Supplementary information

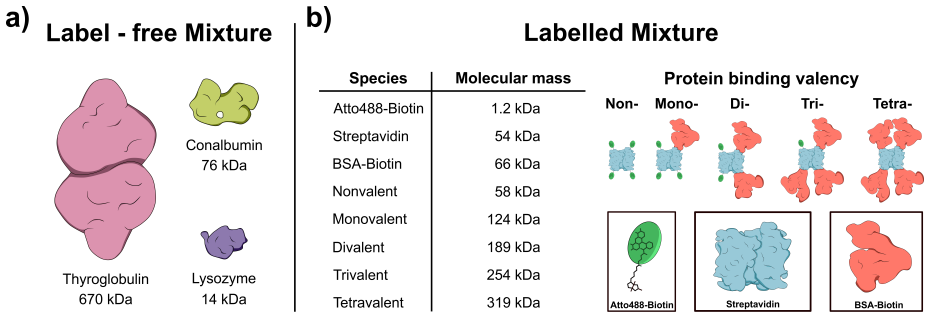

Figure S1: Composition of molecule mixtures used for label-free and Atto488-labeled in-line size exclusion chromatography-electrodiffusion measurements. a) Proteins used for label-free detection were thyroglobulin (bovine), conalbumin (chicken) and lysozyme (chicken). b) For the labeled mixture, biotinylated Atto488, streptavidin and biotinylated bovine serum albumin (BSA) were mixed. The three components form a range of labeled and unlabeled components with different stoichiometry, varying in size from 1.2 kDa to 320 kDa.

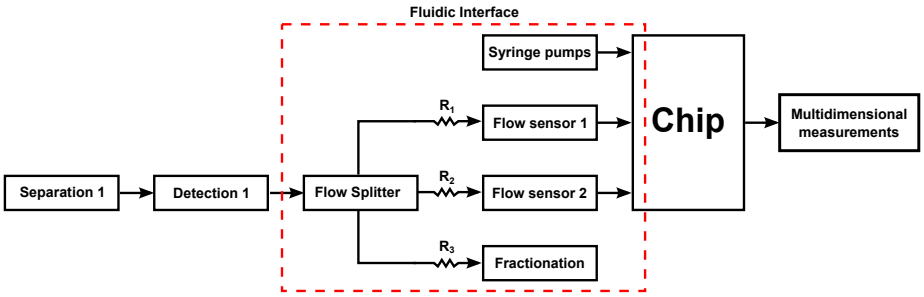

Figure S2: The flow adapter interface contains a 4-way fluidic channel with calibrated PEEK capillaries acting as hydrodynamic resistors. The ratio of the capillary hydrodynamic resistance determines the fluidic adapter flow splitting ratio, which can be monitored with flow sensors.

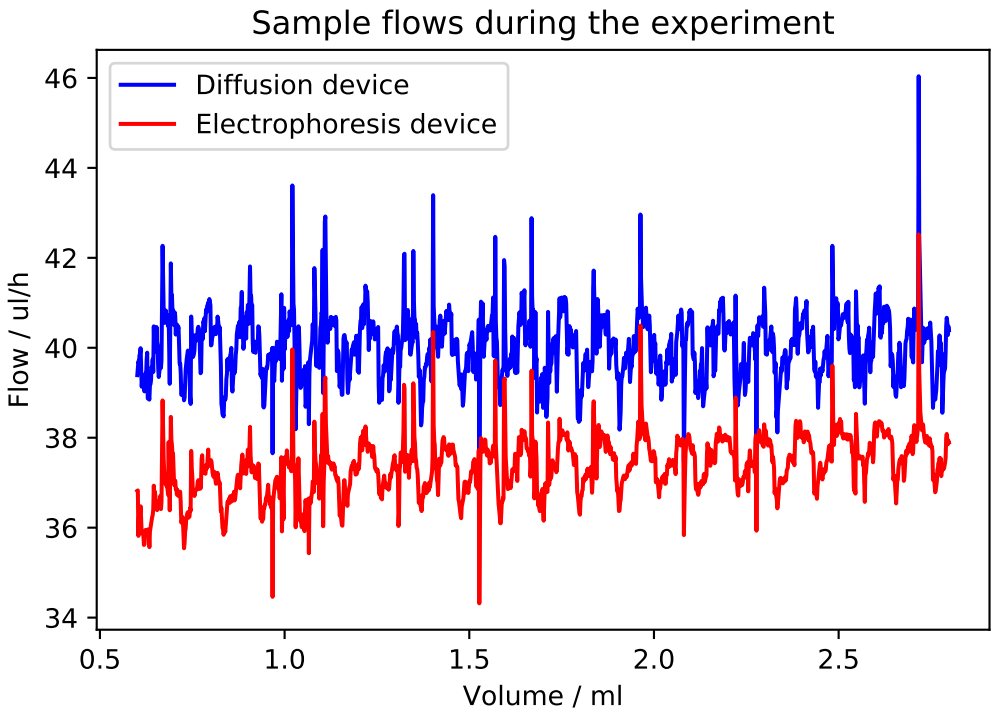

Figure S3: The flow rates at the diffusional sizing and the electrophoresis device remains constant to few percent variation throughout experiments.

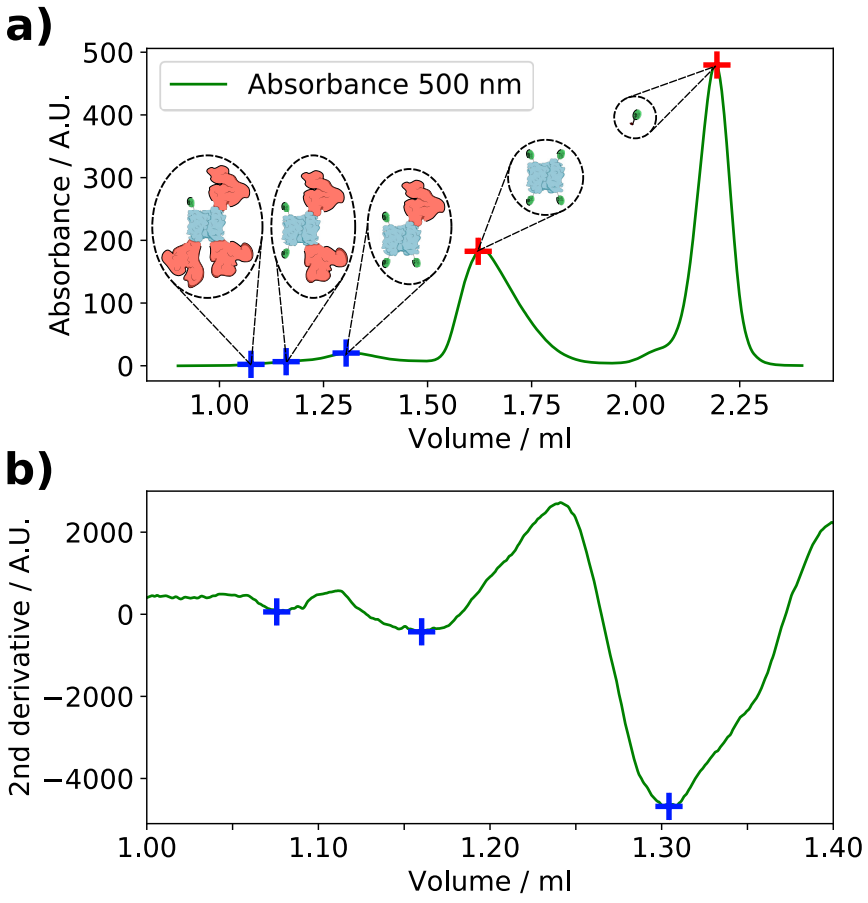

Figure S4: a) Streptavidin, BSA and Atto488 dye complexes absorbance at 500 nm just after the LC separation at pH 8.2. Three distinct regions in the spectra are identified representing the 5 different labelled molecular complexes. b) second derivative of the spectrum between 1-1.4ml reveals the 3 most significant sub-peaks.

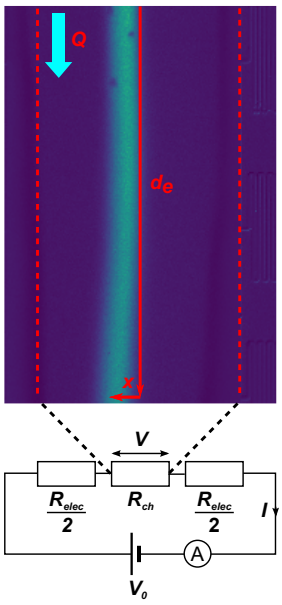

Figure S5: Representation of the electrophoresis device equivalent electronic circuit. During the calibration step, the chamber electric resistance can be neglected, allowing R_{elec} estimation. Due to a high electrode resistance of $R_{elec} \sim 250$ kΩ, the voltage drop was 5% across the main electrophoresis chamber.

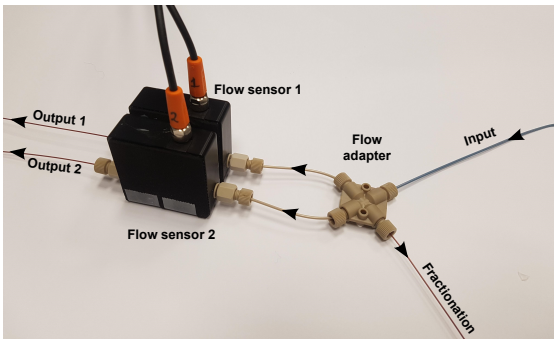

Figure S6: Microfluidic flow adapter matching the flow between liquid chromatography and microfluidic chip over two orders of magnitude. The flow is split between the fractionation outlet and outputs A and B; the flow through the outlets A and B is monitored with flow sensors.

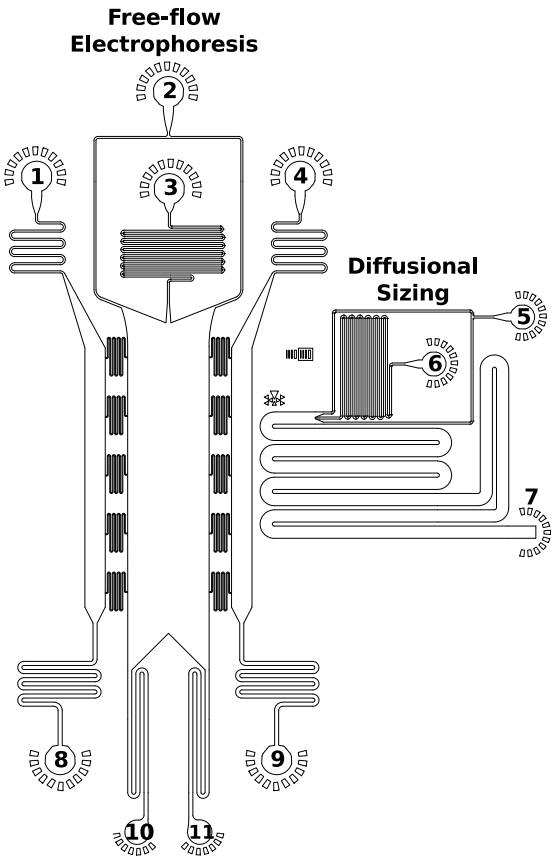

Figure S7: Microfluidic chip design containing two functional blocks: diffusional sizing and free-flow electrophoresis. The device has 11 ports. Port 1 and 4 are electrolyte inlets, 2 and 5 are buffer inlets for the co-flow, 3 and 6 are sample inlets and 7-11 are outlets with 8 and 9 connected to hollow stainless steel electrodes.

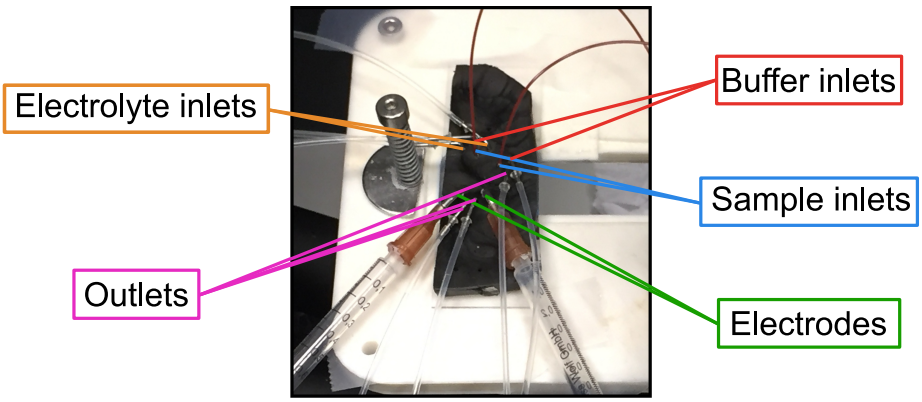

Figure S8: Top view of a black PDMS chip on microscope stage connected to sample, buffer and electrolyte supply and outlet tubing.