# Calvin cycle enzymes knockout in GSM of *Arabidopsis thaliana*

Kailash Adhikari

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# Outline

- Introduction
- Photophosphorylation
- Calvin Cycle
- Oxidative Pentose Phosphate Pathway
- Central Carbon Metabolism

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    - General Model Properties
    - Part-I : Investigation of Calvin cycle enzymes KO
    - Part-II: Investigating energy dissipation mechanism

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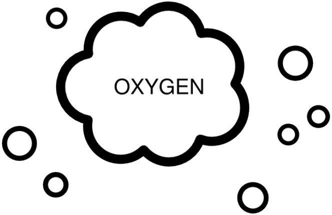
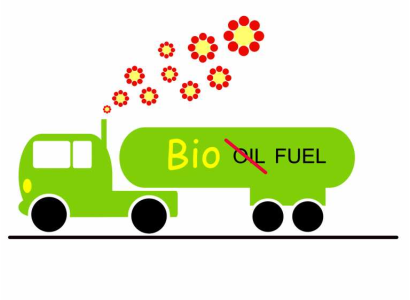
- Conclusions

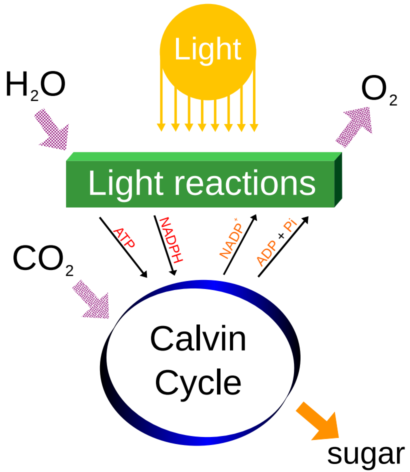
# Importance of Plants



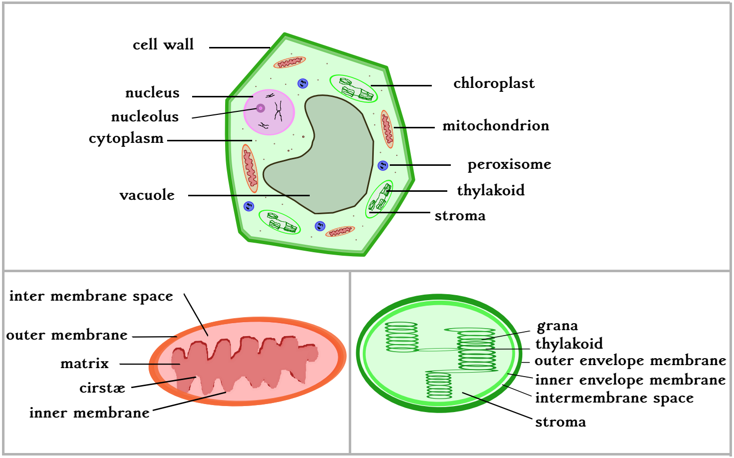




# Photosynthesis

Photosynthesis is the process by which plants, algae and some bacteria use carbon dioxide and light energy to make sugar molecules and oxygen



# Photosynthesis

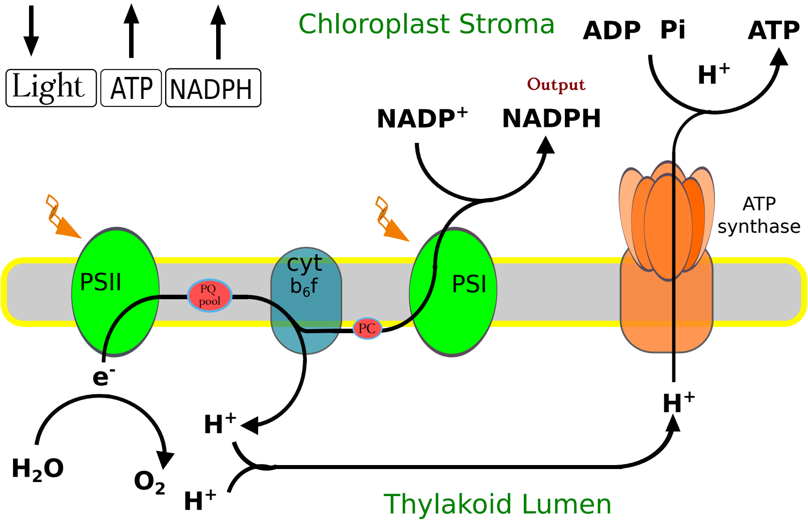


# Photosynthesis

The process of photosynthesis can be broadly classified into two phases:

- Light dependent process: Photophosphorylation
  - Occurs in thylakoid membrane
  - Light energy captured by chlorophyll molecule to produce energy in the form of NADPH and ATP
- Light independent process: Calvin Cycle
  - Occurs in chloroplast stroma
  - Energy produced during the light reaction is used to fix the inorganic carbon to produce sugar

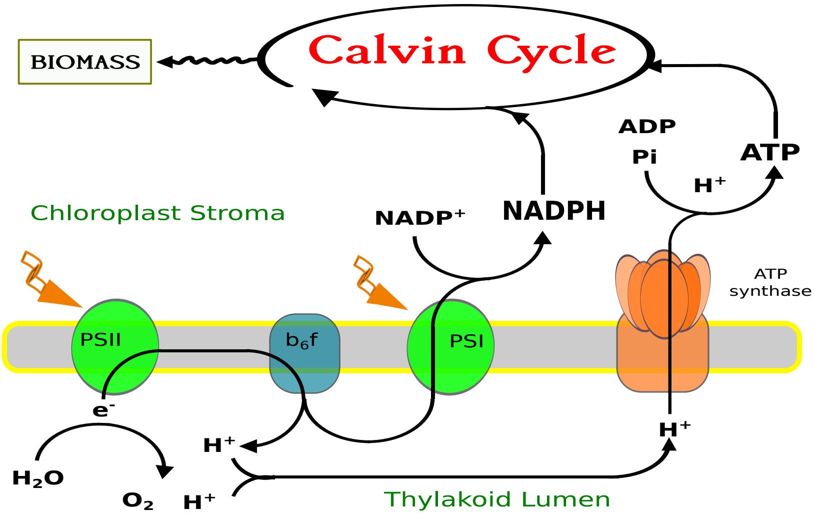
# Photophosphorylation



# Photophosphorylation

- Non-Cyclic Photophosphorylation/Non-Cyclic Light Reaction
  - Both PSI and PSII are involved
  - Electrons travel in a non-cyclic manner
  - Electron from PSI is accepted by NADP
  - Both ATP and NADPH is produced
  - Oxygen is evolved from photolysis
- Cyclic Photophosphorylation/Cyclic Light Reaction
  - Only PSI is involved
  - Electrons travel in a cyclic manner
  - Electron travels back to PSI
  - Only ATP is produced
  - Oxygen is not evolved as photolysis is absent

# Photophosphorylation - Calvin Cycle



# Stoichiometry of Light Reactions

- Light Non-Cyclic Reaction:

**56 Photon + 18 ADP + 14 NADP + 18 Pi + 14 $H_2O$ → 7 $O_2$ + 14 Proton + 14 NADPH + 18 ATP**

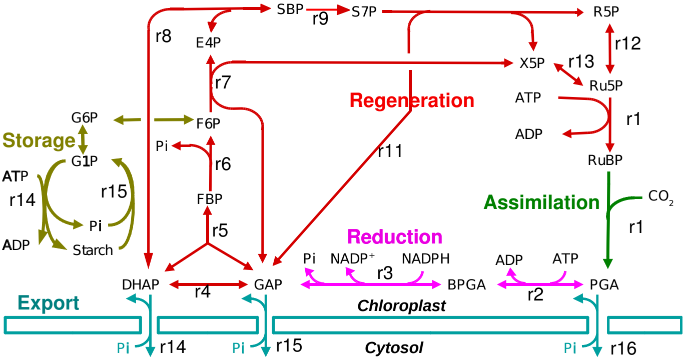
- Light Cyclic Reaction:

**14 Photon + 6 ADP + 6 Pi → 6 ATP + 6 $H_2O$**

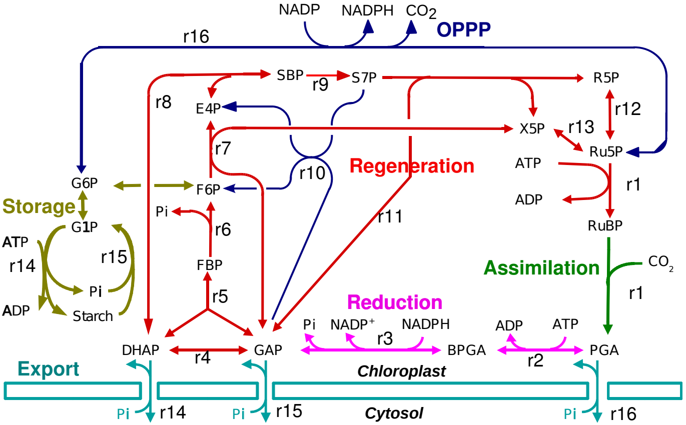
# Calvin Cycle

- The Calvin cycle was discovered by Melvin Calvin, James Bassham, and Andrew Benson at the University of California, Berkeley in 1950
- It is a light independent process that uses the energy from light reactions for carbon fixation
- Phases of Calvin Cycle:

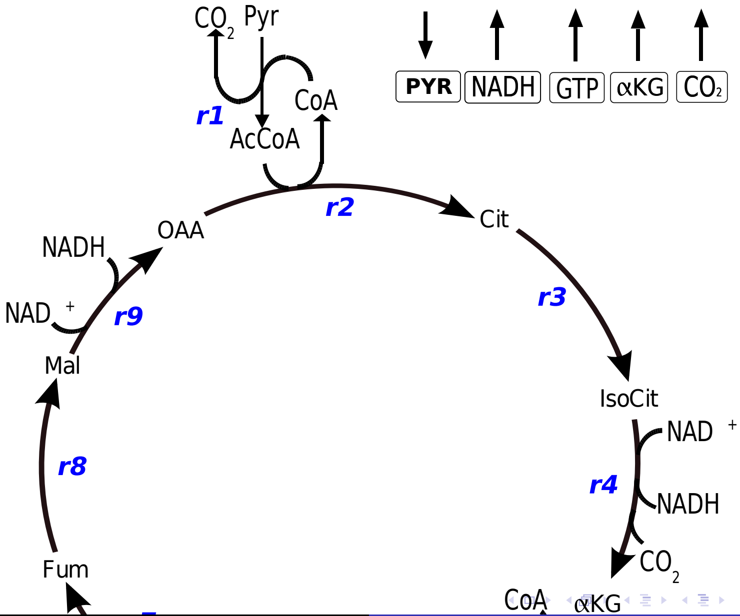
# Calvin Cycle



# Oxidative Pentose Phosphate



# Role of Mitochondria



# Plant metabolism is complex

- Plant metabolism is a complex process involving several enzymes
- The performance and metabolism of plant is significantly affected by several environmental factors such as light, temperature and soil salinity

# Introduction

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  - Identify potential metabolic responses under changing light condition.
  - Investigate the physiological impact of Calvin cycle enzymes knockouts in the plant.

# Construction and validation of GSM

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- Biomass production
  - amino acids
  - nucleotides
  - lipid
  - starch
  - cellulose
  - chlorophyll A and B
  - lignin
- The model is ready for analysis

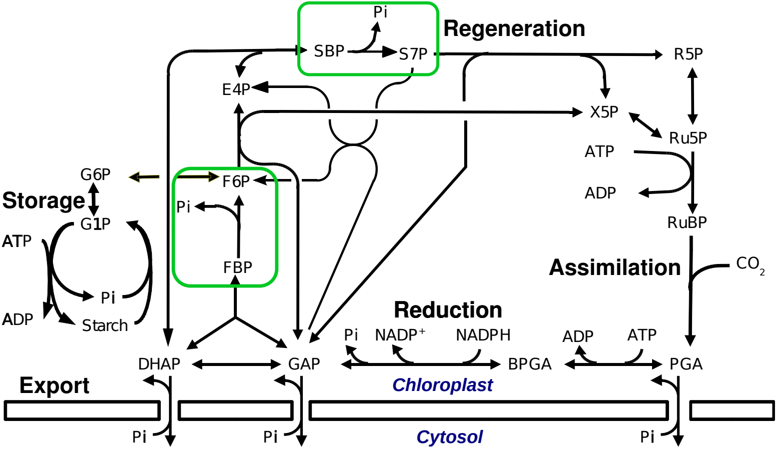
# General properties of the GSM

| Properties | Specification |
|---|---|
| Source of carbon | $CO_2$ |
| Source of energy | Light / Starch |
| Total number of reactions | 2588 |
| Total number of metabolites | 2481 |
| Total number of transporters | 234 |
| External transporters | 55 |
| Inter-compartmental transporters | 179 |
| Compartments | Cytoplasm, Plastid, Mitochondria, Peroxisome, Vacuole |

Total number of reactions required to produce all biomass varies from 331 - 336 reactions depending upon the environmental condition.

# Motivation

- Increasing demand of food in the - compelling need to improve productivity of crop plants
- Many *in vivo* and *in silico* analysis suggest that improving photosynthetic capacity of plants can help improve crop productivity
- Plants with increased or reduced activity of individual enzymes from the Calvin cycle have been used to study the regulation of photosynthetic carbon flow
- Collaborating partners in the AccliPhot consortium at ETH Zurich performed experimental work to knockout Calvin cycle enzymes


Pi
SBP
S7P
Regeneration
R5P
E4P
X5P
Ru5P
ATP
ADP
RuBP
G6P
Storage
G1P
F6P
Pi
ATP
Pi
FBP
ADP
Starch
Assimilation
$CO_2$
Reduction
Pi
NADP+
NADPH
ADP
ATP
DHAP
GAP
BPGA
PGA
Export
Chloroplast
Cytosol
Pi
Pi
Pi

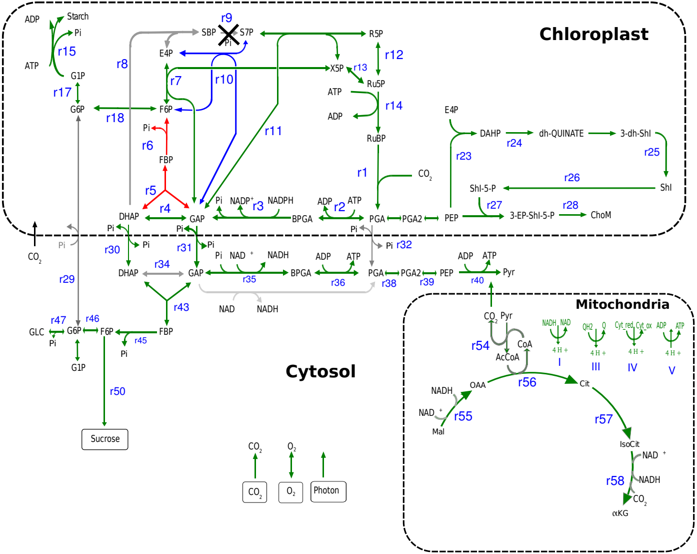
# Methodology

- FBA was used to predict the feasibility of the solution in WT and all the mutants
- Minimisation of total reaction flux was used as objective function.

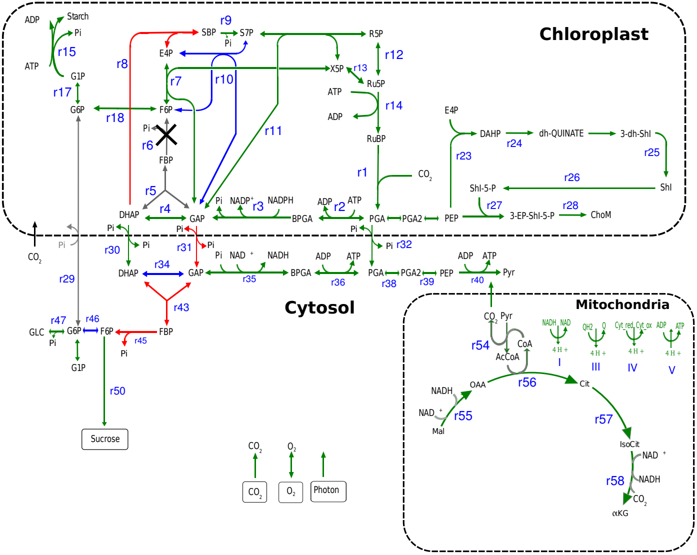
$$
\begin{aligned}
\text{minimise} \quad &: \quad \mathbf{v} \\
\text{subject to} \quad & \begin{cases} \mathbf{N}\mathbf{v} = 0 \\ v_{i..j} = \mathbf{t}_{i..j} \\ v_{\mathrm{ATPase}} = \mathrm{ATPase} \\ v_{\nu} = \nu \\ v_{\mathrm{LightNonCyc}} \geq v_{\mathrm{LightCyc}} \\ v_{\mathrm{RubiscoCarboxylase}} + v_{\mathrm{RubiscoOxygenase}} = 0.4 \\ v_{\mathrm{KO-rxn}} = 0 \end{cases}
\end{aligned} \tag{1}
$$

- The change in reaction fluxes as the effect of knockout was noted.

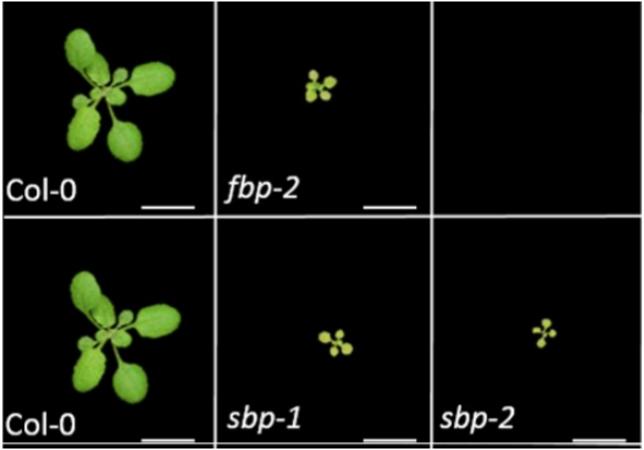
# Central carbon metabolism of SBPase knockout mutant



# Central carbon metabolism of FBPase KO mutant



# Experimental results

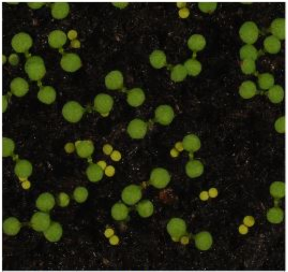



The homozygous plants are compromised in growth, but are viable.

# PRK KO mutant

No feasible LP solution was found for PRK knockout and the experimental evidence also shows that the mutant plants were not viable.

***prk1*** seedlings; homozygous non viable plants are the pale one



***prk2*** seedlings; homozygous non viable plants are the pale one

# Summary of the KO investigation

| Enzyme | Model prediction | Experimental result |
| --- | --- | --- |
| SBPase | Viable | Viable |
| FBPase | Viable | Viable |
| PRK | Non-viable | Non-viable |

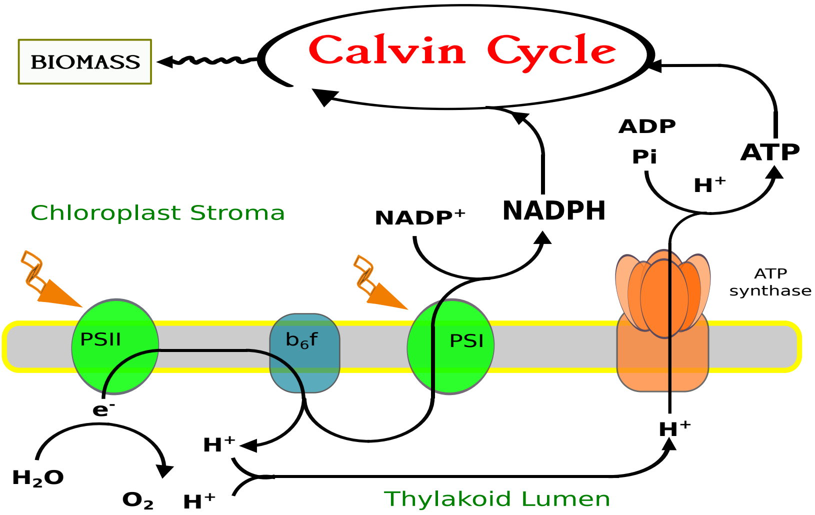
# Conclusion

- Correctly predict the viability or non-viability of all individual KO mutants
- Highlighted a novel role of the enzyme transaldolase in photosynthetic metabolism
- Propose newer features of the metabolic network such as the complementary roles of SBPase and FBPase
- Portrayed the need for further experimental data and development of more theoretical methods to accurately predict the phenotypic impacts of plant mutation

# Part -II

Light Scan Analysis : Investigate energy dissipation mechanism

# Investigating energy dissipation mechanism



# Using GSMs to investigate energy dissipation mechanism

- The changes in light intensity affects rate and regulation of light harvesting, photochemical reactions, electron transport, production of energy components, synthesis of end products like starch, lipids and their use to drive growth and development.

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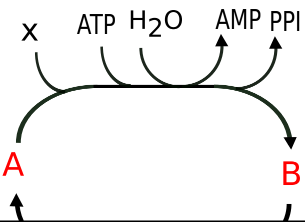
- The turnover of energy components, ATP, NADP and NADPH happens in seconds, as response to the changing light, which ultimately affects the long term acclimation and growth of the organisms.
- Hence, from the modelling point of view, we are interested in identifying all possible metabolic routes that consumes these energy components, the production of which is in high amount under supra optimal light conditions, and act as potential energy dissipation mechanism thus protecting the organisms form photo-damage.

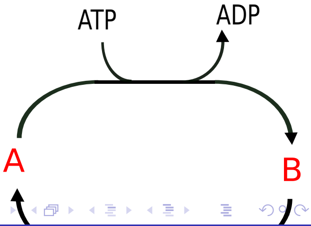
# Methodology

- The models were condensed to photon absorbing core model - reactions that have some degree of correlation with the photon consuming reaction
- reactions that are assumed to be active under light conditions, and are therefore, potentially involved in various energy dissipation processes
- photosynthetic core model was used to generate a metabolic tree representing the relationship between reactions
- The set of reactions that cluster together in such tree are more likely to operate together and have similar biological significance compared to others in the adjoining clusters
- Set of reactions that can operate as metabolic cycles were identified

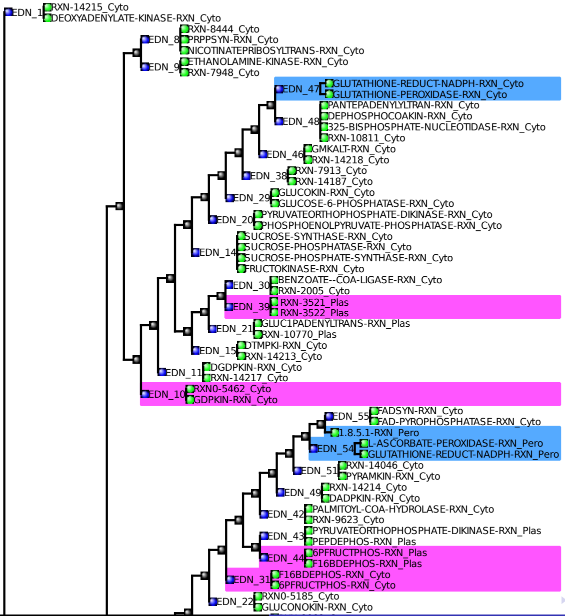
# Metabolic cycles

- sets of reactions operating in a metabolic system, whose net stoichiometry either hydrolyses ATP or oxidises a reductant while the overall net change of the process is only the absorbtion of energy with no net involvement of any other external metabolites other then $\mathrm{H_2O}$ for ATP hydrolysis and $\mathrm{O_2}$ for NADPH oxidation
- They include pathways with reactions operating in cyclic manner such that the cycle dissipates energy but without any net anabolic or catabolic transformation
- only few of them have been identified



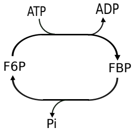



# Correlation tree

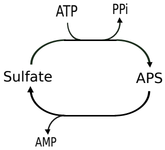


# metabolic cycles

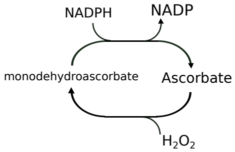
EDN_44
EDN_31 Plas & Cyto



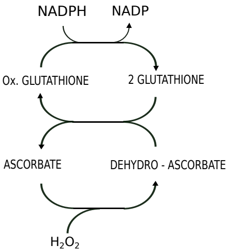

EDN_41 Cyto



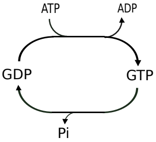

EDN_39 Cyto




EDN_52
EDN_54 Mito & Pero




EDN_10 Cyto

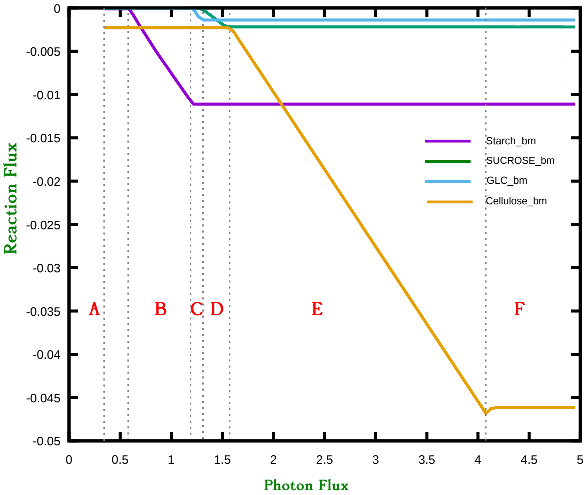



EDN_47 Cyto

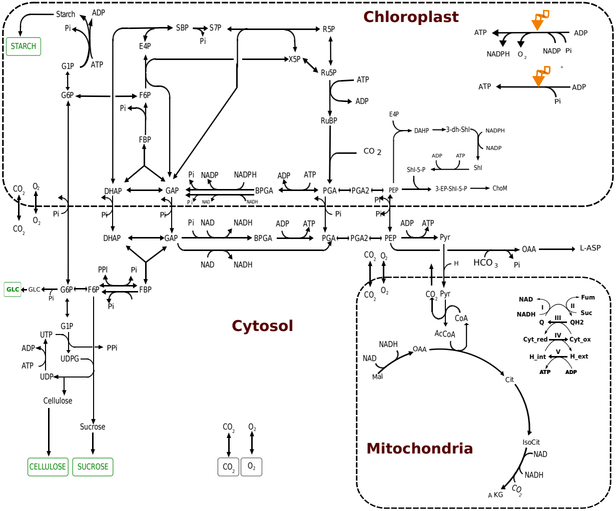
# Validation with proteomics data

- Proteomics expression can be used as a qualitative indicator of the metabolic activity. Thus for each reaction, the corresponding genes were identified and mapped to their respective protein count. In the case of many to one or many to many relationships between genes and reactions, the gene with highest protein count was considered for the comparison
- Proteomics, transcriptomics and metabolomics data (omics) can be used to study condition dependent changes into metabolic activity of the organism using metabolic models as a platform.

# Light Scan Analysis



# Final network diagram



# Discussion

- investigation was particularly focused to comprehensively identify the full range of potential energy dissipating cycles occurring in different part of metabolism in GSMs
- Using correlation coefficient analysis and MILP, it was possible to systematically identify all possible combinations of reactions in the model metabolic networks that could collectively function to dissipate excess energy by hydrolysis of ATP or oxidation of equivalent reductants.
- Some of the cycles identified from both the meth-
ods are more commonly known and have experimental evidence Geig$_9$1, $Rohw_0 1, Koch_0 5 of their in vivo activity while some others have bee$
- experimental support for metabolic cycles identified
- Increase in biomass composition after removing reactions involved in energy dissipation

# Final Conclusion

- GSM represents the metabolic behavior in greater details and can be used to investigate metabolic adjustments under different conditions.
- Genome scale metabolic models can be used in aid with other modelling tools to investigate organism behaviors, under various environmental conditions.

# Acknowledgement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# Thank you!

- Thank you for your attention!