Step-by-Step 13CFLUX2 Test Case - Webservice

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Save the python_scripts directory. Save the Ecoli directory in the python_scripts directory. Open the cmd command window. Switch in the python_scripts directory with cd [path_to_python_script_directory] . Enter the following commands in the cmd-terminal command line:

1. Start with checking the syntax of your FluxML document:
   >> fmllint.py -i Ecoli\Ecoli.fml

   The following error message should appear on stdout:

```
No handlers could be found for logger "suds.client"
XML exception: XML parser (fatal error): expected end of tag
'metabolitepools' in stdin; row: 47, column: 7
     location: #document/fluxml/reactionnetwork/metabolitepools/#text
 node details: #text{
    }
XML exception: element node (reaction) expected.
     location:
 node details: (null)
FluxML parsing error: element node (reaction) expected.
```

   (See also file: stderror1)

2. Fix the error by editing the FluxML file :
   In line 47 replace “`metabolitepols`” by “`metabolitepools`”:

```
...
 <pool atoms="5" id="Val"/>
 <pool atoms="5" id="X5P"/>
</metabolitepols>
<reaction id="uptU">
  <reduct cfg="ABCDEF" id="GLCU"/>
...
```

   (See also file Ecoli2.fml: there this error is fixed)

3. With the corrected Ecoli.fml file (or the Ecoli2.fml file) type again the command:

   >> fmllint.py -i Ecoli\Ecoli(2).fml

   Now the following error message is printed:

```
No handlers could be found for logger "suds.client"
XML exception: cfg "default": element "fluxvalue": illegal value for
attribute "type"
location: #document/fluxml/configuration/simulation/variables/fluxvalue
node details: #<fluxvalue flux="tca6" type="xc"/>
FluxML parsing error: cfg "default": element "fluxvalue": illegal value for
attribute "type"
```

   (See also file: stderror2)

4. Again fix the error by editing the FluxML file:

   In line 698 replace “xc” by “xch”:

```
…
        <fluxvalue flux="emp1" type="xch">0.428571428571</fluxvalue>
        <fluxvalue flux="tca6" type="xc">1.5</fluxvalue>
        <fluxvalue flux="BM_oaa1" type="xch">0.111111111111</fluxvalue>
      </variables>
…
```

   (See also file Ecoli3.fml - there both errors are fixed)

5. If now the command

   >> fmllint.py -i Ecoli\Ecoli(3).fml

   is executed, no error message should appear, only:

```
*** This version of 13CFLUX 2 is licensed to:
*** Demo Version, FZ Juelich
```

6. Next step is the attempt of forward simulation:

   >> fwdsim.py -i Ecoli\Ecoli(3).fml -o Ecoli\Ecoli.fwd

   You should get the following output on stdout:

```
No handlers could be found for logger "suds.client"
Failed to invoke web service:
validating configuration "default" ...
user requests 26 free fluxes (11 free net + 15 free xch).
size of stoichiometry is (35x68); exact rank is 35.
size of NET constraint system is (44x68); numerical rank is 44
size of XCH constraint system is (1x68); numerical rank is 1
```

```
the following NET-fluxes are completely determined by constraint-fluxes:
   BM_oga1.n [= .18523] = .18523
   BM_pep1.n [= .05481] = .05481
   BM_pep2.n [= .05481] = .05481
   BM_pga1.n [= BM_pga1_aux.n] = .144642
   BM_pyr3.n [= BM_pyr4_aux.n] = .064982
   BM_pyr4.n [= BM_pyr4_aux.n] = .064982
validation succeeded!
using the following stoichiometry ...
              uptU: net=     +2.66531 (DEPD), xch=            0 (CONS)
              upt0: net=     +10.6612 (DEPD), xch=            0 (CONS)
               upt: net=     +13.3265 (DEPD), xch=            0 (CONS)
              emp1: net=     +13.2724 (DEPD), xch=           50 (FREE)
              emp2: net=      +13.785 (FREE), xch=            0 (CONS)
              emp3: net=     +13.2332 (DEPD), xch=           50 (FREE)
              emp4: net=     +26.4108 (DEPD), xch=           50 (FREE)
              emp5: net=     +26.2173 (DEPD), xch=           50 (FREE)
              emp6: net=     +18.1537 (FREE), xch=            0 (CONS)
             gneo1: net=    +0.551815 (DEPD), xch=            0 (CONS)
             gneo2: net=     +14.1365 (DEPD), xch=            0 (CONS)
              ppp1: net=   +0.0476512 (FREE), xch=            0 (CONS)
              ppp2: net=   -0.0305639 (DEPD), xch=           50 (FREE)
              ppp3: net=   +0.0782151 (DEPD), xch=           50 (FREE)
              ppp4: net=   -0.0426869 (DEPD), xch=           50 (FREE)
              ppp5: net=   +0.0121231 (DEPD), xch=           50 (FREE)
              ppp6: net=   +0.0121231 (DEPD), xch=           50 (FREE)
              tca1: net=     +13.8254 (DEPD), xch=            0 (CONS)
              tca2: net=     +24.9643 (DEPD), xch=            0 (CONS)
              tca3: net=     +36.3948 (DEPD), xch=            0 (CONS)
              tca4: net=     +36.2014 (DEPD), xch=            0 (CONS)
             tca5a: net=     +12.3855 (DEPD), xch=           50 (FREE)
             tca5b: net=     +12.3855 (DEPD), xch=           50 (DEPD)
              tca6: net=     +3.09543 (FREE), xch=           50 (FREE)
               gs1: net=     -11.4305 (FREE), xch=            0 (CONS)
               gs2: net=     -11.4305 (DEPD), xch=            0 (CONS)
              ana1: net=     +22.5142 (DEPD), xch=            0 (CONS)
              ana2: net=        +0.43 (CONS), xch=            0 (CONS)
              ana3: net=      +10.245 (DEPD), xch=            0 (CONS)
             coOut: net=     +74.9898 (FREE), xch=            0 (CONS)
          BM_pga1: net=    +0.144642 (QCON), xch=           50 (FREE)
      BM_pga1_aux: net=    +0.144642 (CONS), xch=            0 (CONS)
          BM_pyr2: net=   +0.0822769 (DEPD), xch=            0 (CONS)
      BM_pyr2_aux: net=   +0.0822769 (DEPD), xch=            0 (CONS)
          BM_pyr3: net=    +0.064982 (QCON), xch=            0 (CONS)
          BM_pyr4: net=    +0.064982 (QCON), xch=            0 (CONS)
      BM_pyr4_aux: net=    +0.064982 (CONS), xch=            0 (CONS)
          BM_pep1: net=     +0.05481 (QCON), xch=            0 (CONS)
          BM_pep2: net=     +0.05481 (QCON), xch=            0 (CONS)
         BM_pep3a: net=   +0.0171509 (DEPD), xch=            0 (CONS)
         BM_pep3b: net=   +0.0171509 (DEPD), xch=            0 (CONS)
      BM_pep3_aux: net=   +0.0343019 (DEPD), xch=            0 (CONS)
         BM_pep4a: net=   +0.0102541 (DEPD), xch=            0 (CONS)
         BM_pep4b: net=   +0.0102541 (DEPD), xch=            0 (CONS)
      BM_pep4_aux: net=   +0.0205081 (FREE), xch=            0 (CONS)
          BM_oga1: net=     +0.18523 (QCON), xch=           50 (FREE)
      BM_oga1_aux: net=    +0.114557 (DEPD), xch=            0 (CONS)
          BM_oga2: net=    +0.070673 (DEPD), xch=            0 (CONS)
      BM_oga2_aux: net=    +0.070673 (FREE), xch=            0 (CONS)
          BM_oaa1: net=    +0.158655 (DEPD), xch=           50 (FREE)
      BM_oaa1_aux: net=   +0.0899823 (FREE), xch=            0 (CONS)
          BM_oaa3: net=   +0.0686726 (DEPD), xch=           50 (FREE)
      BM_oaa3_aux: net=  +0.00230051 (FREE), xch=            0 (CONS)
          BM_oaa4: net=   +0.0663721 (DEPD), xch=            0 (CONS)
      BM_oaa4_aux: net=   +0.0663721 (FREE), xch=            0 (CONS)
         BM_oaa6a: net=    +0.013136 (DEPD), xch=            0 (CONS)
         BM_oaa6b: net=    +0.013136 (DEPD), xch=            0 (CONS)
      BM_oaa6_aux: net=   +0.0262721 (DEPD), xch=            0 (CONS)
           BM_G6P: net=    +0.006487 (CONS), xch=            0 (CONS)
           BM_F6P: net=     +0.00864 (CONS), xch=            0 (CONS)
           BM_GAP: net=    +0.012921 (CONS), xch=            0 (CONS)
```

```
            BM_PGA: net=    +0.048837 (CONS), xch=           0 (CONS)
            BM_PEP: net=     +0.00624 (CONS), xch=           0 (CONS)
            BM_PYR: net=     +0.04968 (CONS), xch=           0 (CONS)
            BM_R5P: net=    +0.066092 (CONS), xch=           0 (CONS)
             BM_Ac: net=     +0.22656 (CONS), xch=           0 (CONS)
            BM_OAA: net=    +0.030407 (CONS), xch=           0 (CONS)
            BM_OGA: net=     +0.00816 (CONS), xch=           0 (CONS)
tayloring simulation for measurement specifications ...
There are more than 3 free fluxes! (number of free fluxes=26)
There are more than 15 measurements! (number of measurements=194)
This is not allowed in demo version !
```

(See also file fwdsim_error_number_flux_meas)

To use the demo version of 13CFLUX2 you have to reduce the number of measurements and free fluxes:

6.1 Reducing the number of measurements:

In Ecoli(3).fml:

Delete the labeling measurement groups:

ms_group_2, ms_group_3, ms_group_4, ms_group_5, ms_group_7, ms_group_8 and ms_group_10 till ms_group_35. And also delete the m3, m4, m5 and m6 measurements of ms_group_9 (in red):

```
<measurement>
  <model>
      <labelingmeasurement>
        <group id="ms_group_1" scale="auto">
          <textual>Asp[1,2,3,4]#M0,1,2,3,4</textual>
        </group>
        <group id="ms_group_2" scale="auto">
          <textual>Asp[1,2,3,4]#M0,1,2,3,4</textual>
        </group>
        <group id="ms_group_3" scale="auto">
          <textual>Asp[2,3,4]#M0,1,2,3</textual>
        </group>
        <group id="ms_group_4" scale="auto">
          <textual>Asp[2,3,4]#M0,1,2,3</textual>
        </group>
        <group id="ms_group_5" scale="auto">
          <textual>Asp[1,2]#M0,1,2</textual>
        </group>
        <group id="ms_group_6" scale="auto">
          <textual>Glu[1,2,3,4,5]#M0,1,2,3,4,5</textual>
        </group>
        <group id="ms_group_7" scale="auto">
          <textual>Glu[2,3,4,5]#M0,1,2,3,4</textual>
        </group>
        <group id="ms_group_8" scale="auto">
          <textual>Glu[2,3,4,5]#M0,1,2,3,4</textual>
        </group>
        <group id="ms_group_9" scale="auto">
          <textual>Ile[1,2,3,4,5,6]#M0,1,2,3,4,5,6</textual>
        </group>
        <group id="ms_group_10" scale="auto">
          <textual>Ile[2,3,4,5,6]#M0,1,2,3,4,5</textual>
        </group>
        <group id="ms_group_11" scale="auto">
          <textual>Ile[2,3,4,5,6]#M0,1,2,3,4,5</textual>
        </group>
      …
        <group id="ms_group_35" scale="auto">
          <textual>Val[1,2]#M0,1,2</textual>
```

```
  </group>
</labelingmeasurement>
<fluxmeasurement>
     …
```

To delete these measurements correctly, you also have to delete the data to this measurements (in red):

```
<data>
  <datum id="fm_1" stddev="0.2">2.3</datum>
  <datum id="ms_group_1" stddev="0.020" weight="0">0.513</datum>
  <datum id="ms_group_1" stddev="0.020" weight="1">0.214</datum>
  <datum id="ms_group_1" stddev="0.020" weight="2">0.192</datum>
  <datum id="ms_group_1" stddev="0.020" weight="3">0.047</datum>
  <datum id="ms_group_1" stddev="0.020" weight="4">0.012</datum>
  <datum id="ms_group_2" stddev="0.020" weight="0">0.508</datum>
  <datum id="ms_group_2" stddev="0.020" weight="1">0.203</datum>
  <datum id="ms_group_2" stddev="0.020" weight="2">0.192</datum>
  <datum id="ms_group_2" stddev="0.020" weight="3">0.056</datum>
  <datum id="ms_group_2" stddev="0.020" weight="4">0.019</datum>
  <datum id="ms_group_3" stddev="0.020" weight="0">0.556</datum>
  <datum id="ms_group_3" stddev="0.020" weight="1">0.250</datum>
  <datum id="ms_group_3" stddev="0.020" weight="2">0.144</datum>
  <datum id="ms_group_3" stddev="0.020" weight="3">0.034</datum>
  <datum id="ms_group_4" stddev="0.020" weight="0">0.541</datum>
  <datum id="ms_group_4" stddev="0.020" weight="1">0.251</datum>
  <datum id="ms_group_4" stddev="0.020" weight="2">0.142</datum>
  <datum id="ms_group_4" stddev="0.020" weight="3">0.051</datum>
  <datum id="ms_group_5" stddev="0.020" weight="0">0.704</datum>
  <datum id="ms_group_5" stddev="0.020" weight="1">0.143</datum>
  <datum id="ms_group_5" stddev="0.020" weight="2">0.142</datum>
  <datum id="ms_group_6" stddev="0.020" weight="0">0.375</datum>
  <datum id="ms_group_6" stddev="0.020" weight="1">0.244</datum>
  <datum id="ms_group_6" stddev="0.020" weight="2">0.235</datum>
  <datum id="ms_group_6" stddev="0.020" weight="3">0.088</datum>
  <datum id="ms_group_6" stddev="0.020" weight="4">0.026</datum>
  <datum id="ms_group_6" stddev="0.020" weight="5">0.020</datum>
  <datum id="ms_group_7" stddev="0.020" weight="0">0.431</datum>
  <datum id="ms_group_7" stddev="0.020" weight="1">0.297</datum>
  <datum id="ms_group_7" stddev="0.020" weight="2">0.191</datum>
  <datum id="ms_group_7" stddev="0.020" weight="3">0.051</datum>
  <datum id="ms_group_7" stddev="0.020" weight="4">0.010</datum>
  <datum id="ms_group_8" stddev="0.020" weight="0">0.410</datum>
  <datum id="ms_group_8" stddev="0.020" weight="1">0.304</datum>
  <datum id="ms_group_8" stddev="0.020" weight="2">0.199</datum>
  <datum id="ms_group_8" stddev="0.020" weight="3">0.056</datum>
  <datum id="ms_group_8" stddev="0.020" weight="4">0.010</datum>
  <datum id="ms_group_9" stddev="0.020" weight="0">0.380</datum>
  <datum id="ms_group_9" stddev="0.020" weight="1">0.209</datum>
  <datum id="ms_group_9" stddev="0.020" weight="2">0.253</datum>
  <datum id="ms_group_9" stddev="0.020" weight="3">0.082</datum>
  <datum id="ms_group_9" stddev="0.020" weight="4">0.039</datum>
  <datum id="ms_group_9" stddev="0.020" weight="5">0.004</datum>
  <datum id="ms_group_9" stddev="0.020" weight="6">0.001</datum>
  <datum id="ms_group_10" stddev="0.020" weight="0">0.397</datum>
  <datum id="ms_group_10" stddev="0.020" weight="1">0.248</datum>
  <datum id="ms_group_10" stddev="0.020" weight="2">0.220</datum>
  <datum id="ms_group_10" stddev="0.020" weight="3">0.079</datum>
  <datum id="ms_group_10" stddev="0.020" weight="4">0.024</datum>
  <datum id="ms_group_10" stddev="0.020" weight="5">0.020</datum>
  <datum id="ms_group_11" stddev="0.020" weight="0">0.382</datum>
  <datum id="ms_group_11" stddev="0.020" weight="1">0.242</datum>
   ...
  <datum id="ms_group_33" stddev="0.020" weight="2">0.169</datum>
  <datum id="ms_group_33" stddev="0.020" weight="3">0.125</datum>
  <datum id="ms_group_33" stddev="0.020" weight="4">0.022</datum>
  <datum id="ms_group_33" stddev="0.020" weight="5">0.020</datum>
  <datum id="ms_group_34" stddev="0.020" weight="0">0.504</datum>
```

```
        <datum id="ms_group_34" stddev="0.020" weight="1">0.163</datum>
        <datum id="ms_group_34" stddev="0.020" weight="2">0.251</datum>
        <datum id="ms_group_34" stddev="0.020" weight="3">0.036</datum>
        <datum id="ms_group_34" stddev="0.020" weight="4">0.025</datum>
        <datum id="ms_group_35" stddev="0.020" weight="0">0.714</datum>
        <datum id="ms_group_35" stddev="0.020" weight="1">0.098</datum>
        <datum id="ms_group_35" stddev="0.020" weight="2">0.177</datum>
      </data>
```

(See also Ecoli3_meas.fml – in this file all changes are made)

After these changes, first check again the FluxML file with fmllint:

`>> fmllint.py -i Ecoli\Ecoli(3_meas).fml`

You should get no error message.

If you now run fwdsim with this file:

`>> fwdsim.py -i Ecoli\Ecoli(3_meas).fml –o Ecoli\Ecoli.fwd`

You get the following message on standard out:

```
No handlers could be found for logger "suds.client"
Failed to invoke web service:
validating configuration "default" ...
user requests 26 free fluxes (11 free net + 15 free xch).
size of stoichiometry is (35x68); exact rank is 35.
size of NET constraint system is (44x68); numerical rank is 44
size of XCH constraint system is (1x68); numerical rank is 1
the following NET-fluxes are completely determined by constraint-fluxes:
   BM_oga1.n [= .18523] = .18523
   BM_pep1.n [= .05481] = .05481
   BM_pep2.n [= .05481] = .05481
   BM_pga1.n [= BM_pga1_aux.n] = .144642
   BM_pyr3.n [= BM_pyr4_aux.n] = .064982
   BM_pyr4.n [= BM_pyr4_aux.n] = .064982
validation succeeded!
using the following stoichiometry ...
              uptU: net=     +2.66531 (DEPD), xch=            0 (CONS)
              upt0: net=     +10.6612 (DEPD), xch=            0 (CONS)
               upt: net=     +13.3265 (DEPD), xch=            0 (CONS)
              emp1: net=     +13.2724 (DEPD), xch=           50 (FREE)
…

            BM_OAA: net=    +0.030407 (CONS), xch=            0 (CONS)
            BM_OGA: net=     +0.00816 (CONS), xch=            0 (CONS)
tayloring simulation for measurement specifications ...
There are more than 3 free fluxes! (number of free fluxes=26)
This is not allowed in demo version !
```

(See also file fwdsim_error_number_flux)

6.2 Reducing the number of free fluxes:

To reduce the number of free fluxes, fluxes were fixed by adding them into the constraint section. In this example the fluxes are set to values, which results from the optimization of this network with the full unlimited 13CFLUX2 version:

Add the following lines (in red) into ***Ecoli(3_meas).fml:***

```
  <constraints>
    <net>
       <textual>0 &lt;= ppp1; 0 &lt;=gneo1; emp6 &lt;= emp5; 0&lt;=emp6; 0
&lt;= gneo2; gneo2 &lt;= emp6; ana1 &lt;=emp5; ana2 &lt;= emp5; ana3
&lt;=emp5; emp3 &lt;=emp1; gneo1 &lt;= emp1;  4*uptU-upt0=0; tca5a-tca5b=0;
BM_oaa6a-BM_oaa6b=0; BM_pep3a-BM_pep3b=0; BM_pep4a-BM_pep4b=0;
2*BM_pyr2_aux+2*BM_pyr4_aux+BM_oaa4_aux+BM_oaa6_aux=0.387162;
2*BM_pep3_aux+2*BM_pep4_aux=0.109620; BM_oga1_aux+BM_oga2_aux=0.185230;
BM_oaa1_aux+BM_oaa3_aux+BM_oaa4_aux+BM_oaa6_aux=0.184927; 0.0001 &lt;= ana3;
BM_Ac=0.226560; BM_F6P=0.008640; BM_G6P=0.006487; BM_GAP=0.012921;
BM_OAA=0.030407; BM_OGA=0.008160; BM_PEP=0.006240; BM_PGA=0.048837;
BM_PYR=0.049680; BM_R5P=0.066092; BM_pga1_aux=0.144642; BM_pyr4_aux=0.064982;
ana2=0.43; BM_oaa1_aux=0.052037; BM_oaa3_aux=0.052037; BM_oaa4_aux=0.040394;
BM_oga2_aux=0.092615; BM_pep4_aux=0.027405; coOut=4.330657; emp2=1.438056;
gs1=0.832321</textual>
</net>
    <xch>
      <textual>tca5a-tca5b=0; BM_oaa4=0; BM_oaa6a=0; BM_oaa6b=0; BM_oga2=0;
BM_pep1=0; BM_pep2=0; BM_pep3a=0; BM_pep3b=0; BM_pep4a=0; BM_pep4b=0;
BM_pyr2=0; BM_pyr3=0; BM_pyr4=0; ana1=0; ana2=0; ana3=0; emp2=0; emp6=0;
gneo1=0; gneo2=0; gs1=0; gs2=0; ppp1=0; tca1=0; tca2=0; tca3=0; tca4=0;
upt=0; BM_oaa1=100897; BM_oaa3=109865; BM_oga1=78457; BM_pga1=105483;
emp1=120125; emp3=80639;emp4=4.900495e-07; emp5=707227; ppp2=130120;
ppp3=138437; ppp4=1.178837; ppp5=8.200724; ppp6=1.174774; tca5a=4.9008e-07;
tca6=410389</textual>
    </xch>
  </constraints>
```

And delete these lines (in green):

```
    <simulation method="auto" type="auto">
      <variables>
        <fluxvalue flux="BM_oaa1_aux" type="net">0.0899823464901</fluxvalue>
        <fluxvalue flux="BM_oaa3_aux" type="net">0.00230050564996</fluxvalue>
        <fluxvalue flux="BM_oaa4_aux" type="net">0.0663720546518</fluxvalue>
        <fluxvalue flux="BM_oga2_aux" type="net">0.070673028877</fluxvalue>
        <fluxvalue flux="BM_pep4_aux" type="net">0.0205081444022</fluxvalue>
        <fluxvalue flux="coOut" type="net">74.9897690964</fluxvalue>
        <fluxvalue flux="emp2" type="net">13.7850080696</fluxvalue>
        <fluxvalue flux="emp6" type="net">18.1536948609</fluxvalue>
        <fluxvalue flux="gs1" type="net">-11.4304970954</fluxvalue>
        <fluxvalue flux="ppp1" type="net">0.0476511698562</fluxvalue>
        <fluxvalue flux="tca6" type="net">3.09543019274</fluxvalue>
        <fluxvalue flux="BM_oaa1" type="xch">49.9999999884176</fluxvalue>
```

```
        <fluxvalue flux="BM_oaa3" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="BM_oga1" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="BM_pga1" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="emp1" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="emp3" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="emp4" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="emp5" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="ppp2" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="ppp3" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="ppp4" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="ppp5" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="ppp6" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="tca5a" type="xch">49.9999999884176</fluxvalue>
        <fluxvalue flux="tca6" type="xch">49.9999999884176</fluxvalue>
      </variables>
    </simulation>
```

(See also Ecoli3_meas_flux.fml – in this file all changes are made)

Now again first check the FluxML file:

>> fmllint.py -i Ecoli\Ecoli(3_meas_flux).fml

(You should get no error message.)

Then again start the forward simulation:

>> fwdsim.py -i Ecoli\Ecoli(3_meas_flux).fml
–o Ecoli\Ecoli.fwd

You get the following message on standard out:

```
No handlers could be found for logger "suds.client"
Failed to invoke web service:
validating configuration "default" ...
user requests 3 free fluxes (3 free net + 0 free xch).
size of stoichiometry is (35x68); exact rank is 35.
size of NET constraint system is (44x68); numerical rank is 44
size of XCH constraint system is (1x68); numerical rank is 1
the following NET-fluxes are completely determined by constraint-fluxes:
   BM_oaa1.n [= BM_oaa1_aux.n+BM_oaa3_aux.n+BM_oaa4_aux.n] =
.14446799999999999
   BM_oaa3.n [= BM_oaa3_aux.n+BM_oaa4_aux.n] = .092431
  …
   uptU.n [=
.06666666666666667*BM_Ac.n+.19999999999999998*BM_F6P.n+.19999999999999998*BM_
G6P.n+.09999999999999999*BM_GAP.n+.13333333333333333*BM_OAA.n+.16666666666666
```

```
666*BM_OGA.n+.09999999999999999*BM_PEP.n+.09999999999999999*BM_PGA.n+.0999999
99999999999*BM_PYR.n+.16666666666666666*BM_R5P.n+.016666666666666666*BM_oaa1_a
ux.n+.016666666666666666*BM_oaa3_aux.n+.09999999999999999*BM_pga1_aux.n+.0333
3333333333333*BM_pyr4_aux.n+.03333333333333333*coOut.n+.10115298333333334] =
.31019985000000005
the following XCH-fluxes are completely determined by constraint-fluxes:
   tca5b.x [= tca5a.x] = 4.9008e-07
NET ineq. constraint "anonymous" [gneo1>=0] is violated by -1.960119e-02
NET ineq. constraint "anonymous" [-emp5+emp6<=0] is violated by 1.548747e+01
NET ineq. constraint "anonymous" [ana3>=.0001] is violated by -1.043340e+00
some inequalities are violated
using the following stoichiometry ...
              uptU: net=      +0.3102 (QCON), xch=            0 (CONS)
              upt0: net=      +1.2408 (QCON), xch=            0 (CONS)
 …
            BM_OAA: net=    +0.030407 (CONS), xch=            0 (CONS)
            BM_OGA: net=     +0.00816 (CONS), xch=            0 (CONS)
tayloring simulation for measurement specifications ...
configuration "default": inequality constraints violated
validation of configuration failed. Aborting ...
```

(See also file fwdsim_error_wrong_free_flux_values)

By use of these new constraints, the solution space for this mathematical problem has changed. The given error message means, the values of the free fluxes are no longer located in the feasible flux solution space. To get valid free fluxes, sscanner can be used.

7. Setting valid initial free Flux values in the FluxML file with:

>> sscanner.py -i Ecoli\Ecoli(3_meas_flux).fml
–o Ecoli\Ecoli_demo.fml

The sscanner program searches initial free flux values by determining the analytical center (centroid) of the feasible flux space.

The new Ecoli_right.fml is established like the old Ecoli(3_meas_flux).fml file. Only the (values of the) free fluxes have changed (starts at line 399) :

```
 …
 <variables>
    <fluxvalue flux="emp6" type="net">2.2339921051368847</fluxvalue>
    <fluxvalue flux="ppp1" type="net">.10645580452688662</fluxvalue>
    <fluxvalue flux="tca6" type="net">-.1960429620976761</fluxvalue>
  </variables>
```

…

N. b. different calls can lead to different results.

8. Now the forward simulation can be executed successfully:

```
>> fwdsim.py  -i  Ecoli\Ecoli_demo.fml  -o Ecoli\Ecoli.fwd
```

You can find the computed flux values in the Ecoli.fwd file as well as the corresponding norms.

9. If you run the forward simulation with the option "-s" the output file contains additional data like sensitivities of the fluxes:

```
>> fwdsim.py  -i  Ecoli\Ecoli_demo.fml
                                    -o Ecoli\Ecoli_statistic.fwd -s
```

10. Calculating another set of valid initial free flux values with:

```
>> ssampler.py  -i  Ecoli\Ecoli_demo.fml
                                    –o Ecoli\Ecoli_samp.hdf5 –s nx
```

The program ssampler determines several random initial values for the free fluxes. The random initial values were written in an hdf5-file, which is specified by the user. By default 1000 random initial distributions were provided. With the option "-n" [number of samples] the number of initial distributions can be selected. The option "-s nx" forces the sampling of netto and exchange fluxes, by default only the netto fluxes were sampled.

11. You can write one of the random initial distributions, stored in the hdf5 file Ecoli_samp.hdf, into a new FluxML document by the command:

```
>> setfluxes.py  -H Ecoli\Ecoli_samp.hdf5
      -i Ecoli\Ecoli_demo.fml -o  Ecoli\Ecoli_samp.fml -f -l 20
```

The new Ecoli_samp.fml is established like the old Ecoli_demo.fml file. Again just the (values of the) initial/free fluxes have changed. By use of the option "-l 20", the random initial distribution given in line 20 is incorporated in the FluxML file. If ssampler is used without the "-l" option then the random initial distribution given in the first line is incorporated in the FluxML file. Node different calls of ssampler lead to different flux values.

12. Now you can calculate the optimal flux distribution with fitfluxes:

```
>> fitfluxes.py  -i  Ecoli\Ecoli_samp.fml
                                    -o Ecoli\Ecoli_opt.fwd
```

The output you should get on stdout is stored in fitfluxes_stdout.

13. To get statistical data for the optimized flux distribution, first you have to rewrite the optimized flux distribution in the FluxML file. Therefore you can use again the setfluxes program:

```
>> setfluxes.py  -F Ecoli\Ecoli_opt.fwd
            -i  Ecoli\Ecoli_samp.fml -o Ecoli\Ecoli_opt.fml -f
```

The new Ecoli_opt.fml is built like the old Ecoli_samp.fml file. Again just the (values of the) initial/free fluxes have changed.

14. T o get statistical data you can run the forward simulation with fwdsim in combination with the option "-s". Then the output file also contains statistical data like sensitivities of the fluxes:

```
>> fwdsim.py -i  Ecoli\Ecoli_opt.fml
                            -o Ecoli\Ecoli_statistic_opt.fwd  -s
```

Flow Chart for 13C-MFA with 13CFLUX2