Supporting Information

Native MS Analysis of Bacteriorhodopsin and an Empty Nanodisc by Orthogonal Acceleration Time-of-flight, Orbitrap and Ion Cyclotron Resonance

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Bacteriorhodopsin Purification

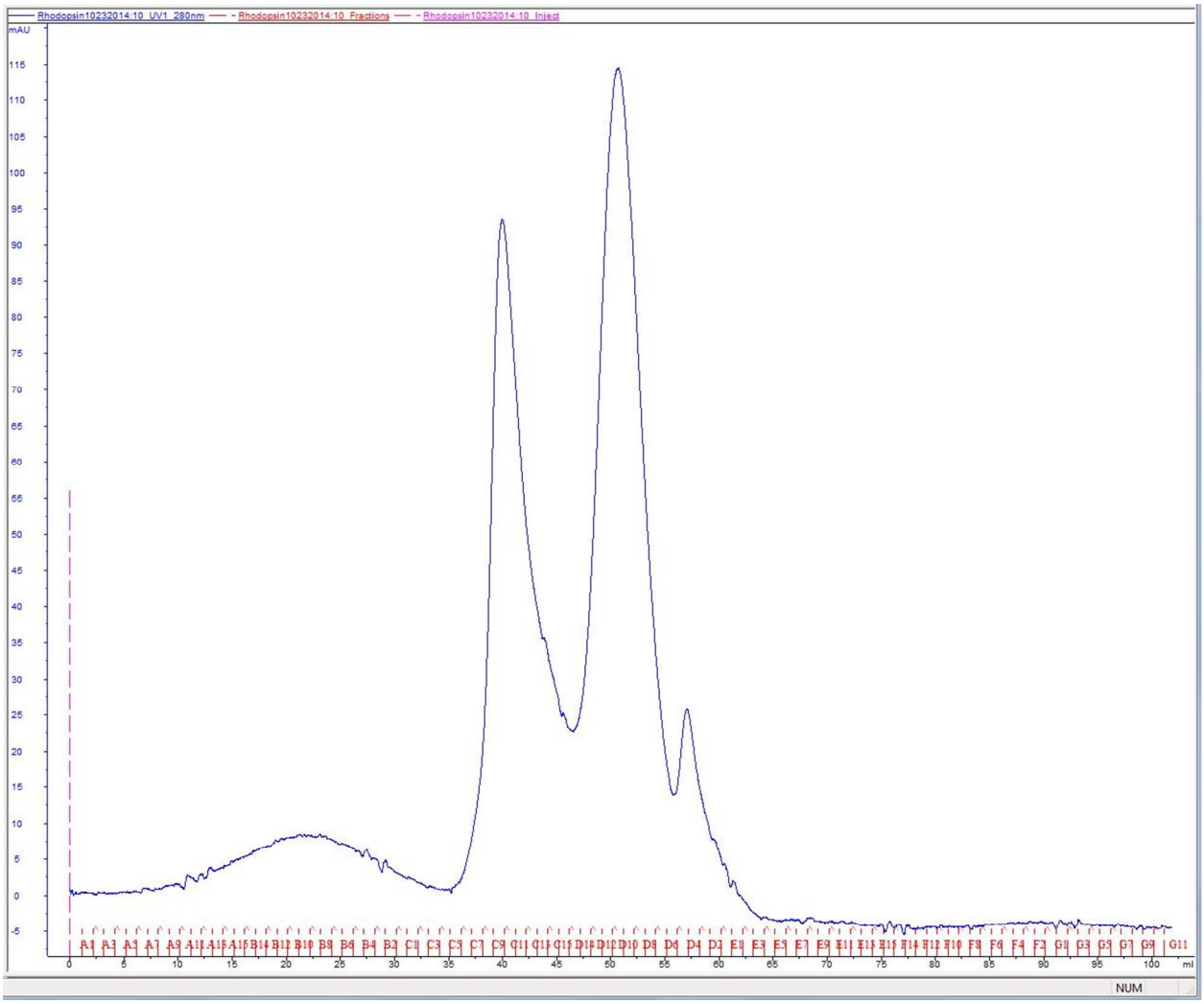

Figure S1. Size exclusion chromatogram (SEC) obtained as the final purification stage of the purple membrane bacteriorhodopsin purification using a Superdex 75 column with a bed volume of 130 mL. The region between 45 and 50 mL was pooled and concentrated to 1.23 ug/uL at 280 nm (extinction coefficient 63,000) and 1.34 ug/uL at 560 nm (extinction coefficient 63,000) using an Amicon Ultra Centrifuge Filter, Ultracel 50K, membrane filtration device (Millipore, Carrigtwohill, CO, IRL). The buffer used for the SEC was 25mM Tris pH 7.5 containing 150mM KCl and 1.2% (w/v) octyl-glucoside.

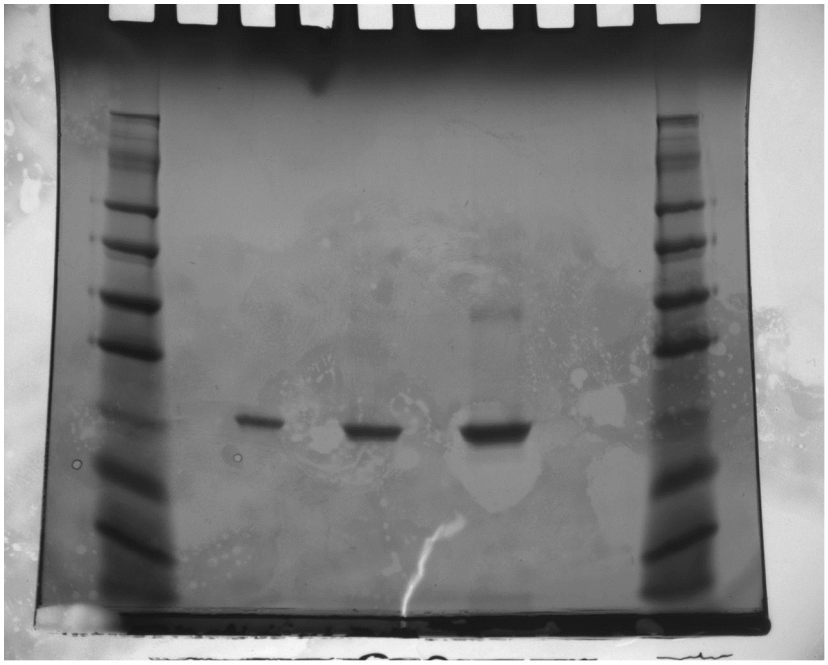

Figure S2. SDS-PAGE (4-20% Tris-glycine; Novex, Life Technologies, Carlsbad, CA) of the post size exclusion chromatographically purified bacteriorhodopsin. Wells 1 and 5 are the SeeBlue Plus2 pre-stained standard molecular weight markers (Novex, Life Technologies, Carlsbad, CA): Myosin 250 kDa, Phosphorylase 148 kDa, BSA 98 kDa, Glutamic dehydrogenase 64 kDa, Alcohol dehydrogenase 50 kDa, Carbonic Anhydrase 36 kDa, Myoglobin Red 22 kDa, Lysozyme 16 kDa, Aprotinin 6 kDa and Insulin B-chain 4 kDa. All approximate protein molecular weights based on measurements made in Tris-glycine. Wells 2, 3 and 4 represent the amounts of 1.2 ug, 2.4 ug and 4.8 ug of loaded bacteriorhodopsin. The SEC purified bacteriorhodopsin appears between markers Myoglobin Red (22 kDa) and Carbonic Anhydrase (36 kDa) therefore its SDS-PAGE estimated molecular weight is approximately 27-30 kDa, which is consistent with expected molecular weight.

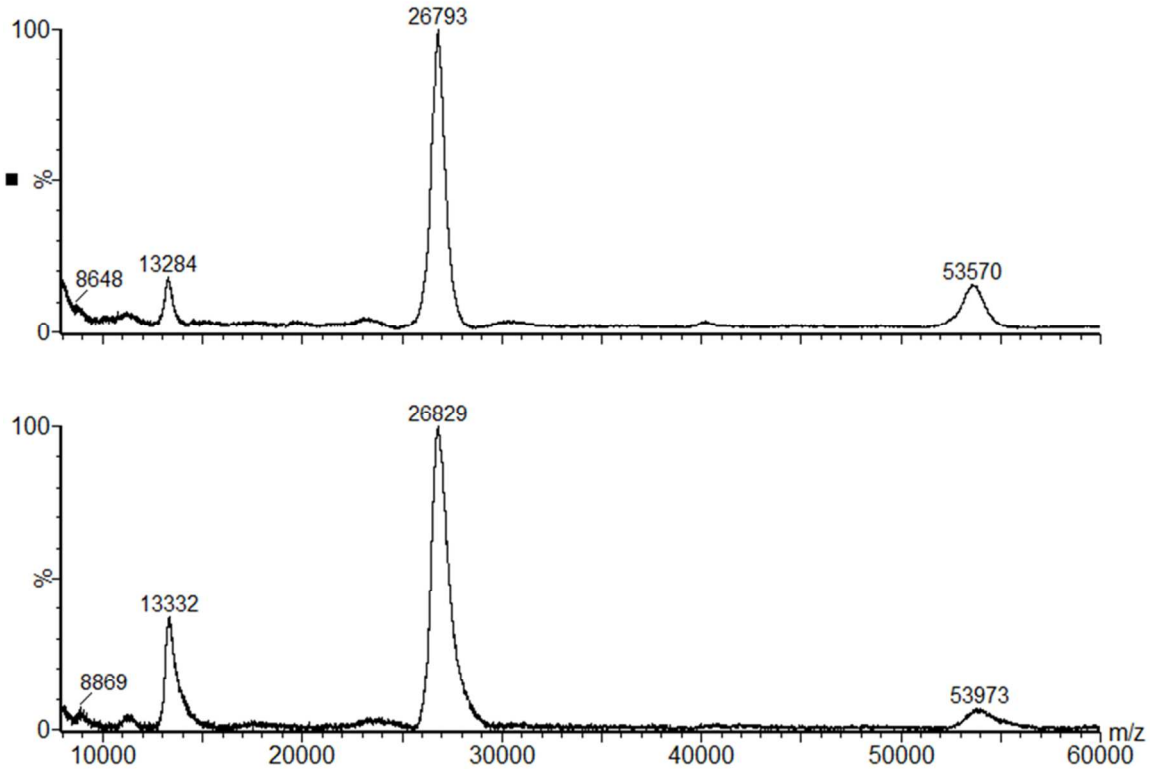

Figure S3. Matrix assisted laser desorption-MS (MALDI-MS) spectra of bacteriorhodopsin. Data represents 1-minute of combined continuum data. Smoothing parameters were: Number of smooths 2; Number of channels 25; Savitzky Golay smoothing method. Subtraction parameters were: Polynomial order 25; % of data below curve 1; Tolerance 0.010. The upper spectrum represents a 1:10 dilution of bacteriorhodopsin to matrix solution. Bacteriorhodopsin was at a concentration of 1.25 ug/uL in the buffer 25 mM NaH_2PO_4/Na_2HPO_4 pH 7.0 containing 1.2% (w/v) n-octyl-β-D-glucopyranoside; matrix solution was 20 ug/uL sinapic acid (Sigma-Aldrich, St. Louis, MO, USA; 49508-10MG-F) in 50% (v/v) acetonitrile containing 0.1% (v/v) trifluoracitic acid. The lower spectrum represents a 1:1 dilution of bacteriorhodopsin to matrix solution, where prior to mixing with the matrix solution, the protein bacteriorhodopsin was buffer exchanged into 200 mM ammonium acetate containing 1.2% (w/v) n-octyl-β-D-glucopyranoside, using a BioRad P6 MicroBio-Spin column (Hercules, CA; 732-6221). Based on the measured molecular weight displayed in the above spectra, bacteriorhodopsin is predominantly in the apo-form.

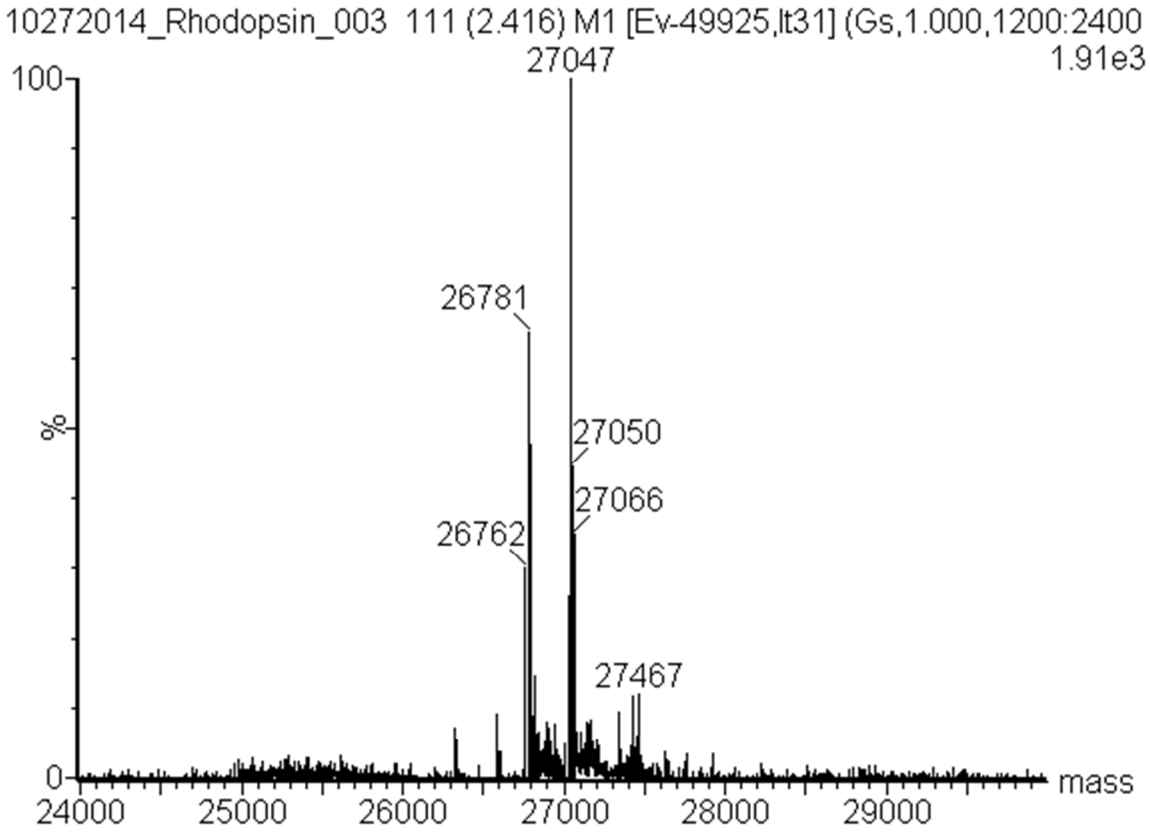

Figure S4. LC-MS spectrum of purified bacteriorhodopsin. The inset shows the deconvoluted spectrum. LC system used was an ACQUITY UPLC operated with a 2.1 x 50mm C4 BEH analytical column at a flow rate of 0.4 mL/min. The MS-system was a Xevo-Q-ToF (Waters MS-Technologies, UK). 26,781 Da corresponds to apo-bacteriorhodopsin. 27,047 Da corresponds to holo-bacteriorhodopsin, where retinal is covalently bound via an acid-labile Schiff base.

The M60A bacteriorhodopsin construct was obtained from Halobacterium using a sucrose gradient to isolate the purple membrane. The construct was then detergent exchanged in to 2x cmc octyl-glucoside (OG), 150 mM sodium chloride and 35 mM Tris-HCl by size-exclusion chromatography on a GE Superdex 200 10/300 GL column. Final buffer exchange was performed for native MS analysis into 200 mM ammonium acetate and containing 2x cmc OG was performed using BioRad Micro Bio-spin 6 Columns.

The empty MSP1D1-Nd preparation and purification have been described elsewhere [1-3] and will not be discussed in detail here. Briefly, the membrane scaffold protein, MSP1D1-D73C28, was licensed from Dr. Stephen Sligar's laboratory (The University of Illinois at Urbana-Champaign) and was expressed and purified in house (Amgen, Thousand Oaks). The amphipathic MSP1D1 protein was biotin labelled using the EZ-link iodoacetyl-PEG2-Biotin kit (ThermoScientific). The phospholipid used was 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Avanti Lipids) unless otherwise stated.

Mass Spectrometer Instrument Parameters

Q-ToF: The Synapt G2 HDMS instrument was operated in positive nanoflowESI mode. All critical instrument voltages and pressures are as follows: capillary voltage 0.8-1.5kV; sample cone 25-100V, extraction cone 1V; source block temperature 30-125°C; trap collision energy 4.0 to 100V; transfer collision energy 3V; trap entrance 3.0V; trap bias 45V; trap DC -2.0V; trap exit 0.0V; IMS entrance 10V; IMS helium cell DC 25V; IMS helium exit -5.0V; IMS Bias 3.0V; IMS exit 0.0V; transfer entrance 4.0V; transfer exit 5.0V; IMS wave velocity 250 m/sec; IMS wave amplitude 19.0 to 21.0V; transfer velocity 47 m/sec; transfer wave amplitude 4.0V; mobility trapping release time 200 usec; trap height 20.0V; extract height 0.0V; source RF-amplitude (peak-to-peak) 450V; triwave RF-amplitudes (peak-to-peak) trap 380V, IMS 250V, transfer 380V; source backing pressure 6.0 mbar; trap/transfer pressure SF_6, $3.25e^{-2}$ mbar (pirani gauge indicated; flow rate 4.5 mL/min); Instrument control and data acquisition was carried out through MassLynx 4.1 SCN 781.

Orbitrap-EMR: Experiments were performed on a modified Exactive Plus instrument (ThermoFisher Scientific, Bremen, Germany) equipped with a nanoflowESI source. All critical instrument voltages and pressures are as follows: Capillary voltage 0.8-1.5kV. Ions formed by nESI are passed through a heated stainless steel capillary (4.25 cm ion transfer tube) maintained at 200^0C into an S-Lens stacked ring ion guide with an applied RF-amplitude (peak-to-peak) of 200V. Ions then travel through a transport multipole and enter the HCD cell where they were stored at a high pressure before they were returned to the C-trap. This feature allows efficient trapping and desolvation of large protein ions and dramatically improves sensitivity. Nitrogen

gas was used in the C-trap as well as the HCD cell. Utilising a trapping gas pressure setting of 7.0 (software determined) the C-trap pressure is approximately $2e^{-4}$mbar and the UHV pressure (Orbitrap analyser) is $7.5e^{-10}$mbar. The voltage offsets on the transport multipoles were manually tuned to increase the transmission of large complexes (C-trap entrance lens; 0V, bent flatapole DC, 4V; inter-flatapole DC 4V; injection flatapole DC, 4V. An in-source CID voltage of 50V to 120V and an HCD voltage of 20V to 100V were required to achieve efficient sample desolvation. Transients detected in the Orbitrap were processed using enhanced Fourier transformation (eFT™) for converting the transients into frequency before *m/z* conversion [4-5 6]. The instrument was set at a nominal resolving power of 70,000 at *m/z* 200 and mass spectra were acquired for 2 minutes by averaging 10 microscans per analytical scan. Data was analyzed using Xcalibur™2.2. No additional data processing (smoothing) was performed.

FT-ICR: The experiments were performed using a 15 Tesla Bruker SolariX FT-ICR-MS instrument possessing an ICR infinity cell. The nESI capillary voltage was set to 0.9~1.2 kV. The temperature of dry gas was 100 ºC and the flow rate was 2.5 L/min. The RF amplitude of the ion-funnels was 300 V_{pp}, and the applied voltages were 210 V and 6 V for funnels 1 and 2, respectively. The voltage of skimmer 1 was 30 V and the skimmer 2 voltage was kept at 20 V. The lowest values of RF frequencies were used in all ion-transmission regions: multipole 1 (2 MHz), quadrupole (1.4 MHz), and transfer hexpole (1MHz). Ions were accumulated for 500 ms in the hexapole collision cell before being transmitted to the infinity ICR cell. The time-of-flight of 2.5 ms was used. Vacuum pressures for different regions were ~2 mbar for the source region, $\sim 2\times10^{-6}$ mbar for the quadrupole region, and $\sim 2\times10^{-9}$ mbar for the UHV-chamber pressure. Various types of activation techniques were performed to disassemble membrane proteins from nanodiscs or detergent micelles. In-source dissociation (ISD) was performed by varying the voltage of skimmer 1 from 30 to 200 V. Collision induced dissociation (CID) was performed in the hexapole collision cell by colliding ions with Ar. IRMPD was performed with a Synrad 30-W CO_2 laser (Mukilteo, WA) that was interfaced to the back of the mass spectrometer. IRMPD experiments were recorded at different laser energy with the irradiation time kept at 0.5 s and the laser power varied from 30% to 80% (30 W). The mass spectrometer was externally calibrated with cesium iodide. 100 scans were averaged for each spectrum and recorded at 256 k data points unless specified otherwise.

During this research, a less massive collision gases (N_2 or Ar) were not investigated on the Q-ToF instrument; conversely, a more massive collision gas (SF_6) were not investigated on the Orbitrap or the FT-ICR instruments, which potentially could result in subtle charge state distribution and center-of mass activation energy differences.

All instrument were externally calibrated using a 50 μg/μL solution of caesium iodode, 50% acetonitrile/water solution.

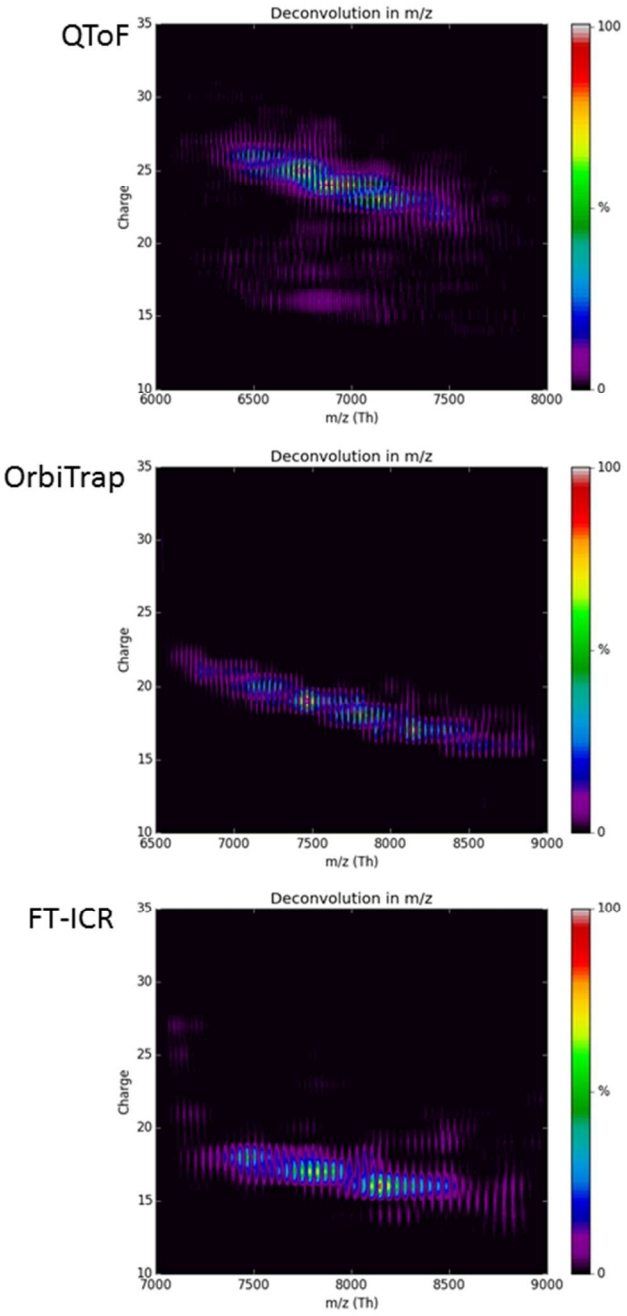

Figure S5. Comparison of empty MSP1D1-Nd charge state distribution and molecular weight, under following instrument voltage parameters: Q-ToF (source temperature 30°C, sample cone 150V, TWIG trap 10V); Orbitrap-EMR (capillary temperature 200°C, source induced dissociation 80V, HCD 20V); FT-ICR-MS (capillary temperature 100°C, skimmer 100V, collision cell 4V).

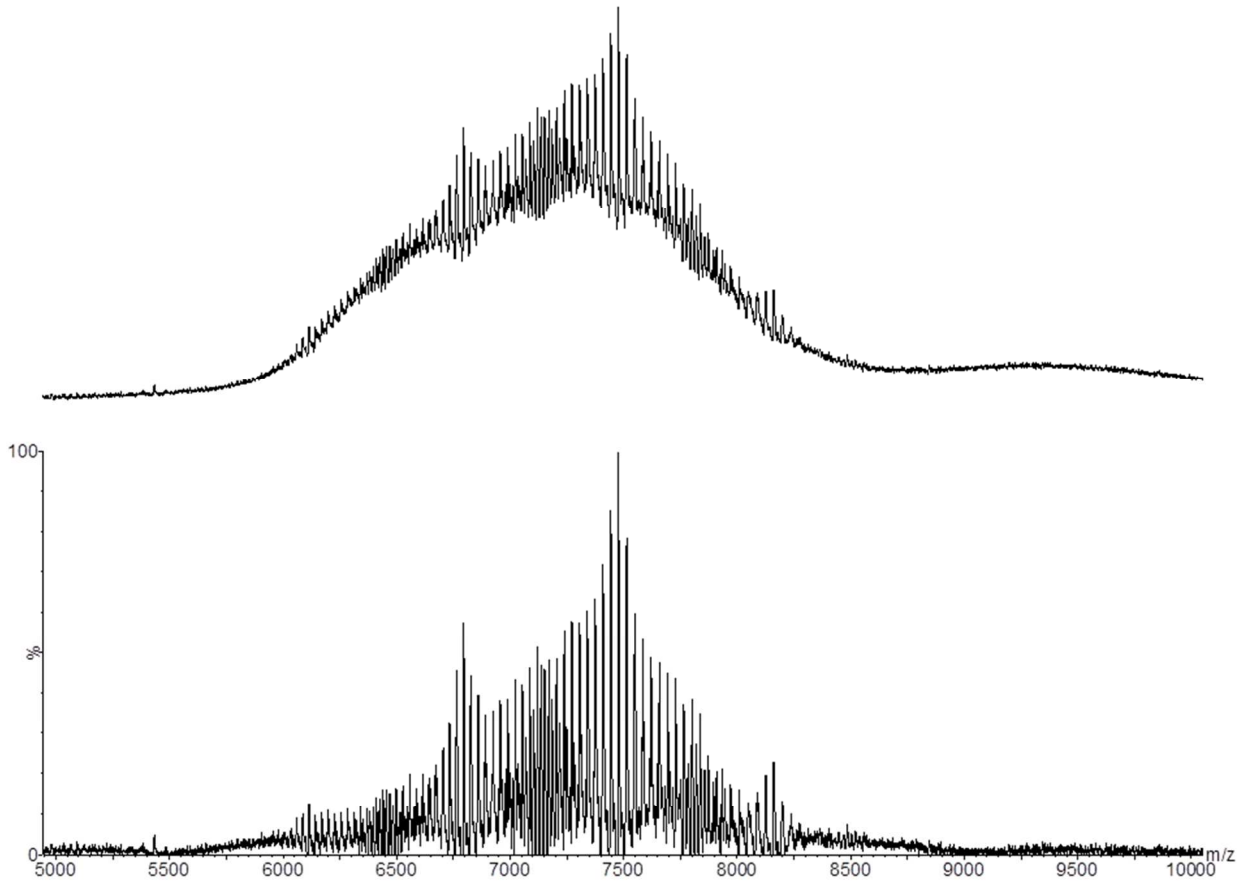

Figure S6. The empty MSP1D1-Nd acquired under native MS and buffer conditions on the Q-ToF. Sample cone 100V, Trap TWIG 10V, SF_6 as the collision gas. Both spectra are smoothed (25 channels x 2, Savitsky-Golay) within MassLynx. The lower spectrum is background subtracted (polynomial order 25, 2 %below curve).

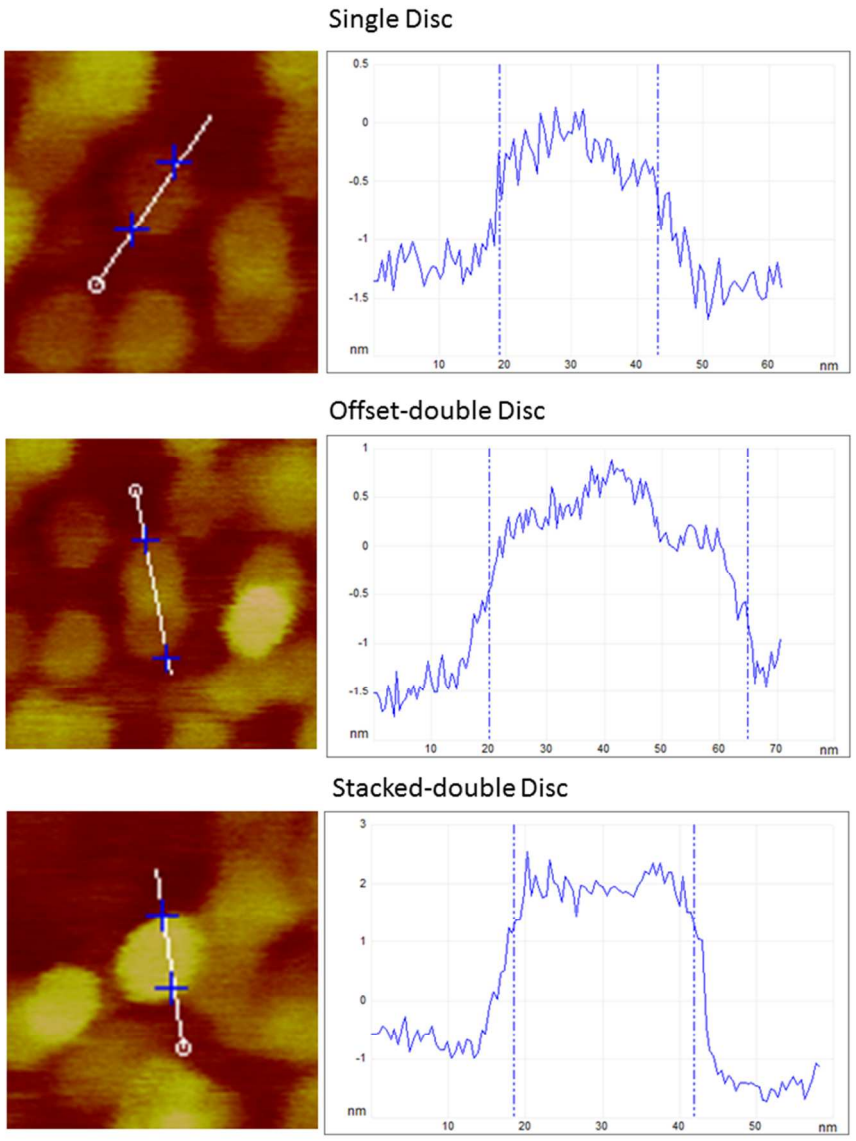

Figure S7. All images were obtained using a Veeco MultiMode 8 (Veeco, Santa Barbara, USA). Nitrite Scanasyst-Fluid tips (k=0.7 Nm) were used in PeakForce Tapping™ mode. A 2 µM suspension of empty MSP1D1 nanodiscs in 25mM Tris buffer, pH 7.5 was used to absorb the Nd on to the surface of freshly cleaved mica for 3 days at 4 °C. The mica surface was then washed with 25mM Tris, pH 7.5 buffer and images were taken using the same Tris solution as the imaging buffer. For AFM analysis, the empty MSP1D1-Nds were absorbed onto a mica surface and the AFM images are presented in Figure S7. Slow absorption at 4 °C results in the MSP1D1-Nd being absorbed in a "flat" position. Relatively high numbers of empty MSP1D1-Nds were absorbed resulting in a population of offset-double disc (overlapping) and multiple

stacked-double discs, in addition to clearly distinguishable single discs. The diameter of Nds obtained from software-generated measurement of single and double discs are approximately 21 to 22 nm which are double of previously published AFM dimensions of 10 nm [7], calculated Stokes radii of 9.8 nm [7-8] and 9.5 nm [3] and theoretically derived values ranging from 9.49 to 10.65 nm, depending on the level of DMPC lipid incorporation [9] within empty MSP1D1-Nd. The thickness obtained by AFM (approximately 1.5 nm) appears to be less than the theoretical value 3.57 nm [9] and previously published AFM dimensions (5.6 to 5.9 nm) [1]. This inaccuracy in thickness is likely due to the possible interactions of probe with sample surface and surface tension, affecting probe scanning in tapping mode.

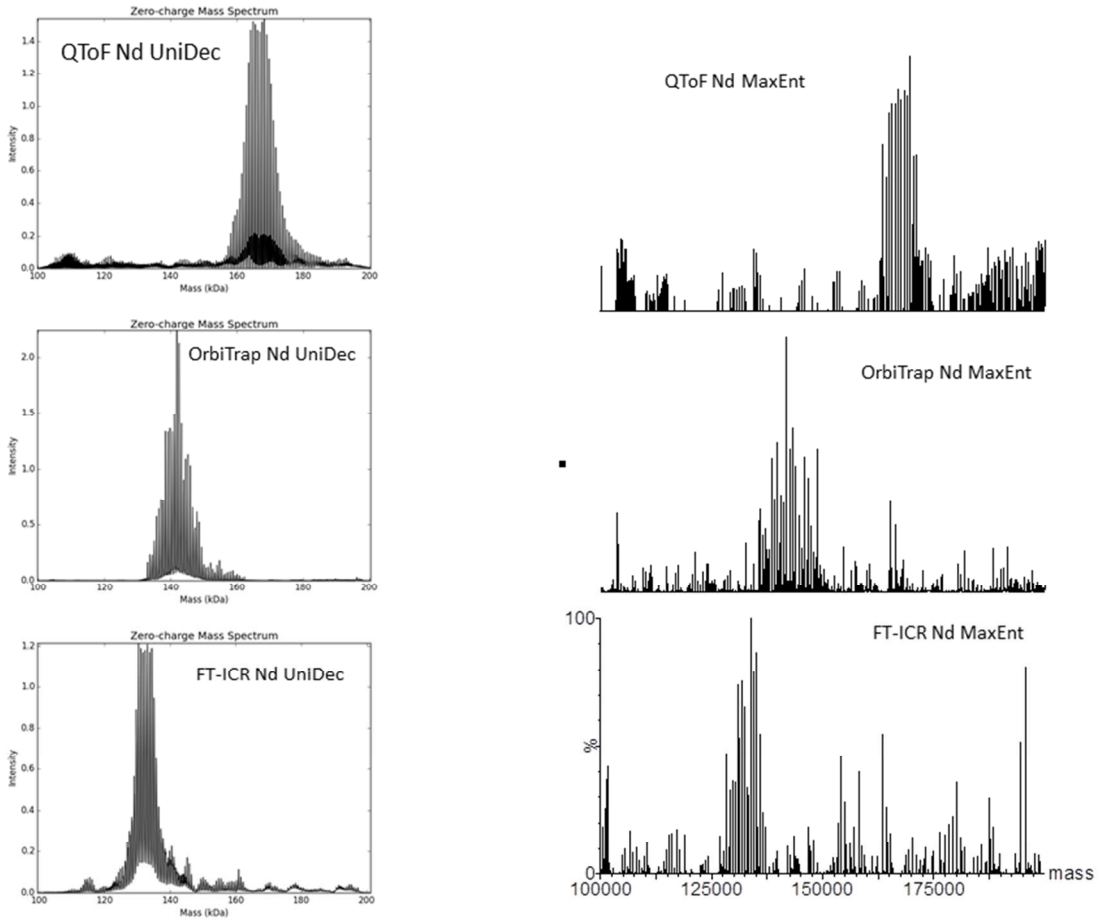

Figure S8. UniDec versus Maximum Entropy deconvolution. Both deconvolution algorithms converge to highly consistent average zero-charge Mw values. For UniDec deconvolution [10-11], all data was exported as a text delimited file composed only of *m/z* value and intensity, prior to deconvolution processing. For Maximum Entropy deconvolution all data files were processed in MassLynx. The FT-ICR (Bruker) and Orbitrap (ThermoScientific) were converted to MassLynx data file format (using DataBridge) prior to Maximum Entropy deconvolution in MassLynx v4.1. Maximum Entropy [12] deconvolution parameters: Ranges: 100,000:200,000; Resolution 10Da/channel; Damage Model Uniform Gaussian Width at Half Height: 7 Da (Q-ToF), 7 Da (Orbitrap), 17 Da (FT-ICR); Minimum intensity ratios: Left 25%, Right 25%; Iterate to Convergence. UniDec Deconvolution parameters: Charge Range 10 to 35; Mass Range 100,000 to 20,000; Sample Mass Every 1 Da; Peak FWHM 3 Da Gaussian (Q-ToF), 3 Da Lorentzian (Orbitrap), 7 Da Lorentzian (FT-ICR); Mass Difference 677.5 Da; Mass Smooth Widths 1.0; Maximum number of Iterations 1000.

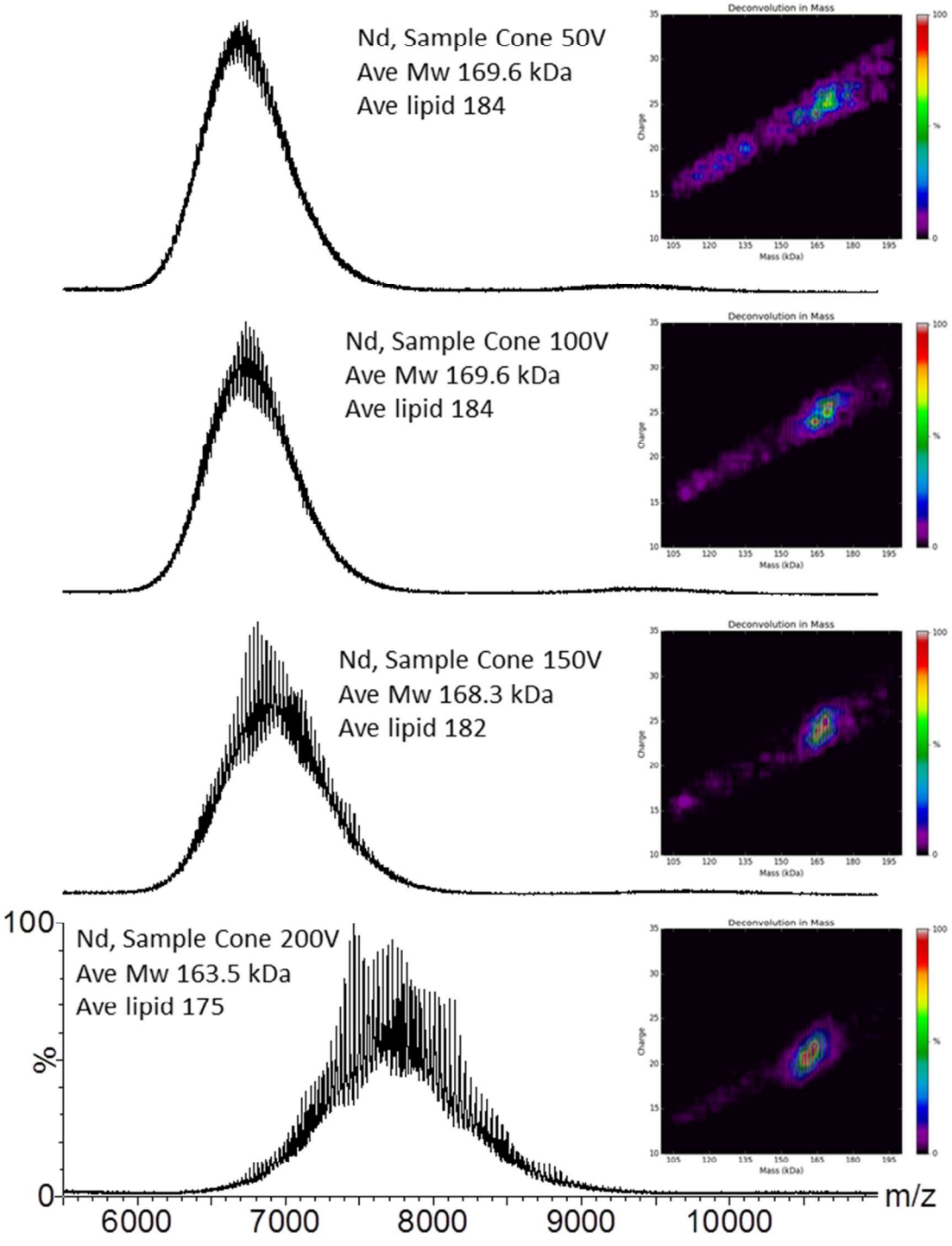

Figure S9. The empty MSP1D1-Nd analyzed on the Q-ToF as a function of source sample cone voltage (50 to 200V). TWIG Trap held constant at 10V. The inset is the UniDec deconvoluted spectrum, displaying the zero-charge Mw on the x-axis and the detected charge states on the y-axis.

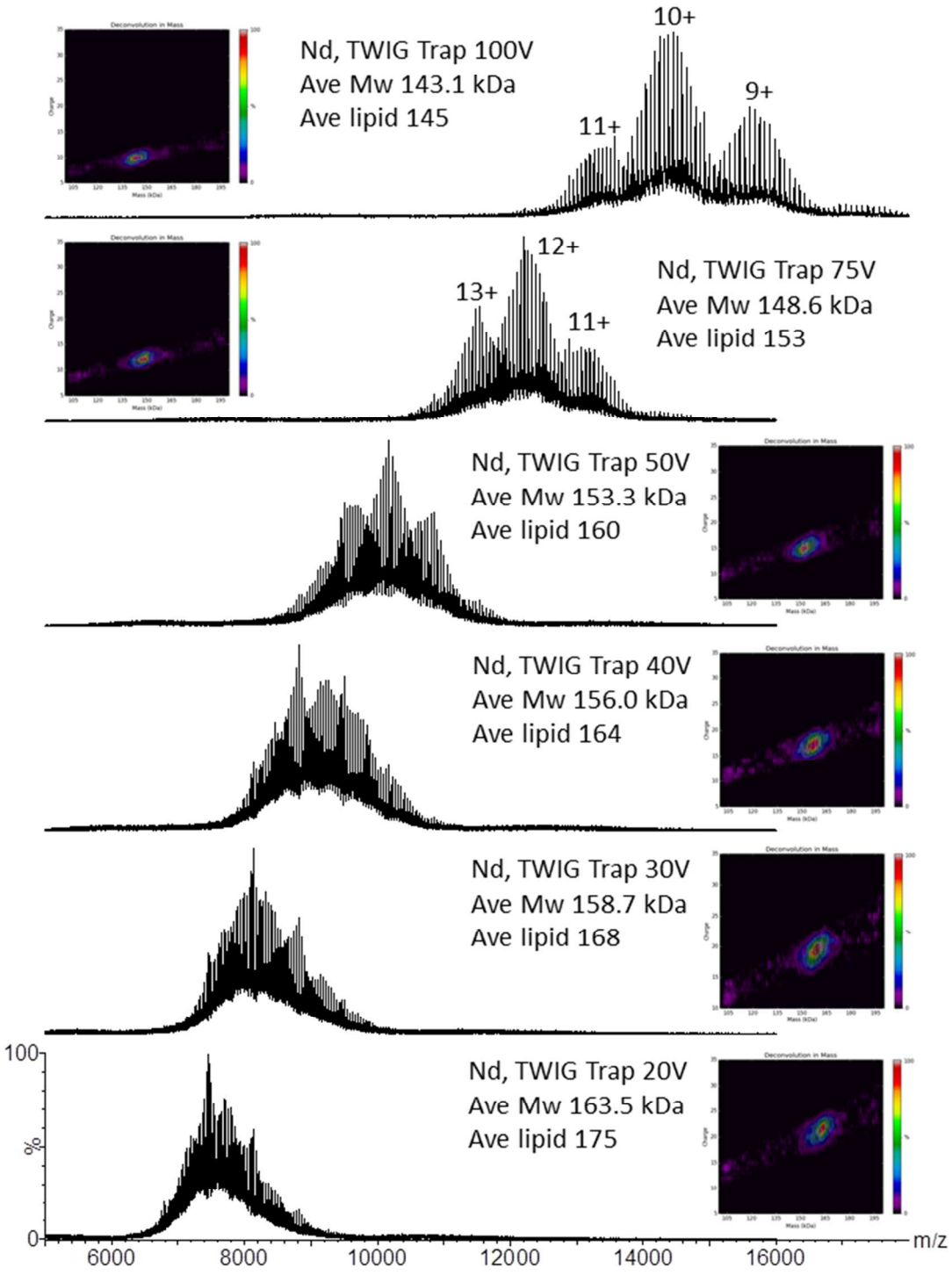

Figure S10. The empty Nd analyzed on the Q-ToF as a function of TWIG Trap voltage (20 to 100V). Sample Cone help constant at 200V. The inset is the UniDec deconvoluted spectrum, displaying the zero-charge Mw on the x-axis and the detected charge states on the y-axis.

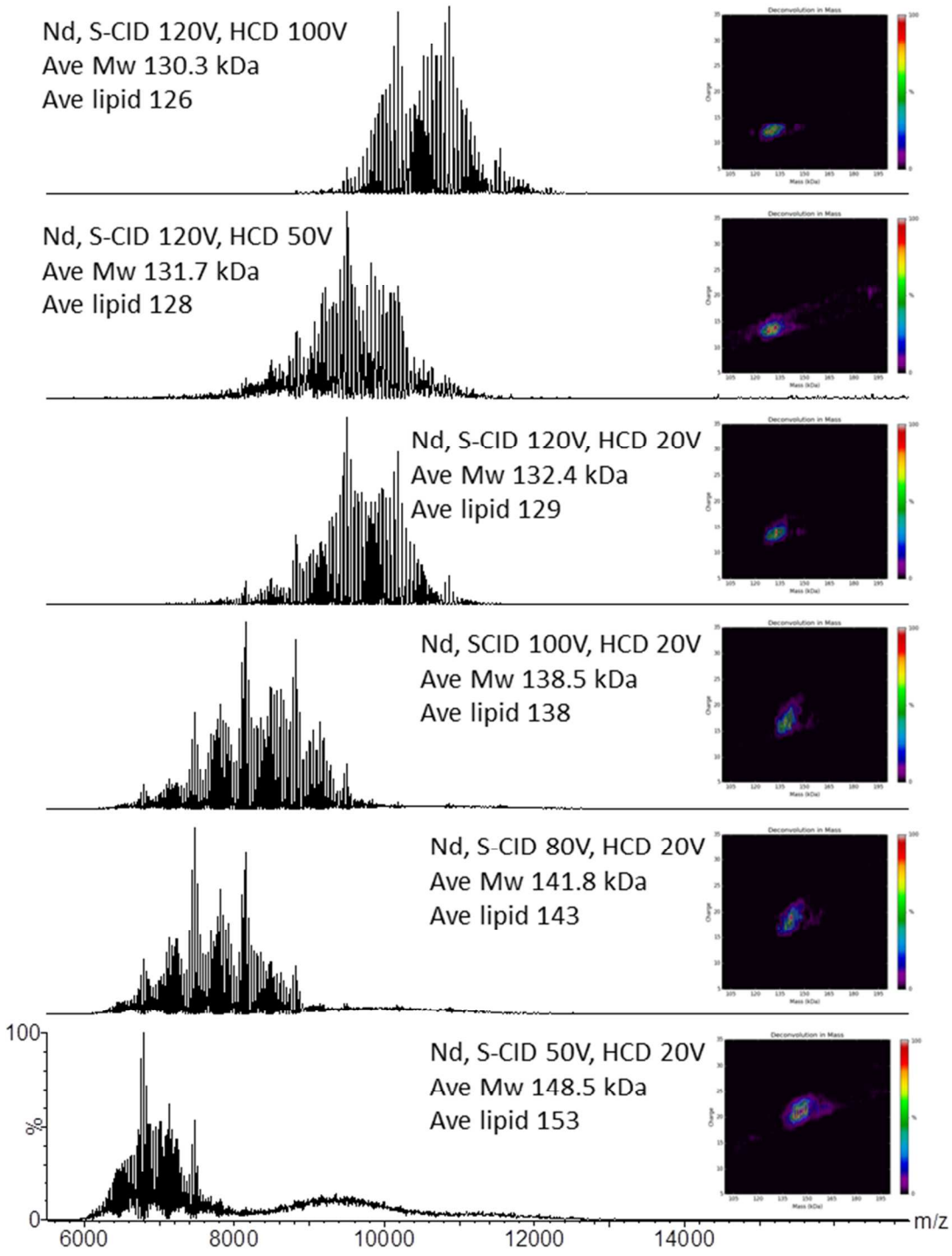

Figure S11. The empty MSP1D1-Nd analyzed on the Orbitrap-EMR as a function of source-CID voltage (20 to 100V) and HCD (20-100V) activation voltage. The inset is the UniDec deconvoluted spectrum, displaying the zero-charge Mw on the x-axis and the detected charge states on the y-axis.

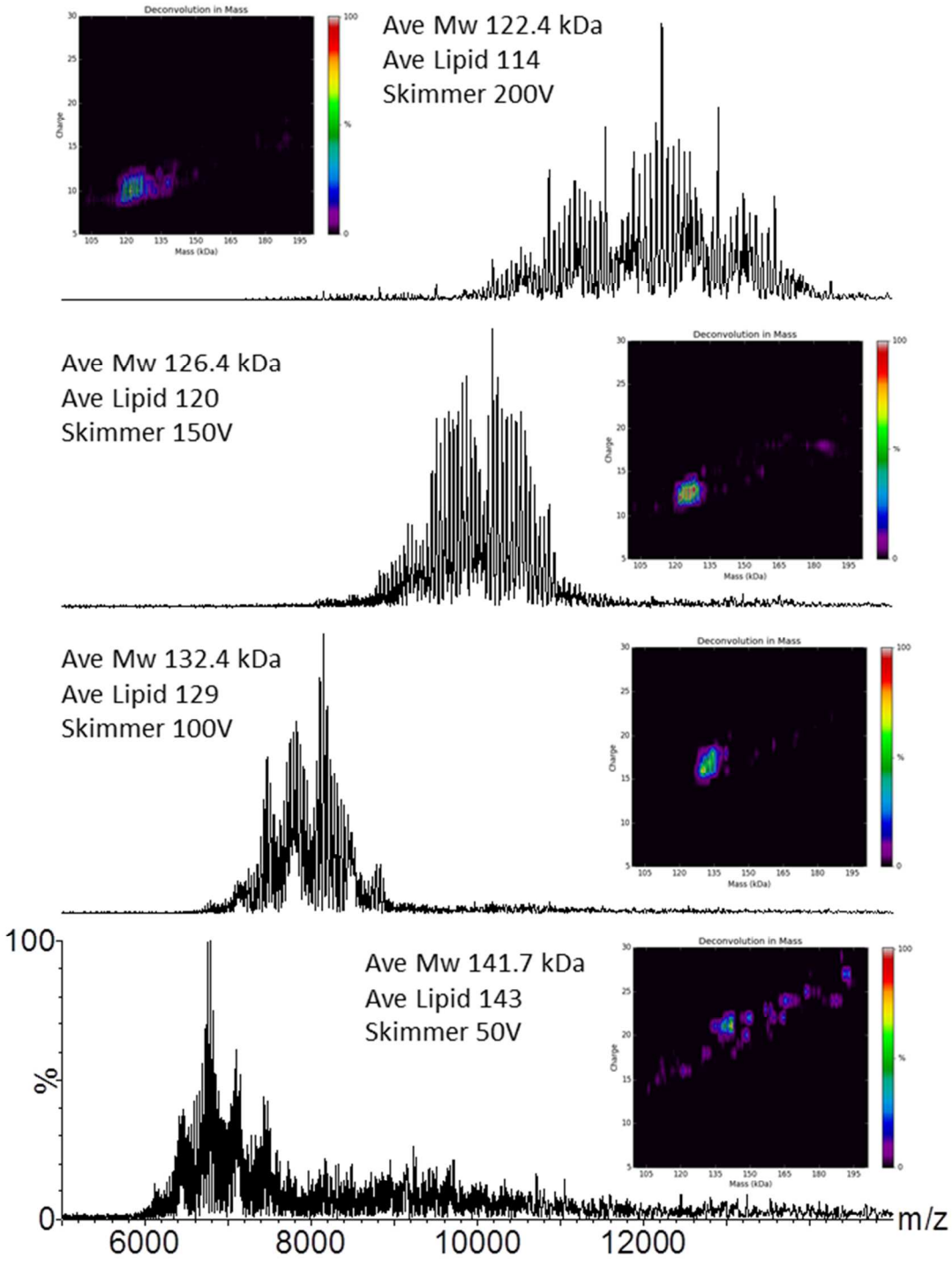

Figure S12. The empty MSP1D1-Nd analyzed on the FT-ICR-MS as a function of source skimmer voltage (50 to 200V). The inset is the UniDec deconvoluted spectrum, displaying the zero-charge Mw on the x-axis and the detected charge states on the y-axis.

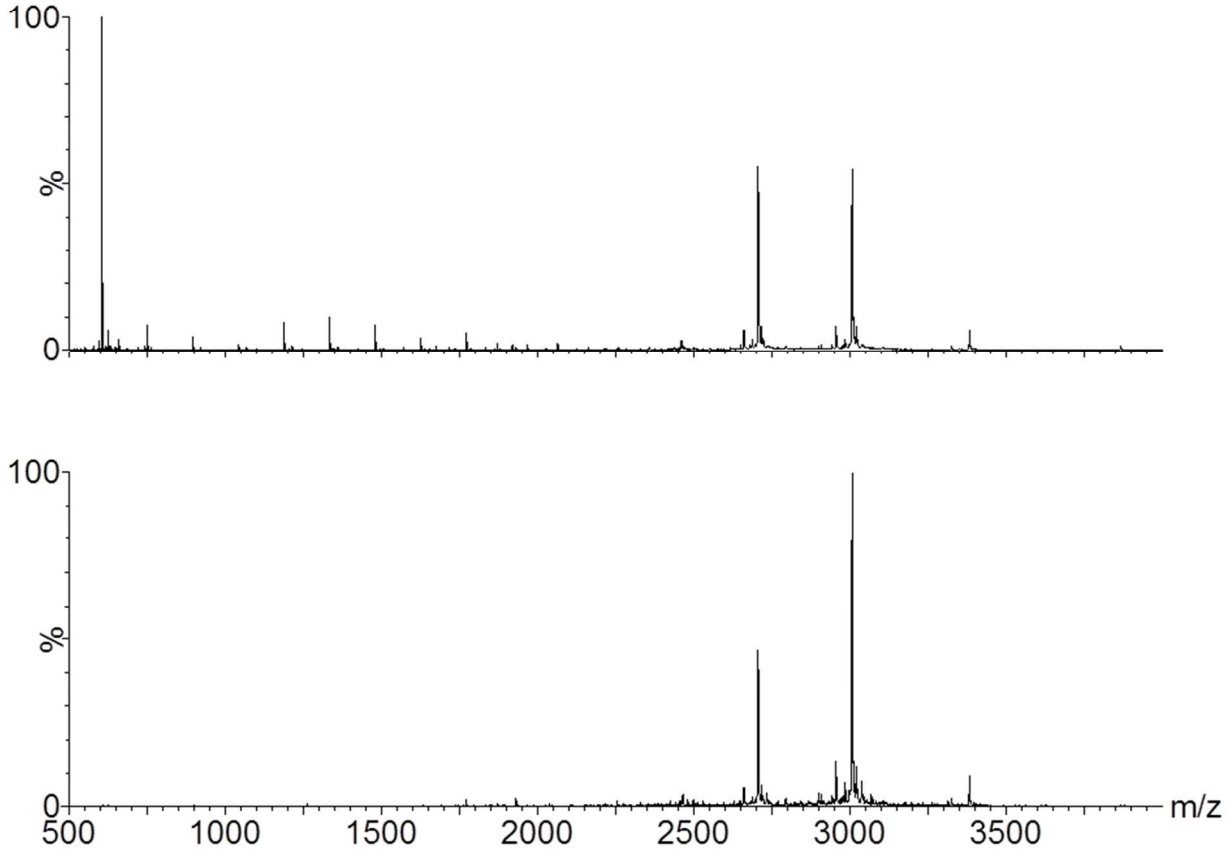

Figure S13. A comparison of a Q-ToF MS (upper) and a tandem-MS of *m/z* 3500 (lower) acquisition of bacteriorhodopsin (B-0184) using a sample cone value of 25V and a TWIG trap value of 100V, for both acquisitions. The lower spectra display far lower detergent related ions in the *m/z* range 50-2000.

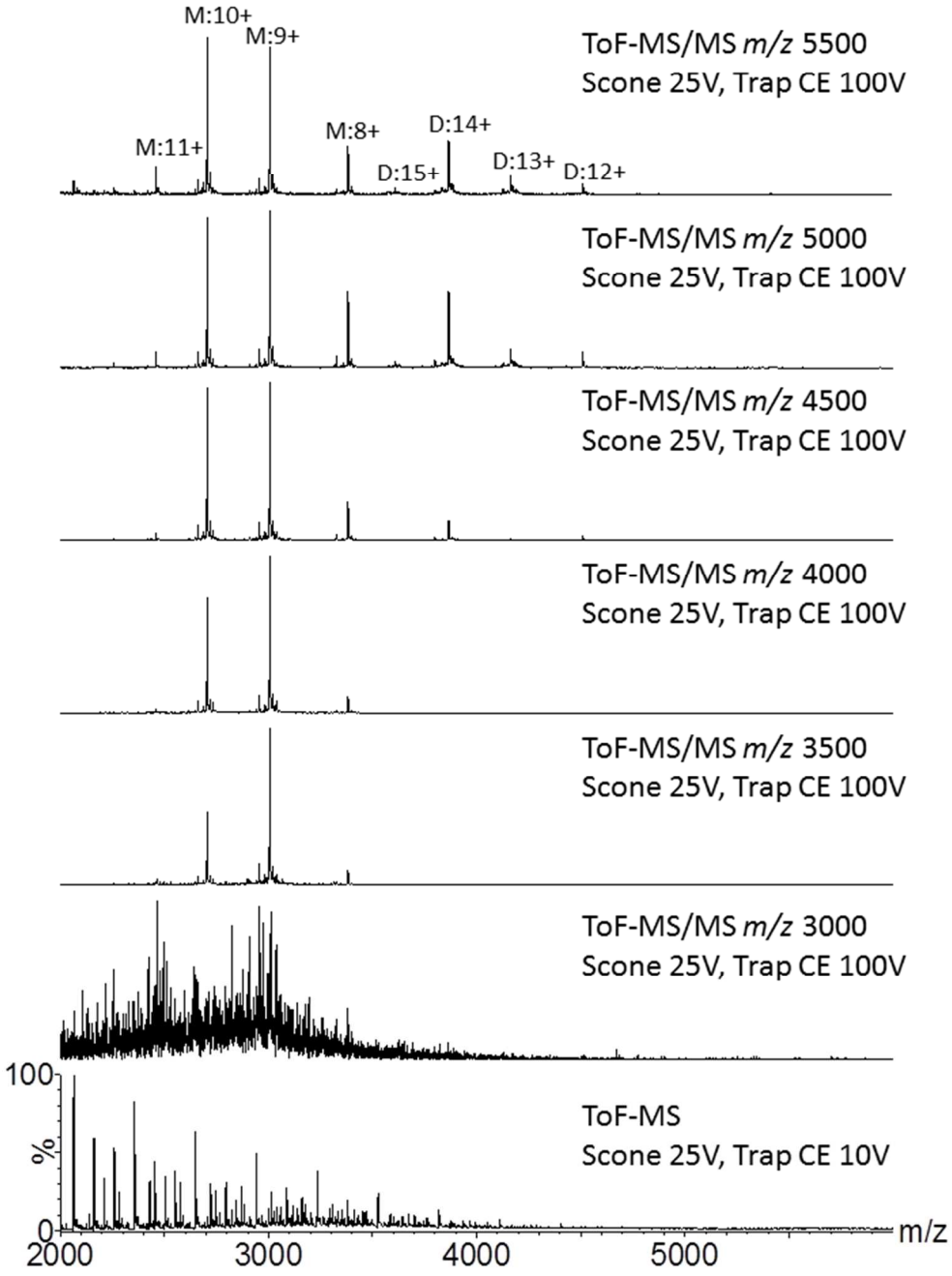

Figure S14. Q-ToF tandem-MS quadrupole selection of the bacteriorhodopsin-OG (B-0184) micelle at *m/z* values 3000, 3500, 4000, 4500, 5000 and 5500. M represents the bacteriorhodopsin monomer; D represents bacteriorhodopsin dimer. Sulphur hexafluoride (SF_6) was used as the collision gas. A TWIG trap voltage of 100V was used to liberate bacteriorhodopsin for the OG-micelle.

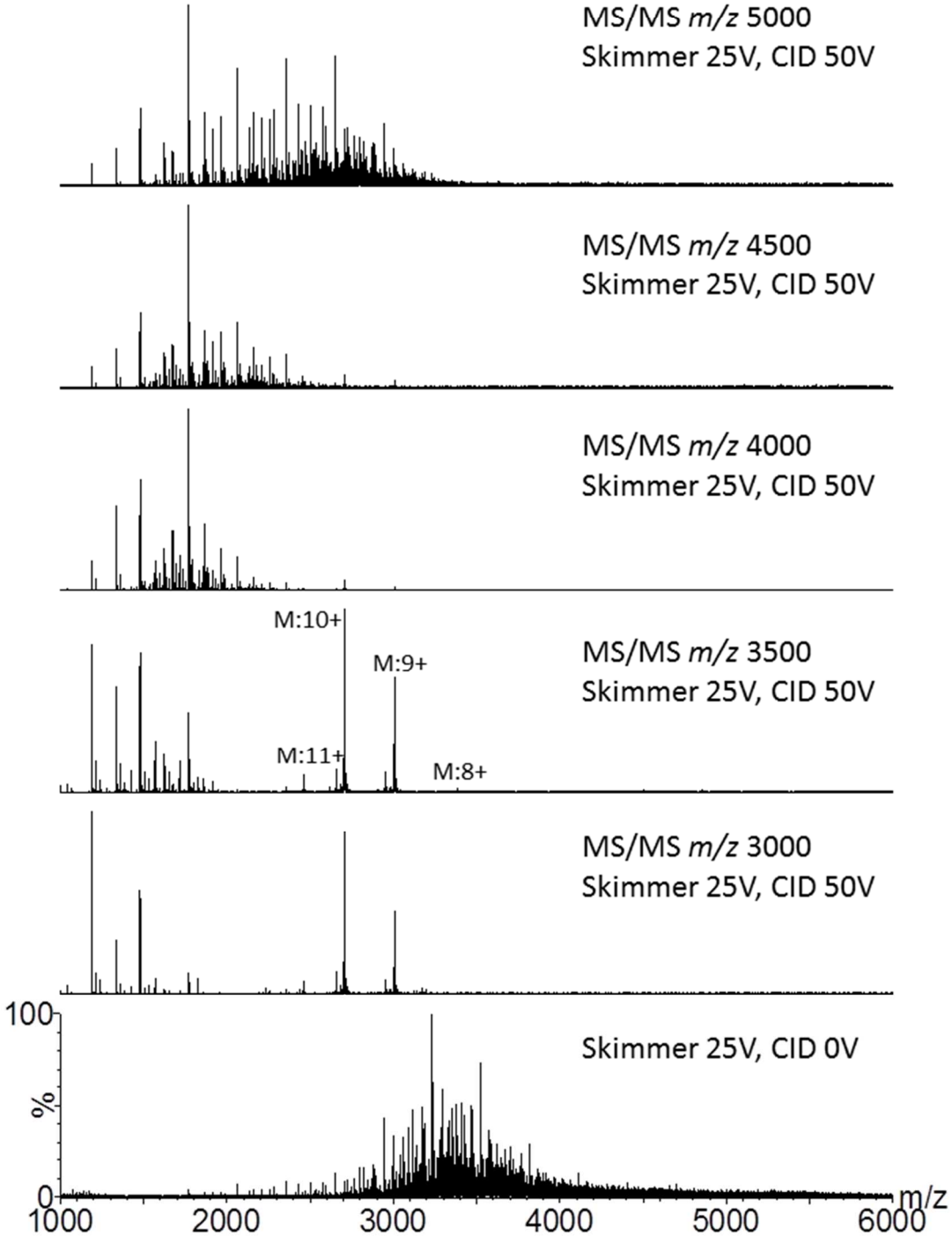

Figure S15. FT-ICR tandem-MS selection of the bacteriorhodopsin-OG (B-0184) micelle at *m/z* values 3000, 3500, 4000, 4500 and 5000. M represents the bacteriorhodopsin monomer. Dimer not detected in this FT-ICR dataset. Argon (Ar) was used as the collision gas. A collision cell CID voltage of 50V was used to liberate bacteriorhodopsin for the OG-micelle.

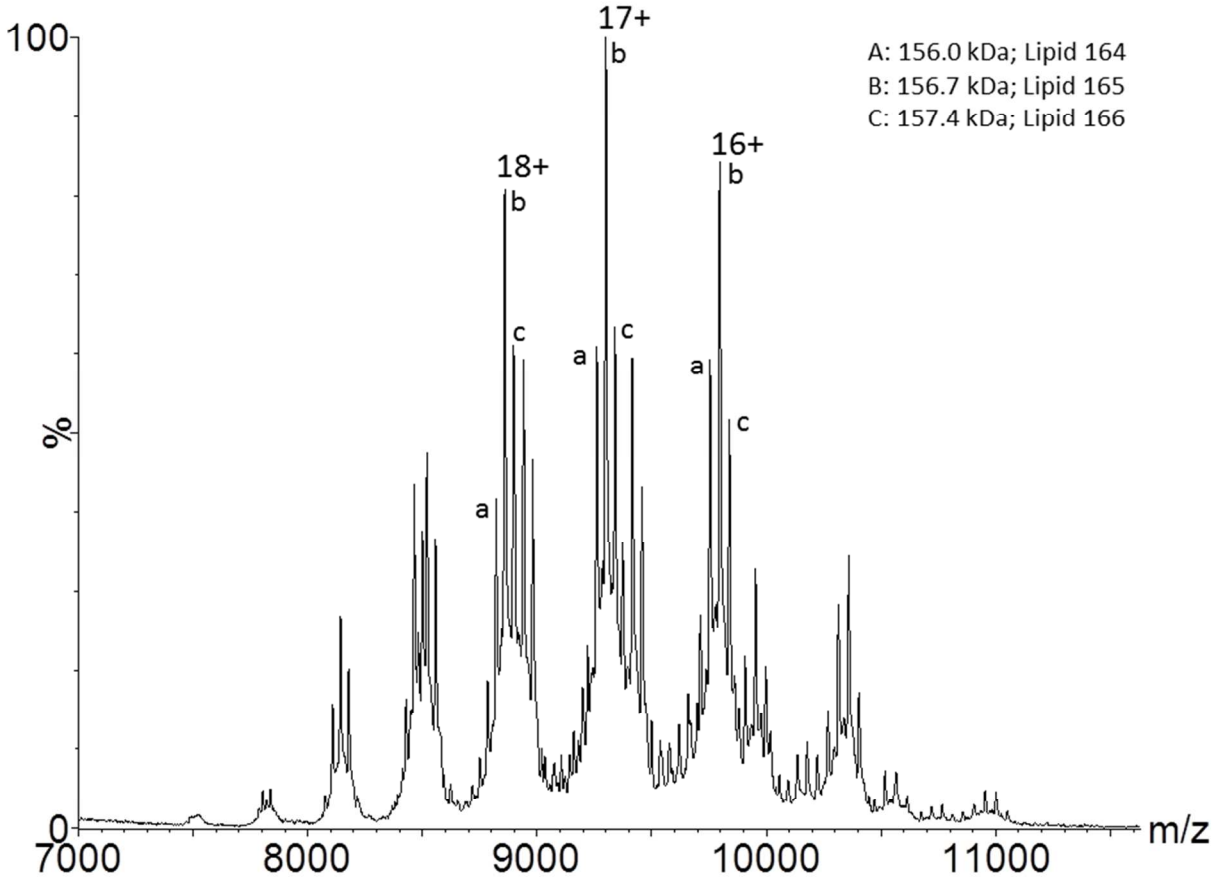

Figure S16. Q-ToF tandem-MS, *m/z* 7500 selection and activation (TWIG Trap 40V) of the empty MSP1D1-Nd. Individual charge states are annotated (21+, 20+ & 19+). Also annotated are the calculated MSP1D1-Nd molecular weight (kDa) and associated phospholipid (lipid) number.

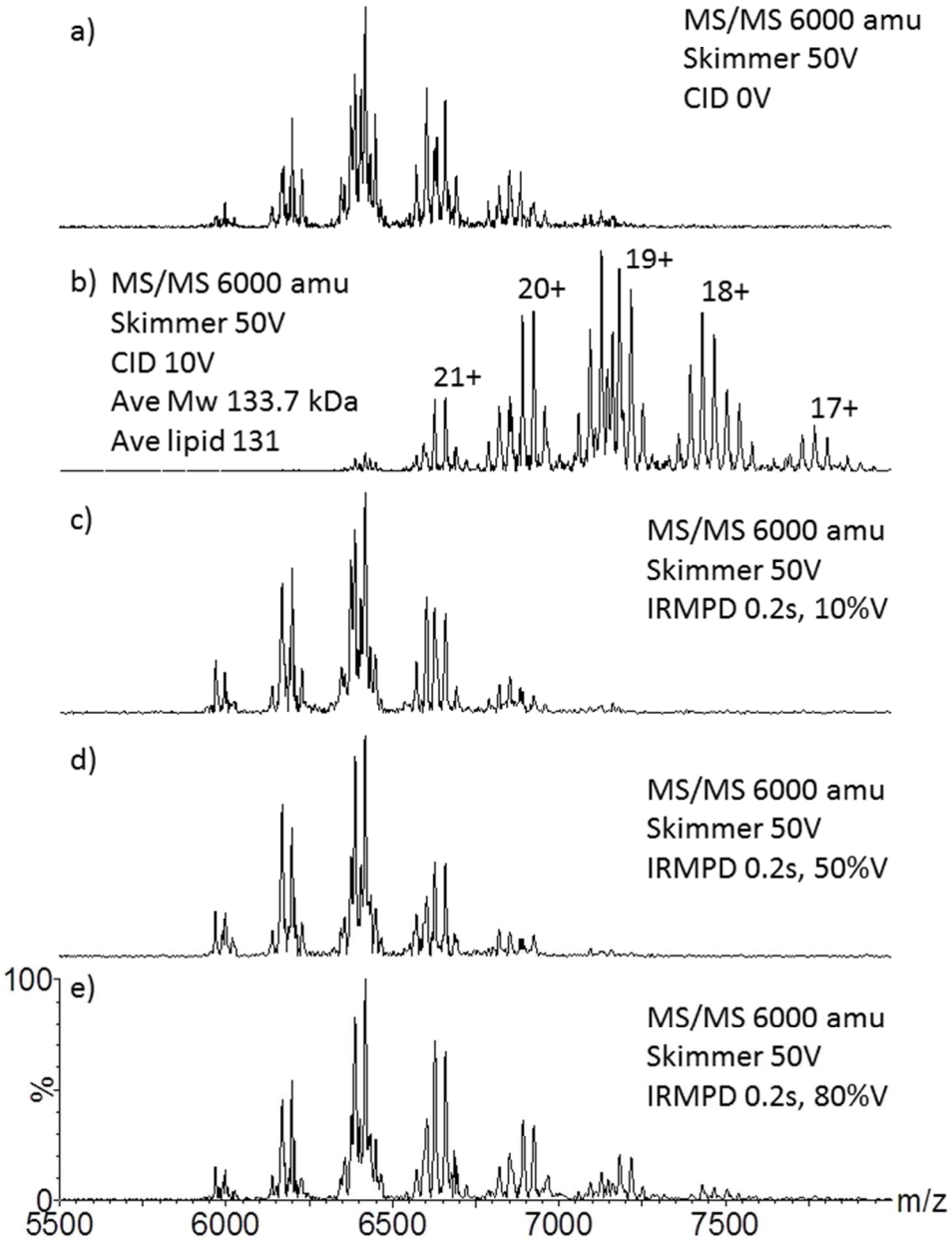

Figure S17. FT-ICR tandem-MS, *m/z* 6000 selection (skimmer 50V), of the empty MSP1D1- Nd and subsequent activation using both collision induced dissociation and IRMPD. a) collision energy 0V; b) collision energy 10V. The average Mw and DMPC lipid count has been calculated for the most intense peak in the identified charge state, corresponding 133.7kDa and 131 DMPC phospholipids; c) IRMPD 0.2s, 10% power; d) IRMPD 0.2s, 50% power; e) IRMPD 0.2s, 80% power. Due to the low *m/z* selection range of the FT-ICR-MS quadrupole (max *m/z* 6000), only limited mass scale selection and subsequent activation was possible.

References

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