UNPRECEDENTED UNDERSTANDING

The LBPA is a CNRS biophysical laboratory situated in the prestigious École Normale Supérieur de Cachan in Paris, as director Dr Malcolm Buckle outlines

Molecular biology is undergoing a revolution. Most of the concepts and technological advances made over the last 60 years have been based upon the elucidation of the DNA structure, the unravelling of the process of gene expression and its regulation at a cellular level. Whilst this has provided an unprecedented understanding of cellular events and a comprehensive toolbox for studying biological objects, the difficulties of transferring from the genome to the proteome, with the accompanying huge increase in degree of complexity, has highlighted the need to separate our attack from the purely structural approach engendered, rather fittingly in this the year of crystallography, by the exponentially increasing obtention of 3D representations of proteins based on X-ray diffraction studies, to develop a more dynamic approach.

It is becoming increasingly evident that macromolecules including proteins and nucleic acids are in constant internal movement, and that the exploration of their surrounding space engendered by this is a direct result of their structure. Both proteins and nucleic acids are polymers that combine to form highly complex structures with functions related to these underlying forms. We are looking at two fundamental repercussions of this structure/function relationship: the first to do with conformational rearrangements in proteins associated with autoimmune diseases; and the second concerning the condensation and organisation of DNA in chromatin and its impact on the regulation of gene expression.

Fig. 1 High energy NdYag ns laser. Photo courtesy of David Arráez www.arraez.com

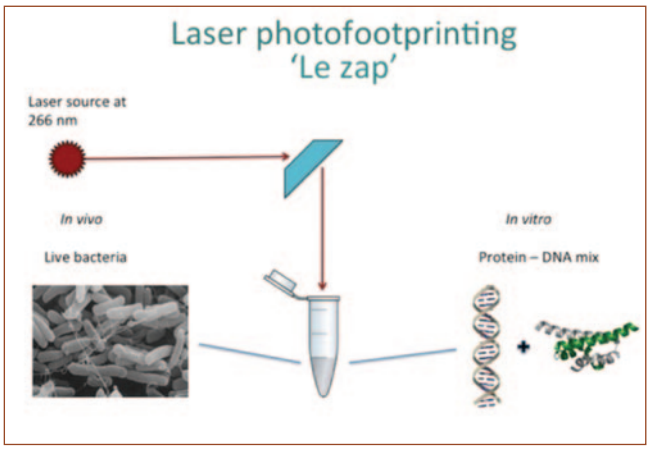

Fig. 2 Schematic for UV laser induced photochemical rearrangement of DNA

At both levels we believe that deciphering the mechanisms involved in these processes will lead to important advances in diagnostic and therapeutic approaches to a range of related human diseases.

An integrative pluridisciplinary biology laboratory

The Laboratoire de Biologie et Pharmacologie Appliquée (LBPA) is a biology laboratory working on dynamic aspects of macromolecular interactions involved in basic biological problems at a molecular and cellular level. Its goal is to integrate the development and utilisation of cutting-edge technologies based essentially upon a biophysical approach in order to elucidate mechanisms involved in key biological events ranging from the onset, propagation and reversion of carcinogenesis, the role of DNA organisation in the regulation of gene expression, the involvement of miRNA in the control of gene expression, the exposure of cryptic epitopes associated with autoimmune diseases and the relationship between transcription and cell replication.

The link that binds these together is unique to LBPA and resides in the approach that is taken, i.e. a dynamic analysis coupled with interdisciplinarity and complementarity of expertise, between, and within teams in the laboratory. LBPA is a founder member of the d'Alembert Institute at the ENSC alongside physicists, chemists, experts in microfluidics and electronics and has access to specialist platforms such as high resolution imagery, clean room facilities and workshops.

Fig. 4 L3 laboratory at LBPA. Photo courtesy of David Arráez www.arraez.com

LBPA consists of six research teams totalling around 93 personnel including 25 CNRS and two INSERM research scientists, and seven university associated research scientists. Each team, although independent in its definition of and pursuit of a specific research goal, is linked to other projects and structures within the LBPA, thus defining a common descriptive of the global research goal of the LBPA as a whole. The three main research fields of the LBPA are fundamental biology; molecular biophysics and health sciences.

Approaches and technologies developed or present in the LBPA

Biosensors

LBPA has been proactive in improving existing, and designing and developing, new biosensors and novel applications for biosensors.[1] We have extended the range of Surface Plasmon Resonance (SPR) devices to include image-based (SPRi). We coupled SPRi approach to a mass spectrometer and demonstrated its potential in proteomics.[2] We have developed custom-designed biosensor surfaces (GLISS) that resolve the problem of non-specific binding.[3] We have developed an extremely sensitive microresonator-based biosensor than can detect atomole quantities of material and can follow structural changes in macromolecules in real-time.[4] Current projects are studying antibody-antigen interactions associated with autoimmune diseases. We have shown that inherent movements in proteins expose cryptic epitopes.[5] We have also identified specific biomarkers in autoimmune diseases such systemic lupus erythematous and discovered a novel mechanism involving anti-IgM and nucleic acids in the innate immune response.[6]

Nanoparticles

We can synthesise, functionalise and characterise a range of nanoparticles, especially gold nanorods, with interesting optical properties involving enhanced light scattering and absorption and induced fluorescent enhancement for use in *in vivo* and *in situ* imagery. We custom design gold nanorods (GNR) functionalised to a given density with macromolecules either on the sides, at the ends or both[7,8] and use induced fluorescent enhancement effects due to plasmon coupling that occur when the fluorescent group is at a precise distance (9-12 nm) from the gold surface.

UV laser photofootprinting

Laser photofootprinting developed in the LBPA uses a high energy nanosecond UV laser to irradiate nucleoprotein complexes and induce intrastrand modifications within the DNA molecule.

The efficiency of the photochemical reaction at a given base is tightly related to the local DNA topology. We have developed a primer extension technique to reveal photochemical changes on the DNA using fluorescent-tagged DNA primers and capillary electrophoresis allowing quantification at base pair resolution.

This technique is being applied to a range of nucleoprotein complexes including nucleosomes, transcription factors, polymerases, helicases and DNA modification enzymes.

Platforms at the LBPA

The LBPA is developing and using cutting-edge, emerging technologies that has lead us to create a number of technological platforms that are open for collaborative use by external groups:

1) **A biology security platform** consisting of five independent structures including one L1 level security laboratory, three L2 level security laboratories and one L3 high level security laboratory. The

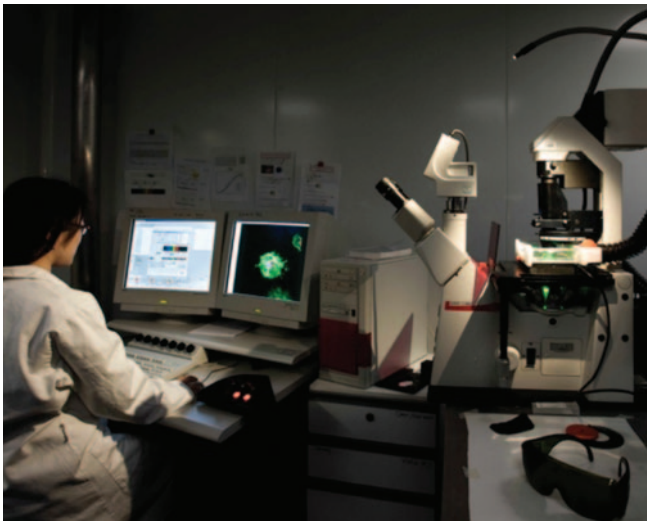

Fig. 3 Confocal microscopy in an L2 environment. Photo courtesy of David Arráez www.arraez.com

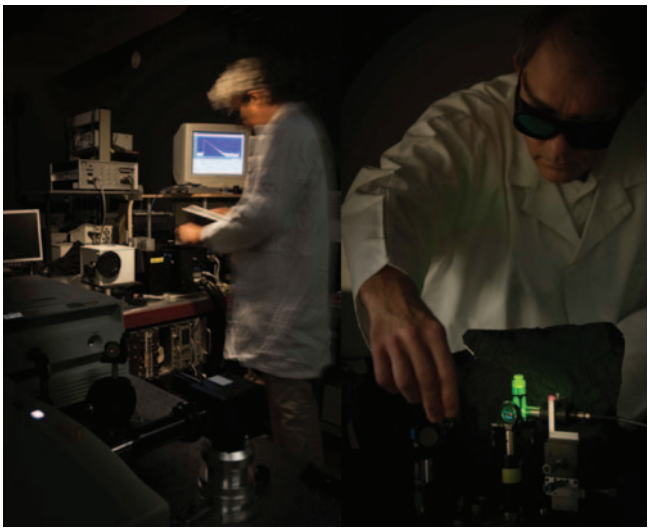

Fig.5 Photos courtesy of David Arráez www.arraez.com

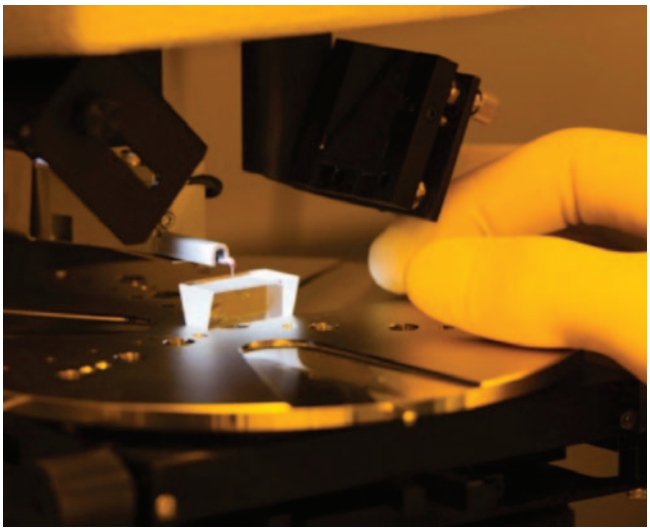

Fig. 6 Preparation of biochips in clean room facility. Photo courtesy of David Arráez www.arraez.com

L1 is equipped for microfluidic work coupled with epifluorescent imagery experiments on cell lines or micro-organisms whereas the L2 is devoted to fluorescent microscopy (confocal or multiphoton) experiments on prokaryotic and eukaryotic cells requiring a class one or two security level.

The L3 level security lab is designed for the manipulation of pathogenic class three micro-organisms that although extremely dangerous to life forms are without risk for the environment thus including infected cells, virus production and transgenesis of eukaryotic cells. The L3 laboratory extends over 60m^2 and is divided into two independent wings allowing the separate manipulation of different pathogens. The L3 is a platform for the local production of viral vectors for use in fundamental studies carried out by private and public research teams. We offer a range of cell systems and molecular biology techniques that are optimised to characterise the effects of antiviral compounds and for examining resistance mechanisms. We have a complete set of viral tools (viral vectors) that we have developed or that are available in our laboratory to modulate gene expression in cell lines or primary cultures. We offer a customised service of new tools in order to reduce costs in projects involving fluorescent imagery. We have developed a range of retroviral vectors that enables differential and selective labelling of specific cellular compartments (nucleus, mitochondria, endoplasmic reticulum, Golgi body) using different fluorescent probes (GFP, RFP, CFP).

2) **An imagery and multiphoton microscopy platform** based on the strong expertise in the lab in the study of dynamics of biological molecules/systems by time-resolved fluorescence and the use of femtosecond lasers.

3) **Clean room and material characterisation facility** – part of the d'Alembert Institute, which allows us access to clean room facilities and a large range of equipment for characterisation (AFM, NMR, MEB, ellipsometry, contact angle). The clean room is a class 100,000 occupying 50m^2 and allows a large range of manipulations to be conducted including biosensor surface production.

This platform is associated with a microfluidics group and is heavily robotised for the robust production of customised products such as biochip surfaces.

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