APPLICATION NOTE 65932

Example Datasets for choosing the optimal data acquisition method

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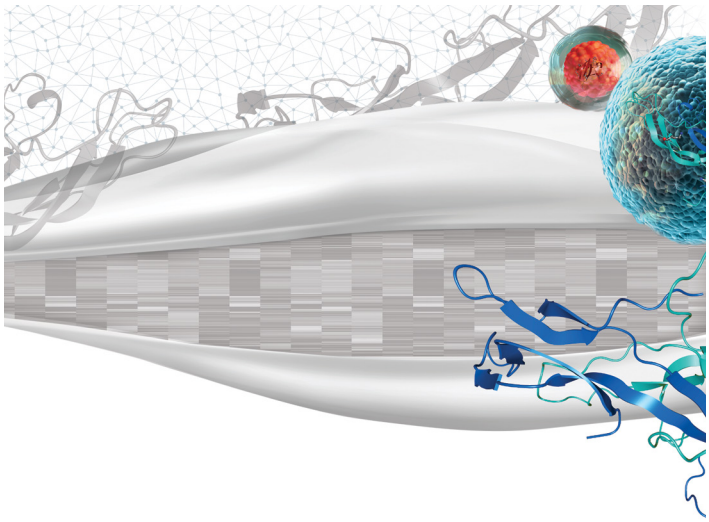
Keywords: Orbitrap Exploris mass spectrometer, FAIMS Pro interface, HRAM, LC-MS/MS, quantitative proteomics, TMT multiplexing, LFQ, DDA, HR-DIA, SureQuant, Single Cell



Your trusted quantitation resources

Biology is a quantitative science. Living organisms dynamically and quantitatively respond to their environment to ensure proper cell growth and determine cell fate. Understanding how the spatial and temporal organization of cellular signaling networks regulate metabolic enzymes is key to revealing its impact on the whole organism. The Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer provides exceptional performance and productivity for proteomic analyses via a range of quantitative methods that allow scientists to choose the strategies best suited to their experiments. Add the Thermo Scientific™ FAIMS Pro™ interface to experience the power of differential ion mobility technology that provides orthogonal gas phase separation and increased selectivity for highest-quality quantitation and proteome depth.

The Thermo Scientific Example Datasets are a trusted resource created to assist scientists in confidently deploying the correct choice of powerful quantitative methods, including the Thermo Scientific™ SureQuant™ method, Thermo Scientific™ Tandem Mass Tags™ (TMT)™ multiplexing, label-free quantification (LFQ) using high resolution (HR)-data independent acquisition (DIA) or data dependent acquisition (DDA), and single-cell proteomic analysis. Depending on study goals, each method has advantages and limitations to consider, including the number of samples to run and time required. Thermo Scientific™ Orbitrap™ high resolution, accurate mass (HRAM) instrumentation supports all of these approaches, providing the user with flexibility, while maintaining high-performance, enhanced quantitation (Figure 1).

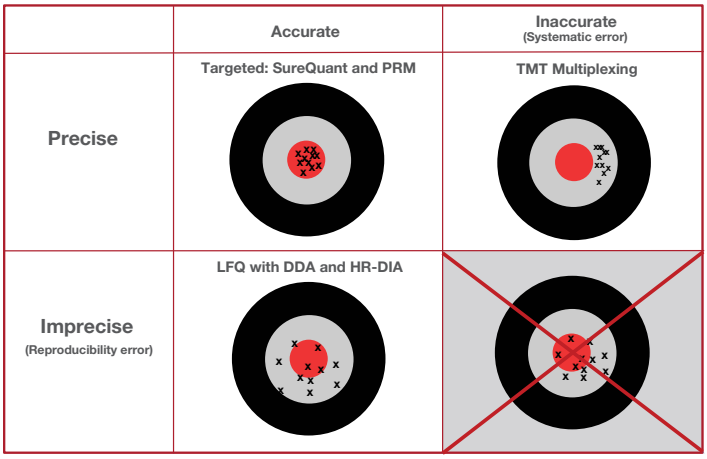


Figure 1. Fit-for-purpose assays for proteomic quantitation using the Orbitrap Exploris 480 mass spectrometer provide flexibility for a range of experimental designs. Higher confidence results can be achieved by avoiding imprecise and inaccurate measurements that can occur when low resolution, low mass accuracy methods are utilized.

Targeted assays like the SureQuant method and parallel reaction monitoring (PRM), also called targeted MS^2 (tMS^2), provide highest quantitation accuracy and precision but are limited to a few hundred candidates. When quantifying thousands of proteins, scientists typically choose between LFQ methods that use either DDA or HR-DIA. Alternatively, isobaric tagging strategies, such as TMT methods, can deliver the highest quantitation precision and enable multiplexing up to 16 samples in a single liquid chromatography-mass spectrometry (LC-MS) analysis. Strategies to maximize identification and proteome depth are supported with a fast 40 Hz scan rate with 7500 resolution @ 200 *m/z* while maintaining improved quantitation (Figure 2).

In addition to describing the quantitative approaches that can be deployed, the Example Datasets demonstrate the quality of quantitative data obtained and efficient processing strategies to accelerate your path to certainty in results, allowing you to spend less time on method optimization and more time on meaningful work. The full documentation provided by the Example Datasets exemplifies Thermo Fisher Scientific's commitment to data transparency.

For each application, the Example Datasets contain:

- Raw data files acquired on an Orbitrap Exploris 480 mass spectrometer.
- Complete experimental methods, along with flexible method templates to enable users to reproduce data and results, while allowing further customization to meet user requirements. The methods include LC-MS instrument parameters and Thermo Scientific™ Proteome Discoverer™ software post-acquisition data processing methods.
- A presentation explaining method use and capabilities for a variety of applications and experiments.
- Other supplemental information to facilitate successful method deployment, including details on sample type, preparation, and commercial availability.

The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface is the platform for highest-quality quantitation. Go faster and dig deeper into your samples than ever before, and enjoy the benefits of robust, precise, and accurate quantification using your preferred methods.

All of the following Example Datasets are available for download after registering for a free account. Additional datasets will be made available in the future.

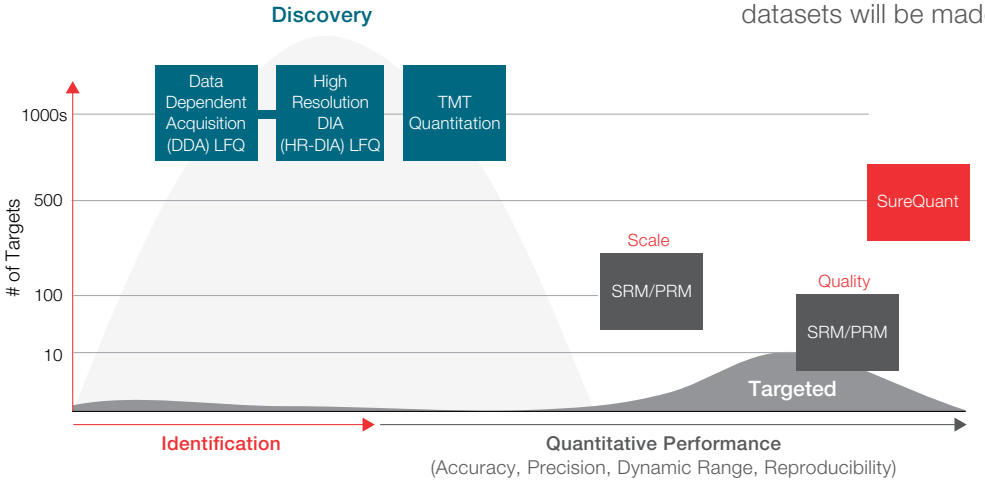


Figure 2. Orbitrap Exploris 480 mass spectrometer delivers high-performance quantitation across multiple methods for experimental flexibility.

Maximum proteome depth method

When used on the Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface, the Maximum Proteome Depth method provides maximum overage and reproducibility across the proteome, even for limited sample amounts (Figure 3). Proteome Discoverer software provides data processing, identification, and quantitation workflows that integrate database searches with high-confidence False Discovery Rates (FDR). Thus, you can choose the appropriate gradient length and sample load to achieve the desired proteome coverage and throughput for your experimental design.

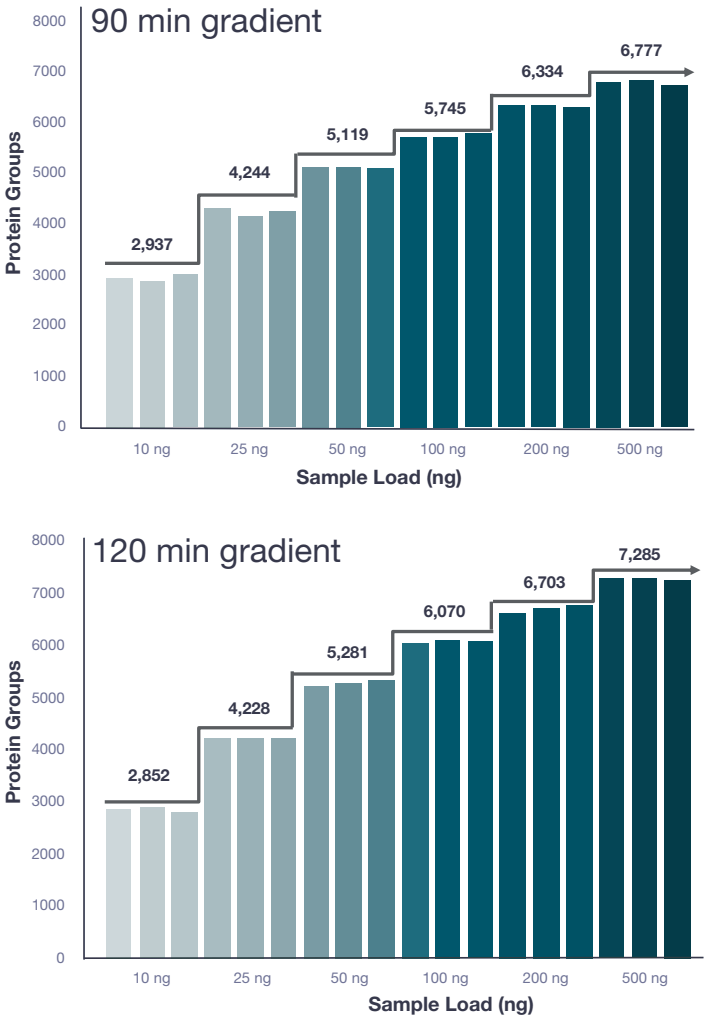


Figure 3. Proteome Coverage from 10 to 1000 ng using a Thermo Scientific Pierce HeLa protein digest standard analyzed with either a 90 min or a 120 min gradient using the Orbitrap Exploris 480 mass spectrometer and the FAIMS Pro interface with intra-analysis CV stepping (CV -50 and -70).

SureQuant IS protein quantitation method

The SureQuant internal standard (IS) targeted protein quantitation method is an intelligent data acquisition strategy that provides sensitive, precise, and reproducible quantitation, independent of retention time scheduling. The method overcomes the limited acquisition efficiency of conventional targeted analyses (selected reaction monitoring or parallel reaction monitoring) that require trade-offs between throughput (scale) and analytical performance (Figure 4). The method supports use of SureQuant Targeted MS Assay Kits and third-party targeted panels like the Biognosys PQ500™ human plasma panel.

Protein	Peptide	PRM 15K-20ms	SureQuant 60K-116ms
GSK3β	TPPEAIALC[Carbamidomethyl]SR	1	20
AKT2	LPFYNQDHER	0	17
IGF1R	AENGPGPGVLVLR	1	15
PTEN	IYNLC[Carbamidomethyl]AER	0	15
GSK3β	LC[Carbamidomethyl]DSGELVAIK	5	15
GSK3β	LLEYTPTAR	0	15
GSK3α	VTTVVATLGQGPER	6	15
AKTS1	GALAEAAR	5	13
AKT1	NDGTFIGYK	0	13
AKT2	SDGSFIGYK	0	13
IGF1R	IDIHSC[Carbamidomethyl]NHEAEK	1	12
PTEN	AQEALDFYGEVR	10	11
MTOR	LFDAPEAPLPSR	11	11
MTOR	TLDQSPELR	2	10
AKT1	YSFQTHDR	0	10
KS6B1	FEISETSVNR	0	9
TSC2	SNPTDIYPSK	0	9
GSK3α	SQEVAYTDIK	1	9
TSC2	GQPEGPLPSSSPR	0	8
AKTS1	SSDEENGPPSSPDLDR	0	8
IGF1R	TTINNEYNYR	0	7
MTOR	ETSFNQAYGR	2	6
MTOR	GNNLQDTLR	0	6
TSC2	GYTISDSAPSR	0	5
PTEN	NNIDDVVR	0	4
KS6B1	DGFYPAPDFR	0	1
AKT3	LVPPFKPQVTSETDTR	0	0
IRS1	SVSAPQQIINPIR	0	0
AKT3	TFHVDTPEER	0	0
IRS1	TGIAAEEVSLPR	0	0

Figure 4. Results from targeted quantitation experiments are color-coded based on the confidence of the quantitation, or the number of MS/MS scans that were collected for the target. The SureQuant method delivers higher sensitivity to accurately measure the profile of the peptide. In comparison, PRM underperforms due to the tradeoff between cycle time and max injection time, which can limit sensitivity.

Tandem Mass Tags (TMT) multiplexed quantitation

Thermo Scientific™ TMTpro™ and TMT workflows integrate reagents, instrumentation, and advanced software to enable rapid identification and quantitation of thousands of proteins from up to 11 or 16 multiplexed samples, respectively, with maximum accuracy and precision—all in a single run. The resolution, selectivity, and specificity of the Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface, Thermo Scientific™ TurboTMT™ technology, and Precursor Fit filter decrease the challenges associated with TMT quantitation, such as co-isolated interferences that mask true differences in protein abundance across samples (Figure 5).

HR-DDA LFQ method

HR-DDA LFQ is a common quantification strategy for comparing proteomes. LFQ is performed on precursor HRAM full-scan data, thereby minimizing sample preparation time. MS/MS spectra and feature mapping are used to identify peptides. Top Speed (TopS) or TopN intelligently determine MS and MS/MS scans across LC peaks, with fixed and variable cycle times, respectively. The FAIMS Pro interface can be added to provide orthogonal gas phase separation and increase identifications. Proteome Discoverer software speeds data processing workflows that include detection, identification, quantitation, and annotation.

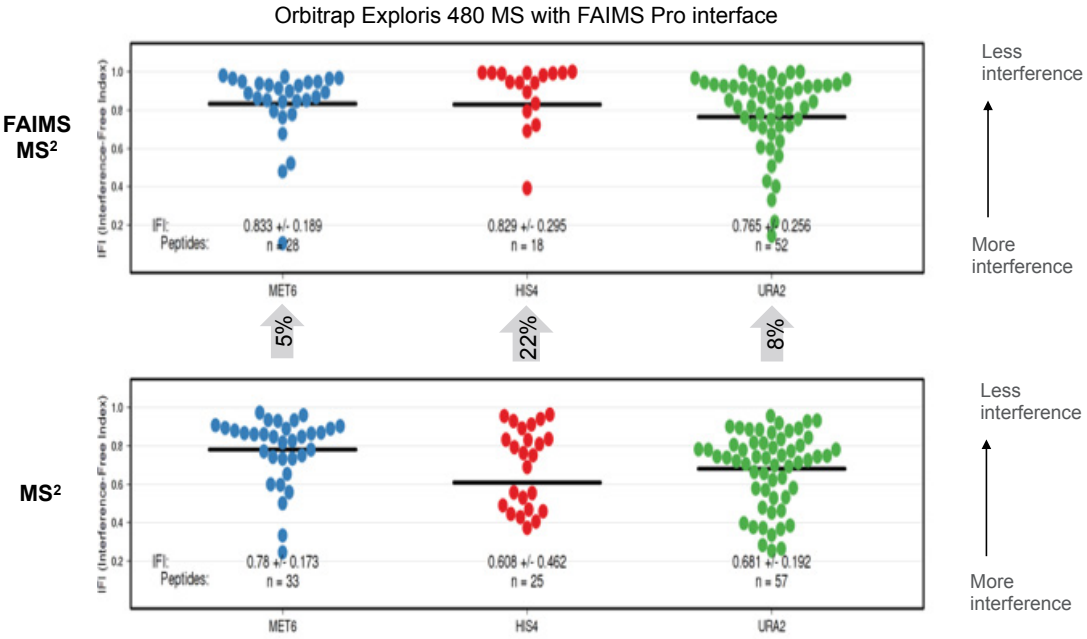


Figure 5. The Orbitrap Exploris 480 mass spectrometer coupled with the FAIMS Pro Interface improves TMT quantitation accuracy by reducing co-isolation interference. Interference free index (IFI) plots shown are from TKOmics.com

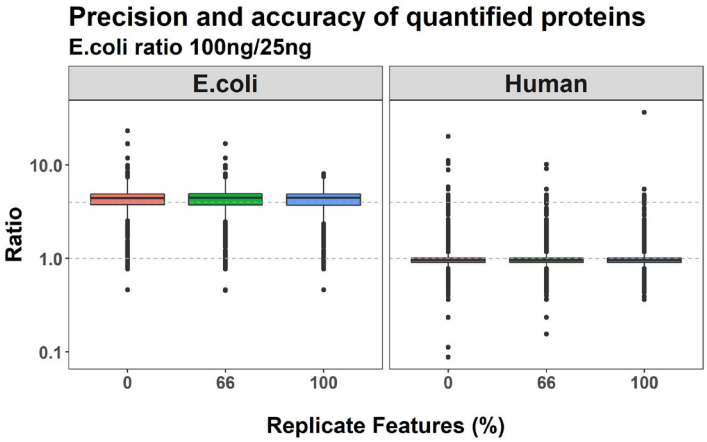


Figure 6. The Orbitrap Exploris 480 mass spectrometer delivers accurate protein quantitation using the HR-DDA LFQ.method. Here, *E.coli* protein digests were mixed with Pierce HeLa protein digest standard at a ratio of 100 ng *E.coli* to 25 ng Hela.

HR-DIA LFQ method

Of LFQ strategies, HR-DIA has the advantage of collecting more MS/MS information on all peptides in a sample, achieving reproducible coverage across a large sample set. HR-DIA method strategies using high resolution MS[1] quantitation balance sensitivity (acquisition window size) with quantitative precision and accuracy (cycle time) for a set of chromatographic conditions (Figure 7). For faster analysis that balances coverage with analysis time, library-free HR-DIA using Biognosys Spectronaut™ directDIA™ workflow provides additional flexibility, saving valuable instrument analysis time.

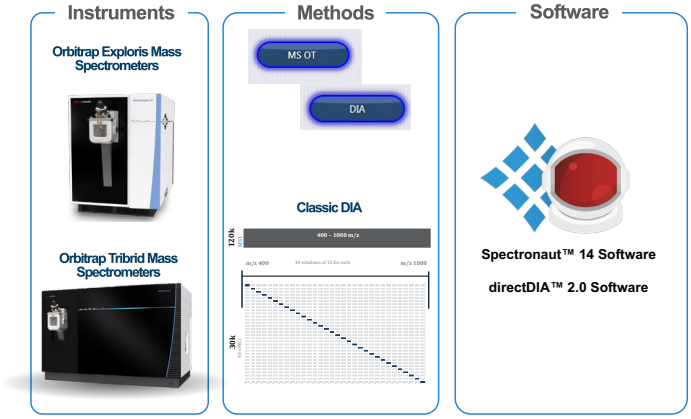


Figure 7. Rapid library-free HR-DIA using Biognosys Spectronaut™ directDIA™ workflow provides additional flexibility to balance coverage with analysis time.

Single-cell proteomics

Ultra-sensitive MS using the Orbitrap Exploris 480 mass spectrometer coupled to the FAIMS Pro interface goes beyond quantitative analysis of limited samples to true single-cell proteomics, facilitating investigation of cellular processes and rare cells (Figure 8). For label-free single-cell experiments, the FAIMS Pro interface increases coverage and signal-to-noise to low nanogram-level sensitivity. The platform also supports high-throughput 11plex and TMTpro 16plex experiments for increased sample throughput and quantitative precision using a booster channel or pooled control. Single-cell sample-preparation methods and data processing are described.[1]

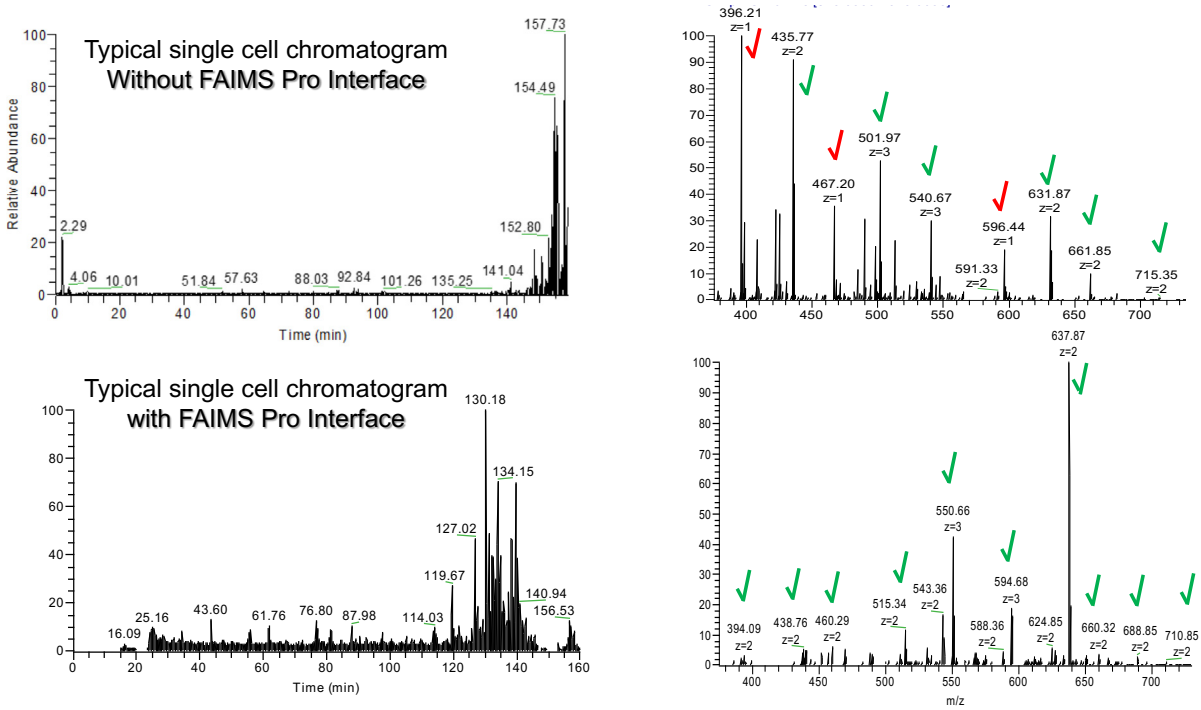


Figure 8. Improving single-cell protein coverage with the FAIMS Pro Interface. High abundance +1 charged contaminants (red check marks) are removed using the FAIMS Pro interface, enabling detection of precursors with a higher charge state (green check marks for +2 or +3 charge) to be detected and improving proteome coverage for low-nanogram to single-cell analysis.

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Summary

For laboratories considering the Orbitrap Exploris 480 mass spectrometer, the Example Datasets provide increased confidence in three important ways. The Example Datasets:

- Empower prospective users to make informed decisions when considering the Orbitrap Exploris mass spectrometer platform for their proteomic workflows including higher proteome coverage for peptide identification, increased sensitivity for single cell analysis, and improved global and targeted quantitation for TMT and SureQuant methods.
- Provide full documentation to support new and current Orbitrap Exploris 480 mass spectrometer users in reproducing the results presented and achieving success.
- Ensure data transparency while enabling users to obtain the highest workflow performance. The Orbitrap Exploris mass spectrometer platform delivers HRAM performance that can be combined with the next-generation differential ion mobility device—the FAIMS Pro interface—to achieve the highest performance proteome coverage, quantitation accuracy and precision, all with exceptional data quality.

Reference

1. Fully Automated Sample Processing and Analysis Workflow for Low-Input Proteome Profiling. Yiran Liang, Hayden Acor, Michaela A. McCown, Andikan J. Nwosu, Hannah Boekweg, Nathaniel B. Axtell, Thy Truong, Yongzheng Cong, Samuel H. Payne, and Ryan T. Kelly; *Anal. Chem. 2020*, https://pubs.acs.org/doi/10.1021/acs.analchem.0c04240

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