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COMMUNICATION

Evaluating Impacts of Bambusuril Pocket Size and Sterics on Anion Binding Trends using ChemFET Sensors

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Chemically-sensitive Field Effect Transistors (ChemFETs) are a useful tool to evaluate aqueous anion affinity of hydrophobic supramolecular scaffolds. More specifically, ChemFETs can be used to probe impacts of receptor modification to aqueous anion affinity. In this study, ChemFETs are used to evaluate the anion affinity of both dodeca-*n*-butyl bambus[6]uril and dodecabenzyl bambus[6]uril to assess steric effects in the chemical selectivity of the sensor membrane. The ChemFETs were evaluated through a series of common anions in the Hofmeister series in order to ascertain the difference in detection limit imparted by the specific functionalization of the bambus[6]uril macrocycles, which are quite sensitive to modest steric effects. Significant improvements to perchlorate and nitrate detection limits were observed via *n*-butyl bambusuril-containing sensor membranes over detection limits recorded with benzyl bambusuril sensors.

The size and configuration of supramolecular host motifs have long been recognized as influential factors for target analyte affinity in host-guest chemistry.^{1–8} Bambus[6]urils are a known supramolecular host scaffold comprising six glycoluril subunits cyclized around methylene bridges.^{9–13} Two of the first reported bambusurils by Sindelar et al. contained *n*-propyl and benzyl functionality, respectively.¹³ Bambusuril crystal structures confirmed the shape of the binding pocket was drastically altered by the different steric bulk of these functional groups.¹³ The dodeca-*n*-propyl bambusuril was roughly cylindrical in shape, binding a single chloride (Figure 1, bottom left).¹⁰ However, replacing each propyl group with bulkier benzyl groups changed the shape from cylindrical to a more hourglass-like shape, resulting in the bambusuril binding two chlorides, one each in two binding pockets (Figure 1, bottom right).¹³

The current study seeks to evaluate the impacts of

bambusuril sterics and binding pocket shape/configuration on anion sensing when the macrocycles are used as an additive in a ChemFET sensor membrane. ChemFETs are similar to ISFETs (Ion-selective FETs), with the addition of a chemically-selective membrane to introduce specificity towards anions of interest. ChemFETs can thus take advantage of host-guest interactions by incorporating supramolecular host scaffolds in the gate oxide membrane, where the affinity for the analyte of interest results in a concentration gradient and resulting potential change that is measurable by the FET (Figure 2).^{14–16} Incorporation of the supramolecular host into the gate oxide membrane facilitates direct measurement of hydrophobic host interaction with

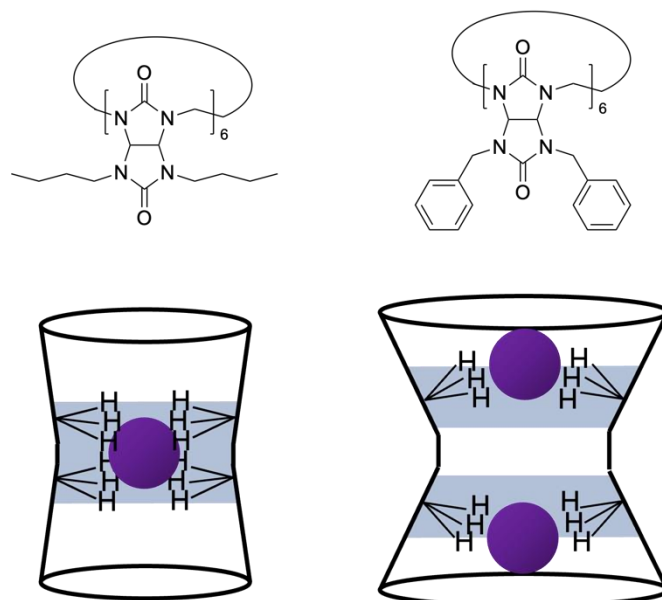


Figure 1. Simplified structural comparison of (left) dodeca-*n*-butyl bambusuril and (right) dodecabenzyl bambusuril. Anions are depicted as blue spheres. (Left) The lower steric bulk of the *n*-butyl groups results in a more cylindrical host shape, a single binding pocket, a 1:1 binding stoichiometry, and directs all 12 CH hydrogen bond donors into the pocket. (Right) The increased steric bulk of the benzyl groups results in an hourglass shape for the host, two binding pockets, 1:2 binding, and only six hydrogen bond donors inwardly directed to each pocket.

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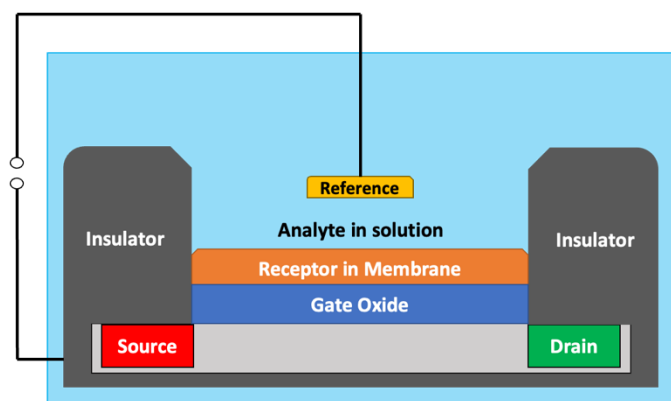


Figure 2. Chemically-sensitive Field Effect Transistor (ChemFET) diagram. FETs are purchased pre-constructed. FET modification includes covering the entire FET with insulating epoxy (aside from gate nitride/oxide surface), development of ionophore-containing membrane to cover the gate oxide surface, and construction of reference electrode. Entire ChemFET and reference electrode are submerged in analyte solution for analysis. Construction and operation follows previously published procedures.¹²

aqueous anion guests. In a ChemFET application, the differences between the more accessible binding pockets found in dodecabenzyl bambusuril and the less accessible central interior binding pocket of dodeca-*n*-butyl bambusuril are of particular interest (Figure 1). For numerous sensing applications, the ability to tune detection limits by modifying the receptor binding pocket size, shape, and configuration is of great interest and could lead to sensors with improved anion binding selectivities and detection limits.^{17,18}

The two bambusurils chosen were the previously characterized dodeca-*n*-butyl bambus[6]uril¹⁶ and dodecabenzyl bambus[6]uril.^{9,12–14} These two bambusurils were chosen due to marked differences in sterics and anion binding pockets resulting from the different external substituents. Additionally, crystal structures of dodeca-*n*-butyl bambusuril showed that, like dodeca-*n*-propyl bambusuril, it was also cylindrical in shape and binds small anions in a 1:1 host-guest stoichiometry.¹⁶ Both bambusurils were evaluated via ChemFETs for affinity to seven common anions found on the Hofmeister series, with careful attention paid to differing aqueous anion detection limits. Any trends in this key figure of merit were expected to be based primarily, if not solely, on steric differences (and the resulting host-guest binding geometry differences) between the otherwise identical bambusurils and ChemFET gate oxide membranes. Affinity was assessed via millimolar detection limit determination.

ChemFET construction was accomplished via previously reported methods (Figure 2).^{14–16} A polyvinyl chloride (PVC)-based membrane system was developed, with the PVC made semi-permeable by the inclusion of *ortho*-nitrophenyl octyl ether (NPOE) as plasticizer. Cationic character was imparted into the semi-permeable membrane by inclusion of tetraoctylammonium nitrate (TOAN).

Synthesis of the dodeca-*n*-butyl bambus[6]uril and dodecabenzyl bambus[6]uril was accomplished via previously reported methods¹⁶ and literature procedures¹³ using commercially available precursors. The sensor membranes were then prepared according to the following formulas, where

the only variable changed between the three formulations was the host macrocycle used (none used in the control membrane):

Control Sensor Membrane (no macrocycle):

- 66 wt. % PVC (69.0 mg), 32 wt. % NPOE (33 mg), and 2 wt. % TOAN (2 mg) in THF (2 mL).

Dodeca-*n*-butyl Bambus[6]uril Sensor Membranes:

- 65 wt. % PVC (68.6 mg), 32 wt. % NPOE (33.2 mg), 2 wt. % TOAN (2 mg), and 1 wt. % dodeca-*n*-butyl bambus[6]uril (1.02 mg) in 50:50 anisole/THF (2 mL).

Dodeca-benzyl Bambus[6]uril Sensor Membranes:

- 65 wt. % PVC (64.7 mg), 32 wt. % NPOE (32.3 mg), 2 wt. % TOAN (2 mg), and 1 wt. % dodeca-benzyl bambus[6]uril (1 mg) in 50:50 anisole/THF (2 mL).

ChemFET evaluation was accomplished via previously reported methods.^{14–16} Each ChemFET evaluation involved the synthesis of the respective bambusuril ionophore, the creation of the membrane formulation, construction of ChemFET sensors, construction of reference electrodes, and performance of the sensor during experimental runs (see ESI for details). Analyte solutions were developed from commercially-obtained reagents. Each anion was evaluated as the potassium salt, using a common counteranion for the sake of consistency. Each salt was used to create a series of 12 solutions, each with a constant concentration of piperazine-*N,N'*-bis-2-ethanesulfonic acid (PIPES) buffer and varying analyte concentration. The concentrations used were 0.100 M, 50.0 mM, 10.0 mM, 5.00 mM, 1.00 mM, 500 μ M, 100 μ M, 50.0 μ M, 10.0 μ M, 5.00 μ M, 1.00 μ M, and 0.500 μ M analyte, each with constant 50.0 mM PIPES. All solutions were fixed to pH 7 using 4.0 M KOH. Each sensor evaluation comprised sensors run through the 12 analyte solutions, in triplicate, using four different identically-constructed sensors. Actual detection limits were calculated using these results, after conversion of concentration to activity (using Davies activity coefficients).¹⁹

The two bambusuril binding motifs appears to engender some significant differences in ChemFET evaluation results (Table 1). Most notably, *N*-butyl bambusuril sensors display one and two orders of magnitude improvement over benzyl bambusuril sensors for nitrate and perchlorate, respectively, while the remaining anions remained very similar between the two membranes featuring the host macrocycle as well as the control sensor lacking any supramolecular host.

A general trend that emerges is that our sensors displayed the most sensitivity to the chaotrope end of Hofmeister series (I^- and ClO_4^-), and the least sensitivity to the kosmotrope end (SO_4^{2-} and F^-) (Figure 3). However, the difference in physical configuration of bambusuril binding pockets caused by bulky

Receptor	Figure of Merit	SO_4^{2-}	F^-	Cl^-	Br^-	NO_3^-	ClO_4^-	I^-
n-BuBU[6]	Detection Limit (mM)	34	6.3	1.9	0.16	0.039	0.0013	0.0086
BnBU[6]	Detection Limit (mM)	30	19	7.5	0.20	0.43	0.14	0.0055

Table 1. ChemFET evaluation results of *n*-butyl and benzyl bambusuril-containing sensors. Both sensors contain 1 weight percent bambusuril. Detection limits measured in mM.

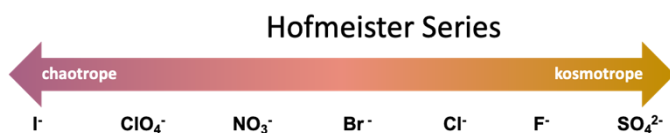


Figure 3. Common anions in the Hofmeister Series. This represents a common reference framework for electrochemical sensors. Electrochemical sensors are commonly more sensitive to chaotropes (left) than kosmotropes (right).

functional groups caused clear impacts on binding trends in the Hofmeister anions evaluated (Figure 4). The larger size of the binding pocket, coupled with less available hydrogen bond donors, resulted in clear differences in Hofmeister trends typically seen in electrochemical sensor evaluations, which tend to simply select for more chaotropic (~lipophilic) anions within the membranes.

The bambus[6]uril family contains 12 hydrogen bond donors; 2 from each of the 6 glycuril subunits. The more sterically-bulky benzyl bambusuril, which binds anions in a 1:2 host:guest stoichiometry, has only half the number of hydrogen bond donors available in each binding pocket. While the more spacious physical configuration of the binding pocket may be more conducive to certain anions, that is countered by the presence of fewer hydrogen bond donors.

This feature may shed light on the impact of the bambusuril structural differences on detection limits for various anions (Figure 4). Of particular interest is that the dodeca-*n*-butyl bambusuril is nearly equal or superior to dodecabenzyl bambusuril as measured by detection limits across the series of anions measured. This result was quite unexpected, as it was thought the binding pockets more at the periphery of the benzyl variant might be more accessible to anions in the semipermeable PVC membrane, and thus show improvement in binding (as measured by detection limits). However, along with the more accessible binding pocket comes the added complication that the number of hydrogen bond donors per pocket is halved, which may play a significant role in the

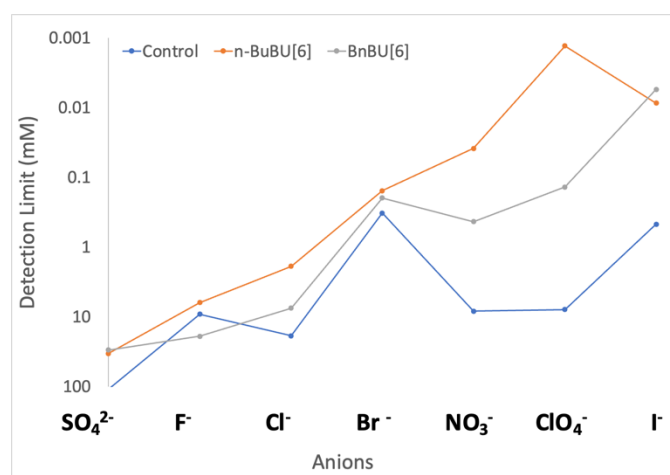


Figure 4. Graphically-depicted results of control (no ionophore) sensors in grey, *n*-butyl bambusuril sensors in orange, and benzyl bambusuril in blue. Lower detection limit (higher on the y-axis) is better. Of particular note is the significant differences in detection limits between the control and both bambusuril derivative-containing sensors at the more lipophilic end of the anion range.

differences observed. Additionally, the more accessible peripheral binding pockets found in benzyl bambusuril may also leave the binding pockets more susceptible to intrusion by the membrane material (PVC) and plasticizer (NPOE).

Other particularly noteworthy observations were that fluoride and bromide show almost no difference between control and ionophore-containing sensors, and perchlorate shows the largest difference (3-4 orders of magnitude, Figure 5). Significantly, although the general trends between *n*-butyl and benzyl bambusuril-containing sensors are similar, benzyl bambusuril shows a marked degradation towards sensing perchlorate (and, to a lesser extent, nitrate). Given that nitrate and perchlorate are larger anions that feature more hydrogen bond acceptors, it appears the more open binding pocket and additional hydrogen bond donors provides a markedly enhanced detection limit for these two anions.

This evaluation also provides a good illustration of the utility of “turning off” sensitivity towards particular analytes through functionalization of the host scaffold. The change of bambusuril functional groups significantly “turned off” (or at least greatly diminished) perchlorate sensitivity. The corollary is likely true also, where strengthening the molecular-level interactions (e.g., strong CH hydrogen bonds) between host and guest would provide even better sensitivity for tightly bonded anions. Taken in tandem, that might suggest this approach could be well-suited towards array-based sensing platforms, where subtle differences in analyte binding within an array can provide robust anion speciation and concentration information in solution.^{20–24}

Much of host-guest chemistry stems from ideas around the lock and key principle, which emphasizes the size, shape, and non-covalent interactions between host and guest. Varying the configuration of the binding pocket thus results in different binding affinities. This concept holds especially true for the shape-persistent bambus[6]uril family of supramolecular host scaffolds, where R groups with different steric bulk result in different anion affinities, and changing bambusuril functionality even allows changing between 1:1 or 1:2 binding configurations. Differences were measured in a ChemFET sensor motif, where both types of bambusuril (*n*-butyl 1:1 binding, and benzyl 1:2 binding) were evaluated against a series of anions to determine the resulting impact of the different binding configurations.

Of particular note was the response to perchlorate, which was demonstrated to be quite sensitive to sensors with one bambusuril configuration, with that same affinity greatly toned down merely by changing the functional group of the bambusuril. This also illustrates the differences between electrochemical sensing and solution state binding, where Sindelar et al. demonstrated remarkably high solution state binding of dodecabenzyl bambus[6]uril with perchlorate.²⁵ We attribute one possible explanation of this difference to the intrusion of polymer and/or plasticizer into the more exposed dual binding pockets of dodecabenzyl bambus[6]uril, a complication not present in solution state ¹H NMR titrations or Isothermal Titration Calorimetry (ITC) determinations of binding constants.

In the field of electrochemical perchlorate sensing, the current best detection limit was reported by Filip and coworkers of 0.46 nM.^{26,27} This ISE-based sensor system required pretreatment of the sensor membrane with perchlorate to attain this record detection limit, and without such pretreatment attained a diminished detection limit of 280 nM. The next lowest reported perchlorate detection limit with an ISE-based sensing system and perchlorate conditioning of the membrane is 3.09 nM by Bhatti and coworkers.^{26,28} In light of this, the 1 μ M detection limit of the n-butyl bambus[6]juril ChemFET system stands out given ChemFETs are robust and low-cost, and importantly, they require no pretreatment or perchlorate-containing reference electrolyte, so that identical sensors can be used for all evaluated anions. Overall, this shows promise for future studies in evaluating impacts of subtle structural changes to host receptors and their effects on sensor performance.

Conflicts of interest

There are no conflicts to declare.

Notes and references

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