Curriculum Vitae

Jeffrey N. Agar, Ph.D.
Northeastern University
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Education/Employment History

Professional Preparation

- University of Michigan, Biochemistry/Chemistry B.Sc., 1995
- University of Georgia Inorganic Chemistry Ph.D., 2000 (Michael K. Johnson)
- McGill University, Montreal Neurological Institute Post-Doctoral Fellow, 2005 (Heather D. Durham)

Work Experience and Appointments

- 1992-1995: Co-op student promoted to chemist Dupont Automotive Products, Flint
- 2005-2013: Assistant Professor of Chemistry, Brandeis University
- 2013: Associate Professor of Chemistry and Pharm. Sci., Northeastern University

Scholarship/Research/Creative Activity

Publications:

Submitted articles

1. EC Randall, Begona GC Lopez , MS Regan, WM Abdelmoula, SS Basu , H Yoon, MC Haigis , **JN Agar**, NL Tran , WF Elmquist, FM White, JN Sarkaria, NYR Agar. : Localized metabolomic gradients in patient-derived xenograft models of glioblastoma. *PNAS.* Submitted 12/7/2018

Refereed articles (h-index 30) *Note: A statement of our contribution is given in italics following any collaborative efforts.*

63. "A five-level classification system for proteoform identifications" Agar JN et. al. *Nature Methods*. Accepted August 2019.
62. DP Donnelly, **JN Agar**, SA Lopez “Nucleophilic substitution reactions of cyclic thiosulfinates are accelerated by hyperconjugative interactions” Chemical Science 10 (21), 5568-5575 (2019).
61. WM Abdelmoula, MS Regan, BGC Lopez, EC Randall, S Lawler, AC Mladek, MO Nowicki, BM Marin, **JN. Agar**, KR Swanson, T Kapur, JN Sarkaria, W Wells, NYR Agar Automatic 3D Non-linear Registration of Mass Spectrometry Imaging and Magnetic Resonance Imaging Data. *Analytical chemistry* 91 (9), 6206-6216 *(*2019). *My group provided all of the MALDI MS imaging data and related write-up, and I revised the manuscript*
60. D Guo, K Bemis, C Rawlins, **JN Agar**, O Vitek “Unsupervised segmentation of mass spectrometric ion images characterizes morphology of tissues” *Bioinformatics* 35 (14), i208-i217 (2019).
59. Daniel P. Donnelly, Catherine M. Rawlins, Caroline J. DeHart, Luca Fornelli, Luis F. Schachner, Ziqing Lin, Jeremy Wolff, Jennifer L. Lippens, Iain D. G. Campuzano, Jared R. Auclair, Ljiljana Paša-Tolić, Julia Chamot-Rooke, Paul O. Danis, Lloyd M.

Smith, Yury O. Tsybin, Joseph A. Loo, Ying Ge, Neil L. Kelleher, Agar J.N. “Best Practices and Benchmarks for Mass Spectrometry of Intact Proteins” Nature Methods 16 (7), 587 (2109). *I designed and oversaw this collaborative effort of the Consortium for Top-Down Proteomics, and wrote the first draft of the manuscript. Other groups provided data, protocols, and significant revisions.*

58. Nicholas Schmitt, Catherine M. Rawlins, Elizabeth C. Randall, Jared R. Auclair, Jane-Marie Kowalski, Paul J. Kowalski, Ed Luther, Nathalie Y.R. Agar, **Agar JN**. Genetically Encoded Fluorescent Proteins Enable High-Throughput Assignment of Cell-cohorts Directly from MALDI-MS Images. *Analytical chemistry* 91 (6), 3810-3817 (2019). *My group designed the study, performed the experiments, and wrote the manuscript. N. Agar’s group and Bruker provided MALDI-TOF (Rapiflex) instrument time and helped perform unsupervised clustering of the MALDI-MS data.*
57. E Randall, G Zadra, P Chetta, B Lopez, S Syamala, S Basu, **JN Agar**, M Loda, C Tempany, F Fennessy, and NYR Agar. “Molecular characterization of prostate cancer with associated Gleason score using mass spectrometry imaging. *Molecular Cancer Research* 17 (5), 1155-1165 (2019). *My group provided all the MALDI MS imaging data and related write-up, and I revised the manuscript.*
56. Clark AR, Calligaris D, Regan MS, Pomeranz Krummel D, **Agar JN**, Kallay L, MacDonald T, Schniederjan M, Santagata S, Pomeroy SL, Agar NYR, Sengupta S.. “Rapid discrimination of pediatric brain tumors by mass spectrometry imaging.” *J Neurooncol.* 2018 Nov;140(2):269-279. doi: 10.1007/s11060-018-2978-2. Epub 2018 Aug 20. PMID:30128689 *My group provided all of the MALDI MS imaging data and related write-up, and I revised the manuscript.*
55. Randall EC, Emdal KB, Laramy JK, Kim M, Roos A, Calligaris D, Regan MS, Gupta SK, Mladek AC, Carlson BL, Johnson AJ, Lu FK, Xie XS, Joughin BA, Reddy RJ, Peng S, Abdelmoula WM, Jackson PR, Kolluri A, Kellersberger KA, **Agar JN**, Lauffenburger DA, Swanson KR, Tran NL, Elmquist WF, White FM, Sarkaria JN, Agar NYR. “Integrated mapping of pharmacokinetics and pharmacodynamics in patient-derived xenograft models of glioblastoma.” Nat Commun. 2018 Nov 21;9(1):4904. doi: 10.1038/s41467-018-07334-3. PMID:30464169. *My group provided all of the MALDI MS imaging data and related write-up, and I revised the manuscript.*
54. Donnelly DP; Dowgiallo, MG; Salisbury, JP; Aluri, KC; Iyengar S; Chaudhari, M; Mathew, M; Miele, I; Auclair, JR; Lopez, SA; Manetsch, R; **Agar J.N.** “Cyclic Thiosulfinates and Cyclic Disulfides Selectively Crosslink Thiols While Avoiding Modification of Lone Thiols” Journal of the American Chemical Society, Jun 20;140(24):7377-7380. doi: 10.1021/jacs.8b01136. Epub 2018 Jun 11. PMID: 29851341. *I invented cyclic disulfide and cyclic thiosulfinate-mediated protein crosslinking, wrote the first draft, and my group performed the in vitro and in cellulo cross-linking experiments. Manetsch’s group contributed considerable mechanistic insight, wrote the synthetic portion of the manuscript, provided extensive review of the entire manuscript, and synthesized more than half of the molecules used in this study. Lopez’s group performed QM calculations and wrote the related sections.*
53. Basu SS, Randall EC, Regan MS, Lopez BGC, Clark AR, Schmitt ND, **Agar JN**, Dillon DA, Agar NYR. Anal Chem. "In Vitro Liquid Extraction Surface Analysis Mass Spectrometry (ivLESA MS) for Direct Metabolic Analysis of Adherent Cells in Culture"

(2018) Analytical Chemistry 2018 Apr 17;90(8):4987-4991. doi: 10.1021/acs.analchem.8b00530. Epub 2018 Apr 2. PMID:29608279 *My group provided and interpreted the ultra-high resolution MS and MS/MS used to identify molecules in this study.*

52. Ruedi Aebersold, **Agar J.N.** , I Jonathan Amster , Mark S Baker , Carolyn R Bertozzi , Emily S Boja, Catherine E Costello , Benjamin F Cravatt , Catherine Fenselau , Benjamin A Garcia, Ying Ge, Jeremy Gunawardena, Ronald C Hendrickson, Paul J Hergenrother, Christian G Huber, Alexander R Ivanov , Ole N Jensen, Michael C Jewett, Neil L Kelleher , Laura L Kiessling , Nevan J Krogan, Martin R Larsen, Joseph A Loo , Rachel R Ogorzalek Loo, Emma Lundberg, Michael J MacCoss, Parag Mallick, Vamsi K Mootha, Milan Mrksich, Tom W Muir, Steven M Patrie, James J Pesavento , Sharon J Pitteri , Henry Rodriguez , Alan Saghatelian, Wendy Sandoval, Hartmut Schlüter , Salvatore Sechi, Sarah A Slavoff, Lloyd M Smith, Michael P Snyder, Paul M Thomas, Matthias Uhlén, Jennifer E van Eyk, Marc Vidal, David R Walt, Forest M White, Evan R Williams, Therese Wohlschlager, Vicki H Wysocki, Nathan A Yates, Nicolas L Young & Bing Zhang. “How many human proteoforms are there?” *Nature Chemical Biology.* Feb 14;14(3):206-214. doi: 10.1038/nchembio.2576 PMID: 29443976 (2018). *A collaborative effort of the Consortium for Top-Down Proteomics. I wrote the first draft of one section and revised this article.*
51. Richard D. LeDuc, Veit Schwämmle, Michael R. Shortreed, Anthony J. Cesnik, Stefan K. Solntsev, Jared B. Shaw, Maria J. Martin, Juan A. Vizcaino, Emanuele Alpi, Paul Danis, Neil L. Kelleher, Lloyd M. Smith, Ying Ge, **Agar J.N.**, Julia Chamot-Rooke, Joseph Loo, Ljiljana Pasa-Tolic, and Yury O. Tsybin. “ProForma: a Standard Proteoform Notation.” *Journal of Proteome Research* Mar 2;17(3):1321-1325. doi: 10.1021/acs.jproteome.7b00851. PMID: 2939773950 (2018). *A collaborative effort spearheaded by LeDuc. I discussed notation methods from the end-user’s standpoint and revised the manuscript.*
50. Pavlopoulos S, Pelekoudas DN, Benchama O, Rawlins CM, **Agar J.N.**, West JM, Malamas M, Zvonok N, Makriyannis A. “Secretion, isotopic labeling and deglycosylation of N-Acylethanolamine acid amidase for biophysical studies.” *Protein Expr Purif.* May;145:108-117. doi: 10.1016/j.pep.2017.12.005. PMID: 2925368849 (2018). *My group provided the intact protein analysis used in this manuscript, and revised the manuscript.*
49. Wang YA, Wu D, Auclair JR, Salisbury JP, Sarin R, Tang Y, Mozdzierz NJ, Shah K, Zhang AF, Wu SL, **Agar J.N.**, Love JC, Love KR, Hancock WS. “Integrated Bottom-Up and Top-Down Liquid Chromatography-Mass Spectrometry for Characterization of Recombinant Human Growth Hormone Degradation Products.” *Anal Chem.* Dec 5;89(23):12771-12777. doi:10.1021/acs.analchem.7b03026. PMID: 29096433 (2017). *My group provided all of the top-down MS data and some of bottom-up MS data for this manuscript, co-supervised graduate students, and revised the manuscript.*
48. Shao G., **Agar, J.N.**, and Giese R.W. Aqueous Acetonitrile Cold Phasing: A New Way to Begin QuEChERS. *J. Chromatography A* Jul 14;1506:128-133. doi: 10.1016/j.chroma.2017.05.045. PMID: 28558907 (2017). *I provided phase separation calculations, wrote the related sections, and revised the manuscript.*

47. Schmitt ND, **Agar J.N.** Parsing Disease-relevant Protein Modifications from Epiphenomena: Perspective on the Structural Basis of SOD1-Mediated ALS. *J Mass Spectrom. Jul;52(7):480-491* doi:10.1002/jms.3953. PMID: 28558143 ***Featured Article and Cover Article*** (2017). *Entirely the Agar laboratory's effort.*
46. Quijada JV, Schmitt ND, Salisbury JP, Auclair JR, **Agar J.N.** Heavy Sugar and Heavy Water Create Tunable Intact Protein Mass Increases for Quantitative Mass Spectrometry in Any Feed and Organism. *Analytical Chemistry* Nov 15; 88(22):11139-11146 (2016). *Entirely the Agar laboratory's effort.*
45. Salisbury, J.P., Sirbulescu, R.F., Moran, B.M., Auclair, J.R., Zupanc, G.K.H. & **Agar, J.N.** The central nervous system transcriptome of the weakly electric brown ghost knifefish (*Apteronotus leptorhychus*): de novo assembly, annotation and proteomics validation. *BMC Genomics* Mar 11;16:166. doi:10.1186/s12864-015-1354-2. (2015). *I designed this study and my postdoctoral fellow performed the majority of the experimental and data analyses. Zupanc and his post-doctoral fellow provided tissue preparations and helped with the manuscript.*
44. Rawlins, C.M., Salisbury, J.P., Feldman, D.R., Isim, S., Agar, N.Y.R., Luther, E., & **Agar, J.N**. Imaging and mapping of tissue constituents at the single cell level using MALDI MSI and Quantitative Laser Scanning Cytometry. *Methods in Molecular Biology* (2015). *Principally the Agar laboratory's effort, with N. Agar revising manuscript.*
43. Salisbury, J.P., Liu, Q. & **Agar, J.N.** QUDeX-MS: hydrogen/deuterium exchange calculations for mass spectra with resolved isotopic fine structure. *BMC Bioinformatics*, 15(1), 403 (2014). *Entirely an Agar laboratory effort.*
42. Dang, X., *et al.,* Brodbelt, J.S., **Agar, J.N.**, Paša-Tolić, L., Kelleher, N.L. & Young, N.L. The First Pilot Project of the Consortium for Top Down Proteomics: A Status Report. *Proteomics,* 14(10), 1130-1140 (2014). *This is a collaborative effort of the Consortium for Top-Down Proteomics. I am a member of the board of directors that designed the study, and my group also generated top-down data for the study.*
41. Auclair, J.R., Salisbury, J.P., Johnson, J.L., Petsko, G.A., Ringe, D., Bosco, D.A., Agar, N.Y., Santagata, S., Durham, H.D. & **Agar, J.N.** Artifacts to avoid while taking advantage of top-down mass spectrometry based detection of protein S-thiolation. Artifacts to avoid while taking advantage of top-down mass spectrometry based detection of protein S-thiolation. *Proteomics.* 14(10), 1152-1157 (2014). *I designed this study, my lab members performed the study, and I wrote the manuscript with post-doctoral fellow Auclair. Nathalie Agar, Heather Durham, and Daryl Bosco provided patient tissues and helped revise the manuscript.*
40. Rotunno MS, Auclair JR, Maniatis S, Shaffer SA, **Agar J.N.**, Bosco DA. Identification of a misfolded region in superoxide dismutase 1 that is exposed in amyotrophic lateral sclerosis. *The Journal of Biological Chemistry.* Oct 10; 289(41):28527-38 (2014). *Collaborator Daryl Bosco designed this study and wrote the manuscript, my group performed crosslinking mass spectrometry analysis to identify sites of protein-antibody interaction (epitope mapping).*
39. Liu, Q., Easterling, M. L., **Agar, J. N.** Resolving isotopic fine structure to detect and quantify natural abundance-and hydrogen/deuterium exchange-derived isotopomers. *Analytical Chemistry, 86*(1), 820–825 (2014). *Entirely the Agar laboratory's effort.*

38. Liu, Q., Cobb, J. S., Johnson, J. L., Wang, Q., **Agar, J. N**. Performance Comparisons of Nano-LC Systems, Electrospray Sources and LC-MS-MS Platforms. *Journal of Chromatographic Science, 52*(2), 120–127 (2014). *Entirely the Agar laboratory's effort.*
37. Salisbury, J. P., Boggio, K. J., Hsu, Y. W., Quijada, J., Sivachenko, A., Gloeckner, G., Kowalski, P. J., Easterling, M. L., Rosbash, M., **Agar, J. N.** A rapid MALDI-TOF mass spectrometry workflow for Drosophila melanogaster differential neuropeptidomics. *Molecular Brain, 6*(1), 60 (2013). *I designed the study and my laboratory performed the analysis and wrote the manuscript. Michael Rosbash provided genetically engineered flies and revised the manuscript.*
36. Liu, X. K., Ide, J. L., Norton, I., Marchionni, M. A., Ebling, M. C., Wang, L. Y., Davis, E., Sauvageot, C. M., Kesari, S., Kellersberger, K. A., Easterling, M. L., Santagata, S., Stuart, D. D., Alberta, J., **Agar, J. N.**, Stiles, C. D., Agar, N. Y. Molecular imaging of drug transit through the blood-brain barrier with MALDI mass spectrometry imaging. *Science Reports, 3*, 2859 (2013). *Nathalie Agar and Chuck Stiles designed this study. The mass spectrometry analysis was performed on NEU's FTICRMS. I optimized the instrument and helped analyze the data.*
35. Auclair, J. R., Johnson, J. L., Liu, Q., Salisbury, J. P., Rotunno, M. S., Petsko, G. A., Ringe, D., Brown, R., Bosco, D. A., **Agar, J. N.** Post-translational modification by cysteine protects cu/zn-superoxide dismutase from oxidative damage. *Biochemistry, 52*(36), 6137–6144 (2013). *I designed the study, my lab members performed the analysis, and wrote the manuscript. Daryl Bosco and Bob Brown provide patient tissues. Petsko and Ringe co-supervised first author Auclair and they helped revise the manuscript.*
34. Auclair, J. R., Brodkin, H. R., D'Aquino, J. A., Petsko, G. A., Ringe, D., **Agar, J. N.** Structural consequences of cysteinylation of cu/zn-superoxide dismutase. *Biochemistry*, 52(36), 6145–6150 (2013). *A collaborative effort of the Agar, Petsko, and Ringe labs, which co-supervised Auclair. Agar's lab prepared the biological materials and purified proteins, and Petsko-Ringe directed the x-ray crystallography. Agar wrote the manuscript.*
33. Smith, L., Kelleher, N. L., Linial, M., Goodlett, D., Langridge-Smith, P., Goo, Y. A., Safford, G., Bonilla, L., Kruppa, G., Zubarev, R., Rontree, J., Chamot-Rooke, J., Garavelli, J., Heck, A., Loo, J., Penque, D., Hornshaw, M., Hendrickson, C., Pasa-Tolic, L., Borchers, C., Chan, D., Young, N., **Agar, J. N.**, Masselon, C., Gross, M., McLafferty, F., Tsybin, Y., Ge, Y., Sanders, I., Langridge, J., Whitelegge, J., Marshall, A. Proteoform: a single term describing protein complexity. *Nature Methods, 10*(3), 186–187 (2013). *A collaborative nomenclature effort of the consortium for top-down proteomics. Smith and Kelleher wrote a draft manuscript, all members revised the manuscript.*

Below This Point are Brandeis-affiliated Refereed Articles

32. Auclair, J. R., Somasundaran, M., Green, K. M., Evans, J. E., Schiffer, C. A., Ringe, D., Petsko, G. A., **Agar, J. N.** Mass spectrometry tools for analysis of intermolecular interactions. *Methods in Molecular Biology, 896*, 387–398 (2012). *Petsko and Ringe co-supervised first author Auclair and helped revise the manuscript.*
31. Kabashi, E., **Agar, J. N.**, Strong, M. J., Durham, H. D. Impaired proteasome function in sporadic amyotrophic lateral sclerosis. *AMYOTROPHIC LATERAL SCLEROSIS,*

13(4), 367-371 (2012). *A study I designed as a post-doc in Durham's laboratory, performed by my graduate student mentee, Edor Kabashi.*

30. Wang, W., Perovic, I., Chittuluru, J., Kaganovich, A., Nguyen, L. T., Liao, J., Auclair, J. R., Johnson, D., Landeru, A., Simorellis, A. K., Ju, S., Cookson, M. R., Asturias, F. J., **Agar, J. N.**, Webb, B. N., Kang, C., Ringe, D., Petsko, G. A., Pochapsky, T. C., Hoang, Q. Q. A soluble a-synuclein construct forms a dynamic tetramer. *Proceedings of the National Academy of Science, 108*(43), 17797–17802 (2011). *I performed the MALDI-MS analysis that demonstrated that synuclein was mainly a tetramer (which is a figure in this manuscript and in the title).*
29. Brodkin, H. R., Novak, W. R., Milne, A. C., D'Aquino, J. Alejandro, Karabacak, N.M., Goldberg, I. G., **Agar, J. N.**, Payne, M. S., Petsko, G. A., Ondrechen, M. J., Ringe, D. Evidence of the Participation of Remote Residues in the Catalytic Activity of Co-Type Nitrile Hydratase from Pseudomonas putida. *Biochemistry, 50*(22), 4923-4935 (2011). *My laboratory performed intact protein mass spectrometry analysis to determine the sequence, metal content, and oxidation state of the protein.*
28. Auclair, J. R., Boggio, K. J., Petsko, G. A., Ringe, D., **Agar, J. N.** Strategies for stabilizing superoxide dismutase (SOD1), the protein destabilized in the most common form of familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Science, 107*(50), 21394–21399 (2010). *Petsko and Ringe co-supervised first author Auclair and helped revise the manuscript.*
27. Agar, N. Y., Kowalski, J. M., Kowalski, P. J., Wong, J. H., **Agar, J. N.** Tissue preparation for the in situ MALDI MS imaging of proteins, lipids, and small molecules at cellular resolution. *Methods in Molecular Biology, 656*, 415–431 (2010). *Principally the Agar laboratory's effort, N. Agar revised manuscript.*
26. Bosco, D. A., Morfini, G., Karabacak, N. M., Song, Y., Gros-Louis, F., Pasinelli, P., Goolsby, H., Fontaine, B. A., Lemay, N., McKenna-Yasek, D., Frosch, M. P., **Agar, J. N.**, Julien, J. P., Brady, S. T., Brown, R. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. *Nature Neuroscience, 13*(11), 1396–1403 (2010). Featured in Nature Neuroscience "News and Views."13(11) 1303-1304 (2010). *My laboratory analyzed the modified proteins by mass spectrometry, and developed methods for creating SOD1 that was only modified at one residue. This protein was used in all biochemical assays and the analysis is two figures in the manuscript.*
25. Li, L., Karabacak, N. M., Cobb, J. S., Wang, Q., Hong, P., **Agar, J. N.** Memory-efficient calculation of the isotopic mass states of a molecule. *Rapid Communications in Mass Spectrometry, 24*(18), 2689–2696 (2010). *Entirely the Agar laboratory's research effort, Hong revised manuscript.*
24. Karabacak, N. M., Easterling, M. L., Agar, N. Y., **Agar, J. N.** Transformative effects of higher magnetic field in Fourier transform ion cyclotron resonance mass spectrometry. *Journal of American Society for Mass Spectrometry, 21*(7), 1218–1222 (2010). *Entirely the Agar laboratory's effort.*
23. Cobb, J. S., Easterling, M. L., **Agar, J. N.** Structural characterization of intact proteins is enhanced by prevalent fragmentation pathways rarely observed for peptides. *Journal of American Society for Mass Spectrometry, 21*(6), 949–959 (2010). *Entirely the Agar laboratory's effort.*

22. Agar, N. Y., Malcolm, J. G., Mohan, V., Yang, H. W., Johnson, M. D., Tannenbaum, A., **Agar, J. N.**, Black, P. M. Imaging of meningioma progression by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Analytical Chemistry, 82*(7), 2621–2625. Accelerated and Featured Publication (2010). *This study was designed by Nathalie Agar while a post-doc in Peter Black's lab. Experiments were performed on my lab's mass spectrometers and I helped analyze the data and write the manuscript.*
21. Molnar, K. S., Karabacak, N. M., Johnson, J. L., Wang, Q., Tiwari, A., Hayward, L. J., Coales, S. J., Hamuro, Y., **Agar, J. N.** A common property of amyotrophic lateral sclerosis-associated variants: destabilization of the copper/zinc superoxide dismutase electrostatic loop. *The Journal of Biological Chemistry, 284*(45), 30965–30973 (2009). *I designed the study and authored the manuscript. Hamuro worked for the CRO that performed the H/D exchange mass spectrometry. Tiwari and Hayward supplied many of the ALS variants used in the study.*
20. Karabacak, N. M., Li, L., Tiwari, A., Hayward, L. J., Hong, P., Easterling, M. L., **Agar, J. N.** Sensitive and specific identification of wild type and variant proteins from 8 to 669 kDa using top-down mass spectrometry. *Molecular and Cellular Proteomics, 8*(4), 846–856 (2009). *I designed the study and my laboratory performed the analysis and wrote the manuscript. Tiwari and Hayward supplied many of the ALS variants used in the study.*
19. Li, L., Kresh, J. A., Karabacak, N. M., Cobb, J. S., **Agar, J. N.**, Hong, P. A hierarchical algorithm for calculating the isotopic fine structures of molecules. *Journal of American Society for Mass Spectrometry, 19*(12), 1867–1874 (2008). *I co-designed the study, provided the data that was to be fit, and wrote the manuscript. Long Li was co-supervised by Pengyu Hong.*
18. Wang, Q., Johnson, J. L., Agar, N. Y., **Agar, J. N.** Protein aggregation and protein instability govern familial amyotrophic lateral sclerosis patient survival. *PLOS BIOLOGY, 6*(7), 1508-1526 (2008). Editors' Choice: PLoS Biology 6 (7), "The Threat of Instability: Neurodegeneration Predicted by Protein Destabilization and Aggregation Propensity" by Elizabeth M Meiering. Article also featured in Forbes, Washington Post, Alzheimer's Forum, etc. *Entirely the Agar laboratory's effort.*
17. Kabashi, E., **Agar, J. N.**, Hong, Y., Taylor, D. M., Minotti, S., Figlewicz, D. A., Durham, H. D. Proteasomes remain intact, but show early focal alteration in their composition in a mouse model of amyotrophic lateral sclerosis. *Journal of Neurochemistry, 105*(6), 2353–2366 (2008). *A study I co-designed as a post-doc in Durham's laboratory, performed by my graduate student mentee, Edor Kabashi.*
16. Morris, A. M., Watzky, M. A., **Agar, J. N.**, Finke, R. G. Fitting neurological protein aggregation kinetic data via a 2-step, minimal/"Ockham's razor" model: the Finke-Watzky mechanism of nucleation followed by autocatalytic surface growth. *Biochemistry, 47*(8), 2413–2427 (2008). *I conceived of this study and contacted Rick Finke, convinced his model for fitting nanocluster growth would work for proteins. I supplied data from neurodegenerative disease protein aggregation, which Finke and Morris fit. We co-wrote the manuscript.*
15. Agar, N. Y., Yang, H. W., Carroll, R. S., Black, P. M., **Agar, J. N.** Matrix solution fixation: histology-compatible tissue preparation for MALDI mass spectrometry imaging. *Analytical Chemistry, 79*(19), 7416–7423 (2007). *A collaboration with*

Nathalie Agar, while a post-doctoral fellow in Peter Black's lab. Nathalie and I are co-inventors on the related patent, designed and performed the experiments together, and co-wrote the manuscript.

14. Taylor, D. M., Gibbs, B. F., Kabashi, E., Minotti, S., Durham, H. D., **Agar, J. N.** Tryptophan 32 potentiates aggregation and cytotoxicity of a copper/zinc superoxide dismutase mutant associated with familial amyotrophic lateral sclerosis. *The Journal of Biological Chemistry, 282*(22), 16329–16335 (2007). *A study that started while I was a post-doc in Durham's laboratory. I developed it further, acquired and analyzed the data, and wrote the manuscript.*
13. Smith, A. D., Jameson, G. N., Dos Santos, P. C., **Agar, J. N.**, Naik, S., Krebs, C., Frazzon, J., Dean, D. R., Huynh, B. H., Johnson, M. K. NifS-mediated assembly of [4Fe-4S] clusters in the N- and C-terminal domains of the NifU scaffold protein. *Biochemistry, 44*(39), 12955–12969 (2005). *Work from my Ph.D.*
12. Taylor, D., Kabashi, E., **Agar, J. N.**, Minotti, S., Durham, H. Proteasome activity or expression is not altered by activation of the heat shock transcription factor Hsf1 in cultured fibroblasts or myoblasts. *Cell Stress & Chaperones, 10*(3), 230-241 (2005). *Post-doctoral work.*
11. Taylor, D., Minotti, S., **Agar, J. N.**, Durham, H. Overexpression of metallothionein protects cultured motor neurons against oxidative stress, but not mutant Cu/Zn-Su peroxide dismutase toxicity. *Neurotoxicology, 25*(5), 779-792 (2004). *Post-doctoral work.*
10. Kabashi, E.*, **Agar, J. N***, Taylor, D. M., Minotti, S., Durham, H. D. Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. *Journal of Neurochemistry, 89*(6), 1325–1335 (2004). **Co-first author. Post-doctoral work.*
9. Antonicka, H; Leary, SC; **Agar, J.N.**; ***et al.*** Mutations in COX10 result in a defect in mitochondrial heme A biosynthesis and account for multiple, early-onset clinical phenotypes associated with isolated COX deficiency. *Human Molecular Genetics* 12(20), 2693-2702 (2003). *Post-doctoral work.*
8. Krebs, C., **Agar, J. N.**, Smith, A. D., Frazzon, J., Dean, D. R., Huynh, B. H., Johnson, M. K.. IscA, an alternate scaffold for Fe-S cluster biosynthesis. *Biochemistry, 40*(46), 14069–14080 (2001). *Work from my Ph.D.*
7. Smith, A. D., **Agar, J. N.**, Johnson, K. A., Frazzon, J., Amster, I. J., Dean, D. R., Johnson, M. K. Sulfur transfer from IscS to IscU: the first step in iron-sulfur cluster biosynthesis. *Journal of the American Chemical Society, 123*(44), 11103–11104 (2001). Editors' Choice: Highlights of the recent literature; "Biochemistry Construction Sites," Science, 295: 5552, (2001). *Work from my Ph.D.*
6. Olson, J. W., **Agar, J. N.**, Johnson, M. K., Maier, R. J. Characterization of the NifU and NifS Fe-S cluster formation proteins essential for viability in Helicobacter pylori. *Biochemistry, 39*(51), 16213–16219 (2000). *Work from my Ph.D.*
5. **Agar, J. N.**, Krebs, C., Frazzon, J., Huynh, B. H., Dean, D. R., Johnson, M. K. IscU as a scaffold for iron-sulfur cluster biosynthesis: sequential assembly of [2Fe-2S] and [4Fe-4S] clusters in IscU. *Biochemistry, 39*(27), 7856–7862 (2000). Accelerated Publication *Work from my Ph.D.*
4. **Agar, J. N.**, Yuvaniyama, P., Jack, R. F., Cash, V. L., Smith, A. D., Dean, D. R., Johnson, M. K. Modular organization and identification of a mononuclear iron-binding

site within the NifU protein. *Journal of Biological Inorganic Chemistry, 5*(2), 167–177 (2000). *Work from my Ph.D.*

3. **Agar, J. N.**; Zheng, LM; Cash, VL; et al. Role of the IscU protein in iron-sulfur cluster biosynthesis: IscS-mediated assembly of a [Fe2S2] cluster in IscU, *Journal of the American Chemical Society,* 122(9), 2136-2137 (2000). *Work from my Ph.D.*
2. Yuvaniyama, P., **Agar, J. N.**, Cash, V. L., Johnson, M. K., Dean, D. R. NifS-directed assembly of a transient [2Fe-2S] cluster within the NifU protein. *Proceedings of the National Academy of Science U.S.A., 97*(2), 599–604 (2000). *Work from my Ph.D.*
1. Goodwin, P. J., **Agar, J. N.**, Roll, J. T., Roberts, G. P., Johnson, M. K., Dean, D. R. The Azotobacter vinelandii NifEN complex contains two identical [4Fe-4S] clusters. *Biochemistry, 37*(29), 10420–10428 (1998). *Work from my Ph.D.*

Non-refereed Book Chapters and Reviews

7. Recent advances in single-cell MALDI mass spectrometry imaging and potential clinical impact. Boggio, K. J., Obasuyi, E., Sugino, K., Nelson, S. B., Agar, N. Y., **Agar, J. N.** *Expert Reviews in Proteomics, 8*(5), 591–604 (2011).
6. "Motor Neuron Disease" H. D. Durham, E. Kabashi, D. M. Taylor, **Agar, J. N.,** in The Proteasome in Neurodegeneration, L. Stefanis and J. N. Keller ed. Springer US, 247-264 (2007).
5. "Relevance of oxidative injury in the pathogenesis of motor neuron diseases" **Agar, J. N.**, H. D. Durham, *Amyotrophic Lateral Sclerorisis and Other Motor Neuron Disorders.* **4**, 232-42 (2003).
4. "Biological Iron-Sulfur Cluster Assembly" **Agar, J. N.**, D. R., Dean, M. K. Johnson, in Biochemistry and Physiology of Anaerobic Bacteria*,* L. G. Ljungdahl, ed., Springer-Verlag, 46-66 (2003).
3. "Biological Iron-Sulfur Cluster Assembly," P. Yuvaniyama, **Agar, J. N.**, M. K. Johnson, and D. R. Dean, *Archives of Microbiology* (2001).
2. "Studies on the Mechanism for the Activation of Iron and Sulfur for Formation of the Nitrogenase Metal Centers," D. R. Dean, P. Yuvaniyama, **Agar, J. N.,** and M. K. Johnson, Nitrogen Fixation: From Molecules to Crop Productivity. *Current Plant Science and Biotechnology in Agriculture* **38**, 3739 (2000).
1. "Activation of Iron and Sulfur for Nitrogenase Metallocluster Formation," D. R. Dean, J. Christianson, P. Yuvaniyama, L. Zheng, V. Cash, **Agar, J. N.,** M. K. Johnson, and D. H. Flint, *Current Plant Science and Biotechnology in Agriculture* **31**, 27-31 (1998).

Other Creative Activity: Patents

5. U.S. Provisional Application No.: 62/530,934 Cyclic Thiosulfinate-Dithiol Click Chemistry (2017).
4. US 9,428,589 Tethering Cysteine Residues Using Cyclic Disulfides, Granted Aug 2016 (Licensed).
3. US 8,945,941 Tissue sample preparation and MALDI MS imaging thereof, Granted Feb 2015.
2. ES2535222 Cross-Linking of Superoxide Dismutase Monomers (US Pending, Europe Granted July 2015). (Licensed)
1. US 8,609,649 Compositions and methods for the diagnosis, treatment, and prevention of amyotrophic lateral sclerosis and related neurological diseases, Granted Dec 2013. (Licensed).

Presentations:

77. University of Wisconsin Madison Chemistry Department ""Target Discovery, Validation, and Therapy Development for ALS" Oct 2019.
76. American Society for Mass Spectrometry "Advanced MALDI Imaging for Neurosurgery and Neurooncology" Keynote lecture Bruker User Meeting. June 2019.
75. American Society for Mass Spectrometry "Metalloprotein Analysis with ExD/UVPD" Invited lecture. June 2019.
74. American Society for Mass Spectrometry "Pick Picking Methods and Perilous Pitfalls" Tutorial Co-organizer and principal lecturer. June 2019.
73. University of Georgia Chemistry "Target Discovery, Validation, and Therapy Development for ALS" Nov 2018.
72. Mass Spectrometry in Biotechnology and Mass Spectrometry (MSBM) 14 July, 2018, Center for Advanced Academic Studies (CAAS) Dubrovnik, Croatia. "Isotopic Fine Structure."
71. Mass Spectrometry in Biotechnology and Mass Spectrometry (MSBM) 11 July, 2018, Center for Advanced Academic Studies (CAAS) Dubrovnik, Croatia. "Top-down MS in Disease."
70. Penn State University Department of Chemistry. "A novel cross-linker that doesn't form dead-end modifications." June 2018
69. U Mass Lowell Dept. of Chemistry, March 2018 "Protein PTMs Inspired Pharmacological Chaperones and Crosslinkers That Minimize Dead-End Modifications."
68. US HUPO, March 2018, Minneapolis MN. Session Chair for Lightning Talks.
67. US HUPO, March 2018, Minneapolis MN. "How Protein PTMs Inspired Pharmacological Chaperones and Crosslinkers That Minimize Dead-End Modifications"
66. Baylor University Department of Chemistry, Jan 2018. "ALS-related Toxic Protein Modifications."
65. ASMS Fall Workshop. Top-Down Proteomics, Nov 2017, Boston MA. Co-session chair. "Sample Preparation for Top-Down Mass Spectrometry."
64. ASMS Fall Workshop. Top-Down Proteomics, Nov 2017, Boston MA. "Top-Down Proteomics Applications in Human Disease."
63. SUNY Medical School Department of Pathology, Oct 2017. "Protein Mass Spectrometry for Evaluating Neurotoxicology and *Vice Versa*."
62. Next Generation Sequencing & Single Cell Analysis Congress, October 2017, Boston. "Mass Spectrometry Methods for Single Neuron Analysis."
61. American Society for Mass Spectrometry, Indianapolis IN, June 2017 "Characterization of Single Fluorescent Motor Neurons in Amyotrophic Lateral Sclerosis (ALS) Mouse Brains via MALDI Mass Spectrometry Imaging (MSI)."
60. 9th International Symposium on Enabling Technologies (ETP 2017) May 4 – 5, 2017, University of Ottawa, Ontario, Canada. "A metabolic labeling approach for intact protein half-live determination in any organism."
59. Internal NEU Development Office Meeting With Potential Donors. Nov 2016 "Methods of Diagnosing and Treating Neurodegenerative Diseases"
58. Internal Parent's Day (NEU), Oct 2016. "Will Our Brains Survive the Aging Pandemic?" *This and the following presentation could also be considered University*

Service, but are shown here. There were another seven more traditional (for colleagues and students) internal lectures that are not listed.

57. NSF BRAIN workshop, Arlington VA, Oct 2016. “The Complexity of the Brain Versus The Current Technology”
56. International Mass Spectrometry Conference, Toronto Aug 2016 “Tracking the Dark Metabolome (also Proteome) with a Novel Isotopic Fine Structure Enabled Metabolic Labeling Strategy.”
55. American Society for Mass Spectrometry, San Antonio TX, June 2016 “Just add Water or Sugar: Methods for Quantitative Lipidomics and Proteomics.”
54. Inorganic Biochemistry Summer Workshop, Penn State University. June 2016 “Resolving Energy Differences Using Ultra High Resolving Power MS.”
53. Asia Pacific Economic Counsel (APEC). Lima, Peru. Feb 2016 “Dynamic Online Course For Regulatory Harmonization”
52. HUPO, Boston March 2016. “Heavy Sugar or Water Create Arbitrary Changes in Isotope Distribution (ACID) For Quantitative MS of Any Biomolecule, in any Organism or Feed.”
51. CASS, Brooklyn New York, Dec 2015. “Top-Down MS to Study Degradation Reactions of Protein Pharmaceuticals.”
50. Consortium for Top-Down Proteomics Meeting, Boston MA, Nov 2015. “Arbitrary Changes in Isotopomer Distribution: Isotope Dilution MS of Proteins, Lipids, and Nucleic Acids, in Any Feed or Organism.”
49. Concordia University, Montreal. Department of Chemistry, Sept 2015. “Development of Cyclic Disulfides as Pharmacological Chaperones.”
48. Montreal Mass Spectrometry Discussion Group, Lavalle University Sep 2015. “ACID, a metabolic labeling technique for quantitative MS that works in all organisms and for all classes of Biomolecules.”
47. NSF WORKSHOP MASS SPECTROMETRY DATA TO KNOWLEDGE, May 2015. “Toxic Protein Modifications.”
46. American Society for Mass Spectrometry National Conference (ASMS), June 2015 “Stochastic SILAC for intact protein quantification.”
45. Sanibel Conference, Ft. Lauderdale 2015. “Top-down mass spectrometry of toxic protein modifications.”
44. MIT Office of Industrial Liaison, July 2014. “Beyond proteomics: Intact protein analysis and deep MS/MS sequencing.”
43. Association of Biomedical Research Facilities, St Louis 2014. “Top-down mass spectrometry.”
42. University of Maryland, Regulatory Sciences. December 2 2014. “Educating the biopharmaceutical workforces.”
41. Session Chair: American Society for Mass Spectrometry National Conference (ASMS), June 2014. “Top-down Protein Analysis”
40. Session Chair (*stand-in for Alexander Ivanov) ASMS, June 2014. “Pharmacoproteomics and toxicoproteomics for drug development”
39. H3 Biomedicine, Cambridge MA, Feb 2014. “Proteoforms Put in Perspective: Toxic, Therapeutic, and Protective.”
38. Brown University Rhode Island Hospital “Toxic protein modifications in ALS”, January 2014

37. Single Cell Analysis Summit, Select Bioscience, San Diego, Sept 2013 "Mass Spectrometry Imaging of Single Cells in Mouse Models of ALS."
36. Tufts University Sackler School of Biomedical Science, December 2013 PPET Seminar, "Mass spectrometry-based biomarker-, drug target-, small molecule binding-, and metabolite identification."
35. American Society for Mass Spectrometry. Use of isotopic fine structure in HDXMS. Qian Liu (Agar Graduate student) invited presentation, June 2013. *Records of all talks from 2012 and some of 2013 (during transition to NEU) were lost.*
34. Rhode Island College, Sept 2011. “The Many Roles of Mass Spectrometry In Drug Discovery” Inviter: Department Chair.
33. Saint Anselm College, Manchester NH. “Mass Spectrometry as a Tool for Drug Discovery.” Inviter Lisa Bonner. Nov 2010.
32. Brandeis National Committee, Deerfield Beach Florida. Internal, "Why University Laboratories Adopted Orphaned Disease Research." Oct 2010.
31. Massachusettes General Hospital, MIND institute. “Structure-based drug development for ALS” Inviter Ippolita Castelvetri. 2010
30. University of Nebraska Lincoln. “ALS-associated Protein Structural Modifications, and Small Molecules That Stabilize Them” Dept of Chemistry Sept 2010 Title TBA.
29. National Institutes of Health. “ALS-associated structural perturbation of SOD1 and strategies for stabilization” Sept 2010, Inviter Sanford Markey, Sr.
28. American Society for Mass Spectrometry. Salt Lake City Utah. June 2010. “Structural Consequences of Loss of Metal from ALS-Associated SOD1 Variant Characterized Using Top-down Mass Spectrometric Hydrogen/Deuterium Exchange” (Former student Qi Wang to present thesis work). Inviter: Organization Committee.
27. American Society for Mass Spectrometry. Salt Lake City Utah. June 2010. “MALDI MS Imaging at Cellular Resolution Across Entire Tissue Sections of ALS Mice and Coregistration Using YFP-containing Fluorescent Neurons.” (Student Kristin Boggio to present thesis work). Inviter: Organization Committee.
26. American Society for Mass Spectrometry. Salt Lake City Utah. June 2010. “Top-Down Proteomics of Isotopically Enriched Yeast Proteins on a LC Timescale using FT-ICR MS with Funnel-Skimmer Dissociation Fragmentation” (Student Jenifer Cobb to present thesis work). Inviter: Organization Committee.
25. March 2010, Harvard Medical School, Brigham and Women’s Hospital “Mass Spectrometry’s Many Roles in the Drug Discovery Process.” Inviter Matt Lavoie.
24. ICOSA 2010, University of Massachusetts Medical School. “Small Molecule Mediated Stabilization of SOD1” Inviter Lawrence Haywood.
23. University of Oregon, Dept. of Biochemistry Colloquium: Feb 2010. “How Post Translational Modifications Change SOD1 structure”. Inviter Joseph Beckman
22. University of Illinois at Chicago, School of Pharmacognesy. Feb 2010. “A Mass Spectrometry-Based Pipeline for Amyotrophic Lateral Sclerosis Drug Discovery.” Inviter: Richard Van Breemen.
21. The University of Michigan, Flint. Nov 2010. “Drug discovery for Lou Gehrig's disease using mass spectrometry.” Inviter Robert Stach.
20. Merrimack College. October 2009. "The many roles of mass spectrometry in drug development." Inviter: Department Chair.

19. Clarke University. October 2009. "The many roles of mass spectrometry in drug development." Inviter: Department Chair.
18. American Society for Mass Spectrometry. Philadelphia PA. June 2009. “MALDI Mass Spectrometry Imaging of Drugs and Metabolites.” (Kathy Kellersberger Presenter of Collaborative Work).
17. American Society for Mass Spectrometry. Philadelphia PA. June 2009. “Methods for MALDI Mass Spectrometry Imaging at Cellular Resolution.” Inviter: Organization Committee.
16. University of Texas at San Antonio Health Center: March 20009. “Properties of SOD1 that effect patient lifespan.” Inviter: P. John Hart.
15. American Society for Mass Spectrometry. Indianapolis IN. June 2008. “Funnel Skimmer Dissociation” (Graduate Student Jennifer Cobb Speaker). Inviter: Organization Committee.
14. Rhode Island College, April 2008. “Biological Mass Spectrometry of ALS” Inviter: Department Chair.
13. Wellesley University. 2008. “Biological Mass Spectrometry of ALS.” Inviter: Department Chair.
12. Colorado State, Dept. of Biochemistry, April 2008. “The physicochemical basis of amyotrophic lateral sclerosis (ALS)” Inviter: Rick Finke.
11. TREAT ALS Forum, Tampa Bay, Florida, Jan 2008. “The potential of advanced glycation endproduct inhibitors as ALS therapeutics.” Inviter: The ALS association of America.
10. Boston Area Mass Spectrometry Discussion Group, Dec 2007. “Top Down for the Masses.” Inviter: Organization Committee.
9. 18th International Symposium on ALS/MND (the major conference in ALS), Toronto, Canada, Dec 2007. “Protein aggregation and thermodynamic stability are risk factors in ALS patient survival.” Inviter: Organization committee.
8. ICOSA 2007, Brookhaven National Laboratory, New York, Sept 2007. “Mechanisms of SOD-1 toxicity in ALS.” Inviter: Diane Cabelli.
7. Human Proteomics Symposium, University of Wisconsin Madison, August 2007. “Sensitive and selective protein identification using top-down mass spectrometry” Inviter: organization committee probably influenced by Bruker Daltonics.
6. Bruker Daltonics Morning Seminar, Keynote Lecture: Cambridge Massachusetts, April 2007, “Methods for the identification of intact proteins using mass spectrometry.” Inviter: Bruker Daltonics.
5. Bruker Daltonics Symposium, Keynote Lecture: March 2007, King of Prussia, Pennsylvania. “Selective protein identification using top-down mass spectrometry.” Inviter: Bruker Daltonics.
4. Mount Sinai Hospital, March 2007, New York: “Selective protein identification using top-down mass spectrometry.” Inviter: department colloquia probably influenced by Bruker Daltonics.
3. New Jersey Mass Spectrometry Users Group, Somerset NJ, Feb 2007. “Top-down protein identification using Big Mascot.” Inviter: organization committee influenced by Bruker Daltonics.

2. American Society for Mass Spectrometry, Bruker Daltonics User Meeting, San Antonio Texas, June 2006. “Characterization of ALS-associated Protein Post Translational Modifications.” Inviter: Bruker Daltonics.
1. American Society for Mass Spectrometry (5000 attendees, the major conference in mass spectrometry), Seattle WA. June 2005. “Superoxide Dismutase Modifications of Potential Therapeutic Relevance for Familial Amyotrophic Lateral Sclerosis.” Inviter: organization committee.

Grant Support

Current Funded

5. ALS Association (ALSA) 10/2017-10/2020
Cyclic Disulfides to Stabilize ALS-Associated SOD1 Variants. Role: PI Manetch: Co PI Goal: Preclinical optimization of a lead SOD1 stabilizing compound. $270 k direct
4. Robert Johnston Foundation 05/2016-07/2020
Cyclic Disulfides as Pharmacological Chaperones for ALS and Parkinson. Role: PI Goal: Bring ALS mouse colony online and assess pharmacodynamics of compound 56224. $120 k direct in May 2016 and an additional $100k direct in May 2018.
3. Dana Farber Cancer Institute 02/2016-1/2019
Clinical and Preclinical MALDI Imaging of cancer drugs repurposed for brain cancer. Role: Subcontract with Nathalie Y.R. Agar Goal: To test the feasibility of drug repurposing for neurooncology via blood-brain-barrier penetration using our MALDI-FTICR MS. $120 k direct.
2. invriCRO 09/2015-1/2019
Brain imaging research subcontracts for Biogen, Alnylam, Bayesian, etc. Role: CoPI with Nathalie Y.R. Agar Goal: *MALDI Imaging of Drug Blood-Brain Barrier Penetration* (Diagnostics). $ > 40k direct.
1. INTERNAL Northeastern University, Tier 1. 07/2018-07/2019
Project title and goal: Cyclic Thiosulfinate-mediated Polymers. Role: PI. $50 K direct.

Completed Funded

19. Ono Pharmaceuticals 08/2015-08.2018 *Development of Pharmaceutical Agents.* Role: PI Goal: Ono small molecule mass spectrometrists spend one year in lab in the role of Agar lab post-doctoral fellow with their supplies provided. In the course of their projects they receive protein MS training. $40k direct
18. **INTERNAL** BioAPEC Center of Excellence. 06/2016-06/2017 *Tier III: Biopharmaceutical Regulatory Harmonization Pilot.* Role: Lead PI, Jared Auclair, Mike Polastri co PIs. Goal: Training FDA-equivalent regulators from APEC member states in pharmaceutical characterization. $150 K direct.
17. **INTERNAL** Northeastern University, Tier 1. 07/2014-07/2016 *Project title and goal: Building a Prototype Hyperspectral Imaging Platform.* Role: PI, Max Diem CoPI $50 K direct.
16. **INTERNAL** Northeastern University, Tier 1. 07/2014-07/2016 *Project title and goal: Systems Biology Approaches to Enable Proteomic Profiling of Single Neurons Isolated from the Brain of an Amyotrophic Lateral Sclerosis (ALS) Mouse Model.* Role: Co PI (50%) with Alexander Ivanov. $ 50 K direct.
15. NIH 1R01NS065263-01 04/2009-05/2015 *Structural consequences of ALS-related modifications of SOD1* Goal: This study characterizes structural changes to SOD1 that occur as a result of familial ALS-associated mutations. Role: PI $1.4 M direct.

14. *The role of systemic LPS in the pathogenesis of schistosomiasis morbidity*, Sponsored by Rhode Island Hospital, National Institutes of Health. (July 1, 2015 - June 30, 2018), Organized Research. Effort: 5%, Subcontract for $20 K/year. PI is Lisa Gangley-Leal.
13. **INTERNAL** "Nanotechnology for the nervous system: Developing injectable materials for nervous system tissue engineering applications" Sponsored by Northeastern University. (July 1, 2013 - June 30, 2014), TIER 1 Sponsored by Northeastern University. CoPI with Tom Webster $50 K direct.
12. **INTERNAL** "Using MALDI mass spectrometry imaging to map spinal cord regeneration in a regeneration-competent vertebrate model system" Sponsored by Northeastern University. (July 1, 2013 - June 30, 2014), TIER 1 Sponsored by Northeastern University. CoPI with Gunter Zupanc $50 K direct
11. *Greater Boston Mass Spec Discussion Group Student Travel Awards* of $2K to grad students Jeniffer Quijada, Kristin Boggio, and Jeniffer Cobb to present at ASMS 2010, 2012, 2015.
10. *Stabilizing fALS SOD1 Variants by Crosslinking Subunits* NIH 1R21NS071256-01 04/2010-04/2012 $275 K direct. Role: PI
9. *Mass Spectrometry Imaging of Motor Neurons and Their Environment.* Amyotrophic Lateral Sclerosis Society of America 60993 09/2010-09/2013 $240 K direct. Role: PI
8. *Mass Spectrometry Imaging Of Motor Neurons and Their Environment* Amyotrophic Lateral Sclerosis Society of America. G1392 08/01/07-08/01/08 Role: PI $40 K direct.
7. *Core Facilities for Neuroscience at Brandeis* P30 NIH NS45713 01/2008-01/2010 Role: coPI (one of many), Michael Rosbash PI. (Agar's portion $224 K)
6. *Sleep in flies and mammals*. This was a project involving seven co-investigators to study the circuitry that control sleep and waking. Funds were acquired through two supplemental funds applications coauthored by Agar. US Army Medical Research and Material Command Contract: W81XWH-04-1-0158 Role: coauthor and coPI of supplements, Michael Rosbash PI. Department of Defense 01/15/04-09/18/09 ($935 K is Agar's portion of the funds).
5. *Advanced Glycation Endproduct Inhibitors, Preclinical Development.* Robert Johnson Foundation 1/2012-1/2013 $52 K direct Role: PI
4. *Physiological and Pathological Implications of the Unique Subcellular distribution of LRRK2* Role: Subcontract to Agar with Matt Lavoie PI. Michael J Fox Foundation 10/2010-10/2012 $40 K direct.
3. *Identification of Molecules for Stabilizing DJ-1*, A Protein Involved In Parkinson and Alzheimer Diseases Role: Mentor/PI, Salisbury (graduate student) PI. Brandeis Sprout Grant 06/2011-06/2012 $5 K direct.
2. **INTERNAL** Identifying Pharmacological Chaperones that Promote Survival in Mouse Models of ALS Role: Mentor/PI, Auclair (post-doc) PI Brandeis Sprout Grant 06/2011-06/2012 $10 K direct.
1. *Mechanisms and Consequences of Altered Protein Solubility in Amyotrophic Lateral Sclerosis (ALS).* Muscular Dystrophy Assoc (US). G203655 06/01/02-6/01/05 Role: PI $150 K direct.

Other research Support: Agreements Leading to Equipment

2. "Donation of Two Holographic Imaging, Holomonitor, Systems" From Phase Holographic Imaging (a second instrument). Ed Luther PI. Agar provided lab space,

animal models, consumables, presentation to Phase Holographic Imaging, and biological preparations. 09/2014-ongoing

1. *Development of Ultrahigh resolution MS methods.* This collaboration agreement results in the continuous upgrade of our FT-ICR MS; $30 k of Bruker electronics shop resources; a two year loan (2013-2015) of a Q-TOF mass spectrometer; travel to ASMS 2016 (San Antonio) and IMSC 2016 (Toronto); as-needed (currently three weeks total) visits from applications chemists; "Donation of Dynamically Harmonized Ion Cyclotron Resonance Cell" in 2015 (retail ~ $100k); and is scheduled to result in the "Donation of Dynamically Harmonized Ion Cyclotron Resonance Cell Redesigned for Two-Omega Acquisition" in Dec 2018. Role PI Bruker Daltons (>$250k, 2005-current collaboration agreement).

Pending Funded

1. NIH R21 AG063369-01 "Modifying Cyclic Thiosulfinates to Facilitate Cross-linking Mass Spectrometry Analysis" $350k direct June 2018

Honors, Awards, and Recognition (post-baccalaureate)

1996-1998	National Science Foundation Fellowship at the Center for Metalloenzyme Studies, UGA
2001, 2002	Conrad F. Harrington postdoctoral fellowship
2002, 2003	Jeanne Timmins Costello postdoctoral fellowship
2002-2005	Muscular Dystrophy Association of America Career Development Grant
2010	Alberta Gotthardt Strage and Henry Strage Award for Aspiring Young Science Faculty

Service and Professional Development

Service to the Institution:

Northeastern University Global Initiatives

- **2016 Asia Pacific Economic Council Pilot Center of Excellence in Regulatory Harmonization.** Co-designer and Co-PI of dynamic release on-line curriculum (~25 hours). Online portions taught in Aug. and Oct. 2017. See appendix "institutional service" for details.
- **2016 Asia Pacific Economic Council Pilot Center of Excellence in Regulatory Harmonization** Co-organizer, co-designer, and lecturer for both four-day on-ground courses. This was a hybrid lecture/lab for experiential learning. The first four-day course taught in Burlington MA, USA in Sept 2016 and the second was taught Dec 2016 in Seoul Korea. With the help of representatives from FDA, Health Canada, and Korean Drug evaluators, as well as every major pharma company, we trained two sets of drug evaluators from 12 APEC nations. I also developed online statistics primer based upon the feedback from the first course and gave Skype recorded lecture for Dec 2016 Pilot CoE in Korea.
- **2017 Asia Pacific Economic Council Center of Excellence in Regulatory Harmonization.** APEC ratified permanent online-onground program. http://news.northeastern.edu/2017/10/northeastern-selected-to-lead-global-biotherapeutics-education-training-center/

2017-2018 Northeastern Institutional Service

- **CCB Department:** Service load reduced in recognition of extra work effort on the APEC CoE.

- **Pharm. Sci Department:** Instrumentation Committee.
- **College/University:** BATL service load eliminated in recognition of extra work effort on the APEC CoE. Two internal seminars given at student recruitment events. Visited Glaxo in Philadelphia and gave research seminar as part of College of Science industrial Ph.D. recruiting group.

2016 Northeastern Institutional Service

- **CCB Department:** Analytical Chemistry Position Search Committee. Hired L. Deravi.
- **Pharm. Sci Department:** Instrumentation Committee.
- **College/University:**
  I. **Taught six, five hour continuing mass spec education lectures at NEU's Burlington Innovation Campus.**
  II. Was the academic driving force for the recently awarded "Asia Pacific Economic Counsel Center of Excellence in Biopharmaceutical Harmonization."
  III. Developed draft curriculum and pilot online course for presentation at APEC Peru.
    a. Attended APEC meeting in Peru. Spent one week lobbying for the Center of Excellence in Biotherapeutic Regulation Harmonization course in Peru with Auclair and Luzzi. Awarded a pilot course.
    b. Developed curriculum for Pilot CoE with Jared Auclair including a 15-25 hour, dynamic online learning module, and four days of on-ground classes.
    c. Led one-week course (and taught classes for pilot CoE) in Burlington. Seven major pharmaceutical companies as well as government regulators from four countries were recruited and taught at this Pilot CoE. Trainees were from regulatory agencies of Chile, China, Indonesia, Malaysia, Mexico, Papua New Guinea, Peru, the Philippines, Russia, Chinese Taipei, Thailand, and Vietnam. Observers from APEC and the World Health Organization also attended. Organized social events and chaperoned guest from 12 nations in trip to Boston. Note that in terms of the number of hours spent, this CoE is the equivalent of developing one course and teaching and additional two courses in 2016.
    d. Based upon excellent student reviews and enthusiasm from APEC constituents, this course has been ratified as a full Center of Excellence. The World Health Organization has been in contact and is interested in extending this curriculum worldwide.
  IV. Performed Mass Spec Service: We have analyzed MS samples and provide letters of support to numerous labs, including Sunny Zhou, Roger Giese, Bill Hancock, Alex Makriyannis, Mansoor Amiji, Carolyn Lee Parsons, Alexander Ivanov, Barry Karger, Diomedes Logathetis.
  V. Spearheaded and Contact PI for Schrodinger Site License. Negotiated from $60k down to $30k, organized 12 faculty members, and garnered support for an unlimited Schrodinger software site license. Also organized training service
  VI. Lectured for NEU student day and for the Office of Development.

2015 Northeastern Institutional Service

- **CCB Department:** Graduate Admissions Committee. Equipment Committee.
- **College/University:**
  I. Evaluated and Beta Tested New Digital Measures Merit Review System.

II. **Taught six, five-hour continuing education Mass spectrometry lectures at BATL.**

2014 Northeastern Institutional Service

- **CCB Department:** Graduate Admissions Committee.
- **College/University:**

I. **Led the establishment of Biotherapeutic Analysis Training Laboratory (BATL) labs.**

a. Helped establish a working laboratory (design and order equipment).
b. Met with Water's and NEU representatives to vet curriculum and procure the necessary materials and protocols.
c. Modernized and maintained the BATL website, helped develop promotional materials, *etc.*
d. Developed curriculum for two, three-day Biopharmaceutical Analysis Training Laboratory (BATL) courses- Protein Mass Spectrometry and Antibody Mass Spectrometry- that are taught at NEU's Burlington Campus (with Jared Auclair, who was at the time my post-doctoral fellow). This curriculum formed the backbone of the new Protein Mass Spectrometry lab (Chem 5617) for NEU students, and five sections of Analytical Lab and Bioengineering courses developed by and taught by Jared Auclair.

II. **Taught three, five-hour MS lectures at BATL.**

Brandeis University Committees

2010-2011: Brandeis Intellectual Property Review Committee. Brandeis Institutional Animal Care and Use Committee. 2009-2010: Chem. Dept. Instrumentation Committee. Chem. Dept. Graduate Admissions Committee. Chem. Dept. Graduate Studies Committee. Brandeis Intellectual Property Review Committee. Brandeis Institutional Animal Care and Use Committee. 2008-2009: Chem. Dept. Instrumentation Committee. Chem. Dept. Graduate Admissions Committee. Brandeis Intellectual Property Review Committee. 2007-2008: Chem. Dept. Graduate Studies Committee. Chem. Dept. Instrumentation Committee. Chem. Dept. Organized chemistry colloquia. Brandeis Intellectual Property Review Committee. 2006-2007: Chem. Dept. Graduate Admissions Committee. Chem. Dept. Instrumentation Committee. 2005-2006: Chem. Dept. Graduate Studies Committee. Chem. Dept. Instrumentation Committee.

Director, Brandeis Mass Spectrometry Facility: 2005-2012

We helped over twenty-five Brandeis faculty with their mass spectrometry projects, mostly on a *pro bono* basis. We frequently contributed figures for grants and quality control of purified proteins and molecules. We offer walk-up instrument use to students having taken Chem147b (mass spec). We have been acknowledged by the Ozerov group in multiple publications; provided quality control (accurate mass) in publications of the Yu and Deng laboratories; structural characterization by MS^4 for the Krauss laboratory, our data has been included as a thesis chapter in Nicole Persky's Ph.D. thesis (Lovett lab); and the Petsko-Ringe group has included us as coauthors in submitted manuscript (Biochemistry) where we provided a figure.

Service to the Discipline/Profession:

Education Outreach

1998, 2000, 2010, 2012, 2014, 2016, 2018. Instructor at the Bioinorganic Chemistry Summer Workshop. Courses taught: Metalloprotein mass spectrometry, electron paramagnetic resonance spectroscopy, magnetic circular dichroism, resonance Raman. For example in Summer 2014 and 2016 I taught eight, three hour labs over the course of one week (pro bono). I was a trainee at this conference in 1996, and have been an instructor since 1998. Every research university can send 1-2 students, and the leading bioinorganic spectroscopist PIs train the students extensively.

Review Service

- Grant Review Activity (Foundations): Burroughs Wellcome Fellowships Reviewer 2008; Motor Neuron Disease Association Reviewer 2009, 2015, 2017; Weston Brain Science Foundation (Toronto), 2013-2015; CIHR Reviewer (Canadian Institute of Health Research) proposals 2014; ALS society of America 2015, 2017; Reviewer ASMS Research Award Committee Dec 2015.
  - Grant Review Activity (Federal): DOD *CPRSM ALS TIA* ALS Therapy Development Awards (**2009-2012**); NIH/CSR *Ad Hoc* Astrocytes (**2012**); NIH/CSR *ZRG1 BCMB-D* Shared MS Instrumentation S10 (**2012**); NIH/CSR *ZRG1-BST* High-End Shared Instrumentation S10 (**2012, 2013**); NIH/CSR *Ad Hoc ZRG1 MCDN-B* Biophysics of Neuronal Systems Study Section (**2011, 2012, 2013a, 2013b**); NIH/CSR *ZRG1-BST-N-40* P41 Centers (**2013, 2014**); NIH/CSR *ZMH1-ERB-B-4* Fellowships Grants (**2014**); NIH/CSR *ZRG1-BDCN-W-2* Special Emphasis Brain Disorders: Trauma, Hydroceph. and Alzheimer (**2014**); NIH/CSR *Ad Hoc* DBD Developmental Brain Disorders Study Section (**2010, 2012, 2014, 2015**); NIH/CSR *ZNS1-SRB-N-08* NINDS P30 review (**2015, 2017, 2018**), ZNS1 SRB-N (08) Research Resource (R24) Review **(2015)**. ASMS Research Award Committee (**2015**). DOE/SBIR grant review (**2018, 2019**); NIH (NINDS ZNS1 SRB-A(23)) POI review (**2019**), NIH BCDN anonymization panel (2019) .
- **Granted continuous submission privileges at NIH 2012-2016 due to high service load.**
- Journal reviewer for Analytical Chemistry, PLoS Biology, Nature Chemical Biology, Nature Methods, Analyst, PNAS, JACS, JBC, JASMS, ACS Neuroscience, Annals of Medicine, *etc*. Ad Hoc Academic Editor PLoS Biology.

Leadership

- Board Member. Consortium for Top-Down Proteomics 2014 – current.
- Committee member NSF WORKSHOP MASS SPECTROMETRY DATA TO KNOWLEDGE, May 2015. This was a think tank organized by John Yates to make recommendations for NSF funding directions.
- Committee member NSF "BRAIN" WORKSHOP, Oct 2016. This was a think tank organized by John Sweedler to make recommendations for NSF funding directions.

Software Provided to Research Community

- QUDeX-MS
- Isotope Calculator

Service to the Community/Public:

- Board member and then **Chairman of the Board**, Centre de la petite enfance (CPE) for Regulated Daycare Oversight Board, Montreal, PQ (August 2003 - May 2005). All

child daycare is regulated in Quebec by this government agency https://www.mfa.gouv.qc.ca/fr/services-de-garde/cpe-garderies/pages/index.aspx The board had operational authority over CPE activities for ~40 daycare facilities on the island of Montreal. This required understanding and application of Quebec's (Napoleonic, French Language) Civil code. We approved new facilities, were responsible for re-approval of existing facilities, liaised with CPE regulators, hired and dismissed workers, and dealt with corrective and punitive action.

- Society for the Advancement of Chicano and Native American Students (SACNAS). Served as mentor, poster competition judge, and attend National Conference.
- Head Coach of 6th grade football at Newton's Brown Middle School (2013).

Professional Development

- 2003- American Society for Mass Spectrometry.

Teaching

Since 2005 student evaluations consistently place my courses in the upper quartile, and generally above the 90th percentile, in instructor effectiveness.

Northeastern Courses Taught

Spring 2018

- CHEM 5615, Online Protein Mass Spectrometry, Credit Hours: 3.000. Enrollment: four students (extra compensation, note the time commitment for this completely automated course is minimal).
- CHEM 5616, Protein Mass Spectrometry, Credit Hours: 3.000. Enrollment: 21 students (extra compensation, note the time commitment for this completely automated course is minimal).

Spring 2017

- Chemistry teaching load reduced in return for teaching APEC CoE in 2016.
- CHEM 5616 02, Online Protein Mass Spectrometry, Credit Hours: 3.000 Enrollment: four students (extra compensation, note the time commitment for this completely automated course is minimal)
- PHSC 6218 01, Biomedical Chemical Analysis, Traditional, Credit Hours: 2.000, Enrollment: 14 students, mean teaching effectiveness 4.8/5.0.

Fall 2016

- Co-developed (with Auclair) and lectured a 15 hour, adaptive release online course for the Asia Pacific Economic Cooperative (APEC) Pilot center of excellence. See NEU service section for details. **(New Course).**
- Co-developed (with Auclair), organized, lectured, and MC'd a four day on-ground course for the Asia Pacific Economic Cooperative (APEC) Pilot center of excellence. See NEU service section for details. Both courses had excellent reviews. **(New Course).**
- Developed statistics online prime and gave lecture for Korean Pilot **(New Course).**

Spring 2016

- CHEM 5616 01, Protein Mass Spectrometry, Traditional, Credit Hours: 3.000, Enrollment: 32.
- CHEM 5616 02, Online Protein Mass Spectrometry, Credit Hours: 3.000.

Spring, Summer, Fall 2016.

- Taught six lectures (5 hours each) for BATL courses. *Note: this was uncompensated and in addition to my normal teaching load.*

Spring 2015

- CHEM 5616 01, Protein Mass Spectrometry, Traditional, Credit Hours: 3.000, Enrollment: 30.
- PHSC 6218 01, Biomedical Chemical Analysis, Traditional, Credit Hours: 2.000.

Spring, Summer, Fall 2015.

- Developed online protein MS course.
- Taught six lectures (5 hours each) for BATL courses.

Fall 2014

- Taught three lectures (5 hours each) for Biopharmaceutical Analysis Training Laboratory (BATL) courses at Burlington Innovation Campus. **(New Course)**

Spring 2014

- CHEM 5616 01, Protein Mass Spectrometry, Traditional, Credit Hours: 3.000, Enrollment: 25. **New Course)**
- PHSC 6218 01, Biomedical Chemical Analysis, Traditional, Credit Hours: 2.000. **(New Course)**

Brandeis Courses Taught

- Chem 142a Quantum Chemistry (Spring 2011, **New Course).**
- Chem 123b Bioinorganic chemistry (Spring 2008, **New Course**).
- Chem 147b Mass Spectrometry (spring 2006, fall 2008 and 2010, **New Course).**

Brandeis Courses Co-Taught

- One lecture. Cont 300B Ethical Practice in Health Sciences.Ethics of collaboration (2008).
- Lecturer Biochemistry 103b Advanced Biochemistry-Information Transfer Mechanisms (multiple lectures, **New Course**).
- Business 261 Technology Strategy, supervised one group of students on their project relating to our Patented Matrix Solution Fixation technology (Spring 2008).
- Lecturer (one lecture) COSI 230A Topics in Computational Biology (2007, **New Course**).
- Lecturer (two lectures) NBIO 146a The Neurobiology of Human Disease (2006, **New Course**).

Mentoring

PhDs Awarded	Term	U.G.	Project	Awards and Support
Jennifer Stroka-Cobb, **Ph.D.** *Employed by Novartis.*	2006-2010	B.S., 2003, Lafayette University	Top-Down mass spectrometry methods development	2009 – International Mass Spectrometry Foundation Travel Award 2009 – Greater Boston Mass Spectrometry Discussion Group Student Travel Award 2009, 2008 – Provost's Dissertation Expense Award 2008 – Graduate Student Association Travel & Research Grant 2007 – Graduate School of Arts & Sciences Outstanding Teaching Fellows Award
Kristin Boggio, **Ph.D.** *Employed by Pfizer*	2006-2011	B.S., 2005 Univ. Massachusetts Boston	Design of ALS therapeutics, MALDI mass spectrometry imaging	2010- Provost's Dissertation Expense Award 2009 – International Mass Spectrometry Foundation Travel Award 2009 – Provost's Dissertation Expense Award 2008 – Graduate Student Association Travel & Research Grant 2008 – Graduate School of Arts & Sciences Outstanding Teaching Fellows Award
Murat Karabacak, **Ph.D.** *Research fellow at Mass. General Hospital*	2006-2010	B.S., 2004 Ankra University	FTICRMS methods development	2009 – Provost's Dissertation Expense Award
Qi Wang, **Ph.D.** *Employed by Waters Inc.*	2005-2010	B.S., 2003 Nankai University	Mechanisms of ALS pathogensis	Graduate school of Arts and Sciences Outstanding Teaching Fellow 2006 *Post-doctoral fellow at the BU School of Medicine.*
Joshua Johnson, **Ph.D.** *Employed by Novartis*	2006-2011	B.S., 2005 Univ. Wisconsin	Mechanisms of familial ALS pathogensis	IGERT Fellow

Qian Liu **Ph.D.** *Employed by STC Biologics*	2008-2012	B.S. Nankai University	Mechanism of ALS pathogenesis.	2010- Provost's Dissertation Expense Award
Joseph Salisbury, **Ph.D.** *Employed by Brain Power, LLC*	2008-2012	B.S/M.S. Rhode Island College	Development of small molecule inhibitors of SOD1 aggregation.	2012 – ASMS Student Travel Award (Greater Boston Mass Spectrometry Discussion Group) 2011 – Brandeis University Virtual Incubator Sprout Grant 2017 – CDMRP Autism Research Program, grant PI.
Jeniffer Quijada, **Ph.D.** *Stanford Post-doc.*	2013-2017	B.S. UC Irvine	Top-Down Protein Quantification	2015 – ASMS Student Travel Award (Greater Boston Mass Spectrometry Discussion Group). 2016- Provost's Dissertation Expense Award
Catherin Rawlins Ph.D. Weighing three post-doctoral offers	2014-2018	B.S. Wisconsin-Stout	MALDI Imaging Studies of ALS Mouse Models	2017 – Wolfgang Goetzinger-Amgen memorial scholarship; ACS Leadership Award 2018- Provost's Dissertation Completion Award; College of Science Graduate Student Excellence Award in Leadership

Current Ph.D. Students

- The following NEU graduate students are members of the Agar laboratory: Nicholas Schmitt, Dan Donnelly, and Amin Hossian, and Industrial Ph.D. students Krishna Aluri (with Alnylam Pharma), Richa Sarin (Biogen), Rutali Brahme (Novartis), Jakal Amin (Charles River).
- Agar co-supervised Di Wu and Yanjun Liu with Bill Hancock, and Zhidan Chen with Paul Vouros.
- Two post-doctoral fellows, Joseph Salisbury and Jared Auclair (promoted to Senior Research Scientist and now running the BATL laboratory and PSM Biotech. program) were mentored.
- David DeFillippo and Haly Raharimampionona received M.S. from the Agar Lab in 2014 and 2015, respectively.
- **Visiting Scientists:** Takuya Nagama, Ono Pharmaceuticals- one year industry sabbatical (Aug 2015-Sept 2016) to learn protein MS

Undergraduate and MS Student's Supervised by Agar Lab

The Agar laboratory is a popular amongst undergraduates and masters students (including entirely paying PSM biotech and Pharm. Sci students). We have a supportive environment and a detailed, formal training program that each student undergoes (tantamount to a lab class) that includes required completion of EH&S safety training, training in micropipette craft, MALDI-TOF operation, laboratory information management systems, SDS-PAGE, cell transformation, cell growth, protein purification, etc). I set aside a few hours and in some cases much more, for personally training each student, and interact with students almost every day they are

in lab. I also keep records of starting salary, and coach each student on the job interview and negotiation process. The students listed below were active researchers, generally 10-20 hours for MS, and five or more hours for UGs.

Northeastern Undergraduates Supervised: Mary Duffy; Molly Blevins; Carrie Brown; Curtis Gong; Danielle DeLooze; Jessica Xu; Elliott Mueller; Caroline Lucas; Joy Horng, Justin Crisafulli, Nathanial Shepard, Jeremy Conway, Nathalie Leung, Isabella Miele, Millie Ness, William Peterson, Sydney Geyer, Nicole Vieira, Joshua Berger.

Northeastern (Paying) M.S. Student Researchers Supervised: Nabila Newaz: Brandeis Chemistry. *All that follow are NEU students:* Meenal Chaudhari (Pharm Sci); Tri Devi Dahal Busfield [PSM Biotech (PSM)]; Janice Ferreira (PSM); Chirag Jain (PSM); Sandeep Kini (Pharm Sci); Fnu Ruchika (PSM); Madhumita Ramesh (PSM); Shreya Sarraf (Pharm Sci); Sneha Shenoy (Pharm Sci); Anirudh Singh (PSM); Vineet Joshi (Pharm Sci), Radhika Barve (PSM), Omkar Bhate (PSM), Senchan Khamboung; Chinmayee Shah (PSM), Shreosi Ghosh (PSM), Merlit Mathew (PSM), Hetvi Desai (Bio-Tech), Shama Pilankar (Bio-Tech), Meet Shaw (Bio-Tech), Durga Sivasankar (Bio-Tech). Each student had job offers upon leaving the lab, and multiple MS students are co-authors on our group's publications.

Brandeis Undergraduates Supervised. **2006** Jennifer Chabra (jenni_chabra@yahoo.com): REU Bottom-up Proteomics. **2007** Josh Agranat (jagranat@brandeis.edu): Ion Trap liquid chromatography mass spec. **2008** Jung Gun "Justin" Song (jsong781@gmail.com) Top-Down Monoisotopic Mass Determination, Sagar Patel: Yeast metalloproteins. Medical Student at A.T. Still University-*Kirksville* College of Osteopathic Medicine. **2009** Rebecca Lazarus (rlazarus@brandeis.edu) Honors **Thesis:** Purifying and Labeling single neurons. Fjodor Melnikov (shlaffen@brandeis.edu): Single neuron purification, Worked with Harvard School of Public Heath and then company Gradient Environmental Health. John Wong (jhwhy@brandeis.edu): Publication in *Methods in Molecular Biology*, see Agar's publication list for details. Labeling single neurons. HehSun Kim (hehsun@brandeis.edu): Slicing flies Applying for Dental School. Jung Gun "Justin" Song (jsong781@gmail.com): from Bowdoin University. Top-Down Monoisotopic. Yeon Hwa "Jennifer" Jung (jyung@smith.edu): REUY from Cornell University. Drug development. Daniel Weisz **Thesis.** Top-Down Monoisotopic Mass Spectrometry. Became a graduate student at University of Washington St Louis, now finished with Ph.D. **2010** Stacey Frisch (sfrisch@brandeis.edu): mouse husbandry, Gabriel Bronk (gbronk@brandeis.edu): Out-Gel Digest mass spectrometry. **2011** Emmanuel Obasuyi (emmaobas@brandeis.edu): High Honors Thesis, Labeling single neurons. Peer reviewed publication in *Expert Review of Proteomics*, see Agar's publication list for details. Nana Sarp (nosarp@brandeis.edu): Top-Down Proteomics. Brian Williams (briwilli@brandeis.edu): High Honors Thesis, Computational docking studies of small molecule SOD1 stabilizers. Nikhil Malik (nikhil.nmalik@gmail.com): Animal husbandry of ALS model mice. Brandon Meiseles (br.meiseles@gmail.com): (Duke University): Proteomics of Parkinson's Brains. Yuewei Tao (yuewei@brandeis.edu): MALDI MS training. **2012** Bhavin Patel: Highest Honors thesis on the role of the proteasome in ALS etiology. Josue Alfaro M.S. 2010-2013 B.S. UC Irvine "MALDI Imaging MS" IGERT Fellow. M.S. Student Tracy Frish.

Student Advising

Northeastern Advising Committees. 2018 Thesis committee: Christina Codden, Stubins group, Ahmed Said, Ivanov Group, Thesis defense committee: Di Wu, Hancock group, Yuanyuan Yao, Giese group, Lihau Yang, Zhou group. 2017 Thesis defense committee: Shanshan Liu, Zhou Group. **(chairperson)** Arseniy Belov, Karger Group. **2016 Thesis committees:** Megha Kamath, Carrier/Amiji Groups. Arseniy Belov, Karger Group. Di, Yanjun, Hancock Group. Di Wu, Hancock Group. Yanjun Liu, Hancock Group. Yu Wang, Hancock Group. Shanshan Liu, Zhou Group. Yuanyuan Yao, Giese Group. Wenjun Di, Heather Clark Group. **2016 Ph.D. Thesis Defense Committees:** Siyuan Liu, Karger Group March 2016. Gregory Pirrone, Engen Lab April 2016. Xianzhe Wang, Karger Group April 2016. Kalli Catcott, Zhou Group Dec 2016. Simion Kramer, Karger Group Dec 2016. **2015 Thesis committees.** Xianzhe Wang, Karger Group. Yuanwei Gao, Karger Group. Siyuan Liu, Karger Group. Arseniy Belov, Karger Group. Di Wu, Hancock Group. Yanjun Liu, Hancock Group. Yu Wang, Hancock Group. Gregory Pirrone, Engen Group. Shanshan Liu, Zhou Group. Yuanyuan Yao, Giese Group. **2015 Ph.D. Thesis Defense Committees:** Christopher Chumsae, Zhou Group April 2015. Fan Zhang, Karger Group May 2015. Ph.D. **External Ph.D. thesis defense committee member:** Meena Kathiresan, Ann English Group, Concordia University Montreal Quebec, Sept 2015. **2014 Thesis committees**. Xianzhe (Jason) Wang, Karger Group. Yuanwei (Abby) Gao, Karger Group. Fan (Anna) Zhang, Karger Group. Gregory Pirrone, Engen Group. Yu (Annie) Wang, Zhou-Hancock Groups. Shanshan Liu, Zhou Group. Yuanyuan Yao, Giese Group. Ph.D. **External Ph.D. thesis defense committee member:** Reddy Sama, Daryl Bosco Group, U Mass Medical School, March 2014.

Brandeis MS or Ph.D. Thesis, Progress, or Proposal Committees (2005-2012). Jon Kay, undergraduate in neuroscience, Honors. Ben Cuifo: graduate student in MCB. Yearly Progress. Wladimir Labeikovsky: graduate student in biochemistry. Proposal. Bo Hong: graduate student in chemistry. Proposal. Marina Dang: graduate student in Chemistry. Proposal. Nicki Persky: graduate student in MCB. Thesis. Chris Hoefler: graduate student in Biochemistry. Yearly Progress. Iva Petrovic: graduate student in Chemistry. Proposal. Chris Knoell: graduate student in MCB: Yearly Progress. Sean O'Toole: graduate student in MCB: Proposal. Duane Winkler (University of Texas San Antonio) Outside Thesis Reviewer. Sai Venkatesh Seetharaman (University of Texas San Antonio) Outside Thesis Reviewer. Aram John Raissi: Proposal. Maria Genco graduate in Neuroscience: Proposal. Sivanne Pearl graduate student in MCB: Proposal.

Teaching Statement:

Teaching Accomplishments and Philosophy:

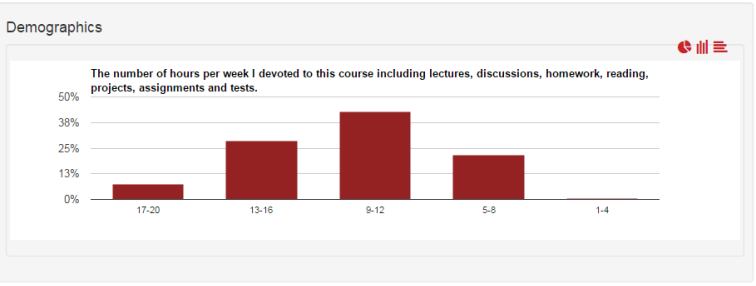
As a result of winning a university-wide Distinguished Tutor Award, I was given the opportunity to teach one-hour chemistry recitation courses while still an undergraduate at The University of Michigan Flint. Starting in my sophomore year I taught as many as 6 classes per semester to classes of 30 college students. I thoroughly enjoyed helping college students learn chemistry—listening to what they considered perplexing and helping them to grasp the underlying simplicity. By the time I had obtained my

bachelor's degree I had a great deal of teaching experience, and the confidence to help anybody learn just about anything having to do with chemistry.

My teaching is predicated upon a deep, contemplative understanding of the subject material; putting the material into modern and historical perspectives; finding methods to make students work hard and contemplate; and respect and empathy for the students. My teaching evaluations have continually improved, even in years where the subject material and my mastery of the subject were unchanged. I am certain that these improvements are due to my finally having learned to translate "on the fly" the words I am speaking in class to the ideas my words invoke in the students. This is possibly a combination of observing the students closely, and fostering a respectful environment where the students can say "I didn't get that at all."

One of my major teaching objectives is to give students the tools that will help them stand out at the next level. Most often that is not rote knowledge. They need to be able solve real-world problems. I expect students to put what they learn in my class into a broader context, stressing historical perspective, social perspective, and scientific perspective. I stress understanding the fundamentals over memorization. I expect students to develop a healthy sense of skepticism. For example, in the final third of a class, I provide many examples of now-defunct or disproven primary literature and allow the students to dissect these papers. The students then scour the primary literature to find additional publications that they are skeptical of and submit this as homework. Their writing is evaluated with great scrutiny, even the grammar and punctuation. I want my students to feel confident that they can have a meaningful conversation and hold court with a world's expert.

I pay close attention to my evaluations and attempt to correct anything perceived by the students as an area for improvement. For example, in my first Brandeis course, Chem 147b, the organization of my individual classes was lacking (more precisely, it was evaluated as average), whereas the overall course structure was well above average. In my second teaching of Chem 147b, I addressed this issue, as reflected by the evaluations. I gave the lectures a visual makeover, organized the online teaching (Latte) environment, made future lectures available so that students with English as a second language could review the material before class. I have continued to improve my Northeastern courses in the same way. In particular, through the use of online tools, I learned to simultaneously remediate my lowest quartile students (on day-one) while challenging the best students. See the 2016 demographics figure below (2018 results are similar)—the lowest quartile student work 13-20 hours per week on the online content, which allows them to catch up with the better students. *Remediating my poorest students while still challenging my best students is my crowning achievement as an educator.*



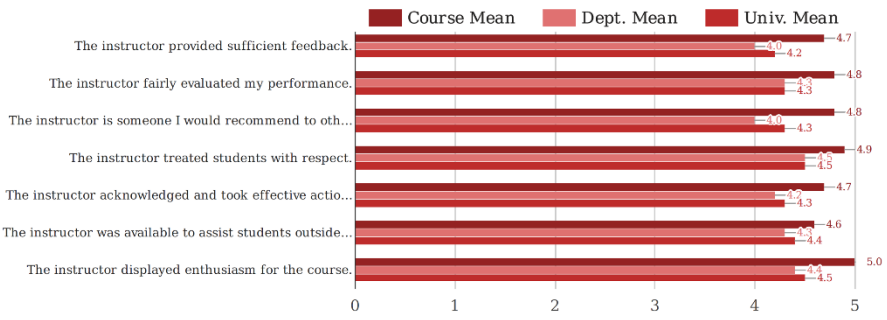
I am a student of teaching. I have attended many workshops and training courses on evidence-based teaching, and have benefited greatly from CATLR (NEU has Ph.D.s on staff devoted to improving our teaching) and NU Global (NEU's

online course development experts). I have watched over ten of Adobe's hour-long videos concerning online education – even the hour concerning fonts. I have studied testing theory. My tests routinely have standard deviations within one or two points of ~18%, which is what I want them to be. To reduce possible grading bias, I grade blinded to student's identities, asking students to put names on the back of exams, and giving them the option of using a student ID (rather than a name) for the entire year. I do not believe in high-stakes testing (the evidence does not support it). I use grades primarily as a motivator—giving weekly quizzes and offering second chances to learn material in return for improved grades. I understand the indifference of standardized test giants like ETS, and avoid inadvertently testing students on their vocabulary, English language comprehension, socioeconomic background, or test-taking ability. I am acutely aware of the challenges faced by ESL students, lower socioeconomic status students, even students with ADH(D). My online lectures are transcribed for ESL students, which also helps other students learn through a second (visual in addition to auditory) channel. I am careful not to cognitively overload my students, and make good use of multimedia assets. I developed every course I have taught from scratch.

A preponderance of evidence from the educational literature indicated that "flipping" my classroom (a matter of giving lecture material ahead of class and creating online tests to assure the material was covered) would get the students to work harder and learn more. I also knew that online teaching methods gave me access to more areas of the student's brain (especially visuospatial channels that lend themselves to understanding the structured world of chemistry); to more of their time (through mobile devices); and to a more personalized and dynamic learning experience. I took an offer to develop an online course with the ulterior motive of using the online course material to flip my on-ground course. After a major time investment, the online course was complete. The resulting flipped on-ground course was a great success (the median instructor effectiveness was 5.0/5.0, the mean was 4.7/5.0 in 2016, and improved to 5.0/5.0 and 4.8/5.0 in 2018, note SD = 0.4). More importantly, students were investing ~9-20 hours per week in the class (see figure above). For these classes I secured a six-month license for Bruker's software suite for each student's laptop, set up five remote servers, and made instructional videos on how to use the software. This gives the students the rare opportunity to apply their knowledge to real-world, cutting edge data and problems, and they are always enthralled. After flipping my classroom, I was elated by how much the students learned compared to previous years—especially the advanced applied materials. I'll allow the students to speak for themselves. The following comments are not "cherry picked" and are representative.

The most common sentiment is that students are grateful for the flipped classroom. Here are a few of their comments, with my favorites underlined. "Teaching this course as a 'reversed course' is an EXCELLENT way to teach science. I skipped every science lecture I could as an undergrad. Sitting for two hours while someone reads through a slide deck is a 95% waste of my time. These concepts are far too complex to absorb on the fly during a PowerPoint lecture at least for someone with my meager mental abilities. That time is much better utilized going over difficult topics/examples and diverging into interesting related issues, like Dr. Agar did. Another aspect of this course that I thought was great is the incorporation of Bruker's software into the

course. Prior to this class I had never had the opportunity to analyze MS data. Using the software to analyze real MS data was, honestly, a real confidence booster for me"; "I found myself exasperated at the difficulty/amount of material about 15-20% of the time which in hindsight seems about right for a graduate level class that costs 4 grand."; "Great course for anyone interested in doing Protein Mass spec. Be ready to work a lot, as this course has a lot of material to cover"; "this is one the best course I have had in northeastern. Dr. Agar is very knowledgeable and expert in the protein mass spec field and I feel its a great pleasure to learn from him"; "Its the best course I have taken in my master's program. It is both challenging and informative"; "The online modules were very helpful. I was able to go through slides for material I found difficult, while fly through the ones I was comfortable with (notably, not many!). Had this not been a flipped classroom, I think I would have done poorly" "Awesome class, very challenging but fair." "I was very skeptical at first of the online lectures vs. in-class lectures but I was wrong. I learned a shit load from the online lectures and it was supplemented well with the in-class discussions." "Best instructor I've had at the university. He was engaging and knowledgeable and always brought his A game. He made my brain hurt with in-class discussions and activities but I walked away feeling like I learned something. There was a lot of material to learn but it was given to us in a way that was manageable."



Those student's comments and the following sum up my philosophy. Be honest with the students—they are going to have to work hard. Be fair with students—make your expectations (learning objectives) and methods of evaluation clear. Inspire and motivate students—lead by example by working hard for them, showing enthusiasm for the subject, and giving examples of the utility/power of knowledge. Impart confidence—prove to the students that they have all of the tools to compete with the best scientists in the world. A few of the comments from my 2018 Protein MS course evaluations are shown in the figure below.

Other teaching highlights. Brandeis Chem 147b, Mass Spectrometry Laboratory. I offered a laboratory where students learned to operate our mass spectrometers, and to analyze their data. ~40 students, post-doctoral fellows, and one professor (Tom Pochapsky) attended or audited this course. They were free to bring their own samples and begin independent projects. A number of students from other laboratories who took the class went on to have successful mass spectrometry projects that contributed to their thesis (Dan Tardiff using Harvard's facility and Nicky Persky, Marina Deng, and Wladimir Labeikovsky using our facility, to name a few). Woong Kim, a Brandeis post-doctoral fellow in Piali Sengupta's laboratory, used his mass spectrometry skills to get a job at Steve Gygi's mass spectrometry lab at Harvard. Paying masters student, Tracey Friss (tracey.friss@gmail.com), secured a job as a mass spectrometrist based solely upon her training in Chem 147, and has since hired

three more of my trainees! I suggested that she attend the Greater Boston Mass Spectrometry User's Group meeting, and offered cab fare to be paid by the department. At this meeting she met her future employers at Berg Diagnostics. Brandeis course Chem 123b, Bioinorganic Chemistry. I invited Steve Lippard, who wrote the textbook we used for Chem 123b, to have lunch with the students and to give a seminar. He signed their textbooks, and had a chat with each of the students.

SYLLABUS Spring 2016

CHEM5616 Protein Mass Spectrometry (3 SH Credit)

INSTRUCTOR: Jeffrey Agar, Ph.D.
417 The Fenway
Tel: 617-373-5909
E-mail: j.agar@neu.edu

CLASS MEETS: Forsyth Building 201. 6-8:30 PM Tuesday

OFFICE HOURS: One hour following class.

TEXTBOOK: Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation, J. Throck Watson, O. David Sparkman, ISBN-10 0470516348

Introduction

Arguably the most popular analytical method in science, mass spectrometry is utilized in fields from sub-atomic physics to biology. This course introduces modern mass spectrometry hardware, methods, and data interpretation and how these are applied to the analysis of proteins. Applications include biomarker discovery, tissue characterization, forensics, drug-discovery, and the characterization of protein-based therapeutics. Given a particular protein chemistry problem, the successful student will be able to choose the appropriate mass spectrometry method and interpret the data. Reading will be assigned with most lectures. *The protein MS lab course, Chem 5617, is complementary (little overlap) and focuses upon preparation of biological samples, operation of liquid chromatography/mass spectrometry instrumentation, and data analysis.*

Course Organization

This will be a "flipped" classroom, which means you'll be given required introductory material and an online quiz before lecture (except for lecture 1) so that we work and master real-world examples in class. The introductory material includes a weekly online module (with multiple lectures) that needs to be completed before class, as well as readings. The lectures should take one-three hours, and upon completion you'll receive credit. After completing the online lecture and the weekly readings you should take the online quiz. There will also be a ~10 min in-class quiz at the end of every class that will include questions relating to the in-class discussion and online lectures. Grade includes quizzes (70%, 35% for online quizzes and 35% for in class quizzes), homework (15%), completing the weekly lessons on time (10%), and final presentation (5%). Homework and final presentation instructions and rubric are available in Blackboard as "Homework Instructions." Homework is to be uploaded via Turnitin and can be accessed under the "assignments" tab in Blackboard. Examples of

exemplar homework and presentations are included in both the course content and modules folders in Blackboard.

Course Grading

Grading for quizzes, homework, and oral presentations are stringent to prepare you for the expectations of the modern workplace. Earned grades are given (no grade inflation).

A	92-100%
A-	90-91
B+	88-89
B	83-87
B-	80-82
C	70-79
D	60-69
F	< 60

Schedule and Topics

Schedule and topics are posted in the "Course Overview and Map" in the *Syllabus and Course Resources* tab within Blackboard. Advanced topics may be added to adapt to new research breakthroughs and student feedback/interest. Any changes will be noted in Blackboard.

Course Objective and Learning Outcomes: Are listed explicitly for each week (module) in blackboard.

Final Examination: I do not believe high-stakes testing is beneficial to the learner. As a result there is no final examination. Your grade for shorter, more tractable, weekly quizzes is the primary method of evaluation.

Course Delivery: This course is delivered online via the Blackboard learning management system. To access your course, login to [blackboard.neu.edu] using your MyNEU credentials. From there, click the "Courses" tab and then select your course.

Course Geography: Each weekly "module" has multiple lectures, assigned reading, and there is a weekly quiz in scope covering that week's material. In the final third of the course there will be three homework assignments, and one presentation

Course Description: Offers students an opportunity to obtain a fundamental understanding of modern mass spectrometers, to conceptually operate these instruments, and the ability to prepare biological samples. Undoubtedly the most popular analytical method in science, mass spectrometry is utilized in fields ranging from subatomic physics to biology. Focuses on the analysis of proteins, with applications including biomarker discovery, tissue characterization, detection of blood doping, drug discovery, and the characterization of protein-based therapeutics. By the end of the course, the student is expected to be able to solve a particular chemistry- or biology-related problem by choosing the appropriate sample preparation methods and mass spectrometer.

Technical and Software Requirements: A computer with a modern webbrowser (this is not a mobile-friendly course). Viewing course videos requires that Adobe Flash Player is installed on your computer or device. Please visit their website https://get.**adobe**.com/**flash**player/ to download and access technical support.

Course Prerequisites: Students should have taken two semesters of introductory (freshman) chemistry, and one (two recommended) organic chemistry course. An introductory mass

spectrometry course is recommended, and will greatly decrease the time commitment necessary for the first seven weeks.

Course Expectations: "Attendance" for this class means completing the online lectures, weekly quizzes, and assignments for a given week, on time. Online discussion is facilitated through blackboard and is encouraged, including explicit discussion of how to solve the check your knowledge questions in weekly lectures. LATE WORK & MISSING CLASS: Completing the weekly lectures and assignments on time is mandatory. If you miss the lectures or the weekly quiz or homework or presentation you will receive a grade of zero, and cannot make up the material later. This is a matter of fairness to the other students, assuring all students are graded based upon the same criteria, and assures academic integrity, since answers to quizzes are posted. Late homework is not accepted for any reason. Please don't submit homework at the last minute- problems with electronic submission can occur but can't be used as an excuse. There will be no exceptions to this policy.

Statement of Time Commitment: This is a rigorous class. The online lecture should take ~1-3 hours to complete, for average students the assigned readings vary from 1-5 hours per week, and additional study time depends upon the student, but should average 2 hours. Students with Disabilities Students needing disability accommodations should visit the Northeastern University Disability Resource Center (DRC).

Student Privacy (FERPA): The Family Educational Rights and Privacy Act (FERPA) affords students certain rights with respect to their education records. For information about these rights, visit the Northeastern University Office of the Registrar.

Blackboard and Technical Support: Northeastern students can get technical, computing, and Blackboard support through the Northeastern University Information Technology Services Student Support site.

Financial Aid: Please visit the Northeastern University Student Financial Services for information about grants and scholarships and how to apply for aid.

Library: For Library services and support, please visit Northeastern University Libraries.

Important Dates: Please review the appropriate Academic Calendar for important Graduate School dates for the current and upcoming semesters.

Diversity: Northeastern University is committed to providing equal opportunity to its students and employees, and to eliminating discrimination when it occurs. For more information, including grievance procedures, please visit the Northeastern University Office of Institutional Diversity and Inclusion.

Academic Integrity: The highest standards of academic integrity are expected. Academic misconduct/dishonesty/cheating are not tolerated and will be acted upon in accordance with University policies, which may include an automatic F in the course, involuntary withdrawal, and/or automatic reporting of offense(s) to the NEU Office of Student Conduct and Conflict Resolution. Work that violates these policies cannot be redone. All students must adhere to the university's Academic Integrity Policy, which can be found on the website of the Office of Student Conduct and Conflict Resolution (OSCCR), at http://www.northeastern.edu/osccr/academicintegrity/index.html. Please be particularly aware of the policy regarding plagiarism. As you probably know, plagiarism involves representing anyone else's words or ideas as your own. It doesn't matter where you got these ideas—from a book, on the web, from a fellow-student, from your mother. It doesn't matter whether you quote the source directly or paraphrase it; if you are not the originator of the words or ideas, you must state clearly and specifically where they came from. Please consult an instructor if

you have any confusion or concerns when preparing any of the assignments so that together. You can also consult the guide "Avoiding Plagiarism" on the NU Library Website at http://www.lib.neu.edu/online_research/help/avoiding_plagiarism/. There are many ways to plagiarize that don't involve copying verbatim, but if you're copying four or more words in a row and not citing the source, you're probably plagiarizing. If an academic integrity concern arises, one of the instructors will speak with you about it; if the discussion does not resolve the concern, we will refer the matter to OSCCR. It is the responsibility of each student to read and understand these policies, including understanding what plagiarism is.

Course Policies: Be sure to review the College of Science Academic Course Policies available at http://www.northeastern.edu/cos/wp-content/uploads/2014/11/Northeastern- COS-Policies-Template.pdf. These policies hold across all courses taught in the College of Science.

Wk.	Short Title	Topic	Main Concepts	Textbook Reading and external content	Assessment
1	MS History and Building Molecules	**Evolution of MS and protein applications. Building molecules: How the mass spectrometer modifies molecules.**	Origins of Advanced Protein MS Technology (L1) and How MS Ionization Modifies Molecules (L2)	Textbook: Preface and pp **1-21**. (Lesson pp **9-21**, Lesson 2 pp 1-8).	Quiz 1
2	Building Spectra	**Building spectra: From elements and their isotopes to molecules and their mass spectra.**	Critical Concepts and Terminology: Resolution, Accuracy, Elemental Composition, Isotopomer Distribution, isotopic fine structure, deconvolution.	Textbook: pp 22-44 (intro); pay extra attention to 22-28 and 269-275 (critical defs); 293-311 (molecular mass to molecular formula)	Quiz 2
3	Building and Interpreting Protein Spectra	**Building and interpreting complex protein mass spectra: how neutrons modify the spectra.**	Developing a (probabilistic) mental model for generating isotopomer distributions (L1). Molecular Mass and Molecular ID From Experimental MS spectra (L2). Apps for MS	Nothing Like L1, most of L2, or L3 in your (or any) book. pp 276-293 (using relative peak intensities in ID'ing molecules).	Quiz 3

			Interpretation (L3).		
4	Ionization and EI Fragmentation	**Mass Spectrometers: Ionization and fragmentation mechanisms and techniques.**	The physical and chemical mechanisms of ionization and protein fragmentation.	Review pp **270-271. 180-181** (CAD-ECD). **317-344** EI mechanism). **405-412** (EI fragmentation mechanism of N compounds). Optional **349-404** (EI interpretation). **486-505** (ESI). **521-553** (MALDI) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC470779/ (ECD, ETD mechanism). **443-448** (EI search)	Quiz 4
5	MS Instrumentation	**Mass Spectrometer: Instrumentation and how it works.**	***How molecules and their fragments are weighed in the mass spectrometer, part I.*** The basics of popular mass analyzers, and tandem mass analyzers (MS/MS).	Read pp 53-131	Quiz 5
6	Advanced Fragmentation (CAD MS/MS)	**Mass Spectrometers: Using isolation and fragmentation wit hin the mass spectrometer for protein and PTM identification and quantification.**	***How molecules and their fragments are made and weighed, part II.*** How to generate and interpret useful CAD MS/MS data (L1). Instrument operational principles for	Primary Literature	Quiz 6

			advanced MS/MS experiments (L2).		
7	Chromatography (LC-MS)	**<u>Mass Spectrometers</u>: Using isolation by chromatography to improve protein mass spectra.**	***How molecules and their fragments are separated before introduction to the mass spectrometer*.** Sample Preparation and Chromatography Techniques for Proteins. Fractionation-based forensics.	Ch 10 (look over), and Ch11 of textbook	Quiz 7
8	Protein Sequencing ID	**<u>Interpretation:</u> Peptide Sequencing and Protein ID.**	Interpreting MS/MS: How to identify peptides, proteins and their primary structural modifications. When and how to use collisional-, electron-, photon-based dissociation, and how to interpret the data (L1).	Primary Literature	Quiz 8

9	Quantification	**<u>Interpretation:</u>** Quantification	Quantification methods: Spectral counting, MRM, Isotope dilution. Biomarker and drug target identification.	Primary Literature	Quiz 9
10	Biochemistry and biologics Student Check-In for Assignments	**<u>Biochemistry, Drug Discovery, and Biologics</u>**	Protein structure and function relationships. MS in drug discovery I. Target ID and characterization. Sample preparation strategies. Characterization of Protein-based therapeutics	Auclair Lecture as Intro	***Quiz 10 discussion board for homework #1***
11	Protein Structure Characterization	**Application I**: Protein 1D, 2D, 3D, and 4-D structure characterization.	Hydrogen-deuterium exchange mass spectrometry, protein footprinting, ion-mobility, native protein mass spectrometry, gas-phase dissociation.	Primary Literature	Quiz 11 **and Homework #1 Due**
12	Disease-Related	**Application II:** Disease related	Protein modifications	Primary Literature	Quiz 12 **and discussion**

	Modifications	protein characterization	of disease relevance. Disease-related post-translational modification. Disease-related, toxic and protective protein mods. Apply MS to drug development efforts: Bioactive compound discovery. Drug binding-mode characterization. Drugs that modify proteins.		**board for homework #2**
13	Imaging	**Application III**: MS Imaging,	Mass Spectrometry Imaging: Direct characterization of tissues by MALDI Imaging (molecular histology) and DESI imaging in the operating room.	Primary Literature	Quiz 13 **discussion board for student presentation and Homework #2 Due**
14	Proteogenomics	**Application IV:** Proteogenomics	Proteogenomics- Combing	Primary Literature	***Student Presentatio***

		and Multiomics	second-generation RNA-seq (deep sequencing) with MS.		***n Due***

HOMEWORK SYLLABUS Spring 2016

Primary objective of this course:

Given a biomedical problem, a particular biological preparation, and instrumentation resources ***propose appropriate analysis tools/technique and recognize their strengths and weaknesses.***

Homework and Final Presentation Objectives:

Become knowledgeable enough with techniques covered in the course to design experiments and recognize poorly performed (bad technique) or poorly conceived (using the wrong technique) research. A second objective of the homework is to increase your proficiency with the primary literature (which is generally years ahead of textbooks and more comprehensive than "Wikipedia"). A third objective is to improve your writing and the advanced critical thinking skills of comprehending and summarizing complex research. Finally, the homework is intended to fine-tune your critical thinking by applying the fundamental knowledge you acquired in class to identify flaws in research (and hopefully avoid making similar mistakes). Examples of excellent homework and presentations (without voiceover) are given in course content.

Homework Instructions:

Find two primary literature manuscripts involving technique(s) (ideally, but not necessarily) taught that week in lecture, including: one article that chooses or applied the technology appropriately, even admirably, and one article that did not. Those that apply the technology/technique appropriately should: 1) require the technology to address the central hypothesis, 2) choose and use appropriate methodology, and 3) interpret the data correctly. Those that did not should have weakness in the experimental approach, could have chosen a better technique, or did not interpret the data correctly. Flawed research will be found if you read multiple scientific articles; or in retractions, corrections, sometimes in articles that cite a research article, and comments to the editor.

Article Discussion

To avoid duplicating effort no two students should report on the same articles, and therefore topics will be first-come-first-serve. Add your topic to the "homework" Discussion Board in Blackboard. Noting the articles you've chosen by first author, year, journal, and PubMedID (PMID). Students should post their articles and a brief description in the discussion board by wed of the week the homework is due. You should thoughtfully respond to at least two fellow student posts by Sunday at midnight of the week the discussion is due.

Written Assignment

Report on "honest mistakes." In other words errors in judgment, as opposed to outright data falsification such as things you'll find on University's Research Misconduct websites. Write a half page summary (no more than 500 words) detailing the scientific problem that was addressed, the hypothesis, and how the method you learned in class was applied to the scientific problem (70% of grade). Spelling and grammar are graded (30% of grade).

Final Presentation/Project Instructions:

The final presentation should be a screencast of a PowerPoint (or other) presentation, of no more than five minutes, where you describe one of your "best" and one of your "worst" articles from the homework. The key to the presentations is including the Figures and text (quotes) from the articles you have chosen, and using these to demonstrate the strengths and weaknesses of the article you have chosen. This presentation will be available for the entire class. Oral presentations will be graded 50% on the appearance of the slides and 50% on the speaker's technique. Students should post their presentation Wednesday of week 15 (finals week). You should thoughtfully respond to at least two fellow student posts by Sunday at midnight.

Research Statement

My ultimate goal has always been to develop disease treatments, and my independent career can be thought of as target-discovery, leading to target-validation, leading to target-engagement. I opted for Ph.D. training that would allow me to understand, in great detail, the chemistry of disease-associated proteins and potential drugs. In particular, to master a variety of biophysical techniques for analyzing protein modifications, and to obtain the quantum mechanical foundations necessary to participate when drug design at the atomic level (i.e. "lock-and-key") evolved to include orbital interactions (as occurs with the cyclic thiosulfinates we developed). The iron-sulfur cluster biosynthesis (ISC) proteins that I characterized during my Ph.D. were not yet disease-associated. I tried but failed to secure a post-doctoral position in the genetics labs with the necessary patient populations (one of these, Eric Shoubridge, eventually associated ISC proteins with disease). My intended career track of characterizing ISC protein mechanisms (Ph.D.), defining ISC disease-association (post-doc.), and pharmacologically modulating ISC proteins (independent career) was unobtainable. This history is noteworthy because it led me to develop a disease research program, de novo, and to understand that "biophysics data" and "disease-relevant data" are orthogonal descriptors. After realizing that defining something as being disease-relevant is the mainly the province of genetics, toxicology, or epidemiology, I searched for and found a post-doctoral fellowship in neurotoxicology (Heather Durham).

My core research is defined by the following questions: "what cause familial ALS?"; "what could cause sporadic ALS"; "how can we treat familial ALS?" I chose to study ALS for my independent career because there were > 150 highly-penetrant (i.e. "disease-causing" as defined by leading researchers) mutations in the gene encoding Cu/Zn-superoxide dismutase, but no consensus regarding their mechanism of toxicity, or regarding the cause of sporadic ALS. The first benefit of having numerous mutations is the ability to use their purified variants to rule-out potential structural changes as being causative (e.g. all mutations do not result in loss of activity, and therefore a "gain-of-toxic" function must exist). The second benefit—one I contend my group was the first to harness for any neurodegenerative disease (and for any disease as far as I know)—is applying epidemiology techniques to assess the disease-relevance of protein modifications. This research is described in the section entitled "*Core research: the biochemical basis of familial ALS.*" Having many biochemically diverse mutations that cause a familial disease supports the hypothesis that certain post-translational

modifications (PTMs) could cause sporadic ALS. This could be considered derivative of Harman's 1960 theory of aging, and similar hypotheses have proposed by a number of ALS researchers—the difficult matter is testing these hypotheses. This line of research is described in the paragraph "*Core research: Toxic and Protective SOD1 PTMS*." Our studies, as well as those of many other ALS researchers, supported the hypothesis that stabilizing the SOD1 dimer is a strategy for treating familial ALS, and potentially even for addressing the role of WT-SOD1 in sporadic ALS. This research is described in "*Core research: Pharmacological stabilization of proteins*." Due to the scope of potential applications and their challenging chemistry, cyclic thiosulfinates have become my major research focus of my research (75% of our effort), and should continue to be for at least the next ten years.

My group's research required a variety of techniques, and in many cases developing or co-developing new techniques (e.g. kinetic models of protein aggregation; physicochemical epidemiology; and ultra-high resolving power, imaging, and top-down mass spectrometry). Much of my core research relies upon on mass spectrometry, which in most cases, was the only technique with the required combination of sensitivity and information content. The mass spectrometry methods my group developed were designed to be applicable to problems beyond my core-research, and some of these are described the section "*Development of MS technology.*"

Core research: the biochemical basis of familial ALS. This line of research was based upon the hypothesis that diverse SOD1 variants shared a common toxic structural aberration. If so, this aberration might be inhibited by small molecules. All of the 13 ALS-associated SOD1 variants we tested do indeed share a common structural aberration, destabilization of the SOD1 electrostatic loop and dimer interface (*Molnar K 2009, click to access; full references can be found above in the CV*). Combined with John Hart's work, which showed that ALS variant's electrostatic loops undergo an aberrant intermolecular interaction, this provides a plausible mechanism for nucleation (the limiting step in aggregation) of fALS SOD1 variants. To determine whether aggregation was potentially toxic (which was controversial at the time) we developed a technique termed physicochemical epidemiology. The idea was to apply well-established epidemiology statistics and test biochemical changes as previous studies would test environmental hazards (e.g. smoking as a hazard for lung cancer). We compiled the largest ALS epidemiology database, the largest protein aggregation (raw data) database, and used Chris Dobson's recent algorithm (after re-deriving it with more data) to calculate aggregation propensities of fALS SOD1 variants. Robust methods for extracting rates of protein aggregation elongation (growth after nucleation) were not available. I contacted an inorganic chemist, Rick Finke, and proposed applying his model of nanoparticle growth to proteins. The resulting manuscript (*Morris, A. 2008*) debuted what became the preeminent model for protein aggregation. Next, my group found that for the 30 SOD1 variants with more than five patient's survival data, ALS progression (time of disease onset to death) was related to a given SOD1 variant's loss of stability and increased propensity to aggregate (*Wang Q 2008, click to access*). For the sake of comparison, the hazard ratio (*c.a.* increased risk of death) of smoking for lung cancer is *c.a.* 12, and the combined hazard ratio for loss of protein stability and aggregation was over 300. Our model could account for c.a. 80% of the variability in fALS SOD1 patient's survival, has since been validated in other ALS models and

applied to models of other neurodegenerative diseases (e.g. Alzheimer), and is among my best research.

Core research: Toxic and Protective SOD1 PTMS. This line of research was based upon the hypothesis that post-translational modification of wild-type SOD1 could account for some of the 90% of ALS that is not inherited (i.e. is sporadic). The in vitro literature, which my neurotoxicology training led me to view with skepticism, showed that SOD1's enzymatic product, peroxide, modified SOD1 at over 10 different locations (amino acids). Rather than perform additional in vitro experiments, we took the approach of analyzing the protein as exists in vivo. These efforts took the form of affinity purification MS from patient's (and control) tissues, followed by MALDI imaging studies of the cells most affected by ALS. The methods we developed place us among the handful of groups that can purify a protein from a few milligrams of human tissue and acquire intact protein MS data of sufficient quality to identify post-translational modifications. Custom-made antibodies were used to purify SOD1 from ALS patients; ultrahigh resolution MS was used to determine that SOD1 was modified and these modifications affected SOD1 structure; and disease-relevant modifications were discovered using neurotoxicology assays [including rescue of SOD1-mediated neuron death (*Taylor D 2007, click to access*), rescue of aggregation; inhibition of axonal transport, *etc. (Bosco D 2010 in collaborative study led by Bosco)*]. At the time, the generation and interpretation of intact protein collisionally-activate fragmentation (which we needed to use with our samples to prevent artifactual modifications) was difficult. We convinced the creator of the most popular bottom-up MS/MS search engine, Matrix Science, to move their floating-point limit, and debuted "Big Mascot" (*Karabacak M 2008*) for automated top-down data interpretation. We characterized gas-phase protein intact protein fragmentation pathways, showed these contained significantly more internal fragments, and combined existing models of peptide fragmentation into a unified model of protein and peptide fragmentation (*Cobb J 2010, click to access)*. We then discovered that SOD1 is post-translationally modified by exogenous cysteine in nervous tissue, and in a manner that protects SOD1 from oxidative damage *(Auclair J 2013 a,b, and Auclair J 2014)*. The above findings are summarized in *(Schmitt N 2017, click to access).* In collaborative efforts led by other groups, we have also applied these techniques to other proteins and diseases *(Pavlopoulos S 2018, Wang Y 2017, Rotunno MS 2014, Wang W 2011, Brodkin H 2011)*.

We are also among a different handful of groups that can analyze single cells by MALDI-MS. Like other neurodegenerative diseases, ALS results from the death of rare neurons that are obscured following tissue homogenization. There were no proteomics tools for studying neurons within tissues, and so we developed them. This collaboration with Nathalie Agar's group resulted in techniques we termed "microinjection with matrix" and "matrix solution fixation." *(Agar NY 2007, Agar NY 2010, Boggio KJ 2011, Rawlins CM 2015)*. A manuscript by Rawlins was just submitted that is the first to allow mammalian motor neurons (for that matter any neuron) to be targeted for high-throughput in situ MS imaging. A second manuscript comparing ALS mice to control mice is in preparation, and shows pronounced changes to particular lipids. Our studies on the basis of neurodegenerative disease continues (25% effort), with my group filling the niche of developing multiomics MS methods for characterizing single mammalian cells. The first paper in this series was just published (Quijada, J. Analytical Chemistry

2016), and a second developing technique to study protein and lipid dynamics is underway.

Core research: Pharmacological stabilization of proteins. Cyclic disulfides and thiosulfinates, a privileged chemistry for protein stabilization. Based upon our research and the research of others, it was clear that stabilizing the SOD1 dimer was a valid therapeutic strategy for ALS. We began by using small molecules crosslinkers (*Auclair J 2011*), which stabilized SOD1 variants by up to 40 °C and restored their enzymatic activity. The molecules we used were maleimides, which like every other crosslinker, are primarily toxic because they modify lone amino acids (in addition to forming crosslinks). We therefore had to solve a very general problem of creating a crosslinker that "only" forms crosslinks. This led to the development of a novel, privileged chemistry involving modified cyclic disulfides *(Donnelly D 2018, click for privilege-dependent access)*. These prevented toxic PTMs and stabilized SOD1 (and DJ-1, IscU, and Caspase), and are applicable to number of other diseases. Cyclic disulfides are generally tolerable in humans at 2-5 g/person/day, are highly resistant to reduction/metabolism, and the first compound we assayed has an EC_{50} of ~5 μm in human cell culture. The chemistry of cyclic disulfides is nuanced and highly tunable (over six orders of magnitude), and as the result of collaboration with our NEU colleagues, we understand this chemistry in great depth.

Development of MS technology. One part of my research output results from finding ways to have the mass spectrometry services I was once expected to provide benefit my research and reputation. An example includes steering studies of circadian rhythm away from traditional LC-MS/MS proteomics and toward native neuropeptidomics, which furthered my group's top-down agenda *(Salisbury J 2013)*. Another was turning instrument qualification into a publication that would benefit other MS users *(Liu Q 2014b)*. The most important benefit of my MS facility analyzing hundreds of different intact protein samples was the development of both a broad perspective and workflows that can be used to analyze just about any protein. This led to a number of co-authored publications arising from my position on the Board of Directors of the Consortium for Top-Down Proteomics *(Aebersold R 2018, LeDuc R 2018, Dang X 2014, Smith L 2013)*, and will culminate in what I believe will be one of my most highly-cited papers (the "Decision Tree" manuscript that received favorable reviews and is being revised for Nature Methods).

We are also among the leading groups in applying ultra-high resolution MS data. Recent advances in mass spectrometry *(Karabacak M 2010 characterized the importance of magnetic field strength)* allow us to distinguish biomolecules that have the same nominal mass (the same # of protons and electrons) using their isotopic fine structure. The analytical power that results from isotopic fine structure can't be underestimated- it allows molecules to be identified with certainty and permits novel methods of quantifying biomolecules and measuring their dynamics. Proof-of-concept experiments at the National High Magnetic Field laboratory (with proteins) led us to resolve isotopic fine structure on the peptide, Substance P *(Li L 2008)*. We learned, to our chagrin, that existing methods were not capable of simulating this complex data, and therefore had to develop a sufficiently accurate and fast algorithm *(Li 2008)*, that we packaged into the "Isotope Calculator" GUI and began distributing *(Li 2010)*. We went

on to show that isotopic fine structure could be observed and inform HDX experiments *(Liu 2014a)*, and developed and now distribute the software suite, QuDEX MS *(Salisbury 2014, click for access)*. This capability has allowed us to distinguish di-oxygen versus sulfur PTMs in our published manuscripts; is the basis of our collaboration on drug distribution with Nathalie Agar's group *(Randell E, submitted, Basu S 2018, Liu X 2013)*; and is required for the multiomics metabolic labeling schemes we are developing. These metabolic labeling methods consolidates previously siloed labelling workflows into a single multiomics experiment, while significantly expanding the number of metabolites and lipids that can be analyzed in a single experiment. Cell culture, fly, and mouse models of familial Amyotrophic Lateral Sclerosis are being tested for changes to lipids and proteins (including post-translational modifications and folding) using ultra-high resolution MS. We are also combining metabolic labeling, transgenic animals with fluorescent neurons, and MALDI MS imaging at high spatial resolution to study individual motor neurons – the cells most affected by ALS -throughout the disease course. We use central metabolic precursors (sugar or water) to label and change the mass of all biomolecules *(Quijada J 2016, click to access)*. Mass shifts depend upon the size of the molecule, resulting in changes in the isotopic fine structure that can be monitored for small molecules, and shifts in entire isotopologue distributions that can be monitored for large molecules. Particular strengths are that the method can be employed without changing the chemical composition of the growth media; that the technique costs less than 1% of SILAC experiments, enabling the testing of large volumes of cell culture; that it enables quantification of intact proteins; and that it enables the detection of molecular half-lives and protein folding by global HDX.

In summary my research defines the molecular basis of SOD1 ALS, generalizes these finding to sporadic ALS, develops small molecules to stop the toxic structural changes we discover, and develops methods as needed. The cyclic thiosulfinates we developed turn out to encode a very powerful and nuanced chemistry with applications beyond ALS and even beyond proteins, for the example bio-compatible synthetic polymers we have already created and are characterizing with our NEU collaborators.

Statement on service:

I have devoted a great deal of time to reviewing for the NIH and other granting agencies. I have been included in two recent NSF think-tanks, helping to define their future funding areas. I am a board member of the Consortium for Top-Down Proteomics, an international organization. My role in performing mass spectrometry service is described in detail in my CV, while at Brandeis there were semesters when 50% of my time and my graduate students time are spent helping other groups. I describe in the research section how I leveraged this required service to the benefit of my research. The one-week course that I teach at the Bioinorganic Summer Workshop (described above) is another important component of my service, but is also means to increase my sphere of influence, to recruit students, gives my own students (free of charge) access to advanced training, and keeps me up to date with advances in bioinorganic chemistry.

I have spent a great deal of time helping to develop our continuing education efforts at NEU's Burlington Innovation Campus. I paid Jared Auclair while he and I designed

course for Biopharmaceutical Analysis Training Laboratory (BATL) and set up the lab. After a string of successful departmental endeavors, Jared was promoted and stopped working for me in the spring of 2014. Together we created a thriving continuing education initiative on NEU's innovation campus in Burlington. We ordered equipment, physically set up the lab space, developed billing systems, created advertising materials, set up the website, developed three different three-day curricula (Intact Protein MS, Antibody MS, and Glycoproteins), and taught the classes. Click https://web.northeastern.edu/batl/ for more information. BATL is now a world-class training laboratory with four UPLCs and two Xevo Q-TOF mass spectrometers, providing advanced training to the Biotech industry and income to NEU. We also managed to bolster Northeastern's relationship with Waters Corporation. The next success was to leverage the working BATL lab to create an advanced mass spectrometry laboratory class (now oversubscribed) for Northeastern graduate students. The BATL curriculum was then adopted by a Bioengineering course (becoming 1/3 or that class) and two semesters of undergraduate analytical chemistry (becoming 1/3 or that class).

Another success was that we offered the equipment at BATL to Northeastern researchers – effectively setting up a mass spectrometry facility. I know of nine Northeastern professors that have used this equipment. Based upon the reputation of BATL and our online teaching, we won a bid to develop a pilot Center of Excellence Course in Regulatory Harmonization for the Asia Pacific Economic Counsel (APEC represents 40% of the world's population and GDP). Working together (on different modules) Jared and I developed a curriculum, a dynamic (adaptive release) online course, and organized a four day on-ground training for drug evaluators. Course reviews were outstanding this Pilot CoE was adopted as a permanent Center of Excellence. https://news.northeastern.edu/2017/10/03/northeastern-selected-to-lead-global-biotherapeutics-education-training-center/ Notably, the APEC CoE has garnered a $300k donation from one major pharmaceutical company, and major commitments from another 5 companies, which I am told now total over $900 k.

My relative service load was higher from 2005-2016 than I would have preferred, but has positively impacted my institution, and with the APEC CoE global health, in ways that I am extremely proud of. With the strong support of NEU's current leadership my 2017 and 2018 service and teaching loads were reduced far below normal levels to allow me to focus on research. I was granted chemistry teaching release in the Spring of 2017 in exchange for developing and teaching the Pilot APEC Center of Excellence, and no longer teach courses at BATL.