Important Concepts of Biochemistry, Biotechnology and Chemical Engineering

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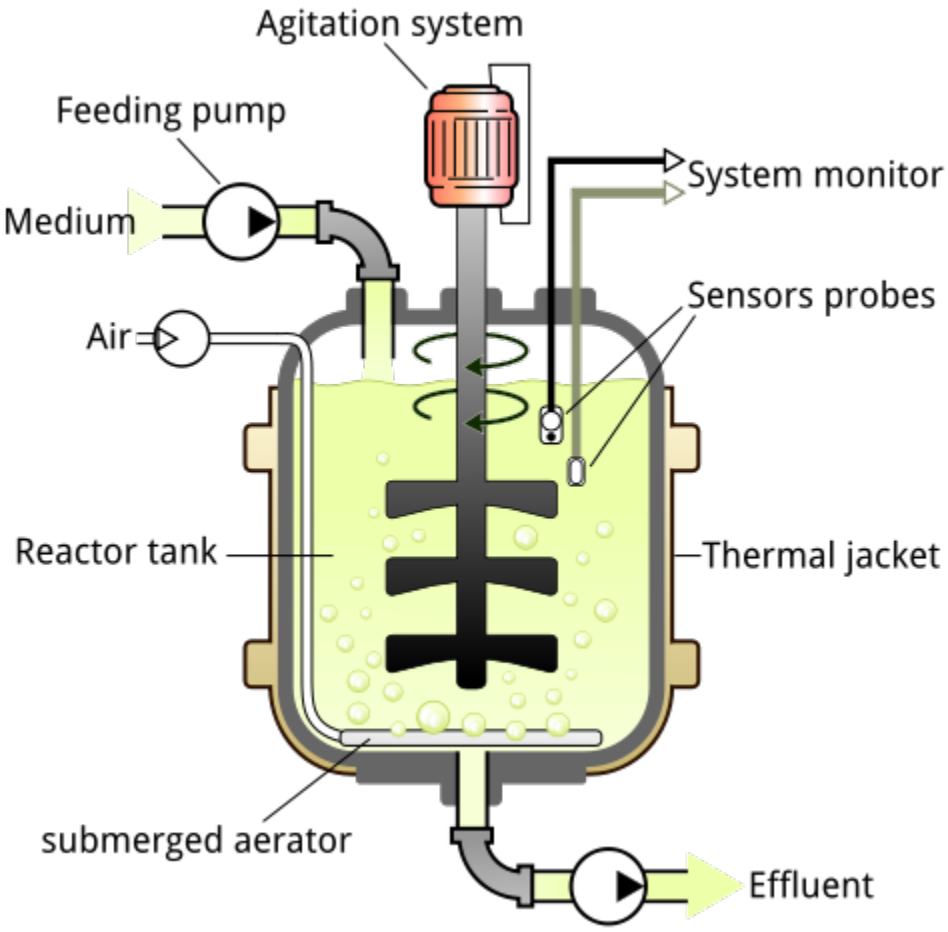


General structure of batch type bioreactor

A **bioreactor** may refer to any manufactured or engineered device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel.

A bioreactor may also refer to a device or system meant to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical engineering.

On the basis of **mode of operation**, a bioreactor may be classified as batch, fed batch or continuous (e.g. a continuous stirred-tank reactor model). An example of a continuous bioreactor is the chemostat.

Organisms growing in bioreactors may be suspended or immobilized. A simple method, where cells are immobilized, is a Petri dish with agar gel. Large scale immobilized cell bioreactors are:

- moving media, also known as Moving Bed Biofilm Reactor (MBBR)
- packed bed
- fibrous bed
- membrane

Bioreactor design

A closed bioreactor used in cellulosic ethanol research

Bioreactor design is a relatively complex engineering task, which is studied in the discipline of biochemical engineering. Under optimum conditions, the microorganisms or cells are able to perform their desired function with a 100 percent rate of success. The bioreactor's environmental conditions like gas (i.e., air, oxygen, nitrogen, carbon dioxide) flow rates, temperature, pH and dissolved oxygen levels, and agitation speed/circulation rate need to be closely monitored and controlled.

Most industrial bioreactor manufacturers use vessels, sensors and a control system networked together.

Fouling can harm the overall sterility and efficiency of the bioreactor, especially the heat exchangers. To avoid it, the bioreactor must be easily cleaned and as smooth as possible (therefore the round shape).

A heat exchanger is needed to maintain the bioprocess at a constant temperature. Biological fermentation is a major source of heat, therefore in most cases bioreactors need refrigeration. They can be refrigerated with an external jacket or, for very large vessels, with internal coils.

In an aerobic process, optimal oxygen transfer is perhaps the most difficult task to accomplish. Oxygen is poorly soluble in water—even less in fermentation broths—and is relatively scarce in air (20.95%). Oxygen transfer is usually helped by agitation, which is also needed to mix nutrients and to keep the fermentation homogeneous. There are, however, limits to the speed of agitation, due both to high power consumption (which is proportional to the cube of the speed of the electric motor) and to the damage to organisms caused by excessive tip speed. In practice, bioreactors are often pressurized; this increases the solubility of oxygen in water.

Photobioreactor

Moss photobioreactor with *Physcomitrella patens*

A photobioreactor (PBR) is a bioreactor which incorporates some type of light source. Virtually any translucent container could be called a PBR, however the term is more commonly used to define a closed system, as opposed to an open tank or pond. Photobioreactors are used to grow small phototrophic organisms such as cyanobacteria, algae , or moss plants . These organisms use light through photosynthesis as their energy source and do not require sugars or lipids as energy source. Consequently, risk of contamination with other organisms like bacteria or fungi is lower in photobioreactors when compared to bioreactors for heterotroph organisms.

Sewage treatment

Bioreactors are also designed to treat sewage and wastewater. In the most efficient of these systems there is a supply of free-flowing, chemically inert media that acts as a receptacle for the bacteria that breaks down the raw sewage. Examples of these bioreactors often have separate, sequential tanks and a mechanical separator or cyclone to speed the division of water and biosolids. Aerators supply oxygen to the sewage and media further accelerating breakdown. Submersible mixers provide agitation in anoxic bioreactors to keep the solids in suspension and thereby ensure that the bacteria and the

organic materials "meet". In the process, the liquids Biochemical Oxygen Demand (BOD) is reduced sufficiently to render the contaminated water fit for reuse. The biosolids can be collected for further processing or dried and used as fertilizer. An extremely simple version of a sewage bioreactor is a septic tank whereby the sewage is left in situ, with or without additional media to house bacteria. In this instance, the biosludge itself is the primary host (activated sludge) for the bacteria. Septic systems are best suited where there is sufficient landmass and the system is not subject to flooding or overly saturated ground and where time and efficiency is not of an essence.

In bioreactors where the goal is to grow cells or tissues for experimental or therapeutic purposes, the design is significantly different from industrial bioreactors. Many cells and tissues, especially mammalian ones, must have a surface or other structural support in order to grow, and agitated environments are often destructive to these cell types and tissues. Higher organisms also need more complex growth media.

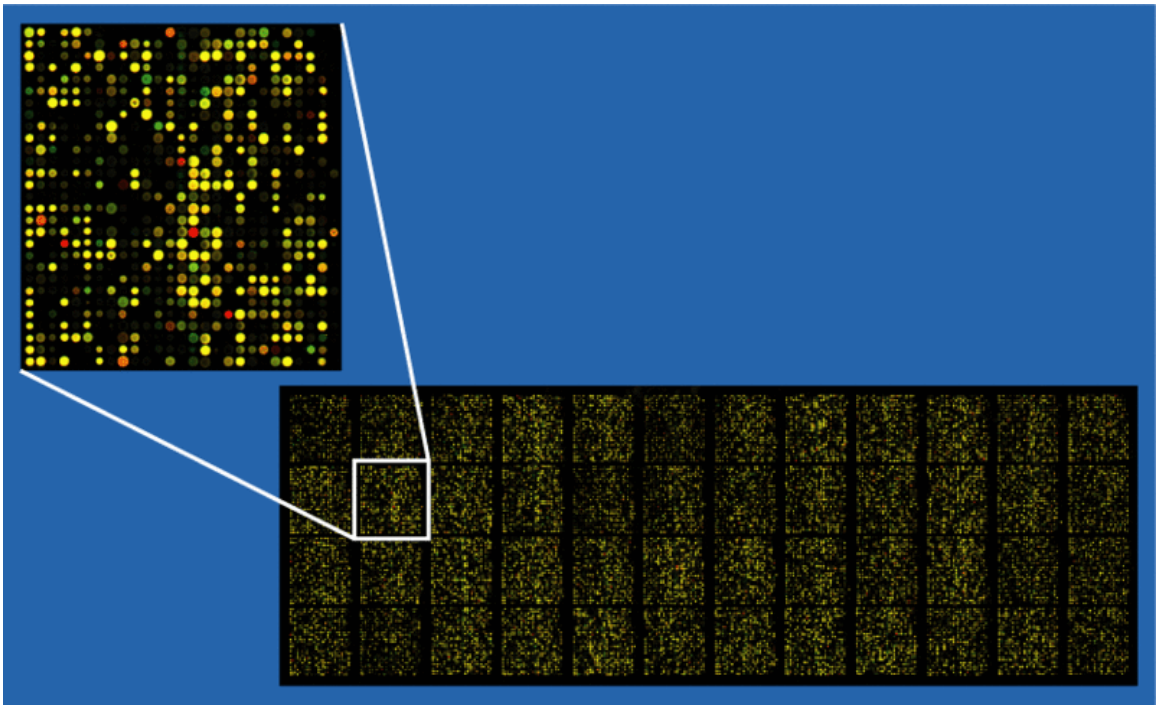
Because they are the engine that drives biological wastewater treatment, it is critical to closely monitor the quantity and quality of microorganisms in bioreactors. One method for this is via 2nd Generation ATP tests.

NASA tissue cloning bioreactor

NASA has developed a new type of bioreactor that artificially grows tissue in cell cultures. NASA's tissue bioreactor can grow heart tissue, skeletal tissue, ligaments, cancer tissue for study, and other types of tissue.

Chapter 2

DNA Microarray



Example of an approximately 40,000 probe spotted oligo microarray with enlarged inset to show detail.

A **DNA microarray** is a multiplex technology used in molecular biology. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles (10^{-12} moles) of a specific DNA sequence, known as *probes* (or *reporters*). These can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA sample (called *target*) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. Therefore arrays have dramatically accelerated many types of investigation.

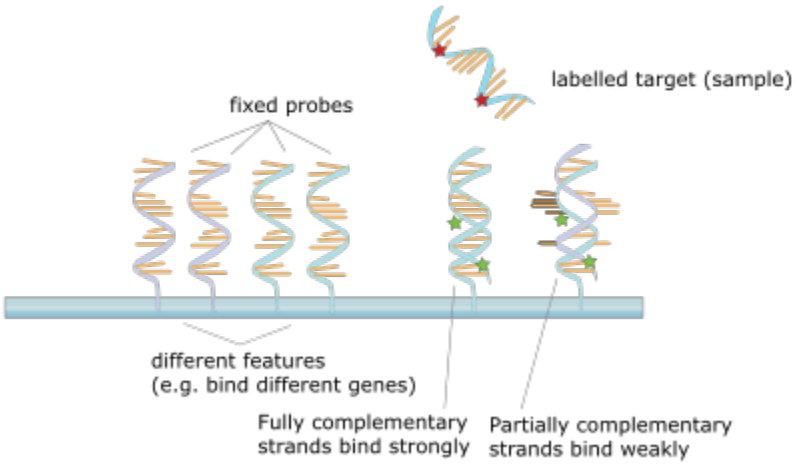
In standard microarrays, the probes are attached via surface engineering to a solid surface by a covalent bond to a chemical matrix (via epoxy-silane, amino-silane, lysine, polyacrylamide or others). The solid surface can be glass or a silicon chip, in which case they are colloquially known as an *Affy chip* when an Affymetrix chip is used. Other microarray platforms, such as Illumina, use microscopic beads, instead of the large solid support. DNA arrays are different from other types of microarray only in that they either measure DNA or use DNA as part of its detection system.

DNA microarrays can be used to measure changes in expression levels, to detect single nucleotide polymorphisms (SNPs), or to genotype or resequence mutant genomes. Microarrays also differ in fabrication, workings, accuracy, efficiency, and cost. Additional factors for microarray experiments are the experimental design and the methods of analyzing the data.

History

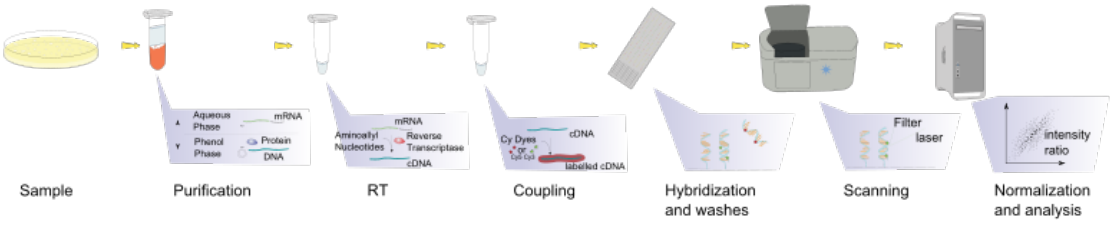
Microarray technology evolved from Southern blotting, where fragmented DNA is attached to a substrate and then probed with a known gene or fragment. Nucleic Acids Res. 1992 Apr 11;20(7):1679-84. Oligonucleotide hybridizations on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesised in situ. Maskos U, Southern EM. The first reported use of this approach was the analysis of 378 arrayed lysed bacterial colonies each harboring a different sequence which were assayed in multiple replicas for expression of the genes in multiple normal and tumor tissue (Augenlicht and Kobrin, Cancer Research, 42, 1088–1093, 1982). This was expanded to analysis of more than 4000 human sequences with computer driven scanning and image processing for quantitative analysis of the sequences in human colonic tumors and normal tissue (Augenlicht *et al.*, Cancer Research, 47, 6017-6021, 1987) and then to comparison of colonic tissues at different genetic risk (Augenlicht *et al.*, Proceedings National Academy of Sciences, USA, 88, 3286-3289, 1991). The use of a collection of distinct DNAs in arrays for expression profiling was also described in 1987, and the arrayed DNAs were used to identify genes whose expression is modulated by interferon. These early gene arrays were made by spotting cDNAs onto filter paper with a pin-spotting device. The use of miniaturized microarrays for gene expression profiling was first reported in 1995, and a complete eukaryotic genome (*Saccharomyces cerevisiae*) on a microarray was published in 1997.

Principle



hybridization of the target to the probe

The core principle behind microarrays is hybridization between two DNA strands, the property of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs. A high number of complementary base pairs in a nucleotide sequence means tighter non-covalent bonding between the two strands. After washing off of non-specific bonding sequences, only strongly paired strands will remain hybridized. So fluorescently labeled target sequences that bind to a probe sequence generate a signal that depends on the strength of the hybridization determined by the number of paired bases, the hybridization conditions (such as temperature), and washing after hybridization. Total strength of the signal, from a spot (feature), depends upon the amount of target sample binding to the probes present on that spot. Microarrays use relative quantization in which the intensity of a feature is compared to the intensity of the same feature under a different condition, and the identity of the feature is known by its position. An alternative to microarrays is serial analysis of gene expression, where the transcriptome is sequenced allowing an absolute measurement.



The step required in a microarray experiment

Uses and types

Two Affymetrix chips

Many types of array exist and the broadest distinction is whether they are spatially arranged on a surface or on coded beads:

- The traditional solid-phase array is a collection of orderly microscopic "spots", called features, each with a specific probe attached to a solid surface, such as glass, plastic or silicon biochip (commonly known as a *genome chip*, *DNA chip* or *gene array*). Thousands of them can be placed in known locations on a single DNA microarray.
- The alternative bead array is a collection of microscopic polystyrene beads, each with a specific probe and a ratio of two or more dyes, which do not interfere with the fluorescent dyes used on the target sequence.

DNA microarrays can be used to detect DNA (as in comparative genomic hybridization), or detect RNA (most commonly as cDNA after reverse transcription) that may or may not

be translated into proteins. The process of measuring gene expression via cDNA is called expression analysis or expression profiling.
Applications include:

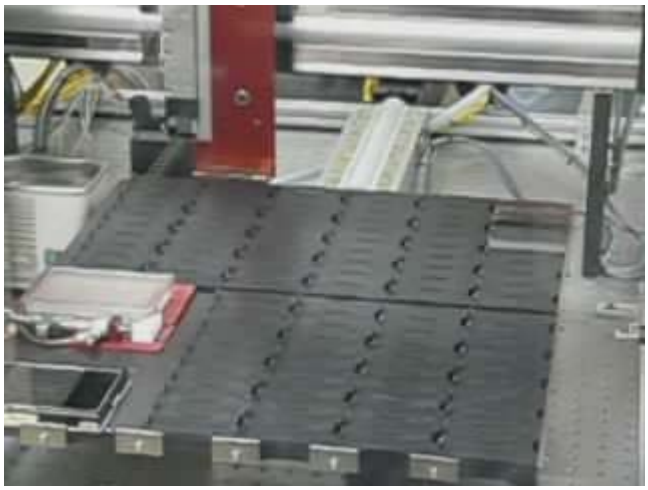
Application or technology	Synopsis
Gene expression profiling	In an mRNA or gene expression profiling experiment the expression levels of thousands of genes are simultaneously monitored to study the effects of certain treatments, diseases, and developmental stages on gene expression. For example, microarray-based gene expression profiling can be used to identify genes whose expression is changed in response to pathogens or other organisms by comparing gene expression in infected to that in uninfected cells or tissues.
Comparative genomic hybridization	Assessing genome content in different cells or closely related organisms.
GeneID	Small microarrays to check IDs of organisms in food and feed (like GMO), mycoplasms in cell culture, or pathogens for disease detection, mostly combining PCR and microarray technology.
Chromatin immunoprecipitation on Chip	DNA sequences bound to a particular protein can be isolated by immunoprecipitating that protein (ChIP), these fragments can be then hybridized to a microarray (such as a tiling array) allowing the determination of protein binding site occupancy throughout the genome. Example protein to immunoprecipitate are histone modifications (H3K27me3, H3K4me2, H3K9me3, etc.), Polycomb-group protein (PRC2:Suz12, PRC1:YY1) and trithorax-group protein (Ash1) to study the epigenetic landscape or RNA Polymerase II to study the transcription landscape.
DamID	Analogously to ChIP, genomic regions bound by a protein of interest can be isolated and used to probe a microarray to determine binding site occupancy. Unlike ChIP, DamID does not require antibodies but makes use of adenine methylation near the protein's binding sites to selectively amplify those regions, introduced by expressing minute amounts of protein of interest fused to bacterial DNA adenine methyltransferase.
SNP detection	Identifying single nucleotide polymorphism among alleles within or between populations. Several applications of microarrays make use of SNP detection, including Genotyping, forensic analysis, measuring predisposition to disease, identifying drug-candidates, evaluating germline mutations in individuals or somatic mutations in cancers,

	assessing loss of heterozygosity, or genetic linkage analysis.
Alternative splicing detection	An '*exon junction array* design uses probes specific to the expected or potential splice sites of predicted exons for a gene. It is of intermediate density, or coverage, to a typical gene expression array (with 1-3 probes per gene) and a genomic tiling array (with hundreds or thousands of probes per gene). It is used to assay the expression of alternative splice forms of a gene. Exon arrays have a different design, employing probes designed to detect each individual exon for known or predicted genes, and can be used for detecting different splicing isoforms.
Fusion genes microarray	A Fusion gene microarray can detect fusion transcripts, *e.g.* from cancer specimens. The principle behind this is building on the alternative splicing microarrays. The oligo design strategy enables combined measurements of chimeric transcript junctions with exon-wise measurements of individual fusion partners.
Tiling array	Genome tiling arrays consist of overlapping probes designed to densely represent a genomic region of interest, sometimes as large as an entire human chromosome. The purpose is to empirically detect expression of transcripts or alternatively splice forms which may not have been previously known or predicted.

Fabrication

Microarrays can be manufactured in different ways, depending on the number of probes under examination, costs, customization requirements, and the type of scientific question being asked. Arrays may have as few as 10 probes or up to 2.1 million micrometre-scale probes from commercial vendors.

Spotted vs. in situ synthesised arrays



A DNA microarray being printed by a robot at the University of Delaware

Microarrays can be fabricated using a variety of technologies, including printing with fine-pointed pins onto glass slides, photolithography using pre-made masks, photolithography using dynamic micromirror devices, ink-jet printing, or electrochemistry on microelectrode arrays.

In *spotted microarrays*, the probes are oligonucleotides, cDNA or small fragments of PCR products that correspond to mRNAs. The probes are synthesized prior to deposition on the array surface and are then "spotted" onto glass. A common approach utilizes an array of fine pins or needles controlled by a robotic arm that is dipped into wells containing DNA probes and then depositing each probe at designated locations on the array surface. The resulting "grid" of probes represents the nucleic acid profiles of the prepared probes and is ready to receive complementary cDNA or cRNA "targets" derived from experimental or clinical samples. This technique is used by research scientists around the world to produce "in-house" printed microarrays from their own labs. These arrays may be easily customized for each experiment, because researchers can choose the probes and printing locations on the arrays, synthesize the probes in their own lab (or collaborating facility), and spot the arrays. They can then generate their own labeled samples for hybridization, hybridize the samples to the array, and finally scan the arrays with their own equipment. This provides a relatively low-cost microarray that may be customized for each study, and avoids the costs of purchasing often more expensive commercial arrays that may represent vast numbers of genes that are not of interest to the investigator. Publications exist which indicate in-house spotted microarrays may not provide the same level of sensitivity compared to commercial oligonucleotide arrays, possibly owing to the small batch sizes and reduced printing efficiencies when compared to industrial manufactures of oligo arrays.

In *oligonucleotide microarrays*, the probes are short sequences designed to match parts of the sequence of known or predicted open reading frames. Although oligonucleotide

probes are often used in "spotted" microarrays, the term "oligonucleotide array" most often refers to a specific technique of manufacturing. Oligonucleotide arrays are produced by printing short oligonucleotide sequences designed to represent a single gene or family of gene splice-variants by synthesizing this sequence directly onto the array surface instead of depositing intact sequences. Sequences may be longer (60-mer probes such as the Agilent design) or shorter (25-mer probes produced by Affymetrix) depending on the desired purpose; longer probes are more specific to individual target genes, shorter probes may be spotted in higher density across the array and are cheaper to manufacture. One technique used to produce oligonucleotide arrays include photolithographic synthesis (Affymetrix) on a silica substrate where light and light-sensitive masking agents are used to "build" a sequence one nucleotide at a time across the entire array. Each applicable probe is selectively "unmasked" prior to bathing the array in a solution of a single nucleotide, then a masking reaction takes place and the next set of probes are unmasked in preparation for a different nucleotide exposure. After many repetitions, the sequences of every probe become fully constructed. More recently, Maskless Array Synthesis from NimbleGen Systems has combined flexibility with large numbers of probes.

Two-channel vs. one-channel detection

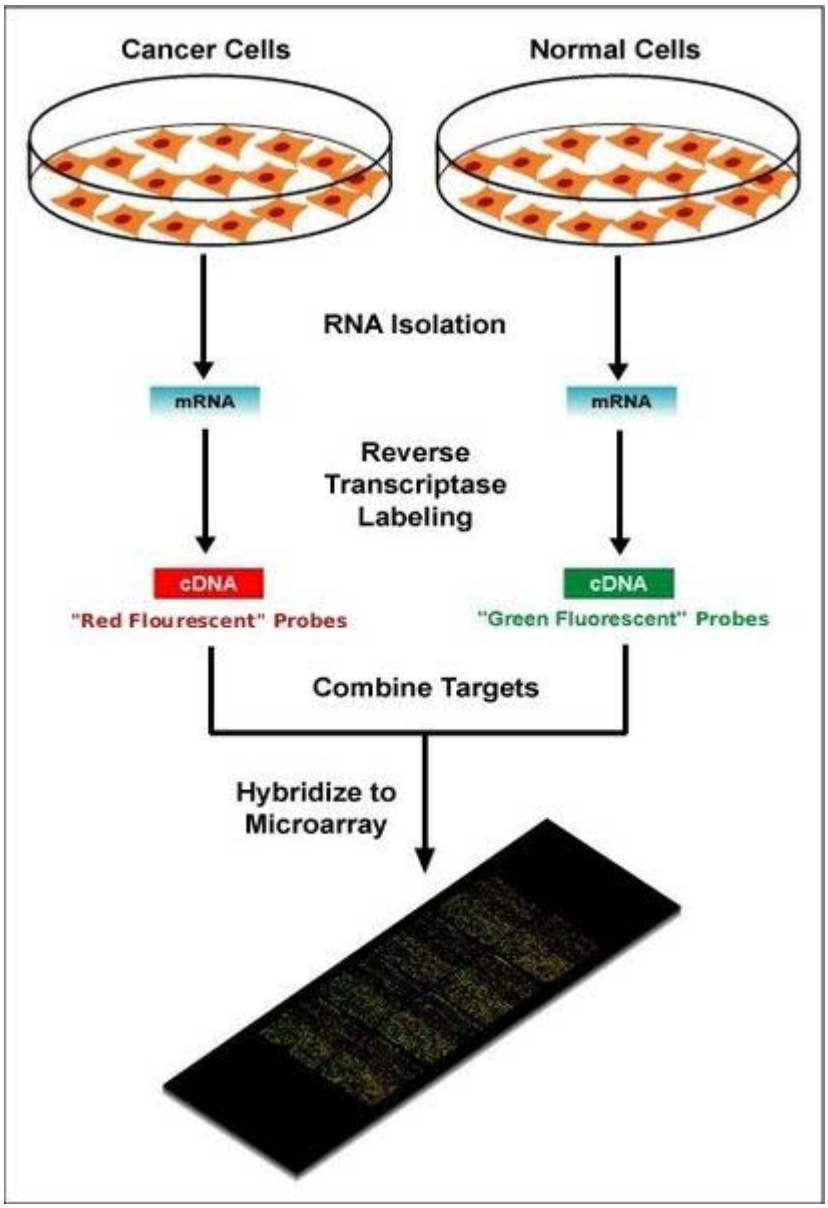


Diagram of typical dual-colour microarray experiment.

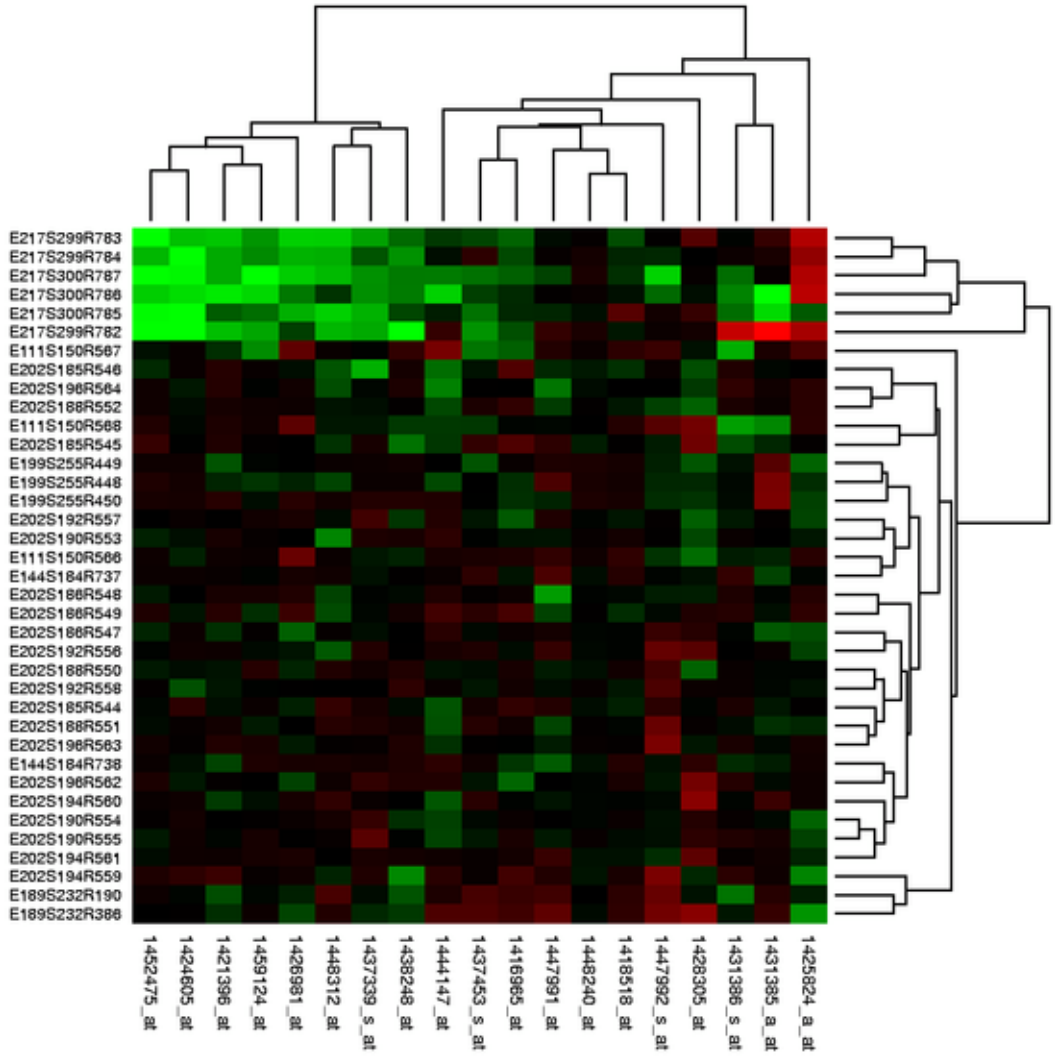
Two-color microarrays or *two-channel microarrays* are typically hybridized with cDNA prepared from two samples to be compared (e.g. diseased tissue versus healthy tissue) and that are labeled with two different fluorophores. Fluorescent dyes commonly used for cDNA labeling include Cy3, which has a fluorescence emission wavelength of 570 nm (corresponding to the green part of the light spectrum), and Cy5 with a fluorescence emission wavelength of 670 nm (corresponding to the red part of the light spectrum). The two Cy-labeled cDNA samples are mixed and hybridized to a single microarray that is then scanned in a microarray scanner to visualize fluorescence of the two fluorophores after excitation with a laser beam of a defined wavelength. Relative intensities of each

fluorophore may then be used in ratio-based analysis to identify up-regulated and down-regulated genes.

Oligonucleotide microarrays often carry control probes designed to hybridize with RNA spike-ins. The degree of hybridization between the spike-ins and the control probes is used to normalize the hybridization measurements for the target probes. Although absolute levels of gene expression may be determined in the two-color array in rare instances, the relative differences in expression among different spots within a sample and between samples is the preferred method of data analysis for the two-color system. Examples of providers for such microarrays includes Agilent with their Dual-Mode platform, Eppendorf with their DualChip platform for colorimetric Silverquant labeling, and TeleChem International with Arrayit.

In *single-channel microarrays* or *one-color microarrays*, the arrays provide intensity data for each probe or probe set indicating a relative level of hybridization with the labeled target. However, they do not truly indicate abundance levels of a gene but rather relative abundance when compared to other samples or conditions when processed in the same experiment. Each RNA molecule encounters protocol and batch-specific bias during amplification, labeling, and hybridization phases of the experiment making comparisons between genes for the same microarray uninformative. The comparison of two conditions for the same gene requires two separate single-dye hybridizations. Several popular single-channel systems are the Affymetrix "Gene Chip", Illumina "Bead Chip", Agilent single-channel arrays, the Applied Microarrays "CodeLink" arrays, and the Eppendorf "DualChip & Silverquant". One strength of the single-dye system lies in the fact that an aberrant sample cannot affect the raw data derived from other samples, because each array chip is exposed to only one sample (as opposed to a two-color system in which a single low-quality sample may drastically impinge on overall data precision even if the other sample was of high quality). Another benefit is that data are more easily compared to arrays from different experiments so long as batch effects have been accounted for. A drawback to the one-color system is that, when compared to the two-color system, twice as many microarrays are needed to compare samples within an experiment.

Microarrays and bioinformatics



Gene expression values from microarray experiments can be represented as heat maps to visualize the result of data analysis.

The advent of inexpensive microarray experiments created several specific bioinformatics challenges:

- the multiple levels of replication in experimental design (Experimental design)
- the number of platforms and independent groups and data format (Standardization)
- the treatment of the data (Statistical analysis)
- accuracy and precision (Relation between probe and gene)
- the sheer volume of data and the ability to share it (Data warehousing)

Experimental design

Due to the biological complexity of gene expression, the considerations of experimental design that are discussed in the expression profiling article are of critical importance if statistically and biologically valid conclusions are to be drawn from the data.

There are three main elements to consider when designing a microarray experiment. First, replication of the biological samples is essential for drawing conclusions from the experiment. Second, technical replicates (two RNA samples obtained from each experimental unit) help to ensure precision and allow for testing differences within treatment groups. The biological replicates include independent RNA extractions and technical replicates may be two aliquots of the same extraction. Third, spots of each cDNA clone or oligonucleotide are present as replicates (at least duplicates) on the microarray slide, to provide a measure of technical precision in each hybridization. It is critical that information about the sample preparation and handling is discussed, in order to help identify the independent units in the experiment and to avoid inflated estimates of statistical significance.

Standardization

Microarray data is difficult to exchange due to the lack of standardization in platform fabrication, assay protocols, and analysis methods. This presents an interoperability problem in bioinformatics. Various grass-roots open-source projects are trying to ease the exchange and analysis of data produced with non-proprietary chips:

- For example, the "Minimum Information About a Microarray Experiment" (MIAME) checklist helps define the level of detail that should exist and is being adopted by many journals as a requirement for the submission of papers incorporating microarray results. But MIAME does not describe the format for the information, so while many formats can support the MIAME requirements, as of 2007 no format permits verification of complete semantic compliance.
- The "MicroArray Quality Control (MAQC) Project" is being conducted by the US Food and Drug Administration (FDA) to develop standards and quality control metrics which will eventually allow the use of MicroArray data in drug discovery, clinical practice and regulatory decision-making.
- The MGED Society has developed standards for the representation of gene expression experiment results and relevant annotations.

Statistical analysis

Microarray data sets are commonly very large, and analytical precision is influenced by a number of variables. Statistical challenges include taking into account effects of background noise and appropriate normalization of the data. Normalization methods may be suited to specific platforms and, in the case of commercial platforms, the analysis may be proprietary. Algorithms that affect statistical analysis include:

- Image analysis: gridding, spot recognition of the scanned image (segmentation algorithm), removal or marking of poor-quality and low-intensity features (called *flagging*).
- Data processing: background subtraction (based on global or local background), determination of spot intensities and intensity ratios, visualisation of data, and log-transformation of ratios, global or local normalization of intensity ratios.
- Identification of statistically significant changes: t-test, ANOVA, Bayesian method Mann–Whitney test methods tailored to microarray data sets, which take into account multiple comparisons or cluster analysis. These methods assess statistical power based on the variation present in the data and the number of experimental replicates, and can help minimize Type I and type II errors in the analyses.
- Network-based methods: Statistical methods that take the underlying structure of gene networks into account, representing either associative or causative interactions or dependencies among gene products.

Microarray data may require further processing aimed at reducing the dimensionality of the data to aid comprehension and more focused analysis. Other methods permit analysis of data consisting of a low number of biological or technical replicates; for example, the Local Pooled Error (LPE) test pools standard deviations of genes with similar expression levels in an effort to compensate for insufficient replication.

Relation between probe and gene

The relation between a probe and the mRNA that it is expected to detect is not trivial. Some mRNAs may cross-hybridize probes in the array that are supposed to detect another mRNA. In addition, mRNAs may experience amplification bias that is sequence or molecule-specific. Thirdly, probes that are designed to detect the mRNA of a particular gene may be relying on genomic EST information that is incorrectly associated with that gene.

Data warehousing

Microarray data was found to be more useful when compared to other similar datasets. The sheer volume (in bytes), specialized formats (such as MIAME), and curation efforts associated with the datasets require specialized databases to store the data.

Chapter 3

Protein Microarray

A **protein microarray**, sometimes referred to as a **protein binding microarray**, provides a multiplex approach to identify protein–protein interactions, to identify the substrates of protein kinases, to identify transcription factor protein-activation, or to identify the targets of biologically active small molecules. The array is a piece of glass on which different molecules of protein or specific DNA binding sequences (as capture probes for the proteins) have been affixed at separate locations in an ordered manner thus forming a microscopic array. The most common protein microarray is the antibody microarray, where antibodies are spotted onto the protein chip and are used as *capture molecules* to detect proteins from cell lysate solutions.

Related microarray technologies also include DNA microarrays, cellular microarrays, antibody microarrays, tissue microarrays and chemical compound microarrays.

Applications

Protein microarrays (also **biochip**, **proteinchip**) are measurement devices used in biomedical applications to determine the presence and/or amount (referred to as relative quantitation) of proteins in biological samples, e.g. blood. They have the potential to be an important tool for proteomics research. Usually a multitude of different capture agents, most frequently monoclonal antibodies, are deposited on a chip surface (glass or silicon) in a miniature array. This format is often also referred to as a **microarray** (a more general term for chip based biological measurement devices).

Types of chips

There are several types of protein chips, the most common being glass slide chips and nano-well arrays.

Production of protein arrays

The production process depends on the type of protein chip.

Protein–Protein array: The proteins can be externally synthesised, purified and attached to the array. Alternatively they can be synthesised in-situ and directly attached to the array. The proteins can be synthesised through biosynthesis, cell-free DNA expression or chemical synthesis. In-situ synthesis is possible with the latter two. With cell-free DNA expression, proteins are attached to the support right after their production. Peptides chemically procured by solid phase peptide synthesis are already attached to the support. Selective deprotection is carried out through lithographic methods or by the so-called SPOT-synthesis.

DNA-Protein array: Double-stranded DNA (the exact binding sequence of the protein)is attached/ spotted on the array.

Artifacts to avoid

- 1) To avoid variability in results, use a very efficient lysis buffer and maintain consistent sample processing conditions;
- 2) Many antibodies don't work well as capture reagents, even if they do work well in western blotting and other denaturing conditions. Some antibodies often bind poorly to intact proteins in a cell extract;
- 3) Different proteins like different solution conditions, so if you do not see binding it doesn't mean that there is no binding between the two partners in physiological conditions;
- 4) Adjust the solute conditions to avoid non-specific association: change salt concentration, pH, add 1% alignate;
- 5) on the array's surface the conjugated protein should be in the right conformation (i.e., folded, NOT denatured), anchored by the same amino acid (in the same orientation), and be kept away from the surface by a linker to avoid steric hindrance.

Types of capture molecules

Capture molecules used are most commonly antibodies; however, antigens are used in applications where antibodies are detected in serum. More recently there has been a push towards other types of capture molecules which are more similar in their nature such as peptides or aptamers. Antibodies have several problems including the fact that there are no antibodies for most proteins and also problems with specificity in some commercial antibody preparations. Nevertheless, antibodies still represent the most well-characterized and effective protein capture agent for microarrays. Recently, nucleic acids, receptors, enzymes, and proteins have been spotted onto chips and used as capture molecules. This allows a vast variety of experiments to be conducted on protein–protein interactions, and all other protein binding substrates.

Detection methods

Although protein microarrays may use similar detection methods as DNA Microarrays, a problem is that protein concentrations in a biological sample may be many orders of magnitude different from that for mRNAs. Therefore, protein chip detection methods must have a much larger range of detection.

The preferred method of detection currently is fluorescence detection. The fluorescent detection method is compatible with standard microarray scanners, the spots on the resulting image can be quantified by commonly used microarray quantification software packages. However, some minor alterations to the analysis software may be needed. Other common detection methods include colorimetric techniques based on silver-precipitation, chemiluminescent and label free Surface Plasmon Resonance.

Chapter 4

Biosensor

A **biosensor** is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component.

It consists of 3 parts:

- the *sensitive biological element* (biological material (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.), a biologically derived material or biomimic) The sensitive elements can be created by biological engineering.
- the *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified;
- associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. This sometimes accounts for the most expensive part of the sensor device, however it is possible to generate a user friendly display that includes transducer and sensitive element.

A common example of a commercial biosensor is the blood glucose biosensor, which uses the enzyme glucose oxidase to break blood glucose down. In doing so it first oxidizes glucose and uses two electrons to reduce the FAD (a component of the enzyme) to FADH2. This in turn is oxidized by the electrode (accepting two electrons from the electrode) in a number of steps. The resulting current is a measure of the concentration of glucose. In this case, the electrode is the transducer and the enzyme is the biologically active component.

Recently, arrays of many different detector molecules have been applied in so called electronic nose devices, where the pattern of response from the detectors is used to fingerprint a substance.. In the Wasp Hound odor-detector, the mechanical element is a video camera and the biological element is five parasitic wasps who have been conditioned to swarm in response to the presence of a specific chemical. Current commercial electronic noses, however, do not use biological elements.

A canary in a cage, as used by miners to warn of gas, could be considered a biosensor. Many of today's biosensor applications are similar, in that they use organisms which respond to toxic substances at a much lower concentrations than humans can detect to warn of the presence of the toxin. Such devices can be used in environmental monitoring, trace gas detection and in water treatment facilities.

Principles of Detection

Photometric

Many optical biosensors based on the phenomenon of surface plasmon resonance are evanescent wave techniques. This utilises a property of gold and other materials; specifically that a thin layer of gold on a high refractive index glass surface can absorb laser light, producing electron waves (surface plasmons) on the gold surface. This occurs only at a specific angle and wavelength of incident light and is highly dependent on the surface of the gold, such that binding of a target analyte to a receptor on the gold surface produces a measurable signal.

Surface plasmon resonance sensors operate using a sensor chip consisting of a plastic cassette supporting a glass plate, one side of which is coated with a microscopic layer of gold. This side contacts the optical detection apparatus of the instrument. The opposite side is then contacted with a microfluidic flow system. The contact with the flow system creates channels across which reagents can be passed in solution. This side of the glass sensor chip can be modified in a number of ways, to allow easy attachment of molecules of interest. Normally it is coated in carboxymethyl dextran or similar compound.

Light of a fixed wavelength is reflected off the gold side of the chip at the angle of total internal reflection, and detected inside the instrument. This induces the evanescent wave to penetrate through the glass plate and some distance into the liquid flowing over the surface.

The refractive index at the flow side of the chip surface has a direct influence on the behaviour of the light reflected off the gold side. Binding to the flow side of the chip has an effect on the refractive index and in this way biological interactions can be measured to a high degree of sensitivity with some sort of energy.

Other evanescent wave biosensors have been commercialised using waveguides where the propagation constant through the waveguide is changed by the absorption of molecules to the waveguide surface. One such example, Dual Polarisation Interferometry uses a buried waveguide as a reference against which the change in propagation constant is measured. Other configurations such as the Mach-Zehnder have reference arms lithographically defined on a substrate. Higher levels of integration can be achieved using resonator geometries where the resonant frequency of a ring resonator changes when molecules are absorbed.

Other optical biosensors are mainly based on changes in absorbance or fluorescence of an appropriate indicator compound and do not need a total internal reflection geometry. For example, a fully operational prototype device detecting casein in milk has been fabricated. The device is based on detecting changes in absorption of a gold layer. A widely used research tool, the micro-array, can also be considered a biosensor.

Nanobiosensors use a immobilized bioreceptor probe that is selective for target analyte molecules. Nanomaterials are exquisitely sensitive chemical and biological sensors. Nanoscale materials demonstrate unique properties. Their large surface area to volume ratio can achieve rapid and low cost reactions, using a variety of designs.

Biological biosensors often incorporate a genetically modified form of a native protein or enzyme. The protein is configured to detect a specific analyte and the ensuing signal is read by a detection instrument such as a fluorometer or luminometer. An example of a recently developed biosensor is one for detecting cytosolic concentration of the analyte cAMP (cyclic adenosine monophosphate), a second messenger involved in cellular signaling triggered by ligands interacting with receptors on the cell membrane. Similar systems have been created to study cellular responses to native ligands or xenobiotics (toxins or small molecule inhibitors). Such "assays" are commonly used in drug discovery development by pharmaceutical and biotechnology companies. Most cAMP assays in current use require lysis of the cells prior to measurement of cAMP. A live-cell biosensor for cAMP can be used in non-lysed cells with the additional advantage of multiple reads to study the kinetics of receptor response.

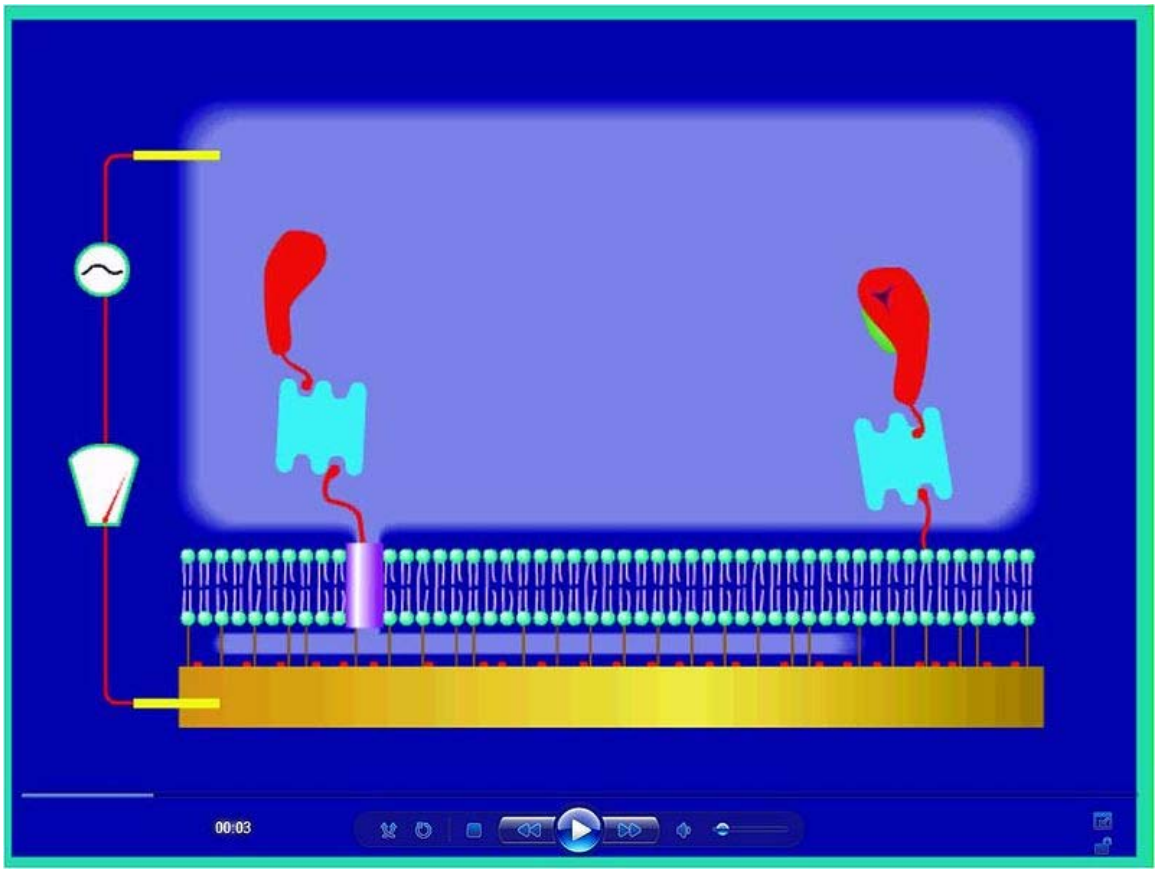
Electrochemical

Electrochemical biosensors are normally based on enzymatic catalysis of a reaction that produces or consumes electrons (such enzymes are rightly called redox enzymes). The sensor substrate usually contains three electrodes; a reference electrode, a working electrode and a sink electrode. An auxiliary electrode (also known as a counter electrode) may also be present as an ion source. The target analyte is involved in the reaction that takes place on the active electrode surface, and the ions produced create a potential which is subtracted from that of the reference electrode to give a signal. We can either measure the current (rate of flow of electrons is now proportional to the analyte concentration) at a fixed potential or the potential can be measured at zero current (this gives a logarithmic response). Note that potential of the working or active electrode is space charge sensitive and this is often used. Further, the label-free and direct electrical detection of small peptides and proteins is possible by their intrinsic charges using biofunctionalized ion-sensitive field-effect transistors.

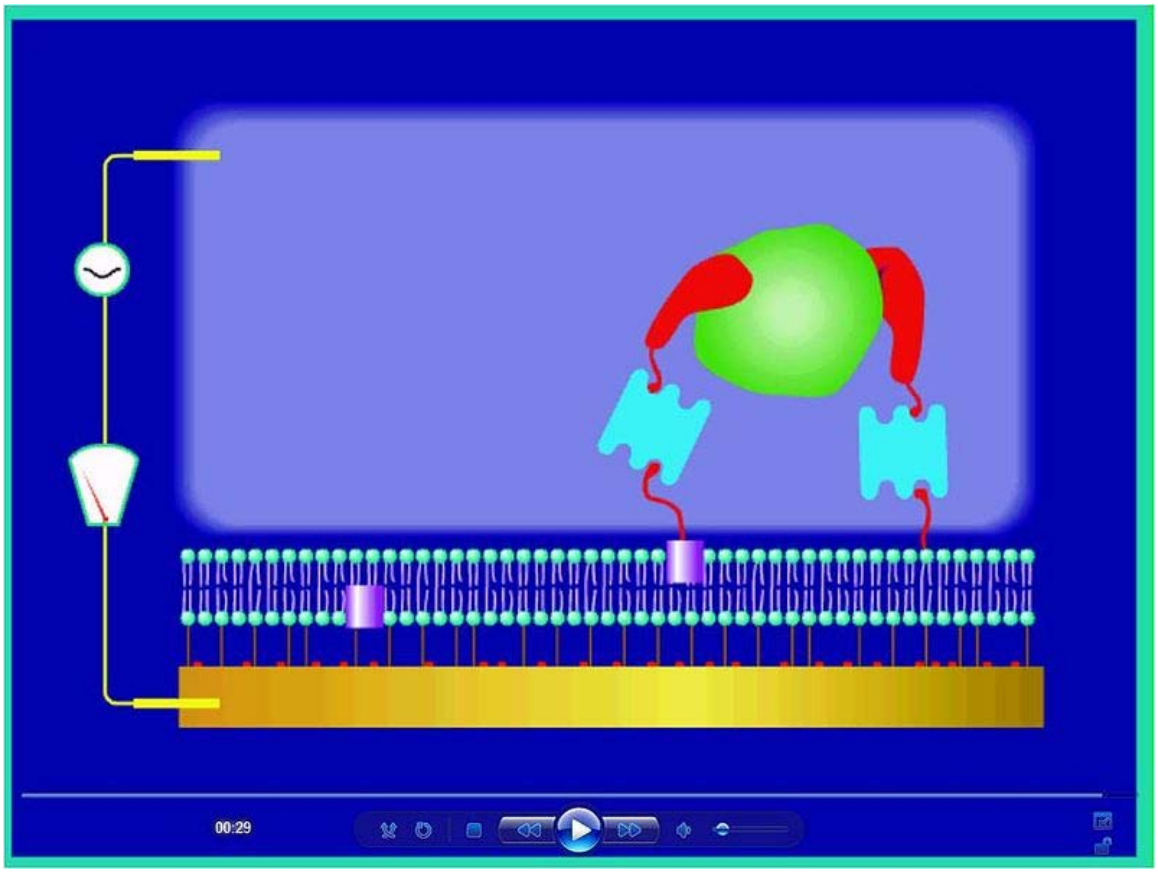
Another example, the potentiometric biosensor, works contrary to the current understanding of its ability. Such biosensors are screenprinted, conducting polymer coated, open circuit potential biosensors based on conjugated polymers immunoassays. They have only two electrodes and are extremely sensitive and robust. They enable the detection of analytes at levels previously only achievable by HPLC and LC/MS and without rigorous sample preparation. The signal is produced by electrochemical and

physical changes in the conducting polymer layer due to changes occurring at the surface of the sensor. Such changes can be attributed to ionic strength, pH, hydration and redox reactions, the latter due to the enzyme label turning over a substrate().

Ion Channel Switch



ICS - channel open



ICS - channel closed

The use of ion channels has been shown to offer highly sensitive detection of target biological molecules. By imbedding the ion channels in supported or tethered bilayer membranes (t-BLM) attached to a gold electrode, an electrical circuit is created. Capture molecules such as antibodies can be bound to the ion channel so that the binding of the target molecule controls the ion flow through the channel. This results in a measurable change in the electrical conduction which is proportional to the concentration of the target.

An Ion Channel Switch (ICS) biosensor can be created using gramicidin, a dimeric peptide channel, in a tethered bilayer membrane. One peptide of gramicidin, with attached antibody, is mobile and one is fixed. Breaking the dimer stops the ionic current through the membrane. The magnitude of the change in electrical signal is greatly increased by separating the membrane from the metal surface using a hydrophilic spacer.

Quantitative detection of an extensive class of target species, including proteins, bacteria, drug and toxins has been demonstrated using different membrane and capture configurations.

Others

Piezoelectric sensors utilise crystals which undergo an elastic deformation when an electrical potential is applied to them. An alternating potential (A.C.) produces a standing wave in the crystal at a characteristic frequency. This frequency is highly dependent on the elastic properties of the crystal, such that if a crystal is coated with a biological recognition element the binding of a (large) target analyte to a receptor will produce a change in the resonance frequency, which gives a binding signal. In a mode that uses surface acoustic waves (SAW), the sensitivity is greatly increased. This is a specialised application of the Quartz crystal microbalance as a biosensor.

Thermometric and magnetic based biosensors are rare.

Applications

There are many potential applications of biosensors of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based techniques in some situations. Some examples are given below:

- Glucose monitoring in diabetes patients ←**historical market driver**
- Other medical health related targets
- Environmental applications e.g. the detection of pesticides and river water contaminants
- Remote sensing of airborne bacteria e.g. in counter-bioterrorist activities
- Detection of pathogens
- Determining levels of toxic substances before and after bioremediation
- Detection and determining of organophosphate
- Routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay
- Determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey.
- Drug discovery and evaluation of biological activity of new compounds.
- Protein engineering in biosensors**.**
- Detection of toxic metabolites such as mycotoxins**.**

Glucose monitoring

Commercially available gluocose monitors rely on amperometric sensing of glucose by means of glucose oxidase, which oxidises glucose producing hydrogen peroxide which is detected by the electrode. To overcome the limitation of ameperometric sensors, a flurry of research is present into novel sensing methods, such as fluorescent glucose biosensors.

Biosensors in food analysis

There are several applications of biosensors in food analysis. In food industry optic coated with antibodies are commonly used to detect pathogens and food toxins. The light system in these biosensors has been fluorescence, since this type of optical measurement can greatly amplify the signal.

A range of immuno- and ligand-binding assays for the detection and measurement of small molecules such as water-soluble vitamins and chemical contaminants (drug residues) such as sulfonamides and Beta-agonists have been developed for use on SPR based sensor systems, often adapted from existing ELISA or other immunological assay. These are in widespread use across the food industry.

Surface Attachment of the biological elements

An important part in a biosensor is to attach the biological elements (small molecules/protein/cells) to the surface of the sensor (be it metal, polymer or glass). The simplest way is to functionalize the surface in order to coat it with the biological elements. This can be done by polylysine, aminosilane, epoxysilane or nitrocellulose in the case of silicon chips/silica glass. Subsequently the bound biological agent may be for example fixed by Layer by layer deposition of alternatively charged polymer coatings Alternatively three dimensional lattices (hydrogel/xerogel) can be used to chemically or physically entrap these (where by chemically entraped it is meant that the biological element is kept in place by a strong bond, while physically they are kept in place being unable to pass through the pores of the gel matrix). The most commonly used hydrogel is sol-gel, a glassy silica generated by polymerization of silicate monomers (added as tetra alkyl orthosilicates, such as TMOS or TEOS) in the presence of the biological elements (along with other stabilizing polymers, such as PEG) in the case of physical entrapment. Another group of hydrogels, which set under conditions suitable for cells or protein, are acrylate hydrogel, which polymerize upon radical initiation. One type of radical initiator is a peroxide radical, typically generated by combining a persulfate with TEMED (Polyacrylamide gel are also commonly commonly used for protein electrophoresis), alternatively light can be used in combination with a photoinitiator, such as DMPA (2,2-dimethoxy-2-phenylacetophenone). Smart materials that mimic the biological components of a sensor can also be classified as biosensors using only the active or catalytic site or analogous configurations of a biomolecule.

Chapter 5

Heat Transfer

Heat transfer is a discipline of thermal engineering that concerns the transfer of thermal energy from one physical system to another. Heat transfer is classified into various mechanisms, such as heat conduction, convection, thermal radiation, and phase-change transfer. Engineers also consider the transfer of mass of differing chemical species, either cold or hot, to achieve heat transfer. All forms of heat transfer may occur in some systems (for example, in transparent fluids like the Earth's atmosphere) at the same time.

Heat conduction, also called diffusion, is the direct microscopic exchange of kinetic energy of particles through the boundary between two systems. When an object is at a different temperature from another body or its surroundings, heat flows so that the body and the surroundings reach the same temperature at thermal equilibrium. Conduction happens in both fluids and solids.

Heat convection only occurs in fluids. It occurs when bulk flow of a fluid (gas or liquid) carries heat along with the flow of matter in the fluid. The flow of fluid may be forced by external processes, or sometimes (in gravitational fields) by buoyancy forces caused when thermal energy expands the fluid (for example in a fire plume), thus influencing its own transfer. The latter process is sometimes called "natural convection". All convective processes also move heat partly by diffusion, as well.

The final major form of heat transfer is by radiation, which occurs in any transparent medium (solid or fluid) but may also even occur across vacuum (as when the Sun heats the Earth). Heat transfer by thermal radiation is the transfer of energy by transmission of electromagnetic radiation described by black body theory.

Overview

Heat is defined in physics as the transfer of thermal energy across a well-defined boundary around a thermodynamic system. It is a characteristics of a process and is not statically contained in matter. In engineering contexts, however, the term *heat transfer* has acquired a specific usage, despite its literal redundancy of the characterization of transfer. In these contexts, *heat* is taken as synonymous to thermal energy. This usage has

its origin in the historical interpretation of heat as a fluid (*caloric*) that can be transferred by various causes, and that is also common in the language of laymen and everyday life.

Fundamental methods of heat transfer in engineering include conduction, convection, and radiation. Physical laws describe the behavior and characteristics of each of these methods. Real systems often exhibit a complicated combination of them. Heat transfer methods are used in numerous disciplines, such as automotive engineering, thermal management of electronic devices and systems, climate control, insulation, materials processing, and power plant engineering.

Various mathematical methods have been developed to solve or approximate the results of heat transfer in systems. Heat transfer is a path function (or process quantity), as opposed to a state quantity; therefore, the amount of heat transferred in a thermodynamic process that changes the state of a system depends on how that process occurs, not only the net difference between the initial and final states of the process. Heat flux is a quantitative, vectorial representation of the heat flow through a surface.

Heat transfer is typically studied as part of a general chemical engineering or mechanical engineering curriculum. Typically, thermodynamics is a prerequisite for heat transfer courses, as the laws of thermodynamics are essential to the mechanism of heat transfer. Other courses related to heat transfer include energy conversion, thermofluids, and mass transfer.

The transport equations for thermal energy (Fourier's law), mechanical momentum (Newton's law for fluids), and mass transfer (Fick's laws of diffusion) are similar and analogies among these three transport processes have been developed to facilitate prediction of conversion from any one to the others.

Mechanisms

The fundamental modes of heat transfer are:

Conduction or diffusion
: The transfer of energy between objects that are in physical contact

Convection
: The transfer of energy between an object and its environment, due to fluid motion

Radiation
: The transfer of energy to or from a body by means of the emission or absorption of electromagnetic radiation

Mass transfer
: The transfer of energy from one location to another as a side effect of physically moving an object containing that energy

Conduction

On a microscopic scale, heat conduction occurs as hot, rapidly moving or vibrating atoms and molecules interact with neighboring atoms and molecules, transferring some of their energy (heat) to these neighboring particles. In other words, heat is transferred by conduction when adjacent atoms vibrate against one another, or as electrons move from one atom to another. Conduction is the most significant means of heat transfer within a solid or between solid objects in thermal contact. Fluids—especially gases—are less conductive. Thermal contact conductance is the study of heat conduction between solid bodies in contact.

Steady state conduction is a form of conduction that happens when the temperature difference driving the conduction is constant, so that after an equilibration time, the spatial distribution of temperatures in the conducting object does not change any further. In steady state conduction, the amount of heat entering a section is equal to amount of heat coming out.

Transient conduction occurs when the temperature within an object changes as a function of time. Analysis of transient systems is more complex and often calls for the application of approximation theories or numerical analysis by computer.

Convection

Convective heat transfer, or convection, is the transfer of heat from one place to another by the movement of fluids. (In physics, the term *fluid* means any substance that deforms under shear stress; it includes liquids, gases, plasmas, and some plastic solids.) Bulk motion of the fluid enhances the heat transfer between the solid surface and the fluid. Convection is usually the dominant form of heat transfer in liquids and gases. Although often discussed as a third method of heat transfer, convection actually describes the combined effects of conduction and fluid flow.

Free, or natural, convection occurs when the fluid motion is caused by buoyancy forces that result from density variations due to variations of temperature in the fluid. *Forced* convection is when the fluid is forced to flow over the surface by external means—such as fans, stirrers, and pumps—creating an artificially induced convection current.

Convection is described by Newton's law of cooling: "The rate of heat loss of a body is proportional to the difference in temperatures between the body and its surroundings."

Radiation

A red-hot iron object, transferring heat to the surrounding environment primarily through thermal radiation.

Thermal radiation is energy emitted by matter as electromagnetic waves due to the pool of thermal energy that all matter possesses that has a temperature above absolute zero. Thermal radiation propagates without the presence of matter through the vacuum of space.

Thermal radiation is a direct result of the random movements of atoms and molecules in matter. Since these atoms and molecules are composed of charged particles (protons and electrons), their movement results in the emission of electromagnetic radiation, which carries energy away from the surface.

Unlike conductive and convective forms of heat transfer, thermal radiation can be concentrated in a small spot by using reflecting mirrors, which is exploited in concentrating solar power generation. For example, the sunlight reflected from mirrors heats the PS10 solar power tower and during the day it can heat water to 285 °C (545 °F).

Mass Transfer

In mass transfer, energy—including thermal energy—is moved by the physical transfer of a hot or cold object from one place to another. This can be as simple as placing hot water in a bottle and heating a bed, or the movement of an iceberg in changing ocean currents. A practical example is thermal hydraulics.

Convection vs. conduction

In a body of fluid that is heated from underneath its container, conduction and convection can be considered to compete for dominance. If heat conduction is too great, fluid moving down by convection is heated by conduction so fast that its downward movement will be stopped due to its buoyancy, while fluid moving up by convection is cooled by conduction so fast that its driving buoyancy will diminish. On the other hand, if heat conduction is very low, a large temperature gradient may be formed and convection might be very strong.

The Rayleigh number (*Ra*) is a measure determining the result of this competition.

$$Ra = \frac{g\Delta\rho L^3}{\mu\alpha} = \frac{g\beta\Delta T L^3}{\nu\alpha}$$

where

- g is acceleration due to gravity
- ρ is the density with $\Delta\rho$ being the density difference between the lower and upper ends
- μ is the dynamic viscosity
- α is the Thermal diffusivity
- β is the volume thermal expansivity (sometimes denoted α elsewhere)
- T is the temperature and
- ν is the kinematic viscosity.

The Rayleigh number can be understood as the ratio between the rate of heat transfer by convection to the rate of heat transfer by conduction; or, equivalently, the ratio between the corresponding timescales (i.e. conduction timescale divided by convection timescale), up to a numerical factor. This can be seen as follows, where all calculations are up to numerical factors depending on the geometry of the system.

The buoyancy force driving the convection is roughly $g\Delta\rho L^3$, so the corresponding pressure is roughly $g\Delta\rho L$. In steady state, this is canceled by the shear stress due to viscosity, and therefore roughly equals $\mu V / L = \mu / T_{conv}$, where V is the typical fluid velocity due to convection and T_{conv} the order of its timescale. The conduction timescale, on the other hand, is of the order of $T_{cond} = L^2 / \alpha$.

Convection occurs when the Rayleigh number is above 1,000–2,000. For example, the Earth's mantle, exhibiting non-stable convection, has Rayleigh number of the order of 1,000, and T_{conv} as calculated above is around 100 million years.

Phase changes

Transfer of heat through a phase transition in the medium—such as water-to-ice, water-to-steam, steam-to-water, or ice-to-water—involves significant energy and is exploited in many ways: steam engines, refrigerators, etc. For example, the Mason equation is an approximate analytical expression for the growth of a water droplet based on the effects of heat transport on evaporation and condensation.

Boiling

Heat transfer in boiling fluids is complex, but of considerable technical importance. It is characterized by an *S*-shaped curve relating heat flux to surface temperature difference.

At low driving temperatures, no boiling occurs and the heat transfer rate is controlled by the usual single-phase mechanisms. As the surface temperature is increased, local boiling occurs and vapor bubbles nucleate, grow into the surrounding cooler fluid, and collapse. This is *sub-cooled nucleate boiling*, and is a very efficient heat transfer mechanism. At high bubble generation rates, the bubbles begin to interfere and the heat flux no longer increases rapidly with surface temperature (this is the departure from nucleate boiling, or DNB). At higher temperatures still, a maximum in the heat flux is reached (the critical heat flux, or CHF). The regime of falling heat transfer that follows is not easy to study, but is believed to be characterized by alternate periods of nucleate and film boiling. Nucleate boiling slows the heat transfer due to gas bubbles on the heater's surface; as mentioned, gas-phase thermal conductivity is much lower than liquid-phase thermal conductivity, so the outcome is a kind of "gas thermal barrier".

At higher temperatures still, the hydrodynamically-quieter regime of film boiling is reached. Heat fluxes across the stable vapor layers are low, but rise slowly with temperature. Any contact between fluid and the surface that may be seen probably leads to the extremely rapid nucleation of a fresh vapor layer ("spontaneous nucleation").

Condensation

Condensation occurs when a vapor is cooled and changes its phase to a liquid. Condensation heat transfer, like boiling, is of great significance in industry. During condensation, the latent heat of vaporization must be released. The amount of the heat is the same as that absorbed during vaporization at the same fluid pressure.

There are several types of condensation:

- Homogeneous condensation, as during a formation of fog.
- Condensation in direct contact with subcooled liquid.

- Condensation on direct contact with a cooling wall of a heat exchanger: This is the most common mode used in industry:
    - Filmwise condensation is when a liquid film is formed on the subcooled surface, and usually occurs when the liquid wets the surface.
    - Dropwise condensation is when liquid drops are formed on the subcooled surface, and usually occurs when the liquid does not wet the surface.

  Dropwise condensation is difficult to sustain reliably; therefore, industrial equipment is normally designed to operate in filmwise condensation mode.

Modeling approaches

Complex heat transfer phenomena can be modeled in different ways.

Heat equation

The heat equation is an important partial differential equation that describes the distribution of heat (or variation in temperature) in a given region over time. In some cases, exact solutions of the equation are available; in other cases the equation must be solved numerically using computational methods. For example, simplified climate models may use Newtonian cooling, instead of a full (and computationally expensive) radiation code, to maintain atmospheric temperatures.

Lumped system analysis

System analysis by the lumped capacitance model is a common approximation in transient conduction that may be used whenever heat conduction within an object is much faster than heat conduction across the boundary of the object.

This is a method of approximation that reduces one aspect of the transient conduction system—that within the object—to an equivalent steady state system. That is, the method assumes that the temperature within the object is completely uniform, although its value may be changing in time.

In this method, the ratio of the conductive heat resistance within the object to the convective heat transfer resistance across the object's boundary, known as the *Biot number*, is calculated. For small Biot numbers, the approximation of *spatially uniform temperature within the object* can be used: it can be presumed that heat transferred into the object has time to uniformly distribute itself, due to the lower resistance to doing so, as compared with the resistance to heat entering the object.

Applications and techniques

Heat transfer has broad application to the functioning of numerous devices and systems. Heat-transfer principles may be used to preserve, increase, or decrease temperature in a wide variety of circumstances.

Insulation and radiant barriers

Heat exposure as part of a fire test for firestop products.

Thermal insulators are materials specifically designed to reduce the flow of heat by limiting conduction, convection, or both. Radiant barriers are materials that reflect radiation, and therefore reduce the flow of heat from radiation sources. Good insulators are not necessarily good radiant barriers, and vice versa. Metal, for instance, is an excellent reflector and a poor insulator.

The effectiveness of an insulator is indicated by its **R-value**, or resistance value. The R-value of a material is the inverse of the conduction coefficient (k) multiplied by the thickness (d) of the insulator. In most of the world, R-values are measured in SI units: square-meter kelvins per watt (m²·K/W). In the United States, R-values are customarily given in units of British thermal units per hour per square-foot degrees Fahrenheit (Btu/h·ft²·°F).

$$R = \frac{d}{k}$$

$$C = \frac{Q}{m\Delta T}$$

Rigid fiberglass, a common insulation material, has an R-value of four per inch, while poured concrete, a poor insulator, has an R-value of 0.08 per inch.

The tog is a measure of thermal resistance, commonly used in the textile industry, and often seen quoted on, for example, duvets and carpet underlay.

The effectiveness of a radiant barrier is indicated by its **reflectivity**, which is the fraction of radiation reflected. A material with a high reflectivity (at a given wavelength) has a low emissivity (at that same wavelength), and vice versa. At any specific wavelength, reflectivity = 1 - emissivity. An ideal radiant barrier would have a reflectivity of 1, and would therefore reflect 100 percent of incoming radiation. Vacuum flasks, or Dewars, are silvered to approach this ideal. In the vacuum of space, satellites use multi-layer insulation, which consists of many layers of aluminized (shiny) Mylar to greatly reduce radiation heat transfer and control satellite temperature.

Critical insulation thickness

Low thermal conductivity (k) materials reduce heat fluxes. The smaller the k value, the larger the corresponding thermal resistance (R) value. Thermal conductivity is measured in watts-per-meter per kelvin ($\mathrm{W \cdot m^{-1} \cdot K^{-1}}$), represented as k. As the thickness of insulating material increases, the thermal resistance—or R-value—also increases.

However, adding layers of insulation has the potential of increasing the surface area, and hence the thermal convection area.

For example, as thicker insulation is added to a cylindrical pipe, the outer radius of the pipe-and-insulation system increases, and therefore surface area increases. The point where the added resistance of increasing insulation thickness becomes overshadowed by the effect of increased surface area is called the critical insulation thickness. In simple cylindrical pipes, this is calculated as a radius:

$$R_{critical} = \frac{k}{h}$$

Heat exchangers

A heat exchanger is a tool built for efficient heat transfer from one fluid to another, whether the fluids are separated by a solid wall so that they never mix, or the fluids are in direct contact. Heat exchangers are widely used in refrigeration, air conditioning, space heating, power generation, and chemical processing. One common example of a heat exchanger is a car's radiator, in which the hot coolant fluid is cooled by the flow of air over the radiator's surface.

Common types of heat exchanger flows include parallel flow, counter flow, and cross flow. In parallel flow, both fluids move in the same direction while transferring heat; in counter flow, the fluids move in opposite directions; and in cross flow, the fluids move at

right angles to each other. Common constructions for heat exchanger include shell and tube, double pipe, extruded finned pipe, spiral fin pipe, u-tube, and stacked plate.

When engineers calculate the theoretical heat transfer in a heat exchanger, they must contend with the fact that the driving temperature difference between the two fluids varies with position. To account for this in simple systems, the log mean temperature difference (LMTD) is often used as an "average" temperature. In more complex systems, direct knowledge of the LMTD is not available, and the number of transfer units (NTU) method can be used instead.

Heat dissipation

A heat sink is a component that transfers heat generated within a solid material to a fluid medium, such as air or a liquid. Examples of heat sinks are the heat exchangers used in refrigeration and air conditioning systems, and the radiator in a car (which is also a heat exchanger). Heat sinks also help to cool electronic and optoelectronic devices such as CPUs, higher-power lasers, and light-emitting diodes (LEDs). A heat sink uses its extended surfaces to increase the surface area in contact with the cooling fluid.

Buildings

In cold climates, houses with their heating systems form dissipative systems. In spite of efforts to insulate houses to reduce heat losses via their exteriors, considerable heat is lost, which can make their interiors uncomfortably cool or cold. For the comfort of the inhabitants, the interiors must be maintained out of thermal equilibrium with the external surroundings. In effect, these domestic residences are oases of warmth in a sea of cold, and the thermal gradient between the inside and outside is often quite steep. This can lead to problems such as condensation and uncomfortable air currents, which—if left unaddressed—can cause cosmetic or structural damage to the property. Such issues can be prevented by use of insulation techniques for reducing heat loss.

Thermal transmittance is the rate of transfer of heat through a structure divided by the difference in temperature across the structure. It is expressed in watts per square meter per kelvin, or W/m^2K. Well-insulated parts of a building have a low thermal transmittance, whereas poorly-insulated parts of a building have a high thermal transmittance.

A thermostat is a device capable of starting the heating system when the house's interior falls below a set temperature, and of stopping that same system when another (higher) set temperature has been achieved. Thus, the thermostat controls the flow of energy into the house, that energy eventually being dissipated to the exterior.

Thermal energy storage

Thermal energy storage refers to technologies that store energy in a thermal reservoir for later use. They can be employed to balance energy demand between daytime and

nighttime. The thermal reservoir may be maintained at a temperature above (hotter) or below (colder) than that of the ambient environment. Applications include later use in space heating, domestic or process hot water, or to generate electricity. Most practical active solar heating systems have storage for a few hours to a day's worth of heat collected.

Evaporative cooling

Evaporative cooling is a physical phenomenon in which evaporation of a liquid, typically into surrounding air, cools an object or a liquid in contact with it. Latent heat describes the amount of heat that is needed to evaporate the liquid; this heat comes from the liquid itself and the surrounding gas and surfaces. The greater the difference between the two temperatures, the greater the evaporative cooling effect. When the temperatures are the same, no net evaporation of water in air occurs; thus, there is no cooling effect. A simple example of natural evaporative cooling is perspiration, or sweat, which the body secretes in order to cool itself. An evaporative cooler is a device that cools air through the simple evaporation of water.

Radiative cooling

Radiative cooling is the process by which a body loses heat by radiation. It is an important effect in the Earth's atmosphere. In the case of the Earth-atmosphere system, it refers to the process by which long-wave (infrared) radiation is emitted to balance the absorption of short-wave (visible) energy from the Sun. Convective transport of heat and evaporative transport of latent heat both remove heat from the surface and redistribute it in the atmosphere, making it available for radiative transport at higher altitudes.

Laser cooling

Laser cooling refers to techniques in which atomic and molecular samples are cooled through the interaction with one or more laser light fields. The most common method of laser cooling is Doppler cooling. In Doppler cooling, the frequency of the laser light is tuned slightly below an electronic transition in the atom. Thus, the atoms would absorb more photons if they moved towards the light source, due to the Doppler effect. If an excited atom then emits a photon spontaneously, it will be accelerated. The result of the absorption and emission process is to reduce the speed of the atom. Eventually the mean velocity, and therefore the kinetic energy of the atoms, will be reduced. Since the temperature of an ensemble of atoms is a measure of the random internal kinetic energy, this is equivalent to cooling the atoms.

Sympathetic cooling is a process in which particles of one type cool particles of another type. Typically, atomic ions that can be directly laser-cooled are used to cool nearby ions or atoms. This technique allows cooling of ions and atoms that cannot be laser cooled directly.

Magnetic cooling

Magnetic evaporative cooling is a technique for lowering the temperature of a group of atoms. The process confines atoms using a magnetic field. Over time, individual atoms will become much more energetic than the others due to random collisions, and will escape—removing energy from the system and reducing the temperature of the remaining group. This process is similar to the familiar process by which standing water becomes water vapor.

Other

A heat pipe is a passive device constructed in such a way that it acts as though it has extremely high thermal conductivity. Heat pipes use latent heat and capillary action to move heat, and can carry many times as much heat as a similar-sized copper rod. Originally invented for use in satellites, they have applications in personal computers.

A thermocouple is a junction between two different metals that produces a voltage related to a temperature difference. Thermocouples are a widely used type of temperature sensor for measurement and control, and can also be used to convert heat into electric power.

A thermopile is an electronic device that converts thermal energy into electrical energy. It is composed of thermocouples. Thermopiles do not measure the absolute temperature, but generate an output voltage proportional to a temperature difference. Thermopiles are widely used, e.g., they are the key component of infrared thermometers, such as those used to measure body temperature via the ear.

A thermal diode or thermal rectifier is a device that preferentially passes heat in one direction: a "one-way valve" for heat.

Chapter 6

Chemical Reactor

In chemical engineering, **chemical reactors** are vessels designed to contain chemical reactions. The design of a chemical reactor deals with multiple aspects of chemical engineering. Chemical engineers design reactors to maximize net present value for the given reaction. Designers ensure that the reaction proceeds with the highest efficiency towards the desired output product, producing the highest yield of product while requiring the least amount of money to purchase and operate. Normal operating expenses include energy input, energy removal, raw material costs, labor, etc. Energy changes can come in the form of heating or cooling, pumping to increase pressure, frictional pressure loss (such as pressure drop across a 90^{o} elbow or an orifice plate), agitation, etc.

Chemical reaction engineering is the branch of chemical engineering which deals with chemical reactors and their design, especially by application of chemical kinetics.

Overview

Cut-away view of a stirred-tank chemical reactor with a cooling jacket

Chemical reactor with half coils wrapped around it

There are a couple main basic vessel types:

- A tank
- A pipe or tubular reactor

Both types can be used as continuous reactors or batch reactors. Most commonly, reactors are run at steady-state, but can also be operated in a transient state. When a reactor is first brought back into operation (after maintenance or inoperation) it would be considered to be in a transient state, where key process variables change with time. Both types of reactors may also accommodate one or more solids (reagents, catalyst, or inert materials), but the reagents and products are typically liquids and gases.

There are three main basic models used to estimate the most important process variables of different chemical reactors:

- *batch reactor* model (batch),
- *continuous stirred-tank reactor* model (CSTR), and
- *plug flow reactor* model (PFR).

Furthermore, catalytic reactors require separate treatment, whether they are batch, CST, or PF reactors, as the many assumptions of the simpler models are not valid.

Key process variables include

- Residence time (τ, lower case Greek tau)
- Volume (V)
- Temperature (T)
- Pressure (P)
- Concentrations of chemical species (C_1, C_2, C_3, ... C_n)
- Heat transfer coefficients (h, U)

A chemical reactor, typically tubular reactor, could be a packed bed. The packing inside the bed may have catalyst to catalyze the chemical reaction. A chemical reactor may also be a fluidized bed.

Chemical reactions occurring in a reactor may be exothermic, meaning giving off heat, or endothermic, meaning absorbing heat. A chemical reactor vessel may have a cooling or heating jacket or cooling or heating coils (tubes) wrapped around the outside of its vessel wall to cool down or heat up the contents.

Types

CSTR (Continuous Stirred-Tank Reactor)

Reactor CSTR. Check of a condition of the case. Note the impeller (or agitator) blades on the shaft for mixing. Also note the baffle at the bottom of the image which also helps in mixing.

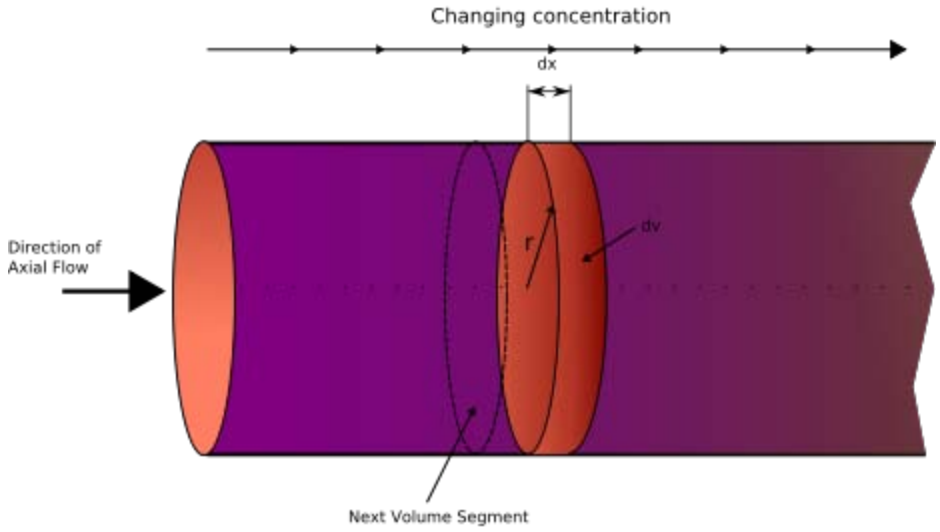
In a CSTR, one or more fluid reagents are introduced into a tank reactor equipped with an impeller while the reactor effluent is removed. The impeller stirs the reagents to ensure proper mixing. Simply dividing the volume of the tank by the average volumetric flow rate through the tank gives the *residence time*, or the average amount of time a discrete quantity of reagent spends inside the tank. Using chemical kinetics, the reaction's expected percent completion can be calculated. Some important aspects of the CSTR:

- At steady-state, the flow rate in must equal the mass flow rate out, otherwise the tank will overflow or go empty (transient state). While the reactor is in a transient state the model equation must be derived from the differential mass and energy balances.
- The reaction proceeds at the reaction rate associated with the final (output) concentration.
- Often, it is economically beneficial to operate several CSTRs in series. This allows, for example, the first CSTR to operate at a higher reagent concentration

and therefore a higher reaction rate. In these cases, the sizes of the reactors may be varied in order to minimize the total capital investment required to implement the process.
- It can be seen that an infinite number of infinitely small CSTRs operating in series would be equivalent to a PFR.

The behavior of a CSTR is often approximated or modeled by that of a Continuous Ideally Stirred-Tank Reactor (CISTR). All calculations performed with CISTRs assume perfect mixing. If the residence time is 5-10 times the mixing time, this approximation is valid for engineering purposes. The CISTR model is often used to simplify engineering calculations and can be used to describe research reactors. In practice it can only be approached, in particular in industrial size reactors.

[[File:===PFR (Plug Flow Reactor)===



Simple diagram illustrating plug flow reactor model

In a PFR, one or more fluid reagents are pumped through a pipe or tube. The chemical reaction proceeds as the reagents travel through the PFR. In this type of reactor, the changing reaction rate creates a gradient with respect to distance traversed; at the inlet to the PFR the rate is very high, but as the concentrations of the reagents decrease and the concentration of the product(s) increases the reaction rate slows. Some important aspects of the PFR:

- All calculations performed with PFRs assume no upstream or downstream mixing, as implied by the term "plug flow".
- Reagents may be introduced into the PFR at locations in the reactor other than the inlet. In this way, a higher efficiency may be obtained, or the size and cost of the PFR may be reduced.

- A PFR typically has a higher efficiency than a CSTR of the same volume. That is, given the same space-time, a reaction will proceed to a higher percentage completion in a PFR than in a CSTR.

For most chemical reactions, it is impossible for the reaction to proceed to 100% completion. The rate of reaction decreases as the percent completion increases until the point where the system reaches dynamic equilibrium (no net reaction, or change in chemical species occurs). The equilibrium point for most systems is less than 100% complete. For this reason a separation process, such as distillation, often follows a chemical reactor in order to separate any remaining reagents or byproducts from the desired product. These reagents may sometimes be reused at the beginning of the process, such as in the Haber process.

Continuous oscillatory baffled reactor (COBR) is a tubular plug flow reactor. The mixing in COBR is achieved by the combination of fluid oscillation and orifice baffles, allowing plug flow to be achieved under laminar flow conditions with the net flow Reynolds number just about 100.]]

Semi-batch reactor

A semi-batch reactor is operated with both continuous and batch inputs and outputs. A fermenter, for example, is loaded with a batch, which constantly produces carbon dioxide, which has to be removed continuously. Analogously, driving a reaction of gas with a liquid is usually difficult, since the gas bubbles off. Therefore, a continuous feed of gas is injected into the batch of a liquid.One chemical reactant is charged to the vessel and a second chemical is added slowly.

Catalytic reactor

Although catalytic reactors are often implemented as plug flow reactors, their analysis requires more complicated treatment. The rate of a catalytic reaction is proportional to the amount of catalyst the reagents contact. With a solid phase catalyst and fluid phase reagents, this is proportional to the exposed area, efficiency of diffusion of reagents in and products out, and turbulent mixing or lack thereof. Perfect mixing cannot be assumed. Furthermore, a catalytic reaction pathway is often multi-step with intermediates that are chemically bound to the catalyst; and as the chemical binding to the catalyst is also a chemical reaction, it may affect the kinetics.

The behavior of the catalyst is also a consideration. Particularly in high-temperature petrochemical processes, catalysts are deactivated by sintering, coking, and similar processes.

Chapter 7

Green Chemistry

Green chemistry, also called sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances. Whereas environmental chemistry is the chemistry of the natural environment, and of pollutant chemicals in nature, green chemistry seeks to reduce and prevent pollution at its source. In 1990 the Pollution Prevention Act was passed in the United States. This act helped create a *modus operandi* for dealing with pollution in an original and innovative way. It aims to avoid problems before they happen.

As a chemical philosophy, green chemistry applies to organic chemistry, inorganic chemistry, biochemistry, analytical chemistry, and even physical chemistry. While green chemistry seems to focus on industrial applications, it does apply to any chemistry choice. Click chemistry is often cited as a style of chemical synthesis that is consistent with the goals of green chemistry. The focus is on minimizing the hazard and maximizing the efficiency of any chemical choice. It is distinct from environmental chemistry which focuses on chemical phenomena in the environment.

In 2005 Ryōji Noyori identified three key developments in green chemistry: use of supercritical carbon dioxide as green solvent, aqueous hydrogen peroxide for clean oxidations and the use of hydrogen in asymmetric synthesis. Examples of applied green chemistry are supercritical water oxidation, on water reactions, and dry media reactions.

Bioengineering is also seen as a promising technique for achieving green chemistry goals. A number of important process chemicals can be synthesized in engineered organisms, such as shikimate, a Tamiflu precursor which is fermented by Roche in bacteria.

There is some debate as to whether green chemistry includes a consideration of economics, but by definition, if green chemistry is not applied, it cannot accomplish the reduction in the "use or generation of hazardous substances."

Principles

Paul Anastas, then of the United States Environmental Protection Agency, and John C. Warner developed 12 principles of green chemistry, which help to explain what the definition means in practice. The principles cover such concepts as:

- the design of processes to maximize the amount of raw material that ends up in the product;
- the use of safe, environment-benign substances, including solvents, whenever possible;
- the design of energy efficient processes;
- the best form of waste disposal: not to create it in the first place.

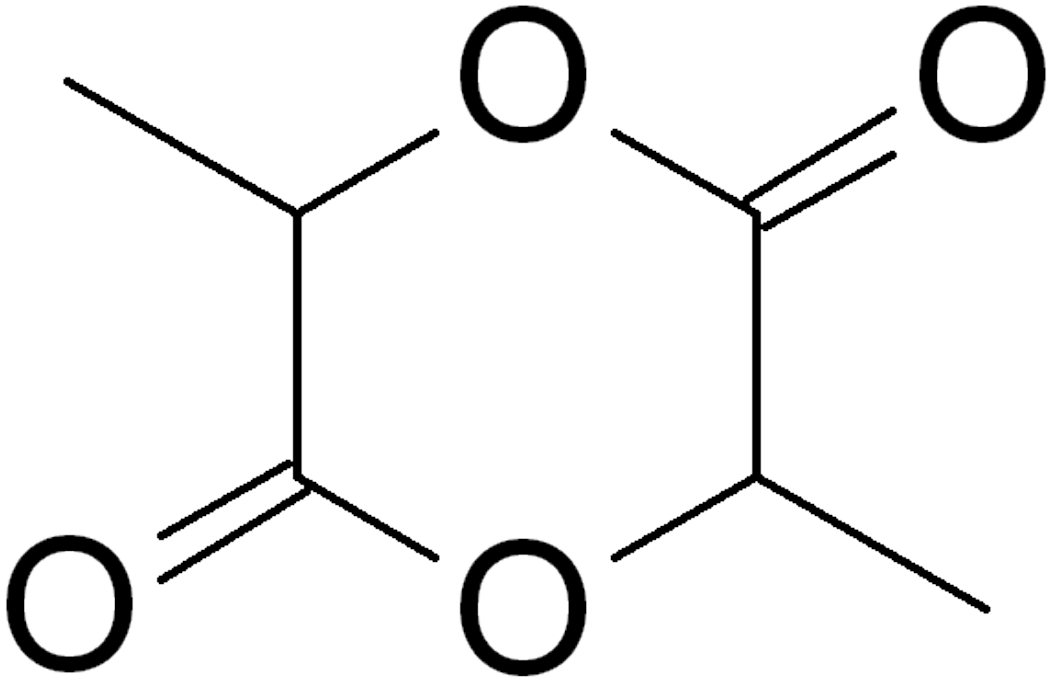
The 12 principles are:

1. It is better to prevent waste than to treat or clean up waste after it is formed.
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
5. The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.
7. A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.
8. Reduce derivatives - Unnecessary derivatization (blocking group, protection/ deprotection, temporary modification) should be avoided whenever possible.
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.
11. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. Substances and the form of a substance used in a chemical process should be chosen to minimize potential for chemical accidents, including releases, explosions, and fires.

Presidential Green Chemistry Challenge Awards

The Presidential Green Chemistry Challenge Awards began in 1995 as an effort to recognize individuals and businesses for innovations in green chemistry. Typically five awards are given each year, one in each of five categories: Academic, Small Business, Greener Synthetic Pathways, Greener Reaction Conditions, and Designing Greener Chemicals. Nominations are accepted the prior year, and evaluated by an independent panel of chemists convened by the American Chemical Society. Through 2006, a total of 57 technologies have been recognized for the award, and over 1000 nominations have been submitted.

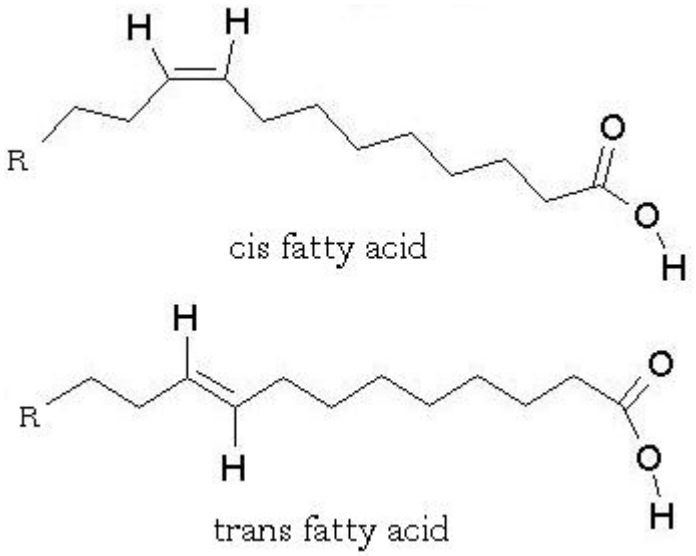
- In 1996, Dow Chemical won the 1996 Greener Reaction Conditions award for their 100% carbon dioxide blowing agent for polystyrene foam production. Polystyrene foam is a common material used in packing and food transportation. Seven hundred million pounds are produced each year in the United States alone. Traditionally, CFC and other ozone-depleting chemicals were used in the production process of the foam sheets, presenting a serious environmental hazard. Flammable, explosive, and, in some cases toxic hydrocarbons have also been used as CFC replacements, but they present their own problems. Dow Chemical discovered that supercritical carbon dioxide works equally as well as a blowing agent, without the need for hazardous substances, allowing the polystyrene to be more easily recycled. The CO_2 used in the process is reused from other industries, so the net carbon released from the process is zero.



Lactide

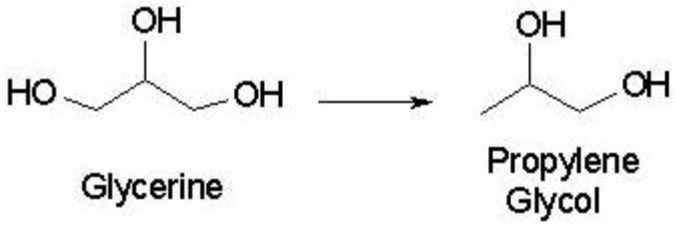
- In 2002, Cargill Dow (now NatureWorks) won the Greener Reaction Conditions Award for their improved polylactic acid polymerization process. Lactic acid is produced by fermenting corn and converted to lactide, the cyclic dimer ester of lactic acid using an efficient, tin-catalyzed cyclization. The L,L-lactide enantiomer is isolated by distillation and polymerized in the melt to make a crystallizable polymer, which has use in many applications including textiles and apparel, cutlery, and food packaging. Wal-Mart has announced that it is using/will use PLA for its produce packaging. The NatureWorks PLA process substitutes renewable materials for petroleum feedstocks, doesn't require the use of hazardous organic solvents typical in other PLA processes, and results in a high-quality polymer that is recyclable and compostable.

- In 2003 Shaw Industries was recognized with the Designing Greener Chemicals Award for developing EcoWorx Carpet Tile. Historically, carpet tile backings have been manufactured using bitumen, polyvinyl chloride (PVC), or polyurethane (PU). While these backing systems have performed satisfactorily, there are several inherently negative attributes due to their feedstocks or their ability to be recycled. Shaw selected a combination of polyolefin resins as the base polymer of choice for EcoWorx due to the low toxicity of its feedstocks, superior adhesion properties, dimensional stability, and its ability to be recycled. The EcoWorx compound also had to be designed to be compatible with nylon carpet fiber. Although EcoWorx may be recovered from any fiber type, nylon-6 provides a significant advantage. Polyolefins are compatible with known nylon-6 depolymerization methods. PVC interferes with those processes. Nylon-6 chemistry is well-known and not addressed in first-generation production. From its inception, EcoWorx met all of the design criteria necessary to satisfy the needs of the marketplace from a performance, health, and environmental standpoint. Research indicated that separation of the fiber and backing through elutriation, grinding, and air separation proved to be the best way to recover the face and backing components, but an infrastructure for returning postconsumer EcoWorx to the elutriation process was necessary. Research also indicated that the postconsumer carpet tile had a positive economic value at the end of its useful life. EcoWorx is recognized by MBDC as a certified Cradle to Cradle design.



Trans and *cis* fatty acids

- In 2005, Archer Daniels Midland (ADM) and Novozymes N.A. won the Greener Synthetic Pathways Award for their enzyme interesterification process. In response to the U.S. Food and Drug Administration (FDA) mandated labeling of *trans*-fats on nutritional information by January 1, 2006, Novozymes and ADM worked together to develop a clean, enzymatic process for the interesterification of oils and fats by interchanging saturated and unsaturated fatty acids. The result is commercially viable products without *trans*-fats. In addition to the human health benefits of eliminating *trans*-fats, the process has reduced the use of toxic chemicals and water, prevents vast amounts of byproducts, and reduces the amount of fats and oils wasted.



Glycerine to propylene glycol

- In 2006, Professor Galen J. Suppes, from the University of Missouri in Columbia, Missouri, was awarded the Academic Award for his system of converting waste glycerin from biodiesel production to propylene glycol. Through the use of a copper-chromite catalyst, Professor Suppes was able to lower the required temperature of conversion while raising the efficiency of the distillation reaction. Propylene glycol produced in this way will be cheap enough to replace the more toxic ethylene glycol that is the primary ingredient in automobile antifreeze.

Other awards

The Royal Australian Chemical Institute (RACI) presents Australia's Green Chemistry Challenge Awards. This awards program is similar to that of the United States Environmental Protection Agency (EPA), although the RACI has included a category for green chemistry education as well as small business and academic or government.

The Canadian Green Chemistry Medal is an annual award given to an individual or group for promotion and development of green chemistry in Canada and internationally. The winner is presented with a citation recognizing the achievements together with a sculpture.

Green Chemistry activities in Italy center around an inter-university consortium known as INCA. Beginning in 1999, the INCA has given three awards annually to industry for applications of green chemistry. The winners receive a plaque at the annual INCA meeting.

In Japan, The Green & Sustainable Chemistry Network(GSCN), formed in 1999, is an organization consisting of representatives from chemical manufacturers and researchers. In 2001, the organization began an awards program. GSC Awards are to be granted to individuals, groups or companies who greatly contributed to green chemistry through their research, development and their industrialization. The achievements are awarded by Ministers of related government agencies.

In the United Kingdom, the Crystal Faraday Partnership, a non-profit group founded in 2001, awards businesses annually for incorporation of green chemistry. The Green Chemical Technology Awards have been given by Crystal Faraday since 2004; the awards were presented by the Royal Society of Chemistry prior to that time. The award is given only to a single researcher or business, while other notable entries are given recognition as well.

The Nobel Prize Committee recognized the importance of green chemistry in 2005 by awarding Yves Chauvin, Robert H. Grubbs, and Richard R. Schrock the Nobel Prize for Chemistry for "the development of the metathesis method in organic synthesis." The Nobel Prize Committee states, "this represents a great step forward for 'green chemistry', reducing potentially hazardous waste through smarter production. Metathesis is an example of how important basic science has been applied for the benefit of man, society and the environment."

Trends

Attempts are being made not only to quantify the *greenness* of a chemical process but also to factor in other variables such as chemical yield, the price of reaction components, safety in handling chemicals, hardware demands, energy profile and ease of product workup and purification. In one quantitative study, the reduction of nitrobenzene to aniline receives 64 points out of 100 marking it as an acceptable synthesis overall

whereas a synthesis of an amide using HMDS is only described as adequate with a combined 32 points.

Green chemistry is increasingly seen as a powerful tool that researchers must use to evaluate the environmental impact of nanotechnology. As nanomaterials are developed, the environmental and human health impacts of both the products themselves and the processes to make them must be considered to ensure their long-term economic viability.

Laws

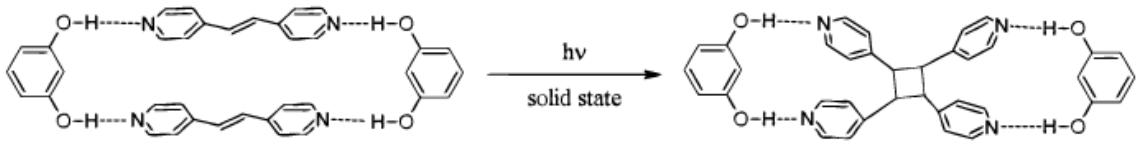
In 2007, Europe put into place the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) program, which requires companies to provide data showing that their products are safe. The US Toxic Substances Control Act, passed in 1976, has similar provisions.

On September 29, 2008 California approved two laws which encourage green chemistry, launching the California Green Chemistry Initiative. The law requires California's Department of Toxic Substances Control to prioritize "chemicals of concern", and puts the burden of testing on the agency rather than industry. The laws were criticized by Paul Anastas, who stated that the laws did not go far enough in encouraging research, education, and industry incentives.

Examples

Supramolecular chemistry

Research is currently ongoing in the area of supramolecular chemistry to develop reactions which can proceed in the solid state without the use of solvents. The cycloaddition of *trans*-1,2-bis(4-pyridyl)ethylene is directed by resorcinol in the solid state. This solid-state reaction proceeds in the presence of UV light in 100% yield.



Cycloaddition of *trans*-1,2-bis(4-pyridyl)ethylene

Natural Product Synthesis

Research is currently also going in the area of natural product synthesis to develop reactions which can proceed involving green chemistry principles. Recently, Atul Kumar has developed an efficient and green method for the synthesis of Tryptanthrin a biologically active natural product employing β-cyclodextrin as a catalyst in aqueous media at room temperature from isatoic anhydride and isatin in excellent yields.

Reducing market barriers

In March 2006, the University of California published a report by Dr. Michael P. Wilson and colleagues, Daniel A. Chia and Bryan C. Ehlers, on potential chemicals policy for the California Legislature entitled, Green Chemistry in California: A Framework for Leadership in Chemicals Policy and Innovation. The report finds that long-standing weaknesses in the U.S. chemical management program, notably the Toxic Substances Control Act (TSCA) of 1976, have produced a chemicals market in the U.S. that discounts the hazardous properties of chemicals relative to their function, price, and performance. The report concludes that these market conditions represent a key barrier to the scientific, technical, and commercial success of green chemistry in the U.S., and that fundamental policy changes are needed to correct these weaknesses. Drs. Wilson and Schwarzman of UC Berkeley published their analysis in the August issue of *Environmental Health Perspectives* (EHP) in an article entitled, Toward a New U.S. Chemicals Policy: Rebuilding the Foundation to Advance New Science, Green Chemistry, and Environmental Health.

Education

The University of Massachusetts, Boston became the first university in the World to offer a Ph.D. in Green Chemistry. In 2005, the University of Oregon's chemistry department unveiled the Greener Education Materials, a database of green chemistry topics. In 2009, Oxford University Press/ACS Symposium Series published *Green Chemistry Education: Changing the Course of Chemistry*. The book contains essays from a broad array of educators who share their best practices for the incorporation of green chemistry into the chemistry and chemical engineering curricula.

There are many green chemistry courses available in the UK at an MSc level. These include MSc in Green Chemistry & Sustainable Industrial Technology at the Green Chemistry Centre of Excellence based at the University of York, MSc Chemical Research in Green Chemistry at Leicester University and MRes in Green Chemistry at Imperial College London. A masters level course in Green Technology, has been introduced by the Institute of Chemical Technology, India in 2010. This is the first of its kind in India and aims at educating students about cleaner & more sustainable mechanisms for a greener tomorrow.

Laboratory chemicals

Several laboratory chemicals are controversial, and alternatives have been explored. The Massachusetts Institute of Technology has created the Green Alternatives Wizard to help identify alternatives. Ethidium bromide, xylene, mercury, and formalin have been identified as "worst offenders" which have alternatives.

Organometallic chemicals

Following green chemistry principles, ionic liquids and microreactors were recently used to synthesize and purify extremely reactive organometallic compounds for atomic layer deposition (ALD) and chemical vapor deposition (CVD) applications, with improved safety in operations and efficient production of ultra-high purity organometallics.

Scientific uncertainty

Following historical analyses of the green chemistry development, there have been green chemistry advocates who see it as an innovative way of thinking. On the other hand, there have been chemists who have argued that green chemistry is no more than a public relations label. In fact, a lot of chemists use the term "green chemistry" independently from the green chemistry paradigm, as proposed by Anastas and Warner. This explains the uncertainty of the scientific status of green chemistry.

Chapter 8

Synthetic Biology

Synthetic biology is a new area of biological research that combines science and engineering. Synthetic biology encompasses a variety of different approaches, methodologies and disciplines, and many different definitions exist. What they all have in common, however, is that they see synthetic biology as the design and construction of new biological functions and systems not found in nature.

A light programmable biofilm made by the UT Austin / UCSF team during the 2004 Synthetic Biology competition, displaying "Hello World"

History of the term

The term "synthetic biology" has a history spanning the twentieth century. The first use was in Stéphane Leducs's publication of « Théorie physico-chimique de la vie et générations spontanées » (1910) and « La Biologie Synthétique » (1912). In 1974, the Polish geneticist Waclaw Szybalski used the term "synthetic biology", writing:

Let me now comment on the question "what next". Up to now we are working on the descriptive phase of molecular biology. ... But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field with the unlimited expansion potential and hardly any limitations to building "new better control circuits" and ..... finally other "synthetic" organisms, like a "new better mouse". ... I am not concerned that we will run out of exciting and novel ideas, ... in the synthetic biology, in general.

When in 1978 the Nobel Prize in Physiology or Medicine was awarded to Arber, Nathans and Smith for the discovery of restriction enzymes, Waclaw Szybalski wrote in an editorial comment in the journal *Gene*:

The work on restriction nucleases not only permits us easily to construct recombinant DNA molecules and to analyze individual genes, but also has led us into the new era of synthetic biology where not only existing genes are described and analyzed but also new gene arrangements can be constructed and evaluated.

Biology

Biologists are interested in learning more about how natural living systems work. One simple, direct way to test our current understanding of a natural living system is to build an instance (or version) of the system in accordance with our current understanding of the system. Michael Elowitz's early work on the Repressilator is one good example of such work. Elowitz had a model for how gene expression should work inside living cells. To test his model, he built a piece of DNA in accordance with his model, placed the DNA inside living cells, and watched what happened. Slight differences between observation and expectation highlight new science that may be well worth doing. Work of this sort often makes good use of mathematics to predict and study the dynamics of the biological system before experimentally constructing it. A wide variety of mathematical descriptions have been used with varying accuracy, including graph theory, Boolean networks, ordinary differential equations, stochastic differential equations, and Master equations (in order of increasing accuracy). Good examples include the work of Adam Arkin, Jim Collins and Alexander van Oudenaarden.

Chemistry

Biological systems are physical systems that are made up of chemicals. Around 100 years ago, the science of chemistry went through a transition from studying natural chemicals

to trying to design and build new chemicals. This transition led to the field of synthetic chemistry. In the same tradition, some aspects of synthetic biology can be viewed as an extension and application of synthetic chemistry to biology, and include work ranging from the creation of useful new biochemicals to studying the origins of life. Eric Kool's group at Stanford, the Foundation for Applied Molecular Evolution, Carlos Bustamante's group at Berkeley, Jack Szostak's group at Harvard, and David McMillen's group at University of Toronto are good examples of this tradition. Much of the improved economics and versatility of synthetic biology is driven by ongoing improvements in gene synthesis.

Engineering

Engineers view biology as a *technology.* Synthetic Biology includes the broad redefinition and expansion of biotechnology, with the ultimate goals of being able to design and build engineered biological systems that process information, manipulate chemicals, fabricate materials and structures, produce energy, provide food, and maintain and enhance human health and our environment. A good example of these technologies include the work of Chris Voigt, who redesigned the Type III secretion system used by *Salmonella typhimurium* to secrete spider silk proteins, a strong elastic biomaterial, instead of its own natural infectious proteins. One aspect of Synthetic Biology which distinguishes it from conventional genetic engineering is a heavy emphasis on developing foundational technologies that make the engineering of biology easier and more reliable. Good examples of engineering in synthetic biology include the pioneering work of Tim Gardner and Jim Collins on an engineered genetic toggle switch, a riboregulator, the Registry of Standard Biological Parts, and the International Genetically Engineered Machine competition (iGEM).

Studies in synthetic biology can be subdivided into broad classifications according to the approach they take to the problem at hand: photocell design, biomolecular engineering, genome engineering, and biomolecular-design. The photocell approach includes projects to make self-replicating systems from entirely synthetic components. Biomolecular engineering includes approaches which aim to create a toolkit of functional units that can be introduced to present new orthogonal functions in living cells. Genome engineering includes approaches to construct synthetic chromosomes for whole or minimal organisms. Biomolecular-design approach refers to the general idea of the de novo design and combination of biomolecular components. The task of each of these approaches is similar: To create a more synthetic entry at a higher level of complexity by manipulating a part of the proceeding level.

Re-writing

Re-writers are Synthetic Biologists who are interested in testing the idea that since natural biological systems are so complicated, we would be better off re-building the natural systems that we care about, from the ground up, in order to provide engineered surrogates that are easier to understand and interact with. Re-writers draw inspiration from refactoring, a process sometimes used to improve computer software. Drew Endy

and his group have done some preliminary work on re-writing (e.g., Refactoring Bacteriophage T7). Oligonucleotides harvested from a photolithographic or inkjet manufactured DNA chip combined with DNA mismatch error-correction allows inexpensive large-scale changes of codons in genetic systems to improve gene expression or incorporate novel amino-acids.

Challenges

Opposition to Synthetic Biology

Opposition by civil society groups to Synthetic Biology has been led by the ETC Group who have called for a global moratorium on developments in the field and for no synthetic organisms to be released from the lab. In 2006 38 civil society organizations authored an open letter opposing voluntary regulation of the field and in 2008 ETC Group released the first critical report on the societal impacts of synthetic biology which they dubbed "Extreme Genetic Engineering".

Safety and Security

In addition to numerous scientific and technical challenges, synthetic biology raises questions for ethics, biosecurity, biosafety, involvement of stakeholders and intellectual property. To date, key stakeholders (especially in the US) have focused primarily on the biosecurity issues, especially the so-called dual-use challenge. For example, while the study of synthetic biology may lead to more efficient ways to produce medical treatments (e.g. against malaria), it may also lead to synthesis or redesign of harmful pathogens (e.g., smallpox) by malicious actors. Proposals for licensing and monitoring the various phases of gene and genome synthesis began to appear in 2004. A 2007 study compared several policy options for governing the security risks associated with synthetic biology. Other initiatives, such as OpenWetWare, diybio, biopunk, biohack, and possibly others, have attempted to integrate self-regulation in their proliferation of open source synthetic biology projects. However the distributed and diffuse nature of open-source biotechnology may make it more difficult to track, regulate, or mitigate potential biosafety and biosecurity concerns.

An initiative for self-regulation has been proposed by the International Association Synthetic Biology that suggests some specific measures to be implemented by the synthetic biology industry, especially DNA synthesis companies. Some scientists, however, argue for a more radical and forward looking approaches to improve safety and security issues. They suggest to use not only physical containment as safety measures, but also trophic and semantic containment. Trophic containment includes for example the design of new and more robust forms of auxotrophy, while semantic containment means the design and construction of completely novel orthogonal life-forms.

Social and Ethical

Online discussion of “societal issues” took place at the SYNBIOSAFE forum on issues regarding ethics, safety, security, IPR, governance, and public perception (summary paper). On July 9–10, 2009, the National Academies' Committee of Science, Technology & Law convened a symposium on "Opportunities and Challenges in the Emerging Field of Synthetic Biology" (transcripts, audio, and presentations available).

Some efforts have been made to engage social issues "upstream" focus on the integral and mutually formative relations among scientific and other human practices. These approaches attempt to invent ongoing and regular forms of collaboration among synthetic biologists, ethicists, political analysts, funders, human scientists and civil society activists. These collaborations have consisted either of intensive, short term meetings, aimed at producing guidelines or regulations, or standing committees whose purpose is limited to protocol review or rule enforcement. Such work has proven valuable in identifying the ways in which synthetic biology intensifies already-known challenges in rDNA technologies. However, these forms are not suited to identifying new challenges as they emerge, and critics worry about uncritical complicity.

An example of efforts to develop ongoing collaboration is the "Human Practices" component of the Synthetic Biology Engineering Research Center in the US and the SYNBIOSAFE project in Europe, coordinated by IDC, that investigated the biosafety, biosecurity and ethical aspects of synthetic biology. A report from the Woodrow Wilson Center and the Hastings Center, a prestigious bioethics research institute, found that ethical concerns in synthetic biology have received scant attention.

In January 2009, the Alfred P. Sloan Foundation funded the Woodrow Wilson Center, the Hastings Center, and the J. Craig Venter Institute to examine the public perception, ethics, and policy implications of synthetic biology. Public perception and communication of synthetic biology is the main focus of COSY: Communicating Synthetic Biology, that showed that in the general public synthetic biology is not seen as too different from 'traditional' genetic engineering. To better communicate synthetic biology and its societal ramifications to a broader public, COSY and SYNBIOSAFE published a 38 min. documentary film in October 2009.

After a series of meetings in the fall of 2010, the Presidential Commission for the study of Bioethical Issues released a report, on December 16, to the President calling for enhanced Federal oversight in the emerging field of sythetic biology. The panel that facilitated the production of the report, composed of 13 scientists, ethicists, and public policy experts, said that the very newness of the science, which involves the design and construction of laboratory-made biological parts, gives regulators, ethicists and others time to identify problems early on and craft solutions that can harness the technology for the public good.

“We comprehensively reviewed the developing field of synthetic biology to understand both its potential rewards and risks,” said Dr. Amy Gutmann, the Commission Chair and

President of the University of Pennsylvania. “We considered an array of approaches to regulation—from allowing unfettered freedom with minimal oversight and another to prohibiting experiments until they can be ruled completely safe beyond a reasonable doubt. We chose a middle course to maximize public benefits while also safeguarding against risks.”

Dr. Gutmann said the Commission’s approach recognizes the great potential of synthetic biology, including life saving medicines, and the generally distant risks posed by the field’s current capacity. “Prudent vigilance suggests that federal oversight is needed and can be exercised in a way that is consistent with scientific progress,” she said.

Key enabling technologies

There are several key enabling technologies that are critical to the growth of synthetic biology. The key concepts include standardization of biological parts and hierarchical abstraction to permit using those parts in increasingly complex synthetic systems. Achieving this is greatly aided by basic technologies of reading and writing of DNA (sequencing and fabrication), which are improving in price/performance exponentially (Kurzweil 2001). Measurements under a variety of conditions are needed for accurate modeling and computer-aided-design (CAD).

DNA sequencing

DNA sequencing is determining the order of the nucleotide bases in a molecule of DNA. Synthetic biologists make use of DNA sequencing in their work in several ways. First, large-scale genome sequencing efforts continue to provide a wealth of information on naturally occurring organisms. This information provides a rich substrate from which synthetic biologists can construct parts and devices. Second, synthetic biologists use sequencing to verify that they fabricated their engineered system as intended. Third, fast, cheap and reliable sequencing can also facilitate rapid detection and identification of synthetic systems and organisms.

Fabrication

A critical limitation in synthetic biology today is the time and effort expended during fabrication of engineered genetic sequences. To speed up the cycle of design, fabrication, testing and redesign, synthetic biology requires more rapid and reliable *de novo* DNA synthesis and assembly of fragments of DNA, in a process commonly referred to as gene synthesis.

In 2000, researchers at Washington University, mentioned synthesis of the 9.6 kbp Hepatitis C virus genome from chemically synthesized 60 to 80-mers. In 2002 researchers at SUNY Stony Brook succeeded in synthesizing the 7741 base poliovirus genome from its published sequence, producing the second synthetic genome. This took about two years of painstaking work. In 2003 the 5386 bp genome of the bacteriophage Phi X 174 was assembled in about two weeks. In 2006, the same team, at the J. Craig

Venter Institute, has constructed and patented a synthetic genome of a novel minimal bacterium, *Mycoplasma laboratorium* and is working on getting it functioning in a living cell.

In 2007 it was reported that several companies were offering the synthesis of genetic sequences up to 2000 bp long, for a price of about $1 per base pair and a turnaround time of less than two weeks. By September 2009, the price had dropped to less than $0.50 per base pair with some improvement in turn around time. Not only is the price judged lower than the cost of conventional cDNA cloning, the economics make it practical for researchers to design and purchase multiple variants of the same sequence to identify genes or proteins with optimized performance.

In 2010, Venter's group announced they had been able to assemble a complete genome of millions of base pairs, insert it into a cell, and cause that cell to start replicating.

Modeling

Models inform the design of engineered biological systems by allowing synthetic biologists to better predict system behavior prior to fabrication. Synthetic biology will benefit from better models of how biological molecules bind substrates and catalyze reactions, how DNA encodes the information needed to specify the cell and how multi-component integrated systems behave. Recently, multiscale models of gene regulatory networks have been developed that focus on synthetic biology applications. Simulations have been used that model all biomolecular interactions in transcription, translation, regulation, and induction of gene regulatory networks, guiding the design of synthetic systems.

Measurement

Precise and accurate quantitative measurements of biological systems are crucial to improving understanding of biology. Such measurements often help to elucidate how biological systems work and provide the basis for model construction and validation. Differences between predicted and measured system behavior can identify gaps in understanding and explain why synthetic systems don't always behave as intended. Technologies which allow many parallel and time-dependent measurements will be especially useful in synthetic biology. Microscopy and flow cytometry are examples of useful measurement technologies.

Chapter 9

Dip-Pen Nanolithography

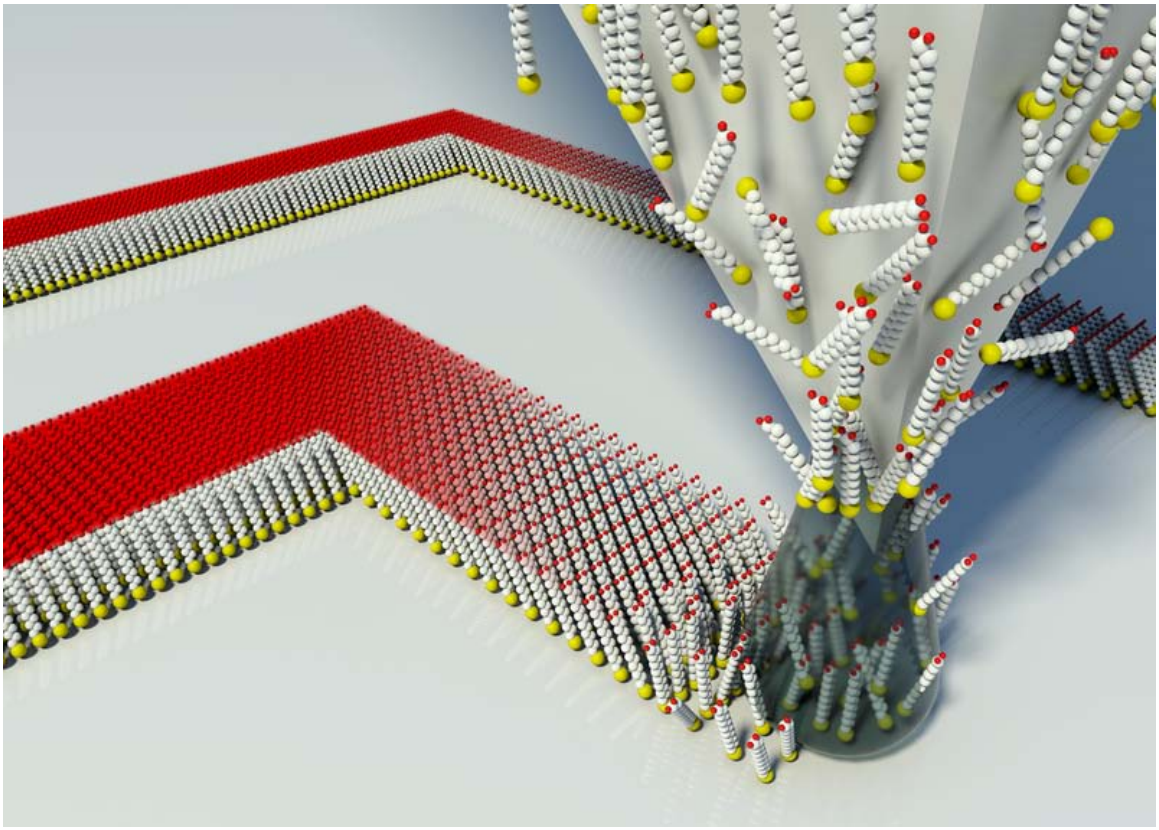
Dip Pen Nanolithography (DPN) began as a scanning probe lithography technique where an atomic force microscope tip was used to transfer alkane thiolates to a gold surface. This technique allows surface patterning on scales of under 100 nanometers. DPN is the nanotechnology analog of the dip pen (also called the quill pen), where the tip of an atomic force microscope cantilever acts as a "pen," which is coated with a chemical compound or mixture acting as an "ink," and put in contact with a substrate, the "paper."

DPN enables direct deposition of nanoscale materials onto a substrate in a flexible manner. Recent advances have demonstrated massively parallel patterning using two-dimensional arrays of 55,000 tips. Applications of this technology currently range through chemistry, materials science, and the life sciences, and include such work as ultra high density biological nanoarrays, and additive photomask repair.

The uncontrollable transfer of a molecular 'ink' from a coated AFM tip to a substrate was first reported by Jaschke and Butt in 1995, but they erroneously concluded that alkanethiols could not be transferred to gold substrates to form stable nanostructures. A research group at Northwestern University led by Chad Mirkin studied the process and determined that under the appropriate conditions, molecules could be transferred to a wide variety of surfaces to create stable chemically-adsorbed monolayers in a high resolution lithographic process they termed "DPN" . Mirkin and his coworkers hold the patents on this process, and the patterning technique has expanded to include liquid "inks". It is important to note that "liquid inks" are governed by a very different deposition mechanism when compared to "molecular inks".

Deposition Materials

Molecular Inks



Classic DPN mechanism: Molecular ink diffusing from a nanoscale tip to a surface through a water meniscus.

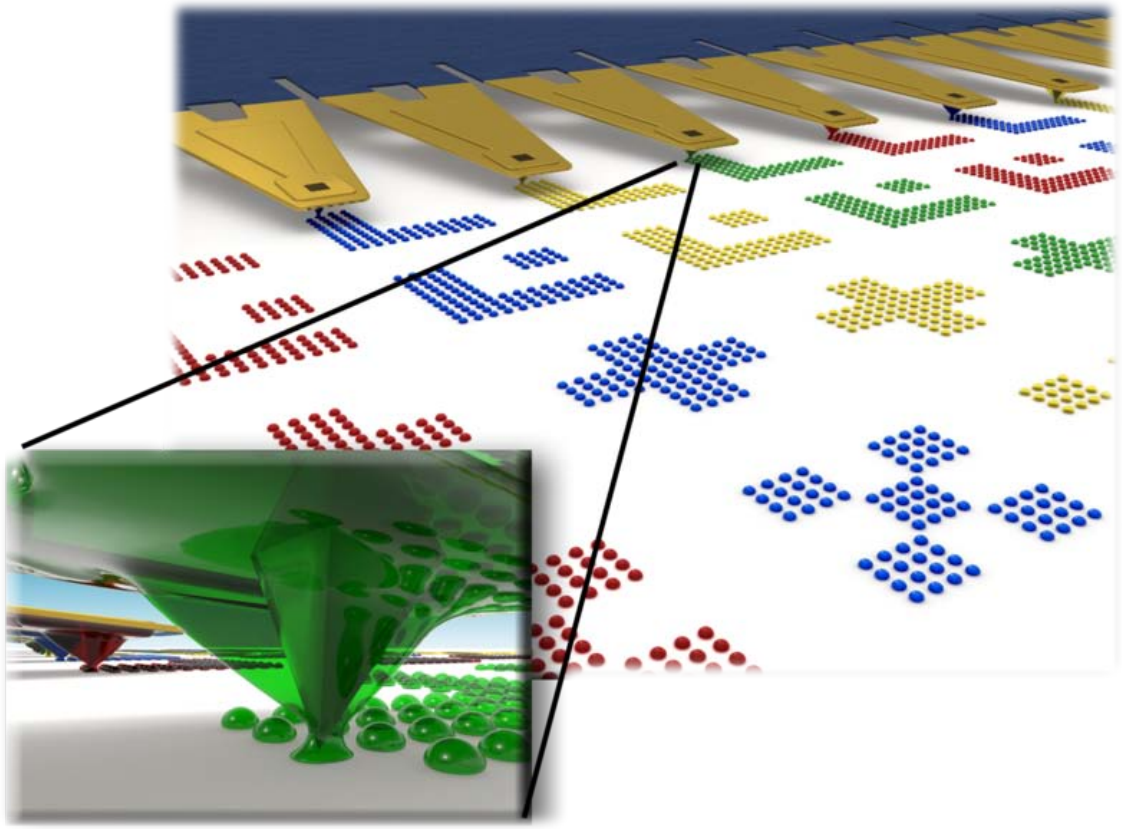
Molecular inks are typically composed of small molecules that are coated onto a DPN tip and are delivered to the surface through a water meniscus. In order to coat the tips, one can either vapor coat the tip or dip the tips into a dilute solution containing the molecular ink. If one dip-coats the tips, the solvent must be removed prior to deposition. The deposition rate of a molecular ink is dependent on the diffusion rate of the molecule, which is different for each molecule. The size of the feature is controlled by the tip/surface dwell-time (ranging from milliseconds to seconds) and the size of the water meniscus, which is determined by the humidity conditions (assuming the tip's radius of curvature is much smaller than the meniscus).

- Water meniscus mediated (exceptions do exist)
- Nanoscale feature resolution (50 nm to 2000 nm)
- No multiplexed depositions
- Each molecular ink is limited to its corresponding substrate

Examples

- Alkane thiols written to gold
- Silanes written to glass or silicon

Liquid Inks



Liquid ink deposition mechanism

Liquid inks can be any material that is liquid at deposition conditions. The liquid deposition properties are determined by the interactions between the liquid and the tip, the liquid and the surface, and the viscosity of the liquid itself. These interactions limit the minimum feature size of the liquid ink to about 1 micron, depending on the contact angle of the liquid. Higher viscosities offer greater control over feature size and are desirable. Unlike molecular inks, it is possible to perform multiplexed depositions using a carrier liquid. For example, using a viscous buffer, it is possible to directly deposit multiple proteins simultaneously.

- 1-10 micron feature resolution
- Multiplexed depositions
- Less restrictive ink/surface requirements
- Direct deposition of high viscosity materials

Examples

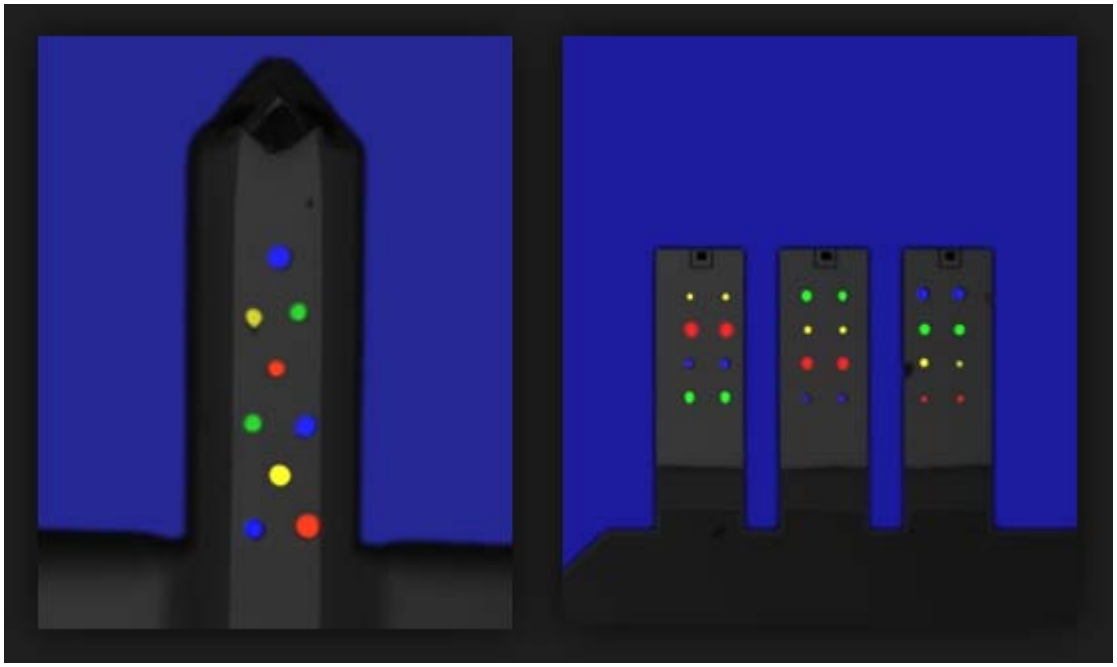
- Protein, peptide, and DNA patterning
- Hydrogels
- Sol gels
- Conductive inks
- Lipids

Applications

In order to define a good DPN application, it is important to understand what DPN can do that other techniques can't. Direct-write techniques, like contact printing, can pattern multiple biological materials but it cannot create features with subcellular resolution. Many high-resolution lithography methods can pattern at sub-micron resolution, but these require high-cost equipment that were not designed for biomolecule deposition and cell culture. Micro contact printing can print biomolecules at ambient conditions, but it cannot pattern multiple materials with nanoscale registry.

Industrial Applications

The following are some examples of how DPN is being applied to potential products.



Cantilever biosensor functionalized with 4 different proteins

1. Biosensor Functionalization - Directly place multiple capture domains on a single biosensor device

2. Nanoscale Sensor Fabrication - Small, high-value sensors that can detect multiple targets
3. Nanoscale Protein Chips - High-density protein arrays with increased sensitivity

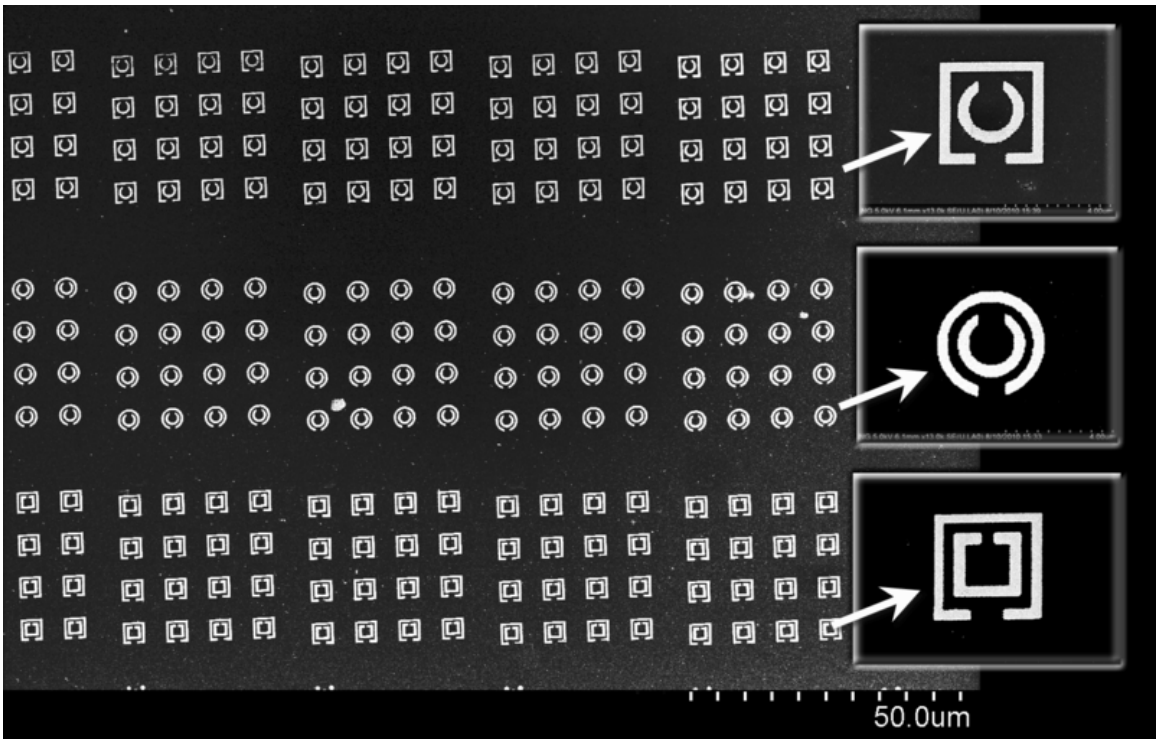
Emerging Applications

Cell Engineering

DPN is emerging as a powerful research tool for manipulating cells at subcellular resolution

- Stem cell differentiation
- Subcellular drug delivery
- Cell sorting
- Surface gradients
- Subcellular ECM protein patterns
- Cell adhesion

Rapid Prototyping



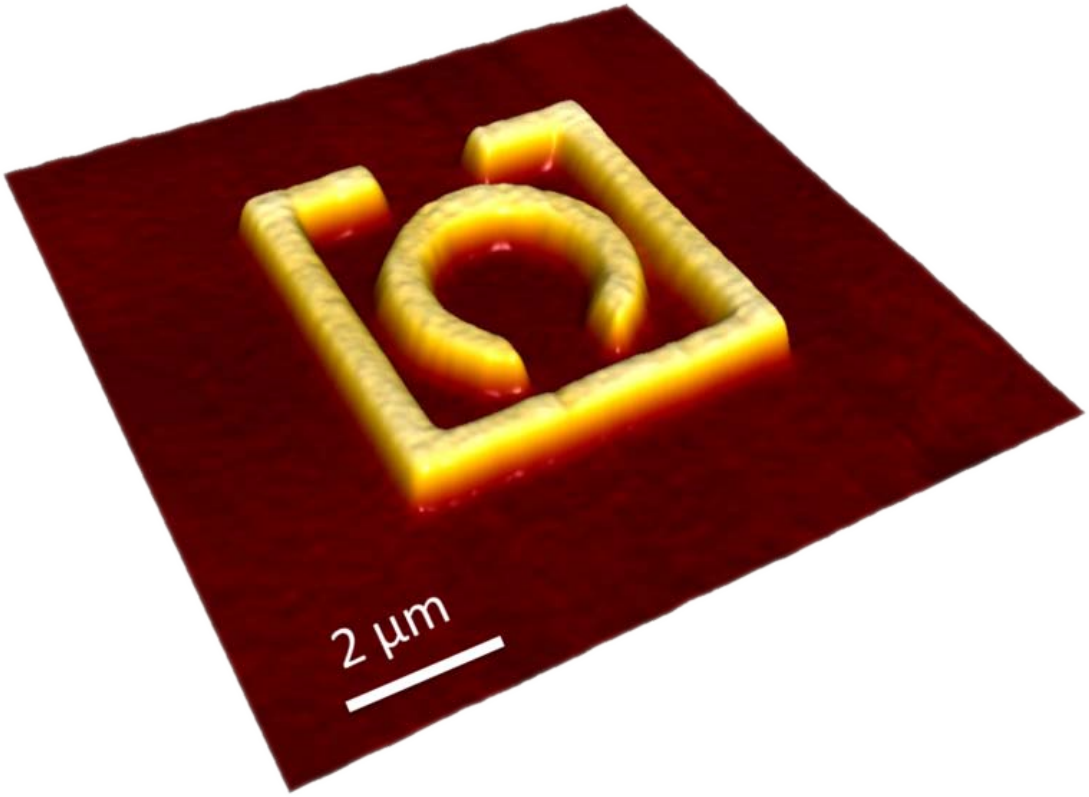
SEM image of DPN fabricated gold metastructure arrays.

- Plasmonics and Metamaterials
- Cell and tissue screening

DPN Properties

Direct Write

DPN is a direct write technique so it can be used for top-down and bottom-up lithography applications. In top-down work, the tips are used to deliver an etch resist to a surface, which is followed by a standard etching process. In bottom-up applications, the material of interest is delivered directly to the surface via the tips.



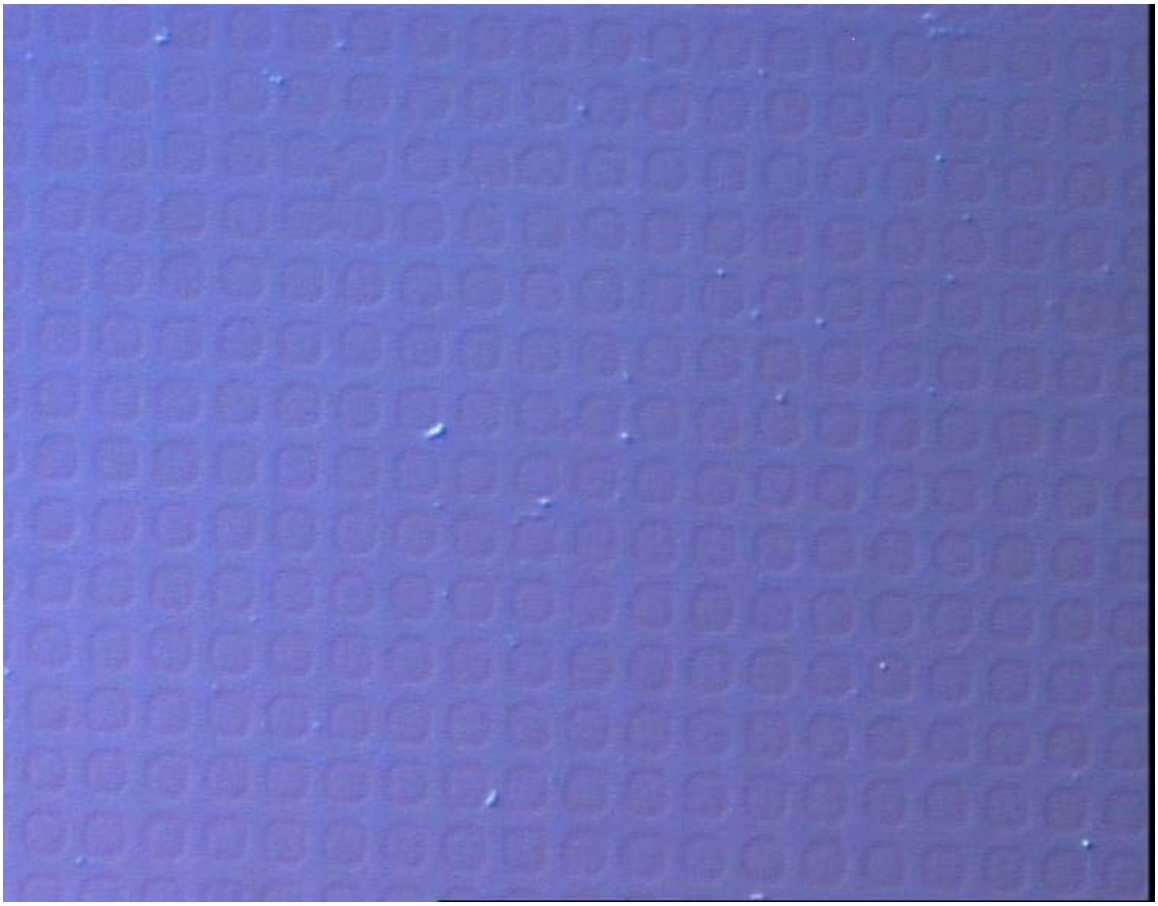
Gold on silicon metastructure fabricated with top-down DPN methods

Unique advantages

- Directed Placement - Directly print various materials onto existing nano and microstructures with nanoscale registry
- Direct Write - Maskless creation of arbitrary patterns with feature resolutions from as small as 50 nm and as large as 10 microns
- Biocompatible - Subcellular to nanoscale resolution at ambient deposition conditions
- Scalable - Force independent, allowing for parallel depositions

Common Misconceptions

Direct comparisons to other techniques



Streptavidin (4nm thickness)deposited using micro contact printing

The criticism most often directed at DPN is the patterning speed. The reason for this has more to do with how it is compared to other techniques rather than any inherent weaknesses. For example, the soft lithography method, micro contact printing (μCP), is the current standard for low cost, bench-top micro and nanoscale patterning, so it is easy to understand why DPN is compared directly to micro contact printing. The problem is that the comparisons are usually based upon applications that are strongly suited to μCP, instead of comparing them to some neutral application. μCP has the ability to pattern one material over a large area in a single stamping step, just as photolithography can pattern over a large area in a single exposure. Of course DPN is slow when it is compared to the strength of another technique. DPN is a maskless direct write technique that can be used to create multiple patterns of varying size, shape, and feature resolution, all on a single substrate. No one would try to apply micro contact printing to such a project because the it would never be worth the time and money required to fabricate each master stamp for each new pattern. Even if they did, micro contact printing would not be capable of aligning multiple materials from multiple stamps with nanoscale registry. The best way to

understand this misconception is to think about the different ways to apply photolithography and e-beam lithography. No one would try to use e-beam to solve a photolithography problem and then claim e-beam to be "too slow". Directly compared to photolithography's large area patterning capabilities, e-beam lithography **is** slow and yet, e-beam instruments can be found in every lab and nanofab in the world. The reason for this is because E-beam has unique capabilities that cannot be matched by photolithography, just as DPN has unique capabilities that cannot be matched by micro contact printing.

Connection to Atomic Force Microscopy

DPN evolved directly from AFM so it is not a surprise that people often assume that any commercial AFM can perform DPN experiments. In fact, DPN does not require an AFM, and an AFM does not necessarily have real DPN capabilities. There is an excellent analogy with scanning electron microscopy (SEM) and electron beam (E-beam) lithography. E-beam evolved directly from SEM technology and both use a focused electron beam, but no one would ever suggest that one could perform modern E-beam lithography experiments on a SEM that lacks the proper lithography hardware and software requirements.

It is also important to consider one of the unique characteristics of DPN, namely its force independence. With virtually all ink/substrate combinations, the same feature size will be patterned no matter how hard the tip is pressing down against the surface. As long as robust SiN tips are used, there is no need for complicated feedback electronics, no need for lasers, no need for quad photo-diodes, and no need for an AFM.

Chapter 10

Biochemistry

Biochemistry, sometimes called **biological chemistry**, is the study of chemical processes in living organisms, including, but not limited to, living matter. Biochemistry governs all living organisms and living processes. By controlling information flow through biochemical signalling and the flow of chemical energy through metabolism, biochemical processes give rise to the incredible complexity of life. Much of biochemistry deals with the structures and functions of cellular components such as proteins, carbohydrates, lipids, nucleic acids and other biomolecules although increasingly processes rather than individual molecules are the main focus. Over the last 40 years biochemistry has become so successful at explaining living processes that now almost all areas of the life sciences from botany to medicine are engaged in biochemical research. Today the main focus of pure biochemistry is in understanding how biological molecules give rise to the processes that occur within living cells which in turn relates greatly to the study and understanding of whole organisms.

Among the vast number of different biomolecules, many are complex and large molecules (called *biopolymers*), which are composed of similar repeating subunits (called *monomers*). Each class of polymeric biomolecule has a different set of subunit types. For example, a protein is a polymer whose subunits are selected from a set of 20 or more amino acids. Biochemistry studies the chemical properties of important biological molecules, like proteins, and in particular the chemistry of enzyme-catalyzed reactions.

The biochemistry of cell metabolism and the endocrine system has been extensively described. Other areas of biochemistry include the genetic code (DNA, RNA), protein synthesis, cell membrane transport, and signal transduction.

History

Originally, it was generally believed that life was not subject to the laws of science the way non-life was. It was thought that only living beings could produce the molecules of life (from other, previously existing biomolecules). Then, in 1828, Friedrich Wöhler published a paper on the synthesis of urea, proving that organic compounds can be created artificially.

The dawn of biochemistry may have been the discovery of the first enzyme, diastase (today called amylase), in 1833 by Anselme Payen. Eduard Buchner contributed the first demonstration of a complex biochemical process outside of a cell in 1896: alcoholic fermentation in cell extracts of yeast. Although the term "biochemistry" seems to have been first used in 1882, it is generally accepted that the formal coinage of biochemistry occurred in 1903 by Carl Neuberg, a German chemist. Previously, this area would have been referred to as physiological chemistry. Since then, biochemistry has advanced, especially since the mid-20th century, with the development of new techniques such as chromatography, X-ray diffraction, dual polarisation interferometry, NMR spectroscopy, radioisotopic labeling, electron microscopy and molecular dynamics simulations. These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell, such as glycolysis and the Krebs cycle (citric acid cycle).

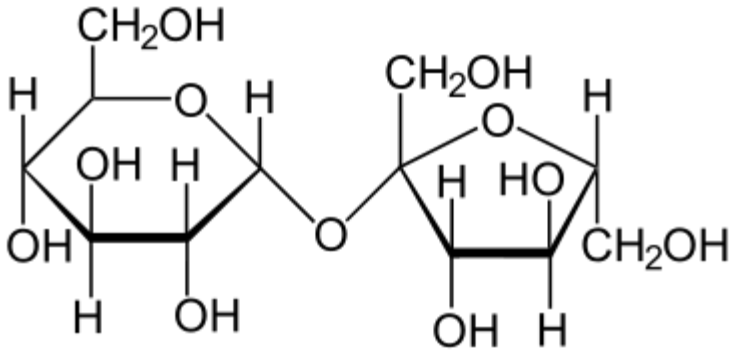
Another significant historic event in biochemistry is the discovery of the gene and its role in the transfer of information in the cell. This part of biochemistry is often called molecular biology. In the 1950s, James D. Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins were instrumental in solving DNA structure and suggesting its relationship with genetic transfer of information. In 1958, George Beadle and Edward Tatum received the Nobel Prize for work in fungi showing that one gene produces one enzyme. In 1988, Colin Pitchfork was the first person convicted of murder with DNA evidence, which led to growth of forensic science. More recently, Andrew Z. Fire and Craig C. Mello received the 2006 Nobel Prize for discovering the role of RNA interference (RNAi), in the silencing of gene expression.

Today, there are three main types of biochemistry. Plant biochemistry involves the study of the biochemistry of autotrophic organisms such as photosynthesis and other plant specific biochemical processes. General biochemistry encompasses both plant and animal biochemistry. Human/medical/medicinal biochemistry focuses on the biochemistry of humans and medical illnesses.

Biomolecules

The four main classes of molecules in biochemistry are carbohydrates, lipids, proteins, and nucleic acids. Many biological molecules are polymers: in this terminology, ***monomers*** are relatively small micromolecules that are linked together to create large macromolecules, which are known as ***polymers***. When monomers are linked together to synthesize a biological polymer, they undergo a process called dehydration synthesis.

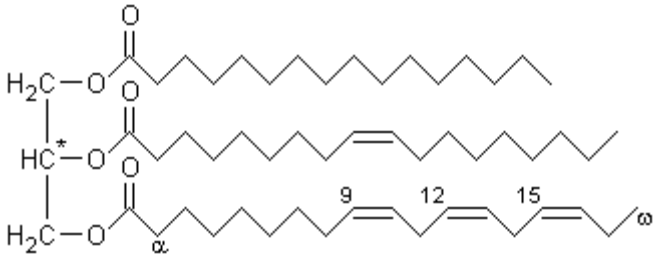
Carbohydrates



A molecule of sucrose (glucose + fructose), a disaccharide.

Carbohydrates are made from monomers called *monosaccharides*. Some of these monosaccharides include glucose ($C_6H_{12}O_6$), fructose ($C_6H_{12}O_6$), and deoxyribose ($C_5H_{10}O_4$). When two monosaccharides undergo dehydration synthesis, water is produced, as two hydrogen atoms and one oxygen atom are lost from the two monosaccharides' hydroxyl group.

Lipids

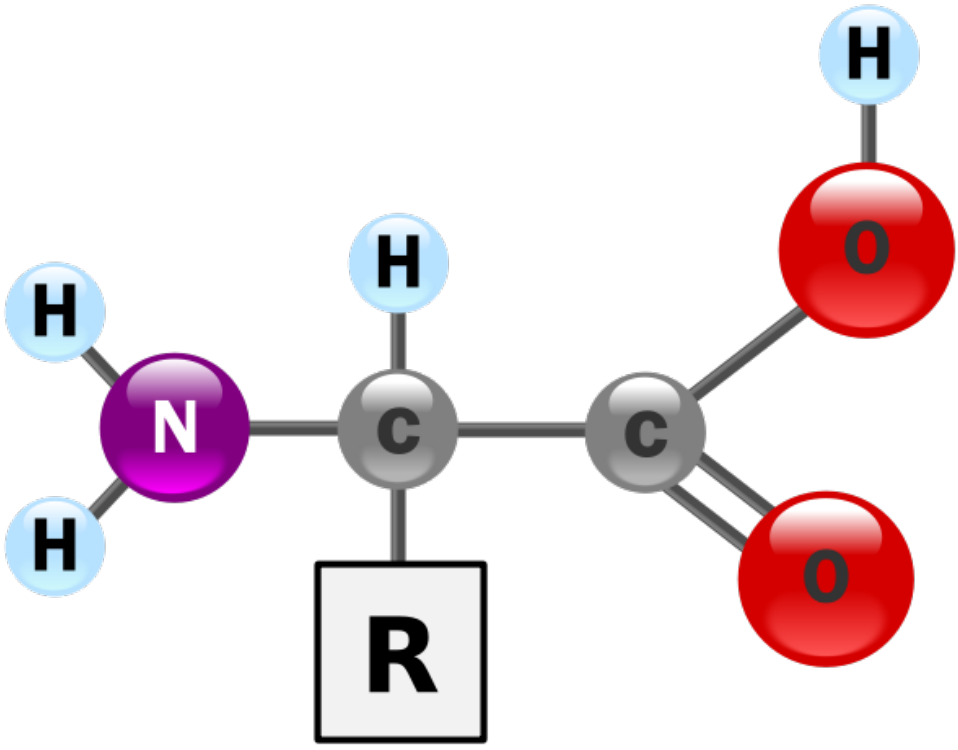


A triglyceride with a glycerol molecule on the left and three fatty acids coming off it.

Lipids are usually made from one molecule of glycerol combined with other molecules. In triglycerides, the main group of bulk lipids, there is one molecule of glycerol and three fatty acids. Fatty acids are considered the monomer in that case, and may be saturated (no double bonds in the carbon chain) or unsaturated (one or more double bonds in the carbon chain).

Lipids, especially phospholipids, are also used in various pharmaceutical products, either as co-solubilisers (e.g. in parenteral infusions) or else as drug carrier components (e.g. in a liposome or transfersome).

Proteins

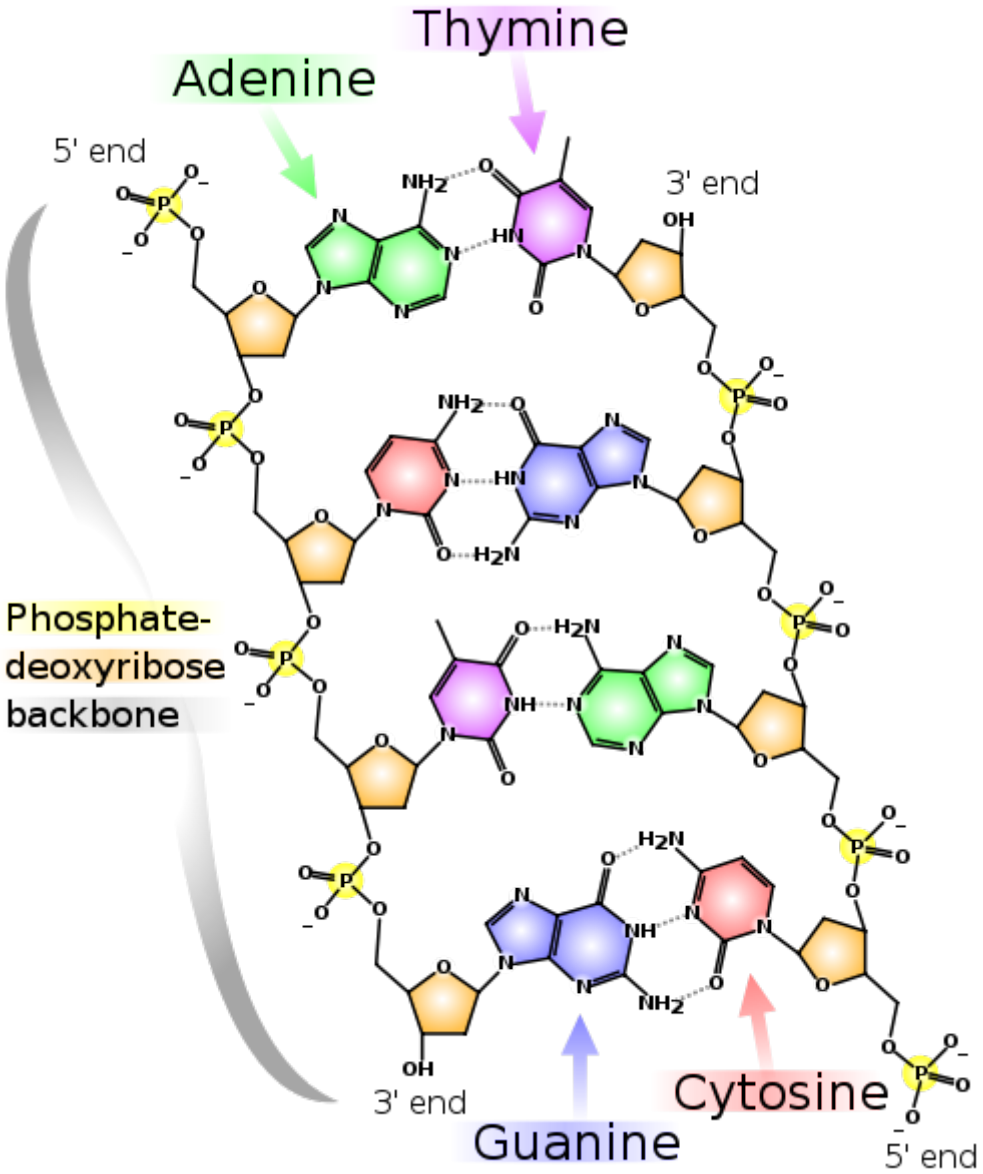


The general structure of an α-amino acid, with the amino group on the left and the carboxyl group on the right.

Proteins are very large molecules – macro-biopolymers – made from monomers called *amino acids*. There are 20 standard amino acids, each containing a carboxyl group, an amino group, and a side chain (known as an "R" group). The "R" group is what makes each amino acid different, and the properties of the side chains greatly influence the overall three-dimensional conformation of a protein. When amino acids combine, they form a special bond called a peptide bond through dehydration synthesis, and become a ***polypeptide***, or protein.

To determine if two proteins are related or in other words to decide whether they are homologous or not, scientists use sequence-comparison methods. Methods like Sequence Alignments and Structural Alignments are powerful tools that help scientist identify homologies between related molecules. The relevance of finding homologies among proteins goes beyond forming an evolutionary pattern of protein families. By finding how similar two protein sequences are, we acquire knowledge about their structure and therefore their function.

Nucleic acids



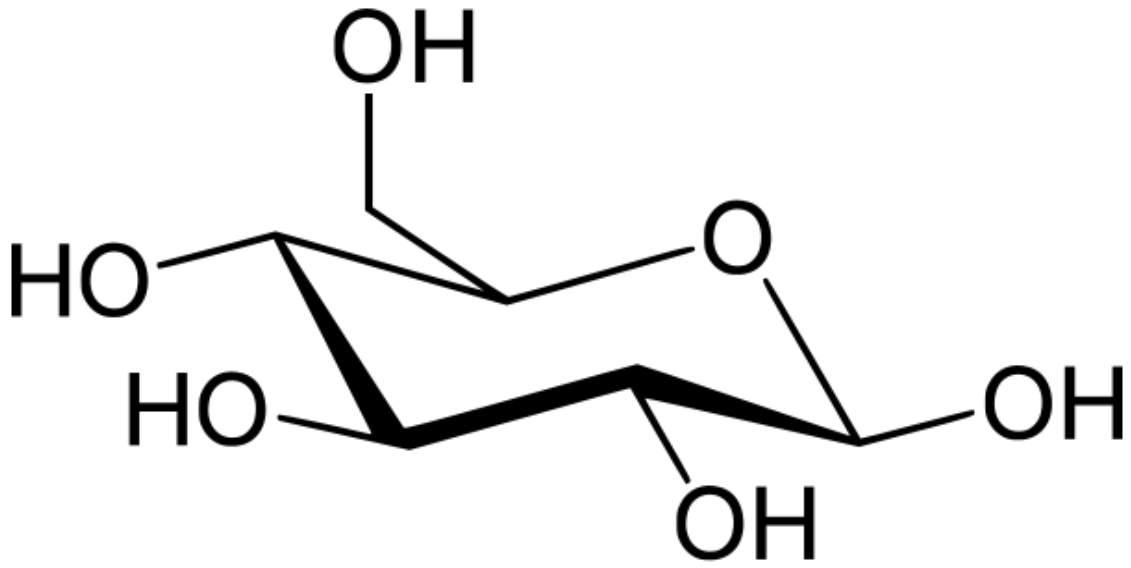
The structure of deoxyribonucleic acid (DNA), the picture shows the monomers being put together.

Nucleic acids are the molecules that make up DNA, an extremely important substance which all cellular organisms use to store their genetic information. The most common nucleic acids are deoxyribonucleic acid and ribonucleic acid. Their monomers are called nucleotides. The most common nucleotides are Adenine, Cytosine, Guanine, Thymine, and Uracil. Adenine binds with thymine and uracil; Thymine only binds with Adenine; and Cytosine and Guanine can only bind with each other.

Carbohydrates

The function of carbohydrates includes energy storage and providing structure. Sugars are carbohydrates, but not all carbohydrates are sugars. There are more carbohydrates on Earth than any other known type of biomolecule; they are used to store energy and genetic information, as well as play important roles in cell to cell interactions and communications.

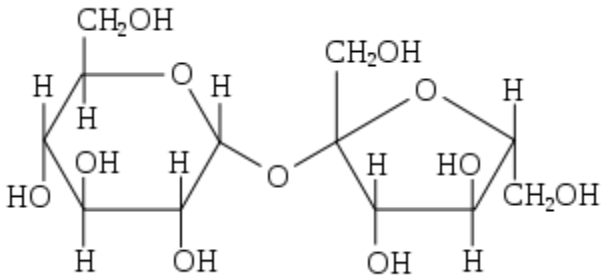
Monosaccharides



Glucose

The simplest type of carbohydrate is a monosaccharide, which among other properties contains carbon, hydrogen, and oxygen, mostly in a ratio of 1:2:1 (generalized formula $C_nH_{2n}O_n$, where n is at least 3). Glucose, one of the most important carbohydrates, is an example of a monosaccharide. So is fructose, the sugar commonly associated with the sweet taste of fruits. Some carbohydrates (especially after condensation to oligo- and polysaccharides) contain less carbon relative to H and O, which still are present in 2:1 (H:O) ratio. Monosaccharides can be grouped into aldoses (having an aldehyde group at the end of the chain, e. g. glucose) and ketoses (having a keto group in their chain; e. g. fructose). Both aldoses and ketoses occur in an equilibrium (starting with chain lengths of C4) cyclic forms. These are generated by bond formation between one of the hydroxyl groups of the sugar chain with the carbon of the aldehyde or keto group to form a hemiacetal bond. This leads to saturated five-membered (in furanoses) or six-membered (in pyranoses) heterocyclic rings containing one O as heteroatom.

Disaccharides

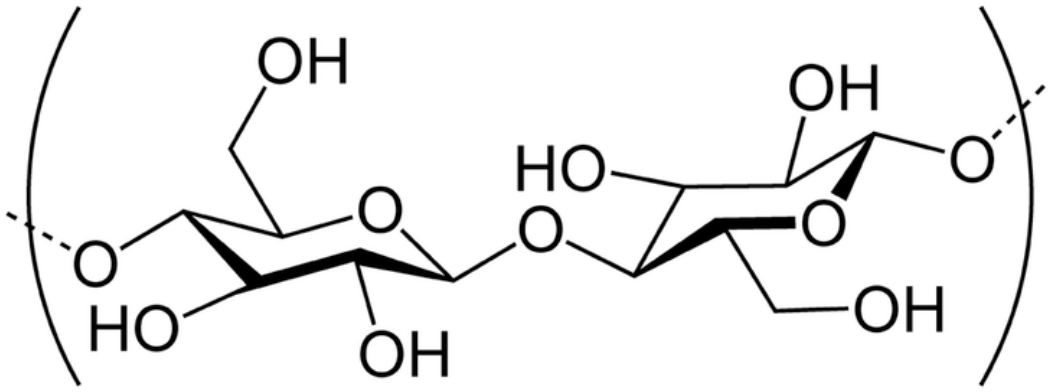


Sucrose: ordinary table sugar and probably the most familiar carbohydrate.

Two monosaccharides can be joined together using dehydration synthesis, in which a hydrogen atom is removed from the end of one molecule and a hydroxyl group (—OH) is removed from the other; the remaining residues are then attached at the sites from which the atoms were removed. The H—OH or H_2O is then released as a molecule of water, hence the term *dehydration*. The new molecule, consisting of two monosaccharides, is called a *disaccharide* and is conjoined together by a glycosidic or ether bond. The reverse reaction can also occur, using a molecule of water to split up a disaccharide and break the glycosidic bond; this is termed *hydrolysis*. The most well-known disaccharide is sucrose, ordinary sugar (in scientific contexts, called *table sugar* or *cane sugar* to differentiate it from other sugars). Sucrose consists of a glucose molecule and a fructose molecule joined together. Another important disaccharide is lactose, consisting of a glucose molecule and a galactose molecule. As most humans age, the production of lactase, the enzyme that hydrolyzes lactose back into glucose and galactose, typically decreases. This results in lactase deficiency, also called *lactose intolerance*.

Sugar polymers are characterised by having reducing or non-reducing ends. A reducing end of a carbohydrate is a carbon atom which can be in equilibrium with the open-chain aldehyde or keto form. If the joining of monomers takes place at such a carbon atom, the free hydroxy group of the pyranose or furanose form is exchanged with an OH-side chain of another sugar, yielding a full acetal. This prevents opening of the chain to the aldehyde or keto form and renders the modified residue non-reducing. Lactose contains a reducing end at its glucose moiety, whereas the galactose moiety form a full acetal with the C4-OH group of glucose. Saccharose does not have a reducing end because of full acetal formation between the aldehyde carbon of glucose (C1) and the keto carbon of fructose (C2).

Oligosaccharides and polysaccharides



Cellulose as polymer of β-D-glucose

When a few (around three to six) monosaccharides are joined together, it is called an *oligosaccharide* (*oligo-* meaning "few"). These molecules tend to be used as markers and signals, as well as having some other uses. Many monosaccharides joined together make a polysaccharide. They can be joined together in one long linear chain, or they may be branched. Two of the most common polysaccharides are cellulose and glycogen, both consisting of repeating glucose monomers.

- *Cellulose* is made by plants and is an important structural component of their cell walls. Humans can neither manufacture nor digest it.
- *Glycogen*, on the other hand, is an animal carbohydrate; humans and other animals use it as a form of energy storage.

Use of carbohydrates as an energy source

Glucose is the major energy source in most life forms. For instance, polysaccharides are broken down into their monomers (glycogen phosphorylase removes glucose residues from glycogen). Disaccharides like lactose or sucrose are cleaved into their two component monosaccharides.

Glycolysis (anaerobic)

Glucose is mainly metabolized by a very important ten-step pathway called glycolysis, the net result of which is to break down one molecule of glucose into two molecules of pyruvate; this also produces a net two molecules of ATP, the energy currency of cells, along with two reducing equivalents in the form of converting NAD^+ to NADH. This does not require oxygen; if no oxygen is available (or the cell cannot use oxygen), the NAD is restored by converting the pyruvate to lactate (lactic acid) (e. g. in humans) or to

ethanol plus carbon dioxide (e. g. in yeast). Other monosaccharides like galactose and fructose can be converted into intermediates of the glycolytic pathway.

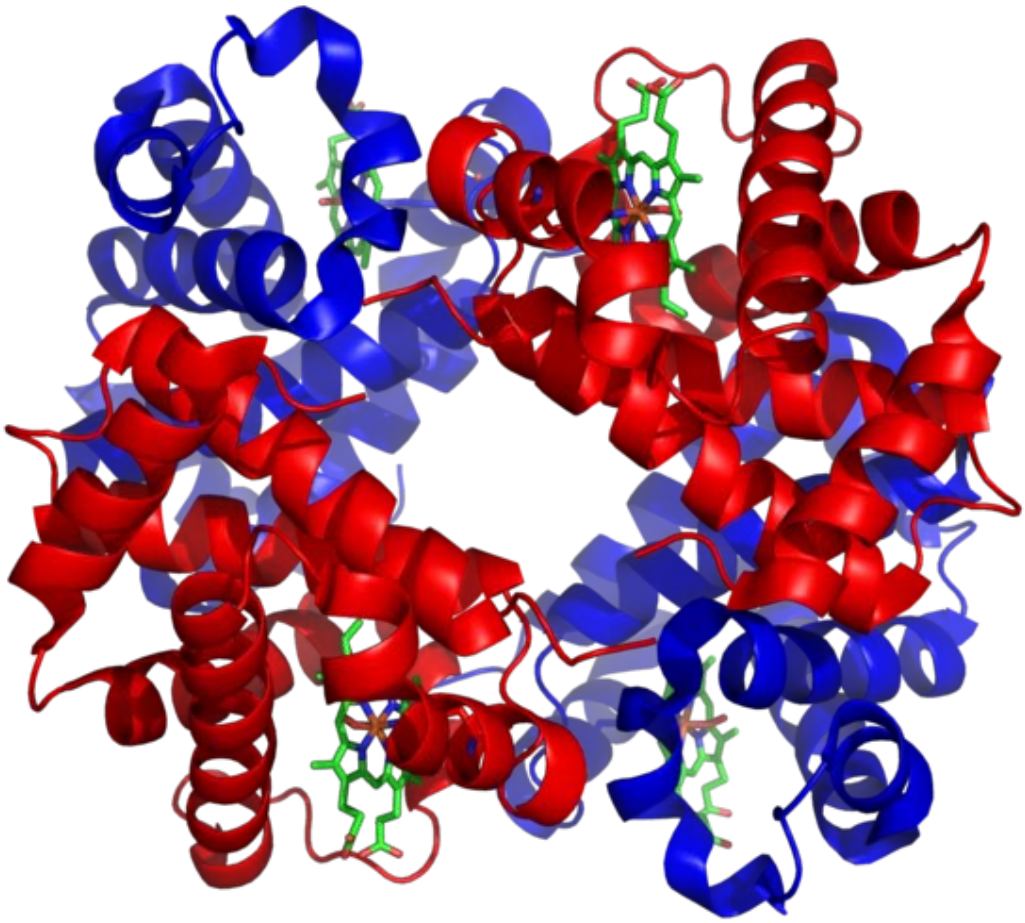
Aerobic

In aerobic cells with sufficient oxygen, like most human cells, the pyruvate is further metabolized. It is irreversibly converted to acetyl-CoA, giving off one carbon atom as the waste product carbon dioxide, generating another reducing equivalent as NADH. The two molecules acetyl-CoA (from one molecule of glucose) then enter the citric acid cycle, producing two more molecules of ATP, six more NADH molecules and two reduced (ubi)quinones (via $FADH_2$ as enzyme-bound cofactor), and releasing the remaining carbon atoms as carbon dioxide. The produced NADH and quinol molecules then feed into the enzyme complexes of the respiratory chain, an electron transport system transferring the electrons ultimately to oxygen and conserving the released energy in the form of a proton gradient over a membrane (inner mitochondrial membrane in eukaryotes). Thereby, oxygen is reduced to water and the original electron acceptors NAD^+ and quinone are regenerated. This is why humans breathe in oxygen and breathe out carbon dioxide. The energy released from transferring the electrons from high-energy states in NADH and quinol is conserved first as proton gradient and converted to ATP via ATP synthase. This generates an additional *28* molecules of ATP (24 from the 8 NADH + 4 from the 2 quinols), totaling to 32 molecules of ATP conserved per degraded glucose (two from glycolysis + two from the citrate cycle). It is clear that using oxygen to completely oxidize glucose provides an organism with far more energy than any oxygen-independent metabolic feature, and this is thought to be the reason why complex life appeared only after Earth's atmosphere accumulated large amounts of oxygen.

Gluconeogenesis

In vertebrates, vigorously contracting skeletal muscles (during weightlifting or sprinting, for example) do not receive enough oxygen to meet the energy demand, and so they shift to anaerobic metabolism, converting glucose to lactate. The liver regenerates the glucose, using a process called gluconeogenesis. This process is not quite the opposite of glycolysis, and actually requires three times the amount of energy gained from glycolysis (six molecules of ATP are used, compared to the two gained in glycolysis). Analogous to the above reactions, the glucose produced can then undergo glycolysis in tissues that need energy, be stored as glycogen (or starch in plants), or be converted to other monosaccharides or joined into di- or oligosaccharides. The combined pathways of glycolysis during exercise, lactate's crossing via the bloodstream to the liver, subsequent gluconeogenesis and release of glucose into the bloodstream is called the Cori cycle.

Proteins

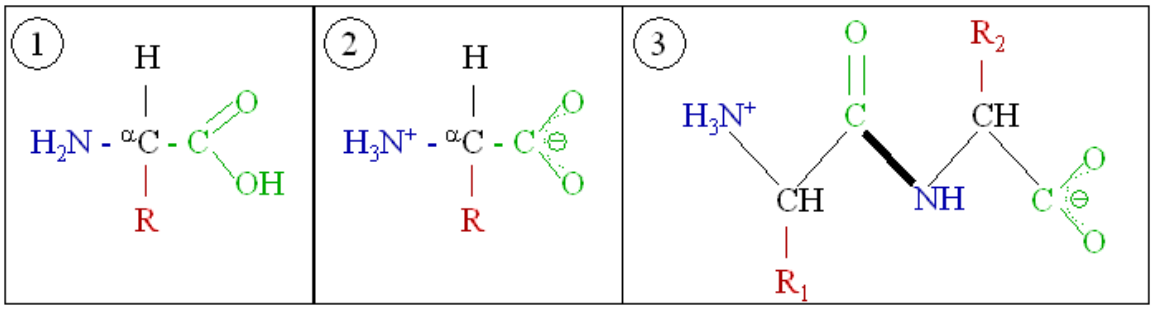


A schematic of hemoglobin. The red and blue ribbons represent the protein globin; the green structures are the heme groups.

Like carbohydrates, some proteins perform largely structural roles. For instance, movements of the proteins actin and myosin ultimately are responsible for the contraction of skeletal muscle. One property many proteins have is that they specifically bind to a certain molecule or class of molecules—they may be *extremely* selective in what they bind. Antibodies are an example of proteins that attach to one specific type of molecule. In fact, the enzyme-linked immunosorbent assay (ELISA), which uses antibodies, is currently one of the most sensitive tests modern medicine uses to detect various biomolecules. Probably the most important proteins, however, are the enzymes. These molecules recognize specific reactant molecules called *substrates*; they then catalyze the reaction between them. By lowering the activation energy, the enzyme speeds up that

reaction by a rate of 10^{11} or more: a reaction that would normally take over 3,000 years to complete spontaneously might take less than a second with an enzyme. The enzyme itself is not used up in the process, and is free to catalyze the same reaction with a new set of substrates. Using various modifiers, the activity of the enzyme can be regulated, enabling control of the biochemistry of the cell as a whole.

In essence, proteins are chains of amino acids. An amino acid consists of a carbon atom bound to four groups. One is an amino group, —NH_2, and one is a carboxylic acid group, —COOH (although these exist as —NH_3^+ and —COO^- under physiologic conditions). The third is a simple hydrogen atom. The fourth is commonly denoted "—R" and is different for each amino acid. There are twenty standard amino acids. Some of these have functions by themselves or in a modified form; for instance, glutamate functions as an important neurotransmitter.



Generic amino acids (1) in neutral form, (2) as they exist physiologically, and (3) joined together as a dipeptide.

Amino acids can be joined together via a peptide bond. In this dehydration synthesis, a water molecule is removed and the peptide bond connects the nitrogen of one amino acid's amino group to the carbon of the other's carboxylic acid group. The resulting molecule is called a *dipeptide*, and short stretches of amino acids (usually, fewer than around thirty) are called *peptides* or polypeptides. Longer stretches merit the title *proteins*. As an example, the important blood serum protein albumin contains 585 amino acid residues.

The structure of proteins is traditionally described in a hierarchy of four levels. The primary structure of a protein simply consists of its linear sequence of amino acids; for instance, "alanine-glycine-tryptophan-serine-glutamate-asparagine-glycine-lysine-…". Secondary structure is concerned with local morphology (morphology being the study of structure). Some combinations of amino acids will tend to curl up in a coil called an α-helix or into a sheet called a β-sheet; some α-helixes can be seen in the hemoglobin schematic above. Tertiary structure is the entire three-dimensional shape of the protein. This shape is determined by the sequence of amino acids. In fact, a single change can change the entire structure. The alpha chain of hemoglobin contains 146 amino acid residues; substitution of the glutamate residue at position 6 with a valine residue changes the behavior of hemoglobin so much that it results in sickle-cell disease. Finally quaternary structure is concerned with the structure of a protein with multiple peptide

subunits, like hemoglobin with its four subunits. Not all proteins have more than one subunit.

Ingested proteins are usually broken up into single amino acids or dipeptides in the small intestine, and then absorbed. They can then be joined together to make new proteins. Intermediate products of glycolysis, the citric acid cycle, and the pentose phosphate pathway can be used to make all twenty amino acids, and most bacteria and plants possess all the necessary enzymes to synthesize them. Humans and other mammals, however, can only synthesize half of them. They cannot synthesize isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. These are the essential amino acids, since it is essential to ingest them. Mammals do possess the enzymes to synthesize alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine, the nonessential amino acids. While they can synthesize arginine and histidine, they cannot produce it in sufficient amounts for young, growing animals, and so these are often considered essential amino acids.

If the amino group is removed from an amino acid, it leaves behind a carbon skeleton called an α-keto acid. Enzymes called transaminases can easily transfer the amino group from one amino acid (making it an α-keto acid) to another α-keto acid (making it an amino acid). This is important in the biosynthesis of amino acids, as for many of the pathways, intermediates from other biochemical pathways are converted to the α-keto acid skeleton, and then an amino group is added, often via transamination. The amino acids may then be linked together to make a protein.

A similar process is used to break down proteins. It is first hydrolyzed into its component amino acids. Free ammonia (NH_3), existing as the ammonium ion (NH_4^+) in blood, is toxic to life forms. A suitable method for excreting it must therefore exist. Different strategies have evolved in different animals, depending on the animals' needs. Unicellular organisms, of course, simply release the ammonia into the environment. Similarly, bony fish can release the ammonia into the water where it is quickly diluted. In general, mammals convert the ammonia into urea, via the urea cycle.

Lipids

The term lipid comprises a diverse range of molecules and to some extent is a catchall for relatively water-insoluble or nonpolar compounds of biological origin, including waxes, fatty acids, fatty-acid derived phospholipids, sphingolipids, glycolipids and terpenoids (e.g. retinoids and steroids). Some lipids are linear aliphatic molecules, while others have ring structures. Some are aromatic, while others are not. Some are flexible, while others are rigid.

Most lipids have some polar character in addition to being largely nonpolar. Generally, the bulk of their structure is nonpolar or hydrophobic ("water-fearing"), meaning that it does not interact well with polar solvents like water. Another part of their structure is polar or hydrophilic ("water-loving") and will tend to associate with polar solvents like water. This makes them amphiphilic molecules (having both hydrophobic and

hydrophilic portions). In the case of cholesterol, the polar group is a mere -OH (hydroxyl or alcohol). In the case of phospholipids, the polar groups are considerably larger and more polar, as described below.

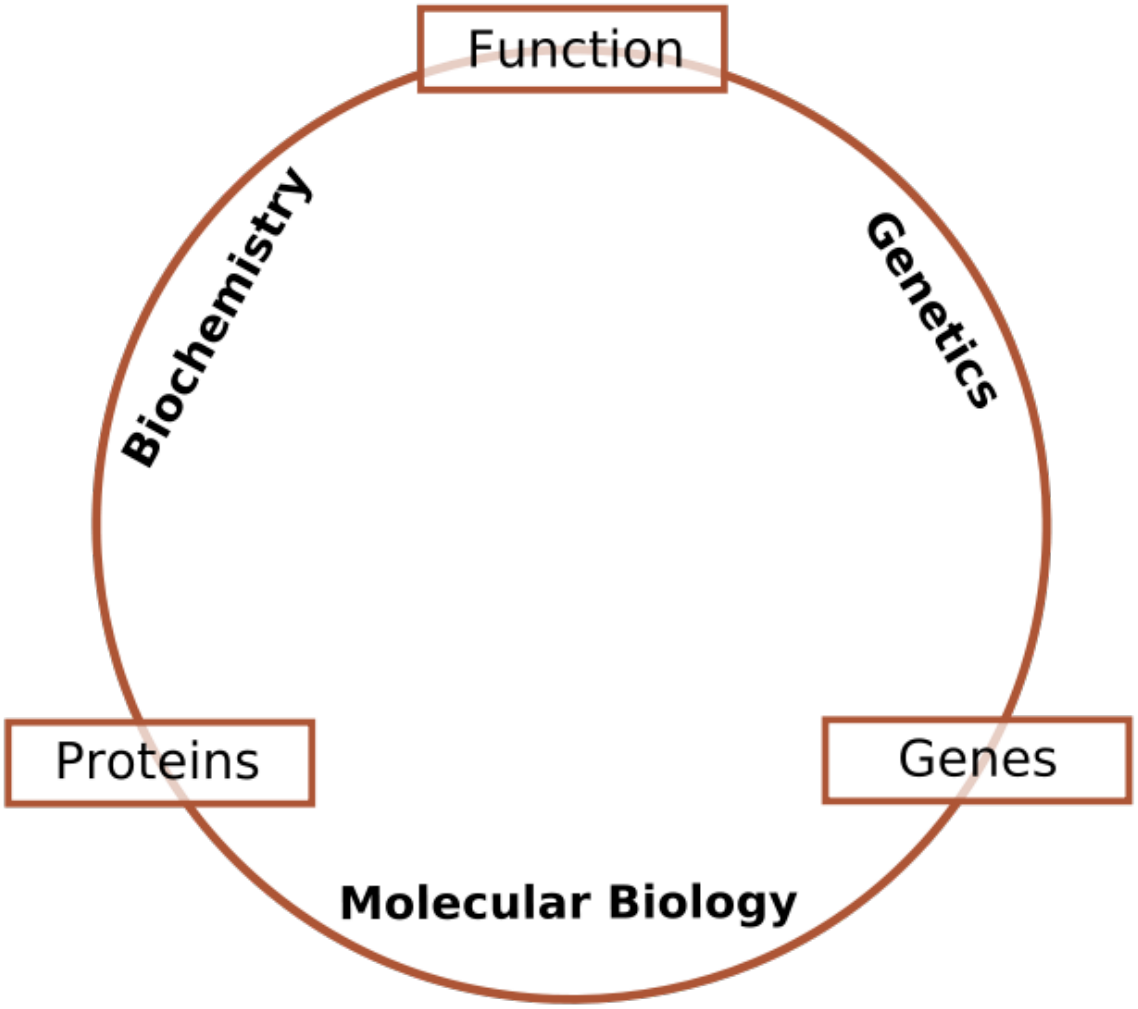
Lipids are an integral part of our daily diet. Most oils and milk products that we use for cooking and eating like butter, cheese, ghee etc., are composed of fats. Vegetable oils are rich in various polyunsaturated fatty acids (PUFA). Lipid-containing foods undergo digestion within the body and are broken into fatty acids and glycerol, which are the final degradation products of fats and lipids.

Nucleic acids

A nucleic acid is a complex, high-molecular-weight biochemical macromolecule composed of nucleotide chains that convey genetic information. The most common nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are found in all living cells and viruses. Aside from the genetic material of the cell, nucleic acids often play a role as second messengers, as well as forming the base molecule for adenosine triphosphate, the primary energy-carrier molecule found in all living organisms.

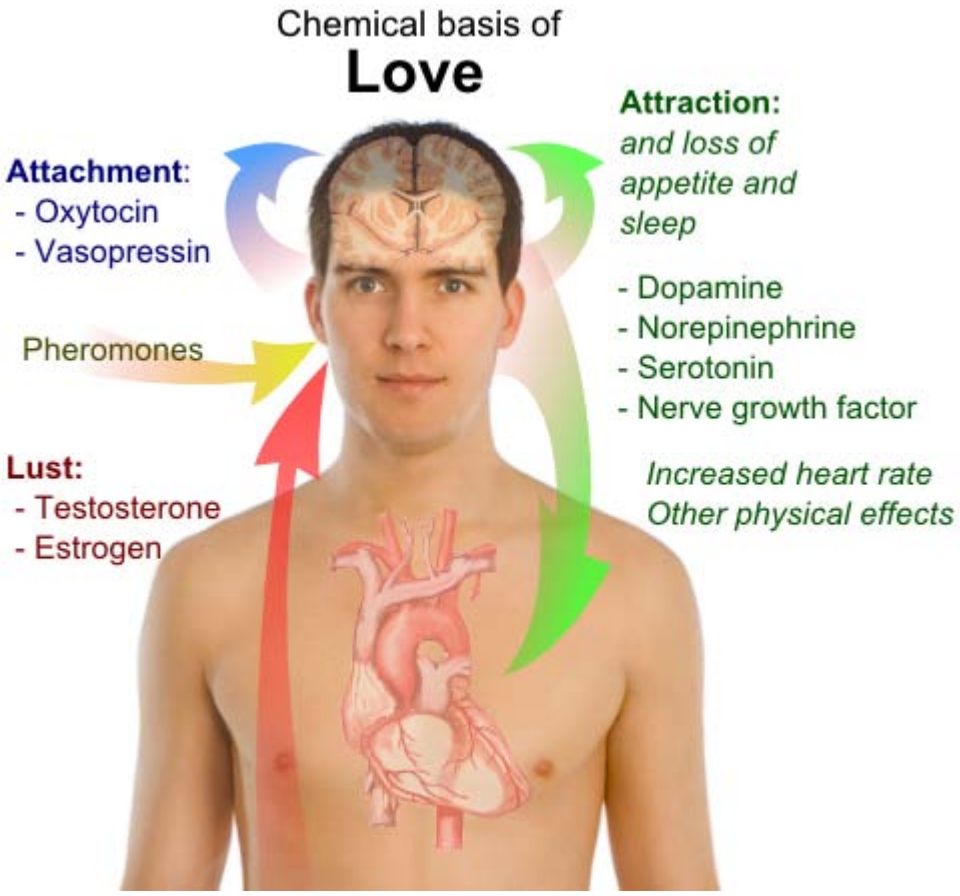
Nucleic acid, so called because of its prevalence in cellular nuclei, is the generic name of the family of biopolymers. The monomers are called nucleotides, and each consists of three components: a nitrogenous heterocyclic base (either a purine or a pyrimidine), a pentose sugar, and a phosphate group. Different nucleic acid types differ in the specific sugar found in their chain (e.g. DNA or deoxyribonucleic acid contains 2-deoxyriboses). Also, the nitrogenous bases possible in the two nucleic acids are different: adenine, cytosine, and guanine occur in both RNA and DNA, while thymine occurs only in DNA and uracil occurs in RNA.

Relationship to other "molecular-scale" biological sciences



Schematic relationship between biochemistry, genetics and molecular biology

Researchers in biochemistry use specific techniques native to biochemistry, but increasingly combine these with techniques and ideas from genetics, molecular biology and biophysics. There has never been a hard-line between these disciplines in terms of content and technique. Today the terms *molecular biology* and *biochemistry* are nearly interchangeable. The following figure is a schematic that depicts one possible view of the relationship between the fields:



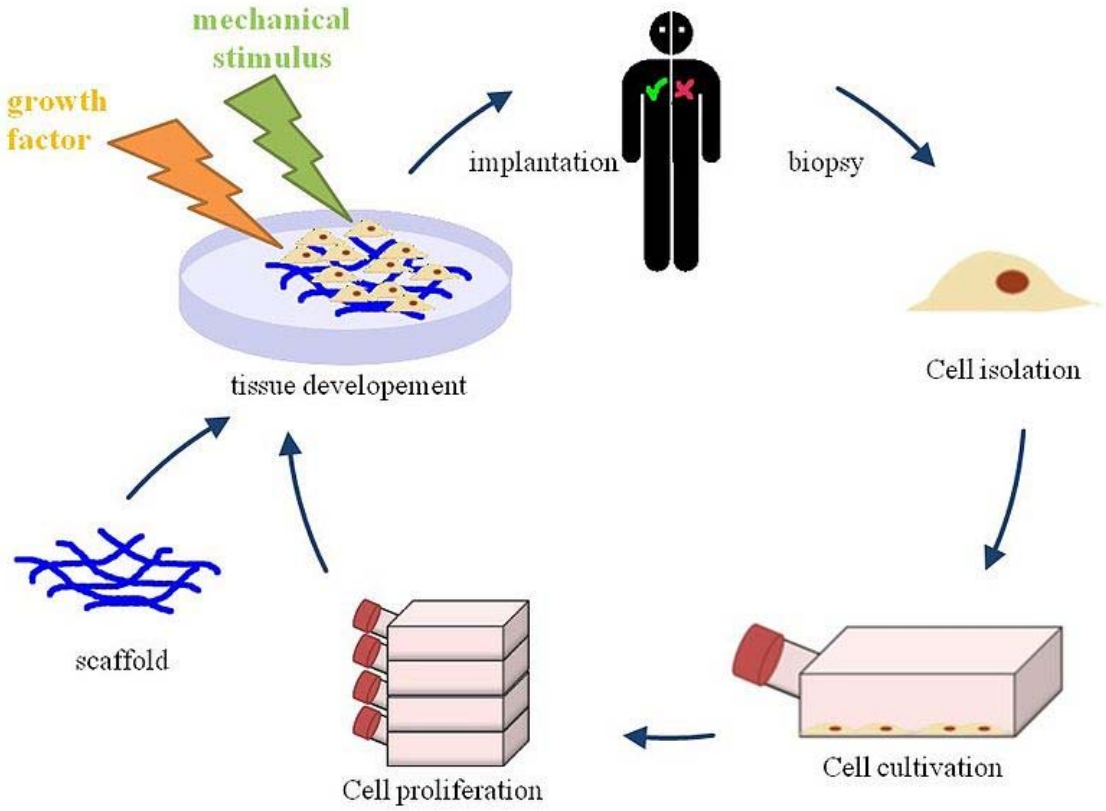
Simplistic overview of the chemical basis of love, one of many applications that may be described in terms of biochemistry.

- *Biochemistry* is the study of the chemical substances and vital processes occurring in living organisms. Biochemists focus heavily on the role, function, and structure of biomolecules. The study of the chemistry behind biological processes and the synthesis of biologically active molecules are examples of biochemistry.
- *Genetics* is the study of the effect of genetic differences on organisms. Often this can be inferred by the absence of a normal component (e.g. one gene). The study of "mutants" – organisms which lack one or more functional components with respect to the so-called "wild type" or normal phenotype. Genetic interactions (epistasis) can often confound simple interpretations of such "knock-out" studies.
- *Molecular biology* is the study of molecular underpinnings of the process of replication, transcription and translation of the genetic material. The central dogma of molecular biology where genetic material is transcribed into RNA and then translated into protein, despite being an oversimplified picture of molecular biology, still provides a good starting point for understanding the field. This picture, however, is undergoing revision in light of emerging novel roles for RNA.
- *Chemical Biology* seeks to develop new tools based on small molecules that allow minimal perturbation of biological systems while providing detailed information

about their function. Further, chemical biology employs biological systems to create non-natural hybrids between biomolecules and synthetic devices (for example emptied viral capsids that can deliver gene therapy or drug molecules).

Chapter 11

Tissue Engineering

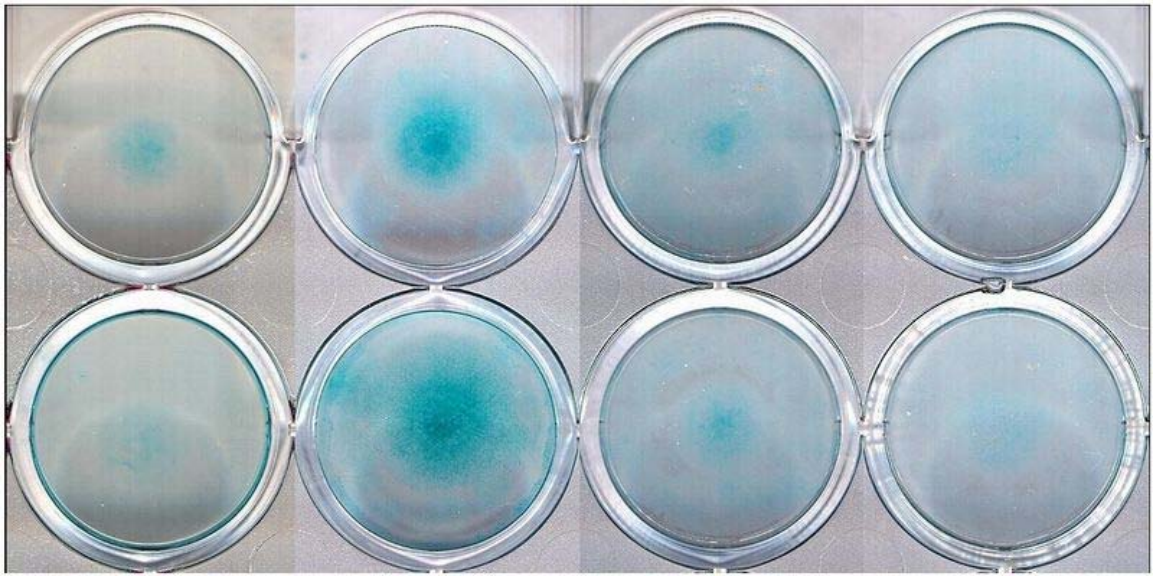


Principle of tissue engineering

Tissue engineering was once categorized as a sub-field of bio materials, but having grown in scope and importance it can be considered as a field in its own right. It is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions. While most definitions of tissue engineering cover a broad range of applications, in practice the term is closely associated with applications that repair or replace portions of

or whole tissues (i.e., bone, cartilage, blood vessels, bladder, skin etc.). Often, the tissues involved require certain mechanical and structural properties for proper functioning. The term has also been applied to efforts to perform specific biochemical functions using cells within an artificially-created support system (e.g. an artificial pancreas, or a bio artificial liver). The term **regenerative medicine** is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues.

Overview



Micro-mass cultures of C3H-10T1/2 cells at varied oxygen tensions stained with Alcian blue.

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ". Tissue engineering has also been defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." A further description goes on to say that an "underlying supposition of tissue engineering is that the employment of natural biology of the system will allow for greater success in developing therapeutic strategies aimed at the replacement, repair, maintenance, and/or enhancement of tissue function."

Powerful developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in

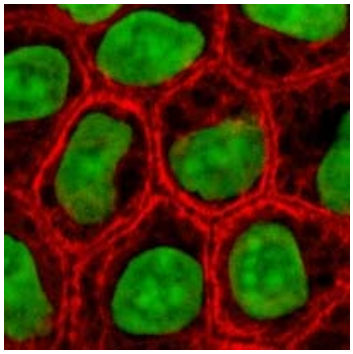
laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell, and developmental biology, as well as materials science and bio informatics.

In 2003, the NSF published a report entitled "The Emergence of Tissue Engineering as a Research Field" , which gives a thorough description of the history of this field.

Examples

- Tissue engineered autologous heartvalves and vessels - workgroup of Dr.med.S.Jockenhoevel at the Department of Applied Medical Engineering (RWTH-Aachen University,Germany)
- In vitro meat — Edible artificial animal muscle tissue cultured *in vitro*.
- Bioartificial liver device — several research efforts have produced hepatic assist devices utilizing living hepatocytes.
- Artificial pancreas — research involves using islet cells to produce and regulate insulin, particularly in cases of diabetes.
- Artificial bladders — Anthony Atala (Wake Forest University) has successfully implanted artificially grown bladders into seven out of approximately 20 human test subjects as part of a long-term experiment.
- Cartilage — lab-grown tissue was successfully used to repair knee cartilage.
- Doris Taylor's heart in a jar
- Tissue-engineered airway
- Artificial skin constructed from human skin cells embedded in collagen
- Artificial bone marrow
- Artificial bone
- Artificial penis

Cells as building blocks



Stained cells in culture

Tissue engineering utilizes living cells as engineering materials. Examples include using living fibroblasts in skin replacement or repair, cartilage repaired with living chondrocytes, or other types of cells used in other ways.

Cells became available as engineering materials when scientists at Geron Corp. discovered how to extend telomeres in 1998, producing immortalized cell lines. Before this, laboratory cultures of healthy, noncancerous mammalian cells would only divide a fixed number of times, up to the Hayflick limit.

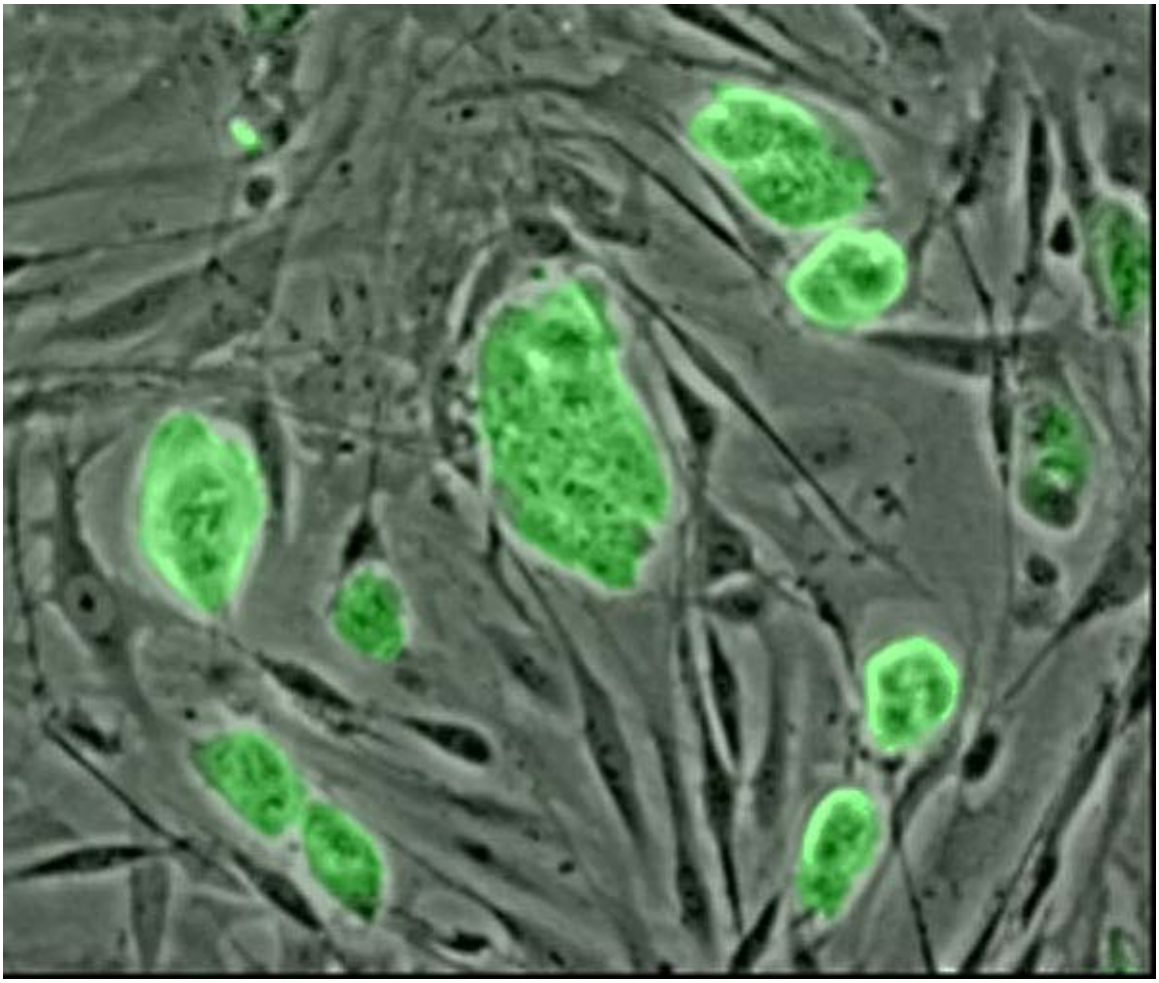
Extraction

From fluid tissues such as blood, cells are extracted by bulk methods, usually centrifugation or apheresis. From solid tissues, extraction is more difficult. Usually the tissue is minced, and then digested with the enzymes trypsin or collagenase to remove the extracellular matrix that holds the cells. After that, the cells are free floating, and extracted using centrifugation or apheresis.
Digestion with trypsin is very dependent on temperature. Higher temperatures digest the matrix faster, but create more damage. Collagenase is less temperature dependent, and damages fewer cells, but takes longer and is a more expensive reagent.

Types of cells

Cells are often categorized by their source:

- **Autologous** cells are obtained from the same individual to which they will be reimplanted. Autologous cells have the fewest problems with rejection and pathogen transmission, however in some cases might not be available. For example in genetic disease suitable autologous cells are not available. Also very ill or elderly persons, as well as patients suffering from severe burns, may not have sufficient quantities of autologous cells to establish useful cell lines. Moreover since this category of cells needs to be harvested from the patient, there are also some concerns related to the necessity of performing such surgical operations that might lead to donor site infection or chronic pain. Autologous cells also must be cultured from samples before they can be used: this takes time, so autologous solutions may not be very quick. Recently there has been a trend towards the use of mesenchymal stem cells from bone marrow and fat. These cells can differentiate into a variety of tissue types, including bone, cartilage, fat, and nerve. A large number of cells can be easily and quickly isolated from fat, thus opening the potential for large numbers of cells to be quickly and easily obtained.



Mouse embryonic stem cells. **More lab photos**

- **Allogeneic** cells come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for *in vitro* studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin.

- **Xenogenic** cells are these isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants.

- **Syngenic** or **isogenic** cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

- **Primary** cells are from an organism.

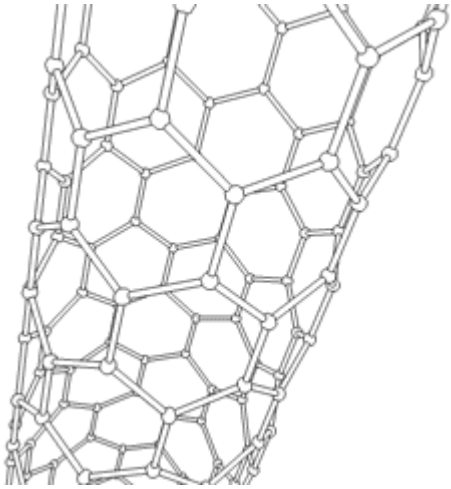
- **Secondary** cells are from a cell bank.

- **Stem cells** are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells. According to their source stem cells are divided into "adult" and "embryonic" stem cells, the first class being multipotent and the latter mostly pluripotent; some cells are totipotent, in the earliest stages of the embryo. While there is still a large ethical debate related with the use of embryonic stem cells, it is thought that stem cells may be useful for the repair of diseased or damaged tissues, or may be used to grow new organs.

Scaffolds

Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds, are often critical, both *ex vivo* as well as *in vivo*, to recapitulating the *in vivo* milieu and allowing cells to influence their own microenvironments. Scaffolds usually serve at least one of the following purposes:

- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients and expressed products
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase



Carbon nanotubes are among the numerous candidates for tissue engineering scaffolds since they are biocompatible, resistant to biodegradation and can be functionalized with biomolecules. However, the possibility of toxicity with non-biodegradable nano-materials is not fully understood.

To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal. The rate at which

degradation occurs has to coincide as much as possible with the rate of tissue formation: this means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down leaving the neotissue, newly formed tissue which will take over the mechanical load. Injectability is also important for clinical uses. Recent research on organ printing is showing how crucial a good control of the 3D environment is to insure reproducibility of experiments and offer better results.

Materials

Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen and some polyesters.

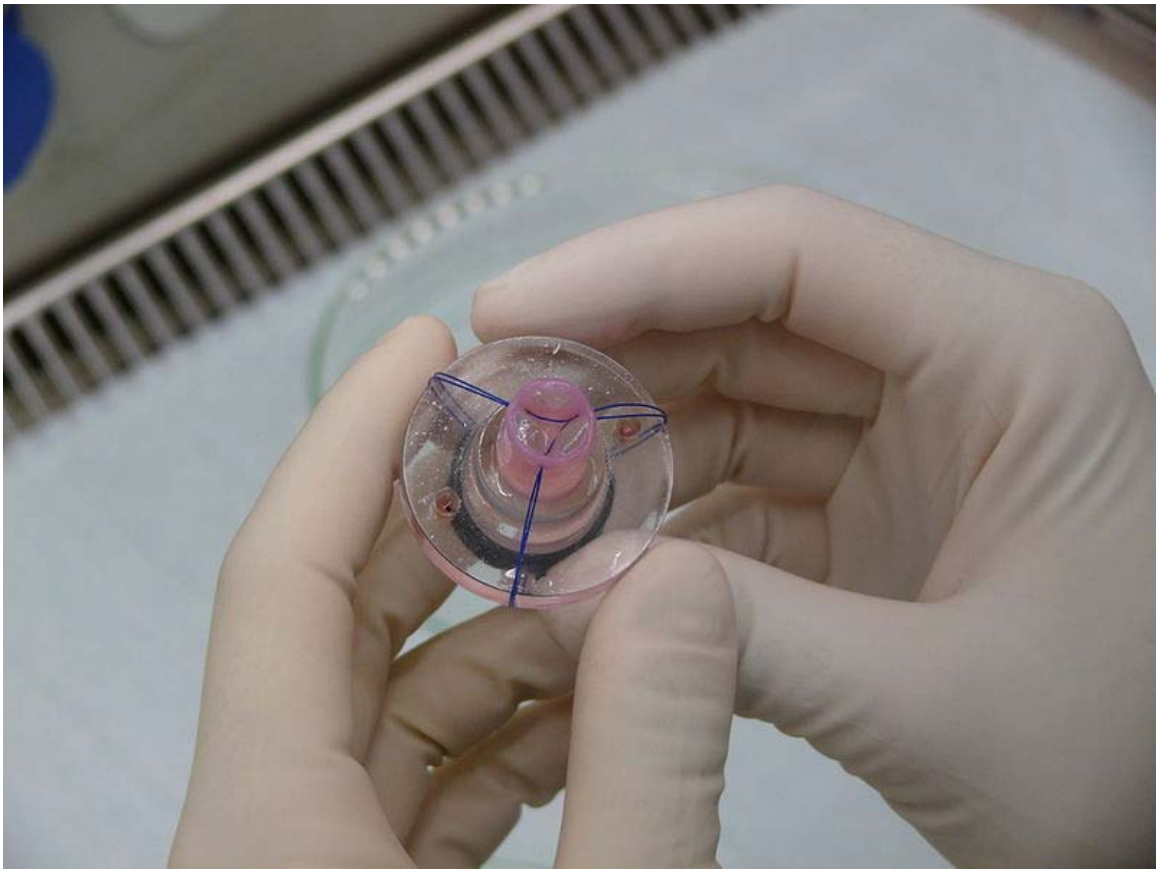
New biomaterials have been engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration, resorption rates, etc. PuraMatrix, originating from the MIT labs of Zhang, Rich, Grodzinsky and Langer is one of these new biomimetic scaffold families which has now been commercialized and is impacting clinical tissue engineering.

A commonly used synthetic material is PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body. Similar materials are polyglycolic acid (PGA) and polycaprolactone (PCL): their degradation mechanism is similar to that of PLA, but they exhibit respectively a faster and a slower rate of degradation compared to PLA.

Scaffolds may also be constructed from natural materials: in particular different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth. Proteic materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Among GAGs hyaluronic acid, possibly in combination with cross linking agents (e.g. glutaraldehyde, water soluble carbodiimide, etc...), is one of the possible choices as scaffold material. Functionalized groups of scaffolds may be useful in the delivery of small molecules (drugs) to specific tissues. Another form of scaffold under investigation is decellularised tissue extracts whereby the remaining cellular remnants/extracellular matrices act as the scaffold.

Synthesis

tissue engineered vascular graft



tissue engineered heart valve

A number of different methods have been described in literature for preparing porous structures to be employed as tissue engineering scaffolds. Each of these techniques presents its own advantages, but none are free of drawbacks.

- **Nanofiber Self-Assembly**: Molecular self-assembly is one of the few methods for creating biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix (ECM). Moreover, these hydrogel scaffolds have shown superiority in in vivo toxicology and biocompatibility compared to traditional macroscaffolds and animal-derived materials.

- **Textile technologies**: These techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers. In particular, non-woven polyglycolide structures have been tested for tissue engineering applications: such fibrous structures have been found useful to grow different types of cells. The principal drawbacks are related to the difficulties in obtaining high porosity and regular pore size.

- **Solvent Casting & Particulate Leaching (SCPL)**: This approach allows for the preparation of porous structures with regular porosity, but with a limited thickness. First, the polymer is dissolved into a suitable organic solvent (e.g.

polylactic acid could be dissolved into dichloromethane), then the solution is cast into a mold filled with porogen particles. Such porogen can be an inorganic salt like sodium chloride, crystals of saccharose, gelatin spheres or paraffin spheres. The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast the solvent is allowed to fully evaporate, then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen: water in the case of sodium chloride, saccharose and gelatin or an aliphatic solvent like hexane for use with paraffin. Once the porogen has been fully dissolved, a porous structure is obtained. Other than the small thickness range that can be obtained, another drawback of SCPL lies in its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold.

- **Gas Foaming**: To overcome the need to use organic solvents and solid porogens, a technique using gas as a porogen has been developed. First, disc-shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold. The discs are then placed in a chamber where they are exposed to high pressure CO_2 for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge-like structure. The main problems resulting from such a technique are caused by the excessive heat used during compression molding (which prohibits the incorporation of any temperature labile material into the polymer matrix) and by the fact that the pores do not form an interconnected structure.

- **Emulsification/Freeze-drying**: This technique does not require the use of a solid porogen like SCPL. First, a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane) then water is added to the polymeric solution and the two liquids are mixed in order to obtain an emulsion. Before the two phases can separate, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure. While emulsification and freeze-drying allow for a faster preparation when compared to SCPL (since it does not require a time consuming leaching step), it still requires the use of solvents. Moreover, pore size is relatively small and porosity is often irregular. Freeze-drying by itself is also a commonly employed technique for the fabrication of scaffolds. In particular, it is used to prepare collagen sponges: collagen is dissolved into acidic solutions of acetic acid or hydrochloric acid that are cast into a mold, frozen with liquid nitrogen and then lyophilized.

- **Thermally Induced Phase Separation (TIPS)**: Similar to the previous technique, this phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example dioxane could be used to

dissolve polylactic acid, then phase separation is induced through the addition of a small quantity of water: a polymer-rich and a polymer-poor phase are formed. Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent, a porous scaffold is obtained. Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying.

- **Electrospinning**: A highly versatile technique that can be used to produce continuous fibers from submicron to nanometer diameters. In a typical electrospinning set-up, a solution is fed through a spinneret and a high voltage is applied to the tip. The buildup of electrostatic repulsion within the charged solution, causes it to eject a thin fibrous stream. A mounted collector plate or rod with an opposite or grounded charge draws in the continuous fibers, which arrive to form a highly porous network. The primary advantages of this technique are its simplicity and ease of variation. At a laboratory level, a typical electrospinning set-up only requires a high voltage power supply (up to 30 kV), a syringe, a flat tip needle and a conducting collector. By modifying variables such as the distance to collector, magnitude of applied voltage, or solution flow rate—researchers can dramatically change the overall scaffold architecture.

- **CAD/CAM Technologies**: Because most of the above techniques are limited when it comes to the control of porosity and pore size, computer assisted design and manufacturing techniques have been introduced to tissue engineering. First, a three-dimensional structure is designed using CAD software, then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer melt.

Assembly methods

One of the continuing, persistent problems with tissue engineering is mass transport limitations. Engineered tissues generally lack an initial blood supply, thus making it difficult for any implanted cells to obtain sufficient oxygen and nutrients to survive, and/or function properly.

Self-assembly may play an important role here, both from the perspective of encapsulating cells and proteins, as well as creating scaffolds on the right physical scale for engineered tissue constructs and cellular ingrowth.

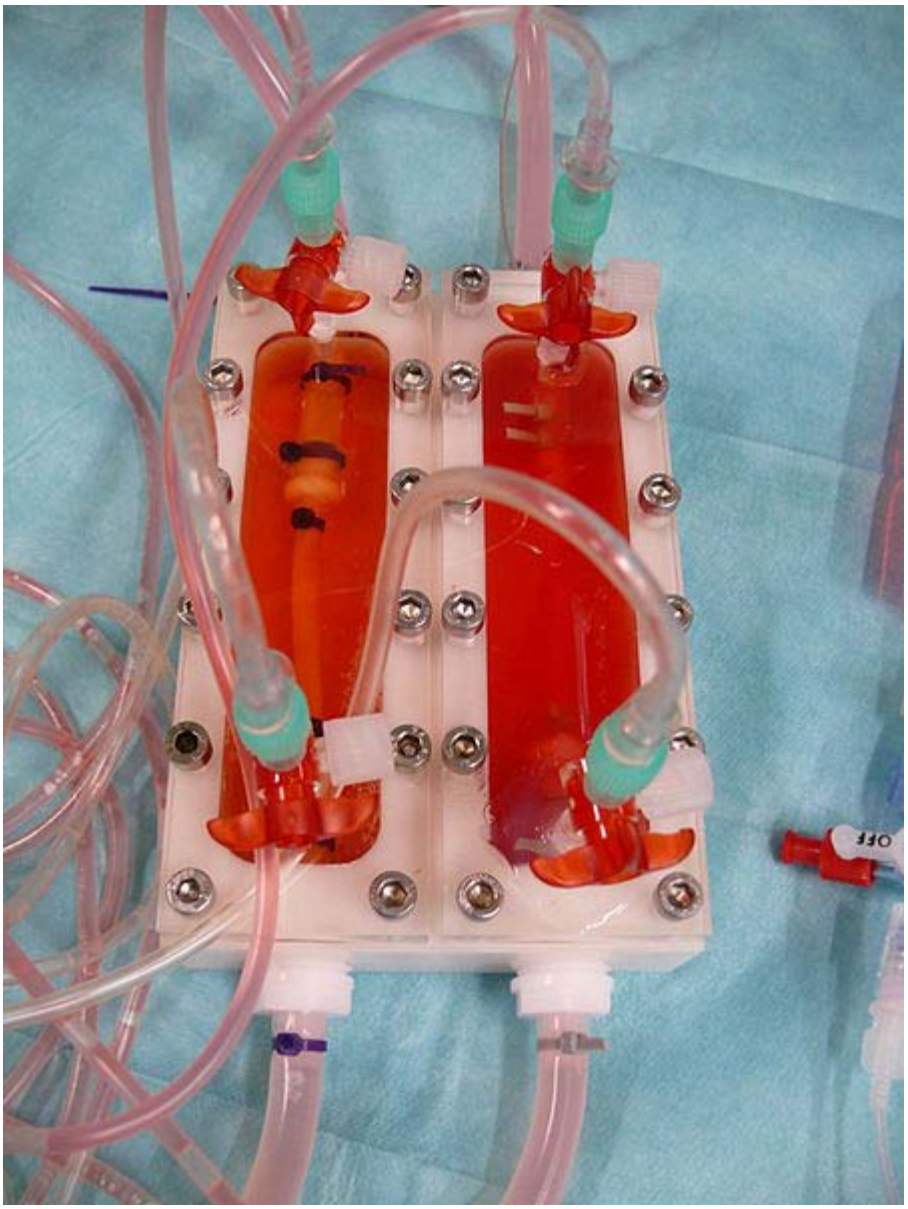
It might be possible to print organs, or possibly entire organisms. A recent innovative method of construction uses an ink-jet mechanism to print precise layers of cells in a matrix of thermoreversable gel. Endothelial cells, the cells that line blood vessels, have been printed in a set of stacked rings. When incubated, these fused into a tube.

Tissue culture

In many cases, creation of functional tissues and biological structures *in vitro* requires extensive culturing to promote survival, growth and inducement of functionality. In

general, the basic requirements of cells must be maintained in culture, which include oxygen, pH, humidity, temperature, nutrients and osmotic pressure maintenance.

Tissue engineered cultures also present additional problems in maintaining culture conditions. In standard cell culture, diffusion is often the sole means of nutrient and metabolite transport. However, as a culture becomes larger and more complex, such as the case with engineered organs and whole tissues, other mechanisms must be employed to maintain the culture, such as the creation of capillary networks within the tissue.



bioreactor for cultivation of vascular grafts

Another issue with tissue culture is introducing the proper factors or stimuli required to induce functionality. In many cases, simple maintenance culture is not sufficient. Growth

factors, hormones, specific metabolites or nutrients, chemical and physical stimuli are sometimes required. For example, certain cells respond to changes in oxygen tension as part of their normal development, such as chondrocytes, which must adapt to low oxygen conditions or hypoxia during skeletal development. Others, such as endothelial cells, respond to shear stress from fluid flow, which is encountered in blood vessels. Mechanical stimuli, such as pressure pulses seem to be beneficial to all kind of cardiovascular tissue such as heart valves, blood vessels or pericardium.

Bioreactors

A bioreactor in tissue engineering, as opposed to industrial bioreactors, is a device that attends to simulate a physiological environment in order to promote cell or tissue growth in vivo. A physiological environment can consist of many different parameters such as temperature and oxygen or carbon dioxide concentration, but can extend to all kinds of biological, chemical or mechanical stimuli. Therefore, there are systems that may include the application of forces or stresses to the tissue or even of electrical current in two- or three-dimensional setups.

In academic and industry research facilities, it is typical for bioreactors to be developed to replicate the specific physiological environment of the tissue being grown (e.g., flex and fluid shearing for heart valve growth). Several general-use and application-specific bioreactors are also commercially available, and may provide static chemical stimulation or combination of chemical and mechanical stimulation.