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SCIENCE AT THE FRONTIERS OF KNOWLEDGE

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RESEARCH SPOTLIGHT

Joint European Torus • Institut Laue-Langevin • European Southern Observatory
European Synchrotron Radiation Facility • The Netherlands Organisation for Scientific Research
Nuclear Physics European Collaboration Committee • Square Kilometre Array • EuroGeoSurveys
Fermilab • Swiss Science and Technology Council • Engineering and Physical Sciences Research Council

Ultrafast studies

Professor Majed Chergui has been at the forefront of ultrafast spectroscopy research for some 20 years now, making major contributions to the development of novel measurement methods

Could you begin by outlining the key aims and objectives of your research?

My research deals with the so-called ultrafast phenomena. Basically, these are phenomena that occur on the timescale of femtoseconds (fs) up to several picoseconds (ps). The aim is to visualise in 'real-time' atomic motion; in our case, mainly in molecules and in proteins. Indeed, these motions are at the core of chemical and biochemical reactions. In a way, we aim at making 'films' of molecular reactions; obviously, the aim is not just to make films but to decompose the structural evolution of a molecular or biological system in order to obtain a better understanding of its reactivity or function.

Two of your major contributions to the field have been in time-resolved X-ray absorption spectroscopy (XAS) and, more recently, two-dimensional ultraviolet (2D UV) spectroscopy. What advantages do these novel methods hold over the more traditional ones?

As far as time-resolved XAS is concerned, the major advantage over optical-based ultrafast spectroscopy is its ability to retrieve information about both the electronic and the molecular structure of the system. Compared to diffraction methods (with X-rays or electrons), it can easily be implemented in the liquid phase, which is the medium in which most of the natural and manmade chemistry and biochemistry occurs. This has helped us answer questions that could not have been tackled by other means.

The UV region below 300 nm is important because this is the region where amino acids in proteins and nucleic acids in DNA absorb. Pushing 2D spectroscopy into this region is a novel development that allows the energy- and electron-transfer processes to be probed as well as the global response of the protein, down to very short timescales (as little as 200 fs). For example, we recently unravelled a hitherto unknown ultrafast electron transfer between tryptophan and haem in myoglobins.

How did you go about pushing XAS into the fs regime?

The development of hard X-ray absorption spectroscopy (XAS) in the fs regime is actually a joint effort of my group together with scientists at the Swiss Light Source (SLS) synchrotron. It is based on the 'slicing' scheme, which involves co-propagating an intense fs laser pulse together with a 50-100 ps long electron bunch in a synchrotron. The electromagnetic interaction between the laser pulse and the electrons slices two wedges from the electron bunch, whose time structure is similar to that of the laser pulse. The sliced fs electron bunches are then used to generate fs X-ray pulses.

Parallel to your work on X-ray spectroscopy, you also develop novel optical spectroscopy tools. In your opinion, do these hold any advantages over X-ray spectroscopy?

It is important to have in mind that no single technique can answer all questions. The best arrangement is to combine as many techniques as possible to get the full picture. Optical spectroscopy can reach very short timescales and it can deliver valuable information about energy relaxation processes in molecular systems. However, it does not yield information about the geometric (or molecular) structure, which is why we resort to XAS.

You are also very interested in the field of solvation dynamics. What have you found with regards to the interplay between these and intramolecular structural dynamics?

The study of solvation dynamics is one of the most active areas of research in chemical physics. The solvent shell around molecules and proteins is known to play a crucial role in modifying, enhancing or hindering chemical reactions. It also influences the energy balance of the system. Thus, in many cases, we need to look at the intra- and the intermolecular structural changes, as well as the interplay between them. In polar solutions (eg. water), for charge transfer reactions, the reorientation of the water molecules is important in stabilising the excited molecule.

Does the future look bright for those developing structural and spectroscopic tools for biological systems?

Absolutely! Firstly, our implementation of the high repetition rate ps XAS scheme is opening new doors for studying the structural dynamics of biosystems in their physiological environment, which was not possible before. Secondly, the development of new, ultrashort intense X-ray pulse sources, such as the X-ray free electron laser, will render these experiments more and more routine. Finally, the advent of 2D UV spectroscopies is offering a new turn in our understanding of protein dynamics and of energy and charge flow within these systems by probing the couplings between chromophores during a biological function.

Below the **surface**

Researchers from **École Polytechnique Fédérale de Lausanne**, Switzerland, have been pushing the boundaries of what is possible in spectroscopy for decades. In so doing, they have opened up new avenues of investigation for themselves and the wider scientific community

PROTEINS ARE RECOGNISED as the building blocks of life and for this reason they have been the subject of extensive research ever since their apt naming, derived from the ancient Greek word for 'primary', in the 19th Century by Jöns Jacob Berzelius. Francis Crick of DNA fame once stated that 'if you want to understand function, study structure', and it is this aphorism that has driven scientists on their perennial quest to understand proteins (but also chemical) dynamics on an atomic level. In 1934, the first observation of a crystalline structure by X-ray diffraction paved the way for a flourishing era of macromolecular investigation. This allowed scientists to observe proteins at a spatial resolution far greater than ever before and, ultimately, gifted us with an improved understanding of their function.

However, as with all aspects of life, proteins are characterised by continuous change. This can be determined by large-scale structural alterations, such as folding, which are, in turn, caused by changes on a smaller, atomic scale. These latter changes, that involve the reorganisation of subatomic particles, happen on an infinitesimal timescale ranging from femtoseconds ($1 \text{ fs} = 10^{-15}$ s) to picoseconds ($1 \text{ ps} = 10^{-12}$ s).

Whilst the visualisation of atoms became possible about a century ago, an atomic-scale resolution of time remained out of reach until the 1980s. Combining the atomic resolution, or space, with that of time would allow the 'real-time' observation of structural changes, and ultimately further our understanding of protein function. Professor Majed Chergui from École Polytechnique Fédérale de Lausanne (EPFL) in Switzerland explains the importance of this in more detail: "If you can decompose the structural evolution of molecular or biological systems, you can better understand their reactivity or function".

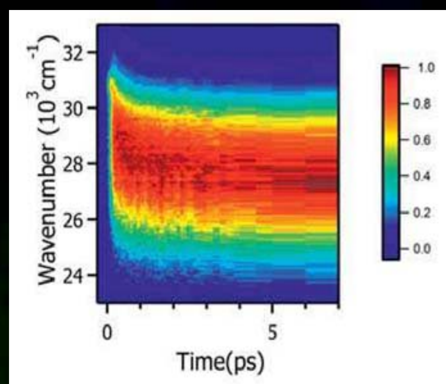

FIGURE 1. Time-wavenumber plot of the UV fluorescence of a tryptophan analogue in water.

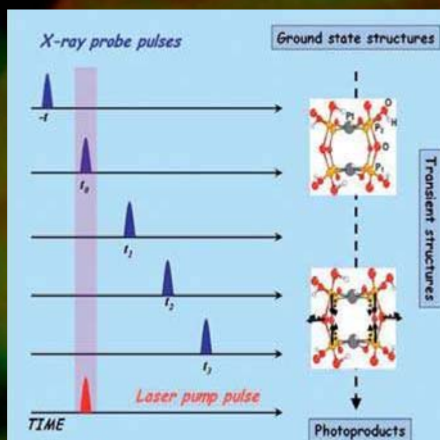

FIGURE 2. Principle of the pump/X-ray probe scheme: at time t_0 a laser pulse excites the system, whose evolution is probed by the X-ray pulse at time delays t_1, t_2, t_3.

X-RAY ABSORPTION SPECTROSCOPY

Femtosecond laser optical spectroscopies led to the fields of femtochemistry and femtobiology in the 1980s which look at molecular phenomena and, particularly, chemical reactions. At the temporal resolution of tens of femtoseconds, it becomes possible to observe molecules, crystal lattices, proteins, etc., at various stages of vibrational distortion or chemical transformation. The emergence of these ultrafast spectroscopies marked the birth of an intense period of research into the dynamics of photoexcited molecular and biological systems, as well as solid materials. However, these methods did not provide structural information and so, with this in mind, researchers began to push traditional structural methods, such as electron and X-ray diffraction or X-ray absorption spectroscopy, into the ultrafast domain.

Having come from a background of vacuum ultraviolet (UV) spectroscopy, Chergui had extensive experience with synchrotron radiation. Using this form of radiation, which can also provide ultrashort pulses of X-ray with a high photon flux, Chergui and his team at EPFL were finally able to adapt X-ray absorption spectroscopy in time domain experiments; first in the picosecond regime, where they achieved groundbreaking results on metal-based molecular complexes, and then in the femtosecond regime. In the latter, they were the first to observe atomic motion in 'real-time' – an incredible feat that has gained Chergui much acclaim and the highest regard within his field. The femtosecond X-ray absorption spectra they obtained also identified the interplay between ultrafast electron spin

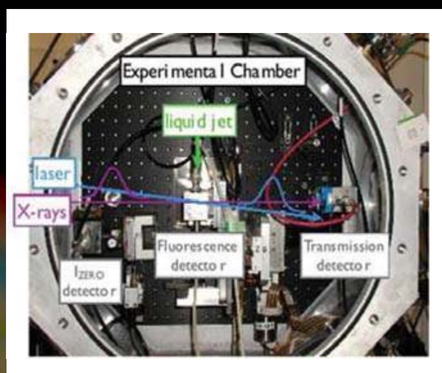

FIGURE 3. Sample chamber for the time-resolved X-ray absorption spectroscopy showing the laser pump pulse, the X-ray probe beam, the sample (liquid jet) and the detectors.

changes and the conformational alterations of the system.

An early hurdle of picosecond X-ray absorption spectroscopy meant that it was only possible to examine highly concentrated samples (typically tens of milliMolars (mMol)). With lower concentrations (2-3 mMol) of biological samples, the major challenge lay in increasing the signal-to-noise ratio. Chergui managed this using higher repetition pump lasers, achieving a significant increase in sensitivity and demonstrating picosecond X-ray absorption spectroscopy of photo-triggered haem protein function in physiological media. Haem proteins are a substantial class of macromolecules that are responsible for a range of biochemical processes, in particular, fixation and transport of oxygen.

ULTRAVIOLET SPECTROSCOPIES

In proteins, the interaction between amino acid residues and the co-factor plays a crucial role in functionality. Because of these interactions, the amino acid residues are often used as local reporters of the intra-protein dynamics. Using the fact that all amino acid residues (as well as nuclei acids) absorb light below 300 nm, Chergui's team developed cutting-edge UV spectroscopic tools such as transient absorption with a broad continuum or ultrafast fluorescence up-conversion, with which he and his group carried out several insightful studies on proteins, amongst others.

Another important tool that is widely used in protein structural dynamics comes in the form of multi-dimensional (2D) infrared and visible spectroscopy. According to Chergui, they have significant advantages: "They result in a fuller picture of protein dynamics *in vivo* through the observation of the mutual interactions between chromophores, in the system and their evolution over time". Wanting to exploit this technique further, Chergui believed it could be used to observe interactions between amino acid residues, and between the bioactive centre and the close-lying amino acid residues.

Pushing 2D spectroscopy into the UV domain was far from trivial due, in particular, to the challenge of having to generate two broad continua (typically 80-100 nm) centred near 300 nm of ultrashort duration. However, this problem was overcome and the first studies of protein dynamics with 2D UV spectroscopy have already revealed hitherto unknown electron-transfer processes between the tryptophan amino acid and the haem in myoglobins.

The availability of this novel method of 2D UV spectroscopy now means that large-scale protein motion can be followed and observed, whilst the monitoring of the interactions and movements between excited amino acid residues can also be recorded. Combined with picosecond X-ray absorption spectroscopy, it has now become possible to monitor protein dynamics in physiological media from the atom to the scaffold, as Chergui and his group recently demonstrated for the first time.

As with all aspects of life, proteins are characterised by continuous change

COMBINATIONS

These invaluable contributions now mean that the world of structural dynamics is a very exciting place to be. However, throughout his extended career, Chergui has continually stated that it is not the invention or development of one single tool that is of the upmost importance – there is no single technique that will allow these structural dynamics to be any better than the next – it is instead the combination of techniques that will gift us with a truly greater comprehension of how proteins and other molecules function below the surface.

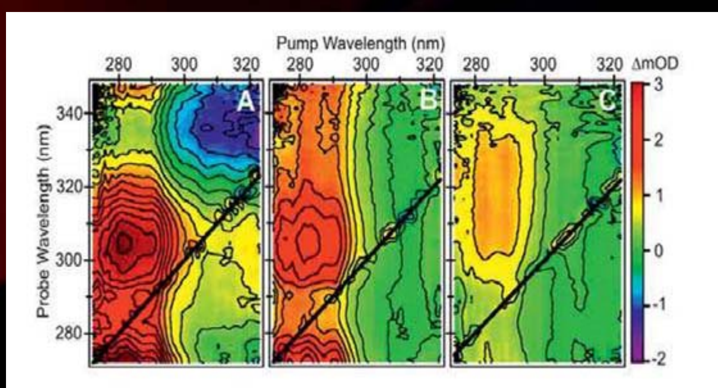

FIGURE 4. 2D spectra of myoglobin at different time delays: 2.5 ps (A), 17 ps (B) and 200 ps (C).

INTELLIGENCE

NOVEL ULTRAFAST OPTICAL AND X-RAY SPECTROSCOPIC STUDIES OF CHEMICAL AND BIOCHEMICAL SYSTEMS

OBJECTIVES

To probe in 'real time' the photoinduced structural changes in molecular and biological systems in solution. Also of importance is the ability to map out the electronic structure changes that underlie the geometric ones

KEY COLLABORATORS

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PROFESSOR MAJED CHERGUI began his career in 1982 as a junior research assistant at the National Centre for Scientific Research in France, after obtaining his PhD at Université Paris-Sud. He received his Habilitation in 1986 and then spent six years at the Free University of Berlin, Germany. In 1993, he was appointed Professor of Physics at the Université de Lausanne, Switzerland, and then moved to the École Polytechnique Fédérale de Lausanne in 2003 as Professor of Physics and Chemistry, a position which he still holds.