

## SUPPORTING INFORMATION

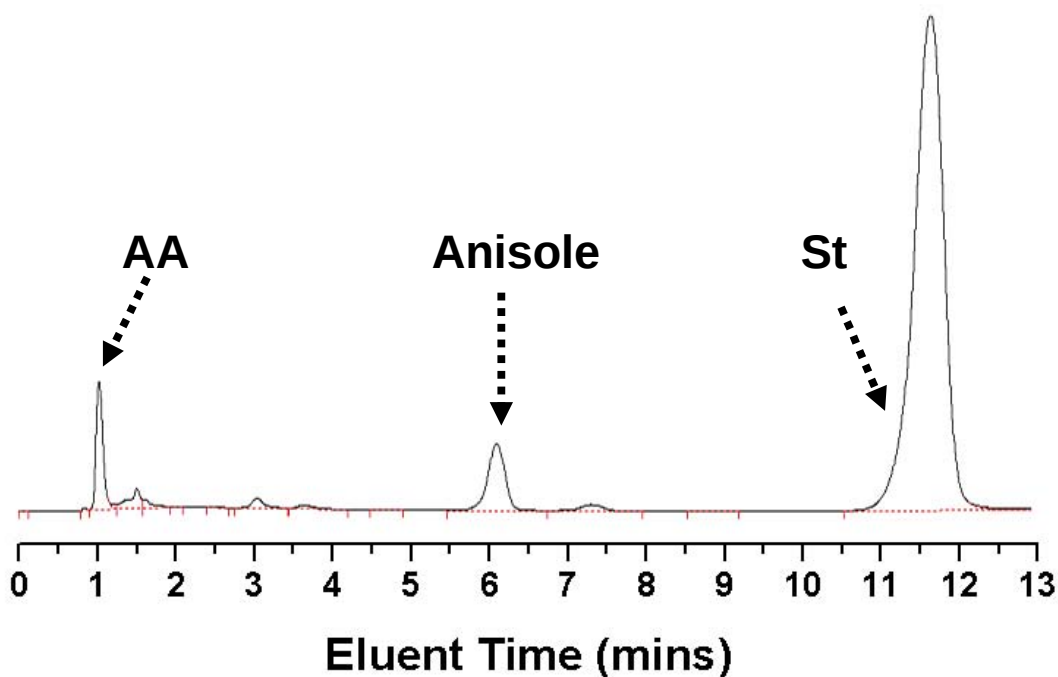
### Kinetics of Two-stage Dispersion Copolymerization for the Preparation of Ln-encoded Polystyrene Microparticles

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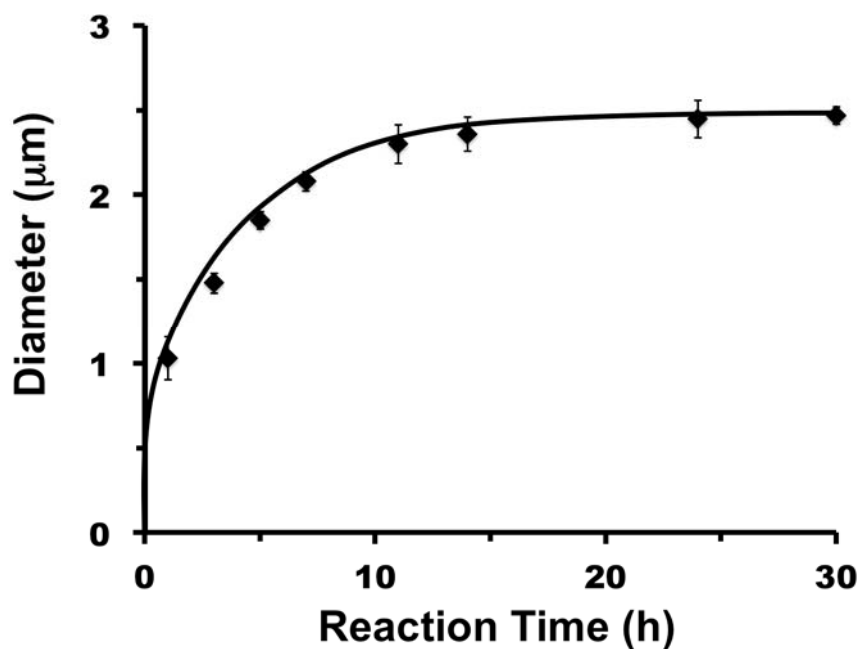
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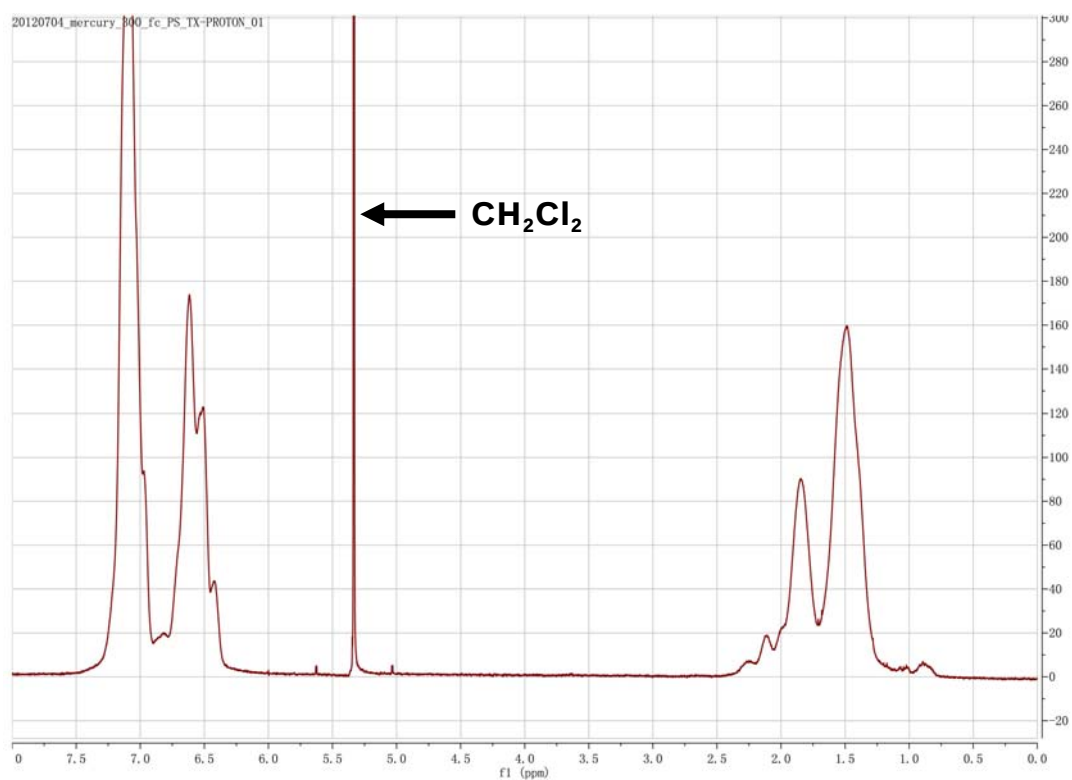
## FIGURES



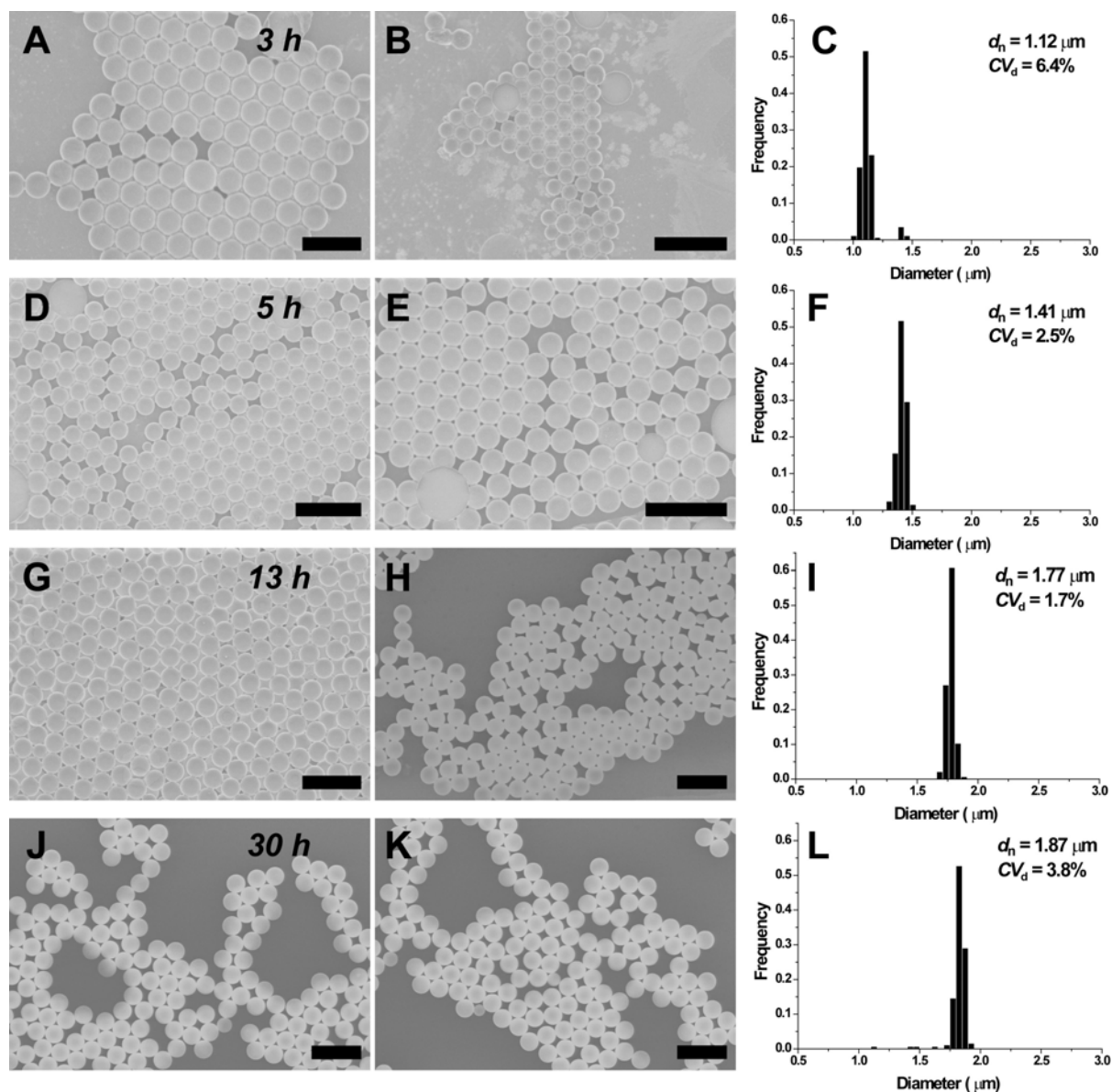
**Figure S1.** HPLC curve of the sample for the dispersion copolymerization of St and AA in presence of 0.50 wt %  $\text{TmCl}_3$ , 14 h after the initiation.



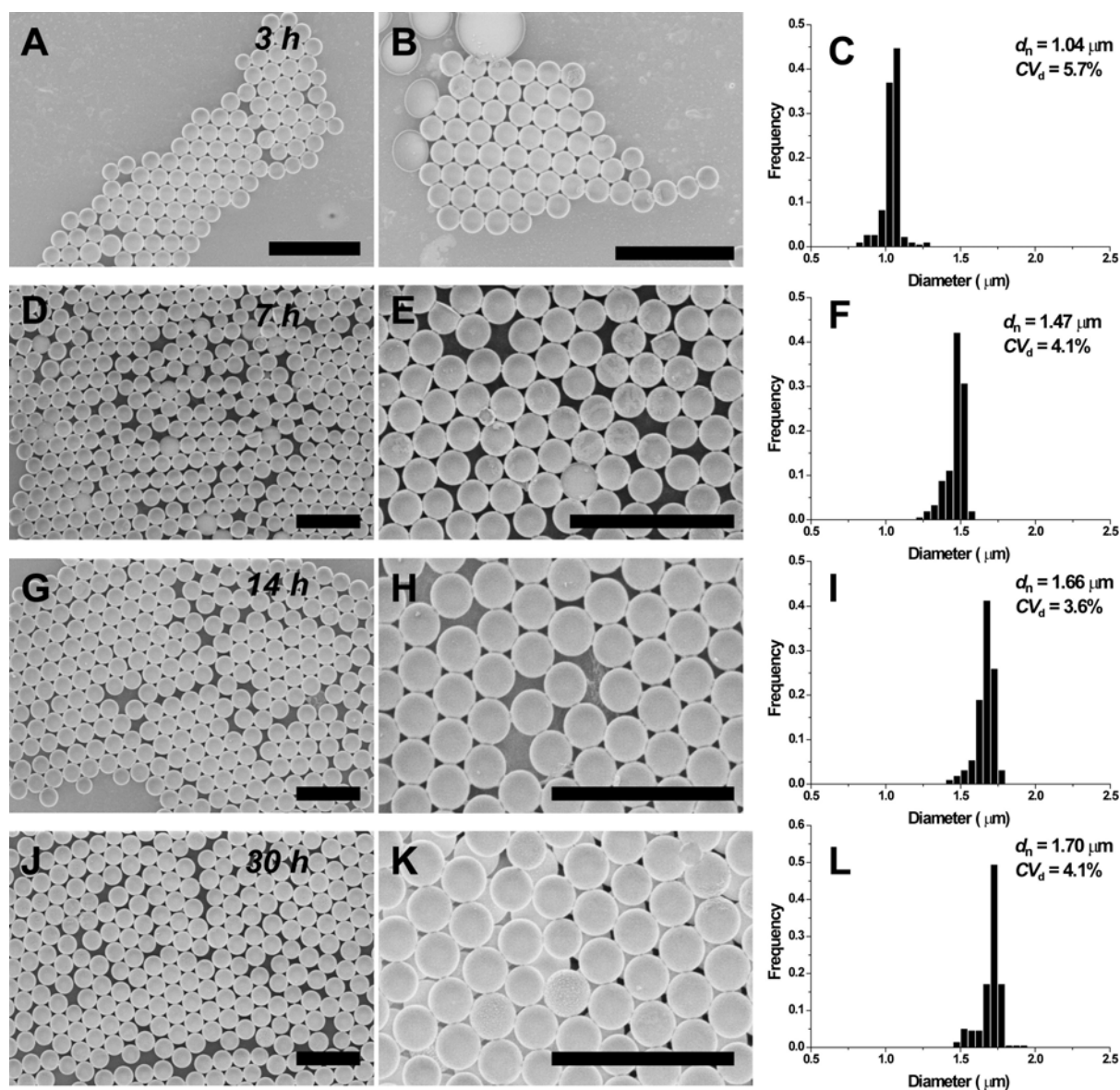
**Figure S2.** Evolution of the number average particle diameter ( $d_n$ ) with time for the two-stage dispersion copolymerization of St and 2.0 wt % AA based on St. The AA solution in ethanol was added during the second stage, 1 h after initiation ( $t = 1$  h). Error bars show the standard deviation of  $d$  determined by measuring approximately 200 particle diameters.



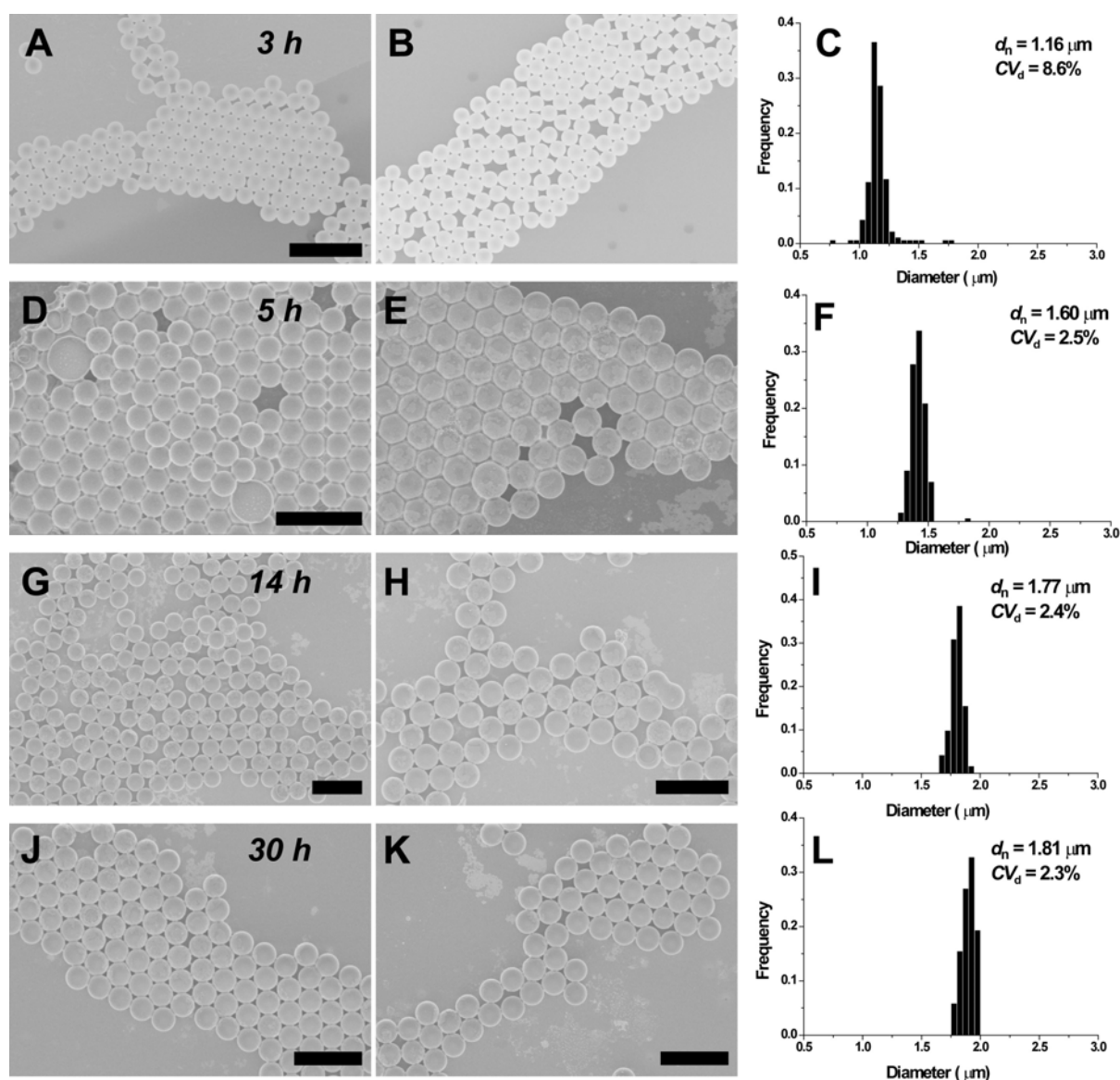
**Figure S3.**  $^1\text{H}$  NMR spectrum of PS-co-PAA in  $\text{CD}_2\text{Cl}_2$ . The polymer was obtained from particles synthesized by two-stage dispersion polymerization with PVP as the stabilizer in the presence of 2.0 wt % AA based on St. The particles were taken after 30 h reaction and were purified by centrifugation and redispersion in ethanol three times and dried in a vacuum oven.

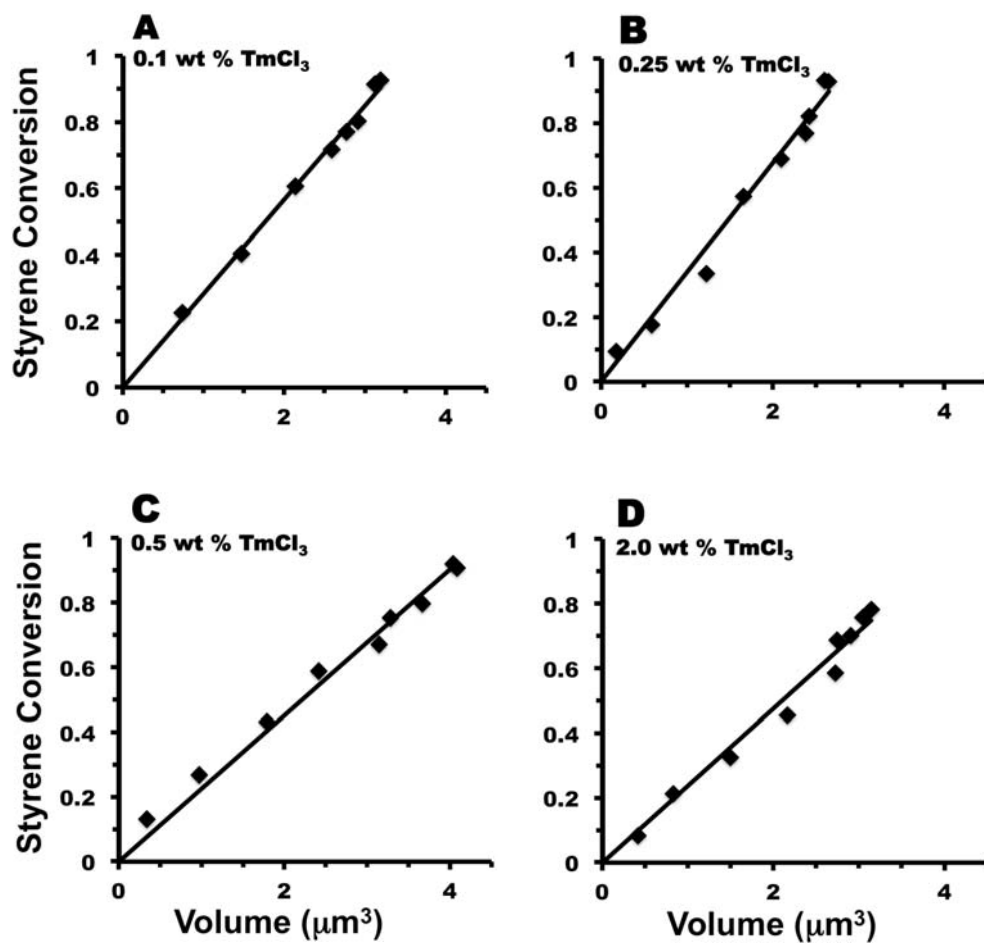


**Figure S4.** SEM images and diameter distribution histograms of the particles at different times after the initiation of the reaction. This sample was synthesized by two-stage dispersion polymerization with PVP as the stabilizer in the presence of 2.0 wt % AA, 0.10 wt %  $\text{TmCl}_3$  relative to the weight of styrene. 3 h (A, B, C); 5 h (D, E, F), 13 h (G, H, I); 30 h (J, K, L). Time zero refers to initiation of the reaction. The second stage reactants (AA and  $\text{TmCl}_3$ ) were added at  $t = 1$  h. The scale bars are 5  $\mu\text{m}$ .

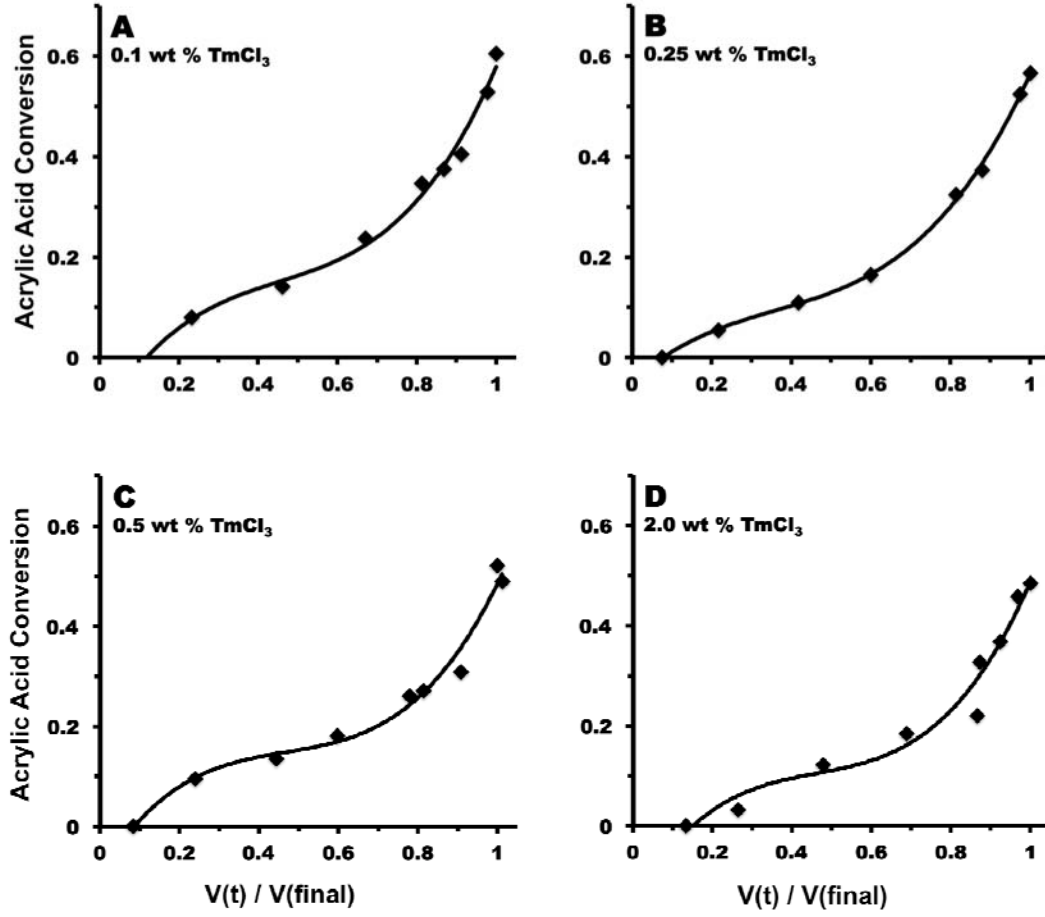


**Figure S5.** SEM images and diameter distribution histograms of the particles at different times after the initiation of the reaction. This sample was synthesized by two-stage dispersion polymerization with PVP as the stabilizer in the presence of 2.0 wt % AA, 0.25 wt % TmCl<sub>3</sub> relative to the weight of styrene. 3 h (A, B, C); 7 h (D, E, F), 13 h (G, H, I); 30 h (J, K, L). Time zero refers to initiation of the reaction. The second stage reactants (AA and TmCl<sub>3</sub>) were added at  $t = 1$  h. The scale bars are 5 μm.





**Figure S7.** Conversion of St versus particle volume for two-stage dispersion polymerization of St and AA with different  $\text{TmCl}_3$  content: (A) 0.10 wt %; (B) 0.25 wt %; (C) 0.50 wt % and (D) 2.00 wt % based on St. The x-axis represents the particle volume calculated from the mean particle diameter  $d_n$  as determined by SEM, and the y-axis indicates the conversion of St obtained by HPLC. AA and  $\text{TmCl}_3$  were added 1 h after initiation.



**Figure S8.** AA conversion versus the normalized particle volume during the growth of the particle with  $\text{TmCl}_3$  content of (A) 0.10 wt %; (B) 0.25 wt %; (C) 0.50 wt % and (D) 2.00 wt % based on St. The x-axis represents the particle volume calculated from the diameter of the particle as measured by SEM and the y-axis indicates the conversion of St obtained by HPLC. AA and  $\text{TmCl}_3$  was added 1 h after initiation.

## ADDITIONAL RESULTS AND DISCUSSION

### *Calculation of the predicted fraction of monomer in solution and in the copolymer*

The Mayo-Lewis equations, eq S1, predict the mole fraction of the monomer in the copolymer,  $F$ , for small changes in the conversion of the monomers for polymerizations in homogenous solution.

$$f_1 = 1 - f_2 = \frac{M_1}{M_1 + M_2}$$

$$F_1 = 1 - F_2 = \frac{r_1 f_1^2 + f_1 f_2}{r_1 f_1^2 + 2 f_1 f_2 + r_2 f_2^2} \quad (\text{S1})$$

Here  $f_1$ ,  $f_2$  are the mole fractions of styrene and acrylic acid, respectively, in solution;  $F_1$ ,  $F_2$  are the mole fractions of these monomers in the copolymer;  $M_1$ ,  $M_2$  are the number of moles of the monomers, and  $r_1 = r_{\text{St}}$ ,  $r_2 = r_{\text{AA}}$  are the reactivity ratios. For the predicted values of  $F$

we used the reactivity ratio for solution polymerization of St and AA in methanol,  $r_{AA} = 0.13$  and  $r_{St} = 1.10$ . We started with the same initial feed of monomer as we had in our experimental dispersion polymerization. When we added AA (0.129 g) 1 h after initiation, the conversion of St was 13.2 % with the starting amount of St (6.265 g). From these values we calculated the initial mole fraction of monomers in solution to be  $f_{AA(0)} = 0.0333$  and  $f_{St(0)} = 0.967$ . Using the above model reactivity ratios, we calculated the mole fractions of monomer in the copolymer to get  $F_{AA(0)} = 0.0296$  and  $F_{St(0)} = 0.9704$  for very small conversion. We then calculated new mole fractions of monomer in solution,  $f$ , for a small steps of total monomer conversion,  $p = 0.001$ . We then calculated new mole fractions of monomer in solution,  $f$ , using the formula:

$$\begin{aligned} f_{AA(n)} &= f_{AA(n-1)} - (0.001 \times f_{AA(n-1)}) \\ f_{St(n)} &= f_{St(n-1)} - (0.001 \times f_{St(n-1)}) \end{aligned} \quad (S2)$$

where  $n$  is the number of iterations.

New values of  $F_{AA(n)}$  and  $F_{St(n)}$  were then calculated using the eq S1. These new values of  $F$  were used to calculate new values of  $f$ . With each iteration, the total conversion increased by 0.001:

$$p_n = n \times 0.001 \quad (S3)$$

where  $n$  is the number of iterations and  $p$  is the total conversion.

The mole fractions of monomers St and AA in solution versus total conversion for the predicted values using the Mayo-Lewis equations are presented in Figure 2A and 2B in the main text.

### ***Calculation of the number of particles per reaction.***

The volume of the particle in each polymerization system during the polymerization could be calculated based on the eq S4, as shown below:

$$Conv(St) = \frac{N \times V \times \rho}{m} \quad (S4)$$

where  $Conv(St)$  is the conversion of St;  $m$  is the amount (g) of St added;  $V$  is the volume of the particle;  $\rho$  is the density of the particle (taken to be the density of polystyrene, 1.05 g/cm<sup>3</sup>), and  $N$  is the number of the particles in the dispersion.

We assumed that  $m$  and  $\rho$  remained constant during the polymerization. Therefore, the slopes of the lines in Figure 1C and in Figure S7 are proportional to the number of particles

in the dispersion. Based on the value of the slope (slope = 0.12) obtained in **Figure 1C**, we could obtain the number of the particles for this reaction to be  $0.71 \times 10^{12}$  particles. The reaction volume for each reaction was  $\sim 50$  mL giving  $1.42 \times 10^{10}$  particles/mL.

$$\begin{aligned} \text{slope} &= \frac{\text{Conv(St)}}{V} \\ N &= \frac{\text{slope} \times m}{\rho} = \frac{(0.12 \mu\text{m}^{-3}) \times (6.2646 \text{g})}{(1.05 \text{g} \cdot \text{cm}^{-3}) \times (1 \times 10^{-12} \text{cm}^3 \cdot \mu\text{m}^{-3})} = 0.71 \times 10^{12} \text{ particles} \\ N(\text{particles} / \text{mL}) &= \frac{0.71 \times 10^{12} \text{ particles}}{50 \text{mL}} = 1.42 \times 10^{10} \text{ particles} / \text{mL} \end{aligned}$$

***Justification of assumption that AA, PVP and TX305 make a negligible contribution to the size of the PS particles.***

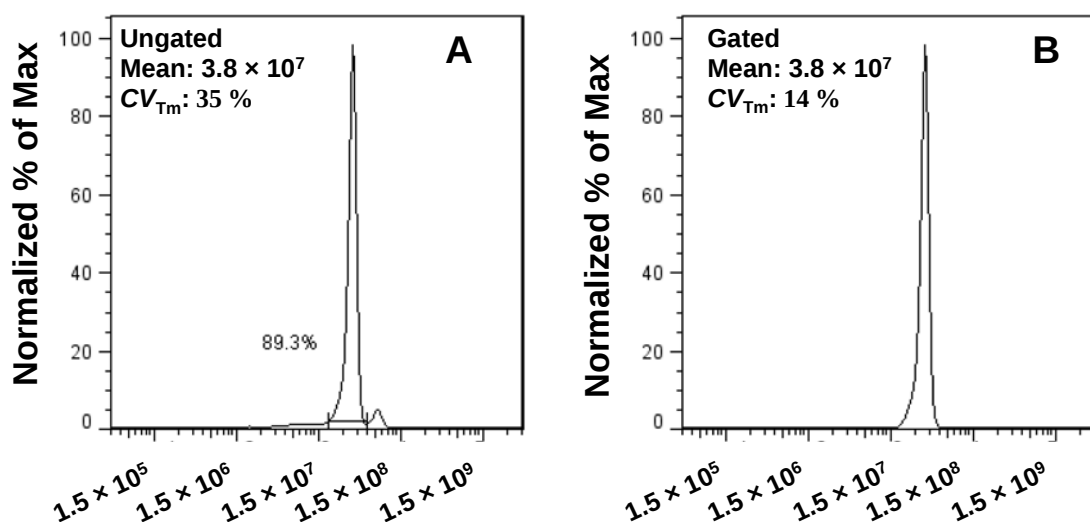
At the end of the reaction, a sample of the particles from the dispersion copolymerization of St and 2.0 wt % AA based on St were washed, dried and then dissolved in  $\text{CDCl}_3$  for analysis by  $^1\text{H}$  NMR. **Figure S3** shows the  $^1\text{H}$  NMR spectrum of the sample. We were unable to detect signals due to the PVP stabilizer. These results are consistent with the predictions of the kinetic model developed by Paine to describe dispersion polymerization of styrene in ethanol using PVP ( $M_w = 40,000$ ) as a stabilizer.<sup>1</sup> This model predicts the minimum and maximum amount of the PVP-g-PS required to prevent particle aggregation and to avoid secondary nucleation, which for these particular conditions were  $\sim 0.1 \text{ mg/m}^2$  and  $\sim 1.0 \text{ mg/m}^2$ , respectively, i.e., on the order of 0.1-1.0 wt %, too small to be detected by  $^1\text{H}$  NMR. We were also unable to detect a signal for the PEG chain of the TX-305 co-stabilizer. Only 62 % of the 2.0 wt % of AA based on the weight of St was incorporated into the particles.

***Mass cytometry data acquisition.***

The particle sample dispersion was washed by three cycles of centrifugation and resuspension in water to remove free ions in the supernatant. The resultant slurry in water (ca.  $10^6$  particles/mL) was then nebulized into the sample introduction system of the mass cytometry, followed by the atomization and ionization of individual particles including the Tm embedded inside the particle by an inductively coupled plasma torch. The formed ion stream is automatically introduced into the time-of-flight mass analyzer and the transient signals corresponding to each particle ionization event were recorded by the detector and stored.

**Figure S9A** is a typical ungated Tm signal intensity obtained from FlowJo software based on the original data from mass cytometry, where the particle sample was taken from

the polymerization in the presence of 0.25 wt %  $\text{TmCl}_3$  combined with 2.0 wt % of AA at 14 hours after the initiation. As shown in **Figure S9A**, an average of  $3.8 \times 10^7$  Tm ions per particle was calculated from the measured ion intensity and the standard calibration solution, with a coefficient of variation of lanthanide distribution ( $\text{CV}_{\text{Tm}}$ ) of 35 %. The signal at the low end of the intensity distribution peak was attributed to particle fragmentation and small particles in the solution. The signal at the high end was attributed to occasional formation of dimmers and large aggregates during injection into the plasma torch by the nebulizer. The gated peak as shown in **Figure S9B** represents 89.3% of the particles and is the value characteristic of the synthesized particle, which gives an average of  $3.8 \times 10^7$  Tm ions per bead with a  $\text{CV}_{\text{Tm}}$  of 14 %. Thus, for the other samples, the number of Tm ions per particle was obtained by using a similar strategy.

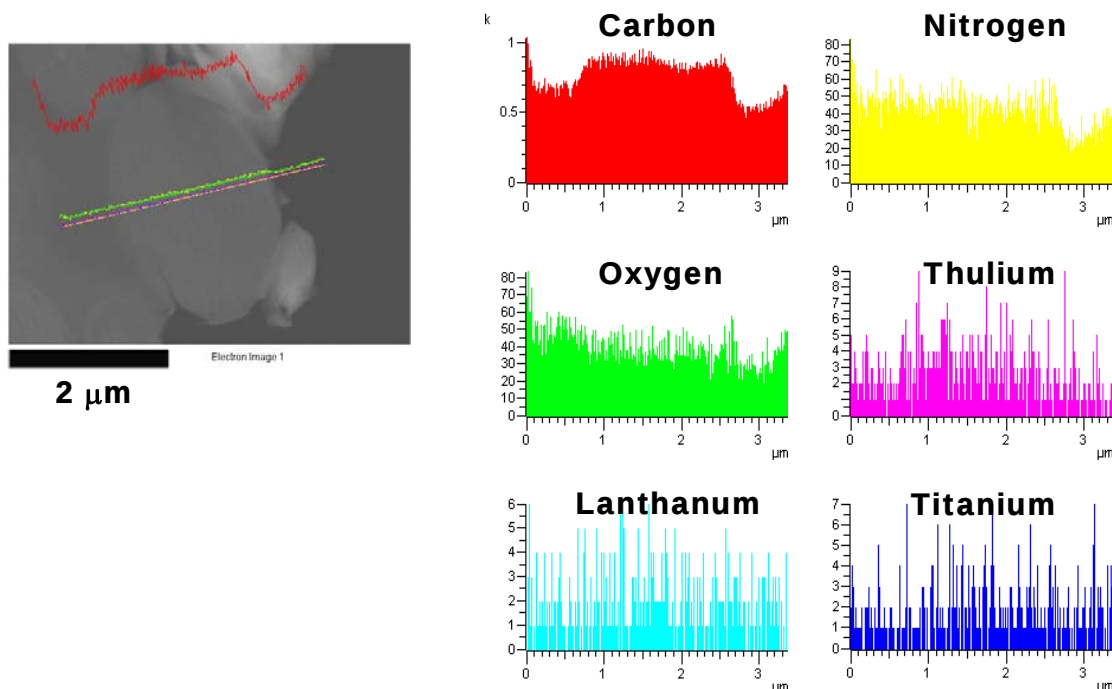


**Figure S9.** Distribution of Tm (A) ungated and (B) gated signal intensity for the particles taken from the polymerization in the presence of 0.25 wt %  $\text{TmCl}_3$  combined with 2.0 wt % of AA based on the weight of St at 14h after initiation.

### **EDX results**

EDX line scans give elemental constituent changes across a region by scanning the electron beam across an interactively defined line. The particle synthesis with adding 2.00 wt % of  $\text{TmCl}_3$  was microtomed with a thickness of 100 nm and the microtomed slices of the particle were used for imaging. The slices of the particle were oblong shape and not spherical due to compression during the microtoming process. However, individual bead slices were clearly visible in the supporting matrix. As shown in **Figure S10**, we could distinguish a slice of the particle by carbon signal since the carbon from the oval shape sample was higher than

the background. However, the X-ray was not sensitive enough to detect the Tm ions in such low concentration in the 100 nm thick slice of the particle.



**Figure S10.** EDX results for a 100 nm section of a particle sample obtained in a synthesis with 2.0 wt % of  $\text{TmCl}_3$  and 2.00 wt % of AA both based on St added in the second stage ( $t = 1$  h), C, O, and N are present in the section, while Tm could not be distinguished from the background compared with La and Ti, which were not present in the particle and serve as a negative control.

## REFERENCES:

- (1) Paine, A. J. *Macromolecules* **1990**, 23, 3109–3117.