The matter of the structure and rules of the universe have been intriguing the mankind from the very beginning. Partially the question answers physics with theories of mutual reactions and connections. But this is as far as we do not want to analyze living creatures - this is where the field of biology and chemistry enters. The proteins are the basic building material of the whole animated world. Learning of the broadly understood life on earth requires understanding how the proteins work on a very deep level. This task is not that easy as one could think - all the environment is dynamic and hard to "catch" in precise. All one can do to try to measure a protein is to perform an experiment and interpret the results. This is where hydrogen-deuterium exchange mass spectrometry comes in handy. It helps distinguish the regions exposed to the outside from the regions of the folded structure. This enables the understanding of the interaction between proteins, as it provides the information where the exchange is likely to happen. The obtained knowledge is particularly important for life or medical sciences and the biopharmaceutical industry in topics of antibodies analysis, epitops mapping, etc.

Hydrogen-deuterium exchange mass spectrometry (HDX-MS) is a way to perform an analysis of a protein. The principal is to observe the behavior of the protein in the native state in the aqueous solution where the hydrogen particles are replaced by the deuterium particles. Isotopes (variants of the same chemical element but with the different number of neutrons) exchanges with themselves very well. Under controlled conditions, there is a spontaneous process of exchange. This kind of experiment can gather very effective data on how the exchange goes in the main chain of the protein - the exchange on the ends of the sequence goes too fast to be correctly measured. This kind of data not only serves the purpose of gaining the basic information about protein structure but also getting some insight into the protein structure of the tertiary and quaternary order.

The analysis process for HDX-MS data is not yet well developed. Software and hardware dedicated to obtaining the raw results from the experiment do only basic data pre-processing and validating. The visualization and interpretation of the data depend strictly on the skills of the experimentator that can be on different levels and from different backgrounds. The lack of official methodology makes this task even more complicated.

What is special about HDX-MS experiments is that they produce unique data that cannot be obtained in any other way. For example, nearly one-third of the proteins are considered as intrinsically disordered protein - lacking a fixed or ordered three-dimensional structure. For those, the crystallography experiments cannot be performed - but HDX-MS can. It is also a suitable solution for analysis of very long proteins - compared to short protein sequences that can be processed using the NMR method. Moreover, HDX-MS can give some insight if the function of the protein is preserved when joined with other substances - that is particularly important when constructing and analyzing new or already existing drugs.

The disadvantage of the HDX-MS method in comparison with crystallography or NMR is gaining data for whole peptide (considered as low resolution) instead of a single amino residue (high resolution). Elimination of this limitation could make HDX-MS method more efficient and competitive with others.

Data from HDX-MS experiments provide a unique insight into the dynamics of protein structure. Such information is a very valuable addition to existing or upcoming models of proteins and protein complexes. They provide data on the mechanics of molecular biological processes, where their dynamics are crucial for understanding their course. However, we believe that HDX-MS data can be analyzed more efficiently and informatively by switching from peptide (low-resolution) to amino acid pairs (high-resolution).

Therefore, the aim of this work is to create reliable biophysical methods for the analysis of high-resolution HDX-MS experiments and their application in the study of unstructured proteins.