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Microfluidics for Ultrafast Spectroscopy

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Abstract

Ultrafast laser technologies became one of the essential tool in the characterization of molecular compounds. Being comprised of spectroscopists, laser scientists, chemists and biologists, the “ultrafast community” is often disconnected and consequently unaware of the developments in microfluidic systems. The challenges of studying limited amount of precious liquid sample by means of ultrafast spectroscopy remains silent and, while no commercial systems are available, each research group is developing its own “home-made” options. This chapter will therefore contribute in filling up the gap that exist between the two communities, that of the ultrafast spectroscopy and that of microfluidics by revealing the importance of this analytical tool as well as the advantages of applying microfluidic technics to it. In this goal, the chapter will focus of the recently developed microfluidic flow-cell. With a minimal volume of about 250 μL , the flow-cell enables the study of precious protein complexes that are simply not available in larger quantities. The multiple advantages of the microfluidic flow-cell will be illustrated by the analysis of the cytochrome *bc₁*. In particular, the study will describe how the capabilities of the microfluidic flow-cell enabled the resolution of the ultrafast electronic and nuclear dynamics of specific embedded chromophores.

Keywords: Microfluidics, Ultrafast Spectroscopy, Liquid Sample

1. Introduction

The aim of this chapter is to address the gap that exists between two research communities: ultrafast spectroscopy and microfluidics. Indeed, the development of pulsed laser systems in the last few decades has ushered in new techniques in ultrafast spectroscopy. These techniques have opened new doors for the study of fundamental photo-chemical and photo-physical behavior of a variety of photosynthetic protein complexes.[1] For methodological reasons, i.e. samples being rare and the use of highly specialized equipment, there existed a pressing need to apply microfluidics systems in ultrafast spectroscopy, two fields that unfortunately

developed separately and whose researchers are rarely knowledgeable in both areas. For the microfluidic readers who may be unfamiliar with this literature, I introduce, in the first part of this chapter, the basic concepts of ultrafast pump-probe spectroscopy. In so doing, I highlight the relevance of this technique in gaining understanding protein dynamics and therefore biological properties and functions. It is in fact due to the laborious procedures that are involved in the purification processes of these proteins complexes that most biological samples are only available in sub-milliliters quantities. In the second part of this chapter, I will therefore expose some of the most common solutions that are in use in order to manipulate liquid samples in ultrafast spectroscopy. Finally, in the third part of this chapter, I describe one of the latest applications to the field of ultrafast spectroscopy—what is now called microfluidics—as it handles micro-liters volumes of a given sample. I illustrate the benefits of the application of such a microfluidic system through an analysis the of the cytochrome bc_1 . I conclude with a discussion on the areas of the present microfluidic flow-cell in need of further research and investigation.

2. The need for microfluidics in ultrafast spectroscopy

In this section I will introduce the field of ultrafast spectroscopy to which the microfluidics systems will be applied. I describe the basics of the technique and its relevance to the current fundamental research efforts in biochemistry and biophysics. In the last section of this chapter, I cover the practical challenges that emerge from such studies, which justify the need for microfluidics.

2.1. Ultrafast transient absorption spectroscopy

Transient absorption is a spectroscopic technique whose aim is to resolve the relaxation dynamics of an excited molecule “simply” by looking at its spectral modifications. The technique involves two principal light beam: a pump and a probe. While the role of the pump is to promote the molecule to a particular excited state, the probe is used to “look” at the state in which this molecule is in. The fact that these light beams are not continuous but are strains of pulses allows one to excite the molecule for a brief instant (the duration of the pulse) and to probe its state at a later time. The light pulses are first produced in a cavity (oscillator) that is built around a doped crystal such as titanium doped sapphire crystals (Ti:S) as shown in Figure 1.[2]

The crystal, when excited, serves as a photon tank that will amplify any pulses passing through, via stimulated emission. The cavity then enters into a “mode locked” state as soon as the enclosed pulse strains are in resonance with the dimension of the cavity itself. The energy trapped within the cavity builds up until the pulses are intense enough to leak through one of the cavity end-mirror. Typical Ti:S cavity produces strains of ~30 fs pulses centered around ~800 nm at 80 MHz repetition rate; each pulse being about tenth of nano-Joules. The extracted pulses are then amplified in a similar fashion by passing in a second cavity comprised of a second Ti:S crystal.[2] The frequency is however decreased to few kHz in order to reach few

Figure 1. Ti:S crystal cavity.

milli-Joules per pulses. It is these amplified pulses that are then split into pump and probe pulses.

Figure 2. Pump-probe experimental scheme.

As illustrated in Figure 2, varying the time delay that separate both pump and probe enables to follow the evolution of the transient excited states over time. This delay is introduced by physically increasing the path of one of the pump or probe arm of the setup via a precision stage. In a typical configuration, a 30 cm stage provides with a temporal window of about 4 ns (round-trip). The time resolution with which we can follow the molecular dynamics is then given by the duration of the light pulses themselves. A regular transient absorption setup is today capable of producing amplified strains of ~ 40 fs pulse with a kHz repetition rate. Such femto-to-nano-second time window corresponds to the dynamics of energy and electron transfer within and between molecules as well as local structural modifications.[3] Further-

more, the high repetition rate and the development in matters of laser stability and detection system gives the ability to monitor the absorption changes of a single molecule out of a thousand (corresponding to changes of $\sim 10^{-4}$ OD) in less than a second of accumulation time. [4] With such setups, it is for example possible to trigger the charge separation in the Photosystem I protein complex and to follow the liberated electron as it progresses from one side of the protein to the other. [4] Another example is the monitoring of the heme-ligand dissociation and rebinding dynamics that results from the absorption of a photon such as it is the case of various types of cytochromes. [5, 6]

The use of non-linear optics, such as in the famous and various kind of optical parametric amplifiers (OPA, Non-collinear OPA and multi-pass OPA) [7] as well as the different pulse shaping devices (in either transmission or reflection), [8] gives the ability to tune both the pump and the probe to the desire wavelength, therefore enabling to excite and to follow a particular molecular transition. For example, in the study of the bacterial reaction centers complexes, being comprised of multiple types of pigments that are spectrally distinct, careful tuning of the pump allows to preferably excite one pigment while leaving the others in their fundamental state. [9]

It is therefore out of the development in laser technology and specifically in tunable table-top pulsed lasers that the field of ultrafast spectroscopy came to know the success it knows today.

2.2. Studying biological samples

As implied in the previous section, transient spectroscopy is best suited for the study of compounds that have distinct spectral features. Fortunately, most organic compounds are made of either aromatic amino acids or incorporate chromophores within their protein structure, each having distinct spectral features. The technique consequently became in the past decade a common analytical tool for biologists and chemists. Ultrafast transient spectroscopy is indeed used for a broad range of investigations: being sensitive to changes in absorption spectrum of the proteins, it is possible to collect data on local conformational deformations, electronic transitions and (low) vibrational modes of oscillation within molecules, intra and inter molecular energy and electron transfers, etc. [10] In the field of solar energy conversion for example, which is one of today's essential topic in our energy savvy societies, this technique allowed to better understand the conversion processes from light to consumable energy. In particular, the study of photosynthesis showed how the specific arrangement of pigment within larger protein structure either favors the absorption and passing of the photon energy, as it takes place in antennae systems, or favors the generation of a charge separated state, as it happens in photosynthetic reaction centers, [4, 9] which results in the liberation of a high energy, and therefore usable, electron. Other chromophores can also serve as electron docking sites and electron carriers. [6] The knowledge gained from such studies is then applied for medical and industrial purposes and used to optimize specific molecular reactions. From these examples, it is possible to understand why the study of biological samples by means of ultrafast transient spectroscopy, among other spectroscopic techniques, became and remains one of the standard analytical tool for the fundamental understanding of a broad range of molecular dynamics.

2.3. The challenges of the application

As discussed above, ultrafast transient spectroscopy is suitable for the study of biological samples. These samples are usually made of purified and solvated proteins. Once a particular molecule is excited, the deposited energy ultimately dissipates into the solvent (so long as the changes are not permanent). It is to remember that the typical repetition rate of the pulses are in the order of the kHz and at this rate the sample is excited about every milliseconds. The risk is that the photo-induced molecular modifications live for a time that is comparable, which will then results in a rapid saturation of the sample. In other words, the excited molecule might not have sufficient time to relax to its fundamental state before the coming of the next light pulse. Saturation thus takes place and as the molecules gets overexcited: they are unable to release the deposited energy quickly enough and end up by “burning”. In order to avoid such consequences, the sample is usually flown in front of the laser beams. The condition is that the flux is high enough so that the sample is refreshed for each laser pulse.

The second major constraint is related to the quantity of the sample available. The sample being made of purified proteins, it is then concentrated in order to reach an optical density that is suitable for spectroscopic analysis. Ideally, transient absorption spectroscopy requires an optical density of about 0.6 for the transition of interest, which in the case of heme protein corresponds to a concentration of hundreds of micro molar. The samples are consequently limited in terms of volumes and sub-milliliters quantities already requires months-long of successive growth of the organisms and protein purification cycles.[11, 12]

The third constraint concerns the susceptibility of the sample in respect to its solvent and atmosphere in which it is enclosed. Indeed some biological samples are hydrophobic and require to be dissolved in various chemicals in order to avoid aggregation and the subsequent scattering of the light pulses, such as it is the case for solvated porphyrins. Many samples are also sensitive to oxygen and therefore require the atmosphere to be controlled. For example, myoglobin is able to effectively bind a variety of diatomic molecule. It however has a high affinity for oxygen, so much that it is not possible to study its deoxygenated state unless in anaerobic conditions.[13]

In conclusion, ultrafast transient spectroscopy is today one of the standard analytical tool for whoever desire to study the local structural, electronic and vibrational photo-induced dynamics. In particular, solvated chromophores and chromophore-containing proteins are well suited for the techniques as they can be specifically targeted via their absorption band. However, these liquid samples are often limited in quantities and have to be flown in order to avoid any photo-damages. From these challenges arise the need for microfluidics so as to flow the limited sample volumes. Furthermore, in order to perform ultrafast spectroscopic measurement, the probe pulses must pass through the sample, therefore through the flow-cell in which it is enclosed. The cell consequently requires adequate windows that do not alter the signal-to-noise ratio nor the temporal and spectral resolution of the apparatus. Additionally, due to the properties and high sensitivity of certain samples, the cell must be resistant to the solvent while providing control of the atmosphere. In such cases, the sample must be hermetically confined within the microfluidic system which therefore has to also play the role of an anaerobic chamber.

3. Most common solutions available

In this section I will review different techniques that are commonly employed to flow the sample in front of the laser beam. I will discuss the advantages and inconveniences of each in terms of their compatibilities with the requirements of ultrafast spectroscopic laser systems.

3.1. Flow cell

The main idea is to flow the sample in between two transparent plates. These windows are usually made of quartz so as to permit the broadband (near UV-Visible-near IR) beams to pass through. This type of cell allows for small path lengths and thin windows, down to 0.02 mm such as the one shown in Figure 3, therefore reducing scattering of the excitation beam through the quartz. The thin windows also have the advantage to minimally alter the pulse duration (limited group velocity dispersion), therefore allowing for an optimal time resolution. Furthermore, the cell is steady and its stability allows for optimal signal to noise levels.

Figure 3. Flow cell from Starna Cell [14]

The quartz cells, by themselves, are commercially available.[14] However, due to their size, these cells already enclose about one milliliter of sample. Furthermore they must be connected to a pump, typically a peristaltic or flow-through pump, in order to generate the flow. Altogether, the flow cell system requires few tenth of milliliters and is consequently not adequate for precious samples that are simply not available in such quantities.

3.2. Liquid micro-jet

The term micro here comes from the diameter of the jet itself, which produces a couple of centimeters-long of regular flow. The advantage of expelling the sample as a jet is to remove the constraints of having it to pass through windows, i.e. avoiding any additional absorption, scattering and group velocity dispersion. It therefore allows pump probe experiment in all spectral region including UV and X-ray region. Associated with an adequate sample collector, as shown in Figure 4, it is also applicable in vacuum chambers and becomes suitable for photo-electron spectroscopy.[15]

Figure 4. Micro-jet implemented for photo-electron spectroscopy. Courtesy of José Ojeda, EPFL.

The inconvenience of having to produce a constant flow rate is that the sample must pass through a sophisticated HPLC pump,[15-17] which consequently requires sample volumes that are larger than our targeted sub-milliliter. Note also that while passing in either the air or in vacuum, the sample's solvent is subjected to evaporation. In such conditions, recycling of the sample results in a change of the sample's concentration and temperature over the course of the experiment.[16] Furthermore, the high speed at which the sample goes through the nozzle induces charging of the liquid and/or of the nozzle, which might alter the measurement.[17]

3.3. Spinning /moving cell

The idea behind the spinning-cell is similar to that of the flow cell as the sample is housed between two circular glass plates that are spaced by the desirable optical path-length.[18] While rotating, the sample either creates a rim at the edge of the cell, or at lower speed, the solution remains at the bottom of the cell and is constantly mixed due to friction with the glass as shown in Figure 5. The advantage is that it typically requires minimal amounts of sample (~0.3 mL) as well as to provide control over the initial atmosphere in which the sample is enclosed since the sample is hermetically confined.

However, because the rotation of the glass plate causes the excitation beam to sweep a large surface, the cleanness of the plates is directly related to the noise. It is consequently burdensome to clean. Furthermore, the fact of having a moving piece of glass in which the beam is focused renders the alignment of the cell crucial, and any slight asymmetry of the glass plates has consequences on the transmitted probe beam. Also, since the glass plates are typically few cm in diameter, the required minimal thickness of the glass lowers the time resolution. Another inconvenience is that once the cell is set it does not allow access to the enclosed sample and each experiment therefore requires its own sample preparation.

In conclusion, the proposed solutions used to flow the sample in front of the laser beam have each distinct advantages and inconveniences, and none ideally respond to all the requirements, i.e. is suitable for sub-milliliter sample volumes; provides a close atmosphere; grants access to the sample once closed; affecting minimally the signal-to-noise ratio and resolution of the

Figure 5. Spinning cell used in few-kHz spectroscopy setups.

apparatus. Through these few examples I hope to have convinced the reader of the need for a development in the application of microfluidic system to the field of ultrafast spectroscopy.

4. Recent improvement and application: the microfluidic flow cell

In this section I will describe the recently developed microfluidic flow-cell in order to illustrate the importance and advantages of applying microfluidic systems to the field of ultrafast spectroscopy. I will show that recent improvements in the field of microfluidics have the capabilities to solve the previously mentioned constraint all at once. I will thus discuss the advantages of the system in light of the other techniques. Following the technical properties I will illustrate the flow-cell's effectiveness through a study done on rare *bc*-cytochrome and conclude with an appeal for further development.

4.1. The microfluidic flow-cell

The microfluidic flow-cell, [19] as illustrated in Figure 6, is composed of three main elements that are connected via flexible tubing of 1-mm diameter:

The decantation chamber is a home-made polymer cylindrical chamber as shown in Figure 6. The 0.5-mm diameter inlet and outlet are at the bottom of the chamber in order to minimize turbulences that are created at high flow rates. The chamber requires a minimal amount of ~50 μL of liquid sample in order to have a continuous flow between the inlet and outlet. Any excess of sample fills up the chamber and allows the bubbles that might be enclosed in the closed circuit to rise to the surface. The bubbles are naturally trapped by the chamber while passing through at low flow-speed. At high flow-speed however, larger sample volume are required in order to avoid the suction of air due to the liquid's turbulences. The top of the chamber is threaded to fit a standard septum screw cap. This allows for the addition of chemicals to the enclosed solution while keeping the confined atmosphere protected and avoiding evaporation of the solvents.

The *capillary window* is made of a square quartz silica capillary bought from Composite Metal Services Ltd (CMS). It has a path-length of 0.5 mm with 0.1-mm thin walls. Knowing that the focus of the laser beam is about 100 μm in diameter, the window can easily be set within the beam path. The capillary is fixed at the center of a xyz-rotation mount that allows for fine adjustment of the angle between the incident beam and the window.

The *turbisc pump* is a design from CSEM.[20] In short, the flow is created by direct friction between a grooved barrel and the liquid. The inner volume that the pump contains is about 100 μL only. Because the housing and the seal are respectively made out of Polyetherimide and of Polyetheretherketone, the pump is relatively resistant to chemicals.

Figure 6. (A) Overall view of the assembled system, with zoom on the capillary junctions. (B) Side view of the bubble chamber and (C) of the capillary window. Reproduced from Ref. [19].

When the pump, the chamber and the capillary are connected, the minimal volume of sample needed for good working conditions is about 250 μL only. This configuration includes a total tubing (1-mm inner diameter) length of ~ 6 cm and a sufficient amount of sample in the cuvette to avoid the suction of bubbles due to turbulences with a minimal flow of ~ 0.1 mL/sec. The flow is proportional to the voltage applied to the pump and under the same configuration the maximum flow rate was measured to be ~ 0.36 mL/sec, as shown in Figure 7.

Figure 7. Measured flow-rate (dots) and its best fit (line, 2nd order polynomial) while the pump is connected to ~6 cm of 1-mm diameter tubing and ~1-cm long 0.5x0.5 mm² square capillary. In order to ensure fresh sample at each laser shot, while assuming a typical laser focus size of 100 μ m, the expected maximum repetition rate of excitation is indicated as a reference only (right axis).

While assuming a typical laser focus diameter of 100 μ m within the 0.5x0.5 mm² square capillary, the flow, when assumed to be uniform, is expected to effectively refresh the sample within the laser focus for each laser shot at an excitation rate of up to ~14 kHz. In practice, it is to remember that the flow is impeded on the edges of the capillary and consequently the value of 14 kHz has to be taken as an upper limit only. Taking into account that the inner volume of the pump is only about 100 μ L, it represents one of the best (if not the actual best) compromise between flow-rate and required volume. The assembled microfluidic flow cell, in working conditions is shown in Figure 8.

Figure 8. Microfluidic flow-cell in action

4.2. Example of application: the study of cytochrome *bc*₁ complexes

In order to illustrate the applicability of the microfluidic flow-cell as well as some of its advantages I present here a unique analysis, that of the Cytochrome (cyt) *bc*₁ complex. [6] The

cyt bc_1 complex is a key player in mitochondrial and bacterial respiratory chains.[21] It is the main actor in the protonmotive Q cycle and results in the formation of a proton gradient across the membrane via a series of embedded hemes as shown in Figure 9.[22-24] The generated potential gradient serves as the driving force for the synthesis of ATP, the universal energy transporter in living organisms. The understanding of the protein complex is therefore of primary importance. However the sample is rare and mainly because of the limited quantity available after each purification process, the heme dynamics were until then never studied by means of ultrafast spectroscopy.

Figure 9. (Left) Structure of the bc_1 complex[25] with the protein backbone being shaded for clear visualization of the key actors in the proton-coupled-electron mechanisms. (Right) Static absorption spectrum of the sequential reduction and oxidation of the cyt bc_1 dimer complex: after pre-reduction by ubiquinol and before (red, c_1 -hemes reduced) and right after (blue, b - and c_1 -hemes reduced) the addition of dithionite, and at the end, after oxidation of the complexes by oxygen (green, c_1 -hemes reduced). The 523-nm excitation pump is indicated as reference. Reproduced from Ref. [6] with permission from the Royal Society of Chemistry.

Thanks to the microfluidic flow-cell, and more particularly to the access it provides to the sample via the septum, the reduction and oxidation of the hemes of interests could be controlled chemically. All data were therefore taken from the same sample preparation within the same experiment, in the same experimental conditions. The different signals that emerge from the reduced states of the hemes could then be directly compared: either the c_1 -hemes can be exclusively reduced, or both c_1 - and b -hemes can be reduced together. Direct subtraction of the two data set enabled the extraction of the sole signal from the b -hemes, as shown in Figure 10.

The dynamics of each heme type could be analysed separately by means of singular value decomposition and global fitting as described in detail elsewhere.[6] The resulting decay associated spectra, shown in Figure 11, revealed the clear differences between the b - and c_1 -hemes behaviours within the bc_1 protein complex: while the c_1 -hemes undergo photo-dissociation of their axial ligand as a result of ultrafast laser excitation, the b -hemes were shown to undergo photo-oxidation with a high (> 0.4) quantum yield that is beyond all expectations.

Figure 10. (A) Spectra at selected time delays while both *b*- and *c*₁-hemes are reduced. (B) Spectra at selected time delays while only the *c*₁-hemes are left reduced. (C) Difference (A-B) corresponding solely to the signal of the ferrous *b*-heme, as discussed in the text. Note that the vertical scales below and above 515 nm differs by a factor of two. Reproduced from Ref. [6] with permission from the Royal Society of Chemistry.

Figure 11. Photo-dissociation of the *c*₁-heme (left) and photo-oxidation of the *b*-heme (right) upon light excitation of the *cyt bc*₁ complex. Reproduced from Ref. [6] with permission from the Royal Society of Chemistry.

The *b*-heme's high electronic reactivity makes sense in the light of cyt *bc*₁ having to efficiently fulfil its role in the Q-cycle: it favours the reduction and oxidization of the ubiquinone and ubiquinol, respectively.[22] The *b*-hemes have to efficiently "process" the electrons, which demand them to easily loose or gain electron. Similar electronic-reactivity would in fact be counterproductive in soluble cytochromes as they would less efficiently keep their electrons from being scavenged by other solutes. The hydrophilic environment of the *bc*₁ core on the other hand preserves the *b*-hemes from unwanted solvated electron carrier and their high electronic reactivity is then an advantage. In contrast to the high electronic reactivity of the *b*-hemes, the high photo-dissociation quantum yield of the *c*₁-hemes can be understood as being an efficient "heat sink" that protects the reduced state of the heme against light excitations.

Overall, this study illustrate that, even though the *b*-hemes in cyt *bc*₁ and in other cyt *b* have similar ligation to their protein backbone; specific structural constraints and amino-acid arrangements result in clearly different responses, and therefore functions. While cytochromes were known to serve only as electron carriers, this study demonstrates that with the appropriate environment, light-induced charge separation can readily be initiated within single heme structures. The use of the microfluidic flow-cell therefore not only enabled the study of this rare protein complex, but allowed to shine light on the relevance of local heme-bonding and structural environment in initiating larger chemical reactions. This particular case study is only one example of how the field of ultrafast spectroscopy can benefit from the application of microfluidics technologies.

4.3. Call for development

As I mentioned, the described microfluidic flow-cell, beside the numerous advantages it provides in respect to the other systems commonly employed, is certainly not perfect, which leaves room for improvement.

For example, in terms of sample volumes, most of the liquid is contained in the pump that is used to generate the flow and in the tubing. Miniaturization of both would allow to use even smaller sample volumes. Already the small turbisc pump that is employed uses a newly developed friction based technologies[20] that is able to flow even viscous samples. The samples studied in ultrafast spectroscopy are however water-like and, not being in need of the actual viscous sample capabilities of the pump. I can therefore imagine that a simplified version of the pump would be sufficient and require even smaller volumes. Concerning the actual 1 mm diameter tubing, that links the pump with the capillary, it could be replaced by other microfluidics technologies that uses micro-channels that are specifically designed for spectroscopy.[26] The goal through these proposed improvements is to reduce the amount of liquid used, keeping in mind that reducing further the diameter of the channels might impede the overall flow rate.

Another field of improvement that I see is that of the control over the inner atmosphere of the cell in which the sample is enclosed. As I mentioned, the actual pumping is done by friction between a grooved barrel and the sample. The spinning motion is however done via a shaft and under high pressure difference between the inner and outer part of the cell, either the sample can leak through or air can be sucked in via the shaft's joints. In order to palliate to this

issue, I can imagine that the shaft would be replaced, not by another mechanical interaction, but remote electromagnetical interaction so as to preserve the inner atmosphere of the sample. Such systems are already implemented for applications in biomedical for example.[27] Furthermore, the microfluidic flow-cell uses epoxy beads in order to hermetically fit the square capillary in the cylindrical tubing. Better seal could be achieved if the capillary themselves were to be made with initial beads on each sides such as it is the case for the larger commercially available flow-cells.[14]

At last, I would like to precise that, due to friction between the sample and the capillary, the capillary does not provide with a flow that is homogeneous. Sample that is the closest to the window has consequently lower flow rate and might not be refreshed for each laser pulse, being therefore subjected to photo-damage. One solution would be to employ the newly developed Electro-Osmotic flow systems that are used to generate homogeneous flows.[28]

In conclusion, the microfluidic flow-cell that I propose is specifically designed to fits the requirement imposed by the ultrafast spectroscopy of quantity-limited and sensitive sample, while it remains cost effective and easy to use. As an illustration of the flow-cell's effectiveness, I showed how its implementation enabled the study of the cytochrome *bc₁*. More precisely, I was able to resolve the ultrafast electronic and nuclear motions that precedes some of the larger physiological function of the protein. The microfluidic flow-cell not only facilitates but also opens the door to the study of a whole range of samples that cannot be purified in large quantities such as it is the case for most organic compound that are extracted from living organisms.

The advantages of this microfluidic flow-cell over other pre-existing solutions are clear; as clear as there is room for development.

5. General conclusion

The implementation of the microfluidic flow-cell to the field of ultrafast spectroscopy can be considered as one of the first attempts to bridge two communities, i.e. that of microfluidics and that of ultrafast spectroscopy. In this chapter I have first described the technique of ultrafast spectroscopy in order to show its requirements in terms of sample as well as the importance of such analytical tool when applied to the study of biological protein complexes. In particular, ultrafast transient spectroscopy became one of the essential approach for whoever desires to understand the local electronic and nuclear modifications that are at the origin of the larger physiological functions of proteins. I then exposed the advantages and inconveniences of the different techniques that are commonly used in order to flow liquid samples in front of the laser beams. The aim of this discussion is to better appreciate how the application of microfluidics technologies is able to responds to the challenges raised by the technique. In this aim, the recently developed microfluidic flow-cell is adequate as it requires only about 250 μL while generating flow rate that are suitable for high repetition rate laser systems. Its steady window and decantation chamber allow for an optimal time and spectral resolution. By providing direct access to the sample while running a single experiment and

monitoring chemical changes in “real time”, the microfluidic system enables studies that were otherwise not possible. The advantages of microfluidics over other usual systems are numerous but as it represents only one of the first attempt, there is ample room for improvement. In this regard, one of the goal of this chapter is to serve as an initial step in an effort to bridge the microfluidics community with that of ultrafast spectroscopy in order to foster new ideas, new applications and new perspectives.

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