2. Ethylene\_biosynthesis\_pathway.spy should include the following reactions:

```
Structural()
"ETHYLENE_tx":
    "x_ETHYLENE" <> "ETHYLENE-CMPD"
    ~
"GUANIDIUM_tx":
    "x_GUANIDIUM" <> "CPD0-1470"
    ~
"RXN-12535":
    3.0 "2-KETOGLUTARATE" + 1.0 "ARG" + 3.0 "OXYGEN-MOLECULE" + 3.0
    "PROTON" -> 2.0 "ETHYLENE-CMPD" + 7.0 "CARBON-DIOXIDE" + 1.0 "SUC" +
    1.0 "CPD0-1470" + "L-DELTA1-PYRROLINE_5-CARBOXYLATE" + 3.0 "WATER"
    ~
```

You will need to add your new submodule to the top-level file MetaReutro.spy, i.e.

Include(AutoReutro.spy,

PHL.spy,
LPS.spy,
FA\_unlumped.spy,
ExtraReacs.spy,
ETC.spy,
Transporters.spy,
Biomass.spy,
Ethylene\_biosynthesis\_pathway.spy)

3.b. The fructose transport reaction is 'FRU\_tx'

3.d. Use lp.SetObjDirec(direc = 'Max') for maximisation

3.e. Set ethylene transporter as the objective using lp.SetObjective(['ETHYLENE\_tx'])

3.g. The maximum theoretical yield is calculated by dividing the ethylene flux value by the fructose flux value. Multiple each flux rate by the number of carbon atoms in the metabolites to calculate the carbon yield.

3.h. The oxygen transport reaction is 'O2\_tx'

4.a. To look for all reactions that produce proline (or its precursors) you can use: for i in m.sm.InvolvedWith('PRO'): print(m.sm.ReacToStr(i)

4.b. To block proline biosynthesis block the following 2 reactions: lp.SetFixedFlux({'GLUTKIN-RXN' : 0.0})

lp.SetFixedFlux({'ORNITHINE-CYCLODEAMINASE-RXN' : 0.0})