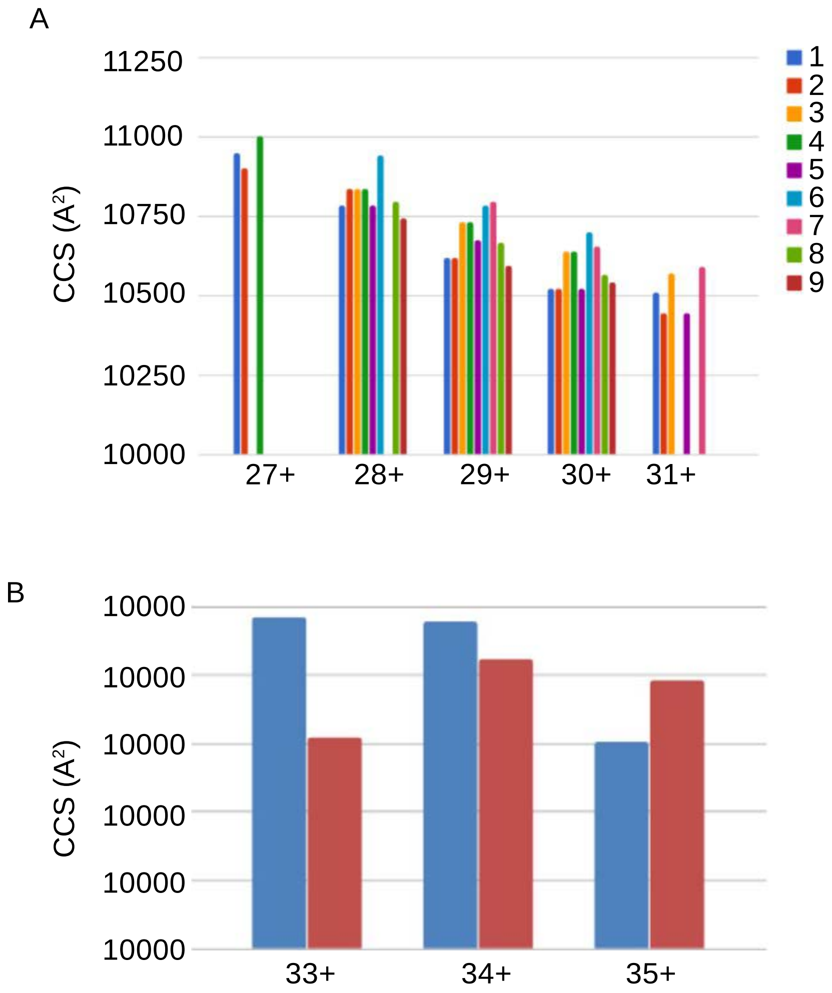

Figure S1. Related to Figure 1 and Figure 2; A) Reproducibility of CCS measurements of the urease $(UreABC)_3$ core complex. The standard deviation of 29+ measurements was 0.6%, with similar deviations for other charge states. B) Reproducibility of duplicate CCS measurements of $(UreABC)_3(MBP\text{-}UreDFG)_1$ complexes. The standard deviation across all charge states is 1%.

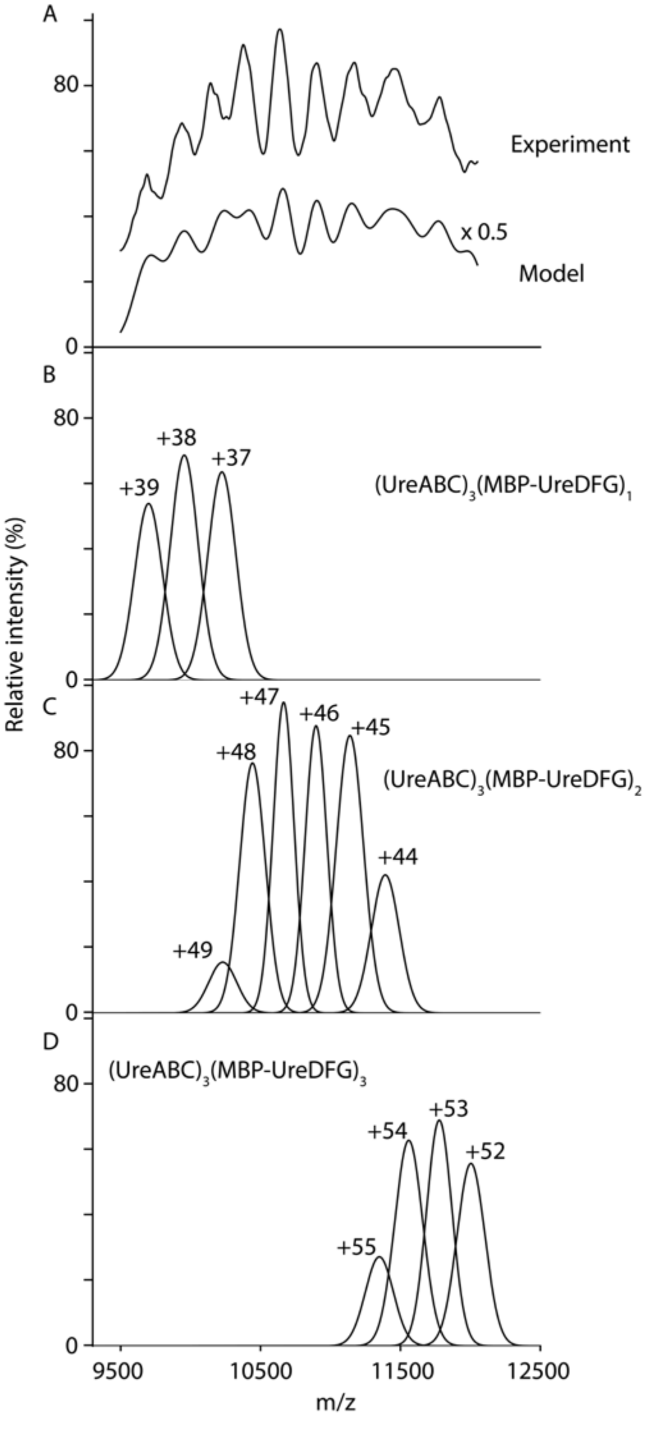

Figure S2. Related to Figure 1; Deconvolution of mass spectrum with Urease complexes. (A) Modeled mass spectrum agrees well with the experiment. Contribution of $(UreABC)_3(MBP\text{-}UreDFG)_1$, $(UreABC)_3(MBP\text{-}UreDFG)_2$, and $(UreABC)_3(MBP\text{-}UreDFG)_3$ species is shown in (B), (C), and (D), respectively.

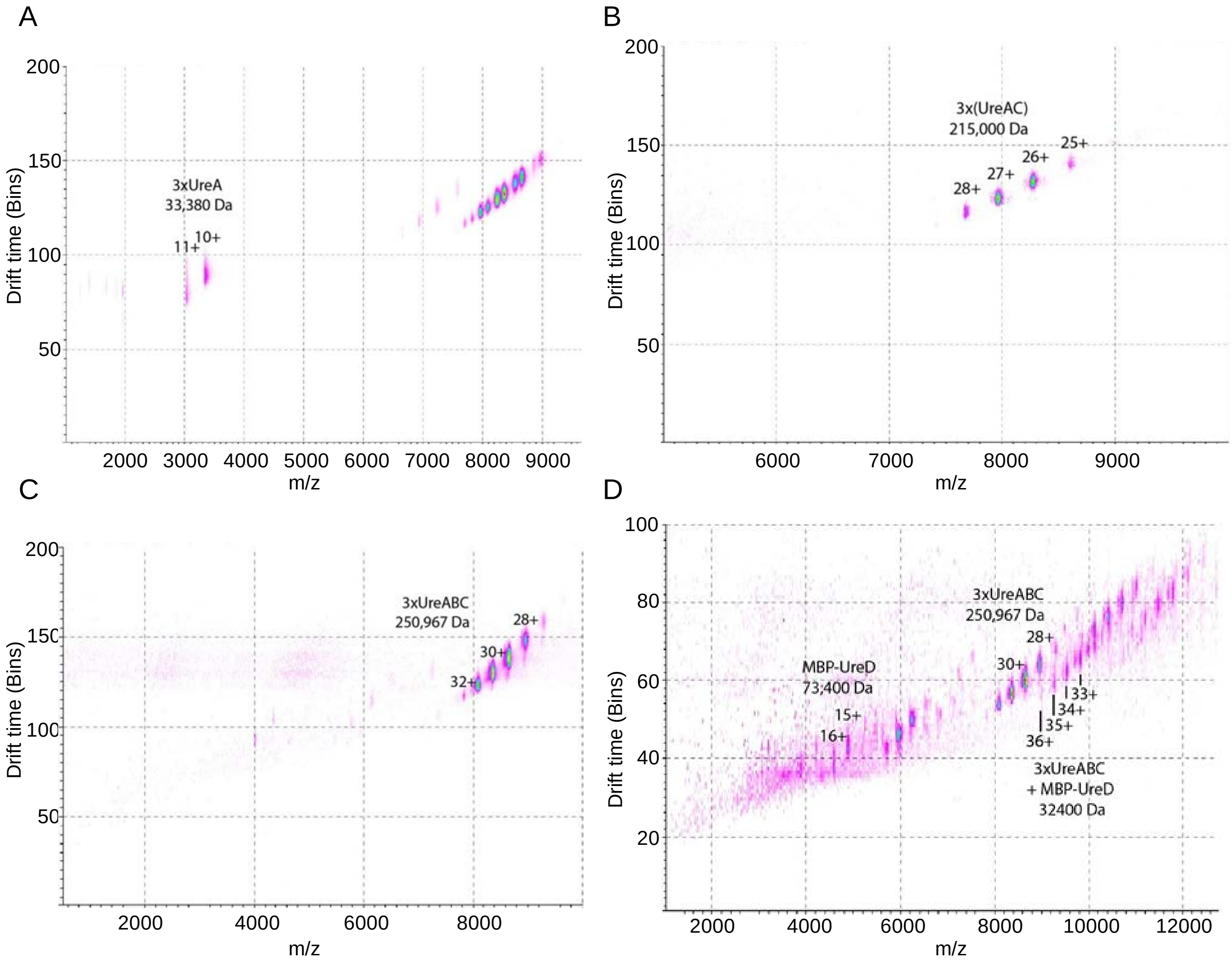

Figure S3.Related to Figure 1; A) Solution-phase disruption reveals the $(UreA)_3$ subcomplex of urease. We sprayed the urease core complex from a solution of 50% isopropanol and 50% 200mM ammonium acetate. The IM-MS spectrum under these conditions reveals the presence of a modular ureA trimer at 10+ and 11+. Also observed in the spectra are various losses of ureB from the urease core complex. Expected mass of $(UreA)_3$: 33,254 Da. B) Expression of urease with a ureB gene knockout yields a homogenous population of $(UreAC)_3$ hexamers at 215,000 Da. Expected mass : 215,024 Da. C) IM-MS of the urease holoenzyme $(UreABC)_3$ reveals predominant signals with a mass in close agreement with the expected mass of 250,308 Da. D) IM-MS of a sample containing all urease accessory components including MBP-ureD. Key complexes are annotated. Although higher order complexes were identified, it was impossible to assign configurations to these different stoichiometries, and thus they were not included in the model.

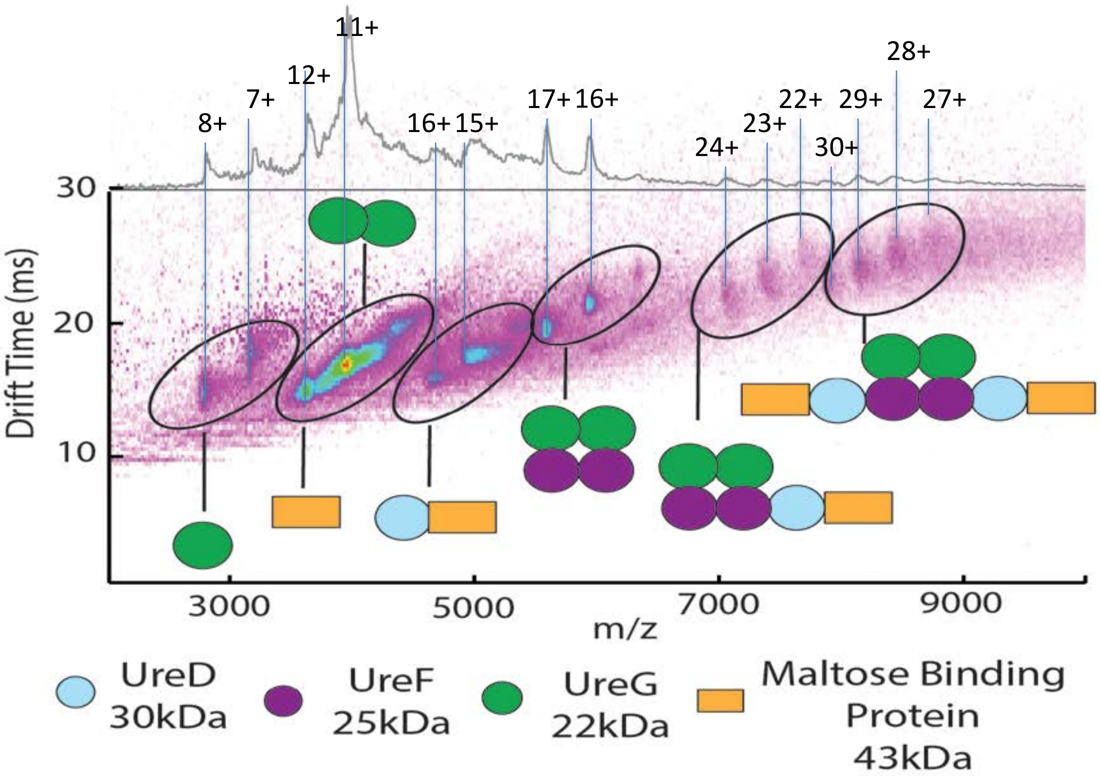

Figure S4. Related to Figure 1; Fully annotated IM-MS spectrum of the $(ureDFG)_2$ complex and its subcomplexes.

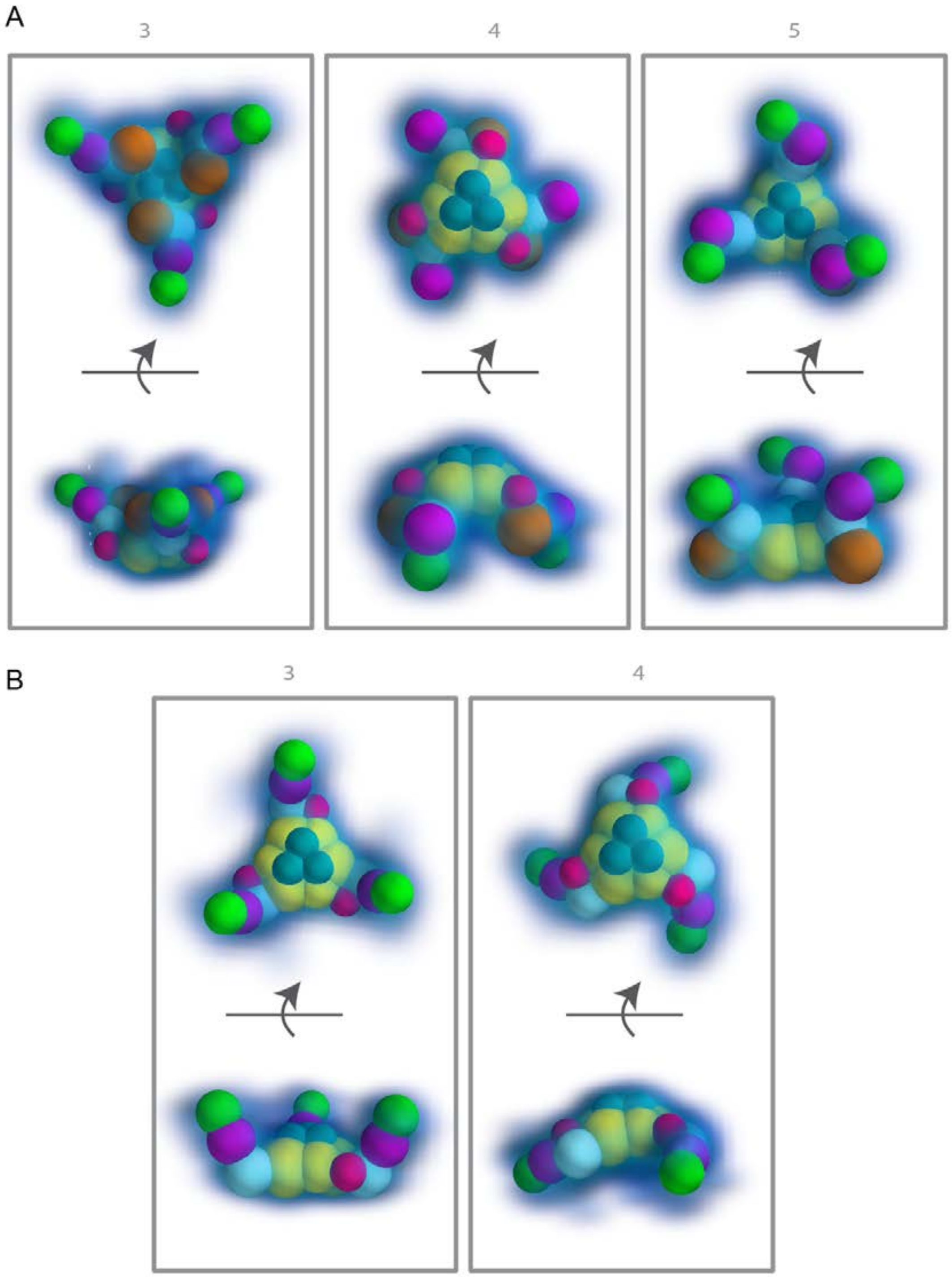

Figure S5. A) Related to Figure 3; The median structure and kernel density from clusters 3, 4, and 5 from Figure 3. B) Related to Figure 4; The median structure and kernel density from clusters 3 and 4 from Figure 4.

Cluster	CCS (nm^2)		
	μ	*median*	*σ*
0	23970	24095	370
1	23955	23793	296
2	23809	23706	359
3	23856	23703	242
4	23642	23723	129
5	23841	23962	166

Table S1**.** Related to Figure 3; Mean (*μ*), median and standard deviation (*σ*) of CCS values calculated using projection approximation method for clusters from Figure 3 including the MBP tag.

Cluster	CCS (A^2)		
	μ	*median*	*σ*
0	21207	20797	608
1	21113	20909	695
2	21006	20317	669
3	21813	21654	415
4	21285	21452	184
5	20518	20906	394

Table S2. Related to Figure 3; Mean (*μ*), median and standard deviation (*σ*) of CCS values calculated using projection approximation method for clusters from Figure 3 for urease-related proteins only.

Cluster	CCS (A^2)		
	μ	*median*	*σ*
0	23944	24712	376
1	23899	24058	318
2	23959	24027	352
3	23970	24093	184
4	23650	23435	256

Table S3. Related to Figure 4; Mean (*μ*), median and standard deviation (*σ*) of CCS values calculated using projection approximation method for clusters from Figure 4 including the MBP tag.

Cluster	CCS (A^2)		
	μ	*median*	*σ*
0	20386	21158	440
1	20926	20949	441
2	20767	21014	445
3	20321	20421	498
4	21285	21452	184
5	20049	21039	435

Table S4. Related to Figure 4; Mean (*μ*), median and standard deviation (*σ*) of CCS values calculated using projection approximation method for clusters from Figure 4 for urease-related proteins only.