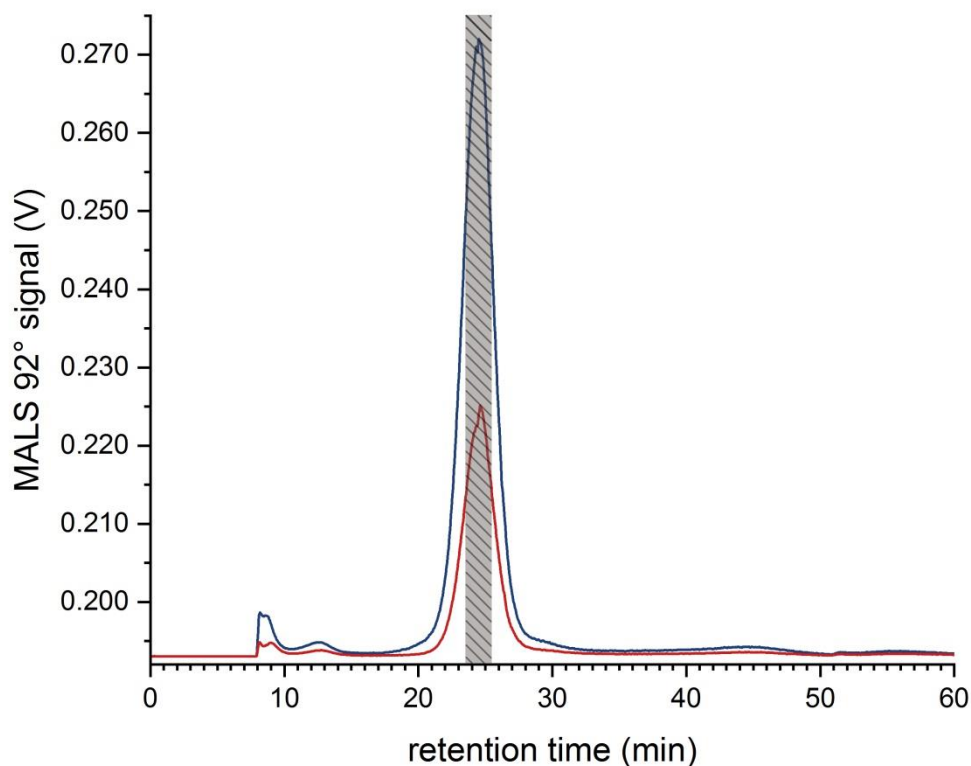
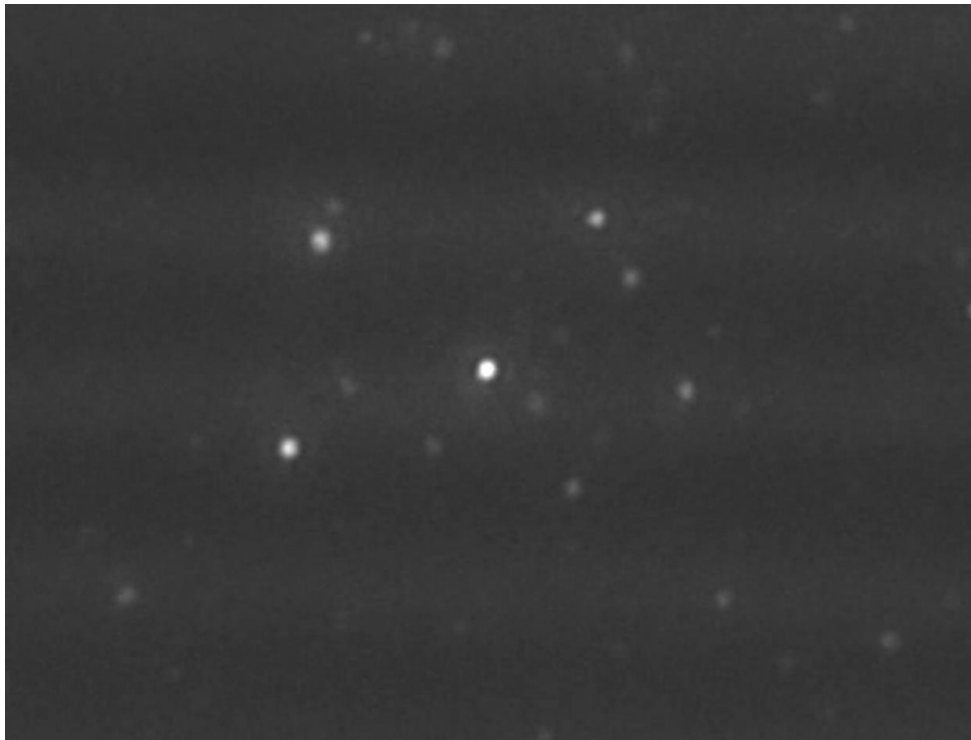


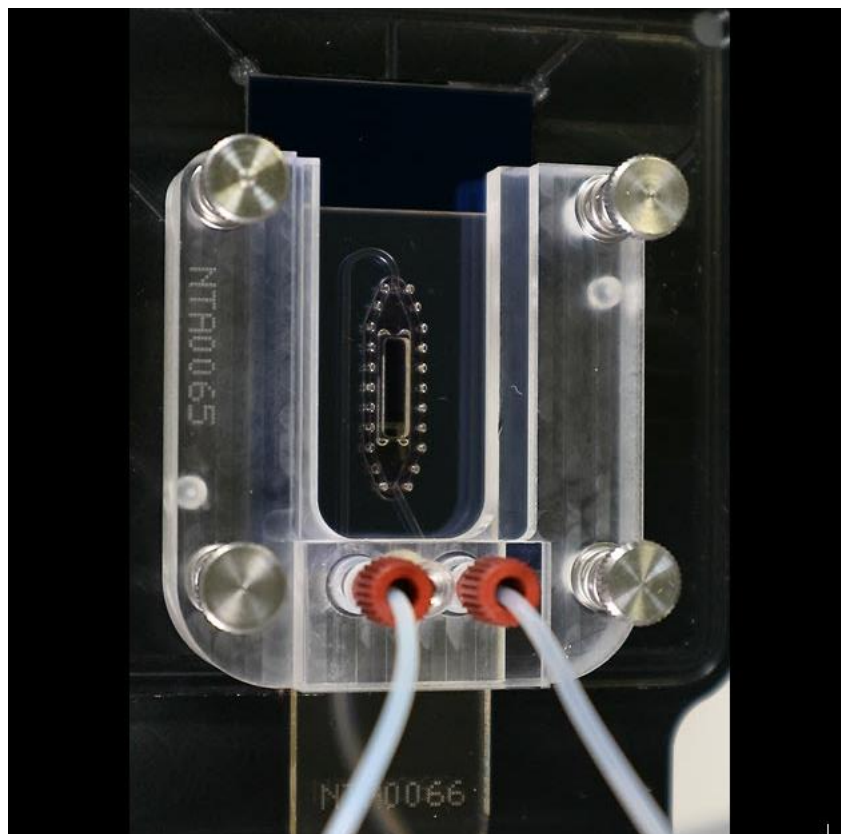
**Figure S1.** EAF4 fractionation principle with the parabolic flow profile across the channel and the illustration of the slot outlet principle in order to remove parts of the sample free channel flow.



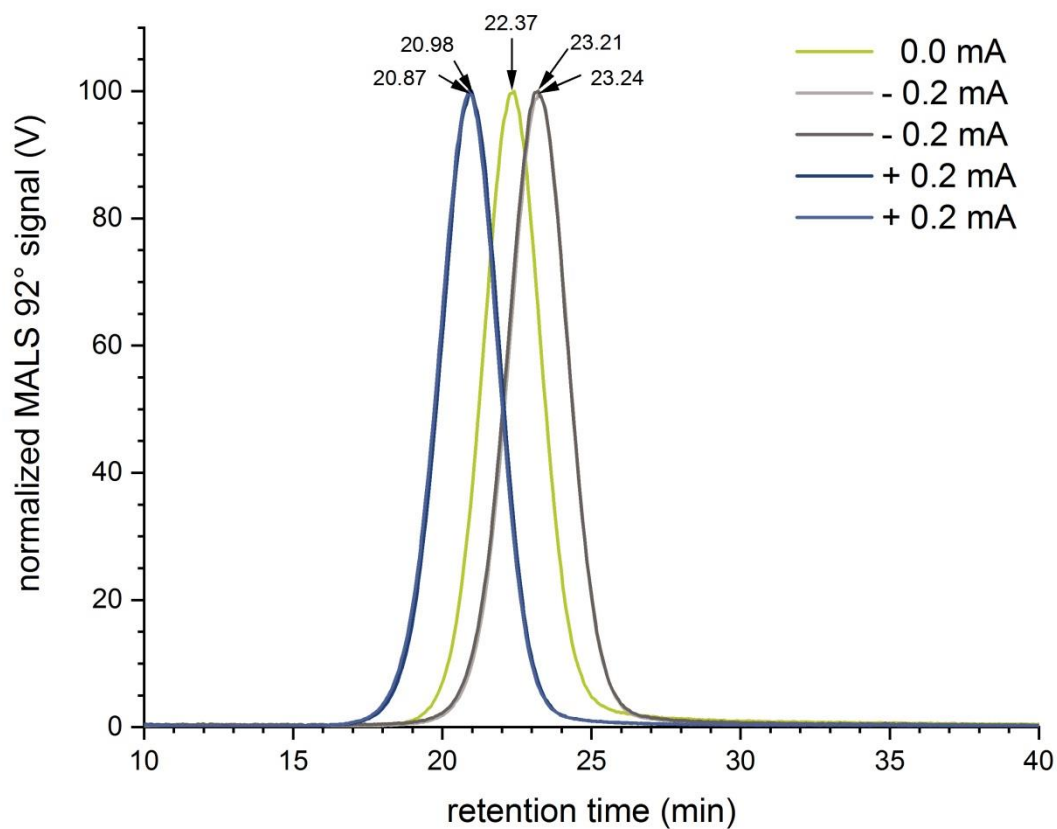
**Figure S2.** EAF4-MALS fractograms of the PS100-DMEM sample ( $V_{inj} = 10 \mu\text{L}$  (red line) and  $V_{inj} = 25 \mu\text{L}$  (blue line)) with the highlighted region (gray-crosshatched area) between 23.5 min and 25.5 min, which was collected by a fraction collector and analyzed offline by NTA. For the PS100 sample the region was changed accordingly to 21.3 min to 23.3 min due to the differences in retention times.



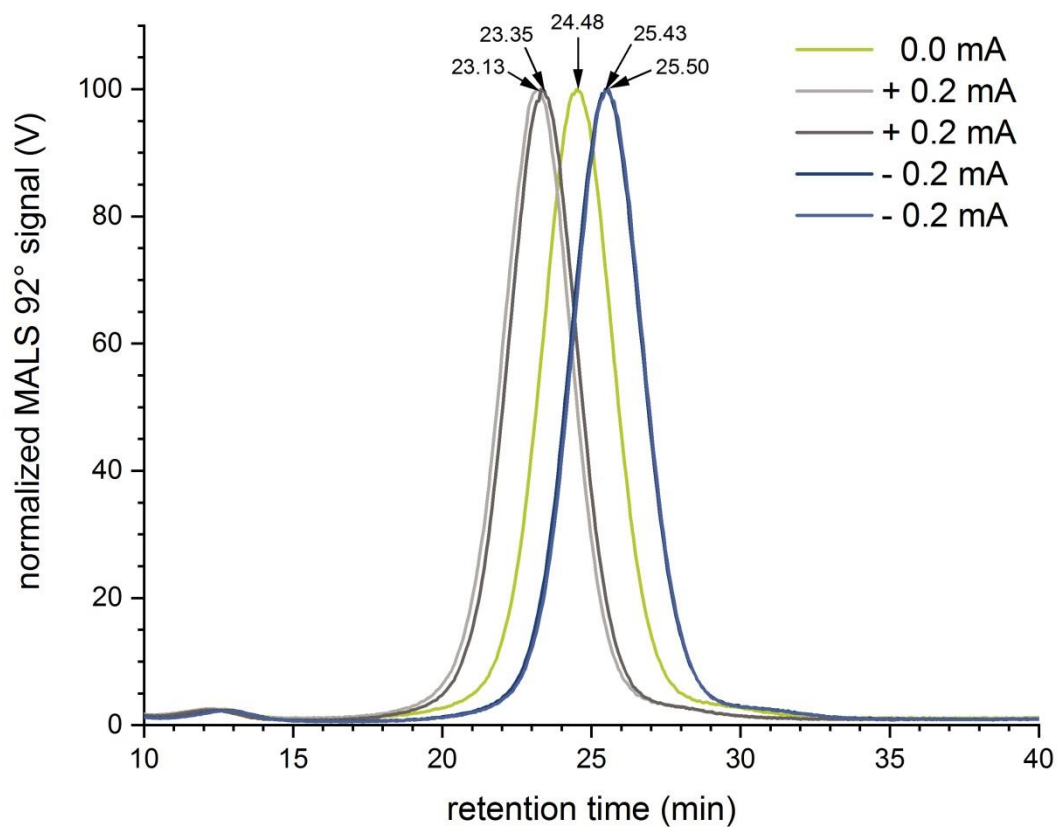
**Figure S3.** Snapshot taken from NTA video of blank DMEM cell culture medium.



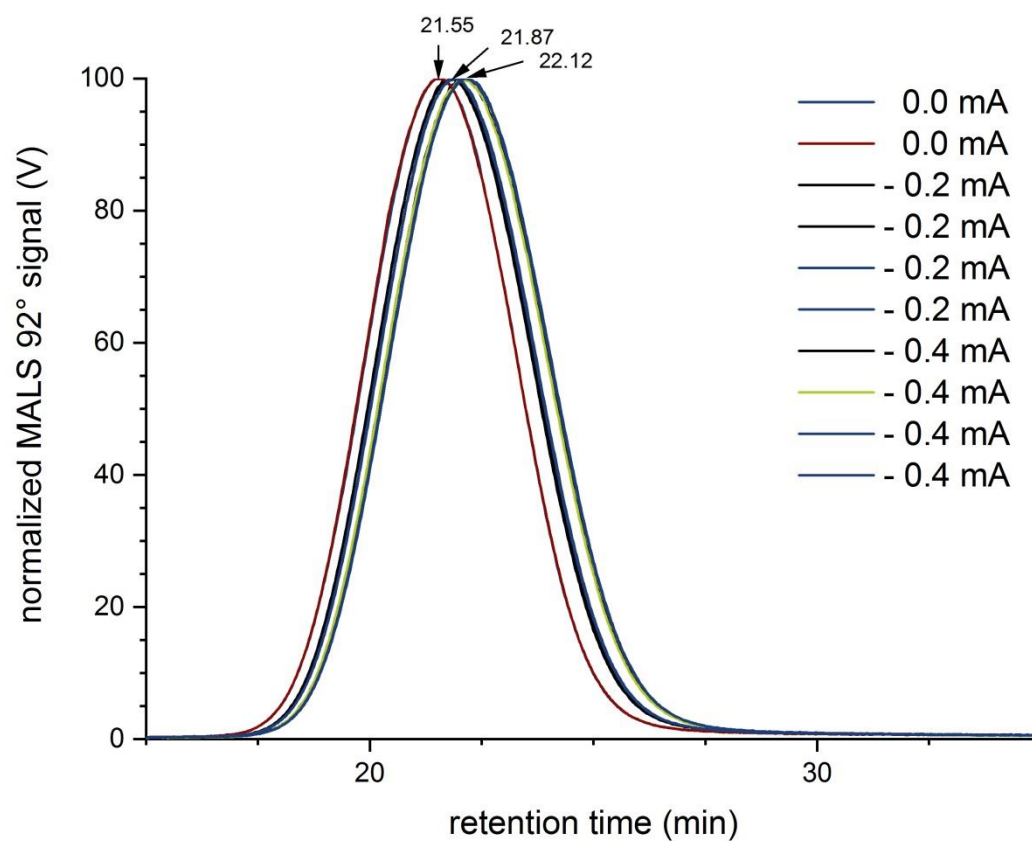
**Figure S4.** LVFC of NTA with the inlet connected to the “short” end, right port.



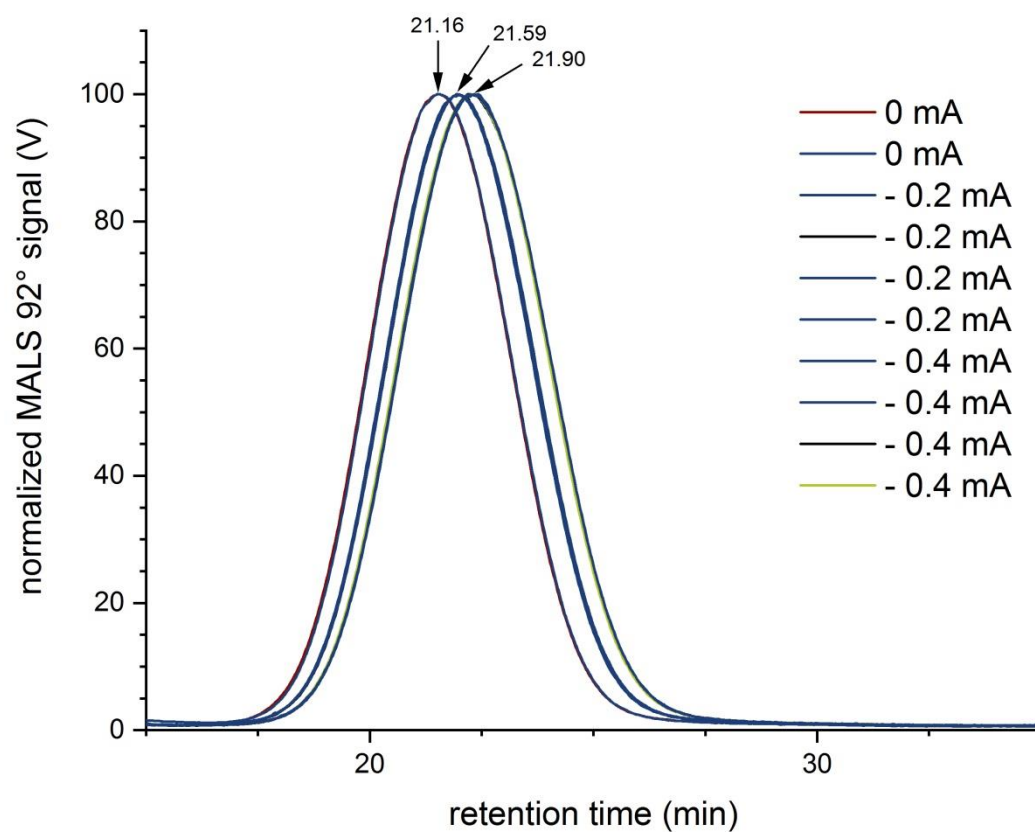
**Figure S5.** EAF4-MALS fractograms displaying the measurements performed at different electrical fields and the induced shifts in retention time of the PS100 sample. The green MALS signal represents the measurement without electrical field (0.0 mA). The measurements with a positive top electrode shifted to smaller retention times (positive signs), in contrast to measurements with a switched polarity (negative sign).



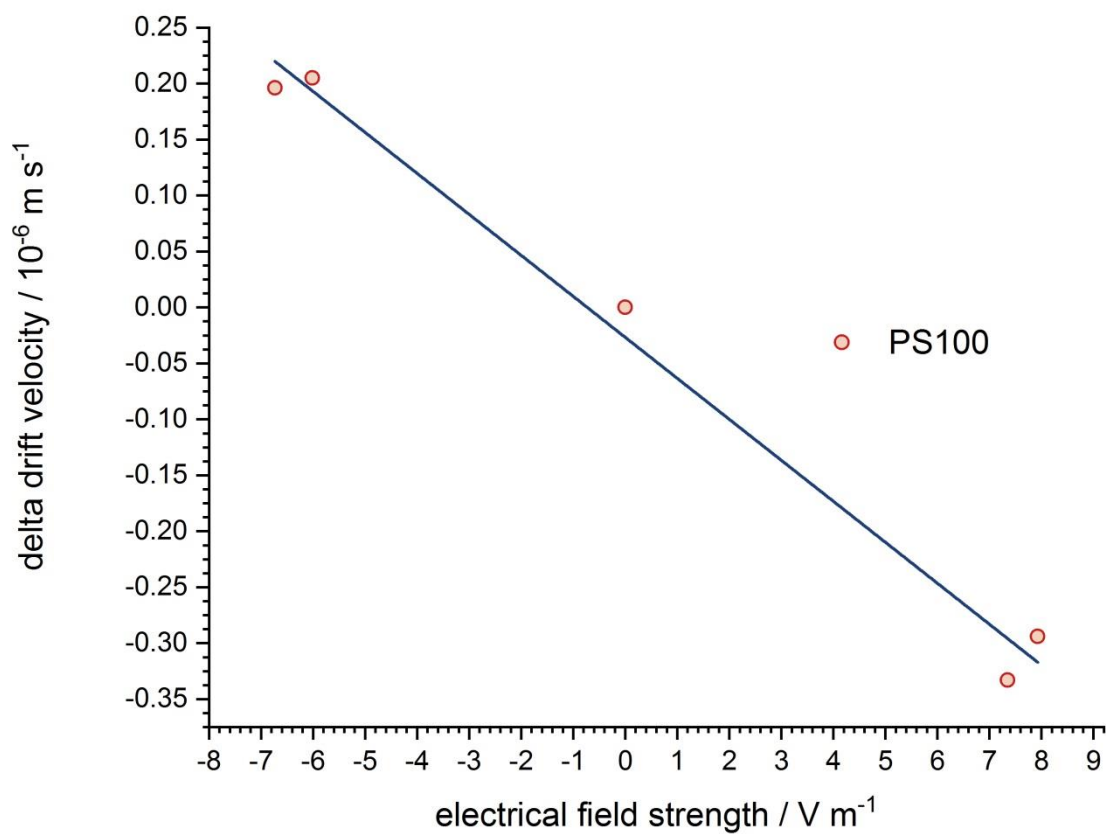
**Figure S6.** EAF4-MALS fractograms displaying the measurements performed at different electrical fields and the induced shifts in retention time of the PS100-DMEM sample. The green MALS signal represents the measurement without electrical field (0.0 mA). The measurements with a positive top electrode shifted to smaller retention times (positive signs), in contrast to measurements with a switched polarity (negative sign).



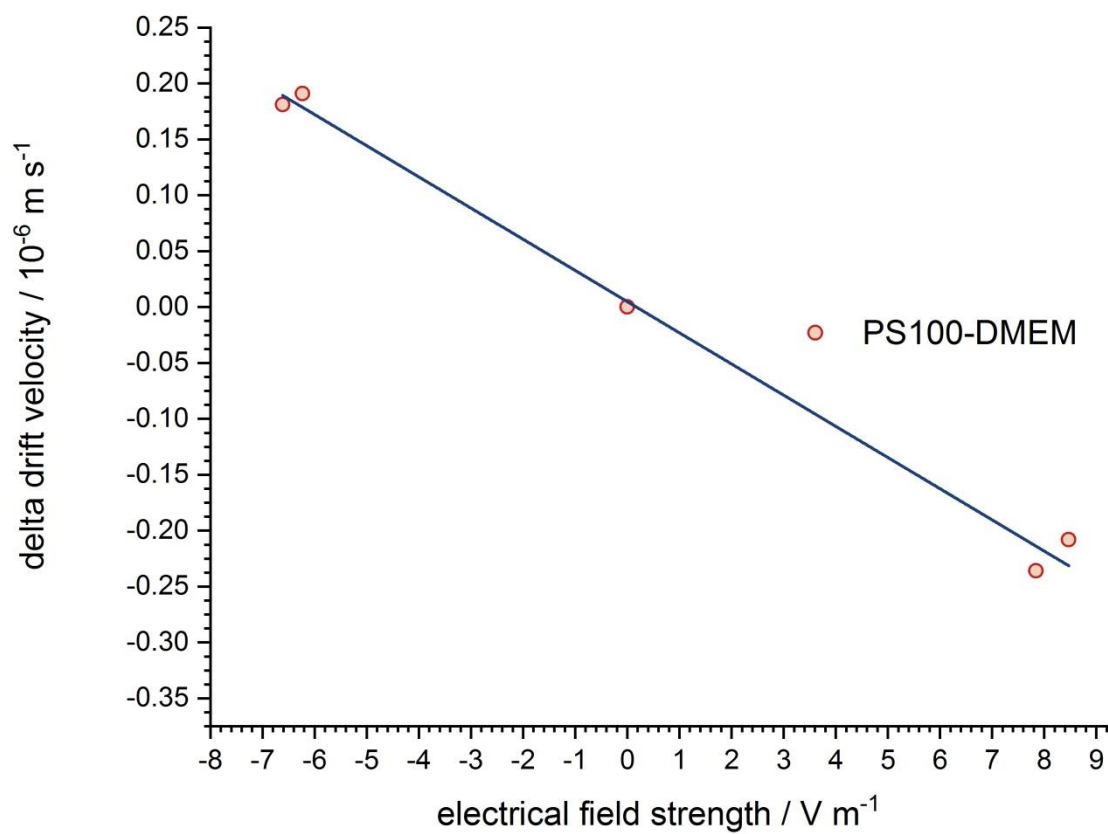
**Figure S7.** EAF4-MALS fractograms displaying the measurements performed at different electrical fields and the induced shifts in retention time of the liposomal Doxorubicin HCl sample. The MALS signals with 0.0 mA represent the measurements without an electrical field. The signals of measurements with a negative top electrode shifted to larger retention times (negative signs).



**Figure S8.** EAF4-MALS fractograms displaying the measurements performed at different electrical fields and the induced shifts in retention time of the liposomal Doxorubicin-DMEM sample. The MALS signals with 0.0 mA represent the measurements without an electrical field. The signals of measurements with a negative top electrode shifted to larger retention times (negative signs).

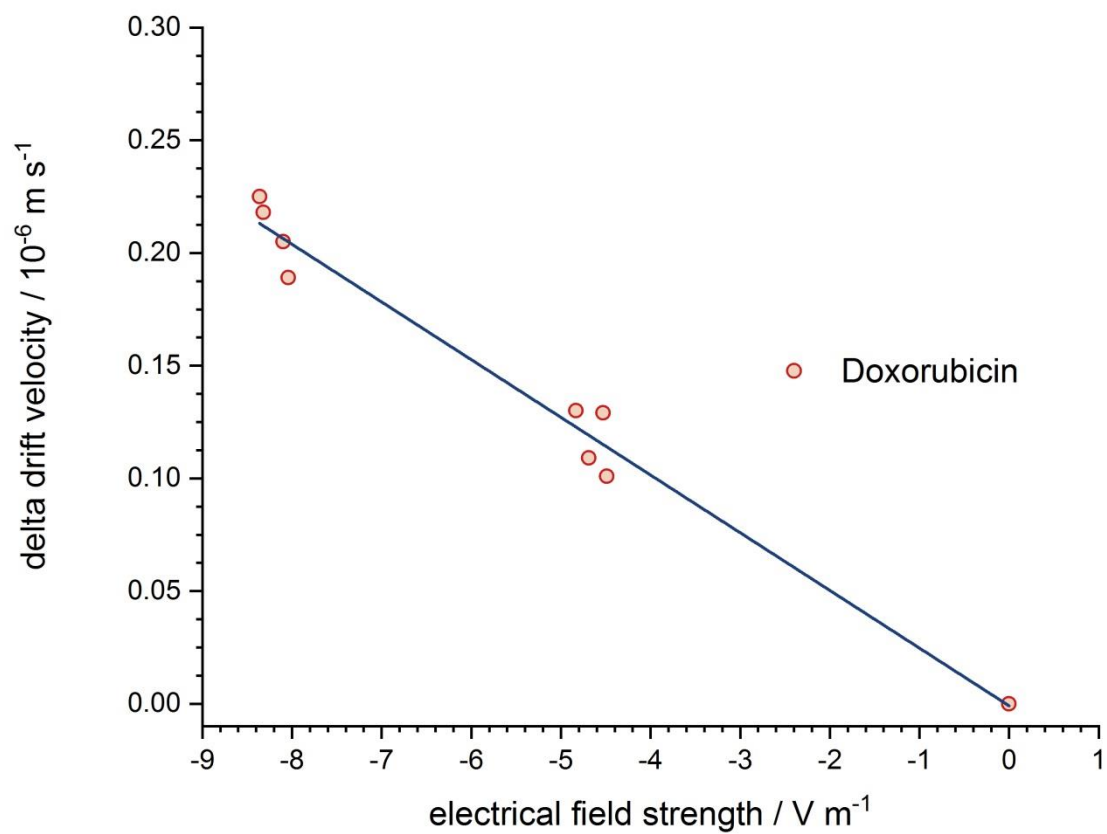


**Figure S9.** Plot of drift velocity against electrical field strength for PS100 beads in UPW with the linear curve obtained from a linear least squares analysis ( $R^2 = 0.9876$ ) yielding an electrophoretic mobility of  $-3.66 \text{ E-}8 \text{ m}^2\text{V}^{-1}\text{s}^{-1} \pm 0.24 \text{ E-}8 \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ .

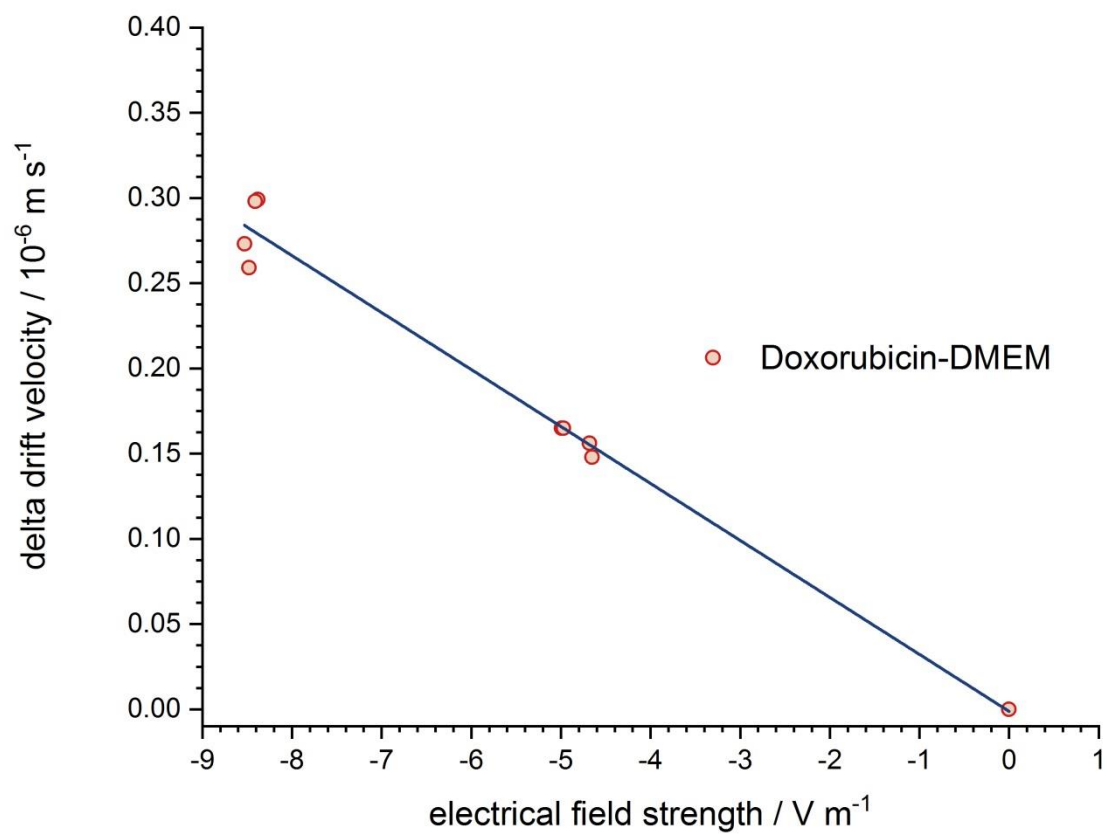


**Figure S10.** Plot of drift velocity versus electrical field strength for PS100 beads in DMEM medium with the linear curve obtained from a linear least squares analysis ( $R^2 = 0.9927$ ) yielding an electrophoretic mobility of  $-2.82 \text{ E-}8 \text{ m}^2\text{V}^{-1}\text{s}^{-1} \pm 0.14 \text{ E-}8 \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ .

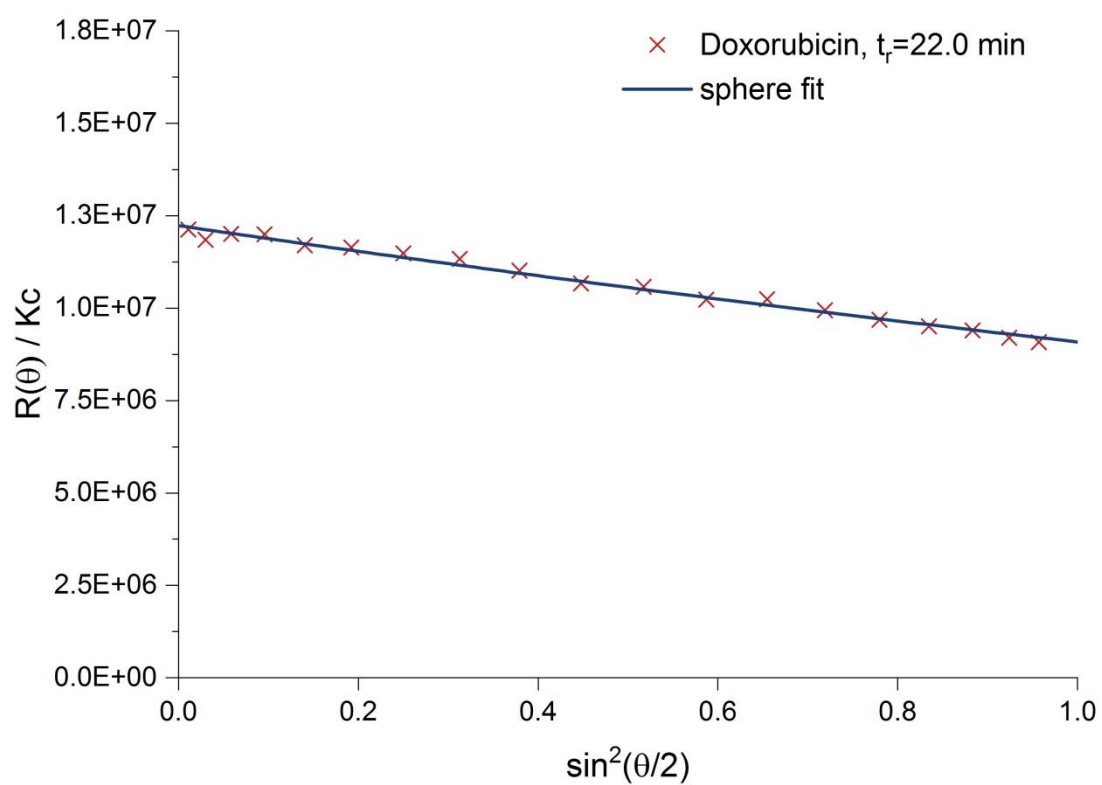




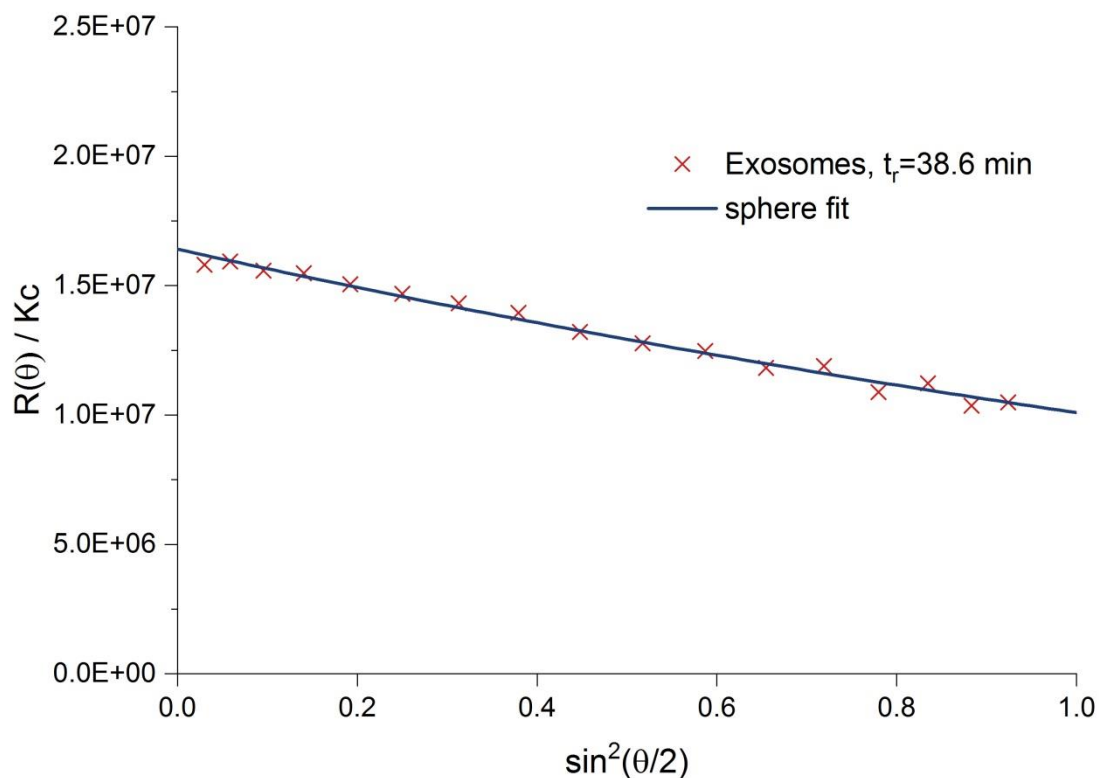
**Figure S11.** Plot of drift velocity versus electrical field strength for liposomal Doxorubicin HCl in the respective carrier solution with the linear curve obtained from a linear least squares analysis ( $R^2 = 0.9856$ ) yielding the electrophoretic mobility.



**Figure S12.** Plot of drift velocity versus electrical field strength for liposomal Doxorubicin in DMEM medium with the linear curve obtained from a linear least squares analysis ( $R^2 = 0.9916$ ) yielding the electrophoretic mobility.



**Figure S13.** Angular dependent light scattering plot at a retention time ( $t_r$ ) of 22 min visualizing the obtained scattering intensities at 19 different angles and the spherical fit for the liposomal Doxorubicin HCl sample.



**Figure S14.** Angular dependent light scattering plot at a retention time ( $t_r$ ) of 38.6 min visualizing the obtained scattering intensities at 17 different angles and the spherical fit for the exosome sample.

**Table 1.** Summary of the applied EAF4 conditions and cross flow rates  $V_x$  for the separation of all investigated samples.

<b>Fractionation Method A</b>			
Injection flow rate (mL/min)	0.08		
Channel flow rate (mL/min)	0.5		
Transition time (min)	0.2		
	duration (min)	$V_x$ (mL/min)	$V_x$ mode
Focusing + injection	7	1	constant
Elution	1	1	constant
	40	1	power decay, exp: 0.2
	2	0.1	constant
Rinse	10	0	constant
<b>Fractionation method B</b>			
Injection flow rate (mL/min)	0.08		
Channel flow rate (mL/min)	0.5		
Transition time (min)	0.2		
	duration (min)	$V_x$ (mL/min)	$V_x$ mode
Focusing + injection	7	1	constant
Elution	30	1	linear
Rinse	10	0	constant
<b>Fractionation method C</b>			
Injection flow rate (mL/min)	0.2		
Channel flow rate (mL/min)	0.5		
Transition time (min)	0.2		

	duration (min)	V <sub>x</sub> (mL/min)	V <sub>x</sub> mode
Focusing + injection	6	1	constant
Elution	25	1	linear
	5	0.28	power decay, exp: 0.8
	5	0.15	power decay, exp: 0.8
	5	0.08	power decay, exp: 0.9
	30	0.05	constant
Rinse	20	0	constant