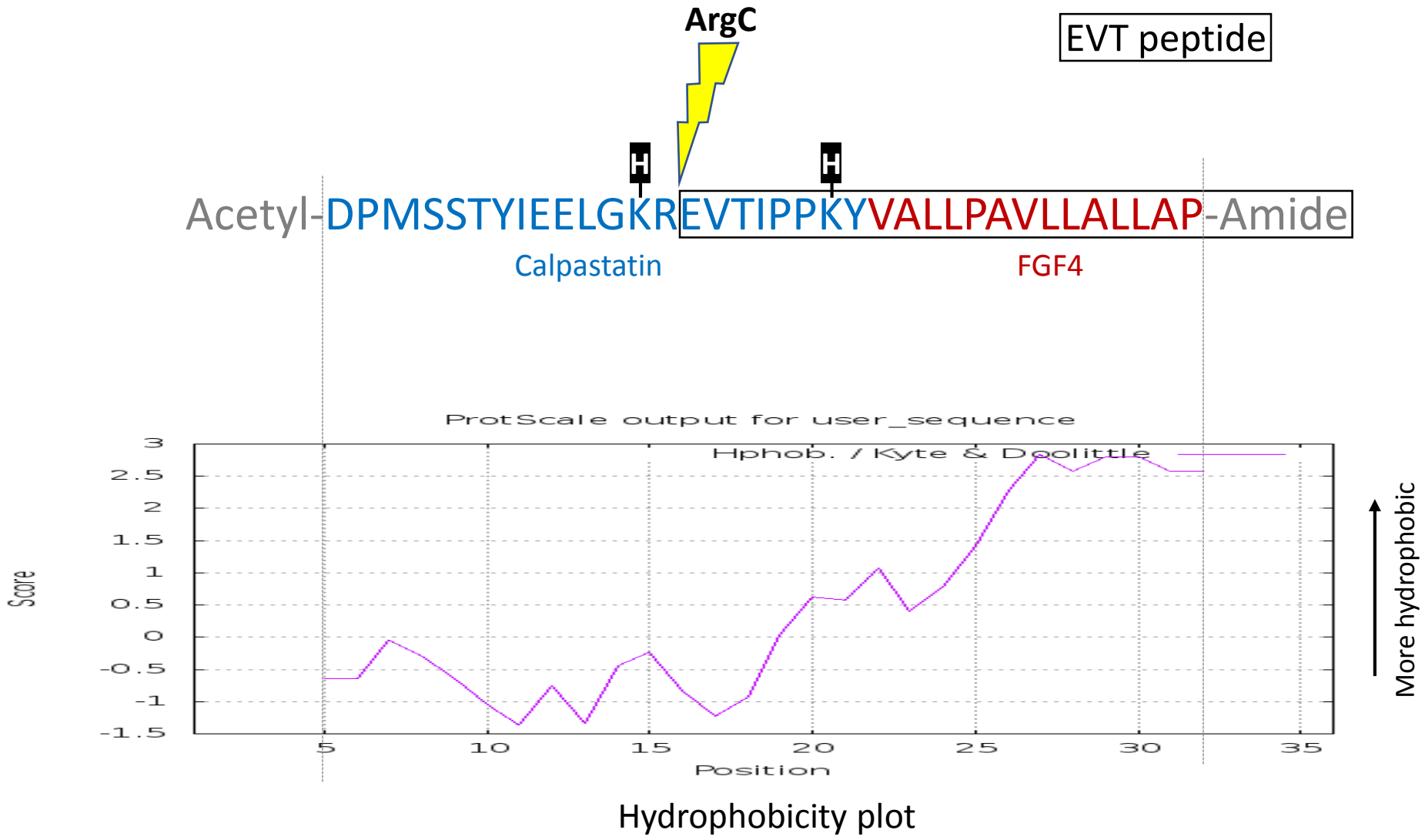
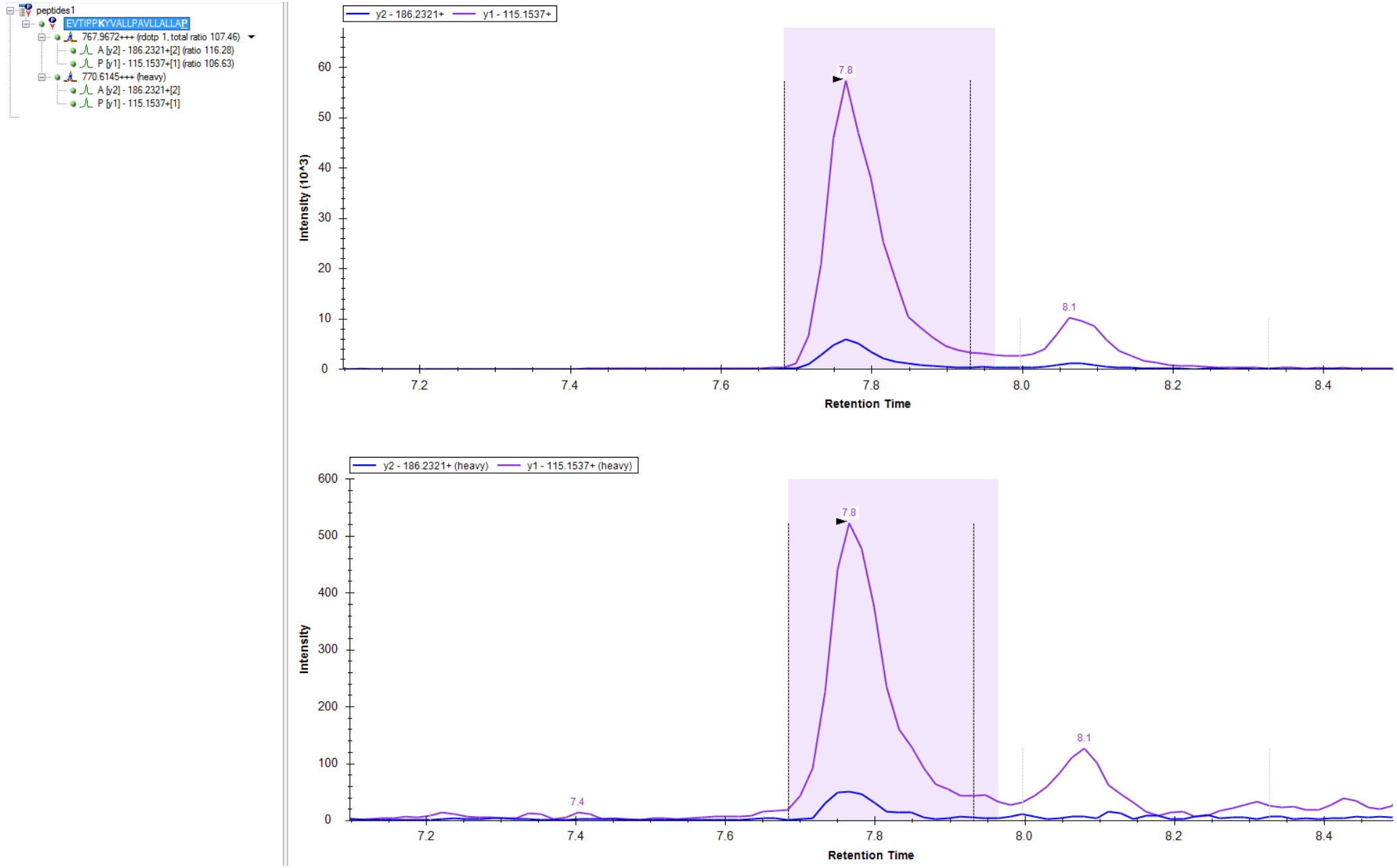


Hydrophobicity plot


peptides1
EVTIPPKYVALLPAVLLALLAP
767.9672+++ (rdotp 1, total ratio 107.46)
A [y2] - 186.2321+[2] (ratio 116.28)
P [y1] - 115.1537+[1] (ratio 106.63)
770.6145+++ (heavy)
A [y2] - 186.2321+[2]
P [y1] - 115.1537+[1]
y2 - 186.2321+
y1 - 115.1537+
Intensity (10^3)
Retention Time
7.8
8.1
y2 - 186.2321+ (heavy)
y1 - 115.1537+ (heavy)
Intensity
Retention Time
7.4
7.8
8.1

Method Development

- The full length peptide could be observed by LC/MS using a 30-90% acetonitrile (ACN) gradient, but it was only stable in solution for about 30 min.
- A tryptic digest was attempted, but trypsin was not ideal since: (i) a short middle peptide; (ii) KR sequence could lead to variability in missed cleavage; and (iii) the C-terminal FGF4 portion does not have heavy label
- It was determined that ArgC digestion would work best since both resulting peptides are a good length for LC/MS and both have a heavy K.
- The C-terminal ArgC peptide (EVT) was chosen since it has a mixture of calpastatin and FGF4 sequences. The N-terminal heavy ArgC peptide could also be observed.
- The EVT peptide is very hydrophobic. It was resuspended in 30% ACN, 0.1% TFA, then run over a 30-95% ACN gradient. It elutes at around 50% ACN. This helps to eliminate background by removing a lot of peptides which elute at lower organic concentrations.
- Both the light and heavy EVT peptides were optimized on the TSQ triple quadrupole, and results were imported into Skyline software for analysis. MS1 for heavy and light are focused in Q1, then 2 transition ions for each peptide are quantified in Q3
- The next step will be to construct a standard curve to determine LOD and LOQ.