Influence of Amphiphilic Block Copolymer Induced Changes in Membrane Ion Conductance on the Reversal of Multidrug Resistance

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Block copolymers are able to reverse multidrug resistance (MDR) of tumor cells by a yet unknown mechanism. The drug efflux system's direct and indirect inhibition mediated by polymer P-glycoprotein (Pgp) interactions or adenosine triphosphate (ATP) depletion, respectively, may be involved in MDR reversal as well as damage to the membrane barrier caused by polymer insertion into the membrane. To test the latter hypothesis, cellular drug accumulation was monitored in the presence of both overexpressed fluorescently labeled Pgp and different block copolymers. Therefore, a new triblock copolymer (poly(ethylene oxide)-*block*-poly(hexafluoropropylene oxide)-*block*-poly(ethylene oxide)) was designed and synthesized by combined polymerization and polymer analogous reaction. Its administration induced drug uptake, whereas control cells with high Pgp expression levels showed no drug accumulation. Drug uptake was even more pronounced in the presence of another triblock copolymer: (poly(perfluorohexylethyl methacrylate)-*block*-poly(ethylene oxide)-*block*-poly(perfluorohexylethyl methacrylate). The latter polymer's lack of ionophoric activity suggests that ion transport facilitation by polymers is not a determinative factor for MDR reversal.

Introduction

Multidrug resistance (MDR)[a] seriously hampers treatment of cancer, HIV, bacterial, parasitic, and fungal diseases. MDR is associated with the upregulation of cellular drug efflux systems like P-glycoprotein (Pgp) and multidrug resistance proteins. However, in the presence of block copolymers (e.g., ABA amphiphilic triblock copolymers of Pluronic type with hydrophilic poly(ethylene oxide) end blocks and hydrophobic poly(propylene oxide) middle block), the cytotoxic activity of chemotherapeutic agents targeting drug resistant cells increases up to 1000 fold.[1] Because the use of these copolymers occurred by coincidence, and because their operative mechanism is unknown, it remains unclear as to how block copolymer structure could be optimized.

It is still a matter of debate as to whether the multidrug resistance reducing effect of block copolymers and other surfactants is due to the alteration of the physicochemical properties of the cytoplasmic membrane[2] or to alteration of Pgp ATPase activity[3] or perhaps both.[4] Among all of the effects polymers may exert on membranes, the disruption of the barrier function is most intriguing. Supportive evidence has been obtained on model systems, whereby these triblock copolymers were found to facilitate the electroneutral movement of cytostatic drugs—a feature that may clearly contribute to their chemosensitizing ability.[5] Moreover, ion channel activity of Pluronic was observed.[5] Given that polymers are able to mimic Pgp substrates,[6] Pgp inhibition may also result from direct interactions with the polymer.

The chemical composition of the block copolymers is modified in the present study such that their ionophoric activity is inhibited while membrane partitioning is retained. The polymer may still inhibit the drug efflux system if equipped with binding motifs common for all Pgp substrates,[6] thereby contributing to MDR reversal (see Figure 1). In contrast, if the ionophoric activity is significant for MDR reversal, the new polymers should not be able to resensitize the drug resistant cells.

In contrast to the triblock copolymers of the Pluronic type, which consist of a poly(propylene oxide) (PPO) middle block and poly(ethylene oxide) (PEO) outer blocks (Figure 1a), the polymers designed for this study contained poly(hexafluoropropylene oxide) (PHFPO) as a substitute for the hydrophobic middle block of PPO (Figure 1b). The biological effects of the new polymers were compared to the respective effects (i) of Pluronics and (ii) of triblock copolymers with reversed architecture (compare Figure 1c[7]), i.e., polymers consisting of a middle hydrophilic block of PEO embedded in between two hydrophobic poly(perfluorohexylethyl methacrylate) (PFMA) blocks.

Chemistry

PHFPO dicarboxylic acid was polymerized from HFPO. By using a polymer analogous reaction, PEO monomethyl ether (MME) with a molecular weight of 2000 g/mol is attached to the carboxylic acid, which forms a PEO-*b*-PHFPO-*b*-PEO triblock copolymer (Scheme 1). This procedure is essential for creating an ether linkage between the PHFPO and the PEO blocks. An ester linkage produced by direct esterification of the

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[a] Abbreviations: MDR, multidrug resistance; Pgp, P-glycoprotein; ATP, adenosine triphosphate; HFPO, hexafluoropropylene oxide; FMA, perfluorohexylethyl methacrylate; MME, monomethyl ether; HFGF, hexafluoroglutaryl difluorid; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); Tris, 2-amino-2-hydroxymethyl-propane-1,3-diol; HEK293, human embryonic kidney cells; FP, fluorescent protein tag; Diglym, glycol dimethyl ether; DMEM, Dulbecco's modified Eagle medium; FCS, fetal calf serum; P/S, penicillin/streptomycin.

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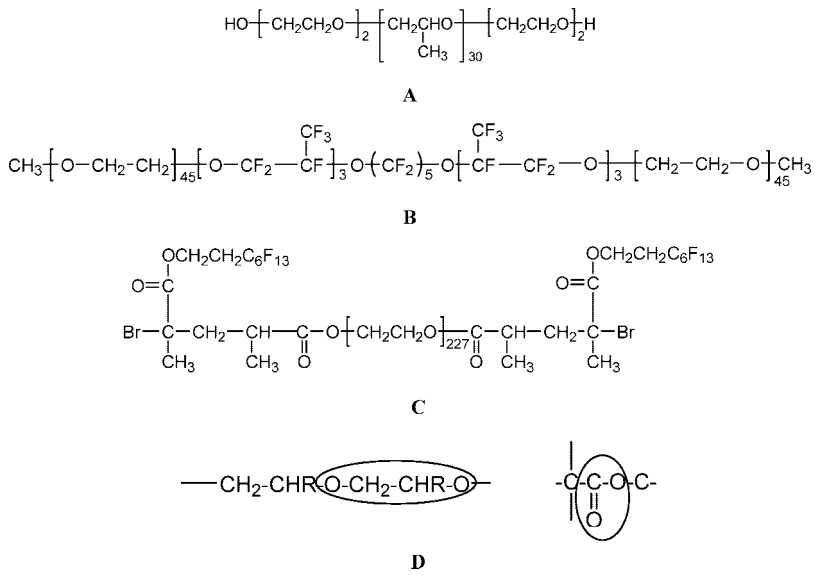
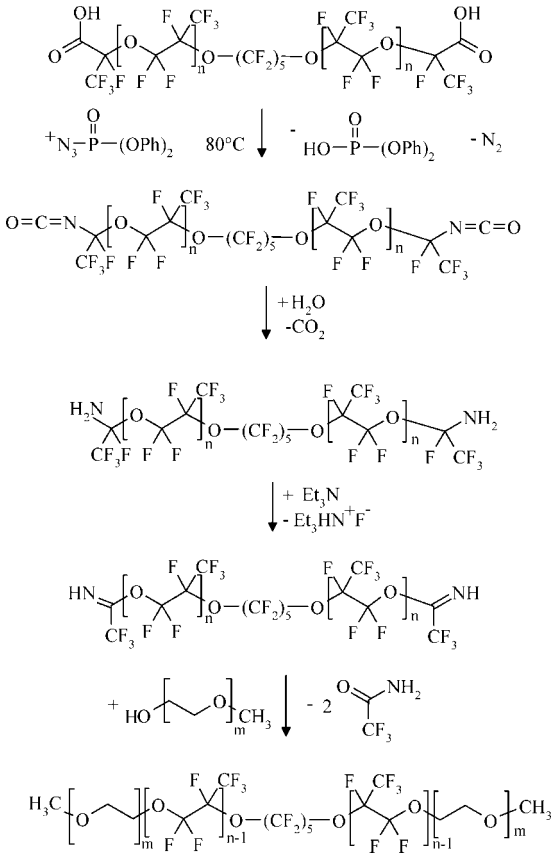


Figure 1. Structure patterns of: Pluronic L61 (A), PEO-*b*-PHFPO-*b*-PEO (B), and PFMA-*b*-PEO-*b*-PFMA (C). Ellipses indicate binding motifs for Pgp substrates of ether (D left) or ester type (D right), which are present in the investigated structures.

Scheme 1. Synthesis of PEO-*b*-PHFPO-*b*-PEO Using Polymer Analogous Reaction



acid with the OH group of PEO would not be stable enough, as the neighboring perfluoro alkyl groups would activate the ester linkage and make it labile to hydrolysis.[8]

Results

Polymerization of HFPO. The literature discusses a number of possible polymerization reactions[9–14] and in particular HFPO's ring opening reactions[15–17] using different carboxylic acid fluorides in the presence of alkali metal fluoride catalysts.

Table 1. Initial Amounts and Molar Masses for PHFPO Obtained

	amounts in mmol			molar mass in g/mol [b]	
substance[a]	HFPO	HFGF	CsF	calculated	measured
PHFPO-1100	40.20	7.72	0.92	1100	1090
PHFPO-1200	33.0	5.96	1.76	1200	1190
PHFPO-1500	80.48	10.96	21.92	1500	1460
PHFPO-2470	16.49	1.25	2.5	2470	2450

[a] Synthesized using the initiator system CsF/HFGF. [b] Determined by titration of carboxylic acid endgroups.

Hexafluoroglutaryl difluoride (HFGF) was used as an initiator in the presence of CsF. Literature deliberates about an anionic mechanism[9,14,18] and a chain transfer polymerization mechanism for this polymerization. To understand this, the polymerization was carried out with identical amounts of HFPO and HFGF, respectively, but with different amounts of CsF (see Table 1). The polymerization experiments were carried out by adding equimolar amounts (with respect to HFGF) or catalytical amounts of CsF for initiator preparation. In the event of anionic polymerization, the molar masses of the polymers should strongly depend on the CsF amounts. However, the experiments show that the molar masses of the polymerization products are independent of the CsF amounts, thus a chain transfer mechanism is likely to occur. During polymerization, small amounts of a fluorinated byproduct occur, but the polymer (or the oligomer) can be purified by vacuum distillation. Termination of the growing PHFPO chain with water leads to the respective PHFPO dicarboxylic acid. This material is used for the synthesis of the respective block copolymers. The molar masses are determined by acid−base titration in trifluoroethanol (see Table 1).

Synthesis of PEO-*b*-PHFPO-*b*-PEO. The respective PHFPO dicarboxylic acid was used for the Curtius rearrangement[19] by adding diphenylphosphoryl azide.[20,21] In a first step, a mixed anhydride is formed, which is acylated by the simultaneously formed azide ions. The acylazid is rearranged to the diisocyanate at 80 °C. The use of equimolar amounts of water leads to the formation of the respective diamine. Then the PEO-MME and triethylamine are added. The addition of triethylamine leads to the reaction of the amine to the imidate, which reacts with the PEO-MME to the triblock copolymers, where the PEO is

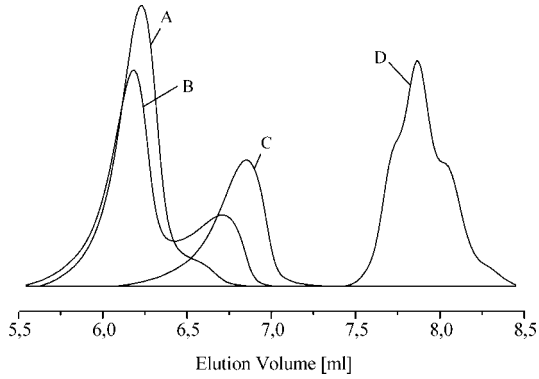


Figure 2. SEC trace of the respective polymers in THF with 3 vol % trifluoroacetic acid. The initial substances are PEO-MME (C) and PHFPO (D). Curve (A) shows the SEC trace of a sample with nearly complete conversion (96%), and only a small shoulder is depicted, which indicates the presence of minuscule amounts of PEO-MME. Trace (B) shows a sample with 75% conversion.

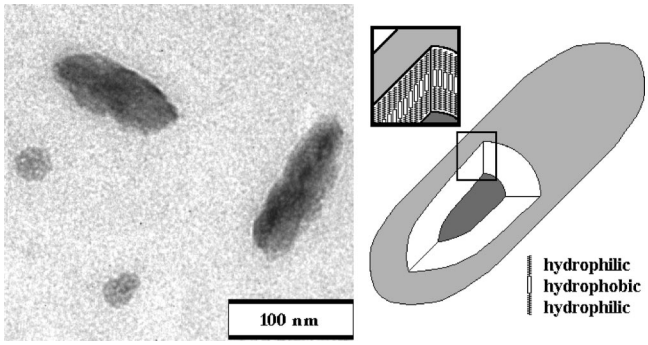


Figure 3. TEM image of the PEO-*b*-PHFPO-*b*-PEO nanostructures transferred from the water solution on a carbon film.

connected to the polymer backbone via ether bonds.[22,23] This can be done in one step. These ether bonds are hydrolytically stable with neighboring CF_2 groups in contrast to the ester and amide bonds.

The ^{1}H NMR spectra show intensity reduction of the OH protons of the PEO-MME end group, confirming the reaction. Following Dust et al.,[24] conversions of up to 96% were obtained. Impurities in the sample, e.g., remaining solvents, were negligibly small (around 0.01 wt %).

The synthesis of the respective block copolymers can also be tracked by making SEC measurements of the respective polymers dissolved in THF with 3 vol % trifluoroacetic acid (see Figure 2). The initial substances are PEO-MME (C) and PHFPO (D). Curve A shows the SEC trace of a sample with essentially complete conversion (96%), and only a small shoulder is depicted, which indicates the presence of very small amounts of PEO-MME. Trace B shows a sample with 75% conversion.

Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). Diluted solutions of 1 wt % of polymer in water were prepared for DLS and TEM measurements. Because of their amphiphilic character, a micellization of the molecules can be expected. DLS measurements indicated small particles with a typical hydrodynamic radius of 10 nm, but also large aggregates with radii of 60 nm were found. This is much larger than expected for micelles, indicating the existence of vesicles. TEM measurements (solution dropped on carbon coated copper grids and the water is evaporated) confirmed the existence of such particles. In Figure 3, a typical

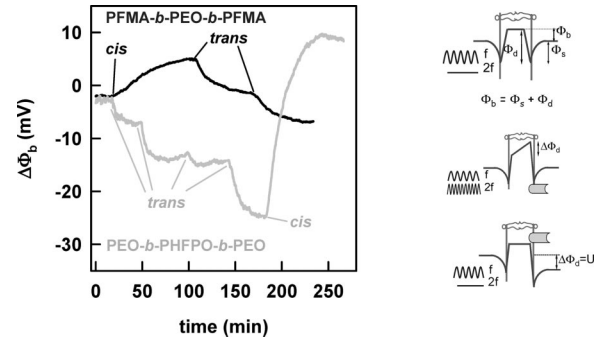


Figure 4. Changes of interfacial membrane boundary potential difference $\Delta\Phi_b$ caused by unilateral membrane adsorption of PEO-*b*-PHFPO-*b*-PEO and PFMA-*b*-PEO-*b*-PFMA. The buffer contained 10 mM HEPES, 10 mM Tris, and 20 mM Choline chloride, pH 7.5.

TEM picture is shown, and the assumed double layer vesicle structure[25] is also depicted.

Polymer Interaction with Membranes. Polymer induced changes of membrane boundary potential $\Delta\Phi_b$ were used to monitor its interaction with the membrane. $\Delta\Phi_b$ represents the sum of $\Delta\Phi_s$ and $\Delta\Phi_d$, the interfacial surface potential difference and the dipole potential difference, respectively. Because PFMA-*b*-PEO-*b*-PFMA and PEO-*b*-PHFPO-*b*-PEO were uncharged, the changes of $\Delta\Phi_b$ that were detected upon their binding to membranes could entirely be attributed to $\Delta\Phi_d$ (Figure 4). Polymer addition to the opposite leaflet reversed the effect. The observed membrane binding is in line with monolayer experiments that also revealed intercalation of PFMA-*b*-PEO-*b*-PFMA into the lipid phase.[7]

Facilitation of Transmembrane Doxorubicin Diffusion. Adsorption of the positively charged doxorubicin increased Φ_s (and consequently Φ_b) of the respective leaflet. In case of facilitated transmembrane doxorubicin transport, Φ_s of the leaflet facing the doxorubicin receiving compartment is expected to increase. As a consequence, the interfacial potential difference $\Delta\Phi_s$ should decrease. However, $\Delta\Phi_s$ failed to decrease when PEO-*b*-PHFPO-*b*-PEO or PFMA-*b*-PEO-*b*-PFMA were added (Figure 5). The observation indicates that, in contrast to Pluronic L61,[5] the fluorine containing polymers are unable to facilitate the transmembrane transport of doxorubicin.

Polymer Ionophoric Activity. Ion conductance of planar lipid bilayers was tested by measurements of current flow upon clamping the electrical potential between the reference electrodes at both sides of the membrane. The addition of PFMA-*b*-PEO-*b*-PFMA had no effect on membrane conductance, *G*. In contrast, PEO-*b*-PHFPO-*b*-PEO augmented *G* from 3.2 nS/cm^2 to 70 nS/cm^2 (Figure 6).

Polymer Effects on Doxorubicin Accumulation in Cells. Human embryonic kidney cells (HEK293) exhibiting a high level of Pgp expression (labeled by a green fluorescent protein tag; FP) did not show doxorubicin accumulation in the nucleus (Figure 7a). With decreasing levels of Pgp expression, doxorubicin accumulation increased (Figure 7b). Treatment of the cells with L61 reversed the protecting effect provided by Pgp and allowed doxorubicin accumulation within the nucleus. As a consequence, the difference in the doxorubicin concentrations between Pgp expressing cells and drug sensitive cells vanished (Figure 7c,d).

PFMA-*b*-PEO-*b*-PFMA enabled only moderate intracellular doxorubicin accumulation. In contrast to L61, the final doxorubicin concentration in cells lacking Pgp was larger than in Pgp expressing cells (Figure 7e,f). The potency of PEO-*b*-PHFPO-*b*-PEO to promote drug accumulation was even lower.

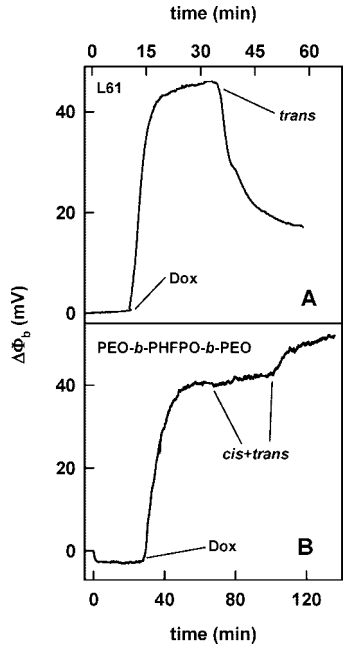


Figure 5. Polymer effect on doxorubicin membrane permeability. (A) Doxorubicin adsorption to one membrane leaflet increased the interfacial membrane boundary potential difference. The effect was reversed by L61, indicating doxorubicin adsorption to the second leaflet, i.e., doxorubicin diffusion through the membrane. (B) In contrast to L61, PEO-*b*-PHFPO-*b*-PEO was unable to facilitate doxorubicin transport. Instead of a drop in $\Delta\Phi_d$, a small increase was observed, probably due to a slight change of the doxorubicin adsorption plane in the membrane or a small change in doxorubicin membrane affinity. The buffer contained 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10 mM Tris (2-amino-2-hydroxymethyl-propane-1,3-diol), and 20 mM choline chloride, pH 7.5, and 0.2 mM doxorubicin were added to one compartment only. The copolymers were added to both sides of the membrane.

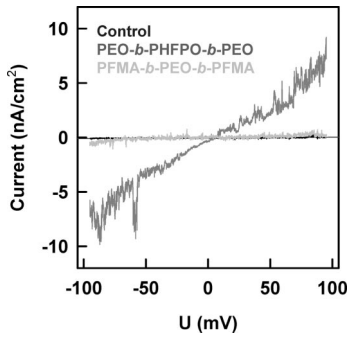


Figure 6. Polymer induced changes of membrane conductivity. A voltage ramp was applied, and the current was recorded without additives (A), in the presence of 10^{-2} % PFMA-B-PEO-B-PFMA (B) or 5×10^{-4} % PEO-B-PHFPO-B-PEO (C). The conductivities were equal to 3.2 nS/cm^2 (A,B) and 70 nS/cm^2 (C). The buffer contained 10 mM HEPES, 10 mM Tris, and 200 mM KCl, pH was equal to 7.5.

It exhibited little effect on cells with high Pgp levels (Figure 7g). If administered to cells with low Pgp expression levels, an increased doxorubicin accumulation was observed (Figure 7h).

A more quantitative analysis of doxorubicin accumulation was made by taking the Pgp expression level into account (Figure 8). Therefore, all pictures were taken at exactly the same filter settings, the same laser power, and with the same detector gain. These settings were adjusted to assign the highest gray scale value of 255 to the highest Pgp expression level and background fluorescence to gray scale values <5. For Pgp fluorescence intensities >100, doxorubicin accumulation was independent of Pgp expression level. After 1 h of exposure to doxorubicin, the difference ΔF_{dox} in the brightness of intracellular doxorubicin fluorescence between these highly Pgp-expressing cells and nonexpressing cells was taken as a measure of polymer efficacy to reverse the cell protecting effect of Pgp. ΔF_{dox} of untreated cells was set to 100%. PEO-*b*-PHFPO-*b*-PEO, PFMA-*b*-PEO-*b*-PFMA, and L61 were respectively restored to 25%, 45%, and 100% of the doxorubicin accumulation capacity that was lost due to Pgp overexpression (Figure 8).

Discussion

The fluorine containing polymers differed in their efficacy to promote intracellular drug accumulation. The more potent promoter PFMA-*b*-PEO-*b*-PFMA lacked ionophoric activity, indicating that hampering the membrane barrier is not a prerequisite for the inhibition of the drug efflux pump by block copolymers. An accompanying compensatory increase in the activity of ion pumps leading to ATP depletion[5] may be ruled out as a possible mechanism of polymer action.

An alternative explanation for the reduced effect of the fluorinated polymers on cellular doxorubicin accumulation is provided by the observation that, in contrast to L61, the fluorinated polymers are unable to facilitate transmembrane doxorubicin transport (Figure 6). If L61 would exhibit the same activity in the plasma membrane, it would not act to inhibit drug efflux but to promote drug influx. However, this hypothesis contrasts with efflux experiments, whereby Pgp-dependent drugs were found to be retained intracellularly by Pluronic block copolymers.[26–28]

Another hypothetical mechanism of MDR reversal would be Pgp inhibition either by affecting the pressure profile within the membrane or by direct polymer binding to Pgp. Because PFMA-*b*-PEO-*b*-PFMA is likely to be anchored in the membrane via the two fluoro alkylated chain extensions, the two ester groups are brought to the lipid water interface. This is an ideal location for binding to Pgp, provided the polymer can flip to the cytosolic side of the membrane. The small change of $\Delta\Phi_b$ observed upon the addition of PFMA-*b*-PEO-*b*-PFMA to lipid bilayers (Figure 4) suggests that flippage occurs. Otherwise, $\Delta\Phi_d$ should be at least an order of magnitude larger, as indicated by Φ_d measurements of lipids with both chains fluoroalkylated.[29] During PFMA-*b*-PEO-*b*-PFMA binding to Pgp, inhibition occurs because the bulky polymers cannot be cycled. Profound differences between polymer affinities to Pgp are not expected, as the major binding motifs of L61 are retained in both PFMA-*b*-PEO-*b*-PFMA and PEO-*b*-PHFPO-*b*-PEO (compare Figure 1). However, it should be noted that the accessibility of these motifs may be different in different polymers, e.g., due to the aggregation of several polymers within the bilayer. Experiments with fluorescently labeled polymers are in the process of proving that hypothesis.

Conclusion

The results show that polymerization using HFPO dicarboxylic acid via the initiator system CsF/HFGF does not follow an anionic mechanism. The acid end groups were transformed to isocyanate groups via Curtius rearrangement. The respective triblock copolymers with PHFPO middle block and PEO end blocks were then realized via ether linkage. The amphiphilic character of these block copolymers in water yields formation of nanoscale aggregates, as demonstrated by dynamic light scattering and transmission electron microscopy. The increased

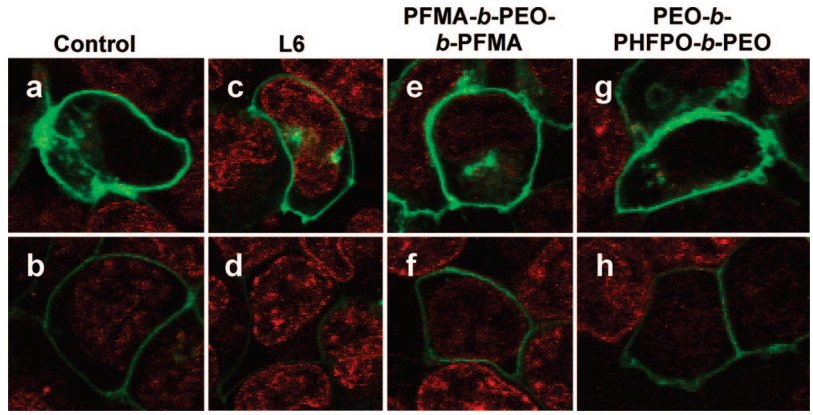


Figure 7. Images of live cells exposed to doxorubicin obtained by confocal laser scanning microscopy. The HEK293 cells were transiently transfected with pEGFP-MDR1 (green) 24 h before analysis and treated with 16 μM doxorubicin (red) for 1 h. The cells were shame-treated (a,b), or exposed to 0.01% L61 (c,d), to 0.05% PFMA-*b*-PEO-*b*-PFMA (e,f), or 0.05% PEO-*b*-PHFPO-*b*-PEO (g,h). The upper (a,c,e,g) and lower (b,d,f,h) rows show cells with, respectively, high and low levels of Pgp expression.

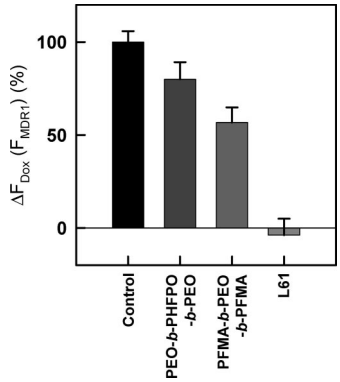


Figure 8. Polymer effect on doxorubicin accumulation in transiently transfected HEK293 cells. After 1 h of exposure to doxorubicin, the difference ΔF_{dox} in the brightness of intracellular doxorubicin fluorescence between highly Pgp-expressing cells and nonexpressing cells was taken as a measure of polymer efficacy to reverse the cell protecting effect of Pgp. ΔF_{dox} of untreated Pgp expressing cells was set to 100%. PEO-*b*-PHFPO-*b*-PEO, PFMA-*b*-PEO-*b*-PFMA, and L61 were respectively restored to 25%, 45%, and 100% of the doxorubicin accumulation capacity that were lost due to Pgp overexpression.

cellular drug accumulation observed upon administration of block copolymers to multidrug resistant cells is not mediated by pore formation in membranes.

Experimental Section

Synthesis. Diethylene glycol dimethyl ether (Diglym, Merck, purity ≥99%) was refluxed over CaH_2 for several hours and then distilled. The middle fraction was refluxed under argon in the presence of benzophenone and sodium. Finally, a vacuum distillation was carried out. During the distillation of benzene, sodium was permanently added to the water-free main fraction until the formation of hydrogen was finished. Then it was refluxed for several hours under argon and again distilled. Poly(ethylene oxide) monomethyl ether (PEO-MME) with a molar mass of 2000 g/mol (Fluka, 81321) was dissolved in 50 mL of benzene (Aldrich, purity ≥99%), and then half of the solvent was removed by distillation. The residual amount of PEO-MME was crystallized in a dry ice/methanol bath, and the solvent was sublimated in a vacuum. Hexafluoropropylene oxide (HFPO, Fluorochem, purity 97%) was dried in a Schlenk tube over CaH_2 at −78 °C. Shortly before polymerization, the HFPO was recondensed in a flask, and from there it was given into the reaction flask. Hexafluoroglutaryl difluoride (Fluorochem, purity 98%) was stirred with KF (Fluka, purity ≥99%)) in order to remove traces of HF, and then it was recondensed. CsF (Chempur, purity 99.999%) was used as supplied.

PHFPO Dicarboxylic Acid. A condenser using dry ice/isopropanol was connected between the argon line and the Schlenk tube. The apparatus was flamed several times prior to use. Then 70 mL of dried Diglym was injected with a syringe, degassed, and purged with argon. Then 1.77 g (7.27 mmol) hexafluoroglutaryl difluoride was added dropwise to a flask connected with a 3-way valve to the suspension of 0.7 g CsF in 10 mL of Diglym under argon at −78 °C. The suspension was stirred and slowly heated for 1 h to reach room temperature. Then the initiator solution (including catalyst) was filtered in a Schlenk flask under argon and injected into the reaction mixture with a syringe. The reaction mixture was cooled to −25 °C and evacuated. Afterward, 6.68 g (40.2 mmol) HFPO was added to the reaction mixture and then kept for another 8 h at −25 °C. Finally, the reaction mixture was mixed with 70 mL of water at 0 °C. Two liquid phases were formed, one water-rich and the other containing the polymer. The polymer phase was washed 3 times with water and finally distilled in vacuum. The molar mass of PHFPO dicarboxylic acid was determined by end group titration. Then 50−60 mg of PHFPO dicarboxylic acid was dissolved in 10−15 mL of trifluoroethanol (Aldrich, purity 99%). Then methyl orange was added and the titration was carried out with 0.01 M solution of KOH in trifluoroethanol.

PEO-*block*-PHFPO-*block*-PEO-Copolymer. First, 0.544 g of PHFPO dicarboxylic acid (M_n = 1100 g/mol, i.e., 0.494 mmol) was dissolved at 80 °C in 40 mL of dried benzene. Then, 0.27 g (0.988 mmol) of diphenylphosphoryl azide (Aldrich, purity 97%) was added, and the solution was stirred overnight at 80 °C. Without further purification, 20 μL of water was added using a microsyringe. The solution was stirred for another 3 h at room temperature. The excess water and part of the benzene were removed by azeotropic distillation. Then 1.98 g (0.988 mmol) of PEO monomethyl ether dissolved in 20 mL of dried benzene were added, followed by the addition of 0.2 g (1.976 mmol) triethyl amine (Fluka, purity 98%). After additional stirring for 8 h at room temperature, the polymer was precipitated in 250 mL of diethyl ether (Fluka, purity ≥99%).

Characterization of the Polymers. ^{1}H NMR spectra were taken in DMSO-d_6 at 27 °C using a Varian GEMINI-400BB at 400 MHz. PHFPO-PEO: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.23 (3H, s, CH_3), 3.32 (1H, t, PEO), 3.37 to 3.60 (181H, m, PEO), 3.66 (1H, t, PEO).

^{19}F NMR measurements were carried out in DMSO-d_6 at 27 °C with a Varian INOVA-500 at 470 MHz. PHFPO: δ −82.35 to −84.56, −86.33, −124.02, −126.81, −130.22 −146.88. PHFPO-PEO: δ −80.87 to −86.69, −124.17, −126.92, −147.00.

Size exclusion chromatography (SEC) was used to measure the molar mass built up during the coupling of different blocks. The SEC measurements were carried out with a Knauer apparatus using a flow rate of 1.0 mL/min. The polymer was dissolved in THF with 3 vol % trifluoroacetic acid. The column was Macherey & Nagel 104-5. The apparatus was equipped with a refractive index detector and the calibration was done with PEO standards.

The structure formation of the block copolymers in water was analyzed by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Prior to the measurements, the samples (1 wt % polymer in water) were filtered through a 0.45 μm pore-size filter into dust-free sample cells. The light-scattering hardware setup consists of commercially available equipment for simultaneous static and dynamic experiments made by ALV-Laser Vertriebsgesellschaft mbH (Langen, Germany). A green Nd:YAG DPSS-200 laser (532 nm) from Coherent with an output of 200 mW was used. The thermostatted sample cell was placed on a motor-driven precision goniometer (±0.01°), which enabled the photomultiplier detector to be moved from 20° to 150° scattering angle. The intensity time-correlation functions (ITCF) $g^2(\tau)$ were recorded with an ALV-5000E multiple tau digital correlator with fast option. The minimal sampling time of this correlator is 12.5 ns. The cylindrical sample cells are made of Suprasil quartz glass by Hellma (Muellheim, Germany) and have a diameter of 10 mm. Transmission electron microscopy was carried out using a TEM EM 900 (Zeiss NTS) with an acceleration voltage of 80 KV. The polymer solution (1 wt % in water) was cast directly on carbon-coated copper grids. No staining was applied due to the sufficient contrast of the polymer containing fluorine.

For biological experiments, a DMSO solution (20 mg/mL) was diluted in water (final concentration 5 mg/mL), stirred overnight at +4 °C, and treated by ultrasound to destroy larger aggregates.

The second type of block copolymer, PFMA-*block*-PEO-*block*-PFMA-copolymer (PFMA-*b*-PEO-*b*-PFMA) was synthesized[30] to a molecular weight of 10000 g/mol and 8.7% (w/w) PFMA. Preparation of aqueous stock solutions (10−20 mg/mL) for biological experiments required (i) continuous stirring (1−2 days) of the suspension that initially formed, (ii) dialysis in the presence of EDTA to get rid of residual Cu^{2+} from ATRP polymerization catalyst, and (iii) and ultrasonic treatment.

Formation of Planar Membranes. Planar bilayers were formed by the monolayer apposition technique[31,32] across an aperture (130−180 μm in diameter) in a Teflon septum (thickness 25 μm) separating two aqueous compartments. The septum was pretreated with 2% solution of hexadecane in hexane. On top of the two aqueous phases, a 20 mg/mL solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids) in hexane was spread to form lipid monolayers. After solvent evaporation, the buffer solution levels in both compartments were raised above the aperture by syringes. Within the aperture, the two monolayers combined spontaneously to a bilayer. The buffer solution was agitated by magnetic bars. The experiments were carried out at room temperature (21−23 °C).

Measurements of Transmembrane Current and Single-Channel Activity. The current was monitored under voltage clamp conditions. Therefore, Ag/AgCl-reference electrodes were immersed into the bathing buffer solutions at both sides of the membrane. The recording filter of the current amplifier (model VA-10, NPI Electronic, Tamm, Germany) was a three-pole Bessel low pass filter with a corner frequency of 100 Hz. The amplified signal was digitalized by a PCI 6025E computer board (National Instruments, Muenchen, Germany) and analyzed by the WINEDR software package (Strathclyde Electrophysiology Software, Strathclyde, UK). Gaussian filters between 1 and 7 Hz were applied to reduce noise.

Monitoring Doxorubicin Transport by Measurements of the Interleaflet Boundary Potential Difference, $\Delta\Phi_b$. Surface charge, and thus membrane boundary potential Φ_b of the leaflet facing the doxorubicin containing compartment, increase upon adsorption of the positively charged drug. Changes of the interfacial differences in boundary potential, $\Delta\Phi_b$, indicated membrane permeation of doxorubicin and its subsequent adsorption to the second monolayer.[5] $\Delta\Phi_b$ was determined by capacitance minimization.[33] The method is based on the observation that, at its minimum, the capacitive current does not contain a signal harmonic to the fundamental frequency.[34] In our setup,[35,36] the second harmonics of the amplified current was detected by a lock-in amplifier (model 7265, Perkin-Elmer, Shelton, CT). The dc offset required to minimize the amplitude of the overtone was adjusted every second and applied along with the sine voltage to the membrane (source: model 33120A, Hewlett-Packard, Loveland, Colorado). It is equal in magnitude to $\Delta\phi_b$ but opposite in sign.

Because $\Delta\Phi_b$ represents the sum of interfacial surface potential difference, $\Delta\Phi_s$, and dipole potential difference, $\Delta\Phi_d$, it can also be used to monitor the adsorption of electrical neutral substances, provided that their dipole moment has a component normal to the membrane plane. The fluorine-containing polymers fulfill this requirement.

Cells. The human HEK293 cell line (purchased from DSMZ, Germany) was cultivated in Dulbecco's modified Eagle medium (DMEM) and 10% fetal calf serum (FCS), supplemented with penicillin/streptomycin (P/S) at 5% CO_2. Cells were seeded on 30 mm coverslips, which were covered with 0.025 mg/mL poly-L-lysine hydrobromide (wt ≥ 300000, Sigma-Aldrich). After 24 h, the cells were transfected[37] with 2 μg plasmid DNA of pEGFP-N1-MDR1 (kindly provided by Sanford M. Simon, Rockefeller University, NY) using Lipofectamine TM2000 (Invitrogen).

Laser Scanning Microscopy. Before analysis, the medium was replaced by modified DMEM (without phenol red) and 10% FCS, supplemented with P/S, containing 16 μM doxorubicin hydrochloride (Fluka, Sigma-Aldrich). LSM images were taken within 1 h using LSM 510 Meta (Zeiss). For determination of fluorescence intensities, ImageJ was used.

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Supporting Information Available: NMR data and dynamic light scattering results are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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