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Bioanalysis – techniques for the characterization of biological material

Everybody who studies natural organisms can be called a bioanalyst. Bioanalysis is therefore as old as mankind. Our biological knowledge is increasing rapidly and, along with it, the range of methods that have become available over the last decades to analyze complex biological samples. Science constantly provides researchers with new challenges biologists and bioanalysts have to deal with, and which come from sources as varied as the ever increasing number of resistant pathogenic bacterial strains or the famine conditions in Third-World countries. In the search for scientific truths, bioanalysis is the development, optimization and application of the entire range of analytical methods available. However, we need to keep in mind that, although this leads to an expansion of knowledge, the truth is only temporary.

Pierre J. Macquer, French physician and chemist who was interested in applying chemical findings to the field of medicine.

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The analysis of biological macromolecules such as proteins, DNA, RNA, carbohydrates and lipids in cells, tissues and blood using state-of-the-art technologies is at the centre of biochemical and molecular research. Science has dealt with the analysis of the structure and function of proteins for more than 200 years now, long before the molecules were actually called proteins.

In 1777, the French chemist Pierre J. Macquer called all substances, including egg white, "albumins" because of their strange ability to change from a liquid to a solid state when they were heated. It was not until the early 19th century that scientists realized that purified proteins were far more complicated than other known organic molecules. The Swedish chemist Jöns J. Berzelius is considered to have coined the term "protein" in 1838. Although 19th century scientists were able to purify proteins using simple separation methods (extraction, acidification or crystallization following the addition of precipitation agents), protein structure nevertheless remained elusive up until the mid-20th century. More effective analytical techniques such as electrophoresis and chromatography eventually enabled the generation of proteins of the required high purity and homogeneity that was crucial for comprehensively studying the composition and function of proteins, sugars, fats and genetic material.

An independent branch of science

3D model showing the tertiary structure of the protein myoglobin.

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The validity of a theory can only be verified and assessed by knowing the method that was used to analyze a certain issue. Analytics has come a long way from being an auxiliary science that is used to confirm data produced by other disciplines, and is now a separate area of expertise that can be used to formulate and answer questions. As an independent science, analytics comes up with methodological developments that play a key role in the study of biological relationships. In fact, it can be safely assumed that it has been the major driver of truly significant scientific progress.

Visions – for example the attempt to understand complex functional relationships in the cell – require the development of technologies with ever increasing levels of performance. Electrophoresis helps scientists to carry out qualitative analyses of protein mixtures and mass spectrometry is used to determine the mass of proteins and peptides. Scientists interested in the primary structure of proteins use Edman sequencing to glean information on the sequence of amino acids that make up the proteins. X-ray crystallography, which is capable of unravelling secondary and tertiary structures, is used to glean information on the conformation of proteins.

Methods are key to progress

Modern techniques and methods are up to ten thousand times faster and more sensitive than their predecessors when they were first used. The further development of light microscopy into confocal microscopy, to name but one example, enables us to study individual molecules in their biological context. Highly parallel next-generation sequencing that enables the rapid sequencing of genomes has long since found its way into the laboratories of today's researcher generation.

The increasing availability of protein and DNA sequences provides the basis for the systematic functional analysis of proteins and nucleic acids. While more and more complicated machines are let loose on smaller and smaller particles, modern bioanalysts need to focus on interdisciplinary thinking more than ever before in order to create useful synergies. Modern analyses are characterized by the combination of numerous individual procedures, which on their own would only have a limited outcome. Moreover, high-throughput methods generate an ever increasing quantity of data, which is why data analysis is also gaining in importance.

The huge amount of data that life scientists can extract from genomes, proteomes and metabolomes in a relatively short period of time, requires methods that enable the intelligent storage, provision and interpretation of their information content, and hence close cooperation with computer scientists. It is only in this way that the information can be compared, linked and assembled in complex networks "in silico", i.e. with computers.

Paradigm shift in modern bioanalysis

Modern bioanalytical investigations have become far more complex than they were in the past. Compared to physico-chemical analyses, this also makes the reproducibility of results more challenging.

Nevertheless, awareness has grown that systematic and primarily data-driven research provides fundamental insights into biological phenomena. A shift from classical targeted and function-oriented approaches to a more holistic view of biological issues is currently occurring.

Modern gel electrophoresis apparatus in a vertical tank.

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Researchers have traditionally focused on a specific biological phenomenon – enzyme activity or phenotypic changes, for example – and associated it with one or a few molecular structures. In most cases, it is the proteins that are investigated in detail using the entire range of bioanalytical methods available: molecular methods for studying protein expression and physical methods for obtaining insights into the structure of the molecules. However, as biological effects are rarely the result of the activity of one single protein, but of activity sequences of different interaction partners, the entire spectrum of methods is also applied to the reaction partners of the molecules under investigation.

Although virtually all of our knowledge results from this type of approach, it is still a lengthy process. It usually takes several years to decrypt an entire pathway. On the other hand, it is quite difficult to elucidate network-like systems and complex reaction processes. The quantitative use of these data is often impossible since they reflect an artificial situation in which the constituents are broken down into subunits and have been removed from the in vivo situation. Systems biology uses holistic strategies that, rather than specifically focusing on the smallest units of a system, they focus on the system as a whole. Perturbation analysis is a technique for analyzing without prejudice changes in a system's performance where specific modifications have been made to the system.

Mathematical descriptions of complex processes with quantitative data produced as described above are possible with the help of computer science. The advantages are that any system change can be traced back to the changed parameter, network connections become clear and we approach the conditions of a real biological system.

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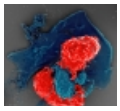
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