J|A|C|S
COMMUNICATIONS

Published on Web 11/22/2006

Adhesive π-Clamping within Synthetic Multifunctional Pores

Hiroyuki Tanaka, Svetlana Litvinchuk, Duy-Hien Tran, Guillaume Bollot, Jiri Mareda, Naomi Sakai, and Stefan Matile*

Department of Organic Chemistry, University of Geneva, Geneva, Switzerland

Received September 25, 2006; E-mail: stefan.matile@chiorg.unige.ch

We report the design, synthesis, and evaluation of multifunctional pores[1] with electron-deficient naphthalenediimide (NDI)[2] π-clamps[3] at their inner surface for molecular recognition by aromatic electron donor−acceptor interactions (i.e., **1**, Figures 1 and 2).[4] Stimuli-responsive synthetic pores attract increasing scientific attention because of their adaptable applicability in domains such as diagnostics (multicomponent sensing), drug discovery (inhibitor screening), and so on.[5] However, the molecular recognition motifs elaborated so far for synthetic pores focus on multiple ion pairing.[5,6] Here, we introduce π-clamping[3] by aromatic electron donor−acceptor interactions[2] as an attractive concept to complement molecular recognition within synthetic multifunctional pores with interactions that are (a) orthogonal to ion pairing[6] and (b) beyond reach with biological multifunctional pores.[7] In pore **1**, NDIs with their sticky, π-acidic surfaces[2,4] were selected as ideal abiotic amino acid side chains (π_A)[8] to "clamp" π-basic guest molecules (Figure 2). Flanking lysines (K) were added for assistance with ion pairing[3a,b] as in the previous pore **2** without π-acidic clamps.[9]

Pore **1** was synthesized in 24 overall steps. The synthesis of artificial NDI amino acids was as unproblematic as expected from previous reports in the literature.[4,8] Solution-phase peptide synthesis as well as coupling to the classical *p*-octiphenyl scaffold,[4,5,9] however, was successful only with the temporary introduction of bulky protecting groups for the primary alcohol in the NDI side chain to solubilize otherwise intractable synthetic intermediates. Detailed procedures are reported in the Supporting Information. To probe the functional relevance of π-clamping within pore **1**, we further synthesized the π-basic dialkoxynaphthalene (DAN) dihydrazide **3** and the π-acidic NDI dihydrazide **4** (Figure 2). Their reaction with ketones such as pyruvate or α-ketoglutarate provided rapid access to DAN and NDI blockers of variable size and charge such as **5**−**8** (Table 1).[10]

Pore **1** was characterized by fluorogenic dye efflux from egg yolk phosphatidylcholine large unilamellar vesicles that were loaded with 5(6)-carboxyfluorescein at concentrations high enough for self-quenching (EYPC-LUVs⊃CF).[5,9] In this assay, the activity of pore **1** is reported as an increase in CF emission in response to CF efflux (Figure 3A, solid line).[11] In the same assay, molecular recognition of blockers **5**−**10** by pore **1** is reported as a change in pore activity in response to chemical stimulation (Figure 3A, dotted line).[5,9] Decreasing pore activity in the resulting dose response curves (Figure 3B) identifies pore blockers and their IC_{50}, the characteristic blocker concentration that reduces pore activity to 50% (Table 1).

According to this method, pore **1** was sensitive to the presence of aromatic anions such as **5**−**10** (Table 1). The same pore did, however, not recognize the presence of the aliphatic control **11** despite comparable bulk and charge (Table 1). Direct comparison of aromatic and aliphatic α-ketoglutarate dihydrazones **7** and **11** revealed a more than 1000-fold increase in blocker efficiency in the presence of DAN (Table 1, entries 3 vs 7). Within the DAN series, blockage efficiency increased with blocker charge and size.

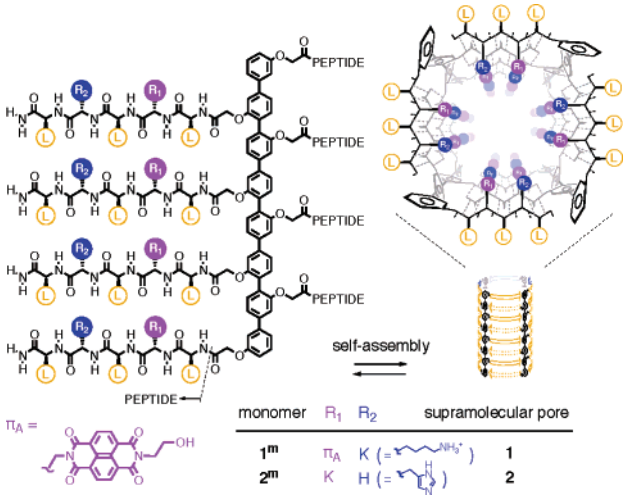


Figure 1. Self-assembly of the rigid-rod β-barrel pore **1** from monomers **1**^{m} and of the control pore **2** from monomers **2**^{m}. β-Sheets are shown as solid (backbone) and dotted lines (hydrogen bonds, top) or as arrows (N→C, bottom); external amino acid residues are dark on white, internal ones white on dark (single-letter abbreviations); see Figure 2 for full structure of β-sheets and π-clamps. All shown suprastructures may be considered as, at worst, simplifying but productive working hypotheses that are compatible with all reported experimental data on function (including ref 11), with molecular dynamics simulations,[12] and with extensive previous structural and functional evidence on the same motif[5,9] (including AFM images of polymers moving through pore **2**).[9b]

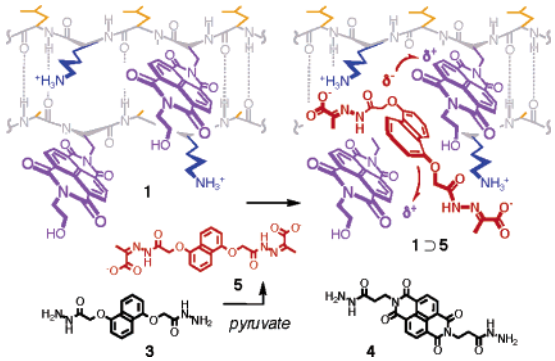


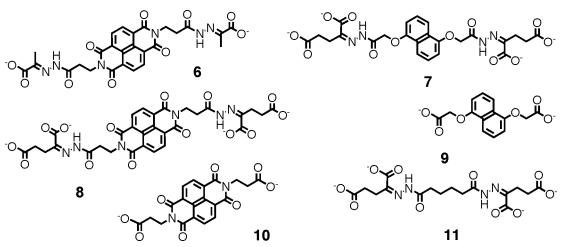
Figure 2. The concept of π-clamping within pore **1** exemplified with blocker **5**, made from hydrazide **3** and pyruvate, to give inclusion complex **1**⊃**5** with operational aromatic electron donor−acceptor interactions (red arrows; see Figure 1 for full structure of **1**).

Effective inhibitory concentrations reached from IC_{50} = 95 μM for the small DAN dianion **9** to IC_{50} = 240 nM for the large DAN tetraanion **7** (Table 1, entries 1−3).

To determine the specific contributions from π-clamping to molecular recognition within pore **1**, comparison with the clamp-free pore **2** was necessary. Clamping factors $\prod = IC_{50}$ (**2**)/IC_{50} (**1**) were introduced to identify negligible ($\prod \approx 1$) or significant ($\prod >> 1$) aromatic interactions.[2,3] As with the overall blocker activities,

10.1021/ja066886+ CCC: $33.50

Table 1. Blockage Data for Pores **1** and **2**[a]



	cpd	IC_{50} (**1**) (μM)[b]	IC_{50} (**2**) (μM)[b]	Π[c]	Π_{DA}[d]
1	**9**	94.7 ± 3.1	2043 ± 103	21.6	4.8
2	**5**[e]	4.5 ± 0.3	136.2 ± 5.8	30.3	3.9
3	**7**[e]	0.24 ± 0.02	9.8 ± 0.4	40.8	3.8
4	**10**	32.7 ± 0.9	146.7 ± 5.7	4.5	
5	**6**[e]	25.7 ± 1.5	196.7 ± 18.3	7.7	
6	**8**[e]	2.5 ± 0.19	26.9 ± 1.2	10.8	
7	**11**[e]	>100	n.d.		

[a] Determined from dose response curves for fluorogenic CF efflux from EYPC-LUVs⊃CF as in Figure 3A. [b] Blocker concentration required for 50% blockage of pore **1** or **2**. Data ± SE are the average value of at least three independent measurements. [c] Clamping factor $\Pi = IC_{50}$ (**2**)/IC_{50} (**1**). [d] Donor−acceptor factor $\Pi_{DA} = \Pi_D/\Pi_A$, i.e., Π_9/Π_{10} (entry 1); Π_5/Π_6 (entry 2); Π_7/Π_8 (entry 3). [e] Accessible from **3**, **4**, or adipic hydrazide and pyruvate or α-ketoglutarate.

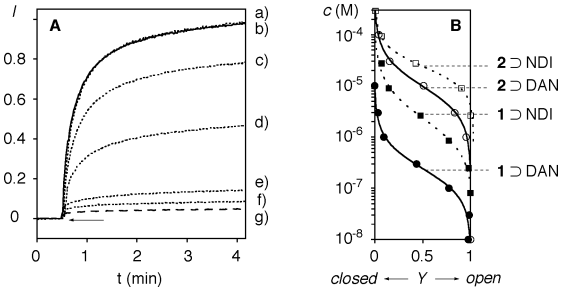


Figure 3. (A) Fractional change in CF emission I (λ_{ex} = 492 nm, λ_{em} = 517 nm) as a function of time after addition of **7** (0.01 (a), 0.03 (b), 0.1 (c), 0.3 (d), 1 (e), 3 (f), and 10 μM (g)) and **1**[m] (375 nM, arrow) to EYPC-LUVs⊃CF (~65 μM EYPC, 10 mM HEPES, 107 mM NaCl, pH 6.5). (B) Dependence of the fractional activity Y of pores **1** (●, ■) and **2** (○, □, both 375 nM monomer) on the concentration c of DAN **7** (●, ○) and NDI **8** (■, □).

clamping factors increased with blocker charge and size in both the DAN and the NDI series (Table 1). Increasing Π factors with increasing blocker charge suggested that proximal ion pairing strengthens rather than weakens π-clamping within pore **1**.

To further identify individual contributions from aromatic electron donor−acceptor interactions, direct comparison of individual IC_{50}'s was naturally insufficient. Contributions from other effects were, however, readily eliminated by comparing clamping factors for π-basic DAN blockers (Π_D) with those for the corresponding π-acidic NDI blockers (Π_A). Donor−acceptor factors $\Pi_{DA} = \Pi_D/\Pi_A$ were introduced to identify absence ($\Pi_{DA} = 1$) or presence ($\Pi_{DA} > 1$) of this eventual "adhesiveness" of π-clamping within pore **1**. Gratifyingly, clamping factors Π for π-basic DAN blockers (Π_D) exceeded those for π-acidic NDI blockers (Π_A) without exception. The found $\Pi_{DA} \approx 4$ values were all significant and roughly independent of blocker charge and size (Table 1, entries 1−3).

In research that focuses on the creation of advanced function, experimental evidence for that function seems to be all that really matters. For π-clamping within synthetic pores, this essential line of evidence consists of (a) similar activity of pores with and without π-clamps, (b) different blockage efficiency with and without π-clamps ($\Pi > 1$), and increasing clamping factors Π with (c) increasing blocker charge (supportive ion pairing), and (d) increasing aromatic electron donor−acceptor interactions ("adhesiveness" $\Pi_{DA} > 1$). Preliminary results confirm that the availability of synthetic pores that respond to chemical stimulation other than ion pairing will greatly expand their practical usefulness as sensors and beyond.[10,12]

Acknowledgment. We thank D. Jeannerat, A. Pinto, and S. Grass for NMR measurements, P. Perrottet and the group of F. Gülaçar for MS, and the Swiss NSF for financial support.

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

References

(1) Selected reviews on synthetic ion channels and pores: (a) Scrimin, P.; Tecilla, P. *Curr. Opin. Chem. Biol.* **1999**, *3*, 730−735. (b) Gokel, G. W.; Mukhopadhyay, A. *Chem. Soc. Rev.* **2001**, *30*, 274−286. (c) Koert, U.; Al-Momani, L.; Pfeifer, J. R. *Synthesis* **2004**, *8*, 1129−1146. (d) Matile, S.; Som, A.; Sordé, N. *Tetrahedron* **2004**, *60*, 6405−6435. (e) Fyles, T. M. *Chem. Soc. Rev.* **2006**, in press. (f) Davis, A. P.; Sheppard, D. N.; Smith, B. D. *Chem. Soc. Rev.* **2006**, in press. (g) Sisson, A. L.; Shah, M. R.; Bhosale, S.; Matile, S. *Chem. Soc. Rev.* **2006**, in press.

(2) Aromatic donor−acceptor interactions with NDIs: (a) Lokey, R. S.; Iverson, B. L. *Nature* **1995**, *375*, 303−305. (b) Vignon, S. A.; Jarrosson, T.; Iijima, T.; Tseng, H. R.; Sanders, J. M. K.; Stoddart, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 9884−9885. (c) Mukhopadhyay, P.; Iwashita, Y.; Shirakawa, M.; Kawano, S.; Fujita, N.; Shinkai, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1592−1595.

(3) Synthetic π-clamps (or tweezers, clips, clefts, jaws): (a) Butterfield, S. M.; Sweeney, M. M.; Waters, M. L. *J. Org. Chem.* **2005**, *70*, 1105−1114. (b) Schneider, H.-J.; Tianjun, L.; Sirish, M.; Malinovski, V. *Tetrahedron* **2002**, *58*, 779−786. (c) Klärner, F. G.; Kahlert, B. *Acc. Chem. Res.* **2003**, *36*, 919−932. (d) Chen, C.-W.; Whitlock, H. W. *J. Am. Chem. Soc.* **1978**, *100*, 4921−4922. (e) Zimmerman, S. C.; Saionz, K. W. *J. Am. Chem. Soc.* **1995**, *117*, 1175−1176. (f) Rowan, A. E.; Elemans, J. A. A. W.; Nolte, R. J. M. *Acc. Chem. Res.* **1999**, *32*, 995−1006. (g) Charlton, A. T.; Davis, A. L.; Jones, D. P.; Lewis, J. R.; Davis, A. P.; Haslam, E.; Williamson, M. P. *J. Chem. Soc., Perkin Trans. 2* **2000**, 317−322. (h) Sun, D.; Tham, F. S.; Reed, C. A.; Chaker, L.; Boyd, P. D. W. *J. Am. Chem. Soc.* **2002**, *124*, 6604−6612. (i) Kerckhoffs, J. M.; ten Cate, M. G.; Mateos-Timoneda, M. A.; van Leeuwen, F. W.; Snellink-Ruel, B.; Spek, A. L.; Kooijman, H.; Crego-Calama, M.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2005**, *127*, 12697−12708.

(4) Different to the present use to close large pores formed by rigid-rod β-barrels, DAN−NDI interactions have been used previously to open up helical rigid-rod π-stacks into small ion channels: (a) Talukdar, P.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2005**, *127*, 6528−6529. Different to the sensing applications of the present β-barrel architecture,[5,10] this conceptually complementary π-stack architecture is of interest for artificial photosynthesis: (b) Bhosale, S.; Sisson, A. L.; Talukdar, P.; Fürstenberg, A.; Banerji, N.; Vauthey, E.; Bollot, G.; Mareda, J.; Röger, C.; Würthner, F.; Sakai, N.; Matile, S. *Science* **2006**, *313*, 84−86.

(5) (a) Das, G.; Talukdar, P.; Matile, S. *Science* **2002**, *298*, 1600−1602. (b) Sakai, N.; Mareda, J.; Matile, S. *Acc. Chem. Res.* **2005**, *38*, 79−87. (c) Das, G.; Matile, S. *Chem.—Eur. J.* **2006**, *12*, 2936−2944.

(6) This limitation is particularly problematic for electric rather than optical detection, where high ionic strength is essential to observe high single-channel currents.

(7) (a) Deamer, D. W.; Branton, D. *Acc. Chem. Res.* **2002**, *35*, 817−825. (b) Astier, Y.; Braha, O.; Bayley, H. *J. Am. Chem. Soc.* **2006**, *128*, 1705−1710.

(8) NDI amino acids: (a) Mokhir, A. A.; Krämer, R. *Bioconjugate Chem.* **2003**, *14*, 877−883. (b) Ashkenasy, N.; Horne, W. S.; Ghadiri, M. R. *Small* **2006**, *1*, 99−102.

(9) (a) 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. (b) Kumaki, J.; Yashima, E.; Bollot, G.; Mareda, J.; Litvinchuk, S.; Matile, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 6154−6157.

(10) π-Clamping of hydrazide blockers is of interest for the multianalyte sensing of aldehydes and ketones in complex matrixes with synthetic multifunctional pores.[5]

(11) Dependence of activity on monomer concentration and pH revealed highly active (EC_{50} = 25 nM), thermodynamically unstable, base-sensitive and acid-insensitive tri- to tetrameric pores (Hill coefficient n = 3.5, compare ref 9a). Single-channel conductance measurements in planar bilayer membranes revealed a quite heterogeneous mixture including high-conducting, either very short-lived or very long-lived pores reminiscent of an Engelman two-state formation process.[12]

(12) Results referred to in ref 11, and the mentioned molecular dynamics simulations will be reported in the full paper on the topic.

JA066886+