

University of Groningen

## Principles of sorting and assembly of peroxisomal alcohol oxidase

Ozimek, Paulina Zofia

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2005

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Ozimek, P. Z. (2005). *Principles of sorting and assembly of peroxisomal alcohol oxidase*. s.n.

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# **Principles of sorting and assembly of peroxisomal alcohol oxidase**

Paulina Zofia Ozimek

Cover design: Paulina Ozimek and Anne Koek,  
Picture: Łukasz Ozimek; Windmill at the lake Paterswoldermeer, Groningen;  
The Netherlands



**This work was performed in the Eukaryotic Microbiology Group of the Groningen Biomolecular Sciences and Biotechnology Institute (GBB) of the University of Groningen, The Netherlands.**

**Printed by JAKS, Wrocław, Poland**

RIJKSUNIVERSITEIT GRONINGEN



# Principles of sorting and assembly of peroxisomal alcohol oxidase

Proefschrift

ter verkrijging van het doctoraat in de  
Wiskunde en Natuurwetenschappen  
aan de Rijksuniversiteit Groningen  
op gezag van de  
Rector Magnificus, dr. F. Zwarts,  
in het openbaar te verdedigen op  
vrijdag 16 december 2005  
om 14:45 uur

door

**Paulina Zofia Ozimek**

geboren op 3 april 1976  
te Zielona Góra, Polen

Promotores: Prof. dr. I. J. van der Klei  
Prof. dr. M. Veenhuis

Beoordelingscommissie: Prof. dr. A.J.M. Driessen  
Prof. dr. P.J.M. van Haastert  
Prof. dr. J. Pronk

## Preface

The thesis is ready... I look at this manuscript wondering how I managed to squeeze the last five years into 120 pages of text and figures. But then I look more carefully and I see all of what is written in between the lines. All the discussions we had, coffee (and cake ☺) breaks, BBQ's, "labuitjes", bowling, but also my visits to the lab when all the others were sleeping, biking in the rain, little frustrations caused for instance by clonings that, for unknown reason, did not work for weeks, etc. All of it is an integral part of this thesis. Of course I shall not forget to add to this list all the small (and bigger) mistakes I made in the lab. But repeating after Niels Bohr: "An expert is a man, who has made all the mistakes, which can be made in a very small field". I am far from considering myself an expert but I have certainly tried to become one.

The thesis is ready... I am filled with pride but at the same time I realize it would not be possible without the help of many people. It is now the time to acknowledge you all.

First my promoters, Ida and Marten, I would like to thank you for giving me the opportunity to perform a research in your lab and for all the support I got from you over these years. You showed me the guidelines I hope to follow in the future.

I would like to mention all the former and current members of the Eukaryotic Microbiology group. Those of you, who formed EM, when I arrived to the Netherlands almost 6 years ago: Adriana, Anita, Anna Rita, Anne, Florian, Gert-Jan, Ineke, Ira, Jan K., Jan Z., Janet, Klaas Nico, Marco, Meis, Ralf, and Richard; the friendly atmosphere you created in the lab compensated for the separation with my family and friends from Poland. You all inspired me to become a Ph.D. student in this group. Also, colleagues that joined the group later: Ania, Agnieszka, Arjen, Arjo, Bart, Christiaan, Dongyuan, Eda, Erwin, Jurre, Kantcho, Kasinath, Kevin, Katja, Klaas, Marcel, Maria, Marleen, Michel, Nancy, Patricia, Raina, Ralph, Rene, Sandra, Shirisha, Steffi, Susan, Torsten, Virginia, and Wieb. Each of you contributed to the fact that I felt really well in the group and you were always there to help. Thank you all.

Several people deserve special acknowledgements. Jan, thank you for endless discussions and all the support you gave me; Nelly, thank you for the beautiful Christmas cards, delicious dinners and cakes you made; Richard, thanks for the time you spent on the Dutch version of the summary of this thesis; Anne, thank you for everything (just to mention your help with fermentors).

Our wonderful secretaries: Bea, Marga and Manon, I would like to acknowledge your inestimable help with all the paperwork I had to go through during all these years. My 'paranimfs', Marta and Anne (again ☺), the preparations for the defence would be very difficult without you. You did a great job.

Also, I would like to mention people, without whom my life in the Netherlands would not be the same. Burzyńscy, Wojciechowscy, Lubelscy, Zuza, Grzesiek, Kasia, Theo, Iza, Ewelina and Kamila, you have been great friends.

I would not get to this point without the support of my family. Thank you for always believing in me. Also thanks to my new families of Ozimek and Sycz, I am grateful for all the support and kindness I received from you ever since I knew you.

Finally, my beloved husband and best friend, Lukasz. Thank you for your love and patience. It is a great honor to share my life with you.

## Contents

Aim and outline of this thesis	9
<b>Chapter 1</b>	11
Alcohol oxidase: a complex peroxisomal, oligomeric protein	
<b>Chapter 2</b>	23
Pyruvate carboxylase is an essential protein in the assembly of yeast peroxisomal oligomeric alcohol oxidase	
<b>Chapter 3</b>	43
A region linking two conserved domains in pyruvate carboxylase is essential for sorting and assembly of peroxisomal alcohol oxidase	
<b>Chapter 4</b>	63
<i>Hansenula polymorpha</i> and <i>Saccharomyces cerevisiae</i> Pex5p's recognize two different, independent peroxisomal targeting signals in alcohol oxidase	
<b>Chapter 5</b>	77
<i>Hansenula polymorpha</i> Swi1p and Snf2p are essential for methanol utilization	
Summary	93
Samenvatting	97
Podsumowanie	101
References	105
List of publications	119



## Aim and outline of this thesis

Peroxisomal matrix proteins are synthesized in the cytosol and posttranslationally imported into peroxisomes. Most of them contain a C-terminal peroxisomal targeting signal (PTS1) that consists of only three amino acids and is recognized by the receptor protein Pex5p.

Alcohol oxidase (AO) from the methylotrophic yeast *Hansenula polymorpha* is a peroxisomal enzyme that catalyses the first step in methanol catabolism, namely the oxidation of methanol into formaldehyde and hydrogen peroxide. Enzymatically active AO is an oligomer and consists of eight identical subunits that each contains one flavin adenine dinucleotide (FAD) non-covalently bound.

AO undergoes an unusual biosynthetic pathway that is exceptional in many aspects. First, AO import, although dependent on Pex5p, is independent of the PTS1 of AO, –LARF.COOH. Instead, another yet unknown PTS in AO is recognized by Pex5p and is most likely formed upon FAD-binding to AO monomers.

The research presented in this thesis aimed at elucidating the molecular mechanisms of the AO biosynthetic pathway.

In **chapter 1** the current knowledge on AO biosynthesis, sorting and assembly is presented.

*H. polymorpha* pyruvate carboxylase (HpPyc1p) is a cytosolic enzyme that replenishes the tricarboxylic acid cycle with oxaloacetate. In **Chapter 2** we show that this protein is also specifically involved in sorting and assembly of AO, most likely being essential for FAD-binding to AO monomers in the cytosol. This function is independent of the enzyme activity of HpPyc1p, because mutations that affect the active site of HpPyc1p do not abolish the function of the protein in AO sorting and assembly.

**Chapter 3** is a logic continuation of the previous chapter and describes the analysis of regions/domains in HpPyc1p that are specifically involved in AO sorting and assembly. By the analysis of HpPyc1p mutants obtained by transposon-mediated mutagenesis and of truncated versions of HpPyc1p, a region was identified that is specifically required for AO sorting and assembly. We speculate that this region is involved in the formation or stabilization of a conformation of AO monomers that allows FAD binding.

**Chapter 4** describes attempts to reconstitute the AO biosynthetic pathway in *Saccharomyces cerevisiae*. Expression of the *H. polymorpha* *AOX* gene in *S. cerevisiae* results in the formation of inactive AO monomers, which are poorly sorted to peroxisomes. We showed that introduction of HpPyc1p is sufficient to mediate assembly of enzymatically active, FAD-containing AO octamers in *S. cerevisiae*. Our attempt to increase the sorting efficiency of AO by replacing ScPex5p by HpPex5p was unsuccessful.

These studies revealed that ScPex5p uses the PTS1 of AO for import into peroxisomes, whereas HpPex5p recognizes an alternative, unknown PTS that is only formed in the presence of HpPyc1p.

*H. polymorpha* Swi1p and Snf2p are homologues of *S. cerevisiae* subunits of the SWI/SNF chromatin remodelling complex. In **chapter 5**, the phenotype of *H. polymorpha* strains deleted for either *SWI1* or *SNF2* are presented. The outcome of these studies suggest that the chromatin remodelling complex that contains HpSwi1p and HpSnf2p is involved in a common regulation machinery for genes involved in methanol metabolism.