

# Boundless Biotech

## New technology fostered the biotechnology revolution.

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Although many people think of the biotechnology revolution as something that has occurred only within the past 30 years, its roots extend back several generations. Many of the tools that were developed for the birth of the nuclear age became the foundation of the biotech age, revolutionizing fields as diverse as medicine, agriculture, and the environment.

### Genetic Signposts

Over a century of research into nucleic acids and genetics preceded the famous elucidation of the structure of DNA by James Watson and Francis Crick in 1953. But it was not until 1956 that Elliot Volkin and colleague Lazarus Astrachan at the labs that eventually became the Oak Ridge National Laboratory found evidence of messenger RNA. They fed phosphorus-32 to a bacterial culture and infected the culture with a DNA-containing virus and showed that RNA isolated from the culture appeared more like viral and less like bacterial RNA. This suggested that during the viral replication, viral protein production moved through an RNA intermediate.

At the University of Cambridge, Watson was similarly pursuing messenger RNA, and in 1960, he met up with Walter Gilbert. Gilbert focused his efforts on elucidating the mechanisms behind protein synthesis and DNA replication, and in 1980, he was awarded the Nobel Prize in Chemistry for his work with Allan Maxam on DNA sequencing. The Maxam-Gilbert method, which selectively chemically modified the bases, was first published in 1977 but continues to be a critical technique for the analysis of DNA sequences bound by proteins or other molecules.

Simultaneously, Cambridge biochemist Frederick Sanger developed a nucleic acid sequencing method that incorporated dideoxy nucleotides into a nascent DNA chain being synthesized on a template strand. Dideoxy incorporation terminates chain elongation. By radiolabeling the molecule and using one specific dideoxy nucleotide per reaction (ddA, ddC, ddG, or ddT), one

can run the reactions on a gel and generate a sequence ladder. In 1977, Sanger published the sequence of the first completed genome, that of the bacterial virus  $\phi$ X174. In 1980, for the development of the dideoxy sequencing method, Sanger was awarded his second Nobel Prize in Chemistry.

In 1986, Lloyd Smith, Mike Hunkapiller, and colleagues at Applied Biosystems replaced the radiolabels with fluorescent labels and introduced the first automated DNA sequencer to the market. Over the years, company engineers modified the process further, and in 1995, they replaced the traditional slab gel electrophoresis with capillary electrophoresis, setting the stage for production-scale sequencing and the Human Genome Project.

### Synthesizing Life

Although the first published account of the synthesis of a dinucleotide was performed by A. M. Michaelson and A. R. Todd in 1955, it was not until the late 1950s that University of Chicago professor Gobind Khorana introduced the phosphodiester method, which became the template of future synthetic methods.

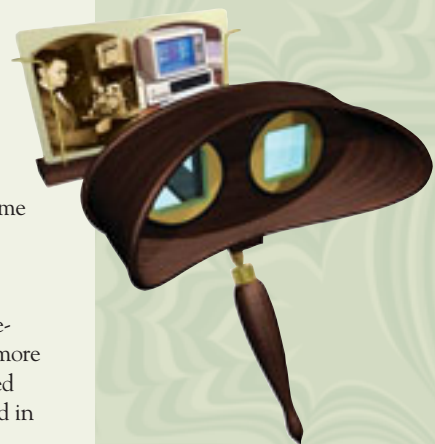
In 1965, Robert Letsinger, a visiting professor from Northwestern University, was able to build simple di- and trinucleotides on solid supports, and by 1969, he had worked out the conditions for a phosphotriester synthesis method that was significantly faster than Khorana's method. The new scheme formed the basis of the first automated DNA synthesizers, which were introduced by Vega Biotechnologies and Biosearch in the 1970s.

Later, Letsinger introduced the phosphotriester synthetic method, which was much more efficient and also opened the door to modified oligonucleotide backbones akin to that found in the recently developed peptide nucleic acids. Biologics, a company that hired numerous



Top: Frederick Sanger, *Luminaries of the Chemical Sciences*, 2002

Center: JEOL Nucleic Acid Analyzer ad, *Analytical Chemistry*, 1966



Letsinger students, and Vega both introduced automated synthesizers based on this technology. But the final step in the evolution of sequencing methods was developed by former Letsinger student Marvin Caruthers.

Caruthers's technique replaced a chloride-leaving group in the phosphite-triester method for an amine—dramatically increasing the shelf life of synthetic intermediates, allowing researchers to prepare them well in advance. Caruthers teamed with Leroy Hood, then professor at CalTech, and the two worked with Hunkapiller at Applied Biosystems to develop the first automated DNA synthesizer using this new technology, which was introduced in 1983.

Several other companies quickly entered the DNA synthesizer fray, such as PerSeptive Biosystems (which had purchased Biosearch), and this process continues now with companies such as TriLink Technologies looking for ways to increase reaction yields and introduce new chemical modifications for use in therapeutics, diagnostics, and high-throughput screening.

#### Gene Genies

In the late 1980s, Stephen Fodor and colleagues at the Affymax Research Institute realized that they could combine semiconductor manufacturing techniques with combinatorial chemistry methods to incorporate short oligonucleotides onto glass slides. In principle, these DNA fragments could then be used to identify the sequences of specific target DNA molecules. In 1990, as this work was progressing at a newly formed subsidiary, Affymetrix, UC–San Francisco professor Ron Davis and newly appointed Stanford University professor Mark Schena were talking about the company's efforts.

"After seeing some unpublished Affymetrix data, the idea of using microarrays for gene expression studies hit me with the force of a thunderbolt," recalls Schena in the preface to his book *Microarray Data Analysis*. "Instead of using short oligonucleotide arrays for sequencing by hybridization, it seemed much more powerful to use microarrays of long oligonucleotides for gene expression analysis."

Schena worked with the Affymetrix group to establish many of the technical parameters, and with other Stanford colleagues, he presented the first gene expression data in the summer of 1995. Months later, in a collaborative effort with Palo Alto-based Synteni, Schena's group presented the first microarray data using human sequences.

Microarrays, whether formatted on the ever-popular glass slide as provided by companies such as ArrayIt (founded in 1996) and Genomic Solutions (1997) or in multiwell dishes as produced by High Throughput Genomics (1997), continue to provide researchers with a revolutionary analysis tool and clinicians with a method to determine the diagnosis and prognosis of disease.

One of the challenges of studying nucleic acids is that they are typically found in low abundance, and at least with humans, it is difficult for the researcher to simply use large sample quantities to offset this problem. In April 1983, Kary Mullis—then a Cetus Corp. employee—realized that if he used deoxynucleotides and two primers that hybridized at opposite ends of the fragment of interest, he could amplify the region between the primers, relying on a series of hybridization, synthesis, and heating cycles. This was the birth of the polymerase chain reaction (PCR).

The standard DNA polymerase was quickly denatured during the heating cycle, requiring the introduction of a new polymerase isolated from a thermostable microbe, *Thermus aquaticus*. Likewise, PCR required three water baths at different temperatures, making the process very labor intensive. To solve this problem, Cetus Instrument Systems developed the thermal cycler, an aluminum block that could be heated and cooled as required. In a joint venture with PerkinElmer in 1987, Cetus introduced Mr. Cycle, the first fully automated PCR unit. In 1991, Chiron Corp. purchased Cetus and sold the rights to PCR to Roche Molecular Systems. Upon its merger with PerkinElmer in 1993, Applied Biosystems obtained the licensing rights to PCR from Roche and has since been instrumental in developing quantitative real-time PCR.

#### Probing Proteins

In the 1950s, researchers were also trying to develop analytical techniques for proteins similar to those being designed for nucleic acids. Working at the University of Cambridge, Frederick Sanger used fluorodinitrobenzene—later known as Sanger's reagent—to cleave amino-terminal residues from the polypeptide chains that comprise insulin, separating the residues via paper chromatography and ion-exchange chromatography. Pehr Edman at the University of Lund used phenylisothiocyanate as a cleavage agent. The Edman degradation method soon replaced Sanger's method and formed the basis of most commercial automated sequencing systems, such as those introduced by Applied Biosystems in 1981 and Agilent Technologies in 1992.

By the late 1950s, William Stein and Stanford Moore, researchers at the Rockefeller Institute for Medical Research, succeeded in automating the process, speeding it up dramatically. They used two columns containing Amberlite IR-120 resin and relied on the introduction of the ninhydrin



#### PARTICLE PARTICULARS

In the mid-1930s, the Coulter brothers, specialists in electrical engineering, founded Coulter Electronics. They developed a concept known as the Coulter Principle, an automated electronic method for counting and measuring the size of microscopic particles. Such knowledge is critical to a variety of fields, including production stream quality control, environmental analysis for pollutants, and medical diagnostics. Initially developed to allow researchers to study red blood cells, the Coulter Counter became one of the fundamental tools for analyzing all cells and led to the development of other technologies such as flow cytometry and particle sorters by companies such as Union Biometrica (1998). The Coulters then extended their efforts beyond electronics, making advances in the production of monoclonal antibodies. In October 1997, Coulter Electronics was acquired by Beckman Instruments, and a year later, the combined company was renamed Beckman Coulter.

**Above:** Model A Coulter Counter, 1953, *Pharmaceutical Century*, 2000

reagent to produce a color reaction detectable by a photometric cell. Their method serves as the basis of automated amino acid analysis to this day. Although numerous precolumn analysis systems exist, including those developed by Waters and Applied Biosystems, according to John C. Anders of AAI International, “Since the mid-1980s, a single ion-exchange system, the Beckman 6300/7300, has dominated postcolumn biopharmaceutical amino acid analysis.”

### Peptide Synthesis

The basic chemistry needed to attach amino acids to a developing polypeptide chain was developed in the late 1800s by Emil Fischer, but the technology was impractical for most syntheses involving more than 10 residues. To address this shortcoming, Rockefeller’s Bruce Merrifield applied a solid-phase approach to the problem, attaching the initial amino acid to a polymer bead and performing subsequent residue attachments on this template, using filtration to purify the product at each step. For this work and its subsequent application to the burgeoning field of combinatorial chemistry, Merrifield was honored with the 1984 Nobel Prize in Chemistry. One of Merrifield’s students, Steve Kent, continued to optimize the synthesis chemistry, and with researchers at Applied Biosystems, introduced an automated reagent-synthesis instrument to the market in 1984.

Garland Marshall, a student who had apprenticed in Merrifield’s lab as an “overnight troubleshooter” for the first automated peptide synthesizer, became an early proponent of computational approaches to protein conformation. Upon taking a position at Washington University in 1967, Marshall began work with his colleague Charles Molnar. Molnar had helped design and construct the first lab computer at the Massachusetts Institute of Technology, called the LINC, a predecessor to the PDP8 and Digital Equipment Co. The two developed CHEMAST, a molecular modeling program, and in 1979, after recruiting other researchers into their project, they founded the modeling company Tripos.

Over the years, Marshall and his team continued to develop modeling programs such as SYBYL and VALIDATE, turning Tripos into a market leader in molecular modeling, alongside such companies as Accelrys (2001) and Chemical Computing Group (1996).

### Putting Peptides into Play

In the 1950s, Rosalyn Sussman Yalow and Solomon Berson conjugated radiolabeled iodine to bovine insulin and introduced the compound to

healthy and diabetic individuals to monitor its rate of clearance. As a result of this research, in 1959, Yalow and Berson published their description of the radioimmunoassay (RIA), for which Yalow won the Nobel Prize in Physiology or Medicine in 1977. They never patented the RIA technology, and numerous companies quickly adapted it into diagnostic assays.

The use of radiolabel meant that RIA reactions could be dangerous and often had to be performed quickly to ensure a strong signal. Peter Perlmann and Eva Engvall, working at the University of Stockholm, replaced the radiolabel with an enzyme—horseradish peroxidase or alkaline phosphatase—that could be detected

through a variety of methods, most often colorimetric. In the process, they gave birth to the enzyme-linked immunosorbent assay, or ELISA (also known as the enzyme immunoassay, or EIA), first described in 1972.

Recent technical developments in EIA by many companies have taken detection levels down to the attomole, almost single-molecule detection.

In 1975, Bayer Diagnostics, through its Ciba Corning subsidiary, developed the first immunoassay using glass beads as a solid-phase support and extended this technology eight years later when it incorporated magnetic bead separation methodology. Likewise, in 1990, it introduced a fully automated chemiluminescence immunoassay system.

Over the years, EIA has also formed the basis of Western blotting technology, with companies such as Bio-Rad, Pierce Biotechnology, and BD Biosciences Pharmingen taking the lead. In this method, proteins are first separated by gel electrophoresis—a technology that was first developed by Arne Tiselius in 1926. They are then transferred from the gels to membranes for antibody-based detection.

More recently, companies such as NextGen Sciences, Affibody AB, and Cambridge Antibody Technology are using antibody-based methods to develop protein arrays. Similarly, the proteome is becoming more accessible through the efforts of companies such as Ciphergen Systems, which coordinates mass spectrometry with protein-binding surfaces to identify proteome constituents, and LumiCyte, which uses a bead-based approach.

Scientists, engineers, and clinicians continue to push for even greater throughputs and lower detection limits for studying biological systems. This means that the need for newer and more sophisticated instruments and tools will continue to increase, maintaining biotechnology demand as a key market force for growth among the instrument industry and its suppliers.



Center: Rosalyn Yalow in lab, *Luminaries of the Chemical Sciences*, 2002

### Systems Biology

Whereas the 20th century can best be described as a reductionist era in biology and medicine, the 21st century is likely to be remembered as a time when the minutiae of life were brought together by scientists to assemble a whole-organism view of the world in what they hope will be a grand unified theory of life. Key to this new approach is systems biology.

**Genomics.** Much as the identification of individual genes and their roles in biology were spawned from genetics experiments of previous centuries, defining and understanding the facets of systems biology are possible because of the achievements of genomics research in the past half-century. Starting with rudimentary methods that relied on bacterial and viral gene shuffling, the science of molecular biology expanded through the 1970s and 1980s into a burgeoning industry of enzyme- and plasmid-supply companies that offered scientists the reagents they needed to isolate DNA fragments for further analysis. Similarly, instrumentation and hardware companies developed various chromatographic and centrifugal tools that allowed researchers to separate the nucleic acid fragments from the cellular debris.

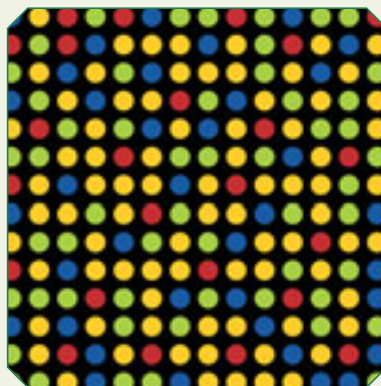
But as molecular biologists moved from bacteria and viruses into model eukaryotic systems,

such as fly, mouse, and human, they found that the absolute quantities of DNA were too small. With the advent of automated thermal cyclers and thermostable enzymes, scientists could amplify their DNA samples using the polymerase chain reaction. Likewise, improvements in capillary elec-

trophoresis and nucleic acid sequencing methodologies led to the development of automated sequencers.

**Transcriptomics.** Of course, genes are merely the templates upon which life is based, and understanding how these templates define a living system has been one of the greatest challenges to scientific understanding. Researchers have developed a panoply of tools and methods to identify where, when, and how genes are expressed in different tissues and organisms. In the process,

these scientists have created the field of transcriptomics. Technical developments critical to this endeavor include the introduction of automated oligonucleotide synthesizers, microarrays, and in situ hybridization technologies using fluorescently or radioactively labeled probes.



**Proteomics.** Where genes provide a map, proteins provide the method for reaching your metabolic destination. From the early days of protein biochemistry and the development of amino acid sequencers and peptide synthesizers to the more recent efforts in proteomics with multidimensional gels and chromatographs, MS, and protein chips, scientists are exploring the machinery of

the cell. In the process, they are identifying new signaling pathways and novel targets for drug discovery.

**Metabolomics.** One of the major goals of these technologies is to identify and characterize a series of markers that allow researchers and clinicians to understand and predict diseases. Key to this analysis, however, is not just proteins but also metabolites that are produced by the myriad cellular enzymes and pathways. Advances in high-sensitivity NMR and in the multitude of hyphenated MS techniques have allowed scientists to peer into the minutest metabolic changes in a cell or organism. Likewise, developments in the application of GC and capillary electrophoresis have opened doors in our understanding of cell activity.

**Informatics.** Each of these fields, however, has generated a flood of data that is only now truly being tapped using informatics tools. Software companies, both large and small, have made it possible for researchers to store and mine large silos of experimental data, while modeling specialists create the tools to put a molecular face on the metabolic players. Meanwhile, many early participants in the bioinformatics revolution are moving into the drug discovery arena, plying their computational trade for themselves and partner companies.

**Cyomics.** Ultimately, though, events that happen in vitro do not always occur in vivo, and thus cell biology or cytomics remains relevant to modern exploration. New developments in confocal microscopy and flow cytometry allow researchers to study the dynamic interplay of cellular metabolism and genetics. Likewise, advances in high-throughput cell assays and model organisms, such as mouse, fly, and zebrafish, are playing a greater role in the screening of new therapeutics for efficacy and tolerance.

As these and other fields mingle into the whole, the systems biology approach will develop, and the 21st-century view of life will continue to change. ♦



Center: Artist's rendering of microarray, *Modern Drug Discovery*, 2003

Left: JEOL Amino Acid Analyzer ad, *Analytical Chemistry*, 1969