

Sample A (5ug) was dissolved in 100 mM of ammonium bicarbonate, reduced/alkylated and digested with 500 ng of trypsin (1:10). Sample B (10 ug) was dissolved in 100 mM of ammonium bicarbonate, reduced/alkylated and digested with 1000 ng trypsin (1:10). Both digests were at 37C for 12 hours. The digests were purified on a C18 column (1mm ID x 10 cm long) and eluted with high acetonitrile.

The purified digests were lyophilized and dissolved (A in 15 uL of 0.2% TFA, B in 30 uL of 0.2% TFA); then 0.5 uL of each was deposited onto a MALDI target for direct peptide analysis.

We examined both spots for overall peptide coverage in a triple-quadrupole reflecting TOF spectrometer. Both spots contained good peptide coverage, with two strong additional peptides noted in the sample B spectrum.

Both samples (10 uL of 15 uL from A, 10 uL of 30 uL from B) were then fractionated using micro-HPLC (water-ACN gradient 0.75% ACN per minute, 0.1% TFA, 3 uL/min flow rate, PepMap™ 100 column) and mixed with matrix, then deposited onto two MALDI targets at 30 second intervals (100 spots each).

For each target, spectra from the 100 spots were acquired on our prototype triple-quadrupole reflecting TOF spectrometer. These were submitted to our in-house peptide mass-fingerprinting tool, which exploits peptide retention time prediction as an additional identification constraint.

In sample A we identified the most prominent protein to be Q15109 (advanced glycosylation end product-specific receptor precursor, human) with observed tryptic peptide coverage of 70% (15 PPM mass accuracy, +-4 minutes observed vs. predicted peptide retention time tolerance). The peptide [CQAMNRDGKETK] (NG->DG) M+H 1437.658 (span 99-110) was manually observed in fraction 38, as was a modified version at M+H 1420.625 was in fraction 45. This was initially missed by our search tool, but was later confirmed once our *in-silico* digestion rules were relaxed.

| MEASURED (intg frac) | COMPUTED ERR | SPAN | RTD | SEQUENCE |
|-----------------------|--------------|--------------|------|--|
| 559.303 (040106 035) | 559.308 -8 | [53 57] | -0.9 | (K)LNTGR(T) |
| 574.309 (122007 064) | 574.311 -4 | [49 52] | +0.9 | (R)LEWK(L) |
| 624.370 (180512 030) | 624.371 0 | [44 48] | -0.2 | (K)KPPQR(L) |
| 633.310 (219686 052) | 633.312 -3 | [58 62] | -0.1 | (R)TEAWK(V) |
| 642.316 (030710 036) | 642.316 0 | [38 43 +] | +2.2 | (K)CKGAPK(K) [Q/C-17] RTC: 15.8->18.9 |
| 659.344 (037551 028) | 659.342 2 | [38 43 +] | -1.8 | (K)CKGAPK(K) |
| 761.291 (152918 046) | 761.295 -4 | [99 104] | +4.4 | (R)CQAMNR(N) [Q/C-17] RTC: 18.6->23.9 |
| 778.318 (157891 037) | 778.321 -4 | [99 104] | -0.1 | (R)CQAMNR(N) |
| 781.437 (307809 046) | 781.444 -9 | [222 228] | -2.3 | (R)TAPIQPR(V) |
| 867.534 (351089 073) | 867.543 -10 | [30 37] | -0.6 | (R)IGEPLVLK(C) |
| 977.581 (001567 037) | 977.577 4 | [40 48 +] | +1.1 | (K)GAPKPPQR(L) |
| 1002.542 (008430 043) | 1002.546 -3 | [170 178 +] | +2.3 | (K)GVSVKEQTR(R) |
| 1061.436 (067548 045) | 1061.438 -1 | [99 107 +] | +4.3 | (R)CQAMNRNGK(E) [N+0.98 Q/C-17] RTC: 18.2->21.4 |
| 1078.468 (057011 036) | 1078.465 3 | [99 107 +] | -0.2 | (R)CQAMNRNGK(E) [N+0.98] |
| 1115.602 (006564 073) | 1115.609 -5 | [49 57 +] | +2.2 | (R)LEWKLNTGR(T) |
| 1121.663 (006682 057) | 1121.667 -3 | [219 228 +] | +0.3 | (R)ALRTAPIQPR(V) |
| 1155.665 (002321 069) | 1155.668 -2 | [30 39 +] | +0.4 | (R)IGEPLVLKCK(G) |
| 1174.608 (035125 059) | 1174.609 -1 | [53 62 +] | -0.1 | (K)LNTGRTEAWK(V) |
| 1180.672 (014270 065) | 1180.672 0 | [44 52 +] | -0.2 | (K)KPPQRLEWK(L) |
| 1196.582 (001320 038) | 1196.578 2 | [105 114 ++] | +2.1 | (R)NGKETKSNYR(V) [N+0.98] |
| 1419.618 (037195 045) | 1419.623 -3 | [99 110 ++] | +6.3 | (R)CQAMNRNGKETK(S) [N+0.98 Q/C-17] RTC: 16.2->19.8 |
| 1436.649 (034692 038) | 1436.650 0 | [99 110 ++] | +2.8 | (R)CQAMNRNGKETK(S) [N+0.98] |
| 1524.750 (370329 074) | 1524.768 -12 | [63 77] | +1.3 | (K)VLSPQGGPWSVAR(V) |
| 1533.881 (002156 064) | 1533.878 2 | [40 52 ++] | +3.3 | (K)GAPKPPQRLEWK(L) |
| 1721.962 (001685 071) | 1721.969 -3 | [44 57 ++] | +1.5 | (K)KPPQRLEWKLNTGR(T) |
| 1730.899 (001447 079) | 1730.910 -6 | [49 62 ++] | +1.2 | (R)LEWKLNTGRTEAWK(V) |
| 1933.890 (335360 076) | 1933.910 -10 | [199 216] | -0.8 | (R)GGDPRPTFSCSFSPGLPR(H) |
| 2126.079 (095898 093) | 2126.083 -1 | [180 198] | -0.2 | (R)HPETGLFTLQSELMVTPAR(G) |
| 2140.074 (003896 085) | 2140.070 1 | [58 77 +] | +1.2 | (R)TEAWKVLSPQGGPWSVAR(V) |

| | | | | |
|-----------------------|--------------|--------------|------|--|
| 2141.082 (001057 087) | 2141.054 13 | [58 77 +] | +2.2 | (R)TEAWKVLSPQGGPWDSVAR(V) [Q+0.98] |
| 2227.059 (001070 072) | 2227.070 -5 | [199 218 +] | -0.6 | (R)GGDPRPTFSCSFSPGLPRHR(A) |
| 2241.203 (008193 108) | 2241.216 -5 | [78 98] | +2.2 | (R)VLPNGSLFLPAVGIQDEGIFR(C) |
| 2242.198 (041911 109) | 2242.200 0 | [78 98] | +2.7 | (R)VLPNGSLFLPAVGIQDEGIFR(C) [N+0.98] |
| 2282.188 (040972 092) | 2282.184 1 | [179 198 +] | +0.3 | (R)RHPETGLFTLQSELMVTPAR(G) |
| 2511.302 (101598 081) | 2511.322 -7 | [117 140] | -1.0 | (R)VYQIPGKPEIVDSASELTAGVPNK(V) |
| 2567.276 (001225 079) | 2567.293 -6 | [199 221 ++] | +1.7 | (R)GGDPRPTFSCSFSPGLPRHRALR(T) |
| 2681.374 (001044 085) | 2681.367 2 | [53 77 ++] | +2.0 | (K)LNTGRTEAWKVLSPQGGPWDSVAR(V) |
| 2766.461 (001232 081) | 2766.491 -10 | [115 140 +] | -1.2 | (R)VRVYQIPGKPEIVDSASELTAGVPNK(V) |
| 2796.421 (001940 089) | 2796.434 -4 | [175 198 ++] | +1.9 | (K)EQTRRHPETGLFTLQSELMVTPAR(G) |
| 3097.516 (039472 084) | 3097.518 0 | [141 169] | +1.0 | (K)VGTVCVSEGSYPAGTLSWHLDGKPLVPNEK(G) |
| 4198.077 (000226 093) | 4198.084 -1 | [179 216 ++] | -1.7 | (R)RHPETGLFTLQSELMVTPARGDPRPTFSCSFSPGLPR(H) |
| 4199.110 (000497 093) | 4199.068 10 | [179 216 ++] | -1.7 | (R)RHPETGLFTLQSELMVTPARGDPRPTFSCSFSPGLPR(H) [Q+0.98] |
| 4333.158 (000214 094) | 4333.132 5 | [274 314] | +3.3 | (K)DGVPLPLPPSPVLILPEIGPQDQGTYSVCVATHSSHGPPQESR(A) [Q+0.98] |
| 4860.484 (004297 103) | 4860.451 6 | [229 273] | +0.1 | (R)VWEPVPLEEVQLVVEPEGGAVAPGGTVTLTCEVPAQPSQIHWMK(D) |
| 5590.833 (000880 089) | 5590.829 0 | [117 169 +] | -2.1 | (R)VYQIPGKPEIVDSASELTAGVPNKVGTVCVSEGSYPAGTLSWHLDGKPLVPNEK(G) |

COVERAGE: 70 percent

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0001 MAAGTAVGAWVLVLSLWGAVVGAQNITAR*****
0061 *****
0121 *****
0181 *****
0241 *****
0301 *****AVSISIIIEPGEEGPTAGSVGSGLGTLLALALGILGGLGTAALLIGV
0361 ILWQRRQRGEERKAPENQEEEEERAEINQSEEPAGESSTGGP
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Sample A also contained three prominent ribosomal proteins from *E. coli* (P0A7K8 @ 45% coverage, P0AA45 @ 61% coverage and P60440 @ 41% coverage).

Sample B was shown to contain the same prominent proteins as Sample A. To explore the differences between the two samples, the mass-hydrophobicity values for all peaks from were compared using both custom software tools and direct user observation of the spectra. The two prominent peptide peaks unique to Sample B that were seen in the non-fractionated spectra appeared as (M+H 1731.862 in fraction 71, intensity integral 315148) and (M+H 1764.941 in fraction 107, intensity integral 104930). These peaks were subjected to MS/MS analysis on our spectrometer and *denovo* sequenced by hand.

For M+H 1764.941 we *denovo* sequenced the peptide [VWEPVPLEEVQLVVE] which is a truncated (span 229-243) version of a tryptic peptide observed in Q15109 (span 229-273). For M+H 1731.862 we *denovo* sequenced the peptide [VYQIPGKPEIVDSASE] which is a truncated (span 117-132) version of a tryptic peptide observed in Q15109 (span 117-140).

Both of these prominent peptide peaks were also fingerprint-confirmed using the “broken peptide” feature of our search software, which permutes through all possible amino acid substrings in a candidate sequence regardless of possible cleavage points. The retention comparison constraint was relaxed from 4 minutes to 5 minutes to account for current limitations in predicting the hydrophobicity of non-tryptic peptides:

| Observed Intensity | frac | theoretical | PPM | span | RT-diff | Sequence |
|--------------------|------|-------------|-----|-----------|---------|------------------------|
| 1730.854 (315148) | 071 | 1730.873 | -10 | [117 132] | -1.0 | (R)VYQIPGKPEIVDSASE(L) |
| 1763.933 (104930) | 107 | 1763.934 | 0 | [229 243] | +4.8 | (R)VWEPVPLEEVQLVVE(P) |

Based on this work sample B appears to be a mixture of a full version and two truncated versions of Q15109, with terminations at location 243 and 132.

Sample A (5ug) was dissolved in 100 mM of ammonium bicarbonate, reduced/alkylated and digested with 500 ng of trypsin (1:10). Sample B (10 ug) was dissolved in 100 mM of ammonium bicarbonate, reduced/alkylated and digested with 1000 ng trypsin (1:10). Both digests were at 37C for 12 hours. The digests were purified on a C18 column (1mm ID x 10 cm long) and eluted with high acetonitrile.

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For each target, spectra from the 100 spots were acquired on our prototype triple-quadrupole reflecting TOF spectrometer. These were submitted to our in-house peptide mass-fingerprinting tool, which exploits peptide retention time prediction as an additional identification constraint.

In sample A we identified the most prominent protein to be Q15109 (advanced glycosylation end product-specific receptor precursor, human) with observed tryptic peptide coverage of 70% (15 PPM mass accuracy, +-4 minutes observed vs. predicted peptide retention time tolerance). The post-translational modifications observed included deamidation (N+0.98 / Q+0.98) and acetylation (Q/C-17).

The peptide [CQAMNRDGKETK] (NG->DG) M+H 1437.658 (span 99-110) was manually observed in fraction 38. The acetylated version at M+H 1420.625 was also observed in fraction 45. This was initially missed by our search tool, but was later confirmed once our *in-silico* digestion rules were relaxed.

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| 633.310 (219686 052) | 633.312 -3 | [58 62] | -0.1 | (R)TEAWK(V) |
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| 977.581 (001567 037) | 977.577 4 | [40 48 +] | +1.1 | (K)GAPKPPQR(L) |
| 1002.542 (008430 043) | 1002.546 -3 | [170 178 +] | +2.3 | (K)GVSVKEQTR(R) |
| 1061.436 (067548 045) | 1061.438 -1 | [99 107 +] | +4.3 | (R)CQAMNRNGK(E) [N+0.98 Q/C-17] RTC: 18.2->21.4 |
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| 1115.602 (006564 073) | 1115.609 -5 | [49 57 +] | +2.2 | (R)LEWKLNTGR(T) |
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| 1419.618 (037195 045) | 1419.623 -3 | [99 110 ++] | +6.3 | (R)CQAMNRNGKETK(S) [N+0.98 Q/C-17] RTC: 16.2->19.8 |
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| 1524.750 (370329 074) | 1524.768 -12 | [63 77] | +1.3 | (K)VLSPPQGGPWSVAR(V) |
| 1533.881 (002156 064) | 1533.878 2 | [40 52 ++] | +3.3 | (K)GAPKPPQRLWK(L) |
| 1721.962 (001685 071) | 1721.969 -3 | [44 57 ++] | +1.5 | (K)KPPQRLWKLNTGR(T) |
| 1730.899 (001447 079) | 1730.910 -6 | [49 62 ++] | +1.2 | (R)LEWKLNTGRTEAWK(V) |
| 1933.890 (335360 076) | 1933.910 -10 | [199 216] | -0.8 | (R)GGDRPTFSCSFSPGLPR(H) |

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| 2126.079 (095898 093) | 2126.083 -1 | [180 198] | -0.2 | (R)HPETGLFTLQSELMVTPAR(G) |
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| 2282.188 (040972 092) | 2282.184 1 | [179 198 +] | +0.3 | (R)RHPETGLFTLQSELMVTPAR(G) |
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| 2567.276 (001225 079) | 2567.293 -6 | [199 221 ++] | +1.7 | (R)GGDPRPTFSCSFSPGLPRHRALR(T) |
| 2681.374 (001044 085) | 2681.367 2 | [53 77 ++] | +2.0 | (K)LNTGRTEAWKVLSPQGGGPWDSVAR(V) |
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| 2796.421 (001940 089) | 2796.434 -4 | [175 198 ++] | +1.9 | (K)EQTRRHPTGLFTLQSELMVTPAR(G) |
| 3097.516 (039472 084) | 3097.518 0 | [141 169] | +1.0 | (K)VGTCVSEGSYPAGTLSWHLDGKPLVPNEK(G) |
| 4198.077 (000226 093) | 4198.084 -1 | [179 216 ++] | -1.7 | (R)RHPETGLFTLQSELMVTPARGDPRPTFSCSFSPGLPR(H) |
| 4199.110 (000497 093) | 4199.068 10 | [179 216 ++] | -1.7 | (R)RHPETGLFTLQSELMVTPARGDPRPTFSCSFSPGLPR(H) [Q+0.98] |
| 4333.158 (000214 094) | 4333.132 5 | [274 314] | +3.3 | (K)DGVPLPLPPSPVLIILPEIGPQDQGTYSVCVATHSSHGPEESR(A) [Q+0.98] |
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| 5590.833 (000880 089) | 5590.829 0 | [117 169 +] | -2.1 | |

(R)VYQIPGKPEIVDSASELTAGVFNKVGTCVSEGSYPAGTLSWHLDGKPLVPNEK(G)
 COVERAGE: 70 percent
 0001 MAAGTAVGAWVLVLSLWGAVVGAQNITAR*****
 0061 *****
 0121 *****
 0181 *****
 0241 *****
 0301 *****AVSISIIIEPGEEGPTAGSVGGSLGTLALALGILGGLGTAALLIGV
 0361 ILWQRQRERGEERKAPENQEEEEERAEINQSEEPAGESSTGGP

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Both of these prominent peptide peaks were confirmed using the “broken peptide” feature of our search software, which permutes through all possible amino acid substrings in a candidate sequence regardless of possible cleavage points. The retention comparison constraint was relaxed from 4 minutes to 5 minutes to account for current limitations in predicting the hydrophobicity of non-tryptic peptides:

| Observed Intensity | frac | theoretical | PPM | span | RT-diff | Sequence |
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| 1730.854 (315148 | 071) | 1730.873 | -10 | [117 132] | -1.0 | (R)VYQIPGKPEIVDSASE(L) |
| 1763.933 (104930 | 107) | 1763.934 | 0 | [229 243] | +4.8 | (R)VWEPVPLEEVQLVVE(P) |

Based on our work, sample B appears to be a mixture of full and two truncated versions of Q15109, with terminations at location 243 and 132.