Procedures to compute elementary flux modes or extreme pathways forming glyceraldehyde 3-phosphate (GAP) from CO_2

Please prepare the 3 text files named Paths_example.txt, perform_expa.bat, and CO2_to_GAP.expa as described below. The following procedure was successful on a Windows PC on August 18, 2021.

1. Go to https://systemsbiology.ucsd.edu/Downloads/ExtremePathwayAnalysis
2. Get expa.zip by clicking "A new ExPA program (02/16/05)".
3. Extract expa.exe from expa.zip.
4. Make a working directory and put the 3 files of expa.exe, perform_expa.bat, and CO2_to_GAP.expa there.
5. Double-click on perform_expa.bat in the directory from Windows Explorer.
6. Find Paths.txt generated in the same directory. The content of Paths.txt must be the same as that of Paths_example.txt. Paths.txt as well as Paths_example.txt carries a matrix, where each of rows corresponds to one elementary flux mode or extreme pathway and each of columns to one of the fluxes defined in CO2_to_GAP.expa. The last 3 rows of the matrix correspond to the 3 columns in Table 2, which correspond to the S7P-removing transaldolase variant, the S7P-forming transaldolase variant, and the canonical Calvin-Benson cycle. The other rows correspond to pathways neither consuming CO_2 nor producing GAP. They include 2 cycles composed of 5 reactions, but those cycles require the unrealistic reverse reaction of FBPase or SBPase. Each row of CO2_to_GAP.expa beginning at '>' corresponds to the reaction with the same name as defined in Table 1, whilst each row of CO2_to_GAP.expa beginning at '<' corresponds to the reverse reaction of the reaction with the same name as defined in Table 1.

Paths_example.txt

```
0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 1 0 0
0 0 0 0 1 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0
0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0
0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0
0 1 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0
0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0
0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0
3 6 6 2 0 0 1 2 2 1 1 2 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 -3 1
3 6 6 2 2 2 1 0 0 1 1 2 3 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -3 1
3 6 6 2 1 1 1 1 1 1 1 2 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -3 1
```

perform_expa.bat

```
expa -v CO2_to_GAP.expa
```

CO2_to_GAP.expa

```
(Internal Fluxes)
>Rubisco                        I       -1 CO2   -1 RuBP     2 PGA
>PGA_kinase                     I       -1 PGA               1 BPG
>GAP_dehydrogenase              I       -1 BPG               1 GAP
>Triose_phosphate_isomerase     I       -1 GAP               1 DHAP
>FBP_aldolase                   I       -1 GAP   -1 DHAP     1 FBP
>FBPase                         I       -1 FBP               1 F6P
>Transketolase                  I       -1 F6P   -1 GAP      1 E4P    1 Xu5P
>SBP_aldolase                   I       -1 E4P   -1 DHAP     1 SBP
>SBPase                         I       -1 SBP               1 S7P
>Transketolase                  I       -1 S7P   -1 GAP      1 R5P    1 Xu5P
>Isomerase                      I       -1 R5P               1 Ru5P
>Epimerase                      I       -1 Xu5P              1 Ru5P
>Phosphoribulokinase            I       -1 Ru5P              1 RuBP
S7P-forming_transaldolase       I       -1 E4P   -1 F6P      1 S7P    1 GAP
<Rubisco                        I        1 CO2    1 RuBP    -2 PGA
<PGA_kinase                     I        1 PGA               -1 BPG
<GAP_dehydrogenase              I        1 BPG               -1 GAP
<Triose_phosphate_isomerase     I        1 GAP               -1 DHAP
<FBP_aldolase                   I        1 GAP    1 DHAP     -1 FBP
<FBPase                         I        1 FBP               -1 F6P
<Transketolase                  I        1 F6P    1 GAP      -1 E4P   -1 Xu5P
<SBP_aldolase                   I        1 E4P    1 DHAP     -1 SBP
<SBPase                         I        1 SBP               -1 S7P
<Transketolase                  I        1 S7P    1 GAP      -1 R5P   -1 Xu5P
<Isomerase                      I        1 R5P               -1 Ru5P
<Epimerase                      I        1 Xu5P              -1 Ru5P
<Phosphoribulokinase            I        1 Ru5P              -1 RuBP
S7P-removing_transaldolase      I        1 E4P    1 F6P      -1 S7P   -1 GAP
(Exchange Fluxes)
CO2                             Input
GAP                             Output
```