Published in final edited form as:

Mol Pharm. 2017 July 03; 14(7): 2333–2339. doi:10.1021/acs.molpharmaceut.7b00197.

Chemical End Group Modified Diblock Copolymers Elucidate Anchor and Chain Mechanism of Membrane Stabilization

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Abstract

Block copolymers can be synthesized in an array of architectures and compositions to yield diverse chemical properties. The triblock copolymer Poloxamer 188 (P188), the family archetype, consisting of a hydrophobic poly(propylene oxide) core flanked by hydrophilic poly(ethylene oxide) chains, can stabilize cellular membranes during stress. However, little is known regarding the molecular basis of membrane interaction by copolymers in living organisms. By leveraging diblock architectural design, discrete end-group chemistry modifications can be tested. Here we

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Author Contributions

E.M.H. performed the physiological experiments and MD simulations. K.J.H., M.K., and W.Z. performed the cellular experiments, copolymer synthesis, and characterization. All authors contributed to analysis, discussions, and writing of the manuscript.

The authors declare the following competing financial interest(s): J.M.M. is on the scientific advisory board of and holds shares in Phrixus Pharmaceuticals Inc., a company developing novel therapeutics for heart failure.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.7b00197. Comparison of hydroxyl vs *tert*-butoxy end groups of PEO₄₈-PPO₁₅ against lengthening contraction-induced force loss in *mdx* mice *in vivo*. PEO-PPO copolymers with methoxy (–C₁) terminated end group on the hydrophobic PPO. Evaluation of a methoxy end group on PEO₇₅-PPO₁₅ against lengthening contraction-induced force loss in *mdx* mice *in vivo*. Evaluation of subcutaneous delivery route on PEO₇₅-PPO₁₅ against lengthening contraction-induced force loss in *mdx* mice *in vivo*. Size exclusion chromatograms of P188 (PEO₇₅PPO₃₀PEO₇₅) and diblock copolymer analogues. ¹H NMR spectra of P188 (PEO₇₅PPO₃₀PEO₇₅), PEO homopolymer (PEO₁₈₂), and diblock copolymers (PDF)

show evidence of an anchor and chain mechanism of interaction wherein titrating poly(propylene oxide) block end group hydrophobicity directly dictates membrane interaction and stabilization. These findings, obtained in cells and animals *in vivo*, together with molecular dynamics simulations, provide new insights into copolymer–membrane interactions and establish the diblock copolymer molecular architecture as a valuable platform to inform copolymer–biological membrane interactions. These results have implications for membrane stabilizers in muscular dystrophy and for other biological applications involving damaged cell membranes.

Graphical abstract

Keywords

block copolymer; materials science; striated muscle; Duchenne muscular dystrophy

INTRODUCTION

Synthetic block copolymers are an expansive and versatile class of soft materials with wide ranging industrial¹ and biological applications.^{2–4} In the biomedical arena, the block copolymers known as Poloxamers (or Pluronics) are composed of a hydrophobic poly(propylene oxide) (PPO) block flanked by hydrophilic poly(ethylene oxide) (PEO) chains. Poloxamer 188 (P188), a PEO–PPO–PEO triblock copolymer containing 20% by weight PPO and an overall molecular weight of 8400 Da, has been proposed as a cell membrane stabilizer, as evidenced by a reduction in the electroporation-induced leakage of carboxyfluorescein dye from cells.⁵ P188 has been further shown to confer membrane protection in a wide range of cells and living systems, including ischemia-reperfusion injury,⁶ thermal³ and radiation burns,⁷ myocardial infarction,⁸ cardiac resuscitation,⁹ and in protecting damaged muscle membranes in Duchenne muscular dystrophy models.^{10–12}

Detailed thermodynamic and dynamic interactions between P188 and lipid bilayers have not been resolved. Numerous experimental^{13–15} and computer simulation studies,^{16,17} however, suggest the structural picture outlined in Figure 1A: the lipophilic portion of the molecule (PPO) adsorbs to the membrane surface with some penetration into the hydrocarbon core, with the hydrophilic tails (PEO) located in the adjacent water rich layer. The strength of this interaction is thought to be delicately balanced by the hydrophilic–lipophilic balance set by

the molecular composition (percent PPO) and molecular weight.^{17,18} We are aware of no reports that assess the physiological efficacy of PEO–PPO diblock copolymers, which is surprising given the well-established importance of this molecular architecture in many applications involving amphiphilic block copolymers.^{19,20}

To gain mechanistic insights, we have designed several diblock copolymer analogues to P188. Here, by exercising precise control over the PPO end group chemistry, as illustrated in Figure 1B, we tested diblock membrane stabilizing performance *in vivo*. A diblock molecular architecture affords unique opportunities to fine-tune the hydrophilic–lipophilic balance by tailoring the PPO end group without impacting the composition or molecular weight of the amphiphile, an option not possible with the triblock architecture. This powerful design strategy has precedence in the surfactancy and polymer literature, wherein terminal functional groups can dramatically influence solution and bulk phase behavior.^{21,22} Figure 1 further illustrates two limiting variants investigated in this work: (1) the terminal hydrophilic hydroxyl and (2) the hydrophobic tertiary butoxy functionalities that we speculate control association of the PPO block with lipid bilayer membranes, as shown.

In this article we demonstrate, for the first time to our knowledge, that diblock PEO–PPO architectures are effective membrane stabilizers *in vivo* and establish that specific PPO end group chemistries play a critical role in defining membrane stabilization. Our data are discussed in the context of a novel “anchor and chain” model of membrane interaction whereby optimal PPO block end group hydrophobicity directly dictates membrane interaction and stabilization.

For these studies we used a Duchenne muscular dystrophy (DMD) animal model, the *mdx* mouse, as a clinically relevant test bed model of membrane destabilization. DMD is a devastating disease of muscle deterioration, resulting from the lack of the cytoskeletal protein dystrophin, which is essential for maintaining the structural integrity of the muscle cell membrane (sarcolemma). The primary pathophysiological defect in DMD is membrane destabilization with marked susceptibility to muscle contraction-induced injury owing to inherited deficits in sarcolemmal barrier function.²³ We and others have previously validated this system to study P188 triblock copolymers and biological membrane stabilization in the context of DMD.^{10–12}

We hypothesized that significant insights into the mechanism of copolymer–membrane interaction could be obtained by modification in block copolymer architecture. It has been proposed that triblock copolymer interactions with lipid membrane could be restrained in part by a chemical mismatch between the PPO block core and steric constraints imposed by the flanking PEO block chains.²⁴ Accordingly, we reasoned that removal of one of the flanking PEO chains to form a diblock PEO–PPO architecture would enable enhanced association of the hydrophobic PPO core with the lipid bilayer, due to reduction in the steric constraints limiting PPO incorporation with the hydrophobic lipid acyl chains.

Therefore, due to the established membrane stabilization activity of P188,^{5,10–12,25} we first made a comparison between triblock P188 (PEO₇₅–PPO₃₀–PEO₇₅) and its diblock analogue (PEO₇₅–PPO₁₅–CH₃; referred to in the text as PEO₇₅–PPO₁₅–C₁). Results showed in

isolated myoblasts and in skeletal muscle *in vivo* that diblock copolymer architectures yield significant protection to muscle from physiological stresses. Next, to gain deeper insight into how the hydrophobic PPO core interacts with lipid membranes, we investigated the effect of specifically altering the hydrophobicity of the PPO terminus of the copolymer. To this end, we synthesized and compared the behavior of *tert*-butoxy-terminated (PEO₇₅–PPO₁₅–C(CH₃)₃; PEO₇₅–PPO₁₅–C₄) and hydroxyl-terminated (PEO₇₅–PPO₁₅–H) diblock copolymers; synthetic details are provided in the Supporting Information. (Here we note that the PEO blocks have hydroxyl and methoxy end groups, respectively. This difference at the terminus of the hydrophilic chains is not expected to affect membrane interactions significantly.) Schematics of the tested diblocks are presented in Figure 1B, and copolymer properties are described in Table 1.

METHODS

Animals

Adult male *mdx* mice (C57Bl/10ScSn-DMD*mdx*) and wildtype *BL/10* mice (C57Bl/10ScSn) aged 2–8 months old were obtained from Jackson Laboratories (Bar Harbor, ME) and housed locally. Only male mice were included in this study as preliminary experiments with female *mdx* mice showed a sex-dependent protective effect against lengthening contraction injury. The procedures used in this study were approved by the University of Minnesota's Institutional Animal Care and Use Committee (IACUC).

Block Copolymers

National Formulary grade of Poloxamer 188 (P188) was generously provided by BASF (Pluronic F68, Wyandotte, MI). PEO₇₅PPO₁₅–C₁ was purchased from Polymer Source (P1861-EOPO, Montreal, Quebec). PEO-8K was purchased from Sigma, St. Louis, MO).

Diblock Copolymer Synthesis

Synthetic methods for preparing PEO–PPO diblock copolymers using sequential anionic polymerization are well established.²⁶ In brief, preparation of PEO₇₅PPO₁₅–C₄ (Table 1) began by reacting a specified amount of propylene oxide with potassium *tert*-butoxide in the presence of 18-crown-6 ether and terminating the polymerization with acidic methanol, resulting in monohydroxyl terminated PPO. Following purification, the PPO chains were reinitiated using potassium naphthalenide and chain extended by addition of a specified amount of ethylene oxide, followed by termination with acidic methanol. PEO₇₅PPO₁₅–H was prepared in the reverse order: poly-(ethylene glycol) methyl ether was purchased from Polymer Source (P5532, Montreal, Quebec) and reacted with potassium naphthalenide followed by addition of a specific amount of propylene oxide and termination with acidic methanol.

Polymer Characterization

Polymers were characterized by a size exclusion chromatograph (SEC, Waters) (Figure S5) equipped with a refractive index detector and tetrahydrofuran as the solvent to determine dispersity ($\bar{\alpha} = M_w/M_n$). ¹H NMR spectroscopy (Bruker AX-400 in deuterated chloroform,

CDCl₃) (Figure S6) was used to determine block compositions and overall number-average molecular weight (M_n) by end-group analysis.

Myoblast Cell Culture

Mouse muscle myoblasts (C2C12; American Type Culture Collection, Manassas, VA) were grown in cell growth media consisting of high glucose DMEM (Gibco Invitrogen, Grand Island, NY), 20% fetal bovine serum (Gibco Invitrogen, Grand Island, NY), and 1% penicillin/streptomycin (Gibco Invitrogen, Grand Island, NY) at 37 °C with humidified atmosphere of 5% CO₂. At 70% confluency, cells were split in order to prevent partial differentiation due to cell–cell contact. The cell culture media was replaced every 2 days.

Hypo-Osmotic Stress Assay

For hypo-osmotic stress assay, around 10⁴ cells were inoculated to each well of a 96-well plate and grown to cover enough well plate surface. When ready to use, the cells were transferred from the growth media to a 310 mOsm isotonic buffer (140 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES) with or without polymers. After incubating for 30 min, the cells were subjected to hypo-osmotic stress by exchanging the isotonic buffer to 133.5 mOsm hypotonic buffer (composition equivalent to 310 mOsm solution but with NaCl reduced to 50 mM) with or without polymers for 50 min. The cells were subsequently re-equilibrated in an isotonic buffer with or without polymers for 30 min. Then, the cells were lysed with 0.01% Triton. In every buffer exchange step, the buffer pulled out of the well was collected and stored at 4 °C before being used for enzyme release assay. Enzyme release was assessed as the amount of lactate dehydrogenase (LDH) released at each step divided by total LDH content per well. The fractional LDH release in the presence of the polymer was further normalized to the fractional LDH release without the polymer (nontreated).

Enzyme Release Assays

Lactate dehydrogenase (LDH) release was assessed at the end of the hypo-osmotic stress protocol. LDH release was assayed using a kit from Pointe Scientific (Canton, MI).

In Vivo Lengthening Contraction Force Loss Protocol

Block copolymers were dissolved in sterile saline to final stock solutions of 150 mg/mL. At least 30 min before the start of the *in vivo* injury protocol, mice received specific dosages of block copolymer or equivalent saline volume intraperitoneally. *In vivo* force measurements of the anterior crural muscle compartment (tibialis anterior, extensor digitorum longus, and extensor hallucis longus) were performed as described previously.²⁷ Mice were anesthetized with a combination of fentanyl citrate (0.2 mg/kg), droperidol (10 mg/kg), and diazepam (5 mg/kg), and the left hindlimb was depilated. The left foot was then secured to an aluminum foot-plate coupled to a servomotor (Model 300B-LR; Aurora Scientific, Aurora, Ontario, Canada). Contractions were induced via stimulation of the peroneal nerve via percutaneously inserted Pt–Ir electrode wires (Model E2–12; Grass Technologies, West Warwick, RI, USA) connected to a stimulator and stimulus isolation unit (Models S48 and SIU5, Grass Technologies). An initial preinjury maximal isometric tetanic force was

determined (250 Hz and 150 ms duration), followed by an injury protocol consisting of 50 lengthening contractions. For the lengthening contractions, the foot underwent 19° passive dorsiflexion at which a prelengthening 100 ms isometric contraction was initiated followed by another 50 ms of stimulation as the foot was actively moved to 19° of plantarflexion (for a total ankle rotation of 38°, which is within typical anatomical range during locomotion in a mouse). Each lengthening contraction was separated by 10 s to prevent fatigue. Maximal force was measured for each lengthening contraction during the course of the injury protocol and presented initialized to the first lengthening contraction force. A final isometric force was measured at the end of the lengthening protocol. $n = 5$ mice for each treatment group of each experiment as it was determined to be the minimum number of animals needed to observe a quantitative physiological significance between control and injured groups.

Molecular Dynamics

All-atom systems of the diblock copolymers with POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (C16:0–18:1PC)) membrane bilayers were generated via molecular modeling using Schrodinger Maestro.²⁸

POPC lipid bilayers containing 280 lipid molecules (140 per leaflet) were generated using the Desmond package^{28–30} with the OPLS 2005 force field³¹ and TIP3P explicit solvation model,³² which is commonly used as the solvent model in all-atom MD systems of various lipid bilayers under different force fields.^{32–34} Counter ions of Na⁺ and Cl[−] (150 mM) were included to charge neutralize the system and to allow the simulations to be carried out under periodic boundary conditions using particle mesh Ewald.³⁵ POPC systems were simulated at physiological temperature (310 K) and atmospheric pressure (1 atm). All molecular dynamics simulations were performed using Desmond³⁰ at constant surface tension (NP γ T ensemble) where $\gamma = 5500 \text{ bar}\cdot\text{\AA}$ to apply mechanical and lateral stretch to the bilayer. The SHAKE³⁶ method was employed to restrain all hydrogen bonds. A 2 fs time step was used with coordinates saved at 1 ps time intervals. Simulations were carried out for 110 ns. Analysis was performed using MEMBPLUGIN.³⁷

Statistics

All results are expressed as mean $\pm$ SEM. Multigroup comparisons for *in vitro* LDH release experiments were assessed using one way analysis of variance (ANOVA) with Tukey posthoc test and $P < 0.05$ considered statistically different. A two-way ANOVA followed by a Bonferroni-post hoc test was used to assess the effect of lengthening contraction numbers and treatment routes across the 50 contractions protocol with $P < 0.05$ considered statistically different. All statistical analysis was carried out using Prism (GraphPad Software).

RESULTS

In functional tests, we first used an established muscle cell membrane injuring assay¹² to compare the efficacy of the hydroxyl (–H), methoxy (–C₁), and *tert*-butoxy (–C₄) terminated diblocks in prevention of stress-induced lactate dehydrogenase (LDH) release from skeletal myoblasts (Figure 2, Figure S2). In C2C12 myoblasts exposed to hypo-osmotic stress and

isotonic recovery, 14 μ M PEO₇₅-PPO₁₅-C₄ significantly decreased LDH release ($78 \pm 4\%$ decrease, $*P < 0.0001$ compared to nontreated group) (Figure 2). However, replacement of the end group with either the less hydrophobic -C₁ ($30 \pm 11\%$ decrease, $*P < 0.0001$ compared to nontreated group) (Figure S2) or hydrophilic -H (terminal hydroxyl -OH) ($30 \pm 8\%$ decrease, $*P < 0.0001$ compared to nontreated group and $*P < 0.0001$ compared to the -C₄ group) led to significant loss of membrane stabilization efficacy.

The mouse model for DMD, the *mdx* mouse, exhibits significant susceptibility to lengthening contraction injury, as evidenced by a marked force loss after successive lengthening contractions *in vivo*.³⁸ We therefore used this assay to evaluate the impact of the molecular architecture (diblock versus triblock) and end group functionality on physiological muscle function of the hindlimb anterior crural muscle group (dorsiflexors). Remarkably, PEO₇₅-PPO₁₅-C₄ exhibited marked protection against lengthening contraction injury (Figure 3A). Additionally, postinjury isometric force was significantly higher in mice that received PEO₇₅-PPO₁₅-C₄ than saline-treated *mdx* mice ($*P < 0.05$), and not significantly different from the C57BL/10 saline group ($P = 0.45$) (Figure 3B). In marked contrast, neither the hydroxy terminated PEO₇₅-PPO₁₅-H (Figure 3) nor methoxy terminated PEO₇₅-PPO₁₅-C₁ (Figure S3) exhibited a significant protective effect *in vivo*. Preinjury, isometric force output, was not altered by copolymers.

These data suggest that specific chemical features of the PPO end group, particularly relative hydrophobicity, significantly modifies the membrane stabilization functionality of the copolymer. This is further substantiated in the comparison of another pair of diblock analogues, PEO₄₈-PPO₁₅-C₄ and PEO₄₈-PPO₁₅-H. Even with an increase in overall diblock hydrophobicity compared to PEO₇₅-PPO₁₅-R (30% PPO vs 20% PPO), a *tert*-butoxy end group conferred significantly greater protection against lengthening contraction force loss compared to its hydroxy terminated analogue (Figure S1).

Previous pharmacodynamic (PD) studies of membrane stabilizing copolymers demonstrate the importance of route of delivery.¹² To ensure that the differential protective effect observed in the C₄ terminated diblock, compared to the -H terminated diblock, was not due to differential PDs due to delivery route, we also performed lengthening contraction experiments using subcutaneous delivery, which we previously demonstrated to be the optimal delivery route for P188 *in vivo*. Here, neither the subcutaneously delivered -H or -C₄ terminated diblocks had protective effects (Figure S4). This result underscores that copolymers, like all drugs, have unique PDs based on molecular weight, architecture, and chemical composition.³⁹ Together, these PD studies further support the critical function of chemical end groups in conferring copolymer-based membrane stabilization.

Motivated by the efficacy of the diblock architecture and the functional impact of end group hydrophobicity, molecular dynamics (MD) simulations were performed to investigate mechanism at an atomic level. Here, detailed end group-bilayer interactions were evaluated using a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) membrane bilayer model (Figure 4). MD analyses demonstrated distinct di-block-membrane interactions between PEO₇₅-PPO₁₅-C₄ and PEO₇₅-PPO₁₅-H, with the -C₄ terminated PPO block inserting significantly farther into the hydrophobic acyl chains of the membrane bilayer,

whereas the –H terminated PPO block preferentially remained at the polar interface headgroup region (Figure 4A). Calculation of hydrogen bonding between the end groups and surrounding water molecules (Figure 4B) provided further evidence that the difference in distribution of the PPO block within the membrane is influenced by the propensity of the hydrophilic –H end group to interact with water, whereas the hydrophobic –C₄ end group is anchored in the hydrophobic region of the membrane bilayer. This is further supported by the significantly different mass density profiles (Figure 4C) of the individual copolymer blocks and end groups (Figure 4D).

DISCUSSION

This study is the first mechanistic investigation into the structure–function relationship of block copolymer architecture and chemistry in terms of defining the basis of copolymer-based membrane stabilization in live cells and animals under physiological conditions. We show here evidence that the diblock copolymer architecture can adeptly confer marked biological membrane stabilization efficacy both *in vitro* and *in vivo*. Importantly, we show that the triblock copolymer architecture is not necessary for effective membrane stabilization. Our experimental data show that the addition of a single hydrophobic *tert*-butoxy end-group to the PPO core significantly enhances membrane protection in dystrophic limb skeletal muscles during *in vivo* mechanical stress. By contrast, substitution of either the relatively less hydrophobic methoxy terminal end group (Figures S2 and S3) or the relatively more hydrophilic hydroxyl end group to the PPO core leads to complete loss of membrane stabilization efficacy *in vivo*.

These findings underscore the striking biological effect of the hydrophobic PPO core terminal end group to membrane stabilization *in vivo* and inform a novel “anchor and chain” model of copolymer–phospholipid membrane interaction whereby the addition of a hydrophobic end group “anchors” the PPO block within the alkyl tail region of the bilayer (Figure 1A). By contrast we propose that a hydrophilic end group, such as a hydroxyl group, would position the end of the PPO block at the lipid head-extracellular fluid interface consistent with a propensity for hydrogen-bonding of the hydroxyl moiety with surrounding water molecules (Figure 1A).

To evaluate this hypothesis, we performed the first, to our knowledge, fully atomistic-based MD simulations of copolymer–bilayer interactions under simulated mechanical stress. Results from our MD simulations show differential partitioning of the copolymer PPO block inside the bilayer for the H-terminated and C₄-terminated diblock copolymers. The –H end group resides more at the bilayer periphery and has significantly more hydrogen-bonding events with surface water molecules than the –C₄ group, which resides near the bilayer center.

Based on these findings, we hypothesize that diblock and triblock molecular architectures both place hydrophilic PEO at the lipid bilayer–water interface and that the diblock design decouples control of the overall amphiphilicity from the composition (PPO/PEO ratio) and molecular weight of the macromolecular surfactant. We further posit that small changes in the structure of the terminal functional group, such as replacing *tert*-butoxy with *n*-butoxy or

increasing (e.g., C₆H₁₃) or decreasing (e.g., C₃H₇) the size of the hydrocarbon tail, will influence the packing and interaction strength with the lipid core. Thus, the molecular weights of the PPO and PEO blocks could be adjusted to optimize the structural and dynamic effects conferred to cell membranes.

In summary, in this article we coupled physiological and molecular dynamics platforms to interrogate the mechanistic basis of copolymer interaction with biological membranes under mechanical stress. Validation across muscle cell *in vitro* and muscle function *in vivo*, together with MD *in silico* studies, establishes the potential directed design of new block copolymers. Here, copolymer–membrane interactions using MD simulations can be implemented to guide copolymer design. Accordingly, this work has the potential to impact diverse chemical and biomedical applications involving conditions whereby cell membrane integrity is compromised, including Duchenne muscular dystrophy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from National Institutes of Health (NIH grant HL122323), the Lillehei Heart Institute (to J.M.M.), American Heart Association predoctoral fellowship (to E.M.H.) the Muscular Dystrophy Association (to J.M.M.), and NIH P30 grant AR057220 (to D.A.L.). The University of Minnesota Supercomputing Institute provided all the necessary computational resources for the MD simulations.

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Figure 1.

Working model of copolymer–membrane interactions. (A) Triblock PEO–PPO–PEO (left), diblock PEO–PPO–H (middle), and PEO–PPO–C(CH₃)₃ (right). Blue represents PEO block; red is PPO block. Water molecules are blue-white. (B) Chemical structures of block copolymers. a and b denote the number of PEO and PPO blocks, respectively. (C) Chemical nomenclatures for P188 and diblock analogues.

Figure 2.

In vitro hypo-osmotic stress assay to screen diblock copolymers for membrane stabilization. LDH release from C2C12 myoblasts exposed to hypo-osmotic stress (* $P < 0.0001$, via one-way ANOVA compared to nontreated group, # $P < 0.0001$ compared to P188 group, and † $P < 0.0001$ compared to PEO₇₅-PPO₁₅-H group) in the presence of 14 μ M copolymers. Mean values are derived from at least three independent experiments. Error bars shown as mean $\pm$ SEM.

Figure 3.

Comparison of *tert*-butoxy vs hydroxyl end groups of PEO₇₅-PPO₁₅ against lengthening contraction-induced force loss in *mdx* mice *in vivo*. (A) Force loss by the anterior crural muscles, in adult *mdx* mice ($n = 6$ per group) treated with 1000 mg/kg of each PEO₇₅-PPO₁₅ copolymer or saline vehicle intraperitoneally at least 30 min before the injury protocol, was assessed over the course of 50 lengthening contractions. Force loss is presented as a fraction of the initial maximal force $\pm$ SEM. Contractions #1–15 show the marked force deficit in *mdx* mice. Results from BL/10 control mice injected with saline are also shown. Inset shows all 50 contractions. (B) *In vivo* isometric force measurements in PEO₇₅-PPO₁₅-treated *mdx* mice immediately postinjury. PEO₇₅-PPO₁₅-H had no significant effect on peak isometric force immediately after injury, whereas the PEO₇₅-

PPO₁₅-C₄ treatment significantly enhanced immediate postinjury isometric force recovery compared to *mdx* saline, * $P < 0.001$, and was not significantly different from *BL/10* saline group.

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Figure 4. Molecular dynamics simulations of *tert*-butoxy vs hydroxyl end groups of PEO₇₅-PPO₁₅. (A) MD snapshots of PEO₇₅-PPO₁₅ depicting membrane interaction of -H (green) and -C₄ (purple) end groups with a POPC lipid bilayer at increased surface tension. (B) Hydrogen bonding of PEO₇₅-PPO₁₅ end groups with water molecules over the course of 110 ns of simulation, * $P < 0.0001$. (C) Mass density profiles of PEO (blue), PPO (red), and of the phosphate groups of the POPC bilayer obtained from the 110 ns simulation trajectories for PEO₇₅-PPO₁₅-H (left) PEO₇₅-PPO₁₅-C₄ (right). (D) Mass density profiles of the -H (green) and -C₄ (purple) end groups.

Table 1

Block Copolymer Chemical Features

polymers	PEO units ^a	PPO units ^d	end group on PPO	total M_n^b	PEO wt % ^c	δ^d
triblock copolymer ^e	PEO ₇₅ PPO ₃₀ PEO ₇₅	150	30	8400	79	1.07
diblock copolymer	PEO ₇₅ PPO ₁₅ -H	75	15	4200	79	1.08
	PEO ₇₅ PPO ₁₅ -C ₁	75	15	4260	78	1.10
	PEO ₇₅ PPO ₁₅ -C ₄	75	15	4430	77	1.05
homopolymer	PEO ₁₉₈	198	198	8700 ^f	100	1.02

^aTotal number of EO or PO monomer units in a polymer chain.

^bNumber-average molecular weight in g/mol determined by ¹H NMR end-group analysis.

^cPEO weight percent to total molecular weight.

^dPolydispersity obtained from size exclusion chromatography using poly(styrene) calibration.

^eReferred to as P188 in the literature and by the manufacturer BASF.

^fNumber average molecular weight by size exclusion chromatography and MALDI mass spectrometry.