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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Table S1. Literature Ω and mobility values in He for soluble calibrant proteins used in this study

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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.

Figure S3. Arrival time distribution overlays for showing the anticipated drift times.

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 $Å^2$ and mobility values in cm^2/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 $Å^2$ 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
MATE, MW: 50,120 Da (charge states 8–10)	
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
AqpZ, MW: 98,870 Da (charge states 12–14)	
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
AmtB, MW: 126,720 Da (charge states 15–17)	
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
MATE, MW: 50,120 Da	
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
AqpZ, MW: 98,870 Da	
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
AmtB, MW: 126,720 Da	
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
MATE, MW: 50,120 Da	
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
AqpZ, MW: 98,870 Da	
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
AmtB, MW: 126,720 Da	
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	***N***	**Mass (kDa)**	***z***	**Ω_{He} (nm^2)**
ADH *Saccharomyces cerevisiae*	4	143	18+	69.3
			19+	69.2
			20+	68.8
			21+	68.5
			22+	67.8
ConA *Canavalia ensiformis*	4	103	14+	56.8
			15+	56.6
			16+	56.3
			17+	56.1
			18+	55.9
			19+	55.6
PKin Rabbit heart	4	237	24+	11.2
			25+	11.1
			26+	10.9
			27+	10.9
			28+	10.8
			29+	10.8

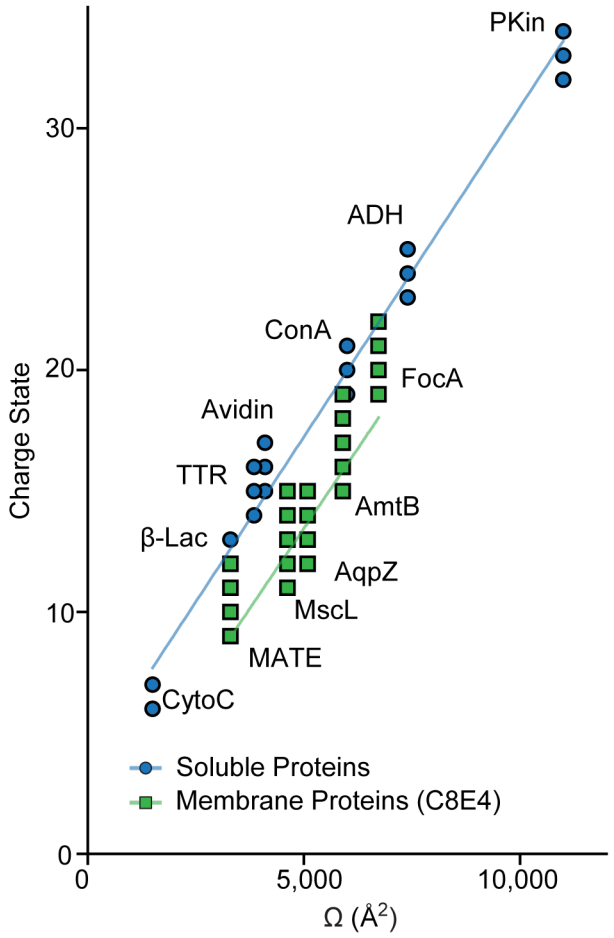

Figure S1. Charge state and Ω of membrane proteins and commonly used soluble calibrant proteins measured by DTIMS.

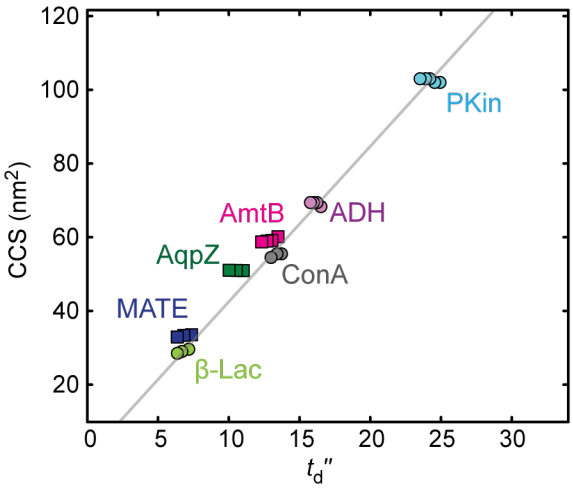

Figure S2. A calibration plotted as the 'doubly-corrected' drift time against Ω corresponding to the data in Figure 2A. The fit is to the soluble protein data points only. Membrane proteins are represented with squares, and soluble proteins by circles. All Ωs are from literature, and measured by DTIMS.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

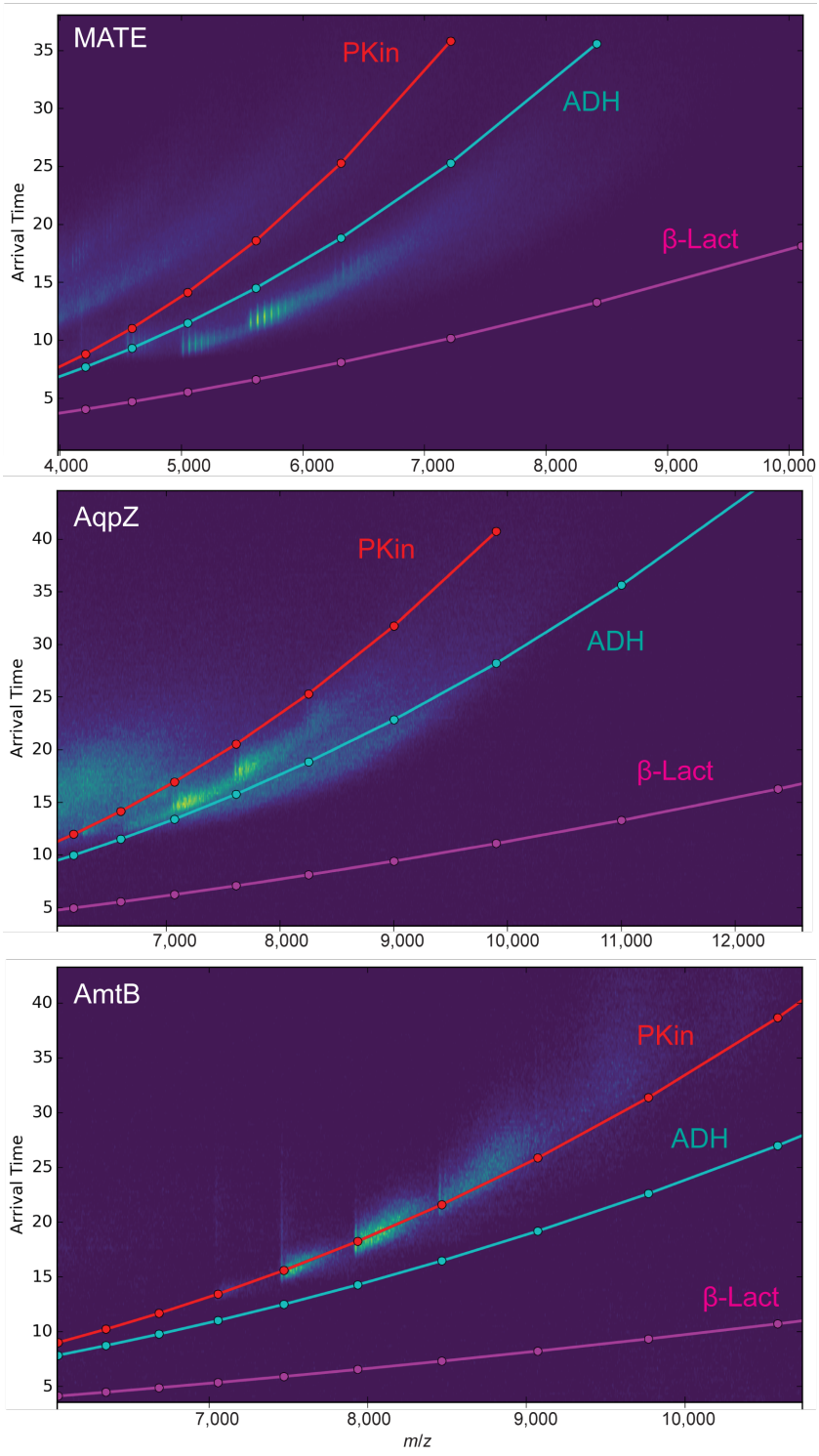

Figure S3. Arrival time distribution overlays for MATE, AqpZ and AmtB showing the anticipated drift times of ions with a Ω of 3,400 Å^2 (MATE), 5,100 Å^2 (AqpZ), or 5,900 Å^2

(AmtB) in each case when only β-lactoglobulin (2,900 $Å^2$), only alcohol dehydrogenase (6,900 $Å^2$) or only pyruvate kinase (10,300 $Å^2$) are used as calibrant ions.

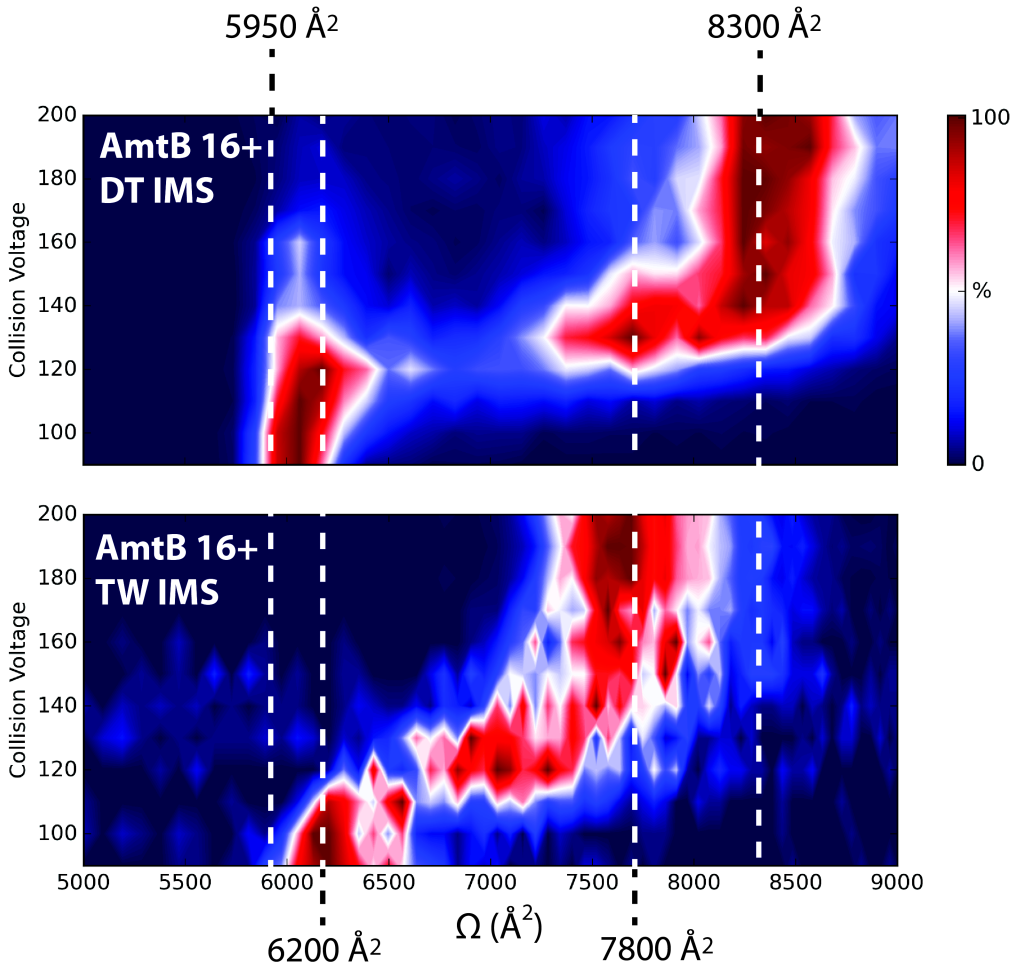

Figure S4. Gas-phase unfolding of the 16+ charge state of AmtB monitored using a Waters Synapt G1 HDMS mass spectrometer equipped either with a travelling wave (top) or a drift tube (bottom) mobility cell. The Ω values from the TWIMS drift times were calculated using PKin as calibrant. Although the calibration 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

(1) Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T. *Analytical Chemistry* **2010**, *82*, 9557-9565.

(2) Reading, E.; Liko, I.; Allison, T. M.; Benesch, J. L. P.; Laganowsky, A.; Robinson, C. V. *Angewandte Chemie-International Edition* **2015**, *54*, 4577-4581.

(3) Laganowsky, A.; Reading, E.; Allison, T. M.; Ulmschneider, M. B.; Degiacomi, M. T.; Baldwin, A. J.; Robinson, C. V. *Nature* **2014**, *510*, 172-175.