Final Scientific/Technical Report

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Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA):
Component 5: Imaging Protein Conformations, Shapes &
Assemblies in Solution & Administration project

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A final report including a detailed summary of accomplishments during the period of this grant, and publications resulting from research supported by the Department of Energy.

I. Executive Summary

We set ambitious goals to examine microorganism communities and measure both their chemical input and output as a read out of specific biochemical activity. These scientific goals are driving the development of sophisticated algorithms to analyze large amounts of experimental measurements made using high throughput technologies to explain and predict how the environment influences biological function at multiple scales and how the microbial systems in tum modify the environment. By examining how bacteria communities rely on symbiotic metabolic relationships for survival and reproductive success, and how these relationships consequently affect their biochemical capabilities. This was accomplished using state-of-the-art mass spectrometry-based methods, and metabolic fingerprinting approaches with a high degree of chemical specificity and sensitivity. During this period the original effort transitioned and was consolidated into what is now ENIGMA, the efforts on technology development expanded to include more untargeted metabolomics and its application to organisms on a systems level.

Towards this end we have made substantial progress towards understanding gaining insight into metabolic potential of model species (Desulfovibrio alaskensis G20, Desulfovibrio vulgaris RCHI) by studying molecular mechanisms that underlie the response to different environmental stressors (e.g. salt stress, metal stress), growth modes (planktonic vs. biofilm) and conditions (e.g. carbon and nitrogen source variations, electron acceptor limitations). One focus in these studies has been to apply existing and design novel technologies to examine the metabolic changes in both, lipid and central carbon metabolism. In biofilm studies, the untargeted LC/MS metabolite profiling is also coupled to multimodal imaging (SEM and MS Imaging). For example, the combined untargeted metabolite profiling and mutant fitness profiling provided a link between genes encoding metabolic enzymes and metabolite production in G20, as an adaptation to high salt environment. The specific gene association to unknown metabolite facilitated the putative identification of modified amino acid (N-acetyl-lysine), produced as an osmolyte.

Another primary question addressed was how the metabolism of one cell differs from the metabolism of a cell grown in a community and why the metabolism of a cell in a community would put itself in a metabolic state that is not optimized. By using mass-based technologies that allow us to examine the metabolism of single cells as well as microbial communities, we investigated the hypothesis that a cell alters its comprehensive metabolism to enable the microbial community to thrive, essentially becoming a component of a multicellular network as opposed to being self-sufficient. By using meta-analysis, we also investigated the chemical nature of the signals used for the cells to communicate within the communities.

A variation on the above experiments was to examine the media hosting the community to observe what effect the media has on other communities. The primary purpose helped to identify key signaling molecules. This was accomplished by growing microbes in a combination of different media and performing a multi-sample comparison by using our state-of-the-art meta-analysis software (Nature Protocols 2011, Anat. Chem. 2011).

Key to the success of these experiments was the development of numerous technologies including metabolomic platforms for quantitative QqQ MRM experiments, LC/MS/MS shotgun high throughput protein analysis as well as novel CESI-MS, capillary electrophoresis mass spectrometry. Multiple technology advancements have been developed. These advances include streamlined data processing using a newly designed autonomous metabolomics approach for simultaneous quantification and identification of up to 1000 metabolite matches, multimodal imaging and pathway deconvolution approach by means of untargeted isotope metabolomics. X13CMS for untargeted, isotope-labeled metabolite analysis is also under development as a complement to XCMS, the most popular cloud-based platform in metabolomics with over 47,000 users worldwide. XCMS, the most cited

LC/MS-based data analysis metabolomics approach, incorporates novel nonlinear retention time alignment and feature detection/comparison.

The lab also developed METLIN, the world's largest metabolite and tandem mass spectrometry (and most widely used) database containing over 850,000 molecular standards across multiple organisms, it also represents a data management system designed to assist in a broad array of metabolite research and metabolite identification by providing public access to its repository of current and comprehensive MS/MS metabolite data.

Overall, these metabotyping approaches (targeted and untargeted LC/MS metabolite profiling) combined with cutting-edge metabolomic technologies (isotope-based metabolomics, MS-imaging) and functional genomics (mutant fitness profiling, transcriptomics and proteomic analyses) have been used to identify alterations in the metabolome that result from specific gene mutation or differential gene expression. Using these technology advancements (streamlined data processing, isotope metabolomics, multimodal imaging) and multi-scale systems approach, we characterized known and identified novel genes and metabolites, associated with pathways/processes that play key physiological roles in microorganisms.

Publications, samples and data delivered:

1. Domingo-Almenara, X.; J.R. Montenegro-Burke, C. Guijas, E.L.-W. Majumder, H.P. Benton and G. Siuzdak (2019) Autonomous METLIN-Guided In-Source Fragment Annotation for Untargeted Metabolomics. American Chemical Society Analytical Chemistry. [doi]:10.1021/acs.analchem.8b03126 {PMID}:30681830 PMCID:PMC6637741 OSTI:1777377
2. Krantz, G.P.; K. Lucas, E.L.-W. Majumder, L.T Hoang, R. Avci, G. Siuzdak and M.W. Fields (2019) Bulk phase resource ratio alters carbon steel corrosion rates and endogenously produced extracellular electron transfer mediators in a sulfate-reducing biofilm. Biofouling. [doi]:10.1080/08927014.2019.1646731 {PMID}:31402749 OSTI:1780729
3. Huan, T.; E.M. Forsberg, D. Rinehart, C.H. Johnson, J. Ivanisevic, H.P. Benton, M. Fang, A. Aisporna, B. Hilmers, F.L. Poole, M.P. Thorgersen, M.W.W. Adams, G. Krantz, M.W. Fields, P.D. Robbins, L.J. Niedernhofer, T. Ideker, E.L. Majumder, J.D. Wall, N.J.W. Rattray, R. Goodacre, L.L. Lairson and G. Siuzdak (2017) Systems biology guided by XCMS Online metabolomics. Nature Methods. [doi]:10.1038/nmeth.4260 {PMID}:28448069 PMCID:PMC5933448 OSTI: 1779087
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6. Montenegro-Burke, J.R.; T. Phommavongsay, A.E. Aisporna, T. Huan, D. Rinehart, F.L. Poole, M.P. Thorgersen, M.W.W. Adams, G. Krantz, M.W. Fields, P.D. Robbins, L.J. Niedernhofer, H.P. Benton and G. Siuzdak (2016) Smartphone Analytics: Mobilizing the Lab into the Cloud for Omic-Scale Analyses. Analytical Chemistry. [doi]:10.1021/acs.analchem.6b02676 {PMID}:27560777 PMCID:PMC5054939 OSTI:1324847

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8. Gowda, H.; J. Ivanisevic, C.H. Johnson, M.E. Kurczy, H.P. Benton, D. Rinehart, T. Nguyen, J. Ray, J. Kuehl, B. Arevalo, P.D. Westenskow, J. Wang, A.P. Arkin, A.M. Deutschbauer, G.J. Patti and G. Siuzdak (2014) Interactive XCMS Online: simplifying advanced metabolomic data processing and subsequent statistical analyses. Analytical Chemistry. [doi]:10.1021/ac500734c {PMID}:24934772 PMCID:PMC4215863 OSTI ID: 1788440
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10. Ivanisevic, J.; Z.-J. Zhu, L. Plate, R. Tautenhahn, S. Chen, P.J. O'Brien, C.H. Johnson, M.A. Marletta, G.J. Patti and G. Siuzdak (2013) Toward 'omic scale metabolite profiling: a dual separation-mass spectrometry approach for coverage of lipid and central carbon metabolism. Analytical Chemistry. [doi]:10.1021/ac401140h {PMID}:23781873 PMCID:PMC3761963 OSTI ID: 1788443
11. Patti, G.J.; R. Tautenhahn, D. Rinehart, K. Cho, L.P Shriver, M. Manchester, I. Nikolskiy, C.H. Johnson, N.G. Mahieu and G. Siuzdak (2013) A view from above: cloud plots to visualize global metabolomic data. Analytical Chemistry. [doi]:10.1021/ac3029745 {PMID}:23206250 PMCID:PMC3716252 OSTI ID: 1788445
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17. Tautenhahn R., Patti G.J., Rinehart D., Siuzdak G. (2012) XCMS Online: A web-based platform to process untargeted metabolomic data. http://xcmsonline.scripps.edu) Analytical Chemistry. 84 (11), 5035-5039. DOI: 10.1021/ac300698c, PMID: 22533540, PMCID: PMC3703953 OSTI ID: 1788449
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There were no published conference papers or other public release of results.

Ill. Products Developed

The website links that reflect the results for the project are:

https://xcmsonline.scripps.edu/

https://metlin.scripps.edu/

The project did generate several network references:

a) METLIN
b) XCMS
c) XCMS Online
d) X13CMS
e) isoXCMS
f) isoMETLIN

IV. Training

Former Postdocs

Caroline Johnson	Assistant Professor, Yale
Xavi Domingo	Assistant Professor, Euro-Catalonia
Julijana Ivanisevic	Assistant Professor, Univ. Lausanne
Martin Giera	Associate Professor, Leiden Univ.
Erica Majumder	Assistant Professor, Univ. Wisconsin
Markus Rinschen	Assistant Professor, Aarhus University
Gary Patti	Professor, Washington University
Tao Huan	Assistant Professor, University of British Columbia
Rafa Montengro	Assistant Professor, University of Toronto
Benedikt Warth	Associate Professor, University of Vienna