NEXT GENERATION GREEN SYNTHESIS: EXPERIMENTAL AND THEORETICAL APPROACHES TO NEW ENZYMES, PATHWAYS AND BIOMIMETIC CATALYSTS

Dr. Harold Bright
Office of Naval Research

Dr. Eric Eisenstadt
Defense Advanced Research Projects Agency

March 24-26, 2000

Hilton Washington Dulles Airport
13869 Park Center Road
Herndon, VA 20171

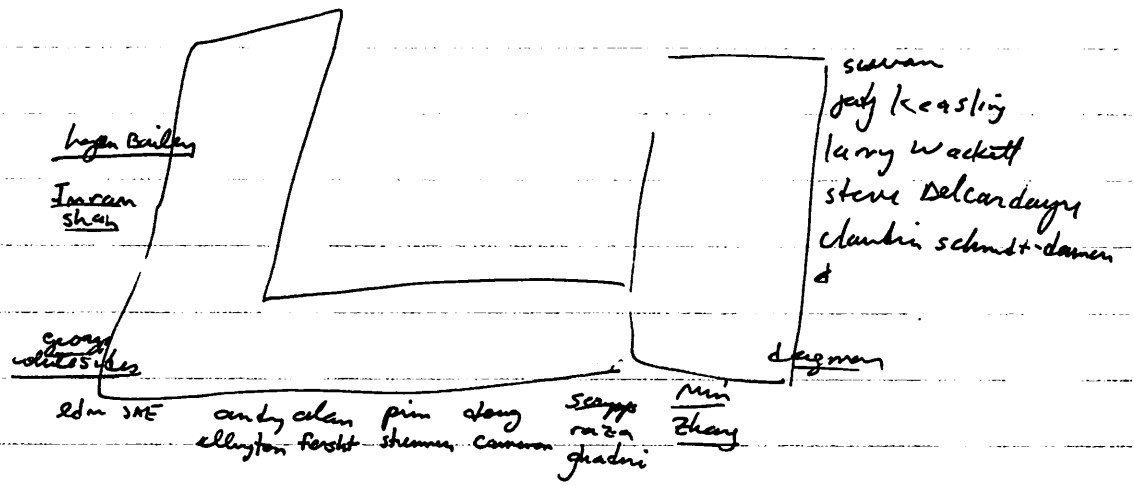
① Genes
② uncultivables
③ why complete
④ how product f(x)

Eric Eisenstadt

Chemicals + Products made by bioprocessing
- biosynthesis main focus
- Andy Ellington asks about biodegradation

Genome
How product f(x)
How understand extremophiles

Harold Bright -
- molecular biosensors at ONR
- sensors, materials, + processes
  - biomimetic catalysts
  - energetic materials
  - microbial fuel cells
- microbial systems
  - bioplastics in microbes - (using them for combinatorial)



genomics — Minimal organism?

cellular engineering to make conditions correct

normal genome

directed evolution

enzymes

pathways

new amino-acids

structural genomics — Sriram Subramaniam to do CryoEM of membrane prots at NCI

Targets

organics

inorganics

biomolecular materials

Other Things

- engineered cells -

- make drugs on-site

- engineered sensors

- bioremediation

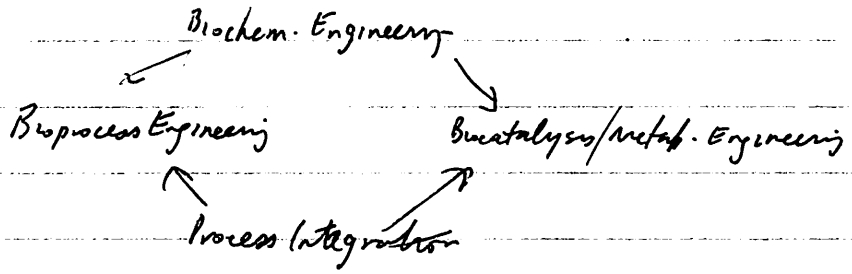
- fuel cells

- weapons

How are pathways segregated w/in cells

Doug Cameron - Cargill - Law + Practice of Biochem. Engineering

- Cargill - large scale producer of various chemicals



J

Biocatalysis

enzymes, cells, whole organisms, communities

Enzymes

- diversity
- enzyme engineering
- directed evolution
- stabilization
- co-factor regeneration

Whole cells

- naturally occurring + mutagenesis/selection
- metabolic engineering
  - host cell or specialized organism
  - pathways + enzymes
- metabolic engineering + mutagenesis/selection (evolutionary engineering)

They have several whole organism group projects.

Bioreactor engineering

yield
titer
mass transfer
heat transfer
contamination

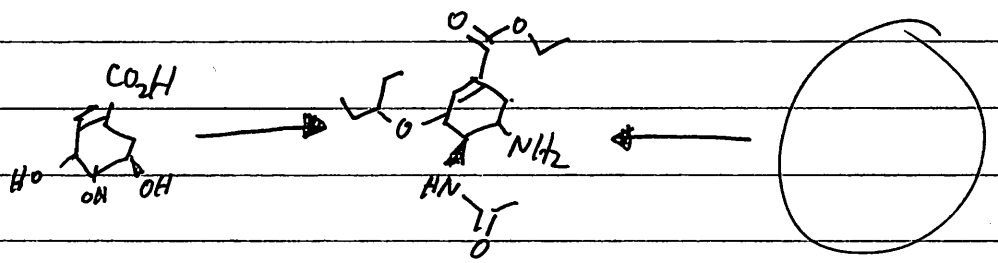
~~synthesis of scarce products from abundant~~

John Frost

1. synthesis of scarce natural products from abundant polyols
2. synthesis of petroleum compounds from renewable carbon

1. Scarce products

- tamiflu (which competes w/ relenza) aka GS4104
- made by roche



shikimic acid — GS4104 — quinic acid

- come from anise in wild ... 1500/kg
- this is what Roche used

- used modification of shikimic acid synthesis pathway

- so did some modifications to maintain plasmid + reduce pathway
- shikimic acid accumulated but so did quinic acid which was bad

Gene + genome shuffling

glucose → → shikimic acid

↓ metabolite

↓

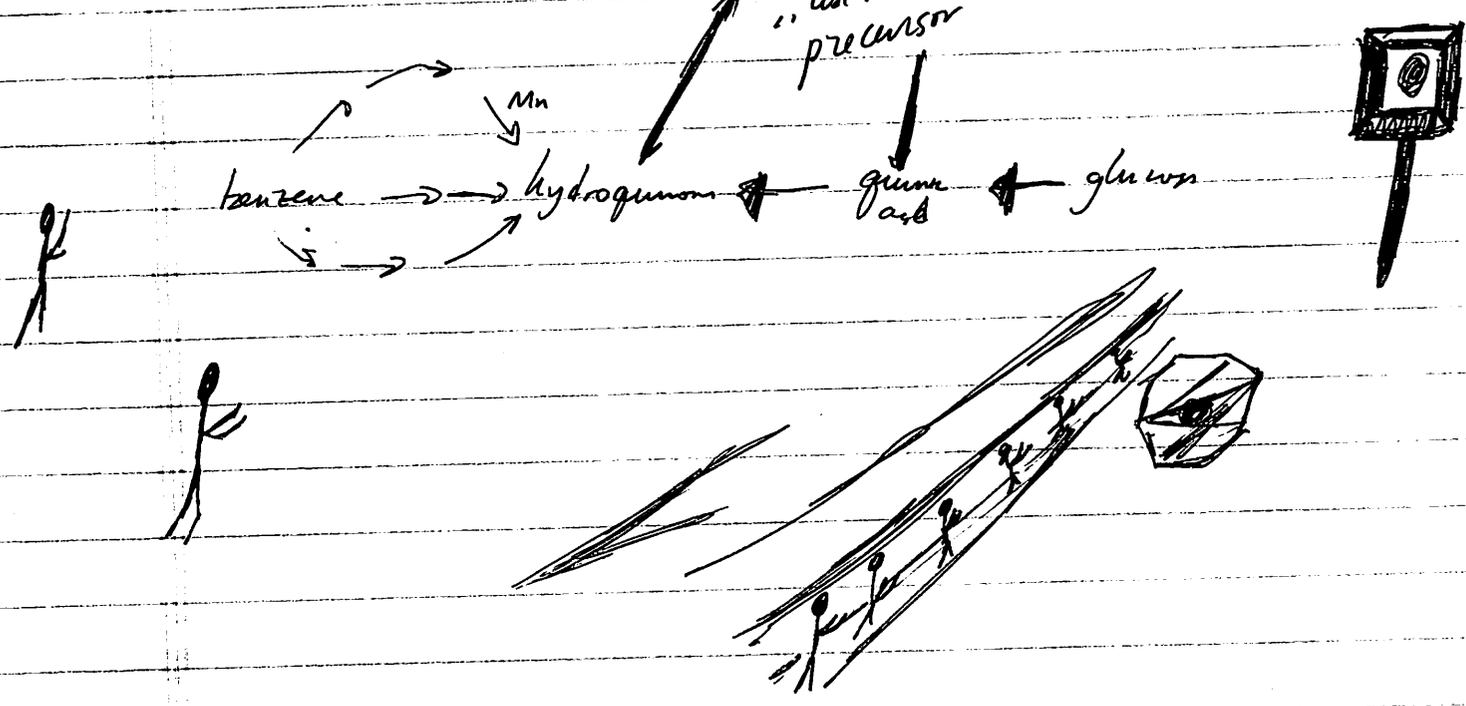
quinic acid

- so use a molecule - that suppresses catabolism of shikimic acid

Biosynthetic diversity

new pathways

novel connections



Amaxcells Ecoli-array

Immobilizing microbial biocatalysts (what is going on at the point of end of synthesis)

- functional genomics
- proteomics

Lignocellulose is a very hard nut to crack

Carbohydrates as a source of C

- e.g. maybe lignocellulose ... want to use things other than starch
- make a plant w/ cellulase engineered into it - and thus could only be active after grinding

Sequencing cellulolytic genes expression and proteomics in small samples

Processing

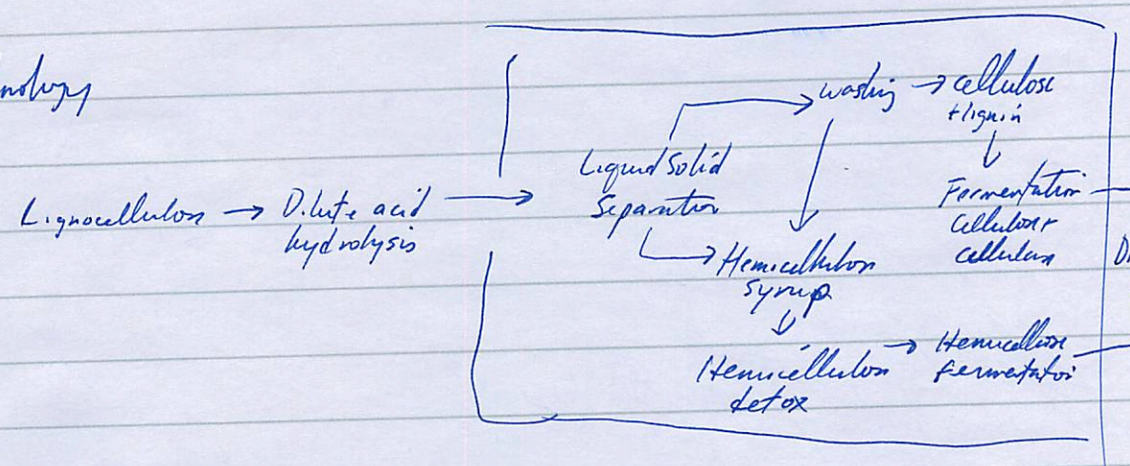
Fermentation / options

Downstream Processing

Process Optimization, miniaturization

If they looked at my post. I would be a dirt. Stay on guy.

A. Current technology



B. Simplification w/ Biocatalysts

Lignocellulose → Dilute acid hydrolysis → Fermentation cellulose + cellulase + hemicellulose syrup → Distillation

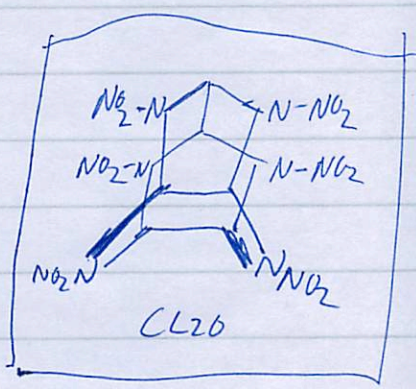
C. Corn → EtOH

Corn → Steam cooker → Fermentation + amylase + ~~glucosylase~~ Glucoamylase → Distillation

ADM → CO_2 from EtOH + Fish shit used for lettuce

Feedback

- put tags into microbes to tell fermenter what to do
- luciferase not good b/c pH dependent
- GFP has 1 hr delay



CL20

How run fermentations under septic conditions

- phage problems
- contamination

Biomass production - what do w/ it - can you feed it back but there is no regulation on use of GMOs for feed to livestock.

Malting - sprout the seed ... and get molecules in media

Corn cob = hard granules

Process optimization

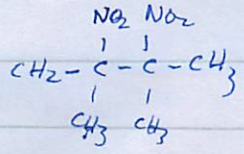
Machinery

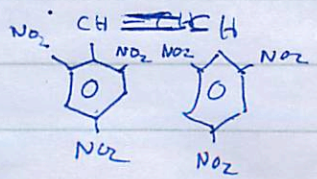
- Bar coding
- Selection in serial
- Libraries of diverse genomes
- Rules for biotransformation

communication testing mobile equipment

Aromatic as synthetic thermophiles

DMNB





HNS

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Time	Activity	Purpose
Friday, 24 March (6-9 PM)	**Arrival and reception**	
Saturday, 25 March		
730	**Continental Breakfast**	
830	**Introductory remarks**	welcome, why we are here, around the table, logistics
	Keynote talks	
845	Hagan Bayley (Texas A&M) Prospects for pathway engineering	To broadly identify research issues and opportunities in metabolic pathway engineering
900	Doug Cameron (Cargill) The language and practice of biochemical engineering	To define terms for the diverse (but technically sophisticated) audience by broadly discussing what we mean by bioprocessing, and drawing comparisons among chemical synthesis via chemistry, enzymology (natural, genetically engineered, or biomimetic), bioreactors and hybrid technologies (combinations of the above)
930	John Frost (Michigan State) Current and imaginable role of biotech in industrial production of chemicals	The future role of biotechnology for chem (and materials) synthesis; introduction to some of the issues that surround (and currently limit) the choice of which biomedium (plants, microbes, biomimetics) and combinations of processes one can use for chemical and materials production
1000	**Break**	
1015	**5 min presentations by invitees (actual order to be determined)**	in which we learn what participants do in relation to metabolic pathway engineering
	Andrew Ellington -- UT Austin	
	Hagan Bayley -- Texas A&M	
	John Frost -- Michigan State	
	Douglas Cameron -- Cargill	
	Jonathan Eisen -- TIGR	
	Alan Fersht -- Cambridge U	
	Reza Ghadiri -- Scripps Institute	
	Jay Keasling -- UC Berkeley	
	Break	
	Dagmar Ringe -- Brandeis	
	Claudia Schmidt-Dannert -- U Minnesota	
	Imran Shah -- ATCC	
	Pim Stemmer/ Steve DelCardayre -- Maxygen	
	Lawrence Wackett -- U Minnesota	

	George Whitesides -- Harvard Min Zhang -- NREL Mark Gerstein -- Yale	
1200	**Lunch**	
Times below are notional; a break will be added between 1400 and 1600	**Facilitated discussions**	
1300	Whitesides and Frost	Useful (from the DOD and national security perspective) things to make via biotechnology
1400	Eisen, Shah, Gerstein	Exploiting bioinformatics and genomics for pathway engineering
1500	Keasling, Schmidt-Dannert, Stemmer, DelCardayre and Zhang	Tools used/needed to engineer organisms to do useful things
1600	Ghadiri, Fersht, Ringe and Wackett	Engineering enzymes via genetics and designing biomimetic catalysts
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Sunday, 26 March		
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830	Frost, Cameron	Bioprocessing: scaling and engineering/production issues
930	Ellington	Cleanup batter; rump session to deal with emerging topics
1030	**wrap-up**	

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Workshop Background: Biocatalytic processing, as opposed to chemical processing, is environmentally friendly because enzymes have ~10Kcal barriers (high temperatures/pressures not required), exhibit high reaction-, regio- and stereo-specificity (protection/deprotection wastes and catalyst poisoning avoided) and control the dielectric constant in their binding pockets (largely eliminating toxic solvents). Performance metrics for the chemical industry in 2020 can only be met through substantial substitution of biocatalytic steps and processes. To meet this challenge there is emerging, on the one hand, bioinformatic modeling of enzymes and pathways through the genomic and structural databases and, on the other, combinatorial gene shuffling protocols of directed evolution and molecular breeding to generate biocatalysts that are precisely tuned for specific processing applications. Enzyme engineering has, until now, been limited to hydrolytic enzymes. The next generation of Green Synthesis, to which this workshop topic is addressed, will greatly expand the reaction repertoire (to include redox and other important transfer processes) as well as exploit the theoretical (including modeling and simulation), evolutionary, and biomimetic protocols now emerging.

Workshop Objective: To characterize gaps in understanding and technology that limit our ability to design enzymes and synthetic pathways. Also, to identify research opportunities that would lead to the creation of new biocatalytic toolboxes for affordable and environmentally friendly synthesis of DoD materials and for energy harvesting processes. The outcome of this workshop is expected to contribute directly to the development of a major new research program at DARPA.

Examples of Research Opportunities: 1) the development and use of predictive tools to identify catalytic properties of enzymes in existing organisms, or theoretical ancestors, from comparative genomic data; 2) the use of directed evolution/molecular breeding, in conjunction with 1), to generate enzymes with novel properties (thermal and solvent stability, tuned substrate/cofactor specificities, resistance to substrate/product inactivation and inhibition, matched pH dependencies); 3) the development of truncated, inexpensive and stable cofactors for group (hydride/phosphoryl/acyl) transfer to replace NAD(P), ATP and CoA, as well as enzymes evolved to use these, and novel schemes for their cyclic regeneration; 4) the assembly of heterologous pathways in conjunction with 1) and 2) for coordinated synthesis (employing *in vitro, in vivo* or permeabilized cell systems) and featuring combinatorial diversification of precursors in the case of polymer synthesis; 5) the development of novel high throughput screens and selections that are generally applicable to target enzymes and pathways; 6) the potential of novel cellular platforms (*e.g.* minimized genomes) for experimental, theoretical (including modeling and simulation) approaches to optimizing pathway design and gene product expression; 7) the use of theoretical and biochemical principles to design and validate biomimetic redox catalysts for alkane activation and H_2 generation and, more generally, to enable hybrid biological/biomimetic processing schemes; 8) the identification of appropriate biological systems and platforms including single cell organisms, animals and plants.

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	Steve DelCardayre -- Maxygen - biocatalysis, shuffling whole genome - protoplast fusion, recursive recombination)	
	Jonathan Eisen -- TIGR	
	Andrew Ellington -- UT Austin	
	Alan Fersht -- Cambridge U	
	John Frost -- Michigan State	
	Mark Gerstein -- Yale genome evolution, comparative genomics	
	Reza Ghadiri -- Scripps Institute non-living → living; need self organization + information transfer; protein-protein interaction v. important	
	Jay Keasling -- UC Berkeley - microbial engineering, controlling gene expression (eg. inducer key & % of cells that induce, not all or nothing)	
	Break	
	Dagmar Ringe -- Brandeis	
	Claudia Schmidt-Dannert -- U Minnesota - molecular breeding of synthetic pathways	
	Imran Shah -- ATCC	
	Pim Stemmer -- Maxygen	
	Lawrence Wackett -- U Minnesota - do unrecognizable genes encode enzymes or old ones)	

	George Whitesides -- Harvard Min Zhang -- NREL - EtOH production + other	renewable sources, may need to adapt strain
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PROSPECTS FOR PATHWAY ENGINEERING

Hagan Bayley, ***Texas A&M University***

NOTES:

THE LANGUAGE AND PRACTICE OF BIOCHEMICAL ENGINEERING

Doug Cameron, ***Cargill, Inc.***

NOTES:

CURRENT AND IMAGINABLE ROLE OF BIOTECH IN INDUSTRIAL PRODUCTION OF CHEMICALS

John Frost, *Michigan State University*

NOTES:

Name: Hagan Bayley

Title: Professor and Head

Organization: Department of Medical Biochemistry and Genetics
Texas A&M University System Health Science Center
TAMU 1114
College Station, TX 77843-1114

Current Position:

Areas of Interests:

HAGAN BAYLEY is Professor and Head in the Department of Medical Biochemistry & Genetics at Texas A&M University, where he is also a Professor of Chemistry. He enjoys working at the interface of chemistry and biology by, for example, developing techniques for protein modification that have applications in both basic science and biotechnology. He received his B.A. in chemistry from the University of Oxford in 1974, while at Balliol College, and his PhD. in chemistry from Harvard University in 1979 in the laboratory of Jeremy Knowles. After postdoctoral work with Gobind Khorana at the Massachusetts Institute of Technology, he was on the faculty at Columbia University and the University of Oxford. From 1988 to 1996, he was at the Worcester Foundation for Experimental Biology in Shrewsbury,Massachusetts.

photo: www.chem.tamu.edu/faculty/bayley
more information: bletchley.tamu.edu/homepage

Recent Publications:

Song, L., Hobaugh, M.R., Shustak, C., Cheley, S., Bayley, H., and Gouaux, J.E. Structure of staphylococcal -hemolysin, a heptameric transmembrane pore. Science, 274, 1859-1865 (1996)

Braha, O., Walker, B., Cheley, S., Kasianowicz, J.J., Song, L. Gouaux, J.E., and Bayley, H. Designed pores as components for biosensors. Chemistry & Biology 4, 497-505 (1997)

Cao, Q., Wang, Y., and Bayley, H. Sequence of abductin, the molluscan "rubber" protein. Current Biology, 7, R677- R678(1997)

Chang, C-Y., Fernandez, T., Panchal, R., and Bayley, H. A caged catalytic subunit of cAMP-dependent protein kinase, J. Am. Chem. Soc. 120, 7661-7662 (1998).

Gu, L., Braha, O., Conlan, S. Cheley, S. and Bayley, H. Stochastic sensing of organic analytes by a pore-forming protein containing a molecular adapter, Nature 398, 686-690 (1999)

Eroglu, A., Russo, M.J., Bieganski, R., Fowler, A., Cheley, S., Bayley, H. and Toner, M. Intracellular trehalose improves the survival of cryopreserved mammalian cells. Nature Biotechnology 18, 163-167 (2000).

Name: Douglas C. Cameron

Title: Director of Biotechnology

Organization: Cargill, Inc., Minneapolis, MN

Current Position:

I manage a newly formed biotechnology research group in Cargill Central Research. The group focuses microbial strain development and metabolic engineering. The group works closely with Cargill business units in industrial chemicals, feed, food and nutraceuticals.

Areas of Interest:

I am interested in the basic and applied aspects of metabolic engineering, genomics, bioinformatics, and microbial physiology. I am also interested in hybrid metabolic engineering/evolutionary methods in strain development.

Recent Publications:

Skraly, F.A., B.L. Lytle, and D.C. Cameron. 1998. Construction and characterization of a 1,3-propanediol operon. Appl. Environ. Microbiol., 64:98-105.

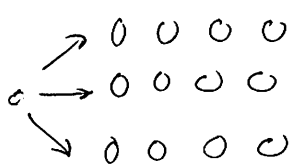
Sauer, U., D.C. Cameron, and J.E. Bailey. In 1998. Metabolic capacity *of Bacillus subtilis* for the production of purine nucleotides, riboflavin, and folic acid. Biotech. Bioeng., 59:227-238.

Cameron, D.C., N.E. Altaras, M.L. Hoffman, and A.J. Shaw. 1998. Metabolic engineering of propanediol pathways. Biotechnol. Prog., 14:116-125.

Altaras, N.E. and D.C. Cameron. 1999. Metabolic engineering of a 1,2-propanediol pathway in *Escherichia coli*. Appl. Environ. Microbiol., 65: 1180-1185.

Continuous growth

- chemostat
- turbidostat (feedback mechanism prevents washout)
- serial culture - automated, can do in parallel



Name: **Stephen B. delCardayré**

Title: **Group Leader Whole Genome Shuffling – Chemicals/Core Technology**

Organization: **Maxygen**

Current Position:

Lead research to develop and apply methods for the rapid evolution of whole cells and organisms. We are integrating gene, pathway, and genome shuffling to create robust industrial microorganisms having novel or improved metabolic pathways.

Areas of Interest:

Technically I am interested in enzymes, enzyme evolution, microbial biochemistry, metabolic engineering, genetics, and molecular evolution. This includes the mechanisms by which new enzyme catalysts arise and are assembled to form functional metabolic pathways, as well as how cells rapidly adapt to new chemical and physical challenges. My primary interest is harnessing the power of biological catalysis from enzymes and whole cells for useful chemical applications.

Recent Publications:

Ness, J., del Cardayré, S.B., Minshull, J. and Stemmer, W.P.C. (2000) "Molecular Breeding – The Natural Approach to Protein Design" In Evolutionary Approaches to Protein Design, A Volume of Advances in Protein Chemistry (in press).

del Cardayré, S.B., et. al. Evolution of Whole Cells and Organisms by Recursive Sequence Recombination. PCT/WO 00/04190.

del Cardayré, S.B., Zhang, Y.X., Huisman, G.W. (2000) Generating New Biocatalysts by Molecular Breeding. Presentation for the National Meeting of the American Chemical Society, San Francisco, CA, March 26-30th, 2000.

Name: Jonathan A. Eisen

Title: Assistant Investigator

Organization: The Institute for Genomic Research

Current Position: Assistant Investigator
The Institute for Genomic Research
Department of Microbial Genomics

Areas of Interest:

Molecular evolution, evolution of multigene families

DNA repair and mechanisms of adaptation to extreme environments

Microbial genomics, evolution of genomes, genome annotation

Recent Publications:

Eisen JA, Hanawalt PC. 1999. A phylogenomic study of DNA repair genes, proteins, and processes. Mutation Research 435(3): 171-213.

White O, Eisen JA, Heidelberg JF, Hickey EK, Peterson JD, Dodson RJ, Haft DH, Gwinn ML, Nelson WC, Richardson DL, Moffat KS, Qin H, Jiang L, Pamphile W, Crosby M, Shen M, Vamathevan JJ, Lam P, McDonald L, Utterback T, Zalewski C, Makarova KS, Aravind L, Daly MJ, Minton KW, Fleischmann RD, Ketchum KA, Nelson KE, Salzberg SL, Smith HO, Venter JC, Fraser CM. 1999. Genome sequence of the radioresistant bacterium Deinococcus radiodurans R1. 1999. Science 286: 1571-1577.

Eisen JA.1999. Mechanistic basis of microsatellite instability. In Microsatellites: Evolution and Application (DB Goldstein and C Schlotterer, eds). Oxford University Press, Oxford.

Eisen JA. 1998. Phylogenomics: improving functional predictions for uncharacterized genes by evolutionary analysis. Genome Research 8(3): 163-167.

Name: Andrew D. Ellington

Title: Associate Professor/ Biochemistry and Chemistry

Organization: University of Texas at Austin

Current Position:

Associate Professor of Chemistry and Biochemistry. Undergraduate advisor for nine undergraduates. Graduate advisor for sixteen graduate students.Post-doctoral advisor for two post-doctoral fellows.

Areas of Interest:

Evolutionary engineering of nucleic acids, proteins, and organisms. We select binding species (aptamers) and catalysts (ribozymes) from random sequence nucleic acid pools. The binding species are engineered to function as biosensors. We are similarly screening and selecting allosteric enzymes as diagnostic reagents. We have most recently begun efforts to engineer the monomer chemistry of entire organisms (bacteria).

Recent Publications:

Matsumura, I., Wallingford, J.B., Surana, N.K., Vize, P.D., and Ellington, A.D. (1999) Directed evolution of the surface chemistry of beta-glucuronidase. *Nature Biotechnology*, **17**(7):696-701.

Robertson, M.P. and Ellington, A.D. (1999) *In vitro* selection of an allosteric ribozyme that transduces analytes into amplicons. *Nature Biotechnology*, **17**(1): 62-66.

Robertson, M.P. and Ellington, A.D. (2000) Design and optimization of effector-activated ribozyme ligases. In press, *Nucleic Acids Research.*

Jhaveri, S., Kirby, R., Conrad, R., Magion, E.J., Glick, G., Ellington, A.D. (2000) Signaling aptamers. In press, *JACS*.

"Irrational" methods

Function based selection
- augmentation
- unnatural organisms

I can cycle them (selection and mutation)

NEED BETTER WAYS TO DO GENOME EVOLUTION

USING NON-UNIFORM DISTRIBUTION OF PRIMERS TO REARRANGE

- Emergent Properties
they you cannot predict

Name: Alan Fersht

Title: Professor

Organization: University of Cambridge

Current Positions:

Herchel Smith Professor of Organic Chemistry; Director of the Cambridge University/Medical Research Council Centre for Protein Engineering.

Areas of Interest:

Protein Folding, Stability and Design. Protein Misfolding and Cancer. Protein Refolding Technology.

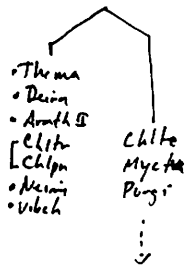
Recent Publications:

Directed evolution of new catalytic activity using the a/b-barrel scaffold

Myriam M. Altamirano, Jonathan M. Blackburn, Cristina Aguayo and Alan R. Fersht Nature 403, 617-622 (2000).

Oxidative Refolding Chromatography: Folding of the Scorpion Toxin Cn5
M. M. Altamirano, C. García, L. D. Possani and A. R. Fersht
Nature Biotechnology 17, 187-191 (1999).

Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding (W. H. Freeman & Co., 1998)



Cannot design enzymes de-novo still b/c don't understand structure-f(x).

Too many mutants to assay.

If you burn all your bridges you don't have to burn anything.

Name: John W. Frost

Title: Professor

Organization: Michigan State University

Current Position:

Professor with joint appointment in the Department of Chemistry and Department of Chemical Engineering

Areas of Interests:

Frost group research focuses on the integration of microbe-based biocatalysis with organic chemistry. The goal is to create new synthetic routes to ultrafine, fine, pseudocommodity, and commodity chemicals based on the use of nontoxic carbohydrate starting materials derived from renewable starch, hemicellulose, and cellulose

Recent Publications:

"Shikimic Acid and Quinic Acid: Replacing Isolation from Plant Sources with Recombinant Microbial Biocatalysis" Draths, K. M.; Knop, D. R.; Frost, J. W. J. Am. Chem. Soc. 1999, 121, 1603.

"Fed-Batch Fermentor Synthesis of 3-Dehydroshikimic Acid Using Recombinant Escherichia coli" Li, K.; Mikola, M.R.; Draths, K. M.; Worden, R. M.; Frost, J. W. Biotechnol. Bioeng. 1999, 64, 61.

Name: Mark Gerstein

Title: Asst. Prof.

Organization: Yale University

Current Position:

Teach and do research in Molecular Biophysics and Biochemistry Dept -- also, joint appointment in CS Dept.

Areas of Interest:

We do research in the new field of bioinformatics. Broadly, we are interested in large-scale analyses of the rapidly expanding number of genome sequences and macromolecular structures. It is hoped that these will allow us to address a number of overall, statistical questions about proteins, relating to their physical properties, cellular function, and phylogenetic distribution. More specifically, we have two research foci. The first is comparative genomics. We compare genomes in terms of protein folds, biochemical pathways, and patterns of gene expression. We are also developing methods to cluster proteins into fold families and predict structure and function from sequence similarity. The second focus is the analysis of macromolecular geometry. Specific Interest in the following topics: Bioinformatics, Genomics, Whole-genome Expression Analysis, Structure Analysis, Molecular Simulation, Packing, Macromolecular Motions, Tree Construction, Structural Genomics, Metabolic Pathways.

Recent Publications:

Wilson CA, Kreychman J, Gerstein M. J Mol Biol 2000 Mar 17;297(1):233-249
Assessing Annotation Transfer for Genomics: Quantifying the Relations between Protein Sequence, Structure and Function through Traditional and Probabilistic Scores.

Jansen R, Gerstein M. Nucleic Acids Res 2000 Mar 15;28(6):1481-1488
Analysis of the yeast transcriptome with structural and functional categories: characterizing highly expressed proteins.

H Hegyi & M Gerstein (1999). "The Relationship between Protein Structure and Function: a Comprehensive Survey with Application to the Yeast Genome," J Mol. Biol. 228: 147-164.

M Gerstein & H Hegyi (1998). "Comparing Microbial Genomes in terms of Protein Structure: Surveys of a Finite Parts List," FEMS Microbiology Reviews 22: 277-304

M Gerstein (1997). "A Structural Census of Genomes: Comparing Bacterial, Eukaryotic, and Archaeal Genomes in terms of Protein Structure," Journal of Molecular Biology 274: 562-576.

Name: M. Reza Ghadiri, Ph.D.

Title: Professor

Organization: The Scripps Research Institute

Current Position:

Departments of Chemistry and Molecular Biology and Member of Skaggs Institute for Chemical Biology

Areas of Interest:

Design of functional artificial metalloproteins and enzymes, peptide nanotubes and related biomaterials, artificial transmembrane ion and molecular channels, transition metal nanocomposite and catalyst engineering, biosensors, self-replicating molecular systems, self-organized nonlinear chemical networks, and the study of the early events of protein folding.

Recent Publications:

Bong, D. T.; Steinem, C.; Janshoff, A.; Johnson, J. E.; Ghadiri, M. R. "A Highly Membrane Active Peptide in Flock House Virus: Implications for the Mechanism of Nodavirus Infection". *Chem. Biol.* **1999**, *6*, 473-481.

Clark, T. D.; Kobayashi, K.; Ghadiri, M. R. "Covalent Capture and Stabilization of Cylindrical sheet Peptide Assemblies", *Chem.Eur. J.* **1999**, *5*, 782-792.

Janshoff, A.; Dancil, K.-P. S.; Steinem, C.; Greiner, D. P.; Lin, V. S.-Y.; Gurtner, C.; Motesharei, K.; Sailor, M. J.; Ghadiri, M. R. "Macroporous *p*-Type Silicon Fabry-Perot Layers. Fabrication, Characterization, and Applications in Biosensing", *J. Am. Chem. Soc.* **1998**, *120*, 12108-12116.

Hartgerink, J. D.; Ghadiri, M. R. "Peptide Nanotubes and Beyond", *Chem. Eur. J.* **1998**, *4*, 1367-1372.

Lee, D. H.; Severin, K.; Ghadiri, M. R. "Autocatalytic Networks: The Transition From Molecular Self-Replication to Ecosystems", *Curr. Op. Chem. Biol.* **1997**, *1*, 491-496.

Severin, K.; Lee, D. H.; Kennan, A. J.; Ghadiri, M. R. "A Synthetic Peptide Ligase", *Nature* **1997**, *389*, 706-709.

Name: Jay D. Keasling

Title: Associate Professor

Organization: University of California at Berkeley

Current Position:

Associate Professor of Chemical Engineering at the University of California at Berkeley

Areas of Interest:

The research in the Keasling Laboratory focuses on the metabolic engineering of microorganisms for degradation of environmental contaminants or for environmentally friendly synthesis. To that end, we have developed a number of new genetic and mathematical tools to allow more precise and reproducible control of metabolism. These tools are being used in such applications as synthesis of biodegradable polymers, accumulation of phosphate and heavy metals, and degradation of chlorinated and aromatic hydrocarbons, biodesulfurization of fossil fuels, and complete mineralization of organophosphate nerve agents and pesticides.

Recent Publications: (3-4 sentences)

J. D. Keasling. 1999. "Gene-expression tools for the metabolic engineering of bacteria." *Trends in Biotechnology* **17**:452-460.

P. L. Trelstad, P. Purdhani, W. Geibdorfer, W. Hillen, and J. D. Keasling. 1999. "Polyphosphate kinase of *Acinetobacter* sp. Strain ADP1: purification and characterization of the enzyme and its role during changes in extracellular phosphate." *Appl. Environ. Microbiol.* **65**(9):3780-3786.

T. A. Carrier and J. D. Keasling. 1999. "Library of synthetic 5' secondary structures to manipulate mRNA stability in *Escherichia coli*." *Biotechnol. Prog.* **15**:58-64.

Name : Dagmar Ringe

Title: Lucille P. Markey Professor of Biochemistry and Chemistry

Organization: Brandeis University

Current Position:

I am on the faculty of two departments and a member of the Rosenstiel Basic Medical Sciences Research Center. I have a research group of approximately 12 people, some of whom I share with other collaborators. This year I teach a course in Advanced Organic Chemistry in the Chemistry Department.

Areas of Interest:

My research is focussed on the use of structure to elucidate function, whether enzymatic or other. Thus, I have several projects aimed at structural enzymology, and one aimed at determination of protein/DNA recognition. One of the goals of these studies is to "convert" one enzymatic activity into another using structural information as a guide for the transformation. A general goal of these studies is to use crystallographic methods to understand the basis of function and to exploit that information for enzyme or inhibitor design.

Recent Publications:

Babbitt et al., The enolase superfamily: a general strategy for enzyme-catalyzed abstraction of the a-porotons of carboxylic acids, Biochemistry 35, 16489 (1997).

Schlichting et al., The catalytic pathway of cytochrome P450 at atomic resolution, Science, 287, 1615 (2000).

Ringe and Mattos, Analysis of the binding surfaces of proteins, Medicinal Research Review, 19, 321 (1999).

Zhang et al., The role of water in the catalytic efficiency of triosephosphate isomerase, Biochemistry 38, 4389 (1999).

White and Ringe, Metal-ion activation of transcription in Iron Metabolism (Ferreira, Moura, Franco, eds) Wiley-VCH, p359 (1999).

Van Ophem et al., Effects of the E177K mutation in D-amino acid aminotransferase: Studies of an essential coenzyme anchoring group that contributes to stereochemical fidelity, Biochemistry 38, 1323 (1999).

Finding the chemistry
specificity comes last

Name: Claudia Schmidt-Dannert

Title: Assistant Professor

Organization: University of Minnesota

Current Position:

Assistant Professor, Department of Biochemistry, Molecular Biology and Biophysics

Areas of Interest:

The research in my laboratory focuses on tailoring new metabolic pathways for the recombinant production of complex, biologically active molecules for medical and biotechnological applications by combining techniques of metabolic engineering and molecular evolution. To 'breed' new biosynthetic pathways genes from different sources, even from unrelated metabolic routes, can be mixed and matched and at the same time new biosynthetic functions created by random mutagenesis, recombination and selection, all in the absence of detailed information on enzyme structure or catalytic mechanism.

Recent Publications:

C. Schmidt-Dannert, D. Umeno and F.H. Arnold (2000) Molecular breeding of carotenoid biosynthetic pathways, submitted; C. Schmidt-Dannert (1999) . Microbial lipases for biotechnological applications.

Bioorgan. Med. Chem. 7: 2123-2130; C. Schmidt-Dannert and F. H. Arnold (1999) Directed evolution of industrial enzymes. Trends Biotechnol. 17:135-136.; U. Schwaneberg, C. Schmidt-Dannert, J. Schmitt and R.D. Schmid (1999) A continuous spectrophotometric assay for P-450 BM-3, a fatty acid hydroxylating enzyme, and its mutant F87A. Anal. Biochem; 269: 359-366; U. Schwaneberg, A. Sprauer, C. Schmidt-Dannert, J. Schmitt, and R.D. Schmid (1999) P450 monooxygenase in biotechnology – I. Single-step, large-scale purification method for cytochrome P450BM-3 by anion-exchange chromatography. J. Chromatogr. A 848:149-159. S. Lutz-Wahl, P. Fischer, C. Schmidt-Dannert, W. Wohlleben, B. Hauer and R.D. Schmid (1998) Stereo- and regioselective hydroxylation of α-ionone by Streptomyces strains. Appl. Environ. Microbiol. 64:3878-3881.

Name: Imran Shah

Title: Dr. Until 4/7/00

Organization: American Type Culture Collection

Current Position:

Research Scientist Bioinformatics

From 4/8/00 — New Organization: University of Colorado, Health Sciences Center

New Position: Assistant Professor

Areas of Interest:

Machine learning methods for correlating enzyme function and protein sequence

Computational methods for predicting novel biochemical pathways

Bioinformatics tools for organizing and integrating metabolic knowledge.

Recent Publications:

Shah, I. and Hunter, L., Visual management of large scale data mining projects, *Pacific Symposium on Biocomputing*, 5:275-287, 2000.

Shah, I. and Hunter, L., Identification of Divergent Functions in Homologous Proteins by Induction of Conserved Modules, *Intelligent Systems for Molecular Biology*, 6:157-164, 1998.

Shah, I. and Hunter, L., Visualization based on the Enzyme Commission nomenclature, *Pacific Symposium on Biocomputing*, 3:142-152, 1998.

Shah, I. and Hunter, L., Predicting Enzyme Function from Sequence: A Systematic Appraisal, *Intelligent Systems for Molecular Biology*, 5:276-283, 1997.

Bioinformatics - gives in silico approach to organize + integrate data.

Name: Dr. Willem (Pim) Stemmer

Title: Vice President of Research and Development

Organization: Maxygen, Inc.

Current Position:

Vice President of Research and Development

Areas of Interest:

Molecular Breeding, Directed evolution, Complex systems engineering, Metabolic pathways, regulatory networks

Recent Publications:

Ness, J., Welch, M., Giver, L., Bueno, M., Cherry, J., Borchert, T., Stemmer, W.P.C., Minshull, J. (1999) DNA Shuffling of subgenomic sequences of subtilisin. Nature Biotechnology 17:893-896

Chang, C., Chen, T., Cox, B., Dawes, G. Stemmer, W.P.C., Punnonen, J., Patten, P. (1999) Evolution of a cytokine using DNA family shuffling. Nature Biotechnology 17:793-797

Minshull, J., Stemmer, W.P.C. (1999) Protein evolution by molecular breeding. Current Opinion in Chemical Biology 3:284-290

Christians, F.C., Scapozza, L., Crameri, A., Folkers, G., Stemmer, W.P.C. (1999) Directed evolution of thymidine kinase for AZT phosphorylation using DNA family shuffling. Nature Biotechnology 17:259-264

Crameri, A., Bermudez, E., Raillard, S. and Stemmer, W.P.C. (1998) DNA shuffling of a family of genes from diverse species accelerates directed evolution. Nature 391:288-291

Enzymes → NEW ACTIVITIES: BINDING
→ EXON SHUFFLING
→ COMBIZYMES

Pathways → Distributed pathways
→ Exon shuffling
→ Natural products

Episomes - multifactorial control of pathways

Bacterial whole genome shuffling (WGS)
Yeast WGS
Plant WGS - in conjunction w/ metabolic libraries
mammalian WGS

microbial community shuffling - maybe stimulate nature mechanisms

minimal genomes
- fast buy
- complexity is useful
- WGS can KO genes

non-natural aa

Name: Lawrence P. Wackett

Title: Professor

Organization: University of Minnesota

Current Position:

Lawrence Wackett is the Head of the Microbial Biochemistry and Biotechnology Division of the Department of Biochemistry, Molecular Biology and Biophysics. He is also a faculty member of the BioProcess Technology Institute.

Areas of Interest:

Microbial biotransformations;see: http://www.labmed.umn.edu/umbbd/

predicting catabolic reactions, see:
http://www.labmed.umn.edu/umbbd/predictbt/

There is a great need in the biodegradation community to predict the metabolic fate of new chemicals before they are released into the environment. Like predicting the weather, the outcome does not have to be 100% accurate to be useful. However, it should be based on sound scientific principles, backed up by experimental results. The knowledge required to predict biodegradation pathways with high accuracy is very broad, beyond that of any single human being. Thus we propose the PredictBT project to collect this knowledge and make it available via a computerized predictive system. Experts make predictions about biodegradation pathways using the scientific literature, unpublished knowledge, and (perhaps unconscious) heuristic rules about how to apply this knowledge. It is the goal of PredictBT to extract these heuristics from the experts and use them for automated biodegradation prediction.

Recent Publications:

Wackett, L.P., M.J. Sadowsky, L.M. Newman, H-G. Hur and S. Li (1994) Metabolism of polyhalogenated compounds by a genetically engineered bacterium. *Nature* **368:**627-629.

Ellis, L.B.M., C.D. Hershberger and L.P. Wackett (1999) The University of Minnesota Biocatalysis/Biodegradation Database: Specialized metabolism for functional genomics. *Nucl. Acids Res.* 27:373-376.

Wackett, L.P., L.B.M. Ellis, S.M. Speedie, C.D. Hershberger, H-J. Knackmuss, A.M. Spormann, C.T. Walsh, L.J. Forney, W.F. Punch, T. Kazic, M. Kanehisa, and D.J. Berndt (1999) Predicting microbial biodegradation pathways. *ASM News* 65:87-93.

Brim, H., S.C. McFarlan, J.K. Fredrickson, K.W. Minton, M. Zhai, L.P. Wackett and M.J. Daly (2000) Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nature Biotech.* 15:85-90.

Name: George Whitesides

Title: Mallinckrodt Professor

Organization: Harvard University

Current Position:

Mallinckrodt Professor of Chemistry at Harvard University

Areas of Interest:

Present research interests include materials science, biochemistry, surface chemistry, drug design, optics, and self-assembly.

Recent Publications:

enzymes

what has worked + not-worked - in enzymes that are cell free

- Can't do enzymes
- carbohydrate synthesis
- Enzymes as components in molecular

phytase from a thermophile

what are the opportunities

- find products from complex metabolic pathways
- computers w/ cells - interfaced
- metabolic predictions frequently wrong

Name: Min Zhang

Title: Senior Molecular Biologist

Organization: National Renewable Energy Laboratory
Biotechnology Center for Fuels and Chemicals
1617 Cole Blvd.,
Golden CO 80401

Current Position:

Team leader for Strain Development Team. Lead a group of 8 staff scientists/biochemical engineers to develop microbial biocatalysts for efficient conversion of biomass to ethanol and other chemicals using metabolic engineering. Our research efforts have targeted *Zymomonas mobilis*, *Lactobacillus sp.*, and *Saccharomyces cerevisiae*.

Areas of Interest:

Metabolic Pathway Engineering, Gene Expression, Strain Improvement, Protein Engineering, Fermentation, Biomass Conversion.

Recent Publications:

Zhang, M., Y. C. Chou, S. K. Picataggio and M. Finkelstein. 1998. *Single Zymomonas mobilis* strain for xylose and arabinose fermentation. U.S. Patent No. 5,843,760, issued December 1, 1998.

Picataggio, S.K., M. Zhang, M. A. Franden, J. D. McMillan and M. Finkelstein. Recombinant Lactobacillus for fermentation of xylose to lactic acid and lactate. 1998. U.S. Patent No. 5,798,237, issued August 25, 1998.

Deanda, K., M. Zhang, C. Eddy and S. Picataggio. 1996. Development of an arabinose fermenting *Zymomonas mobilis* strain by metabolic engineering. *Appl. Envirron. Microbiol.*, 62:4465-4470.

Zhang, M., C. Eddy, K. Deanda, M. Finkelstein and S. Picataggio. 1995. Metabolic engineering of a pentose metabolism pathway in *Zymomonas mobilis. Science.* 289: 240-243.

USEFUL (FROM THE DOD AND NATIONAL SECURITY PERSPECTIVE) THINGS TO MAKE VIA BIOTECHNOLOGY

George Whitesides, ***Harvard University***
John Frost, ***Michigan State University***

NOTES:

New capability
Availability
Critical foreign sovereignty
IP/proprietary position
Cost
Raw material exploitation
Technology exploitation (e.g. GMO)
Market exploitation
Regulatory/Political

War Fighting
- signature mgmt
- fouling
- corrosion enhancement

Combat medicine
- hyaluronic acid (surgery)
- lewis X (anti-inflammatory)
- proteins (serum, Ab, ...)
- anticaries, antifungal
- defensins
- enteric infections
- small molecule signalling (density f(x) in bacteria)

Peacekeeping
- GMO for public health
- power, H_2, EtOH, guayule
- H_2O

Logistics / Supply
- lubricants
- antifouling
- marine signalling chemicals
- fuel upgrading
- improved nutrition

BWD - Biological warfare defense
Antivirals
Antibiotics -- concern is that Ab^R will be built in so should hold in reserve
Potatoes/food vaccines
Adjuvants
Plants resistant to bio warfare

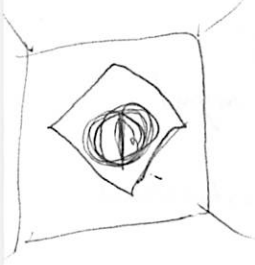
Decontamination
Heavy metals
Petroleum
Plant/fungi for cleaning
anti-biofilm phages
chemoreactive agents
biodegradable polymers
thermostable/salt stable

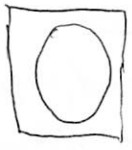
Sensors
Intel
Recognition elements
Alternatives to GFP
BWD
Amplify outputs

How prevent these getting in hands
- decomp

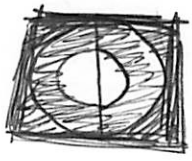
Unconventional Countermeasures

- need rapid characterization of organisms





John mekelores - phage agst Vibrio



need to process

methane sources

Plants

Carbon

CO_2

CH_3

C

EXPLOITING BIOINFORMATICS AND GENOMICS FOR PATHWAY ENGINEERING

Jonathan Eisen, ***The Institute for Genomic Research***
Imran Shah, ***American Type Culture Collection***
Mark Gerstein, ***Yale University***

NOTES:

TOOLS USED/NEEDED TO ENGINEER ORGANISMS TO DO USEFUL THINGS

Andrew Ellington, ***University of Texas at Austin***
Jay Keasling, ***University of California at Berkeley***
Claudia Schmidt-Dannert, ***University of Minnesota***
Williem (Pim) Stemmer, ***Maxygen, Inc.***
Stephen delCardayre, ***Maxygen, Inc.***
Min Zhang, ***National Renewable Energy Laboratory***

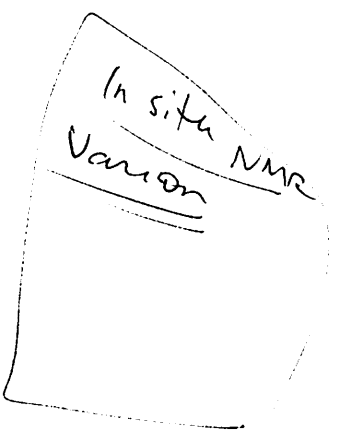
NOTES:

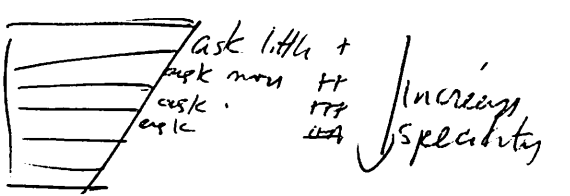
Platform organisms
- optimizing existing
- creating new ones

Streptomyces
Ecol.
Pseudomonas
Bacillus
Saccharomyces
Arabidopsis, Tobacco
Fil. fungi
Baculovirus
Insect
CHO - HeLa
Yeast
Archaea

Non-standard organisms
- transformation
  - speed
  - methods
- vectors
- expression systems
- engineered endosymbiosis
- Rhizobium engineering
- transfer

Screening (not selection)

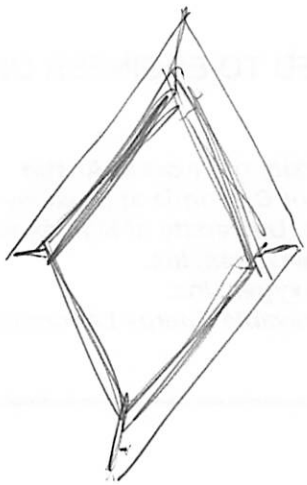




- mimic scale in small scale

- optimize function

- metomics
- where are the metabolites ?



Andy Ellington's Idea

metabolic screen in environment

oligo

↑ protein that binds particular metabolite

- metomics

- Species barriers

Complex phenotypes

- Tools

Catalytic antibodies?
- turnover # too low

Cofactor synthesis

Production tools

Alter internal of organisms

Post translational modifications

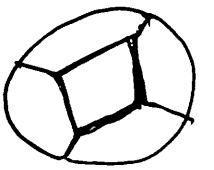
methylation

Ten Commendments

① Thou shall not alter thy hydrophobic core

ENGINEERING ENZYMES AND VIA GENETICS AND DESIGNING BIOMIMETIC CATALYSTS

Reza Ghadiri, ***The Scripps Research Institute***
Alan Fersht, ***University of Cambridge***
Dagmar Ringe, ***Brandeis University***
Lawrence Wackett, ***University of Minnesota***



NOTES:

Enzymes

Protein engineering
- stability
- pH
- refolding
- specificity

Biomimetic - what can you learn from enzymes that can be incorporated into smaller things

BIOPROCESSING: SCALING AND ENGINEERING/PRODUCTION ISSUES

John Frost, ***Michigan State University***
Douglas Cameron, ***Cargill, Inc.***

NOTES:

RUMP SESSION TO DEAL WITH EMERGING TOPICS

Designated Hitter

NOTES:

DARPA/ONR Metabolic Engineering Workshop
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March 24 – 26, 2000

Hagan Bayley
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Seeing Green

For Martek, Turning Algae Into Profits Has Been an Evolutionary Process

By Terence Chea
Washington Post Staff Writer
Monday, September 3, 2001; Page E01

Industry Watch
Pharmaceuticals

Industry Watch
Biotechnology

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As far back as 1993, when Martek Biosciences Corp. went public, the company has been promising investors big profits on sales of its algae-based nutritional oils for infant formula. But every time the Columbia firm thought it was about to hit it big, something got in the way.

Formula makers challenged the product's money-making potential, the medical community questioned its health benefits, government regulators wanted more proof of its safety. Martek officials hadn't realized how long it would take to persuade a deeply conservative and cautious industry to adopt a new ingredient.

"It was real missionary work to change the way people thought about nutrition," said Henry "Pete" Linsert Jr., Martek's chairman and chief executive, sitting in his sparsely furnished corner office in Columbia.

A banker by trade, Linsert is an unlikely missionary. Martek became his charge in 1989, and the company's early discoveries and subsequent setbacks would have turned off most in his risk-averse profession. Linsert, a 60-year-old former Marine who runs almost every day on the nature trail behind his office, admits, "It was a much larger effort than we ever imagined."

But the effort appears, finally, to be paying off. Martek still posts quarterly losses, but the profits it has been forecasting for years may not be far away for the 16-year-old company, whose nutritional oils have been shown to boost an infant's mental development.

All companies want to be profitable, but it is a notoriously rare feat for biotech ventures, especially those developing heavily regulated products such as drugs, diagnostic tests and food additives. Maryland has about 300 biotech companies, but the majority are research firms burning through large sums of cash to develop products that may never reach the market.

Investors know biotech companies are risky investments, but they're attracted to the huge potential payoff of a blockbuster product. For example, Maryland's most profitable biotech company, MedImmune Inc., struggled for more than a decade before it became a success story with its flagship drug Synagis, which last year generated $541 million in sales. But the Gaithersburg company is still a rarity in the fast-growing industry.

Martek on its surface is not much different from the unprofitable hundreds of its fellow biotech companies here. The company employs 160 people, including 90 workers at its corporate headquarters and research laboratory in a nondescript office complex in Columbia, which the company has occupied since 1989. The rest work at Martek's production facility in Winchester, Ky.

But this company may soon join the elite ranks of profitable biotech firms. Sales of infant formula spiked with Martek's oils are climbing steadily overseas in the more

Workshop on CO_2 Sequestration Schemes and Markets for Carbon Trading in US Agricultural and Energy Sectors
Washington, DC
November 20, 1998

A panel of scientists advising the United Nations has predicted the Earth's average surface temperature will rise 2 to 6 degrees Fahrenheit over the next century if emissions of greenhouse gases, particularly carbon dioxide, are not reduced. This, they predict, will cause shifts in climatic and agricultural zones, exacerbating both rainfall and drought.

"The onset of global climate change will require significant, and costly adjustments on the part of the agricultural community. A lack of preparation could have serious consequences for production, particularly in the case of extreme weather events. But if we can develop incentives for farmers and other landowners to help reduce levels of CO_2 in the atmosphere, global warming could be a blessing rather than a curse for U.S. agriculture."

David Zilberman, Professor of Agriculture and Resource Economics
Director, Center for Sustainable Resource Development
University of California, Berkeley

On November 20, 1998 a workshop entitled "CO_2 Sequestration Schemes and Markets for Carbon Trading in US Agricultural and Energy Sectors," will be held in Washington D.C. Funded through a grant from the USEPA and with the support of the Farm Foundation, it is being organized by the University of California's Center for Sustainable Resource Development, located on the Berkeley campus.

The workshop, which is in follow-up to the May 28 workshop on global climate change (also funded by the USEPA and with support from the Farm Foundation), has two practical aims. First, it will discuss post-Kyoto carbon sequestration schemes, their design and operation, and how they might impact US agriculture and elements of the energy sector here. Second, the workshop seeks to explore the structure and mechanics of setting up markets for trading of carbon credits around and beyond the sequestration schemes, such as:

- How practical is trading in carbon credits produced from or around carbon sequestration schemes?
- What can we learn from trading in environmental pollution permits?
- What should be the criteria for establishing sequestration schemes and markets? For example, should they focus on carbon alone or include other greenhouse gases?
- What about monitoring of the sequestration schemes? Can it be done with existing technology? What mechanisms (e.g., incentives) are there that can be applied to induce actual sequestration?
- What is the magnitude of the prices associated with markets for carbon trading? Who will benefit and who will lose from these markets?
- How important will global climate change be in the future of US agriculture, especially what is the importance to that sector of carbon sequestration schemes?
- What are the prospects with respect to legislation regarding US carbon sequestration schemes and markets?

Directed to a wide cross-section of the US agricultural sector, including participation from public utilities and others representing energy interests, the one-day workshop was held on Friday, November 20, 1998 at the Phoenix Park Hotel, 520 North Capitol Street, N.W., Washington D.C

Workshop Publications - Publications resulting from this workshop are in Adobe Acrobat format (PDF) Make sure you have the Adobe Acrobat Reader to read these files. If you do not have Adobe Acrobat Reader, you can download it at the Adobe website.

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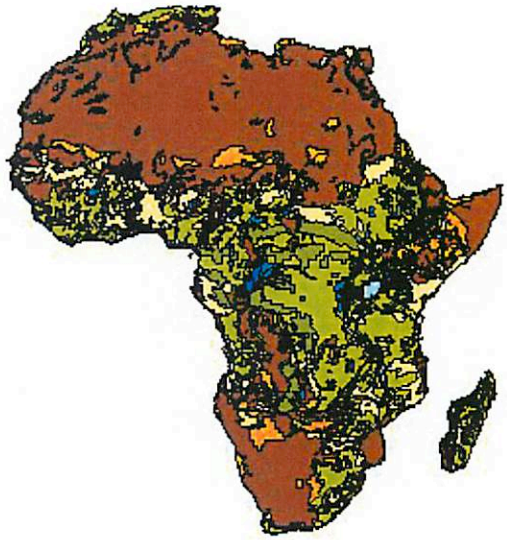

Click the map of Africa to see a larger image/Zoom.

Summary Conclusions

The following workshop report summarizes the proceedings of the workshop, Carbon Sequestration in Soils and Carbon Credits: Review and Development of Options for Semi-Arid and Sub-Humid Africa. This report includes the agenda, lists of presenters and attendees, abstracts of the presentations, and a synopsis of the breakout sessions.

The following points are a summation of a set of overarching conclusions emerging from the larger workshop group. Workshop participants concluded that:

- Farms, grasslands, and savannas in Africa have the potential to store carbon in the soil and the people have a great need for the land practices that improve soil carbon storage and productivity. Desertification and land degradation reduce soil quality, which then leads to declines in agricultural productivity. Declining soil quality occurs when soil organic carbon is reduced as the carbon moves from the soil to the atmosphere, thus exacerbating climate change. Fortunately, restoring degraded soils through improved agricultural practices reverses this process, thus increasing agricultural productivity and slowing climate change. The workshop stressed that while energy-sector emissions are the predominant contributor of the climate change problem globally, agricultural practices have emitted an estimated 55 Pg of carbon (IPCC 1995). Conversely, practicing conservation tillage, improving agricultural productivity, reducing soil erosion, and improving water management improve soil quality and increase the carbon stored in soil. In total, it is estimated that these practices have the potential to restore between 40 to 112 Pg of carbon globally.

- Successful soil sequestration projects and activities in Africa must have a strong sustainable development component, such that the project improves the livelihood of farmers by improving agricultural productivity, reducing the risk of crop failure, providing access to better agricultural inputs, such as organic fertilizers.

- Successful soil sequestration efforts in Africa are more likely to succeed if they build upon existing institutions, initiatives, organizations, and land management practices.

- Changes in soil carbon can be monitored and measured, however, because carbon sequestration is a new field some technical challenges remain. A good first step to addressing these challenges will be the development of a measurement and monitoring Manual. The Manual should build upon the existing work of the Intergovernmental Panel on Climate Change, be drafted by a small group of experts, then widely circulated to experts and stakeholders for review.

- A demonstration pilot project will help create monitoring and measuring protocols for soil carbon, illustrate the economic benefit of such efforts to landowners, and the carbon benefits of such projects to potential investors.

- While the majority of land use projects to date have been in the forest sector, soil carbon projects in semi-arid and sub-humid Africa provide the following unique opportunities:
  - The land has relatively low opportunity cost relative to humid tropical forests, where in many cases climate mitigation may not be able to compete with logging or agricultural land demands. Large areas of degraded and desertified lands are in need of technical assistance and capital for restoring farmlands, grasslands, and savannas. While exact estimates of desertification are difficult to obtain, estimates range from 3.47 to 3.97 billion hectares of desertified land (Lal et al 1998).
  - Therefore while the tons of carbon per hectare is relatively small relative to forests, the overall potential for cost effective climate mitigation is quite large.
  - Arid regions of Africa have very low rates of energy emissions, so they do not present great opportunities for reductions in their energy sector, Nor do they have large areas of humid tropical forests, so they do not qualify for many forest-based projects. Soil carbon projects offer an opportunity for semi-arid and sub-humid regions of Africa to meaningfully participate in climate mitigation, while improving human well being. Sub-Saharan Africa comprises 52 countries, with a total land area of 2.39 billion hectares. Crop productivity is extremely low, ranging from 1.5 Mg/ha for corn, 0.8 mg/ha for sorghum, and 0.7 Mg/ha for millet. The productivity is low due to land degradation and desertification.

Workshop Invitation

Carbon Sequestration in Soils and Carbon Credits:
Review and Development of Options for Semi-Arid and Sub-Humid Africa
May 19-21, 1999 (COMPLETED)
U.S. Geological Survey, EROS Data Center
Sioux Falls, South Dakota, USA

I am pleased to send you this letter of invitation to a workshop entitled **CARBON SEQUESTRATION IN SOILS AND CARBON CREDITS: REVIEW AND DEVELOPMENT OF OPTIONS FOR SEMI-ARID AND SUB-HUMID AFRICA** to be held at the EROS Data Center, Sioux Falls, South Dakota, **May 19 —21** , 1999. This workshop is a joint effort of the USGS, USAID, the World Resources Institute, and the Bradley Fund for the Environment of Sand County Foundation.

The main purposes of the conference are: 1) to extend our understanding of the potential roles of land use and land management in the sequestration of carbon in soil and 2) to identify mechanisms for developing country implementation. Our focus will be on:

- semi-arid and sub-humid areas, grasslands, savannas and agricultural lands with emphasis in developing countries in Africa;

- defining the potential for C sequestration, the economic value, its importance for sustainability, and possible implementation mechanisms; and

- insuring appropriate participation in carbon crediting opportunities stemming from the Kyoto Protocol for Climate Change (KPCC) and subsequent conferences.

This workshop effectively builds upon the recent seminar held at St. Michaels, MD, which established that substantial C sequestration could be achieved with some modifications in land use or land management. The two-day workshop is designed with key presentations and working group sessions to achieve a robust set of goals.

GOALS:

1. **Review the new agreements, policy implications, and developing opportunities for joint US and African participation in programs arising from the KPCC.**
2. **Support participation by African specialists and insure that the potential contributions by developing countries and small holders are realized.**
3. **Describe the potential for C sequestration in parts of Africa; confirm measurement, monitoring and verification procedures; and review financial instruments to assure transfer of accrued funds to individuals.**
4. **Help equip industrial representatives, landowners and other stakeholders to understand both the science and the opportunities.**
5. **Review selected C sequestration projects and industry participation, encourage networking, and consider a well-monitored demonstration project.**

The workshop will support presentations by leading technical and policy experts, representatives from Africa, and representation by U.S. industry. It will define opportunities for C sequestration and possible credit trading, especially in the framework of the Clean Development Mechanism. The potential carbon and economic opportunities will be evaluated in the context of implementation plans at the level of the small landholder. The interactive nature will result in a proceedings that outlines the potential role of carbon sequestration to support sustainable economic development in Africa. Although all major land cover types, projects and sectors will be reviewed, the emphasis will be on C accretion in soils and semi-arid and sub-humid Africa.

We hope that you will be able to participate in this event. You will find all details pertaining to logistics listed on the Registration Form (also attached). Please acknowledge receipt of this e-mail by returning the completed Registration Form to include your current mailing address, telephone number, fax number, and a correction if we have not used your preferred e-mail address. We would appreciate your response as soon as possible, **but no later than April 5** , as to whether you wish to attend. We intend to limit the conference to 75 participants.

The workshop opens with a "Mixer" on the evening of May 19, continues with two days of presentations and working sessions, and concludes at 4:30 p.m. on May 21. Registrants will receive an Agenda.

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FETC Calendar of Events

Industry-University-Government Stakeholders Workshop on Carbon Sequestration Options

Boston, MA *(at the Massachusetts Institute of Technology's Energy Lab)*
June 22-23, 1998

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Industry-University-Government Stakeholders Workshop on Carbon Sequestration Options
Boston, MA (at the Massachusetts Institute of Technology's Energy Lab)

Hotel Accomodations

A block of hotel rooms has been reserved at the Cambridge Marriott Hotel, which is only a few minutes walk from the workshop venue. A special rate of $199.00 per night has been secured for these rooms. Attendees are requested to make their reservations directly. Please refer to the "Carbon Sequestration Workshop" when making your reservation. In order to secure this rate, reservations need to be made by June 1.

Cambridge Marriott Hotel
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Telephone: 617-494-6600
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FETC Calendar of Events

Industry-University-Government Stakeholders Workshop on Carbon Sequestration Options

Boston, MA *(at the Massachusetts Institute of Technology's Energy Lab)*
June 22-23, 1998

Agenda

Monday, June 22, 1998

8:00 - **Registration and Continental Breakfast**

9:00 - **Plenary Session I -**
Opening Session Chair: Jefferson Tester, Director, *MIT Energy Laboratory*

Welcoming Remarks

Keynote Address
Rita Bajura, Director, *Federal Energy Technology Center*

Technology s Role in Carbon Management
Henry (Jake) Jacoby, William F Pounds Professor of Mgmt, *MIT*

10:30 - **Break**

11:00 - **Plenary Session II- Sequestration Technologies**
Chair: Elisabeth Drake, Associate Director, *MIT Energy Laboratory*

Overview of Technologies for CO2 Sequestration
Howard Herzog, Principal Research Engineer, *MIT*

The Technological Response in Norway
Olav Falk-Pedersen, *Kvaerner Oil & Gas*

12:00 - **Lunch**

2:00 - **Plenary Session III - Terrestrial Sequestration**
Chair: Roger Dahlman, Program Manager for Carbon Cycle Research, *Office of Energy Research, US Department of Energy*

The Ameriflux Initiative and the Role of Forests as Sinks for CO2
Steven Wofsy, Professor, *Department of Atmospheric Sciences, Harvard University*

Soil Sequestration of Carbon
Keith Paustian, Research Scientist, *Natural Resource Ecology Laboratory, Colorado State University*

Terrestrial Sequestration Policy Opportunities and Implementation Issues
Mark Trexler, President, *Trexler and Associates, Inc.*

3:30 - **Break**

4:00 - **Plenary Session IV - International Outlook**
Chair: Perry Bergman, *Federal Energy Technology Center*

Opportunities for International Collaborative R&D
Kelly Thambimuthu, *Natural Resources Canada*

4:30 - **Charge to Breakout Sessions**
Charles Schmidt, Environment Product Manager, *Federal Energy Technology Center*

4:45 - **Breakout Session I**
Coal Breakout Chair: John Wootten, Vice President, *Peabody Group*
Electricity A Breakout Chair: C. V. Mathai, Principal Scientist, *Arizona Public Service*
Electricity B Breakout Chair: *To be determined*
Oil & Gas Breakout Chair. Mark Northam, Technology Consultant, *Mobil Oil*

5:45 - **Adjournment**

6:30 - **Reception**

7:30 - **Dinner**

8:30 - **The Future of Fossil Energy**
Morris Adelman, Professor Emeritus of Economics, *MIT*

9:15 - **Adjournment**

Tuesday, June 23, 1998

8:00 - **Continental Breakfast**

8:45 - **Feedback from initial breakout sessions -- resolution of questions or issues that arose**

9:00 - **Breakout Session II**

12:00 - **Lunch**

1:15 - **Plenary Session V - Wrap-up**

Reports from breakout sessions

Concluding Remarks

2:30 - **Adjournment**

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DARPA Workshop

NEXT GENERATION GREEN SYNTHESIS: EXPERIMENTAL AND THEORETICAL APPROACHES TO NEW ENZYMES, PATHWAYS AND BIOMIMETIC CATALYSTS

Workshop Background: Biocatalytic processing, as opposed to chemical processing, is environmentally friendly because enzymes have ~10Kcal barriers (high temperatures/pressures not required), exhibit high reaction-, regio- and stereo-specificity (protection/deprotection wastes and catalyst poisoning avoided) and control the dielectric constant in their binding pockets (largely eliminating toxic solvents). Performance metrics for the chemical industry in 2020 can only be met through substantial substitution of biocatalytic steps and processes. To meet this challenge there is emerging, on the one hand, bioinformatic modeling of enzymes and pathways through the genomic and structural databases and, on the other, combinatorial gene shuffling protocols of directed evolution and molecular breeding to generate biocatalysts that are precisely tuned for specific processing applications. Enzyme engineering has, until now, been limited to hydrolytic enzymes. The next generation of Green Synthesis, to which this workshop topic is addressed, will greatly expand the reaction repertoire (to include redox and other important transfer processes) as well as exploit the theoretical (including modeling and simulation), evolutionary, and biomimetic protocols now emerging.

Workshop Objective: To characterize gaps in understanding and technology that limit our ability to design enzymes and synthetic pathways. Also, to identify research opportunities that would lead to the creation of new biocatalytic toolboxes for affordable and environmentally friendly synthesis of DoD materials and for energy harvesting processes. The outcome of this workshop is expected to contribute directly to the development of a major new research program at DARPA.

Examples of Research Opportunities: 1) the development and use of predictive tools to identify catalytic properties of enzymes in existing organisms, or theoretical ancestors, from comparative genomic data; 2) the use of directed evolution/molecular breeding, in conjunction with 1), to generate enzymes with novel properties (thermal and solvent stability, tuned substrate/cofactor specificities, resistance to substrate/product inactivation and inhibition, matched pH dependencies); 3) the development of truncated, inexpensive and stable cofactors for group (hydride/phosphoryl/acyl) transfer to replace NAD(P), ATP and CoA, as well as enzymes evolved to use these, and novel schemes for their cyclic regeneration; 4) the assembly of heterologous pathways in conjunction with 1) and 2) for coordinated synthesis (employing *in vitro, in vivo* or permeabilized cell systems) and featuring combinatorial diversification of precursors in the case of polymer synthesis; 5) the development of novel high throughput screens and selections that are generally applicable to target enzymes and pathways; 6) the potential of novel cellular platforms (*e.g.* minimized genomes) for experimental, theoretical (including modeling and simulation) approaches to optimizing pathway design and gene product expression; 7) the use of theoretical and biochemical principles to design and validate biomimetic redox catalysts for alkane activation and H_2 generation and, more generally, to enable hybrid biological/biomimetic processing schemes; 8) the identification of appropriate biological systems and platforms including single cell organisms, animals and plants.

DARPA/ONR Metabolic Engineering Workshop
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March 24 – 26, 2000

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DARPA/ONR Metabolic Engineering Workshop
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Title: Professor and Head

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Current Position:

Areas of Interests:

HAGAN BAYLEY is Professor and Head in the Department of Medical Biochemistry & Genetics at Texas A&M University, where he is also a Professor of Chemistry. He enjoys working at the interface of chemistry and biology by, for example, developing techniques for protein modification that have applications in both basic science and biotechnology. He received his B.A. in chemistry from the University of Oxford in 1974, while at Balliol College, and his PhD. in chemistry from Harvard University in 1979 in the laboratory of Jeremy Knowles. After postdoctoral work with Gobind Khorana at the Massachusetts Institute of Technology, he was on the faculty at Columbia University and the University of Oxford. From 1988 to 1996, he was at the Worcester Foundation for Experimental Biology in Shrewsbury,Massachusetts.

photo: www.chem.tamu.edu/faculty/bayley
more information: bletchley.tamu.edu/homepage

Recent Publications:

Song, L., Hobaugh, M.R., Shustak, C., Cheley, S., Bayley, H., and Gouaux, J.E. Structure of staphylococcal -hemolysin, a heptameric transmembrane pore. Science, 274, 1859-1865 (1996)

Braha, O., Walker, B., Cheley, S., Kasianowicz, J.J., Song, L. Gouaux, J.E., and Bayley, H. Designed pores as components for biosensors. Chemistry & Biology 4, 497-505 (1997)

Cao, Q., Wang, Y., and Bayley, H. Sequence of abductin, the molluscan "rubber" protein. Current Biology, 7, R677- R678(1997)

Chang, C-Y., Fernandez, T., Panchal, R., and Bayley, H. A caged catalytic subunit of cAMP-dependent protein kinase, J. Am. Chem. Soc. 120, 7661-7662 (1998).

Gu, L., Braha, O., Conlan, S. Cheley, S. and Bayley, H. Stochastic sensing of organic analytes by a pore-forming protein containing a molecular adapter, Nature 398, 686-690 (1999)

Eroglu, A., Russo, M.J., Bieganski, R., Fowler, A., Cheley, S., Bayley, H. and Toner, M. Intracellular trehalose improves the survival of cryopreserved mammalian cells. Nature Biotechnology 18, 163-167 (2000).

Name: Douglas C. Cameron

Title: Director of Biotechnology

Organization: Cargill, Inc., Minneapolis, MN

Current Position:

I manage a newly formed biotechnology research group in Cargill Central Research. The group focuses microbial strain development and metabolic engineering. The group works closely with Cargill business units in industrial chemicals, feed, food and nutraceuticals.

Areas of Interest:

I am interested in the basic and applied aspects of metabolic engineering, genomics, bioinformatics, and microbial physiology. I am also interested in hybrid metabolic engineering/evolutionary methods in strain development.

Recent Publications:

Skraly, F.A., B.L. Lytle, and D.C. Cameron. 1998. Construction and characterization of a 1,3-propanediol operon. Appl. Environ. Microbiol., 64:98-105.

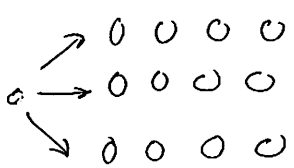
Sauer, U., D.C. Cameron, and J.E. Bailey. In 1998. Metabolic capacity *of Bacillus subtilis* for the production of purine nucleotides, riboflavin, and folic acid. Biotech. Bioeng., 59:227-238.

Cameron, D.C., N.E. Altaras, M.L. Hoffman, and A.J. Shaw. 1998. Metabolic engineering of propanediol pathways. Biotechnol. Prog., 14:116-125.

Altaras, N.E. and D.C. Cameron. 1999. Metabolic engineering of a 1,2-propanediol pathway in *Escherichia coli*. Appl. Environ. Microbiol., 65: 1180-1185.

Continuous growth

- chemostat
- turbidostat (feedback mechanism prevents washout)
- serial culture - automated, can do in parallel



Name: **Stephen B. delCardayré**

Title: **Group Leader Whole Genome Shuffling – Chemicals/Core Technology**

Organization: **Maxygen**

Current Position:

Lead research to develop and apply methods for the rapid evolution of whole cells and organisms. We are integrating gene, pathway, and genome shuffling to create robust industrial microorganisms having novel or improved metabolic pathways.

Areas of Interest:

Technically I am interested in enzymes, enzyme evolution, microbial biochemistry, metabolic engineering, genetics, and molecular evolution. This includes the mechanisms by which new enzyme catalysts arise and are assembled to form functional metabolic pathways, as well as how cells rapidly adapt to new chemical and physical challenges. My primary interest is harnessing the power of biological catalysis from enzymes and whole cells for useful chemical applications.

Recent Publications:

Ness, J., del Cardayré, S.B., Minshull, J. and Stemmer, W.P.C. (2000) "Molecular Breeding – The Natural Approach to Protein Design" In Evolutionary Approaches to Protein Design, A Volume of Advances in Protein Chemistry (in press).

del Cardayré, S.B., et. al. Evolution of Whole Cells and Organisms by Recursive Sequence Recombination. PCT/WO 00/04190.

del Cardayré, S.B., Zhang, Y.X., Huisman, G.W. (2000) Generating New Biocatalysts by Molecular Breeding. Presentation for the National Meeting of the American Chemical Society, San Francisco, CA, March 26-30th, 2000.

Name: Andrew D. Ellington

Title: Associate Professor/ Biochemistry and Chemistry

Organization: University of Texas at Austin

Current Position:

Associate Professor of Chemistry and Biochemistry. Undergraduate advisor for nine undergraduates. Graduate advisor for sixteen graduate students.Post-doctoral advisor for two post-doctoral fellows.

Areas of Interest:

Evolutionary engineering of nucleic acids, proteins, and organisms. We select binding species (aptamers) and catalysts (ribozymes) from random sequence nucleic acid pools. The binding species are engineered to function as biosensors. We are similarly screening and selecting allosteric enzymes as diagnostic reagents. We have most recently begun efforts to engineer the monomer chemistry of entire organisms (bacteria).

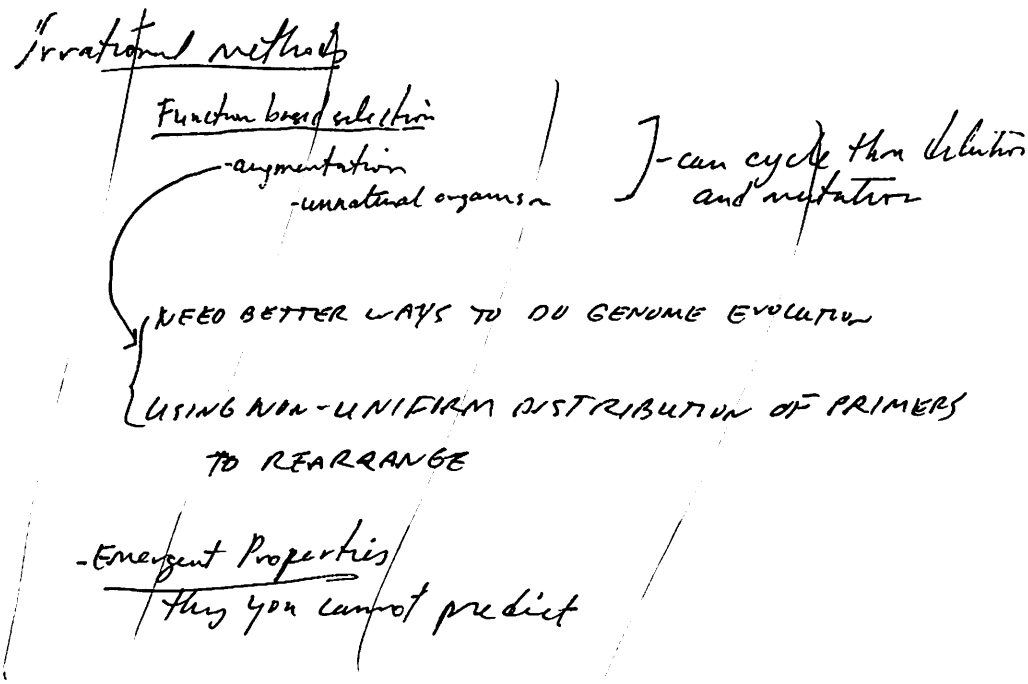
Recent Publications:

Matsumura, I., Wallingford, J.B., Surana, N.K., Vize, P.D., and Ellington, A.D. (1999) Directed evolution of the surface chemistry of beta-glucuronidase. *Nature Biotechnology*, **17**(7):696-701.

Robertson, M.P. and Ellington, A.D. (1999) *In vitro* selection of an allosteric ribozyme that transduces analytes into amplicons. *Nature Biotechnology*, **17**(1): 62-66.

Robertson, M.P. and Ellington, A.D. (2000) Design and optimization of effector-activated ribozyme ligases. In press, *Nucleic Acids Research.*

Jhaveri, S., Kirby, R., Conrad, R., Magion, E.J., Glick, G., Ellington, A.D. (2000) Signaling aptamers. In press, *JACS.*



Name: Alan Fersht

Title: Professor

Organization: University of Cambridge

Current Positions:

Herchel Smith Professor of Organic Chemistry; Director of the Cambridge University/Medical Research Council Centre for Protein Engineering.

Areas of Interest:

Protein Folding, Stability and Design. Protein Misfolding and Cancer. Protein Refolding Technology.

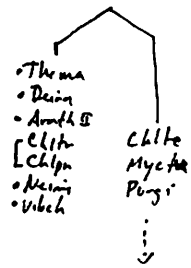
Recent Publications:

Directed evolution of new catalytic activity using the a/b-barrel scaffold

Myriam M. Altamirano, Jonathan M. Blackburn, Cristina Aguayo and Alan R. Fersht Nature 403, 617-622 (2000).

Oxidative Refolding Chromatography: Folding of the Scorpion Toxin Cn5
M. M. Altamirano, C. García, L. D. Possani and A. R. Fersht
Nature Biotechnology 17, 187-191 (1999).

Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding (W. H. Freeman & Co., 1998)



Cannot design enzymes de-novo still b/c don't understand structure-fn/.

Too many mutants to assay.

If you burn all your bridges you don't have to burn anything.

Name: John W. Frost

Title: Professor

Organization: Michigan State University

Current Position:

Professor with joint appointment in the Department of Chemistry and Department of Chemical Engineering

Areas of Interests:

Frost group research focuses on the integration of microbe-based biocatalysis with organic chemistry. The goal is to create new synthetic routes to ultrafine, fine, pseudocommodity, and commodity chemicals based on the use of nontoxic carbohydrate starting materials derived from renewable starch, hemicellulose, and cellulose

Recent Publications:

"Shikimic Acid and Quinic Acid: Replacing Isolation from Plant Sources with Recombinant Microbial Biocatalysis" Draths, K. M.; Knop, D. R.; Frost, J. W. J. Am. Chem. Soc. 1999, 121, 1603.

"Fed-Batch Fermentor Synthesis of 3-Dehydroshikimic Acid Using Recombinant Escherichia coli" Li, K.; Mikola, M.R.; Draths, K. M.; Worden, R. M.; Frost, J. W. Biotechnol. Bioeng. 1999, 64, 61.

Name: M. Reza Ghadiri, Ph.D.

Title: Professor

Organization: The Scripps Research Institute

Current Position:

Departments of Chemistry and Molecular Biology and Member of Skaggs Institute for Chemical Biology

Areas of Interest:

Design of functional artificial metalloproteins and enzymes, peptide nanotubes and related biomaterials, artificial transmembrane ion and molecular channels, transition metal nanocomposite and catalyst engineering, biosensors, self-replicating molecular systems, self-organized nonlinear chemical networks, and the study of the early events of protein folding.

Recent Publications:

Bong, D. T.; Steinem, C.; Janshoff, A.; Johnson, J. E.; Ghadiri, M. R. "A Highly Membrane Active Peptide in Flock House Virus: Implications for the Mechanism of Nodavirus Infection". *Chem. Biol.* **1999**, *6*, 473-481.

Clark, T. D.; Kobayashi, K.; Ghadiri, M. R. "Covalent Capture and Stabilization of Cylindrical sheet Peptide Assemblies", *Chem.Eur. J.***1999**, *5*, 782-792.

Janshoff, A.; Dancil, K.-P. S.; Steinem, C.; Greiner, D. P.; Lin, V. S.-Y.; Gurtner, C.; Motesharei, K.; Sailor, M. J.; Ghadiri, M. R. "Macroporous *p*-Type Silicon Fabry-Perot Layers. Fabrication, Characterization, and Applications in Biosensing", *J. Am. Chem. Soc.* **1998**, *120*, 12108-12116.

Hartgerink, J. D.; Ghadiri, M. R. "Peptide Nanotubes and Beyond", *Chem. Eur. J.* **1998**, *4*, 1367-1372.

Lee, D. H.; Severin, K.; Ghadiri, M. R. "Autocatalytic Networks: The Transition From Molecular Self-Replication to Ecosystems", *Curr. Op. Chem. Biol.* **1997**, *1*, 491-496.

Severin, K.; Lee, D. H.; Kennan, A. J.; Ghadiri, M. R. "A Synthetic Peptide Ligase", *Nature* **1997**, *389*, 706-709.

Name: Jay D. Keasling

Title: Associate Professor

Organization: University of California at Berkeley

Current Position:

Associate Professor of Chemical Engineering at the University of California at Berkeley

Areas of Interest:

The research in the Keasling Laboratory focuses on the metabolic engineering of microorganisms for degradation of environmental contaminants or for environmentally friendly synthesis. To that end, we have developed a number of new genetic and mathematical tools to allow more precise and reproducible control of metabolism. These tools are being used in such applications as synthesis of biodegradable polymers, accumulation of phosphate and heavy metals, and degradation of chlorinated and aromatic hydrocarbons, biodesulfurization of fossil fuels, and complete mineralization of organophosphate nerve agents and pesticides.

Recent Publications: (3-4 sentences)

J. D. Keasling. 1999. "Gene-expression tools for the metabolic engineering of bacteria." *Trends in Biotechnology* **17**:452-460.

P. L. Trelstad, P. Purdhani, W. Geibdorfer, W. Hillen, and J. D. Keasling. 1999. "Polyphosphate kinase of *Acinetobacter* sp. Strain ADP1: purification and characterization of the enzyme and its role during changes in extracellular phosphate." *Appl. Environ. Microbiol.* **65**(9):3780-3786.

T. A. Carrier and J. D. Keasling. 1999. "Library of synthetic 5' secondary structures to manipulate mRNA stability in *Escherichia coli*." *Biotechnol. Prog.* **15**:58-64.

Name: Claudia Schmidt-Dannert

Title: Assistant Professor

Organization: University of Minnesota

Current Position:

Assistant Professor, Department of Biochemistry, Molecular Biology and Biophysics

Areas of Interest:

The research in my laboratory focuses on tailoring new metabolic pathways for the recombinant production of complex, biologically active molecules for medical and biotechnological applications by combining techniques of metabolic engineering and molecular evolution. To 'breed' new biosynthetic pathways genes from different sources, even from unrelated metabolic routes, can be mixed and matched and at the same time new biosynthetic functions created by random mutagenesis, recombination and selection, all in the absence of detailed information on enzyme structure or catalytic mechanism.

Recent Publications:

C. Schmidt-Dannert, D. Umeno and F.H. Arnold (2000) Molecular breeding of carotenoid biosynthetic pathways, submitted; C. Schmidt-Dannert (1999) . Microbial lipases for biotechnological applications.

Bioorgan. Med. Chem. 7: 2123-2130; C. Schmidt-Dannert and F. H. Arnold (1999) Directed evolution of industrial enzymes. Trends Biotechnol. 17:135-136.; U. Schwaneberg, C. Schmidt-Dannert, J. Schmitt and R.D. Schmid (1999) A continlous spectrophotometric assay for P-450 BM-3, a fatty acid hydroxylating enzyme, and its mutant F87A. Anal. Biochem; 269: 359-366; U. Schwaneberg, A. Sprauer, C. Schmidt-Dannert, J. Schmitt, and R.D. Schmid (1999) P450 monooxygenase in biotechnology – I. Single-step, large-scale purification method for cytochrome P450BM-3 by anion-exchange chromatography. J. Chromatogr. A 848:149-159. S. Lutz-Wahl, P. Fischer, C. Schmidt-Dannert, W. Wohlleben, B. Hauer and R.D. Schmid (1998) Stereo- and regioselective hydroxylation of α-ionone by Streptomyces strains. Appl. Environ. Microbiol. 64:3878-3881.

Name: Lawrence P. Wackett

Title: Professor

Organization: University of Minnesota

Current Position:

Lawrence Wackett is the Head of the Microbial Biochemistry and Biotechnology Division of the Department of Biochemistry, Molecular Biology and Biophysics. He is also a faculty member of the BioProcess Technology Institute.

Areas of Interest:

Microbial biotransformations;see: http://www.labmed.umn.edu/umbbd/

predicting catabolic reactions, see:
http://www.labmed.umn.edu/umbbd/predictbt/

There is a great need in the biodegradation community to predict the metabolic fate of new chemicals before they are released into the environment. Like predicting the weather, the outcome does not have to be 100% accurate to be useful. However, it should be based on sound scientific principles, backed up by experimental results. The knowledge required to predict biodegradation pathways with high accuracy is very broad, beyond that of any single human being. Thus we propose the PredictBT project to collect this knowledge and make it available via a computerized predictive system. Experts make predictions about biodegradation pathways using the scientific literature, unpublished knowledge, and (perhaps unconscious) heuristic rules about how to apply this knowledge. It is the goal of PredictBT to extract these heuristics from the experts and use them for automated biodegradation prediction.

Recent Publications:

Wackett, L.P., M.J. Sadowsky, L.M. Newman, H-G. Hur and S. Li (1994) Metabolism of polyhalogenated compounds by a genetically engineered bacterium. *Nature* **368:**627-629.

Ellis, L.B.M., C.D. Hershberger and L.P. Wackett (1999) The University of Minnesota Biocatalysis/Biodegradation Database: Specialized metabolism for functional genomics. *Nucl. Acids Res.* 27:373-376.

Wackett, L.P., L.B.M. Ellis, S.M. Speedie, C.D. Hershberger, H-J. Knackmuss, A.M. Spormann, C.T. Walsh, L.J. Forney, W.F. Punch, T. Kazic, M. Kanehisa, and D.J. Berndt (1999) Predicting microbial biodegradation pathways. *ASM News* 65:87-93.

Brim, H., S.C. McFarlan, J.K. Fredrickson, K.W. Minton, M. Zhai, L.P. Wackett and M.J. Daly (2000) Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nature Biotech.* 15:85-90.

Name: Min Zhang

Title: Senior Molecular Biologist

Organization: National Renewable Energy Laboratory
Biotechnology Center for Fuels and Chemicals
1617 Cole Blvd.,
Golden CO 80401

Current Position:

Team leader for Strain Development Team. Lead a group of 8 staff scientists/biochemical engineers to develop microbial biocatalysts for efficient conversion of biomass to ethanol and other chemicals using metabolic engineering. Our research efforts have targeted *Zymomonas mobilis*, *Lactobacillus sp.*, and *Saccharomyces cerevisiae*.

Areas of Interest:

Metabolic Pathway Engineering, Gene Expression, Strain Improvement, Protein Engineering, Fermentation, Biomass Conversion.

Recent Publications:

Zhang, M., Y. C. Chou, S. K. Picataggio and M. Finkelstein. 1998. *Single Zymomonas mobilis* strain for xylose and arabinose fermentation. U.S. Patent No. 5,843,760, issued December 1, 1998.

Picataggio, S.K., M. Zhang, M. A. Franden, J. D. McMillan and M. Finkelstein. Recombinant Lactobacillus for fermentation of xylose to lactic acid and lactate. 1998. U.S. Patent No. 5,798,237, issued August 25, 1998.

Deanda, K., M. Zhang, C. Eddy and S. Picataggio. 1996. Development of an arabinose fermenting *Zymomonas mobilis* strain by metabolic engineering. *Appl. Environ. Microbiol.*, 62:4465-4470.

Zhang, M., C. Eddy, K. Deanda, M. Finkelstein and S. Picataggio. 1995. Metabolic engineering of a pentose metabolism pathway in *Zymomonas mobilis. Science.* 289: 240-243.

- sequence + predict f(x)
  - genes
  - genome
  - example uncultivables

- combining evolution + genomics

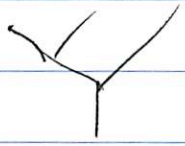
- uncultivables + extremophiles

Genome Sequences → comparative studies

↓ inferred analysis

Biology/Evolution
- Pathogenesis
- Radiation resistance
- Communities
- Symbiosis
- Extremophiles

Evolution
- predict f(x)
- duplications
- mutations
-



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Hilton
Washington Dulles Airport

Problem

- extremophiles
- clone homologs or genetics

- Microarray
- KOIs
- Whole genome info

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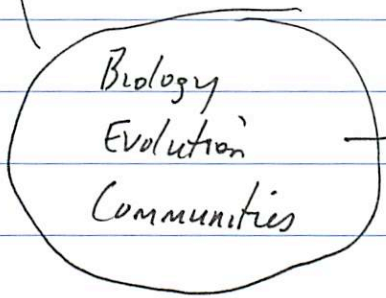
Genomes would solve this — but not simple

① predict f(x) hard
② can't predict NOVEL functions well
③ unusual evolution



"Minimize"

"can't create that transformation don't exist - have to but can assist those that do"

PHYLOGENOMICS
- evols tells a lot
- genomes tell a lot
- p6 profiles

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