Programming ionic pore accessibility in zwitterionic polymer modified nanopores

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ABSTRACT:

Creating switchable and gradually tunable pores or channels that display transport control similar to biological pores remains a major challenge in nanotechnology. It requires the generation and manipulation of complex charge situations at the nanoscale and the understanding how confinement influences chemistry and transport. Here, two different pore sizes, ~100 nm and less than 10 nm, functionalized with varying amounts of responsive zwitterionic polycarboxybetaine methylacrylate (PCBMA) give fascinating insight into the confinement controlled ionic transport of pores functionalized with pH-dependent zwitterionic polymers. Under basic conditions, the zwitterionic state offers complex, strongly pore-size dependent ionic permselectivity characteristics. For mesoporous films with pore sizes smaller than 10 nm, complete ion exclusion is observed after reaching a critical zwitterionic polymer amount, clearly indicating an electrostatic behavior of “bipolar charged” pores. This ion exclusion is not observed for pore diameters of ~ 100 nm. In addition, the solution pH of equal pore accessibility for oppositely charged ions and pore sizes smaller than 10 nm shifts with increasing polymer amount from a pH of 2.5 to 8.2, and the quantity of ions accessing the pores depends on the pore size. These observations clearly show the potential of controlling pore
accessibility based on controlled functional composition at the nanoscale without changing the components themselves as well as the influence of spatial confinement on pore accessibility in the presence of complex (zwitterionic) charged states.

**TOC**

**INTRODUCTION**

The creation of switchable and gradually tunable nanodevices that display transport properties similar to those observed in biological pores is a major challenge in nanotechnology that requires an understanding of how confinement influences chemistry and transport phenomena. Biological ion channels or pores controlling mass transport are based on a sophisticated combination of structure and chemical function at the nanoscale, and can exhibit complex charge situations within the pores with precise nanoscale local control of functional group arrangement. One such notable example from nature is the nuclear pore complex.¹ Numerous synthetic pores using different functionalization strategies have been reported in attempts to mimic these exceptional transport control. In case the nanopore size is comparable to the Debye Screening Length, ion selectivity,²⁻⁴ ion concentration polarization, e.g. in
electrokinetic ion transport, and gated ionic pore accessibility have been observed. In addition to electrostatic effects pore shape is important for transport control. In this context asymmetric single pores are reported for their rectification characteristics and recently as well for their potential as ion pumps. Besides asymmetric pore shapes concentration gradients are reported to induce reversal of transport direction, for example based on ion gradients, and are discussed for energy harvesting from sea water. A promising approach in terms of transport control based on electrostatics and volume effects is the combination of ceramic or ion track etched pores with responsive polymers. Recently investigated aspects are externally responsive pores, gradually adjustable charge density and thus transport properties, or asymmetric pore design. The polymers investigated to-date are often limited to those that display only two different and well-defined charged states. However, zwitterionic polymers possess higher potential to express more complex charged states; they exhibit fascinating swelling properties, and interact with biological species already present on a planar surface. These properties have led to extensive research into their potential application in anti-fouling materials. For example, by confining such switchable zwitterions into brushes at a planar surface, the complex charge switching and its impact on its swelling behavior and non-fouling characteristics were elucidated. Recently, the pH-dependent surface charge of zwitterionic brushes on a planar surface was even modulated by adding a strong cationic polyelectrolyte methacryloyloxyethyltrimethyl ammonium chloride (METAC) into zwitterionic polysulfobetaine methacrylate polymer brushes.

Implementing zwitterionic polymers into the spatial confines of nanopores is expected to generate a complex charge situation, influencing the ionic pore accessibility and transport characteristics of such materials. The spatial confinement, and thus the pore size, should significantly influence the polymer characteristics and transport properties. It is well-known that acid-base equilibria are strongly affected by spatial confinement. Besides, as well the polymer molecular weight grafted to a pore wall is crucial. A recent study investigating zwitterionic brushes in carbon nanotube membranes stated a decrease in pore size upon the reduction of salt concentration due to chain stretching. Furthermore, switchable zwitterionic polymers in principle allow charge inversion of pores and thus offer the potential for active complex ionic transport control. The first examples of zwitterionically-functionalized pores show a complex interplay of polymer and pore charges at relatively low polymer amounts exists. In larger
(1.65 µm base diameter) lysine-functionalized conical pores a transition between three charged states could be observed. Recent theoretical studies, e.g. on lysine-functionalized nanopores, explain pH-dependent electrokinetic ion transport including charge conversion which might be of interest e.g. for biomolecule separation or DNA sequencing. Polymer functionalization is thereby carried out using free radical polymerization techniques. Via this approach the functional density within the pores can be severely hindered and is usually not controlled. To the best of our knowledge the effect of polymer density and thus zwitterionic charge density in pores is not investigated up to date. This aspect is essential for the rational design of bioinspired transport phenomea which are based on a sophisticated spatial arrangement of chemical function at the nanoscale. To advance the complex transport control and enhance our understanding of complex charge situations within the spatial confinement of pores, the charge quantity must be tunable and responsive. Our group recently reported the gradual variation of polymer amount in pores, and on the application of controlled initiator initiated polymerization to adjust the amount of zwitterionic polymer within the pores. Investigating the transport characteristics of a pH-dependent zwitterionically- or positively-charged carboxybetaine methacrylate (CBMA)-based polymer, we observed a clear influence of spatial confinement and polymer amount on the charged state and its transition indicating a confinement controlled adjustment of pore charge without changing the chemical components, which is an interesting and new perspective on nanoscale chemistry.

RESULTS AND DISCUSSION

To understand the influence of these parameters we functionalized porous films with two different pore sizes (< 10 nm and ~100 nm) with a pH-responsive positively or zwitterionically-charged carboxybetaine methacrylate polymer (PCBMA) (Figure 1) and systematically investigated the ionic permselectivity by cyclic voltammetry (CV). As described in detail in a previous work mesoporous films classified having pore sizes < 10 nm (template Pluronic® F127) are characterized by pore sizes of 6–7 nm, pore connections (necks) of ~3 nm, and a film thickness between 150–250 nm. The surface area was determined via krypton BET adsorption to be 36 m² per 1 m² substrate surface. Films templated with polystyrene nanoparticles (diameter of 115 nm), classified as pore size ~ 100 nm, show a specific surface area of 132–265 m²/g, as determined from SEM images.
**Figure 1.** Schematic overview on the complex pH-dependent charge situation within porous films with pore sizes of approximately 100 nm and below 10 nm. The exact charge amount depends on the functionalization process used, and thus on the polymer amount. In general, PCBMA functionalization results in positively-charged pores under acidic conditions, and zwitterionically-charged pores under basic conditions.

The polymer functionalization was carried out in the presence of a CBMA monomer concentration of 0.3 M or 1.3 M and was monitored by IR spectroscopy\(^{33}\) as summarized in Figure S1. Using a CO\(_2\) plasma treatment\(^{14,34}\), initiator at the outer mesoporous film surface can be destroyed resulting in exclusive polymer formation inside the mesopores instead of inside the mesopores and on the outer surface. The iniferter density was determined by XPS and BET measurements giving a density of approximately 0.2 molecules per nm\(^2\) after binding at 70°C.\(^{33}\) A maximum average pore volume filling up to 35-40 vol% for a 1.3 M CBMA monomer solution after 240 min of polymerization was achieved as determined by ellipsometry\(^{35}\) corresponding to a PCBMA chain length of 2-5 monomers per chain as determined after degrafting. The generated PCBMA amount for mesoporous (< 10 nm) films is determined to be increased by a factor of approximately 1.5 by using 1.3 M CBMA instead of
0.3 M CBMA for polymer functionalization although the mesoporous films functionalized in the presence of 0.3 M CBMA present PCBMA both inside the pores, as well as on the external surface. The influence of pore size, pH, and polymer amount on the ionic permselectivity of PCBMA-functionalized films was studied systematically in order to understand the effect of spatial confinement. The pH-dependent pore accessibility for relatively low polymer amount (polymerization in the presence of 0.3 M CBMA) and polymer formation inside the pores and on the outer surface are summarized in Figure 2, the ones for a higher final PCBMA amount (polymerization in the presence of 1.3 M CBMA) and polymer formation exclusively inside the pores are summarized in Figure 3.

**Figure 2.** Peak current density ($j_p$) determined by cyclic voltammetry in dependence of solution pH for a) PCBMA-functionalized ~ 100 nm pore-sized films in the presence of $[\text{Fe(CN)}_6]^{3-/4-}$ and b) in the presence of $[\text{Ru(NH}_3)_6]^{2+/3+}$. c) PCBMA-functionalized mesoporous films (pore size <10 nm) in the presence of $[\text{Fe(CN)}_6]^{3-/4-}$ and d) in the presence of $[\text{Ru(NH}_3)_6]^{2+/3+}$. Porous films were functionalized inside the mesoporous as well as on the outer planar surface (no CO$_2$ plasma treatment) using a monomer concentration of 0.3 M.

**Influence of polymer amount and pore size on ionic permselectivity**
In general, an increasing PCBMA amount leads to a change in the ionic permselectivity from a silica-like behavior to a polymer (PCBMA) charge dominated behavior as reflected in the peak current density ($j_p$).

**Porous silica of different pore size.** Unmodified mesoporous silica is neutral under acidic conditions ($\text{pH} < 3$) (Figure S8), and does not electrostatically discriminate between $[\text{Fe(CN)}_6]^{3/-4}$ and $[\text{Ru(NH}_3)_6]^{2+/3+}$ (Figure 2c, d black, pH 3). This corresponds to literature results for comparable mesoporous silica materials$^{29}$ and is reflected by the comparable peak current densities for both probe molecules at pH 3. Under basic conditions, the silanol groups, present on the silica pore walls, are deprotonated and silica is negatively charged (Figure S8). This leads to an electrostatic exclusion of $[\text{Fe(CN)}_6]^{3/-4}$, and an electrostatic preconcentration of $[\text{Ru(NH}_3)_6]^{2+/3+}$ within mesoporous silica with pore sizes below 10 nm (Figure 2c, d black pH 9). This is reflected in the CV traces where the $j_p$ decreases for $[\text{Fe(CN)}_6]^{3/-4}$, and a significant increase in $j_p$ is observed for $[\text{Ru(NH}_3)_6]^{2+/3+}$ in combination with signal broadening in the mesoporous silica films with pore sizes < 10 nm (Figure S2a and Figure 2c, d black). For larger pores i.e., in the ~100 nm porous films, exclusion effects are not observed at basic pH for $[\text{Fe(CN)}_6]^{3/-4}$. This result indicates the pore size is significantly larger than the Debye screening length (Figure 2a, b, black, Figure S2b). Pre-concentration effects can still be observed in these pores due to the electrostatic attraction in the pore wall region (Figure 2a, violet, 90 minutes, pH 3). But these effects are generally less significant due to the free space for molecule diffusion in the center of the pore, which is in accordance with literature reports.$^{36}$

**Pre-concentration of countercharged ions $[\text{Fe(CN)}_6]^{3/-4}$ under acidic conditions.** At an acidic pH of 3, PCBMA-functionalized pores with sufficiently high PCBMA amounts (polymerization time of 90-240 min) are expected to be positively-charged (Figure 1, Figure S8). In addition, a variation of PCBMA polymer amount (e.g. shorter functionalization times) is expected to result in a variation of the number of positive charges at acidic pH. Due to the
electrostatic attraction between the PCBMA-functionalized pore charge and countercharged probe molecules this results in a gradual increase of $j_p$ for countercharged $[\text{Fe(CN)}_6]^{3-/4-}$ with increasing PCBMA amount at pH $\leq 3$. In accordance with this expectation, an increasing $j_p$ for the negatively charged $[\text{Fe(CN)}_6]^{3-/4-}$ probe molecule is observed for both pore sizes (Figure 2a,c) with increasing PCBMA amount (polymerization time) at pH 3. Increasing the CBMA monomer concentration to 1.3 M (Figure 3a, c) and thus further increasing the PCBMA polymer amount, the $j_p$ in the presence of $[\text{Fe(CN)}_6]^{3-/4-}$ at pH 3 increases even further. Under these conditions the $j_p$ measured at pH 3 (Figure 2a, c and Figure 3a, c) results in a continuously increasing electrostatic attraction with increasing PCBMA amount up to a pre-concentration and thus a higher $j_p$ as compared to unfunctionalized pores (Figure 2a, c black) or the unmodified ITO electrode. This is even better reflected in plotting $j_p$ for $[\text{Fe(CN)}_6]^{3-/4-}$ at pH 3 in dependence of the C=O stretching vibrational band at 1728 cm$^{-1}$ (Figure 4 dark blue traces).

The PCBMA amount deduced from infrared spectroscopy (Figure S1) increases approximately by a factor of 1.5 comparing the largest PCBMA amount in the presence of a 0.3 M CBMA monomer solution to the largest PCBMA amount achieved in the presence of a 1.3 M CBMA solution. This translates into an approximate $j_p$ increase of a factor of 2 at pH 3 for $[\text{Fe(CN)}_6]^{3-/4-}$ (Figure 4 dark blue traces at high PCBMA content). The peak current density ($j_p$) for $[\text{Fe(CN)}_6]^{3-/4-}$ at pH 3 for the highest PCBMA content (Figure 3a black, grey) exhibits an $j_p$ that is by a factor of 2 higher as the $j_p$ for unmodified neutral silica films under identical conditions. For films with ~100 nm sized pores a factor of 6 higher $j_p$ is observed comparing the maximum PCBMA pore filling with unmodified pores (Figure 3c black, grey).

Another interesting observation is the presence of an initial decrease in the $j_p$ at low PCBMA concentrations (short polymerization times) for $[\text{Fe(CN)}_6]^{3-/4-}$ at pH 3 in comparison to the unmodified silica films (Figure 2a, pH 3). This likely arises due to an increase in hydrophobicity and a slight decrease in pore size after initiator binding. After reaching a critical PCBMA
amount a gradual increase in pre-concentration \( (j_p \) higher than that of unmodified porous silica) of countercharged \([\text{Fe(CN)}_6]^{3+/4-}\) probe molecules occurs with an increasing PCBMA content within the porous silica films under acidic conditions (Figure 3a, c, pH 3). This behavior is more pronounced in larger pores (Figure 3a) most probably due to the higher PCBMA amount relative to the surface area of the silica surface, and thus the higher concentration of positive charges. This is in accordance with previous studies conducted in our group investigating strong polyelectrolytes.\(^{17}\) Additionally, this observation indicates that electrostatic interactions are dominant over possible steric hindrance due to polymer pore filling, as already stated based on theoretical studies for small molecules.\(^{27}\)

Figure 3. Peak current density \( (j_p) \) determined by cyclic voltammetry in dependence of solution pH for a) PCBMA-functionalized ~ 100 nm sized pores in the presence of \([\text{Fe(CN)}_6]^{3+/4-}\) and b) in the presence of \([\text{Ru(NH}_3)_6]^{2+/3+}\). c) PCBMA-functionalized mesoporous films (pore size <10 nm) in the presence of \([\text{Fe(CN)}_6]^{3+/4-}\) and d) in the presence of \([\text{Ru(NH}_3)_6]^{2+/3+}\). A higher monomer concentration of 1.3 M as compared to Figure 2 resulting in a higher PCBMA amount as compared to Figure 2. PCBMA is only present inside the pores due to the destruction of initiator on the external planar surface by a CO\(_2\) plasma treatment\(^{14, 34}\).
Acidic pH and electrostatic exclusion for [Ru(NH$_3$)$_6$]$^{2+}$/3+. The electrostatic situation at pH 3 for PCBMA-modified pores results in the electrostatic exclusion of positively-charged [Ru(NH$_3$)$_6$]$^{2+}$/3+ probe molecules when the pore size is in the range of the Debye screening length (mesoporous films with pores < 10 nm), and at a sufficiently high PCBMA amounts and thus sufficiently high charge density (Figure 3d pH 3, Figure 4d blue trace). As expected a transition from silica-like behavior (neutral pores at pH ≤ 3) to PCBMA-dominated behavior (positively charged) is observed which results in the complete exclusion of the positively-charged [Ru(NH$_3$)$_6$]$^{2+}$/3+ probe molecule in PCBMA-functionalized mesoporous silica thin films (Figure 4 b, d blue traces) at this acidic pH.

For films with larger pores of ~ 100 nm, this electrostatic exclusion is not observed (Figure 4a c blue traces) because the pore size being larger than the Debye screening length. Although by electron microscopy the films with ~ 100 nm sized pores are observed to have filled pores after a polymerization time of 240 min using a monomer concentration of 1.3 M (Figure S1 corresponding to Figure 3a, b) no exclusion of positively-charged probe molecules is observed for these films under acidic conditions. This may be attributed to the polymer conformation or differences between dry and wet polymer state, and the ions taking part in the charge equilibrium of the pores, which permits the ions to enter these relatively large pores, or to a limited number of unfunctionalized pores. However, these observations indicate that pore filling with polymer is not the determining parameter to control the pore accessibility in polymer-functionalized porous materials. Films with pore sizes of ~ 100 nm only show a slight decrease in $j_p$ at pH 3 (Figure 4 c blue trace, Figure 3b) from $3.5 \times 10^{-4}$ A cm$^{-2}$ (IR C=O absorption of 0.01, Figure S1) to $1.5 \times 10^{-4}$ A cm$^{-2}$ (IR C=O absorption of 0.13, Figure S1) for the positively-charged probe molecule [Ru(NH$_3$)$_6$]$^{2+}$/3+ upon PCBMA functionalization. Mesoporous films with pore sizes < 10 nm show a decrease in $j_p$ (Figure 4d blue trace, Figure 3d) from $0.75 \times 10^{-4}$ A cm$^{-2}$ (IR C=O absorption of 0.05, Figure S1) to 0 (IR C=O absorption
Figure S1) - displaying complete electrostatic exclusion at this PCBMA amount corresponding to 2-5 monomers per chain as described above.

Figure 4. Peak current density ($j_p$) in dependence of PCBMA amount encoded by the C=O stretching band intensity at 1728 cm$^{-1}$ extracted from Si-O-Si stretching band at 1070 cm$^{-1}$ normalized infrared spectra. 

- **a)** For ~100 nm sized pores after PCBMA functionalization in the presence of 0.3 M CBMA,
- **b)** for <10 nm sized mesopores after PCBMA functionalization in the presence of 0.3 M CBMA,
- **c)** for ~100 nm sized pores after PCBMA functionalization in the presence of 1.3 M CBMA and a CO$_2$ plasma treatment resulting in PCBMA formation exclusively inside the pores and not on the planar outer surface, and
- **d)** for <10 nm sized mesopores after PCBMA functionalization in the presence of 1.3 M CBMA and a CO$_2$ plasma treatment resulting in PCBMA formation exclusively inside the pores and not on the planar outer surface. Lines are a guide to the eye. The grey scale indicates three categories of PCBMA amount used in Figure 5.

**Pore accessibility in the zwitterionic state.** By increasing the pH to 9, the residual free silanol groups of the silica mesopore walls, as well as the PCBMA carboxyl groups are expected to be deprotonated resulting in a zwitterionically-charged PCBMA (Figure 1, Figure S8). Interestingly, films with pore sizes of ~100 nm show higher absolute $j_p$ values at pH 9 for [Ru(NH$_3$)$_6$]$^{2+/3+}$ (Figure 3b pH 9, Figure 4c, d orange) as for [Fe(CN)$_6$]$^{3-/4-}$ (Figure 3a pH 9, Figure 4a, c red). This likely indicates that the silica pore walls are still visible for the probe
molecules to a certain extent due to the polymer conformation and grafting density and/or inhomogeneous functionalization of the pore walls. This influence of residual silanolate groups is much less pronounced for mesoporous silica films for the same functionalization conditions resulting in highest PCBMA amount achieved in this study. For this PCBMA amount both molecules are almost completely excluded from the PCBMA functionalized mesopores at basic pH and thus in the presence of a zwitterionic PCBMA (Figure 4d, orange and red). Comparing $j_p$ at pH 9 to the one at acidic pH 3, as expected, a smaller peak current for the negatively-charged $[\text{Fe(CN)}_6]^{3-\Delta^-}$ at pH 9 (Figure 4 red) than at acidic pH (Figure 4 dark blue) is observed. Comparing this result with theoretical studies,\textsuperscript{27, 37} we can attribute this observation to the zwitterionic state and polymer filling the majority of the pore diameter under these conditions. This state results in an electrostatic equilibrium within the pore in the absence of further counterions. Reaching a critical confined polymer amount in the zwitterionic state, the polymer interaction free energy contributions appear to dominate over the entropy contributions of mixing or electrostatic interactions. For lower polymer amounts (Figure 3 polymerization times $< 30$ min), the positively-charged probe molecule is still able to access the pores under basic conditions. This likely results from pore wall effects and PCBMA amounts smaller than the concentration of silanolate functional groups at the pore wall. The zwitterionic polymer, PCBMA, appears to result in an electrostatically “bipolar-charged” pore due to the close proximity of charges with the spatial confinement of a pore. In contrast to the typical Donnan exclusion in which confined negative charges repel anions and confined positive charges repel cations in case the charges electrostatically span the entire pore, here the simultaneously confinement of negative and positive charges leads to a very particular set of exclusion conditions. This particular phenomenon of “bipolar” environments has previously been observed in electrostatic ion chromatography,\textsuperscript{38} and was also noted in a study using relatively low polymer amounts resulting in a zwitterionic pore due to a positively-charged polymer in the presence of the simultaneously negatively-charged silica pore wall.\textsuperscript{29} A comparable effect
was observed in bipolar, covalently crosslinked LBL-assembled polyelectrolyte multilayers. At a solution pH of 7 where both PEL-carrying aminogroups and carboxyl groups are ionized, the film excluded cations and anions. Interestingly, non-homogeneous counterion and ligand distributions have also been reported in modeling studies of polymeric adsorbent media for bioseparation applications. A dependence of ion distribution and ion exclusion from polymeric dextran-based structures on counterion shape, size, charge and structure inducing local non-electroneutrality, and strongly affecting ligand loading is reported. The slightly higher $j_p$ observed for the positively-charged probe molecule $[\text{Ru(NH}_3)_6]^{2+/3+}$ under these zwitterionic conditions for the larger ~ 100 nm pores may result from the presence of individual unfunctionalized pores, and thus the negatively charged silica pore walls remain accessible.

**Influence of PCBMA amount and pore size on the pH-induced transition of ionic pore accessibility**

**~ 100 nm-sized pores.** Unmodified mesoporous silica films with pore sizes < 10 nm show a pK$_a$ of 2.5$^{29}$ and an isoelectric point of ~6, whereas PCBMA exhibits a pK$_a$ of ~4 and an isoelectric point of ~8 (Figure S8). For the $[\text{Fe(CN)}_6]^{3-/4-}$ probe molecule, the observed $j_p$, and thus the pore accessibility for PCBMA-modified 100 nm-sized porous films decreases with increasing pH (Figure 2a, 3a) down to almost zero for basic pH > 8. At pH 3 an increasing PCBMA amount in ~ 100 nm sized pores (Figure 3a) leads to an increasing pre-concentration of $[\text{Fe(CN)}_6]^{3-/4-}$ as well as a broadening pH dependent transition of $j_p$. Going from acidic to basic pH a transition from PCBMA amount dependent $[\text{Fe(CN)}_6]^{3-/4-}$ pre-concentration to the PCBMA amount independent almost constant state of exclusion is observed (Figure 3a). Additionally, this pH-dependent $j_p$ decrease between acidic and basic pH is shifted to higher pH-values for higher PCBMA contents (Figure 3a, 0-240 minutes). An increase in the PCBMA content (Figure 3a, 0-240 minutes) leads to a further shift in this pH transition, with a significant decrease in the $j_p$ already observed at pH 5-6, and still continuing until a pH of 8 (Figure 3a).

To summarize, the pH-dependent pore accessibility for negatively-charged $[\text{Fe(CN)}_6]^{3-/4-}$ probe...
molecules with increasing PCBMA content in the films with ~ 100 nm pores, two effects are observed: first, a significant broadening of the pH transition with increasing polymer amount going from a pre-concentration at acidic pH due to electrostatic attraction to exclusion at pH 9-10; second, a shift in the pH transition is observed starting at lower pH values. This is a remarkable effect due to the combination of the porous structure and zwitterionic polymer functionalization of the spatially confined space that has not been observed systematically to-date, but restricted to theoretical predictions. These results show that the pore accessibility can be tuned over a wide range by the gradual adjustment of the polymer content within a pore, not by changing the chemical composition. Therefore, changing the functional density, and thus the degree of confinement, the permselectivity can be easily tuned. This not only supports previous theoretical calculations, but also opens up a completely new playground of confinement-controlled chemistry.

**Mesoporous silica.** In contrast to the films with ~ 100 nm sized pores, PCBMA-modified mesoporous films with < 10 nm sized pores exhibit an even broader pH-dependent transition of $j_p$ for the negatively-charged $[\text{Fe(CN)}_6]^{3/-4}$ probe molecules with a decreasing $j_p$ already observed between pH 3 and 4 (Figure 3c, 90-240 minutes). This corresponds to a shift of 4 pH units compared to free PCBMA in solution (Figure S8b). The decrease in $j_p$ finished at a pH of 7 for low polymer amounts (Figure 2c, Figure 3c). For higher PCBMA contents the decrease in $j_p$ almost finishes until a pH of 5 (Figure 3c) followed by a further decrease to zero between pH 5 and 8. This change between pH 5 and 8 is more pronounced when polymerized in the presence of 1.3 M CBMA monomer solution (Figure 3c) resulting in a factor of two higher maximum amount of PCBMA (at long polymerization times of 90-240 minutes). This higher PCBMA amount is also associated with an increase in the $j_p$ (from $1.5 \times 10^{-4}$ A cm$^{-2}$ to $3.8 \times 10^{-4}$ A cm$^{-2}$) by a factor of 2.5 due to pre-concentration under acidic conditions (pH 3). For these < 10 nm pore size mesoporous films, the silica pore wall may have a stronger influence on the measured
Due to the smaller pore diameter and the larger surface area. This is indicated by the pre-
concentration of the positively-charged [Ru(NH₃)₆]²⁺/³⁺ probe molecules under basic conditions
for low polymer amounts (Figure 2d, Figure 3d). Furthermore, the clearly visible confinement
effects that are observed through the variation in the pore accessibility that are pH- and PCBMA
content dependent, are especially pronounced in the small mesopores. With increasing amount
of PCBMA a different pH-dependent behavior as compared to the larger ~ 100 nm pores results.
This is especially visible by the difference in pH-dependent jₚ transition and their changes with
increasing PCBMA amount for [Ru(NH₃)₆]²⁺/³⁺ (Figure 2d, 3d). Before reaching a critical
PCBMA amount that compensates the number of silanolate groups at basic pH a transition from
[Ru(NH₃)₆]²⁺/³⁺ exclusion at acidic pH to accessible mesopores is observed. This behavior
changes to constant pH-independent exclusion of [Ru(NH₃)₆]²⁺/³⁺ after reaching sufficiently
high PCBMA amounts (Figure 3d). This constant exclusion can be explained by electrostatic
repulsion at acidic pH and a “bipolar” pore at basic pH. For 100 nm sized pores no exclusion
of [Ru(NH₃)₆]²⁺/³⁺ at acidic pH is observed most probably due to the larger pore sizes not being
in the range of the Debye screening length. This observation is in accordance with theoretical
work from Szleifer and co-workers that states that for pore diameters larger than approximately
30 nm, a pKₐ comparable to the polymer in solution is observed, whereas, the pKₐ is shifted for
smaller pore diameters.²⁷

**pH of equal probe molecule concentration.** The above mentioned cross influence of pH, pore
size, and polymer amount becomes more pronounced when comparing the pH values at which
the jₚ for [Ru(NH₃)₆]²⁺/³⁺ and [Fe(CN)₆]³⁻/⁴⁻ are identical, and thus where the pH-dependent jₚ
curves cross each other (Figure 5).
Figure 5. Peak current density ($j_p$) determined from cyclic voltammetry in dependence of solution pH for PCBMA-functionalized ~100 nm pore sized films in the presence of [Fe(CN)$_6$]$_{3/-4}$ (filled symbols) and [Ru(NH$_3$)$_6$]$^{2+/3+}$ (empty symbols) for a) a low polymer amount (polymerization time of 4 and 8 min), b) an intermediate polymer amount (polymerization time of 15-60 min), and for c) high polymer amounts after polymerization times of 90-240 min. Accordingly, the results for the <10 nm pore-sized mesoporous films are shown for d) low polymer amounts (polymerization time of 4-8 min), e) intermediate polymer amounts (polymerization time of 15-30 min) and f) relatively high polymer amounts after polymerization times of 60-240 min. The pH at which the $j_p$ for [Fe(CN)$_6$]$_{3/-4}$ and [Ru(NH$_3$)$_6$]$^{2+/3+}$ are comparable are indicated with a dotted line and an arrow. Low, intermediate and high PCBMA amount corresponds to the grey scale in Figure 4c,d. All porous films are CO$_2$-plasma treated and thus are PCBMA functionalized exclusively inside the pores.

The peak current at this pH is approximately 2.5 and 3 A cm$^{-2}$ for ~100 nm pore sized films, and close to 0 for mesoporous films with pore sizes <10 nm (Figure 5). At the pH of identical $j_p$ for [Ru(NH$_3$)$_6$]$^{2+/3+}$ and [Fe(CN)$_6$]$_{3/-4}$, the concentration of positively and negatively charged...
probe molecule within the pore is equal. The pH at which this equal concentration of 
$[\text{Ru(NH}_3\text{)}_6^{2+/3+}]$ and $[\text{Fe(CN)}_6]^{3-/4-}$ inside the pore is observed shifts significantly to higher pH-values with increasing polymer amount. For the ~ 100 nm pores, the $j_p$ for $[\text{Ru(NH}_3\text{)}_6^{2+/3+}]$ and $[\text{Fe(CN)}_6]^{3-/4-}$ is observed at a pH of around 6, and shifts to a pH of 6.5-6.7 for intermediate PCBMA amounts until a pH of 7.9 - 8.2 for high PCBMA amounts (Figure 5a-c). For mesoporous (< 10 nm pores) PCBMA-functionalized films, the equal $j_p$ pH, in this case where the $j_p$ is almost zero, shifts from between 5 and 5.5 for low polymer amounts, to 5.7 and 7 for intermediate polymer amounts, and to approximately 7.3 for the highest obtained polymer amounts (Figure 5d-f). This polymer amount-dependent shift in the solution pH to reach an equivalent $j_p$ for positively- and negatively-charged probe molecules indicates the complex charge situation of residual silanol groups and pH-responsive polymer, but also shows that increasing the polymer amount requires higher pH values to reach ion exclusion indicating the zwitterionic state. Considering electrostatics and deprotonation of carboxyl groups in a confined space exclusively, this observation is in accordance with theoretical considerations making it more and more difficult deprotonating and thus increasing the charge density in a confined space with an increasing number of charges. On the other hand, the final state within the investigated system is not negatively charged, but a zwitterionically charged one. In terms of confinement effects, a shift towards uncharged segments in order to minimize local electrostatic repulsions would be expected as observed and theoretically explained for charged polymers baring a single charge. Another effect that may be relevant here is the mixing entropy of mobile ions from solution into the PCBMA-functionalized pore. In the zwitterionic state, all ions independent of their charge are excluded. Nevertheless, it is a very interesting observation that although the pK$_a$ of PCBMA in solution is approximately 4, and the isoelectric point is approximately 8 (Figure S8), the oppositely-charged probe molecules equal their concentration, depending on pore size and polymer amount over a very large pH range between pH 2.5 and 8.2. This experimental observation is possible due to controlled pore
CONCLUSIONS

By controlling pore functionalization and zwitterionic polymer amount, a gradual tuning of ionic pore accessibility, and a solution pH-shift of equal pore accessibility for positively and negatively charged probe molecules between a pH of 2.5-8.2 was achieved, and an unexpected complete ion exclusion in the zwitterionic polymer state independent of molecular charge was observed for nanometer-sized pores. An increasing PCBMA amount results in a polymer amount proportional to the preconcentration of countercharged [Fe(CN)₆]³⁻/⁴⁻ ions at acidic pH, and thus in a positively-charged pore and a reduced accessibility reaching an electrostatic exclusion of equally charged [Ru(NH₃)₆]²⁺/³⁺. Besides this observation, which has already been observed in strong non-zwitterionic polyelectrolytes¹⁷, the zwitterionic state of the PCBMA used here under basic conditions offers complex pore charge and transport that is dependent on the polymer amount and pore size resulting in a charge-independent ion exclusion for a sufficiently high polymer amount when pore dimensions are below 10 nm. This impressively shows the effect of spatial confinement and complex charged states on pore accessibility. This effect of spatial confinement and complex zwitterionic charge is even more prominent when investigating the pH transition from a positively-charged polymer to the zwitterionically-charged polymer state. The pH transition broadens with increasing polymer amount. Additionally, the pH of equal concentration for [Ru(NH₃)₆]²⁺/³⁺ and [Fe(CN)₆]³⁻/⁴⁻ shifts within a pH range from 2.5 to 8.2, dependent on PCBMA amount and pore size. This broad pH range clearly shows the potential to control the transport characteristics by understanding and using confinement effects. Consequently, a fascinating field of nanoscale chemistry may allow the
design of material properties by exclusively controlling nanoscale organization of functional components without changing the components themselves.

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EXPERIMENTAL SECTION

Synthesis of N,N-(Diethylamino)-dithiocarbonylbenzyl(trimethoxy)silane (SBDC). SBDC was synthesized according to a previously published protocol. Briefly, sodium N,N-diethyldithiocarbamate trihydrate was recrystallized from methanol before use. Then, the sodium N,N-diethyldithiocarbamate (1.46 g, 8.59 mmol) was dissolved in 10 mL of dry THF before being dropped into a solution of p-(chloromethyl)phenyltrimethoxysilane (1.89 mL, 8.59 mmol) in 10 mL of dry THF. The solution was stirred for 3 h at room temperature before being filtered to remove the NaCl that formed during the reaction. The THF was evaporated under reduced pressure and the product was dried under high vacuum to yield a yellow oil (22% yield). The product was characterized by $^1$H-NMR. $^1$H-NMR (300 MHz, CDCl₃) δ 7.60 (d, J = 8.1 Hz, 2H, C₆H₄), 7.42 (d, J = 8.1 Hz, 2H, C₆H₄), 4.55 (s, 2H, CH₂S), 4.04 (q, J = 6 Hz, 2H, NCH₂), 3.73 (q, J = 6 Hz, 2H, NCH₂), 3.61 (s, 9H, Si(OCH₃)₃), 1.28 (t, J = 7.2 Hz, 6H, CH₃).
Mesoporous film preparation (< 10 nm pores). Mesoporous silica films were synthesized via a sol-gel method based on the oxide precursor tetraethoxysilane (TEOS) in the presence of the template Pluronic® F127. The precursor solutions were stirred for 24 h and used to produce films by evaporation induced self-assembly (EISA)\textsuperscript{41} on ITO, glass, or silicon wafer substrates at 40-50 % relative humidity and 298 K at a withdrawal speed of 2 mm/s. The precursor solution was prepared using the following molar ratios 1 TEOS : 0.0075 F127 : 24 EtOH : 5.2 H\textsubscript{2}O : 0.28 HCl. Freshly deposited films were stored at 50 % relative humidity in a climate chamber for 1 h. Then a stabilizing thermal treatment was carried out in two successive 1 h steps at 60 and 130 °C. The temperature was then increased to 350 °C at a rate of 1 °C/min. The films were then stabilized at 350 °C for 2 h. Subsequently, the films were rinsed with ethanol and stored under ambient conditions. The resulting films were 150-300 nm thick with a porosity of ~50 % as measured by ellipsometry.\textsuperscript{35}

~ 100 nm pore-sized film were prepared according to work from Retsch et al.\textsuperscript{42}. Porous silica films with a pore size of~ 100 nm were prepared using polystyrene nanoparticles (PS-NP) as template. A solution containing 0.1 mL 30 wt% LUDOX silica nanoparticles (Sigma Aldrich), 8.94 mL (1.12 wt%) PS-NP (diameter 115 nm), and 0.96 mL water (milli Q) was used for vertical lifting deposition onto ITO-coated glass substrates using a withdrawl speed of 600 nm/s at 50 % relative humidity. Subsequently, the films were heated up to 500 °C using a 1°C/min temperature gradient. The films were then stabilized at 500 °C for 3 h. Finally, the films were rinsed with ethanol and stored under ambient conditions.

Surface grafting of SBDC and subsequent plasma treatment. The prepared mesoporous films were inserted into a 1 mM solution of SBDC prepared in dry THF. The films were then stored in this solution for either 24 h at room temperature (for subsequent polymerization with a monomer concentration of 0.3 M CBMA) or for 4 h at 70 °C (in case of a subsequent polymerization with a monomer concentration of 0.3 M CBMA). Subsequently, the films were
extracted in THF for 30 min. The subsequent CO2-plasma treatments were performed according to a protocol from Babu et al.\textsuperscript{34} using a Diener Electronic Femto equipment (13.56 MHz, 0-50 W), at a pressure of 0.4 mbar and a power of 20 % for 12 s.

**Synthesis of Carboxybetaine Methacrylate (CBMA).** CBMA was synthesized according to a published protocol.\textsuperscript{43} N,N-Dimethylaminoethyl methacrylate (30 mL, 0.18 mol) was dissolved in 130 mL of dry acetone before being cooled to 0 °C. Then β-propiolactone (10.87 mL, 0.17 mol) was added, and the mixture was stirred at 0 °C for 2 h. The white precipitate was filtered off and dried under reduced pressure to obtain the final CBMA monomer, which was stored at 4 °C (79 % yield). The product was characterized by \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR, and mass spectrometry (ESI). \textsuperscript{1}H-NMR (500 MHz, D\textsubscript{2}O) δ 6.22 (s, 1H, =CH\textsubscript{2}), 5.85 (s, 1H, =CH\textsubscript{2}), 4.71 (t, 2H, J = 5Hz, CH\textsubscript{2}), 3.86 (t, 2H, J = 5Hz, CH\textsubscript{2}), 3.75 (t, 2H, J = 5Hz, CH\textsubscript{2}), 3.26 (s, 6H, CH\textsubscript{3}), 2.81 (t, 2H, J = 5Hz, CH\textsubscript{2}), 2.01 (s, 3H, CH\textsubscript{3}). \textsuperscript{13}C-NMR (126 MHz, D\textsubscript{2}O) δ 176.1, 168.4, 135.2, 127.8, 62.5, 62.2, 58.5, 51.4, 30.6, 17.6. MS (ES)\textsuperscript{+}: m/z (%) = 1146.7 (45) [5M+H]\textsuperscript{+}, 917.6 (100) [4M+H]\textsuperscript{+}, 688.4 (35) [3M+H]\textsuperscript{+}, 459.3 (30) [2M+H]\textsuperscript{+}, 230.1 (40) [M+H]\textsuperscript{+}.

**Polymerization of Carboxybetaine Methacrylate (CBMA).** SBDC-functionalized mesoporous silica films were degassed by several consecutive vacuum–nitrogen cycles and immersed into a degassed aqueous solution of CBMA (0.3M (Figure 2) or 1.3 M (Figure 3)) under a nitrogen atmosphere. To initiate the polymerization, the samples were irradiated (Vilber Lourmat Bio-Link BLX cross-linker) at 365 nm (P = 2 mW cm\textsuperscript{-2}). After performing the polymerization, the films were extracted overnight at room temperature in deionized water to remove non-covalently attached monomer and polymer.

**Infrared spectroscopy measurements (IR).** IR was performed on a Spectrum One (Perkin Elmer) instrument in attenuated total reflection (ATR) mode. Mesoporous films were scratched from both sides of the substrate, and the spectra recorded between 4000 and 600 cm\textsuperscript{-1}. The measured spectra were automatically background corrected and normalized to the Si-O-Si band
at \( \sim 1070 \text{ cm}^{-1} \). Out of two measurements an average absorption value including standard deviation was determined using the absorption of the C=O signal at 1728 cm\(^{-1}\).

**Ellipsometry.** The film thickness and refractive index was determined from films prepared on silicon wafers using a Nanofilm EP3 imaging ellipsometer. One zone angle of incidence (AOI) variation measurements were captured between an AOI of 40 and 80° with a 658 nm laser. The apparent film thickness and refractive indices were calculated from the measured \( \Psi \) and \( \Delta \) angles using the EP4 analysis software supplied with the instrument. The fitting parameters for the silicon oxide layer thickness on the wafer substrate (\( d(\text{SiOx}) = 2.8 \text{ nm} \)) were measured separately. The measured data was fitted with a one-layer box model. Multilayer models present increasing uncertainty, and do not yield better results. The fitting program was allowed to vary the film thickness of the mesoporous silica thin films between 100-500 nm, and the refractive index between 1.1-1.7. All films were measured at three identical marked positions after each modification step. Changes were calculated for each specific position. To determine the porosity from the measured refractive indices, the Brüggemann effective medium approximation was used as discussed elsewhere.\(^{35, 44} \)

**Cyclic Voltammetry.** Quantitative variations in permselectivity were studied by following the changes in the voltammetric peak currents associated with cationic \( \text{Ru(NH}_3)_6^{2+/3+} \) and anionic \( \text{Fe(CN)}_6^{4-/3-} \) redox species, diffusing across the mesoporous film. These measurements were recorded with an Autolab PGSTAT302N (Metrohm). Mesoporous films modified with PMETAC were prepared on bare indium tin oxide (ITO) electrodes. The measurements were performed with a 2 mM solution of the probe molecule in a 0.1 M aqueous solution of potassium chloride. The pH was adjusted with either sodium hydroxide or hydrochloric acid and measured by using pH-paper (Roth) or a pH-electrode (VWR pH 110). A Ag/AgCl electrode (BASi RE-6) was used as the reference electrode, and various scan rates between 25 and 1000 mV s\(^{-1}\) were measured. The measured electrode area was 0.21cm\(^2\). Preconcentration observed in these measurements is defined as measured \( j_p \) PCBMA modified pores > \( j_p \) unmodified pores.
REFERENCES

