National Science Foundation Workshop Report on

Design of Catalyst Systems for Biorenewables

"Harnessing Catalysis for a New Carbon Economy"

American Chemical Society Headquarters
Washington, D.C.
June 23 and 24, 2005

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Workshop Website: **www.cbe.iastate.edu/nsfbioren/**

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Executive Summary

This report provides an overview of the National Science Foundation and American Chemical Society-sponsored workshop held on June 23 and 24, 2005, in conjunction with the ACS Green Chemistry and Engineering Conference in Washington, D.C. The workshop included 12 invited participants, who attended the entire workshop, and 16 part-time participants, who were attendees at the ACS conference. The biorenewable theme for the workshop was follow up to a previous NSF-sponsored workshop entitled "Catalysis for Biorenewable Conversion" (www.egr.msu.edu/apps/nsfworkshop), which was held in April, 2004. This second workshop focused more specifically on the design of catalysis systems with particular emphasis on engaging academic and industrial researchers already working on constructing catalyst systems for use in biorenewable conversions to chemicals.

In contrast to the first workshop, which largely drew participants from the heterogeneous catalysis community, participants in this workshop were selected from both the biocatalysis and chemocatalysis (catalysis materials other than enzymes or whole cells) communities. As these two catalysis communities typically do not hold joint research discussions, an important objective of the workshop was to initiate the examination of synergies between these different catalytic approaches to determine if a common framework could be established for their application to biorenewable conversions. A question posed early in the conference was whether there was in fact value in jointly engaging the biocatalysis and chemocatalysis communities for the biorenewables area. The strong consensus from the participants was that this joint engagement was important for advancing biorenewable conversion technology, since both types of catalysis will be required.

The grand challenge developed in the workshop for the utilization of renewable carbon and development of the catalyst technology underlying this new carbon source was *harnessing catalysis for a new carbon economy*. Application of this grand challenge to the design of catalysis systems led to the development of three overarching thematic areas in the workshop:

a) design and engineering of individual catalysts for biorenewables;

b) hybrid catalyst systems for biorenewables and new carbon materials;
c) multiscale modeling and characterization of structure/function relationships in biorenewables process streams.

This report further describes scientific and technical challenges underlying these thematic areas and provides a number of vignettes illustrating examples of approaches that have been taken to address these challenges.

The first thematic area is further divided into four areas including issues of catalytic site design, issues of catalyst selectivity, issues of catalyst production and issues of catalyst application. In many respects the research required to address these issues for individual catalysts in biorenewable conversions is unique to the development of either biocatalysts or chemocatalysts and, therefore, requires different approaches for designing an efficient catalyst system. However, an important aspect that is relevant to both catalyst types is the cross-fertilization of concepts between them such as examining the incorporation of metal sites within proteins or constructing organic-inorganic hybrid catalytic sites that can mimic features of the catalytic site environments in proteins.

The hybrid systems discussed in the second thematic area concerns designing catalyst systems that can lead to a cascade of reactions. These reaction cascades could be developed to accomplish reactions in a series of several reactors in series or to achieve multiple reactions in the same reactor. The hybrid catalyst systems could consist of either biocatalysts or chemocatalysts or involve a combination of the two catalyst classes. A key question in developing multiple catalyst systems is how to incorporate the best features of biocatalysts and chemocatalysts to create a synergistic reaction system that is most efficient to produce the desired product.

An important underpinning to the design of individual catalysts or hybrid catalyst systems is multiscale modeling and characterization, which is the topic of the third thematic area. Understanding structure/function relationships of both the catalytic site and the reaction substrate will be needed to deal with the complexity of converting substrates that contain multiple functional groups. However, this molecular-level analysis also needs to connect to a systems-level analysis. Examples of this systems-level requirement include integrated metabolic pathways in whole cell catalysts and condensed-phase chemical reactions with impure process streams

The workshop served to initiate the interaction between biocatalysis and chemocatalysis researchers to address the challenges involved in biorenewable conversions. The need for this interaction was validated and supported by the participants. While this report provides the start of a common catalysis framework, there remains a need to further refine it to exploit the synergies between the two catalyst classes so that effective catalyst systems for biorenewable conversions can be designed.

Workshop Overview and Objectives

The workshop, which was held in conjunction with ACS Green Chemistry and Engineering Conference, occurred on June 23 and 24, 2005 at the American Chemical Society headquarters in Washington, D.C. A primary goal of the workshop was to bring together researchers from the biocatalysis and chemocatalysis communities who are actively involved in the design of catalyst systems for biorenewables conversion. Appendix A provides a list of the invited participants. Given the experience of the 12 invited participants, the workshop was able to focus on the interaction between biocatalysis and chemocatalysis rather than including extensive background information on biorenewable chemicals. The format for the workshop, which is given in Appendix B, began with presentations on the design of catalyst systems specifically for biorenewables. These presentations were open to attendees at the ACS Green Chemistry and Engineering Conference. The presentations can be found on the workshop website at www.cbe.iastate.edu/nsfbioren/.

After the presentations several working sessions were held with the following objectives:

a) determine if there is value in jointly engaging the biocatalysis and chemocatalysis communities in developing the challenges involved with biorenewables;
b) establish a grand challenge for the biorenewable area that would provide a common connecting point for these communities;
c) identify significant catalysis-related challenges for progress in the biorenewables area;
d) establish a forward plan for creating synergy opportunities between biocatalysis and chemocatalysis science and technology development as applied to biorenewables.

Summary Recommendations

The central objective of the workshop was to gather experts in both biocatalysis and chemocatalysis, who have worked on catalyst design for biorenewable conversions, to discuss whether biorenewables research would be aided by jointly engaging these catalysis communities. A strong consensus from the participants was that joint engagement was important for moving forward catalysis science as related to biorenewables and for accomplishing the grand challenge of *harnessing catalysis for a new carbon economy*. Once this basis was established, more specific technological challenges were discussed that could be grouped into three broad thematic areas. Given below are the thematic areas as well as more specific challenges that were identified.

Design and Engineering of Individual Catalysts for Biorenewables

Methods for enzyme design need to be expanded to encompass learning from chemocatalysts where metal sites could be incorporated within enzymes.

There is a need to develop enzyme catalysts that are robust to higher operating temperatures, which could be facilitated by understanding the amino acid sequences that lead to stability in proteins that are active at elevated temperatures.

The incorporation of non-natural amino acids into biocatalysts has applications in structural research, but also provides the potential for investigating protein function and stability.

A challenge is how to develop new methods of confining organic and organometallic catalytically active sites, which will allow for increasing selectivity in catalysis.

The ability to organize active sites in synthetic organic or inorganic catalyst systems would provide a means for replicating the functional group cooperativity that can occur with enzymes.

Catalysts that act on insoluble substrates, e.g., protein particles, biomass materials, or starch, provide a unique opportunity for improving the efficiency of the biorenewables industry.

Many reactions will be carried out in an aqueous system so there is a need to understand adsorption, interaction and cavity effects in an aqueous environment.

The design of chemocatalysts in which the active site structure and composition is the same throughout the catalyst is important in pursuing high selectivity.

An important issue in using chemocatalysts for converting biorenewables is how to perform selective chemistry on a single functional group when the molecule to be converted contains multiple functional groups.

Olefin metathesis is a powerful method for the catalytic transformation of fatty acids and vegetable oils into new families of products, but further catalyst development will require higher efficiency in the form of high turnover numbers.

A limited number of primary organisms are available for use as host systems, so new platform organisms still need to be developed.

To improve the efficiency of whole cell biocatalysis, there is a need to be able to uncouple cell mass production from product synthesis.

Microbial conversions under extreme conditions, such as high temperature, extreme pH and high salt or ionic concentrations would be desirable for some processes.

A challenge in the conversion of many biorenewable conversions is the delivery of hydrogen. The development of new enzyme cofactors that mimic those in biological systems could enhance this transport.

Long-lived and renewable catalysts will be needed for biorenewable systems that can deal with fouling by protein, phenolics, nucleic acids, and naturally-occurring materials and that have support materials that are stable in aqueous solutions at high or low pH.

Hybrid Catalyst Systems for Biorenewables and New Carbon Materials

For hybrid catalyst systems, an approach needs to be developed that determines where is the best place for the chemistry to occur such as within the plant itself or at a biorefinery.

Process integration is needed that will enable biocatalytic systems to carry out synthesis reactions that are not well suited for chemocatalysis and that will exploit chemocatalysis for high throughput rates and concentrations which cannot be readily achieved in a biological system.

Reaction systems could be designed so that parallel/sequential reactions could be accomplished in a one-pot synthesis thereby obviating the need for purification between reaction steps.

Examine the potential for using biocatalysts to remove poisons that would inactivate chemocatalysts.

Combination of catalyst types could be used such that either enzymes or chemocatalysts are used as delivery systems for one another.

The potential exists to design plant feedstocks for better subsequent processing in a biorefinery by improved integration of genetic engineering and targeted breeding.

Metabolic engineering needs to be further developed so that economically attractive concentrations of products can be achieved by directing the metabolic pathways in whole cell biocatalysts.

Cooperative catalysis needs to be developed at both the microscopic length scale, such as organized catalytic active site, and the macroscopic length scale in which synergistic combinations of different catalyst types are used.

There is a need to further develop the concept of consolidate bioprocessing in which several biological processes combined into one step.

New conversion chemistries will need to be developed to enable transformation of by-products from biorenewable reactions into useful products.

Multiscale Modeling and Characterization of Structure/Function Relationships in Biorenewables Process Streams

Spectroscopic techniques need to be developed for *in situ* imaging for catalyzed biorenewable reactions.

Chemical reaction theory should be developed for the unique process conditions involved with biorenewables, which includes condensed phase and impure process streams.

There is a need for fundamental studies of enzymatic catalysis in highly concentrated solutions and in relation to protein-surface interactions.

Characterization and modeling techniques need to be developed for highly organized assemblies of enzymes such as the cellulosome to better understand the enzyme cooperativity.

Methods are needed to characterize organized catalytic sites that are synthesized in chemocatalysts.

Models need to be developed for the interaction of metal ions and metal catalysts with sugars, lignin, proteins, and other materials in biorenewable feed streams.

Introduction

Multiple incentives including carbon dioxide emission reductions, dwindling resources, and national security concerns drive the need to diminish society's dependence on carbon derived from nonrenewable fossil fuels. However, transitioning from a crude oil-based chemical industry to one that relies primarily on renewable feedstocks will require fundamental changes in conversion routes to industrial chemicals. Due to the economic ramifications of these changes, any proposed renewables-based replacements – whether new routes to existing materials or new materials that functionally substitute for established products – must offer compelling advantages over the status quo. The chemical industry has accumulated a 100-year technical knowledge base for a petroleum foundation; the process needs to be jump started for the biorenewable feedstock approach. The utilization of renewable carbon and developing the catalyst technology underlying this new carbon source led to posing the grand challenge of *harnessing catalysis for a new carbon economy*.

The economic conversion of biorenewables to desirable chemical products will require harnessing biocatalyst and chemocatalyst technology for the unique large volume and low cost demands dictated by commodity chemicals. The chemical properties inherent in biobased feedstocks will likely necessitate the use of both biocatalysis and chemocatalysis in a biorenewable-based chemical industry. Chemocatalysis has been successfully exploited in the petrochemical industry to meet the commodity chemical demands, but fundamentally new technical challenges will need to be conquered to extend broadly the use of chemocatalysts into biorenewables. While biocatalysis has made limited inroads into the production of commodity chemicals, commercial biocatalysis technology has predominantly focused on high value specialty and fine chemicals. Therefore, a new carbon economy based on renewable carbon will require significant technical advancements in biocatalysis and chemocatalysis.

To better define the underlying technological issues that need to be addressed in advancing the catalysis and reaction engineering science associated with converting biorenewables to industrial chemicals, the National Science Foundation (NSF) sponsored a workshop entitled Catalysis for Biorenewables Conversion on April 13-14, 2004. The focus of the workshop was bringing together engineers and scientist who worked in research areas that were relevant to biorenewables conversions, but who had not generally applied their expertise directly to the area. The workshop served an important role in engaging the broader catalysis community (predominantly the chemocatalysis community) and initiating dialogue on identifying the critical technological needs in catalysis for biorenewable conversions. The workshop report (found at http://www.egr.msu.edu/apps/nsfworkshop) provides an excellent overview of the workshop discussions. There was general agreement from the participants that the workshop was successful in initiating the discussion on catalysis for biorenewable conversions, but that more in-depth treatment was needed as well as stronger engagement with the biocatalysis community. This need was addressed in a second workshop, Design of Catalyst Systems for Biorenewables, held on June 23-24, 2005 in Washington, DC. This document constitutes the final report from the 2005 workshop.

From the perspective of this workshop, the challenge discussed was the design of catalysts and catalytic systems capable of efficient transformation of renewable materials into value-added products and new carbon compounds that will supplant or supplement those derived from petrochemical sources. Complementary and interdisciplinary thrusts is one of the requirements of meeting this challenge, whereby scientists and engineers in the chemistry, engineering, biotechnology, and biology communities will work together to address the scientific answers needed to catalyze a transformation from predominantly petroleum sources of carbon compounds, to a scenario that includes biorenewables. To initiate the interdisciplinary efforts, the workshop gathered participants with experience in the design of both biocatalysts and chemocatalysts for the conversion of biorenewables to industrial chemicals.

Virtually all current industrial chemical products are synthesized from a small group of platform chemicals derived from petroleum and natural gas such as ethylene, benzene, etc. The path from crude oil to final commercial product thus requires two basic steps: building block extraction and/or synthesis, then subsequent conversion to the final target. This basic model has been extremely successful, and it will continue as the foundation for the chemical industry for the foreseeable future. What *will* change, however, are the origins and identities of the high-volume building blocks. Agricultural products provide a broad array of materials in complex feedstocks, and some of these have the realistic potential to replace or supplement petroleum-derived materials in the production of commodity chemicals. Since it is very likely that only a handful of biorenewables-derived building blocks will attain platform chemical status, it is useful to initially focus attention primarily on the production and further transformations of those that seem most likely to succeed. After considering more than 300 potential carbohydrate-derived candidates, the authors of a 2004 DOE report (*Top Value Added Chemicals from Biomass* found at http://www.nrel.gov/docs/fy04osti/35523.pdf) suggested that 12 had the greatest potential as building blocks for a chemical industry based on "biorefineries" (Figure 1) as determined through an extensive analysis. Following current industry practice, these can be classified by carbon number, ranging from C_3 to C_6.

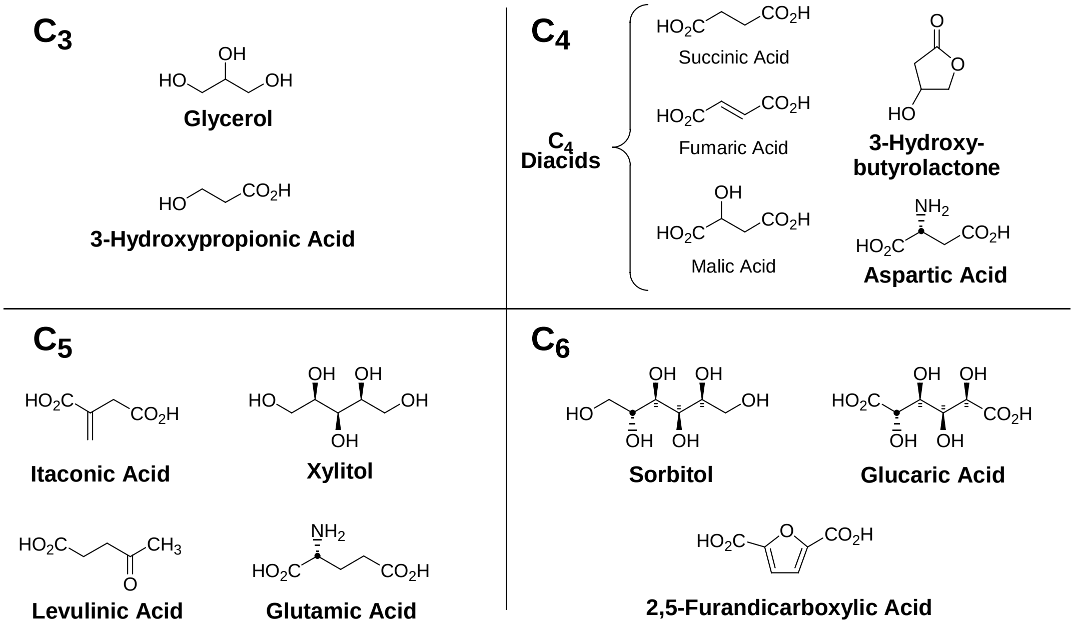


Figure 1. Twelve carbohydrate-derived compounds selected as most likely to be developed into biorenewables-derived platform chemicals. (*Top Value Added Chemicals from Biomass*, U.S. DOE, 2004).

The NSF can play an important role in the transition from a petroleum-based chemical economy to one using significant amounts of biorenewables by targeted grant support. This will have two beneficial effects. First, the intellectual property developed with NSF support may directly benefit the emerging new carbon economy. Also, this academic work will catalyze private sector research (either in collaboration with academic groups or independently) that will yield economically viable processes. A workforce trained in the concepts of a biorefinery-based chemical industry will be the even more important result of targeted NSF funding. This agency has a longstanding tradition of emphasizing graduate education and providing hands-on training in laboratories focused on converting biorenewables to platform chemicals and further conversions to final targets will ensure that the research workforce of tomorrow will have the tools to succeed in the new chemical economy.

To provide initial focus to the workshop, talks were planned around two themes: catalysis in the production of ethanol and new monomer building blocks to replace petrochemically-derived counterparts. Copies of these presentations are available at the web site for the conference. While the talks only directly addressed a few of the projected building blocks described above as well as several additional molecules, having speakers with diverse backgrounds presenting their views on the same topics helped to uncover synergistic approaches combining chemistry, biology and chemical engineering.

Harnessing Catalysis for a New Carbon Economy

Meeting the grand challenge will require the development of a new catalysis knowledge base that will result in fundamentally new catalysts and unique implementation of existing ones in order to address feedstocks that are thermally labile, highly functionalized, and renewable, i.e., biorenewable resources. The challenges are to process these molecules in a cost-effective manner using environmentally compatible catalysts that can result in a sustainable carbon economy. The overarching thematic areas that the workshop participants developed were:

a) design and engineering of individual catalysts for biorenewables;
b) hybrid (syncretic) catalyst systems for biorenewables and new carbon materials;
c) multiscale modeling and characterization of structure/function relationships in biorenewables process streams.

There are numerous technical barriers that must be overcome for the development of robust, sustainable, and cost-effective catalytic systems targeted to the processing of biorenewables. Catalysts must have properties including selective conversion, multifunctionality, transparent functioning, minimum complexity, and ultimate utility either directly or indirectly utilizing CO_2 in order to make advanced molecules and materials. Within this context, the workshop participants converged on advanced concepts, which can be realized assuming that an interagency, multidisciplinary, cross-cutting research program is initiated to integrate concepts that are as diverse as self-disassembling materials that are catalytically degraded to their building blocks for re-use and the bioinspired design of catalysts and materials that mimic many of the benefits of nature with respect to clean synthesis and a complete life cycle where the key building block (carbon) is recycled.

Examples of key concepts include: syncretic catalysts and catalyst systems that combine the best of chemical and of biological catalysis; new enzyme co-factors that serve as sources and storage of hydrogen for use in chemical synthesis; chemocatalysts that function successfully in the aqueous phase; advanced computer modeling that would enable molecules to be designed starting at an atomic level, and then developed into materials whose structure, function, and characteristics could be mathematically estimated. These approaches would enable rapid prototyping and design of new materials and new catalytic systems that could be tested on a larger scale.

The concepts include engaging agriculture to produce feedstocks that are designed specifically for industrial processing and chemicals, and which may contain catalysts for their transformation into useful products, where these catalysts are activated at non-biological conditions (high temperature, high pressure, organic solvents) in an industrial processing plant. The interaction between agriculture and the biorenewables area is an underlying theme, since agriculture will provide the renewable resources required to develop a new carbon economy. The new carbon economy will initially meld the best of both worlds, the renewability of certain biorenewable resources and the established science base and efficiency of petrochemical processing methods.

The societal impacts will include the development or evolution of a new economy which is based on renewable resources. This is a gradual process that will require, and be based upon, the cooperative efforts between the renewable and the petrochemical communities and will examine areas of traditional catalysis where improvements are made using bio-inspired designs. In addition, the best characteristics of certain types of catalysts that have already been developed for the petrochemical industry will also be implemented by designing new types of polymeric materials and biocatalysts that are based on proteins derived from biological sources. This effort will build upon the rapidly growing liquid fuels sector which utilizes biorenewable resources in order to make transportation fuels in facilities that are located near the sources of the sugars. This program will benefit not only the existing areas of concentrated petrochemical and high-tech industries in the United States, but also will create jobs in areas of the country where agriculture and biorenewable resources will provide the source of raw material to drive the industry.

Thematic Areas

Thematic areas that would form a national initiative on harnessing catalysis for a new carbon economy encompass the design and engineering of individual catalysts for biorenewable transformations, hybrid catalyst systems for biorenewables and the generation of new carbon materials, and the multiscale modeling and characterization of structure and function of both biorenewables and materials derived from them. The benefits of carrying out research, the needs for obtaining a fundamental science base, and developing students to work in these areas is described under each of the areas outlined below.

A. Design and Engineering of Individual Catalysts for Biorenewables.

The scientific and technical challenges highlighted in this section were further sub-divided into four areas: issues of the catalytic site, issues of catalyst selectivity, issues of catalyst production and issues of catalyst application.

Catalyst design issues

- **Methods for enzyme design and learning from chemical catalysts to design new biocatalysts.** As increasing knowledge regarding the interaction of transition metals with biorenewable building blocks is obtained, these results will feed back into related biochemical processes. Mechanistic understanding of chemical processes at the molecular level will provide information of use to biochemical transformations. What unique mechanistic features of a selective, metal catalyzed oxidation can be used to guide development of new biocatalysts? In analogy to labeling of DNA with metal centers through hybridization, can a biocatalyst bind to a biorenewable and selectively deliver a chemical catalyst to a single site? Conversely, could the much smaller chemical catalysts bind to sites not accessible to biocatalysts, but simultaneously deliver the biocatalyst to a remote site? Could such hybrid catalysts be used in tandem to carry out simultaneous chemical and biochemical transformations at different sites?

  New enzymes can be developed by mapping the chemistry of transition states for inorganic or silica-based metal supported catalysts. In this case the enzymes would mimic the characteristics of the inorganic catalysts, and thereby lead to new pathways. The key example would, for example, be the use of gold. The metal, such as gold, which is supported on the catalyst, would be integrated into a protein catalyst to make it more efficient.

- **Robust protein catalysts.** Robust protein catalysts are needed that can operate at temperatures of up to 80°C. Some of these can be isolated from thermophilic microorganisms. Using these enzymes or other proteins with inherent thermal stability, their structure and function as well as genetic templates can be studied in order to better understand the amino acid sequence that leads to their stability. Once this is achieved, other catalysts can be developed which are more robust, and resistant to higher thermal conditions, although these conditions would not approach the temperatures traditionally seen for inorganic catalysis.

Microfluidic Biocatalysis

Microfluidic channels with a characteristic cross sectional width of 50 to 80 microns form when poly(dimethylsiloxane) (PDMS) sheets are pressed onto fibers placed on C18 coated SiO_2 glass slides. Particles with ion-exchange or affinity functionalities, adsorbed onto the fiber may provide a platform onto which enzymes may be immobilized. The resulting monolayer of particles results in a microfluidic

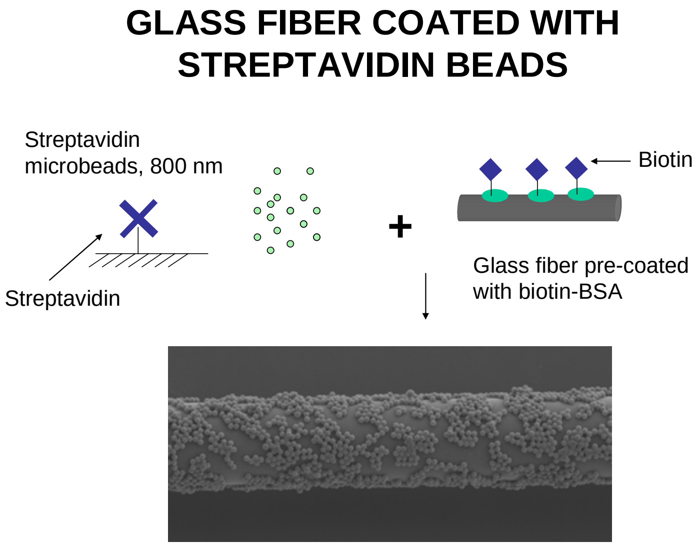
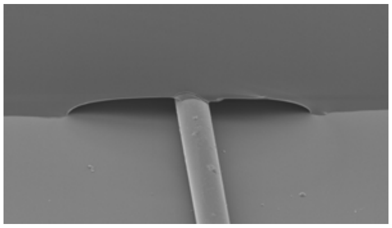


Figure 2. . *Left:* Schematic and SEM showing immobilization of streptavidin beads on surface of fiber. *Below*: Channel formed by fiber.



biocatalytic reactor. For example, beads with streptavidin surfaces, coupled to a biotinylated fiber surface, could anchor biotinylated enzymes, while blocking non-specific adsorption of other macromolecules. The contact angles of the surfaces that make up the channel induce the fluid to form a narrow boundary layer next to the fiber, where it directs molecules to the biocatalyst surface. Rapid screening of catalysts in an aqueous flow reactor is enabled, since devices of this type may be constructed in less than an hour. Huang *et al.* (Huang, T. T.; Geng, T.; Akin, D.; Chang, W-J.; Sturgis, J.; Bashir, R.; Bhunia, A. K.; Robinson, J. P.; Ladisch, M. R. *Biotechnol. Bioeng*. **2003**, 416-427, Huang, T.; Chang, W-J.; Akin, D.; Gomez, R.; Bashir, R.; Mosier, N.; Ladisch, M. R. *AIChE Journal* **2003**, *49*, 2984-2987, Huang, T. T.; Taylor, D. G.; Sedlak, M.; Mosier, N. S.; Ladisch, M.R. *Analytical Chemistry* **2005**, *77*, 3671-3675) summarize the benefits. "Formation of microfluidic features using microfibers is an attractive research tool because it places a rapid prototyping capability for microfluidic devices within the reach of many laboratories. Access to glass slides, an optical microscope, digital camera, tweezers, and PDMS is sufficient to initiate studies on microfluidic devices for study of mixing, separation, microfluidic transport, reaction, and sensing."

- **Confined organic and organometallic active sites.** Confinement has been recognized to be extremely important for controlling enantioselectivity in many chemical catalytic systems; it is also important for controlling adsorption. Increased confinement of an active site typically increases the enantioselectivity of a chemical catalyst. Confinement could also be used to control regioselectivity of chemical reactions, as well as general shape selectivity. Zeolites are an excellent example of the power of confinement for catalysis; however, there are few methods currently available for confining organic and organometallic active sites in heterogeneous catalysts. The challenge is in how to develop new methods for confining catalytically active sites, which will allow new applications for increasing selectivity in catalysis. Three (of many) references on the benefits of enantioselectivity upon confinement in synthetic heterogeneous systems: 1) Corma, A.; Iglesias, M.; del Pino, C.; Sanchez, F. J. *Chem. Commun.* **1991**, 1253-1255; 2) Jones, M. D.; Raja, R.; Thomas, J. M.; Johnson, B. F. G.; Lewis, D. W.; Rouzaud, J.; Harris, K. D. M. *Angew. Chem. Int. Ed. Engl.* **2003**, *43*, 4326-4331. 3) Raja, R.; Thomas, J. M.; Jones, M. D.; Johnson, B. F. G.; Vaughan, D. E. W. *J. Am. Chem. Soc.* **2003**, *125*, 14982-14983. A reference contrasting confinement in synthetic and biological systems: Brunet, E. *Chirality* **2002**, *14*, 135-143.

Design of Confined Sites

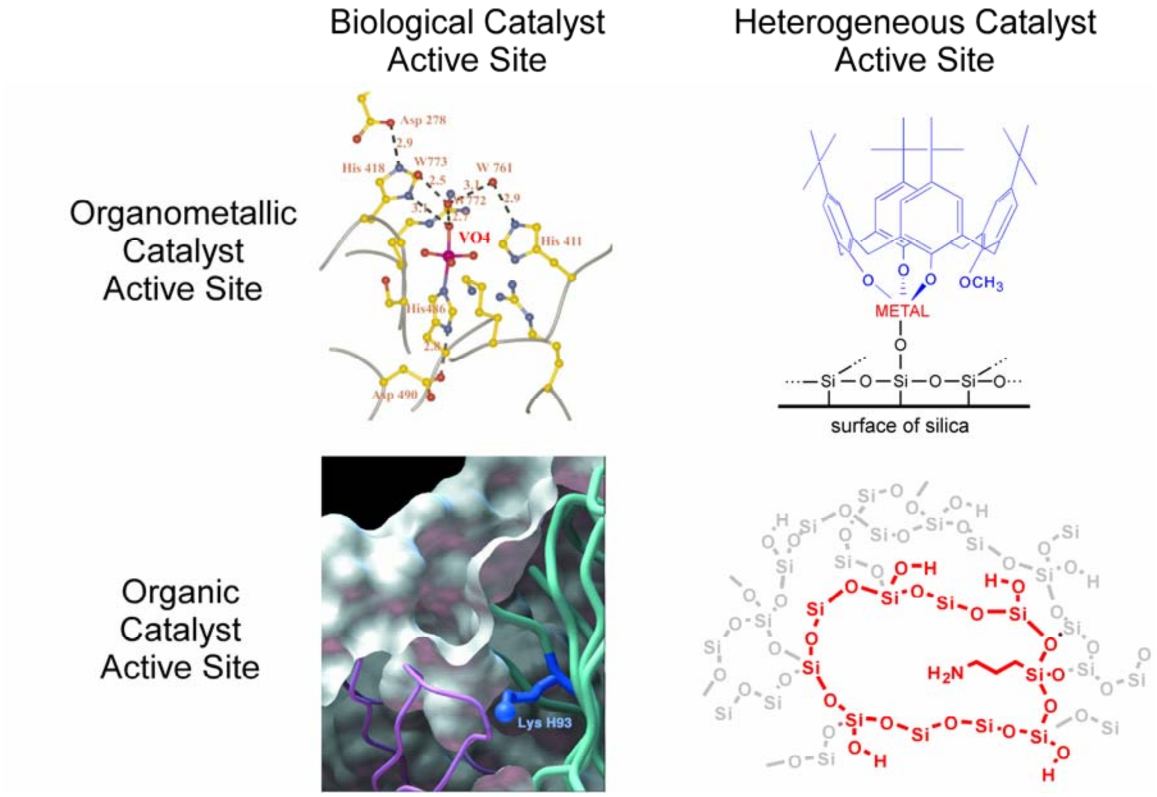


Figure 3. Demonstration of synthetic and biological catalysts consisting of similar organic and organometallic active sites. Note the confined environment surrounding both biological catalysts, which results from the hydrophobic interior of the enzyme. It is this confinement that is responsible for the high enantioselectivity of both biological catalysts. This confinement has been successfully replicated in the

synthetic equivalents of the biological active sites shown on the right-hand-side of the figure. The structure of the biological active sites has been determined using single-crystal X-ray diffraction (Weyand, M.; Hecht, H. –J.; Kieß, M.; Liaud, M. –F.; Vilter, H; Schomburg, D. *J. Molec. Biol.* **1999**, *293*, 595-611 for the organometallic active site and Barbas III, C. F.; Heine, A.; Zhong, G.; Hoffmann, T.; Gramatikova, S.; Bjornestedt, R.; List, B.; Anderson, J.; Stura, E.; Wilson, I. A.; Lerner, R. A.. *Science* **1997**, *278*, 2085-2092 for the organic active site). The structure of the synthetic heterogeneous catalysts has been determined using a combination of spectroscopy, probe molecule adsorption, and catalysis. The synthetic organic catalyst (lower right hand corner) represents the first hybrid acid-base bifunctional hybrid organic-inorganic catalyst (Bass, J.D., Anderson, S.L.; Katz, A. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 5219-5222); whereas the organometallic synthetic catalyst represents an example of a single-site heterogeneous catalyst that encompasses a new concept for confinement, in which the metal center is organized between a calixarene and silica metal surface (Notestein, J.M.; Iglesia, E.; Katz, A *J. Am. Chem. Soc.* **2004**, *126*, 16478-16486). Both synthetic catalysts show unprecendented high activity due to the specific synergy of the active site with chemical functional groups in the surrounding outer-sphere (organic) and inner-sphere (organometallic), and provide a platform for the incorporation of selectivity-by-design into these active sites.

- **Replacing proteins with organic and inorganic polymeric catalysts that lack nitrogen and sulfur that are active under a wider range of process conditions.** Enantioselective catalysis (one of the current roles played by enzymes for biorenewables catalysis) is an area where synthetic heterogeneous systems may replace natural catalysts (see enantioselectivity entry above). Metalloenzymes for selective oxidations can be replaced if they could be accomplished with a synthetic system. For example, consider the system of a V-oxo metalloenzyme catalyst for enantioselective oxidation as described in Andersson, M.; Willetts, A.; Allenmark, S. *J. Org. Chem.* **1997**, *62*, 8455-8458. If this and other enzymatic catalysts could be replaced with a synthetic heterogeneous enantioselective catalyst for enantioselective oxidation, it could have large economic repercussions. Another area is multifunctional catalysis for greater activity/selectivity, where specific adsorption and catalysis due to functional group organization in an active site results. Hydrolysis of oligo- and poly-sacharides represents one such area in which enzymes are currently being used and are advantageous. How can synthetic systems be made with active sites that fulfill some of the general functions that biological catalysts accomplish (i.e. functional group cooperativity, etc.)? Templating strategies for synthesis of synthetic heterogeneous catalysts may be useful here for organizing the active sites in the synthetic systems.

- **Catalysts that act upon insoluble substrates.** Catalysts that act on insoluble substrates, be it protein particles, biomass materials, starch, or other biorenewable resources, provide a unique opportunity for improving the efficiency of both the biorenewables industry and also leading to development of new carbon products. For example, the processing of starch to monosaccharides, where the monosaccharides form a starting feedstock for various types of fermentation, are currently carried out on starch that is cooked in water at high temperature. Significant energy savings could be achieved if enzymes are developed that can directly act on the starch substrate without prior cooking of the starch. Such enzymes are being developed and tested in the industry. Using this as an example, we could further extrapolate this characteristic to imagine catalysts that are either protein or organically based that can adsorb onto solid substrates such as cellulosic materials, to disassemble them into their unit components. This would then enable feedstocks to be created which could be used in the existing infrastructure of the chemical industry, as well as lead to new processes and products for the new carbon economy.

Diacid Catalysts for Cellulose Hydrolysis

The rapid and complete hydrolysis of cellulosic materials can be achieved by using large amounts of cellulase enzymes or mineral acids at relatively high temperatures. In the case of mineral acids, degradation products also readily form which make this method less attractive than enzyme hydrolysis where product yields are essentially 100% fermentable sugars. However, in the case of enzymes, the amount of catalyst which must be used is so expensive that it is not practical in many cases. An approach that is inspired by knowledge of enzyme structure and function, while still being based on acid catalysis, is given by the dicarboxylic acid maleic acid ($C_4H_4O_4$). In this case, the difunctional nature of the acid, enables it to have hydrolytic activity (low pK_a) while at the same time minimizing degradation of the monosaccharides that are formed (Mosier, N. S.; Wilker, J. J.; Ladisch, M. R. *Biotech. Bioeng*. **2004**, *86*, 756-764, Mosier. N.S.; Ladisch, C.M.; Ladisch, M.R. *Biotech. Bioeng.* **2002**, *79*, 610-618, Mosier, N.S.**;** Sarikaya, A.; Ladisch, C.M.; Ladisch, M.R. Biotech. Prog. **2001**, *17*, 474-480). This leads to a hypothesis that covalently linking a selective acid catalyst, such as maleic acid, to a cellulose binding domain could result in a catalyst that has sorptive selectivity that approaches that of an enzyme, while at the same time being made from an organic structure which should be biodegradable once the use of the acid has been completed.

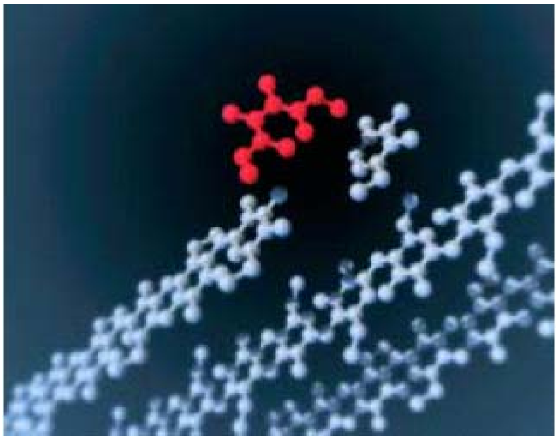


Figure 4. Maleic acid (red) hydrolyzing cellulose chain (photo courtesy of Purdue University College of Engineering)

- **Understanding adsorption, interaction and cavity effects in an aqueous environment.** One of the challenges in developing catalysis for a new carbon economy is the likelihood that many of the reactions will be carried out in an aqueous environment. Many of the transformations that enzymes carry out indeed reflect a very hydrophobic interior of the protein molecule which is stably presented to the substrate in an aqueous solvent. The mechanism of action involves the change in confirmation of the protein, transport of the substrate or substrates into the active site of the enzyme. Fundamental knowledge of how these different reaction pathways occur, and how hydrophobic cavities are able to carry out catalysis in aqueous solvents deserves study. This will help to develop a fundamental knowledge base which can then be used to design other catalysts.

  Biological catalysts (enzymes) and receptors (antibodies) rely on the design of intricate cavities decorated with precise organic functionality on their interior periphery for activating reactants for chemical reaction via use of controlled adsorption from aqueous solution, involving many simultaneous chemical interactions. It is generally unknown how to do the same in synthetic heterogeneous catalysts and adsorbents. This reduces to a question of how to control the steric and electronic environment (including in an enantioselective sense) of a molecule in its adsorbed state, with water acting as solvent? Although there are numerous examples of functional synthetic adsorbents that rely on a single type of interaction for adsorption (i.e. electrostatic in ion-exchange resins, dispersive interactions in hydrophobic carbons and zeolites for removing trace organics from water), there are relatively few examples where these interactions are working synchronously for causing adsorption by design (i.e. examples where there are hydrogen bonding, electrostatic, and/or dispersive interactions happening simultaneously for achieving a desired goal in adsorption). An open question is what is the role of water for systems involving adsorption onto a solid by the different mechanisms above (versus organic solvents or gas phase environment)? Applications of understanding adsorption include synthesis of heterogeneous catalyst active sites that are surrounded by a hydrophobic shell, which can protect the active site from water and disfavors partitioning of polar species, such as catalyst poisons (see Bass, J.D.; Katz, A. *Chem. Mater.* **2003**, *15*, 2757-2763). A better understanding of adsorption on solids can also be useful also for controlling chemoselective catalysis, where one portion of a bifunctional molecule is left unreacted whereas only the other portion is reacted (i.e. example of sugar oxidation to acid where desire only terminal OH's for reaction).

Designing Hydrophobic Catalytic Domains

Unlike catalyzed petrochemical reactions, biorenewable conversions will typically occur in the condensed phase. The reaction performance of enzymes in the condensed phase is enhanced by the relative hydrophobicity/hydrophilicity of their catalytic domains. The advent of uniformly structured materials such supramolecular templated mesoporous metal oxides provide scaffolds in which the relative hydroprobicity of catalytic domains can be systematically modified. Diaz *et al.* have demonstrated that adding alkyl groups to acid functionalized mesoporous silica can improve the esterification efficiency of lauric acid with glycerol to monolaurin (Diaz, I.; Marquez-Alvarez, C.; Mohino, F.; Perez-Pariente, J.; Sastre, E. *J. Catal.* **2000**, *193*, 295-302). By appropriately selecting of the alkyl group as well as the alkyl group loading, the hydrophobicity of the catalytic pores was tuned to increase activity and selectivity to the monoglyceride. Modification of pore hydrophobicity was also found to enhance the activity of fatty acid esterification with alcohol (Mbaraka, I. K.; Shanks, B. H. *J. Catal.* **2005**, *299*, 365-373). The choice of alkyl

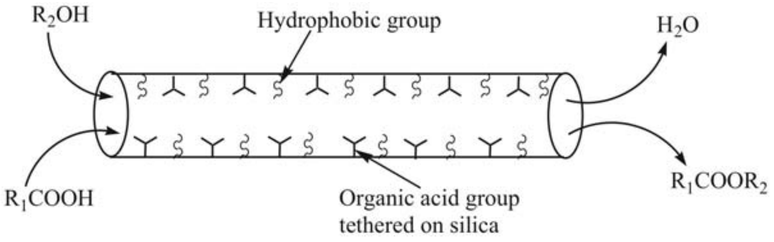


Figure 5. Schematic of mesoporous silica functionalized with organosulfonic acid and hydrophobic groups for fatty acid esterification with alcohol.

group and the location of the alkyl groups were found to significantly impact the resulting catalytic activity.

- **Single-site catalysis.** This represents a goal for making "heterogeneous" catalysts – solid catalysts – "homogeneous", in the sense that the active site structure and composition is the same throughout the catalyst. This can be important for applications when high selectivity is desired, because if there is a population of different types of active sites, some of which are non-selective, the overall selectivity can become poor due to amplification by the turnover frequency. Thus there is a need to prepare heterogeneous catalysts that behave as through there is one entity rather than a mixture of active sites on the surface. This is true for both organometallic and organic active sites. A hallmark of single-site catalysts is that their catalytic activity and selectivity do not depend on the local coverage of active sites on the heterogeneous catalyst surface (Notestein, J.M.; Iglesia, E.; Katz, A. *J. Am. Chem. Soc.* **2004**, *126*, 16478-16486).

Catalyst selectivity issues

- **Site-selective chemocatalysis on polyfunctional molecules**. A wide body of chemical catalysis knowledge has been developed for converting petrochemical species that have little functionalization. In contrast, biological molecules have a high degree of functionalization, which introduces a new set of challenges for chemocatalysts. An important question in using chemocatalysts for converting biorenewables is how to perform selective chemistry on a single functional group when the molecule to be converted contains multiple functional groups.

Selective Hydrogenolysis Catalysis

Catalyst and process conditions have been developed to convert sorbitol or xylitol through hydrogenolysis to ethylene glycol and propylene glycol. It would be desirable to increase the selectivity of these reactions to the desired glycol products.

However, little information is available on the interaction of polyhydroxylated molecules with metal surfaces. The initial step in the reaction system is dehydrogenation of the sugar alcohol to an aldehyde or ketone. A key question is the relative reactivity of the different hydroxyl groups to this dehydrogenation reaction, e.g., is the hydroxyl on the terminal carbon more or less prone to dehydrogenation than an interior hydroxyl. This reaction system can be better understood by examining the conversion and selectivity behavior of stereoisomer families of sugar alcohols. Within a stereoisomer family, the initial reaction rate over a Ru catalyst was found to correlate with the configuration of the molecule whereby fewer inversions in the hydroxyl location gave higher reaction rates (Lahr, D. G.; Shanks, B. H. manuscript in preparation, **2006**). The selectivity patterns for the stereoisomers suggested that the terminal hydroxyls were equally likely as the internal hydroxyls to be dehydrogenated by the Ru catalyst, but upon sulfur addition the reactivity of the terminal hydroxyls were significantly attenuated and the overall selectivity to the glycols improved (Lahr, D. G.; Shanks, B. H. *J. Catal.* **2005**, *232*, 386-394, Lahr, D. G.; Shanks, B. H. manuscript in preparation, **2006**). It is clear that more information is needed about the interaction of metal surfaces with biorenewables molecules to be able to design improved catalysts.

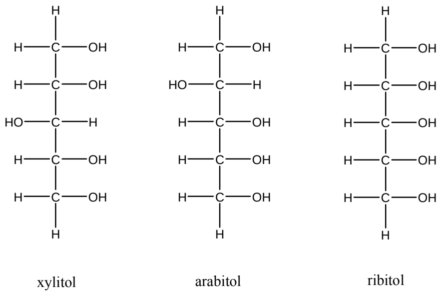


Figure 6. C5 stereoisomers.

Polyol	Reaction Rate	Inversions	Polyol Length
Glycerol	10.3	0	3
Erythritol	10.1	0	4
Ribitol	11.0	0	5
Threitol	5.0	1	4
Arabitol	9.5	1	5
Mannitol	7.8	1	6
Xylitol	6.4	2	5
Sorbitol	6.7	2	6
Galactical	3.5	2	6

- **Enantioselectivity in oxidations, reductions, aminations and C-C bond formation and breaking.** This topic is one example of a broader, fundamental need in catalysis for biorenewables. For the most part, chemical catalysis is optimized for hydrocarbons, and has not been widely applied to highly oxygenated renewable feedstocks, such as carbohydrates or lignin. Breakthrough technology will be identified by development of catalytic processes that can accommodate the structural features of these new feedstocks. As an example, carbohydrates present catalysts with a group of –OH groups exhibiting similar reactivity. Fundamental research will lead to new chemical catalysts able to differentiate these sites with high selectivity. Chemical catalysis in the petrochemical industry is able to induce a broad range of highly selective processes, such as selective C-C bond making/breaking processes, chemoselective and regioselective oxidations and reductions, introduction of stereochemically defined centers (diastereoselectivity), and sites of optical activity. Basic research probing the interaction of transition metal centers with the functional groups inherent to biorenewables will lead to processes of similar selectivity and power within the biorefinery.

- **Processing of vegetable oils.** The most available but least explored functional group of fatty acids are the aliphatic C-H bonds. Modern chemical or biochemical techniques are currently unable to effectively select and functionalize a specific C-H bond from among many similar bonds in the absence of neighboring activating groups. New metal catalyzed processes are beginning to develop the understanding necessary to effectively carry out these types of transformations. Most work on functionalization of C-H bonds has been carried out on simple hydrocarbons. The advantage possessed by fatty acids for implementation of this type of chemistry is the presence of additional functional groups that may be used to direct C-H bond functionalization (olefins, -COOH, -OH groups).

  Vegetable oils are a potential source of a diesel substitute known as biodiesel. When the triglycerides in vegetable oils are reacted with alcohol (methanol or ethanol), methyl or ethyl esters are formed. Catalysis for processing of vegetable oils require hybrid systems, since the coproduct of this reaction is glycerol, and must be processed to other value-added products in order to achieve an economic system. Catalyst systems that can do both types of conversions, or which would transform an inhibitory product into a useful one should be studied.

Designing Individual Catalysts for Biorenewables

The power of catalysis within the nonrenewable chemical industry is evidenced by more than 85% of all chemical processes using at least one catalytic step (Holderich, W. F.; Roseler, J.; Heitmann, G.; Liebens, A. T.; *Catal. Today*, **1997**, *37*, 353). Catalysis is employed because of its ability to promote reactions adding one molecular fragment to another, with incorporation of most or all of the atoms in both fragments in the final product. Catalysis thus embodies the goal of eliminating the stoichiometric reagent and its associated waste from chemical manufacturing. As the biorefinery develops, catalysis will play a similar role in conversion of biorenewables.

Yet conversion of biorenewables presents challenges rarely faced by catalytic processes optimized for hydrocarbons. Oleochemicals concentrate unfunctionalized and undifferentiated methylene groups in long chains. Sugars present several –OH groups with similar reactivity, but surprisingly poor activity as metal ligands (Gyurcsik, B.; Nagy, L. *Coord. Chem. Rev.* **2000**, *203*, 81). Lignin offers an almost random array of functionality spread over a large number of substructural units. The ability to address these new kinds of functional group challenges is at the very heart of organometallic chemistry and catalyst design. Fundamental knowledge will be necessary to develop processes with scope and utility equivalent to processes available for nonrenewables. How do biorenewables interact with metal centers? How are the structural features of biomass incorporated? Will processes work in water? How must catalyst structure be modified for biomass? How can the processes be controlled?

Catalytic processes, both homo- and heterogeneous, are starting to develop for biorenewables (Figure 7). Recent research activities are exploring what features of existing organometallic systems can be applied to biorenewables, and what types of new catalysts need to be designed to accommodate the unique structural features of these building blocks.

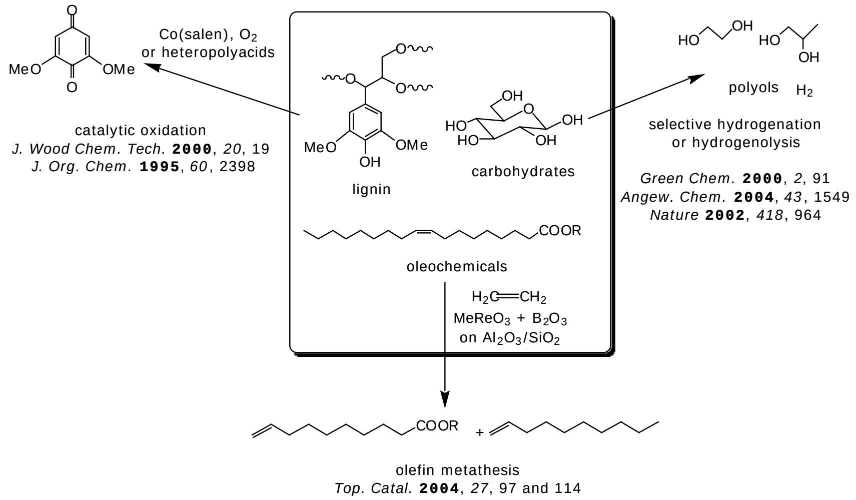


Figure 7. Representative catalytic transformations of biorenewable building blocks.

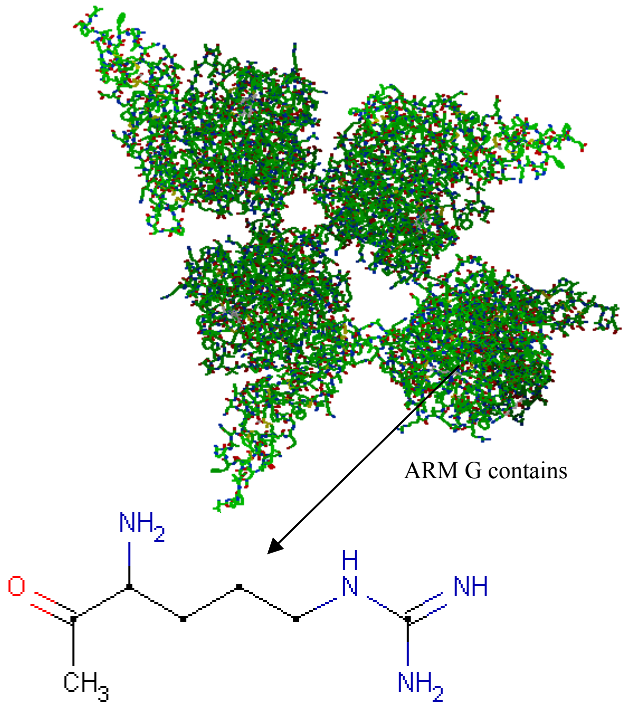
In this context, it is reasonable to assume that transition metal promoted reactions of biorenewables will find a key role in the use of renewable carbon. Organometallic processes will also lead to new types of reactivity with biorenewables not usually observed with conventional organic chemistry. Of central importance to NSF program development is that these types of transformations of biorenewables are largely unexplored, with surprisingly little interface between the two fields.

- **Catalysts for isomerizing olefins, metathesis of vegetable oils and immobilizing these catalysts.** Olefin metathesis has become an extremely powerful method for the catalytic transformation of fatty acids and vegetable oils into new families of products. Investigation of the actual metathesis process may be of less importance in an NSF context than other processes. There are a number of broadly applicable catalysts already available (in particular, Grubbs homogeneous Ru alkylidene complex and its growing family of derivatives). In addition, a considerable amount of fundamental knowledge regarding metathesis in general has been generated. However, a higher level challenge remains. Catalyst development requires processes exhibiting high efficiency, in the form of high turnover numbers. Processes for the metathesis of fatty acids frequently exhibit reasonable TONs, but only a very few are exceptional (see Mol, J. C. *Top. Catal.* **2004**, *27*, 97-104 for a review). Fundamental research into catalyst deactivation pathways, especially in processes involving biorenewable building blocks will lead to larger numbers of high activity, long-lived systems able to be used for industrial scale production. Further, immobilization of these catalysts on appropriate supports is a growing opportunity. Grubbs-type catalysts have been immobilized (Hallbach, T. S.; Mix. S.; Fischer, D.; Maechling, S.; Krause, J. O.; Sievers, C.; Blechert, S.; Nuyken, O.; Buchmeiser, M. R. *J. Org. Chem.* **2005**, *70*, 4687-4694). Understanding the interaction of sterically large molecules, such as oleochemicals, with these supported systems will lead to easily recoverable catalysts with high activity and selectivity.

Catalyst production issues

- **More platform organisms still need to be developed.** Currently yeast and *E. coli* are the primary organisms used as hosts. New platform organisms, such as those strive at high temperature, extreme pH levels, high ionic concentration and high osmotic pressure, still need to be developed.

Figure 8. Thrombin-Thrombomodulin complex example.



- **Incorporating non-natural amino acids into biocatalysts.** Incorporation of non-natural amino acids into proteins has become an increasingly important field, not only because of the applications in structural research, but also for investigating protein function and stability with tools not provided by nature. A powerful benefit of the approach is the coupling of site specific chemical modification and labeling of proteins during their translation.

Considerable progress has been made in this field following the pioneering work by Schultz (Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182-188) and Chamberlin (Bain, J. D.; Glabe, C. G.; Dix, T. A.; Chamberlin, A. R.; Diala, E. S. *J Am Chem Soc* **1989**, *111*, 8013-8014) in expanding types of amino acids, finding new methods of tRNA aminoacylation, and in extending genetic codes for directing insertion of new amino acids. To fully explore the potential of this new science, deeper understanding of protein and peptide structure with respect to incorporation of non natural chemistries must be acquired.

- **Uncoupling of cell mass production from product synthesis**. The cost of cell mass formation may be a serious economic issue in some cases. In order to maximize biomass utilization, it is highly desirable to uncouple the cell mass accumulation phase with that of the product synthesis phase. Optimization of this two-stage production process may lead to much improved overall process performance. Another related issue is how to prolong the life of the biocatalyst during the product synthesis stage.

Catalyst application issues

- **Exceptionally long-lived and reusable catalysts.** Long-lived and renewable catalysts will be needed for biorenewable systems since the potential for fouling by protein, phenolics, nucleic acids, and other naturally-occurring materials is significant. These catalysts will need to be designed to minimize adsorption of species that are not part of the catalytic reaction pathway, and which can be cleaned without using functionality.
- **Microbial conversion under extreme conditions.** Microbial conversion under extreme conditions, such as high temperature, extreme pH and high salt or ionic concentrations, may be very desirable for some processes. Some of the potential benefits include higher conversion rate, reduced contamination risk and potentially enhanced separation efficiency.
- **Chemical conversion in condensed phases with variable pH.** A significant portion of biorefinery operation will produce building blocks in aqueous solution (sugars). In some cases, these solutions (for example, those formed from certain bioprocesses) may be dilute. Therefore, biorefinery operations will require high efficiency

conversion processes that take place in such media over a range of substrate concentrations. Fundamental knowledge is needed in developing new catalysts (homo- and heterogeneous) that can operate with aqueous solutions of these materials. Studies on the stability and reactivity of heterogeneous supports in aqueous acidic media will lead to high efficiency processes for the conversion of biorenewables. Investigation of alternative condensed phases, such as biphasic systems and ionic liquids, will be synergistic with catalyst development by providing reaction media optimized for these new types of catalysts.

- **Robust catalyst supports.** The raw materials that will be derived from biorenewable resources may be chemically complex, and contain biologically-derived components (such as proteins, oils, and cell debris) that rapidly foul catalysts. In order to achieve effective transformation of biorenewables to value-added products, catalyst supports that resist fouling, can be cleaned between uses, and are generally stable in aqueous solutions at either high or low pH must be developed.
- **Keeping the organism of interest dominant in a long-term bioconversion.** Maintaining the desired culture characteristics is an important factor in maintaining proper bioprocess performance in the long run. The culture must be stable genetically over time. In addition, the strain must be resilient and be able to out-compete other potential contaminants. These attributes will lead to process robustness.
- **New enzyme cofactors.** The new carbon economy, particularly for transformation of CO_2 and other oxidized compounds, will require hydrogen to carry out the transformations. In biological systems, the hydrogen is supplied in a chemical form such as NADH, NADPH, and FADH. Using biology as a template, research should be carried out to study the fundamentals of developing organic co-factors that could be used in analysis using either biological or inorganic catalysts, and which would chemically transport the hydrogen for purposes of carrying out a reaction. This directed approach would mimic the sort of reaction that occurs in a cell, or which can be carried out with cell-free systems. The development of new co-factors that may be stable at higher temperatures, which will disassociate more freely than biological systems, or which will have higher solubility is proposed.

- **Reactor design and catalysts to allow ester or amide bond formation in dilute aqueous solutions.** The formation of esters or amides from natural materials in aqueous systems continues to be a challenge. For example, in the production of soy diesel through transesterification of fatty acids with short chain alcohols (methanol or ethanol), exclusion of water from the reaction catalyzed by sodium or potassium hydroxide to prevent saponification (hydrolysis and catalyst neutralization) is a significant challenge. Once the reaction is completed and alkyl-esters are formed, a solution phase of glycerol and catalyst (sodium hydroxide) results, which must be removed and properly disposed of. This research area would address the design of organic or other types of catalysts which can be synthesized in a solid form, packed in a reactor, and then used to carry out esterification reactions. Other approaches might include the study of organic hydrophobic pockets in catalytic systems that are dispersed in an aqueous solution containing the substrates.

B. Hybrid Catalyst Systems for Biorenewables and New Carbon Materials.

Crop-related issues

- **Where is the best place for the chemistry to occur? Within the plant itself or at the biorefinery?** The expression of catalysts in plants and other living organisms could be carried out post-harvest. In this way, the plant could be engineered or grown to contain the genesis of a catalytic system which is activated after the crop or component is harvested, and sent to the plant. Benefits of this approach include the storage of the raw material in a stable form until it is ready to undergo catalysis, and the *in situ* location of catalysts that could be used to efficiently carry out transformations without the usual mass transfer or steric limitations for catalysts that might be added to solid materials from an external source. The catalysts themselves would be selected to be directly active or indirectly active (i.e., a procatalytic system that activates the desired catalysts) in transforming the material into its component building blocks. Once these building blocks are obtained, further catalysis could be carried out
- **Process integration and process design.** Biological systems are adapted for carrying out complex sequences of inter-related reaction steps in order to conserve

energy. There are three types of fermentations: those where metabolite production is a function of the energy requirements of the cell, transformations that are indirectly related to the energy of the cell, and metabolites that are not linked to cell energetics (such as antibiotics). In some cases the transformation of intermediates derived from a fermentation system may more efficiently be carried out using chemical catalysis. An example of this would be the formation of antibiotics from 6 APA (penicillic acid). Integrated chemical/biocatalytic processes are envisioned that will enable biological or biochemical systems to carry out synthesis reactions that are not well suited for chemical catalysis. At the same time, chemical catalysis will be used where high throughput rates and high concentrations are needed for process economics or efficiency which cannot be achieved in a biological system. In this manner, chemical and biocatalysis will be integrated, and processes will be designed that combine the two steps.

- **Design of feedstocks.** Traditional agriculture has domesticated and bred many crops for extreme production of one co-product, such as maize producing high yield corn (starch). Today, the composition of existing energy plants may be improved by integration of genetic engineering and targeted breeding. Perennial plants with higher cellulose and hemicellulose contents, and reduced lignin, may be better candidates for the lignocellulosic biorefinery (Taliaferro, C. M.; Vogel, K. P.; Bouton, J. H.; McLaughlin, S. B.; Tuskin, G. A. *Proc. 4th Biomass Conf. Americas*

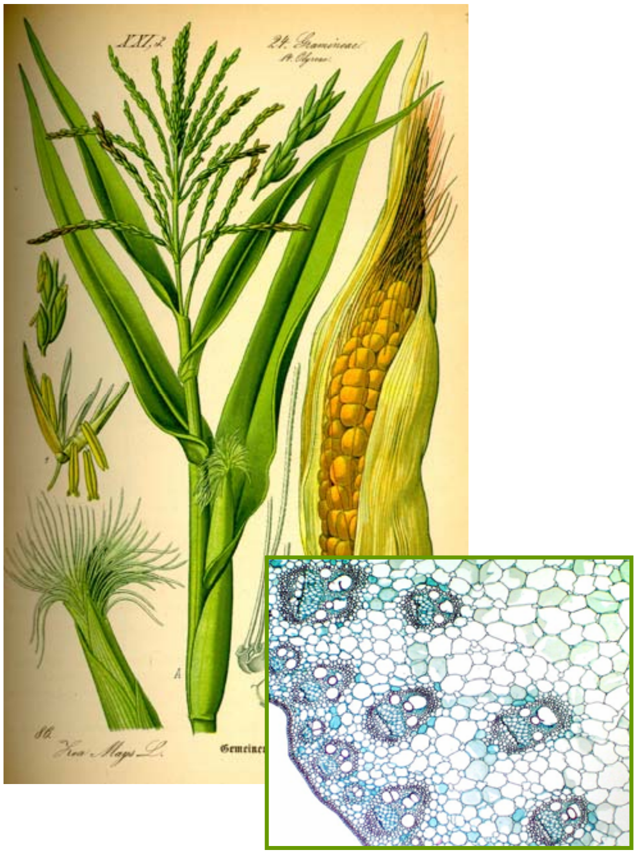


Figure 9. Cross section of a cornstalk

Overend, R. P.; Chornet, E. (Eds.), **1999,** 147-153). It may also be possible to directly produce cellulase and other polysaccharideases in the plants themselves (Dai, Z.Y.; Hooker, B.S.; Anderson, D.B.; Thomas, S.R. *Molecular Breeding* **2000**, *6*, 277-285). To enable this outcome, a better understanding of plant expression of heterologous genes must be acquired. These enzymes could lay dormant until a biological or process trigger enables their action. Of course, other features of these plants would have to suit existing agronomic and marketing requirements as well.

Processing and conversion-related issues

- **Design for parallel reactions and one-pot synthesis.** A good example would be combining two different steps into one, obviating the need for purification in-between, and enabling reaction in a single pot. A nice demonstration of this has been performed recently using two heterogeneous catalysts that would be incompatible with each other in solution (acids + bases: Gelman, F.; Blum, J.; Schumann, H.; Avnir, D. *J. Sol-Gel Sci. Tech.* **2003**, *26*, 43-46; biocatalyst and synthetic heterogeneous catalyst: Gelman, F.; Blum, J.; Avnir, D. *J. Am. Chem. Soc.* **2002**, *124*, 14460-14463; oxidation + reduction: Gelman, F.; Blum, J.; Avnir, D. *New J. Chem.* **2003**, *27*, 205-207).

  Parallel reactions have different implications in microbial catalysis. At a branch node where parallel reactions competing for the same substrate (metabolite), it is important to manipulate the relative fluxes of these two pathways in order to channel the resources down to each pathway properly. For example, in secondary metabolite production, a proper balance must be maintained between the primary metabolic pathway for cell growth (and energy generation) and the secondary metabolite biosynthesis pathway.

- **Metabolic engineering.** The direction of metabolic pathways in living cells can result in economically attractive concentrations of products which would form monomers or polymers with a broad array of uses. In order to achieve this type of engineering, the metabolism of the organism must be defined, the genetic basis of the pathway must be delineated, and molecular manipulation of the microorganisms' genetic make-up must be carried out in order to address synthetic bottlenecks in the metabolic pathway. Metabolic engineering is generally defined as the targeted modification of intermediary metabolism to produce desirable cellular properties by designing and implementing rational genetic modifications using recombinant DNA techniques. Metabolic engineering often involves the deletion of existing pathway(s) or addition of new pathways(s). As such, it is a very powerful tool for the design of organisms that can improve existing processes or produce new products.

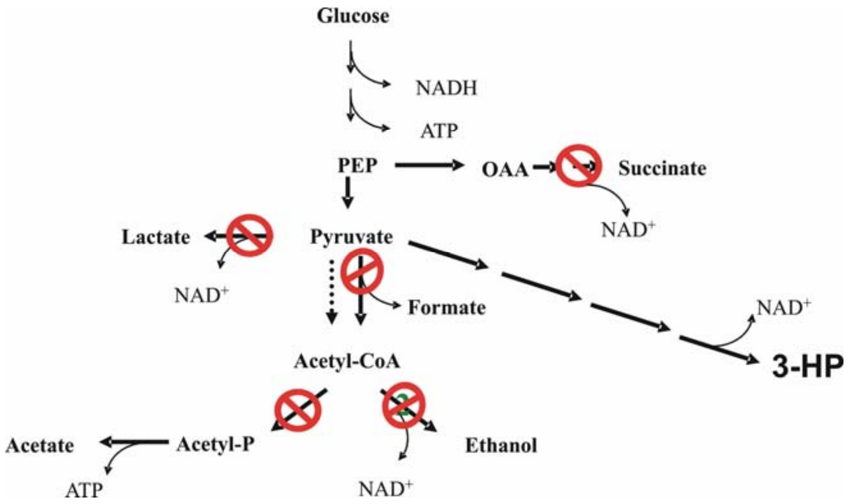


Figure 10. Host strain engineering for 3-hydroxypropionic acid production (Cameron, Cargill).

Rational Pathway Design and Synthesis

Recent rapid advances in a number of areas provide a unique opportunity to rationally design and synthesize synthetic metabolic pathway network for efficient product formation. These advances include the rapid accumulation of biological information (such as genomic, enzymatic and metabolic pathways) of a large set of organisms in an ever-increasing speed; the construction of various powerful databases that render this information readily available and usable in a searchable format over the Internet; enormous increase in DNA synthesis capability where large gene clusters (currently more than 13 Kb) can be readily synthesized using micro-fluidic technology; new and improved molecular biology techniques that greatly enhance our genetic manipulation ability; and rapid improvement in our measurement capability at the cellular and molecular levels. While these advances in the above areas provide the necessary enabling technology to perform rational design of novel synthetic metabolic pathway network by potentially choosing optimal combinations of enzymes (genes) from various organisms, to implement the genetic manipulations in the production strains, and to characterize the genetically engineered strains, more research, however, is needed to render this opportunity into reality.

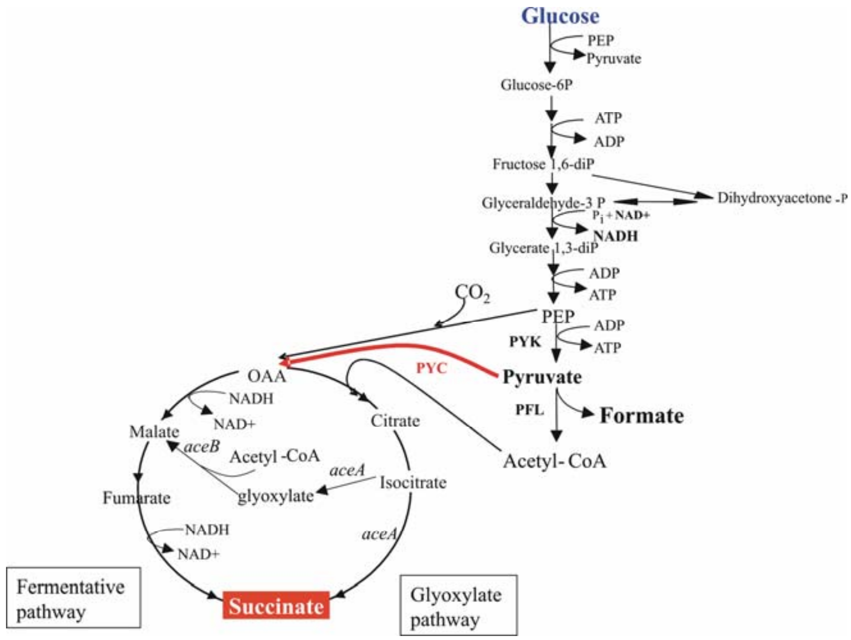


Figure 11. Designed pathway for succinate production.

- **Cooperative catalysis.** This concept has meanings both in macroscopic and microscopic length scales. In microscopic, this refers to making organization in catalytic active sites work together within a catalytic mechanism (i.e. acid-base bifunctional catalysis, or combination of sterics and activity for highly selective catalysis). This will require development of new methodology for the organization of functional groups in synthetic heterogeneous catalyst active sites, on a length scale of less than 2 nm. The goal is to synthesize these different functional groups so that there is enhancement of catalytic activity/selectivity. For the case of activity enhancement via cooperativity, the two groups must contribute a rate enhancement significantly greater than the sum of the individual parts. In a more macroscopic view, this can refer to combinations of different types of catalysts, *i.e.*, bio and non-bio for enabling removal of contaminants from fermentation broth as above or acid heterogeneous with base heterogeneous for enabling one-pot conversions.

The in vitro transformation of substrates to specific products may require numerous enzymes in a sequential pathway. In this case, catalysts, whether biological or chemical in nature, can be designed in order to carry out this process where a reactant will undergo a series of distinct transformations within the same reactor. This can manifest itself in one-pot synthesis, as well as sequential reaction steps in a packed bed reactor. In order to carry out this cooperative catalysis, which could also occur on a single particle of catalyst, the interactions, the mechanisms, and co-factor reactions must be understood.

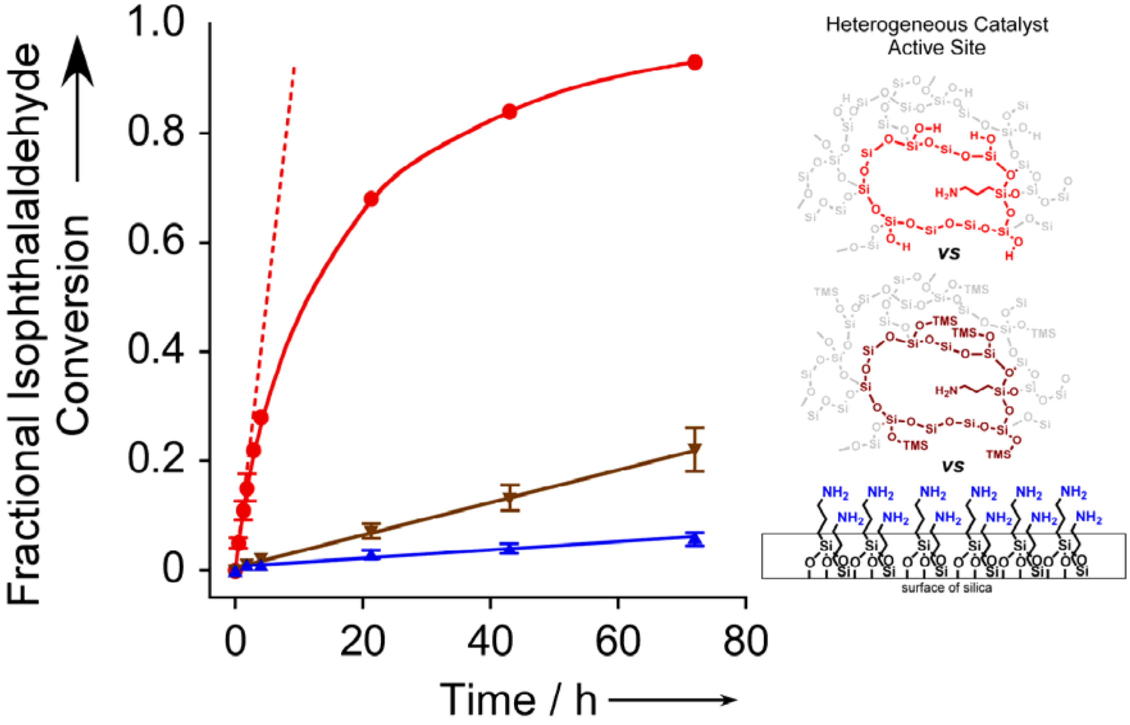


Figure 12. Effect of chemical functional group organization on C-C bond forming catalysis involving Knoevenagel condensation of ethyl cyanoacetate and isophthalaldehyde (*Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 5219-5222). The slowest material (▲) is a commercially available surface-functionalized material consisting of a monolayer of aminopropyl groups grafted onto the surface of silica. It represents the conventional amine-on-silica material in which there is no site isolation and amines are immediately adjacent to other amine active sites. The material shown in brown (▼) represents a single-site catalyst in which isolated anchored amines are surrounded by a hydrophobic framework. Both the conventional and hydrophobic outer-sphere act as monofunctional catalysts and are of comparable rate. The exception to this is the material shown in red (●), which also represents a single-site catalyst in which isolated anchored amines are surrounded by an acidic outer sphere

comprising silanol functionality. The silanols function cooperatively with the basic amine active sites for producing a push-pull or bifunctional catalytic effect. This material is 90-fold more active than the conventional monofunctional material for catalyzing this reaction, demonstrating the power of functional group organization and cooperativity in a catalyst active site, and is the first example of an acid-base bifunctional catalyst in a hybrid organic-inorganic material. The single-site materials were prepared by a templating process, which allowed rigorous investigation of the effect of outer-sphere on catalysis, without changing the composition or degree of isolation of the active site. Note that "TMS" represents a trimethylsilyl functional group.

- **Reactive separation systems: bioproduction of non-toxic precursors that would then be converted by chemical methods to the final products.** Reactive separation systems will remove products of metabolism which may inhibit the catalysts or the microorganisms used to produce it. An example of a microbial system would be yeast, which is inhibited by ethanol, a product of glycolysis and which is formed as a consequence of the energy needs of the cell. An example would be cellulase enzyme, which is inhibited by the type of product it forms, such as cellobiose (a dimer of glucose) or glucose itself. In either case, the removal of the product by separation, or preferably by formation of another species which is not inhibitory and which is a desired product is envisioned. Biocatalytic systems, which would benefit from such an approach should be investigated, and reactions to final (and desirable) products being pursued.
- **Using biocatalysis to remove poisons that would inactivate chemocatalysts.** One of the problems for biorenewables is trace contaminants in fermentation broths that poison active sites in synthetic heterogeneous catalysts, for subsequent processing steps. This involves using biocatalysts to remove these dilute contaminants before treatment with synthetic catalyst for avoiding poisoning.

- **Either enzymes or chemocatalysts as delivery systems for one another.** One serves as a recognition element for the other. Use a protein to make a small molecule catalyst that then acts on the substrate. This is a strategy used by peroxidases and by microorganisms to attack substrates that are inaccessible directly to enzymes. Fungi use long tubes (hyphae) to deliver catalysts to specific areas. Plant pathogenic fungi use this strategy.
- **Consolidated bioprocessing.** Our ultimate vision for truly advanced conversion involves consolidating as many separate biological processes in one step as possible. This calls for developing new organisms that can produce and use enzymes to hydrolyze hemicellulose and cellulose to sugars and ferment the full breadth of sugars contained in the biomass. One notable example of CBP technology has been proposed for the *Clostridium thermocellum* case (see Zhang, Y.-H. P.; Lynd, L. R. *PNAS* **2005,** *102*, 7321-7325). For *C. thermocellum*, the bioenergetic benefits specific to growth on cellulose result from the efficiency of oligosaccharide uptake combined with intracellular phosphorolytic cleavage of β-glucosidic bonds. Zhang and Lynd believe that these benefits exceed the bioenergetic cost of cellulase synthesis, supporting the feasibility of anaerobic processing of cellulosic biomass without added saccharolytic enzymes. Another option for CBP is to enable yeast, already ethanologenic, to produce cellulases (VanRensburg, P.; Zyl, W. H. V.; Pretorius, I. S. *Yeast* **1998,** *14*, 67-76). In this case, expression of some active and effective cellulases from yeast has proven challenging (Godbole, S.;

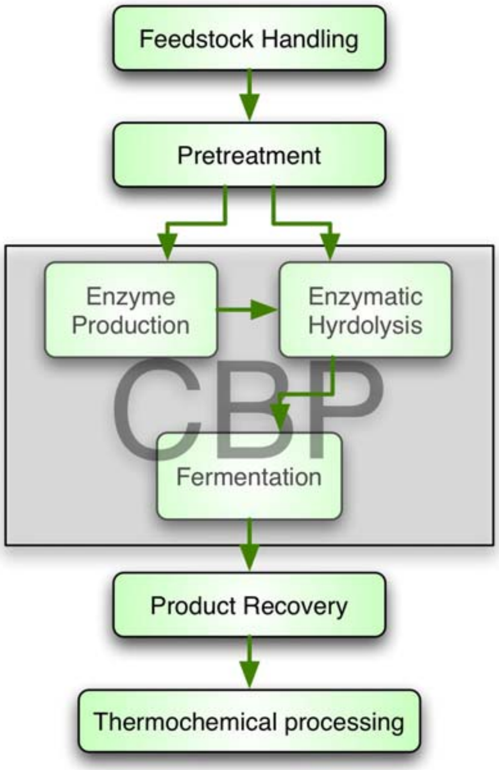


Figure 13. Conceptual schematic for consolidated bioprocessing.

Decker, S. R.; Nieves, R. A.; Adney, W. S.; Vinzant, T. B.; Baker, J. O.; Thomas, S. R.; Himmel, M. E. *Biotech. Prog.* **1999,** *15*, 828-833); however, endoglucanses and beta-glucosidases appear more amenable to yeast processing (VanRooyen, R.; Hahn-Hagerdal, B.; Grange, D. C. L.; VanZyl, W. H. *Yeast* **2003,** *20*, S223-S223).

- **Innovative new chemistries that utilize co-products such as glycerol and designing for by-products.** The conversion of biorenwable feedstocks can lead to the production of by-products that will need to be effectively converted to valuable products to enable an economical overall process. Examples include glycerol resulting from transesterification of vegetable oils in alkyl ester production, acetone resulting from fermentation to produce butanol, and distillers dry grains resulting from the dry mill production of ethanol.

C. Multiscale Modeling and Characterization of Structure/Function

A better understanding of catalyst systems

- **Fundamental studies of enzymatic catalysis in highly concentrated solutions as well as under immobilized conditions; protein-surface interactions and the tools to manipulate immobilization.** Microbial depolymerization and breakdown of solid materials including lignin and proteins often occurs by fungi that direct enzyme mixtures to the surfaces in very high concentrations. Hydrolysis also occurs when catalytic domains link to a binding domain are adsorbed onto a solid substrate which is then degraded by the catalytic domain. In both cases, concentrated protein solutions indeed are in operation in order to catalyze the depolymerization or formation of monomeric units from the solid material. The chemistry of achieving this effect, and the study of reactions where the ratio of catalyst to substrate is much greater than a catalytic amount, and actually approaches stoichiometric proportions, is needed. This is an important first step toward design of solid phase catalysts which would carry out similar transformations.

  Catalysis at surfaces will be a very important part of any catalytic system for transforming biorenewables to new carbon compounds. Many of the biorenewable materials that must be acted-upon by catalysts are solid, and require adsorption of the catalysts in some form, and reactions to occur at the surface of the substrate. The fundamental knowledge base of how proteins interact with such materials and enhance their activities as a consequence of these interactions needs greater examination.

- **Spectroscopic characterization and *in situ* imaging of active sites in non-crystalline active sites, especially for systems that don't have model systems, *e.g.*, XAFS. Modeling of these systems by computational methods.** This relates to the general problem of being able to characterize functional group organization in active sites for catalysis that contribute to highly cooperative catalysis. Such sites do not have long-range periodicity or order, but they do contain highly specific short-range order and organization. Because of this, it becomes necessary to use spectroscopic and microscopic methods, such as EXAFS, solid-state NMR spectroscopy, and SEM/TEM, to name a few. Yet many of these methods require the development of models and the techniques themselves for meaningful characterization. An example would be how to characterize a confined active site containing an organometallic metal complex by EXAFS. It is necessary to first study related model compounds with known oxygen to metal distances/coordination before being able to conduct EXAFS studies of catalytic materials containing such active sites.
- **Chemical reaction theory under process conditions, especially under condensed phases and with impure process streams that are closer to what would be found in the real world (third- and fourth-order component effects) with complex reaction streams.** This relates to the general need for modeling the active sites of biorenewables catalysts on a microscopic level of detail, which will enable modifications in silico, both to the catalyst and optimization. This requires modeling of the catalyst active site and its interaction with the transition state structure, taking the role of the solvent (water) into account. Recent good examples of this type of modeling relevant to acid catalysts for biorenewables would be Janik, M. J.; Davis, R. J.; Neurock, M. *J. Am. Chem. Soc.* **2005**, *127*, 5238-5245.
- **Cellulosome is a highly organized assembly that carries out a sequence of reactions where the active sites cooperate and channel intermediates.** The cellulosome shown is an extracellular, multi-protein complex that is produced by a wide range of cellulolytic microorganisms. It is believed to have the feature of

"collecting" and "positioning" cellulose degrading enzymes onto a substrate (Bayer, E. A.; Morag, E.; Lamed, R. *Trends in Biotechnology* **1994**, *12*, 378-386). The functional unit of the cellulosome is the "scaffolding," which is a non-catalytic protein containing repetitive domains (cohesins) for specific interaction with other protein domains, called dockerins. Cellulosomal enzymes contain both a catalytic domain and a binding domain (dockerin). The cellulosome then self-assembles by type-specific recognition of cohesin/dockerin pairs. The scaffoldins can also contain the carbohydrate-binding module (CBM) which serves as an attachment device for harnessing the cellulosome to the cell surface and/or for its targeting to substrate. The study of the cellulosome is in its infancy compared to that of "soluble" cellulases produced by fungi and some non-rumen bacteria.

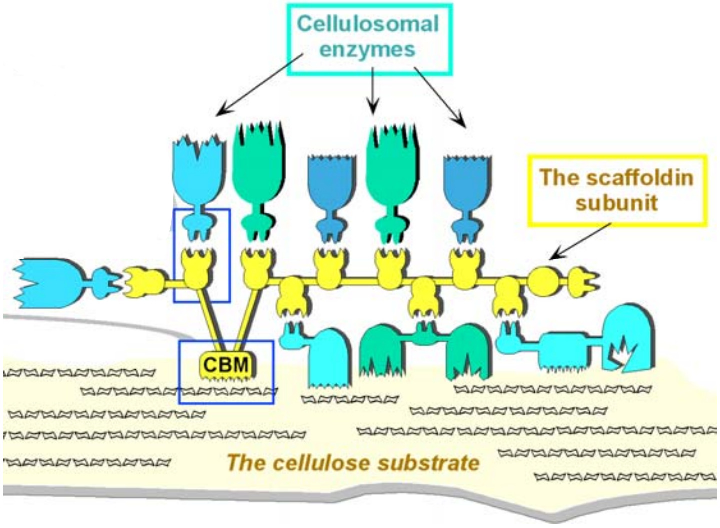


Figure 14. Cellulosome multi-protein complex.

- **Organized catalytic sites.** Because biological catalysts are known to achieve high chemical selectivity in catalyzing chcemical reactions and because it is known that much of this chemical selectivity arises from intricate functional group organization at the active site, an appropriate goal for synthetic heterogeneous catalysts is to translate some of these organizational motifs found in biological catalysts into functional synthetic heterogeneous ones. An example would be translating push-pull catalysis, which requires an acid-base pair, into a synthetic heterogeneous catalyst (Bass, J.D., Anderson, S.L.; Katz, A. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 5219-5222). Another example would be to create a confined environment in the immediate vicinity of a metal active site (Notestein, J.M.; Iglesia, E.; Katz, A. *J. Am. Chem. Soc.*

2004, *126*, 16478-16486), which is a useful foundation for developing heterogeneous enantioselective oxidation catalysis. These are both examples of organized catalytic active sites.

- **A model-based design and screening approach.** The screening of different types of catalysts requires a significant amount of laboratory effort. A mathematical modeling approach should be developed in which the catalysts, for known structure, would be evaluated for catalytic activity in order to determine the probable success of the interaction. This will require a fundamental database starting at the atomic level, so that molecular interactions between substrate and catalysts might be predicted or at least estimated. This would be a useful tool for rapidly screening different prospects, without running experiments. If successful, such a method would enable a researcher to quickly identify which structures are unlikely to catalyze the desired reaction. This will help to increase the rate at which discovery is carried out.

A better understanding of substrates

- **Can we use fundamental chemical and physical properties to model these systems mathematically?** Quantum mechanics has enabled significant modeling to be done at the atomic and molecular scale for various types of chemical compounds. A unique approach is proposed in which data and computational algorithms will be developed for determining fundamental properties on a mesoscale system starting from first principles. This sort of modeling approach would assist a researcher by identifying probable catalytic moieties, while discarding ones that are unlikely to be successful. While a creative invention may be lost this way, such a tool if used by an intelligent researcher, could be very helpful in screening out catalysts that are unlikely to be successful. The evaluation of a catalyst in ester that has been synthesized is one of the most time-consuming steps.
- **How do metal ions and metal catalysts interact with sugars, lignin, proteins and other materials in the biorenewables feed stream?** Conversion of biorenewables presents challenges rarely addressed by catalytic processes optimized for hydrocarbons. The breadth of these challenges is illustrated by the wide variety of functional groups clustered within biorenewable building blocks. Oleochemicals offer fatty acids, a completely unfunctionalized and undifferentiated chain of

methylene groups, or glycerol, a three carbon unit possessing three –OH groups of similar reactivity. Sugars present an even larger number of –OH groups concentrated within a single molecule. Alternatively, lignin offers an almost random array of functionality spread over a large number of substructural units. At present, our ability to carry out selective transformations on these types of molecules is extremely limited. New research activities will learn how chemical catalysts interact with these various feedstocks. Complexation of metal centers at single and multiple sites on sugars will lead to understanding of the bonds most active within these building blocks. The impact of the different sugar diastereomers on catalyst complexation will be understood.

- **Controlled disassembly of polymers.** New materials are envisioned that are either biorenewable or chemically synthesized. Such materials would have imbedded in the catalyst which could be activated by an external and extreme condition so that the polymer or the material would disassemble itself into its starting components. In designing such a system, complete recycle of a material could be achieved by breaking down the polymers to component systems, which in turn would be used to synthesize more of the same polymers. The key issue would be encapsulation of the catalysts in non-active (or pro-active) form, and designing the chemical composition of the catalysts so that its activity could be triggered by an external event which is far removed from the conditions at which the product would be used. Once activated, the catalyst would begin to disintegrate or hydrolyze the polymers that are in its vicinity. This methodology would further contribute to a new carbon economy, since it would provide a method by which the carbon could be recycled.

Appendix A: Invited Workshop Participants

Joe Bozell	University of Tennessee
Mark Burk	Diversa
Doug Cameron	Cargill
Rich Gross	Polytechnic University
Mike Himmel	USDOE National Renewable Energy Laboratory
Alex Katz	University of California – Berkeley
Mike Ladisch	Purdue University
Nathan Mosier	Purdue University
Ka-Yiu San	Rice University
Glenn Schrader	National Science Foundation
Brent Shanks	Iowa State University
Jon Stewart	University of Florida

Appendix B: Workshop Program

Thursday, June 23

8:15-8:45	**Introduction** Glenn Schrader (NSF) Jon Stewart (University of Florida)
8:45-9:15	**Biological and Chemical Catalysis for New Monomers from Carbohydrates and Vegetable Oils** Doug Cameron (Cargill)
9:15-9:45	**TBA** Rick Gross (Polytechnic University)
9:45-10:15	**Generating Value through Biorenewable Feedstocks** Mark Burk (Diversa)
10:15-10:30	Break
10:30-11:00	**Biomass Recalcitrance: The Lignocellulosic Biorefinery Industry** Mike Himmel (NREL)
11:00-11:30	**Design of Biocatalysts for the Synthesis Biorenewable-Derived Platform Chemicals** Ka-Yiu San (Rice University)
11:30-12:00	**Selective Conversion of Carbohydrates and their Derivatives with Chemical Catalysts** Brent Shanks (Iowa State University)
12:00-1:30	Lunch
1:30-2:00	**Catalysis for Biorenewables Conversion to Transportation Fuels and Bioproducts** Mike Ladisch (Purdue University)
2:00-2:45	**Working Session 1** Is there value in jointly engaging the biocatalysis and chemical catalysis communities?
2:45-3:00	Break

3:00-5:00	**Working Session 1, continued** Is there value in jointly engaging the biocatalysis and chemical catalysis communities?

Friday, June 24

8:00-10:00	**Working Session 2** What are the most significant catalysis-related obstacles to progress in this area?
10:00-10:15	Break
10:15-12:00	**Working Session 3** Developing an action plan
12:00-1:30	Lunch
1:30-4:00	**Working Session 4** Workshop report development