

Label	Value
<b>Core Facility Name</b>	Mass Spectrometry and Proteomics Facility
<b>Last Name</b>	Robert
<b>First Name</b>	Cole
<b>Email</b>	<a href="mailto:rcole@jhmi.edu">rcole@jhmi.edu</a>
<b>Phone</b>	410-614-6968
<b>Amount of Funding Requested</b>	\$25000
<b>Briefly describe the core services you offer:</b>	<p>The Johns Hopkins University School of Medicine Mass Spectrometry and Proteomics Facility assists investigators with identifying and quantifying proteins and their modifications that are differentially expressed in cells, tissues or body fluids, with tracking interactions with binding partners during changes in signal transduction, and with identifying proteolytic cleavage sites and mapping post-translational modifications by providing the following proteomic services:</p> <p>Consultation: The Core Director, Core Proteomics Specialist and, when appropriate, a Biostatistician, will have pre- and post-analysis discussions with investigators on project goals, experimental design, sample preparation procedures and data analysis as required for successful mass spectrometry analysis.</p> <p>Sample preparation: The Core staff will advise, teach or assist investigators' students and fellows in the current protein extraction and sample preparation techniques, including buffer exchange, column chromatography, proteolytic digestion and stable isotope labeling, to ensure high quality, reproducibility and continuity of sample preparation.</p> <p>Protein Identification: The Core will identify and characterize proteins from complex mixtures of proteins in solution, in gel bands or spots, by liquid chromatography interfaced with tandem MS (LCMS/MS) using the single or multi-dimensional protein identification technology (MuDPIT). Investigators will receive an interactive results file containing all proteins identified, are taught how to navigate through their data and are advised on methods to verify the presence of identified proteins.</p> <p>Protein Modifications: The Core staff will perform or train investigators to enrich samples for modified peptides using chemical (e.g. TiO<sub>2</sub> for Ser/Thr/Tyr phosphorylated peptides) or immunoprecipitation methods (e.g. antibodies to Tyr phosphorylated, acetylated or ubiquitinated peptides). The Core has successfully identified and mapped acetylation, citrullination, O-GlcNAcylation, phosphorylation, proline hydroxylation and S-nitrosation, ubiquitination sites, as well as cleavage and crosslinking sites. Techniques, however, are often customized to each project after discussion with the investigator.</p> <p>Protein Quantification: The Core offers three types of relative quantitative proteomic analyses: (a) label free spectral counting with spiked in standards to compare individual sample analyses; (b) chemical labeling with isobaric mass tags to compare up to 10 samples per experiment using Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) or Tandem Mass Tags (TMT); and (c) metabolic labeling with stable isotope labeled Arg or Lys to compare up to 3 samples per experiment using Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC).</p> <p>Protein Modification Quantification: The Core combines the above protein</p>

	<p>quantification methods with enrichment strategies for modified peptides to quantify post-translational modifications. Thousands of modified peptides may be identified and quantified, and changes in the abundance of a modified site due to protein expression versus site occupancy is determined for modified proteins identified before and after enrichment.</p> <p>High Resolution Mass Analysis: The Core uses liquid chromatography interfaced with mass spectrometry (LCMS) to separate and accurately determine (with better than 100 ppm or 10 Da mass accuracy) the intact mass of proteins (typically up to 50 kDa) in complex mixtures to (a) detect modified forms of a protein; (b) detect proteins in a functional complex; and (c) determine cleavage sites if the N- or C-terminus is known.</p> <p>Data Analysis and Bioinformatics: The Core has several bioinformatics software packages for protein identification, quantification and characterization. Investigators can download free viewers of all Core bioinformatics software from the vendor's web site and, after reviewing with and instruction from Core staff, drill deeper into their results. As needed, biostatistics/bioinformatics consultation with the Facility's collaborators is available for Principal Component Analysis (PCA) and Volcano plots to identify proteins of interest, map proteomic results to canonical pathways using Ingenuity Gene Ontology analysis software or to compare or integrate their proteomic data with other publically available 'omic' data sets.</p> <p>Self-Service Equipment: The Facility's MALDI-TOF mass spectrometer, 2D gel electrophoresis and Typhoon gel imaging equipment are available to investigators after successfully completing a training workshop. Investigators may reserve Core self-service equipment via the Facility's iLabs website (<a href="https://skcccjhmi.corefacilities.org/account/login">https://skcccjhmi.corefacilities.org/account/login</a>).</p>
<p><b>What specific services do you plan to offer as part of this RFA?</b></p>	<p>We plan to offer Protein Quantification and Protein Modification Quantification services using isobaric mass tags as part of this RFA. These services will be offered for global quantitative proteomic experiments as well as quantitative experiments focusing on a specific protein or the protein's modifications.</p>
<p><b>How do these services address the goals of the pilot program?</b></p>	<p>Offering Protein Quantification and Protein Modification Quantification services as part of this pilot program will address the goal of the pilot program by providing basic, clinical and translational research investigators, who are not currently funded, a new mechanism to access these Proteomics Core services.</p> <p>Quantitative proteomic experiments, especially ones that involve quantifying changes in protein modifications, require many hours of mass spectrometer time and core staff effort. The cost can range from \$5,000 to \$10,000 to globally compare the relative abundance of proteins or their modifications in 10 samples. The pay back is quantifying 5,000 to 10,000 proteins or specific protein modifications across 10 samples, and identifying potential metabolic pathways or biomarkers of affected normal and disease cellular processes. Even less global quantitative experiments, focusing on changes in a specific protein or the protein's modifications can become costly, typically starting at \$1000.</p> <p>The cost of these quantitative proteomic experiments is often a major barrier to investigators needing these services but not currently funded or with insufficient funds request them. Thus, the Core Coins will provide these investigators a rapid, targeted funding mechanism for Protein Quantification or Protein Modification Quantification, and encourage them to use the Proteomics Core to acquire the preliminary, proof of principal or even confirmation data to fill gaps in their research that are critical for publication or for strengthening their grant applications.</p>
<p><b>How would you select recipients to receive</b></p>	<p>As soon as the Core Coins mechanism is in place within iLabs, a broadcast email will be sent to the Hopkins research community announcing this Core Coins Pilot Program and</p>

<p><b>core services? Please describe the process and criteria you might use.</b></p>	<p>that the Proteomics Core is seeking proposals to supplement pilot projects that would benefit from our Protein Quantification or Protein Modification Quantification services. Investigators should submit no more than a 1 page application describing how quantifying proteins or modifications globally or on a specific protein in up to 10 samples will advance their research program by providing data for publication, to secure additional funding for completing, expanding or validating these proteomics experiments, or to fund additional Proteomics Core services. The investigator's NIH biosketch should accompany the application. Preference will be given to hypothesis driven projects requiring quantitative proteomics by faculty who do not currently have sufficient funds to support these experiments and who need to generate preliminary, proof of principal or confirmation data that are critical for publication or for a grant application to be submitted within a year of receiving the data. New users of the Proteomics Core, junior faculty and faculty in the last year of their funding will be encouraged to apply. Applications will be due two months after issuing the announcement.</p> <p>A committee consisting of myself and two other faculty will, within two weeks from the application due date, review and rank the proposals based on novelty, impact, feasibility and weighted for potential success in competing for future funding. Drs. John Groopman and Hui Zhang have agreed to serve on the review committee. Drs. Groopman and Zhang use mass spectrometry in their research, have used many of the Proteomics Core services and have published quantitative proteomics data generated by the Proteomics Core. The goal will award 4 to 6 investigator quantitative proteomics projects by three months of receiving the core coins.</p>
<p><b>How do you plan to allocate the amounts available to individual investigators?</b></p>	<p>Projects requiring Protein Quantification or Protein Modification Quantification services will receive \$2,500, \$5,000 or \$7,500 awards to compare protein or protein modifications on a specific protein or proteins globally in up to 10 samples in one TMT 10-plex quantitative experiment. These quantitative experiments currently cost \$2,000 to \$3,500 to quantify a specific protein or the protein's specific modification, \$5,750 to globally compare proteins, or \$7,830 to globally compare a specific modification in 10 samples. At least one award at each of the three dollar amounts will be funded by the Core Coins. Thus, the \$25,000 Core Coins, therefore, will support up to six investigators (one \$7,500, two \$5,000 and three \$2,500 awards), to supplement their quantitative experiments. However, other award combinations will be considered and are dependent on the applications received.</p> <p>One year after receiving these Core Coins the investigators will be surveyed to determine the publications and submitted grants that were supported by the data generated in this Core Coins program.</p>