# Package 'GRmetrics'

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Type Package

Title Calculate growth-rate inhibition (GR) metrics

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TimeCourse, Visualization

**Description** Functions for calculating and visualizing growth-rate

inhibition (GR) metrics.

License GPL-3

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BugReports https://github.com/uc-bd2k/GRmetrics/issues

LazyData TRUE

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GRbox

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Boxplots of a given GR metric

## Description

Given a SummarizedExperiment object created by GRfit, this function creates boxplots according to the parameters below.

## Usage

```
GRbox(
  fitData,
  metric,
  groupVariable,
  pointColor,
  factors = "all",
  wilA = NULL,
  wilB = NULL,
  plotly = TRUE
)
```

## Arguments

fitData metric	a SummarizedExperiment object, generated by the GRfit function. the GR metric (GR50, GRinf, h_GR, GRmax, GEC50, or GR_AOC) or tradi-
	tional metric (IC50, Einf, h, Emax, EC50, or AUC) that will be used for the boxplot.
groupVariable	the name of the variable from data (e.g. drug, cell-line, etc.) to select factors from.
pointColor	a variable that defines the coloring of the points overlayed on the boxplot.
factors	a vector of values of "groupVariable" of data that define which variables to make
	boxplots for. By default, a separate boxplot is made for each unique value of groupVariable.

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wilA	one value or a vector of values from "factors", i.e. a subset of the boxplots. If specified, a one-sided Wilcoxon rank sum test (wilcox.test) will be performed between "wilA" and "wilB" and the results will be displayed on the figure. The null hypothesis that the values from "wilA" and "wilB" have the same mean will be tested against the alternative hypothesis that the mean of the "wilB" values is greater than that of the "wilA" values.
wilB	one value or a vector of values from "factors", i.e. a subset of the boxplots (not overlapping "wilA").
plotly	a logical value indicating whether to output a ggplot2 graph or an interactive ggplotly graph

#### **Details**

Given a SummarizedExperiment object created by GRfit, this function creates boxplots of a given GR metric (GR50, GRmax, etc.) or traditional metric (IC50, Emax, etc.) for values of the grouping variable. The results can be viewed in a static ggplot image or an interactive plotly graph.

By default, a boxplot is created for all unique values of the grouping variable. The "factors" parameter can be used to specify a smaller subset of values for which to create boxplots. Points are overlayed on the boxplots and they can be colored by the variable specified in the pointColor parameter. If pointColor is set to NULL, the points will all be black. The results can be viewed in a static ggplot image or an interactive plotly graph.

#### Value

ggplot2 or ggplotly boxplots of the factors along the x-axis, with points colored by the given variable

#### Author(s)

Nicholas Clark

#### See Also

To create the object needed for this function, see GRfit. For other visualizations, see GRdrawDRC and GRscatter. For online GR calculator and browser, see <a href="http://www.grcalculator.org">http://www.grcalculator.org</a>.

```
# Load Case A (example 1) input
## Not run:
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line', 'agent', 'perturbation', 'replicate',
   'time'))
GRbox(output1, metric ='GRinf',
groupVariable = 'cell_line', pointColor = 'agent' , factors = c('BT20',
   'MCF10A'))
GRbox(output1, metric ='GRinf',
```

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```
groupVariable = 'cell_line', pointColor = 'cell_line' ,
factors = c('BT20', 'MCF10A'), plotly = FALSE)
GRbox(output1, metric = 'GR50', groupVariable = 'cell_line',
pointColor = 'cell_line', wilA = "BT20", wilB = c("MCF7", "MCF10A"))
## End(Not run)
```

GRdrawDRC

Dose-Response Curves

## Description

Given a SummarizedExperiment object created by GRfit, this function plots either the growth-rate inhibition (GR) dose response curves or the traditional dose response curves for a given set of data.

## Usage

```
GRdrawDRC(
  fitData,
  metric = "GR",
  experiments = "all",
  min = "auto",
  max = "auto",
  points = TRUE,
  curves = TRUE,
  plotly = TRUE
)
```

# Arguments

fitData	an element of class SummarizedExperiment, generated by the GRfit function.
metric	either "GR" for GR dose response curves or "rel_cell" for traditional dose response curves based on relative cell count.
experiments	the names of the experiments to plot (or "all")
min	the minimum concentration to plot (for curves)
max	the maximum concentration to plot (for curves)
points	a logical value indicating whether points (individual GR values) will be plotted
curves	a logical value indicating whether sigmoidal dose-response curves will be plotted
plotly	a logical value indicating whether to output a ggplot2 graph or a ggplotly graph

#### **Details**

Given a SummarizedExperiment object created by GRfit, this function plots these GR values (versus concentration) and/or the sigmoidal curves fitted to the sets of points. The results can be viewed in a static ggplot image or an interactive plotly graph.

The "min" and "max" parameters control the concentration values for which the curves are plotted. They are automatically set to the minimum and maximum concentrations of the data, but can be set by the user as well. "min" and "max" take raw values (not log transformed) for concentration.

By default, curves and points are plotted for all experiments. To specify a smaller set of experiments, use the "experiments" parameter. To see the names of individual experiments for a GRfit object fit\_example, see colData(fit\_example). See the examples below.

#### Value

ggplot2 or ggplotly graphs of Growth-rate inhibition dose-response curves

#### Author(s)

Nicholas Clark

#### See Also

To create the object needed for this function, see GRfit. For other visualizations, see GRbox and GRscatter. For online GR calculator and browser, see http://www.grcalculator.org.

#### **Examples**

```
# Load Case A (example 1) input
data("inputCaseA")
# Run GRfit function with case = "A"
drc_output = GRfit(inputCaseA,
groupingVariables = c('cell_line','agent'))
GRdrawDRC(drc_output, experiments = c('BT20 drugA', 'MCF10A drugA',
'MCF7 drugA'), min = 10^(-4), max = 10^2)
GRdrawDRC(drc_output, plotly = FALSE)
```

GRfit

Extract GR parameters from a dataset

## **Description**

This function takes in a dataset with information about concentration, cell counts over time, and additional grouping variables for a dose-response assay and calculates growth-rate inhibition (GR) metrics as well as traditional metrics (IC50, Emax, etc.) for each experiment in the dataset. The data must be in a specific format: either that specified by case "A" or case "C" described in the details below.

#### **Usage**

```
GRfit(inputData, groupingVariables, case = "A", force = FALSE, cap = FALSE)
```

#### **Arguments**

case

inputData a data table in one of the specified formats (Case A or Case C). See details below

for description. See data(inputCaseA) or data(inputCaseC) for example input data frames. See help files for inputCaseA and inputCaseC for description

of these examples.

groupingVariables

a vector of column names from inputData. All of the columns in inputData

except for those identified here will be averaged over.

either "A" or "C", indicating the format of the input data. See below for descrip-

tions of these formats.

force a logical value indicating whether to attempt to "force" a sigmoidal fit, i.e.

whether to allow fits with F-test p-values greater than .05

cap a logical value indicating whether to cap GR values (or relative cell counts) at 1.

If true, all values greater than 1 will be set to 1.

#### **Details**

Calculation of GR values is performed by the function .GRcalculate according to the "Online Methods" section of Hafner and Niepel et al. (2016, http://dx.doi.org/10.1038/nmeth.3853).

The fitting of the logistic curve is performed by the .GRlogisticFit function, which calls the drm function from the drc package to solve for the curve parameters. The GR curve fit function is given by  $f(c) = GRinf + (1 - GRinf)/(1 + (c/GEC50)^h_GR)$  where c is concentration. The fit is performed under following constraints:  $h_GR$  in [.1, 5], GRinf in [-1, 1], and GEC50 in  $[min(c)^*1e^2, max(c)^*1e^2]$  (c is concentration). The initial conditions for the fitting algorithm are  $h_GR = 2$ , GRinf = 0.1 and GEC50 = median(c). The fitting of the traditional dose response curve is done using the same formula, replacing GRinf with Einf, GEC50 with EC50, and  $h_GR$  with  $h_GR$  with  $h_GR$  is performed on the relative cell counts instead of  $h_GR$  values. Also, since the traditional dose response curve is bounded between 0 and 1 whereas the  $h_GR$  dose response curve is bounded between -1 and 1, we restrict Einf to the range  $h_GR$  is performed on the range  $h_GR$  in  $h_GR$  with  $h_GR$  with  $h_GR$  with  $h_GR$  with  $h_GR$  is performed on the relative cell counts instead of  $h_GR$  values. Also, since the traditional dose response curve is bounded between -1 and 1, we restrict Einf to the range  $h_GR$  is  $h_GR$ .

The parameters of the GR dose response curves (and traditional dose response curves) for each experiment are fitted separately. An F-test is used to compare the sigmoidal fit to a flat line fit. If the p-value of the F-test is less than .05, the sigmoidal fit is accepted. If the p-value is greater than or equal to .05, a flat horizontal line fit is given, with y equal to the mean of the GR values (or relative cell counts in the case of the traditional dose response curve). For each flat fit, GEC50 (or EC50) is set to 0, h\_GR (or h) is set to 0.01, GRinf (or Einf) is set to the y value of the flat fit, and GR50 (or IC50) is set to +/-Inf depending on whether GRinf (or Einf) is greater or less than .5.

The mandatory columns for inputData for Case "A" are the following as well as other grouping columns.

- 1. concentration column with concentration values (not log transformed) of the perturbagen on which dose-response curves will be evaluated
- 2. cell\_count column with the measure of cell number (or a surrogate of cell number) after treatment

3. cell\_count\_\_time0 - column with initial (Time 0) cell counts - the measure of cell number in untreated wells grown in parallel until the time of treatment

4. cell\_count\_\_ctrl - column with the Control cell count: the measure of cell number in control (e.g. untreated or DMSO-treated) wells from the same plate

All other columns will be treated as additional keys on which the data will be grouped (e.g. cell\_line, drug, time, replicate)

The mandatory columns for inputData for Case "C" are the following as well as other grouping columns.

- 1. concentration column with concentration values (not log transformed) of the perturbagen on which dose-response curves will be evaluated
- 2. cell\_count column with the measure of cell number (or a surrogate of cell number)
- 3. time column with the time at which a cell count is observed

All other columns will be treated as additional keys on which the data will be grouped (e.g. cell\_line, drug, replicate)

GR values and dose-response curves/metrics can also be computed using division times for (untreated) cell lines in the place of time zero cell counts, using the first formula in the Supplement of Hafner et al. (2017, http://dx.doi.org/10.1038/nbt.3882).

To use division rate instead of initial cell count, inputData should not have any initial cell counts (i.e. For Case "A", no "cell\_count\_\_time0" column. For Case "C", no values of 0 in the "time" column) and should instead have two columns "treatment\_duration" and "division\_time".

In the first column, "treatment duration", one should have the duration of the assay between time of treatment and the final cell counts (e.g. 72 for hours in a typical 3-day assay). In the second column, "division\_time", one should have the time it takes for one cell doubling to occur in each (untreated) cell line used under the conditions of the experiment. These two columns must contain numbers (no units), but need to refer to the same units (e.g. hours). In most cases, all experiments of a particular cell line would have the same "division\_time", however if the division rate of untreated cells varied on another parameter, for example seeding density, it would be appropriate to measure and input division times based on cell line/seeding density pairs.

#### Value

A SummarizedExperiment object containing GR metrics (GR50, GRmax, etc.) and traditional metrics (IC50, Emax, etc.) as well as goodness of fit measures is returned. The object also contains, in its metadata, a table of the original data converted to the style of "Case A" (with calculated GR values and relative cell counts for each row) and a vector of the grouping variables used for the calculation.

#### Note

To see the underlying code, use (getAnywhere(.GRlogistic\_3u)), (getAnywhere(.rel\_cell\_logistic\_3u)), (getAnywhere(.GRcalculate)), and (getAnywhere(.GRlogisticFit))

#### Author(s)

Nicholas Clark

#### References

Hafner, M., Niepel, M., Chung, M., and Sorger, P.K., "Growth Rate Inhibition Metrics Correct For Confounders In Measuring Sensitivity To Cancer Drugs". *Nature Methods* 13.6 (2016): 521-527. http://dx.doi.org/10.1038/nmeth.3853

Hafner, M., Niepel, M., Sorger, P.K., "Alternative drug sensitivity metrics improve preclinical cancer pharmacogenomics". *Nature Biotechnology* 35.6 (2017): 500-502. http://dx.doi.org/10.1038/nbt.3882

#### See Also

See drm for the general logistic fit function that solves for the parameters GRinf, GEC50, and h\_GR. See drmc for options of this function. Use the functions GRdrawDRC, GRbox, and GRscatter to create visualizations using the output from this function. For online GR calculator and browser, see http://www.grcalculator.org.

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line', 'agent', 'perturbation', 'replicate',
'time'))
# Overview of SummarizedExperiment output data
output1
## Not run:
# View GR metrics table
View(GRgetMetrics(output1))
# View descriptions of each metric (or goodness of fit measure)
View(GRgetDefs(output1))
# View table of original data (converted to style of Case A) with GR values
# and relative cell counts
View(GRgetValues(output1))
# View vector of grouping variables used for calculation
GRgetGroupVars(output1)
## End(Not run)
# Load Case C (example 4) input
# Same data, different format
data("inputCaseC")
head(inputCaseC)
output4 = GRfit(inputData = inputCaseC,
groupingVariables = c('cell_line', 'agent', 'perturbation', 'replicate',
'time'),
case = "C")
# Extract data tables and export to .tsv or .csv
## Not run:
# Write GR metrics parameter table to tab-separated text file
write.table(GRgetMetrics(output1), file = "filename.tsv", quote = FALSE,
sep = "\t", row.names = FALSE)
```

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```
# Write original data plus GR values to comma-separated file
write.table(GRgetValues(output1), file = "filename.csv", quote = FALSE,
sep = ",", row.names = FALSE)
## End(Not run)
```

GRgetDefs

GR metrics definitions

## **Description**

Given a SummarizedExperiment object created by GRfit, this function returns a table with the definition of each GR metric and traditional metric that is calculated.

## Usage

```
GRgetDefs(fitData)
```

#### **Arguments**

fitData

a SummarizedExperiment object, generated by the GRfit function.

#### **Details**

Given a SummarizedExperiment object created by GRfit, this function returns a table containing the definition of each GR metric, traditional metric, and goodness of fit measure in the object. This is simply a convenient accessor function, equivalent to rowData(fitData).

#### Value

A table of definitions of GR metrics and traditional metrics

#### Author(s)

Nicholas Clark

## See Also

To create the object needed for this function, see GRfit.

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent'))
defs = GRgetDefs(output1)
```

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```
# See all definitions (some will be truncated)
## Not run: View(defs)
# See the first six descriptions
head(defs)
# Look at a specific definition
defs[15,]
```

GRgetGroupVars

Grouping Variables

## **Description**

Given a SummarizedExperiment object created by GRfit, this function returns a vector of the grouping variables used to create the object.

## Usage

```
GRgetGroupVars(fitData)
```

#### **Arguments**

fitData

a SummarizedExperiment object, generated by the GRfit function.

#### **Details**

Given a SummarizedExperiment object created by GRfit, this function returns a vector of the grouping variables used to create the object. These are the variables in the dataset that are not averaged over. This is simply a convenient accessor function, equivalent to metadata(fitData)[[2]].

#### Value

A vector of grouping variables

## Author(s)

Nicholas Clark

#### See Also

To create the object needed for this function, see GRfit.

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent'))
groupVars = GRgetGroupVars(output1)
groupVars
```

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GRgetMetrics

GR metrics

## **Description**

Given a SummarizedExperiment object created by GRfit, this function returns a table of GR metrics (as well as traditional metrics) for each experiment in the dataset.

## Usage

```
GRgetMetrics(fitData)
```

#### **Arguments**

fitData

a SummarizedExperiment object, generated by the GRfit function.

#### **Details**

Given a SummarizedExperiment object created by GRfit, this function returns a table of GR metrics and traditional metrics along with goodness of fit measures. It also identifies each fit as flat or sigmoidal. This is simply a convenient accessor function, equivalent to cbind(as.data.frame(colData(fitData)), as.data

## Value

A table with GR metrics and goodness of fit measures

#### Author(s)

Nicholas Clark

#### See Also

To create the object needed for this function, see GRfit.

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent'))
metrics = GRgetMetrics(output1)
## Not run: View(metrics)
head(metrics)
```

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GRgetValues

GR values

#### **Description**

Given a SummarizedExperiment object created by GRfit, this function returns a table of the original data (in the form of "Case A") with columns for GR values and relative cell counts.

## Usage

```
GRgetValues(fitData)
```

#### **Arguments**

fitData

a SummarizedExperiment object, generated by the GRfit function.

#### **Details**

Given a SummarizedExperiment object created by GRfit, this function returns a table of the original data (in the form of "Case A") with columns for GR values and relative cell counts. This is simply a convenient accessor function, equivalent to as.data.frame(metadata(fitData)[[1]]).

#### Value

A table with GR values and relative cell counts

#### Author(s)

Nicholas Clark

#### See Also

To create the object needed for this function, see GRfit.

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent'))
GRvalues = GRgetValues(output1)
head(GRvalues)
## Not run: View(GRvalues)
```

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GRscatter	Scatterplot of a given GR metric	

## **Description**

Given a SummarizedExperiment object created by GRfit, this function creates a scatterplot according to the parameters below.

#### Usage

```
GRscatter(fitData, metric, variable, xaxis, yaxis, plotly = TRUE)
```

#### **Arguments**

fitData	a SummarizedExperiment object, generated by the GRfit function.
metric	the GR metric (GR50, GRinf, h_GR, GRmax, GEC50, or GR_AOC) or traditional metric (IC50, Einf, h, Emax, EC50, or AUC) that will be used for the scatterplot.
variable	The name of the variable from data (e.g. drug, cell-line, etc.) to select factors from.
xaxis	a vector of values of "variable" of data to be used for the scatterplot's x-axis
yaxis	a vector of values of "variable" of data to be used for the scatterplot's y-axis
plotly	a logical value indicating whether to output a ggplot2 graph or a ggplotly graph

## Details

Given a SummarizedExperiment object created by GRfit, this function creates a scatterplot of a given GR metric (GR50, GRmax, etc.) or traditional metric (IC50, Emax, etc.) with the "xaxis" values plotted against the "yaxis" values. The results can be viewed in a static ggplot image or an interactive plotly graph.

The xaxis and yaxis vectors must be of the same length or at least one must be of length one. For each pair of values xaxis[i] and yaxis[i], the function will create a scatterplot (all on one graph) of the specified GR metric. If a vector is of length one, it will be repeated to the length of the other vector.

### Value

a ggplot2 or ggplotly scatterplot of the x-axis variable(s) versus the y-axis variable(s) for the given GR metric

#### Author(s)

Nicholas Clark

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#### See Also

To create the object needed for this function, see GRfit. For other visualizations, see GRdrawDRC and GRbox. For online GR calculator and browser, see <a href="http://www.grcalculator.org">http://www.grcalculator.org</a>.

#### **Examples**

```
# Load Case A (example 1) input
data("inputCaseA")
head(inputCaseA)
# Run GRfit function with case = "A"
output1 = GRfit(inputData = inputCaseA,
groupingVariables = c('cell_line','agent', 'perturbation','replicate',
'time'))
GRscatter(output1, 'GR50', 'agent', c('drugA','drugD'), 'drugB')
GRscatter(output1, 'GR50', 'agent', c('drugA','drugD'), 'drugB',
plotly = FALSE)
```

inputCaseA

Simulated dose-response assay data (Case A)

#### **Description**

This is an example dataset corresponding to Case A. The data was generated by the python script "generate\_data.py" found in the "inst/scripts/" directory. This object is meant to be used as the "inputData" argument for the GRfit function when "case" is equal to "A" (the default option). The input data for "Case A" of the GRfit function must be in this format with columns named "concentration", "cell\_count\_\_ctrl", and "cell\_count\_\_time0" as well as columns for other key variables. The columns "cell\_line", "agent", "perturbation", "replicate", and "time" are simply examples of these key variables. It is not necessary that your input data frame include these exact column names or the same number of columns.

#### Usage

inputCaseA

#### Format

A data frame with 1008 rows and 9 variables:

- cell line: the cell-line used (MCF10A, MCF7, BT20)
- agent: the drug used (drugA, drugB, drugC, drugD)
- pertubation: an example of another key variable (e.g different media, a co-treatment, etc.) (0, 1)
- replicate: replicate number, (1, 2, 3)
- time: time of assay measured in hours (48, 72)
- concentration: concentration of the perturbagen on which dose-response curves will be evaluated (not log transformed)

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• cell\_count: the measure of cell number (or a surrogate of cell number) after treatment at the end of the assay

- cell\_count\_\_ctrl: the measure of cell number in control (e.g. untreated or DMSO-treated) wells from the same plate at the end of the assay
- cell\_count\_\_time0: the measure of cell number in untreated wells grown in parallel until the time of treatment

#### Value

An example dataset in the form of "Case A" generated for use with GRfit

#### Source

/inst/scripts/generate\_data.py

inputCaseC

Simulated dose-response assay data (Case C)

#### **Description**

This is an example dataset corresponding to Case C. The dataset is equivalent to that of Case A, but in a different form. The data was generated by the python script "generate\_data.py" found in the "inst/scripts/" directory. This object is meant to be used as the "inputData" argument for the GRfit function when "case" is equal to "C". The input data for "Case C" of the GRfit function must be in this format with columns named "concentration", "cell\_count", and "time" as well as columns for other key variables. The columns "cell\_line", "agent", "perturbation", and "replicate", are simply examples of these key variables. It is not necessary that your input data frame include these exact column names or the same number of columns.

#### Usage

inputCaseC

#### **Format**

A data frame with 1352 rows and 7 variables:

- cell\_line: the cell-line used (MCF10A, MCF7, BT20)
- agent: the drug used (-, drugA, drugB, drugC, drugD)
- pertubation: an example of another key variable (e.g different media, a co-treatment, etc.) (0, 1)
- replicate: replicate number, (1, 2, 3)
- time: time of assay measured in hours (0, 48, 72)
- concentration: concentration of the perturbagen on which dose-response curves will be evaluated (not log transformed)
- cell\_count: measure of cell number or a surrogate of the number of cells.

inputCaseC

## Value

An example dataset in the form of "Case C" generated for use with GRfit

# Source

inst/scripts/generate\_data.py

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