Supporting Information for:

Bond-specific fragmentation of oligopeptides via electronic stopping of swift heavy ions in molecular films

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Table S1: Fragment species observed in SHI-irradiated angiotensin II samples. Specific fragments are labeled according to the nomenclature introduced by Biemann [1, 2]. Fragments resulting from double cleavage of the peptide backbone are labeled according to Fig. S1. Reactive fragments are frequently observed at $\Delta m/z = +2$ with respect to their nominal mass; they are denoted by $+2H$ indicating the uptake of two further hydrogen atoms. The table includes both specific and non-specific fragments which are not highlighted in Fig. 3 in the main article since they exhibit intensities below 3% of the intensity of the peak corresponding to the intact molecule.

| $m/z$     | Fragment species  |
|-----------|-------------------|
| 506.3     | a₄               |
| 523.8     | [M+2H]$^{2+}$    |
| 526.3     | non-specific      |
| 534.3     | b₄               |
| 539.4     | x₄               |
| 551.4     | c₄               |
| 613.4     | non-specific      |
| 619.3     | a₅               |
| 647.3 / 649.3 | b₅ / b₅+2H |
| 664.4     | c₅               |
| 741.4     | non-specific      |
| 756.4 / 758.4 | a₆ / a₆+2H |
| 758.4     | z₆               |
| 784.4     | b₆               |
| 853.4 / 855.4 | a₇ / a₇+2H |
| 872.5     | non-specific      |
| 883.5     | b₇+2H            |
| 898.4     | c₇               |
| 916.5     | z₇+2H            |
| 926.5     | non-specific      |
| m/z      | Fragment     |
|----------|--------------|
| 931.5    | y7           |
| 942.5    | x7 NH        |
| 957.5    | x7           |
| 972.5    | non-specific |
| 985.5 / 987.5 | a8-z8+H / a8-z8+3H |
| 1000.5 / 1002.5 | a8 / a8+2H   |
| 1018.5   | non-specific |
| 1031.5   | z8+2H        |
| 1046.5   | [M+H]^+      |

Figure S1: Example for the nomenclature of fragments resulting from double cleavage of the peptide backbone. The fragment name is composed of the names of the fragments corresponding to the two cleavage sites.
Figure S2: DINEC mass spectra of angiotensin II in the low m/z range between 50 and 300 (a) prior to, and (b) after SHI irradiation (compare Figs. 3 (a) and (b) in the main article). Most peaks observed in the irradiated sample are already present in the measurement prior to irradiation. Two major additional peaks are observed after SHI irradiation (orange arrows) which are also observed in bradykinin samples irradiated together with this sample. Moreover, samples from other irradiation series do not show these peaks at all. They probably have to be attributed to sample contamination during transfer or irradiation. In particular, no significant fragment peaks are observed in this m/z range.
Figure S3: Signal intensity (peak maximum) of the peaks associated with intact angiotensin II and bradykinin molecules in SHI-irradiated samples as a function of applied SHI flux. Total accumulated fluence was $3 \times 10^{11}$ ions/cm$^2$ for all samples; the signal of each irradiated sample was normalized to the signal of the same sample prior to irradiation. The signal is largely independent of the flux. Dashed lines correspond to the respective mean values of the relative signal. For angiotensin II, a rather low signal is observed in the case of $3 \times 10^7$ ions/cm$^2$ which might be in part explained by some inhomogeneity of the irradiation resulting in a in total higher effective fluence on the sample. Most importantly, higher SHI flux does not lead to higher damage (i.e., lower signal intensity of the intact molecules) in the sample. Therefore, the fragmentation observed in the irradiated samples is attributed to direct SHI-molecule-interaction rather than secondary effects such as heating of the sample.
Figure S4: Stopping power of Au ions in hydrocarbon material (0.8 g/cm$^3$) as a function of kinetic energy according to SRIM-2013 calculations [3]. The Au ions used for irradiation in this study exhibit a kinetic energy of 946 MeV (indicated by the arrow). The initial energy loss amounts to $dE/dx \approx 11$ keV/nm and is attributed almost exclusively to electronic stopping. The range of the ions in hydrocarbon material is about 100 µm, i.e., much higher than the thickness of the oligopeptide films investigated.
Table S2: Fragment species observed in SHI-irradiated neurotensin samples. The nomenclature is the same as used in Tab. S1.

| m/z  | Fragment species          |
|------|---------------------------|
| 393.2| $z_3+2\text{H}$          |
| 407.2| non-specific              |
| 571.3| non-specific              |
| 585.3| non-specific              |
| 602.3| non-specific              |
| 646.4| $z_5+2\text{H}$          |
| 661.4| $y_5$                    |
| 689.4| $x_5+2\text{H}$          |
| 757.3| non-specific              |
| 779.9| c$_{12}+\text{H}^+$      |
| 802.5| $z_6+2\text{H}$          |
| 814.9| a$_{13}+2\text{H}+\text{H}^+$ |
| 828.3| a$_7$                    |
| 836.9| [M+2H]$^{2+}$            |
| 845.4| $x_6+2\text{H}$          |
| 847.9| [M+H+Na]$^{2+}$          |
| 873.4| c$_7$                    |
| 914.5| $y_7$                    |
| 942.5| $x_7+2\text{H}$          |
| 986.5| a$_8+2\text{H}$          |
| 999.5| non-specific              |
| 1012.5| b$_8$                   |
| 1023.6| non-specific             |
| 1027.6| $z_8+2\text{H}$         |
| 1042.6| $y_8$                   |
| Mass (m/z) | Description               |
|-----------|---------------------------|
| 1046.5    | angiotensin II            |
| 1068.6 / 1070.6 | x₈ / x₈+2H                |
| 1139.6 / 1141.6 | z₉ / z₉+2H                |
| 1156.7    | y₉                        |
| 1168.7    | b₉                        |
| 1184.7    | x₉+2H                     |
| 1237.6 / 1239.6 | a₁₀ / a₁₀+2H              |
| 1270.7    | z₁₀+2H                    |
| 1282.7    | c₁₀                       |
| 1402.7    | a₁₁+2H                    |
| 1433.8    | z₁₁+2H                    |
| 1445.7    | c₁₁                       |
| 1474.7    | x₁₁                       |
| 1515.8    | a₁₂+2H                    |
| 1543.8    | b₁₂+2H                    |
| 1546.8    | z₁₂+2H                    |
| 1558.8    | c₁₂                       |
| 1575.8    | b₁₃-x₁₂+3H                |
| 1587.8    | x₁₂                       |
| 1600.8    | non-specific               |
| 1611.8    | a₁₃-z₁₃+H                 |
| 1628.8    | a₁₃+2H                    |
| 1644.8    | non-specific               |
| 1656.8    | b₁₃+2H                    |
| 1672.8    | [M+H]⁺                     |

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Table S3: Specific fragments observed in SHI-irradiated bradykinin samples. The nomenclature is the same as used in Tab. S1.

| \(m/z\) | Fragment species               |
|--------|-------------------------------|
| 407.2  | non-specific                  |
| 530.8  | \([M+2H]^{2+}\)              |
| 545.2  | non-specific                  |
| 555.3  | \(b_5\)                      |
| 572.3  | \(c_5\)                      |
| 585.2  | non-specific                  |
| 600.3  | non-specific                  |
| 653.3  | \(y_5\)                      |
| 710.3  | \(y_6\)                      |
| 756.4  | \(c_7\)                      |
| 775.4  | \(x_8-c_8+2H\)               |
| 784.4  | non-specific                  |
| 807.4  | \(y_7\)                      |
| 858.4  | \(a_8\)                      |
| 886.4  | \(b_8 / x_8-a_9+H\)          |
| 887.4  | \(z_8\)                      |
| 903.4  | \(c_8\)                      |
| 904.4  | \(y_8\)                      |
| 932.5  | \(x_8+2H\)                   |
| 973.5  | non-specific                  |
| 999.5  | \(z_9-a_9+H\)                |
| 1016.5 | \(a_9+2H\)                   |
| 1042.5 | \(b_9\)                      |
| 1043.5 | \(z_9\)                      |
| 1060.5 | \([M+H]^+\)                  |
References

[1] Roepstorff, P.; Fohlman, J. Proposal for a Common Nomenclature for Sequence Ions in Mass Spectra of Peptides. *Biomed. Mass Spectrom.* **1984**, *11*, 601.

[2] Biemann, K. Contributions of Mass Spectrometry to Peptide and Protein Structure. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99 – 111.

[3] Ziegler, J. F.; Ziegler, M. D.; Biersack, J. P. SRIM - The stopping and range of ions in matter. *Nucl. Inst. Meth. Phys. Res. B* **2010**, *268*, 1818 – 1823.