Response Envelope Analysis Toolbox

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1 Introduction

Response envelope analysis (REA) enables the determination of drug combination effects at local, regional, and global levels without knowing the inhibition mechanism of drugs. Compared to other existing methods to determine drug combination effects, REA has the following advantages. First, REA is based on rigorous physical models. Second, REA does not require multi-parameter fitting which may be inaccurate due to overfitting. Moreover, REA is more accurate and robust than other existing methods.

The structure of the toolbox is shown in Figure 1. All the ".m" files are MATLAB files, and the ".csv" files are example data sets.

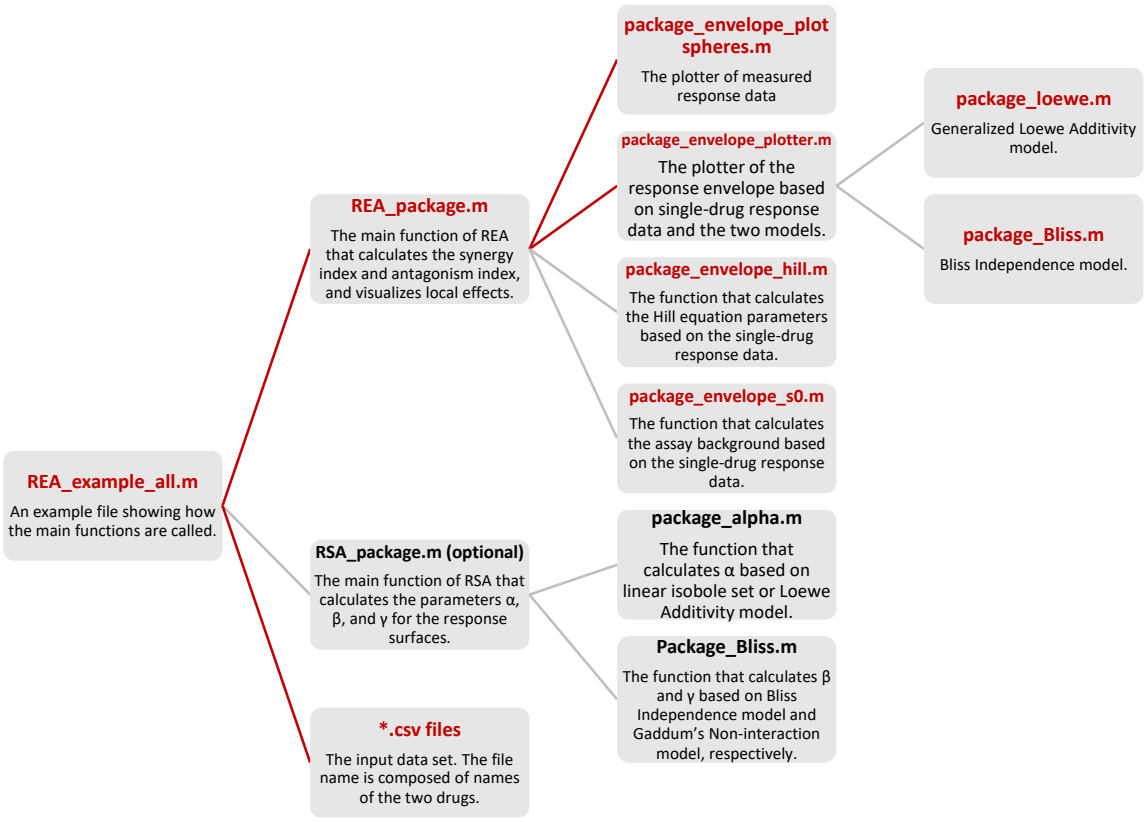

Figure 1 The structure of the toolbox. The red files are required, and the black ones are optional.

2 Prerequisites

The toolbox was developed under MATLAB computing environment (MATLAB 2016b). Though it is suggested that MATLAB 2016b or later version is used, most of the functions used in the toolbox have been available for decades. One exception is the "strsplit.m" function used in the example file "REA_example_all.m". It is a built-in MATLAB function since 2014. However, for MATLAB earlier than 2014a, one can download a string toolkit in MATLAB File Exchange at https://www.mathworks.com/matlabcentral/fileexchange/21710-string-toolkits.

The data format used in the ".csv" files are the standard input format for REA and should always be used. The format specifies column 1 to be concentrations of drug 1, column 2 to be concentrations of drug 2, and column 3 to be corresponding survival rates in percentage.

3 Applications

3.1 REA

"REA_package.m" is the main function of response envelope analysis. The function requires four input parameters and prefers another three to be specified. The output of the function includes the synergy index and antagonism index and the pipeline used to obtain these two indexes. The meaning of the input and output is shown in Table 1.

Table 1 The input and output of REA. The red font represents required, and the black optional.

Input	Meaning
data	Data matrix to be analyzed by REA
trim	Margin of the plot beyond existing measurements
ft	Font size
lw	Line width
drug1	Name of Drug 1
drug2	Name of Drug 2
custom_label	Whether custom axis labels are used. If not customized, logarithm of axis labels will be shown.
Output	**Meaning**
siai	Synergy index (SI) and antagonism index (AI) that reflect regional combination effects, and the difference between these two suggests global effects. SI>AI, SI=AI, and SI<AI represents synergy, additivity, and antagonism.
A Figure Object	Pipeline used to obtain these two indexes.

Among the four required input parameters, only "data" is functional, and the other three are rather aesthetical. The effect of customized label is shown in Figure 2. The customization of axis labels and tick labels allows one to present the pipeline on an arithmetic scale, whereas the original input data are converted to a logarithmic scale for calculation of SI and AI in REA. The example data set shown in Figure 2 is "ibrutinib_nintedanib.csv" [1] and another data set "pentamidine_chlorpromazine.csv" [2] is also included in the package for testing purposes. Figures 2A and 2B are generated using the following syntaxes, respectively:

```
REA_package(data,0.1,9,3, 'ibrutinib', 'nintedanib',1);
REA_package(data,0.1,9,3, 'ibrutinib', 'nintedanib',0);
```

Here “csvread.m”, a built-in MATLAB function is used to read the “*.csv” files into “data” in the syntaxes, while alternative functions such as “readtable.m” can also be used. The output can be omitted while the SI and AI are shown in the last step in the REA pipeline panel. One can also obtain these indexes using a specified output:

```
output = REA_package(data,0.1,9,3, 'ibrutinib', 'nintedanib',0)
```

The syntax results in a pipeline panel and an output:

```
output =

    0.0400    0.0025
```

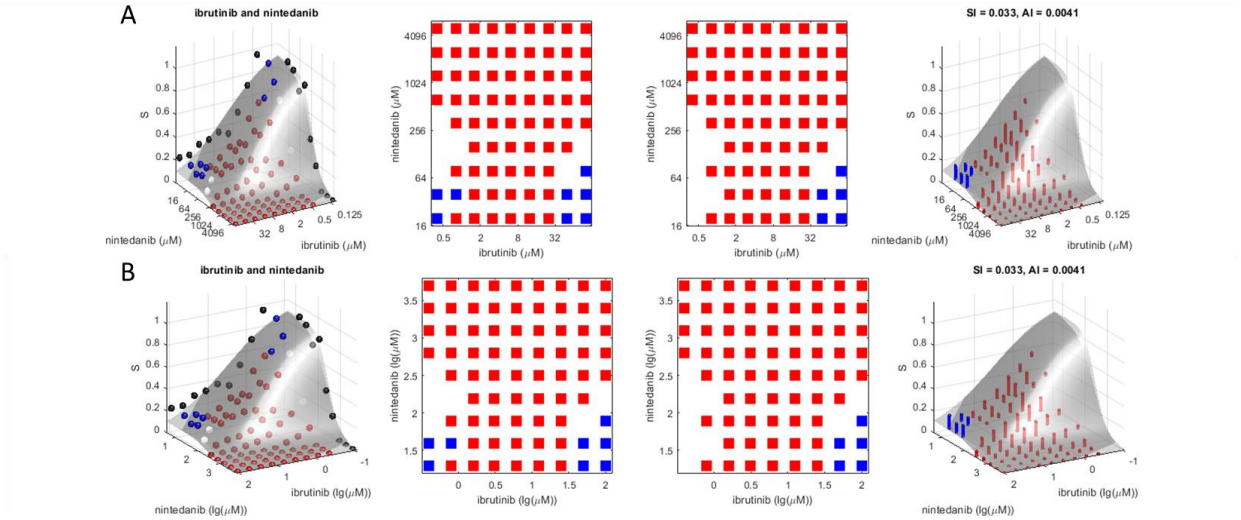

Figure 2 The customized label for (A) an arithmetic scale and (B) a logarithmic scale.

3.2 RSRA

"RSRA_package.m" is the main function of response surface regression analysis (RSRA) in comparison to REA. RSRA generally fits data obtained from experiments or simulations onto a response surface. The response surface is constructed from one of the three models: Loewe Additivity model (linear isobole set) [3], Bliss Independence model [4], and Gaddum's Non-interaction model [4]. The fitting parameter to quantify combination effect is α, β, and γ for those three models, respectively. Since RSRA only performs regression to investigate the global combination effect, no graphical output is involved. . The meaning of the input and output is shown in Table 2.

Table 2 The input and output of RSRA. All input parameters are required.

Input	Meaning
data	Data matrix to be analyzed by REA
ca	Initialization of α, typically 0 for additivity
cb	Initialization of β and γ, typically 1 for additivity
Output	**Meaning**
alpha	Fitting parameter of Loewe Additivity model (linear isobole set). $\alpha > 0$, $\alpha = 0$, and $\alpha < 0$ represents synergy, additivity, and antagonism.
beta	Fitting parameter of Bliss Independence model. $\beta < 1$, $\beta = 1$, and $\beta > 1$ represents synergy, additivity, and antagonism.
gamma	Fitting parameter of Gaddum's Non-interaction model. $\gamma < 1$, $\gamma = 1$, and $\gamma > 1$ represents synergy, additivity, and antagonism.

The following syntax can be used to calculate the three fitting parameters:

```
[alpha,beta,gamma] = RSRA_package(data,0,1)
```

The output is:

```
alpha = -0.4775

beta = 0.9803

gamma = 0.8445
```

Different parameters suggest different combination effects. α based on Loewe Additivity model (linear isobole set) suggests antagonism, β based on Bliss Independence model suggests additivity (an interval of [0.95,1.05] is typically taken as additivity in practice [1]), and γ based on Gaddum's Non-interaction model suggests additivity. These inconsistent results from different parameters make it difficult for one to draw a conclusion about combination effect.

4 References

1. Mathews Griner, L.A. et al. High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 2349-2354 (2014).
2. Borisy, A.A. et al. Systematic discovery of multicomponent therapeutics. *Proceedings of the National Academy of Sciences* **100**, 7977-7982 (2003).
3. Greco, W.R., Park, H.S. & Rustum, Y.M. Application of a New Approach for the Quantitation of Drug Synergism to the Combination of cis-Diamminedichloroplatinum and 1-β-D-Arabinofuranosylcytosine. *Cancer research* **50**, 5318-5327 (1990).
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