OptForce Tutorial

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INTRODUCTION:

In this tutorial we will run optForce. For a detailed description of the procedure, please see [1]. Briefly, the problem is to find a set of interventions of size "K" such that when these interventions are applied to a wild-type strain, the mutant created will produce a particular target of interest in a higher rate than the wild-type strain. The interventions could be knockouts (lead to zero the flux for a particular reaction), upregulations (increase the flux for a particular reaction) and downregulations (decrease the flux for a particular reaction).

For example, imagine that we would like to increase the production of succinate in Escherichia coli. Which are the interventions needed to increase the production of succinate? We will approach this problem in this tutorial and we will see how each of the steps of OptForce are solved.

MATERIALS

EQUIPMENT

1. MATLAB
2. A solver for Mixed Integer Linear Programming (MILP) problems. For example, Gurobi.

EQUIPMENT SETUP

Use `changeCobraSolver` to choose the solver for MILP problems.

PROCEDURE

The proceduce consists on the following steps

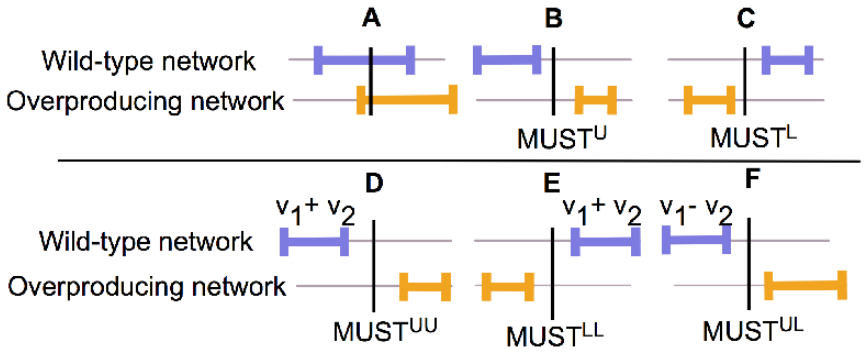
1) Maximize specific growth rate and product formation.

2) Define constraints for both wild-type and mutant strain:

3) Perform flux variability analysis for both wild-type and mutant strain.

4) Find must sets, i.e, reactions that MUST increase or decrease their flux in order to achieve the phenotype in the mutant strain.

Figure 1.



5) Find the interventions needed that will ensure a increased production of the target of interest

Now, we will approach each step in detail.

STEP 1: Maximize specific growth rate and product formation

First, we load the model. This model comprises only 90 reactions, which describe the central metabolism of E. coli [2].

Then, we change the objective function to maximize biomass ("R75"). We also change the lower bounds, so E. coli will be able to consume glucose, oxygen, sulfate, ammomium, citrate and glycerol.

```
global TUTORIAL_INIT_CB;
if ~isempty(TUTORIAL_INIT_CB) && TUTORIAL_INIT_CB==1
    initCobraToolbox
    changeCobraSolver('gurobi','all');
end

pathTutorial = which('tutorial_OptForce.mlx');
pathstr = fileparts(pathTutorial);
cd(pathstr)

load('AntCore');
model.c(strcmp(model.rxns,'R75')) = 1;
model = changeRxnBounds(model, 'EX_gluc', -100, 'l');
model = changeRxnBounds(model, 'EX_o2', -100, 'l');
model = changeRxnBounds(model, 'EX_so4', -100, 'l');
model = changeRxnBounds(model, 'EX_nh3', -100, 'l');
model = changeRxnBounds(model, 'EX_cit', -100, 'l');
model = changeRxnBounds(model, 'EX_glyc', -100, 'l');
```

Then, we calculate the maximum specific growth rate and the maximum production rate for succinate

```
growthRate = optimizeCbModel(model);
fprintf('The maximum growth rate is %1.2f', growthRate.f);

model = changeObjective(model, 'EX_suc');
maxSucc = optimizeCbModel(model);
fprintf('The maximum production rate of succinate is %1.2f', maxSucc.f);
```

TIP: The biomass reaction is usually set to 1%-10% of maximum theoretical biomass yield when running the following steps, to prevent solutions with not biomass formation

1. maximizing product formation

2. finding MUST sets of second order
3. finding FORCE sets

STEP 2: Define constraints for both wild-type and mutant strain

TIMING: This step should take a few days or weeks, depending on the information available for your species.

CRITICAL STEP: This is a manual task, so you should search for information in articles or even perform your own experiments. You can also make assumptions for describing the phenotypes of both strains which will make this task a little faster but make sure to have two strains different enough, because you should be able to find differences in reactions ranges.

First, we load the model. This model comprises only 90 reactions, which describe the central metabolism of E. coli [2].

Then, we change the objective function to maximize biomass ("R75"). We also change the lower bounds, so E. coli will be able to consume glucose, oxygen, sulfate, ammomium, citrate and glycerol.

We define constraints for each strain

```
constrWT = struct('rxnList', {{'R75'}}, 'rxnValues', 14, 'rxnBoundType', 'b');
constrMT = struct('rxnList', {{'R75', 'EX_suc'}}, 'rxnValues', [0, 155.55], 'rxnBoundType', 'b
```

Step 3: Flux Variability Analysis

TIMING: This task should take from a few seconds to a few hours depending on the size of your reconstruction

We run the FVA analysis for both strains

```
[minFluxesW, maxFluxesW, minFluxesM, maxFluxesM, ~, ~] = FVAOptForce(model, constrWT, constrMT
disp([minFluxesW, maxFluxesW, minFluxesM, maxFluxesM]);
```

Now, the run the next step of OptForce.

Step 4: Find Must Sets

TIMING: This task should take from a few seconds to a few hours depending on the size of your reconstruction

First, we define an ID for this run. Each time you run the functions associated to the optForce procedure, some folders can be generated to store inputs used in that run. Outputs are stored as well. These folder will be located inside the folder defined by your run ID. Thus, if your runID is "TestOptForce", the structure of the folders will be the following:

```
├── CurrentFolder
| ├── TestOptForce
| | ├── Inputs
| | └── Outputs
```

To avoid the generation of inputs and outputs folders, set `keepInputs = 0`, `printExcel = 0` and `printText = 0`.

Also, a report of the run is generated each time you run the functions associated to the optForce procedure. So, the idea is to give a different `runID` each time you run the functions, so you will be able to see the report (inputs used, outputs generated, errors in the run) for each run.

We define then our `runID`

```
runID = 'TestOptForceM';
```

Fow now, only functions to find first and second order must sets are supported in this third step. As depicted in **Figure 1**, the first order must sets are MUSTU and MUSTL; and second order must sets are MUSTUU, MUSTLL and MUSTUL

A) Finding first order must sets

We define constraints

```
constrOpt = struct('rxnList', {{'EX_gluc', 'R75', 'EX_suc'}}, 'values', [-100, 0, 155.5]');
```

We then run the functions `findMustL` and `findMustU` that will allow us to find `mustU` and `mustL` sets, respectively.

i) MustL Set:

```
[mustLSet, pos_mustL] = findMustL(model, minFluxesW, maxFluxesW, 'constrOpt', constrOpt, ...
                                  'runID', runID, 'outputFolder', 'OutputsFindMustL', ...
                                  'outputFileName', 'MustL' , 'printExcel', 1, 'printText', 1,
                                  'printReport', 1, 'keepInputs', 1, 'verbose', 0);
```

Note that the folder "TestOptForceM" was created. Inside this folder, two additional folders were created: "InputsMustL" and "OutputsMustL". In the inputs folder you will find all the inputs required to run the the function `findMustL`. Additionally, in the outputs folder you will find the `mustL` set found, which were saved in two files (.xls and .txt). Furthermore, a report which summarize all the inputs and outputs used during your running was generated. The name of the report will be in this format "report-Day-Month-Year-Hour-Minutes". So, you can mantain a chronological order of your experiments.

We display the reactions that belongs to the `mustL` set

```
disp(mustLSet)
```

ii) MustU set:

```
[mustUSet, pos_mustU] = findMustU(model, minFluxesW, maxFluxesW, 'constrOpt', constrOpt, ...
                                  'runID', runID, 'outputFolder', 'OutputsFindMustU', 'outputF
                                  'MustU' , 'printExcel', 1, 'printText', 1, ...
                                  'printReport', 1, 'keepInputs', 1, 'verbose', 0);
```

Note that the folders "InputsMustU" and "OutputsFindMustU" were created. These folders contain the inputs and outputs of `findMustU`, respectively.

We display the reactions that belongs to the `mustU` set

```
disp(mustUSet)
```

B) Finding second order must sets

First, we define the reactions that will be excluded from the analysis. It it suggested to include in this list the reactions found in the previous step as well as exchange reactions

```
constrOpt = struct('rxnList', {{'EX_gluc', 'R75', 'EX_suc'}}, 'values', [-100, 0, 155.5]');
exchangeRxns = model.rxns(cellfun(@isempty, strfind(model.rxns, 'EX_')) == 0);
excludedRxns = unique([mustUSet; mustLSet; exchangeRxns]);
```

Now, we run the functions for finding second order must sets

i) MustUU:

```
[mustUU, pos_mustUU, mustUU_linear, pos_mustUU_linear] = ...
    findMustUU(model, minFluxesW, maxFluxesW, 'constrOpt', constrOpt, ...
               'excludedRxns', excludedRxns,'runID', runID, ...
               'outputFolder', 'OutputsFindMustUU', 'outputFileName', 'MustUU', ...
               'printExcel', 1, 'printText', 1, 'printReport', 1, 'keepInputs', 1, ...
               'verbose', 1);
```

Note that the folders "InputsMustUU" and "OutputsFindMustUU" were created. These folders contain the inputs and outputs of `findMustUU`, respectively.

We display the reactions that belongs to the `mustUU` set

```
disp(mustUU);
```

ii) MustLL:

```
[mustLL, pos_mustLL, mustLL_linear, pos_mustLL_linear] = ...
    findMustLL(model, minFluxesW, maxFluxesW, 'constrOpt', constrOpt, ...
               'excludedRxns', excludedRxns,'runID', runID, ...
               'outputFolder', 'OutputsFindMustLL', 'outputFileName', 'MustLL', ...
               'printExcel', 1, 'printText', 1, 'printReport', 1, 'keepInputs', 1, ...
               'verbose', 1);
```

Note that the folders "InputsMustLL" and "OutputsFindMustLL" were created. These folders contain the inputs and outputs of `findMustLL`, respectively.

We display the reactions that belongs to the `mustLL` set. In this case, `mustLL` is an empty array because no reaction was found in the `mustLL` set.

```
disp(mustLL);
```

iii) MustUL:

```
[mustUL, pos_mustUL, mustUL_linear, pos_mustUL_linear] = ...
    findMustUL(model, minFluxesW, maxFluxesW, 'constrOpt', constrOpt, ...
               'excludedRxns', excludedRxns,'runID', runID, ...
               'outputFolder', 'OutputsFindMustUL', 'outputFileName', 'MustUL', ...
               'printExcel', 1, 'printText', 1, 'printReport', 1, 'keepInputs', 1, ...
               'verbose', 1);
```

Note that the folders "InputsMustUL" and "OutputsFindMustUL" were created. These folders contain the inputs and outputs of `findMustUL`, respectively.

We display the reactions that belongs to the `mustUL` set. In this case, `mustUL` is an empty array because no reaction was found in the `mustUL` set.

```
disp(mustUL);
```

TROUBLESHOOTING 1: "I didn't find any reaction in my must sets"

TROUBLESHOOTING 2: "I got an error when running the `findMustX` functions (X = L or U or LL or UL or UU depending on the case)"

Step 5: OptForce

TIMING: This task should take from a few seconds to a few hours depending on the size of your reconstruction

We define constraints and we define `K` the number of interventions allowed, `nSets` the maximum number of sets to find, and `targetRxn` the reaction producing the metabolite of interest (in this case, succinate).

Additionally, we define the `mustU` set as the union of the reactions that must be upregulated in both first and second order must sets; and `mustL` set as the union of the reactions that must be downregulated in both first and second order must sets .

```
mustU = unique(union(mustUSet, mustUU));
mustL = unique(union(mustLSet, mustLL));
targetRxn = 'EX_suc';
biomassRxn = 'R75';
k = 1;
nSets = 1;
constrOpt = struct('rxnList', {{'EX_gluc','R75'}}, 'values', [-100, 0]);

[optForceSets, posOptForceSets, typeRegOptForceSets, flux_optForceSets] = optForce(model, targ
                                                                                   minFluxesW,
                                                                                   'k', k, 'nS
                                                                                   'runID', ru
                                                                                   'outputFile
                                                                                   'printRepor
```

Note that the folders "InputsOptForce" and "OutputsOptForce" were created. These folders contain the inputs and outputs of `optForce`, respectively.

We display the reactions found by `optForce`

```
disp(optForceSets)
```

The reaction found was "SUCt", i.e. a transporter for succinate (a very intuitive solution).

Next, we will increase `k` and we will exclude "SUCt" from upregulations to found non-intuitive solutions.

TIP: Sometimes the product is at the end of a long linear pathway. In that case, the recomendation is to also exclude most reactions on the linear pathway. Essential reactions and reactions not associated with any gene should also be excluded.

We will only search for the 20 best solutions, but you can try with a higher number.

We will change the runID to save this second result (K = 2) in a diffetent folder than the previous result (K = 1)

```
k = 2;
nSets = 20;
runID = 'TestOptForceM2';
excludedRxns = struct('rxnList', {{'SUCt'}}, 'typeReg','U');
[optForceSets, posOptForceSets, typeRegOptForceSets, flux_optForceSets] = ...
    optForce(model, targetRxn, biomassRxn, mustU, mustL, ...
             minFluxesW, maxFluxesW, minFluxesM, maxFluxesM, ...
             'k', k, 'nSets', nSets, 'constrOpt', constrOpt, ...
             'excludedRxns', excludedRxns, ...
             'runID', runID, 'outputFolder', 'OutputsOptForce', ...
             'outputFileName', 'OptForce', 'printExcel', 1, 'printText', 1, ...
             'printReport', 1, 'keepInputs', 1, 'verbose', 1);
```

Note that the folders "InputsOptForce" and "OutputsOptForce" were created inside TestOptForce2. These folders contain the inputs and outputs of `optForce`, respectively.

We display the reactions found by `optForce`

```
disp(optForceSets)
```

TIMING

1. STEP 1 ~ 1-2 seconds
2. STEP 2: ~ 2-5 seconds
3. STEP 3: ~ 10-20 seconds
4. STEP 4: ~ 10-20 seconds

TROUBLESHOOTING

1) problem: "I didn't find any reaction in my must sets"

possible reason: the wild-type or mutant strain is not constrained enough.

solution: add more constraints to your strains until you find differences in your reaction ranges. If you don't find any differences, it is better to change the approach and use another algorithm.

2) problem: "I got an error when running the `findMust` functions"

possible reason: inputs are not defined well or solver is not defined

solution: verify your inputs, use `changeCobraSolver`, verify that the global variable `CBT_MILP_SOLVER` is not empty. It should containg the identifier for a MILP solver.

ANTICIPATED RESULTS

In this tutorial some folders will be created inside the folder called "runID" to store inputs and outputs of the optForce functions (findMustU.m, findMustL.m, findMustUU.m, findMustLL.m, findMustUL.m, optForce.m)

In this case runID = 'TestOptForce', so inside this folder the following folders will be created:

```
├── CurrentFolder
| ├── TestOptForceM
| | ├── InputsFindMustL
| | ├── OutputsFindMustL
| | ├── InputsFindMustU
| | ├── OutputsFindMustU
| | ├── InputsFindMustLL
| | ├── OutputsFindMustLL
| | ├── InputsFindMustUU
| | ├── OutputsFindMustUU
| | ├── InputsFindMustUL
| | ├── OutputsFindMustUL
| | ├── InputsOptForce
| | └── OutputsOptForce
```

The input folders contain inputs (.mat files) for running the functions to solve each one of the bilevel problems. Output folders contain results of the algorithms (.xls and .txt files) as well as a report (.txt) summarizing the outcomes of the steps performed during the execution of the optForce functions.

The optForce algorithm will find sets of reactions that should increase the production of your target. The first sets found should be the best ones because the production rate will be the highest. The last ones should be the worse because the production rete will be slower. Be aware that some sets could not guarante a minimum production rate for your target, so you always have to check the minimum production rate. You can do this using the function testOptForceSol.m. Some sets could allow a higher growth rate than others, so keep in mind this too when deciding which set is better.

Acknowledgments

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References

[1] Ranganathan S, Suthers PF, Maranas CD (2010) OptForce: An Optimization Procedure for Identifying All Genetic Manipulations Leading to Targeted Overproductions. PLOS Computational Biology 6(4): e1000744. https://doi.org/10.1371/journal.pcbi.1000744.

[2] Maciek R. Antoniewicz, David F. Kraynie, Lisa A. Laffend, Joanna González-Lergier, Joanne K. Kelleher, Gregory Stephanopoulos, Metabolic flux analysis in a nonstationary system: Fed-batch fermentation of a high yielding strain of E. coli producing 1,3-propanediol, Metabolic Engineering, Volume 9, Issue 3, May 2007, Pages 277-292, ISSN 1096-7176, https://doi.org/10.1016/j.ymben.2007.01.003.