Ditopic Ion Transport Systems: Anion-π Interactions and Halogen Bonds at Work

VARGAS JENTZSCH, Rodrigo Andreas, *et al*.

Abstract

Single-atom exchange series are introduced to extract the individual contributions of halogen bonds and anion–π interactions to the transport of anions across lipid bilayer membranes (see picture). Known cation binding sites are used for counterion activation of the neutral calix[4]arene transporters. The experimental evidence for anion transport with halogen bonds is unprecedented.

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Ditopic Ion Transport Systems: Anion-π Interactions and Halogen Bonds at Work**

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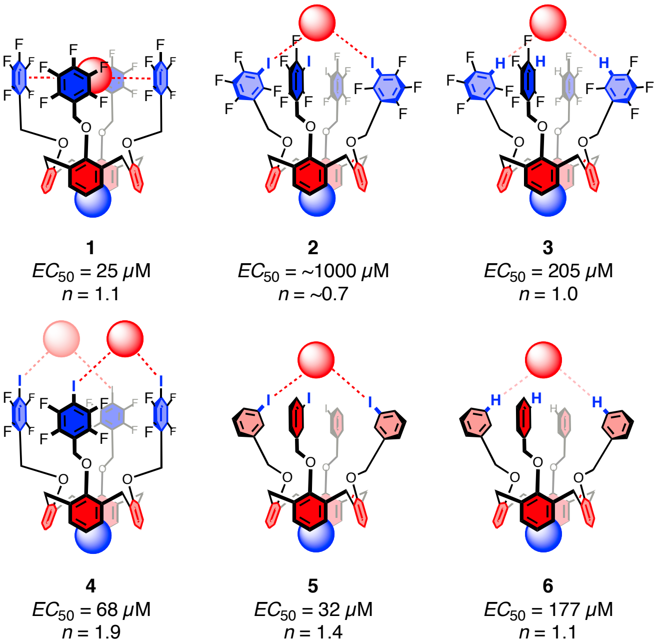
Ion transport systems that operate in lipid bilayer membranes[1-3] are emerging as attractive tool to probe the functional relevance of weak interactions that are otherwise difficult to observe.[1] This approach builds on the notion that transport and catalysis operate best with weaker interactions than the ones that required for detection in binding studies. Earlier important work in theory and in bulk membranes has confirmed that transport efficiency follows "Goldilocks principle", with the stronger binders not being the best transporter.[4] Here, we introduce single-atom mutation series with a new ditopic ion transport system to demonstrate the general functional relevance of anion-π interactions and to achieve, for the first time, anion transport with halogen bonds.

The rarity of functional halogen bonds[5-10] compared to the frequency of hydrogen bonds is reminiscent to the situation with anion-π interactions[11] compared to the ubiquitous cation-π interactions. Increasing in strength with halogen atom polarizability, halogen bonds are best with iodine substituents.[5,6] They depend strongly on the environment, including contributions from solvation/desolvation, and are strongest in non-competitive hydrophobic solvents.[7] Halogen bonds have been studied extensively in solid-state crystal engineering.[5] Whereas pioneering examples for rational drug design[8] and anion binding with halogen bonds in solution exist,[9] their application to catalysis[10] and particularly to transport is essentially unexplored.

Calix[4]arenes **1**-**6** were designed to dissect the individual contributions of halogen bonds, hydrogen bonds and anion-π interactions to anion transport (Figure 1). Calix[4]arene cones[2,12-14] were selected for this study because 1) they offer convenient construction sites at the lower rim, 2) bind ammoniun cations at the upper rim,[15] and 3) are well explored as transport systems in lipid bilayer membranes.[2] These features indicated their suitability for the construction of ditopic transporters with a tetramethylammonium (TMA) binding site near a modular anion binding site, where nature and number of possible anion-π (**1**), halogen-bonding (**2**, **4**, **5**) or hydrogen-bonding (**3**, **6**) interactions with anions can be varied systematically and without global structural changes. Ditopic transporters are interesting because they can operate by anion/cation symport and thus avoid any temporary transmembrane separation of anions and cations during transport. Their straightforward synthesis is described in the Supporting Online Information (Schemes S1-S2, Figures S10-S20).[16]



1
EC_{50} = 25 μM
n = 1.1

2
EC_{50} = ~1000 μM
n = ~0.7

3
EC_{50} = 205 μM
n = 1.0

4
EC_{50} = 68 μM
n = 1.9

5
EC_{50} = 32 μM
n = 1.4

6
EC_{50} = 177 μM
n = 1.1

Figure 1. Ditopic ion transport systems made to study anion-π interactions and halogen bonds at work, with effective concentrations EC_{50} and Hill coefficients *n* to summarize the transport activities found. Red colors indicate electron-rich, blue colors electron-poor regions. Red balls indicate anions (e.g., chloride or hydroxide), blue balls TMA cations, and dotted red lines possible anion-π (**1**), halogen-bonding (**2**, **4**, **5**) or hydrogen-bonding (**3**, **6**) interactions between anions and transporters. These interactions are purely speculative and shown with the only intention to illustrate the design (compare models, Figures 3b, S21, S22).

The transport activity of calix[4]arenes **1**-**6** was evaluated with the 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS) assay.[1,16,17] In this assay, large unilamellar vesicles composed of egg yolk

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

phosphatidylcholine (EYPC LUVs) are loaded with the pH-sensitive fluorophore HPTS, and a pH gradient is applied.[17] The ability of transport systems to accelerate the dissipation of this pH gradient is then reported ratiometrically by the intravesicular internal pH probes. In this assay, ditopic calix[4]arene transporters were expected to cause the collapse of pH gradients by chloride/hydroxide antiport.[17] Results described in the following support that this antiport occurs by chloride/TMA and hydroxide/TMA symport, with TMA participating as counterion activator of the transporter that neither adds nor removes transmembrane gradients.

The HPTS assay is convenient because anion/cation symport, anion/anion antiport and cation/cation antiport are detected.[1,17] In a typical experiment, HPTS-loaded LUVs were first exposed to a base pulse. Then, the potential transporter was added, and the change in HPTS emission was recorded. At the end of each experiment, the pH was equilibrated with excess gramicidin D to calibrate for 100% fluorescence change (Figures 2c■, S1).

Under these conditions, several promising candidates for chloride/hydroxide transport with halogen bonds were inactive (not shown). Inactivity of analogs of **1**-**6** with tert-butyl groups at the upper rim was consistent with previous reports on interfering conformational constraints (not shown).[2] Calix[4]arenes **1**-**6** were almost inactive as well in the presence of all cations tested except for the TMA cation (Figures S1, S5). This superb cation selectivity confirmed that the known TMA complexes at the upper rim are essential for function (Figure 1).[2,15] Similar counterion activation has been observed with cesium-activated calix[4]pyrrole anion transporters[18] and with many polyion transporters.[19]

Hill analysis of dose response curves of TMA-activated calix[4]arene **1** gave an effective concentration EC_{50} = 25 μM and a Hill coefficient n = 1.1 (Figures 1 and 2a□). The EC_{50} describes the effective concentration needed to reach 50% activity and can be similar or proportional to the dissociation constant K_D of the transport-active complex.[17] The Hill coefficient n can describe cooperativity and can indicate the number of monomers needed for activity, although other contributions can complicate the situation (*e.g.*, the thermodynamic stability of the active complex).[17,20]

Single-atom F→I mutation from fluorines to iodines in the anion binding site of **2** caused nearly complete inactivation (Figures 1 and 2△). Single-atom substitution to hydrogens in the anion binding site of **3** partially restored activity (Figures 1 and 2a○).

The obtained activity sequence demonstrated that the anion-π binding site in calix[4]arene **1** generates highest activity (Figures 1 and 2a□). Perfluorophenyls are classical π-acids. Their quadrupole moment has with Q_{zz} = +9.5 B about half the strength of NDI transporters with Q_{zz} = +19 B.[11] In calix[4]arene **1**, this reduced π-acidity should, however, be compensated by the preorganized tetravalent array along the macrocycle.[12-14]

The dramatic drop in activity from calix[4]arene **1** to **2** demonstrated that F→I mutation destroys the operational anion-π active site in calix[4]arene **1** (Figures 1 and 2△). This abrupt change suggested that chloride/hydroxide binding by four proximal activated halogen bond donors in calix[4]arene **2** (1) occurs but (2) does not lead to transport. ^{19}F NMR titrations in dry acetone-d_6 revealed detectable chloride binding to the inactive **2** (K_D = 18.0 ± 1.0 mM for tetrabutylammonium (TBA) chloride in dry acetone-d_6) but not to the active **1** and **3** (Figures 3, S7-S9, Table S1)[21,22] Similar transport inhibition by excessive binding has been observed previously.[1a,4] To transport anions with halogen bonds, either the thermodynamic or the kinetic stability of the chloride/hydroxide complex of **2** would therefore have to be reduced.

Thermodynamic destabilization of chloride/hydroxide binding to **2** was achieved without global structural changes by replacing the π-acidic with π-basic arenes (Figure 1). With the weaker halogen-bond donors in calix[4]arene **5**, chloride binding became undetectable in ^{19}F NMR titrations in dry acetone-d_6,[16] whereas chloride/hydroxide transport activity increased more than 30-times (Figures 1, 2■, S1). The EC_{50} = 32 μM of the halogen-bond transporter **5** was as good as that of anion-π transporter **1** (Figures 1, 2). More than 5-times decreasing activity in response to I→H mutation in calix[4]arene **6** provided corroborative evidence that transporter **5** indeed uses halogen bonds to transport chloride/hydroxide anions (Figures 1, S1).

Kinetic destabilization was envisioned by moving the halogen-bond donors from *meta*-position in **2** to *para*-position in **4**.[22] This constitutional isomerization should increase the distance between halogen bond donors and move the active site to the periphery of the complex (Figure 1). Consistent with mainly kinetic complex destabilization, TBACl binding by *para*-isomer **4** remained detectable in ^{19}F NMR titrations (K_D = 13.3 ± 0.6 mM for TBACl in dry acetone-d_6, Figures S7-S9), whereas transport activity increased 15-times to an EC_{50} = 68 μM (Figures 1, 2●, S1).[21]

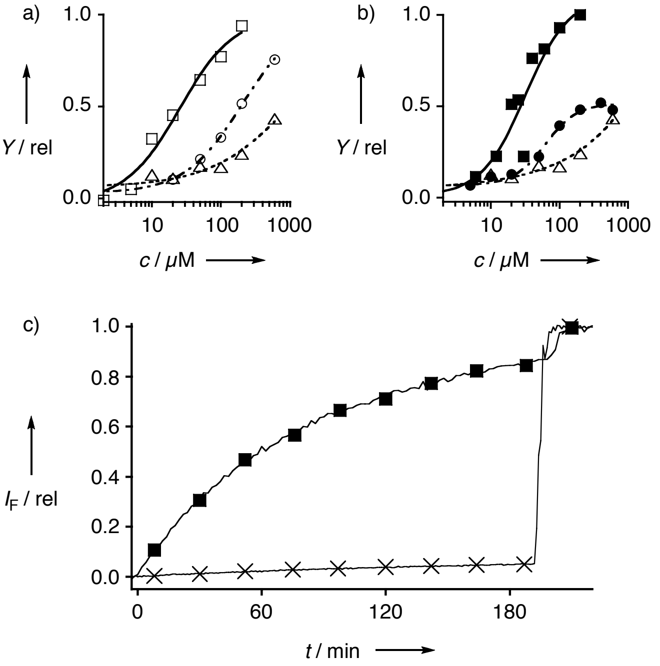

Figure 2. Anion transport activity Y as a function of the concentration of (a) **1** (□), **2** (△) and **3** (○), and (b) **2** (△), **4** (●) and **5** (■) with curve fit to Hill equation (a, b). (c) Original data for anion transport (■) and non-specific leakage (×) mediated by **5** (■, 60 μM; ×, 600 μM). These original data show (■) fractional emission I_F during the addition of NaOH (20 μl, 0.5 M) and **5** to EYPC-LUVs⊃HPTS in TMACl (200 mM, 10 mM Hepes buffer, pH 7), with final calibration with gramicidin D (200 s), and (×) the same for **5** added to EYPC-LUVs⊃CF.

Reduced proximity of the halogen-bond donors in *para*-isomer **4** further caused an increase from the usual n ~ 1 to a Hill coefficient n ~ 2 (Figures 1, 2●). This change can suggest that, with peripheral active sites, two calix[4]arenes are required to sufficiently surround and transport one chloride/hydroxide anion, whereas the number of proximal halogen-bond donors in *meta*-isomers is already sufficient in 1:1 complexes.[20] Job plots of ^{19}F NMR titrations in dry acetone-

d_6 supported the 1:1 stoichiometry of complexes with *meta*-isomers such as **2**, whereas in acetone, *para*-isomers **4** could bind around two chlorides (Figure S9b). This inversion of stoichiometry with *para*-isomers **4** provided compelling evidence that binding of TBACl in acetone and transport of TMACl across lipid bilayers must be compared with highest caution.[7,21,23]

Replacement of one or two haloaryl substituents in the too efficient binder **2** with one or two methoxy groups to reduce the number of halogen-bond donors did not afford active transporters.[16,S5] This persistent inactivity was meaningful because neither power nor proximity of the individual halogen-bond donors are significantly changed by this modification. Moreover, the essential cation binding site could possibly be perturbed by the appearance of stable calix[4]arene isomers.[12,13] Detectability of TMA-independent chloride binding by homologs of **2** without one or two haloaryl substituents by ^{19}F NMR spectroscopy in dry acetone-d_6 confirmed that reduction in multivalency is insufficient to significantly reduce the thermodynamic stability of the halogen-bond chloride complexes.[S5]

Density functional theory (DFT) modeling at the PBE1PBE/6-311G** level of theory showed that out of four aryls, only three participate in the anion-π interactions of the TMACl complex of calix[4]arene **1** (Figures 3b, S21), resulting in a binding energy of -58.3 kcal mol^{-1}.[23] For the *meta*-isomer **2,** DFT models confirmed the reinforcement of the binding energy (-70.9 kcal mol^{-1}) with the formation of two halogen bonds, whereas the other two iodoarene donors rest nearby to, we speculated, possibly increase probability effects and inertness of the complex (Figure S21). In the absence of perfluorinated iodoarenes, the two halogen bonds of the TMACl complex of calix[4]arene **5** were maintained (Figure S22). However, the binding energy clearly decreased to -59.7 kcal mol^{-1}, which correlates with the observed regain in the transport activity with **5**.

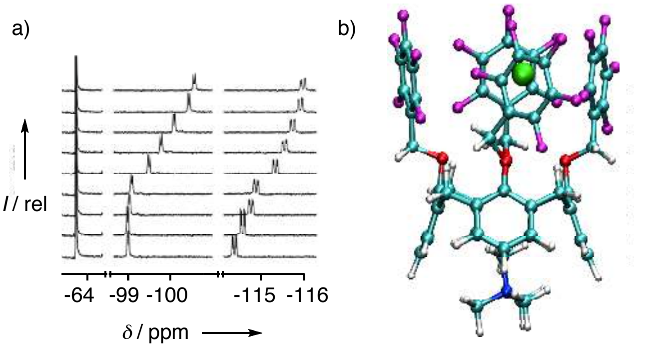

Figure 3. a) Part of the ^{19}F NMR spectra in dry acetone-d_6 of the least active transporter **2** and α,α,α-trifluorotoluene (-63.72 ppm) in dry acetone-d_6 in the presence of increasing concentrations of TBACl (0 – 500 mM, bottom to top). b) Density functional theory (PBE1PBE/6-311G**) optimized TMACl complex of the most active transporter **1**; TBACl binding by **1** was not detectable by ^{19}F NMR spectroscopy in dry acetone-d_6 (in ball-and-stick, cyan; H, white; O, red; N, blue; Cl anion, green; F, magenta; I, gold).

All pertinent control experiments confirmed that calix[4]arenes **1-6** act as counterion-activated anion transporters with operational halogen bonds and anion-π interactions. Namely, external anion exchange produced changes in transport activity, and revealed a general selectivity for chloride (Figure S4). This responsiveness to external anion exchange indicated that weak anion binding occurs and matters for transport in all cases.[1,17] The nonconformity to the Hofmeister topology suggested that the cost of at least partial anion desolvation is compensated by binding.[17] Inability to transport 5(6)-carboxyfluorescein (CF) excluded the occurrence of non-specific leakage through larger defects in the membrane (Figures 2c✕, S6).

In summary, the absolute transport activity of calix[4]arene transporters was about as modest as expected from the literature.[2, 3] However, the calix[4]arene scaffold was very useful to orchestrate transport with halogen bonds and anion-π interactions. Best transport activity was obtained for anion binding at the focal point of four pentafluorobenzyl π-acids, demonstrating that the functional relevance of anion-π interactions is general, independent of the structural motif involved. Direct substitution of these π-acids by halogen-bond donors did not afford active anion transporters, whereas anion binding was clearly detectable. Thermodynamic and kinetic destabilization of this too good binder by weakening of the strength and the proximity of the halogen-bond donors, respectively, provided rational approaches to active anion transporters. These remarkably consistent results confirm synthetic transporters as unique systems to explore the functional relevance of weak interactions that are otherwise difficult to detect. This is the first time anion transport in lipid bilayers has been achieved with halogen bonds.

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[1] a) R. E. Dawson, A. Hennig, D. P. Weimann, D. Emery, S. Gabutti, J. Montenegro, V. Ravikumar, M. Mayor, J. Mareda, C. A. Schalley, S. Matile, *Nat. Chem.* **2010**, *2*, 533-538; b) J. Misek, A. Vargas Jentzsch, S. Sakurai, D. Emery, J. Mareda, S. Matile, *Angew. Chem. Int. Ed.* **2010**, *49*, 7680-7683.

[2] a) O. Lawal, K. S. J. Iqbal, A. Mohamadi, P. Razavi, H. T. Dodd, M. C. Allen, S. Siddiqui, F. Fucassi, P. J. Cragg, *Supramol. Chem.* **2009**, *21*, 55-60; b) J. C. Iglesias-Sánchez, W. Wang, R. Ferdani, P. Prados, J. de Mendoza, G. W. Gokel, *New J. Chem.* **2008**, *32*, 878-890; c) O. A. Okunola, J. L. Seganish, K. J. Salimian, P. Y. Zavalij, J. T. Davis, *Tetrahedron* **2007**, *63*, 10743-10750; d) Y. Tanaka, Y. Kobuke, M. Sokabe, *Angew. Chem. Int. Ed.* **1995**, *34*, 693-694;

[3] a) J. K. Chui, T. M. Fyles, *Chem. Soc. Rev.* **2011**, DOI: 10.1039/C1CS15099E; b) S. Matile, A. Vargas Jentzsch, A. Fin, J. Montenegro, *Chem. Soc. Rev.* **2011**, *40*, 2453-2474; c) U. Devi, J. R. Brown, A. Almond, S. J. Webb, *Langmuir* **2011**, *15*, 1448-1456; d) F. Otis, C. Racine-Berthiaume, N. Voyer, *J. Am. Chem. Soc.* **2011**, *133*, 6481-6483; e) H. Cho, L. Widanapathirana, Y. Zhao, *J. Am. Chem. Soc.* **2011**, *133*, 141-147; f) J. T. Davis, P. A. Gale, O. A. Okunola, P. Prados, J. C. Iglesias-Sanchez, T. Torroba, R. Quesada, *Nat. Chem.* **2009**, *1*, 138-144; g) A. P. Davis, D. N. Sheppard, B. D. Smith, *Chem. Soc. Rev.* **2007**, *36*, 348-357.

[4] J.-P. Behr, M. Kirch, J.-M. Lehn, *J. Am. Chem. Soc.* **1985**, *107*, 241-246.

[5] P. Metrangolo, F. Meyer, T. Pilati, G. Resnati, G. Terraneo, *Angew. Chem. Int. Ed.* **2008**, *47*, 6114-6127.

[6] P. Politzer, J. S. Murray, T. Clark, *Phys. Chem. Chem. Phys.* **2010**, *12*, 7748-7757.

[7] Y. Lu, H. Li, X. Zhu, W. Zhu, H. Liu, *J. Phys. Chem. A* **2011**, *115*, 4467-4475.

[8] a) L. A. Hardegger, B. Kuhn, B. Spinnler, L. Anselm, R. Ecabert, M. Stihle, B. Gsell, R. Thoma, J. Diez, J. Benz, J. M. Plancher, G. Hartmann, D. W. Banner, W. Haap, F. Diederich, *Angew. Chem. Int. Ed.* **2011**, *50*, 314-318; b) P. Auffinger, F. A. Hays, E. Westhof, P. S. Ho, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 16789-16794.

[9] a) M. G. Chudzinski, C. A. McClary, M. S. Taylor, *J. Am. Chem. Soc.* **2011**, *133*, 10559-10567; b) A. Caballero, N. G. White, P. D. Beer, *Angew. Chem. Int. Ed.* **2011**, *50*, 1845-1848; c) M. G. Sarwar, B. Dragisic, L. J. Salsberg, C. Gouliaras, M. S. Taylor, *J. Am. Chem. Soc.* **2010**, *132*, 1646-1653; d) A. Mele, P. Metrangolo, H. Neukirch, T. Pilati, G. Resnati, *J. Am. Chem. Soc.* **2005**, *127*, 14972-14973.

[10] a) S. Walter, F. Kniep, E. Herdtweck, S. Huber, *Angew. Chem. Int. Ed.* **2011**, *50*, 7187-7191; b) S. Dordonne, B. Crousse, D. Bonnet-Delpon, J. Legros, *Chem. Commun.* **2011**, *47*, 5855-5857; c) D. A. Kraut, M. J. Churchill, P. E. Dawson, D. Herschlag, *ACS Chem. Biol.* **2009**, *4*, 269–273; d) A. Bruckmann, M. A. Pena, C. Bolm, *Synlett* **2008**, *6*, 900-902.

[11] a) A. Frontera, P. Gamez, M. Mascal, T. J. Mooibroek, J. Reedijk, *Angew. Chem. Int. Ed.* **2011**, DOI: 10.1002/anie.201100208; b) L. M. Salonen, M. Ellermann, F. Diederich, *Angew. Chem. Int. Ed.* **2011**, *50*, 4808-4842; c) H. T. Chifotides, B. L. Schottel, K. R. Dunbar, *Angew. Chem. Int. Ed.* **2010**, *49*, 7202-7205; d) O. B. Berryman, V. S. Bryantsev, D. P. Stay, D. W. Johnson, B. P. Hay, *J. Am. Chem. Soc.* **2007**, *129*, 48-58; e) D. Quinonero, C. Garau, C. Rotger, A. Frontera, P. Ballester, A. Costa, P. M. Deya, *Angew. Chem. Int. Ed.* **2002**, *41*, 3389-3392; f) M. Mascal, A. Armstrong, M. D. Bartberger, *J. Am. Chem. Soc.* **2002**, *124*, 6274-6276; g) I. Alkorta, I. Rozas, J. Elguero, *J. Am. Chem. Soc.* **2002**, *124*, 8593-8598.

[12] Stable calix[4]arene isomers appeared in the ^{1}H, ^{13}C and ^{19}F NMR spectra only upon removal of at least one bulky substituent at the lower rim.[13] The energetics of conformational changes of the flexible sidechains has been determined in rigidified cavitand analogs.[14]

[13] P. F. Hudrlik, A. M. Hudrlik, L. Zhang, W. Arasho, J. Cho, *J. Org. Chem.* **2007**, *72*, 7858-7862.

[14] V. A. Azov, A. Beeby, M. Cacciarini, A. G. Cheetham, F. Diederich, M. Frei, J. K. Gimzewski, V. Gramlich, B. Hecht, B. Jaun, T. Latychevskaia, A. Lieb, Y. Lill, F. Marotti, A. Schlegel, R. R. Schlittler, P. J. Skinner, P. Seiler, Y. Yamakoshi, *Adv. Funct. Mater.* **2006**, *16*, 147-156.

[15] a) H.-J. Schneider, D. Guettes, U. Schneider, *J. Am. Chem. Soc.* **1988**, *110*, 6449-6454; b) J. M. Harrowfield, W. R. Richmond, A. N. Sobolev, A. H. White, *J. Chem. Soc., Perkin Trans. 2* **1994**, 5-9; c) W. Abraham, *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *43*, 159-174; d) L. M. Salonen, C. Bucher, D. W. Banner, W. Haap, J.-L. Mary, J. Benz, O. Kuster, P. Seiler, W. B. Schweizer, F. Diederich, *Angew. Chem. Int. Ed.* **2009**, *48*, 811-814.

[16] See Supplementary Information.

[17] S. Matile, N. Sakai, *The Characterization of Synthetic Ion Channels and Pores. Analytical Methods in Supramolecular Chemistry* (Ed.: C. A. Schalley), Wiley, Weinheim, **2007**, 391-418.

[18] C. C. Tong, R. Quesada, J. L. Sessler, P. A. Gale, *Chem. Commun.* **2008**, *44*, 6321-6324.

[19] T. Takeuchi, J. Montenegro, A. Hennig, S. Matile, *Chem. Sci.* **2011**, *2*, 303-307.

[20] S. Bhosale, S. Matile, *Chirality* **2006**, *18*, 849-856.

[21] Chloride binding studies by ^{19}F NMR spectroscopy were not possible with TMACl because of mutually incompatible solubilities at higher concentrations. Successful binding studies with tetrabutylammonium chloride (TBACl) in dry acetone-d_6 have to be interpreted carefully because TBA is too large for central binding to the macrocycle,[15] a feature that is essential for transport. Moreover, binding studies were highly solvent dependent, with binding constants decreasing rapidly with traces of water in acetone.[7,23] K_D values were determined by nonlinear fitting of $\Delta\delta$ vs TBACl concentration as described in the literature.[9a,16]

[22] The synthesis of the *ortho*-isomer was not considered because the interior is too overcrowded for hosting and external chloride binding was already covered with **4**.

[23] Gas phase calculations were preferred because they presumably mimic the situation in the apolar core of the lipid bilayer more closely than results from competing polar solvents.[1,7,21] In general, computational results that fail to include the changing complex environments experienced during transport in lipid bilayers have to be considered with appropriate reservation. For the same reasons, efforts to characterize complexes in solid state[5,8-10] appeared less meaningful in the context of this study.

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Halogen Bonds

Andreas Vargas Jentzsch, Daniel Emery, Jiri Mareda, Pierangelo Metrangolo, Giuseppe Resnati and Stefan Matile*

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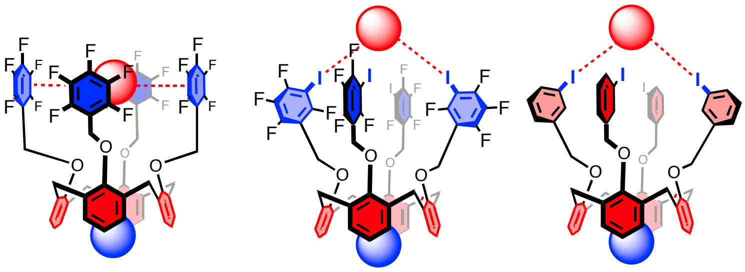

anion-π interactions

halogen bonds

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