Label	Value
Core Facility Name	Mass Spectrometry and Proteomics Facility
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Amount of Funding Requested	$25,000
Briefly describe the core services you offer:	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: 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. 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. 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. Protein Modifications: The Core staff will perform or train investigators to enrich samples for modified peptides using chemical (e.g.TiO2 for Ser/Thr/Tyr phosphorylated peptides) or immunoprecipitation methods (e.g. antibodies to Tyr phosphorylated, acetylated, citrullinated 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 used, however, are often customized to each project after discussion with the investigator. 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 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). Protein Modification Quantification: The Core combines the above protein 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. High Resolution Mass Analysis (HRMS): The Core uses liquid chromatography (LC) and, now, capillary electrophoresis (CE) interfaced with mass spectrometry (MS) to separate and accurately determine within 100 ppm or 5 Da mass accuracy the intact mass of proteins in complex mixtures. HRMS analysis is typically, but not exclusively, performed on proteins up to 50 kD for applications that include (a) detecting the forms and stoichiometry of a modified protein; (b) detecting proteins in a functional complex; (c) identifying cleavage sites if the N- or C-terminus is known; and (d) to create a spectral library of a protein. 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. 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 iLab Solutions website (https://johnshopkins.corefacilities.org/landing/42).
What specific services do you plan to offer as part of this RFA?	We plan to offer Protein Quantification and Protein Modification Quantification services using isobaric mass tags and High Resolution Mass Analysis 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.
How do these services address the goals of the pilot program?	Offering Protein Quantification, Protein Modification Quantification and High Resolution Mass Analysis services as part of this pilot program will address the goals 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. For Hopkins investigators considering similar services at other institutions, offering these services with supplemental funding will encourage them to use the Hopkins core. Successful mass spectrometry analysis will encourage them to continue working our cores. 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 just to create a spectral library of a protein. 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, or who have not previously worked with the core. Thus, the Core Coins will provide these investigators a rapid, targeted funding mechanism for Protein Quantification, Protein Modification Quantification or High Resolution Mass Analysis, 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. Our successful 2016 Core Coins application. provided quantitative proteomic services to 9 Hopkins investigators from a pool of 37 applications. The three member Review Committee gave preference to junior faculty, faculty in the last year of funding, and new users of the core. The resulting 9 Core Coins awardees were equally represented by rank (33% Assistant, 33% Associate, 33% Full Professor) and gender (54% female, 44% male) with 78% of these awardees being new core users. The awards ranged from $2,500 to $4,000 to maximize the number of labs funded. Award amounts demographics closely paralleled the awardee rank, gender and core user demographics.
How would you select recipients to receive core services? Please describe the process and criteria you might use.	A week after of the Core Coins award is in place within core's iLab Solutions center, a broadcast email will be sent to the Hopkins research community announcing this Core Coins Pilot Program and that the Proteomics Core is seeking proposals to supplement pilot projects that would benefit from our Protein Quantification, Protein Modification Quantification or High Resolution Mass Analysis 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. The Proteomics Core Coins awardees from the previous year are not eligible. Applications will be due two months after issuing the announcement. A committee consisting of myself and two other faculty will, within three weeks from the application due date, review and rank the proposals based on novelty, impact, feasibility and weighted for potential success in completing a publication or 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, use many of the Proteomics Core services and have published quantitative proteomics data generated by the Proteomics Core. The goal is to award 4 to 9 investigator quantitative proteomics projects by three months of receiving the core coins award.
How do you plan to allocate the amounts available to individual investigators?	Projects requiring Protein Quantification, Protein Modification Quantification or High Resolution Mass Analysis 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 $5,000 to quantify a specific protein or the protein’s specific modification, $6,000 to globally compare proteins, or $8,000 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. For example, last year the Review Committee distributed the Core Coins award to support 9 investigators. One year after receiving data generated by these Core Coins award, the investigators will be surveyed to determine the publications and submitted grants that were supported by the data generated in this core coin program. Four awardees from last year are still

	generating data. However, 5 of the 9 core coins awardees are in the process of analyzing their data for publications or grant applications.