Electronic Supplementary Material (ESI) for ChemComm.

Insights into the association and aggregation of the Metamorphic Chemokine Lymphotactin with Fondaparinux.

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Supporting information

Experimental details

TEM studies

For transmission electron microscopy (TEM) studies samples were prepared at 50 µM protein concentration at a 1:1 ratio of Ltn:Fx.. In all cases samples were prepared in 20 mM AmAc at pH 6.8 and left to incubate for 30 minutes before grid preparation. In addition individual solutions of WT Ltn and Fx, at 50 µM in 20 mM AmAc were studied as controls. All transmission electron microscopy (TEM) experiments were performed on a Philips CM120 Biotin transmission electron microscope (Philips, Eindhoven, Netherlands) with an operating voltage of -80 kV. The preparation of TEM grids for analysis was as follows; 3 µL of sample was spotted onto a 3.05 mm, 200 mesh copper grid (Taab, Aldermaston, UK) coated with formvar and carbon and left for five minutes. The grid was then dried and rinsed once with 10 µL high purity water, after which the excess water was wicked off. The sample was then stained using 4 µL 1 % uranyl acetate (w/v) and left for 30 seconds before the excess stain was wicked off. The grid was then left to dry overnight.

MS studies

In all cases samples were prepared in 20 mM AmAc, at pH 6.8 and analysed immediately after sample preparation. For all Ltn samples individual protein plus Fx solutions were prepared at 50 µM protein concentration at a 1:1 ratio of Ltn:Fx. WT Ltn was additionally studied at a 1:1 ratio at 25 and 10 µM, and at a 2:1 ratio (Ltn:Fx) at a 25 µM Ltn concentration. WT 1-72 was also studied at 1:1 and 2:1 ratios (Ltn:Fx) at a 25 µM protein concentration. The interactions of WT 72-93 plus WT 1-72 with Fx were also considered using MS. n-ESI was utilized for all MS and DT IM-MS experiments. Sample solutions were ionized through a potential applied to a thin platinum wire inserted in a glass capillary. Nanoelectrospray tips were made in-house using thin-walled glass capillaries (i.d.0.5 mm) using a Flaming/Brown micropipette puller (Sutter Instrument Company, Novato, CA, U.S.A.).

DT IM- MS studies

For determination of CCS for Ltn:Fx species WT, CC3 (monomer mutant) and W55D (dimer mutant) constructs were prepared at 100 µM protein concentration in 20 mM AmAc at a 1:1 (Ltn:Fx) ratio. WT 1-72 was studied at 50 µM in 20 mM AmAc at a 2:1 ratio with fondparinux. All DT IM-MS measurements were performed on an in-house-modified Q-TOF (Micromass U.K. Ltd.), adapted in order to carry out separations based on an ion's mobility and to enable the temperature dependent CCS to be determined from the measured m/z selected arrival time distributions (ATDs). This is possible via the inclusion of a

5.1 cm long copper drift cell and supplementary ion optics situated post source but before the quadrupole analyzer. The instrument and its operation have been described in detail elsewhere[1]

TEM

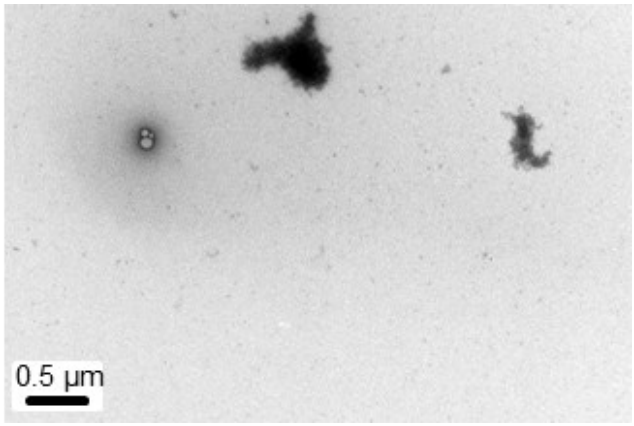

Fig S1: Representative TEM image for Fx at 50 µM concentration in 20 mM ammonium acetate.

MS

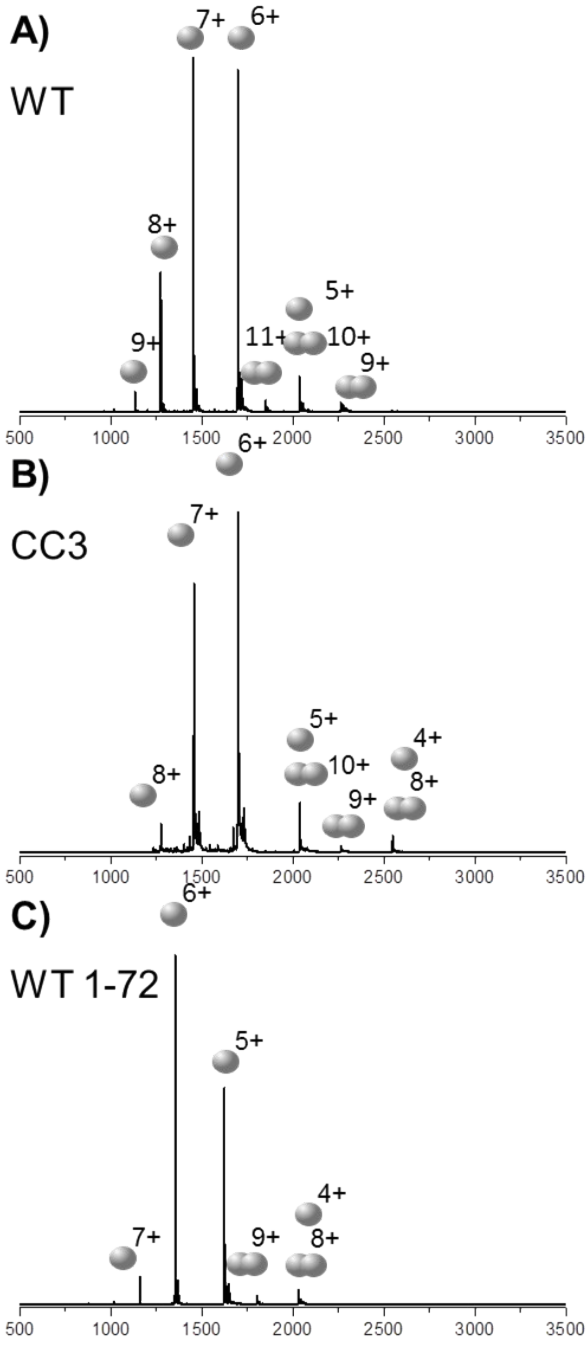

Fig S2: Spectra acquired for Ltn, at 50 µM protein concentration in 20 mM ammonium acetate A) WT ltn B) CC3 C) structural core, WT 1-72 and D) ID tail, WT 72-93

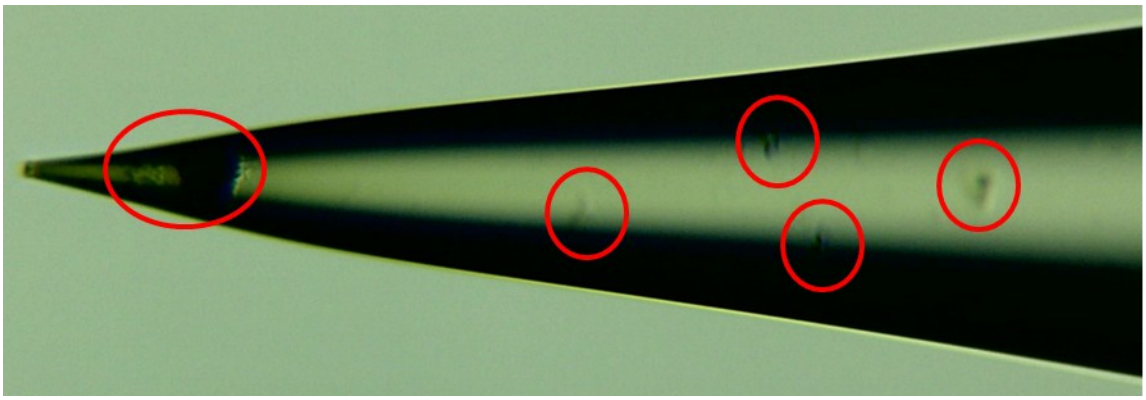

Fig S3: Large visible aggregates circled in red are observed upon addition of Fx to Ltn solutions, representative image shown here for WT 1-72 Ltn plus Fx prepared at a 1:1 ratio of Ltn:Fx, at 50 μM protein concentration in 20 mM ammonium acetate

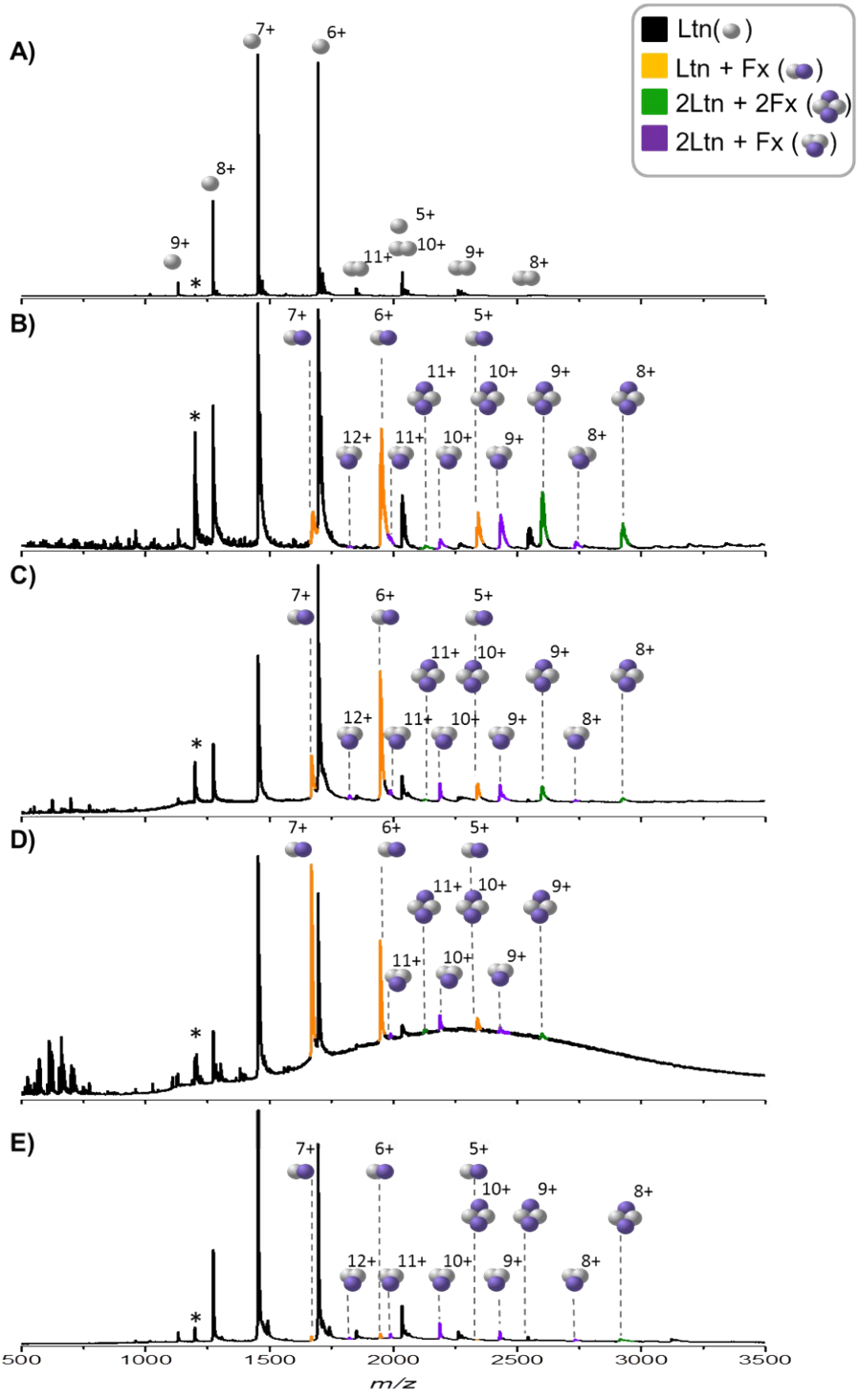

Fig. S4: Spectra obtained for WT Ltn A) 50 µM B) 50 µM plus 50 µM Fx (1:1, Ltn:Fx6587) C) 25 µM plus 25 µM Fx (1:1) D) 10 µM plus 10 µM Fx (1:1) and E) 25 µM plus 12.5 µM Fx (2:1) * denotes a species observed in all spectra presumably a contaminant left over after purification. In panel A all Ltn species are labelled. For figure clarity in panels B-E only GAG bound species are labelled.

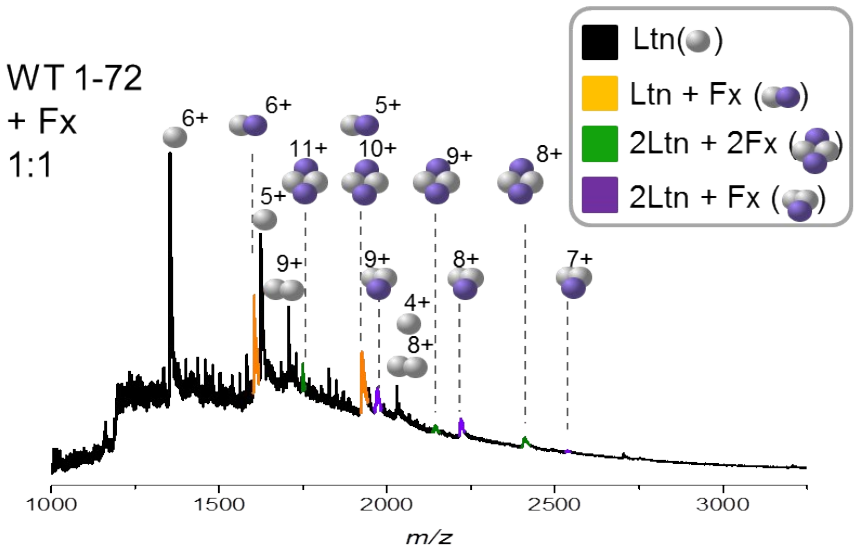

Fig. S5: nESI mass spectrum acquired from a 1:1 mixture of WT 1-72 plus Fx, at 50 µM protein concentration in 20 mM ammonium acetate.

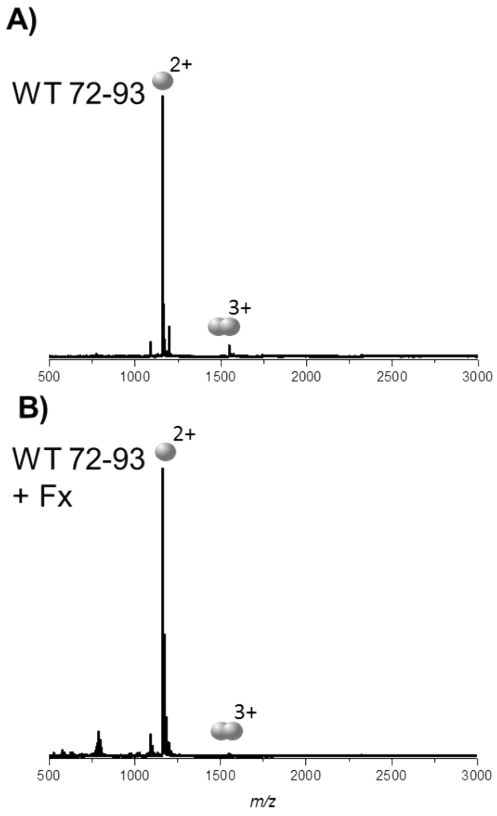

Fig. S6: nESI mass spectrum acquired for A) WT 72-93 and B) WT 72-93 plus Fx, at 50 µM protein concentration in 20 mM ammonium acetate. For Fx studies a stoichiometric concentration of Fx was used.

Species	CCS/ Å² WT	CC3	WT 1-72
$[M+4H]^{4+}$	757±4	-	856±4
$[M+5H]^{5+}$	967±13	1012±2	916±8
$[M+6H]^{6+}$	1021±21	1014±4	950±9
$[M+7H]^{7+}$	1100±63	1163±4	1037,1229±12
$[M+8H]^{8+}$	1528±8	1243±5	1416±8
$[M+9H]^{9+}$	1560±40,1685	-	-
$[D+8H]^{8+}$	1376±25	-	1404±6
$[D+9H]^{9+}$	1605±12,1534	1611±2	1425±8
$[D+10H]^{10+}$	1778±47	1802±5	1603
$[D+11H]^{11+}$	1822	-	1751±10,1872±4
$[D+12H]^{12+}$	1894	-	1861±8
$[D+13H]^{13+}$	1902	-	-
$[M+Fx+4H]^{4+}$	-	-	946
$[M+Fx+5H]^{5+}$	845±27	833±7	995±16
$[M+Fx+6H]^{6+}$	1004±8	1075±21	1232±46
$[M+Fx+7H]^{7+}$	1247±54	1105±13	-
$[D+Fx+7H]^{7+}$	-	-	1403±25,1660±12
$[D+Fx+8H]^{8+}$	-	1424±28	1420±19,1788±5
$[D+Fx+9H]^{9+}$	1530±1	1524±16	1624±20,1884±48,
	-	-	2153±24
$[D+Fx+10H]^{10+}$	1602±7	1716±4	-
$[D+2Fx+8H]^{8+}$	1426±31,1591±37	1423	-
$[D+2Fx+9H]^{9+}$	-	1624±13	-
$[D+2Fx+10H]^{10+}$	1651	1712±5	-

Table S1. Experimental CCS for unbound (determined in absence of Fx[2, 3]) and Fx bound WT, CC3 and WT 1-72 Ltn as shown in Fig. 3. Errors reported as standard deviation between three different day repeats.

Species	CCS/ Å²	
	Absence of Fx	Presence of Fx
$[M_{WT}+6H]^{6+}$	1021 ± 21	1034 ± 47
$[M_{WT}+7H]^{7+}$	1100 ± 63	1077 ± 34
$[D_{WT}+12H]^{12+}$	1894*	1893 ± 21

Table S2: Experimentally determined average CCS for all unbound WT species observed in the absence and presence of Fx, the error is reported as the standard deviation between repeats. *species observed in all repeats but only resolvable in one.

Species	CCS/ Å²	
	Absence of Fx	Presence of Fx
$[M_{CC3}+6H]^{6+}$	1014 ± 4	1030 ± 28
$[M_{CC3}+7H]^{7+}$	1163 ± 4	1181 ± 23

Table S3: Experimentally determined average CCS for all unbound CC3 species observed in the absence and presence of Fx, the error is reported as the standard deviation between repeats.

Species	*CCS/ Å²*	
	Absence of Fx	*Presence of Fx*
$[M_{1-72}+5H]^{5+}$	916 ± 8	909 ± 13
$[M_{1-72}+6H]^{6+}$	950 ± 9	973 ± 20
$[M_{1-72}+6H]^{6+}$	--	1209 ± 13
$[M_{1-72}+7H]^{7+}$	1037*	1142 ± 18
$[M_{1-72}+7H]^{7+}$	1229 ± 12	1211 ± 3

Table S4: Experimentally determined average CCS for all unbound WT 1-72 species observed in the absence and presence of Fx, the error is reported as the standard deviation between repeats. *species observed in all repeats but only resolvable in one.

References

1. B. J. McCullough, J. Kalapothakis, H. Eastwood, P. Kemper, D. MacMillan, K. Taylor, J. Dorin and P. E. Barran, *Analytical chemistry*, 2008, **80**, 6336-6344.
2. S. R. Harvey, M. Porrini, A. Konijnenberg, D. J. Clarke, R. C. Tyler, P. R. Langridge-Smith, C. E. MacPhee, B. F. Volkman and P. E. Barran, *The Journal of Physical Chemistry B*, 2014.
3. S. R. Harvey, M. Porrini, R. Tyler, C. MacPhee, B. Volkman and P. Barran, *Physical Chemistry Chemical Physics*, 2015.