Predicting Ion Mobility-Mass Spectrometry Trends of Polymers using the Concept of Apparent Densities

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Supplementary Information

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Materials & Methods

Chemicals.

The solvents toluene, tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2), used for the synthesis of (poly(2-ethoxy-1,3,2-dioxaphospholane 2-oxide); poly(ethoxyphosphate)[1], PEtP), were dried under vacuum before use. 2-Chloro-1,3,2-dioxaphospholane 2-oxide (COP) was purchased from Acros, diethyl ether, methanol (analytical grade) and calcium hydride (CaH_2) (Aldrich) were used as received. Ethanol (Aldrich), benzyl alcohol (Aldrich), *N,N*-diethylethanamine (or triethylamine, TEA) (Aldrich), and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) (Aldrich) were dried over calcium hydride at room temperature, followed by distillation under vacuum before use. 1-1-[3,5-Bis(trifluoromethyl)phenyl]-3-cyclohexyl-2-thiourea (TU) was synthesized as described elsewhere[2].

Synthesis of the monomer (EP).

The monomer (2-ethoxy-1,3,2-dioxaphospholan 2-oxide, EP) for the PEtP synthesis was synthesized as described by Clément *et al.*[1]. The reaction is based on an esterification reaction of the chlorinated cyclic precursor 2-chloro-2-1,3,2-dioxaphospholan 2-oxide (COP).

The solvent and reagents used were dried either by a drying apparatus or by fresh distillations. COP (0.7 mol) in 200 mL of dry tetrahydrofurane (THF) was added dropwise (during 1-2 h) under N_2 atmosphere to a mixture of ethanol (EtOH 0.7 mol), triethylamine (TEA) (0.7 mol) in 200 mL of dry THF. The final conditions were COP/EtOH/TEA 1/1/1. The reaction flask was

kept at 0 °C under constant stirring during the reaction time of 4 to 5 hours. After the reaction was completed, the reaction medium was filtered under ambient atmosphere in order to remove the precipitated salt. The product was dried under vacuum and kept under N_2 atmosphere at -20 °C for one night. Afterwards, a fractional distillation was performed under vacuum (~3 h, oil bath at 130 °C - 140 °C). The observed boiling point of the monomer was around 74 °C.

Synthesis of the poly(phosphoester) (poly(2-ethoxy-1,3,2-dioxaphospholane 2-oxide; PEtP).

The polymer synthesis was inspired by Clément and coworkers[1]. The polymers were synthesized by Ring-Opening Polymerization (ROP) with a dual organo-catalysis system (1,8-diazobicyclo[5.4.0]undec-7-ene DBU and 1-1-[3,5-bis(trifluoromethyl)phenyl]-3-cyclohexyl-2-thiourea TU) as initially described by Hedrick and coworkers[3,4]. Several linear poly(2-ethoxy-1,3,2-dioxaphospholane 2-oxide) (poly(ethoxyphosphate); PEtP) (PEtP 1200 g/mol, 3000 g/mol, 4500 g/mol, 5500 g/mol; Figure SI1) were produced.

For the 1200 g/mol and 3000 g/mol polymers, 2-ethoxy-1,3,2-dioxaphospholan 2-oxide (EP) (39.4 mmol) was mixed with TU (5% of EP, 1.97 mmol) in dry CH_2Cl_2 (40 mL) in order to obtain a 1 M solution. Before adding the solvent (CH_2Cl_2), three azeotropic distillations with anhydrous toluene were performed to eliminate residual traces of water. The freshly distilled initiator was then added (benzyl alcohol, BzOH, 1.97 mmol). The ratio EP/BzOH was fixed according to the intended Degree of Polymerization (DP). The polymerization-initiating catalyst, DBU, was then added (2.7 mmol) to the reaction medium at 0 °C under constant stirring. The quantity of DBU was chosen according to the molar ratio DBU/BzOH to lie within 1.2 and 1.5. After 15 minutes for PEtP 1200 g/mol or after 40 minutes for PEtP 3000 g/mol, the polymerizations were stopped by precipitating the reaction medium in cold diethyl ether under

heavy stirring. The polymers were then recuperated while being solid. They were dried under vacuum and put under N_2 atmosphere.

The longer polymers (4500 g/mol and 5500 g/mol) were obtained using a slightly different procedure. Toluene was used as solvent and the monomer concentration was raised to 4 M. The ratio DBU/TU was set to 1/1. The ratio DBU/BzOH was kept at 1.5 equivalents of DBU towards 1 equivalent of the initiator. Instead of the azeotropic distillations, TU was dried overnight under vacuum. After the polymerization, the polymer was precipitated in cold diethyl ether and was dried under vacuum in order to be stored under N_2 atmosphere.

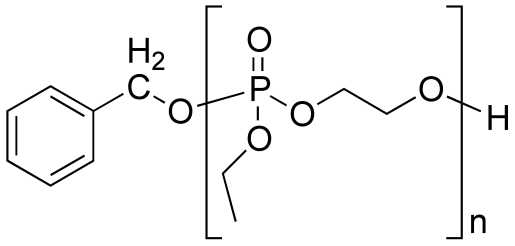

Figure SI1: Poly(ethoxyphosphate) (poly(2-ethoxy-1,3,2-dioxaphospholane 2-oxide), PEtP).

Ion Mobility-Mass Spectrometry.

The PEtP and PEO samples were infused at a flow rate of 4 µL/min, using a syringe pump and a 250 µL Thermo Scientific syringe, into a SYNAPT G2 HDMS Ion Mobility-Mass Spectrometer (Waters, Manchester, UK) which was used to determine the Arrival Time Distributions. This IM-MS is fitted with an Electrospray Ionization (ESI) source and a stacked ring Traveling Wave (T-Wave) Ion Mobility cell. The capillary voltage was set to 3 kV, the sampling cone voltage was 40 V and the extraction cone was set to 4 V. The source and desolvation temperatures were 100 °C and 200 °C respectively. No cone gas flow was used and the desolvation gas flow was set to 500 L/h. The voltages for the trap and the transfer Collision Energies (CE) were set to 4 V and

2 V, respectively and the trap bias was set to 45 V. The IM wave height was 40 V and the wave speed was set to 1200 m/s. The trap Ar gas flow was set to 2 mL/min, the He gas flow was 180 mL/min and the N_2 pressure in the IM cell was set to 2.6 mbar.

Collision Cross-Section (CCS)/Ion Mobility calibration.

As Traveling Wave Ion Mobility cells need to be calibrated in order to convert drift time measurements into CCS values, the calibration methodology described by Ruotolo and coworkers was followed[5] using proteins and peptides as calibrants (bradykinin[6], tryptic digest of Bovine Serum Albumin (BSA)[7], ubiquitin[8], myoglobin[9], cytochrome C[10] and lysozyme[11]; see Figure SI2, Figure SI3 and Table SI1).

PCL calibration curves are to be found in the paper of Morsa *et al.*[12].

Even if the T-Wave (Traveling Wave Ion Mobility) conditions were identical for PEtP and PEO, two different calibration sets were used.

The blue lines in Figure SI2 and Figure SI3 correspond to the 95% confidence bands; the red lines correspond to the 95% prediction bands. Not all the molecules described in Table SI1 were used for both calibrations.

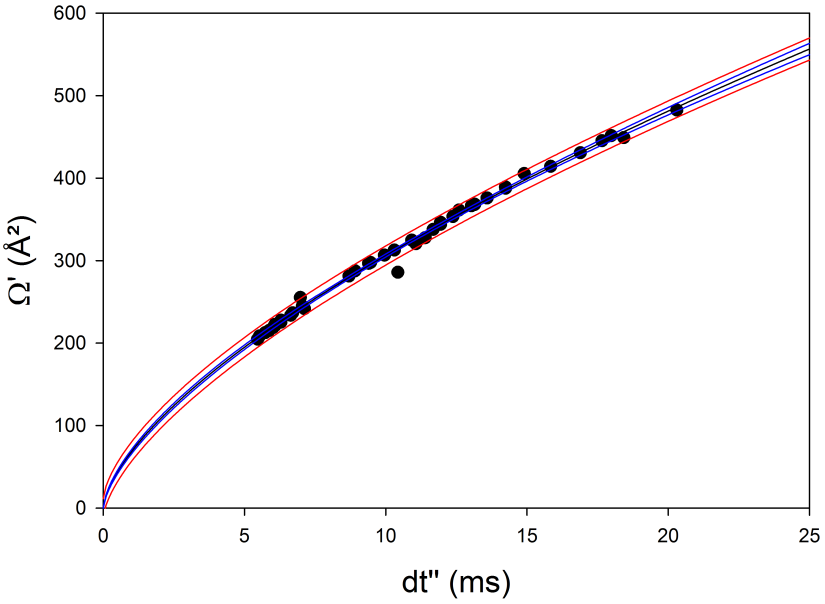

Figure SI2: Calibration curve to convert drift times into CCS values (as T-Wave derived CCS) for PEtP polymer complexes. The power fit function is used to establish the calibration curve ($\Omega'=a.dt''^{b}$). The fit parameters of the calibration curve are $a=68.3367$ and $b=0.6516$; the fit parameters of the 95% prediction band are $a=61.6784$ and $b=0.6773$.

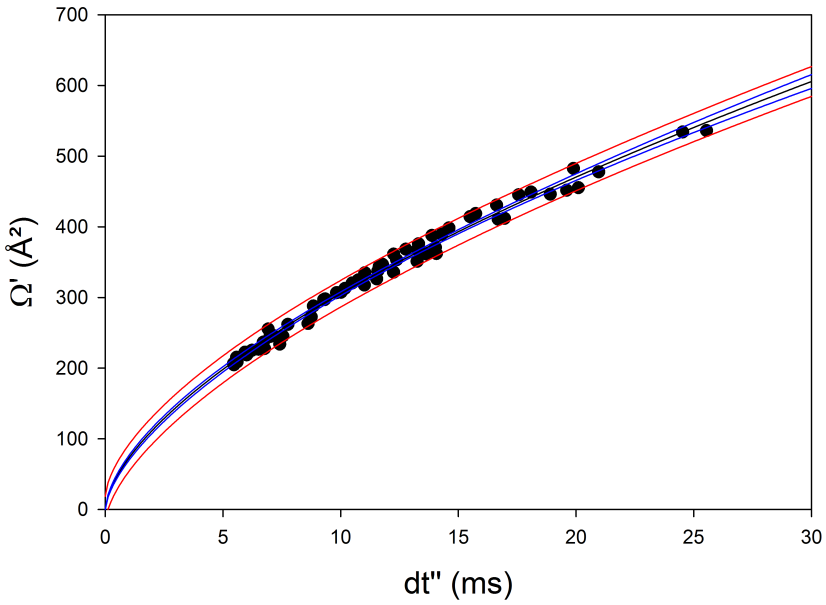

Figure SI3: Calibration curve to convert drift times into CCS values (as T-Wave derived CCS) for PEO polymer complexes. The power fit function is used to establish the calibration curve ($\Omega'=a.dt''^{b}$). The fit parameters of the calibration curve are $a=72.6837$ and $b=0.6234$; the fit parameters of the 95% prediction band are $a=62.1956$ and $b=0.6606$.

Table SI1: Table of proteins and peptides used to calibrate the Traveling Wave Ion Mobility.

Molecule	**Mass (Da)**	**z**	**m/z**	**Ω (Å^2, CCS) in He**
Bradykinin	1059.62	2	530.81	246
FPK	390.23	1	391.23	123.14
YTK	438.23	1	439.23	131.96
VGTR	431.26	1	432.26	136.71
ADLAK	516.29	1	517.29	159.31
AFDEK	608.29	1	609.29	168.36
IETMR	648.33	1	649.33	181.29
KFWGK	664.37	1	665.37	185.85
VASLR	544.34	1	545.34	163.7
SEIAHR	711.37	1	712.37	181.53
TPVSEK	659.35	1	660.35	175.98
ATEEQLK	817.42	1	818.42	206.4
LVTDLTK	788.46	1	789.46	205.76
AEFVEVTK	921.49	1	922.49	223.42
YLYEIAR	927.00	1	927.99	228.03
LVVSTQTALA	1001.58	1	1002.58	239.27
LVTDTDLTK	788.5	2	395.25	205
GACLLPK + CAM	757.48	2	379.74	207
ATEEQLK	817.46	2	409.73	209
LCVLHEK+CAM	897.52	2	449.76	228
AEFVEVTK	921.52	2	461.76	219
YLYEIAR	926.52	2	464.26	237
LVVSTQTALA	1001.62	2	501.81	234

QTALVELLK	1013.66	2	507.83	245
LVNELTEFAK	1162.68	2	582.34	262
HLVDEPQNLIK	1304.78	2	653.39	288
TVMENFVAFVDK	1398.72	2	700.36	297
SLHTLFGDELCK + CAM	1418.76	2	710.38	297
YICDNQDTISSK + CAM	1442.7	2	722.35	298
LGEYGFQNALIVR	1478.86	2	740.43	307
DDPHACYSTVFDK + CAM	1553.72	2	777.86	313
MPCTEDYLSLILNR + CAM	1723.88	2	862.94	338
YNGVFQECCQAEDK + CAM	1748.72	2	875.36	325
HLVDEPQNLIK	1304.76	3	435.92	319
SLHTLFGDELCK + CAM	1291.65	3	431.55	334
TCVADESHAGCEK+CAM	1461.75	3	488.25	340
DDPHACYSTVFDK+CAM	1248.63	3	417.21	323
KVPQVSTPTLVEVSR	1638.99	3	547.33	338
DAFLGSFLYEYSRR	1722.75	3	575.25	383
DAIPENLPPLTADFAEDKDVCK+CAM	2460.2	3	820.07	422
QNCDQFEKLGEYGFQNALIVR	2474.2	3	824.73	429
NECFLSHKDDSPDLPK	1900.96	4	476.24	442
Ubiquitin	8560.8	7	1223.82	1580
Ubiquitin	8560.8	8	1070.97	1622
Ubiquitin	8560.8	9	952.2	1649
Ubiquitin	8560.9	10	857.09	1732
Ubiquitin	8561.08	11	779.28	1802
Myoglobin	16950.93	12	1413.58	3044
Myoglobin	16977.09	13	1304.93	3136
Myoglobin	16978.22	14	1211.73	3143

Myoglobin	16981.05	15	1131.07	3230
Myoglobin	16982.08	16	1060.38	3313
Myoglobin	16951.10	17	998.12	3384
Myoglobin	16950.6	18	942.7	3489
Myoglobin	16947.81	19	892.99	3570
Myoglobin	16948	20	848.4	3682
Myoglobin	16947	21	808	3792
Myoglobin	16940	22	771	3815
Cytochrome c	12230.07	5	2447.01	1340
Cytochrome c	12229.11	6	2039.18	1602
Cytochrome c	12371.9	10	1236.19	2226
Cytochrome c	12371.9	11	1123.9	2303
Cytochrome c	12375.96	12	1030.33	2335
Cytochrome c	12352.6	13	951.2	2391
Cytochrome c	12348.7	14	883.05	2473
Cytochrome c	12354.75	15	824.65	2579
Cytochrome c	12187.49	16	762.72	2679
Cytochrome c	12385.86	17	727.58	2723
Cytochrome c	12326.4	18	685.8	2766
Lysozyme	14303.73	6	2384.96	1355

Results & Discussions

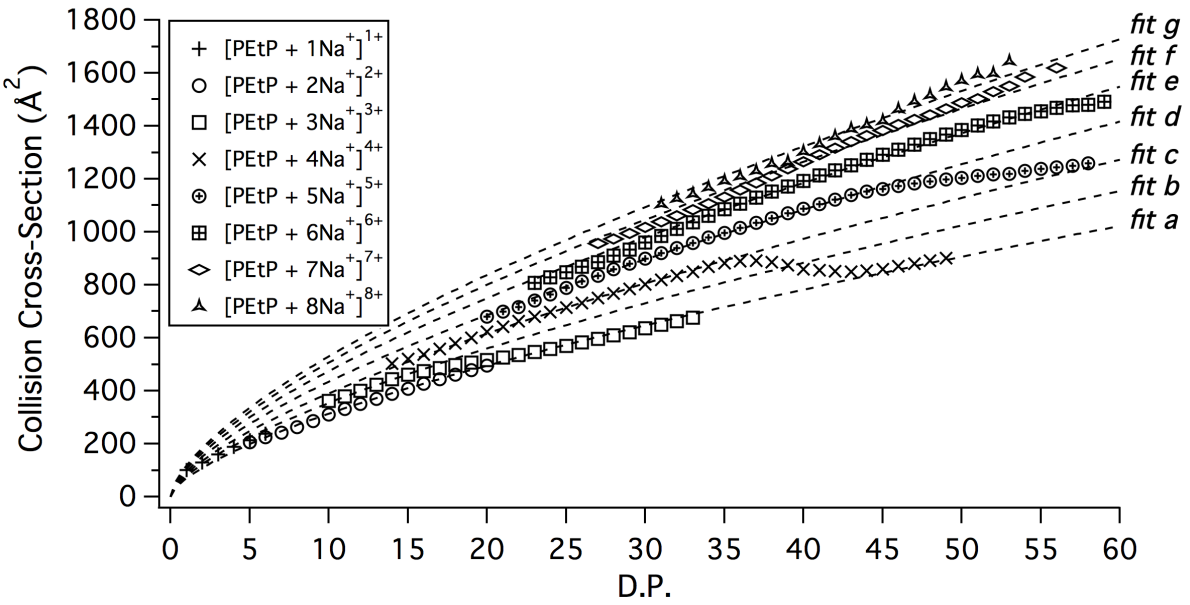

Figure SI4: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) versus the DP (Degree of Polymerization) of sodiated poly(ethoxyphosphate) (PEtP) polymer ions at different charge states (from 1+ to 8+). The constrained power fit functions ($CCS = A.DP^{pow}$, $pow=0.66$) for the different CCS trends before and after the tipping points are described as *fit a* to *fit g*.

Table SI2: Fitting parameters of the trend lines for poly(ethoxyphosphate) PEtP, poly(ethylene oxide) PEO and different topologies of poly(caprolactone) PCL polymer-cation complexes. The unconstrained fits ($CCS = A.DP^{pow}$, Equation 2) are calculated with free A and pow parameters.

Polymer	Fitting Curve	Charge State Description	pow[1]	A[1]
PEtP[2]	*Fit a*	Common trend line (2+ to 4+)	0.67 ± 0.01	65.1 ± 2.1
	Fit b	3+	0.61 ± 0.04	88.4 ± 9.0
	Fit c	4+	0.63 ± 0.00	94.5 ± 1.4
	Fit d	5+	0.68 ± 0.01	88.9 ± 2.8
	Fit e	6+	0.71 ± 0.01	87.3 ± 3.2
	Fit f	7+	0.74 ± 0.01	80.5 ± 3.1
	Fit g	8+	0.76 ± 0.02	78.7 ± 7.4
PEO[2]	*Fit a*	Common trend line (3+)	0.64 ± 0.02	35.2 ± 4.1
	Fit c	4+	0.50 ± 0.03	93.6 ± 12.5
	Fit e	5+	0.58 ± 0.03	81.9 ± 13
	Fit f	6+	0.71 ± 0.01	49.6 ± 2.4
	Fit g	7+	0.83 ± 0.01	28.8 ± 1.8
	Fit h	8+	0.92 ± 0.01	18.8 ± 0.7
	Fit i	9+	0.93 ± 0.01	18.8 ± 0.7
Linear PCL[3]	*Fit a*	Common trend line (2+ to 4+)	0.75 ± 0.01	44.4 ± 1.1
	Fit b	2+	0.68[4]	67.9[4]
	Fit c	3+ and 4+ trend	0.64 ± 0.04	87.4 ± 12.4
	Fit d	4+	0.63[4]	101.8[4]
6-arm star PCL[3]	*Fit a*	Common trend line (2+ to 4+)	0.70 ± 0.01	53.6 ± 2.4
	Fit b	3+ and 4+ trend	0.63 ± 0.03	81.3 ± 10.6
	Fit c	4+	0.60[4]	103.6[4]

[1] The values of the pow and A fitting parameters are given with their 95% confidence interval.
[2] see Figure 1 and Figure 2.
[3] see Supplementary Information (Figure SI9 and Figure SI10).
[4] Fitted on small amount of data points; no 95% confidence interval calculation.

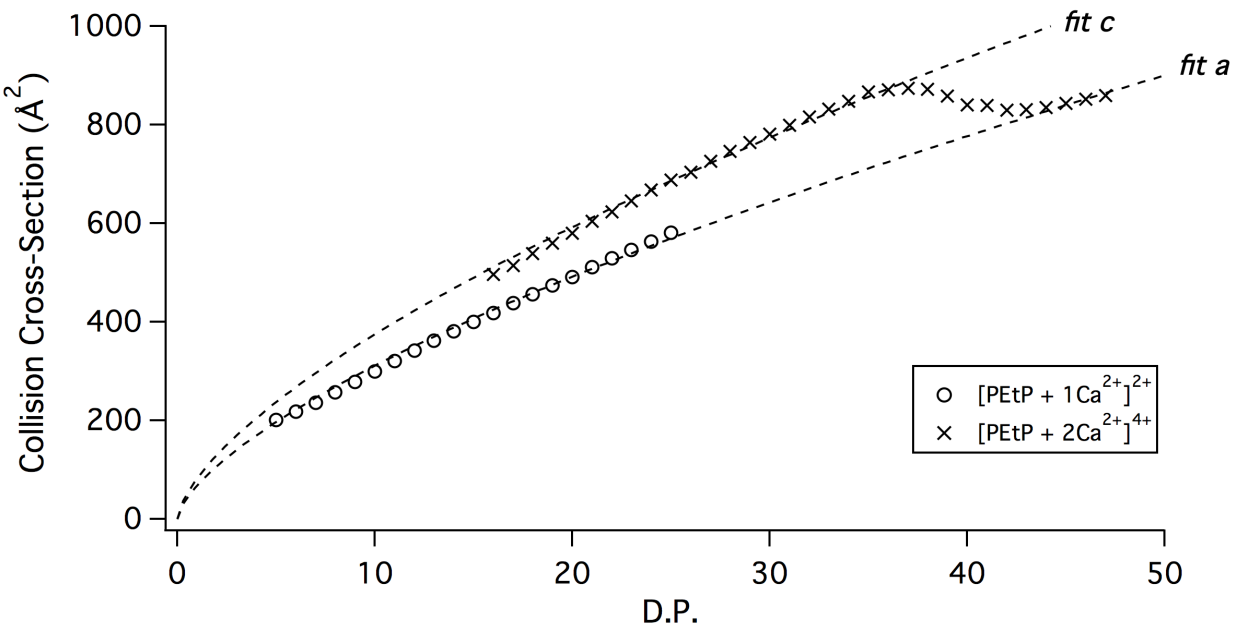

Figure SI5: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) versus the DP (Degree of Polymerization) of poly(ethoxyphosphate) (PEtP) polymers with one or two calcium cations. The constrained power fit functions ($CCS = A.DP^{pow}$, $pow=0.66$) for the two CCS trends are described as *fit a* (i.e. the common trend line) and *fit c*.

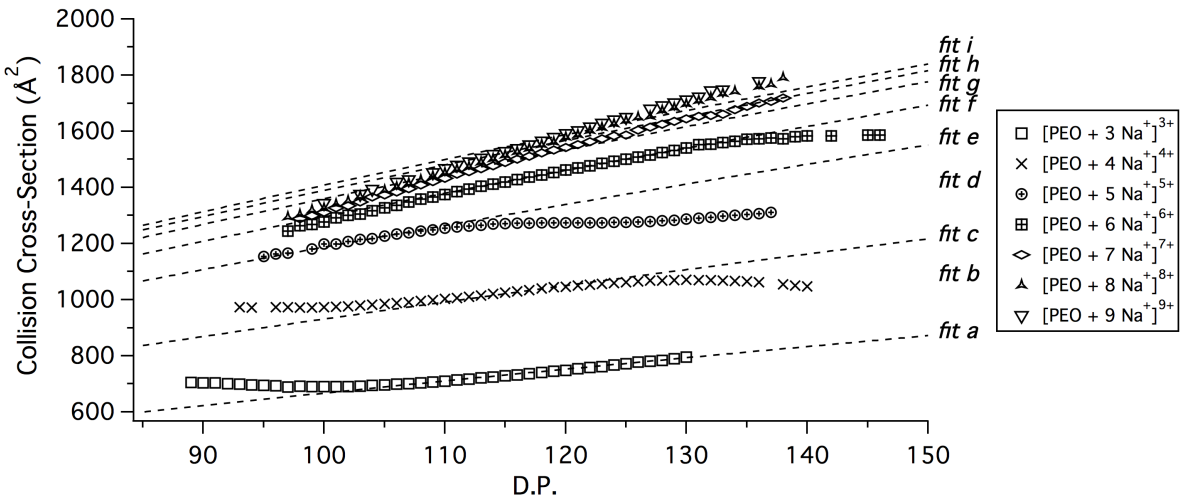

Figure SI6: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) as a function of the DP (Degree of Polymerization) of sodiated poly(ethylene oxide) (PEO) polymer ions (from 3+ to 9+). The constrained power fit functions ($CCS = A.DP^{pow}$, $pow=0.66$) for the different CCS trends before and after the tipping points are described as *fit a* to *fit i*. The fit functions *fit b* and *fit d* are not shown but are still accounted for in the plot as they denote the CCS trends missed in the analyzed DP range.

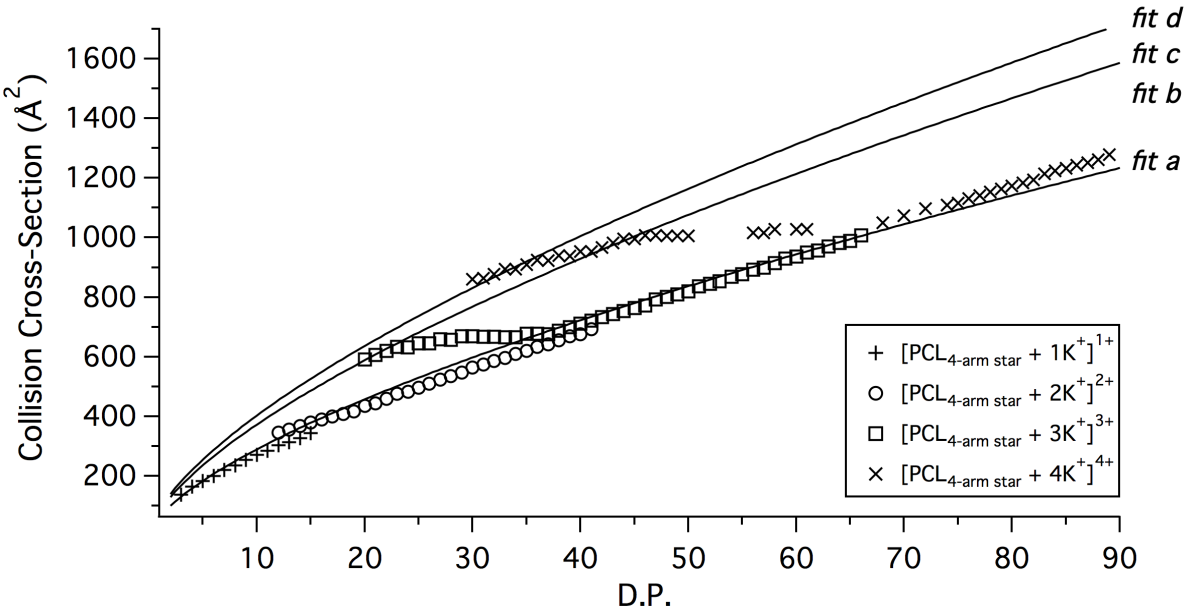

Figure SI7: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) as a function of the DP (Degree of Polymerization) of 4-arm star poly(caprolactone) (PCL) polymers with varying numbers of potassium cations used to reach different charge states (from 1+ to 4+). The constrained power fit functions ($CCS = A.DP^{pow}$, $pow=0.66$) for the different CCS trends before and after the tipping points are described as *fit a* to *fit d*. The fit function *fit b* is not shown but is still accounted for in the plot as it denotes the CCS trend of the 2+ complexes which did not yet reach the plateau (< DP 12). Figure SI15 highlights the data points used for performing the data fits.

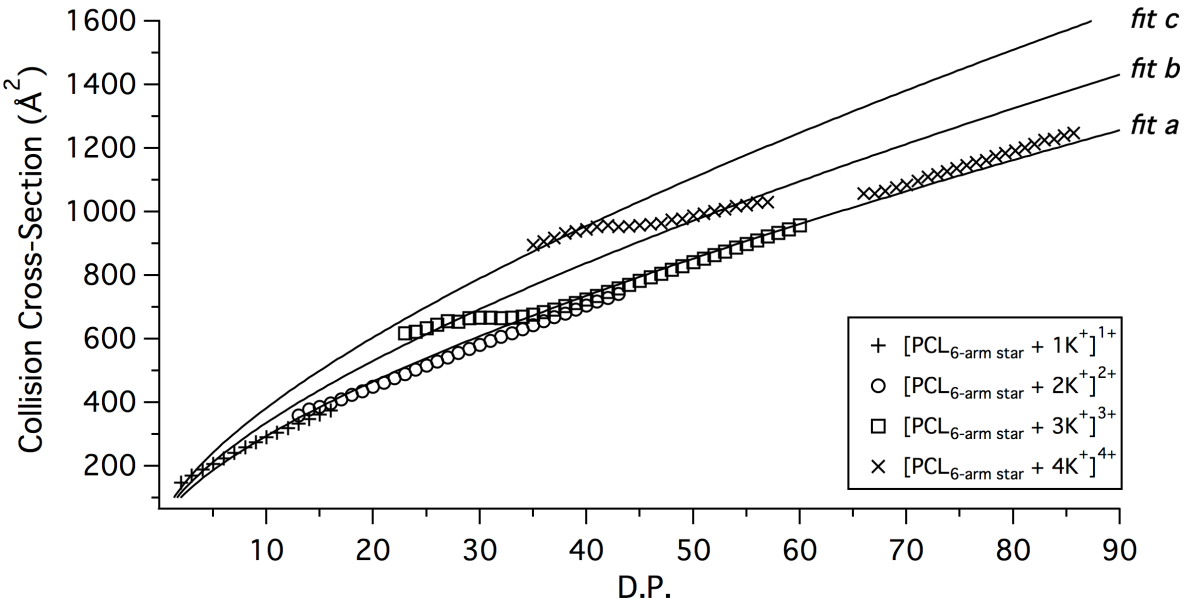

Figure SI8: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) as a function of the DP (Degree of Polymerization) of 6-arm star poly(caprolactone) (PCL) polymers with varying numbers of potassium cations used to reach different charge states (from 1+ to 4+). The constrained power fit functions ($CCS = A.DP^{pow}$, $pow=0.66$) for the different CCS trends are described as *fit a* to *fit c*. No new trend is yielded by the 2+ complexes which do not show a structural rearrangement. Figure SI16 highlights the data points used for performing the data fits.

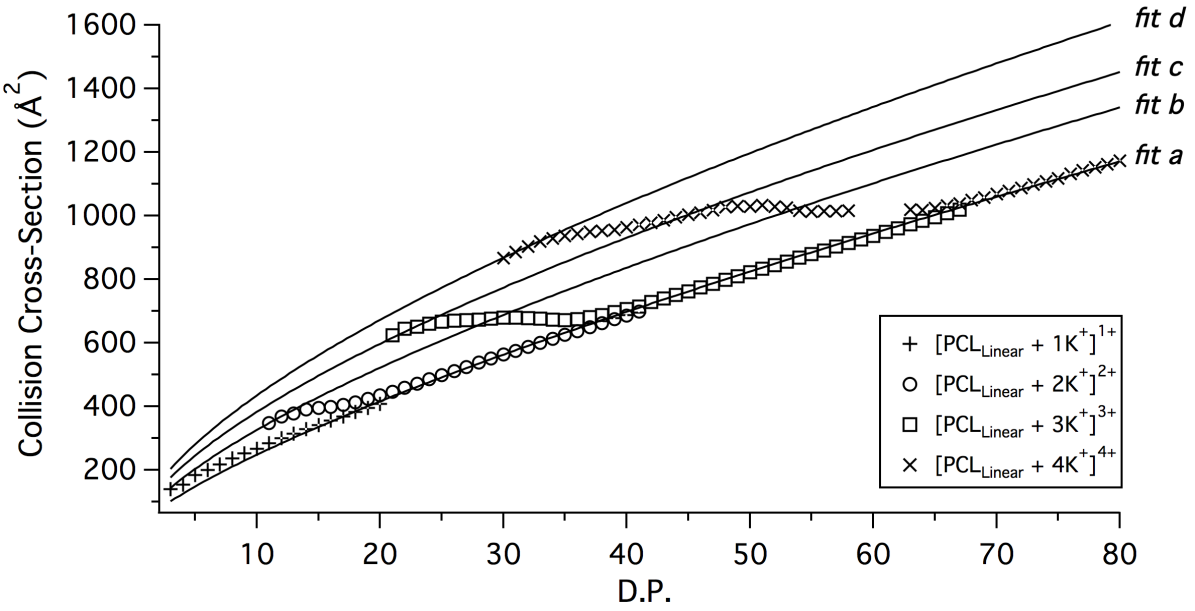

Figure SI9: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) versus the DP (Degree of Polymerization) of linear poly(caprolactone) (PCL) polymers with varying numbers of potassium cations used to reach different charge states (from 1+ to 4+). The unconstrained power fit functions ($CCS = A.DP^{pow}$, Equation 2) for the different CCS trends are described as *fit a* to *fit d*.

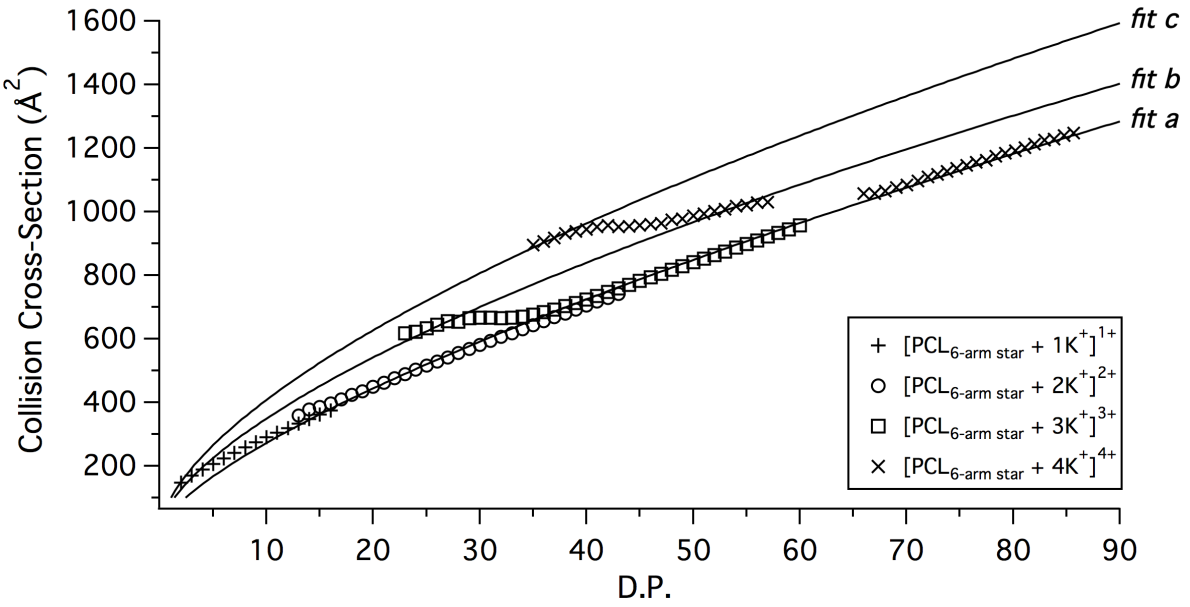

Figure SI10: Plot of the Collision Cross-Section (CCS, He-derived T-Wave$_{N2}$ CCS) as a function of the DP (Degree of Polymerization) of 6-arm star poly(caprolactone) (PCL) polymers with varying numbers of potassium cations used to reach different charge states (from 1+ to 4+). The unconstrained power fit functions ($CCS = A.DP^{pow}$, Equation 2) for the different CCS trends are described as *fit a* to *fit c*.

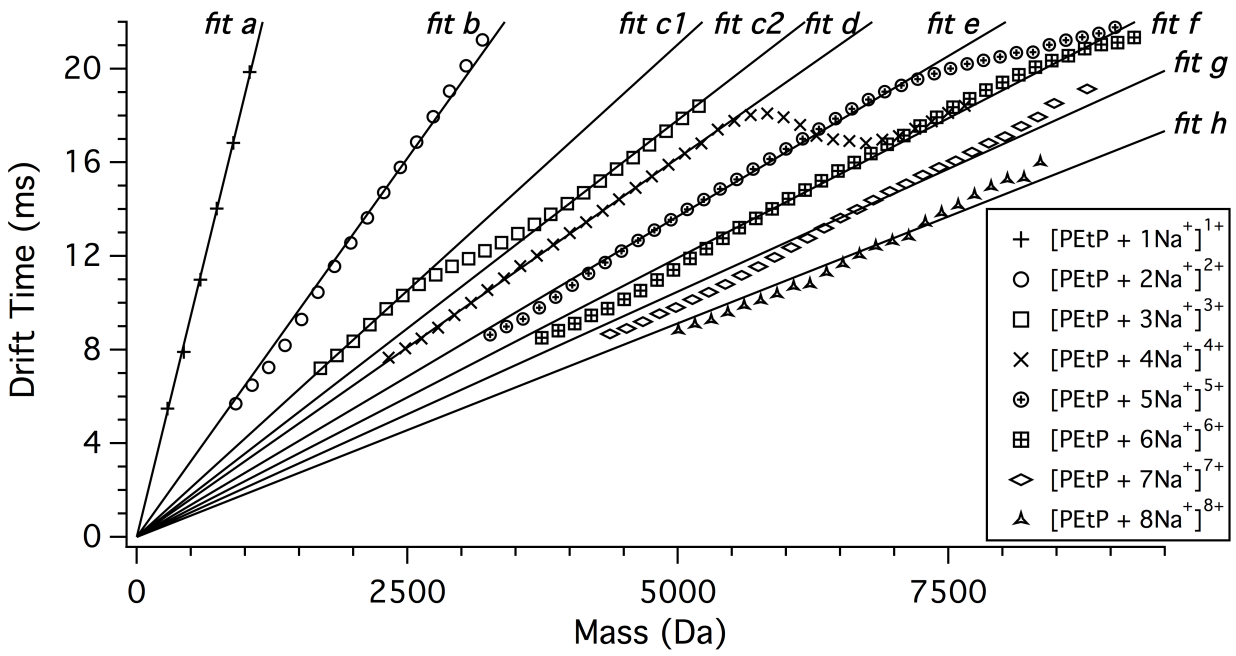

Figure SI11: Plot of the measured drift time as a function of the mass of poly(ethoxyphosphate) (PEtP) polymers with varying numbers of sodium cations used to reach different charge states (from 1+ to 8+). The constrained linear fit functions ($y = y_0+m.x$ and $y_0=0$) for the different CCS trends are described as *fit a* to *fit h*.

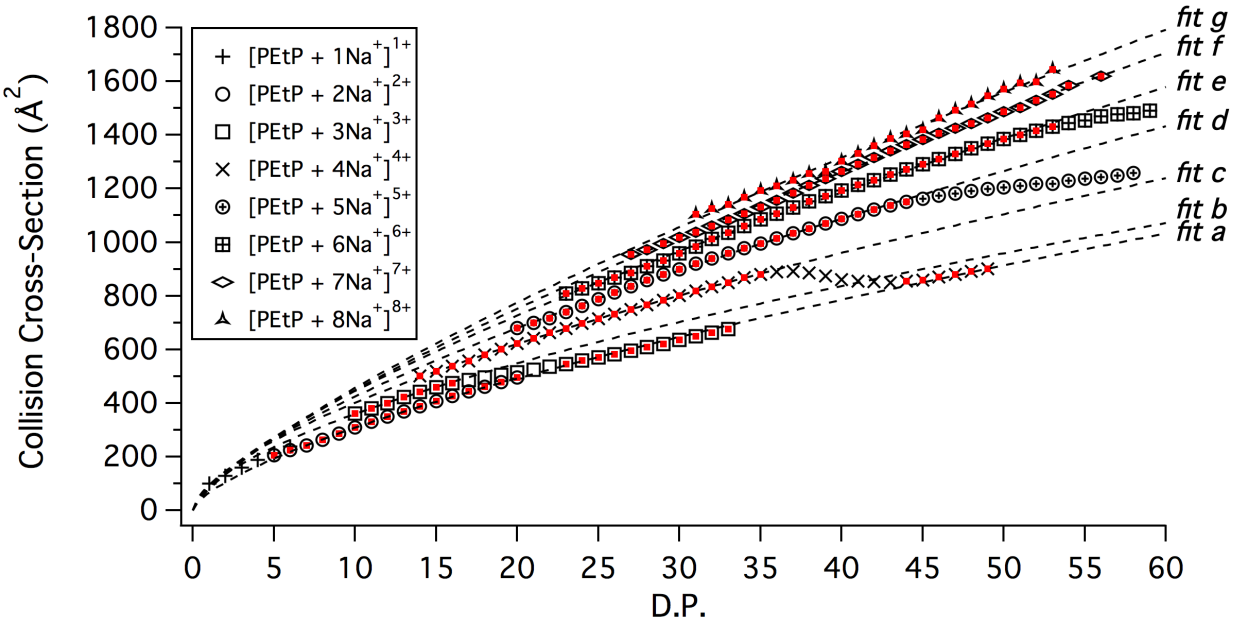

Figure SI12: Figure 1 (CCS vs. DP of PEtP) with highlighted data points (red dots) used for performing the data fits ($CCS = A.DP^{pow}$, *pow* unconstrained).

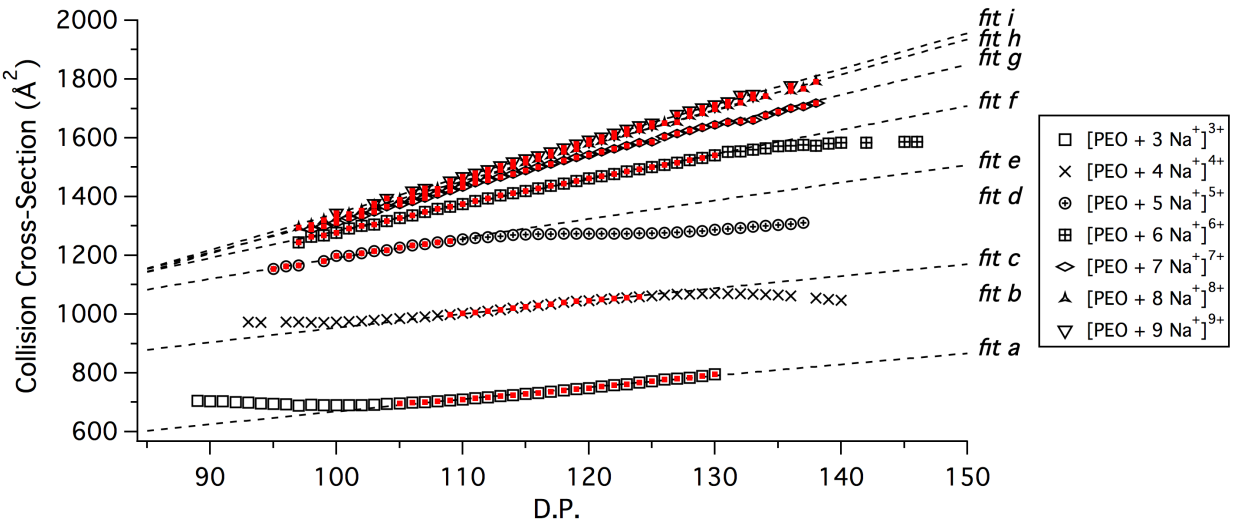

Figure SI13: Figure 2 (CCS vs. DP of PEO) with highlighted data points (red dots) used for performing the data fits ($CCS = A.DP^{pow}$, *pow* unconstrained).

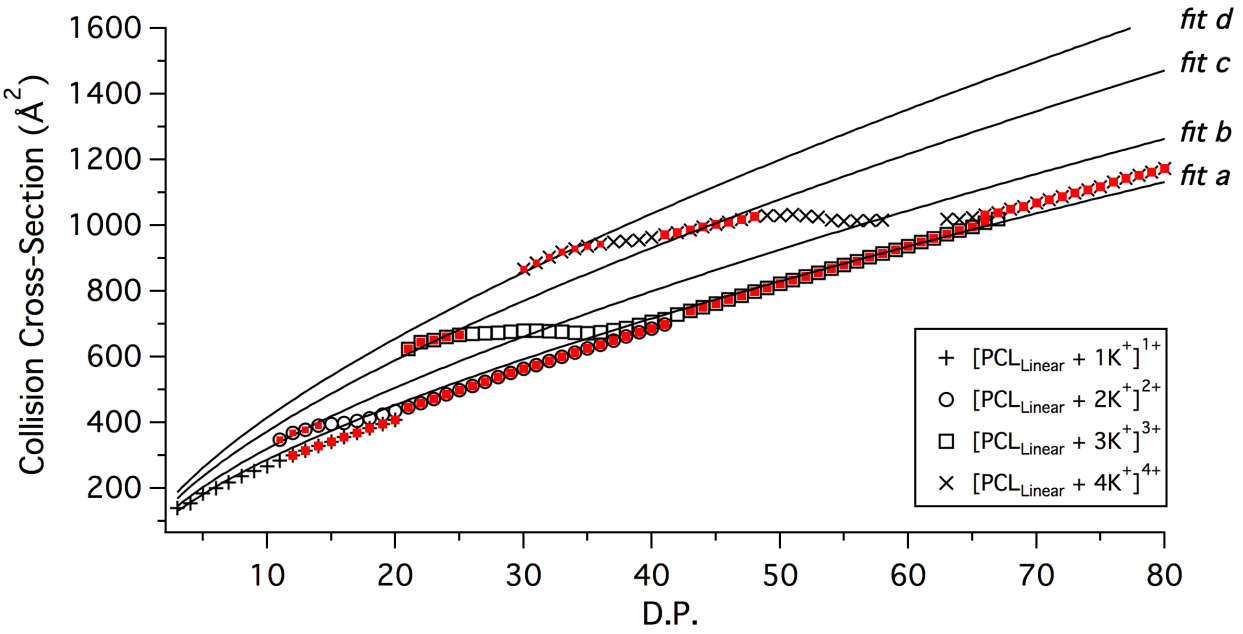

Figure SI14: Figure 3 (CCS vs. DP of linear PCL) with highlighted data points (red dots) used for performing the data fits ($CCS = A.DP^{pow}$, $pow=0.66$).

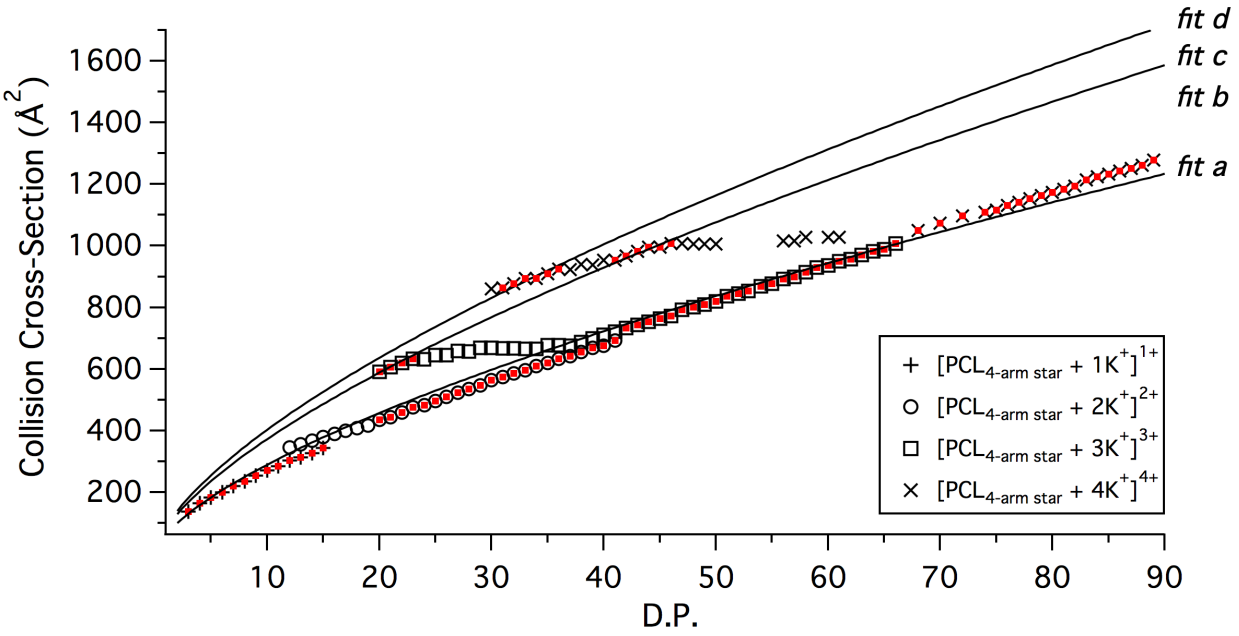

Figure SI15: Figure SI7 (CCS vs. DP of 4-arm star PCL) with highlighted data points (red dots) used for performing the data fits ($CCS = A.DP^{pow}$, $pow=0.66$).

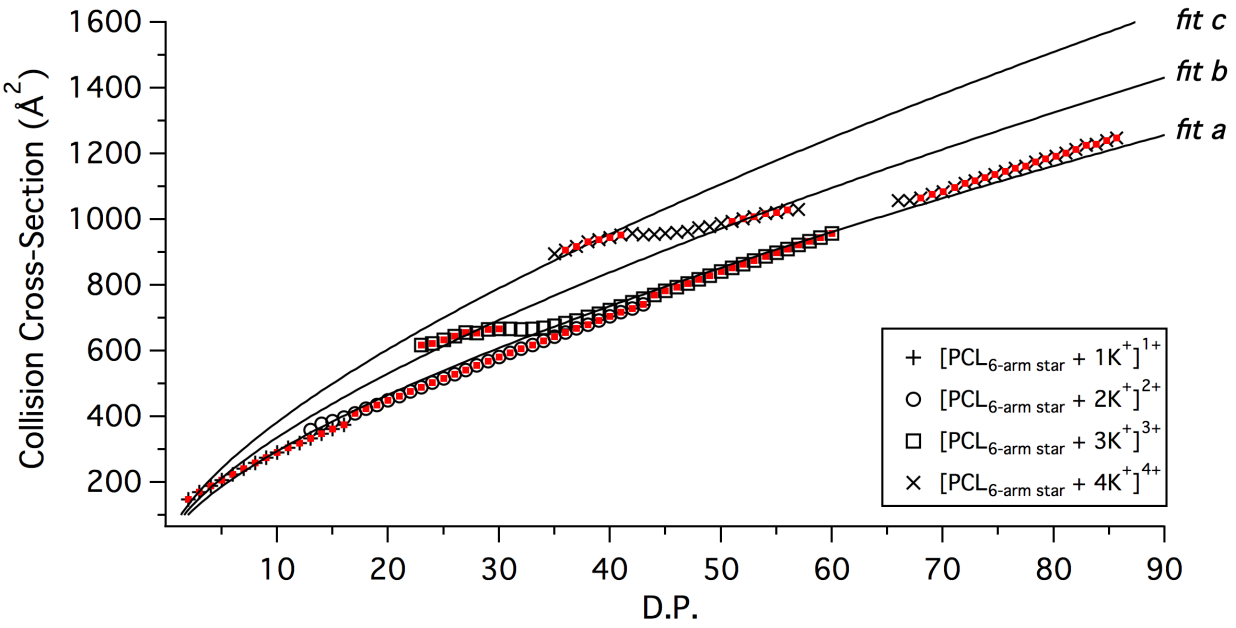

Figure SI16: Figure SI8 (CCS vs. DP of 6-arm star PCL) with highlighted data points (red dots) used for performing the data fits ($CCS = A.DP^{pow}$, $pow=0.66$).

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