An algal Baeyer-Villiger monooxygenase is produced as an active enzyme in the cyanobacterium *Synechocystis* sp. PCC6803

Elisabetta Bergantino

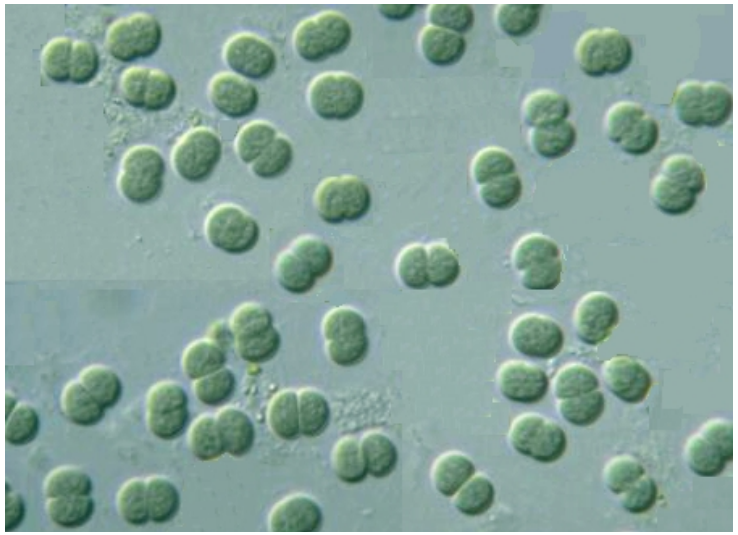
Department of Biology
University of Padova
ITALY

Complesso Interdipartimentale A. Vallisner

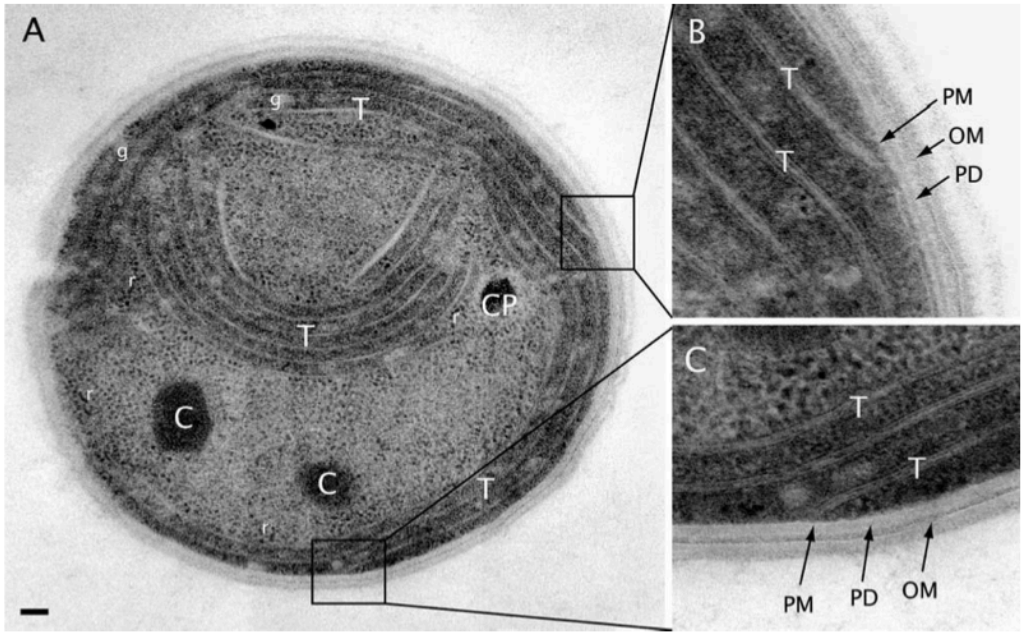
Università degli Studi di Padova

Import and Export of Small Molecules for Whole Cell Biocatalysis
September 12th and 13th 2017, Edinburgh

Synechocystis PCC 6803



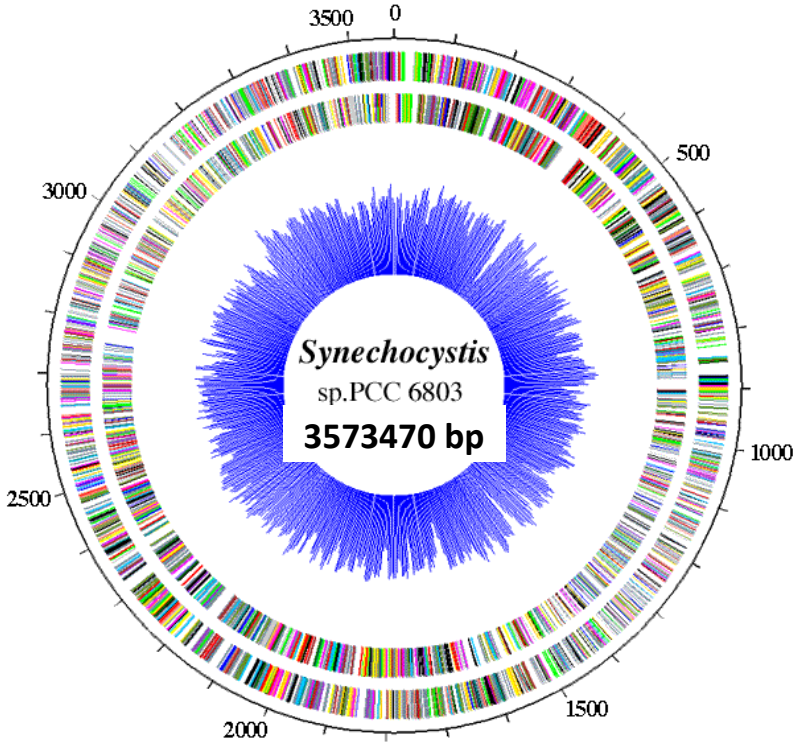
- Able to grow **both photoautotrophycally** and heterotrophically providing an organic carbon source (glucose)
- Spontaneously transformable, DNA integrates in the genome by homologous recombination ➔ **model organism**



Spherical-shape cell
average Φ 1,5-2 mm

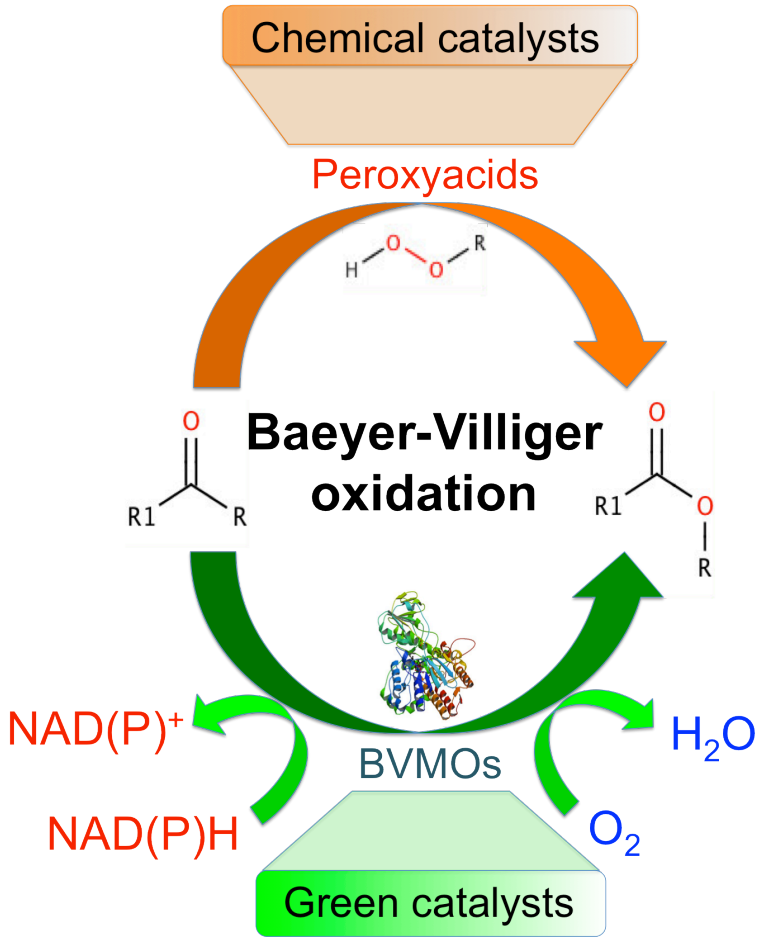
Synechocystis PCC 6803 : the 3573470 bp genome

- Multiple copies of the genome (20 on average; 4-60 copies estimated, depending growth phase and physical and chemical environmental parameters) (Zerulla & al., 2016)
- 87% of the genome is protein coding
- 64 % of the Transcriptional Start Sites code for ncRNAs (Mitsche & al., 2011)
- ➔Heteroplasmic clones are frequently obtained and analysed

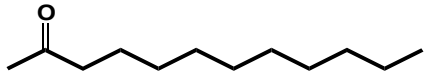
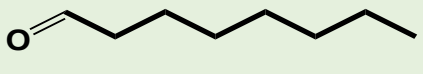
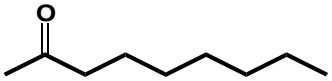
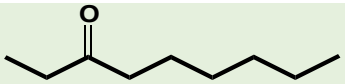


- Lack of handy plasmids, currently only one broad-host-range shuttle vector, RSF1010
- ➔Episomal vectors need helper plasmids for coniugation, are genetically unstable, require high selective pressure

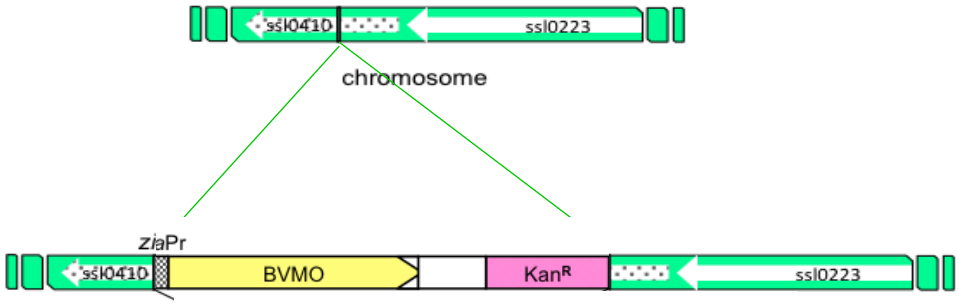
Baeyer-Villiger monooxygenases in biocatalysis



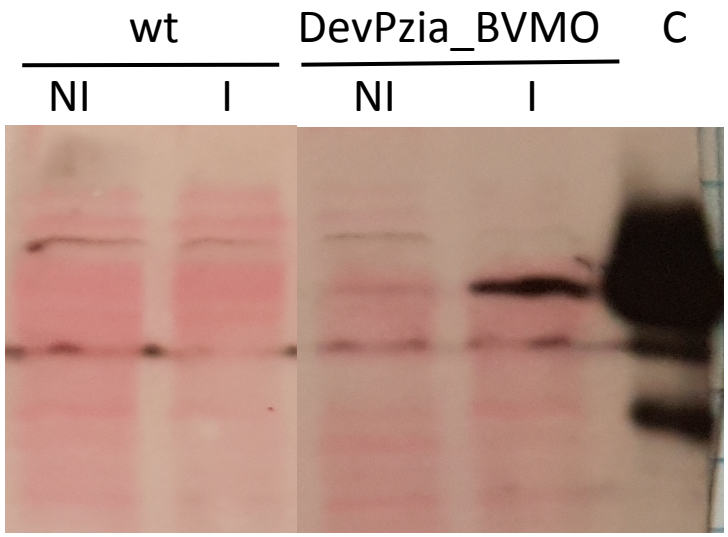
- Valuable alternative to peroxyacids, with a great potential in biocatalysts
- Flavoenzymes: bound FAD cofactor
- Lead to enantiomerically pure products
- We had previously characterized a BVMO from the red alga *Cyanidioschyzon merolae* (*Cm*BVMO) (Beneventi & al., 2013)

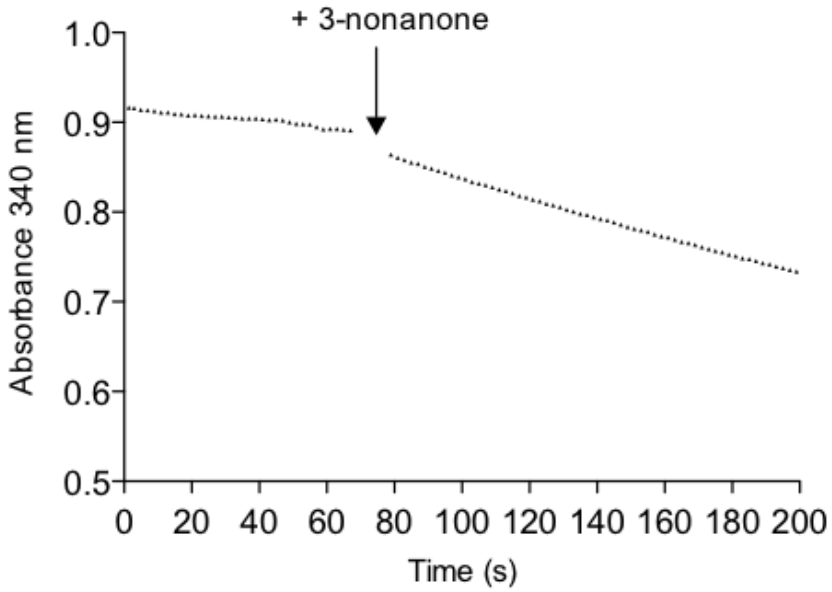
	Kcat/Km[s-1 mM]
	**90,8 ± 0.018**
	25,3 ± 0.043
	22,7 ± 0.031
	70 ± 0.006

BVMO from *Cyanidioschyzon merolae* expressed in *Synechocystis*

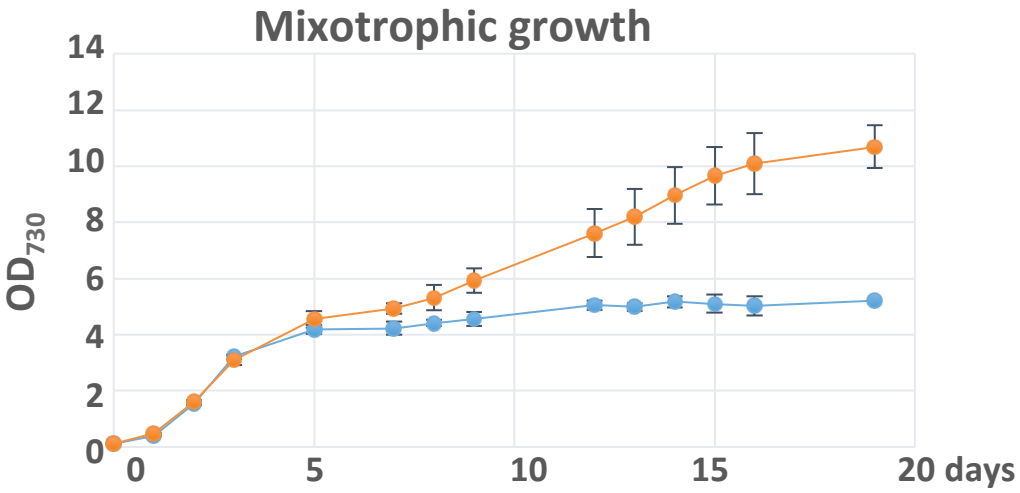


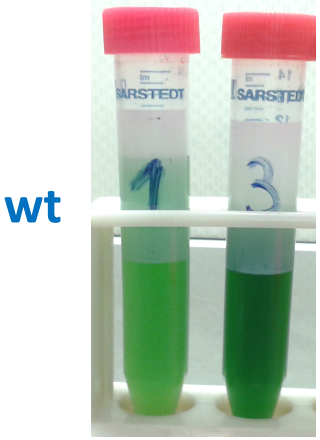
DevPzia_BVMO chromosome

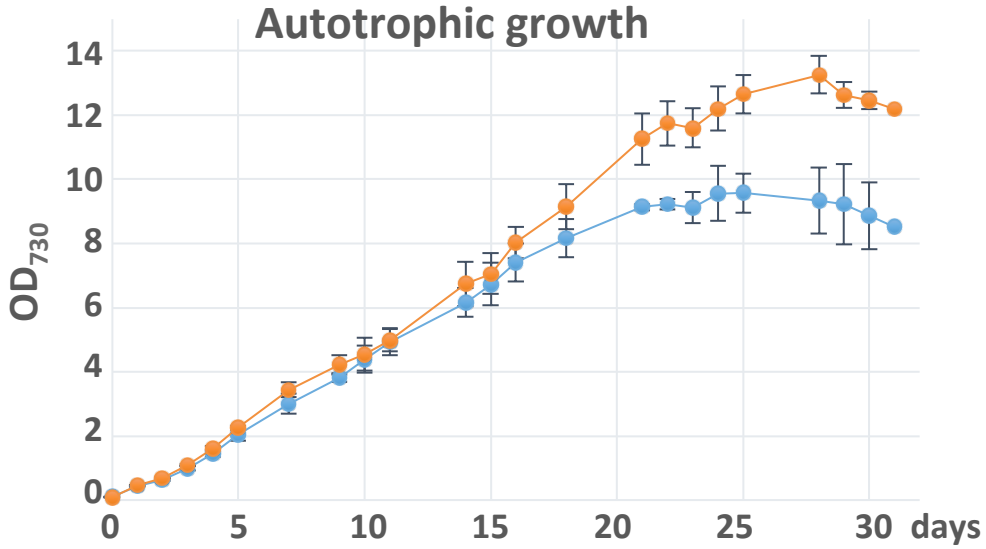


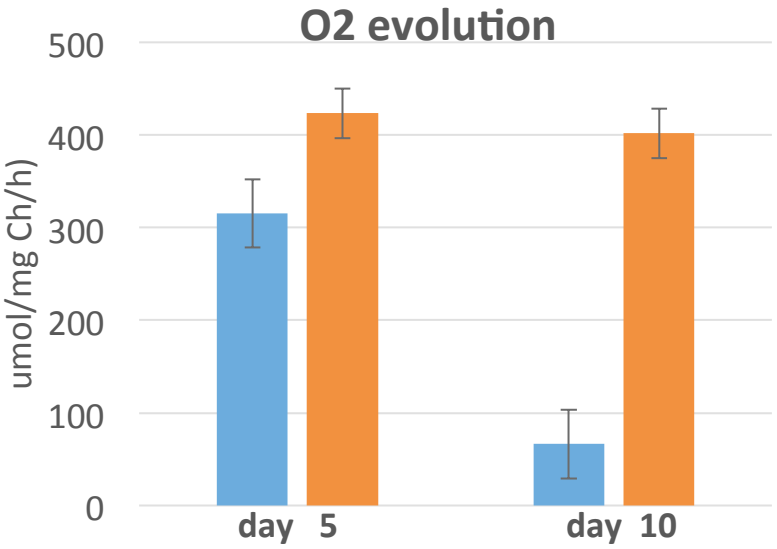


Phenotype of the strain expressing the BVMO

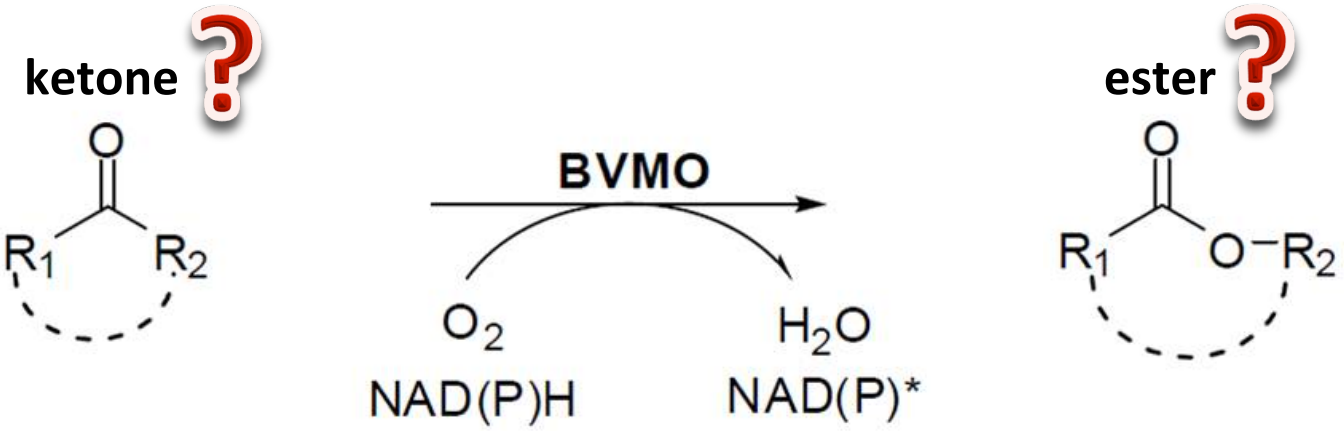








How does the BVMO increase cyanobacterial growth?

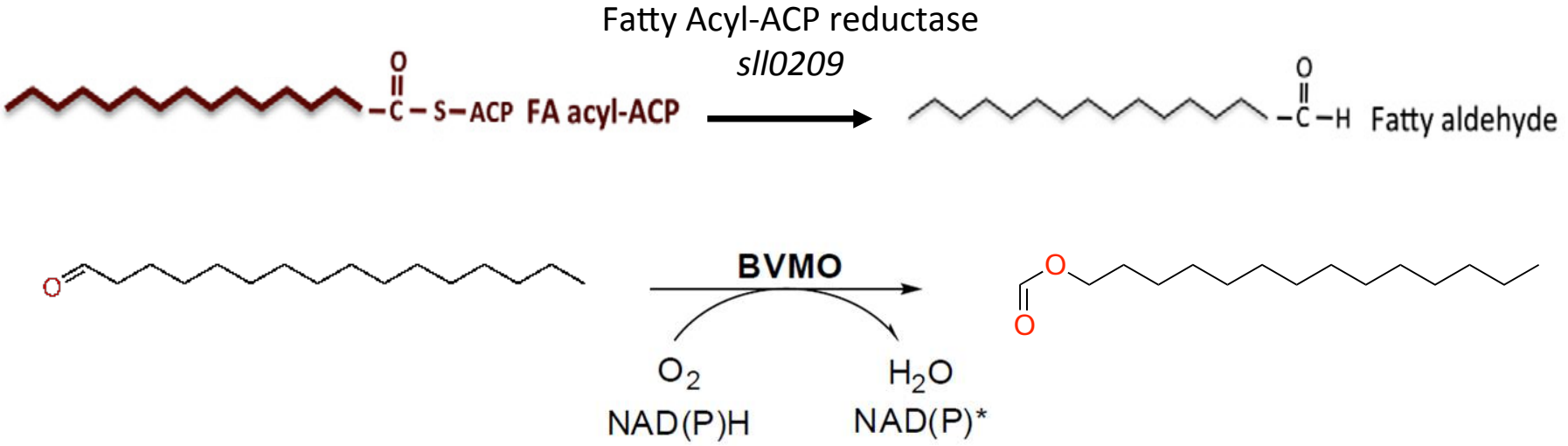


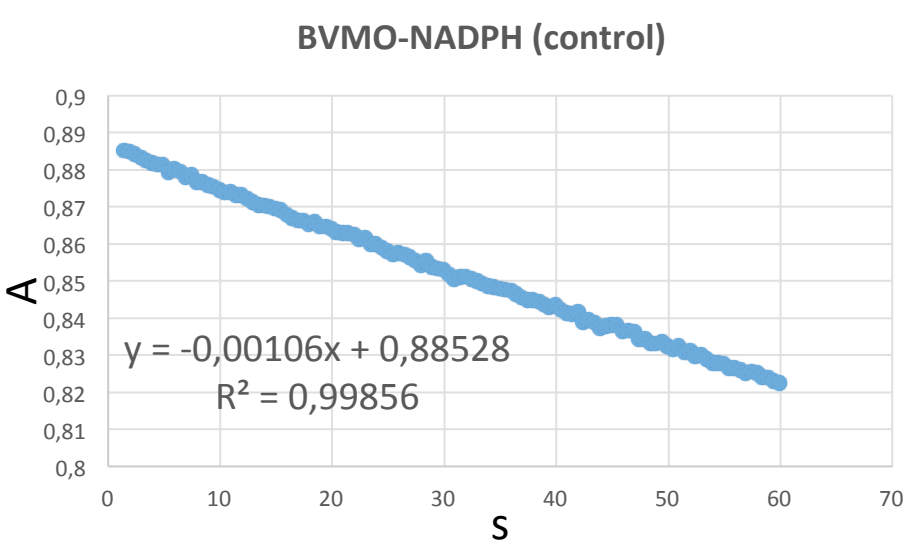
*Cm*BVMO is able to act on an unknown substrate accumulating into the cells

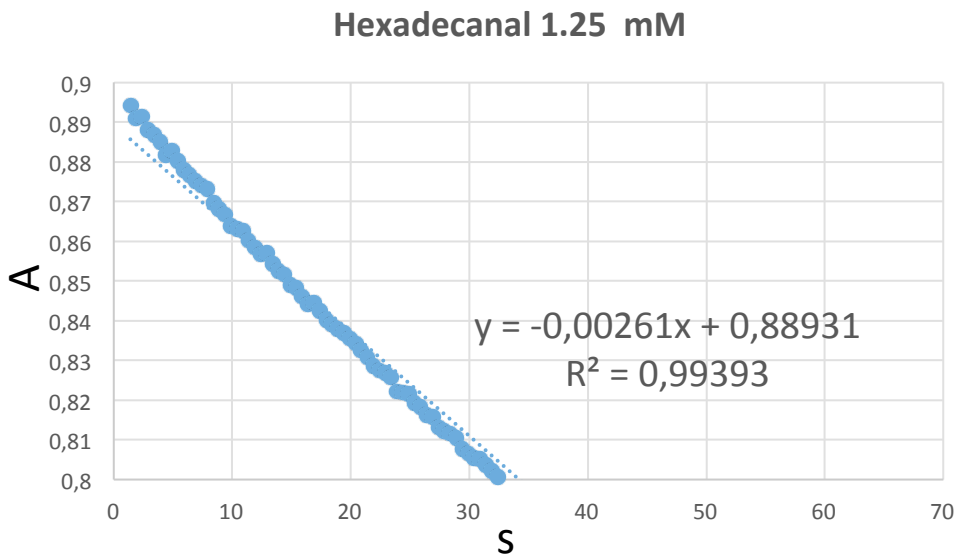
release of a molecule reusable as energy source

increased consumption of NADPH

A possible substrate for *Cm*BVMO in *Synechocystis*







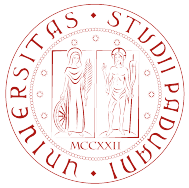
Conclusions & outlooks

- The BVMO Baeyer-Villiger monooxygenase from the red alga *Cyanidioschyzon merolae* is produced as an active enzyme in the *Synechocystis*
- The heterologous enzyme somehow increases photosynthetic efficiency and biomass accumulation

- In progress: whole-cell transformations using identified substrates of *Cm*BVMO ➔ import and export of small molecules across ?
- Disruption of the gene coding for the fatty acyl-ACP reductase in both the wild type and the BVMO strain ➔ growth profiles of these two mutants will be compared.

Thanks to

Mattia Niero
PhD



Università degli Studi di Padova

university of groningen

Marco Fraaije
Molecular Enzymology group, Groningen

iBPC

université PARIS DIDEROT PARIS 7

Francesca Zito
Bruno Miroux
Laboratory of Physical and Chemical Biology of Membrane Proteins, Paris