

Nanostructural characterization is of critical importance for understanding the complex relationship between chemical composition, structure, property, and function for advanced materials design. Many modern imaging methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), provide sub-nanometer resolutions, but each technique has limitations including high vacuum requirements, poor electron contrast, or difficulty in probing sub-surface features. In the past decade, single-molecule super-resolution microscopy has become a standard tool for visualizing nanostructures in the life sciences under native conditions. In this technique, a small fraction of spatially-separated fluorophores are activated from a non-emissive to an emissive state. Images of these bright molecules are then fit to their point-spread function, which determines their positions with tens of nanometer precision, after which the molecules are then de-activated to their non-emissive state. By repeating this process, a super-resolution image is reconstructed from the positions of all identified emitters over many thousands of frames. While this method has been routinely used in the biological community, super-resolution imaging in polymer science is much less reported. A key barrier to widespread adoption is the lack of suitable fluorophore chemistry in organic systems which are common in polymers. A photoswitchable fluorophore that can be easily covalently incorporated into polymers can promote super-resolution microscopy in this community, particularly for investigating nanoscale polymer dynamics in real space and real time, which is difficult through other characterization methods.

In our first year of ACS PRF support, we have developed a photoswitchable fluorophore for super-resolution microscopy that can be directly copolymerized into polymer chains, which greatly streamlines labeling strategies into a single step. We demonstrated this scheme by polymerizing with radical monomers such as styrene and methyl methacrylate, which are labeled with our fluorophore with no further post-functionalization reactions or purifications needed. The molecule is based on recently explored disulfone derivatives of diarylethenes that are highly photostable and compatible with organic environments. These fluorophores can adopt two thermodynamically stable isomers, only one of which absorbs at visible wavelengths and emits with excellent fluorescence quantum yield. Unlike many aqueous fluorophore systems that require special buffers to promote transient dark states, switching between the two diarylethene states is controlled by relative intensities of ultraviolet and visible illumination, enabling super-resolution imaging through a photoactivated localization microscopy (PALM) scheme. We apply this technique to image the nanostructures of fluorophore-labeled polymer blends and validate these images through correlation with AFM of the same region. As each individual fluorophore can be switched several times, multiple time-lapse images can be acquired for observing the dynamic nanostructural evolution, which we demonstrate using polymer blends swollen with solvent vapor.

This photoswitchable fluorophore is shown in Fig. 1 and is called **V-DAE** throughout this report. Upon irradiation with 375 nm light, the initially colorless fluorophore isomerizes into a bright yellow state *via* cyclization of a central six-membered ring, which can revert back to its colorless state when exposed to 473 nm light (Fig. 1). The vinyl group in **V-DAE** enables copolymerization of this fluorophore with standard monomers through conventional controlled free-radical polymerization, significantly simplifying fluorophore-labeling strategies. We demonstrate this by successful synthesis of functionalized polystyrene (PS) and poly(methyl methacrylate) (PMMA) *via* reversible addition-fragmentation polymerization (RAFT). The resulting polymers maintained low polydispersities of less than 1.2 and the dye incorporation was constant with monomer conversion over time, signifying a true random living copolymerization.

To demonstrate this fluorophore in super-resolution imaging of self-assembled features, we used a model system of PS ($M_n=40,500$ g/mol, PDI: 1.3) /PMMA ($M_n=120,000$ g/mol) homopolymer blends

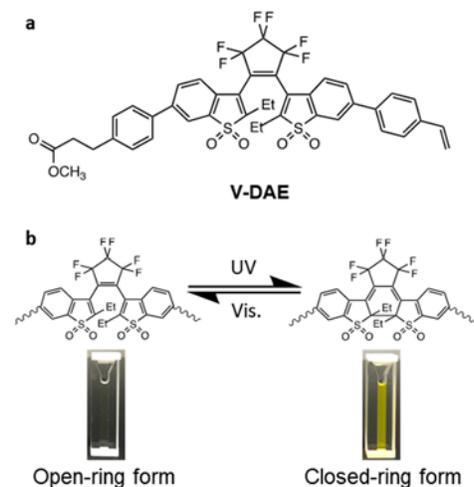


Figure 1. a) Structure of **V-DAE**. **b)** Two photoisomers of **V-DAE**.

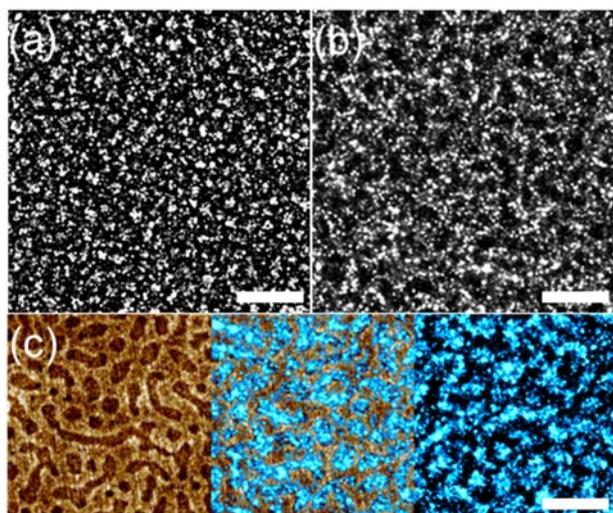


Figure 2. Super-resolution images of thin films of PS/PMMA blends with mass ratios of (a) 1:3 and (b) 3:1. c) Images of a 1:2 PS/PMMA blend. Left is an AFM phase image, right is a super-resolution image and they are partially overlaid in the middle. Film thickness for imaging is 28 nm and scale bars are 1 μ m.

where PS is selectively labeled at approximately 0.48 wt% fluorophore concentration. Fig. 2 shows a super-resolution image of PS/PMMA (1:3 by mass) blends cast in a film of 28 nm thickness, in which white spherical domains are fluorophore-labeled PS and the dark matrix is non-fluorescent PMMA. Inverting the weight ratio of PS/PMMA to 3:1 led to a structure of dark voids (PMMA) embedded in a bright continuous PS phase (Fig. 3b). A more direct confirmation of the nanostructure was achieved by comparing with AFM of PS/PMMA blends at a mass ratio of 1:2 in the same region (Fig. 2). Here, the AFM phase image was directly superimposable with the super-resolution image with near perfect matching.

To visualize dynamic evolution of polymer nanostructures at different length scales using this technique, films of PS/PMMA (1:2 by mass) blends were annealed under two different conditions. Annealing a film with unsaturated anisole vapor ($p/p_0 = 0.95$, where p_0 is the anisole vapor pressure at room temperature) limits the large-size

coarsening behavior of polymer blends with the average feature sizes consistently under optical diffraction limit (~ 200 nm) even after hours of annealing time. Fig. 3 shows the morphological evolution from an as-cast film composed of locally-ordered cylindrical and spherical patterns, where both local coalescence and fission of the bright PS domains are easily visible. The current acquisition time of 50 s is sufficiently fast to observe the dynamic phenomena relevant in this process, though further improvements are easily possible by increasing the frame rate and illumination intensity, or improving the fluorophore kinetics. These results confirm that this photoswitchable fluorophore is particularly promising for observing polymer self-assembly *in-situ* by super-resolution imaging.

Funding from the ACS PRF was received within the first year of the PI's faculty position, enabling a fast start to his research program and relieving significant early financial pressure. The proposed project is a core interest area in the group, and this support has seeded several related lines of research. Through ACS PRF funding, students and postdocs have learned techniques in optics, polymer chemistry, and nanoscale characterization in order to conduct their work. They are now largely independent researchers and will undoubtedly be successful in further efforts in the group and beyond. The work described above has been compiled into a submitted manuscript.

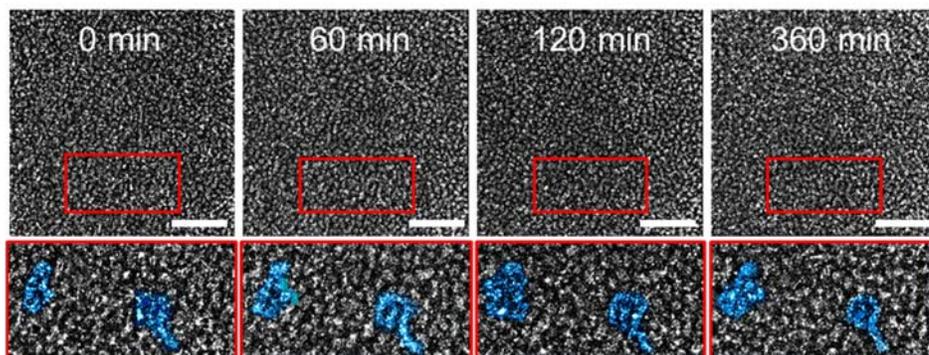


Figure 3. Time lapse super-resolution images of PS/PMMA blends thin film (1/2; w/w) under anisole vapor annealing ($p/p_0=0.95$) for 0, 60, 120, and 360 min. All scale bars are 4 μ m. The corresponding zoomed-in regions below are magnified two-fold from the red areas in the above images, with highlighted features in blue visualizing specific features that evolve or remain fixed during annealing. The film thickness is approximately 28 nm.