Transmission Electron Microscopy of Proteins and Single Particle 3D Reconstruction

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During the first half of the XXth century a great number of new ideas and experimental achievements established the corner stones of our actual fundamental and technological approaches concerning both materials science as well as molecular biology. All these achievements in both physics and biology were possible by the improving of our experimental techniques such as ultra high-vacuum techniques, synchrotron radiation facilities, plasma techniques, LASER, several light and electron spectroscopies as well as near and far field microscopies. For the last three decades solid-state physics has explored new materials with novel mechanic and optoelectronic properties based on organic molecules as carbon fullerenes, nanotubes, phthalocyanines and polymers. These studies have convinced us that carbon-based materials are indeed very promising and have begun to familiarize physicists with the idea that biological material as DNA/RNA or proteins, essentially composed by carbon and nitrogen, are worth to be studied in detail.

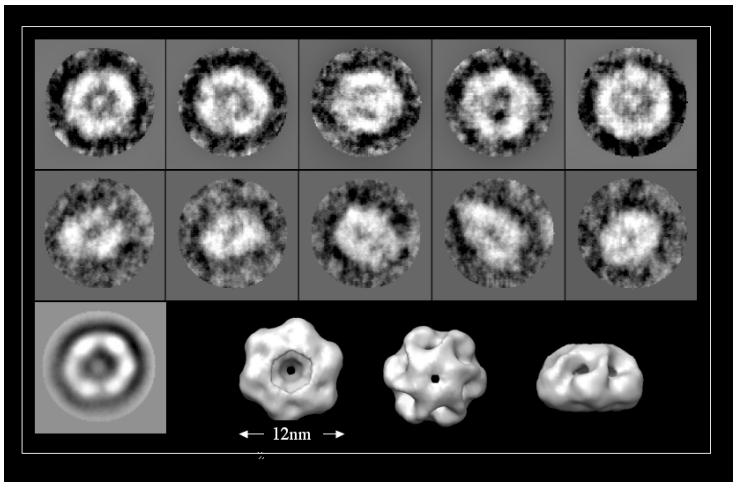
Proteins are large linear polymers composed by smaller molecules called peptides. The exact series of peptides in a protein sequence is coded by RNA/DNA strands. Each protein is folded in a unique way. This folding is responsible for its structural or catalytic properties. There are two kinds of proteins. The ones used as building blocks of living organisms (structural proteins) and those used during the cell-cycle for replication or metabolism (non structural proteins). For example, replication proteins "read" the genetic code in order to make "copies", which in turn are translated to proteins on cellular ribosomes. This kind of proteins is characterized by extremely selective and efficient substrate adsorption and catalytic sites for hydrolysis, protein-membrane binding, RNA/DNA binding or proton/metal atom transfer.

Concerning applications in the domain of materials science, the study of structural proteins could be useful in order to elaborate novel materials which mimic the organization of living building blocks as fibers, porous materials, various kinds of membranes etc. On the other hand, there are many things to learn from non structural proteins concerning catalysis, "clever" self-assembling, or nanostructures with novel functions and properties as molecular devices, nanorobots etc.

From the physicist's point of view, the first thing to know about a protein is its 3D structure. A great amount of proteins have been crystallized until now and their 3D structure has been solved by X-ray diffraction techniques from crystals or recently from micro-crystal powders [1]. However, in many cases crystallization is not possible or very difficult to achieve, especially in the case of protein complexes or very large proteins. In that case other techniques as small angle X-ray scattering (SAXS), dynamic light scattering (DLS), electron and nuclear magnetic resonance (EMR, NMR), transmission electron microscopy (TEM) and atomic force microscopy (AFM) may be used in order to extract structural information. One application of major importance concerning structural knowledge is in the field of "drug design". For example knowing the topology and chemistry of a protein's active site permits the design of small molecules, which after adsorption onto the active site inhibits the protein function. One of the best examples is the HIV protease inhibition achieved this way and used in patients in the case of AIDS therapies.

Here we present a structural study of the viral non-structural protein 2C by means of TEM [2]. The protein 2C under study is the putative helicase of the echovirus 30, a virus which belongs to the *Picornaviridae* family. Picorna viruses are RNA viruses responsible for a large spectrum of human diseases such as hepatitis A, poliomyelitis, various kinds of meningitis and the common cold. Protein 2C is one of the most conserved non structural viral proteins within the *Picornaviridae* family which means that a possible drug targeting its action and designed for hepatitis A could also be used against the common cold. The 2C protein is believed to be endowed with helicase activity based on signature sequences containing conserved motifs, found in NTP-binding proteins as well as in helicase super family 3 (SF3) [3, 4]. The protein shows ATPase activity but no helicase activity has ever been demonstrated neither any structural data are available for any 2C protein. Apart from its putative helicase activity, 2C protein also functions as a membrane-anchoring protein as well as an agent for virus self-assembling and structural rearrangements of intracellular membranes. A stable and soluble construct of the 2C protein was selected and studied here by means of negative staining TEM, SAXS, AFM and DLS. Only the TEM and AFM

measurements showed that the protein adopts a hexameric shape reminiscent to that of SF3 helicases. Indeed, our measurements show that the main fraction of the purified protein tends to aggregate leaving behind a small fraction of monomers and an even smaller fraction of hexamers impossible to observe with SAXS or DLS. Typical TEM micrographs of the hexamer particle are shown in the figure together with a 3D reconstruction of the hexamer particle calculated out of 1500 collected particles. Surprisingly, the hexameric structure observed here by TEM is in line with results obtained from other SF3 helicases of DNA viruses whose crystal structure is known and which form hexameric oligomers.



We will discuss the above results and especially insist in some technical aspects concerning protein preparation (genetic cloning) and handling of protein samples, the problem of protein adsorption on carbon coated microscope grids, the preparation of the samples by negative staining techniques, the effects of the electron beam on the samples and experimental conditions to respect, the acquisition of TEM images on photographic plates their subsequent digitalization and numerical treatment and finally the procedure that permits to reconstruct the 3D structure of a single particle out of a large number of TEM images.

Finally we will discuss some interesting properties of the helicase molecules. Helicases unwind double DNA or RNA strands and also are used by the replication complex as motors (molecular motors) [5]. The 2C protein is also believed to transport other viral proteins and participate in the self-assembling of progeny virus before their take off from the infected cells.

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