Supporting information

Polyvinylpyridinium-type gradient porous membranes: synthesis, actuation and intrinsic cell growth inhibition

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1. Materials and Methods

1.1. Materials

Isopthalic acid (99%) was purchased from Acros, 1,2,3-tricarboxyl benzoic acid (99%) from Alfa Aesar, 1,2,3,4,5-pentacarboxyl benzoic acid (98%) was purchased from TCI, 1,2,4,5-tetracarboxyl benzoic acid (96%), mellitic acid (99%) and poly(4-vinyl pyridine) (P4VP) (M_w ~ 60 kDa) from Sigma-Aldrich. Bromoacetonitrile (97%) was obtained from TCI. All chemicals were used without further purification. The solvents used were of analytical grade.

1.2. Synthesis of PCMVPy-Tf_2N

Poly[1-cyanomethyl-4-vinylpyridinium bis(trifluoromethane sulfonyl)imide] (PCMVPy-Tf_2N) was synthesized as previously reported[1] by postmodification of P4VP (M_w ~ 60 kDa). In a typical experiment, P4VP (5 g, 47.55 mmol) was dissolved in 60 mL of a mixture of dimethyl sulfoxide (DMSO) / ethylene glycol (ETG) (40 mL:20 mL). After purging the solution for 20 min with nitrogen, it was heated to 90 °C and bromoacetonitrile (17.13 g, 142.28 mmol) was added dropwise. The mixture was stirred at 90 °C overnight. The polymer was purified by precipitation in tetrahydrofuran and dialysis in water (MWCO ~ 14kDa). After drying, 3 g (13.33 mmol) of the as obtained polymer were collected and re-dissolved in 200 mL of water for the anion exchange. Lithium bis(trifluoromethane sulfonyl)imide (7.6 g, 26.48 mmol) was dissolved in 10 mL of water and added dropwise to the stirred polymer solution. The precipitate was filtered off, washed extensively with water and dried under high vacuum at 80 °C. 4.621 g (10.86 mmol, 81.5 %) of PCMVPy-Tf_2N was collected. The chemical structure was confirmed by ^{1}H-NMR (Fig. S1).

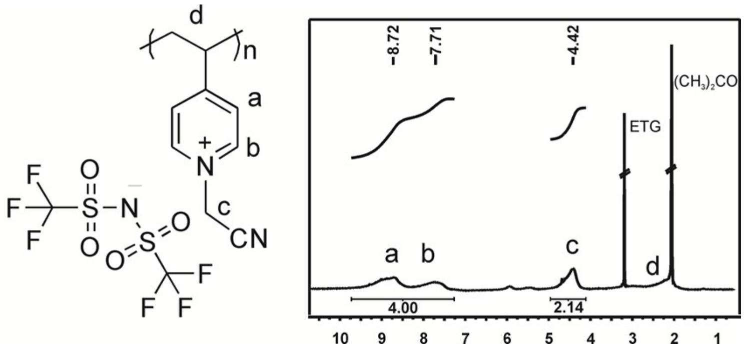
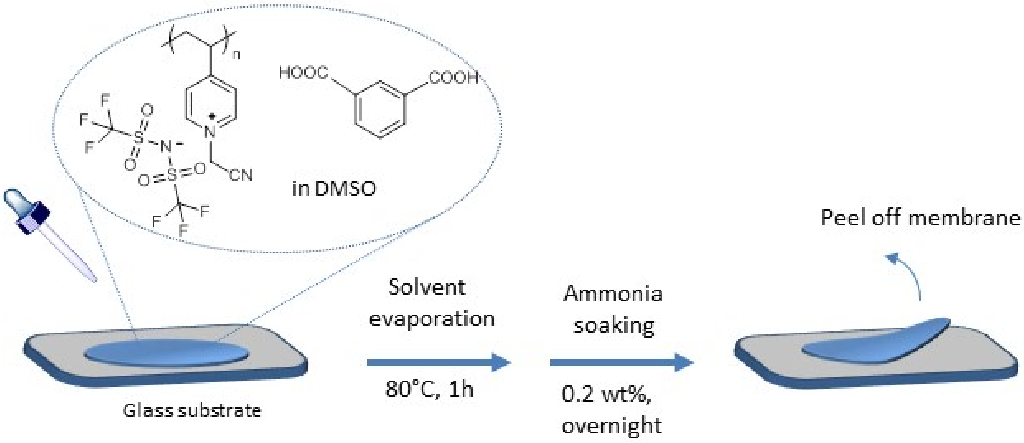


Figure S1: ^{1}H-NMR spectrum of PCMVPy-Tf_2N in acetone-d_6.

1.3. Membrane fabrication

PCMVPy-Tf_2N (106.33 mg, 0.25 mmol) and the according acid (0.25 mmol of -COOH units) were dissolved in DMSO and stirred for 2 h until dissolved at the molecular level. The black solution was then dripped onto a glass plate and the solvent was evaporated at 80 °C in an oven. The resulting non-porous dry polymer film was subsequently immersed into aqueous ammonia (0.2 wt%) overnight for pore formation and electrostatic complexation. The membrane was detached easily from the glass plate and washed several times with water.



Scheme S1. Schematic preparation of a PCMVPyTf_2N-PAA membrane actuator.

1.4. Cell toxicity assay

Cell viability tests were done using the Chinese hamster ovary (CHO) cell line and a murine fibrosarcoma cell line (L929). The cells were incubated in an incubator at 37 °C, 95% relative humidity and at a CO_2 level of 5%. A CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) was performed according to the manufacturer's instructions. The optimal cell number was determined as of 2.5×10^4 cells/well. 100 µL of the respective cells where incubated on the previously prepared membranes (see above) in 96 well plates for 15 h. Subsequently, 20 µL of the tetrazolium compound was added and incubated for another 30 min. To clear the medium from debris, 100 µL supernatant were carefully collected into a cone shaped 96 well plate, pelleted at 2000 g for 10 min and transferred into a 96 well polystyrene clear plate (Corning Life Sciences, Amsterdam). The absorption at 490 nm was measured using an Infinite 200 PRO Tecan Plate reader (Männedorf, Switzerland).

1.5. Characterization methods

Scanning electron microscopy (SEM) was performed with a GEMINI LEO 1550 microscope at 3 kV. Samples were sputtered with a thin layer of gold beforehand. The averaged pore sizes of the membranes were determined on the basis of SEM images using the software ImageJ. EDX experiments were performed on a field emission SEM Type Jeol JSM-7500F at 15 kV. In order to determine the relative sulfur content in the cross-section of the membranes, EDX measurements were performed at 5 different points on the cross-section. Membranes were sputtered with a thin layer of gold beforehand. Attenuated total reflection Fourier transformed infra-red (ATR-FTIR) spectra were recorded on a Nicolet^{TM}iS5 instrument from Thermo Scientific. The samples were measured in the solid state. Combustion elemental analysis was done with a varioMicrocube apparatus from elementar. ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a Bruker DPX-400 spectrometer at 400 MHz. Deuterated acetone was used as the solvent.

2. Additional data

2.1. FTIR measurements of the water in which the membranes were stored

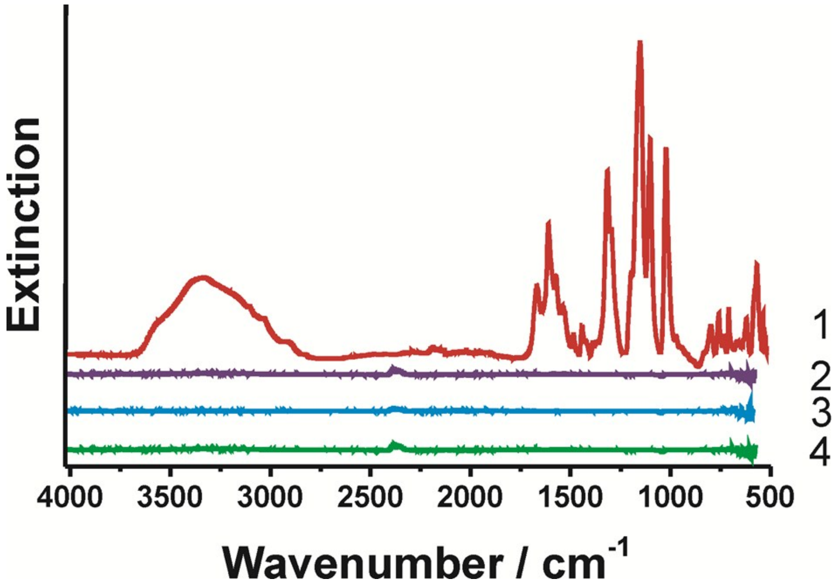


Figure S2: ATR-FTIR spectra of 1) PCMVPy-Tf_2N membrane with acid **2**. 2-4) membrane storing water of PCMVPy-Tf_2N membrane with 2) acid **5** after storing for 2 month. 3) acid **2** after storing for 3 month. 4)acid **2** after storing for 2 month. For 2-4) the background was taken with MilliQ water.

2.2. Bending angle determination

In order to determine the bending angle α of the dry membranes, a circle was placed into the loop and α (unit in rad) was determined from the center of this circle (Figure S3).

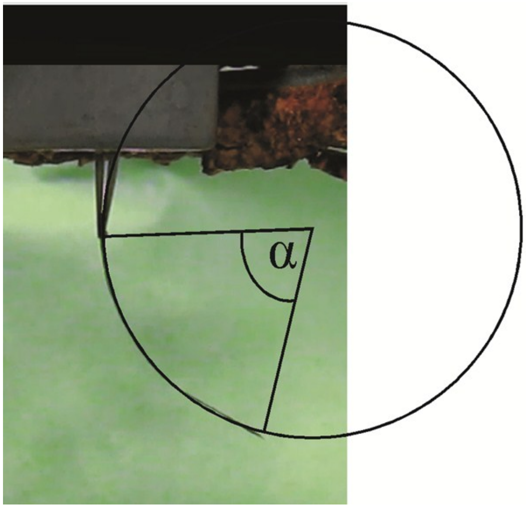
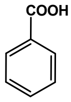
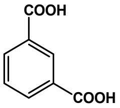
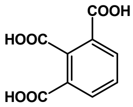
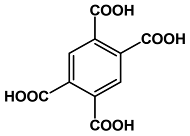
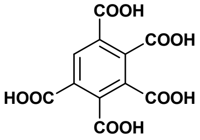
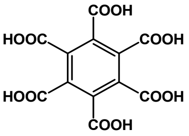
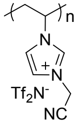
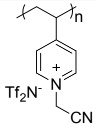


Figure S3: Determination of the bending angle by placing a circle into the membrane's loop and taking the angle from its center.

Table S1: Comparison of the **pore sizes in nm** of membranes from the imidazolium and pyridinium based PIL. The data from polyvinylimidazolium-type membranes are obtained from ref. 22.

Acid / Polymer						
	1	2	3	4	5	6
	-	**2500**	**960**	**220**	**200**	**80**
	-	**460**	**270**	**155**	**174**	**100**

Equation S1:

$$F = \frac{Q_1 Q_2}{4\pi\epsilon_0 \epsilon r^2} \tag{S1}$$

Relationship between the electrostatic attraction F and the dielectric constant ϵ of the surrounding medium.

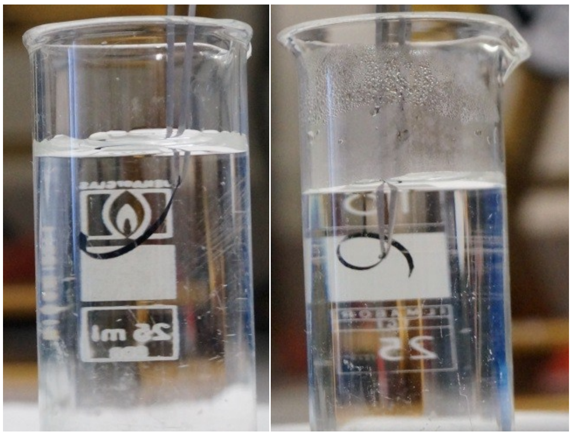


Figure S4: Left: Membrane in water at 22 °C (left) and at 60 °C (right).

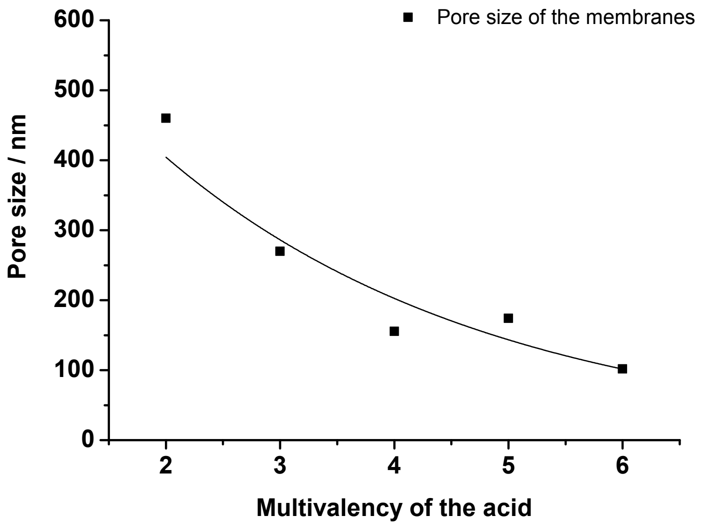


Figure S5: Average pore size of the membranes as a function of the multivalency of the benzoic acid derivatives employed for crosslinking.

1 Yuan, J.; Marquez, A. G.; Reinacher, J.; Giordano, C.; Janek, J.; Antonietti, M., *Polymer Chemistry* **2011,** *2* (8), 1654-1657.