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Sensing Penicillin

**Design and Construction
of Metabolite Biosensors**

Esther Magano Volz

The research described in this thesis was carried out in the Genetics Department of the DSM Biotechnology Center Delft, The Netherlands, and in the Department of Molecular Microbiology, part of the Groningen Biomolecular Science and Biotechnology Institute (GBB), University of Groningen, The Netherlands.

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Images

Cover: Cytoplasm of a cell. Adapted from David S. Goodsell¹
Chapter 1: TPP riboswitch (PDB ID: 3D2V)²
Chapter 2: Spinach RNA aptamer in complex with DFHBI (PDB ID: 4TS0)²
Chapter 3: TcaR in complex with penicillin G (PDB ID: 3KP2)²
Chapter 4: Ribosome (#121)³
Chapter 5: Model of RNA polymerase in action (#40)³
Chapter 6: Citric acid cycle (#154)³

1. doi: [10.2210/rcsb_pdb/goodsell-gallery-006](https://doi.org/10.2210/rcsb_pdb/goodsell-gallery-006)
2. Goodsell DS, Autin L, Olson AJ (2019) Illustrate: Software for Biomolecular Illustration. doi: [10.1016/j.str.2019.08.011](https://doi.org/10.1016/j.str.2019.08.011)
3. <http://pdb101.rcsb.org/motm/#>

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Sensing Penicillin

Design and Construction of Metabolite Biosensors

PhD thesis

to obtain the degree of PhD at the
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on the authority of the
Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

Friday 15 May 2020 at 12.45 hours

by

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Für meine Familie

TABLE OF CONTENTS

Aim and scope of the thesis	8
Chapter 1	11
Strategies to detect secondary metabolite production of filamentous fungi using biosensors	
Chapter 2	35
Aptamers for biosensing of penicillin in fungal cultures: a feasibility study	
Chapter 3	57
Interactions of the bacterial multi-drug transcriptional regulator TcaR with DNA and β -lactam antibiotics	
Chapter 4	87
Interaction analysis of the transcription factor TcaR with its promoter DNA using a cell-free transcription-translation system	
Chapter 5	109
Engineering of a prokaryotic transcriptional repressor as metabolite biosensor in filamentous fungi	
Chapter 6	147
Summary	148
Samenvatting	154
Zusammenfassung	160
Acknowledgements	166
About the Author	170

Aim and scope of the thesis

The aim of this thesis was to investigate different strategies for the development of biosensors to detect secondary metabolites from filamentous fungi. We selected the β -lactam antibiotic penicillin, which is produced by the filamentous fungus *Penicillium chrysogenum* on industrial scale, as a model metabolite for sensor development.

Chapter 1 briefly outlines the benefits and challenges of secondary metabolite production from filamentous fungi, presents the mechanisms of established nucleic acid, protein, and whole sensors for the detection of small molecules, and proposes different strategies for the development of penicillin biosensors.

Chapter 2 assesses the feasibility of DNA aptamers to sense penicillin in fungal cultures. We reevaluated the binding properties of published β -lactam aptamers and performed SELEX experiments to enrich for new penicillin aptamers. However, neither the analysis of published aptamers nor the selection of new aptamers resulted in suitable penicillin aptamers for sensor development. In addition, the analysis of DNA aptamer stability in *P. chrysogenum* culture samples revealed that aptamers cannot be applied for metabolite detection in fungal cultures as they are rapidly degraded by fungal enzymes.

Chapter 3 focuses on the interactions of the transcription factor TcaR with its ligands to lay the basis for the development of TcaR-based penicillin biosensors. The interactions of TcaR with DNA and β -lactam antibiotics were characterized with Thermal Shift Assays and Microscale Thermophoresis. Multiple TcaR-ligand interactions were quantified, thereby proving a solid foundation for sensor development. Additionally, targeted site-directed mutagenesis of the TcaR penicillin-binding pocket resulted in TcaR mutants with improved penicillin and DNA binding properties.

Chapter 4 explores the development of a cell-free penicillin biosensor using the TcaR system and a GFP reporter system. We demonstrated that TcaR can repress GFP expression in a reconstituted cell-free system. Due to an adverse effect of high penicillin concentrations on translation, we found the TcaR system to be unsuitable for the development of a cell-free penicillin biosensor. Nevertheless, the chapter presents a new microtiter plate-based method to study transcription factor-DNA binding in cell-free systems.

In **Chapter 5** we transplanted the bacterial transcription factor TcaR into the genome of *P. chrysogenum* to construct a whole-cell penicillin biosensor. It was demonstrated that the choice of promoter and codon usage significantly impacts TcaR expression levels *in vivo*. A fungal strain showing high TcaR expression levels was found to detect high concentrations of penicillin using a fluorescent reporter cassette. Different fungal growth phases were classified to dissect a specific, penicillin-dependent transcriptional regulation by TcaR from gene expression noise.

Chapter 6 summarizes the findings from this thesis and provides an outlook on the prospects of metabolite biosensor in fungal biotechnology.