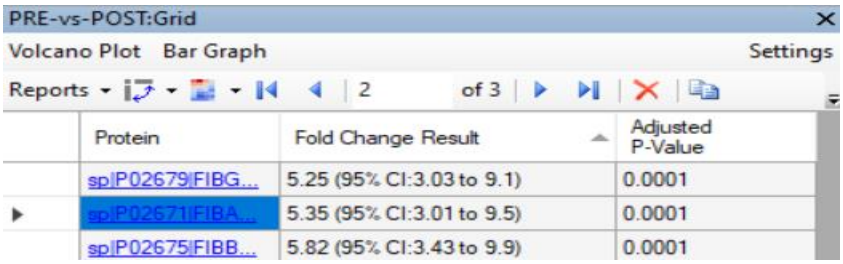
PIERRE WENSEL: PROTEOMICS PRACTICAL




| Protein | Fold Change Result | Adjusted P-Value |
|---|---|---|
| sp\|P02679\|FIBG... | 5.25 (95% CI:3.03 to 9.1) | 0.0001 |
| sp\|P02671\|FIBA... | 5.35 (95% CI:3.01 to 9.5) | 0.0001 |
| sp\|P02675\|FIBB... | 5.82 (95% CI:3.43 to 9.9) | 0.0001 |

1.The effect of the experimental drug was to increase fibrinogen protein levels in patients by up to 5.82x. This was a statistically significant increase with adjusted p-value<0.0001

2. Yes, I first used a library built with the neural network prediction tool PROSIT, which advantageously precludes the need to perform experiments to obtain the MSMS library which is useful when heavy peptides are not available as in large-scale DIA projects. Subsequently, only the precursors (with charge of 2 and 3) and transitions that have been identified in the library were displayed. I later can build an MS/MS spectral library from experimental data (search engine output file is in pep.xml format and the raw data in the standard mzXML format) obtained from a mixture of the target peptides of interest isotopically labelled (heavy peptides) which have been acquired in the mass spectrometer in DDA mode and have been searched against a protein database for identification. Spectra from both can be mirrored.

3.I used 90 transitions to quantify each 18 precursors for 5 peptides of 3 proteins. This quantification was enabled by activation of PROSIT spectral library. Only the precursors and transitions identified in the library are displayed.

4. The coefficient of variation (CV) of the peak area for each peptide in each group indicates good reproducibility between replicates when it is at low values (<20%). Based on my histogram for “heavy” setting, the CV for peptide K.VAQLEA...was extremely high (81%). With “no normalization” setting, that of peptide R.HPDEAA... is well below 20% at 9% for one control PRE-condition vs. POST-condition.

5. We are targeting specific peptides with PRM (parallel reaction monitoring) whereas DIA collects data from wide, evenly-spaced precursor isolation windows. Parallel Reaction Monitoring enables qualitative and quantitative analysis through secondary full scans, exhibiting strong resistance to interference and high resolution in complex backgrounds. Synthetic standard peptides can be selectively incorporated as needed, saving experimental costs

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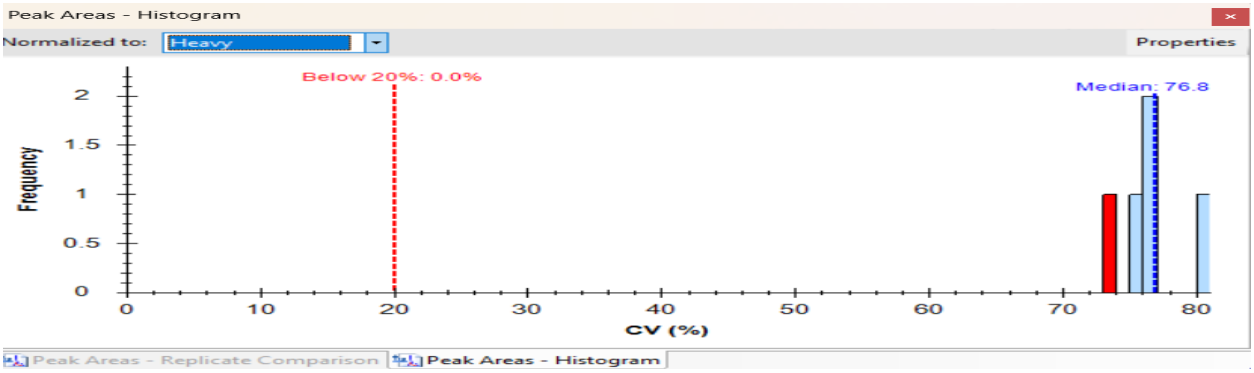


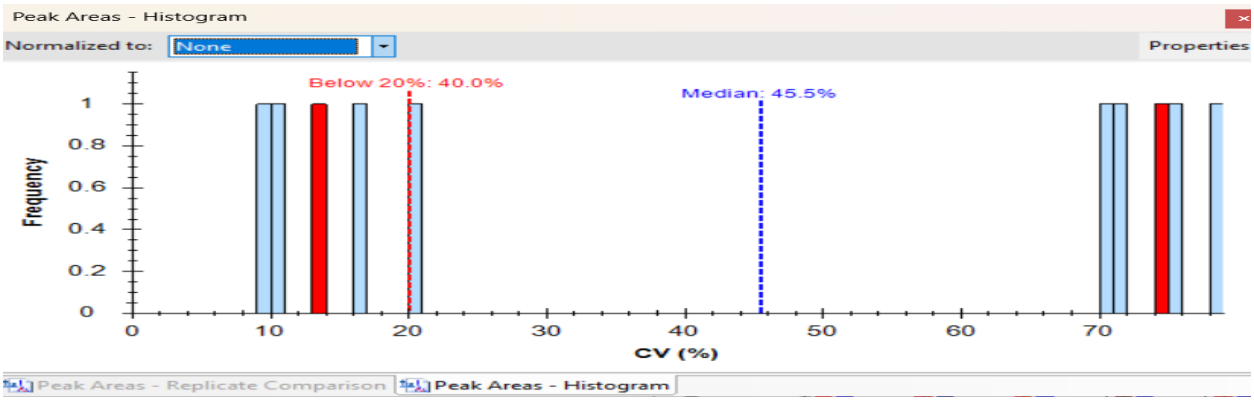


| Peptide | Protein | Replicate | Peptide Peak Found Ratio | Peptide Retention Time | Ratio To Standard | Quantification |
|---|---|---|---|---|---|---|
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | POST_REP1 | 0.5 | 40.94 | 49.3823 | 493.8229 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | POST_REP2 | 0.5 | 41.17 | 34.8128 | 348.1282 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | POST_REP3 | 0.5 | 40.93 | 26.3942 | 263.9415 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | POST_REP4 | 0.5 | 41.05 | 29.8667 | 298.6667 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | POST_REP5 | 0.5 | 40.97 | 36.3135 | 363.135 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | PRE_REP1 | 0.5 | 41.31 | 3.8337 | 38.3373 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | PRE_REP2 | 0.5 | 41.77 | 3.1977 | 31.9772 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | PRE_REP3 | 0.5 | 41.53 | 15.0102 | 150.1016 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | PRE_REP4 | 0.5 | 41.55 | 7.5185 | 75.1846 fmol |
| HPDEAAFFDTASTGK | sp\|P02671\|FIBA_HUMAN | PRE_REP5 | 0.5 | 41.39 | 5.9531 | 59.5311 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | POST_REP1 | 0.5 | 21.04 | 705.1334 | 7051.3342 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | POST_REP2 | 0.5 | 21.33 | 662.3981 | 6623.9811 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | POST_REP3 | 0.5 | 21.23 | 385.3939 | 3853.9387 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | POST_REP4 | 0.5 | 21.36 | 568.0972 | 5680.9725 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | POST_REP5 | 0.5 | 21.36 | 748.4415 | 7484.4154 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | PRE_REP1 | 0.5 | 21.53 | 91.5872 | 915.8722 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | PRE_REP2 | 0.5 | 21.48 | 63.3751 | 633.7512 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | PRE_REP3 | 0.5 | 21.83 | 160.8562 | 1608.5624 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | PRE_REP4 | 0.5 | 21.38 | 139.3115 | 1393.115 fmol |
| ESSSHHPGIAEFPSR | sp\|P02671\|FIBA_HUMAN | PRE_REP5 | 0.5 | 21.68 | 124.502 | 1245.0203 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | POST_REP1 | 1 | 59.31 | 46.9288 | 469.2882 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | POST_REP2 | 1 | 59.63 | 40.9065 | 409.0655 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | POST_REP3 | 1 | 59.44 | 50.0206 | 500.206 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | POST_REP4 | 1 | 59.39 | 42.318 | 423.1796 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | POST_REP5 | 1 | 59.27 | 43.0401 | 430.4006 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | PRE_REP1 | 1 | 59.86 | 5.6366 | 56.3661 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | PRE_REP2 | 1 | 59.78 | 4.4357 | 44.3573 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | PRE_REP3 | 1 | 59.64 | 16.2284 | 162.2838 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | PRE_REP4 | 1 | 59.69 | 9.8097 | 98.0968 fmol |
| HQLYIDETVNSNIPTNLR | sp\|P02675\|FIBB_HUMAN | PRE_REP5 | 1 | 59.76 | 6.6938 | 66.9378 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | POST_REP1 | 0.5 | 37.31 | 664.3157 | 6643.1574 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | POST_REP2 | 0.5 | 37.64 | 452.0409 | 4520.4094 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | POST_REP3 | 0.5 | 37.59 | 350.5555 | 3505.5549 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | POST_REP4 | 0.5 | 37.6 | 419.3267 | 4193.2672 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | POST_REP5 | 0.5 | 37.6 | 450.0322 | 4500.3218 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | PRE_REP1 | 0.5 | 38.05 | 67.9395 | 679.3945 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | PRE_REP2 | 0.5 | 38.14 | 51.8583 | 518.583 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | PRE_REP3 | 0.5 | 38.29 | 175.4244 | 1754.2442 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | PRE_REP4 | 0.5 | 38.04 | 102.9552 | 1029.5516 fmol |
| YEASILTHDSSIR | sp\|P02679\|FIBG_HUMAN | PRE_REP5 | 0.5 | 38.15 | 81.2699 | 812.6991 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | POST_REP1 | 0.5 | 22.98 | 615.4501 | 6154.5011 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | POST_REP2 | 0.5 | 23.32 | 400.5629 | 4005.6286 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | POST_REP3 | 0.5 | 23.34 | 265.6409 | 2656.4092 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | POST_REP4 | 0.5 | 23.25 | 338.828 | 3388.2803 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | POST_REP5 | 0.5 | 23.3 | 359.357 | 3593.5697 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | PRE_REP1 | 0.5 | 23.54 | 51.4211 | 514.2115 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | PRE_REP2 | 0.5 | 23.46 | 44.9138 | 449.1377 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | PRE_REP3 | 0.5 | 23.77 | 159.3392 | 1593.3919 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | PRE_REP4 | 0.5 | 23.5 | 87.6849 | 876.8491 fmol |
| VAQLEAQCQEPCK | sp\|P02679\|FIBG_HUMAN | PRE_REP5 | 0.5 | 23.77 | 58.6816 | 586.8163 fmol |