

# Refinement of an algorithm for High-Resolution HDX-MS data analysis combined with HaDeX - online software for HDX data representation



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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 workflow. We propose **HaDeX**, a novel tool for processing, analysis and visualisation of HDX-MS experiments. **HaDeX** covers the whole analytic process, including preliminary data exploration, quality control and generation of publication-quality figures. The reproducibility of the whole procedure is ensured with advanced reporting functions.

## HaDeX web server overview



The HaDeX web servers has tools to view and analyze the results of HDX-MS data. (A) Comparison of different states (including protein modifications); (B) sequence coverage; (C) Woods plots.

## Differential plot

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 comparison plot 1. **HaDeX** provide both experimental and theoretical levels of deuteration.

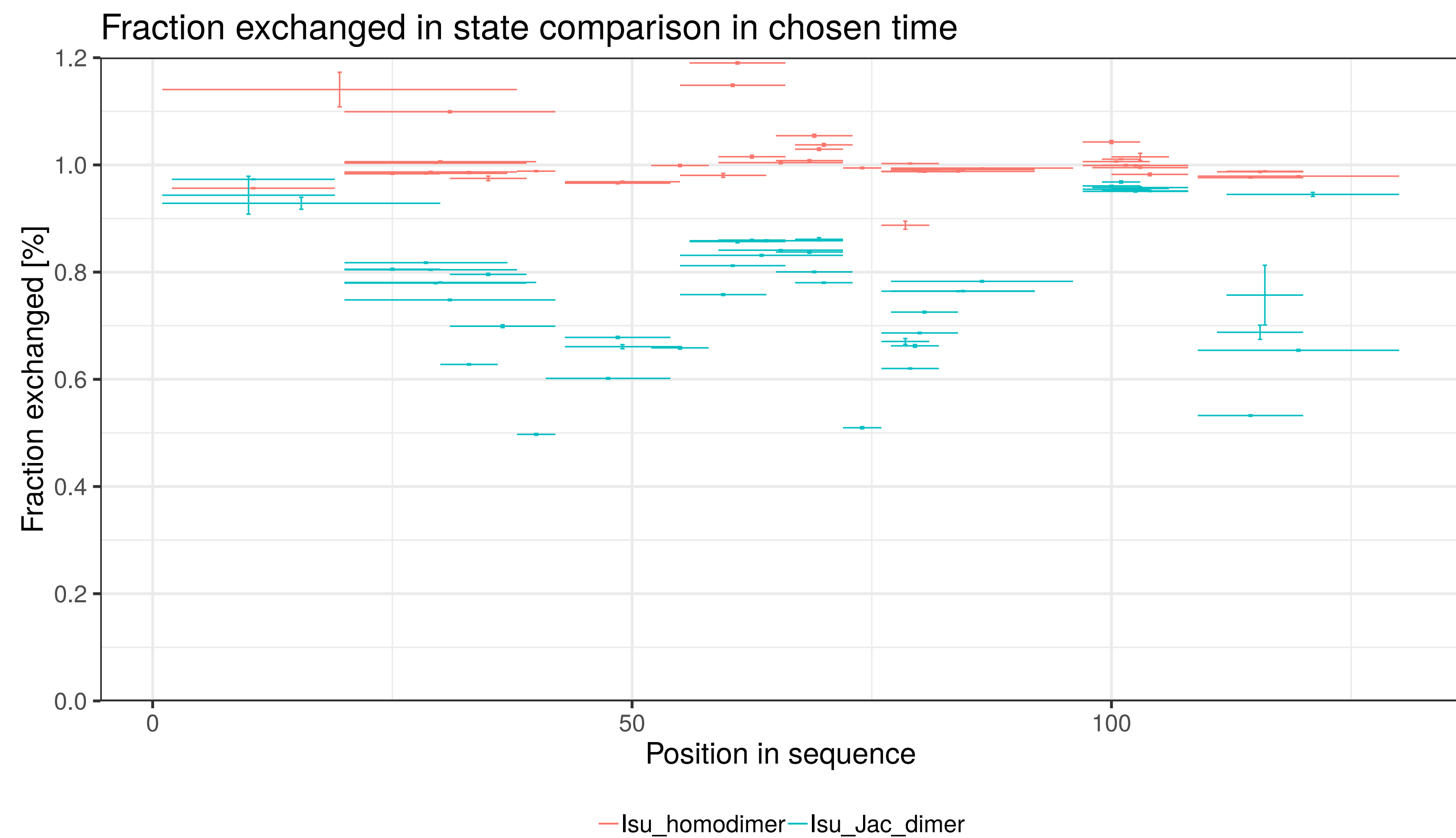


Figure 1: Comparison plots - comparing experimental and theoretical calculations, based on example data.

## Woods plot

Woods plot shows a 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 2 we get uncertainty described by equation 5.

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

Results of calculations described above are shown on the Woods below.

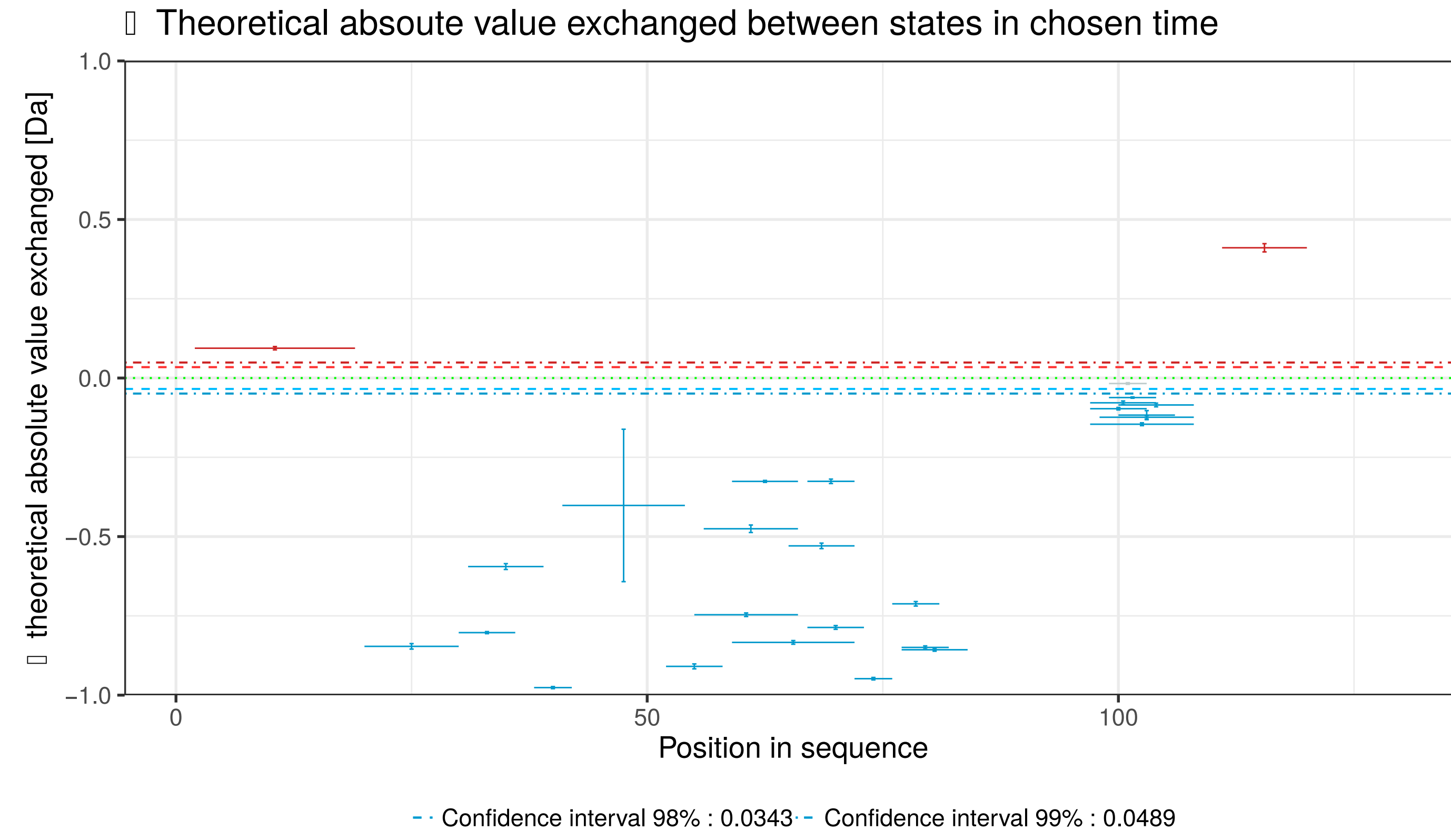


Figure 2: Plots in Woods format - comparing experimental and theoretical calculations, based on example data.

## Quality control

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

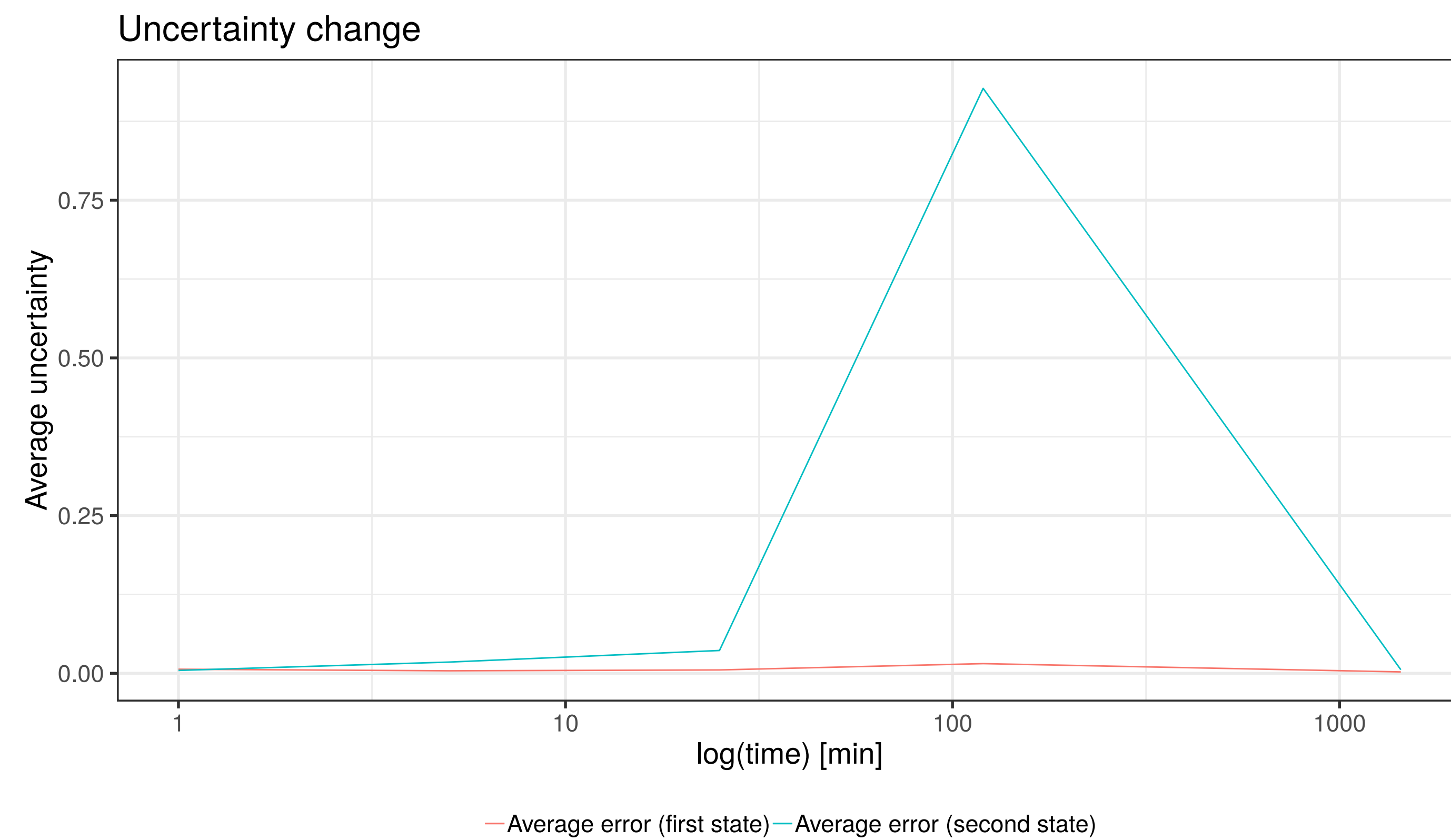


Figure 3: Plot illustrating uncertainty change depending on used out parameter

## 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 4 are shown available elements of the report - that can be chosen by user and limited strickly for user needs.

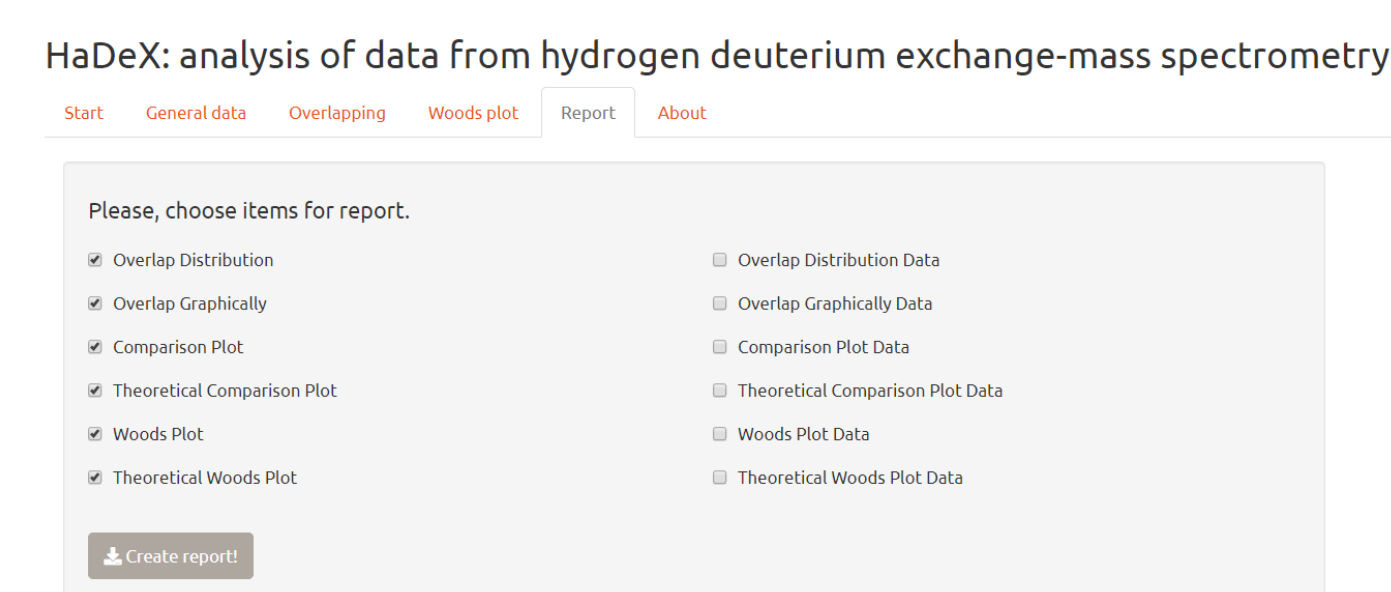


Figure 4: Screenshot of **HaDeX** - reporting page

## Availability

<http://mslab-ibb.pl/shiny/HaDeX/>



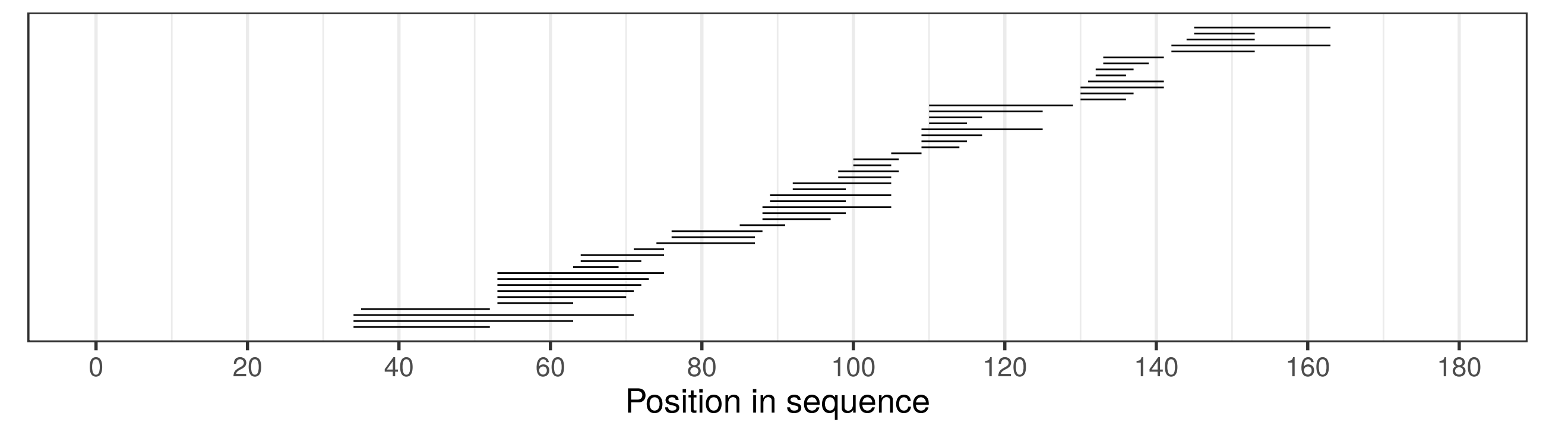
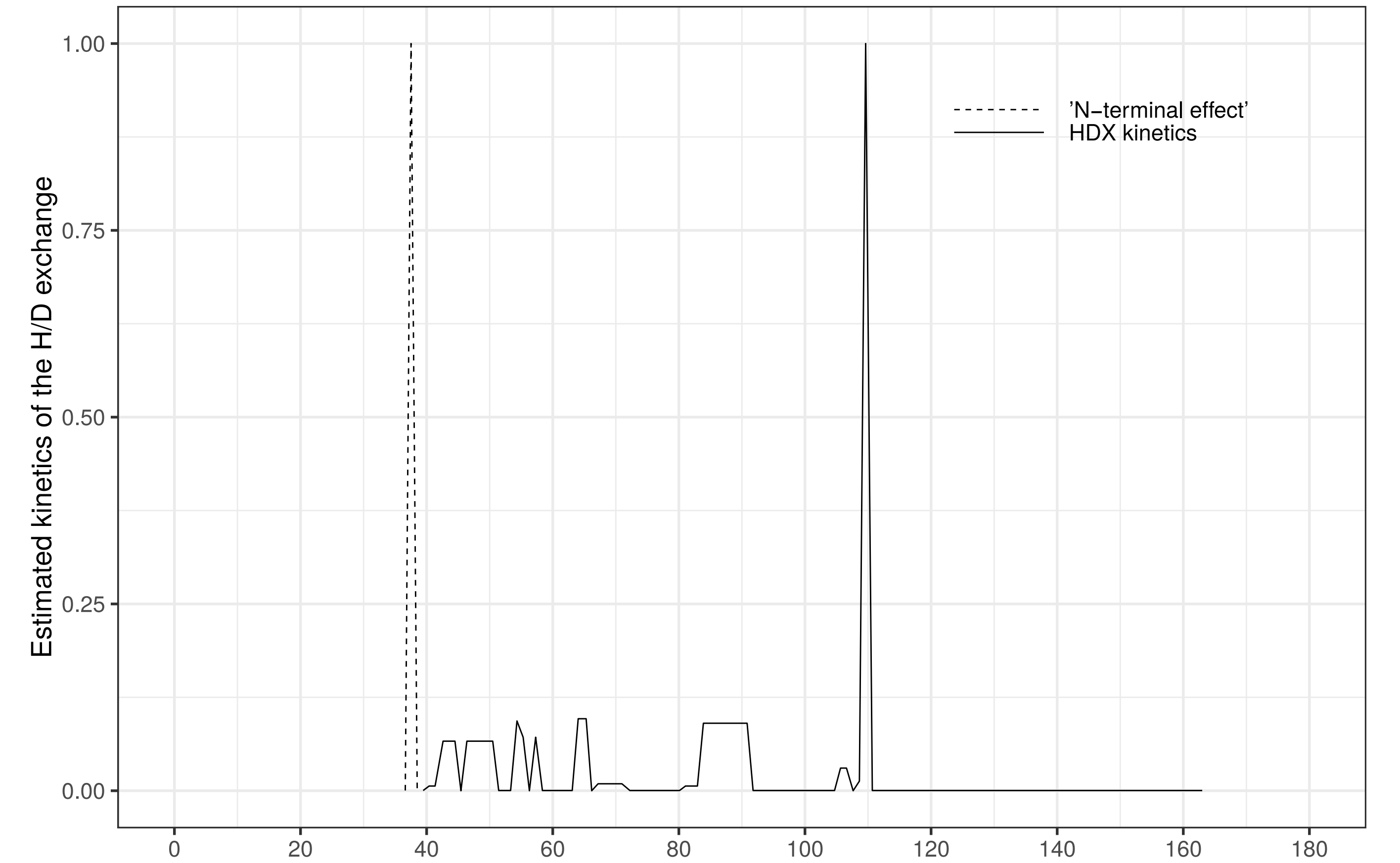
HaDeX is also available as the R package:

```
source("https://install-github.me/HaDeXversum/HaDeX")
```

## High-resolution HDX

Improvements of the algorithm proposed by Gessner et. al. (2017):

- a unified software available from the command line,
- ultimately a web server enriched with numerous data visualization possibilities,
- consistent implementation in C++,
- optimization of computational efficiency (simplification of functions, implementation in C++),
- simple parameterization in an external configuration file (specification of experimental conditions),
- experimental data in any format (the need to indicate the right columns from the input file in the configuration file),
- used solvers (linear: lsqin and nonlinear: nlssrc) are direct analogs of matlab functions, implemented in the OCTAVE library (substantive/conceptual compatibility),
- simplifying and standardizing the problem.



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