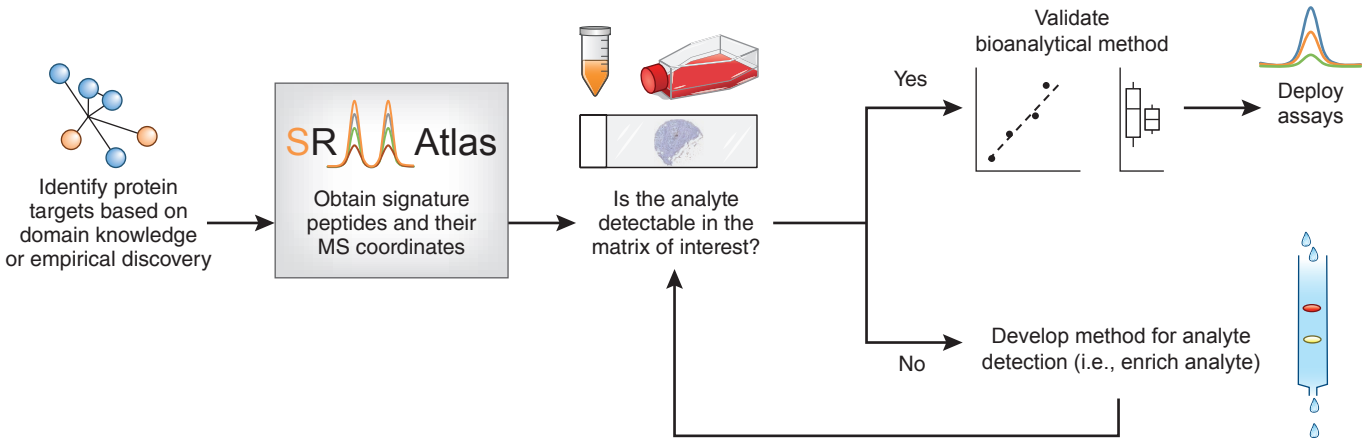

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Figure 1 Workflow for development and implementation of targeted MS assays. Proteotypic peptides uniquely representing proteins of interest can be identified from SRMAtlas, along with fragment ions arising from these peptides that can be monitored by SRM. The next step is to determine whether the selected peptide analytes can be detected by SRM in the biological matrix of interest (e.g., plasma, cell line or tissue lysates). If the peptides are present but not detected by SRM, additional analytical method development is required, such as chromatographic enrichment of the target peptide (e.g., affinity enrichment). Once a procedure is developed that enables peptide detection by SRM, stable isotope–labeled standards are developed and used to perform bioanalytical method validation of the assay using a fit-for-purpose approach in the biological matrix of interest. During assay deployment, quantitative rigor is maintained through the use of QA/QC standards.

peptides in SRMAtlas were not observed in empirical data). This is especially true for studies of complex matrices like plasma or cell lysates, in which many endogenous peptides must be enriched to be quantified by SRM. Furthermore, interferences (e.g., SRM signals that do not arise from the analyte of interest) can be encountered when applying SRM coordinates to complex biological matrices, so it is critical to confirm the specificity of the SRM signal to avoid artifacts.

Guidelines for developing well-characterized targeted MS-based assays, analogous to robust protocols in other areas of quantitative measurement, have been established by the community[9]. To have confidence in experimental results, basic performance metrics of the assay must be characterized, such as imprecision, repeatability, reproducibility, bias, linearity, limit of quantification, matrix effects and selectivity, and analyte stability[10]. An open-source repository of well-characterized targeted MS assays is available[11] (https://assays.cancer.gov/), as is open-source software for data analysis[12]. Integration of these tools will undoubtedly expand the toolbox for all biological researchers, improving the way we study, diagnose, and treat disease. The inclusion of post-translational modifications beyond *N*-glycosylation, such as phosphorylation, will also greatly broaden the utility of the database.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Kusebauch, U. *et al.* Human SRMAtlas: a resource of targeted assays to quantify the complete human proteome. *Cell* **166**, 766–778 (2016).
2. Mertins, P. *et al.* Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature* **534**, 55–62 (2016).
3. Zhang, B. *et al.* Proteogenomic characterization of human colon and rectal cancer. *Nature* **513**, 382–387 (2014).
4. Zhang, H. *et al.* Integrated proteogenomic characterization of human high-grade serous ovarian cancer. *Cell* **166**, 755–765 (2016).
5. Baker, M. Reproducibility crisis: blame it on the antibodies. *Nature* **521**, 274–276 (2015).
6. Hoofnagle, A.N. & Wener, M.H. The fundamental flaws of immunoassays and potential solutions using tandem mass spectrometry. *J. Immunol. Methods* **347**, 3–11 (2009).
7. Kennedy, J.J. *et al.* Demonstrating the feasibility of large-scale development of standardized assays to quantify human proteins. *Nat. Methods* **11**, 149–155 (2014).
8. Hoofnagle, A.N. *et al.* Recommendations for the generation, quantification, storage, and handling of peptides used for mass spectrometry-based assays. *Clin. Chem.* **62**, 48–69 (2016).
9. Carr, S.A. *et al.* Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. *Mol. Cell Proteomics* **13**, 907–917 (2014).
10. Grant, R.P. & Hoofnagle, A.N. From lost in translation to paradise found: enabling protein biomarker method transfer by mass spectrometry. *Clin. Chem.* **60**, 941–944 (2014).
11. Whiteaker, J.R. *et al.* CPTAC Assay Portal: a repository of targeted proteomic assays. *Nat. Methods* **11**, 703–704 (2014).
12. Sharma, V. *et al.* Panorama: a targeted proteomics knowledge base. *J. Proteome Res.* **13**, 4205–4210 (2014).

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