Soft Matter

PAPER

View Article Online
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Cite this: *Soft Matter*, 2024, **20**, 4127

Stretched or wrinkled? Looking into the polymer conformation within polymersome membranes†

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Self-assembly of amphiphilic block-copolymers into polymersomes is a well-established concept. In this membrane, the hydrophilic part is considered to be loosely assembled towards the solvent, and the hydrophobic part on the inside of the membrane is considered to be more densely packed. Within the membrane, this hydrophobic part could now have a stretched conformation or be a random coil, depending on the available space and also on the chemical nature of the polymer. We now analysed the literature for works on polymersomes that determined the membrane thickness *via* cryo-TEM and analysed the hydrophobic part of their polymers for their conformation. Over all available block-copolymers, a variety of trends became obvious: the longer a hydrophobic block, the more coiled the conformation and the bulkier the side chains, the more stretched the polymer became. Polymers with less conformational freedom like semi-crystalline ones were present in a more stretched conformation. Both trends could be exemplified on various occasions in this cross-literature *meta*-study. This overview hence provides additional insight into the physical chemistry of block-copolymer membranes.

Received 22nd February 2024,
Accepted 29th April 2024

DOI: 10.1039/d4sm00239c

rsc.li/soft-matter-journal

Introduction

Since their discovery in 1999, vesicles of amphiphilic block-copolymers have quickly found their way into modern research.[1–3] Such vesicles are also called polymersomes and are a hollow sphere that is surrounded by a bilayer of the polymeric amphiphile. One great advantage of polymersomes is their ability to carry a variety of bioactive payloads like enzymes, DNA or RNA. For this purpose, polymersomes are branded as stable compartments that transport their payload safely to the target, where it is delivered upon an external trigger.[4–7] Most of these promises rely on a stable hydrophobic part as the chemical versatile building material for the membrane that prohibits premature leakage.[7–10] Their lipid counterparts, the liposomes, usually cannot be held to the same standard following decreased mechanical stability.[11]

One major argument for the superior mechanical stability of polymersomes over liposomes is the ability of polymers to coil up, effectively supporting the membrane better than lipids in a fully stretched conformation. A typical lipid membrane stretches 4–5 nm[12,13] and considering an average hydrophobic lipid of 18 carbon–carbon bonds, this means that 36 carbon–carbon bonds are aligned within the membrane. Extending this thought to polymersomes, one realises that things are little different there. The membrane formed by PG_{14}-*b*-PBO_{27}, for example, spans 11 nm (2.5 times the size of a lipid membrane), but the polymer contains 81 bonds in the hydrophobic block (4.5 times the amount of bonds found in a lipid).[14] This underpins the aforementioned assumption that polymers are present in a coiled state, which then contributes to their mechanical stability. It has also been shown that polymers can change their conformation if an inserted protein, for example, demands it.[15]

As they can change the conformation, this raises the question of the equilibrium conformation of a hydrophobic polymer within a native polymersome membrane. Understanding the conformation, what drives it and how it could be altered within a given block-copolymer system, or how changing the polymer affects the polymer conformation, is hence key to design mechanically robust polymersomes. Within a typical depiction, the hydrophobic parts of the polymers (red in Fig. 1) meet each other in the middle of the membrane and then a wobbled line is drawn towards the outside of the membrane, where the hydrophilic part of the polymer (blue in Fig. 1) takes over. This line can be shown in a stretched conformation (Fig. 1A) or in a coiled conformation (Fig. 1B), depending on the original artist. Behind such sketches lies the scientific question on whether the polymer is present in a stretched conformation or in a perfectly random coil. A standard assumption could be that the actual conformation is "in between". In previous studies with the group of late Wolfgang Meier, we have had a look at this and calculated the theoretical maximum length of the polymer as well as the dimensions of a perfectly random coil.[16] To the best of our knowledge, no other group has looked into the

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4sm00239c

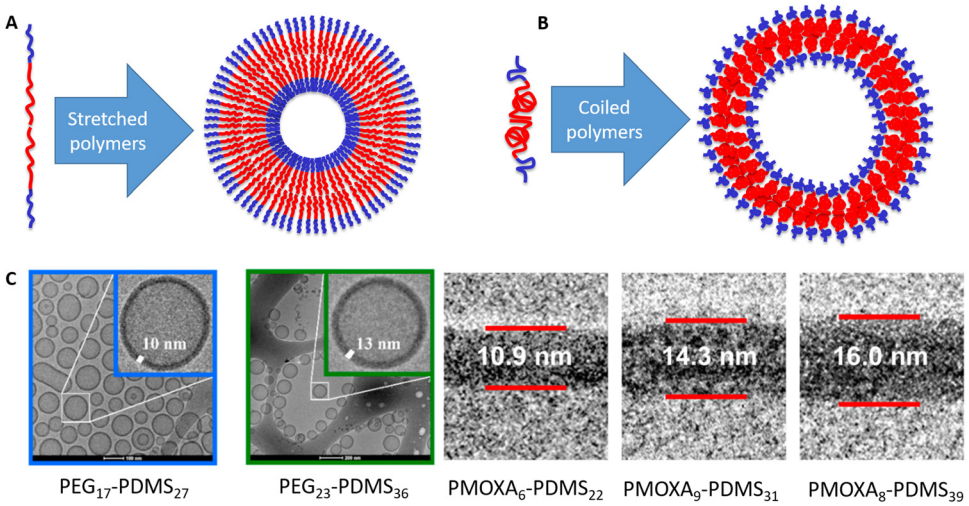

Fig. 1 Amphiphilic block-copolymers (blue = hydrophilic, red = hydrophobic) can self-assemble into polymersomes. (A) Amphiphilic block-copolymer and polymersome with a stretched polymer conformation. (B) Amphiphilic block-copolymer and polymersome with a coiled polymer conformation. (C) Examples for cryo-TEM images that the respective authors used to measure the membrane thickness. The examples for PEG–PDMS are from the study by Fauquignon *et al.*[18] reproduced using the creative commons licence CC BY 4.0. The examples for PMOXA–PDMS were adapted with permission from Itel *et al.*[19] Copyright {2019} American Chemical Society.

polymer conformation within the polymersome membrane so far. Initially, we only looked into the boundaries of a stretched molecule and random coil and noted that the actual conformation was in between both extremes for all noted self-assemblies (micelles, multi-compartment-micelles, vesicles and similar). In the follow-up work on PEG–PEHOx (all polymer acronyms are explained in Table 1), we noted that polymer conformations tend to become less stretched with increasing block length, going from 25% stretched (48 repeating units) to 17% stretched (138 repeating units).[17] The less hydrophobic PG–PBO block-copolymers even went up to being 51% stretched for 27 repeating units of PBO.[14] However, all of these were isolated measurements and calculations and no further comparison was investigated.

In this work, we thus compared polymer conformations within polymersomes obtained from amphiphilic AB-diblock-copolymers across the literature. Polymersomes of ABA or ABC triblock-copolymers were looked at separately as they impose a conformation restriction on the hydrophobic block (polymer spans through the membrane) and also because there is much less data available on that. If the self-assembly of these di-and triblock-copolymers into polymersomes was confirmed by cryo-TEM (examples shown in Fig. 1C) and the membrane thickness was thus determined, the degree of stretching within the hydrophobic block could be calculated. The approach allowed for a *meta*-study across the literature of the conformation of a polymer within a membrane. Effects of the degree of polymerisation (same polymer), different hydrophilic blocks (same hydrophobic block), and the influence of polymeric properties like melting temperature amongst others, could now be looked into. Our evaluation of almost 90 block-copolymers promised insights into how polymers actually look within a membrane, what determines their conformation and hence be a viable basis to improve vesicle models in the future.

Results & discussion

Theoretical considerations

In order to qualify this *meta*-study, the original research had to meet the following criteria: (i) published in a peer-review journal, (ii) vesicles were proven by cryo-TEM (examples shown in Fig. 1C), (iii) the membrane thickness was determined *via* cryo-TEM, (iv) the chemical composition of the hydrophobic block had to be retrievable in terms of chemical structure and repeating unit. Since dispersity values are not always reported and are only relevant for the hydrophobic block here, they have been left out of the discussion but were mentioned when they could notably contribute to measurement errors. It was also required for the analytical data of the block-copolymers to be available for verification purposes. This excluded all commercially sourced amphiphilic block-copolymers, where the authors did not validate the chemical composition after purchase. Focussing on membrane thicknesses determined *via* cryo-TEM allowed to assume a comparatively similar approach by different authors to determine the membrane thickness, as it is a directly measurable read-out from a recorded image. Small-angle X-ray scattering (SAXS), for example, does require a specially trained co-worker to record and interpret relative data and may hence be subjected to a larger measurement and evaluation error across different publications. Small subjective differences by ±1 nm cannot be ruled out for cryo-TEM as well but were considered to be smaller than for other methods like SAXS. If all data were present, the hydrophobic block of the amphiphilic block-copolymers could be analysed as follows. At first, the contour length of the polymer was calculated using the following eqn (1):[14]

$$L_{\text{contour}} = b \times n \times d \times \sin\left(\frac{\theta}{2}\right) \quad (1)$$

Table 1 All mentioned acronyms of the mentioned polymers as well as their long name

Polymer acronym	Long name	Bonds per repeating unit + notable deviations
PA444	Poly((400-acryloxybutyl) 2,5-di(40-butyloxybenzoyloxy) benzoate)	2
PA6ester1	Poly(4′-methoxyphenyl 4-(6″-(acryloyloxy)hexyloxy) benzoate)	2
PAA	Poly(acrylic acid)	2
PAGE	Poly(allyl glycidyl ether)	3
PBD	Poly(butadiene)	2
PBO	Poly(butylene oxide)	3
PCL	Poly(ε-caprolactone)	7
PCMA	Poly(coumarin methacrylate)	2
PDEAEMA	Poly(diethylaminoethyl methacrylate)	2
PDEAMA	Poly(diethylaminoethyl methacrylate)	2
PDMAEMA	Poly(dimethylaminoethyl methacrylate)	2
PDMIBMA	Poly(dimethylmaleimidobutyl methacrylate)	2
PDMIHMA	Poly(6-(3,4-dimethylmaleimidio)hexyl methacrylate)	2
PDMS	Poly(dimethylsiloxane)	2 (SI–O bond: 164 pm, bond angle: 126.5°)[20]
PDPA	Poly(diisopropylaminoethyl methacrylate)	2
PDPAEMA	Poly(2-(*N*,*N*′-diisopropylamino)ethyl methacrylate)	2
PDPAMA	Poly(diisopropylamino ethyl methacrylate)	2
PEE	Poly(ethylethylene)	2
PEG (=PEO)	Poly(ethylene glycol)	3
PEHOx	Poly(2-ethylhexyl oxazoline)	3
PEO	Poly(ethylene oxide) → always noted as PEG throughout the study for consistency	3
PEtOz	Poly(2-ethyl-2-oxazoline)	3
PFcMA	Poly(2-(methylacryloyloxy)ethyl ferrocene carboxylate)	2
PG	Poly(glycidol)	3
PGlyMA	Poly(glycidyl methacrylate)	2
PGMA	Poly(glycerol monomethacrylate)	2
PHPMA	Poly(2-hydroxypropyl methacrylate)	2
PMA	Poly(methyl acrylate)	2
PMAzo444	Poly(4-butyloxy-20-(400-methacryloyloxybutyloxy)-4-(4-butyloxybenzoyloxy)azobenzene)	2
PMeSPG	Poly(*N*-3-(methylthio)propyl glycine)	3
PMOXA	Poly(methyl oxazoline)	3
PNIPAM	Poly(*N*-isopropylacrylamide)	2
PNAM	Poly(*N*-acryloylmorpholine)	2
PNAT	Poly(*N*-acryloylthiomorpholine)	2
PPDMI	Poly(perylene diester monoimide)	2
PPS	Poly(propylene sulphide)	3
PS	Polystyrene	2
PSS	Poly(styrene sulfonate)	2
PtBGE	Poly(*tert*-butyl glycidyl ether)	3
PTMC	Poly(trimethylene carbonate)	6
PTPEMA	Poly(tetraphenylethene methacrylate)	2
PVCL	Poly(*N*-vinylcaprolactam)	2

where $L_{contour}$ (L_c) is the contour length of the polymer, b is the number of bonds per repeating unit, n is the number of repeating units, d is the bond length and θ is the bond angle. Similar to previous studies, for any bond between a carbon, nitrogen and oxygen atom, a bond length of 145 pm will be assumed. As all bonds are single bonds, this is a reasonable value.[14,16,17] Unless stated otherwise, a complete sp^3 hybridisation with a tetrahedral angle of 109.5 degrees will be assumed as the bond angle θ. Deviations of this procedure are noted in Table 1. Even though small deviations may be present, these would eventually even out over the entire length of the polymer. As n represents the number of bonds, it is the amount of bonds per repeating unit (specified for each polymer in Table 1) multiplied with the amount of repeating units within the hydrophobic block. The other extreme conformation, the random coil, was assessed using the following eqn (2):[14]

$$L_{coil} = \sqrt{\frac{1-\cos\theta}{1+\cos\theta}} \times b \times n \times d \quad (2)$$

With L_{coil} representing the mean end-to-end distance of chain ends in a random coil, it should be noted that this equation assumes a random walk of the chain after each chemical bond. Random means that the next chemical bond can continue in any direction as long as the bond angle θ is not violated. This will inevitably result in a lower end-to-end distance than the real one because in reality, a *gauche*-conformation is usually preferred. It is still a reasonable assumption, as this affects all polymer chains equally and still allows for a comparison between the different polymers. A real polymer will now have a conformation that is somewhere in between these extreme values. All polymer chains will hence be stretched by x% and coiled by $(100 - x)$%. This will be referred to as the effective length (L_{eff}), which can be expressed using eqn (3):[14]

$$L_{eff} = x \times L_{contour} + (1 - x) \times L_{coil} \quad (3)$$

For all AB diblock-copolymers, L_{eff} will be determined as half of the corresponding membrane thicknesses as the other half is

occupied by the opposing AB diblock-copolymer. For all ABA and ABC triblock copolymers, the entire membrane thickness will be taken as L_{eff} because the polymer spans through the membrane. In eqn (3), x represents the dimensionless factor of how much the polymer represents a stretched conformation and is the aimed-for value of this study. Eqn (3) hence needs to be reformed to yield the final formula for x, which is provided in the following eqn (4):[14]

$$x = \frac{L_{eff} - L_{coil}}{L_{contour} - L_{coil}} \quad (4)$$

This set of equations was now applied to the examples of polymersomes of amphiphilic-*block*-copolymers, which met the criteria stated above. A sample calculation has been done in a previous publication.[14] An overview of all 70 AB diblock-copolymers and 18 ABA + ABC triblock-copolymers that were selected, can be found in Table 2 and the extended table with all information (bond length, bond angle, L_{coil}, L_{eff}) can be found in the ESI.†

This discussion will now be grouped into AB-diblock-copolymers and ABA/ABC triblock-copolymers and it will focus on general trends that can be derived from the obtained data for almost 90 block-copolymers. Similar to Table 2, this discussion will follow polymers with similar or comparable hydrophobic blocks in order to make general trends more easily visible.

AB diblock-copolymers

PDMS is one of the most common hydrophobic polymers in self-assembly and was also one of the first ones used. Consequently, a number of block-copolymers combining PDMS either with PEG or PMOXA have been reported. Generally speaking, short PDMS blocks are in a much more stretched conformation than long ones. In PEG_8-*b*-$PDMS_{14}$,[18] for example, PDMS is 79% stretched and in $PMOXA_6$–$PDMS_{22}$[19] it is quite similar with 78% stretching. In longer ones, this number drops to 50% stretching for PEG_{23}-*b*-$PDMS_{36}$[18] and notably further to 27% for $PMOXA_{11}$-*b*-$PDMS_{68}$.[19] Even though there are no big outliers within the PEG and PMOXA series, the PDMS bits notably exhibit greater stretching when combined with PMOXA. They appear to reach a plateau of about 48% stretching for 23–36 repeating units, when combined with PEG, and drop from 79% to 61% stretching for 22 and 39 repeating units, respectively, when combined with PMOXA (Fig. 2A). As no other hydrophobic block exhibited the transition from PEG to PMOXA, this trend of more stretching with PMOXA could not be generalised.

The series of PBD and PEE as saturated counterparts then extends this series of polymers with a relatively simple structure in their repeating unit. Here, the trend of polymers that become less stretched with increasing length becomes once again very much apparent. The series starts at 32% stretching for PEG_{40}–PEE_{37}[3,22,23] and goes down to 11% stretching for the considerably longer PEG_{150}–PBD_{250},[23] hence strongly underpinning the previously observed trend (Fig. 2B).

As for PEG derivatives as a hydrophobic block, only a limited number of polymers with PBO (3 examples)[14] and PPS (1 example)[24] were available. Within these four datasets, all hydrophobic blocks were of similar length (26–30 repeating units), making them comparable between each other. While the PBO blocks were around 50% stretched, the PPS chain was only 24% stretched. The ethyl side chain present in PBO, but not in PPS, could be a reason for this as a side chain can prevent polymer folding for sterical reasons and consequently lead to a more stretched polymer conformation. Both previously reported block-copolymers PEG_{45}-*b*-$PEHOx_{95}$ and PEG_{45}-*b*-$PEHOx_{128}$[16] technically also fall into this category with 3 atoms per repeating in their main chain. Likely owing to their long hydrophobic parts, the degrees of stretching are very similar with both 15% and 17% being relatively low.

Testing the argument for the side chain, polymers with rather bulky or very long side chains (more than 10 C or O atoms) were examined next. With repeating units as low as seven in PEG_{45}-*b*-$PA444_7$,[25] the linker moiety between the hydrophilic and hydrophobic block and most crucially, the dispersity of the polymer now became relevant as well and can explain the calculated yet impossible stretching of over 250%. However, a notable measurement error seems to be apparent with this kind of polymers as the same group of authors reported 6 nm and 11 nm of membrane thickness in different publications.[26,27] It is reasonable to assume that for shorter numbers of repeating units, bend side chains partially present longer chains (dispersity) and the linker moieties extend the hydrophobic part of the membrane. As a consequence, the calculated degree of stretching becomes formally too high, which explains the calculated numbers of over 400% degrees of stretching. For an increasing number of repeating units like for PEG_{45}-*b*-$PA6ester1_{20}$,[26] a realistic number of 100% stretching could be calculated. Both examples, however, strongly suggest that the trend stated above is correct and polymer side chains do prevent dense coiling and support a stretched conformation.

Semi-crystalline polymers or those with a high glass-transition temperature behaved in the exact opposite way. These polymers either have a high incentive for close packing (building crystalline domains) or lack the mobility to leave their energetically preferred coiled state (high glass transition temperature). All polymers with PCL,[31] PTMC[32] or PS[28–30] in their hydrophobic blocks preferred coiled conformations, ranging between 3% and 17% stretching. Having 300 and more atoms in the main chain of their hydrophobic block made all of them long polymers, giving another incentive for low degrees of stretching. It is hence not entirely clear if the lack of mobility or the high degree of polymerisation caused the low degree of stretching. For the polymers with a comparable amount of atoms in their main chain, PEG_{44}-*b*-PS_{292} (584 atoms)[30] and PEG_{45}-*b*-$PTMC_{96}$ (576 atoms),[32] the PS chain is more stretched (13% stretching) than the PTMC chain (3–4% stretching), again strongly underpinning the argument that side chains prevent ideally coiled structures.

Several other methacrylic derivatives have been synthesised as well but are difficult to evaluate for a series, but this opened the opportunity to look into different trends. For example,

Table 2 All di- and triblock copolymers evaluated for this study, sorted in the same order as they are discussed in the main text. The original publication is cited in the first column

Ref.	Polymer by type of hydrophobic block[a]	Bonds in hydrophobic part	L_{eff}/nm[b]	% stretched[c]	Self-assembly technique[d]
	AB diblock copolymers				
	PDMS				
18	PEG_8-*b*-$PDMS_{14}$	28	3.6	79	Film
18	PEG_{13}-*b*-$PDMS_{23}$	46	4.3	47	Film
18	PEG_{17}-*b*-$PDMS_{27}$	54	5.0	48	Film
18	PEG_{23}-*b*-$PDMS_{36}$	72	6.6	49	Film
19	$PMOXA_6$–$PDMS_{22}$	44	5.5	78	Electro
19	$PMOXA_9$–$PDMS_{31}$	62	7.2	72	Electro
19	$PMOXA_8$–$PDMS_{39}$	78	8.0	61	Electro
19	$PMOXA_{14}$–$PDMS_{65}$	130	10.7	46	Electro
21	$PMOXA_{11}$-*b*-$PDMS_{68}$	136	8.0	27	Film
	No heteroatoms				
3, 22 and 23	PEG_{40}–PEE_{37}	74	4.0	32	Electro, film
23	PEG_{26}–PBD_{46}	92	4.8	32	Film
22 and 23	PEG_{50}–PBD_{55}	110	5.3	29	Electro, film
22 and 23	PEG_{80}–PBD_{125}	250	7.4	16	Electro, film
23	PEG_{150}–PBD_{250}	500	10.5	11	Film
	PEG derivatives				
14	(*R*/*S*)-PG_{14}-*b*-(*R*/*S*)-PBO_{26}	78	5.6	50	Cosolvent
14	(*R*)-PG_{14}-(*R*)-PBO_{26}	78	5.8	54	Cosolvent
14	(*S*)-PG_{14}-*b*-(*S*)-PBO_{27}	81	5.5	47	Cosolvent
24	PEG_{17}-*b*-PPS_{30}	90	4.5	24	Film
16	PEG_{45}-*b*-$PEHOx_{95}$	285	9.0	15	Film, cosolvent
16	PEG_{45}-*b*-$PEHOx_{128}$	384	12.1	17	Film, cosolvent
	Bulky side chain in hydrophobic block				
25	PEG_{45}-*b*-$PA444_7$	14	3.0	251[e]	Emulsion
26 and 27	PEG_{45}-*b*-$PA444_7$	14	5.3	504[e]	Emulsion, nanoprec.
26 and 27	PEG_{45}-*b*-$PMAazo444_{12}$	24	7.3	340[e]	Emulsion, nanoprec.
26	PEG_{45}-*b*-$PA6ester1_{20}$	40	5.0	101	Emulsion
26	PEG_{91}-*b*-(PB_{33}-*g*-Chol)	66	6.8	83	Emulsion
	Semi-crystalline or high T_g hydrophobic polymers				
28	PEG_{45}-*b*-PS_{206}	412	11.0	15	Cosolvent
29	PEG_{45}-*b*-PS_{230}	460	13.0	17	Cosolvent
30	PEG_{44}-*b*-PS_{292}	584	13.0	13	Cosolvent
31	PEG_{45}–PCL_{44}	308	8.8	16	Rehydration
32	PEG_{45}-*b*-$PTMC_{96}$	576	7.3	4	Cosolvent
32	PEG_{45}-*b*-$PTMC_{144}$	864	8.8	3	Cosolvent
32	PEG_{45}-*b*-$PTMC_{170}$	1020	9.6	3	Cosolvent
	Non-bulky (meth)acrylates				
33	PEG_{43}-*b*-P($NIPAM_{21}$-*co*-$PDMI_9$)	60	4.0	44	Cosolvent
33	PEG_{43}-*b*-P($NIPAM_{21}$-*co*-$PDMI_9$)	60	4.8	58	Cosolvent
33	PEG_{43}-*b*-P($NIPAM_{21}$-*co*-$PDMI_9$)	60	5.5	70	Cosolvent
33	PEG_{43}-*b*-P($NIPAM_{21}$-*co*-$PDMI_9$)	60	7.2	101	Cosolvent
34	PEG_{43}-*b*-P($NIPAM_{23}$-*co*-$PDMI_{19}$)	84	5.0	39	Cosolvent
35	$PNAM_{25}$-*b*-$PNAT_{25}$	50	6.5	112	PISA
35	$PNAM_{25}$-*b*-$PNAT_{50}$	100	8.6	67	PISA
35	$PNAM_{25}$-*b*-$PNAT_{70}$	140	9.7	51	PISA
36	PEG_{45}-*b*-$PMeSPG_{17}$	51	4.5	66	Nanoprec.
36	PEG_{45}-*b*-$PMeSPG_{71}$	213	6.5	16	Nanoprec.
37	PEG_{16}-*b*-PMA_{70}	140	6.2	27	Rehydration
37	PEG_{45}-*b*-PMA_{70}	140	5.6	22	Rehydration
37	PAA_{10}-*b*-PMA_{70}	140	5.5	21	Rehydration
	Photo cross-linked membranes				
38	PEG_{45}-*b*-P($DEAEMA_{36}$-*co*-$TPEMA_6$)	84	7.4	68	Nanoprec.
39	PEG_{45}-*b*-P($FcMA_{17}$-*co*-$DEAEMA_{48}$-*co*-$DMIHMA_{16}$)	162	7.0	26	Emulsification
40	PEG_{45}-*b*-P($DPAEMA_{59}$-*co*-$DMIHMA_{24}$)	166	9.8	42	pH switch
41	PEG_{45}-*b*-P($DPAEMA_{57}$-*co*-$DMIHMA_{27}$)	168	13.5	63	pH switch
41	PEG_{45}-*b*-P($DEAEMA_{70}$–$DMIBMA_{20}$)	180	8.1	29	pH switch
42	PEG_{45}-*b*-P($DEAEMA_{73}$-*s*-$DMIBMA_{19}$)	184	8.8	32	pH switch
43	PEG_{45}-*b*-P($DEAEMA_{77}$-*s*-$DMIBMA_{18}$)	190	9.5	34	pH switch
44	PEG_{45}-*b*-($PDEAEMA_{49}$-*co*-$PDMAEMA_{27}$-*co*-$PDMIBMA_{24}$)	200	5.3	11	pH switch
45	PEG_{45}-*b*-P($DEAEMA_{78}$-*s*-$DMIBMA_{23}$)	202	8.0	24	pH switch
46	PEG_{45}-*b*-P($DEAEMA_{82}$-*s*-$DMIBMA_{20}$)	204	7.3	20	pH switch
47	PEG_{45}-*b*-P($DEAEMA_{81}$-*co*-$DMIBMA_{23}$)	208	7.0	19	pH switch
48	PEG_{45}-*b*-P($DEAEMA_{83}$–$DMIBMA_{23}$)	212	7.0	18	pH switch
44	PEG_{45}-*b*-($PDEAEMA_{49}$-*co*-$PDMAEMA_{31}$-*co*-$PDMIBMA_{29}$)	218	5.7	12	pH switch
40	PEG_{45}-*b*-P($DEAMA_{83}$-*co*-$DMIBMA_{28}$)	222	8.6	24	pH switch
49	PEG_{45}-*b*-P($DEAEMA_{89}$-*s*-$DMIBMA_{24}$)	226	7.5	19	pH switch
49	PEG_{45}-*b*-P($DMEAEMA_{45}$–$DEAEMA_{45}$–$DMIBMA_{24}$)	228	7	16	pH switch

Table 2 (*continued*)

Ref.	Polymer by type of hydrophobic block[a]	Bonds in hydrophobic part	L_{eff}/nm[b]	% stretched[c]	Self-assembly technique[d]
39	PEG_{45}-*b*-P($FcMA_{19}$-*co*-$DEAEMA_{83}$-*co*-$DMIBMA_{33}$)	270	6.5	11	Emulsification
50	$PEG_{77.5}$N3-*b*-P($DEAEMA_{130}$-*co*-$DMIBMA_{32}$)	324	13.0	27	pH switch
	Polymers from PISA				
51	PEG_{113}-*b*-P($HPMA_{320}$-*co*-$GlyMA_{80}$)	800	14.0	9	PISA
52	PEG_{113}-*b*-$PHPMA_{400}$	800	12.5	8	PISA
53	$PGMA_{59}$–$PHPMA_{400}$	800	14.0	9	PISA
54	$PGMA_{62}$–$PHPMA_{600}$	1200	21.4	11	PISA
54	$PGMA_{62}$–$PHPMA_{700}$	1400	25.0	11	PISA
54	$PGMA_{62}$–$PHPMA_{800}$	1600	26.7	10	PISA
54	$PGMA_{62}$–$PHPMA_{900}$	1800	29.9	10	PISA
54	$PGMA_{62}$–$PHPMA_{1000}$	2000	35.1	12	PISA
	Triblock-copolymers				
	ABA triblock-copolymers				
55	PEG_{22}-*b*-P(S-*stat*-$CMA)_{118}$-*b*-PEG_{22}	236	14.0	44	Cosolvent
55	PEG_{45}-*b*-P(S-*stat*-$CMA)_{206}$-*b*-PEG_{45}	412	21.0	38	Cosolvent
19	$PMOXA_3$–$PDMS_{19}$–$PMOXA_3$	38	6.0	114	Electro
19	$PMOXA_6$–$PDMS_{34}$–$PMOXA_6$	68	9.2	91	Electro
19	$PMOXA_6$–$PDMS_{44}$–$PMOXA_6$	88	10.7	79	Electro
19	$PMOXA_7$–$PDMS_{49}$–$PMOXA_7$	98	12.1	81	Electro
19	$PMOXA_{12}$–$PDMS_{63}$–$PMOXA_{12}$	126	13.4	67	Film
56	$PMOXA_{17}$–$PDMS_{67}$–$PMOXA_{17}$	134	11.7	51	Film
19	$PMOXA_{12}$–$PDMS_{87}$–$PMOXA_{12}$	174	16.2	57	Electro
56	$PVCL_{10}$–$PDMS_{65}$–$PVCL_{10}$	130	14.6	72	Film
57	$PEG_{16}PPS_{50}PEG_{16}$	150	8.0	30	Film
	ABC triblock copolymers				
58	PEG_{45}–$PDPA_{85}$–PSS_{22}	170	13.9	64	pH switch
17	PEG_{45}-*b*-$PEHOx_{48}$-*b*-$PEtOz_{10}$	144	6.3	26	Cosolvent
17	PEG_{45}-*b*-$PEHOx_{62}$-*b*-$PEtOz_{35}$	186	8.2	28	Cosolvent
17	PEG_{45}-*b*-$PEHOx_{65}$-*b*-$PEtOz_{19}$	195	7.8	24	Cosolvent
17	PEG_{45}-*b*-$PEHOx_{87}$-*b*-$PEtOz_{10}$	261	9.9	21	Film
17	PEG_{45}-*b*-$PEHOx_{139}$-*b*-$PEtOz_{10}$	417	12.9	19	Film
59	PEG_{42}-*b*-$PAGE_{COOH12}$-*b*-$PtBGE_{22}$	36	4.1	95	Cosolvent

[a] All polymers were prepared using controlled radical polymerisation or a living polymerisation method such as ring-opening polymerisation. [b] As per cryo-TEM reported in the noted reference. [c] Calculated using the formula mentioned in the main text. [d] Cosolvent = cosolvent technique = solvent switch, electro = electroformation, emulsification = emulsification and solvent diffusion method, emulsion = inverted emulsion, film = film rehydration, nanoprec = nanoprecipitation, PISA = polymerisation induced self-assembly, pH switch = pH switch from acidic to basic, rehydration = rehydration without film formation. All details can be found in the respective publications. [e] These degrees of stretching are physically impossible and likely originate from the large side chains that contribute to the membrane thickness as discussed in the main text.

PEG_{43}-*b*-P($NIPAM_{21}$-*co*-$PDMI_9$)[33] has 4 reported values, ranging from 44% to 100% of stretching when altering the amount of tetrahydrofuran (THF) during self-assembly. Taking our method, the most amount of THF leads to the most stretched polymers, most likely because of high chain mobility in the good solvent THF. While this is an interesting observation, it cannot be verified further as more data from different polymer systems are missing. Of some interest is also the mini-series of $PEG_{10\text{-}45}$-*b*-PMA_{70}[37] as it is the only one with an altering length of the hydrophilic polymer, while maintaining a constant length of the hydrophobic polymer. With 21–27% of stretching for all polymers and no apparent trend, this influence seems to be negligible. Albeit from a low sample size, the mini-series in $PNAM_{25}$-*b*-$PNAT_{25–70}$ (from about 100% to 50% of stretching)[35] and PEG_{45}-*b*-$PMeSPG_{17–71}$ (66% to 16% stretching)[36] follow the general trend that the polymers with a low degree of polymerisation prefer a more stretched conformation (Fig. 2B).

The photo cross-linked polymersome membranes studied by Appelhans and Voit *et al.* have been studied widely over the past 15 years and thankfully provided the largest cohesive data set for this analysis. To keep everything comparable, only block-copolymers with PEG_{45} were taken into consideration. As it was the longest block-copolymers in this series, an exception was made for $PEG_{77.5}$N3-*b*-P($DEAEMA_{130}$-*co*-$DMIBMA_{32}$)[50] to extend the series as much as possible. Plotting all of them into one graph revealed the same tendency as previously observed that stretching decreased notably with increasing degree of polymerisation with the hydrophobic part of the membrane. Neither the alkyl residue on the pH responsive part (methyl, ethyl, iso-propyl) nor the spacer in the photo cross-linker (butyl or hexyl) appeared to have notable impact on the degree of stretching. It decreased from 68% stretching for PEG_{45}-*b*-P($DEAEMA_{36}$-*co*-$TPEMA_6$; 42 RU)[38] over 42% for PEG_{45}-*b*-P($DPAMA_{59}$-*co*-$DMIHMA_{24}$; 83 RU)[40] and 24% of PEG_{45}-*b*-P($DEAMA_{83}$-*co*-$DMIBMA_{28}$; 111 RU)[40] to 11% for PEG_{45}-*b*-P($FcMA_{19}$-*co*-$DEAEMA_{83}$-*co*-$DMIBMA_{33}$; 135 RU).[39] The latter is especially notable as even the ferrocene residue did not alter the overall trend in the degree of stretching for high degrees of polymerisation (Fig. 2C).

A similar approach can be used to assess the conformation in polymers obtained from the polymerisation-induced self-assembly (PISA). All of the ones with a measured membrane thickness in an aqueous system are from PHMPA and have a high degree of polymerisation (800–2000)[52–54] and a low degree

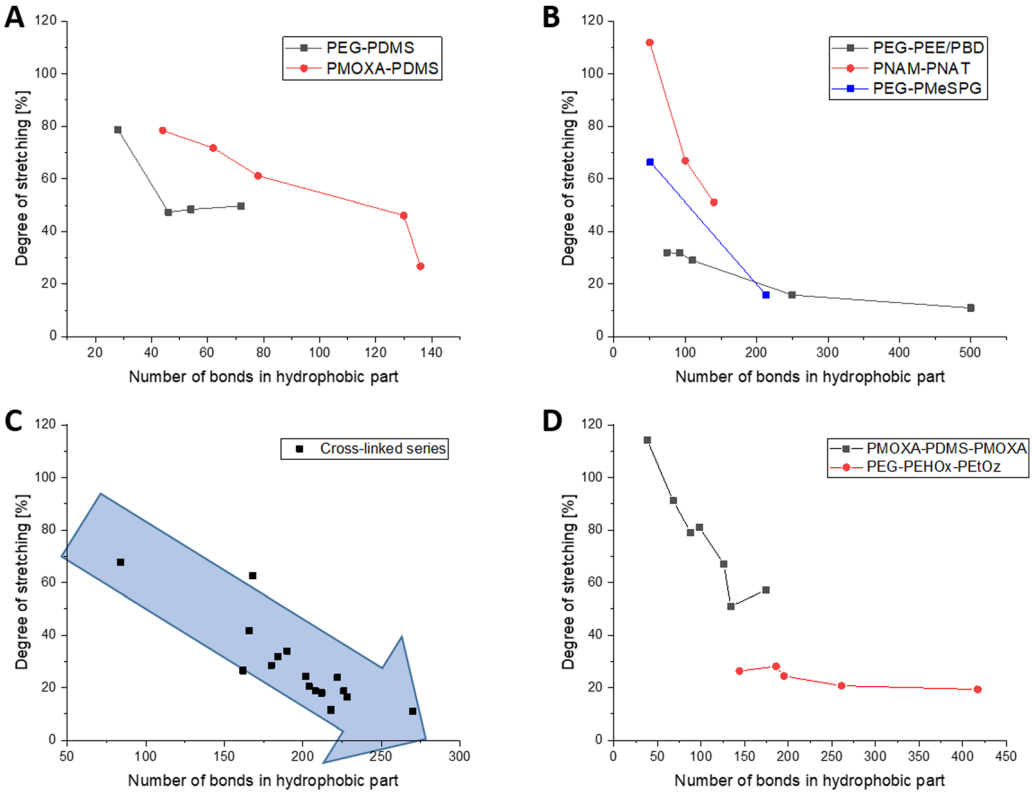

Fig. 2 Development of the degree of stretching for the various groups of polymers, always dependent on the amount of chemical bonds in the backbone of the hydrophobic part. (A) Diblock-copolymers with PDMS as the hydrophobic part. (B) Series of AB diblock copolymers, where more than one polymer have been reported, see the inserted caption for polymer names. (C) All photo cross-linked and pH sensitive polymersomes. The blue arrow has been added to highlight the decreasing degree of stretching with increasing length of the hydrophobic block. (D) ABA and ABC triblock copolymers, where a series of polymers have been reported.

of stretching with 8–11% stretched polymer chains. Following the argument of previously mentioned polymers, this follows the trend of polymers with a high degree of polymerisation exhibiting a low degree of stretching. While this could be expected, the argument should be treated with caution with PISA as the PISA process within the membrane may not necessarily result in alignment along the cross-section of the membrane. For the same reason, these polymers are not in an energetically relaxed state because tensions due to the polymerisation were never released from the system. The real degree of stretching of polymers from PISA may hence be determined using the polymerisation method and not by the degree of polymerisation. Owing to the generally high degrees of polymerisation, however, the exact effect of PISA as a simultaneous polymerisation and self-assembly method cannot be determined from the available data.

ABA and ABC triblock-copolymers

There are notably less publications on the self-assembly of ABA or ABC triblock-copolymers (A and C hydrophilic), let alone ones that have all information to be considered for this *meta*-study. Generally speaking, an ABA or ABC triblock copolymer can be in an I-shape (A and C (second A) on opposite sides) or in a U-shape (A and C (second A) on the same side).[60] For the sake of simplicity, an I-shape will be assumed for this overview as the general trends would remain the same for the U-shape, and only the degrees of stretching would be cut by half. There are two interesting series of publications from the group of the late Wolfgang Meier, which cover PDMS as well as PEHOx in such triblock-Copolymers. Within both series, the trend of more stretched polymers at a lower amount of repeating units does extend. For PDMS, this decreases from about 100% stretching for 19 repeating units to about 50% stretching for 67 repeating units (Fig. 2D, chemical bonds shown).[19,56] These are generally higher than the ones for AB-diblock copolymers of PDMS, which saw 78% stretching for 22 repeating units and 27% for 68 repeating units. Because the hydrophilic parts are now on the opposite side of the membrane, this understandably pulls the hydrophobic part further apart.

The trend for decreasing stretching with increasing chain length also holds true for the PMOXA–PEHOx–PEtOz system, although not as pronounced. Stretching here decreased from 26% for 48 repeating units to 19% for 139 repeating units (Fig. 2D).[17] Compared to their AB-diblock counterparts with 95 and 128 repeating units of PEHOx and 15% and 17% of stretching, respectively,[16] the triblock-copolymers with 87 and 139 repeating units of PEHOx also showed a larger degree of stretching (20% and 19%, respectively). Although notably less different than for PDMS, the triblock copolymers are still more stretched. Following the relatively high degree of

polymerisation for PEHOx, the generally less stretched chains can be expected to show a lower difference in absolute terms.

Conclusion

Generally spoken, the longer a polymer became, the more coiled it became. This held true for all polymers in this series, regardless of if they had additional functional groups, were cross-linked or involved diblock- or triblock copolymers. Polymers with a longer side chain had the tendency to be less coiled following the steric restrictions of the side chain. Conversely, polymers with a high glass transition temperature or semi-crystalline polymers showed a low level of stretching as the chains lack the mobility to rearrange into a stretched conformation. There was also an indication that PDMS-containing polymers were more stretched in ABA triblock-copolymers than in AB diblock-copolymers, although this could not be verified with a second polymer system.

It can hence be hypothesised that a polymer is more stretched towards the hydrophilic part of the membrane and begins to coil up once it penetrates deeper into the hydrophobic block. This is reasonable, considering that a hydrophobic polymer would always minimise the contact area with the hydrophilic surroundings of the solvent. A direct or stretched pathway to the hydrophobic part of the membrane would serve this purpose. Shorter hydrophobic blocks hardly reach this stage and are hence more stretched.

With these results, it is now better explainable, why polymers with entirely different packing parameters, *i.e.* different hydrophilic-to-hydrophobic balances like PEG_{40}–PEE_{37}[3,22,23] and PEG_{45}-*b*-$PTMC_{170}$,[32] can both form polymersomes. While the first example has a mass ratio of 0.85 (1800 g mol^{-1} to 2100 g mol^{-1}), the latter has a ratio of 0.12 (2000 g mol^{-1} to 17 000 g mol^{-1}), and they exhibit decisively different degrees of stretching with 32% and 3%, respectively. Polymer conformation is hence a factor to consider when designing polymersomes.

We hope that our study motivates more researchers to take a closer look into the conformation of their polymers and it is certain that this *meta*-study already provides a valuable insight into polymer conformations within the membrane of polymersomes.

Author contributions

Christiane Effenberg: methodology, formal analysis, Investigation, data curation, writing – review and editing. Jens Gaitzsch: conceptualisation, Methodology, Resources, writing – original draft, writing – review and editing, Supervision, project administration.

Conflicts of interest

The authors have no competing financial interests to declare.

Acknowledgements

The authors thank Dr. Riccardo Wehr and Dr. Davy Daubian, whose discussions helped setting up this train of thought in the first place and also thank Prof. Giuseppe Battaglia and Prof. Nico Bruns for helpful discussions.

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