Supplementary Information

Calibration curves for the conversion of conductivities into fractions of feed buffer concentration remaining in the retentate

The conversion of conductivities measured by an online sensor in the retentate into residues of the original feed buffer after diafiltration was conducted by the help of calibration curves determined from standards of known concentrations. In all experiments the feed solution contained 30 mM NaH_2PO_4 and 100 mM NaCl. In the initial series of diafiltration experiments, also the diafiltration buffer contained 30 mM NaH_2PO_4 but only 5 mM NaCl. Therefore, even a 100% exchange of the original feed buffer with diafiltration buffer does not reduce the conductivity of the retentate down to values close to zero. This fact was taken into account by preparing the standards by dilution with increasing amounts of diafiltration buffer (Fig. S1A). At a later point in the study pure water was used as diafiltration buffer, because even at very low ionic strength no detrimental effect onto the dissolved BSA could be observed. On the one hand this simplifies the preparation of calibration standards, on the other hand the calibration curve had to be divided into two sections (see Fig. S1B), because a single straight line is not a good fit of the required correlation if the conductivity stretches over several orders of magnitude.

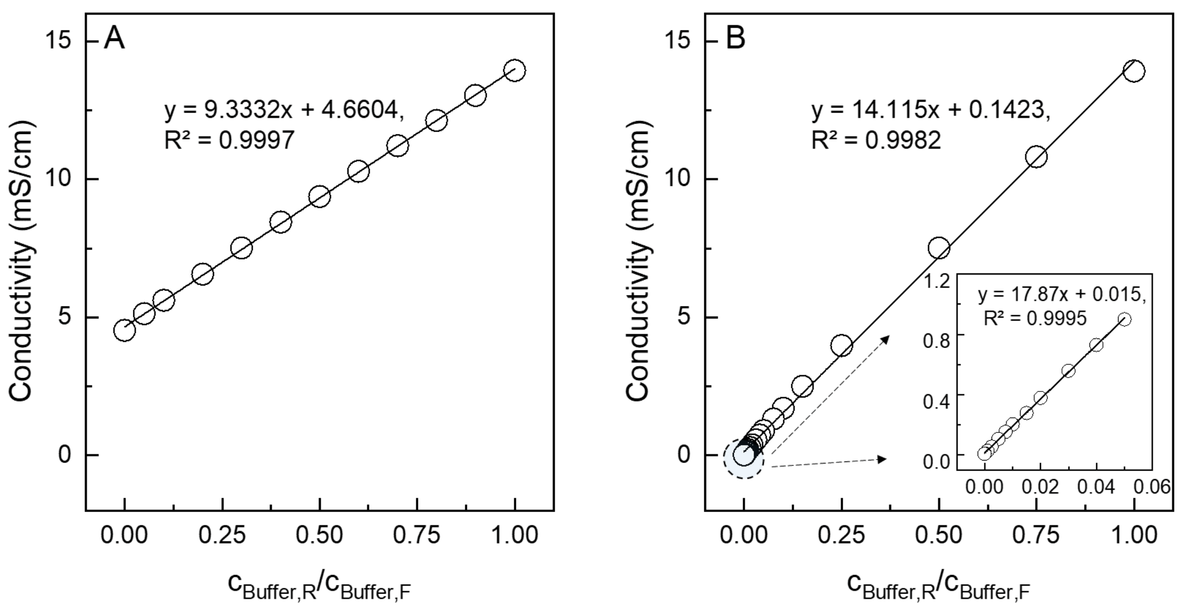

Fig. S1. Calibration curves for the conversion of conductivities into fractions of feed buffer concentration remaining in the retentate

Exemplary concentration factors resulting from different settings of pumps A and B of the chromatography system

The Äkta system was used in a way that pump A controlled the feed rate and pump B controlled the retentate rate. In the Äkta software the two feed rates are defined by the sum-flow of pumps A and B and the percentage X defining how much of this sum-flow is pumped by pump B. The pump rate of pump A is automatically set to (100 -X)% of the sum-flow. Dividing the resulting flow rates of pumps A and B will give the theoretical values of the concentration factor listed in Table S1.

Table S1. Theoretical concentration factors controlled by adjusting the percentages of pump A and pump B of the Äkta system.

Percentage of pump A (%)	Percentage of pump B (%)	Concentration factor
50	50	1
66.7	33.3	2
75	25	3
80	20	4

Details of measured and theoretical buffer exchange in the experiments applying co-current diafiltration with unidirectional and alternating flow of the diafiltration buffer

The theoretical values listed in Table S2 and Table S3 are calculated using two idealized models based on the assumption of (i) complete mixing and (ii) plug flow.

Table S2. Measured and theoretical buffer exchange in the experiment applying co-current diafiltration with unidirectional flow

Flow rate ratio Q_{DF}/Q_F (-)	Experimental result, buffer exchange (%)	SD	Complete mixing, buffer exchange (%)	Plug flow, buffer exchange (%)
0	0	0.000	0	0
0.2	15.7	0.000	16.7	18.1
0.4	31.6	0.049	28.6	33.0
0.6	41.5	0.043	37.5	45.1
0.8	53.2	0.031	44.4	55.1
1.0	62.3	0.000	50.0	63.2

Table S3. Measured and theoretical buffer exchange in the experiment applying co-current diafiltration with alternating flow of the diafiltration buffer

Flow rate ratio Q_{DF}/Q_F (-)	Experimental result, buffer exchange (%)	SD	Complete mixing, buffer exchange (%)	Plug flow, buffer exchange (%)
0.2	18.9	0.003	16.7	18.1
0.4	31.0	0.010	28.6	33.0
0.6	34.6	0.040	37.5	45.1
0.8	44.5	0.047	44.4	55.1
1.0	53.5	0.043	50.0	63.2
1.2	58.7	0.054	54.5	69.9
1.4	62.7	0.042	58.3	75.3
1.6	66.0	0.045	61.5	79.8

Co-current diafiltration with alternating DF flow direction: degree of buffer exchange as a function of switching interval times

For co-current diafiltration with alternating DF flow direction Fig. S2 shows the influence of the applied switching interval time onto the buffer exchange and the maximum pressure build-up. For low Q_{DF} the dependency shows the expected order, with longer switching intervals resulting in a higher pressure build-up but at the same time slightly better buffer exchange. The higher buffer exchange is assumed to be related to the detrimental mixing effects originating from the switching event. However, above a Q_{DF} of 0.7 ml/min, the buffer exchange order changes, saying the buffer exchange of the experiment with the shorter switching interval is slightly higher. The reason for this unexpected behavior is not clear yet.

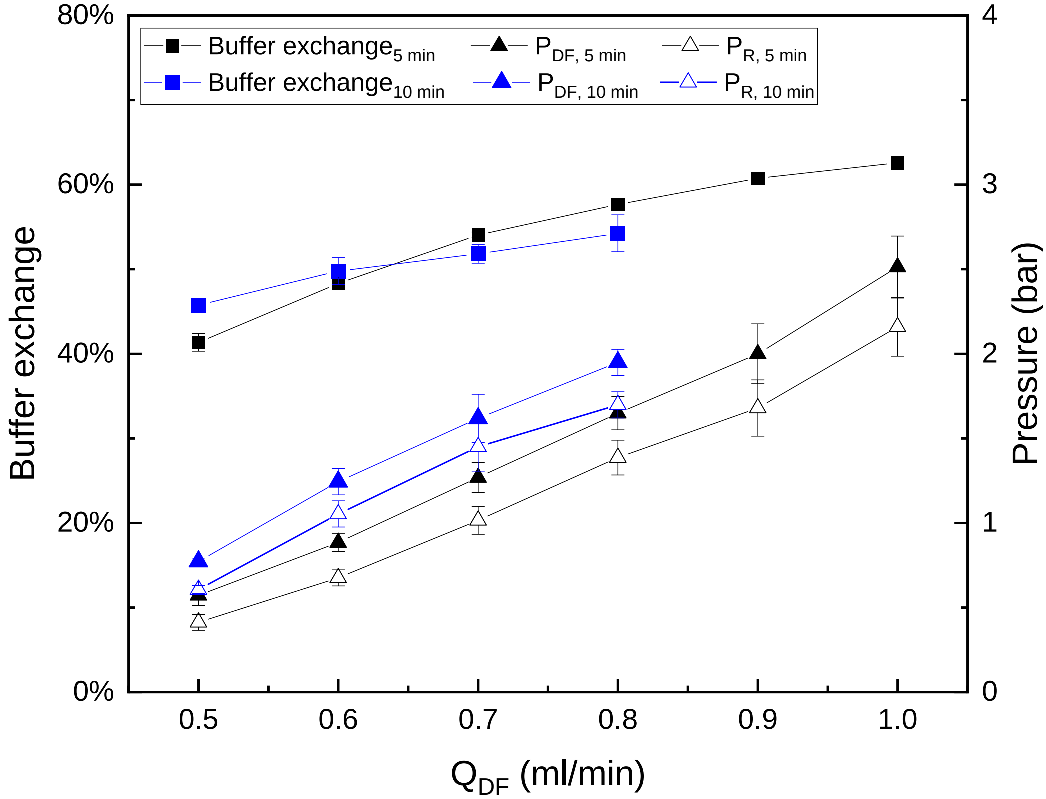

Fig. S2. Degree of buffer exchange and pressures in the membrane module as a function of various switching interval times. $Q_F = Q_R = 0.5$ ml/min (56.4 L m^{-2} h^{-1}), flushing time 15 s at 10 ml/min.

Co-current diafiltration with alternating DF flow direction: Detailed time courses of the degree of buffer exchange as well as P_R and P_{DF} within the small and scaled-up module

For co-current diafiltration with alternating DF flow direction Fig. S3 shows the detailed time courses of buffer exchange and the pressures P_R and P_{DF} for long-term experiments in the small and scaled-up module.

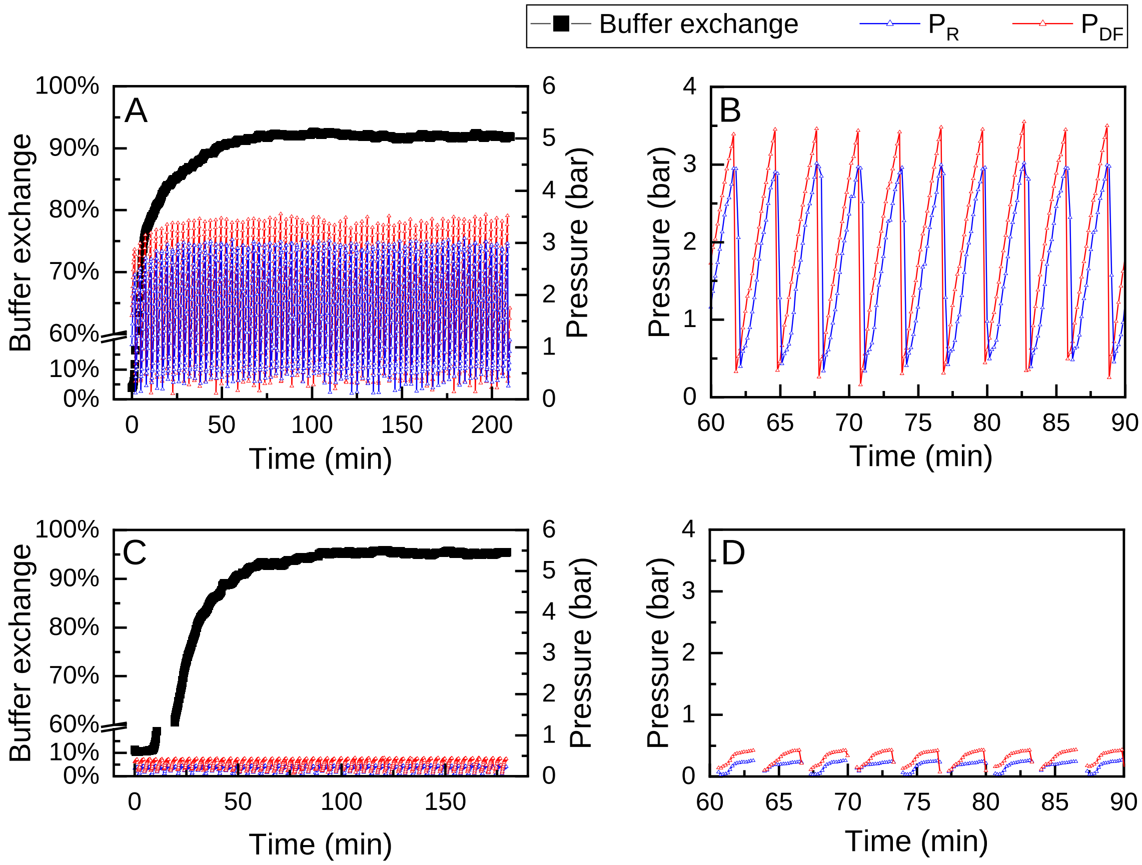

Fig. S3. Detailed time courses of buffer exchange as well as P_R and P_{DF} during co-current diafiltration with alternating DF flow direction. $Q_F = Q_R = 0.25$ ml/min, $Q_{DF} = 1.8$ ml/min (7.2 DV), switching interval 3min, flushing time 15 s; (A) full-time course of the experiment using the small module, (B) zoomed out the section of the time course of the experiment using the small module, (C) full-time course of the experiment using the scaled-up module, (D) zoomed out section of the time course of the experiment using the scaled-up module.

Co-current diafiltration with alternating DF flow direction: Detailed time courses of the degree of buffer exchange as well as P_R and P_{DF} within the scaled-up module

Fig. S4 shows the results of an experiment applying co-current diafiltration with alternating direction of the permeate flow. During the experiment, operation conditions applying 7.2, 12, and 14.4 diavolumes were tested.

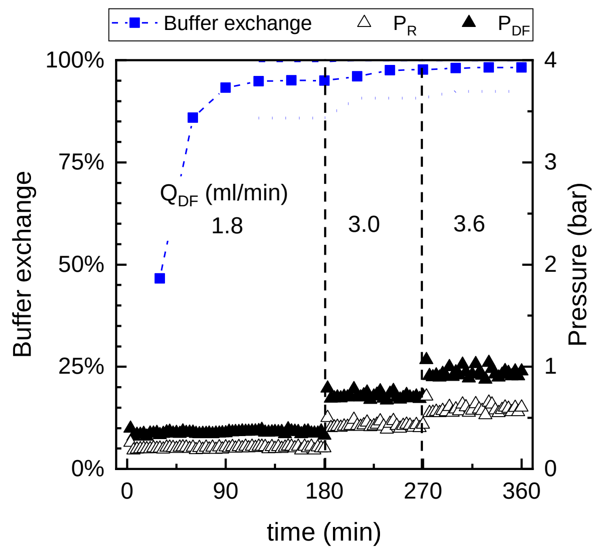

Fig. S4. Time course of the buffer exchange and the pressures in the middle (P_R) and the lateral (P_{DF}) part of the module for co-current diafiltration with alternating DF flow direction in the scaled-up membrane module. The diafiltration flow flux was increased from 1.8 to 3.6 ml/min during the experiment. The dashed lines represent the theoretical buffer exchange value corresponding to the idealized models of complete mixing (▪▪▪▪▪▪) and plug-flow (▬ ▬ ▬), respectively.

Counter-current diafiltration with alternating DF flow direction: Detailed time courses of the degree of buffer exchange as well as P_R and P_{DF} within the scaled-up module

For counter-current diafiltration with alternating DF flow direction Fig. S5 shows the detailed time courses of the buffer exchange and the pressures P_R and P_{DF} for long-term experiments with and without flushing step as well as different feed flow rates in the scaled-up module.

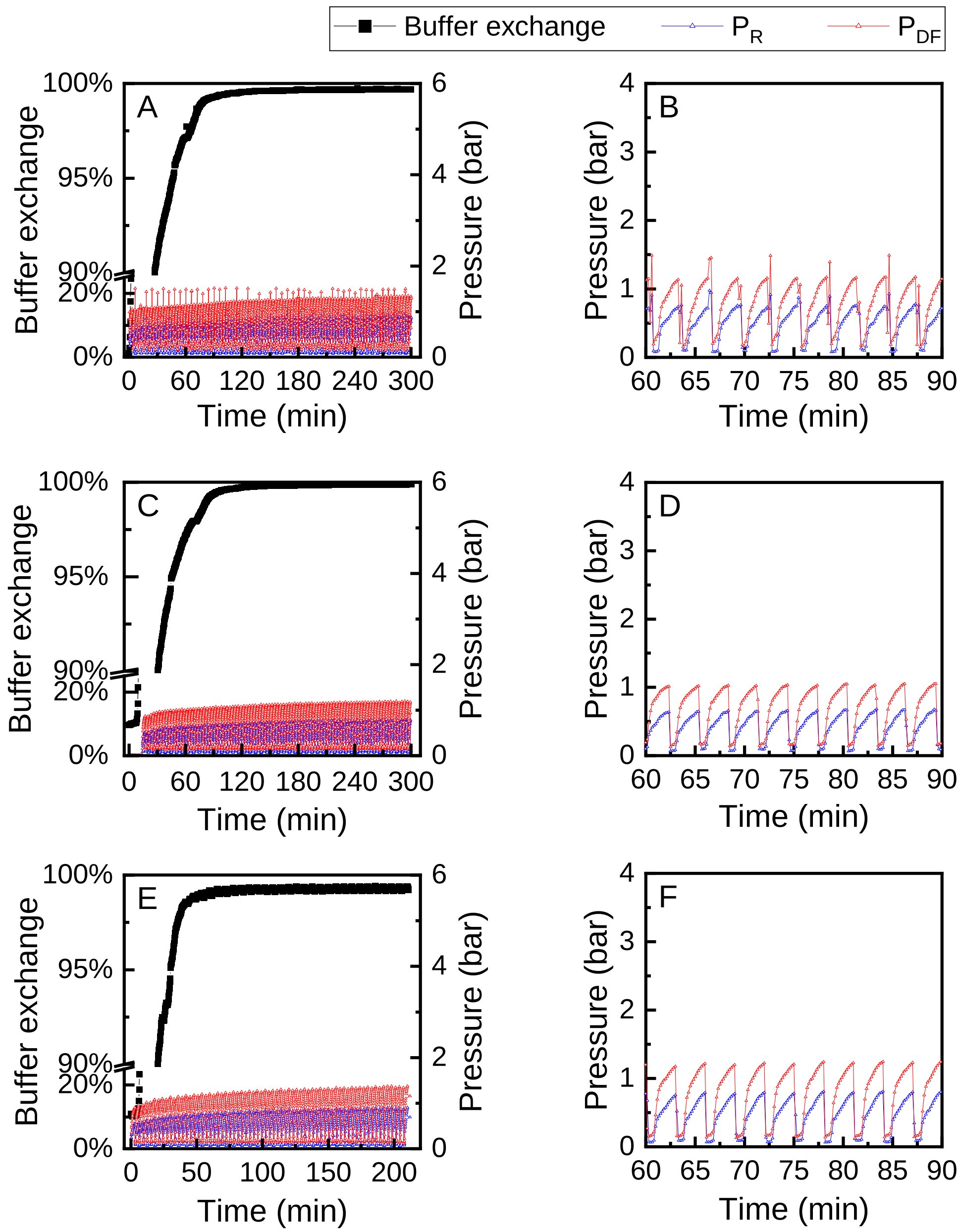

Fig. S5. Detailed time courses of buffer exchange as well as P_R and P_{DF} during counter-current diafiltration with alternating DF flow direction using the scaled-up module. The flow rate Q_{DF} and switching interval were 3.6 ml/min and 3 min respectively in these experiments. A and B: With flushing steps, Q_F = Q_R = 0.25 ml/min (5.05 L m^{-2} h^{-1}), DV 21.3), (A) full-time course of the experiment, (B) zoomed out section of the time course of pressures in the module. C and D: Without flushing steps, Q_F = Q_R = 0.25 ml/min (DV 14.4), (C) full-time course of the experiment, (D) zoomed out the section of the time course of pressures in the module. E and F: Without flushing steps, Q_F = Q_R = 0.5 ml/min (10.1 L m^{-2} h^{-1}), DV 7.2), (E) full-time course of the experiment, (F) zoomed out the section of the time course of pressures in the module.