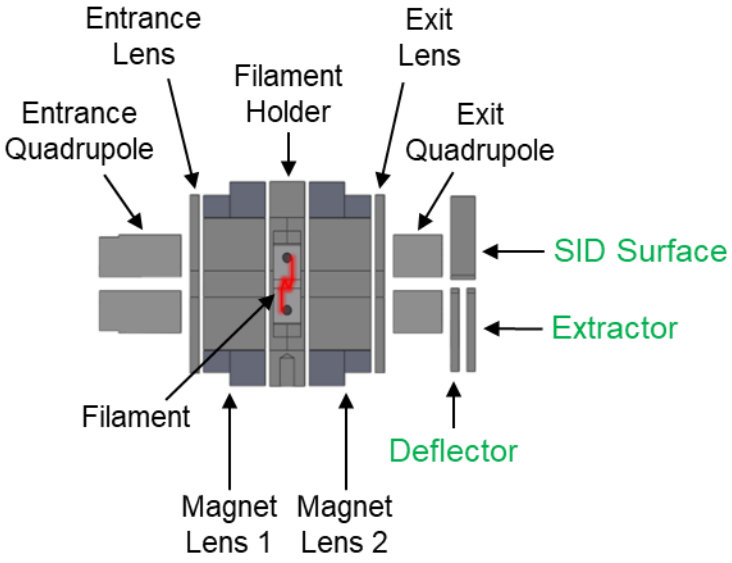
Supporting information:

Protein complex heterogeneity and structure revealed by native mass spectrometry with electron capture charge reduction and surface induced dissociation.

Table S1. Typical voltages applied to the ExD cell for electron capture charge reduction. The ExD-SID cell of Figure 1 is repeated here for comparison with the descriptors in the Table.

	ECCR 2V	ECCR 4V
Entrance quadrupole	0 V	0 V
Entrance lens	-30 V	-30 V
Magnet lens 1	2 V	4 V
Filament holder	3 V	5 V
Filament Bias	-0.5 V	-0.5 V
Magnet lens 2	2 V	4 V
Exit quadrupole	-6 V	-6 V
Exit lens	-30 V	-30 V
Filament current	2.2 A	2.2 A
Surface	-8 V	-8 V
Extractor	-8 V	-8 V
Deflector	-8 V	-8 V



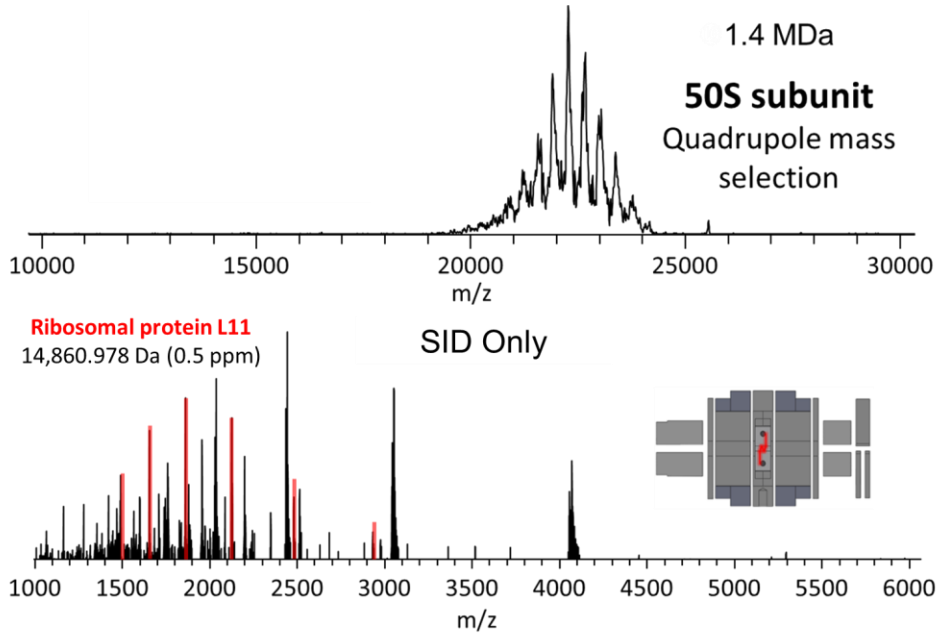


Figure S1. Surface induced dissociation of the 50S ribosome, using the SID feature of the ExD device and illustrating the measurement of the 3x trimethylated ribosomal protein L11 at 0.5 ppm accuracy (peaks marked in red).

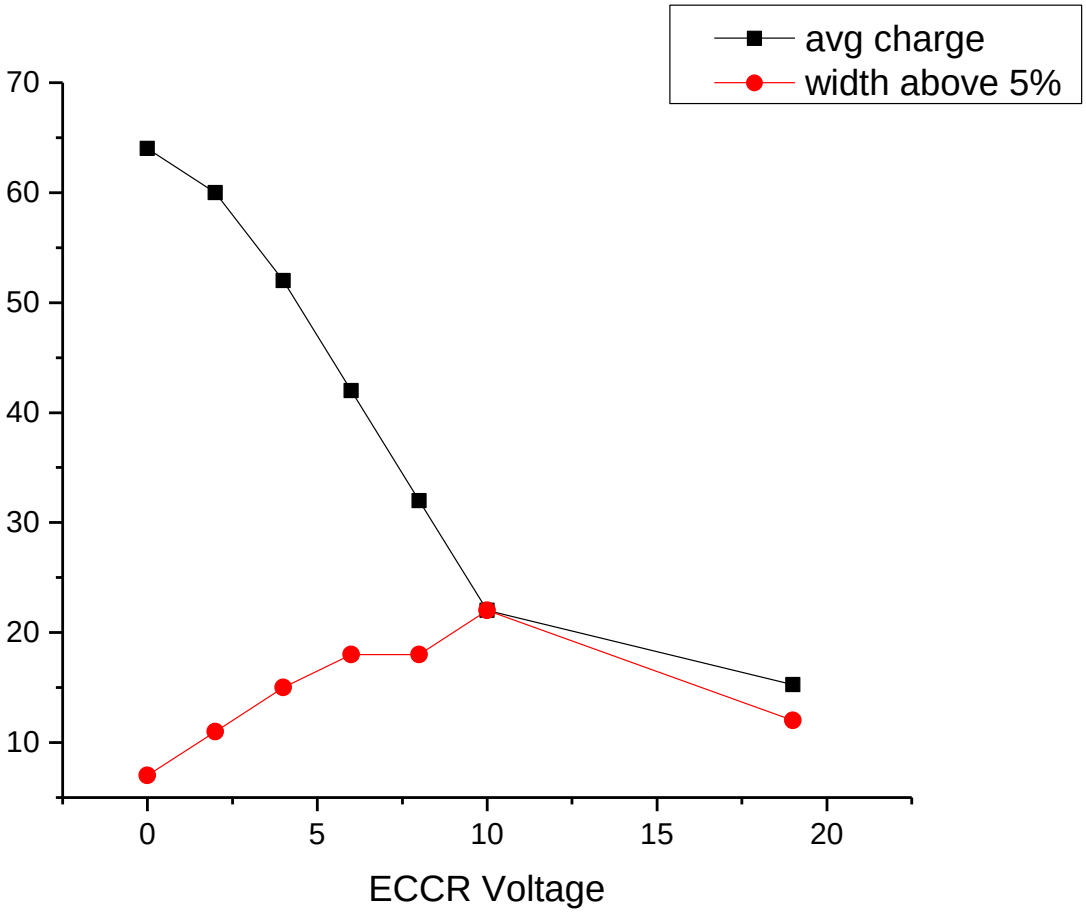


Figure S2. Average charge of GroEL with tunable ECCR.

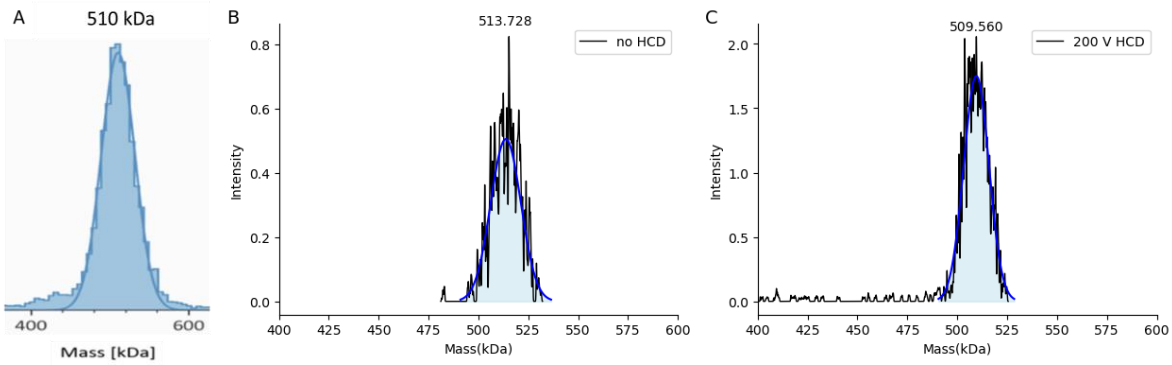


Figure S3. A) Mass photometry (Refeyn TwoMP) of VFLIP spike protein, showing average mass of 510 kDa ± 5%. Charge detection mass spectrometry of heterogeneous VFLIP spike protein with an in-source trapping voltage of -50 V and B) no HCD or C) 200 V HCD. HCD removes some salt and/or solvent adducts but we cannot rule out minor covalent losses. The average mass from Fig S3C agrees with the average in S3A.

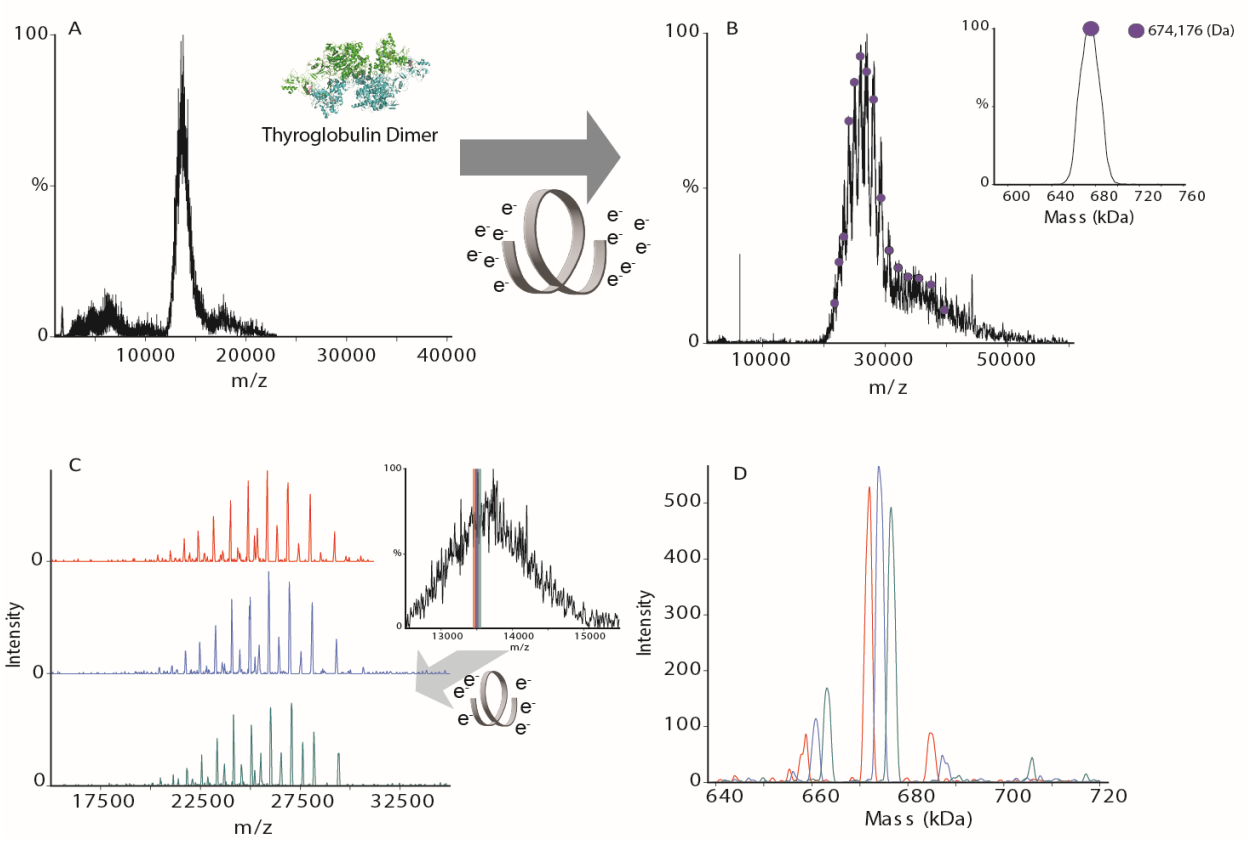


Figure S4. A) An unresolved native mass spectrum of dimeric bovine TG. PDB: 7QTQ(B) A charge state-resolved native mass spectrum of the bovine TG with electron capture charge reduction (ECCR, voltage 7 V). The isolation region is 12k-17.5k m/z. The average mass is 674 kDa deconvolved using UniDec.[48] (C) Overlay of three narrow window isolations (each highlighted in a different color) covering the *m/z* range of 13426-13599, windows share an overlap of 1 *m/z*, inset showing the position of three narrow quadrupole window selections. (D) Deconvolved mass spectrum of the three narrow window isolations shown in C, deconvolved using UniDec.[48]

To further assess the ability of ECCR to provide insight into heterogeneous glycoproteins we also considered the glycoprotein thyroglobulin. Thyroglobulin (Tg) is a dimeric protein complex that exhibits multiple PTMs, which includes glycosylation, phosphorylation, and iodinated tyrosine. While both chains in the dimer have the same sequence of amino acids, they may exhibit differences in their PTMs. The sequence mass of bovine thyroglobulin is ~602 kDa, however, it is commonly referred to as a dimer of ~670 kDa partially as a result of extensive glycosylation (~10% of its mass being attributed to glycosylation). When Tg is introduced into the mass spectrometer under native like conditions, like with VFLIP, it is not possible to resolve the charge states and determine the mass (Figure S3A). However, after gas-phase charge reduction of the entire charge state envelope, an average mass of 674 kDa was determined. As with VFLIP, when narrow window isolation is used, the complexity and heterogeneity of the sample becomes more apparent, as shown in Figures S3C and D.