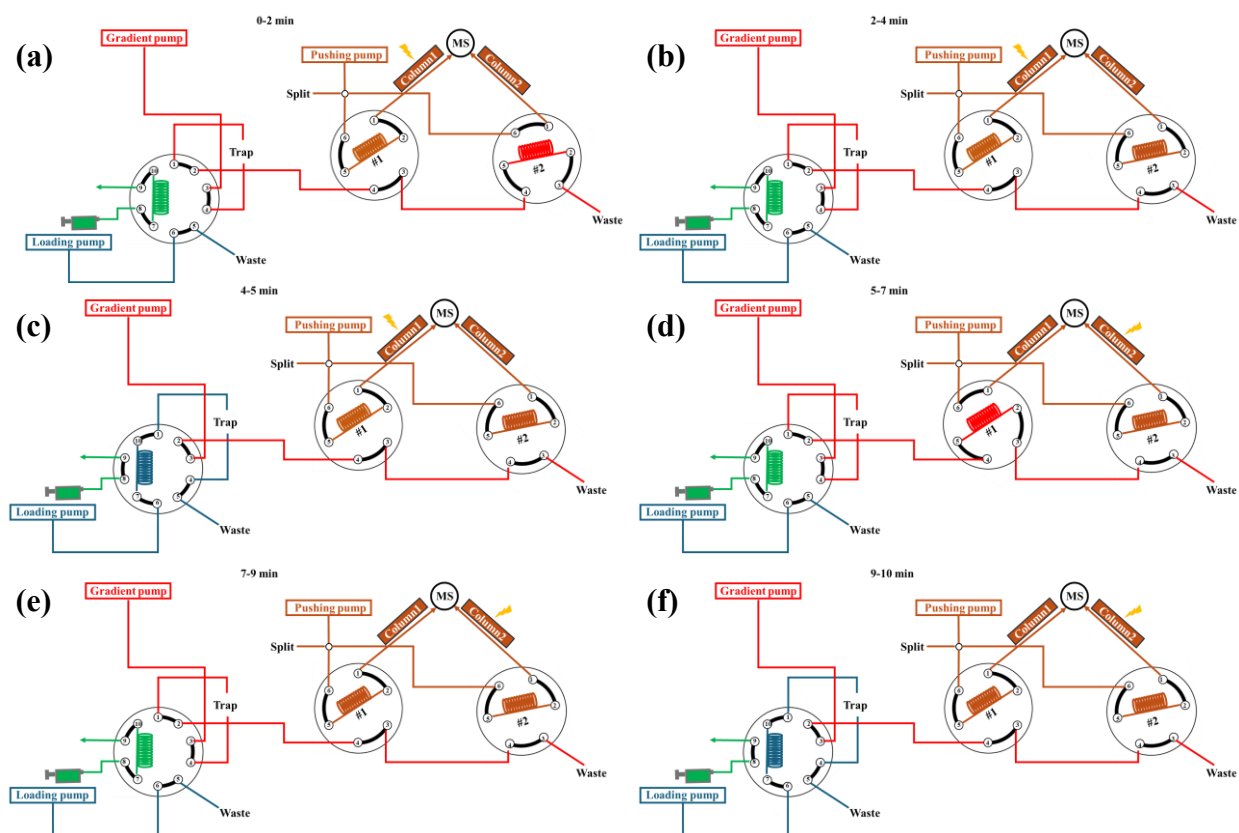
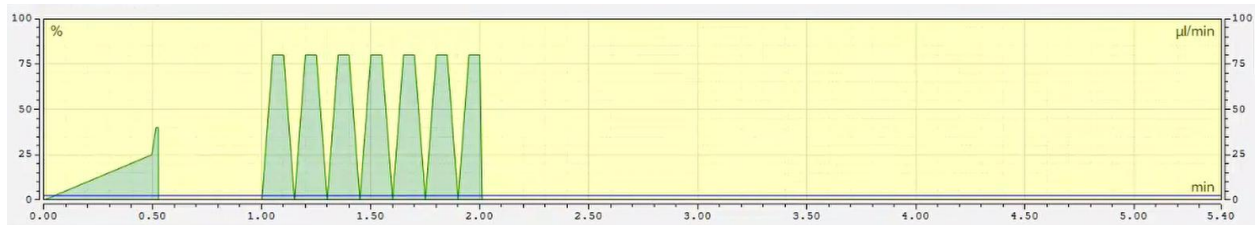
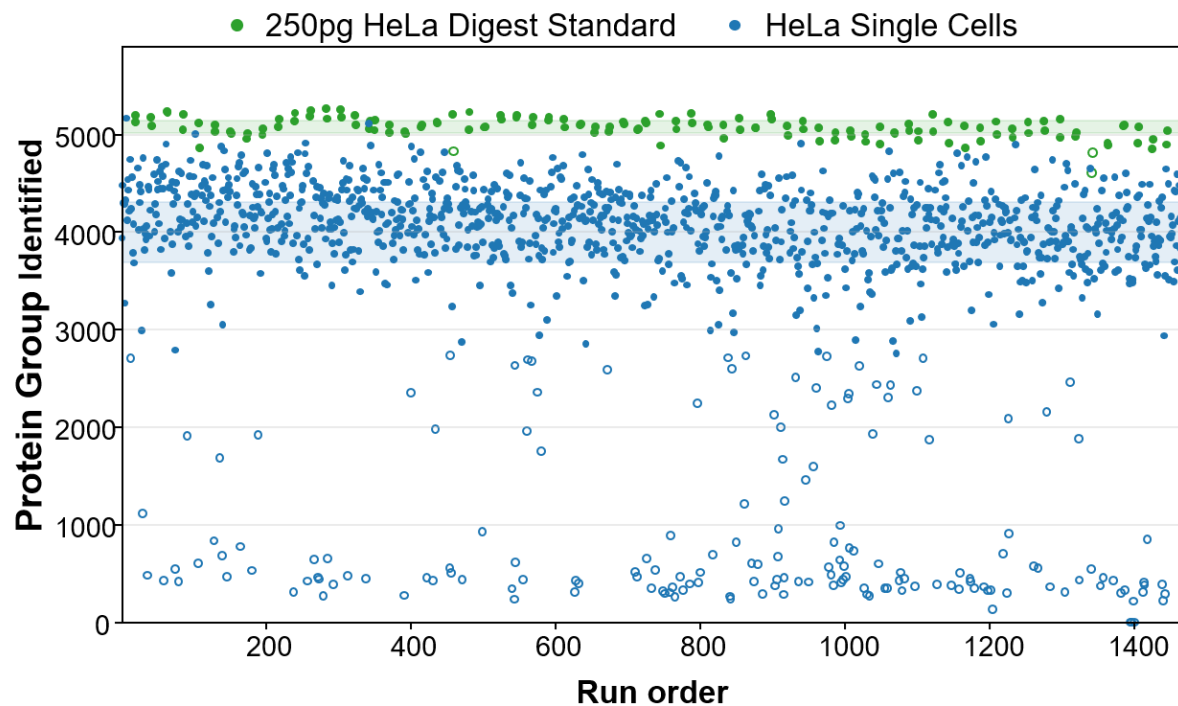




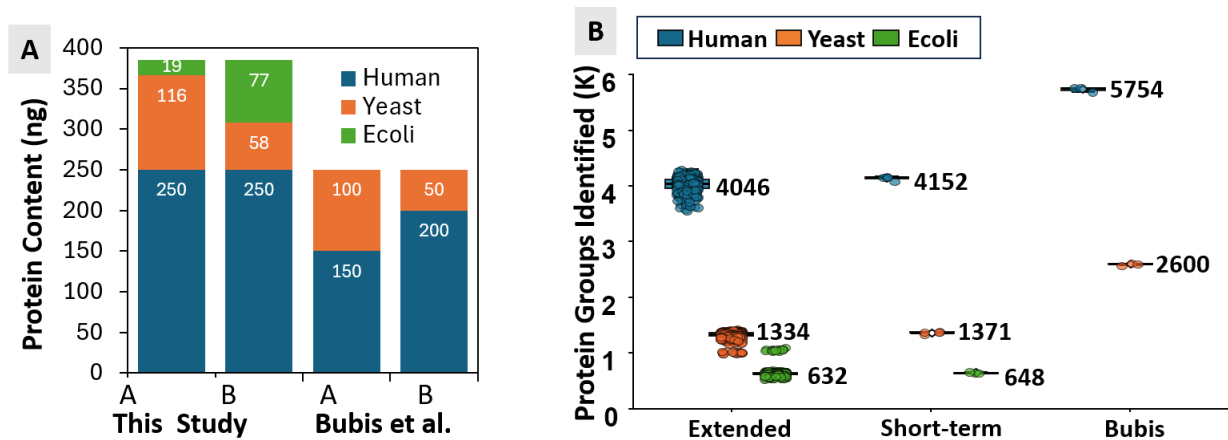
Supplementary Figure 1. Minute-by-minute schematic diagrams of the multicolumn LC system.



Supplementary Figure 2. Illustration of the gradient curve used for trap column cleanup.



Supplementary Figure 3. Complete run-order plot before outlier exclusion. The number of protein groups identified per run is plotted as a function of acquisition order for single HeLa cells (blue) and periodically injected 250 pg HeLa digest standards used as quality-control samples (green) over six days ($n = 1230$ biological replicates for 250 HeLa single cells and $n = 138$ technical replicates for 250 pg HeLa digest standard). Samples excluded by the $1.5\times$ IQR filter are shown as open circles.



Supplementary Figure 4. HYE standard composition and protein-identification distributions. (A) Protein composition of the HYE mixtures used in this study and by Bubis et al¹. (B) Distributions of protein groups identified for each of the Human, Yeast, and *E. coli* species in the extended-acquisition ($n = 578$), short-term reference ($n = 4$), and Bubis et al ($n = 3$). datasets. Each point represents one LC–MS HYE A run.

References

1. Bubis, J.A. et al. Challenging the Astral mass analyzer to quantify up to 5,300 proteins per single cell at unseen accuracy to uncover cellular heterogeneity. *Nat Methods* 22, 510-519 (2025).