

# **La mise en phase gazeuse de molécules d'intérêt biologique pour les Nuls avec quelques références pour ceux qui souhaiteraient en savoir plus.**

## ***Généralités***

La vaste majorité des biomolécules possèdent de très faibles tensions de vapeur à température ordinaire et sont thermiquement fragiles. For example, aminoacids sublimate and their vapour pressure at their temperature threshold for evaporation range from  $9 \cdot 10^{-10}$  atm (L-Cys) down to  $1.8 \cdot 10^{-10}$  atm (L-Phe) [1]. Different types of sources have been devised to bring intact neutral [2-5] or charged biomolecules in the gas-phase while still avoiding rapid degradation from heating. Some methods, such as electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI), are since a long time very widely used in mass-spectrometry and have been recently for spectroscopic studies of ionic species. Others have been specially devised in order to produce cold neutral beams suitable for spectroscopy and will be first examined in this chapter. The separation between production of either neutral or charged species is sometimes arbitrary since some sources, such as matrix-assisted laser desorption (MALD) can deliver both.

## **1 Production d'espèces neutres à partir de jets thermiques ou supersoniques**

The difficulty for setting molecules of biological interest into gas-phase is somewhat dependent upon the sensitivity of the used experimental set-up. According to the spectroscopic method and its sensitivity, a more or less large density of neutrals is required. Among molecules, a fraction of them can be simply heated in order to obtain sufficiently high vapour pressure before reaching decomposition. For example, among nucleobases, reasonable partial vapour pressures allowing for some spectroscopic measurements concerning uracil, thymine, adenine and cytosine [6] can be reached while guanine often requires more elaborate methods than simple heating [7-9].

Immediately after vaporization, the molecular temperature is still rather high and different methods are available for cooling molecular systems down to a temperature such that only a restricted number of low-lying conformations are populated. For that purpose, free jet expansions and deposition on helium clusters are used for neutrals. Carrier gases (usually helium or argon) can be seeded by the molecular systems of interest either or after the source nozzle creating the supersonic expansion.

Prior to the expansion, one can assume that a Boltzmann population distribution exists among the different conformers corresponding to the temperature, typically in between 400 and 550°C. The question is then whether this initial distribution is maintained or not during the cooling process induced by the supersonic expansion. The number of collisions per second  $z_{coll}$  in the supersonic jet depends upon the reservoir density  $n_0$ , the nozzle diameter  $D$ , the collision cross-section  $\sigma$  with the carrier gas and the local Mach number  $M$  (ratio of the beam velocity to the local speed of sound) and the heat capacity ratio  $\gamma=C_p/C_v$ .

$$z_{coll} = \sqrt{2} n_0 \sigma \bar{v}_0 \left[ 1 + 1/2(\gamma - 1)M^2 \right]^{-1/2[(\gamma+1)/(\gamma-1)]} \quad (3.1.1)$$



Figure 1. (a) The carrier gas of a supersonic expansion can be seeded by biomolecular systems in the sample reservoir situated before the supersonic expansion (b) practical realization of a hyperthermal source producing pulsed supersonic expansion with a sample reservoir held at 750°C. (HS heat shield, SR sample reservoir, H heater, T thermocouple wire, P stainless steel poppet, BS buffer spring, MS main spring, SW solenoid wire, WL water-cooled line) (c) practical realization of a laser vaporization source combined with a pulsed supersonic expansion. The sample is mixed with graphite powder and pressed into a pellet that is illuminated by the second harmonic of a pulsed YGG laser through a multimode fiber

The energy loss  $\Delta E$  in each collision of a heavy biomolecule of energy  $E$  with a light carrier gas atom can be approximated by

$$\Delta E = \lambda(E - E_{ZPE}) \quad (3.1.2)$$

where  $E_{ZPE}$  is the zero-point energy of the lowest energy conformation. The local Mach number  $M$  depends upon the ratio  $x/D$  of the distance from the nozzle  $x$  and  $D$  and varies along the beam as:

$$M = 3.26(x/D - 0.075)^{0.67} - 0.61(x/D - 0.075)^{-0.67} \approx C(x/D)^{\gamma-1} \quad (3.1.3)$$

If the temperature of the reservoir is  $T$ , the temperature in the beam  $T$  varies with distance  $x$  from the nozzle as:

$$T = T_0 / (1 + (\gamma - 1)M^2 / 2) \quad (3.1.4)$$

The dynamics of conformational isomerization in a jet expansion has been experimentally [10] and theoretically [11] studied into details in relationship with the systematic exploration of the PES (see 1.5 and 2.1.4.4). The change of the occupation probability  $P_i(t)$  of a given minimum  $i$  of the PES depends upon the occupation probabilities  $P_j(t)$  of the other populated minima (figure 3.1.2) and the rate constants  $k$  summed over the different transition states directly connecting minima between themselves.

$$\frac{dP_i(t)}{dt} = \sum_{j \neq i} [k_{ij}(E)P_j(t) - k_{ji}(t)P_i(t)] \quad (3.1.5)$$

The cooling process along the supersonic expansion can then be simulated once the different minima and transition state energies have been estimated by a systematic exploration of the molecular PES conducted with a force-field.

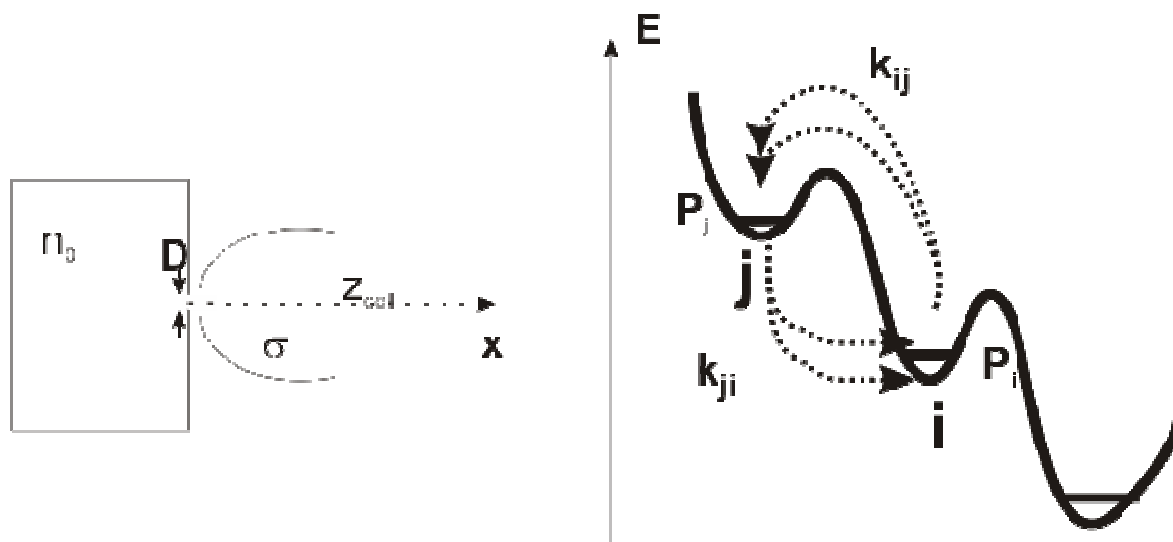


Figure 3.1.2. Gauche. Parameters of a supersonic expansion used in simulation of the cooling process. Droite. Schematic diagram representing minima of a potential energy surface and transition rates between those minima corresponding to different transition states.

An example of the variation of an experimentally observed laser-induced fluorescence (LIF) spectrum recorded at different distances from a beam nozzle is represented in figure 4.3.3. The variation of the temperature along the expansion (equation 3.1.4) can be estimated by comparison between the intensities of the  $v''=0 \rightarrow v'=1$  cold band and the  $v''=1 \rightarrow v'=0$  hot band according to the relationship.

$$\frac{I_{0 \rightarrow 1}}{I_{1 \rightarrow 0}} = \exp(-\Delta E / kT) \quad (3.1.6)$$

In the case of a flexible molecule such as N-acetyl-tryptophan methyl amide, a vibrational temperature down to 10-15°K can be obtained.

A “rule of thumb” has been proposed by J.P.Simons [12]. When barriers between conformers in the potential energy surface (PES) are larger than 12-15 kJ/mol, the initial Boltzmann population distribution remains unchanged. On the contrary, in presence of energy barriers lower than 5-6 kJ/mol, collisional relaxation brings the molecular systems down to their lowest zero-point energy conformations.

## 2 Production d'espèces neutres à l'aide de désorption laser

Thermal degradation can be avoided by means of a very rapid heating of biomolecules. Biomolecules can be either bare [9,18] or deposited on surfaces such as graphite [19,20]. They can also be embedded in a carbon [8], cellulose or nicotinic acid [21] matrix. Fast laser irradiation of the surrounding medium introduces a very fast expansion before thermal degradation of the system of interest. This method can be considered as relatively mild since seeded supersonic beams mostly containing intact parents as well as heterogeneous clusters of matrix molecules and aminoacids can be obtained [22]. Expansion conditions (e.g. backing pressure) must then be carefully controlled to avoid pyrolysis. However, getting non-fragmented molecules can sometimes become a problem. For example, nucleosides can have

different behaviors. Guanosines do not fragment whereas some substituted adenosines do [23].

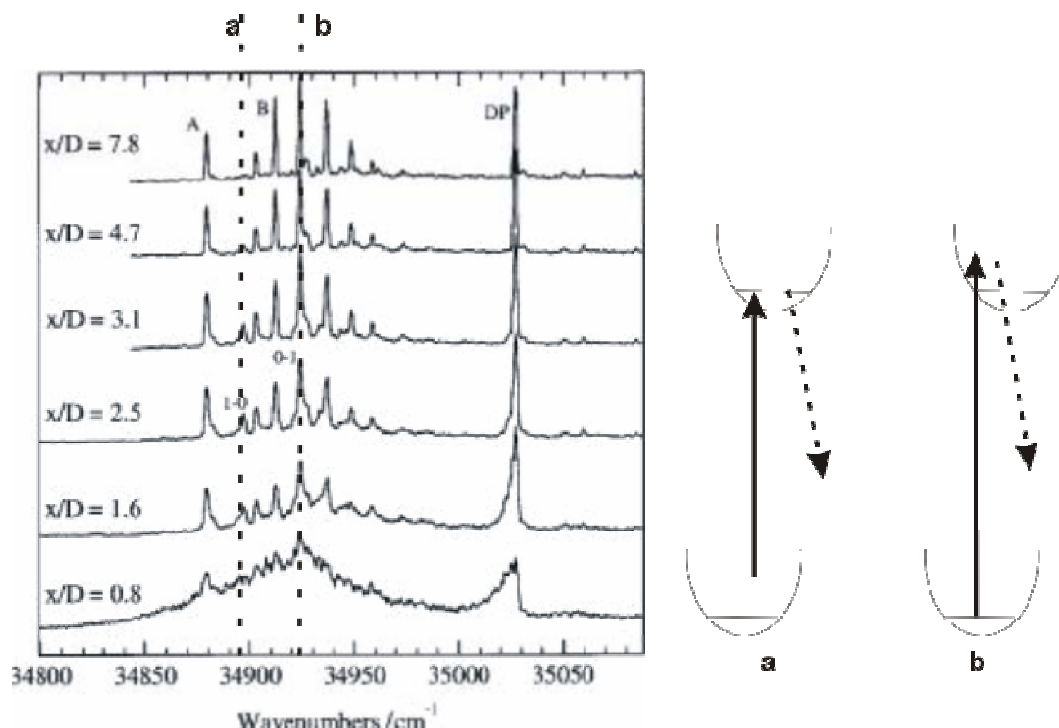


Figure 3. Variation of the laser-induced fluorescence (LIF) spectrum of N-acetyl-tryptophan methyl amide recorded at different distances  $x$  from the beam nozzle of diameter  $D$ . The temperature is estimated from the ratio of the intensities of the hot (a) and cold (b) bands. For experiments requiring long interaction paths between photon beams and molecular systems due to weak absorption in the mid- and far-infrared region, slit nozzles [13-15] or “ragout-type” [16,17] beams are employed. Very large buffer chambers up to  $23 \text{ m}^3$  are then used to feed oversized slit nozzles up to 60 cm long and gas pulses up to 1 s.

Most spectroscopic experiments using laser-desorption sources are run at very low repetition rates, typically in between 1 and 30 Hz. Some experiments such as photoelectron-photoion coincidence (PEPICO) spectroscopy require less than one particle per laser shot in order to avoid false coincidences and high repetition rates must then be employed for both laser and source. A design of a 1kHz source used for the generation of a jet-cooled molecular beam of guanine [24] is shown in figure 3.1.4.

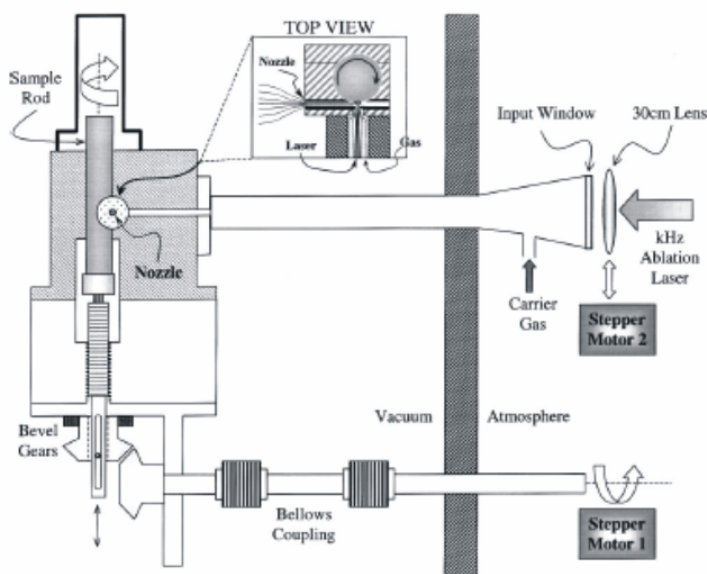


Figure 4. Schematic representation of a high-repetition rate laser-desorption source. The beam of a Nd:YLF laser at 527 nm illuminates a translated rotating cylindrical target of 6 mm diameter

### 3 Deposition d'espèces neutres sur des gouttelettes d'hélium

Molecules, even possessing very low vapour pressures, can be deposited on the surface or embedded inside very small droplets of liquid  $^4\text{He}$  typically containing  $10^3$ - $10^5$  atoms (see 2.1.3.2.3) [25-31]. Those droplets are produced by cryogenic nozzle expansion. Pressures range from 1 to 100 bars and nozzles with diameters from 5 to  $20\mu\text{m}$  are cooled from 10 to  $30^\circ\text{K}$ . The equilibrium temperature achieved inside the superfluid droplets has been evaluated to  $0.37 \pm 0.05^\circ\text{K}$  by fitting the rotational contour of embedded  $\text{SF}_6$  [32]. In exceptional cases, very costly  $^3\text{He}$  can be used to attain temperatures down to  $0.15^\circ\text{K}$ . After collimation, the droplets are seeded with the molecule of interest (dopant) by passing them through a pick-up cell (figure 3.1.5). If  $L$  is the path length through the cell,  $n$  the density of the gas in the cell and  $\sigma$  the attachment cross-section, the ratio between  $L$  and the mean free path  $\lambda = n\sigma L$  represents the expected number of sticking events. The probability that a passing cluster will pick up  $k$  molecules (admitting a sticking probability equal to 1) is given by the Poisson distribution:

$$P(k) = \lambda^k e^{-\lambda} / k! \quad (3.1.7)$$

The initial kinetic and internal energies of the dopant molecules are rather high since the dopant must be heated for sublimation. Each helium atom evaporating takes off an energy equivalent to approximately 5 to  $7^\circ\text{K}$ . Several hundred He atoms evaporate due to dissipation of kinetic and internal energy of a very small dopant molecule during the pick-up process. Thousands of He atom evaporations are required for large molecules, requiring the use of large droplets. A droplet containing  $N$  helium atoms has a very large cross-section equal to  $15.5 N^{2/3} \text{ \AA}$  and a dopant vapour pressure in between  $10^{-5}$  and  $10^{-4}$  mbar is sufficient to pick-up a single molecule. The pick-up process results in a Poisson distribution of the number of dopants per droplet.

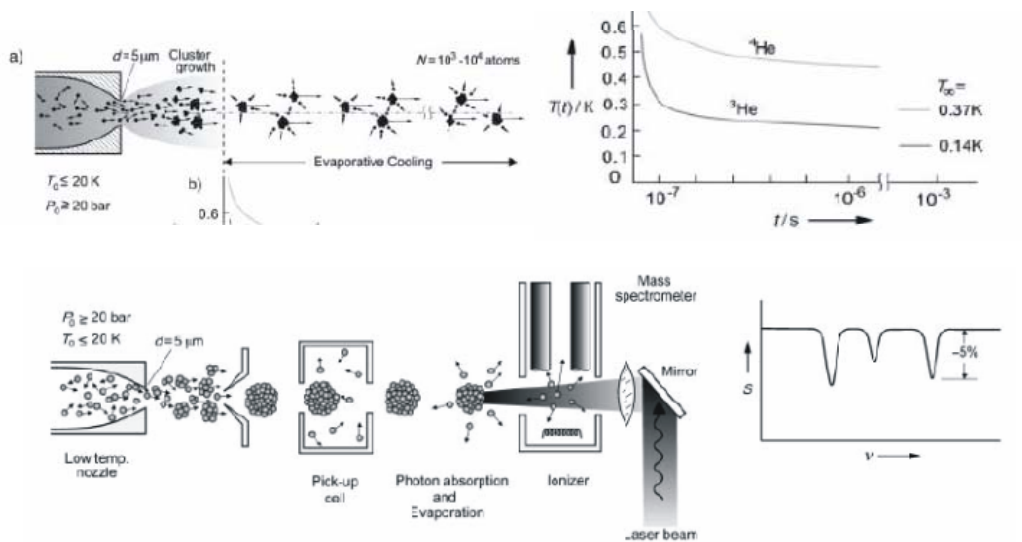


Figure 3.1.5. Haut Left Free jet expansion leading to production of helium clusters. Gaseous helium held in a reservoir at a pressure in between 20 and 100 bar and a temperature 5-20°K is expanded through a several microns diameter nozzle. The extensive cooling in the expansion leads to formation of droplets in the cluster growth region. Droplets then evaporate in vacuum and carry on cooling respectively down to 0.4 and 0.15°K for  $^4\text{He}$  and  $^3\text{He}$ . Right Calculated dependence of the droplet temperature as function of time for  $^4\text{He}$  and  $^3\text{He}$  after they have left the cluster growth region. Bas Set-up used for pick-up and laser depletion spectroscopy of molecules embedded in helium droplets. The absorption of resonant infrared photons results in evaporation of a large fraction of helium atoms that is detected as a decrease of an ion signal following ionization, mass-selection and detection

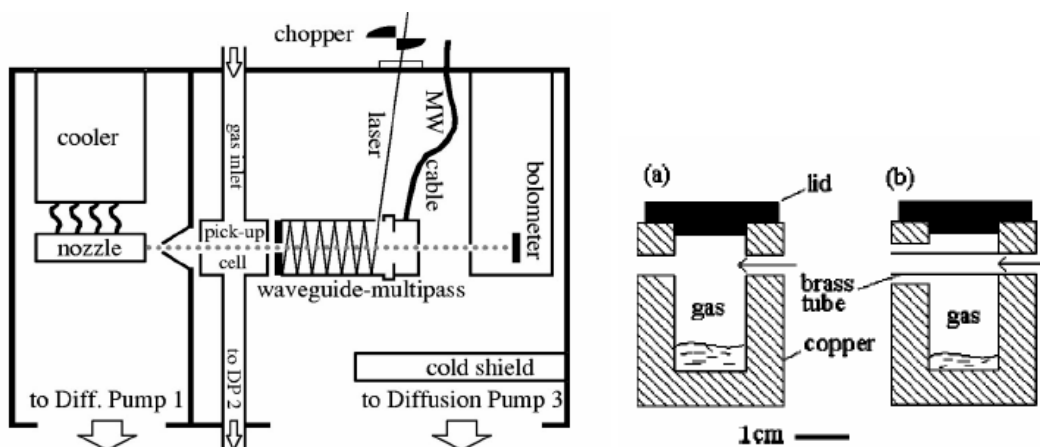


Figure6. Gauche Typical design of a helium droplet experiment for high-resolution spectroscopy with a conventional pick-up cell Droit (a) conventional pick-up cell (b) modified pick-up cell reducing cluster deflections and improving detection of doped clusters.

## 4 Jets liquides and supercritiques, microjets, goutelettes liquides

### Jets liquides

Neutral and ionized biomolecular systems can be directly injected from their natural medium, i.e. water, into the gas-phase. Continuous liquid beams can be produced into vacuum by injection of a liquid through a 10-20  $\mu\text{m}$  aperture in diameter at a pressure in between 10 and 100 bars leading to a flow speed of 10-100 m/s [35,36]. Although a rather large amount of water is introduced in vacuum, the background pressure in the vacuum chamber can still be maintained in the  $10^{-5}$ - $10^{-6}$  Torr region by capturing the liquid beam in a liquid nitrogen trap. The high-speed flow renews the liquid beam surface that remains clean. The conditions experienced by molecules lying on a liquid surface have been studied by means of molecular dynamics simulation and experiments with metastable atoms [37]. Due to evaporation, the surface of the liquid beam drops rapidly at a rate of  $5^\circ\text{K}/\text{mm}$  down to  $220^\circ\text{K}$  [38]. Alcohols are easily used as solvents but water may present some difficulties due to a rapid freezing [39]. Mechanical chopping of the ice beam can then be necessary. The studied biomolecular systems introduced in the solvent are ejected from the liquid beam by means of a pulsed nanosecond infrared laser exciting stretching vibrations of the solvent (e.g. the C-O stretch vibration of methanol at  $9.66\mu\text{m}$  or the O-H stretch vibration of water in the  $3000\text{-}3800\text{ cm}^{-1}$  region) [40-42]. A first explanation of the observation of ion ejection from the liquid beam is the following [43]. After pulsed IR photon absorption by the solvent molecules, the vibrational energy relaxation produces within a few picoseconds an extremely rapid heating of the solvent, in the order of  $10^{10}\text{ }^\circ\text{K}/\text{s}$ . This raises the pressure up to several hundred bars and brings the solvent in a supercritical state (for water,  $T_c=647^\circ\text{K}$ ,  $p_c=221\text{ bars}$ ,  $\rho_c=322\text{ kg/m}^3$ ). The dielectric constant then drops suddenly and most ions recombine except few "survivors". Those were highly solvated or far from counter ions and can then escape into vacuum. By varying the IR laser frequency and power, it is possible to modify its penetration depth in the liquid beam. Fast neutral clusters are also ejected from the beam surface while slow ones are ejected from the bulk region inside the beam [44]. An alternative explanation [45] implies a strong shock wave produced by the fast energy deposition leading to an explosive thermal volume expansion. The liquid is then dispersed into very tiny droplets too small for supporting more than a single charge. In contrast with ESI (see 3.1.6), only singly or doubly charged ions are observed. Polydisperse nanoparticles of 150 nm can also be obtained by atomizing solutions of aminoacids. A heater and a diffusion dryer are then used to evaporate the solvent. The dry particles are entrained by a nitrogen flow and pass through a skimmer. A copper heater then produces the neutral aminoacids in the gas-phase [46].

In the case of aminoacids, both protonated and negatively charged arginine ions have been observed in the gas-phase. It is supposed that following absorption of more than four IR photons, water molecules dissociate into  $\text{H}^+$  and  $\text{OH}^-$  which then react with arginine to produce the observed ions [47,48]. Interestingly, in the case of large biomolecules such as cytochrome c or bovine serum albumine desorbed from a liquid beam, it has been shown that the relative intensities of the mass peaks reflects the relative concentration in the solution [45]. Liquid beams have also been used for near-edge X-ray absorption fine structure (NEXFS) studies of aqueous proline and diglycine [49].

Fast liquid beams (100-500 m/s, 1-20  $\mu\text{m}$  diameter) can be created from modified DESI sources (see 3.1.6) by using a pulled quartz capillary nozzle. Aqueous solvent systems are then introduced into the nozzle at 300-400 bars. The jet desorption/ionization method (JeDI) allows in-depth analysis of frozen tissue samples [50,51].

## Expansions supercritiques

Supercritical fluids have large solvent power. Beams of small molecules such as adenine, caffeine, guanine or vitamin K3 [52,53] can be generated by seeding carbon dioxide ( $T_c=304^\circ\text{K}$ ,  $p_c=73.8$  bars). The solvent power can be adjusted between that of gas- or liquid-phase with only moderate changes in pressure. Moreover, carbon dioxide is compatible with ultrahigh conditions. Supercritical fluids are also used in high performance liquid chromatography (HPLC). Intermediate size peptides such as bradykinin or angiotensin can then be dissolved in a  $\text{CO}_2$  /methanol mobile phase with addition of trifluoroacetic acid to suppress deprotonation of carboxylic groups and to protonate amino groups. The corresponding ions are then electrospayed (see 3.1.6) [54].

## Goutelettes liquides

The laser induced liquid beam ionization/desorption (LILBID) process is very mild and allows the study of non-covalent complexes complexes (see 4.5) [55,56]. However, the main drawback of this method is that a large amount of solute is required due to the production of continuous liquid beams while ions are set into gas-phase by means of very short-pulsed lasers. An alternative possibility has thus been devised to considerably reduce the consumption by increasing the duty cycle and bringing it close to unity. Microdroplets [57,58] are introduced into vacuum by means of a piezoelectric driven generator synchronized with an infrared [59] or UV [60] desorption laser. The irradiated droplets are submitted to a shockwave [61] and explode, leading to a small fraction ( $10^{-4}$ ) of the dissolved ions they contain being detected in the gas-phase. In ESI, droplets created at atmospheric pressure evaporate slowly and produce highly charged and totally desolvated species. In the microdroplet method, droplets evaporate in less than 100ns. Only low charge states are observed and some solvent molecules can be retained or removed, if required, by a second evaporation laser shot. The method is also less sensitive than ESI to the presence of salts. The absolute amount of analyte that can be detected from a single droplet is in the attomolar range [59].

This mild method allows the observation of specific non-covalent complexes (see 4.5) such as the minor groove binder distamycin A bound to the single strand Dickerson dodecamer [62] or cytochrome c complexes of bacteria [63]. It also allows the measurement of the stoichiometry of membrane protein complexes. Often, membrane complexes are oligomers and their quaternary structure can be ascertained only by mass-spectrometry (see 4.8.1). At very low laser intensities, use of the microdroplet method provides detection of the complete assembly of the non-covalently bound subunits. At higher laser intensities, partial rupture of the non-covalent bonds leads to individual detection of subunits. Protein membranes play a crucial role for cells since they are responsible for communications between the intracellular medium and the cytoplasm. Mass-spectrometric methods encounter problems since those proteins are highly hydrophobic and cannot be solubilised in water or any polar solvent [64,65] The necessary use of surface active detergents strongly reduces the ionization efficiency in electrospray (see 3.1.6) and make difficult crystallization in MALDI (see 3.1.5). In contrast, the laser-ablated droplet method is much more tolerant to solutions containing buffers and detergents make the method particularly interesting for the study of membrane proteins [63].

## 5 Matrix Assisted Laser Adsorption Ionization (MALDI)

## Processus d'ionisation

For a long time, the most widely used ionization process has been electron impact (EI see 2.3.1). For reproducibility of the mass-spectra, the used electron energy was in the range of 50-80eV, much above ionization energies of molecules, and this exaggerated supplied energy lead to extensive fragmentation. Much milder ionization processes using free electrons leading to electron capture followed by non-dissocialise (RET) or specific dissocialise (ECD) processes are respectively considered in chapters 2.3.2.2 and 4.7. EI is limited to rather low masses and other ionization processes have since been devised for nearly any molecular masses. One or several protons can be added to bimolecular systems and reside on the most basic sites. Those protonated sites must be sufficiently separated in order to limit Coulomb repulsion and it is usually admitted that, for example, in peptides, the most basic amino acids (Arg, Lys) must be distant from at least three or four units. However, in some cases, the  $\pi$ -electron cloud of an aromatic side chain offers screening between positive charges and doubly-charged peptides can be observed. Removal of protons is another ionization process useful in the case of acidic compounds. In some cases, fragmentation can be minimized by replacing protons by metal cations. For example,  $\text{Na}^+$  or  $\text{K}^+$  are common adducts for carbohydrates (see 4.4).

## Fonctionnement du MALDI

In matrix-assisted laser desorption/ionization (MALDI), analytes are embedded in a surplus of a matrix of small organic molecules [66,67] or ultrafine metal powder [68] absorbing the beam of UV lasers usually delivering pulses in the ns range. IR lasers are also used but to a less extent [69]. Following absorption of a laser beam by the matrix, a plume is produced and ejects the analytes into the gas phase. Only a rather small fraction of ejected material is detected as ions in UV-MALDI, in between  $10^{-2}$  and  $10^{-5}$ , and further mass-analyzed. During preparation, the analyte molecules and the matrix are dissolved, for example in water or in a 1:1 mixture of water and methanol, and deposited as small spots of a few  $\mu\text{l}$  on a stainless steel, either at atmospheric pressure or under vacuum conditions [70]. In the latter case, vacuum sublimation purifies the matrix compound and an extremely pure layer of small matrix crystals with diameter less than 300nm is obtained. For analytical purposes, biomolecular solutions are at in between  $10^{-5}$  M and  $10^{-3}$  M concentrations. For spectroscopic purposes, larger amounts of material are required since the number of laser shots can be larger than  $10^4$  for example in order to record a full IR spectrum [71]. Most often, MALDI sources are preferentially associated to time-of-flight (TOF) mass-spectrometers (see 3.2.1.1) and the acquisition time of mass-spectra can be very short when high-repetition rate lasers are used. For example, a rate of 2 spots per second, each spot being analyzed with 800 laser shots, can be obtained allowing a rapid on-line coupling to a liquid chromatograph (LC) [72]. MALDI sources in combination with ion trapping devices can also be used for long spectroscopic scans [73-75] lasting several hours.

The choice of a matrix molecule containing a UV chromophore, such as *trans*-cinnamic acid or 2,5-dihydroxybenzoic acid (DHB), is dictated by the analyte according to the matrix ability to ionize and form adducts [76]. The matrix plays several roles. The first one is isolation to prevent formation of analyte aggregates. In the matrix, analytes are incorporated in the charge state already existing in the solution, in presence of their counter ions to ensure neutrality. Once the UV laser photon energy is electronically deposited very locally in the matrix during on a time scale of a few ns much shorter than the time required for energy redistribution by thermal diffusion, two physical regimes can occur. At low laser fluences, neutral and ionic species are individually set into gas-phase in a desorption process. When the laser fluence

increases, the matrix layer explodes in an ablation regime and ejects molecular clusters as well as individual species. The modelling of the MALDI process involves a large number of processes occurring on three different time scales. The initial excitation by the UV laser of matrix molecules containing chromophores in their first excited state  $S_1$  is redistributed by hopping on a picosecond time scale to neighbouring molecules. Those excited molecules interact through their aromatic rings and can thus acquire enough energy (energy pooling) to overcome their ionization limit, providing ions and free electrons with a mean free path of 10nm in the matrix. Those electrons can escape the matrix leaving it with a positively charged surface. The electronic energy is also converted into vibrational energy and a fast heating, within nanoseconds, leads to ejection of a surface region of the irradiated target and formation of the plume. A large number of reactions take place within microseconds in the plume and produce mostly singly-charge ions. [77,78].

The analyte ions have a high initial velocity  $v_0$  typically in between 300 and 800 m/s, with a considerable spread ( $v_0 \pm 0.5v_0$ ). In order to improve mass-resolution, a technique called time-lag focusing is introduced. A time delay is applied between ionization and extraction of the ions out of the ion source into the drift tube of the analyzer. Ions are extracted from the source by an electric pulse after the time delay expires in order to minimize the arrival time distribution of the ions at the detector. When a second time-dependent field is applied in the second extraction region of the TOF, the time-lag method becomes mass-independent over a broad mass range.

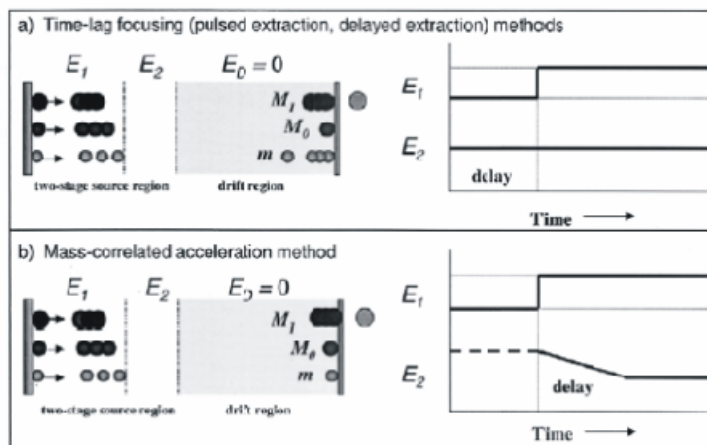
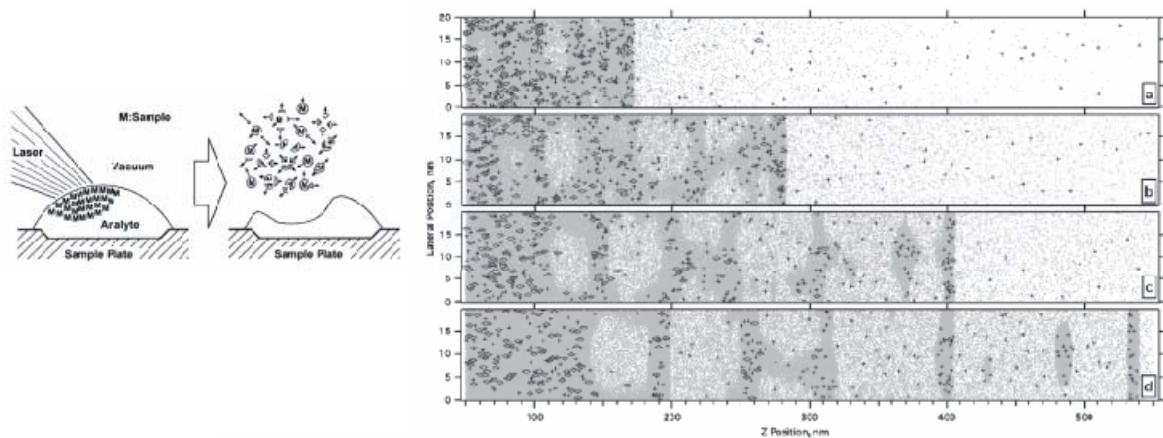


Figure 7. Haut Gauche Matrix-assisted laser desorption/ionization Droit. Snapshots of a simulation showing ablation and cluster formation comprising 1,022,976 molecules at 500 (a), 750 (b), 1000 (c) and 1250 ps after a 30 ps laser shot. Neutral molecules are denoted as gray points, positive ions as crosses and negative ions as diamonds. Bas. Time-lag extraction in a MALDI-TOF device with a single pulse (a) or a second time-dependent pulse (b)

MALDI can be used for a wide variety of biomolecular systems such as DNA [81], peptides [82], proteins [83] or oligonucleotides [84]. It is also a mild process leading to intact non-covalent complexes (see 4.5) in the gas-phase [85,86]. When combined with a time-of-flight mass spectrometer, a high mass accuracy ( $<10$  ppm) can be obtained up to very high masses ( $>10^6$ ) [87], with a subfemtomole sensitivity and the possibility to mass-analyze more than a thousand spots in a single run.

## Bioaérosols

In order to reduce the amount of analyte and increase the sensitivity of MALDI, it is possible to replace matrix-spots with typical mm diameter by calibrated aerosol droplets with  $\mu\text{m}$  diameter [88]. Lasers or synchrotron radiation [89] can be used for ionization. In off-line experiments, the matrix and the analyte are premixed in solution prior to aerosolization and the droplets composition reflects that of the solution. In on-line experiments, analyte particles enter a chamber coated with the matrix and particles containing an analyte core with a matrix coating are produced. A detection limit of 14 zeptomoles, corresponding to 8400 molecules, has been reached with this method.

Aminoacids such as glycine, phenylalanine or tryptophan and peptides such as  $\beta$ -carotene or phenylalanine-glycine-glycine can be set into gas-phase from aerosol dry nanoparticles that are further thermally vaporized in high vacuum. The resulting vapor that contains neutral molecules can then be ionized by tunable VUV synchrotron radiation with negligible fragmentation. Particles are obtained by atomizing solutions containing 0.5 to 1 g of aminoacids or peptides in 1 liter of  $\text{H}_2\text{O}$ , methanol or ethanol. The liquid droplets forming the bioaerosols are then entrained in a nitrogen carrier gas, preheated to  $50^\circ\text{C}$  and dried at room pressure to form solid particles. The mean size of those particles is typically 100 nm and the concentration ca.  $10^7$  particles/ $\text{cm}^3$ . Those particles enter vacuum by passing through a 200  $\mu\text{m}$  diameter hole and five apertures acting as an aerodynamic lens. The focused beam enters the first region of a time-of-flight (TOF, see 3.2.1.1) mass spectrometer where they hit a 3mm diameter heated copper tip at a temperature in between 300 and  $850^\circ\text{K}$ . The beam particles are thus vaporized and the expanding gas acts as a localized molecular source at the entrance of the TOF set-up [90].

## 6 Electrospray

The electrospray (ESI) process transfers large unvolatile molecules from liquid phase into the gas phase as highly-charged isolated entities [91]. It is a very mild process as demonstrated by the possibility to observe for example intact protein quaternary structures (see 4.8) [92] or non-covalent drug binding sites [93,94]. A solution containing the analyte is introduced by means of a syringe in a capillary. The application of a high electric field to the capillary accumulates ions attracted by a counter electrode at the surface of the capillary tip [95]. The meniscus at the tip acquires the shape of a so-called Taylor cone that continuously breaks into droplets [57,96]. Those droplets are enriched in ions (e.g. cations in the case of a positive tip) when the applied electric field exceeds a threshold value in between 700 eV and several keV. Their diameter varies from microns at flow rates in between 1 and 100  $\mu\text{L}/\text{min}$  down to 100

nm for nL/min rates in nanospray.[97,98]. At large flow rates, the spraying process is generally pneumatically assisted by a coaxial dry gas possibly heated. The introduction of the ions in the mass spectrometer uses a dry nitrogen “curtain” or “sheath” gas. A heated capillary interface aids in desolvation and de-clustering of ions from neutrals. The mist of highly charged droplets initially at atmospheric pressure [58,99] pass down an electrostatic potential and pressure gradient towards the high vacuum of a mass-analyzer. In the nano-spray regime (nano-ESI) 1-10  $\mu\text{m}$  capillary exits and nanoliters/min rates are used without the need of pushing syringe and sheath gas. The electric field at the tip is alone sufficient for droplet formation and desolvation [100]. A very short distance (mm) can be applied between the tip of the capillary and the entrance of the mass-spectrometer. A high efficiency, defined as the ratio of the flux of ions reaching the ion detector after mass-selection and the flux of ions leaving the tip, of typically 1% and up to 12% can then be reached. Droplets reduce in size by evaporation of the solvent and Coulomb explosion during their passage through the desolvation chamber which is usually kept at room temperature.

Two mechanisms are mainly invoked for explaining the fate of the charged droplets (figure 3.1.8). Reviews can be found in references [101,102]. The droplets undergo a sequence of solvent evaporation and Coulomb explosion cycles. In the “ion evaporation” model (IEM), it is assumed that the high electric field at the surface lifts ions from the solute medium into vacuum when the droplets, following successive evaporations, acquire a radius less than 10nm. In the “charge-residue” model (CR), droplets sequentially evaporate their solvent molecules. When their radius becomes too small (“Rayleigh limit”) [58,99], their surface charge-density provides an electrostatic force overcoming their surface tension. For example, the critical radius for a droplet carrying 200 charges is 12nm. Finally, a bare charged molecule is left without any solvent. It seems that in nano-ESI where very small droplets are formed, the CR mechanism dominates for large species while the IEM mechanism is favoured in ESI in the case of small ions.

Mass spectra obtained with electrospray show that after evaporation the charged droplets leave intact sample molecules containing a rather large number of charges [103,104]. The  $m/Z_e$  ratio is usually limited to 3000 although values up to 30.000 can still be observed [105](see 4.8). Thus, ESI is well-adapted to mass spectrometers with a limited  $m/Z_e$  range [101,106].

Mass-spectrometry is widely used to obtain structural information of biomolecules through their fragmentation pattern. In the case of MS/MS (see 3.2.2), a spatial or temporal sequence of events is required to first isolate parent ions, dissociate them by collision (CID) with a neutral gas and analyse their fragments. In an electrospray source, a collisional exchange occurs between the translational and the internal (vibrational plus rotational) energies of the biomolecular ions. By increasing the applied acceleration voltage leading to their entrance in the mass-spectrometer, it is possible to raise the ion internal energy and thus to increase their fragmentation rates. Above a critical energy, the different fragmentation pathways are characterized by different variations of the dissociation rates as a function of the ion internal energy. This so-called source-CID has been studied into details and it has been shown that it can be used to test the kinetic stability of non-covalent complexes, providing for example, insights about binding properties of drugs to DNA [109,110].

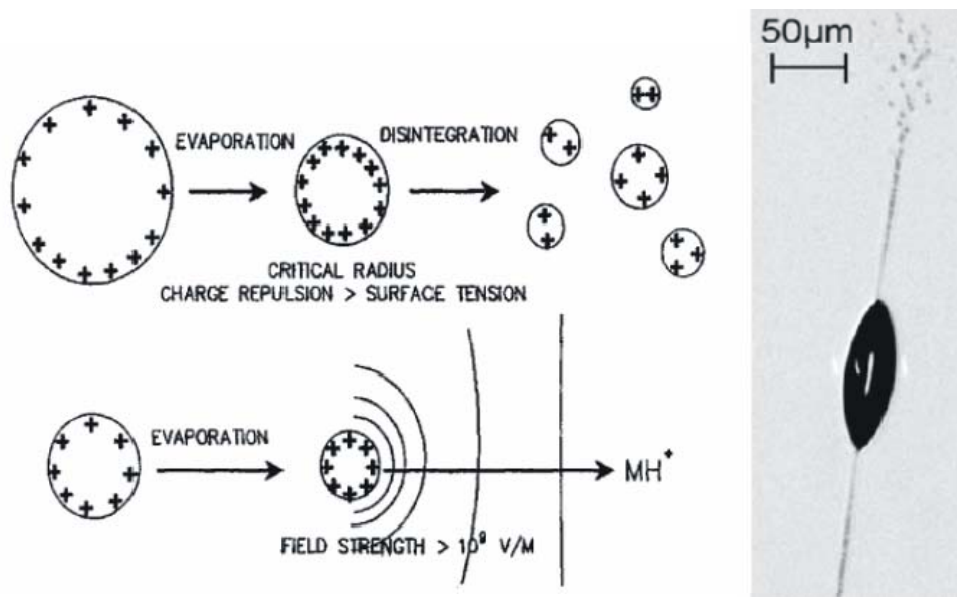


Figure.8. Gauche Haut “Charged-residue model”(CR). Offspring droplets resulting from a first Rayleigh instability continue to evaporate solvent until they become too small and undergo a second Rayleigh instability and explode. Following a sequence of evaporation-explosion, very small droplets only containing a single solute molecule are produced. Bottom. In the “ion evaporation model”(IEM), before ultimate droplets are formed, the electric field at the droplet surface becomes large enough to expel solute ions. Droplet implosion of a glycerol droplet investigated by ultra fast microscopy. In the moment of instability, the deformed droplets have a spindle-like shape from which two highly-charged liquid jets are emitted. The great versatility of electrospray allows to combine two ESI sources for producing ions of both signs and studying ion/ion reactions (figure 3.1.9) [107]

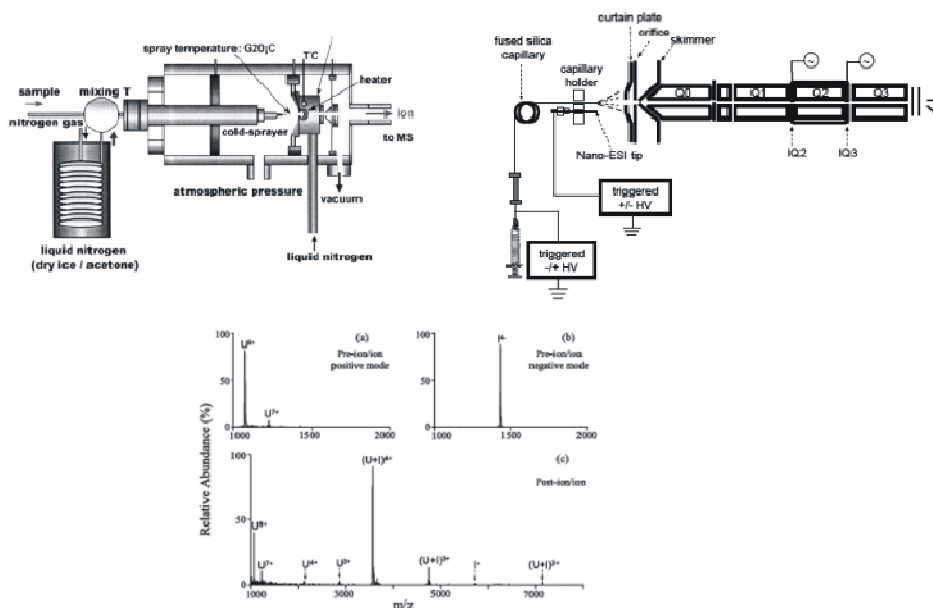


Figure.9. Haut.Gauche. Electrospray source with a desolvation chamber held at low temperature (-80-10°C) in order to reduce breaking of non-covalent interactions. Droit.. Pulsed dual electrospray source and triple quadrupole/linear trap tandem mass-spectrometer (see 3.2). Each ESI source is pulsed alternatively and ions of opposite signs penetrate separately in Q1 where they are isolated. During mutual storage in Q2 and Q3, ions of opposite charges react. Following ejection of the undesired ions of a given sign, the other ions are mass-analyzed. Bas. Mass spectra derived from the pulsed dual electrospray source. Positive bovine ubiquitin (U)[M+8H]<sup>8+</sup> ions (a) and [M-4H]<sup>4-</sup> bovine insulin (I) ions (b) react during 300ms and preferentially form the protein-protein complex [U+I]<sup>4+</sup> complex (c).

Other methods related to electrospray are desorption sonic spray ionization (DeSSI) [111-113] or laser spray [114] using a laser beam in presence of a weaker electric field [115] to produce droplets. In atmospheric pressure chemical ionization (APCI), vaporization of the solution containing the analyte is assisted by a strong gas flow at a high temperature (ca 500°C) in absence of high voltage. The solvent is excited and ionized by a corona discharge and either transfers or removes protons from the analyte which becomes ionized. In a photo spray, the gaseous mixture is transferred through a heated quartz tube. An added dopant is then ionized by 10eV photons issued from a Kr lamp and in turn ionizes the analyte [116-119]. Synchrotron radiation can also be used to obtain a far better control of ionization conditions.

### **Desorption electrospray ionization (DESY) et electrospray-assisted laser desorption/ ionization (ELDI)**

Analyte samples do not necessarily need to be manipulated into solution and further desolved. The desorption electrospray ionization (DESI) allows the production of ions directly in the ambient environment [120-123]. In DESI [113,123-126], charged droplets of a solvent, produced by an electrospray emitter (figure 3.1.10) impact at few hundred m/s on a surface and cover an area having a diameter 3-10 times larger than their initial diameter, producing offspring droplets. Ionization of the analyte deposited on the surface takes place by a heterogeneous charge-transfer mechanism and a droplet pick-up mechanism. Ions of both signs can be obtained. For example, DESI mass spectra of protonated alkaloids, polar lipids or carbohydrates have been directly obtained from plant tissues at atmospheric pressure. [127]. DESI combines advantages of both MALDI and ESI for protein sequencing in proteomics. As ESI, it provides multiply-charged ions more easily identified by MS/MS than the singly-charged ions produced in MALDI. Since it does not require any chromatographic step, it is as fast and easy to automate as MALDI.

ESI and MALDI can be combined for ambient mass spectrometry in electrospray-assisted laser desorption/ ionization (ELDI). In MALDI, the number of desorbed neutral far exceeds the number of ions and post-ionization methods have thus been devised. Among them, one can find electron ionization, REMPI or corona-discharge atmospheric pressure chemical ionization (APCI) [128,129]. In ELDI [130], ESI is used to post-ionize neutral proteins desorbed from a metallic plate by means of a laser beam.

The direct analysis in real time method (DART) [122] uses a plasma of helium or nitrogen that possess high ionization potentials, much larger than that of biomolecules. Metastable atoms or molecules  $M^*$  are separated from ions and impinge upon the analyte that is ionized by the Penning process  $M^* + a \rightarrow M + a^+ + e$ .

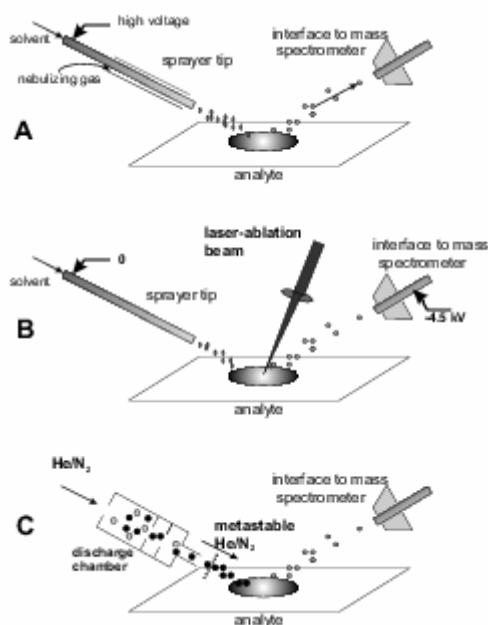


Figure 10. A Schematic view of a desorption electrospray ionization (DESI) source B Schematic view of a electrospray-assisted laser desorption/ ionization (ELDI) source. The laser-ablated analyte molecules issued from the MALDI matrix are ionized by the electrospray solvent plume C Schematic view of a direct analysis in real time (DART) source.

## Sonic spray

In a sonic spray nebulizer, the sample solution is introduced in a silica capillary fixed in the middle of an orifice drilled in a chamber. Nitrogen introduced in the chamber exits through the orifice with the sample solution and disperses it. There is no need to apply a potential drop between the sample introduction capillary and the quadrupole mass analyzer entrance. As in ESI, produced droplets evaporate and form ions. The ion signal intensity is then two orders of magnitude less than in usual ESI. A stable ion yield is obtained with a sample flow rate as low as  $1\mu\text{l}/\text{min}$  [131,132]. Such an ion source has been used to produce  $[m\text{Serine} + nH]^{n+}$  serine nanoparticles with  $m$  up to 600 and  $n$  in between 1 and 10. The chiral selectivity observed in  $[8\text{Serine} + H]^+$  ions (see 4.6) is still present in the large nanoparticles [133].

## 7 Laser-induced acoustic desorption

An alternate method that can be run in quadrupole-trap (3.2.1.2) or FT-ICR (3.2.1.4) mass-spectrometers is laser-induced acoustic desorption. A high-intensity laser irradiates on one side a thin ( $10\mu\text{m}$ ) metal foil or thick silicium ( $0.5\text{mm}$ ) wafer. Ablation of the metal creates an acoustic shockwave that propagates through the foil and induces gentle desorption on the opposite side. This method can be used for example bringing peptides in an FT-ICR cell and letting them further react to produce radicals [134]. It can also be used for desorbing very large biomolecular systems such as bacteria or cells (see 4.8) [135]. The advantage with respect to electrospray is then avoiding the production of aerosols when dealing with pathogenic bioparticles.

## 8 Production d'espèces hydratées

In order to establish a link between the behavior of biological species under isolated conditions and in the crowded cellular medium (see 5.1), a large number of experiments are devoted to study of species in presence of a progressive number of water molecules. Those studies are described in chapter 5 and only sources of hydrated species are considered here. In the case of supersonic expansions, the rare gas carrier (He, Ne or Ar) is seeded by passing over a reservoir containing water, generally at room temperature.[6,136-142].

Hydrated neutral species can also be set in gas-phase by dissolving them in water and then freezing drops of the solution at low temperature. A plume of desorbed hydrated clusters is then produced by resonant absorption of ice by 3.1  $\mu\text{m}$  radiation. Neutral tryptophan-(water)<sub>N<11</sub> clusters have been generated with this method [143]. Tryptophan-(water)<sub>N<16</sub> clusters can also be obtained from a pick-up source by deposition of tryptophan on a water cluster beam [144].

In electrospray sources, ions can be prepared from a water/methanol solution and different possibilities are offered [145]. One can operate the ESI source under conditions where ions are not totally dehydrated [146-148]. It is also possible to rehydrate ions either in specific ESI sources designed with two chambers [149-151] or by further exposing ions to water vapor, for example in a drift-cell [145].

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