

An Approach to identifying Proteins from Microbial Communities

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The lack of reliable genome sequences currently limits the effectiveness of proteomics studies of microbial communities because of the difficulty in identifying peptides. Characterizing the proteomics of microbial communities is an inverse modeling problem of a complex system that requires (1) the computational interpretation and integration of high-throughput experimental data, (2) the leveraging of existing sources of knowledge from multiple domains, and (3) searching for solutions that meet criteria on multiple levels in a large search space. Our goal is to develop novel methods needed to describe the proteins and metagenomic functional processes occurring within unsequenced microbial communities being investigated as part of DOE's missions in carbon sequestration, bioremediation and bioenergy research.

A New Multi-Modal Imaging Technology: High Numerical Aperture Cryogenic Light Microscopy with Correlated Soft X-ray Tomography

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In this poster we will present instruments and methods for carrying out correlated high numerical aperture immersion light microscopy on cryogenic specimens, together with recent results. This new imaging modality greatly increases the fluorescence lifetime of all fluorescent probes, including those commonly used for protein localization studies, while retaining the ability to image the specimen with high fidelity and spatial resolution. The novel use of a cryogenic immersion fluid also minimizes the refractive index mismatch between the sample and lens, leading to a more efficient coupling of the light from the sample to the image forming system. This enhancement is applicable to both fluorescence and transmitted light microscopy techniques. The design concepts used for the cryogenic microscope can be applied to almost any existing light-based microscopy technique. This prospect is particularly exciting in the context of 'super-resolution' techniques, where enhanced fluorescence lifetime probes are especially useful. The techniques described can be used in conjunction with other imaging modalities in correlated studies. We have developed instrumentation to perform cryo-light imaging together with soft x-ray tomography on the same cryo-fixed specimen as a means of carrying out high content, quantifiable correlated imaging analyses. The cryo-stage for the new soft x-ray microscope constructed by the National Center for X-ray Microscopy has an integrated high resolution cryo-light microscope.

Additional technologies are being developed to enable processing and multimodal imaging of virtually any specimen; from whole cells to cryo-sectioned tissue, biofilms or even lignocellulosic material. These developments are therefore of general interest to a significant number of researchers working towards achieving the missions and goals of the DOE.

Reference:

Le Gros, M.A. *et al.* Larabell, C.A. (2009) High aperture cryogenic light microscopy. *J. Microscopy* (in press).

Parkinson, D.Y., G. McDermott, L.D. Etkin, M.A. Le Gros, C.A. Larabell, (2008) Quantitative 3-D imaging of eukaryotic cells using soft X-ray tomography. *J Structural Biology* **162**, 380-6.

For more information on the National Center for X-ray Tomography visit

<http://ncxt.lbl.gov>

Isotropic Soft X-ray Tomography of Eukaryotic Cells.

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In this poster we will present recent results using correlated soft x-ray tomography and fluorescence microscopy to image eukaryotic cells and their sub-cellular architecture. In soft x-ray tomography cells are imaged fully hydrated. The technique does not require that the specimen be stained, chemically fixed or otherwise treated prior to being imaged. The cells are simply and rapidly transferred from their growth environment to a specimen holder for immediate vitrification. This results in images of cells in their native state. Soft X-ray Tomography is a quantitative technique; the extent to which x-rays are absorbed is a direct consequence of the chemical species present. For example, in the region of the spectrum used for this type of imaging, water absorbs soft x-ray photons an order of magnitude less strongly than biological materials such as membranes, or lipid droplets. This physical characteristic gives rise to images containing exquisite contrast between architectural features inside the cell. This poster will demonstrate this in terms of tomographic images of a number of different yeast cell types of interest to the DOE, in particular to those researchers working on the development of new methods for the productions of biofuels.

In combination with correlated cryogenic fluorescence microscopy (see poster by Le Gros et al), it is also possible to accurately position labelled molecules into the tomographic reconstructions of these cells. This opens up the capability of carrying out live cell studies to a point of specific interest, then immediately cryo-fixing the cells for imaging by a number of modalities. Thus allowing for unambiguous identification of organelles, or the localization of labeled molecules in the context of the tomographic reconstruction. As a further benefit, these types of analyses can be carried out rapidly. For example, it takes less than 3 minutes to collect a tomographic data set on a field of view containing upwards of five yeast cells. Thus allowing imaging to be carried out on large numbers of cells in a short space of time. This is ideally suited to providing realtime feedback on the morphological consequences of different growth conditions, mutations, and other parameters under consideration.

Reference:

Le Gros, M.A. *et al.* (2009) High aperture cryogenic light microscopy. *J. Microscopy* (In press).

For more information on the National Center for X-ray Tomography visit <http://ncxt.lbl.gov>

Soft X-ray Tomography: A New Tool for Imaging Prokaryotic Cells

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In this poster we will present recent results using correlated soft x-ray tomography and fluorescence microscopy to image prokaryotic cells. The instruments and techniques being developed at the National Center for Tomography are well suited to imaging a broad range of organisms and specimen types to produce quantitative information on cell morphology and the sub-cellular architecture. In this poster, we will present our latest images of *Caulobacter crescentus*, *E. coli*, *Haloferax elongans*, and *Haloferax volcanii*. Using our newly developed suite of correlated imaging modalities it is possible, for the first time, to tomographically image prokaryotic cells in large numbers. For example, a single field of view in this microscope contains upwards of 200 individual *Caulobacter crescentus* cells. In all cases, the signal-to-noise in a calculated soft x-ray tomogram is isotropic and good enough to allow automated segmentation and quantitation. Therefore, using this technique it is trivial to obtain accurate information on cellular and sub-cellular morphological characteristics, such as volumes, densities, and distributions. Using correlated cryogenic fluorescence microscopies (see poster by Le Gros et al), it is also possible to accurately position labelled molecules into the tomographic reconstructions of the cell. Taken together this exciting new suite of multimodal imaging tools is capable of imaging virtually any specimen; from whole cells to cryo-sectioned tissue, biofilms or even lignocellulosic material, and their interactions with communities of prokaryotic organisms. These developments are therefore of general interest to a significant number of researchers and to the missions and goals of the DOE.

Reference:

Le Gros, M.A. *et al.* (2009) High aperture cryogenic light microscopy. *J. Microscopy* (In press).

Ashcroft, J.M., W. Gu, T. Zhang, S.M. Hughes, K.B. Hartman, C. Hofmann, A.G. Kanaras, D.A. Kilcoyne, M. Le Gros, Y. Yin, A.P. Alivisatos, C.A. Larabell, 2008. TiO₂ nanoparticles as a soft X-ray molecular probe. *Chemical Communications*, 2471-2473.

Tang, Y.J.J., J.M. Ashcroft, D. Chen, G.W. Min, C.H. Kim, B. Murkhejee, C. Larabell, J.D. Keasling, F.Q.F. Chen, 2007. Charge-associated effects of fullerene derivatives on microbial structural integrity and central metabolism. *Nano Letters* 7, 754-760.

For more information on the National Center for X-ray Tomography visit

<http://ncxt.lbl.gov>

Assessing population genetic structure of diploid alfalfa germplasm as a prelude to association mapping

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Cultivated alfalfa is an tetrasomic tetraploid, which has limited the development and application of genomics tools. Fortunately, diploid germplasm exists and it has rarely been used for cultivar improvement. Our objective was to categorize the genetic diversity in diploid germplasm as a prelude to association mapping for yield and composition traits. A collection of 384 individual genotypes derived from 123 unimproved diploid accessions from the USDA National Plant Germplasm System was selected to represent the diploid *Medicago sativa-falcata* complex, including *M. sativa* subspecies *caerulea*, subsp. *falcata*, and subsp. *hemicycla*. The accessions were screened with 89 polymorphic SSR loci distributed throughout the genome in order to estimate genetic diversity, infer the genetic bases of current morphology-based taxonomy, and determine population structure. High levels of variation were detected with a mean of 18.4 alleles per locus and a mean heterozygosity across 89 loci of 0.485. A model-based clustering analysis of the genomic data identified two clearly discrete subpopulations, corresponding to the morphologically defined subspecies *falcata* and subspecies *caerulea*. The hybrid nature of subspecies *hemicycla* has also been confirmed based on its genome composition. Subsequent hierarchical population structures indicated that two distinct subpopulations exist within subspecies *caerulea* (northern vs. southern) and subspecies *falcata* (lowland vs. upland). When considering these five populations, about 81% of the estimated genetic variance resided within the populations leaving only 19% among populations. We have analyzed these populations for biomass yield and agronomic traits, and will have a compositional analysis in hand by spring. The biomass yield and agronomic trait data did not reveal any clustering, unlike the SSR results. This suggests that good alleles exist for different traits within the divergent germplasm, offering the opportunity to mine good alleles from this material for cultivated alfalfa improvement. We are also in the process of identifying SNP variants within genes in the lignin biosynthetic pathway, and of identifying SNP in the transcriptomes derived from stems of selected individuals. These markers will enable us to conduct association mapping for biomass yield and composition accounting for population structure.

Tetrahymena thermophila as a novel platform for overexpression of membrane and secretory proteins.

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Recombinant proteins have become increasingly important in a wide range of applications that extend from alternative fuel production to the treatment of human and animal disease. Manufacture of genetically engineered enzymes, therapeutic proteins, vaccines, and biopolymers constitutes a multibillion dollar-per-year industry, independent of the large market for recombinant proteins in basic research. Despite this, many important targets, particularly **membrane and secretory proteins**, are often difficult to produce using current manufacturing platforms. To address this problem, **Tetragenetics Inc.** is using the common pondwater ciliate, *Tetrahymena thermophila*, as a new system for the production of difficult to express proteins. The first “animal-like” cell to be grown in pure culture, *Tetrahymena* belongs to a large and diverse phylum (ciliata) whose members display a structural and functional complexity comparable to that of metazoa. At the same time, *T. thermophila* is remarkably easy to grow and reaches high cell densities in a short time on a variety of inexpensive media. Unlike bacteria and yeast, however, *Tetrahymena* has no cell wall devotes a large part of its metabolism toward membrane protein production owing to its hundreds of surface-associated cilia. Finally, *Tetrahymena* has numerous pathways for protein secretion that can be targeted using appropriate signal peptide domains. Our Phase I SBIR project will use the H5 hemagglutinin (HA) of avian influenza virus as a model membrane protein to demonstrate the feasibility of this targeting approach. Preliminary studies indicate that *Tetrahymena* can provide a robust platform for the production and rapid purification of membrane and secretory proteins from a wide range of organisms and may be ideally suited to functional analysis of gene products identified in high-throughput sequencing of efforts sponsored by the GTL program.

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Control of *Shewanella oneidensis* MR-1 biofilms by c-di-GMP and environmental factors

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ABSTRACT

Environmental conditions significantly influence the physiology of cells within a biofilm. Microbial biofilms are dynamic structures that respond to changing environments, enabling continued survival of the bacterial population. One such response is the dissolution of a biofilm community, termed detachment or dispersal, the consequence of which is release of a viable population of cells that may subsequently colonize new environments. *Shewanella oneidensis* is a gram-negative facultative bacterium that can respire many insoluble electron acceptors in the absence of oxygen, implying the importance of biofilm formation in its growth. Aerobically grown *S. oneidensis* biofilms readily exhibit biofilm detachment in response to oxygen deprivation, as well as to a decrease in intracellular cyclic-di-GMP concentration. Biofilm formation in *S. oneidensis* is thought to be controlled by the bacterial second messenger cyclic diguanylate (c-di-GMP). Our previous work suggested that the *mxl* operon is involved in both biofilm formation and c-di-GMP metabolism. In this work, we present evidence that the MxlA protein indirectly increases intracellular c-di-GMP concentration, rather than possessing diguanylate cyclase activity itself. We present a physiological characterization that indicates that biofilm stability in the region closest to the bulk liquid phase requires metabolic energy; that the detachment response is controlled post-translationally; and that cells disperse from the biofilm autonomously. We also present preliminary results of an investigation into the impact on c-di-GMP metabolism of a number of *S. oneidensis* proteins containing GGDEF (diguanylate cyclase), EAL (phosphodiesterase) and PAS (sensory) domains.

Meeting the Demand for Biofuels: Impact on Land Use and Carbon Mitigation

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Abstract

This integrated, interdisciplinary research project investigates the impact of meeting the biofuel mandates on land use, crop production, social welfare and the environment in the U.S. over a 15 year horizon. To this end, we conduct a variety of research activities including: (1) examination of the optimal allocation of existing cropland for feedstock production, the mix of feedstocks that should be produced, and the spatial pattern of land use in the U.S. to meet specified levels of renewable fuel production under a variety of policy scenarios; (2) determination of the productivity in terms of yield and greenhouse gas mitigation benefits for each type of feedstock both in the form of soil carbon sequestration and displacement of carbon emissions from gasoline; and (3) identification of the optimal size and location of biorefineries and the transportation network that is consistent with regional feedstock production patterns and the location of demand for ethanol.

To date, using a supply-side dynamic agricultural sector model, we have examined: (1) the economically viable mix of cellulosic feedstocks and resulting spatial pattern of land use in Illinois to meet biofuel targets; (2) the implications for crop production, food and fuel prices of large scale biofuel production; (3) the environmental effects of meeting biofuel targets for greenhouse gas emissions and nitrogen use; (4) the optimal policies for achieving greenhouse gas mitigation; and (5) the social costs of existing policies (subsidies, tariff and mandates) and (5) the location and size of biofuel refineries to meet biofuel targets at least cost.

Our preliminary research results suggest that for Illinois to produce 20% of the national renewable fuels mandate, total cropland use in Illinois increases by 5% and cropland under corn increases from current 47% to 53%-55% in 2022 relative to that with no biofuels. Over the same time period, soybean acres decrease from current 45% to 29% while 14% of the cropland is used to grow miscanthus as feedstock for cellulosic ethanol. Additionally, 56% of corn will be used to produce ethanol and 100% of available corn stover will be used to produce cellulosic ethanol in Illinois in 2022. The mix of viable cellulosic feedstocks varies spatially and temporally with corn stover and miscanthus co-existing in the state; corn stover is viable mainly in central and northern Illinois while miscanthus acres are primarily located in southern Illinois. Corn prices are expected to increase by 44% and soybean prices to increase by 7% over 2007 levels. Biofuel production and resulting land use change lead to a reduction in cumulative greenhouse gas emissions by 54% but an increase in nitrogen use by 25% between 2007 and 2022. A multi-year transshipment and facility location model is then developed to determine the optimal size and time to build biofuel refineries in the region, and to identify the transportation routes of both feedstocks and biofuels. In order to meet given biofuel targets for Illinois, we find that 7 corn ethanol refineries and 19 cellulosic ethanol refineries should be constructed by 2022, and that capacity of 10 existing corn ethanol refineries should be expanded. The average optimal capacity of corn-ethnaol refineries is estimated to be 176 million gallons per year while that of cellulosic refineries is estimated to be 221 million gallons per year.

Using fuel cell-mediated hydrogen conversion in photosynthetic bioreactors

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With the ultimate goal of producing ready-to use energy from biological sources, we intend to couple our photosynthetic bioreactors with electrode assemblies. The reactors consisted in batch cultures of *Rhodobacter sphaeroides* grown in anaerobic conditions under the presence of light. Under optimal medium and using wild type organisms, these reactors were able to produce up to 0.1 mmol of hydrogen per ml of culture. The oxidation of hydrogen into protons, which corresponds to the anodic reaction in our fuel cells, was coupled with the reduction of oxygen present in the air. We assessed the fuel cell performance (i.e., the amount of biohydrogen converted into electricity), in different reactor and cathode configurations.

We evaluated the straightforward approach, consisting in connecting the gas outlet of the reactor into a separate chamber equipped with a thin membrane electrode assembly (MEA), which has one side facing the chamber and the other one open to the air. Under this configuration, we were able to convert up to 40% of the biohydrogen into electricity. As biogas production and hydrogen consumption presented good correlations, we intend to explore this system as an on-line sensor, as a cheap alternative to evaluate the presence and proportion of hydrogen in the biogas mixture produced in the reactors, instead of using an off-line method such as gas chromatography.

We also considered a second approach for hydrogen utilization, consisting in de-coupling the hydrogen oxidation from the oxygen reduction in separate assemblies. Transparent anodes were made of a conductive glass coated with platinum and titanium dioxide applied using sol-gel techniques. The cathodes consisted in commercially available platinum-sputtered porous graphite paper. We evaluated the effects of increasing loads of the TiO₂-Pt addition on the anodic performance. We demonstrated the ability of these transparent anodes to oxidize hydrogen, although in a much lower rate than in the MEAs. Along with these anodes, we expect to develop transparent, non-porous cathodes, with the goal of making light-permitting, redox-active bioreactors in a single chamber.

"Microbial Synthetic Biology at the Great Lakes Bioenergy Research Center: Screening, Fermentation, and Reiterative Engineering"

Wesley D. Marner II - Great Lakes Bioenergy Research Center

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The production of liquid fuels from cellulosic biomass is an important milestone in instituting a sustainable energy program. The Great Lakes Bioenergy Research Center conducts basic, genomics-based research to design the microbial and plant systems needed to realize the potential of biofuels. Combining innovative science, a critical mass of natural assets, and the corporate horsepower to build and advance a new bioenergy economy, the Great Lakes Bioenergy Research Center will become a worldwide center of excellence for research and development of cellulosic ethanol and other bioenergy products.

Towards this goal, we are engineering the model organisms *Escherichia coli* and *Saccharomyces cerevisiae* as energy production platforms. While strategies for the production of ethanol and other fuels have been devised for simple sugar inputs, there has been little progress developing systems to consume cellulosic feedstocks. The challenges for cellulosic fuels include lignocellulose depolymerization, sugar transport, pentose sugar metabolism, lignotoxin tolerance, metabolic flux balancing, and product fuel tolerance.

Over the past year, the Microbial Synthetic Biology (MiSynBio) group within GLBRC has implemented a reiterative engineering cycle consisting of strain engineering, high-throughput screening, advanced fermentation, and sophisticated multi-omic analyses to generate robust microorganisms capable of meeting the challenges in cellulosic fuel production. Here, we describe our approach to the strain engineering cycle, the capabilities of the MiSynBio facilities, and our early experimental work.

Agricultural Considerations in Region Selection for Biofuel Economic Impact Modeling

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As part of our larger project to quantify economic and social impacts that might be expected from efforts to satisfy biofuel production mandates in the Pacific Northwest from in-region supply, careful attention to region selection is required. Apart from determination of functional economic regions in rural areas based on traditional measures such as labor markets, demographic factors, and other economic statistics, agricultural factors should be considered.

Agricultural factors include those attributes associated with the growth of biofuel feedstocks that may have spatial significance and that are independent from other attributes used in economic region identification. Such attributes may include soil characteristics, availability of irrigation, traditional cropping patterns, and historical yields. These attributes are further affected by variable weather patterns and growing-season considerations.

Identification of specific agronomic regions using agricultural characteristics data is analogous to identifying economic regions using socioeconomic data. Data such as soil taxonomy can be spatially disaggregated to a very fine resolution such as individual soil types or aggregated to groupings of soil types (associations) with similar characteristics with respect to cultivation of biofuel feedstock crops. Spatial description of relevant soils, availability of irrigation, growing season, historical yields, and other agronomic considerations permits the identification of relevant geographies for feedstock crop production.

To inform the study of potential economic and social impacts of satisfying biofuel production mandates in the Pacific Northwest, agronomic regions are being identified for a range of biofuel feedstocks using this spatial description approach. Maps depicting agronomic regions will be laid over base maps featuring economic regions identified by aggregations of counties. This will illustrate the feedstock potential in the vicinity of existing economic regions. Regions then will be selected based on the diversity of feedstocks that can be modeled for economic and social impacts. Feedstock diversity will be evaluated based on potentially economically viable cellulosic crops, as outcomes from the GTL program are expected to impact the viability of these crops in the future.

Abstract Title: Not So Fast Please: New Analysis Gives Much Lower Carbon Debt for Biofuels

Authors: Bruce E. Dale and Seungdo Kim, OE Great Lakes Bioenergy Research Center and Michigan State University

Greenhouse gas release from land use change (the so-called “carbon debt”) has been identified as a potentially significant contributor to the environmental profile of biofuels. The time required for biofuels to overcome this carbon debt due to land use change and begin providing cumulative greenhouse gas benefits is referred to as the “payback period” and has been estimated to be 100-1000 years depending on the specific ecosystem involved in the land use change event. Two mechanisms for land use change exist: “direct” land use change, in which the land use change occurs as part of a specific supply chain for a specific biofuel production facility, and “indirect” land use change, in which market forces act to produce land use change in land that is not part of a specific biofuel supply chain, including, for example, hypothetical land use change on another continent.

Existing land use change studies did not consider many of the potentially important variables that might affect the greenhouse gas emissions of biofuels. We examine here several variables that have not yet been addressed in land use change studies. Our analysis shows that cropping management is a key factor in estimating greenhouse gas emissions associated with land use change. Sustainable cropping management practices (no-till and no-till plus cover crops) reduce the payback period to 2 years for the grassland conversion case and to 14 years for the forest conversion case. It is significant that no-till and cover crop practices also yield higher soil organic carbon (SOC) levels in corn fields derived from former grasslands or forests than the SOC levels that result if these grasslands or forests are allowed to continue undisturbed.

The United States currently does not hold any of its domestic industries responsible for its greenhouse gas emissions. Thus the greenhouse gas standards established for renewable fuels such as corn ethanol in the Energy Independence and Security Act (EISA) of 2007 set a higher standard for that industry than for any other domestic industry. Holding domestic industries responsible for the environmental performance of their own supply chain, over which they may exert some control, is perhaps desirable (direct land use change in this case). However, holding domestic industries responsible for greenhouse gas emissions by their competitors worldwide through market forces (via indirect land use change in this case) is fraught with a host of ethical and pragmatic difficulties. Greenhouse gas emissions associated with indirect land use change depend strongly on assumptions regarding social and environmental responsibilities for actions taken, cropping management approaches, and time frames involved, among other issues.

Developed proteomics scale solution X-ray scattering (SAXS) tools applied to metabolic networks of interest from the MAGGIE program project.

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Abstract:

Macromolecular functions involve shape-dependent interactions; yet, even structural genomics initiatives (SGI) are challenged by the scale of metagenomic and proteomic results. Here we present an efficient pipeline enabling proteomics-scale small angle X-ray scattering (SAXS) analyses to rapidly interrogate macromolecular structures in physiologically-relevant solution contexts. We tested this pipeline on 16 representative recombinant proteins from *Pyrococcus furiosus*. The results revealed 11 (69%) multimeric structures in solution, distinguished aggregated and unfolded proteins, defined global structural parameters and oligomeric states for most samples, disclosed shapes and similar structures for all five unknown structures, and described envelopes for 14 proteins (87%). The pipeline combines automated sample handling of micro-liter volumes, temperature and anaerobic control, rapid data collection, data analysis decision tree, and coupled structural analyses with automated archiving. SAXS with high throughput capabilities promises to be an important enabling technology that may change the way SGI and proteomics research is done.

In addition to the proteins described above several of the MAGGIE components are dedicated to purifying protein complexes from native bio-mass. We are applying the described SAXS pipeline to pathways of importance. Of particular interest are the glycolytic pathway of *Pyrococcus furiosus* which is coupled to Hydrogenase and the catalytic cycle of the thermosome in *Sulfolobus Solfataricus*. The analysis of several complexes from these systems are also described here.

Growth in Biofuel Markets: Long Term Environmental & Socioeconomic Impacts

Nicholas Kalaitzandonakes, Seth Meyer and Wyatt Thompson, with James Kaufman presenting.
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Introduction

Over the last few years, concerns over volatile oil prices, energy security, and the environment have motivated policy mandates and aggressive goals in the US and EU. However, it is quickly becoming evident that the biofuel agendas set by US and EU policymakers will require complex adjustments in order to best deliver on their intended goals. For example, these adjustments could have unanticipated environmental and social consequences in land utilization and deforestation, as well as increased food prices and concomitant changes in food consumption. At the same time, these impacts could be eased or reversed by the introduction of promising innovations in the production of agricultural commodities and their conversion into biofuels. To delineate these impacts from current policy mandates, one must sort out the complex interrelationships that exist in the global production and distribution of relevant agricultural commodities. To this end, the project leverages existing FAPRI models and constructs a model specifically designed to evaluate international biofuel markets.

Research Results

The poster highlights a selection of interesting findings from recent projects categorized here into three main areas:

The effects of US policy on national and international agricultural markets

Wyatt Thompson, Seth Meyer, Nicholas Kalaitzandonakes, & James Kaufman consider the potential expiration of the ethanol tariff and the change of the RFS mandate to increase the use of feedstocks other than corn (including imported sugar-based ethanol). Back-of-the-envelope analysis leads to an expectation of greater imported ethanol, less domestically produced ethanol, and, thus, a lower impact on corn prices. However, the interaction between commodity markets and policies produce some unintuitive results that suggest the complexity of moderating such price impacts.

Thompson, Meyer, and Pat Westhoff (2009, February) investigate how biofuel-use mandates affect agricultural market volatility. They find the biofuel-use mandates of the Energy Independence and Security Act of 2007 can sever the links across markets or exacerbate the effects of some factors that cause variability.

Thompson, Meyer, and Westhoff (2008, December) look at the biofuel and commodity markets through the market for Renewable Identification Numbers (RINs). Using an economic model that represents the supply and demand for biofuels, the value of RINs is estimated under various market conditions, including changes in petroleum prices and

corn yields. Further experiments are conducted to consider how different regulations to implement US policies could affect markets, as measured by RIN values.

The effects of technology on the sustainability and impact of biofuel use

Biotechnology offers the potential to increase the efficiency of corn production as well as the conversion of corn to ethanol. Kalaitzandonakes, Kaufman, Meyer and Thompson (2009) find that these impacts are noteworthy in improving the energy balance, petroleum consumption, and GHG use of corn ethanol. Kalaitzandonakes, Kaufman, Meyer and Thompson (2008) find that such technologies can facilitate increased ethanol production, while easing pressure on land and corn prices. However, policies such as the RFS have the potential to complicate the realization of benefits, potentially leading to lowered technological adoption by the corn ethanol industry.

The effects of policy changes on international land-use

Thompson, Meyer, and Westhoff (2009) find that simulation model analysis over a range of parameters governing the responsiveness of land use to relative prices leads to a corresponding range of possible land effects following a hypothetical change in US biofuel policy. Moreover, the results are sensitive to other model parameters. For example, Brazilian ethanol exports to the US can be increased in response to a higher US price either by greater production or lower consumption -which in practice is likely to be some combination of the two.

Selected References

- Wyatt Thompson, Seth Meyer, Nicholas Kalaitzandonakes, & James Kaufman. Ethanol Policy Changes to Ease Pressures in Corn Markets: Could They Work?
- Wyatt Thompson, Seth Meyer, & Pat Westhoff. (2009, February). "How Does Petroleum Price and Corn Yield Volatility Affect Ethanol Markets with and without an Ethanol Use Mandate?" *Energy Policy*, 37(2), 745-749.
- Wyatt Thompson, Seth Meyer, & Pat Westhoff. (2008, December). "Mandate Economics Applied to US Biofuel Policies." Presented at the annual meeting of the International Agricultural Trade Research Consortium. Scottsdale, AZ.
- Nicholas Kalaitzandonakes, James Kaufman, Seth Meyer, & Wyatt Thompson. (2009). The Impact of Biotech Corn Traits on Ethanol Production. In M. Khanna (Ed.), *Transition to a Bioeconomy: Environmental and Rural Development Impacts* (Proceedings of Farm Foundation/USDA Conference, St. Louis, Missouri, October 15-16, 2008). Oak Brook, IL: Farm Foundation.
- Nicholas Kalaitzandonakes, James Kaufman, Seth Meyer, & Wyatt Thompson. (Forthcoming). "Technologies for Biofuel Production and Relevant Impacts. Invited Paper for conference: Economics of Ethanol: Costs, Benefits and Future Prospects of Biofuels." Federal Reserve Bank and Washington University, November 13th, 2008.
- Wyatt Thompson, Seth Meyer, & Pat Westhoff. (2009). "Policy Risk for the Biofuels Industry." In B. English (Ed.), *Risk, Infrastructure and Industry Evolution* (Proceedings of Farm Foundation/ USDA Conference, Berkeley, California, June 24-25, 2008), Oak Brook, IL: Farm Foundation.

Science Literacy Training for Public Radio Journalists
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To help radio journalists explain accelerating scientific advances and their profound social implications, SoundVision is offering another series of Science Literacy Training workshops. The goal of these workshops is to shrink the widening knowledge gap between the scientific community and the general public by increasing the quality and number of science stories on the radio and the web. During the project's three weeklong workshops and one weekend session, experts will teach public radio reporters about science, science journalism and the creative use of radio and online technologies to produce compelling and accurate science stories.

SoundVision selects twelve applicants to attend each weeklong Science Literacy Training intensive. We continue to hear from more impressive candidates than we can accommodate, including journalists from NPR, *The World*, *Studio 360*, *Living on Earth*, *Radio Lab* and the major market radio stations in the United States. We've even attracted inquiries from as far away as Singapore and China. To be eligible, applicants must have contributed frequently to news or public affairs programs on national, regional or local public radio outlets and represent stations or programs that reach broad and diverse audiences. As a result of broadcasters' growing commitment to science stories, the majority of our recent applicants are now science, environmental, health or technology reporters. We've also had a large influx of young Internet-savvy news people applying to the program, some of whom work exclusively in their stations' online departments, and we've broadened the curriculum to include more Internet reporting and production techniques. In addition, we've created an intensive weekend workshop to accommodate reporters and producers from small, often ethnic or rural, stations that can't spare key staff members for a full week. The first weeklong workshop in this series is scheduled for April 2009 in the San Francisco Bay Area.

Curriculum: Workshop leaders train participants in several key areas: science, including basic biology, chemistry, physics, statistics and nanotechnology, with a strong focus on energy-related issues; science journalism, including how to interview scientists, how to handle controversy and the standards and ethics of science reporting; radio craft, from story telling to audio and production tools, and web techniques and podcasting. Participants also go on field trips, attend informal gatherings with scientists and learn creative ways to deal with the unique limitations and advantages of radio and web production. Session leaders also teach participants how scientific methods differ from journalistic practices and show them how to explore new research and fact check stories on tight deadlines. Finally online librarians show journalists how to make the best use of the Internet for research and identify reliable sources online.

The Science Literacy Training project also includes a web site that provides online resources, "tip sheets," and transcripts and selected audio from the training sessions. (For sample Tip Sheets, see www.scienceliteracyproject.org.)

Major stations around the country are eager to host the workshops, but considering the time, expense and logistical challenges of planning a workshop in a new city, it might be most productive and cost-effective to hold most or all of the workshops at previous conference sites in San Francisco, Boston and Austin, Texas, and possibly to schedule the workshops at a full-service conference center.

Evaluation: Our workshops are evaluated by Rockman *et al*, a well-established San Francisco firm with expertise in assessing media projects and the impact of training on journalistic practice. Pre-workshop evaluations help us tailor presentations to participants' needs, and as part of our daily evaluations during the workshops, we ask participants after each presentation to list the key scientific concepts they have just learned to find out what scientific information they've retained.

In previous post-project reviews we've learned that many radio reporters return to their stations and give their own workshops using some of our handouts. They also produce stories using the tools and techniques they acquired in the workshops, and a large number keep in touch with each other, sharing editorial advice and support. Most important, at the end of the Science Literacy Training workshops, participants feel confident that they can handle complex scientific stories well. "I am a better reporter because of what I learned at that workshop," one said. "I use what I learned there almost every day."

Targeting Bioenergy, Environment and New Protein Families: Community Nominated JGI/MCSG Structural Genomics Pilot Project

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The structural genomics high-throughput pipeline developed at the Midwest Center for Structural Genomics (MCSG) as a part of the Protein Structure Initiative (PSI) comprises: (1) classifying all available genomic sequences to establish a prioritized target set of proteins, (2) cloning and expressing proteins of microbial and eukaryotic origin, (3) purifying and crystallizing native and derivatized proteins for X-ray crystallography, (4) collecting data and determining structures, (5) analyzing structures for fold and function assignment, and homology modeling of related proteins. The structural genomics pipeline takes advantage of significant advances in molecular and structural biology including synchrotron facilities, dedicated PX beamlines, advanced software and computing resources. The long-term goal of PSI is to provide structural coverage of major protein superfamilies with sufficient granularity to allow 3D homology modeling of all proteins using only computational methods. The ultimate goal is to build a foundation for 21st century structural biology where the structures of virtually all proteins will be found in the Protein Data Bank (PDB) or derived by computational methods. The structural genomics technologies can be applied to a wide range of protein targets and are very well suited for proteins originating from microbial communities. The majority of MCSG targets are from large protein families with no structural representative, biomedically important pathogens and higher eukaryotes, metagenomics projects and also include community nominated targets. Recently the Joint Genome Institute (JGI) User Community and JGI scientists nominated a large set of targets from more than 50 microbial species. Nearly 70 scientists proposed more than 700 targets for structure determination. The list of species include many relevant to DOE missions, namely important in global carbon cycling, microbial communities or single species that play a role in the degradation of lignocellulosic material, targets from species with large metabolic potential, as well as targets from new protein families discovered as a part of the Genomic Encyclopedia of Bacteria and Archaea (GEBA) project. This poster will summarize the results from the first batch of targets processed to date. Some of these proteins are targeted to bacterial micro-compartments and the structural biology provides an in-depth insight into the function and potential mechanism of action of these proteins.

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The DNA Files®

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SoundVision's highly acclaimed radio series *The DNA Files III*® was National Public Radio's most-broadcast news series last year. The series, which makes complex science stories clear and exciting to a general audience, was aired on more than 300 stations around the United States—nearly twice as many as originally planned—in nine out of ten major U.S. markets. It reached listeners from downtown Manhattan to Alaska's Kenai Peninsula and was broadcast internationally in Australia, New Zealand, Canada, Berlin, and even Tbilisi in the Republic of Georgia. Our outreach partner Radio Bilingue made *The DNA Files* available in Latin America; NPR aired it on their international service, and Armed Forces Radio broadcast it to servicemen and women around the world.

The DNA Files series includes five hour-long, nationally distributed public radio documentaries: *Beyond Human*; *The Heat is On: Evolution in Action*; *Rewriting Heredity: Environment and the Genome*; *Designing the Garden: Food in the Age of Biotechnology*, and *Minding the Brain*. The project also includes support for eleven "outreach partner stations" as they create related local programming and community activities, an ethnic media project, a related science museum workshop with online lesson plans, and an interactive multimedia website.

The DNA Files Online: In response to the explosion of online possibilities, SoundVision created a new, information-packed website that includes downloadable podcasts of our programs and excerpts of interviews with scientists, policy makers and concerned citizens; links to the latest scientific discoveries and issues; audio slide shows; in-depth articles related to the five documentaries, and background information for editors and reporters. It offers program descriptions, transcripts and broadcast information; biographies of our interviewees, producers and staff members; conversations between leading scientists; photographs, video and downloadable audio; producer commentaries, and reports from our ethnic media and station partners. In addition, we plan to add more interactive question-and-answer sessions with scientists like our provocative week-long feature "Chimp Chat," an offshoot of the "Beyond Human" program. Introduced by the startling question, "If my DNA is 98.8 percent chimp, am I just 1.2 percent human?" Chimp Chat enabled web site visitors to ask researchers about the similarities and differences between chimpanzees and human beings.

Outreach Partner Stations: To help local radio journalists make science stories clear and relevant to their diverse rural and urban audiences, SoundVision chose eleven outreach partner stations around the country and gave them \$12,000 to produce their own *DNA Files*-related stories and develop community activities that would interest their audiences in science.

To get them started, we held an intensive three-day science journalism workshop for participating journalists (and several ethnic media representatives) that gave them a stronger foundation in the basics of science, genetics, and science reporting as well as a deeper enthusiasm for and commitment to the project. According to one participant, "I received more formal training in these two days than in the past ten years." In the months that followed, we provided individual support and coaching as the energized partners tackled far more ambitious projects than we'd expected. When they'd finished, our eleven outreach partner stations and other associates had produced more than 40 science-related features (typically run during *Morning Edition* and *All Things Considered*) and another eight hours of specials. They also coordinated their stories with local newspapers, developed a full-featured dedicated web presence (making sure the content and methods of distributing stories were also relevant to younger and minority audiences), and developed partnerships with a total of 39 other organizations.

Community activities: Our outreach partner stations also sponsored a wide range of successful activities including a series of large standing room only community events; forums hosted by John Hockenberry in Philadelphia and Ira Flatow in San Antonio; field trips, including one sponsored by WBEZ-Boston that enabled 175 inner-city students to work with scientists featured in *The DNA Files* in their labs; science

camp for 100 rural teens in upstate New York, and a Utah program helping students produce on-air science news stories. And after Radio Bilingue held a *DNA Files*-related genetics career event at California State University in Fresno, a number of students called the school to ask about enrolling.

Maybe most important, our Outreach Partners have been changed and inspired by *The DNA Files* Outreach Program. Several, including WSIU in Carbondale, Illinois, the Radio Bilingue network and Georgia Public Broadcasting, have significantly increased the time and resources devoted to science stories, while Chicago Public Radio, WHYI in Philadelphia and KWMU in St. Louis have hired full-time science and technology reporters. The new KWMU reporter is the only science radio journalist in the St. Louis metropolitan area.

The impact of our program continues to expand. When the Corporation for Public Broadcasting mandated that public television's National Center for Outreach promote and support public radio outreach efforts they used *The DNA Files*' outreach program as a model.

Ethnic Media Fellowships: SoundVision awarded \$500 fellowships to ten journalists from ethnic print media or wire services to write 750-word articles on *The DNA Files*-related issues for their outlets. New America Media (NAM) helped us select journalists to compete for the grants and we provided them with editorial assistance and training in the basic protocols of science journalism. Their stories have appeared in ethnic media serving Chinese, Vietnamese, African-American and Latino communities throughout the country and around the world.

Educational Programs: San Francisco's world-renowned Exploratorium science education museum translated *The DNA Files* content into five *DNA Files*-related workshops for use in science centers and museums around the country, and modified four of them for online use by parents, teachers, home schoolers and other educators who lack the equipment available to museums.

Evaluation: After conducting online user surveys and focus groups to gauge their understanding and retention of *The DNA Files III*'s key themes, an independent evaluation firm concluded that the series had "significantly increased" listeners' understanding of the material covered in the programs. They concluded the series' style and format were highly effective.

The DNA Files has won numerous awards, including the George Foster Peabody Award, the Alfred I. DuPont-Columbia University Award, the American Association for the Advancement of Science Journalism Award, the Robert Wood Johnson Foundation Award, the American Institute of Biological Sciences Broadcast Award, and the Society of Professional Journalists Excellence in Journalism Public Service Broadcast Award.