Practical 6: Identifying pathways for TAG synthesis in *Phaeodactylum tricornutum*

Here, we will investigate the genome-scale metabolic model of *P. tricornutum* to identify pathways for TAG synthesis. For further details you can refer to Villanova V *et al* (2021) Boosting Biomass Quantity and Quality by Improved Mixotrophic Culture of the Diatom *Phaeodactylum tricornutum*. *Front. Plant Sci.* 12:642199. doi: 10.3389/fpls.2021.642199

1. Download the archive containing the model and extract the files.

a) Start ScrumPy in the folder containing the .spy files and load the top-level model file Phaeo.spy to create a model object. Note that the model is created in a modular fashion, and the top-level file will load the different components of the model and open a window for each.

b) Can you explain why there are more modules in this model compared to Campylobacter model?

2. Set up and solve an LP problem where the objective is to minimise total flux (see previous practical), while producing 1 unit flux of TAG (Hint: use SetFixedFlux() function on reaction 'TAG_synthesis_Cyto').

a) What is the source of energy in your LP solution ?

b) Examine the source of carbons.

3. Now we will perform lipid scan analysis under mixotrophic condition. We will perform this analysis under various growth conditions so better to write the steps into python method, for re-usability, in a text file and save as python module (MyLipidScan.py) in Analysis directory (Note: Model directory contains the model definition files i.e .spy files and analysis contains the python modules ie. .py files you will need for this practical)

a) Generate LP problem where the objective is to minimise total flux. Constrain the maximum Rubisco flux and glycerol transporter flux to 400 and 20 respectively (make use of SetFluxBounds() function).

```
def BuildLP(m):
    lp = m.GetLP()
    lp.SetObjective(m.sm.cnames)
    lp.SetFluxBounds({'RIBULOSE-BISPHOSPHATE-CARBOXYLASE-RXN_Plas': (0,400.0)})
    lp.SetFluxBounds({"GLYCEROL_Cyto_tx":(0,20)})
    return lp
```

b) Solve this LP repeatedly (using for loop) while increasing flux in TAG synthesis reaction in range between 1 to 20. Save each of the solution in a dataset.

```
Import numpy
from ScrumPy.Data import DataSets
```

```
def LipidScan(m, lp=None, lo=1.0, hi=20.0):
    ds = DataSets.DataSet()
    ranges = numpy.arange(lo,hi)
    if lp == None:
        lp = BuildLP(m)
    for t in ranges:
        lp.SetFixedFlux({"TAG_synthesis_Cyto":t})
        lp.Solve()
        if lp.GetStatusMsg() == "optimal":
            sol = lp.GetPrimSol()
            ds.UpdateFromDic(sol)
    return ds
```

c) Examine the flux pattern in Rubisco reaction with respect to increasing flux in TAG synthesis. What is the maximum flux in Rubisco reaction?

```
ds.SetPlotX("TAG_synthesis_Cyto") #setting x-axis
ds.AddToPlot("RIBULOSE-BISPHOSPHATE-CARBOXYLASE-RXN_Plas")
```

d) Add inorganic carbon transporters (Hint: “CO2_Cyto_tx” and “HCO3_Cyto_tx”) and organic carbon transporter (“GLYCEROL_Cyto_tx”) to the plot

e) What is the maximum flux in TAG synthesis?

4. As you would have noticed TAG synthesis in above example is through mixotrophic mode (I.e model uses light energy and organic carbon, glycerol, for lipid production). As you remember from the lecture, *P. tricornutum* can grow under phototrophic condition (I.e in the absence of glcerol). As you have saved the method in ../Analysis/MyLipidScan.py. We will import the module (as shown below) and repeat the above analysis for phototropic condition. For this, constrain the flux in glycerol transporter to zero.

```
import sys
sys.path.append('../Analysis')
import MyLipidScan
res = MyLipidScan.LipidScan(m)
```

a) Plot reactions as above. Examine the difference in flux patterns.

b) What is the maximum feasible flux in TAG synthesis under phototrophic condition?

c) Is is higher or lower than that in mixotrophic condition (in question 3)?

5. Find the reactions that are active in mixotrophic condition but not in phototrophic condition?

```
from ScrumPy.Util import Set
Set.Complement(ds.cnames,res.cnames)
```

Can you identify which pathways these reactions belong to? Refer to network diagram in lecture slides for convenience or visit MetaCyc. Note that _Cyto suffix is added to differentciate compartmentalisation in the model and is not part of MetaCyc identifier.