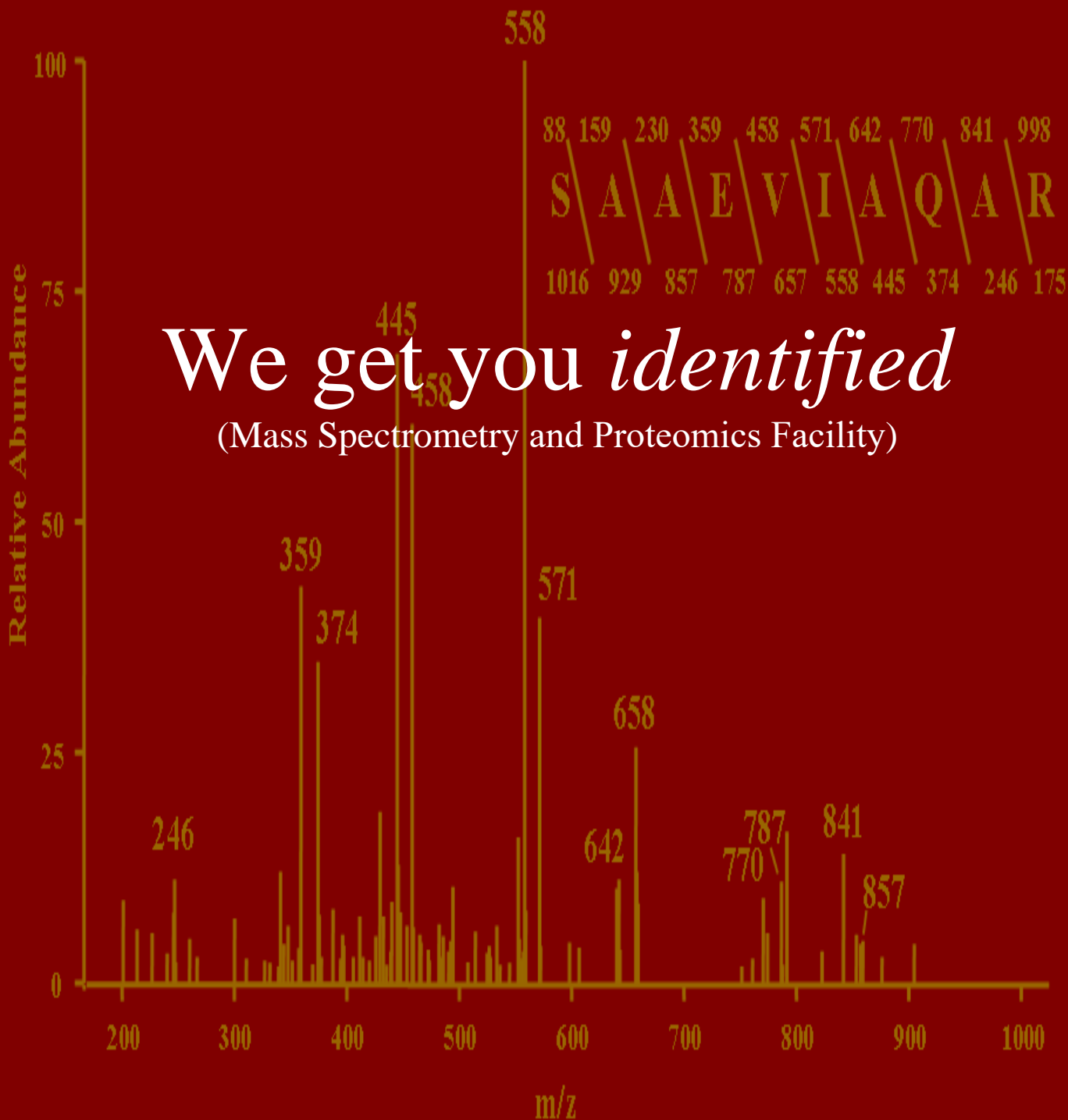


P47942

Dihydropyrimidinase-related protein 2 (DRP-2) (Turned on after division, 64 kDa protein) (TOAD-64) (Collapsin response mediator protein 2) (CRMP-2)  
gi|1351260|sp|P47942|DPYL2\_RAT[1351260]



## THE JOHNS HOPKINS SCHOOL OF MEDICINE MASS SPECTROMETRY AND PROTEOMICS FACILITY

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Co-Director of Mass Spectrometry Molecular Imaging and Multi-Omics Shared Resource  
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### Services:

- **Consultation:** Pre- and Post-analysis on Experimental Design and Data Analysis
- **Sample preparation:** Buffer exchange; Column Chromatography (fractionation, enrichment, depletions); Proteolytic digestion; Isobaric Mass Tag (TMT) labeling
- **Protein Identification:** Proteins (solution, gel bands/spots, complex protein mixtures, proximity, IPs), DDA (Data Dependent Acquisition), DIA (Data Independent Acquisition)
- **Protein Modifications:** Acetylation, AMPylation, biotin, citrullination, glycosylation, phosphorylation, proline hydroxylation, nitrosation, ubiquitination and novel cleavage sites, etc. Ever growing list of known and custom modifications
- **Protein Quantification:**  
Relative Quantification: Tandem Mass Tags (TMT 10, 18 and 32 plexes), SILAC\*  
Targeted Quantification: Parallel Reaction Monitoring (PRM), Internal Standard Triggering PRM (IS-PRM or SureQuant), Absolute Quantification (AQUA), Protein Standard Absolute Quantification (PSAQ)
- **High Resolution Mass Analysis:** Protein characterization and their modifications
- **Biostatistical/Bioinformatic analysis:** PCA analysis, Volcano plots, Heat Maps, Limited gene ontology; Bioinformatics consultation
- **Training Workshops:** MALDI
- **Self Service:** MALDI, Gel Electrophoresis, Scanners, Bioinformatic Software

**Description:** The Johns Hopkins University (JHU) School of Medicine (SOM) Mass Spectrometry and Proteomics Facility couples mass spectrometry to one and two dimensional separations by column chromatography or gel electrophoresis to identify, quantify or characterize proteins and their post-translational modifications, that are present in complex protein extracts or isolated in solution, gel bands or spots from cells, tissues or body fluids.

**Service Costs:** The costs of services are ala carte (Table 1) and depend of what is required to acquire high quality data. Table 2 provides estimates based on straight forward analysis of samples. However, because each sample and project has it's own unique challenges, costs may vary due to additional procedures required for successful mass spectrometry analysis. **FY2024 rates will increase by 3% after July 1, 2024.**

**TABLE 1: FY2024 HOPKINS RATES FOR CORE STAFF PROVIDED SERVICES**

Service	Charge	Unit
Consultation	Free	
Buffer Exchange	\$ 46	per sample
Proteolysis: Solution, In-gel, On-bead	\$ 62	per sample
Proteolysis: FASP	\$ 87	per sample
Labeling: Chemical	\$ 142	per sample
Fractionation: Liquid Chromatography	\$ 970	per sample
Fractionation: Step Fractionation	\$ 113	per sample
Fractionation: Phosphopeptide Enrichment (TiO2)	\$ 57	per sample
Fractionation: Immunodepletion	\$ 128	per sample
Fractionation: Gel Electrophoresis	\$ 128	per sample
Fractionation: HFIP Elution from streptavidin	\$ 30	per sample
MS Analysis: Gradient, 1 hr	\$ 67	per sample
MS Analysis: Gradient, 2 hr	\$ 134	per sample
Data Analysis: Database Searching	\$ 12	per sample
Data Analysis: Custom Database	\$ 84	per sample
Data Analysis: Deconvolution	\$ 84	per sample
Data Analysis: Targeted	\$ 84	per sample
Data Analysis: Custom Data Analysis	\$ 84	per database
Data Reporting	\$ 5	per analysis
Training: MALDI	\$ 190	per person
Self Service: MALDI (per hour)	\$ 140	per hour
Method Development:	Inquire	per method

**TABLE 2: ESTIMATED COSTS FOR COMMON ANALYSES AT FY2024 RATES**

Service	Unit	Estimated Cost
Protein ID (solution), DDA, DIA or PRM	per sample	\$258 to \$304
Protein ID (gel bands), DDA, DIA or PRM	per band	\$258 to \$304
PTM Mapping with Enrichment	per sample	\$460
Proteomic Comparison without TMTs	per sample	\$4,575
	24 fractions	
Proteomic Comparison with TMTs	per 18 samples	\$8,995
	24 fractions	
Phosphoproteomic Comparison with TMTs	per 18 samples	\$11,419
	24 fractions proteome	
	12 fractions phosphopeptide	

**Description of Services:**

**(1) Consultation (Pre/Post Data Acquisition):** Requests for services begins with an initial consultation between the investigator, Core Director, Core Proteomics Specialist and, when appropriate, the Bioinformatician to discuss the project goals, experimental design and sample preparation procedures required for successful mass spectrometry analysis. These consultations provide an opportunity to gauge the difficulty of the analysis, eliminate any confusion about sample preparation and, most importantly, increase the chances for successful analysis. At these meetings, Core handouts are provided explaining how to prepare and submit samples. The Core's Proteomics Specialists will advise, teach and assist the investigator's students and fellows with the current sample preparation techniques. After collecting the data, the Core Director and staff will meet with investigators to help with data interpretation, instruct fellows or graduate students in using software for re-interrogating their data, work with the Biostatistician/Bioinformatician to identify significant changes in proteins, modifications or pathways, and develop a plan for the next step in their research requiring proteomic services and/or validation of proteomic findings.

**(2) Sample Preparation and Proteolytic Digestions:** To increase quality, reproducibility and continuity of sample preparation in the investigators' lab, the Core's staff will advise, teach or assist the investigator's students and fellows in the current protein extraction and sample preparation techniques. Alternatively, the Core staff will provide the following sample preparation services: buffer exchange (TCA/acetone precipitation, affinity or filtration spin columns or resin filled pipet tips); reduction and alkylation of proteins; proteolytic digestion; isobaric mass tag labeling (TMTs); enrichment protocols for modified peptides; or peptide fractionation by strong cation exchange (SCX) or basic reverse phase (bRP) chromatography. When requested, the Core staff will gladly train the investigator's students interested in performing these techniques.

**(3) Mass Measurement (MALDI-TOF or High Resolution Mass Analysis):** The Core uses MALDI-TOF or liquid chromatography interfaced with mass spectrometry to separate and accurately determine the intact mass of peptides or proteins 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. The high resolution methods can measure masses up to 50 kDa using 10 pmol per protein, with mass accuracy of at least 100 ppm (or 10 Da). Although higher masses are more challenging and highly protein dependent, the core has successfully measured masses above 50 kD.

**(4) Protein Identification:** The Core routinely identifies and characterizes 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). Proteome Discoverer extracts and searches MS data against protein databases using the Core's three license Mascot server or Sequest software to identify proteins in investigator samples. Mascot or Sequest search results are imported into Scaffold for reporting results to the investigator. A Scaffold viewer is available free of charge to investigators at <http://www.proteomesoftware.com/products/free-viewer>. Core staff review Scaffold results with investigators to assist with determining significant protein identifications based on the standard criteria of > 2 peptides identified from the same protein with individual peptide scores >95% confidence probability score at 1% FDR and sub 3 ppm mass error. Investigators received an interactive Scaffold file containing all proteins identified, are taught how to use Scaffold to navigate through their data and are advised on methods to verify the presence of MS identified proteins.

**(4) Protein Modification Identification:** 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). Immunoprecipitations are performed in the client lab. The Core has successfully identified and mapped acetylation, AMPylation, citrullination, O-GlcNAcylation, phosphorylation, proline hydroxylation and S-nitrosation, ubiquitination sites, as well as cleavage and crosslinking sites. Techniques used to determine the modification and amino acids modified are customized to each project after discussing options with the investigator.

**(5) Protein Quantification (Discovery or Relative Quantitative Proteomics):** The Core offers label and label-free relative quantitative proteomic analyses: (a) Label approach couples Multi-Dimensional Protein Identification Technology (**MuDPIT**) basic C18 reverse phase (bRP) chromatograph followed by acidic C18 reverse chromatography with Tandem Mass Tags (**TMT**) for chemical labeling with isobaric mass tags to compare up to 18 samples per experiment. 32 plex will be available Fall 2024. Analysis includes experimental design, protein digestions, TMT labeling, bRP separation in up to 24 fractions, tandem MS, protein identification and quantification. Similar analysis is available for Stable Isotope Labeling of Amino Acids in Cell Culture (**SILAC**) using metabolic labeling with stable isotope labeled Arg or Lys to compare up to 3 samples per experiment. However, SILAC labeling is performed in the investigators lab. Investigators receive an interactive Proteome Discoverer (PD) file containing quantification of all proteins and modified peptides identified, Volcano plots, PCA plots, Heat Maps, and are taught how to navigate through their data using a free PD viewer (<http://portal.thermo-brims.com/>). Core staff will advise on methods to validate fold changes of MS identified proteins. TMT and SILAC all provide relative protein quantification between multiple samples during a single experiment. B) Label-free approach used Data Independent Analysis (**DIA**) to capture and identify all ions in a wide isolation windows across the entire mass range survey. Quantification is based on summing the intensity of the peptide fragmentations. MSFragger or Chimerys is used to link the coisolating precursor peptide masses to their corresponding fragment ions for identification and quantification. For projects requiring measuring the amount of a protein in a sample, the core has workflows for Absolute Quantification or Protein Standard Absolute Quantification using stable isotope labeled synthetic peptides or recombinant proteins, respectively, as reference standards (see service 7).

**(6) Protein Modification Quantification:** The Core combines the above protein quantification methods with enrichment strategies for modified peptides to quantify post-translational modifications. These workflows start with 0.1 to 1 mg of total protein. After labeling, 5% of the sample is analyzed before enrichment and the remaining 95% is analyzed after enrichment. With this approach, thousands of modified peptides may be identified and quantified for investigators. Changes in the abundance of a modified site due to protein expression versus site occupancy is also determined for modified proteins identified before and after enrichment, or if the time course of the experiment precludes changes due to protein synthesis or inter-compartment trafficking. The Core is experienced with quantifying many protein modifications, most notably, phosphorylation and acetylation sites.

**(7) Targeted (or Absolute) Quantitative Proteomics:** The Proteomics Core offers three targeted (often called "absolute") quantitative proteomic workflows. Product Reaction Monitoring (PRM), Absolute Quantification (AQUA) and Protein Standard Absolute Quantification (PSAQ), using heavy stable isotopic peptides or recombinant proteins, respectively, to trigger and quantify specific proteins of interest in investigators samples. These techniques allow an investigator to (a) validate changes in proteins or modifications determined from relative abundance data in DIA, TMT or SILAC experiments, especially when antibodies are not available or prohibitively expensive; (b) measure the protein of interest's concentration in samples so as to correlate protein 'biomarker' concentration with specific disease phenotypes; and (c) follow changes in proteins specific to pathways associated with a disease. Because this is a highly customized service, consultation between the Core Director and Investigator is required to determine practical and economic aspects as well as division of labor.

**(8) Data Analysis and Bioinformatics:** The Core has several bioinformatics softwares for protein identification, quantification and characterization. Investigators can download free viewers of all Core proteomic software from the vendor's web site and, after reviewing with and instruction from Core staff, drill deeper into their results. Following the Core's proteomic analysis, the investigators are informed that they can work with Connie Talbot in the HIT Center for more advanced Principal Component Analysis (PCA) and Volcano plots to identify proteins of interest, map proteomic results to canonical pathways using Ingenuity Gene Ontology analysis software. Core will staff transfer data to and work with the investigator's bioinformaticist (such as Conover Talbot) and assist investigators with uploading MS data and results to public web sites.

**(9) Training and Self-Service Equipment:** In addition to working with each Investigator on individual projects, the Core offers workshops to use the Core's MALDI-TOF mass spectrometer and gel electrophoresis equipment. MALDI-TOF training consists of three hours hands-on instruction in sample preparation, spectra acquisition and analysis using the facility Voyager DE-STR and is offered monthly for up to four attendees per session.