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Induced-Fit Recognition by a Calixarene–Pyrene Conjugate for Single- and Dual-Wavelength Fluorescence Sensing in Water

Joana N. Martins[1] | Márcia Pessêgo[2] | Luis Garcia-Rio[2] | João Carlos Lima[1] | Nuno Basílio[1]

[1]Laboratório Associado para a Química Verde (LAQV), Rede De Química e Tecnologia (REQUIMTE), Departamento De Química, Faculdade De Ciências e Tecnologia, Universidade NOVA De Lisboa, Caparica, Portugal | [2]Departamento De Química Física, Facultad De Química, Universidad De Santiago, Santiago, Spain

Correspondence: Nuno Basílio (nuno.basilio@fct.unl.pt)

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ABSTRACT

The discovery of fluorescence receptors that selectively bind small molecules with high affinity in aqueous media is critical for the development of new sensing and bioimaging assays. We herein report a new fluorescence receptor-dye chemosensor based on the monofunctionalization of *p*-sulfonatocalix[4]arene (SC4) receptor with pyrene (**SC4-4Py**). The **SC4-4Py** conjugate was found to retain the exceptional binding properties of SC4, allowing the high-affinity recognition of neurotransmitters, amino acids, and biogenic amines in aqueous solution and their optical detection. The **SC4-4Py** chemosensor operates through a photoinduced electron transfer (PET) mechanism triggered by conformational changes in the calixarene structure upon binding, allowing single-wavelength fluorescence detection of target analytes and its expansion into a dual-wavelength ratiometric indicator displacement assay (IDA) that relies on competitive PET between the pyrene and non-covalently bound lucigenin dye.

1 | Introduction

The development of molecular sensors and probes capable of detecting small molecules and ions in aqueous environments is essential for a wider understanding of many biological and environmentally relevant processes. Sensing strategies based on supramolecular chemosensors explore molecular recognition and reversible self-assembly phenomena to create stimuli-responsive systems that can detect target analytes (e.g. disease biomarkers, pollutants, etc) and monitor their concentration fluctuations within specific environments [1–6]. Water-soluble macrocyclic receptors featuring hydrophobic cavities decorated with polar functional groups offer particularly useful recognition elements for the construction of supramolecular chemosensors [7–10]. Their successful recognition properties emerge from a combination of factors that include the establishment of complementary non-covalent interactions with the analytes of interest, the hydrophobic effect, and size- and shape selectivity associated to their highly preorganized and relatively rigid structures, which frequently enhances their affinity and selectivity with respect to their acyclic counterparts [11–13].

p-Sulfonatocalix[n]arenes (SCn), a widely explored class of water-soluble calixarene macrocycles, have been shown to be particularly useful synthetic receptors for the binding of ammonium ions in aqueous solution, presenting high affinity and selectivity toward relevant targets such as neurotransmitters, post-translational trimethylated lysine residues, arginine- and lysine-rich peptides, biogenic amines, among other molecules of interest [14–22]. The use of SCn as receptors in the construction of optical chemosensors has been dominated by the so-called indicator displacement assay (IDA) [23–30]. This approach explores

dynamic receptor-dye assemblies to detect target analytes via competitive binding, using the fluorescence or colorimetric signal given by the replacement of the dye from the receptor binding site [31, 32]. IDA based on self-assembled SCn-dye chemosensors have been demonstrated to be highly useful and versatile molecular tools in the development of sensing assays for the monitoring and measurement of enzymatic activities, membrane transport or the uptake of small molecules in living cells [33–36]. In contrast to SCn-dye self-assembled chemosensors, alternative sensing approaches involving the covalent modification of SCn with dye molecules remain practically unexplored. A rare example was reported by Hof and coworkers, who developed fluorescent *p*-sulfonatocalix[4]arene (SC4) derivatives integrating merocyanine dyes into their macrocyclic structure which self-assemble into nonemissive homodimers in aqueous solution [37]. These dimers can be competitively disassembled upon binding of analytes to form emissive host-guest complexes, enabling a turn-on fluorescence sensing mechanism to detect relevant targets such as methylated lysine or drugs in biological fluids. [37, 38] Despite the success of these strategies, nearly all SC4-based chemosensors rely on single-wavelength fluorescence signaling, which is highly sensitive to environmental conditions and probe concentration. This dependence can compromise quantitative accuracy and result in false positives or negatives in qualitative analysis. In contrast, ratiometric readout systems address these limitations by incorporating an internal reference signal, enabling correction for such variations. This approach enhances the reliability of quantitative measurements and reduces the need for extensive controls in qualitative applications [39, 40]. Host–guest ratiometric chemosensors typically employ dye-functionalized macrocycles to achieve fluorescence resonance energy transfer (FRET) between complementary encapsulated dyes [41–43]. However, in SC4-based architectures, this strategy can be severely hindered by significant dye quenching by this receptor and frequent aggregation of the resulting host–guest complexes [7, 30, 44, 45].

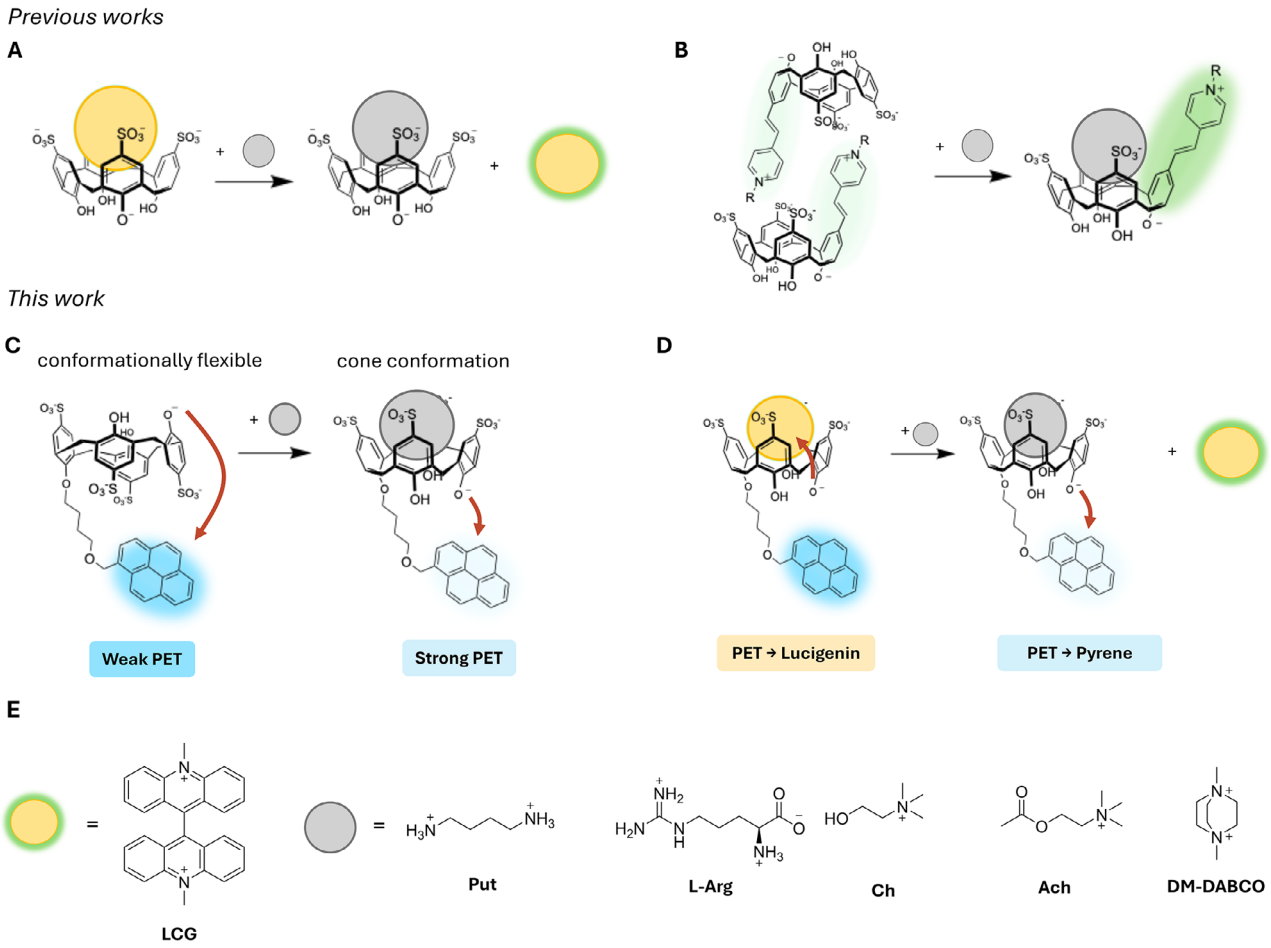
Herein we report a new monofunctionalized SC4-Pyrene conjugate (**SC4-4Py**) to introduce an optical sensing design that explores the conformational mobility of SC4 along with photoinduced electron transfer (PET) phenomenon (Scheme 1). Our approach leverages the ability of the conformationally mobile **SC4-4Py** to adopt a fixed cone conformation upon binding target guest molecules. This process brings the phenolate anion closer to the pyrene unit, thereby enhancing the PET quenching efficiency. Notably, this sensing mechanism can be extended to a dual-wavelength ratiometric indicator displacement assay (IDA) through competitive PET, using lucigenin (LCG) as a reporter dye for the detection of neurotransmitters, amino acids, and biogenic amines.

2 | Results and Discussion

SC4-4Py was synthesized by direct monoalkylation of SC4 with the respective pyrene derivative in basic media (see SI, Scheme S1), as previously reported for the monofunctionalization of SC4 with azobenzene units [46]. The novel macrocyclic receptor is soluble in aqueous solution and presents the characteristic absorption and emission spectra of the pyrene dye (see SI, Figure S5). A pH titration monitored by fluorescence emission (Figure 1A) shows a strong quenching on the pyrene emission when increasing the pH from pH 3 to pH 10. Fitting the fluorescence intensity versus pH data allows the determination of a $pK_a = 5.6$, which can be assigned to deprotonation of one phenol unit in the **SC4-4Py** macrocycle. This relatively strong acidity is consistent with the significant stabilization of the resulting phenolate provided by intramolecular hydrogen bonding with the vicinal phenols and in good agreement with what is observed for the parent SCn [47, 48]. Importantly, the observed pH-dependent quenching is indicative of a PET mechanism from the phenolate to the excited pyrene dye. This is supported by the exergonic Gibbs free energy for the PET process, ΔG = -0.5 eV, which can be roughly estimated from the Rehm-Weller equation [49] using the reduction potential of pyrene (−2.09 V versus SCE) [50], the oxidation potential of the SC4 unit (0.86 V vs Ag/AgCl, 0.83 V vs SCE) [28] and the $E_{0,0}$ = 3.45 eV for the singlet excited state of pyrene.

In order to evaluate the optical response of **SC4-4Py** toward relevant analytes, a family of known SC4 guests with biological interest was chosen (Scheme 1). Direct fluorescence titration of **SC4-4Py** with putrescine, a biogenic amine known to bind SC4 with μM affinity, caused a gradual quenching of the pyrene emission (see Figure 1B), approaching saturation at ca. 40 μM. The fluorescence intensity vs concentration data can be satisfactorily fitted to a 1:1 host-guest model with a binding constant of $K = 1.6 \times 10^5$ M^{-1}, which compares well with the value previously reported for the parent SC4 [29]. Control experiments conducted under acidic conditions (pH = 3.4) showed unsignificant quenching effects upon addition of putrescine, pointing out to the critical importance of the anionic phenolate unit for the sensing mechanism (see SI, Figure S6). Similar fluorescence titration experiments were conducted for choline, acetylcholine and arginine at neutral pH which also resulted in partial quenching of the pyrene fluorescence emission upon binding to **SC4-4Py** (see SI, Figure S7). Once again, data fitting retrieved the respective K values for this set of biologically relevant analytes (see Table 1), which align reasonably well with those previously reported for SC4 [19, 24]. In addition, fluorescence titration of **SC4-4Py** with the high-affinity SC4 binder DM-DABCO [51] led to a $K > 1 \times 10^6$ M^{-1} (see SI, Figure S7), further supporting the notion that the recognition properties of SC4 are conserved in **SC4-4Py**. To rule out any contribution from complexation-induced deprotonation of the **SC4-4Py** phenol unit to the observed fluorescence quenching, the titration of **SC4-4Py** with DM-DABCO was also performed at pH 10, reproducing the same changes observed at pH 7.4 (see SI, Figure S8).

Having demonstrated that **SC4-4Py** can be employed for the direct fluorescence detection of typical SC4 guests, we turned our attention to the mechanism behind the observed fluorescence quenching phenomena upon guest binding. Because PET efficiency is distance-dependent, we anticipated that this quenching could be assigned to structural alterations induced by host-guest association. The ^{1}H-NMR spectrum of **SC4-4Py** in D_2O (see SI, Figure S9) shows significant signal broadening for all aliphatic protons and for part of the aromatic protons assigned to the SC4 moiety, supporting the hypothesis that this calixarene is conformationally mobile, alternating between different conformations at intermediate exchange rate in the ^{1}H-NMR chemical shifts



SCHEME 1 | – Previous works on self-assembled chemosensors using sulfonatocalix[4]arene receptors: A—Conventional indicator displacement assay and B—Hof's dimeric assembly. The approach introduced in this work: C—The conformationally flexible SC4-4Py adopts different conformations resulting from the free rotation of the unsubstituted phenol units through the macrocyclic annulus. This leads to an averaged weaker PET due to the conformations that place the phenolate oxygen distant to the pyrene dye. Binding of target guest molecules blocks SC4-4Py in the cone conformation, bringing the phenolate closer to the pyrene thus increasing the PET efficiency. D—Combining the lucigenin dye with SC4-4Py enabled a dual-wavelength ratiometric displacement assay. E—Structures of the lucigenin dye and guest molecules explored in this work.

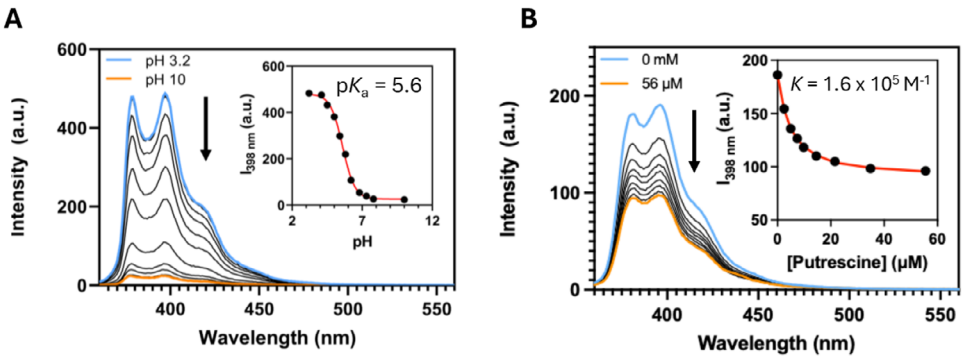


FIGURE 1 | – A. Fluorescence spectra **of SC4-4Py** at different pH in 5 mM phosphate buffer. B. Direct fluorescence titration of **SC4-4Py** (2 μM) with putrescine at pH = 7.4 in 5 mM phosphate buffer.

timescale. Though it was not possible to unequivocally assign the exchanging conformations due to signal broadening, the unusual high stabilization of one phenolate unit of **SC4-4Py** (pK_a = 5.6, $\Delta\Delta G \sim 20.5$ kJ.mol^{-1}) relative to the 4-hydroxybenzenosulfonate monomer (pK_a = 9.2) [48] strongly suggests that the *cone* and *partial cone* conformers should predominate, as these structures allow the formation of bifurcated hydrogen bonds with the phenolate oxygen donor. Furthermore, the ^{1}H-NMR spectrum of **SC4-4Py** at 5°C shows two upfield shifted signals in the aliphatic region at 1.4 and 0.1 ppm, indicating that alkyl chain linker is partially included in the pseudo-aromatic cavity of the *partial cone* conformer. Finally, it is also worth noting that previous lower

TABLE 1 | Affinity constants (K / M^{-1}) of the several analytes, obtained by ratiometric IDA (rIDA), by fitting of the ratio between lucigenin and **SC4-4Py** emission, and by direct titration of the **SC4-4Py** receptor with the analytes and following the resulting quenching of pyrene emission and by ITC, at 25°C. All experiments were carried out in 5 mM phosphate buffer, pH = 7.4. ITC isotherms can be found in the Supplementary Information. The fluorescence switching ratios (as I_f/I_0) are also presented.

Analyte	K / M^{-1} [a]	I_f/I_0	K / M^{-1} [b]	K / M^{-1} [c]	ΔG / kJmol^{-1}	ΔH / kJmol^{-1}	−TΔS / kJmol^{-1}
L-Arginine	4.2×10^3	0.64	5.8×10^3	2.6×10^3	−19.5	−17.1	−2.4
Choline	2.0×10^4	0.67	3.9×10^4	1.1×10^4	−23.1	−21.4	−1.7
Acetylcholine	3.3×10^4	0.66	6.8×10^4	1.2×10^4	−23.2	−24.3	1.1
Putrescine	1.6×10^5	0.51	2.7×10^5	1.9×10^5	−30.2	−17.8	−12.4
DM-DABCO	5.9×10^6	0.43	$> 10^6$	3.0×10^6	−37.0	−21.6	−15.4

[a]Obtained by ratiometric IDA.
[b]Obtained by direct fluorescence titration.
[c]Obtained by ITC.

rim substituted SC4 derivatives also favor the *cone* and *partial cone* conformation [52].

To investigate possible structural changes upon binding, the spherically shaped high-affinity binder, DM-DABCO, was selected for ^{1}H-NMR studies. As can be observed from Figure 2, upon addition of the guest, the signals assigned to the **SC4-4Py** become sharp and well resolved, indicating that the initial dynamic structure of the receptor is blocked into a single conformation. Furthermore, the appearance of two pairs of doublets assigned to the axial and equatorial protons of the methylene CH_2 bridges of the macrocycle and together with ^{13}C-NMR signals at 29.59 and 32.48 ppm for same groups (see SI, Figure S10), show that **SC4-4Py** adopts a *cone* conformation upon binding [53]. This conformation was also supported by a ROESY experiment, showing strong ROE cross signals between the equatorial protons of the CH_2 bridges and meta protons of the phenolic units (see SI, Figure S12). It is worth noting that, the downfield shifts observed for the signals of the O-CH_2-Py group and its vicinal aromatic proton upon complexation (blue arrows in Figure 2B), support pseudo-inclusion of the alkyl chain linker in the *partial cone* conformation, as discussed above. In addition, the ^{1}H-NMR spectrum of DM-DABCO in the presence of 1.3 equiv. of **SC4-4Py** clearly shows large upfield shifts (see SI, Figure S13), demonstrating the inclusion of the guest in the aromatic binding pocket of **SC4-4Py**.

Although less resolved, due to weaker binding, similar changes in the ^{1}H-NMR spectrum of **SC4-4Py** are observed in the presence of other guests, such as Ach (see Figure S14 in the SI). Overall, this evidence supports the proposed PET mechanism controlled by an induced fit model in which guest binding leads to a conformational alteration of **SC4-4Py**, bridging the phenolate unit closer to pyrene dye. This improves PET efficiency, resulting in the observed fluorescence quenching in the presence of guest molecules. It is important to emphasize that the PET mechanism requires short contact distances between the donor and acceptor for efficient quenching, a condition that is much more readily achieved in the cone conformation. This contrasts with, for example, the FRET mechanism, which operates via dipole–dipole interactions and can occur over much longer through-space distances (typically up to ~10 nm).

After establishing the mechanism of sensing for the **SC4-4Py**, the LCG dye was titrated with this host, yielding almost complete quenching of LCG emission (Figure 3A) under saturation concentrations. The changes in fluorescence intensity were fitted to a 1:1 host-guest binding model to obtain an affinity constant of $K = 1.7 \times 10^7$ M^{-1}, which is within the same order of magnitude of those previously reported by our group for other mono-O-substituted SC4 [46]. The high affinity of LCG towards **SC4-4Py**, the significant quenching observed upon complexation and the fact that both LCG and **SC4-4Py** can be simultaneous excited at 340 nm, offered ideal conditions for the construction of a ratiometric IDA (rIDA). Competitive assays were performed with >90% **SC4-4Py**-LCG complex, monitoring both pyrene (360-400 nm) and lucigenin (450-600 nm) emissions. Figure 3 B shows the spectroscopic changes observed for the rIDA using putrescine as the analyte (see the SI for analog experiments conducted for the remaining analytes, Figure S16). As observed, the emission of LCG is enhanced upon the addition of putrescine, which promotes its competitive release from the cavity, while the pyrene emission is quenched. This ratiometric signal variation is consistent with a PET-based quenching mechanism involving both LCG and pyrene dyes. When LCG occupies the **SC4-4Py** binding site, PET predominantly targets LCG due to its superior electron-accepting ability and closer proximity to the phenolate. However, upon its competitive release, PET is redirected toward the pyrene dye, leading to the observed quenching of pyrene emission and the simultaneous enhancement of LCG fluorescence.

Analysis of the LCG displacement experiments reveal that its emission seems to reach a plateau at lower intensity than expected, most likely due to the documented quenching of this dye by halide anions, present as the counterions of the analytes (see SI, Figure S15) [54]. Nevertheless, the mathematical approach employed to obtain the affinity constants from the ratiometric emission data ($I_{505\,nm}/I_{398\,nm}$), was based in a modified version of competitive model that also accounts for anion quenching of LCG emission. As can be observed from Table 1, the K values were in the same order of magnitude of those obtained by direct fluorescence titration of **SC4-4Py**, validating the rIDA introduced in this work.

Finally, the binding properties of **SC4-4Py** were independently validated by ITC experiments (see SI, Figure S17). The ITC

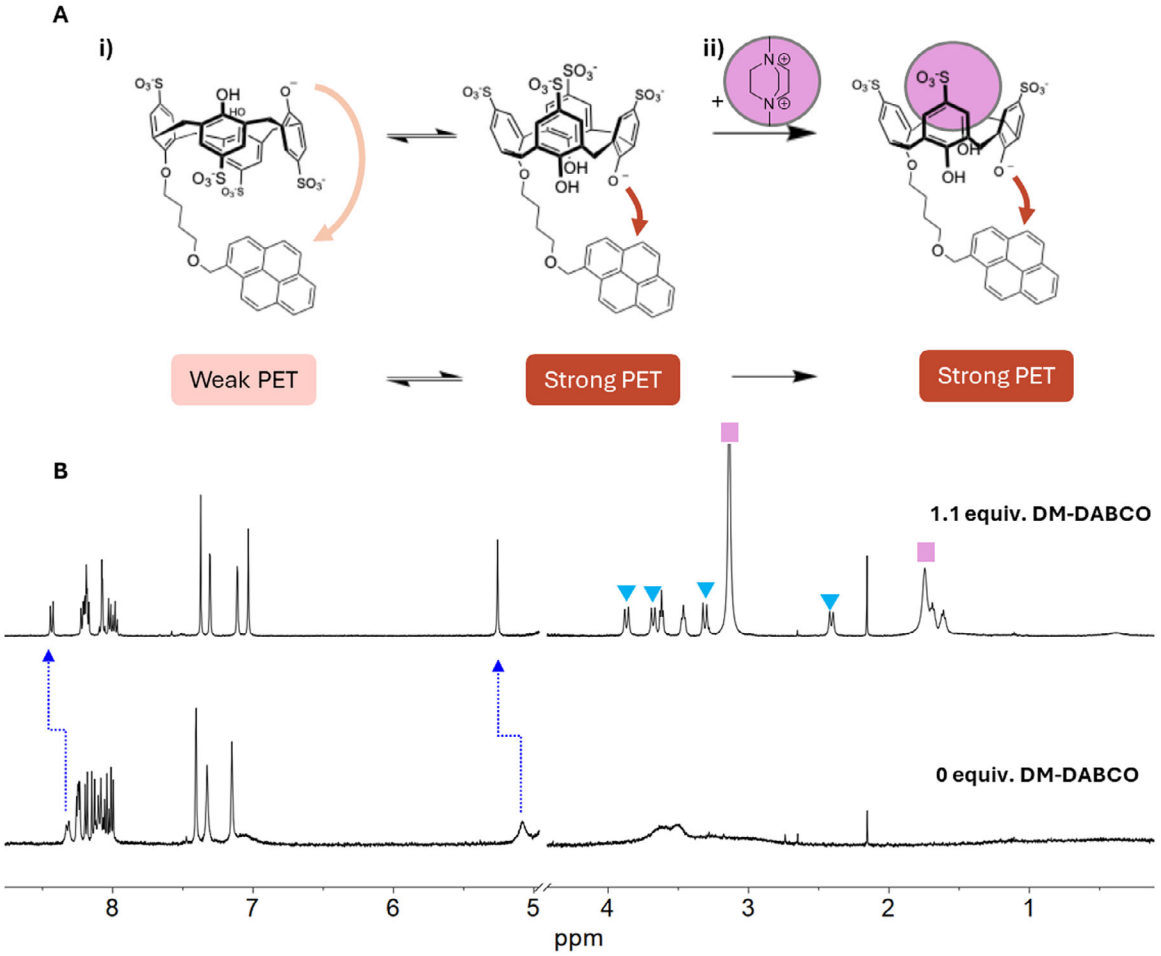


FIGURE 2 | – A. Schematization of the blocking of the cone conformation by guest binding to **SC4-4Py**. In the absence of guests, the **SC4-4Py** receptor exists in dynamic conformational equilibrium. Please note that no direct experimental evidence supports the deprotonation of this specific phenol unit; it is therefore arbitrary, and any of the other two phenol groups could equally have been chosen for illustrative purposes. B. ^{1}H-NMR (500 MHz) spectra of **SC4-4Py** at 0.2 mM in D2O, in the absence and presence 1.2 equivalents of DM-DABCO (signals identified by pink squares). A considerable sharpening of the signals assigned to the methylene bridges (blue triangles) and aliphatic chain signals is observed in the presence of the guest, concordant with decrease in flexibility of **SC4-4Py**. ^{1}H-^{13}C HSQC (se SI, Figure S10) shows the methylene bridge carbon chemical shifts between 30 and 32 ppm, which supports the locking of the calixarene in cone formation upon binding the guest.

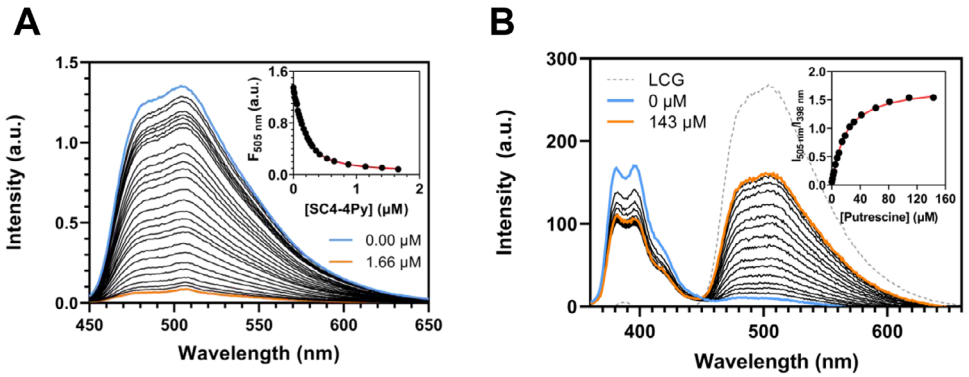


FIGURE 3 | – A. Direct titration of LCG, 0.19 μM, with **SC4-4Py**. B. Displacement assay of putrescine, done with LCG at 0.5 μM and **SC4-4Py** at 3 μM and corrected for quenching of LCG emission.

results (see Table 1) are also consistent with the 1:1 host:guest complexes showing binding affinities in reasonable agreement to those obtained by spectroscopic titrations. The thermodynamic parameters demonstrate that the complexation of the investigated guest molecules is enthalpy driven (ΔH values $\sim -20 \pm 4$ kJ.mol^{-1}), which is consistent with previous reported data for the binding of organic ammonium ions with parent SC4 [16]. Noteworthy, the higher affinity of **SC4-4Py** towards the dicationic guests (putrescine and DM-DABCO) is mostly determined by favorable entropic contributions, which can be tentatively assigned to the release of hydration water molecules upon additional ionic interactions between the oppositely charged groups of the host and guest.

3 | Conclusion

To conclude, we introduced a novel sulfonatocalix[4]arene-pyrene conjugate (**SC4-4Py**) which retains the excellent binding properties of the parent sulfonatocalix[4]arene receptor toward biologically relevant analytes, while enabling their direct detection by fluorescence emission. Our design strategy leveraged the intrinsic conformational flexibility of calixarene receptors and the enhanced acidity of their phenolic units. To achieve fluorescence signaling, we employed a bioinspired induced-fit mechanism, wherein the flexible **SC4-4Py** receptor adopts a *cone* conformation upon guest binding. This conformational transition positions the phenolate unit within the macrocyclic ring closer to the pyrene dye, thereby increasing the efficiency of photoinduced electron transfer (PET) and leading to stronger fluorescence quenching. The **SC4-4Py** conjugate was further utilized to construct a self-assembled chemosensor with the lucigenin dye, enabling dual-wavelength ratiometric fluorescence sensing of SC4 targets through an indicator displacement assay. Unlike most ratiometric fluorescence chemosensors based on receptor–dye conjugates that operate via Förster resonance energy transfer (FRET), our system relies on a competitive PET process. This strategy offers a simple yet effective alternative to FRET-based approaches, which are often difficult to implement in SC4-based systems due to complexation-induced quenching and aggregation of host–dye complexes commonly observed with this class of receptors. Given the demonstrated potential of the **SC4-4Py** conjugate for developing new optical assays and chemosensing schemes, as well as the ease with which our design strategy can be extended to other dye conjugates, we anticipate promising new applications of calixarene-based receptors in bioimaging and optical sensing.

Supporting Information

The authors have cited additional references within the Supporting Information [55, 56].

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. J. Krämer, R. Kang, L. M. Grimm, L. De Cola, P. Picchetti, and F. Biedermann, "Molecular Probes, Chemosensors, and Nanosensors for Optical Detection of Biorelevant Molecules and Ions in Aqueous Media and Biofluids," *Chemical Reviews* 122 (2022): 3459–3636, https://doi.org/10.1021/acs.chemrev.1c00746.

2. R. Pinalli, A. Pedrini, and E. Dalcanale, "Biochemical Sensing with Macrocyclic Receptors," *Chemical Society Reviews* 47 (2018): 7006–7026, https://doi.org/10.1039/C8CS00271A.

3. J. Wu, B. Kwon, W. Liu, E. V. Anslyn, P. Wang, and J. S. Kim, "Chromogenic/Fluorogenic Ensemble Chemosensing Systems," *Chemical Reviews* 115 (2015): 7893–7943, https://doi.org/10.1021/cr500553d.

4. L. You, D. Zha, and E. V. Anslyn, "Recent Advances in Supramolecular Analytical Chemistry Using Optical Sensing," *Chemical Reviews* 115 (2015): 7840–7892, https://doi.org/10.1021/cr5005524.

5. D. Wu, A. C. Sedgwick, T. Gunnlaugsson, E. U. Akkaya, J. Yoon, and T. D. James, "Fluorescent Chemosensors: The Past, Present and Future," *Chemical Society Reviews* 46 (2017): 7105–7123, https://doi.org/10.1039/C7CS00240H.

6. M. A. Beatty and F. Hof, "Host–guest Binding in Water, Salty Water, and Biofluids: General Lessons for Synthetic, Bio-targeted Molecular Recognition," *Chemical Society Reviews* 50 (2021): 4812–4832, https://doi.org/10.1039/D0CS00495B.

7. R. N. Dsouza, U. Pischel, and W. M. Nau, "Fluorescent Dyes and Their Supramolecular Host/Guest Complexes With Macrocycles in Aqueous Solution," *Chemical Reviews* 111 (2011): 7941–7980, https://doi.org/10.1021/cr200213s.

8. W. Zhong and R. J. Hooley, "Combining Excellent Selectivity with Broad Target Scope: Biosensing With Arrayed Deep Cavitand Hosts," *Accounts of Chemical Research* 55 (2022): 1035–1046, https://doi.org/10.1021/acs.accounts.2c00026.

9. L. Escobar and P. Ballester, "Molecular Recognition in Water Using Macrocyclic Synthetic Receptors," *Chemical Reviews* 121 (2021): 2445–2514, https://doi.org/10.1021/acs.chemrev.0c00522.

10. J. N. Martins, J. C. Lima, and N. Basílio, "Selective Recognition of Amino Acids and Peptides by Small Supramolecular Receptors," *Molecules (Basel, Switzerland)* 26 (2020): 106, https://doi.org/10.3390/molecules26010106.

11. F. Biedermann, W. M. Nau, and H.-J. Schneider, "The Hydrophobic Effect Revisited—Studies With Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force," *Angewandte Chemie International Edition* 53 (2014): 11158–11171.

12. F. Biedermann and H.-J. Schneider, "Experimental Binding Energies in Supramolecular Complexes," *Chemical Reviews* 116 (2016): 5216–5300, https://doi.org/10.1021/acs.chemrev.5b00583.

13. F. Chen, R. Fu, Z. Gong, C. Li, D. Guo, and K. Cai, "Ultrahigh-Affinity Molecular Recognition in Water and Biomedical Applications," *Angewandte Chemie International Edition* 64 (2025): e202500916, https://doi.org/10.1002/anie.202500916.

14. S. Shinkai, K. Araki, T. Matsuda, et al., "NMR and Crystallographic Studies of a p-sulfonatocalix[4]Arene-guest Complex," *Journal of the American Chemical Society* 112 (1990): 9053–9058, https://doi.org/10.1021/ja00181a004.

15. S. Shinkai, K. Araki, and O. Manabe, "NMR Determination of Association Constants for Calixarene Complexes. Evidence for the Formation of a 1:2 Complex With Calix[8]Arene," *Journal of the American Chemical Society* 110 (1988): 7214–7215, https://doi.org/10.1021/ja00229a046.

16. D.-S. Guo, K. Wang, and Y. Liu, "Selective Binding Behaviors of p-sulfonatocalixarenes in Aqueous Solution," *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 62 (2008): 1–21, https://doi.org/10.1007/s10847-008-9452-2.

17. D.-S. Guo and Y. Liu, "Supramolecular Chemistry of p -Sulfonatocalix[n]Arenes and Its Biological Applications," *Accounts of Chemical Research* 47 (2014): 1925–1934, https://doi.org/10.1021/ar500009g.

18. F. Perret and A. W. Coleman, "Biochemistry of Anionic Calix[n]Arenes," *Chemical Communications* 47 (2011): 7303.

19. M. A. Romero, P. Mateus, B. Matos, et al., "Binding of Flavylium Ions to Sulfonatocalix[4]Arene and Implication in the Photorelease of Biologically Relevant Guests in Water," *Journal of Organic Chemistry* 84 (2019): 10852–10859, https://doi.org/10.1021/acs.joc.9b01420.

20. C. S. Beshara, C. E. Jones, K. D. Daze, B. J. Lilgert, and F. Hof, "A Simple Calixarene Recognizes Post-translationally Methylated Lysine," *Chembiochem* 11 (2009): 63–66, https://doi.org/10.1002/cbic.200900633.

21. Y.-C. Pan, A. Barba-Bon, H.-W. Tian, et al., "An Amphiphilic Sulfonatocalix[5]arene as an Activator for Membrane Transport of Lysine-rich Peptides and Proteins," *Angewandte Chemie International Edition* 60 (2021): 1875–1882, https://doi.org/10.1002/anie.202011185.

22. S. Peng, A. Barba-Bon, Y.-C. Pan, W. M. Nau, D.-S. Guo, and A. Hennig, "Phosphorylation-Responsive Membrane Transport of Peptides," *Angewandte Chemie International Edition* 56 (2017): 15742–15745, https://doi.org/10.1002/anie.201707979.

23. K. N. Koh, K. Araki, A. Ikeda, H. Otsuka, and S. Shinkai, "Reinvestigation of Calixarene-Based Artificial-Signaling Acetylcholine Receptors Useful in Neutral Aqueous (Water/Methanol) Solution," *Journal of the American Chemical Society* 118 (1996): 755–758, https://doi.org/10.1021/ja951488k.

24. H. Bakirci and W. M. Nau, "Fluorescence Regeneration as a Signaling Principle for Choline and Carnitine Binding: A Refined Supramolecular Sensor System Based on a Fluorescent Azoalkane," *Advanced Functional Materials* 16 (2006): 237–242, https://doi.org/10.1002/adfm.200500219.

25. N. Korbakov, P. Timmerman, N. Lidich, B. Urbach, A. Sa'ar, and S. Yitzchaik, "Acetylcholine Detection at Micromolar Concentrations with the Use of an Artificial Receptor-Based Fluorescence Switch," *Langmuir* 24 (2008): 2580–2587, https://doi.org/10.1021/la703010z.

26. Y.-J. Zhang, W.-X. Cao, and J. Xu, "Interaction of Sulfonated Calix[n]Arenes with Rhodamine B and Its Application to Determine Acetylcholine in a Real Neutral Aqueous Medium," *Chinese Journal of Chemistry* 20 (2002): 322–326, https://doi.org/10.1002/cjoc.20020200406.

27. T. Jin, "A New Fluorometric Method for the Detection of the Neurotransmitter Acetylcholine in Water Using a Dansylcholine Complex with p-Sulfonated Calix[8]Arene," *Journal of inclusion phenomena and macrocyclic chemistry* 45 (2003): 195–201, https://doi.org/10.1023/A:1024506714332.

28. D.-S. Guo, V. D. Uzunova, X. Su, Y. Liu, and W. M. Nau, "Operational Calixarene-based Fluorescent Sensing Systems for Choline and Acetylcholine and Their Application to Enzymatic Reactions," *Chemical Science* 2 (2011): 1722–1734, https://doi.org/10.1039/c1sc00231g.

29. A. S. Pires, K. Droguett Muñoz, V. de Freitas, N. Basílio, and L. Cruz, "Host–Guest Chemosensor Ensembles based on Water-Soluble Sulfonated Calix[n]arenes and a Pyranoflavylium Dye for the Optical Detection of Biogenic Amines," *Journal of Agricultural and Food Chemistry* 72 (2024): 3719–3729, https://doi.org/10.1021/acs.jafc.3c08695.

30. N. Basílio, Á. Piñeiro, J. P. Da Silva, and L. García-Río, "Cooperative Assembly of Discrete Stacked Aggregates Driven by Supramolecular Host–Guest Complexation," *Journal of Organic Chemistry* 78 (2013): 9113–9119.

31. S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne, and E. V. Anslyn, "Teaching Old Indicators New Tricks," *Accounts of Chemical Research* 34 (2001): 963–972, https://doi.org/10.1021/ar9600796.

32. A. C. Sedgwick, J. T. Brewster, T. Wu, et al., "Indicator Displacement Assays (IDAs): The Past, Present and Future," *Chemical Society Reviews* 50 (2021): 9–38, https://doi.org/10.1039/C9CS00538B.

33. A. Hennig, H. Bakirci, and W. M. Nau, "Label-free Continuous Enzyme Assays with Macrocycle-fluorescent Dye Complexes," *Nature Methods* 4 (2007): 629–632, https://doi.org/10.1038/nmeth1064.

34. A. Barba-Bon, Y.-C. Pan, F. Biedermann, D.-S. Guo, W. M. Nau, and A. Hennig, "Fluorescence Monitoring of Peptide Transport Pathways Into Large and Giant Vesicles by Supramolecular Host–Dye Reporter Pairs," *Journal of the American Chemical Society* 141 (2019): 20137–20145.

35. S. Pangeni, J. D. Prajapati, J. Bafna, et al., "Large-Peptide Permeation Through a Membrane Channel: Understanding Protamine Translocation Through CymA from Klebsiella Oxytoca **," *Angewandte Chemie International Edition* 60 (2021): 8089–8094, https://doi.org/10.1002/anie.202016943.

36. A. Norouzy, Z. Azizi, and W. M. Nau, "Indicator Displacement Assays Inside Live Cells," *Angewandte Chemie International Edition* 54 (2015): 792–795, https://doi.org/10.1002/anie.201407808.

37. M. A. Beatty, J. Borges-González, N. J. Sinclair, A. T. Pye, and F. Hof, "Analyte-Driven Disassembly and Turn-On Fluorescent Sensing in Competitive Biological Media," *Journal of the American Chemical Society* 140 (2018): 3500–3504, https://doi.org/10.1021/jacs.7b13298.

38. M. A. Beatty, A. J. Selinger, Y. Li, and F. Hof, "Parallel Synthesis and Screening of Supramolecular Chemosensors That Achieve Fluorescent Turn-On Detection of Drugs in Saliva," *Journal of the American Chemical Society* 141 (2019): 16763–16771, https://doi.org/10.1021/jacs.9b07073.

39. X. Huang, J. Song, B. C. Yung, X. Huang, Y. Xiong, and X. Chen, "Ratiometric Optical Nanoprobes Enable Accurate Molecular Detection and Imaging," *Chemical Society Reviews* 47 (2018): 2873–2920, https://doi.org/10.1039/C7CS00612H.

40. M. H. Lee, J. S. Kim, and J. L. Sessler, "Small Molecule-based Ratiometric Fluorescence Probes for Cations, Anions, and Biomolecules," *Chemical Society Reviews* 44 (2015): 4185–4191, https://doi.org/10.1039/C4CS00280F.

41. S. Zhang, K. I. Assaf, C. Huang, A. Hennig, and W. M. Nau, "Ratiometric DNA Sensing With a Host–guest FRET Pair," *Chemical Communications* 55 (2019): 671–674.

42. K. L. Kim, G. Sung, J. Sim, et al., "Supramolecular Latching System Based on Ultrastable Synthetic Binding Pairs as Versatile Tools for Protein Imaging," *Nature Communications* 9 (2018): 1712, https://doi.org/10.1038/s41467-018-04161-4.

43. A. F. Sierra, D. Hernández-Alonso, M. A. Romero, J. A. González-Delgado, U. Pischel, and P. Ballester, "Optical Supramolecular Sensing of Creatinine," *Journal of the American Chemical Society* 142 (2020): 4276–4284, https://doi.org/10.1021/jacs.9b12071.

44. D.-S. Guo, B.-P. Jiang, X. Wang, and Y. Liu, "Calixarene-induced Aggregation of Perylene Bisimides," *Organic & Biomolecular Chemistry* 10 (2012): 720–723, https://doi.org/10.1039/C2OB06973C.

45. K. Wang, D.-S. Guo, and Y. Liu, "Temperature-Controlled Supramolecular Vesicles Modulated by p -Sulfonatocalix[5]Arene with Pyrene," *Chem—A Eur J* 16 (2010): 8006–8011.

46. J. N. Martins, B. Raimundo, A. Rioboo, Y. Folgar-Cameán, J. Montenegro, and N. Basílio, "Photoswitchable Calixarene Activators for Controlled Peptide Transport Across Lipid Membranes," *Journal of the American Chemical Society* 145 (2023): 13126–13133, https://doi.org/10.1021/jacs.3c01829.

47. G. Arena, R. Cali, G. G. Lombardo, et al., "Water Soluble Calix[4]Arenes. A Thermodynamic Investigation of Proton Complex Formation," *Supramolecular Chemistry* 1 (1992): 19–24, https://doi.org/10.1080/10610279208027437.

48. K. Suga, T. Ohzono, M. Negishi, K. Deuchi, and Y. Morita, "Effect of Various Cations on the Acidity of p -sulfonatocalixarenes," *Supramolecular Science* 5 (1998): 9–14, https://doi.org/10.1016/S0968-5677(97)00074-6.

49. D. Rehm and A. Weller, "Kinetics of Fluorescence Quenching by Electron and H-Atom Transfer," *Israel Journal of Chemistry* 8 (1970): 259–271, https://doi.org/10.1002/ijch.197000029.

50. T. Kubota, K. Kano, B. Uno, and T. Konse, "Energetics of the Sequential Electroreduction and Electrooxidation Steps of Benzenoid Hydrocarbons," *Bulletin of the Chemical Society of Japan* 60 (1987): 3865–3877, https://doi.org/10.1246/bcsj.60.3865.

51. H.-X. Zhao, D.-S. Guo, and Y. Liu, "Binding Behaviors of p -Sulfonatocalix[4]Arene With Gemini Guests," *Journal of Physical Chemistry B* 117 (2013): 1978–1987, https://doi.org/10.1021/jp312744d.

52. A. Shaurya, G. A. E. Garnett, M. J. Starke, et al., "An Easily Accessible, Lower Rim Substituted Calix[4]Arene Selectively Binds N, N -dimethyllysine," *Organic & Biomolecular Chemistry* 19 (2021): 4691–4696, https://doi.org/10.1039/D1OB00524C.

53. C. Jaime, J. De Mendoza, P. Prados, P. M. Nieto, and C. Sanchez, "Carbon-13 NMR Chemical Shifts. A Single Rule to Determine the Conformation of Calix[4]Arenes," *Journal of Organic Chemistry* 56 (1991): 3372–3376, https://doi.org/10.1021/jo00010a036.

54. K. D. Legg and D. M. Hercules, "Quenching of Lucigenin Fluorescence," *Journal of Physical Chemistry* 74 (1970): 2114–2118, https://doi.org/10.1021/j100909a011.

55. N. Basilio, L. García-Río, and M. Martín-Pastor, "Counterion Binding in Solutions of p -Sulfonatocalix[4]Arene," *Journal of Physical Chemistry B* 114 (2010): 7201–7206, https://doi.org/10.1021/jp101474a.

56. Q. Dai, W. Liu, X. Zhuang, J. Wu, H. Zhang, and P. Wang, "Ratiometric Fluorescence Sensor Based on a Pyrene Derivative and Quantification Detection of Heparin in Aqueous Solution and Serum," *Analytical Chemistry* 83 (2011): 6559–6564, https://doi.org/10.1021/ac2008724.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: chem70685-sup-0001-SuppMat.docx