Electronic Supplementary Material (ESI) for ChemComm.

Controlling release, unfolding and dissociation of membrane protein complexes in the gas phase through collisional cooling

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Supplementary Methods

Lipid preparations and purification of AmtB and NapA D157N and were performed as described.[1-3] Samples were introduced into the mass spectrometer using gold-coated borosilicate capillaries. Mass spectra were recorded on a Waters Synapt G1 mass spectrometer (Waters, Milford, MA) equipped with a travelling-wave ion mobility cell and modified for transmission of large complexes.[4] Instrument settings were: Capillary voltage 1.5 V, cone voltage 130 V, extraction voltage 4 V, collision voltages in the trap raging between 20 and 200 V, and transfer collision voltage 50 V. The pressure was adjusted between 1.8 to 7.5 mbar as measured by the built-in sensor on the rough pumping foreline and displayed in the MassLynx tunepage. Adjustments were made by gradually opening or closing the speedivalve (BOC Edwards, Crawley/Sussex, UK) that connects the first pumping stage between the sample cone and the extractor cone with the rotary pump, and in this manner controls the pumping efficiency in this region.[4] Ion mobility settings were: wave velocity 300 m/s with a wave height of 13 (IMS cell), wave velocity of 248 m/s with a wave height of 13 (transfer). Nitrogen was used as drift cell gas with a pressure of 1.6 torr. Collisional cross-section (CCS) calibrations were performed as described[4] using pyruvate kinase, yeast alcohol dehydrogenase and concanavalin A (all Sigma) as calibrants. The 12+ charge state of NapA was found to have a CCS of 4600 $Å^2$, suggesting a slightly compacted native-like conformation compared to the calculated value of 4900 $Å^2$. Spectra were analyzed

using Mass Lynx 4.1 software (Waters, Milford, MA). Peak intensities were extracted using mMass 3.9.[5] Unfolding trajectory plots were generated using an in-house developed software.[6]

References

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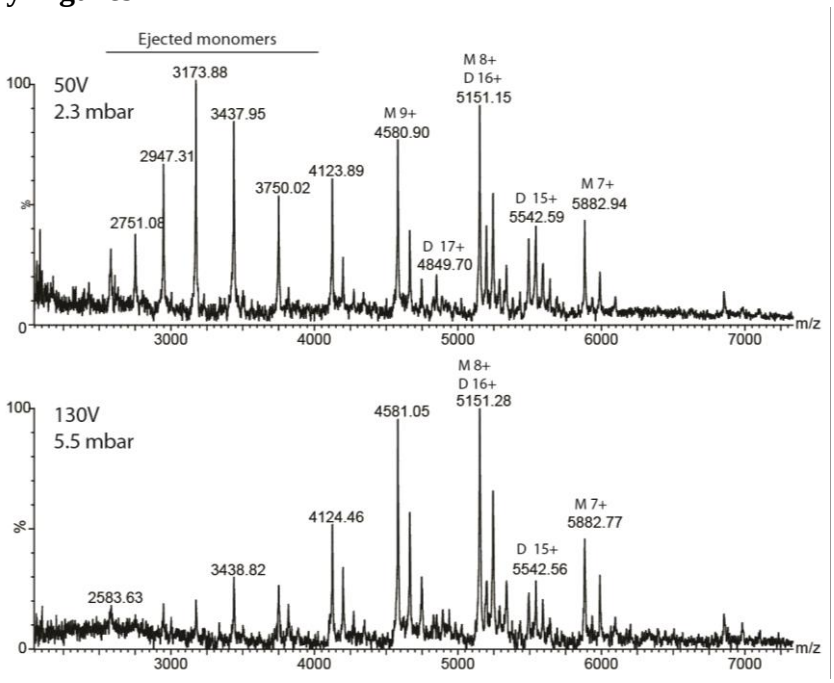
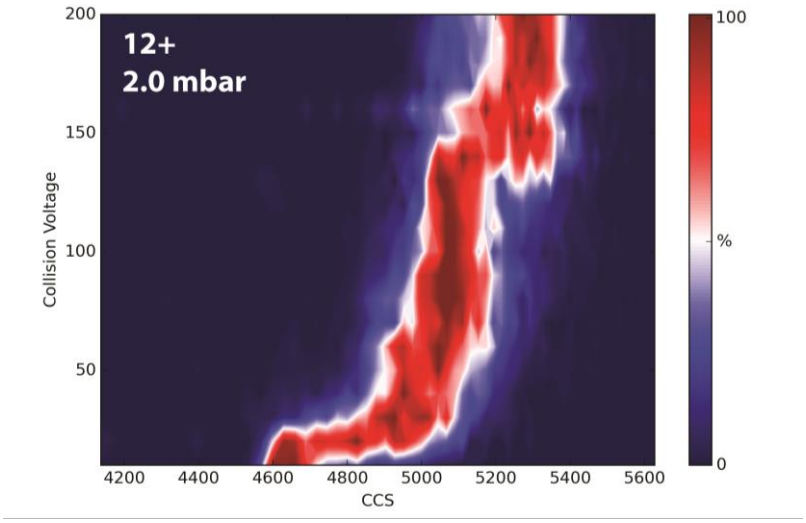
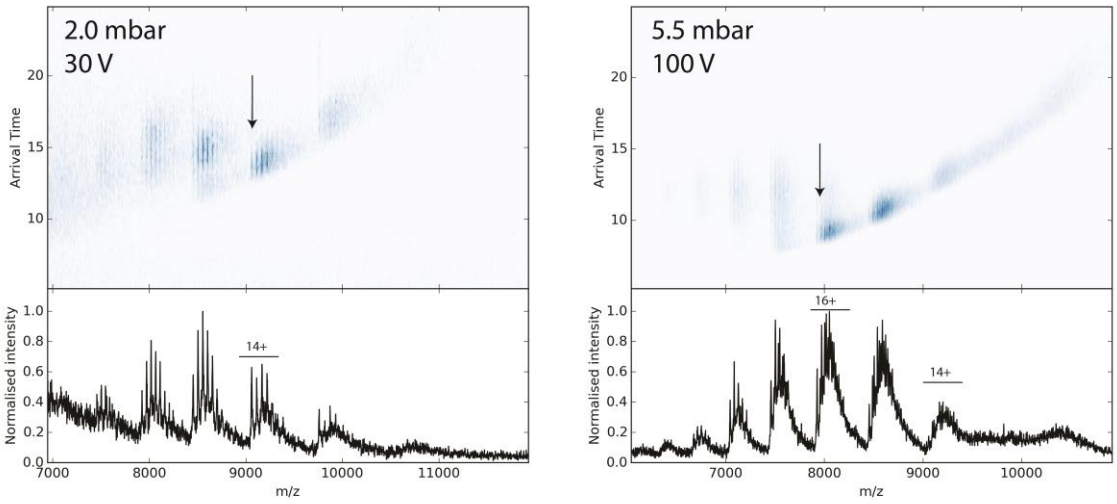
Supplementary Figures



Supplementary Figure 1. Reduced source pressure facilitates the release of NapA from DDM micelles. At low source pressure, ejected monomers as well as intact NapA dimers bound to PG can be detected at significantly lower collision voltage.



Supplementary Figure 2. At reduced source pressure, compact conformations indicative of native-like folding states can only be observed at low collision voltages. The unfolding trajectory of NapA at a source pressure of 2.0 mbar shows a compact conformation at a collision voltage of 10-20 V.



Supplementary Figure 3. IMMS of AmtB shows that the 14+ charge state is the highest charge state to maintain a compact conformation (arrow) when the source pressure is reduced to 2.0 mbar, even if the collision voltage is reduced to 30 V. At a source pressure of 5.5 mbar and a collision voltage of 100 V, the 16+ charge state is the highest charge state that retains a compact conformation (arrow).