**Artificial neural networks for solution small angle scattering data analysis**

Abstract

Small angle X-ray scattering (SAXS) experiments are widely used for the characterization of biological macromolecules in solution. We propose a novel method of SAXS data analysis based on the application of interconnected neural networks (perceptrons). For a given experimental data from proteins or DNA/RNA, our stack of networks evaluates major SAXS model-free parameters: molecular weight, maximum intraparticle distance, a radius of gyration, pair-distance distribution function p(r), and a noise-free scattering curve. The neural networks were trained on a synthetic dataset simulated from PDB databank and outperforms other conventional methods. This completely automatic approach does not require manual adjustment of parameters and has proved to be robust against experimental errors. The method was implemented as a publicly available web service with a graphical interface, providing the possibility to inspect and download the results (<https://dara.embl-hamburg.de/gnnom.php>).

Introduction

Small-angle scattering (SAS) of X-rays and neutrons from biological macromolecules in solution is a powerful tool, providing information on molecular structures and dynamics under a wide range of conditions [Grawert & Svergun, 2020; Koch, 2003; Glatter & Kratky, 1982]. Due to relatively soft requirements to sample preparation for the SAS experiment and to the intense development of synchrotron radiation sources, the technique became high throughput and publicly available even to the non-specialists in the area. Despite the huge success in the development of data analysis software [ATSAS, new], [SASTBX (J. Appl. Cryst. (2012). 45, 587-593)], [BioXTAS], calculation of crucial primary SAS-derived parameters such as radius of gyration Rg, maximum intraparticle distance Dmax, molecular weight MW, and pair-distance distribution function p(r) still strongly depends on the quality of the experimental data and sometimes stays non-trivial task. The p(r) function represents a histogram of distances between pairs of points in the particle, weighted by the product of their scattering contrasts [Glatter & Kratky, 1982]. Mathematically, the p(r) function is closely related to the scattering intensity versus the momentum transfer I(s) via well-known transformation [Debye, 1915]:

(1)

(2)

The limited angular range of discretely recorded experimental data, as well as the presence of experimental noise, makes the evaluation of p(r) an ill-posed problem. As estimation of Dmax, as well as reconstruction of a macromolecular model, is typically based on the p(r) function, therefore it is crucial to reliably and non-ambiguously compute p(r) from available experimental data. The method of solving this problem by so-called indirect Fourier transformation (IFT) has been originally proposed by Glatter [Glatter, 1977], and further enhanced by Svergun [Svergun, 1992] and Hansen [Vestergaard & Hansen, 2012]. In IFT approach a guess on the Dmax must be given, the p(r) function is expressed as a sum of some analytical functions (e.g. cubic splines), and a classical regularization procedure [Tikhonov, 1943] is applied such that p(r) i) agrees to experimental data and ii) ensures satisfaction to the imposed constraints. Most commonly the constraint is the smoothness of p(r), so that termination effects are reduced or idially completely removed. However, in all of these approaches, the choice of the final solution remains a subjective criterion left to the discretion of the user.

Recently, neural networks (NN) technologies applied to structural biology have experienced a sudden leap with regard not only to the business and industrial applications but also to academic research [Senior et al, 2019], [Armenteros et al, 2019], [Liu et al, 2020]. Inspired by these works, we developed a novel method of SAXS data analysis based on state-of-the-art machine learning principles. We employed a stack of interconnected NN trained on synthetic SAXS data from thousands of models from PDB databank [Berman et al, 2000] to perform principal data analysis. For a given experimental data from proteins, RNA, or DNA our stack of networks independently evaluates overall SAXS parameters: molecular weight (MW), Dmax, Rg; as well as p(r) function and a noise-free scattering curve. Here we demonstrate the robustness of our method and test accuracy of predicted parameters for synthetic data with different levels of added noise against the parameters estimated by major well-established methods. This completely automatic approach accounts for the whole angular range of experimental data, takes into consideration a shape of molecule and appears to be the only one (to the best of our knowledge) that applies both to amino-acid and nucleotide-based molecules. The method was implemented as a publicly available web service with a graphical interface, providing the possibility to inspect and download the results (<https://dara.embl-hamburg.de/gnnom.php>).

Methods

**Training set**. In order to train the neural networks on the most realistic data set, we used the real models of deposited proteins and nucleotides from the worldwide protein databank PDB ([www.rcsb.org](http://www.rcsb.org)) for simulation. Generally, for the better performance of NNs it is recommended to prepare unbiased training set, ideally evenly distributed over the parameters for prediction. As is clearly seen from the histogram in fig.1, the deposited PDB molecules are skewed towards small and globular proteins.



(a)

(b)

*numbers*

*numbers*

Fig.1. Distribution of (a) all deposited in PDB proteins and (b) sampled proteins for the training set by their radii of gyration and molecular weights.

Intuitively, it is clear that the vast majority of the structures were solved by crystallographic methods, thus the overall distribution tends to be biassed towards small and globular proteins simply due to their ability to form a crystal. Preliminary computer experiments confirmed, that NNs trained on the whole amount of PDB data works well for the small and globular proteins and underperform for bigger and elongated models. These results, however commendable, would fall short of the SAXS community aspirations, as one of the strongest sides of the method is its ability to analyze unfolded and even intrinsically disordered proteins. Thus, we decided to expand the training set to make it as diverse and complete as possible. Among other techniques, the most successful one turned out to be a Latin Hypercube sampling [McKay, Beckman, et al, 1979] – a statistical method for generating a near-random sample of parameter values from a multidimensional space. In our case, we employed it to pick several thousands of models in two-dimensional space (MW, Rg) out of more than 150000 available to date in PDB. As is demonstrated in Fig.1, the chosen dataset of 1015 models has almost a step-like distribution across (MW,Rg) indicating drastically more diverse by size and shape models.

For each model, a pair of (simulated curve – predicted parameter) was prepared using CRYSOL [crysol paper] in order to apply classical supervised training of NNs. For each of the required parameters (MW, Dmax, p(r)) the dedicated NN was developed and trained. The calculation of p(r) from experimental data is typically fairly ambiguous operation due to the limited angular range of SAS data and the presence of experimental noise. The big advantage of using synthetic data for training NN is the possibility to simulate a noiseless SAS curve in an unrealistically wide range of angles. For such “ideal” data it is unnecessary to apply IFT as eq.2 readily gives a smooth and importantly unique solution. As a double-check, the Rg, Dmax, and smooth SAXS curve are computed on the fly from the predicted p(r) function and compared with the parameters estimated by more classical methods.

**Neural network architectures.** We exploited interconnected neural networks (perceptrons) with one hidden layer (fig.2) for predicting the above-mentioned SAS parameters. Since the expected output of the NNs is a number and not a discrete value, we encounter a classical regression task for machine learning.

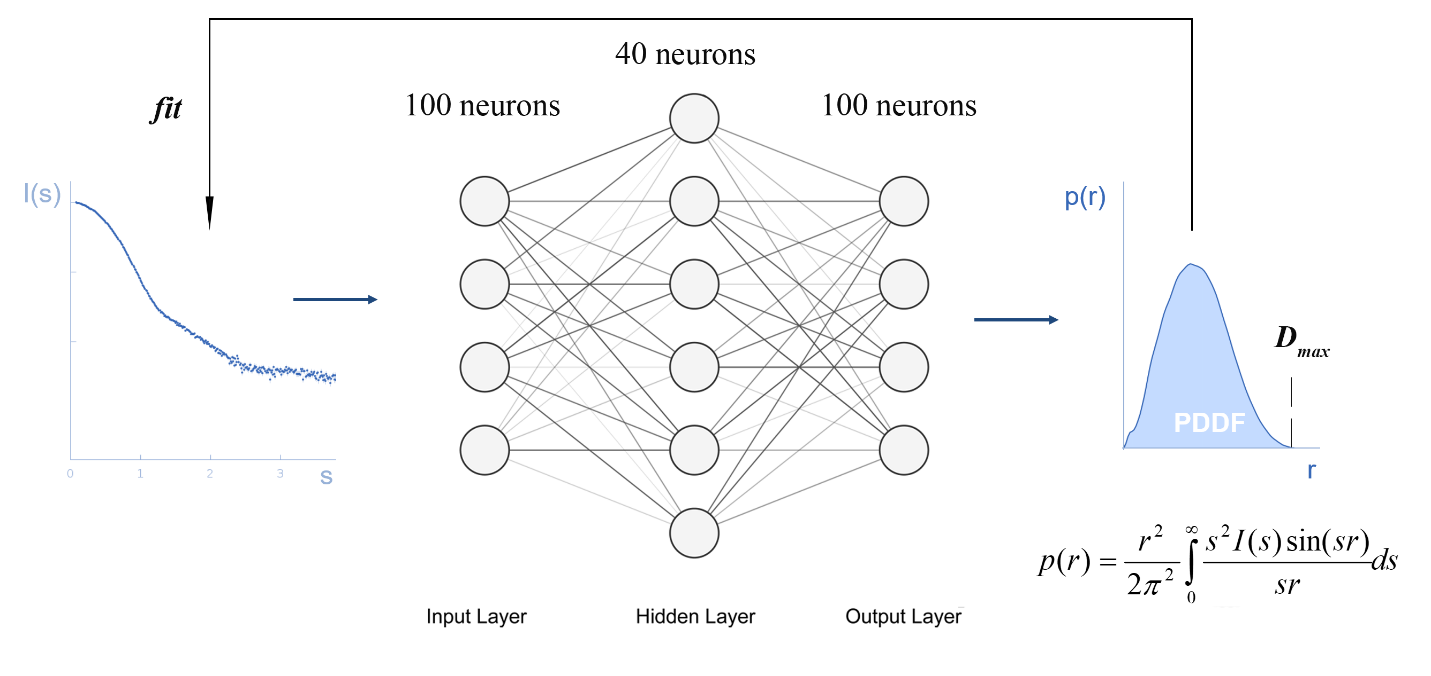
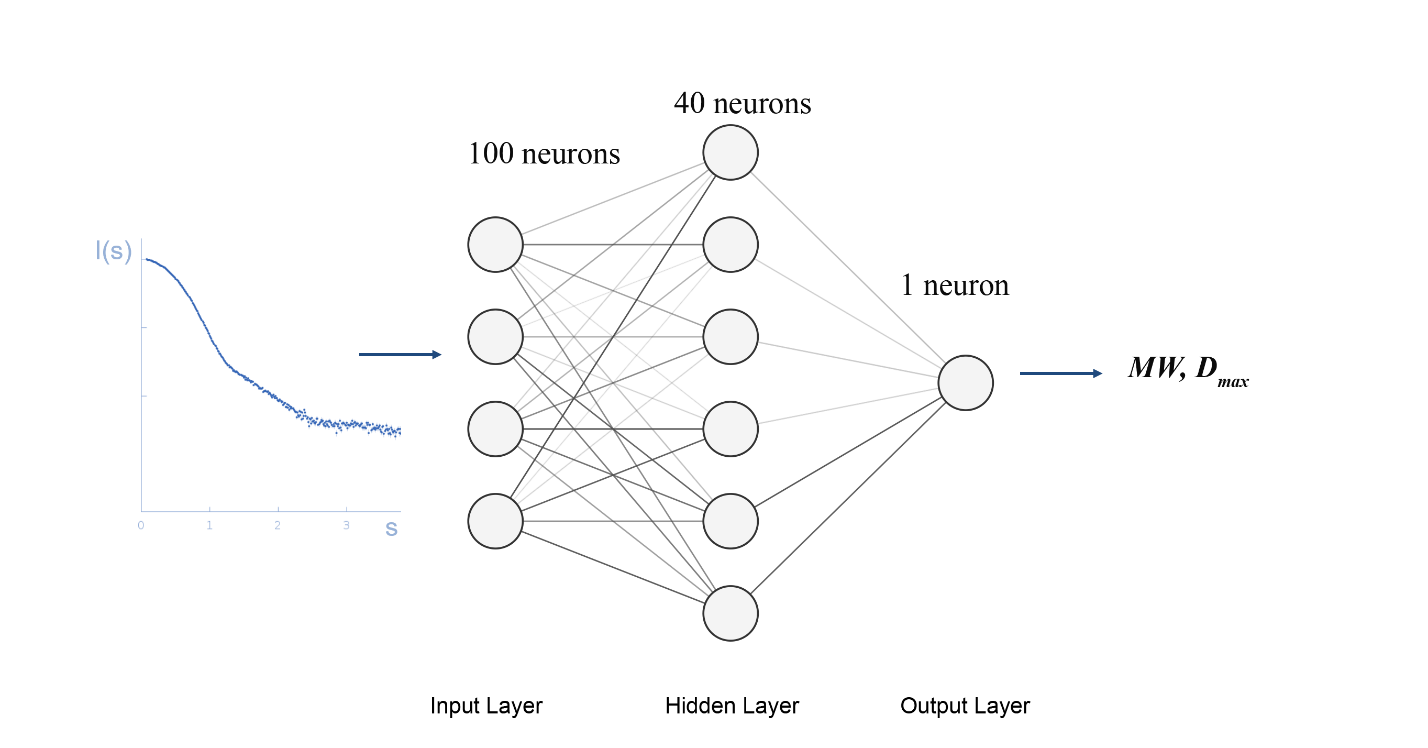


Fig.2 Architectures of neural networks used for primary SAXS data analysis: i) NNs with a scalar output of MW or Dmax and ii) output of one-dimensional p(r) function, that as a byproduct provides Rg and Dmax values and denoised SAXS curve.

According to the Universal approximation theorem (see e.g. [G.Cybenko, 1989]), a feed-forward artificial network with a single hidden layer containing a finite number of neurons can approximate any continuous function, under mild assumption on the activation function. In particular, it was recently shown [Lu, Zhou, et al. "The expressive power of neural networks: A view from the width." *Advances in neural information processing systems*. 2017.] that NNs utilizing the rectified Linear Unit (ReLU) activation function with a width (number of neurons in one layer) of n+1 is capable to approximate any continuous convex function of n-dimensional input variables to any desired degree of precision [Hanin, 2019]. In SAXS one typically analyzes a hugely oversampled curve, that in fact contains only up to 15-35 Shannon channels [Moore, 1980]. Therefore, in the context of SAS with an angular range smax < 5 nm-1 it is sufficient to use a NN with only one hidden layer and the width of ~40 neurons. The attempts to introduce more layers and neurons led us to increased learning time and higher instability without any gain in the NNs productivity.

**Molecular weight prediction**. Estimation of the MW of irradiated macromolecules in solution is one of the most important overall parameters that can be derived from SAS experiment. To date, there are several well-established techniques for MW estimation, that nominally could be subdivided into concentration-dependent and concentration-independent methods. Former methods account for the dependence of forwarding scattering I(0) on the MW and rely on the scattering from calibrants (e.g. known protein or water) [Mylonas, Svergun, 2007]. The latter methods utilize a single background-subtracted curve and require no additional *a priori* information on sample concentration. The historically first concentration-independent method [Porod, paper] calculates hydrated volume (also known as the Porod volume) from the ratio of I(0) to a Porod invariant Qp, thus estimating MW as an empirical relation between protein volume and mass [Petoukhov et al, 2012]. Subsequently, the major contributions were done to improve the accuracy of MW determination by varying integration range over *s* [Fisher, 2010] and by introducing a more complicated empirical dependence between the dimensionless volume of correlation and MW [Rambo&Tainer, 2013]. The recent development pushes precision of MW estimation even further, as it accounts not only for molecular size contribution but also for its shape using the classical support vector machine approach [Franke et al, 2018]. At roughly the same time, there was an attempt to combine all described methods employing Bayesian statistics to enable one to find the most probable molecular weight and assess its credibility interval [Hajizadeh et al, 2018].

Application of neural networks is a very promising novel method, that potentially makes corrections for protein shape and conformational state (folded/unfolded), as it takes the whole SAS curve for calculation. Moreover, it appears to be the first method for concentration-independent estimation of MW from SAS data applicable to DNA/RNA samples, and not only to proteins.

**Maximum intraparticle distance Dmax.** The common and as a matter of fact the only available method to determine Dmax from SAS scattering profile is via inspection of corresponding p(r) function. Upon certain restrictions, such as non-negativity and zeroing p(0) = p(r ≥ Dmax) = 0, it is possible to find the Dmax as a first negative point of p(r) distribution. Importantly, small deviations of Dmax values typically do not significantly affect the fit to the data (inverse Fourier transform of p(r)), thus precise estimation of Dmax is left to the discretion of a human.

Application of NNs gear human independent way of Dmax calculation, based solely on the one-to-one correspondence (SAS profile – Dmax). From the physical point of view, experimental SAS data do not contain a single Dmax value, as the scattering is always averaged over the huge ensemble of slightly different (even in case of near-to-perfect monodisperse solution) particles. Additionally, hydration layers may vary among different particles and introduce uncertainties to Dmax to some extent. As we demonstrate below, our method seems to be working best on synthetic data and robust against experimental errors.

**Pair-distance distribution function: Dmax, Rg, and noiseless curve**. Pair distance distribution function contains the same scattering information as the original curve and intuitively more comprehensive, as it represents the histogram of distances between pairs of intraparticle points in real space. Here we employed the same NN architecture of 1 hidden layer with 40 neurons, except for the output layer was extended to 100 units to represent a p(r) function. Apart from the fit to the data (eq. 1), that can be interpreted as a noiseless SAS curve, the algorithm finds a Dmax value as the first negative point in p(r) and cuts off the p(r > Dmax). The radius of gyration is estimated as half the second moment of predicted p(r) function:

(3)

.a as a byproduct we get another way of calculation of Dmax and Rg

*Results*

Comparison of accuracy of predicted SAXS parameters with the other available methods.

Fig.3 Comparison of performances of different methods.

Fig.4 Web interface.

*Conclusion.*

A novel completely independent method for the estimation of primary SAXS parameters was developed. The comparison with well-established methods demonstrated higher accuracy and robustness of the method against the experimental noise.

*References.*

11