Supporting Information

to

Ring-Opening Metathesis Polymerization-Derived Large-Volume Monolithic Supports for Reversed-Phase and Anion-Exchange Chromatography of Biomolecules

by

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EXPERIMENTAL

Materials. Trimethylolpropane triacrylate (TMPTA), dicyclopentadiene, methanol, toluene, 2-propanol (2-PrOH), N,N-dimethyl-N-allylamine, methyl iodide, pyridine, norborn-2-ene, CH_2Cl_2, N,N-dimethylformamide (DMF), ethyl vinyl ether (EVE), tetrahydrofuran (THF), the 2nd-generation Grubbs initiator [$RuCl_2(PCy_3)(IMesH_2)(CHPh)$] ($PCy_3$=tricyclohexylphosphine, $IMesH_2$=1,3-bis(2,4,6-trimethylphenyl)imidazolin-2-ylidene) and the 1st-generation Grubbs initiator $RuCl_2(PCy_3)_2(CHPh)$ (**1**) were purchased from Sigma-Aldrich (Munich, Germany). [$RuCl_2(Py)_2(IMesH_2)(CHPh)$] (**2**) was prepared according to a published protocol.[55] Bicyclo[2.2.1]hept-2-ene-5-trichlorosilane was purchased from ABCR GmbH & Co. KG. (Karlsruhe, Germany). Poly(styrene) (PS) standards $580<M_n<2750000$ g/mol used for inverse size exclusion chromatography (ISEC) were purchased from Polymer Standards Service, PSS (Mainz, Germany). A mixture of 5'-phosphorylated oligodeoxythymidylic acids, $[d(pT)]_{12\text{-}18}$, as well as insulin, cytochrome *C*, lysozyme, conalbumin and ß-lactoglobulin were purchased from Sigma-Life Sciences (Munich, Germany). NMR data were obtained at 250.13 MHz for proton and 62.90 MHz for carbon in the indicated solvent at 25°C on a Bruker Spectrospin 250 and are listed in parts per million downfield from tetramethylsilane for proton and carbon. Scanning electron micrographs

(SEM) were recorded with a Zeiss Auriga with 1.74 or 2.40 kV. The samples were sputtered with Pt/Pd before analysis.

Preparation of Buffers. HPLC-analyses were performed using deionized-water, acetonitrile (ACN) and trifluoroacetic acid purchased from Sigma-Aldrich (Munich, Germany). NaH_2PO_4, trizma, ammonium acetate and NaOH from Sigma-Aldrich (Munich, Germany) were used for buffer preparation. The NaH_2PO_4 buffers (0.05 M, pH 7 and 8) were prepared by adjusting the solution with NaOH to the required pH. Tris-HCl (Trizma) was used for buffer preparation (0.05M, pH 9) by adjusting the pH with HCl.

HPLC System. Preparative experiments were carried out on a preparative gradient HPLC system consisting of two preparative K-1800 pumps allowing for flow rates up to 1000 mL/min, a preparative K-2500 UV detector set to 214 nm, a preparative mixing chamber and an injection valve with a 1-mL SS sample loop. All equipment was connected with 1.5-mm I.D. PEEK capillary tubes. A HPLC data acquisition and control station from Knauer (Berlin, Germany) was used. Analytical-scale separations were carried out on an Agilent Technology HPLC-system (Böblingen, Germany). The HPLC system consisted of a binary HPLC pump, a diode array UV-Vis detector, an auto sampler, a column oven and a sample thermostat. Proteins and 5`-phosphorylated oligodeoxythymidylic acids fragments $d[pT]_{12\text{-}18}$ were detected at λ=214 nm, 280 nm and 254 nm, respectively.

Bicyclo[2.2.1]hept-5-en-2-yl)-methyl-N,N-dimethylamine (3). A mixture of N,N-dimethylallylamine (18.2 g, 0.214 mol), freshly distilled cyclopentadiene (15.5 g, 0.235 mol) and hydroquinone (50 mg, 0.45 mmol) was heated to 175^{o}C in a sealed tube for 48 h. The tube was cooled in ice bath and the crude black reaction mixture was fractionally distilled under reduced pressure to afford a pale yellow liquid. Yield: 20.8 g (64 %). ^{1}H NMR

($CDCl_3$): δ = 6.09-5.90 (m, 2H), 2.81 (s, 1H), 2.73 (s, 1H), 2.19 (s, 6H), 2.05-1.98 (m, 1H), 1.89-1.81 (m, 2H), 1.38-1.35 (m, 1H), 1.21-1.13 (m, 2H), 0.53-0.49 (m, 1H). ^{13}C NMR ($CDCl_3$): δ = 137.0, 136.8, 136.7, 132.6, 66.3, 64.7, 49.6, 46.1, 45.4, 45.1, 44.9, 42.5, 41.8, 36.9, 31.8, 31.3; IR (ATR mode): 3067 (m), 2965 (s), 2864 (m), 2814 (m), 2768 (s), 2358 (m), 1456 (s), 1377 (m), 1208 (m), 1037 (s) 838 (s), 718 (s) cm^{-1}; GC-MS (EI) m/z calculated for $C_{10}H_{17}N$, m/z=151.25; found: 151.1 (*exo*/*endo* mixture, t_{R1}=5.997 min; t_{R2}=6.005 min).

Bicyclo[2.2.1]hept-5-en-2-yl)-methyl-N,N-dimethylammonium hydrochloride (4). Compound **3** (7.00 g, 0.046 mol) was dissolved in 50 mL of diethyl ether and treated drop wise with 3.0 mL of 36% of hydrochloric acid at 0°C, then the mixture was stirred for 2 h. The resulting white suspension was allowed to stand for another 1 h and finally filtered, washed with 50 mL of diethyl ether and then dried *in vacuo*. Yield: 8.2 g (94 %). ^1H NMR ($CDCl_3$): δ = 12.09 (s, 1H), 6.23-6.03 (m, 2H), 3.10 (s, 1H), 2.92-2.86 (m, 2H), 2.78-2.77 (m, 6H), 2.66-2.54 (m, 2H), 2.09-2.02 (m, 1H), 1.50-1.47 (m, 1H), 1.30-1.26 (m, 1H), 0.78-0.73 (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 139.0, 137.3, 135.9, 131.7, 63.3, 62.6, 50.0, 45.7, 45.5, 44.1, 43.7, 42.7, 42.2, 34.8, 34.7, 32.4; IR (ATR mode): 3452 (m), 3071 (m), 2966 (b), 2500-2700 (b), 1571 (m), 1474 (s) 1341 (m), 1246 (m), 1169 (s), 965 (s), 829 (s), 787 (m), 720 (s) cm^{-1}; Elem. Anal.: Calcd for $C_{10}H_{18}ClN$: C, 63.99; H, 9.67; N, 7.46; Found: C, 63.97; H, 9.68, N, 7.40.

Bicyclo[2.2.1]hept-5-en-2-yl)methyl-N,N,N-trimethylammonium iodide (5). Compound **3** (7.00 g, 0.046 mol) was dissolved in 50 mL of diethyl ether and treated drop wise with CH_3I (7.3 g, 0.051mol) at 0°C; then the mixture was stirred for 4 h. The resulting white suspension was filtered, washed with 50 mL of diethyl ether and then dried *in vacuo*. Yield: 13 g (96 %). ^1H NMR ($CDCl_3$): δ = 6.27-6.01 (m, 2H), 3.22-3.25 (m, 1H), 2.98 (m, 9H), 2.98-2.83 (m, 2H), 2.67 (s, 1H), 2.59 (s, 1H), 2.12-2.06 (m, 1H), 1.35-1.28 (m, 2H), 0.77-0.73 (m, 1H); ^{13}C

NMR ($CDCl_3$): δ = 138.5, 137.08, 136.1, 131.5, 71.1, 70.1, 52.6, 52.6, 52.5, 49.0, 47.1, 46.0, 45.6, 42.0, 41.8, 33.6, 33.5, 32.6; IR (ATR mode): 3434 (m), 3052 (m), 2958 (b), 2867 (s), 1630 (m), 1478 (s), 1334 (s), 1249 (m), 1142 (s), 1082 (m), 961 (s), 904 (s), 827 (s), 770 (m), 715 (s) cm^{-1}; Elem. Anal.: Calcd for $C_{11}H_{20}IN$: C, 45.06; H, 6.88; I, 43.28 N, 4.78; Found: C, 45.11; H, 7.08, N, 4.76.

Trimethylolpropane-tris-(5-norbornene-2-yl-carboxylate) (**CL**, **6**). A solution of (100 g, 0.33 mol) of trimethylolpropane triacrylate (TMPTA) in methylene chloride (400 mL) was flushed with nitrogen for 15 min. Then, freshly cracked cyclopentadiene (70 g, 1.06 mol) was added drop wise and the reaction mixture was stirred for 16 h at 40°C. Then it was consecutively washed with 500 mL of saturated $NaHCO_3$ solution and with 500 mL of water. Finally, the organic layer was dried over anhydrous $MgSO_4$ and concentrated, then high vacuum was applied for 16 h to obtain the pure product. Yield: 155 g (93%). ^{1}H-NMR (250 MHz, $CDCl_3$) δ=0.82-0.89 (m, 3H), 1.22-1.26 (m, 2H), 1.33-1.46 (m, 9H), 1.82-1.92 (m, 3H), 2.17.2.22 (m, 1H), 2.88-2.98 (m, 6H), 3.16 (s, 2H), 3.92-4.09 (m, 6H), 5.83-5.87 (m, 2H), 6.07-6.18 (m, 4H); ^{13}C-NMR (250 MHz, $CDCl_3$) δ=7.3, 22.8, 26.8, 29.0, 30.2, 40.5, 40.6, 40.7, 41.5, 42.4, 43.1, 42.4, 43.2, 45.7, 46.4, 49.6, 63.5, 63.8, 135.5, 138.0, 139.9, 174.3, 175.8. IR (ATR mode): υ=3061 (m), 3032 (m), 2979 (s), 2865 (m), 1731 (s), 1466 (m), 1338 (s), 1278 (m), 1173 (s), 1120 (s), 1030 (m), 702 (m) cm^{-1}. Elem. Anal.: Calcd for $C_{30}H_{38}O_6$: C, 72.85; H, 7.74; Found: C, 72.98; H, 7.91.

Preparation of Monoliths. PEEK columns were cleaned, rinsed and sonicated in a 1:1 mixture of ethanol and acetone, then dried for 2 h *in vacuo*. All glass columns were etched at 45°C with 2 *M* ethanolic KOH overnight. After repeated washing with water, columns were dried *in vacuo*. Silanization was performed at 45°C overnight using a mixture of pyridine, toluene and bicyclo[2.2.1]hept-2-ene-5-yltrichlorosilane (molar ratio 3:30:1). Then, these

columns were washed consecutively with acetone, water, and ethanol and dried at 40°C for 2 h.

The pretreated HPLC columns were closed at one end with end fittings and placed in an ice-cold water bath. Two different solutions (A, B) were prepared and cooled to -15°C. Solution A consisted of the cross-linker, NBE and 2-propanol, while solution B consisted of initiator **1** in toluene. Both solutions were merged at -15°C and mixed for ~30 s. The column was filled with the polymerization mixture, sealed with Teflon caps and kept at 0°C for 15 minutes. After rod formation was finished, the column was removed from the ice bath and stored at room temperature for 8 h. In order to remove initiator and non-reacted monomers, the column open ends were cleaned and closed with end fittings and attached to HPLC system, then flushed with a mixture of ethyl vinyl ether (EVE, 20 vol.-%) in a 1:1 mixture of DMSO and THF.

Functionalization of Monoliths. For the generation of weak or strong anion-exchange functionalities, initiator **2** (0.5 mg, 6.86×10^{-4} mmol) was dissolved in 1 mL of CH_2Cl_2 or DMF and injected into the monolith, then the monolith kept at room temperature for 1 h. In order to remove any unattached initiator from the monolithic support, columns were washed with two column volumes of CH_2CL_2 or DMF, respectively. Monoliths were treated with one column volume of a solution of monomer **4** (32 mg/mL, 0.17 mmol in CH_2Cl_2) or monomer **5** (32 mg/mL, 0.109 mmol in DMF) then sealed and left at 45°C for 2h. Finally, the column was cooled to room temperature and flushed with two column volumes of 20 vol.-% of EVE in 1:1 mixture of DMSO and THF, then with methanol and water. The amount of grafted monomer was determined by elemental analysis.

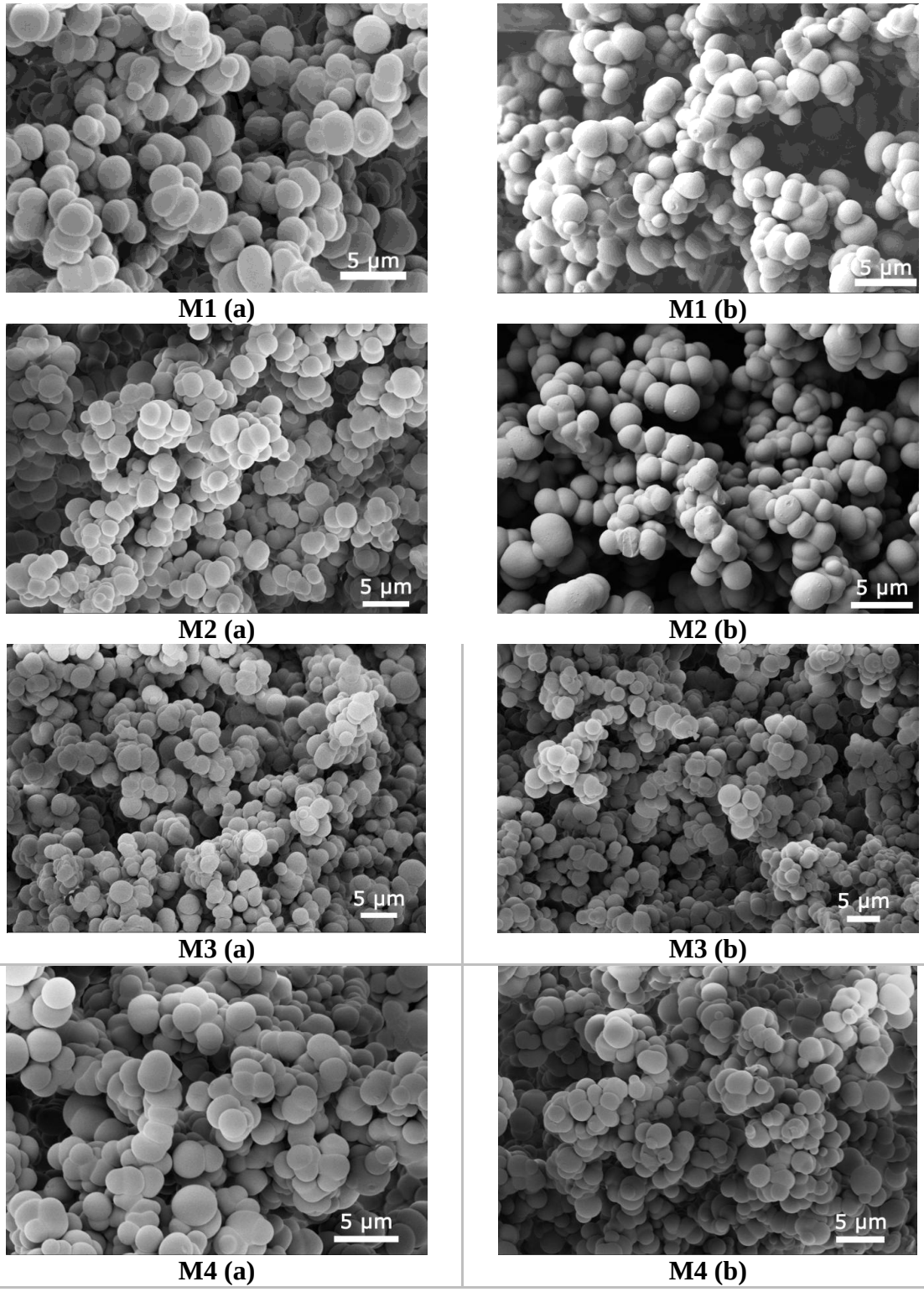

Figure S1. SEM images of monoliths **M1**-**M4**. (a) top and (b) center parts of the monoliths.

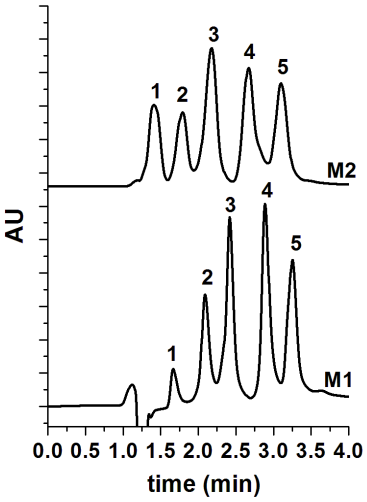

Figure S2. Separation of a mixture of five proteins on monoliths **M1** and **M2**. Column dimensions: 1.0 x 10 cm I.D. Chromatographic conditions; mobile phase A: 0.1% TFA in water; mobile phase B: 0.1% TFA in ACN; gradient: 25-60 % B in 5 min. flow rate 5 mL/min; sample for **M1**, 1.0 mg/mL insulin (1), 2.3 mg/mL cytochrome *C* (2), 3.5 mg/ mL lysozyme (3), 3.5 mg/mL conalbumin (4) and 3.0 mg/mL lactoglobulin (5); sample for **M2**, 2.0 mg/mL insulin (1), 2.3 mg/mL cytochrome *C* (2), 3.5 mg/ mL lysozyme (3), 3.5 mg/mL conalbumin (4) and 3.0 mg/mL lactoglobulin (5) were dissolved in water; injection volume, 50 uL; 25°C; detection: UV, 214 nm.

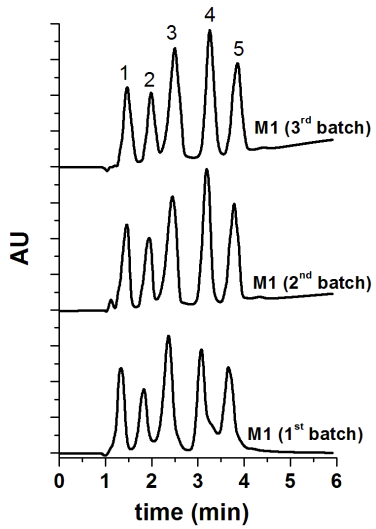

Figure S3. Separation of a mixture of five proteins on monolith **M2** (1.0 x 10 cm PEEK column). Chromatographic conditions; mobile phase A: 0.1% TFA in 100 % water; mobile phase B: 0.1% TFA in 100 % acetonitrile; gradient: 0-5 min 25-50 % B in 5 min. flow rate 5 mL/min; sample, 3 mg/mL insulin (1), 2.0 mg/mL cytochrome *C* (2), 4.0 mg/ mL lysozyme (3), 4.0 mg/mL conalbumin (4) and 3.0 mg/mL lactoglobulin (5) dissolved in water; injection volume, 50 uL; 25°C; detection: UV, 214 nm. 25°C; detection: UV, 214 nm.

Table S1. Separation efficiencies for a mixture of 5 proteins on monoliths **M1** and **M2** (1 x 10 cm I.D.) presented as retention times (t_R), peak widths at peak half height ($\omega_{0.5}$) and resolution (R_s).

	monolith **M1**			monolith **M2**		
fragment	t_R (min)	$\omega_{0.5}$ (s)	R_s	t_R (min)	$\omega_{0.5}$ (s)	R_s
1	1.668	2.9	4.24	1.410	5.0	2.40
2	2.088	3.0	3.20	1.789	4.4	2.41
3	2.417	3.1	4.60	2.175	5.1	2.90
4	2.890	3.0	3.31	2.667	5.0	2.62
5	3.251	3.5		3.098	4.9	

Table S2. Separation efficiency for a mixture of 5 proteins on monoliths **M3**, **M4** and **M5** presented as retention times (t_R), peak width at peak half height ($\omega_{0.5}$) and resolution (R_s).

	monolith **M3**			monolith **M4**			monolith **M5**		
fragment	t_R (min)	$\omega_{0.5}$ (s)	R_s	t_R (min)	$\omega_{0.5}$ (s)	R_s	t_R (min)	$\omega_{0.5}$ (s)	R_s
1	1.05	8.8	1.18	3.633	33.6	1.24	5.783	36	1.15
2	1.50	14.1	0.91	5.267	45	1.00	7.217	38	1.06
3	1.933	14.4	1.86	6.817	48	1.51	8.617	40	1.45
4	2.817	14.1	0.99	8.883	33.9	1.12	10.467	36	1.08
5	3.3	15	-	10.367	45.6	-	11.767	36	-

Table S3. Influence of various pH values (phosphate buffers and acetate buffers) on the speed and efficiency of separation $d(pT)_{12-18}$ by applying a ROMP-derived weak anion-exchange monolith **M6** (100x3 mm I.D.) presented as retention times (t_R), peak width at peak half height ($\omega_{0.5}$) and resolution (R_s).

	M6 (pH 5)			**M6 (pH 7)**			**M6 (pH 8)**		
fragment	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**
1	38.567	38.76	2.02	38.267	41.52	2.05	35.253	41.04	2.04
2	41.045	34.77	2.22	40.987	37.79	2.10	37.733	31.86	2.15
3	43.500	31.31	2.07	43.453	32.39	2.09	40.007	33.30	2.05
4	45.661	31.29	2.07	45.780	34.08	2.14	42.820	28.82	1.975
5	47.660	26.49	2.05	47.760	23.6	1.61	43.820	22.42	1.798
6	49.500	27.35	2.01	49.307	33.99	2.47	45.513	34.05	0
7	51.267	25.38	-	51.4	17.74	-	45.513	34.05	-

Table S4. Separation of 5'-phosphorylated oligodeoxythymidylic acids fragments on a ROMP-derived weak anion-exchange monolith **M6** (100x3 mm I.D.) and on strong anion-exchange monoliths **M7** (100x3 mm I.D.) and **M8** (100x10 mm I.D.); presented as retention times (t_R), peak width at peak half height ($\omega_{0.5}$) and resolution (R_s).

	M6 (pH 7)			**M7 (pH 7)**			**M8 (pH 7)**		
fragment	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**
1	29.073	23.58	2.48	23.367	12.88	3.26	36.233	56.28	2.811
2	31.000	22.95	2.41	24.771	12.88	3.09	41.233	50.4	2.586
3	32.747	20.44	2.27	26.046	11.86	2.799	45.553	49.78	2.662
4	34.302	20.67	2.24	27.203	12.99	2.412	49.707	43.82	2.650
5	35.774	18.67	2.182	28.259	13.26	2.219	53.587	42.88	2.948
6	37.080	17.23	2.16	29.231	13.01	2.01	57.653	39.84	2.495
7	38.340	17.67	-	30.124	13.62	-	61.793	59.7	-

Table S5. Influence of various pH values (phosphate buffers and TRIS-HCl buffers) on the speed and efficiency of separation $d(pT)_{12-18}$ applying a ROMP-derived strong anion-exchange monolith **M7** (100x3 mm I.D.) presented as retention times (t_R), peak width at peak half height ($\omega_{0.5}$) and resolution (R_s).

	M7 (pH 7)			**M7 (pH 8)**			**M7 (pH 9)**		
fragment	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**
1	30.241	16.82	3.11	29.460	16.27	3.90	31.255	15.30	3.84
2	32.478	26.22	2.30	31.348	12.69	3.64	32.901	10.87	4.054
3	34.040	14.44	3.70	31.127	16.60	3.30	34.443	11.94	3.5
4	35.650	11.66	3.19	34.710	12.17	3.68	35.849	12.17	3.00
5	37.140	16.30	2.54	36.150	11.26	3.60	37.123	13.31	2.56
6	38.513	16.09	2.41	37.497	11.17	2.70	38.290	13.95	2.57
7	39.751	14.65	-	38.707	15.51	-	39.395	11.75	-

Table S6. Separation efficiency for a mixture of 5 proteins on three ROMP-derived **M2**-type monoliths (1x10 cm I.D.) presented as retention times (t_R), peak widths at half height ($\omega_{0.5}$) and resolution (R_s).

	M2 (1st batch)			**M2 (2nd batch)**			**M2 (3rd batch)**		
protein	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**	**t_R (min)**	**$\omega_{0.5}$ (s)**	**R_s**
1	1.413	5.5	2.56	1.544	4.5	2.88	1.549	5.6	2.74
2	1.911	6	2.46	2.038	5.8	2.10	2.068	5.7	2.29
3	2.445	6.8	3.18	2.529	6.5	3.07	2.578	6.6	3.15
4	3.154	6.5	2.55	3.280	6.3	2.72	3.335	6.7	2.58
5	3.738	7.2	-	3.858	6.4	-	3.937	7.3	-

proteins: insulin (1), 2 cytochrome *C* (2), lysozyme (3), conalbumin (4), lactoglobulin (5).