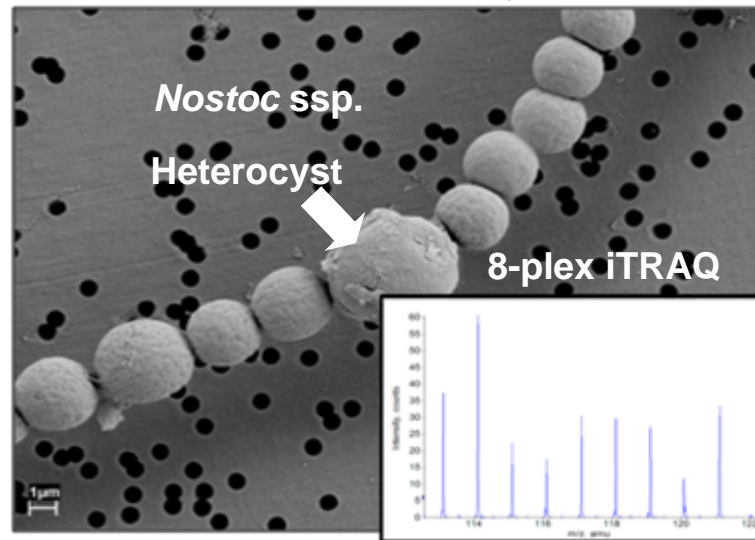




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# Proteome dynamics in *Nostoc* sp. PCC 7120 and in *Nostoc punctiforme* ATCC 29133

Karin Stensjö



2008-Molecular Bioenergetics of Cyanobacteria



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# Characteristics of the strains

## ***Nostoc* sp. strain PCC 7120:**

Genome 7.2 Mb

6252 ORFs (NCBI jan 2008)

Nitrogenase, uptake hydrogenase and bidirectional hydrogenase

Free living

## ***Nostoc punctiforme* strain ATCC 29133 (PCC 73102):**

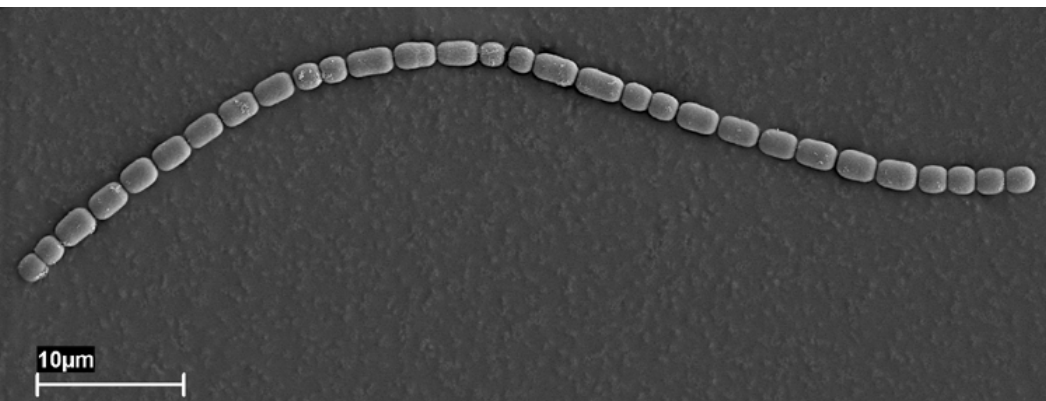
Genome 9.2 Mb

7771 ORFs (NCBI jan 2008)

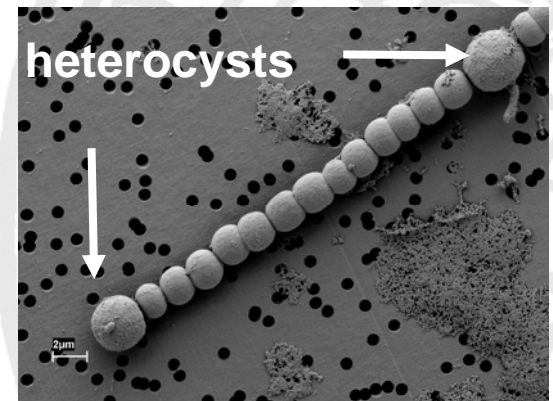
Nitrogenase and uptake hydrogenase

From symbiotic origin

Cultured with ammonium



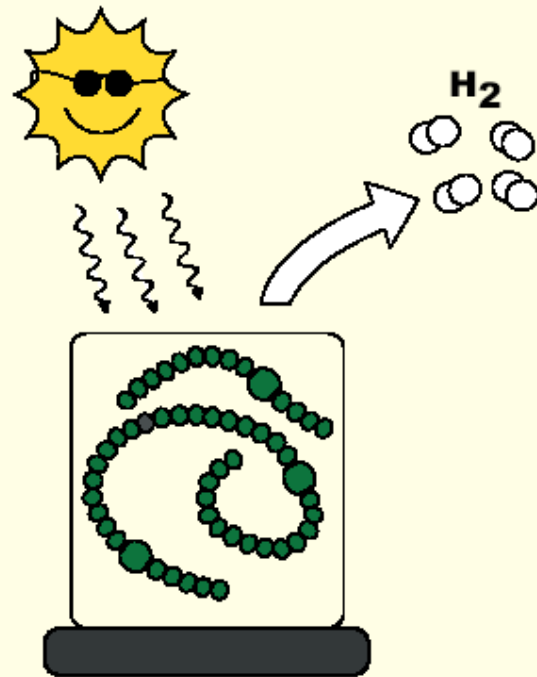
Cultured without ammonium, N<sub>2</sub> fixing



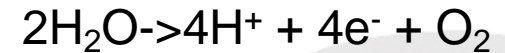


# The ultimate goal

- To use cyanobacteria for photobiological production of  $H_2$



Photosynthesis



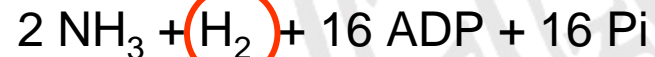
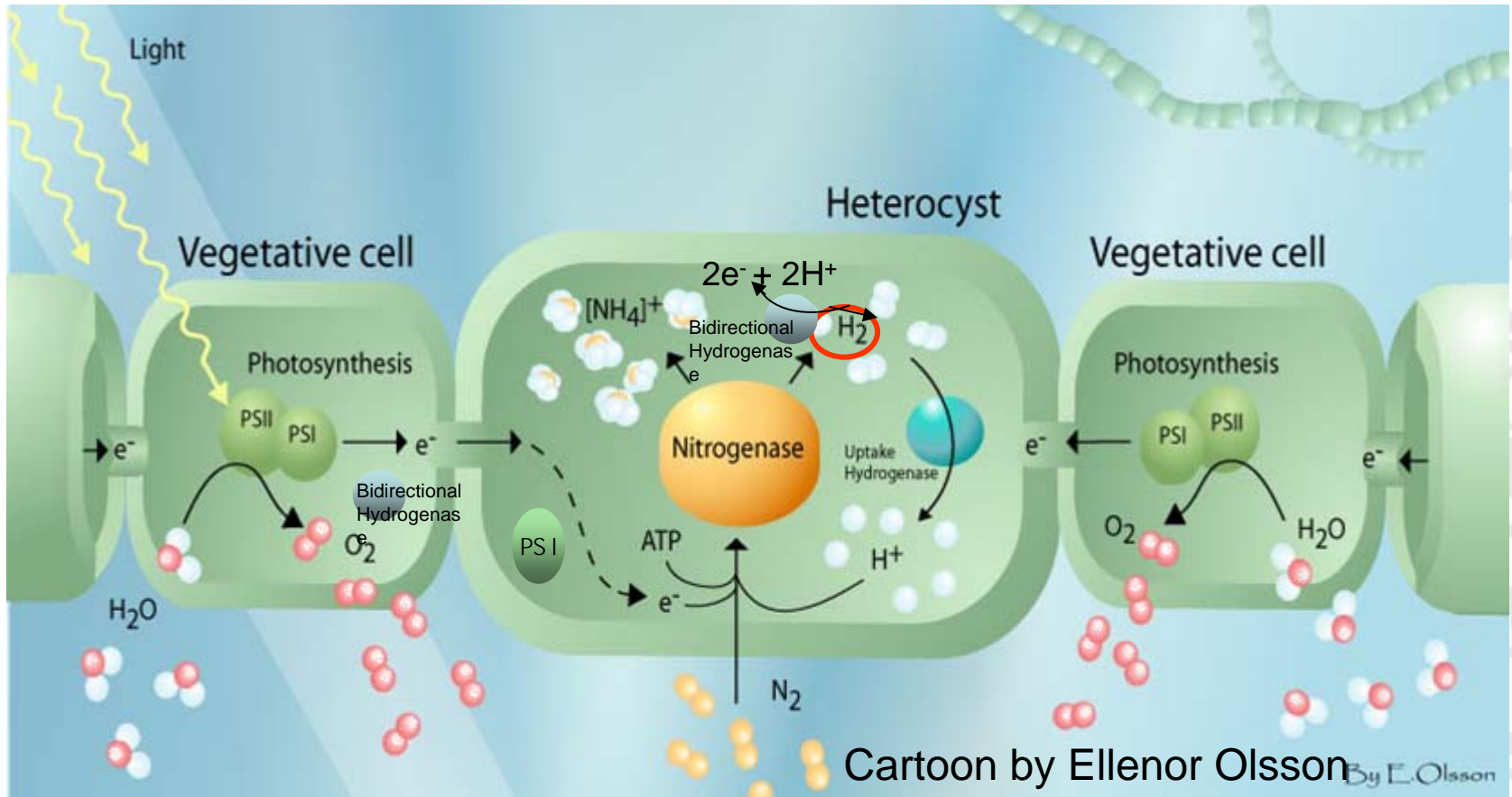
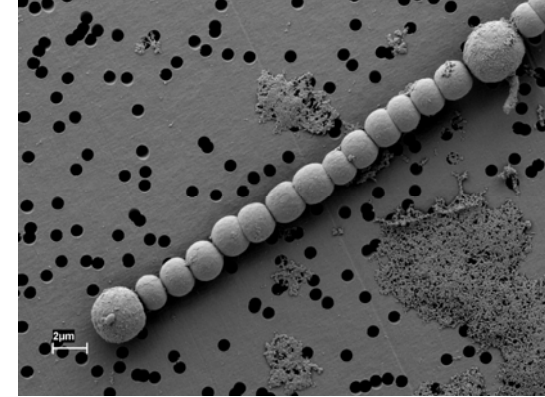
Hydrogenase





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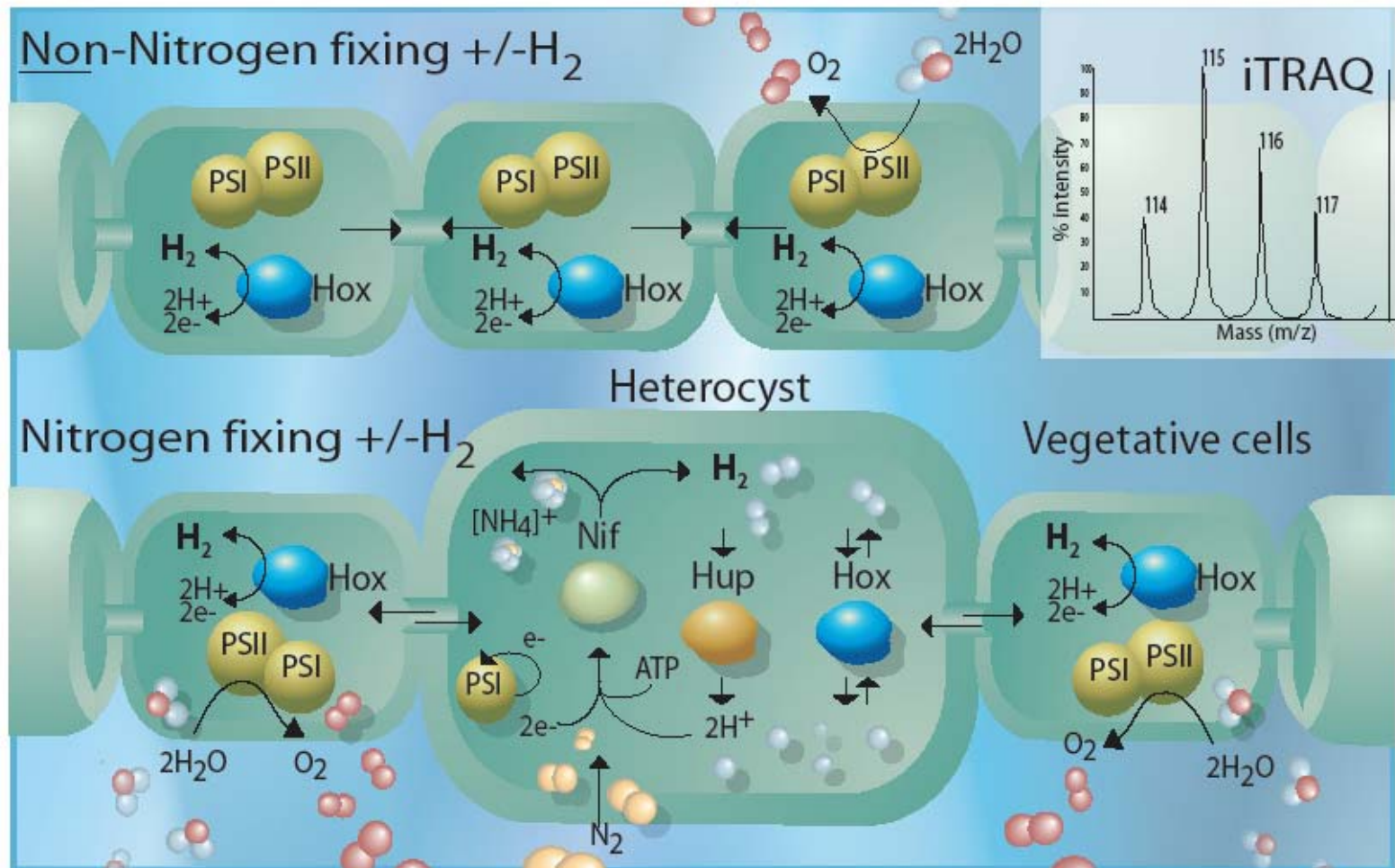
# H<sub>2</sub> production from N<sub>2</sub> fixing cyanobacteria



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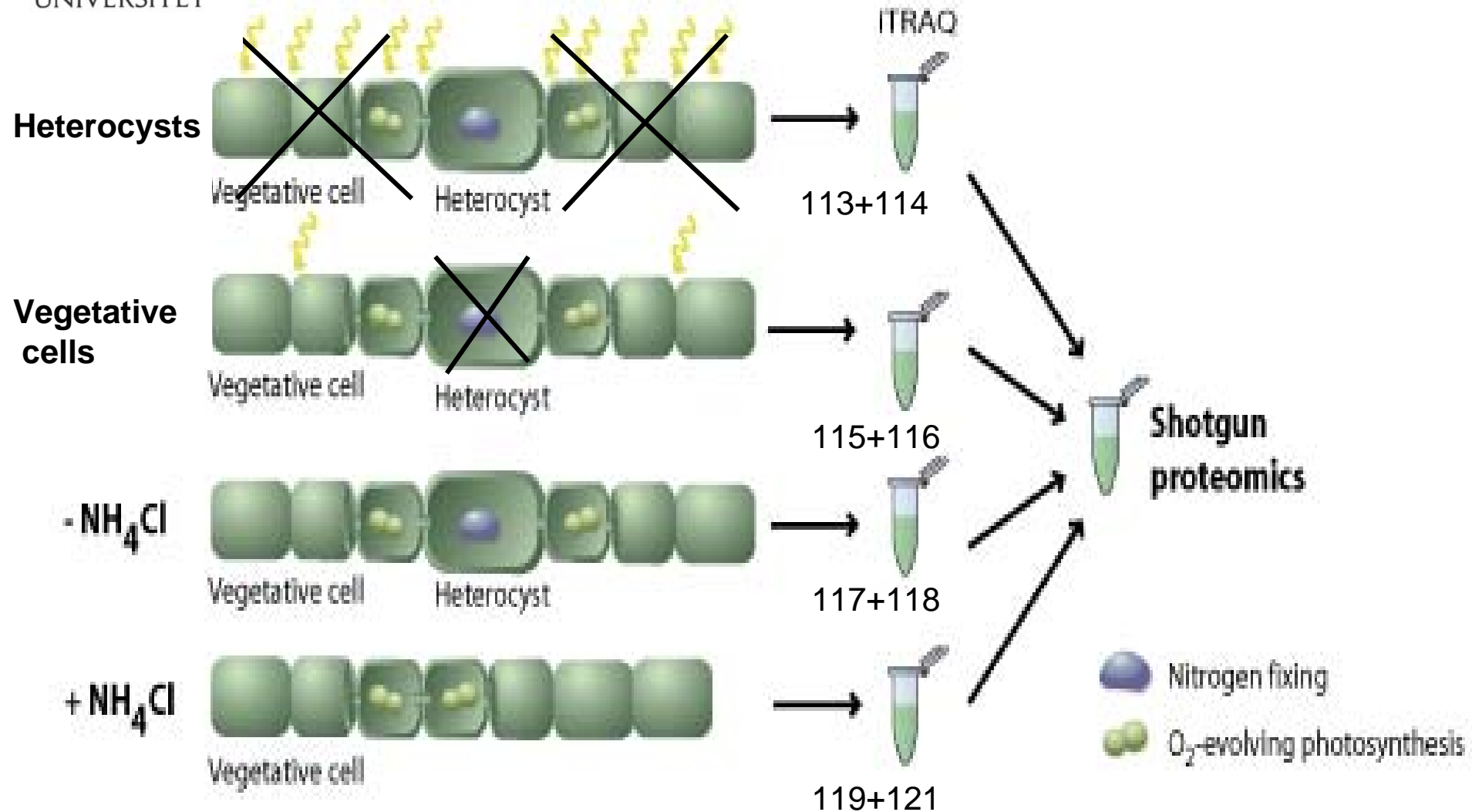
# Overall understanding of processes involved in $H_2$ metabolism and identification of novel proteins





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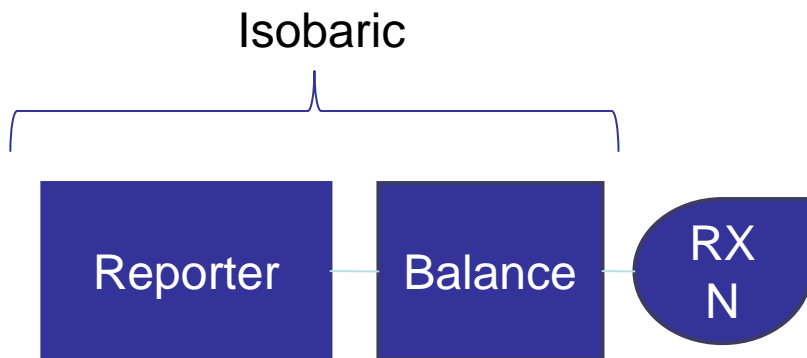
# Quantitative shotgun proteomics





# iTRAQ - Isobaric Tag for Relative and Absolute Quantification

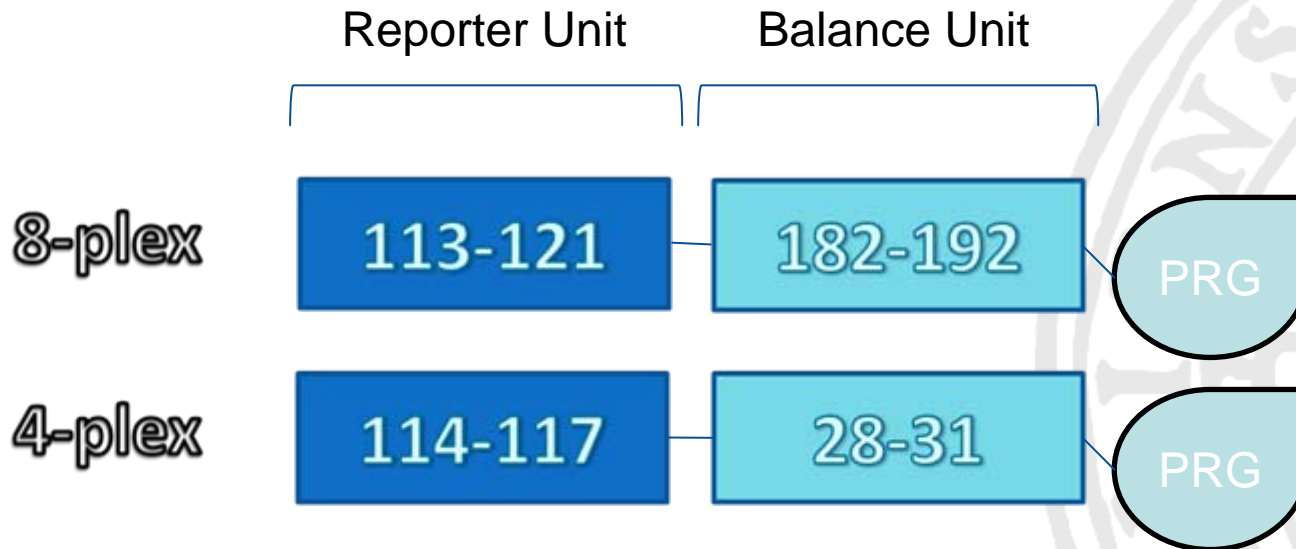
- iTRAQ use isobaric labels
- Varies between the mass of 'Reporter' and 'Balance'



Reporter		Balance	Total
114	~	31	145
115	~	30	145
116	~	29	145
117	~	28	145



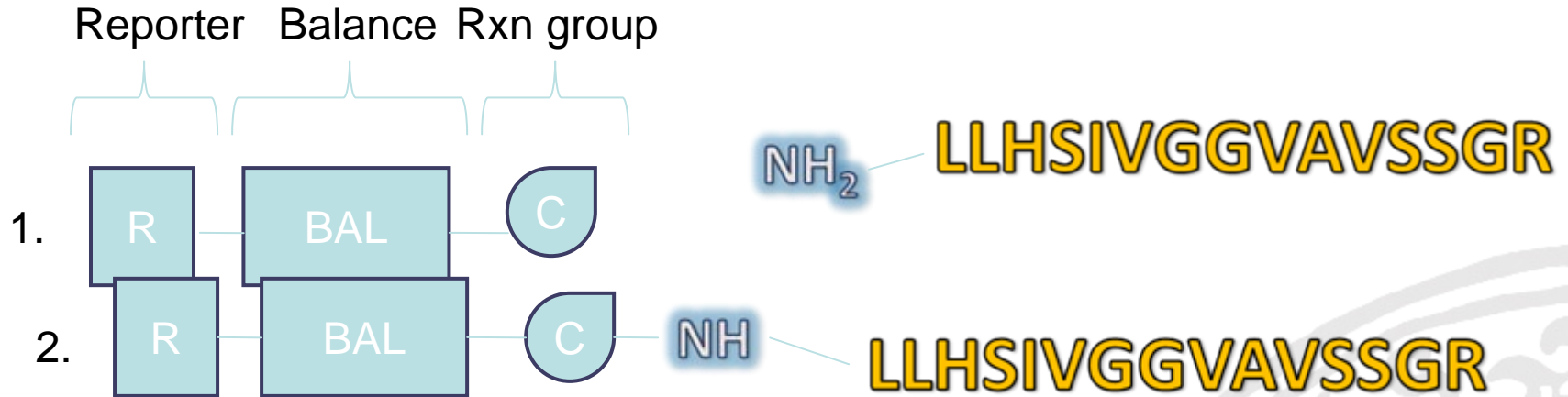
- 8 plex iTRAQ reporter now spans 113-121 and 121



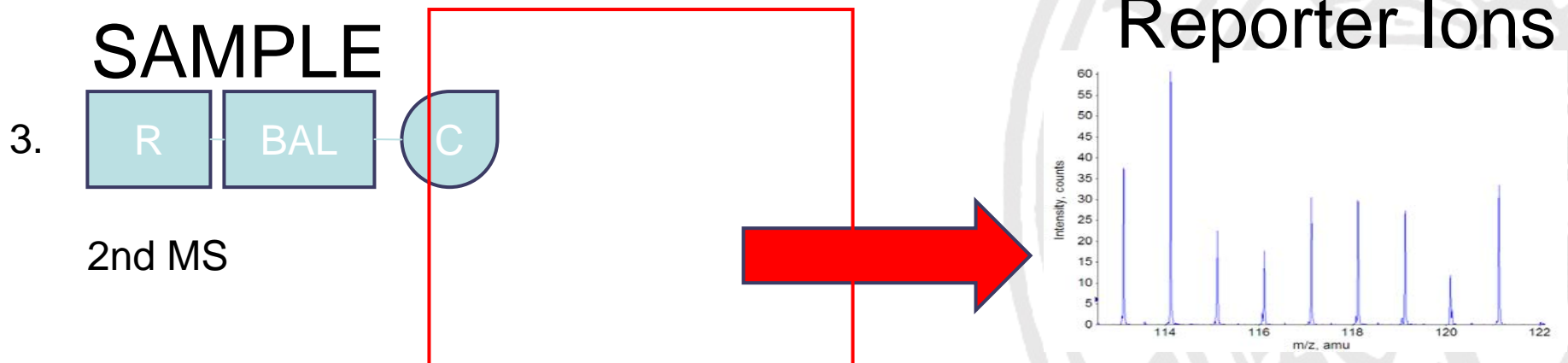




# iTRAQ Methodology



## Tandem MS Fragmentation





# Experimental design for the 8(4)-plex study

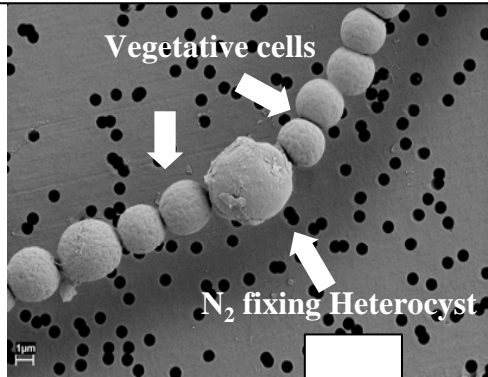
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*Nostoc* sp. PCC 7120  
*Nostoc punctiforme* ATCC 29133

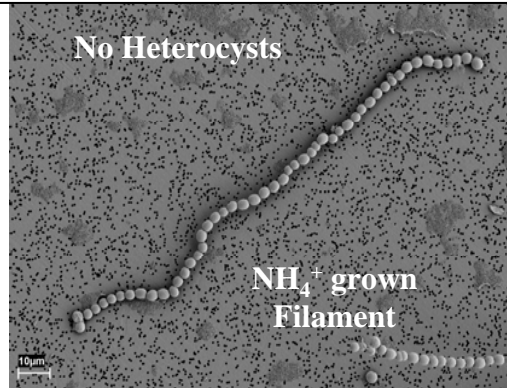
Filaments grown under  $N_2$   
fixing conditions

Filaments grown with  $NH_4^+$   
supplementation

**Study B**  
Whole Filament  
Study



SEM Micrograph



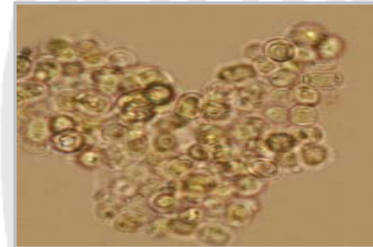
SEM micrograph by Marie Holmqvist

Cellular subpopulations  
are isolated and purified

Vegetative Cells

$N_2$  fixing Cells:  
**Heterocysts**

**Study A**  
Purified Heterocyst  
Study

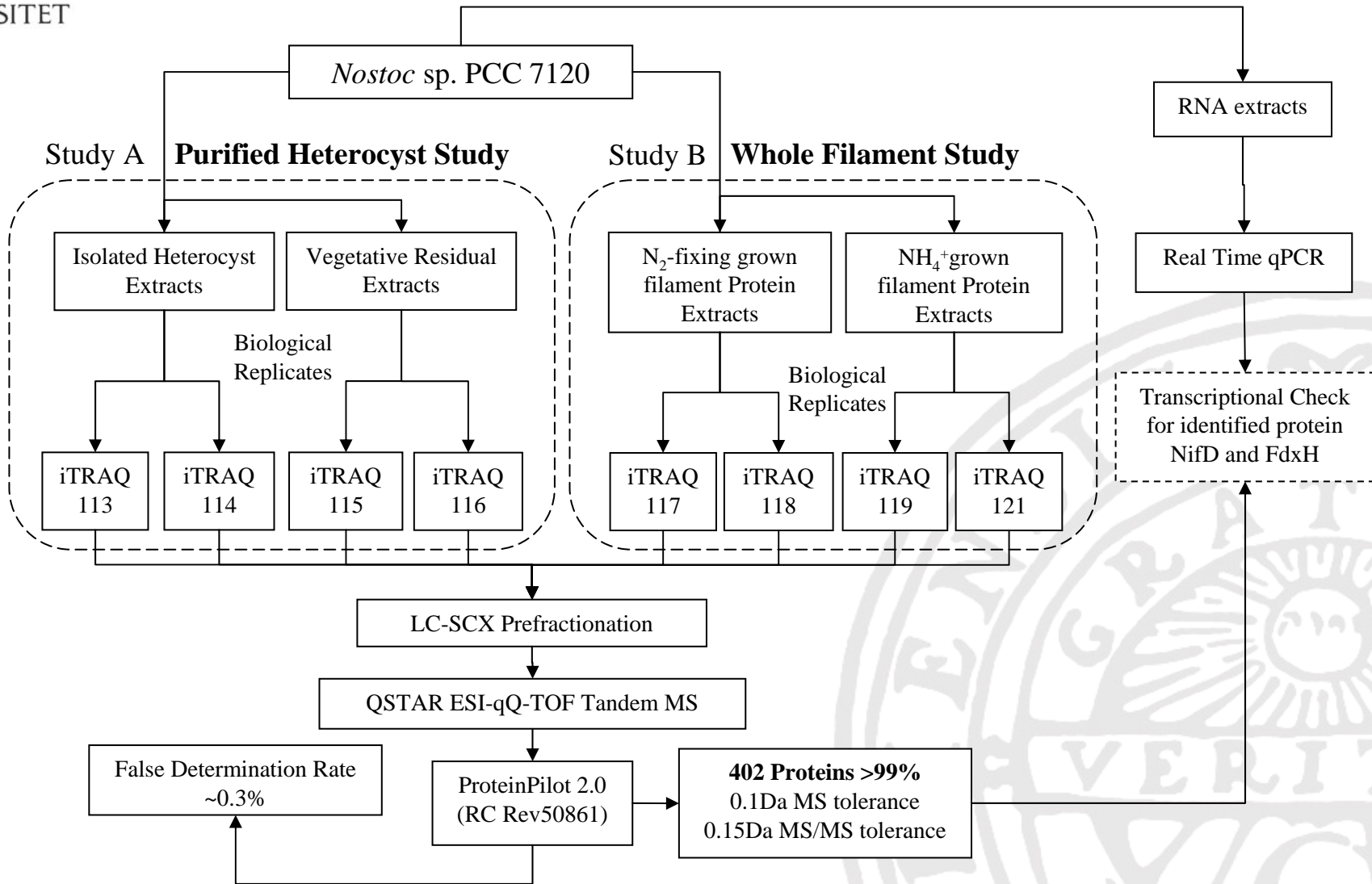


Stensjö et al 2007, JPR  
Ow et al 2008, JPR  
Ow et al 2008, JPR



B

# 8-plex iTRAQ experimental design





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## Phenotypes



### Protein Quantification



100µg total protein (each phenotype)

### Reduction & Cys blocking



### Trypsin Digestion (16 hrs)



### Digested Peptides



Label<sub>114</sub>

Label<sub>115</sub>

Label<sub>116</sub>

Label<sub>117</sub>

Pool Labels

2 x Fragment Scan (65-  
2000 m/z)

Precursor Scan (300-1800 m/z)

LC/MS Analysis – Tandem MS  
QSTAR XL Hybrid QTOF-MS

Buffer Substitution  
0.1% FA, 3% ACN

HPLC Ion Exchange  
Prefractionation  
(N fractions)

Primary Search Engine

Protein Pilot v2.0  
QSTAR DATA

99% Confidence Interval Filter

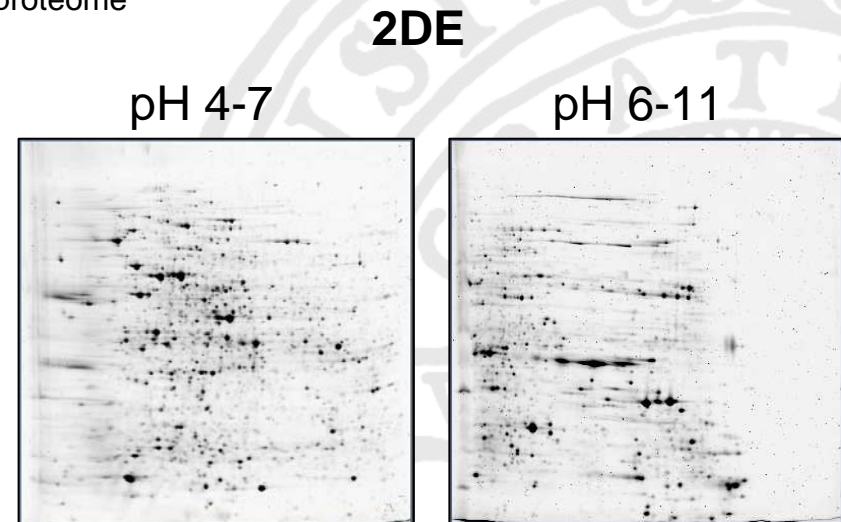
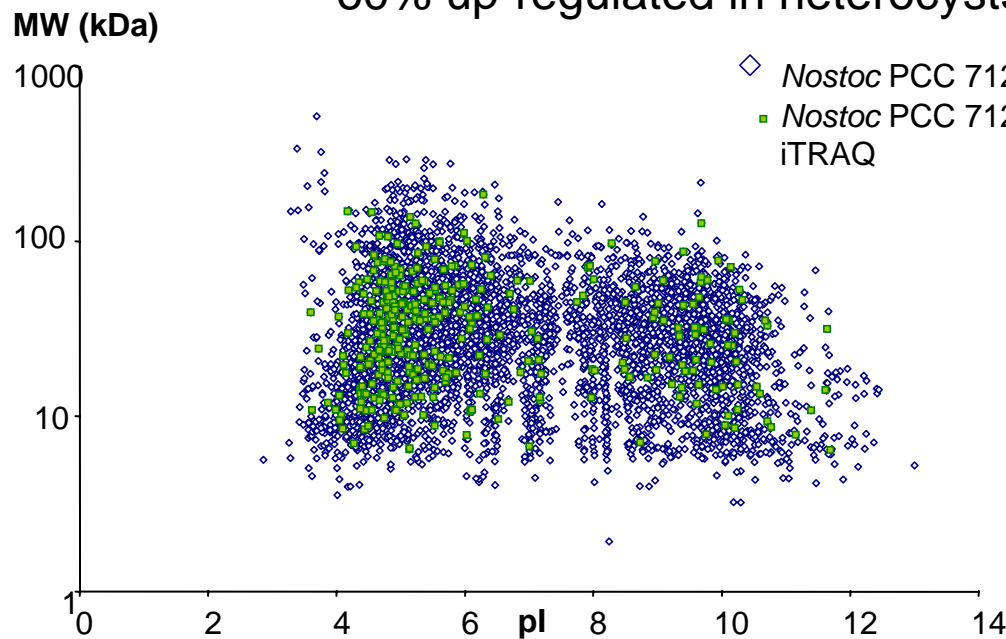
Abundance Threshold  
±1.6 fold

Identifications &  
Quantifications



# Summary of protein identification *Nostoc* sp PCC 7120

- *Nostoc* sp. PCC 7120: 486 (506) different proteins quantified
- *Nostoc punctiforme* 722 proteins quantified
- 30% hypothetical proteins
- 30% significantly ( $>1.8$  and  $<0.6$ ) up/down regulated filaments
- 60% up-regulated during  $N_2$  fixing versus  $NH_4^+$  grown
- 60% up-regulated in heterocysts versus vegetative cells

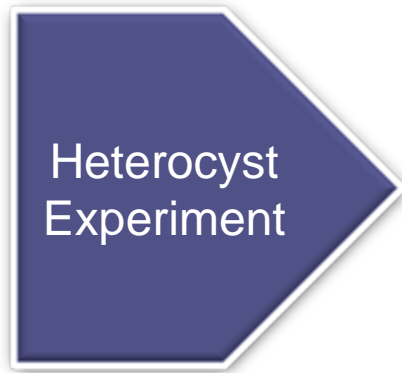






# Summary of results *Nostoc punctiforme*

## In Heterocyst



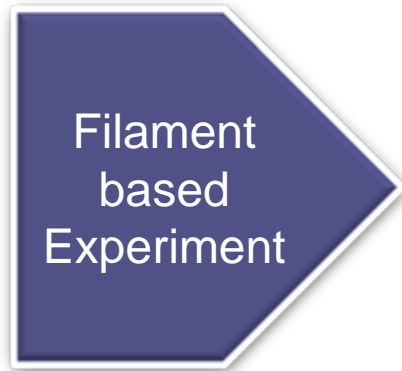
**516 Proteins**  
Quantified  
46566 spectra



117



121



**643 Proteins**  
Quantified  
77449 spectra

## In N<sub>2</sub> fixing Filaments

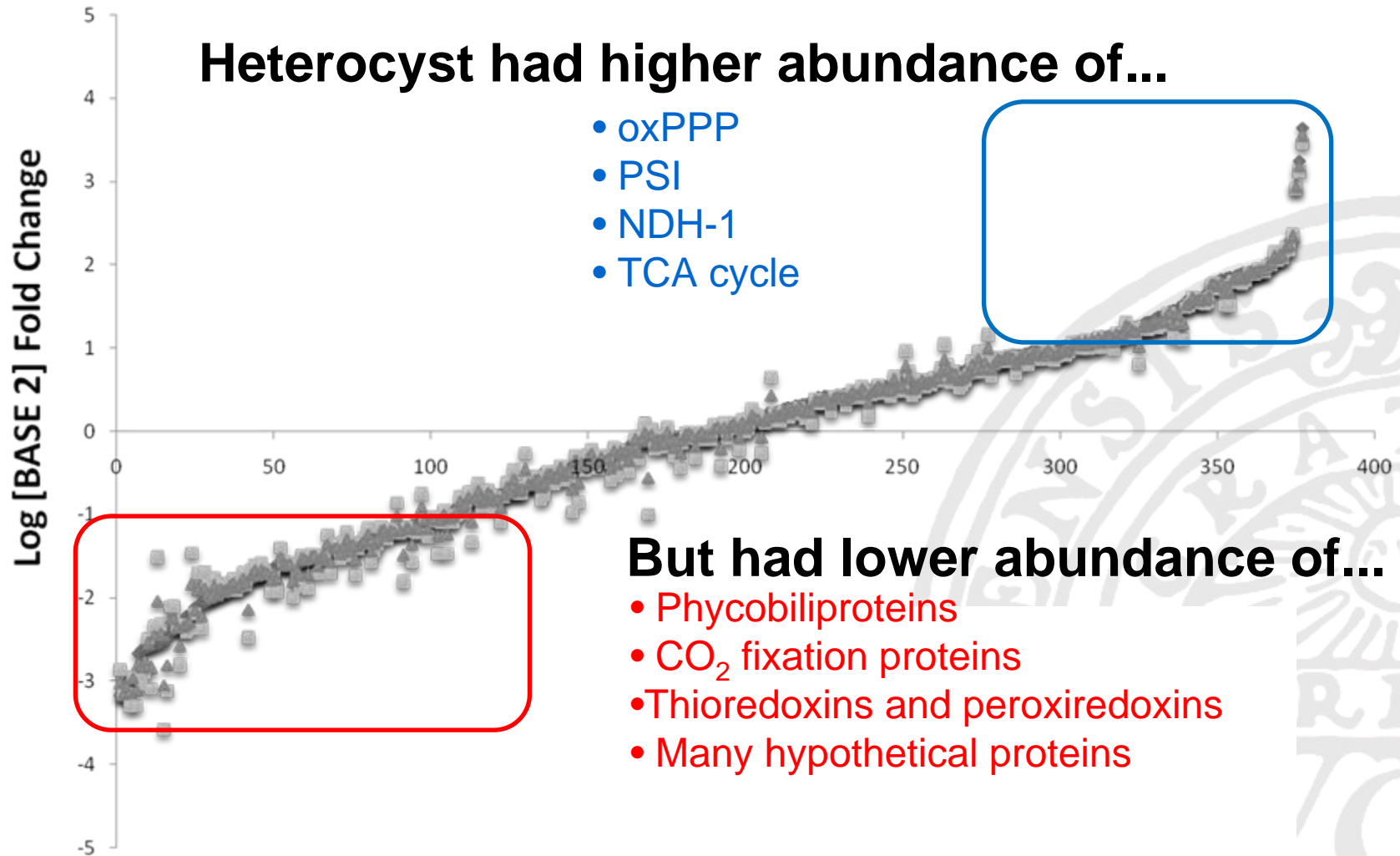


29



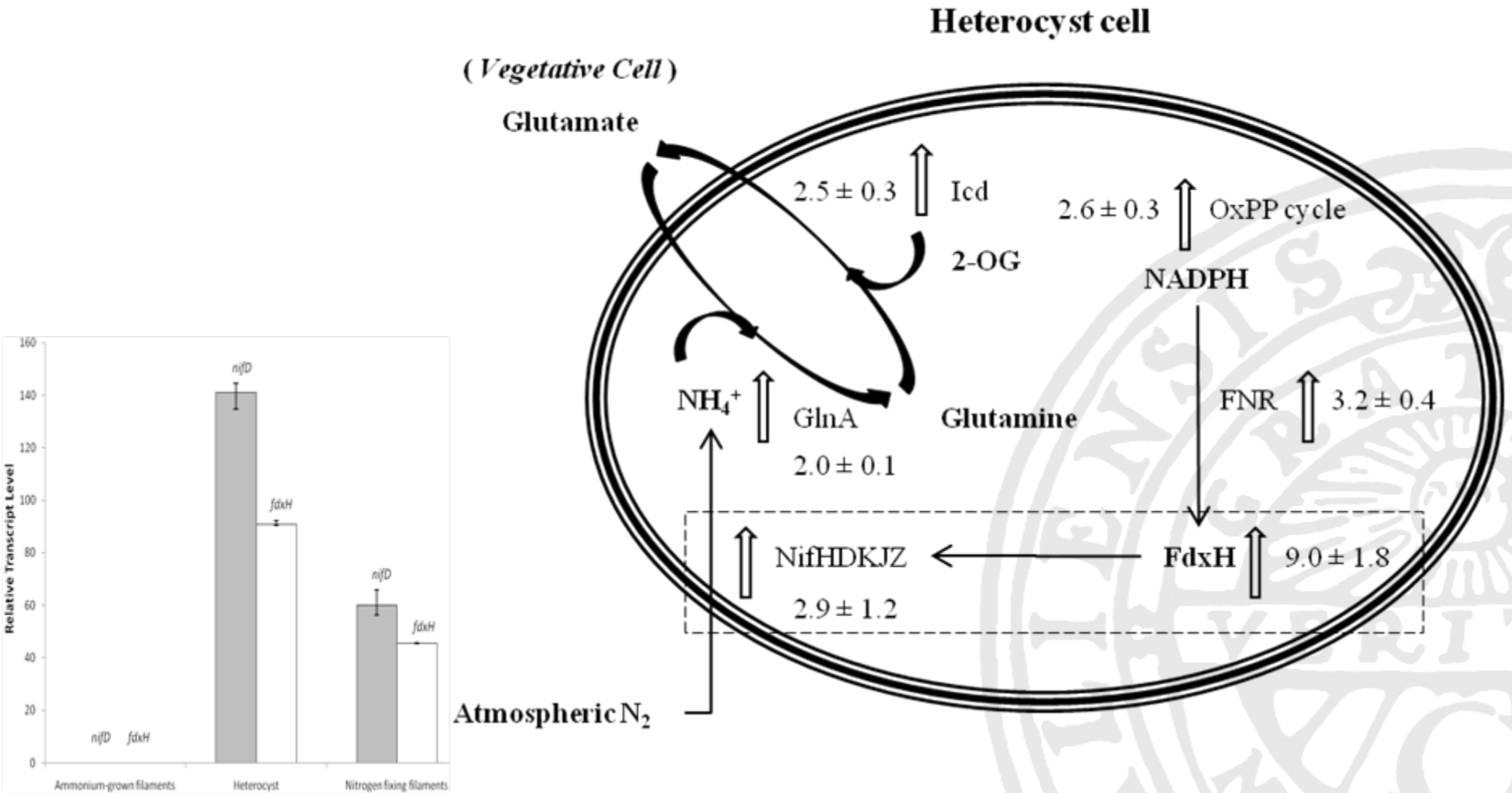
42

# Heterocysts versus N<sub>2</sub> fixing filaments in *Nostoc punctiforme*



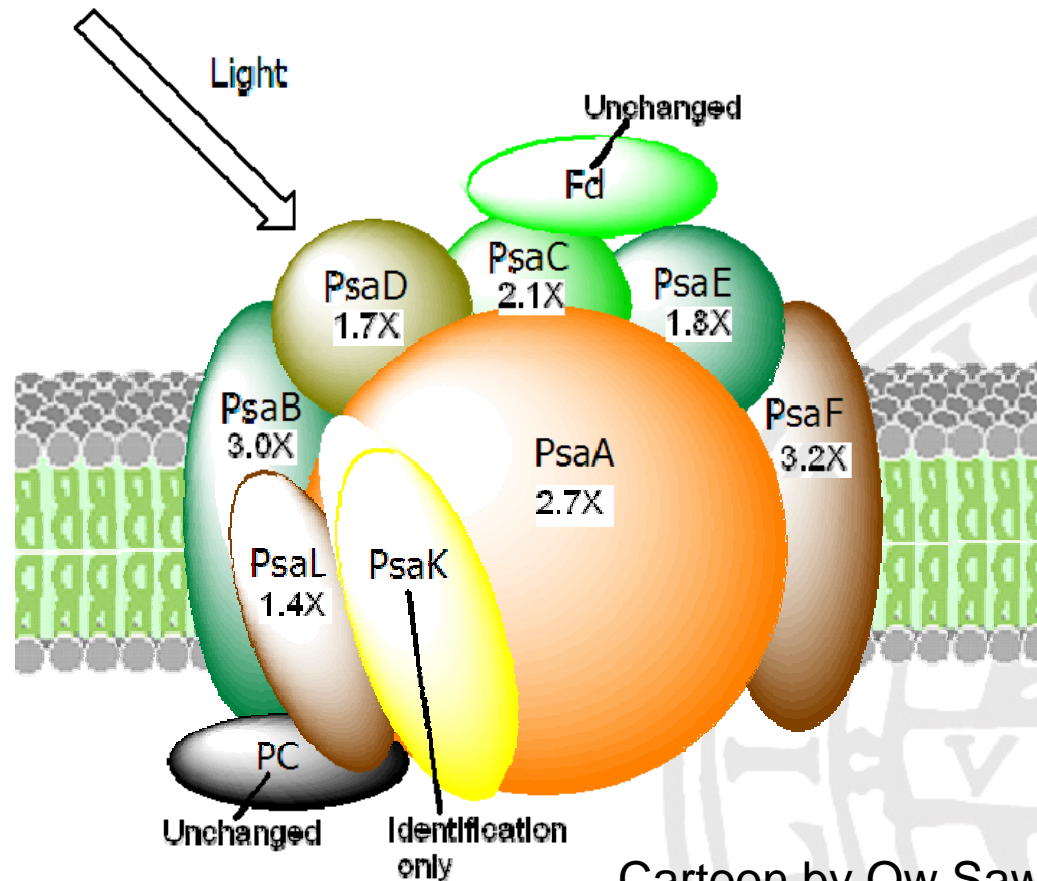


# Overview of nitrogen assimilation





# Photosystem I more highly abundant in heterocysts

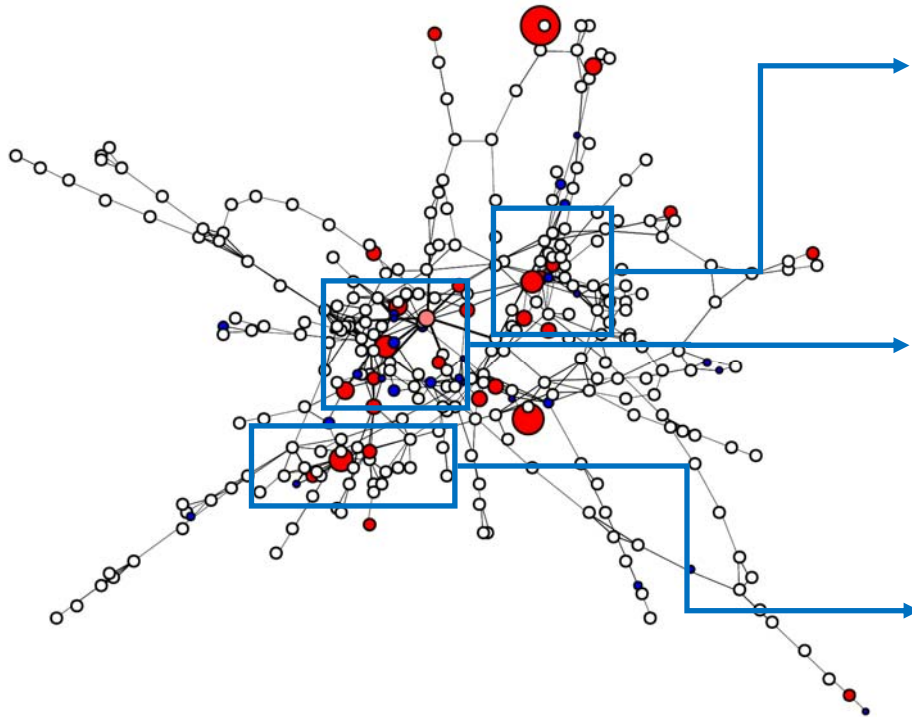


Cartoon by Ow Saw Yen 2006



# metabolic network reconstruction

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## Glycolysis/Gluconeogenesis

8 candidate proteins quantified  
Average 2.1 fold higher (Gluconeogenesis)  
Average 3.9 fold lower (Glycolysis)

## TCA Cycle and Nitrogen Assimilation

11 candidate proteins quantified  
Average 1.8 fold higher ( $N_2$  assimilation)  
Average 2.1 fold higher (TCA cycle)

## Pentose Phosphate Pathway

13 candidate proteins quantified  
Average 2.7 fold higher (oxPP cycle)  
Average 2.2 fold lower (non-ox cycle)

KEGG (<http://www.genome.jp/anonftp/>)

JGI ([http://genome.jgi-psf.org/draft\\_microbes/nospu/nospu.home.html](http://genome.jgi-psf.org/draft_microbes/nospu/nospu.home.html))

E.C. (enzyme code) numbers were obtained from JGI and inserted into KEGG map





# Conclusions

- 8-plex iTRAQ shotgun proteomics has demonstrated similar levels of reliability as a tool for large scale proteome relative abundance profiling.
- Analysis of purified heterocyst have shown predominant localisation of certain protein candidates amongst the cellular species.
- We need to quantify larger no. of proteins. For this pre-fractionation on protein level will be done.
- Generate hypothesis to be experimentally tested.

# Thanks!



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Martin Ekman**

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BioModularH<sub>2</sub>