



# Analysis of data from hydrogen-deuterium exchange mass spectrometry experiments

Weronika Puchała<sup>1,\*</sup>, Michał Burdukiewicz<sup>2</sup>, Michał Kistowski<sup>1</sup>, Katarzyna A. Dabrowska<sup>1</sup>, Aleksandra E. Badaczewska-Dawid<sup>3</sup>, Dominik Cysewski<sup>1</sup>, Michał Dadlez<sup>1</sup>  
\*puchala.weronika@gmail.com



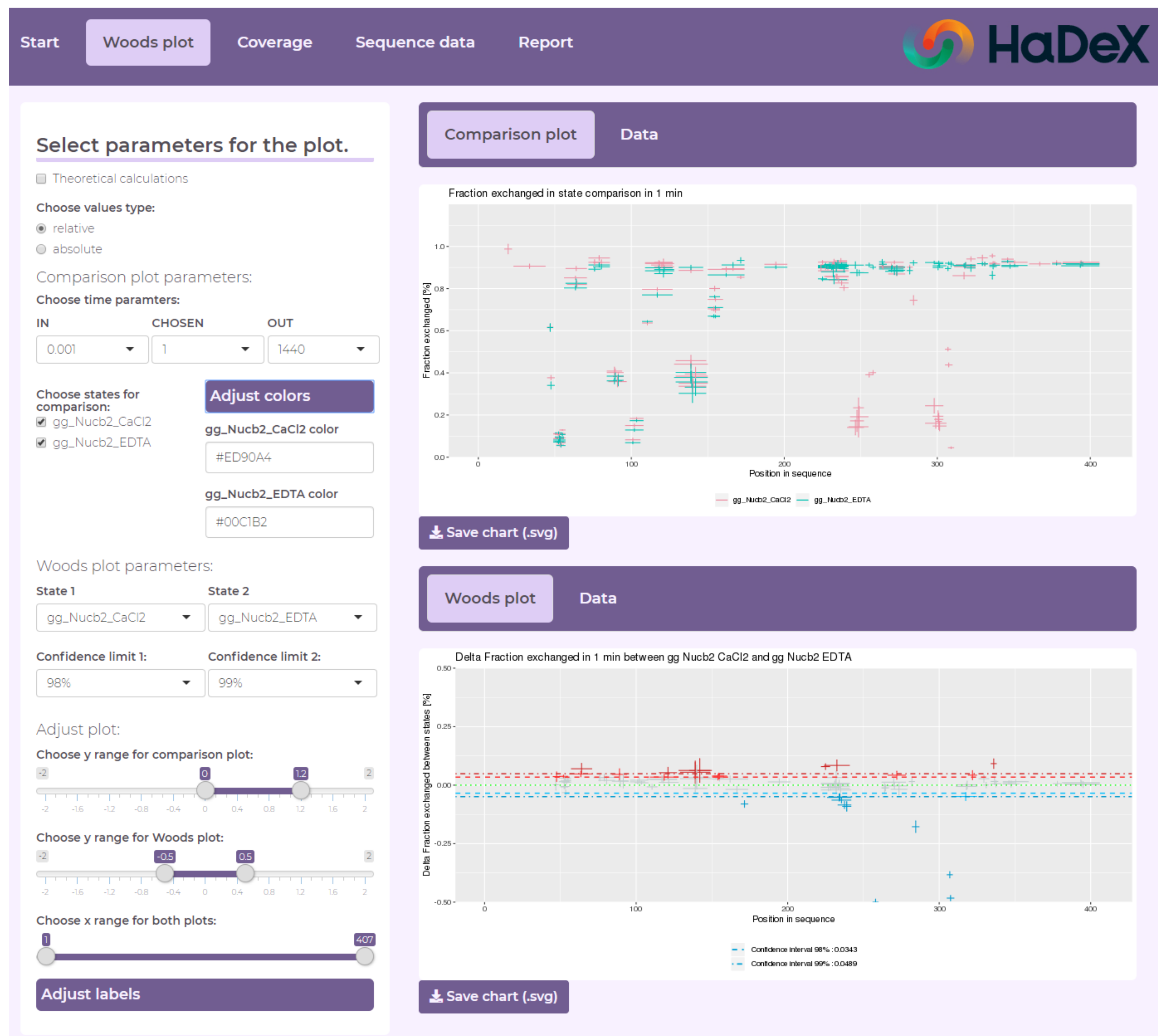
<sup>1</sup>Institute of Biochemistry and Biophysics Polish Academy of Sciences, Poland, <sup>2</sup>Faculty of Mathematics and Information Science, Warsaw University of Technology, Poland, <sup>3</sup>Faculty of Chemistry, Biological and Chemical Research Center, University of Warsaw, Poland.

## Introduction

Hydrogen-deuterium mass spectrometry (HDX-MS) is a staple tool for monitoring dynamics and interactions of proteins. Due to the sheer size of the HDX-MS results, the data analysis require a dedicated software suite. However, the majority of existing tools provides only point-and-click interfaces to black-box models or does not offer a complete analytic workflow. We propose **HaDeX**, a novel tool for processing, analysis and visualisation of HDX-MS experiments. **HaDeX** covers the whole analytic process, including quality control, ISO-based uncertainty and publication-quality figures. The reproducibility of the whole procedure is ensured with advanced reporting functions. It is important to us that our analytic methodology is transparent and understandable for the users so it is consulted with international experts and discussed in-depth in the package vignette.

## Main functionality

The main audience for **HaDeX** is HDX-MS practitioners whose area of expertise doesn't include programming and advanced data analysis skills. To help them with their work, **HaDeX** is also available as a Shiny web server with a wide range of clickable customizable options. To ensure publication-quality figures all the plots are fully editable by the user and processed data is easily downloadable in every step.



## Calculations and uncertainty

### Comparison Plot

An important aspect of analysing data is a comparison of peptide deuteration in different states. A relative deuteration level used by **HaDeX** is defined by the equation 1.

$$D = \frac{D_t - D_0}{D_{100} - D_0} \quad (1)$$

The levels of deuteration along with uncertainty intervals are shown on figure 1. **HaDeX** provide both experimental and theoretical levels of deuteration in two variants: with relative or absolute values.

### Woods Plot

Woods plot format shows difference between results of two different states as described by equation 2.

$$diff = D_1 - D_2 \quad (2)$$

Described calculations lead to results shown on Woods plot format - figure 1.

### Uncertainty of the measurement

The equation 1 and 2 are functions of multiple variables (each with its own uncertainty), so there is need to use the Law of Propagation of Uncertainty, defined in equation 3:

$$u_c(y) = \sqrt{\sum_k \left[ \frac{\partial y}{\partial x_k} u(x_k) \right]^2} \quad (3)$$

The Law of Propagation of Uncertainty, as described by equation 3 is applied to both 1 and 2. Uncertainty calculated this way is compatible with guidelines developed by International Organization for Standardization.

## Availability

**HaDeX** is available as a web server: <http://mslab-ibb.pl/shiny/HaDeX/>

or a standalone software: <https://sourceforge.net/projects/hadex/>

The documentation and methodology is available on:

<https://hadexversum.github.io/HaDeX/articles/overview.html>



## Overview of the sequence

The **HaDeX** provides the tools to view and analyse the results of HDX-MS data. Obtained data is used to show information in different ways.

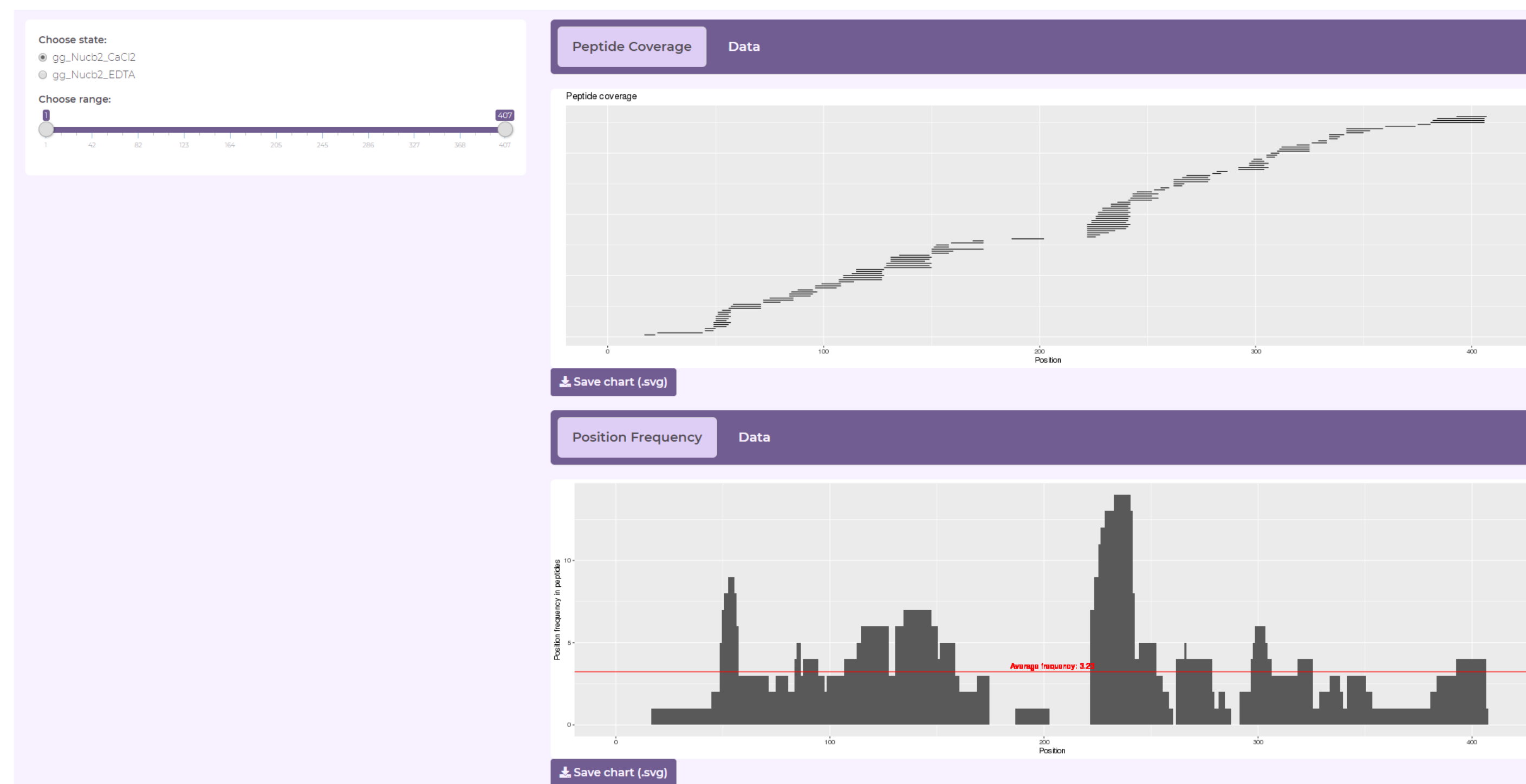


Figure 2: Sequence coverage shown in two variants

## Kinetics of the peptides

**HaDeX** has a module for analyzing deuteration uptake change in time for given peptide in given time. For comparison, choosing multiple combinations of peptides and their states is possible for the plot.

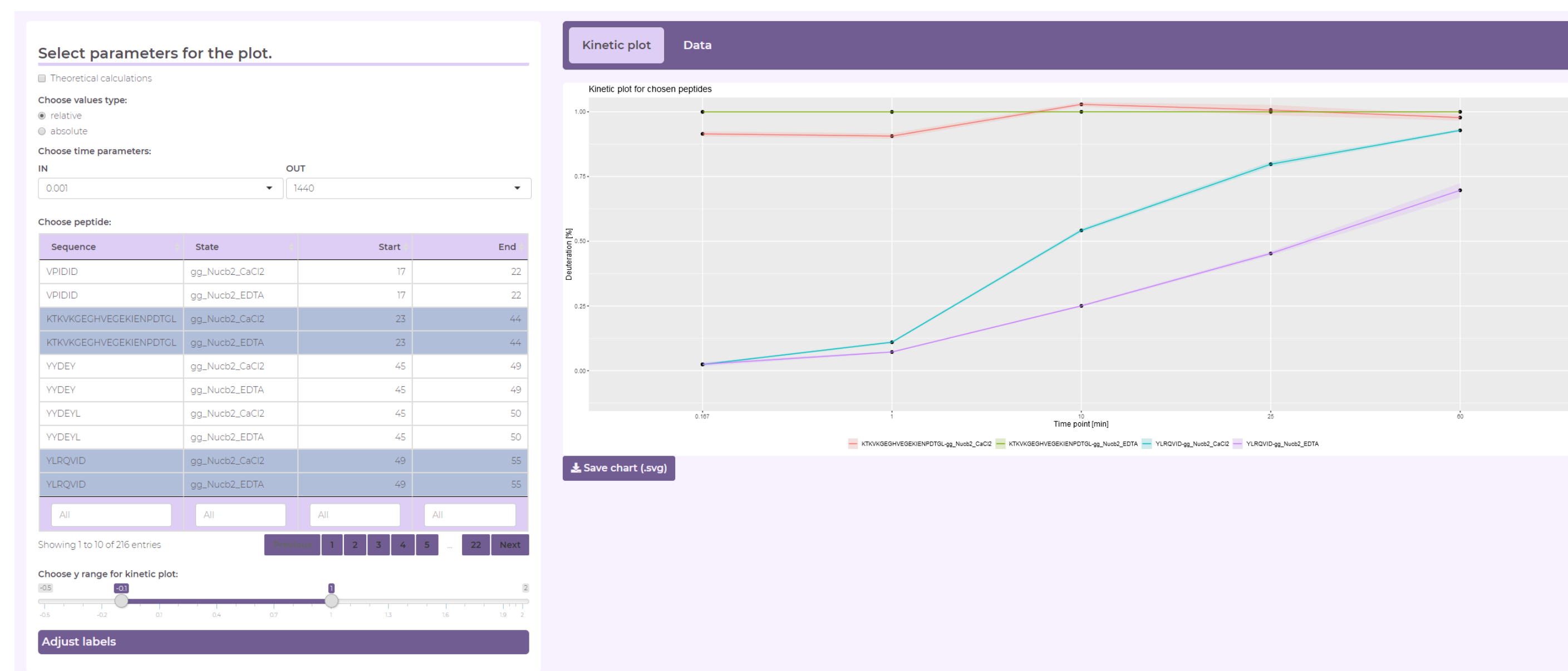


Figure 3: Kinetics plot for chosen peptides

## Recommendations for performing an experiment

**HaDeX** follows the recently published recommendations for performing, interpreting and reporting HDX-MS experiments. One aspect of that is summary of the data - for quality control purposes - as shown on the figure 4. The summary table can be downloaded in csv/pdf form, like all of the available data tables.

Name	Value
HDX time course	8
Number of peptides	42
Sequence coverage	0.9836
Average peptide length	14.6829
Redundancy	4.5606
Replicates	4
Significant differences in HDX	0.98 / 0.99

Figure 4: Summary table

## Report function

**HaDeX** provides report generation - with parameters chosen by user so is fully personalizable. On figure 5 are shown available elements of the report - that can be chosen by user and limited strictly for user needs.

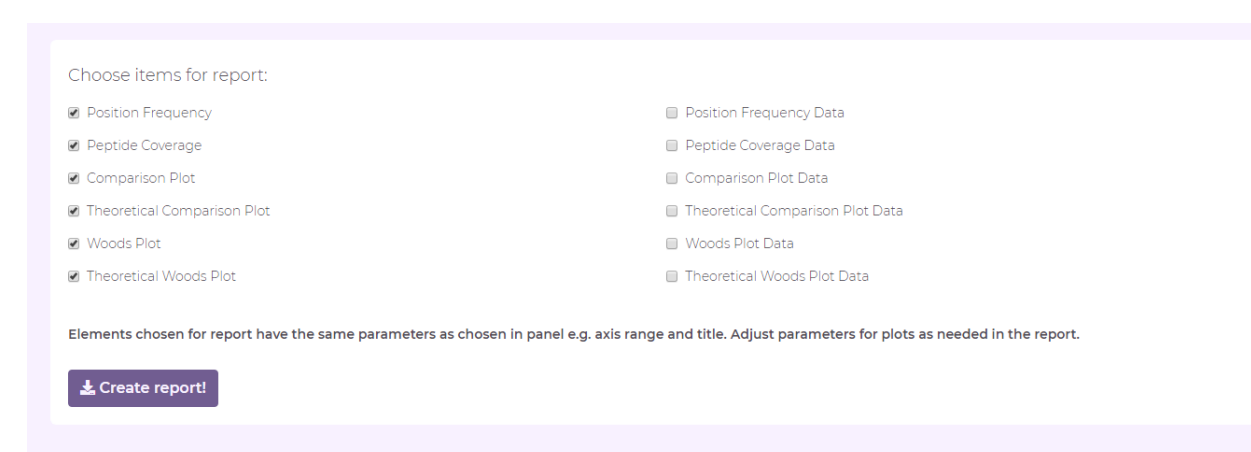


Figure 5: Screenshot of **HaDeX** - reporting page

## Acknowledgements

The project is carried out within the TEAM TECH programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.(TEAM TECH CORE FACILITY/2016-2/2) Mass Spectrometry of Biopharmaceuticals-improved methodologies for qualitative, quantitative and structural characterization of drugs, proteinaceous drug targets and diagnostic molecules"



## Bibliography

- 1 Start2Fold: a database of hydrogen/deuterium exchange data on protein folding and stability. Pancsa R, Varadi M, Tompa P, Vranken WF. Nucleic Acids Res. 2016 Jan 4;44(D1):D429-34. doi: 10.1093/nar/gkv1185. Epub 2015 Nov 17
- 2 MEMHDX: an interactive tool to expedite the statistical validation and visualization of large HDX-MS datasets. Hourdel V, Volant S, O'Brien DP, Chenal A, Chamot-Rooke J, Dillies MA, Brier S. Bioinformatics. 2016 Nov 15;32(22):3413-3419. Epub 2016 Jul 13
- 3 Deuterios: software for rapid analysis and visualization of data from differential hydrogen deuterium exchange-mass spectrometry. Andy M C Lau, Zainab Ahdash, Chloe Martens, Argyris Politis, Bioinformatics, btz022, <https://doi.org/10.1093/bioinformatics/btz022>
- 4 ISO, Guide to the Expression of Uncertainty in Measurement (International Organization for Standardization, Geneva, Switzerland, 1993)
- 5 The Utility of Hydrogen/Deuterium Exchange Mass Spectrometry in Biopharmaceutical Comparability Studies. Houde, D. et al.(2011). Journal of pharmaceutical sciences,100(6), 2071-2086
- 6 Masson GR, Burke JE, Ahn NG, Anand GS, Borchers C, Brier S, et al. Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments. Nat Methods. 2019 Jul;16(7):595-602.