EMSL

Pacific Northwest NATIONAL LABORATORY
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U.S. DEPARTMENT OF ENERGY

Science & Technology Opportunities at EMSL

David W. Koppenaal
Chief Technology Officer

JGI Genomics Technologies Workshop - March 24, 2015

BIOLOGY

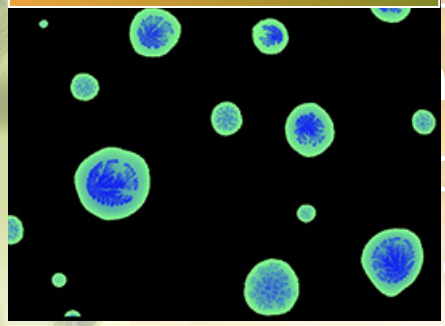
ENVIRONMENT

ENERGY

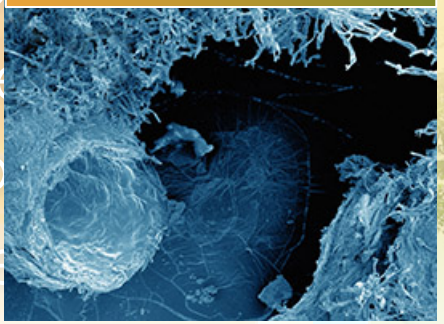
SCIENCE THEMES

Biosystem Dynamics & Design

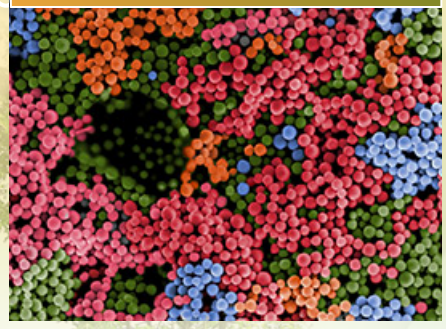
Atmospheric Aerosol Systems



Terrestrial & Subsurface Ecosystems



Energy Materials & Processes

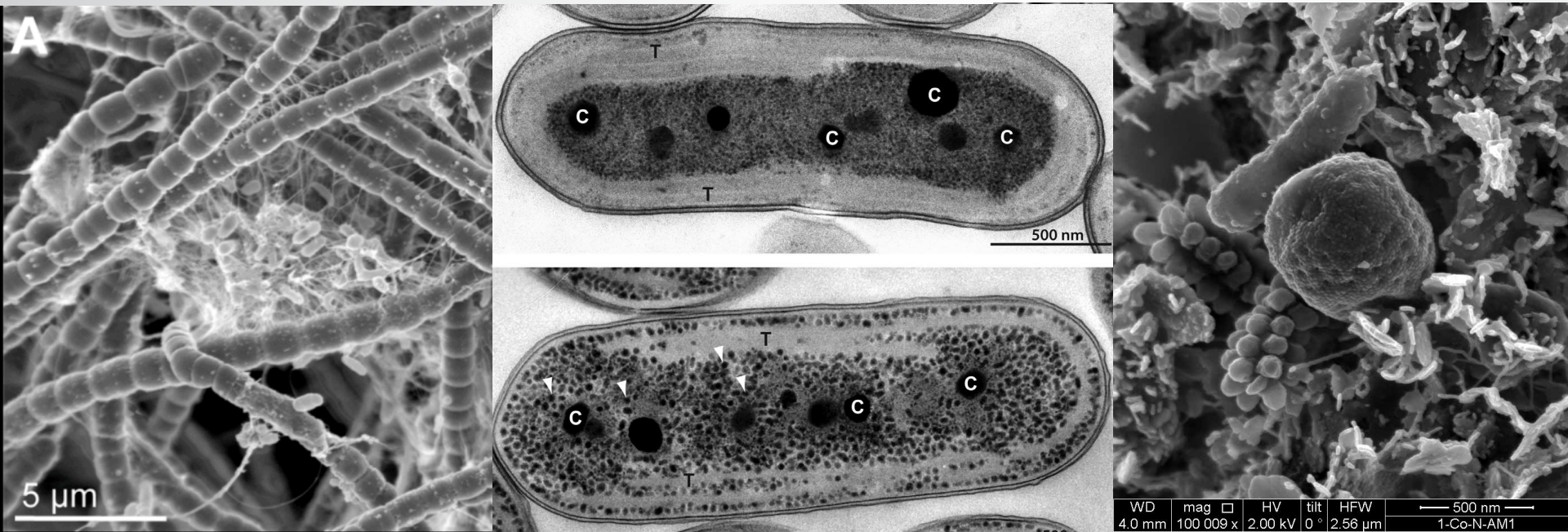


CAPABILITY AREAS

- Mass Spectrometry
- NMR & EPR
- Molecular Science Computing
- Microscopy
- Spectroscopy & Diffraction
- Deposition & Microfabrication
- Subsurface Flow & Transport
- Cell Isolation & Systems Analysis

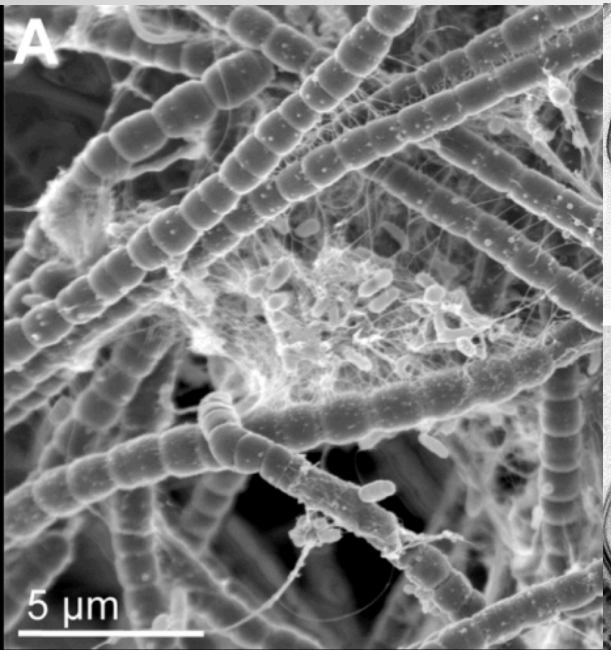
Today's topics

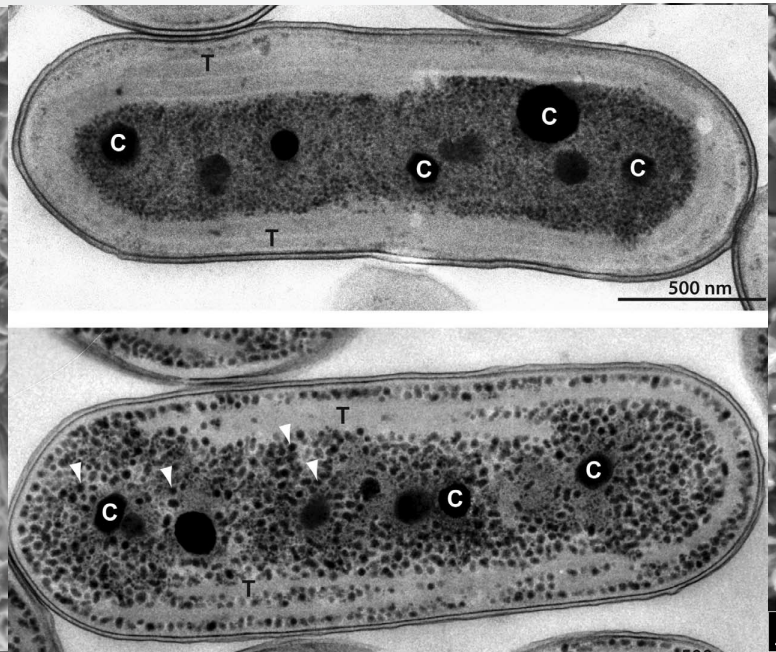
- EMSL-JGI Joint User Call – status and overview
- Science highlights
- New capabilities

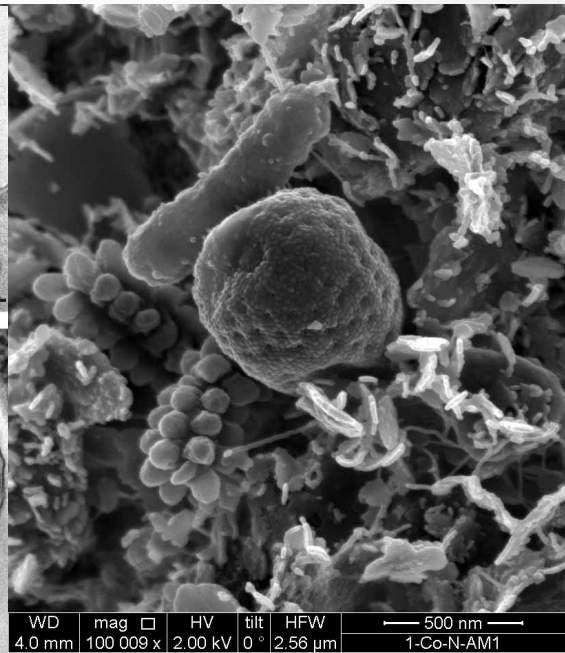


EMSL-JGI Joint Science Calls

- EMSL-JGI Joint Science Call established in 2014 to promote collaborative use
- Of 58 proposals submitted, 20 accepted in 2014-2015
- Focus on bioenergy, terrestrial carbon, microbial dynamics

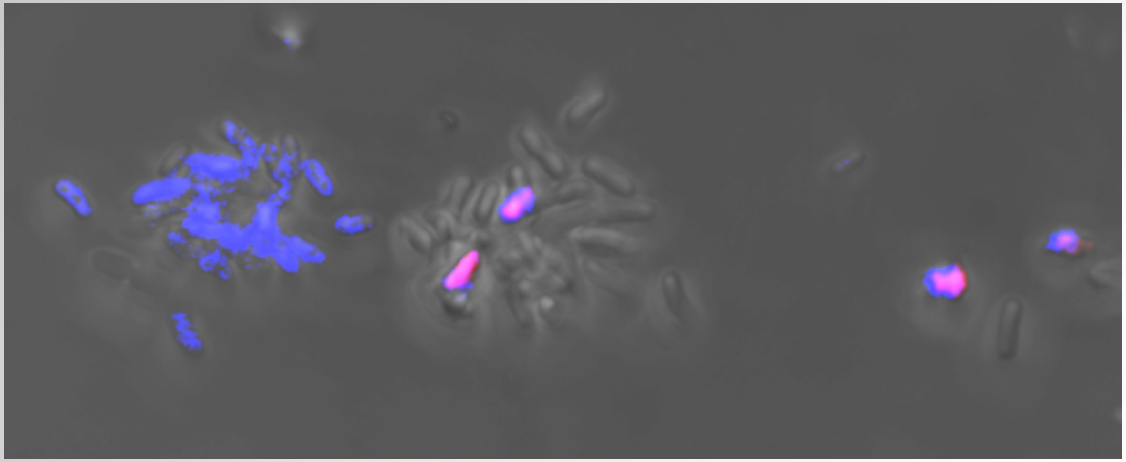


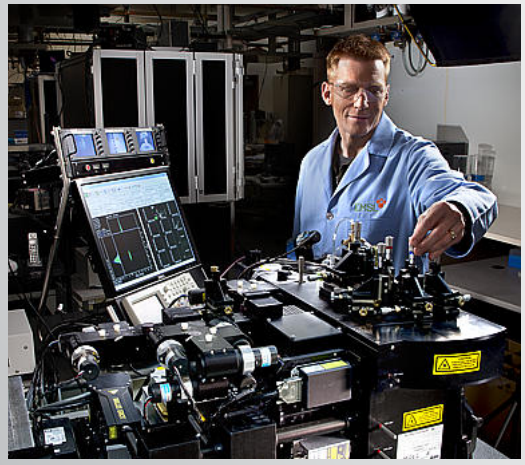




JGI-EMSL Project : Isolate cellulose-assimilating cells from soil microbial communities

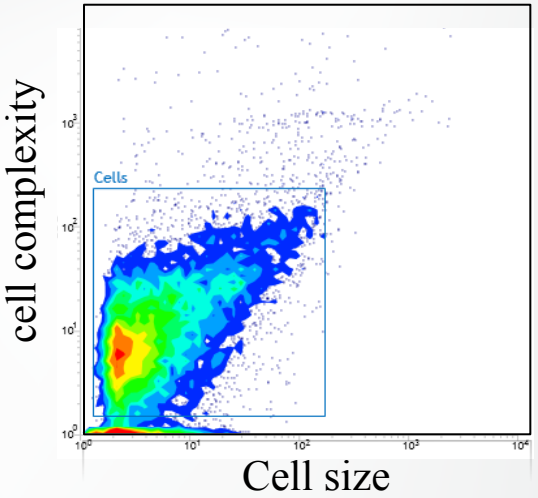
Goal: Identify key organisms involved in soil carbon cycling (e.g. cellulose) and determine their metabolic functions by single cell- and meta-genomic/transcriptomic analyses

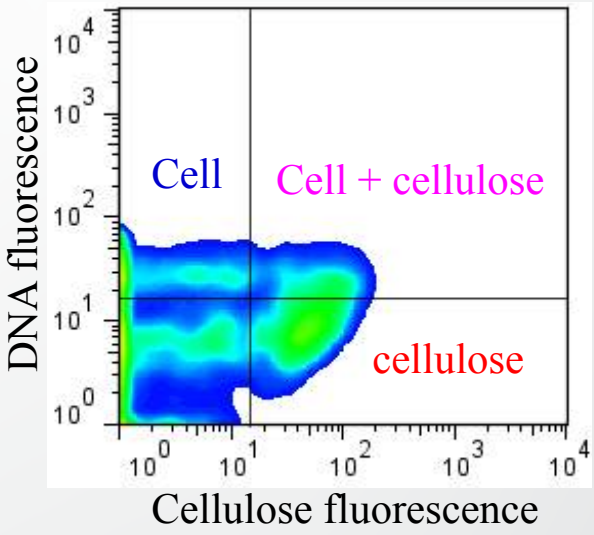




Blue – fluorescent DNA probe
Red – fluorescent cellulose
Magenta – co-localized blue and red

Influx Flow Cytometer



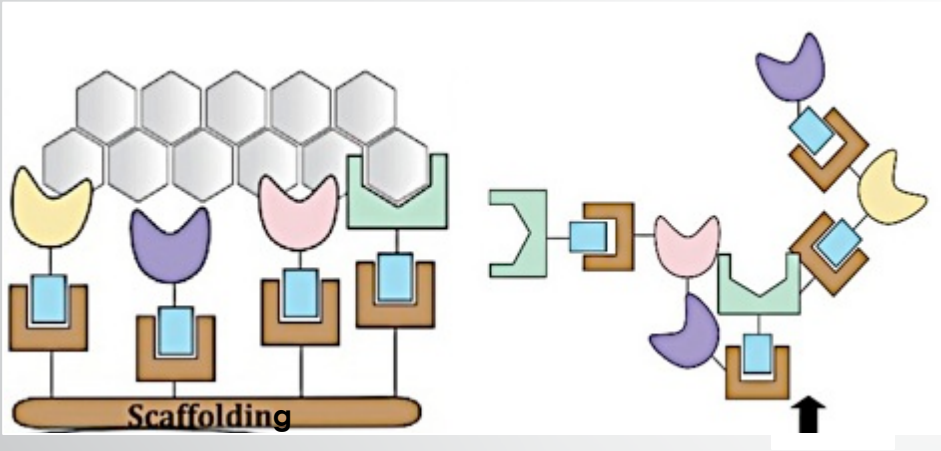


Kirsten Hofmockel, Iowa State University

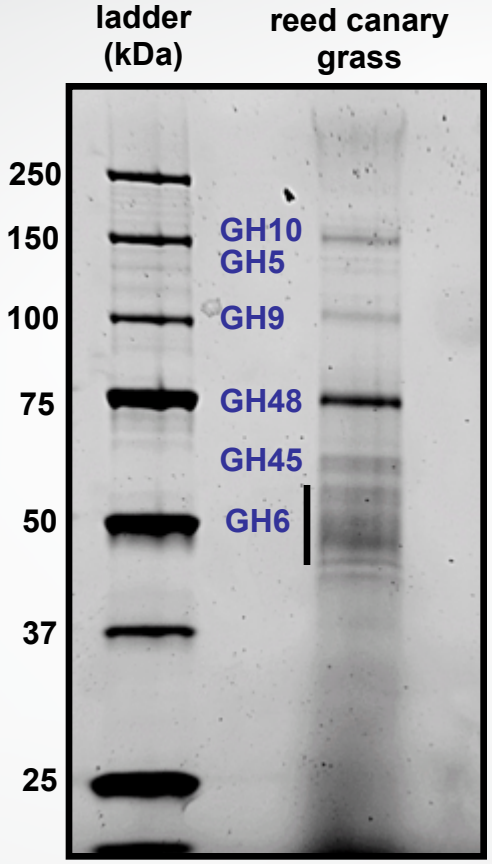
Deciphering the composition of fungal cellulosomes

- Fungal cellulosomes from *Piromyces sp finn* are readily precipitated from anaerobic cultures
- Cellulosome complexes are large, and have 10+ putative subunits

What is the structure/composition of these fungal cellusomes?



Fungal Cellulosome Architecture?

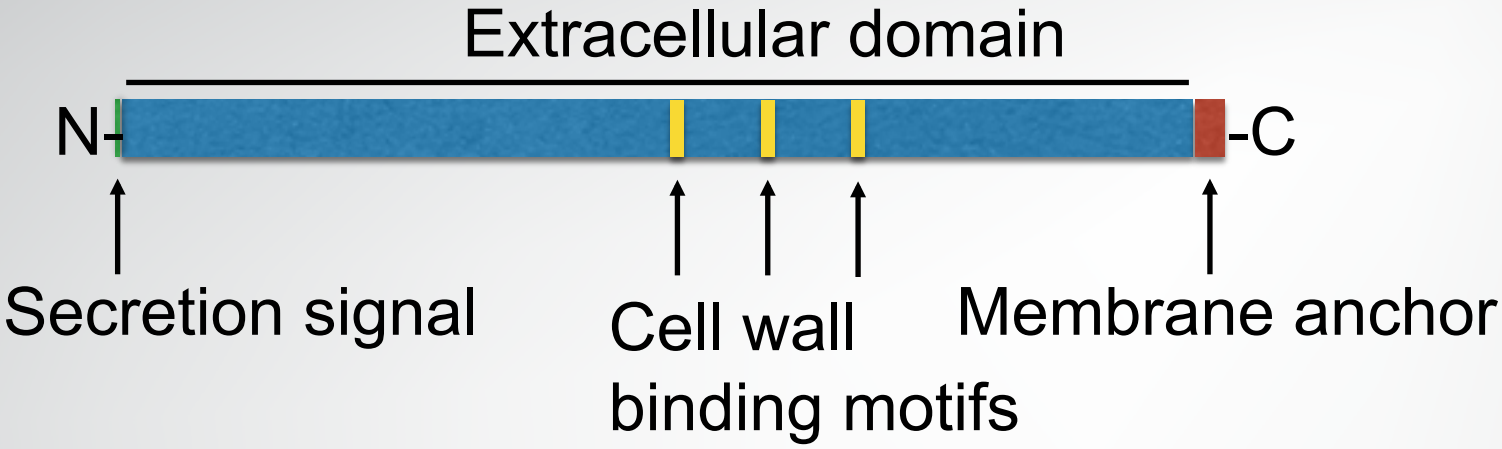


Fungal Cellulosome Components

All proteins were predicted by the transcriptome and contain dockerin domains

Michelle O'Malley, Charles Haitjema, UCSB

Proteomic studies reveal a putative scaffold in gut fungi

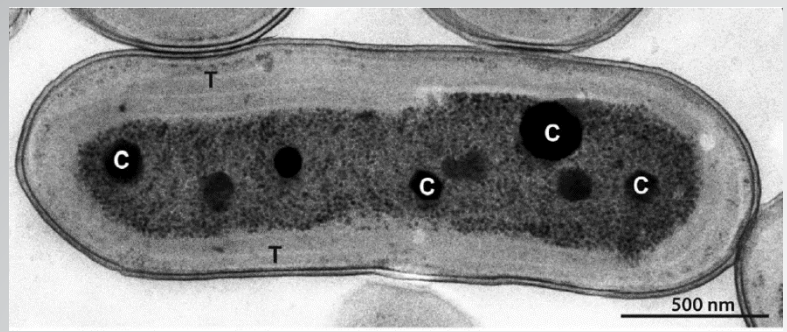


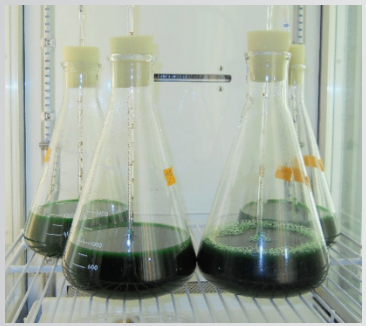
- ✓ ~ 600 kDa protein with interspersed “repeat” motifs
- ✓ Motif is also found in GH48 and GH6
- ✓ Downregulated on glucose
- ✓ Conserved across all gut fungal genera we have isolated and characterized
- ✓ No homology to other sequences in the NCBI database

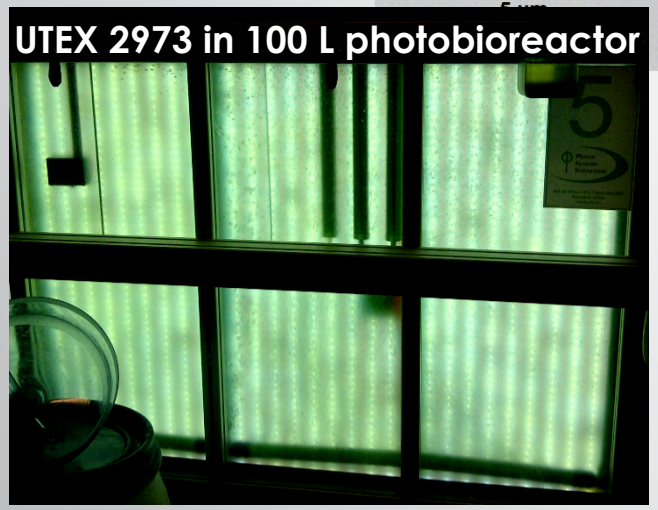
Michelle O’Malley, Charles Haitjema, UCSB *Aaron Wright, Sam Purvine, PNNL*

EMSL Research Campaign: Cyanobacterial Synthetic Biology

C = carboxysome, T = thylakoid membrane







- *Synechococcus elongatus* - UTEX 2973
- Unicellular, oxygenic photosynthetic microbe
- Most rapid growth recorded for a cyanobacterium to date
- Ideal characteristics for synthetic biology chassis

Himadri Pakrasi, WUSTL

Washington University in St. Louis · 1853 · Per Veritatem Vis

UTEX The Culture Collection of Algae

Pacific Northwest NATIONAL LABORATORY

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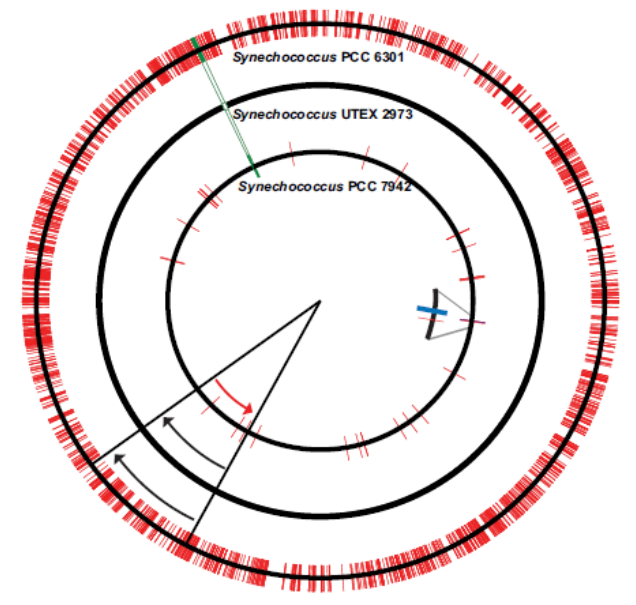
Limited number of genomic changes confers high growth rate for UTEX 2973 compared to PCC 7942

- Closest genomic relative, *Synechococcus elongatus* sp. PCC 7942 was used as comparator strain
- 99.8% sequence identity between UTEX 2973 and PCC 7942
- Yet markedly faster growth…how/why?



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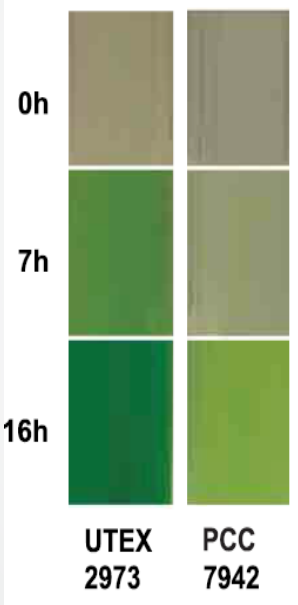
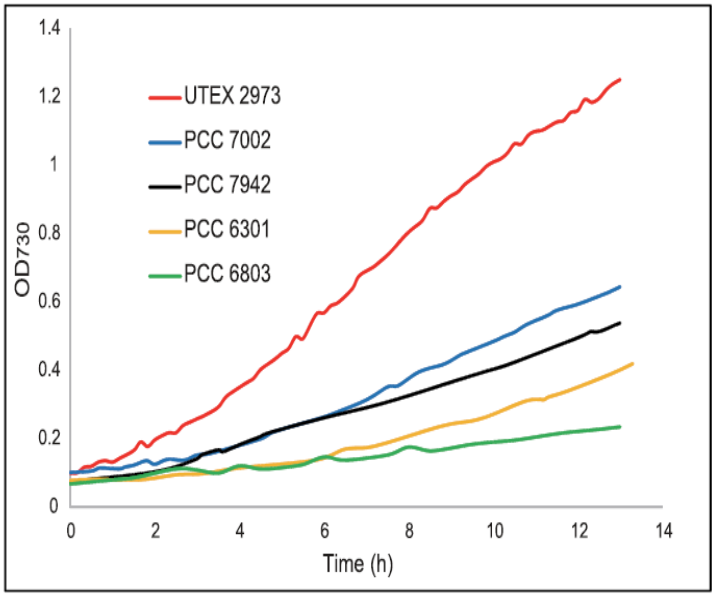


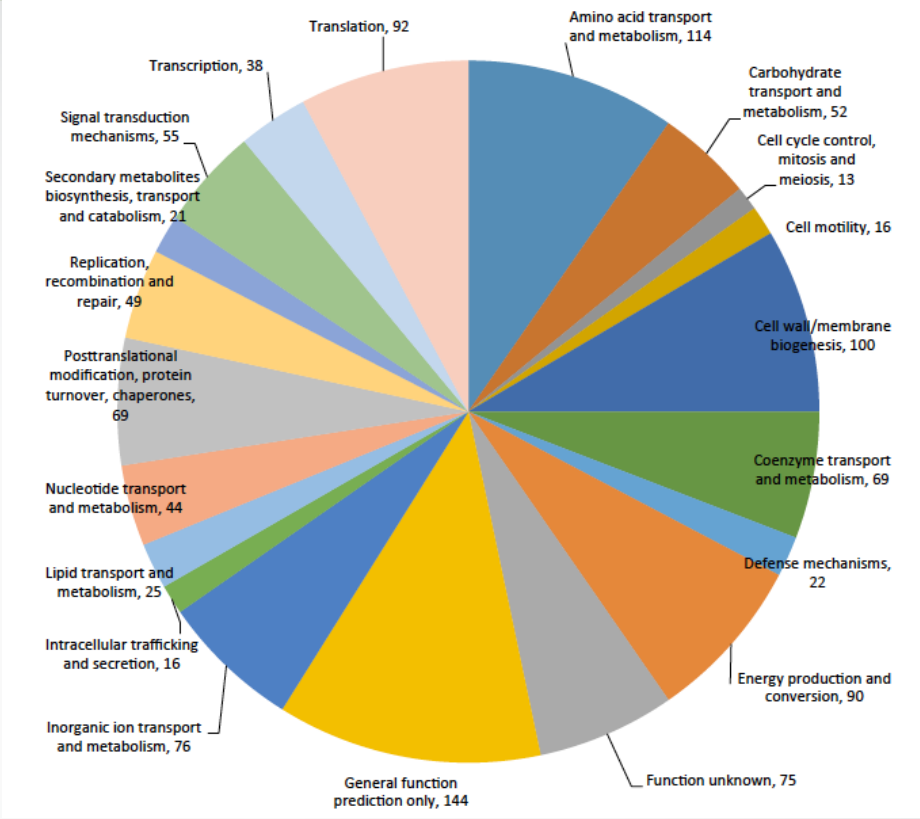


Visual culture densities across 16 hrs; Growth curves of strains at optimum conditions.

Yu, J. *et al.* (2015) *Scientific Reports* 5, doi:10.1038/srep08132

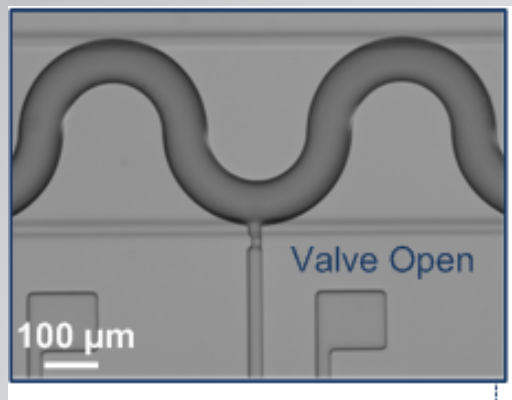
EMSL proteomic capabilities helping to explain

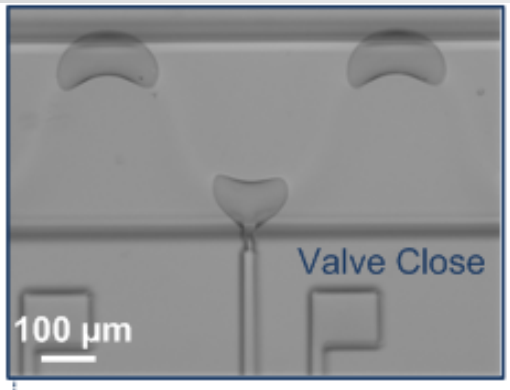
- Proteomics validated specific SNPs, and confirmed key protein abundances
  - 1754 proteins; 66% coverage
  - Peptide sequence validation of 5 detected UTEX 2973 SNPs
  - Quantitative validation of 5 of 6 unannotated genes expressed in PCC 7942 but deleted from UTEX 2973
- Proteomics a vital tool in linking protein expression with physiological observations
- Top-down proteomics can help to decipher regulatory mechanisms behind fast growth

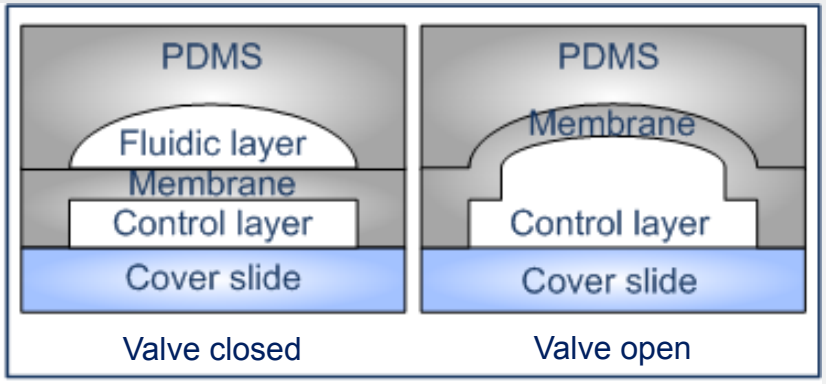


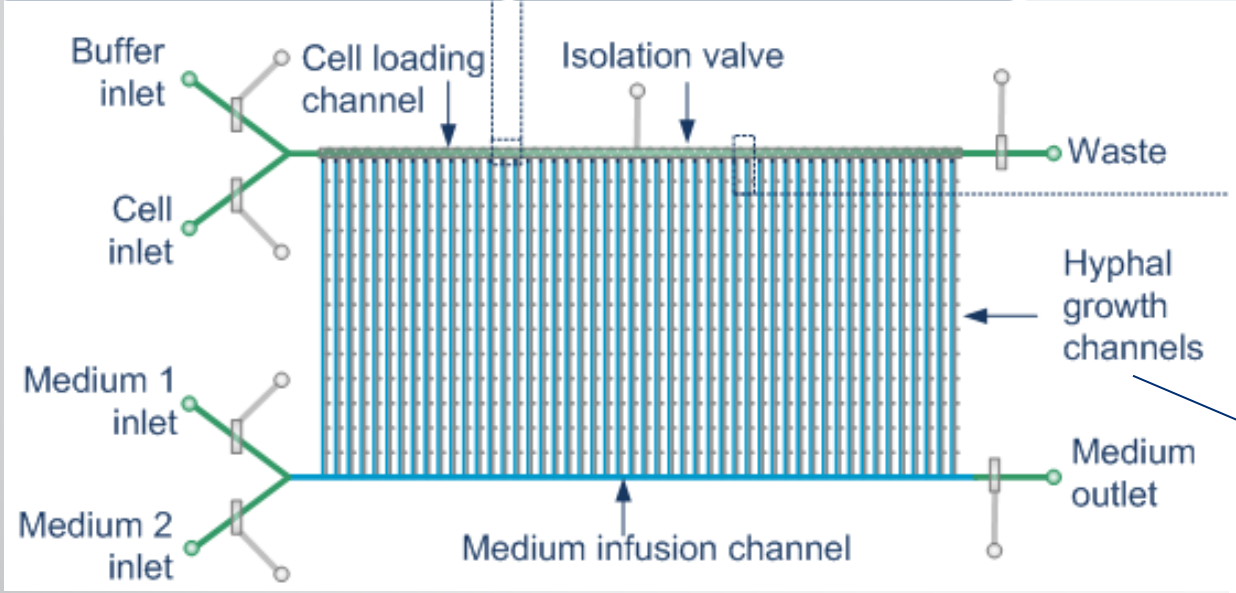
Yu, J. *et al.* 2015

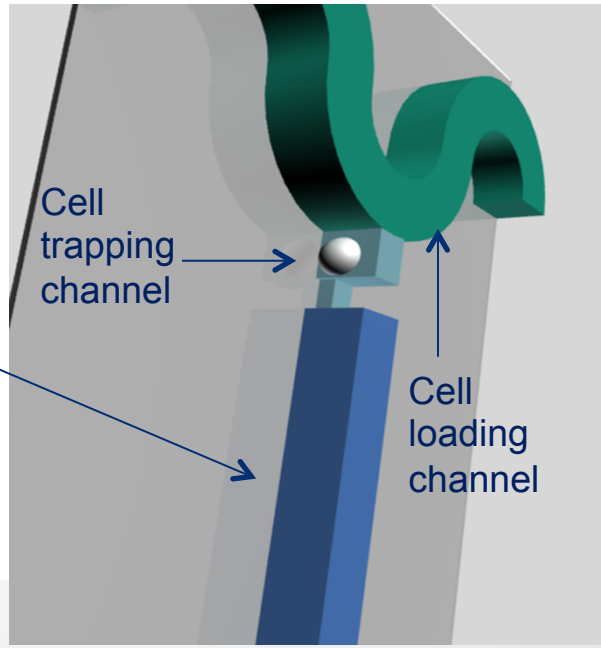
Probing single-cell fungi growth with microfluidic cell culture arrays







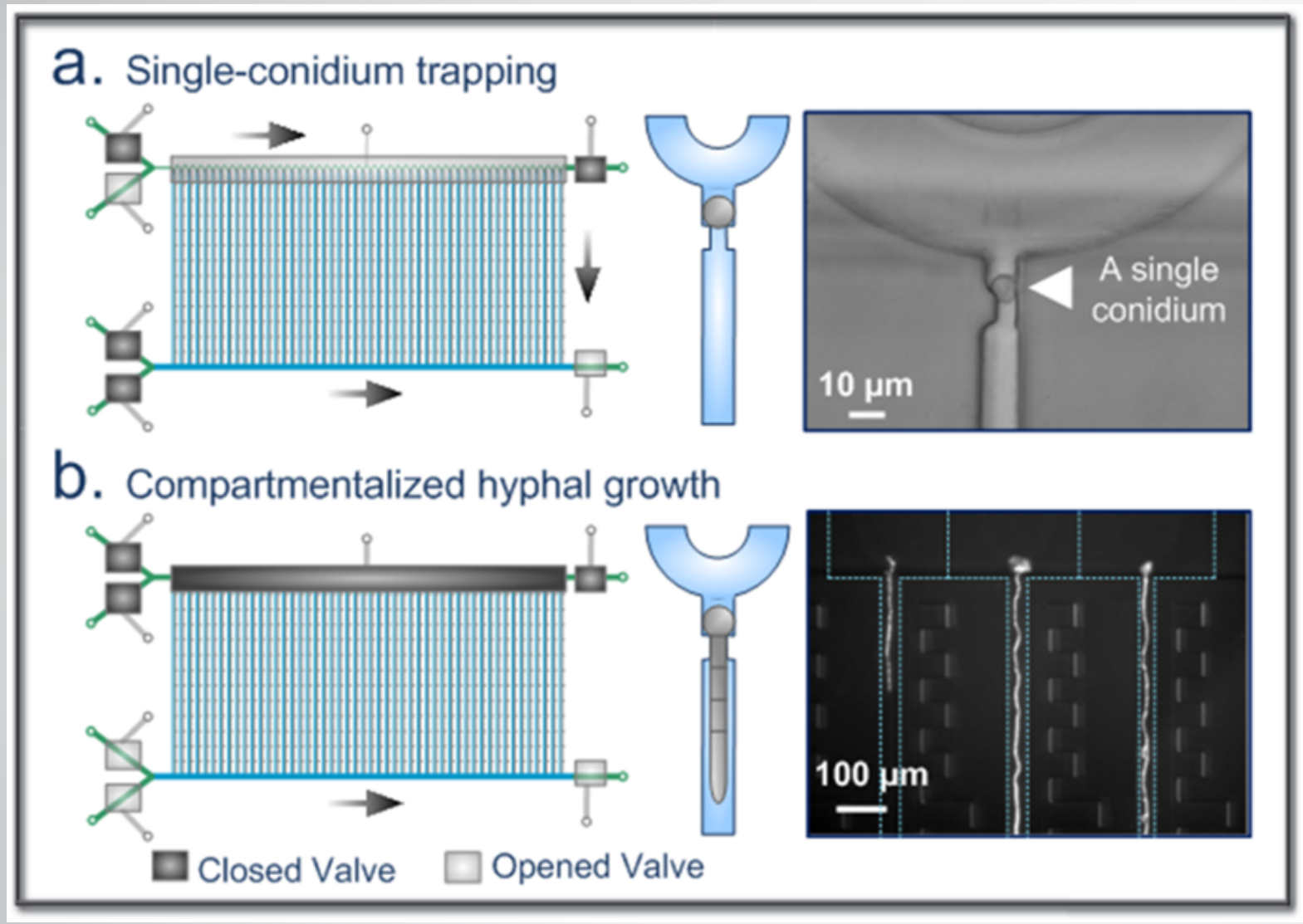




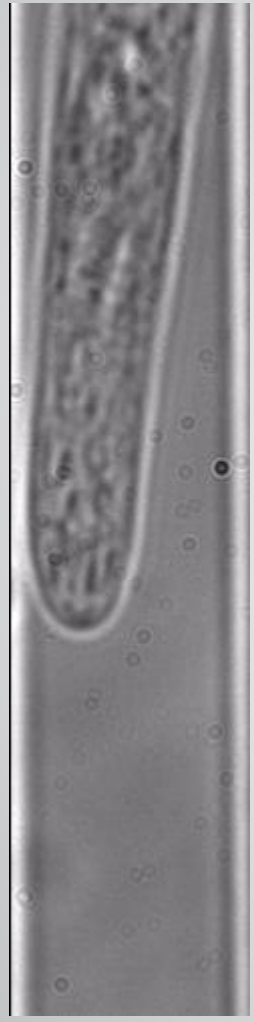
Hyphal growth channel: 2.5 mm X 10 µm X 10 µm

Cell trapping channel: 10 µm X 7 µm X 2 µm

Single-conidium trapping and compartmentalized hyphal growth

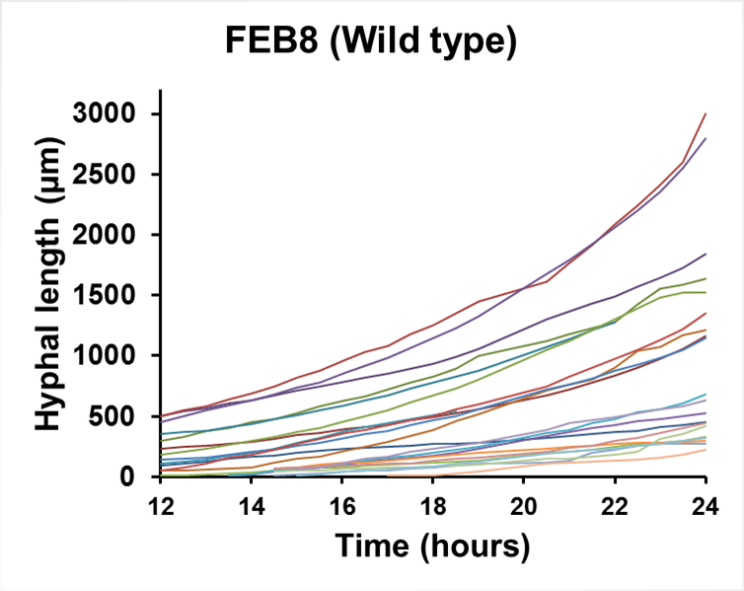


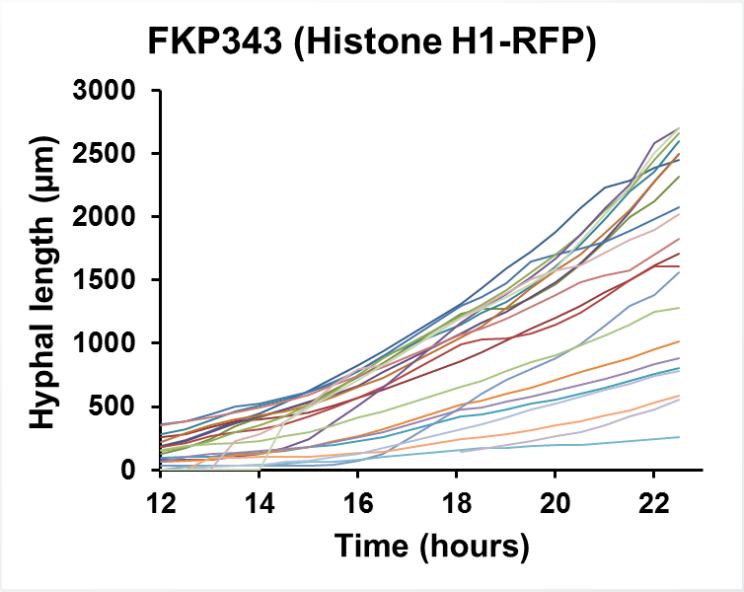
Hyphal growth observation - Neurospora crassa



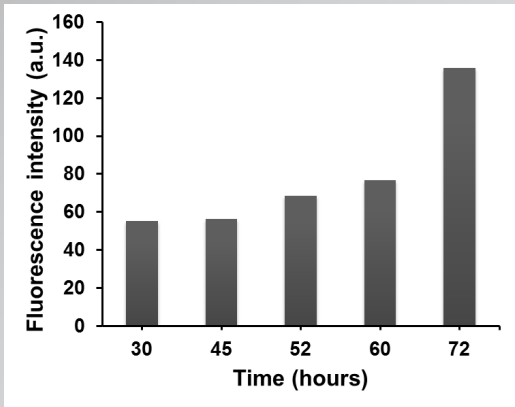
2.5 mm long, 10 µm wide channels

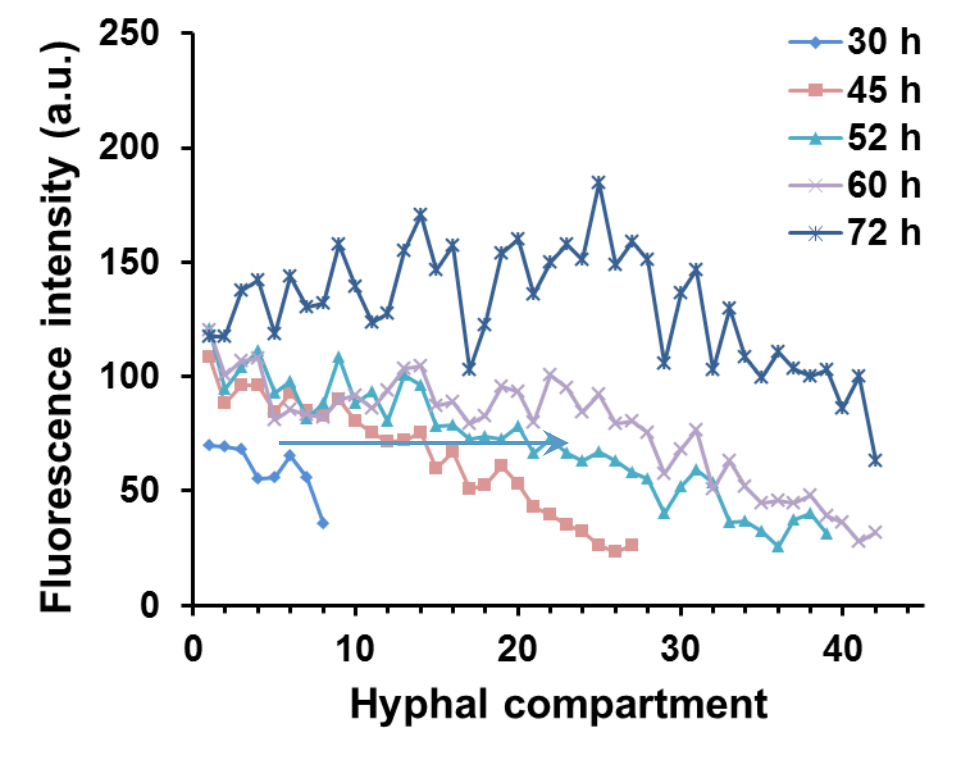
In both strains, single hyphae exhibit distinct germination time and growth rate behavior (cellular heterogeneity).

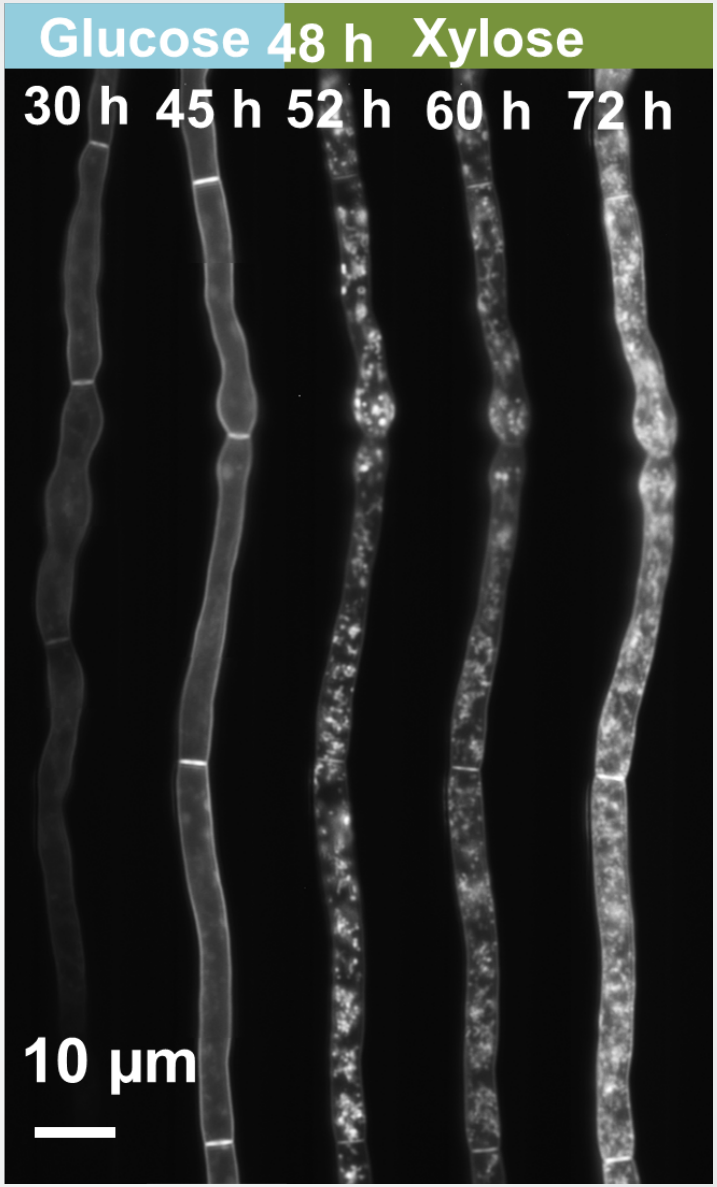




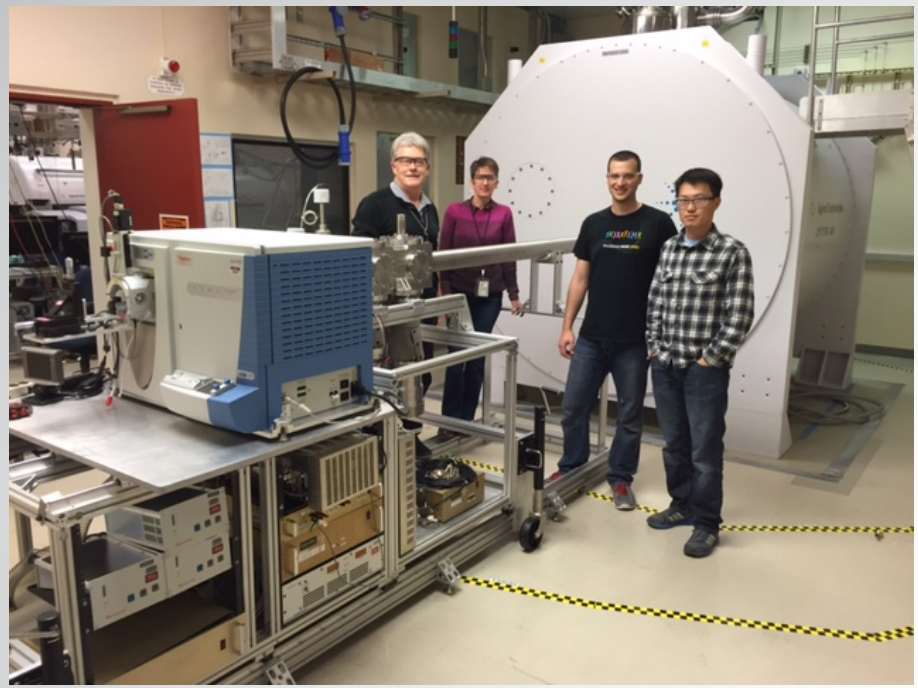
Long-term observation of xylose transporter-GFP expression in response to carbon source change

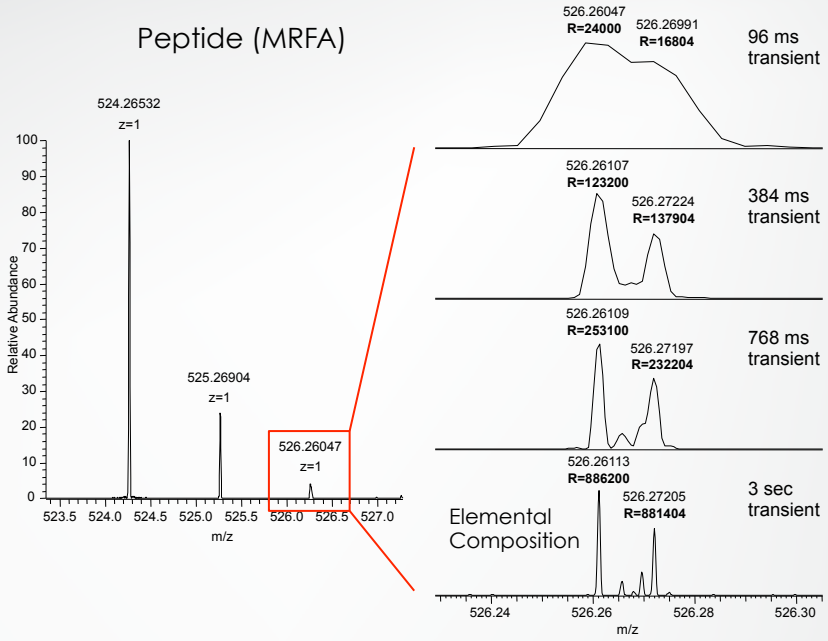






21T FTICR MS: A game changer for omics & MS imaging





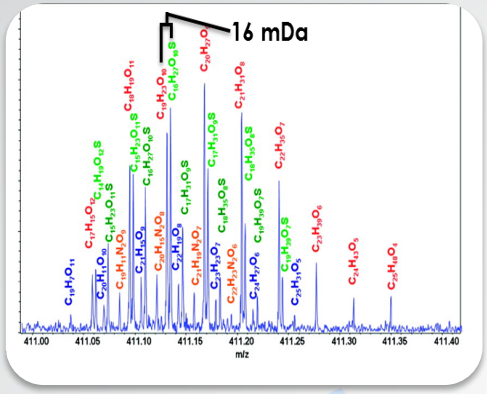
First Spectra!

HRMAC (21T FTICR MS) – A new EMSL capability

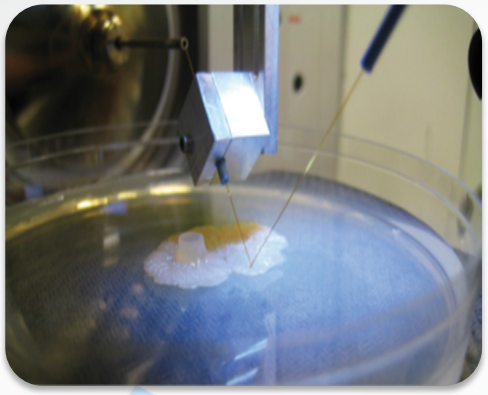
- Ultra-high resolving power and mass accuracy will provide near unequivocal biomolecular species identification
- Improved ability detect & monitor intact level protein transformations
- Increased sensitivity will provide higher imaging resolution (< 1μ)

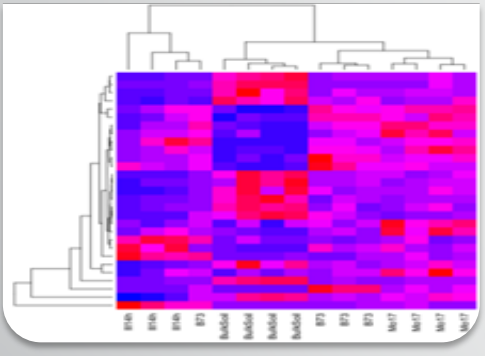
HRMAC will address environmental and biological complexity at the molecular level

Natural Organic Matter Analysis

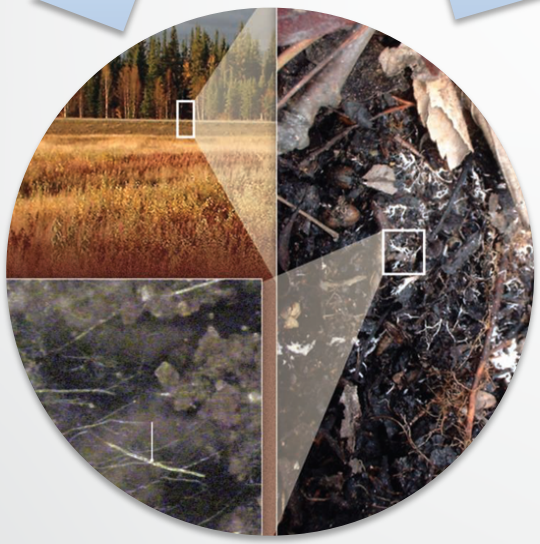


MS Imaging

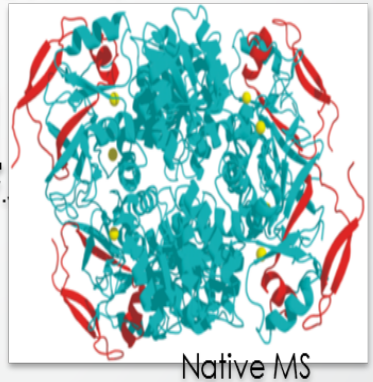




Proteomics, Metabolomics



Top Down MS



Native MS

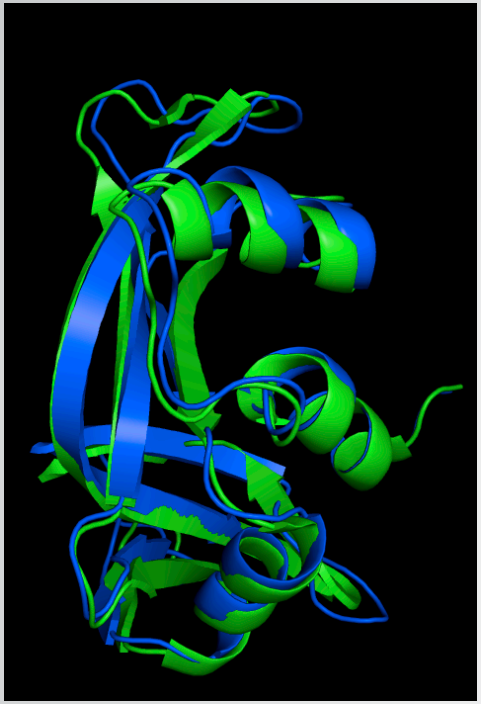
Computing at EMSL

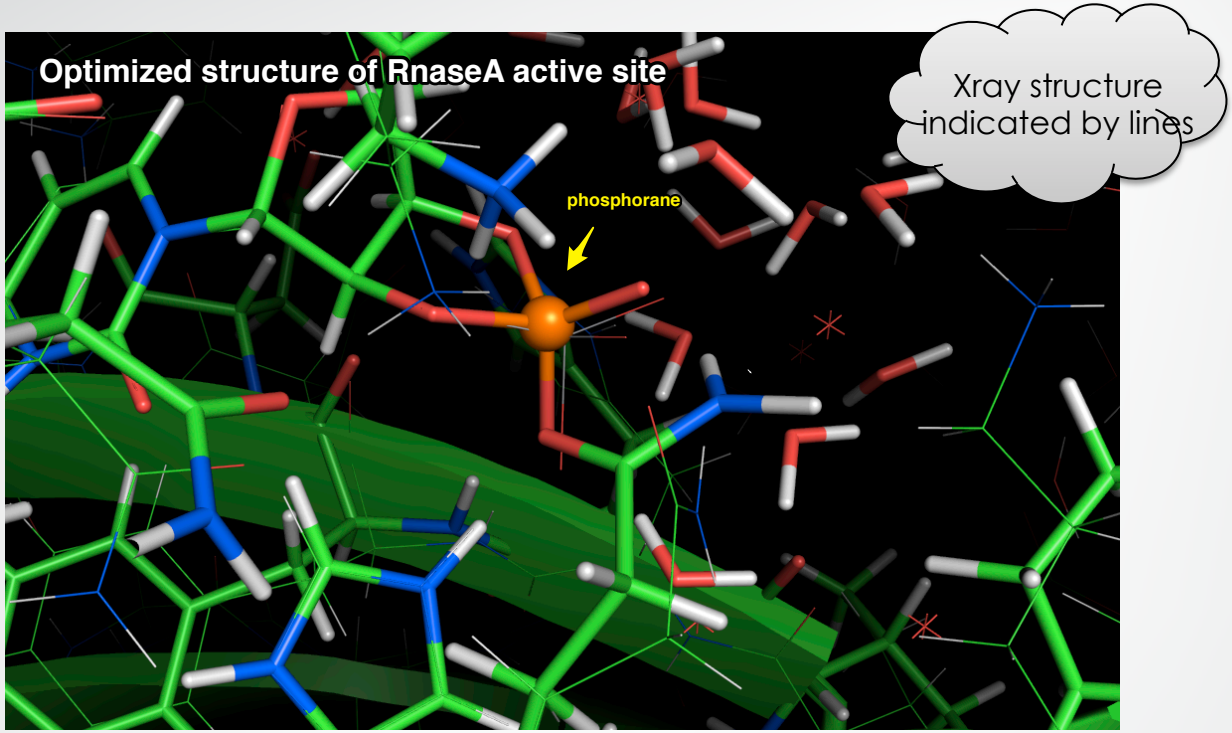
- Computation modeling is an integral part of many research projects
- Molecular Science Computing Facility - integrated production computing environment
  - Chinook supercomputer - 18,480 processor cores (163 teraflops peak performance)
  - Data archive - 7.5 petabytes of storage capacity
  - High performance software development – NWChem
- **Unique environment for integration of experiment and simulation to address complex molecular science problems**

NWCHEM
HIGH-PERFORMANCE COMPUTATIONAL CHEMISTRY SOFTWARE

Computationally aided structural characterization

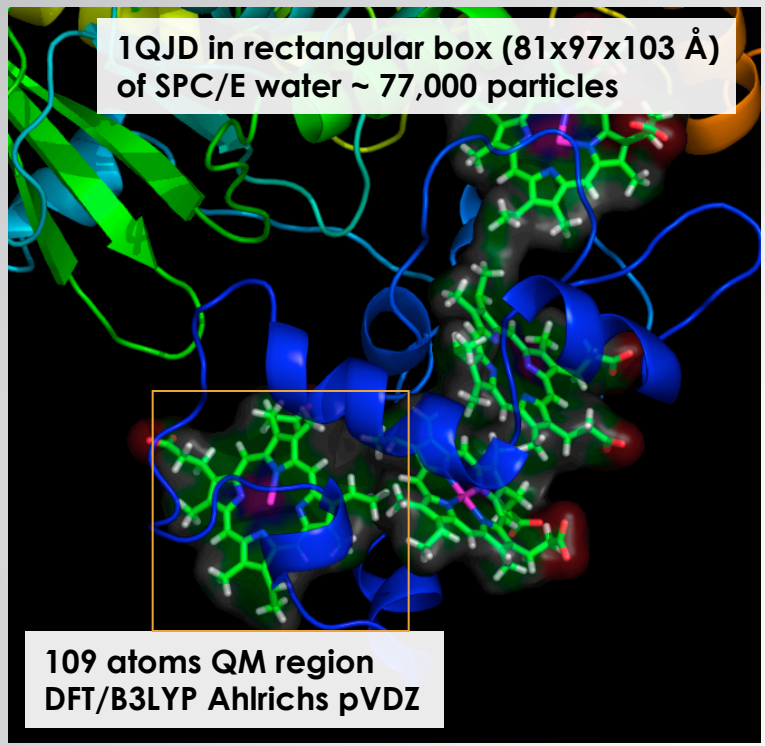
Ribonuclease A (RNase A) protein B. Elsasser, M. Valiev, and J. H. Weare, JACS(2009).



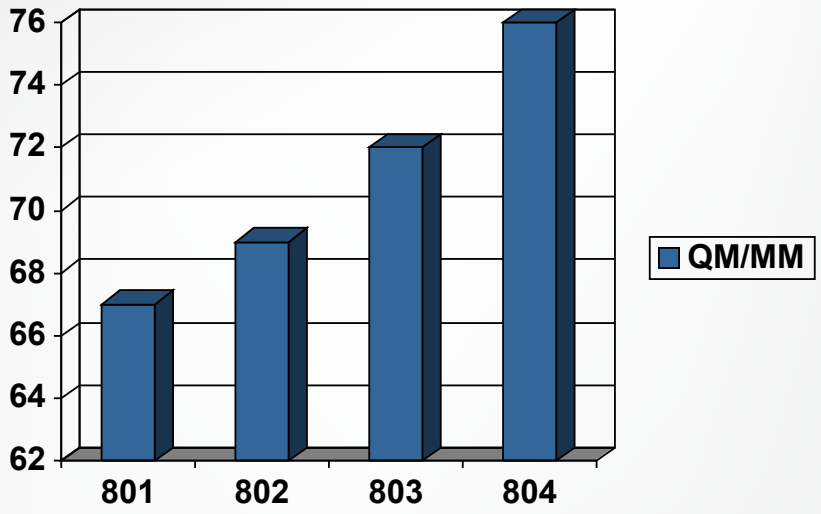


- Starting guess came from 1RUV (RNaseA -uridinium vanadate complex)
- While protein backbone changed little, there are significant changes in the active site

Calculation of Redox Potentials



Redox Potential of heme groups (kcal/mol)



- Important for bioremediation efforts (metal-ion reduction)
- Four iron heme groups facilitate electron transfer

EMSL combines multiple approaches and high-performance computing for complex molecular science studies

What we offer:

- Expert staff
- Specialized facilities
- Unique instrumentation
- Science opportunity

Call for Proposals Open:

- Science Theme research
  - Opens in December/January
- JGI-EMSL Collaborative Science research
  - Letters of intent due *April 6*

http://www.emsl.pnl.gov

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Questions?

ENVIRONMENTAL MOLECULAR SCIENCES LABORATORY