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Biomanufacturing: A State of the Technology Review

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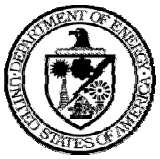

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Biomanufacturing: A State of the Technology Review

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Abstract

Biomanufacturing has the potential to be one of the defining technologies in the upcoming century. Research, development, and applications in the fields of biotechnology, bioengineering, biodetection, biomaterials, biocomputation and bioenergy will have dramatic impact on both the products we are able to create, and the ways in which we create them. In this report, we examine current research trends in biotechnology, identify key areas where biomanufacturing will likely be a major contributing field, and report on recent developments and barriers to progress in key areas.

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Acknowledgments

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Executive Summary

This white paper was requested by the Manufacturing Sciences & Technology Center of Sandia National Laboratories with the goal of educating the organization about developments in biomanufacturing technology and applications that would assist it in planning new programs. Specifically we address the following tasks:

- Examine current research trends in biotechnology
- Identify key areas where biomanufacturing will likely be a major contributing field
- Report on recent developments and barriers to progress in key areas, including fields that Sandia traditionally has not largely contributed to

The ultimate goal of this report is to inform readers about emerging technologies and opportunities for Sandia to contribute to this emerging field of biomanufacturing.

The starting point of this study was a general definition of biomanufacturing and an identification of the key contributing areas. **The most basic definition of biomanufacturing is the use of living organisms to manufacture a product.** However, this definition is too general and broad to be of much practical use. With this definition, biomanufacturing covers such well-established trades as wine, cheese and bread making, but it also excludes several potential key disciplines like biodetection and biomimetics. Therefore **we have revised this definition to encompass manufacturing methods that use a biological organism, or parts of a biological organism, in an unnatural manner to produce a product, as well as products designed to detect, modify, maintain and study biological organisms for use as new manufacturing agents.** This definition qualifies the use of biological organisms to processes that are unnatural, meaning that the organism being used has been modified on a molecular or genetic level to perform a function that it could not perform in its unaltered state. This allows us to hold to the convention of exempting traditional manufacturing trades (bread making etc.) from being considered as biomanufacturing. Even with a definition, biomanufacturing is not a field with strictly defined boundaries. Rather, it may best be described by the disciplines it derives from and what fields contribute to it shown schematically in Figure 1. The following fields could reasonably be considered key to the science of biomanufacturing:

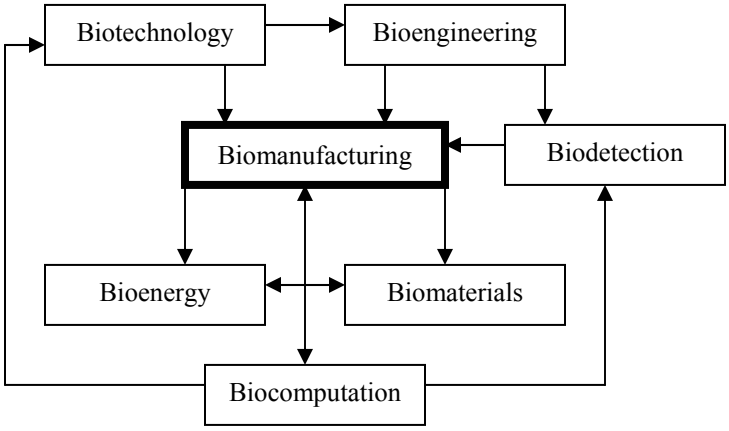


Figure 1: Simple illustration of the connections between biomanufacturing and key contributing disciplines

- **Biotechnology**
- **Bioengineering**
- **Biodetection**
- **Biomaterials**
- **Biocomputation**
- **Bioenergy**

Biotechnology and bioengineering are both considered the parent disciplines of biomanufacturing while the remaining four fields are considered use areas.

Any discoveries in the biotechnology or bioengineering fields will most likely trickle into areas of biomanufacturing. **Biotechnology** research gives a complete picture of the fundamental processes involved in genetic expression. Traditionally associated with the pharmaceutical industry, its applications are becoming more commercialized. Biotechnology includes such fields as Genomics, the study of the genetic material of life, and Proteomics, the study of proteins. Most of the technologies used in modern Genomic studies are byproducts of the Human Genome Project and are fairly well established. Proteomics, on the other hand, remains in need of more advanced research.

Bioengineering studies the mechanisms of biological processes and utilizes the knowledge of those mechanisms to modify organisms or to create new products. Although the definition of bioengineering sounds very similar to the definition of biomanufacturing, they differ in that bioengineering concentrates on modifying organisms for unnatural use and biomanufacturing is concerned with optimizing the application of unnatural organisms, as well as creating new technologies that improve the efficiency of creating unnatural organisms. Research in this area generally falls into one of five categories:

- Metabolic Engineering
- Tissue Engineering
- Cellular Engineering
- Molecular Engineering
- Nanoengineering (Nanotechnology)

Metabolic engineering involves the direct modification of cellular metabolism through modification of protein production. It is based upon the idea that optimal production within a cellular environment can be obtained through the modification of metabolic pathway. The applications of metabolic engineering all revolve around the optimization of bioengineering. The major barrier to progress in this field is the lack of basic research. The more that we understand about the way a cell controls its own manufacturing processes, the finer our control will be in tailoring those processes to our needs.

Tissue engineering involves the controlled growth of living tissues and organs on three-dimensional support structures, or scaffolds. As with metabolic engineering, one of the major barriers to progress in this field is basic research. Tissues tend to self-assemble and the methods by which they accomplish this are not well understood. At this time,

tissue engineering is firmly in the realm of medicine and has applications in the creation of artificial organs, blood substitutes, neurological implants, and orthopedic devices. There are, however, opportunities to make this field more of a commercial venture. One example is research into preventing the body's rejection of implants. An understanding of how tissues determine place and identify their own cells will assist in designing implants that do not initiate strong immune responses.

Cellular engineering deals with directed modification of cell growth, differentiation, and division. Cellular engineering also has strong ties to the pharmaceutical industry, and it is currently held back by a lack of basic research. Any research into the process of cell growth will be useful in optimizing processes dependant on cellular reproduction. Processes that depend on cellular reproduction include DNA sequencing and many pharmaceutical production processes. There are also applications in fields where optimized cellular processes would be extremely advantageous. Examples would be in energy fields where optimized cellular replication could lead to improved biomass sources.

Molecular engineering is the study of biomolecules and biomolecular processes to discover and create new high value biomolecules and to develop enabling technologies to produce them on a commercial scale. This field has obvious ties to the pharmaceutical industry, but other applications will come in the form of optimized production of commercial products by biological means. One often cited example is the production of ethanol. However, microorganisms create much more complicated products every day. Basic research into the chemistry that a microorganism naturally uses affect the way in which industry synthesizes its chemicals.

Nanoengineering (Nanotechnology) is probably one of the most talked about fields in research today. Many of the manufacturing applications being studied in this field have corresponding processes in microorganisms. As in molecular engineering, basic research into the processes by which cells create complex molecules will yield information that industry can use to optimize its own processes.

Any research in biotechnology and bioengineering will have dramatic influences on biomanufacturing. Both are rapidly developing fields that will give scientists the ability to create a whole new range of products that will improve the quality of human life. The rest of the key disciplines are sciences that contribute to, and are affected by, biomanufacturing, and as such their major developmental barriers need to be adequately addressed.

Biodetection is concerned with methods that identify and quantify biological organisms, their processes, and responses. It relates to biomanufacturing in that any research or development in biodetection will contribute to the understanding and control of organisms used in biomanufacturing processes. Biodetection also has a number of applications in national security. Currently at Sandia there are several efforts underway to develop new biosensors specifically for use as bioweapon detectors. But security is not the only field that would benefit from new biodetection technology. Other fields that

would benefit include the medical, biotechnology, and bioengineering fields. One major need in this field is for efficient manufacturing methods that would make currently developed biosensors commercially available.

Biomaterials can be considered direct applications of biomanufacturing principles and products. There are literally hundreds of potential applications for biomaterials, including adhesives, new synthetic materials, and new medical devices. The major fields of research involved with biomaterials are biomimetics, biomineralization, and bioadhesion. Biomimetics is the study and mimicry of advantageous surface properties or processes of living organisms for materials science and technology. Biomineralization is concerned with how organisms form minerals. Finally, bioadhesion is concerned with how biological organisms create membranes or substances that can be modified to promote or prevent adhesion to a given surface. Nature has developed hundreds of ways to use surface, mineral, and structural properties, yet we understand only a few of them. The major barriers to advancement in these fields are a lack of basic research and failure to apply research that has already been conducted.

Biocomputation is quite possibly the key field to all of the preceding fields. Bioinformatics is the largest biocomputation application. It contributes to continuing research in biomanufacturing in that it creates a repository of reference material that will affect all other fields related to biomanufacturing. One major barrier to progress is the need for faster computers and more efficient algorithms to handle the sheer volume of data being produced by all of the bio-related fields. It also includes biomanufacturing processes relating to computers. There are potential computing applications for biomolecules, DNA, and RNA, but most of the research in this field has not been considered for commercial production.

Finally, **bioenergy** is an application of biomanufacturing research that is of great interest to the Department of Energy. Bioenergy technologies have been explored for a number of years, but are not in commercial production because of their lack of efficiency when compared to current energy sources. There is a definite need in this field for application and optimization of current technologies and processes.

Biomanufacturing is an advanced manufacturing technology that involves the use of chemical, physical, and biological processes performed by living cells for use in other applications. Biomanufacturing enables the manufacture of products with unique characteristics and has significant potential in the manufacture of several products such as polymers, energy sources, chemicals, food products, pharmaceuticals, and biodegradation (remediation) of hazardous materials.

Perhaps the most unique and advantageous aspect of biomanufacturing is the excellent control that may be afforded during fabrication. In particular, sequence-by-sequence building of polymeric materials may be possible. Biological species can be used to synthesize polymers of more uniform chain lengths or chain branching than those produced by conventional synthesis techniques. Additionally, biosynthesis could be used to produce specialty copolymers that are not available through traditional synthesis

methods. These applications are of particular interest to SNL as we strive to understand polymers and nanoparticles in terms of their thermal, mechanical, optical, and electrical properties for use in nuclear weapons, satellites, and homeland defense applications.

Other biomanufacturing areas of interest include fabrication of sensors and encryption tools. It may be possible to utilize this technology to manufacture sensors that offer superior recognition of chemical and biological agents. Currently, it is possible to manufacture sensors that are able to detect only one or a few agents. However, development of the appropriate bioprocessing techniques will enable manufacture of sensors that are able to detect all materials of interest at once. This is of tremendous interest in detecting and neutralizing potential terrorist attacks using these agents. Additionally, it may be possible to use biosequencing to provide encryption and subsequent decoding of complex, sensitive data.

Biomanufacturing has the potential to be one of the defining technologies in the upcoming century. Research, development, and applications in the fields of biotechnology, bioengineering, biodetection, biomaterials, biocomputation and bioenergy will have dramatic impact on both the products we are able to create, and the ways in which we create them. Sandia National Laboratories has the expertise to contribute to any one of these fields.

Biomanufacturing: An Introduction

Biomanufacturing is both a very old and a very new science. For centuries, man has been using biological organisms to produce desirable products such as wine, cheese and bread. These products all result from microorganism processes. Only in the last decade have we been able to understand and directly manipulate these microorganisms to create desirable products they would not normally be able to produce. Until recently, this type of manipulation has been considered a part of the biotechnology industry with close ties to pharmaceutical production. Now the same techniques of biological manipulation and utilization are finding their way into other disciplines. The expansion of these techniques has led to the emergence of biomanufacturing as a science that is both very similar and very different from its parent disciplines, biotechnology and bioengineering. It also encompasses such varied fields as bioengineering, biodetection, biomaterials, biocomputation, and bioenergy.

Statement of Task

This white paper was requested by the Manufacturing Sciences and Technology Center of Sandia National Laboratories with the goal of educating the organization about developments in biomanufacturing technology and applications that would assist it in planning new programs. Specifically, the following tasks were completed:

- Examined trends in biotechnology, including research activities occurring in industry, universities and other national laboratories.
- Identified key areas where biomanufacturing will likely be a major contributing field.
- Reported on recent developments in key areas, including fields that Sandia traditionally has not largely contributed to, as well as any barriers to progress in those fields.

All of the information provided in this report will serve the ultimate goal of informing readers about emerging technologies and opportunities for Sandia to contribute to this emerging field of biomanufacturing.

Definition of Biomanufacturing

The starting point of this study was a general definition of biomanufacturing and an identification of the key contributing areas. As background research progressed it became increasingly apparent that biomanufacturing is not a field with strictly defined boundaries, but one that is best described by the fields that contribute to this science as shown in Figure 1. In general, **biomanufacturing is the use of living organisms to manufacture a product.** However, this definition is too broad to be of much practical use. With this definition, biomanufacturing covers such well-established trades as wine, cheese and bread making, but it also excludes several potential key disciplines like biodetection and biomimetics. Therefore **we have revised this definition to encompass manufacturing methods that use a biological organism, or parts of a biological**

organism, in an unnatural manner to produce a product, as well as products designed to detect, modify, maintain and study biological organisms for use as new manufacturing agents. This definition qualifies the use of biological organisms to processes that are unnatural, meaning that the organism being used has been modified on a molecular or genetic level to perform a function that it normally would not in its natural state. This allows us to hold to the convention of exempting traditional manufacturing trades (bread making etc.) from being considered as biomanufacturing.

With this definition in hand, we can begin to define key fields that will contribute to the field of biomanufacturing. We determined that the following fields could reasonably be considered key to the science of biomanufacturing:

- **Biotechnology**
- **Bioengineering**
- **Biodetection**
- **Biomaterials**
- **Biocomputation**
- **Bioenergy**

Biotechnology and bioengineering are both considered the parent disciplines of biomanufacturing, and any research into or discoveries from these fields will most likely trickle into areas of biomanufacturing. **Biotechnology** research gives a complete picture of the fundamental processes involved in genetic expression. **Bioengineering** studies the mechanisms of biological processes and utilizes the knowledge of those mechanisms to modify organisms or to create new products. Although the definition of bioengineering sounds very similar to the definition of biomanufacturing, they differ in that bioengineering concentrates on modifying organisms for unnatural use and biomanufacturing is concerned with optimizing the application of unnatural organisms, as well as creating new technologies that improve the efficiency of creating unnatural organisms.

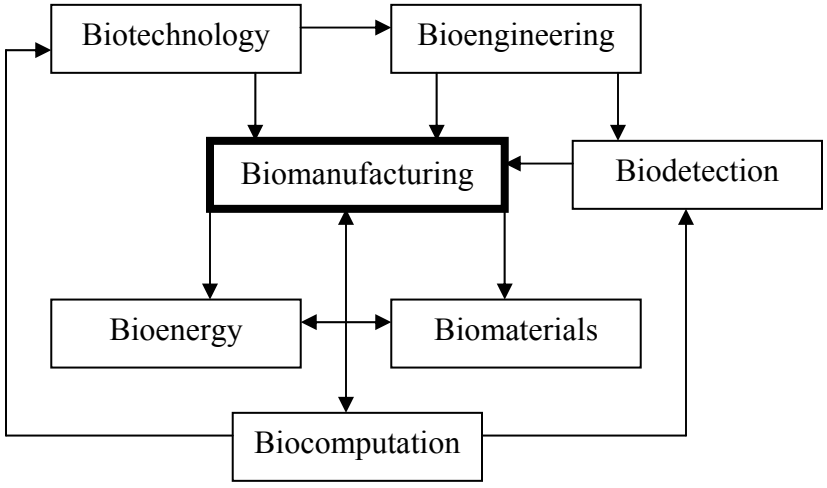


Figure 1: Simple illustration of the connections between Biomanufacturing and key contributing disciplines

The rest of the key disciplines are sciences that contribute to, and are affected by, biomanufacturing. **Biodetection** is concerned with methods that identify and quantify biological organisms, their processes and responses. It relates to biomanufacturing in that any research or development in biodetection will contribute to the understanding and control of organisms used in biomanufacturing processes. **Biomaterials** can be considered direct applications of biomanufacturing principles and

products. **Biocomputation** contributes to ongoing research in biomanufacturing in that it creates a repository of reference material. In addition, it is a product of biomanufacturing processes as they relate to computers. Finally, **bioenergy** is an application of biomanufacturing research that explores the use of biological processes for energy production.

Report Organization

The goal of this report is to present relevant, unbiased information on the field of biomanufacturing and its contributing disciplines. This paper has been divided by key disciplines. Each section will define and discuss one of the above listed key disciplines. Each section will also include an example of current research, a list and brief explanation of key technologies in that field and a discussion of limitations to development in the field.

Biotechnology

Biotechnology is often seen as one of the parent disciplines to the many new and rapidly expanding bio-related disciplines. While much has been accomplished in this field in the last decade, biotechnology is not a new discipline. Man has been manipulating living organisms to improve his way of life and to solve problems for thousands of years. The oldest discipline that could be considered biotechnology is the field of Agriculture, followed closely by a variety of fermentation and aging processes. It was not until the late 1800's that man began to take a more direct role in manipulating living organisms. The greatest progress began when Gregor Mendel established the first rules of genetic inheritance and when Von Leewenhoek became the first person in history to see a microorganism. As the twentieth century dawned, new discoveries united the fields of industry and agriculture, while new fermentation methods allowed the industrial synthesis of common solvents and the first pharmaceuticals. The term biotechnology was originally coined by a Hungarian engineer named Karl Ereky in 1919. Ereky used the term to describe all lines of work by which products are produced from raw materials with the aid of living organisms. As with biomanufacturing, biotechnology does not include traditional trades (bread making, etc.). Throughout the twentieth century, biotechnology has continued to expand through intensive investigation of organisms for antibodies and vaccines as well as biological warfare. Today, **biotechnology has come to refer to the use of living organisms or their products to modify human health and the human environment.**[1]

The two sub-disciplines that have contributed the most to the field of biotechnology are the fields of Genomics and Proteomics.

Genomics

The history of genomics can be traced back to the 1960's when scientists began to closely examine the structure of DNA. The actual term "genomics" was not coined until 1986 by Thomas H. Roderick during a discussion about the name of a new planned journal. The

Journal (*Genomics*) was to include articles that discussed gene sequencing data, gene mapping, new gene discovery, new genetic technologies, and genetic comparison of various species. **Today genomics is viewed as the methodical analysis of DNA, within the context of the entire genome, as well as a comparison of genes to one another** without respect for the products that result when those genes are expressed. The most famous study in Genomics is the Human Genome Project. The Human Genome Project is a huge coordinated effort to map the human genome, identify all of its genes, and determine the entire nucleotide sequence. This project includes work done in industry, universities, and national laboratories to meet the target date for completion of this project in 2005. The Human Genome Project has done more to advance the technologies of gene sequencing, mapping, and database management than any other project in the history of mankind.

One important point when discussing this field is the difference between Genetics and Genomics. While both are concerned with an organism's hereditary material (DNA) they concentrate on different aspects. The study of Genetics first began in the 1850's when Gregor Mendel published his work on genetic inheritance. As such, **Genetics is concerned with the relationship between the genetic material and its physical expression.** Genomics did not begin until the 1950's when Watson and Crick published their paper on the structure of DNA. **Genomics is concerned with the structure of genes, DNA, and their relative positions and values when compared with one another.** Both fields focus on DNA as the hereditary material of a cell, but Genetics is concerned with how DNA is used and Genomics is interested in what DNA is.

Technologies of Genomics

Some of the most important technologies currently used in Genetics were advanced by the Human Genome Project, and include: DNA synthesis, Gene Amplification, DNA sequencing, DNA genotyping, Expression profiling, Imaging, and Microarrays. We will briefly discuss each of these technologies with the exception of Microarrays, as they are better explained in the chapter on biodetection.

The first step is to isolate the sequence of interest, which involves separating the fragment of interest from a host of other potential fragments. For this, researchers use a Southern blot. This involves separating the fragments by gel electrophoresis and then running it through a nitrocellulose filter paper. Care is taken to make sure the fragments are deposited on the filter paper in the same positions they occupied on the gel. The filter paper is then exposed to a solution full of radioactively-labeled probes, sequences designed to bind to a specific order of base pairs. The filter is then dried, overlaid with X-ray film, and developed. This gives the researcher a print of exactly which DNA fragments in the gel are of interest.

DNA Synthesis

DNA synthesis is a process carried out every day in every replicating cell on earth. Replicating cells have all of the necessary equipment to synthesize one copy of the entire piece of DNA, but the ability to replicate DNA is also necessary in order to map any genome or simply study the properties of DNA. There are two procedures used to

produce the short piece of DNA (an oligonucleotide) needed for any given experiment: recombinant DNA cloning and automated DNA synthesis.

Today most DNA sequences are synthesized by a DNA synthesizer, called a thermocycler, using a solid-phase synthesis technique. Solid-phase synthesis involves attaching a primary residue, the 3' end of a nucleotide, to a substrate and then programming a sequence into a master control unit. The synthesizer, a sophisticated fluid-handling device, then adds individual nucleotides in the desired order. This method is different from the process ordinary cells use to replicate DNA in that nucleotides are attached to the 5' end of the newly-synthesized DNA instead of the standard 3' end. There are advantages and drawbacks to using this method. One of the major advantages is the ease of use and specificity of this technique. The major drawback lies in the fact that the sequence must be known before the DNA can be cloned. This makes automated DNA synthesis useless when trying to clone novel DNA sequences.

Recombinant DNA involves cutting and pasting existing DNA to form a novel DNA sequence. Recombinant DNA cloning would then logically involve isolating a desired DNA sequence and inserting it into another larger piece of DNA. The larger piece of DNA, called a cloning vector, is then placed in a desired microorganism, usually *Escherichia coli (E.coli)*. After the cloning vector is inserted, the bacteria are allowed to reproduce. As the bacteria reproduce, the cloning vector containing the desired DNA segment is also reproduced. These are the four general stages to recombinant DNA cloning:

1. DNA Cleavage
2. Synthesis of cloning vector
3. Cloning
4. Screening

DNA cleavage involves the use of Restriction Endonucleases (RE) to cut the DNA strands into small fragments that have 'sticky ends'. RE's are a type of enzyme that can cut completely through double-stranded DNA. They are useful in that certain RE's will cut between certain base pairs, allowing scientists to control exactly where the DNA is cut. 'Sticky ends' are portions of unpaired bases at the ends of one strand of the double-stranded DNA. The nature of these ends is predictable based on the RE used to cut the DNA, and they will match up with the sticky ends of other fragments that have been cut using the same RE.

The second step is the synthesis of a cloning vector. The vector is chosen based on the size of the DNA to be cloned. Usually a plasmid, a piece of circular DNA found in bacteria, is used as the vector, but plasmids are only suitable for small DNA pieces. The two other options for cloning vectors are bacterial artificial chromosomes (BAC's) or Yeast artificial chromosomes (YAC's). Once a suitable plasmid is selected, a section of it is removed using the same RE used to cut the desired piece of DNA. Then using another enzyme, DNA ligase, the sticky end of the desired DNA is bound to the reciprocal sticky end of the cut plasmid. Once the sticky ends have bonded, the recombinant DNA vector is ready for use.

The third step in this process is inserting the cloning vector into the desired microorganism. There are three options available to the researcher: Transformation, the direct uptake of DNA from the cell's environment; Conjugation, the direct transfer of DNA from one cell to another; or Transduction, the indirect transfer of DNA from one bacterial cell to another via a virus. The most commonly used methods are transformation and transduction. After the cloning vector is successfully inserted, the bacteria are allowed to replicate.

Not every bacterium will successfully incorporate the vector, so there are several very clever ways to screen DNA clones for those that have successfully incorporated the vector. One method involves including an additional antibiotic resistance gene on the cloning vector. When the bacteria are grown in the presence of the antibiotic, only the clones with the resistance factor will survive. The other option is to insert a gene on the cloning vector that will produce a specific enzyme that turns the bacteria containing the cloning vector blue.[2,3]

Gene Amplification

Gene amplification is carried out when large quantities of a DNA sequence are required for a given experiment. Either one can purchase the desired number of oligonucleotides, or one can use a process called the Polymerase Chain Reaction (PCR) to quickly replicate more than enough oligonucleotides. Karry Millis developed it in 1983 while acting as a staff chemist at Chetus Corporation. In 1993, he won the Nobel Prize for chemistry. There are three steps to PCR shown schematically in Figure 2:

1. Denaturation
2. Annealing of Primers
3. Primer Extension

Denaturation is the breakdown of structure, in this case of DNA, from a double-helical structure to a single strand. This process is accomplished by adding excess primer and heating the solution to 90^{o} C. The primers are short, complementary strands of DNA that bind to a specific section of the DNA to be copied. The solution is then cooled from 90°C to 60˚C to allow the primer to combine with the complementary single strands of DNA. The primers finish as borders to the portion of the single strand to be amplified. A solution of the four DNA nucleotides and very heat-stable enzymes, called DNA polymerases, are added to synthesize the complementary sequences that will fill the gaps on the single strand of DNA between the primers. A complementary copy of the original single-stranded fragment is then produced and there will remain two copies of the original fragment. These steps are repeated and after 20 cycles, more than 1 million (2^{20}) copies are produced, and will continue to be produced exponentially as the steps are repeated. These steps can take some time when performed by hand which is why PCR, these days, is entirely an automated process.

DNA Sequencing

One of the most important technologies in any sequencing operation is Gel Electrophoresis. In this procedure, DNA pieces are added to a gelatinous solution and an electrical field is applied. The DNA pieces separate according to size with the smaller pieces making it farther down the gel than the larger pieces. The electric field hastens the separation process. Gel electrophoresis is valuable because it can discriminate between DNA fragments that differ by a single base, known as nested fragments.[4]

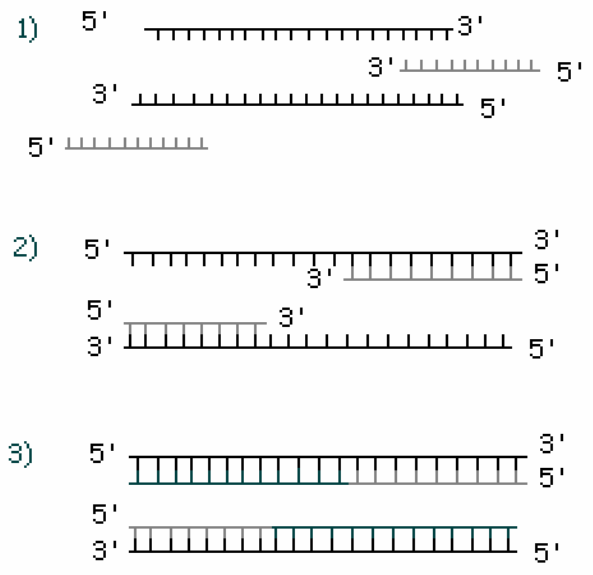


Figure 2 PCR steps: 1) Denaturation of DNA (Black) 2) Binding of promoters (light gray) 3) Primer Extension (dark gray)

DNA sequencing is the process used to determine the exact order of base pairs in an oligonucleotide. There are two types of sequencing available: Sanger sequencing and Maxam-Gilbert sequencing.

Maxam-Gilbert sequencing uses brute-force chemistry, while Sanger sequencing, also known as chain termination or dideoxy method, relies on enzymes to synthesize chains that vary in length by one base. A desired piece of DNA is isolated and replicated thousands of times. It is then divided into four test tubes. Each test tube is given a set volume of one of the four types of dideoxynucleotides, ddATP, ddGTP, ddTTP, or ddCTP, one for each of the four nucleic acids, Adenine, Guanine, Thymine, or Cytosine, respectively. Dideoxynucleotides differ from regular nucleotides in that they lack a functional group that would allow the next base in the sequence to be added. When the dideoxy nucleotides are added to a solution along with the DNA polymerase, and an excess of the other three nucleotides, it guarantees that replication will terminate with the dideoxy nucleotide. All four tubes are then separated using gel electrophoresis. The end product is a gel column that, when developed, yields a grid showing DNA pieces separated by a number of nucleotides and the identity of the last nucleotide in the given DNA piece, usually designated with a radioactive marker. Most modern DNA sequencing machines use this method, but they use fluorescent markers instead of radioactive markers. That eliminates the need for a developing step and allows the computer to automate the process.[5]

DNA genotyping

Once a gene is sequenced, genotyping is necessary to determine where the sequence fits into the entire genome, and to mark it for genetic studies. One of the most common genotyping methods lies in detecting single nucleotide polymorphisms (SNPs). SNPs are common DNA sequence variations between individuals. They occur at a high rate in the human genome (approximately one SNP per 1,000 base pairs). To date, over 1.5 million

SNPs have been characterized, and can be used to advance our ability to understand and treat human disease.

Genotyping technologies have proliferated rapidly in recent years. Currently there are around one hundred methods available for detecting the genotypes of individual SNPs. The ideal SNP genotyping system would offer assay uniformity, high-throughput capacity, ease of assay design, and most importantly, accurate and reliable results. Currently, there is no one method that satisfies all of these requirements. All of the current SNP detection techniques fall into three categories: 1) few SNPs, many samples, 2) many SNPs, few samples, and 3) many SNPs, many samples. Different techniques are more effective depending upon the application. High-throughput analysis has only recently been optimized. Even so, there is still no "gold standard" and different companies have devised their own technologies for genotyping.[6]

One example of genotyping is a method developed in 2001 at Lawrence Berkeley Lab. Their process is a high-throughput haplotyping that can be used for a variety of medical applications. Samples are scanned for haplotypes, SNPs that occur on each chromosome in a homologous pair. The value of this new technique lies in the fact that it allows direct detection of all haplotypes present in the sample, without the need for statistical inference or the need for cloning PCR products.[7]

Proteomics

While DNA stores all of the blueprints for a cell, the proteins are the actual workhorses in a cell. Serious study of proteins began with the Molecular Anatomy Program at Oak Ridge National Laboratories in the 1960's. Its goal was to produce a complete inventory of cells at the molecular level. The intent was to discover and purify new pathogenic viruses, but the completion of the program would have impacts on many other medical fields. In 1975, the program was moved to Argonne National Lab in response to new applicable technologies, including methods for large-scale vaccine purification, systematic fractionation of proteins, high-pressure liquid chromatography, high-speed computerized clinical analyzers, and 2D (two-dimensional) electrophoresis. In 1980, interest in proteins was growing and an attempt was made to launch the Human Protein Index Project (HPI), but the proposal did not receive public backing. The attempt was made again in 1983, but this time the HPI project was coupled with the Human Genome Project. In 2001, a new wave of support for Proteomics arose, and currently there is a program called the Human Proteome project. The first annual meeting for this program occurred in January 2003.[8] Proteomics today is the study of proteins, but it also includes the identification, quantification, function, and location within the cell. Current research in this field can be divided into three distinct subsets: protein profiling, protein-protein interaction, and structural biology.

Just as in Genomics, the first step in any proteomic study is the ability to separate and identify the protein of interest. The most popular technique is gel electrophoresis. This process is the same as was discussed in the Genomics section in that the proteins must be denatured before they are separated. This can pose additional problems when studying protein structure, as one must disrupt that structure to separate the protein. There is also

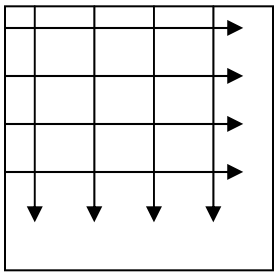


Figure 3 2D Gel Electrophoresis

another option when dealing with proteins. One can separate them by standard electrophoresis or by Iso-electric focusing (IEF) which uses a pH gradient to separate them instead of an electric current. There is also an additional technique, called 2D electrophoresis, that is used only with proteins. This involves running two samples of protein on the same gel with each sample offset by 90° as shown schematically in Figure 3. After separation, the protein of interest must be identified. There are two options here: Northern blotting and Western blotting. Both use the same process as Southern blotting (described in more detail in the Genomics section), but they isolate RNA and Proteins, respectively.

Protein profiling concentrates on describing the origin and structure of a protein. One major method of research in this field is the study of gene expression. The basics of transcription (DNA to RNA) and translation (RNA to Proteins) are fairly well known. What is currently being done is a large-scale study of translation, by simply taking a piece of RNA and studying the protein produced.

Protein-protein interaction is, as suggested by the term, the study of protein interactions. Protein interactions involve every aspect of a cell, from signal pathways, to the overall metabolism. One method of studying these interactions is to monitor protein expression under varying circumstances. This type of study is often performed for medical reasons. Structural biology concentrates on the relationship between the structure of a protein molecule and its function within a cell. In an attempt to better understand this relationship, protein modeling has become a major contributor to this field. With the completion of the Human Genome Project, protein research is in a prime position to be launched. New advances in assay technology and mass spectrometry will contribute to the success of this project, but there is widespread opinion that new technology will be necessary if the project is ever to be completed.[9]

Bioengineering

The other parent discipline of biomanufacturing is bioengineering, the actual application of biotechnology. It is often closely associated with biomedical engineering, but is slowly being commercialized beyond the medical field. **Bioengineering studies the mechanisms of biological processes and utilizes the knowledge of those mechanisms to modify organisms or to create new products.** Although the definition of bioengineering sounds very similar to the definition of biomanufacturing, they differ in that **bioengineering concentrates on modifying organisms for unnatural use while biomanufacturing is concerned with optimizing the application of unnatural organisms, as well as creating new technologies, that improve the efficiency of creating unnatural organisms.** Research in this area generally falls into one of five categories:

- Metabolic Engineering
- Tissue Engineering

- Cellular Engineering
- Molecular Engineering
- Nanoengineering (Nanotechnology)
- Biomedical Engineering

Metabolic Engineering

Metabolic Engineering involves the direct modification of cellular metabolism through modification of protein production. It is based on the premise that optimal production within a cellular environment can be obtained through the modification of metabolic pathways. Examples of such modifications include the alteration of the cells' feedback systems to inhibit cellular production of a particular product or to eliminate those agents that consume the product. The applications of metabolic engineering all revolve around the optimization of bioengineering. The major barrier to progress in this field is the lack of basic research. The more that we understand about the way a cell controls its own manufacturing processes, the finer our control will be in tailoring those processes to our needs.

Applications include:

- Production of metabolites and other products that are already made by the altered organism. (e.g. the alteration of E. coli to optimize the production of ethanol)
- Production of new metabolites and other products not already made by the organism (e.g. the production of antibiotics)
- Increasing the range of substrate utilization (e.g. production of ethanol by S. cerevisiae using starch)
- Improvement or the design of new metabolic pathways for chemical degradation (e.g. degradation of benzene by Pseudomonas putida)
- Altering the cell properties that control bioprocessing (e.g. enhanced fermentation)

Metabolic flux analysis is based on stoichiometric balances of metabolic species under the assumption of steady state. This analysis is used to better achieve the applications listed above by presenting cell models that will aid in providing a more precise genetic modification of the microbes utilized.

Amino acids are important targets in plant metabolic engineering because they provide chemical constituents essential to human and animal life. In addition, they provide mechanical support to the plant. Many of the genetic pathways that govern the production of amino acids in plants have been studied and can now be manipulated via metabolic engineering techniques to achieve desired properties.

Another major developing area of research involves alteration of plant metabolic pathways to produce carbon molecules with useful functional properties. To do this, scientists and engineers will need to obtain a better understanding of a plant's metabolism, including the regulation of carbon flow. As gene technology improves, better control of plants at the genetic level will be achieved. This will make this technology much more feasible for practical, everyday usage.

It is understood that improved plant productivity will need to be achieved. Chemical consistency studies will need to take place. In addition, an improved understanding of the factors and theory of sustainable agriculture will provide a stronger foundation for this technology.

Metabolic engineering presents to the scientific community new insights into bioprocess optimization through its goal to analyze, use, and control metabolic pathways. This new paradigm utilizes a broad range of sciences including biochemistry, molecular biology, cell physiology, and biochemical engineering. Bioinformatics and new discoveries in genomics and proteomics will also act to catalyze growth and development in this growing field in that it will allow researchers the capability to produce custom enzymes and metabolic pathways suitable to the desired task.

Tissue Engineering

Tissue Engineering involves the controlled growth of living tissues and organs on three-dimensional support structures, or scaffolds. As with metabolic engineering one of the major barriers to progress in this field is the lack of basic research. Tissues tend to self-assemble and the methods by which they accomplish this are not well understood. At this time, tissue engineering is firmly in the realm of medicine and has applications in the creation of artificial organs, blood substitutes, neurological implants, and orthopedic devices. There are, however opportunities to make this field more of a commercial venture. One example is research into preventing the body's rejection of implants. An understanding of how tissues determine place and identify their own cells will assist in designing implants that do not initiate strong immune responses.

Because of recent technological breakthroughs, the engineering of medical devices can be done using naturally occurring biological substances such as cells and biopolymers. It has been common practice in medicine to treat organ and tissue failure by replacing the failed organ or tissue if it cannot be repaired through surgical reconstruction. The problem with organ transplants is that donor organs are in short supply, their use may cause disease, and surgical replacement can require more than one procedure causing pain and loss of time. Synthetic medical devices have been constructed, e.g. kidney dialyzers, to help to replace some of the function lost in tissue or organ failure. These devices cannot compensate for the total loss of function. Thus, tissue engineering applies principles from current technologies to develop biological substitutes for failed organs and tissue.

One of the first real successes in tissue engineering was the development of artificial skin. Scientists are now capable of providing skin grafts that will aid in healing wounds. Methods have been developed to minimize rejection of transplanted skin from a donor to a recipient. One method involves the removal of the patient's epidermis before transplantation. This technique completely bypasses the need for immunosuppression.

Other tissue engineering applications include:

- Bioartificial Organs

- Blood Substitutes
- Neurological Implants
- Tissue Engineered Vascular Grafts
- Orthopedic Devices

One method of tissue engineering involves the use of cultured cells in contact with a supporting substrate. The substrate may be entirely synthetic or synthetic with an extra cellular matrix of the normal adhesive proteins that are involved in the extra cellular environment of the cells. Needless to say, the interaction of cells with the matrices is important to tissue engineering. Cells become oriented in response to the underlying substrate. This interaction is commonly referred to as contact guidance and is affected by the following physicochemical surface properties:

- Surface Composition
- Surface Charge
- Surface Energy
- Surface Oxidation
- Curvature
- Morphology

Collagen is one popular example of the many extracellular matrix (ECM) macromolecules used as substrate for the attachment and growth of cells. The ECM material can affect:

- Cell Shape
- Cytoskeleton
- Cell Migration
- Control of Cell Growth
- Cell Differentiation

The ECM interacts with the cytoskeleton of the cell and binds growth factors. This interaction allows signals to cross the cell membrane. Some of those signals may affect cell shape and protein synthesis. Cells are attached to the ECM through cell surface proteoglycans, integrins, and cell attachment glycoproteins. Attachment of cells to the ECM substratum is affected by the presence of specific macromolecules and interaction between ECM and the cell membrane. The ECM is very important for the attachment and proper growth of cells. Much work is still being done to understand the exact nature of the interactions between cells and the ECM and could provide information useful in the development of synthetic materials meant to mimic the ECM.

Bioartificial organs can be a hybrid of synthetic materials and living cells. Work has been done to produce an artificial pancreas (Sambanis), liver (Regenerex), nervous system (Bellamkonda), and many other organs. One common limitation of organ engineering is the lack of techniques that would enable scaffolding in three dimensions.

In bypass surgery, a saphenous vein or a mammary artery is implanted to bypass obstructed blood vessels near the heart. The flow of blood causes a pressure on the vessel wall as well as a frictional shear force. Much work is being done to tissue

engineer blood vessel substitutes for those people who do not have a usable vessel of their own for bypass surgery.

Tissue engineering holds future benefits in medicine that can be used to provide superior medical treatment of troops deployed in combat situations and in that way, benefits the security of this nation.

Cellular Engineering

Cellular engineering includes directed modification of cell growth, cell differentiation and division, and cell-to-cell interactions and their physical properties. Cellular engineering also has strong ties to the pharmaceutical industry, and it is currently limited by a lack of basic research. Research into the process of cell growth will be useful in optimizing processes that are dependent on cellular reproduction. Processes that depend on cellular reproduction include DNA sequencing and many pharmaceutical production processes. There are also applications in fields where optimized cellular processes would be extremely advantageous. Examples include improved biomass sources for energy and greater predictability of drug effectiveness in the pharmaceutical industry.

Some of the important technologies involved in cellular engineering are standard tissue-cultivation techniques, force measurement in cultivated cells, and measurements of cell adhesion. Tissue-cultivation techniques involve taking a tissue sample, placing it on a suitable growth medium, and allowing the cell to grow and divide on its own. The growth media must be carefully considered, as well as the cell type. There are many cell types, especially fetal cells, whose differentiation we do not fully understand.

Cell-adhesion measurements are extremely important to any study of cell dynamics. Many biological molecules and drugs affect cell adhesion, and thereby affect the organism. Most adhesion assays are static, qualitative, or quasi-quantitative, but techniques used by Cellular Engineering Technologies, Inc., allow quantitative cell-adhesion measurements. They accomplish this by passing a current through the cell from a microscopic electrode and measuring the transcellular impedance. There is also the matter of identifying the cell adhesion sites and the mechanisms by which biomolecules regulate cell-to-cell and cell-to-matrix adhesion. The first issue that makes this identification complex is that the cytoskeleton of the cell is an integrated three-dimensional network of filaments and adhesion proteins. This physical framework allows mechanical forces to be passed to remote sites within the cell and even from one cell to another. The second issue is that cell adhesion proteins are not uniform. They respond differently to stimuli depending on where they are located in the cell. Changes in cell adhesion can occur rapidly, so the measurements of cell-to-cell and cell-to-matrix adhesion need to be made separately but compared simultaneously.

Cell force measurements are also an important part of studying cell dynamics. As with cell adhesion, cell force is a primary target for both drugs and diseases. Currently, the effects of drugs on cellular force are studied using animal models, but this process can be expensive, time-consuming, and imprecise. One technique being used for laboratory study is to measure force in reconstituted artificial tissues consisting of cultured cells and

connective tissue to approximate a physiological model. This technique is widely used in studies of muscular biology, wound healing, and pulmonary medicine.[10]

Molecular Engineering

Molecular Engineering is the study of biomolecules and biomolecular processes to discover and create new high-value biomolecules and to develop enabling technologies to produce them on a commercial scale. This field has obvious ties to the pharmaceutical industry, but other applications will come in the form of optimized production of commercial products by biological means. One often cited example is the production of ethanol, but microorganisms create much more complicated products every day. Basic research into the chemistry a microorganism uses will affect the way industry synthesizes chemicals.

Molecular engineering has been growing as a field since before the beginning of the Human Genome Project. The characterization of biomolecules led to the development of the field of biochemistry, but it was the ability to synthesize those molecules that gave rise to the field of Molecular engineering. Molecular engineering deals with biomolecules, molecules produced by biological organisms, and molecular processes to discover and create new high-value biomolecules at the molecular scale. The goal is to develop enabling technology for the production of biomolecules to improve the quality of life and the environment. Some of the enabling biological processes and scientific disciplines include directed genome evolution, metabolic-pathway engineering, protein engineering, analysis of functional genomics and proteomics, and high-throughput screening. The development of the enabling bioprocess technologies for the production of high-value biomolecules of medical, agricultural, and economic importance is also a high priority.

The design of novel biomolecules concentrates mostly on structure-stability and structure-activity relationships, which are based on the following five areas. The interrelationships between these five areas are shown schematically in Figure 4.

- Bioinformatics – includes functional genomics and proteomics.
- Protein chemistry and protein engineering - deals primarily with protein structure-function and structure-activity relationships, structure-based design, and prediction of designed protein structure.
- Recombinant techniques – includes random mutation, DNA shuffling, and phage-display techniques.
- Metabolic-pathway engineering – includes the metabolic flux analysis.
- Bioprocess engineering – used to develop enabling technology to produce the desired high-value biomolecules.

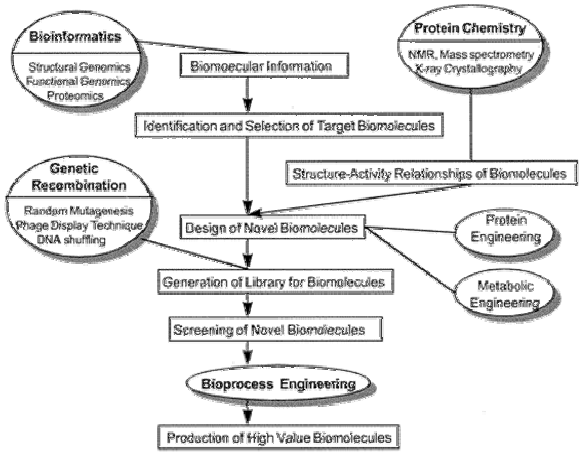


Figure 4 Important components of biomolecular engineering and flow sheet for generation of novel biomolecules.[1]

Bioinformatics include databases related to functional genomics and proteomics. These databases provide the most current genetic sequence and function data, protein sequence and function data, and metabolic process data. Use of bioinformatics databases has resulted in the design of many protein/peptide and metabolic molecules. For more information on bioinformatics please refer to the segment of this paper with that title.

Protein engineering, the application of proteomics, deals with both protein structure-based design and prediction of a designed protein's structure. Both the function and the activity level of a protein are factors in protein structure. Current research centers on characterizing and archiving protein structure, as well as characterizing and modeling protein structure-function and structure-activity relationships.

Recombinant DNA techniques provide the capability of design, modification, and engineering of natural biomolecules. Recombinant techniques include:

- Oligonucleotide-directed Mutagenesis
- DNA Shuffling
- Phage-display Techniques

Oligonucleotide-directed mutagenesis is the site-directed mutation of DNA with a designed oligonucleotide primer, with the intention of producing a specific novel protein. This method allows the researcher to design and modify a protein structure, but is limited by current knowledge of protein structure-function relationships and the approximate nature of current computer-graphic modeling technology. Oligonucleotide-directed mutagenesis is also limited to the synthesis of one novel biomolecule at a time, a limitation that researchers have attempted to compensate for by creating expanded combinatorial libraries. Using error-prone Polymerase Chain Reactions (PCR) to induce random point mutations as the DNA is replicated creates novel DNA combinations. This method is limited by the enormous screening necessary to determine the results of the random point mutations.

The DNA shuffling technique mimics the process of natural, sexual DNA recombination. A group of related genes is randomly fragmented and subjected to denaturation and hybridization, followed by the extension of the potential fragment binding ends. As the DNA is replicated with high fidelity, some of the fragments bind together, forming novel DNA combinations. This process allows more direct

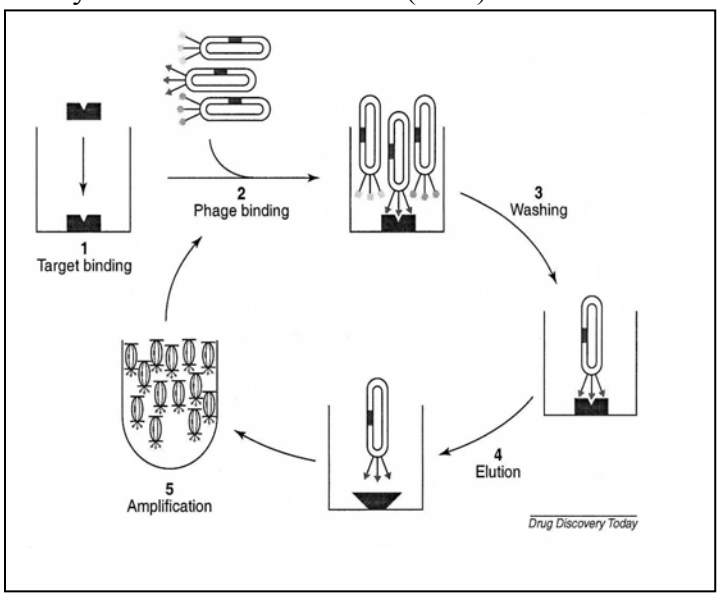


Figure 5 The process for affinity selection of phage-displayed peptides.[11]

recombination of all beneficial mutations from each completion of a PCR cycle, giving the researcher the ability to generate multi-step mutants with dramatically improved phenotypes.

Phage-Displayed Peptide Library Screening was initially developed for isolation of a monoclonal antibody, an antibody that recognizes only one type of antigen. The typical process for phage screening is shown in Figure 5. This process begins with the target being immobilized on either a surface of beads or the wells of a microtiter plate (Step 1). A variety of possible matches to the target are placed in the solution and allowed to incubate with the target. This gives the phages time to bind to the target (Step 2). The plate is then washed to remove any unbound phages (Step 3). The bound phages are eluted when the target is *denatured,* the process of partial or total alteration of the native structure of a macromolecule resulting from the loss of tertiary or tertiary and secondary structure that is a consequence of the disruption of stabilizing weak bonds. The phages are then freed into solution (Step 4). The eluted phages are then put through an amplification process, usually a PCR-type process (Step 5). The amplified phage can then be re-used in the process until only the phages that most closely match the target remain.[11]

Metabolic-pathway engineering is a new approach to understanding and using metabolic processes. It is the targeted and purposeful alteration of metabolic pathways found in an organism in order to better understand and use cellular pathways for chemical transformation, energy transduction, and supramolecular assembly. It typically involves the alteration of cellular activities by the manipulation of the enzymatic transport and regulatory functions of the cell through the use of recombinant DNA, metabolic-flux analysis, and other genetic techniques. Metabolic-flux analysis is the detailed quantification of all metabolic fluxes in the central metabolism of a microorganism and is a combination of measured extracellular fluxes and measured intracellular labeling information. It is used to determine targets for genetic manipulation and to determine the results a genetic manipulation will have on cellular metabolism.[12] Metabolic engineering challenges include the need for methods to effectively express target proteins at desired levels, for improved tools to coax production of materials normally not produced or synthesized at extremely low levels, and for methods to provide insight into the complex interactions that exist between metabolic pathways.

Bioprocess engineering includes both fundamental and applied research and development in the process engineering fields of bioreactors, biocatalysis measurements and control, and downstream processing. It involves multidisciplinary approaches for integrative process design based on the hierarchical structure and decomposability of biosystems leading to analysis and synthesis. New approaches for rational and evolving design of cellular systems are emphasized by taking into account the environment and constraints of technical production processes, integration of recombinant technology and process design, as well as new hybrid disciplines, such as bioinformatics and process systems engineering.

The molecular engineering paradigm involves the construction of beneficial materials and molecular agents at the micro-to-nano scale in a top-to-bottom fashion as opposed to nanoscale engineering that involves a bottom-up approach. It draws from its contributing disciplines, i.e. genetic, protein, metabolic, and bioprocess engineering, what it needs to help accomplish this goal and proves to still be a very useful manufacturing methodology.

Nanoengineering (Nanotechnology)

Nanotechnology is probably one of the most talked-about fields in research today. Many of the manufacturing applications currently under investigation have corresponding processes in microorganisms. As in molecular engineering, basic research into the processes by which cells create complex molecules will yield information industry can use to optimize its own processes.

According to the 1999 Interagency Working Group on Nanoscience, Engineering and Technology Report, **nanotechnology is two things: 1) the creation of useful materials, devices, and systems through the control of matter on the nanometer scale, and 2) the exploitation of novel properties and phenomena developed at that scale.**[13] If properly developed, this technology will forever change the face of human existence, enabling us to manipulate atomic elements to create any desired material without the need of machining.

Nanotechnology has the potential to take biomanufacturing to an entirely new scale. Efforts to enhance this technology have been hindered by the lack of international and even domestic cooperative efforts. Increasingly, the U.S. government is stimulating academia, national labs, and the private sector to investigate this technology through massive funding and supporting National policy. For example, the National Institute for Science and Technology (NIST) announced that it will support U.S. industry in "moving nanomanufacturing technologies into production within this decade by concurrently developing the scientific and engineering foundations necessary to support measurements and standards required to achieve effective and validated nanoscale product and process performance." Also, the National Science Foundation (NSF) released a request for proposals outlining its efforts to support nanotechnology for FY2003. Attempts are being made at this time to encourage academic institutions to create courses on this subject to stimulate professional awareness and educate professionals to meet the growing demand for experts in this area.

The idea of nanotechnology is not new. The origin of this idea can be traced back to Richard Feynman's talk[14] at the California Institute of Technology entitled "There's plenty of room at the bottom". In his talk Feynman stated his belief that science would turn to the nanoscale, and outlined the need for better electron microscopes and supporting technologies for biologists and engineers who will eventually deal with systems at this scale. Improvements in the use of scanning force microscopy to manipulate molecules individually and lithography for use in microelectronics and microelectromechanical systems (MEMS) are providing evidence that nanofabrication is feasible.

There are three primary areas of interest in nanotechnology:

- Nanomeasurement
- Nanomanipulation
- Nanomanufacturing

The third activity hints at the concept of "molecular manufacturing" using the first two technologies as the tools. Many believe this may become feasible in the next 10-20 years. Molecular manufacturing could take place in nanoscale molecular-assembly factories, which will take basic chemical elements and produce a final product. These factories may even have sensors or actuators that will respond to commands or environmental changes. However, advancements in biotechnology, chemistry, computational tools, electrical engineering, and physics will need to take place in order to enable development of nanotechnology.

The properties of electrons and atomic interactions within matter are influenced by material variations on the nanoscale. It may be possible to control properties of materials by engineering them at the nanoscale. Manipulation of the nanostructure of materials would allow scientists and engineers control over these properties without changing the actual chemical composition of the materials. This manipulation may be done inside of a biological cell using the principles of self-assembly. This bypasses the need to use miniature robots or devices, since the information necessary for assembly is already contained within the system.

Biomedical Engineering

One of the best-known branches of bioengineering is biomedical engineering (BME). Although the terms are often used synonymously, bioengineering is slowly moving away from being a strictly medical discipline. BME involves "the acquisition of new knowledge and understanding of living systems through the innovative and substantive application of experimental and analytical techniques based on the engineering sciences."[15] It also involves "the development of new devices, algorithms, processes, and systems that advance biology and medicine and improve medical practice and health care delivery." BME includes not only the relevant applications of engineering to medicine but also to the basic life sciences.[14]

Fueled by advances in the understanding of basic biological and cellular mechanisms that have great potential in benefiting public health and thus producing general improvements in the quality of life, bioengineering is on the upsurge. Before leaving office, President Clinton signed a bill establishing the National Institute of Biomedical Imaging and Bioengineering, the newest member of National Institutes of Health (NIH). This is a major contribution to the science in that it introduces a new element to the NIH structure, which tends to focus on distinct disease processes and organ systems.

Increasingly, universities and other academic institutions are making enormous efforts to add bioengineering programs. According to David Gough, professor and chair of the department of bioengineering, University of California San Diego, "A department of

bioengineering has been inaugurated at a major university nearly every month for the last several years." Contributing to this growth are improvements in public perception of bioengineering and the high-profile advances in genomic sciences. Students are graduating in increased numbers thus producing a large community of engineers to meet industrial demand as this field continues to grow.

Biodetection

There are many motivating factors in the recent development of "biosensors" and other detection devices. Historically, the motivation has been medical with the primary goal to use sensors to diagnose disease accurately and to avoid human error and misdiagnosis. In more recent times, research has shifted to the detection of biological toxins that could be used in terrorist or military attacks on both military and civilian targets. This report will distinguish between those sensors that have strictly medicinal applications and those that have tactical applications.

All sensors have three major components: a receptor to receive information from the surrounding environment, a transducer to translate that information into a usable format, and a signal conditioner to analyze the information before it is output to a display. Biosensors are a specific type of sensor that use scientific principles to detect mechanical, chemical, and physiological events for medicinal purposes. Additionally, they can use biodetection methods to identify biological agents. **Biodetection typically refers to the use of biomolecular recognition principles to "sense" biological agents.** Biomolecular recognition depends on the binding of ions, small-molecular-weight organics, and biological macromolecules to biological receptors. Mechanisms for detecting and measuring the recognition event depend primarily upon the action of the biological receptor. These receptors can be grouped functionally as biocatalytic (i.e. enzymes), bioaffinity (e.g. receptors, antibodies, and DNA), or microbial (e.g. bacteria) in nature. When these bimolecular recognition elements are directly coupled to signal transducers (e.g. electrochemical, optical, or acoustic), they can generate an electrical signal proportional to the target analyte concentration and are typically referred to as biosensors.[16]

The types of receptors and transducers will be discussed in this section:

Receptors	**Transducers**
Bioaffinity	Electrochemical
Biocatalytic	Piezoelectric
Microbial	Optical
	Thermal

Receptors

Bioaffinity Receptors

Bioaffinity sensors are a class of biosensors that use immobilized receptors to detect specific substances. A schematic of a general biosensor is shown in Figure 6.

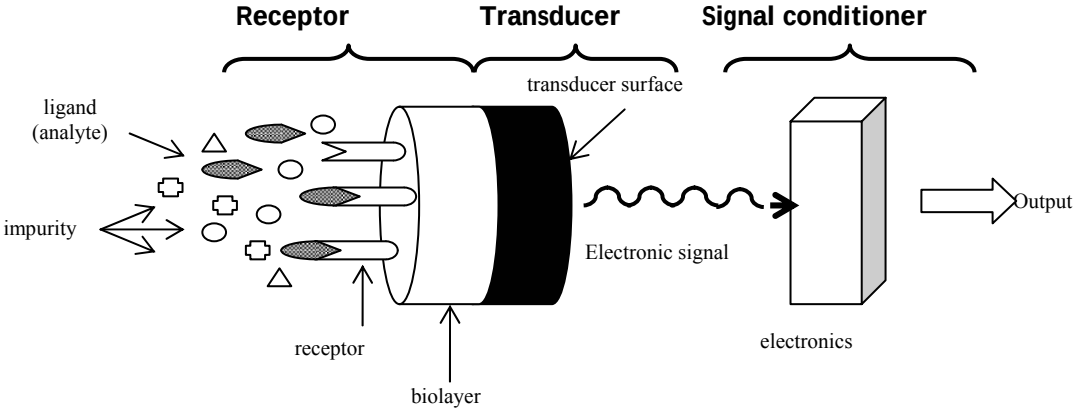


Figure 6 Schematic configuration of a biosensor

There are four general types of biosensors:

1. DNA- and RNA-Based Probes that bind to strands of complementary nucleic acids from pathogens.

2. Antibody-Based Probes ("Immunosensors") that use antibodies to recognize very specific antigens or sites on cellular components (epitopes). Antibodies for a specific microbe need to be obtained in pure culture adding to production costs. Antibodies must be screened for their binding characteristics such as affinity, and on and off rates. It must also be known what epitope a specific antibody will recognize.

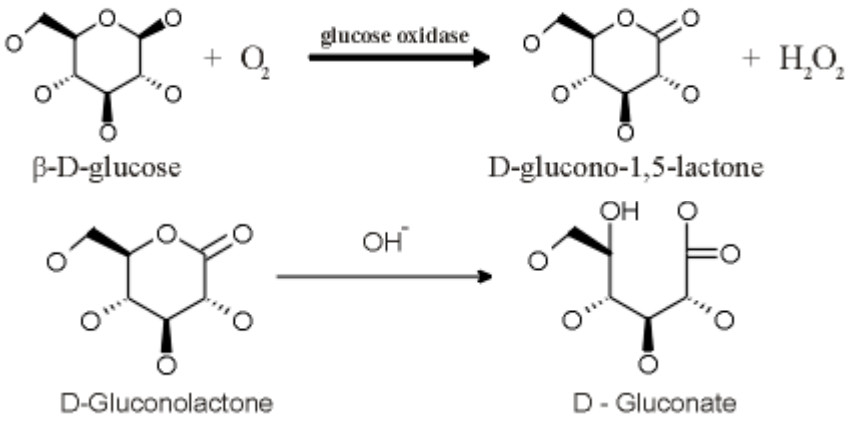
3. Antigen-Based Probes that are similar to antibody-based probes, but use antigens to detect antibodies.

4. Ligand-Based probes in which every cell has cell surface proteins that bind to other specific molecules and ligands that are specific to a particular microbial serotype or common to related groups.

Biocatalytic Receptors

Biocatalytic receptors expose specific substances, like a specific protein or toxin, to a catalyst that will break the substance into specific products. Enzymes are the most popular biocatalysts because they are highly selective and efficient. Depending on the enzyme, it can be very difficult or very simple to purify. Enzymes can be used to break down polymers into their monomers, which can be detected, and can cause many other reactions to occur in which the products or reagents can be monitored.

The most common enzyme that is used in biosensors is glucose oxidase. Monitoring either the oxygen or hydrogen production performs glucose measurements. This enzyme catalyzes the oxidation of glucose as shown schematically in Figure 7.



(*Glucose Oxidase*)

Glucose + O_2 + H_2O → gluconic acid + H_2O_2

Figure 7 Oxidation of glucose via glucose oxidase catalyst

Microbial Receptor

Microbial biosensors consist of a transducer combined with immobilized viable or non-viable microbial cells. Non-viable cells are obtained from a process called *permeabilisation* in which the cell is exposed to an organic solvent that creates minute pores in the cell membrane. This process allows the free diffusion of small-molecular-weight substrates or products across the cell membrane, in addition to killing the cell. Another type of microbial biosensor involves engineering a cell that transports its intracellular enzymes into the periplasmic space. In these cells the exported enzymes avoid membrane transport problems. Non-viable cells or whole cells containing periplasmic enzymes can be used as an economical substitute for enzymes. Viable cells make use of the respiratory and metabolic functions of the cell, where the analyte to be monitored is either a substrate or an inhibitor of the cell's metabolic processes.

An application of this technology is a genetically-engineered cell-based biosensor (GECBB) that can be used to classify biologically active agents. Conventional immunoassays traditionally use some indicator (radiological, fluorescent, electrical, etc.) to measure the antigen or antibody concentration in a solution, while cell-based biosensors indicate a cell's physiologic response to some bioactive stimuli. This GECBB sensor combines the technologies of conventional immunoassays with that of cell-based biosensors. Two cells, one naturally occurring and one genetically modified, are used as primary transducers and are placed on a sensor. The genetically-modified cell is missing a specific membrane-bound receptor, while the natural cell has not been modified. Both cells, by virtue of their location, are exposed to similar environmental conditions. The combination of responses, either positive or negative, from both cells will tell the operator both the activity and the mode of entry of a particular unknown agent. This setup has the potential to be run in parallel with many modified cells and one natural type

cell. Limitations include the number of membrane-bound receptors that can be removed before the cell is inactivated and the number of unknowns that can be run simultaneously.

Biological agents must be immobilized in order to interact with the transducer. There are four methods of immobilization:

- Adsorption
- Direct cross-linking or Entrapment
- Encapsulation or confining
- Covalent Binding

In adsorption, there is weak binding (i.e. hydrogen or charge-to-charge) most often to cellulose, collagen, or collodion, which may be reinforced with a cross-linking agent such as glutaraldehyde.

In entrapment, the biological agent is entrapped in a gel by formation of a cross-linked network around the agent.

Encapsulation and confining most often involve an enzyme system. The agent is first enclosed within a semi-permeable spherical microcapsule or the agent is placed directly on the transducer where it is held in place by a semi-permeable membrane.

Finally, covalent binding typically involves a substrate hybridized with hydroxyl (OH), amine (NH_2), carboxyl (COOH), and Sulfahydryl (SH) groups. The biological agent is then covalently bonded to one or more of the groups on the substrate.

Transducers

Electrochemical Transducers

Electrochemical transducers use enzymes to generate an electrochemical signal, either amperometric or potentiometric (amperometric being more sensitive). For example, Field Effect Transistors (FETs) have their conductance modulated by the gate voltage through the field effect of a semiconductor. Ion-sensitive FETs (ISFETs) are most often used and allow for the detection of acidic or alkaline species during enzyme reactions.

Piezoelectric Transducers

Piezoelectric transducers use piezoelectric crystals that change dimensions when subjected to current or voltage. These are coated with a receptor that will alter the frequency of crystal vibration when a biological agent attaches to the receptor material coating. This is an example of an acoustic sensor that indicates changes in mass or viscosity on the surface layer. Acoustic sensors also make use of the piezoelectric properties of a substrate to indicate changes in mass or viscosity of the surface layer.

Optical Transducers

Optical transducers can use light scattering or absorbance, but usually use fluorescent or other luminescence spectroscopy. For example, fiber-optic biosensors result from the interaction of a reagent phase that incorporates an immobilized biological agent with a transducing system comprising an optical fiber connected to a light detector.

Thermal Sensors
Thermal sensors can be used as thermistors to detect exothermic heat generated from an enzyme reaction.

Sandia National Laboratories is currently developing a biosensor based on current immunoassay technology coupled with acoustic transducers. The receptor is composed of OH-terminated alkanethiol layer bound to the substrate. Then phospholipid vesicles are added to form a phospholipid bilayer, on which streptavidin and then biotinylated antibodies can be bound. This receptor layer is self-assembling and can be easily removed by washing the lipid bilayer in a detergent, allowing the operator to use the same sensor more than once. This particular Sandia-patented device[17], the novel magnetically-driven flexural-plate wave device (mag-FPW), is used as the acoustic transduction element upon which the receptor membrane is bound. This particular transducer can be tuned to maximize its mass and viscoelastic sensitivity, is used in both liquid- and vapor-phase detection, and be easily miniaturized and integrated into an array.

Biomaterials

Complicated nanosystems can be designed through use of biomimetic principles. **Living systems and their molecular structures are observed by researchers and can be modeled by using applied biological sciences. Biomimetics entails researching structure and organization of biomolecules, cells, tissues and biological systems, and then designing based upon these observations.** For example, a ribosome is a powerful manufacturing tool used by the body to synthesize products valuable to physiological health. Its structure, function, and information flow may be studied and used for inspiration of a nano-device made of synthetic materials that will not coagulate in, or be affected by, environmental extremes.[18] The possibilities of biologically-inspired designs are endless when coupled with other science and technologies, especially nanotechnology.

Biomimetic materials use the biological world for the inspiration of new materials for use in a wide range of fields including:

- Aerospace Engineering
- Bioengineering
- Computer Science Engineering
- Electrical Engineering
- Materials Science Engineering
- Mechanical Engineering

Biomimetic materials have been used in the design of aircraft and swimwear intended to mimic the unique qualities of sharkskin in order to reduce form drag in the water and air by up to 8%.[19] This design has also been used in trains whose outer coverings have ribs parallel to the flow of air in order to reduce drag.

Composites currently are being designed to mimic some of the biocomposites found inside the human body. Bone, connective tissue, and mucus are very good examples. Non-human biologics have also contributed to many applications ranging from analysis of abalone shells to design composites with similar mechanical properties for architectural applications.

Velcro is a commonly-used material whose design was inspired by the observation of burred seed shells that cling to hair. New "smart" adhesives have been designed that have molecular hooks that attach to small corresponding springs. These adhesives are comparable to Velcro on a molecular scale. They are sensitive to heat, loosening grip at around 80ºC.[20]

Smart hydrogels also are biomimetic materials that are important for use in biochemical manufacturing. They consist of hydrogels that are able to respond to changes found in their environment. One way they respond is by changing their level of swelling (swelling ratio). The stimulus that causes change could be a change in pH, temperature, electric field, ionic strength, salt type, solvent, stress, light, or pressure.[21] Changes in the swelling ratio could be used to set up a feedback system for the delivery of a drug or other agent.

Biomimetic design is not limited to the mechanical aspects of natural systems. For example, genetic algorithms are based upon random mutation as well as natural selection, but are not themselves models of evolution. This does not suggest that biological systems are optimized systems. However, they have the potential to help inspire good engineering designs.[22]

There is much to learn by observing the way that biological systems make what they need or change the properties of what they already have. The most visual representation of biomimetic design is structure. In biological systems, structure is very closely related to function. By knowing more about this relationship, we may be able to mimic some of the more useful structures that are already available in nature. It is also important to understand what properties of a system or material we are trying to mimic.[23]

Nature produces some very complicated biomolecular structures that science does not fully understand, i.e. proteins. To mimic biomolecules, additional research into protein/biomolecule structure-function and synthesis techniques will need to take place. Science continues to build on the phenomena exhibited by nature. Manufacturers can learn from the complex interactions that have brought nature to its current level of efficiency.

Biocomputation

Biocomputation is a combination of computer science and biology that attempts to build computational models of real biological systems. Experts use the tools and concepts of information science. One goal is to see biological systems from a different paradigm. Another is to use biological systems or processes as a metaphor, or inspiration. It may also act to enable and catalyze the development of new computing technologies as well as new areas of computer science.

Biocomputing should not be confused with bioinformatics (data handling) or computational biology (simulations). Rather biocomputing focuses on the intersection between computer science and biology. Biocomputation is involved with:

- Computational properties of cells
- DNA Computation
- DNA Self Assembly
- Cellular and DNA Logic Gates
- Computer Immune Systems
- Artificial Intelligence (AI)
- Artificial Neural Nets
- Genetic and Evolutionary Algorithms

Cells can serve as models for sensors or digital logic gates. Currently, cellular logic gates are being produced that use cellular inverters made up of proteins that either suppress or activate the production of other proteins as an on/off switch. Additionally, genetic regulatory networks can be simulated using computer science concepts and knowledge of how cells process information, yielding benefits to molecular biology. In general, applying computer science techniques to cellular events is helping biologists to understand these events.

Protein-based electronic devices were first developed in the former Soviet Union around 1975. The goal was to create a three-dimensional (3D) memory system that would far exceed the capacity of western memory systems. One major Soviet development was the creation of biochrome, a real-time photochromic and holographic film based on chemically-modified polymer films containing Bacteriorhodopsin (BR). A review of this research can be found in the papers of Vsevolodov and Poltoratskii.[24]

Bacteriorhodopsin (BR) is a protein that has unique photophysical properties. It is known to be highly stable under thermal and photo-physical extremes. The protein has good light-to-energy conversion properties and is thus well-suited for a number of computer and data storage applications. BR is isolated from the salt marsh archaebacteria called *Halobacterium salinarium*. This protein is known for its ability to maintain its structure and function at temperatures as high as 140°C, a temperature that would cause most proteins to lose function and/or coagulate.[25]

To be more specific, BR is a photon-driven ion pump that consists of a seven-helical transmembrane protein with a retinal co-factor. In the parent bacterium *H. salinarium*,

BR converts light to a proton gradient, which is then used by a second membrane protein called *ATP synthase* to generate chemical energy in the form of ATP (adenosine 5'-triphosphate). The cell then uses ATP to drive a multitude of vital processes.[26]

There is a protein in the human eye called Rhodopsin that enables us to detect light. Although this protein has similar structural properties to BR, it is very different in function. Rhodopsin would not be a good choice for computing because it is destroyed during usage. Bacteriorhodopsin can be used many times, however, before it denatures.

The protein BR cycles through a series of spectrally distinct intermediates upon absorption of light. Chromophores, a light-absorbing group embedded in the protein matrix, convert light energy into a complex series of molecular events that store energy. This complex series of reactions causes changes in the optical and electronic properties of the protein. BR has good holographic properties because of the large (65%), highly-efficient change in refractive index. This protein is known to be 10 times smaller than the wavelength of light. Therefore, the resolution of the thin film is determined by the diffraction limit of the optical geometry rather than by the graininess of the film. BR absorbs two photons at a time and consequently it can be used to store information in three dimensions by using two-photon architectures. Because BR was naturally engineered to function in conditions of high temperature and light intensity, it can be used to create very robust devices.

BR, like any protein, can be engineered to enhance its natural properties or at least tailor them to a specific task. Therefore, the BR protein can be used as a template to design novel proteins through genetic engineering principles for use in biomolecular-electronic devices. In essence, this protein is the prototype for future protein-based devices.

Because of its unique properties, BR has been used in the design and development of holographic and volumetric 3D memories. Holographic memories are based upon the protein's ability to form an M-state and thus produce a large change in refractive index. In fact, films of BR can generate diffraction efficiencies of about 8%.[27] This property makes the protein capable of performing holographic data storage. With the manipulation of the protein through mutation, it has become competitive with photorefractive polymers. Thin and thick films of BR can be prepared in variable concentrations for optimal optical architecture.

Holographic memories can be made to operate effectively in three dimensions using proteins. Data can be stored in such a holographical system using a sequential pair of one-photon processes, making possible the use of diode lasers to source one bit into the long-lived Q state.[28] Such a memory system can store 3 times the amount of information normally stored in the same amount of space. Such memories are extremely robust and can withstand fierce gravitational forces, electromagnetic radiation, and cosmic rays. These memories are also low cost, lightweight, and resistant to moisture. These devices can be submerged in water for extended periods of time without loss of data.

Because BR is a protein, its development is bottlenecked by the same factors affecting advancements in protein engineering. Limitations include the lack of satisfactory molecular modeling tools and understanding of the structure-to-function relationships presented by the natural complexity. It may not be realistic to expect a design team to use protein-engineering tools to enhance the holographic properties of this protein without some unforeseen difficulties. At this time, protein engineering is still done by trial and error.

Researchers have also been able to use BR in the construction of neural computer memory design as the photoactive holographic media in thin films.[29] The idea is to create associative memories like the memory believed to function in the human brain. Associative memories operate by scanning the entire memory for the data block that matches the input. It may also be capable of locating the closest match.

Artificial neural nets are computer algorithms that mimic the way neurons in the brain process information. They are used in an iterative methodology where real data are used to train the artificial neural net to recognize certain patterns. Genetic and evolutionary algorithms are computer programs based upon the concept of simulating evolution through natural selection, mutation, and reproduction. Artificial intelligence studies natural life by attempting to recreate biological phenomena using computers and other artificial media.

Bioinformatics

Bioinformatics is one of the most important contributors to biomanufacturing. It involves the handling of all of the data produced by any of the bio-related fields. It can also include the application of statistical techniques to the management of information, creation and management of databases, and analysis of DNA sequence data. In general, bioinformatics describes any use of computers to handle biological information. To most people, bioinformatics is a synonym for computational molecular biology, the use of computers to characterize the molecular components of living things.[30] There are a number of subcategories within the field of bioinformatics including:

- Comparative genomics, a search for similarities and differences in all the genes of multiple species,
- Cheminformatics, the analysis and database maintenance of information from the study of molecular-level chemistry, and
- Medical informatics, the management of all biomedical experimental data.

Bioinformatics tools can be used to obtain sequences of genes or proteins of interest, either from material obtained directly from researchers or from repositories of previously prepared and investigated sequences. Analysis of sequence data can be accomplished in a number of ways.

One of the most difficult tasks that falls under the field of bioinformatics is the assembly of gene sequence data. Genetic sequencing can only be done on small pieces of the genome at a time, so the product of all of the sequencing data is a huge array of overlapping sequences. Bioinformatics can be used to assemble those pieces into one

continuous code using gene mapping. Gene mapping determines where the restriction enzymes have cut the genome, and where they can be pasted back together. Genes that look remarkably similar to one another, called homologues, are likely to be related to one another. This relationship between proteins is used to trace evolutionary family trees, and can be used to track the changes in biomolecules through time. The sheer volume of data makes sequence assembly extremely laborious, requiring extensive and expensive computing power.

Once a homologue has been uncovered, then the newly discovered protein may be modeled. Molecular modeling and structural biology are growing fields that are considered part of bioinformatics. The model, a three-dimensional structure of the gene product, can be predicted without the need for laboratory experiments. In this way, bioinformatics is used to predict the function of gene products. There are, for example, tools that allow a user (often via the Net) to make reasonably good predictions of the secondary structure of proteins arising from a given amino acid sequence. These predictions are based on known "solved" structures and other sequenced molecules acquired by structural biologists. Structural biologists use bioinformatics to handle the vast and complex data from X-ray crystallography, nuclear magnetic resonance (NMR), and electron microscopy investigations, and to create the 3D models of molecules that are commonly seen in journal articles.

There are other disciplines of biologically-inspired computation that include genetic algorithms, artificial intelligence, and neural networks. Often these areas interact in strange ways. Neural networks, inspired by crude models of the functioning of nerve cells in the brain, are used in a program called PHD to predict, somewhat accurately, the secondary structures of proteins from their primary sequences. What almost all bioinformatics has in common is the processing of large amounts of biologically-derived information, whether it consists of DNA sequences or breast X-rays.[31]

Bioenergy

Fossil fuels represent 80% of the energy that we consume at this time. **Biobased products and bioenergy from crops, trees, and agriculture, as well as industrial, municipal, and forestry waste, hold great promise for the future. We can use the energy and molecular binding blocks of plants to produce fuels, provide chemical feedstocks, and generate electrical power.** Each year around 100 billion tons of biomass are created worldwide, which contain an energy value equal to five times the current total global energy consumption. Most biomass is currently burned or left to biodegrade, and little is turned into useful energy or other products. This is because it is widely dispersed and difficult to capture. In the U. S. biomass supplies only 3% of the nation's total energy consumption. In the future, experts anticipate that biomass will play a far greater role.[32]

The following useful terms are defined in section 303(2) of the Biomass Research and Development Act of 2000:[33]

- **Biomass** – Any organic matter that is available on a renewable or recurring basis, including agricultural crops and trees, wood and wood wastes and residues, plants (including aquatic plants), grasses, residues, fibers, and animal wastes, municipal wastes, and other waste materials.

- **Biobased industrial products** – Fuels, chemicals, building materials, or electric power or heat produced from biomass.

- **Biofuels** – Liquid fuels produced from biomass that are used in stationary and mobile applications.

- **Biopower** – Includes new installations using 100 percent biomass or a blend of biomass and fossil fuel; the installation of co-firing systems at existing fossil fuel-based generating station; and repowering options involving biomass.

There are substantial benefits to fully utilizing these potential bioenergy sources including:

- Enhanced national energy security
- Improved environmental protection
- Rural economic growth
- Sustainable global development

The fast development of the bioengineering of plants as well as the "synergistic production" of agricultural and chemical feedstocks, and the conversion of wastes and other biomass into energy is underway. Plant-based materials may soon be designed to yield novel polymers or to maximize inherent energy. Technologies may also tap the possibilities of using aquatic biomass. In time, engineered processes might mimic plant photosynthetic processes and act to directly convert the sun's rays into high-energy materials.[32]

One option for future energy production that reduces the use of petroleum products is the use of coal. Coal is in abundance and can be liquefied or gasified to replace petroleum-based products. There are, however, environmental problems associated with the use of coal, such as emission of nitrogen oxides, sulfur dioxides, particulates, and carbon dioxide.[32]

Biomass can be used to generate electricity, provide liquid fuels and chemical feedstocks, and extract hydrogen from water. However, there are numerous potential technical issues. For example, the cultivation of biomass energy sources must be balanced with other demands on the land and water. In addition, the long-term safety of genetically engineering plants must be assessed.

Usually the term "biomass" refers to lignin and cellulosics. Development in the advanced gasification of such resources may result in more efficient energy retrieval.

Also, enzymatic conversion of lignocellulosic biomass is revolutionizing the way we use agricultural crops, as well as fast growing trees and other plant parts, to produce ethanol.

Dramatic scientific developments in such areas as genomics of transgenic plants are creating new possibilities for development of new biomass resources. These new types of plants and microorganisms could potentially yield novel polymers, high-energy-content hydrocarbons, and other important products. However, genetic biotechnologies remain controversial. They have resulted in impressive gains in productivity and yields in agriculture, but the long-term ecological and health effects of genetic biotechnologies are the subjects of much debate.

One major developing area of research involves alteration of plant metabolic pathways to produce carbon molecules with useful functional properties. To do this scientists and engineers will need to obtain a better understanding of not just the actual plant metabolism but also the regulation of carbon flow. There is also a need to address the concern that this technology will have a negative impact upon the ecosystem. As gene technology is improved, there will be better control of plants at the genetic level. This will enable use of this technology for practical, every-day usage.

Plant productivity also needs to be improved. Chemical consistency studies will need to take place. Also, an improved understanding of the factors and theory of sustainable agriculture will provide a stronger foundation for this method of biobased product and bioenergy production.

Bio-refineries have the potential to produce energy and biobased products. Agricultural, forestry, and methane products would be used as the raw material in such refineries. It is also possible to exploit landfill, animal, and plant wastes as potential bioenergy sources. Another possibility lies in the conversion of light into energy using natural photosynthetic processes coupled with special enzymes. This combination could be used to design a solar panel to capture solar energy for use in carbon fixing reactions aimed at the production of energy-rich products.

Processing and conversion involves the separation and conversion of biomass materials into power, fuels, and products.[32] R&D is needed to develop the following technologies:

- More economical separation techniques
- Enzymatic and thermal processing
- Biomass material conversion processes
- Improved fermentation processes
- Improved reduction chemistry
- Capital cost reduction strategies
- Biopower systems
- Advanced processing strategies in biorefineries

In addition, The DOE Advisory Committee made the following recommendations for research and development of biofuels: [32]

- Advanced methods to overcome the resistance of agricultural, forest-based, and urban feedstocks to enzymatic and fermentation treatments.
- An understanding of the fundamental structure of lignocellulosic materials, including the chemistry of its cell wall structures, transport properties, and genetic properties, to improve growth rates and processing characteristics. Agronomic, economic, and environmental impacts of harvesting lignocellulosic material must be established to ensure that the use of these materials results in beneficial lifecycle impacts.
- Pretreatment technologies that make utilization of both current and new feedstocks more effective and less expensive.
- Traditional thermo-chemical and catalytic processing to convert starches, sugars, and cellulosic material into fuel. This fits into broader biorefinery concepts.
- Sensors for quick, cost-effective systems for on-line, real-time analysis and maintenance of feedstocks.
- Biorefineries that use complex strategies to efficiently produce a diverse and flexible mix of conventional products, fuels, electricity, heat, chemicals, and material products from all available, environmentally appropriate biomass feedstocks. Current biorefineries need improved processing efficiency.
- Research in utilization that examines the fundamental properties of biofuels in pure form and in combination with petroleum-based fuels.
- Research in systems management that examines the systems that compose the biofuels industry that include feedstock production and harvesting, feedstock transportation, fuel production, transportation of finished products, and distribution to end-users.

In order to use these biofuels, additional research and development is required in the fields of thermochemical conversion, co-firing, direct combustion, thermal gasification, anaerobic fermentation gas production, modular systems, and feedstocks.[34] Investigation into these areas could enable the U. S. to use bioenergy sources to supply the majority of its energy requirements.

Conclusions and Recommendations

Biomanufacturing is an advanced manufacturing technology that involves the use of chemical, physical, and biological processes performed by living cells for use in other applications. Biomanufacturing enables the manufacture of products with unique characteristics and has significant potential in the manufacture of several products such as polymers, energy sources, chemicals, food products, pharmaceuticals, and biodegradation (remediation) of hazardous materials. Biomanufacturing encompasses technologies such as:

- **Biotechnology**
- **Bioengineering**
- **Biodetection**
- **Biomaterials**
- **Biocomputation**

- **Bioenergy**

Perhaps the most unique and advantageous aspect of biomanufacturing is the excellent control that may be afforded during fabrication. In particular, sequence-by-sequence building of polymeric materials may be possible. Biological species can be used to synthesize polymers of more uniform chain lengths or chain branching than those produced by conventional synthesis techniques. For example, biomolecules could be used in place of metallocene catalysts in the production of commodity polymers (e.g. polyethylene and polypropylene), resulting in improved performance at a reduced cost and energy consumption. Additionally, biosynthesis could be used to produce specialty copolymers that are not available through traditional synthesis methods. These applications are of particular interest to SNL as we strive to understand polymers and nanoparticles in terms of their thermal, mechanical, optical, and electrical properties for use in nuclear weapons, satellites, and homeland defense applications.

Biomineralization, a bioprocess that involves the controlled agglomeration of atoms or molecules, is also of interest as it enables the fabrication of unique fillers and nanoparticles. This process can produce organized structures more efficiently than current technology that uses self-assembled structures. The organized structures can be used in templating that can be used to produce clear, high-strength composites and high-refractive-index materials for solid-state-lighting applications.

Other biomanufacturing areas of interest include sensors, encryption tools, and adhesives. It may be possible to utilize this technology to manufacture sensors that offer superior recognition of chemical and biological agents. Currently, it is possible to manufacture sensors that are able to detect only one or a few agents. However, development of the appropriate bioprocessing techniques will enable manufacture of sensors that are able to detect all materials of interest at once. This is of tremendous interest in detecting and neutralizing potential terrorist attacks using these agents. Additionally, it may be possible to use biosequencing to provide encryption and subsequent decoding of complex, sensitive data.

Biomanufacturing has the potential to be one of the defining technologies in the upcoming century. Research, development, and applications in the fields of biotechnology, bioengineering, biodetection, biomaterials, biocomputation and bioenergy will have dramatic impact on both the products we are able to create, and the ways in which we create them. Sandia National Laboratories has the expertise to contribute to any one of these fields.

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