Supporting Information for:

C-Terminal Residue of Ultrashort Peptides Impacts on Molecular Self-Assembly, Hydrogelation, and Interaction with Small-Molecule Drugs

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1. Live-Dead assay of peptides 1B, 1C, 2B, and 2C

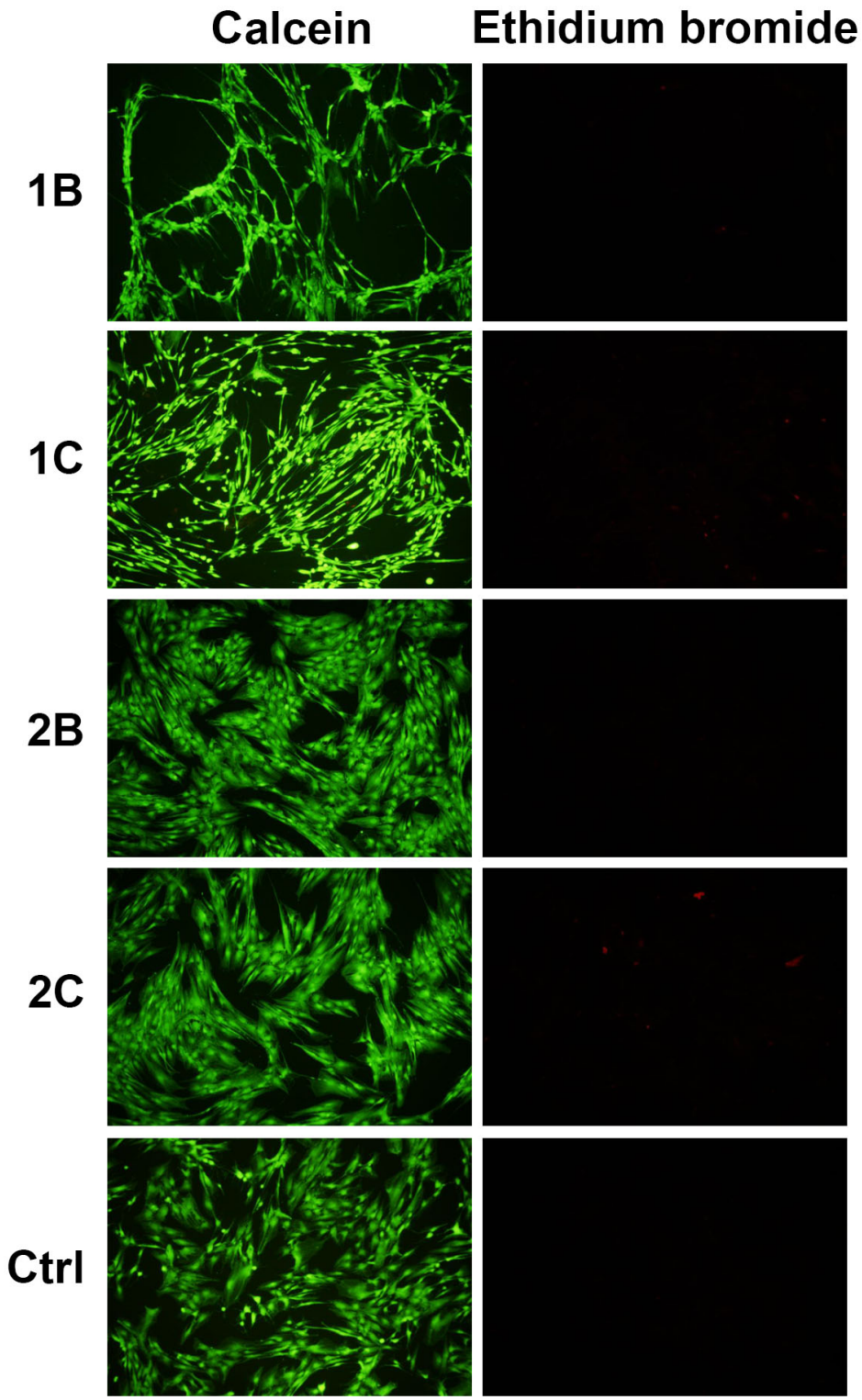


Figure S1. Calcein and ethidium bromide staining of human adipose-derived stem cells (hASCs) in the presence of peptides 1B, 1C, 2B, and 2C. The control refers to no addition of peptides. The calcein fluorescence images indicate that the presence of the peptides do not affect the metabolic activity of the hASCs while the ethidium bromide fluorescence images indicate that the peptides 1B, 1C, and 2B did not compromise the membrane integrity of the hASCs; only peptide 2C led to the entry of a very small amount of ethidium bromide (red fluorescence) into the hASCs.

2. Angular frequency sweep studies of the peptide hydrogels

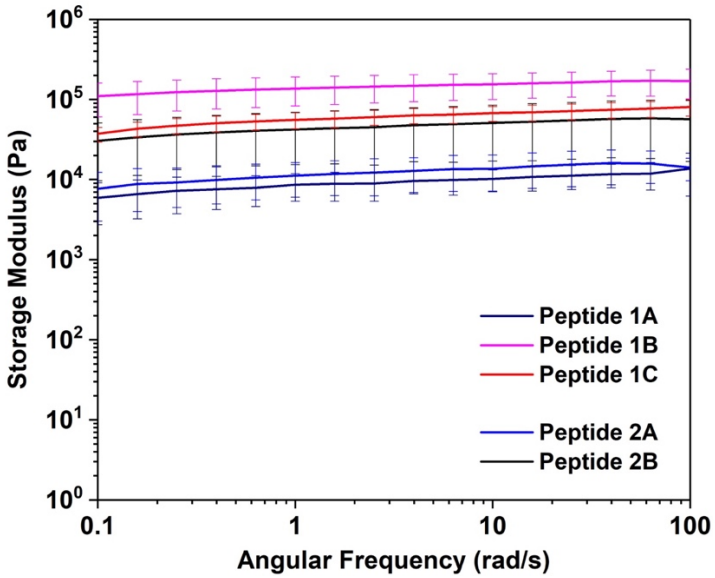


Figure S2. The rheological studies were carried out at a constant strain of 0.1 %. The error bars represent the standard deviation of three separate measurements. The graph shows that despite the consistent backbones, the stiffness of the hydrogels within each group can vary considerably: 7-100 kPa for the hexapeptides (1A, 1B, 1C) and 5-20 kPa for the tripeptides (2A, 2B). This shows that the C-terminal residues influence hydrogelation (i.e. interaction of the peptide fibrils with water) profoundly.

3. Elution of small-molecule drugs (SMDs) from peptide hydrogels

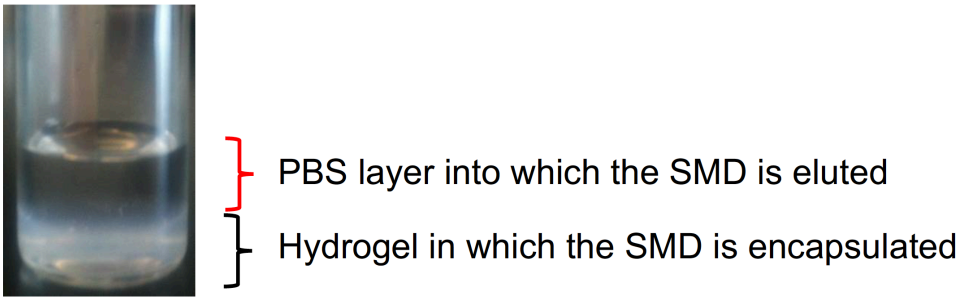


Figure S3. Experimental set-up to measure the rate and extent of elution of various SMDs from various peptide hydrogels.

4. Intra-protofibril binding of doxorubicin by peptide 1B

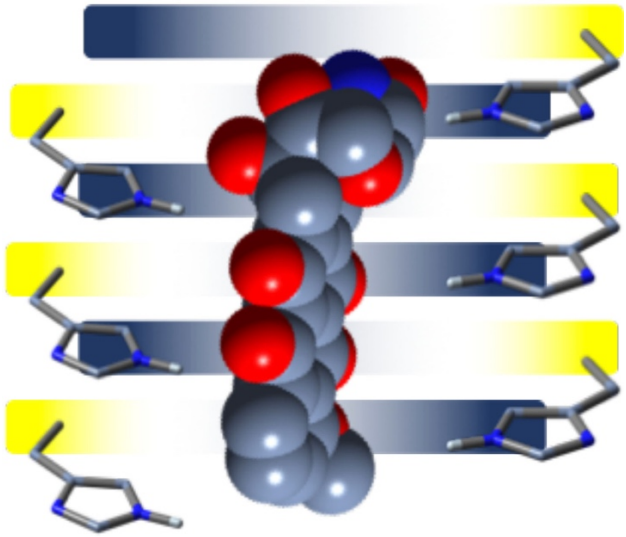


Figure S4. A proposed model of how intra-protofibril binding of doxorubicin by peptide 1B might occur. Except for the imidazole N-H, all other hydrogen atoms on the imidazole side chain of His are removed for clarity. The imidazole N-H can point towards the aromatic faces of doxorubicin and bind the SMD via N-H mediated cation-π interactions.

5. Field emission scanning electron microscopy studies of peptide hydrogel with SMDs

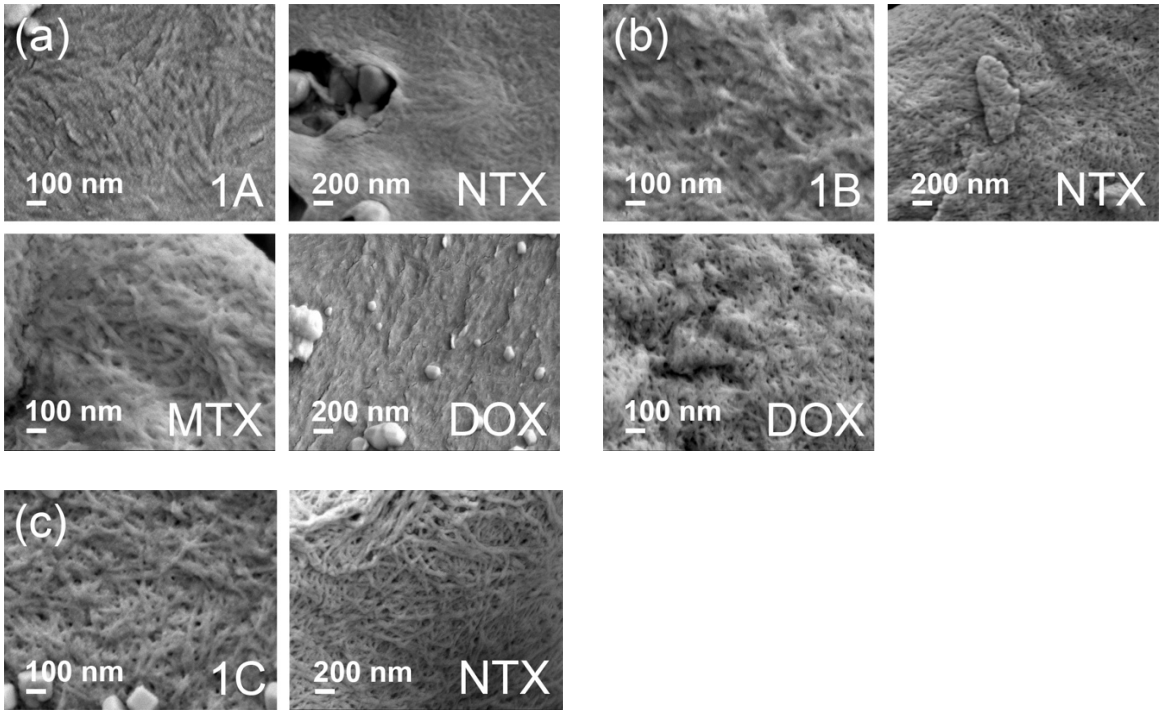
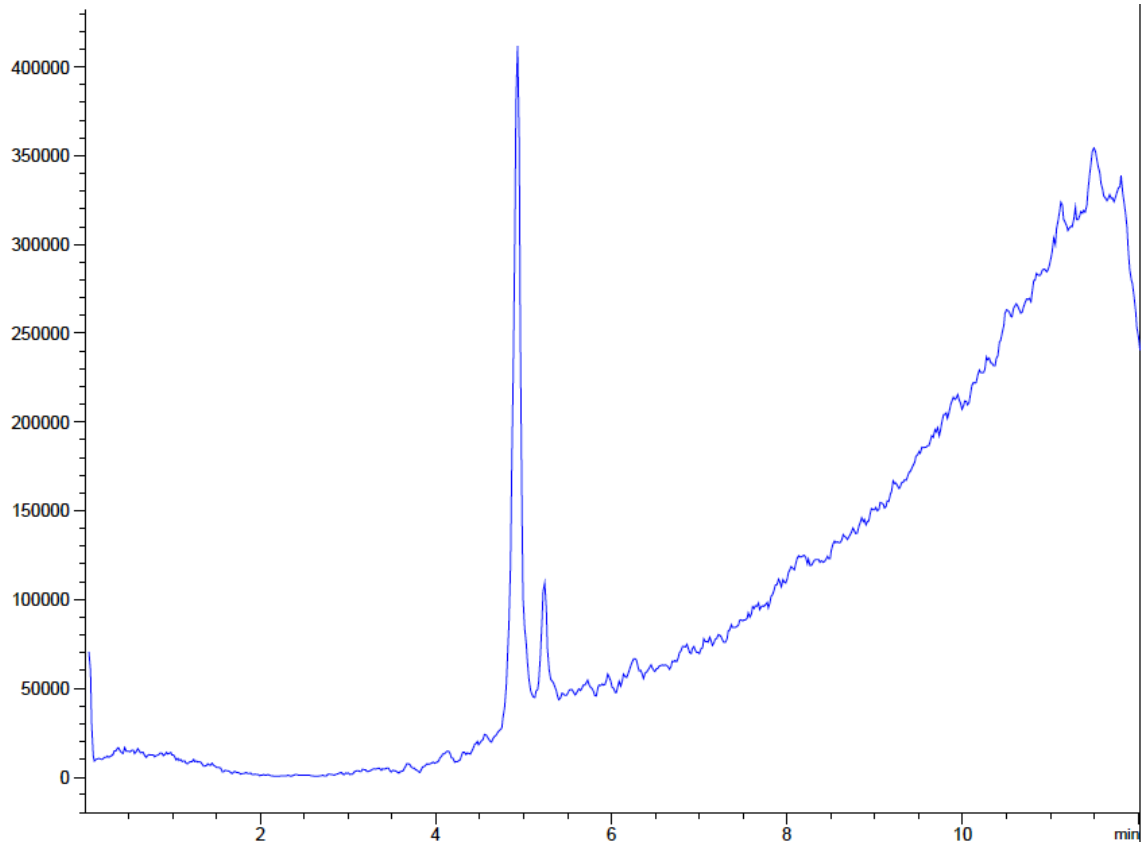
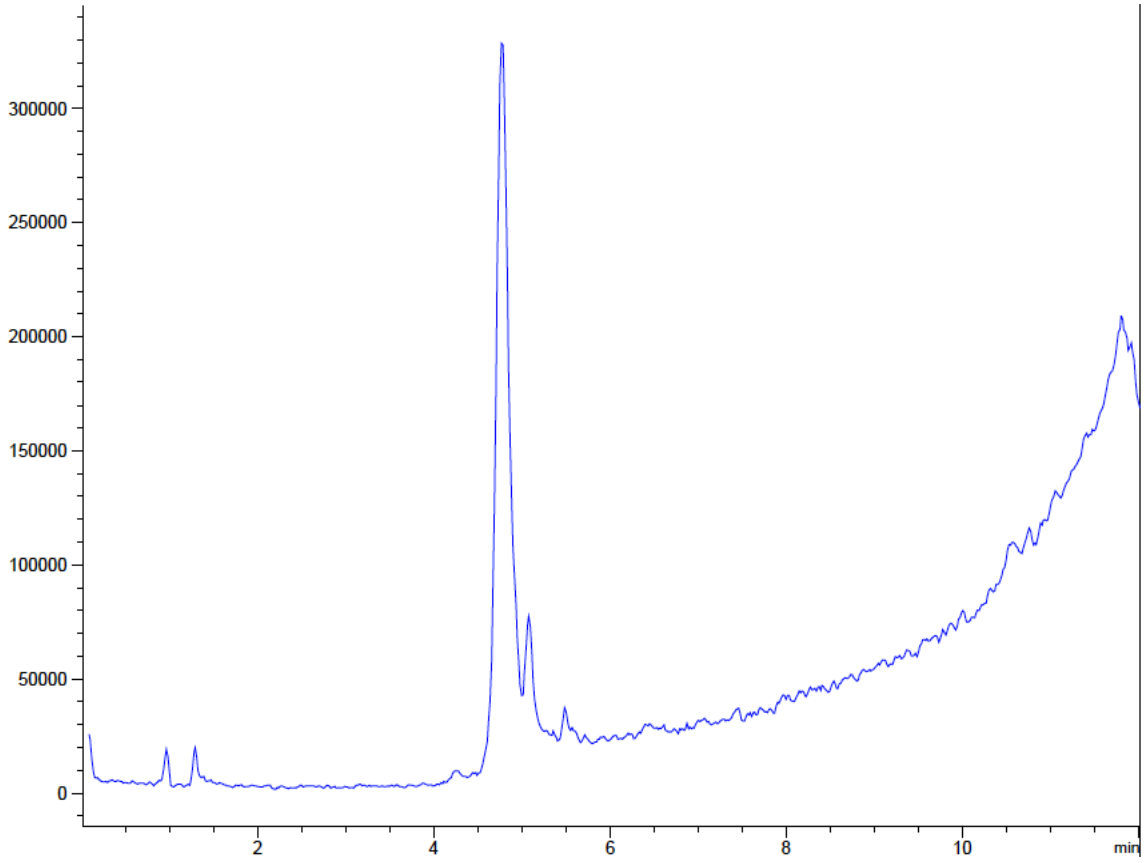


Figure S5. Scanning electron micrographs of various freeze-dried peptide hydrogels in the presence of various SMDs (10 molar %). (a) Peptide 1A hydrogels with naltrexone (NTX), methotrexate (MTX), and doxorubicin (DOX). (b) Peptide 1B with naltrexone (NTX) and doxorubicin (DOX). (c) Peptide 1C hydrogel with naltrexone (NTX). The electron micrographs show that the presence of the SMDs do not significantly affect the morphology of the peptide hydrogels.

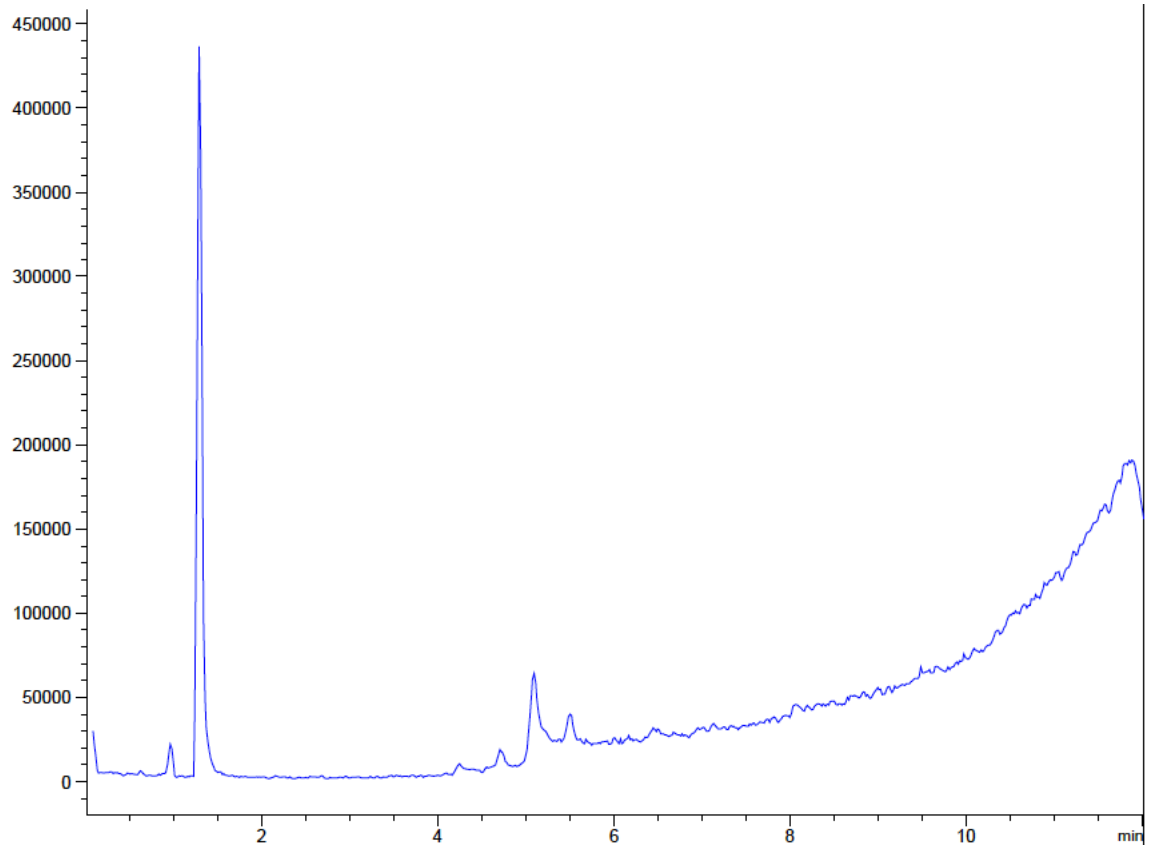
6. High performance liquid chromatography data of new peptides



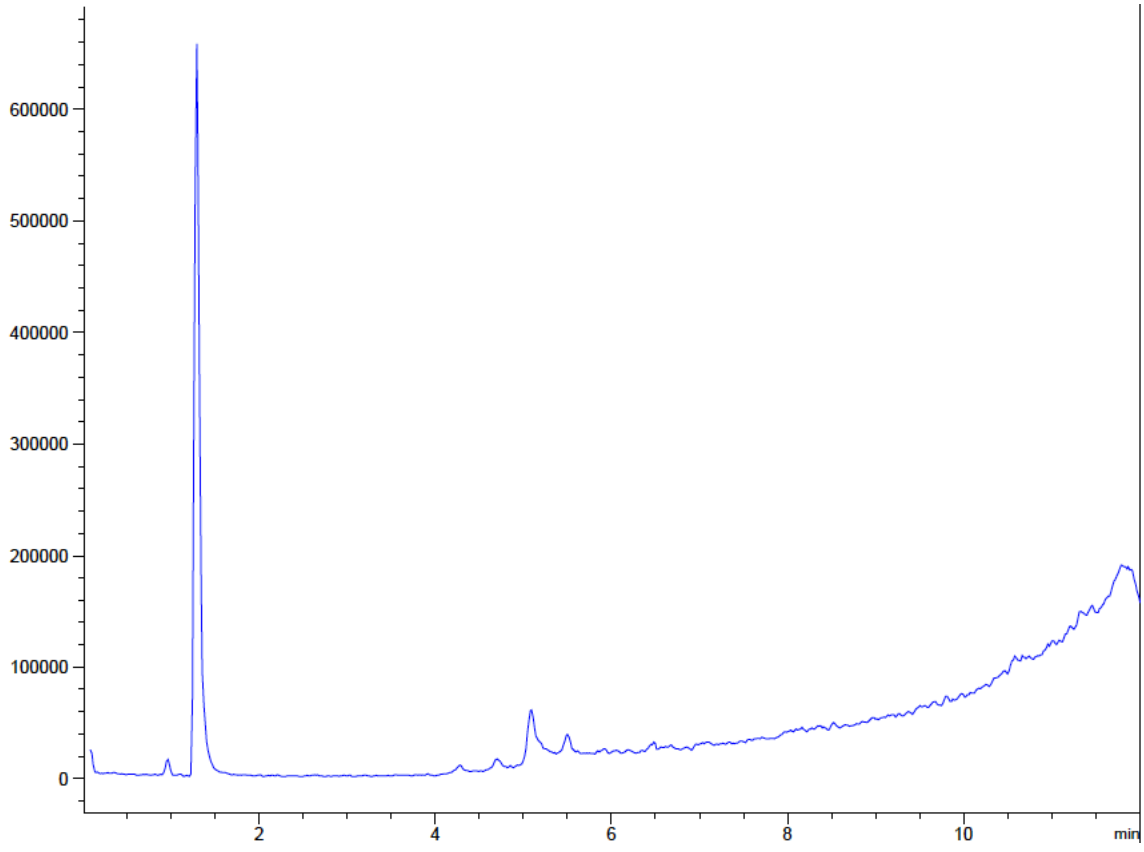
High performance liquid chromatogram of peptide 1B



High performance liquid chromatogram of peptide 1C.

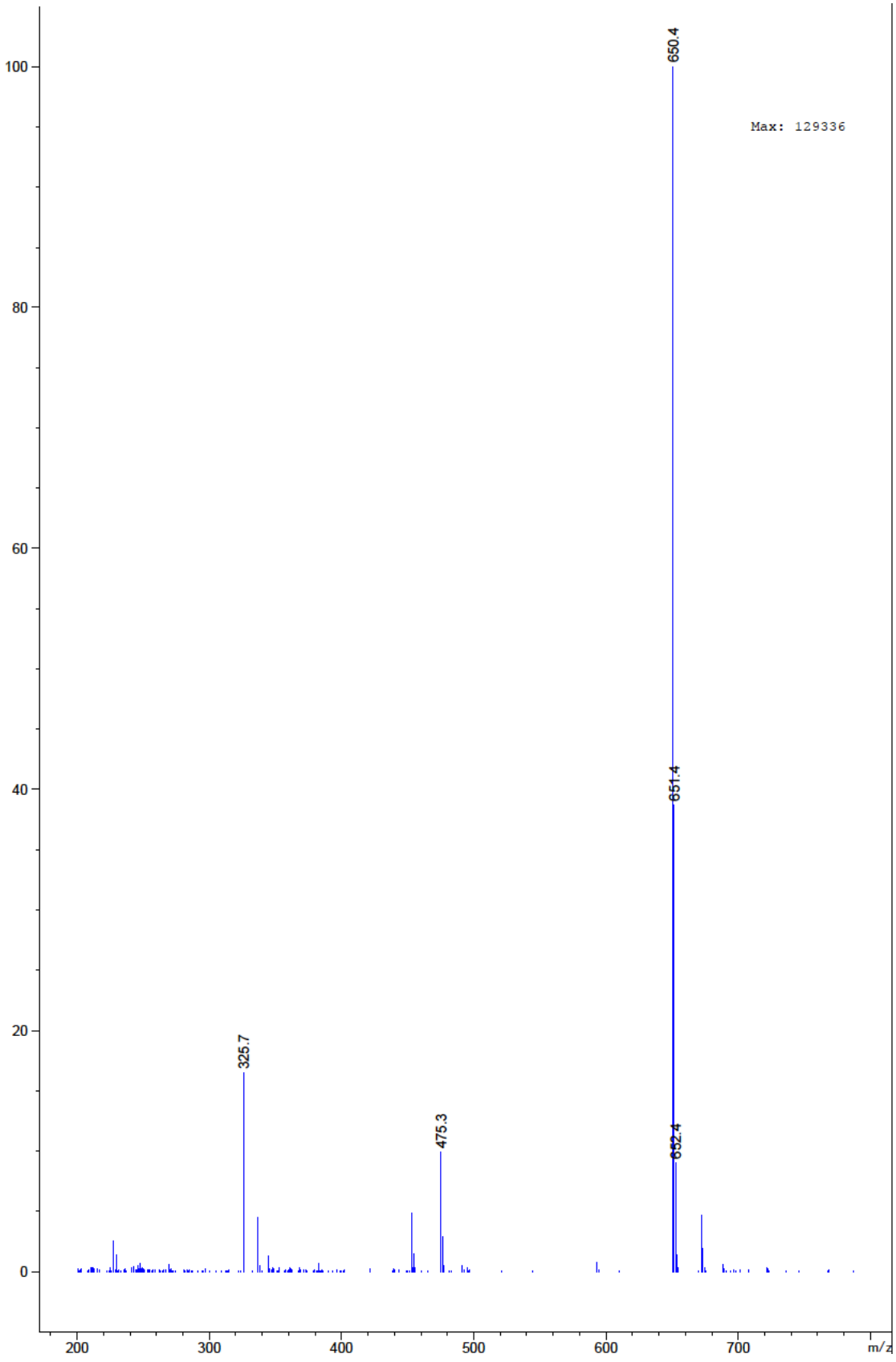


High performance liquid chromatogram of peptide 2B.

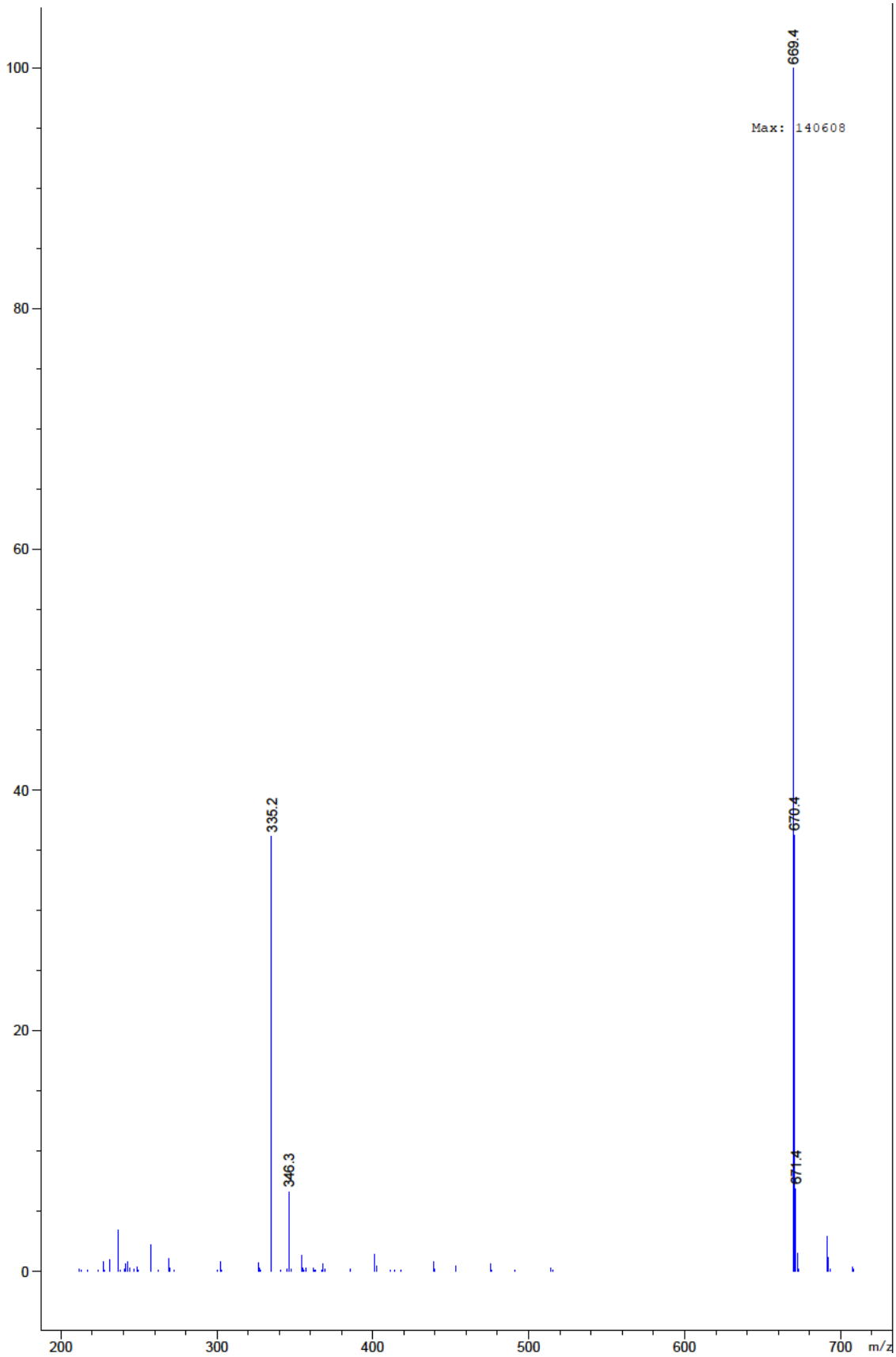


High performance liquid chromatogram of peptide 2C.

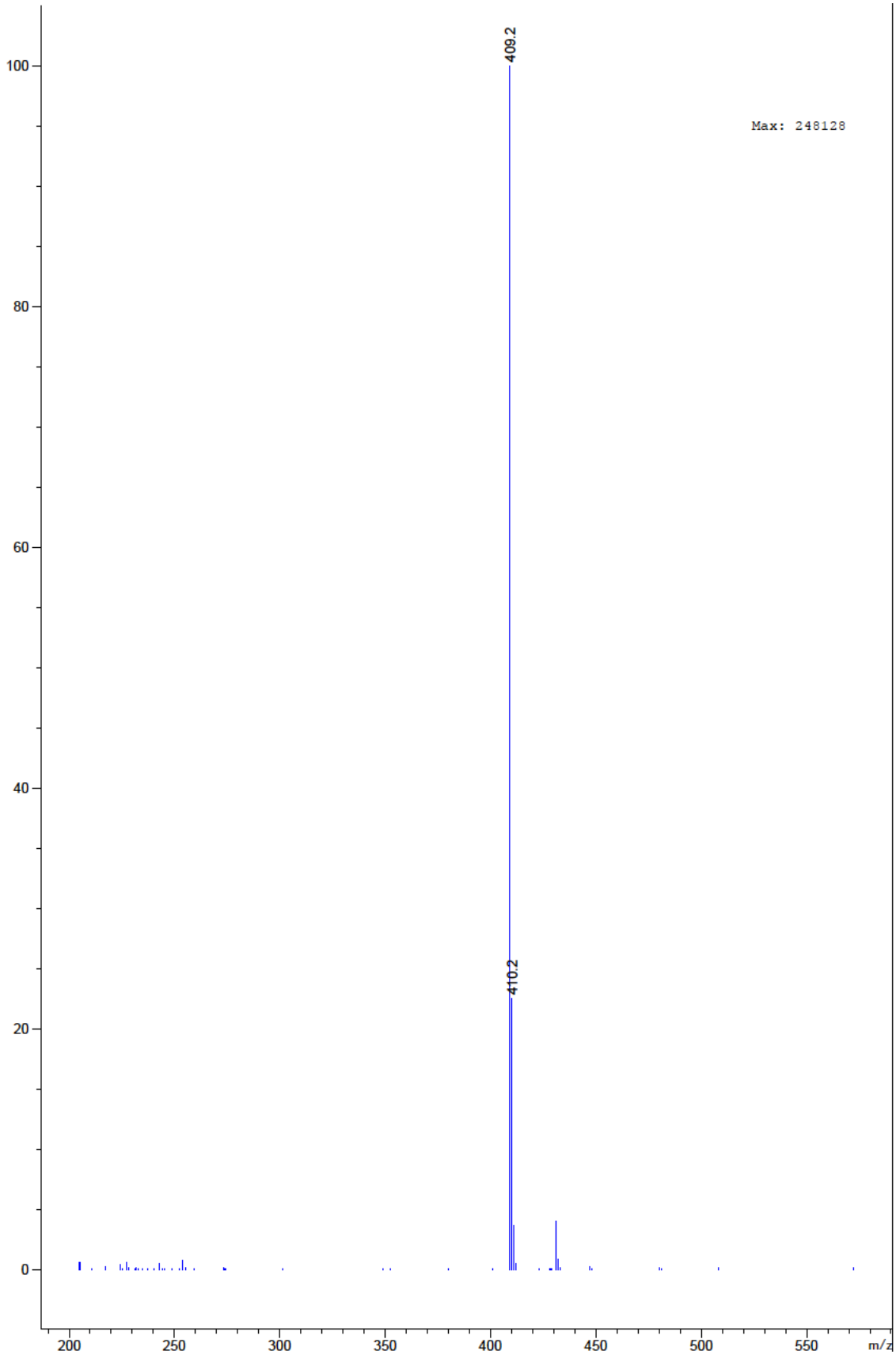
7. High performance liquid chromatography data of new peptides



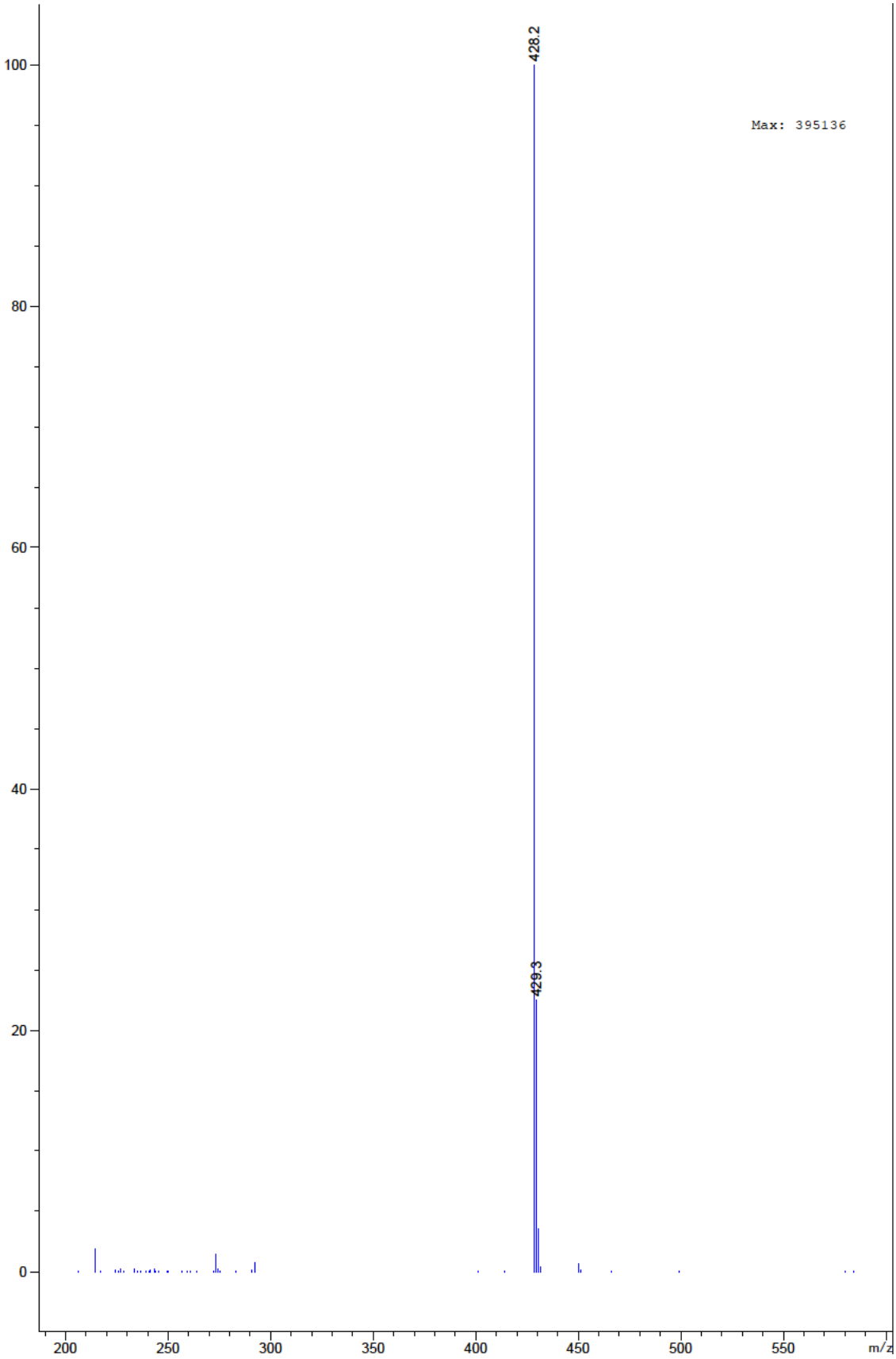
Electrospray ionisation mass spectrum of peptide 1B.
Calctd for $C_{30}H_{51}N_9O_7$, 649.79; found, 650.4 ($[M + H]^+$), 325.7 ($[M + 2H]^{2+}$).



Electrospray ionisation mass spectrum of peptide 1C.
Calctd for $C_{30}H_{56}N_{10}O_7$, 668.83; found, 669.4 ($[M + H]^+$), 335.2 ($[M + 2H]^{2+}$).



Electrospray ionisation mass spectrum of peptide 2B.
Calctd for $C_{19}H_{32}N_6O_4$, 408.50; found, 409.2 ($[M + H]^+$).



Electrospray ionisation mass spectrum of peptide 2C.
Calctd for $C_{19}H_{37}N_7O_4$, 427.54; found, 428.2 ($[M + H]^+$).