Supporting Information

Photodissociation of TEMPO-modified peptides: New approaches to radical-directed dissociation of biomolecules

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Table S1: Peptides investigated in this study, and the charge states for which ions arising from TEMPO loss are observed upon photodissociation by 266 nm wavelength photons.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>m/z</th>
<th>[M - TEMPO]^{n/-}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>[M + H]^+</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>RPPGFSPFR</td>
<td>1177.4</td>
<td>589.2</td>
</tr>
<tr>
<td>Kinetensin</td>
<td>IARRHPYFL</td>
<td>n.d.*</td>
<td>645.3</td>
</tr>
<tr>
<td>YGGFMRF</td>
<td>YGGFMRF</td>
<td>994.1</td>
<td>497.2</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>DRVYIHPF</td>
<td>n.d.*</td>
<td>582.3</td>
</tr>
<tr>
<td>Substance P†</td>
<td>RPKPQQFFGLM-NH₂</td>
<td>n.d.*</td>
<td>733.1</td>
</tr>
</tbody>
</table>

n.d.: Product ion not detected upon PD_{266}

* Precursor ion containing TEMPO not detected in sufficient abundance for isolation and photodissociation.

† An additional ion at m/z 948 is also observed in the full ESI mass spectrum, due to TEMPO-Bz coupling at lysine and the N-terminus. This doubly charged ion also exhibits TEMPO loss upon PD_{266} (m/z 870).
**Figure S1:** a) Photodissociation spectrum of modified bradykinin $[M + 2H]^{2+}$ ions, irradiated with a single laser pulse of 266 nm wavelength photons. The spectrum has been normalised to the abundance of $[M + 2H – TEMPO]^{2+}$ ions (*cf.* Figure 1b, main text), exhibiting similar features as the CID spectrum of the same precursor ion (Figure 1a, main text). b) Photodissociation spectrum of unmodified bradykinin (RPPGFSPFR) $[M + 2H]^{2+}$ ions, irradiated with a single laser pulse of 266 nm wavelength photons, exhibiting formation of $b_n^+$ and $y_n^+$ ions in minor abundance.

**Figure S2:** Collision-induced dissociation of $[M + 3H]^{3+}$ ions of TEMPO-modified bradykinin (RPPGFSPFR), highlighting loss of selectivity at high charge states, due to protonation of the piperidinyl nitrogen of the TEMPO-Bz moiety.
Figure S3: Charge-state dependence of radical ion photoproduct yields upon PD$_{266}$ of the TEMPO-modified peptide YGGFMRF: (a) [M + H]$^+$; (b) [M – H]$^-$; (c) [M + 2H]$^{2+}$. The photoproduct and neutral loss in each spectrum are indicated with a horizontal arrow. Note the magnification of product ions in Figure S3(b) and S3(c). All spectra were acquired with the same laser fluence.
**Figure S4**: MS$^3$ spectrum obtained following CID of [M + 2H – TEMPO]$^{2+}$ radical ions of bradykinin, generated by CID of doubly charged TEMPO-modified bradykinin ions (cf. Figure 3(c), main text). Major peptide sequence ions and radical mediated side chain losses are assigned, with the identified amino acid in parentheses. The precursor ion is marked with an asterix (*).

**Figure S5**: MS$^3$ spectrum obtained following CID of [M + 2H – TEMPO]$^{2+}$ radical ions of kinetensin, generated by CID of doubly charged TEMPO-modified kinetensin ions (cf. Figure 3(d), main text). Major peptide sequence ions and radical mediated side chain losses are assigned, with the identified amino acid in parentheses. The precursor ion is marked with an asterix (*).
Figure S6: Photodissociation action spectrum of TEMPO-Bz modified bradykinin (RPPGFSPFR) deconvolved into the $[\text{M} + 2\text{H} - \text{TEMPO}]^{2+}$ and $y_8^+$ product ion signals.
Figure S7: Representative PD mass spectra at varying wavelengths used to compile the PD action spectrum of TEMPO loss from bradykinin [M + 2H]$^{2+}$ ions (Figure 4, main text): (a) $\lambda$ = 225 nm; (b) $\lambda$ = 250 nm; (c) $\lambda$ = 275 nm; (d) $\lambda$ = 300 nm. Each spectrum is the
accumulation of at least 50 MS scans. Note the increasing magnification required to observe the photoproduct radical ion \((m/z \ 589)\). Raw ion abundances are corrected for power variations over the wavelength range before compiling the PD action spectrum.
**Figure S8:** Representative PD mass spectra at varying wavelengths used to compile the PD action spectrum of TEMPO loss from kinentensin \([M + 2H]^{2+}\) ions (Figure 4, main text): (a) \(\lambda = 232\) nm; (b) \(\lambda = 250\) nm; (c) \(\lambda = 275\) nm; (d) \(\lambda = 300\) nm. Each spectrum is the accumulation of at least 50 MS scans. Note the increasing magnification required to observe the photoproduct radical ion \((m/z 645)\). Raw ion abundances are corrected for power variations over the wavelength range before compiling the PD action spectrum.
Figure S9: (a) MS³ spectrum obtained following CID of [M – H – 91]⁻ radical ions (1a), resulting from CID of (1); (b) MS³ spectrum obtained following CID of [M – H – 91]⁻ radical ions (1a), resulting from PD_{266} of (1); (c) CID spectrum of [M – H]⁻ anions of standard (1a).