BIOSYSTEMS DESIGN RESEARCH OPPORTUNITIES

EMSL provides distinctive staff expertise and capabilities for design and analysis of biodesign and synthetic systems. Users can characterize and monitor biological processes with ultra-high resolution mass spectrometry (MS), spatiotemporally resolved omics, flux measurements of dynamic systems, and extensive imaging resources. EMSL integrates theory and simulation with experiment to provide unmatched multimodal analytical capabilities and feedback for development of designed systems, interrogating biological system performance, and applied cell biology.

Omics

https://www.emsl.pnl.gov/emslweb/capabilities/mass-spectrometry
https://www.emsl.pnl.gov/emslweb/capabilities/nmr_epr
https://www.emsl.pnl.gov/emslweb/capabilities/cisa

EMSL scientists and users are advancing the identification and interpretation of molecular signals within and between cells, building understanding and use of pathways for bioproduct formation and stress tolerance. Researchers can use extensive data from these efforts to construct network analyses, as well as to foster a deeper understanding of organism genotype-to-phenotype associations and the fundamental rules that govern cellular life. EMSL houses a suite of measurement capabilities for metametabolomics, metaproteomics, and metatranscriptomics in support of systems biology and multi-omics integration efforts. These include high-end MS instrumentation (i.e. Orbitraps) for quantitative meta-proteomics, and next-generation sequencing systems such as the Ion Proton™ system for massively parallel unbiased sequencing and metatranscriptome analysis of complex tissues and microbial communities, as well as transcriptome analysis of small, spatially defined bacterial or fungal colonies, isolated by laser capture microdissection and other cell isolation approaches. EMSL also offers an ultra-high-performance 21 Tesla Fourier transform ion cyclotron resonance MS – one of only two available worldwide. It has unmatched ability to identify and quantify diverse proteins in fungal secretomes (top-down proteomics), allowing access to the functional capacity of the system and analytes in complex environmental mixtures (i.e., dissolved or soil organic matter).

Top-down Proteomics

Top-down proteomics is aimed at functional characterization of intact proteins from natural or engineered fungal, plant, or microbial systems to understand the effects of post-translational modifications and proteolytic processing events on their activity and stability. In contrast, the traditional bottom-up strategy, which involves the analysis of protein fragments, often destroys critical information about chemical modifications that alter protein structure and function. Similarly, activity-based proteomics enables highly specific tagging of microbial populations that participate in different metabolic/functional roles of interest (e.g., lignocellulose degradation) detected by activity-based probes. Coupling activity-based probes with fluorescent tags allows for imaging of distinct populations with high spatial and chemical resolution, or partitioning using, for example, fluorescence-activated cell sorting for further interrogation using multi-omics. This approach can determine identities and functional potential of distinct subpopulations and their specific roles within consortia. Advanced microfluidic capabilities help users perform omics measurements with limited amounts of material (down to very small populations such as hundreds of microbial cells or a single eukaryotic cell). EMSL has been at the forefront of proteomics measurements at this level of sensitivity, opening new possibilities such as proteomics of an engineered consortia, or a synthetic chassis organism coupled with transcriptome analysis using approaches specifically developed for small amounts of starting RNA. Overall, this suite of capabilities lets EMSL scientists and users test hypotheses about the functional response of microorganisms, microbial consortia, and microbe-by-environment interactions. This work informs complex biological system organization, identifies unique biological features, and addresses bioproduct engineering challenges.

emsl.pnl.gov
Integrated Metabolomics

EMSL offers integrated, state-of-the-art nuclear magnetic resonance (NMR) spectroscopy and MS of various types – ion mobility spectrometry, gas chromatography, and Fourier transform ion cyclotron resonance MS. These approaches provide increased identification coverage of the metabolomes of microbes or microbial consortia, their products, and their environments to identify mechanisms by which metabolites involved in communication and interaction are generated, transported, and sequestered within microbial organisms. Scientists at EMSL and Pacific Northwest National Laboratory couple these measurements with molecular dynamics simulations using the NWChem code (http://www.nwchem-sw.org/index.php/Main_Page) to accurately identify metabolites without the use of chemical standards.

Stable Isotope Probing

Stable isotope probing (SIP) measurements are used to measure key elemental fluxes (e.g., C, N, O) and provide critical insights into microbial processes, including nutrient flow through metabolic pathways, eukaryotic/microbe interactions (e.g., fungi/bacteria), and the ultimate fate of nutrients within a cell population. EMSL offers a suite of SIP measurement technologies for omics (RNA, proteins, metabolites via RNA-Seq, MS, and NMR) and multimodal imaging, as well as software for steady state metabolic flux analysis. Because SIP experiments are inherently temporal in nature, this approach addresses both the spatial and temporal aspects of organism interaction and dynamics.

Dynamic Multimodal Imaging

https://www.emsl.pnl.gov/emslweb/capabilities/microscopy

EMSL offers distinctive capabilities for dynamic multimodal imaging of metabolites, lipids, and proteins in microbes and consortia with (sub)cellular resolution, based on fluorescence and MS-based imaging. Ambient ionization MS imaging approaches facilitate in-situ molecular imaging of plant and microbial cells and organelles to directly map chemical distributions of intact molecular species (metabolites, proteins). The approaches employ liquid extraction surface analysis, laser ablation electrospray ionization, and nano-desorption electrospray ionization with 1-200 micron spatial resolution. Super resolution fluorescence imaging and nanoscale secondary ion mass spectrometry (nanoSIMS), with combinatorial fluorescence in-situ hybridization (FISH), can help quantify expression of multiple genes of interest in individual cells to reveal mechanistic details about elemental flux within fungi and other microbes with submicron resolution. Combined MS, FISH, and nanoSIMS experiments enable linking biota with processes at the submicron scale.

Next-generation RNA Sequencing

RNA-Seq using the SOLiD sequencing systems, together with the Ion Proton™ system for massively parallel unbiased sequencing, enables single-cell transcriptomics of fungal and other eukaryotic cells.

Modeling and Simulation

https://www.emsl.pnl.gov/emslweb/capabilities/computing

The spatial organization of biosignature organisms, communities, and their interactions, can be simulated using individual-based modeling. In such simulations, users can combine the reaction processes of microbes (inferred from genome data) with transport processes (such as diffusion of nutrients) to query the cell-by-cell and cell-by-environment interactions. Depending on the level of detail needed and the computing resources available, each microbe can be represented by reactions that summarize cellular metabolism, or with complete metabolic models. Software such as BioCellion powers these types of discrete, individual-based modeling simulations to run on high-performance computing systems (using the Cascade high-performance computer at EMSL).

Multiscale fungal or other organismal modeling is used for mechanistic understanding of how the environment and genotype shape metabolite flux through compartmentalized cellular space. Moreover, modeling can anticipate the phenotype of a chassis organism and how genotypic information is translated to traits and responses across levels, from molecules to interaction partners to designed systems. A predictive computational model is being developed to describe how genomic and environmental variables inform predictions of traits and responses at different scales, from molecules to cells to biosystems.

For more information about Biosystems Design research opportunities contact Scott Baker 1 (509) 372-4759, scott.baker@pnnl.gov.

For more information about EMSL: www.emsl.pnl.gov