Model Interrogation

Part II

Learning Objectives

Each student should be able to:

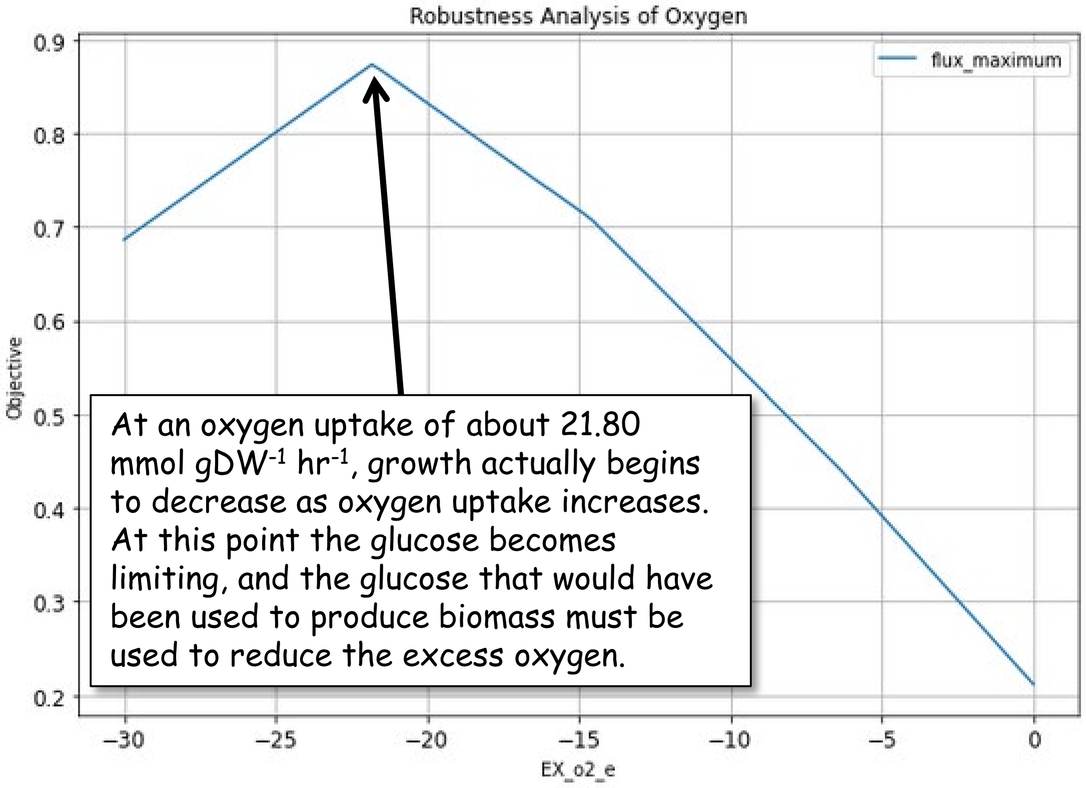
- Explain robustness analysis
- Understand the iJN678 *Synechocystis sp. PCC6803 COBRA model.*
- Describe respiration in the iJN678 model.
- Describe photosynthesis in the iJN678 model.
- Describe the results of autotrophic operation in the iJN678 model.
- Describe the results of heterotrophic tropic operation in the iJN678 model.
- Describe the results of mixotrophic operation in the iJN678 model.

Lesson Outline

- Robustness Analysis
- *Synechocystis sp. PCC6803* (Cyanobacteria) Overview
- Understanding the iJN678 *Synechocystis sp. PCC6803* Model
- iJN678 Energy & Reducing Power
  - ✓ Respiration
  - ✓ Photosynthesis
- iJN678 Cellular Operation
  - ✓ Autotrophic
  - ✓ Heterotrophic
  - ✓ Mixotrophic

Robustness Analysis

- Flux is varied through one reaction while the optimal objective value is calculated for each value of this flux.
- Describes the sensitivity the objective function to the reaction.
- Example: Determine the effect of varying oxygen uptake on growth



Robustness_analysis_core.ipynb

Robustness Analysis: *E.coli* core model

Load appropriate Python and COBRApy packages

In [1]:
```
import cobra.test
import numpy as np
import pandas as pd
from cobrapy_bigg_client import client
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 500)
```

Load model and set constraints

In [2]:
```
model_orig = client.download_model('e_coli_core', save=False) # Loading the model to the simulation
model_orig.reactions.EX_o2_e.lower_bound = -20
model_orig.reactions.EX_glc__D_e.lower_bound = -10
model = model_orig.copy()
```

```
Set parameter Username
Academic license - for non-commercial use only - expires 2022-10-10
Read LP format model from file C:\Users\hinton\AppData\Local\Temp\tmpqo0i0khf.lp
Reading time = 0.01 seconds
: 72 rows, 190 columns, 720 nonzeros
```

Robustness Analysis Using "envelope" COBRApy method¶

Robustness analysis for oxygen

Robustness_analysis_core.ipynb

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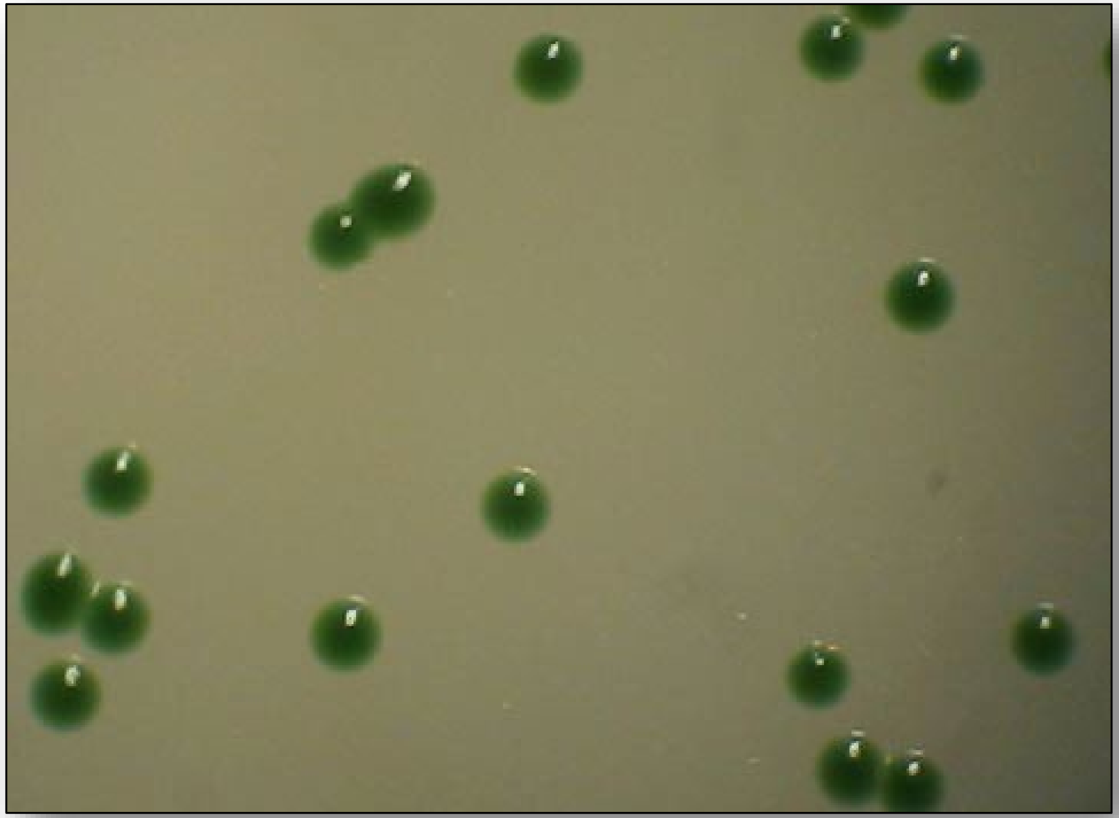
Synechocystis sp. PCC6803 (Cyanobacteria)

- *Synechocystis* is a genus of cyanobacteria represented by the strain *Synechocystis sp. PCC6803*.
- *Synechocystis sp. PCC6803* lives in freshwater and is capable of both phototrophic growth by oxygenic photosynthesis in sunlight and heterotrophic growth by glycolysis and respiration during dark periods.
- It is able to effectively anticipate transitions of light and dark phases by using a circadian clock.
- *Synechocystis sp. PCC 6803* was the first photosynthetic organism that the entire genome sequence was determined.



Colonies of *Synechocystis sp. PCC 6803*

http://synechocystis.asu.edu/

Synechocystis sp. PCC6803

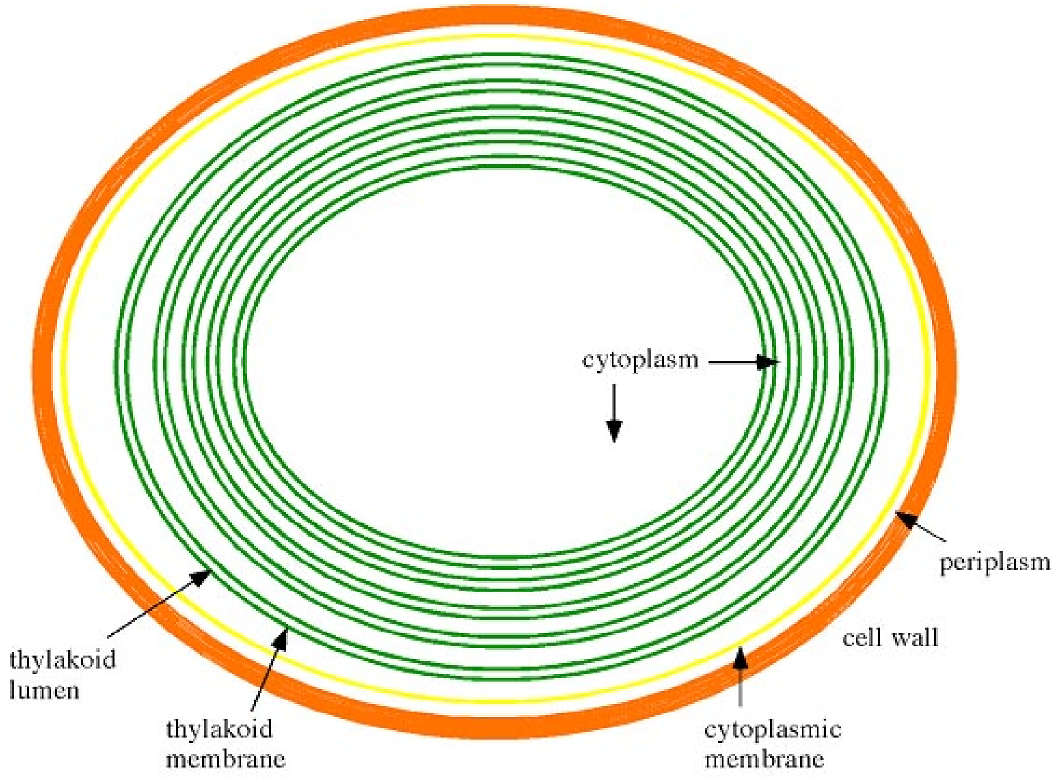


Figure 5 : Schematic overview of a cyanobacterial cell. Indicated are: thylakoid membranes (green) that contain photosynthetic and respiratory complexes and that separate the cytoplasm from the lumen; the cytoplasmic membrane (yellow) that separates the cytoplasm from the periplasm; and the outer membrane and cell wall

http://synechocystis.asu.edu/index.htm

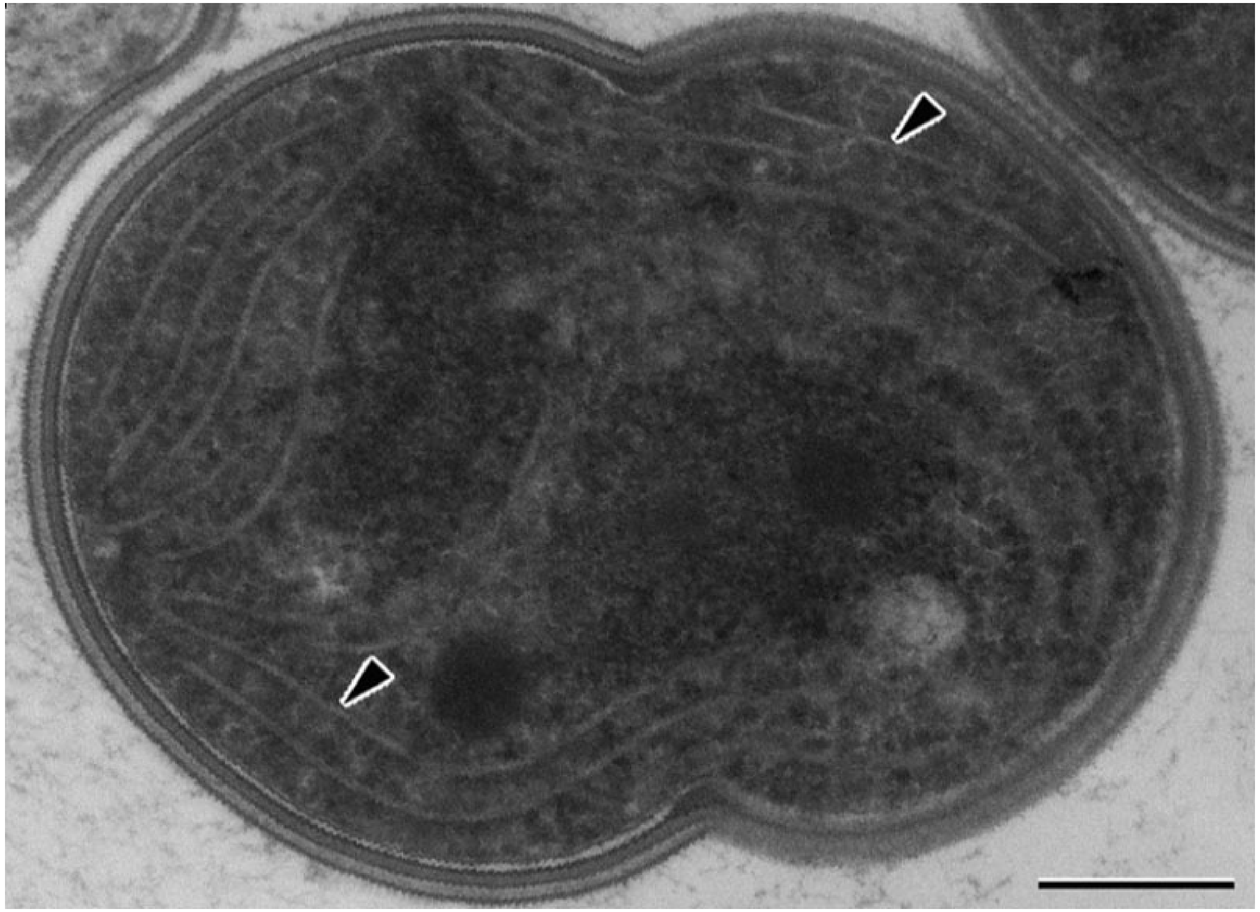


Figure 6 : Transmission electron micrograph of a dividing *Synechocystis* cell illustrating thylakoid membranes (arrowheads) that occur along the periphery of the cytoplasm. Scale bar = 400 nm

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    - ✓ Mixotrophic

Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis

Juan Nogales[1], Steinn Gudmundsson, Eric M. Knight, Bernhard O. Palsson, and Ines Thiele[2]

Center for Systems Biology, University of Iceland, 101 Reykjavik, Iceland

Edited by Robert Haselkorn, University of Chicago, Chicago, IL, and approved December 28, 2011 (received for review November 8, 2011)

Photosynthesis has recently gained considerable attention for its potential role in the development of renewable energy sources. Optimizing photosynthetic organisms for biomass or biofuel production will therefore require a systems understanding of photosynthetic processes. We reconstructed a high-quality genome-scale metabolic network for *Synechocystis* sp. PCC6803 that describes key photosynthetic processes in mechanistic detail. We performed an exhaustive in silico analysis of the reconstructed photosynthetic process under different light and inorganic carbon (*Ci*) conditions as well as under genetic perturbations. Our key results include the following. (*i*) We identified two main states of the photosynthetic apparatus: a *Ci*-limited state and a light-limited state. (*ii*) We discovered nine alternative electron flow pathways that assist the photosynthetic linear electron flow in optimizing the photosynthesis performance. (*iii*) A high degree of cooperativity between alternative pathways was found to be critical for optimal autotrophic metabolism. Although pathways with high

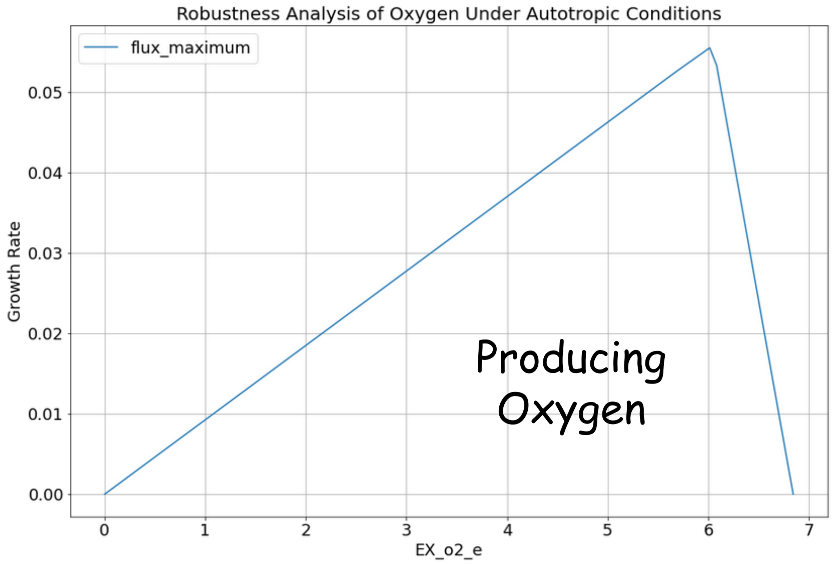
desirable for systematic understanding of the photosynthetic process (2, 4). To date, analysis of photosynthetic processes at the genome-scale has received limited attention (7).

The cyanobacterium *Synechocystis* sp. PCC6803, henceforth called *Synechocystis*, is a model photosynthetic prokaryotic organism capable of growing phototrophically because of oxygenic photosynthesis and heterotrophically at the expense of reduced carbon sources in the dark. These features make *Synechocystis* an interesting microorganism for biotechnology applications. Although several central-metabolic and genome-scale reconstructions are available for *Synechocystis* (8–12), they do not describe photosynthetic and heterotrophic metabolism in detail. Moreover, pathways relevant for biotechnological endeavors, such as the biosynthesis of lipids and photosynthetic pigments, are currently poorly represented. To fill these gaps, we reconstructed a high-quality genome-scale metabolic network of *Synechocystis* named *i*JN678. Here we describe *i*JN678 and the properties of the metabolic network it represents.

Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

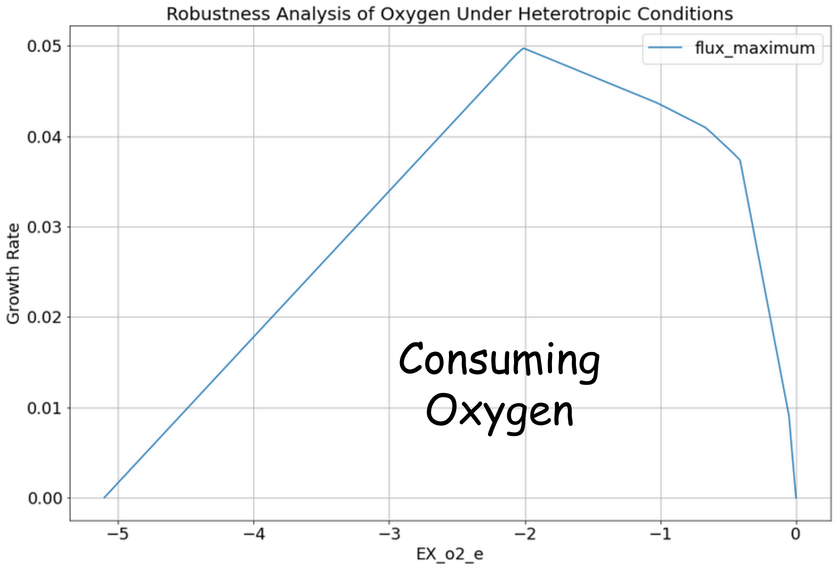
Oxygen Robustness Analysis

Autotrophic Conditions



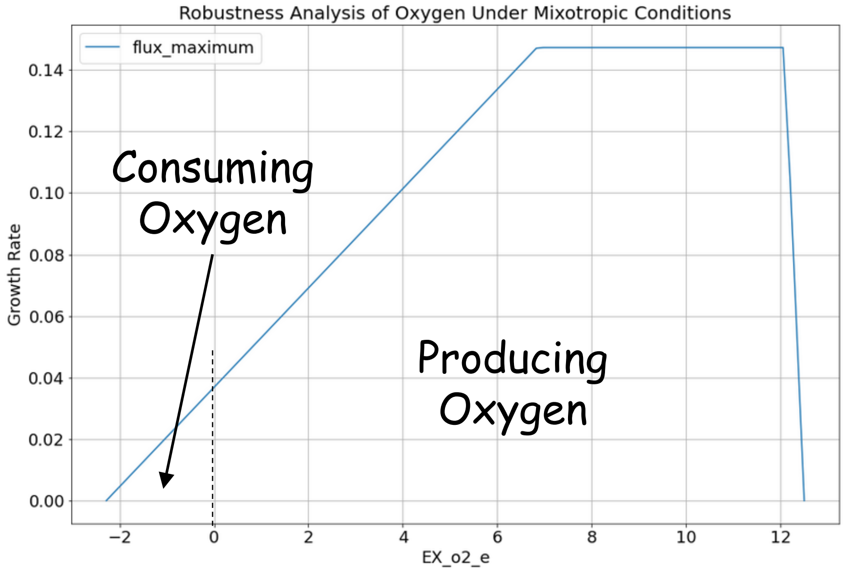
iJN678_Autotrophic.ipynb

Heterotrophic



iJN678_Heterotrophic.ipynb

Mixotrophic



iJN678_Mixotrophic.ipynb

Synechocystis sp. PCC6803 iJN678 Model Attributes

Set the environment

```
In [1]: import cobra.test
        import pandas as pd
        import numpy as np
        import pandas as pd
        import escher
        from escher import Builder
        from cobra.sampling import sample
        import matplotlib.pyplot as plt
        from cobrapy_bigg_client import client
        pd.set_option('display.max_rows', 1000)
        pd.set_option('display.width',1000)
        pd.set_option('display.max_colwidth',None)
```

Load the model

```
In [2]: model = client.download_model('iJN678', save=False) # Loading the model to the simulation
```

```
Set parameter Username
Academic license - for non-commercial use only - expires 2022-10-10
```

Model Attribute Summary

```
In [3]: model
```

Out[3]:

Name	iJN678
Memory address	0x01b02f11fca0
Number of metabolites	795
Number of reactions	863
Number of groups	0
Objective expression	1.0*BIOMASS_Ec_SynHetero - 1.0*BIOMASS_Ec_SynHetero_reverse_5d8af
Compartments	cytosol, periplasm, thylakoid, extracellular space

iJN678_Model_Attributes.ipynb

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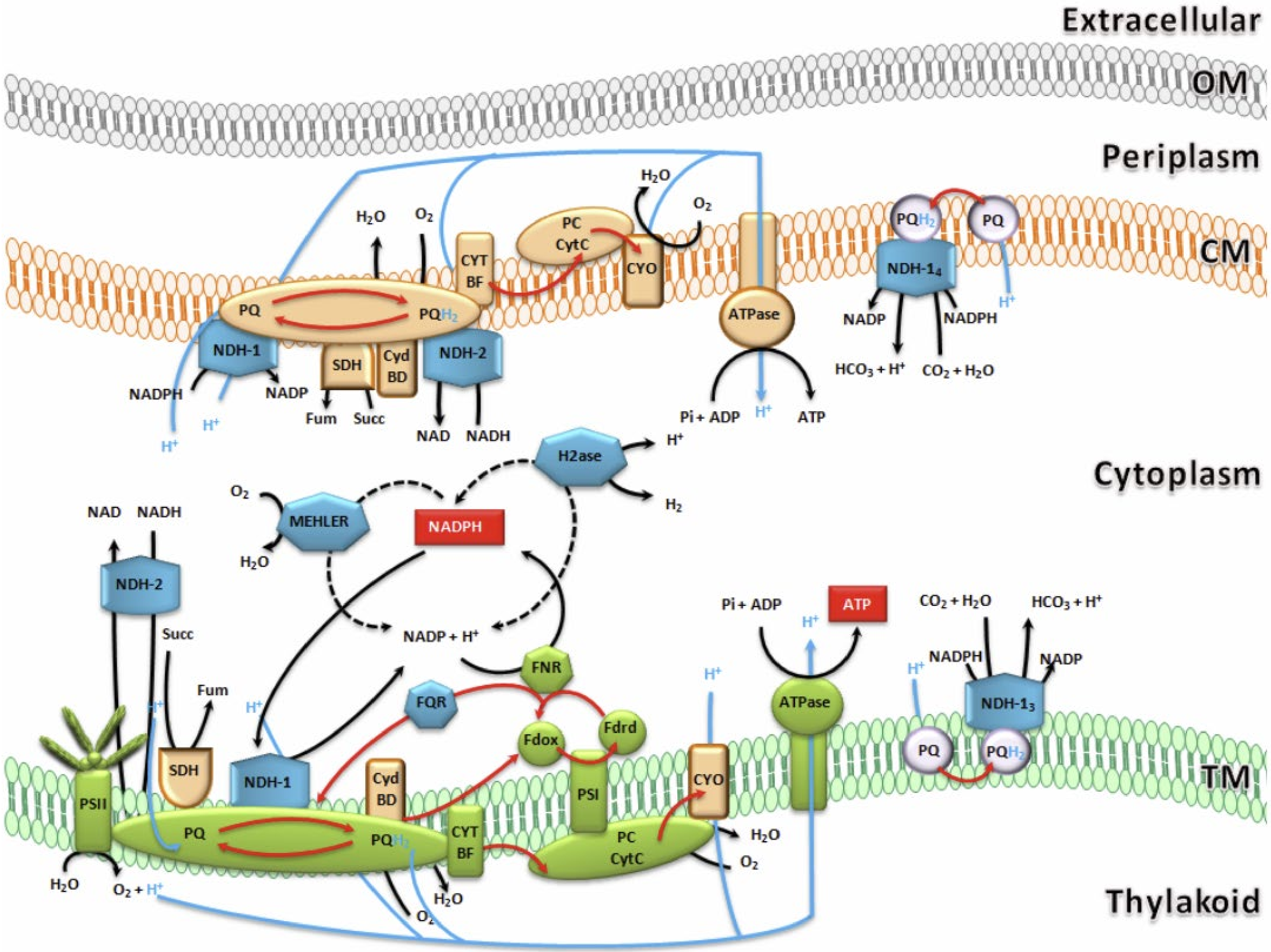
Modeling of the oxidative phosphorylation and photosynthetic pathways included in iJN678.



Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

Respiration

- The main method by which energy is generated for growth-related physiological processes such as biosynthesis and solute transport in respiring prokaryotes is by coupling the flow of electrons in membranes to the creation of an electro-chemical proton gradient. Electrons flow spontaneously down a potential energy gradient (electron transport chain) towards acceptors that have a more positive electrode (reduction) potential.
- The electrons flow from primary electron donors to terminal electron acceptors through a series of electron carrier proteins and a class of lipids called quinones.
- Electron flow via electron carriers in membranes is called as **respiration**. If the terminal electron acceptor is oxygen, then electron flow is called ***aerobic respiration***. If it is not oxygen, it is called ***anaerobic respiration***.

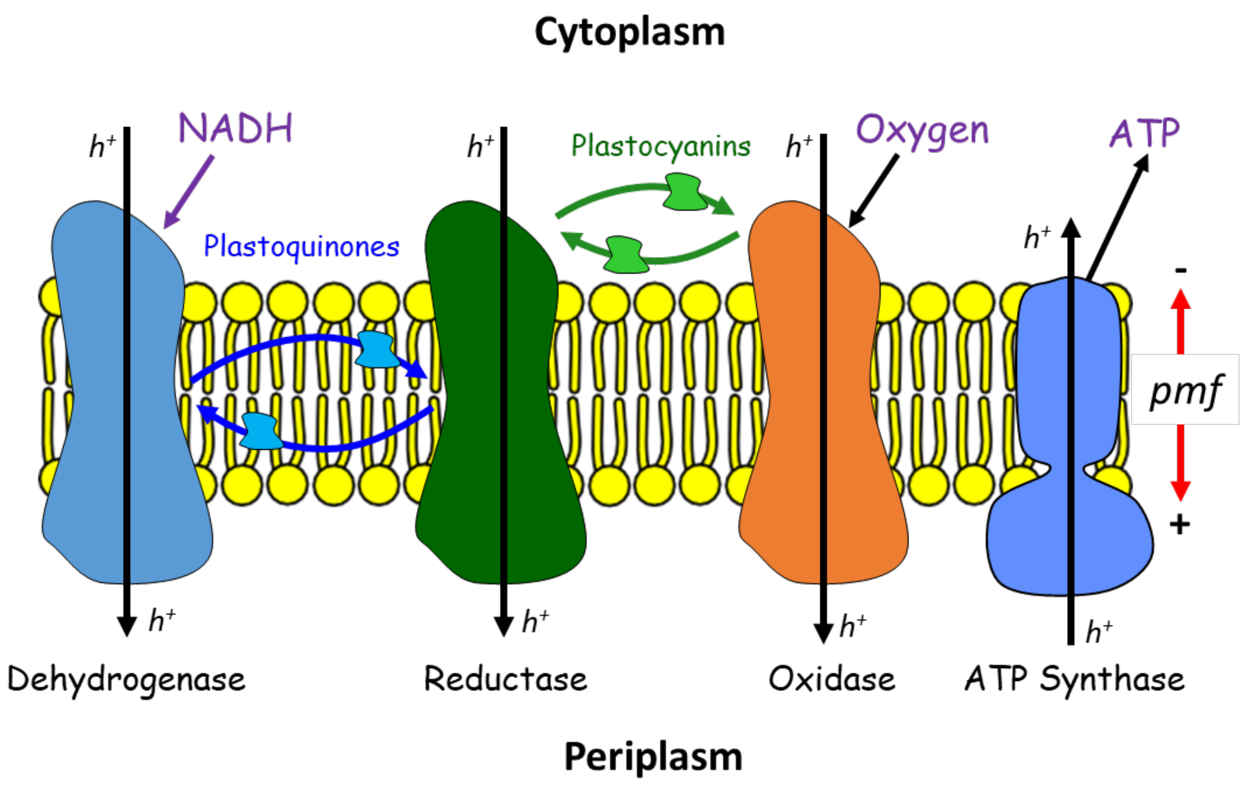
D. White, "The Physiology and Biochemistry of Prokaryotes," 3rd Edition.

Aerobic Respiration

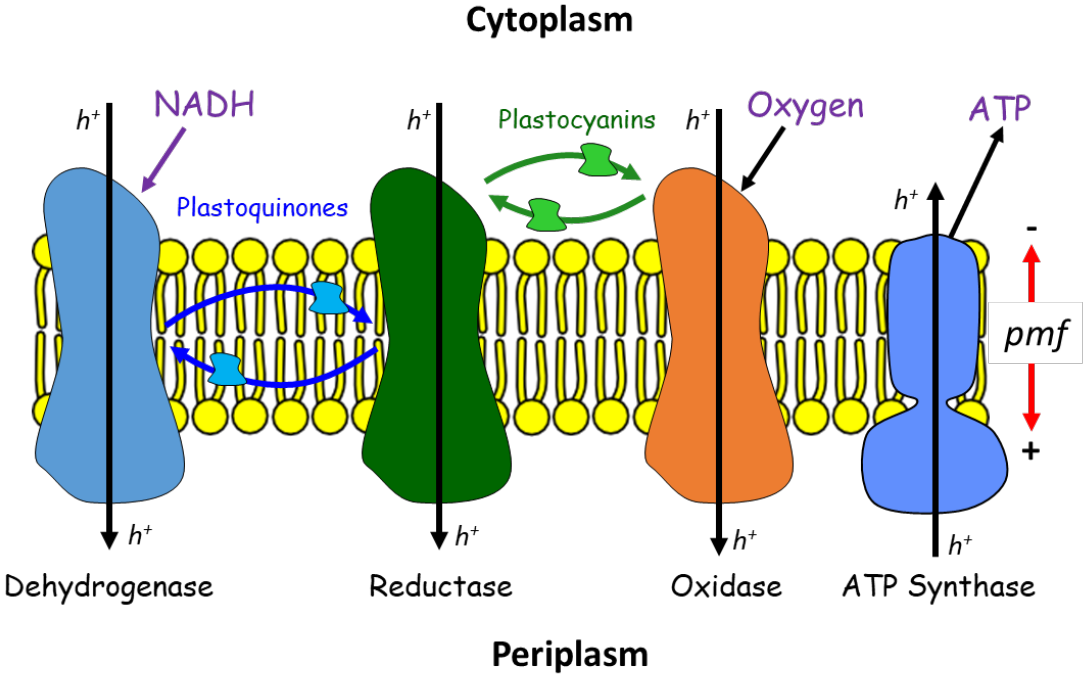
Aerobic respiration is a cellular process for harvesting energy. Electrons are extracted from an electron donor (NADH) and transferred to O_2 as the terminal electron acceptor. This process generates a membrane potential across the cytoplasmic membrane termed proton motive force (pmf). The pmf is then used to drive ATP synthesis via the membrane-bound ATP synthase (electron transport phosphorylation).

Key Concepts

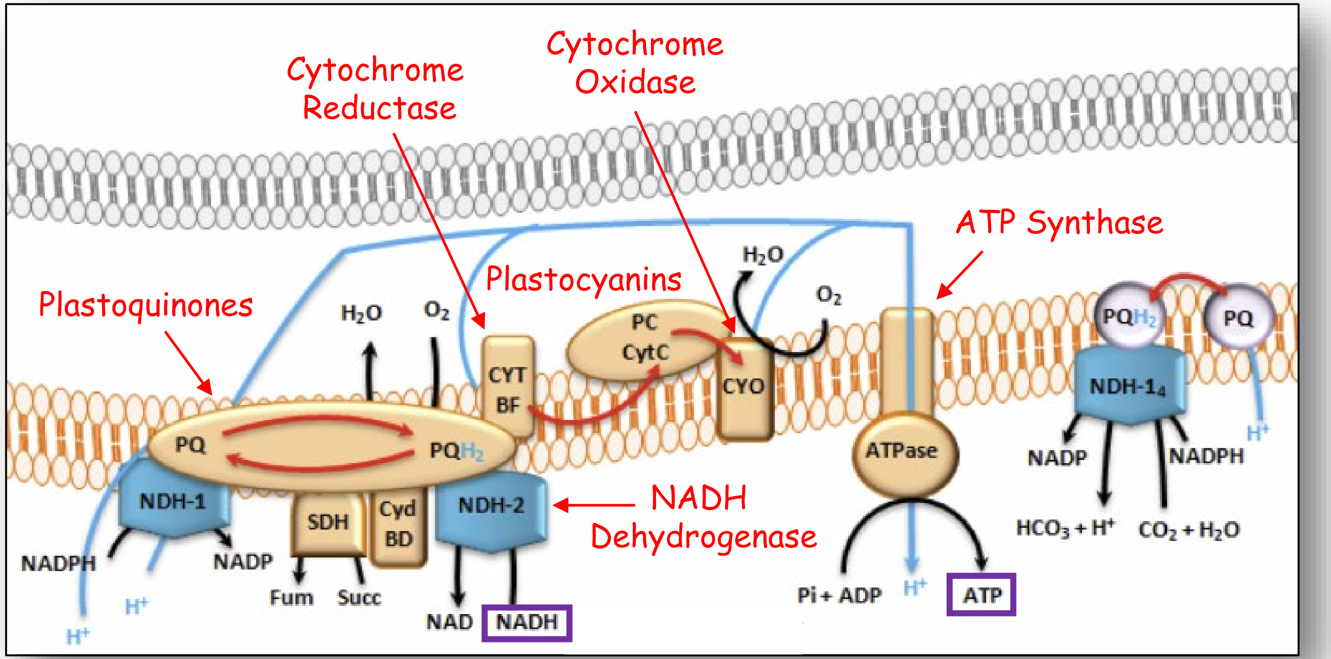
- Aerobic respiratory chains are located in the cytoplasmic membrane and are used to generate a proton motive force (pmf).
- Aerobic electron transport chains contain a ***dehydrogenase, reductase, and oxidase enzymes*** that pump protons into the periplasmic space.
- The ***oxidase*** reduces O_2 to H_2O.
- ***Plastoquinones*** mediate electron transfer between the ***dehydrogenase and reductase***.
- ***Plastocyanins*** mediate electron transfer between the ***reductase*** and the ***oxidase***.
- The ***proton motive force drives ATP synthesis*** via the membrane-bound ATP synthase.
- Cytochrome oxidases with different affinities for O_2 allow organisms to grow under aerobic and microaerophilic conditions.



Modeling the Aerobic Respiration Pathways Included in iJN678



ECOCYC: The Escherichia Coli Student Portal
http://ecolistudentportal.org/article_aerobic_respiration#_



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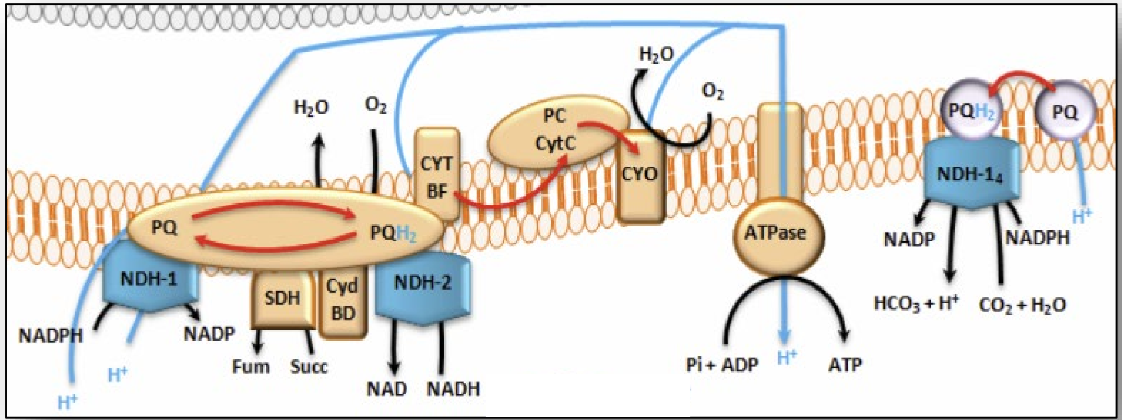
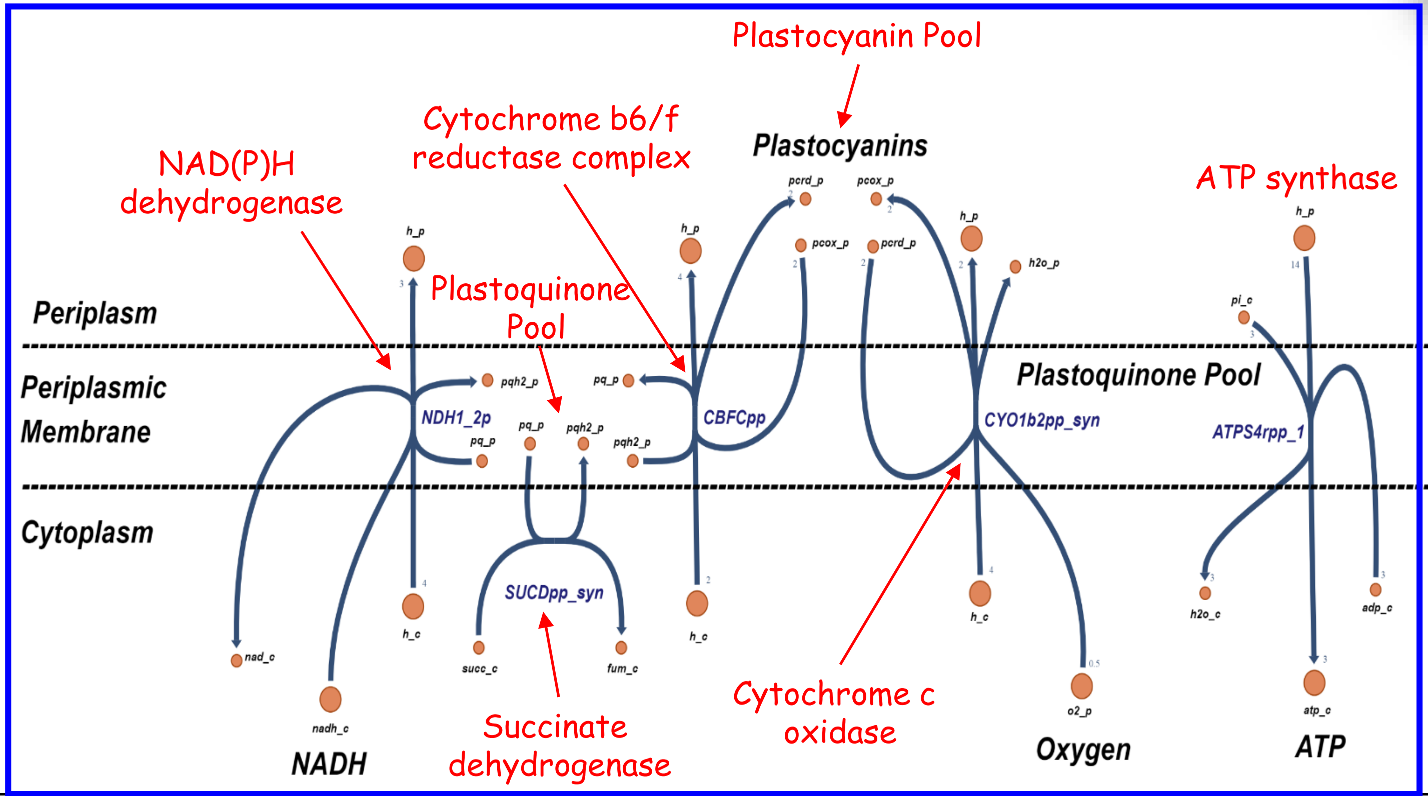
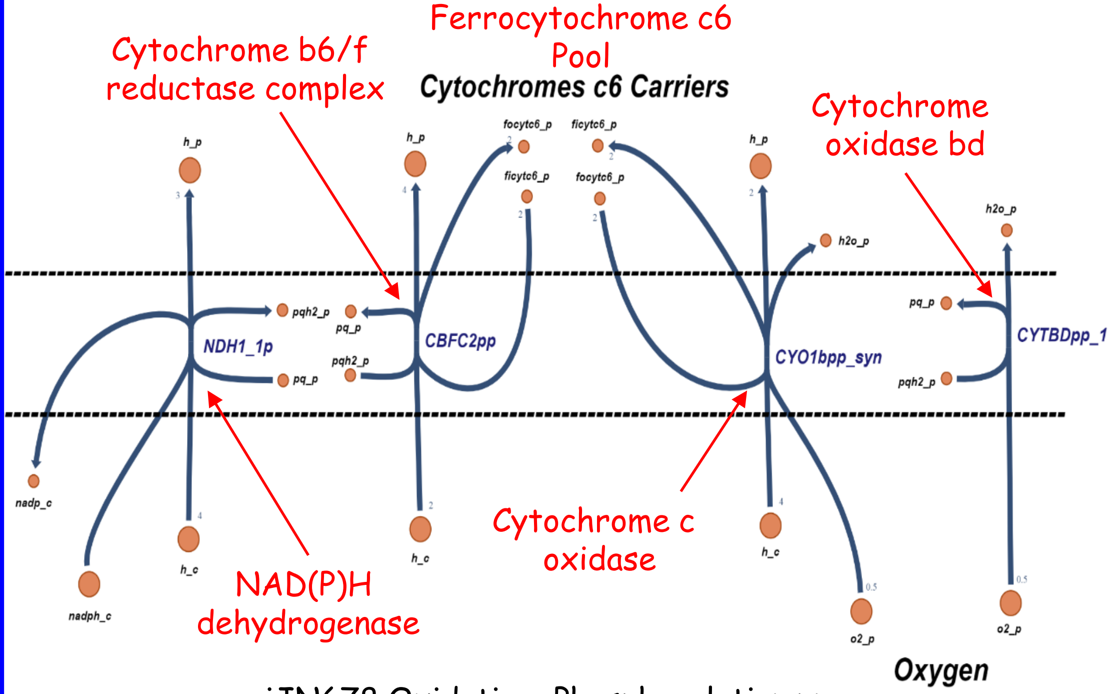
Aerobic Respiration

Synechocystis sp. PCC6803



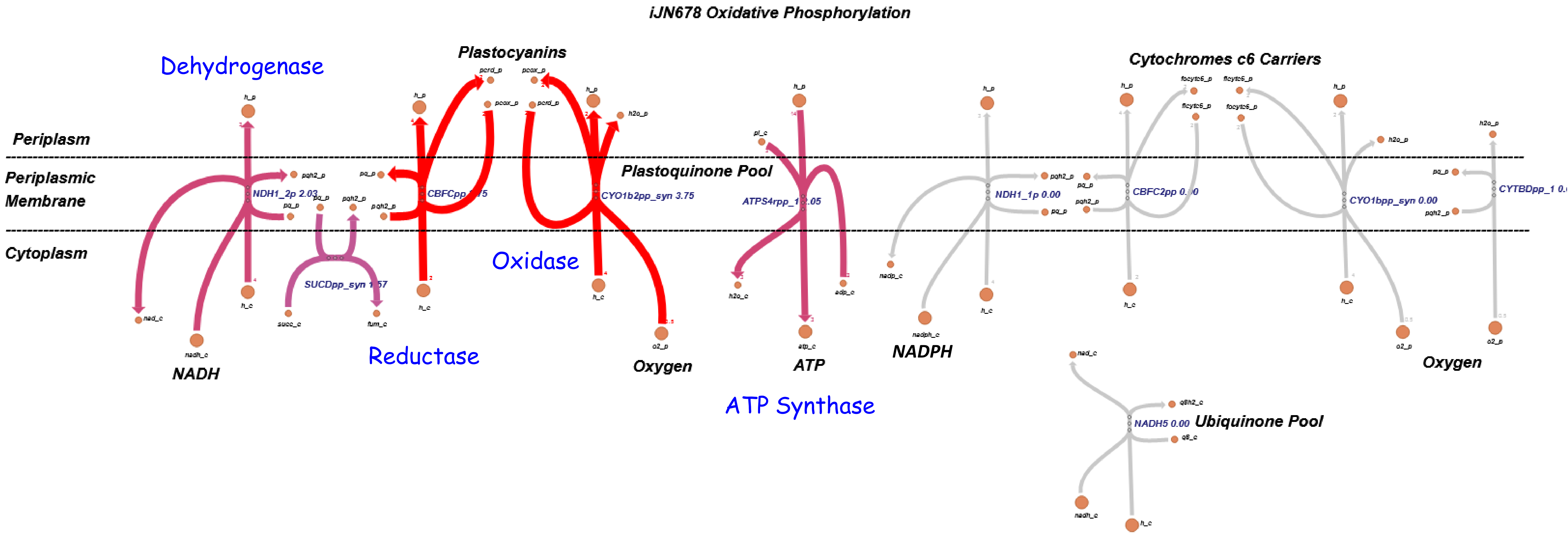
Nogales, J., S. Gudmundsson, et al. (2012). 2678-2683.





iJN678.Oxidative_Phosphorylation.png

Aerobic Respiration in iJN678



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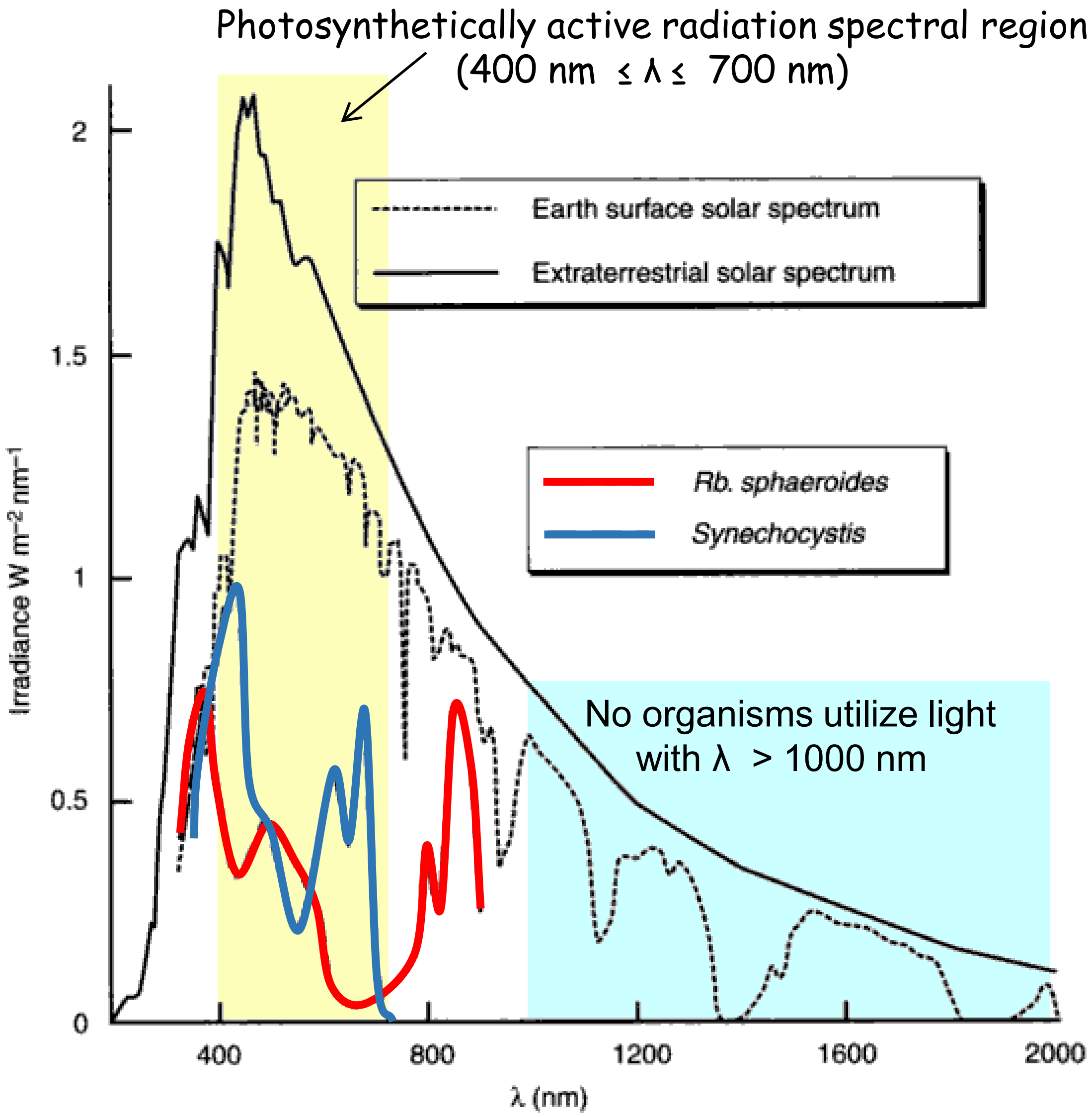
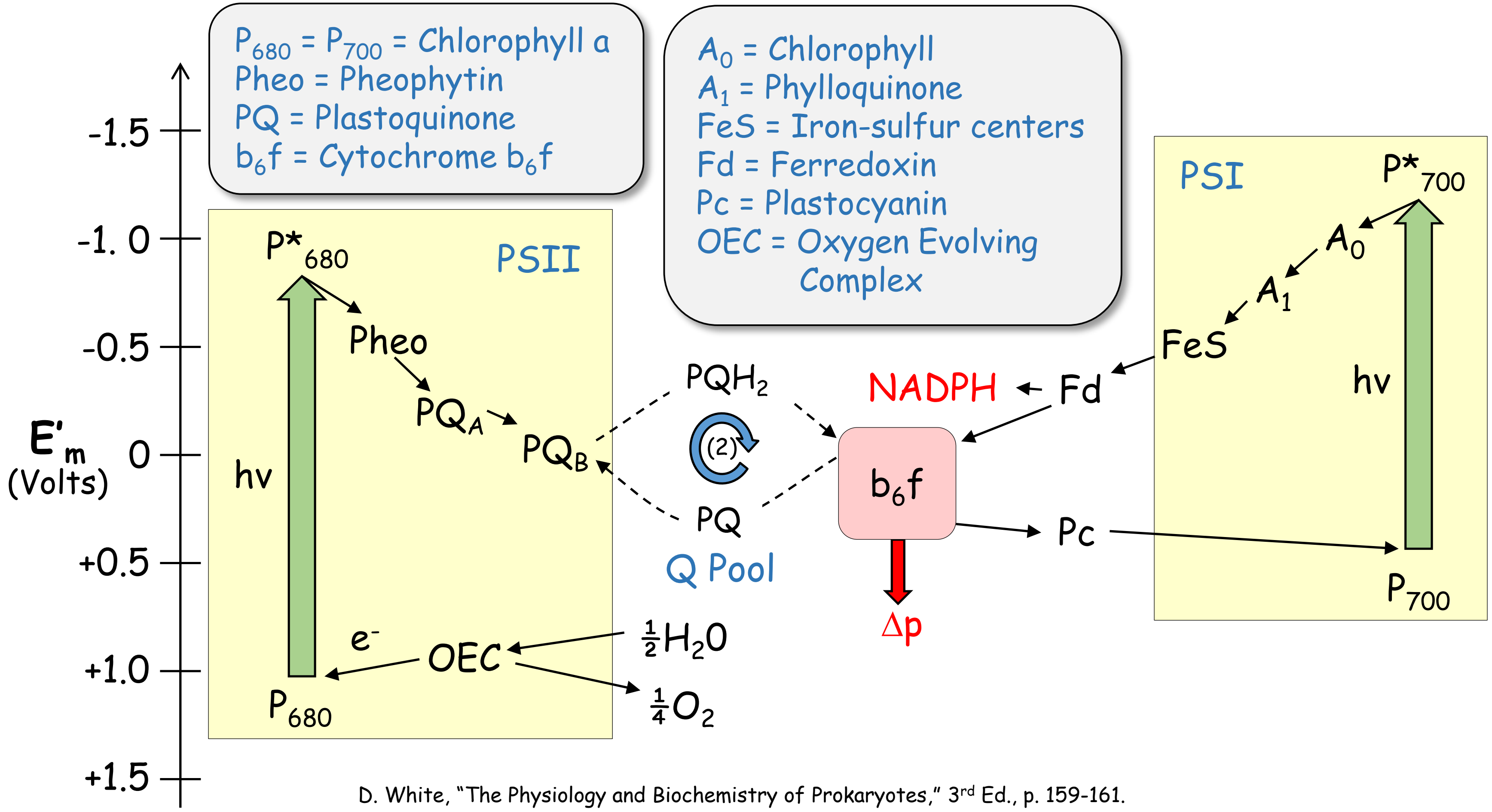
Spectra of Photosynthetic Organisms

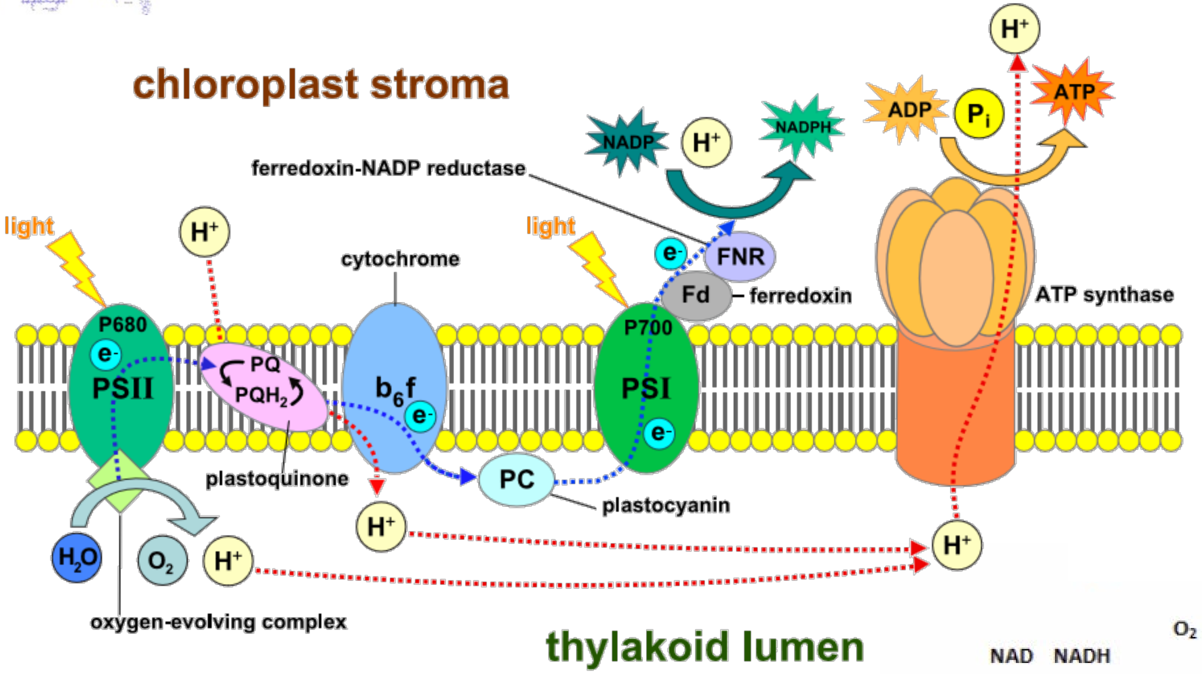
"Molecular Mechanisms of Photosynthesis,"
R. E. Blankenship, Figure 1.1



Electron Flow for Oxygenic Photosynthesis

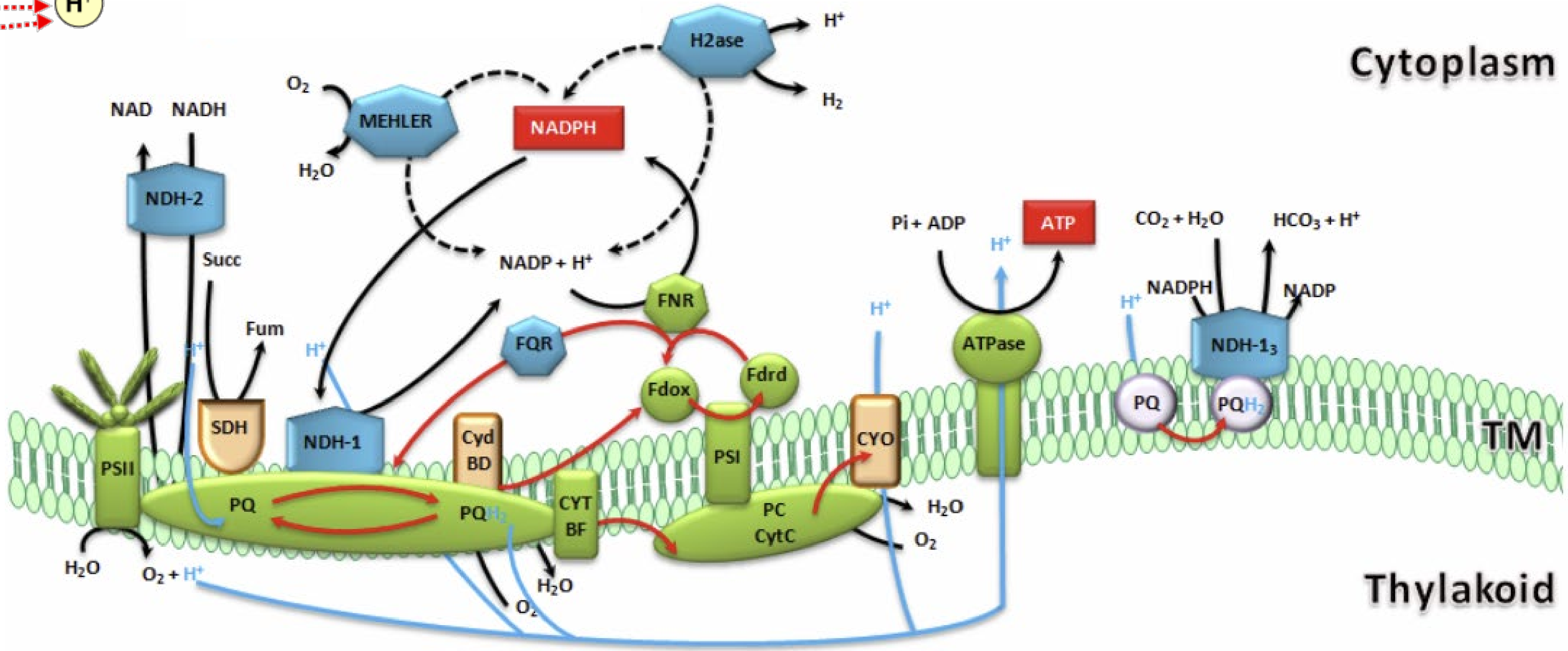


D. White, "The Physiology and Biochemistry of Prokaryotes," 3rd Ed., p. 159-161.



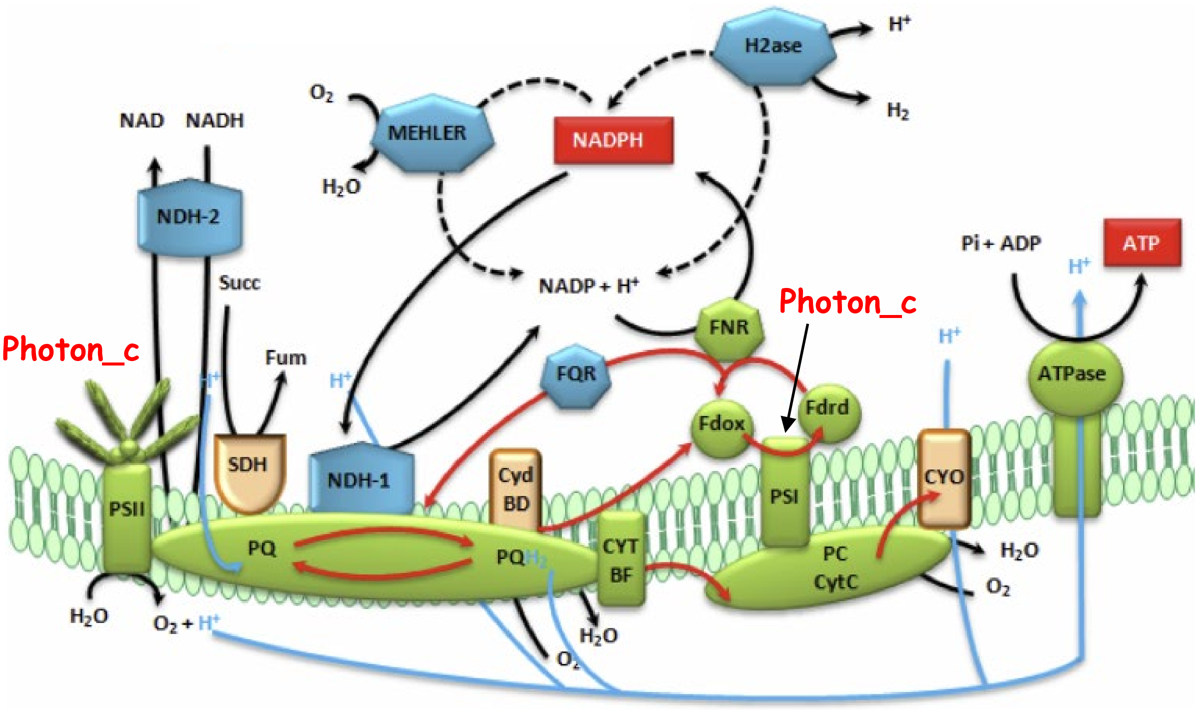
https://en.wikipedia.org/wiki/Photosynthesis

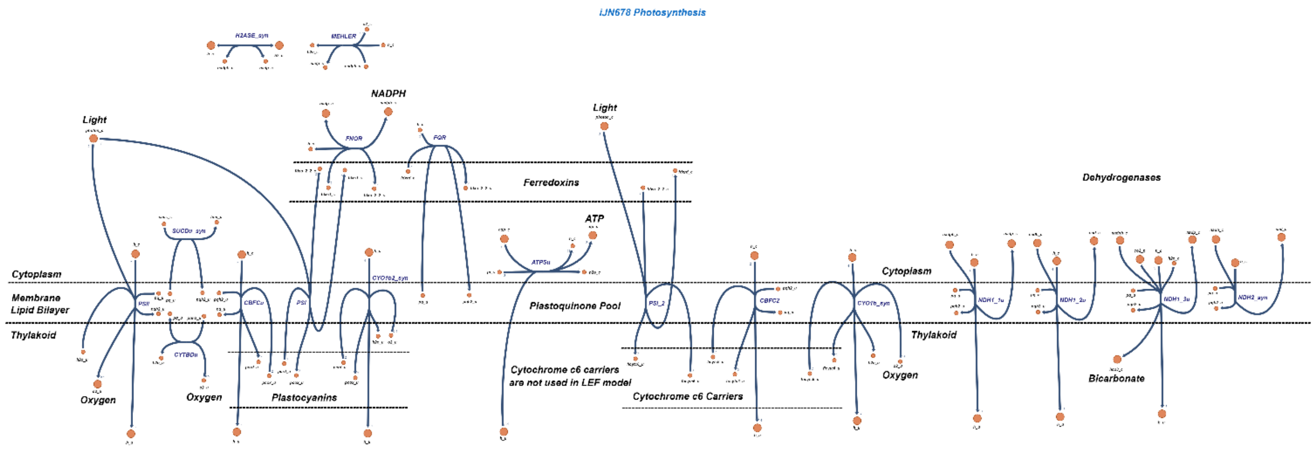
Modeling the Photosynthetic Pathways Included in iJN678.



Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

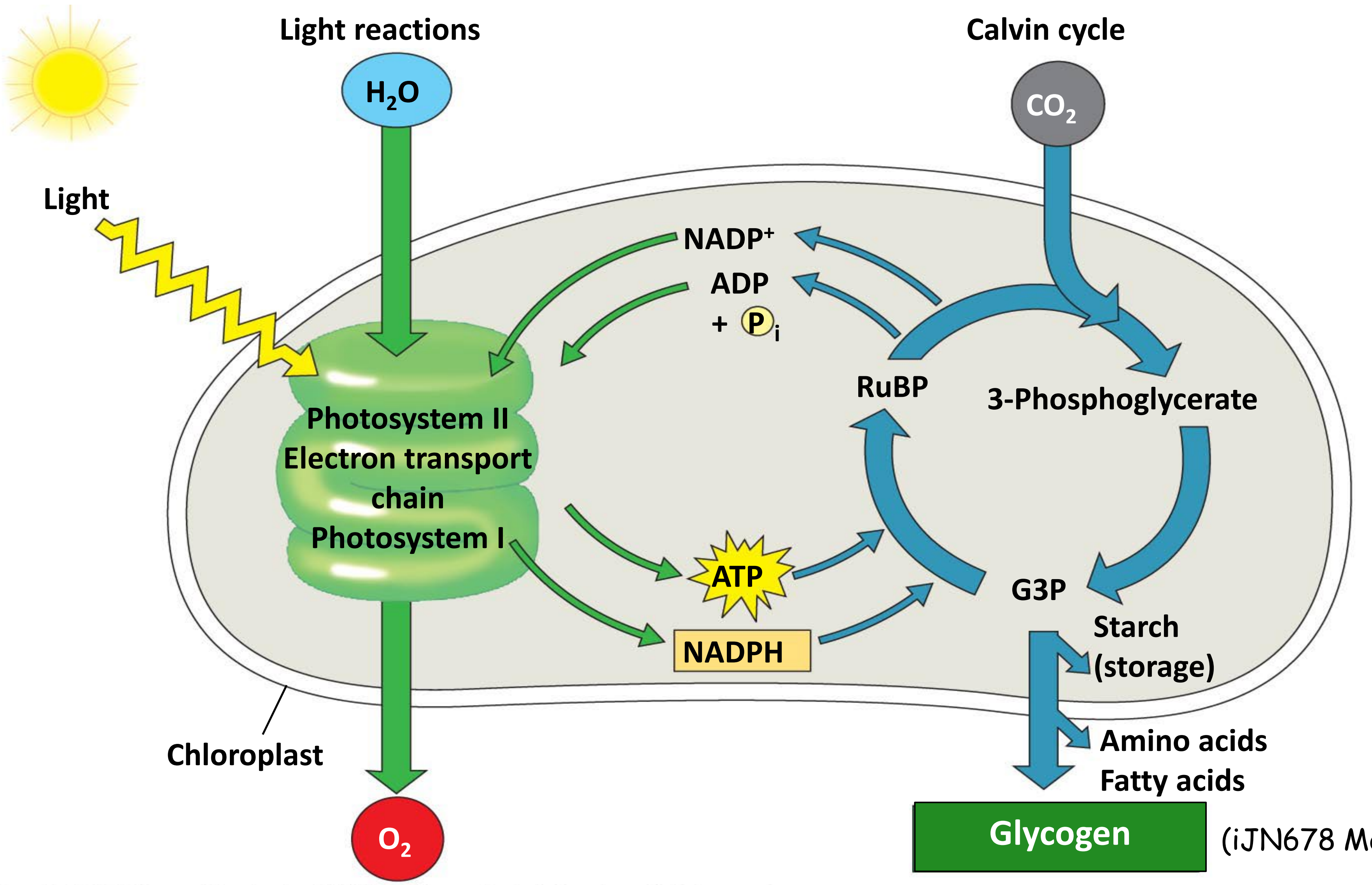
Photosynthesis Subsystem for iJN678





iJN678.Photosynthesis.json

Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.



(iJN678 Model)

Biology, Campbell & Reese, 7ed

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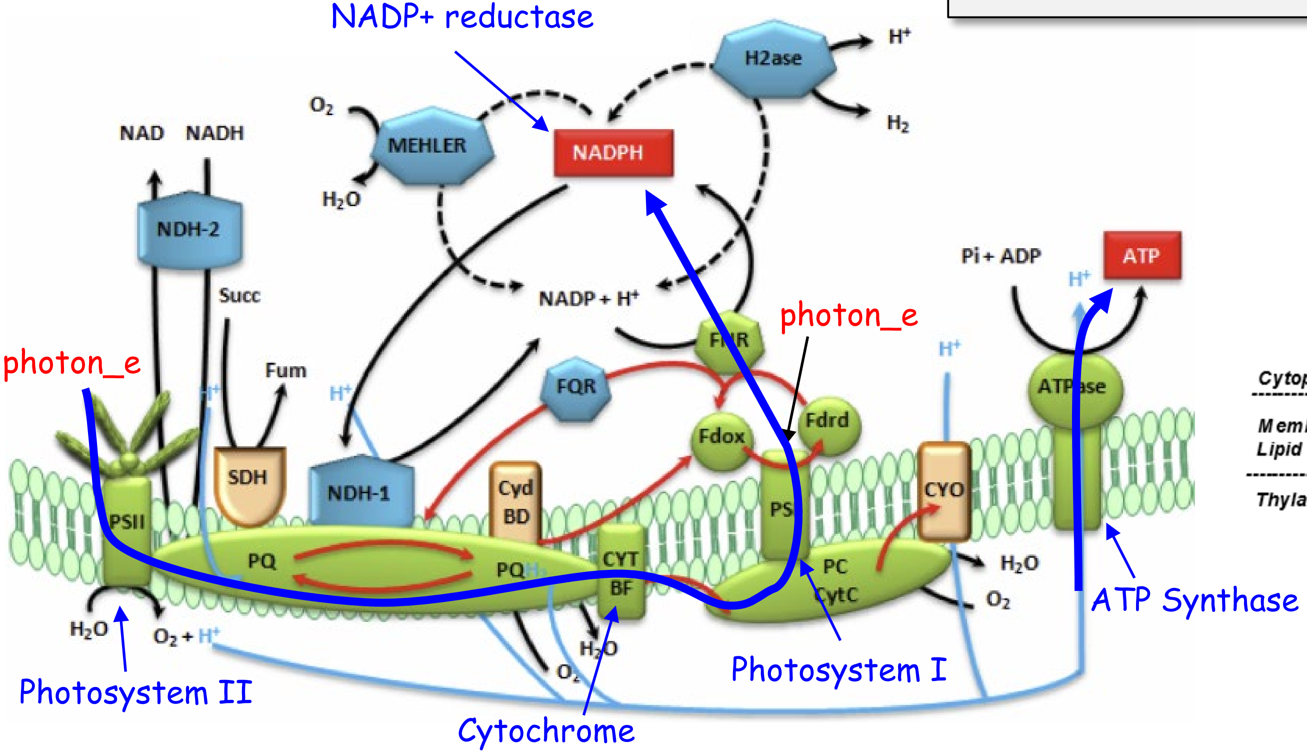
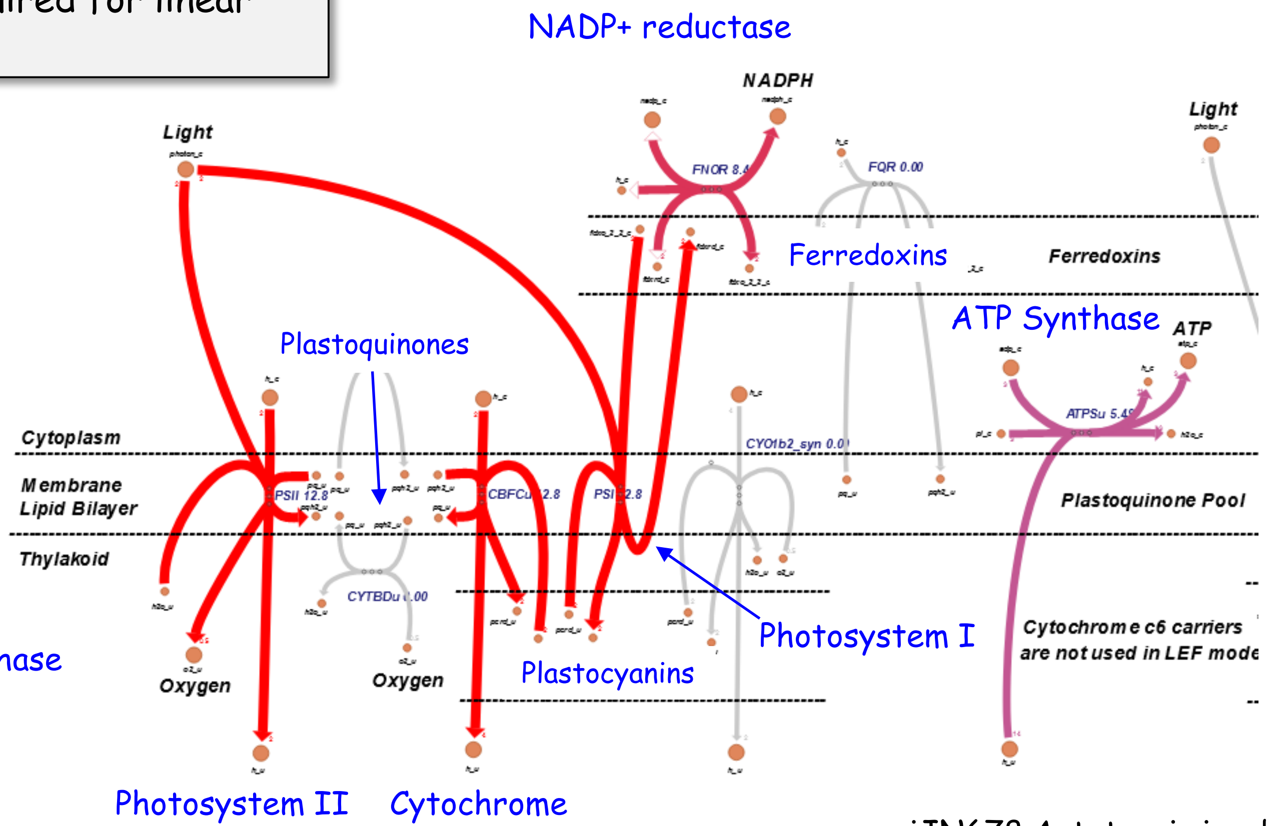
Linear Electron Flow (LEF)

Alternate Electron Flow (AEF) involves using other reactions than those required for linear electron flow.





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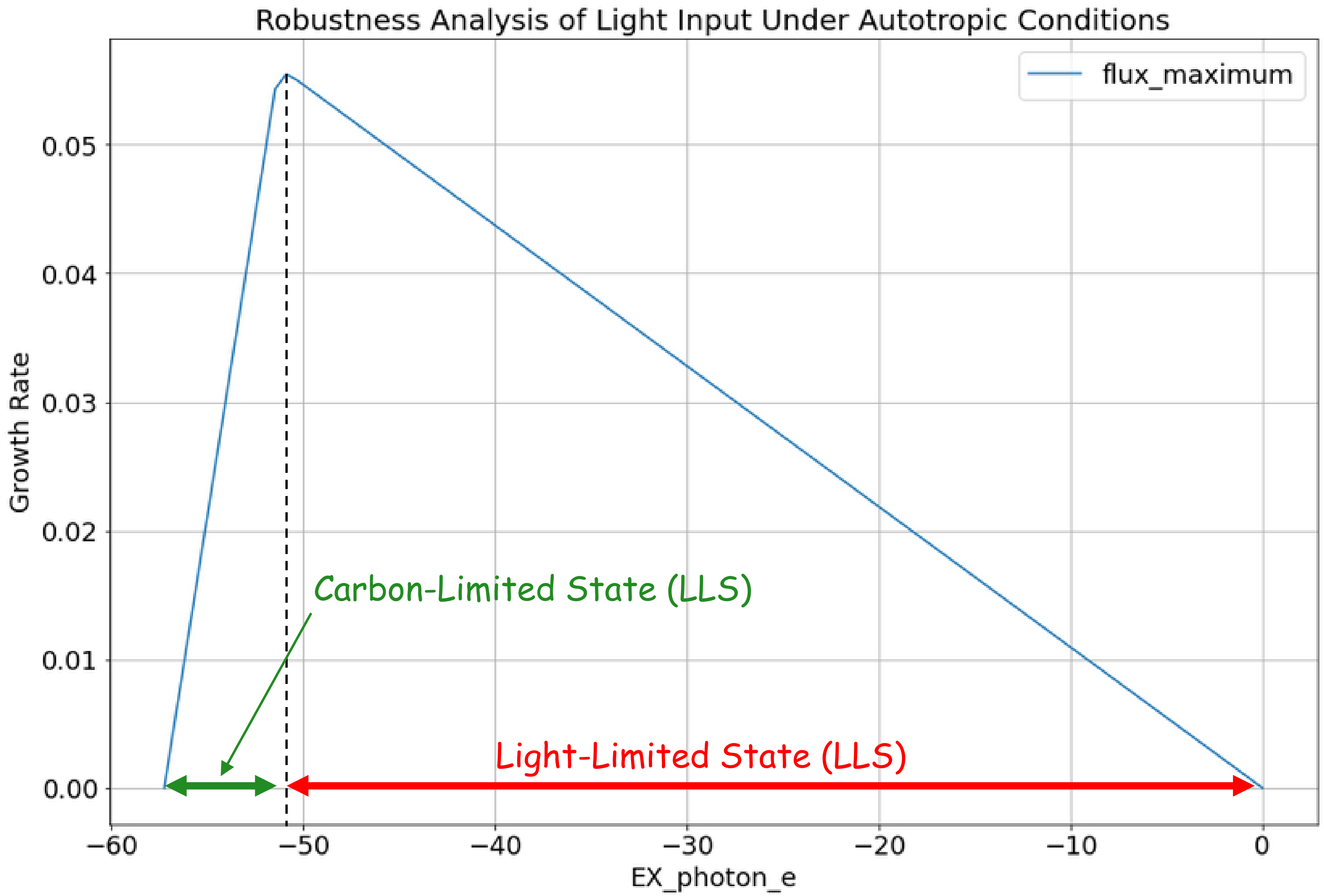
iJN678.Autotropic.ipynb

Light-Limited States (LLS) and Carbon-Limited States (CLS)

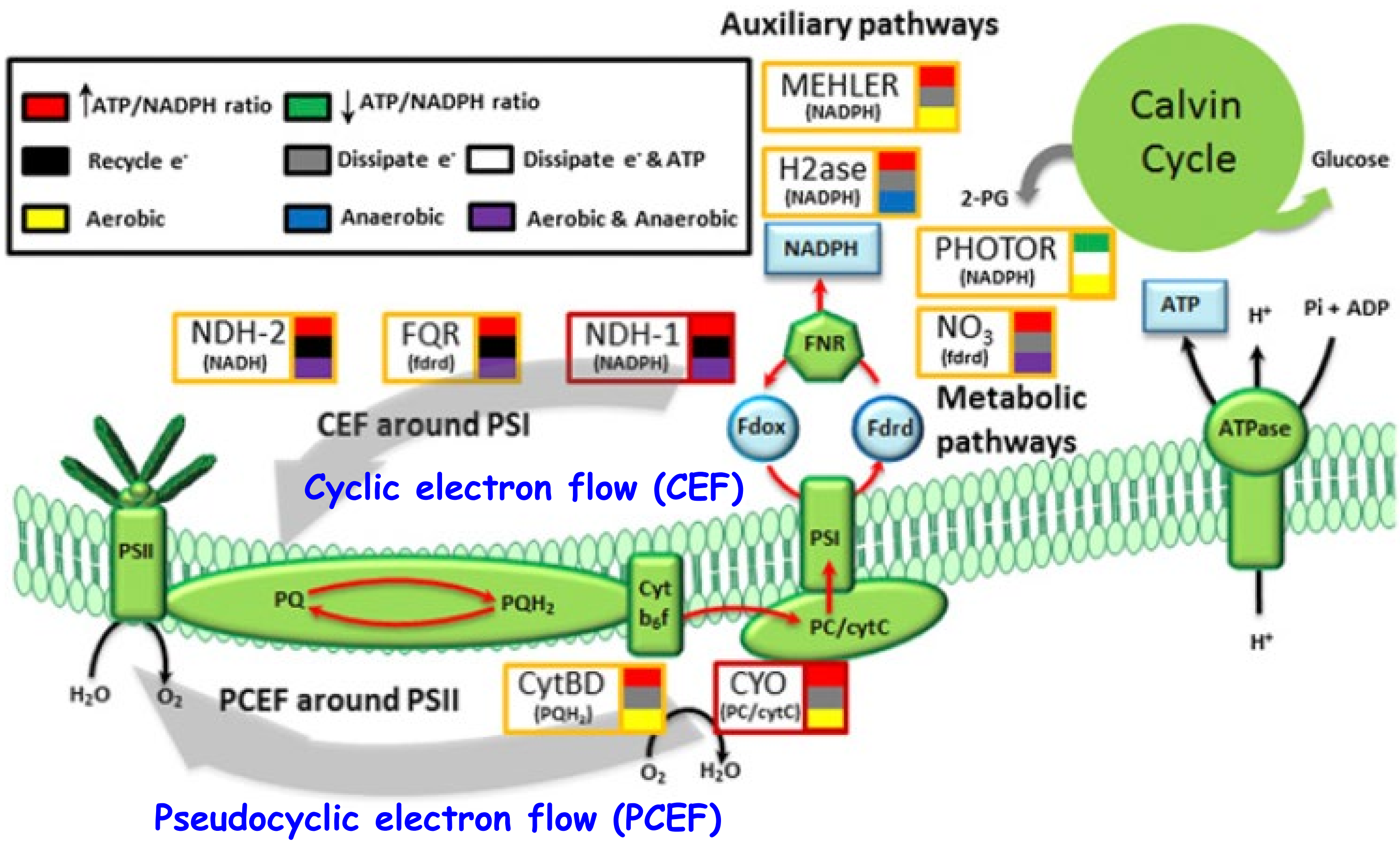
- Light-limited state (LLS): the initial state in which light, not inorganic carbon (C_i – HCO_3 or CO_2), was the growth-limiting factor.
- Carbon-limited state (CLS): the final state in which C_i was growth-limiting factor.
- Under LLS, the fluxes through the LEF pathway, ATPase, and the HCO_3 or CO_2 uptake increase with light availability.
- The attainment of maximum C_i uptake [3.7 mmol·gDW^{-1}·h^{-1}] marks the beginning of CLS, which was characterized by light excess and C_i limitation.
- Increasing light availability leads to more photosynthetic activity and, thus, to higher levels of reducing equivalents (PSII, PSI, or FNR) and ATP.



Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

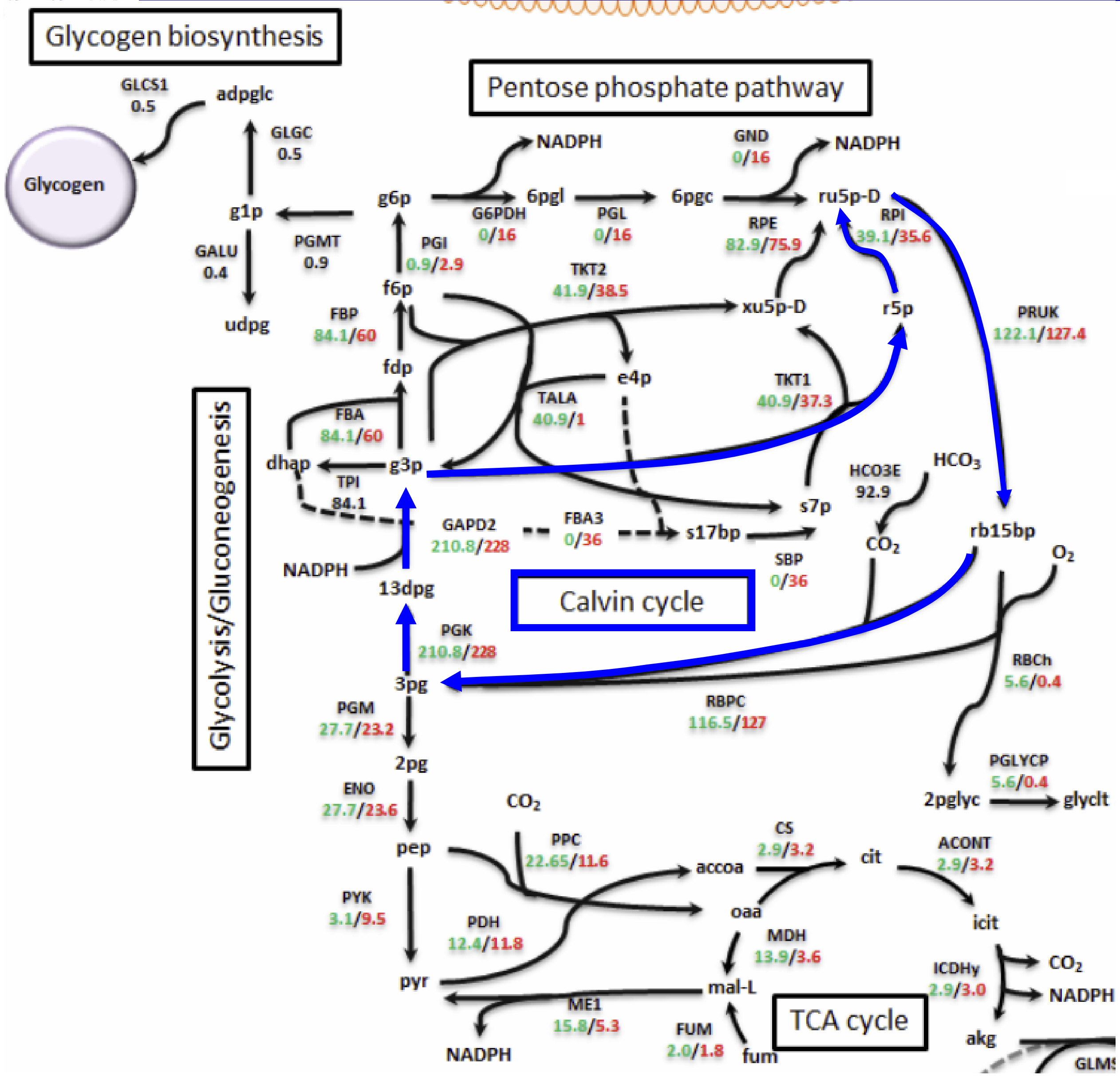
Alternate Electron Flow (AEF)

- The LEF pathways alone do not enable maximal growth under suboptimal light conditions, AEF pathways are also required.
- Under LLS, with AEF pathways guarantees optimal growth by consuming reducing equivalents and increasing the ATP levels.
- Under CLS, AEF pathways primarily act as photoenergy-dissipation pathways by consuming reducing equivalents and dissipating more than 50% of available photons (hv_i/hv_o).
- Cyclic electron flow (CEF) around PSI has been suggested to be the main AEF pathway in phototrophs and to be primarily responsible for supplying the additional ATP required for CO_2 fixation and ATP/NADPH balancing.
- Under CLS, PCEF can be classified as high and low photosynthetic yield pathways, they also enable optimal growth by acting as an electron sink, indirectly consuming more than 53% of the available photons and preventing internal overreduction.

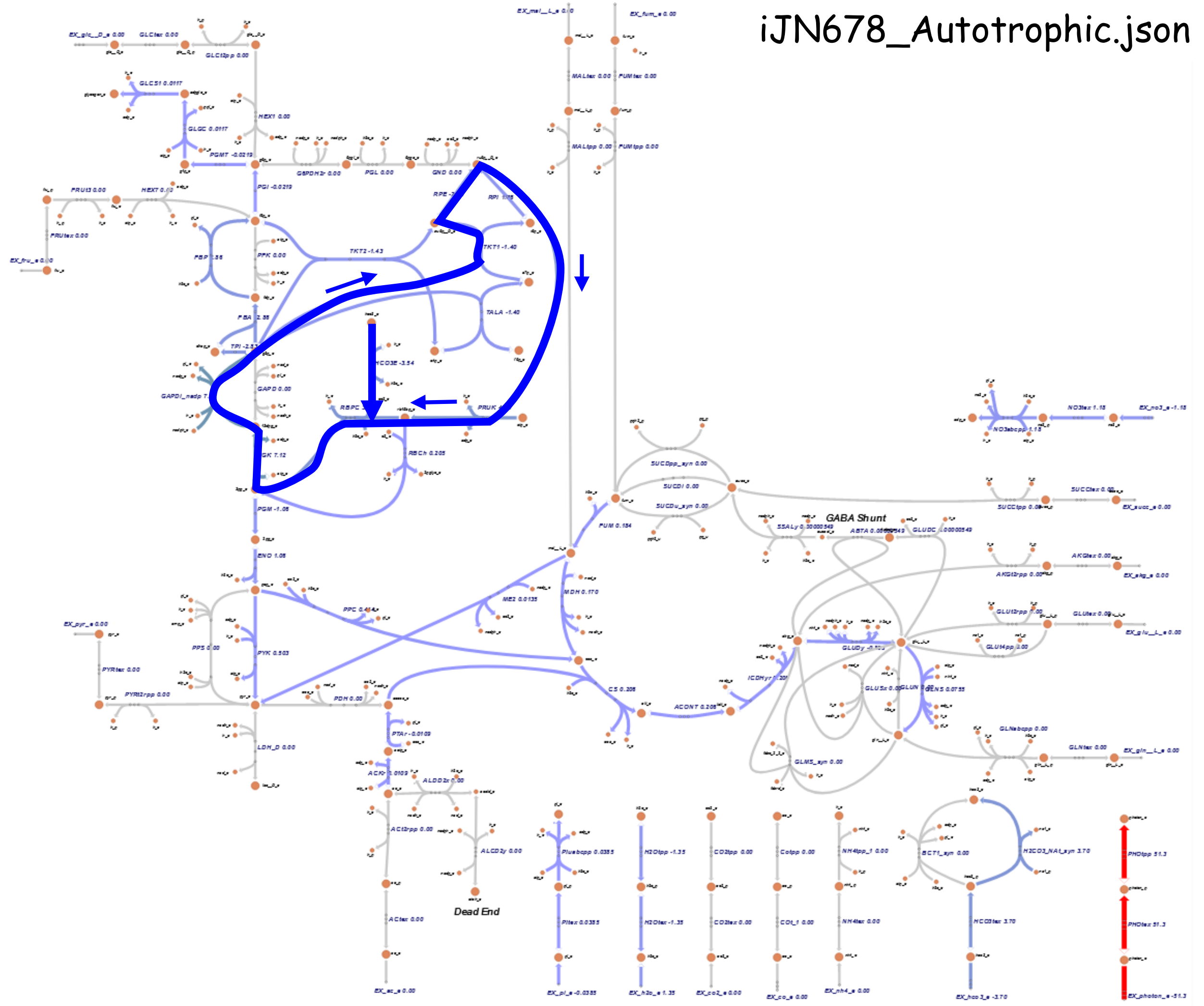


Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

Autotrophic Conditions



iJN678_Autotrophic.json



Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

iJN678 Autotrophic Condition

iJN678_Autotrophic.json

Set the environment

In [1]:
```
import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
import matplotlib.pyplot as plt
from cobrapy_bigg_client import client
from cobra.flux_analysis.loopless import add_loopless, loopless_solution

pd.set_option('display.max_rows', 1000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
```

Load and save the model

In [2]:
```
import cobra
cobra_config = cobra.Configuration()
model_orig = client.download_model('iJN678', save=False) # Loading the model to the simulation
#model_orig.solver = "glpk_exact" # Takes a long time
model_orig.solver = 'glpk'
model = model_orig.copy()
```

```
Set parameter Username
Academic license - for non-commercial use only - expires 2022-10-10
```

Model Attribute Summary

In [3]:
```
model
```

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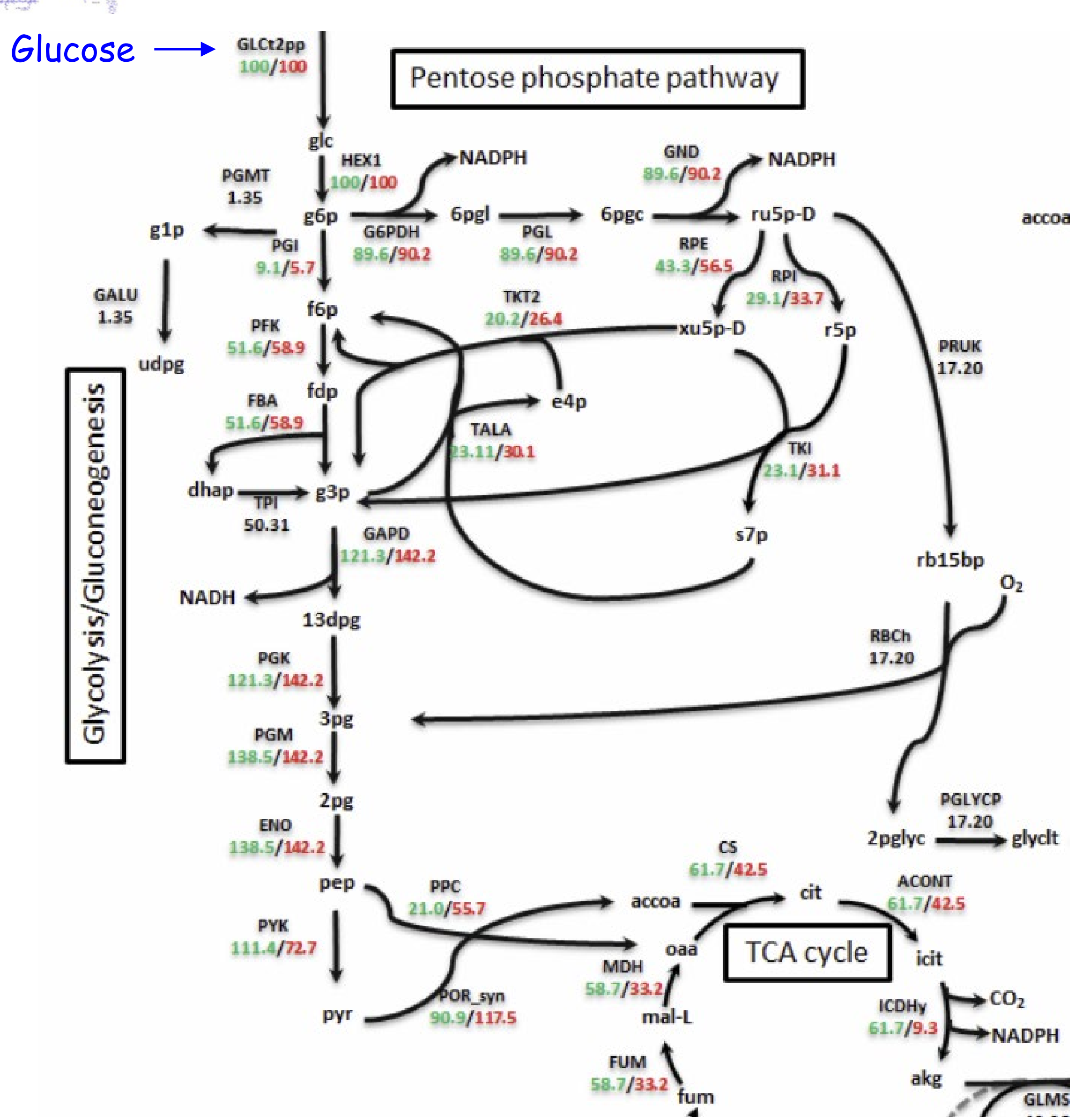
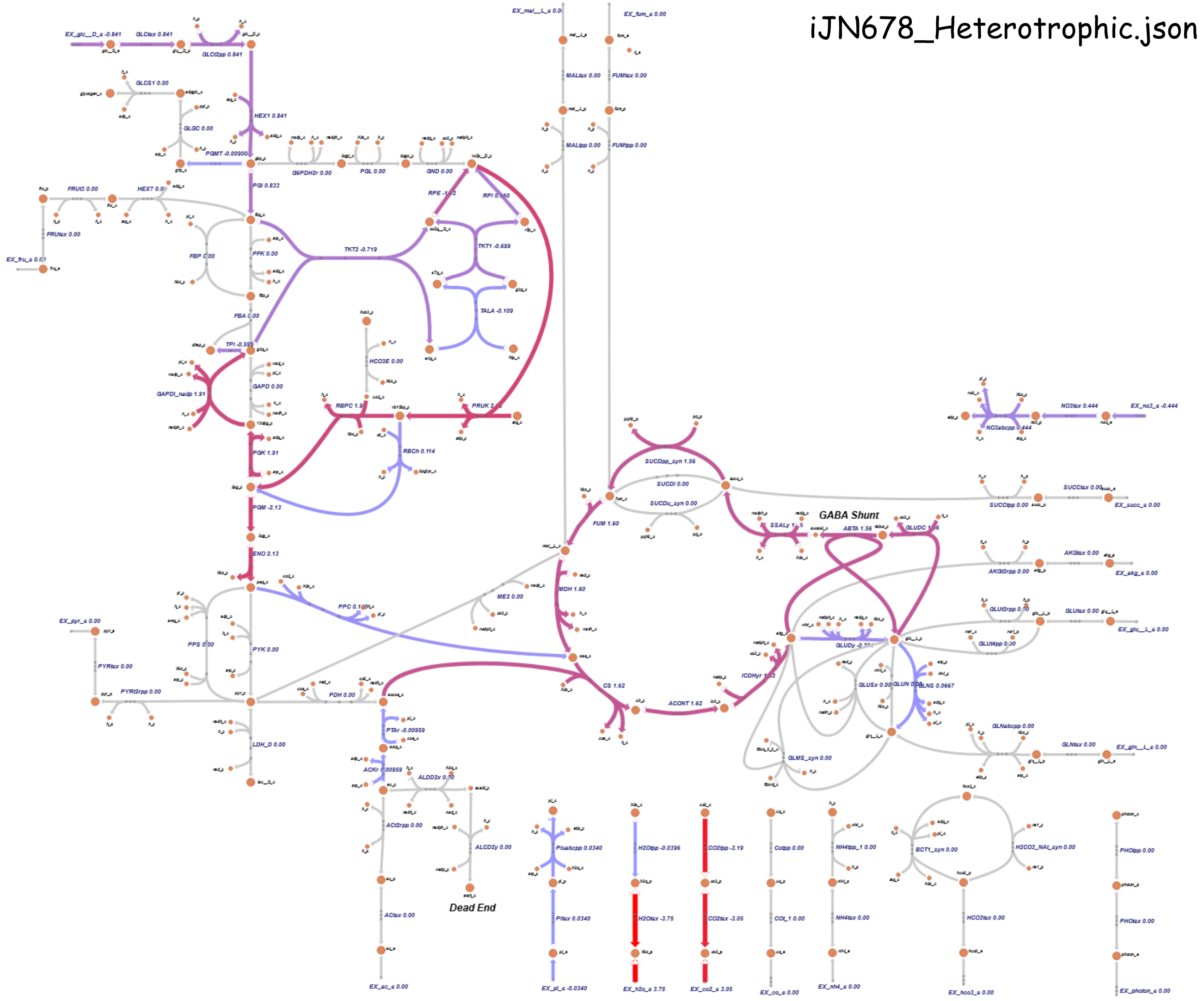
Heterotrophic Conditions



iJN678_Heterotrophic.json



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iJN678 Heterotrophic Operation

iJN678_Heterotrophic.json

Set the environment

In [1]:
```
import cobra.test
import pandas as pd
import numpy as np
import escher
from escher import Builder
import matplotlib.pyplot as plt
from cobrapy_bigg_client import client
pd.set_option('display.max_rows', 1000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
```

Load and save the model

In [2]:
```
model_orig = client.download_model('iJN678', save=False) # Loading the model to the simulation
model = model_orig.copy()
```

```
Set parameter Username
Academic license - for non-commercial use only - expires 2022-10-10
Read LP format model from file C:\Users\hinton\AppData\Local\Temp\tmpm2dy6uh1.lp
Reading time = 0.01 seconds
: 795 rows, 1726 columns, 8316 nonzeros
```

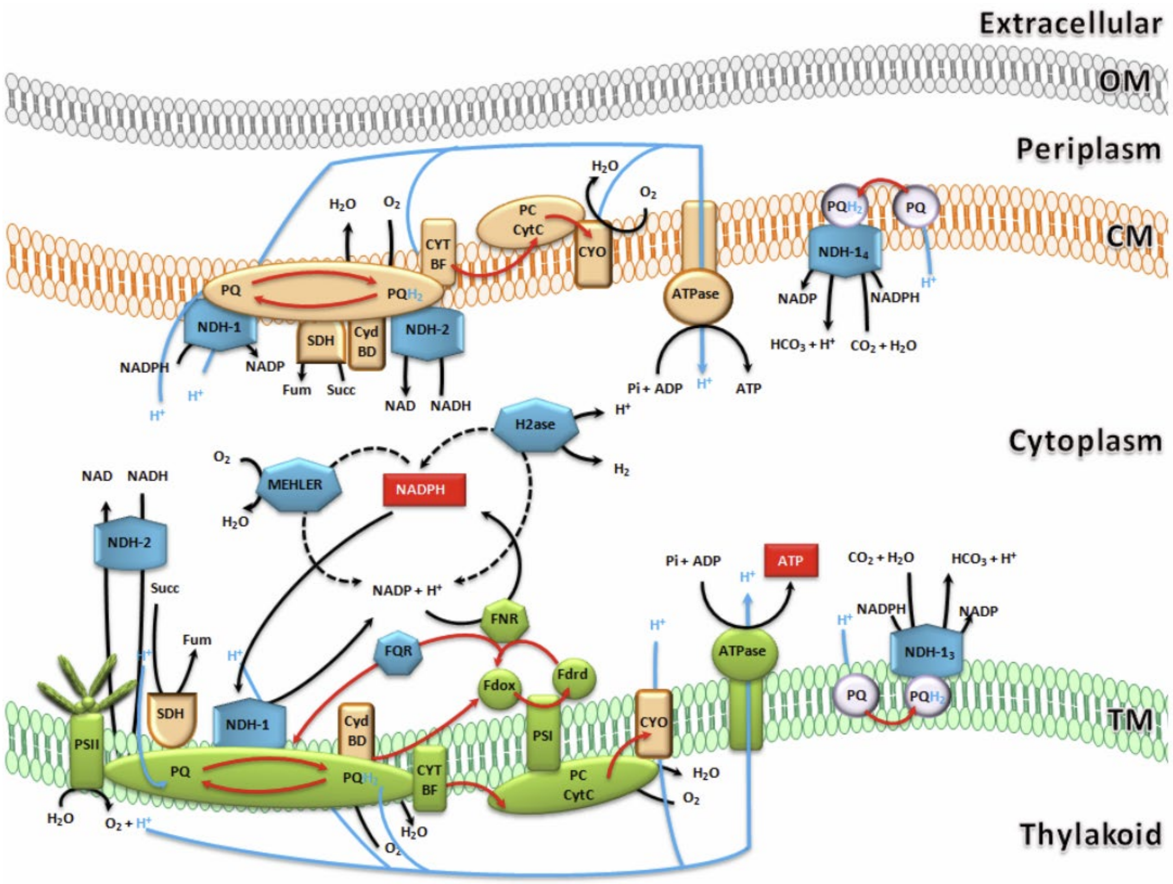
Model Attribute Summary

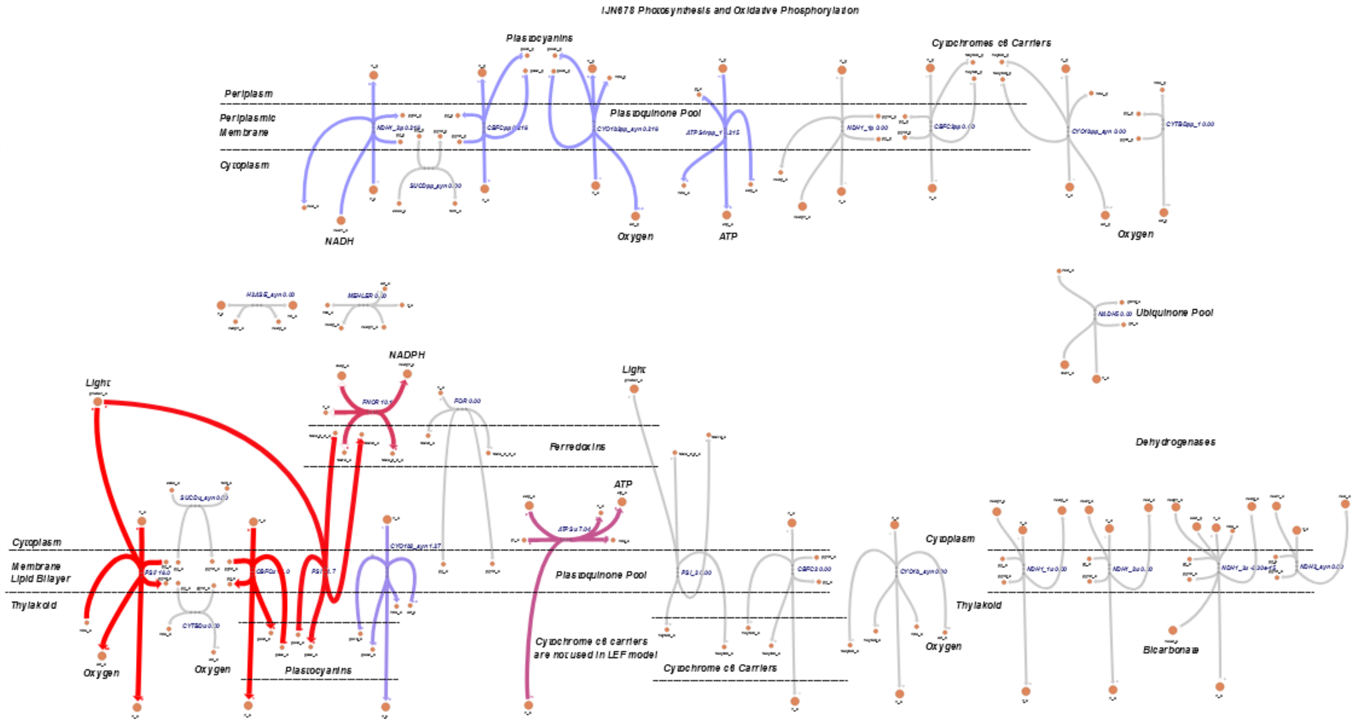
In [3]:
```
model
```

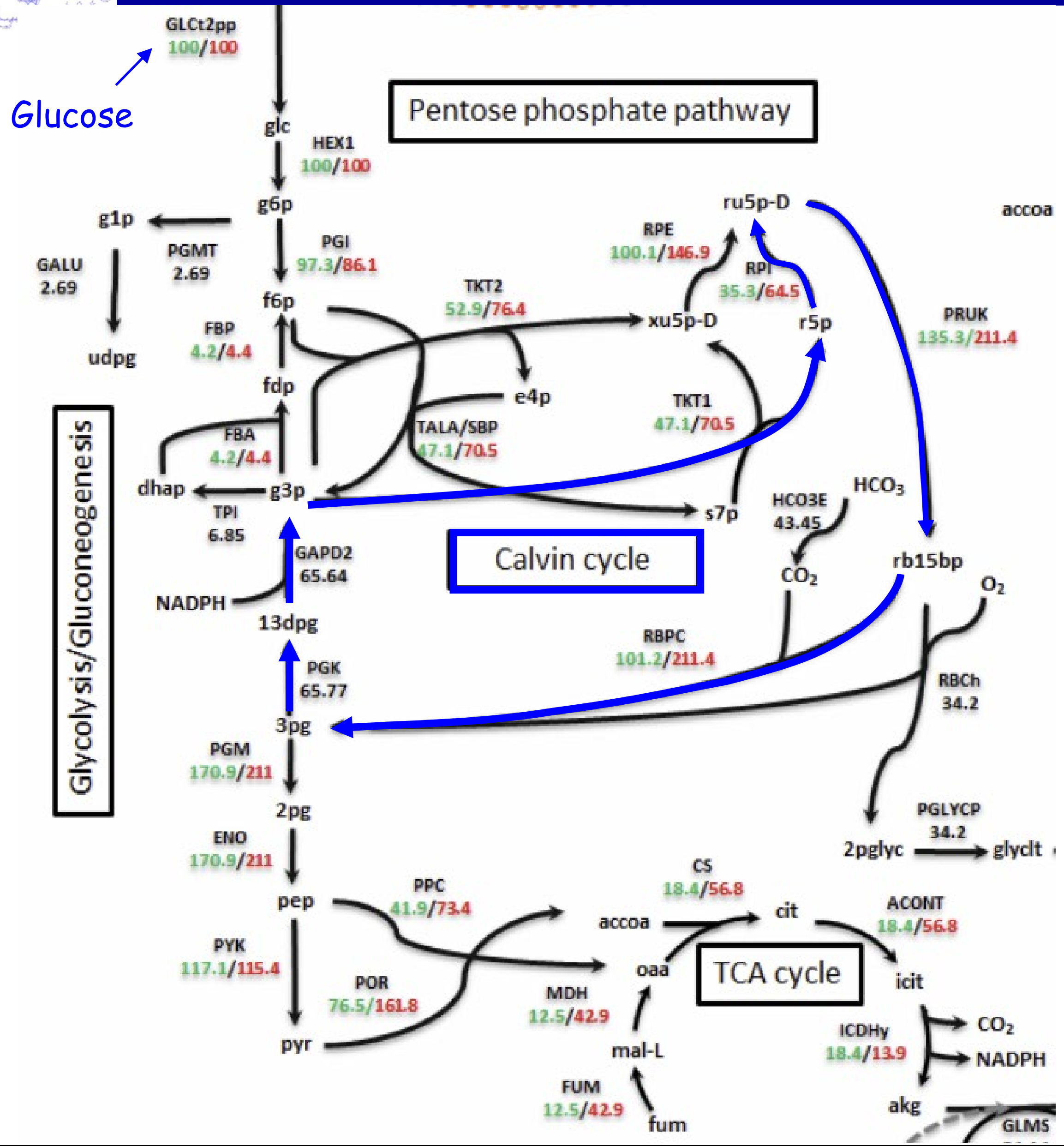
Lesson Outline

- Robustness Analysis
- *Synechocystis sp. PCC6803* (Cyanobacteria) Overview
- Understanding the iJN678 *Synechocystis sp. PCC6803* Model
- iJN678 Energy & Reducing Power
  - ✓ Respiration
  - ✓ Photosynthesis
- iJN678 Cellular Operation
  - ✓ Autotrophic
  - ✓ Heterotrophic
  - ✓ Mixotrophic

Mixotropic Respiration & Photosynthesis

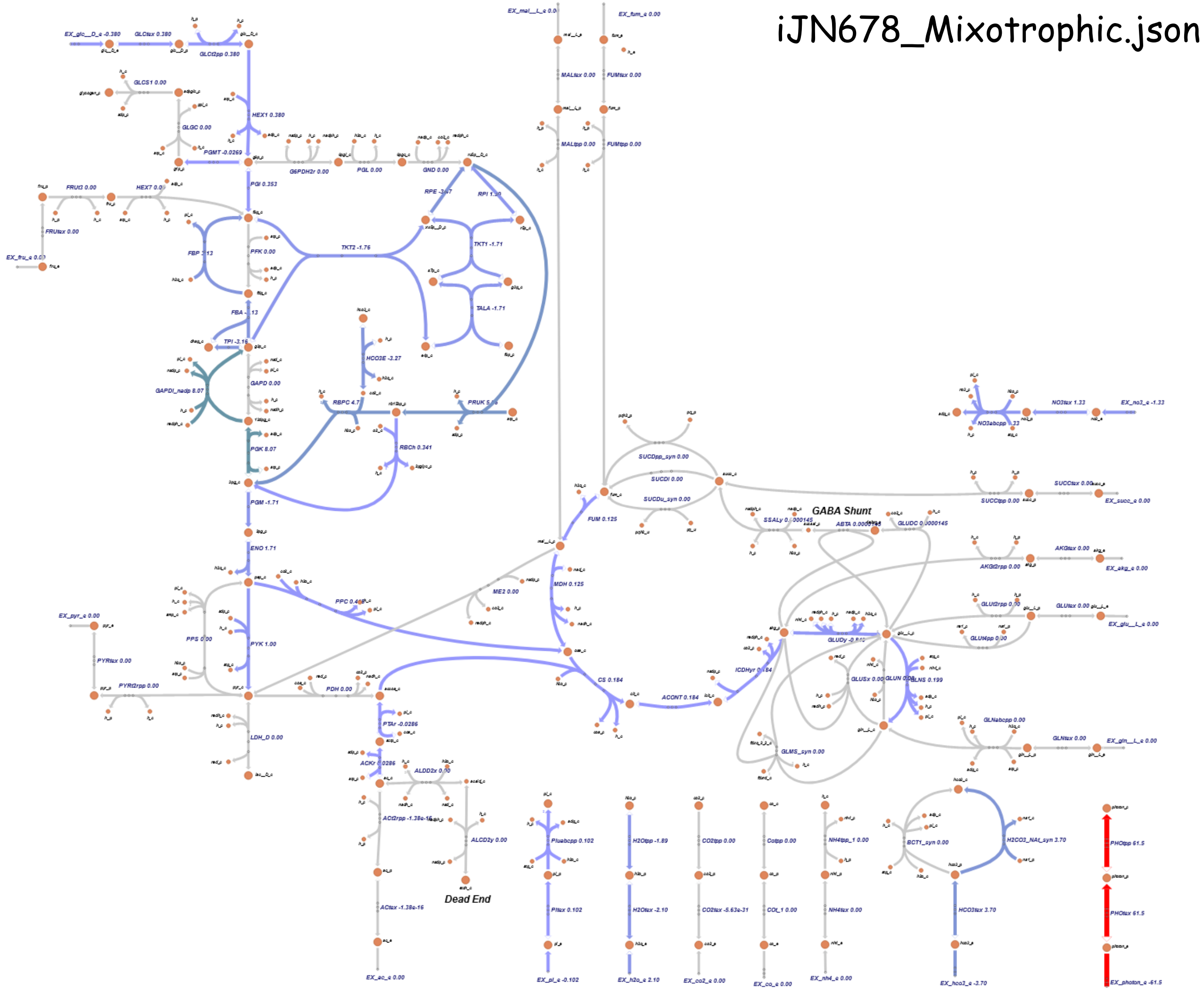






Mixotropic Conditions

iJN678_Mixotrophic.json



Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.

iJN678 Mixotrophic Operation

iJN678_Mixotrophic.json

Set the environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import escher
from escher import Builder
import matplotlib.pyplot as plt
from cobrapy_bigg_client import client
from cobra.flux_analysis.loopless import loopless_solution

pd.set_option('display.max_rows', 1000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
```

Load and save the model

```
In [2]: model_orig = client.download_model('iJN678', save=False) # Loading the model to the simulation
model_orig.solver = 'glpk'
model = model_orig.copy()
```

```
Set parameter Username
Academic license - for non-commercial use only - expires 2022-10-10
```

Model Attribute Summary

```
In [3]: model
```

Lesson Outline

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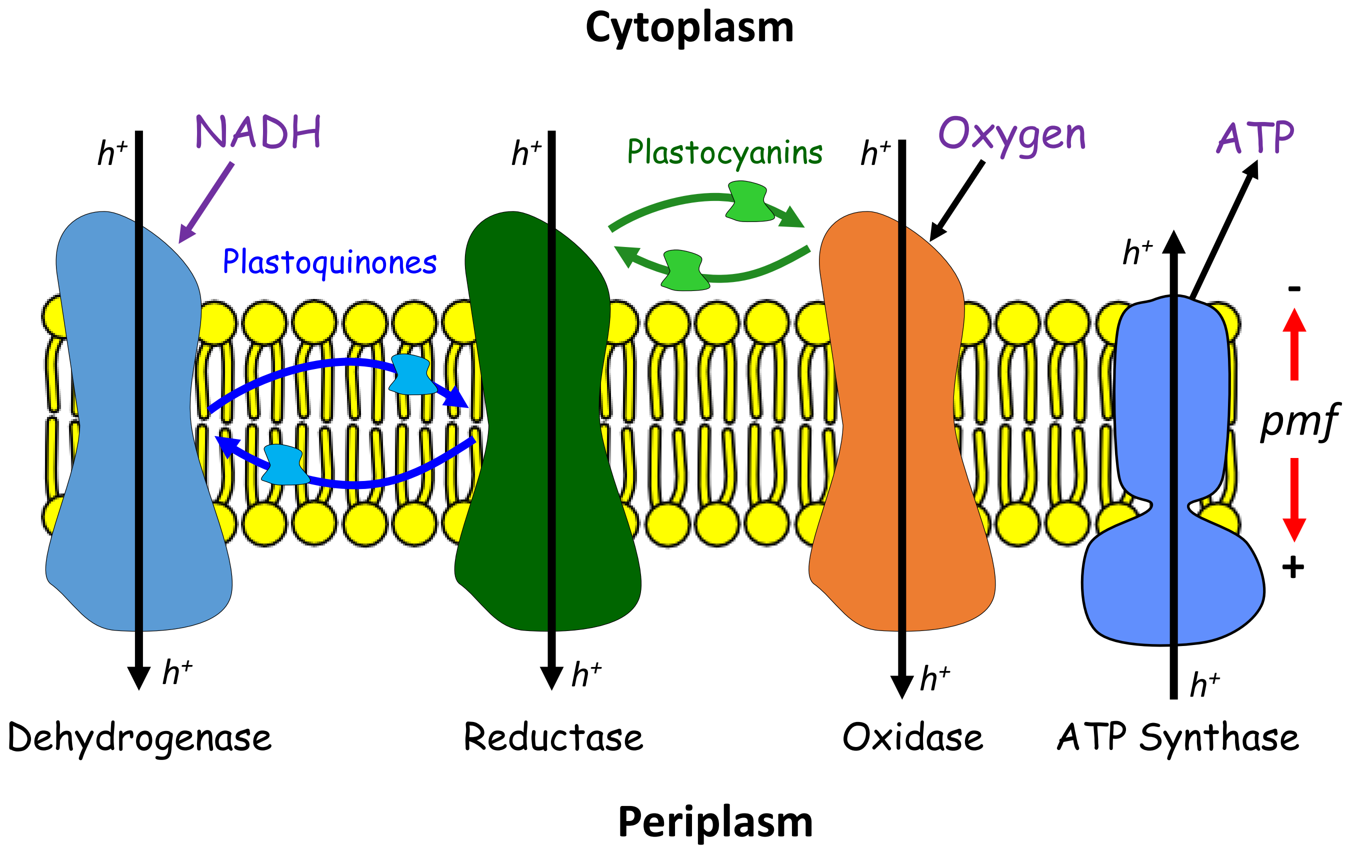
  - ✓ Heterotrophic
  - ✓ Mixotrophic

Reflective Questions

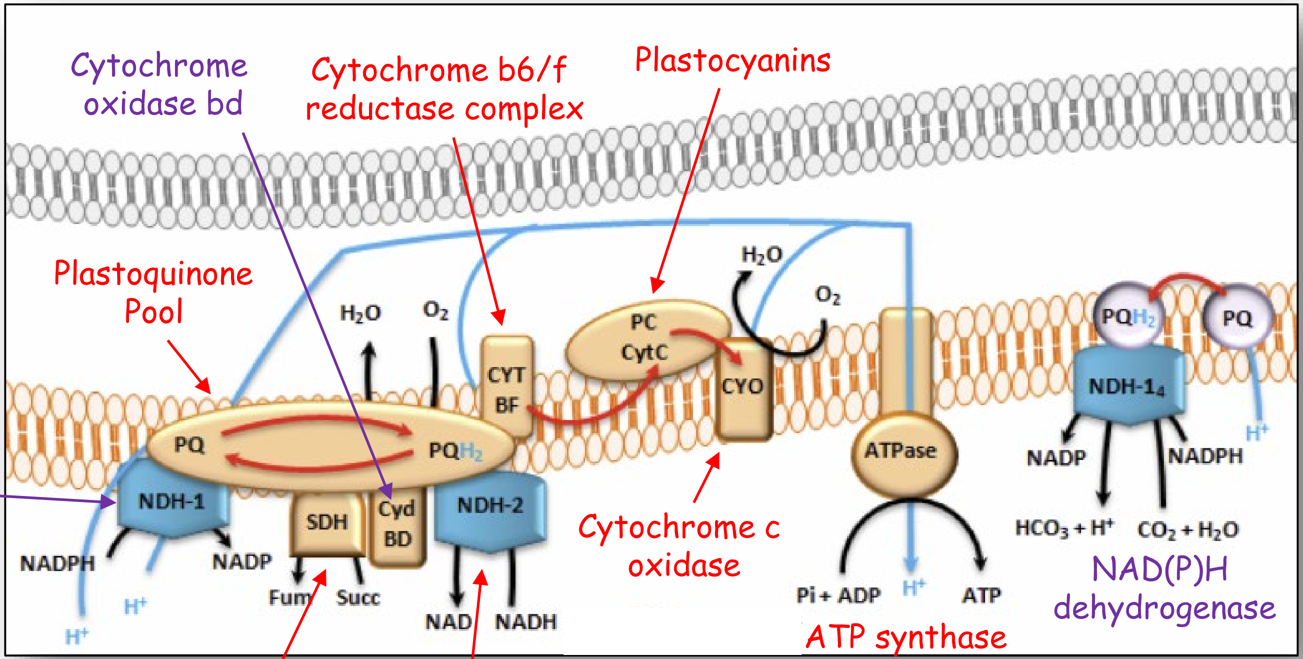
1. What is robustness analysis
2. What is oxidative phosphorylation?
3. What is photosynthesis?
4. What does autotrophic mean?
5. What does heterotrophic mean?
6. What does mixotrophic mean?
7. What is the purpose of the Calvin cycle?
8. What is the purpose of an ATP synthetase?
9. What is the proton-motive force?
10. What is the purpose of multiple biomass functions for a single model?
11. What is an LEF pathway?
12. What is an AEF pathway?
13. What is the electron transport chain?

Cynobacteria ETC



Modeling the Oxidative Phosphorylation Pathways Included in iJN678

NAD(P)H dehydrogenase



Succinate dehydrogenase

NAD(P)H dehydrogenase

Nogales, J., S. Gudmundsson, et al. (2012). "Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2678-2683.