Bioconductor packages for analysis of Thermal Proteome Profiling data

Nils Kurzawa[1,*], Dorothee Childs[1,2], Holger Franken[2], Mikhail M. Savitski[1] & Wolfgang Huber[1]

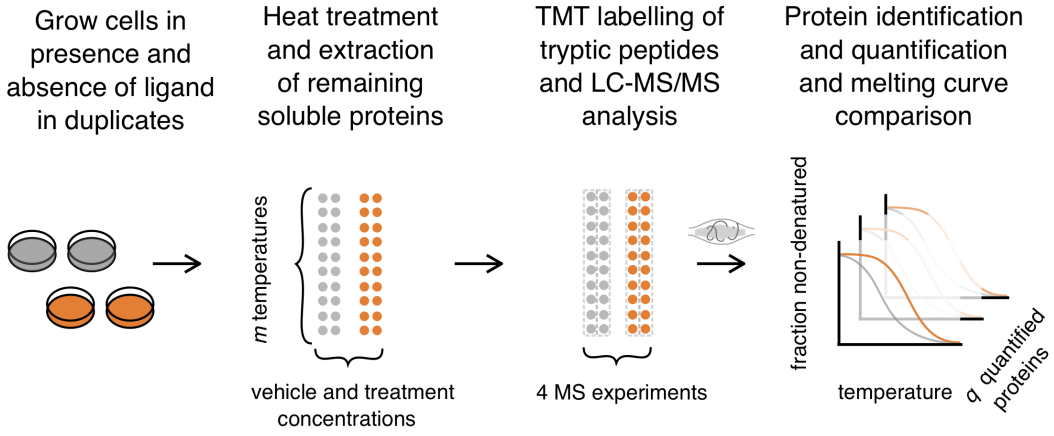
[1] *Genome Biology Unit, European Molecular Biology Laboratory*
[2] *Cellzome GmbH, GlaxoSmithKline*

[*]contact: nils.kurzawa@embl.de

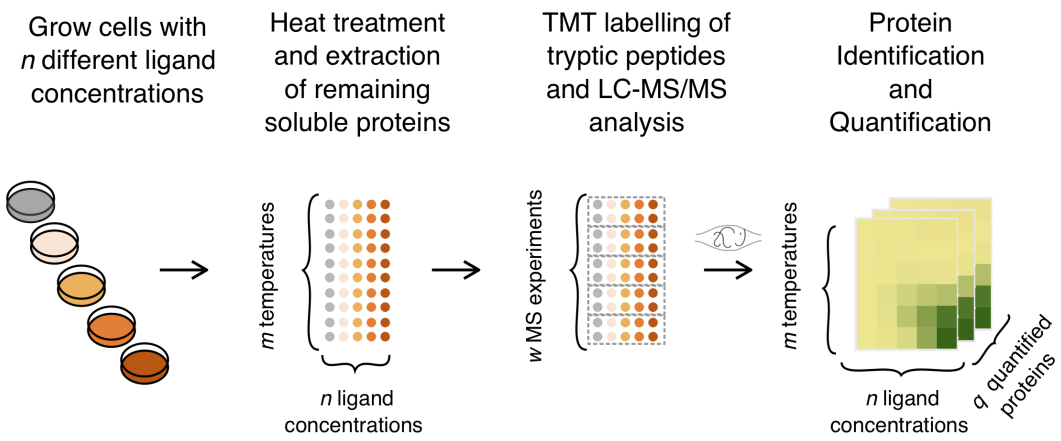
Thermal proteome profiling (TPP) is a mass spectrometry-based technology which was originally developed to identify drug (off-) targets on a proteome-wide scale. The assay is built on the principle of the cellular thermal shift assay which is that proteins inside living cells may be modulated in their thermal stability by ligand binding. This can be read out by shifts of melting profiles of respective proteins. Soon after TPP was established, a first Bioconductor package `TPP` was released, which implemented a method to infer ligand-protein interactions using shifts of melting points. The realization that this procedure lacks sensitivity to detect proteins with e.g. very high thermal stability lead to the development of the `NPARC` package. Further, it was realized that TPP data can also inform on protein-protein interaction (PPI) dynamics. Another Bioconductor package `Rtpca` was thus implemented to accomodate respective analyses. Lastly, the experimental setup of TPP experiments was expanded to profile ligand dose and temperature ranges together to improve sensitivity. To accomodate the different data structure and different required statistical analysis the package `TPP2D` was created. Here, the different functionalities of these packages are presented.

Thermal Proteome Profiling (TPP)

TPP over a temperature range (TPP-TR) [1]

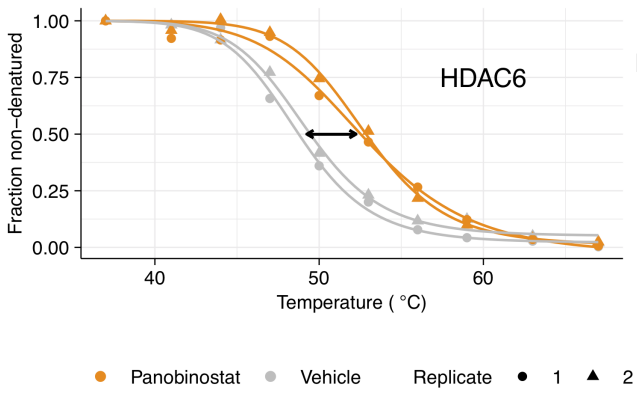


Ligand dose and temperature range TPP (2D-TPP) [2]



TPP-TR analysis with `TPP` [3]

Melting point-centric data analysis.

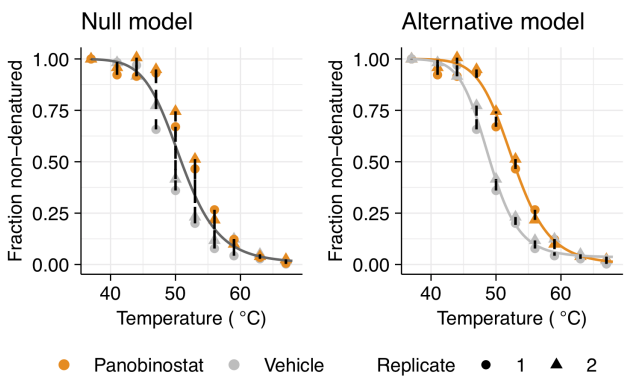


Features of the `TPP` package:

- TPP-TR data import into ExpressionSet structure
- data normalization and melting curve fitting, detection of melting point shifts
- analysis of isothermal concentartion range TPP experiments

TPP-TR analysis with `NPARC` [4]

Nonparametric analysis of response curves comparing exploiting all measurements.

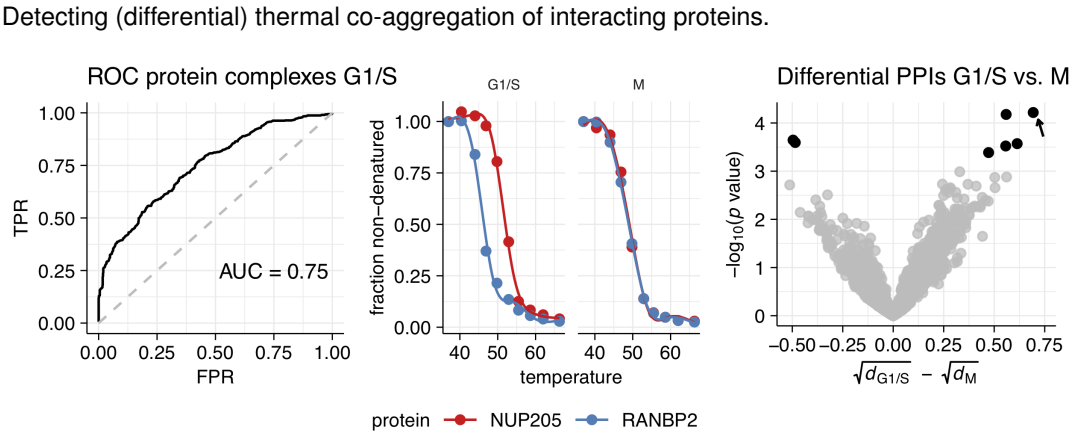


Features of the `NPARC` package:

- light-weight package for performing sensitive differential melting curve analysis
- nested model comparison: one sigmoidal across conditions vs. sigmoidals per condition
- F-statistic based on explained variance of each model
- method for calibration of retrieved F-statistics

Detecting differential PPIs with `Rtpca` [5]

Detecting (differential) thermal co-aggregation of interacting proteins.

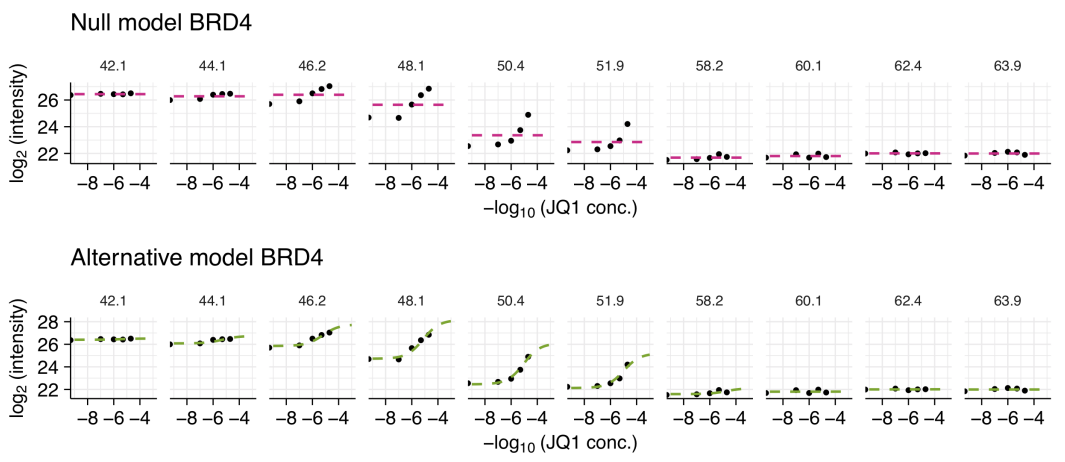


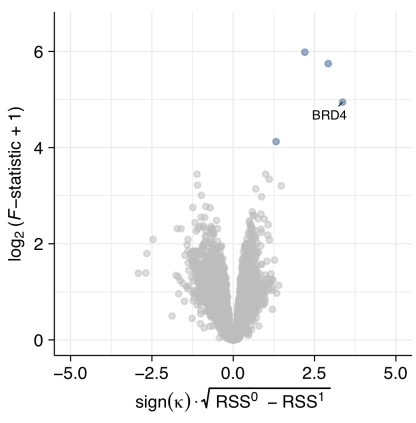
Features of the `Rtpca` package:

- test for co-aggregation of annotated protein-protein interactors (PPI), compute receiver operating characteristics (ROC)
- perform differential testing to find changes in PPIs between different conditions

2D-TPP data analysis with `TPP2D` [6]

FDR-controlled detection of ligand-protein interactions from 2D thermal profiles.





Features of the `TPP2D` package:

- hypothesis test on curves: intercept model vs. dose-response model contrained across temperatures
- bootstrapping approach to calibrate F-statistic in terms of false discovery-rate (FDR)
- several functions for data visualization

References

[1] M. M. Savitski, F. B. M. Reinhard, H. Franken, T. Werner, M. F. Savitski, D. Eberhard, D. Martinez Molina, R. Jafari, R. B. Dovega, S. Klaeger, B. Kuster, P. Nordlund, M. Bantscheff, and G. Drewes, "Tracking cancer drugs in living cells by thermal profiling of the proteome.," *Science*, vol. 346, no. 6205, p. 1255784, 2014.

[2] I. Becher, T. Werner, C. Doce, E. A. Zaal, I. Tögel, C. A. Khan, A. Rueger, M. Muelbaier, E. Salzer, C. R. Berkers, P. F. Fitzpatrick, M. Bantscheff, and M. M. Savitski, "Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat.," *Nat Chem Biol*, vol. 12, no. 11, pp. 908–910, 2016.

[3] H. Franken, T. Mathieson, D. Childs, G. M. A. Sweetman, T. Werner, I. Tögel, C. Doce, S. Gade, M. Bantscheff, G. Drewes, F. B. M. Reinhard, W. Huber, and M. M. Savitski, "Thermal proteome profiling for unbiased identification of direct and indirect drug targets using multiplexed quantitative mass spectrometry.," *Nat Protoc*, vol. 10, no. 10, pp. 1567–1593, 2015.

[4] D. Childs, K. Bach, H. Franken, S. Anders, N. Kurzawa, M. Bantscheff, M. Savitski, and W. Huber, "Non-parametric analysis of thermal proteome profiles reveals novel drug-binding proteins.," *Mol Cell Proteomics*, 2019.

[5] N. Kurzawa, A. Mateus, and M. M. Savitski, "Rtpca: an r package for differential thermal proximity coaggregation analysis.," *Bioinformatics*, 2020.

[6] N. Kurzawa, I. Becher, S. Sridharan, H. Franken, A. Mateus, S. Anders, M. Bantscheff, W. Huber, and M. M. Savitski, "A computational method for detection of ligand-binding proteins from dose range thermal proteome profiles.," *Nat Commun*, vol. 11, no. 1, p. 5783, 2020.

This work was funded by EMBL and GSK. The poster is available at https://github.com/nkurzaw/EuroBioc2020Poster.