

P-SCOPE: Carrier-enabled low-input phosphoproteomics by mass spectrometry

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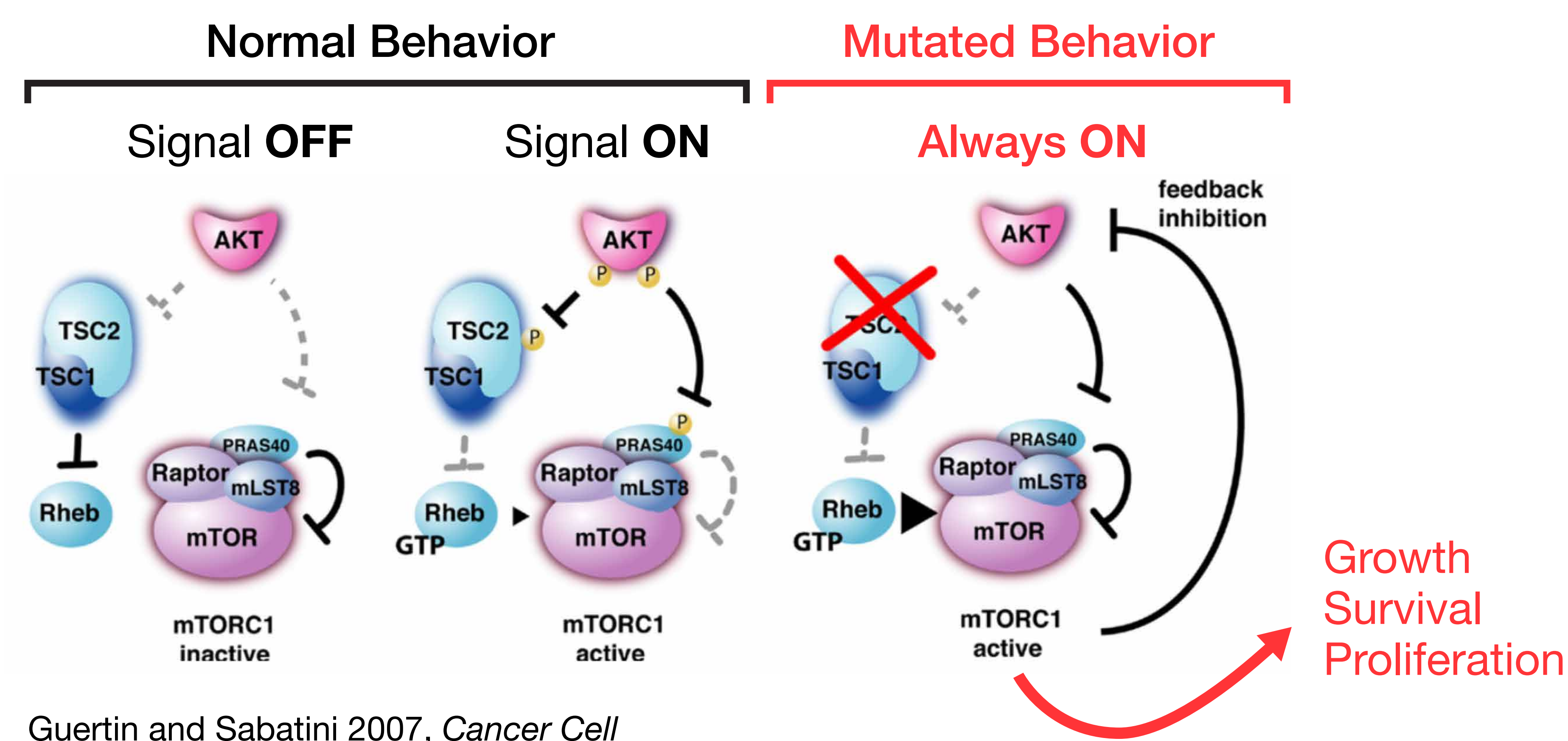


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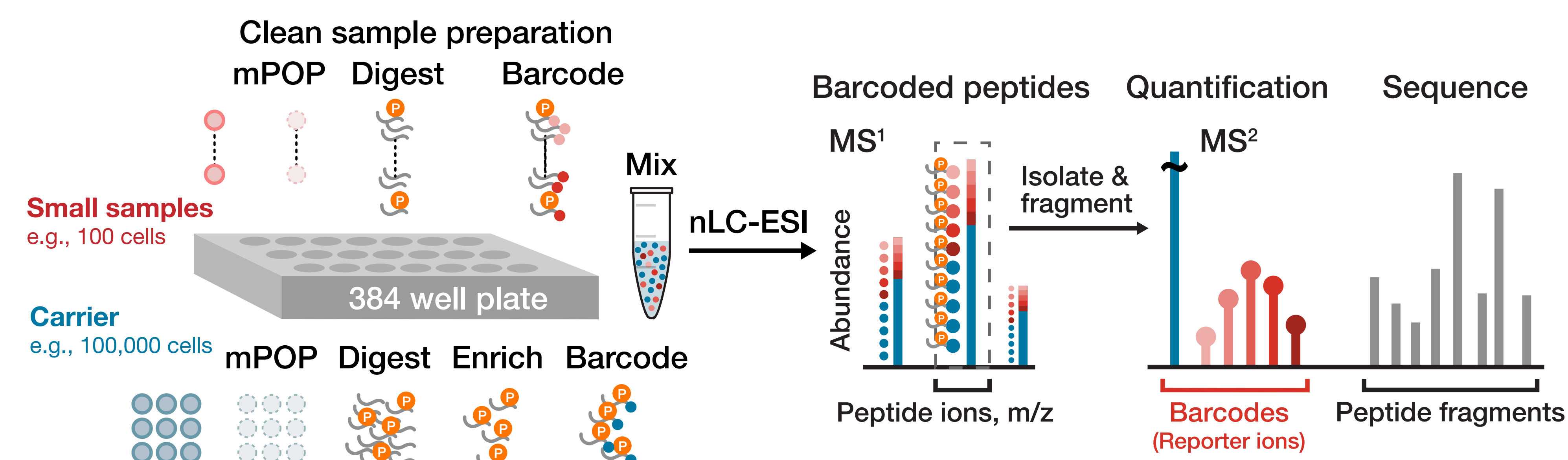
Summary

- Cellular signaling pathways are primarily mediated by phosphorylation, and dysregulation of these pathways is the cause of many diseases such as cancer
- Understanding signaling pathways and developing targeted clinical therapies would benefit from measuring every component of the pathway.
- Current measurements are limited to large samples or a handful of proteins
- P-SCOPE allows measuring hundreds-thousands of phosphoproteins for low-abundance samples
- P-SCOPE's carrier design can be extended to other post-translational modifications, such as glycosylation or ubiquitination

Dysregulated signaling pathways can lead to diseases such as cancer

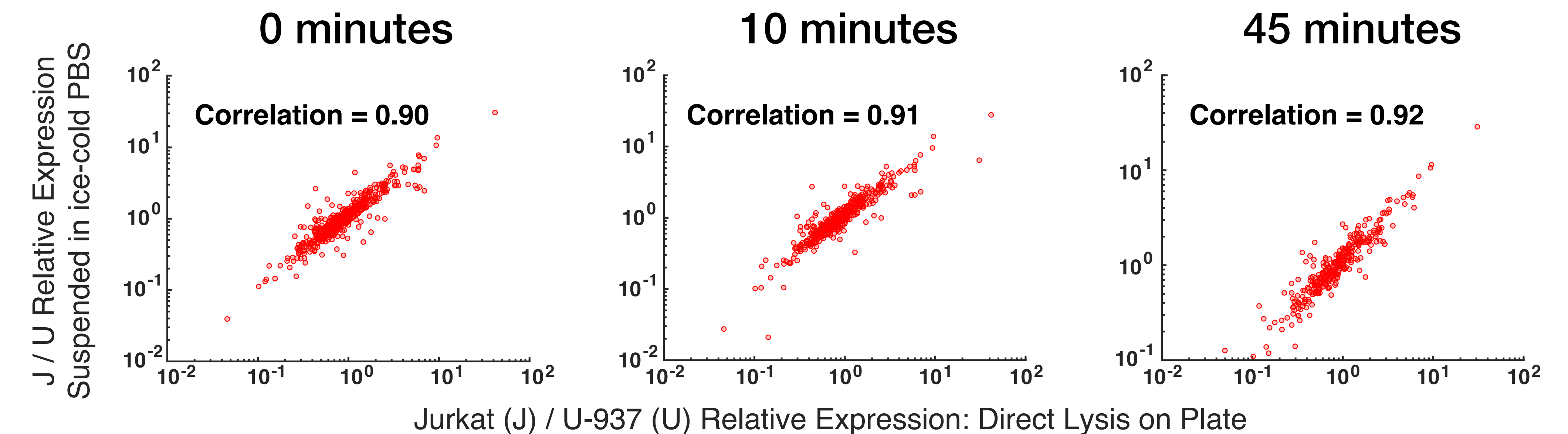


P-SCOPE Mass Spectrometry Method



Thanks to members of the Slavov Laboratory. This work was funded by the National Institute of Health to N.S. under Project Number 1DP2GM123497-01

P-SCOPE preserves protein phosphorylation

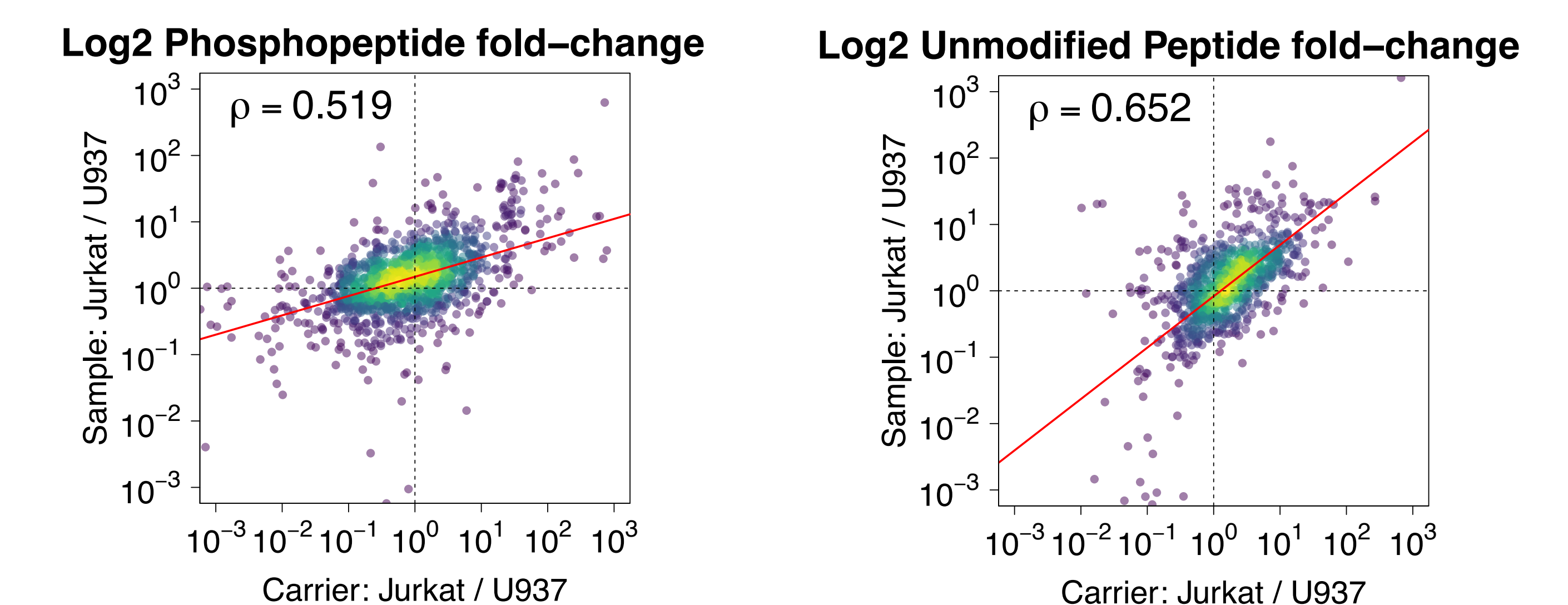


- Phosphorylation motifs in small samples are not lost during realistic cell sorting timelines (< 45 min, suspended in PBS, on ice), compared to cell lysis on plate

Measuring differential expression of phosphopeptides between T-cells and monocytes

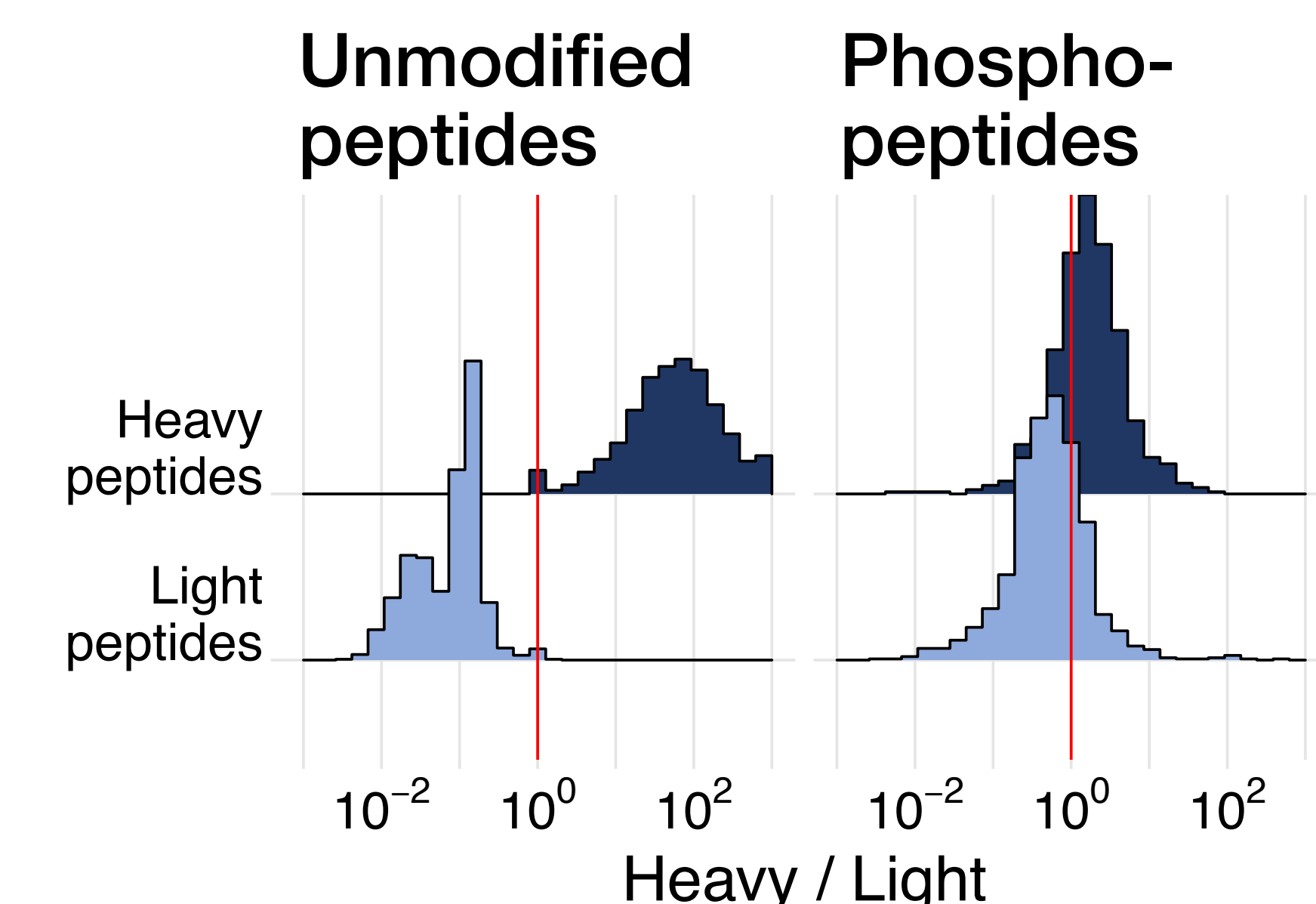
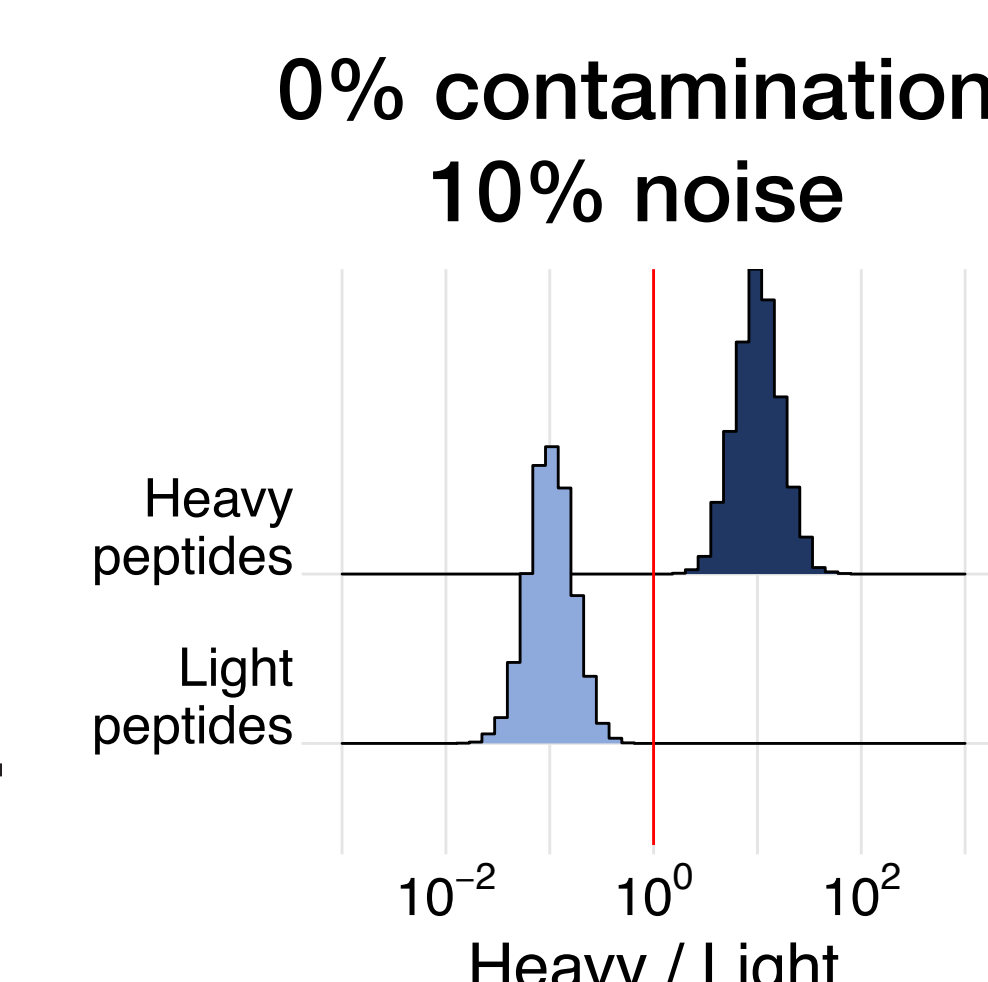
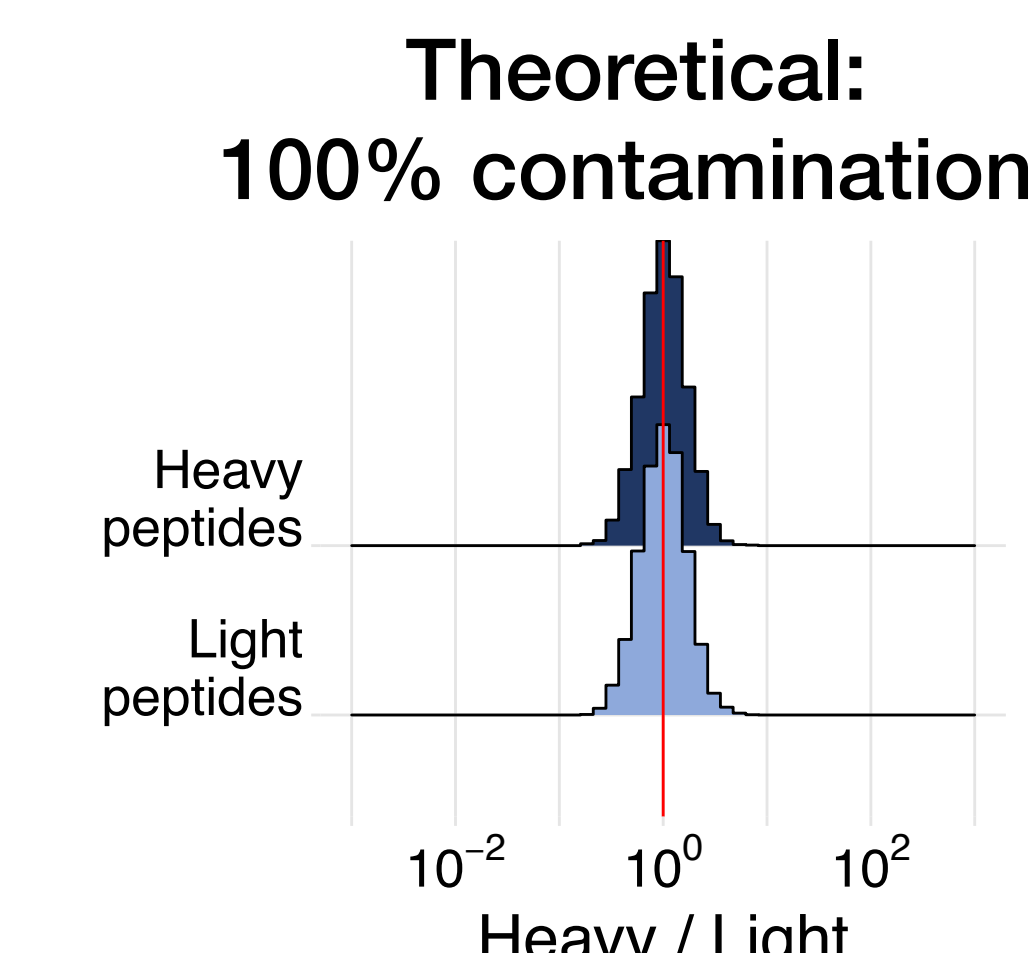
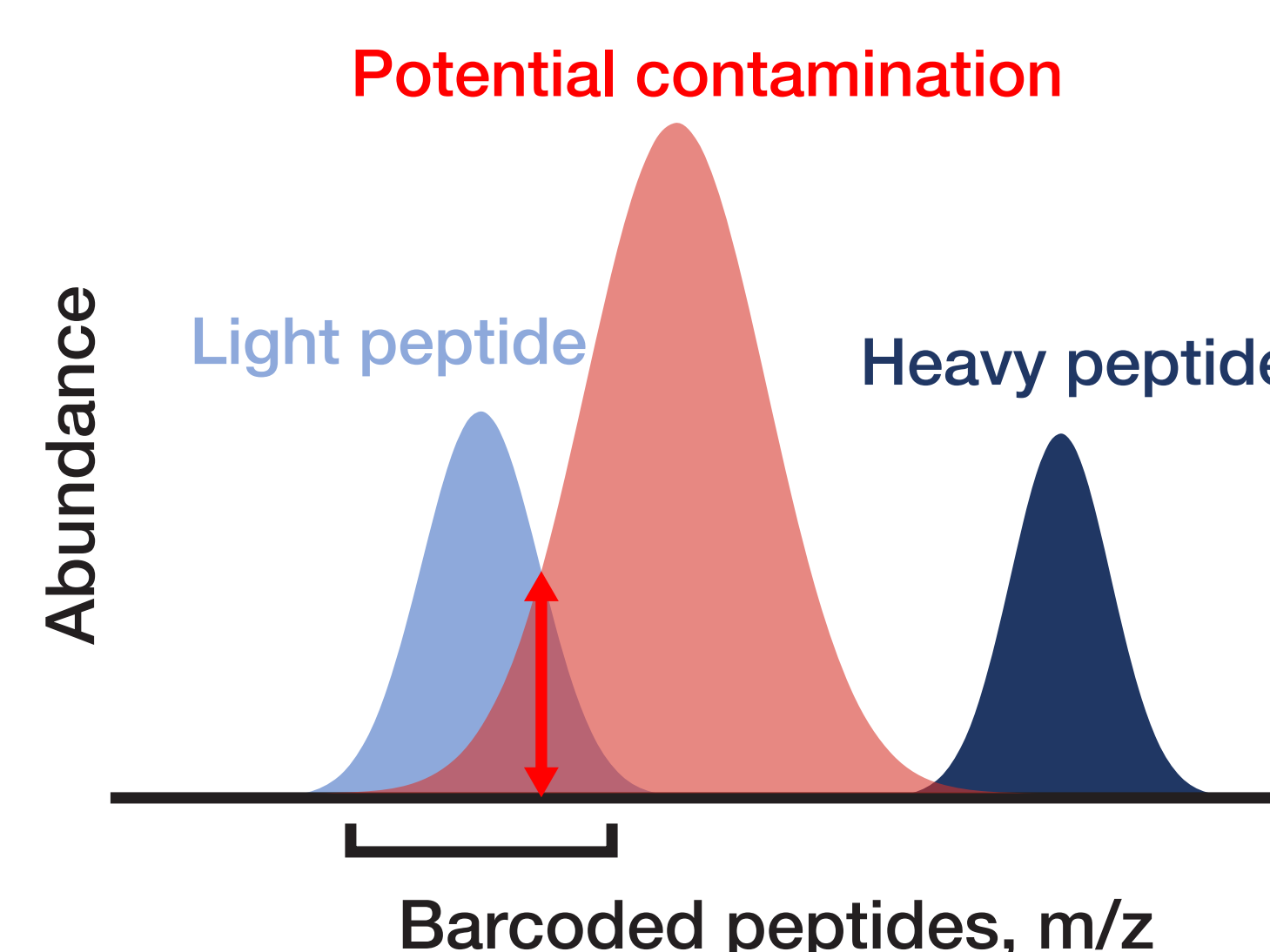
Label (TMT tag)	P-SCOPE set: T-cells & monocytes
126	Carrier
127N	10,000 Jurkat cells (enriched)
128N	10,000 U-937 cells (enriched)
127C	empty
128C	1,000 Jurkat cells
129N	1,000 U-937 cells
129C	1,000 Jurkat cells
130N	1,000 U-937 cells
130C	empty
131N	empty
131C	empty

Jurkat = T-cell, U937 = monocyte



Measuring signal-to-noise and potential contamination

Label (TMT tag)	P-SCOPE set: Light & Heavy U-937
126	Carrier
127	10,000 Light U-937 cells (enriched) + 10,000 Heavy U-937 cells (enriched)
128	empty
129	1,000 Light U-937 cells
130	1,000 Heavy U-937 cells
131	empty
	1,000 Light U-937 cells



- Additional modifications to preparatory, analytical, and computational methods needed to reduce noise and potential contamination