Supporting Information

Ion Mobility-Derived Collision Cross Section as an Additional Measure for Lipid Fingerprinting and Identification

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Figure S2. Composite ion map for the brain lipidome. After isotopic and adduct deconvolution, over 4,000 lipid species were annotated in human brain (black dots). A search against the accurate mass database in LipidMaps and the CCS database lead to the identification of 137 lipids (red dots).

Procedure for manually deriving CCS using a Synapt HDMS

1. Measure the drift time of the ions (t_d) in ms.

2. Subtract instrumental offsets. This is a correction factor (c) which can be found in the Tune page under System, Acquisition Settings EDC delay (mass <5000 Da). This value is set-up for each individual instrument.

3. *Corrected drift time* $t_d' = t_d - (c\sqrt{\ (m/z\ (ion)\ /\ 1000}$) ms

4. To obtain calibration coefficients use published cross-section data (Ω).

5. Correct published cross sections by taking into account reduced mass and charge state.
   Reduced mass $\mu = (M_{ion}\ x\ m_{gas}\ /\ M_{ion}\ +\ m_{gas})$

6. *Normalised cross-section* $\Omega' = (\Omega\ x\ \sqrt{\ }(\mu)\ )/z$

7. Plot t_d' *versus* Ω'.

8. Fit a linear trendline of the form y = ax + b or power trendline of the form $y = Ax^b$ to the data to obtain a calibration curve. (The best fit trendline will depend on the range of the calibration).

9. Convert experimental effective drift times to estimated collision cross-sections using the calibration curve.

Table S1. List of natural lipids and lipid extracts used in this study.

Natural lipids	**Abbreviations**	**Avanti Catalog Number**
L-α-phosphatidylcholine (Brain, Porcine)	PC	840053
L-α-phosphatidylethanolamine (Brain, Porcine)	PE	840022
L-α-lysophosphatidylcholine (Egg, Chicken)	LPC	830071
L-α-lysophosphatidylethanolamine plasmalogen (Brain, Porcine)	LPE	850095
L-α-phosphatidylinositol (Liver, Bovine)	PI	840042
L-α-phosphatidylserine (Brain, Porcine)	PS	840032
L-α-phosphatidylglycerol (Egg, Chicken)	PG	841138
Ceramide (Brain, Porcine)	Cer	860052
Sphingomyelin (Brain Porcine)	SM	860062
Cerebrosides (Brain, Porcine)	HexCer	131303
Sulfatides (Brain, Porcine)	ST	131305
Lipid Extracts		
Porcine brain		131101
E.Coli		100500
Yeast (*S. cerevisiae*)		190000

Table S2. Summary of MS Settings used to measure CCS.

ES^+	Capillary Voltage (kV)	Cone Voltage (V)	Source Temperature (°C)	Desolvation Temperature (°C)	Desolvation Gas Flow (L/hr)	IMS gas (mL/min)	Wave Velocity (m/s)	Wave Height (V)	EDC Delay Coefficient (V)
Synapt 1	2.5	40	120	500	600	90	600	40	1.58
Synapt 2	2.5	30	100	250	200	90	650	37	1.58
Synapt 3	2.5	30	110	200	400	90	650	40	1.44

ES^-	Capillary Voltage (kV)	Cone Voltage (V)	Source Temperature (°C)	Desolvation Temperature (°C)	Desolvation Gas Flow (L/hr)	IMS gas (mL/min)	Wave Velocity (m/s)	Wave Height (V)	EDC Delay Coefficient (V)
Synapt 1	2.5	30	120	500	1000	90	900	40	1.58
Synapt 2	2.9	45	100	250	200	90	790	40	1.58
Synapt 3	3.0	40	110	200	400	80	600	40	1.41

Table S3. Collision cross-sections (CCS) reference values for singly charged protonated and deprotonated oligomers of polyalanine in nitrogen (1).

Sequence	Molecular Weight	Positive Ionization		Negative Ionization	
		$[M+H]^+$	CCS ($Å^2$)	$[M-H]^-$	CCS($Å^2$)
A_3	231.122	232.130	151	230.114	150
A_4	302.159	303.167	166	301.151	165
A_5	373.196	374.204	181	372.188	179
A_6	444.233	445.241	195	443.225	195
A_7	515.270	516.278	211	514.262	209
A_8	586.307	587.315	228	585.300	223
A_9	657.345	658.352	243	656.337	238
A_{10}	728.382	729.390	256	727.374	253
A_{11}	799.419	800.427	271	798.411	267
A_{12}	870.456	871.464	282	869.448	279
A_{13}	941.493	942.501	294	940.485	294
A_{14}	1012.530	1013.538	306	1011.522	308

Reference

1. Bush, M. F., I. D. Campuzano, and C. V. Robinson. 2012. Ion mobility mass spectrometry of peptide ions: effects of drift gas and calibration strategies. *Analytical chemistry* **84**: 7124-7130.

Table S4. CCS database for lipid cations in nitrogen. CCS values may represent a combination of structurally similar isomers that remain unresolved.

Lipid	*m/z*	Ion	CCS
PC 32:1	732.554	$[M+H]^+$	287
PC 32:0	734.569	$[M+H]^+$	291
PC 34:3	756.554	$[M+H]^+$	293
PC 34:2	758.569	$[M+H]^+$	293
PC 34:1	760.585	$[M+H]^+$	295
PC 34:0	762.601	$[M+H]^+$	297
PC 36:5	780.554	$[M+H]^+$	296
PC 36:4	782.569	$[M+H]^+$	298
PC 36:3	784.585	$[M+H]^+$	299
PC 36:2	786.601	$[M+H]^+$	301
PC 36:1	788.616	$[M+H]^+$	305
PC 36:0	790.632	$[M+H]^+$	305
PC 38:7	804.554	$[M+H]^+$	300
PC 38:6	806.569	$[M+H]^+$	300
PC 38:5	808.585	$[M+H]^+$	302
PC 38:4	810.601	$[M+H]^+$	305
PC 40:8	830.569	$[M+H]^+$	304
PC 40:7	832.585	$[M+H]^+$	306
PC 40:6	834.601	$[M+H]^+$	309
PC 40:5	836.616	$[M+H]^+$	311
PC 40:4	838.632	$[M+H]^+$	312

Lipid	*m/z*	Ion	CCS
Cer d18:1/14:0	492.478	$[M-H_2O+H]^+$	251
Cer d18:1/16:0	520.509	$[M-H_2O+H]^+$	258
Cer d18:1/18:0	548.540	$[M-H_2O+H]^+$	266
Cer d18:1/20:0	576.571	$[M-H_2O+H]^+$	273
Cer d18:1/22:0	604.603	$[M-H_2O+H]^+$	279
Cer d18:1/24:1	630.618	$[M-H_2O+H]^+$	285
Cer d18:1/24:0	632.634	$[M-H_2O+H]^+$	287
Cer d18:1/26:1	658.650	$[M-H_2O+H]^+$	293
Cer d18:1/26:0	660.665	$[M-H_2O+H]^+$	295
Cer d18:0/16:0	540.535	$[M+H]^+$	261
Cer d18:0/18:0	568.566	$[M+H]^+$	272
Cer d18:0/20:0	596.598	$[M+H]^+$	277
Cer d18:0/22:0	624.629	$[M+H]^+$	284
Cer d18:0/24:0	652.66	$[M+H]^+$	293
Cer d18:0/24:1	650.645	$[M+H]^+$	291
Cer d18:0/26:0	680.692	$[M+H]^+$	299
Cer d18:0/26:1	678.676	$[M+H]^+$	297
SM d18:1/16:0	703.575	$[M+H]^+$	291
SM d18:1/18:0	731.606	$[M+H]^+$	297
SM d18:1/20:0	759.637	$[M+H]^+$	305
SM d18:1/22:1	785.653	$[M+H]^+$	307
SM d18:1/22:0	787.669	$[M+H]^+$	311

PCp 34:1;PCe 34:2	744.590	$[M+H]^+$	295
PCe 34:1;PCp 34:0	746.606	$[M+H]^+$	298
PCe 34:0	748.621	$[M+H]^+$	299
PCe 34:3;PCp 34:2	742.575	$[M+H]^+$	294
PCe 36:1;PCp 36:0	774.637	$[M+H]^+$	301
PCe 36:2;PCp 38:1	772.621	$[M+H]^+$	303
PCe 36:3;PCp 36:2	770.606	$[M+H]^+$	301
PCe 36:4;PCp 36:3	768.590	$[M+H]^+$	299
PCp 36:4; PCe 36:5	766.575	$[M+H]^+$	297
PCe 38:1;PCp 38:0	802.668	$[M+H]^+$	305
PCe 38:2;PCp 38:1	800.653	$[M+H]^+$	303
PCe 38:3;PCp 38:2	798.637	$[M+H]^+$	301
PCe 38:4;PCp 38:3	796.621	$[M+H]^+$	304
PCe 38:5;PCp 38:4	794.606	$[M+H]^+$	304
PCe 38:6;PCp 38:5	792.590	$[M+H]^+$	303
PCe 40:6;PCp 40:5	820.621	$[M+H]^+$	312
PC(O-16:0/0:0)	482.361	$[M+H]^+$	234
PC(P-16:0/0:0)	480.345	$[M+H]^+$	230
PC(P-18:0/0:0)	508.376	$[M+H]^+$	232
PC(O-18:0/0:0)	510.392	$[M+H]^+$	241
LPC 14:0	468.308	$[M+H]^+$	227
LPC 16:1	494.324	$[M+H]^+$	229
LPC 16:0	496.340	$[M+H]^+$	236
LPC 18:1	522.355	$[M+H]^+$	240
LPC 18:0	524.371	$[M+H]^+$	246
LPC 20:4	544.340	$[M+H]^+$	242
LPC 20:3	546.355	$[M+H]^+$	249

SM d18:1/24:1	813.684	$[M+H]^+$	315
SM d18:1/24:0	815.700	$[M+H]^+$	316
SM d18:1/26:0	843.731	$[M+H]^+$	324
SM d18:1/26:1	841.716	$[M+H]^+$	320
SM d18:0/16:0	705.591	[M+H]+	291
SM d18:0/18:0	733.622	[M+H]+	299
SM d18:0/20:0	761.653	[M+H]+	305
SM(d18:0/22:1(OH))	801.648	[M+H]+	313
SM d18:0/22:0	789.684	[M+H]+	312
SM d18:0/24:0	817.716	[M+H]+	320
SM d18:0/26:0	845.747	[M+H]+	326
HexCer d18:1/12:0	644.51	[M+H]+	277
HexCer d18:1/16:0	700.572	[M+H]+	284
HexCer d18:1/18:0	728.604	$[M+H]^+$	286
HexCer d18:1/h18:0	744.598	$[M+H]^+$	288
HexCer d18:1/20:0	756.635	$[M+H]^+$	291
HexCer d18:1/h20:0	772.630	$[M+H]^+$	293
HexCer d18:1/22:1	782.650	$[M+H]^+$	297
HexCer d18:1/h22:0	800.661	$[M+H]^+$	298
HexCer d18:1/23:0	798.682	$[M+H]^+$	296
HexCer d18:1/h23:0	814.677	$[M+H]^+$	303
HexCer d18:1/24:1	810.682	$[M+H]^+$	301
HexCer d18:1/h24:1	826.677	$[M+H]^+$	304
HexCer d18:1/24:0	812.697	$[M+H]^+$	304
HexCer d18:1/h24:0	828.692	$[M+H]^+$	306
HexCer d18:1/26:0	840.729	$[M+H]^+$	309

LPC 20:2	548.371	$[M+H]^+$	250
Cholesterol	369.352	$[M-H_2O+H]^+$	205

HexCer d18:1/h26:0	856.724	$[M+H]^+$	312
HexCer d18:1/26:1	838.713	$[M+H]^+$	307
HexCerd18:1/h26:1	854.708	$[M+H]^+$	313

Abbreviations: Ether-linked isobaric species of plasmanyl (e) and plasmenyl (p) analogues of glycerophospholipids.

Table S5. CCS database for lipid anions in nitrogen. CCS values may represent a combination of structurally similar isomers that remain unresolved.

Lipid	*m/z*	Ion	CCS
FA C16:0	255.232	$[M-H]^-$	170
FA C17:0	269.248	$[M-H]^-$	174
FA C18:0	283.264	$[M-H]^-$	178
FA C20:0	311.295	$[M-H]^-$	187
FA C22:0	339.326	$[M-H]^-$	191
FA C24:0	367.358	$[M-H]^-$	200
FA C18:1	281.248	$[M-H]^-$	175
FA C18:2	279.232	$[M-H]^-$	172
FA C18:3	277.217	$[M-H]^-$	170
FA C20:1	309.279	$[M-H]^-$	183
FA C22:1	337.311	$[M-H]^-$	189
FA C24:1	365.342	$[M-H]^-$	195
FA C20:3	305.248	$[M-H]^-$	178
FA C20:4	303.232	$[M-H]^-$	182
FA C20:5	301.217	$[M-H]^-$	184
FA C22:4	331.264	$[M-H]^-$	188
FA C22:5	329.248	$[M-H]^-$	188
FA C22:6	327.232	$[M-H]^-$	187
PEe 34:3; PEp 34:2	698.513	$[M-H]^-$	270
PEe 34:2;PEp 34:1	700.528	$[M-H]^-$	272
PEe 34:1;PEp 34:0	702.544	$[M-H]^-$	274

Lipid	*m/z*	Ion	CCS
PG 34:2	745.503	$[M-H]^-$	279
PG 34:1	747.518	$[M-H]^-$	281
PG 34:0	749.534	$[M-H]^-$	283
PG 36:4	769.503	$[M-H]^-$	285
PG 36:3	771.518	$[M-H]^-$	287
PG 36:2	773.534	$[M-H]^-$	289
PG 36:1	775.549	$[M-H]^-$	290
PG 36:0	777.565	$[M-H]^-$	292
PG 38:6	793.503	$[M-H]^-$	290
PG 38:5	795.518	$[M-H]^-$	291
PG 38:4	797.534	$[M-H]^-$	293
PG 38:3	799.549	$[M-H]^-$	294
PG 40:7	819.518	$[M-H]^-$	296
PG 40:6	821.534	$[M-H]^-$	297
PG 40:5	823.549	$[M-H]^-$	299
PG 40:4	825.565	$[M-H]^-$	301
PS 34:2	758.498	$[M-H]^-$	280
PS 34:1	760.513	$[M-H]^-$	282
PS 34:0	762.529	$[M-H]^-$	283
PS 36:5	780.482	$[M-H]^-$	282
PS 36:4	782.498	$[M-H]^-$	283

PE 34:2	714.507	$[M-H]^-$	271
PE 34:1	716.523	$[M-H]^-$	272
PE 34:0	718.533	$[M-H]^-$	274
PEe 36:6;PEp 36:5	720.497	$[M-H]^-$	274
PEe 36:5;PEp 36:4	722.513	$[M-H]^-$	275
PEe 36:3;PEp 36:2	726.544	$[M-H]^-$	277
PEe 36:2;PEp 36:1	728.560	$[M-H]^-$	279
PE 36:5	736.492	$[M-H]^-$	273
PE 36:4	738.507	$[M-H]^-$	274
PE 36:3	740.523	$[M-H]^-$	276
PE 36:2	742.539	$[M-H]^-$	279
PE 36:1	744.555	$[M-H]^-$	281
PEe 38:7;PEp 38:6	746.513	$[M-H]^-$	279
PEe 38:6;PEp 38:5	748.529	$[M-H]^-$	280
PEe 38:5;PEp 38:4	750.544	$[M-H]^-$	282
PEe 38:4;PEp 38:3	752.560	$[M-H]^-$	284
PEe 38:3;PEp 38:2	754.576	$[M-H]^-$	285
PEe 38:2;PEp 38:1	756.591	$[M-H]^-$	287
PE 38:6	762.508	$[M-H]^-$	280
PE 38:6	762.508	$[M-H]^-$	280
PE 38:5	764.524	$[M-H]^-$	283
PE 38:4	766.539	$[M-H]^-$	284
PEe 40:8;PEp 40:7	772.529	$[M-H]^-$	284
PEe 40:7;PEp 40:6	774.544	$[M-H]^-$	286
PEe 40:6;PEp 40:5	776.560	$[M-H]^-$	288
PEe 40:5;PEp 40:4	778.576	$[M-H]^-$	289

PS 36:3	784.513	$[M-H]^-$	285
PS 36:2	786.529	$[M-H]^-$	288
PS 36:1	788.545	$[M-H]^-$	290
PS 38:6	806.498	$[M-H]^-$	288
PS 38:5	808.513	$[M-H]^-$	290
PS 38:4	810.529	$[M-H]^-$	294
PS 38:3	812.545	$[M-H]^-$	296
PS 38:2	814.56	$[M-H]^-$	298
PS 38:1	816.576	$[M-H]^-$	300
PS 40:7	832.513	$[M-H]^-$	298
PS 40:6	834.529	$[M-H]^-$	300
PS 40:5	836.545	$[M-H]^-$	302
PS 40:4	838.56	$[M-H]^-$	303
PS 40:3	840.576	$[M-H]^-$	305
PS 40:2	842.592	$[M-H]^-$	308
PI 32:0	809.519	$[M-H]^-$	288
PI 32:1	807.503	$[M-H]^-$	286
PI 32:2	805.487	$[M-H]^-$	284
PI 34:0	837.550	$[M-H]^-$	294
PI 34:1	835.534	$[M-H]^-$	292
PI 34:2	833.519	$[M-H]^-$	292
PI 36:0	865.581	$[M-H]^-$	303
PI 36:1	863.565	$[M-H]^-$	304
PI 36:2	861.55	$[M-H]^-$	300
PI 36:3	859.534	$[M-H]^-$	297

PEe 40:4;PEp 40:3	780.591	$[M-H]^-$	291
PEe 40:3;PEp 40:2	782.607	$[M-H]^-$	293
PEe 40:2;PEp 40:1	784.623	$[M-H]^-$	294
PE 40:7	788.524	$[M-H]^-$	285
PE 40:6	790.539	$[M-H]^-$	288
PE 40:5	792.555	$[M-H]^-$	290
PE 40:4	794.571	$[M-H]^-$	295
PE 40:3	796.586	$[M-H]^-$	295
PE 40:2	798.602	$[M-H]^-$	297
LPE 16:1	450.263	$[M-H]^-$	210
LPE 16:0	452.278	$[M-H]^-$	212
LPE 18:1	478.294	$[M-H]^-$	217
LPE 18:0	480.310	$[M-H]^-$	219
LPE 20:4	500.278	$[M-H]^-$	219
LPE 20:3	502.294	$[M-H]^-$	220
LPE 20:2	504.310	$[M-H]^-$	222
LPE 20:1	506.326	$[M-H]^-$	224
LPE 20:0	508.341	$[M-H]^-$	225
LPE 20:5	498.263	$[M-H]^-$	219
LPE 22:6	524.278	$[M-H]^-$	223
LPE 22:5	526.294	$[M-H]^-$	224
LPE 22:4	528.309	$[M-H]^-$	225
PE (P-18:1/0:0)	462.298	$[M-H]^-$	215
PE(O-18:0/0:0)	466.330	$[M-H]^-$	215
PE(O-16:0/0:0)	438.298	$[M-H]^-$	209

PI 36:4	857.519	$[M-H]^-$	295
PI 36:5	855.503	$[M-H]^-$	292
PI 38:2	889.581	$[M-H]^-$	312
PI 38:3	887.565	$[M-H]^-$	309
PI 38:4	885.55	$[M-H]^-$	308
PI 38:5	883.534	$[M-H]^-$	306
PI 38:6	881.519	$[M-H]^-$	303
PI 40:4	913.581	$[M-H]^-$	319
PI 40:5	911.565	$[M-H]^-$	317
PI 40:6	909.55	$[M-H]^-$	315
PI 40:7	907.534	$[M-H]^-$	313
C18 Sulfatide	806.546	$[M-H]^-$	292
C16 Sulfatide	778.514	$[M-H]^-$	286
C16-OH Sulfatide	794.509	$[M-H]^-$	292
C18-OH Sulfatide	822.541	$[M-H]^-$	298
C20-OH Sulfatide	850.572	$[M-H]^-$	302
C22 Sulfatide	862.608	$[M-H]^-$	306
C20 Sulfatide	834.577	$[M-H]^-$	296
C22-OH Sulfatide	878.603	$[M-H]^-$	302
C24 Sulfatide	890.64	$[M-H]^-$	313
C24-OH Sulfatide	906.635	$[M-H]^-$	317
C24:1 Sulfatide	888.624	$[M-H]^-$	309
C24:1-OH Sulfatide	904.619	$[M-H]^-$	312

PE(P-16:0/0:0)	436.283	$[M-H]^-$	211
PE(P-18:0/0:0)	464.314	$[M-H]^-$	217

Abbreviations: Ether-linked isobaric species of plasmanyl (e) and plasmenyl (p) analogues of glycerophospholipids.

Table S6. Comparison of CCS values reported in our database with those of previously published reports.

Lipid Species	CCS ($Å^2$) in N_2				CCS ($Å^2$) in He		
	May et al., 2014 (1) †	Damen et al., 2014 (2)*	Database *		Kim et al., 2009 (3) ‡	Fenn et al., 2009 (4) † #	Database *
PC 34:2	291.3 $[M+K]^+$		292.8 $[M+H]^+$		218.7 $[M+H]^+$	217.4 $[M+H]^+$	213.4 $[M+H]^+$
PC 36:2		302.8 $[M+H]^+$	301.3 $[M+H]^+$		224.4 $[M+H]^+$	222.6 $[M+H]^+$	220.9 $[M+H]^+$

Abbreviations: PC, phosphatidylcholine; N_2, Nitrogen; He, Helium.
*Values calculated TWIM-MS instrument using power fit calibration curve for polyalanine in Nitrogen(5) or Helium(6);
† Values generated in drift tube instrument;
‡ Values generated in TWIM-MS instrument using power fit calibration curve for peptides and lipids in Helium;
# MALDI-generated data.

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Table S7. Representative brain lipid species were matched against our database for either just *m/z* (at 5 ppm and 10ppm) or both *m/z* and CCS.

Measured *m/z*	**Measured CCS (Å^2)**	**# of matches at 5ppm**	**# of matches at 10ppm**	**# of matches at 10ppm + 2% CCS**	**Database matches**	**Database *m/z***	**Database CCS (Å^2)**
731.6091	305	1	1	0	SM d18:1/18:0	731.6061	297
731.6097	300	0	1	1	SM d18:1/18:0	731.6061	297
834.6021	313	1	1	1	PC 40:6	834.6007	309
834.6064	301	0	1	0	PC 40:6	834.6007	309
834.6083	248	0	1	0	PC 40:6	834.6007	309

Missed Match
Mismatch

Table S8. Comparison of different tolerance criteria applied to identify brain lipids against our database.

Tolerance Criteria	**A**	**B**	**C**
	Without CCS	**Without CCS**	**With CCS**
m/z tolerance (±)	5ppm	10 ppm	10 ppm
CCS tolerance (±)	-	-	2%
Matched IDs	21	55	34
Missed matches	13	0	0
Mismatched	9	21	0

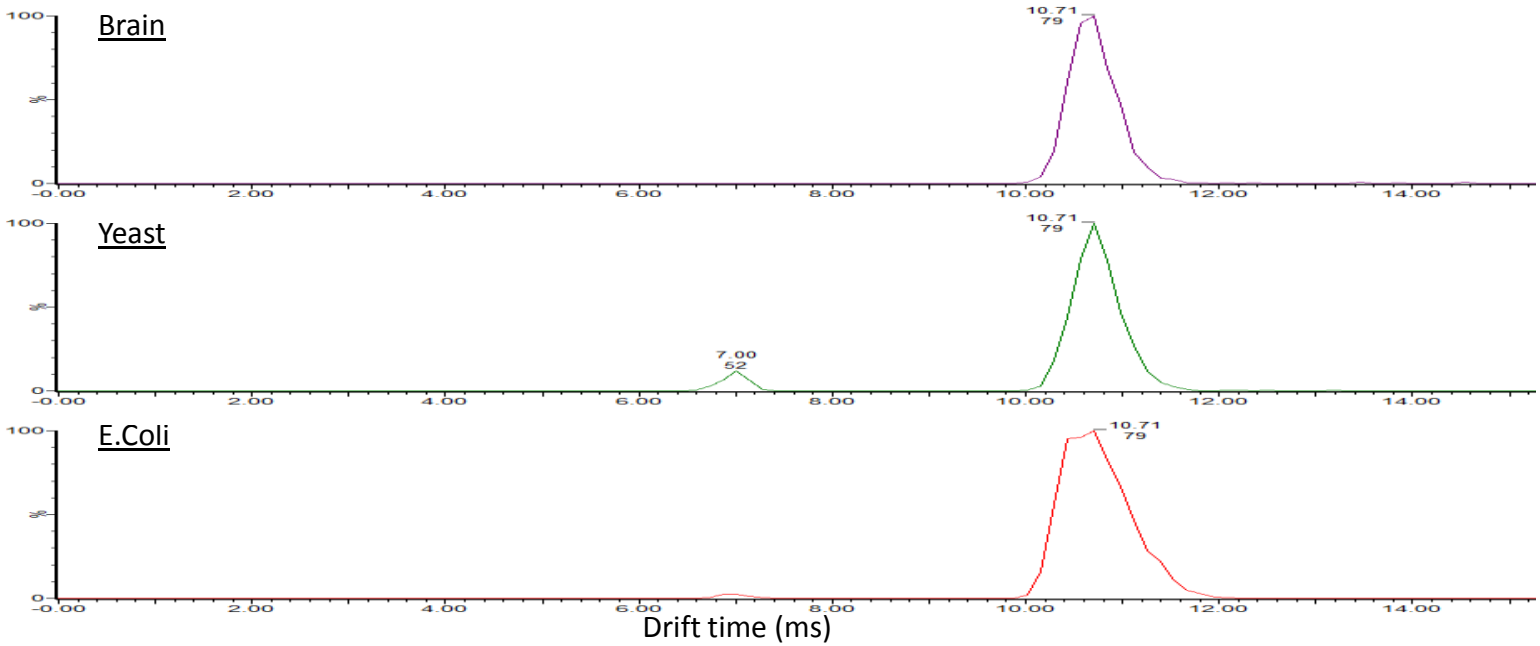
Supplementary Figure Legends

Figure S1. a, Reproducibility of the drift time separation for PE 34:1 in lipid extracts from porcine brain, yeast and E.Coli. **b,** CCS determinations for various lipid species across the range of lipid extracts and comparison with the values reported in our CCS database.

Figure S2. Composite ion map for the brain lipidome. After isotopic and adduct deconvolution, over 4,000 lipid species were annotated in human brain (black dots). A search against the accurate mass database in LipidMaps and the CCS database lead to the identification of 137 lipids (red dots).

Fig. S1

a



b

			CCS (Å²)			
Lipid Species	***m/z***	**ESI**	**E.Coli**	**Yeast**	**Brain**	**Database**
PC 34:2	758.57	$[M+H]^+$	296	298	296	**293**
PE 34:1	716.52	$[M-H]^-$	272	272	272	**272**
PG 34:1	747.52	$[M-H]^-$	283	279	277	**281**

Fig. S2