21st Century Advances in Fluorescence Techniques to Characterize Glass-Forming Polymers at the Nanoscale

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Characterization of polymers at the nanometer-length scale has become increasingly important with the growth and expansion of nanotechnology. Due to limitations of sensitivity and specificity that persist with traditional materials characterization techniques, there is a growing need to develop new tools to measure the properties of confined polymer systems. Within the past 20 years, fluorescence characterization techniques have emerged to address this challenge. This review focuses on the employment of fluorescence techniques such as temperature- and time-dependent steady-state intensity, fluorescence recovery after photobleaching, and nonradiative energy transfer to study polymer behavior at the nanoscale. Properties discussed include glass transition temperature, physical aging, diffusion and mobility, and mechanical response. Particular attention is given to the unique insight gained through these techniques as well as opportunities for future studies. The ability of fluorescence to obtain location- and component-specific measurements with nanometer resolution for a variety of polymer chemistries and geometries on a bench-top instrument provides a valuable contribution to the characterization and understanding of polymers at the nanoscale.

1. Overview

Recent advances in polymer nanotechnology for applications in renewable energy,[1] electronics,[2–4] and healthcare[5] drive improved characterization of nanoscale polymer systems. The thermal and mechanical properties of confined polymers have been observed to deviate substantially from bulk properties, making them a subject of extensive investigation over the past few decades.[6–8] The development and application of fluorescence techniques to investigate the behavior of confined polymers has provided valuable insights into how surfaces and interfaces can lead to dramatic modifications in key material properties.[9,10] While fluorescence has been used extensively to characterize bulk properties of polymers, including glass transition temperature ($T_g$)[11–13] and polymerization conversion,[13] it is particularly well-suited to study polymers at the nanoscale. This is largely due to the fact that fluorescence techniques enable sensitive and location-specific measurements with high resolution.

In this review, we focus on the use of both steady-state and transient fluorescence techniques to characterize physical properties of polymers over nanoscale dimensions. This length scale can refer to film thickness in single-component polymer films, interparticle spacing in nanocomposites, domain spacing in phase-separated block copolymers, and distance from the polymer–polymer interface in multicomponent immiscible blends. Several geometries characterized via fluorescence are shown in Figure 1. Prior to delving into specific contributions, the basic principles and techniques of fluorescence are presented as well as a brief introduction to the behavior of confined polymers. We then present recent advances in fluorescence techniques for polymer physical characterization under confinement with regards to the glass transition temperature, physical aging, mobility and diffusion, and mechanical response. Through this examination of the literature, we illustrate the unique ability of fluorescence to characterize polymers in confined and complex geometries, providing insights into the influences of interfaces and nanostructure on material properties through sensitive, location-specific measurements. We conclude with a view toward future areas of research in which fluorescence has the potential to be impactful.

2. Introduction to Fluorescence

When a molecule absorbs energy via light, an electron is excited to a higher energy state, returning to the ground state through either radiative or nonradiative deactivation. This electronic excitation and return to the ground state is a combination of three photophysical processes: absorption, luminescence, and nonradiative decay.[13] Fluorescence is a luminescent process in which the excited-state electron returns to the ground state by emitting a photon, i.e., light. As shown in a simplified Jablonski
diagram in Figure 2a. Absorption of energy promotes an electron to one of several vibrational levels of the excited state, from which it undergoes internal conversions to return to the lowest excited vibrational state (S1v0). From this energy level, the molecule can return the excited-state electron to the ground state through emission of a photon (fluorescence) or by nonradiative deactivation in the form of rotational and vibrational motions. These competing deactivation pathways enable fluorescing molecules, i.e., fluorophores, to probe the properties of their surrounding environments due to the sensitivity of nonradiative motion to density changes in the polymer matrix.[10]

Fluorescence spectroscopy excites fluorophores with light at a specific wavelength and records the emitted light with a photodetector. As mentioned above, fluorescence deactivates electrons from the lowest vibrational level of the excited state (S1v0) to vibrational levels of the ground state (S0v). These transitions between different energy levels correspond to the emission of photons at different wavelengths. Collecting these photons gives a characteristic spectrum for each fluorophore, as shown in Figure 2b. The intensity of the spectrum is reflective of the number of photons emitted with each wavelength. The fluorescence emission spectrum occurs at longer wavelengths than the corresponding absorption spectrum due to the loss of energy through internal conversion. The number of photons emitted via fluorescence relative to the number absorbed by the molecule is referred to as its quantum yield, and fluorophores with high quantum yield are generally desired for high signal and sensitivity.

Kinetically, the rate equation for unimolecular fluorescence can be described as

$$\frac{d[M^*]}{dt} = I_0 - (k_i + k_f)[M^*]$$  \hspace{1cm} (1)

where \([M^*]\) is the concentration of excited molecules, \(I_0\) is a constant reflective of the spectrofluorimeter properties, and \(k_i\) and \(k_f\) are the rates of fluorescence and nonradiative deactivation, respectively.[33] Fluorescence can be measured through steady state or transient experiments, which will be described in more detail in Section 4. Steady-state measurements are time-averaged over the fluorophore’s fluorescence lifetime. They use a continuous light source, e.g., a xenon lamp, to excite a sample and collect fluorescence spectra at photostationary conditions. This allows for the assumption that \(d[M^*]/dt = 0\), thus the steady-state fluorescence intensity, \(I_F\), at a particular wavelength can be described as

$$I_F = k_f \left[ M^* \right] = I_0 \frac{k_i}{k_i + k_f} = I_0 \phi_f$$  \hspace{1cm} (2)

where \(\phi_f\) is the quantum efficiency.[33] This equation clearly illustrates the dependence of fluorescence intensity on relative rates of fluorescence and nonradiative deactivation.

Transient, or fluorescence lifetime, measurements use a pulsed light source and collect emitted intensity as a function of time. Kinetic Equation (1) still applies to transient measurements, without the assumption that \(d[M^*]/dt = 0\). The fluorescence intensity as a function of time can then be expressed as

$$I_F (t) = M_0 A_1 e^{-(k_i + k_f) t} = F_0 e^{-(k_i + k_f) t}$$  \hspace{1cm} (3)

where \(A_1\) and \(F_0\) are constants.[33] The fluorescence lifetime (\(\tau_F\)) can be defined as

$$\tau_F = \frac{1}{(k_i + k_f)}$$  \hspace{1cm} (4)

These transient measurements are advantageous over steady-state measurements, as they are less sensitive to instrumentation factors.[33]
The selection of fluorophores is also important in designing a fluorescence experiment. The wide variety of fluorescing molecules available enables optimization of their selection for each experimental system and technique. Dyes sensitive to minute changes in the density of their surrounding matrix have been used to measure $T_g$ and physical aging, while dyes with high degrees of anisotropy are desired for dye rotation experiments. Dye pairs can also be incorporated into samples as electron donors and acceptors for energy transfer experiments. Specific fluorophores used in relevant studies will be discussed briefly in Section 4. In all cases, high quantum yield improves the sensitivity of the measurements and reduces the necessary

![Diagram showing different geometries probed via fluorescence](image)

**Figure 1.** Schematic showing different geometries that have been probed via fluorescence for the reader’s general reference: a) supported films, b) freestanding films, c) capped films, d) substrate layer, e) free-surface layer, f) multilayer films, g) polymer–polymer interfaces, h) micelles, i) nanocomposites, j) patterned films, k) irreversibly adsorbed layers, l) grafted brushes. Fluorescently labeled/doped polymer is shown in green against neat polymer layers. Arrows indicate dimensions that can be adjusted.

![Diagram showing simplified Jablonski diagram](image)

**Figure 2.** a) Simplified Jablonski diagram illustrating fluorescence as a competing energy deactivation pathway with internal conversion (vibrational or rotational motion resulting in heat loss). Phosphorescence is neglected here due to its low occurrence in the systems of interest. b) Photons corresponding to specific energy levels and wavelengths combine to form a fluorescence emission spectrum. Adapted with permission. Copyright 2000, Elsevier.
concentration of dye, thus enabling minimal perturbation to polymer matrices.

Several factors promote fluorescence as a good technique for characterizing polymers at the nanoscale. Its high sensitivity gives strong signals relative to the amount of fluorophores, enabling measurements at low concentrations, reflective of an unperturbed matrix. This sensitivity is also ideal for measuring nanoscale samples. The timescale of fluorescence, ranging from $10^{-12}$ to $10^{-6}$ s, enables the capture of dynamic processes occurring in polymeric systems. The tradeoff between fluorescence and nonradiative energy deactivation enables probing properties of the surrounding polymer matrix. Furthermore, the instrumentation required is readily accessible, and experiments can be conducted in-house.

### 3. Confined Polymer Properties

Over the past few decades, there has been substantial investigation into the deviation from bulk properties observed in polymers when confined to the nanoscale.$^{[8,15]}$ By restricting one or more dimensions to less than hundreds of nanometers, researchers have observed dramatic changes in $T_g^{*}$ physical aging,$^{[9,48]}$ mobility,$^{[49]}$ fragility,$^{[50]}$ diffusion,$^{[18,19,51,52]}$ viscosity,$^{[53-57]}$ elastic modulus,$^{[58,59]}$ and rubbery stiffening,$^{[60]}$ among others, with very few exceptions.$^{[61]}$ The magnitude and direction of these deviations, as well as the confinement length scale necessary to induce changes vary for specific polymer systems.$^{[6]}$ These deviations have largely been attributed to the enhanced influence of surrounding surfaces and interfaces due to the extreme surface-to-volume ratios that result from physical confinement to the nanoscale.$^{[8]}$ Here, we describe in more detail properties of particular emphasis in the literature and how they deviate from bulk properties upon confinement. In doing so, we lay the foundation for understanding to be gained through the contributions from fluorescence characterization.

#### 3.1. The Glass Transition

The glass transition occurs when a liquid, supercooled below crystallization, is kinetically arrested in an amorphous state.$^{[62]}$ This transition is accompanied by a many-orders-of-magnitude increase in viscosity and a change from rubbery or liquid-like behavior to the solid-like behavior of a glass. This dramatic change in material properties makes the temperature at which this transition occurs, known as the $T_g$, an important classification of a material. The $T_g$ is often graphically depicted, as shown in Figure 3, as a change in the slope of a thermal property proportional to the second derivative of free energy, such as specific volume or enthalpy, against temperature. Due to the fact that many polymers are amorphous, $T_g$ strongly impacts their performance and selection for applications. The $T_g$ in bulk polymer systems can be measured using conventional tools such as differential scanning calorimetry (DSC), viscometry, thermal mechanical analysis (TMA), dynamic mechanical analysis (DMA), and dilatometry. However, confined geometries pose challenges to applying these techniques due to sensitivity limits and sample geometry requirements.

![Image](https://www.advancedsciencenews.com/)

**Figure 3.** Schematic of the glass transition (shown in blue) and physical aging (shown in red) on a plot of specific volume or enthalpy vs temperature. $T_m$ is the temperature at which a liquid, supercooled below its melting temperature ($T_m$), falls out of equilibrium and into a glassy state. Physical aging is the gradual relaxation of a glass towards equilibrium when annealed at a temperature below $T_g$.

The first observation of a size-dependent effect on $T_g$ was reported by Jackson and McKenna when they measured a depressed calorimetric $T_g$ in ortho-terphenyl confined to nanopores.$^{[63]}$ This size dependence of $T_g$ was extended in 1994 to confined polymers in a seminal work by Keddie et al.$^{[35]}$ Measuring film thickness as a function of temperature via ellipsometry enabled the determination of $T_g$ of PS films supported on Si. They observed a reduction in film $T_g$ with decreasing thickness, down to several tens of degrees below bulk in =10 nm thick films. The deviations from bulk, starting at =40 nm were much larger than those previously observed for small molecules, suggesting that the long-chain nature of polymers magnified their confinement behavior. They concluded that these deviations were due to the strong influence of a liquid-like layer at the polymer–air (free) interface that grew in proportion to the film with decreasing film thickness. This study was expanded upon by using the same technique to measure the $T_g$ of supported poly(methyl methacrylate) (PMMA) thin films.$^{[41]}$ Contrary to PS results, PMMA was found to increase in $T_g$ with decreasing thickness when supported on Si, which was attributed to hydrogen bonding with the native-oxide layer on the Si. Measurements of PMMA supported on gold showed a negative $T_g$ deviation with decreasing thickness,$^{[41]}$ confirming that both polymer–air and polymer–substrate interactions contribute in determining the direction and magnitude of the change in $T_g$ for confined polymers.

The balance of interfacial influences continued to be a leading hypothesis for confined $T_g$ behavior in polymer thin films for much of the next decade, and additional techniques...
were adapted to achieve further insight. Brillouin light scattering (BLS)\cite{42,46,64} and transmission ellipsometry\cite{39,43,65} enabled the measurement of free-standing thin film $T_g$. The $T_g$ of $\approx$40 nm thick freestanding films of PS and PMMA with similar molecular weight measured 55 K\cite{65} and 15 K\cite{47} below their respective bulk values. This persistent difference in the magnitude of their deviations, even in an unsupported geometry, suggested that the relative strength of substrate interactions could not be entirely responsible for the difference in their confinement behavior. Furthermore, the presence of two free surfaces, and hence a lack of substrate interactions, enhanced negative $T_g$ deviations beyond those measured in supported films. The correlation between these larger deviations and the increased number of free surfaces was clarified in a study by Bäumchen et al. where freestanding PS films were measured before and after placement onto substrates.\cite{39} The latter measurements of the same films had much smaller $T_g$ reductions with respect to bulk, in line with those reported for directly spin-coated films.

Understanding and prediction of confined polymer $T_g$ is critical to advancing the application of polymers in nanotechnology. Interest in this field has produced a multitude of research, and we refer the reader to several studies and review articles to gain a broader appreciation for the subject.\cite{6–8,44,73,74} Positron annihilation lifetime spectroscopy (PALS)\cite{75} and neutron reflectivity\cite{76,77} have also been used to characterize thin film $T_g$. Although the majority of experimental studies attribute confined $T_g$ behavior to strong interfacial influences, these studies relied on models and indirect measurements of local $T_g$ to support their conclusions.\cite{15,46,78} Recent simulation and theoretical work show strong evidence of interfacial effects on $T_g$\cite{79–81} averaged across films, lacking the ability to provide direct, location-specific measurements.

### 3.2. Physical Aging

Physical aging has also been shown to deviate from bulk behavior upon confinement, and the requirement that physical aging occurs below $T_g$ suggests that physical aging under confinement would be associated with confined $T_g$. The relationship between these two properties at the nanoscale is an ongoing area of research. Similar to with $T_g$ measurements, conventional tools for characterizing physical aging in bulk samples, such as calorimetry and PALS, have limitations to nanoscale characterization due to sensitivity and accessibility. A substantial body of work exists examining the effects of confinement on physical aging of polymer thin films.\cite{32,22,84,86}

Kawana et al. used thickness vs. temperature data measured via ellipsometry to derive expansivity overshoots for films of different thickness aged at 70 °C and 80 °C for up to 7 d.\cite{85} They observed smaller overshoots in thinner films, indicative of a decrease in the extent of physical aging. These experiments were conducted at temperatures above the predicted confined $T_g$ for 30 and 18 nm films, but they still observed expansivity overshoots indicative of physical aging. They attributed the presence of physical aging in these films to heterogeneous behavior where a liquid-like layer at the free interface remains above the $T_g$ but the remainder of the film undergoes physical aging. The lack of detectable overshoot in 10 nm films and a thickness-independent temperature of the overshoot in other samples combined to suggest a two-layer model with a 10 nm mobile layer above a bulk-like underlayer. The existence of a free-surface mobile layer was consistent with predictions made in $T_g$ studies, but direct measurement was still not possible.

Calorimetric measurements of confined PS films also supported deviations in physical aging due to reduced $T_g$. Although mass requirements prevented measurements of single ultrathin films via conventional DSC, Koh, and Simon used this technique to measure confined structural relaxation in stacked PS thin films. In this study, they made an important distinction between the time to reach equilibrium and aging rate as indications of physical aging.\cite{69} These two different methods to quantify physical aging are both used in the literature to describe physical aging under confinement, so attention should be given to which method is employed.\cite{87} Koh and Simon reported faster time to reach equilibrium and slower physical aging rates in thin films compared to bulk when annealed at identical temperatures. However, annealing confined and bulk samples relative to their respective $T_g$s yielded slightly longer
times to achieve equilibrium in confined films and similar aging rates. This suggested that deviations in physical aging of ultrathin films were solely an effect of their reduced \( T_g \), since the driving force for physical aging is largely determined by proximity of aging temperature to \( T_g \).[65] As with \( T_g \) measurements obtained by stacking films, extensive sample preparation limits wide implementation of this technique, with the exception of a study by Boucher et al. in which they also measured faster times to equilibrium in confined PS films.[88] Nanocalorimetry shows promise for characterizing single ultrathin films, but it has thus far mostly been used to expand the range of aging times and temperatures of structural recovery measurements in films \( >1 \) \( \mu \)m thick.[89,90] Very recent work by Koh and Simon examined aging in a single 20 nm thick PS film and found faster aging rates, shorter time to equilibrium, and a broader distribution of relaxation rates compared to bulk.[91] The examination of a wider range of thicknesses would elucidate thickness-dependent trends.

Physical aging in nanocomposites, where a polymer matrix is confined between nanoparticles with interparticle spacings on the order of \( \approx 100 \) nm, has also been studied extensively by DSC.[87,92–94] At high nanoparticle loadings \( \approx 10 \) wt\%, the time to reach the equilibrium enthalpy plateau was consistently lower than with neat polymer. The magnitude of this depression in equilibration time increased with increasing the surface area/volume ratio of nanoparticle fillers, regardless of the interaction strength between polymer and nanoparticles. However, accompanying measurements of nanocomposite segmental mobility by broadband dielectric spectroscopy (BDS) showed no deviation from bulk.[92,94] This suggested a separation of structural relaxation and segmental mobility under confinement, which was rationalized by the free volume holes diffusion (FVHD) model.[93,94]

Despite initial evidence, the relationship between physical aging and \( T_g \) at the nanoscale remains unclear. Pye et al. studied thickness and temperature dependence of the aging rates of supported thin PS films via ellipsometry, calculating aging rates by plotting normalized film thickness against log aging time.[84] Similar to previous studies, they observed a decrease in aging rate with film thickness at the temperature of peak aging rate in bulk. However, measuring confined film aging rates at different temperatures showed not just a shift due to reductions in \( T_g \), but that confined films had consistently lower aging rates at a range of temperatures. They interpreted their data in terms of a two-layer and gradient model and saw good agreement with literature that the mobile layer increased in thickness with decreasing temperature. This implied that aging behavior and \( T_g \) under confinement derive from a similar mechanism, but aging is not simply reflective of the shift in \( T_g \).

Their application in membrane technology has drawn attention to physical aging behavior in freestanding thin films. While researchers largely report suppressed physical aging in supported thin films,[63,84,85] free-standing films—characterized by transmission ellipsometry,[86] PALS,[95] and gas permeativity—have been widely observed to exhibit enhanced physical aging.[96–98] Furthermore, these enhanced physical aging rates were observed in films over 1 \( \mu \)m thick—much thicker than typically associated with confined behavior. However, work by Pye and Roth suggests that this behavior, in films thicker than 200 nm, might be due to thickness-dependent stress put on the sample by differences in thermal expansivity with the sample holder.[86] Using transmission ellipsometry, they observed no thickness dependence of aging rates for films held by rigid holders over a 220–1800 nm thickness range.

The difference in film thickness for which deviations from bulk \( T_g \) and physical aging behavior are measured in thin films is an ongoing area of research. As with \( T_g \) layer models have been used extensively to interpret observed deviations in physical aging rates and extents.[84,85] However, direct measurement of these properties has been limited and correlation between fitted thickness and index of refraction limit aging rate measurements on films \( <30 \) nm via ellipsometry.[84]

3.3. Diffusion and Mobility

Due to the long chain nature of polymers, relaxations occur on many different length scales and therefore many different time scales, from segmental relaxation to whole-chain reptation and diffusion. Polymer glasses, and other glass formers, exhibit complex dynamics as the glass transition is approached due to these relaxation events.[62] Many theoretical models and data from both experiments and simulations exist to try to describe these phenomena, the details of which are outside the scope of this review. There are many excellent reviews on the fundamentals of relaxation in glass formers[62,99–103] and under confinement[8,74,104] some of which are given here for the interested reader.

Upon cooling from above \( T_g \), segmental, or \( \alpha \), relaxations deviate from an Arrhenius temperature dependence such that the energy barrier for segmental rearrangement grows with decreasing temperature, diverging at \( T_c \).[62,105] Even above \( T_g \), many nearby polymer segments must also move to allow a given segment to break out of its “cage” of neighbors. This cooperativity is thought to introduce dynamic heterogeneity, where at different times, individual groups of polymer segments relax faster or slower than the ensemble average.[99] Near and above \( T_g \), measurements of a relaxation function \( r(t) \) are well-fit by the Kohlrausch-Williams-Watt (KWW) form

\[
r(t) = e^{-(t/\tau)\beta}
\]

where \( \tau \) is a characteristic relaxation time and \( \beta \) is the stretching exponent associated with the breadth of the relaxation time distribution.[62]

Several experimental methods can probe polymer chain motion under confinement such as dewetting,[106,107] hole growth,[44,108,109] nanoeembedding,[110] and various surface relaxation techniques.[111–113] These studies, either probing the free-surface or averaging dynamics across the whole polymer film thickness, have shown enhanced mobility in free-surface layers propagating tens of nanometers into the polymer film, though the length-scale and extent of enhanced mobility varies depending on polymer, experimental temperature, and measurement technique. Neutron scattering has been used...
extensively to measure diffusivity and mobility in polymers confined between nanoparticles within nanocomposites, as reviewed by ref. [114], and has recently been applied to diffusion perpendicular to interfaces with different degrees of polymer attraction.[115] However, the required access to a neutron source and geometric and polymer constraints present limits to their accessibility and broad application. This warrants a technique to measure diffusion and mobility in nanoscale polymer systems that retains sensitivity but does not impose beam-line requirements or restraints on samples to be measured.

Experimental techniques including BDS,[116,117] nuclear magnetic resonance imaging,[118] and a combination of BDS and small angle X-ray scattering,[119] have been used to great effect to understand confined polymer segmental dynamics in thin films and within nanocomposites. For example, BDS has been used to measure $T_g$, the temperature at which the $\alpha$-relaxation shows a maximum in isochronal conditions. Although this peak is associated with $T_g$ in bulk polymers, $T_g$ is constant or slightly decreasing with decreasing thickness in confined systems—even in systems where $T_g$ increases in the doubly-supported, or capped, geometry required for BDS.[117]

### 3.4. Mechanical Response

The deformation of polymer glasses in bulk is well studied by classical mechanical testing tools.[120,121] However, yielding in a glassy polymer could be viewed as “mechanically passing the glass transition temperature” and implies association with the molecular (segmental) mobility of polymer chains.[128,122] Characterizing how externally applied stress and resulting deformation induces mobility in polymers is a question of increasing importance.[123] At the atomic level, chemical bond and conformational changes occur for some specific classes of polymers.[124] At the nano- and microscale, plastic deformation results in voids, cavitation, and craze or crack formation that manifest as large-scale viscoelastic deformation.[120,121,125] Experimental findings from conventional mechanical testing under small strain[120,121,126] were indirectly interpreted in terms of enhanced segmental motion in polymers and several simulation studies addressed stress-induced molecular mobility in glassy polymers.[127–129] However, none of these experiments directly characterized nanoscale segmental mobility under small scale deformation (pre-yield) in thin films, since their molecular relaxation rate was derived from large-scale plastic flow and yielding.

Several techniques have been developed to measure the mechanical properties of ultrathin polymer films and have largely been used to elucidate deviations from bulk stiffness[9,60,130–136] and residual stress.[132,137] The experimental temperatures at which these studies were performed proved important in revealing different confinement behaviors below and above bulk $T_g$: glassy softening[9,111] and rubber softening.[118,119] Tensile testing of films supported on water[113] and flat-punch nanoindentation[114] also revealed reduced elastic modulus and yield stress in confined glassy films less than $\sim$30 nm thick. Buckling-based techniques on elastomer-supported thin glassy films showed reductions in elastic modulus for both PS and PMMA upon decreasing film thickness below $\sim$40 nm.[59,131] Dewetting[129,130] and wrinkling[139] studies showed a decrease in elastic modulus for thin glassy PS films, though they report higher and lower values of residual stress, respectively. By using nanobubble inflation to measure creep compliance of free-standing poly(vinyl acetate) and polystyrene films in the rubbery regime, O’Connell and McKenna revealed a stiffening of modulus with decreasing thickness for sub-200 nm films.[60,136] Karim and McKenna later used nanoparticle embedding techniques to measure stiffness at the free surface in poly($\alpha$-methylstyrene) (PMoMS).[135] They noted a softening of the surface well into the glassy state, consistent with previously mentioned confined polymer studies.[59,130–132] However, they observed an onset of surface-stiffening 20 K below $T_g$, which they attributed to the uniquely high density and stiffness of PMoMS and its previously reported lack of a surface mobile layer.[140] Location-specific measurements within films would allow for connections to be made between this surface behavior and deviations in the average moduli of films from bulk, allowing for a comparison of free-surface influence and perturbation length scales in the glassy and rubbery regime.

### 4. Methodology

#### 4.1. Glass Transition Temperature

Much of the work described in the next two sections relies on the ability of extrinsic fluorescent probes to detect changes in the density of the surrounding polymer matrix in order to measure $T_g$ via fluorescence. These probes are primarily derivatives of pyrene or 4-tricyanovinyl-[N-(2-hydroxyethyl)-N-ethyl]-aniline (TC1) dyes; representative forms are seen in the insets of Figures 4a and 5a, respectively. As mentioned previously, these probes rely on a tradeoff between fluorescence emission and nonradiative energy dissipation through molecular motion to characterize their environment. Pyrene’s nonradiative deactivation largely occurs via vibrational motion of the probe, while TC1 has a significant rotational motion component.[22] The temperature dependence of their fluorescence response has been observed to be sensitive to $T_g$ due to relative changes in the density and rigidity of the polymer matrix above and below $T_g$.[9,10,17] Figure 4a displays the difference in emission intensity of pyrene with a 100 K difference in temperature.[9] At higher temperatures, increased mobility and reduced density in the polymer matrix allows for a larger fraction of the probe’s energy to be dissipated via motion—resulting in lower fluorescence intensity. This holds true for both pyrene (shown) and TC1 probes.

Each of these fluorophores can be incorporated into a polymer matrix by doping or labeling at dilute concentrations, to avoid plasticization or perturbation of the polymer. Slight modification of the dye is required to label the polymer chain by covalently attaching the probe. While the key molecular structure of a given dye is not altered for labeling, it is linked to a methacrylate monomer via a short carbon chain. The length of this linker chain, typically methyl or butyl, depends on the technique used.[10,17,23] The dye-labeled monomers can then be incorporated at low concentrations (<1 mol%) in a bulk free
radical polymerization to produce labeled polymer.\textsuperscript{[10,22]} While both doping and labeling enable average $T_g$ measurements in thin films, fluorescently labeled polymer has a significant advantage: it can be combined with unlabeled, neat, polymer to study site-specific phenomena, having precise control of label location. This technique will be discussed in Section 5.

Films are prepared by either spincoating or dropcasting a solution of labeled polymer or neat polymer with dye, onto silica substrates. These substrates are made of quartz specifically chosen to not transmit light at the wavelengths of interest (depending on the fluorophore) so as not to interfere with the fluorescence emission spectra. Thickness of the films is controlled via solution concentration and spin speed, and films are carefully dried to remove residual solvent.

The measurement of $T_g$ requires the combination of a spectrofluorimeter with a front-facing sample collection setting and a temperature-controlled sample holder. Steady-state fluorescence measurements are conducted by collecting spectra upon cooling, after holding the films well above $T_g$ to erase any thermal history. Excitation wavelength and the wavelength range for emission spectra collection depend on the probe used, noting that probes modified for labeling will also require different settings than their doped counterparts. The precise method for determining $T_g$ from these spectra varies somewhat depending on the system and study. For supported films, either peak intensity or integrated spectra intensity is plotted against temperature. An example of peak intensity data is shown in Figure 4b for labeled PS films. Distinct slopes pertaining to the rubbery and glassy regions are fit to the intensity data with their intersection being $T_g$. These slopes reflect the difference in the temperature dependence of probe mobility due to changes in the density of the polymer matrix.\textsuperscript{[16]} While $T_g$ measured by peak intensity and integration are found to be in good agreement within experimental error,\textsuperscript{[15]} integration is reflective of total quantum yield and is considered a more robust approach.\textsuperscript{[141]}

These $T_g$ measurements have also been adapted to measure $T_g$ in freestanding polystyrene films. Films of pyrene-labeled PS are first spin-coated onto mica or salt disk substrates before transferring to a nylon washer via a water floating technique.\textsuperscript{[23,24]} The excitation beam is then focused on the section of the film suspended over the hole in the washer. While freestanding film fluorescence spectra are collected upon cooling as with supported films, they cannot

**Figure 4.** a) Pyrene fluorescence emission spectrum in a 350 nm thick polystyrene film at 298 K (bold curve) and 398 K (thin curve). Data have been normalized by the maximum intensity of the spectrum at 298 K. Also shown is the molecular structure of pyrene. Reproduced with permission.\textsuperscript{[9]} Copyright 2002, Springer. b) Fluorescence intensity measurements of 545 nm- (squares) and 17 nm- (diamond) thick pyrene-labeled PS films plotted against temperature. Reproduced with permission.\textsuperscript{[10]} Copyright 2003, Macmillan Publishers Ltd.

**Figure 5.** a) Fluorescence emission spectrum for a 39 nm thick P(BMA-MATC1) film taken $\approx 2$ min after quenching from 343 to 298 K (dashed curve) and after 6.5 h of aging at 298 K (solid curve). Also shown is the molecular structure of TC1. Reproduced with permission.\textsuperscript{[9]} Copyright 2002, Springer. b) Normalized fluorescence intensity with aging time of TC1-doped PS films: 500 nm aged at $T_{g,\text{bulk}} - 10$ K ($\Delta$) and $T_{g,\text{bulk}} - 71$ K ($\circ$), and 20 nm films at $T_{g,\text{bulk}} - 10$ K ($\square$) and $T_{g,\text{bulk}} - 71$ K ($\star$). Reproduced with permission.\textsuperscript{[48]} Copyright 2005, American Chemical Society.
be analyzed using this same method due to the influence of film rippling during cooling on the surface area of the film exposed to fluorescence excitation.\[24\] Instead, their spectra are analyzed using a self-referencing technique that compares the intensities of the first and third emission peak, occurring at wavelengths of $375 \pm 3.5$ nm and $386 \pm 3.5$ nm, respectively.\[23,24\] Plotting their $I_3/I_1$ ratio against temperature gives plots similar to that shown in Figure 4b to determine $T_g$. Of note, it was found that only pyrene covalently linked to the polymer chain via a methyl linkage is sensitive to film $T_g$ using this method.\[23\]

In addition to dye incorporation in extrinsic fluorescence studies, the intrinsic fluorescence of PS has been used to characterize $T_g$ in thin films of PS and P(S-r-MMA).\[20\] Polystyrene exhibits fluorescence due to the interaction of its phenyl groups. This technique relies on the combination of monomer and excimer fluorescence emission peaks, originating from single and stacked phenyl rings, respectively.\[9,20\] Spectra from these samples, despite smaller signal-to-noise ratios than with extrinsic fluorescence, also show a temperature dependence that is sensitive to $T_g$.

### 4.2. Physical Aging

Most of the physical aging fluorescence studies described in this review use TC1 derivatives, either doped or covalently attached to the polymer chain to measure physical aging rates. The additional rotational motion TC1 undergoes via nonradiative deactivation provides greater sensitivity to the small density changes that accompany physical aging, thus making it better suited to physical aging experiments than pyrene.\[9\]

Films for aging experiments are prepared much in the same way as they are for $T_g$ measurements. However, spectra for aging experiments are collected isothermally as a function of time after quenching to a certain temperature. Figure 5a shows the effect of isothermal aging on the system. Here, aging for 6.5 h at 298 K densifies the polymer matrix, restricting nonradiative energy dissipation of the probe, which results in an increased fluorescence intensity. Figure 5b shows the normalized maximum fluorescence intensity as a function of aging time for different thicknesses and aging temperatures of TC1-doped PS films. Relative changes in fluorescence intensity over time are an indication of the degree of physical aging undergone. These intensities can also be used to calculate aging rates through the equation:

$$r = \frac{1}{F_0} \left( \frac{dF}{d \log t} \right)$$

where $F$ is the fluorescence intensity at aging time $t$, and $F_0$ is the initial fluorescence intensity.\[22\]

### 4.3. Diffusion and Mobility

A variety of fluorescence-based techniques exist to quantify mobility and diffusion in polymeric systems. Here, we will outline two photobleaching procedures for quantifying (1) segmental mobility from the rotational anisotropy of fluorescent dyes after photobleaching and (2) translational diffusion from fluorescence recovery after photobleaching (FRAP). Other techniques include second harmonic generation, fluorescence lifetime studies, single molecule particle tracking, and Förster resonance or nonradiative energy transfer (FRET or NRET), and will be briefly discussed in Section 8.

To quantify dye rotational mobility, fluorescence emission is monitored following photobleaching with linearly polarized light. A pattern or a spot can be photobleached by selectively exposing part of the polymer sample to bright light that permanently inhibits the fluorophore from fluorescing. In the case of photobleaching with linearly polarized light, the fluorophores oriented parallel to the incident light will be bleached.\[140,142,143\] This creates an anisotropy in the polarization of fluorescent light immediately following photobleaching. At a constant temperature or as temperature is increased through and above $T_g$, the fluorescent dye molecules will reorient—if the temperature is below $T_g$ there may not be enough mobility for the dye to reorient during the timescale of the experiment.\[140,142,143\] The rotational anisotropy, $r(T,t)$, can be defined as:

$$r(T,t) = \frac{(\Delta I_\parallel(T,t) - \Delta I_\perp(T,t))}{(\Delta I_\parallel(T,t) + 2\Delta I_\perp(T,t))}$$

where $\Delta I_\parallel(T,t)$ and $\Delta I_\perp(T,t)$ are the differences in fluorescence intensities between the bleached and unbleached regions for parallel and perpendicular components of fluorescence, measured either as a function of time or temperature.\[140,142,143\]

Figure 6a gives an example of fluorescent dye reorientation as a function of temperature. Freestanding polystyrene films containing trace levels of N,N'-bis(2,5-di-tert-butylphenyl) 3,4,9,10perylenedicarboximide (BTBP) (shown in the inset of Figure 6a) were bleached well below the glass transition then heated through $T_g$.\[140\] For bulk PS films, the rotational anisotropy does not relax until $T_g$ bulk is approached. The results for confined films will be discussed in detail later. At a constant temperature, the anisotropy recovers over time (Figure 6b) and for bulk films (not shown) is well fit by the Kohlrausch-Williams-Watts stretched exponential\[140,143\] given in Equation (5). Reorientation times from this technique agree well with segmental mobility measured by BDS for bulk PS.\[142\]

Thin films are best fit by the sum of two KWW stretched exponentials, as will be discussed further in Section 8.\[140,144\]

Polymer self-diffusion has been studied via fluorescence recovery after photobleaching (FRAP)\[145\] utilizing polymers covalently labeled with a fluorescent dye. To measure self-diffusion from FRAP, portions of polymer films covalently labeled with a fluorescent dye, such as nitrobenzofurazan,\[18,19,146\] were exposed to bright light to permanently bleach the fluorescent dyes within the exposed portion of the film. Commonly, a grating is used to create a pattern of alternating bleached and unbleached lines, as shown in Figure 7a.\[18,19,146\] Over time, the intensity of the bleached region recovers as polymers with unbleached fluorophores diffuse into the region and bleached fluorophores diffuse out. The intensity across the image is monitored as a function of time (Figure 7b) and the diffusion coefficient can be extracted from the decay in amplitude vs. time (Figure 7c) from the relation:  

\[ \text{Equation for diffusion coefficient} \]
At A Dt A t π

\[ \lambda() = - \left( \frac{4\pi^2Dt}{\lambda^2} \right) = A(0)\exp\left( - \frac{t}{\tau(\lambda)} \right) \]

where \( D \) is the in-plane diffusivity, \( \lambda \) is the wavelength of the patterned photobleach, and \( \tau \) is the characteristic relaxation time measured for several values of \( \lambda \).\[18,19\]

5. Fluorescence to Measure \( T_g \) and Physical Aging in Single-Component Confined Systems

The high sensitivity of fluorescence, combined with its ability to conduct location-specific measurements, has enabled its large contribution to understanding \( T_g \) and aging in confined single-component systems. It has been applied to study homopolymer and random copolymer thin films in both supported\[9,10,14–17,20–22\] (Figure 1a) and freestanding\[23,24\] (Figure 1b) geometries, nanocomposites\[21,25\] (Figure 1i), patterned\[30\] (Figure 1j), and nanostructured films such as brushes\[32\] (Figure 1l) and irreversibly adsorbed layers\[31\] (Figure 1k). This section will provide an overview of fluorescence’s contribution to the understanding of such systems and showcase its unique abilities to probe properties radiating from interfaces and in nanostructured films.

Torkelson and co-workers pioneered the application of fluorescence to study confined thin films, illustrating its ability to contribute to the field through the measurement of \( T_g \) as a function of film thickness for several polymer systems.\[9,17\] These measurements were consistent with those measured via ellipsometry\[35\] and other techniques. Furthermore, \( T_g \) measured by fluorescence in bulk films was found to be consistent with the bulk \( T_g \) measured by DSC.\[9,10\] Due to the broad applicability of this technique, they were also able to conduct measurements on supported films of a variety of polymers and gain insight into how small modifications in repeat units,\[15\] the incorporation of plasticizers,\[16,21\] and polymer–substrate interactions,\[25,26\] impact confined \( T_g \). Through this method, they were also able to observe an increase in the breadth of the transition under confinement, which was studied in more detail and compared to ellipsometric measurements.\[141\] This study suggested that due to the nature of fluorescence measurements, the technique may slightly over weigh contributions from the free surface in measuring average \( T_g \) of supported films, while ellipsometry...
might slightly under weigh the same contributions.\cite{141} This discrepancy likely accounts for the slightly more pronounced reductions in average film $T_g$ measured by fluorescence.

Intrinsic fluorescence techniques, described in Section 4, enabled researchers to measure $T_g$ in P(S-r-MMA) thin films as a function of thickness,\cite{35,46,56,57} which proved to be in good agreement with ellipsometric measurements of $T_g$ in similar systems by Park et al.\cite{147} They also found consistent measurements with extrinsic fluorescence studies, supporting the argument that the low concentration of fluorescent dye does not impact the measured $T_g$ and thus only probes properties of the polymer matrix.\cite{20}

As outlined in Section 3, interfaces have long been thought to play a strong role in determining deviations from bulk $T_g$.\cite{35,41,46} But their direct measurement and correlation with average confined $T_g$ was not well documented. Several researchers have hypothesized layer models to describe $T_g$ and dynamics in thin films.\cite{35,46,56,57} While some techniques, such as gold nanoembedding,\cite{110,148,149} enabled direct probing of mobility and dynamics at the free surface, equivalent measurements of surface $T_g$ were lacking. These measurements were particularly valuable given growing evidence of a separation of $T_g$ and dynamics behavior at the nanoscale.\cite{8} Ellison and Torkelson used fluorescence to provide the first direct measurement of free-surface $T_g$.\cite{10} By selectively placing a layer of fluorescently labeled polymer within a neat polymer film, they observed a 32 K depression from bulk $T_g$ in a 14 nm thick surface layer incorporated atop a 270 nm thick film. This large $T_g$ depression at the free surface was in direct contrast to the bulk $T_g$ measured in an identical 14 nm layer when sandwiched between two 270 nm layers.\cite{10} Comparison of the $T_g$ of these two labeled layers verified their ability to produce multilayer films with continuous properties (i.e., that behaved as one film) and the demonstrated difference in $T_g$ of the free surface layer from the bulk. The different manifestations of this multilayer film technique are shown in Figure 1d–f. This technique was extended to observe a gradient in $T_g$, with deviations radiating from the free surface resulting in the large average $T_g$ depression seen in polystyrene thin films. This gradient was present near the free surface in bulk systems, but it only dominated average film $T_g$ to show depressions below a threshold total film thickness of ∼40 nm. The $T_g$ in a 14 nm thick free-surface layer was found to be independent of total film thickness down to 50 nm. However, decreasing total film thickness below 50 nm (shown in the inset of Figure 8) caused free-surface $T_g$ to increase until aligned with average film $T_g$ below ∼30 nm. This increase and subsequent decrease in free surface $T_g$ shown in Figure 8, demonstrated that the $T_g$ at the free surface could be impacted by the presence of the substrate if required to maintain a continuous gradient throughout the film.

Their enhanced free-surface effect and resulting dramatic confinement behavior, combined with their unexplained molecular weight dependence of $T_g$, differentiate freestanding films from those supported on substrates and warrant separate investigation into freestanding confinement behavior. Kim et al. provided the first measurement of freestanding film $T_g$ via fluorescence using the self-referencing technique described in Section 4.\cite{23} They saw good agreement with previously reported confinement effects and molecular weight dependencies for freestanding films.\cite{65} The self-referencing fluorescence technique was later combined with multilayer film studies to measure $T_g$ of 14 nm thick free surface layers in freestanding films.\cite{24} They saw that, absent any influence from the opposite interface (i.e., when the free surface was measured on top of bulk films) the free surface layer $T_g$ was identical for freestanding and supported films. They also observed a decrease in the free surface $T_g$ of freestanding films below a threshold total thickness, indicating the influence of the opposing free surface penetrating through the film. This was consistent with how the substrate could cause free-surface layer $T_g$ to maintain a smooth gradient in supported films,\cite{10} and it supported the hypothesis that below certain length scales interfacial influences compete and combine effects more directly.\cite{26} This combination of influences likely caused the larger $T_g$ depression seen in freestanding films. This study was also able to provide evidence against de Gennes’ sliding motion mechanism,\cite{150} one of the major theories for the molecular weight dependence of $T_g$ in freestanding films in which free volume is transported along polymer chains from the free surface into the interior of the film. Molecular weight, i.e., length, of the chains would then dictate the distance the free volume was transported and thus the magnitude of $T_g$ depression. Their ability to recover previously reported molecular-weight dependent trends\cite{65} in $T_g$ depression for interior labeled layers, where chains did not extend from the free surface, suggested that the molecular-weight-dependent behavior could not be due to chain bridging.\cite{24} This work demonstrated that fluorescence not only provides insight into interfacial influences, but also fundamental processes occurring in confined systems.

The multilayer film technique was also employed to investigate interfacial effects on physical aging in PMMA thin films. By measuring fluorescence intensity as a function of aging time, Priestley and co-workers were able to isolate aging rates at the free surface, center, and substrate interface in PMMA thin films.\cite{22} At 305 K, aging rates were highest in the center of
Complex, nanostructured systems prove another challenge to measuring polymer properties under confinement. Ellipsometry, capillary dilatometry, and X-ray reflectivity require relatively flat, continuous films to accurately measure $T_g$. While DSC has been used to study 2D-confined (where two dimensions are restricted to the nanoscale) polymer systems through anodized aluminum oxide (AAO) template-supported nanorods and nanotubes, sample geometry and interfacial interactions were somewhat limited by template constraints. Additionally, these techniques do not enable location-specific measurements in the presence of a free surface without costly and time-intensive scattering and reflectivity studies. Fluorescence has been employed to study both using only in-house benchtop instruments.

Advances in lithography have enabled the expansion of using polymers for patterning, and incorporation into devices. These nonuniform films have a larger free surface area than their flat counterparts. Given the large impact of the free surface and resulting deviations from bulk properties, characterizing these structures and accounting for this difference in geometry (where the xy plane is no longer infinite) is an important step prior to their application. Its relative lack of geometric constraints and resulting ability to measure polymer properties in a variety of geometries makes fluorescence uniquely suited to study this system. Work by Munda et al. on patterned PMMA films supported on silica investigated the impact of patterning and resulting 2D confinement on $T_g$.

They used electron beam lithography to remove sections of the polymer films down to the substrate, leaving long strips or “lines” of polymer confined by two dimensions (Figure 1j). Smaller line width (corresponding to the width of polymer film strips remaining after etching across the substrate) resulted in increasing fraction of free surface to substrate interaction and significant $T_g$ depression from bulk. This is in contrast to the positive deviation expected in PMMA thin films due to strong interactions with the substrate, indicating domination of the free surface influence due to the larger exposed area. Without fluorescence, this detailed comparison of the free surface influence to that of the substrate in nanopatterned films would not be possible.

Additional systems that provide challenges to other benchtop technologies are brushes, created by chemically grafting one end of a polymer brush to the substrate. While the $T_g$ of isolated brushes and their effect on overall polymer films has been studied by ellipsometry, in situ measurements of brush $T_g$ had not been measured prior to Lan and Torkelson’s study of densely grafted brush layers. They were able to measure average brush $T_g$ within a polymer film (Figure 1l) as well as at specific locations from the substrate within the brushes. In contrast to the reduction in $T_g$ observed for PS supported films below $40\, \text{nm}$, they saw invariant average brush $T_g$ down to $11\, \text{nm}$. However, they measured a dramatic gradient in the $T_g$ of chain segments within the brush layer, ranging from $36\, \text{K}$ above bulk $T_g$ near the substrate to $14\, \text{K}$ below bulk $T_g$ near the free surface. Finally, through bilayer experiments, they were able to measure the influence of brush thickness on overlayer $T_g$ and overlayer thickness on brush $T_g$. This study directly illustrates how fluorescence can complement confinement studies done by ellipsometry and contribute additional insight.
through site specific measurements that cannot be otherwise easily measured.

6. SPOTLIGHT: Measuring the $T_g$ of Exposed and Buried Irreversibly Adsorbed Layers

Fluorescence has previously been applied by this group to characterize properties of unique structural features within thin films in the context of irreversible adsorption. This physical adsorption of polymer chains to the substrate during melt-state annealing can lead to an adsorbed layer of polymer with slower chain dynamics that remains on the substrate after washing with a good solvent. The growth of this layer with annealing has been observed to impact properties of thin films, such as $T_g$ diffusion, thermal expansion and viscosity. In order to further explore the relationship between irreversible adsorption and $T_g$ in thin films, we examined the effect of the free interface on adsorbed layer $T_g$ (Figure 1k). The extremely thin nature of these adsorbed layers makes their $T_g$ difficult to measure via other techniques. Thus, the high sensitivity of fluorescence is necessary to measure the $T_g$ of adsorbed layers <3 nm thick in an exposed geometry (possessing both a free surface and substrate interface), another unique contribution to the field. In previous studies, these exposed measurements were largely restricted due to limitations in the measurement technique: capacitance dilatometry was used to measure $T_g$ but it necessitated a capped geometry, prohibiting the examination of the free surface effect—which is known to be particularly strong with polystyrene. In our study, the glass transition temperatures of exposed PS adsorbed layers were measured and compared to their thicknesses.

As seen in Figure 10, there is a good correlation with adsorbed layer growth and $T_g$. When plotted separately against annealing time, $t_{ads}$, both adsorbed layer thickness and $T_g$ gradually increase until saturating, as seen in blue in Figure 11 and the stacked films were briefly annealed to consolidate into one film. This effectively eliminated the free surface while still restricting fluorescence measurements to only the adsorbed layer, now submerged in a bulk film. This was the first measure of adsorbed layer $T_g$ submerged within a film, enabled by the ability of fluorescence to conduct location-specific measurements. Comparing the $T_g$ in these exposed and submerged films, depicted in Figure 11, clearly shows two opposing trends.

For $T_g$. These trends are consistent with existing literature for $T_g$ of capped adsorbed layers. However, the fact that the exposed adsorbed layer $T_g$ was consistently less than bulk was unique to our system. We attributed this depression in adsorbed layer $T_g$ to the presence of a free surface preventing the recovery of bulk $T_g$ observed in capped adsorbed layers.

In order to isolate the effect of the free surface, we compared the exposed adsorbed layers to those capped with an unlabeled bulk film of PS (shown in the upper inset of Figure 11). Unlabeled PS films were floated on top of exposed adsorbed layers and the stacked films were briefly annealed to consolidate into one film. This effectively eliminated the free surface while still restricting fluorescence measurements to only the adsorbed layer. This trend was attributed to a weaker influence of the upper interface on adsorbed layers at longer $t_{ads}$ due to tighter chain adsorption and conformations. This reduction of free surface influence with $t_{ads}$ was further investigated through application of the free volume holes diffusion model, which suggested that healing of the polymer–polymer interface occurs with further annealing in the bilayer geometry, resulting in recovery of bulk $T_g$. The amount of bilayer annealing required to recover bulk $T_g$ was dependent on $t_{ads}$ further suggesting that chain adsorption dominates over the free-surface and top-interface in adsorbed layers at long $t_{ads}$.

Figure 11. Influence of the free surface on adsorbed layer $T_g$ decreases with annealing time, $t_{ads}$. $T_g$ of exposed (closed diamonds) and submerged (open diamonds) adsorbed layers are measured via fluorescence and compared. Lines illustrate fits to the data by the free volume holes diffusion model. Insets show geometries measured for exposed (lower) and submerged (upper) adsorbed layers. Adapted with permission. Copyright 2016, American Chemical Society.

Figure 10. A positive trend is observed between exposed adsorbed layer $T_g$ (measured via fluorescence) and adsorbed layer thickness (measured via ellipsometry). Adapted with permission. Copyright 2016, American Chemical Society.
The application of fluorescence techniques to a wide variety of thin film geometries has been studied to measure $T_g$ and physical aging. These experiments have provided direct measurement of $T_g$ and physical aging at the free surface and substrate interface, giving insight into gradients in confinement behavior radiating from interfaces. Fluorescence has also enabled the isolated measurement of features in nanostructured films. Within the confined single-component field, opportunities exist for the expansion of these techniques to address the different length scales associated with $T_g$ and physical aging perturbations in thin films. Multilayer fluorescence experiments could expand upon the work of Pye et al.[84] to investigate the temperature dependence of aging rates at different locations within thin films. This investigation could provide limits to the dependence of aging gradients on confined $T_g$ gradients proposed by Priestley et al.[22] Elucidation of aging gradients is of particular interest in the freestanding geometry, given the abundance of studies on physical aging in membrane-relevant polymers[95,96,98] and somewhat contradictory results.[96,86] Furthermore, physical aging of polystyrene could be investigated to see how the limited suppression in aging rates seen in supported polystyrene[48] correspond to the length scale of interfacial perturbations.

7. Fluorescence to Measure $T_g$ in Multicomponent Polymer Systems

Multicomponent polymer systems have scientific and technological relevance due to the ability to tailor user-defined properties based on the nature of the individual components. For the simplest case of a binary system, a polymer of type A will be miscible in polymer B at infinite dilution. From the viewpoint of the dilute polymer heterogeneous compositions are encountered at the distance of a monomer segment. Upon increasing the concentration of the dilute polymer, miscibility will be preserved if the Flory interaction parameter ($\chi$) between the two polymers is negative or zero. However, the repulsive interactions typical between chemically dissimilar polymer segments lead to blends where $\chi$ is positive and increasing the concentration of the dilute component results in phase separation with domains of compositions rich in polymer A or B.[165] These domains are separated by a polymer–polymer interface where large and symmetric composition gradients exist, which span thicknesses on the order of several nanometers.[166] The length scales governing phase separation between these domains range from tens of nanometers to macroscopic distances, depending on details of the polymer system.

An immiscible blend can exhibit rich dynamic behavior based on the contrast in $T_g$ between the homopolymer components and the length scale over which $T_g$ is quantified. At macroscopic or bulk length scales $T_g$ is a combination of contributions from the unperturbed value of the individual components, the interdependence of dynamics between the components, and the presence of the polymer–polymer interface. Decoupling the different contributions to $T_g$ requires an ability to independently measure the $T_g$ of each component over length scales ranging from that of a few segments where the interdependence of dynamics is defined, to that of confined length scales where the presence of the interface may act to perturb $T_g$. At the interface the composition gradients present may setup gradients in $T_g$ in a manner analogous to the free surface effect observed in thin films.[169] Characterizing the length scales associated with the gradients requires a location-specific measurement of $T_g$ relative to the interface.

Conventional characterization tools (e.g. DSC, rheometry, and ellipsometry) have provided powerful insights into the nature of perturbations to the bulk $T_g$ of a polymer as caused by interfaces or when present in a blend. However, these tools face limitations when demand for a location-specific measure of $T_g$ or for sensitivity to small sample volumes exists. As described previously, the use of fluorescence spectroscopy to characterize $T_g$ is highly sensitive; hence, small sample volumes do not pose a barrier to inquiry. Synthetically labeling a polymer with a fluorescent probe enables location-specific measurements of $T_g$. These two features of fluorescence spectroscopy enable the experimenter to circumvent the limitations of the conventional tools.

Examples of the free surface or polymer/substrate interface acting to perturb $T_g$ of a confined homopolymer are abundant in the literature.[7] In most cases $T_g$ is characterized in thin films via temperature-dependent ellipsometry, a dilatometric technique which spatially averages polymer properties over the entire thickness of the film. In multilayered, multicomponent, polymer films, the properties of each layer have different temperature dependencies, limiting the utility of ellipsometry to characterize $T_g$. Alternatively, $T_g$ can be characterized via calorimetry. A demonstration of this capability used coextrusion to control the thickness of each layer in a bilayer geometry.[167] One limitation of coextrusion was the inability to independently control the thickness of each layer. The location-specific nature of fluorescence labeling and spectroscopy to characterize $T_g$ provides the opportunity to independently probe the dynamics of each layer or relative to the polymer–polymer interface in a multicomponent system and offers access to a parameter space inaccessible to conventional characterization tools.

Multilayer thin films can also be used as model systems to study immiscible polymer blends by annealing layers of thin films of polymers A and B together to form one continuous film. The facile thickness control afforded by independently spin coating each layer offers a route to systematically characterize the magnitudes and length scales governing perturbations to $T_g$ caused by the polymer–polymer interface or a combination of interfaces. Evans et al. investigated the ability of the polymer–polymer interface to perturb film $T_g$ using a trilayer geometry, in which pyrene-labeled PS films were confined between a series of bulk polymer films whose $T_g$ spanned a range from 30 K below to 50 K above the bulk $T_g$ of PS.[168] Characterization of the thickness dependence of the confined PS film’s average $T_g$ revealed that $T_g$ was tunable by the nature of the confining polymer and bulk $T_g$ was recovered over length scales ranging from 80 to 150 nm.[168] The ability of the confining layer to perturb $T_g$ of PS was correlated to the relative fragility of the confining layer where polymers with smaller fragility indices relative to PS perturbed $T_g$ to a greater extent.

As in single-component systems, interfaces perturb $T_g$ through the generation of gradients in $T_g$ which originate at the interface and propagate toward the interior of the film.
Multilayered film geometries have been used to characterize gradients created by the polymer–polymer interface in multicomponent systems. The experimental system created by Baglay and Roth consisted of a multilayer film geometry where a pyrene-labeled film of PS or poly(n-butyl methacrylate) (PnBMA) was selectively inserted into bulk films of PS or PnBMA to mimic a bilayer system. A representative schematic of this geometry is shown in Figure 1g. The location of this labeled layer was precisely controlled to give local measurements of $T_g$ relative to the PS–PnBMA interface. These polymers have a $\sim$80 K difference in bulk $T_g$. Characterization of the location dependence of $T_g$ revealed an asymmetric distribution of $T_g$ across the interface with a larger gradient existing in the high-$T_g$ PS layer than the low-$T_g$ PnBMA layer. This asymmetry in $T_g$ gradient is shown in Figure 12.

In a subsequent study investigating the generality of the asymmetric gradient in $T_g$ about the polymer–polymer interface, PS was kept as one layer while varying the polymer in the second layer of the bilayer configuration. The second polymer was chosen to vary $T_g$ from 80 K below to 80 K above the bulk $T_g$ of PS, thus making PS either the glassy or rubbery polymer, respectively. Repeating location-specific $T_g$ measurements revealed that in all cases $T_g$ was found to be asymmetrically distributed about the interface in the same manner as observed with PS/PnBMA. Larger gradients in $T_g$ were consistently observed in the high-$T_g$ (glassy) layer than the low-$T_g$ (rubbery) layer. The length scales governing the perturbation to the bulk $T_g$ of PS ranged from 100 to 250 nm. Both the asymmetric $T_g$ profile and the large distances associated with perturbations to $T_g$ caused by a finite interface are in agreement with theoretical predictions. Characterization of the effect of the polymer–polymer interface revealed a system-dependent perturbation to $T_g$ reminiscent of previous confinement studies where the effect of the free surface or the polymer/substrate interface was demonstrated. A comparison between the composition gradients and $T_g$ gradients about the polymer–polymer interface revealed two things: present, gradients in $T_g$ were (1) asymmetric and (2) spanned distances many times larger than the symmetric composition gradients.

The multilayered films discussed previously were created via annealing layers of different homopolymers together. Lamella-forming diblock copolymers also represent a multilayered immiscible blend. The main difference between these two systems arises from heterogeneity in composition within the chain of a diblock copolymer resulting in multilayers with a covalent bond present across the interface. The comparison of $T_g$ of bilayered films of homopolymers to diblock copolymers of an equivalent chemical identity and layer thickness enables an assessment of the ability of chain connectivity across the polymer–polymer interface present in a diblock copolymer to perturb $T_g$. The presence of the covalent bond was reported to have a negligible effect on free surface perturbations to $T_g$ in a case where the difference in $T_g$ between each layer was <20 K. It remains to be seen if this effect will persist for polymer pairs with a larger $T_g$ contrast.

The lamella-forming morphology of diblock copolymers represents one of several possibilities in a composition-dependent phase diagram. Within this phase diagram, diblock copolymer domains exist over length scales of 10–100 nm where the polymer–polymer interface may perturb $T_g$. An example of the importance of characterizing location specific measures of $T_g$ was demonstrated using amphiphilic diblock copolymers which micellize in the presence of a selective solvent for one of the blocks (Figure 1h). They are actively researched for use as nanoparticle drug carriers. The micelle core $T_g$ is an important design parameter as drug diffusion is controlled by the mobility of the core. Conventional tools such as rheology or DSC have been successfully employed to characterize micelle core $T_g$s at relatively high concentrations. In the limit of low micelle concentration, where small sample volumes are probed, these techniques lose sensitivity to micelle material properties. Here, fluorescence labeling and spectroscopy, which have been routinely used to characterize the critical micelle transition (CMT) of block copolymer micelles, can be readily expanded to characterize the micelle core $T_g$.

Lodge and co-workers demonstrated both the sensitivity limitations of DSC and the ability of fluorescence spectroscopy to complement these sensitivity limits in a study characterizing micelle core $T_g$. The chosen micelle system was a poly(styrene–b-ethylene oxide) (PS–PEO) diblock copolymer suspended in an ionic liquid where PS was the core-forming block. The reported breadth of $T_g$ as characterized by DSC and fluorimetry were in agreement within experimental error over micelle concentrations ranging from 10–30 wt%. For a 2 wt% micelle concentration which is beyond the sensitivity limit of DSC $T_g$ as determined by fluorimetry was found to be suppressed relative to higher concentrations. The glass transition temperature has been characterized in other micellar systems at concentrations as low as 0.1 wt% where a strong sensitivity of $T_g$ to the nature of the selective solvent was observed. Overall the sparse reports of the characterization of micelle-core $T_g$ in dilute suspensions reveal a nontrivial interplay between concentration, block components, the selective solvent, and the

**Figure 12.** Measured local $T_g(z)$ as a function of the pyrene-labeled layer’s position from the PS/PnBMA interface (positive $z$ = glassy-PS side, negative $z$ = rubbery-PnBMA side). $T_g(z)$-profile fit to hyperbolic tangent functions and $T_g$ values for PS and PnBMA at the bulk. Gray, dashed curve indicates local $T_g(z)$ contrast. The location of this labeled layer was precisely controlled to give local measurements of $T_g$ relative to the PS–PnBMA interface. These polymers have a $\sim$80 K difference in bulk $T_g$. Characterization of the location dependence of $T_g$ revealed an asymmetric distribution of $T_g$ across the interface with a larger gradient existing in the high-$T_g$ PS layer than the low-$T_g$ PnBMA layer. This asymmetry in $T_g$ gradient is shown in Figure 12.
resulting core $T_g$. Fluorescence’s ability to selectively measure core $T_g$ with high sensitivity enables a better understanding and further potential application of micelles at low concentrations.

Even for the simplest example of a multicomponent polymer system (i.e., a binary blend of miscible polymers), if the components have dissimilar values of $T_g$, the blend $T_g$ cannot be unambiguously predicted based on knowledge of the pure component values. The theory of self-concentration was proposed to estimate the dynamics of a component in a binary blend. Due to the connectivity of similar monomer segments, a polymer exhibits self-concentration so that its dynamics is determined by a concentration greater than its average value in the blend. Characterizing the value of self-concentration for a polymer component in a blend requires the use of a technique, which is sensitive only to that component. Self-concentration studies that employ NMR or BDS to characterize the segmental relaxation time of a given component in polymer melts have previously been reported. Fluorescence spectroscopy has also been demonstrated as a tool to characterize self-concentration in binary blends. The ability to directly characterize $T_g$ using fluorescence spectroscopy provides an added advantage beyond the capabilities of NMR or BDS.

8. Fluorescence Measurements of Diffusion and Mobility

As discussed above, fluorescence spectroscopy is a versatile tool to measure the local $T_g$ of polymers under a variety of confinement conditions. Here, we will discuss advances fluorescence spectroscopy and microscopy have made in directly and indirectly quantifying polymer dynamics. Specifically, we will highlight work regarding segmental mobility and small molecule diffusion, self-diffusion, and polymer chain conformation when polymers are confined in melts and glasses. One of the fundamental questions to be addressed in confined systems is whether the relationships that exist between dynamics and thermodynamics (such as $\alpha$ relaxation and $T_g$) that exist in bulk polymer systems persist when the polymer is perturbed by confinement. Increased understanding of segmental motions, diffusion, and polymer conformations under confinement are important to developing a complete description of the glass transition.

Small molecular fluorescent dye rotation, probing segmental dynamics, has been used to observe glassy dynamics in polymeric and organic small molecule glass formers, for example elucidating dynamic heterogeneity in supercooled bulk glass formers. Fluorescent dye rotation can be observed in any polymeric system and confinement geometry, whereas many of the experimental techniques described in Section 3.3 limit the choice of polymer and geometry.

Paeng et al. first utilized dye reorientation after photobleaching with polarized light to quantify molecular mobility in confined freestanding polymer films. In the glassy state, dye molecules did not reorient on the experimental time scale, but the rotational anisotropy began to decay as temperature increased. For films thinner than 30 nm, the temperature at which anisotropy began to decay shifted lower by as much as 20 K, comparable to $T_g$ reductions measured by Brillouin light scattering. Isothermal anisotropy recovery curves of confined freestanding polymer films were best fit to two stretched exponentials, of the form of Equation (5), indicating the presence of a bulk-like and a more mobile population. The latter was interpreted to be a free-surface mobile layer. They extracted the thickness of this mobile free-surface layer from the ratio of the anisotropy decay in confined films compared to bulk at a given temperature. At bulk $T_g$, the mobile layer of PS was equal to 7 nm. Fluorescence lifetime measurements and nanosecond measures of dye reorientation in supported PS also revealed a comparable free-surface mobile layer. Dye reorientation after photobleaching on other polymers has shown that the mobile free-surface layer thickness depends on chemical composition. For example, poly(ethyl methylestyrène) showed no evidence of a mobile layer.

In contrast, other studies have observed no effect of confinement on dye reorientation. In both PiBMA and PMMA, little change in the average relaxation time measured via second harmonic generation was observed for films as thin as 7 nm thick at temperatures ranging from $T_g$ - 10 K to $T_g$ + 30 K. However, a broader distribution of relaxation times, as quantified by a smaller value of the stretching exponent $\beta$ in Equation (5), was observed for films less than 150 nm thick, indicating that thinner films had a larger portion of quickly rotating dyes than bulk films. Oba and Vacha used a cylindrical lens to split incident light to create an astigmatic image from which the depth of the dye in the film could be ascertained to within 12 nm. At $T_g$ + 13 K for supported poly(methyl acrylate) they observed no depth dependence to the rotational mobility of a large perylene diimide dye. Araoz et al. observed enhanced rotational anisotropy decay in poly(butyl methacrylate) and poly(ethyl methacrylate) films at room temperature but no change in mobility in PMMA. Caution is required when interpreting dye reorientation studies. The choice of dye is important as some dyes may interact with the polymer matrix differently, for example resulting in dye-dependent VFT scaling of dye rotational mobility above $T_g$. Additionally, polarized photobleaching techniques may preferentially bleach fast rotating dyes, removing the fastest relaxations from the analysis.

In addition to rotational motion, translational diffusion of molecular dyes has been studied at temperatures near and above $T_g$. While this is not directly related to the diffusion or mobility of polymer chains, the diffusion of small molecules within a particular polymer matrix could be an important characterization for the polymer’s application in drug delivery and membranes. Flier et al. tracked the translational diffusion of single free perylene diimide derivative dyes in supported PS thin films. As film thickness was reduced, the fraction of dyes mobile at temperatures down to $T_g$ - 25 K increased. The breadth of the transition from all dyes being immobile to all being mobile as temperature increased was broader for thinner films than for bulk, indicating greater dynamic heterogeneity in confined PS films.

Longer length-scale relaxations, associated with the whole chain motion responsible for self-diffusion and viscosity, can also be examined with fluorescence techniques. Polymer self-diffusion has been studied via fluorescence recovery after
Frank et al. measured the self-diffusion of dye, as previously discussed in Section 4.3. Frank et al. measured the self-diffusion of PS at $T_g,\text{bulk} + 40$ K. For supported films less than 100 nm thick, the diffusion coefficient decreased by 50% from the bulk value.[146] Contrary to expectations from experimental measures of $T_g[15]$ and mobility from dye reorientation.[148] Granick and co-workers reported a similar decrease in self-diffusion coefficient of PDMS supported on or sandwiched between mica measured using FRAP.[195,196] For poly(iso-butyl methacrylate) (PiBMA) that exhibits no $T_g$ reduction upon confinement[20] the diffusivity was also found to be constant for supported films.[19]

Perhaps more puzzling than the discrepancy between confined $T_g$ and diffusivity measurements are the discrepancy between confined diffusivity and viscosity. Tsui and co-workers recently used FRAP to compare self-diffusion of PS and PiBMA in supported films to their effective viscosity measured with AFM.[18] Although both polymers exhibit a decrease in viscosity upon confinement,[18] the diffusivity decreases by 50% for PS[146] and is constant for PiBMA films down to 20 nm thick.[18] To account for differences in viscosity and diffusivity vs. thickness, they employ a three-layer model where a more mobile, less viscous free-surface layer with thickness on the order of the polymer end-to-end distance and an $\approx 1$ nm thick adsorbed, less mobile layer at the buried interface contribute to the film-average viscosity and diffusivity. In the three-layer model, they assume that diffusion and viscosity scale with local viscosity differently and can fit both using the same values for the thicknesses and viscosities of the three layers.[18] However, no unification was made with the thickness dependence of $T_g$ measurements.

Nonradiative or Förster resonance energy transfer (NRET or FRET) is another fluorescence technique that can be used to uniquely observe the distance between two complementary dye molecules. When an acceptor and donor dye are brought together, energy can be transferred from the donor to the acceptor via dipole–dipole coupling. The intensity of light emitted by the donor and acceptor when only the donor is excited can yield the average distance between dye molecules as the efficiency of the energy transfer is proportional to the distance between the dyes to the sixth power.[144] Itagaki et al. used a FRET pair of dyes covalently labeled to separate PMMA chains blended together to measure entanglement density upon confinement.[157] Their fluorescence spectra are depicted in Figure 13a. From the quenching ratio of one of the dyes, the average distance between the dye pairs on separate PMMA chains could be calculated, as shown in Figure 13b. For films thicker than 12 nm, this average distance was 1.9 nm, the same as in bulk films and less than the polymer radius of gyration, $R_g \approx 5.5$ nm, indicating the presence of entanglements. For films thinner than 12 nm, an increase in the apparent average distance was measured, indicating a decrease in entanglement density. However, as the film thickness approaches the Förster radius, interpretation of the quenching ratio became difficult. Mechanical measurements of freestanding PS films[198] as well as simulations[199] and theory[200] suggest that entanglement density decreases for films thinner than the $R_g$ of the polymer. While this perturbation length scale is smaller than the 12 nm threshold observed by Itagaki et al., Barbaro and Steiner suggest that lower entanglement density could be responsible for reductions in viscosity measured in films as thick as 100 nm.[201] Contrary to this, the quenching ratio of dyes attached to separate PS or PnBMA chains confined to AAO nanopores have been shown to increase with decreasing pore size indicating greater entanglement density under cylindrical hard confinement.[202,203]

Fluorescence techniques have been utilized to study the complex dynamics of polymer systems over a variety of length and timescales. Rotational mobility and reorientation of dyes provided insight into the dynamic heterogeneities that broaden upon confinement, also illustrating the presence of a high mobility surface layer. Some of the papers discussed above tracked individual dye molecules within the polymer matrix.[187,188,193,194] Future studies more closely examining reorientation statistics over many dyes could further quantify dynamic heterogeneities in polymer glass formers as has been studied in small molecule glass formers.[204] With regards to longer length scale interactions, sophisticated synthesis techniques allowing the placement of different dye molecules along the length of a single polymer chain[205,206] could be used to study changes in polymer chain conformation upon confinement. While valuable insight has been gained through fluorescence techniques, the multitude of techniques and dyes used makes comparison between studies and arriving at overarching conclusions difficult. As such, development of more complementary techniques and experiments is warranted.

9. Fluorescence to Measure Mechanical Response

We have previously discussed how molecular mobility associated with the glass transition and polymer chain diffusion...
has been probed by fluorescence techniques. As described in Section 3.4, the mechanical responses of confined polymers have also been seen to deviate substantially from bulk. Here we focus on the use of fluorescence spectroscopy to study the molecular level motion of confined polymers in response to either preparation-induced residual stresses\cite{125,207} or externally applied stresses.\cite{133,134}

While most of the experiments in thin, spin-coated, glassy polymer films involve a thermal annealing protocol above the glass transition to at least partially erase the preparation induced residual stresses, some out-of-equilibrium conformations of polymer chains are believed to remain. These conformations are known to affect the total strain and deformation of such films.\cite{130} Using similar experimental approaches to those developed to determine $T_g$\cite{17,31} and structural relaxation\cite{22} by fluorescence, Torkelson and co-workers recently investigated several aspects of residual stresses and stiffening in pyrene-labeled polystyrene single-layer films.\cite{208,209} Although the ratio of the first to third vibronic peak intensities of the pyrenyl dye fluorescence spectra (displayed in Figure 14a) can be plotted against temperature to give $T_g$ (as shown for two different film thicknesses in Figure 14b), individual isothermal measurements of this ratio were found to be related to a “caging” mechanism involving sequential segmental motion of the polymer.\cite{208} This “caging” was further dependent on the stiffness and residual stress relaxation of the film.\cite{208,209}

Measuring the $I_3/I_1$ ratio for films of different thicknesses supported on silica at 60 °C, 100 °C, and 140 °C allowed Askar and Torkelson to probe average stiffness in both the glassy and rubbery regimes.\cite{208} As shown in Figure 14c, they observed that stiffness in glassy films was nearly invariant with film thickness down to 63 nm, whereas the rubbery state thickness threshold was 240 nm.\cite{208} Below these thresholds, stiffness was enhanced in both the glassy and rubbery regimes, as indicated by an increase in the $I_3/I_1$ ratio. While increased stiffness in the rubbery regime was qualitatively in accordance with previous experimental observations,\cite{60,210} stiffening in the glassy regime was not consistent with previous reports mentioned in Section 3.4. The glassy behavior in this study was likely due to an increased rigidity of the substrate compared to the freestanding, water-, and elastomer-supported films described previously.\cite{59,130,131,133} Bilayer film measurements were also employed, revealing a reduction in stiffness at the free surface and enhancement of stiffness at the substrate in both the glassy and rubbery regime.\cite{208} However, the length scale and magnitude of these perturbations strongly depended on temperature and interfaces. Films in the rubbery regime exhibited a significantly weaker free-surface deviation and a larger perturbation length scale of the substrate influence than those in the glassy state. These differences could explain the differences in surface\cite{135} and average film\cite{60,211} behavior between the two states.

In agreement with previous dewetting studies to probe residual stress relaxation in thin polystyrene films,\cite{117,207} Askar et al. found Arrhenius temperature dependence of stress relaxation times, having low activation energy comparable to a fast β-like relaxation process.\cite{209} However, the $T_g$ of bulk PS films was unaffected by residual stress relaxation.\cite{209} Such relaxations closely resembled a segmental scale, local process and had a notable impact on crack and craze formation in polystyrene thin films.\cite{125,207} Similarly, relaxation of intrinsic stresses was found to be important, leading to enhanced FRET characteristics in a donor–acceptor filled glassy polymer layers due to cracking and local stress release.\cite{211}

Direct quantitative probing of segmental mobility during active deformation was introduced by Ediger and co-workers using an optical photobleaching method which measures the reorientation of fluorescence dye in lightly cross-linked bulk PMMA glasses under tensile creep deformation.\cite{212–214} They found in general the polymer mobility increased with increasing stress, strain, and strain rate, while removal of stress caused gradual disappearance of mobility. While the classical Eyring model described this mobility enhancement at low stress well, it failed after the plastic flow onset leading to a narrowing of the relaxation time distribution (measured by KWW β-parameter, as described in Equation (5)).\cite{211,214} They clearly differentiated mobility in the subyield and postyield regime with further correlation to physical aging and rejuvenation, saying postyield plastic-flow is necessary for rejuvenation or to restart the physical aging clock.\cite{211} Additionally, the problem of stress induced mobility and stability in polymer films has been addressed as a function of thermal stress by probing film thickness via ellipsometry.\cite{215} To the best of our knowledge similar
quantitative experiments in thin nanoconfined films do not exist yet, largely due to the limitation of imposing small-scale tensile stress on such small volume. However, with the development of recent tensile tools for polymer thin films,[13] such photobleaching experiments should be possible.

Several studies probed intrinsic fluorescence of conjugated polymers incorporated at dilute concentrations within thin glassy films to examine the effect of mechanical deformation on those conjugated polymers. Changes in their intrinsic fluorescence were interpreted as a result of altered conjugation.[216–218] The deformation of these conjugated polymers upon application of mechanical stress was interpreted to reflect deformations of the glassy matrix.[216,217,219] This interpretation was based on an assumption that the glassy matrix and conjugated polymer had identical mechanical properties. While these studies illustrate the ability of fluorescence to characterize semicrystalline conjugated polymers, we will not discuss them further, as this review focuses on characterizing glassy polymers.

While we have mostly discussed fundamental aspects of mechanical deformation in confined thin polymer films using fluorescence, the underlying principles could be easily propagated to technological advancements of a range of stimuli-responsive smart applications in thin confined layers, including pressure/damage sensors,[211,220] self-healing materials,[221] stretchable/wearable electronics.[222]

10. Conclusions and Future Outlook

Rapid advances in and propagation of nanotechnology motivate an improved understanding of polymer physical properties at the nanoscale. The studies described above highlight how fluorescence techniques, ranging from steady-state temperature-dependent measurements to techniques tracing fluorescence anisotropy of rotating dyes, have advanced polymer characterization at the nanoscale. In doing so, they provided insight into how interfacial interactions and polymer structure can greatly impact polymer properties. Its bench-top availability, high sensitivity, and location-specificity make fluorescence an excellent technique for characterizing polymers in confined geometries. Most recent research has focused on characterizing thermal properties such as \( T_g \) and physical aging in both single-component systems, confirming the presence of gradients in these properties in thin films. However, unanswered questions persist regarding the relationship of \( T_g \) and physical aging under confinement and the origin of their different gradient length scales. Additional studies probing the temperature dependence of physical aging gradients could give valuable insight into the interplay between \( T_g \) and physical aging. Fluorescence probing of nanostructured and multicomponent systems illustrated the great promise of fluorescence in characterizing location-specific material properties in complex synthetic and biologically relevant systems. While a lack of standardization of dyes and methods currently prevent meaningful comparison of mobility and diffusion results, incorporation of a distribution of dye sizes could probe dynamic heterogeneity. Fundamental studies of mechanical deformation and stress response illustrate how fluorescent probes can provide in situ characterization of polymer properties during their use in stimuli-responsive applications.

The location-specificity, sensitivity, broad applicability, and geometric flexibility of fluorescence-based polymer characterization promote it as a valuable tool for advancing polymer innovation at the nanoscale.

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Conflict of Interest

The authors declare no conflict of interest.

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