

# Introduction to the *TPP* package for analyzing Thermal Proteome Profiling data: 2D-TPP experiments

***Dorothee Childs, Nils Kurzawa***

European Molecular Biology Laboratory (EMBL),  
Heidelberg, Germany  
dorothee.childs@embl.de

***TPP* version 3.32.0**

## Abstract

Thermal Proteome Profiling (TPP) combines the cellular thermal shift assay concept [1] with mass spectrometry based proteome-wide protein quantitation [2]. Thereby, drug-target interactions can be inferred from changes in the thermal stability of a protein upon drug binding, or upon downstream cellular regulatory events, in an unbiased manner.

The package *TPP* facilitates this process by providing executable workflows that conduct all necessary data analysis steps. Recent advances in the field have lead to the development of so called 2D Thermal Proteome Profiling (2D-TPP) experiments [3]. Recent advances in the field have lead to the development of so called 2D Thermal Proteome Profiling (2D-TPP) experiments [3]. Similar as for the TPP-TR and the TPP-CCR analysis, the function `analyze2DTPP` executes the whole workflow from data import through normalization and curve fitting to statistical analysis. Nevertheless, all of these steps can also be invoked separately by the user. The corresponding functions can be recognized by their suffix `tpp2d`.

Here, we first show how to start the whole analysis using `analyze2DTPP`. Afterwards, we demonstrate how to carry out single steps individually.

For details about the analysis of 1D TR- or CCR experiments [2, 4], please refer to the vignette `TPP_introduction_1D`.

**Note:** There is a new package *TPP2D* available focused on FDR- controlled analysis of 2D-TPP datasets. This workflow is maintained for backwards compatibility, but *TPP2D* is now recommended for analysis. You can find it [here](#)

## Contents

1	Installation . . . . .	2
1.1	Special note for Windows users . . . . .	2
2	Analyzing 2D-TPP experiments. . . . .	3
2.1	Overview . . . . .	3
2.2	Performing the analysis . . . . .	3

## 1 Installation

---

To install the package, type the following commands into the *R* console

```
if (!requireNamespace("BiocManager", quietly=TRUE)){  
  install.packages("BiocManager")  
}  
BiocManager::install("TPP")
```

The installed package can be loaded by

```
library("TPP")
```

### 1.1 Special note for Windows users

The *TPP* package uses the *openxlsx* package to produce Excel output [5]. *openxlsx* requires a zip application to be installed on your system and to be included in the path. On Windows, such a zip application is not installed by default, but is available, for example, via [Rtools](#). Without the zip application, you can still use the 'TPP' package and access its results via the dataframes produced by the main functions.

## 2 Analyzing 2D-TPP experiments

### 2.1 Overview

Before you can start your analysis, you need to specify information about your experiments:

The mandatory information comprises a unique experiment name, as well as the isobaric labels and corresponding temperature values for each experiment. The package retrieves this information from a configuration table that you need to specify before starting the analysis. This table can either be a data frame that you define in your R session, or a spreadsheet in .xlsx or .csv format. In a similar manner, the measurements themselves can either be provided as a list of data frames, or imported directly from files during runtime.

We demonstrate the functionality of the package using the dataset `Panobinostat_2DTPP_smallExampleData`. It contains an illustrative subset of a larger dataset which was obtained by 2D-TPP experiments on HepG2 cells treated with the histone deacetylase (HDAC) inhibitor panobinostat in the treatment groups and with vehicle in the control groups. The experiments were performed for different temperatures. The raw MS data were processed with the Python package `isobarQuant`, which provides protein fold changes relative to the protein abundance at the lowest temperature as input for the TPP package [3].

### 2.2 Performing the analysis

First of all, we load an example data set:

```
data(panobinostat_2DTPP_smallExample, package = "TPP")
```

Using this command we load two objects:

1. `Panobinostat_2DTPP_smallExampleData`: a list of data frames that contain the measurements to be analyzed,
2. `hdac2D_config`: a configuration table with details about each experiment.

```
config_tpp2d <- panobinostat_2DTPP_config
data_tpp2d <- panobinostat_2DTPP_data
```

```
config_tpp2d
```

##	Compound	Experiment	Temperature	126	127L	127H	128L	128H	129L	129H
## 1	Panobinostat	X020466	42.0	5	1	0.143	0.02	0	-	-
## 2	Panobinostat	X020466	44.1	-	-	-	-	-	5	1
## 3	Panobinostat	X020467	46.2	5	1	0.143	0.02	0	-	-
## 4	Panobinostat	X020467	48.1	-	-	-	-	-	5	1
## 5	Panobinostat	X020468	50.4	5	1	0.143	0.02	0	-	-
## 6	Panobinostat	X020468	51.9	-	-	-	-	-	5	1
## 7	Panobinostat	X020469	54.0	5	1	0.143	0.02	0	-	-
## 8	Panobinostat	X020469	56.1	-	-	-	-	-	5	1
## 9	Panobinostat	X020470	58.2	5	1	0.143	0.02	0	-	-
## 10	Panobinostat	X020470	60.1	-	-	-	-	-	5	1
## 11	Panobinostat	X020471	62.4	5	1	0.143	0.02	0	-	-
## 12	Panobinostat	X020471	63.9	-	-	-	-	-	5	1
##	130L	130H	131L	RefCol	Path					
## 1	-	-	-	128H						

```
## 2 0.143 0.02 0 131L
## 3 - - - 128H
## 4 0.143 0.02 0 131L
## 5 - - - 128H
## 6 0.143 0.02 0 131L
## 7 - - - 128H
## 8 0.143 0.02 0 131L
## 9 - - - 128H
## 10 0.143 0.02 0 131L
## 11 - - - 128H
## 12 0.143 0.02 0 131L

data_tpp2d %>% str(1)

## List of 6
## $ X020466: 'data.frame': 484 obs. of 15 variables:
## $ X020467: 'data.frame': 478 obs. of 15 variables:
## $ X020468: 'data.frame': 448 obs. of 15 variables:
## $ X020469: 'data.frame': 372 obs. of 15 variables:
## $ X020470: 'data.frame': 306 obs. of 15 variables:
## $ X020471: 'data.frame': 261 obs. of 15 variables:
```

The data object `Panobinostat_2DTPP_smallExampleData` is organized as a list of data frames which contain the experimental raw data of an 2D-TPP experiment. The names of the list elements correspond to the different multiplexed experiments. Each experimental dataset contains the following columns:

```
data_tpp2d$X020466 %>% colnames

## [1] "clustername"          "representative"
## [3] "msexperiment_id"      "qupm"
## [5] "qusm"                  "sumionarea_protein_126"
## [7] "sumionarea_protein_127L" "sumionarea_protein_127H"
## [9] "sumionarea_protein_128L" "sumionarea_protein_128H"
## [11] "sumionarea_protein_129L" "sumionarea_protein_129H"
## [13] "sumionarea_protein_130L" "sumionarea_protein_130H"
## [15] "sumionarea_protein_131L"
```

In order to perform the complete workflow we can now simply use:

```
tpp2dResults <- analyze2DTPP(configTable = config_tpp2d,
                             data = data_tpp2d,
                             compFc = TRUE,
                             idVar = "representative",
                             intensityStr = "sumionarea_protein_",
                             nonZeroCols = "qusm",
                             addCol = "clustername",
                             methods = "doseResponse",
                             createReport = "none")

tpp2dResults %>% mutate_if(is.character, factor) %>% summary

## Protein_ID norm_rel_fc_protein_0_unmodified
```

```
## X020466_42_IPI00000001.2: 1 Min. :1
## X020466_42_IPI00000005.1: 1 1st Qu.:1
## X020466_42_IPI000000690.1: 1 Median :1
## X020466_42_IPI000000811.2: 1 Mean :1
## X020466_42_IPI000000875.7: 1 3rd Qu.:1
## X020466_42_IPI00001466.2: 1 Max. :1
## (Other) :4650
## norm_rel_fc_protein_0.02_unmodified norm_rel_fc_protein_0.143_unmodified
## Min. :0.1767 Min. :0.2612
## 1st Qu.:0.9192 1st Qu.:0.9364
## Median :1.0000 Median :1.0000
## Mean :1.0035 Mean :1.0105
## 3rd Qu.:1.0727 3rd Qu.:1.0632
## Max. :4.6565 Max. :5.8855
##
## norm_rel_fc_protein_1_unmodified norm_rel_fc_protein_5_unmodified
## Min. : 0.2422 Min. : 0.2512
## 1st Qu.: 0.9344 1st Qu.: 0.9337
## Median : 1.0000 Median : 1.0000
## Mean : 1.0163 Mean : 1.0259
## 3rd Qu.: 1.0654 3rd Qu.: 1.0589
## Max. :10.0240 Max. :17.0405
##
## norm_rel_fc_protein_0_normalized_to_lowest_conc
## Min. :1
## 1st Qu.:1
## Median :1
## Mean :1
## 3rd Qu.:1
## Max. :1
##
## norm_rel_fc_protein_0.02_normalized_to_lowest_conc
## Min. :0.1767
## 1st Qu.:0.9192
## Median :1.0000
## Mean :1.0035
## 3rd Qu.:1.0727
## Max. :4.6565
##
## norm_rel_fc_protein_0.143_normalized_to_lowest_conc
## Min. :0.2612
## 1st Qu.:0.9364
## Median :1.0000
## Mean :1.0105
## 3rd Qu.:1.0632
## Max. :5.8855
##
## norm_rel_fc_protein_1_normalized_to_lowest_conc
## Min. : 0.2422
## 1st Qu.: 0.9344
## Median : 1.0000
```

```

## Mean      : 1.0163
## 3rd Qu.: 1.0654
## Max.      :10.0240
##
## norm_rel_fc_protein_5_normalized_to_lowest_conc
## Min.      : 0.2512
## 1st Qu.: 0.9337
## Median : 1.0000
## Mean      : 1.0259
## 3rd Qu.: 1.0589
## Max.      :17.0405
##
## norm_rel_fc_protein_0_transformed norm_rel_fc_protein_0.02_transformed
## Min.      :0.000                      Min.      : -0.884
## 1st Qu.:0.000                      1st Qu.: -0.154
## Median :1.000                      Median : 0.297
## Mean      :0.621                      Mean      : 0.302
## 3rd Qu.:1.000                      3rd Qu.: 0.614
## Max.      :1.000                      Max.      : 2.542
## NA's      :4421                      NA's      :4421
## norm_rel_fc_protein_0.143_transformed norm_rel_fc_protein_1_transformed
## Min.      : -1.201                      Min.      : -0.961
## 1st Qu.: 0.086                      1st Qu.: 0.095
## Median : 0.376                      Median : 0.313
## Mean      : 0.400                      Mean      : 0.400
## 3rd Qu.: 0.662                      3rd Qu.: 0.652
## Max.      : 3.294                      Max.      : 2.925
## NA's      :4421                      NA's      :4421
## norm_rel_fc_protein_5_transformed      pEC50      slope
## Min.      :0.000                      Min.      :5.728      Min.      : -50.000
## 1st Qu.:0.000                      1st Qu.:6.696      1st Qu.: -12.934
## Median :0.000                      Median :7.769      Median : -1.000
## Mean      :0.379                      Mean      :7.344      Mean      : -8.342
## 3rd Qu.:1.000                      3rd Qu.:8.126      3rd Qu.: 1.159
## Max.      :1.000                      Max.      :8.126      Max.      : 50.000
## NA's      :4421                      NA's      :4421      NA's      :4421
##      R_sq      plot      compound_effect      meets_FC_requirement
## Min.      : -0.068      NA's:4656      destabilized: 146      Mode :logical
## 1st Qu.: 0.545                      stabilized : 89      FALSE:4537
## Median : 0.723                      NA's      :4421      TRUE :119
## Mean      : 0.675
## 3rd Qu.: 0.881
## Max.      : 1.000
## NA's      :4421
## passed_filter      pEC50_outside_conc_range      model_converged
## Mode :logical      Mode :logical      Mode:logical
## FALSE:4601      FALSE:112      TRUE:235
## TRUE :55      TRUE :123      NA's:4421
##      NA's :4421
##
##
##

```

```

##
##      pEC50_quality_check sufficient_data_for_fit protein_identified_in
## 5.72818301656452: 14      Mode:logical      Mode:logical
## 6.07074587494624: 6      TRUE:235          TRUE:4656
## 7.44099730847312: 5      NA's:4421
## 5.86139125662412: 1
## 5.86343472719295: 1
## (Other)          : 85
## NA's             :4544
##      representative      qupm      qusm      clustername
## IPI00000001.2: 12      Min.   : 1.000      Min.   : 1.00      A2M      : 12
## IPI00000005.1: 12      1st Qu.: 3.000      1st Qu.: 5.00      ABHD10   : 12
## IPI00000690.1: 12      Median : 7.000      Median : 11.00      ACAA1    : 12
## IPI00000811.2: 12      Mean   : 9.149      Mean   : 19.57      AC01     : 12
## IPI00000875.7: 12      3rd Qu.:12.000      3rd Qu.: 23.00      AC02     : 12
## IPI00001914.1: 12      Max.   :87.000      Max.   :263.00      ACTC1    : 12
## (Other)          :4584      (Other):4584
## sumionarea_protein_5 sumionarea_protein_1 sumionarea_protein_0.143
## Min.   :2.063e+05      Min.   :3.819e+05      Min.   :3.579e+05
## 1st Qu.:7.696e+07      1st Qu.:7.604e+07      1st Qu.:8.079e+07
## Median :2.511e+08      Median :2.512e+08      Median :2.591e+08
## Mean   :7.182e+08      Mean   :7.542e+08      Mean   :7.554e+08
## 3rd Qu.:7.382e+08      3rd Qu.:7.682e+08      3rd Qu.:7.857e+08
## Max.   :2.125e+10      Max.   :2.138e+10      Max.   :1.924e+10
##
## sumionarea_protein_0.02 sumionarea_protein_0 temperature      experiment
## Min.   :4.335e+05      Min.   :2.925e+05      Min.   :42.0      X020466:968
## 1st Qu.:8.401e+07      1st Qu.:7.345e+07      1st Qu.:46.2      X020467:950
## Median :2.739e+08      Median :2.574e+08      Median :50.4      X020468:894
## Mean   :8.100e+08      Mean   :8.599e+08      Mean   :51.6      X020469:738
## 3rd Qu.:8.331e+08      3rd Qu.:8.554e+08      3rd Qu.:56.1      X020470:600
## Max.   :2.249e+10      Max.   :2.644e+10      Max.   :63.9      X020471:506
##
## rel_fc_protein_5 rel_fc_protein_1 rel_fc_protein_0.143 rel_fc_protein_0.02
## Min.   : 0.3487      Min.   :0.2985      Min.   :0.3887      Min.   : 0.1882
## 1st Qu.: 0.7894      1st Qu.:0.8231      1st Qu.:0.8156      1st Qu.: 0.8413
## Median : 0.8964      Median :0.9197      Median :0.9415      Median : 0.9601
## Mean   : 0.9935      Mean   :0.9753      Mean   :1.0187      Mean   : 1.0974
## 3rd Qu.: 1.0878      3rd Qu.:1.0588      3rd Qu.:1.1447      3rd Qu.: 1.2027
## Max.   :17.1835      Max.   :8.6463      Max.   :6.2354      Max.   :10.0917
##
## rel_fc_protein_0
## Min.   :1
## 1st Qu.:1
## Median :1
## Mean   :1
## 3rd Qu.:1
## Max.   :1
##

```

Moreover, we can also invoke the single functions of the workflow manually. Therefore, we start with importing the data. Using the import function the data is subsequently imported and stored in a single dataframe containing all the required data columns and those that the user likes to take along through the analysis to be displayed together with the results of this workflow.



## TPP

```
data2d <- tpp2dImport(configTable = config_tpp2d,
  data = data_tpp2d,
  idVar = "representative",
  intensityStr = "sumionarea_protein_",
  nonZeroCols = "qusm",
  addCol = "clustername")

head(data2d)

##   representative qupm qusm clustername sumionarea_protein_5
## 1 IPI00000001.2   15  25      STAU1          1193994914
## 2 IPI00000001.2   15  25      STAU1          1272771185
## 3 IPI00000001.2   13  22      STAU1          1482437522
## 4 IPI00000001.2   13  22      STAU1          1157290962
## 5 IPI00000001.2   15  24      STAU1          396823892
## 6 IPI00000001.2   15  24      STAU1          345169960
##   sumionarea_protein_1 sumionarea_protein_0.143 sumionarea_protein_0.02
## 1          1337957734                1375948494          1956350223
## 2          1473572092                1273285951          1669312103
## 3          1513181000                1284434575          1487032006
## 4          1050288621                1110810226          1128507681
## 5           458022616                453860821          412257039
## 6           350182409                352193788          344410388
##   sumionarea_protein_0 temperature experiment          unique_ID
## 1          1801848318          42.0      X020466 X020466_42_IPI00000001.2
## 2          1404292404          44.1      X020466 X020466_44.1_IPI00000001.2
## 3          1422365645          46.2      X020467 X020467_46.2_IPI00000001.2
## 4           999666282          48.1      X020467 X020467_48.1_IPI00000001.2
## 5           439399665          50.4      X020468 X020468_50.4_IPI00000001.2
## 6           309019704          51.9      X020468 X020468_51.9_IPI00000001.2

attr(data2d, "importSettings")

## $proteinIdCol
## [1] "representative"
##
## $uniqueIdCol
## [1] "unique_ID"
##
## $addCol
## [1] "clustername"
##
## $intensityStr
## [1] "sumionarea_protein_"
##
## $qualColName
## [1] "qupm"
##
## $nonZeroCols
## [1] "qusm"
##
## $fcStr
## NULL
```

If we haven't computed fold changes from the raw "sumionarea" data, as it is the case in this example, we can invoke the function `tpp2dComputeFoldChanges` in order to do so:

```
fcData2d <- tpp2dComputeFoldChanges(data = data2d)
```

Thereon the function adds additional columns to our dataframe containing corresponding fold changes:

```
head(fcData2d)

## representative qupm qusm clustername sumionarea_protein_5
## 1 IPI00000001.2 15 25 STAU1 1193994914
## 2 IPI00000001.2 15 25 STAU1 1272771185
## 3 IPI00000001.2 13 22 STAU1 1482437522
## 4 IPI00000001.2 13 22 STAU1 1157290962
## 5 IPI00000001.2 15 24 STAU1 396823892
## 6 IPI00000001.2 15 24 STAU1 345169960
## sumionarea_protein_1 sumionarea_protein_0.143 sumionarea_protein_0.02
## 1 1337957734 1375948494 1956350223
## 2 1473572092 1273285951 1669312103
## 3 1513181000 1284434575 1487032006
## 4 1050288621 1110810226 1128507681
## 5 458022616 453860821 412257039
## 6 350182409 352193788 344410388
## sumionarea_protein_0 temperature experiment unique_ID
## 1 1801848318 42.0 X020466 X020466_42_IPI00000001.2
## 2 1404292404 44.1 X020466 X020466_44.1_IPI00000001.2
## 3 1422365645 46.2 X020467 X020467_46.2_IPI00000001.2
## 4 999666282 48.1 X020467 X020467_48.1_IPI00000001.2
## 5 439399665 50.4 X020468 X020468_50.4_IPI00000001.2
## 6 309019704 51.9 X020468 X020468_51.9_IPI00000001.2
## rel_fc_5 rel_fc_1 rel_fc_0.143 rel_fc_0.02 rel_fc_0
## 1 0.6626501 0.7425474 0.7636317 1.0857463 1
## 2 0.9063434 1.0493342 0.9067100 1.1887212 1
## 3 1.0422338 1.0638481 0.9030270 1.0454640 1
## 4 1.1576773 1.0506392 1.1111810 1.1288844 1
## 5 0.9031047 1.0423827 1.0329112 0.9382279 1
## 6 1.1169837 1.1332041 1.1397130 1.1145257 1
```

We can then normalize the data by performing a median normalization on the fold changes, in order to account for experiment specific noise.

```
normData2d <- tpp2dNormalize(data = fcData2d)
head(normData2d)

## representative qupm qusm clustername sumionarea_protein_5
## 1 IPI00000001.2 15 25 STAU1 1193994914
## 2 IPI00000001.2 15 25 STAU1 1272771185
## 3 IPI00000001.2 13 22 STAU1 1482437522
## 4 IPI00000001.2 13 22 STAU1 1157290962
## 5 IPI00000001.2 15 24 STAU1 396823892
## 6 IPI00000001.2 15 24 STAU1 345169960
## sumionarea_protein_1 sumionarea_protein_0.143 sumionarea_protein_0.02
## 1 1337957734 1375948494 1956350223
```

## TPP

```
## 2      1473572092      1273285951      1669312103
## 3      1513181000      1284434575      1487032006
## 4      1050288621      1110810226      1128507681
## 5      458022616      453860821      412257039
## 6      350182409      352193788      344410388
##      sumionarea_protein_0 temperature experiment      unique_ID
## 1      1801848318      42.0      X020466      X020466_42_IPI00000001.2
## 2      1404292404      44.1      X020466      X020466_44.1_IPI00000001.2
## 3      1422365645      46.2      X020467      X020467_46.2_IPI00000001.2
## 4      999666282      48.1      X020467      X020467_48.1_IPI00000001.2
## 5      439399665      50.4      X020468      X020468_50.4_IPI00000001.2
## 6      309019704      51.9      X020468      X020468_51.9_IPI00000001.2
##      rel_fc_5      rel_fc_1      rel_fc_0.143      rel_fc_0.02      rel_fc_0      norm_rel_fc_5
## 1 0.6626501 0.7425474 0.7636317 1.0857463 1 1.107187
## 2 0.9063434 1.0493342 0.9067100 1.1887212 1 1.114453
## 3 1.0422338 1.0638481 0.9030270 1.0454640 1 1.187727
## 4 1.1576773 1.0506392 1.1111810 1.1288844 1 1.249516
## 5 0.9031047 1.0423827 1.0329112 0.9382279 1 1.123552
## 6 1.1169837 1.1332041 1.1397130 1.1145257 1 1.171933
##      norm_rel_fc_1      norm_rel_fc_0.143      norm_rel_fc_0.02      norm_rel_fc_0
## 1 1.059331 1.105019 1.244416 1
## 2 1.164559 1.022695 1.195813 1
## 3 1.229422 1.078735 1.211522 1
## 4 1.147406 1.108487 1.329434 1
## 5 1.268366 1.267164 1.256324 1
## 6 1.176446 1.158041 1.163566 1
```

To run the TPP-CCR main function on our 2D-TPP data we now invoke:

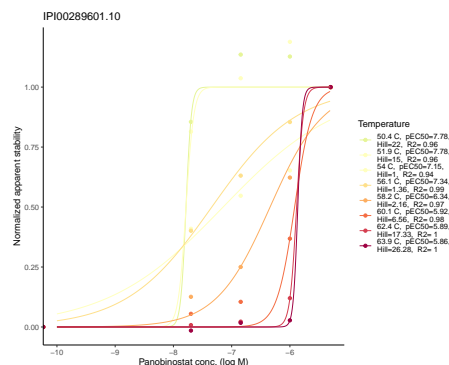
```
ccr2dResults <- tpp2dCurveFit(data = normData2d)
```

Now we can plot the curves for any of the proteins for which at least one CCR curve could be fitted. In this case we choose HDAC2:

```
drPlots <- tpp2dCreateDRplots(data = ccr2dResults, type = "good")
```

```
# Find IPI id for HDAC2 (in column representative):
IPI_id_HDAC2 <- unique(filter(ccr2dResults, clustername == "HDAC2")$representative)

# Show corresponding plot:
drPlots[[IPI_id_HDAC2]]
```



And we can also plot the single curves for each of the proteins with:

## 2.3 Quality control analyses

In order to start this workflow the first thing we need to do, is to generate a cell line specific TR reference object. We also need to specify the result path where this object should be stored:

We then load the TPP TR example data and modify it to use only replicate 1 of the vehicle condition as our reference experiment:

```
tp2dCreateTPPTRreference(trConfigTable = trConfig,
                        trDat = hdacTR_data[1:2],
                        resultPath = resultPath,
                        outputName = "desired_file_name",
                        createFCboxplots = FALSE)
```

Calling the function will generate a couple of output files in the indicated output directory.

## TPP

- The `tppRefData.RData` is a dataset that can be used to assess temperature-range melting behavior of proteins captured with 2D-TPP e.g. for checking whether stabilization effects happen at temperatures close to the melting point of individual proteins. When loaded in *R* the object `tppRefData` represents a list with the following elements:
  - `tppCfgTable`: the TPP-TR configtable which was used for generating this object
  - `sumResTable` a list of two elements:
    - `detail`: the exact result data from the TR analysis and
    - `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)
  - `temperatures`: a table listing the temperatures which were used in the TR experiment in the different replicates
  - `lblsByTemp`: a table matching each temperature to an isobaric label
- An excel file which summarizes the data present in `tppRefData` on different sheets
- Textfiles representing the sheets of the excel file as plain text
- `normalizedData.RData` containing the TPP-TR data after normalization
- `resultTable.RData` containing the TPP-TR analysis result table

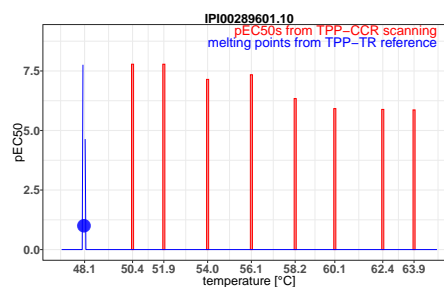
Secondly, we can generate plots which visualize the melting point temperatures of the 2D-TPP data in comparison to the TR reference data. Here we demonstrate this function on a subset of the proteins:

```
# set the system path for the HepG2 TR reference data set:
trRef <- file.path(system.file("data", package="TPP"),
  "TPPTR_reference_results_HepG2.RData")

plotData <- ccr2dResults %>% filter(clustername == "HDAC2")

pEC50QC_HDAC1 <- tpp2dPlotQCpEC50(resultTable = plotData,
  resultPath = NULL,
  trRef = trRef,
  idVar = "representative")

pEC50QC_HDAC1[[1]]
```



We have therefore used the `ccr2dResults` data frame which we previously generated by invoking the TPP-CCR routine and the the respective configTable.

Moreover, we can generate plots that visualize the distributions of fold changes over the different treatment concentrations and temperatures and how the normalization affected them (of course only if we previously performed a normalization). The function automatically also visualizes various other characteristics of the data, such as how proteins behave in neighboring temperatures which are multiplexed. It can be invoked as follows:

```
tpp2dPlotQChist(configFile = config_tpp2d,  
                resultTable = ccr2dResults,  
                resultPath = resultPath,  
                trRef = trRef,  
                idVar = "representative")
```

## References

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