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a. REPORT	b. ABSTRACT	c. THIS PAGE			Akos Vertes
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as of 01-May-2020

Agency Code:

Proposal Number: 64972LSDRP **Agreement Number: W911NF-14-2-0020**
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Report Date: 12-Oct-2019 Date Received: 11-Oct-2019
Final Report for Period Beginning 13-Jan-2014 and Ending 12-Jul-2019
Title: New Tools for Comparative Systems Biology of Threat Agent Action Mechanisms
Begin Performance Period: 13-Jan-2014 **End Performance Period:** 12-Jul-2019
Report Term: 0-Other

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: **STEM Participants:**

Major Goals: This project aims to develop novel methodologies and technologies for the rapid identification of the mechanism of action (MoA) for emerging chemical and biological threat agents. Our approach integrates systems biology tools to detect and identify differential component levels in the cellular transcriptome, proteome and metabolome following exposure to a threat agent. Knowledge-based network inference and mathematical Bayesian network inference models are utilized to interpret the changes measured by the multi-omics experiments to construct the MoA of the challenge agent. Confirmatory assays are utilized to validate the MoA.
The aims of Technical Area 1 are to detect cellular biomolecules within a mass range of 50 to 200,000 Da. In the Base Period, and Option Periods 1, 2, and 3, the objectives are to detect proteins present between 50 and 3×107 copies per cell, and other molecules (transcripts and metabolites) present between 4×103 and 4×1010 molecules per cell. Cells are treated with the challenge agent and sampled at several time-points between 2 s and 48 h, followed by transcriptomics, proteomics, and metabolomics analyses. The untargeted detection of proteins and metabolites is to be performed using liquid chromatography and mass spectrometry.
The goals of Technical Area 2 are to identify components and events. During the base period, the objective of Technical Area 2 is to identify components and events from minutes to days within whole cells and the cytoplasm compartment. In Option Period 1, the aim is to identify components and events in the time range from seconds to days from whole cells and from the cytoplasm compartment. In Option Period 2, the aim is to identify components and events in the time range from seconds to days from whole cells, and from the cytoplasm and nuclear compartments. In Option Period 3, the aim is to identify components and events in the time range from seconds to days from whole cells, and from the cytoplasm, membrane, and nuclear compartments.
Identification of proteins and metabolites is accomplished using search engines against curated and reviewed databases. Quantitation of proteins is performed using tandem mass tags (TMT) and that of metabolites is performed label-free. Identification and quantitation of transcripts is accomplished using Affymetrix microarrays. Experiments are run in triplicates and statistical significance of the regulated component is generated using ANOVA and t-test.
The final goal of Technical Area 3 is to reconstruct at least 95% of the MoA of a threat agent simulant in less than 30 days. In the Base Period, the goal was to reconstruct at least 20% of the MoA for a challenge agent, with the degree of reconstruction in Option Periods 1, 2, and 3, increasing to 60%, 80%, and 95%, respectively.
In Option Period 3, the major goals included the development of additional causal network prediction algorithms, evaluation of the algorithms and analysis workflow in a new biological context, preparation of papers documenting our results, and preparing the database containing all the experimental data (and metadata) collected during the process. The causal network algorithms discover potential causal relations between timeseries expression profiles, typically measuring change of expression levels of cellular components in response to an external perturbation. Different algorithms typically look at different features and thus give different views of the relations between time

series profiles. The long-term aim is to obtain a better understanding of what these relations are capturing, where they agree, and what distinguishes each one. Confirmatory assays are then used to confirm the biological processes that were identified computationally. SRI developed and maintained a project data base for storing and sharing the experimental data collected during the project.

Accomplishments: All project metrics were accomplished, and the following goals were achieved under Technical Area 1.
In GW Task 1, culturing and exposure of cells, we established different cell lines such as hepatocytes (HepG2/C3A and HepG2), and neuroblastoma (SK-N-AS). Cultures were regularly monitored for quality and viability and tested for contamination. During the program, we studied several challenge compounds, e.g., rotenone, forskolin, nocodazole, and bendamustine. For each compound we determined the GI50, and the relevant dose for the challenge agent. Samples were delivered to different omics tasks for further analysis. On average ~3×109 cells were cultured and treated for each 30-day demonstration period. In the last phase of our program we investigated the downstream effects of the binary mixture of methamidophos/atropine as agonist/antagonist. We also explored different RNA extraction methods and optimized them for different cell cultures, such as hepatocytes, neuroblastomas, and bacteria.
In GW Task 2, proteomics, we have analyzed protein regulation in HepG2/C3A and SK-N-AS cells and have identified and quantified more than 5,000 protein groups at several treatment time-points.
In GW Task 3, LAESI/UPLC-IMS-MS/MS metabolomics, we harvested, prepared for analysis, and analyzed 80 polar sample fractions and 80 nonpolar fractions of cells for each challenge agent throughout the project. We have also applied UPLC-MS/MS to expand the coverage of metabolites.
In GW Task 4, Metabolomics by NAPA-MS, we have provided metabolomics data for interpretation to the computational tasks. We have developed workflows allowing for high-throughput processing and analysis of metabolomics samples using NAPA- and MALDI-MS to maximize molecular coverage, with experiments during the most recent test period producing 600+ data files from 100+ biological samples, and all sample handling, acquisition, and analysis completed in ~23 days.
In GW Task 5, subcellular fractionation, we performed the compartmental fractionation of HepG2/C3A and SK-N-AS cells resulting in proteome profiling of cytoplasmic, membrane, and nuclear fractions at 8 time-points for each challenge agent.
In SRI Task 4, transcriptomics, we performed the transcriptomics workflow on 22 separate occasions (904 samples total) using whole genome microarray technology. Differential RNA expression data from challenge agent-treated vs. control-treated cells (HepG2/C3A or SK-N-AS cells) at 10 time points spanning 0.5 to 48 h post-exposure and at time zero were delivered for each of the 10 challenge agents we tested over the course of this program, and repeatedly delivered to the team in less than 14 calendar days after receipt of the harvested cell pellets.
All project metrics were accomplished, and the following goals were achieved under Technical Area 2.
In SRI task 1, Master Database, we defined an RTA database schema, providing storage for experimental raw data, metadata, analyses, and related documents. We implemented graphically-based software for data entry, search, and retrieval. We augmented the database access software to allow the automatic entry of data via spreadsheets. Using this infrastructure, we processed data for 1146 experiments using a total of 10 challenge agents. We implemented a mirrored server infrastructure with one server located at GWU and one at SRI, and we modified the server configuration to provide data access beyond the completion of the project.
In SRI Task 2, logical network inference, an analysis workflow was developed that combined results from abstract shape analysis and a suite of algorithms based on Gaussian process (GP) models of timeseries omics data. Two main classes of algorithm were developed: ranking and network synthesis. Ranking algorithms rank GP timeseries profiles on a scale from anomalous/atypical to typical behavior were developed using both autoencoders and Generative Adversarial Networks techniques. Network synthesis algorithms identify causal relations among features of GP timeseries profiles. Network synthesis algorithms were developed using techniques including convolutional autoencoders, Siamese twin networks, and wide/deep prediction networks. Finally, we developed a Probabilistic Tensor Logic and applied it to causal network inference. The analysis workflow, in different stages of development, was applied to transcriptomics, proteomics, and metabolomics data from six challenges (three internal and three provided by DARPA) plus variations of two of the challenges. In each case key components of the MoA were identified.
In SRI Task 3, Bayesian network inference, we used bootstrap resampling to allow us to determine whether a compound was up, down, nominal, or too noisy to distinguish. We developed a variant of Bayesian network inference, biological time warp. It uses an analog of a genetic sequence alignment algorithm to align events in time without a fixed relationship between cause and effect. Biological time warp results are demonstrably superior to n-order, dynamic Bayesian network results. We also developed a number of criteria to select important areas of the network to view. Our algorithm was able to detect all the observable cause-effect relationships in the MoA of each of the challenge agents with which we experimented.

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All project metrics were accomplished, and the following goals were achieved under Technical Area 3.
At GW, we have evaluated de novo network construction based on time-profile similarities measured by Pearson and Spearman correlation. Following network mapping, we have partitioned the network using modularity classes followed by Gene Set Enrichment Analysis (GSEA) of each class to reconstruct the MoA of the challenge agents. Furthermore, we have identified the biomolecules with the highest betweenness centrality in the network. These biomolecules are central to the propagation of the MoA. Targeting these biomolecules might allow for an efficient countermeasure.
In GE Task 1, Confirmatory Assays, we applied biological assays to downselect and validate the MoA proposed based on the analyses of the integrated omics data. We were successful at identifying >95% of the MoA of all challenge agents which were analyzed.
In GE Task 2, Fractionation/microfluidics, we have focused on 2 main goals: (1) Integrating fluidic device development for subcellular fractionation of suspension cells and 2) integration and automation of component technologies for adherent cell subcellular fractionation. The devices and disposables assembled at GE remain available for future transition work.

Training Opportunities: FY1
Graduate Students: 2
- Lombard, Camille
- Stopka, Sylwia,

Post-Doctoral Scientists: 2
- Kiss, Andras
- Sahab, Ziad

FY2
Graduate Student: 1
- Lombard, Camille

Post-Doctoral Scientists: 3
- Sahab, Ziad
- Andrew Korte
- Yuan, Wei

Undergraduate Student: 1
- Kimmey, Samuel

FY3
Graduate Student: 1
- Li, Hang (Ph.D. awarded)

Post-Doctoral Scientists: 3
- Korte, Andrew
- Hwang, Sunil
- Li, Hang

FY4
Graduate Students: 2
- Lombard-Banek, Camille,
- Zhang, Linwen,

Post-Doctoral Scientists: 4
- Arul, Albert
- Avar, Peter
- Korte, Andrew
- Li, Hang

FY5
Graduate Student: 1
- Zhang, Linwen (Ph.D. awarded)
- Stopka, Sylwia (Ph.D. awarded)

Post-Doctoral Scientists: 3
- Arul, Albert
- Avar, Peter
- Zhang, Linwen

FY6
Graduate Student: 1
- Fincher, Jarod (Ph.D. awarded)

Post-Doctoral Scientist: 1
- Avar, Peter

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Results Dissemination: (a) Papers published in peer-reviewed journals: 11

- Jarod A. Fincher, Derek R. Jones, Andrew R. Korte, Jacqueline E. Dyer, Paola Parlanti, Anastas Popratiloff, Christine A. Brantner, Nicholas J. Morris, Russell K. Pirlo, Victoria K. Shanmugam, and Akos Vertes, “Mass Spectrometry Imaging of Lipids in Human Skin Disease Model Hidradenitis Suppurativa by Laser Desorption Ionization from Silicon Nanopost Arrays,” Sci. Rep., 2019, accepted.
- Jarod A. Fincher, Andrew R. Korte, Jacqueline E. Dyer, Sridevi Yadavilli, Nicholas J. Morris, Derek R. Jones, Victoria K. Shanmugam, Russel K. Pirlo, and Akos Vertes, “Mass Spectrometry Imaging of Triglycerides in Biological Tissues by Laser Desorption Ionization from Silicon Nanopost Arrays,” J. Mass Spectrom., 2019, accepted. http://dx.doi.org/10.1002/jms.4443
- Andrew R. Korte, Nicholas J. Morris, and Akos Vertes, “High Throughput Complementary Analysis and Quantitation of Metabolites by MALDI- and Silicon Nanopost Array-Laser Desorption/Ionization-Mass Spectrometry,” Anal. Chem., 2019, 91, 3951-3958. http://dx.doi.org/10.1021/acs.analchem.8b05074
- Jarod A. Fincher, Jacqueline E. Dyer, Andrew R. Korte, Sridevi Yadavilli, Nicholas J. Morris, and Akos Vertes, “Matrix-free Mass Spectrometry Imaging of Mouse Brain Tissue Sections on Silicon Nanopost Arrays,” J. Comp. Neurol., 2019, 527, 2101-2121. https://doi.org/10.1002/cne.24566
- Linwen Zhang, Nikkita Khattar, Ildiko Kemenes, Gyorgy Kemenes, Zita Zrinyi, Zsolt Pirger, and Akos Vertes, “Subcellular Peptide Localization in Single Identified Neurons by Mass Spectrometry,” Sci. Rep., 2018, 8, 12227. http://dx.doi.org/10.1038/s41598-018-29704-z
- Sylwia A. Stopka, Xavier A. Holmes, Andrew R. Korte, Laine R. Compton, Scott T. Retterer, and Akos Vertes, “Trace Analysis and Reaction Monitoring by Nanophotonic Ionization Mass Spectrometry from Elevated Bowtie and Silicon Nanopost Arrays,” Adv. Funct. Mater., 2018, 28, 1801730. http://dx.doi.org/10.1002/adfm.201801730
- Linwen Zhang, Christopher J. Sevinsky, Brian M. Davis, and Akos Vertes, “Single-Cell Mass Spectrometry of Subpopulations Selected by Fluorescence Microscopy,” Anal. Chem., 2018, 90, 4626-4634. http://dx.doi.org/10.1021/acs.analchem.7b05126
- Linwen Zhang, and Akos Vertes, “Single?Cell Mass Spectrometry Approaches to Explore Cellular Heterogeneity,” Angew. Chem. Int. Ed., 2018, 57, 4466-4477. http://dx.doi.org/10.1002/anie.201709719
- Hang Li, and Akos Vertes, “Solvent Gradient Electrospray for Laser Ablation Electrospray Ionization Mass Spectrometry,” Analyst, 2017, 142, 2921-2927. http://dx.doi.org/10.1039/C7AN00819H
- Andrew R. Korte, Sylwia A. Stopka, Nicholas Morris, Trust Razunguzwa, and Akos Vertes, “Large-Scale Metabolite Analysis of Standards and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays,” Anal. Chem., 2016, 88, 8989-8996. (Cover Page Article) http://dx.doi.org/10.1021/acs.analchem.6b01186
- Nicholas J. Morris, Heather Anderson, Brian Thibeault, Akos Vertes, Matthew J. Powell and Trust T. Razunguzwa, “Laser desorption ionization (LDI) silicon nanopost array chips fabricated using deep UV projection lithography and deep reactive ion etching,” RSC Advances, 2015, 5, 72051-72057. http://dx.doi.org/10.1039/c5ra11875a

(b) Papers published in non-peer-reviewed journals: 4

- Mark-Oliver Stehr, Minyoung Kim, Carolyn L. Talcott, Merrill Knapp, Akos Vertes, “Probabilistic Approximate Logic and its Implementation in the Logical Imagination Engine,” arXiv preprint arXiv:1907.11321, 2019. https://arxiv.org/abs/1907.11321
- Mark-Oliver Stehr, Peter Avar, Andrew R. Korte, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, Maria I. Zavodszky, Akos Vertes, “Learning Causality: Synthesis of Large-Scale Causal Networks from High-Dimensional Time Series Data,” arXiv preprint arXiv:1905.02291, 2019. https://arxiv.org/abs/1905.02291
- Mark-Oliver Stehr, “Probabilistic Approximate Logic and its Implementation in the Logical Imagination Engine”, 2019. http://www.csl.sri.com/users/stehr/RTA/palo-and-lime.pdf
- Akos Vertes, Peter Avar, Andrew R Korte, Lida Parvin, Ziad J Sahab, Deborah I Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Mark-Oliver Stehr, Carolyn L Talcott, Brian M Davis, Christine A Morton, Christopher J Sevinsky, and Maria I Zavodszky, “Transcriptional Response of SK-N-AS Cells to Methamidophos”, 2019. http://www.csl.sri.com/users/clt/XYZ/methamidophosX.pdf

d) Manuscripts: 2

- Sylwia A. Stopka and Akos Vertes, “Metabolomic Profiling of Adherent Mammalian Cells In situ by LAESI-MS

with Ion Mobility Separation," in: Ion Mobility Mass Spectrometry: Methods and Protocols, Methods in Molecular Biology, Giuseppe Paglia and Giuseppe Astarita (eds), Springer, New York, 2018, submitted.

- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky, "Network Integration of Time-dependent Omics Data for the Identification of Off-target Effects for Drug Candidates", 2019, in preparation.

e) Edited Book: 1

- Single Cell Metabolism, Bindesh Shrestha (ed), Methods in Molecular Biology, vol 2064. Humana, New York, NY, 2020, pp. 9-18. ISBN-13: 978-1-4939-9829-6, https://doi.org/10.1007/978-1-4939-9831-9

f) Book Chapters: 3

- Sylwia A. Stopka, Akos Vertes, "Toward Single Cell Molecular Imaging by Matrix-Free Nanophotonic Laser Desorption Ionization Mass Spectrometry," in: Single Cell Metabolism, Bindesh Shrestha (ed), Methods in Molecular Biology, vol 2064. Humana, New York, NY, 2020, pp. 135-146. ISBN-13: 978-1-4939-9829-6. https://doi.org/10.1007/978-1-4939-9831-9_11
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky and Maria I. Zavodszky, "Transcriptional response of SK-N-AS cells to methamidophos," in: Computational Methods in Systems Biology, Bortolussi L., Sanguinetti G. (eds) Lecture Notes in Computer Science, vol 11773. Springer, 2019, pp. 368-372. ISBN-13: 978-3-030-31303-6. https://doi.org/10.1007/978-3-030-31304-3_29
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Hang Li, Peter Nemes, Lida Parvin, Sylwia Stopka, Sunil Hwang, Ziad J. Sahab, Linwen Zhang, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Sean R. Dinn, Christine A. Morton, Christopher J. Sevinsky, Maria I. Zavodszky, "Inferring Mechanism of Action of an Unknown Compound from Time Series Omics Data," in: Computational Methods in Systems Biology, Milan Ceska and David Safranek (eds), Springer International Publishing, 2018, pp. 238-255. ISBN 13: 978 3 319 99428 4. http://dx.doi.org/10.1007/978-3-319-99429-1_14

Honors and Awards: • April 20, 2016 – Distinguished Researcher Award, awarded to Akos Vertes by The George Washington University

- May 23, 2017 – WBMSDG Travel Award, awarded to Linwen Zhang by the Washington-Baltimore Mass Spectrometry Discussion Group (WBMSDG)
- Nov 8, 2018 – 1st place prize awarded to poster titled "Mechanism of Action Identification of Threat Agents within 30 Days" presented by Deborah Bunin at the SRI Biosciences' Internal Annual Poster Day Event.

Protocol Activity Status:

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Technology Transfer: Filed Patent Applications: 2

- Title: “Laser desorption ionization-mass spectrometry analysis of biomolecules in cellular compartments captured on functionalized silicon nanopost arrays” Inventors: Akos Vertes (The George Washington University), Andrew Korte (The George Washington University), Brian Davis (GE Global Research), and Sean Dinn (GE Global Research) Filing date: provisional patent application filed on May 27, 2016 with the USPTO
- Title: “System and Method for Laser Desorption Ionization-Mass Spectrometry Analysis of Biomolecules in Cellular Compartments Captured on Functionalized Silicon Nanopost Arrays”, Inventors: Akos Vertes and Andrew R. Korte (The George Washington University); Brian Davis and Sean Dinn (GE Global Research), Date of non-provisional patent application: May 26, 2017

Patents awarded: 6

- United States Patent US 8,809,774 B2, Date of Patent: 8/19/2014, Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry (Continuation of United States Patent US 8,487,244 B2), Inventors: Akos Vertes, and Peter Nemes
- United States Patent US 8,829,426 B2, Date of Patent: 9/9/2014, Title: Plume Collimation for Laser Ablation Electrospray Ionization Mass Spectrometry, Inventors: Akos Vertes, and Jessica A. Stolee
- United States Patent US 8,901,487 B2, Date of Patent: 12/2/2014, Title: Subcellular Analysis by Laser Ablation Electrospray Ionization Mass Spectrometry, Inventors: Akos Vertes, Jessica A. Stolee, and Bindesh Shrestha
- United States Patent US 9,000,361 B2, Date of Patent: 04/7/2015, Title: Nanophotonic Production, Modulation and Switching of Ions by Silicon Microcolumn Arrays, Inventors: Akos Vertes and Bennett N. Walker
- United States Patent US 9,362,101 B2, Date of Patent: 06/07/2016, Title: Plume Collimation for Laser Ablation Electrospray Ionization Mass Spectrometry, Inventors: Akos Vertes and Jessica Stolee
- United States Patent US 9,490,113 B2, Date of Patent: 11/08/2016, Title: Tailored Nanopost Array (NAPA) for Laser Desorption Ionization in Mass Spectrometry, Inventors: Akos Vertes, Bennett N. Walker, Jessica Stolee, and Scott Retterer

PARTICIPANTS:

Participant Type: PD/PI
Participant: Akos Vertes
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
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Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Deborah Bunin
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
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Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Co PD/PI
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Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Participant: Sean Dinn
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Jessica Karp
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Vandana Keskar
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Andras Kiss
Person Months Worked: 8.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Merrill Knapp
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Andrew R. Korte
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Adriana Larriera
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Hang Li
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Annie Liang
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Camille Lombard
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Nicholas Morris
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Christine Morton
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Anthony Murray
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Faculty
Participant: Peter Nemes
Person Months Worked: 7.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Denise Nishita
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Technician
Participant: Lida Parvin
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N

Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Andrew Poggio
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Matthew J. Powell
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Christopher Puleo
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Trust Razunguzwa
Person Months Worked: 14.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Co PD/PI
Participant: Ziad Sahab
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Christopher Sevinsky
Person Months Worked: 8.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:

National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Kashan Shaikh
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Co PD/PI
Participant: Bindesh Shresha
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Mark-Oliver Stehr
Person Months Worked: 13.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Sylwia Stopka
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Co PD/PI
Participant: Carolyn L. Talcott
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Andrew Tilton
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:

International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Timothy Toepfer
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Abraham Wang
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Maneesh Yadav
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Wei Yuan
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Lisa Zassowski
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Maria Zavodszky
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:

International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Linwen Zhang
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Yun Zou
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

ARTICLES:

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: RSC Adv.
Publication Identifier Type: DOI Publication Identifier: 10.1039/C5RA11875A
Volume: 5.0E+000 Issue: 8.8E+001 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Laser desorption ionization (LDI) silicon nanopost array chips fabricated using deep UV projection lithography and deep reactive ion etching
Authors:
Keywords: mass spectrometry, trace analysis, nanofabrication
Abstract: Deep UV projection lithography (DUV-PL) and deep reactive ion etching (DRIE) processes are used to fabricate silicon nanopost surfaces for laser desorption ionization mass spectrometry (LDI-MS). Described here is a fabrication process that is amenable to mass production of silicon nanopost array (NAPA) devices optimized for laser desorption ionization mass spectrometry of small molecules less than 2 kDa, suitable for pharmaceutical and metabolomics applications. The resulting devices exhibit excellent performance for analysis and quantitation of pharmaceutical drugs over at least four orders of magnitude dynamic range, with very good limits of detection and lower limits of quantitation. For metabolite analysis, these devices also exhibit improved spectral quality over MALDI-MS which su?ers from noise from the chemical matrix. With the ability to perform a one-step sample spotting, these devices become extremely useful for high throughput work? ows a?orded b
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Volume: 88 Issue: 18 First Page #: 8989
Date Submitted: 11/14/16 12:00AM Date Published: 7/11/16 8:00AM
Publication Location: Washington, DC
Article Title: Large-Scale Metabolite Analysis of Standards and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays
Authors: Andrew R. Korte, Sylwia A. Stopka, Nicholas Morris, Trust Razunguzwa, Akos Vertes
Keywords: Amino Acids, Carbohydrates, Lipids, Metabolic Pathways, Metabolomics, NAPA-LDI-MS, Nucleotides, Silicon Nanopost Array, Xenobiotics
Abstract: The unique challenges presented by metabolomics have driven the development of new mass spectrometry (MS)-based techniques for small molecule analysis. Here we apply NAPA-LDI-MS to the large-scale acquisition of high-resolution mass spectra and tandem mass spectra from a collection of metabolite standards covering a range of compound classes. In untargeted analysis of metabolite standard mixtures, detection was achieved for 374 compounds and useful MS/MS spectra were obtained for 287 compounds, without individual optimization of ionization or fragmentation conditions. Metabolite detection was evaluated in the context of 31 metabolic pathways, and NAPA-LDI-MS was found to provide detection for 63% of investigated pathway metabolites. Individual, targeted analysis of the 20 common amino acids provided detection of 100% of the investigated compounds, demonstrating that improved coverage is possible through optimization and targeting of individual analytes or analyte classes.....
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Analyst
Publication Identifier Type: DOI Publication Identifier: http://dx.doi.org/10.1039/c7an00819h
Volume: 142 Issue: First Page #: 2921
Date Submitted: 5/2/19 12:00AM Date Published: 7/7/17 12:00PM
Publication Location:
Article Title: Solvent Gradient Electrospray for Laser Ablation Electrospray Ionization Mass Spectrometry
Authors: Hang Li, Akos Vert
Keywords: Laser Ablation Electrospray Ionization, LAESI, Mass Spectrometry
Abstract: Most electrospray based ambient ionization techniques, e.g., laser ablation electrospray ionization (LAESI), utilize a fixed spray solution composition. Complex samples often contain compounds of different polarity that exhibit a wide range of solubilities in the electrospray solvent. Thus, the fixed spray solution composition limits the molecular coverage of these approaches. Two-barrel theta glass capillaries have been used for the rapid mixing of two solutions for manipulating fast reactions including protein folding, unfolding, and charge state distributions. Here, we present a new variant of LAESI mass spectrometry (MS) by scanning the high voltages applied to the two barrels of a theta glass capillary containing two different solvents. In the resulting gradient LAESI (g-LAESI), the composition of the spray solution is ramped between the two solvents in the barrels to facilitate the detection of compounds of diverse polarity and solubility.
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Journal: Journal of Comparative Neurology
Publication Identifier Type: DOI Publication Identifier: 10.1002/cne.24566
Volume: Issue: First Page #:
Date Submitted: 5/2/19 12:00AM Date Published: 12/1/18 10:00AM
Publication Location:
Article Title: Matrix-free mass spectrometry imaging of mouse brain tissue sections on silicon nanopost arrays
Authors: Jarod A. Fincher, Jacqueline E. Dyer, Andrew R. Korte, Sridevi Yadavilli, Nicholas J. Morris, Akos Verte
Keywords: brain, laser desorption ionization, lipidomics, MALDI, mass spectrometry imaging, nanopostarrays
Abstract: Mass spectrometry imaging (MSI) is capable of detection and identification of diverse classes ofcompounds in brain tissue sections, whereas simultaneously mapping their spatial distributions.Given the vast array of chemical components present in neurological systems, as well as theinnate diversity within molecular classes, MSI platforms capable of detecting a wide array ofspecies are useful for achieving a more comprehensive understanding of their biological rolesand significance. Currently, matrix-assisted laser desorption ionization (MALDI) is the method ofchoice for the molecular imaging of brain samples by mass spectrometry. However, nanostruc-tured laser desorption ionization platforms, such as silicon nanopost arrays (NAPA), are emerg-ing as alternative MSI techniques that can provide complementary insight into moleculardistributions in the central nervous system. In this work, the molecular coverage of mouse brainlipids afforded by NAPA-MSI is compared to that of MALDI-MSI ...
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Scientific Reports
Publication Identifier Type: DOI Publication Identifier: 10.1038/s41598-018-29704-z
Volume: 8 Issue: 1 First Page #:
Date Submitted: 5/2/19 12:00AM Date Published: 8/1/18 4:00AM
Publication Location:
Article Title: Subcellular Peptide Localization in Single Identified Neurons by Capillary Microsampling Mass Spectrometry
Authors: Linwen Zhang, Nikkita Khattar, Ildiko Kemenes, Gyorgy Kemenes, Zita Zrinyi, Zsolt Pirger, Akos Vertes
Keywords: Capillary Microsampling, Mass Spectrometry, Single Cell
Abstract: ingle cell mass spectrometry (MS) is uniquely positioned for the sequencing and identification of peptides in rare cells. Small peptides can take on different roles in subcellular compartments. Whereas some peptides serve as neurotransmitters in the cytoplasm, they can also function as transcription factors in the nucleus. Thus, there is a need to analyze the subcellular peptide compositions in identified single cells. Here, we apply capillary microsampling MS with ion mobility separation for the sequencing of peptides in single neurons of the mollusk Lymnaea stagnalis, and the analysis of peptide distributions between the cytoplasm and nucleus of identified single neurons that are known to express cardioactive Phe-Met-Arg-Phe amide-like (FMRFamide-like) neuropeptides. Nuclei and cytoplasm of Type 1 and Type 2 F group (Fgp) neurons were analyzed for neuropeptides cleaved from the protein precursors encoded by alternative splicing products of the FMRFamide....
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Advanced Functional Materials
Publication Identifier Type: DOI Publication Identifier: 10.1002/adfm.201801730
Volume: 28 Issue: 29 First Page #: 1801730
Date Submitted: 5/2/19 12:00AM Date Published: 7/1/18 4:00AM
Publication Location:
Article Title: Trace Analysis and Reaction Monitoring by Nanophotonic Ionization Mass Spectrometry from Elevated Bowtie and Silicon Nanopost Arrays
Authors: Sylwia A. Stopka, Xavier A. Holmes, Andrew R. Korte, Laine R. Compton, Scott T. Retterer, Akos Vertes
Keywords: bowtie arrays, laser desorption, mass spectrometry, nanophotonic ionization, nanopost arrays
Abstract: Silicon nanopost arrays (NAPA) are used in trace analysis by mass spec- trometry (MS) because they enable highly efficient ion production from small molecules and thin tissue sections by UV laser desorption ionization (LDI). Such nanophotonic ionization of adsorbates relies on localized interactions between a nanostructured substrate and laser radiation. In LDI from NAPA, only the component of the oscillating electric field vector that is parallel with the posts couples the laser energy into the nanostructure. Enhancements in control over adsorbate ionization and fragmentation are expected if the surface-parallel component can also interact with the nanostructure. Here, an alternative nanophotonic ionization platform is introduced for LDI-MS, the elevated bowtie (EBT) array by adding triangular chromium features on top of silicon post pairs to form bowties....
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Journal: Analytical Chemistry
Publication Identifier Type: DOI Publication Identifier: 10.1021/acs.analchem.7b05126
Volume: 90 Issue: 7 First Page #: 4626
Date Submitted: 5/2/19 12:00AM Date Published: 3/1/18 5:00AM
Publication Location:
Article Title: Single-Cell Mass Spectrometry of Subpopulations Selected by Fluorescence Microscopy
Authors: Linwen Zhang, Christopher J. Sevinsky, Brian M. Davis, Akos Vertes
Keywords: Single Cell, Mass Spectrometry, Fluorescence Microscopy
Abstract: Specific subpopulations in a heterogeneouscollection of cells, for example, cancer stem cells in a tumor,are often associated with biological or medical conditions.Fluorescence microscopy, based on biomarkers labeled withfluorescent probes, is a widely used technique for thevisualization and selection of such cells. Phenotypic differencesfor these subpopulations at the molecular level can beidentified by their untargeted analysis by single-cell massspectrometry (MS). Here, we combine capillary micro-sampling MS withfluorescence microscopy for the analysisof metabolite and lipid levels in single cells to discern theheterogeneity of subpopulations corresponding to mitoticstages. The distributions of ATP, reduced glutathione (GSH), and UDP-N-acetylhexosamine (UDP-HexNAc) levels in mitosisreveal the presence of 2?3 underlying subpopulations. Cellular energy is found to be higher in metaphase compared toprometaphase and slightly declines in anaphase, telophase, and cytokinesis...
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Angewandte Chemie International Edition
Publication Identifier Type: DOI Publication Identifier: 10.1002/anie.201709719
Volume: 57 Issue: 17 First Page #: 4466
Date Submitted: 5/2/19 12:00AM Date Published: 4/1/18 12:00AM
Publication Location:
Article Title: Single-Cell Mass Spectrometry Approaches to Explore Cellular Heterogeneity
Authors: Linwen Zhang, Akos Vertes
Keywords: bioanalysis; mass spectrometry; metabolomics; proteomics; single-cell analysis
Abstract: Compositional diversity is a fundamental property in cell populations. Single-cell analysis promises new insight into this cellular heterogeneity on the genomic, transcriptomic, proteomic, and metabolomic levels. Mass spectrometry (MS) is a label-free technique that enables the multiplexed analysis of proteins, peptides, lipids, and metabolites in individual cells. The abundances of these molecular classes are correlated with the physiological states and environmental responses of the cells. In this Minireview, we discuss recent advances in single-cell MS techniques with an emphasis on sampling and ionization methods developed for volume-limited samples. Strategies for sample treatment, separation methods, and data analysis require special considerations for single cells. Ongoing analytical challenges include subcellular heterogeneity, non-normal statistical distributions of cellular properties, and the need for high-throughput, high molecular coverage and minimal perturbation.
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Journal: Analytical Chemistry
Publication Identifier Type: DOI Publication Identifier: 10.1021/acs.analchem.8b05074
Volume: 91 Issue: 6 First Page #: 3951
Date Submitted: 5/2/19 12:00AM Date Published: 2/1/19 5:00AM
Publication Location:
Article Title: High Throughput Complementary Analysis and Quantitation of Metabolites by MALDI- and Silicon Nanopost Array-Laser Desorption/Ionization-Mass Spectrometry
Authors: Andrew R. Korte, Nicholas J. Morris, Akos Vertes
Keywords: SMALL-MOLECULE ANALYSIS; ION FORMATION; IONIZATION; METABOLOMICS; NANOSTRUCTURES; FRAGMENTATION; PRODUCTS; PLATFORM; PLUME
Abstract: Silicon nanopost array (NAPA) structures have been shown to be effective substrates for laser desorption/ionization-mass spectrometry (LDI-MS) and have been used to analyze a variety of samples including peptides, metabolites, drugs, explosives, and intact cells, as well as to image lipids and metabolites in tissue sections. However, no direct comparison has yet been conducted between NAPA-MS and the most commonly used LDI-MS technique, matrix-assisted laser desorption/ionization (MALDI)-MS. In this work, we compare the utility of NAPA-MS to that of MALDI-MS using two common matrices for the analysis of metabolites in cellular extracts and human urine. Considerable complementarity of molecular coverage was observed between the two techniques. Of 178 total metabolites assigned from cellular extracts, 68 were uniquely detected by NAPA-MS and 62 were uniquely detected by MALDI-MS....
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CONFERENCE PAPERS:

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Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 63rd ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 01-Jun-2015 Date Published: 02-Jul-2015
Conference Location: Saint Louis, MO
Paper Title: Construction of a Metabolite MS/MS Library for Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays
Authors: Andrew R. Korte, Nicholas Morris, Trust Razunguzwa, and Akos Vertes
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 63rd ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 01-Jun-2015 Date Published:
Conference Location: Saint Louis, MO
Paper Title: Tandem mass spectrum and collision cross section libraries for high-throughput identification of metabolites in adherent hepatocytes by LAESI mass spectrometry
Authors: Wei Yuan, Bindesh Shrestha, Akos Vertes
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 63rd ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 04-Jun-2015 Date Published:
Conference Location: Saint Louis, MO
Paper Title: Early Detection of the Mechanism of Action for Toxins by High-Throughput Proteomics
Authors: Ziad J. Sahab, Camille Lombard, Lida Parvin, Peter Nemes, Akos Vertes
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 63rd ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 03-Jun-2015 Date Published:
Conference Location: Saint Louis, MO
Paper Title: REDIchips for Rapid Quantitation of low Molecular Weight Pharmaceutical Drugs using Laser Desorption Ionization Mass Spectrometry
Authors: Heather Anderson, Matthew J Powell, Nicholas J. Morris, Trust T. Razunguzwa
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 63rd ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 02-Jun-2015 Date Published:
Conference Location: Saint Louis, MO
Paper Title: Metabolomic Profiling of Biofluids Using Laser Desorption Ionization on Nanopost Array Devices (REDIchipsTM)
Authors: Trust T. Razunguzwa, Heather Anderson, Nicholas J. Morris, Matthew Powell
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 64th ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 05-Jun-2016 Date Published: 07-Jul-2016
Conference Location: San Antonio, TX
Paper Title: Analysis of Lipids from Biological Samples by Laser Desorption Ionization from Silicon Nanopost Arrays
Authors: Andrew R. Korte, Akos Vertes
Acknowledged Federal Support: **Y**

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Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 64th ASMS Conference on Mass Spectrometry and Allied Topics
Date Received: 10-Aug-2016 Conference Date: 05-Jun-2016 Date Published: 07-Jul-2016
Conference Location: San Antonio, TX
Paper Title: Rapid Exploration of the Mechanism of Action of Forskolin via Untargeted Proteomics and Knowledge-based Pathway Analysis
Authors: Ziad J. Sahab, Bindesh Shrestha, Lida Parvin, Peter Nemes, Akos Vertes
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: ASBMB 2016 Annual Meeting
Date Received: 10-Aug-2016 Conference Date: 02-Apr-2016 Date Published: 02-Apr-2016
Conference Location: San Diego, CA
Paper Title: Mechanism of Action Identified in 30 Days: a Systems Biology Approach
Authors: Akos Vertes, Andrew R Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Binde
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: HUPO 11th Annual Conference, Next Generation Proteomics
Date Received: 10-Aug-2016 Conference Date: 15-Mar-2015 Date Published: 15-Mar-2015
Conference Location: Tempe, AZ
Paper Title: High-Throughput Proteomics as a Critical Tool to Rapidly Discover the Mechanism of Action for Toxic Compounds
Authors: Ziad J. Sahab, Camille Lombard, Lida Parvin, Peter Nemes, Akos Vertes
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: HUPO 12th Annual Conference, From New Technology to New Biology
Date Received: 10-Aug-2016 Conference Date: 13-Mar-2016 Date Published: 13-Mar-2016
Conference Location: Boston, MA
Paper Title: Integrative Systems Biology Approach to Identify Mechanisms of Action
Authors: Akos Vertes, Andrew R. Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J. Sahab, Bind
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 0-Other
Conference Name: 21st International Mass Spectrometry Conference (IMSC 2016)
Date Received: Conference Date: 20-Aug-2016 Date Published:
Conference Location: Toronto, Ontario, Canada
Paper Title: Rapid Identification of Mechanisms of Action Through Systems Biology
Authors: Akos Vertes, Andrew R Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Binde
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Proceedings of the 65th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics
Date Received: Conference Date: 04-Jun-2017 Date Published: 04-Jun-2017
Conference Location: Indianapolis, IN
Paper Title: Metabolite Fragmentation in Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation
Authors: Ziad J. Sahab, Sylwia Stopka, Bindesh Shrestha, Hang Li, Wei Yuan, Lida Parvin, Akos Vertes
Acknowledged Federal Support: **Y**

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Conference Name: Proceedings of the 65th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics
Date Received: Conference Date: 04-Jun-2017 Date Published: 04-Jun-2017
Conference Location: Indianapolis, IN
Paper Title: Adjustable In-Source Fragmentation of Metabolites and Lipids in Laser Desorption/Ionization from Silicon Nanopost Arrays
Authors: Andrew Korte, Akos Vertes
Acknowledged Federal Support: **Y**

PATENTS:

Intellectual Property Type: Patent Date Received:
Patent Title: System and Method for Laser Desorption Ionization-Mass Spectrometry Analysis of Biomolecules in Cellular Compartments Captured on Functionalized Silicon Nanopost Arrays
Patent Abstract: System and Method for Laser Desorption Ionization-Mass Spectrometry Analysis of Biomolecule
Patent Number: US 62/342,418
Patent Country: USA
Application Date: 26-May-2017 Application Status: 1
Date Issued:

Intellectual Property Type: Patent Date Received:
Patent Title: Tailored Nanopost Array (NAPA) for Laser Desorption Ionization in Mass Spectrometry
Patent Abstract: The production and use of semiconducting nanopost arrays made by nanofabrication is describe
Patent Number: US 9,490,113 B2
Patent Country: USA
Application Date: 07-Apr-2010 Application Status: 3
Date Issued: 08-Nov-2016

Final Report

Period covered: January 13, 2014 – July 12, 2019

BAA number: DARPA-BAA-13-34
Technical area: Rapid Threat Assessment (RTA)

Lead Organization: The George Washington University
Type of business: Educational

Team member: SRI International
Type of business: Nonprofit

Team member: GE Global Research
Type of business: Large Business

Title: **New Tools for Comparative Systems Biology of Threat Agent Action Mechanisms**

Principal Investigator: Professor Vertes, Akos
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E-mail: sahab@gwu.edu

Program start date: January 13, 2014
Submission date: October 11, 2019

Report Documentation

1 Major Goals

This project aims to develop novel methodologies and technologies for the rapid identification of the mechanism of action (MoA) for emerging chemical and biological threat agents. Our approach integrates systems biology tools to detect and identify differential component levels in the cellular transcriptome, proteome and metabolome following exposure to a threat agent. Knowledge-based network inference and mathematical Bayesian network inference models are utilized to interpret the changes measured by the multi-omics experiments to construct the MoA of the challenge agent. Confirmatory assays are utilized to validate the MoA.

The aims of Technical Area 1 are to detect cellular biomolecules within a mass range of 50 to 200,000 Da. In the Base Period, and Option Periods 1, 2, and 3, the objectives are to detect proteins present between 50 and 3×10^7 copies per cell, and other molecules (transcripts and metabolites) present between 4×10^3 and 4×10^{10} molecules per cell. Cells are treated with the challenge agent and sampled at several time-points between 2 s and 48 h, followed by transcriptomics, proteomics, and metabolomics analyses. The untargeted detection of proteins and metabolites is to be performed using liquid chromatography and mass spectrometry.

The goals of Technical Area 2 are to identify components and events. During the base period, the objective of Technical Area 2 is to identify components and events from minutes to days within whole cells and the cytoplasm compartment. In Option Period 1, the aim is to identify components and events in the time range from seconds to days from whole cells and from the cytoplasm compartment. In Option Period 2, the aim is to identify components and events in the time range from seconds to days from whole cells, and from the cytoplasm and nuclear compartments. In Option Period 3, the aim is to identify components and events in the time range from seconds to days from whole cells, and from the cytoplasm, membrane, and nuclear compartments.

Identification of proteins and metabolites is accomplished using search engines against curated and reviewed databases. Quantitation of proteins is performed using tandem mass tags (TMT) and that of metabolites is performed label-free. Identification and quantitation of transcripts is accomplished using Affymetrix microarrays. Experiments are run in triplicates and statistical significance of the regulated component is generated using ANOVA and t-test.

The final goal of Technical Area 3 is to reconstruct at least 95% of the MoA of a threat agent simulant in less than 30 days. In the Base Period, the goal was to reconstruct at least 20% of the MoA for a challenge agent, with the degree of reconstruction in Option Periods 1, 2, and 3, increasing to 60%, 80%, and 95%, respectively.

In Option Period 3, the major goals included the development of additional causal network prediction algorithms, evaluation of the algorithms and analysis workflow in a new biological context, preparation of papers documenting our results, and preparing the database containing all the experimental data (and metadata) collected during the process. The causal network

algorithms discover potential causal relations between timeseries expression profiles, typically measuring change of expression levels of cellular components in response to an external perturbation. Different algorithms typically look at different features and thus give different views of the relations between time series profiles. The long-term aim is to obtain a better understanding of what these relations are capturing, where they agree, and what distinguishes each one. Confirmatory assays are then used to confirm the biological processes that were identified computationally. SRI developed and maintained a project data base for storing and sharing the experimental data collected during the project.

2 Accomplished Under Goals

All project metrics were accomplished, and the following goals were achieved under Technical Area 1.

In GW Task 1, culturing and exposure of cells, we established different cell lines such as hepatocytes (HepG2/C3A and HepG2), and neuroblastoma (SK-N-AS). Cultures were regularly monitored for quality and viability and tested for contamination. During the program, we studied several challenge compounds, e.g., rotenone, forskolin, nocodazole, and bendamustine. For each compound we determined the GI50, and the relevant dose for the challenge agent. Samples were delivered to different omics tasks for further analysis. On average ~3×10^9 cells were cultured and treated for each 30-day demonstration period. In the last phase of our program we investigated the downstream effects of the binary mixture of methamidophos/atropine as agonist/antagonist. We also explored different RNA extraction methods and optimized them for different cell cultures, such as hepatocytes, neuroblastomas, and bacteria.

In GW Task 2, proteomics, we have analyzed protein regulation in HepG2/C3A and SK-N-AS cells and have identified and quantified more than 5,000 protein groups at several treatment time-points.

In GW Task 3, LAESI/UPLC-IMS-MS/MS metabolomics, we harvested, prepared for analysis, and analyzed 80 polar sample fractions and 80 nonpolar fractions of cells for each challenge agent throughout the project. We have also applied UPLC-MS/MS to expand the coverage of metabolites.

In GW Task 4, Metabolomics by NAPA-MS, we have provided metabolomics data for interpretation to the computational tasks. We have developed workflows allowing for high-throughput processing and analysis of metabolomics samples using NAPA- and MALDI-MS to maximize molecular coverage, with experiments during the most recent test period producing 600+ data files from 100+ biological samples, and all sample handling, acquisition, and analysis completed in ~23 days.

In GW Task 5, subcellular fractionation, we performed the compartmental fractionation of HepG2/C3A and SK-N-AS cells resulting in proteome profiling of cytoplasmic, membrane, and nuclear fractions at 8 time-points for each challenge agent.

In SRI Task 4, transcriptomics, we performed the transcriptomics workflow on 22 separate occasions (904 samples total) using whole genome microarray technology. Differential RNA expression data from challenge agent-treated vs. control-treated cells (HepG2/C3A or SK-N-AS cells) at 10 time points spanning 0.5 to 48 h post-exposure and at time zero were delivered for each of the 10 challenge agents we tested over the course of this program, and repeatedly delivered to the team in less than 14 calendar days after receipt of the harvested cell pellets.

All project metrics were accomplished, and the following goals were achieved under Technical Area 2.

In SRI task 1, Master Database, we defined an RTA database schema, providing storage for experimental raw data, metadata, analyses, and related documents. We implemented graphically-based software for data entry, search, and retrieval. We augmented the database access software to allow the automatic entry of data via spreadsheets. Using this infrastructure, we processed data for 1146 experiments using a total of 10 challenge agents. We implemented a mirrored server infrastructure with one server located at GWU and one at SRI, and we modified the server configuration to provide data access beyond the completion of the project.

In SRI Task 2, logical network inference, an analysis workflow was developed that combined results from abstract shape analysis and a suite of algorithms based on Gaussian process (GP) models of timeseries omics data. Two main classes of algorithm were developed: ranking and network synthesis. Ranking algorithms rank GP timeseries profiles on a scale from anomalous/atypical to typical behavior were developed using both autoencoders and Generative Adversarial Networks techniques. Network synthesis algorithms identify causal relations among features of GP timeseries profiles. Network synthesis algorithms were developed using techniques including convolutional autoencoders, Siamese twin networks, and wide/deep prediction networks. Finally, we developed a Probabilistic Tensor Logic and applied it to causal network inference. The analysis workflow, in different stages of development, was applied to transcriptomics, proteomics, and metabolomics data from six challenges (three internal and three provided by DARPA) plus variations of two of the challenges. In each case key components of the MoA were identified.

In SRI Task 3, Bayesian network inference, we used bootstrap resampling to allow us to determine whether a compound was up, down, nominal, or too noisy to distinguish. We developed a variant of Bayesian network inference, biological time warp. It uses an analog of a genetic sequence alignment algorithm to align events in time without a fixed relationship between cause and effect. Biological time warp results are demonstrably superior to n-order, dynamic Bayesian network results. We also developed a number of criteria to select important areas of the network to view. Our algorithm was able to detect all the observable cause-effect relationships in the MoA of each of the challenge agents with which we experimented.

All project metrics were accomplished, and the following goals were achieved under Technical Area 3.

At GW, we have evaluated de novo network construction based on time-profile similarities measured by Pearson and Spearman correlation. Following network mapping, we have partitioned the network using modularity classes followed by Gene Set Enrichment Analysis (GSEA) of each class to reconstruct the MoA of the challenge agents. Furthermore, we have identified the biomolecules with the highest betweenness centrality in the network. These biomolecules are central to the propagation of the MoA. Targeting these biomolecules might allow for an efficient countermeasure.

In GE Task 1, Confirmatory Assays, we applied biological assays to downselect and validate the MoA proposed based on the analyses of the integrated omics data. We were successful at identifying >95% of the MoA of all challenge agents which were analyzed.

In GE Task 2, Fractionation/microfluidics, we have focused on 2 main goals: (1) Integrating fluidic device development for subcellular fractionation of suspension cells and 2) integration and automation of component technologies for adherent cell subcellular fractionation. The devices and disposables assembled at GE remain available for future transition work.

3 Training Opportunities

FY1

Graduate Students: 2

- Lombard, Camille
- Stopka, Sylwia,

Post-Doctoral Scientists: 2

- Kiss, Andras
- Sahab, Ziad

FY2

Graduate Student: 1

- Lombard, Camille

Post-Doctoral Scientists: 3

- Sahab, Ziad
- Andrew Korte
- Yuan, Wei

Undergraduate Student: 1

- Kimmey, Samuel

FY3

Graduate Student: 1

- Li, Hang (Ph.D. awarded)

Post-Doctoral Scientists: 3

- Korte, Andrew
- Hwang, Sunil
- Li, Hang

FY4

Graduate Students: 2

- Lombard-Banek, Camille,
- Zhang, Linwen,

Post-Doctoral Scientists: 4

- Arul, Albert
- Avar, Peter
- Korte, Andrew
- Li, Hang

FY5

Graduate Student: 1

- Zhang, Linwen (Ph.D. awarded)
- Stopka, Sylwia (Ph.D. awarded)

Post-Doctoral Scientists: 3

- Arul, Albert
- Avar, Peter
- Zhang, Linwen

FY6

Graduate Student: 1

- Fincher, Jarod (Ph.D. awarded)

Post-Doctoral Scientist: 1

- Avar, Peter

4 Results Dissemination

(a) Papers published in peer-reviewed journals: **11**

- Jarod A. Fincher, Derek R. Jones, Andrew R. Korte, Jacqueline E. Dyer, Paola

Parlanti, Anastas Popratiloff, Christine A. Brantner, Nicholas J. Morris, Russell K. Pirlo, Victoria K. Shanmugam, and Akos Vertes, "Mass Spectrometry Imaging of Lipids in Human Skin Disease Model Hidradenitis Suppurativa by Laser Desorption Ionization from Silicon Nanopost Arrays," *Sci. Rep.*, **2019**, accepted.

- Jarod A. Fincher, Andrew R. Korte, Jacqueline E. Dyer, Sridevi Yadavilli, Nicholas J. Morris, Derek R. Jones, Victoria K. Shanmugam, Russel K. Pirlo, and Akos Vertes, "Mass Spectrometry Imaging of Triglycerides in Biological Tissues by Laser Desorption Ionization from Silicon Nanopost Arrays," *J. Mass Spectrom.*, **2019**, accepted. http://dx.doi.org/10.1002/jms.4443
- Andrew R. Korte, Nicholas J. Morris, and Akos Vertes, "High Throughput Complementary Analysis and Quantitation of Metabolites by MALDI- and Silicon Nanopost Array-Laser Desorption/Ionization-Mass Spectrometry," *Anal. Chem.*, **2019**, *91*, 3951-3958. http://dx.doi.org/10.1021/acs.analchem.8b05074
- Jarod A. Fincher, Jacqueline E. Dyer, Andrew R. Korte, Sridevi Yadavilli, Nicholas J. Morris, and Akos Vertes, "Matrix-free Mass Spectrometry Imaging of Mouse Brain Tissue Sections on Silicon Nanopost Arrays," *J. Comp. Neurol.*, **2019**, *527*, 2101-2121. https://doi.org/10.1002/cne.24566
- Linwen Zhang, Nikkita Khattar, Ildiko Kemenes, Gyorgy Kemenes, Zita Zrinyi, Zsolt Pirger, and Akos Vertes, "Subcellular Peptide Localization in Single Identified Neurons by Mass Spectrometry," *Sci. Rep.*, **2018**, *8*, 12227. http://dx.doi.org/10.1038/s41598-018-29704-z
- Sylwia A. Stopka, Xavier A. Holmes, Andrew R. Korte, Laine R. Compton, Scott T. Retterer, and Akos Vertes, "Trace Analysis and Reaction Monitoring by Nanophotonic Ionization Mass Spectrometry from Elevated Bowtie and Silicon Nanopost Arrays," *Adv. Funct. Mater.*, **2018**, *28*, 1801730. http://dx.doi.org/10.1002/adfm.201801730
- Linwen Zhang, Christopher J. Sevinsky, Brian M. Davis, and Akos Vertes, "Single-Cell Mass Spectrometry of Subpopulations Selected by Fluorescence Microscopy," *Anal. Chem.*, **2018**, *90*, 4626-4634. http://dx.doi.org/10.1021/acs.analchem.7b05126
- Linwen Zhang, and Akos Vertes, "Single-Cell Mass Spectrometry Approaches to Explore Cellular Heterogeneity," *Angew. Chem. Int. Ed.*, **2018**, *57*, 4466-4477. http://dx.doi.org/10.1002/anie.201709719
- Hang Li, and Akos Vertes, "Solvent Gradient Electrospray for Laser Ablation Electrospray Ionization Mass Spectrometry," *Analyst*, **2017**, *142*, 2921-2927. http://dx.doi.org/10.1039/C7AN00819H
- Andrew R. Korte, Sylwia A. Stopka, Nicholas Morris, Trust Razunguzwa, and Akos Vertes, "Large-Scale Metabolite Analysis of Standards and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays," *Anal. Chem.*, **2016**, *88*, 8989-8996. (Cover Page Article) http://dx.doi.org/10.1021/acs.analchem.6b01186
- Nicholas J. Morris, Heather Anderson, Brian Thibeault, Akos Vertes, Matthew J.

Powell and Trust T. Razunguzwa, "Laser desorption ionization (LDI) silicon nanopost array chips fabricated using deep UV projection lithography and deep reactive ion etching," *RSC Advances*, **2015**, *5*, 72051-72057. http://dx.doi.org/10.1039/c5ra11875a

(b) Papers published in non-peer-reviewed journals: 4

- Mark-Oliver Stehr, Minyoung Kim, Carolyn L. Talcott, Merrill Knapp, Akos Vertes, "Probabilistic Approximate Logic and its Implementation in the Logical Imagination Engine," *arXiv preprint arXiv:1907.11321*, **2019**. https://arxiv.org/abs/1907.11321
- Mark-Oliver Stehr, Peter Avar, Andrew R. Korte, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, Maria I. Zavodszky, Akos Vertes, "Learning Causality: Synthesis of Large-Scale Causal Networks from High-Dimensional Time Series Data," *arXiv preprint arXiv:1905.02291*, **2019**. https://arxiv.org/abs/1905.02291
- Mark-Oliver Stehr, "Probabilistic Approximate Logic and its Implementation in the Logical Imagination Engine", **2019**. http://www.csl.sri.com/users/stehr/RTA/palo-and-lime.pdf
- Akos Vertes, Peter Avar, Andrew R Korte, Lida Parvin, Ziad J Sahab, Deborah I Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Mark-Oliver Stehr, Carolyn L Talcott, Brian M Davis, Christine A Morton, Christopher J Sevinsky, and Maria I Zavodszky, "Transcriptional Response of SK-N-AS Cells to Methamidophos", **2019**. http://www.csl.sri.com/users/clt/XYZ/methamidophosX.pdf

(c) Presentations: **30**

- Ziad J. Sahab, Camille Lombard, Lida Parvin, Peter Nemes, Akos Vertes, "High-Throughput Proteomics as a Critical Tool to Rapidly Discover the Mechanism of Action for Toxic Compounds" oral presentation at the US HUPO Conference – Next Generation Proteomics, Tempe, AZ, March 15 – 18, 2015.
- Andrew Korte, Nicholas Morris, Trust Razunguzwa, Akos Vertes, "Construction of a Metabolite MS/MS Library for Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays" Proceedings of the 63rd ASMS Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 31 – June 4, 2015.
- Ziad Sahab, Camille Lombard, Lida Parvin, Peter Nemes, Akos Vertes, "Early Detection of the Mechanism of Action for Toxins by High-throughput Proteomics" Proceedings of the 63rd ASMS Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 31 – June 4, 2015.
- Wei Yuan, Bindesh Shrestha, Akos Vertes, "Tandem mass spectrum and collision cross section libraries for high-throughput identification of metabolites in adherent

hepatocytes by LAESI mass spectrometry," Proceedings of the 63rd ASMS Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 31 – June 4, 2015.

- Trust Razunguzwa, Heather Anderson, Nicholas Morris, Matthew Powell, "Metabolomic profiling of biofluids using laser desorption ionization on nanopost array devices (REDIchips)," Proceedings of the 63rd ASMS Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 31 – June 4, 2015.
- Trust Razunguzwa, Heather Anderson, Nicholas Morris, Matthew Powell, "REDIchips™ for Rapid Quantitation of low Molecular Weight Pharmaceutical Drugs using Laser Desorption Ionization Mass Spectrometry," Proceedings of the 63rd ASMS Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 31 – June 4, 2015.
- Akos Vertes, Andrew R Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Bindesh Shrestha, Sylwia A Stopka, Wei Yuan, Deborah I. Bunin, Merrill Knapp, Ian Mason, Denise M Nishita, Andrew Poggio, Carolyn L Talcott, Maneesh Yadav, Brian M Davis, Adriana I Larriera, Christine A Morton, Christopher J Sevinsky, Maria I Zavodszky, Nicholas J Morris, Heather R Anderson, Matthew J Powell, Trust T Razunguzwa, "Integrative Systems Biology Approach to Identify Mechanisms of Action", US HUPO Conference – Proteomics: From New Technology to New Biology, Boston, MA, March 13-16, 2016.
- Akos Vertes, Andrew R Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Bindesh Shrestha, Sylwia A Stopka, Wei Yuan, Deborah I. Bunin, Merrill Knapp, Ian Mason, Denise M Nishita, Andrew Poggio, Carolyn L Talcott, Maneesh Yadav, Brian M Davis, Adriana I Larriera, Christine A Morton, Christopher J Sevinsky, Maria I Zavodszky, Nicholas J Morris, Heather R Anderson, Matthew J Powell, Trust T Razunguzwa, "Mechanism of Action Identified in 30 Days: a Systems Biology Approach", Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Diego, CA, April 2-6, 2016.
- Andrew R. Korte, Akos Vertes, "Analysis of Lipids from Biological Samples by Laser Desorption Ionization from Silicon Nanopost Arrays" Proceedings of the 64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, June 5-9, 2016.
- Ziad J. Sahab; Bindesh Shrestha; Lida Parvin; Peter Nemes; and Akos Vertes, "Rapid Exploration of the Mechanism of Action of forskolin via Untargeted Proteomics and Knowledge-based Pathway Analysis" Proceedings of the 64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, June 5-9, 2016.
- Akos Vertes, Andrew R Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Bindesh Shrestha, Sylwia A Stopka, Wei Yuan, Deborah I. Bunin, Merrill Knapp, Ian Mason, Denise M Nishita, Andrew Poggio, Carolyn L Talcott, Maneesh Yadav, Chrystal Chadwick, Brian M Davis, Adriana I Larriera, Christine A Morton, Christopher J Sevinsky, Maria I Zavodszky, Nicholas J Morris, Heather R Anderson, Matthew J Powell, Trust T Razunguzwa, "Rapid Identification of Mechanisms of

Action Through Systems Biology", 21st International Mass Spectrometry Conference (IMSC 2016), Toronto, ON, August 20-26, 2016.

- Andrew R. Korte and Akos Vertes, "Metabolomics of Hepatocytes and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays", Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) SciX 2016, Minneapolis, MN, September 18-23, 2016.
- Andrew R. Korte and Akos Vertes, "Fluence-Dependent Metabolite and Lipid Fragmentation in Silicon Nanopost Array Laser Desorption/Ionization", The Pittsburgh Conference & Exhibition (Pittcon) – Pittcon Conference & Expo 2017, Chicago, IL, March 5-9, 2017.
- Akos Vertes, Andrew R Korte, Sunil Hwang, Hang Li, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Bindesh Shrestha, Sylwia A Stopka, Wei Yuan, Deborah I. Bunin, Merrill Knapp, Ian Mason, Denise M Nishita, Andrew Poggio, Carolyn L Talcott, Maneesh Yadav, Brian M Davis, Adriana I Larriera, Christine A Morton, Christopher J Sevinsky, Maria I Zavodszky, Nicholas J Morris, Heather R Anderson, Matthew J Powell, Trust T Razunguzwa, "Identification of the Mechanisms of Action of Toxins in Less Than 30 Days", Society of Toxicology (SOT) – 56th Annual Meeting and ToxExpo, Baltimore, MD, March 12-16, 2017.
- Akos Vertes, Andrew R Korte, Hang Li, Peter Nemes, Lida Parvin, Sylwia Stopka, Sunil Hwang, Ziad J Sahab, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Carolyn L Talcott, Brian M Davis, Christine A Morton, Christopher J Sevinsky, Maria I Zavodszky, Nicholas J Morris, Matthew J Powell, "Time-Dependent Metabolomics in Systems Biology Context for Mechanism of Action Studies", US Human Proteome Organization (HUPO) Conference – Proteomics: From Genes to Function, San Diego, CA, March 19-22, 2017.
- Ziad J. Sahab, Sylwia Stopka, Bindesh Shrestha, Hang Li, Wei Yuan, Lida Parvin, Akos Vertes, "Metabolite Fragmentation in Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation", Proceedings of the 65th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics, Indianapolis, IN, June 4-8, 2017.
- Andrew R. Korte and Akos Vertes, "Adjustable In-Source Fragmentation of Metabolites and Lipids in Laser Desorption/Ionization from Silicon Nanopost Arrays", Proceedings of the 65th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics, Indianapolis, IN, June 4-8, 2017.
- Akos Vertes, Andrew R. Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J. Sahab, Bindesh Shrestha, Sylwia A. Stopka, Wei Yuan, Deborah I. Bunin, Merrill Knapp, Ian Mason, Denise M. Nishita, Andrew Poggio, Carolyn L. Talcott, Maneesh Yadav, Chrystal Chadwick, Brian M. Davis, Adriana I. Larriera, Christine A. Morton, Christopher J. Sevinsky, Maria I. Zavodszky, Nicholas J. Morris, Heather R. Anderson, Matthew J. Powell, and Trust T. Razunguzwa, "Identification of the Cellular

Mechanism of Action Induced by Exposure to an Emerging Threat Agent", *Military Health System Research Symposium (MHSRS),* Kissimmee, FL, Aug 27-30, 2017.

- Akos Vertes, Andrew R. Korte, Hang Li, Peter Nemes, Lida Parvin, Sylwia Stopka, Sunil Hwang, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Sean R. Dinn, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky, "Mechanism of Action Identification of Threat Agents Within 30 Days", *Society of Toxicology (SOT) – 57th Annual Meeting and ToxExpo,* San Antonio, TX, March 11-15, 2018.
- Akos Vertes, Albert B. Arul, Andrew R. Korte, Hang Li, Peter Nemes, Lida Parvin, Sylwia Stopka, Sunil Hwang, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky, "Novel High-Throughput Metabolomic Techniques and Mainstream Tools for the Discovery of Drug Mechanism of Action", *US Human Proteome Organization (HUPO) Conference – Technology Accelerating Discovery*, Minneapolis, MN, March 11-14, 2018.
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Hang Li, Peter Nemes, Lida Parvin, Sylwia Stopka, Sunil Hwang, Ziad J. Sahab, Linwen Zhang, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Sean R. Dinn, Christine A. Morton, Christopher J. Sevinsky, Maria I. Zavodszky, "Inferring Mechanism of Action of an Unknown Compound from Time Series Omics Data," in: *Computational Methods in Systems Biology*, Milan Ceska and David Safranek (eds), Springer International Publishing, **2018**, pp. 238-255. ISBN-13: 978-3-319-99428-4, http://dx.doi.org/10.1007/978-3-319-99429-1_14
- Akos Vertes, Albert B. Arul, Peter Avar, Andrew R. Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J. Sahab, Bindesh Shrestha, Sylwia Stopka, Wei Yuan, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky, "Systems Biology Approach for Mechanism of Action Identification in 30 Days", *Proceedings of the 66th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics*, San Diego, CA, June 3-7, 2018.
- Andrew R. Korte and Akos Vertes, "Complementary Metabolite Analysis by Combined MALDI- and Silicon Nanopost Array-LDI-MS", *Proceedings of the 66th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics*, San Diego, CA, June 3-7, 2018.
- Jacqueline E. Dyer, Jarod A. Fincher, Andrew R. Korte, Nicholas J. Morris, Matthew J. Powell, Derek Jones, Victoria Shanmugam, Sridevi Yadavilli, Russell K. Pirlo, and Akos Vertes, "Complementary Features of Laser Desorption Ionization from Silicon Nanopost Arrays and MALDI for Mass Spectrometry Imaging", *Proceedings of the 66th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics*, San Diego, CA, June 3-7, 2018.

- Linwen Zhang, Nikkita Khattar, Ildiko Kemenes, Gyorgy Kemenes, Zita Zrinyi, Zsolt Pirger, and Akos Vertes, "Subcellular Analysis of Neuropeptides in Single Identified Neurons by Mass Spectrometry", *Proceedings of the 66th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics*, San Diego, CA, June 3-7, 2018.
- Peter Avar, Andrew R. Korte, Hang Li, Lida Parvin, and Akos Vertes, "Performance Comparison of High-Throughput and Conventional Metabolomics Methods Based on Mass Spectrometry", *Proceedings of the 66th American Society of Mass Spectrometry (ASMS) Conference on Mass Spectrometry and Allied Topics*, San Diego, CA, June 3-7, 2018.
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R Korte, Camille Lombard-Banek, Peter Nemes, Lida Parvin, Ziad J Sahab, Bindesh Shrestha, Sylwia A Stopka, Wei Yuan, Deborah I Bunin, Merrill Knapp, Andrew Poggio, Carolyn L Talcott, Brian M Davis, Christine A Morton, Christopher J Sevinsky, and Maria I Zavodszky, "Integrative Omics Approach for the Rapid Identification of the Mechanism of Action of Emerging Threat Agents" *Military Health System Research Symposium (MHSRS)*, Kissimmee, FL, Aug 20-23, 2018.
- Jarod A Fincher, Jacqueline E Dyer, Andrew R Korte, Nicholas J Morris, Sridevi Yadavilli, and Akos Vertes, "Mass Spectrometry Imaging of Triacylglycerides in Biological Tissue Sections Using Laser Desorption Ionization on Silicon Nanopost Arrays", *Imaging Mass Spectrometry Society (IMSS II)*, Charleston, SC, November 11-14, 2018.
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Peter Nemes, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky "Broad Time-dependent Proteomic and Metabolomic Effects of Atorvastatin on Hepatocytes", *US HUPO Conference – Proteomic at the Frontier of Biology and Medicine*, Washington, DC, March 3-6, 2019.
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Peter Nemes, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky "Network Integration of Omics Data for Fast-track Identification of the Mechanism of Action for Drug Candidates*", US HUPO Conference – Proteomic at the Frontier of Biology and Medicine*, Washington, DC, March 3-6, 2019.

d) Manuscripts: 2

- Sylwia A. Stopka and Akos Vertes, "Metabolomic Profiling of Adherent Mammalian Cells In situ by LAESI-MS with Ion Mobility Separation," in: Ion Mobility Mass Spectrometry: Methods and Protocols, *Methods in Molecular Biology*, Giuseppe Paglia and Giuseppe Astarita (eds), Springer, New York, **2018**, submitted.

- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky, and Maria I. Zavodszky, "Network Integration of Time-dependent Omics Data for the Identification of Off-target Effects for Drug Candidates", **2019**, in preparation.

e) Edited Book: 1

- *Single Cell Metabolism*, Bindesh Shrestha (ed), Methods in Molecular Biology, vol 2064. Humana, New York, NY, **2020**, pp. 9-18. ISBN-13: 978-1-4939-9829-6, https://doi.org/10.1007/978-1-4939-9831-9

f) Book Chapters: 3

- Sylwia A. Stopka, Akos Vertes, "Toward Single Cell Molecular Imaging by Matrix-Free Nanophotonic Laser Desorption Ionization Mass Spectrometry," in: *Single Cell Metabolism*, Bindesh Shrestha (ed), Methods in Molecular Biology, vol 2064. Humana, New York, NY, **2020**, pp. 135-146. ISBN-13: 978-1-4939-9829-6. https://doi.org/10.1007/978-1-4939-9831-9_11
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Lida Parvin, Ziad J. Sahab, Deborah I. Bunin, Merrill Knapp, Denise Nishita, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Christine A. Morton, Christopher J. Sevinsky and Maria I. Zavodszky, "Transcriptional response of SK-N-AS cells to methamidophos," in: *Computational Methods in Systems Biology*, Bortolussi L., Sanguinetti G. (eds) Lecture Notes in Computer Science, vol 11773. Springer, **2019**, pp. 368-372. ISBN-13: 978-3-030-31303-6. https://doi.org/10.1007/978-3-030-31304-3_29
- Akos Vertes, Albert-Baskar Arul, Peter Avar, Andrew R. Korte, Hang Li, Peter Nemes, Lida Parvin, Sylwia Stopka, Sunil Hwang, Ziad J. Sahab, Linwen Zhang, Deborah I. Bunin, Merrill Knapp, Andrew Poggio, Mark-Oliver Stehr, Carolyn L. Talcott, Brian M. Davis, Sean R. Dinn, Christine A. Morton, Christopher J. Sevinsky, Maria I. Zavodszky, "Inferring Mechanism of Action of an Unknown Compound from Time Series Omics Data," in: *Computational Methods in Systems Biology*, Milan Ceska and David Safranek (eds), Springer International Publishing, **2018**, pp. 238-255. ISBN-13: 978-3-319-99428-4. http://dx.doi.org/10.1007/978-3-319-99429-1_14

5 Honors and Awards: 3

- April 20, 2016 – **Distinguished Researcher Award**, awarded to Akos Vertes by The George Washington University
- May 23, 2017 – WBMSDG Travel Award, awarded to Linwen Zhang by the Washington-Baltimore Mass Spectrometry Discussion Group (WBMSDG)
- Nov 8, 2018 – 1st place prize awarded to poster titled "Mechanism of Action Identification of Threat Agents within 30 Days" presented by Deborah Bunin at the SRI Biosciences' Internal Annual Poster Day Event.

6 Technology Transfer

Filed Patent Applications: 2

- **Title:** "Laser desorption ionization-mass spectrometry analysis of biomolecules in cellular compartments captured on functionalized silicon nanopost arrays" **Inventors:** Akos Vertes (The George Washington University), Andrew Korte (The George Washington University), Brian Davis (GE Global Research), and Sean Dinn (GE Global Research) **Filing date:** provisional patent application filed on May 27, 2016 with the USPTO
- **Title:** "System and Method for Laser Desorption Ionization-Mass Spectrometry Analysis of Biomolecules in Cellular Compartments Captured on Functionalized Silicon Nanopost Arrays", **Inventors:** Akos Vertes and Andrew R. Korte (The George Washington University); Brian Davis and Sean Dinn (GE Global Research), **Date of non-provisional patent application:** May 26, 2017

Patents awarded: 6

- **United States Patent US 8,809,774 B2,** Date of Patent: 8/19/2014, Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry (Continuation of United States Patent US 8,487,244 B2), Inventors: Akos Vertes, and Peter Nemes
- **United States Patent US 8,829,426 B2,** Date of Patent: 9/9/2014, Title: Plume Collimation for Laser Ablation Electrospray Ionization Mass Spectrometry, Inventors: Akos Vertes, and Jessica A. Stolee
- **United States Patent US 8,901,487 B2,** Date of Patent: 12/2/2014, Title: Subcellular Analysis by Laser Ablation Electrospray Ionization Mass Spectrometry, Inventors: Akos Vertes, Jessica A. Stolee, and Bindesh Shrestha
- **United States Patent US** 9**,000,361 B2,** Date of Patent: 04/7/2015, Title: Nanophotonic Production, Modulation and Switching of Ions by Silicon Microcolumn Arrays, Inventors: Akos Vertes and Bennett N. Walker

- **United States Patent US 9,362,101 B2,** Date of Patent: 06/07/2016, Title: Plume Collimation for Laser Ablation Electrospray Ionization Mass Spectrometry, Inventors: Akos Vertes and Jessica Stolee
- **United States Patent US 9,490,113 B2,** Date of Patent: 11/08/2016, Title: Tailored Nanopost Array (NAPA) for Laser Desorption Ionization in Mass Spectrometry, Inventors: Akos Vertes, Bennett N. Walker, Jessica Stolee, and Scott Retterer

7 Supported Personnel

FY1

Graduate Students: 2

- Lombard, Camille, 17% supported, 100% FTE
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Post-Doctoral Scientists: 2

- Kiss, Andras, 66% supported, 100% FTE
- Sahab, Ziad, 86% supported, 100% FTE

Faculty: 2

- Nemes, Peter, 31% supported, 100% FTE
- Vertes, Akos, 100% supported, 0% FTE

Other Research staff: 28

- Al-Kofahi, Yousef, 100% supported, 0.3% FTE
- Bodla, Karthik, 100% supported, 5.0% FTE
- Bunin, Debbie, 100% supported, 16.0% FTE
- Davis, Brian, 100% supported, 25.0% FTE
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- Galligan, Craig, 100% supported, 10.0% FTE
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- Knapp, Merrill, 100% supported, 41.0% FTE
- Kovacs, Ernest, 100% supported, 0.1% FTE
- Liu, Xiaofeng, 100% supported, 0.5% FTE
- Morris, Nicholas, 100% supported, 100.0% FTE
- Ostroverkhov, Victor, 100% supported, 0.1% FTE
- Parvin, Lida, 90% supported, 100.0% FTE
- Poggio, Andy, 100% supported, 57.0% FTE
- Puleo, Chris, 100% supported, 10.0% FTE
- Razunguzwa, Trust, 100% supported, 50.0% FTE
- Santamaria-Pang, Alberto, 100% supported, 0.5% FTE
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- Shrestha, Bindesh, 100% supported, 100.0% FTE
- Steche, Thomas, 100% supported, 1.0% FTE

- Talcott, Carolyn, 100% supported, 16.0% FTE
- Tilton, Andrew, 100% supported, 9.0% FTE
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- Zassowski, Lisa, 100% supported, 1.0% FTE
- Zavodsky, Maria, 100% supported, 1.0% FTE

FY2

Graduate Students: 1

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Post-Doctoral Scientists: 3

- Sahab, Ziad, 14.2% supported, 100% FTE
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- Yuan, Wei, 100% supported, 100% FTE

Faculty: 2

- Nemes, Peter, 8.3% supported, 100% FTE
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Undergraduate Students: 1

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Other Research staff: 31

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- Bunin, Deborah, 100% supported, 28.0% FTE
- Davis, Brian, 100% supported, 39.4% FTE
- Galligan, Craig, 100% supported, 14.5% FTE
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- Knapp, Merrill, 100% supported, 54.0% FTE
- Kovacs, Ernest, 100% supported, 10.8% FTE
- Larriera, Adriana, 100% supported, 24.2% FTE
- Morris, Nicholas, 100% supported, 100.0% FTE
- Morton, Christine, 100% supported, 8.3% FTE
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- Nishita, Denise, 100% supported, 4.5% FTE
- Parvin, Lida, 100% supported, 100.0% FTE
- Poggio, Andrew, 100% supported, 54.0% FTE
- Puleo, Chris, 100% supported, 15.0% FTE
- Razunguzwa, Trust, 100% supported, 50.0% FTE
- Sahab, Ziad, 85.8% supported, 100% FTE
- Sevinsky, Chris, 100% supported, 25.1% FTE
- Shrestha, Bindesh, 100% supported, 100.0% FTE
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- Yuan, Wei, 91.7% supported, 100% FTE
- Zavodsky, Maria, 100% supported, 8.5% FTE
- Zassowksi, Lisa, 100% supported, 1.3% FTE
- Zou, Yun, 100% supported, 5.5% FTE

FY3

Graduate Students: 1

- Li, Hang, 100% supported, 50% FTE

Post-Doctoral Scientists: 2

- Korte, Andrew, 100% supported, 100% FTE
- Hwang, Sunil, 100% supported, 100% FTE
- Li, Hang, 8.3% supported, 100% FTE

Faculty: 2

- Vertes, Akos, 100% supported, 13.6% FTE
- Nemes, Peter, 100% supported, 8.3% FTE

Other Research staff: 42

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- Castle, Jason, 100% supported, 0.7% FTE
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- Conway, Kenneth, 100% supported, 0.1% FTE
- Corwin, Alex, 100% supported, 0.4% FTE
- Davis, Brian, 100% supported, 28.0% FTE
- Dinn, Sean, 100% supported, 25.0% FTE
- Galligan, Craig, 100% supported, 11.5% FTE
- Hale, Jared, 100% supported, 1.1% FTE
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- Liang, Annie 100% supported, 5% FTE
- Lowery, Lisa, 100% supported, 0.1% FTE

- Mcdonough, Elizabeth, 100% supported, 0.4% FTE
- Morton, Christine, 100% supported, 2.3% FTE
- Neculaes, Vasile, 100% supported, 1.6% FTE
- Nishita, Denise 100% supported, 6% FTE
- Parvin, Lida, 100% supported, 100.0% FTE
- Poggio, Andrew 100% supported, 45% FTE
- Powell, Matthew, 50% supported, 8.3% FTE
- Puleo, Christopher, 100% supported, 0.2% FTE
- Raghunath, Sushravya, 100% supported, 1.3% FTE
- Razunguzwa, Trust 50% supported, 25% FTE
- Sahab, Ziad, 100% supported, 100% FTE
- Sevinsky, Christopher, 100% supported, 8.0% FTE
- Shaikh, Kashan, 100% supported, 0.7% FTE
- Shrestha, Bindesh, 100% supported, 16.6% FTE
- Smith, Scott, 100% supported, 0.7% FTE
- Stecher, Thomas, 100% supported, 0.8% FTE
- Sui, Yunxia, 100% supported, 1.8% FTE
- Talcott, Carolyn 100% supported, 15% FTE
- Wood, Nichole, 100% supported, 1.7% FTE
- Yuan, Wei, 100% supported, 16.6%FTE
- Zassowski, Lisa, 100% supported, 1.2% FTE
- Zavodszky, Maria, 100% supported, 0.7% FTE

FY4

Graduate Students: 2

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Post-Doctoral Scientists: 4

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- Korte, Andrew, 100% supported, 100% FTE
- Li, Hang, 100% supported, 16.66% FTE

Faculty: 2

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- Nemes, Peter, 100% supported, 8.3% FTE

Other Research staff: 45

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- Chadwick, Chrystal, 100% supported, 3.5% FTE
- Corwin, Alex, 100% supported, 0.3% FTE
- Davis, Brian, 100% supported, 34.8% FTE
- Dinn, Sean, 100% supported, 22.8% FTE
- Galligan, Craig, 100% supported, 15.5% FTE
- Hale, Jared, 100% supported, 1.3% FTE
- Hammond, Tyler, 100% supported, 3.7% FTE
- Hotaling Jr, John, 100% supported, 0.2% FTE
- Hwang, Sunil, 100% supported, 25% FTE
- Kapusta, Christopher, 100% supported, 0.05% FTE
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- Klopman, Jonathan, 100% supported, 0.1% FTE
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- Knapp, Merrill 100% supported, 39.40% FTE
- Knussman, Michael, 100% supported, 1.4% FTE
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- Mason, Ian, 100% supported, 0.43% FTE
- Matthew Powell, 50% supported, 100%FTE
- Morris, Nicholas, 50% supported, 58.33% FTE
- Morton, Christine, 100% supported, 6.6% FTE
- Nelson, John, 100% supported, 0.2% FTE
- Nishita, Denise, 100% supported, 7.01% FTE
- Parvin, Lida, 100% supported, 100.0% FTE
- Poggio, Andrew, 100% supported, 58.78% FTE
- Powell, Matthew, 50% supported, 100% FTE
- Puleo, Christopher, 100% supported, 0.1% FTE
- Raghunath, Sushravya, 100% supported, 1.4% FTE
- Sahab, Ziad, 100% supported, 100% FTE
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- Sevinsky, Christopher, 100% supported, 9.7% FTE
- Shaikh, Kashan, 100% supported, 12.2% FTE
- Smith, Scott, 100% supported, 0.9% FTE
- Stecher, Thomas, 100% supported, 2.9% FTE
- Stehr, Mark-Oliver, 100% supported, 21.03% FTE
- Sui, Yunxia, 100% supported, 3.1% FTE
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- Toepfer, Timothy, 100% supported, 0.5% FTE

- Wood, Nichole, 100% supported, 0.3% FTE
- Wyko, Wayne, 100% supported, 0.2% FTE
- Zassowski, Lisa, 100% supported, 1.8% FTE
- Zavodszky, Maria, 100% supported, 2.3% FTE

FY5

Graduate Students: 1

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Post-Doctoral Scientists: 3

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- Zhang, Linwen, 100% supported, 25% FTE

Faculty: 1

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Other Research staff: 26

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- Davis, Brian, 100% supported, 34.3% FTE
- Dinn, Sean, 100% supported, 13.9% FTE
- Galligan, Craig, 100% supported, 8.6% FTE
- Hammond, Tyler, 100% supported, 0.9% FTE
- Hotaling Jr, John, 100% supported, 0.2% FTE
- Knapp, Merrill 100% supported, 30% FTE
- Korte, Andrew 100% supported, 100% FTE
- Morris, Nicholas, supported, 100% supported, 7% FTE
- Morton, Christine, 100% supported, 8.5% FTE
- Nishita, Denise, 100% supported, 5% FTE
- Parvin, Lida, 100% supported, 100.0% FTE
- Poggio, Andrew, 100% supported, 65% FTE
- Raghunath, Sushravya, 100% supported, 2.6% FTE
- Sahab, Ziad, 100% supported, 100% FTE
- Schyberg, Shannon, 100% supported, 0.8% FTE
- Sevinsky, Christopher, 100% supported, 6.5% FTE
- Shaikh, Kashan, 100% supported, 6.3% FTE
- Stecher, Thomas, 100% supported, 0.3% FTE
- Stehr, Mark-Oliver, 100% supported, 54% FTE
- Talcott, Carolyn, 100% supported, 24% FTE
- Toepfer, Timothy, 100% supported, 4.9% FTE

- Zassowski, Lisa, 100% supported, 4.3% FTE
- Zavodszky, Maria, 100% supported, 16.5% FTE

FY 6

Post-Doctoral Scientists: 2

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Faculty: 1

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Other Research staff: 12

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- Davis, Brian, 12.2% supported, 100% FTE
- Dinn, Sean, 0.4% supported, 100% FTE
- Galligan, Craig, 1.5% supported, 100% FTE
- Knapp, Merrill, 35% supported, 100% FTE
- Morton, Christine, 1.5% supported, 100% FTE
- Nishita, Denise, 1% supported, 100% FTE
- Parvin, Lida, 100% supported, 100.0% FTE
- Poggio, Andrew, 20% supported, 100% FTE
- Sahab, Ziad, 20% supported, 100% FTE
- Stehr, Mark-Oliver, 33% supported, 100% FTE
- Talcott, Carolyn, 22% supported, 100% FTE

8 Attached Report

Scientific Progress and Accomplishments for Specific Tasks

1 GW Tasks

1.1 Culturing and Exposure

- Measured the GI50 at 48 h, cultured and treated ~5×10^9 HepG2/C3A or SK-N-AS cells with each of the 7 challenge agents.
- Provided cultures to the different omics tasks to profile transcripts, proteins, and metabolites.
- Hepatocytes were successfully cultured in stable isotope labeled media for isotopic ratio outlier analysis (IROA).
- Established fluorimetric assays for intracellular calcium measurements.
- Established and optimized RNA extraction protocols for mammalian and bacterial cells.

1.2 Proteomic Analysis

- All methods related to the proteomics task have been established.
- Profiled proteins from whole HepG2/C3A or SK-N-AS cell extracts at 8 time-points following exposure to each of the challenge agents.
- Expanded protein identification to 3,963 protein groups in SK-N-AS and to more than 5,000 protein groups in HepG2/C3A cell extracts at 8 time-points following exposure to the challenge agent.

1.3 Metabolomics - LAESI-MS

- Successfully applied the metabolomics workflow, including the cell extraction, separation, and small molecular analysis on HepG2/C3A and SK-N-AS cell lines that were exposed to the challenge agents.
- Analyzed time dependent metabolic changes in SK-N-AS cell culture media challenged with methamidophos and atropine sulfate.
- Utilized LAESI-MS and later complemented this technique with UPLC-IMS-MS/MS, which allowed for capturing a higher number of metabolites leading to the identification of 3,565 metabolites.

1.4 Metabolomics - NAPA-MS

- Developed a methodology for lysis, extraction, and NAPA- and MALDI-MS analysis of polar and nonpolar metabolites from hepatocyte and neuron cell lines.
- Developed an expandable in-house tandem MS library containing 338 spectra (251 in positive ion mode, 250 in negative ion mode), covering 287/618 tested compounds, for metabolite identification.
- NAPA-MS detection of markers (e.g., lipid signals and nucleobases) for the capture of cellular material on antibody-conjugated NAPA chips.
- Evaluated in-source fragmentation in laser desorption ionization from NAPA, including fluence and compound dependence.
- Demonstrated complementary coverages for NAPA- and MALDI-MS of cell extracts and tissue samples, with NAPA providing improved coverage for low-MW compounds and MALDI for higher-MW compounds; similar complementarity extends to the coverage of various lipid classes.
- Observed improved quantitative reproducibility for NAPA-MS relative to MALDI-MS, for metabolites detected by both techniques.
- Demonstrated complementary MS imaging by NAPA- and MALDI-MS for a range of tissue samples including human skin and various mouse organs.

1.5 Fractionation – Conventional

- All the proteomics fractionation methods related to this task have been finalized.
- Analyzed the regulation of proteins in different compartments leading to the profiling of cytoplasmic, membrane and nuclear fractions collected during the final 30-day internal demonstration period.

- Identified 2,355 protein groups in cytoplasmic; 2,664 in membrane; and 2,034 in nuclear fractions extracted from SK-N-AS cells treated with methamidophos/atropine.

2 SRI Tasks

2.1 Master Database

- Created a new metabolite thesaurus to accommodate change in the Human Metabolite Database.
- Converted GWU database server to operate independently from SRI database server by disabling mirroring and shut down the database on SRI server.
- Modified server monitoring software to monitor database availability in the absence of mirroring.
- Created account on GWU database server to provide access for SRI staff.

2.2 Logical Network Inference

- Developed a GAN (generative adversarial networks) based model to rank compounds by how atypical/anomalous or typical/real they appear to a trained discriminator.
- Developed several additional algorithms to find relations between compounds with time series data including algorithms based on convolutional Siamese twin neural networks, auto-encoders, and a petri-net based prediction algorithm.
- Carried out plausibility checks of causality predictions using TRRUST (http://www.grnpedia.org/trrust) and CMap (https://portals.broadinstitute.org/cmap) data.
- Completed analysis of methamidophos transcriptomics data. Identified several processes that appeared to be upregulated in cells treated with methamidophos including unfolded protein response, response to cAMP, calcium ion response, and cell-cell signaling. Transcripts with potentially key roles were identified and causal networks relating these transcripts were inferred using two different computational methods.
- Summarized the graphs synthesized by four different algorithms and compared the results across the five unknowns studied in the project. Work is in progress to identify the roles of transcripts forming large connected components in different contexts. For example, in the case of atorvastatin, an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) the main connected components for several algorithms contain the responsive transcripts for cholesterol anabolic enzymes.
- Prepared draft papers documenting (i) the different algorithms developed to infer causal relations (graph synthesis) from time series expression data; and (ii) Palo-Lime a first implementation of combining logic and Machine learning with application to time series data. There are also applications to financial data, social networks, and many more.
- The manuscripts on methamidophos findings will be revised and submitted to a journal.

2.3 Bayesian Network Inference

- Augmented RTA analysis software to show volcano plots for all data types.

- Completed analysis of methamidophos/atropine experiment data.
- Produced a summary of analysis results from challenge agents 1, 3, 4, 5, and 6 for a meta-analysis of the effectiveness of different means of identifying compounds changed by challenge agents and the causes of those changes.
- Produced a description of bootstrap resampling and time warp causal analysis for a technical paper.
- Produced an analysis variant of the methamidophos/atropine experiment data analysis for a technical paper.

2.4 Transcriptomics

- Delivered transcriptomics fold change and raw intensity value data from cells exposed to challenge agents and binary mixtures (e.g., methamidophos/atropine) corresponding to 10 timepoints (0.5 h to 48 h post-exposure) plus a time zero.
- Wrote text describing transcriptomics methods and results for inclusion in a technical manuscript submitted for peer-review.
- Completed all milestones for this task.

3 NAPA development Task

3.1 NAPA Development

- Fabricated and provided GW and GE with NAPA chips to support ongoing project efforts and 30-day demos and tests.
- Delivered batches of imaging NAPA chips to GWU.
- Delivered batches of DUV-PL chips to GWU.
- Finalized report detailing optimized production protocols for all types of NAPA chips.

4 GE Tasks

4.1 Confirmatory Assays

- Quantification of acetylcholine levels in SK-N-AS cells estimates its concentration at ~24 µM. Acetylcholine levels were similar in lysed and unlysed cells suggesting that acetylcholine is actively secreted from cells.
- Confirmed that methamidophos inhibits endogenous acetylcholine esterase activity in SK-N-AS cells. At 2 mg/mL (14.2 µM) methamidophos for 15 min inhibited resorufin production by ~85%.
- Observed reduction in intracellular calcium after methamidophos for 40 minutes. At 14.2 µM methamidophos, incomplete AChE inhibition was observed. At 71 µM methamidophos, complete AChE inhibition was observed.
- If we assume that 71 µM methamidophos inhibits all AChE activity, then choline:acetylcholine ratio is ~1:3 (t=20 min) to ~1:2 (t=70 min).

- Developed flow cytometry assay to quantify intracellular calcium in methamidophos treated cells and measured longer term methamidophos response. Up to 40 minutes after exposure, increasing methamidophos exposure causes a reduction in intracellular calcium levels. Increasing atropine in 1.4 mg/ml methamidophos causes a minor increase in intracellular calcium.
- Tested and troubleshot a new flow cytometry assay to measure immediate impact on intracellular calcium levels after addition of methamidophos but data showed no change over multiple experiments. Additional assay development and/or different equipment would be required.
- CellDistinguisher evaluation of methamidophos treated cells showed effects related to RNA binding, RNA splicing, RNA processing, nucleosome, cell-cell adhesion, zinc-finger, and transcription regulation. Observed changes in cell morphology and cell-cell interactions confirm the GO term related to cell-cell adhesion effects.
- Applied Cell Distinguisher algorithm for cell cycle analysis on challenge agent treated synchronized and asynchronized cells. This partial deconvolution approach utilizes a 67-transcript panel that classifies and quantifies cells in G1/S and G2/M phases of the cell cycle.
- Applied Cell Distinguisher to evaluate methamidophos, bendamustine, atorvastatin, and forskolin transcriptomics data by complete deconvolution. Challenge agent atorvastatin (Unk5) showed strong effects on cholesterol and lipid metabolism. Bendamustine (Unk3) GO terms included p53, DNA damage and cell-cell adhesion.
- The CellDistinguisher manuscript is nearing completion pending final edits. Lee Newberg is actively completing the manuscript, with assistance from Maria Zavodszky. The manuscript will be submitted to PLOSOne.
- The topics of the paper include: 1) use of complete deconvolution for drug response after treatment with bendamustine, atorvastatin, forskolin and methamidophos, and 2) partial deconvolution for cell cycle analysis on synchronized/asynchronized cells and cell cycle effects after drug treatment.

4.2 Fractionation/Microfluidics

- All experimental work has been completed.
- The two manuscripts on suspension and adherent fluidic approaches for rapid cell fractionation and analysis are nearing completion.
- The topics of the suspension fractionation paper include a description of the component technologies developed/applied and their performance, as well as the design and performance of the integrated device.
- The topics of the adherent fractionation paper include systems level process workflow, automation and performance of the system.
- Manuscripts will be submitted to SLAS Tech.

Lessons Learned and Future Directions

1. Workflow

We developed and refined the workflow in **Figure 1** to successfully accomplish the goals of this project, i.e., the rapid identification of the MoA of challenge agents. The cell line used for most of this work, HepG2/C3A, was exposed to each of the challenge agents and was sampled at several lengths of treatment.

When cells are exposed to a drug candidate and dynamic changes are measured over multiple time-points for thousands of transcripts, proteins, and metabolites, diverse bioinformatics tools are needed to extract the relevant information from the vast array of possible interactions, and to identify the key elements of the molecular response. Knowledge-based tools, e.g., Ingenuity Pathway Analysis (IPA), are important to identify the canonical pathways affected by the exposure but they were not the ultimate focus of our work because of the scarcity of information for a truly novel emerging threat. Development of tools to generate de novo molecular networks based on the correlation of molecular profiles are also necessary to facilitate the interpretation of large datasets, and to efficiently visualize the results. We explored and compared the two approaches to identify the MoA as well as the off-target and downstream effects of challenge agents. The final step consists of confirmatory assays to downselect the MoA options and validate the findings.

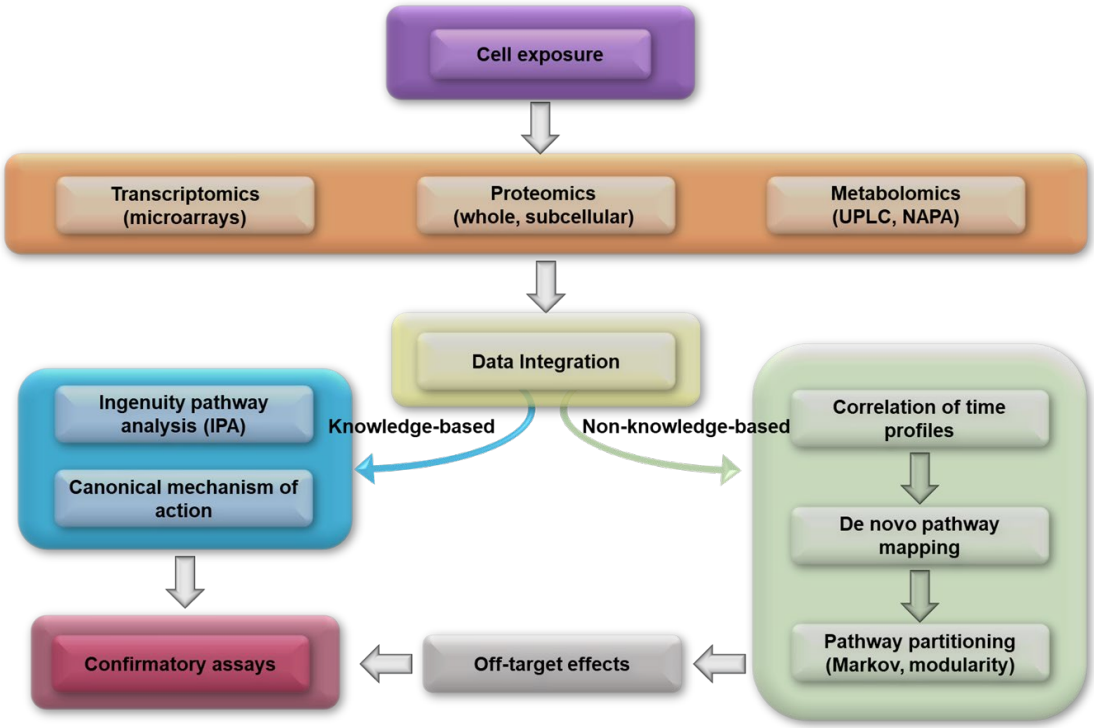

Figure 1: Workflow to identify MoA and off-target effects of challenge agents starting with cell exposure and harvesting at multiple time-points, omics data acquisition, knowledge-based and de novo data analyses to identify canonical pathways and off-target effects.

2. Importance of Metabolomics

Metabolomics is somewhat behind transcriptomics and proteomics due to the lack of standardized protocols for the identification and quantitation of metabolites. The core challenge is the diversity of metabolite structures, when compared with transcripts that are a combination of only four nucleobases, and protein structures that are based on just 20 amino acid residues. The rapid turnover and degradation of metabolites during sample preparation is an additional challenge facing the quantitative measurement of metabolite concentrations. The wide dynamic range of metabolites abundances results in the suppression of the signals from low-level species. However, the use of metabolomics is a must for the discovery of some MoA, e.g., in the case of statins which inhibit the cholesterol biosynthesis pathway, proteomics and transcriptomics data pointed to the activation of this pathway which was in fact a feedback effect of the inhibition of HMG-CoA reductase (**Figure 2**). In the case of statins, only metabolomics analysis was able to accurately capture the MoA. Therefore, although metabolomics is challenging and still an emerging method compared to proteomics and transcriptomics, in some cases it is the only method that can reveal the MoA.

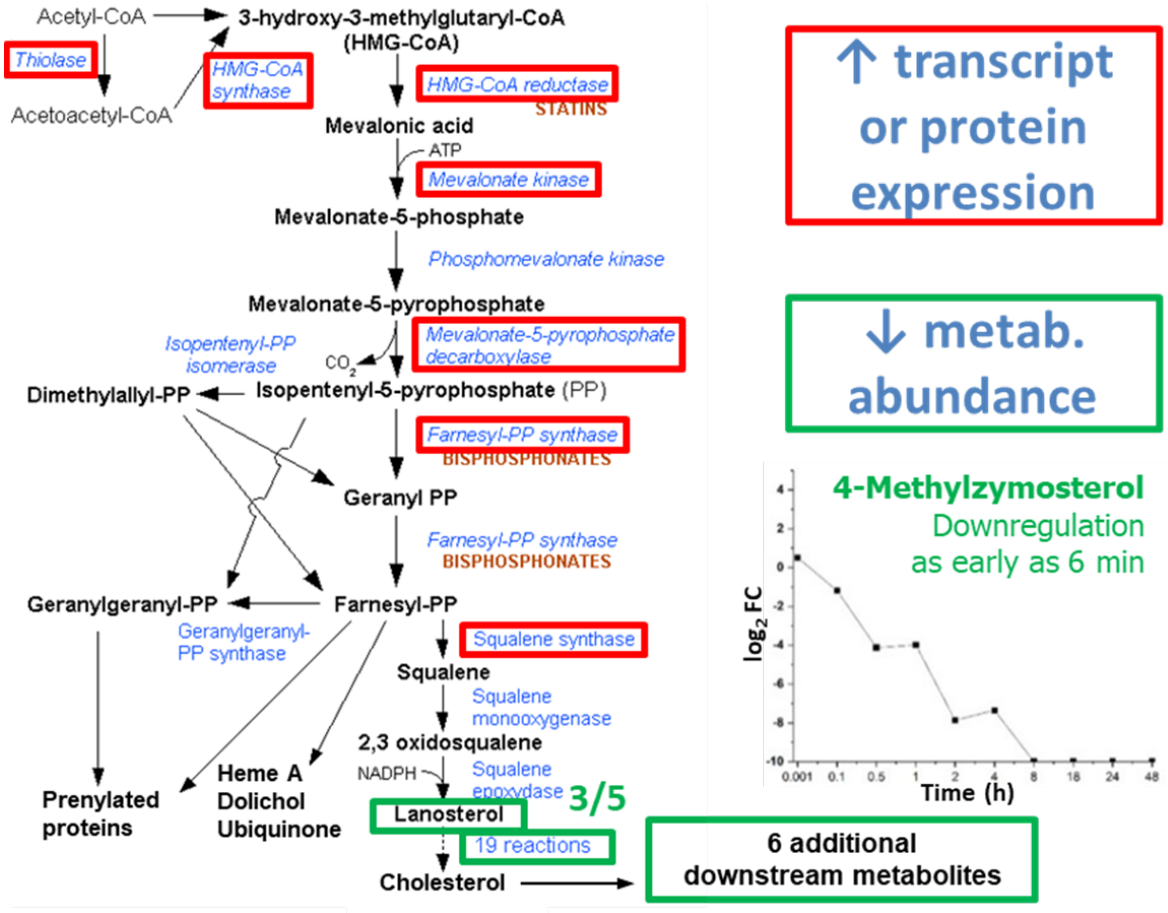

Figure 2: Proteins in cholesterol biosynthesis pathway are all upregulated in response to atorvastatin treatment, whereas metabolites are downregulated. In this case, metabolites correctly identified the MoA of atorvastatin, whereas proteins abundances were controlled by a feedback loop caused by the inhibition of HMG-CoA reductase.

3. One Size Does Not Fit All

There is no single solution to approach the complexity of identifying the MoA for all possible challenge agents. In the case of nocodazole and bendamustine, IPA succeeded at identifying the MoA. Yet in other cases (e.g., Nexturastat A), our bioinformatics approach based on the correlation of time profiles was the most successful at identifying the time-resolved MoA. In other cases (e.g., atorvastatin), none of the explored bioinformatics tools succeeded at identifying the MoA and we had to adopt a heuristic approach to identify the MoA based on mathematical clustering of the biomolecules followed by manual assignment of the clusters to events up-stream or downstream from the target.

4. A Bioinformatics Tool Understandable by Non-informaticians

Bioinformatics tools are important to extract biological information from large datasets. When knowledge-based tools are not able to pinpoint to potential biological processes, a hybrid approach based on de novo network mapping followed by network partitioning and Gene Set Enrichment Analysis of each cluster may be successful at identifying the MoA. This approach is illustrated in **Figure 3**.

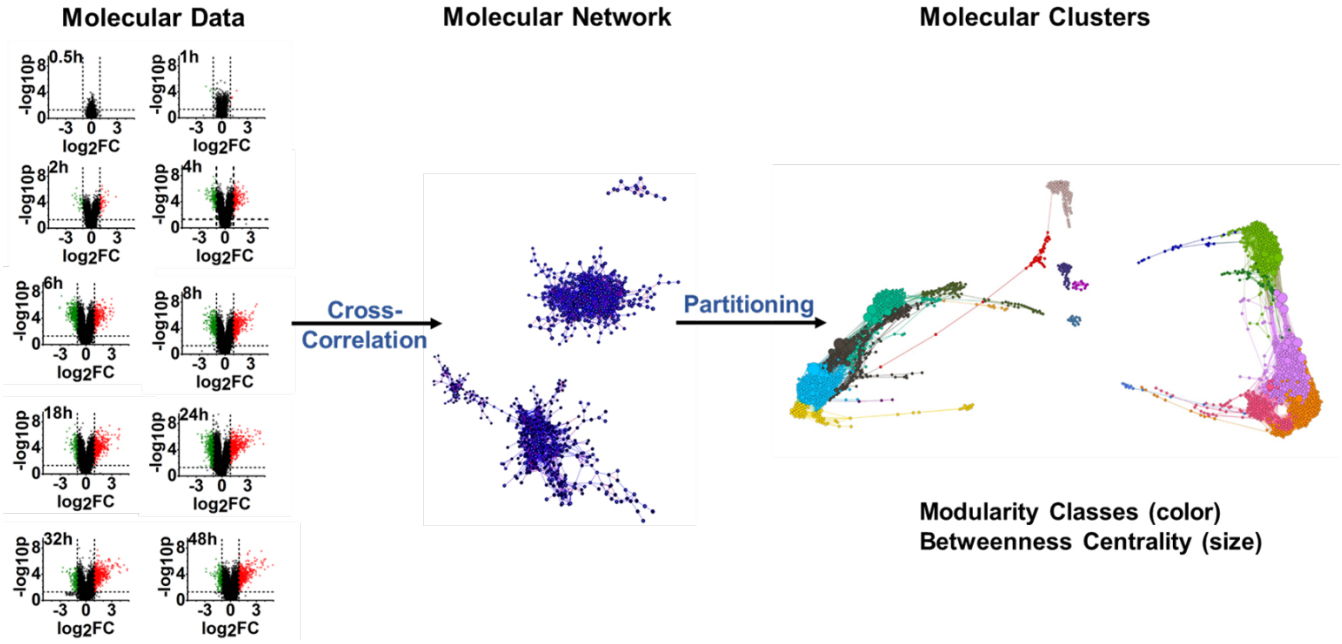

Figure 3: De novo bioinformatics approach using data filtration, network mapping based on cross-correlation of time-profiles, Markov clustering, and partitioning based on modularity classes.

We applied this approach successfully to identify the time-resolved events that constitute the MoA of Nexturastat A (**Figure 4**).

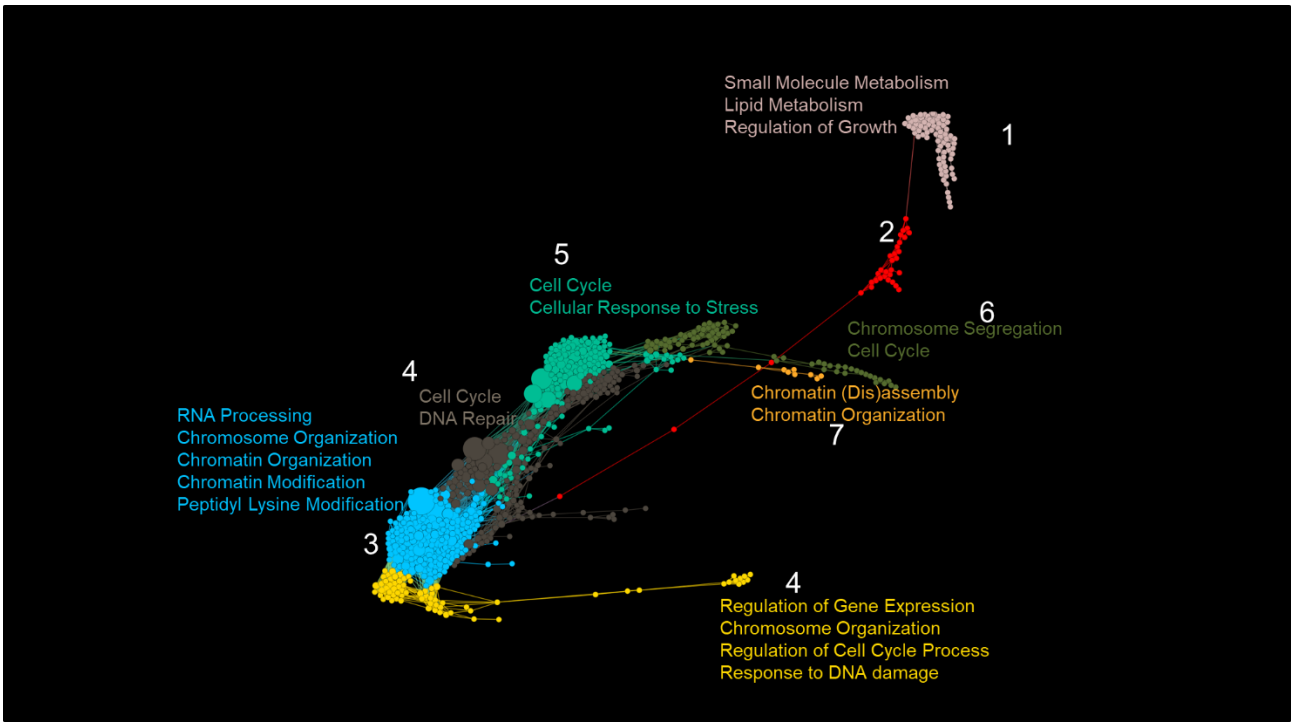

Figure 4: Network generated for Nexturastat A exhibited highest number of regulated biomolecules among all tested challenge agents consisting of 1,880 nodes and >20,000 edges. Morphological observations of the exposed cells revealed the regulation of cell growth and accumulation of lipids in the cells. These two biological processes appeared in Modularity Class 1 along with small molecule metabolism. Going from Class 1 to Class 7, we were able to trace the MoA and the biological events induced by Nexturastat A.

5. Confirmatory Assays

Bioinformatics tools can be successful at pinpointing a number of potential biological events, which might be induced by the challenge agent. Once these are identified, targeted and orthogonal biological assays are crucial to validate the MoA. The example illustrated in **Figure 5** confirms the MoA of bendamustine, a nitrogen mustard agent.

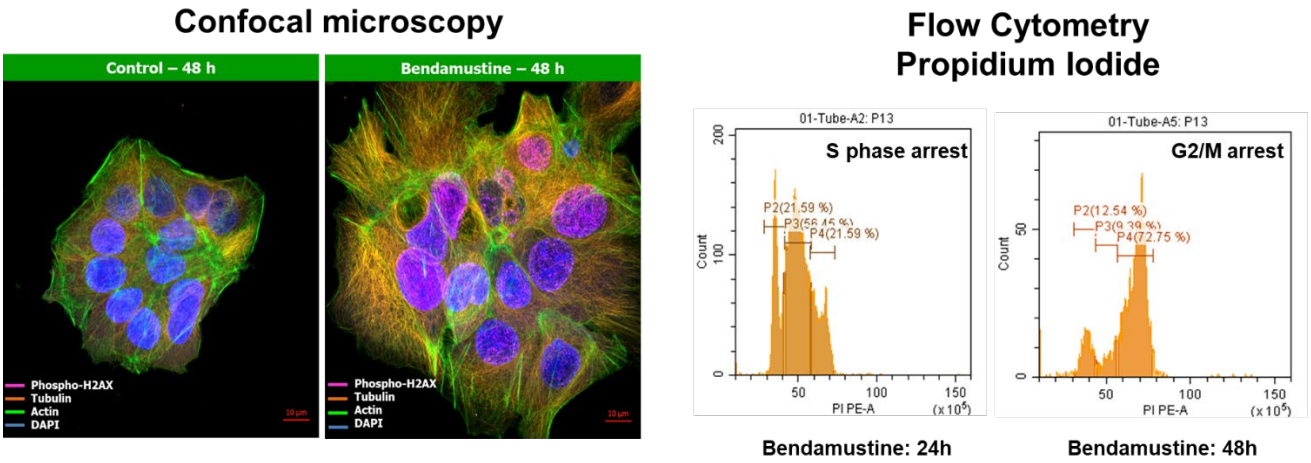

Figure 5: (left) Confirmatory assays were performed to validate DNA damage as demonstrated by increase in phosphorylated histone 2AX (orange signal) after 48 h of exposure to bendamustine. Cell cycle arrest is confirmed by the bigger cell morphology in the treated versus control and by tubulin and DAPI staining which show no evidence for entry into mitosis. (right) To identify the phase of the cell cycle arrest, propidium iodide assay showed an increase in cell population in the S phase at 24 h and an increase in G2 cells at 48 h indicating a G2/M phase arrest following 48 h of bendamustine exposure.

6. Future Directions: Countermeasures

Identification of the MoA might help with the mitigation of the harm caused by the challenge agent. One of the approaches that can be utilized here is to target or protect the critical nodes identified as central in the mapped MoA. Controlling the nodes that exhibit the highest betweenness centrality might allow to block the sequence of events caused by the challenge agent.