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Supporting information

Unravelling the thermo-responsive evolution from single-chain to multiple-chain nanoparticles by thermal field-flow fractionation

Upenyu L. Muza^a, Chelsea D. Williams^b and Albena Lederer^{a,b}*

^aLeibniz-Institut für Polymerforschung Dresden e.V., Center Macromolecular Structure Analysis, Hohe Straße 6, 01069 Dresden, Germany
^bStellenbosch University, Department of Chemistry and Polymer Science, 7602 Matieland, South Africa

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1. Materials and chemicals

The standards as outlined in Table SI1 were purchased from Polymer Source (Canada) and Polymer Standards Service (Germany), and were used as received. No filtering was performed prior to ThFFF injection for analysis.

Table SI.1.	Standards	used
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Sample (g/mol)	Code name	Vendor
PS _{102 000}	PS ₁₀₂	Polymer Standards Service
PS ₁₀₂ 000-PEO ₃₄ 000	PS ₁₀₂ -PEO ₃₄	Polymer Source

99.8+ % Toluene, for analysis (Thermo Scientific, Germany) was used as both solvent and carrier liquid.

2. Instrumentation

2.1 Thermal Field Flow Fractionation (ThFFF)

The TF2000 model (Postnova Analytics, Germany) was used to perform all ThFFF separations. The ThFFF system was coupled to three detectors from (Wyatt Technology Corporation, USA: (1) Dawn Heleos II multiangle light scattering (MALS) with one of the photodiodes performing dynamic light scattering (DLS) detector (2) Viscostar viscosity detector (Visco) and (3) an Optilab T-rEX differential refractive index detector. The operating temperatures for the three respective detectors were 30, 25 and 25 °C.

The channel was 250 microns thick, 2.0 cm wide, 45.6 cm in length from the inlet to the outlet, as composed by clamping a polyimide spacer between two metallic blocks. The top and bottom blocks are the hot and cold walls, respectively. An external chiller at 5 °C was used to maintain the cold wall at sub-room temperatures between 10 - 20 °C, depending on the temperature program in use. The sample loop capacity was 1.0 μ L, and toluene was the sole carrier solvent for all measurements at flow rates between 0.1 - 0.4 mL/ min. The solvent was degassed using PN7520 Solvent Degasser (Postnova Analytics, Germany), prior to being pumped for injection and elution by the PN7140 Pump (Postnova Analytics, Germany).

Differential refractive index increment (dn/dc): Assuming 100% mass recovery, know concentrations were injected into the channel without any focus time or ΔT , and the integral of the resultant peaks was used to back-calculate the dn/dc.

Batch DLS: Using glass cuvettes, the average size distributions were measure from the Zetasizer Nano Series (Nano-ZS, Malvern Panalytical, UK).

2.2 Working principles in ThFFF

Separations in ThFFF proceed inside a microthin channel composed of an upper hot wall, and a lower cold wall, which allows for the existence of a transverse thermal gradient as the separation force-field. The channel is analogous to a column in liquid chromatography, however, no column material is required, which allows for the separation of large and delicate structures such as selfassemblies and aggregates. A carrier solvent is pumped across the channel, and the because of the large aspect therein, a parabolic flow profile is prevalent. The thermal gradient allows for the masstransfer of molecules from the hot to the cold-wall, as measured by their thermal diffusion coefficient (D_T). The accumulation of molecules at the cold plate initiates a counteract masstransfer of molecules by normal diffusion coefficient (D) down a concentration gradient. Smaller molecules have higher rates of D, and thus move away faster from the cold/ accumulation wall. Eventually, an equilibrium is established between D and D_T, whereby, smaller molecules are distributed at higher elevations from the cold wall, and flow dynamics become the most effective aspect of the separation, as expressed in Schematic SI1. The parabolic flow profile along the channel is composed of faster moving streamlines at higher elevations from the cold/ accumulation wall. Notably, D and D_T are sensitive to size and composition, respectively; which explains why separations in ThFFF are characteristically sensitive to both size and composition.^{1,2}



Schematic SI.1. Separation mechanism in ThFFF inside a microthin channel composed of a hot-(red) and called cold-wall (blue), and a carrier solvent flowing within. The injection, separation and elution steps are highlighted.

3. Results and discussion



Figure SI.1. Tyndall scattering effect is observed for PS_{102} -PEO₃₄ and not for PS_{102} , which highlights the presence of colloidal particles in PS_{102} -PEO₃₄.



Figure SI.2. (A) MALS (olive) and dRI (black) signals for the ThFFF separation of PS₁₀₂-PEO₃₄ at Δ T 80 °C, where the outlined demarcations (i-iii) are subpopulations detectible by the MALS detector; (B) Visco signal (red) for the ThFFF separation of PS₁₀₂-PEO₃₄ at Δ T 80 °C.

The hydrodynamics related sizes (R_V and R_H) remain comparable (Figures 9A and -B), which is why the retention times are also comparable. However, there is a drastic surge in the R_G and M_W for the MCNP nanostructures from 0- to 2-weeks equilibration (Figures 9C and -D). This plausibly describes a time-dependent paradigm, whereby, the MCNP nanostructures maintain a constant hydrodynamic size, whilst increasing their degree of aggregation, and thereby becoming more compact. Overall, this reaffirms furthermore the prescribed dynamic nature of the MCNP nanostructures, with equilibrium time being validated as an additional variable.

Ultimately, the tailing of the elution profile diminishes overtime, which highlights the gradual diminishing of larger MCNP nanostructures overtime. Moreover, this also signifies a generic transitioning from broader to narrower distributions in both molecular size and M_W . However, major disparities are not so prevalent about the peak apex (Table 3), where narrower and well-defined distributions are generally observed for both samples as a function of equilibrium time.

Table SI.2. Equilibrium time-dependent ThFFF-triple detection results (over 2 weeks) for PS_{102} -PEO₃₄ MCNP nanostructures in toluene at a ΔT of 30 °C for 1-min elution time, with a programmed linear temperature decay to 0 °C over 80 mins, at a flow of 0.1 mL/ min. The dn/dc value of 0.10 mL/g is used for all M_w calculations. The recorded parameters are at peak apex.

Time (Weeks)	$R_G(nm)$	$R_H(nm)$	$R_v(nm)$	T_R	M_w	Ð
				(min)	(kg/mol)	
0	60.3	46.7	59.5	116.8	16 269	1.00
2	78.6	47.7	61.8	116.0	22 739	1.00



Figure S1.3. Equilibrium time-dependent ThFFF results for PS_{102} -PEO₃₄ MCNP nanostructures at a ΔT of 30 °C for 1-min elution time, with a programmed linear temperature decay to 0 °C over 80 mins, at a flow of 0.1 mL/ min: The parameters are recorded vs. T_R as follows; (A) M_W, (B) R_G, (C) R_V and (D) M_H.

4. References

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