

HaDeX: an R package and web-server for analysis of data from hydrogen-deuterium exchange mass spectrometry experiments

Weronika Puchała^{1,*}, Michał Burdukiewicz², Michał Kistowski¹, Katarzyna Dabrowa-Dabrowska¹, Aleksandra E. Badaczewska-Dawid³,

Dominik Cysewski¹, Michał Dadlez¹

*puchala.weronika@gmail.com

¹Institute of Biochemistry and Biophysics Polish Academy of Sciences, Poland, ²Faculty of Mathematics and Information Science, Warsaw University of Technology, Poland, ³Faculty 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. For users operating on sensitive data standalone application is available. To ensure publication-quality figures all the plots are fully editable by the user and processed data is easily downloadable in every step.

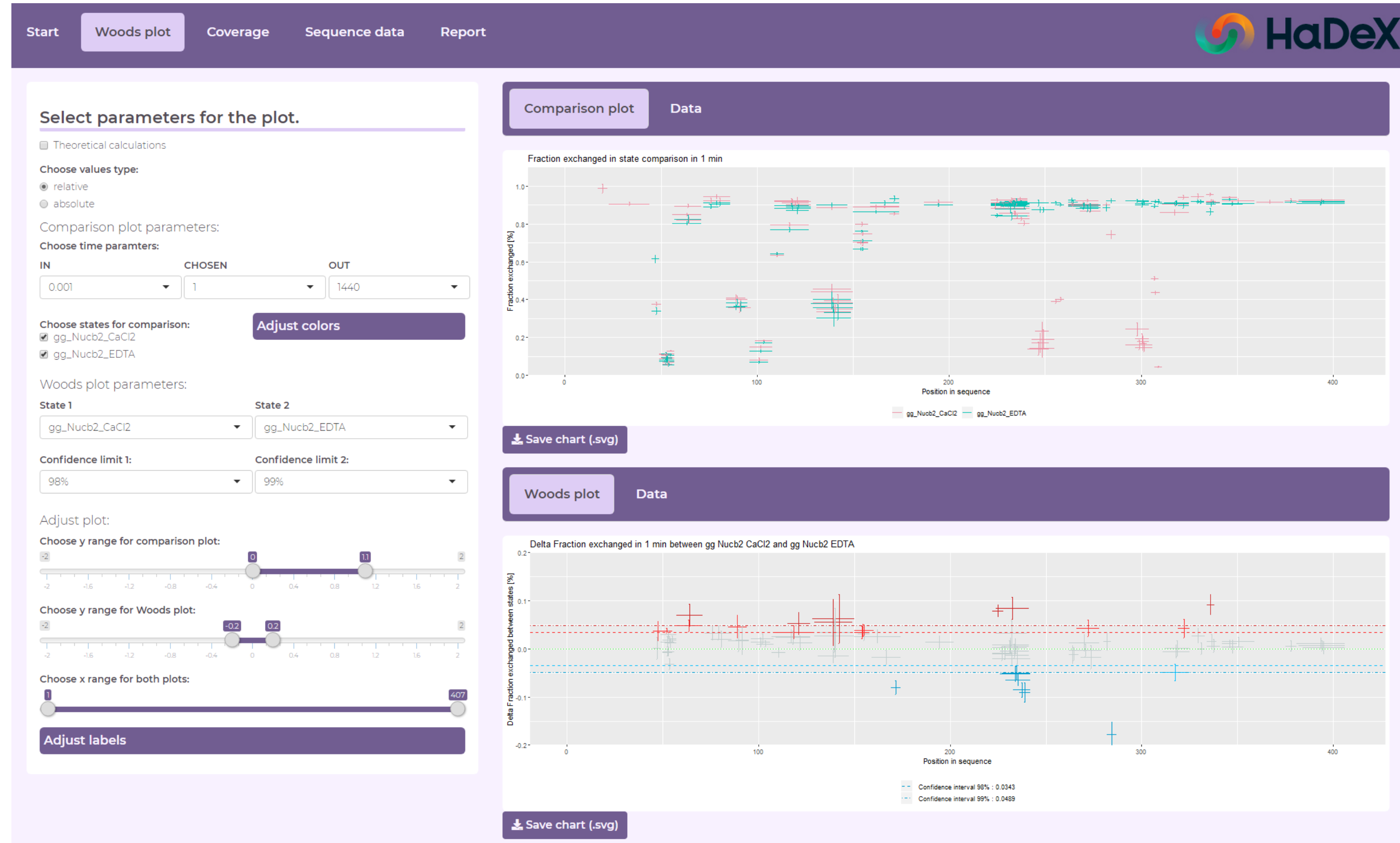


Figure 1: Overview of **HaDeX** app

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.

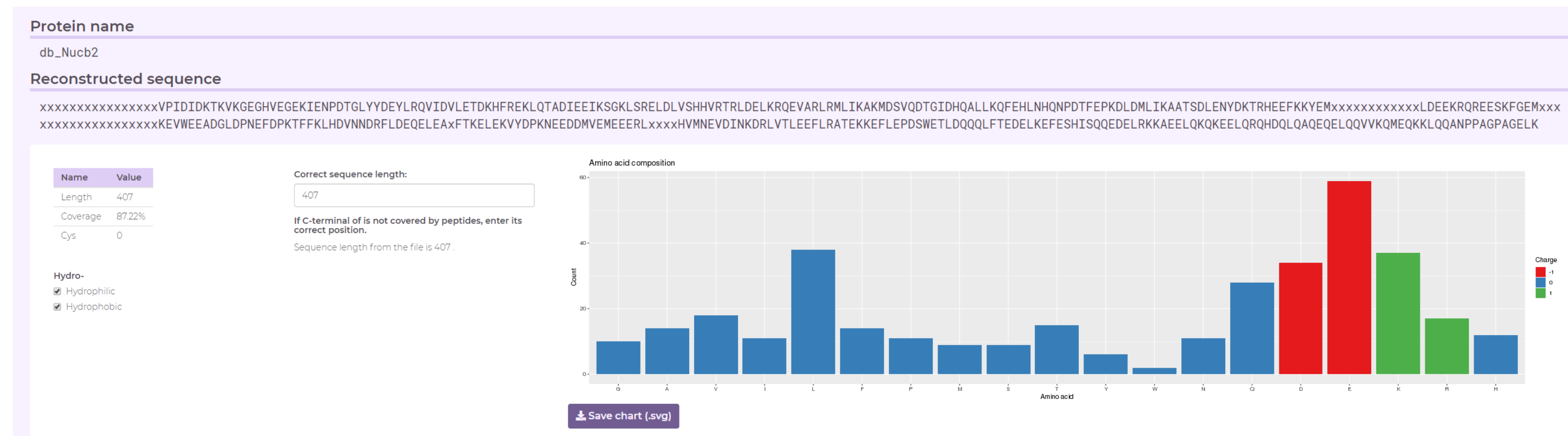


Figure 2: Sequence reconstruction and basic information

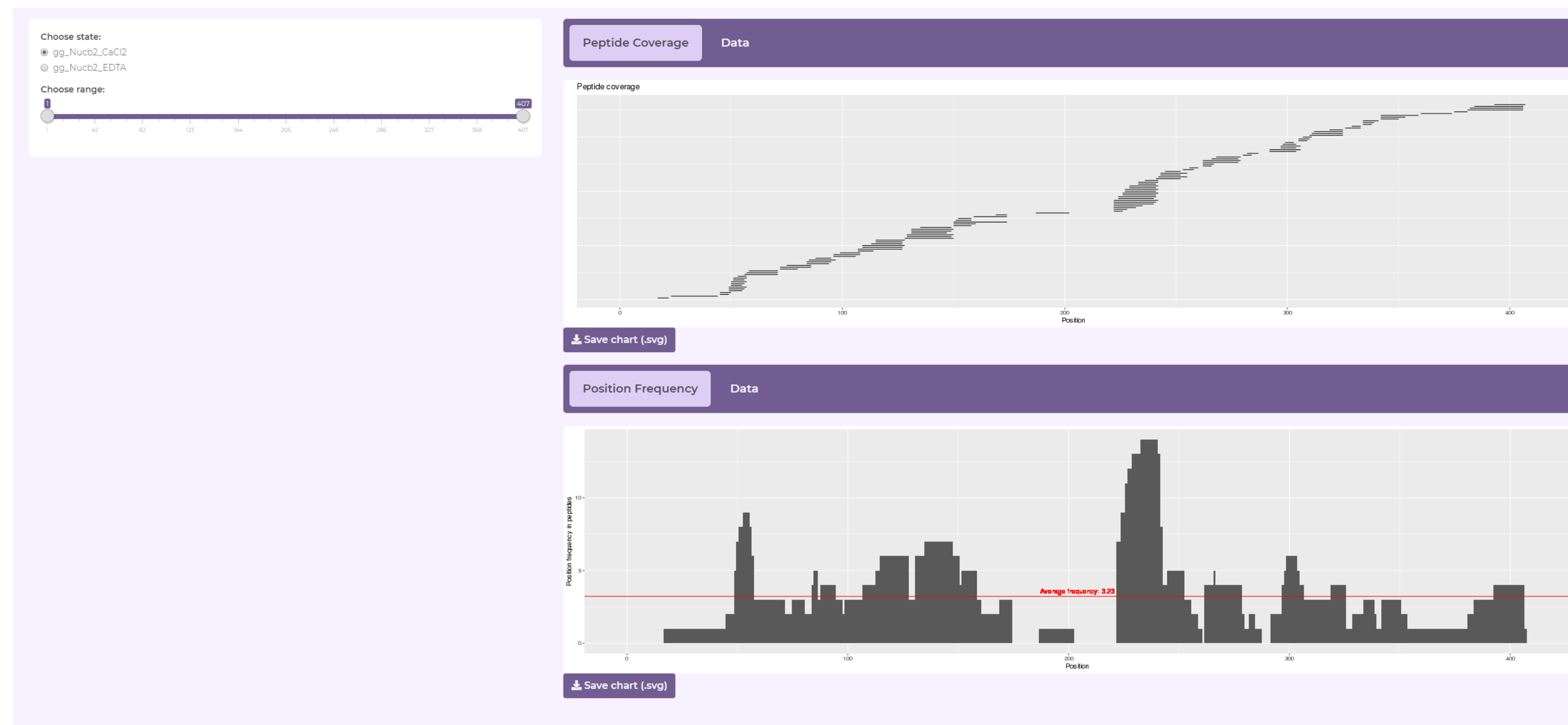


Figure 3: Sequence coverage shown in two variants

Availability

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



as the R package: <https://cran.r-project.org/web/packages/HaDeX/index.html>



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



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 equation 1 is a function of three variables (each with its own uncertainty), so there is need to use the Law of Propagation of Uncertainty, defined in equation 2:

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

Applying equation 2 to equation 1, we get deuteration level uncertainty described by equation 3:

$$u_c(D) = \sqrt{\left[\frac{1}{D_{100} - D_0} u(D_t) \right]^2 + \left[\frac{D_t - D_{100}}{(D_{100} - D_0)^2} u(D_0) \right]^2 + \left[\frac{D_0 - D_t}{(D_{100} - D_0)^2} u(D_{100}) \right]^2} \quad (3)$$

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 4.

$$diff = D_1 - D_2 \quad (4)$$

Equation 4 is a function of two variables, so applying equation 2 we get uncertainty described by equation 5.

$$u_c(diff) = \sqrt{u(D_1)^2 + u(D_2)^2} \quad (5)$$

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

Quality control

HaDeX provides additional tools for assessment of experiments. For example, the quality control function shows how the mean uncertainty per peptide changes with time points of an experiment.

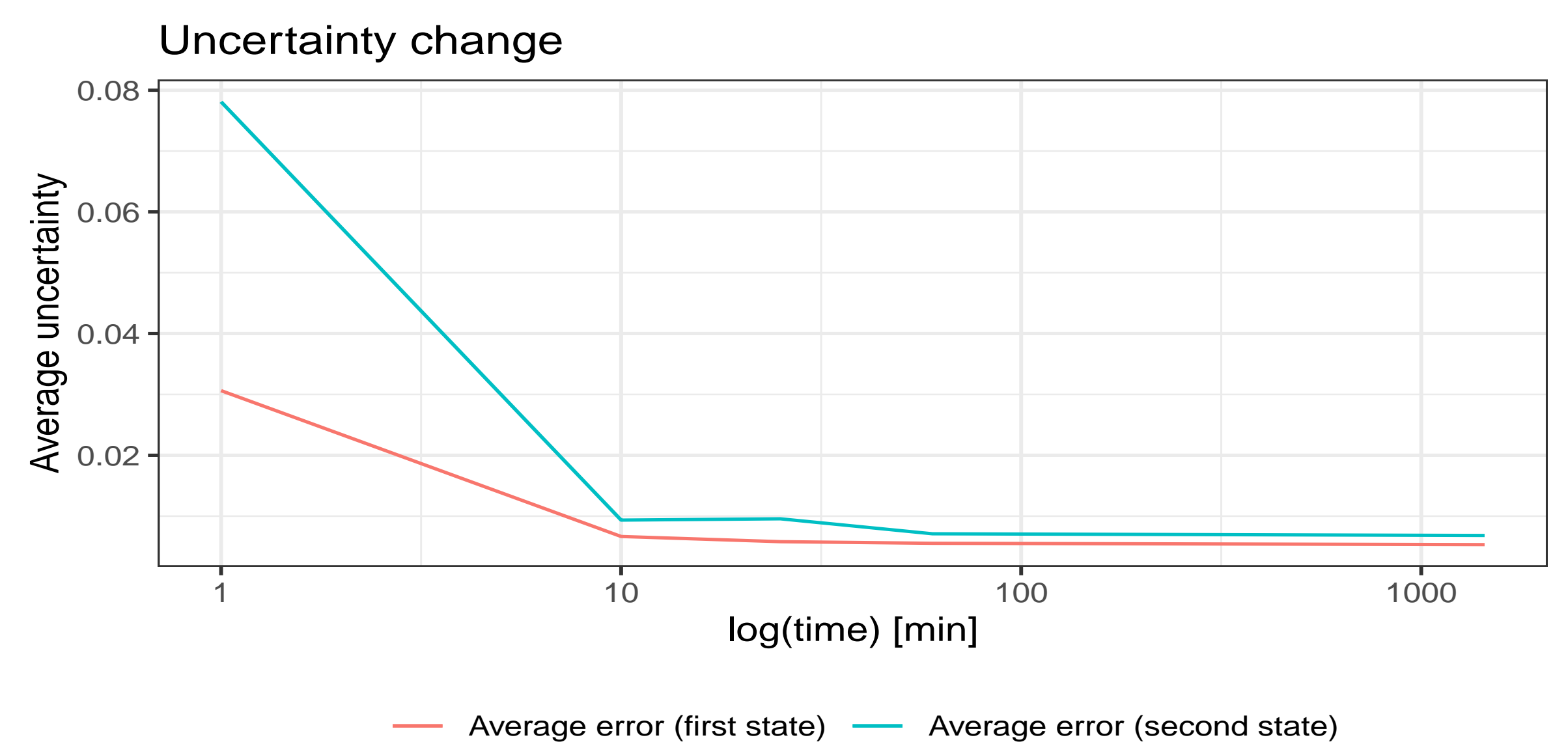


Figure 4: Plot illustrating uncertainty change depending on used out parameter

Report function

HaDeX provides report generation - with parameters chosen by user so is fully personalizable. Additionally, calculated data for plots can be downloaded not only in report form but also in csv/pdf file in panel next to every plot. On figure 5 are shown available elements of the report - that can be chosen by user and limited strictly for user needs.

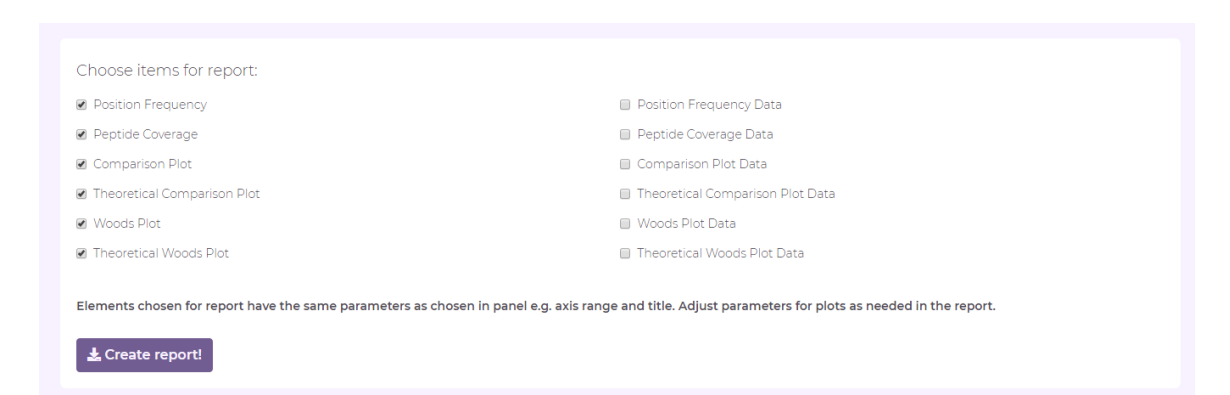


Figure 5: Screenshot of **HaDeX** - reporting page

Acknowledgements

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