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Complementarity of small-angle x-ray and neutron scattering: solvation effects and quaternary structure of proteins in solution

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Small-angle X-ray (SAXS) and neutron (SANS) scattering are complementary techniques that are largely exploited to determine the structure of complex biological macromolecules in solution, such as proteins. Since solution proteins are randomly oriented particles, the scattering signal is purely one-dimensional so that the basic challenge is to develop methods able to extract three-dimensional structures from experimental data. X-rays interact with the electron clouds of each atom, neutrons are scattered by atomic nuclei (with different power for the isotopes of the same element): a wisely chosen combination of SAXS and SANS experiments on the same sample is a strategy to increase the structural information that can be derived from data analysis methods.

Two examples of the application of small-angle scattering on protein structure investigation are shown. The first example is a SANS study of the solvation properties of lysozyme dissolved in water/glycerol mixtures (2). To make detectable the characteristics of protein-solvent interface, 35 different experimental conditions (i.e., protein concentration, water/glycerol fraction in the solvent, content of deuterated compounds) have been considered and fitted with a global fit approach.

In the second example the new QUAFIT method for determining the quaternary structure of proteins assemblies by analysing SAXS or SANS data is presented (2-3). The method is based on the idea that asymmetric monomers, formed by rigid domains of known atomic structure are assembled according to a point group symmetry combined with a screw axis. In order to avoid any overlap among domains, the “contact distance” between two asymmetric domains is determined as a function of their orientation by a novel algorithm. QUAFIT has been applied to study the structure of hemocyanin from Octopus vulgaris, a high molecular weight protein that shows a particular self-assembling pattern, characterized by a hierarchical organization of monomers. A dataset of SAXS and SANS curves has been recorded under different pH values, buffer compositions, H2O/D2O ratios and Hofmeister’s salts. The structures of the decamer and of the dissociated “loose” monomer have been identified by analysing SAS curves in the most and the least aggregative conditions, respectively. Afterwards, all the other curves have been analysed through QUAFIT, by considering heterogeneous mixtures composed of the entire decamer, the dissociated “loose” monomer and all the intermediate dissociation products.

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Summary

Presenter(s) : Dr SPINOZZI, Francesco (University of the Le Marche)

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