

# Package ‘TPP’

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**Type** Package

**Title** Analyze thermal proteome profiling (TPP) experiments

**Version** 3.35.0

**Author** Dorothee Childs, Nils Kurzawa, Holger Franken, Carola Doce, Mikhail Savitski and Wolfgang Huber

**Maintainer** Dorothee Childs <d.childs@posteo.de>

**Depends** R (>= 3.4), Biobase, dplyr, magrittr, tidyr

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**Suggests** BiocStyle, testthat

**Description** Analyze thermal proteome profiling (TPP) experiments with varying temperatures (TR) or compound concentrations (CCR).

**License** Artistic-2.0

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**biocViews** ImmunoOncology, Proteomics, MassSpectrometry

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analyze2DTPP	<i>Analyze a 2D-TPP experiment</i>
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## Description

Performs the whole analysis workflow for 2D-TPP experiment by invoking routines for data import, data processing, fold change computation, median normalization, TPP-CCR curve fitting, plotting and production of the result table.

## Usage

```
analyze2DTPP(
  configTable,
  data = NULL,
  resultPath = NULL,
  idVar = "gene_name",
  fcStr = NULL,
  intensityStr = "signal_sum_",
  naStrs = c("NA", "n/d", "NaN", "<NA>"),
  methods = "doseResponse",
  qualColName = "qupm",
  compFc = TRUE,
  normalize = TRUE,
  addCol = NULL,
  nCores = 1,
  nonZeroCols = "qssm",
  fcTolerance = 0.1,
  r2Cutoff = 0.8,
  fcCutoff = 1.5,
  slopeBounds = c(1, 50),
  fractAbund = FALSE,
  xlsExport = TRUE,
  plotAll = FALSE,
  plotAllR2 = FALSE,
  plotSingle = FALSE,
  trRef = NULL,
  refFcStr = "norm_rel_fc_",
  addInfo = FALSE,
  createReport = "none",
  paletteName = "Spectral",
```

```
    configFile
  )
```

### Arguments

configTable	dataframe, or character object with the path to a file, that specifies important details of the 2D-TPP experiment. See Section details for instructions how to create this object.
data	single dataframe, containing fold change measurements and additional annotation columns to be imported. Can be used instead of specifying the file path in the configTable argument.
resultPath	location where to store dose-response curve plots and results table.
idVar	character string indicating which data column provides the unique identifiers for each protein.
fcStr	character string indicating which columns contain the actual fold change values. Those column names containing the prefix fcStr will be regarded as containing fold change values. Only relevant if compFC = FALSE.
intensityStr	character string indicating which columns contain the actual sumionarea values. Those column names containing the prefix intensityStr will be regarded as containing sumionarea values.
naStrs	character vector indicating missing values in the data table. When reading data from file, this value will be passed on to the argument na.strings in function read.delim.
methods	vector of character strings that indicate which methods should be used for the analysis (default: c("doseResponse"), alternative: c("splineFit") or c("doseResponse", "splineFit"))
qualColName	character string indicating which column can be used for additional quality criteria when deciding between different non-unique protein identifiers.
compFc	boolean flag which indicates whether to perform fold change computation regarding reference column from sumionareas (default: TRUE)
normalize	perform median normalization (default: TRUE).
addCol	character vector indicating which additional columns to include from the input data
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
nonZeroCols	character string indicating a column that will be used for filtering out zero values.
fcTolerance	tolerance for the fcCutoff parameter. See details.
r2Cutoff	Quality criterion on dose response curve fit.
fcCutoff	Cutoff for highest compound concentration fold change.
slopeBounds	Bounds on the slope parameter for dose response curve fitting.
fractAbund	boolean variable, if set to TRUE additional information concerning sumionarea fractional abundance and dmsol vs. dmsol2 of adjacent temperatures is added to the output table

<code>xlsxExport</code>	produce results table in xlsx format and store at the location specified by the <code>resultPath</code> argument.
<code>plotAll</code>	boolean value indicating whether all dose response curves should be generated. Deactivating plotting decreases runtime.
<code>plotAllR2</code>	boolean value indicating whether all dose response curves which fulfill the demanded criteria (Rsquared, maximum plateau) should be generated. Deactivating plotting decreases runtime.
<code>plotSingle</code>	boolean value indicating whether all dose response curves which fulfill the demanded criteria (Rsquared, maximum plateau) should be generated. Deactivating plotting decreases runtime.
<code>trRef</code>	character string containing a valid system path to a previously generated TPP-TR reference object
<code>refFcStr</code>	character string indicating which columns in the reference data set contain the fold change values
<code>addInfo</code>	boolean variable, if set to TRUE additional information on counts of stabilization and destabilization of each protein is added to the output table
<code>createReport</code>	character string indicating whether a markdown report should be created and which format it have (default: "html_document", alternative: "pdf_document" or "none")
<code>paletteName</code>	color palette (see details).
<code>configFile</code>	DEPRECATED

## Details

Invokes the following steps:

1. Import data using the `tpp2dImport` function.
2. Remove zero sumionarea values.
3. Compute fold changes from raw data (sumionarea)
4. Perform normalization by fold change medians (optional) using the `tpp2dNormalize` function. To perform normalization, set argument `normalize=TRUE`.

`paletteName` specifies the color palette to be used by the `brewer.pal` function from the `RColorBrewer` package to assign a separate color to each concentration.

## Value

A data frame in which the model results (slopes and pEC50 values) are stored row-wise for each protein and administered temperatures.

## References

Becher, I., Werner, T., Doce, C., Zaal, E. A., Berkers, C. R., T"ogel, I., Salzer, E., Bantscheff, M., Savitski, M. M. (2016) Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat. *Nature Chemical Biology*, 12(11), 908-910.

**Examples**

```

data(panobinostat_2DTPP_smallExample)
config_tpp2d <- panobinostat_2DTPP_config
data_tpp2d <- panobinostat_2DTPP_data
tpp2dResults <- analyze2DTPP(configTable = config_tpp2d,
                             data = data_tpp2d,
                             methods=c("doseResponse"),
                             createReport="none",
                             nCores=1,
                             idVar = "representative",
                             addCol = "clustname",
                             intensityStr = "sumionarea_protein_",
                             nonZeroCols = "qusm")

```

---

analyzeTPPCCR

*Analyze TPP-CCR experiment*


---

**Description**

Performs analysis of a TPP-CCR experiment by invoking routines for data import, data processing, normalization, curve fitting, and production of the result table.

**Usage**

```

analyzeTPPCCR(
  configTable,
  data = NULL,
  resultPath = NULL,
  idVar = "gene_name",
  fcStr = "rel_fc_",
  naStrs = c("NA", "n/d", "NaN", "<NA>"),
  qualColName = "qupm",
  normalize = TRUE,
  ggplotTheme = tppDefaultTheme(),
  nCores = "max",
  nonZeroCols = "qssm",
  r2Cutoff = 0.8,
  fcCutoff = 1.5,
  slopeBounds = c(1, 50),
  plotCurves = TRUE,
  verbose = FALSE,
  xlsxExport = TRUE,
  fcTolerance = 0.1
)

```

## Arguments

configTable	dataframe, or character object with the path to a file, that specifies important details of the TPP-CCR experiment. See Section details for instructions how to create this object.
data	single dataframe, containing fold change measurements and additional annotation columns to be imported. Can be used instead of specifying the file path in the configTable argument.
resultPath	location where to store dose-response curve plots and results table.
idVar	character string indicating which data column provides the unique identifiers for each protein.
fcStr	character string indicating which columns contain the actual fold change values. Those column names containing the suffix fcStr will be regarded as containing fold change values.
naStrs	character vector indicating missing values in the data table. When reading data from file, this value will be passed on to the argument na.strings in function read.delim.
qualColName	character string indicating which column can be used for additional quality criteria when deciding between different non-unique protein identifiers.
normalize	perform median normalization (default: TRUE).
ggplotTheme	ggplot theme for dose response curve plots.
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
nonZeroCols	character string indicating a column that will be used for filtering out zero values.
r2Cutoff	Quality criterion on dose response curve fit.
fcCutoff	Cutoff for highest compound concentration fold change.
slopeBounds	Bounds on the slope parameter for dose response curve fitting.
plotCurves	boolean value indicating whether dose response curves should be plotted. Deactivating plotting decreases runtime.
verbose	print name of each fitted or plotted protein to the command line as a means of progress report.
xlsxExport	produce results table in xlsx format and store at the location specified by the resultPath argument.
fcTolerance	tolerance for the fcCutoff parameter. See details.

## Details

Invokes the following steps:

1. Import data using the [tppccrImport](#) function.
2. Perform normalization by fold change medians (optional) using the [tppccrNormalize](#) function. To perform normalization, set argument `normalize=TRUE`.
3. Fit and analyze dose response curves using the [tppccrCurveFit](#) function.





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analyzeTPPTR	<i>Analyze TPP-TR experiment</i>
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## Description

Performs analysis of a TPP-TR experiment by invoking routines for data import, data processing, normalization, curve fitting, and production of the result table.

## Usage

```
analyzeTPPTR(
  configTable,
  data = NULL,
  resultPath = NULL,
  methods = c("meltcurvefit", "splinefit"),
  idVar = "gene_name",
  fcStr = "rel_fc_",
  ciStr = NULL,
  naStrs = c("NA", "n/d", "NaN", "<NA>"),
  qualColName = "qupm",
  normalize = TRUE,
  normReqs = tpptrDefaultNormReqs(),
  ggplotTheme = tppDefaultTheme(),
  nCores = "max",
  startPars = c(P1 = 0, a = 550, b = 10),
  splineDF = c(3:7),
  maxAttempts = 500,
  plotCurves = TRUE,
  fixedReference = NULL,
  pValMethod = "robustZ",
  pValFilter = list(minR2 = 0.8, maxPlateau = 0.3),
  pValParams = list(binWidth = 300),
  verbose = FALSE,
  xlsExport = TRUE
)
```

## Arguments

configTable	dataframe, or character object with the path to a file, that specifies important details of the TPP-TR experiment. See Section details for instructions how to create this object.
data	single dataframe, or list of dataframes, containing fold change measurements and additional annotation columns to be imported. Can be used instead of specifying the file path in the configTable argument.
resultPath	location where to store melting curve plots, intermediate results, and the final results table.

methods	statistical methods for modeling melting behavior and detecting significant differences between experimental conditions. Ich more than one method are specified, results will be computed for each and concatenated in the result table (default: meltcurvefit).
idVar	character string indicating which data column provides the unique identifiers for each protein.
fcStr	character string indicating which columns contain the actual fold change values. Those column names containing the suffix fcStr will be regarded as containing fold change values.
ciStr	character string indicating which columns contain confidence intervals for the fold change measurements. If specified, confidence intervals will be plotted around the melting curves.
naStrs	character vector indicating missing values in the data table. When reading data from file, this value will be passed on to the argument na.strings in function read.delim.
qualColName	character string indicating which column can be used for additional quality criteria when deciding between different non-unique protein identifiers.
normalize	perform normalization (default: TRUE).
normReqs	list of filtering criteria for construction of the normalization set.
ggplotTheme	ggplot theme for melting curve plots.
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
startPars	start values for the melting curve parameters. Will be passed to function nls for curve fitting.
splineDF	degrees of freedom for natural spline fitting.
maxAttempts	maximal number of curve fitting attempts if model does not converge.
plotCurves	boolean value indicating whether melting curves should be plotted. Deactivating plotting decreases runtime.
fixedReference	name of a fixed reference experiment for normalization. If NULL (default), the experiment with the best R2 when fitting a melting curve through the median fold changes is chosen as the reference.
pValMethod	Method for p-value computation. Currently restricted to 'robustZ' (see Cox & Mann (2008)).
pValFilter	optional list of filtering criteria to be applied before p-value computation.
pValParams	optional list of parameters for p-value computation.
verbose	print name of each fitted protein to the command lin as a means of progress report.
xlsxExport	boolean value indicating whether to produce result table in .xlsx format (requires package openxlsx and a zip application to be installed).

## Details

Invokes the following steps:

1. Import data using the `tpptrImport` function.
2. Perform normalization (optional) using the `tpptrNormalize` function. To perform normalization, set argument `normalize=TRUE`. The normalization will be filtered according to the criteria specified in the `normReqs` argument (also see the documentation of `tpptrNormalize` and `tpptrDefaultNormReqs` for further information).
3. Fit melting curves using the function `tpptrCurveFit`.
4. Produce result table using the function `tpptrAnalyzeMeltingCurves`.
5. Export results to Excel using the function `tppExport`.

The default settings are tailored towards the output of the python package `isobarQuant`, but can be customized to your own dataset by the arguments `idVar`, `fcStr`, `naStrs`, `qualColName`.

If `resultPath` is not specified, the location of the first input file specified in `configTable` will be used. If the input data are not specified in `configTable`, no result path will be set. This means that no output files or melting curve plots are produced and `analyzeTPPTR` just returns the results as a data frame.

The function `analyzeTPPTR` reports intermediate results to the command line. To suppress this, use `suppressMessages`.

The `configTable` argument is a dataframe, or the path to a spreadsheet (tab-delimited text-file or xlsx format). Information about each experiment is stored row-wise. It contains the following columns:

- `Path`: location of each datafile. Alternatively, data can be directly handed over by the data argument.
- `Experiment`: unique experiment names.
- `Condition`: experimental conditions of each dataset.
- `Label columns`: each isobaric label names a column that contains the temperatures administered for the label in the individual experiments.

The argument `methods` can be one of the following: More than one method can be specified. For example, parametric testing of melting points and nonparametric spline-based goodness-of-fit tests can be performed sequentially in the same analysis. The results are then written to separate columns of the output table.

If `methods` contains "meltcurvefit", melting curve plots will be stored in a subfolder with name `Melting_Curves` at the location specified by `resultPath`. If `methods` contains "splinefit", plots of the natural spline fits will be stored in a subfolder with name `Spline_Fits` at the location specified by `resultPath`.

The argument `nCores` could be either 'max' (use all available cores) or an upper limit of CPUs to be used.

If `doPlot = TRUE`, melting curve plots are generated separately for each protein and stored in separate pdfs. Each file is named by the unique protein identifier. Filenames are truncated to 255 characters (requirement by most operation systems). Truncated filenames are indicated by the suffix "\_truncated[d]", where [d] is a unique number to avoid redundancies. All melting curve plots are stored in a subfolder with name `Melting_Curves` at the location specified by `resultPath`.

If the melting curve fitting procedure does not converge, it will be repeatedly started from perturbed starting parameters (maximum iterations defined by argument `maxAttempts`).

Argument `splineDF` specifies the degrees of freedom for natural spline fitting. As a single numeric value, it is directly passed on to the `splineDF` argument of `splines::ns`. Experience shows that `splineDF = 4` yields good results for TPP data sets with 10 temperature points. It is also possible to provide a numeric vector. In this case, splines are fitted for each entry and the optimal value is chosen per protein using Akaike's Information criterion.

### Value

A data frame in which the fit results are stored row-wise for each protein.

### References

Savitski, M. M., Reinhard, F. B., Franken, H., Werner, T., Savitski, M. F., Eberhard, D., ... & Drewes, G. (2014). Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science*, 346(6205), 1255784.

Franken, H, Mathieson, T, Childs, D. Sweetman, G. Werner, T. Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. *Nature protocols* 10(10), 1567-1593.

### See Also

`tppDefaultTheme`, `tpptrImport`, `tpptrNormalize`, `tpptrCurveFit`, `tpptrAnalyzeMeltingCurves`

### Examples

```
data(hdacTR_smallExample)
tpptrResults <- analyzeTPPTR(configTable = hdacTR_config, data = hdacTR_data,
                             methods = "meltcurvefit", nCores = 1)
```

---

`hdacCCR_config`      *The configuration table to analyze [hdacCCR\\_data](#).*

---

### Description

The configuration table to analyze [hdacCCR\\_data](#).

### Details

`hdacCCR_config` is a data frame that specifies the experiment names, isobaric labels, and the administered drug concentrations at each label.

### References

Franken, H, Mathieson, T, Childs, D. Sweetman, G. Werner, T. Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. *Nature protocols* 10(10), 1567-1593.

**See Also**

[hdacCCR\\_smallExample](#), [hdacCCR\\_data](#)

---

hdacCCR\_data

*TPP-CCR example dataset (replicates 1 and 2)*

---

**Description**

Example subset of a Panobinostat TPP-CCR dataset (replicates 1 and 2)

**Details**

A list with two subsets of a dataset obtained by TPP-CCR experiments to investigate drug effects for HDAC inhibitor Panobinostat. It contains 7 HDACs as well as a random selection of 493 further proteins.

You can use this dataset to explore the [TPP](#) package functionalities without invoking the whole time consuming analysis on the big dataset.

The original dataset is located in the folder 'example\_data/CCR\_example\_data' in the package's installation directory. You can find it on your system by the R command `system.file('example_data', package = 'TPP')`. The measurements were generated by four separate multiplexed TMT experiments with 10 TMT labels each. Quantitative values per protein were obtained by the python software `isobarQuant` and converted to fold changes relative to the lowest temperature. The raw data before quantification can be found in the `proteomicsDB` database (<http://www.proteomicsdb.org/#projects/4221/3102>) with the following sample mapping:

- Panobinostat\_1: MS-experiment numbers P97404B02-B10
- Panobinostat\_2: MS-experiment numbers P97414B02-B10

**References**

Franken, H, Mathieson, T, Childs, D, Sweetman, G, Werner, T, Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. *Nature protocols* 10(10), 1567-1593.

**See Also**

[hdacCCR\\_smallExample](#), [hdacTR\\_config](#)

---

hdacCCR_smallExample	<i>Example subsets of a Panobinostat TPP-CCR dataset (replicates 1 and 2) and the corresponding configuration table to start the analysis.</i>
----------------------	--

---

### Description

Example dataset obtained by TPP-CCR experiments for analysis by the TPP-package. It contains all necessary arguments to start the analysis (config table and list of data frames).

### References

Franken, H, Mathieson, T, Childs, D, Sweetman, G, Werner, T, Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. Nature protocols 10(10), 1567-1593.

### See Also

[hdacCCR\\_data](#), [hdacCCR\\_config](#)

---

hdacTR_config	<i>The configuration table to analyze <a href="#">hdacTR_data</a>.</i>
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---

### Description

The configuration table to analyze [hdacTR\\_data](#).

### Details

hdacTR\_config is a data frame that specifies the experiment name, isobaric labels, and the administered temperatures at each label.

### References

Franken, H, Mathieson, T, Childs, D, Sweetman, G, Werner, T, Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. Nature protocols 10(10), 1567-1593.

### See Also

[hdacTR\\_smallExample](#), [hdacTR\\_data](#)

---

hdacTR_data	<i>TPP-TR example dataset.</i>
-------------	--------------------------------

---

## Description

Example subset of a dataset obtained by TPP-TR experiments to investigate possible targets for HDAC inhibitor Panobinostat.

## Details

hdacTR\_data is a list of data frames that contain measurements for HDACs as well as a random selection of 500 further proteins.

You can use this dataset to explore the [TPP](#) package functionalities without invoking the whole time consuming analysis on the whole dataset.

The original dataset is located in the folder 'example\_data/TR\_example\_data' in the package's installation directory. You can find it on your system by the R command `system.file('example_data', package = 'TPP')`.

The measurements were generated by four separate multiplexed TMT experiments with 10 TMT labels each. Quantitative values per protein were obtained by the python software isobarQuant and converted to fold changes relative to the lowest temperature. The raw data before quantification can be found in the proteomicsDB database (<http://www.proteomicsdb.org/#projects/4221/3101>) with the following sample mapping:

- Panobinostat\_1: MS-experiment numbers P85192B02-B10
- Panobinostat\_2: MS-experiment numbers P85881B02-B10
- Vehicle\_1: MS-experiment numbers P85202B02-B10
- Vehicle\_2: MS-experiment numbers P85891B02-B10

## References

Franken, H, Mathieson, T, Childs, D, Sweetman, G, Werner, T, Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. Nature protocols 10(10), 1567-1593.

## See Also

[hdacTR\\_smallExample](#), [hdacTR\\_config](#)

---

hdacTR\_resultsTable\_smallExample

*Example of a TPP-TR result table.*

---

**Description**

Example of a TPP-TR result table.

**Details**

Contains the data object [resultTable](#).

---

hdacTR\_smallExample

*Example subset of a Panobinostat TPP-TR dataset and the corresponding configuration table to start the analysis.*

---

**Description**

Example dataset obtained by TPP-TR experiments for analysis by the TPP-package. It contains all necessary arguments to start the analysis (config table and list of data frames).

**References**

Franken, H, Mathieson, T, Childs, D, Sweetman, G, Werner, T, Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. Nature protocols 10(10), 1567-1593.

**See Also**

[hdacTR\\_data](#), [hdacTR\\_config](#)

---

panobinostat\_2DTPP\_config

*The configuration table to analyze [panobinostat\\_2DTPP\\_data](#).*

---

**Description**

The configuration table to analyze [panobinostat\\_2DTPP\\_data](#).

**Details**

panobinostat\_2DTPP\_config is a data frame that specifies the experiment names, isobaric labels, and the administered drug concentrations at each label.

**See Also**

[panobinostat\\_2DTPP\\_data](#), [panobinostat\\_2DTPP\\_smallExample](#)



---

panobinostat\_2DTPP\_data

*2D-TPP-CCR example dataset*

---

### Description

Example subset of a Panobinostat 2D-TPP dataset

### Details

A list with two subsets of a dataset obtained by 2D-TPP experiments to investigate drug effects for HDAC inhibitor Panobinostat. The experiment was performed on living HepG2 cells (see Becher et al. (2016). Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat. Nature Chemical Biology, (September)) It contains 7 HDACs as well as a random selection of 493 further proteins.

You can use this dataset to explore the [TPP](#) package functionalities without invoking the whole time consuming analysis on the big dataset.

### See Also

[panobinostat\\_2DTPP\\_config](#), [panobinostat\\_2DTPP\\_smallExample](#)

---

panobinostat\_2DTPP\_smallExample

*Example subsets of a Panobinostat 2D-TPP dataset and the corresponding configuration table to start the analysis.*

---

### Description

Example dataset obtained by 2D-TPP experiments for analysis by the TPP-package. It contains all necessary arguments to start the analysis (config table and list of data frames).

### See Also

[panobinostat\\_2DTPP\\_data](#), [panobinostat\\_2DTPP\\_config](#)

---

resultTable	<i>Example of a TPP-TR result table.</i>
-------------	--

---

**Description**

Example of a TPP-TR result table.

**Details**

resultTable is a data frame that contains the measurements of several TPP-TR experiments, the fitted melting curve parameters, as well as p-values and the results of additional quality checks for each protein. It can be used as input for the function [tppQCPlotsCorrelateExperiments](#).

---

TPP	<i>Thermal proteome profiling (TPP)</i>
-----	---

---

**Description**

TPP is a toolbox for analyzing thermal proteome profiling (TPP) experiments.

**Usage**

```
.onLoad(libname, pkgname)
```

**Arguments**

libname	a character string giving the library directory where the package defining the namespace was found. Passed to .onLoad function.
pkgname	a character string giving the name of the package. Passed to .onLoad function.

**Details**

In order to start a TPP-TR analysis, use function [analyzeTPPTR](#). For a TPP-CCR analysis, use function [analyzeTPPCCR](#). See the vignette for detailed instructions.

**Value**

No return value defined for this document.

**References**

Savitski, M. M., Reinhard, F. B., Franken, H., Werner, T., Savitski, M. F., Eberhard, D., ... & Drewes, G. (2014). Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science*, 346(6205), 1255-1259.

Franken, H., Mathieson, T., Childs, D., Sweetman, G., Werner, T., Huber, W. & Savitski, M. M. (2015). Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. *Nature protocols* 10(10), 1567-1593.

---

TPP-defunct

*Defunct functions in package 'TPP'*

---

**Description**

These functions are defunct and no longer available.

**Usage**

tpp2dPlotCCRGoodCurves()

tpp2dPlotCCRA11Curves()

tpp2dPlotCCRSingleCurves()

tpp2dEvalConfigTable()

tpp2dRemoveZeroSias()

tpp2dReplaceColNames()

tpp2dCreateCCRConfigFile()

**Details**

Defunct functions are: tpp2dPlotCCRGoodCurves, tpp2dPlotCCRA11Curves, tpp2dPlotCCRSingleCurves, tpp2dEvalConfigTable, tpp2dRemoveZeroSias, tpp2dReplaceColNames, tpp2dCreateCCRConfigFile

**Value**

No value returned

---

TPP-deprecated

*Deprecated functions in package 'TPP'*

---

**Description**

These functions are deprecated and no longer available.

**Value**

No value returned

tpp2dAddAdditionalInfo

*Add additional info to 2D-TPP CCR output data*

---

### Description

Adds additional info to 2D-TPP CCR output data, like counts on how often a certain protein was stabilized or destabilized

### Usage

```
tpp2dAddAdditionalInfo(data, idVar = "gene_name")
```

### Arguments

data	output table returned by the tpp2dCurveFit function
idVar	character string indicating which column of the data table contains unique protein ids

### Value

A data frame to which additional data like how often a protein has been (de-)stabilized has been attached

### Examples

```
load(system.file("example_data/2D_example_data/shortCCRresults.RData", package="TPP"))
shortCCRresults <- tpp2dAddAdditionalInfo(data = shortCCRresults, idVar="representative")
```

---

tpp2dCalcFractAbundance

*Calculate fractional abundance and DMSO ratio of successive sumionareas (usage of function is only reasonable when at least two temperatures are multiplexed!)*

---

### Description

Calculates fractional abundance and DMSO ratio of successive sumionareas and creates respective columns which are added two the data frame which is handed over

**Usage**

```
tpp2dCalcFractAbundance(  
  configTable = NULL,  
  data,  
  intensityStr = NULL,  
  idVar = NULL  
)
```

**Arguments**

configTable	DEPRECATED
data	data frame of TPP-CCR results (e.g. obtained by run2DTPPCCR).
intensityStr	DEPRECATED
idVar	DEPRECATED

**Value**

Data frame that was handed over with additional columns of fractional abundance and DMSO1 vs DMSO2 ratio

**Examples**

```
data(panobinostat_2DTPP_smallExample)  
  
# Import data:  
datIn <- tpp2dImport(configTable = panobinostat_2DTPP_config,  
  data = panobinostat_2DTPP_data,  
  idVar = "representative",  
  addCol = "clustname",  
  intensityStr = "sumionarea_protein_",  
  nonZeroCols = "qusm")  
  
# View attributes of imported data (experiment infos and import arguments):  
attr(datIn, "importSettings") %>% unlist  
attr(datIn, "configTable")  
  
# Compute fractional abundance:  
datDMSORatio <- tpp2dCalcFractAbundance(data = datIn)  
colnames(datDMSORatio)
```

---

tpp2dComputeFoldChanges

*Compute 2D-TPP fold changes*

---

**Description**

Computes fold changes by calculating fold changes of the sumionarea relative to the reference column.

**Usage**

```
tpp2dComputeFoldChanges(
  configTable = NULL,
  data,
  intensityStr = NULL,
  fcStr = NULL,
  newFcStr = "rel_fc_"
)
```

**Arguments**

configTable	DEPRECATED
data	dataframe that contain the data for the 2D-TPP experiment
intensityStr	DEPRECATED
fcStr	DEPRECATED
newFcStr	character string indicating how columns that will contain the actual fold change values will be called. The suffix newFcStr will be pasted in front of the names of the experiments.

**Value**

A data.frame with additional columns with constitute fold changes calculated with respect to the intensity values of the zero treatment column

**Examples**

```
# Preparation:
data(panobinostat_2DTPP_smallExample)

# Import data:
datIn <- tpp2dImport(configTable = panobinostat_2DTPP_config,
  data = panobinostat_2DTPP_data,
  idVar = "representative",
  addCol = "clustername",
  intensityStr = "sumionarea_protein_",
  nonZeroCols = "qusm")

# View attributes of imported data (experiment infos and import arguments):
attr(datIn, "importSettings") %>% unlist
attr(datIn, "configTable")

# Compute fold changes:
datFC <- tpp2dComputeFoldChanges(data = datIn)
```

```
# View updated attributes. Now contain field 'fcStrNorm' indicating prefix
# of the fold change columns after normalization.
attr(datFC, "importSettings")["fcStr"]
```

---

tpp2dCreateDRplots      *Create dose response curve plots for 2D-TPP data*

---

## Description

Generates a list of dose response curve plots per protein and temperature point.

## Usage

```
tpp2dCreateDRplots(
  data = NULL,
  type = "all",
  verbose = FALSE,
  paletteName = "Spectral"
)
```

## Arguments

data	the data that should be plotted.
type	string defining which curves to display (see details).
verbose	boolean variable stating whether a print description of problems/success for plotting of each protein should be printed.
paletteName	color palette (see details).

## Details

data is a data frame in wide table format returned by function [tpp2dCurveFit](#). Its attributes contain information about the experiment names, temperatures, isobaric labels, as well as instructions on how to find the relevant columns in the wide table.

type defines which curves to display per plot. Possible values are:

- "all": Create one plot per protein. This plot simultaneously displays the curves for all available temperatures for this protein (the default).
- "good": Create one plot per protein. This plot displays all dose response curves with a high goodness-of-fit. Choose this option to save runtime by focusing only on the reliable fits.
- "single": Create one separate plot per protein and temperature. This plot displays all dose response curves with a high goodness-of-fit.

paletteName specifies the color palette to be used by the [brewer.pal](#) function from the RColorBrewer package to assign a separate color to each concentration.

**Value**

A list of successfully generated plot objects of class 'ggplot'

**See Also**

[tpp2dCurveFit](#) [brewer.pal](#)

**Examples**

```
data(panobinostat_2DTPP_smallExample)

# Import data:
datIn <- tpp2dImport(configTable = panobinostat_2DTPP_config,
                    data = panobinostat_2DTPP_data,
                    idVar = "representative",
                    addCol = "clustername",
                    intensityStr = "sumionarea_protein_",
                    nonZeroCols = "qusm")

# Compute fold changes:
fcData2d <- tpp2dComputeFoldChanges(data = datIn)
normData2d <- tpp2dNormalize(data = fcData2d)
ccr2dResults <- tpp2dCurveFit(data = normData2d)
allCurves <- tpp2dCreateDRplots(data = ccr2dResults, type = "all")
allCurves[["HDAC1"]]
```

---

tpp2dCreateReport

*Create Report of 2D-TPP analysis*

---

**Description**

Creates a markdown pdf file that summarizes the 2D-TPP analysis by reporting e.g. R version and package versions used

**Usage**

```
tpp2dCreateReport(
  data = NULL,
  configFile = NULL,
  resultPath = NULL,
  documentType = "html_document",
  configTable = NULL,
  normalize = TRUE,
  methods = c(""),
  idVar = "gene_name",
  fcStr = "rel_fc_",
```



```

    fcStrUpdated = "norm_rel_fc_",
    intensityStr = "signal_sum_",
    addCol = NULL,
    fcTolerance = NA,
    r2Cutoff = NA,
    fcCutoff = NA,
    slopeBounds = c(NA, NA),
    fTest = FALSE,
    trRef = "none"
)

```

### Arguments

data	output data frame from an 2D-TPP analysis
configFile	character string containing a valid system path to a file which summarizes the experimental details of the 2D-TPP experiment or respective data frame
resultPath	character string containing a system path to where the report should be written
documentType	character string indicating which document type the report should have default: "html_document", alternatives: "pdf_document"
configTable	data frame summarizing the experimental details of the 2D-TPP experiment
normalize	boolean flag indicating whether median normalization has been performed
methods	vector of characters which indicate which methods have been used
idVar	unique protein identifier prefix
fcStr	fold change identifier prefix
fcStrUpdated	character string matching the fold change columns after normalization has been performed
intensityStr	intensity values prefix
addCol	vector of strings indicating which additional data columns were imported
fcTolerance	tolerance for the fcCutoff parameter
r2Cutoff	Quality criterion on dose response curve fit.
fcCutoff	Cutoff for highest compound concentration fold change
slopeBounds	Bounds on the slope parameter for dose response curve fitting
fTest	boolean variable stating whether an fTest was performed
trRef	character string containing a valid system path to a previously generated TPP-TR reference object

### Value

A pdf or html report which summarizes all parameters that were set

---

tpp2dCreateTPPTRreference

*Create TPP-TR reference for 2D-TPP experiment*


---

## Description

Performs a reference analysis of a TPP-TR experiment and generates boxplots for the distribution of fold changes at the different temperatures if desired.

## Usage

```
tpp2dCreateTPPTRreference(
  trConfigTable = NULL,
  trDat = NULL,
  resultPath = NULL,
  outputName = NULL,
  createFCboxplots = FALSE,
  idVar = "gene_name",
  fcStr = "rel_fc_",
  qualColName = "qupm",
  normalize = TRUE
)
```

## Arguments

trConfigTable	config file for a reference TR dataset
trDat	list of dataframes, containing fold change measurements and additional annotation columns to be imported. Can be used instead of specifying the file path in the configTable argument.
resultPath	character string containing a valid system path to which folder output files will be written
outputName	character string which will be used as name of the output folder
createFCboxplots	boolean flag indicating whether quality control boxplots are to be plotted
idVar	character string indicating which column of the data table contains the unique protein ids
fcStr	character string indicating which columns contain fold changes
qualColName	character string indicating which column contain protein identification quality measures
normalize	boolean argument stating whether the data should be normalized or not

**Value**

A TPP-TR reference object for a certain cell line with different supporting files in a desired output directory. The main object which is of interest for further analysis is the `trRefData.RData` file. This is the file to which a referencing system path has to be indicated when a function as [tpp2dSplineFitAndTest](#) require to input a TPP-TR reference object. The RData file consists of list carrying four different items:

1. `tppCfgTable`: the TPP-TR configtable which was used for generating this object
2. `sumResTable` a list of two elements 1. detail: the exact result data from the TR analysis and 2. summary. a summary of the analyzed TR data comprising the median and standard deviation values of the measurements at the different temperatures (encoded by the isobaric labels)
3. `temperatures` a table listing the temperatures which were used in the TR experiment in the different replicates
4. `lblsByTemp` a table matching each temperature to an isobaric label

---

tpp2dCurveFit

*Run TPP-CCR analysis for 2D-TPP experiment*


---

**Description**

Performs analysis of a TPP-CCR experiment by invoking the routine for TPP-CCR curve fitting for each temperature of the sample.

**Usage**

```
tpp2dCurveFit(
  configFile = NULL,
  data,
  nCores = 1,
  naStrs = NULL,
  fcStr = NULL,
  idVar = NULL,
  nonZeroCols = NULL,
  r2Cutoff = 0.8,
  fcCutoff = 1.5,
  slopeBounds = c(1, 50),
  fcTolerance = 0.1
)
```

**Arguments**

<code>configFile</code>	DEPRECATED
<code>data</code>	data frame that contains the data of the 2D-TPP experiment for each temperature.
<code>nCores</code>	numeric value stating how many cores are to be used for computation

naStrs	DEPRECATED
fcStr	DEPRECATED
idVar	DEPRECATED
nonZeroCols	DEPRECATED
r2Cutoff	Quality criterion on dose response curve fit.
fcCutoff	Cutoff for highest compound concentration fold change.
slopeBounds	Bounds on the slope parameter for dose response curve fitting.
fcTolerance	tolerance for the fcCutoff parameter. See details.

**Value**

A data frames in which the fit results are stored row-wise for each protein.

**Examples**

```
# Preparation:
data(panobinostat_2DTPP_smallExample)

# Import data:
datIn <- tpp2dImport(configTable = panobinostat_2DTPP_config,
                    data = panobinostat_2DTPP_data,
                    idVar = "representative",
                    addCol = "clustername",
                    intensityStr = "sumionarea_protein_",
                    nonZeroCols = "qusm")

# Compute fold changes:
datFC <- tpp2dComputeFoldChanges(data = datIn)

# Perform median normalization:
datNorm <- tpp2dNormalize(data = datFC)

# View updated attributes. Now contain field 'fcStrNorm' indicating prefix
# of the fold change columns after normalization.
attr(datNorm, "importSettings")["fcStrNorm"]

# Perform dose response curve fitting and pEC50 calculation:
datFit <- tpp2dCurveFit(data = datNorm)
```

---

tpp2dExport

*Produce Excel table of 2D-TPP experiment.*


---

**Description**

Produce Excel table of 2D-TPP experiment analysis results.

**Usage**

```
tpp2dExport(
  configTable = NULL,
  tab,
  resultPath = NULL,
  idVar = NULL,
  fcStr = NULL,
  intensityStr = NULL,
  outputPath,
  addCol = NULL,
  normalizedData = NULL,
  trRef = NULL,
  addPlotColumns = TRUE
)
```

**Arguments**

configTable	DEPRECATED
tab	Table with results of the 2D-TPP analysis.
resultPath	DEPRECATED
idVar	DEPRECATED
fcStr	DEPRECATED
intensityStr	DEPRECATED
outputPath	path for storing results table
addCol	additional names of columns which are to be attached to the result table
normalizedData	DEPRECATED
trRef	character string containing a valid system path to a TPP-TR reference RData file
addPlotColumns	boolean variable indicating whether paths to plot files should be generated and checked for validity. De-activate if no dose-response curve plots were produced during the analysis.

**Value**

Creates excel file of the TPP-CCR analysis of the 2D-TPP data.

**Examples**

```
data(panobinostat_2DTPP_smallExample)
load(system.file("example_data/2D_example_data/shortData2d.RData", package="TPP"))
# tpp2dExport(configTable = panobinostat_2DTPP_config, tab=shortData2d,
#             outputPath=getwd(),
#             idVar="representative", fcStr="norm_rel_fc_protein_",
#             intensityStr="sumionarea_protein_", addCol=NULL)

data(panobinostat_2DTPP_smallExample)
# cfgRaw <- panobinostat_2DTPP_config
```

```
# datRaw <- panobinostat_2DTPP_data
# datIn <- tpp2dImport(cfgIn, datRaw, fcStr = NULL)
# datFC <- tpp2dComputeFoldChanges(data = datIn)
# datNorm <- tpp2dNormalize(data = datFC)
# cfgCCR <- convert_2D_cfgTable_to_CCR_cfgTable(cfgIn)
# datFitted <- tpp2dCurveFit(datNorm, nCores = 2)
# tpp2dCreateReport(getwd(), cfgIn, resultTable = datFitted, idVar = "representative",
#                   intensityStr = "sumionarea_protein_")
# tpp2dExport(tab = datFitted, outPath = getwd(), addPlotColumns = FALSE)
```

---

tpp2dExportPlots      *Export plots for 2D-TPP experiment.*

---

## Description

Exports plots into plots/ directory in the resultPath

## Usage

```
tpp2dExportPlots(plotList, resultPath, type = "none")
```

## Arguments

plotList	list of ggplots returned from one of the plotting functions
resultPath	path for storing results
type	character string specifying which type of plot is to be exported

## Details

Creates pdf files of the afore created plots by plot\_2D\_data\_on\_temperature\_range or tpp2dCreateDRplots

## Value

None

tpp2dImport

*Import 2D-TPP data***Description**

Imports data from 2D-TPP experiments by parsing a configTable and reading in corresponding data file or data frames containing raw data (sumionarea values) and creating a big data frame comprising all samples with respective fold changes

**Usage**

```
tpp2dImport(
  configTable = NULL,
  data = NULL,
  idVar = "gene_name",
  addCol = NULL,
  intensityStr = "signal_sum_",
  qualColName = "qupm",
  nonZeroCols = "qssm",
  fcStr = NULL
)
```

**Arguments**

configTable	dataframe, or character object with the path to a file, that specifies important details of the 2D-TPP experiment. See Section details for instructions how to create this object.
data	single dataframe, containing raw measurements and if already available fold changes and additional annotation columns to be imported. Can be used instead of specifying the file path in the configTable argument.
idVar	character string indicating which data column provides the unique identifiers for each protein.
addCol	additional column names that specify columns in the input data that are to be attached to the data frame throughout the analysis
intensityStr	character string indicating which columns contain the actual sumionarea values. Those column names containing the suffix intensityStr will be regarded as containing sumionarea values.
qualColName	character string indicating which column can be used for additional quality criteria when deciding between different non-unique protein identifiers.
nonZeroCols	character string indicating a column that will be used for filtering out zero values.
fcStr	character string indicating which columns contain the actual fold change values. Those column names containing the suffix fcStr will be regarded as containing fold change values.

**Value**

A dataframe comprising all experimental data

**Examples**

```
# Preparation:
data(panobinostat_2DTPP_smallExample)

# Import data:
datIn <- tpp2dImport(configTable = panobinostat_2DTPP_config,
                    data = panobinostat_2DTPP_data,
                    idVar = "representative",
                    addCol = "clustername",
                    intensityStr = "sumionarea_protein_",
                    nonZeroCols = "qusm")

# View attributes of imported data (experiment infos and import arguments):
attr(datIn, "importSettings") %>% unlist
attr(datIn, "configTable")
```

---

tpp2dMerge2dRef

*Merge 2D-TPP result data with TPP-TR reference data*


---

**Description**

Merges 2D-TPP result data with TPP-TR reference data to generate a big table including both results

**Usage**

```
tpp2dMerge2dRef(
  resultTable_2D,
  referenceDataSummary,
  refIDVar = "Protein_ID",
  idVar = NULL,
  data = NULL,
  trRef = NULL
)
```

**Arguments**

resultTable\_2D dataframe containing the 2D-TPP results

referenceDataSummary

summarized reference data results. See details.

refIDVar

character string indicating name of the columns containing the unique protein identifiers in the reference data set



idVar	DEPRECATED
data	DEPRECATED
trRef	DEPRECATED

### Details

referenceSummary contains summary statistics like median fold changes and is produced by the function [tpp2dCreateTPPTRreference](#). It summarizes the results of a TPP-TR analysis of a reference data set. A reference data set is the a output of a TR experiment without drug treatment on the same cell line as resultTable\_2D.

### Value

A data frame with results merged from 2D-TPP and TPP-TR reference

### See Also

[tpp2dCreateTPPTRreference](#)

### Examples

```
data(panobinostat_2DTPP_smallExample)
config_tpp2d <- panobinostat_2DTPP_config
data_tpp2d <- panobinostat_2DTPP_data
tpp2dResults <- analyze2DTPP(configTable = config_tpp2d,
                             data = data_tpp2d,
                             methods=c("doseResponse"),
                             createReport="none",
                             nCores=1,
                             idVar = "representative",
                             addCol = "clustername",
                             intensityStr = "sumionarea_protein_",
                             nonZeroCols = "qusm")

trRef <- file.path(system.file("data", package="TPP"),
                  "TPPTR_reference_results_HepG2.RData")

annotatedTable <- tpp2dMerge2dRef(resultTable_2D = tpp2dResults,
                                 referenceDataSummary = trRef)
```

---

tpp2dNormalize

*Median normalization of protein fold changes of 2D-TPP data*

---

### Description

Normalizes fold changes retrieved from 2D-TPP experiment by dividing by the median fold change

**Usage**

```
tpp2dNormalize(configTable = NULL, data, fcStr = NULL)
```

**Arguments**

configTable	DEPRECATED
data	data frame that contains the data for the 2D-TPP experiment
fcStr	DEPRECATED

**Value**

A dataframe identical to the input dataframe except that the columns containing the fold change values have been normalized by their median.

**Examples**

```
# Preparation:
data(panobinostat_2DTPP_smallExample)

# Import data:
datIn <- tpp2dImport(configTable = panobinostat_2DTPP_config,
                    data = panobinostat_2DTPP_data,
                    idVar = "representative",
                    addCol = "clustername",
                    intensityStr = "sumionarea_protein_",
                    nonZeroCols = "qusm")

# Compute fold changes:
datFC <- tpp2dComputeFoldChanges(data = datIn)

# Perform median normalization:
datNorm <- tpp2dNormalize(data = datFC)

# View updated attributes. Now contain field 'fcStrNorm' indicating prefix
# of the fold change columns after normalization.
attr(datNorm, "importSettings")["fcStrNorm"]
```

---

tpp2dPlotQChist

*Plot quality control histograms*

---

**Description**

Plots quality control histograms of pEC50 values of reference dataset and indicates the pEC50 values of the 2D-TPP experiment

**Usage**

```
tpp2dPlotQChist(
  configFile = NULL,
  resultTable = NULL,
  resultPath = NULL,
  trRef = NULL,
  fcStr = "rel_fc_",
  idVar = "gene_name",
  qualColName = "qupm"
)
```

**Arguments**

configFile	data frame or system path to table that specifies important details of the 2D-TPP experiment
resultTable	data.frame containing the results of a CCR analysis of 2D-TPP data
resultPath	character string containing a valid system path to which the qc plots will be written
trRef	character string with a link to a TPP-TR reference object RData file
fcStr	character string indicating how columns that will contain the actual fold change values are called.
idVar	character string indicating name of the columns containing the unique protein identifiers
qualColName	character string indicating which column contain protein identification quality measures

**Value**

A pdf with various quality control plots for a specified 2D-TPP data set

---

tpp2dPlotQCpEC50      *Plot quality control pEC50 plots*

---

**Description**

Plots quality control plots which indicate at which temperatures the pEC50 values of the treatment curves lie in comparison to those of the reference data

**Usage**

```
tpp2dPlotQCpEC50(
  resultTable = NULL,
  resultPath = NULL,
  trRef = NULL,
  idVar = "gene_name"
)
```

**Arguments**

resultTable	data.frame containing the results of a CCR analysis of 2D-TPP data
resultPath	character string containing a valid system path to which the the qc plots will be written
trRef	character string with a link to a TPP-TR reference object RData file
idVar	character string indicating how the column that contains the unique protein identifiers is called

**Value**

A folder with plots for each identified protein that compare melting points in the reference data set with the 2D-TPP data set

---

tpp2dSplineFitAndTest *Fit splines and perform f-Test*

---

**Description**

Fit splines through TR reference dataset and extrapolates relative 2D-TPP datapoints, then compares spline fits of different treatments with non-treatment with an f-test

**Usage**

```
tpp2dSplineFitAndTest(
  data_2D = NULL,
  data,
  trRefDataPath = NULL,
  dataRef,
  refIDVar = "Protein_ID",
  refFcStr = "norm_rel_fc_",
  resultPath = NULL,
  doPlot = TRUE,
  verbose = FALSE,
  nCores = "max",
  ggplotTheme = NULL
)
```

**Arguments**

data_2D	DEPRECATED
data	result data.frame from a 2D-TPP CCR analysis
trRefDataPath	DEPRECATED
dataRef	reference data from a TPP TR analysis on the same cell line as
refIDVar	character string indicating name of the columns containing the unique protein identifiers in the reference data set

refFcStr	character string indicating which columns contain the actual fold change values in the reference data. The suffix fcStr will be pasted in front of the names of the experiments.
resultPath	location where to store dose-response curve plots and results table.
doPlot	boolean value indicating whether protein-wise plots should be produced Deactivating plotting decreases runtime.
verbose	print description of problems for each protein for which splines fits could not be performed
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
ggplotTheme	DEPRECATED

## Details

dataRef can either be a tidy data frame of TPP-TR reference data, a list with TPP-TR reference data and additional information produced by [tpp2dCreateTPPTRreference](#), or a character string with a link to the data in one of the described formats.

## Value

None

## Examples

```
data(panobinostat_2DTPP_smallExample)
config_tpp2d <- panobinostat_2DTPP_config
data_tpp2d <- panobinostat_2DTPP_data
trRef <- file.path(system.file("data", package="TPP"),
  "TPPTR_reference_results_HepG2.RData")
datIn <- tpp2dImport(configTable = config_tpp2d,
  data = data_tpp2d,
  idVar = "representative",
  addCol = "clustername",
  intensityStr = "sumionarea_protein_",
  nonZeroCols = "qusm")
fcData2d <- tpp2dComputeFoldChanges(data = datIn)
normData2d <- tpp2dNormalize(data = fcData2d)
analysisResults <- tpp2dSplineFitAndTest(data = normData2d,
  dataRef = trRef,
  refIDVar = "Protein_ID",
  refFcStr = "norm_rel_fc_protein_",
  doPlot = FALSE,
  nCores = 1)
```

---

tpp2dSplinePlot      *Fit splines and generate ggplot visualizations*


---

### Description

Fit splines through TR reference dataset and extrapolates relative 2D-TPP datapoints, then compares spline fits of different treatments with non-treatment with an f-test

### Usage

```
tpp2dSplinePlot(
  data_2D = NULL,
  trRef = NULL,
  fcStr = NULL,
  idVar = NULL,
  refIdVar = "Protein_ID",
  methods = c("doseResponse", "splineFit"),
  refFcStr = "norm_rel_fc_protein_",
  verbose = FALSE
)
```

### Arguments

data_2D	result data.frame from a 2D-TPP CCR analysis
trRef	character string of a valid system path to a TPP-TR reference RData object
fcStr	character string indicating how columns that will contain the actual fold change values will be called. The suffix fcStr will be pasted in front of the names of the experiments.
idVar	character string indicating name of the columns containing the unique protein identifiers in the 2D data set
refIdVar	character string indicating name of the columns containing the unique protein identifiers in the reference data set
methods	vector of character strings that indicate which methods has been used for the previous analysis (default: c("doseResponse"), alternative: c("splineFit") or c("doseResponse", "splineFit"))
refFcStr	character string indicating how columns that will contain the fold change values in the reference data set
verbose	print description of problems for each protein for which splines fits could not be performed

### Value

A list of ggplots which can be accessed via the unique protein ids in the idVar column

**Examples**

```
load(system.file("example_data/2D_example_data/shortData2d.RData", package="TPP"))
trRef <- system.file("example_data/2D_example_data/referenceNormData.RData", package="TPP")
```

---

tpp2dTRReferenceObject

*TPP-TR reference object*

---

**Description**

Definition of a TPP-TR reference object

**Usage**

```
tpp2dTRReferenceObject(  
  tppRefData = NULL,  
  tppRefPath = NULL,  
  fcStr = "norm_rel_fc",  
  qualColName = "qupm"  
)
```

**Arguments**

tppRefData	TPP-TR reference object that can be directly passed to the function
tppRefPath	character string containing a system path to a RData file containing an TPP-TR reference object
fcStr	character string indicating which columns contain the fold changes
qualColName	character string indicating which column contain protein identification quality measures

**Value**

A TPP-TR reference object

**Examples**

```
trRef <- system.file("example_data/2D_example_data/referenceNormData.RData", package="TPP")
tpp2dTRReferenceObject(tppRefPath=trRef)
```

---

tppccrCurveFit	<i>Fit dose response curves</i>
----------------	---------------------------------

---

## Description

tppccrCurveFit fits logistic dose response curves to fold change measurements of a TPP-CCR experiment.

## Usage

```
tppccrCurveFit(
  data = NULL,
  fcTable = NULL,
  cpdEffects = NULL,
  slopeBounds = c(1, 50),
  nCores = "max",
  verbose = FALSE
)
```

## Arguments

data	list of expressionSet objects containing protein fold changes for dose response curve fitting.
fcTable	optional long table with fold changes for each experiment. Can be provided instead of the input argument data.
cpdEffects	optional long table of compound effects per protein and experiment. Can be provided instead of the input argument data.
slopeBounds	bounds on the slope parameter for dose response curve fitting.
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
verbose	print name of each fitted protein to the command line as a means of progress report.

## Details

data is a list of expressionSet objects created by [tppccrImport](#). If desired, it can be already preprocessed by [tppccrNormalize](#) or [tppccrTransform](#). It contains the isobaric labels and administered drug concentrations in the phenoData and user-defined protein properties in the featureData. Protein IDs are stored in the featureNames.

Measurements and compound effects for curve fitting can be provided by the arguments fcTable and cpdEffects, instead of being stored in expressionSets in data.

If specified, fcTable needs to be a long table with column names "id" (the protein names), "concentration" (the fold changes), "labelName" (the isobaric label to each measurement), and "experiment" (e.g. "Vehicle\_1" or "Panobinostat\_1").



If specified, `cpdEffects` needs to be a long table with column names "id" (the protein names), "cpdEff" (character vector of compound effects, may contain NAs), and "experiment" (e.g. "Vehicle\_1" or "Panobinostat\_1").

## Value

A list of `expressionSet` objects storing fold changes, the fitted curve parameters, as well as row and column metadata. In each `expressionSet` `S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein `expNames` can be accessed by `featureNames(S)`. Isobaric labels and the corresponding concentrations are returned by `S$label` and `S$concentration`. The fitted curve parameters are stored in `codefeatureData(S)`.

## See Also

[tppccrImport](#), [tppccrNormalize](#), [tppccrTransform](#)

## Examples

```
data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config,
                          data=hdacCCR_data)
tppccrNorm <- tppccrNormalize(data=tppccrData)
tppccrTransformed <- tppccrTransform(data=tppccrNorm)
tppccrFitted <- tppccrCurveFit(data=tppccrTransformed, nCores=1)
```

---

tppccrImport

*Import TPP-CCR dataset for analysis by the [TPP](#) package.*

---

## Description

`tppccrImport` imports a table of protein fold changes and stores them in an `ExpressionSet` for use in the [TPP](#) package.

## Usage

```
tppccrImport(
  configTable,
  data = NULL,
  idVar = "gene_name",
  fcStr = "rel_fc_",
  naStrs = c("NA", "n/d", "NaN", "<NA>"),
  qualColName = "qupm",
  nonZeroCols = "qssm"
)
```

**Arguments**

<code>configTable</code>	either a dataframe or the path to a spreadsheet. In both cases it specifies necessary information of the TPP-CCR experiment.
<code>data</code>	dataframe containing fold change measurements and additional annotation columns to be imported. Can be used instead of specifying the file path in <code>configTable</code> .
<code>idVar</code>	character string indicating which data column provides the unique identifiers for each protein.
<code>fcStr</code>	character string indicating which columns contain the actual fold change values. Those column names containing the suffix <code>fcStr</code> will be regarded as containing fold change values.
<code>naStrs</code>	character vector indicating missing values in the data table. When reading data from file, this value will be passed on to the argument <code>na.strings</code> in function <code>read.delim</code> .
<code>qualColName</code>	character string indicating which column can be used for additional quality criteria when deciding between different non-unique protein identifiers.
<code>nonZeroCols</code>	character string indicating a column that will be used for filtering out zero values.

**Details**

The imported dataset has to contain measurements obtained by a TPP-CCR experiment. Fold changes need to be pre-computed using the lowest concentration as reference.

The dataset can be specified by filename in the `configTable` argument, or given directly in the `data` argument

The default settings are adjusted to analyze data of the python package `isobarQuant`. You can also customize them for your own dataset.

The `configTable` argument is a dataframe, or the path to a spreadsheet (tab-delimited text-file without quoted strings, or `xlsx` format). Information about each experiment is stored row-wise. It contains the following columns:

- `Path`: location of the datafile. Alternatively, data can be directly handed over by the `data` argument.
- `Experiment`: unique experiment name.
- `Label columns`: each isobaric label names a column that contains the concentration administered for the label in the individual experiments.

During data import, proteins with NAs in the data column specified by `idVar` receive unique generic IDs so that they can be processed by the package.

**Value**

ExpressionSet object storing the measured fold changes, as well as row and column metadata. In each ExpressionSet `S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein `expNames` can be accessed by `featureNames(S)`. Isobaric labels and the corresponding concentrations are returned by `S$label` and `S$concentration`.

**See Also**

[tpptrImport](#), [tppccrCurveFit](#)

**Examples**

```
data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config,
data = hdacCCR_data)
```

---

tppccrNormalize

*Normalize data from TPP-CCR experiments*

---

**Description**

Normalize each fold change column by its median.

**Usage**

```
tppccrNormalize(data)
```

**Arguments**

data            list of expressionSets with measurements to be normalized

**Value**

List of expressionSet objects storing the normalized fold changes, as well as row and column meta-data. In each expressionSet *S*, the fold changes can be accessed by `Biobase::exprs(S)`. Protein names can be accessed by `featureNames(S)`. Isobaric labels and the corresponding concentrations are returned by `S$label` and `S$concentration`.

**Examples**

```
data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config, data = hdacCCR_data)
tppccrNorm <- tppccrNormalize(data=tppccrData)
head(Biobase::exprs(tppccrNorm[[1]]))
```

---

tppccrNormalizeToReference

*Normalize fold changes of TPP-CCR experiment to a reference column*


---

### Description

Normalize fold changes of TPP-CCR experiment to a reference column (usually that with the lowest concentration) to ensure that the transformation by `tppccrTransform` yields values between 0 and 1.

### Usage

```
tppccrNormalizeToReference(data, refCol = NULL)
```

### Arguments

<code>data</code>	expressionSet object containing the data to be normalized
<code>refCol</code>	column number to use as a reference. Will contain only 1s after the normalization.

### Value

List of expressionSet objects storing the normalized fold changes, as well as row and column meta-data. In each expressionSet *S*, the fold changes can be accessed by `Biobase::exprs(S)`. Protein expNames can be accessed by `featureNames(S)`. Isobaric labels and the corresponding concentrations are returned by `S$label` and `S$concentration`.

### Examples

```
data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config, data = hdacCCR_data)
tppccrNorm <- tppccrNormalize(data=tppccrData)
# Normalize to lowest concentration (in the first column):
tppccrNormToRef <- tppccrNormalizeToReference(data=tppccrNorm, refCol=1)
# Obtain results per replicate:
refTransf_replicate1 <- tppccrNormToRef$Panobinostat_1
head(Biobase::exprs(refTransf_replicate1))
# Perform transformation:
tppccrTransformed <- tppccrTransform(data=tppccrNormToRef)
# Obtain transformed measurements per replicate:
transf_replicate1 <- tppccrTransformed$Panobinostat_1
transf_replicate2 <- tppccrTransformed$Panobinostat_2
# Inspect transformed data in replicate 1:
effects_replicate1 <- Biobase::featureData(transf_replicate1)$compound_effect
newData_repl1 <- data.frame(Biobase::exprs(transf_replicate1),
                           Type=effects_replicate1[!is.na(effects_replicate1),])
```

---

tppccrPlotCurves      *Plot dose response curves*

---

### Description

tppccrPlotCurves plots the logistic dose response curves, as well as the underlying fold change measurements for each TPP-CCR experiment in a study.

### Usage

```
tppccrPlotCurves(
  data = NULL,
  fcTable = NULL,
  curvePars = NULL,
  resultPath = NULL,
  ggplotTheme = tppDefaultTheme(),
  nCores = "max",
  verbose = FALSE
)
```

### Arguments

data	list of expressionSet objects containing protein fold changes, as well as fitted curve parameters.
fcTable	optional long table with fold changes for each experiment. Can be provided instead of the input argument data.
curvePars	optional long table of curve parameters per protein and experiment. Can be provided instead of the input argument data.
resultPath	location where to store dose-response curve plots.
ggplotTheme	ggplot theme for dose response curve plots.
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
verbose	print name of each plotted protein to the command line as a means of progress report.

### Details

data is a list of expressionSet objects created by [tppccrCurveFit](#). It contains the isobaric labels and administered drug concentrations in the phenoData and user-defined protein properties (including dose response curve parameters) in the featureData. Protein IDs are stored in the featureNames.

Measurements and compound effects for curve fitting can be provided by the arguments fcTable and cpdEffects, instead of being stored in expressionSets in data.

If specified, `fcTable` needs to be a long table with column names "id" (the protein names), "concentration" (the fold changes), "labelName" (the isobaric label to each measurement), and "experiment" (e.g. "Vehicle\_1" or "Panobinostat\_1").

If specified, `curvePars` needs to be a long table with column names "id" (the protein names), "param" (curve parameter per protein and experiment, see `TPP:::drCurveParamNames(names=TRUE, info=FALSE)` for possibilities), and "experiment" (e.g. "Vehicle\_1" or "Panobinostat\_1").

The dose response curve plots will be stored in a subfolder with name `DoseResponse_Curves` at the location specified by `resultPath`.

### Value

A list of `expressionSet` objects storing fold changes, as well as row and column metadata. In each `expressionSet S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein `expNames` can be accessed by `featureNames(S)`. Isobaric labels and the corresponding concentrations are returned by `S$label` and `S$concentration`. Paths to the produced plots are stored in `codefeature-Data(S)$plot`.

### See Also

[tppccrCurveFit](#), [tppDefaultTheme](#)

### Examples

```
data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config,
                        data=hdacCCR_data)
tppccrNorm <- tppccrNormalize(data=tppccrData)
tppccrTransformed <- tppccrTransform(data=tppccrNorm)
tppccrFitted <- tppccrCurveFit(data=tppccrTransformed, nCores=1)
hdacSubset <- sapply(tppccrFitted, function(d)d[grepl("HDAC", rownames(d)),])
tppccrPlotted <- tppccrPlotCurves(hdacSubset, resultPath=getwd(), nCores = 1)
```

---

tppccrResultTable	<i>Summarize results of a TPP-CCR study</i>
-------------------	---

---

### Description

`tppccrResultTable` summarizes the outcomes of a TPP-CCR study in a results table and includes quality information about the estimated dose response curves.

### Usage

```
tppccrResultTable(data, r2Cutoff = 0.8)
```

**Arguments**

- data** list of expressionSet objects containing protein fold changes, as well as fitted curve parameters.
- r2Cutoff** quality criterion on dose response curve fit.  
 @details data is a list of expressionSet objects created by [tppccrCurveFit](#) or [tppccrPlotCurves](#). It contains the isobaric labels and administered drug concentrations in the phenoData and user-defined protein properties (including dose response curve parameters) in the featureData. Protein IDs are stored in the featureNames.  
 If data is the output of [tppccrPlotCurves](#), plot locations are given in the plot column of the featureData.

**Value**

A data frame in which the results are stored row-wise for each protein, together with the original annotation from the input files.

**See Also**

[tppccrCurveFit](#), [tppccrPlotCurves](#)

**Examples**

```
data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config,
                          data=hdacCCR_data)
tppccrNorm <- tppccrNormalize(data=tppccrData)
tppccrTransformed <- tppccrTransform(data=tppccrNorm)
tppccrFitted <- tppccrCurveFit(data=tppccrTransformed, nCores=1)
tppccrResults <- tppccrResultTable(data=tppccrFitted)
subset(tppccrResults, passed_filter_Panobinostat_1 & passed_filter_Panobinostat_2)
```

---

tppccrTransform	<i>Transform fold changes of TPP-CCR experiment</i>
-----------------	---

---

**Description**

Transform fold changes of TPP-CCR experiment to prepare them for dose response curve fitting.

**Usage**

```
tppccrTransform(data, fcCutoff = 1.5, fcTolerance = 0.1)
```

**Arguments**

**data** expressionSet object containing the data to be transformed.  
**fcCutoff** cutoff for highest compound concentration fold change.  
**fcTolerance** tolerance for the fcCutoff parameter. See details.

**Details**

Only proteins with fold changes bigger than  $[fcCutoff * (1 - fcTolerance)]$  or smaller than  $1 / (fcCutoff * (1 - fcTolerance))$  will be used for curve fitting. Additionally, the proteins fulfilling the fc-Cutoff criterion without tolerance will be marked in the output column `meets_FC_requirement`.

**Value**

List of expressionSet objects storing the transformed fold changes, as well as row and column metadata. In each expressionSet `S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein `expNames` can be accessed by `featureNames(S)`. Isobaric labels and the corresponding concentrations are returned by `S$label` and `S$concentration`.

**Examples**

```

data(hdacCCR_smallExample)
tppccrData <- tppccrImport(configTable=hdacCCR_config, data = hdacCCR_data)
tppccrNorm <- tppccrNormalize(data=tppccrData)
# Perform transformation:
tppccrTransformed <- tppccrTransform(data=tppccrNorm)
# Obtain transformed measurements per replicate:
transf_replicate1 <- tppccrTransformed$Panobinostat_1
transf_replicate2 <- tppccrTransformed$Panobinostat_2
# Inspect transformed data in replicate 1:
effects_replicate1 <- Biobase::featureData(transf_replicate1)$compound_effect
newData_repl1 <- data.frame(Biobase::exprs(transf_replicate1),
                           Type=effects_replicate1[!is.na(effects_replicate1),])

```

---

tppDefaultTheme

*Default ggplot theme for melting curve plots.*


---

**Description**

Default theme to be passed to the gplots produced by the TPP package.

**Usage**

```
tppDefaultTheme()
```



**Details**

Internally, the theme is used as an argument for the function `ggplot2::theme_set` in order to specify the appearance of the melting curve plots.

The specified plot properties include bold font and increased font size for axis labels and title, as well as a 90 degree angle for y axis labels.

**Value**

ggplot theme with default settings for melting plot appearance.

**Examples**

```
# Import data:
data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable=hdacTR_config, data=hdacTR_data)
# Obtain template with default settings:
normRequirements <- tpptrDefaultNormReqs()
print(normRequirements)
# Relax filter on the 10th fold change column for
# normalization set production:
normRequirements$fcRequirements[3,3] <- 0.25
# Perform normalization:
tpptrNorm <- tpptrNormalize(data=tpptrData, normReqs=)
```

---

tppExport

*Produce Excel table of TPP-TR or TPP-CCR experiment.*


---

**Description**

Produce Excel table of TPP-TR or TPP-CCR experiment out of the data frame returned by [tpptrAnalyzeMeltingCurves](#)

**Usage**

```
tppExport(tab, file, expNames = NULL, expColors = NULL)
```

**Arguments**

tab	Table with results of the TPP analysis.
file	path for storing results table
expNames	character vector of experiment names of the same length as expColors.
expColors	character vector of background colors to group the result columns belonging to different experiments.

**Value**

No value returned.

**Examples**

```
data(hdacTR_resultsTable_smallExample)
tppExport(resultTable, "tpptr_example_results.xlsx")
```

---

```
tppQCPlotsCorrelateExperiments
```

*Visually compare fold changes of different TPP experiments.*

---

**Description**

Plot pairwise relationships between the proteins in different TPP experiments.

**Usage**

```
tppQCPlotsCorrelateExperiments(
  tppData,
  annotStr = "",
  path = NULL,
  ggplotTheme = tppDefaultTheme()
)
```

**Arguments**

tppData	List of expressionSets with data to be plotted.
annotStr	String with additional information to be added to the plot.
path	Location where to store resulting plot.
ggplotTheme	ggplot theme for the created plots.

**Value**

List of plots for each experiment.

**See Also**

[tppDefaultTheme](#)

**Examples**

```
data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable=hdacTR_config, data=hdacTR_data)
# Quality control (QC) plots BEFORE normalization:
tppQCPlotsCorrelateExperiments(tppData=tpptrData,
  annotStr="Non-normalized Fold Changes")
# Quality control (QC) plots AFTER normalization:
tpptrNorm <- tpptrNormalize(data=tpptrData, normReqs=tpptrDefaultNormReqs())
tpptrDataNormalized <- tpptrNorm$normData
```

```
tppQCPlotsCorrelateExperiments(tppData=tpptrDataNormalized,
  annotStr="Normalized Fold Changes")
```

---

tppRefData

*Example of a reference dataset for 2D-TPP experiments.*


---

### Description

Reference dataset obtained by TPP-TR experiments without drug treatment on HepG2 cell lines.

### Details

tppRefData is a list of data frames that contains TPP-TR measurements for a large number of proteins in wide format. The experiments were performed in two replicates. It can be used as a reference for normalization of 2D-TPP data. See the vignette for the 2D workflow for details.

---

tpptrAnalyzeMeltingCurves

*Analyze fitted curve parameters to detect significant shifts in melting points.*


---

### Description

Compute p-values for the pairwise comparisons of melting curve shifts between different conditions.

### Usage

```
tpptrAnalyzeMeltingCurves(
  data,
  pValMethod = "robustZ",
  pValFilter = list(minR2 = 0.8, maxPlateau = 0.3),
  pValParams = list(binWidth = 300)
)
```

### Arguments

data	list of ExpressionSets containing fold changes and metadata. Their featureData fields contain the fitted melting curve parameters.
pValMethod	Method for p-value computation. Currently restricted to 'robustZ' (see Cox & Mann (2008)).
pValFilter	optional list of filtering criteria to be applied before p-value computation.
pValParams	optional list of parameters for p-value computation.

**Details**

The `pValParams` argument is a list that can contain optional parameters for the chosen p-value computation `pValMethod`. The following options are available:

1. `pValMethod = "robustZ"`:  
`pValParams=list(binWidth=[your_binWidth])`.

**Value**

A data frame in which the fit results are stored row-wise for each protein.

**References**

Cox, J., & Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized ppb-range mass accuracies and proteome-wide protein quantification. *Nature biotechnology*, 26(12), 1367-1372.

**Examples**

```
data(hdacTR_smallExample)
tpptrData <- tpptrImport(hdacTR_config, hdacTR_data)
tpptrNorm <- tpptrNormalize(data=tpptrData,
                           normReqs=tpptrDefaultNormReqs())
normalizedData <- tpptrNorm$normData
## Not run:
# Fit melting curves to each protein
# (can take some time depending on device used):
fittedData <- tpptrCurveFit(normalizedData, nCores=1)
resultTable <- tpptrAnalyzeMeltingCurves(fittedData)
subset(resultTable, fulfills_all_4_requirements)$Protein_ID

## End(Not run)
```

---

tpptrCurveFit

*Fit melting curves to all proteins in a dataset.*

---

**Description**

Fit melting curves to all proteins in a dataset.

**Usage**

```
tpptrCurveFit(
  data,
  dataCI = NULL,
  resultPath = NULL,
  ggplotTheme = tppDefaultTheme(),
```

```

doPlot = TRUE,
startPars = c(P1 = 0, a = 550, b = 10),
maxAttempts = 500,
nCores = "max",
verbose = FALSE
)

```

### Arguments

data	list of ExpressionSets with protein fold changes for curve fitting.
dataCI	list of ExpressionSets with protein fold change confidence intervals for curve fitting. Default to NULL.
resultPath	location where to store the melting curve plots.
ggplotTheme	ggplot theme for melting curve plots.
doPlot	boolean value indicating whether melting curves should be plotted, or whether just the curve parameters should be returned.
startPars	start values for the melting curve parameters. Will be passed to function <a href="#">nls</a> for curve fitting.
maxAttempts	maximal number of curve fitting attempts if model does not converge.
nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
verbose	plot name of each fitted protein to the command lin as a means of progress report.

### Details

If the melting curve fitting procedure does not converge, it will be repeatedly started from perturbed starting parameters (maximum iterations defined by argument `maxAttempts`)

If `doPlot = TRUE`, melting curves are be plotted in individual files per protein. Each file is named by its unique identifier. Filenames are truncated to 255 characters (requirement by most operation systems). Truncated filenames are indicated by the suffix "\_truncated[d]", where [d] is a unique number to avoid redundancies.

The melting curve plots will be stored in a subfolder with name `Melting_Curves` at the location specified by `resultPath`.

### Value

A list of ExpressionSets storing the data together with the melting curve parameters for each experiment. Each ExpressionSet contains the measured fold changes, as well as row and column metadata. In each ExpressionSet `S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein expNames can be accessed by `featureNames(S)`. Isobaric labels and the corresponding temperatures are returned by `S$label` and `S$temperature`.

### See Also

[tppDefaultTheme](#)

**Examples**

```

data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable=hdacTR_config, data=hdacTR_data)
tpptrNorm <- tpptrNormalize(data=tpptrData, normReqs=tpptrDefaultNormReqs())
normalizedData <- tpptrNorm$normData
hdacSubsets <- lapply(normalizedData,
                      function(d) d[grepl("HDAC", Biobase::featureNames(d))])
tpptrFittedHDACs <- tpptrCurveFit(hdacSubsets, nCores=1)
# Show estimated parameters for vehicle and treatment experiments:
Biobase::pData(Biobase::featureData(tpptrFittedHDACs[["Vehicle_1"]]))
Biobase::pData(Biobase::featureData(tpptrFittedHDACs[["Panobinostat_1"]]))

```

---

tpptrDefaultNormReqs    *Default filter criteria for fold change normalization*

---

**Description**

Filter criteria as described in the publication.

**Usage**

```
tpptrDefaultNormReqs()
```

**Value**

List with two entries: 'fcRequirements' describes filtering requirements on fold change columns, 'otherRequirements' contains criteria on additional metadata columns.

**Examples**

```

data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable=hdacTR_config, data=hdacTR_data)
tpptrNorm <- tpptrNormalize(data=tpptrData, normReqs=tpptrDefaultNormReqs())

```

---

tpptrFitSplines        *Perform spline fitting*

---

**Description**

Fit natural splines to all proteins in a dataset.

**Usage**

```

tpptrFitSplines(
  data,
  factorsH1,
  factorsH0 = character(0),
  splineDF = 3:7,
  computeAUC = NULL,
  returnModels = TRUE,
  nCores = "max"
)

```

**Arguments**

<code>data</code>	the data to be fitted
<code>factorsH1</code>	which factors should be included in the alternative model?
<code>factorsH0</code>	which factors should be included in the null model?
<code>splineDF</code>	degrees of freedom for natural spline fitting.
<code>computeAUC</code>	DEPRECATED
<code>returnModels</code>	should the linear models be returned in a column of the result table? Activation increases memory requirements.
<code>nCores</code>	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).

Argument `splineDF` specifies the degrees of freedom for natural spline fitting. As a single numeric value, it is directly passed on to the `splineDF` argument of `splines::ns`. Experience shows that `splineDF = 4` yields good results for TPP data sets with 10 temperature points. It is also possible to provide a numeric vector. In this case, splines are fitted for each entry and the optimal value is chosen per protein using Akaike's Information criterion.

**Value**

A table containing the fitted models per protein

**See Also**

[ns](#), [AICc](#)

**Examples**

```

data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable = hdacTR_config, data = hdacTR_data)
normResults <- tpptrNormalize(data = tpptrData,
  normReqs = tpptrDefaultNormReqs())
normData_eSets <- normResults$normData
normData_longTable <- tpptrTidyUpESETS(normData_eSets)
hdacSubset <- subset(normData_longTable, grepl("HDAC", uniqueID))
hdacSplineFits <- tpptrFitSplines(data = hdacSubset,
  factorsH1 = c("condition"),

```

nCores = 1)

---

tpptrFTest

*Analyze spline fits to detect differential behavior over time*

---

### Description

Analyze fitted natural spline models and look for differential behaviour between conditions by a moderated F-test.

### Usage

```
tpptrFTest(fittedModels, doPlot = FALSE, resultPath = NULL)
```

### Arguments

`fittedModels` a table of fitted spline models (produced by `tpptrFitSplines`).

`doPlot` boolean value indicating whether QC plots should be produced. Currently, QC plots comprise distributions of the F statistics, and the p-values before/ after Benjamini Hochberg adjustment.

`resultPath` location where to store QC plots, if `doPlot = TRUE`.

### Details

If `doPlot` is `TRUE`, but no `resultPath` is specified, the plots will be prompted to the active device. The moderated F-statistic is calculated by the following equation: ...

### Value

A long table containing the hypothesis test results per protein.

### See Also

[ns](#), [squeezeVar](#)

### Examples

```
data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable = hdacTR_config, data = hdacTR_data)
normResults <- tpptrNormalize(data = tpptrData, normReqs = tpptrDefaultNormReqs())
normData_eSets <- normResults$normData
fitData <- tpptrTidyUpESets(normData_eSets)
fits <- tpptrFitSplines(data = fitData, factorsH1 = "condition", nCores = 1, splineDF = 4:5)
testResults <- tpptrFTest(fittedModels = fits)
```



---

 tpptrImport
 

---



---

*Import TPP-TR datasets for analysis by the [TPP](#) package.*


---

### Description

tpptrImport imports several tables of protein fold changes and stores them in a list of Expression-Sets for use in the [TPP](#) package.

### Usage

```
tpptrImport(
  configTable,
  data = NULL,
  idVar = "gene_name",
  fcStr = "rel_fc_",
  naStrs = c("NA", "n/d", "NaN"),
  qualColName = "qupm",
  outputFormat = "eSetList"
)
```

### Arguments

configTable	either a dataframe or the path to a spreadsheet. In both cases it specifies necessary information of the TPP-CCR experiment.
data	single dataframe, or list of dataframes, containing fold change measurements and additional annotation columns to be imported. Can be used instead of specifying the file path in configTable.
idVar	character string indicating which data column provides the unique identifiers for each protein.
fcStr	character string indicating which columns contain the actual fold change values. Those column names containing the suffix fcStr will be regarded as containing fold change values.
naStrs	character vector indicating missing values in the data table. When reading data from file, this value will be passed on to the argument na.strings in function read.delim.
qualColName	character string indicating which column can be used for additional quality criteria when deciding between different non-unique protein identifiers.
outputFormat	output format. Either "eSetList" to obtain output in the same way as previously (will be deprecated soon), or "tidy" to obtain a

### Details

The imported datasets have to contain measurements obtained by TPP-TR experiments. Fold changes need to be pre-computed using the lowest temperature as reference.

An arbitrary number of datasets can be specified by filename in the `Path`-column of the `configTable` argument, or given directly as a list of dataframes in the `data` argument. They can differ, for example, by biological replicate or by experimental condition (for example, treatment versus vehicle). Their names are defined uniquely by the `Experiment` column in `configTable`. Experimental conditions can be specified by an optional column in `configTable`.

The default settings are adjusted to analyze data of the python package `isobarQuant`. You can also customize them for your own dataset.

The `configTable` argument is a dataframe, or the path to a spreadsheet (tab-delimited text-file without quoted strings, or `xlsx` format). Information about each experiment is stored row-wise. It contains the following columns:

- `Path`: location of each datafile. Alternatively, data can be directly handed over by the `data` argument.
- `Experiment`: unique experiment names.
- `Condition`: experimental conditions of each dataset.
- `Label columns`: each isobaric label names a column that contains the temperatures administered for the label in the individual experiments.

Proteins with NAs in the `data` column specified by `idVar` receive unique generic IDs so that they can be processed by the package.

### Value

A list of `ExpressionSets` storing the imported data for experiment. Each `ExpressionSet` contains the measured fold changes, as well as row and column metadata. In each `ExpressionSet S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein `expNames` can be accessed by `featureNames(S)`. Isobaric labels and the corresponding temperatures are returned by `S$label` and `S$temperature`

### See Also

[tppccrImport](#)

### Examples

```
data(hdacTR_smallExample)
tpptrData <- tpptrImport(hdacTR_config, hdacTR_data)
```

---

tpptrNormalize

*Normalize protein fold changes*

---

### Description

Normalizes fold changes determined by TPP-TR experiments over different experimental groups.

**Usage**

```

tpptrNormalize(
  data,
  normReqs = tpptrDefaultNormReqs(),
  qcPlotTheme = tppDefaultTheme(),
  qcPlotPath = NULL,
  startPars = c(P1 = 0, a = 550, b = 10),
  maxAttempts = 1,
  fixedReference = NULL
)

```

**Arguments**

<code>data</code>	List of ExpressionSets with protein fold changes to be normalized.
<code>normReqs</code>	List of filtering criteria for construction of the normalization set.
<code>qcPlotTheme</code>	ggplot theme for the created plots
<code>qcPlotPath</code>	location where plots of the curves fitted to the normalization set medians should be stored.
<code>startPars</code>	start values for the melting curve parameters. Will be passed to function <code>nls</code> for curve fitting.
<code>maxAttempts</code>	maximal number of curve attempts to fit melting curve to fold change medians when computing normalization factors.
<code>fixedReference</code>	name of a fixed reference experiment for normalization. If NULL (default), the experiment with the best R2 when fitting a melting curve through the median fold changes is chosen as the reference.

**Details**

Performs normalization of all fold changes in a given list of ExpressionSets. The normalization procedure is described in detail in Savitski et al. (2014). Whether normalization needs to be performed and what method is best suited depends on the experiment. Here we provide a reasonable solution for the data at hand.

We distinguish between filtering conditions on fold changes and on additional annotation columns. Correspondingly, `normReqs` contains two fields, `fcFilters` and `otherFilters`. Each entry contains a data frame with three columns specifying the column to be filtered, as well as upper and lower bounds. An example is given by `tpptrDefaultNormReqs`.

**Value**

A list of ExpressionSets storing the normalized data for each experiment. Each ExpressionSet contains the measured fold changes, as well as row and column metadata. In each ExpressionSet `S`, the fold changes can be accessed by `Biobase::exprs(S)`. Protein `expNames` can be accessed by `featureNames(S)`. Isobaric labels and the corresponding temperatures are returned by `S$label` and `S$temperature`

## References

Savitski, M. M., Reinhard, F. B., Franken, H., Werner, T., Savitski, M. F., Eberhard, D., ... & Drewes, G. (2014). Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science*, 346(6205), 1255784.

Franken, H, Mathieson, T, Childs, D, Sweetman, G, Werner, T, Huber, W. & Savitski, M. M. (2015), Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. *Nature protocols* 10(10), 1567-1593.

## See Also

[tpptrImport](#)

## Examples

```
data(hdacTR_smallExample)
tpptrData <- tpptrImport(hdacTR_config, hdacTR_data)
tpptrNorm <- tpptrNormalize(data=tpptrData, normReqs=tpptrDefaultNormReqs())
names(tpptrNorm)
```

---

tpptrPlotSplines

*Plot spline fits per protein*

---

## Description

Plot spline fits per protein

## Usage

```
tpptrPlotSplines(  
  data,  
  factorsH1 = NULL,  
  factorsH0 = NULL,  
  fittedModels,  
  testResults,  
  resultPath = NULL,  
  individual = TRUE,  
  overview = FALSE,  
  returnPlots = FALSE,  
  control = list(nCores = "max", maxRank = 500, highlightBelow = 0.05),  
  maxRank = NULL,  
  highlightBelow = NULL,  
  plotIndividual = NULL,  
  plotAlphabetical = NULL  
)
```

**Arguments**

<code>data</code>	long table of proteins measurements that were used for spline fitting.
<code>factorsH1</code>	DEPRECATED
<code>factorsH0</code>	DEPRECATED
<code>fittedModels</code>	long table of fitted models. Output of <code>tpptrFitSplines</code> .
<code>testResults</code>	long table of p-values per protein. Output of <code>tpptrFTest</code> .
<code>resultPath</code>	an optional character vector with the name of the path where the plots should be saved.
<code>individual</code>	logical. Export each plot to individual files?
<code>overview</code>	logical. Generate summary pdfs?
<code>returnPlots</code>	logical. Should the ggplot objects be returned as well?
<code>control</code>	a list of general settings.
<code>maxRank</code>	DEPRECATED
<code>highlightBelow</code>	DEPRECATED
<code>plotIndividual</code>	DEPRECATED
<code>plotAlphabetical</code>	DEPRECATED Contains the following fields: <ul style="list-style-type: none"> <li><code>nCores</code>: number of CPUs for parallel production of plots per protein if <code>individual = TRUE</code> (default: "max")</li> <li><code>maxRank</code>: how many of the top hits should be plotted if <code>overview = TRUE</code> (default: 500)</li> <li><code>highlightBelow</code>: maximum adjusted p-value for which a protein is highlighted by a different background color if <code>overview = TRUE</code> (default: 0.05)</li> </ul>

**Details**

Plots of the natural spline fits will be stored in a subfolder with name `Spline_Fits` at the location specified by `resultPath`.

Exporting each plot to individual files (`individual = TRUE`) can cost runtime and the resulting files can be tedious to browse. If you just want to browse the results, use `overview = TRUE` instead.

If `overview = TRUE`, two summary PDFs are created that enable quick browsing through all results. They contain the plots in alphabetical order (`splineFit_alphabetical.pdf`), or ranked by p-values (`splineFit_top_xx.pdf`, where `xx` is the maximum rank defined by `overviewSettings$maxRank`).

**Value**

None

**See Also**

[ns](#), [AICc](#), [tpptrFitSplines](#), [tpptrFTest](#)

**Examples**

```

data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable = hdacTR_config, data = hdacTR_data)
tidyData <- tpptrTidyUpESets(tpptrData)
splineFits <- tpptrFitSplines(data = tidyData, nCores = 1, splineDF = 4:5,
                             factorsH1 = "condition", returnModels = TRUE)
testResults <- tpptrFTest(fittedModels = splineFits, doPlot = FALSE)
tpptrPlotSplines(data = tidyData, fittedModels = splineFits,
                  individual = FALSE,
                  testResults = testResults, resultPath = getwd())

```

---

tpptrSplineFitAndTest *Perform spline fitting and analyze by moderated F-test*

---

**Description**

A wrapper function around the functions `tpptrFitSplines`, `tpptrFTest`, `tpptrPlotSplines`, which fits natural splines to all proteins in a dataset and detect differential behavior between conditions by a moderated F-test. The results are formatted as a wide table with one row per protein. This table contains all the original data, the test results, and (optionally) additional annotation columns for each protein.

**Usage**

```

tpptrSplineFitAndTest(
  data,
  factorsH1,
  factorsH0 = character(),
  resultPath = NULL,
  doPlot = TRUE,
  nCores = "max",
  splineDF = 3:7,
  additionalCols = NULL,
  verbose = NULL,
  ggplotTheme = NULL
)

```

**Arguments**

<code>data</code>	the data to be fitted.
<code>factorsH1</code>	which factors should be included in the alternative model?
<code>factorsH0</code>	which factors should be included in the null model?
<code>resultPath</code>	location where to store the spline plots per protein.
<code>doPlot</code>	boolean value indicating whether melting curves should be plotted, or whether just the curve parameters should be returned.

nCores	either a numerical value given the desired number of CPUs, or 'max' to automatically assign the maximum possible number (default).
splineDF	degrees of freedom for natural spline fitting.
additionalCols	additional annotation per protein to append to the result table.
verbose	DEPRECATED
ggplotTheme	DEPRECATED.

## Details

Plots of the natural spline fits will be stored in a subfolder with name `Spline_Fits` at the location specified by `resultPath`.

Argument `data` can either be long table, or a list of `expressionSets` as returned by `tpptrImport`. If a long table, it needs to contain the following columns: 'uniqueID' (identifier), 'x' (independent variable for fitting, usually the temperature) and 'y' (dependent variable for fitting, usually the relative concentration).

Argument `splineDF` specifies the degrees of freedom for natural spline fitting. As a single numeric value, it is directly passed on to the `splineDF` argument of `splines::ns`. Experience shows that `splineDF = 4` yields good results for TPP data sets with 10 temperature points. It is also possible to provide a numeric vector. In this case, splines are fitted for each entry and the optimal value is chosen per protein using Akaike's Information criterion.

## Value

A data frame in wide format with one row per protein. It contains the smoothing spline parameters and F-test results obtained by comparing the null and alternative models.

## See Also

[ns](#), [AICc](#)

## Examples

```
data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable = hdacTR_config, data = hdacTR_data)
fitData <- tpptrTidyUpEsets(tpptrData)
hdacSplineFits <- tpptrSplineFitAndTest(data = fitData,
                                       factorsH1 = "condition",
                                       nCores = 1,
                                       splineDF = 4:5,
                                       doPlot = FALSE)

# Show estimated splines for HDAC1:
filter(hdacSplineFits, Protein_ID == "HDAC1")
# -> Which proteins showed significant condition effects?
hdacSplineFits %>% filter(p_adj_NPARC <= 0.01) %>% select(Protein_ID, p_adj_NPARC)
# Quality control: test for replicate-specific effects:
testResults <- tpptrSplineFitAndTest(data = fitData,
                                       factorsH1 = "replicate",
                                       nCores = 1,
                                       splineDF = 4,
```

```

                                doPlot = FALSE)
# -> Which proteins showed significant replicate effects?
testResults %>% filter(p_adj_NPARC <= 0.01) %>% select(Protein_ID, p_adj_NPARC)

```

---

tpptrTidyUpESets	<i>Tidy up expressionSets</i>
------------------	-------------------------------

---

## Description

Convert list of expressionSets (intermediate output of several TPP-TR functions) to tidy tables.

## Usage

```
tpptrTidyUpESets(tppESetList, returnType = "exprs")
```

## Arguments

tppESetList	A list of expressionSets, returned by most TPP-TR functions.
returnType	A string with two possible values: "exprs", "featureData".

## Details

expressionSet lists are for example produced by [tpptrImport](#), [tpptrNormalize](#), [tpptrCurveFit](#).

## Value

Either the fold changes per protein across all experiments (if returnType = "exprs"), or the additional annotation per protein and experiment (if returnType = "featureData"). For example, the peptide counts per identified protein can be found [here](#).

## Examples

```

data(hdacTR_smallExample)
tpptrData <- tpptrImport(configTable = hdacTR_config, data = hdacTR_data)
concentrations <- tpptrTidyUpESets(tpptrData)
additionalInfos <- tpptrTidyUpESets(tpptrData, returnType = "featureData")
summary(concentrations)

```



---

TPPTR\_reference\_results\_HepG2

*Example of a reference dataset for 2D-TPP experiments.*

---

### **Description**

Reference dataset obtained by TPP-TR experiments without drug treatment on HepG2 cell lines.

### **Details**

Contains the data object [tppRefData](#).

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