Self-Assembly and Dynamics Driven by Oligocarbonate–Fluorene End-Functionalized Poly(ethylene glycol) ABA Triblock Copolymers

Guangmin Wei, ‡ Shrinivas Venkataraman, † Yi Yan Yang, † James L. Hedrick, ‡ and Vivek M. Prabhu* ‡⊥⊥

‡ Material Measurement Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, Maryland 20899, United States
⊥ IBM Almaden Research Center, 650 Harry Road, San Jose, California 95120, United States
‡ Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669, Singapore
⊥⊥ Material Measurement Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, Maryland 20899, United States

Supporting Information

ABSTRACT: The closed assembly transition from polymers to micelles and open assembly to clusters are induced by supramolecular π–π stacking in model oligocarbonate–fluorene (F-TMC) end-group telechelic polymers. The critical micelle concentration (CMC) depends on the F-TMC degree of polymerization that further controls the weak micelle association and strong clustering of micelles regimes. Clustering follows a multistep equilibria model with average size scaling with concentration reduced by the CMC as \( R \sim (c/CMC)^{1/4} \). The F-TMC packing that drives the supramolecular self-assembly from polymers to micelles stabilizes these larger clusters. The clusters are characterized by internal relaxations by dynamic light scattering. This signifies that while F-TMC groups drive the clustering, the micelles interconnected via F-TMC bridging interactions remain coupled to the extent that the clusters relax via Rouse–Zimm dynamics, reminiscent of microgels.

INTRODUCTION

Polymeric micelles self-assembled from amphiphilic block copolymers are employed in aqueous media as nanocarriers for hydrophobic drugs with high loading capacity and physical stability. The hydrophobic core provides a reservoir for encapsulation of water-insoluble therapeutics, and the hydrophilic shell ensures dispersion in aqueous media. As new functional groups are incorporated, different micellar morphologies are observed due to the interplay of driving forces including electrostatic assembly, hydrogen bonding, π–π stacking interactions, liquid crystalline packing, and crystallization.

Agrawal et al. performed small-angle neutron scattering (SANS) to understand the self-assembly of poly(lactide)–poly(ethylene oxide)–poly(lactide) (PLA–PEO–PLA) in aqueous solutions and gels as a function of block length and stereochemistry of PLA. They found that \( d/\ell \)-lactic acid blocks form amorphous, spherical core micelles, whereas polymers with \( l/\ell \)-lactic acid blocks form crystalline, lamellar micelles. In this case, the molecular packing within the micellar core provides flexibility when designing advanced materials. In another example, strongly associating cholesterol-functionalized PEO triblock copolymers form versatile gels through the supramolecular assembly of disk-like micelles, where smectic order appears in related cholesterol-functionalized diblock system. The micelle structure, local chain conformation, and connectivity of micelles through bridging will determine the transient network necessary for thermoreversible rheological properties of injectable gels.

The interplay between micellization and clustering in systems with anisotropic and strong interactions may be differentiated from polyoxyalkylenes. Poly(oxybutylene)–poly(oxyethylene)–poly(oxybutylene) (B_E_E_B_m) displays unimer to flower-like micelle structure in dilute solution with critical micelle concentration determined by the insoluble block length. The solution aggregation of micelles was observed by SANS, but not as a function of concentration and end-block length. A related system, poly(oxypropylene)–poly(oxyethylene)–poly(oxypropylene) (P_E_P_m), did not show micellization at room temperature but did show micellization and clustering at elevated temperature. Similarly, \( E_P_m_m \) exhibits a drug-loading-dependent micellization. Interestingly, even PEO exhibits clustering. The molecular origins of this clustering were modeled by considering PEO as a copolymer composed of hydrophilic ether and hydrophobic ethylene groups. Hammouda devised a copolymer theory to quantify the clustering from SANS. However, clustering also depends upon the specific PEO end groups. Therefore, clustering properties in ABA block copolymers and telechelic polymers may have multiple origins, inherent in the main chain, as well as enhanced by the end-group functionality.

We provide a systematic study of model aqueous solutions of oligocarbonate-fluorene (F-TMC) end-functionalized poly(ethylene glycol) triblock copolymer, \( P(F-TMC)_m-P(EG)_n-P \).
were measured by a PerkinElmer UV WinLab spectrophotometer in 0.01 mg/mL. The absorption spectrum of water was used as baseline and subtracted correspondingly. The polymer concentration was 0.625 mg/mL were used for F13.5-E445-F13.5 and F13.5-E445-F1.2. Similarly, 0.80–0.04 mg/mL was used for the remaining. The larger dn/dc value for F15.3-E445-F15.3 may be related to a higher packing density of F-TMC within the dispersion, but we were unable to independently verify this.

**Experimental Methods**

We refer to the series in the following manner Fm-EFm to represent P(F-TMC)n-P(E)n-P(F-TMC)m as shown in Scheme 1. E represents the middle poly(ethylene glycol), and F is the symmetrical substituted oligocarbonate fluorene group with degree of polymerization number n and m, respectively. This avoids confusion from prior notation of the series as ABA-F1, ABA-F2, ABA-F3, and ABA-F4. These notations are provided in Table 1 for cross-consistency with previous work.30,31 along with polymer characteristics. These polymers, with the hydrophobic group number m = 15.3, 5.6, 2.0, and 1.2, are named as F15.3-E445-F15.3, F5.6-E445-F5.6, F2-E445-F2, and F1-E445-F1.2, respectively. The selective solvent used in this study is 18 MΩ·cm resistivity water.

**Scheme 1. Chemical Structure of Fm-EFm Triblock Copolymer**

Sample Preparation. F13.5-E445-F13.5 and F5.6-E445-F5.6 block copolymers (2.0 mg) were dissolved in 2.0 mL of inhibitor-free tetrahydrofuran in a clean glass vial and stirred for ≈2 h. These clear polymer solutions were transferred to a prewashed dialysis membrane with molecular mass cutoff of 3500 Da and dialyzed at room temperature against water (1 L) that was changed after 3, 18, and 21 h. Typically, the final concentration of the polymer solution after dialysis was 0.6 mg/mL, which could form some sediment. These F13.5-E445-F13.5 and F5.6-E445-F5.6 solutions were vortexed and then serial diluted. The final concentration was between 1.5×10⁻⁵ and 0.5 mg/mL. Sample F2-E445-F5.6 was filtered by 0.45 μm PVDF membrane, whereas F13.5-E445-F13.5 were not filtered. F13.5-E445-F1.2 and F5.6-E445-F2 block copolymers were dissolved directly in water as clear solutions of 10 mg/mL with stirring. After approximately 24 h, the solutions were diluted to the required concentrations 0.13–10 mg/mL and each solution was filtered by 0.45 μm PVDF membrane before light scattering experiments.

**Ultraviolet–Visible (UV–Vis) Spectroscopy.** UV–vis spectra were measured by a PerkinElmer UV WinLab spectrophotometer in the spectral range of 180–600 nm in water using quartz cuvettes of 10 mm path length at room temperature. The polymer concentration was 0.01 mg/mL. The absorption spectrum of water was used as baseline and subtracted correspondingly.

**dn/dc Measurement.** The refractive index increment, dn/dc, of Fm-EFm is in 18 MΩ·cm resistivity water was measured by a Wyatt Optilab rEX at 685 nm using a flow rate of 0.5 mL/min and provided in Table 1. Five concentrations spanning 10–0.625 mg/mL were used for F13.5-E445-F13.5 and F13.5-E445-F1.2. Similarly, 0.80–0.04 mg/mL was used for the remaining. The larger dn/dc value for F15.3-E445-F15.3 may be related to a higher packing density of F-TMC within the dispersion, but we were unable to independently verify this.

**Light Scattering.** Static and dynamic laser light scattering measurements were performed with a modified Brookhaven BI-200SM using a wavelength of 532 nm laser light from a Coherent VERDI diode-pumped solid state laser operating in TEM₀₀ mode. Glan-laser polarizer and analyzer (Thorlabs) were used under the vertical polarizer and vertical analyzer condition for all experiments for samples thermostatically controlled to 25.0 °C by a recirculating bath to control the decimetric nature of the decimetric matching bath. The laser power was finely adjusted by neutral density filters. A pinhole before the photomultiplier tube detector was adjusted for static (1 mm) or dynamic (100 μm) light scattering. The intensity was corrected for refraction, scattering volume variation with angle, and refraction by standard methods. The excess scattering above the solvent level was placed onto Rayleigh ratio units using toluene as a reference.

In static polarized laser light scattering the excess Rayleigh ratio, Rₚ(q), is measured as a function of scattering wave vector (q), where q = (4π/λ) sin(θ/2) and θ is the scattering angle. Rₚ(q) from dilute polymer solutions is related to the mass-average molecular mass, Mₐυ, by 54

\[
\frac{H_{c}}{R_{\pi}(q, \theta)} = \frac{1}{M_{r}P(q)} + 2A_{c}q^2 + 3A_{c}q^2 + \ldots
\]

where c is the polymer concentration. The optical constant, H = 4π²(n²dndc)²/λN₀, combines the solvent refractive index (n), refractive index increment (dn/dc), wavelength of light (λ) in a vacuum, and Avogadro’s number (N₀). The concentration dependence of the excess scattering estimates the second and third virial coefficients, A₁ and A₂, respectively. The form factor, P(q), measures the characteristic particle size and shape from the angular dependence of the scattered light. When qRₑ < 1, where Rₑ is the polymer radius of gyration, the particle form factor has the following form:

\[
P(q) = 1 + \frac{q^2(R_e^2)}{3} + \ldots
\]

A Zimm plot of Hₑ/Rₑ(q) against q² + kₑ, with k being a scaling constant, results into the two limiting lines Hₑ/Rₑ(q = 0) and Hₑ/Rₑ(q = 0). The first yields 1/Mₑ + P(q), and the latter gives 1/Mₑ + 2Aₑq² + 3Aₑq² + ... where q = 0 is obtained by extrapolation.35 These double extrapolations to obtain Mₑ, Rₑ, and Aₑ are not always simple if curvature appears in Hₑ/Rₑ(q). In these cases, a Berry plot was used, where the square root of Hₑ/Rₑ(q) is plotted versus q² + kₑ. This method was applied recently to characterize block copolymer vesicles.36

Dynamic laser light scattering (DLS) measures the intensity–time correlation function g₁(q, t) through the Siegert relation.39 Herein, two different procedures were used to analyze g₁(q, t). For F13.5-E445-F13.5 and F5.6-E445-F2 at higher polymer concentration, two modes were confirmed by the inverse Laplace transform method of CONTIN.39 In the presence of two modes and in order to facilitate comparison to a

| Table 1. Polymer Characteristics of Fm-EFm Triblock Copolymer Studied |
|--------------------------|----------------|----------------|----------------|----------------|
| Sample                  | Fm-EFm         | PEG Mₜυ (g/mol) | n, PEG⁵        | Mc (g/mol)     | dn/dc (cm³/g) |
| ABA-F1                  | F13.5-E445-F13.5 | 19610          | 445           | 15.3           | 27247         | 0.14 ± 0.002  |
| ABA-F2                  | F5.6-E445-F5.6  | 19610          | 445           | 5.6            | 22358         | 0.126 ± 0.004 |
| ABA-F3                  | F2-E445-F2      | 19610          | 445           | 2.0            | 20544         | 0.1355 ± 0.0006 |
| ABA-F4                  | F1.2-E445-F1.2  | 19610          | 445           | 1.2            | 20141         | 0.1371 ± 0.0002 |

⁴Number-average molecular mass data based on hydroxyl value from supplier. ⁵Calculated from the supplier data. ⁶Based on ²H NMR spectroscopy. ⁷Refractive index increment with 18 MΩ·cm resistivity water.
theory, \( g^{(1)}(q, r) \) was fit by a sum of a single exponential and a stretched exponential function

\[
g^{(1)}(q, r) = a_1 \exp(-\Gamma_1 r) + a_2 \exp(-|\Gamma_2 r|^\beta)
\]

where \( a_1 \) and \( \Gamma_1 \) are the amplitude and characteristic decay rate of the i mode, respectively, and \( \beta \) is a stretched exponent. The translational diffusion coefficient is obtained from \( D = \Gamma_1/\lambda^2 \). For \( F_{1.2}E_{445}F_{1.2} \) and \( F_{1.2}E_{445}F_{2} \) at very low polymer concentration, as well as \( F_{5.6}E_{445}F_{1.2} \) and \( F_{1.2}E_{445}F_{1.3} \) in the entire polymer concentration range, only one mode was observed by CONTIN. In this case, the quadratic cumulant method\(^{40} \) was used to evaluate \( g^{(1)}(q, r) \) to estimate the average diffusion coefficient (\( D = \Gamma_1/\lambda^2 \)) from the mean decay rate \( \Gamma_1 \) and size polydispersity (\( \langle \mu_2/\lambda^2 \rangle \)). The hydrodynamic radius \( R_h \) was estimated using the Stokes–Einstein relation, where \( R_h = k_BT/6\pi \eta_0D \) with known Boltzmann constant \( (k_B) \), solvent viscosity \( (\eta_0) \), and temperature (\( T \)).

Uncertainties (error bars) are estimated by one standard deviation from the mean by fits to static light scattering and DLS data by least-squares minimization. While error bars are shown, they may be smaller than the symbols.

### RESULTS AND DISCUSSION

**Molecular Association of F-TMC Groups.** The UV–vis spectrum provides direct evidence for \( \pi-\pi \) stacking of F-TMC groups. The F-TMC monomer has an absorption peak at 257 nm in chloroform.\(^{33} \) When F-TMC groups associate, the UV–vis spectrum may show a red-shift of the primary absorption peak\(^{42,43} \), as well as introduction of multiple peaks due to J- or H-coupling.\(^{44} \) The red-shifted peak shows that the side-chain fluorene groups are stacked close enough to cause \( \pi-\pi \) interaction in the ground state.\(^{42,43} \) In Figure 1, the absorption

\[
H_c/R_w(q = 0) = 1/M_w^{0.2} + M_w^{0.2} + 2A_1c + 15/8A_1^2 M_w^{0.2}c^2
\]

where \( c \) is the total polymer concentration and \( c_i \) is the concentration of unassociated polymer (unimer). The details may be found in Nolan et al.,\(^{48} \) but briefly the first term on the right is obtained by expanding the mass-average molecular mass, \( M_w \), into a component due to the unimer, \( M_w^{0.2} \) and micelle, \( M_w^{0.2}c \). The second and third terms are contributions from the second and estimated third virial coefficient. Equation 4 was fit to each data set in Figure 2 with fit parameters \( c_i, M_w^{0.2} \), and \( A_1 \).

Far below the CMC, the polymers are unassociated with concentration of unimer equals to that of the polymer. Above the CMC, micelles form, and the concentration of micelles is \( c - c_i \). For a monodisperse block copolymer, \( c_i \) remains constant for \( c \) greater than the CMC. However, for polydisperse block copolymers, \( c_i \) may increase above the CMC.\(^{50} \) In either case, the contribution of micelles causes \( H_c/R_w \) to decrease with polymer concentration. Considering that the CMC is effectively the concentration at which the presence of micelles is just detectable by static light scattering, the CMC can be taken as the point below which the inverse molecular mass remains constant. We assign \( c_i \) as CMC, using the simplifying assumption that the concentration of unassociated polymers remains constant above the CMC.

The CMC obtained from \( c_i \) is shown as the filled circles (●) in Figure 3. These data are in quantitative agreement with the CMC determined by a pyrene fluorescence-quenching method for \( F_{2}E_{445}F_{2} \) and \( F_{1.2}E_{445}F_{1.2} \) shown as the open squares (□), confirming the applicability of eq 4. The CMC values successively decrease with increasing F-TMC group number (\( M_w \)) as summarized in Table 2.

The free energy of micellization for a monodisperse block copolymer is given by \( \Delta G^\circ (\text{mic}) = (N_kb_0)T \ln \text{CMC} \).\(^{50} \) For alkylene surfactants, \( \Delta G^\circ (\text{mic}) \) is proportional to the number of methylene groups (\( N \)) and generally follows \( \log \text{CMC} = a'N + b' \), where \( a' \) and \( b' \) are constants.\(^{52} \) However, block copolymers deviate from this expression due to the interfacial energy associated with chain packing at the core and corona interface.\(^{53,54} \) Classical predictions\(^{56} \) fit log CMC \( \sim N^{2/3} \). Fits to the CMC data (Figure 3) find log CMC = \( -1.77 \pm 0.43 \) (0.47 \pm 0.08) + (1.29 \pm 0.045), which falls between limits\(^{50,55} \) observed by polymeric micelles of \( N^{2/3} \) and \( N^{1/3} \).

**Identifying Weakly Associating Micelles and Strong Clustering Regimes.** In dilute solutions (\( c \approx \text{CMC} \)), when excess light scattering was just detectable, only one mode was observed by DLS. For \( F_{2}E_{445}F_{2} \) and \( F_{1.2}E_{445}F_{1.2} \) at higher concentrations two well-separated modes were observed. In this case, established methods were applied to separate the excess light scattered intensity into two populations from the DLS relative amplitudes \( a_1 \) (fast mode) and \( a_2 \) (slow mode).\(^{50} \) These two populations at \( c > \text{CMC} \) are due to the presence of micelles, \( I_1(q) = a_1(q)R_w(q) \), and clusters, \( I_2(q) = a_2(q)R_w(q) \),
as initially communicated. Figure 4 shows two regimes in the evolution of the reduced scattered intensities versus \( c/\text{CMC} \). The shaded region represents a weak micelle association regime followed by a strong clustering regime distinguished by the presence of micelles and clusters of micelles. The weak micelle association and strong clustering regimes occur within the diagram of Figure 3 as will be discussed.

The second virial coefficient obtained by the Zimm plots for F1-4Fs and F2-4Fs, and Berry plots for F5-6Fs and F15-3Fs within the strong clustering regime are provided in Table 2. \( A_2 \) is smaller than the parent polymer (PEG of \( M_w = 19610 \text{ g/mol} \)), which is on the order of \( 10^{-3} \text{ mol·cm}^3/\text{g}^2 \). Smaller \( A_2 \) values for the series F15-3Fs to F1-2Fs in comparison to the unfunctionalized homopolymer are consistent with the formation of polymeric micelles and aggregation. The very small and negative \( A_2 \) implies weak attraction between clusters. From positive \( A_2 \), we can reliably estimate the cluster overlap concentration by \( c^* = 1/(A_2M_w) \) for F2-4Fs, leading to \( c^* = 35 \text{ mg/mL} \). The mass-averaged molecular mass shown in Table 2 are in agreement with those determined by eq 4 (Table S1). For F2-4Fs and F2-4Fs, the aggregation number, \( N_{\text{agg}} \), calculated from the extrapolated \( M_w \), corresponds well to the aggregation number of micelles and is consistent with previous estimates. For F15-3Fs and F5-6Fs, the value of \( N_{\text{agg}} \) is too high for isolated micelles. We will show that the high \( N_{\text{agg}} \) of F15-3Fs and F5-6Fs is due to kinetically frustrated clusters without clear separation into free micelles by DLS.

Concentration Dependence of the Radius of Gyration of Clusters. In the strong clustering regime, \( \langle R_g \rangle \) was estimated from F2(q) (Figure S2) by \( \langle R_g^2 \rangle = 3 \frac{d}{dq^2} \left[ \frac{H_c}{I_2(q, c)} \right]_{q=0, c=0} \) (eq 5).

In the case of F15-3Fs and F5-6Fs, \( \langle R_g \rangle \) was estimated from \( [R_g(q)]^{1/2} \) (Figure S3). Figure 5 shows the apparent \( \langle R_g \rangle \) as a function of \( c/\text{CMC} \) within the strong clustering regime. While all the data do not collapse onto a single master curve, when normalized by the CMC, the scaling is compared to a 1/4
The probability of micelle-to-micelle contact increases with the probability of the end group being strongly localized into a central Flory-TMC group being buried into the driving force for partitioning of F-TMC into micelles. If F-TMC solubility of the end groups increases with water, therefore, the segregation strength and selective power law. The onset of the strong clustering regime occurs at smaller c/CMC for m = 1.2, when compared to m = 2 in Figure 4 and also observed by $R_g$ in Figure 5. At higher m the value of the segregation strength $\chi_{SM}$ increases, where $\chi_{SM}$ is the Flory-Huggins interaction parameter between F-TMC and water. Therefore, the segregation strength and selective solubility of the end groups increase with m, further increasing the driving force for partitioning of F-TMC into micelles. If F-TMC groups are buried into flower-like micelles, then the probability of the end group is strongly localized into a central core with reduced probability to the micelle periphery. The probability of micelle-to-micelle contact increases with $c$, regardless of $\chi_{SM}$, but the probability of end groups fluctuating from core to solution should decrease with increasing $\chi_{SM}$. This trend overall shifts the expected cluster growth concentration dependence to higher c/CMC. The lattice simulations by Nguyen-Misra and Mattice demonstrate that the loop fraction increases and dangling end fraction decreases as polymer concentration increases or solvent-end group compatibility decreases. Furthermore, the fraction of loops also depends upon the middle block stiffness with flexible chains exhibiting only 0.25 fraction of loops in a molecular dynamics study of strongly associating telechelic polymers. So, the true situation of all loop-like micelles is not expected, but a combination of loops and dangling or bridging chains ends that facilitates cluster formation.

**Dynamics of Micelles and Clusters.** The angular dependence of dynamic laser light scattering provides insight into the F-TMC degree of polymerization and polymer concentration dependence within the weakly associating micelle and strong clustering regimes. For $F_{1.2}E_{445}F_{1.2}$ and $F_{2}E_{445}F_{2}$ the normalized DLS spectrum observe one broad mode as shown for one concentration in Figures 6a and 6b (lower inset). The diffusion coefficient ($D = \Gamma / q^2$) in the upper inset shows a plateau with a positive deviation to higher q in the case of $F_{2}E_{445}F_{2}$. This deviation is also observed in the lower m systems and contains intracluster dynamics information. Typical normalized DLS for $F_{1.2}E_{445}F_{1.2}$ and $F_{2}E_{445}F_{2}$ are shown for one concentration in Figure 7a,b. Within the strong clustering regime as shown in Figure 7, the micelle diffusion coefficient was determined from $D_2 = \Gamma_2 / q^2$, while the cluster effective diffusion coefficient $D_2 = \Gamma_2 / q^2$ determined from the low q region. Systematic positive deviation in $D_2$ is shown in the upper inset. This is attributed to concentration fluctuations within clusters. Such behavior was recently observed in supramolecular assembled fibers. An example of the separated scattered intensities by the cluster and micelle are provided in the lower inset. $I(q)$ exhibits a q-dependence, fit by eq 2, whereas $I(q)$ typically did not display a systematic $q$-dependence, and estimates to obtain the free micelle $R_g$ are

<table>
<thead>
<tr>
<th>$F_nE_mF_m$</th>
<th>CMC (mg/mL)</th>
<th>$M_e$ (g/mol)</th>
<th>$A_2$ (mol·cm⁻³·g⁻²)</th>
<th>$N_{agg}$</th>
<th>$R_g$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1.2}E_{445}F_{1.2}$</td>
<td>0.231 ± 0.011^a</td>
<td>(1.77 ± 0.08) × 10⁻³</td>
<td>(2.15 ± 24.0) × 10⁻⁷</td>
<td>9 ± 0.4^d</td>
<td>39 ± 1^d</td>
</tr>
<tr>
<td>$F_{2}E_{445}F_{2}$</td>
<td>0.050^b</td>
<td>(4.51 ± 0.25) × 10⁻³</td>
<td>(6.41 ± 1.58) × 10⁻⁷</td>
<td>22 ± 1.2^d</td>
<td>36 ± 2^d</td>
</tr>
<tr>
<td>$F_{1.2}E_{445}F_{1.5}$</td>
<td>0.002216 ± 0.000442^a</td>
<td>(1.34 ± 0.25) × 10⁻³</td>
<td>(−7.38 ± 0.65) × 10⁻⁸</td>
<td>49180^c</td>
<td>226 ± 1^c</td>
</tr>
<tr>
<td>$F_{2}E_{445}F_{5.6}$</td>
<td>0.0021 ± 0.0003^c</td>
<td>(6.99 ± 0.36) × 10⁻³</td>
<td>(−1.30 ± 0.16) × 10⁻⁵</td>
<td>3126^d</td>
<td>94 ± 1^e</td>
</tr>
</tbody>
</table>

^a CMC obtained from eq 4. ^b CMC by pyrene fluorescence-quenching method. ^c Berry plot from $R_g$ (Figure S1). ^d Zimm plot from $I(q)$ (Figure S1). ^e $N_{agg} = M_e/M_w$ using $M_w$ from Table 1.

**Figure 4.** Concentration-normalized extrapolated intensity versus reduced concentration obtained from Zimm plots. The gray shaded region highlights a regime of weakly associating micelles (O) that transitions to strong micelle clustering with populations of micelle ($I_1(q)$, ●) and clusters ($I_2(q)$, ■).

**Figure 5.** Normalized concentration dependence of the apparent radius of gyration of clusters ($R_g$) for $F_{1.2}E_{445}$ to $F_{2}E_{445}F_{1.2}$ as indicated in the legend including data in D₂O from ref 31.
not within the resolution of light scattering, but neutron scattering.29

Figure 8 shows the concentration dependence of the hydrodynamic radius estimated by the plateau values of $D$ for $F_{15.3}E_{445}F_{15.3}$ and $F_{5.6}E_{445}F_{5.6}$. The $R_h$ values show a larger polydispersity near the CMC with error bars representing the polydispersity from the cumulant analysis. These results suggest that the average size is due to the formation of clusters without clear separation into single micelles by DLS. The mean $R_h$ values of $F_{15.3}E_{445}F_{15.3}$ in $20 < c/\text{CMC} < 200 \approx 255 \pm 10$ nm and $F_{5.6}E_{445}F_{5.6} \approx 100 \pm 1$ nm show a nearly concentration-independent scaling of the cluster size with $c/\text{CMC}$ that do not dissociate substantially upon dilution.

$F_{2}E_{445}F_{2}$ and $F_{1.2}E_{445}F_{1.2}$ were studied by small-angle neutron scattering and modeled as star-like micelles with hydrophobic oligocarbonate fluorene point-like core and hydrophilic PEG outer shell that form chain-like clusters.29 As shown in Figure 9, $R_h$ determined from micelles ($D_1$) remains constant, whereas the $R_h$ from the clusters ($D_2$) increases with concentration. For $F_{2}E_{445}F_{2}$, micelle $R_h \approx 16.4 \pm 0.1$ nm and cluster $R_h$ increases from 38 to 101 nm as concentration increases from 2.0 to 10 mg/mL. For $F_{1.2}E_{445}F_{1.2}$, micelle $R_h \approx 11.8 \pm 0.8$ nm and cluster size $R_h$ increases from 36 to 92 nm within the concentration range 1.25–10 mg/mL. The concentration dependence of the cluster $R_h$ shows an equilibrium between micelles and clusters within the strong clustering regime defined by Figure 4. As concentration increases, the equilbrium shifts toward the clusters as confirmed by the reduced scattered intensities in Figure 4 and the DLS amplitudes (Figure S4). This behavior is a signature of self-assembly.64,65
given with step length $\mu$. If for each step $\mu = \mu$. This prediction depends in the text.

$\text{cluster size polydispersity obtained from the cumulant analysis.}$

Figure 9. Concentration dependence of hydrodynamic radius ($R_h$) of the micelle and cluster for (a) $F_{1.3}E_{445}F_{15.3}$ and (b) $F_{3.6}E_{445}F_{1.3}$. Black circles represent the data fit by single exponential function. Black spheres represent the data fit by stretched exponential function. Gray spheres represent the data fit by cumulant method when single modes are observed. Dotted line is mean field open assembly model described in the text.

**Strong Clustering Regime: Closed Assembly Coupled to Open Assembly.** The observation of micellization and micelle clustering implies a hierarchical assembly enabled by F-TMC. The self-assembly of small-molecule or polymer amphiphiles into micelles is a closed assembly process.66 In this case a narrow distribution of associated species is present in finite size, such that $N M_N = M_N$, where $N$ is the association number.51,66 This closed assembly model57 assumes a CMC, below which only unimers are present and above which micelles can be detected with equilibrium constant $K_N = [M_{N}] / [M_{1}]^N$. If further association of micelles occurs, then a hierarchical assembly model is necessary. As an approximation, we assume that the micelles remain intact and follow a multistep equilibrium.56,67 The formation of an $L$-mer of micelles occurs via a stepwise association with equilibrium constant $K_L$ given by $K_L = [M_{N-L}] / [M_{N-L-1}] [M_L]$. If for each step $K_i$ is approximately equal, the associated species would have a broad size distribution. This case is referred to as open assembly in the polymer and small molecule surfactant literature. Mukerjee67 showed that with this model the average degree of association, $L$, proportional to mass-average molecular mass, scales as $L = 2\sqrt{K/M} / \sqrt{c / \text{CMC}}$. This prediction depends upon the ratio of two equilibrium constants one to form a dimer ($K_d$) and equal equilibria ($K$) for all other higher-order stepwise associations. This prediction may be compared to the experimental data in Figures 5 and 9 by invoking Gaussian statistics for the cluster size, $R_h^2 \sim L^2$ with step length $l$ leading to $R \sim (c / \text{CMC})^{1/4}$.

Figure 9 shows the general agreement with the equilibrium open assembly model shown as the dotted line and compared to the scaling in Figure 5. Therefore, the equilibrium assembly of $F_{1.3}E_{445}F_{15.3}$ and $F_{3.6}E_{445}F_{1.3}$ is a closed, coupled to open assembly process. In the strong clustering regime, as the polymer concentration increases, more micelles form, and these micelles reversibly associate into clusters mediated by F-TMC groups via bridging interaction.

In the absence of a hierarchical multiequilibria model, the worm-like micelle theory of Cates and Candau predicts the mean contour length, $L$, follows the concentration scaling $L \sim (E / k_B T)\exp(\eta / c / \text{CMC})$. Within Gaussian statistics, $R_h \sim c^{1/4}$, which is identical to the multiequilibria model. Branching may be included in either estimate as discussed by Burchard.71

**Observations of Intracluster Relaxations.** Internal relaxations could be observed if the structures are sufficiently flexible in the $q R_h > 1$ limit.72 The systematic DLS study over a wide concentration and $q$-range of $F_{1.3}E_{445}F_{15.3}$ reveals internal dynamics of clusters. The analysis for $F_{1.3}E_{445}F_{15.3}$, $F_{3.6}E_{445}F_{1.3}$ and $F_{1.3}E_{445}F_{1.3}$ observes a deviation in the effective di

Figure 10 shows the dimensionless plot of $(Γ_z / q^2) / (k_B T / \eta_0)$ as a function of $(q(R_h))^2$ for $F_{1.3}E_{445}F_{15.3}$ and $F_{3.6}E_{445}F_{1.3}$ at $c > \text{CMC}$. The dimensionless plot for the normalized decay rate by the correct Rouse–Zimm variables eliminates the solvent viscosity and temperature dependence. Further, the normalized $q(R_h)$ axis shows the probe scale less than or greater than the $(R_h)$ of the cluster. The values of $(Γ_z / q^2) / (k_B T / \eta_0)$ plateau near $(q(R_h))^2 > 1.5$. This plateau for linear polymer solutions is typically between 0.05 and 0.064–0.14 and frequently lower than the theoretical prediction of 0.071 and 0.079 with and without a preaveraged Oseen tensor, respectively.77

We consider two possibilities to this lower plateau. A lower plateau was explained by Wu et al. as related to a key assumption in the dynamics theory that energetically favorable internal motions are not observable in DLS at a particular $q(R_h)$ due to finite cutoff observation length scale $1/q$.5 Therefore, if internal motions do not contribute to the measured spectrum,
the calculated \( \Gamma \) should be smaller than the predicted Rouse–Zimm model. This smaller \( \Gamma \) will lead to a lower plateau value. The consideration of the dynamics using a stretched exponential integrates over all normal modes is an approximate model.\(^\text{73} \) In Figure 10, the observed plateau values of \( (\Gamma/q^3)/(k_B T/\eta_0) \) are in the range of 0.035–0.055. Wu et al. observed similar behavior for well-defined PNIPAM covalently cross-linked microparticles when compared to linear chains.\(^\text{76} \) The plateau value of PNIPAM microparticles is \( \approx 3.5 \) times lower than that of linear PNIPAM (\( \approx 0.05 \)), since the internal motions of the microparticles are suppressed by covalent cross-linking. In the present case, interconnected micelles within a cluster serves as effective beads connected by entropic bridging chains. This view complements the observations with microgels synthesized through covalent cross-linking. Observations by Wu et al.\(^\text{76} \) with well-defined microparticles additionally show that a linear association is not necessary to observe Zimm-like dynamics.

Another consideration for the concentration dependence of the plateau is that the local solvent viscosity is not that of the bulk. The plateau of \( (\Gamma/q^3)/(k_B T/\eta_0) \) shows a systematic concentration dependence. A plot of the \( q^3 k_B T/\Gamma_2 \) plateau versus \( c \) provides an apparent local solvent viscosity (Figure S7). However, the magnitude of the local viscosity increase does not seem reasonable to be the only source of the fractional increase in the apparent local solvent viscosity, when considering the literature data for polymers in organic and halogenated solvents.\(^\text{78,79} \) Gisser et al. showed that the relative diffusivity of tetrahydrofuran solvent does slow down in the presence of polystyrene.\(^\text{80} \) However, in order to observe an \( \approx 10\% \) slowing down in solvent diffusivity the volume fraction of polymer would need to be \( \approx 0.07 \). A conservative estimate for the volume fraction of polymers within the clusters, using the Zimm plot results, is between 0.02 and 0.05. This subtle effect requires molecular dynamics simulations to understand the local hydrodynamic coupling between solvent and polymer or, in this case, micelle within cluster and solvent. Recent studies on PEO by Mondal et al. would provide insight when focused on the water dynamics in the vicinity of the polymer.\(^\text{81} \)

**Microgel-like Clusters Become More Isotropic at Higher Concentration.** The ratio \( \rho = (R_g)/R_h \) quantifies shape anisotropy.\(^\text{82} \) Estimates for \( \rho \) were updated using modern numerical methods to calculate the frictional and hydrodynamic properties of objects formed by a variety of association mechanisms as reviewed by Mansfield and Douglas.\(^\text{83} \) These theoretical considerations conclude that equilibrium self-similar clusters tend to be anisotropic, whereas nonequilibrium clusters are more isotropic. Figure 11 summarizes \( \rho \) as a function of \( c/\text{CMC} \) within the strong clustering regime. \( F_{1.2}E_{445}F_{1.2} \) and \( F_{1.2}E_{445}F_{2} \) at lower \( c/\text{CMC} \) have initially higher \( \rho \) values that decrease to near unity. \( F_{5.6}E_{445}F_{5.6} \) is near unity across the entire concentration range, and \( F_{15.3}E_{445}F_{15.3} \) has globular values of \( \rho \) that increases with concentration. A universal feature is a tendency toward a similar \( \rho \) with increasing \( c/\text{CMC} \).

Just above the CMC, highly anisotropic clusters are observed for lower \( m \) that transitions to more compact clusters as \( m \) increases from 1.2 to 15.3. This appears to correlate with the clusters that display internal relaxation modes (\( m = 1.2, 2, \) and 5.6) and those which are not apparent (\( m = 15.3 \)). Perhaps the most compact clusters do not exhibit the internal relaxation modes visible to DLS length scales due to the high extent of micelle interconnectivity as concluded by the microgel studies.
of Wu and Zhou.\textsuperscript{76} Measurements that unambiguously quantify the interconnectivity are needed.

The observed shape anisotropy of the clusters connects to theoretical predictions (Figure 11). Self-similar equilibrium clusters predict anisotropic shapes with larger $\rho$. This is observed for lower c/CMC of $F_{1.2}E_{445}F_{1.2}$ and $F_{2}E_{445}F_{2}$. It is not unreasonable to deduce that at lower concentrations the clusters appear more anisotropic, then as the extent of clustering increases, the clusters plateau at a lower value of $\rho$, implying less anisotropy, still far from the isotropic uniform sphere value of 0.775 expected for nonequilibrium clusters. $F_{3.6}E_{445}F_{3.6}$ exhibits very little dependence with c/CMC with a plateau value of $\rho \approx 1$. $F_{15.3}E_{445}F_{15.3}$ has a larger variability but lower values in $\rho$ at low concentration that rises to between 0.78 and 1.0. $F_{18.3}E_{445}F_{18.3}$ has the most compact and isotropic structures better described by nonequilibrium clusters (e.g., diffusion-limited aggregation), whereas $F_{3.6}E_{445}F_{3.6}$, $F_{2}E_{445}F_{2}$, and $F_{1.2}E_{445}F_{1.2}$ are self-similar and equilibrium clusters (e.g., percolation clusters and lattice animals).

Upon increasing the symmetric end-group degree of polymerization the window of micelle and clusters formation grows. However, within the $m\rightarrow c$ state diagram of Figure 3, the clustering mechanisms are different. The formation of clusters having similar shape anisotropy with increasing c/CMC was a universal feature. By comparing the cluster size and anisotropy ratio to mean-field models, one can begin to quantify the nonequilibrium and equilibrium cluster structures. In our system, we note that telechelic polymers with highly hydrophobic ends may have limited water solubility, which may be more common that often discussed in block copolymer micelles and gels for drug encapsulation and delivery. A common route to form micelles is to predissolve in a common organic solvent and dialyze\textsuperscript{84} to obtain desired structures as was done with $F_{15.3}E_{445}F_{15.3}$ and $F_{3.6}E_{445}F_{5.6}$, $F_{3.6}E_{445}F_{5.6}$ exhibited a slight increase in the ($R_\text{g}$) of the cluster with increasing concentration, while $F_{15.3}E_{445}F_{15.3}$ did not. This is most likely the persistence of flower-like micelles,\textsuperscript{85,86} whereby the enthalpic penalty to permit free ends and kinetics of assembly are prohibitively slow making self-assembly kinetically controlled. However, $F_{2}E_{445}F_{2}$ and $F_{1.2}E_{445}F_{1.2}$ are immediately soluble and reversibly assembled, a desirable feature for drug encapsulating particles and injectable gels. Figure 3 needs to consider the interaction parameters. For instance, the strong self-association of the fluorene groups suggests a large Flory–Huggins interaction parameter. This not only drives the selected solubility and micellization but also kinetically prevents dissolution and formation of well-defined structures. Figure 3 may be better considered and made universal by the product $\chi f_R$. This work is ongoing with selective deuterium labeling and small-angle neutron scattering.

**CONCLUSION**

The supramolecular assembly of $F_nE_{445}F_m$ telechelic (triblock) polymers exhibits a classical critical micelle concentration. This closed-assembly process becomes coupled to micelle clustering with increasing concentration and end-group degree of polymerization, $m$. The concentration dependence for the cluster size was consistent with mean-field descriptions derived for worm-like chain assembly and multistep equilibrium models. Without any adjustment, experimental data follow the mean-field scaling $R \sim (c/\text{CMC})^{1/4}$. At low $m$, clusters are anisotropic near the CMC and become more isotropic with increasing concentration as quantified by the anisotropy ratio. Moreover, low $m$ clusters resemble soft microgels through their internal dynamics when compared to well-defined cross-linked microgels. The internal dynamics of these clusters are consistent with Rouse–Zimm dynamics. This behavior is strongly dependent on $m$. Higher $m$ values do not show the $q^4$ deviations in the $q^2$ decay rate law expected by translational diffusion. These tend to be irreversible isotropic clusters through their anisotropy ratio. These observations demonstrate that incorporation of F-TMC groups leads to classical micelles that are coupled to hierarchical assembly (clustering of micelles). This closed, coupled-to open assembly is a general process readily probed by angular-dependent static and dynamic light scattering. Since solution and gel properties (viscosity, shear modulus) and delivery properties (encapsulation, release, loading) are always dependent upon the microstructure, these experiments provide a detailed view on the fundamentals of supramolecular driven assembly.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.6b02524.

Parameters from static light scattering by eq 4, Berry plot from $R_\chi(cq)$, Zimm plot from $I_1(cq)$, angular dependence of the scattered light intensity $I_2(q)$ and $[R_\chi(q)]^{1/2}$, concentration dependence of the relative amplitude of micelle ($a_1$) and cluster ($a_2$), $q$ dependence of the stretched exponent $\beta$, local solvent viscosity, and examples of CONTIN results (PDF)

### AUTHOR INFORMATION

**Corresponding Author**

*E-mail vprabhu@nist.gov, Tel (301) 975-3657 (V.M.P.).*

**ORCID**

Yi Yan Yang: 0000-0002-1871-5448

James L. Hedrick: 0000-0002-3621-9747

Vivek M. Prabhu: 0000-0001-8790-9521

**Notes**

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

Y.Y. and S.V. acknowledge funding support from the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore). V.M.P and G.W. thank Jack Douglas (NIST) for selective deuterium labeling and W. Vreeland (NIST) for assistance in the refractive index measurement.

### ADDITIONAL NOTE

\textsuperscript{4}Certain commercial equipment and materials are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendations by the National Institute of Standards and Technology (NIST) nor does it imply that the material or equipment identified is necessarily the best available for this purpose.


Phenomena in Block Polyelectrolyte Solutions.


Range Interactions in Aqueous Solutions of Poly (Ethylene Oxide).


