

Supplementary Information for
Integrated Platform with Combination of Online Digestion and ^{18}O Labeling for
Proteome Quantification via Immobilized Trypsin Microreactor

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Figure S1. Evaluation of the back-exchange of ^{18}O labeling. After labeling using IMER, samples were analyzed immediately (A) and again after 1 week at room temperature (B) by MALDI-TOF-MS. The amino acid sequences of peptide A, B depicted in all figure are YLYEIAR and LGEYGFQNALIVR.

Figure S2. The LC-MS/MS spectrum of the platform.

(A) The TIC MS spectrum of three LC-MS/MS analysis of the yeast sample analyzed by the platform

(B) A typical MS spectrum of yeast sample

Figure S3. Evaluation of the quantitative accuracy and reproducibility of the integrated platform by 100 ng ^{18}O and ^{16}O online labeled yeast sample at a mixing ratio of 1:1

(C) The observed \log_2 (H/L) ratios of proteins quantified in all of three technical replicates.

(D) The RSD distribution of the ratios of proteins quantified in all three technical replicates.

Figure S4. Box plots showing the ratios measured at mixing ratios of 1:1, 1:5 and 1:10.

Figure S5. The \log_2 (H/L) ratios distribution of peptides with or without miss-cleavage sites from the first run of 1:1 mixed yeast sample.

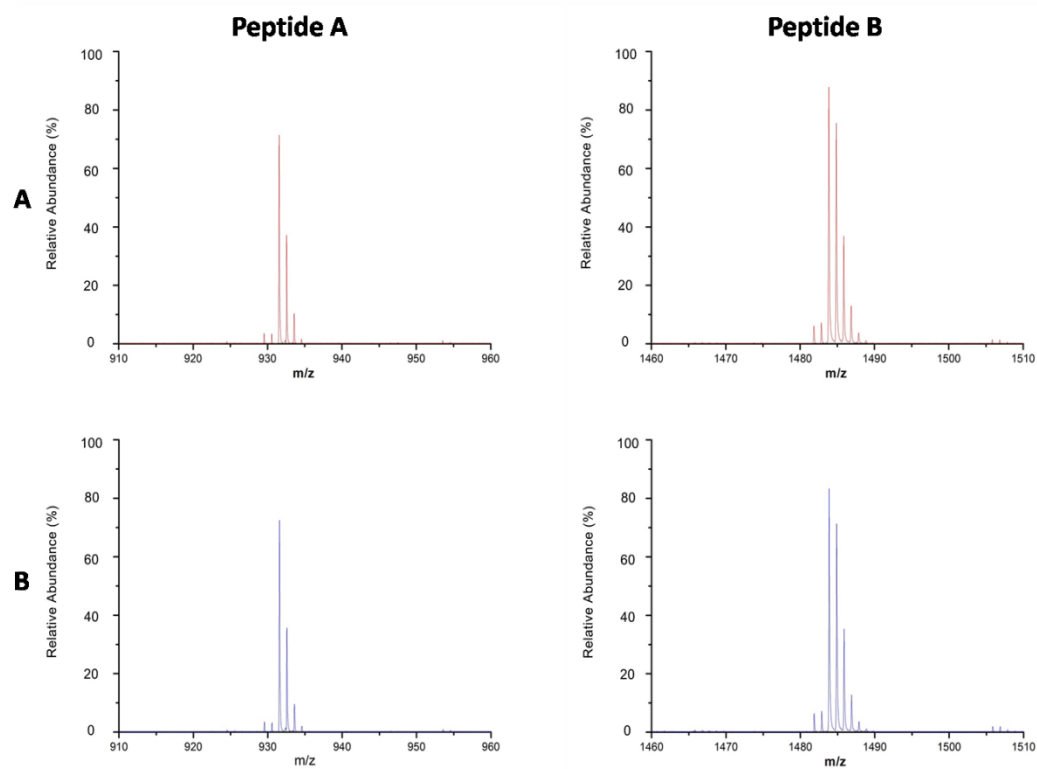


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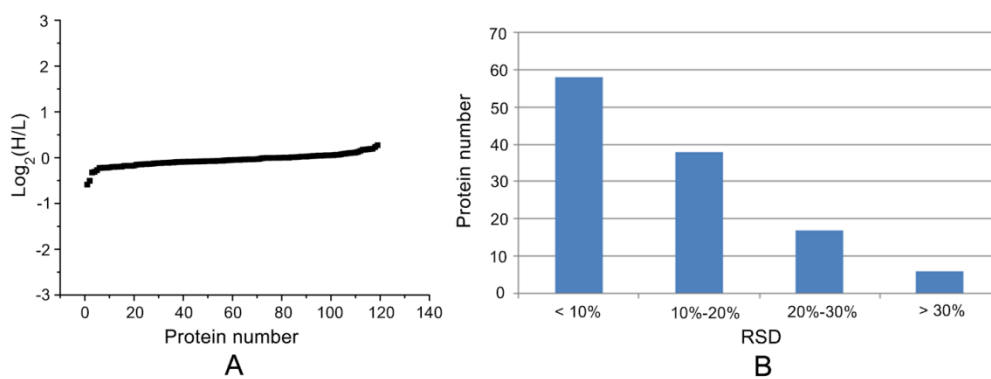


Figure S3. Evaluation of the quantitative accuracy and reproducibility of the integrated platform by 100 ng ^{18}O and ^{16}O online labeled yeast sample at a mixing ratio of 1:1

(A) The observed $\log_2(H/L)$ ratios of proteins quantified in all of three technical replicates.

(B) The RSD distribution of the ratios of proteins quantified in all three technical replicates.

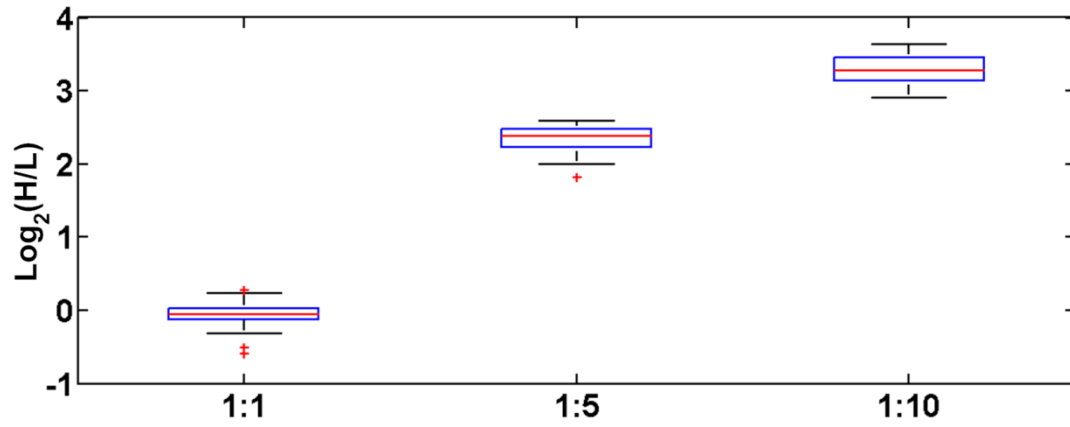


Figure S4. Box plots showing the ratios measured (box and whiskers) at mixing ratios of 1:1, 1:5 and 1:10.

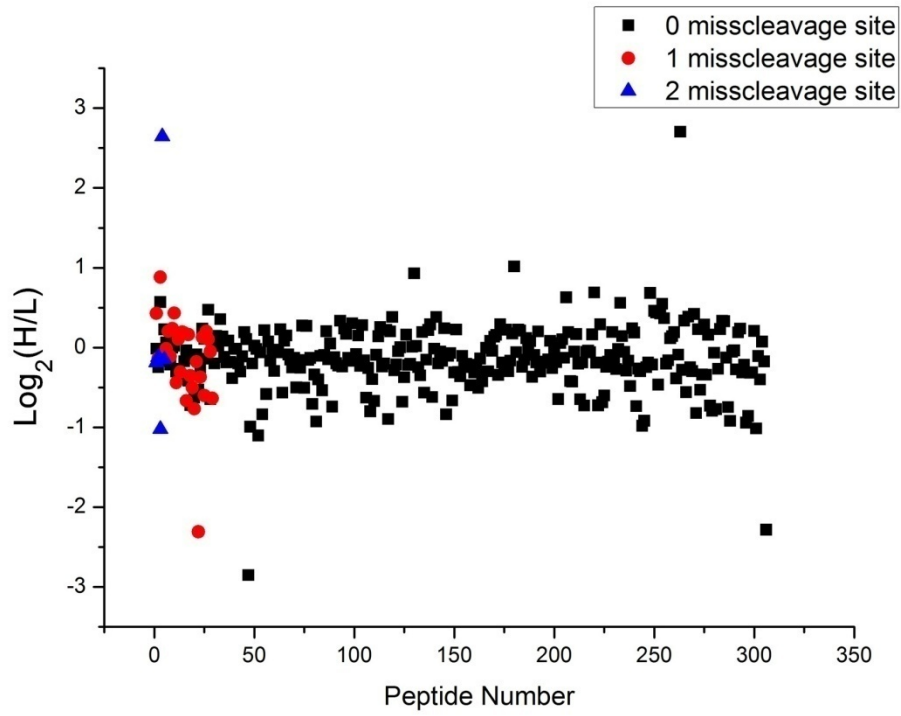


Figure S5. The $\log_2(H/L)$ ratios distribution of peptides with or without misscleavage sites from the first run of 1:1 mixed yeast sample.

Table S1. Differentially expressed proteins in Hca-F and Hca-P cell lines.

IPI	Protein Name	Ratio (Hca-F/Hca-P)
IPI00124700	Transferrin receptor protein 1	2.47 ± 0.13
IPI00123639	Calreticulin	2.44 ± 0.04
IPI00123281	Leucine-rich repeat-containing protein 59	2.27 ± 0.02
IPI00129519	Brain acid soluble protein 1	2.18 ± 0.06
IPI00126072	Synaptic vesicle membrane protein VAT-1 homolog	2.16 ± 0.03
IPI00279079	Fibrinogen beta chain	0.41 ± 0.02
IPI00553798	Ahnak protein	0.39 ± 0.01
IPI00469114	Hemoglobin subunit alpha	0.33 ± 0.03
IPI00885793	Fibrinogen A-alpha-chain	0.27 ± 0.01
IPI00122312	Fibrinogen gamma chain	0.21 ± 0.02