Biological analysis of **tomorrow**: towards personalized medicine

The biological analysis of tomorrow will move out of the precinct of the specialist laboratory, to enter right into the practitioner's consulting room, and everyday life. There will be a requirement to carry out ever more rapid, more numerous analyses, on ever smaller samples. Humans will no longer be faced with a world where, for a given pathology, one single drug holds sway, for every patient the world over. On the contrary, the medical practitioner will be in the position of having the duty, or the ability, to select, from a veritable diversified therapeutic arsenal, the molecule that is most appropriate with respect to his or her patient's characteristics. Which thus brings the hope of truly individually adjusted therapy.

L. Godart/CEA

Owing to their performance, and ease of use, immunoassays have penetrated into all areas of biological analysis. Tomorrow's biological analyses will move out of the precinct of the specialist laboratory.

Ever since they first endeavored to understand the living world, humans have striven to analyze the composition of its constituent objects. Now that it is clearly established that life is the outcome of the arrangement, and coordinated operation of large numbers of **molecules**, some of which are solely to be found in living entities, biological analysis is become molecular analysis. The object of that analysis takes extremely varied forms, ranging from inorganic molecules (metals, **ions**) to biological macromolecules (**proteins**, **nucleic acids**, **complex sugars**), through small organic molecules (sugars, **lipids**, **hormones**, vitamins, neurotransmitters,(1) natural **metabolites**, drugs…). A small proportion of these targets is measured by means of techniques coming from chemical analysis (metals, ions, sugars, total proteins), however the greater part, by far, involve more recent techniques, more specifically suited to biological molecules.

(1) Neurotransmitter: a small molecule, released by a nerve cell, ensuring the transfer of nerve impulses to another nerve cell (by means of a chemical transmission, as opposed to electrical transmission, which propagates the nerve impulse along nerve fibers). Neurotransmitters are most commonly active at synapses (specialized extracellular structures, putting two nerve cells into contact).

A concatenation of basic building blocks

One of the most astonishing specific features of the living world is its ability to generate a multiplicity of different structures, and hence different functions, from a few basic components. Thus, all proteins found on the face of the Earth, the numbers of which may be estimated to run to several millions, or billions – depending on whether or not proteins are taken into account that feature **sequences** yielded by combinatorial arrangements, such as antibodies – result from the linear assembly of 20 distinct **amino acids**. More spectacularly still, the equivalent number of **genes**, and nucleic acids is yielded by linear assembly of four basic **nucleotides**. Thus, the fundamental properties of these biological molecules are not related to their elemental chemical composition, which is virtually the same for all proteins, or all nucleic acids, rather it is the outcome of the concatenation of the basic building blocks (amino acids, nucleotides), which determines the macromolecule's function. In the case of proteins, the influence of the primary sequence essentially consists in dictating the three-dimensional structure, on which biological activity depends.

Present tools for the investigation of the living world

To explore this particular, special world, suitable tools were required, differing from those developed for, and by, chemistry. After more than 50 years of modern biological analysis, it comes as no surprise that these tools were provided, essentially, by the living world itself. Thus, to analyze proteins, biologists nowadays mainly use antibodies, i.e. other proteins. Subsumed under the range of *immunoanalytical methods*, methods based on use of antibodies cover a large area of modern biological analyses. Likewise, nucleic acids are, most commonly, detected by means of other nucleic acids. In all of these techniques, biological molecules are employed as "probes," allowing the detection, and quantification of other biological molecules with which they form specific **complexes**.

Immunoanalytical methods

These methods rely, to a large extent, on the remarkable properties of antibodies, and on the concurrent use of **labeled molecules**. Antibodies are proteins produced by the immune system of higher vertebrates, as a response to the introduction into an organism of an "antigen," perceived as a "foreign" body by the organism. The immune system has the ability to produce billions of different antibodies, to fight toxic or infectious agents (bacteria, **viruses**, **toxins**). It is this fantastic fund that is put to use by biologists, to carry out "immunoassays," for virtually any type of biological molecule (hormones, viruses, bacteria, neurotransmitters, cytokines,(2) drugs…).

Such assays rely on the "antigen–antibody" interaction, which is characterized by high specificity, and high affinity. Antibody specificity, i.e. their ability to form complexes only with the one antigen against which they have been selected for, is an essential property. It is this which allows antibodies to be used as veritable probes, having the ability to go out and recognize, in an often highly complex biological environment, those molecules that are of interest, and those only. The affinity of an antibody for a given antigen is equally a fundamental parameter, since this determines the sensitivity of the measurement being carried out. Indeed, whichever technique is used, an immunoassay will only detect a substance if there is production of an antigen–antibody complex. Now, generation of such complexes is governed by the laws of thermodynamics, which entail that these will only be formed, to any significant level, provided the dissociation constant(3) of these complexes (K_D) allows it (practically, K_D must be lower than 10^{-9} **M**).

Over the 50 years, or more, that have elapsed since research workers learnt to use antibodies for analytical purposes, a considerable number of techniques have been described. There can be no question of presenting a detailed survey of such techniques here. Some of these methods allow the localized detection of an antigen, in order to gain structural information at cell, subcellular, or molecular level (immunohistochemistry, immunocytochemistry,(4) Western blot(5)). They are widely employed in research work. However, most commonly, immunoassays are used to effect a quantitative measurement of concentration for an antigen or an antibody, in a given biological environment. In such situations, the measurement is achieved through the use, concurrently, of a "labeled molecule," also known as a tracer, allowing antigen–antibody complex formation to be quantified. These labeled molecules consist in antibody or antigen molecules, onto which a marker has been fixed, delivering a signal that may be readily measured by means of a sensitive physi-

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Samples being deposited into the wells of a microassay plate, to carry out an extensive series of immunoassays. Such assays rely on the interaction between an antigen and an antibody.

(2) Cytokine: a protein or peptide (a molecule of the protein type, composed of a small number of amino acids) involved in extracellular signaling, acting as local messenger in intercellular communication.

(3) The dissociation constant is equal to: ([antigen concentration] × [antibody concentration])/ [concentration of the antigen–antibody complex].

(4) Immunohistochemistry, immunocytochemistry: methods used for the detection of tissue antigens (immunohistochemistry) or cell antigens (immunocytochemistry), by means of specific, labeled antibodies, marked either with fluorochromes – molecules that absorb light of a given wavelength, and respond by emitting light of greater wavelength – or enzymes.

(5) Western blot: a technique whereby proteins are separated by electrophoresis, and fixed onto a synthetic substrate, and subsequently analyzed by means of a labeled antibody.

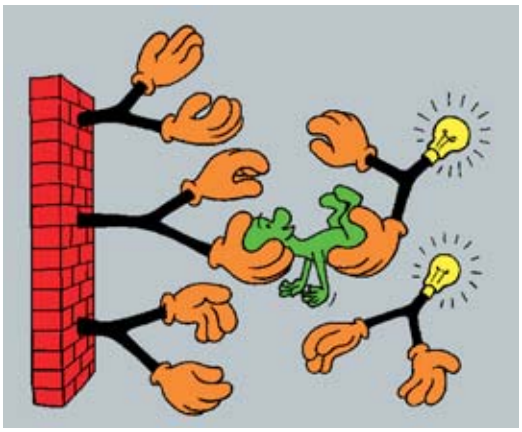

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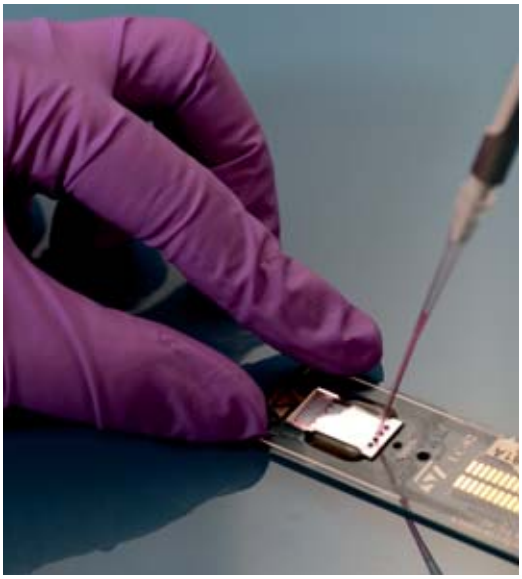
Figure 1. Principle of a two-site immunometric assay (sandwich assay). The hands represent the antibody binding sites, the green manikin the molecule that is being assayed, and the lightbulbs a marker (enzyme, **fluorophor**) fixed onto one of the two antibodies involved in the assay. In this type of assay, the target molecule is simultaneously bound by two antibodies, recognizing two compatible binding sites on the molecule. One of the antibodies, known as the *capture antibody*, is secured to a solid support (tube, sphere, plate). The other one, known as the *tracer* (or *detection*) *antibody*, is labeled, and allows detection of the immobilized complexes. After eliminating, by washing, unfixed labeled antibodies, measurement of the signal associated to the solid support enables a measure of the target molecule concentration to be arrived at.

cal–chemical method (**radioactivity**, **fluorescence**, **luminescence**, **enzymatic** activity). In most cases (heterogeneous assays), use of such markers entails a separation step, in order to differentiate between such labeled molecules as are involved in antigen–antibody complexes, and those that remain free in the solution. The principle of the so-called *sandwich* assay is shown, by way of example, in Figure 1.

Emerging over 50 years ago, immunoassays have, in the meantime, penetrated all areas of biological analysis (research, diagnosis, agrifood), owing to the performance they offer, and ease of use. They are to be found equally in highly sophisticated automated analysis devices, carrying out over 400 analyses per hour, with an analysis time of less than 30 minutes, and a detection threshold close to one **attomole**, as in fast tests, or home tests, suitable for use by anybody, with no prior training (pregnancy tests, for instance).

Molecular hybridization assays

With the spectacular developments witnessed in molecular biology, from the 1970s on, other analytical methods emerged, specifically dedicated to nucleic acids. To a large extent, these take their cue from immunoanalytical methods, while relying on the **hybridization** properties of two complementary nucleic acid strands. This hybridization reaction is used in the same manner as the antigen–antibody interaction, to detect, and quantify specific nucleic acid (**DNA** or **RNA**) sequences, most commonly through the concurrent use of labeled molecules. The domains of application for these techniques, often complementary as they are to immunoanalytical techniques, essentially lie in the areas of genetic analysis, and the detection of infectious agents (viruses or bacteria). Molecular hybridization assays have the advantage of being easier to develop, as a rule, than immunoassays. Indeed, it is incomparably easier to synthesize a nucleic acid probe than it is to select, and produce, a suitable antibody. Further, these methods can draw on a considerable asset, with the rise of nucleic acid amplification techniques, most notably **PCR**. With the latter technique, considerable target amplification may be achieved, commonly by a factor of 10^9 (one billion), which, obviously, greatly facilitates analysis, through a spectacular easing of constraints, in terms of sensitivity. At the same time, in the areas of **genome** and **transcriptome** analysis, molecular hybridization has shown itself to be peculiarly suitable with respect to the deployment of massively parallel analyses (**DNA chip** arrays), involving in some cases more than 100,000 analyses carried out concurrently, on a few square centimeters.



P. Stroppa/CEA

Dedicated device, developed under the aegis of an STMicroelectronics–CEA contract, for genetic diagnosis. It brings together DNA sample preparation by the enzymatic pathway (PCR), and sample analysis by hybridization, on a DNA chip.

The emergence of mass spectrometry

Over the past few years, **mass spectrometry** techniques have experienced a heady rise, taking pride of place in biological analysis. Initially developed for the purposes of **isotopic** analysis, or the analysis of small organic molecules, drugs in particular, these techniques are now proving increasingly suitable for the analysis of a growing number of molecules of biological interest, particularly proteins. These advances are mainly linked to improvements in **ionization techniques** (ESI, MALDI), but equally to coupling with **high-performance liquid chromatography** (**HPLC**), and improved electronic performance in the equipment used. In the areas of **proteome** and **metabolome** analysis, such techniques now provide invaluable tools. While often still restricted, in terms of sensitivity, compared to immunoanalytical methods, they do afford, on the other hand, superior adaptability, and higher specificity.

From the laboratory to the patient's bedside

The evolutions outlined above clearly announce the future trends, concerning the biological analysis of tomorrow. On the one hand, biological analysis will move out of the specialist laboratory, to enter the doctor's consulting room, or appear at the patient's bedside. And, on the other hand, it will be required to rise to the limits envisaged for this technique, by allowing the massively parallel analysis of an impressive number of parameters, while enabling a notable size reduction for biological samples, whether for tissue or blood samples.

More rapid analyses

From our own personal experience, it is apparent that, quite often, diagnosis of a pathological condition, and the consequent therapeutic decision, do not take place during the initial consultation with a practitioner,

P. Avavian/CEA

Protein ESI–Q–TOF mass spectrometer, coupled to a high-performance liquid chromatograph, and associated to an automated sample loader. This instrument exhibits high sensitivity, together with outstanding resolution, and high mass precision.

rather, in many cases, after a more or less protracted interval, required to carry out one or more biopsies (blood, urine, or in some cases tissue samples), followed by specific analysis, performed by a specialist laboratory. An interesting instance is found, where this lead-time can be brought down. Indeed, medical practitioners do already have one available rapid diagnostic test for streptococcal pharyngitis, taking a few minutes to carry out in the practitioner's own consulting room. The test thus allows antibiotics prescriptions to be restricted to those rare, but serious cases only, of bacterial infection, thus avoiding a needless course of antibiotics, in the more numerous, less serious cases of pharyngitis of purely viral origin. One may reasonably surmise that many diagnostic tests, relying on an ultrarapid analysis in the consulting room, or at the patient's bedside, may be developed in the near future, for other pathologies. Such a trend is justified, in particular, in a highly constricted economic environment, as regards health expenditure. In this type of application, the requisite will be that biological analyses be amenable to extremely swift completion, and in any event within a time interval compatible with the length of a consultation. One consequence of that evolution will be the possibility for the patient to carry out himself, in the home, a growing range of biological measurements. This will thus contribute to the patient's taking direct charge of his own pathology, always a factor for progress in the course of an illness.

Rising numbers of markers, of higher sensitivity

Ever since biological analysis has become one of the pillars of medical practice, biologists have sought to base diagnosis on specific, sensitive markers, as far as possible of strong prognostic value. In the coming years, it is more than probable that the number of available markers will keep rising, with improved knowledge of the underlying molecular mechanisms of pathologies, and of cell biology.

It is highly likely that the next generation of markers will occur in biological samples in even smaller concentrations than their forerunners. While historic markers were metabolites circulating in high concentrations, e.g. glucose, for which blood level is of the order of one gram per liter, nowadays the requirement is for the ability to quantify hormones or proteins, at concentrations close to 10 **ng**/L (10^{-8} g/L). Doubtless, a factor 1,000 will still need to be gained with the coming markers.

A large number of analyses, on ever smaller samples

The manifold, complex relations prevailing between biological processes inside a cell are beginning to be better understood, and described. It is becoming apparent that a pathology, or cell or organ malfunction may not be narrowed down to qualitative or quantitative variations of any one single molecule (a protein, a nucleic acid, a metabolite…). To achieve a biological action, the cell may well not follow a single, unambiguous path, rather it may call on many paths, acting simultaneously, or successively. The cell is a complex biological system. It will thus be understood that basing diagnosis, or therapy, for all complex pathologies, on the one measurement of a single marker will not remain the only approach to be explored.

The biological analysis of tomorrow will require the ability to analyze large numbers of molecules simultaneously, on a single sample. In that case, rather than speaking of the high, or low, value of a biological marker, as e.g. a high blood sugar count in cases of diabe-

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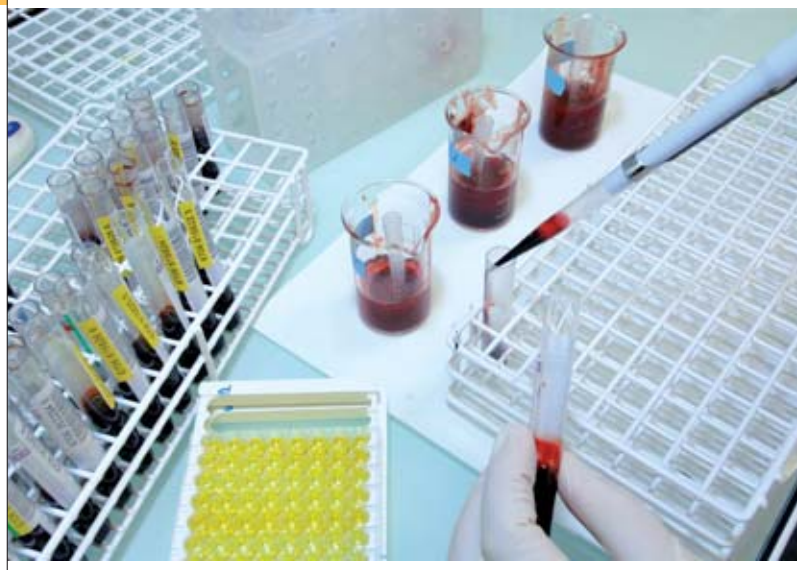
Purification, by means of **liquid–solid chromatography**, of fluorescent tracers. Next-generation markers will be even more numerous, and will occur in biological samples in even lower concentrations.



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Blood samples. Tomorrow's biological analyses will enable large numbers of molecules to be analyzed simultaneously, on a single, ever smaller sample.

tes, the biologist will base himself on the analysis of a profile, summing up the presence, and value, of a multiplicity of molecules (possibly 10, or 100). Only the knowledge of that ensemble of molecules will enable a reliable diagnosis, sound therapeutic decision, and accurate prognosis. These large numbers of analyses will be carried out on samples of ever smaller size, since there can be no question, in order to analyze 100 or 1,000 molecules, of requiring samples larger than a few **micrograms**.

The contribution made by biological analysis is not restricted to diagnosis alone. In future years, it will also play a key part in the choice of drugs to be administered to the patient. The patient and his condition form, in theory, a unique couple. Each of us reacts differently to any one pharmaceutical. For instance, capacity to eliminate a drug may vary with the individual, on a basis that may be, in some cases, genetically conditioned. Ascertaining, by means of appropriate tests, reactions to a drug (undesirable side-effects, elimination capacity…) would direct treatment to this or that drug.

(6) On this topic, see *Clefs CEA* No. 52 (Summer 2005), pp. 107–110.

P. Chagnon/BSIP-CEA

Multi-Patch sensor, miniaturized down to biochip format, allowing the recording, in fully automated manner, of very weak ion currents, in parallel, on a large number of cells. Its design combines the performances of microtechnologies, microelectronics, and microfluidics. Tomorrow's biological analysis needs must rely extensively on micro- and nanotechnologies.

Towards self-standing, portable analysis

One final trend, a strong, emerging trend in fact, is the requirement to carry out *in situ* analyses, on an ongoing, portable basis. In various chronic pathologies, in particular, or in a variety of therapeutic situations (intensive care units, or accident and emergency services), the therapist may wish to receive information in real time, on a continuous basis. The patient must then be "instrumented," i.e. be implanted with devices having the ability to measure a relevant biological parameter, and transmit that information to a data processing or storage device. Such biosensors needs must, of course, be as little invasive, and as biocompatible, as feasible, if they are to be tolerated over extended periods, years even.

The contribution from physics and micro- and nanotechnologies

Such a program may only be carried through by way of the convergence of a number of scientific disciplines, and technologies. Historically, physicists have often provided biologists with innovative instruments to probe living matter. Physicists were the first to gain an understanding, and make use, of the interactions between light, radiation or particles, and matter, and they supplied biologists with **spectroscopic** methods. The use of **ultrasound** should also be mentioned, in medical echography. It is essential that progress along this path be pushed forward, to secure novel measurement methods involving no chemical labeling, or sampling, or that may even be carried out remotely, in noninvasive fashion. Physics laboratories are experimenting currently on the principles on which tomorrow's biological analyses will be based.

Further, it is apparent that micro- and nanotechnologies will take up a major part in this revolution. Reduction in reaction volumes will, obviously, result in gains in terms of sample size. A sample may be split up, to effect a multiplicity of measurements. At the same time, a size reduction can result in appreciable gains in analysis time. Indeed, the speed of a biological reaction varies with the square of the distance the reaction partners must travel, on average, before interacting. For example, if a biological reaction between two proteins takes 24 hours to occur in a 1-cm-diameter test-tube, it will only take a few seconds in a microtube 10 **µm** in diameter.(6)

The future, for biological analysis, will thus hinge on its ability to take in the advances achieved in physics, and to make use of the micro- and nanotechnologies developed in the context of information technologies, putting them to work in the service of our health.

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D Spectroscopy and spectrometry

Spectrometric methods are subdivided, as a whole, into two main categories, radiation spectrometry – itself comprising absorption spectrometry, emission spectrometry, Raman scattering spectrometry, and nuclear magnetic resonance spectrometry – and mass spectrometry.

Radiation spectroscopy and spectrometry[1] cover a ensemble of analytical methods allowing the composition and structure of matter to be ascertained, based on investigation of the spectra yielded by the interaction between atoms and molecules, and various types of electromagnetic radiation, emitted, absorbed, or scattered by the former.

Depending on their energy, photons interact selectively with the various electron shells, or levels, making up the electronic structure of the atom, or molecule. The electrons involved are *core electrons* (close to the atom's nucleus), for X-rays,[2] *peripheral electrons* (furthest from the nucleus, and involved in chemical bonds) for light absorbed, or emitted, in the near ultraviolet and visible region. In the infrared radiation region, it is the leap from one *molecular vibration* level to another that is involved, the switch from one molecular *rotation* level to another for microwave radiation, and atomic nucleus spin for NMR.

Absorption spectrometry

Those spectroscopy methods that rely on absorption make use of the Beer–Lambert law, setting out the proportional relation between the intensity of light absorbed, and the amount of absorbing matter:

$$A = \log (I_0/I) = \varepsilon \, l \, C,$$

where A stands for the absorbance of the medium traversed, I_0 for incident light intensity, I for transmitted light intensity, ε is the characteristic molar extinction coefficient, for a given wavelength, for the substance investigated – expressed in L mol^{-1} cm^{-1} – while l stands for the thickness passed through, expressed in centimeters, and C is the concentration, in moles per liter.

By measuring the medium's absorbance, for a given wavelength, the concentration of a substance, in a sample, may thus be determined.

In an absorption spectrum, as recorded by means of a spectrometer, *absorption peaks* correspond to the wavelengths the medium is able to absorb. Just as the spectrum from the Sun's light is obtained by making it pass through a prism, which breaks it up, spectrometers analyze the spectral distribution of the whole range of electromagnetic radiations, separating them out according to wavelength, by means of a reflection diffraction grating. Spectra exhibit peaks, each one corresponding to a specific wavelength.

Depending of the type of sample to be analyzed, and the performance level being sought, in the laboratory, absorption spectrometry is used either on molecules in liquid or gaseous phase, or on atomic vapor, obtained through thermal breakdown of liquid or solid samples.

Molecular absorption spectroscopy, in the UV–visible region, affords simplicity of use, however it is only applicable to samples of moderate complexity, since, owing to the width of *molecular absorption bands*, absorption spectra, as a rule, do not allow specific discrimination of every constituent, in a complex mixture.

In infrared (IR) spectrometry, absorption is the outcome of molecular vibration and rotation processes. Infrared absorption spectra thus allow the nature of chemical bonds to be determined, that make up a molecule, by ascertaining the bond's elasticity constant (influencing vibration frequency, as for a spring), thus confirming structural hypotheses.

As the number of atoms increases, the spectrum rapidly exhibits growing complexity, and interpretation becomes highly problematical, especially for organic compounds.

Atomic absorption spectrometry, in this respect, brings higher performance, since absorption by atoms yields very narrow absorption lines. Very precise measurements are thus feasible, even when the sample consists in a complex assembly of chemical elements. Atomic absorption is a reference technique for the analysis of trace elements in a wide variety of samples, in particular for biological samples.

Emission spectrometry

Atoms or molecules brought to an excited state may deexcite by emitting radiation, known as *emission radiation*. When the excitation is caused by selective absorption, by the atoms or molecules to be analyzed, of electromagnetic radiation, this represents a fluorescence emission (or a phosphorescence emission, depending on the electron excitation state involved).

As with absorption, fluorescence may be applied, in the UV–visible radiation region, to molecules, or atoms. X-ray fluorescence spectrometry, on the other hand, refers to the X radiation emitted by atoms excited by absorption of X-radiation. Fluorescence techniques are more complex to implement than is the case for absorption techniques, since they entail that the particle subjected to analysis be selectively excited by a monochromatic radiation. On the other hand, since the radiation emitted is likewise specific to the particle, fluorescence spectrometry involves a double selectivity, resulting in very low background noise, thus making it peculiarly well suited for the measurement of very low concentrations.

Emission of radiation may also occur when atoms are thermally excited, in an environment brought to high temperatures. Emission spectroscopy is based on the fact that atoms, or molecules excited to high energy levels deexcite to lower levels, by emitting radiation (emission, or luminescence). This differs from fluorescence spectrometry in that excitation is not applied selectively, rather it involves indiscriminately all of the particles making up the medium. Emission lines thus correspond to radiation directly emitted by a body brought to a high temperature, and the emission spectrum allows the detection, and quantification, of all atoms present in the emission source.

Raman spectrometry

Interactions between matter and electromagnetic radiation also give rise to scattering processes, such as elastic scattering, and inelastic scattering. Scattering may occur when the interface between

(1) The term "spectrometry," initially used only to refer to recording and measurement techniques, has tended to become synonymous with "spectroscopy," as the eye was supplanted, for observation purposes, by other receptors and instruments, while the visible region now only formed one special region, in analytical terms.

(2) It should be noted, at the same time, that X-ray crystallography is not deemed to be a spectroscopy method, in the strict sense of the term.

two media is encountered, or as a medium is passed through. This process, in most cases, is an "elastic" one, in other words it takes place with no change in frequency for the radiation forming the beam involved. Elastic scattering of solar radiation by the atmosphere is, for instance, responsible for the blueness of the sky, observed when the eye is not directed towards the Sun (*Tyndall effect*). Indeed, scattered intensity is all the greater, the shorter the radiation wavelength, which, in the case of the solar spectrum, corresponds to the color blue.

As regards spectrometry, the main use of scattering concerns *Raman spectrometry*. This involves the inelastic scattering of incident radiation by the molecules making up the sample. The difference between scattered radiation frequency, and incident radiation frequency allows the identification of the chemical bonds involved. Raman spectrometry is a technique that is widely used for structural analysis, to complement infrared spectrometry, and mass spectrometry.

Nuclear magnetic resonance spectrometry

The principle of nuclear magnetic resonance (NMR) is based on the fact that an atom has a *magnetic moment*, just like a spinning charge acting as a tiny magnet, governed by quantum mechanics, aligning in a magnetic field as the needle of a compass in the Earth's magnetic field. The principle of NMR consists in inducing, and detecting, the transition, for the nuclear magnetic moment, from the lowest energy level to the highest energy level, through absorption of electromagnetic radiation of a wavelength lying in the radiofrequency region: when the energy of the photon precisely matches the energy difference between the two levels, absorption occurs. Nuclei having numbers of protons, and neutrons that are both even exhibit zero spin. Carbon 12 and oxygen 16 atoms, which are very widespread in nature, thus have zero spin. On the other hand, hydrogen only has one single proton, and its nuclear magnetic moment equals 1/2: it may thus take on two possible energy states, corresponding to the two orientation states of its spin, relative to the magnetic field. Measuring the resonance frequency in the electromagnetic field allowing transition from one of these energy states to the other enables the molecules to be analyzed. This frequency is fixed, however the various nuclei in a molecule do not all resonate at the same frequency, since their magnetic environment is modified by their chemical (electronic) environment.

Many NMR spectra exhibit more peaks than there are protons in the nucleus, owing to the interactions between protons and their neighbors. Two nuclei may interact within the molecule, though they are separated by several chemical bonds: this is known as interatomic coupling. This interaction endows the NMR spectrum with a fine structure.

C. Dupont/CEA

Spectromètre de masse d'ions secondaires utilisé au CEA pour réaliser des mesures isotopiques rapides sur un échantillon par exemple prélevé sur une installation aux activités nucléaires suspectes.

Mass spectrometry

Mass spectrometry is a highly sensitive *detection* and *identification* technique, allowing determination of molecular structures, and thus of a sample's composition. This is not, strictly speaking, a form of spectrometry, since it is not concerned with discrete energy levels. What is its principle? A compound introduced into the device is vaporized, and subsequently ionized by an electron bombardment source (at 70 eV). The ion thus obtained, termed a molecular ion, allows the compound's molar mass to be determined. Breaking chemical bonds within the compound may yield characteristic fragment ions. These are then sorted according to their mass/charge ratio in an *analyzer*, through application of a magnetic and/or electric field, then collected by a *detector*, which amplifies the signal associated to the ions, which arrive with varying delays. A data processing system converts the information from the detector into a mass spectrum, readout of which, by comparing it with reference spectra, allows the identity details of the molecule to be drawn up. Through use of a high-resolution mass spectrometer, the exact mass of the compound may be determined, together with isotope percentages for each constituent atom.

Choice of ionization method is directly related to the nature of the sample, and the type of analysis. If mass spectrometry has gradually adapted to meet the growing demands from chemists, and biologists (separation of increasingly complex, highly polarized mixtures, determination of ever higher molecular masses on samples of ever more constricted sizes), this is essentially due to advances in *ionization techniques*, these including secondary ion mass spectrometry (SIMS), chemical ionization, thermospray ionization, and fast atom bombardment (FAB) sources, further comprising, from the 1980s, matrix-assisted laser desorption ionization (MALDI), and electrospray ionization (ESI), together with advances in *detection techniques*, from time-of-flight (TOF) measurement to "ion traps" (ITs), through quadrupoles (MS or Q).

In proteomics, for instance, only MALDI, ESI and SELDI (surface-enhanced laser desorption ionization) are employed.

Ion mobility spectrometry (IMS) is a chemical analysis technique in the gaseous phase, which consists in subjecting a gas to an electric field. Ionized molecules acquire a velocity that is characteristic for the ion, since this depends on mass, and charge. Arrival of the ions on one of the plates generating the field results in a current, which is recorded. The length of time after which a peak occurs can be related to the nature of the ion causing it.

Scientists often make use of a coupling of devices each belonging to one of the two main families of analytical techniques (see Box E, *What is chromatography?*), e.g. of a chromatograph with a mass spectrometer (or an electron-capture detector [ECD]), particularly for the investigation of trace complex mixtures.

B Fundamental interactions and elementary particles

The standard model of particle physics is the reference theoretical framework describing all known elementary particles (see Table 1) and the fundamental interactions these particles are involved in (see Table 2). The basic constituents of matter, known as fermions, are partitioned into two main categories, as determined by their participation in the fundamental interactions, or forces (the gravitational, electromagnetic, weak, and strong forces), which are mediated by vector bosons, the fundamental particles which carry out the transmission of the forces of nature[1] (see Table 2). Whether a particle belongs to the category of fermions, or to that of bosons depends on its spin (i.e. its intrinsic angular moment, or internal rotation moment), depending on whether it exhibits half-integer spin (fermions) or integer spin (bosons).

At the same time, to every constituent of matter is associated its antiparticle, a particle having the same *mass*, but the opposite *charge*. The positron is thus the positively charged antiparticle of the electron, which exhibits a negative charge.

Leptons and quarks

Fermions include, on the one hand, leptons, which may travel freely and do not participate in the *strong interaction*, which ensures the cohesion of atomic nuclei (it is consequently termed a *nuclear* interaction), and, on the other hand, quarks, which participate in all interactions but are not individually observed, enmeshed and confined as they are within hadrons, the particles susceptible to strong interaction, of which they are the constituents.[2]

In the lepton category, charged leptons participate in the *electromagnetic interaction* (which ensures the cohesion of atoms and molecules, and in the *weak interaction* (which underlies decay processes, in particular β radioactivity). Neutral leptons, or neutrinos, for their part, participate in the weak interaction only. Exhibiting very low mass, there is one type of neutrino for each type of charged lepton.

Independently from their involvement in interactions, the basic constituents of matter are classified into three *generations*, or *families*, of particles. From one family to the next, quarks and leptons having the same charges only differ by their mass, each family being heavier than the preceding one.

The electron, up quark (symbolized *u*) and down quark (symbol *d*), which belong to the first generation, are the lightest massive particles, and are stable. These are the sole constituents of normal matter, so-called baryonic matter (a baryon is an assembly of quarks), which is made up of protons and neutrons, this however only accounting for 4% of the Universe's energy content!

Particles in the other two families are heavier, and are unstable, except for neutrinos, which on the other hand exhibit non-zero mass, but are stable.

These latter particles may only be observed or detected in the final states resulting from collisions effected in accelerators, or in cosmic radiation, and rapidly decay into stable first-generation particles. This is why all the stable matter in the Universe is made up from constituents from the first family.

According to quantum mechanics, for an interaction to take place between particles of normal matter, at least one elementary particle, a boson, must be emitted, absorbed, or exchanged. The photon is the intermediate (or vector) boson for the electromagnetic interaction, the W^+, W^- and Z are the intermediate bosons for the weak interaction, and gluons are those of the strong interaction, acting at quark level.

As to the graviton, the putative vector for the gravitational interaction, it has not so far been empirically discovered. The gravitational force, which acts on all fermions in proportion to their mass, is not included in the standard model, due in particular to the fact that quantum field theory, when applied to gravitation, does not yield a viable scheme, as it stands. While gravitational effects are negligible in particle physics measurements, they become predominant on astronomical scales.

Interaction ranges

Quarks and charged leptons exchange photons. The photon having no electric charge, these particles conserve their electric charge after the exchange. Since the photon's mass is zero, the electromagnetic interaction has an infinite range. Having no electric charge, neutrinos are the only elementary fermions that are not subject to electromagnetic interaction.

In the electroweak theory (a unification of the weak and electromagnetic interactions), the weak interaction has two aspects: charged-current weak interaction, for which the interaction vectors are the W^+ and W^-; and neutral-current weak interaction, for which the mediator is Z^0. These two forms of weak interaction are active between all elementary fermions (quarks, charged leptons and neutrinos). The mass of these bosons being very large (about 80 GeV/c^2 for $W^\pm$, 91 GeV/c^2 for Z^0), the range of the weak interaction is tiny – of the order of 10^{-18} m. Since $W^\pm$ bosons have a non-zero electric charge, fermions exchanging such bosons undergo a change in electric charge, as of nature (*flavor*). Conversely, since the Z^0 boson has no electric charge, fermions exchanging one undergo no change in nature. In effect, neutral-current weak interaction is somewhat akin to exchanging a photon. As a general rule, if two fermions are able to exchange a photon, they can also exchange a Z^0. On the other hand, a neutrino has the ability to exchange a Z^0 with another particle, though not a photon.

Only those quarks that have a color charge[1] exchange gluons, these in turn being bearers of a color charge. Thus,

(1) The participation of basic constituents in fundamental interactions is governed by their *interaction charges* (electric charge, color charge), or "conserved quantum numbers." *Color charge*, a quantum number that determines participation in strong interactions, may take one of three values: "red," "green," or "blue" (these colors bearing no relation to visible colors). Every quark bears one of these color charges, every antiquark one of the three anticolor charges. Gluons are endowed with double color–anticolor charges (eight combinations being possible).

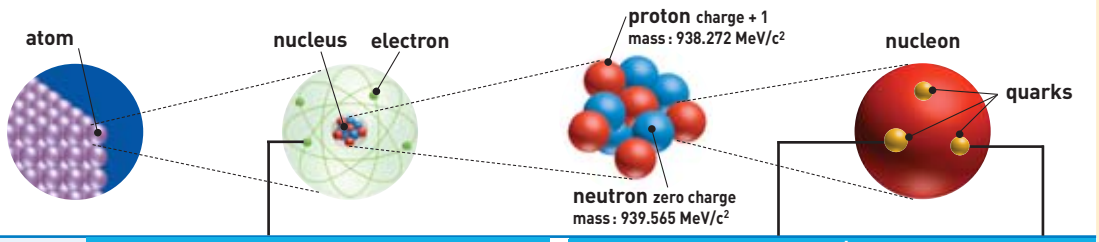
(2) To take e.g. nucleons: the proton holds two up quarks and one down quark, the neutron two down quarks and one up quark. A meson is made up of just two quarks (one quark and one antiquark).

B (cont'd)

when a gluon exchange takes place between quarks, the latter exchange their respective colors. Gluons have zero mass, however, since they do bear a color charge, they are able to interact together, which greatly complicates theoretical treatment of this interaction. The range of the strong interaction is consequently very restricted – of the order of 10^{-15} m.

The quest for unification

The theoretical framework for the standard model is quantum field theory, which allows a quantitative description to be made of the fundamental interac-



		leptons (able to move freely)		quarks (assembled into triplets, or quark–antiquark pairs, to form the many subatomic particles)	
Fermions Normal matter is made up of particles from this group. Most of these particles were around just after the Big Bang. Presently only to be found in cosmic rays, and around accelerators.	First family	**electron (e)** responsible for electricity and chemical reactions charge: - 1 mass : 0.511 MeV/c²	**electron neutrino (ν_e)** has no electric charge, and interacts very seldom with the ambient medium.	**down (*d*)** electric charge: - 1/3 the proton holds one, the neutron two mass : 4 – 8 MeV/c²	**up (*u*)** electric charge: + 2/3 the proton holds two, the neutron one mass : 1.5 – 4 MeV/c²
	Second family	**muon (μ)** a more massive companion to the electron. mass : 105.658 MeV/c²	**muon neutrino (ν_μ)** properties similar to those of the electron neutrino.	**strange (*s*)** a heavier companion to "up" mass : 80 – 130 MeV/c²	**charmé (*c*)** a heavier companion to "down" mass : 1.15 – 1.35 GeV/c²
	Third family	**tau particle (τ)** heavier still. masse : 1,776.99 ± 0.29 MeV/c²	**tau neutrino (ν_τ)** properties similar to those of the electron neutrino.	**beauty (*b*)** tau particle. mass : 4.1 – 4.4 GeV/c²	**top (*t*)** heaviest in the family (observed in 1995) mass : 171.4 ± 2.1 GeV/c²
Vector bosons Fundamental particles carrying out transmission of natural forces.		**photon** elementary grain of light, vector for the electromagnetic force	**gluon** bearer of the strong force between quarks	**$W^{\pm}$, Z^0** bearers of the weak force, responsible for some forms of radioactive decay	
Higgs boson?		responsible for "electroweak symmetry breaking"			

Tableau 1.
Table showing the twelve elementary constituents for which the standard model describes the interactions involved. The three charged leptons (electron e-, muon, μ^-, tau particle τ^-) are subject to electromagnetic and weak interactions, neutrinos (ν_e, ν_μ, ν_τ) are only affected by weak interaction, and the six quarks (up, charm, top – or *u*, *c*, *t* – bearing a charge of 2/3; and down, strange, bottom – *d*, *s*, *b* – bearing a charge of – 1/3) are subject to all three interactions. Every elementary constituent has its antiparticle, having the same mass, and algebraic quantum numbers (such as electric charge) of the opposite sign.

tions between elementary particles, while respecting the principles of *special relativity*, as those of quantum mechanics. According to the latter theory, if one seeks to observe a microscopic structure at high temporal and spatial resolution, this entails transferring to it an amount of energy–momentum, the greater, the higher the resolution being sought. However, according to the theory of relativity, such an energy–momentum transfer is liable to undergo transformation, yielding particles not present in the initial state: fermions may be generated, or annihilated, in particle–antiparticle pairs, while bosons may be so in any arbitrary number.

All processes involving one and the same fundamental interaction are interrelated. The quantum field theory approach, in which properties of **symmetry** play a fundamental part, seeks to describe all of the processes relating to each fundamental interaction, within overarching theoretical constructions.

The strong and electromagnetic interactions are formalized, respectively, in the theories of **quantum chromodynamics**, and **quantum electrodynamics**. The weak interaction, for its part, is not subject to a separate description, being described jointly with the electromagnetic interaction, in the unified formalism of **electroweak theory**. Theories of the *grand unification* of all fundamental interactions do exist, however they remain as yet lacking any experimental validation.

All the predictions of the standard model have been corroborated by experiment, except for just one, to wit, the existence of the **Higgs boson(s)**, which particle (particles?), it is hoped, will be discovered with LHC. The **Higgs mechanism** is thought to be responsible for the mass exhibited by elementary particles, the eponymous boson making it possible for zero-mass fermions interacting with it to be endowed with mass. This would allow the unification, at high energies, of the weak and electromagnetic interactions within the electroweak theory, while effectively accounting for the **breaking** of this **electroweak symmetry** at low energies, taking the form of two interactions, which may be seen as distinct at that energy level (see *The electroweak interaction from one accelerator to the next: the LHC roadmap and the yardstick of LEP measurements*, p. 23).

Going beyond, or completing the standard model?

The standard model features a set of parameters (such as the masses of elementary particles, or the intensities of fundamental forces) which are "anchored" in experimental findings. It is, in any event, a theory that is liable to be improved, or further elaborated, or even surpassed and left behind. It does not account in any way for the classification of the constituents of matter into three generations of particles, whereas it is precisely the existence of these three generations which makes it possible to account for **CP** (charge–parity) **invariance violation** (meaning that a physical process involving the weak interaction is not equivalent to its own mirror image), a violation that is in all likelihood the source of the matter–**antimatter** imbalance, running in favor of the former, in the primordial Universe. The model neither allows quantum treatment of gravitation, nor does it fully account for the fundamental property of *confinement*, which prevents quarks from propagating freely outside hadrons.

To go beyond, or to complete the standard model, research workers are mainly exploring two avenues:

– **supersymmetry** (widely known as SUSY) would associate, to every particle (whether a boson or a fermion) in the standard model, a partner from the other series, respectively a fermion or a boson. Supersymmetric partners would, at first blush, be highly massive, the lightest of them being a particle interacting very weakly only. This would be an ideal candidate to account for the **hidden matter** (or **dark matter**) in the Universe, accounting as it does for some 21% of the Universe's energy content, the remainder (close to 75%) consisting in a **dark energy**, the nature of which likewise remains to be determined. These WIMPs (acronym for "weakly interacting massive particles") are actively being sought (see *EDELWEISS II, the quest for dark matter particles*);

– the **substructure** path assumes there could be a new level of elementarity, underlying the particles in the standard model (or some of them). This would lead to a veritable blossoming of new, composite particles, analogous to hadrons, but exhibiting masses two to three thousand times heavier.

It should be noted that, whereas supersymmetry theories yield predictions that agree with the precision measurements carried out at LEP, the theories propounding substructures (or their simpler variants, at any rate) fail to do so. As for the more complex variants, these are encountering difficulties at the theoretical level.

fundamental interaction	associated particles (messengers)	actions
gravitation	graviton?	having an infinite range responsible for the mutual attraction of any two masses and for the law of falling bodies
electromagnetic interaction	photon	having an infinite range responsible for the attraction between electrons and atomic nuclei, hence for the cohesion of atoms and molecules
weak interaction	W^+, W^-, Z^0	responsible for β^- and β^+ radioactivity, reactions involving particles as neutrinos
strong interaction	gluons (there are 8 gluons)	ensures the cohesion of the atomic nucleus

Tableau 2.
Fundamental interactions, their vectors, and effects.

E What is chromatography?

Chromatography, together with the various forms of spectroscopy and spectrometry (see Box D, *Spectroscopy and spectrometry*), represent the two major basic analytical techniques, the former serving for the separation, the latter for the identification of the constituents of a substance.

Chromatography (from the Greek *khrôma*, "color," and *graphein*, "to write"), allows the *separation* of the constituents of a mixture in a homogeneous liquid or gaseous phase, as blotting paper might spread out in concentric rings a liquid poured onto it.

A chromatograph comprises a sample injection device, a *column*, a detector, and a recording and analysis system. Its principle is based on the equilibrium of compound concentrations, between two phases coming into contact: the *stationary phase*, in the column, and the *mobile phase*, which moves across it. Separation relies on the differential displacement of constituents inside the column, passing through in times that are proportional to their size, or depending on their structure, or affinity for the stationary phase (polarity...). As they reach the far end of the column, a *detector* measures, on a continuous basis, the quantities of each constituent.

The most common form of chromatography is **gas chromatography**, carried out on gaseous samples, or samples that may be vaporized without incurring breakdown. The mobile phase is a gas (helium, nitrogen, argon, or hydrogen), constantly sweeping through the column, which is placed in a thermostat oven. Detectors allow the selective analysis and identification of highly complex mixtures.

If the stationary phase is a nonvolatile, or not highly volatile liquid, exhibiting solvent properties for the compounds to be separated, the process is termed **gas–liquid chromatography**, or *partition chromatography*. If the stationary phase is an **adsorbent** solid (silica, alumina, zeolites, or **polymers**), this is **gas–solid chromatography**. Within this same family, of **adsorption** chromatography processes, **liquid–solid chromatography** is characterized by its stationary phase, this being a polar solid.

In **high-performance liquid chromatography** (**HPLC**), the sample must be wholly soluble in the mobile phase (elution solvent). The latter must be kept at high pressure (hence the alternative name of *high-pressure* liquid chromatography), to ensure a constant flow rate inside the column, and preclude any loss of head. HPLC involves solute–mobile phase–stationary phase exchange mechanisms, based on partition or adsorption coefficients, depending on the nature of the phases in contact.(1)

A chromatographic analysis yields a **chromatogram**, this being a graphical representation of the evolution of a parameter (intensity of the detector signal), related to instantaneous solute concentration, as function of time. This exhibits *peaks*, rising above the *baseline*, which obtains in the absence of any compounds (see Figure).

(1) There are two further types of liquid chromatography, *ion chromatography*, and *exclusion chromatography*.

N.B: This Box reproduces a number of excerpts from a presentation by Pascale Richardin, head of the Datation Group at the Research and Restoration Center of the French National Museums Administration (Musées de France), taken from the pages dealing with analytical methods, as posted on the site : ttp://www.culture.gouv.fr/culture/conservation/fr/biblioth/biblioth.htm

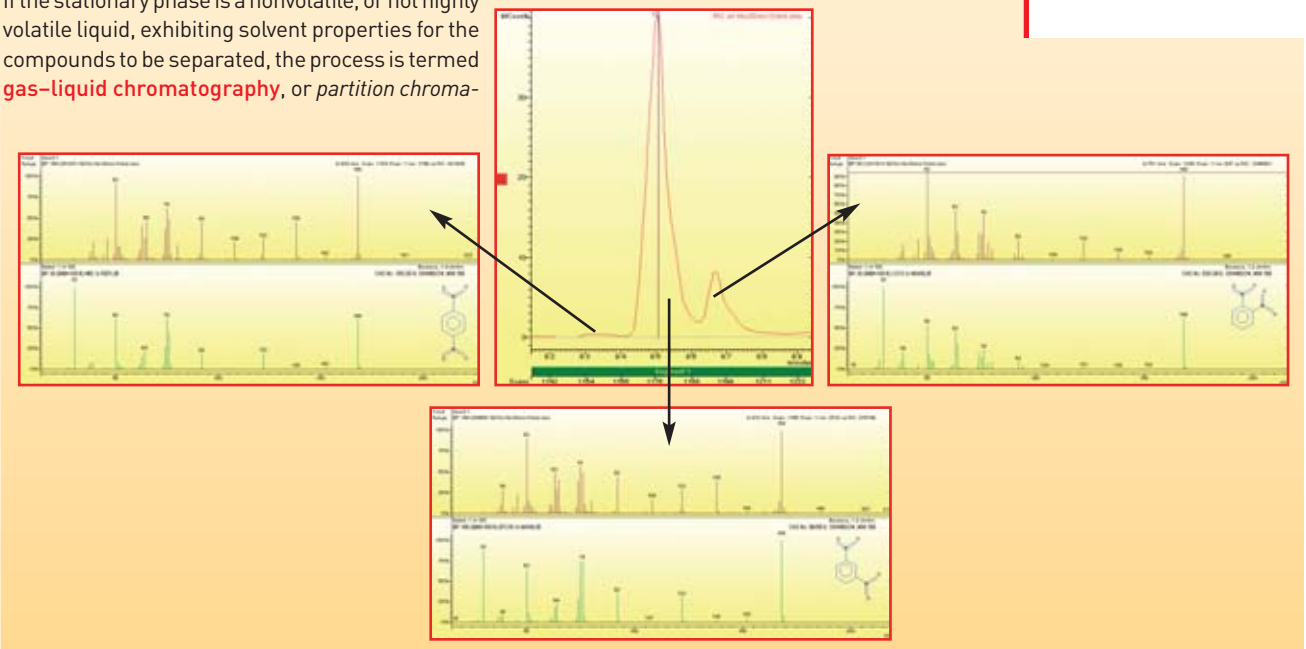


Figure.
An example of the combined use of mass spectrometry and chromatography: the separation of isomers ("sister molecules") of an explosive molecule (dinitrobenzene [DNB]), after solid-phase microextraction sampling, by gas chromatography, and their detection by mass spectrometry (SPME–GC–MS).