Supporting information

Classification and characterization of multimodal nanoparticle size distributions by size-exclusion chromatography

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SI1. Determination of the pore size distribution of the stationary phase



Figure S1: Hg intrusion and extrusion curves of the column material (a). Calculated cumulative pore volume distribution of the column material (b). Since SEC takes place in the pore volume, the interparticle volume distribution is not shown in the diagram.

SI2. Chromatograms of silver and silica nanoparticles



Figure S2: Normalized chromatograms of silver (a) and silica (b) nanoparticles. Chromatograms were measured at an ionic strength of 10 mM and at 450 nm for silver nanoparticles and at 260 nm for silica nanoparticles.

SI3. Characterization of nanoparticles by DLS

Before chromatographic experiments, all nine gold and seven silver standard dispersions after surface modification were characterized by DLS. In Table S1, Table S2 and Table S3, the modal values $x_{3,mod}$ are summarized for gold, silver and silica nanoparticles, respectively. Each value is presented as the mean value of triplicate

measurements. Zeta potentials of all gold and silver dispersions from five consecutive measurements are shown in Figure S3.

Gold		5 nm	10 nm	15 nm	20 nm	30 nm	40 nm	50 nm	60 nm	80 nm
X _{3,mod}	/	9.0 ±	13.1 ±	16.5 ±	22.3 ±	32.0 ±	44.3 ±	52.9 ±	61.2 ±	89.9 ±
nm		1.3	0.0	0.3	0.4	0.1	0.8	0.3	0.9	1.5

Table S1: Modal nanoparticle sizes for all gold standard dispersions measured by DLS

Table S2: Modal nanoparticle sizes of all silver standard dispersions measured by DLS

Silver	10 nm	1	20 nm	١	30 nm	ו	40 nm	۱	50 nm	١	60 nm	١	80 nm	l
x _{3,mod} / nm	11.7	±	22.7	±	34.0	±	43.9	±	48.5	±	61.9	±	75.4	±
	1.8		0.8		0.8		1.2		1.0		1.4		1.1	

Table S3: Modal nanoparticle sizes of all silica standard dispersions measured by DLS

SiO ₂	10 nm	50 nm
x _{3,mod} / nm	9.6 ± 0.3	40.2 ± 0.7



Figure S3: Zeta potentials of MUA-stabilized gold (a) and silver (b) nanoparticles. Error bars indicate the standard deviation of five consecutive measurements.

SI4. Chromatograms of gold standards at different ionic strengths



Figure S4: Overlaid chromatograms of single gold nanoparticle standards (5 nm - 80 nm) with a 2.5 mM (a) and 5 mM (b) ammonium acetate solution as mobile phase.

SI5. Generalization of calibration curves

Since electrostatic repulsive forces between the nanoparticles and the stationary phase material arise due to equal charges, retention volumes increase with increasing ionic strength of the mobile phase. In order to evaluate the influence of the double layer thickness on the retention behavior of nanoparticles, we generalized the calibration curves of gold nanoparticles by taking into account the reduction in Debye lengths at different ionic strengths. Starting from Figure 2(a), we first calculated the calibration points as a function of the partition coefficient K_{SEC} by rearranging eq. (2):

$$K_{SEC} = \frac{V_R - V_0}{V_t - V_0}$$

The total liquid volume inside the column V_t was determined to be 12.4 ml by injecting 0.5 μ l toluene using 96 % Heptane and 4 % isopropanol as mobile phase at a flow rate of 1 ml/min and 25 °C. The interstitial volume V₀ = 6.1 ml was determined by injecting 20 μ l of a gold dispersion with 100 nm particle size using 2.5 mM ammonium acetate as mobile phase at a flow rate of 0.5 ml/min and 25 °C. The resulting calibration points as a function of K_{SEC} are shown in Figure S5.



Figure S5: Calibration points as a function of the partition coefficient K_{SEC} *for different ionic strengths* Since K_{SEC} describes the pore volume fraction that is accessible for a certain nanoparticle size, the smallest penetrable pore diameter can be determined from Figure S1 (b) at the respective 1- K_{SEC} value of a given hydrodynamic diameter. The accessible pore diameters are plotted in Figure S6 (a) as a function of hydrodynamic diameters and ionic strengths.



Figure S6: Accessible pore diameter as a function of hydrodynamic diameter and ionic strength (a). Effective pore diameters corrected by four times the Debye length (b) In order to take the influence of the electrostatic double layer into account, the Debye length $1/\kappa$ is calculated by eq. (10) for $\varepsilon_r = 80$ and T = 298.15 K. For ionic strengths of 2.5 mM, 5 mM and 10 mM the Debye lengths are 6.1 nm, 4.3 nm and 3.0 nm, respectively. Since both the nanoparticles and the stationary phase material are

negatively charged, four times the Debye length is subtracted from the accessible pore diameters resulting in the effective pore diameters given in Figure S6 (b). With the aid of the cumulative pore volume distribution, the effective pore diameters are transferred to the generalized calibration points as shown in Figure S7. By eq. (2), the respective retention volumes are then calculated (see Figure 2 (b)).



Figure S7: Generalized calibration points in consideration of Debye lengths at different ionic strengths plotted against K_{SEC} values

The interactions between nanoparticles and the stationary phase material were calculated according to DLVO theory by summation of attractive vdW and the electric double layer repulsion. Since the diameter of the stationary particles is large compared to the size of the nanoparticles, a sphere-plate configuration is considered. The DLVO interaction potential φ_{DLVO} is given by¹:

 $\varphi_{DLVO} = \varphi_{vdW} + \varphi_{EDL}$

$$\varphi_{DLVO} = -\frac{A_{132}R}{6D} + RZe^{-\kappa D}$$

where A_{132} describes the Hamaker constant for gold (1) interacting with silica (2) across water as the surrounding medium (2). It is approximated from the Hamaker constants of gold ($A_{11} = 41.1^{*}10^{-20}$ J)², silica ($A_{22} = 6.5^{*}10^{-20}$ J)¹ and water ($A_{33} = 3.7^{*}10^{-20}$ J)¹ by:

$$A_{132} = \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right) \left(\sqrt{A_{22}} - \sqrt{A_{33}}\right)$$

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Moreover, R denotes the nanoparticle radius, D the distance between nanoparticle and the stationary phase and Z the interaction constant:

$$Z = 64\pi\varepsilon_0\varepsilon_r \left(\frac{k_BT}{e}\right)^2 tanh^2 \left(\frac{e\psi_0}{4k_BT}\right)$$

with e as the elementary charge and Ψ_0 as the surface potential which is assumed as the mean value of the nanoparticle zeta potentials given in Figure S3 (a) and the zeta potential of the stationary phase particles. The maxima of DLVO potentials $\Psi_{DLVO,max}$ normalized by the thermal energy k_BT are shown in Figure S8 as a function of nanoparticle size and ionic strength of the mobile phase.



Figure S8: Maxima of DLVO potentials $\Psi_{DLVO,max}$ normalized by the thermal energy k_BT as a function of ionic strength and particle size



SI6: PSDs of gold standards determined by SEC and AUC

Figure S9: Extinction weighted particle size distributions of gold nanoparticle standards (15 nm - 60 nm) determined by SEC (black lines) and AUC (red lines)

SI7. Relative masses for classification experiments as well as chromatograms

and PSDs of coarse and fine fractions for STs 2 and 3

Table S4: Relative masses of coarse and fine fractions for classification experiments at three STs determined from peak areas of both fractions in the chromatogram

	A _{coarse} / mAU*ml	A _{fine} / mAU*ml	m _g / -	m _f / -
ST1	33.69	65.84	0.34	0.66
ST2	51.92	47.69	0.52	0.48
ST3	66.94	32.73	0.67	0.33



Figure S10: Chromatograms of coarse and fine fraction after classification for STs 2 (a) and 3 (b)



Figure S11: Particle size distributions and separation efficiency curves for STs 2 (a) and 3 (b)

SI8. Relative deviations in q_{ext} for all STs



Figure S12: Calculated relative deviations in q_{ext} for all STs. Relative deviations were calculated as the ratio of the deviation f(x) to the PSD of the initial mixture q_{F} . The dotted lines represent a deviation of ± 15 %.

References

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