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

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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 exectuable 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`.

Contents

1 Installation

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

```
source("http://bioconductor.org/biocLite.R")
biocLite("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 ist 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 directlyfrom 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

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

```
## 11   128H
## 12   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 constains the following columns:

```
data_tpp2d$X020466 %>% colnames
```

```
##  [1] "clustername"             "representative"          "msexperiment_id"
##  [4] "qupm"                    "qusm"                    "sumionarea_protein_126"
##  [7] "sumionarea_protein_127L" "sumionarea_protein_127H" "sumionarea_protein_128L"
## [10] "sumionarea_protein_128H" "sumionarea_protein_129L" "sumionarea_protein_129H"
## [13] "sumionarea_protein_130L" "sumionarea_protein_130H" "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")
```

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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_IPI00000690.1:   1   Median :1
##  X020466_42_IPI00000811.2:   1   Mean   :1
##  X020466_42_IPI00000875.7:   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 norm_rel_fc_protein_0_transformed
##  Min.   : 0.2512                                 Min.   :0.000
##  1st Qu.: 0.9337                                 1st Qu.:0.000
##  Median : 1.0000                                 Median :1.000
##  Mean   : 1.0259                                 Mean   :0.621
##  3rd Qu.: 1.0589                                 3rd Qu.:1.000
##  Max.   :17.0405                                 Max.   :1.000
##                                                  NA's   :4421
##  norm_rel_fc_protein_0.02_transformed norm_rel_fc_protein_0.143_transformed
##  Min.   :-0.884                       Min.   :-1.201
##  1st Qu.:-0.154                       1st Qu.: 0.086
##  Median : 0.297                       Median : 0.376
##  Mean   : 0.302                       Mean   : 0.400
##  3rd Qu.: 0.614                       3rd Qu.: 0.662
##  Max.   : 2.542                       Max.   : 3.294
##  NA's   :4421                         NA's   :4421
##  norm_rel_fc_protein_1_transformed norm_rel_fc_protein_5_transformed     pEC50
##  Min.   :-0.961                    Min.   :0.000                     Min.   :5.728
##  1st Qu.: 0.095                    1st Qu.:0.000                     1st Qu.:6.696
##  Median : 0.313                    Median :0.000                     Median :7.778
##  Mean   : 0.400                    Mean   :0.379                     Mean   :7.346
##  3rd Qu.: 0.652                    3rd Qu.:1.000                     3rd Qu.:8.126
##  Max.   : 2.925                    Max.   :1.000                     Max.   :8.126
##  NA's   :4421                      NA's   :4421                      NA's   :4421
##      slope              R_sq          plot          compound_effect meets_FC_requirement
##  Min.   :-50.000   Min.   :-0.068   NA's:4656   destabilized: 146   Mode :logical
##  1st Qu.:-10.804   1st Qu.: 0.545               stabilized  :  89   FALSE:4537
##  Median : -1.000   Median : 0.723               NA's        :4421   TRUE :119
##  Mean   : -8.302   Mean   : 0.675
##  3rd Qu.:  1.159   3rd Qu.: 0.881
##  Max.   : 50.000   Max.   : 1.000
##  NA's   :4421      NA's   :4421
##  passed_filter   pEC50_outside_conc_range model_converged       pEC50_quality_check
##  Mode :logical   Mode :logical            Mode:logical    5.72818301656452:  12
##  FALSE:4601      FALSE:111                TRUE:235        6.07074587494624:   6
##  TRUE :55        TRUE :124                NA's:4421       7.44099730847312:   6
##                  NA's :4421                               6.75587159170968:   2
##                                                           5.83469502048232:   1
##                                                           (Other)         :  84
##                                                           NA's            :4545
##  sufficient_data_for_fit protein_identified_in       representative      qupm
##  Mode:logical            Mode:logical          IPI00000001.2:  12   Min.   : 1.000
##  TRUE:235                TRUE:4656             IPI00000005.1:  12   1st Qu.: 3.000
##  NA's:4421                                     IPI00000690.1:  12   Median : 7.000
##                                                IPI00000811.2:  12   Mean   : 9.149
##                                                IPI00000875.7:  12   3rd Qu.:12.000
##                                                IPI00001914.1:  12   Max.   :87.000
##                                                (Other)      :4584
##       qusm          clustername   sumionarea_protein_5 sumionarea_protein_1
##  Min.   :  1.00   A2M    :  12   Min.   :2.063e+05    Min.   :3.819e+05
##  1st Qu.:  5.00   ABHD10 :  12   1st Qu.:7.696e+07    1st Qu.:7.604e+07
```

```
##  Median : 11.00   ACAA1  :  12   Median :2.511e+08    Median :2.512e+08
##  Mean   : 19.57   ACO1   :  12   Mean   :7.182e+08    Mean   :7.542e+08
##  3rd Qu.: 23.00   ACO2   :  12   3rd Qu.:7.382e+08    3rd Qu.:7.682e+08
##  Max.   :263.00   ACTC1  :  12   Max.   :2.125e+10    Max.   :2.138e+10
##                   (Other):4584
##  sumionarea_protein_0.143 sumionarea_protein_0.02 sumionarea_protein_0  temperature
##  Min.   :3.579e+05        Min.   :4.335e+05       Min.   :2.925e+05    Min.   :42.0
##  1st Qu.:8.079e+07        1st Qu.:8.401e+07       1st Qu.:7.345e+07    1st Qu.:46.2
##  Median :2.591e+08        Median :2.739e+08       Median :2.574e+08    Median :50.4
##  Mean   :7.554e+08        Mean   :8.100e+08       Mean   :8.599e+08    Mean   :51.6
##  3rd Qu.:7.857e+08        3rd Qu.:8.331e+08       3rd Qu.:8.554e+08    3rd Qu.:56.1
##  Max.   :1.924e+10        Max.   :2.249e+10       Max.   :2.644e+10    Max.   :63.9
##
##    experiment  rel_fc_protein_5  rel_fc_protein_1 rel_fc_protein_0.143
##  X020466:968   Min.   : 0.3487   Min.   :0.2985   Min.   :0.3887
##  X020467:950   1st Qu.: 0.7894   1st Qu.:0.8231   1st Qu.:0.8156
##  X020468:894   Median : 0.8964   Median :0.9197   Median :0.9415
##  X020469:738   Mean   : 0.9935   Mean   :0.9753   Mean   :1.0187
##  X020470:600   3rd Qu.: 1.0878   3rd Qu.:1.0588   3rd Qu.:1.1447
##  X020471:506   Max.   :17.1835   Max.   :8.6463   Max.   :6.2354
##
##  rel_fc_protein_0.02 rel_fc_protein_0
##  Min.   : 0.1882     Min.   :1
##  1st Qu.: 0.8413     1st Qu.:1
##  Median : 0.9601     Median :1
##  Mean   : 1.0974     Mean   :1
##  3rd Qu.: 1.2027     3rd Qu.:1
##  Max.   :10.0917     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.

```
data2d <- tpp2dImport(configTable = config_tpp2d,
                      data = data_tpp2d,
                      idVar = "representative",
                      intensityStr = "sumionarea_protein_",
                      nonZeroCols = "qusm",
                      addCol = "clustername")
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head(data2d)
##   representative qupm qusm clustername sumionarea_protein_5 sumionarea_protein_1
## 1  IPI00000001.2   15   25       STAU1           1193994914           1337957734
## 2  IPI00000001.2   15   25       STAU1           1272771185           1473572092
## 3  IPI00000001.2   13   22       STAU1           1482437522           1513181000
## 4  IPI00000001.2   13   22       STAU1           1157290962           1050288621
## 5  IPI00000001.2   15   24       STAU1            396823892            458022616
## 6  IPI00000001.2   15   24       STAU1            345169960            350182409
##   sumionarea_protein_0.143 sumionarea_protein_0.02 sumionarea_protein_0 temperature
## 1               1375948494              1956350223           1801848318        42.0
## 2               1273285951              1669312103           1404292404        44.1
## 3               1284434575              1487032006           1422365645        46.2
## 4               1110810226              1128507681            999666282        48.1
## 5                453860821               412257039            439399665        50.4
## 6                352193788               344410388            309019704        51.9
##   experiment                  unique_ID
## 1    X020466   X020466_42_IPI00000001.2
## 2    X020466 X020466_44.1_IPI00000001.2
```

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

```
## 3         1
## 4         1
## 5         1
## 6         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 sumionarea_protein_1
## 1  IPI00000001.2   15   25       STAU1           1193994914           1337957734
## 2  IPI00000001.2   15   25       STAU1           1272771185           1473572092
## 3  IPI00000001.2   13   22       STAU1           1482437522           1513181000
## 4  IPI00000001.2   13   22       STAU1           1157290962           1050288621
## 5  IPI00000001.2   15   24       STAU1            396823892            458022616
## 6  IPI00000001.2   15   24       STAU1            345169960            350182409
##   sumionarea_protein_0.143 sumionarea_protein_0.02 sumionarea_protein_0 temperature
## 1               1375948494              1956350223           1801848318        42.0
## 2               1273285951              1669312103           1404292404        44.1
## 3               1284434575              1487032006           1422365645        46.2
## 4               1110810226              1128507681            999666282        48.1
## 5                453860821               412257039            439399665        50.4
## 6                352193788               344410388            309019704        51.9
##   experiment                  unique_ID  rel_fc_5  rel_fc_1 rel_fc_0.143 rel_fc_0.02
## 1    X020466   X020466_42_IPI00000001.2 0.6626501 0.7425474    0.7636317   1.0857463
## 2    X020466 X020466_44.1_IPI00000001.2 0.9063434 1.0493342    0.9067100   1.1887212
## 3    X020467 X020467_46.2_IPI00000001.2 1.0422338 1.0638481    0.9030270   1.0454640
## 4    X020467 X020467_48.1_IPI00000001.2 1.1576773 1.0506392    1.1111810   1.1288844
## 5    X020468 X020468_50.4_IPI00000001.2 0.9031047 1.0423827    1.0329112   0.9382279
## 6    X020468 X020468_51.9_IPI00000001.2 1.1169837 1.1332041    1.1397130   1.1145257
##   rel_fc_0 norm_rel_fc_5 norm_rel_fc_1 norm_rel_fc_0.143 norm_rel_fc_0.02 norm_rel_fc_0
## 1        1      1.107187      1.059331          1.105019         1.244416             1
## 2        1      1.114453      1.164559          1.022695         1.195813             1
## 3        1      1.187727      1.229422          1.078735         1.211522             1
## 4        1      1.249516      1.147406          1.108487         1.329434             1
## 5        1      1.123552      1.268366          1.267164         1.256324             1
## 6        1      1.171933      1.176446          1.158041         1.163566             1
```

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

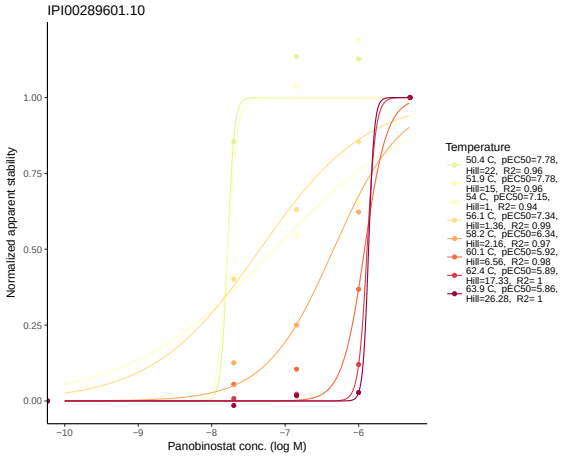
```
ccr2dResults <- tpp2dCurveFit(data = normData2d)
```

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:

```
drPlotsByTemperature <- tpp2dCreateDRplots(data = ccr2dResults, type = "single")
drPlotsByTemperature[[IPI_id_HDAC2]][["54"]]
```

2.3 Quality control analyses

In order to access the quality of the experimental 2D-TPP data set acquired in a specific cell line, we recommend to compare the data with vehicle TR experiments (at least two replicates) of the same cell line. For the analysis of this data we supply a QC-workflow that enables comparison of treatment and non-treatment samples with reference data.

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:

```
resultPath = file.path(getwd(), 'Panobinostat_Vignette_Example_2D')
if (!file.exists(resultPath)) dir.create(resultPath, recursive = TRUE)
```

```
trConfig <- file.path(system.file("example_data", package="TPP"),
                      "2D_example_data/panobinostat_ex_confg.csv")

tpp2dCreateTPPTRreference(trConfigTable = trConfig,
                          resultPath = resultPath,
                          outputName = "desired_file_name",
                          createFCboxplots = FALSE)
```

For the purpose of explaining this worflow, we will use a reference data set of a HepG2 cell line supplied with this package. Originating from this object we can now perform various quality control steps. First of all by setting the *createFCboxplots* flag to true, we can generate box plot melting curves of the reference data which are first of all informative of the quality of the reference data and illustrate melting behavior of all proteins without any treatment.

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

- The `tppRefData.RData` file ist the most important one. This is the file that has to be referenced by indication of a system path to this file when calling functions to generate the 2D-TPP spline plots and perform an F test. 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 %in% IPI_id_HDAC2)

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

print(pEC50QC_HDAC1)
```

```
## named list()
```

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")
dir(resultPath)
```

```
## [1] "qc_Histograms"
```

2.4 Spline fits of treatment effects over temperature

In order to access whether the drug treatment has a significant impact on altering the thermal stability of specific proteins a function was implemented which illustrates the course of stability of a certain protein over different temperatures based on a reference data set. A natural cubic spline fitted to the reference data is then used to infer the relative stability curves of proteins with different concentrations of treatment which are in turn fitted by natural cubic splines. The cubic spline with n degrees of freedom on $[a, b]$ obeys:

- $S(x) \in C^2[a, b]$
- $a = t_0 < t_1 < ... < t_n = b$

and:

$$S(x) = \begin{cases} S_0(x) = a_0x^3 + b_0x^2 + c_0x + d_0, & t_0 \leq x \leq t_1 \\ S_1(x) = a_1x^3 + b_1x^2 + c_1x + d_1, & t_1 \leq x \leq t_2 \\ . \\ . \\ . \\ S_{n-1}(x) = a_{n-1}x^3 + b_{n-1}x^2 + c_{n-1}x + d_{n-1}, & t_{n-1} \leq x \leq t_n \end{cases} \quad (1)$$

a *natural cubic spline* additionally contrains that it's function has to be linear beyond the boundary knots with constrains that both the first and the last section of the cubic spline has to be linear.

The function to perform this analysis can be invoked by:

```
analysisResults <- tpp2dSplineFitAndTest(data = normData2d,
                                         dataRef = trRef,
                                         refIDVar = "Protein_ID",
                                         refFcStr = "norm_rel_fc_protein_",
                                         doPlot = FALSE,
                                         resultPath = resultPath,
                                         nCores = 1)

head(analysisResults)
```

```
##   representative qupm qusm clustername sumionarea_protein_5 sumionarea_protein_1
## 1  IPI00000001.2   15   25       STAU1           1193994914           1337957734
## 2  IPI00000001.2   15   25       STAU1           1272771185           1473572092
## 3  IPI00000001.2   13   22       STAU1           1482437522           1513181000
## 4  IPI00000001.2   13   22       STAU1           1157290962           1050288621
## 5  IPI00000001.2   15   24       STAU1            396823892            458022616
## 6  IPI00000001.2   15   24       STAU1            345169960            350182409
##   sumionarea_protein_0.143 sumionarea_protein_0.02 sumionarea_protein_0 temperature
## 1               1375948494              1956350223           1801848318        42.0
## 2               1273285951              1669312103           1404292404        44.1
## 3               1284434575              1487032006           1422365645        46.2
## 4               1110810226              1128507681            999666282        48.1
## 5                453860821               412257039            439399665        50.4
## 6                352193788               344410388            309019704        51.9
##   experiment                  unique_ID  rel_fc_5  rel_fc_1 rel_fc_0.143 rel_fc_0.02
## 1    X020466   X020466_42_IPI00000001.2 0.6626501 0.7425474    0.7636317   1.0857463
## 2    X020466 X020466_44.1_IPI00000001.2 0.9063434 1.0493342    0.9067100   1.1887212
## 3    X020467 X020467_46.2_IPI00000001.2 1.0422338 1.0638481    0.9030270   1.0454640
## 4    X020467 X020467_48.1_IPI00000001.2 1.1576773 1.0506392    1.1111810   1.1288844
## 5    X020468 X020468_50.4_IPI00000001.2 0.9031047 1.0423827    1.0329112   0.9382279
## 6    X020468 X020468_51.9_IPI00000001.2 1.1169837 1.1332041    1.1397130   1.1145257
##   rel_fc_0 norm_rel_fc_5 norm_rel_fc_1 norm_rel_fc_0.143 norm_rel_fc_0.02 norm_rel_fc_0
## 1        1      1.107187      1.059331          1.105019         1.244416             1
## 2        1      1.114453      1.164559          1.022695         1.195813             1
## 3        1      1.187727      1.229422          1.078735         1.211522             1
## 4        1      1.249516      1.147406          1.108487         1.329434             1
## 5        1      1.123552      1.268366          1.267164         1.256324             1
## 6        1      1.171933      1.176446          1.158041         1.163566             1
##   F_statistic F_moderated F_scaled residual_df_H1 prior_df_H1 df1 df2 df2_moderated
## 1    6.006917    188.3243 9.416217             35    2.581745  20  35      37.58174
## 2    6.006917    188.3243 9.416217             35    2.581745  20  35      37.58174
## 3    6.006917    188.3243 9.416217             35    2.581745  20  35      37.58174
## 4    6.006917    188.3243 9.416217             35    2.581745  20  35      37.58174
## 5    6.006917    188.3243 9.416217             35    2.581745  20  35      37.58174
## 6    6.006917    188.3243 9.416217             35    2.581745  20  35      37.58174
##   posterior_var_H1      p_NPARC p_adj_NPARC
## 1     0.0003455887 3.140386e-09 3.50153e-07
## 2     0.0003455887 3.140386e-09 3.50153e-07
## 3     0.0003455887 3.140386e-09 3.50153e-07
## 4     0.0003455887 3.140386e-09 3.50153e-07
## 5     0.0003455887 3.140386e-09 3.50153e-07
## 6     0.0003455887 3.140386e-09 3.50153e-07
```

Moreover, these fits can be used then, in order to access confidence on whether the curves fitting the relative treatment data points represent the data better than a model which does not distinguish between the different treatment concentrations. The confidence assessment is thereby based on a moderated F statistic adapted from a method by Storey and others [6] which they developed for microarray time course data. The method calculates a

moderated F statistic following:

$$F = \frac{\mathrm{SS}_0 - \mathrm{SS}_1}{\widetilde{s}^2(\sigma^2, \mathrm{df}_2)} \tag{2}$$

with SS_0 representing the sum of squares of the null model (fitting the data without distinguishing between different treatment concentrations) and SS_1 those of the full model (which fits the data by in this case 5 different splines for every treatment concentration respectively). With $\widetilde{s}^2$ representing the empirical Bayes estimator for SS_1, with $\mathrm{df}_2 = n - \nu_1$, where ν_1 denoted the parameters of the full model and n denotes the number of data points.

```
analysisResults %>% filter(representative == IPI_id_HDAC2) %>%
  select(temperature, p_NPARC, p_adj_NPARC)
```

```
##    temperature p_NPARC p_adj_NPARC
## 1         42.0       0           0
## 2         44.1       0           0
## 3         46.2       0           0
## 4         48.1       0           0
## 5         50.4       0           0
## 6         51.9       0           0
## 7         54.0       0           0
## 8         56.1       0           0
## 9         58.2       0           0
## 10        60.1       0           0
## 11        62.4       0           0
## 12        63.9       0           0
```

By defining the `methods` argument to include `"splineFit"`, one prompts the main function `analyze2DTPP` to directly perform spline fits and a moderated F-test for each protein in the data set.

References

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[2] Mikhail M Savitski, Friedrich BM Reinhard, Holger Franken, Thilo Werner, Maria Fälth Savitski, Dirk Eberhard, Daniel Martinez Molina, Rozbeh Jafari, Rebecca Bakszt Dovega, Susan Klaeger, et al. Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science*, 346(6205):1255784, 2014.

[3] Isabelle Becher, Thilo Werner, Carola Doce, Esther A Zaal, Cecilia R Berkers, Ina Tögel, Elsa Salzer, Marcus Bantscheff, and Mikhail M Savitski. Comprehensive thermal and chemoproteomics profiling identifies phenylalanine hydroxylase as a potent off-target of the histone deacetylase inhibitor panobinostat. *in submission*, 2016.

[4] Holger Franken, Toby Mathieson, Dorothee Childs, Gavain Sweetman, Thilo Werner, Wolfgang Huber, and Mikhail M Savitski. Thermal proteome profiling for unbiased identification of drug targets and detection of downstream effectors. *Nature protocols*, 10(10):1567 – 1593, 2015.

[5] Alexander Walker. *openxlsx: Read, Write and Edit XLSX Files*, 2015. R package version 2.4.0. URL: http://CRAN.R-project.org/package=openxlsx.

[6] John D Storey, Wenzhong Xiao, Jeffrey T Leek, Ronald G Tompkins, and Ronald W Davis. Significance analysis of time course microarray experiments. *Proceedings of the National Academy of Sciences of the United States of America*, 102(36):12837–42, 2005. URL: http://www.pnas.org/content/102/36/12837.abstract, doi:10.1073/pnas.0504609102.