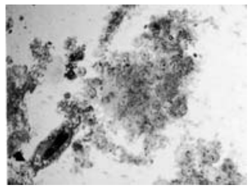
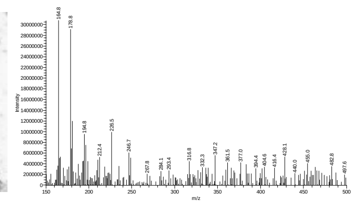
Toward Understanding Dynamic Microbiological Responses to Chemical Stress:
Elucidating Biomarkers for Use in Upset Early Warning Systems





Metropolitan Water Reclamation District of Greater Chicago
June 15, 2007

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Acknowledgements

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Dr. Pedro Mendes, Virginia Bioinformatics Institute
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Dr. Ann Stevens, Biological Sciences, Virginia Tech

WERF
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FOR INNOVATION IN APPLIED WATER QUALITY RESEARCH
THE PAUL L. BUSCH AWARD

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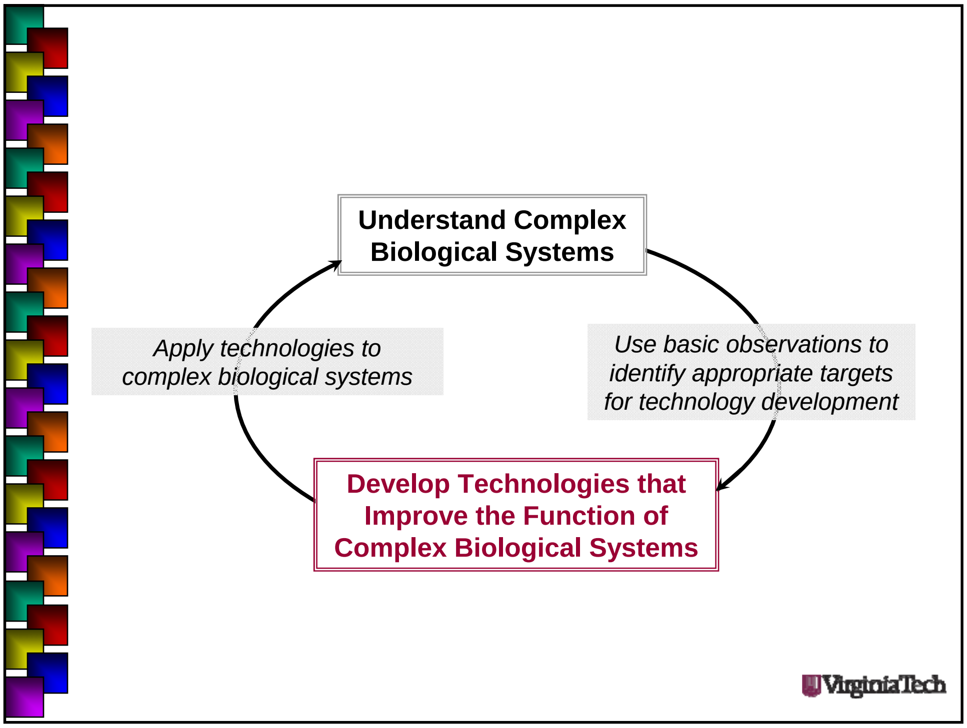
Understand Complex Biological Systems
Use basic observations to identify appropriate targets for technology development
Develop Technologies that Improve the Function of Complex Biological Systems
Apply technologies to complex biological systems
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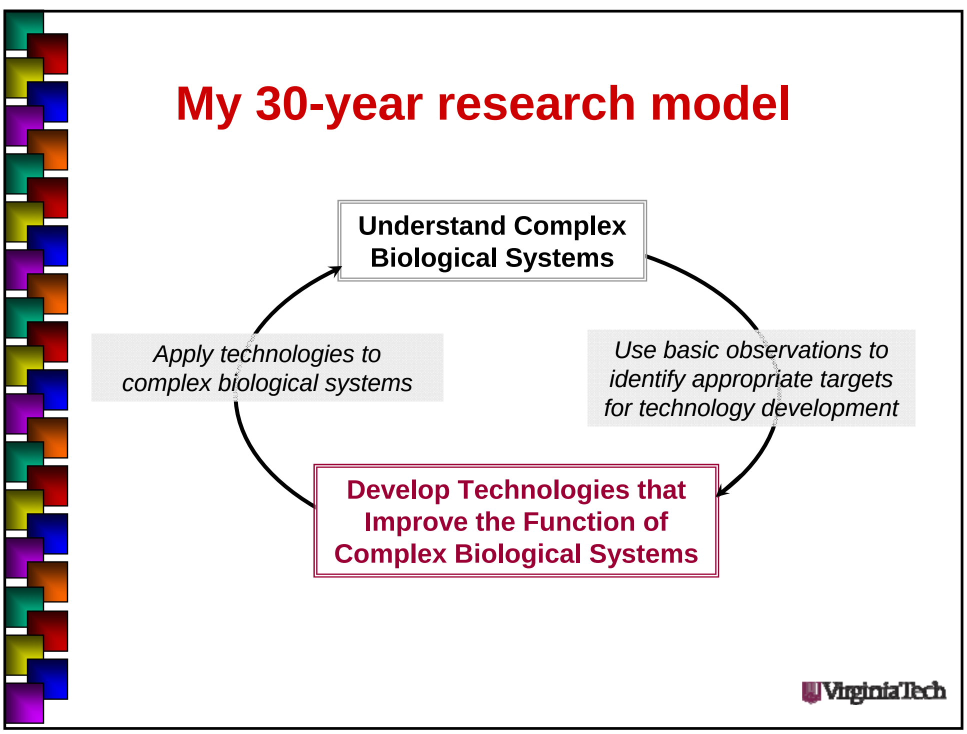
My 30-year research model
Understand Complex Biological Systems
Use basic observations to identify appropriate targets for technology development
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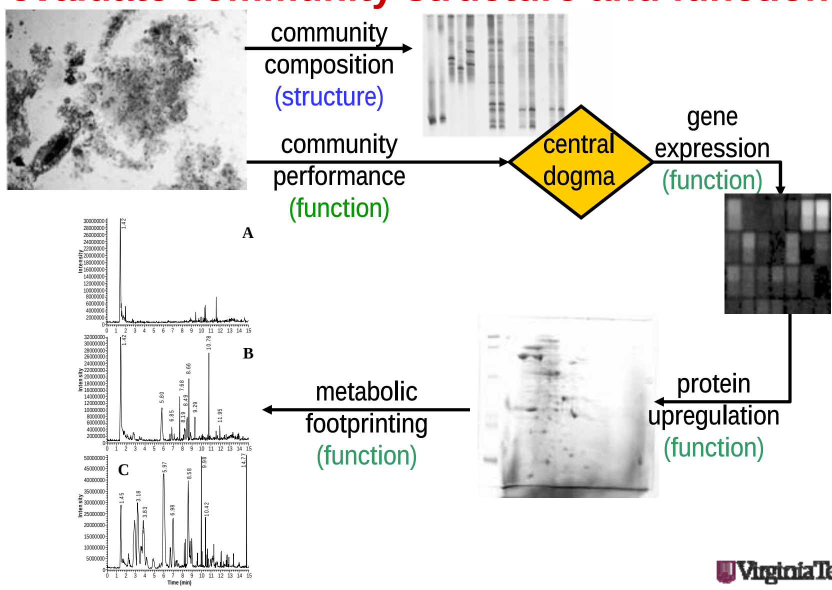
We can use a range of analytical tools to evaluate community structure and function



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Our functional studies have focused on chemical stress of activated sludge systems

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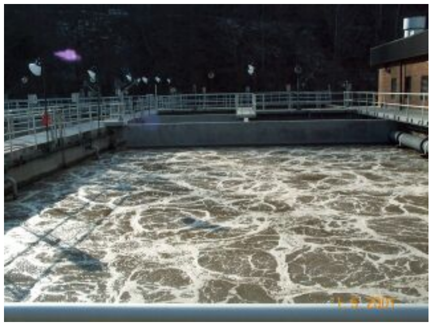

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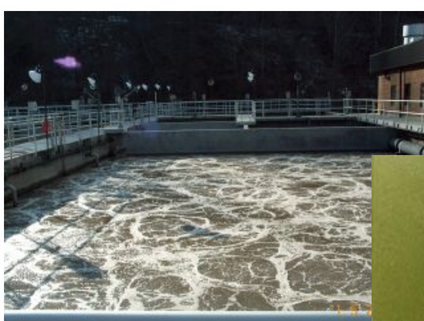

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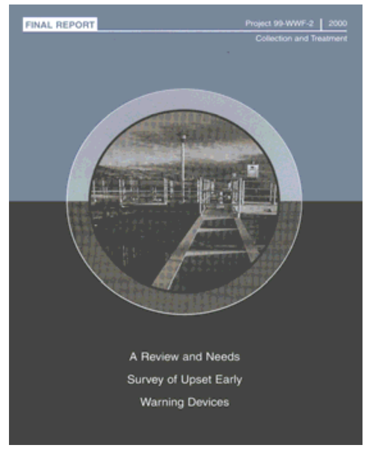
Survey indicates that stressed conditions at plants are common

Over 90% of respondents had experienced process upset.

83% of respondents thought that development of new sensor technologies was important or very important.

"Our monitoring technologies are primitive, at best."



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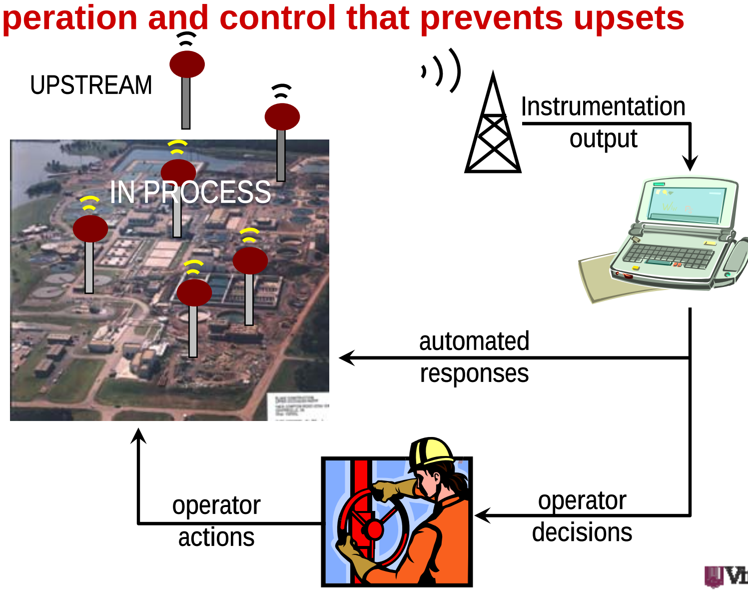
Ideally, our systems should be well instrumented to provide real-time information for improved operation and control that prevents upsets



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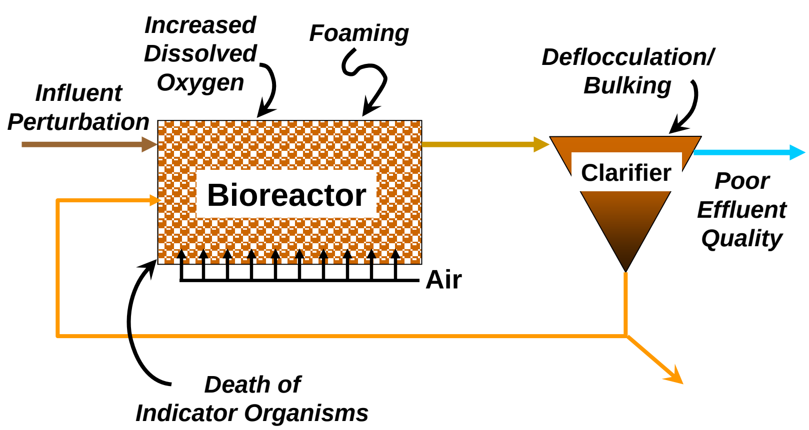
Traditional indicators of activated sludge process stress are valuable, but provide limited information about corrective action



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We proposed a unifying concept for understanding the role of microbial stress in environmental monitoring

SOURCE
Perturbation by Chemical(s)

↔

CAUSE
Biochemical
Physical
Chemical

↔

EFFECT
Poor Ecosystem Performance or Function

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Thesis: An understanding of Source-Cause-Effect relationships is needed to enable:

- proactive design, operation and management
- development of "intelligent" sensors
- development of rapid prevention/corrective action strategies linked with decision support systems

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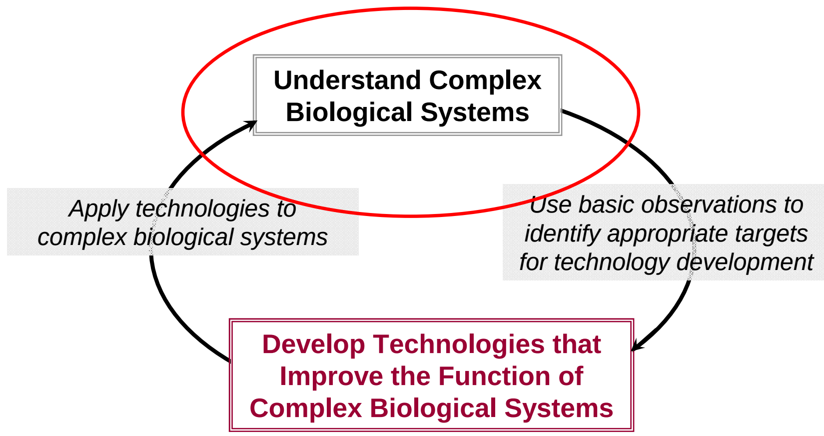

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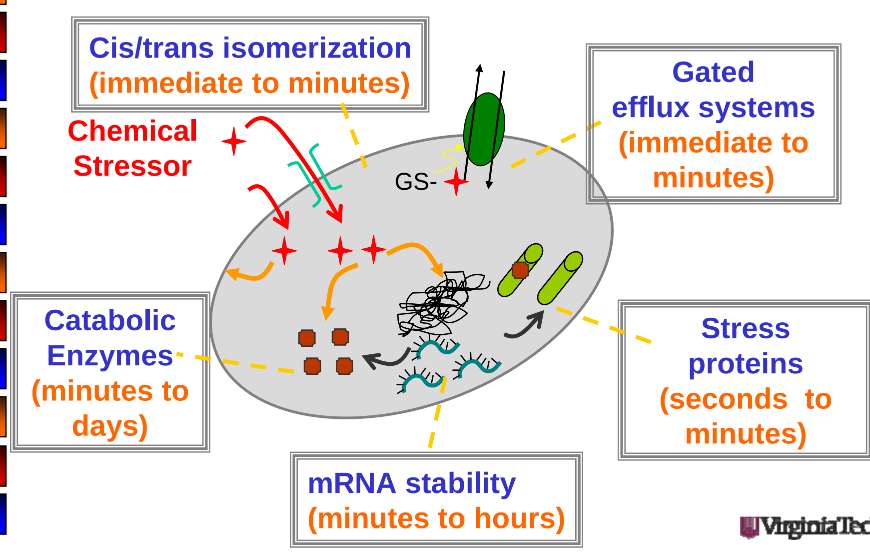
Hypothesis: Activated sludge bacteria rapidly adapt to chemical stressors, and the adaptation response influences system-level performance



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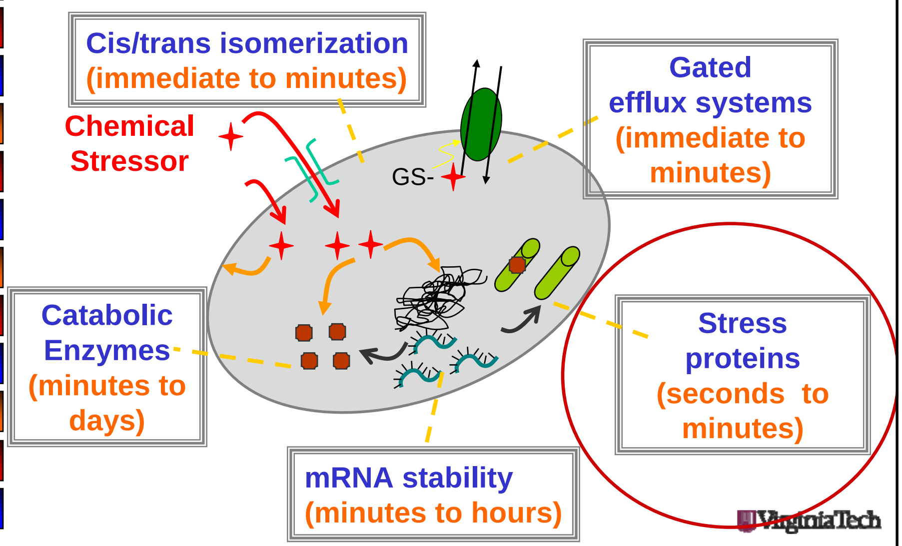

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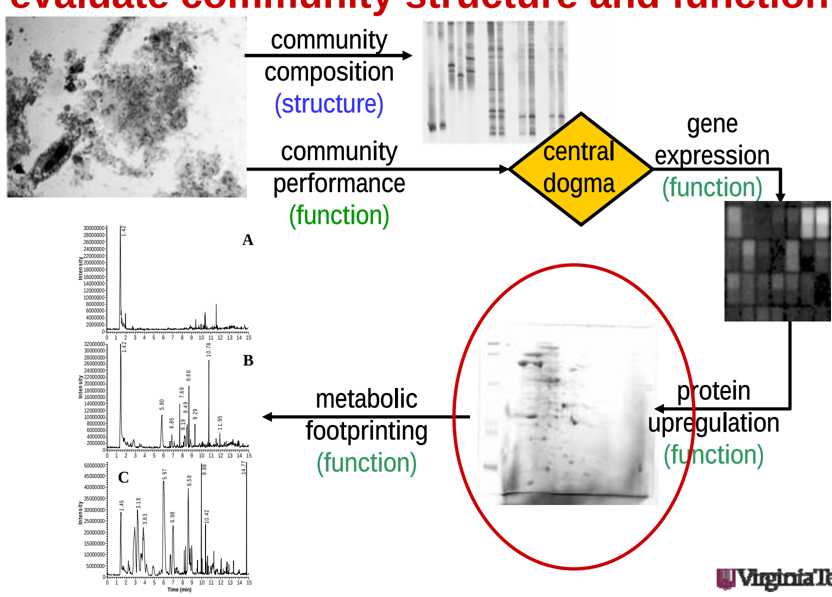
We can use a range of analytical tools to evaluate community structure and function



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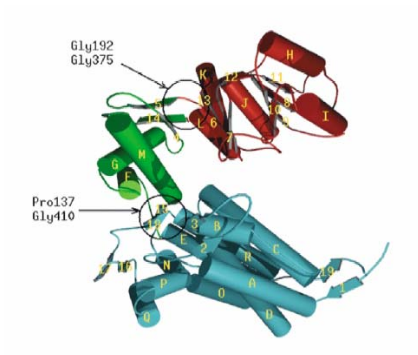
We focused on GroEL (Hsp60), a heat shock protein known to upregulate in response to chemical stressors <u>and</u> starvation



Stan et al. (2003) *Biophy. Chem.* 100:453-467.

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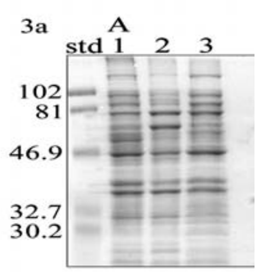
Detecting stress proteins in communities

SDS-PAGE →

A. E. coli



1. Unstressed control
2. Heat Shock
3. Heat + Chloramphenicol

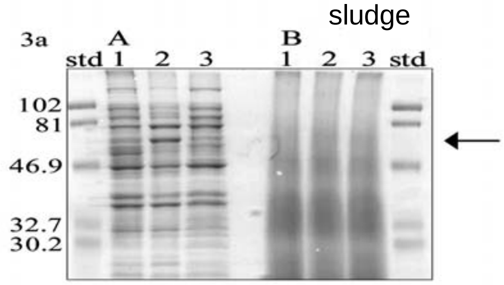
Duncan, Terlesky, Bott and Love, 2000, LAM

Detecting stress proteins in communities

SDS-PAGE

A. E. coli

B. Activated sludge



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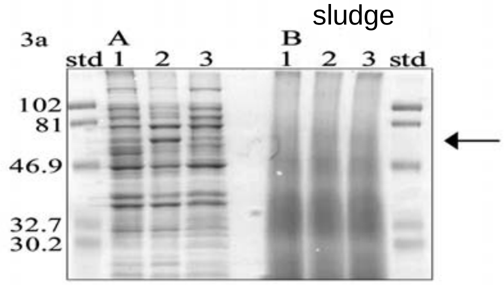

1. Unstressed control
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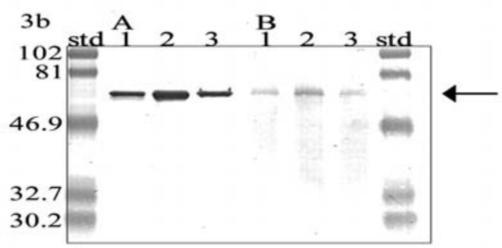
Western blot

(Anti-Hsp60)



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Lessons learned from the protein work:

- It is possible to detect differential expression of a specific protein fingerprint in fresh activated sludge cultures exposed to toxins using traditional biochemical methods

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- It is possible to detect differential expression of a specific protein fingerprint in fresh activated sludge cultures exposed to toxins using traditional biochemical methods

- Is the effort worth the climb using these methods?

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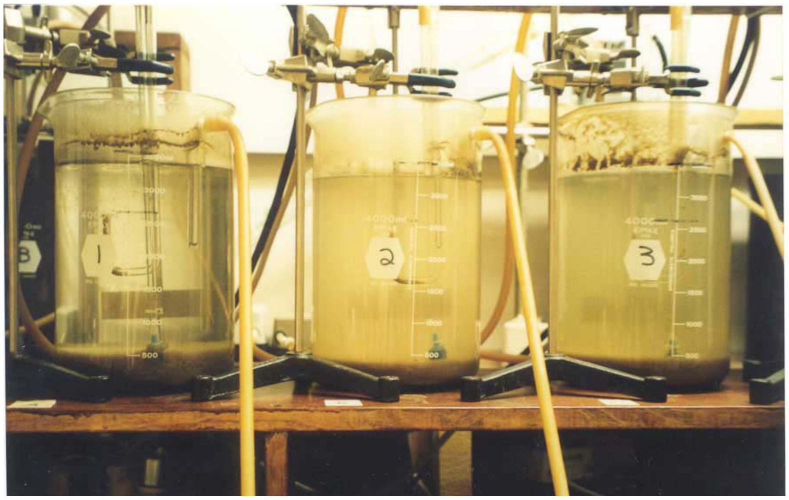
Deflocculation of activated sludge due to toxic shock with ~IC_{25} concentrations



Control NEM CDNB

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Lessons learned from the protein work:

- It is possible to detect differential expression of a specific protein fingerprint in fresh activated sludge cultures exposed to toxins using traditional biochemical methods.
- Is the effort worth the climb using these methods?
- It is not clear that Hsp60 is a primary causal mechanism that explains the prevalent deflocculation observed.

Bott, C. B. and Love, N. G. 2001. The immunochemical detection of stress protein expression in activated sludge exposed to toxic chemicals. *Water Research*, 35:91-100

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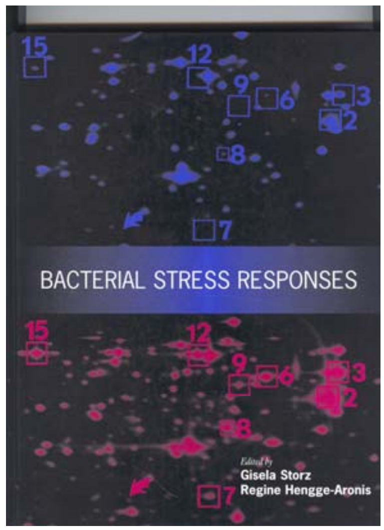
An epiphany at the 2000 Gordon Research Conference on Microbial Stress Responses



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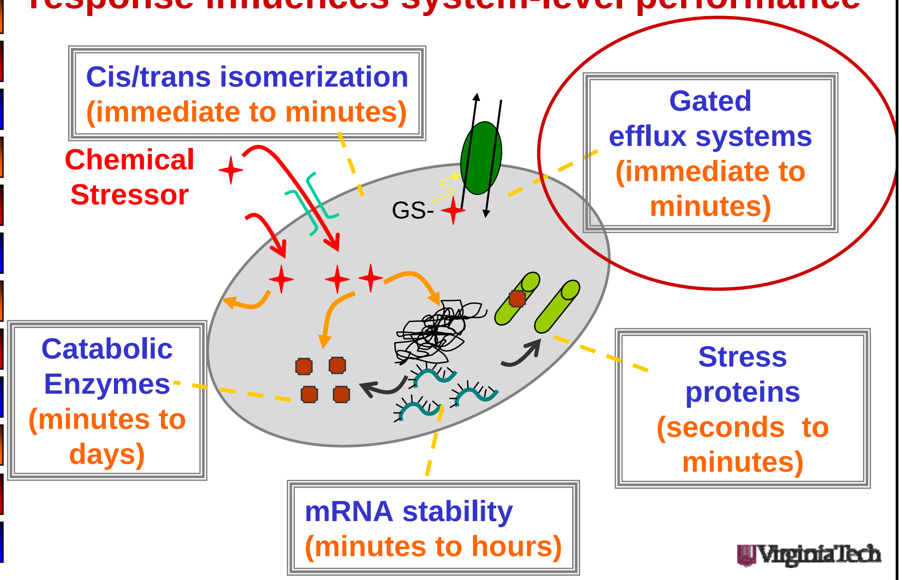
Hypothesis: Activated sludge bacteria rapidly adapt to chemical stressors, and the adaptation response influences system-level performance



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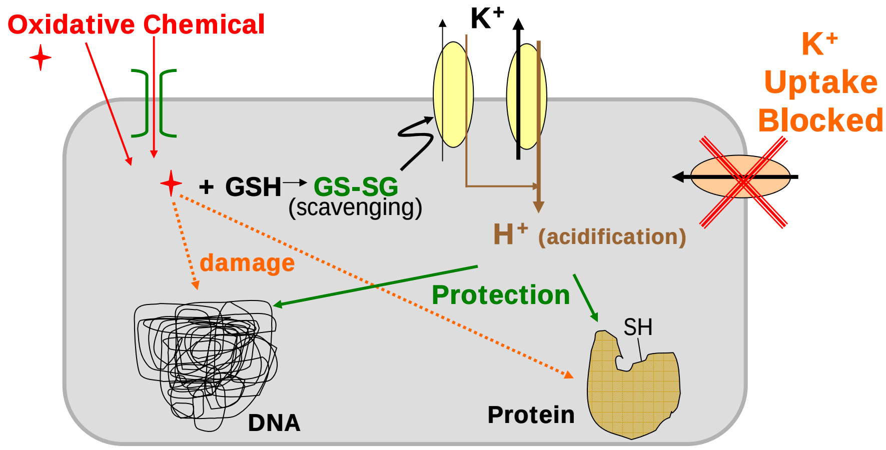
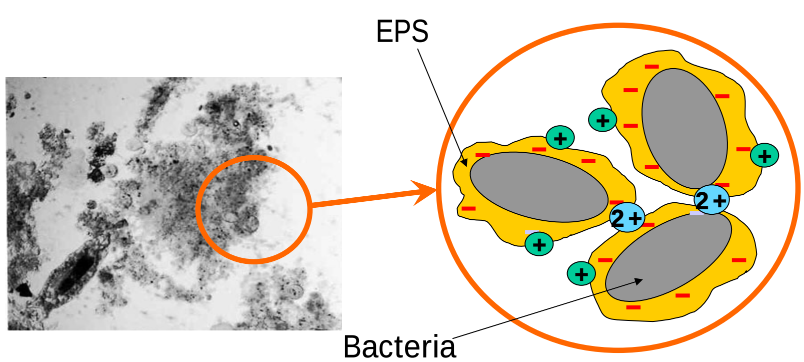
Hypothesis: the Glutathione Gated Potassium Efflux (GGKE) response causes deflocculation by thiol-reactive chemicals



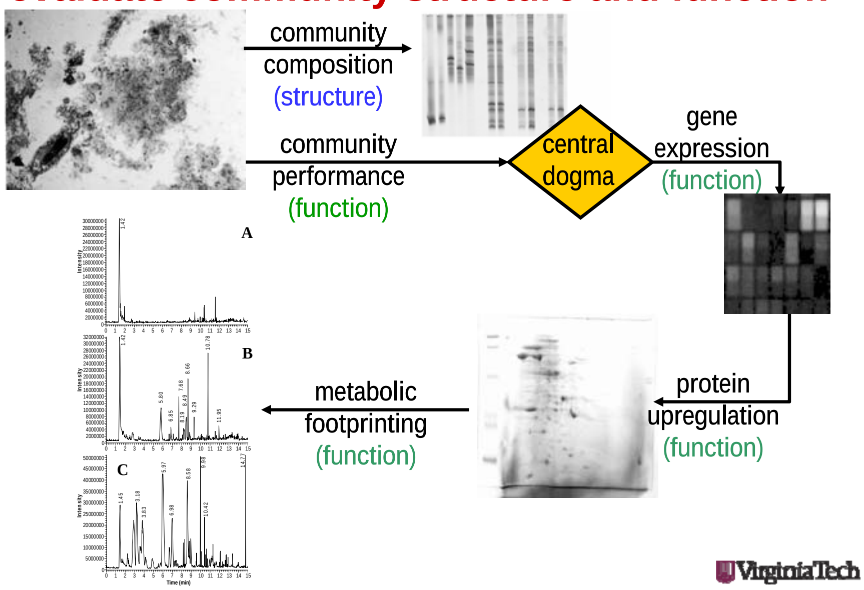
Bakker and Mangerich (1982); Meury and Robin (1990); Ferguson and Booth (1993 – 1998)

Hypothesis: High concentrations of GGKE-generated intrafloc K^+ cations cause weak floc structure that leads to deflocculation



Bott and Love, 2002, *Water Environment Research*

We can use a range of analytical tools to evaluate community structure and function



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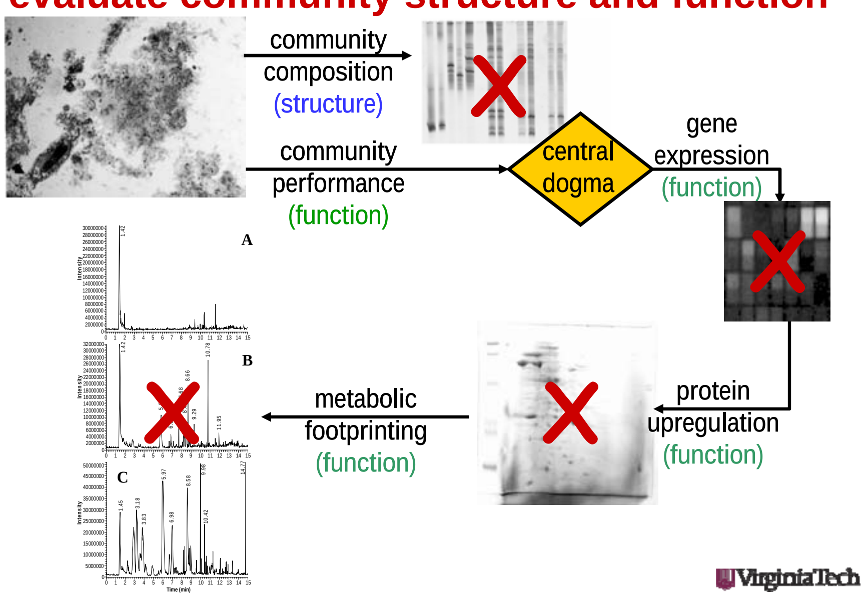
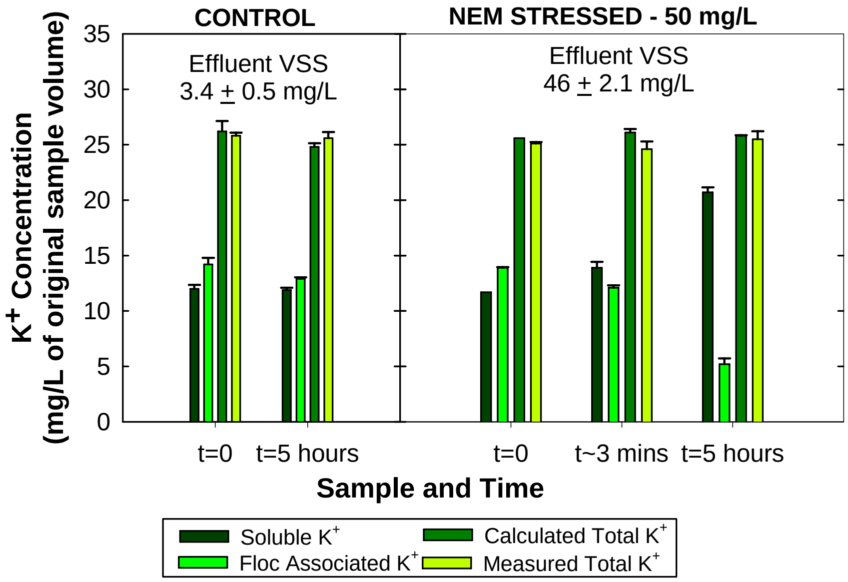


K^+ effluxes from flocs to liquid



Bott and Love, 2002, *Water Environment Research*

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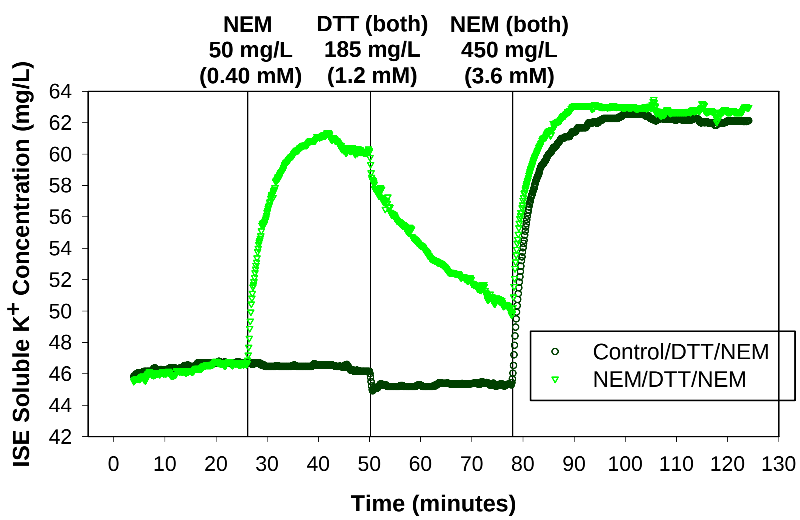
K^+ efflux is reversible in pure culture

(Novosphingomonas capsulatum)



Bott and Love, 2004, *Applied and Environmental Microbiology*

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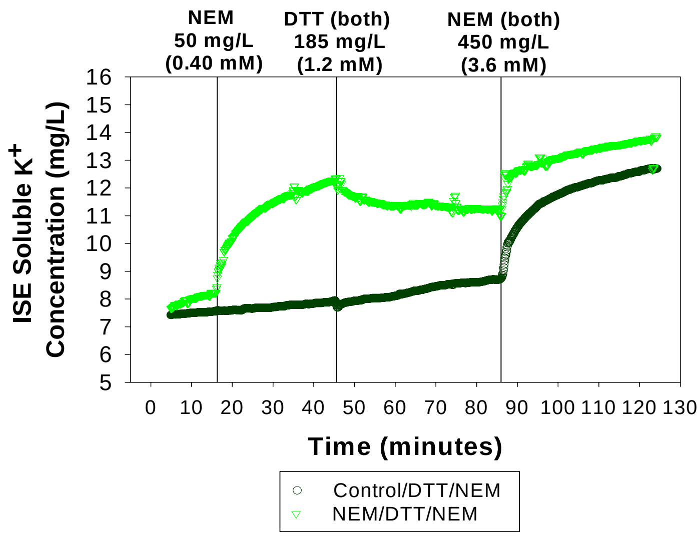
K^+ efflux is reversible with activated sludge



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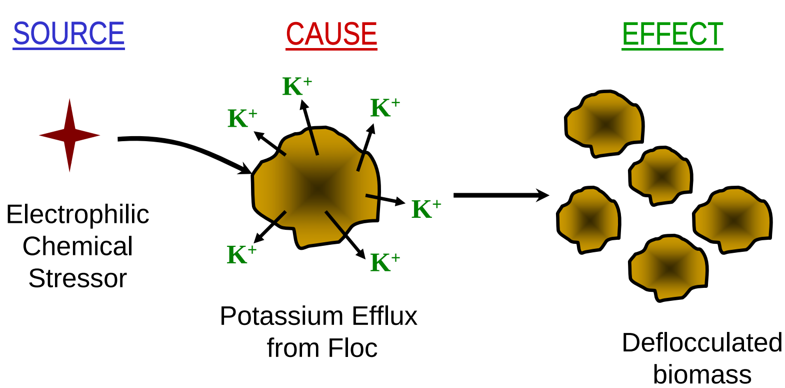
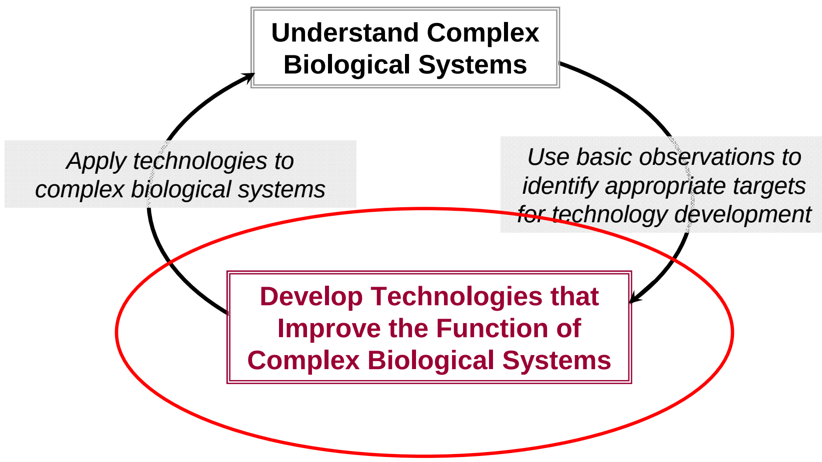
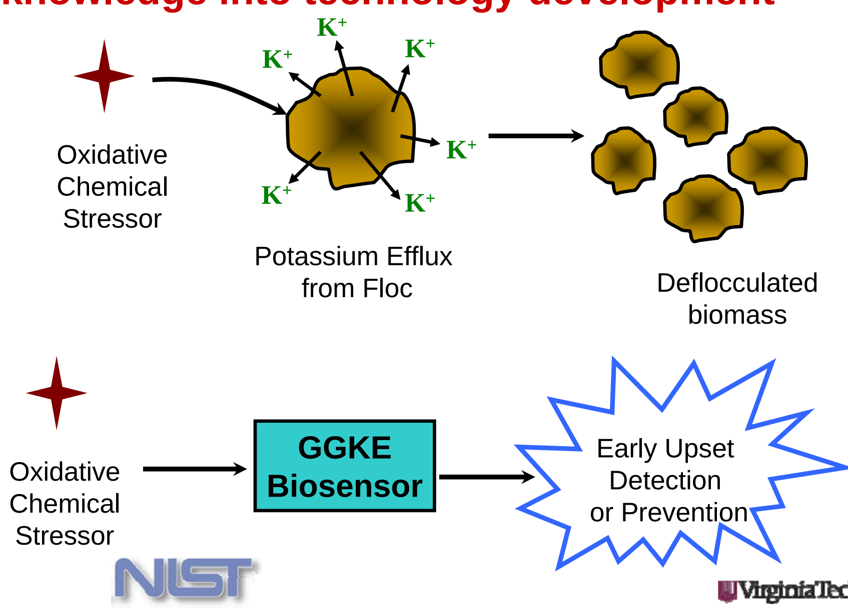
Conclusion: GGKE is a causal mechanism for deflocculation



My 30-year research model



We are converting source-cause-effect knowledge into technology development



Is a glutathione-based, bacterial-based sentinel biosensor best for environmental health applications?

USACEHR

Collaboration with
Dr. Beverly Rzigalinski,
VCOM Pharmacologist

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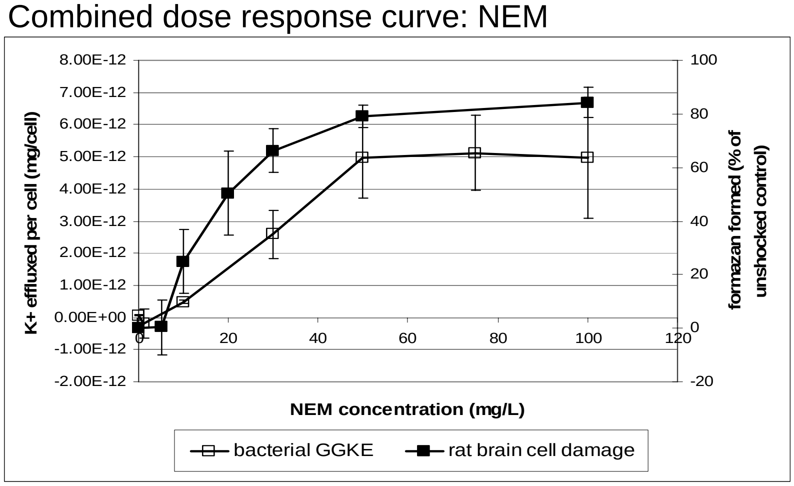
Bacterial and rat brain cells show similar dose-dependent damage in response to NEM

Combined dose response curve: NEM



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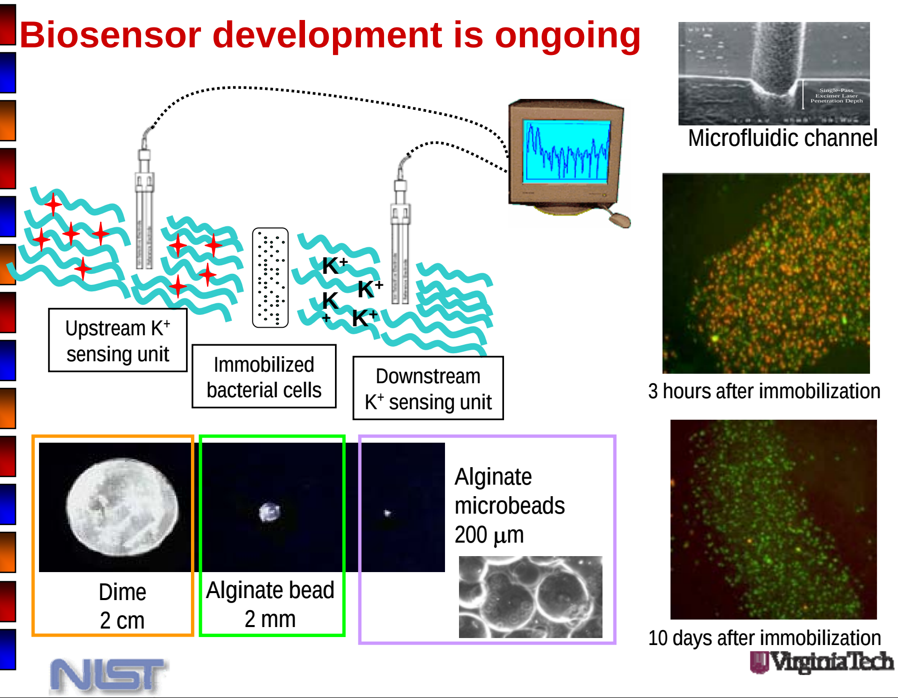
Biosensor development is ongoing



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- proactive design, operation and management
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Summary of process effects for a nitrifying biomass using a pseudo-quantitative scale

Measurement	Observed Process Effect Relative to Control	Hypothesized Causal Mechanism	Ammonium ~ 400 mg/L	Cadmium	CDNB	Cyanide	DNP	Octanol	pH 11
Effluent TSS/VSS	Increase in effluent TSS/VSS	Flocs deteriorate and cause smaller, less dense particles (deflocculation)	0	↓↓↓↓	↓↓↓	0	↓↓	+	↓↓↓↓
Effluent COD	Decrease in COD removal	Inhibition of metabolic pathways	0	↓↓↓↓	↓↓↓	↓	↓↓	+	↓↓↓↓
SOUR	Decrease in SOUR	Inhibition of catabolic pathways	0	↓↓↓↓	↓↓↓	↓↓	↓↓/++	↓↓	↓↓↓
Soluble Potassium	Increase in soluble K^+ concentration	Glutathione-gated K^+ efflux	0	↓↓	↓↓↓	0	0	0	X
Inorganic N effluent conc. and NGR	Nitrification inhibition	Varies	↓	↓↓	↓↓↓	↓↓↓	↓↓	↓↓	↓↓↓↓
SVI	Increase in SVI	Poor biosolids compression, or settleability	0	++	+	↓	0	0	+
CST	Increase in CST	Retention of bound water, leading to poor dewaterability	0	0	0	0	0	0	0

The qualitative scale reflects the intensity of the effect for IC_{50}-shocked reactors and the indicated NH_3 and pH shock level, in comparison to a negative control. The intensity scale ranges from ↓↓↓↓ (most intense process deterioration effect), 0 (no effect), and ++++ (most intense process improvement effect).
X means inconclusive results

Henriques, Kelly, Dauphinais, and Love. In press. *Water Environment Research*

Effluent TSS and COD removal deterioration almost always correlated

Measurement	Observed Process Effect Relative to Control	Hypothesized Causal Mechanism	Ammonium ~ 400 mg/L	Cadmium	CDNB	Cyanide	DNP	Octanol	pH 11
Effluent TSS/VSS	Increase in effluent TSS/VSS	Flocs deteriorate and cause smaller, less dense particles (deflocculation)	0	↓↓↓↓	↓↓↓	0	↓↓	+	↓↓↓↓
Effluent COD	Decrease in COD removal	Inhibition of metabolic pathways	0	↓↓↓↓	↓↓↓	↓	↓↓	+	↓↓↓↓
SOUR	Decrease in SOUR	Inhibition of catabolic pathways	0	↓↓↓↓	↓↓↓	↓↓	↓↓/++	↓↓	↓↓↓
Soluble Potassium	Increase in soluble K^+ concentration	Glutathione-gated K^+ efflux	0	↓↓	↓↓↓	0	0	0	X
Inorganic N effluent conc. and NGR	Nitrification inhibition	Varies	↓	↓↓	↓↓↓	↓↓↓	↓↓	↓↓	↓↓↓↓
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Henriques, Kelly, Dauphinais, and Love. In press. *Water Environment Research*

An epiphany at the 2004 Gordon Research Conference on Environmental Chemistry

(Remember: Ardern and Lockett were chemists!)

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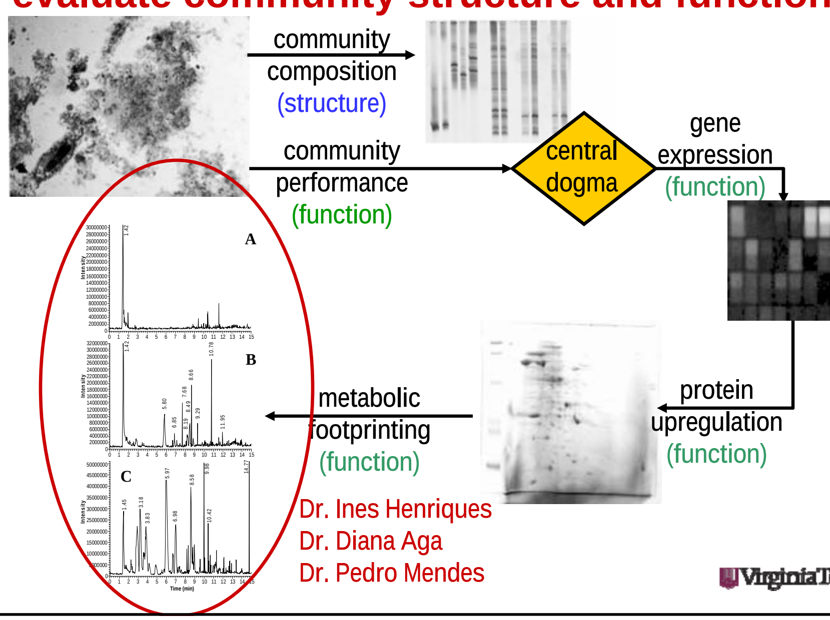
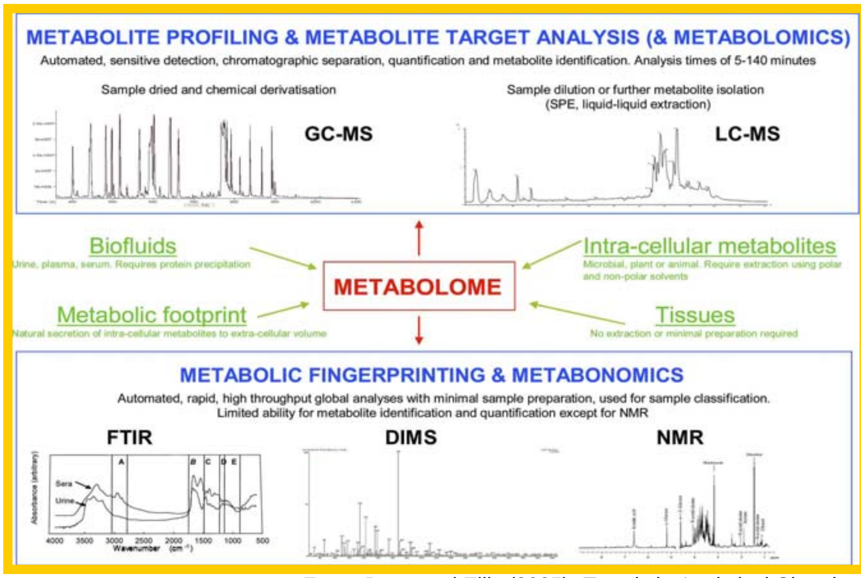
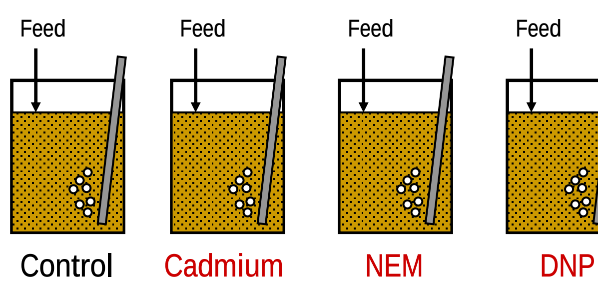


Can metabolomics' techniques be applied to complex environmental matrices ?



From: Dunn and Ellis (2005), *Trends in Analytical Chemistry*

Fingerprint the effluent COD from chemically-stressed SBRs



Sampling at:
- < 5 mins
- 45 mins
- 3 hours
- 5 hours

Henriques, Aga, Mendes, and Love. In press. *Environmental Science and Technology*.

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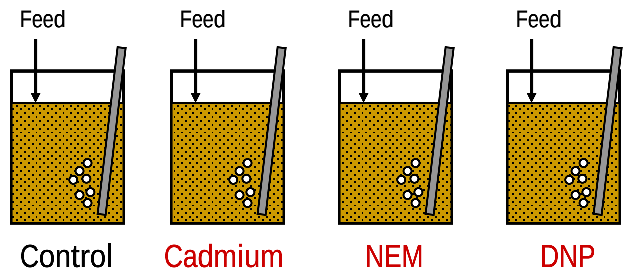
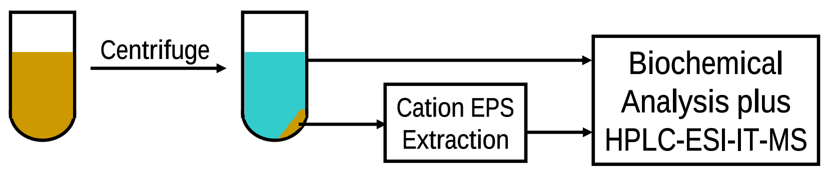


Sampling at:
< 5 mins
45 mins
3 hours
5 hours



Henriques, Aga, Mendes, and Love. In press. *Environmental Science and Technology*.

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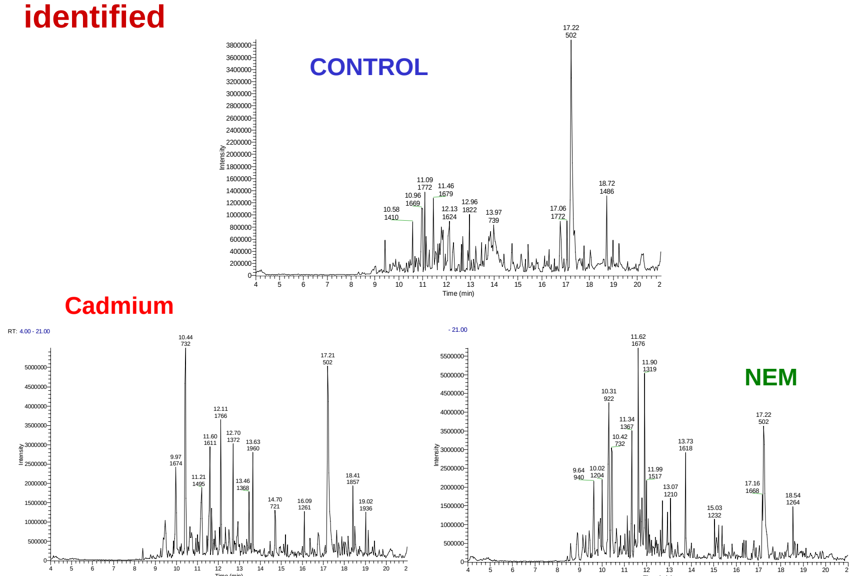
Chromatograms from stressed and control reactors were complex but differences could still be visually identified



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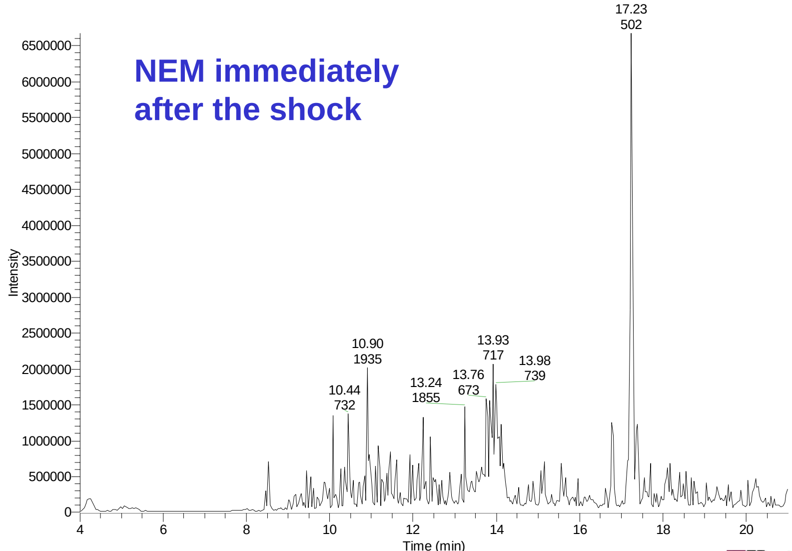
The intensity of the peaks from shocked reactor samples increased in a time-dependent manner



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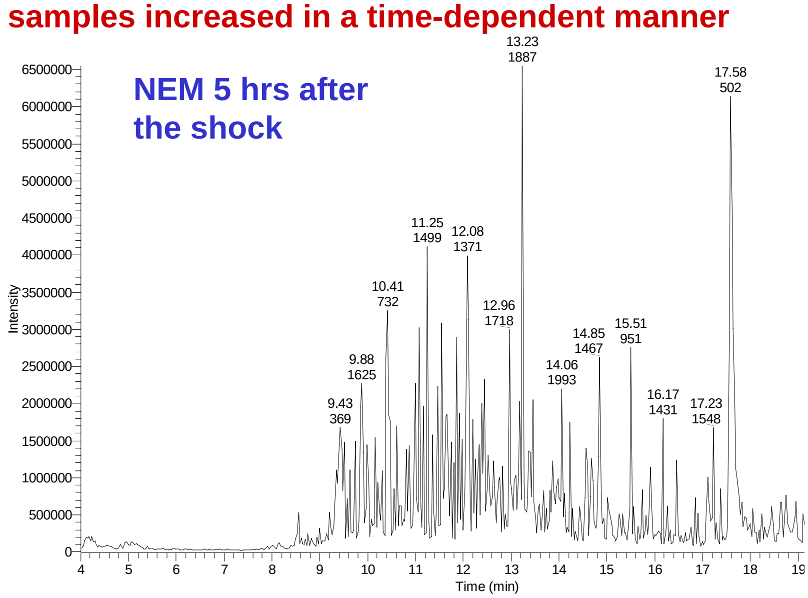
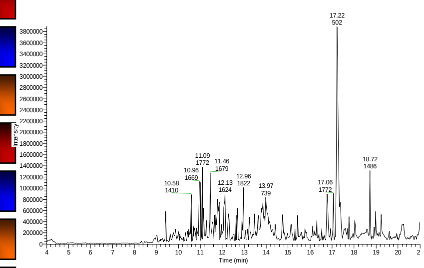
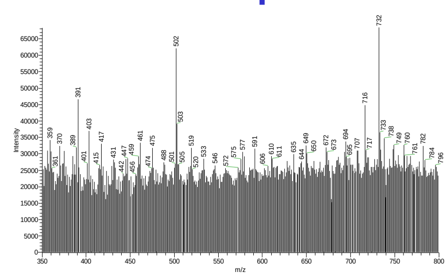


Mass spectra data were used to run statistical analysis

Chromatogram VS. **Mass spectrum**





Peak retention times are not the same for all the samples (alignment problems)

m/z ratios provide a global picture of the substances present in the sample

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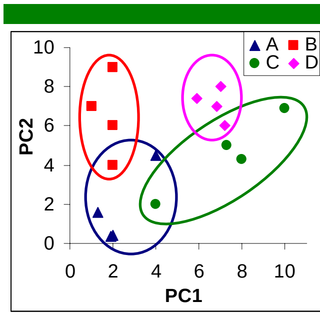
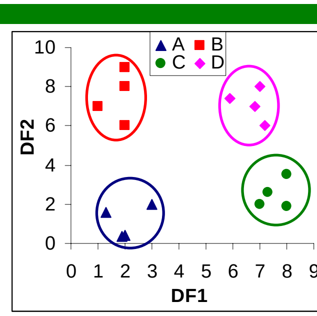
Principal Component Analysis (PCA)

- Unsupervised method
- Maximizes variance between samples
- Does not allow identification of differences between samples



Multiple Discriminant Function Analysis (DFA)

- Supervised method
- Maximizes variance between classes and minimizes variance within a class
- DFA with GA, allows identification of main differences (m/z) between classes



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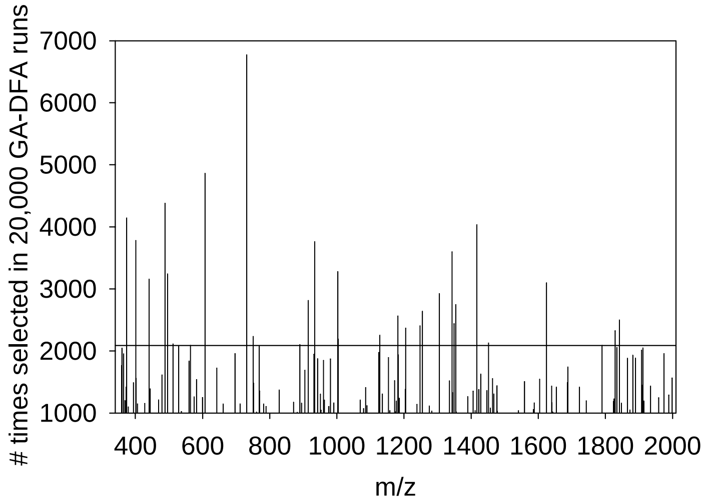
Mass specta data were used to perform Multiple Discriminant Function Analysis with Genetic Algorithm Variable Selection (GA-DFA)



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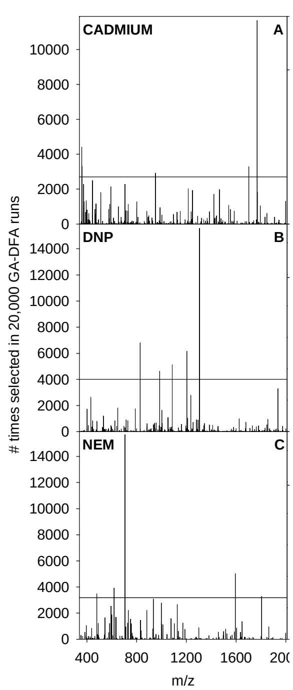

Multiple runs of GA-DFA allow identification of the m/z ratios responsible for the differences between the samples

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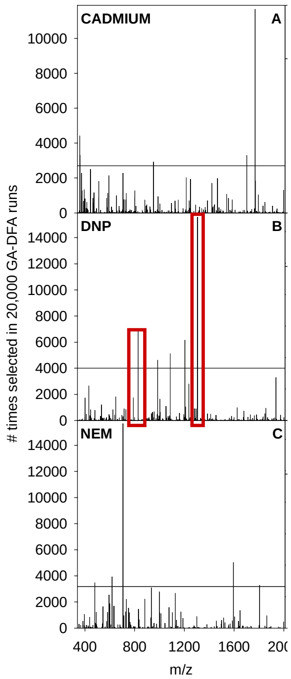

Multiple runs of GA-DFA allow identification of the m/z ratios responsible for the differences between the samples

Example
m/z ratios 828 and 1306 are discriminating biomarkers that reveal the presence of inhibiting levels of 2,4-DNP

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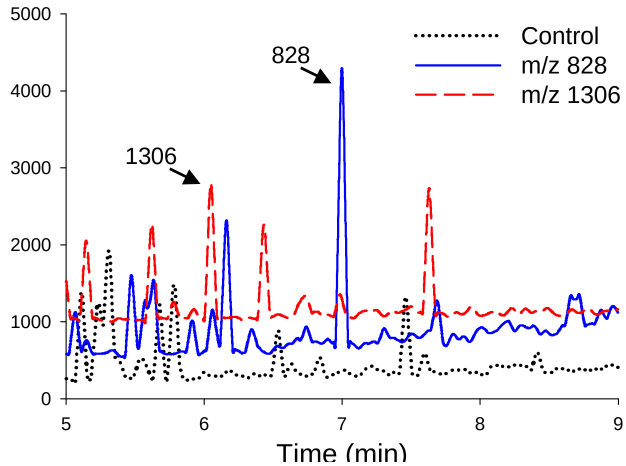
We have correlated 2,4 DNP biomarkers from activated sludge with *Pseudomonas aeruginosa* exudates



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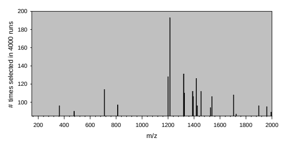
How can this be applied or used?

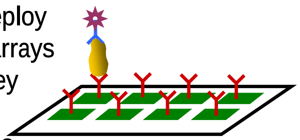
Key m/z fingerprints from mass spectrometry data



Deploy mini mass spectrometers for real time field monitoring with micro total analysis systems (µTAS)

(http://aemc.jpl.nasa.gov/activities/mms.cfm)

Develop and deploy antibody microarrays that target key fingerprint biomolecules



Identify fingerprints and compare to those in sequenced pure cultures (true metabolomic studies)

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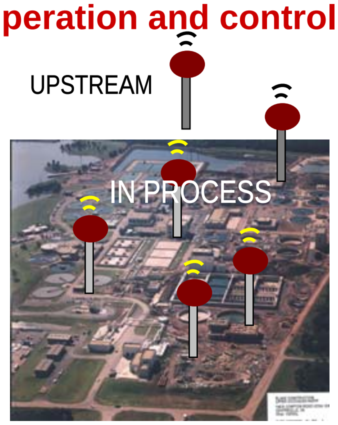
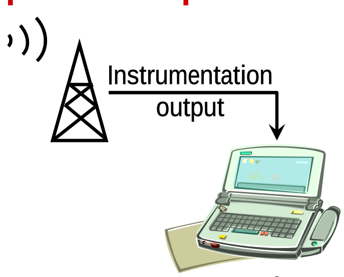
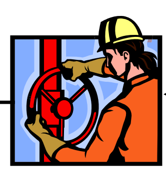
Ideally, our systems should be well instrumented to provide real-time information for improved operation and control that prevents upsets







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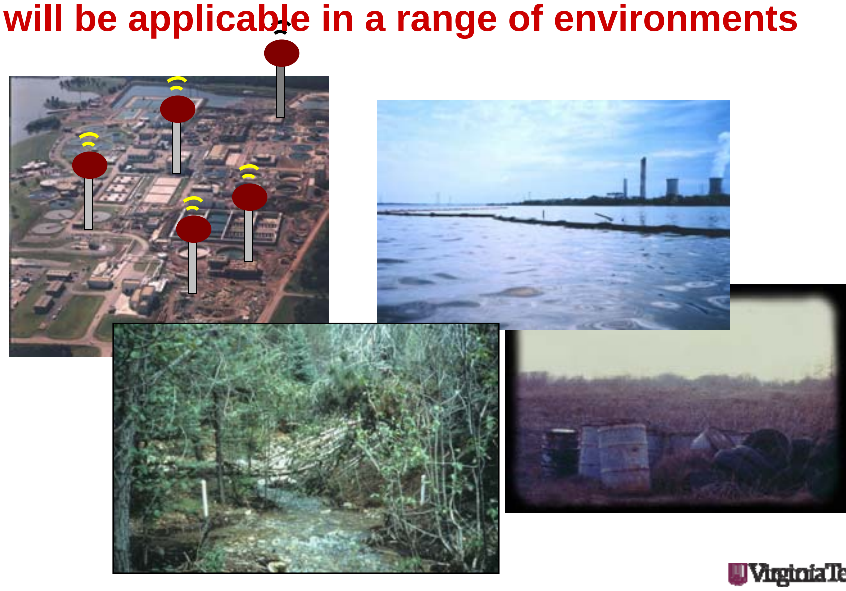
Hypothesis: Stress response-focused sensors will provide useful functional information and will be applicable in a range of environments



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Is there a link between stress and antibiotic resistance?

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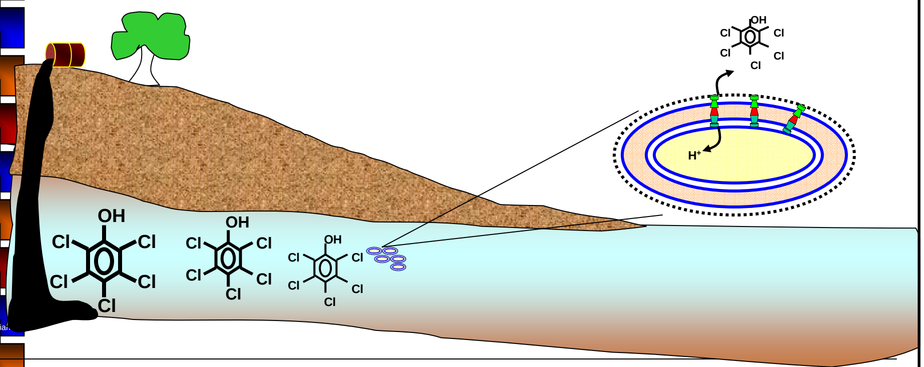
The Role of Multidrug Efflux Pumps in the Stress Response of *Pseudomonas aeruginosa* to Organic Contamination



J. Fraga Muller, Ph.D. Dissertation

Muller, Stevens, Craig, and Love. In press. *Applied and Environmental Microbiology*.
Muller, Stevens and Love. In review. *Science*

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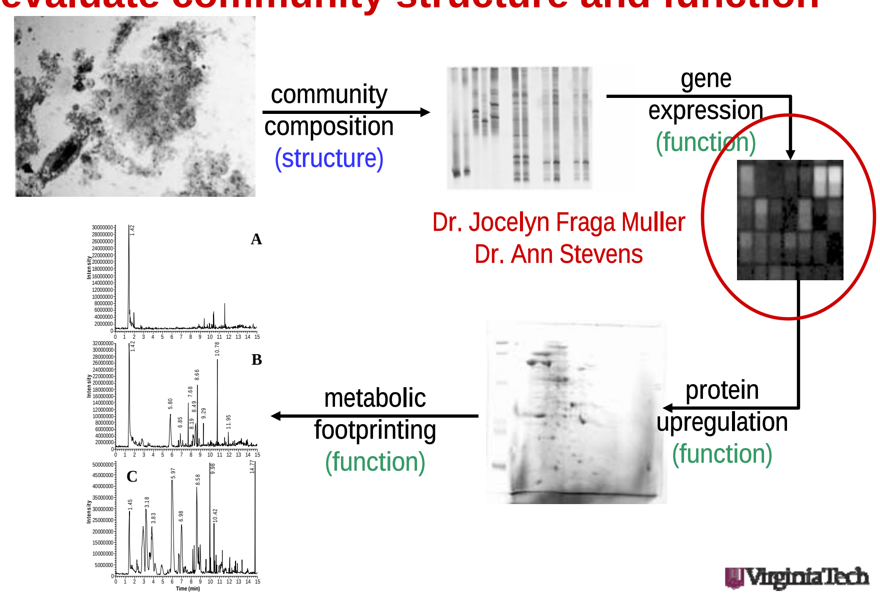
We can use a range of analytical tools to evaluate community structure and function



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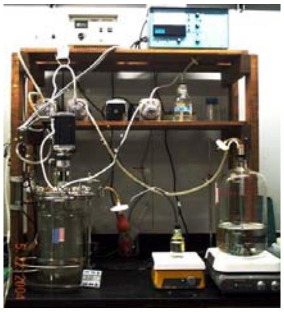
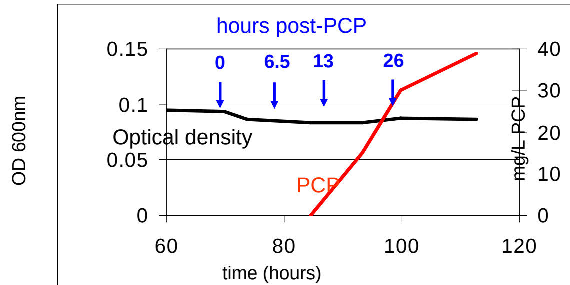


A *Pseudomonas aeruginosa* chemostat was sampled over time for changes in cell physiology & gene expression after perturbation with pentachlorophenol (PCP)



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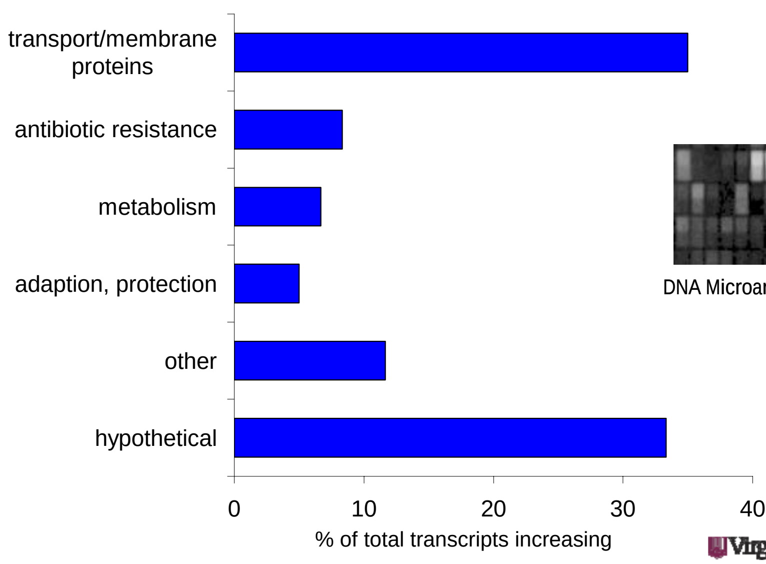
60 transcripts increased, with transport the most represented functional class



DNA Microarray

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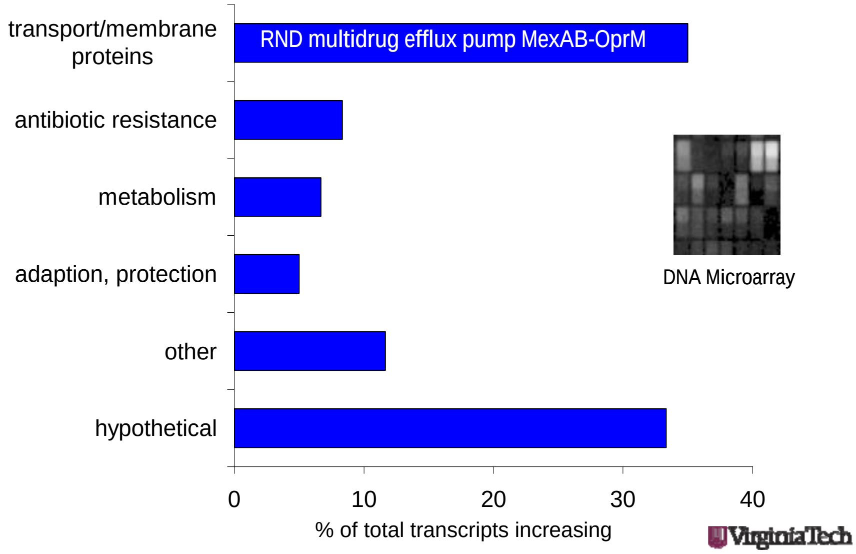
60 transcripts increased, with transport the most represented functional class



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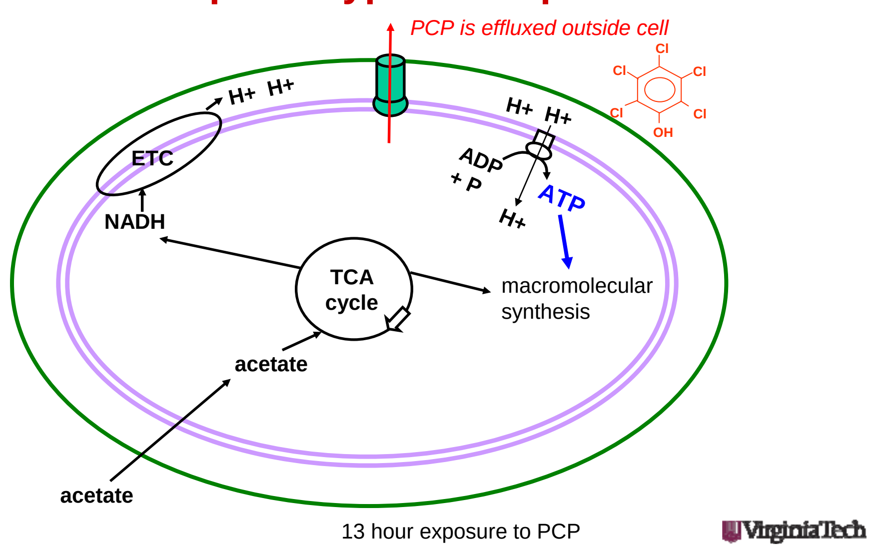
P. aeruginosa upregulates multi-drug efflux pumps and demonstrates an antibiotic resistance phenotype in the presence of PCP



13 hour exposure to PCP

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“Antibiotic resistance has been called one of the world's most pressing public health problems”
http://www.cdc.gov/drugresistance/community/faqs.htm

Environmental factors contributing to antibiotic resistance:

- Overuse of antibiotics (human and animal)

Applied and Environmental Microbiology, Sept. 2005, p. 5383–5390 Vol. 71, No. 9
Occurrence and Relatedness of Vancomycin-Resistant Enterococci in Animals, Humans, and the Environment in Different European Regions
Inger Kühn,[1]* Aina Iversen,[1] Maria Finn,[2] Christina Greko,[2] Lars G. Burman,[3] Anicet R. Blanch,[4] Xavier Vilanova,[4] Albert Manero,[4] Huw Taylor,[5] Jonathan Caplin,[5] Lucas Domínguez,[6] Inmaculada A. Herrero,[6] Miguel A. Moreno,[6] and Roland Möllby[1]

- Natural soil community structure

Sampling the Antibiotic Resistome
SCIENCE VOL 311 20 JANUARY 2006
Vanessa M. D'Costa,[1] Katherine M. McGrann,[1] Donald W. Hughes,[2] Gerard D. Wright[1]*

- ***Anthropogenic contamination***

Does pollution in the environment contribute to antibiotic resistance?

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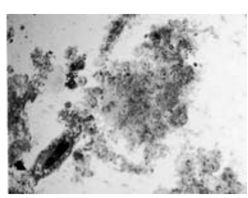
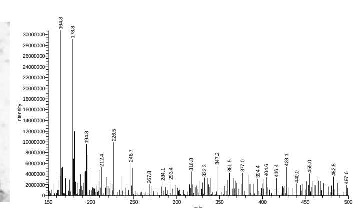
Toward Understanding Dynamic Microbiological Responses to Chemical Stress:
Elucidating Biomarkers for Use in Upset Early Warning Systems

Metropolitan Water Reclamation District of Greater Chicago
June 15, 2007

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Thank You!

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