vesicles can be extrapolated to other catecholaminergic vesicles,[25] vesicles containing less than about 20000 molecules and with radii less than about 25 nm (such as small synaptic vesicles) should never reach full fusion and thereby be released only through their fusion pores. Conversely, larger ones (such as large, dense core vesicles) should always fully fuse unless another mechanism closes the pore before about 20000 molecules have been released. It is interesting that these values correspond precisely to the molecule content ($\sim$10000) and size (20–30 nm) of many catecholaminergic neuronal vesicles. In this perspective, these vesicles would represent the optimum balance between a nonfusional release and a maximum load of neurotransmitter.

*Experimental Section*

All experiments and procedures were identical to those previously described, either for the stimulation of chromaffin cells and monitoring of chronoamperometric traces,[26, 27] or for the deconvolution of the chronoamperometric data.[1, 16] In this study we treated 596 representative events. All events were used in the kinetic treatment of the second half of the full-fusion stage. However, to perform a significant kinetic treatment of the initial half of full fusion, several of these events could not be used, either because insufficient data points ($<5$) were available in the time range of interest or because residual foot features altered the spike rise. This reduced the series to 232 meaningful events, but the data presented here are consistent with all the discarded events.

Received: October 27, 1999 [Z14189]
Revised: February 10, 2000

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[23] The use of a simple pore model in our treatment neglects the fact that the connection of the cell and vesicle membranes creates a "bottle neck" invagination. Therefore, a negative additional energy (termed the Gaussian curvature energy; see, for example: U. Seifert, R. Lipowsky in *Structure and Dynamics of Membranes* (Eds.: R. Lipowsky, E. Sackmann), Elsevier, Amsterdam, **1995**, pp. 409–411, must be added to $W(r)$. The modulus of Gaussian curvature energies is predicted to be about 300 $k_BT$, which compares excellently with the mean value of $E_0 \approx 120\ k_BT$ obtained here. Note that considering that the edge energy of the initial fusion pore is 2.5 $k_BT$, as is implicitly assumed here, would afford exactly $E_0 = 300\ k_BT$.
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## Giant Artificial Ion Channels Formed by Self-Assembled, Cationic Rigid-Rod $\beta$-Barrels**

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*Dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday*

The interior of toroidal biomacromolecules is a privileged site for molecular recognition, translocation, and transformation.[1–4] Giant ion channels (or nanopores) are a particularly

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[**] We thank Dr. E. Buck (Warner Instrument Corp., Hamden, CT) and R. Marclay (University of Geneva) for installation of a BLM workstation, Dr. E. Buck for BLM instructions, A. Pinto, J.-P. Saulnier, the group of Prof. Gülaçar, and Dr. H. Eder for NMR, MS, and elemental analyses, respectively (University of Geneva), and the Swiss NSF and Suntory Institute for Bioorganic Research (SUNBOR Grant) for financial support.

  0570-0833/00/3911-1955 $ 17.50+.50/0 

appealing class of toroidal biomacromolecules because of the additional spatial compartmentalization provided by the bilayer membrane, which gives vectorial accessibility to their confined interior and allows rapid stochastic detection of intratoroidal processes.[5–7] This unique feature has stimulated inspired expansions of intratoroidal chemistry beyond biological function and has culminated so far in stochastic recognition of organic molecules[5] and oligonucleotides.[6] The conceivable intriguing perspectives for future stochastic intratoroidal chemistry make the construction of synthetic nanopores highly attractive. It seemed, however, questionable whether or not large intratoroidal space of variable chemical nature could be established using small synthetic molecules[8–17] instead of biomacromolecules.[5–7] Here, we report direct experimental evidence for the first synthetic toroidal supramolecule capable of providing a) large, b) transmembrane, and c) functionalized intratoroidal space of d) remarkable stability.

The design of intratoroidal "nanospace" was based on electrostatic repulsion at the internal surface of rigid-rod $\beta$-barrels (Figure 1). These very recently devised synthetic

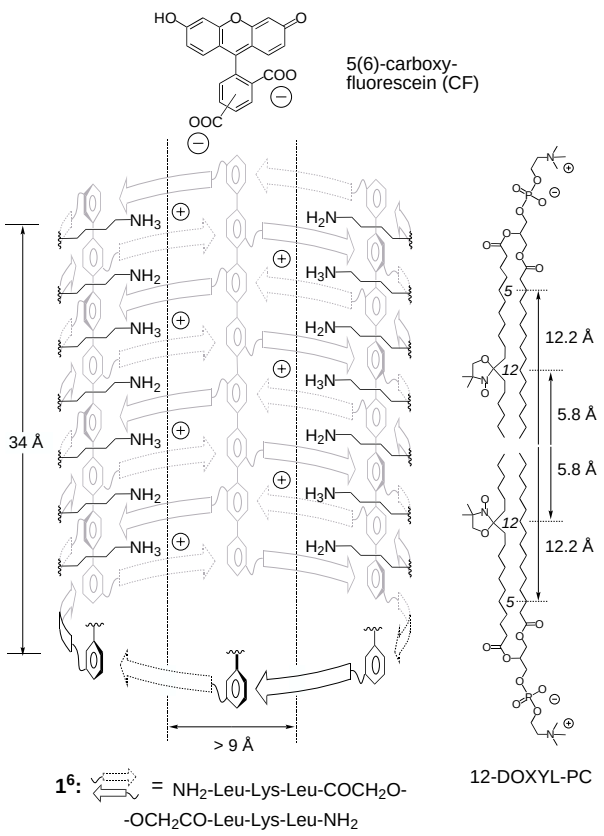
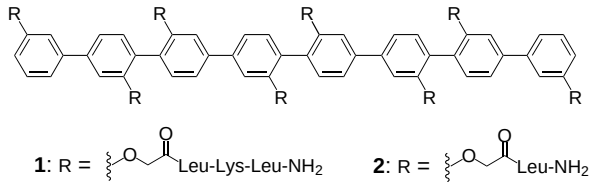



Figure 1. Schematic structure of rigid-rod $\beta$-barrel **1**$^6$ formed by Leu-Lys-Leu-rods **1** in EYPC bilayers and the probes used to determine the transmembrane barrel orientation, barrel size, and internal diameter.[27] The distribution of intratoroidal amines and ammonium cations in **1**$^6$ is unknown and depicted arbitrarily to illustrate the concept.

nanobarrels[1, 17] are composed of preorganizing rigid-rod scaffolds[18–20] that are connected through short, antiparallel $\beta$-sheets[16, 21–24] formed by interdigitating lateral peptide strands. To assure supramolecular organization, despite the internal destructive interactions introduced to maintain central space, overcompensating constructive interactions were needed at the hydrophobic exterior. In rigid-rod $\beta$-barrel **1**$^6$ this situation is created by repulsive, partially protonated Lys-residues[21–24] at the inner and attractive hydrophobic Leu residues at the outer surface of the barrel.

We thus synthesized Leu-Lys-Leu-rod **1** using our previously established, general method for the preparation of peptide-rods.[1] The CD spectrum of **1** in uniformly-sized EYPC-SUVs was indicative of the formation of rigid-rod $\beta$-barrels.[1, 17, 25] A similar CD spectrum obtained in $CHCl_3$ indicated the presence of a similar supramolecular organization of **1**, but poor solubility prevented further structural studies by less sensitive methods.[17, 1] In clear contrast to the previously described self-assembly of Leu-rod **2** into ionophoric "minimalist" $\beta$-barrels **2**$^2$ no evidence for $\beta$-barrel **1**$^6$




was found in other solvents and by elctrospray ionization mass spectrometry (ESI-MS).[17] This observation is also in excellent agreement with the operation of destructive internal electrostatic interactions.

The interaction of rigid-rod $\beta$-barrel **1**$^6$ with hydrophobically matching EYPC bilayers was studied by fluorescence depth quenching; this method provides the most precise information on binding to and orientation in bilayer membranes at relevant concentrations of around 1 μM or less.[26–30] For orientational studies using depth quenching, the emission intensities of fluorophore **1** were measured in EYPC-SUVs containing either 8.7 % of 12-DOXYL-PC or 5-DOXYL-PC. These labeled membranes thus carry quencher $Q_1$ and $Q_2$ at defined distances $d_1$ (5.8 Å) and $d_2$ (12.2 Å) from the center of the EYPC bilayer, respectively (Figure 1).[26, 27] Since collisional quenching is proportional to the average distance between the fluorophore and the quencher,[26, 27] a reduced quenching efficiency of $Q_1$ relative to $Q_2$ ($Q_1 < Q_2$) indicates the location of the fluorophore near the membrane surface.[28, 29] On the other hand, $Q_1 > Q_2$ is consistent with a central location,[28] while $Q_1 \sim Q_2$ directly reveals a transmembrane orientation in the unique case of rigid-rod fluorophores.[29, 30] The latter situation ($Q_1 \sim Q_2$, Figure 2a) was found for **1** (namely, rigid-rod $\beta$-barrel **1**$^6$, Figure 1). The changes of oligo(*p*-phenylene) emission as a function of relative concentrations and time further revealed that about sixty lipids per rod **1** are needed to assure complete incorporation (Figure 2c) and that this process is finished in less than a minute (Figure 2b).[29]

These findings provide a concise description of the interaction of polyamine **1** with lipid bilayers and may further contribute to clarifying the question as to whether or not polyamines in general are capable of spanning bilayer membranes or rather act by supramolecular disorganization

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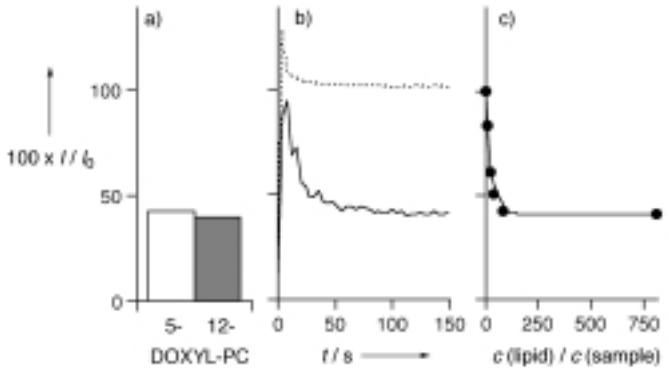


Figure 2. Fluorescence quenching of the emission intensity of oligo(*p*-phenylene) **1** in spin-labeled EYPC-SUVs ($\lambda_{ex} = 328$ nm, $\lambda_{em} = 390$ nm, 10 mM potassium phosphate buffer, 100 mM KCl, pH 6.4). a) Emission intensities ($I/I_0$) of **1** (100 nM) with 0.25 mM of EYPC-SUVs containing 8.7% 5- and 12-DOXYL-PC ($I$) relative to identical measurements with unlabeled EYPC-SUVs ($I_0$). b) Time course of the emission intensity of **1** (100 nM) after addition to 0.25 mM of unlabeled (- - - -) and 12-DOXYL-PC-labeled EYPC-SUVs (——). c) Dependence of the quenching of **1** by 12-DOXYL-PC as a function of lipid/rod ratio at constant lipid concentration.[29]

at the membrane/water interface.[8, 30, 31] Namely, the above direct orientational evidence proved that the permanent location of polyamines within the hydrophobic core of bilayer membranes is possible at biologically relevant concentrations.

Insights into the chemical nature of the intratoroidal space of barrel **1**$^6$ were obtained using EYPC-SUVs loaded with self-quenching 5(6)-carboxyfluorescein (CF).[29] Expanded $\beta$-barrels **1**$^6$ (but not contracted $\beta$-barrels **2**$^2$)[17] were capable of mediating rapid CF-efflux at nanomolar concentrations as judged from the increase in the CF-emission intensity as a function of time (Figure 3). Facile efflux of anionic fluorophores of more than 9 Å minimal diameter implied the formation of at least hexameric barrels ($d \sim 20$ Å; Cerius2/Insight II Discover)[1] with a cationic interior.

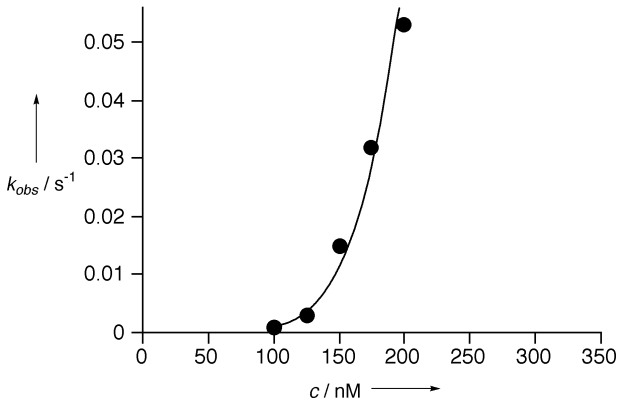


Figure 3. Observed rate constants of CF-efflux as a function of the concentration of oligo(*p*-phenylene) **1** (EYPC-SUVs, 10 mM HEPES, 50 mM $CF_{in}$, 10 mM $NaCl_{in}$, 107 mM $NaCl_{out}$, pH 7.4). The solid line represents the best fit curve ($n = 6.1 \pm 0.7$). Efflux rates were obtained from observed changes in fluorescence intensity $(I_t - I_0)/(I_\infty - I_0)$ of EYPC-SUV-entrapped CF as a function of time ($\lambda_{em} = 514$ nm, $\lambda_{ex} = 492$ nm).

Kinetic information on transmembrane ion flux directly relates to the suprastructure of the mediating nanopore. Namely, the dependence of the CF-efflux rates on pore concentration is given by Equation (1), where $K_D$ is the dissociation constant of supramolecule **1**$^n$ composed of $n$ monomers **1**, $k_0$ the rate constant for CF-efflux in the absence of **1**, and $k_{int}$ an intrinsic rate constant.[8]

$$k_{obs} = k_0 + k_{int}[\text{monomer}]^n/K_D \quad (1)$$

Application of Equation (1) to CF-efflux mediated by rigid-rod $\beta$-barrel **1**$^n$ revealed a sixth-order dependence of $k_{obs}$ on the concentration of peptide-rod **1** (Figure 3). This corroborated the self-assembly of **1** into active hexamers, namely, the rigid-rod $\beta$-barrel **1**$^6$ (Figure 1).

The presence of rigid-rod $\beta$-barrel **1**$^6$ in black lipid membranes (EYPC-BLMs) caused the fluctuating appearance of single channel currents of 178 pA at $-50$ mV applied voltage (Figure 4).[32] Their unusually high magnitude is consistent with a minimal diameter of nanopore **1**$^6$ that is in the range of the paradigmatic natural $\beta$-barrel formed by $\alpha$-hemolysin, a self-assembled polypeptide containing 293 amino acids.[5–7] The "infinite" lifetime of these high-conductance, supramolecular synthetic nanopores exceeded those of the best low-conductance, unimolecular artificial ion channels by far.[14] Together with the absence of transient "bursts" frequently seen with artificial ion channels,[12, 13, 15, 33] this result revealed the remarkable stability of the roughly ohmic nanopore **1**$^6$.

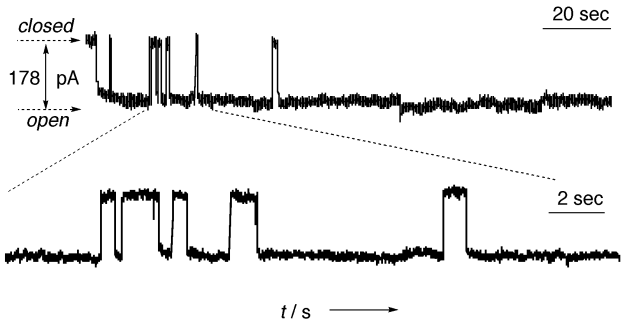


Figure 4. Conductance of EYPC-BLMs at $-50$ mV as a function of time in the presence of oligo(*p*-phenylene) **1**.

In summary, a combination of the unique advantages of self-assembled, rigid-rod oligo(*p*-phenylene)s with tunable electrostatic repulsion was used to design artificial nanopores of remarkable stability and intratoroidal amine/ammonium functional groups. Direct evidence for the stoichiometry, transmembrane orientation, large internal diameter, and long lifetime of rigid-rod $\beta$-barrels **1**$^6$ was obtained under comparable experimental conditions. These superb properties strongly suggest that future ion-channel engineering within functionalized intratoroidal space will not be restricted to biomacromolecules.

*Experimental Section*

Leu-Lys-Leu-rod **1** was obtained from tripeptide H-Leu-Lys(Boc)-Leu-$NH_2$ (Boc = *tert*-butoxycarbonyl) that was made in four steps (overall 23%) from commercial amino acid derivatives (Bachem, Calbiochem-Novabiochem) as described for other sequences.[1] H-Leu-Lys(Boc)-Leu-

$NH_2$ was coupled with benzotriazole-1-yl-oxy-tris(pyrrolidinophosphonium)hexafluorophosphate/*N*,*N*-diisopropylethylamine to the octiphenylene scaffold (55%) and deprotected with trifluoroacetic acid to give **1** (quant.) following previously described protocols without significant changes.[1] Selected data: H-Leu-Lys(Boc)-Leu-$NH_2$: $[\alpha]_D^{20} = -29.5$ ($c = 1.00$ in MeOH); m.p. 179.5–180.1 °C; elemental analysis calcd for $C_{23}H_{45}N_5O_5$: C 58.57, H 9.62, N 14.85; found: C 58.47, H 9.65, N 14.71. Leu-Lys(Boc)-Leu-rod (**1**-Boc): ESI-MS (MeOH): *m*/*z* (%): 1633 (100) $[M+3Na]^{3+}$, 2438 (85) $[M+2Na]^{2+}$. Leu-Lys-Leu-rod (**1**): CD (EYPC-SUVs, pH 6.4): $\lambda_{max}$ [nm] ($\Delta\varepsilon_{max}$ [$M^{-1}cm^{-1}$]) = 333 (+6.4), 307 (−5.1), 252 (−3.7), 237 (+32.6), 215 (−56.0); ESI-MS (MeOH): *m*/*z* (%): 1008 (100) $[M+4H]^{4+}$, 1344 (13) $[M+3H]^{3+}$. Before use, spectroscopically pure **1** was repurified by RP-HPLC (YMC pack ODS-A, 10 × 250 mm coupled with a Jasco PU-980 pump and a Jasco UV-970 UV/Vis detector, acetonitrile: methanol:trifluoroacetic acid = 49.5:49.5:1, $R_t = 4.10$ min) until constant activity was confirmed (dye efflux).

Fluorescence depth quenching (Figure 2) and dye efflux (Figure 3) were performed as described.[29] To measure the conductances, a bilayer of EYPC was formed by painting a solution of EYPC (Northern Lipids Inc.) in *n*-decane (33 mg $mL^{-1}$) containing 0–0.5 mol% of **1** on an orifice ($d = 150$ μm) in a polystyrene cup separating two chambers of a bilayer apparatus (BCH-13, Warner Instrument Corp.). These chambers contained 1 mL saline buffer (5 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 2 M NaCl, pH 7.4), a magnetic stirring bar, and a glass KCl (1M) agar bridge connection to Ag/AgCl electrodes. Oligophenylenes **1** (0.8 mmol in MeOH, 0–30 μL) were added to the stirred *cis* compartment. Formation of EYPC-BLM in the presence of **1**, and addition of **1** to final EYPC-BLM gave comparable results. Currents were recorded at different holding potentials (*trans* at $I = 0$) in a home-made Faraday cage with a bilayer clamp amplifier (BC-525c, Warner Instrument Corp.), low-pass filtered with an 8-pole Bessel filter at 1 kHz (LPF-8, Warner Instrument Corp.), converted (Digipack 1200-2, Axon Instruments), and sampled at 2 kHz by computer. Data were analyzed with pClamp 8.0 software (Axon Instruments).

Received: January 17, 2000 [Z14548]


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## A Novel Catalytic System for the Mannich-Type Reaction of Silyl Enolates: Stereoselective Synthesis of β-Aminoketones**

Katsukiyo Miura, Kentaro Tamaki, Takahiro Nakagawa, and Akira Hosomi*

The Lewis acid promoted addition of ester silyl enolates to imines and iminium salts enables the stereoselective construction of β-aminoesters and β-lactams.[1] However, it is not widely known that the Mannich-type reaction with ketone silyl enolates reveals high stereoselectivity in terms of the relative configuration between the newly formed chiral centers.[2, 3] Here, we describe that dimethylsilyl (DMS) enolates react with *N*-sulfonylimines in the presence of $H_2O$ and a catalytic amount of a base to afford β-aminoketones with high levels of diastereoselectivity.

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[**] Studies on Organosilicon Chemistry, Part 148. This work was partly supported by Grants-in-Aid for Scientific Research, Grants-in-Aid for Scientific Research on Priority Areas No. 706 (Dynamic Control of Stereochemistry) from the Ministry of Education, Science, Sports, and Culture, Japan, by Hitachi Chemical Industries, Inc., and by Pfizer Pharmaceuticals, Inc. Part 147: H. Ito, A. Hosomi, *J. Syn. Org. Chem. Jpn.* **2000**, *58*, in press.

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