Interactive graphical system for small-angle scattering analysis of polydisperse systems

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Abstract. A program suite for one-dimensional small-angle scattering analysis of polydisperse systems and multiple data sets is presented. The main program, *POLYSAS*, has a menu-driven graphical user interface calling computational modules from *ATSAS* package to perform data treatment and analysis. The graphical menu interface allows one to process multiple (time, concentration or temperature-dependent) data sets and interactively change the parameters for the data modelling using sliders. The graphical representation of the data is done via the Winteracter-based program *SASPLOT*. The package is designed for the analysis of polydisperse systems and mixtures, and permits one to obtain size distributions and evaluate the volume fractions of the components using linear and non-linear fitting algorithms as well as model-independent singular value decomposition. The use of the *POLYSAS* package is illustrated by the recent examples of its application to study concentration-dependent oligomeric states of proteins and time kinetics of polymer micelles for anticancer drug delivery.

1. Introduction

Small-angle scattering (SAS) is an effective technique for structural characterization of compounds of different nature: gels, films, microemulsions, solutions of biological macromolecules, polymers, nanoparticles. Especially SAS method has advantages for the studies of biological macromolecules as it does not require special sample preparation, allows one to measure the samples at native physiological conditions and is applicable in the wide range of molecular weights (from 5 kDa to 50 MDa). The progress in instrumentation and software development during two last decades permitted solution SAS technique to solve important questions about the structure of macromolecules and their biological functions. At the same time it enormously increased the amount of the measured data and thus the efforts for the data analysis.

There are a number of software packages developed for the analysis of SAS data, such as ATSAS [1,2], GIFT [3], IRENA [4], IMP [5], etc. They permit to obtain low resolution shapes of the macromolecules, refine the quaternary structure of macromolecular complexes, restore missing portions and characterize protein flexibility, estimate size distributions of the particles. However, most

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of the programs deals with single experimental data curves and for multiple data sets (e.g. time, concentration or temperature scans) the data processing becomes tedious.

Here we present a program suite for one-dimensional small-angle scattering analysis of polydisperse systems. It includes the support for processing of multiple data sets as well as the possibility for interactive modeling.

2. The graphical interactive system *POLYSAS* and its functionality

The main program, *POLYSAS*, has a menu-driven graphical user interface calling computational modules from *ATSAS* package to perform data treatment and analysis. The graphical menu interface allows one to process multiple (time, concentration or temperature-dependent) data sets and interactively change the parameters for the data modeling using sliders. The graphical representation of the data is done via the Winteracter-based program *SASPLOT* [6].

For multiple data sets POLYSAS permits to automatically estimate overall structural parameters such as the radius of gyration (R_g), the maximum size of the particle (D_{max}), the molecular weight (MW) and the excluded hydrated volume of the particle (V_{Porod}). For these purposes POLYSAS calls the computational modules DATRG, DATGNOM and DATPOROD [7] from ATSAS package. Estimated parameters are plotted using SASPLOT in real time and can be easily saved in an ASCII format or as a picture file. The list of the loaded experimental curves can be modified and reprocessed using the buttons in the graphical menu.

In the case of polydisperse systems POLYSAS can provide the size distributions of the particles. Depending on the type of the particles it estimates the following size distributions: 1) distance distributions functions $p(r)$ for the monodisperse particles of arbitrary shape; 2) volume size distributions of spherical particles; 3) cross-section size distributions for the rod-like particles; 4) length size distributions for cylindrical particles. It is performed by calling GNOM module [8] from ATSAS package. The smearing effects for different geometrical setups can be taken into account and the desmeared curves can be obtained. The graphical menu of POLYSAS allows one to interactively change the structural parameters of the particle and/or the geometrical parameters of the experimental setup and monitor how it influences the quality of the fitted curve and the calculated size distribution function.

There are several options for the analysis of mixtures in the POLYSAS. In the case when the high resolution models of the mixture components are available (e.g. in PDB format), POLYSAS permits to fit experimental data sets with the linear combinations of the scattering curves calculated from the selected PDB models. The volume fractions of the components are calculated using OLIGOMER module [6] from ATSAS package and the results are displayed via SASPLOT.

POLYSAS has the option for the non-linear analysis of the multi-component systems using the simple geometrical bodies of three types (spheres, cylinders and dumb-bells) and for maximum of five components. It is possible to take into account the polydispersity effects and interparticle interactions (the latter can be applied for the spherical particles within sticky hard sphere potential approximation). The refined parameters are calculated by MIXTURE module [6] from ATSAS package. The parameters of the models can be interactively changed using the sliders and spin buttons and the fit from the model to the experimental data will be updated. The upper and lower limits of the parameters for each component can be set before the minimization process. If the upper and lower limits coincide with each other, the corresponding parameter will be fixed.

The model independent analysis using the singular value decomposition (SVD) method is also available in POLYSAS. It can be applied for the systems measured at different conditions (different pH, temperature, buffer type, sample and salt concentration, ligand addition etc.). The eigenvalues and the eigenvectors of the SVD method are calculated and the number of independent components in the system is estimated using the statistical test for the non-random oscillations of the eigenvectors. The SVDPLOT module [6] from ATSAS package is called by POLYSAS. The graphical menu of the POLYSAS provides options for interactive selection of the full data sets and partial data subsets of the

experimental curves and allows one to check the stability of the estimated number of components in the system.

3. Applications of POLYSAS to experimental data

The usage of POLYSAS can be illustrated on several experimental *X*-ray data sets that were recorded in collaborative user projects on the X33 beamline of the EMBL [9] at the storage ring DORIS-III (DESY, Hamburg).

The first example concerns the studies of the complex equilibrium mixture of the AXH domain of the Ataxin-1 protein [10]. Ataxin-1 is a human protein responsible for ataxia type 1, a hereditary disease associated with protein aggregation and misfolding. The AXH domain of Ataxin-1 displays a dimer of dimers arrangement in the crystal asymmetric unit. In solution, the domain is present as a complex equilibrium mixture of monomeric, dimeric, and higher molecular weight species. This behavior, together with the tendency of the AXH fold to be trapped in local conformations, makes the AXH domain an unusual example of a chameleon protein whose properties bear potential relevance for the aggregation properties of ataxin-1 and thus for disease.

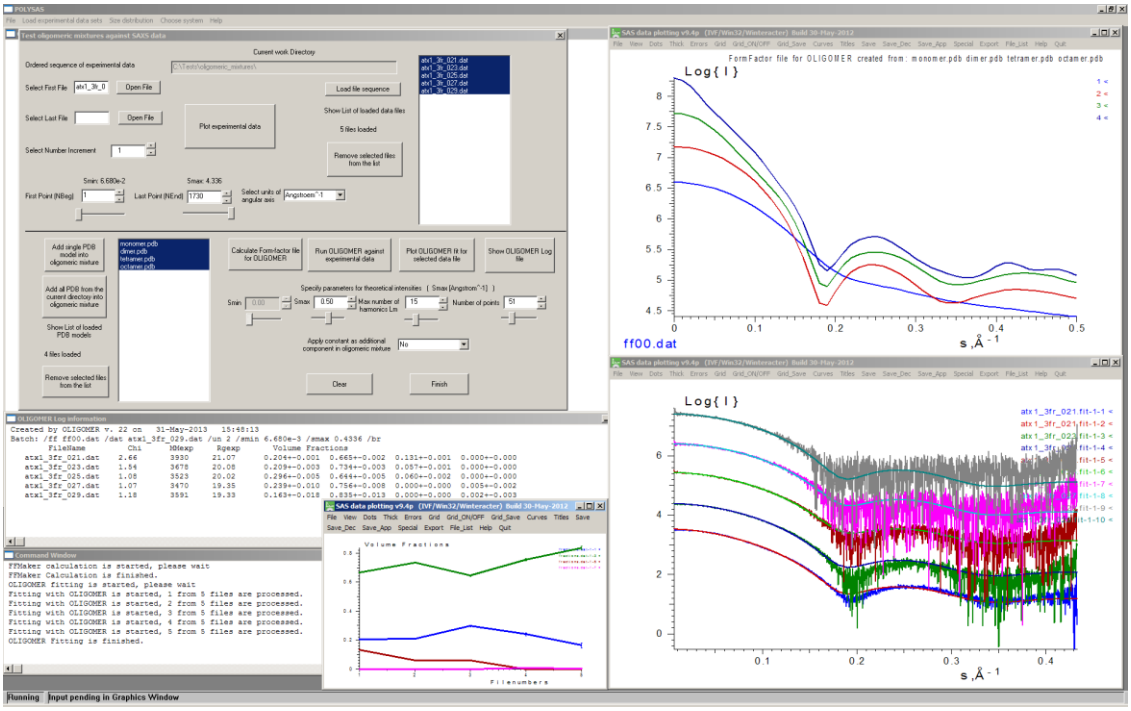

Figure 1. POLYSAS GUI for SAXS data modelling of Ataxin-1 using an oligomeric mixture

In Figure 1 the graphical user interface of POLYSAS is presented for modelling of Ataxin-1 in solution as an oligomeric mixture. The graph with the theoretical curves calculated from PDB models (monomers, dimers, tetramers and octamers) is displayed in the top-right corner of the screen, the graph with the fits to the experimental data set is shown in the bottom-right corner of the screen and the graph with the estimated volume fractions of the components is displayed in the bottom-left corner. The usage of POLYSAS enables to quantitatively characterize the system and provides the opportunity for interactive monitoring of different hypotheses during the data modelling.

The second example is devoted to time-dependent studies of hydrolytically degradable polymer micelles intended for controlled drug delivery [11]. Highly hydrophobic cholesterol moieties as well as the anticancer drug doxorubicin were attached to the polymer backbone by a pH-sensitive hydrazone bond. Time-dependent SAXS measurements were performed after changing pH from a typical blood value (pH=7.2) to that of tumor cells (pH=5.0) to characterize the drug release and changes in particle size and shape. It was found that nanoparticles composed of the conjugates

containing Dox were generally bigger than the drug-free ones. For most conjugates, nanoparticle growth or decay was observed in the time range of several hours. The growth/decay rate and the steady-state size of nanoparticles depended on the spacer structure.

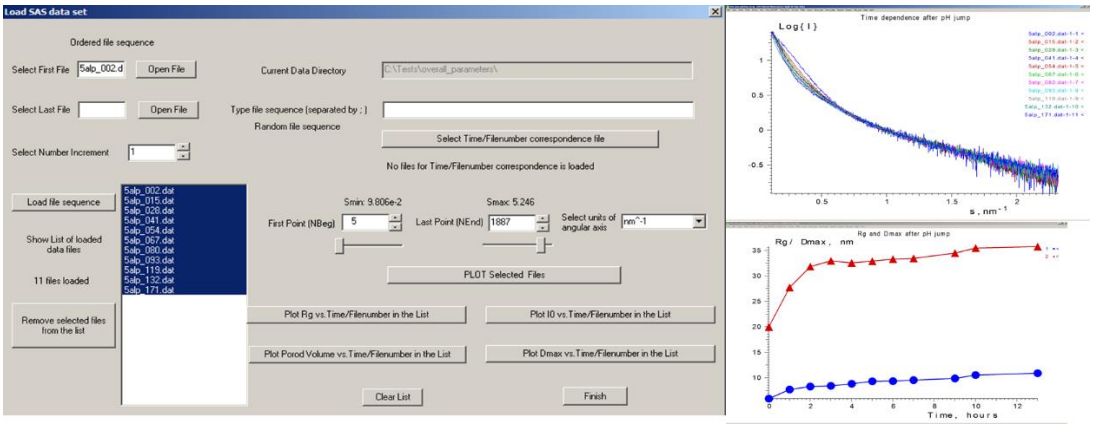

Figure 2. POLYSAS GUI for time-dependent SAXS data studies of hydrolytically degradable polymer micelles bearing the anticancer drug

In Figure 2 the graphical user interface of POLYSAS is shown that was applied for modelling of time dependence of the overall parameters (the radius of gyration R_g and the maximum size of the particles D_{max}) of polymer micelles with the anticancer drug. The graph with the experimental time dependent data set is displayed in the top-right corner of the screen, the time dependence of R_g and D_{max} is shown in the bottom-right corner of the screen. This dependence was automatically obtained by clicking on a single button in POLYSAS GUI.

Thus, we have demonstrated that POLYSAS provides enhanced and convenient options for structural studies of macromolecular solutions using small-angle scattering data. The program POLYSAS is included in the ATSAS package (http://www.embl-hamburg. de/biosaxs/software.html), is freely available to academic users, together with other ATSAS programs as from the 2.6 release.

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