Re-analysis of JQ1 lysate dataset by Savitski et al, 2018

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Package

TPP2D 1.3.10

Contents

1 Step-by-step walk through the anlysis

```
# This script uses the development version of TPP2D
if(require("BiocManager"))
  install.packages("BiocManager")
BiocManager::install("nkurzaw/TPP2D")
```

Load required libraries

```
library(TPP2D)
## Loading required package: dplyr
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##     filter, lag
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
library(dplyr)
library(tidyr)
library(ggplot2)
library(readxl)
```

Define plot style

```
theme_paper <- theme_bw(base_size = 6) +
  theme(legend.background = element_blank(),
        legend.key = element_blank(),
        panel.background = element_blank(),
        panel.grid.major = element_line(colour = "grey92", size = 0.25),
        panel.grid.minor = element_line(colour = "grey92", size = 0.15),
        panel.border = element_blank(),
        strip.background = element_blank(),
        plot.background = element_blank(),
        complete = TRUE,
        axis.line = element_line(color = "black", size = 0.25),
        text = element_text(size = 7),
        axis.ticks = element_line(color = "black", size = 0.25),
        axis.title = element_text(size = 8),
        axis.text = element_text(size = 6))
```

Download the supplementary excel table (Supplementary Dataset S1) by Savitski et al. (2018)

```
if(!file.exists("Savitski_et_al_Figure_3/Supplementary Dataset 2_2D-TPP.xlsx")){
    download.file(
      url = "https://data.mendeley.com/datasets/8pzhg2tdyb/1/files/115f60c9-01d1-4213-9abb-aa095d70a626/Savit
      destfile = "Savitski_et_al_Figure_3.zip")
    unzip(zipfile = "Savitski_et_al_Figure_3.zip",
        exdir = "Savitski_et_al_Figure_3")
    system("rm Savitski_et_al_Figure_3.zip")
```

```
}
```

Read in the data and reformat to a data frame as would be obtained after import of the raw data:

```
jq1_lys_raw <- read_xlsx("Savitski_et_al_Figure_3/Supplementary Dataset 2_2D-TPP.xlsx", sheet = 3, skip = 1)
  dplyr::select(representative = `Accession No.`,
                clustername = `protein name`,
                qupm = QUPM,
                qusm = QUSM,
                temperature,
                matches("sumionarea"),
                -matches("total"),
                matches("rel_fc_protein"),
                -matches("transformed"),
                -matches("orig"))  %>%
  gather(key, value, matches("sumionarea"), matches("rel_fc_protein")) %>%
  mutate(conc = as.numeric(gsub("uM", "", gsub(".+_protein_[0-9,H,L]+_[0-9,H,L]+_", "", key))),
         temperature = as.numeric(gsub("C", "", temperature)),
         key = case_when(grepl("sumionarea", key) ~ "raw_value",
                         grepl("rel_fc", key) ~ "rel_value")) %>%
  spread(key, value) %>%
  arrange(representative, temperature, conc) %>%
  group_by(clustername, temperature, conc) %>%
  filter(qupm == max(qupm),
         qusm == max(qusm),
         raw_value == max(raw_value)) %>%
  filter(!duplicated(clustername)) %>%
  ungroup %>%
  mutate(log2_value = log2(raw_value),
         log_conc = log10(conc/1e6)) %>%
  filter(qupm > 1)

# resolve ambiguous protein names
jq1_lys_fil <- resolveAmbiguousProteinNames(jq1_lys_raw)

# recompute reporter ion signal from robust Isobarquant fold changes
jq1_lys_df <- recomputeSignalFromRatios(jq1_lys_fil)
```

Compute null and alternative model fits and extract parameters

```
jq1_params_df <- getModelParamsDf(jq1_lys_df, maxit = 500)
saveRDS(jq1_params_df, file = "../pre_run_data/jq1_params_df.rds")
```

Compute F statistics

```
jq1_fstat_df <- computeFStatFromParams(jq1_params_df)
```

Get B datasets expected under the null model and perform model fitting and compute F statistics to obtain a null distribution for FDR calibration:

```
set.seed(12, kind = "L'Ecuyer-CMRG")
jq1_null_df <- bootstrapNullAlternativeModel(
  df = jq1_lys_df, params_df = jq1_params_df,
  maxit = 500, B = 100,
  BPPARAM = BiocParallel::MulticoreParam(workers = 20, progressbar = TRUE),
  verbose = FALSE)
saveRDS(jq1_null_df, file = "../pre_run_data/jq1_null_df.rds")
```

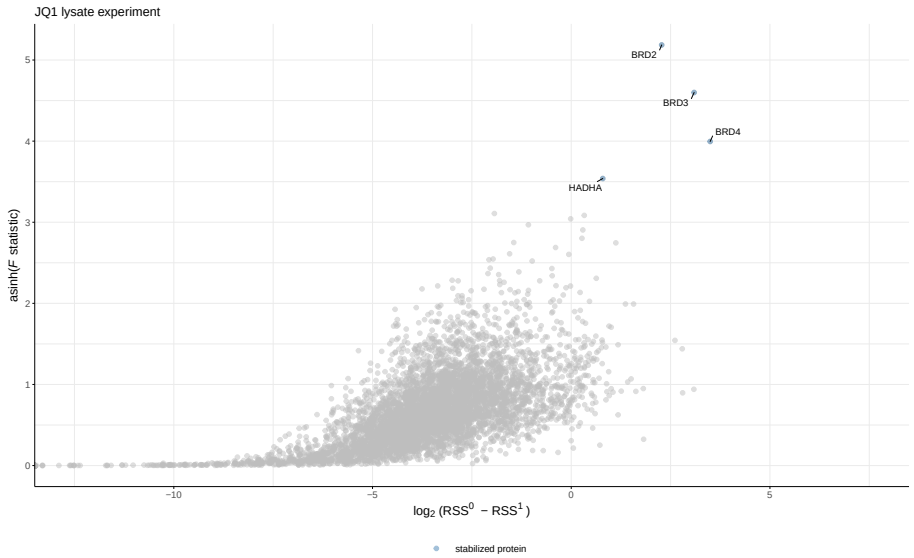
Compute FDR and find hits:

```
jq1_fdr_df <- getFDR(df_out = jq1_fstat_df,
                     df_null = jq1_null_df)

jq1_hits_df <- findHits(jq1_fdr_df, alpha = 0.1)
```

```
ggplot(jq1_fdr_df %>%
           filter(dataset == "true") %>%
           mutate(group = case_when(slopeH1 > 0 ~ "stabilized protein",
                                    slopeH1 < 0 ~ "destabilized protein")),
       aes(log2(rssH0 - rssH1), asinh(F_statistic))) +
  geom_point(color = "gray", alpha = 0.5, size = 1) +
  geom_point(aes(color = group), alpha = 0.5,
             size = 1,
             data = jq1_hits_df %>%
                 mutate(group = case_when(
                     slopeH1 > 0 ~ "stabilized protein",
                     slopeH1 < 0 ~ "destabilized protein"))) +
  ggrepel::geom_text_repel(
    aes(label = clustername),
    data = jq1_hits_df,
    size = 2, segment.size = 0.2, min.segment.length = unit(2, "pt")) +
  scale_color_manual("", values = c("steelblue", "orange")) +
  labs(x = expression('log'[2]~'(RSS'^0~' - RSS'^1~')'),
       y = expression('asinh('*italic(F)*' statistic)')) +
  ggtitle("JQ1 lysate experiment") +
  coord_cartesian(xlim = c(-12.5, 7.5)) +
  theme_paper +
  theme(legend.position = "bottom")
```

Re-analysis of JQ1 lysate dataset by Savitski et al, 2018




```
ggplot(jq1_fdr_df %>%
           filter(dataset == "true") %>%
           mutate(group = case_when(slopeH1 > 0 ~ "stabilized protein",
                                    slopeH1 < 0 ~ "destabilized protein")),
       aes(log2(rssH0 - rssH1), asinh(F_statistic))) +
  geom_point(color = "gray", alpha = 0.5, size = 1) +
  geom_point(aes(color = group), alpha = 0.5,
             size = 1,
             data = jq1_hits_df %>%
                 mutate(group = case_when(
                     slopeH1 > 0 ~ "stabilized protein",
                     slopeH1 < 0 ~ "destabilized protein"))) +
  ggrepel::geom_text_repel(
    aes(label = clustername),
    data = jq1_hits_df,
    size = 2, segment.size = 0.2, min.segment.length = unit(2, "pt")) +
  scale_color_manual("", values = c("orange", "steelblue")) +
  facet_wrap(~nObsRound, scales = "free", ncol = 5) +
  labs(x = expression('log'[2]~'(RSS'^0~' - RSS'^1~')'),
       y = expression('asinh('*italic(F)*' statistic)')) +
  ggtitle("JQ1 lysate experiment") +
  coord_cartesian(xlim = c(-12.5, 7.5)) +
  theme_paper +
  theme(legend.position = "bottom")
```

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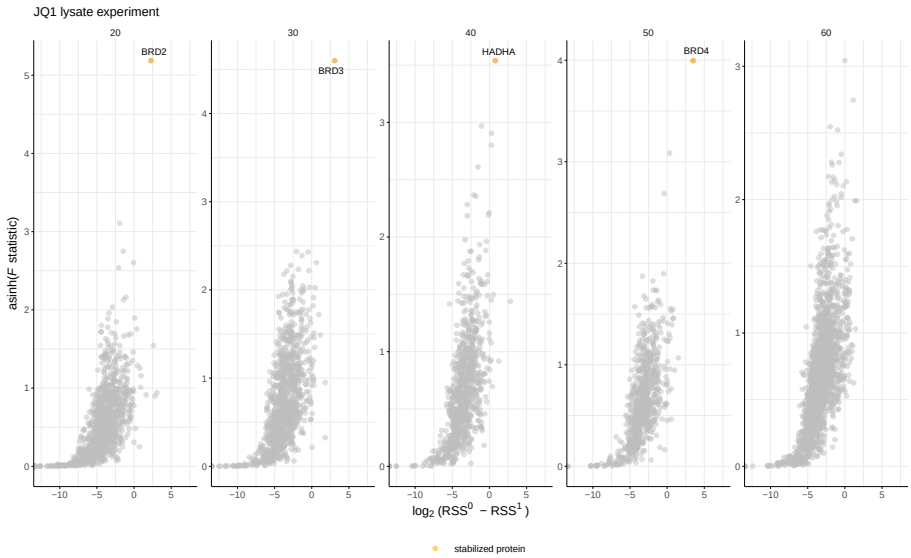
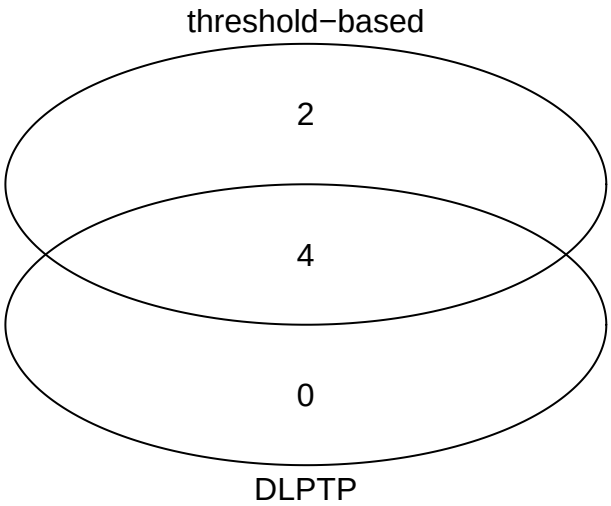



```
library(gplots)
##
## Attaching package: 'gplots'
## The following object is masked from 'package:stats':
##
##     lowess

jq1_thres_df <- read_xlsx("Savitski_et_al_Figure_3/Supplementary Dataset 2_2D-TPP.xlsx", sheet = 3, skip = 1)
  filter(QUPM > 1)

#stabilization
venn(list("DLPTP" = (jq1_hits_df %>% filter(slopeH1 > 0))$clustername,
          "threshold-based" = (jq1_thres_df %>% filter(protein_stabilized_neighb_temp_good_curves_count > 1)
```




```
#destabilization
venn(list("DLPTP" = (jq1_hits_df %>% filter(slopeH1 < 0))$clustername,
          "threshold-based" = (jq1_thres_df %>% filter(protein_destabilized_neighb_temp_good_curves_count > 1
```

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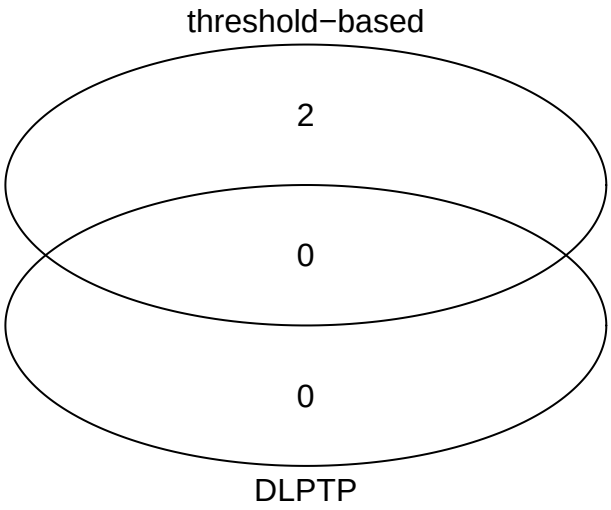
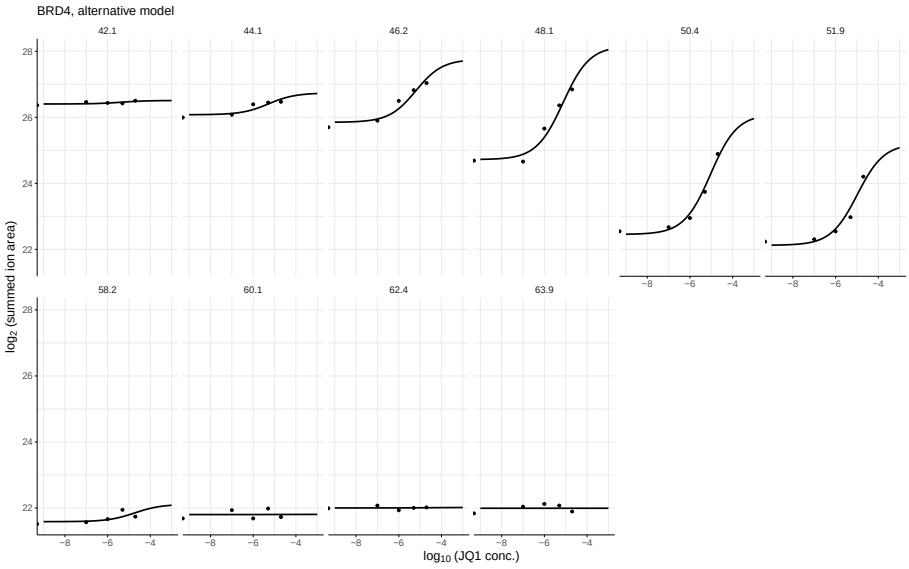


BRD4 profile

```
brd4_fit <- plot2dTppFit(jq1_lys_df, "BRD4", "H1")$data

brd4_df <- filter(jq1_lys_df, clustername == "BRD4")

ggplot(brd4_fit, aes(log_conc, y_hat)) +
  geom_line() +
  geom_point(aes(log_conc, log2_value),
             data = brd4_df, size = 0.5) +
  facet_wrap(~temperature, ncol = 6) +
  labs(x = expression('log'[10]~ '(JQ1 conc.)'),
       y = expression('log'[2]~ '(summed ion area)')) +
  ggtitle("BRD4, alternative model") +
  theme_paper
```



```
sessionInfo()
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Mojave 10.14.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] gplots_3.0.1.2   readxl_1.3.1     ggplot2_3.2.1    tidyr_1.0.0
## [5] TPP2D_1.3.10     dplyr_0.8.3      BiocStyle_2.12.0
##
## loaded via a namespace (and not attached):
##  [1] gtools_3.8.1        tidyselect_0.2.5    xfun_0.10
##  [4] purrr_0.3.3         colorspace_1.4-1    vctrs_0.2.0
##  [7] htmltools_0.4.0     yaml_2.2.0          rlang_0.4.1
## [10] pillar_1.4.2        glue_1.3.1          withr_2.1.2
## [13] BiocParallel_1.18.1 foreach_1.4.7       lifecycle_0.1.0
## [16] stringr_1.4.0       munsell_0.5.0       gtable_0.3.0
## [19] cellranger_1.1.0    zip_2.0.4           caTools_1.17.1.2
## [22] codetools_0.2-16    evaluate_0.14       labeling_0.3
## [25] knitr_1.25          doParallel_1.0.15   parallel_3.6.1
## [28] Rcpp_1.0.2          KernSmooth_2.23-16  scales_1.0.0
## [31] backports_1.1.5     BiocManager_1.30.9  gdata_2.18.0
## [34] digest_0.6.22       stringi_1.4.3       openxlsx_4.1.0.1
## [37] bookdown_0.14       ggrepel_0.8.1       grid_3.6.1
## [40] tools_3.6.1         bitops_1.0-6        magrittr_1.5
## [43] lazyeval_0.2.2      RCurl_1.95-4.12     tibble_2.1.3
## [46] crayon_1.3.4        pkgconfig_2.0.3     zeallot_0.1.0
## [49] MASS_7.3-51.4       ellipsis_0.3.0      assertthat_0.2.1
## [52] rmarkdown_1.16      iterators_1.0.12    R6_2.4.0
## [55] compiler_3.6.1
```

References

Savitski, M.M., Zinn, N., Faelth-Savitski, M., Poeckel, D., Gade, S., Becher, I., Muelbaier, M., Wagner, A.J., Strohmer, K., Werner, T., et al. (2018). Multiplexed Proteome Dynamics Profiling Reveals Mechanisms Controlling Protein Homeostasis. Cell 1–15.