High-Throughput Bioconjugation for Enhanced 193 nm Photodissociation via Droplet-Phase Initiated Ion/Ion Chemistry using a Frontend Dual Spray Reactor

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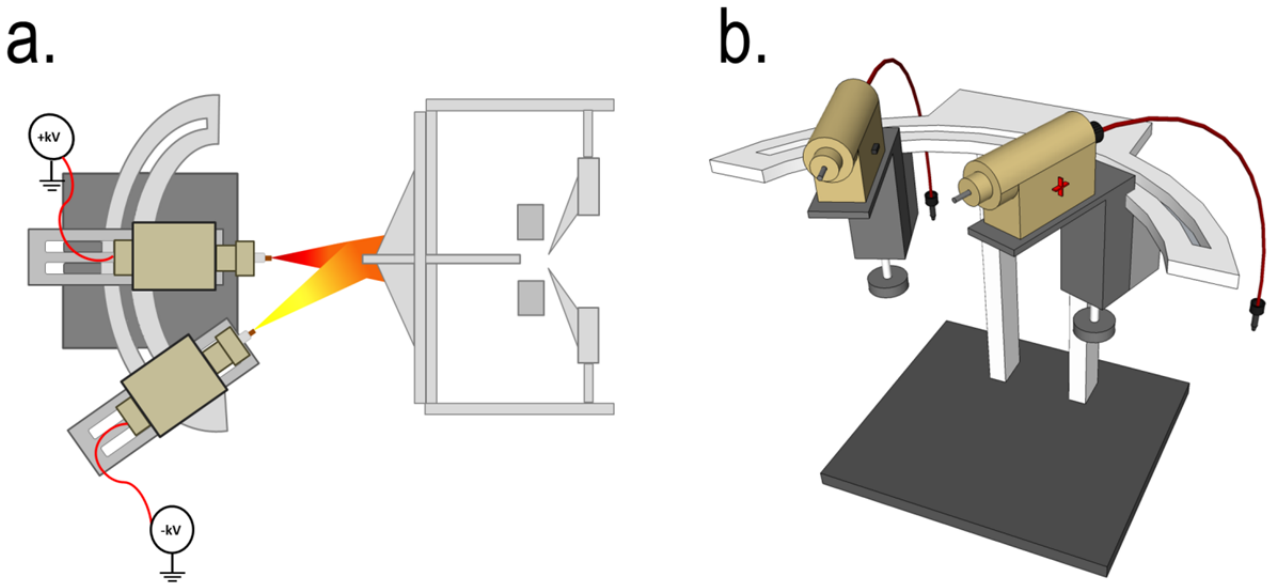
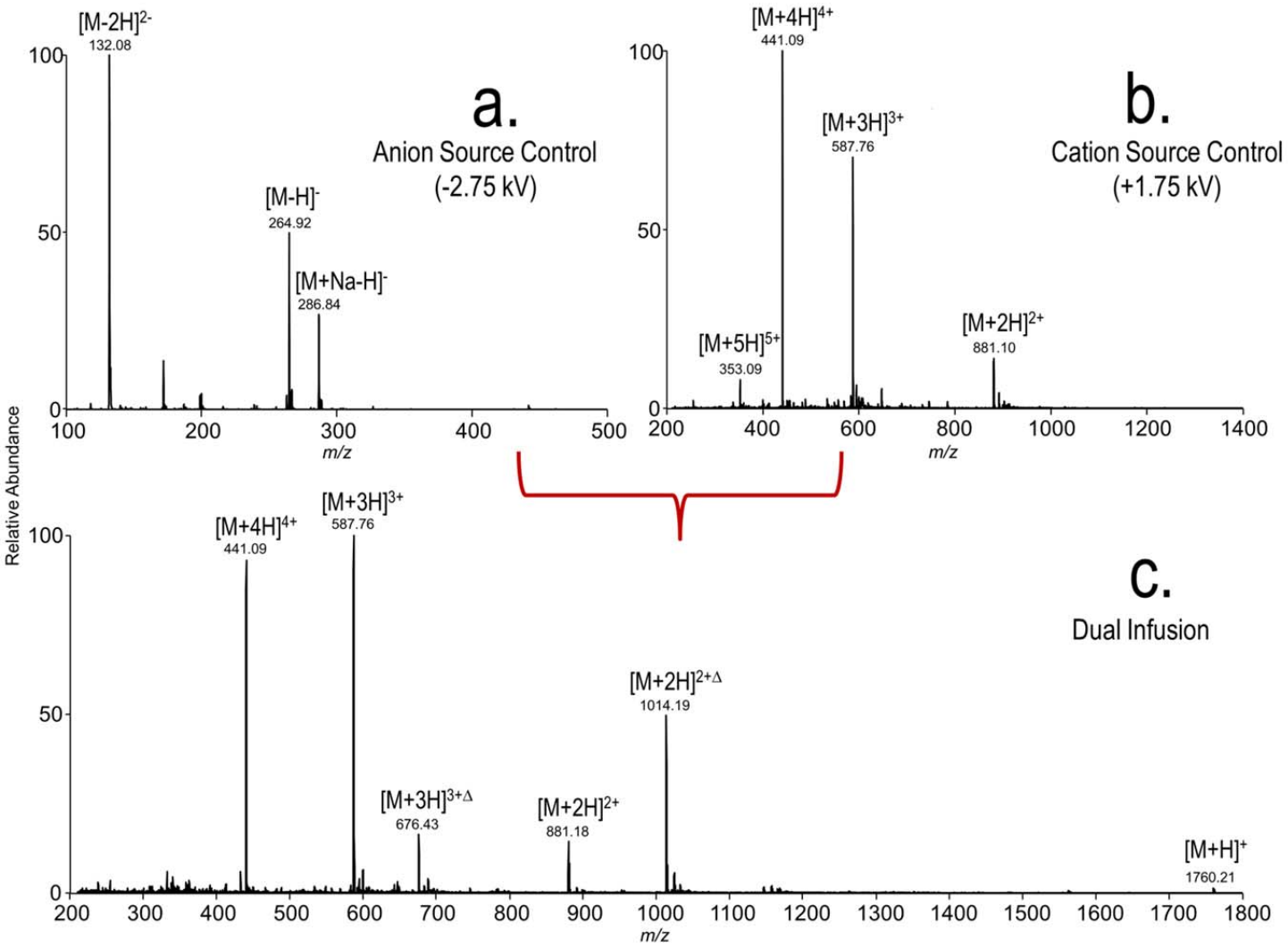
Supplemental Information: The contents of the Supporting Information include diagrams of the dual spray reactor design, control and dual spray reaction mass spectra for direct infusion and chromatographically separated peptides, comparative CID and UVPD mass spectra for Schiff base modified peptides, and energy variable CID plots for unmodified and Schiff base reacted peptides.

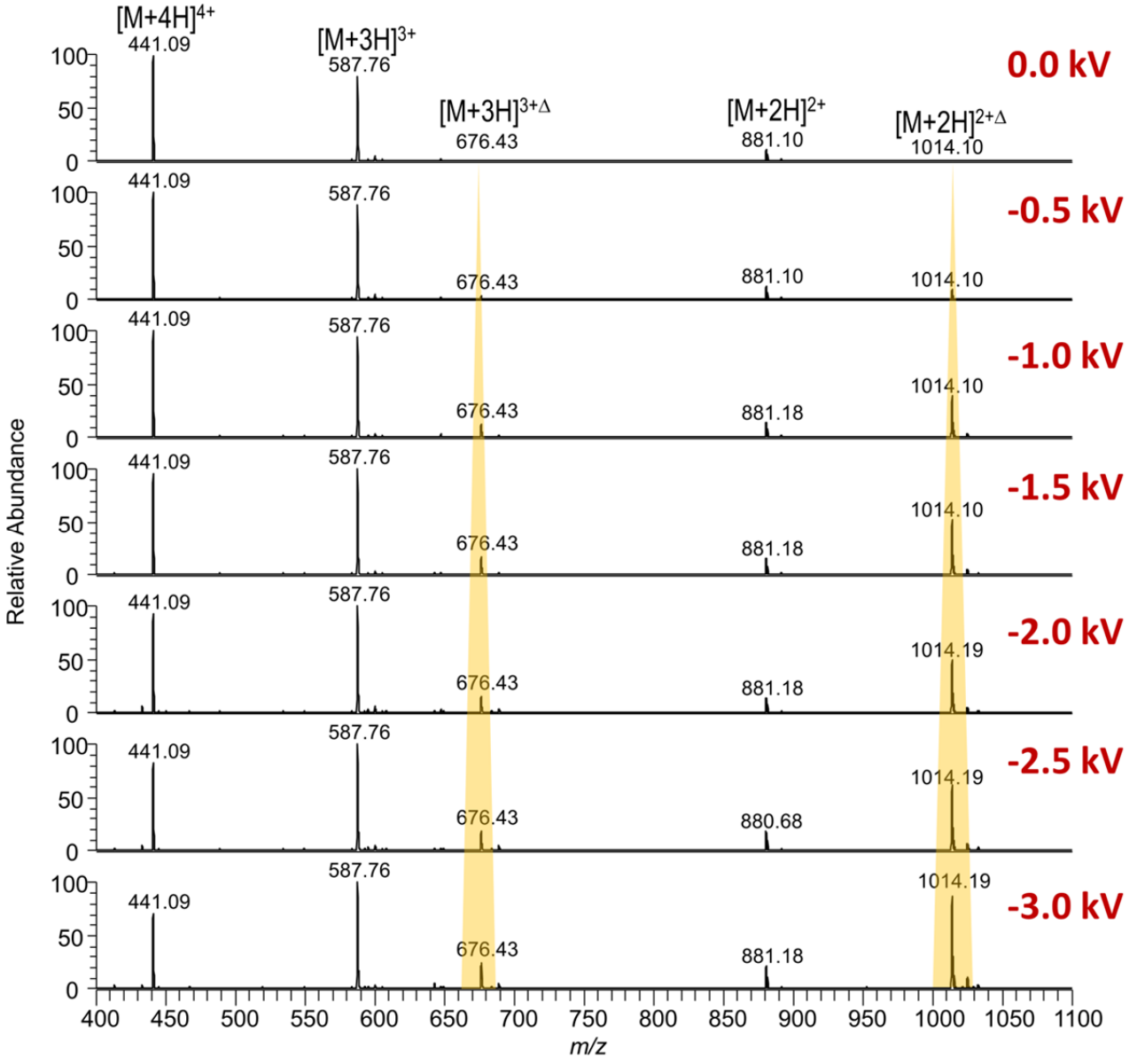
Supplemental Figure S1. (a) Aerial view of dual source reactor mounted at the front-end of a mass spectrometer and (b) free-standing design for facile adaptation to multiple instrument platforms.



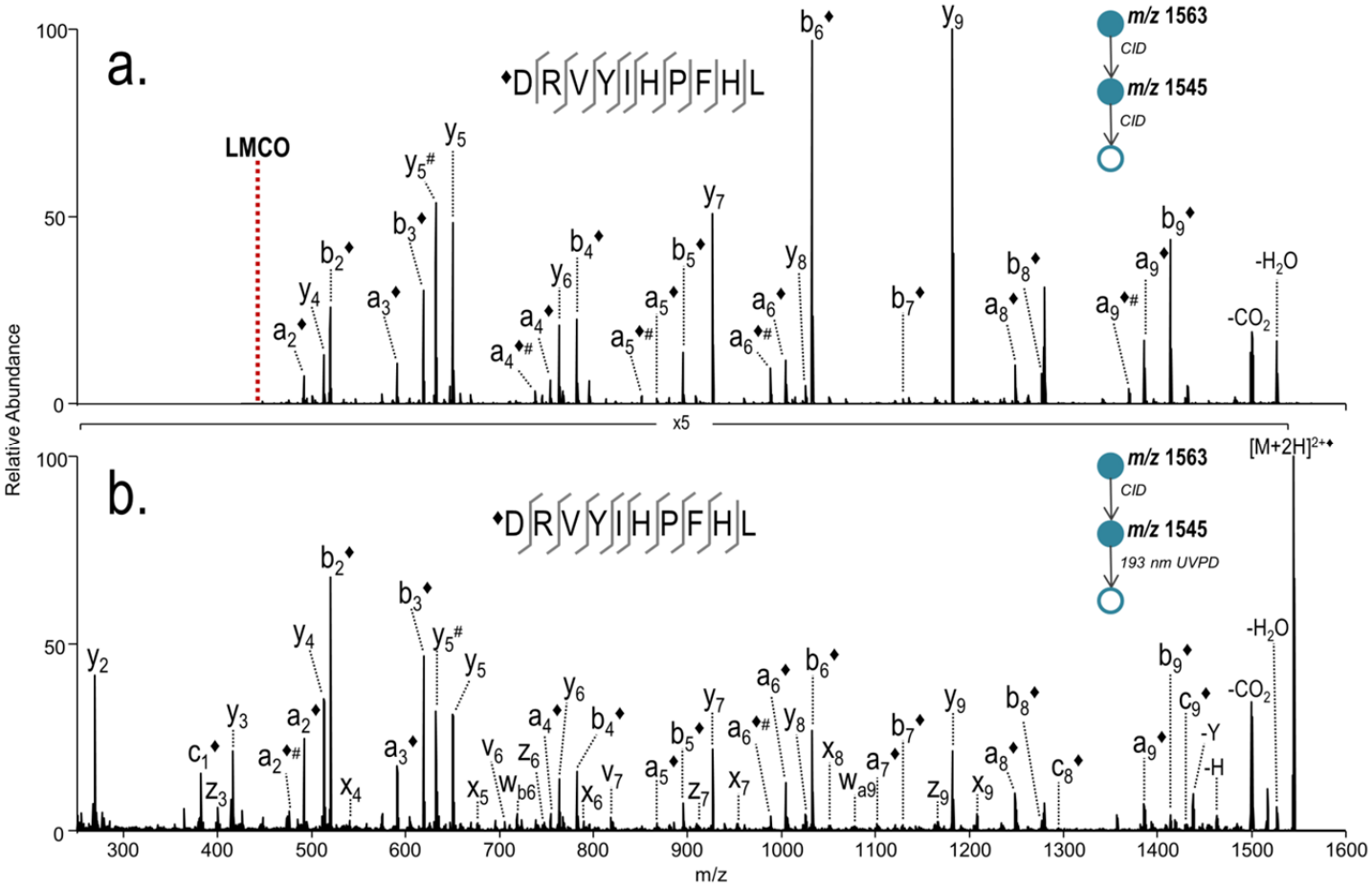
Supplemental Figure S2. (a) Negative mode ESI control spectrum for FBDSA and (b) positive mode ESI control spectrum for DRVYIHPFHLVIHN. (c) Dual spray spectrum for simultaneously infused DRVYIHPFHLVIHN (+) and FBDSA (-). Electrostatic DRVYIHPFHLVIHN/FBDSA complexes are denoted by Δ.



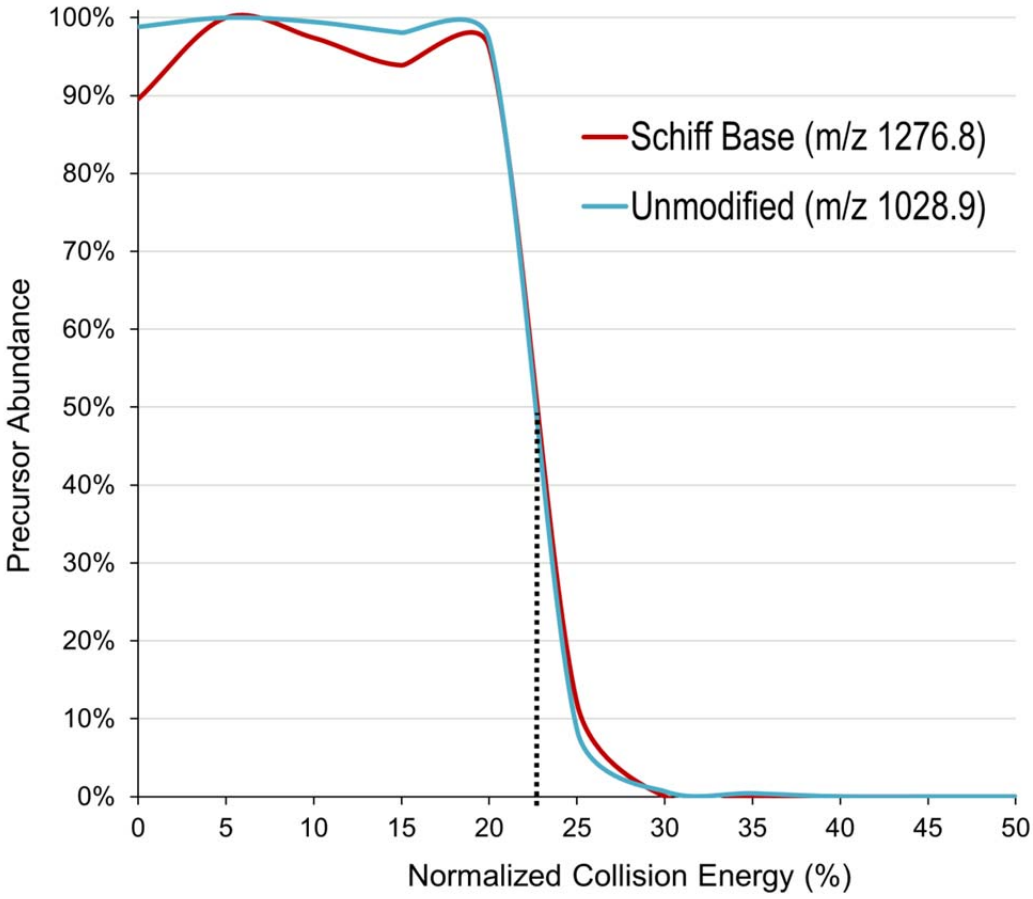
Supplemental Figure S3. Anion source voltage optimization: normalized abundance of unmodified DRVYIHPFHLVIHN and DRVYIHPFHLVIHN/FBDSA complex as a function of anion source voltage.



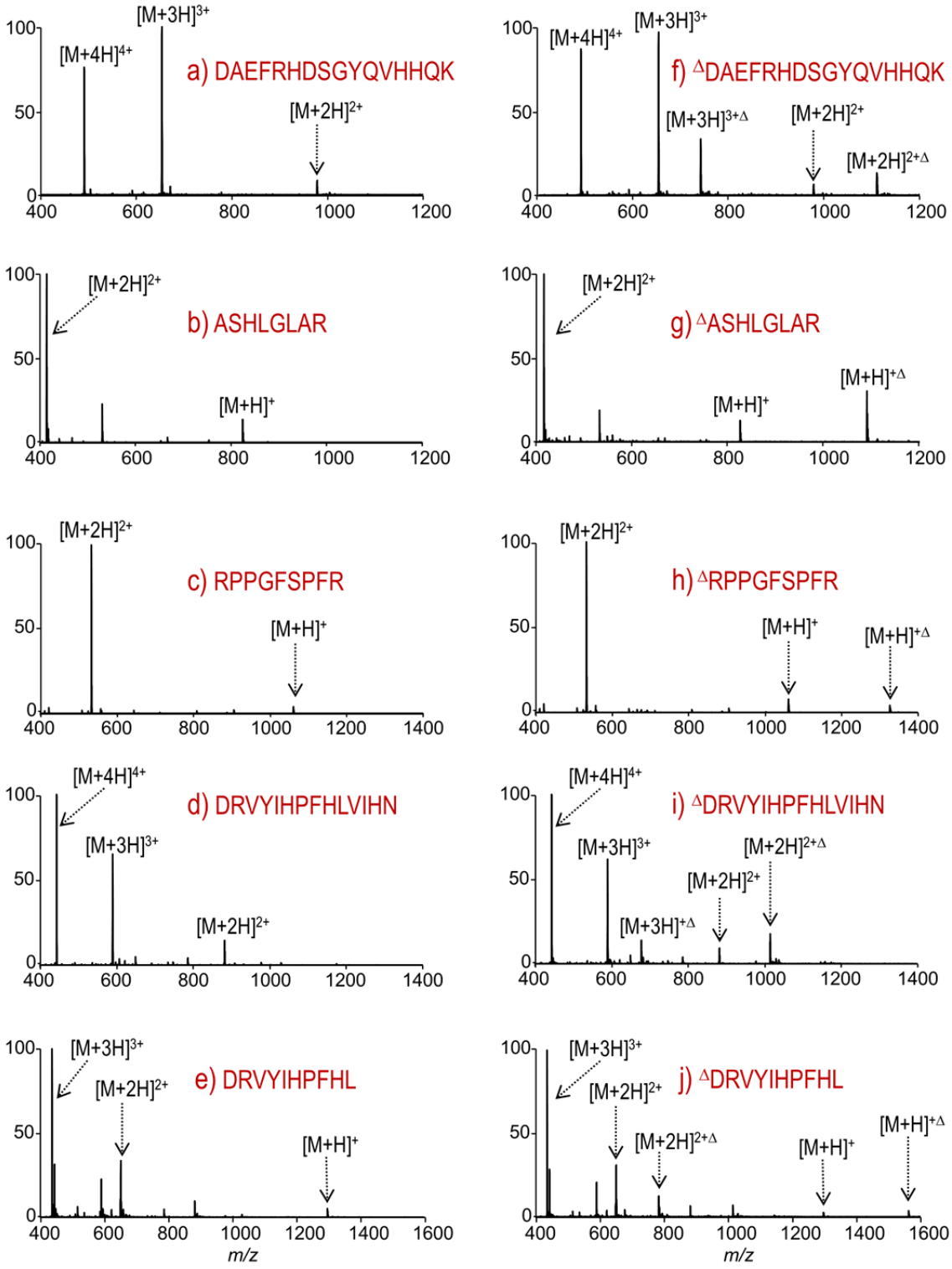
Supplemental Figure S4. Comparison of (a) CID and (b) 193 nm UVPD of FBDSA-modified DRVYIHPFHL following ion/ion mediated bioconjugation using the front-end dual spray reactor. LMCO = low mass cutoff restriction observed for resonant CID activation.



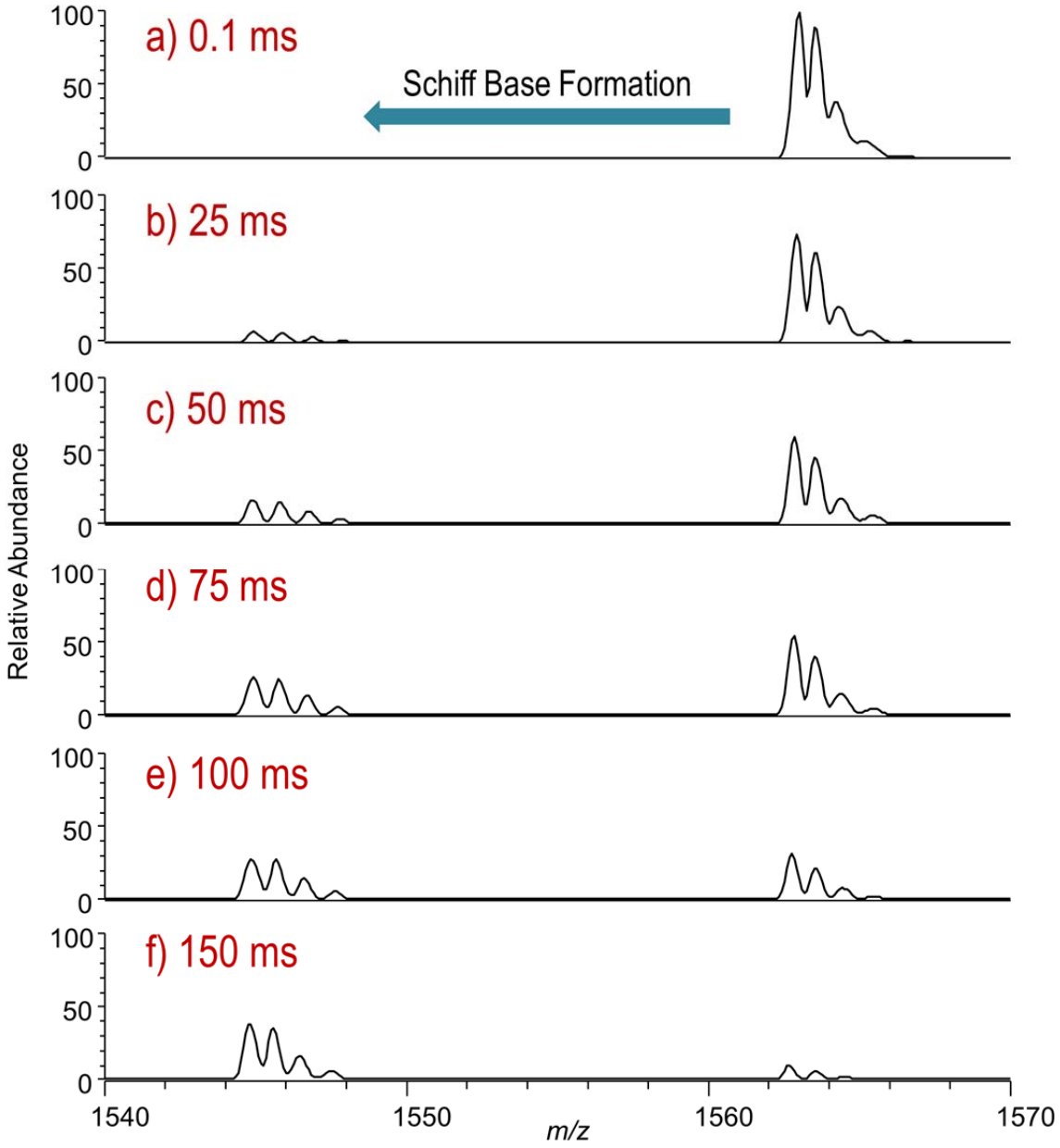
Supplemental Figure S5. Variable energy collision analysis of unmodified and Schiff base labeled KLVANNTRL (1+). Normalized precursor abundances are plotted as a function of increasing collision energy. The dashed line represents the point of 50% precursor dissociation.



Supplemental Figure S6. (a-e) Control and (f-j) FBDSA-reacted LC mass spectra for each peptide of a five peptide mixture. For all data shown, the peptide source voltage was held constant at 1.75 kV. Dual source initiated ion/ion reactions were carried out using an anion source voltage of -2.5 kV. Note that spectra are shown in order of elution.



Supplemental Figure S7. Evaluation of MS2 reaction time on covalent conversion of DRVYIHPFHL/FBDSA (1+) complexes using a fixed normalized collision energy of 18%.



Supplemental Figure S8. 193 nm UVPD spectrum of unmodified ASHLGLAR (1+).