

SUPPLEMENTARY INFORMATION FOR

Low charge and reduced mobility of membrane protein complexes has implications for calibration of collision cross section measurements

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Figure S1. Charge state and Ω of membrane and commonly used soluble calibrant proteins.

Figure S2. Linear representation of a calibration showing deviation of membrane proteins.

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Figure S4. Gas-phase unfolding of the 16+ charge state of AmtB

Table S1. Literature Ω values and calculated mobility values in He for soluble calibrant proteins.¹ All Ω values are given in Å² and mobility values in cm²/Vs.

β-lactoglobulin (dimer)		
MW: 36,550 Da		
z	Ω (He)	Mobility
11	2,850	2.251×10^{-2}
12	2,900	2.414×10^{-2}
13	2,960	2.562×10^{-2}
Concanavalin A (tetramer)		
MW: 103,000 Da		
z	Ω (He)	Mobility
19	5,550	1.997×10^{-2}
20	5,550	2.102×10^{-2}
21	5,550	2.207×10^{-2}
22	5,480	2.342×10^{-2}
Alcohol dehydrogenase (dimer)		
MW: 143,000 Da		
z	Ω (He)	Mobility
23	7,420	1.808×10^{-2}
24	7,450	1.879×10^{-2}
25	7,440	1.960×10^{-2}
Pyruvate kinase (tetramer)		
MW: 237,000 Da		
z	Ω (He)	Mobility
30	10,300	1.699×10^{-2}
31	10,300	1.755×10^{-2}
32	10,300	1.812×10^{-2}
33	10,200	1.887×10^{-2}
34	10,200	1.944×10^{-2}

Table S2. Membrane protein Ω values from literature and calculated mobility values in He.^{2,3}
All Ω values are given in \AA^2 and mobility values in cm^2/Vs .

AmtB (trimer)		
MW: 126,720 Da		
z	Ω (He)	Mobility
14	5,870	1.391×10^{-2}
15	5,890	1.485×10^{-2}
16	5,910	1.579×10^{-2}
17	6,010	1.650×10^{-2}
AqpZ (tetramer)		
MW: 98,870 Da		
z	Ω (He)	Mobility
11	5,030	1.276×10^{-2}
12	5,100	1.372×10^{-2}
13	5,090	1.490×10^{-2}
14	5,120	1.595×10^{-2}
MATE (monomer)		
MW: 50,120 Da		
z	Ω (He)	Mobility
8	3,290	1.419×10^{-2}
9	3,340	1.572×10^{-2}
10	3,350	1.742×10^{-2}

Table S3. The percentage difference to DTIMS Ω values for membrane proteins using different combinations of *charge-reduced* soluble protein calibrants with wave height setting of 13 V. The difference is calculated as the average absolute value across all native-like folded charge states. ADH: Alcohol dehydrogenase (charge states 18–22+); ConA: Concanavalin A (charge states 14–19+); PKin: Pyruvate kinase (charge states 26–29+). Ω values determined by DTIMS for the charge-reduced soluble proteins are listed in Table S7.

% Difference	Calibrant combination
<i>MATE, MW: 50,120 Da (charge states 8–10)</i>	
5.5%	ConA
5.8%	ConA, PKin
7.7%	ADH, ConA
7.8%	ADH, ConA, PKin
9.1%	PKin
9.3%	ADH, PKin
9.3%	ADH
<i>AqpZ, MW: 98,870 Da (charge states 12–14)</i>	
0.9%	ADH, ConA
0.9%	ADH, ConA, PKin
1.4%	PKin
1.4%	ADH
1.4%	ADH, PKin
1.5%	ConA, PKin
2.9%	ConA
<i>AmtB, MW: 126,720 Da (charge states 15–17)</i>	
6.4%	PKin
6.4%	ADH, PKin
6.4%	ADH
7.3%	ADH, ConA, PKin
7.5%	ConA, PKin
8.8%	ADH, ConA
11.1%	ConA

Table S4. The percentage difference to DTIMS Ω values for membrane proteins using different combinations of soluble protein calibrants with wave height setting of 13 V. The difference is calculated as the average absolute value across all native-like folded charge states. ADH: Alcohol dehydrogenase (charge states 23–25+); BLac: β -lactoglobulin (charge states 11–13+); ConA: Concanavalin A (charge states 19–22+); PKin: Pyruvate kinase (charge states 29–34+).

% Difference	Calibrant combination
<i>MATE, MW: 50,120 Da</i>	
6.1%	ConA
6.8%	ADH
8.9%	ADH, ConA
12.8%	BLac, PKin
13.1%	ADH, ConA, PKin
13.2%	ADH, PKin
13.3%	ConA, PKin
13.4%	ADH, BLac, PKin
13.8%	BLac, ConA, PKin
14.0%	ADH, BLac, ConA, PKin
14.2%	PKin
15.6%	ADH, BLac
16.9%	ADH, BLac, ConA
18.8%	BLac, ConA
43.8%	BLac
<i>AqpZ, MW: 98,870 Da</i>	
0.8%	ADH, ConA
3.9%	ConA
4.5%	ADH
6.2%	PKin
8.1%	ADH, ConA, PKin
8.2%	ADH, PKin
8.2%	ConA, PKin
10.7%	BLac, PKin
11.2%	ADH, BLac, PKin
11.3%	BLac, ConA, PKin
11.5%	ADH, BLac, ConA, PKin
14.3%	ADH, BLac
15.8%	ADH, BLac, ConA
18.3%	BLac, ConA
69.3%	BLac

% Difference	Calibrant combination
<i>AmtB, MW: 126,720 Da</i>	
1.2%	ADH, ConA, PKin
1.3%	ADH, PKin
1.4%	PKin
1.4%	ConA, PKin
4.5%	BLac, PKin
5.0%	ADH, BLac, PKin
5.1%	BLac, ConA, PKin
5.3%	ADH, BLac, ConA
7.1%	ADH, ConA
8.5%	ADH, BLac
10.1%	ADH, BLac, ConA
12.4%	ConA
12.9%	BLac, ConA
13.2%	ADH
84.8%	BLac

Table S5. The percentage difference to DTIMS Ω values for membrane proteins using different combinations of soluble protein calibrants with wave height setting of 15 V. The difference is calculated as the average absolute value across all native-like folded charge states. ADH: Alcohol dehydrogenase (charge states 22–25+); BLac: β -lactoglobulin (charge states 11–13+); ConA: Concanavalin A (charge states 19–21+); PKin: Pyruvate kinase (charge states 30–34+).

% Difference	Calibrant combination
<i>MATE, MW: 50,120 Da</i>	
2.1%	PKin
4.8%	ADH
4.9%	ConA
5.4%	ADH, ConA, PKin
5.6%	ConA, PKin
5.7%	ADH, BLac
6.2%	ADH, PKin
6.9%	ADH, ConA
7.2%	ADH, BLac, PKin
7.6%	ADH, BLac, ConA, PKin
7.6%	BLac, PKin
8.0%	ADH, BLac, ConA
8.3%	BLac, ConA, PKin
12.7%	BLac, ConA
56.5%	BLac
<i>AqpZ, MW: 98,870 Da</i>	
2.6%	ConA, PKin
2.9%	ADH, ConA, PKin
3.8%	ADH, BLac
3.9%	ADH, PKin
5.8%	ADH, BLac, PKin
6.1%	ADH, BLac, ConA,
6.3%	BLac, PKin
6.6%	ADH, BLac, ConA
6.9%	BLac, ConA, PKin
7.5%	PKin
12.4%	ADH
12.5%	BLac, ConA
13.3%	ConA
16.1%	ADH, ConA
76.7%	BLac

% Difference	Calibrant combination
<i>AmtB, MW: 126,720 Da</i>	
1.3%	ADH, BLac, ConA
1.4%	BLac, ConA, PKin
1.4%	BLac, PKin
1.4%	ADH, BLac, ConA, PKin
1.6%	ADH, BLac, PKin
2.8%	ADH, BLac
2.8%	ADH, PKin
4.7%	ADH, ConA, PKin
5.1%	ConA, PKin
6.7%	BLac, ConA
16.2%	PKin
21.0%	ADH
22.2%	ConA
25.3%	ADH, ConA
91.7%	BLac

Table S6. Selected charge states of proteins plotted in Figure 2. ADH: Alcohol dehydrogenase; BLac: β -lactoglobulin; ConA: Concanavalin A; PKin: Pyruvate kinase.

Panel	Protein	Charge States
a	PKin	30–34+
	ADH	22–25+
	ConA	19–21+
	BLac	11–13+
	MATE	8–10+
	AqpZ	12–14+
	AmtB	14–17+
b	PKin	29–34+
	ADH	23–25+
	ConA	19–22+
	BLac	11–13+
	MATE	8–10+
	AqpZ	11–14+
	AmtB	13–17+
c	PKin	30–33+
	ADH	21–25+
	ConA	19–21+
	BLac	11–13+
	MATE	8–10+
	AmtB	15–17+
e	ConA	19–22+
	ConA-Z-Reduced	14–17+
	AqpZ	11–14+
f	PKin	29–34+
	AmtB	13–17+

Table S7. DTIMS-measured Ω values of charge-reduced soluble protein calibrants. The drift cell gas was helium. ADH: Alcohol dehydrogenase; ConA: Concanavalin A; PKin: Pyruvate kinase. *N*: oligomeric state.

Protein	<i>N</i>	Mass (kDa)	<i>z</i>	Ω_{He} (nm ²)
ADH <i>Saccharomyces cerevisiae</i>	4	143	18+	69.3
			19+	69.2
			20+	68.8
			21+	68.5
			22+	67.8
ConA <i>Canavalia ensiformis</i>	4	103	14+	56.8
			15+	56.6
			16+	56.3
			17+	56.1
			18+	55.9
PKin Rabbit heart	4	237	19+	55.6
			24+	11.2
			25+	11.1
			26+	10.9
			27+	10.9
			28+	10.8
			29+	10.8

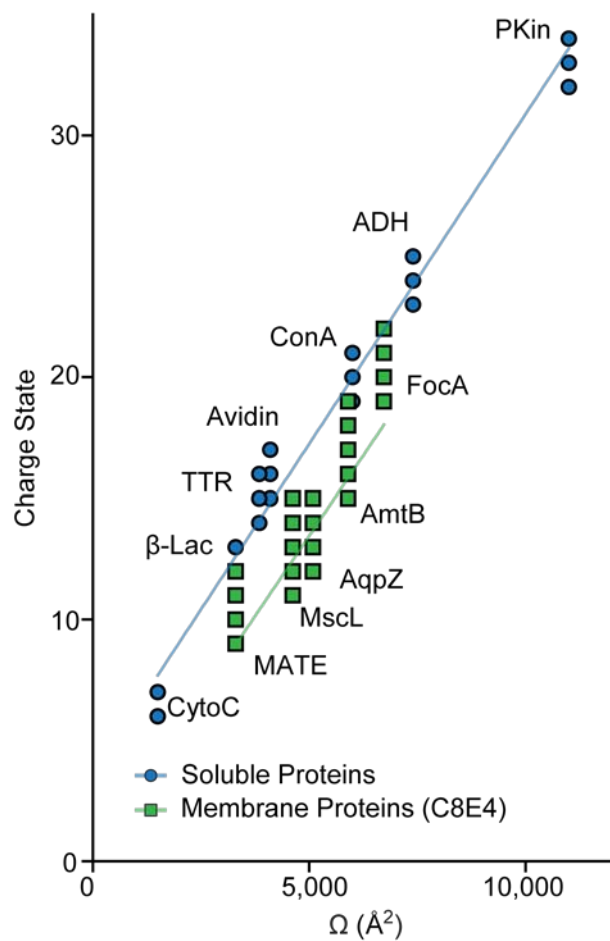


Figure S1. Charge state and Ω of membrane proteins and commonly used soluble calibrant proteins measured by DTIMS.

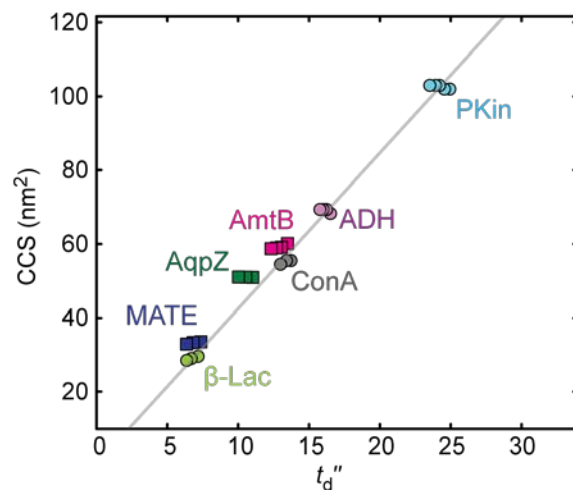


Figure S2. A calibration plotted as the ‘doubly-corrected’ drift time against Ω corresponding to the data in Figure 2A. The fit is to the soluble protein data points only. Membrane proteins are represented with squares, and soluble proteins by circles. All Ω s are from literature, and measured by DTIMS. Drift times were measurements were performed at a wave height of 15 V.

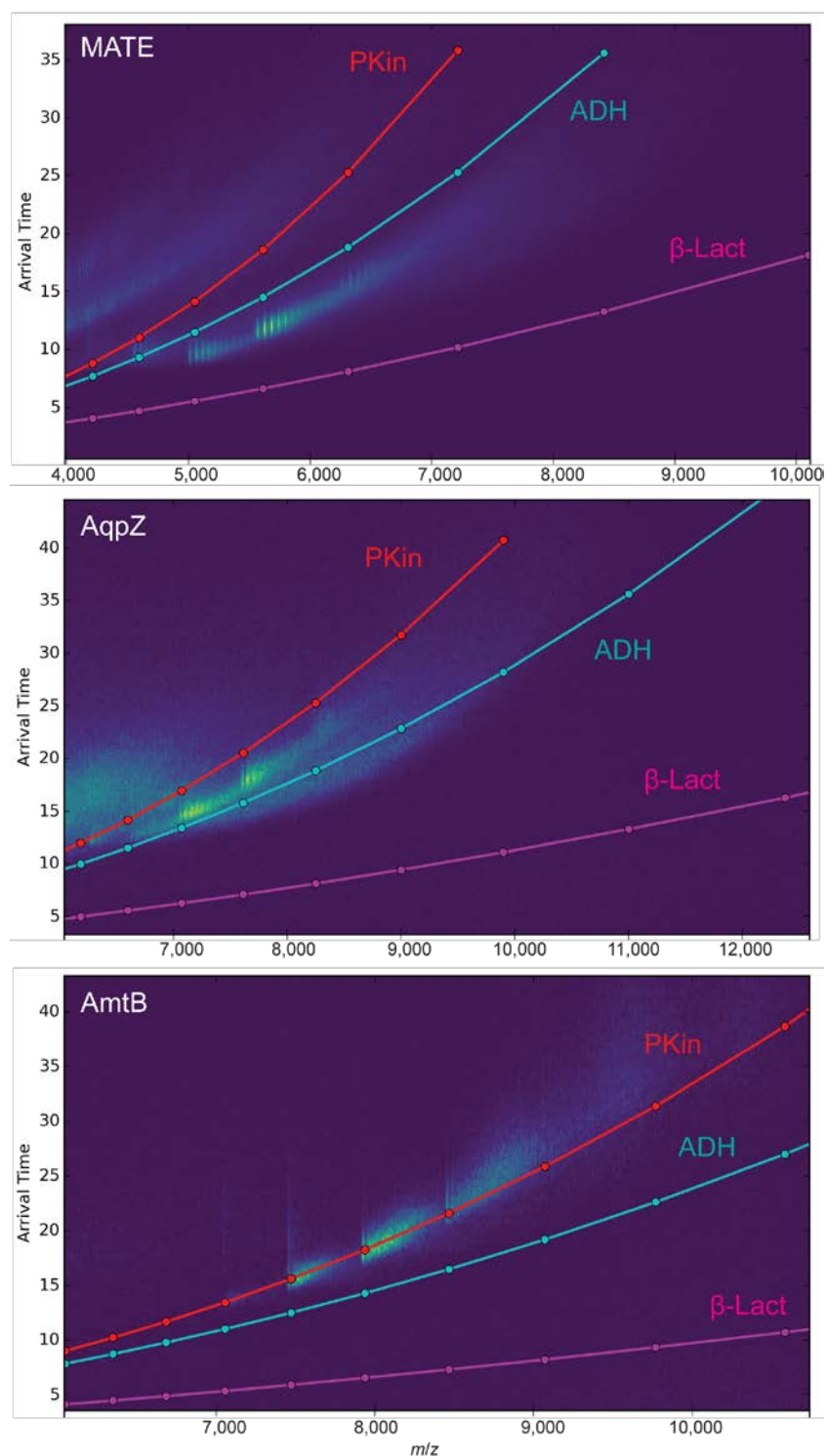


Figure S3. Arrival time distribution overlays for MATE, AqpZ and AmtB showing the anticipated drift times of ions with a Ω of 3,400 \AA^2 (MATE), 5,100 \AA^2 (AqpZ), or 5,900 \AA^2 (AmtB) in each case when only β -lactoglobulin (2,900 \AA^2), only alcohol dehydrogenase (6,900 \AA^2) or only pyruvate kinase (10,300 \AA^2) are used as calibrant ions.

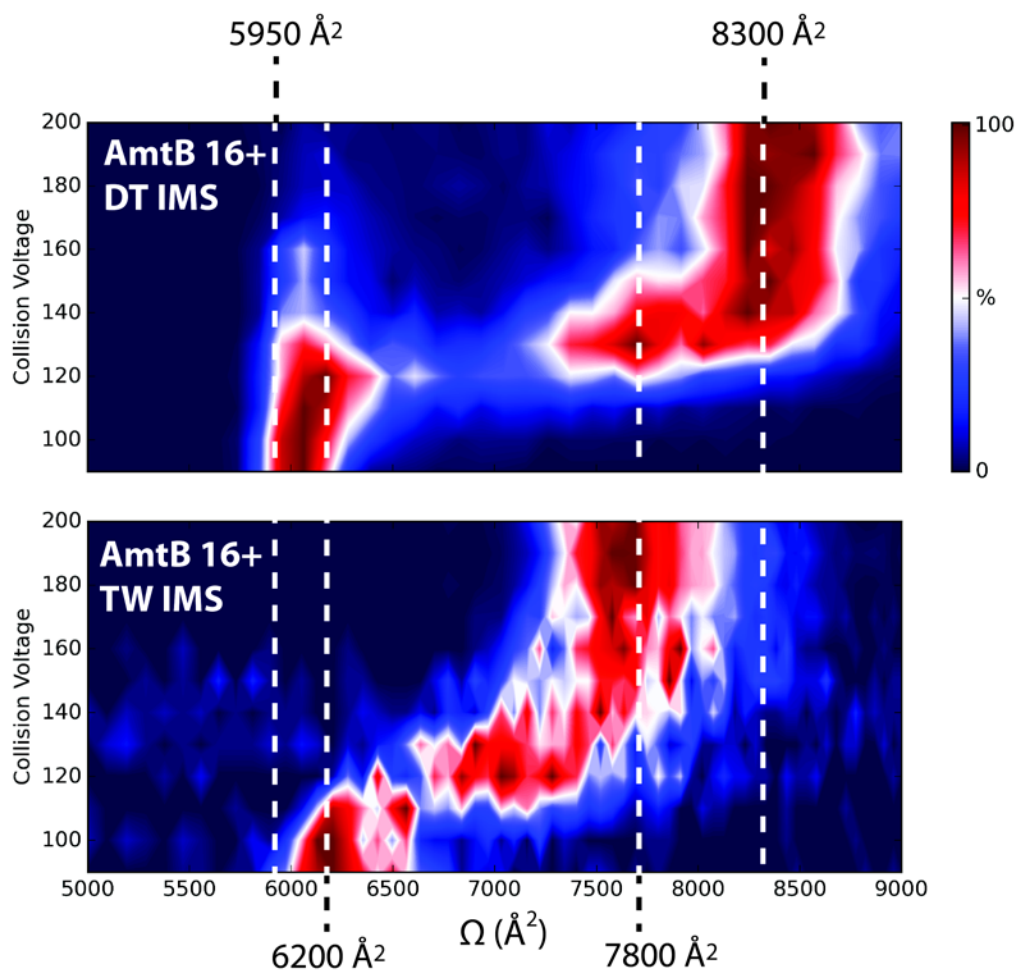


Figure S4. Gas-phase unfolding of the 16+ charge state of AmtB monitored using a Waters Synapt G1 HDMS mass spectrometer equipped either with a travelling wave (top) or a drift tube (bottom) mobility cell. The Ω values from the TWIMS drift times were calculated using PKin as calibrant. Although the calibration yields accurate Ω values for the native-like state, the reduced mobility of the unfolded states leads to a significant underestimation of the Ω (bottom), as evident from the much higher values determined by DTIMS (top).

References

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