Supplementary Information For:

Transport of calcium ions through a bulk membrane by use of a dynamic combinatorial library

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General remarks

*Trace*SELECT® Water (Fluka) and Aquamerck® Calcium Test (Merck) were obtained commercially. 4-Methoxypyridine-2,6-dicarbaldehyde [1] and 3,6,9-trioxa-1,11-undecanediamine [1] were synthesized according to literature. NMR spectra were recorded with Bruker DRX 500 or AV 600 instruments. ESI mass spectra were recorded with an Applied Biosystem Mariner Spectrometry Workstation. All the glassware used for the experiments were first washed with ultra-pure water (purified by mean of ELSA Purelab Plus) and then washed with *Trace*SELECT® Water to prevent any contamination by calcium ions which are always present in distilled water. All experiments were repeated at least three times each. For a complete characterization of 14-methoxy-6,9,12-trioxa-3,15-diaza-1(2,6)-pyridinahexadecacyclophan-2,15-diene see [2].

Experiment **A** (bi-phasic, no calcium ions):

In a test tube, 8.2 mg (0.050 mmol) of 4-methoxypyridine-2,6-dicarbaldehyde (**2**) was dissolved in 5 mL of $CDCl_3$ (or CD_2Cl_2). A solution of 9.6 mg (0.050 mmol) of 3,6,9-trioxa-1,11-undecanediamine (**1**) in 5 mL of D_2O was added to the organic phase to form the bi-phasic system. The test tube was closed with a stopper and the air was exchanged three times by nitrogen. Every 48 h, 600 µL of both layers were taken and the respective ^{1}H-NMR spectra were recorded. A well defined amount of a standard solution of dimethyl terephthalate (DMT) was added into each organic solution to follow the evolution of the experiment.

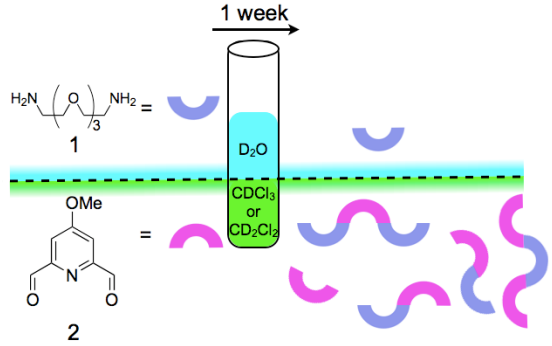

Figure 1: Schematic set-up of experiment **A**. The colored half circles symbolize the diamine and dialdehyde building blocks **1, 2** and the respective products.

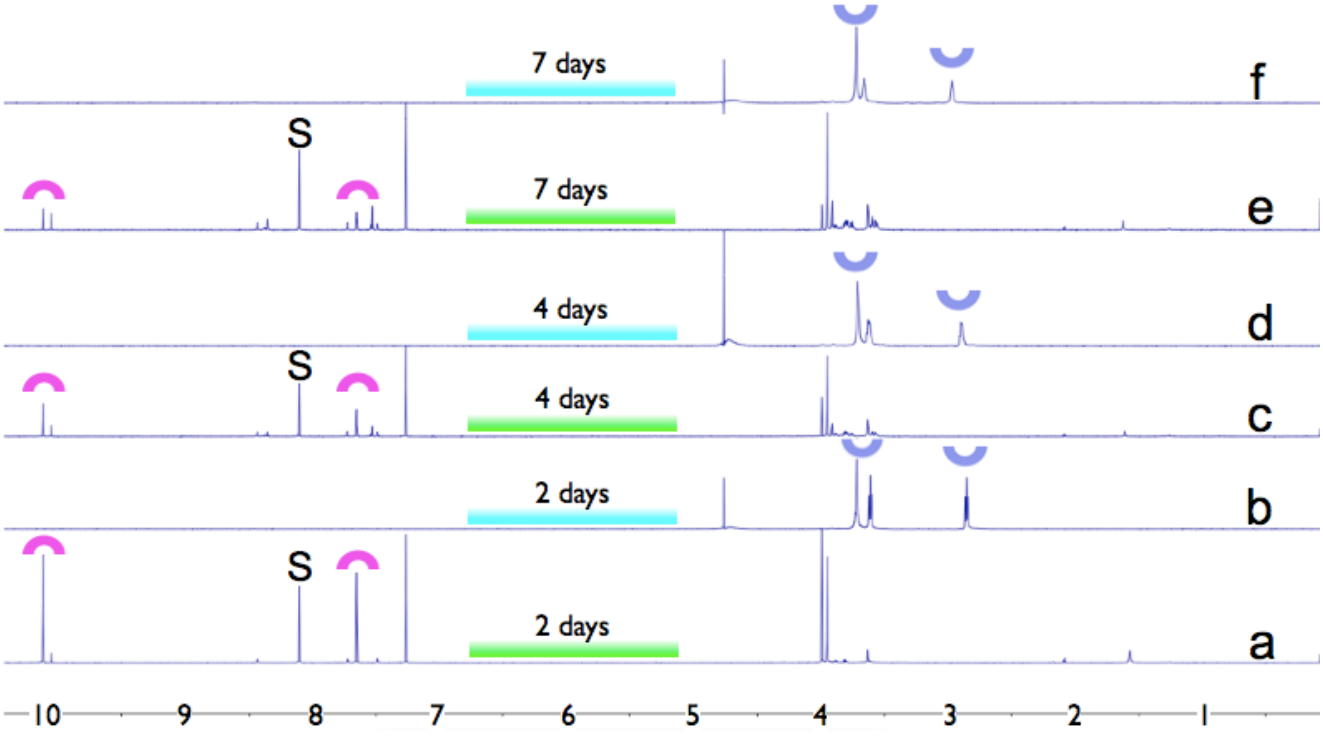

Figure 2. ^{1}H-NMR (500 MHz, 298 K). a), c) and e) $CDCl_3$; b), d) and f) D_2O. S = internal standard (DMT). Peaks are labeled with symbols as defined in Figure 1.

Experiment **B** (bi-phasic, with calcium ions):

In a test tube, 8.2 mg (0.050 mmol) of 4-methoxypyridine-2,6-dicarbaldehyde (**2**) was dissolved in 5 mL of $CDCl_3$ (or CD_2Cl_2). A solution of 9.6 mg (0.050 mmol) of 3,6,9-trioxa-1,11-undecanediamine (**1**) in 5 mL of D_2O was added to the organic phase to form the bi-phasic system. To the water layer, 5.5 mg (0.050 mmol) of $CaCl_2$ was added. The test tube was closed with a stopper and the air was exchanged three times by nitrogen. Every 48 h, 600 µL of both layers were taken and the respective ^{1}H-NMR spectra were recorded. A well defined amount of a standard solution of dimethyl terephthalate (DMT) was added into each organic solution to follow the evolution of the experiment. 14-Methoxy-6,9,12-trioxa-3,15-diaza-1(2,6)-pyridinahexadecacyclophan-2,15-diene in the water phase: 62-65% yield.

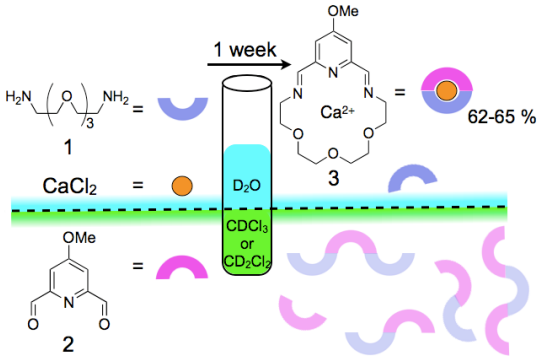

Figure 3: Schematic set-up of experiment **B**. The colored half circles shall symbolize the diamine and dialdehyde building blocks **1, 2** and the respective products (macrocycle **3** and polymers). Calcium ions are shown as orange circles.

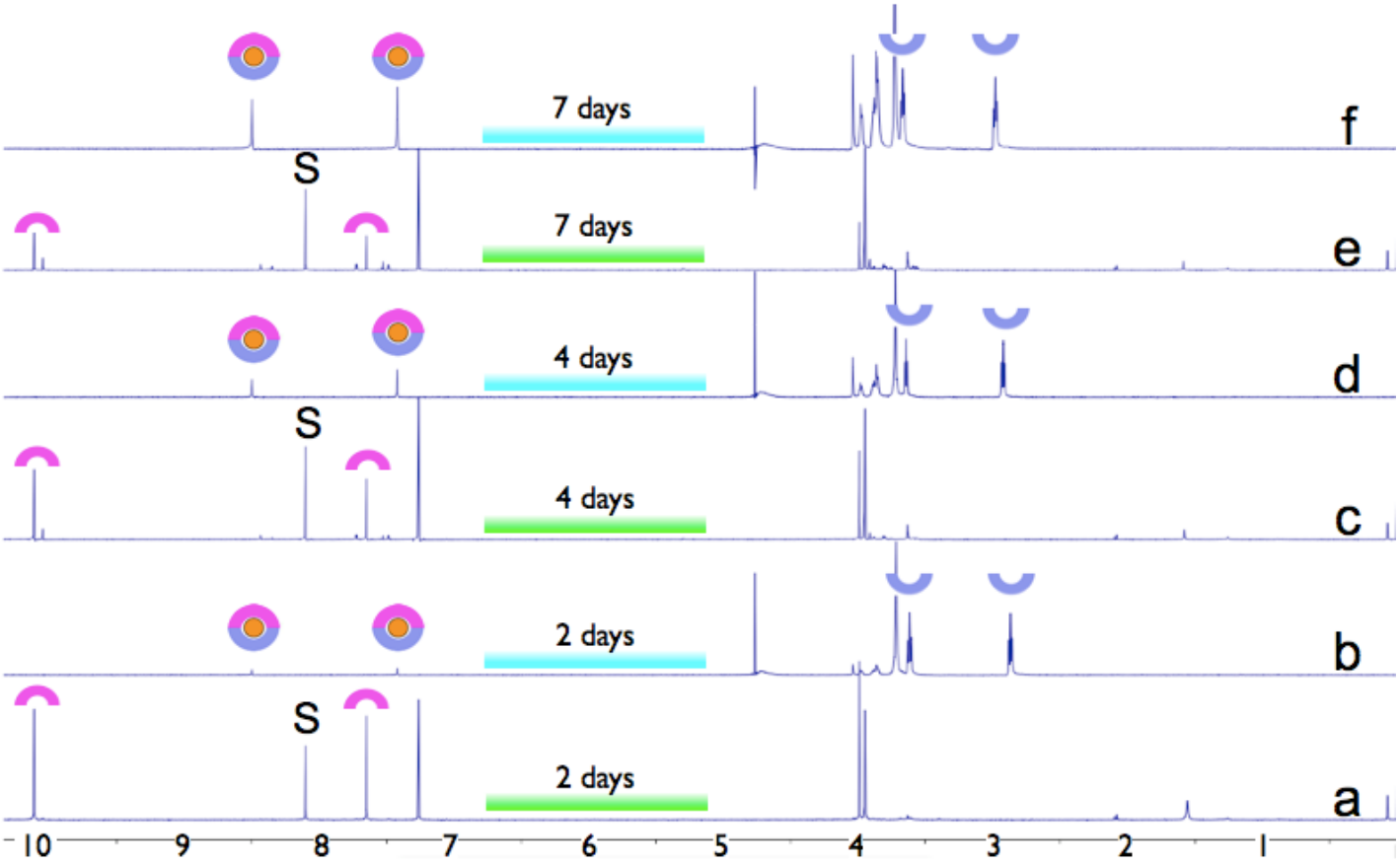

Figure 4. ^{1}H-NMR (500 MHz, 298 K). a), c) and e) $CDCl_3$; b), d) and f) D_2O. S = internal standard (DMT). Peaks are labeled with symbols as defined in Figure 1 or 3.

Experiment **C** (carrier experiment):

Water source phase: 20 mL of Fluka *Trace*SELECT® Water, 49 mg (0.30 mmol) of 3,6,9-trioxa-1,11-undecanediamine (**1**) and 33 mg (0.30 mmol) of $CaCl_2$.

Bulk membrane: 60 mL of CH_2Cl_2, 49 mg (0.30 mmol) of 4-methoxypyridine-2,6-dicarbaldehyde (**2**).

Water receiver phase: 20 mL of Fluka *Trace*SELECT® Water.

Each phase was left under gentle stirring for 1 week. After 1 week, the three phases were analyzed by ^{1}H-NMR. 550 µl of the water source phase and 550 µl of the organic phase were taken. 50 µl of deuterated solvent (D_2O for the water phase and CD_2Cl_2 for the organic phase) were added and the ^{1}H-NMR spectra were recorded. The solvent of the aqueous receiver phase was evaporated by a flux of nitrogen. Then the residue was dissolved in CD_3OD and a ^{1}H-NMR spectrum was recorded. The aqueous receiver phase was also analyzed by means of ESI-MS and with a titration method (Aquamerck® Calcium-Test). Macrocycle **3** and calcium ions were found in the receiver phase with 5 - 7% of yield.

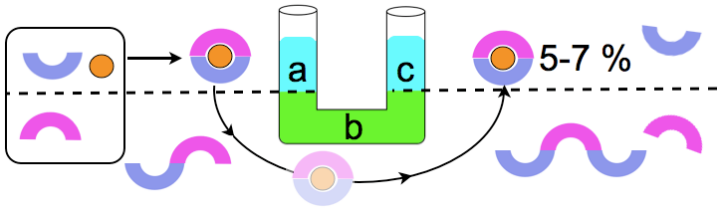

Figure 5: Schematic set-up of experiment **C**. For symbols, see Figure 1 or 3.

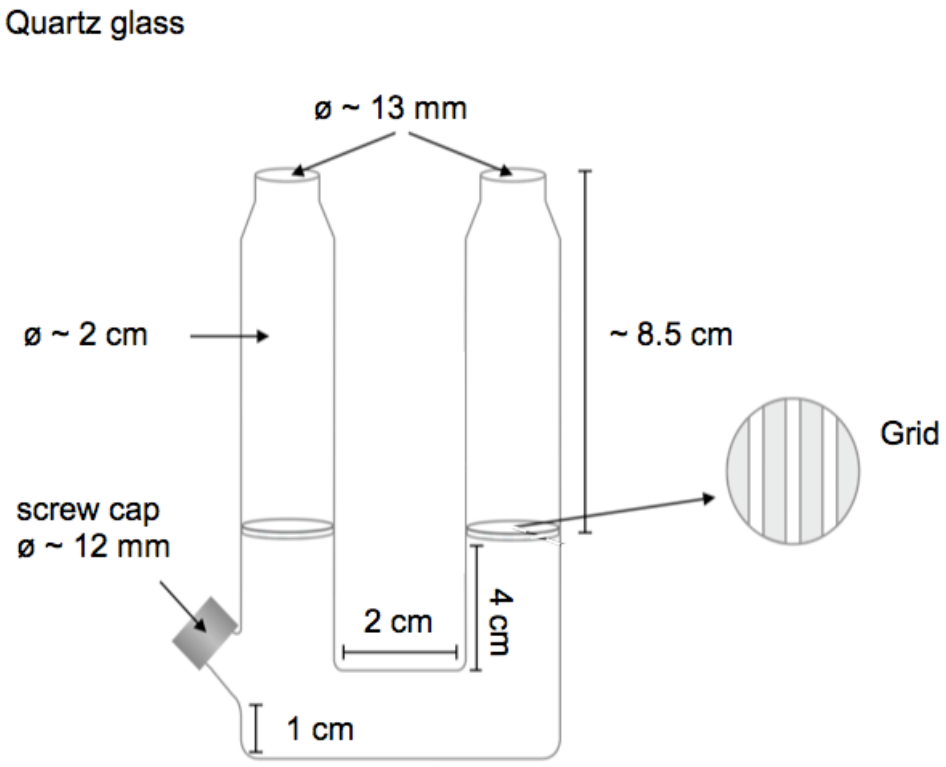

Figure 6: Glassware used in experiment **C**. All three compartments were gently stirred by magnetic stirring bars.

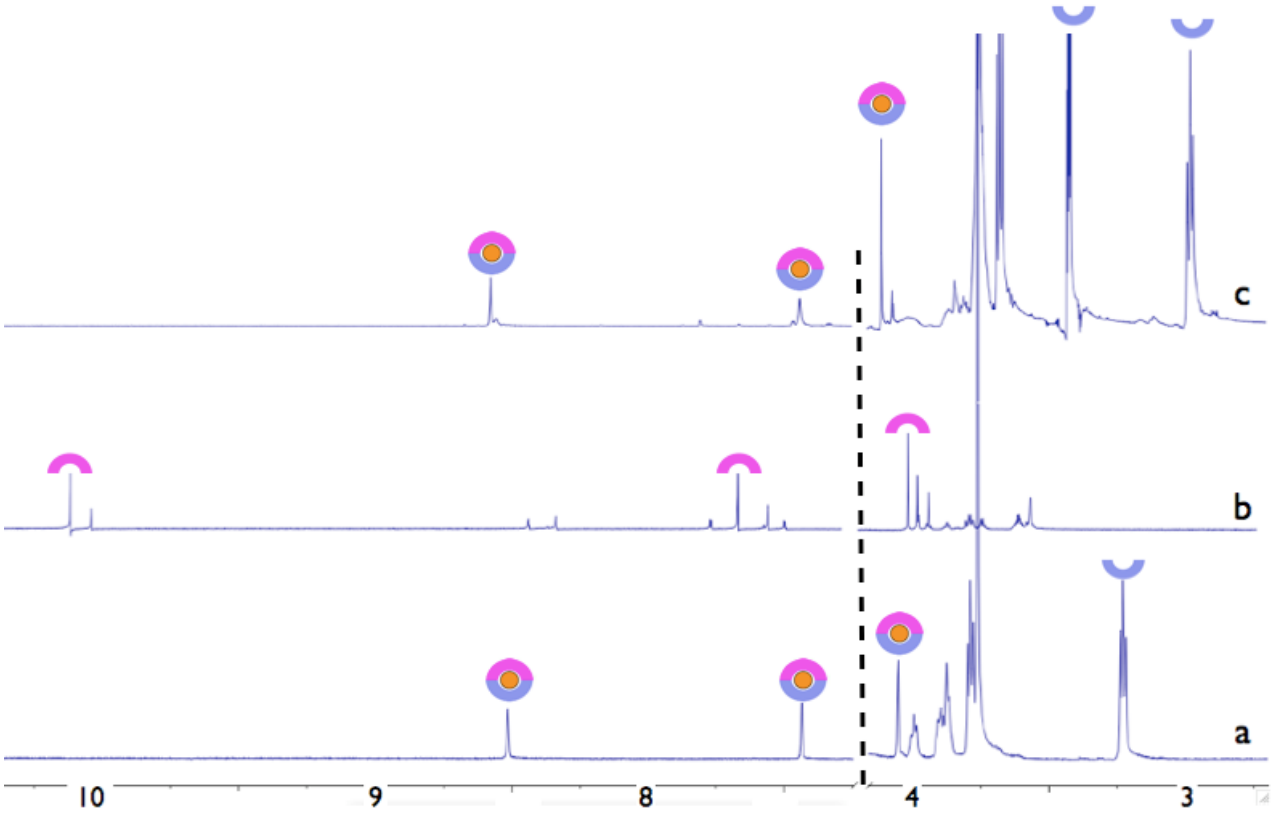

Figure 7. ^{1}H-NMR (500 MHz, 298 K). a) source phase H_2O + 10 % D_2O; b) bulk membrane CH_2Cl_2 + 10 % CD_2Cl_2; c) receiver phase evaporated, CD_3OD. Solvent residue peaks are removed for clarity. For symbols, see Figure 1 or 3.

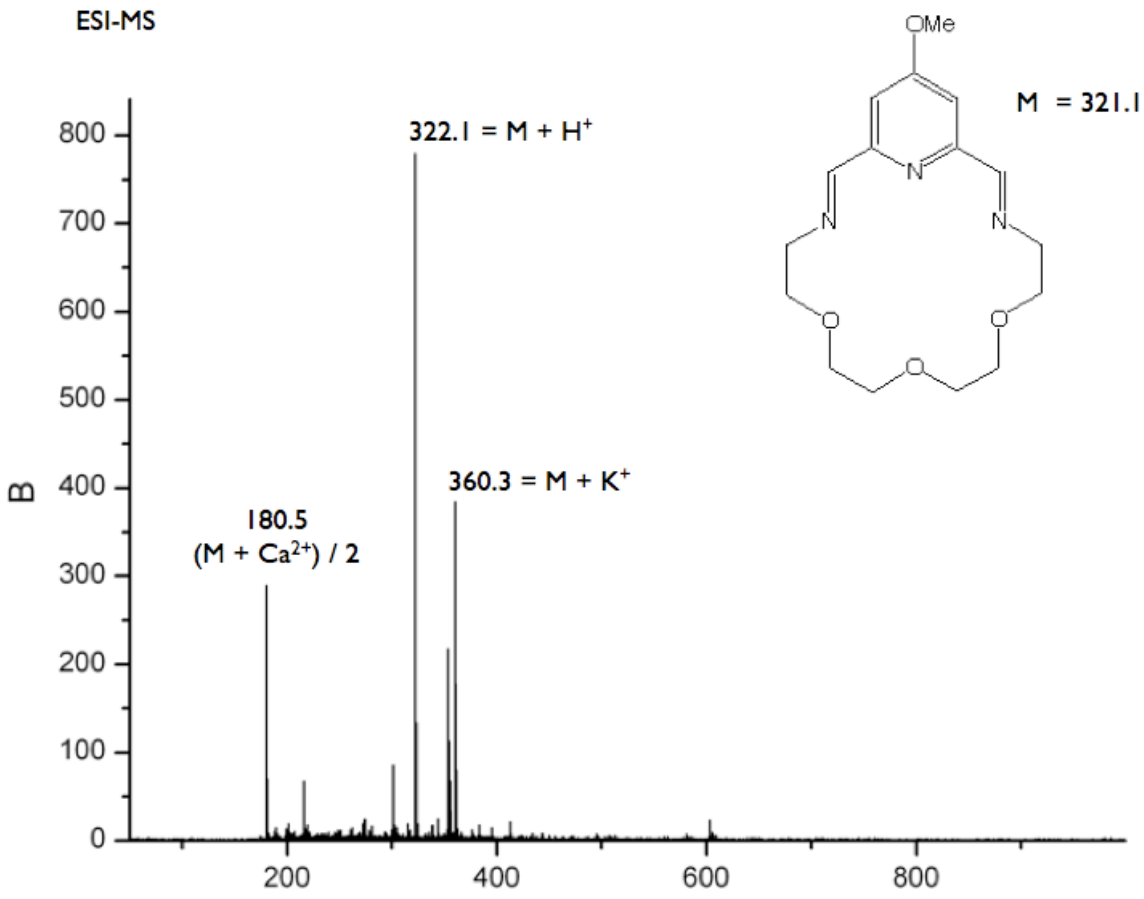

Figure 8. ESI-MS of the receiving phase.

Colorimetric Titration

5 ml were taken from the aqueous receiver phase. 10 drops of Ca-1 solution (Aquamerck® Calcium-Test) were added to this solution followed by a small amount of indicator Ca-2 (Aquamerck® Calcium-Test). The solution was stirred and titrated drop by drop using a titration pipette with a solution of Ca-3 (Aquamerck® Calcium-Test). When the color changed from red-violet to blue-violet the titration was complete. From the volume of the Ca-3 solution used, the amount of calcium was calculated to be 99 mg/L (1.98 mg/20 mL receiver phase, 6 %) which is in agreement with the NMR determined 5 - 7 % of calcium complex carried from the source to the receiver phase.

[1] U. Lüning, R. Baumstark, K. Peters, H. G. v. Schnering, *Liebigs Ann. Chem*., **1990**, 129-143.
[2] V. Saggiomo, U. Lüning, *Eur. J. Org. Chem*., **2008**, 4329-4333.