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Flexible Microdomain Specific Staining of Block Copolymers for 3D Optical Nanoscopy

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Supporting Information

The precise control of structure on the nanoscale, with its consequent promise of targeted properties, can be a formidable challenge. Materials such as block copolymers (BCP) that self-organize into patterns on this size scale are thus of considerable research interest.[1] Fundamentally linked to the study of such nanostructures is our ability to image them. Techniques that noninvasively yield three-dimensional (3D) and dynamic structural information can shorten the process of establishing structure−property relationships.

While far-field (confocal) optical microscopy can provide 3D images noninvasively, its use in the imaging of BCPs nanostructures has been hampered by the diffraction resolution barrier, limiting it to ultrahigh molecular weights.[2] Providing subdiffraction resolution, far-field optical nanoscopy based on the on−off switching of the fluorescence ability of markers[3] has emerged as a viable candidate for the imaging of polymeric nanostructures. In particular, a powerful implementation of stimulated emission depletion (STED) microscopy[4] known as isoSTED[5] has been used to demonstrate 3D *in situ* imaging of BCP nanostructures.[6]

Given the central role of (fluorescent) markers in enabling nanoscopy with focused visible light, the conception of a generally applicable staining scheme assumes great importance. In this paper we demonstrate precisely such a scheme for BCPs. Microdomain specific fluorescence contrast is achieved while leaving the parent BCP unmodified. The staining is flexible in terms of both the phases that can be targeted and the fluorophore tags employed. We exemplify the scheme by the nondestructive cross-sectional imaging of three different BCP microdomains. Additionally, the robustness of the procedure allows us to induce microphase separation by solvent annealing as well as thermal annealing.

Two broad approaches for obtaining fluorescence contrast are the recording of variations in spectroscopic properties of fluorophores in the different phases and the targeted confinement of dyes to specific phases. Here we use the second method by exploiting the preferential solubility of homopolymers of a particular phase in the corresponding microdomain of a BCP.[7] Homopolymers are tagged by incorporation of reactive functional groups during synthesis that are subsequently reacted with complementarily functionalized dye molecules. To ensure efficient and selective coupling, we used variants of two common click chemistry reactions, viz. the thiol−ene and the alkyne−azide Huisgen cycloaddition reactions. (For reasons of brevity, dye-tagged polymers coupled by the thiol−ene and the alkyne−azide reactions are designated as TEC and AAC, respectively.)

The synthesis of the functionalized polymers was performed via RAFT polymerization.[8] Two equally viable synthesis routes were undertaken. The first resulted in a thiol end-functionalized polymer. The second scheme yielded a statistical copolymer with a small number of units containing an exposed alkyne side group.

As a proof of principle, we imaged a poly(styrene-*block*-2-vinylpyridine) (PS−P2VP) system. This system was used in the first *in situ* 3D imaging of BCP nanostructures.[6] (Note: the lamellar morphology observed was also corroborated by transmission electron microscopy in the same work.) In that instance a material specific means, i.e., hydrogen bonding between a carboxyl group functionalized dye and the nitrogen atom of the pyridine group, was utilized to confine the dye to the 2-vinylpyridine (2VP) phase. Although the hydrogen bonding strategy is not applicable to other polymers, PS−P2VP provided a useful platform to initially establish the viability of our tagging strategy.

Thiol- and alkyne-functionalized P2VP were synthesized and tagged as per the reaction schemes shown in Scheme 1 to yield P2VP-TEC and P2VP-AAC, respectively. For the P2VP-TEC, cumyldithiobenzoate (CDB)[8,9] was used as a RAFT agent. The CDB was split by aminolysis[10] to yield P2VP with a thiol end group, which was then reacted with a maleimide-functionalized version of the dye ATTO647N (A647N). For the P2VP-AAC, statistical poly(2-vinylpyridine-*co*-4-(3′-trimethylsilylpropargyloxy)styrene) was first synthesized, using a comonomer suited for azide−alkyne cycloaddition.[11] The alkyne group was deprotected and coupled[12] with an azide-tagged A647N. (For synthesis details see Supporting Information.)

The PS−P2VP used had equal number-average molar masses, $\overline{M}_n$, of both the PS and P2VP blocks (190 kDa each). Blends of PS−P2VP containing 0.1−2 wt % of the dye-tagged polymer were spin-coated onto coverslips, the surfaces of which had previously been treated with (3-aminopropyl)triethoxysilane. Phase separation was induced by solvent annealing in a vapor of pure $CHCl_3$ at 45 °C for 24 h. Non destructive, all-optical cross-sectional images taken with an isoSTED microscope dutifully reveal the expected lamellar morphology for both synthesis routes (Figure 1). (Note: all STED images shown, except Figure 1d, are of 2 wt % blends.)

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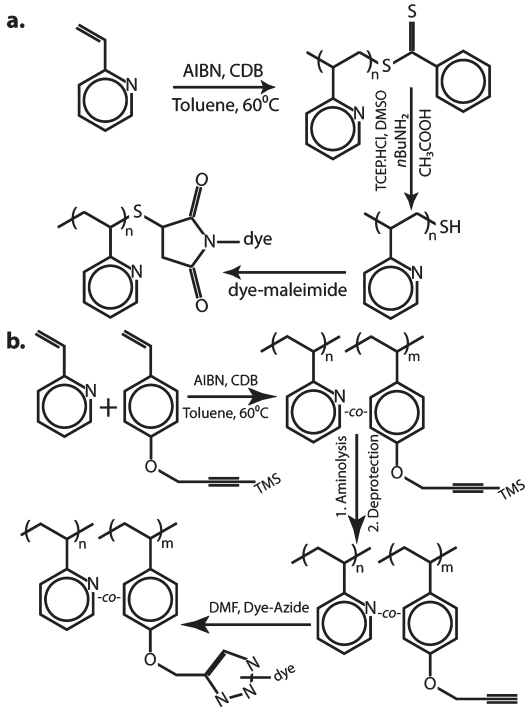
Scheme 1. Synthesis of Dye-Functionalized P2VP[a]



[a] (a) Synthesis of dye-tagged P2VP-TEC. 2-Vinylpyridine monomer is polymerized using a RAFT agent and subsequently cleaved by an amine to yield a thiol group. Polymer dye coupling is effected via a thiol−ene reaction. (b) Synthesis of dye-tagged P2VP-AAC. A statistical copolymer of 2-vinylpyridine and 4-(3′-trimethylsilylpropargyloxy)styrene is synthesized. The alkyne group is deprotected and the dye coupled via an alkyne−azide−Huisgen cycloaddition reaction.

To demonstrate the generality of the staining method, we varied the phase targeted and, separately, the fluorophore used. Specifically, A647N-tagged poly(methyl methacrylate) (A647N-PMMA-TEC) was synthesized using the thiol−ene scheme and used to stain the PMMA domain of a PS−PMMA with almost equal weight fractions of the two blocks ($\overline{M}_{n,PS}$ = 170 kDa, $\overline{M}_{n,PMMA}$ = 168 kDa). The resultant lamellar morphology (see Supporting Information for TEM micrograph) is shown in Figure 2a. Fluorophore flexibility was trivially demonstrated by substituting ATTO633 for the A647N in the thiol−ene coupling step of Scheme 1a. Figure 2b shows a cross-sectional image of PS−P2VP in which the P2VP phase was stained using A633-P2VP-TEC.

In recognition of the care that might be necessary when utilizing dyes and polymers with strongly dissimilar solubility parameters, we examined the combination of PS with the comparatively polar A647N dye. Two parameters related to such cases are the concentration of functional units within the polymer backbone and the average molecular weight ($\overline{M}_n$) of the polymer; the dye should not dominate the solubility properties of the parent macromolecule, the $\overline{M}_n$ of which must ideally be within the wet brush regime.[13] Figure 3a shows a cross-sectional image of PS−P2VP in which the styrene phase is doped with A647N-PS-AAC. To confirm the specificity of the technique, the nanostructured film was first immersed in methanol and subsequently in a solution containing 2,2′-thiodiethanol (97 wt %) and H_2O. The alcohols swell the P2VP domain significantly. The isoSTED cross section (Figure 3b) shows fluorescent PS lamellae

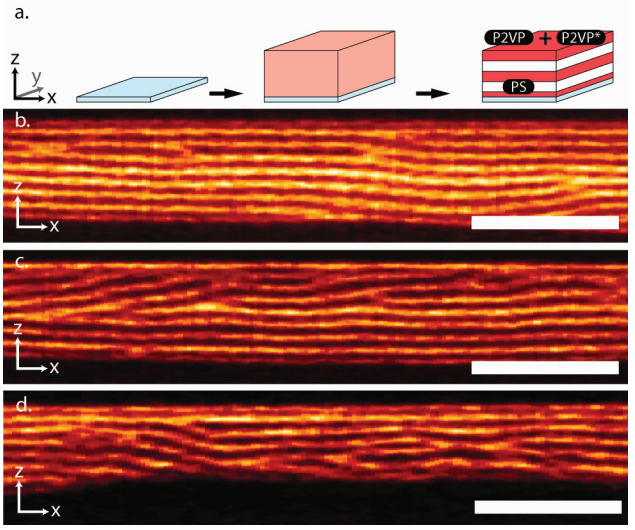


Figure 1. (a) Processing of BCP films. A blend of BCP and dye-tagged polymer is spin-coated onto surface-functionalized glass. Phase separation is induced by solvent annealing in an atmosphere of pure $CHCl_3$ at 45 °C. (b−d) In-situ cross-sectional isoSTED images of PS−P2VP. The P2VP phase is stained using (b) A647N-P2VP-AAC and (c, d) A647N-P2VP-TEC (2 and 0.1 wt %, respectively). Scale bar: 1 μm.

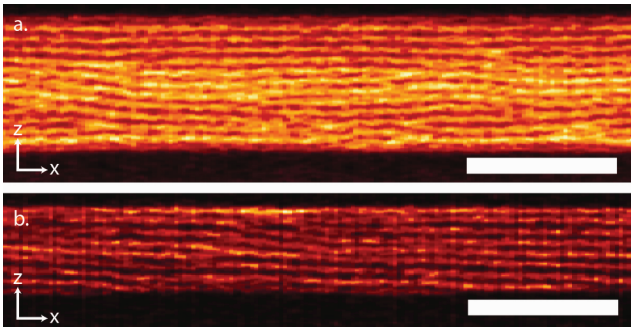


Figure 2. Cross-sectional isoSTED image of (a) PS−PMMA. The PMMA phase is stained using A647N-PMMA-TEC. (b) PS−P2VP. The P2VP phase is stained with A633-P2VP-TEC. Scale bar: 1 μm.

separated by dark swollen domains, confirming that the dye-tagged polymer is preferentially located in the PS domain, as opposed to the more polar P2VP phase.

A final comment is related to the processing of BCPs. Although solvent vapor annealing is increasingly widespread due to the low energy intensity and the high chain mobility achieved, thermal annealing above the glass transition temperature remains a common means for inducing phase separation. We examined the viability of imaging after such a thermal annealing step. Specifically, a film of PS−P2VP was annealed for 3 days at 170 °C. Although there is a reduction in the signal due to the prolonged exposure to high temperatures, the dye clearly is sufficiently robust to allow for imaging (see Supporting Information).

In summary, we have demonstrated a simple and flexible means for staining BCP microdomains that is suitable for far-field fluorescence nanoscopy. Targeted staining was achieved by leveraging the preferential solubility of dye-tagged homopolymers in the corresponding BCP microdomain. This staining scheme circumvents the need to modify the parent BCP being

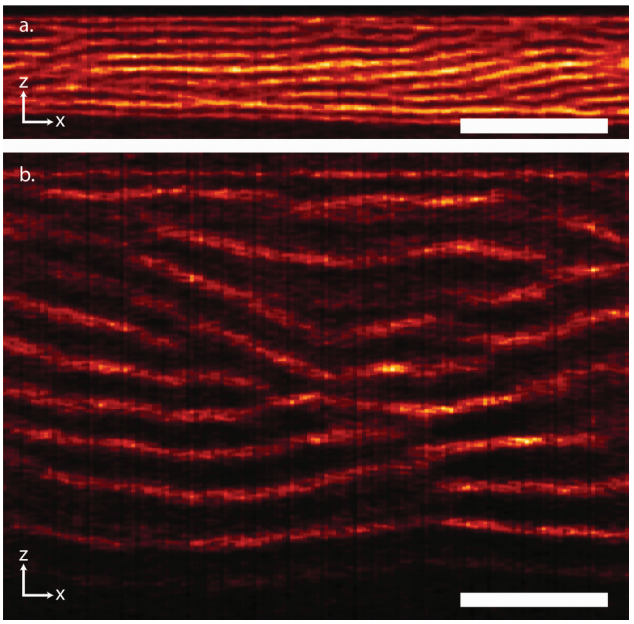


Figure 3. Cross-sectional isoSTED images of PS−P2VP. (a) The PS phase is stained using A647N-PS-AAC. (b) Contact with a solution of 2,2′-thiodiethanol (97 wt %) and H_2O causes preferential swelling of the P2VP phase. The bright unswollen PS domains confirm the preferential nature of the staining protocol. Scale bar: 1 μm.

investigated. The tagged polymers contained reactive functional groups that were incorporated during their synthesis by RAFT polymerization. Coupling of the dye to the functional groups was achieved by two equally viable click chemistry reactions. The suitable selection of dye concentration and molecular weights was combined with the 3D nanometric resolution of an isoSTED microscope to take *in situ* cross-sectional images of BCP nanostructures. The generality of the dye-tagged homopolymer staining scheme was exemplified by staining three chemically distinct BCP phases. The scheme presented here greatly enhances the number of BCP systems that can be imaged by fluorescence nanoscopy. The flexibility of the technique with respect to both phases stained and dyes interrogated opens up the possibility of multicolor imaging of BCPs, a specific strength of using fluorescence microscopy. While we have used fluorescence spectra as the discriminator in this instance, other spectral properties such as lifetime could conceivably be used.[14] The broad palette of available colors would be useful in the case of copolymers consisting of multiple blocks. It is important to note that although we use a specific implementation of far-field fluorescence nanoscopy, the methods presented in this paper are immediately applicable to other nanoscopy forms, including those based on single molecule switching with stochastic readout. The established benefits of conventional far-field fluorescence microscopy include the ability to take *in situ* 3D multicolor dynamic images. With the breaking of the diffraction barrier and the advent of nanometric 3D optical resolution, this method should now play an increasingly important role in the morphological studies of polymeric nanostructures.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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■ REFERENCES

(1) Park, C.; Yoon, J.; Thomas, E. L. *Polymer* **2003**, *44*, 6725.
(2) Yoon, J.; Lee, W.; Thomas, E. L. *Adv. Mater.* **2006**, *18*, 2691.
(3) Hell, S. W. *Science* **2007**, *316*, 1153.
(4) Hell, S. W.; Wichmann, J. *Opt. Lett.* **1994**, *19*, 780.
(5) Schmidt, R.; Wurm, C. A.; Jakobs, S.; Engelhardt, J.; Egner, A.; Hell, S. W. *Nature Methods* **2008**, *5*, 539.
(6) Ullal, C. K.; Schmidt, R.; Hell, S. W.; Egner, A. *Nano Lett.* **2009**, *9*, 2497.
(7) Tanaka, H.; Hasegawa, H.; Hashimoto, T. *Macromolecules* **1991**, *24*, 240.
(8) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559.
(9) Convertine, A. J.; Sumerlin, B. S.; Thomas, D. B.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2003**, *36*, 4679.
(10) Qiu, X. P.; Winnik, F. M. *Macromol. Rapid Commun.* **2006**, *27*, 1648.
(11) Fleischmann, S.; Komber, H.; Voit, B. *Macromolecules* **2008**, *41*, 5255.
(12) Quemener, D.; Le Hellaye, M.; Bissett, C.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *J. Polym. Sci., Part A* **2008**, *46*, 155.
(13) Hashimoto, T.; Tanaka, H.; Hasegawa, H. *Macromolecules* **1990**, *23*, 4378.
(14) Nakashima, K.; Winnik, M. A.; Dai, K. H.; Kramer, E. J.; Washiyama, J. *Macromolecules* **1992**, *25*, 6866.