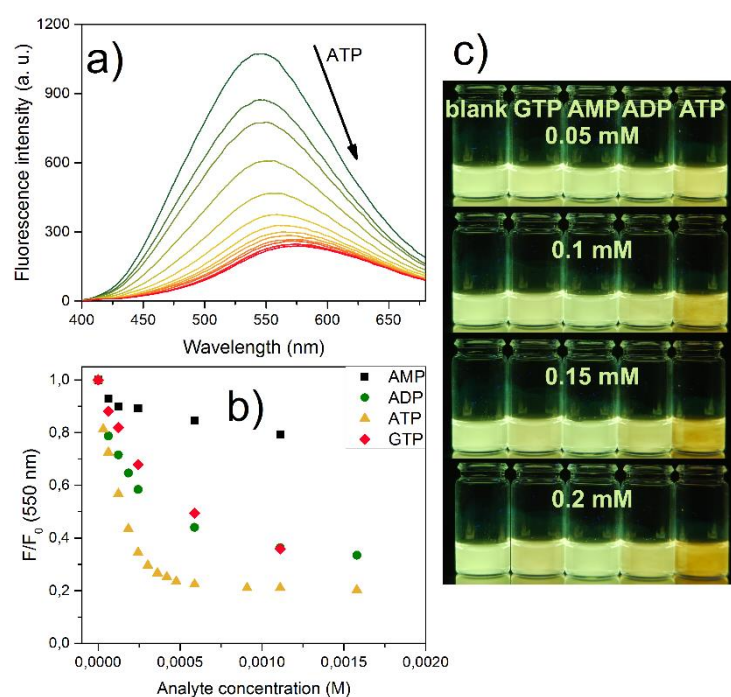


Fig. 4 Energy-minimized structures of the AP6-DSS complex. The guest DSS is displayed as space filling model, the host AP6 as sticks. AP6-DSS<sub>A</sub> is the minimum energy, AP6-DSS<sub>B</sub> is the second lowest energy structure

Further insight into the nature of the **AP6·DSS** complex was gained by theoretical calculations (see ESI Section 5 for details). Moving the **DSS** guest through the cavity of the AP6 host, two local energy minima were identified. The lowest energy conformer is the **AP6·DSS<sub>A</sub>** complex, in which the **DSS** molecule is attached only to the rim of the macrocycle (Fig. 4.). In contrast, in the second lowest energy conformer, **AP6·DSS<sub>B</sub>**, the **DSS** guest penetrates the cavity of the **AP6** host. The interaction energy of the **AP6·DSS<sub>A</sub>** form of the complex is -486 kcal/mol, a high value, in accordance with the strong Coulombic forces acting between the charged components. According to the NMR studies, it is believed that **AP6·DSS<sub>A</sub>** structure should be closer to the real complex structure. To visualize the charge distribution on the **DSS** guest, the electrostatic potential (ESP) on the Van der Waals surface of the dye was also calculated. As expected, the lowest ESP was located on the sulfonato group (Fig. S10 in the Supporting Information).

### 3.2. Indicator Displacement



**Fig. 5. Fluorescent indicator displacement. a)** Fluorescence spectra (excitation at 364 nm) of **AP6·DSS** (5  $\mu$ M **DSS**, 25 equiv. of **AP6**, 90% of **DSS** in complexed form) upon addition of ATP (0 to 1.5 mM) in 0.02 M HEPES buffer (pH 7.4). **b)** fluorescence intensity changes at 550 nm (excitation at 364 nm) of **AP6·DSS** upon addition of various nucleotides in different concentrations (5  $\mu$ M **DSS**, 25 equiv. of **AP6**) **c)** photographs of the **AP6·DSS** (5  $\mu$ M **DSS**, 25 equiv. of **AP6**) system with various nucleotides in different concentration under UV lamp (365 nm)

Upon the addition of ATP to the **AP6-DSS** system (5  $\mu\text{M}$  **DSS**, 25 equiv. of **AP6**, 90% of **DSS** in complexed form, as obtained from  $K_a = 7.62 \cdot 10^4 \text{ M}^{-1}$ ), the indicator was displaced which resulted in the expected turn-off response in the emission spectra (Fig. 5a). A practically complete displacement of **DSS** was reached at a few equivalents of ATP (related to the concentration of the macrocycle) that suggests a strong binding of this nucleotide to **AP6**.

The selectivity of the **AP6-DSS** system as ATP sensor was evaluated over ADP, AMP and GTP. As can be seen in Fig. 5b, ADP and AMP displaced the indicator in much lower ratios, i. e. the latter nucleotides were bound substantially weaker to **AP6** than ATP. This selectivity is the consequence of the different charges and lengths of the phosphate chains in these guests: in the pseudorotaxane-type complexes, in which the adenine units are encapsulated by the pillararene macrocycles, only the triphosphate unit of ATP is long enough to interact strongly with the oppositely charged ammonium groups of **AP6**. Surprisingly, selectivity was observed even over guanosine-5'-triphosphate (GTP), which can be rationalized by the larger guanine nucleobase that is sterically less favorable in the complexation than the adenine of ATP. The selective response towards ATP was visible even to the naked eye, the drop of the fluorescence intensity and the minor change in the emission color are shown on the photographs in Fig. 5c. To characterize the ATP selectivity of the FID system quantitatively, the association constants for the **AP6-nucleotide** complexes were calculated by least square fittings to the fluorescence spectra of the **AP6 – DSS – nucleotide** systems (Supporting Information Section 2). As can be seen in Table 2, the  $K_a$  value for the binding of ATP is about an order of magnitude higher than for the binding of the ADP and GTP.

nucleotide	$K_{a, \text{analyte}} (\text{M}^{-1})$
ATP	$4.45 \cdot 10^5$
GTP	$4.37 \cdot 10^4$
ADP	$5.45 \cdot 10^4$
AMP	$1.79 \cdot 10^2$

*Table 2 Association constants of the nucleotides and **AP6** in HEPES (0.02 M, pH 7.4) calculated from the indicator displacement experiments*



## 4. Conclusions

In conclusion, we have investigated the complexation of the cationic, water soluble ammonium-pillar[6]arene **AP6** with Dapoxyl sodium sulfonate. Large fluorescence enhancement was observed upon partial inclusion, which was confirmed by NMR measurements and calculations. This system was successfully used as a fluorescent indicator displacement assay for the detection of ATP. Large signal modulation and a selective response towards ATP amongst other nucleotides were observed. To our knowledge, this has been the first **AP6**-based FID system and the first cationic pillararene-based FID assay for analytes with biological importance.

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