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Supplementary Material

Efficient Flow Synthesis of Human Antimicrobial Peptides

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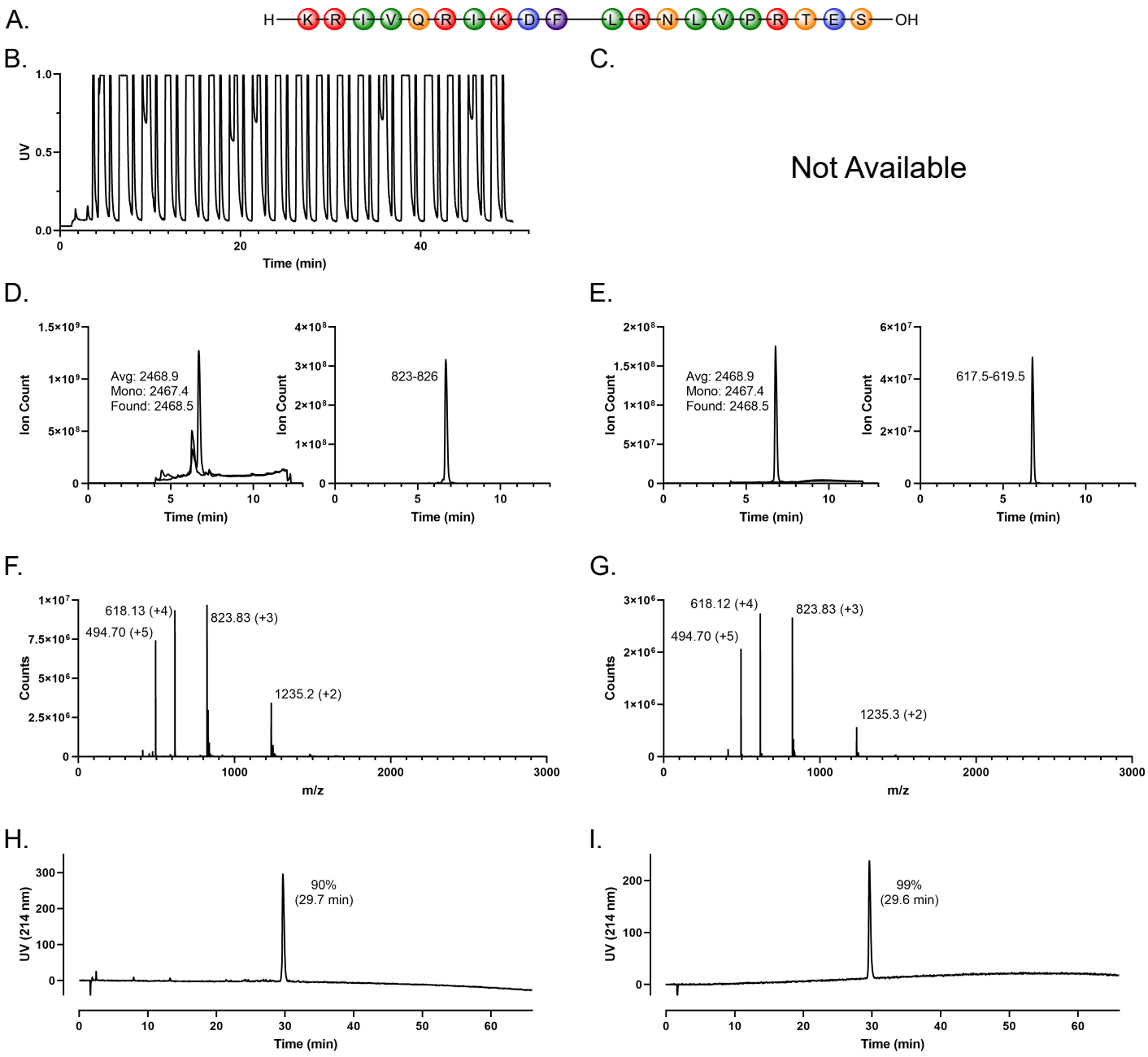
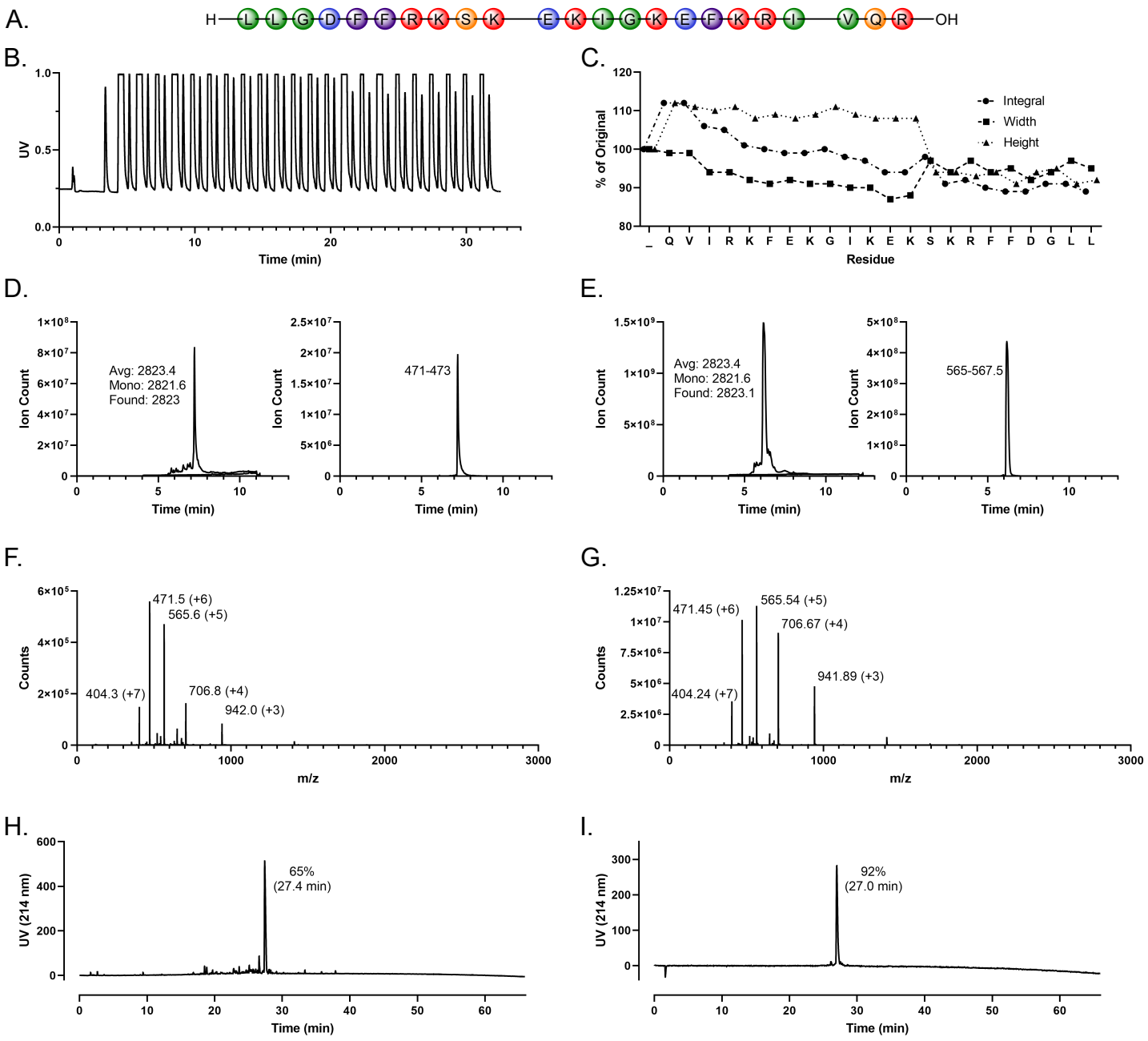
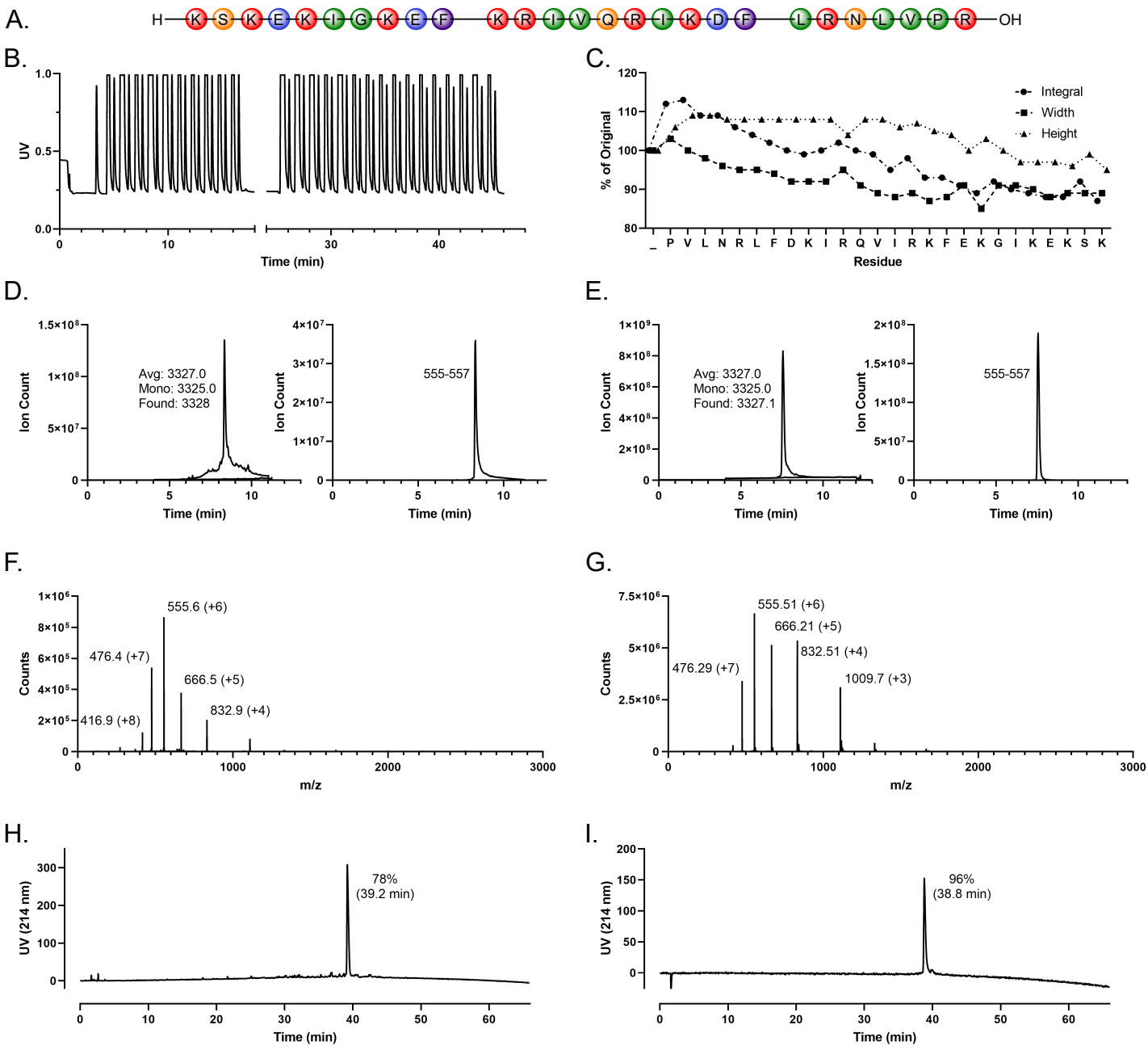


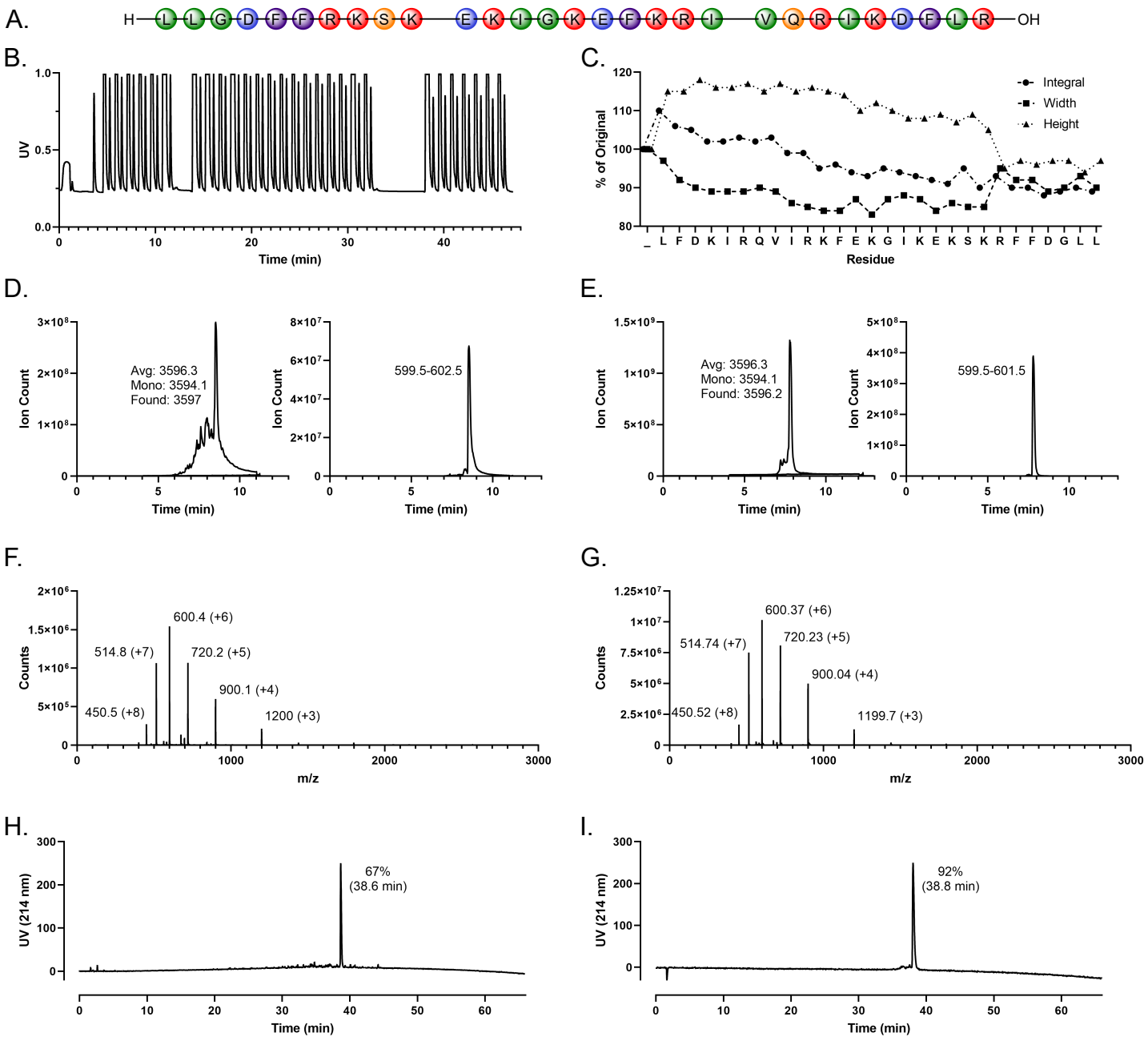
Supplementary Figure S1: A. KR-20 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 4th Generation – Length. **C.** Integrals unavailable for this sequence. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the indicated integral percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



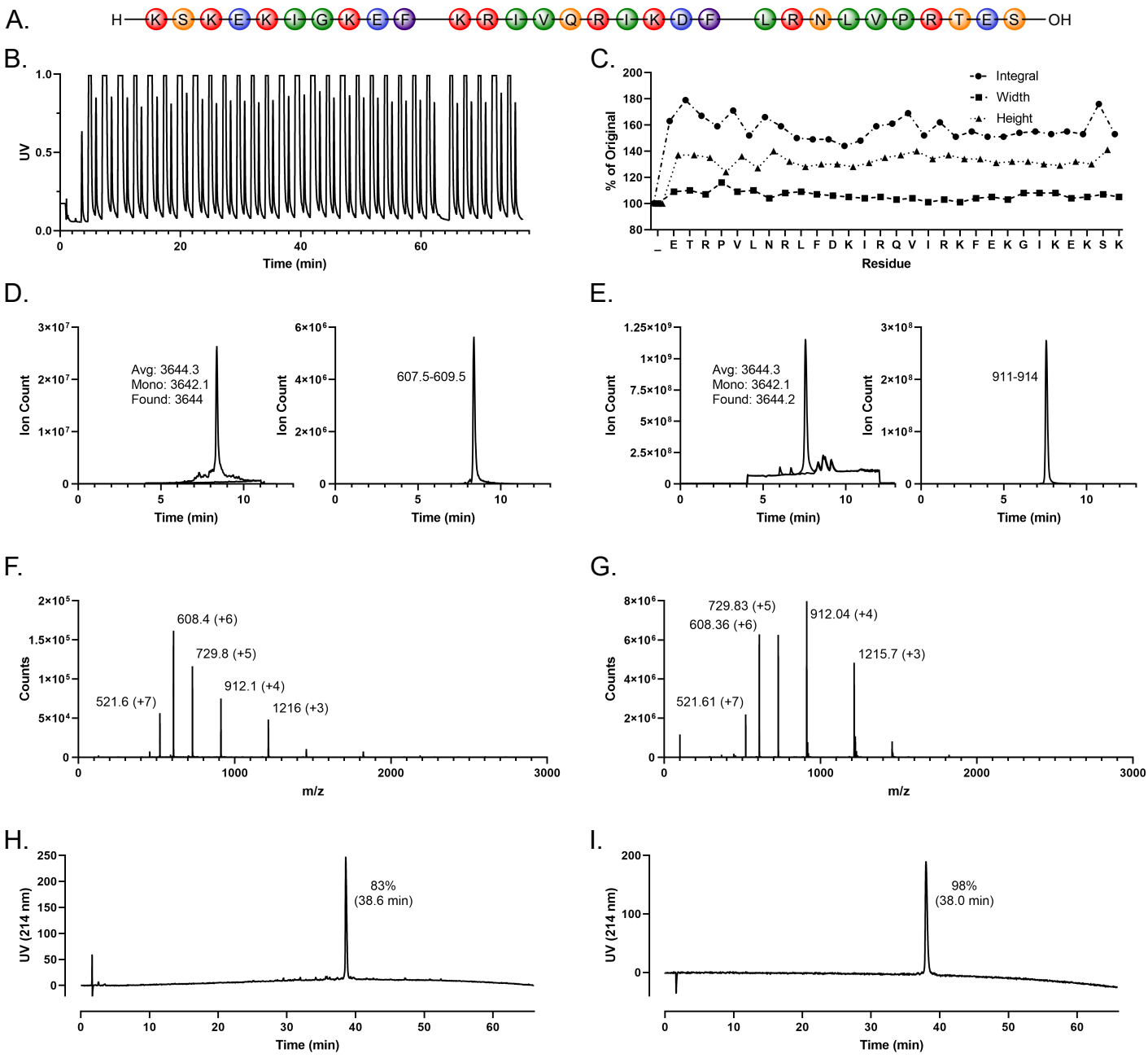
Supplementary Figure S2: A. LL-23 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the indicated integral percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



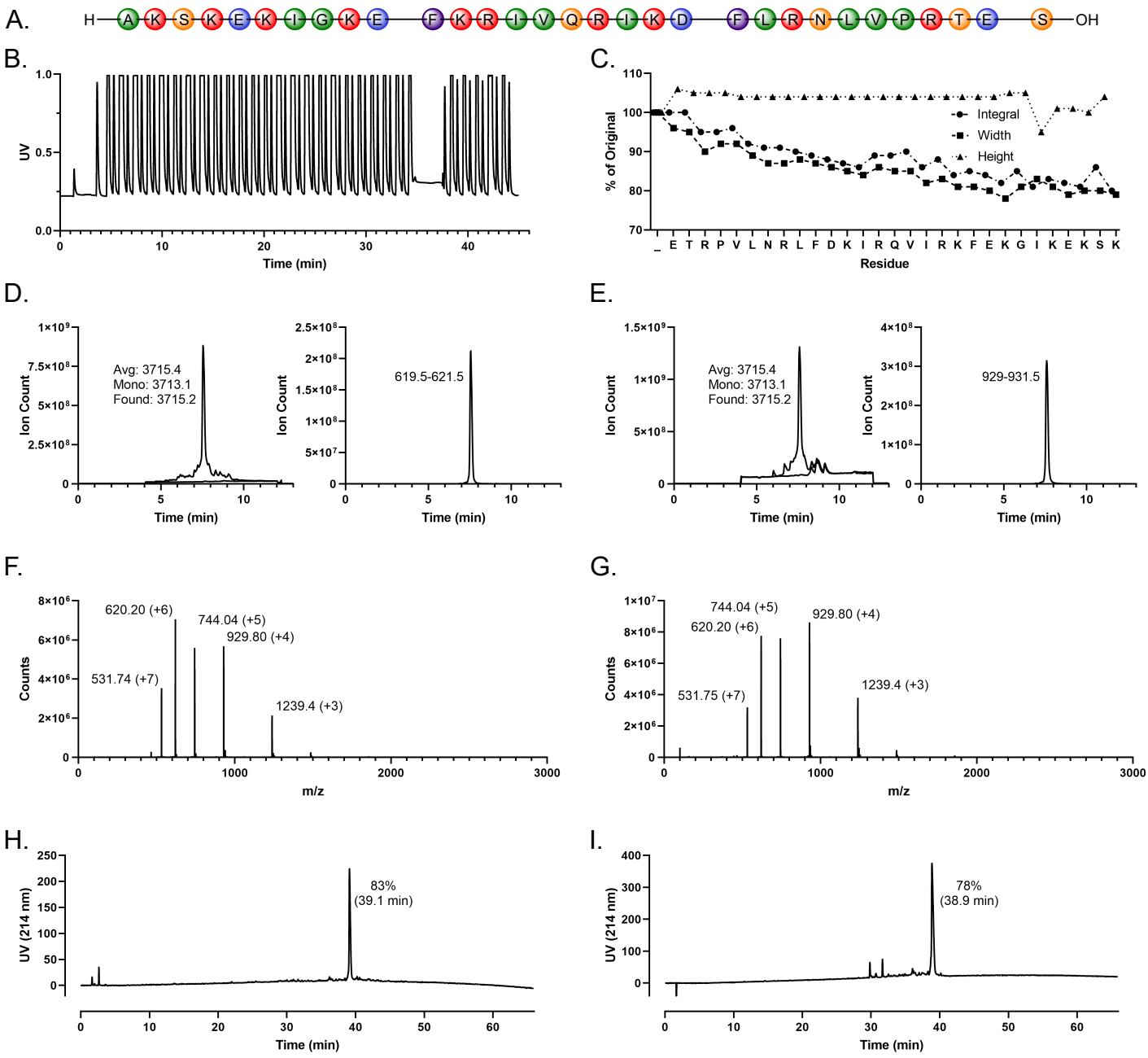
Supplementary Figure S3: A. KS-27 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. The x-axis is cut at a user-initiated pause; total time graphed includes the pause time. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



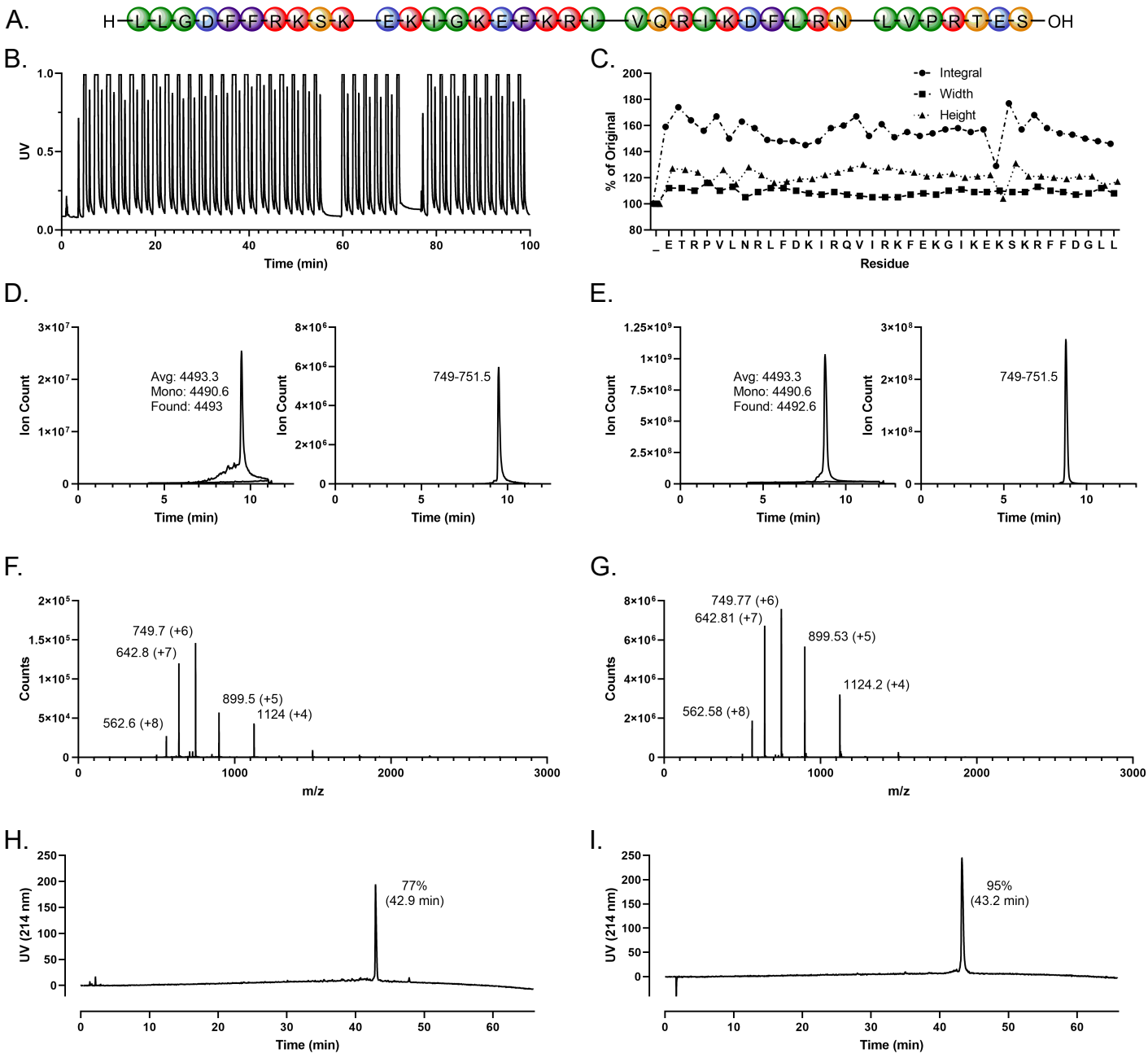
Supplementary Figure S4: A. LL-29 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. Spaces in the x-axis represent user-initiated pauses. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



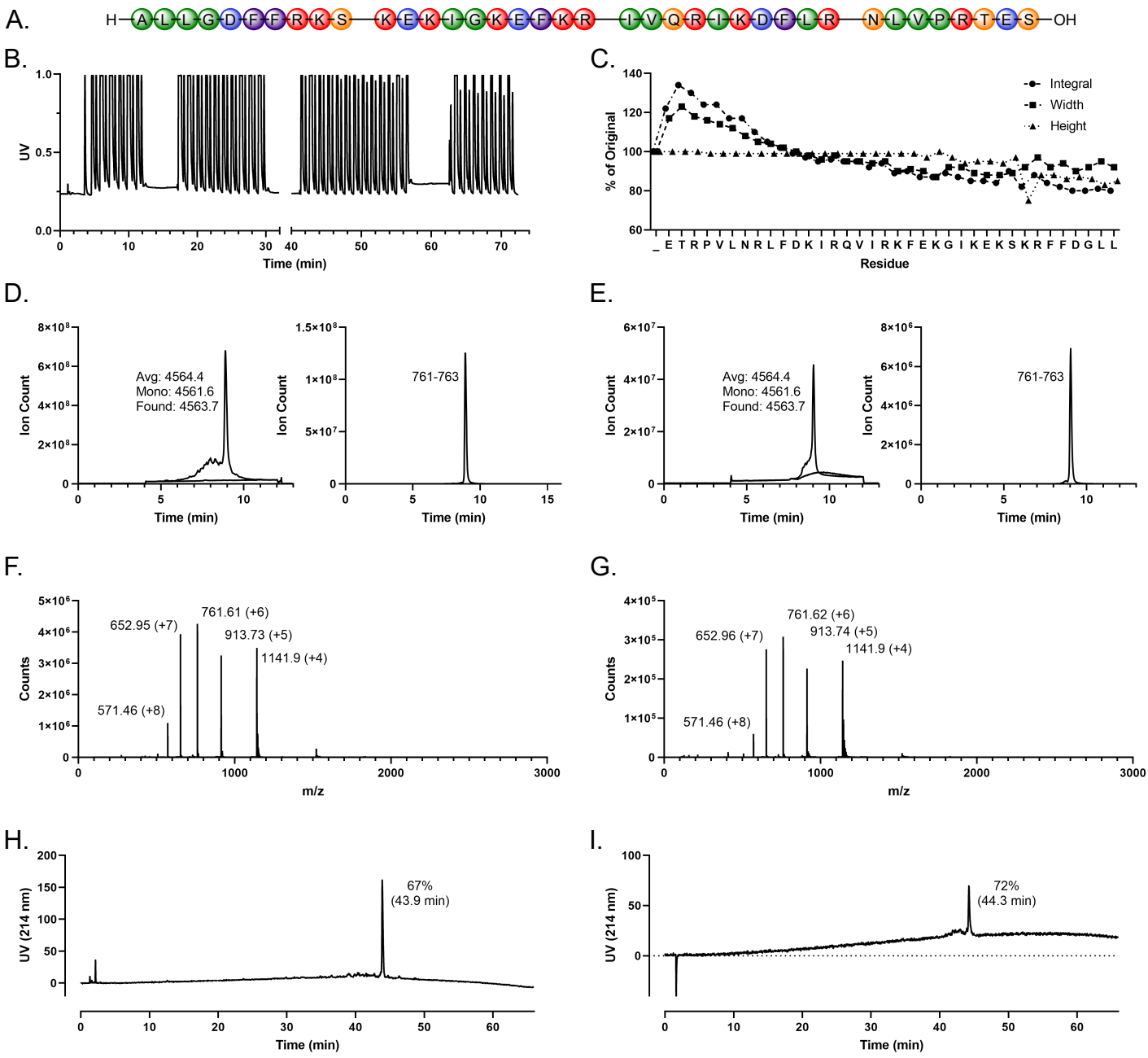
Supplementary Figure S5: A. KS-30 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Length. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



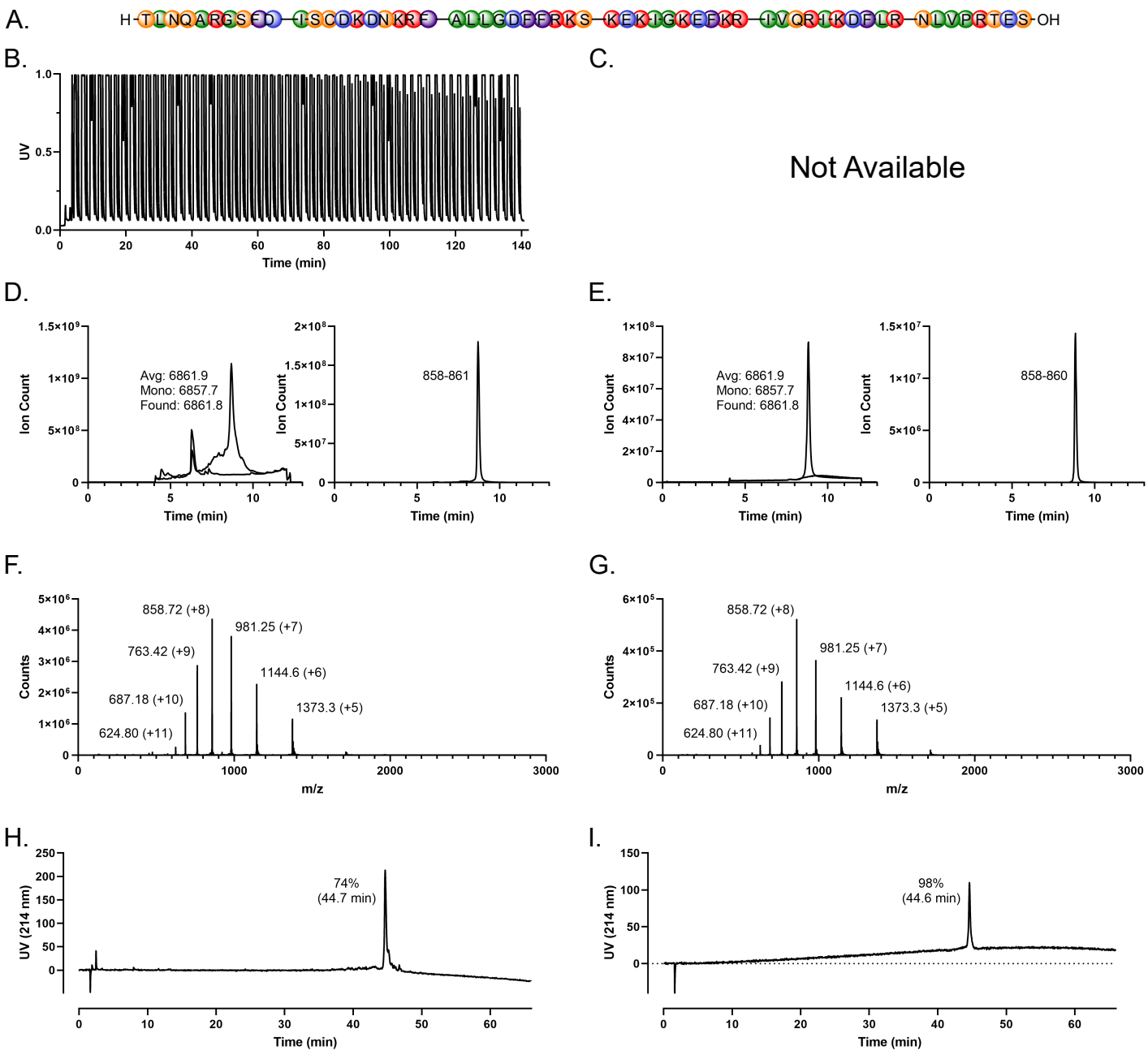
Supplementary Figure S6: A. RK-31 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed with batch addition of the N-terminal amino acid to a prior flow synthesis of KS-30 (not the one shown in **Supplementary Figure 5**). Spaces in the x-axis represent user-initiated pauses. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



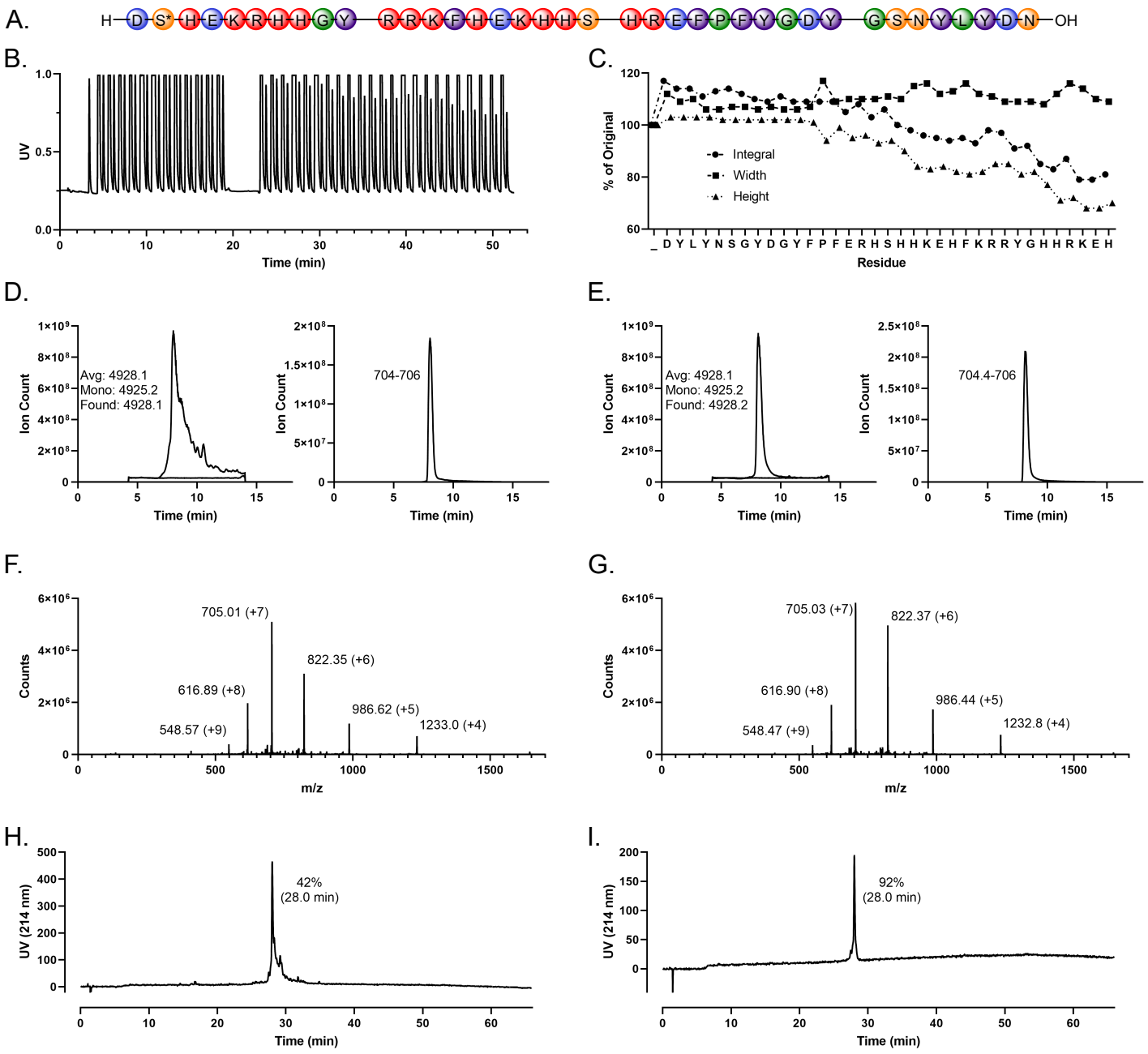
Supplementary Figure S7: A. LL-37 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Length. Spaces in the x-axis represent user-initiated pauses. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



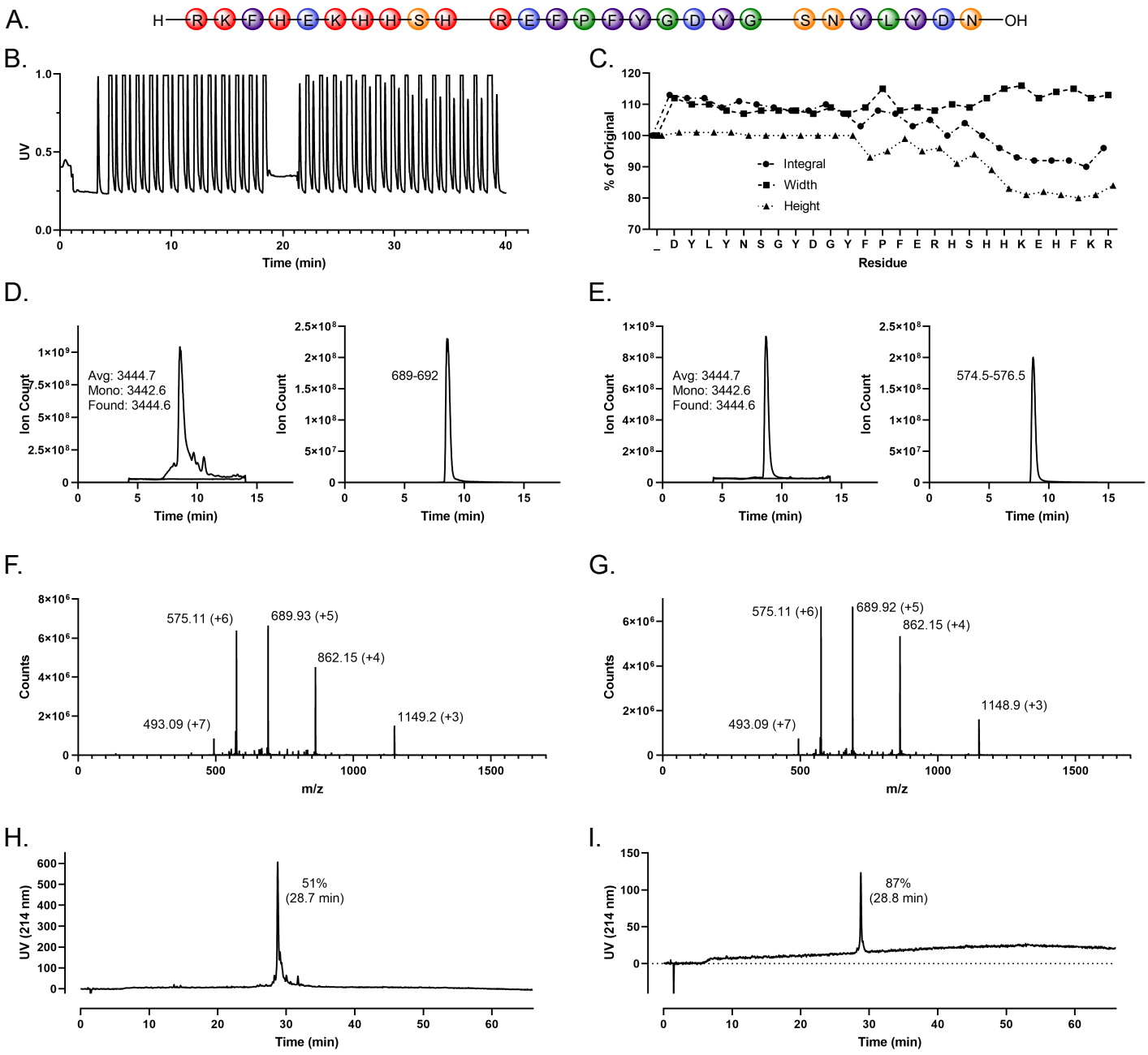
Supplementary Figure S8: A. ALL-38 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed with batch addition of the N-terminal amino acid to a prior flow synthesis of LL-37 (not the one shown in **Supplementary Figure 7**). Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer user-initiated pause; total time graphed includes the pause time. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



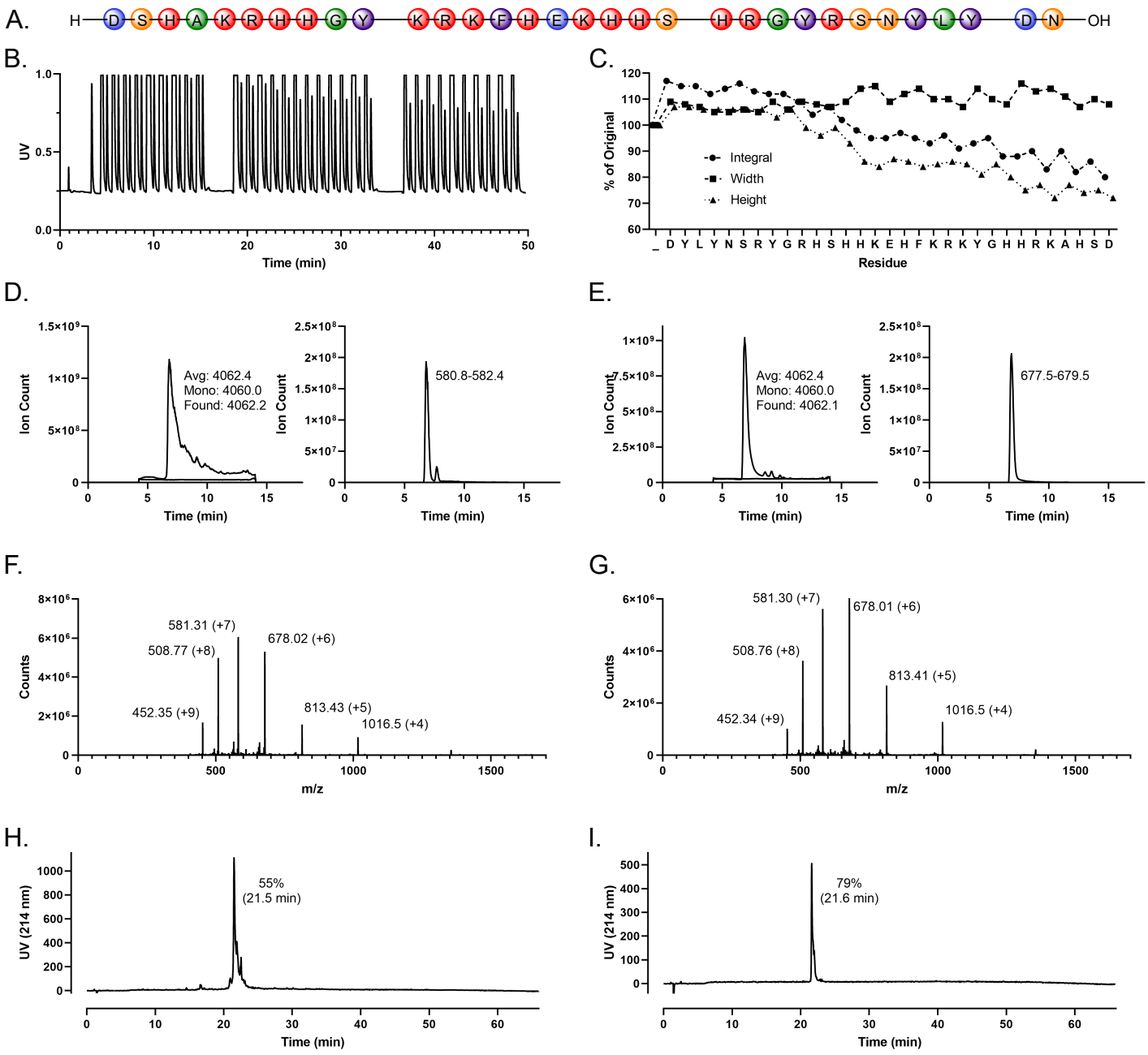
Supplementary Figure S9: A. TLN-58 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 4th Generation – Length. **C.** Integrals unavailable for this sequence. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



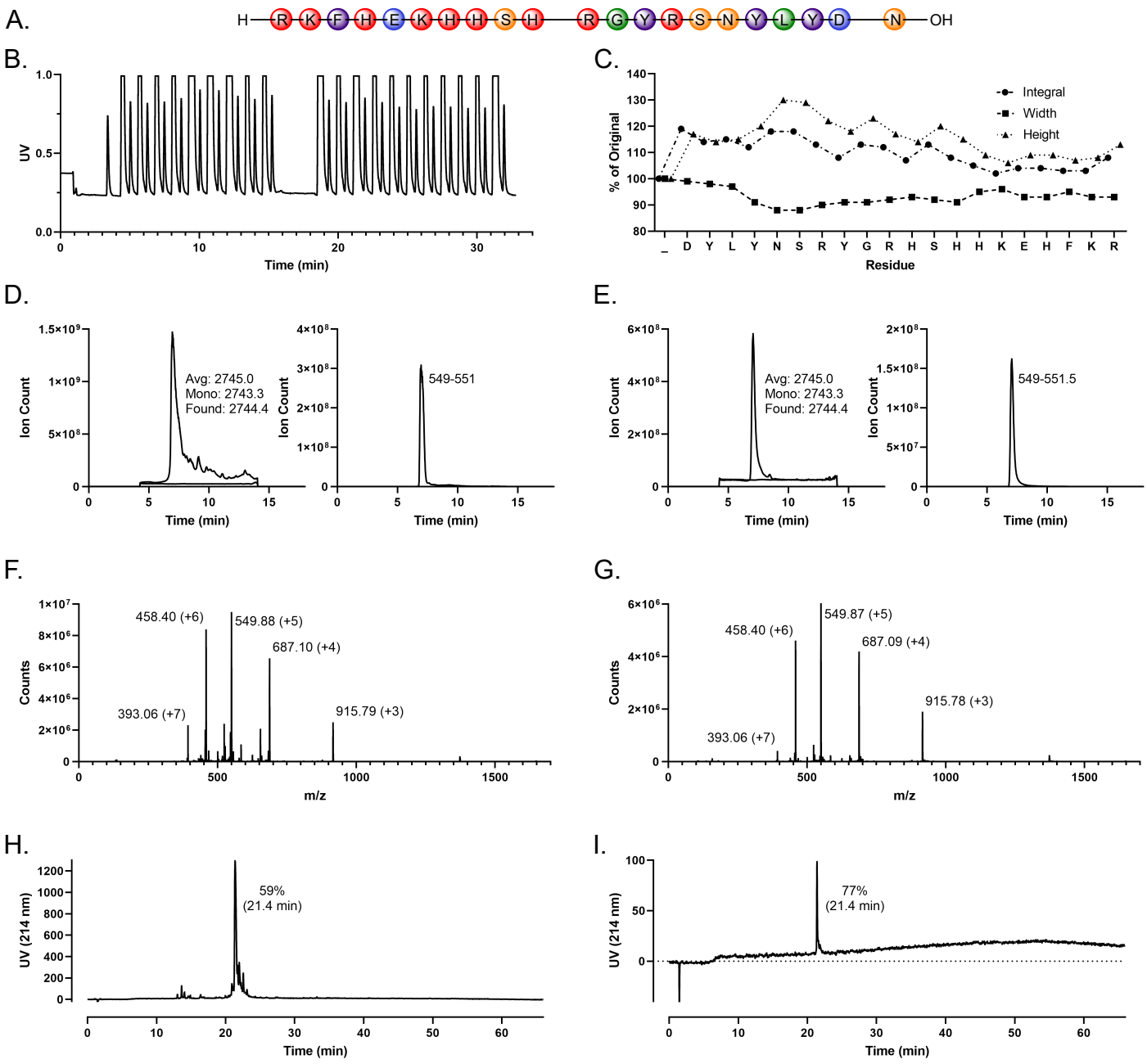
Supplementary Figure S10: A. Histatin 1 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed with batch addition of phospho-Ser2 and N-terminal Glu. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



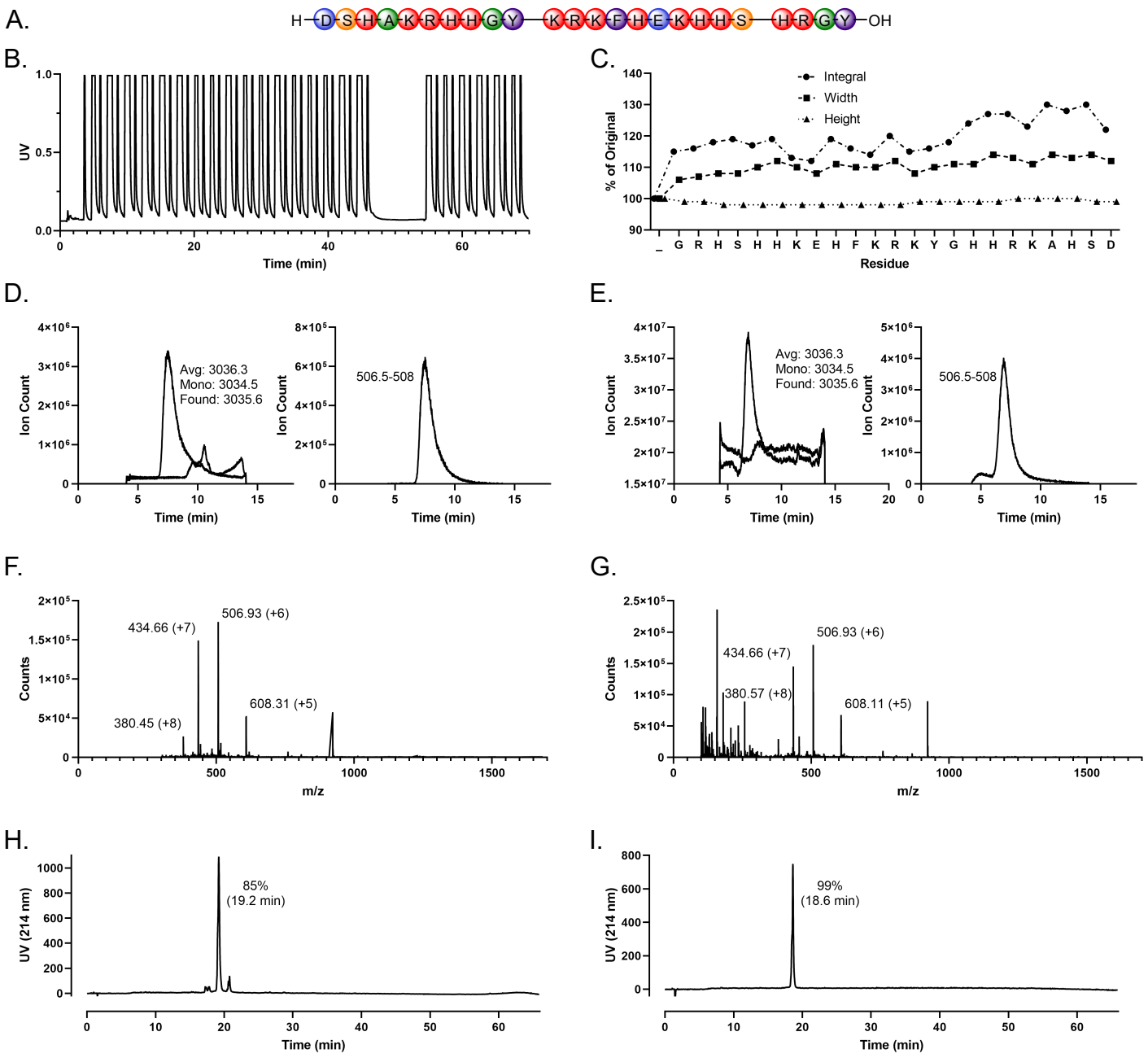
Supplementary Figure S11: A. Histatin 2 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



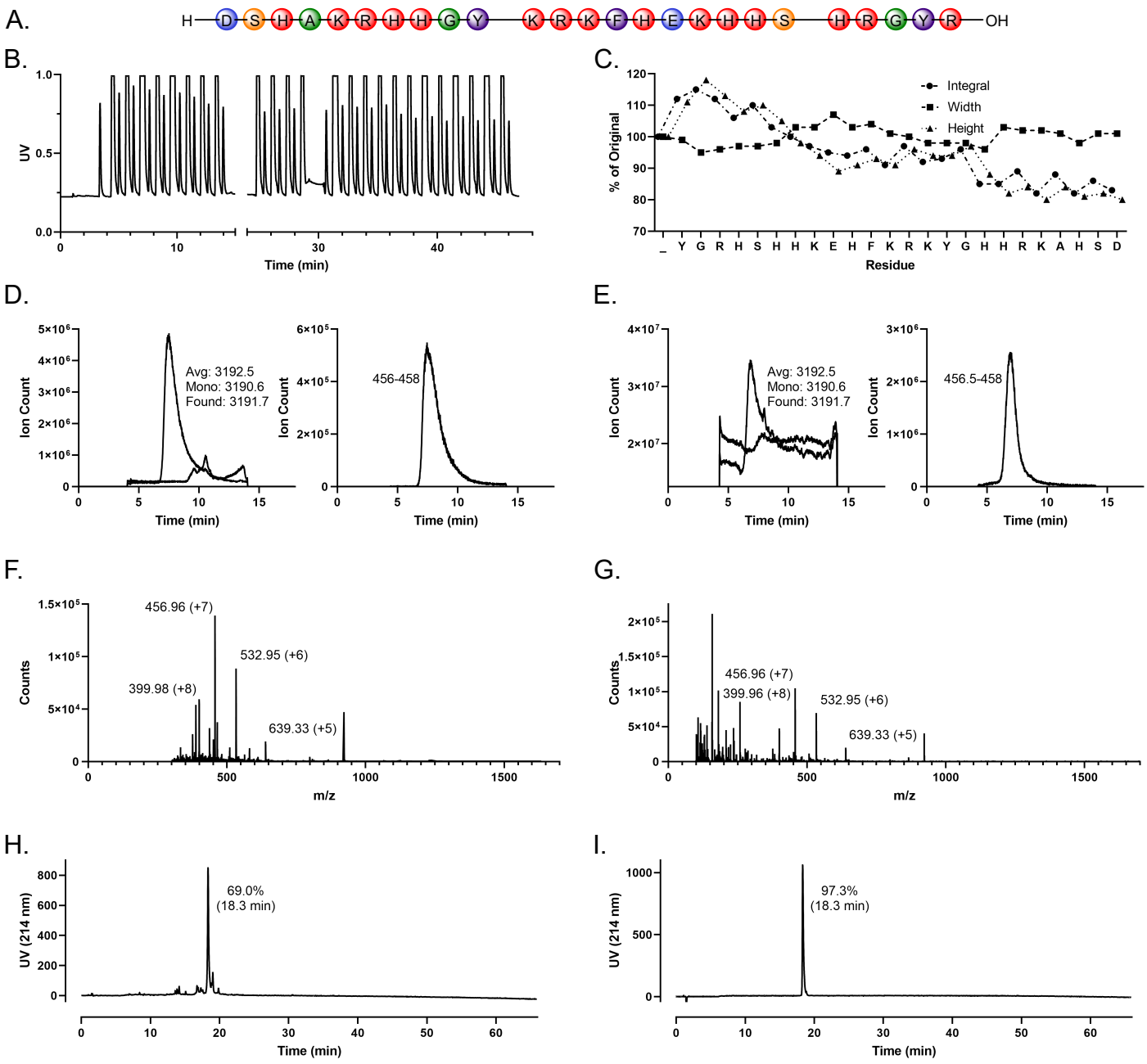
Supplementary Figure S12: A. Histatin 3 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



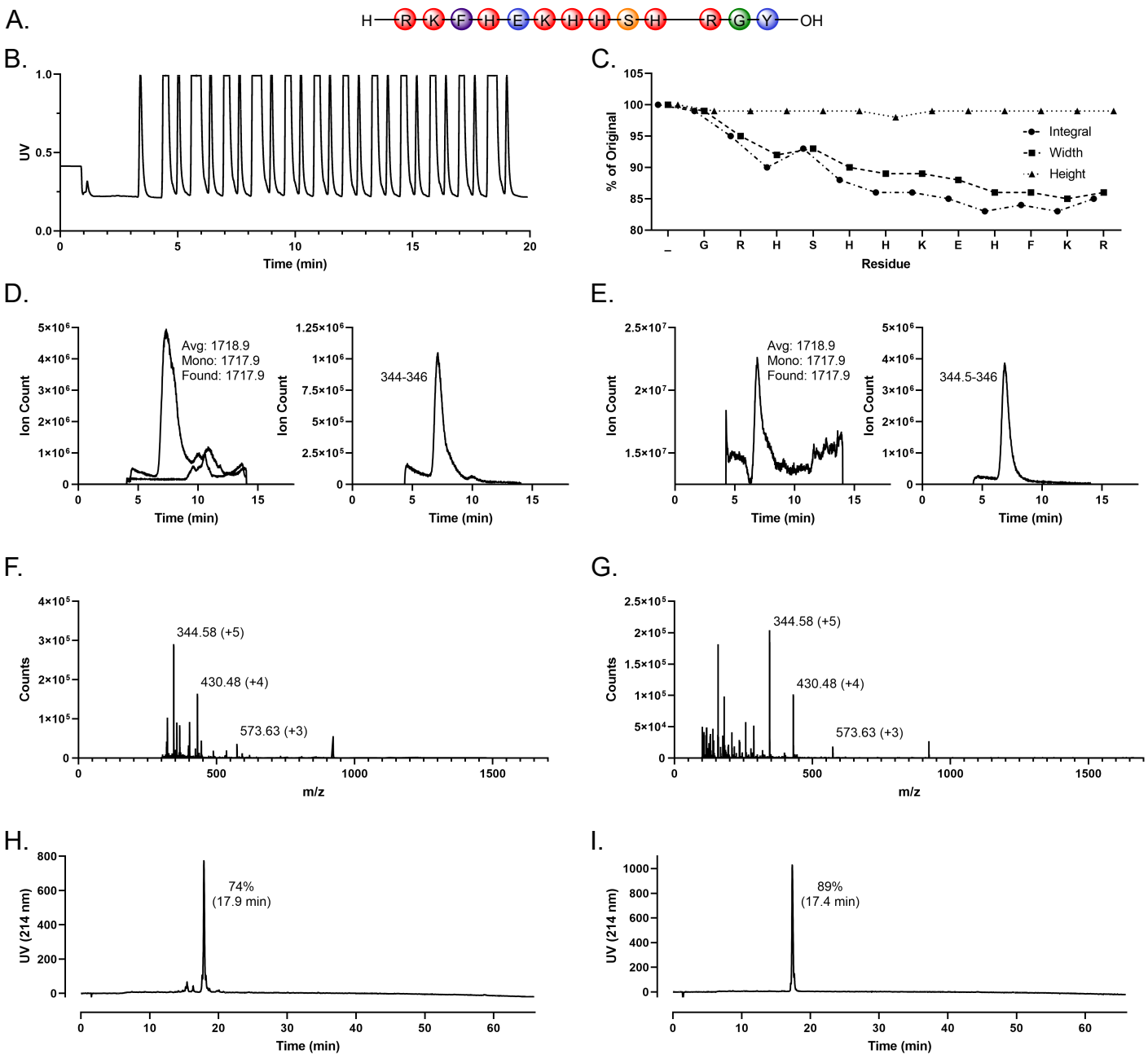
Supplementary Figure S13: A. Histatin 4 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



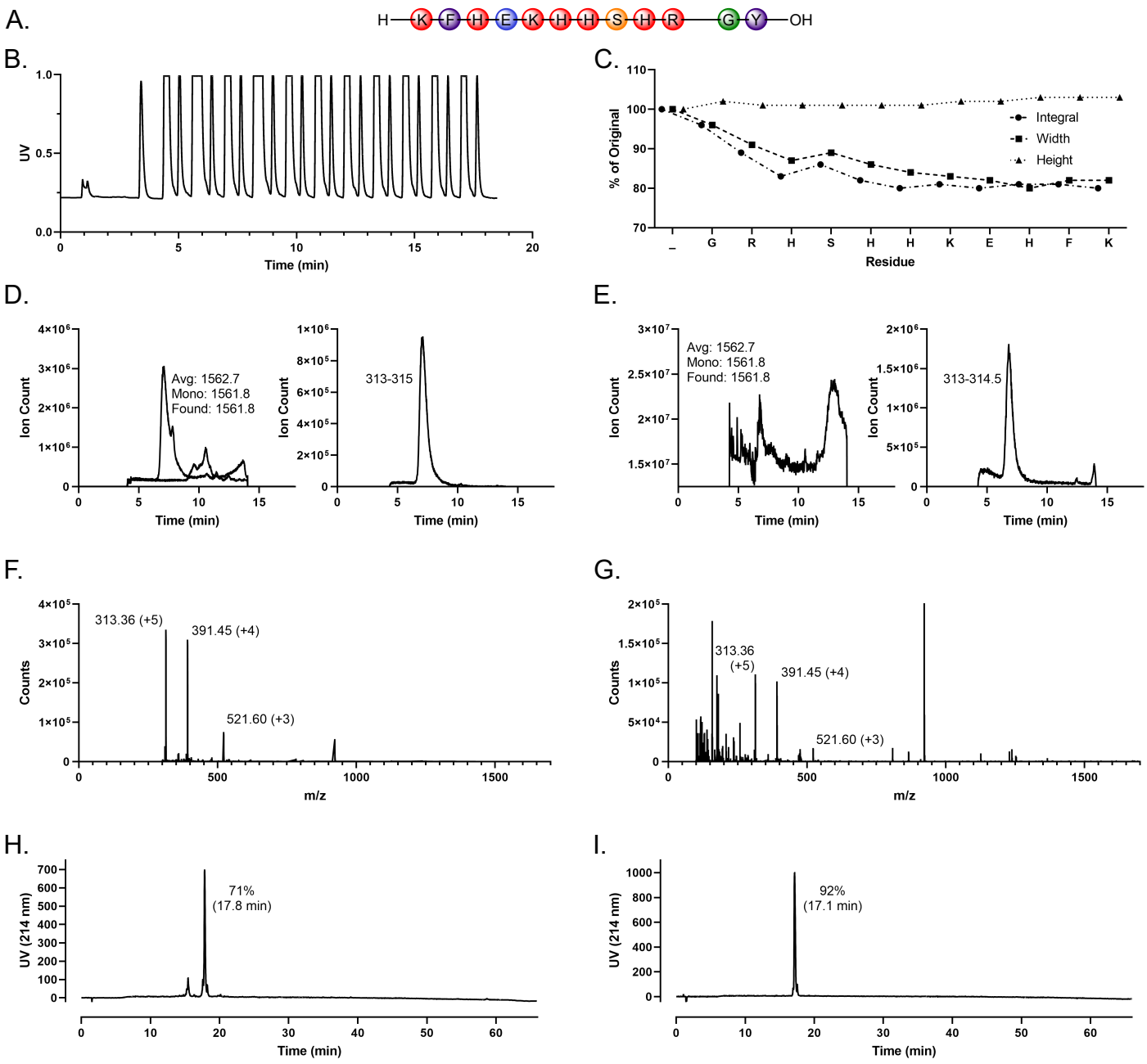
Supplementary Figure S14: A. Histatin 5 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Length. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



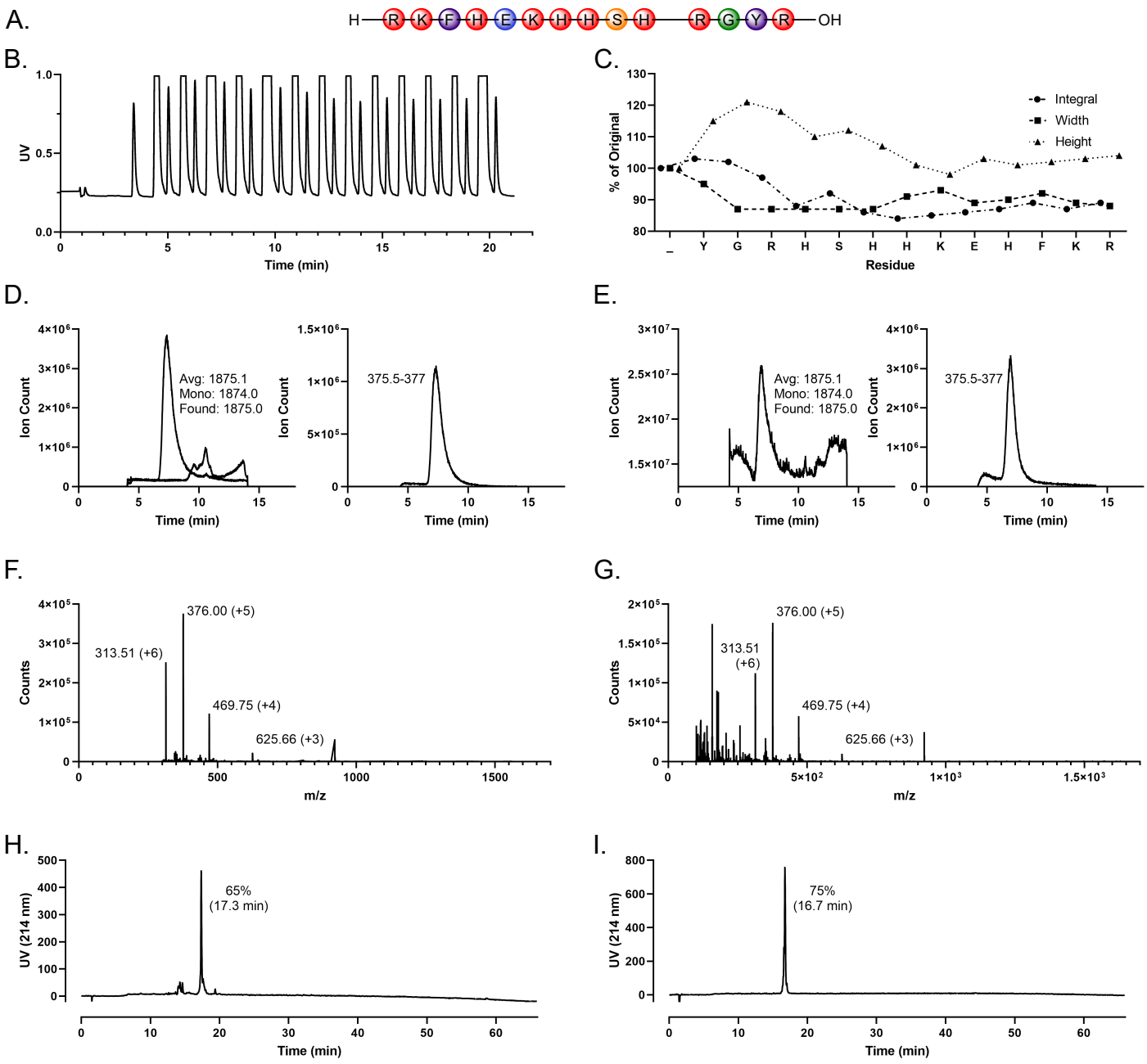
Supplementary Figure S15: A. Histatin 6 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer user-initiated pause; total time graphed includes the pause time. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



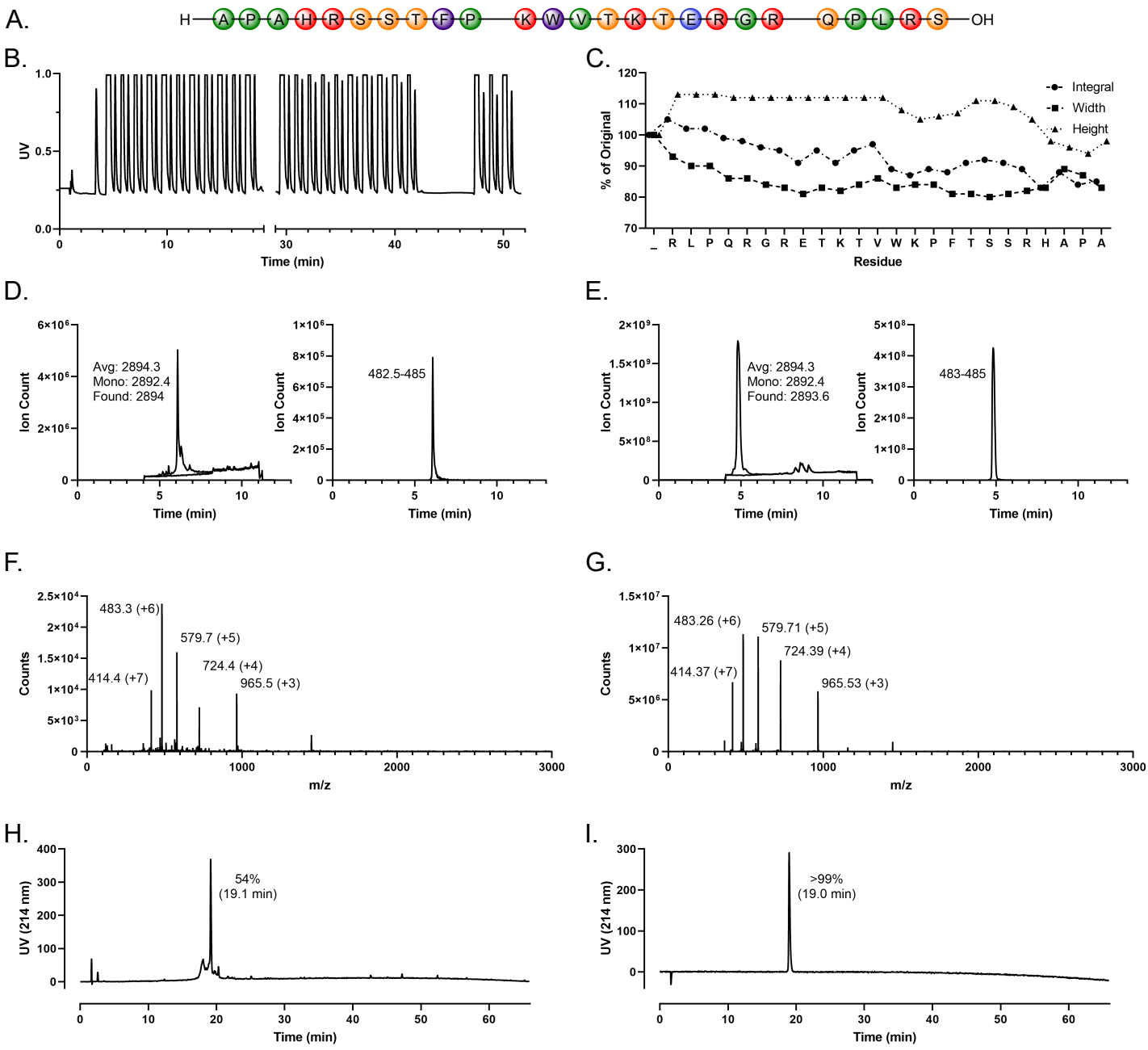
Supplementary Figure S16: A. Histatin 7 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. Blanks omitted from **D** and **E** due to poor baseline; see also main text. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



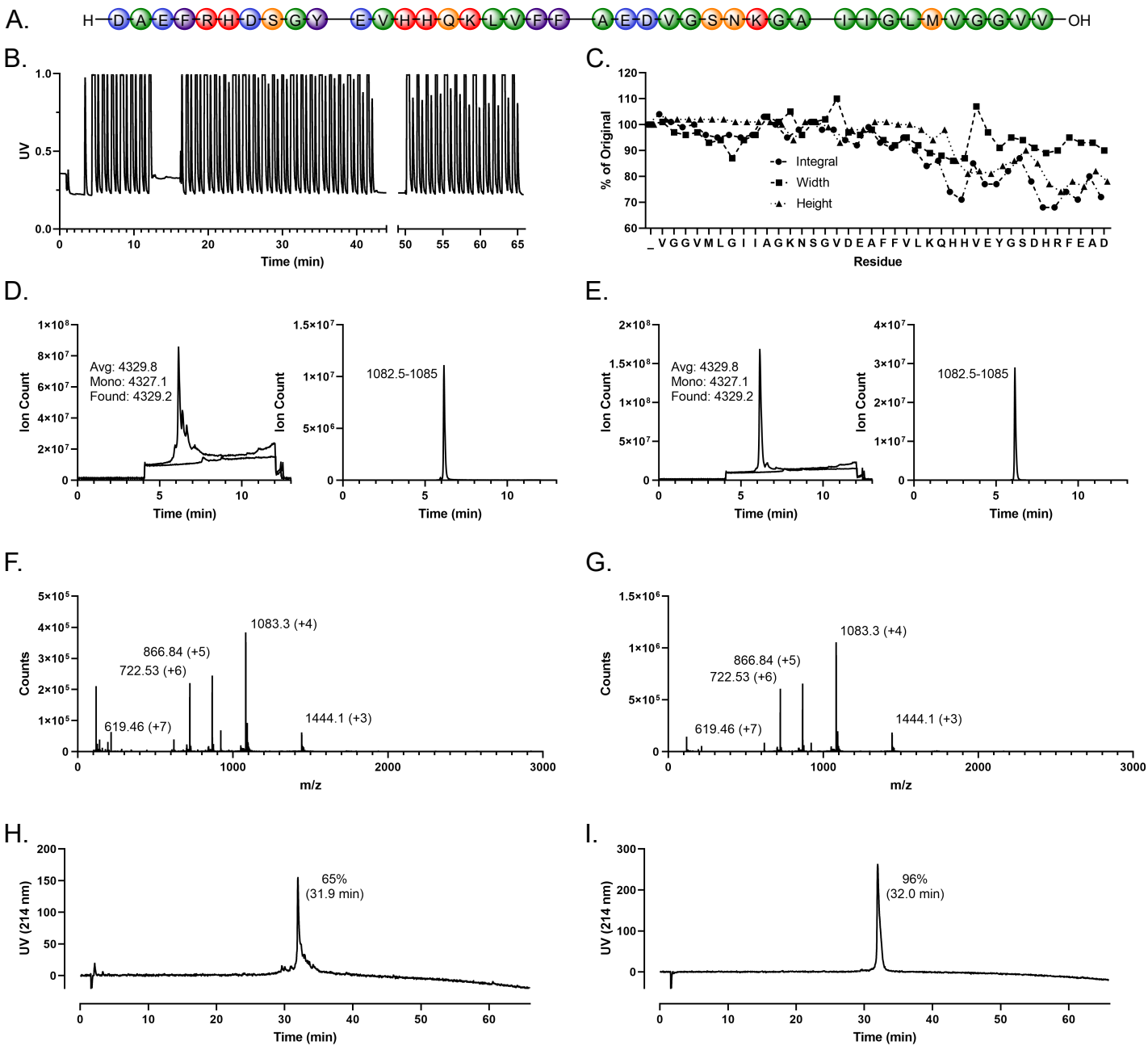
Supplementary Figure S17: A. Histatin 8 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. Blanks omitted from **D** and **E** due to poor baseline; see also main text. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



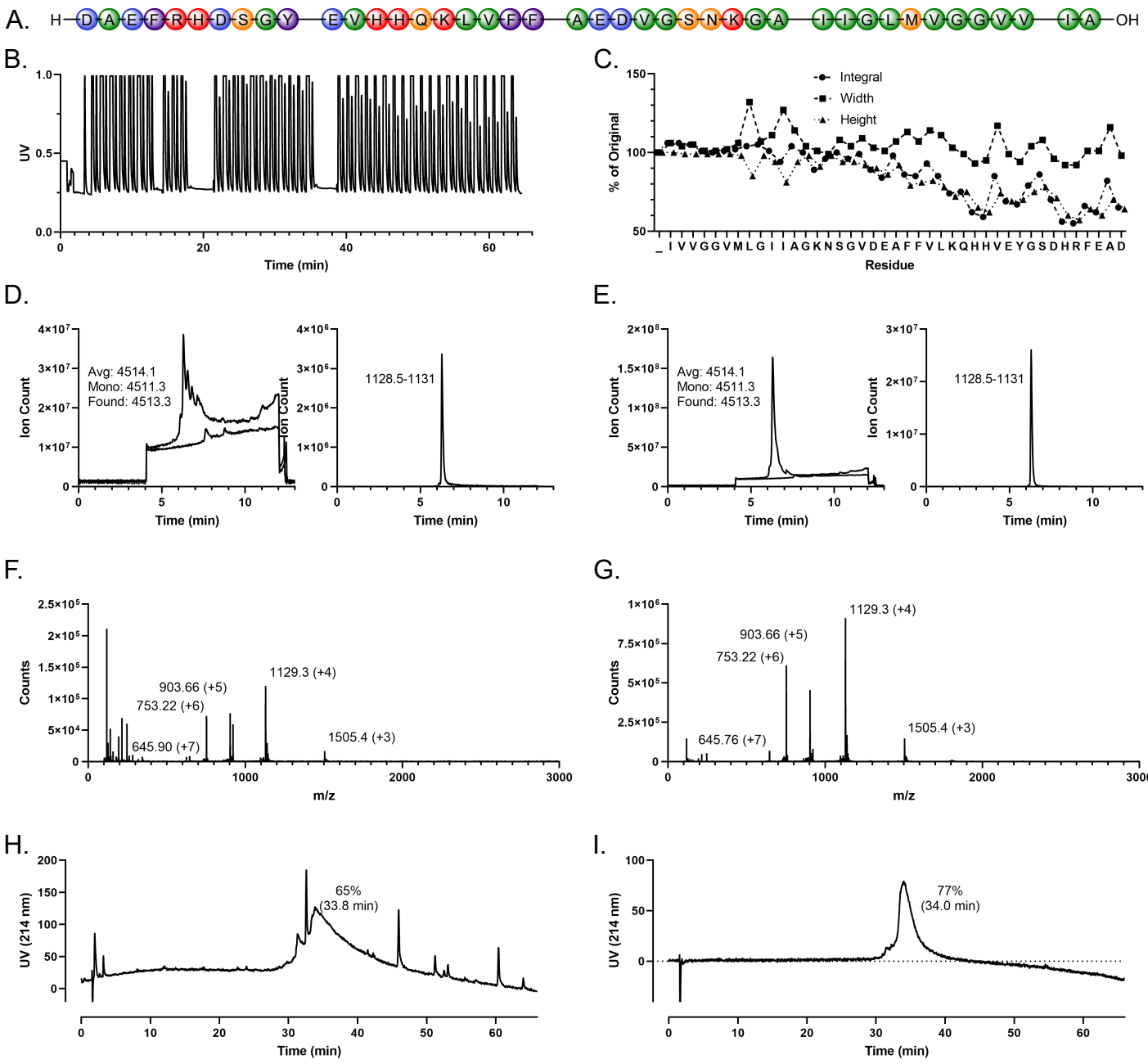
Supplementary Figure S18: A. Histatin 9 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. Blanks omitted from **D** and **E** due to poor baseline; see also main text. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. The charge states of the labeled ions are indicated in parentheses. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



Supplementary Figure S19: A. Alarin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer pause; total time graphed includes the pause time. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



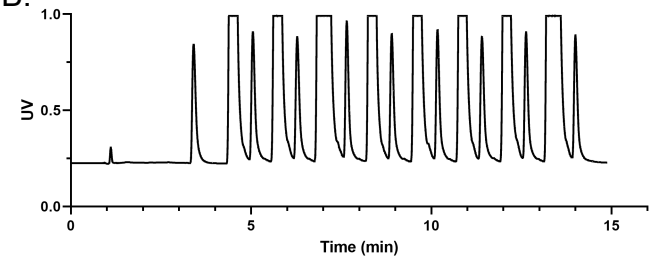
Supplementary Figure S20: A. Amyloid β 1-40 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer pause; total time graphed includes the pause time. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCM S Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



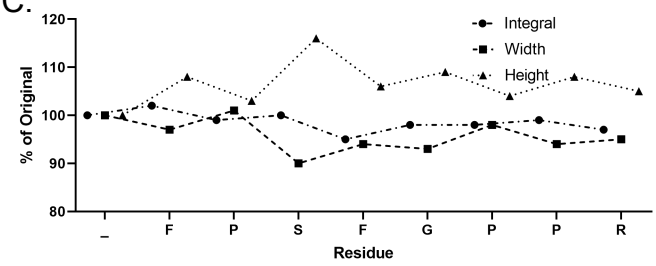
Supplementary Figure S21: A. Amyloid β 1-42 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.

A.

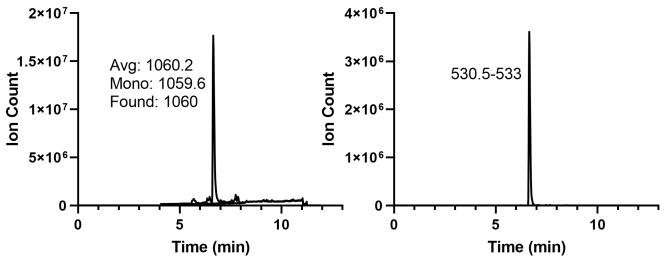
B.



C.



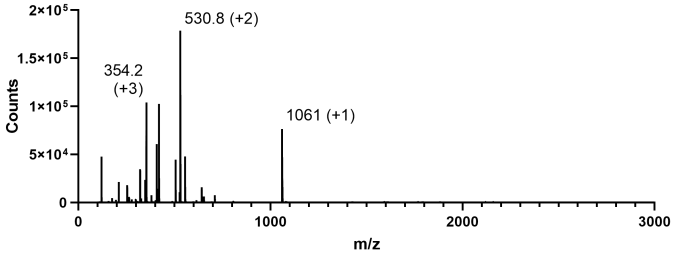
D.



E.

Not Available

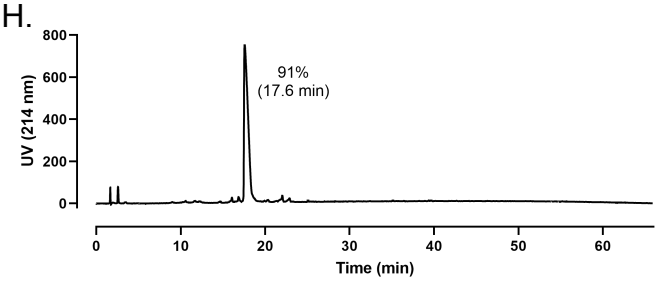
F.



G.

Not Available

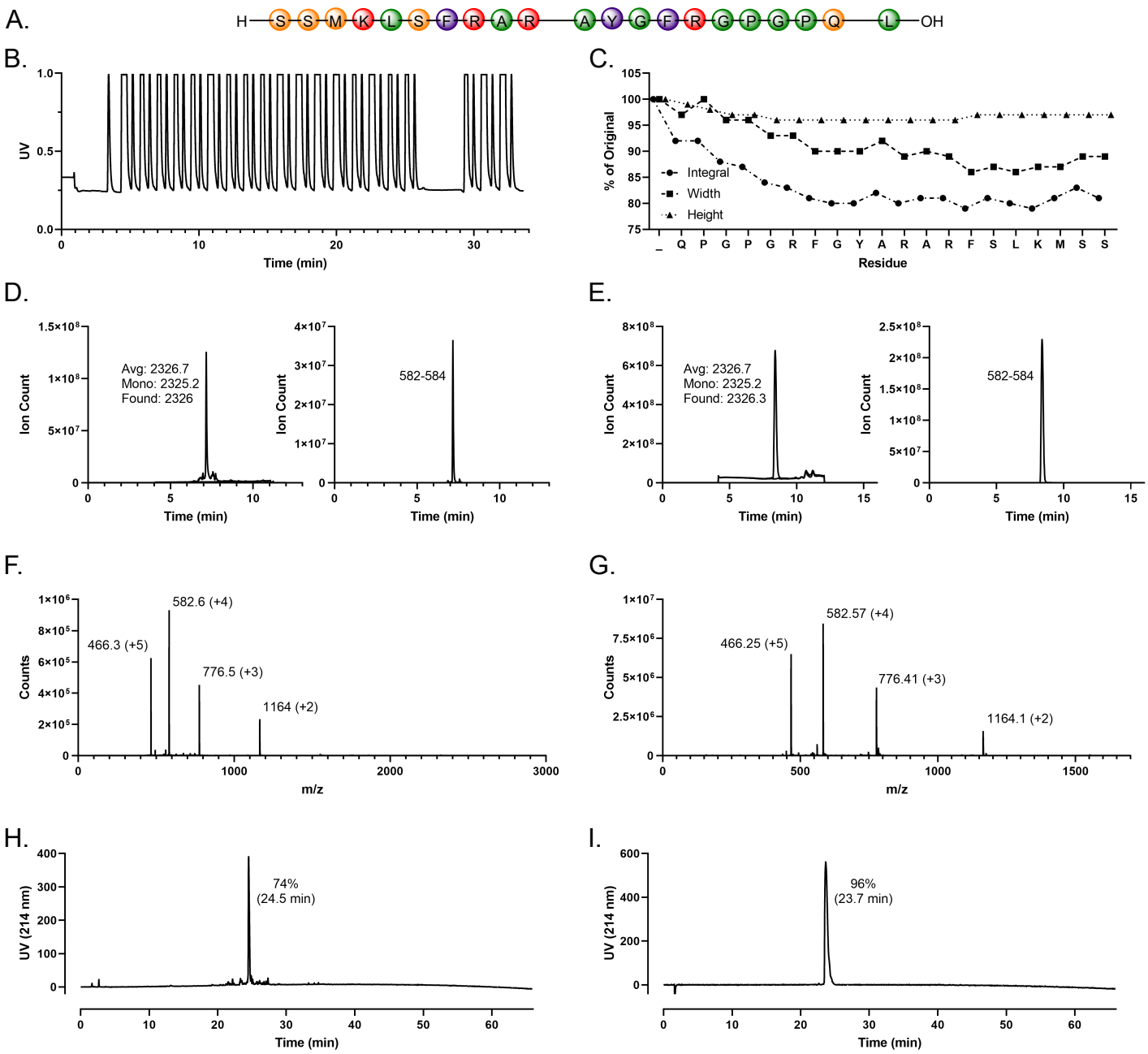
H.



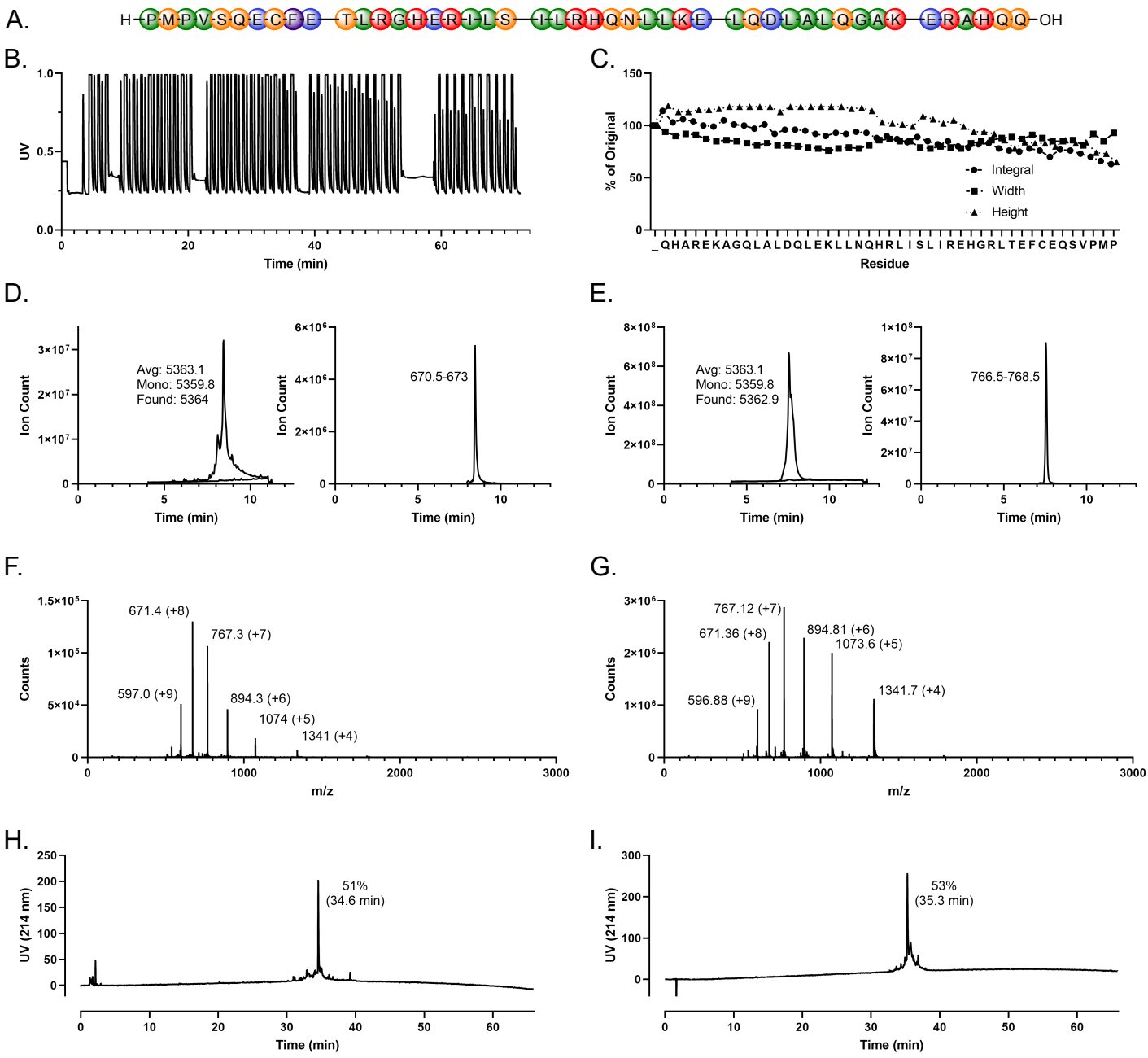
I.

Not Available

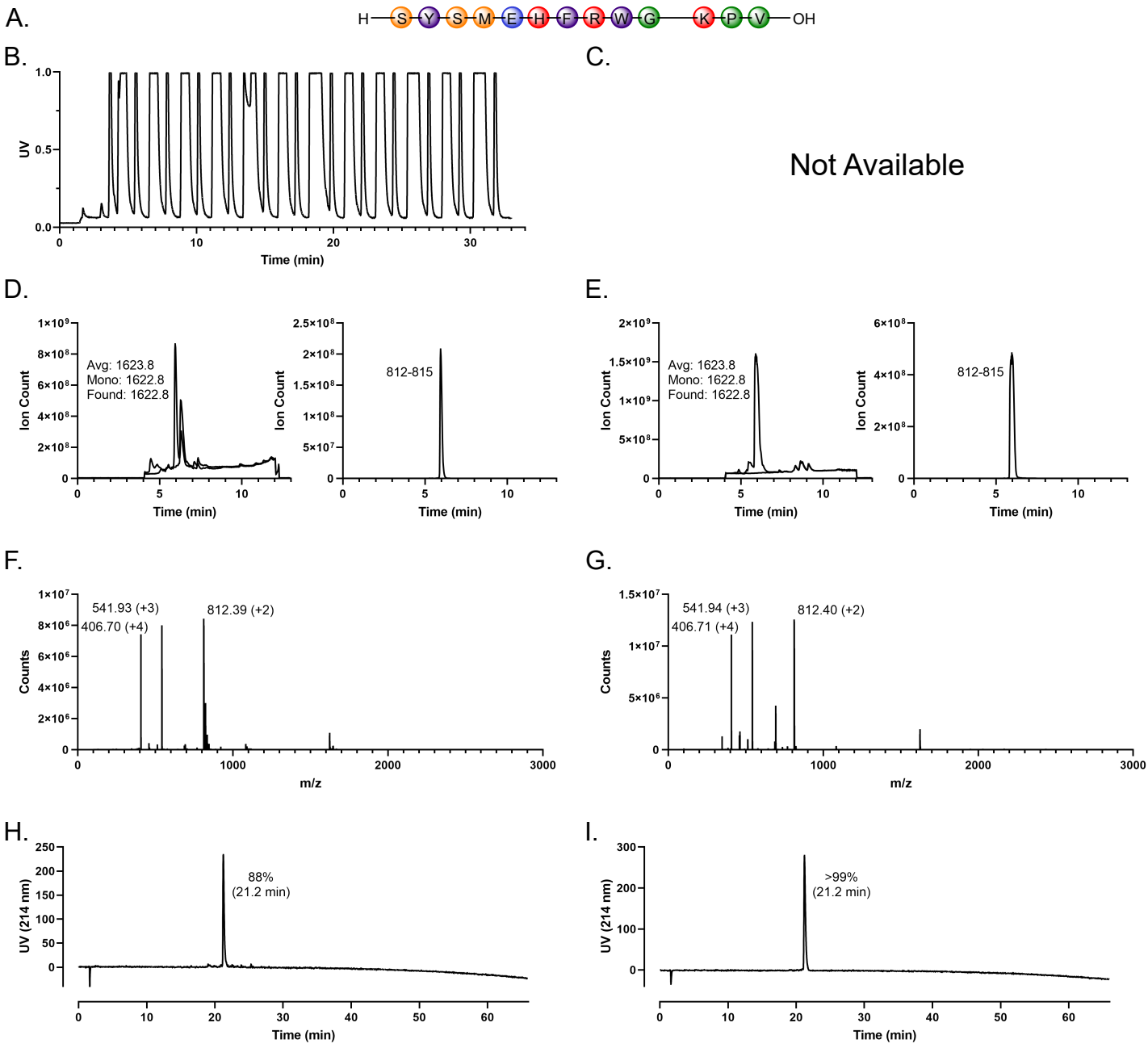
Supplementary Figure S22: A. Bradykinin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** Not purified. **F.** Mass spectrum associated with the dominant peak of **D. G.** Not purified. **H.** Analytical HPLC trace of crude peptide with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1. **I.** Not purified.



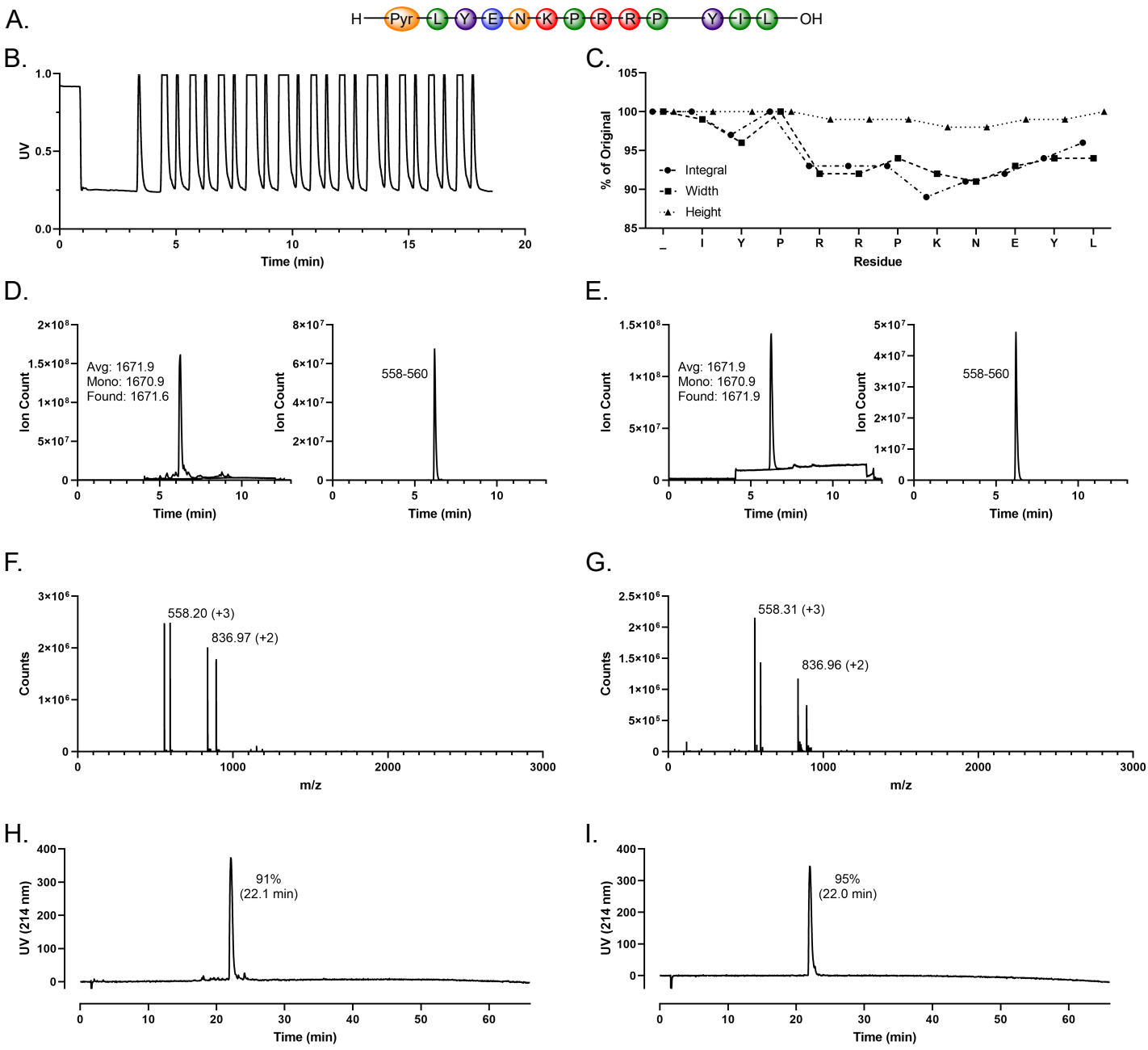
Supplementary Figure S23: A. Catestatin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. Spaces in the x-axis represent user-initiated pauses. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 1. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



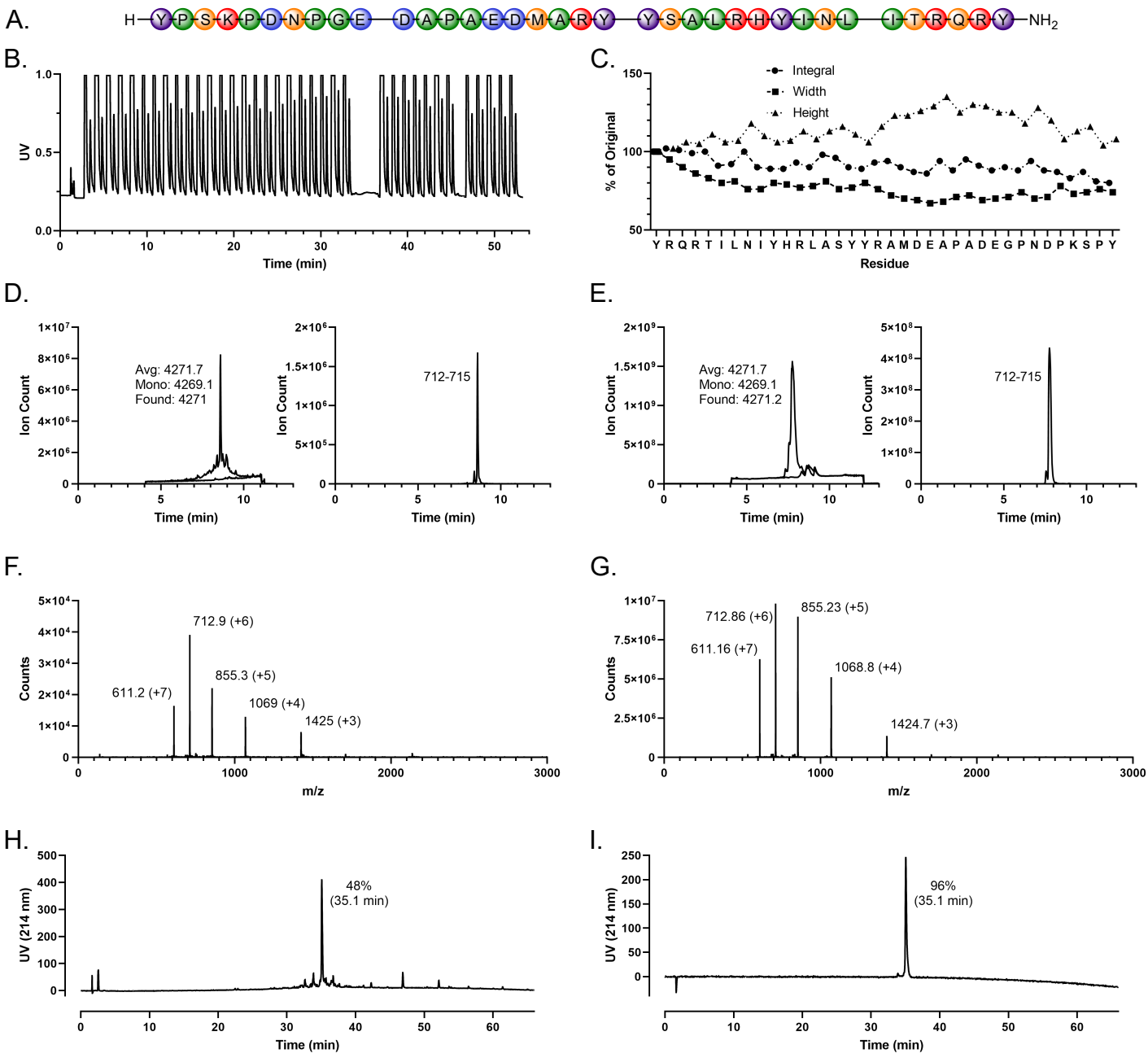
Supplementary Figure S24: A. CGA-N46 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



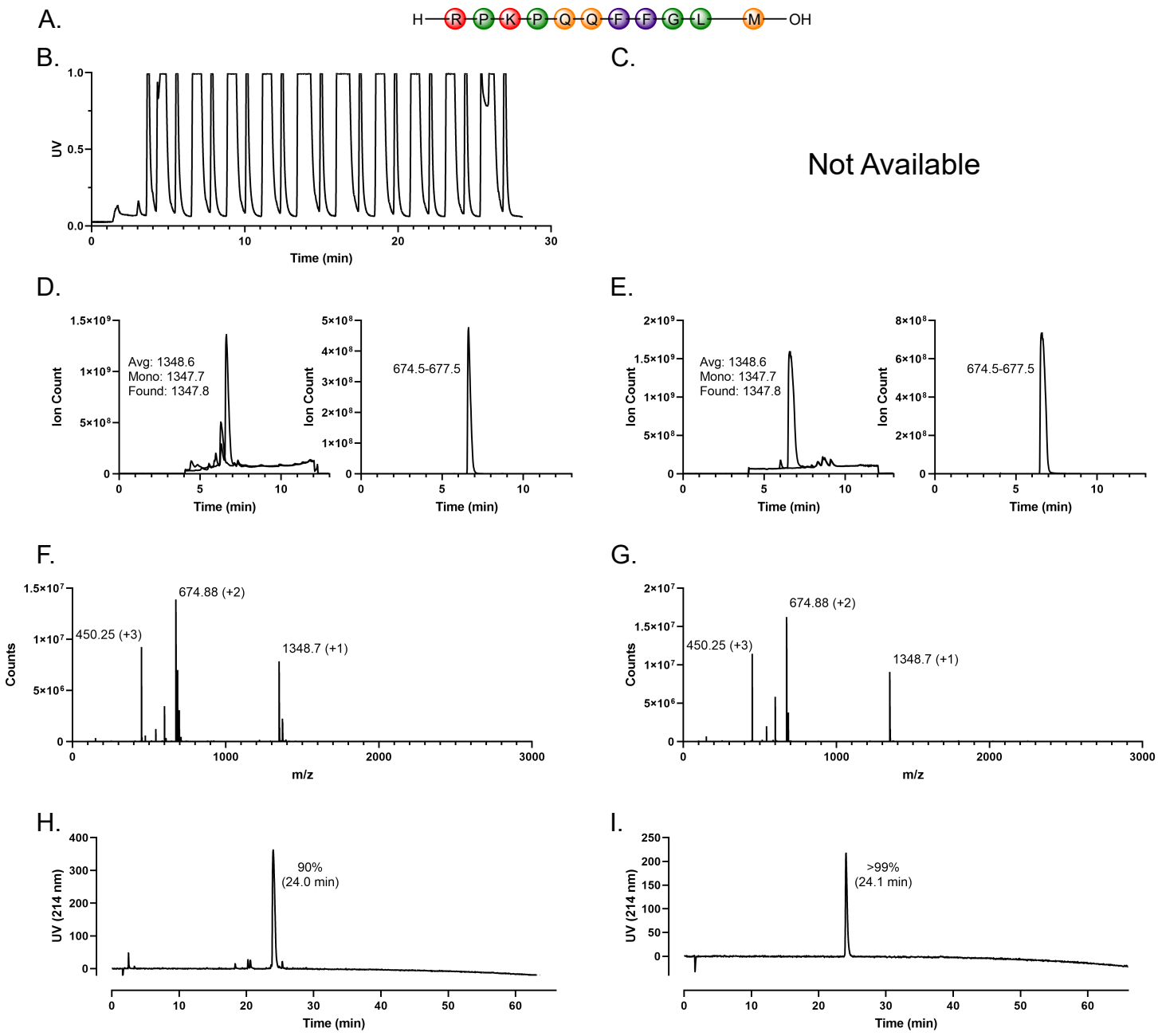
Supplementary Figure S25: A. α MSH sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 4th Generation – Length. **C.** Integrals unavailable for this sequence. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



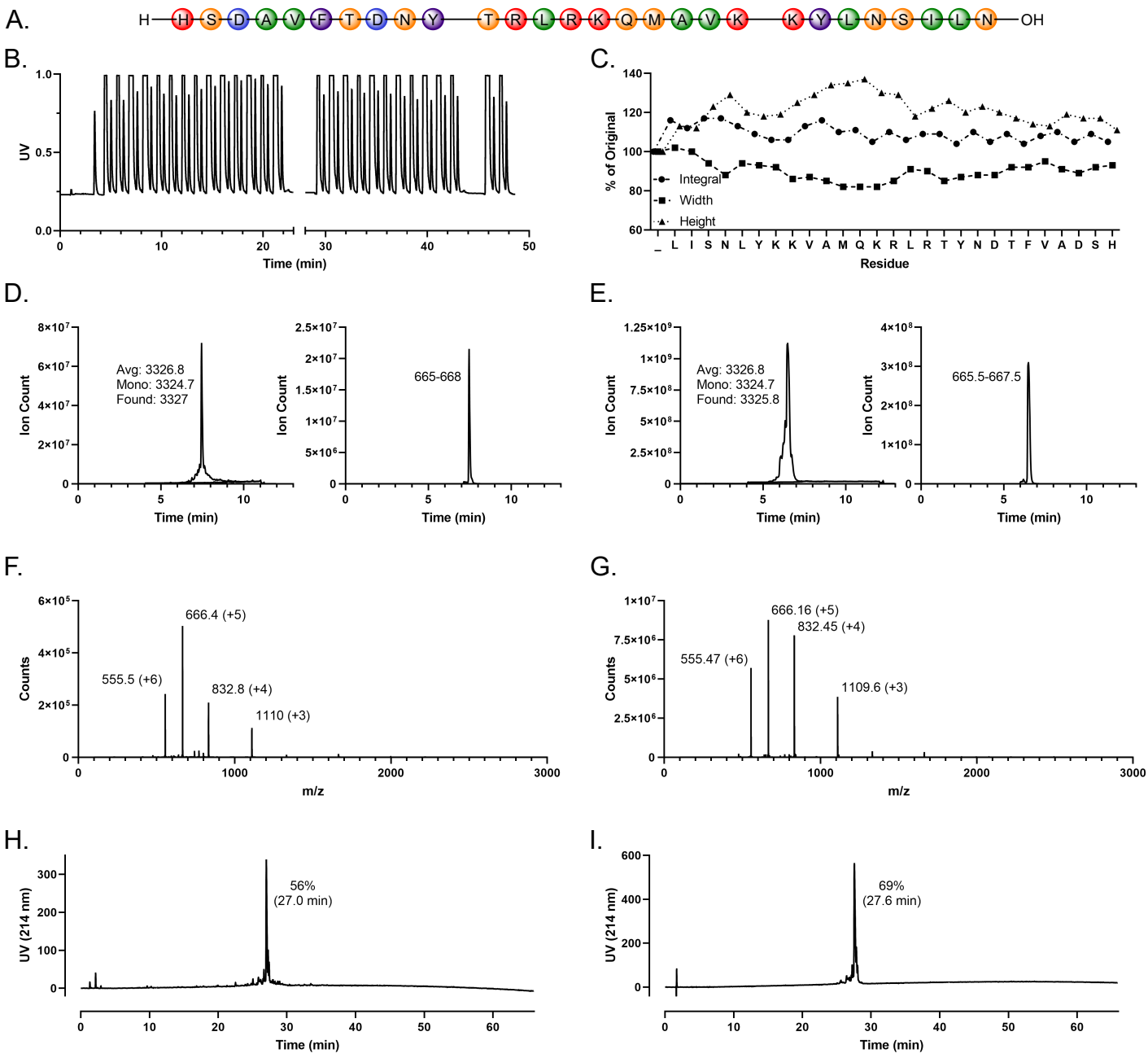
Supplementary Figure S26: A. Neurotensin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic); see main text for discussion pertaining to this synthesis. **B.** Synthesizer UV trace. 3rd Generation – Speed with batch addition of N-terminal pyroglutamate. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



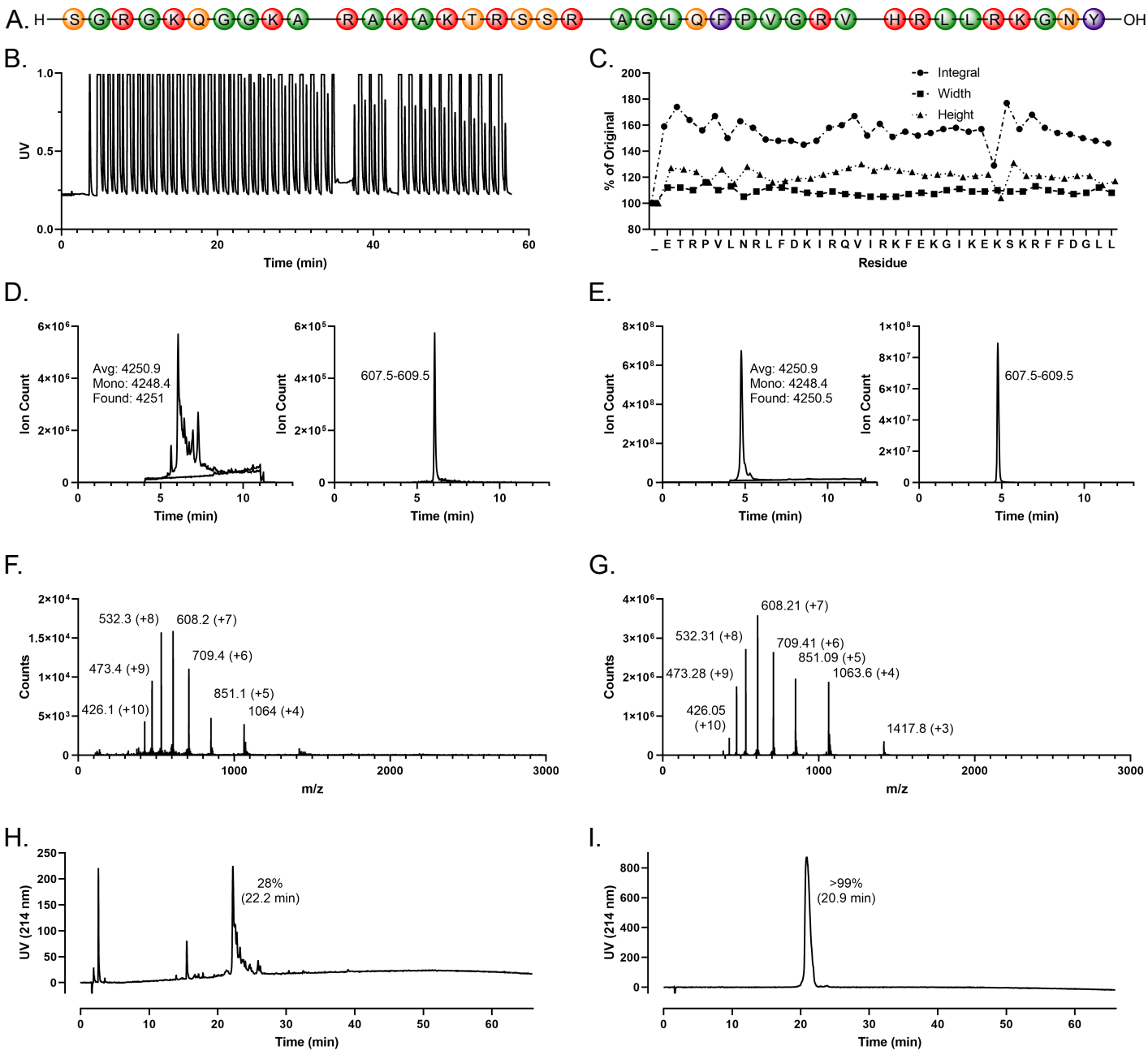
Supplementary Figure S27: A. Neuropeptide Y (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed on Rink Amide to yield C-terminal amide on acid cleavage. Spaces in the x-axis represent user-initiated pauses. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



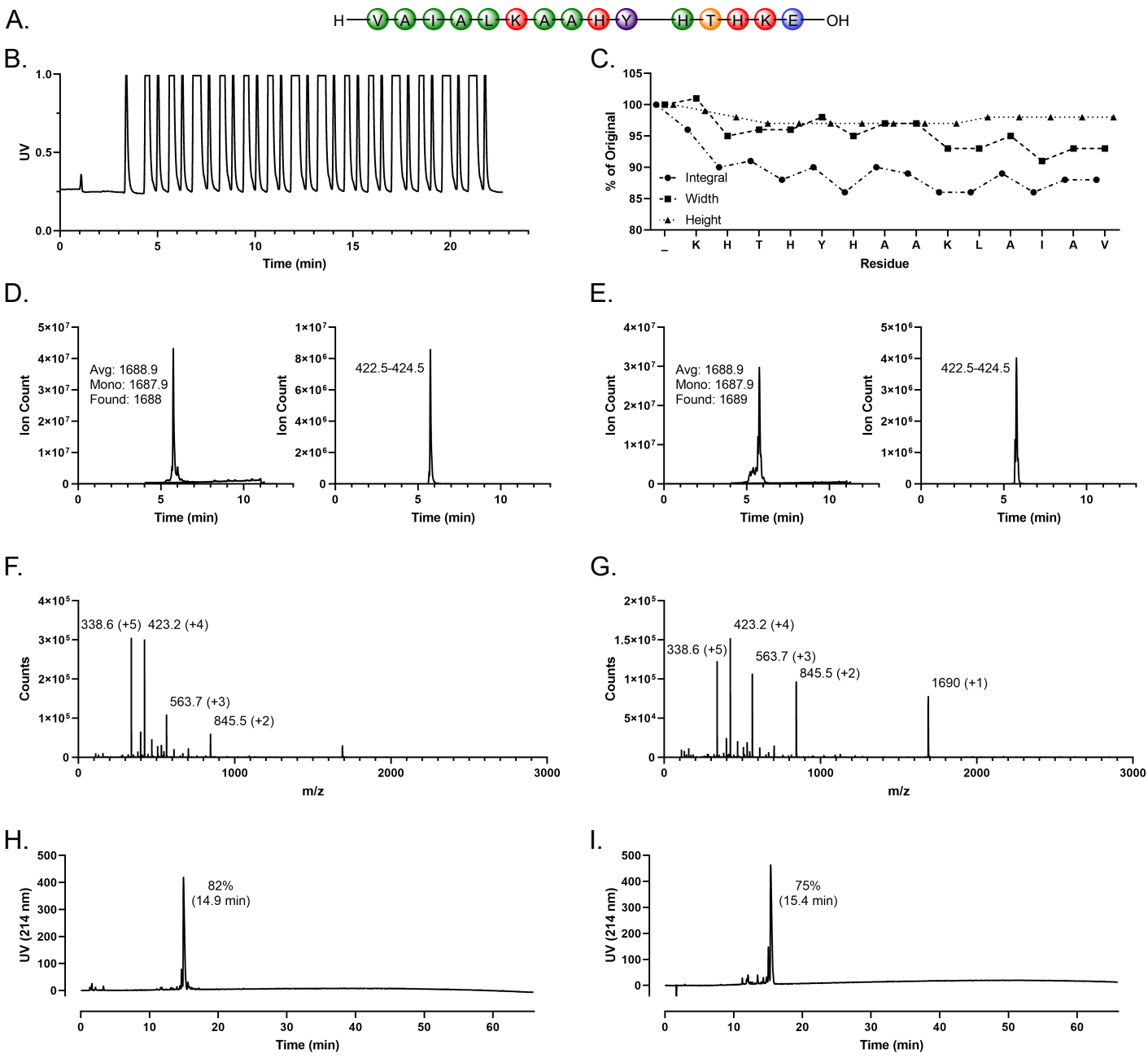
Supplementary Figure S28: A. Substance P sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 4th Generation – Length. **C.** Integrals unavailable for this sequence. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



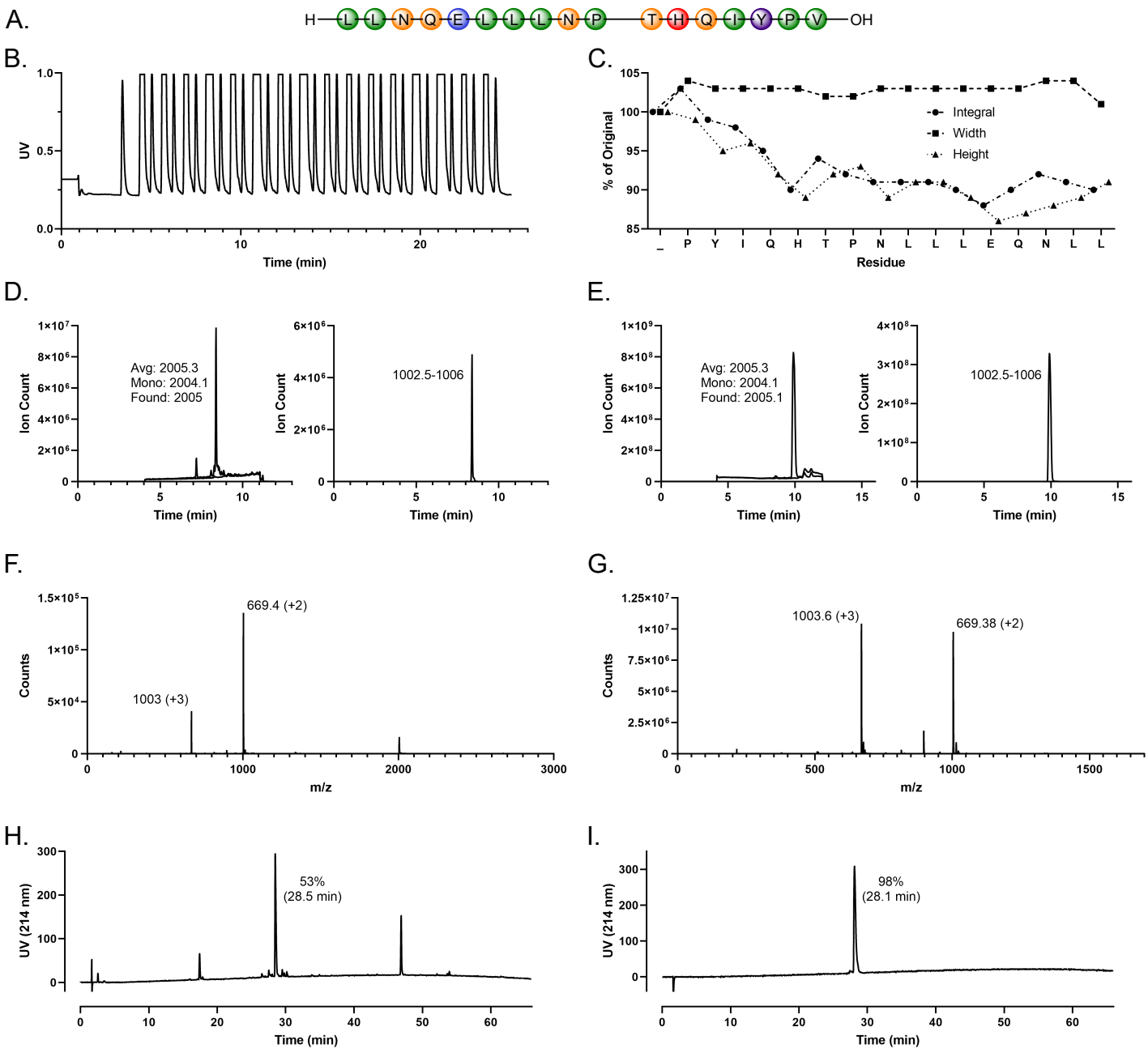
Supplementary Figure S29: A. Vasoactive Intestinal Peptide sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer pause; total time graphed includes the pause time. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



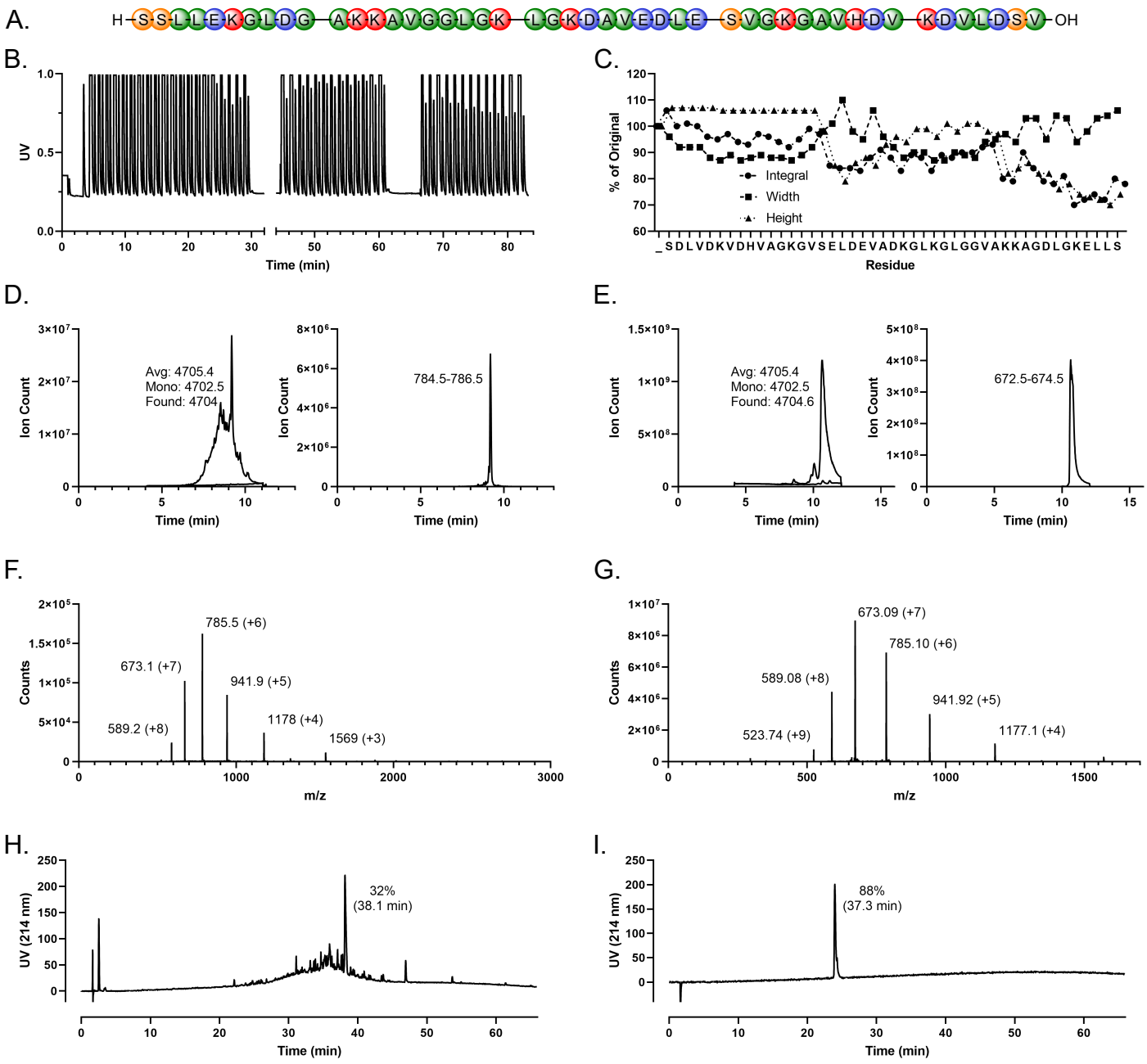
Supplementary Figure S30: A. Buforin I sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



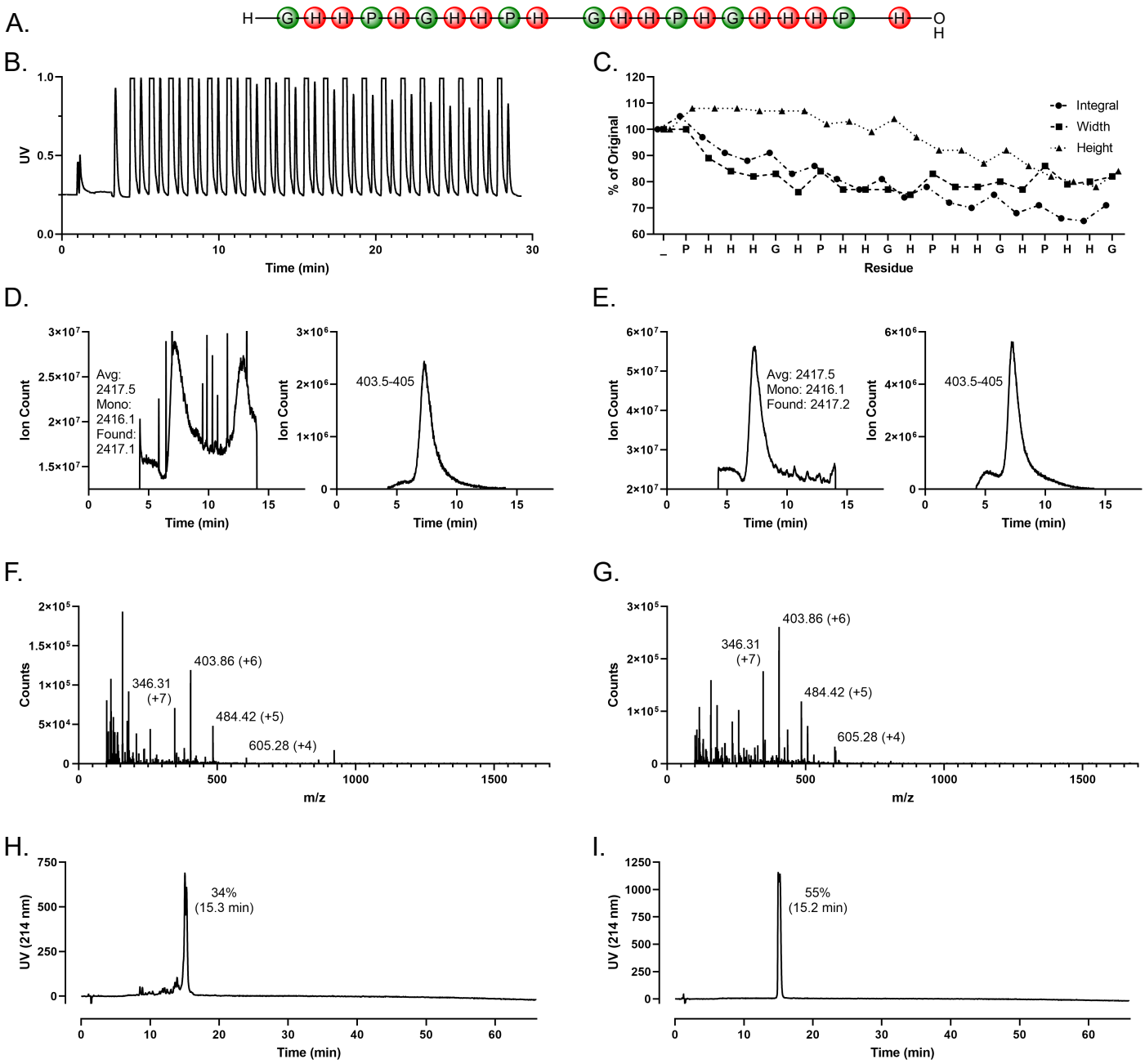
Supplementary Figure S31: A. Calcitermin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 5. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



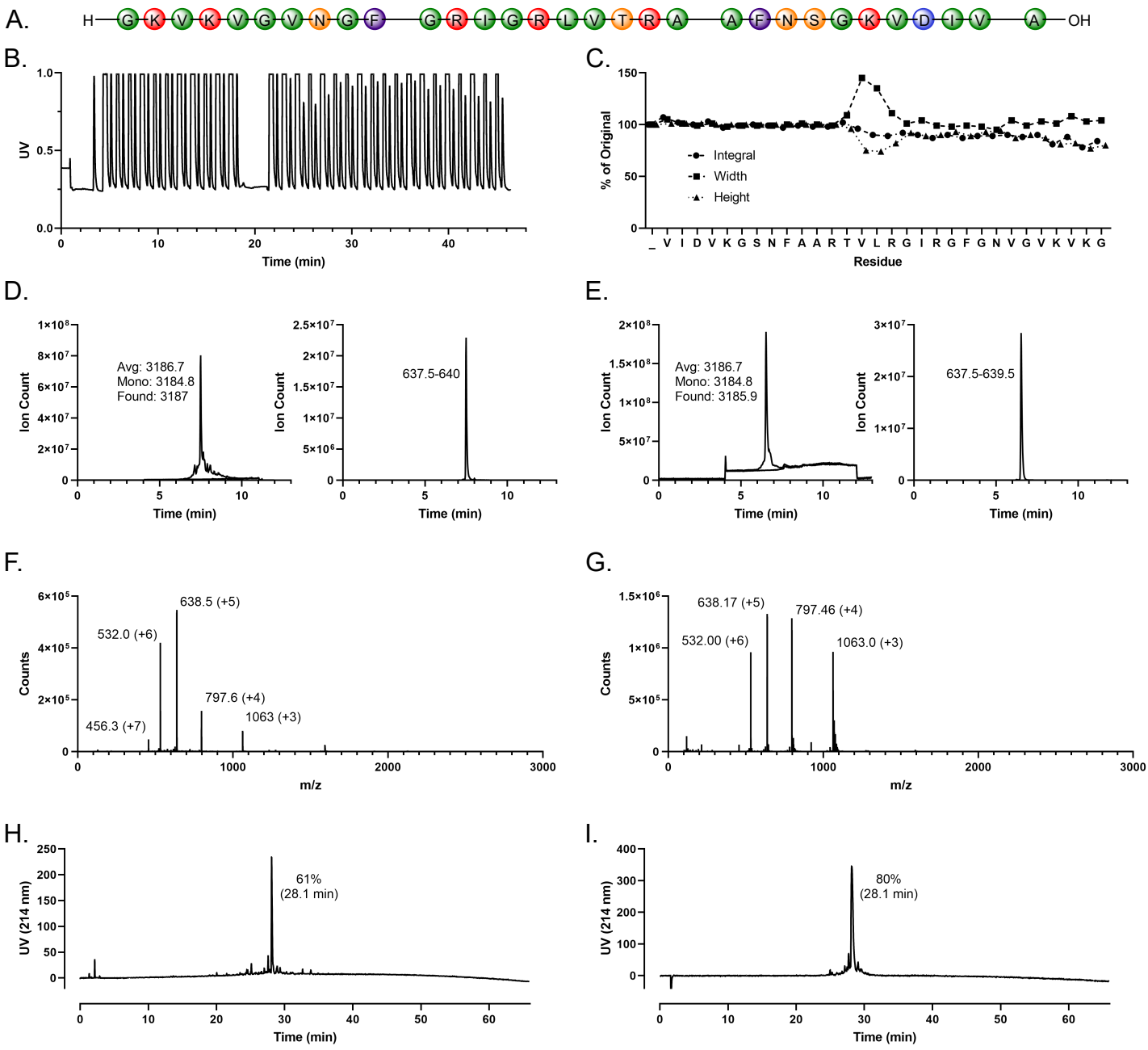
Supplementary Figure S32: A. β-casein 197 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 1. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



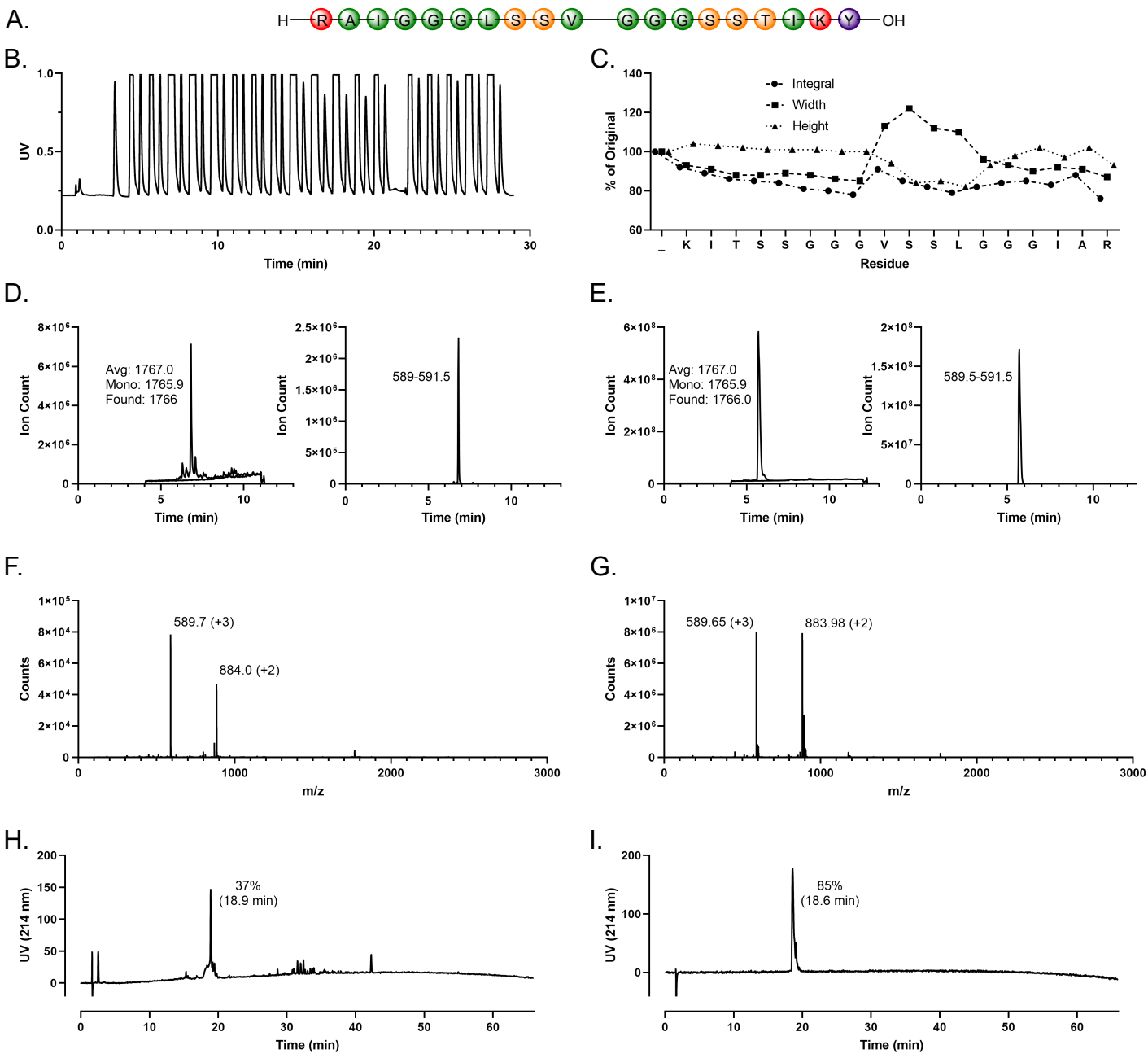
Supplementary Figure S33: A. Dermcidin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer pause; total time graphed includes the pause time. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCM S Method 1. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



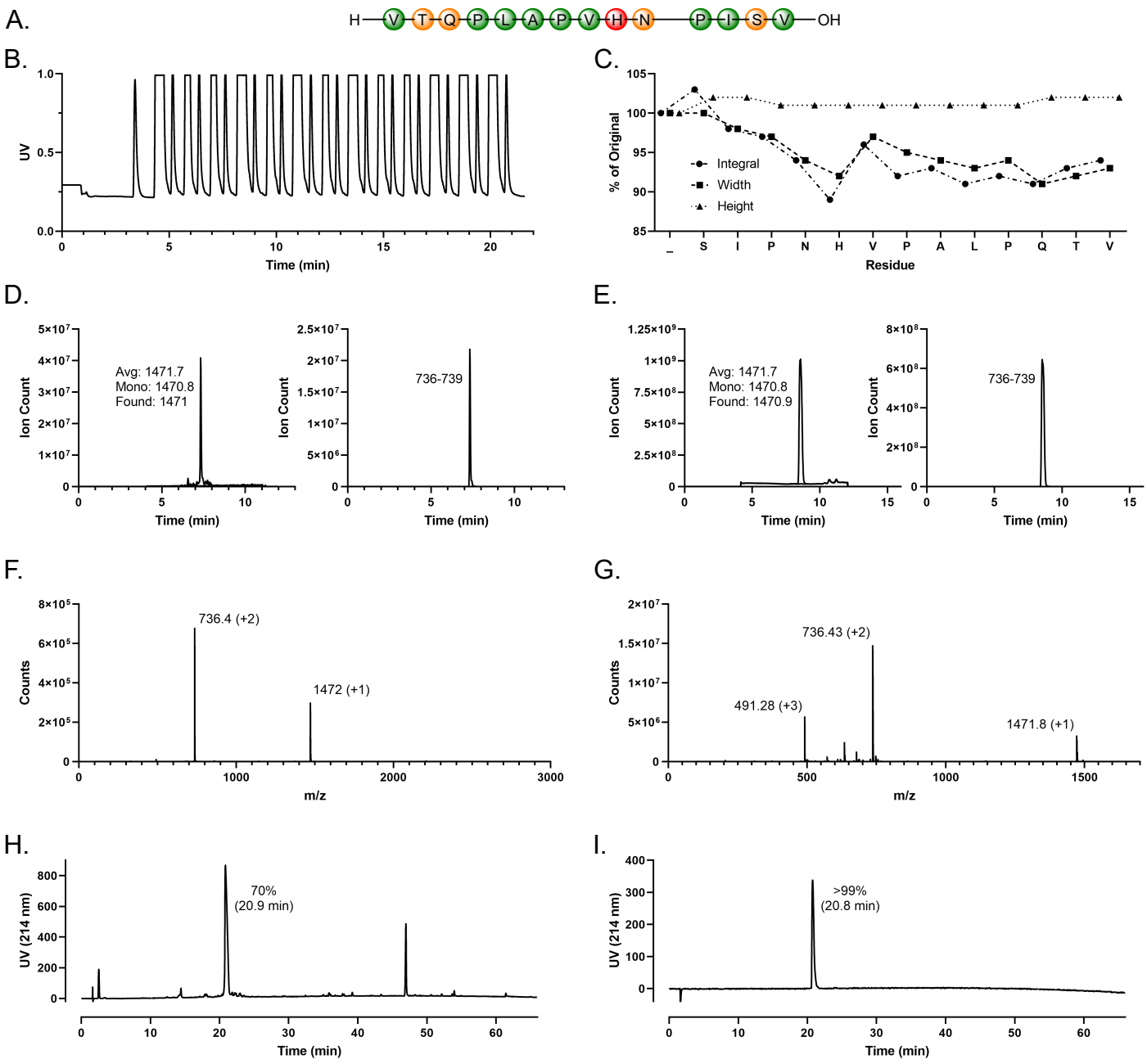
Supplementary Figure S34: A. GHH20 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. Blanks are omitted from panels **D** and **E** due to separate issues with each (MS spiking similar to the sample shown in **D** and substantial debris from prior runs in **E**). **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2.



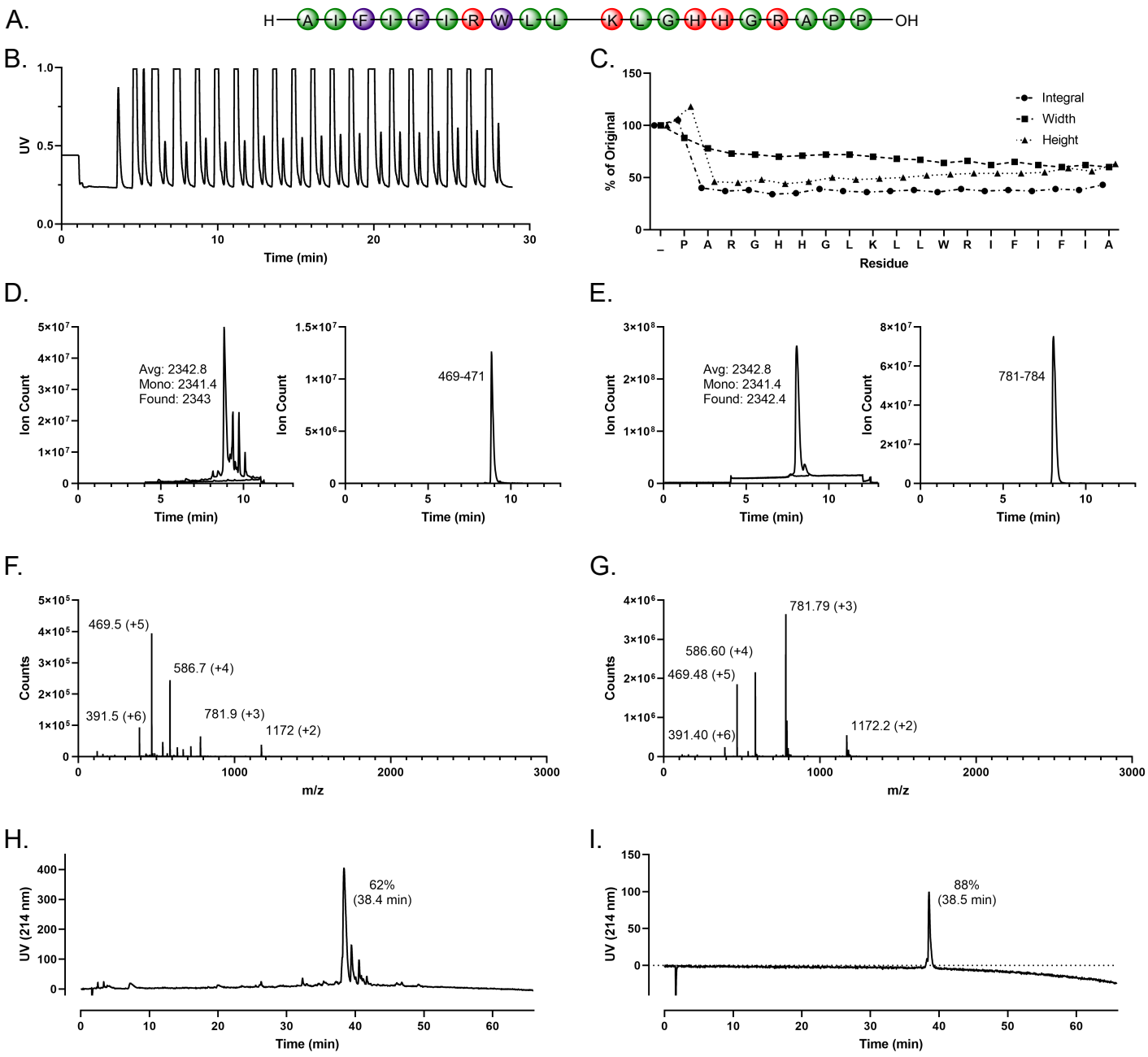
Supplementary Figure S35: A. hGAPDH sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



Supplementary Figure S36: A. KDAMP 19-mer sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



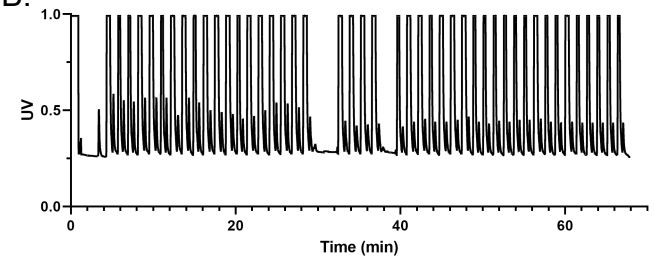
Supplementary Figure S37: A. PDC213 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 1. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.



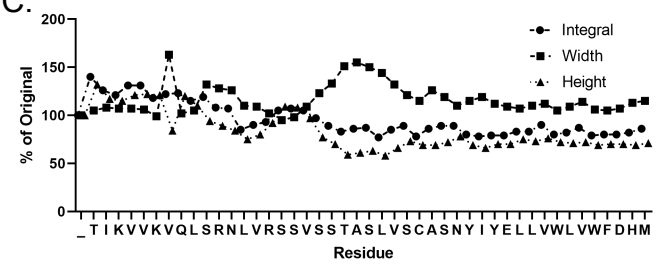
Supplementary Figure S38: A. Salusin β sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 3. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.

A.

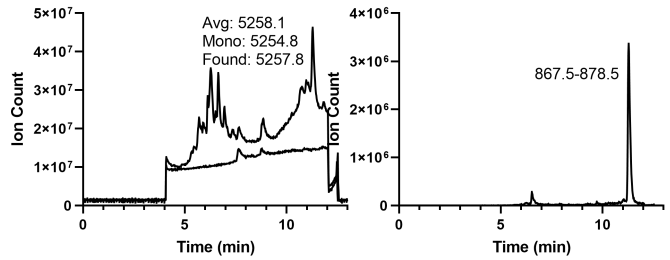
B.



C.



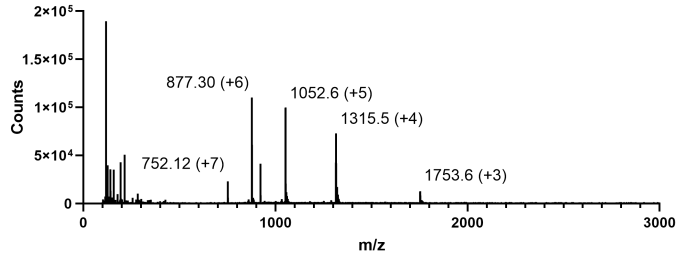
D.



E.

Not Available

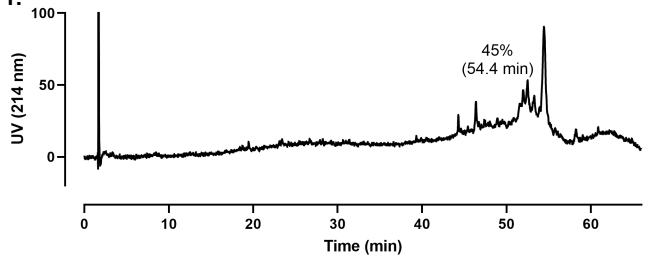
F.



G.

Not Available

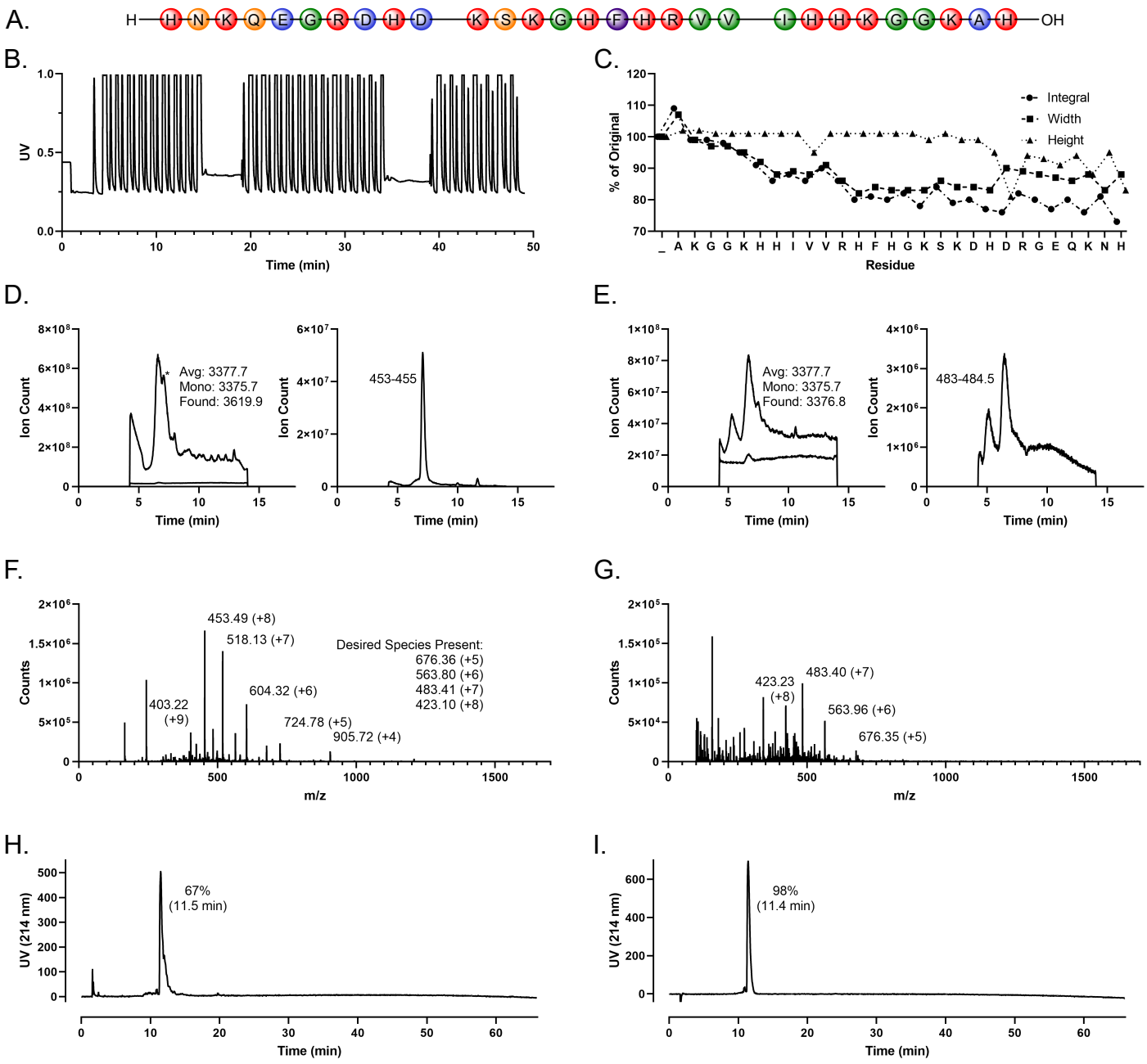
H.



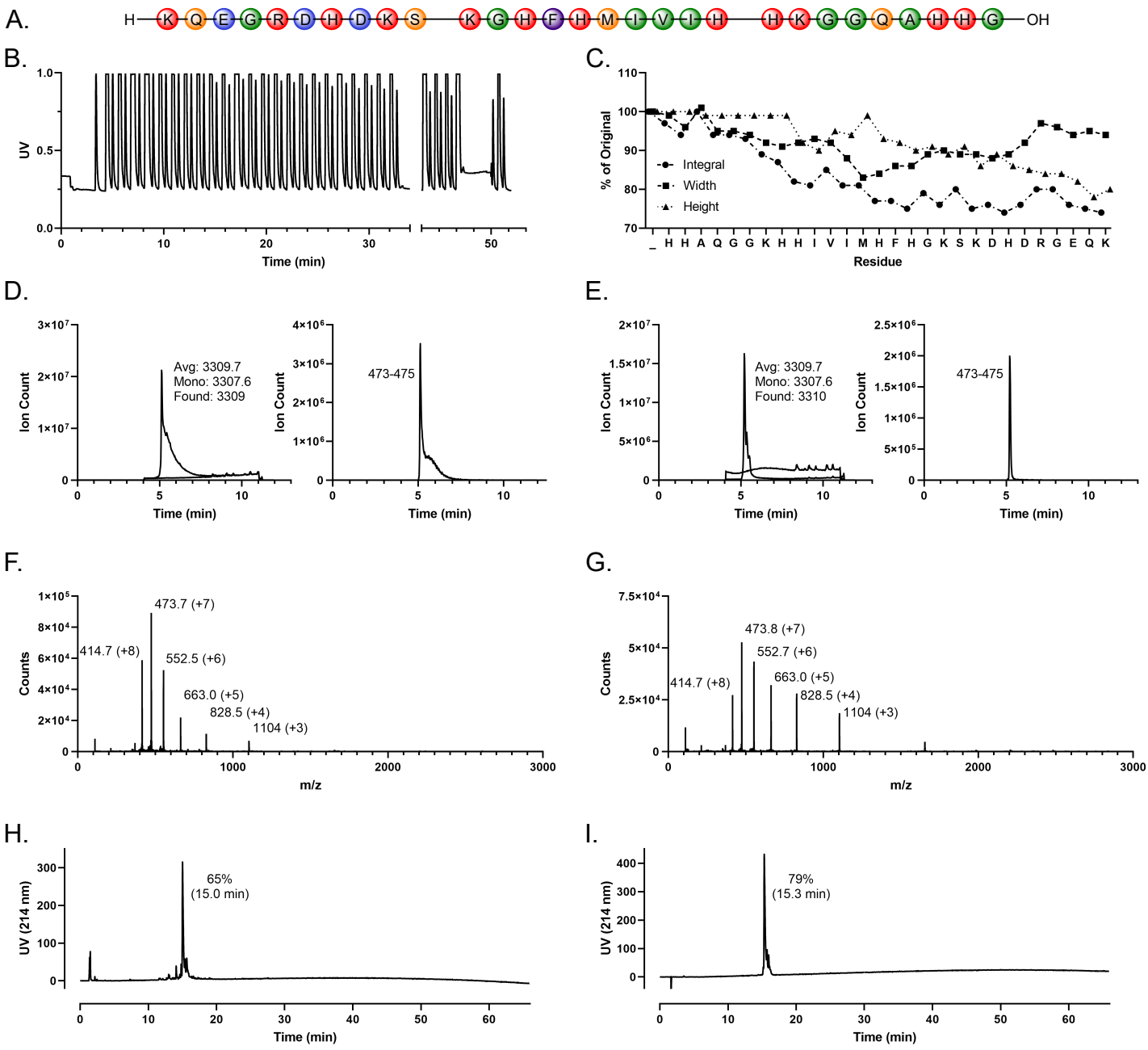
I.

Not Available

Supplementary Figure S39: A. Salvic sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace unavailable. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 3. Right panel EIC of crude AMP for the specified *m/z* range. **E.** Not completed. **F-G.** Mass spectra associated with the dominant peak of **D**. **E.** Not completed. **H.** Analytical HPLC trace of crude peptide with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1. **I.** Not completed.

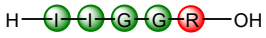


Supplementary Figure S40: A. SgI-29 sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion of the right shoulder of the dominant peak (starred), LCMS Method 2. Right panel EIC in the specified *m/z* range, centered on the most abundant ion in the spectrum shown in **F**. **E.** Left panel TIC of purified AMP overlaid on Blank run, LCMS Method 2. Right panel EIC in the specified *m/z* range, centered on the most abundant ion in the spectrum shown in **G**. **F-G.** Mass spectra associated with the aforementioned peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.

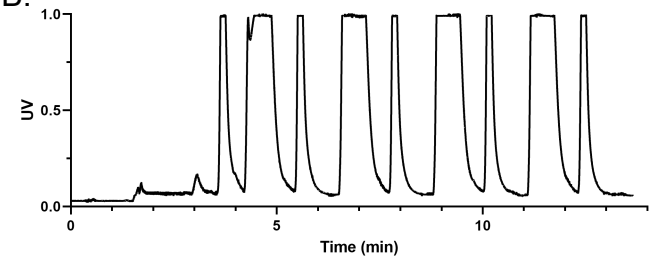


Supplementary Figure S41: A. SgII Peptide A sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. The x-axis is cut at a longer pause; total time graphed includes the pause time. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 5. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 5. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1.

A.



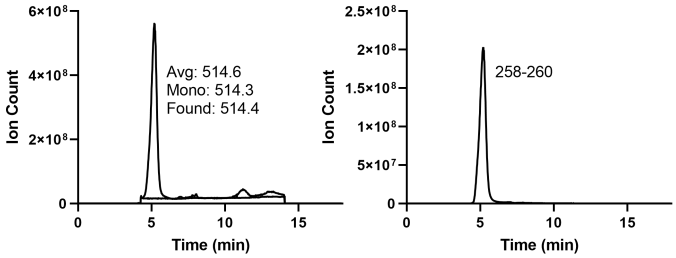
B.



C.

Not Available

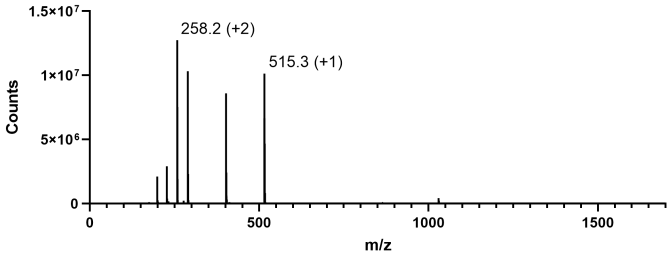
D.



E.

Not Available

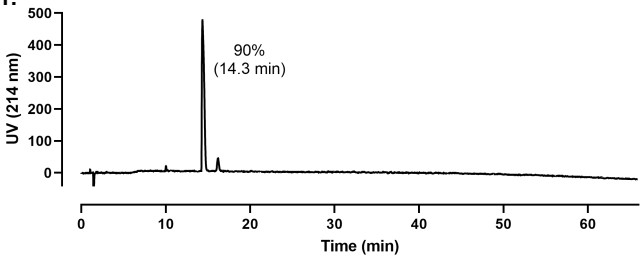
F.



G.

Not Available

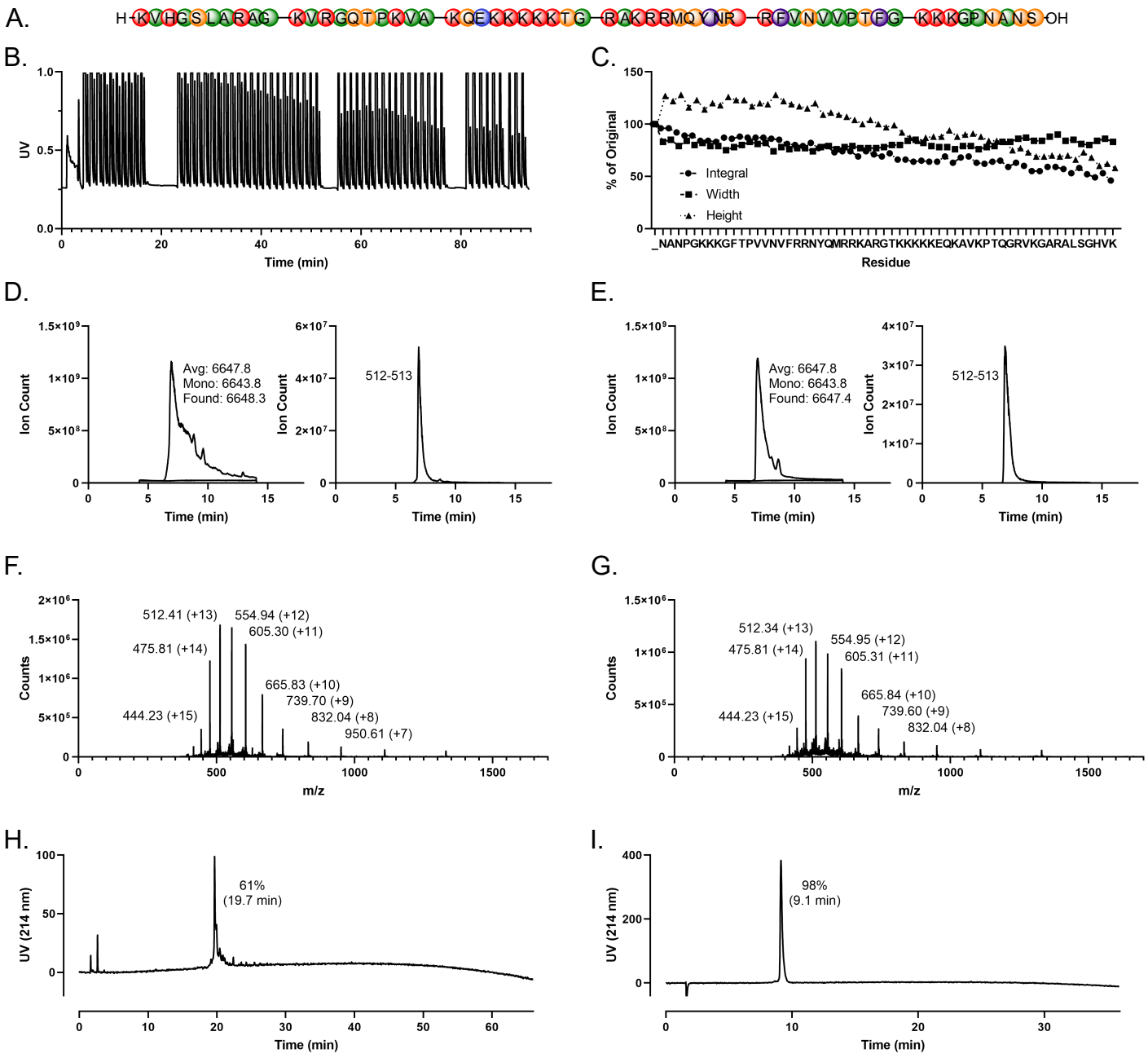
H.



I.

Not Available

Supplementary Figure S42: A. Cathepsin G sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). **B.** Synthesizer UV trace. 4th Generation – Length. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** Not completed. **F.** Mass spectrum associated with the dominant peak of **D**. **E.** Not completed. **H.** Analytical HPLC trace of crude peptide with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 2. **I.** Not completed.



Supplementary Figure S43: A. Ubiquicidin sequence (red = cationic, blue = anionic, orange = polar, green = nonpolar, purple = aromatic). Spaces in the x-axis represent user-initiated pauses. **B.** Synthesizer UV trace. 3rd Generation – Speed. **C.** Integrals and peak width and height expressed as percentages relative to the first cycle. **D.** Left panel TIC of crude AMP overlaid on Blank run with the predicted average and monoisotopic masses as well as the observed mass as calculated from the most abundant ion, LCMS Method 2. Right panel EIC of crude AMP for the specified *m/z* range. **E.** TIC and EIC of purified AMP, LCMS Method 2. **F-G.** Mass spectra associated with the dominant peaks of **D** and **E**, respectively. **H-I.** Analytical HPLC traces of crude and purified peptide, respectively, with the integrated percentage of the dominant peak (retention time in parentheses), HPLC Method 1a.

A.

```
KR-20       ------------------------------------KRIVQRIKDFLRNLVPRTES  20
KS-30       ----------------------------KSKEKIGKEFKRIVQRIKDFLRNLVPRTES  30
RK-31       ----------------------------RKSKEKIGKEFKRIVQRIKDFLRNLVPRTES  31
LL-37       --------------------LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES  37
ALL-38      ------------------ALLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES  38
TLN-58      TLNQARGSFDISCDKDNKRFALLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES  58
LL-23       --------------------LLGDFFRKSKEKIGKEFKRIVQR--------------  23
LL-29       --------------------LLGDFFRKSKEKIGKEFKRIVQRIKDFLR--------  29
KS-27       ----------------------------KSKEKIGKEFKRIVQRIKDFLRNLVPR---  27
                                                 ******
```

B.

```
Histatin-1      DSHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN      38
Histatin-2      -----------RKFHEKHHSHREFPFYGDYGSNYLYDN      27
Histatin-3      DSHAKRHHGYKRKFHEKHHSHRGYR------SNYLYDN      32
Histatin-4      -----------RKFHEKHHSHRGYR------SNYLYDN      21
Histatin-5      DSHAKRHHGYKRKFHEKHHSHRGY--------------      24
Histatin-6      DSHAKRHHGYKRKFHEKHHSHRGYR-------------      25
Histatin-7      -----------RKFHEKHHSHRGY--------------      13
Histatin-8      ------------KFHEKHHSHRGY--------------      12
Histatin-9      -----------RKFHEKHHSHRGYR-------------      14
                           **********
```

C.

```
β Amyloid 1-42      DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA    42
β Amyloid 1-40      DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV--    40
                    ****************************************
```

D.

```
SgI-29      HNKQEGRDHDKSKGHFHRVVIHHKGGKAH--     29
SgII        --KQEGRDHDKSKGHFHMIVIHHKGGQAHHG     29
              *************** :*******:**
```

Supplementary Figure S44: A. Cathelicidin alignment. **B.** Histatin alignment. **C.** β amyloid alignment. **D.** Semenogelin-derived peptide alignment.