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Editorial

Methodological trends in structural biology

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Structural biology has witnessed tremendous developments from the dawn of the new millennium. Macromolecular crystallography, which since its foundation around the '50s of the twenties century has been the stronghold of the technique, has finally reached its full maturity, thanks to several major technical improvements: the building of third (and now forth) generation synchrotron sources and the use of very fast high-resolution detectors allow to collect an entire diffraction data set in a matter of seconds; thanks to the dramatic improvements of computing power, these data can be processed very quickly and often (despite not in all cases) the structure can be solved and refined in days or weeks; the phase problem, the major stumbling block towards a fully automatized solution of the structure, is still present, but it has been overcome in the vast majority of cases thanks to the use of various techniques, from molecular replacement to anomalous scattering [1–5]. The only aspect in which developments have not kept pace is the crystallization process that, despite some significant improvements, still represents the greatest obstacle on the way towards a fast and fully automatized determination of the 3D structure of a macromolecule. Growing crystals can be in most cases a very long and painful process that often requires a significant amount of starting pure material [6].

Electron microscopy, a technique invented nearly one century ago [7], has been for a very long time able only to define an overall shape, but unable to reach atomic or near-atomic resolution. The situation has drastically changed in the last ten years with the development of the so-called "cryo-EM technique". Thanks to several major improvements, including direct electron detectors, new software, a new generation of electron microscopes designed to operate with cryogenic samples, it is nowadays possible to obtain routinely 3D structures at a resolution between 3 and 4 Å, and at a resolution better than 2 Å in the more favorable cases [8–10]. It must be considered the two greatest advantages of cryo-EM with respect to crystallography: the sample does not need to be in crystalline form, but it is represented by a nano-drop of frozen solution, and there is no phase problem. This has allowed the

determination of the structure of large macromolecular complexes, in particular of membrane proteins, very hard or even impossible to obtain in the crystalline state. The major drawback of cryo-EM is the limit in the size of the sample, since macromolecules with a molecular mass smaller than 100,000 Da are hard to detect in the grid. Moreover, the technique is still improving and possibly the technical limits have not been reached yet.

The electron microscope can be used not only for imaging, but also to perform electron diffraction on macromolecular crystals. The advantages over X-ray diffraction are that the electron beam size can easily be made very small compared to an X-ray beam, so that a very small portion of the entire crystal can be selected for diffraction. One of the limits of the technique is that, since electron are less penetrating than X-rays, crystals must be very flat [11].

Another classical technique of structural biology, Nuclear Magnetic Resonance (multidimensional NMR), remains still limited by the size of the sample, since for macromolecules with molecular weight beyond 100,000 Da the line broadening effect brings as a consequence poor spectra sensitivity or even to absence of NMR signal. Specific methods can be used to overcome the size problem in some cases, but this represents possibly an exception rather than the norm [12].

X-ray free electron laser (XFEL) is the most recent among the macromolecular structural techniques [13,14]. The availability of an X-ray laser beam would allow in principle the direct imaging of a single macromolecule at atomic resolution. This has been demonstrated as a proof of principle, but several practical aspects make it difficult the achievement of this goal. In particular, the fact that the biological sample is destroyed after one single pulse of radiation and the three-dimensional structure must be recovered from several two-dimensional projections from different orientations [15,16]. XFEL has been used successfully to determine the structure of protein nano-crystals using the so-called "serial crystallography" method [17], a technique that nowadays is being developed also at fourth-generation synchrotrons [18].

In conclusion, nowadays researchers in the field of structural biology have several alternatives and they can select the technique that best meets their needs. This topic issue is aimed at the description of the more recent developments of these techniques, fundamental both in basic and applied sciences.

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