Yale/NIDA Neuroproteomics Center Newsletter

(Established 8/23/2004/Supported by NIH/NIDA Grant DA018343) March, 2019

External Advisory Board Meeting (EAB)

The Center's next EAB Meeting will be on Wednesday, May 1, 2019. This meeting, which is open to the Yale community, will extend from 9:00 AM to about 4:00 PM and will feature >15 talks by Core staff, Center investigators and/or their staff who have publications during the last four years that utilized Core analyses, and Pilot Project awardees. The EAB Meeting will be preceded by a continental breakfast beginning at 8:40 AM and will include a buffet lunch. A detailed program will be distributed via email to all Center Members as soon as it is available. Since the EAB submits a written report to NIDA that will be incorporated into our next competing grant application, which will be submitted for the 9/26/19 deadline, and since NIH/NIDA staff often attend our EAB meetings; we ask that as many Center Investigators and members of their laboratories as well as Pilot Project Grant Awardees as possible please attend this meeting. Attending the EAB Meeting will give Center members a unique opportunity to learn more about the Center's research, technologies available from its Cores, and to interact with other Center Investigators, Core Directors and their staff. We hope that all of you will continue to support the Yale/NIDA Neuroproteomics Center by attending this very important meeting!

***New* Mass Spectrometry-Based Assays for Quantifying Postsynaptic Density (PSD) Proteins**

The Center has developed novel, mass spectrometry-based approaches for quantifying protein expression changes in mouse/rat PSD proteins. The Data Independent Acquisition (DIA) assay relatively quantifies 14,273 peptides that derive from 2,134 mouse PSD proteins at 2 or more peptides/protein and 1% false discovery rate (FDR). This DIA assay can be used for rat and other species after generating a spectral library for the species of interest. The Parallel Reaction Monitoring (PRM) assay uses internal standard (heavy) synthetic peptides to absolutely quantify 138 peptides and 50 mouse/rat PSD proteins at 1-3 peptides/protein. We anticipate that the PRM assay, which can be expanded to include additional proteins and/or their post-translational modifications, will often be used to validate changes observed initially with the DIA assay. If you would like more information and/or if you are interested in using either or both of these assays to support your Center research project, please contact Dr. Rashaun Wilson (rashaun.wilson@yale.edu) from the Discovery Proteomics Core.

Pilot Projects Grants Opportunity

The Center is delighted to announce that five Pilot Project grants have been awarded in the current Grant Year 14 (**Table 1** below) and that applications are now being accepted for Pilot Projects for Grant Year 15. The goals of this program are to encourage young investigators in our Center's laboratories to embark on careers in substance abuse research, disseminate the Center's core technologies to researchers investigating the neurobiology of addiction who are not yet using neuroproteomics technologies, and to expand the technical abilities of the Center. Pilot Grants provide short term funding to obtain preliminary data so applicants can apply for longer term grant support. These awards provide $7,500 D.C. to help pay for the cost of preparing samples for analysis in the Center's Cores and free access to all of the Center's Cores. Applications are accepted from: 1) Center investigators and 2) their Graduate Students, Postdoctoral Fellows and higher level research staff, 3) non-Center investigators expert in substance abuse with interests in initiating research in neuroproteomics, 4) non-Center investigators with expertise in cellular and molecular aspects of neuronal signaling with interests in initiating neuroproteomics research. Awards will be for one year for research that is directly related to the Center's theme of the "Proteomics of Altered Signaling in Addiction" and that propose to apply existing technologies from the Cores or to develop new technologies. Awards to non-Center investigators will be accompanied by Center membership for the term of the award. Priority will be given to new projects that are related to our Center's theme, to collaborative projects involving multiple investigators, and to Technology Development projects. The receipt deadline for Grant Year 15 applications is May 1, 2019. Additional information: **http://medicine.yale.edu/keck/nida/general/pilot_grants.aspx.** Please contact the Director of the Pilot Projects Core, Dr. Marina Picciotto (marina.picciotto@yale.edu), to ensure that new projects and potential pilot project proposals qualify for Center support.

***New* ProteomicsBrowser: MS/Proteomics Data Visualization and Investigation**

As described in Gang et al. (Bioinformatics, 2018 Nov 21 [Epub ahead of print] PMID:30462190), the first fully functional version of ProteomicsBrowser is now available for download from the Yale/NIDA Neuroproteomics Center website: https://medicine.yale.edu/keck/nida/proteomicsbrowser.aspx. Large-scale, quantitative proteomics data

are being generated at ever increasing rates by high-throughput, mass spectrometry technologies. However, due to the complexity of these large datasets as well as the increasing numbers of post-translational modifications (PTMs) that are being identified, developing effective methods for proteomic visualization has been challenging. ProteomicsBrowser was designed to meet this need for comprehensive data visualization. Using peptide information files exported from mass spectrometry search engines or quantitative tools as input, the peptide sequences are aligned to an internal protein database such as UniProtKB. As described in the User Guide, which is also available for download at the above site, after importing LC/MS/MS data from a study that typically contains multiple "control" versus "experimental" samples, ProteomicsBrowser organizes the project into two sections: Data and Browser. The Data section allows users to view the peptide and protein data in a table and to perform some statistical analyses. The key function of ProteomicsBrowser is its Browser that was designed to help users visualize peptides in a protein of interest in any one of the samples in the project being analyzed. As shown in **Fig. 1**, the Browser depicts each peptide as a horizontal gray bar (box) above the corresponding sequence. The peptide ions are aligned along the parent protein with overlapping peptides being stacked vertically as shown in **Fig. 1**. The intensity of the gray color of each bar indicates the relative peptide abundance with color-coded vertical lines in the bars indicating the positions and type of PTMs that were identified in the selected protein. A particularly novel feature of the Browser is its ability to simplify the depiction of related peptides by combining peptide ions (**Fig. 1**) based on their sequence, charge, and/or specific PTMs. This simplified depiction based on sequence, charge, and/or PTM should greatly aid in the analysis and interpretation of complex LC/MS/MS data sets. In addition, the unique "Quantify PTM" option can be used to rapidly determine the extent of post-translational modification at a selected residue in a protein of interest by combining all of the overlapping peptide ions that contain the PTM of interest into one group and all of the overlapping peptide ions that do not contain that PTM into a second group. The integrated areas of the peptides with the selected PTM are summed and depicted as a single peptide box. Similarly, the overlapping peptides without the PTM of interest are combined into a different peptide box. The extent of modification then can be easily calculated from the ratio of the sum of the integrated peak areas of the combined PTM peptides to the sum of the combined PTM + non-PTM peptides. Another unique feature of ProteomicsBrowser is its ability to export a text file or figure that shows the amino acid frequencies around a PTM of interest similar to a BlockLogo tool.

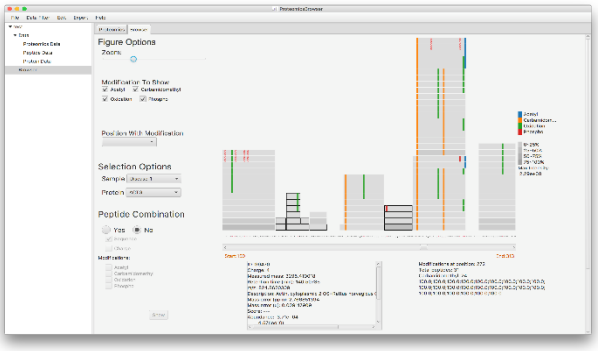

Fig. 1. GUI of ProteomicsBrowser. Peptide ions identified in the ACTG protein from the Disease-1 sample. The left sidebar allows the user to customize and control the analysis procedure, including using the Selection Options to choose a particular sample, a particular protein in that sample, and changing the scale of visualization with the Zoom control. The center panel presents the overall visualization of the alignment of the identified and quantified peptides. As shown, with the Zoom control near the mid position, it is possible to visualize residues 150-313 as designated by the red font "Start 150" on the left and the "End 313" on the right. From Peng et al., 2019

All of the results and Browser views can be exported to text files or figures to facilitate publication. We believe that ProteomicsBrowser will leverage recent advances in LC/MS/MS instrumentation by facilitating the optimal selection of proteotypic peptide ions for designing targeted proteomics analyses such as Parallel Reaction Monitoring (PRM), and in the determination of the overall extent of modification of individual PTMs. These capabilities are augmented by the inclusion in the ProteomicsBrowser of selected statistical analyses tools and a wide range of options for filtering the data. We will continue to improve the ProteomicsBrowser and to add new features based on user feedback. If you have questions about the ProteomicsBrowser or have suggestions for improving it please contact Dr. Gang Peng (gang.peng@yale.edu) in the Bioinformatics and Biostatistics Core (BBC)

Special Issue of the Journal *Proteomes* on Neuroproteomics

With 15 published articles and one manuscript in press, this Special Issue on Neuroproteomics that was Co-Edited by Co-Directors Angus Nairn and Kenneth Williams is nearing completion. As described in more detail here: https://www.mdpi.com/journal/proteomes/special_issues/neuroproteomics, this issue contains articles by Investigators Thomas Biederer, Lakshmi Devi, Elizabeth Eipper, Marina Picciotto, Jane Taylor, Mary Torregrossa, and Angus Nairn and Pilot Project Awardees Becky Carlyle, Drew Kiraly, and Deborah Schechtman. Included among the other contributors are Steven Arnold, Anthony Baucum, Seth Grant, Fernanda Laezza, Joongkyu Park, Junmin Peng, Jeffrey Savas, and John Yates.

Table 1: Grant Year 14 Pilot Projects

PI	Title	Institution	Center Investigator	Non-Center Investigator Mentor	Project Title
Caleb Browne	Postdoctoral Fellow	Mount Sinai School Medicine	Eric Nestler	NA	Proteomic profiling of NAc synaptosomes during early and extended withdrawal from self-administered cocaine
Ayanabha Chakraborti	Senior Research Associate	U. Alabama	James Bibb	NA	Profiling the Nucleus Accumbens proteome in an experimental model of inflammatory bowel disease
Tina Franklin	Postdoctoral Associate	Yale U.	NA	Ron Duman	Stress-induced dysregulation in microglial HMGB1 signaling
Stephanie Groman	Associate Research Scientist	Yale U.	Jane Taylor	NA	A 'targeted' approach to identify the proteins underlying the biobehavioral mechanisms of addiction
Angela Lee	MD/Ph.D. Student	Yale U.	Marina Picciotto	NA	Sex differences in nicotine-induced changes of the mouse brain proteome

Table 2: Yale/NIDA Neuroproteomics Center Cores

Core	Name	Role	Email
Administrative	Angus Nairn	Co-Director/PI	angus.nairn@yale.edu
	Kenneth Williams	Co-Director/PI	kenneth.williams@yale.edu
Discovery Proteomics	TuKiet Lam	Core Director and Protein Identification and Profiling	tukiet.lam@yale.edu
	Pietro DeCamilli	Analysis of Phosphoinositides, Their Interactions, Metabolism, and Transport; Recombinant Protein Expression and Purification	pietro.decamilli@yale.edu
	Ewa Folta-Stogniew	Biophysics	ewa.folta-stogniew@yale.edu
	Angus Nairn	Recombinant Protein Expression and Purification	angus.nairn@yale.edu
Targeted Proteomics	Kenneth Williams	Core Director	kenneth.williams@yale.edu
Bioinformatics and Biostatistics Core (BBC)	Angus Nairn	Core Director	angus.nairn@yale.edu
	Robert Bjornson	High Performance Computing	robert.bjornson@yale.edu
	Mark Gerstein	Bioinformatics	mark.gerstein@yale.edu
	Hongyu Zhao	Biostatistics	hongyu.zhao@yale.edu
Pilot Research Projects	Marina Picciotto	Core Director	marina.picciotto@yale.edu

2018 Publications from the Yale/NIDA Neuroproteomics Center

Please acknowledge the Center's grant, DA018343, in all publications that were supported by the Center's Cores and/or Pilot Project Grants

1. Benedetti, L., Barentineb, A., Messa, M., Wheeler, H., Bewersdorf, J., De Camilli, P. (2018) Light-activated protein interaction with high spatial subcellular confinement. Proc. Natl. Acad. Sci. 115(10):E2238-E2245 (PMCID: PMC5877946, PMID: 29463750)
2. Bertholomey, M.L., Stone, K.L., Lam, T.T., Bang, S., Wu, W., Nairn, A.C., Taylor, J.R., Torregrossa, M.M. (2018) Phosphoproteomic analysis of the amygdala response to adolescent glucocorticoid exposure reveals G-protein coupled receptor kinase 2 (GRK2) as a target for reducing motivation for alcohol. Proteomes Special Issue on Neuroproteomics 6(4), 41 (PMCID: PMC6313880, PMID:30322021).
3. Bian, X., Saheki, Y., and De Camilli, P. (2018) Ca2+ releases E-Syt1 autoinhibition to couple ER-plasma membrane tethering with lipid transport. EMBO J. 37(2) :219-234 (PMCID: PMC5770786 (Available on 2019-01-17), PMID: 29222176)
4. Carlyle, B.C., Kitchen, R.R., Zhang, J., Wilson, R., Lam, T.T., Rozowsky, J.S., Williams, K., Sestan, N., Gerstein, M.B., Nairn, A.C. (2018) Isoform level interpretation of high-throughput proteomic data enabled by deep integration with RNA-seq. J. Proteome Research 17(10), 3431-3444 (PMCID:PMC6392456 available 10/5/19, PMID: 30125121).
5. Cijsouw, T., Ramsey, A., Lam, T., Carbone, B., Blanpied, T., Biederer, T. (2018) Mapping the Proteome of the Synaptic Cleft Through Proximity Labeling Reveals New Cleft Proteins. Proteomes Special Issue on Neuroproteomics 6(4), 48 (PMCID: PMC6313906, PMID:30487426).
6. Dong, R., Zhu, T., Benedetti, L., Gowrishankar, S., Deng, H., Cai, Y., Wang, X., Shen, K., De Camilli P. (2018) The inositol 5-phosphatase INPP5K participates in the fine control of ER organization J. Cell Biol. 217(10):3577-3592 (PMCID:PMC6168264 (Available on 2019-04-01), PMID: 30087126).
7. Kumar, N., Leonzino, M., Hancock-Cerutti, W., Horenkamp, F., Li, P., Lees, J., Wheeler, H., Reinish, K., De Camilli, P. (2018) VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. J. Cell Biology 217(10):3625-3639 (PMCID: PMC6168267 (Avail. 2019-04-01), PMID:30093493).
8. Levy, A., Xiao, X., Shaw, J., Devi, S., Katrancha, S., Bennett, A., Greer, C. Howe, J., Machida, K., and Koleske, A. (2018) Noonan Syndrome-Associated SHP2 Dephosphorylates GluN2B to Regulate NMDA Receptor Function. Cell Reports 24, 1523-1535 (PMCID: PMC6234505, PMID: 30089263).
9. Li, D., Musante, V., Zhou, W., Picciotto, M.R., Nairn, A.C. (2018) Striatin-1 is a B subunit of protein phosphatase PP2A that regulates dendritic arborization and spine development in striatal neurons. J.Biol.Chem. 293(28):11179-11194 (PMCID: PMC6052221 (Available on 2019-07-13), PMID: 29802198).
10. Luxmi, R., Blaby-Haas, C., Kumar, D., Rauniyar, N., King, S., Mains, R., Eipper, B. (2018) Proteases shape the Chlamydomonas secretome: Comparison to classical neuropeptide processing machinery. Proteomes Special Issue on Neuroproteomics 6(4), 36 (PMCID: PMC6313938, PMID: 30249063).
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12. Miller, M.B., Wilson, R.S., Lam, T.T., Nairn, A.C., Picciotto, M.R., (2018) Evaluation of the phosphoproteome of mouse alpha 4/beta 2-containing nicotinic acetylcholine receptors in vitro and in vivo. Proteomes Special Issue on Neuroproteomics 6(4), 42 (PMC6313896, PMID: 30326594).
13. Milovanovic, D., Wu, Y., Bian, X. De Camilli, P. (2018) A liquid phase of synapsin and lipid vesicles. Science 361, 604-607.(PMCID: PMC6191856, PMID:29976799).
14. Park, J. (2018) Phosphorylation of the AMPAR-TARP complex in synaptic plasticity. Proteomes Special Issue on Neuroproteomics 6(4), 40 (PMCID: PMC6313930, PMID: 30297624).
15. Peng, G., Wilson, R., Tang, Y., Lam, T. Nairn, A., Williams, K., Zhao, H. (2018) ProteomicsBrowser: MS/Proteomics Data Visualization and Investigation, Bioinformatics, 2018 Nov 21. doi: 10.1093/bioinformatics/bty958 Epub ahead of print on Nov. 21, 2018 (PMID: 30462190).
16. Rao, V., Zavala, G., Roy, A., Mains, R., Eipper, B. (2018) A pH-sensitive luminal His-cluster promotes interaction of PAM with V-ATPase along the secretory and endocytic pathways of peptidergic cells. J. Cellular Physiology (PMCID:PMC6395498 [Available on 2020-06-01], PMID: 30317586)
17. Wilson, R., Nairn, A.C. (2018) Cell-type-specific proteomics: A neuroscience perspective. Proteomes Special Issue on Neuroproteomics 6(4), 51 (PMCID:PMC6313874, PMID:30544872).
18. Wilson, R.S. and Nairn, A.C. (2018) Making brain proteomics true to type. Nature Biotechnology 36: 149-150. (PMID: 29406511).