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Metal–Organic Transmembrane Nanopores

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Supporting Information

ABSTRACT: A stable tetraporphyrin metallacycle with Re(I) corners (**1**) is capable of forming nanopores in a liposomial membrane, provided that the porphyrin units are properly functionalized with peripheral carboxylic acid residues that, by establishing an hydrogen bond network, allow the formation of dimers that span the depth of the membrane.

Transmembrane molecular nanopores have attracted considerable attention for their potential application in several fields, ranging from sensing to catalysis.[1] Natural nanopores are normally made by complex protein architectures, with α-Hemolysin (αHL) being a well-known and representative example.[2] This bacterial toxin—a monomeric, 293-residue, polypeptide—forms robust and wide (about 2 nm inner diameter) heptameric pores in lipid bilayers that allow permeation of large molecules and ions showing high electrical conductance.[3] Functionalization of the inner walls of this pore led to systems capable of sensing diverse substrates, ranging from transition metal ions to biologically relevant molecules such as DNA.[4] Taking inspiration from the natural examples, the classical approach to synthetic ion channels is based on the self-assembling of cyclic or linear oligomers.[5] However, only few systems capable of forming large and stable pores are known.[6] The most prominent example is the barrel-stave pore formed by the self-assembling of *p*-octiphenyl based monomers described by Matile and co-workers.[7] The size and stability make this artificial channel really competitive with the natural ones, whereas the synthetic tunability of its inner chemical space allowed to design several catalytically active or stimuli-responsive systems.[8]

To this respect, the possibility that the walls of the pore contain porphyrin moieties is particularly attractive as these molecules, beside defining a relatively large, rigid and flat aromatic surface, can be easily functionalized at the periphery as well as metallated at the inner core. Recently, Kobuke and co-workers described a tris-porphyrin covalent system that self-assembles to form a cylindrical shaped macrocycle with an inner diameter and a height of about 2.1 nm.[9] The upper and lower rims of the macrocycle are decorated with carboxylic acid residues that promote a hydrogen-bonding driven dimerization in the lipid bilayer resulting in a large transmembrane pore.

The metal-mediated directional-bonding approach is a convenient alternative to the covalent one for the construction of complex and functional molecular architectures.[10] Indeed, the combination of appropriate metal and organic fragments allows for a precise control of the geometry, shape, kinetic and thermodynamic stability of the resulting metal–organic framework.[11] However, coordination chemistry has been used only occasionally for the design of synthetic ion channels and pores and there are very few reports on the application of the so called "Fujita-Stang" motif in this field.[12] With the exception of the copper based metal–organic polyedra reported by Kim,[13] in all the systems so far investigated by the groups of T. M. Fyles and S. J. Webb, the metal ion used is Pd(II), which gives kinetically labile complexes.[14] Usually, this feature ensures that the thermodynamically most stable adduct is formed, but in the complex membrane environment, in which the concentration of the different species is influenced by their partition between bulk water and membrane, this may lead to complex mixtures of species with different stoichiometry. For example, recently, Webb and co-workers reported ionophoric activity in the presence of a bis(*meso*-3-pyridyl)porphyrin and Pd(II) in liposomes.[14b] However, it was impossible to identify the active species among the different linear and cyclic adducts that may form upon self-assembly of the porphyrin with the palladium center.

On this ground, and taking inspiration from the work of Kobuke, we decided to investigate the formation of transmembrane nanopores using stable metal-mediated macrocycles of porphyrins. In our design, we chose a 10,20-*meso*-dipyridylporphyrin, a linear ligand that upon binding to a *cis*-coordinating metal fragment forms a 4 + 4 metallacycle about 2 nm in size. As corners we used low spin d^6 *fac*-$\{Re^{I}Br(CO)_3\}$ metal fragments that are known to form kinetically inert and thermodynamically stable bonds with pyridyl ligands.[15] The neutral metallacycle is therefore expected to be robust enough as to preserve its structure intact also in the phospholipid membrane, avoiding ligand exchange or scrambling. Moreover, the bulky substituents on the two other *meso* positions of each porphyrin hinder the rotation around the metal–pyridine bond ensuring an average parallel disposition of the four tetrapyrrolic rings, thus roughly defining an empty cube that spans ca. half of the thickness of the phospholipid bilayer. Therefore, to promote an efficient dimerization of the metallacycle and, thus, the formation of a robust transmembrane nanopore, each porphyrin unit was equipped with two peripheral 4-carboxyphenyl groups at the 5,15-*meso* positions. In the resulting tetraporphyrin metallacycle **1**, four carboxylic groups should be pointing upward and four downward, therefore, assisting a hydrogen-bonding driven dimerization of the metallacycle once it is inserted in the lipophilic membrane (Figure 1). Here, we report on the synthesis, characterization, and ionophoric properties of **1** in comparison with its methyl-ester analogue **2** (Scheme 1).

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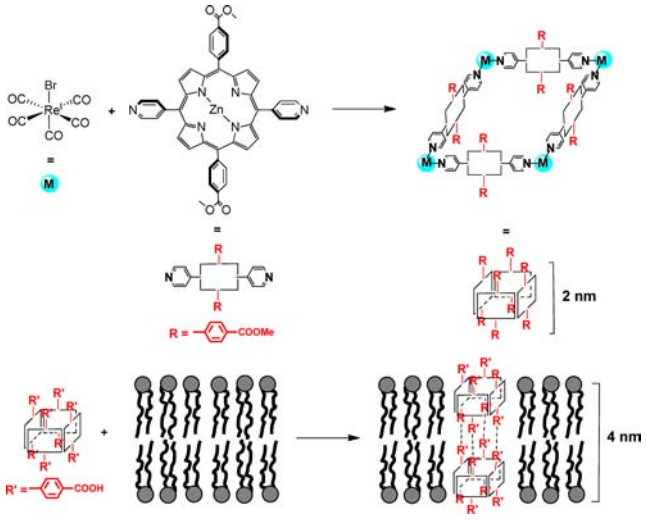
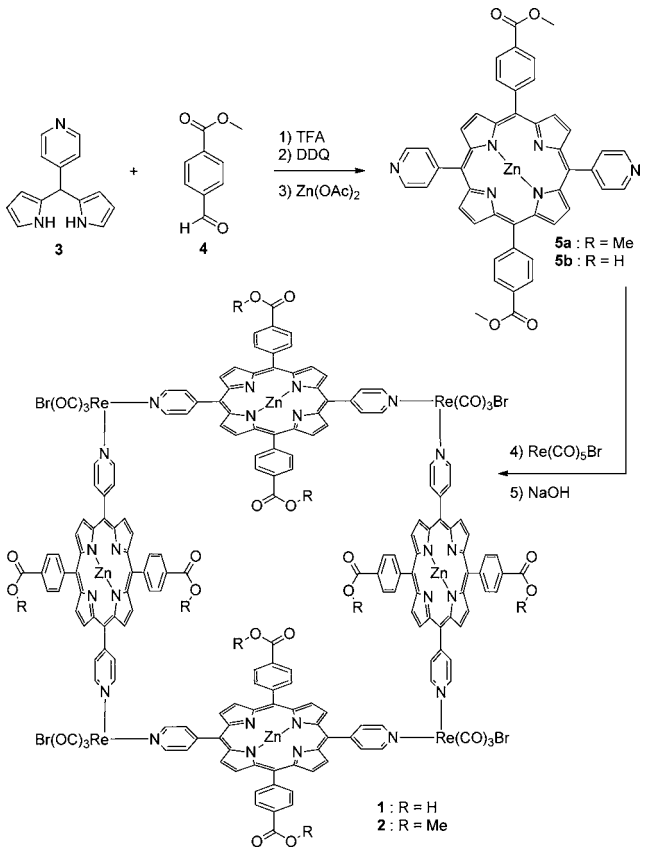


Figure 1. Schematic representation of the porphyrin metallacycle and formation of a transmembrane nanopore upon hydrogen-bonding driven dimerization.

Scheme 1. Synthesis of the Tetraporphyrin Metallacycle 1[a]



[a]Conditions: (1) trifluoroacetic acid (TFA, 43 equiv), dichloromethane (DCM), 0 °C, 20 min; (2) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2 equiv), rt, 1 h (39% from **3**); (3) $Zn(OAc)_2$ (3.2 equiv), $CHCl_3$, rt, overnight (93%); (4) $Re(CO)_5Br$ (1 equiv), THF/toluene 4:1, reflux, 48 h; (5) NaOH (8 equiv), THF/methanol 2:1, rt, overnight (48%).

The synthesis of the tetraporphyrin metallacycle **1** started with the preparation of the 5,15-bis(4-carboxymethylphenyl)-10,20-dipyridylporphyrin. This was obtained in good yield (39 %) from the 5-(4-pyridyl)dipyrromethane (**3**) and methyl 4-formylbenzoate (**4**) under optimized conditions using an excess of TFA, followed by oxidation with DDQ. The porphyrin was then treated with Zn(II) acetate to obtain the [5,15-bis(4-carboxymethylphenyl)-10,20-bis(pyridyl)porphinato]zinc(II) (**5a**). The synthesis of **1** followed the procedure reported by Hupp and coworkers for similar assemblies.[16] Porphyrin **5a** was treated with $ReBr(CO)_5$ in a 4:1 tetrahydrofuran/toluene mixture under reflux for 48 h. After purification by recrystallizzation and size exclusion chromatography, the desired product **2** was recovered in almost quantitative yield. The carboxylate derivative **1** was eventually obtained by alkaline hydrolysis of the methyl esters in 48 % yield. Both metallacycles **1** and **2** were fully characterized by NMR, IR, and UV−vis spectroscopy and the data are in good agreements with those reported by Hupp and co-workers for similar systems (see Supporting Information (SI)).[16,17] The neutral tetraporphyrin metallacycles are poorly soluble in many solvents, including water. Improved solubility was obtained using zincated porphyrins instead of the free-bases. Moreover, the presence of the Zn(II) centers may assist ion transport by making the inner walls of the channel more hydrophilic.

The ionophoric activity of compounds **1** and **2** was investigated with a standard base-pulse assay.[18] The pH-sensitive dye HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid, pK_a = 7.2) was trapped inside 100 nm diameter liposomes buffered at pH = 7. An aliquot of ionophore solution in DMSO was added and, after 20 min of incubation time, a pH-gradient of 0.6 units was applied by external addition of NaOH. The collapse of the transmembrane pH-gradient implies basification of the liposome inner water pool, which is signaled by an increase of the HPTS fluorescence emission. Therefore, the assay gives direct information on the transportation of H^+ (efflux) or OH^- (influx) and indirect information on the correlated symport/antiport of counterions. Figure 2a shows the typical kinetic profiles obtained with this experiment: at 200 s, the base-pulse is applied, and at 1400 s, an excess of

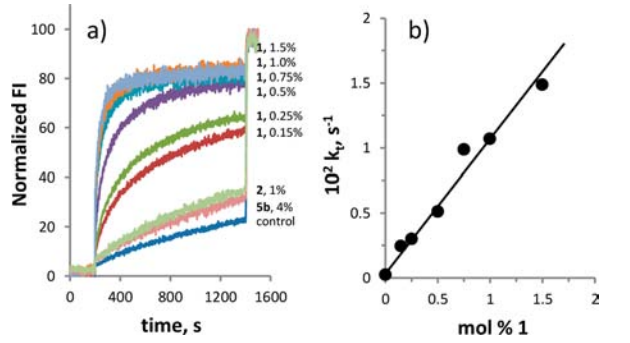


Figure 2. (a) Normalized fluorescence change in HPTS emission as a function of time after addition of the base (50 μL of 0.5 M NaOH) to 95:5 EYPC/EYPG LUVs (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.17 mM total lipid concentration, 25 mM HEPES, 100 mM NaCl, pH 7.0, total volume 3 mL), in the presence of metallacycles **1**, **2** and porphyrin **5b**. The concentrations of ionophores, given in percent with respect to the total concentration of lipids, are indicated along the corresponding traces. (b) Dependence of the observed rate constant for the transport process (k_t, s^{-1}) vs concentration of **1** (HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; EYPC, egg yolk phosphatidyl choline; EYPG, egg yolk phosphatidyl glycerol).

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TRITON X-100 is added, which lyses the liposomes leading to the complete collapse of the pH gradient.

The tetraporphyrin metallacycle **1** shows high ionophoric activity: the pH gradient is fully discharged after less than 2 min in the presence of 1% ionophore and the activity is still remarkable even at a concentration 10 times lower. Although the different experimental conditions make comparisons difficult, the kinetic profiles of **1** are similar to those reported by Matile and co-workers for a barrel-stave channel,[19] and its activity is intermediate between those of amphotericin B and gramicidin D, taken as examples of pore forming natural compounds (Table S1). In contrast, the corresponding methyl ester **2** (1%) and the single wall of metallacycle **1**, that is, the bis(4-carboxyphenyl)porphyrin **5b** (4%), are almost ineffective and the corresponding kinetic traces are close to the control experiment recorded in the absence of ionophore. Thus, both the preorganization of the metallacycle and the presence of the properly oriented peripheral carboxylic acid groups are crucial for the observed activity. The fitting of the kinetic traces obtained at different concentrations of **1** gives the apparent first-order rate constants (k_i, s^{-1}) for the transport process, which show a roughly linear increase with the concentration of metallacycle (Figure 2b). This linear dependence in the activity/concentration profile may suggest that the active species is either a monomer or a dimer with a dimerization equilibrium largely shifted toward the dimer.[20] In the first case, the different activity of **1** compared to **2** might be related to a better amphiphilic character of the acid derivative with respect to the corresponding ester. However, control experiments on the model porphyrins **5a** and **5b** show that the ester derivative **5a** is more active than the parent acid **5b** (Figure S7), thus reasonably excluding a positive effect of the different amphiphilicity on the ionophoric activity. Therefore, considering all the experimental evidences, and in analogy with the model proposed by Kobuke,[9] these results indicate that **1** is indeed capable of forming the proposed transmembrane nanopore via hydrogen bonding dimerization (Figure 1). In this model, each metallacycle has four carboxylic groups pointing inside the membrane involved in the hydrogen bonding network that ensures dimerization of **1**, while the other four point outside toward the (inner or outer) bulk water and are most likely deprotonated, thus efficiently directing the correct orientation of the metallacycle in the membrane. As expected for a nanopore with a large diameter and an empty internal space, the system does not show selectivity toward first group cations or inorganic anions (Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, see SI). Conversely, the large polyanionic calcein dye remains trapped inside the liposome most likely as a consequence of unfavorable electrostatic interactions with the negatively charged portal of the channel (i.e., the deprotonated carboxylic acids, Figure S10). Moreover, this last experiment suggests that the ionophoric activity observed for **1** is specifically related to the formation of a channel and is not due to lytic effects on the membrane or to leakage of the HPTS dye.

To gain further insight into the nanopore formation, we investigated the possibility to block the channel activity using a cystamine core PAMAM dendrimer[21] (Figure 3).[22] Addition of the **G2**-dendrimer, at 0.5 molar ratio with respect to the tetraporphyrin metallacycle **1**, blocks almost completely the ionophoric activity, which is totally suppressed by increasing the concentration of **G2**-dendrimer to a 1:1 molar ratio (the kinetic trace is practically superimposable to the control experiment). We found that the blockage is strongly correlated

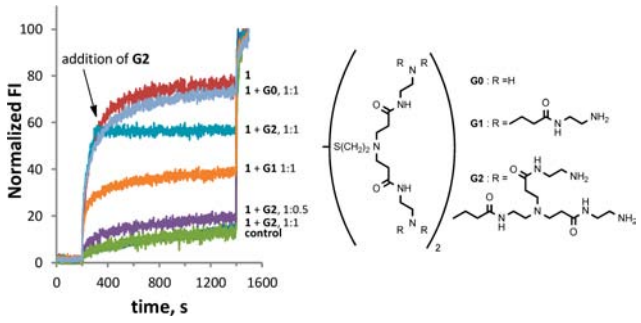

Figure 3. Channel blockage experiments using a cystamine core PAMAM dendrimer. Left: kinetic traces for the transport process registered in the presence of the metallacycle **1** (0.75 %) and a G0−G2 dendrimer (1:1 or 0.5:1 molar ratio with respect to **1**). In all the runs, the dendrimers are added before the incubation time, with the exception of the blue trace resulting from the addition of **G2**-dendrimer at 300 s. For experimental conditions see Figure 2. Right: structures of the generation 0-2 PAMAM dendrimers.

to the dimension of the PAMAM dendrimer and to the number of ionizable amino groups: **G0** (1:1 molar ratio, diameter ca. 1.5 nm) is almost ineffective, **G1** (1:1 molar ratio, diameter ca. 2.2 nm) reduces only partially the activity, while **G2** (1:1 molar ratio, diameter ca. 2.9 nm) completely inactivates the channel. Interestingly, the addition of **G2** during the kinetic run blocks immediately the transport process (blue trace in Figure 3) indicating that the dendrimer has a very rapid effect.

These experiments clearly show that **G2**, and in part **G1**, act as channel blockers. At pH close to 7, the amino groups of the dendrimers are protonated, while the carboxylic groups of porphyrin metallacycle **1** are deprotonated and exposed to the bulk water. Therefore, most likely, a favorable electrostatic interaction between the polycationic dendrimers and the anionic portal of the channel leads to the observed blockage effect. The dendrimer occupies the entrance of the channel acting as a stopper and thus hampering the ion flux. Clearly, **G0** (about 1.5 nm wide) is smaller and, having also a lower charge, binds less efficiently to the channel. Therefore, it is unable to occlude the channel, whereas the effect becomes more and more important with increase of the size and the number of ionizables groups of the dendrimer, until the complete channel blockage.

In conclusion, we have described the first example of a stable tetraporphyrin metallacycle (**1**), obtained via the metal-mediated directional-bonding approach, capable of forming nanopores in a liposomial membrane. The Re(I) corners ensure that **1** is thermodynamically stable and kinetically inert. The pore is likely to be dimeric and it is stabilized by an hydrogen bond network between the appended carboxylic acid residues on each porphyrin of the metallacycle. Efforts are being made to establish the detailed mechanism behind the formation of the transmembrane nanopore and the ion transport activity. We are also modifying the structure of the porphyrin units in order to further stabilize the dimeric assembly and to exploit the functionalization of the inner space of the nanopore (e.g., with metal ions other than Zn^{2+}) for application in the field of sensing. The intrinsic modular nature of the metallacycle will, in principle, enable us to obtain a library of compounds by changing the nature of the porphyrin unit and of the metal corners. We strongly believe that our work has demonstrated the suitability of the "Fujita-Stang" approach for the construction of stable self-assembled nanostructures which are

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active in biological membranes and that it may stimulate new developments in the field of artificial ion transporters.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details of synthesis and characterization of porphyrin components and tetraporphyrin metallacycles. Procedure for the HPTS assay and results for cation and anion selectivity of the nanopore. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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