

HADEx: AN R PACKAGE AND WEB-SERVER FOR ANALYSIS OF DATA FROM HDX-MS EXPERIMENTS

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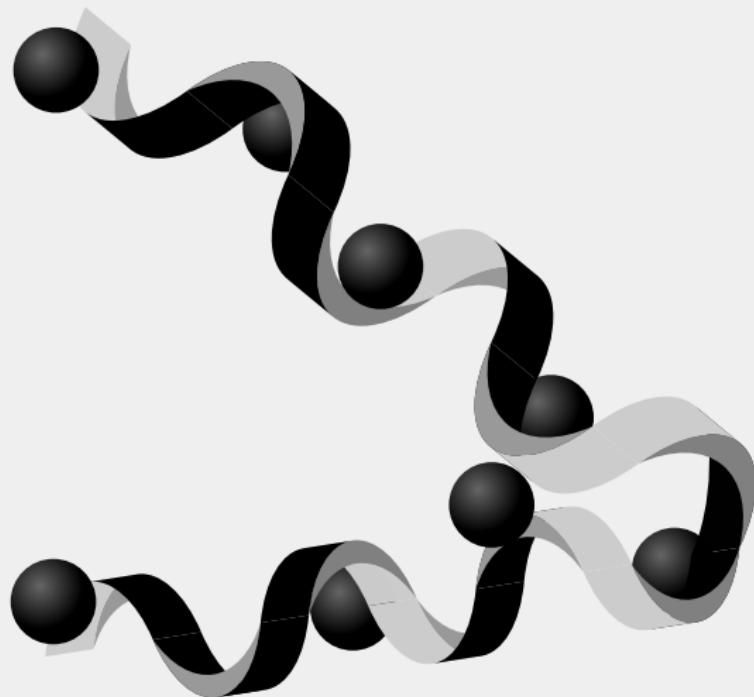
INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS, PAS
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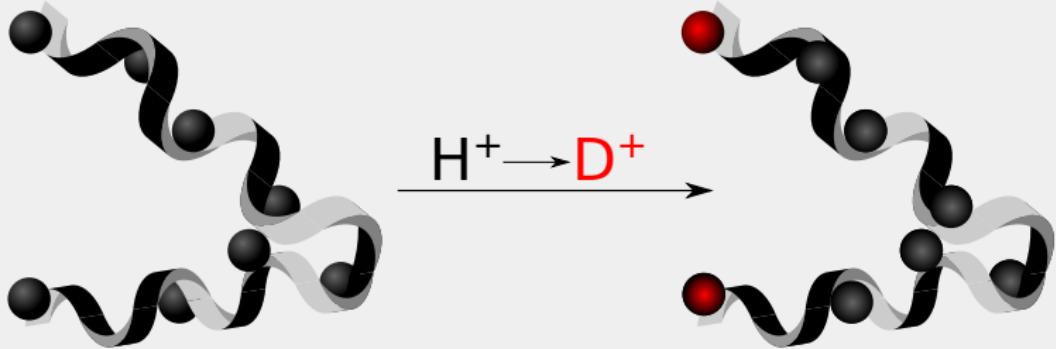
OUTLINE

1 HDX-MS

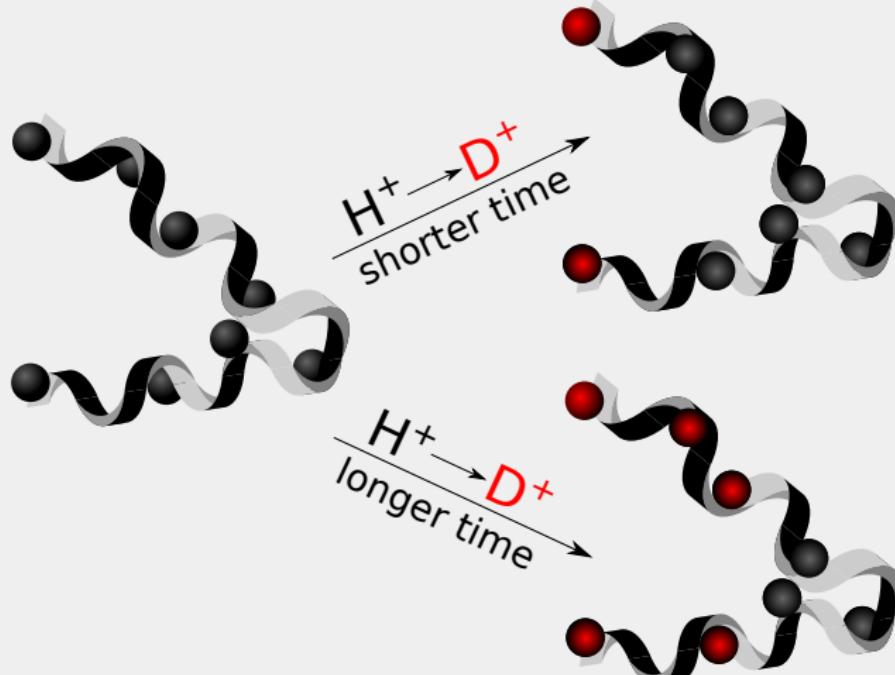
2 HaDeX workflow

HDX-MS





After the incubation in heavy water (D_2O), the most exposed amide hydrogens of the protein backbone are being replaced by deuters.



The longer incubation time, the more protected hydrogens are being replaced by deuters.

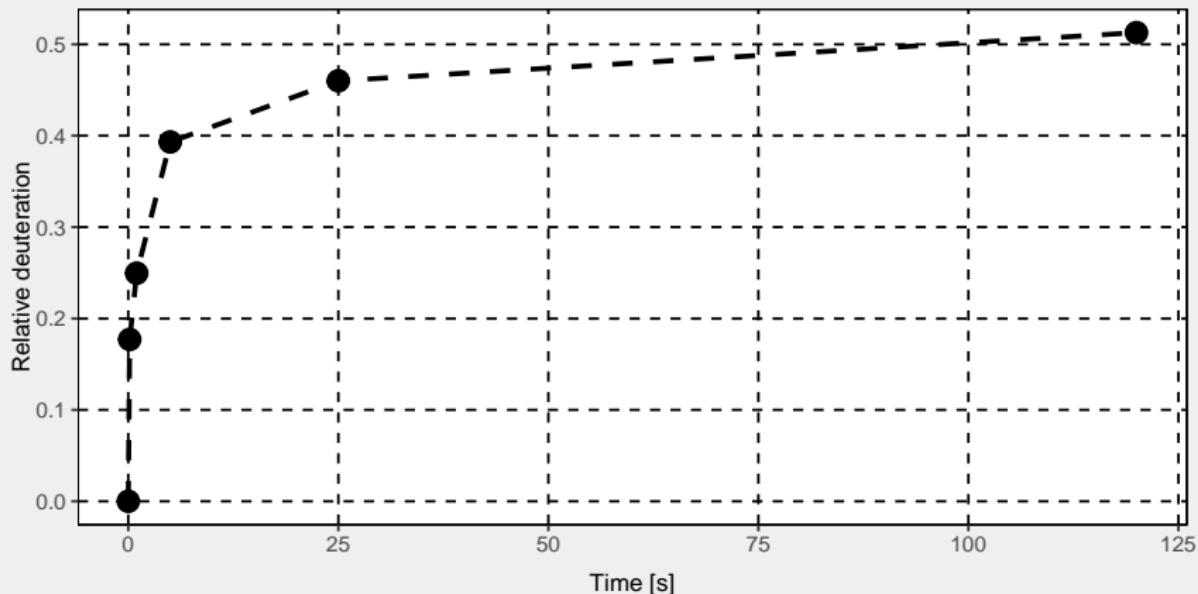
shorter incubation time



longer incubation time

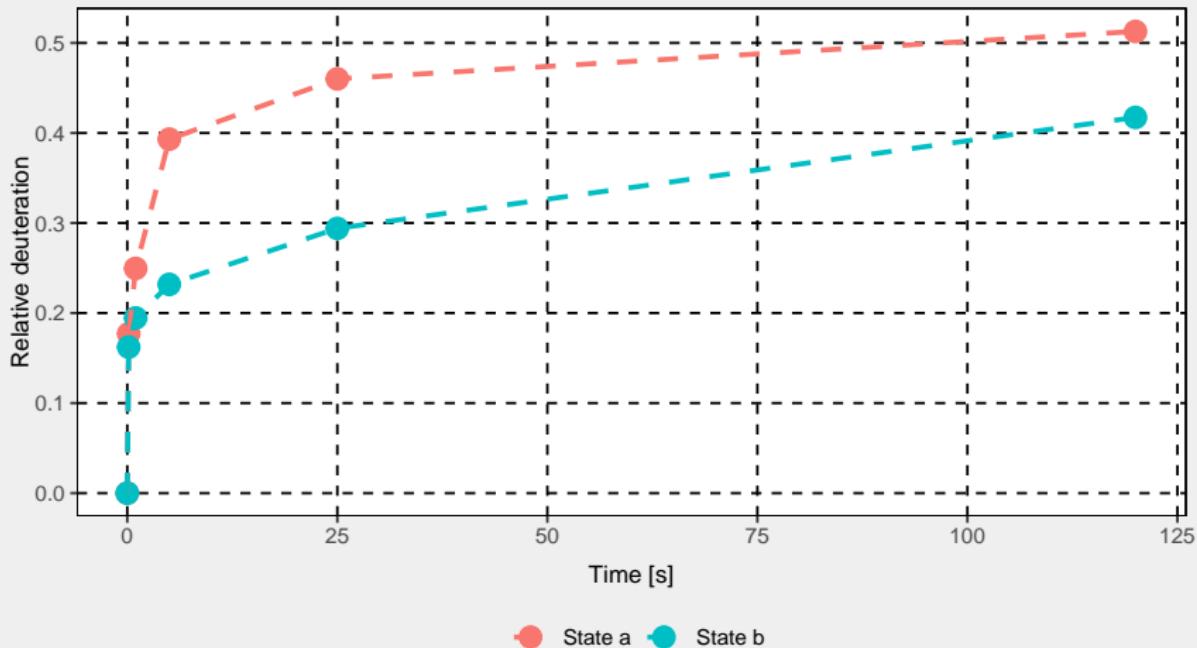


After the incubation, proteins are digested by a combination of proteases.



Thanks to the mass spectrometry, we are able to compute the m/z of each peptide depending on the duration of the incubation, thus we can compute a relative deuteration of a peptide.

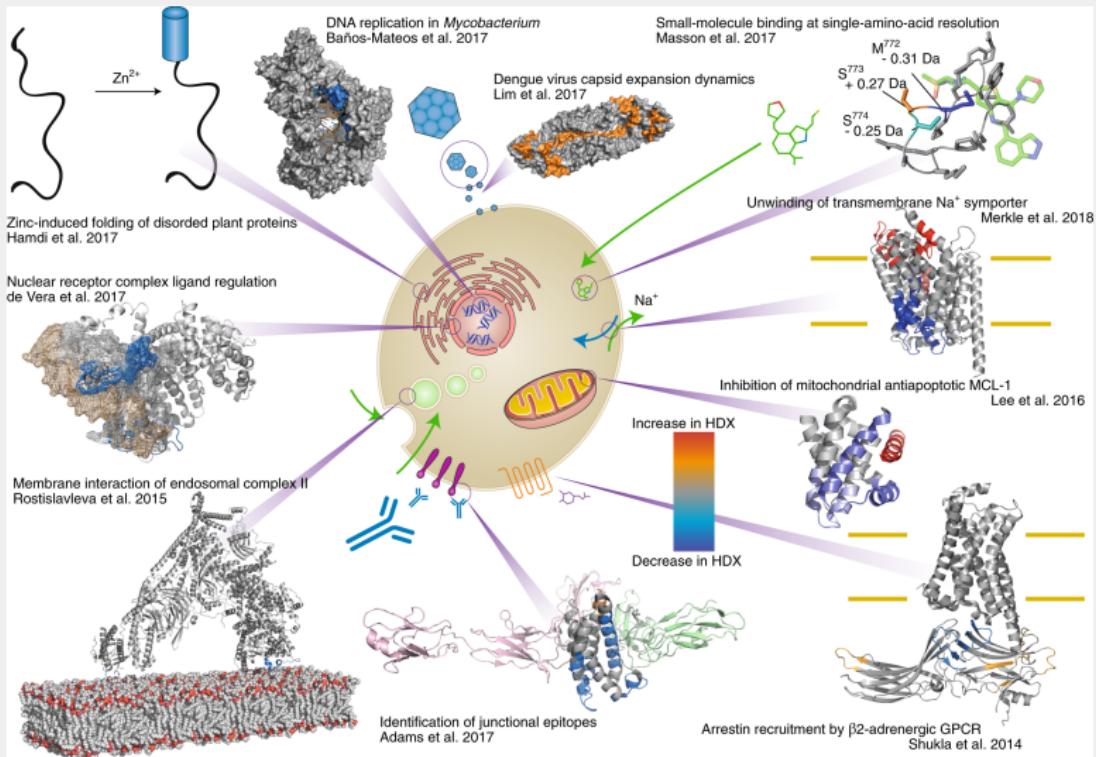
MULTI-STATE ANALYSIS



Peptides may come from proteins in different states, i.e. bounded by different cofactors.

During the analysis of the HDX-MS data we have to consider both time of the incubation and position of a peptide in a sequence.

APPLICATIONS



Source: Masson et al. (2019).

HADEx WORKFLOW

AIMS

HaDeX:

- easy,
- comprehensive,
- reproducible.

Welcome!

Upload your file. Otherwise you will see example data.

Choose file:

No file selected

File status:

Example file: KD_380110_CD160_HVEM.csv

Please be aware that loading data [including example file] may take a while. Be patient.

Currently HaDeX supports files with only one protein.

Accepted file extensions: csv, xsl, xslx.

In order for program to behave correctly, please make sure supplied file fulfills following requirements:

About

The HaDeX web server relies on functions from the [HaDeX R package](#). For more information check our [documentation](#). In case of any question or suggestion don't hesitate to contact us.

Authors

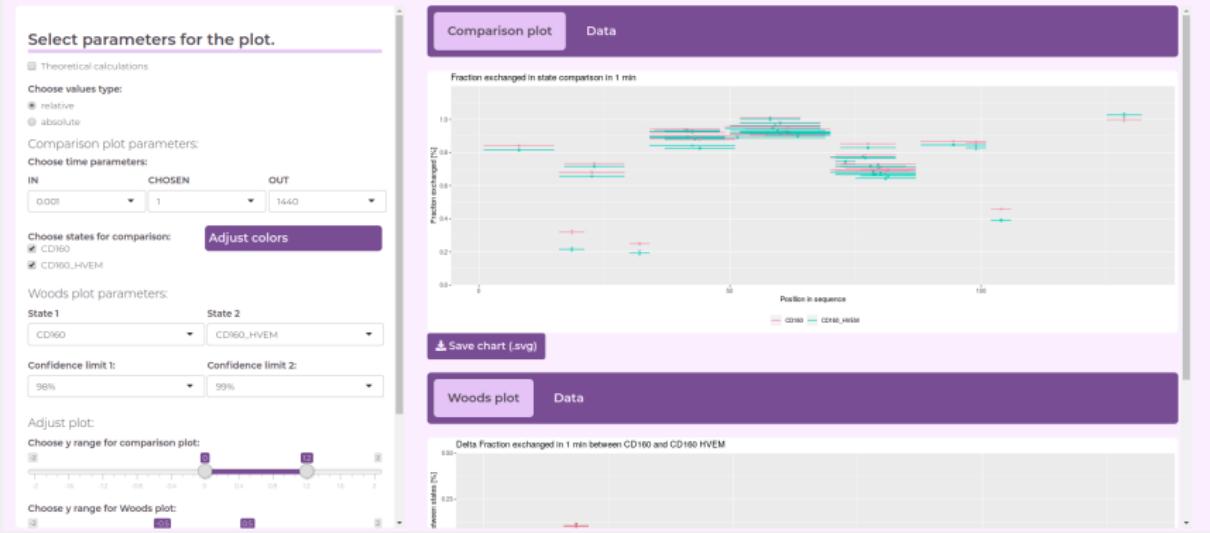
- Weronika Puchala
- Michał Burdakiewicz

Citation

Puchala W, Burdakiewicz M, Kistowski M, Dabrowska KA, Badaczewska-Dawid AE, Cysewski D and Dadlez M (2019). HaDeX: Analysis and Visualisation of Hydrogen/Deuterium Exchange Mass Spectrometry Data. R package version 1.0.

Funding

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Start Woods plot Coverage Sequence data Kinetics Summary Report

 HaDeX

Select parameters for the plot.

Theoretical calculations

Choose values type:
 relative
 absolute

Comparison plot parameters:

Choose time parameters:
 IN CHOSEN OUT
 0.001 1 1440

Choose states for comparison:
 CD160
 CD160_HVEM

Woods plot parameters:
 State 1 State 2
 CD160 CD160_HVEM

Confidence limit 1: Confidence limit 2:
 98% 99%

Adjust plot:
 Choose y range for comparison plot:


Choose y range for Woods plot:

Comparison plot Data

Sequence	State	Start	End	Frac Exch	Err Frac Exch
INTTSSASQEGTRLN	CD160	1	15	0.842	0.0041
INTTSSASQEGTRLN	CD160_HVEM	1	15	0.8154	0.0066
ICTVW	CD160	16	21	0.3199	0.0107
ICTVW	CD160_HVEM	16	21	0.2153	0.0095
ICTVWHKKEEAEG	CD160	16	29	0.6804	0.0053
ICTVWHKKEEAEG	CD160_HVEM	16	29	0.6565	0.0032
ICTVWHKKEEAEG	CD160	17	29	0.7331	0.0038
ICTVWHKKEEAEG	CD160_HVEM	17	29	0.7158	0.0046
FVFL	CD160	30	34	0.2496	0.006
FVFL	CD160_HVEM	30	34	0.1946	0.0128

All All All All All All

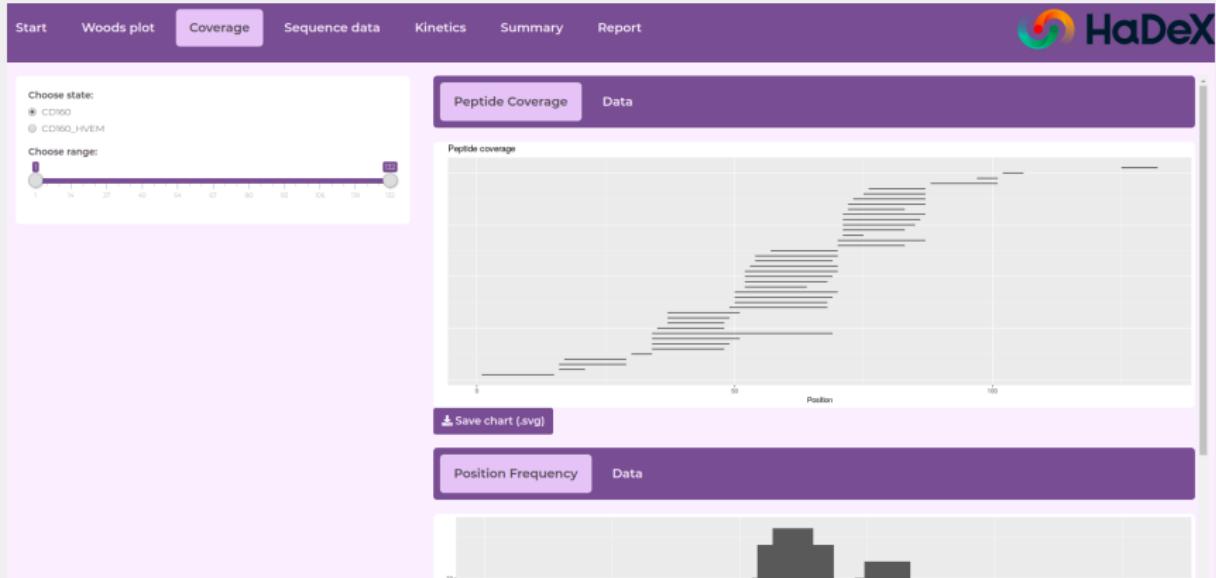
Excel PDF

Showing 1 to 10 of 82 entries

Woods plot Data

1 2 3 4 5 ... 9 Next

Uncertainties derived by error propagation (Joint Committee for Guides in Metrology, 2008).



Protein name

db_CD160

Reconstructed sequence

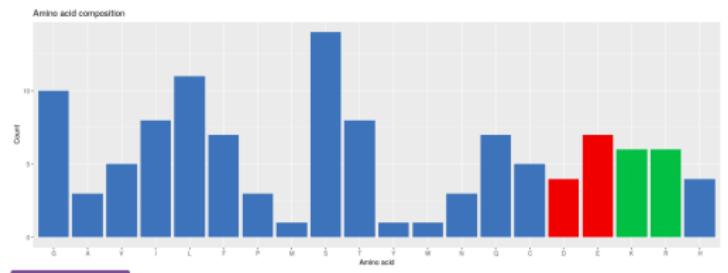
INITSSASQEGTRLNLICTVWHKKEEAEGFVVFLCKDRSGDCSPETSLKQLRLKRDPGIDGVGEISSQLMFTISQVTPLHSGTYQCCARSQKSGIRLQGHFFSILFxXXXXXXXXXXXXFSHNEGTL

Name	Value
Length	132
Coverage	86.36%
Cys	5

Correct sequence length:
132

If C-terminal is not covered by peptides, enter its correct position.
Sequence length from the file is 132.

- Hydro-
 Hydrophilic
 Hydrophobic



Select parameters for the plot.

Theoretical calculations

relative

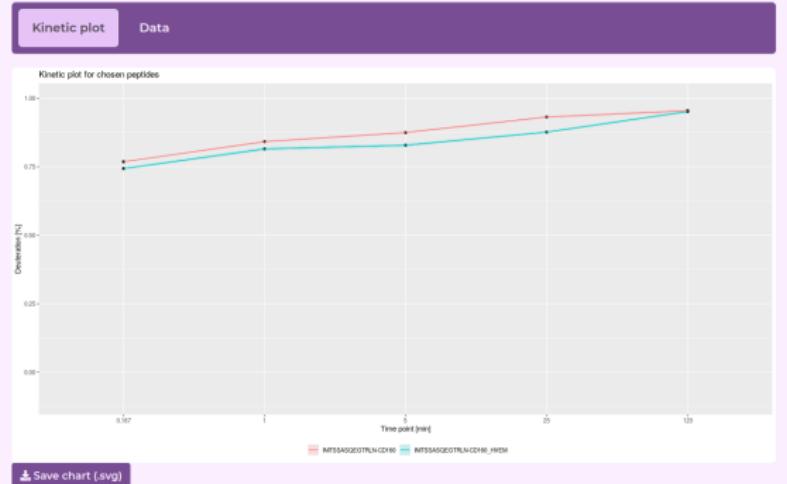
absolute

Choose time parameters:

IN	OUT
0.001	1440

Choose peptide:

Sequence	State	Start	End
INIT5SASQETRLN	CD160	1	15
INT5SASQETRLN	CD160_HVEM	1	15
ICTVW	CD160	16	21
ICTVW	CD160_HVEM	16	21
ICTVWHKKEEAEG	CD160	16	29
ICTVWHKKEEAEG	CD160_HVEM	16	29
ICTVWHKKEEAEG	CD160	17	29
ICTVWHKKEEAEG	CD160_HVEM	17	29
FVFL	CD160	30	34
FVFL	CD160_HVEM	30	34



Name	Value
HDX time course	0
Number of peptides	41
Sequence coverage	0.8636
Average peptide length	14.6829
Redundancy	4.5606
Replicates	4
Significant differences in HDX	0.98 0.99

All

All

[Excel](#) [PDF](#)

Showing 1 to 7 of 7 entries

This table was adapted from the HDX Data Summary (Masson, G.R., Burke, J.E., Ahn, N.G., Anand, G.S., Borchers, C., Brier, S., Bou-Assaf, G.M., Engen, J.R., Englander, S.W., Faber, J., et al. [2019]. Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments. Nat Methods 16, 595–602).

Choose items for report:

- | | |
|---|---|
| <input checked="" type="checkbox"/> Position Frequency | <input type="checkbox"/> Position Frequency Data |
| <input checked="" type="checkbox"/> Peptide Coverage | <input type="checkbox"/> Peptide Coverage Data |
| <input checked="" type="checkbox"/> Comparison Plot | <input type="checkbox"/> Comparison Plot Data |
| <input checked="" type="checkbox"/> Theoretical Comparison Plot | <input type="checkbox"/> Theoretical Comparison Plot Data |
| <input checked="" type="checkbox"/> Woods Plot | <input type="checkbox"/> Woods Plot Data |
| <input checked="" type="checkbox"/> Theoretical Woods Plot | <input type="checkbox"/> Theoretical Woods Plot Data |
| <input type="checkbox"/> Kinetic Plot | <input type="checkbox"/> Kinetic Plot Data |
| <input type="checkbox"/> Theoretical Kinetic Plot | <input type="checkbox"/> Theoretical Kinetic Plot Data |

Elements chosen for report have the same parameters as chosen in panel e.g. axis range and title. Adjust parameters for plots as needed in the report.

 [Create report!](#)

AVAILABILITY

- a web-server (<http://mslab-ibb.pl/shiny/HaDeX/>),
- the R package
(<https://CRAN.R-project.org/package=HaDeX>),
- a standalone software
(<https://sourceforge.net/projects/HaDeX/>).

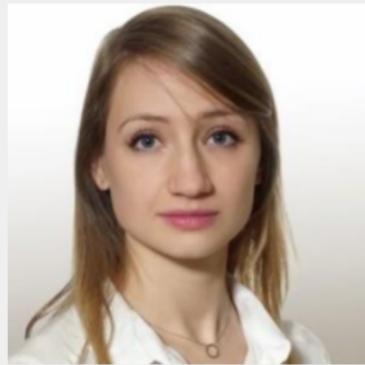
SUMMARY

- rapidly developing technologies require a flexible framework,
- the methods of data analysis should follow the development of both technology and expectations of its users.

ACKNOWLEDGEMENTS

HaDeX developers:

- Weronika Puchala (main developer),
- Dominik Rafacz (frontend developer).



ACKNOWLEDGEMENTS

- Mass Spectrometry Lab,
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and Biophysics, PAS.
- MI² Data Lab, Faculty of
Mathematics and
Information Science,
Warsaw University of
Technology.



Contact: michalburdukiewicz@gmail.com.

Presentation:

[https://github.com/HaDeXversum/Talks-and-posters.](https://github.com/HaDeXversum/Talks-and-posters)

FUNDING

- Foundation of Polish Science 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.

REFERENCES I

- Joint Committee for Guides in Metrology (2008). JCGM 100: Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement. Technical report, JCGM.
- Masson, G. R., Burke, J. E., Ahn, N. G., Anand, G. S., Borchers, C., Brier, S., Bou-Assaf, G. M., Engen, J. R., Englander, S. W., Faber, J., Garlish, R., Griffin, P. R., Gross, M. L., Guttman, M., Hamuro, Y., Heck, A. J. R., Houde, D., Iacob, R. E., Jørgensen, T. J. D., Kaltashov, I. A., Klinman, J. P., Konermann, L., Man, P., Mayne, L., Pascal, B. D., Reichmann, D., Skehel, M., Snijder, J., Strutzenberg, T. S., Underbakke, E. S., Wagner, C., Wales, T. E., Walters, B. T., Weis, D. D., Wilson, D. J., Wintrode, P. L., Zhang, Z., Zheng, J., Schriemer, D. C., and Rand, K. D. (2019). Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments. Nature Methods, 16(7):595–602.