Supporting Information

MdFDIA: A Mass Defect Based Four-Plex Data-Independent Acquisition Strategy for Proteome Quantification

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Contents of SI Materials

Liquid chromatography-mass spectrometry.

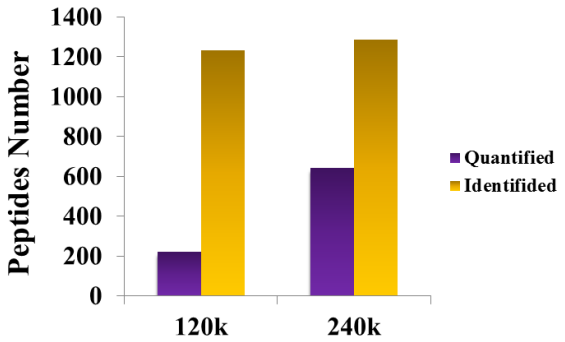
Lysine Isotope Incorporation Analysis. $^{13}C_6{}^{15}N_2$- lysine and D_8- lysine labeled proteins were extracted from MCF-7 cells, digested with Lyc-C, and mixed at a ratio of 1:1 by weight. Mixed samples were resuspended in 98:2 buffer A [98% water, 2% acetonitrile, 0.1% formic

acid]:buffer B[2% water,98% acetonitrile, 0.1% formic acid], and analyzed using EASY-nLC system (Thermo Fisher Scientific, San Jose, CA) coupled to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA). All experiments were performed using the C18 columns which were fabricated in-house with C18 particles (75 μm × 150 mm, 3 μm, 120 Å, ReproSil-pur 120 C18-AQ, Dr. Maisch). And the column was kept in RT during all experiments. After loading onto the column with a flow rate of 0.8 μL/min, peptides were eluted using following gradient: from 2-25% buffer B 110 min, from 25-35% buffer B 20min, from 35-90% buffer B 4min which was held for 5 min, from 90-2% 10 min. The survey scan (350-1800 m/z) was analyzed in the Orbitrap at a resolution of 240 K. k. For all DDA experiments, tandem mass scans were collected at top speed mode with 3 s cycles and the dynamic exclusion duration was 60 s. Precursors were isolated with an isolation window of 1.6 m/z, fragmented, and analyzed in the Orbitrap at a resolving power of 15 K in centroid mode. AGC targets were 4×10^5 for tandem MS acquisition.

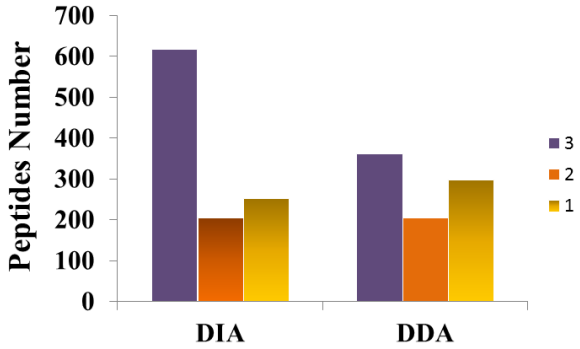
DDA-MS² Quantification. For the comprehensive assessment of quantification efficacy for DDA-MS²- based quantification and MdFDIA, four different isotope labeled samples were mixed at a ratio of 1:1:1:1 by weight. Samples were resuspended in 97:3 buffer A [98% water, 2% acetonitrile, 0.1% formic acid]:buffer B[2% water,98% acetonitrile,0.1% formic acid], and then were loaded onto column in buffer A [98% water, 2% acetonitrile, 0.1% formic acid] at a flow rate of 0.8 μL/min. Peptides gradient elution was performed as follows: an increase from 3% to 8% B[2% water,98% acetonitrile,0.1% formic acid] over 3 min, followed by a 133 min linear gradient to 38% B, followed by a 10 min 1inear gradient to 100% B and held for 3 min. All the following analyses were performed using the gradient above. DDA-MS² quantification was analyzed using EASY-nLC system (Thermo Fisher Scientific, San Jose, CA) coupled to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Full MS scans (m/z 350–1800) were performed in the Orbitrap at a resolution of 120 K (m/z 200), and the AGC targets were 4×10^5. Peptides were isolated at 1.6 m/z with the quadrupole, subjected to HCD fragmentation (NCE of 32%), and analyzed in the Orbitrap at a resolution 240 K (m/z 200). AGC targets were 4×10^5 for tandem MS acquisition.

MdFDIA Quantification. Before DIA experiment, spectral library was constructed first within DDA mode. DDA analysis consisted of full MS scan (m/z 350–1800) performed in the Orbitrap at a resolution of 120 K (m/z 200). Maximum injection time was 50 ms, and AGC targets were 4×10^5. Precursors were isolated at 1.6 m/z with the quadrupole, subjected to HCD fragmentation (NCE of 32%). Tandem mass scans were performed in the Orbitrap at a resolution of 15 K in order to identify peptides/proteins as many as possible. AGC targets were 4×10^5. After DDA experiment, DIA experiment was performed. Each DIA analysis consisted of two interleaved scan experiments, a survey scan (350-1800 m/z) which was analyzed in the Orbitrap at a resolution of 120 K, followed by a series of tandem scans (HCD,NCE of 32%) performed in the Orbitrap at a resolution of 240 K (350-2000 m/z), with the AGC target of 5×10^5 and maximum injection time of 50 ms. The data type was centroid. Variable DIA isolation windows were performed as previously described.[1] For the optimization of resolution, we analyzed ten discrete ranges in total: 349.5-450.5 m/z,

449.5-510.5 m/z, 509.5-570.5 m/z, 569.5-630.5 m/z, 629.5-690.5 m/z, 689.5-750.5 m/z, 749.5-810.5 m/z, 809.5-890.5 m/z, 889.5-1010.5 m/z, 1009.5-1250.5 m/z. For the standard MCF-7 experiments, we analyzed ten discrete ranges in total: 367-456 m/z, 456-508 m/z, 508-549 m/z, 549-595 m/z, 595-633 m/z, 633-675 m/z, 675-722 m/z, 722-790 m/z, 790-908 m/z, 908-1171 m/z. For the quantitative proteomes of four different breast cancer cell lines, we analyzed ten discrete ranges in total: 350-483 m/z, 483-540 m/z, 540-586 m/z, 586-625 m/z, 625-666 m/z, 666-709 m/z, 709-758 m/z, 758-816 m/z, 816-894 m/z, 894-1060 m/z.



Supplementary Figure 1. The number of quantified peptides under different MS^2 resolution.



Supplementary Figure 2. Bar graphs illustrate the number of MCF-7 peptides that were quantified across one, two, or all three of the replicate DIA and DDA-MS^2 experiments.

Reference

[1]Zhang, Y.; Bilbao, A.; Bruderer, T.; Luban, J.; Strambio-De-Castillia, C.; Lisacek, F.; Hopfgartner, G.; Varesio, E. *J. Proteome Res.***2015**,*14*,4359-4371.