Molecular weight and radius of gyration determined by a MALS detector

The Rayleigh theory describes the relationship between the intensity of the scattered light and the size and molecular weight of the respective scatterer. Using the Rayleigh ratio and the analyte concentration the average molecular weight can be determined with the Zimm equation (1).

$$\frac{R_\theta}{Kc} = \frac{P_\theta M_W}{1+2A_2cM_w} \quad (1)$$

with: θ: Scattering angle; R_θ: Rayleigh ratio; c: Concentration of the analyte; A_2: Second virial coefficient

K is a more complex term defined as (2):

$$K = \frac{4\pi^2}{\lambda_0^4 N_A}(n_0 \frac{dn}{dc})^2 \quad (2)$$

with: n_0: refractive index of the solvent; dn/dc: refractive index increment of the analyte in solvent: λ_0: wavelength of the light; N_A: Avogadro's number

P(θ) is the factor describing the angular dependence of the scattered light, which can be described as (3):

$$\frac{1}{P_\theta} = 1 + \frac{16\pi^2 n_0^2 R_g^2}{3\lambda_0^2} sin^2(\frac{\theta}{2}) \quad (3)$$

with: R_g: radius of gyration

Normally, the Zimm equation (1) is solved by a Debye plot ($\frac{R_\theta}{Kc}$ vs $sin^2\frac{\theta}{2}$)

The y – intercept of the plot (which equates to an angle of 0°) is M_W and the radius of gyration can be obtained from the initial slope of the line (4):

$$\frac{dy}{dx} = \frac{16\pi^2 n_0^2 R_g^2}{3\lambda_0^2} \quad (4)$$

The Debye plot of the peak at 40 min (Figure 3) is shown below.

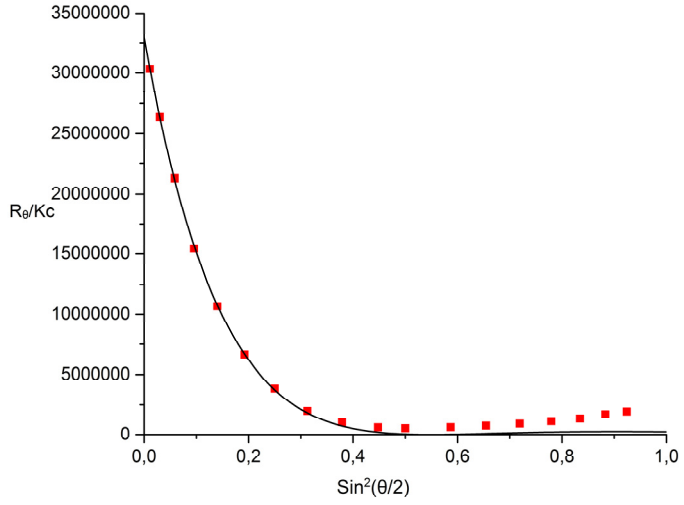

Figure S1. Debye plot (sphere fit, angles from 12° to 148°) for the peak at 40 min shown in Figure 3.

Reproducibility of measurements

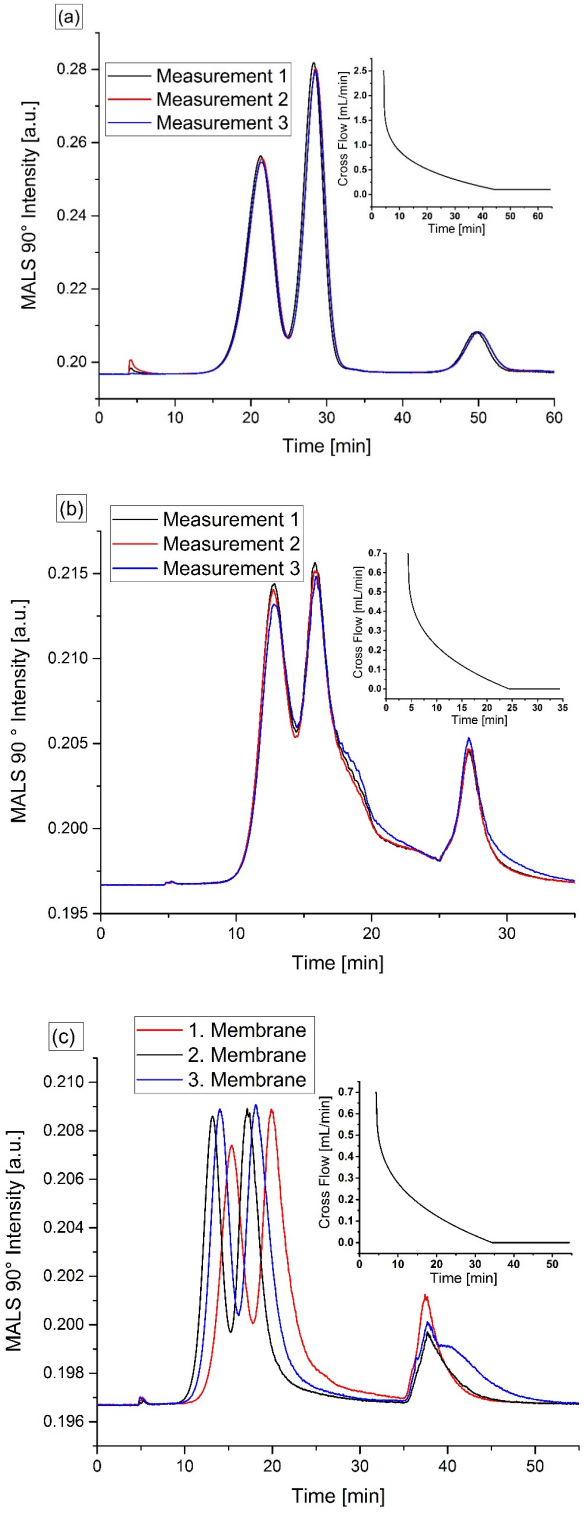

Figure S2. Triplicate measurements using a 10 kDa PES membrane and a conventional (a, PS NP mixture 3, cross flow 2.5 mL min^{-1}, injection time 4 min, elution exponent 0.2, total elution time 60 min) and miniaturized channel (b, PS NP mixture 7, cross flow 0.7 mL min^{-1}, injection time 4 min, elution exponent 0.3, total elution time 30 min), and repeat measurements performed with three different 10 kDa PES membranes in a miniaturized channel (c, PS NP mixture 7, cross flow 0.7 mL min^{-1}, injection time 4 min, elution exponent 0.3, total elution time 30 min), MALS 90° detector

Table S1: Comparison of chromatographic parameters of PS NP mixture 7 separation using 3 different 10 kDa PES membranes in a miniaturized channel

10 kDa PES Membrane	Retention time $t_{R50\ nm}$	Retention ration $R_{50\ nm}$	Plate number $N_{50\ nm}$	Resolution R_S	Recovery rate $R_{e\ 50\ nm}$ %
1	15.4	0.34	98.0	0.73	57.2
2	13.2	0.39	100.8	0.74	64.5
3	14.0	0.37	107.9	0.69	68.4

System calibration and normalization with NIST gold nanoparticles

AF4-UV-Vis-MALS system calibration was performed with certified NIST 10, 30 and 60 nm gold nanoparticles. An optimal behavior was detected as a linear calibration curve was obtained as shown in Figure S3.

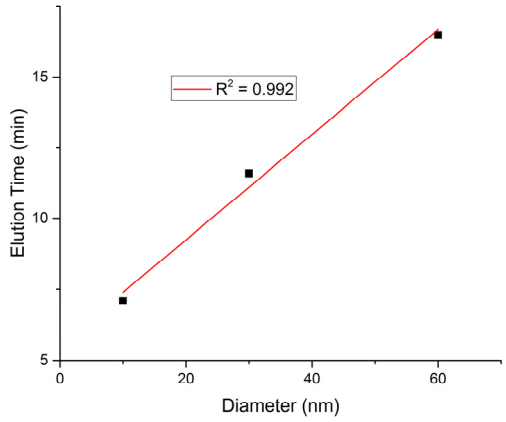

Figure S3. Calibration curve obtained using NIST gold nanoparticles

Fractogram of LOD and LOQ determination using 10 nm gold nanoparticles with different concentrations

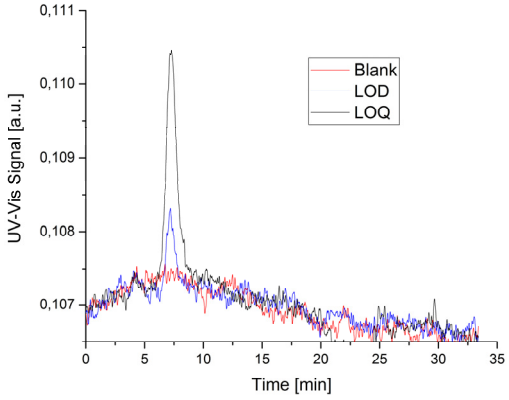

Figure S4. Fractograms of 10 nm gold nanoparticles with different particle concentrations in a miniaturized channel

For the determination of LOD and LOQ the concentration of gold nanoparticles was reduced stepwise and used for separation with the standard method. The concentrations at which the *S*/*N* ratios

equal to 3 and 10, respectively, were defined as LOD and LOQ. In this case, LOD for 10 nm gold nanoparticles is 0.2 μg L^{-1} (2×10^{10} Particle L^{-1}) and LOQ is 0.7 μg L^{-1} (7×10^{10} Particle L^{-1}).