Dendritic Nanopores

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Functional Group Density and Recognition in Polymer Nanotubes**

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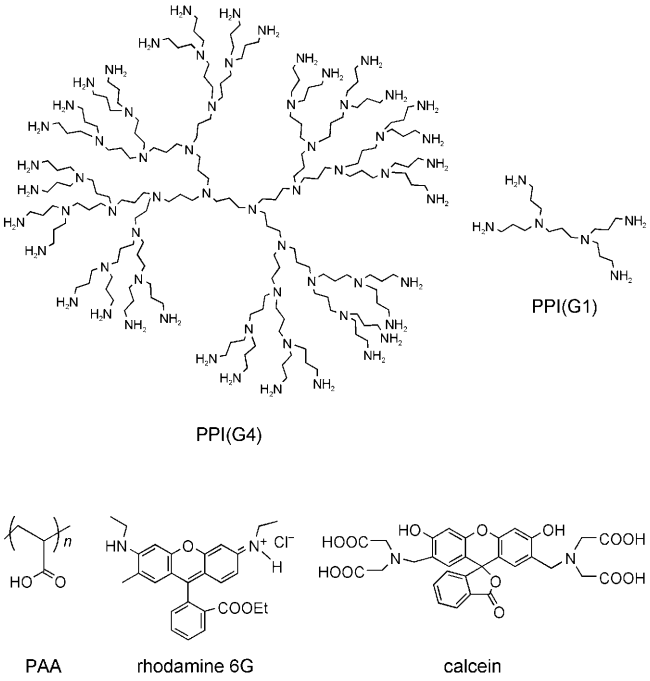
Control of the placement of functional groups within confined nanospaces is of interest because of its potential in applications such as sensing and separations.[1–12] For effective recognition of analytes within nanopores, it is necessary that control over two factors—pore size and functional group presentation—be achieved. While pore-size changes in nanopores and their effects upon recognition have been reported,[2,13–15] it is also important to investigate the relative effect of binding-site density upon molecular recognition inside these nanopores. Herein, we report the effect that the density of functional groups within polymeric nanotubes could have upon complementary analyte molecules that pass through membrane nanopores. Since dendrimers are known as a class of artificial macromolecules that can have a high density of functional groups with a significant degree of control,[16–20] we hypothesized that these molecules are ideal candidates for this study. Even though dendrimer-based "test-tube-like" nanostructures, where one end of the tube is closed, have been obtained using alumina membranes, the possibility of molecular recognition within such nanotubes has not been investigated.[21,22] Recently, we described a very simple and versatile methodology by which polymer-based functionalities can be incorporated inside nanoporous polycarbonate membranes by using polyvalent interactions.[23] We have utilized this methodology to achieve the formation of dendrimer-functionalized nanotubes.

Since polypropyleneimine (PPI) dendrimers[24] are easily accessible, water-soluble, and charged, we used these molecules to test our hypotheses (Scheme 1). Our methodology for functionalization of the membrane nanopores uses electrostatic interactions and the PPI dendrimers are positively charged. Therefore, it is necessary that the predendrimer-functionalized membrane nanopores be negatively charged. To achieve this, we first incorporated Sn^{2+} ions into the pore walls of the membrane by using its poly(vinylpyrrolidone) functionalities. The Sn^{2+} ions were used to introduce a layer of an anionic polymer, polyacrylic acid (PAA). We have shown that the vacuum-filtration-based incorporation of polymers is uniform throughout the membrane.[23] This anionic polymer-functionalized membrane interior was then utilized to incorporate the cationic PPI dendrimers. The schematic illustration of membrane functionalization using PPI dendrimers is shown in Figure 1.



Scheme 1. Structures of PPI(G4), PPI(G1), PAA, rhodamine 6G, and calcein.

Prior to investigating how this functionalization effected recognition of the analytes that passed through the membrane, we needed to characterize the nanotubes formed in this process. We were first interested in assessing the change in pore size of the membranes upon dendrimer functionalization. While we were able to previously demonstrate drastic pore-size changes by using our self-assembling polymers, systematic control over the size of the final nanopores was not demonstrated.[23] We envisaged that dendrimers would provide a significant amount of control over the nanopore dimensions. We tested this possibility by assessing the pore-diameter change induced by the dendrimer functionalization; this was carried out by using a calibration curve obtained from

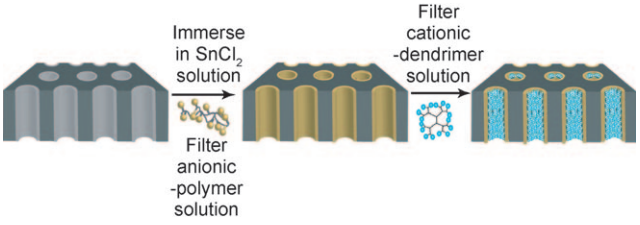


Figure 1. Schematic illustration of the modification of polycarbonate membranes with dendrimers.

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commercially available membranes with different pore diameters. The diameter of the bare membrane was reduced from 30 nm to 28 nm upon incorporation of PAA. When this anionic membrane was then functionalized with dendrimers, we found that the pore size reduced systematically with increasing dendrimer generation (G); the pore sizes for PPI(G1)-PPI(G4) functionalized membranes were found to be 26 nm, 23 nm, 19 nm, and 13 nm, respectively (Table 1).

Table 1: Pore sizes, diffusion rates of molecules through the pores, and separation factors for dendrimer-modified membranes.

Dendron	Size [nm][a]	D_{R6G} [$\times 10^6$][b]	$D_{calcein}$ [$\times 10^6$][b]	Separation factor (α)[c]
none	30	1.0	1.0	1.0
PAA	28	1.0	0.6	0.6
PPI(G1)	26	1.0	0.9	0.9
PPI(G2)	23	0.8	0.8	1.0
PPI(G3)	19	0.05	0.1	2.0
PPI(G4)	13	0.06	0.3	5.0
PPI(G5)	–[d]	–[e]	0.1	–[e]
PC11	9	0.35	0.1	0.3
none	10	0.8	0.7	0.9
BE(G1)	8.0	0.8	0.5	0.6
BE(G2)	6.6	0.7	0.3	0.4
BE(G3)	4.5	0.03	0.01	0.3

[a] Pore sizes measured against a calibration curve generated using the pore sizes of commercial membranes. [b] Diffusion rates D measured from the concentration of the dye in the reservoir. [c] The separation factor (α) is the ratio of the D value of the two dye molecules. [d] The pore size could not be measured because of consistent membrane leakage. [e] The D vaue was too small to be measured for calcein, therefore the separation factor was immeasurably high.

We next investigated the possibility of visualizing the formation of nanotubes by using transmission electron microscopy (TEM). This is interesting because it provides 1) information on whether the functionalization is uniform throughout the membrane, in which case the length of the nanotubes should correspond to the thickness of the membrane and 2) qualitative information about the generation-dependent stability of the PAA–PPI-based nanotubes. Since we were not able to observe any nanotube structures of PAA alone by TEM, it is reasonable to assume that isolation of nanotubes from the PAA–PPI combination is an indication of the dendrimer's ability to stabilize the nanotube assembly. The nanotubes were liberated by dissolving the membrane with dichloromethane on a TEM grid. We were able to isolate nanotubule structures even for lower-generation dendrimers (Figure 2a). However, we also noticed that a greater number of nanotubes can be isolated from higher-generation dendrimers than can be isolated from lower-generation dendrimers (Figure 2a–c), which suggests that higher-generation dendrimers provide better stabilization than the lower-generation dendrimers. This is understandable, since polyvalent interactions play an important role in layer-by-layer deposition,[25,26] therefore higher-generation dendrimers should provide a better affinity for the polyanionic PAA.

The above studies show that the dendrimers play a key role in systematically changing the size of the pores and the

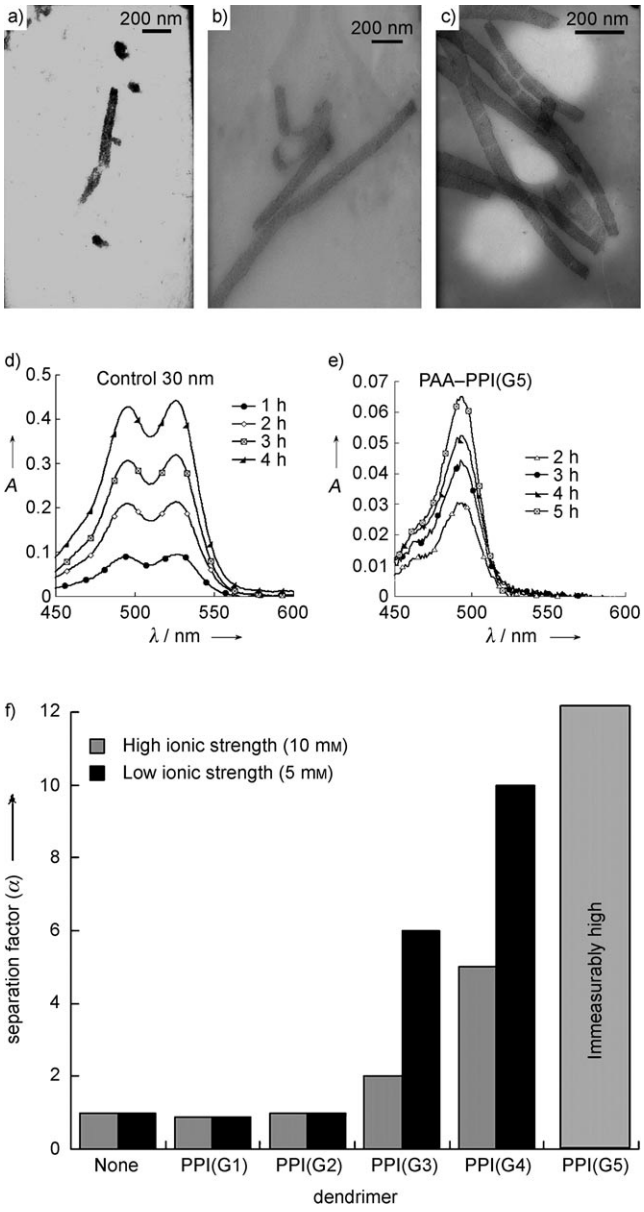

Figure 2. TEM images of a) PAA–PPI(G1), b) PAA–PPI(G4), and c) PAA–PPI(G5) nanotubes. Absorption spectra obtained from the reservoir compartment of a U tube separated by d) a bare 30 nm membrane and e) a PAA–PPI(G5)-functionalized 30 nm membrane. f) Separation factors obtained for calcein from modified and unmodified membranes.

stability of the nanotubes. We were subsequently interested in addressing the effect of functional group density upon recognition of the analyte molecules within the nanotubes. For this purpose, we used the differential transport of dye molecules, calcein and rhodamine 6G (Scheme 1), as an indicator for the recognition process. A buffer solution containing an equimolar mixture of these two dye molecules was added to the feed compartment of a U tube that was separated by the functionalized nanoporous membrane, while the reservoir contained the same buffer solution without any dye molecules. Since the positively charged PPI dendrimers decorate the interiors of the nanopores, it is reasonable to

expect that the negatively charged calcein would diffuse through the pores more rapidly compared to the positively charged rhodamine 6G. In the control experiment with the unfunctionalized membrane, there was no observable difference in the diffusion rates of the analytes (the ratio of the diffusion rates is represented as the separation factor α, Figure 2d and Table 1). When the membrane was functionalized with PAA, the diffusion of rhodamine 6G was slightly faster than that of calcein. This is understandable because rhodamine 6G is positively charged and PAA provides the negatively charged interior for the membrane. However, upon functionalization with PPI(G1) and PPI(G2), there was no discernible difference between the rates of diffusion of the two dye molecules and that of the unfunctionalized membrane. (Table 1). This suggests that these two dendrimers have essentially neutralized the negative charge of PAA, but were not able to provide the surface functionalization with an overall positive charge. When the PAA-functionalized membranes were treated with PPI(G3)–PPI(G5), significant differences in the rates of diffusion were observed, with calcein being favored. It is interesting to note that the α value increases as the dendrimer generation increases, with values of 2.0 for PPI(G3), 5.0 for PPI(G4), and an immeasurably high value for PPI(G5) (Figure 2e,f and Table 1).

At higher generations, dendrimers are expected to make a transition from a linear to a globular shape with a concomitant increase in the functional group density (that is, the amino functionalities).[17,18,20] It is easy to imagine a linear small molecule (PPI(G1) and PPI(G2)) involved in an electrostatic complex with PAA, where most of the amino functionalities are engaged in binding to the carboxylic acid. However, when a globular molecule is bound to a negatively charged surface, it is difficult to imagine a conformation where all the amino functionalities are engaged in the complex (see Figure 3a for an illustration). Therefore, there will be a significant number of positively charged functionalities left for selective recognition of calcein. To test this hypothesis, we first investigated whether this is indeed an electrostatic discrimination by simply varying the ionic strength of the solutions. If the lower-generation dendrimers provide only the charge neutralization, then the ionic-strength change should have no effect on the separation factor α. On the other hand, this factor should significantly increase at lower ionic strengths for higher-generation dendrimers, which was indeed found to be the case. While the α value was unchanged for PPI(G1) and PPI(G2), it increased to 6.0 and 10.0 for PPI(G3) and PPI(G4), respectively (Figure 2f), thus supporting our hypothesis.

The lack of separation in the first two generations, but significant separation in the third generation and above, can be attributed to the structural transition from a linear to a globular structure. The large differences in the separation efficiency among the higher-generation dendrimers (G3–G5) is attributed to the amino functional group density. However, an alternative explanation for the enhanced separation factor is that it is simply due to the fact that higher generation dendrimers provide a greater change in the pore size and thus better recognition. To test whether the functional group density plays a significant role in the observed enhancement

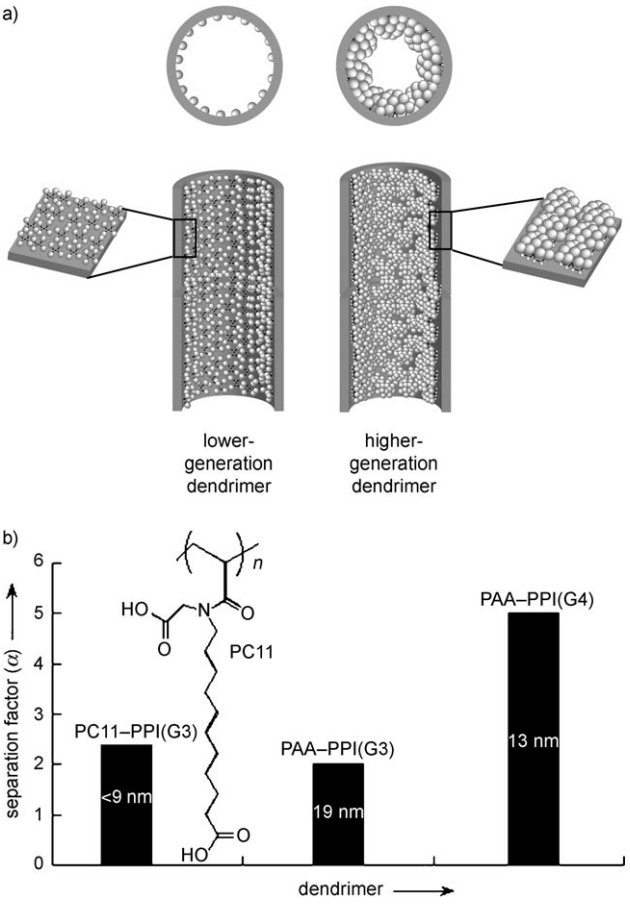

Figure 3. a) Schematic illustration of generation dependence. Lower-generation dendrimers provide charge neutralization, while globular, higher-generation dendrimers modify the functional group display. b) Separation factors for calcein obtained from PC11–PPIG dendrimer-modified 30 nm membranes and structure of PC11.

in separation, we first functionalized the membrane with PC11, a self-assembling amphiphilic polymer[27] that has been shown to directly decrease the 30 nm membrane to about 9 nm.[23] Since PC11 is an anionic polymer, this also provides an anionic base for the membrane, but with a smaller size than even the PAA-PPI(G4) combination (13 nm). Therefore, it is reasonable that the incorporation of PPI dendrimers in the PC11-decorated membrane will afford pores that are smaller than 9 nm. Considering this, it is interesting to note that PC11–PPI(G3) had an α value of 2.4 whereas PAA–PPI(G3) had an α value of 2.0. Even though the expected size for PC11–PPI(G3) is much smaller than PAA–PPI(G3), only small improvements in the separation was observed (Figure 3b). Even more interestingly, we noted that PAA–PPI(G4) provided much better separation with an α value of 5, despite having a larger pore size (13 nm) than PC11–PPI(G3) (expected size of less than 9 nm; Figure 3b). These experiments clearly illustrate that size reduction alone does not account for the improved separation efficiency and suggest that binding-site density plays a greater role in recognition inside these nanopores.

In order to test the versatility of our process further, we were interested in using a structurally different dendrimer for

decorating the nanopores, which would 1) test whether our observations are broadly applicable and 2) identify the minimum generation of a dendron that is needed to obtain functionalized nanotubes without the support of the polyanionic base, PAA. Therefore, we used anionic benzyl ether dendrons (BE(G1–G3), Figure 4a)[28] to decorate the pores of the 10 nm membranes. In a similar fashion to PPI dendrimers, the pore size was also systematically decreased by increasing the generation of the dendron (see Table 1). The unmodified 10 nm membrane did not show any separation, whereas the dendron-modified membranes favored the diffusion of the cationic rhodamine 6G so that the separation factor α increased with increasing dendron generation (Figure 4b–d). Interestingly, dendrimer nanotubes could be isolated even with the G1 dendron, although not with the small molecule G0. Unlike the PPI dendrimers, reasonable separation factors for rhodamine 6G were observed, even with lower-generation dendrons. This is attributed to the fact that there is no polyelectrolyte prelayer for these dendrons to neutralize the charge and that the inherent pore sizes are much smaller. Therefore, the charges might be more available for recognition of the analytes passing through the membrane.

In summary, we have shown that, firstly, the interaction between the dendritic functionalities and the analyte molecules is enhanced at higher generations. This is attributed to the increased binding site density in higher generation dendrons. Secondly, the size of the pores also does play a role in the ability of these functionalities to discriminate the analyte molecules passing through the pores. However, the binding site density plays a more dominant role. Thirdly, dendrimers, as one would expect, can be used to systematically tune the size of the nanopores in polycarbonate membranes. Finally, even smaller-generation dendrons and dendrimers are capable of providing functionalized nanotubes. However, qualitative evidence suggests that the stability of these nanotubes does increase with generation. We believe that the present work will significantly enhance the repertoire of dendrimers as scaffolds for molecular recognition in confined nanoscale environments, as these are among the most versatile scaffolds for presenting a high density of binding sites.

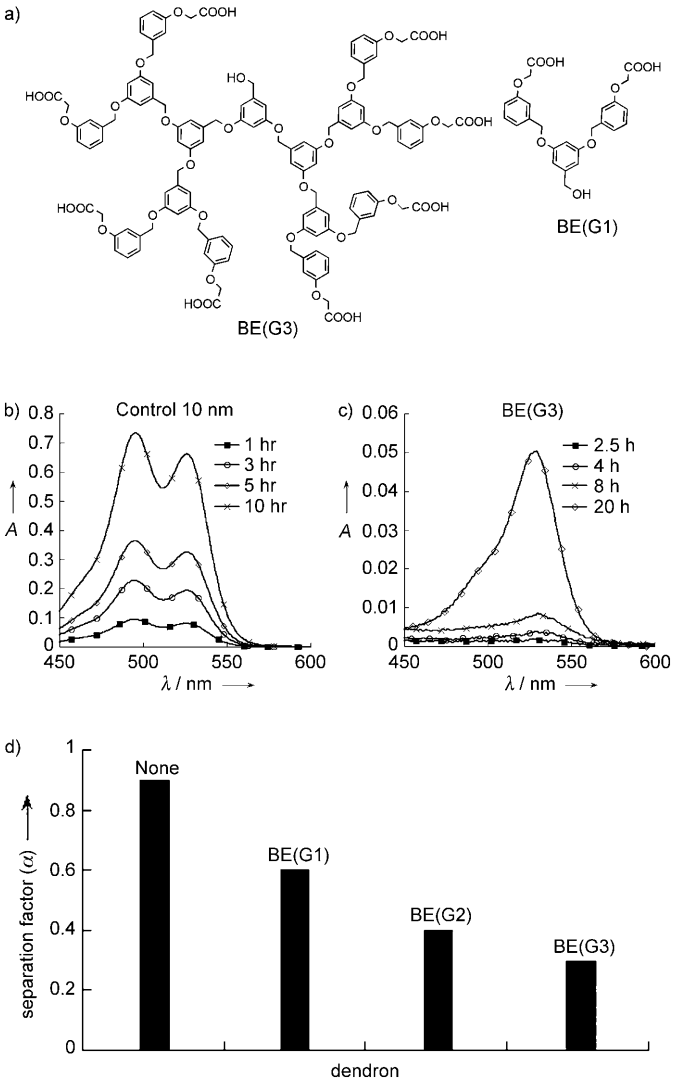

Figure 4. a) Structures of BE(G3) and BE(G1). Absorption spectra obtained from the reservoir compartment of a U tube separated by b) a bare 10 nm membrane and c) BE(G3). d) Separation factors for calcein obtained from modified and unmodified 10 nm membranes.

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