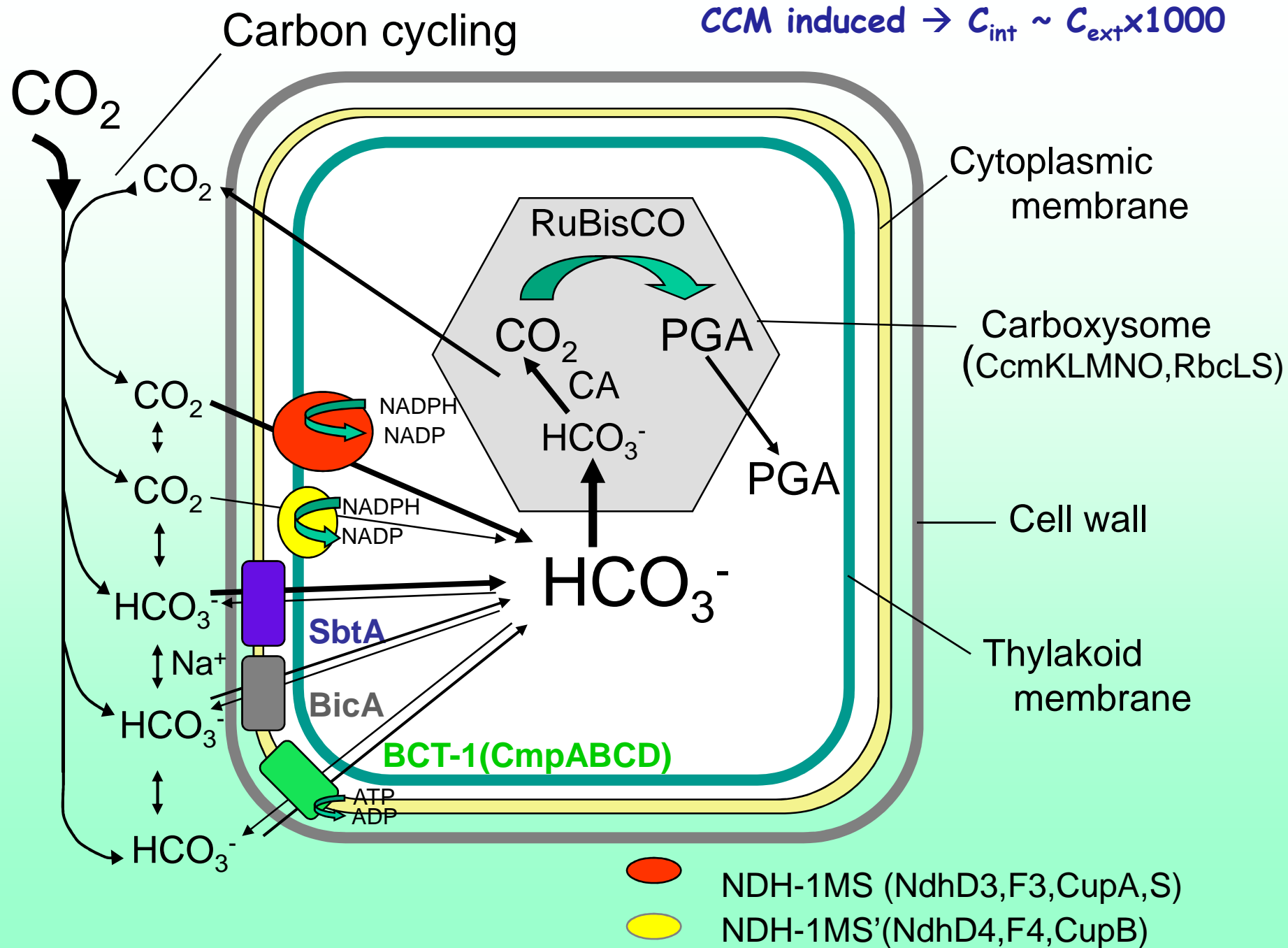# Proteomic approaches to study acclimation of *Synechocystis* cells to low CO_2 environment
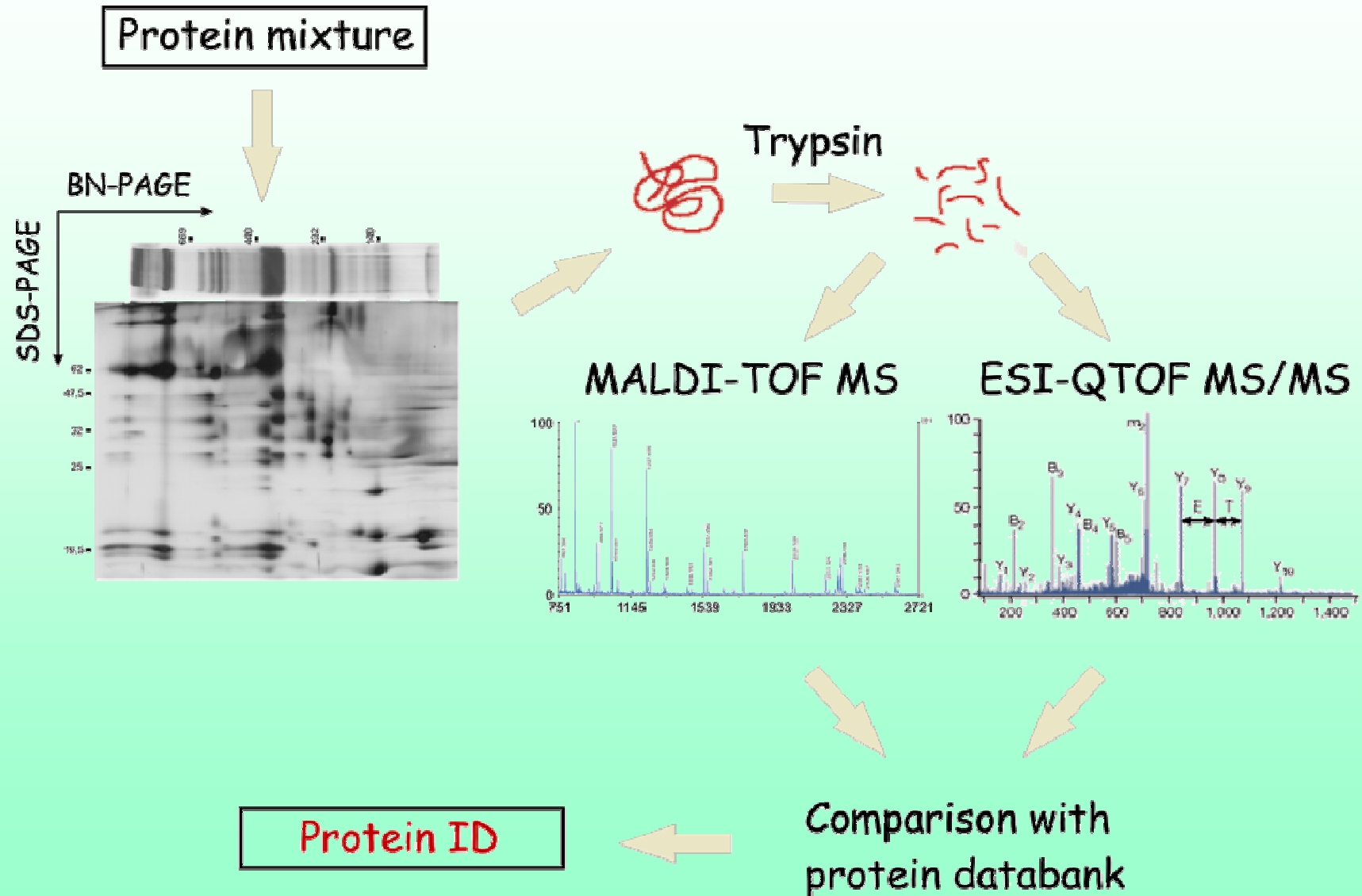
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Carbon cycling

CCM induced → $C_{int} \sim C_{ext} \times 1000$

CO_2

CO_2

CO_2

CO_2

HCO_3^-

Na$^+$

HCO_3^-

HCO_3^-

NADPH
NADP

NADPH
NADP

SbtA

BicA

BCT-1(CmpABCD)

ATP
ADP

RuBisCO

CO_2
CA
HCO_3^-

PGA

PGA

HCO_3^-

Cytoplasmic membrane

Carboxysome
(CcmKLMNO,RbcLS)

Cell wall

Thylakoid membrane

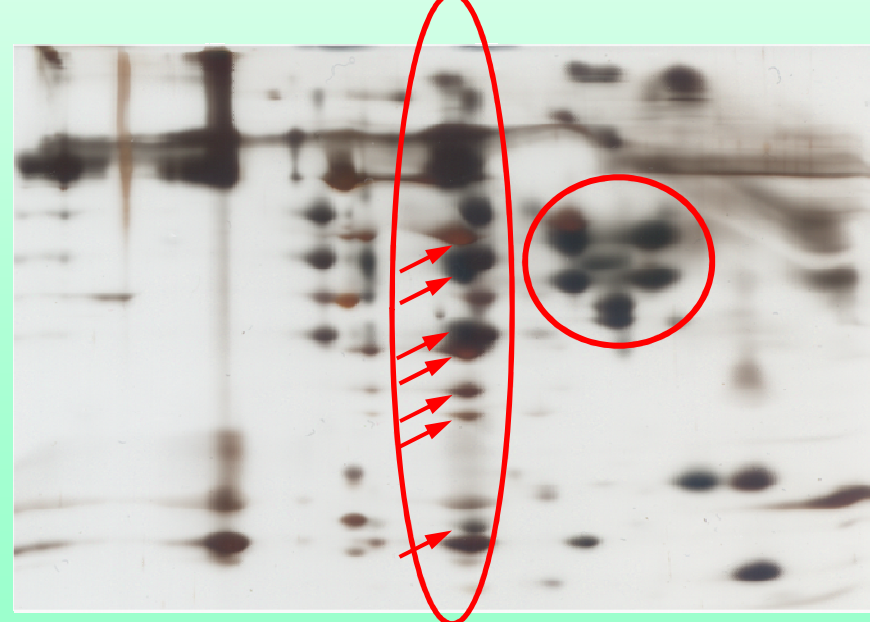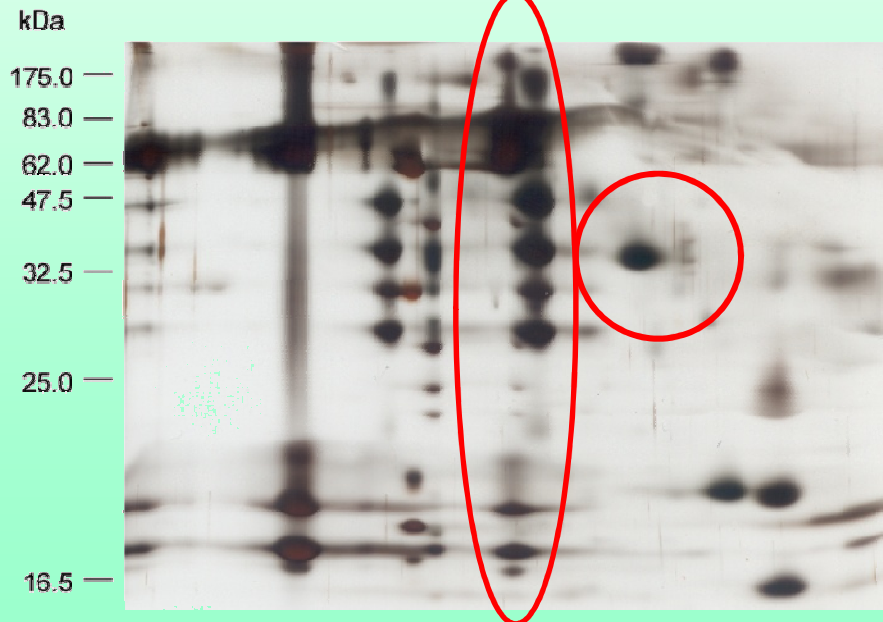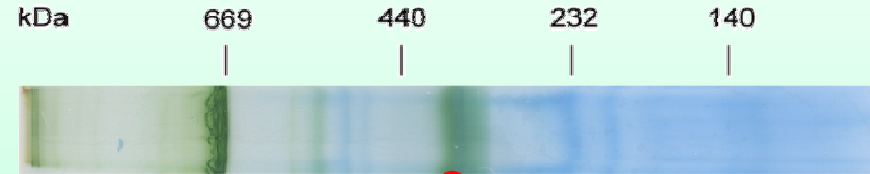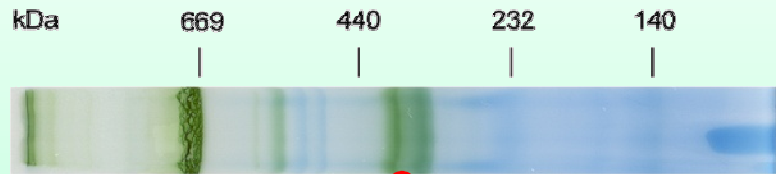NDH-1MS (NdhD3,F3,CupA,S)

NDH-1MS'(NdhD4,F4,CupB)

I. Proteomics of membrane protein complexes using Blue-Native/SDS PAGE

# Membrane proteome of *Synechocystis* Dynamically Responses to CO_2 Supply

NDH-1 genes in *Synechocystis*

| *Synechocystis*	MW	mem	*E. coli*	Predicted function
-	-	-	NuoE	1×[2Fe-2S]
-	-	-	NuoF	NADH-binding; FMN; 1×[4Fe-4S]
-	-	-	NuoG	1×[4Fe-4S]; 1 (2*)×[2Fe-2S]
NdhA	40.5	8	NuoH	Ubiquinone-binding
NdhB	55.4	14	NuoN	
NdhC	13.7	3	NuoA	
NdhD1-D6	52.1-61.0	12	NuoM	
NdhE	11.2	3	NuoK	
NdhF1,F3,F4	66.6-74.4	14	NuoL	
NdhG	20.6	5	NuoJ	
NdhH	45.4	0	NuoD	
NdhI	22.2	0	NuoI	2×[4Fe-4S]
NdhJ	18.6	0	NuoC	
NdhK	27.3	0	NuoB	1×[4Fe-4S]
NdhL	9.3	2	-	
NdhM	14.1	0	-	
NdhN	17.6	0	-	
NdhO	8.3	0	-	
CupA	50.0	0	-	Carbon uptake (inducible)
CupS	14.1	0	-	Carbon uptake (inducible)
CupB	51.2	0	-	Carbon uptake (constitutive)
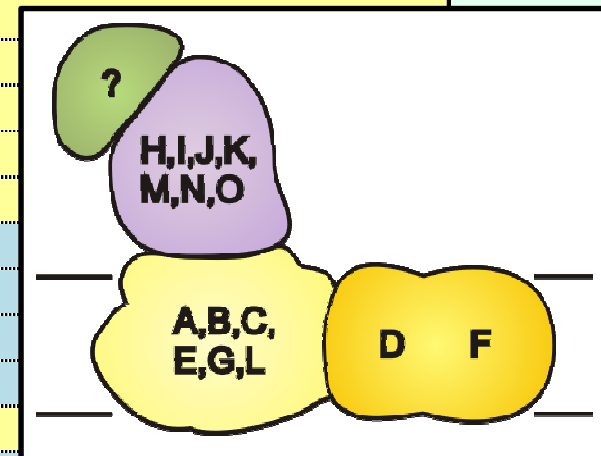
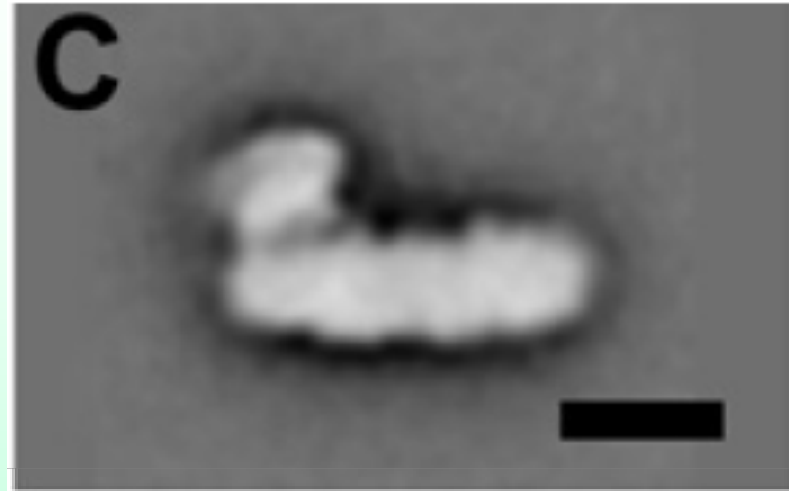# NDH-1L, -1M and -1S complexes of *Synechocystis* 6803
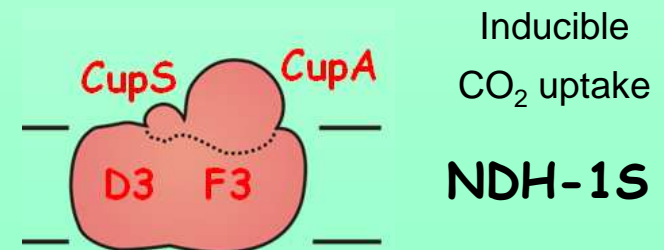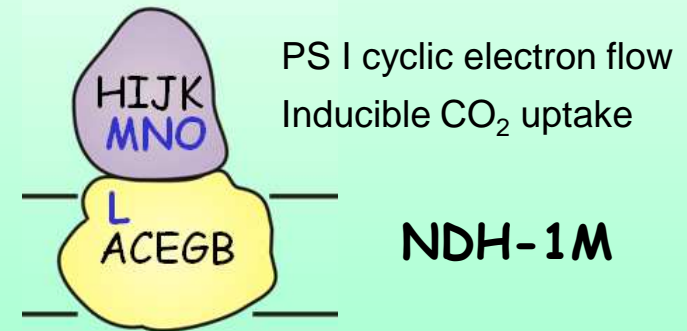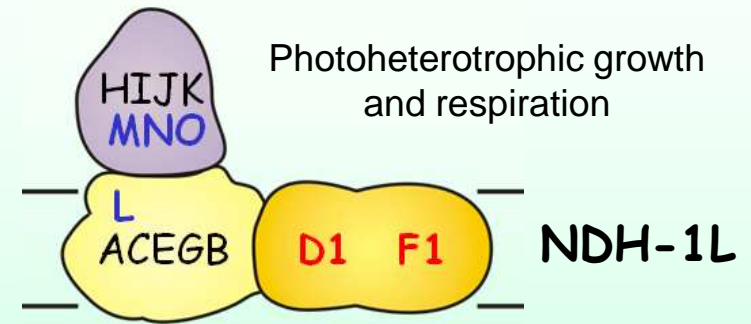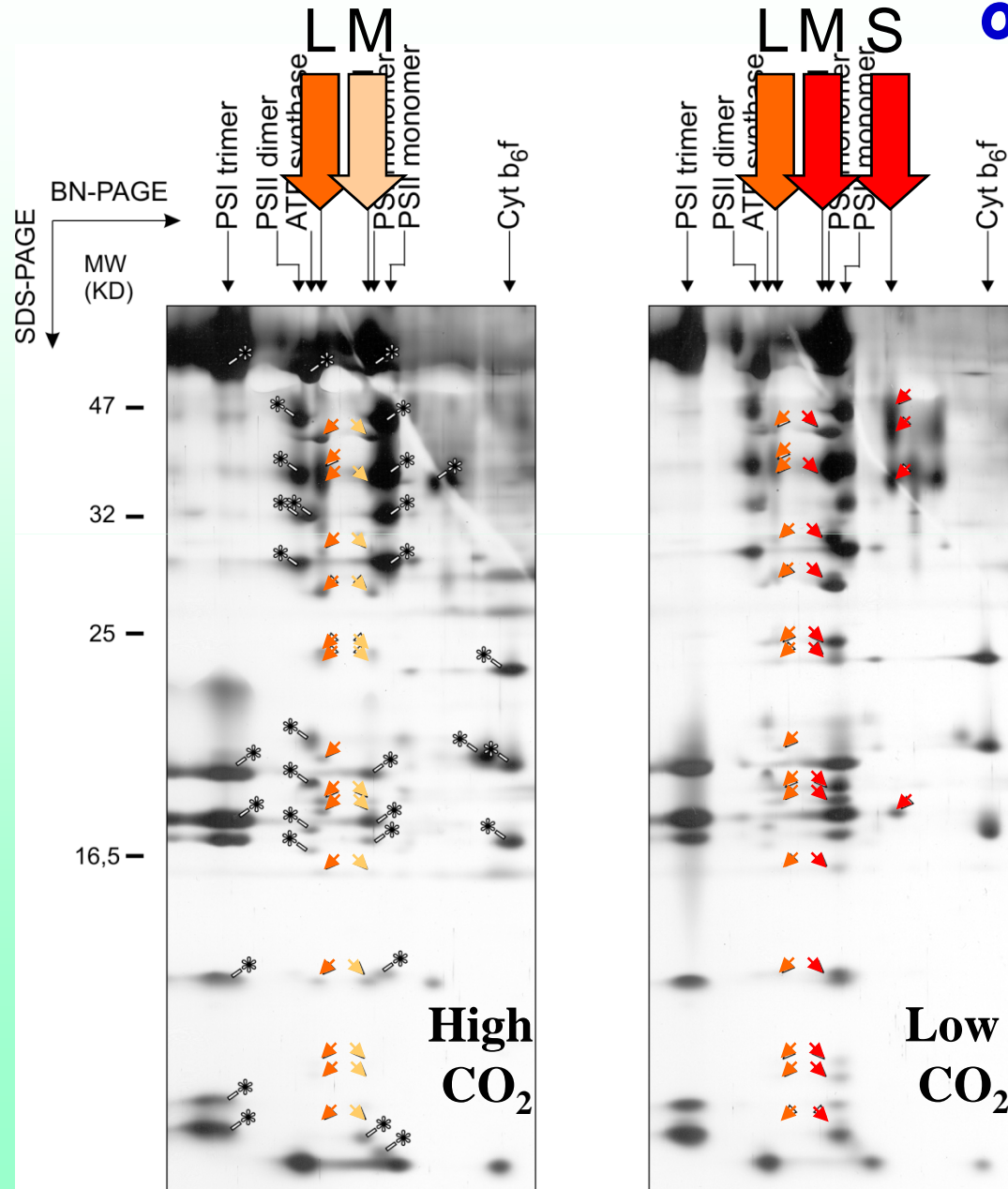
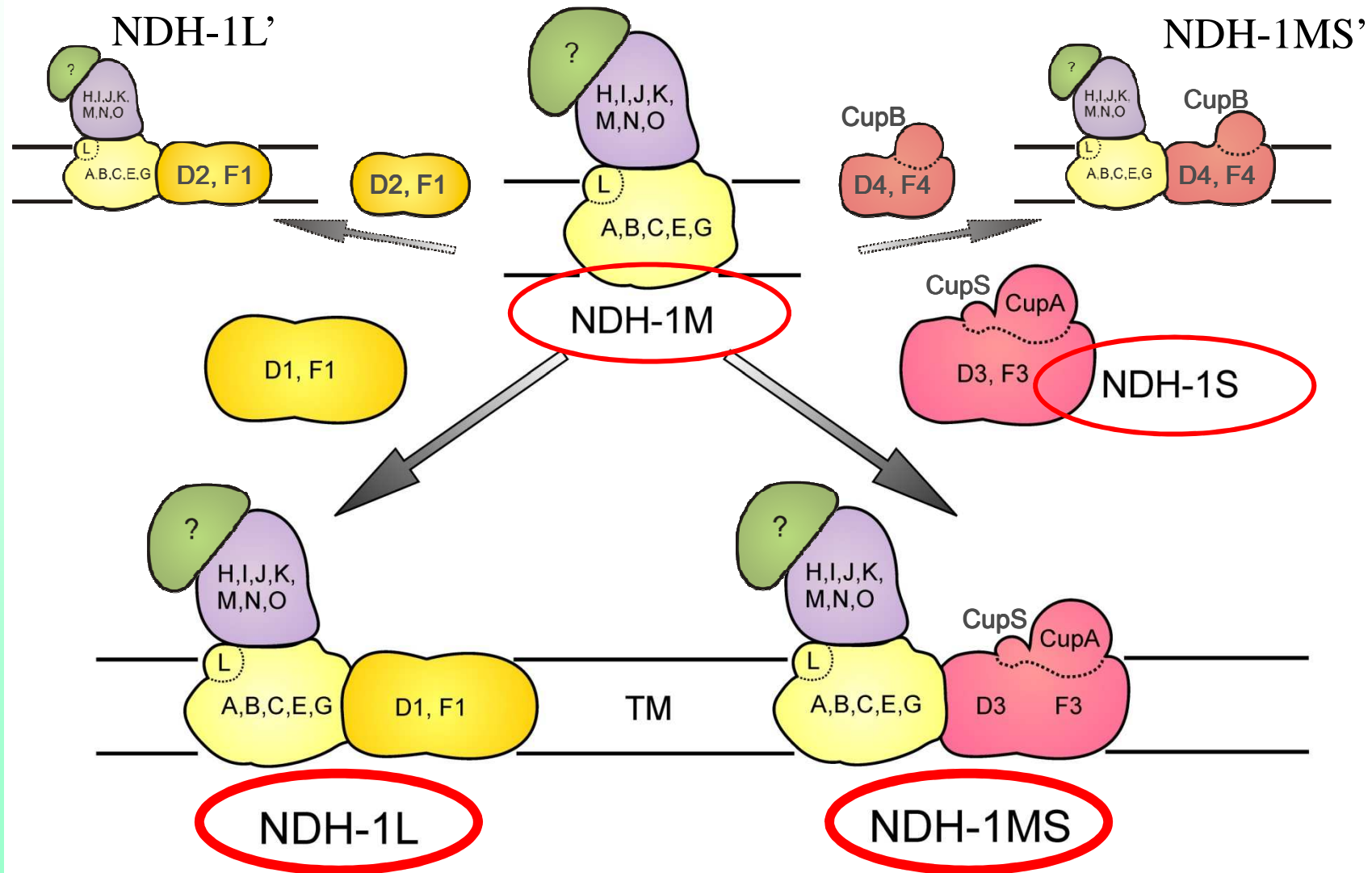# The NDH-1MS supercomplex in *Thermosynechococcus elongatus*
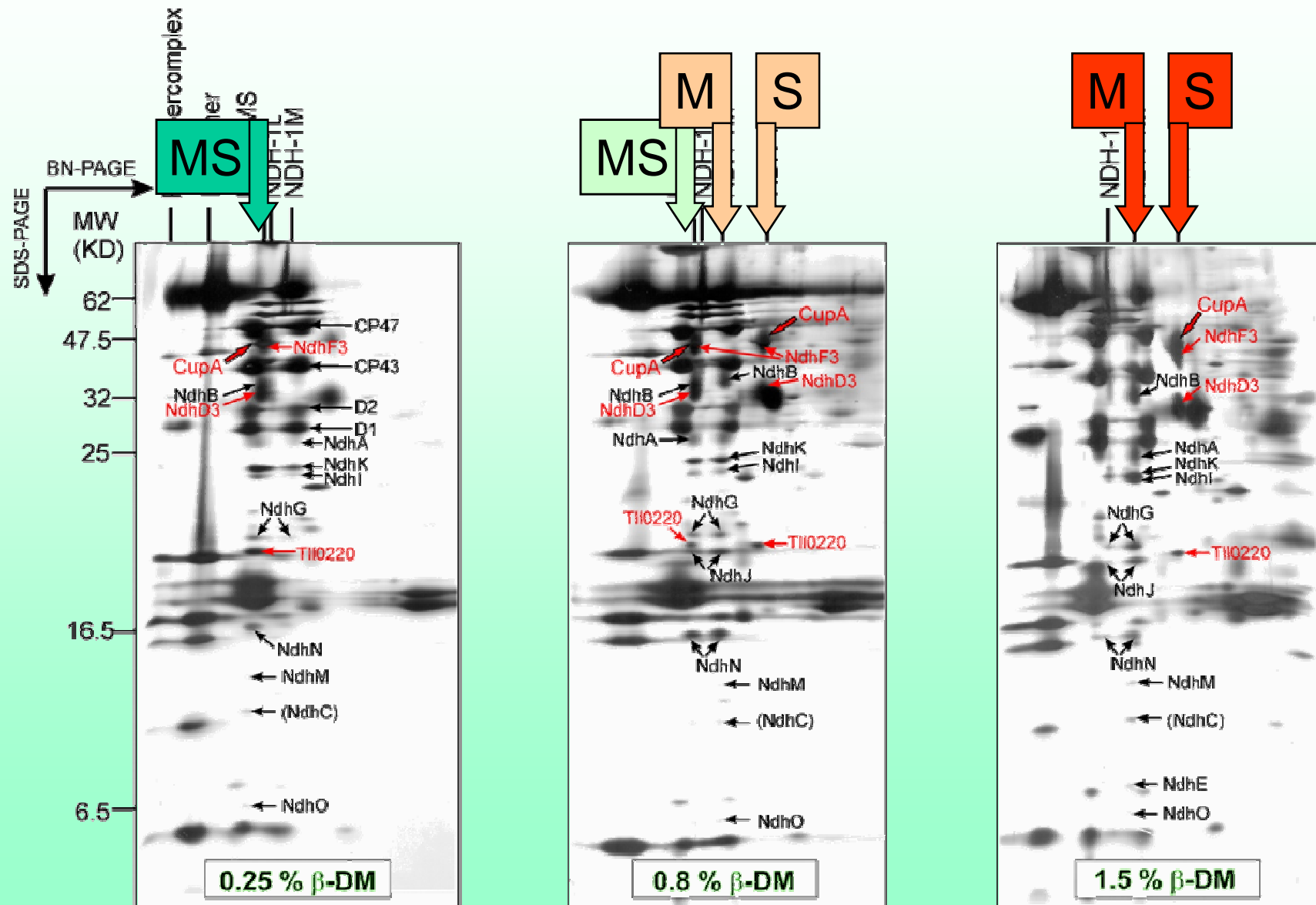
Ndh-1MS
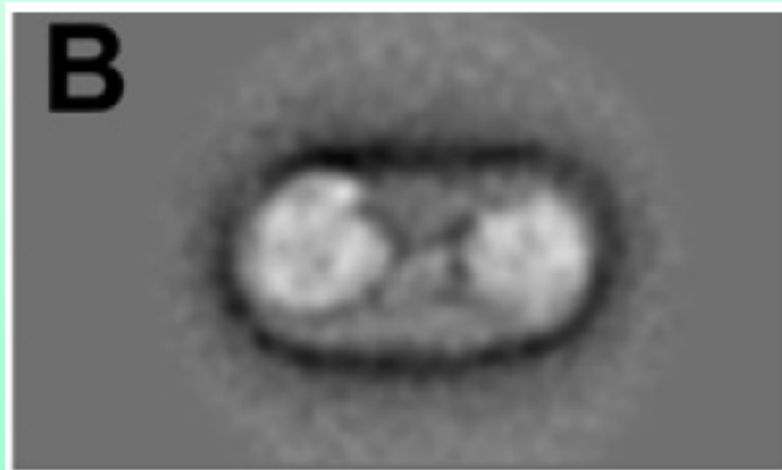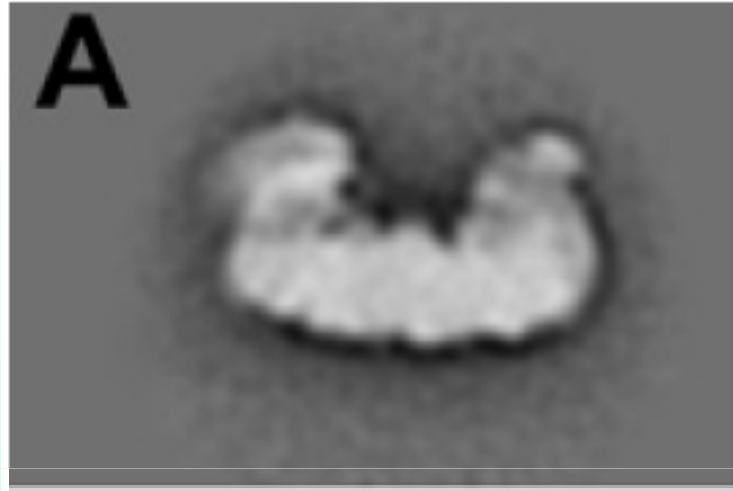
Ndh-1L

Single particle analysis of NDH-1 from *T. elongatus*
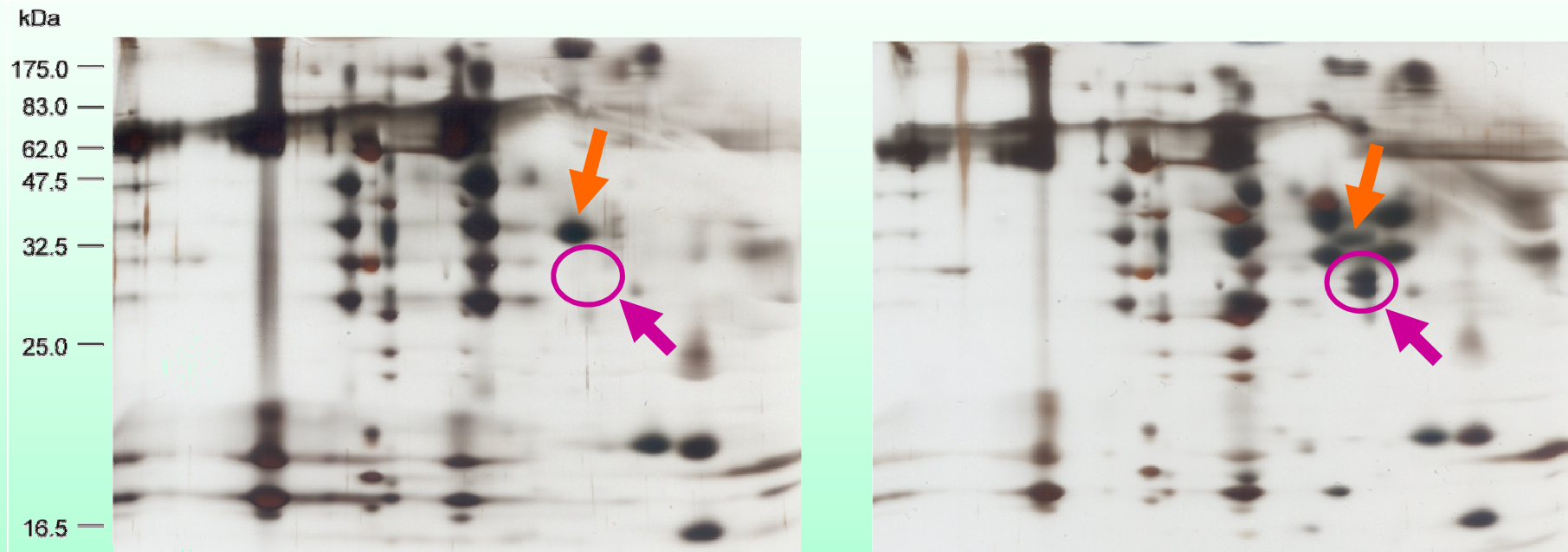
Folea et al., FEBS Lett 2008

Multiple NDH-1 complexes in *Synechocystis*

NDH-1L'

NDH-1MS'

CupB

CupS CupA

NDH-1M

NDH-1S

D1, F1

TM

NDH-1L

NDH-1MS

Respiration/Cyclic electron flow

Inducible CO$_2$ uptake

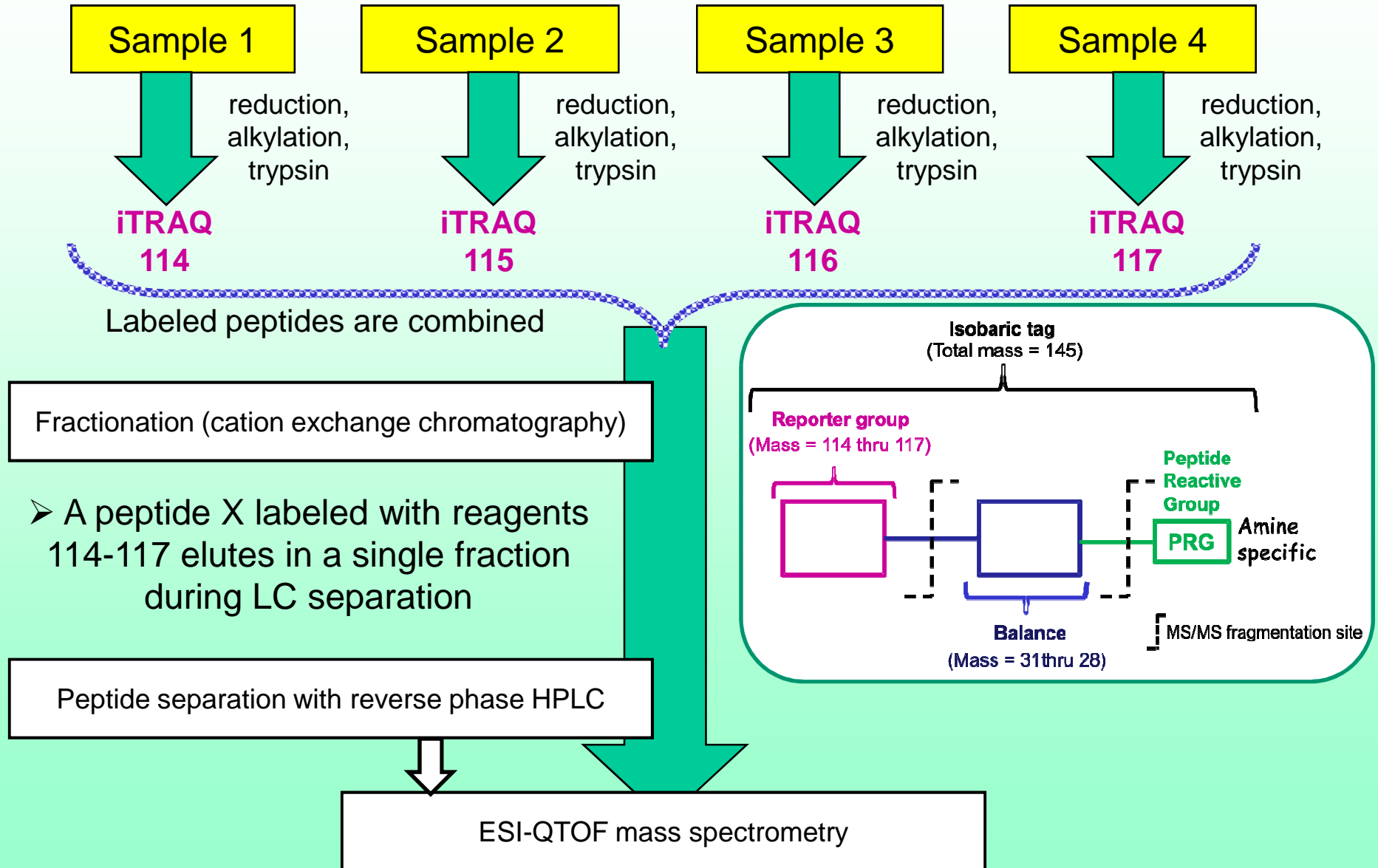# Sodium-bicarbonate transporter (Slr1512, SbtA) is induced at low CO_2
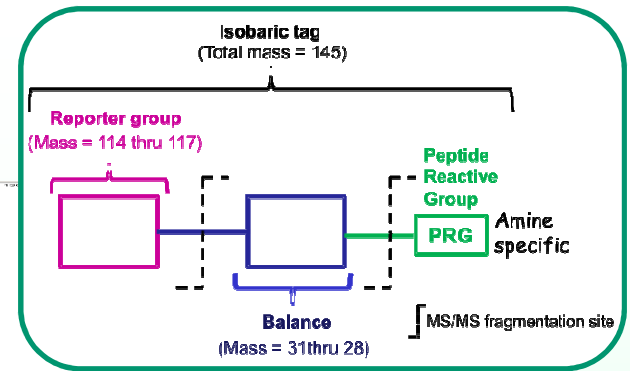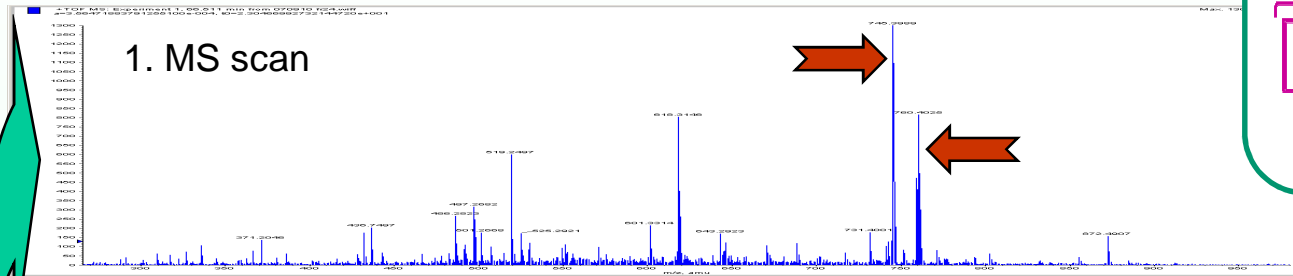

High CO_2

Low CO_2

# Ammonium/methylammonium permease (Sll0108, Amt1) is repressed at low CO_2

II. iTRAQ quantification

In MS scan analysis, a peptide X labeled with reagents 114-117 is detected as one, the same peptide X

Isobaric tag
(Total mass = 145)

Reporter group
(Mass = 114 thru 117)

Peptide
Reactive
Group

PRG   Amine
specific

Balance
(Mass = 31thru 28)

MS/MS fragmentation site

1. MS scan

During MS/MS fragmentation, generated reporter group ions 114-117 appear as peaks in the low-mass region between m/z 113 and m/z 119

2. MS/MS of the 1st most intensive ion

3. MS/MS of the 2nd most intensive ion
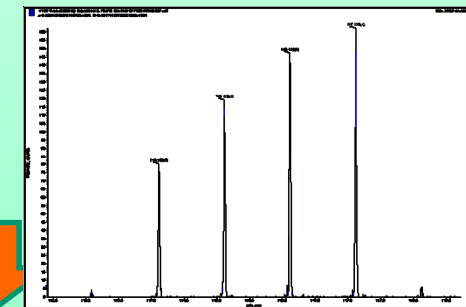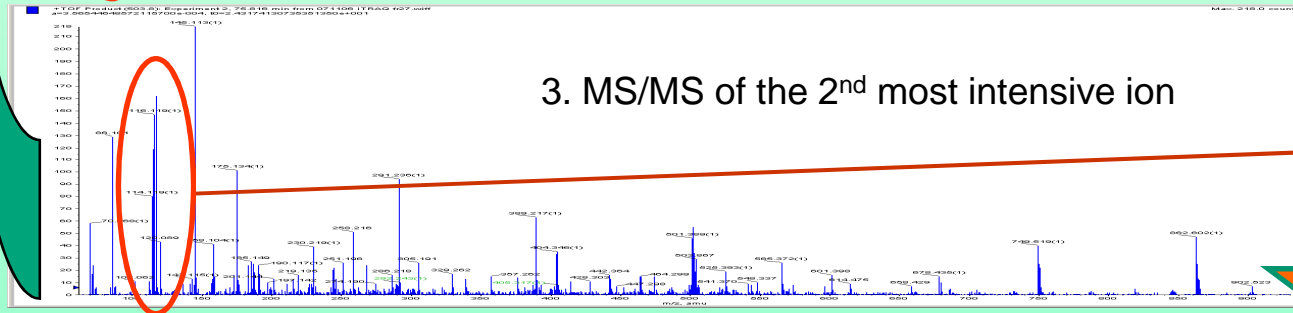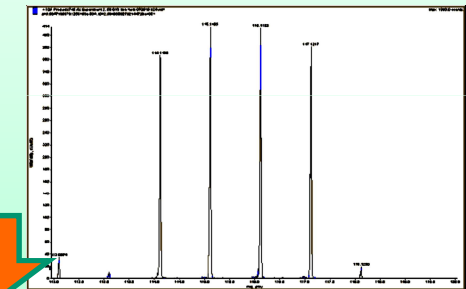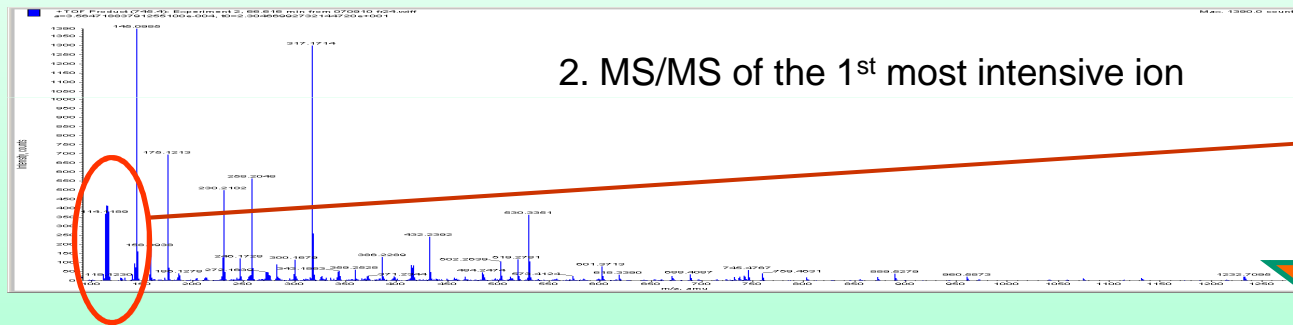
The ratio of one peak area to another represents the relative amount of a given peptide in each of the corresponding sample digests

Major challenges in iTRAQ quantification

➢ Complexity (many peptides in various forms for a protein)
➢ Diversity of properties (hydrophobicity, solubility in solvents, etc)
➢ Broad range on protein abundancy

1. Highly abundant peptides (from the pigment proteins belonging to the phycobilisome family, and Rubisco) reduce the chances to identify peptides belonging to low abundant proteins
2. Multiple injection replicates are necessary
   → Due to autoselection in MS/MS analysis, this approach significantly improves the protein coverage and enhances the number of identified peptides
   → Multiple MS/MS scans of the same peptide improve the consistency of quantification
3. The technique is biased towards hydrophilic proteins