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The protein structure by the combination of SAXS, light scattering and simulations

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The knowledge of the proteins and the nucleic acids structure is crucial to understanding how these molecules are involved in a extremely complex network of interactions related to the life processes of all living organisms. Therefore for decades the high-resolution experimental techniques determining the spatial arrangement of all elements of the biological molecules with a precision of individual atoms have been developed. Despite the fact that in 30 years of existence Protein Data Bank nearly 120,000 of the spatial structures were solved, the number of known and predicted protein is many times greater. Many of these structures cannot be solved either by conventional diffraction techniques or NMR spectroscopy. Sometimes, however, even general information about the shape of the molecule is sufficient to reproduce a more detailed picture of its construction. Sufficient information can provided by low-resolution techniques based on small angle scattering like for example SAXS or hydrodynamic techniques like dynamic light scattering (DLS), fluorescence correlation spectroscopy (FCS), viscosimetry and analytical ultracentrifugation (AUC).

The techniques classified as low-resolution allow only to determine the overall shape and size of molecules in solution. However, a combination of these techniques can significantly extend the interpretation of the experimental results. This statement was tested on protein with known three-dimensional structure as well as wide set of hydrodynamic and structural parameters. As a simple example the combination of hydrodynamic radius of lysozyme with data on hydration and partial specific volume is presented. The partial specific volume, thickness and density of hydration layer determine the radius of the spherical model of the molecule, R_h. The ratio of hydrodynamic radius, R_H, to R_h evaluates a sphericity of lysozyme and the spherical model can be replacement by the model of ellipsoid. The length of semi-axis, *a* and *b*, of "dry" prolate ellipsoid corresponded with the dimensions known from the crystallographic structure of lysozyme. Afterwards the combination of hydrodynamic radius, partial specific volume, hydration with crystallographic structure of lysozyme was chosen to clarify possible changes in the protein structure under elevated pressure. The R_H of lysozyme was measured at an elevated pressure and information on the density of hydration layer in these conditions were find. The compilation of collected parameters showed decrease in dimensions of ellipsoid describing particle at pressure of 150 MPa in comparison to the structure under ambient conditions. A significant reduction concerned the major semi-axes. The most likely explanation assumes the collapse of a deep crevice known from 3D-structure, which divides the protein into two domains.

The radius of gyration, R_G, and hydrodynamic radius, R_H, are associated with shape and dimension of biological molecule in a similar manner. Both the measurement of radius of gyration and hydrodynamic radius of small molecules in solution require high precision and often are marred by an error resulting from interpartical interactions (impact of the structure factor). In addition, for a single radius a whole family of divers models can be offered. However, the use of the relation linking R_H, R_G and ratio *b/a*, can significantly reduce the number of solutions and cross-check the dimensions designated by different experimental techniques (Fig.1).

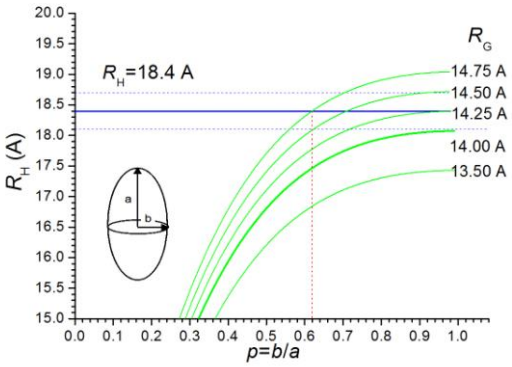

Figure 1. The dependence of hydrodynamic radius vs. ratio of semi-axis for constant values of gyration radius of prolate ellipsoid. The horizontal line indicates the experimental value of R_H.

At present a variety of methods for design of more specific structural models of the molecules in solution are developed. The starting point for more complex models are experimental form factors and/or hydrodynamic parameters in combination with either the high-resolution structure or an *ab initio* modeling [1-3]

The easiest way to build a detailed model of the protein is the distribution of its elements in a space defined by the coordinates of atoms of the experimentally solved or predicted structure. This type of model can be used among other to investigate the structure associates and the polydispersity of experimentally tested solution. While coupling model simulation of the internal dynamics of the protein chain allows to recognize conformational changes, for example, at different conditions of solvent.

The algorithms allowing to random motions of model of the polypeptide chain are the basis for modeling the structure of the intrinsically disordered proteins. This group of proteins discovered nearly 20 years ago constitutes a serious challenge for all known experimental techniques of the spatial structure determination. One of the tactics used for analysis of the structure of random coil is coupling simulation of the

free movement of the chain with the measurements of its overall structural parameters (Fig. 2).

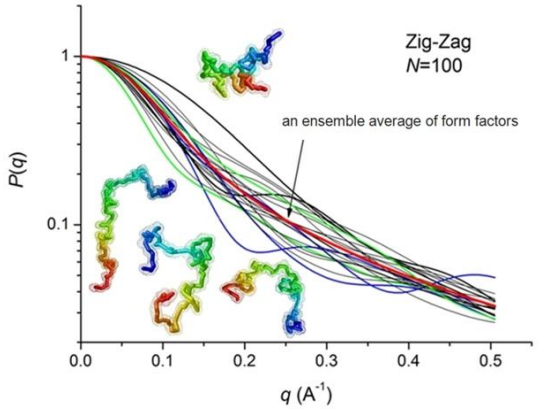

Figure 2. Experimentally measured form factor of the unfolded protein chains corresponds to the ensemble average of all conformations. The ensamble average – indicated by the arrow, at the same time all form factors corresponding to particular chain conformations are obtained - all other lines.

For example based on a large population of equilibrium conformations generated by the simulations the hydrodynamic parameters, the gyration radii and the form factors of the protein chains can be determined. The result of the simulations is an ensemble averaged set of values that could be compared with the experimental data. The population of positively verified models can be used chain by chain for the analysis of chains' local trends.

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