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From Membranes to Melts, Rouse to Reptation: Diffusion in Polymersome versus Lipid Bilayers

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Block copolymers and lipids are well-known for the microphases they form, from complex gyroid morphologies to lamellae.[1] Mobilities within as well as transitions between such bulk ordered phases have generally been considered in terms of either Rouse dynamics or entanglement release, i.e., reptation.[1−5] In the Rouse regime suitable for short enough polymers, the total hydrodynamic friction on a chain is just the cumulated friction ζ on each of N monomers or subsegments: $D_{\mathrm{Rouse}} = k_BT/N\zeta$. In bulk, clear demonstrations of such Rouse scaling ($D \sim N^{-1}$) are generally complicated by D being a tensor while microphase segregation leads to imperfect domains of often unknown relative orientation.[3] Such difficulties have long motivated efforts to control orientational order with shear or solvent casting,[6] but new opportunities now seem presented by block copolymer "polymersome" vesicles that constitute self-limited lamellar domains.[7] The two diblocks studied here, $EO_{37}-EE_{40}$ (designated OE7)[8] and $EO_{80}-BD_{130}$ (designated OB18),[8] are prototypical vesicle formers and, notably, lipidlike in their hydrophilic fractions (Table 1). These two diblocks possess, however, number-average molecular weights M_n which exceed those of any membrane-forming lipid by factors of ∼5−15. The implications of this increased size to $D(T)$, Rouse or otherwise, as well as comparisons to previous melt mobilities are the focus here.

Compared to phospholipid vesicles such as those composed of SOPC,[9] copolymer vesicles have already proven far more robust under strain and less permeable to water, reflecting the increased membrane thickness of these strongly segregating "super"-amphiphiles (Figure 1).[7,10] Rouse scaling, if applicable, would further suggest that lateral diffusion in these copolymer membranes ought to be relatively low. With lipid membranes in the fluid phase, prior measurements tentatively suggest that self-diffusion indeed decreases with acyl chain length. However, the range of lipid M_n's studied thus far varies by less than about 0.3 kDa;[11] moreover, the most common technique for membrane diffusion measurements is fluorescence recovery after photobleaching (FRAP)[12] which makes use of fluorescent probes of significant size compared to lipids. Such limitations that arise with lipid size are mitigated in the present study of block copolymers.

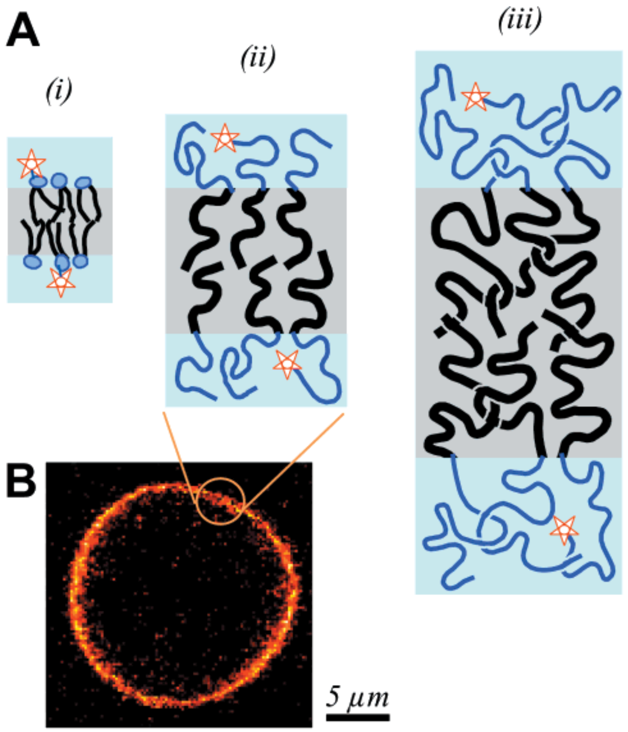

Figure 1. Lipid or copolymer membrane systems studied by fluorescence recovery after photobleaching or FRAP. (A) Self-assembled membranes with fluorescent probes: (i) phospholipid bilayer, (ii) OE7 diblock copolymer bilayer, or (iii) OB18 diblock copolymer bilayer. Relative membrane thicknesses are illustrated to scale.[7] (B) Confocal microscope image of an equatorial section through a giant, unilamellar vesicle of OE7.

Table 1. Membrane-Forming Amphiphiles[8,9] and Their Diffusivities at T = 25 °C (Hydrophilic Volume Fraction, $f_{\mathrm{hydrophilic}}$; Number of Measurements, n)

amphiphile	$f_{\mathrm{hydrophilic}}$	M_n (kDa)	D(25 °C) (μm^2/s)
SOPC	0.31	0.79	3.80 ± 0.20 (n = 6)
OE7	0.39	3.9	0.12 ± 0.047 (n = 3)
OB18	0.29	10.4	0.0024 ± 0.0012 (n = 5)

For FRAP (Figure 2), the necessary starting point is to mix a minimal percentage of fluorescent amphiphile into a vesicle membrane (Figure 1). SOPC is a benchmark lipid[13] used here: it has two 18-carbon acyl chains—one saturated and the other unsaturated—and serves for comparison to well-known liquid crystalline phase D's of ∼10^{-8} cm^2/s (= 1 μm^2/s). For the polymersome measurements, fluorescent probes were covalently attached to PEO's hydroxyl terminus.[13] Given that BD is simply the unsaturated homologue of EE, little difference in intrinsic physical properties might be expected. However, a surprising sensitivity to chain hydrogenation appears in the reported entanglement molecular weight M_e, above which interchain constraints add to Rouse. For bulk homopolymer near 298 K, $M_{e,\mathrm{EE}} \approx 10$ kDa whereas $M_{e,1,2-\mathrm{BD}} \approx 3.5$ kDa.[14] Bulk PEO has $M_{e,\mathrm{PEO}} \approx 1.7$ kDa (at 413 K),[14] but recent scattering studies of the EO brush's volume fraction, ϕ, on micelles of OE7/OB18-like diblocks indicate considerable hydration with a max[ϕ] of ∼50% that decays away from the base of the brush.[15] Added complexity in

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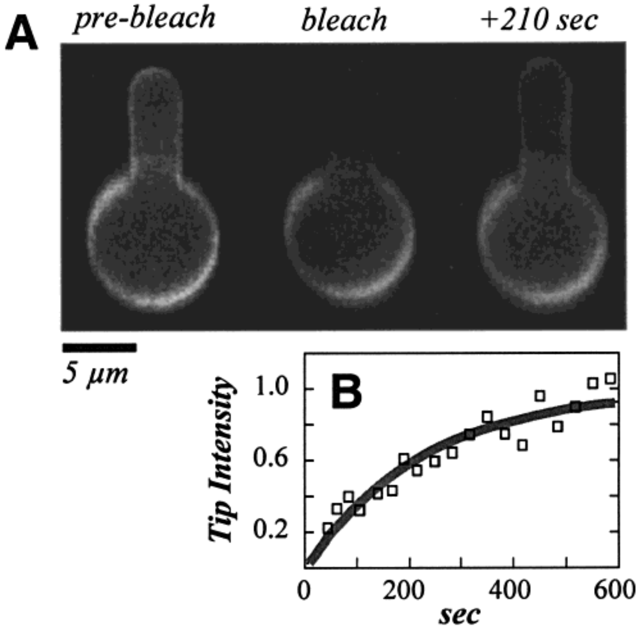

Figure 2. FRAP method applied to an OE7 vesicle pulled into a glass micropipet. (A) Laser bleaching of the fluorescent membrane probe creates a one-dimensional gradient on the projection of length L_P. (B) The tip intensity, normalized by the unbleached outer sphere, was directly determined by imaging. This was used to determine the time constant, τ, and thereby $D = L_P^2/2\tau$. The recovered intensities were generally asymptotic to 100% of the initial state. For longer time or higher T measurements, a sealed chamber without pipet access was used, and a small edge of a vesicle was photobleached.[28]

hydration has been proposed: hydrogen bonding within compressed monolayers of PEO-conjugated lipid has been postulated to explain orders of magnitude increases in measured viscosity and elasticity vs pure lipid layers.[16] However, these latter results did not fully consider the innate tendency of PEO lipid ($f_{\mathrm{hydrophilic}} > 0.4$) to form micelles and the possibility of interfacial structuring through hemi-micellization. Moreover, for a range of polymersomes that include OE7[7] and OB18 (Bermudez et al., submitted), static measures of interfacial elasticity reveal area elastic moduli that are all nearly the same and are also comparable to moduli generic to PC lipids.[17] While such results hint at fluid interfaces, measurements of D (T = 25 °C) in Table 1 together with observations of fully recovered bleached intensities (Figure 2B) clearly demonstrate fluidity. The listed D's also immediately suggest that lateral mobility decreases with increasing M_n.

In the many previous studies of pure phospholipid membrane systems, Rouse scaling of diffusivities does not appear to have been noted. Nonetheless, $D(T)$ of at least two distinct and well-studied phospholipids, POPC and DLPC, [9,19] are very suitably rescaled by total molecular weights: that is, $\tilde{D}_{\mathrm{DLPC}} \equiv D_{\mathrm{DLPC}}\ (M_{\mathrm{DLPC}}/M_{\mathrm{POPC}})$ yields excellent overlap with $D(T)$ for POPC (Figure 3). Similar rescaling of the measured D_{SOPC} (Table 1) also overlaps the POPC results (Figure 3). Pure phospholipids thus appear to follow relatively simple Rouse dynamics in membrane diffusion. This conclusion appears unaffected by the broad range in gel−liquid transition temperatures (~10 °C) exhibited by the phospholipids compared here.[19]

In dense melts of diblocks, either lamellar or disordered, Rouse scaling of $D(T)$, though based on overall M_n's, has already been demonstrated.[1,2] The order−disorder transition has further been shown to have a minimal effect on the smoothness of $D(T)$,[1] allowing full range fits of free volume-based Williams−Landel−Ferry (WLF) relationships. For two OE7-like copolymers, specifically OE12 (M_n = 2.2 kDa, $f_{\mathrm{EO}} = 0.52$) and OE13 (M_n = 2.5 kDa, $f_{\mathrm{EO}} = 0.73$),[1] previous bulk measurements of $D(T)$ performed above PEO's crystallization temperature ($T_m \approx 60$ °C)[8] are here rescaled by M_n relative to OE7. For OE7, $D(T)$ was measured only below 60 °C (to avoid convection, etc.). By extrapolation, this rescaling collapses the previous melt diffusion results onto our membrane diffusion results (Figure 3). Importantly, rescaling of $D(T)$'s by M_{EO} or M_{EE} does not unify the data in any comparable fashion. The extrapolation based on overall M_n—from the copolymer melts down to vesicle membranes with selectively hydrated PEO[15,17]—is consistent, however, with Rouse's model of localized friction wherein monomeric segments are simply replaceable by solvent. The results imply a ϕ-independent ζ in agreement with reports of homopolymer solutions of $\phi > 20\%$.[5]

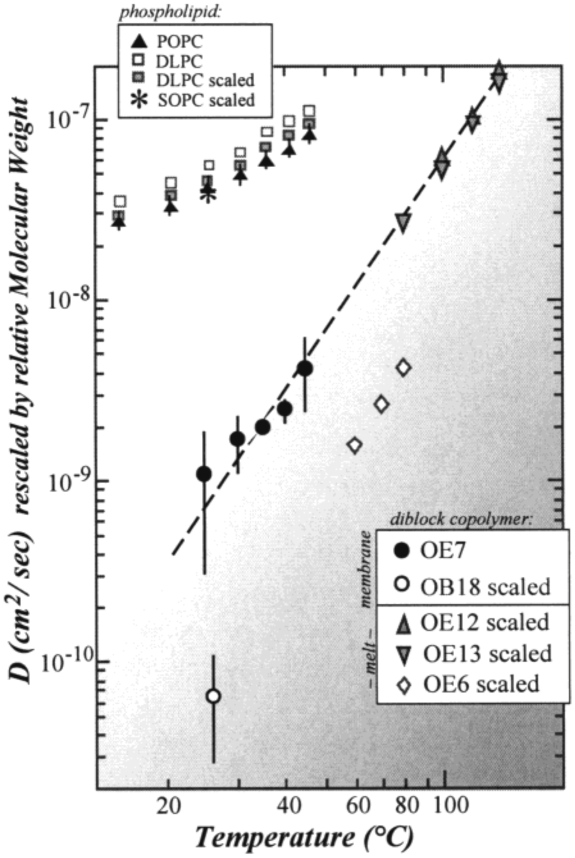

Figure 3. Diffusivities of various phospholipids and diblock copolymers[28] as a function of temperature. For the phospholipids,[19] all D's are rescaled by M relative to POPC, collapsing all of the lipid data onto a single curve and consistent with Rouse scaling. Measurements (average ± S.D. of $n = 3$ measurements) of all diblock copolymer D's, membrane or melt,[1] are rescaled according to the total molecular weight, M_n, of OE7. Collapse of the diffusivities for OE7, OE12, and OE13 is again consistent with Rouse scaling; results for the other diblocks, OE6 and OB18, are indicative of impeded diffusion such as reptation.

With Rouse scaling separately applicable to membrane mobilities of phospholipid as well as OE7 size copolymers, a comparison of monomeric friction factors proves insightful. The simplest Stokes hydrodynamics is assumed: $\zeta = 2d\pi\eta a$, where d is the dimensionality of the system and η and a are respectively the effective viscosity seen by a monomer and its hydrodynamic radius. To facilitate comparison, we assume a common monomer molecular weight so that the number N of effective monomers is simply proportional to M_n. Applying Rouse, a ratio of friction factors is readily obtained from M_n and the measured D(25 °C): $\zeta_{OE7}/\zeta_{SOPC} \approx 6.4$, which implies that even at the monomer level $(d\eta a)_{OE7} \approx 6.4\ (d\eta a)_{SOPC}$. This suggests that monomers making up the thicker copolymer membranes experience higher d hydrodynamics and/or more viscous interactions as well as possibly having larger effective radii. Compared to the thicker OE7 membrane and its extended brush of PEO, the thinner phospholipid membrane is certainly more confining and two-dimensional and the adjacent acyl chains are more compact and likely to be more hydrodynamically coupled, thus experiencing less drag. The simple Rouse model of chain mobility thus proves sufficiently general for a broad range of unentangled systems.

Theoretical explanations for the temperature dependence of $D(T)$ in membrane as well as liquid crystalline systems have been successfully based in the past on free volume thermodynamics;[20] alternative models using continuum hydrodynamics[21] have failed, in contrast, to capture the trends.[19,22] In polymer and membrane physics, free volume ideas have respectively developed into WLF relationships[23] and "extended" free area models.[19] A key parameter in all such approaches is the transition temperature, $T = T_c$, at which molecular mobility is zero (on experimental time scales) due to a lack of accessible volume or area. Similarities of OE7 membranes to phospholipid membranes favor application of a homologous free area model that introduces a temperature-independent friction factor, f.[19] With lipids, f has been attributed to drag at both the membrane–water interface and the bilayer midplane; the Rouse dynamics above suggest a broader interpretation. In the form of an activated process

$$D(T) = (k_B T/f)\ \exp\{-\kappa^*/[\beta + \alpha_T(T - T_c)]\} \qquad (1)$$

where κ^* incorporates several geometric factors that account for free area and molecular overlap and β defines the fraction of free area at T_c. For phospholipid membranes, $\kappa^* \approx 0.4$ and $\beta \approx 0.1$;[19] since WLF relationships are based in part on the idea that such geometric factors are independent of composition, the same κ^* and β will be assumed for OE7 membranes. From previous measurements the thermal area expansivity is known to be $\alpha_T = 2 \times 10^{-3}$ °C^{-1}.[10] f and T_c are thus the primary fit parameters.[24]

Fitting eq 1 to $D(T)$ for OE7 (Figure 3) yields a friction factor $f = 5 \times 10^{-7}$ erg s/cm^2 and a transition temperature $T_c = -33$ °C (residual: $R^2 = 0.92$). The latter is essentially PEE's glass transition temperature in bulk, $T_g^{bulk} \approx -35$ °C [8] (rather than the T_m of bulk PEO). This determination not only is consistent with the PEO brush being highly solvated[15] but also suggests that this self-assembled membrane, as a sort of *free-standing* polymeric thin film, does not have a significantly perturbed T_g. In studying very different nanofilms of spin-coated polystyrene (of low M_n), Forrest and co-workers[25] have suggested that the outermost interfacial nanometers of such films have a significantly lower $T_g = T_g^{surf}$ which effectively reduces the film's overall T_g. The results here suggest that this interfacial length scale may not be so universally "intrinsic to the glass transition" or independent of polymer structure as conjectured.[25] Our fitted value of f is also about 10-fold greater than that typically reported for phospholipid membranes ($f \approx (5 \pm 1.5) \times 10^{-8}$ erg s/cm^2). The difference appears consistent with a higher monomeric friction factor for OE7 (as calculated from $\zeta = N^{-1} f \exp\{y\}$ where $y = \kappa^*/[\beta + \alpha_T(T - T_c)] > 0$).

For the larger block copolymer OB18, the M_n's of both the EO and BD segments are greater than their respective entanglement weights. Rouse dynamics is therefore not expected, at least for bulk diffusion. $\tilde{D}_{OB18}$ indeed falls more than an order of magnitude below D_{OE7}(25 °C) (Figure 3). Importantly, this is independent of rescaling by either total copolymer M_n or the M_n for one block or the other since the $f_{hydrophilic}$ for OE7 and OB18 differ very little (Table 1). The only possible way to achieve Rouse scaling (and make $\tilde{D}_{OB18} = D_{OE7}$) is to propose that the monomeric friction factors for OE7 and OB18 differ by a factor of $\zeta_{OB18}/\zeta_{OE7} \approx 20$(!). This seems unphysical since OB18 is only 2.7-fold larger (in M_n) than OE7. The lack of simple Rouse scaling for OB18 thus suggests additional constraints and is nominally consistent with entanglements.

Despite orientational and interfacial constraints on any super-amphiphile in an ordered state, findings similar to those above led Lodge and co-workers[1] to "tentatively" conclude that an OE7-like copolymer OE6 (M_n = 4.1 kDa, $f_{EO} = 0.42$) also experiences chain entanglements (Figure 3). Clearly, the segment interface could be viewed as pinning the center of a two-armed star so that activated retraction of entangled arms is a needed first step in translational diffusion.[4d] The mechanism is predicated on chain reptation,[26] which asserts a common time scale for both polymer translation along a backbone-defined tube and polymer center-of-mass motion. For homopolymers, this leads to $D_{rep} = D_{Rouse} M_e / M_n \sim M_n^{-\nu}$, where $\nu = 2$. In the strong segregation limit (SSL) of entangled block copolymers,[4d] however, an arm must retract beyond the nearest entanglement, suggesting $D_{\parallel\text{-SSL}} = D_{rep}\ exp\{-\alpha^*\ M_n/M_e\}$ where α^* (< 1) is a fractional retraction. Ignoring the exponential factor as well as compositional differences, extrapolation of the rescaled-OE6 melt results to 25 °C allows a determination of an apparent reptation exponent for lateral mobility of $\nu_{\parallel\text{-app}} = 2.5$ which is essentially the same as $\nu = 2.28 \pm 0.05$ for various large homopolymers.[27] This suggests that $\alpha^* \ll 1$ in the present system. However, combining the various diffusion models with the present experiments on membranes, $\tilde{D}_{OB18} = 0.02 = (M_{OE7}/M_{OB18})(\bar{M}_e/M_{OB18})\ exp\{-\alpha^*\ M_{OB18}/\bar{M}_e\}$ yields $\alpha^* = 0.5$. More definitive statements will clearly require diffusion studies of several more high M_n copolymers of the same composition. The main difficulty with such studies is the long time needed for micron-scale mobility measurements. Whole new regimes of vesicle response, including protracted postporation dynamics in electromechanical breakdown,[29] are nonetheless implicated by the present results.

In sum, a broad range of free-standing bilayer membranes exhibit $D(T)$'s that conform to Rouse scaling. In addition, although monomeric friction factors generally depend on composition, copolymer membrane mobilities extrapolate to dynamics in the dense melt state of like copolymers. Moreover, the T dependence of copolymer D's is well-described by a free volume theory long applied to lipid membranes and further suggests that T_g of a self-assembled nanothin membrane differs very little from the same quantity in the bulk or melt. Last, block copolymers of $M_n > M_e$ appear less mobile than expected from any Rouse-type dynamics. Initial indications are that reptation-type motions are thus possible in such block copolymer membranes, even though such dynamics appear well beyond those of any phospholipid membrane.

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