TEM and NMR as tools in Biology

Ilana Sabanay
Tali Scherf
Vera Shinder
Eyal Shimony
Sharon Wolf

Department of Chemical Services

Tel. 972 8 934 3915 **Fax.** 972 8 934 4161
E-mail: varda.katzir@weizmann.ac.il

Transmission Electron Microscopy in Biology

The major task of biological electron microscopy is to provide the structural information with which one may correlate structure and function. Electron microscopy is the only method, which provides information about the complex hierarchical architecture of biological matter from the cellular and subcellular levels to the level of macromolecular assemblies, within the context of the living organism. The integration of this structural information complements with the biochemical and molecular biological techniques.

The structural elucidation of isolated large macromolecular complexes, such as ribosomes, protein-DNA complexes or viruses by 3D electron microscopy became feasible in recent years due to advances in cryo electron microscopy, computerized control of the TEM, combined with digital image capturing and algorithms for image analysis.

Advanced TEM cryo-immobilization techniques together with improved chemical fixation methods enable the preservation of biological specimens in a state, which better reflects their native structure and better preserves immunological determinants.

NMR as a Tool to Study Protein-Ligand Interactions

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the principal experimental techniques of structural biology, with the ability to determine atomic resolution structures of biological macromolecules in semi-physiological conditions.

Structure determination of proteins with a molecular weight (MW) up to 10kDa requires assigning the two-dimensional (2D) proton NOE spectra that correlates between specific pairs of protons that are closer than 5x in space. For larger proteins (MW <35kDa), uniform enrichment with stable isotopes (13C, 15N, 2H) in combination with multi-dimensional heteronuclear NMR techniques are required.

In addition to structure determination, NMR provides a mean to study protein dynamics; a particular aspect of it is that of ligand binding. There are several important aspects of protein-ligand interactions that can be characterized by NMR. At the most basic level, information regarding the overall binding affinities, whereas at the most detailed level it is possible to precisely define the atomic coordinates of the complete protein-ligand complex. In between these extremes is a wide variety of other information of considerable biological importance: location of the binding site on the protein, mapping the epitope on the ligand, conformation of the bound ligand and conformational dynamics. NMR is particularly useful for deriving information on binding sites since if changes in particular NMR signals of a protein are observed on binding, these groups are likely to be close to the protein-ligand interface.