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Dendritic Folate Rosettes as Ion Channels in Lipid Bilayers

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We report that π-stacked supramolecular rosettes[1] can form synthetic ion channels.[2] These esthetically pleasing supramolecules[1−5] can self-assemble from heteroaromatic "sectors" with directional hydrogen-bond donor−acceptor arrays such as folate **1**,[1,3] guanines (G),[4] and so on.[5] H-bond directed circular self-assembly of these sectors gives cyclic oligomers such as quartet **2**, which, in turn, may self-assemble on top of each other to give π-stacks such as **3** (Figure 1).[1] Templation of this process by cation binding to carbonyl lone pairs in the rosette centers accounts for the confirmed ionophoric properties of many π-stacked supramolecular rosettes in bulk liquid membranes (Figure 1).

It was recognized early on that the central string of cations and the hydrophobic periphery of ionophoric rosettes are reminiscent of an ion channel.[4] The compatibility of π-stack architecture with ion channel formation as well as π-stack chemistry such as intercalation and charge-transfer complexation with ion channel function has been demonstrated last year.[6] Compared to other sectors, π-stacked folate rosettes **3** were particularly promising candidates to form ion channels because they can exist in hydrophobic media with and without central ion templates.[1] Moreover, the surrounding hydrogen-bonded amide network, as well as the dendritic[7] periphery, may cushion[8] the dynamic rosette "breathing" motions[4c] during ion hopping along the central string of ions.

The synthesis of folate dendrimer **1** has been reported previously.[1b] Routine picrate extractions confirmed ionophoric properties of folate **1** in bulk chloroform membranes. The found folate−potassium ratio of 6.7:1 supported the expected[1,3,4] binding of about one cation between two rosettes (Figure 1).

Ion transport activity in lipid bilayers was explored first using EYPC-LUVs⊃HPTS[9] exposed to a pH gradient. In this classical assay,[6a] the ability of folate **1** to eliminate the applied proton gradient is reported as change in emission of the internal HPTS in response to external folate addition (Figure 2A). Dependence of activity on the concentration of folate monomer (c_M) showed saturation behavior with Hill coefficient $n = 0.8$ (Figure 2B). As elaborated elsewhere,[10] $n < 1$ can indicate the exergonic self-assembly of the active supramolecule and poor partitioning into the bilayer. Inactivity in the ANTS/DPX assay[8−10] under nearly identical conditions suggested that the "effective" inner diameter of π-stacked helical rosettes **3** is large enough to mediate the exchange of inorganic ions measured in the HPTS assay but too small to allow the translocation of molecules as large as ANTS or DPX (Figure 2B, ● vs ○).

Structural studies under reasonably relevant conditions revealed π-stacked helical rosettes **3** as active suprastructure and corroborated poor partitioning due to exergonic self-assembly. Namely, the addition of dendrimer **1** to a buffer without EYPC LUVs resulted

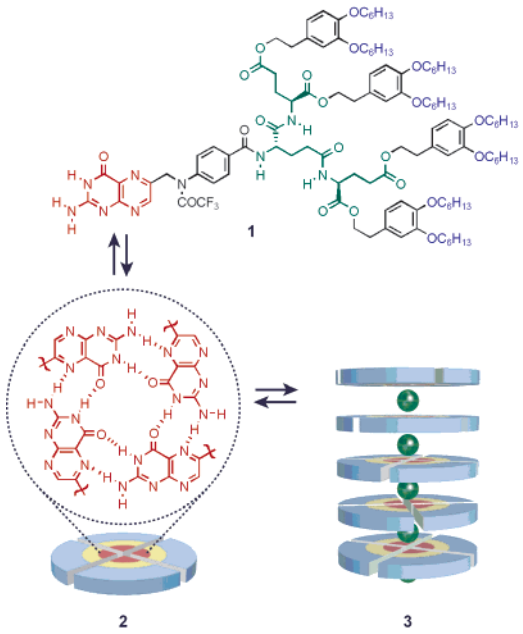

Figure 1. Self-assembly of folate **1** into rosette **2** and π-stack **3** with a central string of cations (green). The precise number of folates per ion channel is unknown.

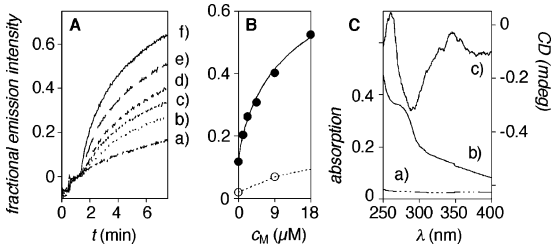

Figure 2. Activity (A, B) and structure (C) of folate rosettes **3** in spherical lipid bilayers. (A) Fractional change in HPTS emission I (λ_{ex} 450 nm, λ_{em} 510 nm) as a function of time during the addition of base (30 s, 20 μL of 0.5 M NaOH) and folate **1** (0 (a), 1.1 (b), 2.3 (c), 4.5 (d), 9 (e), and 18 μM (f)) to EYPC-LUVs⊃HPTS (65 μM EYPC, 10 mM HEPES, 100 mM NaCl, pH 7.0). (B) Dependence of channel activity in the HPTS assay (fractional emission 3 min after folate addition in A) on the concentration of monomer **1** with fit to Hill equation (●), compared to activity in the ANTS/DPX assay (○). (C) Absorption (a, b) and CD spectra (c) of **1** added to the buffer (a), or incorporated in EYPC LUVs (b, c).

in folate concentrations below the detection limit in the absorption spectrum (Figure 2Ca). However, detectable concentration of folate (Figure 2Cb) as well as the known exciton-coupled CD signature[1a] of π-stacked helical rosettes **3** (Figure 2Cc) could be readily observed when EYPC LUVs were prepared in the presence of folate **1**.

In planar bilayer conductance measurements,[6,8,10−14] the dendritic folate rosettes **3** could induce the appearance of distinct, remarkably

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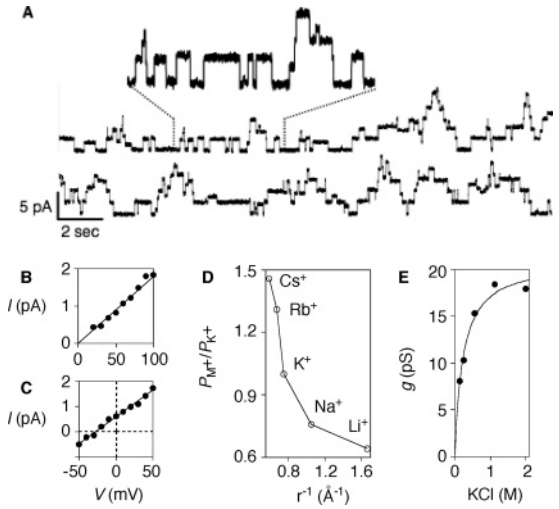

Figure 3. Activity of folate rosettes **3** in planar lipid bilayers. (A) Planar bilayer conductance in the presence of **1** (1 mol % in EYPC) at +100 mV (trans at ground) in 2 M KCl. (B) Single-channel *IV* profile of the most frequent (59%) out of three dominant conductance levels with linear curve fit in symmetric 2 M KCl. (C) Same in asymmetric 2 → 0.25 M KCl (cis→trans). (D) Permeability ratios for monovalent cations as a function of their reciprocal radius. (E) Single-channel conductance as a function of KCl concentration with curve fit to Michaelis−Menten equation.

homogeneous, and long-lived single-channel currents (Figure 3A). The ohmic behavior found in the current−voltage relationship (*IV* profile) was as expected for symmetric supramolecules such as rosette **3** (Figure 3B). Exposed to a salt gradient, rosette **3** revealed the expected preference for cations over anions (Figure 3C). A permeability ratio $P_{K^+}/P_{Cl^-} = 4.9$ was calculated[11] from the reversal potential $V_r = -27$ mV using the Goldman−Hodgkin−Katz equation. Similar analysis of reversal potentials obtained under M^+/K^+ gradients gave the cation selectivity topology (Figure 3D). The found Eisenman I sequence contradicts the known Na > K selectivity of folate aggregates[3] and implies dominance of cation dehydration energies,[12] that is the absence of strong and selective cation binding sites within stacked rosette **3**.[1,3,4] Similarly, dehydration energy is believed to account for K > Na selectivity of G quartets.[4d]

The ionophoric properties revealed by picrate extraction were confirmed by saturation of the single-channel conductance at high salt concentration (Figure 3E).[11,13] Michaelis−Menten analysis revealed that the expected blockage by the permeant cation occurred with an effective cation concentration $EC_{50} = 0.23$ M for K^+ with a maximal single-channel conductance of $g_{MAX} = 21$ pS. With blockage by the permeant cation,[13] application of Hille's equation to estimate the inner channel diameter from the single-channel conductance is meaningful only at concentrations well below the EC_{50}. The corrected[14] Hille[11] diameter $d_{Hille} = 3.7$ Å obtained from the estimated conductance at 100 mM KCl (Figure 3E) was consistent with the size of the central cavity of the folate quartet and compatible with size exclusion experiments in vesicles.

One of the scientifically attractive questions concerning ion channels formed by π-stacked supramolecular rosettes concerns cation hopping along the central string of ions. On the level of covalent macrocycles such as crown ethers or calixarenes,[2] this paradoxical "tunneling" of big ions through small holes is not possible. Because their dynamic nature allows for the required breathing, the possibility of central ion tunneling through supramolecular macrocycles exists and has been experimentally confirmed.[4c] The characteristics found for ion channels formed by stacked folate rosettes **3** are in support of the occurrence of such central, dynamic ion "tunneling." Namely, structural (CD) studies confirmed stacked rosettes as active channel structures. Single-channel homogeneity, conductance, selectivity, and particularly saturation by permeant cations are all in agreement with dynamic "tunneling" of big cations through small holes along the central string of ions, rather than through channels in the dendritic periphery. Nevertheless, structural variations to improve rosette partitioning will be necessary to fully appreciate and exploit the here reported breakthrough.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kamikawa, Y.; Nishii, M.; Kato, T. *Chem. Eur. J.* **2004**, *10*, 5942−5951. (b) Kato, T.; Matsuoka, T.; Nishii, M.; Kamikawa, Y.; Kanie, K.; Nishimura, T.; Yashima, E.; Ujiie, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 1969−1972.
(2) (a) Matile, S.; Som, A.; Sordé, N. *Tetrahedron* **2004**, *60*, 6405−6435. (b) Mitchell, K. D. D.; Fyles, T. M. In *Encyclopedia of Supramolecular Chemistry*; Atwood, J. L.; Steed, J. W., Eds.; Marcel Dekker: New York, 2004; pp 742−746. (c) Koert, U., Ed. Synthetic Ion Channels. *Bioorg. Med. Chem.* **2004**, *12*, 1277−1350. (d) Boon, J. M.; Smith, B. D. *Curr. Opin. Chem. Biol.* **2002**, *6*, 749−756. (e) Gokel, G. W.; Mukhopadhyay, A. *Chem. Soc. Rev.* **2001**, *30*, 274−286.
(3) Ciuchi, F.; Di Nicola, G.; Franz, H.; Gottarelli, G.; Mariani, P.; Bossi, M. G. P.; Spada, G. P. *J. Am. Chem. Soc.* **1994**, *116*, 7064−7071.
(4) (a) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668−698. (b) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060−4067. (c) Hud, N. V.; Schultze, P.; Sklenar, V.; Feigon, J. *J. Mol. Biol.* **1999**, *285*, 233−243. (d) Hud, N. V.; Smith, F. W.; Anet, F. A.; Feigon, J. *Biochemistry* **1996**, *35*, 15383−15390. (e) Ghoussoub, A.; Lehn, J. M. *Chem. Commun.* **2005**, *40*, 5763−5765. (f) An excellent study on unimolecular G-quartet ion channels prepared by covalent capture appeared during the reviewing process of this report: Kaucher, M. S.; Harrell, W. A.; Davis, J. T. *J. Am. Chem. Soc.* **2006**, *128*, 38−39.
(5) (a) ten Cate, M. G. J.; Crego-Calama, M.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2004**, *126*, 10840−10841. (b) Fyles, T. M.; Knoy, R.; Müllen, K.; Sieffert, M. *Langmuir* **2001**, *17*, 6669−6674. (c) Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37−44.
(6) (a) Talukdar, P.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2005**, *127*, 6528−6529. (b) Talukdar, P.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S. *Chem. Eur. J.* **2005**, *11*, 6525−6532.
(7) Tomalia, D. A.; Frechet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 2719−2728.
(8) Gorteau, V.; Bollot, G.; Mareda, J.; Pasini, D.; Tran, D.-H.; Lazar, A. N.; Coleman, A. W.; Sakai, N.; Matile, S. *Bioorg. Med. Chem.* **2005**, *13*, 5171−5180.
(9) (a) EYPC-LUVs⊃HPTS: Large unilamellar vesicles (LUVs) composed of egg yolk phosphatidylcholine (EYPC) and loaded with the pH-sensitive dye HPTS (8-hydroxy-1,3,6-pyrenetrisulfonate). (b) ANTS/DPX-assay: Efflux of fluorophore 8-amino-1,3,6-naphthalenetrisulfonate (ANTS) or quencher *p*-xylene-bis-pyridinium bromide (DPX) from EYPC-LUVs⊃ANTS/DPX[8,9] is monitored.
(10) Litvinchuk, S.; Bollot, G.; Mareda, J.; Som, A.; Ronan, D.; Shah, M. R.; Perrottet, P.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2004**, *126*, 10067−10075.
(11) Hille B. *Ionic Channels of Excitable Membranes*, 3rd ed.; Sinauer: Sunderland, MA, 2001.
(12) Eisenman, G.; Horn, R. *J. Membr. Biol.* **1983**, *76*, 197−225.
(13) Qu, Z.; Hartzell, H. C. *J. Gen. Physiol.* **2000**, *116*, 825−844.
(14) Smart, O. S.; Breed, J.; Smith, G. R.; Sansom, M. S. P. *Biophys. J.* **1997**, *72*, 1109−1126.

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