

# Supporting Information

## **Quantitative accuracy and precision in multiplexed single-cell proteomics.**

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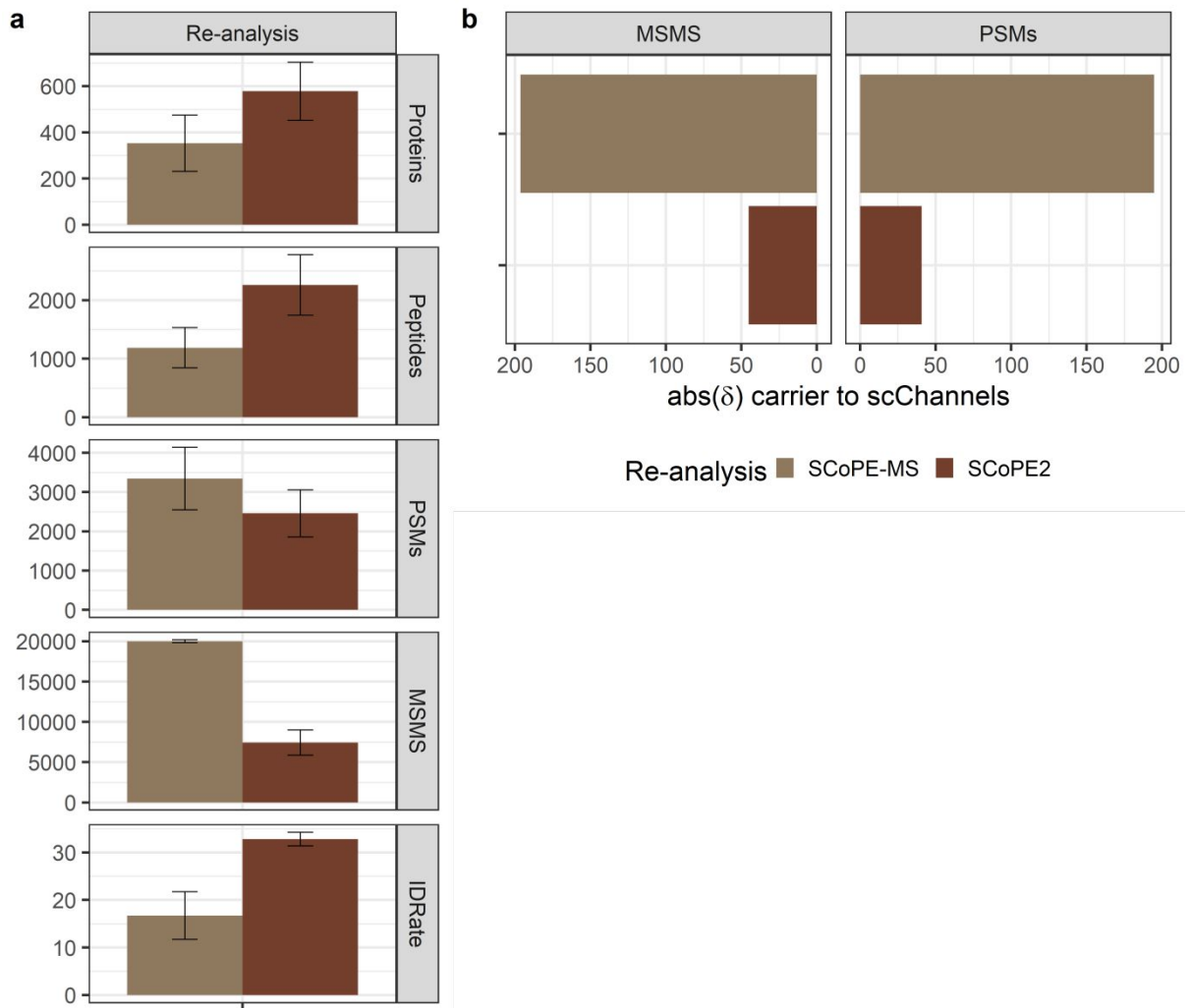
Figure S2: *Ratio compression for SCoPE, TMT10-plex and TMTpro samples at various carrier spikes.*

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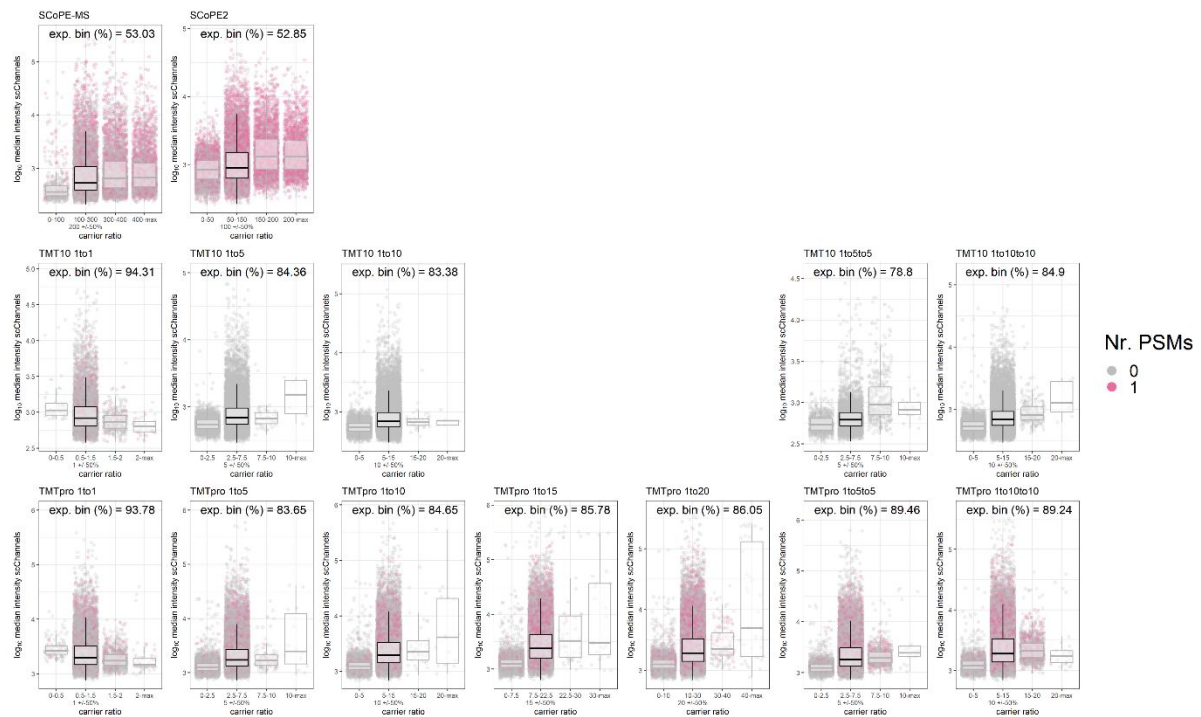
Figure S4: *Measurement stability and variance of public SCoPE data.*

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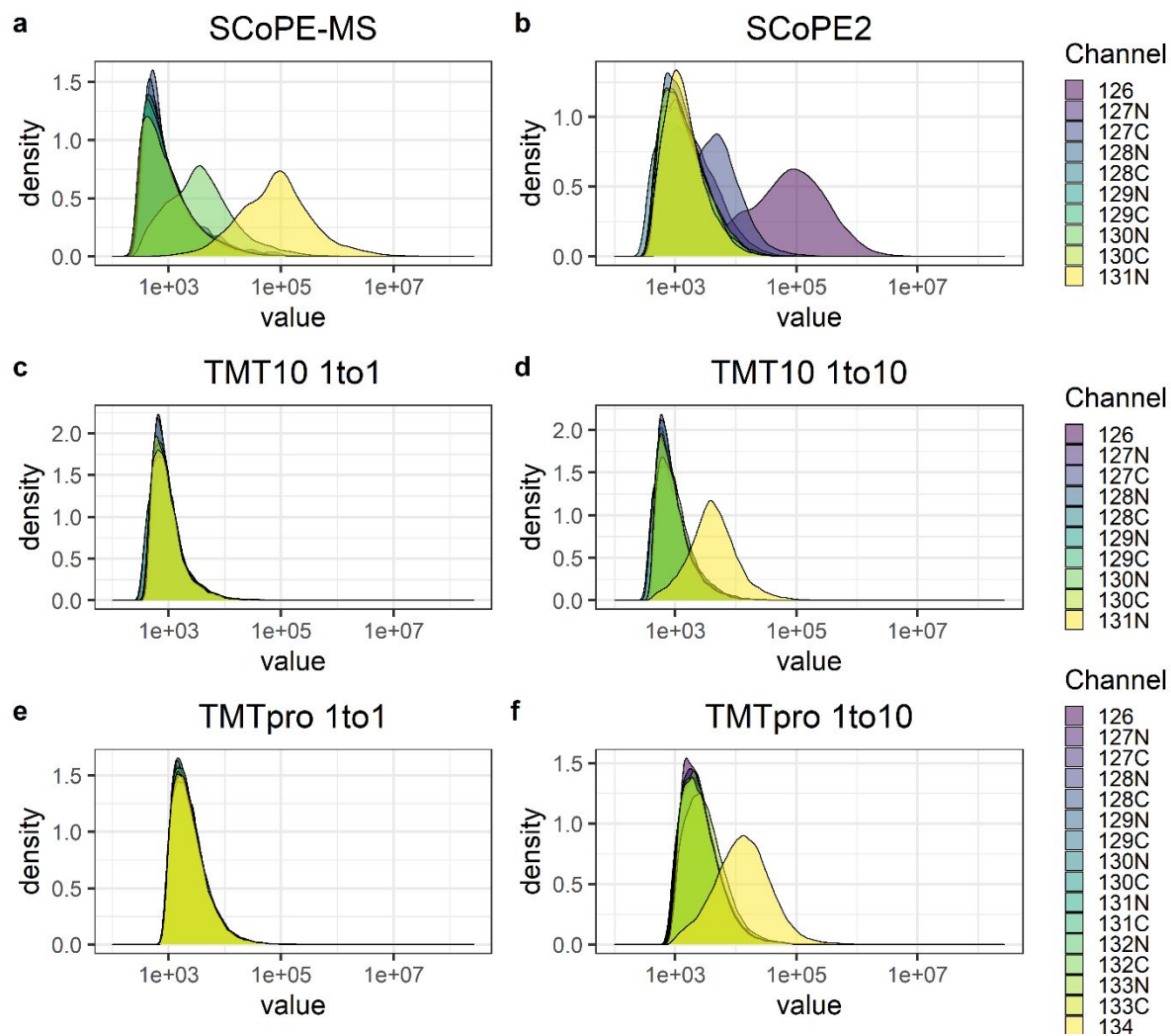
Figure S6: *Ratio compression in DIA-TMT experiments.*



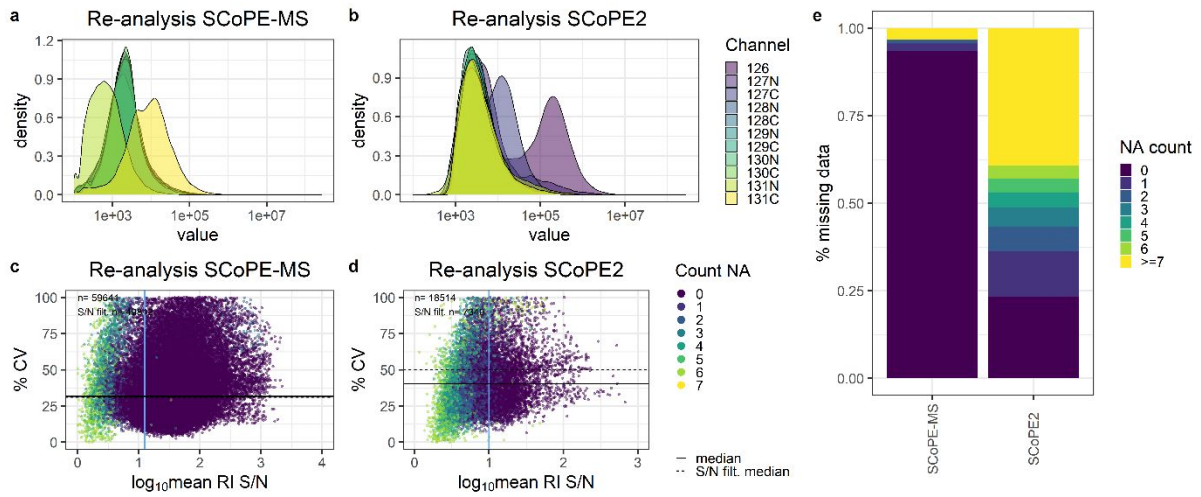
**Figure S1: Re-analysis of public single cell data, (a)** Identified proteins, peptide groups, PSMs, number of MS/MS scans, ID-rates and **(b)** delta between expected and acquired carrier to single cell ratio across all MS/MS scans or PSMs of published SCoPE-MS (light brown) and SCoPE2 (dark brown). Median and median absolute deviation (mad) is shown.



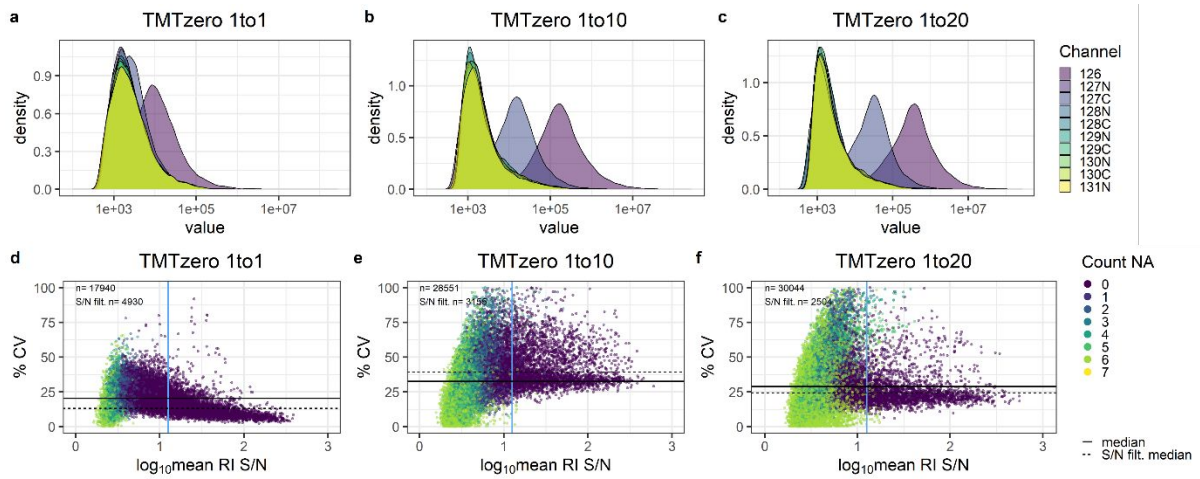
**Figure S2: Ratio compression for SCoPE, TMT10-plex and TMTpro samples at various carrier spikes.** Log<sub>10</sub> median RI intensity of all MS/MS scans and binned ratios between ‘single cells’ and the carrier is displayed. Identified and unidentified MS/MS scans are indicated in grey or pink, respectively. The expected bin (carrier +/- 50 %) is highlighted in black, and the percent of MS/MS scans within those is indicated.



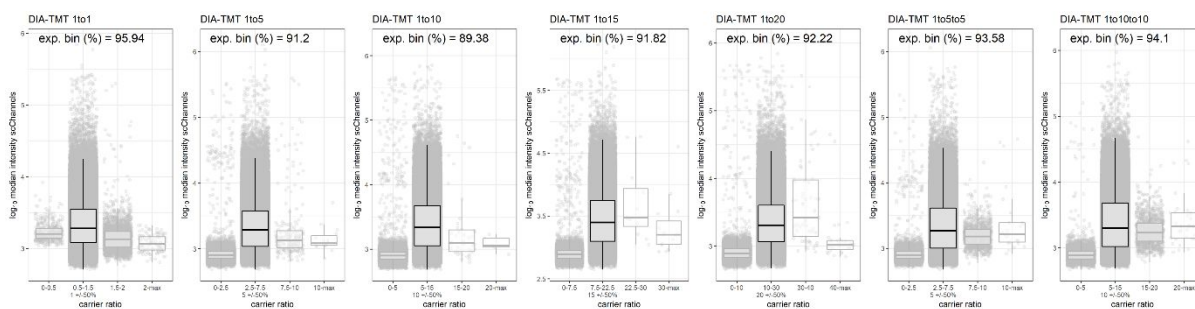
*Figure S3: Measurement stability and RI variability at various carrier ratios. RI intensity distributions based on all MS/MS scans for (a-b) SCoPE, (c-d) TMT10-plex and (e-f) TMTpro experiments at indicated carrier spikes.*



**Figure S4: Measurement stability and variance of public SCoPE-MS and SCoPE2 data. (a-b)** RI intensity distributions based on all MS/MS scans and **(c-d)** percent CV across 'single cell' channels and log<sub>10</sub> mean RI S/N for publicly available SCoPE data. The horizontal solid line and dashed lines indicate median S/N across all MS/MS scans or post-S/N filtering, respectively. The vertical blue line specifies the S/N filter cut-off. Colors reflect the number of missing 'single cell' RIs per MS/MS scan. **(e)** Percent missing quantitative data in publicly available SCoPE data per PSM.



**Figure S5: Measurement accuracy of  $TMT_{zero}$  experiments. (a-c)** RI intensity distribution across all MS/MS scans in  $TMT_{zero}$  experiments. Colors indicate different TMT channels. **(d-f)** Median percent CV and  $\log_{10}$  mean RI S/N at indicated  $TMT_{zero}$  carrier spikes. Horizontal solid and dashed lines indicate median S/N across all MS/MS scans or post-S/N filtering, respectively. The vertical blue line indicates the S/N filter cut-off. Colors indicate the number of missing 'single cell' RIs per MS/MS scan.



**Figure S6: Ratio compression in DIA-TMT experiments.** Log<sub>10</sub> median RI intensity across all MS/MS scans is displayed with binned ‘single cell’ to carrier channels ratios. The expected ratio (carrier +/- 50%) is highlighted in black, and percent of MS/MS scans within this bin is indicated.