Analysis of data from hydrogen-deuterium exchange mass spectometry experiments

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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.

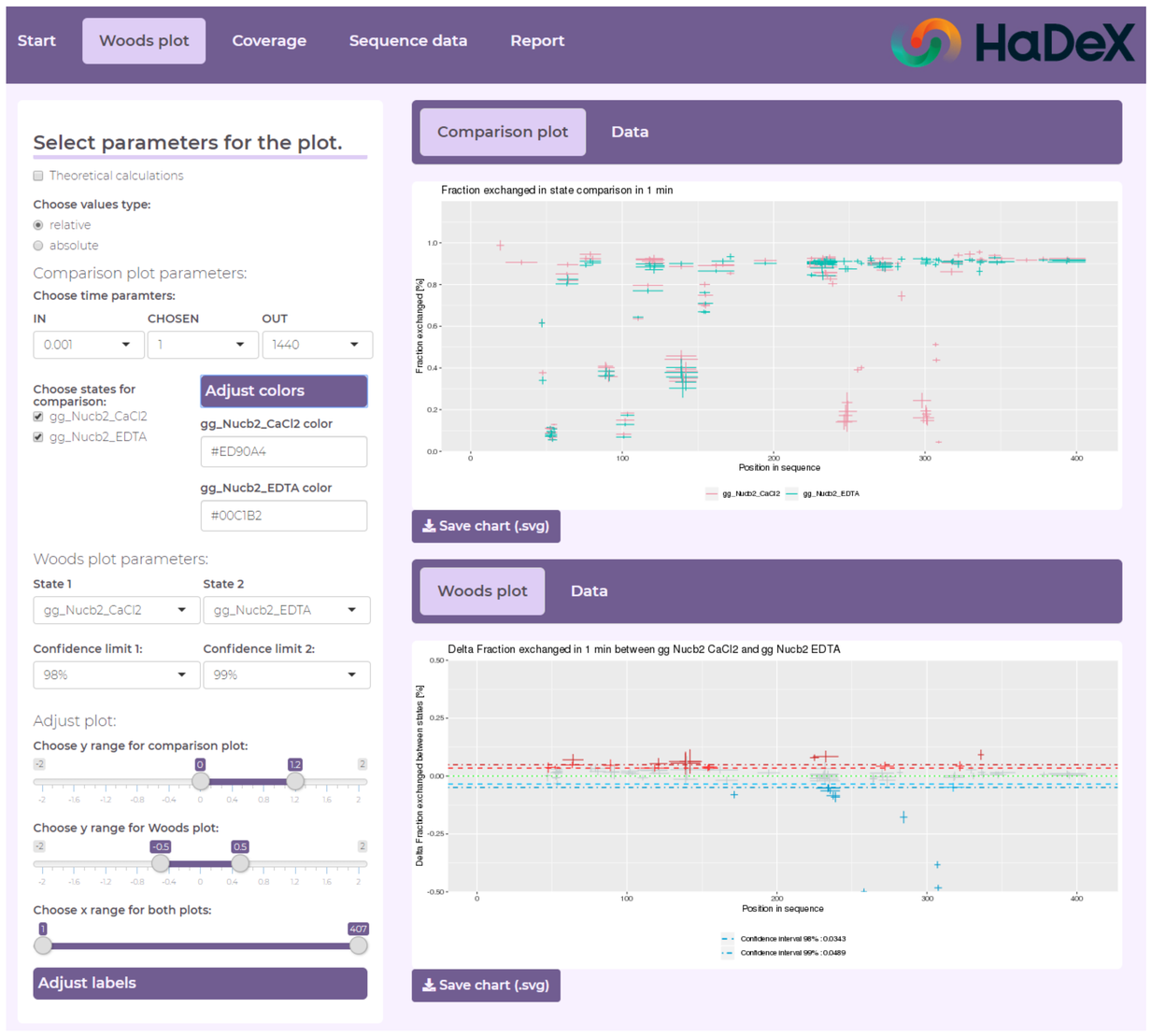

Figure 1: Overview of **HaDeX** app

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.

$$D = \frac{D_t - D_0}{D_{100} - D_0} \tag{1}$$

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

Woods Plot

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

$$diff = D_1 - D_2 \tag{2}$$

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:

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

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

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/`

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.

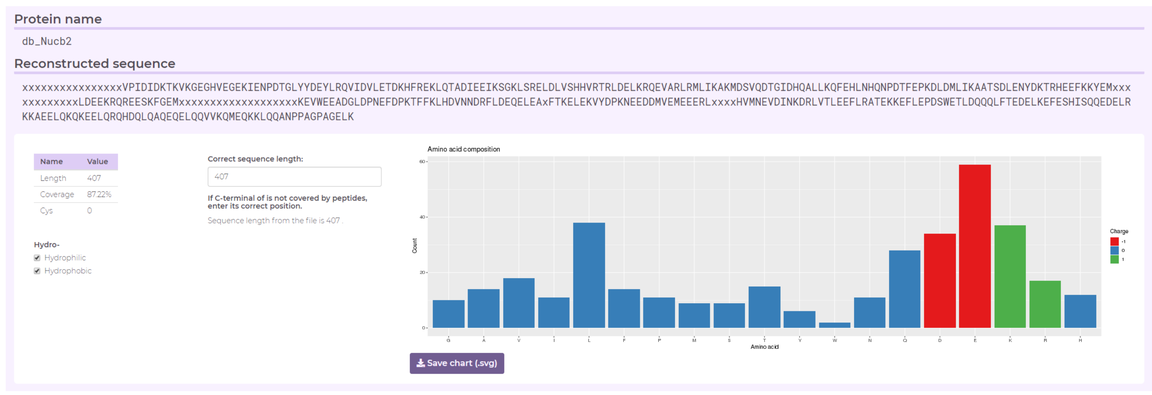

Figure 2: Sequence reconstruction and basic information

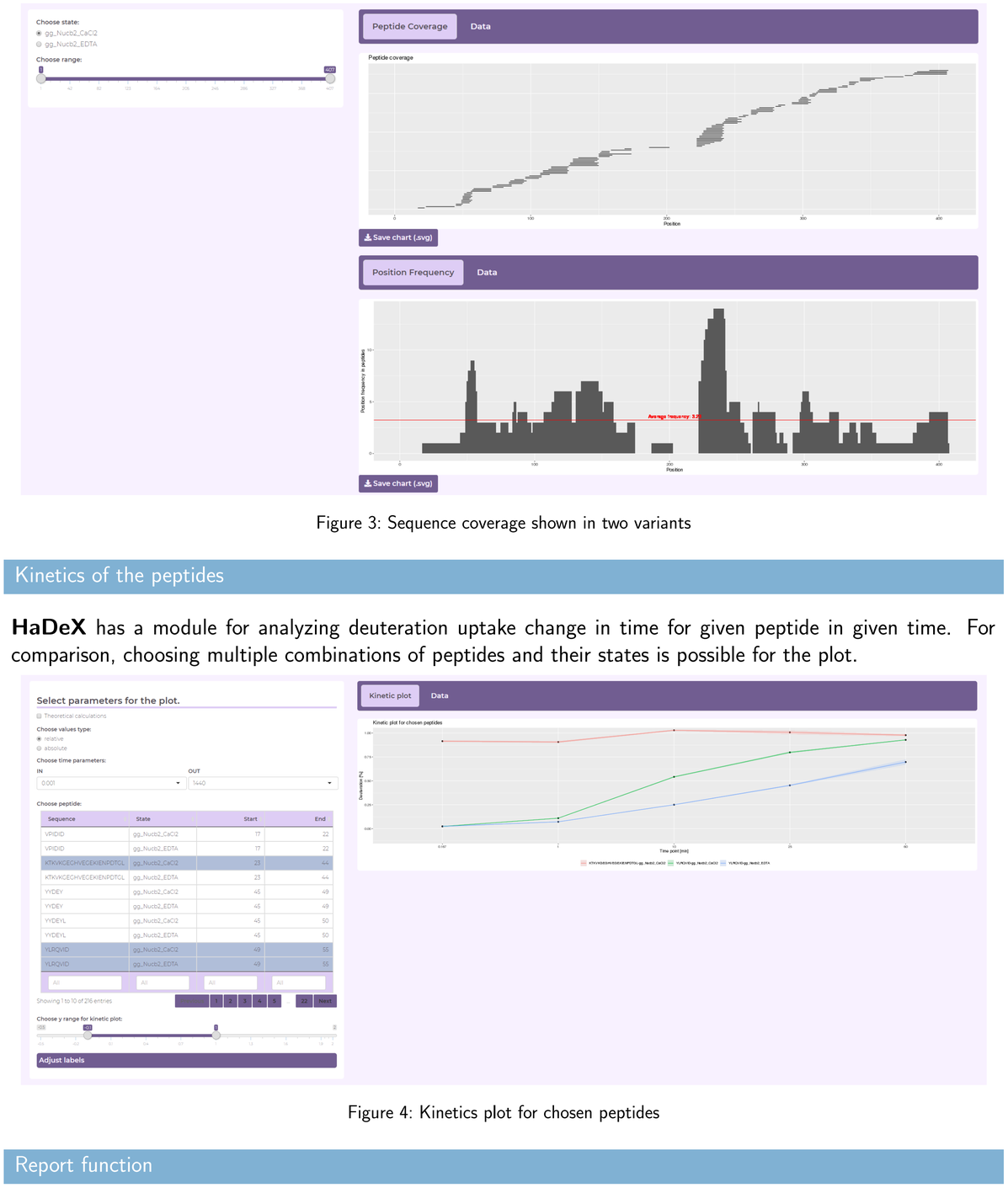

Figure 3: Sequence coverage shown in two variants

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 4: Kinetics plot for chosen peptides

Report function

HaDeX provides report generation - with parameters chosen by user so is fully personalizable. Additionaly, 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 strickly for user needs.

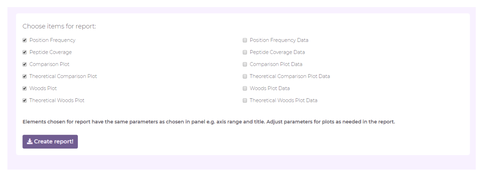

Figure 5: Screenshot of **HaDeX** - reporting page

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