# Supporting Information

## Exploring Charge-Detection Mass Spectrometry on Chromatographic Time Scales

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# Experimental

**Table S1:** Instrument parameter settings used for static nano-ESI based native MS of β-galactosidase and transferrin.

| Parameter | ß-galactosidase | Transferrin |
|---|---|---|
| Spray voltage | 0.9 kV | 1.0 kV |
| Capillary temperature | 250˚C | 250˚C |
| S-lens RF level | 200 | 200 |
| Resolution setting at m/z 400 | 6,250 | 12,500 |
| Trapping gas | Nitrogen | Nitrogen |
| UHV pressure (mbar) | 5.2e-10 | 1.7e-10 |
| IST desolvation voltage | -200 V | -150 V |
| Acquisition time | 2 min | 2 min |
| Averaging | on | on |

**Table S2:** Instrument parameter settings used for static nano-ESI based DMT mode analysis of β-galactosidase.

| Parameter | ß-galactosidase |
|---|---|
| Spray voltage | 0.9 kV |
| Capillary temperature | 250˚C |
| S-lens RF level | 200 |
| Resolution setting at m/z 400 | 50,000 |
| Trapping gas | Nitrogen |
| UHV pressure (mbar) | 2.6e-11 |
| IST desolvation voltage | -200 V |
| Acquisition time | 32 min |
| Averaging | off |
| Detector m/z optimization | High |
| Ion transfer target | High |
| Ion Injection | IT (50 ms) |

**Table S3:** Instrument parameter settings used for LC-DMT mode analysis of β-galactosidase, transferrin, etanercept and AGP.

| Parameter | ß-galactosidase | Transferrin | Etanercept | AGP |
|---|---|---|---|---|
| Spray voltage | 3.8 kV | 3.8 kV | 3.8 kV | 3.8 kV |
| Capillary temperature | 300˚C | 300˚C | 300˚C | 300˚C |
| Probe heater temp. | 150 ˚C | 150 ˚C | 150 ˚C | 150 ˚C |
| S-lens RF level | 200 | 200 | 200 | 200 |
| IST desolvation | -150 V | -100 V | -200 V | -200 V |
| Ion Flux Control | AIC (200%) | AIC (100%) | AIC (100%) | AIC (100%) |
| Resolution at m/z 400 | 50,000 | 100,000 | 100,000 | 100,000 |
| Trapping gas | Nitrogen | Nitrogen | Nitrogen | Nitrogen |
| Trapping gas pressure (mbar) | 5.2e-10 | 5.0e-11 | 6.7e-11 | 6.7e-11 |
| Detector m/z optimization | High | High | High | High |
| Ion transfer target | High | High | High | High |

# ß-galactosidase charge misassignments

ß-galactosidase spectra were assigned to several different charge ranges resulting in a true mass spectrum as is depicted in Figure S1. The most intense peak corresponds to the protein with the expected molecular mass of ~466 kDa. Halo peaks appear as repeating patterns suggesting that signals are caused by charge misassignments. Charge heatmaps of the three dominant peaks are shown in Figure S2. It becomes apparent that signals are identical but are shifted by +/-1 charges, respectively. The +/-1 charge assignment will cause a misassignment of the mass by +/- of the detected *m/z* ratio. In consequence, halo peaks are spaced by 9-10 kDa.

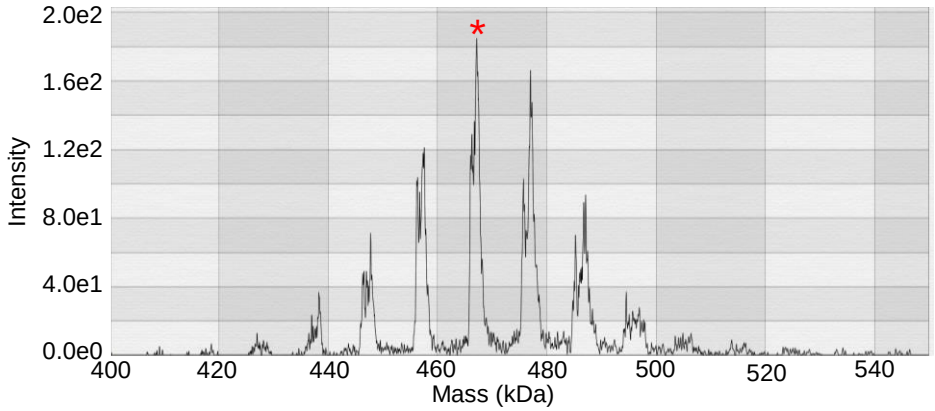


**Figure S1:** Repeating pattern obtained for the direct mass spectrum of ß-galactosidase. The peak at ~466 kDa (labelled in red) represents the expected molecular mass.

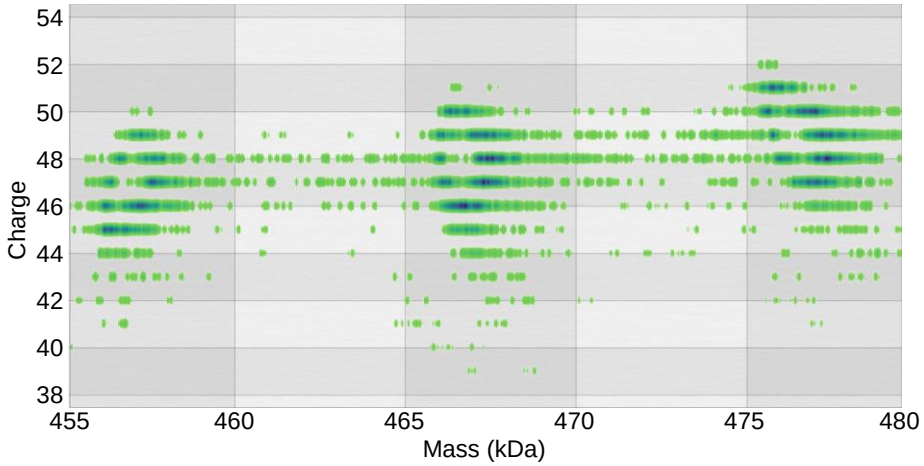


**Figure S2:** Charge heat map of the three most abundant peaks from Figure S1. The pattern seen is the same across all three peaks with only the charge shifting by one, indicating that the same signals were assigned to multiple charges.

## True mass spectrum of β-gal based on 50 DMT mode spectra

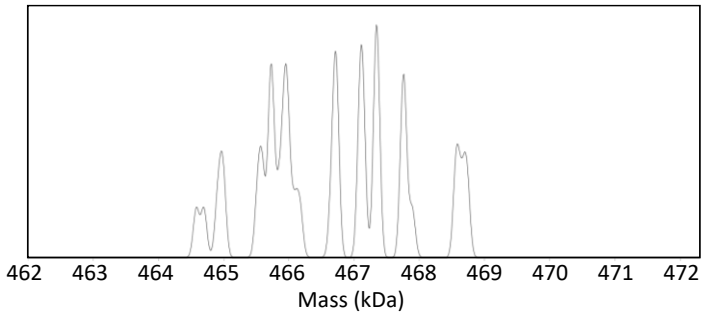


**Figure S3:** True mass spectrum obtained after processing of 50 DMT mode spectra of β-gal.

## Averaged LC-DMT mode spectra of transferrin & β-gal

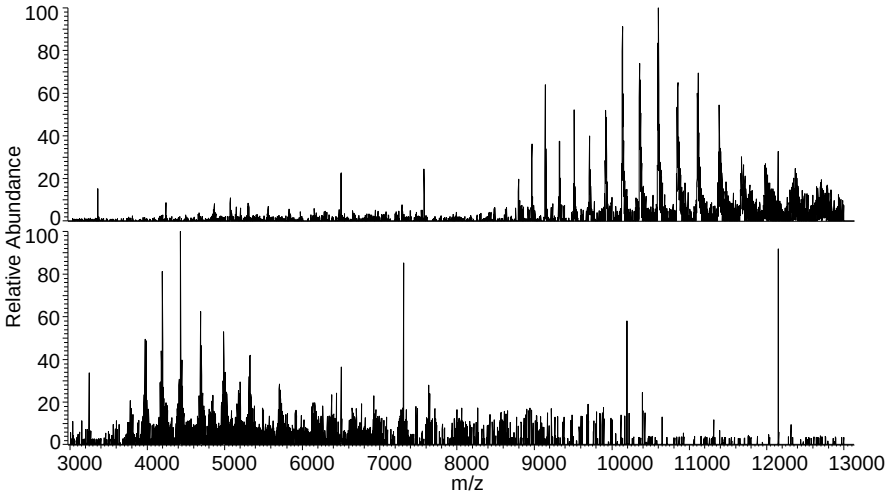


**Figure S4:** Averaged MS spectra of ß-galactosidase (top) and transferrin (bottom) after full width at half maximum peak integration.

# Similarity assessment of transferrin & β-gal LC-DMT mode runs

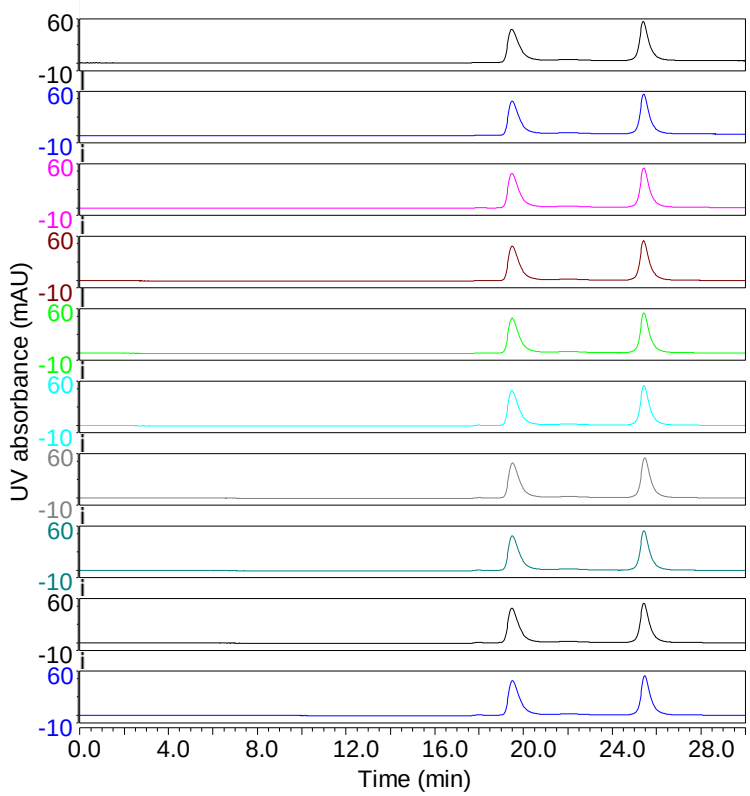


**Figure S5:** Full chromatographic time scale for ten replicate runs of a ß-galactosidase and transferrin mixture.

# Native MS-based annotation of transferrin

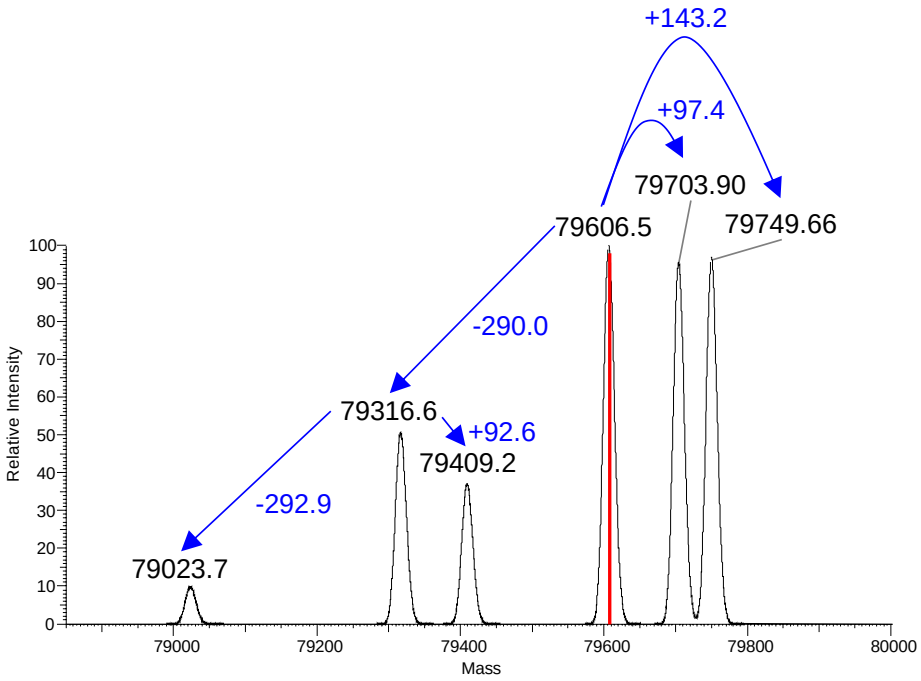


**Figure S6:** Deconvoluted spectrum of transferrin. Mass shifts in relation to the main peak are outlined in blue. Mass shifts are proposedly corresponding to desialylation (-291 Da), fucosylation (+146 Da) and sulphuric or phosphoric acid adduction (+98 Da). The theoretical mass calculated for the most abundant transferrin peak (79,606.5 Da) is shown in red and amounts to 79,607.3 Da. The calculation was based on the primary sequence mass, 19 disulphide bonds, two N-glycans of the A2G2S2 type and a single bound $Fe^{3+}$.

# LC-DMT mode analysis of etanercept and AGP

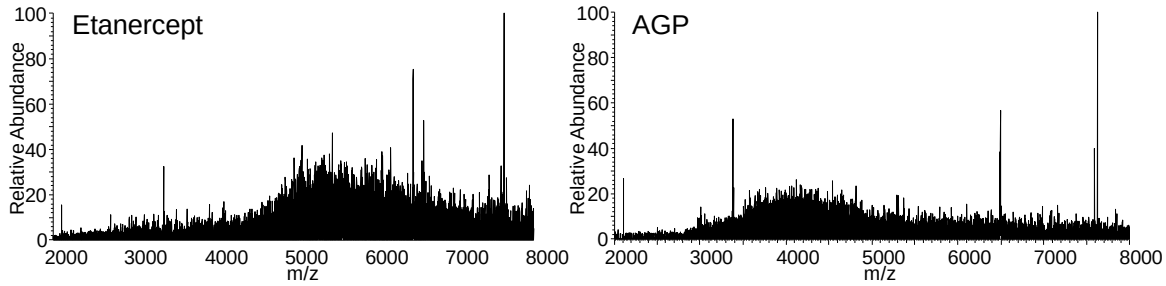


**Figure S7:** Averaged mass spectra obtained for both proteins after chromatographic peak integration.

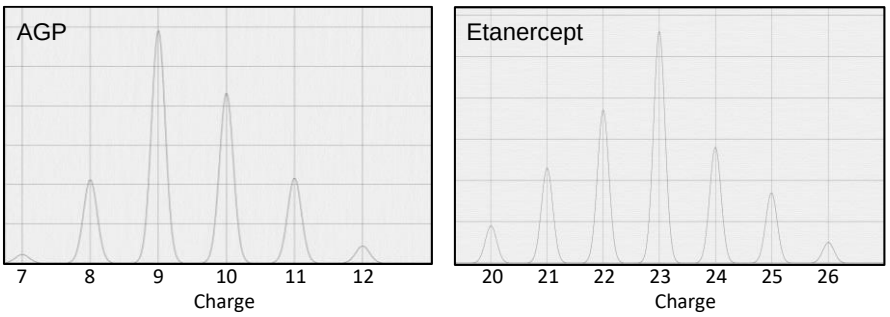


**Figure S8:** Charge state distribution of AGP and etanercept.