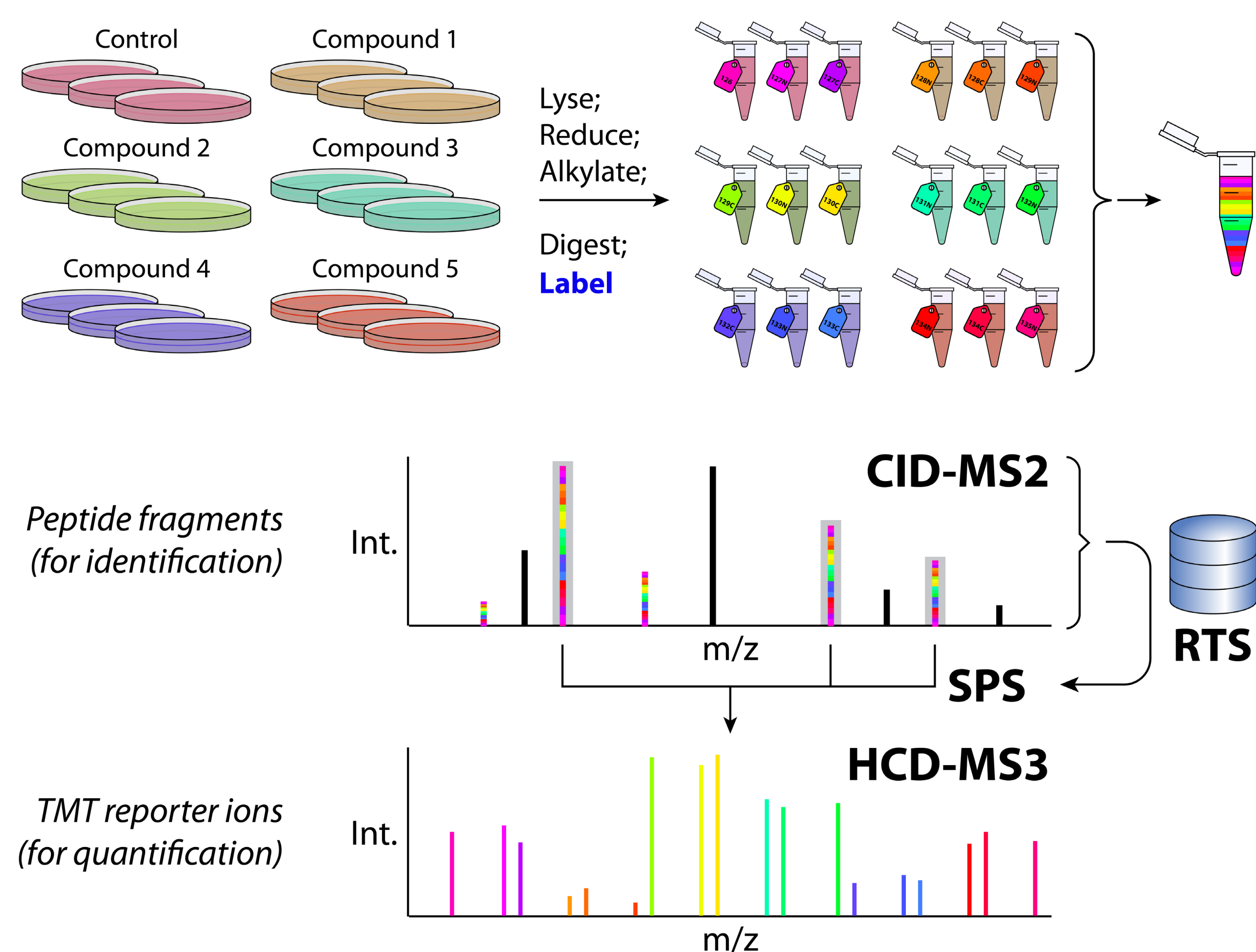


High-Depth Multiplexed Compound Profiling with the Orbitrap Ascend Tribrid Mass Spectrometer

Steven R. Shuken,¹ Graeme C. McAlister,² William D. Barshop,² Jesse D. Canterbury,² David Bergen,² Jingjing Huang,² Romain Hugué,² João A. Paulo,¹ Amanda E. Lee,² Vlad Zabrouskov,² Steven P. Gygi,¹ Qing Yu¹
¹Department of Cell Biology, Harvard Medical School, Boston, MA, USA. ²Thermo Fisher Scientific, San Jose, CA, USA.

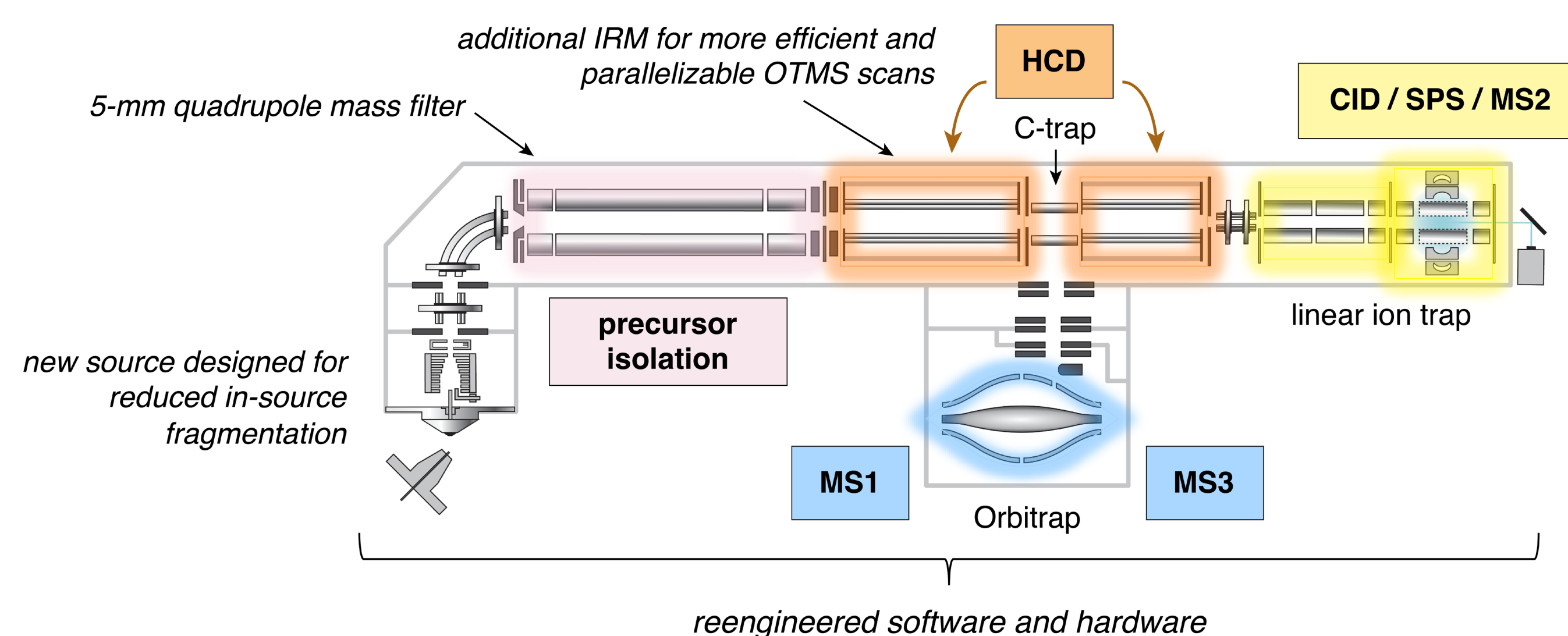
Tandem Mass Tag (TMT) Proteomics with Real-Time Search

- Biological sample pooling enables **high sample throughput**
- Real-time search (RTS) **increases proteomic depth (Ref. 1)**
- **Fractionation-based deep proteomic profiling with TMT is being used to profile compounds (Ref. 2)**

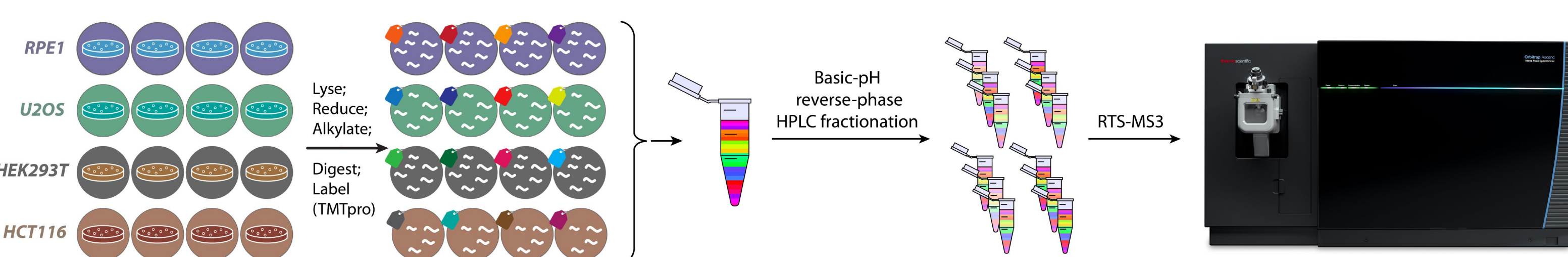


The Orbitrap Ascend Tribrid Mass Spectrometer

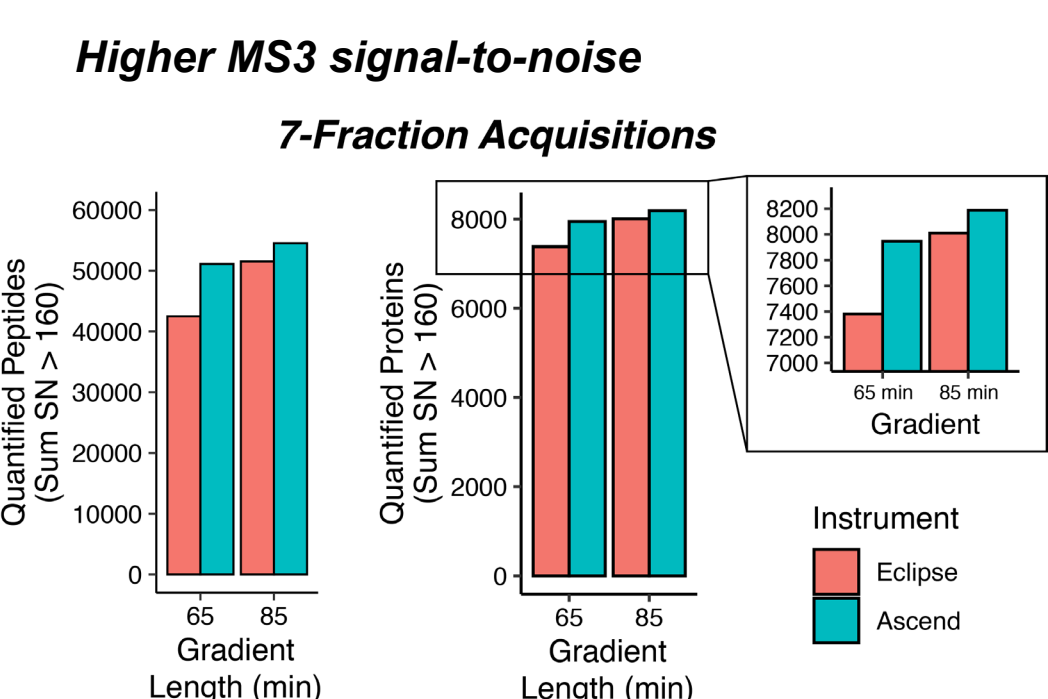
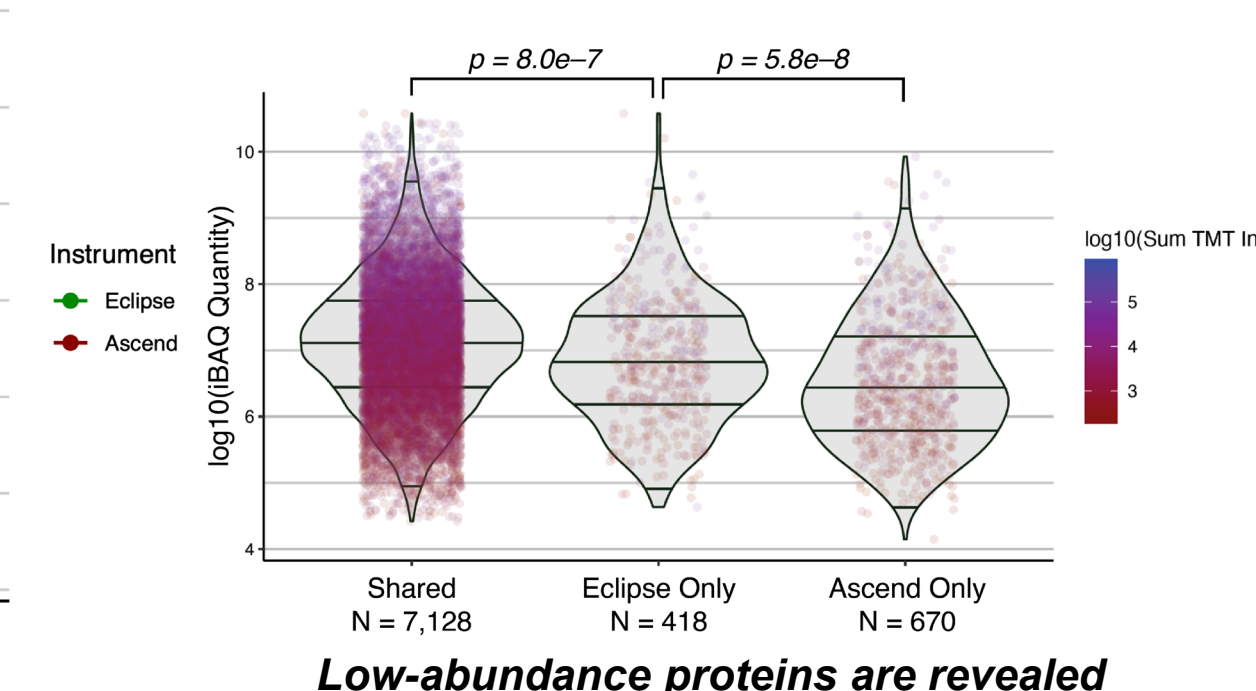
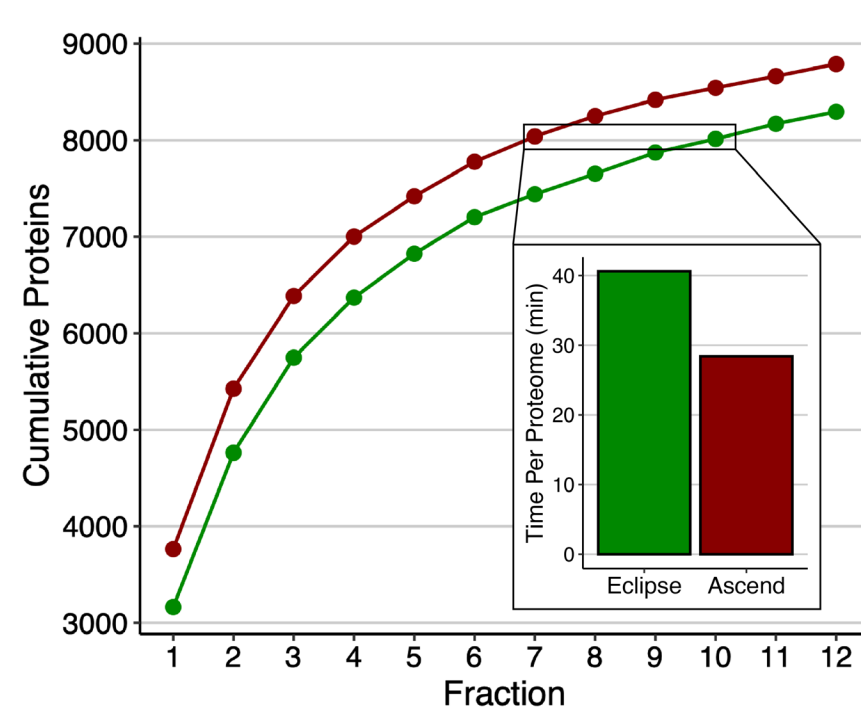
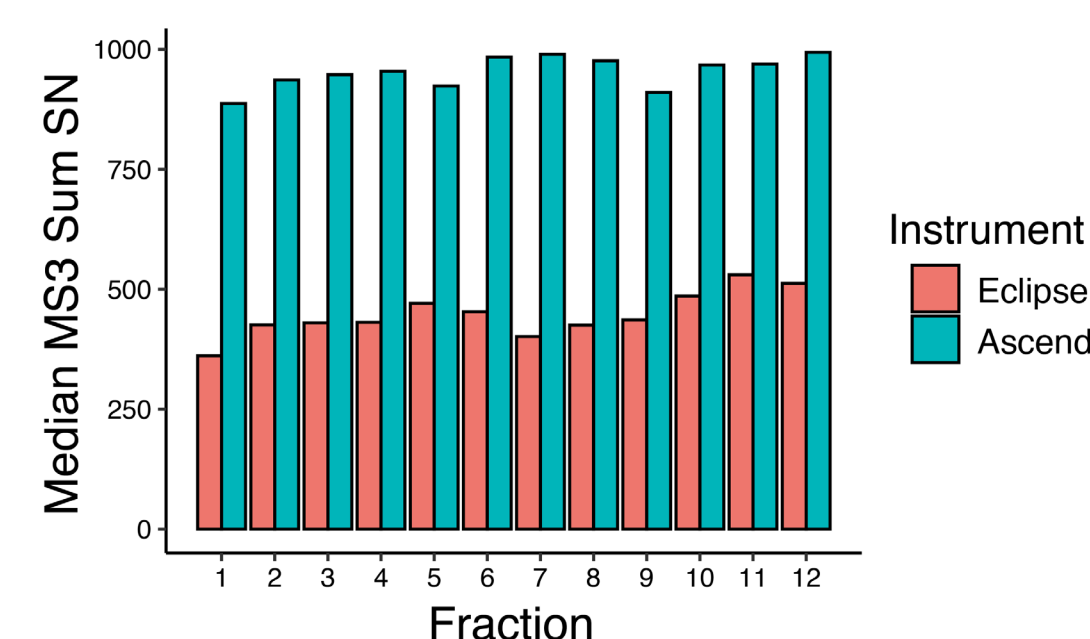
- Designed for **greater sensitivity and speed**
- Sensitivity + speed → **proteomic depth**
- Sensitivity → **quantitative accuracy and precision**



Four-Cell-Line Experiment: Orbitrap Ascend Quantifies More Proteins in Less Time

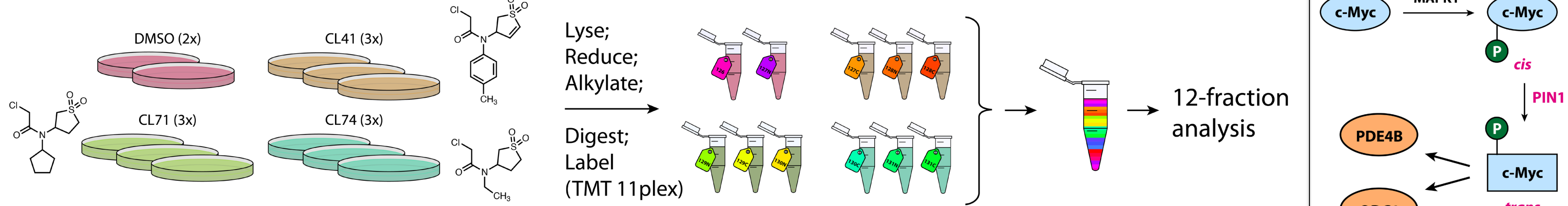


Instrument	Total MS3	Unique Peptides	Quantifiable Peptides (Sum SN > 160)	Protein IDs	Quantifiable Proteins (Sum SN > 160)
Eclipse	131,487	91,080	68,755	9,614	8,294
Ascend	133,613	95,095	83,386	9,853	8,788

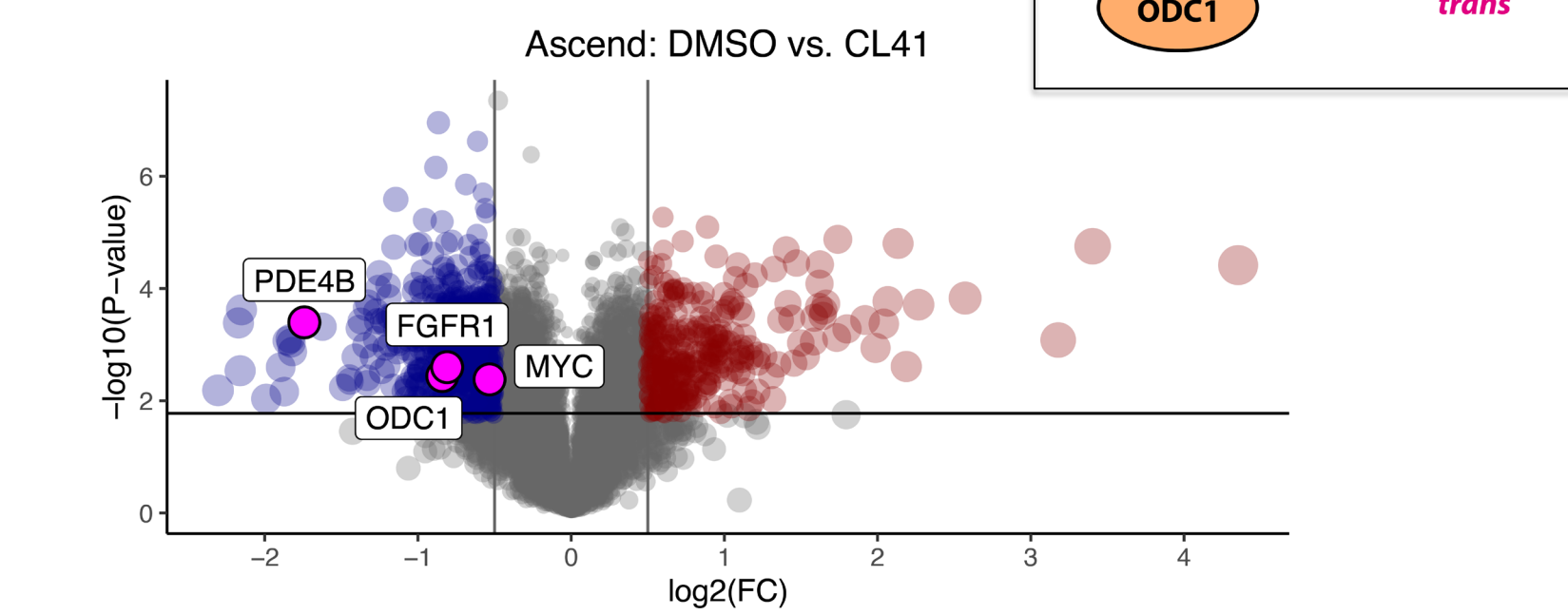


Proteomic Profiling of Novel Covalent PIN1 Inhibitors

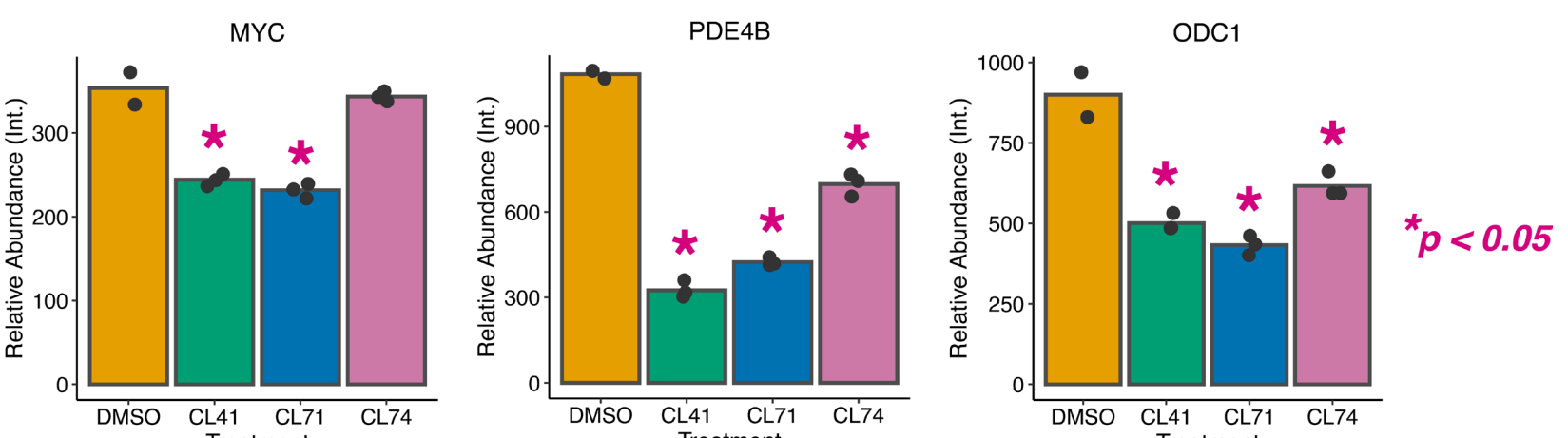
- PIN1: phosphoprotein *cis-trans* isomerase
- Promising cancer target via covalent inhibition (Ref. 3)



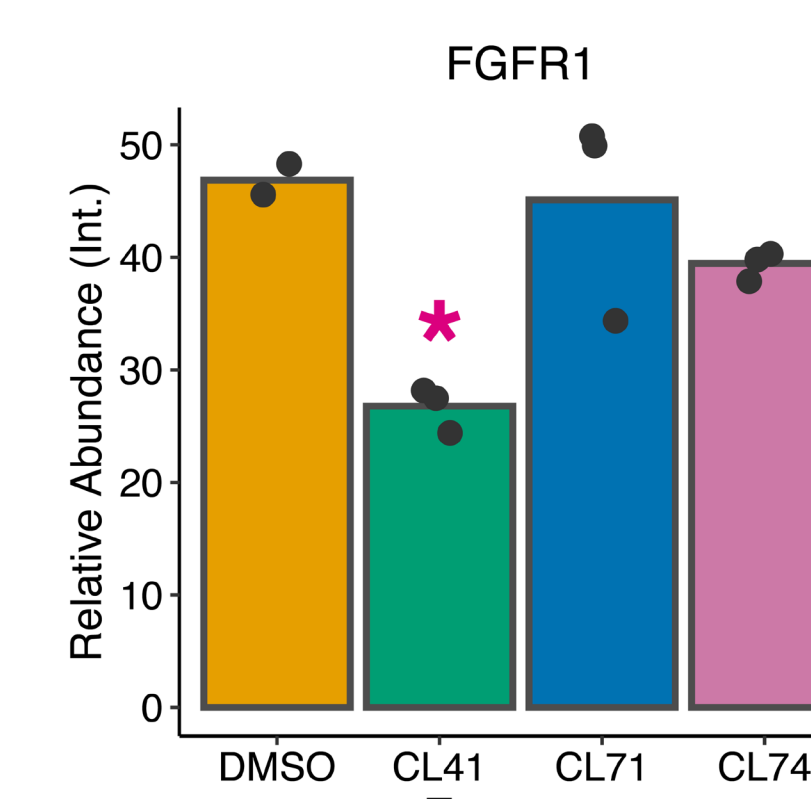
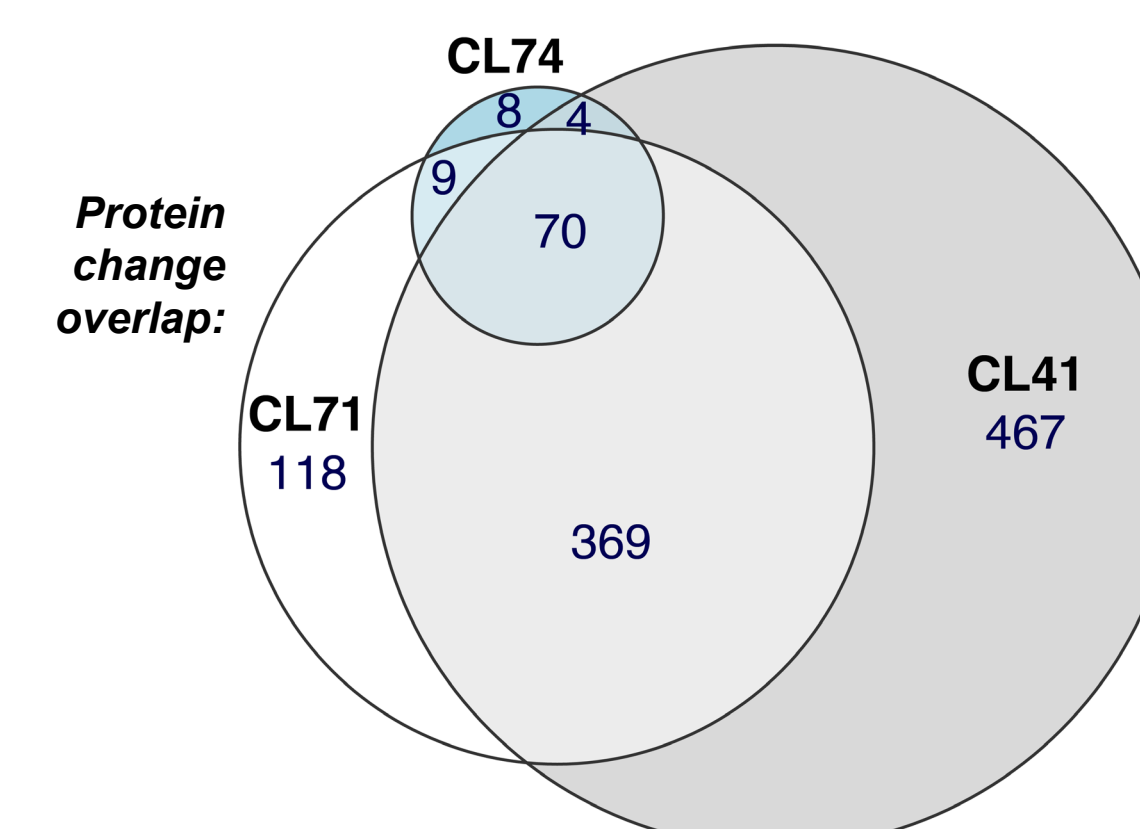
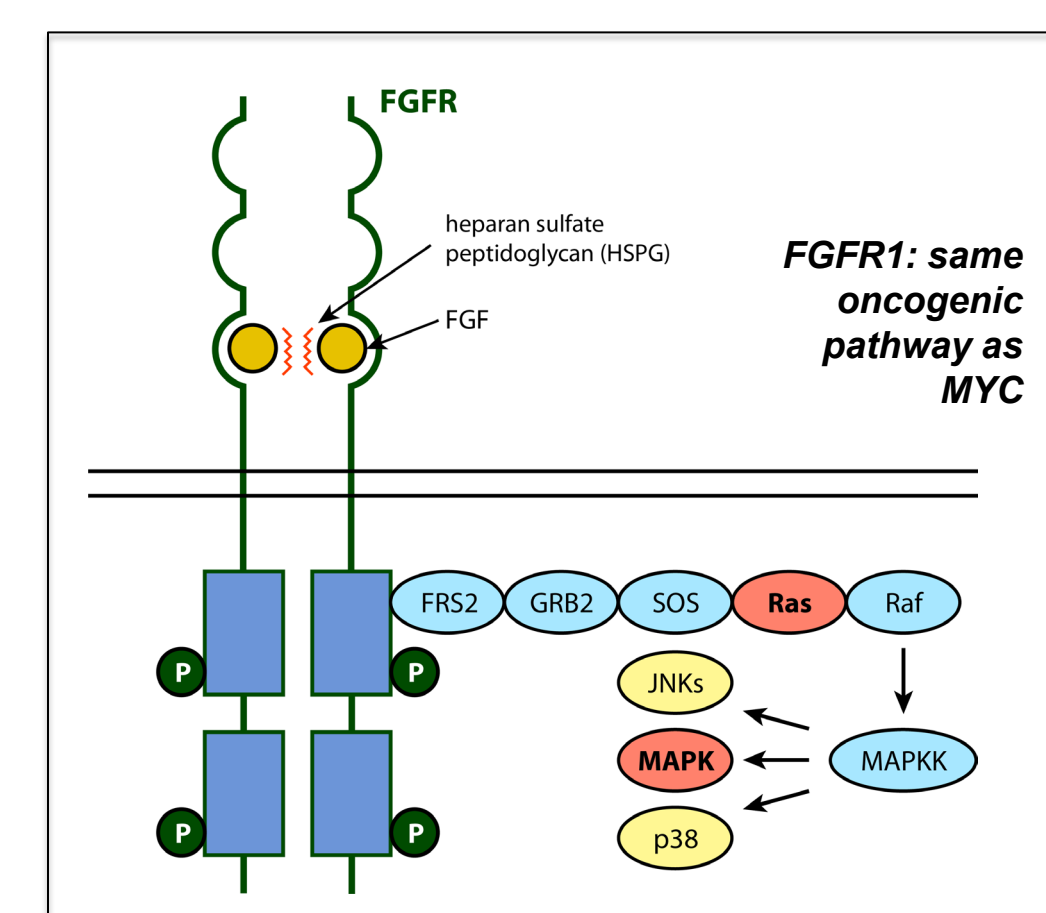
Instrument	Unique Peptides	Quant Peptides	Protein IDs	Quant Proteins
Eclipse	81,592	61,501	9,466	8,216
Ascend	98,328	78,698	10,128	8,881



Instrument	Proteins Up	Proteins Down	Total Changes
Eclipse	817	575	1,392
Ascend	949	618	1,567



Biological Effects of PIN1 Inhibitors



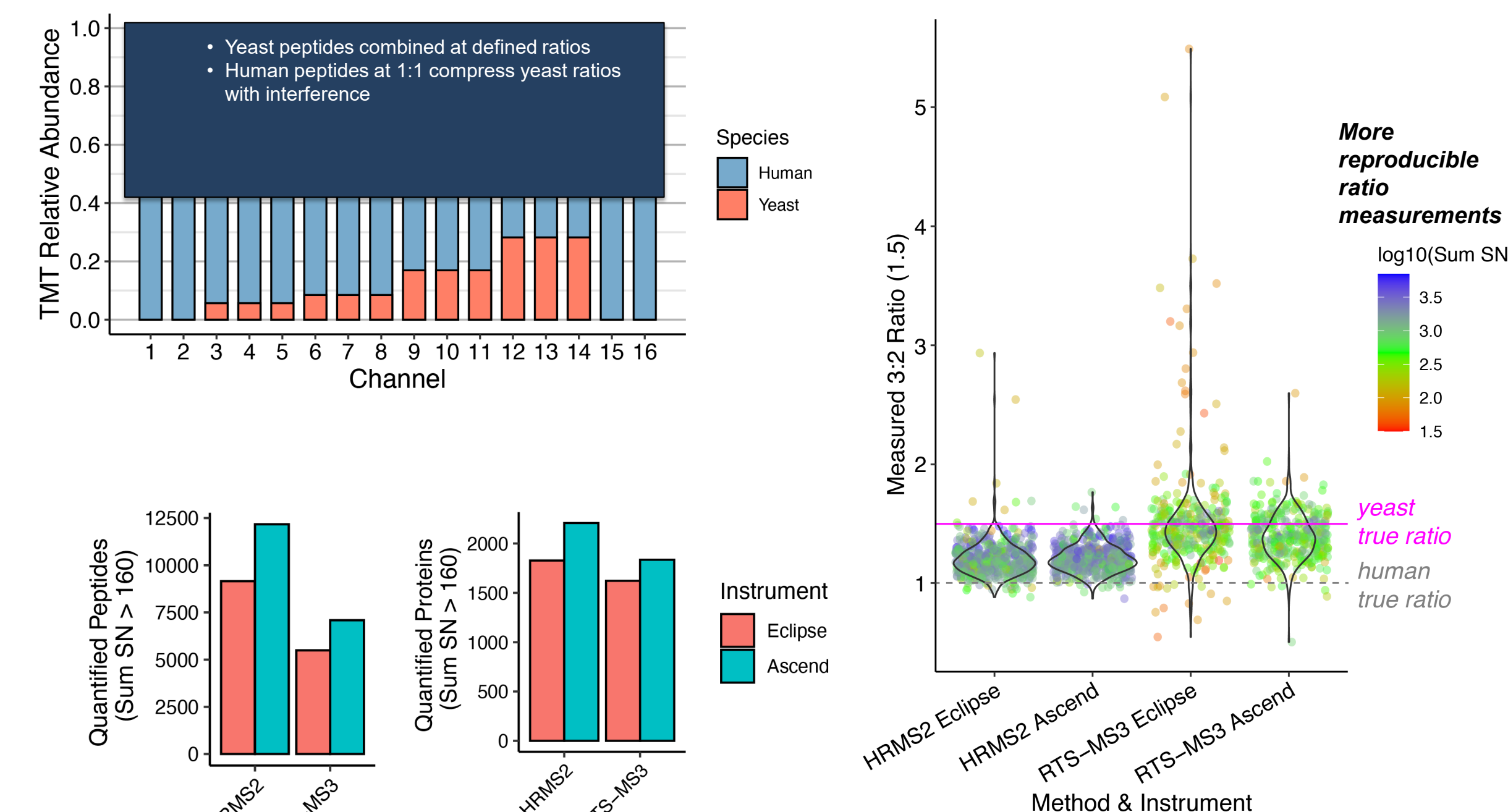
Top GO Pathways enriched in CL41 and CL71 changes:

- Gene expression
- RNA processing and metabolism

Top GO Pathways enriched with CL41 only:

- Translation
- Proliferation and development

Quantitative Precision



Acknowledgments

- NIH NIGMS
- Thermo Fisher Scientific



References

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- Mitchell, D. C., et al. "A proteome-wide atlas of drug mechanism of action." *Nature Biotechnol.* 2023, <https://www.nature.com/articles/s41587-022-01539-0>.
- Kulavri, M., et al. "Rearranging high-throughput profiling of reactive cysteines for cell-based screening of large electrophile libraries." *Nature Biotechnol.* 2024, <https://doi.org/10.1038/s41587-024-0044-4>.

