Supporting Information

Amphipols outperform dodecylmaltoside micelles in stabilizing membrane protein structure in the gas phase

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Table S1: Collision cross sections (CCSs) determined either experimentally (from ESI-IMS-MS analysis of proteins solubilised with DDM or A8-35), or theoretically from crystal structures (PDB accession codes are indicated) using the Projection Superposition Approximation.[1] Experimental data from the lowest energy MS conditions required to observe the protein are shown. – indicates the charge state was not observed or the ATDs were of poor signal to noise. Multiple measured CCSs for the same charge state are indicated where multiple features were present in ATDs.

Protein	Charge State	Experimentally determined CCS (Å^2)		Theoretical CCS (Å^2)	Trap Collision Energy Used (V)[a]	
		DDM	A8-35		DDM	A8-35
PagP	5+	1857	1877	2290[2]	50	60
	6+	1937	1937			
	7+	1956	1984			
	8+	2324	2353			
	9+	2584	2584			
OmpT	5+	-	2722	3017 (1I78)	130	135
	6+	-	2783			
	7+	2841	2814			
	8+	2883	-			
	9+	2933	-			
	9+	3167	-			
	10+	3477	-			
	11+	3686	-			
	12+	3759	-			
	13+	3896	-			
	14+	4388	-			
	15+	4498	-			
	16+	4658	-			
Mhp1	7+	-	3916	3753 (2JLN)	180	180
	8+	-	4002	3771 (2X79)		
	9+	-	4121			
	9+	-	4415			
	10+	-	4616			
	11+	-	4892			
	12+	4939	-			
	12+	5358	-			
	13+	5588	-			
	14+	5684	-			
GalP	7+	-	3028	3530	180	180
	8+	-	3062			
	9+	-	3130			
	10+	-	3950			
	11+	-	4260			
	12+	-	4507			
	13+	4657	4714			
	13+	5148	-			
	14+	5216	-			
	14+	5990	-			
	15+	5631	-			
	15+	6253	-			

[a]CCS data shown are for spectra acquired using the lowest energy mass spectral settings that gave resolvable protein peaks (using the collision energy indicated).

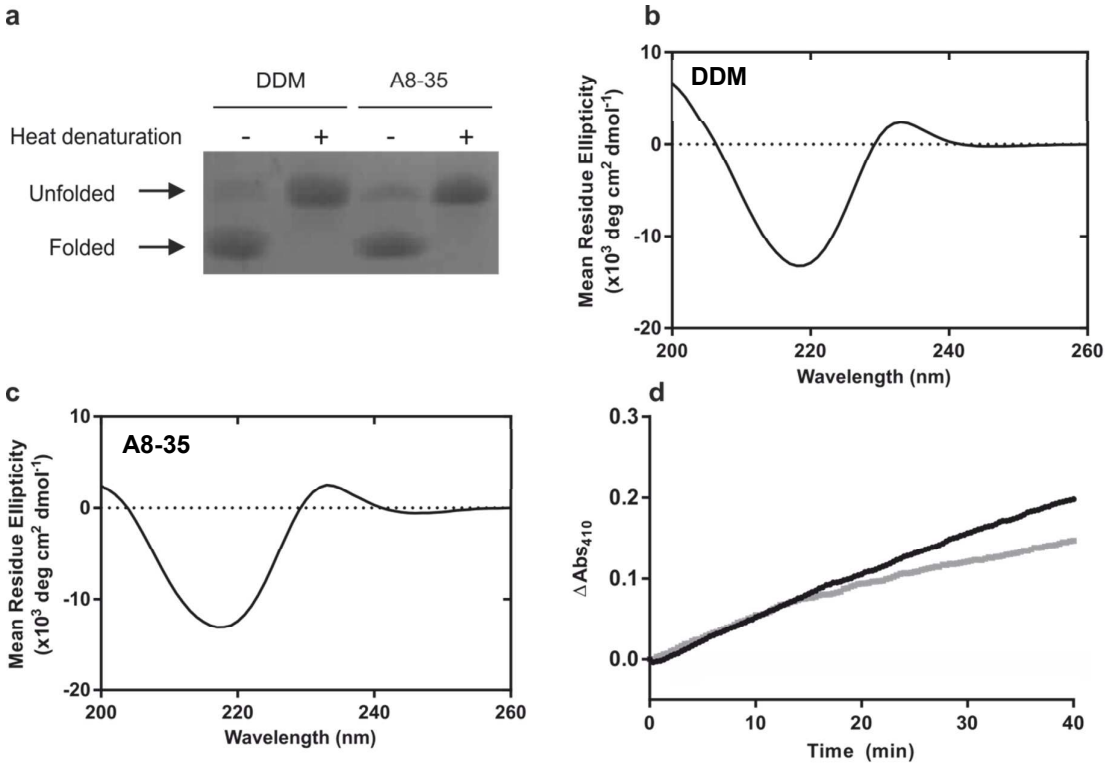

Figure S1: *PagP structure and function.* ***(a)*** *SDS-PAGE of PagP solubilised with either 0.02 % (w/v) DDM or 1:5 (w/w) A8-35, with (+) or without (-) heat denaturation;* ***(b, c)*** *far-UV CD spectra of PagP in* ***(b)*** *0.02 % (w/v) DDM or* ***(c)*** *1:5 (w/w) A8-35 containing 100 mM NH_4HCO_3, pH 8.0;* ***(d)*** *functional assay showing the increase in absorbance at 410 nm due to the hydrolysis of p-NPP to p-NP at a PagP concentration of 5 μM in 0.02 % (w/v) DDM (grey) or 1:5 (w/w) A8-35 (black) containing 100 mM NH_4HCO_3, pH 8.0. The increase in absorbance as a function of time indicates substrate turnover, and that the enzyme is active. DDM solubilised PagP has reduced activity compared with the A8-35 solubilised protein.*

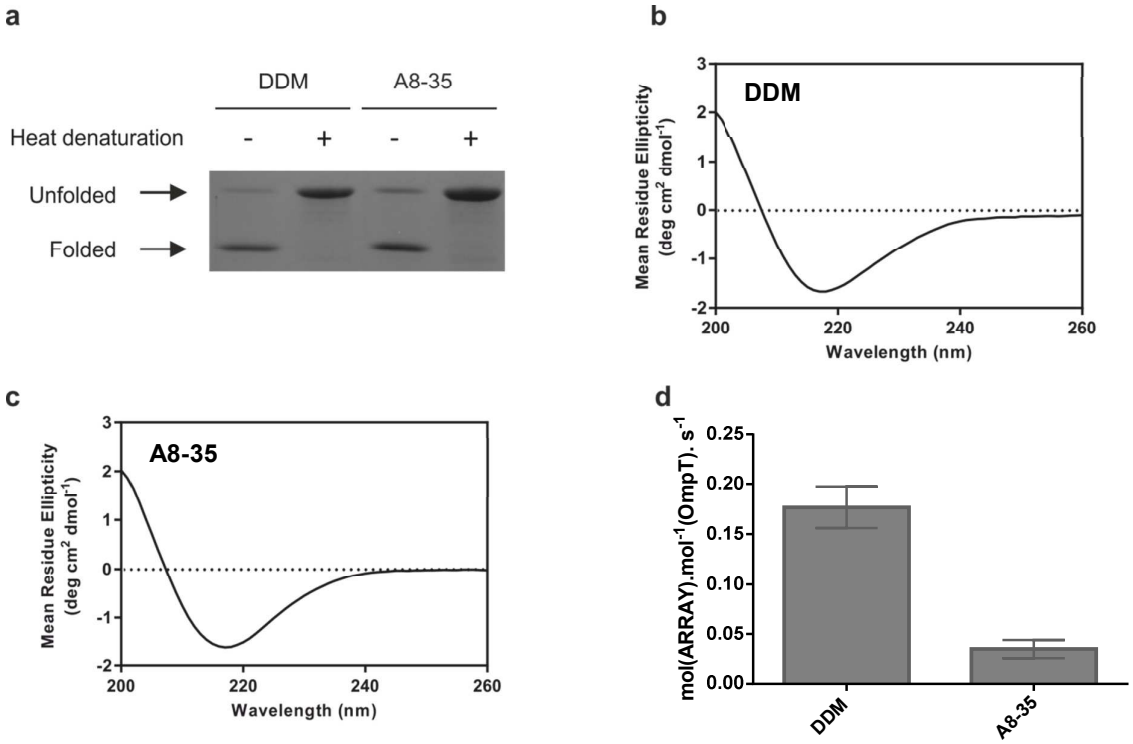

Figure S2: *OmpT structure and function.* ***(a)*** *SDS-PAGE of OmpT solubilised with either 0.02 % (w/v) DDM or 1:5 (w/w) A8-35, with or without heat denaturation;* ***(b, c)*** *far-UV CD spectra of OmpT in* ***(b)*** *0.02 % (w/v) DDM or* ***(c)*** *1:5 (w/w) A8-35 containing 100 mM NH_4HCO_3, pH 8.0;* ***(d)*** *functional assay showing the specific activity of OmpT toward cleavage of the self-quenching Abz-ARRAY-NO_3 peptide (n=12) in 0.02 % DDM (w/v) or 1:5 (w/w) A8-35 containing 100 mM NH_4HCO_3, pH 8.0.*

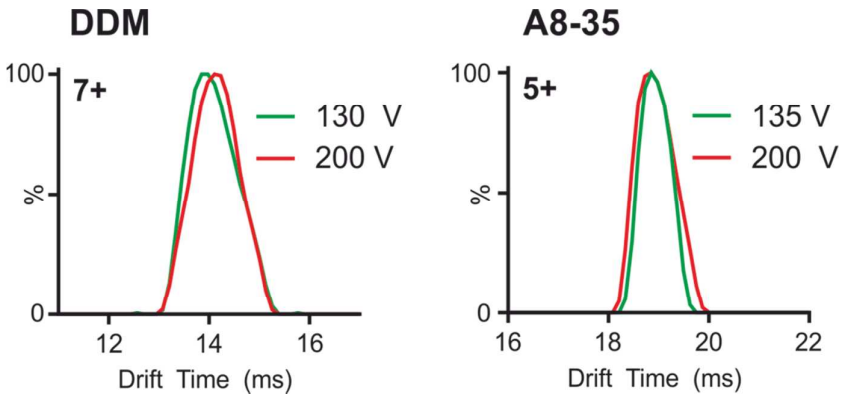

Figure S3: *ATDs for the lowest observed charge state of OmpT in both DDM and A8-35 at the lowest energy required to observe a resolved mass spectrum and at a significantly higher energy.*

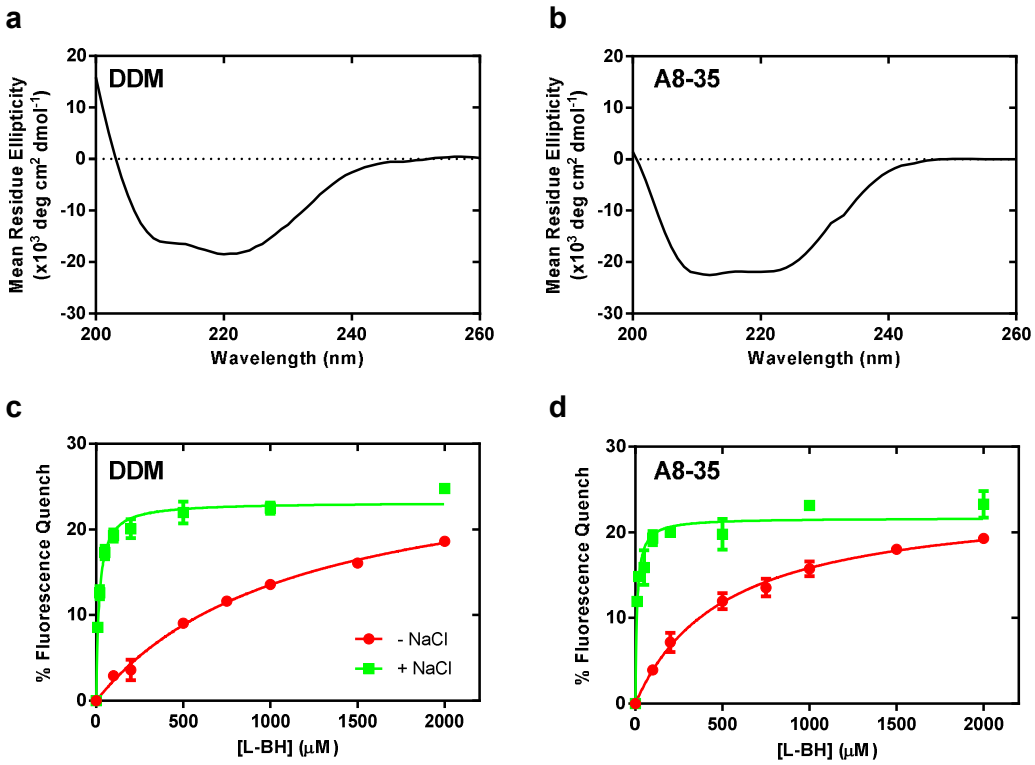

Figure S4: *Mhp1 structure and function.* ***(a,b)*** *Far-UV CD spectra of Mhp1 in* ***(a)*** *0.02 % (w/v) DDM or* ***(b)*** *1:5 (w/w) A8-35 containing 100 mM NH_4HCO_3, pH 8.0.* ***(c,d)*** *Tryptophan fluorescence quenching of Mhp1 solubilised in* ***(c)*** *0.02 % (w/v) DDM or* ***(d)*** *1:5 (w/w) A8-35 upon titration with L-benzylhydantoin, in the presence or absence of NaCl. Error bars indicate the SEM of three measurements. K_d values were determined to be 1.1 ± 0.1 mM (DDM) and 515 ± 69 µM (A8-35) in the absence of Na^+, and 17.6 ± 1.8 µM (DDM) and 10 ± 1.9 µM (A8-35) in the presence of Na^+.*

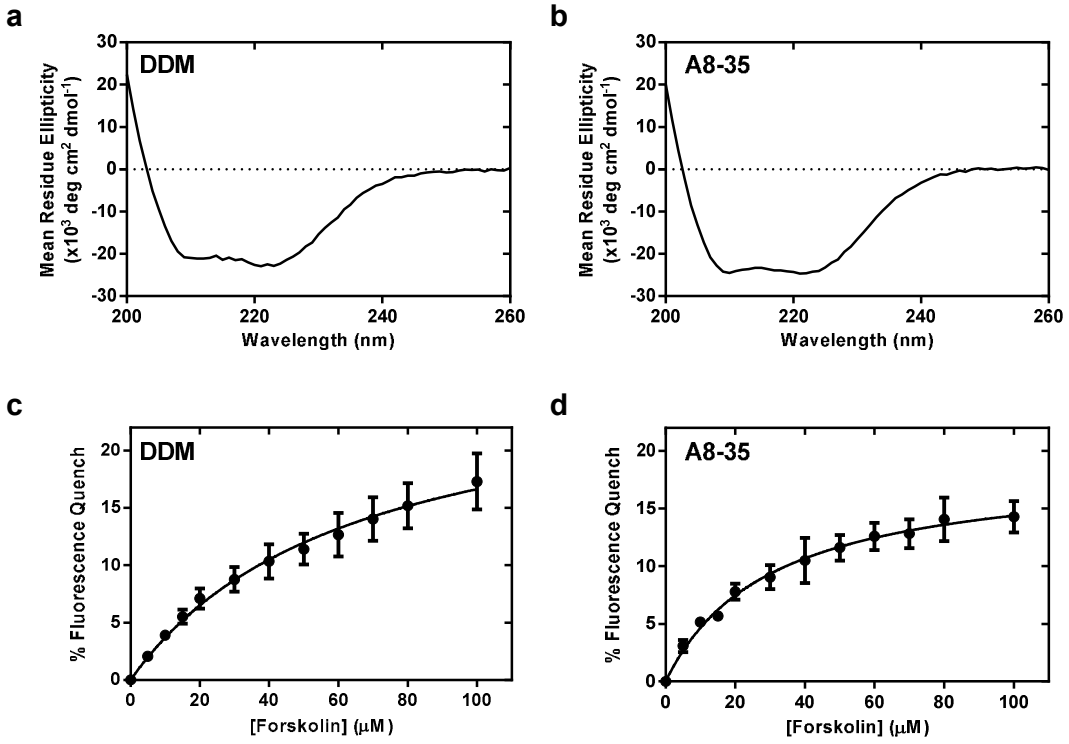

Figure S5: *GalP structure and function.* ***(a,b)*** *Far-UV CD spectra of GalP in* ***(a)*** *0.02 % (w/v) DDM or* ***(b)*** *1:5 (w/w) A8-35 containing 100 mM NH_4HCO_3, pH 8.0.* ***(c,d)*** *Tryptophan fluorescence quenching of GalP solubilised in* ***(c)*** *0.02 %(w/v) DDM or* ***(d)*** *1:5 (w/w) A8-35 upon titration with forskolin. Error bars indicate the SEM of three measurements. K_d values were determined to be 63 ± 19 μM (0.02 % DDM) and 30 ± 6 μM (1:5 (w/w) A8-35).*

Supplementary References

(1) Bleiholder, C.; Wyttenbach, T.; Bowers, M. T. *Int J Mass Spectrom* **2011**, *308*, 1-10.
(2) Borysik, A. J.; Hewitt, D. J.; Robinson, C. V. *J Am Chem Soc* **2013**, *135*, 6078-6083.