Thermoresponsive polymer assemblies via variable temperature liquid-phase transmission electron microscopy and small angle X-ray scattering

Joanna Korpanty 1, Lucas R. Parent 2, Nicholas Hampu 1, Steven Weigand 3 & Nathan C. Gianneschi 1,4,5

Herein, phase transitions of a class of thermally-responsive polymers, namely a homopolymer, diblock, and triblock copolymer, were studied to gain mechanistic insight into nanoscale assembly dynamics via variable temperature liquid-cell transmission electron microscopy (VT-LCTEM) correlated with variable temperature small angle X-ray scattering (VT-SAXS). We study thermoresponsive poly(diethylene glycol methyl ether methacrylate) (PDEGMA)-based block copolymers and mitigate sample damage by screening electron flux and solvent conditions during LCTEM and by evaluating polymer survival via post-mortem matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). Our multimodal approach, utilizing VT-LCTEM with MS validation and VT-SAXS, is generalizable across polymeric systems and can be used to directly image solvated nanoscale structures and thermally-induced transitions. Our strategy of correlating VT-SAXS with VT-LCTEM provided direct insight into transient nanoscale intermediates formed during the thermally-triggered morphological transformation of a PDEGMA-based triblock. Notably, we observed the temperature-triggered formation and slow relaxation of core-shell particles with complex microphase separation in the core by both VT-SAXS and VT-LCTEM.
hermoresponsive polymers are used in numerous technological applications, including biomedicine, insulator materials, and tissue engineering. The most widely studied thermoresponsive polymers exhibit a lower critical solution temperature (LCST) in water. Upon heating above the LCST, such polymers undergo an entropically driven phase separation that coincides with a coil-to-globule transformation. The resulting phase transition induces morphological and rheological changes, attracting attention as so-called “smart” materials for stimuli-responsive drug carriers, nanoreactors, and industrial coatings.

Despite the ubiquity of thermoresponsive polymeric materials, currently, we lack well-established, direct techniques for elucidating their elevated temperature, solution-phase, nanoscale morphologies, and dynamics. Presently, the accepted workflow for analyzing solvated thermoresponsive soft nanomaterials at elevated temperatures consists of scattering techniques in combination with static imaging via electron microscopy. Scattering techniques, including variable temperature dynamic light scattering (VT-DLS) and variable temperature small angle X-ray scattering (VT-SAXS), require prior assumptions about the system so that raw data can be fit to models, often creating challenges in assigning nanostructure morphologies. Conversely, cryogenic (cryo) TEM samples can be used to directly image nanostructures and assess structural dynamics. Herein, we examine three such phase transitions for thermoresponsive polymeric materials with an emphasis on VT-LCTEM.

Results and discussion

VT-LCTEM study of PDEGMA. First, we synthesized PDEGMA (Fig. 2). We measured a cloud point of 45 °C via VT-DLS that remained stable upon further heating (Fig. 2a, b, Supplementary Fig. 2). For PDEGMA and aqueous organic systems generally, a critical consideration for LCTEM is careful electron flux (e− Å−2 s−1) selection. While a higher flux increases signal-to-noise and improves contrast, beam-induced phenomena often become significant with increasing instantaneous flux and cumulative fluence (e− Å−2).

To minimize such artifacts, we screened flux, fluence, and solvent conditions over separate LCTEM experiments and evaluated PDEGMA survival via post-mortem matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS, Supplementary Movies 1-11). We chose to screen hydroxyl radical (-OH) scavengers, such as isopropanol (IPA)29, t-butanol (t-BuOH)28, and dimethyl sulfoxide (DMSO)30, as -OH is generally the most destructive radiolysis product towards polymers. Additionally, we tested deuterated28 and deaged water, which are hypothesized to mitigate radiolytic damage compared to water. Post-mortem MALDI-IMS indicated that PDEGMA survived at higher fluxes and fluences in 5% t-BuOH, 5% IPA, and pure D2O (Fig. 2c, Supplementary Figs. 3, S4).

As PDEGMA also survived LCTEM imaging under purely aqueous conditions at a flux of 0.8 e− Å−2 s−1 and a low fluence (< 10 e− Å−2 s), we employed these conditions with stroboscopic imaging to probe the purely aqueous LCST-transition (Fig. 2d-f). To ensure the LCST would occur in the diffusion-limited, confined liquid-cell, we used 20 mg mL−1 PDEGMA and heated it to 60 °C. With heating, we visualized polymer phase separation, demonstrating the ability of VT-LCTEM to directly image a solvated LCST-type polymer at temperature (Fig. 2f). Cooling did not completely redissolve the sample during the 60 min it was monitored, likely due to diffusion constraints and

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**Fig. 1 Thermal transitions in LCST-type polymers.** Thermally triggered transformations in an LCST-type polymer for a homopolymer undergoing precipitation (left), a diblock copolymer undergoing assembly (center), and a triblock copolymer undergoing a morphological transformation (right). Note that these transformations may show partial reversibility.
the propensity for polymers to stick to SiNx, which occurred even in unimaged controls (Supplementary Fig. 5).

VT-LCTEM study of PEG-b-PDEGMA. Having optimized imaging conditions to study PDEGMA, we sought to visualize assembly of aqueous PEG-b-PDEGMA (Fig. 3). The formation of nano-assemblies upon heating was confirmed via VT-DLS (Fig. 3a, b). Using a flux of $0.8 \text{ e}^{-\text{Å}^{-2s^{-1}}}$, the same conditions where PDEGMA survived, PEG-b-PDEGMA assemblies formed at 50 °C from an aqueous solution without pre-formed structures (Fig. 3c, d). The predominant morphology by VT-LCTEM was small (30-50 nm) micellar nanoparticles with several larger (~ 200 nm) vesicular structures.

We measured the average background intensity for several regions surrounding the assemblies and found the intensity increased upon heating above the LCST and decreased upon cooling (Supplementary Fig. 6). This change in background intensity, which reflects a change in mass-thickness, was likely caused by liquid exclusion upon heating, as the PDEGMA became more hydrophobic and expelled surrounding solvent, followed by local rehydration upon cooling.

With the PEG-b-PDEGMA system, we demonstrate the first example of a thermoresponsive diblock copolymer assembly being directly observed in the solution. Though PEG-b-PDEGMA was too large to ionize by MALDI-IMS, PDEGMA survival under the same imaging conditions suggests the diblock likewise survives. Moreover, VT-LCTEM control experiments showed similar assemblies formed when the sample was left unimaged until reaching the LCST, indicating observed assemblies were thermally driven, not beam-induced (Supplementary Fig. 5).

VT-LCTEM study of PEG-b-PDEGMA-b-PHPMA. Next, we sought to study a more complex triblock system, where the polymer is amphiphilic at room temperature with the addition of a non-responsive hydrophobic block (Fig. 4). Heating prompts a morphological transformation, as PDEGMA alters the hydrophilic-to-hydrophobic balance of the polymer (Fig. 4a). At
room temperature, the dry state TEM of the triblock solution showed micellar nanoparticles \( \sim 60 \text{ nm} \) in diameter (Fig. 4b). VT-DLS indicated a transformation from small assemblies (30–60 nm) into larger structures (200–300 nm) upon heating (Fig. 4c).

We directly imaged a concentrated solution (15 wt%) of thermo-responsive PEG-b-PDEGMA-b-PHPMA via VT-LCTEM at the same imaging conditions where PDEGMA survived (Fig. 3d, e, Supplementary Fig. 8). Using a flux of 0.8 e\(^{-2}\text{s}^{-1}\), at room temperature, we observed small micelles, \( \sim 60 \text{ nm} \), which we analyzed using image processing (Fig. 4d, Supplementary Fig. 9). Heating the sample prompted the formation of larger, higher contrast intermediates at \( t = 32 \text{ min} \), because of the LCST-transition of the PDEGMA block. The transition drove PDEGMA into the particle core with PHPMA (Fig. 4d). The intermediates that formed immediately after heating above the LCST were associated with a significantly increased background liquid intensity, indicating a decrease in the surrounding liquid thickness, which could have been caused by water exclusion from the concentrated assemblies (Supplementary Fig. 6).

Continuing to maintain a temperature of 60 °C, the formation of high contrast intermediates was followed by the appearance of a more dispersed halo surrounding the dense core and a return of the initial liquid thickness at \( t = 40 \text{ min} \) (Supplementary Fig. 6). The clear core-shell structure observed at \( t = 40–50 \text{ min} \) likely reflected exclusion of PHPMA from the core by PDEGMA, causing an increased shell contrast, as PHPMA is hydrophobic. Exclusion of PHPMA from the core was likely caused by enthalpically unfavorable interfacial contacts between PHPMA and PDEGMA upon the PDEGMA LCST-transition. It is also possible that the intermediates observed at \( t = 40–50 \text{ min} \) reflect incomplete packing of PDEGMA into the core, leaving the PDEGMA block partially packed into the dense core and partially exposed to the surrounding water.

Upon cooling, the initial morphology was not restored, as observed by both VT-DLS and VT-LCTEM (Fig. 4d, Supplementary Fig. 10). It is likely that the PHPMA vitrified before the PDEGMA could reswell, due to the glass transition temperature \( (T_g) \) of hydrated PHPMA, at 47 °C\(^{[41]} \), being below the LCST of PDEGMA, \( \sim 50 \text{ °C} \). The observed liquid thinning in the VT-LCTEM experiment may have resulted from PDEGMA excluding surrounding water, an inherent component of the LCST-transition (Supplementary Fig. 6)\(^{[8]} \). Since the LCST-transition is a manifestation of polymer–solvent interactions, this observation exemplifies the unique ability of LCTEM to probe the subtleties of polymer–solvent interactions.

To further analyze LCTEM data, we utilized image processing (Fig. 4e, f). Image processing highlighted the initial presence of small particles, which likely aggregated upon the onset of the PDEGMA LCST (Fig. 4d, e, first frame). This aggregation led to the formation of a larger, more hydrophobic core (Fig. 4e, second and third frames). As the PDEGMA block expelled more water during its LCST-transition, it likely excluded PHPMA from the core to minimize unfavorable interactions, yielding an intermediate contrast shell (Fig. 4d, fourth frame). The intermediate contrast of the shell was better captured using false coloring, whereby each pixel value range was assigned a red, green, or blue value. Applying a 6-shade coloring
highlighted the formation of the disperse shell surrounding the high contrast core (Fig. 4f, fourth frame). Cooling the sample then led to reduced density of the core and shell (Fig. 4e, f, fifth and sixth frames). Despite what appeared as full re-solvation of the corona, the core appeared trapped, and the original morphology was not restored over the time monitored. That is, we did not observe fission into small particles. In addition to image processing, we measured the radial profile centered on the particle core (Fig. 4g, h). This profile highlighted the increased contrast of the core and shell upon heating with an increase in contrast from PDEGMA and exclusion of PHPMA from the core. Cooling then led to decreased contrast in the core and even more so in the shell.

VT-SAXS study of PEG-b-PDEGMA-b-PHPMA. To gain a deeper understanding of the transformations directly observed by VT-LCTEM, we utilized VT-SAXS (Fig. 5). SAXS has been used extensively to study thermal transitions of soft matter, polymerization induced self-assembly, lipoprotein phase transitions, and responsive hydrogels. Interpretation of SAXS data relies upon fitting shape-dependent models to experimental data to understand nanostructure morphology. However, selecting the appropriate model for data fitting is non-trivial, and commonly, TEM data inform model selection. Even for previous studies that employed direct imaging to guide SAXS interpretation, only static snapshots were obtained and mechanisms for transformations of individual nanostructures were inferred. Accordingly, here we employed VT-SAXS (Fig. 5a) with VT-LCTEM to gain insight into the dynamics of individual nanostructures through both in situ imaging and scattering.

With the initial hypothesis that the triblock forms spherical micelles with a PHPMA core, a PDEGMA shell, and a PEG corona below the LCST of PDEGMA, we fit the experimental scattering data acquired at 30 °C to a core-shell-shell sphere form factor. The fitted curve captured the experimental data reasonably well for intermediate and high q, although significant deviation was observed in the low q region, which we attribute to the presence of loose micellar aggregates (Fig. 5b). The fit yielded a core radius of 18 nm, a
PDEGMA inner shell thickness of 1.5 nm, and a PEG outer shell thickness of 4.2 nm. These sizes are reasonably consistent with the DLS and TEM measurements, supporting the robustness of our fitting process and demonstrating the potential for using SAXS as a complementary technique to LCTEM (Table S1).

Heating the sample to 60 °C resulted in the formation of a broad peak in the high \(q\) region of the SAXS data, which supports the LCTEM observation that a morphological change occurs above the PDEGMA LCST. Based on the low degree of polymerization of the PDEGMA block compared to the PHPMA block, we speculated that the micelle core would restructure above the LCST and relax the large entropic penalty for arranging the shorter PDEGMA block on the convex side of the core-shell interface. The LCST of PDEGMA is higher than the \(T_g\) of PHPMA, allowing the mobile PHPMA core to accommodate the collapsed PDEGMA domain at 60 °C. Furthermore, the persistence of the broad peak even after cooling back to 30 °C suggests that the PDEGMA domain is trapped within a vitriﬁed PHPMA matrix and is unable to rehydrate below the LCST. Using this hypothesized PDEGMA core, PHPMA inner shell, and PEG outer shell conﬁguration as our initial model, we attempted to ﬁt the 60 °C SAXS data to the same core-shell-shell sphere form factor that we successfully used for the 30 °C data. However, a satisfactory ﬁt was unobtainable using physically relevant ﬁtting parameters. To conﬁrm that the ﬁtting challenge was unrelated to our choice of the initial model, we also attempted to ﬁt the data to the core-shell-shell form factor assuming a PHPMA core, a PDEGMA inner shell, and a PEG outer shell. Again, a satisfactory fit was unobtainable. Therefore, we speculated that the true micelle structure was more complex than the concentric spheres assumed by the core-shell-shell model. Instead, we hypothesized that the PHPMA and PDEGMA domains might be microphase segregated within the micelle core. To test this hypothesis, we then ﬁt the experimental scattering data obtained at 60 °C to the sum of a spherical and broad peak form factor, where the spherical form factor captures the overall micelle shape, and the broad peak form factor captures the phase segregated core (Fig. 5c).

This choice of scattering model yielded an acceptable ﬁt to the experimental data with a micelle radius of 95 nm and a 30 nm spacing between scattering inhomogeneities within the micelle core. The ﬁtted values for the micelle radius correspond well to those measured by LCTEM and VT-DLS, while the 30 nm length scale is reasonably consistent with the size of the high contrast region observed in the micelle interior by LCTEM.

By VT-SAXS, the triblock did not fully relax to its original structure even 30 min after cooling (Fig. 5a). The slow relaxation suggests that enthalpically unfavorable mixing of PDEGMA and PHPMA in the core upon heating led to the exclusion of PHPMA from the inner core so that isolated PDEGMA domains were surrounded by a PHPMA shell (Fig. 6). This result, which was ambiguous by VT-LCTEM in isolation, shows the value of coupling VT-LCTEM with VT-SAXS, as the combination of the two techniques allowed us to propose a mechanism for the triblock thermal transformation (Fig. 6). These results highlight a new potential workflow for characterizing stimuli-responsive soft materials, where insights from SAXS can reﬁne and clarify ambiguous LCTEM features and vice versa, offering a powerful set of new strategies to enhance our current understanding of complex soft nanomaterials.

In summary, we have gained insight into complex polymeric nanostructures and their dynamics by studying thermoresponsive poly(diethylene glycol methyl ether methacrylate) (PDEGMA)-based polymers. We established general guidelines for observing thermoresponsive polymeric materials and processes in situ. Notably, we highlight the importance of optimizing LCTEM conditions coupled with post-mortem analysis. Particularly, for the PDEGMA triblock system, optimized VT-LCTEM conditions enabled us to gain direct mechanistic insight and observe intermediate structures formed during the thermally triggered
morphological transformation of the polymer. Correlating VT-LCTEM with VT-SAXS for the triblock provided key insights into an intricate phase transition, showing the value of leveraging the techniques in tandem. Our general workflow shows the potential of LCTEM, coupled with SAXS, to answer fundamental questions about functional and responsive nanomaterials, and our approach can be extended to study nanoscale processes of high scientific importance, such as drug encapsulation and release from nanocarriers that may occur in a phase-dependent fashion, influencing carrier design and synthesis.

### Methods

#### General information

All materials were purchased from Sigma or TCI chemicals. All monomers were filtered through basic alumina to remove inhibitors, and all other materials were used as received unless otherwise noted. The synthetic details are available in the supplementary information.

#### LCTEM imaging

The Protechos Poseidon Select Heating holder was used to collect LCTEM data. Milli-Q water was used to pre-clean the chips with 50-nm-thick, 200 µm × 50 µm window SiNx membranes. The synthetic details are available in the supplementary information.

#### General information

Two-dimensional scattering patterns were obtained from 10 s exposure using a fixed area for each timepoint (Fig. 5a). We performed image processing using the software Fiji (Fig. 5).

#### MALDI-IMS

LCTEM chips, with their SiNx membranes facing upwards were adhered to the conductive face of an ITO-coated glass slide with 70–100 ohms resistivity (Bruker Daltonics), using ~0.5 µl nail polish and allowed to dry. To equalize the height difference from SiNx chips on the slide (~0.25 mm), four pieces of Scotch tape were adhered to the back of the slide on the same side. As a control, uncoated PEGMA in deionized water was drop coated onto a clean liquid-cell chip. All chips were coated with trans-2-[3-(4-tert-butylyphenyl)-2-methyl-2-propenylidene]mal- ononitrile (DCIT) matrix in 20 mg mL⁻¹ in acetonitrile.

#### VT-SAXS

SAXS experiments were conducted at the 5-ID-D beamline of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) at the Advanced Photon Source, Argonne National Laboratory. Samples of 1% w/v polymer in water were prepared and loaded into 1.5 mm quartz capillaries. The capillaries were then sealed with epoxy to prevent solvent evaporation prior to data acquisition. Capillaries were loaded into a multicapillary holder and scattering patterns were first acquired at room temperature. Samples were then heated to 60 °C at a rate of 1 °C/min. After reaching the temperature set point, patterns were obtained every 2 min for 30 min. Samples were then cooled to 30 °C at 1 °C/min. After reaching the set point, patterns were acquired every 2 min for 30 min.

Two-dimensional scattering patterns were obtained from 10 s exposure using a Rayonix MX170-HS CCD area detector using a 0.5 s exposure time to X-rays with a wavelength of λ = 0.7293 Å and a sample-to-detector distance of 8.5 m. The 2D data were azimuthally averaged to yield 1D scattering patterns as intensity versus q. Incoherent background scattering was measured by acquiring scattering patterns for a water-loaded capillary in the absence of polymer. The solvent data was fit to a power law of the form I(q) = A + Bj⁻m + Cj⁻q and subtracted from the polymer data. Select scattering patterns were fit to a model comprising the sum of a spherical form factor and a broad Lorentzian peak.

### Data availability

We declare that all other data supporting the findings of this study are available within the article and supplementary information files and are also available from the corresponding author upon request. Source data files are available for the data shown in Figs. 2b, 2e, 3b, 4c, 5a, 5b, and 5c. Source data are provided with this paper.

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Author contributions
J.K., L.R.P. and N.C.G. devised the project. J.K. performed LCTEM, MALDI-IMS, synchrotron SAXS and N.H. aided in SAXS data analysis. All authors wrote and edited the manuscript. All authors have given approval to the final version of the manuscript.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Nathan C. Gianneschi.

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