

At the LAU, we use the methods of molecular biophysics to study life in its finest details, without forgetting that the aim of knowledge is to serve humanity.

Like each living being, each cell and even each molecule is unique. The electron microscope is the instrument of choice for studying biological structures in their details and their specificity.

Living matter is also mobile; it is fluid. Typically, it is composed of \_ water, with everything floating and moving. The finer the detail, the more it shakes. However, the electron microscope functions in a vacuum. In a vacuum, there is no liquid water. Without water, there is only dry and dead matter that was revealed by 50 years of classical electron microscopy.

Since the 1980s, cryo-electron microscopy has made it possible to observe living matter with its waater, immobilized by the cold. The group of the LAU is a pioneer in the development of these methods. We are continuing in the same direction.

Our second line of research concerns the topology of DNA and recombination.

### **Vitrification**

The idea of using freezing to preserve living matter seems to be simple and evident. Unfortunately, cooled water normally transforms into ice. This destroys specimens even more surely than dehydration. The discovery that it is possible to cool water sufficiently rapidly that it does not have time to crystallize – this process is called vitrification – is an important discovery that marks the beginning of the rapid development of cryo-electron microscopy. (Dubochet and McDowell, 1981; Mayer and Brüggeller, 1980 )(Dubochet et al., 1982). Note that this discovery came as a surprise since numerous previous studies had concluded that the vitrification of pure water was fundamentally impossible. Even today, the question of the nature of vitrous water remains unanswered. Its solution could well require a profound revision of what we know about water in general.

### **Cryo-électron microscopy of thin layers of biological suspensions**

Preparing a biological specimen of which the thickness does not exceed a fraction of a  $\mu\text{m}$  and observing its molecular details is difficult. Doing the same below  $-140\text{ }^{\circ}\text{C}$  seems impossible. Yet not long after the discovery of vitrification, it became evident that cryo-microscopists are lucky. For example: vitrification requires an extraordinarily high cooling speed; but the cooling speed increases when the size of the sample decreases; furthermore, the electron microscopists work with very thin specimens; by a stroke of luck, it is just at this dimension that vitrification becomes easy! Making a fine layer of solution so thin that it can be used in an electron microscope seemed to be quite impossible; on the contrary, experiments show that an auto-supporting film is amazingly stable during the moment necessary to prepare its freezing. In brief, it has been possible to put into practice a simple, rapid and relatively inexpensive technique for preparing and observing any biological solution vitrified in thin layers. As expected, the preservation of the biological structures in thin film of vitrified solution is almost perfect. What nobody expected is that, in water, and in the absence of stain, it is possible to see finer and often better contrasted details than were obtained with traditional methods (Adrian et al., 1984)(1984, 1985, 1986, 1994, 1995a, 2000a, 2002a)\*.

Thus cryo-electron microscopy of the thin layers of biological solutions became the standard method of observing biological macromolecules, their complexes, viruses,

organelles and even certain small cells (Dubochet and al., 1988) thus opening, in combination with the techniques of image processing and 3D reconstruction, new avenues to new biology (1986, 1994, 2002a)\*(Abbott, 2002; Baumeister and Steven, 2000).

### **DNA and recombination**

The visualization of DNA is one of the historical successes of classic electron microscopy. It requires the molecule to be dried on a supporting film and contrasted. In cryo-electron microscopy, double stranded DNA can be seen in its native state, floating in water, without stain. A skilful microscopist can even record two successive images at different angles so as to reconstruct the shape of the filament in three dimensions (1992b, 1994, 1995a)\*. The possibility of thus directly observing all of the forms that a given molecule can take opens new possibilities: beyond the structure, the images reveal what physicists call the partition function, the Holy Grail of the system, which reveals all of its thermodynamic, energy, potential function and entropy properties. But this is not a simple business; 10 years after the first measurements, it is still taking its first steps.

Andrzej Stasiak played a decisive part in the introduction of topology into the study of DNA (Krasnow at al, 1983)(1995b)\*. Since then, observations resulting from cryo-microscopy, combined with knot theory, have led to results ranging from fundamental mathematics and knot physics (1997a, 1998, 1999b, 2002b, 2004a and c)\* to the structure of chromatin and the mechanisms of replication (1999a and b)\*. A continuous effort focuses on recombination by the RecA protein and its many assistants (1997, 2000a and B, 2001, 2002b, 2004a, 2005a)\*.

### **CEMOVIS**

Most biological specimens cannot be squeezed in a layer not exceeding a fraction of a  $\mu\text{m}$  thickness. To extend cryo-electron microscopy to tissues, cells and most organelles, it was necessary to learn how to vitrify much larger volumes and to cut them into thin vitrified sections. Until recently, the possibility of carrying out these operations effectively appeared doubtful at best, although preliminary experiments had given us hope (McDowall at al., 1983). Freezing under high pressure, which multiplies tenfold the attainable depth of vitrification, constituted a decisive advance which we were able to adopt. Many studies were also necessary to master the properties of vitreous water sufficiently and to understand the cutting mechanism. After the most optimistic hopes were confirmed by some remarkable micrography (Leforestier at al., 2001; Sartori Blanc at al., 2001), it still took a few years for CEMOVIS (cryo-electron microscopy of vitreous sections) to become a reasonably effective and reproducible method applicable to almost any tissue (Al-Amoudi at al., 2004)(2004d)\*. Today CEMOVIS makes it possible to see the structure of cells and tissues in a state which is closer to reality and finer than was previously possible. Obviously, we are delighted to note that what CEMOVIS reveals is very different from what was known until now. A considerable effort is underway to make CEMOVIS become a routine method in many other laboratories (<http://www.3demonoe.org/about/index.html>).

### **Biology and Society.**

Can a laboratory of biological research in a university devote a significant portion of its resources towards promoting a better relationship between the sciences and society? At

the LAU we answer strongly in the affirmative; as members of the public service, our role of researcher is inseparable from that of citizen. We are therefore engaged in the Biology and Society course, which is obligatory for all biology students. A question particularly close to our hearts is: how can we bridge the gap between the humanities and biological science? With the Determinism and Freedom program, we have the ambition, within the framework of the Anthropos project, to provide new answers in the next three years.

\* The dates in brackets refer to work from our laboratory illustrating the cover of the reviews below.

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