Researchers develop easier and faster way to quantify, explore therapeutic proteins

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Researchers at New Jersey Institute of Technology in collaboration with Ohio University and Merck & Co. Inc. recently developed a new efficient method for targeted protein analysis—one they say could speed

up processes for disease testing, drug discovery and vaccine development.

The research, published in the journal *Analytical Chemistry*, highlights the team's new coulometric mass spectrometric (CMS) approach for determining the quantity of proteins in biological samples, potentially opening new doors for exploring proteins in the human body that may only be expressed at low levels, but which could serve important biological functions or roles as disease biomarkers, drug targets or therapeutic antibodies.

Researchers say the new mass spectrometry and electrochemistry-based approach—capable of accurately quantifying a spectrum of small proteins to large monoclonal antibody drugs—is an advance on current methods in the field of absolute protein quantitation, which typically require time-consuming and costly preparation of synthesized standard material for analysis.

"Measurement of the molecular changes in disease-associated processes and pathways is critical to our understanding of pathogenesis and discovery of new biomarkers for diagnosis and treatment of diseases," said Hao Chen, professor at NJIT's Department of Chemistry and Environmental Sciences, and the corresponding author of the paper. "With our new method, we've shown we can quantify a range of biomolecules accurately and quickly."

"This approach could benefit a range of life sciences research including combating the COVID-19 pandemic for instance, as it could be used to more quickly quantify various antibodies from patients to probe infection stage and to assist vaccine development," Chen added.

In proteomics, mass spectrometry analysis can offer researchers a way of quantifying thousands of proteins in a single experiment, under various

conditions or stimuli. It can also be used to help uncover details about how certain proteins function, interact and change over time in healthy and disease cell states, and can reveal more about level changes of antibodies produced by the immune system to combat antigens, such as viruses or bacteria.

Pengyi Zhao, a Ph.D. researcher in Chen's lab and first author of the paper, says protein quantitation has been typically done through liquid chromatography-mass spectrometry methods that involve preparing synthetic isotope-labeled peptides. These labeled peptides are usually spiked in known concentration into samples to help determine the amount of a protein of interest based on the intensities of the protein's associated peptides relative to the added standards. "The expense and time it takes to synthesize these isotope-labeled peptide standards is a big issue hindering quantitative analysis in research and drug development," explained Zhao.

Chen says the team's new CMS approach instead quantifies proteins based on the electrochemical signature produced during mass spectrometry analysis. "In this method, absolute protein quantitation is based on the electrochemical oxidation of a surrogate peptide from target protein combined with mass spectrometric measurement of the oxidation yield ... this breakthrough opens a new door to investigate many proteins where no standard is available for analysis."

The team demonstrated their new method by analyzing several proteins such as model proteins β-casein and apomyoglobin. In collaboration with Yong-Ick Kim's research group at NJIT, they also successfully quantified a key protein involved in the circadian clock, called KaiB. The team used tyrosine-containing peptides as surrogate peptides for quantitation, finding the results of the CMS analysis comparable in accuracy to the results produced by traditional isotope-labeling methods overall.

"Currently, through this proof-of-concept we've shown this method can accurately quantify various proteins from apomyoglobin to therapeutic antibodies," said Chen. "As our method does not need standards, it would enable a large-scale absolute quantitation analysis of proteins in blood, tissues, or organs, which would need thousands of expensive heavy isotope-labeled peptide standards otherwise. In the next steps, we'll apply this new method for large scale protein quantitation in different biological samples, for disease biomarker discovery."

More information: Pengyi Zhao et al, Absolute Quantitation of Proteins by Coulometric Mass Spectrometry, *Analytical Chemistry* (2020). DOI: 10.1021/acs.analchem.0c01151

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