

Integrative Approach to Promote Hydroxylations with Novel P450 Enzymes for Industrial Processes

Project acronym: HyPerIn

Project no: EIB.12.026

Prof. Dr. Vlada B. Urlacher

ERA-IB-2 Final conference, Berlin, 16./17.02.2016

Project partners

UCL
Prof. Gary Lye

HEINRICH HEINE UNIVERSITÄT DÜSSELDORF
Prof. Vlada Urlacher

c-LEcta enzymes and strains
Dr. Andreas Vogel

Fraunhofer
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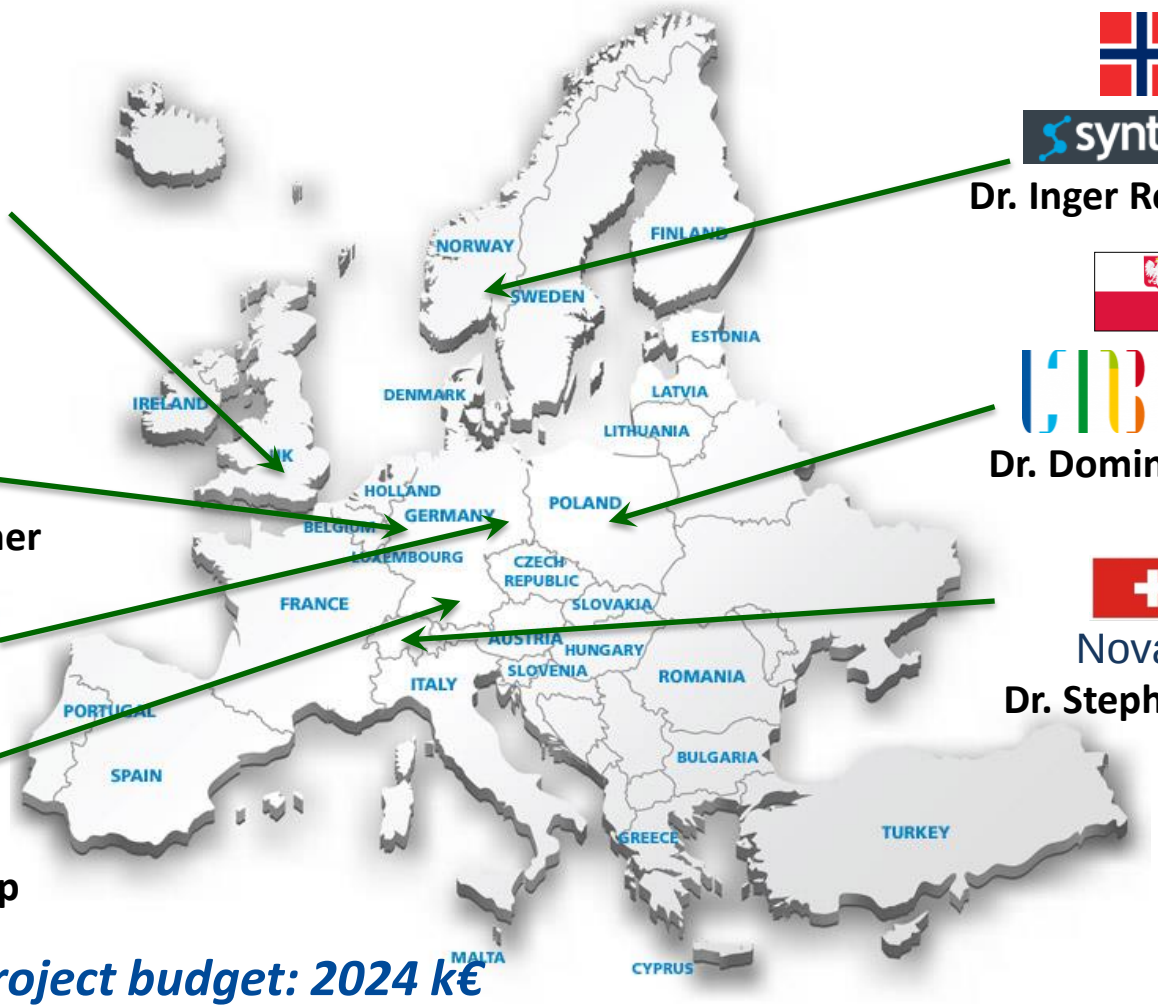


synthetica
Dr. Inger Reidun Aukrust

LABORATORY of THEORY of BIOPOLYMERS
Dr. Dominik Gront

Novartis
Dr. Stephan Luetz

- ***Total project budget: 2024 k€***

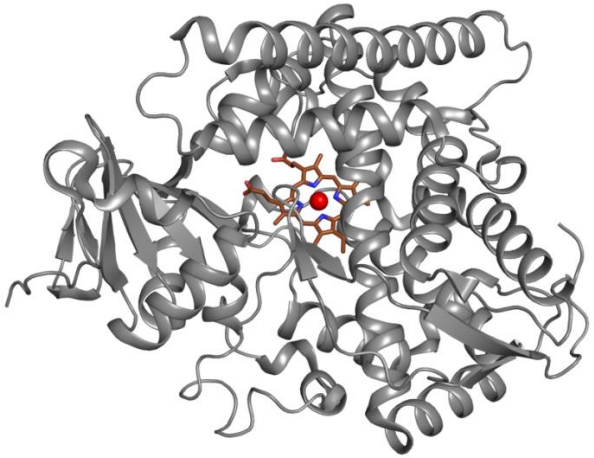
Introduction



Cytochrome P450 monooxygenases

$$NAD(P)H + RH + O_2 + H^+ \rightarrow R{-}OH + NAD(P)^+ + H_2O$$

- Heme *b* containing oxidoreductases
- > 30 types of oxidation reactions
- Hydroxylation of non-activated C-atoms
- Accept a vast variety of substrates



Limitations for application

- Requirement of electron transfer partner(s)
- Limited access to suitable P450 biocatalysts
- Inefficient biotransformation processes

Introduction

- *HyPerIn objectives:*
  - *To provide a platform of novel cytochrome P450 biocatalysts*
  - *To overcome bottlenecks for the application of cytochrome P450 monooxygenases*

- *General project approach:*
  - *Discovery of novel P450s through genome mining*
  - *Construction of designer biocatalysts with optimized P450 systems in recombinant hosts*
  - *Development of a high-throughput platform for P450 biocatalyst evaluation*
  - *Representative process examples at a lab- and a preparative scale*

Technical overview



WP 6 Overall project management

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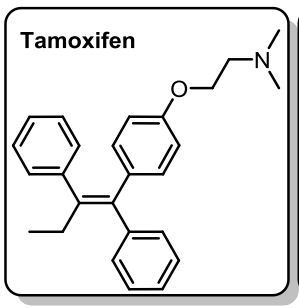
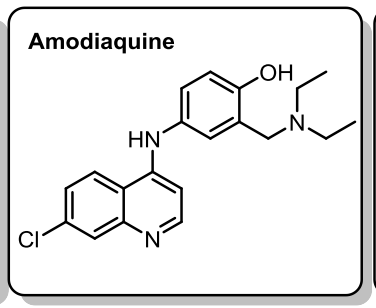
WP 1	WP 2	WP 3	WP 4	WP 5
Target compound and strain identification & Assay development	New enzyme identification through genome mining	Enzyme expression & Development of whole-cell biocatalysts	High-throughput P450 bioprocess development & Optimization	Synthetic application & Process evaluation
Novartis	Fraunhofer	HEINRICH HEINE UNIVERSITÄT DÜSSELDORF	UCL	Novartis
synthetica	LABORATORY of THEORY of BIOPOLYMERS	c-LEcta enzymes and strains	HEINRICH HEINE UNIVERSITÄT DÜSSELDORF	Fraunhofer
HEINRICH HEINE UNIVERSITÄT DÜSSELDORF	c-LEcta enzymes and strains			UCL
c-LEcta enzymes and strains				

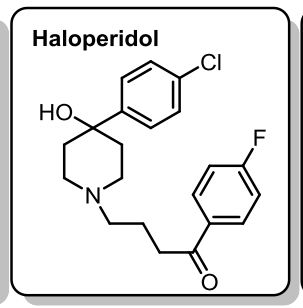
Technical overview

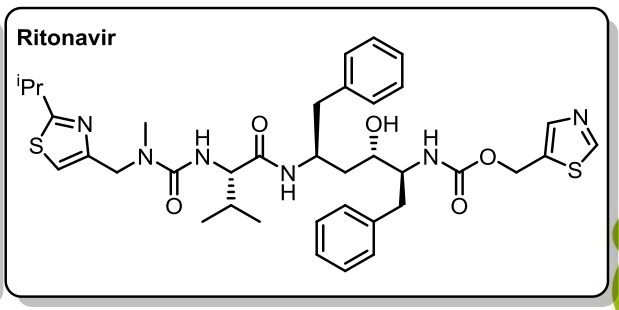
- *Identification of target compounds and synthesis of reference standards*

Tamoxifen	Amodiaquine	Haloperidol	Ritonavir
Estrogen receptor antagonist	Histamine N-methyltransferase inhibitor	Dopamine receptor inhibitor	HIV-1 protease inhibitor
Breast cancer	Malaria	Psychosis	HIV infection





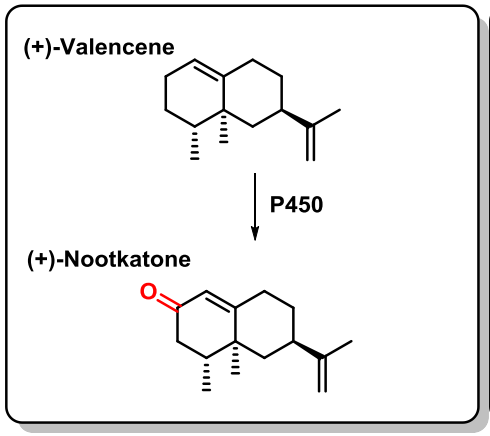


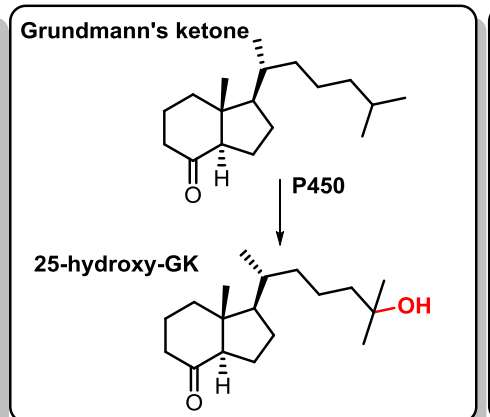


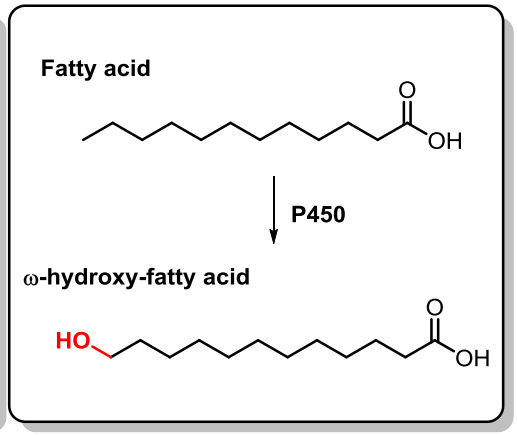
Technical overview

- *Identification of target compounds and synthesis of reference standards*

Valencene	Grundmann's ketone	Fatty acids
Ingredient of orange peel oil	Precursor for vitamin D3 derivatives	ω-hydroxylation
Grapefruit flavor	Synthesis of medicines	Polymer synthesis

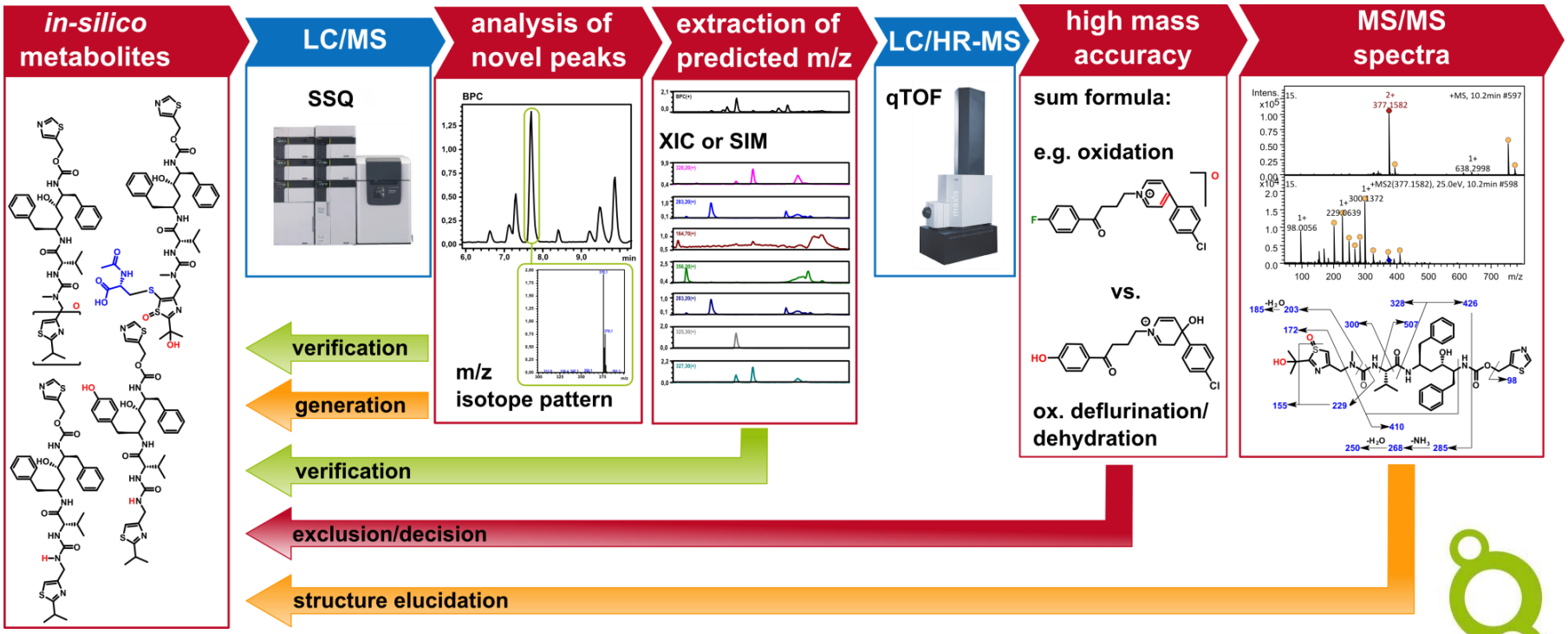






Technical overview

- *Development of analytical methods*



Technical overview



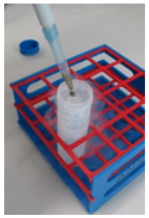
- *Screening of strains with P450 activity*

Strain		Number of target compound hits					
No.	ID	VAL	AM	HAL	TAM	RIT	GK
1	AAU			1		4	
2	SHR					1	
3	SHY		2	2			
4	SPL	2	3	4	3	9	
5	SER			3		5	
6	TBF		3				1
7	MVI			1	3	1	
8	RST		2	3	2	2	

Technical overview

- *DNA-sequencing of strains with P450 activity*

I. Sample collection



-Novartis

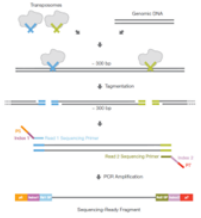
-c-LEcta

II. Nucleic acid isolation

-DNA

-mRNA

III. Library preparation



-whole genome shotgun

-mate pair

-strand-specific RNA

IV. Sequencing

-Illumina HiSeq2500

-Roche GSjunior

V. Bioinformatic analysis

-assembly

-ORF prediction

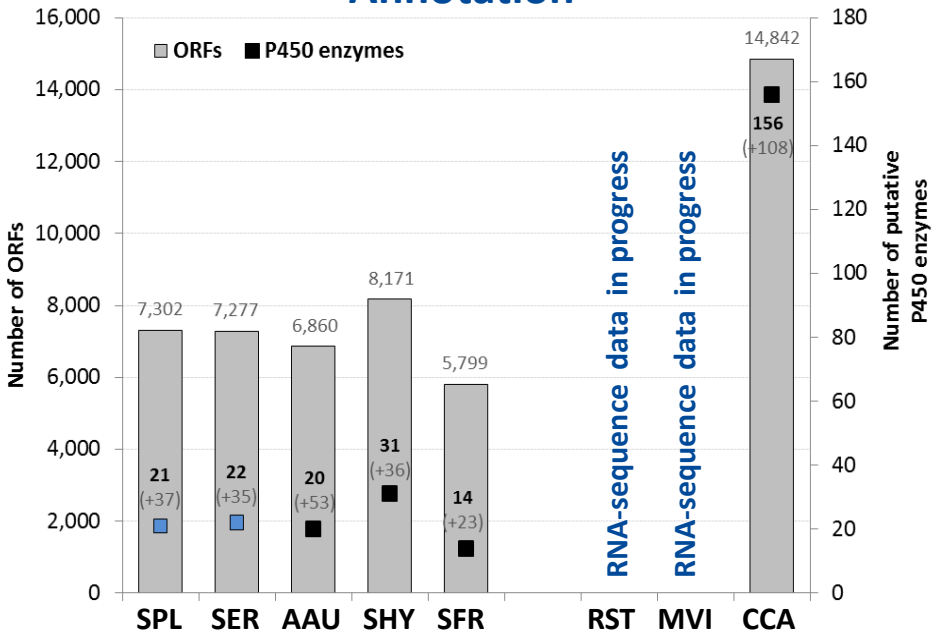
-annotation

-gene expression

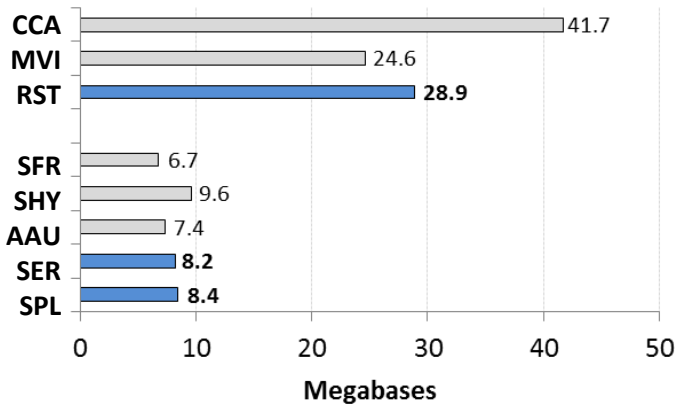
Technical overview

- *DNA-sequencing of strains with P450 activity*
- *Transcriptomics analysis*

Annotation



Genome sizes



108 prokaryotic P450 genes

~ 150 putative redox partners

> 300 eukaryotic P450 genes

Technical overview

LABORATORY of THEORY of BIOPOLYMERS

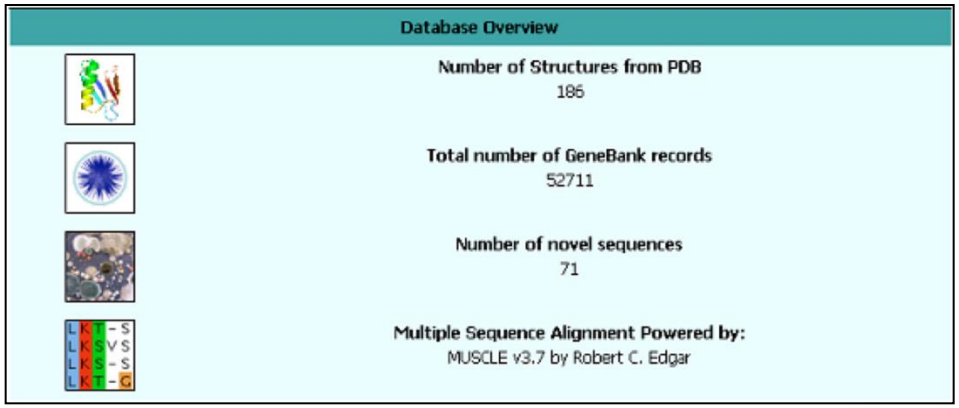


- *Establishment of a P450 database*

Internet-based platform:

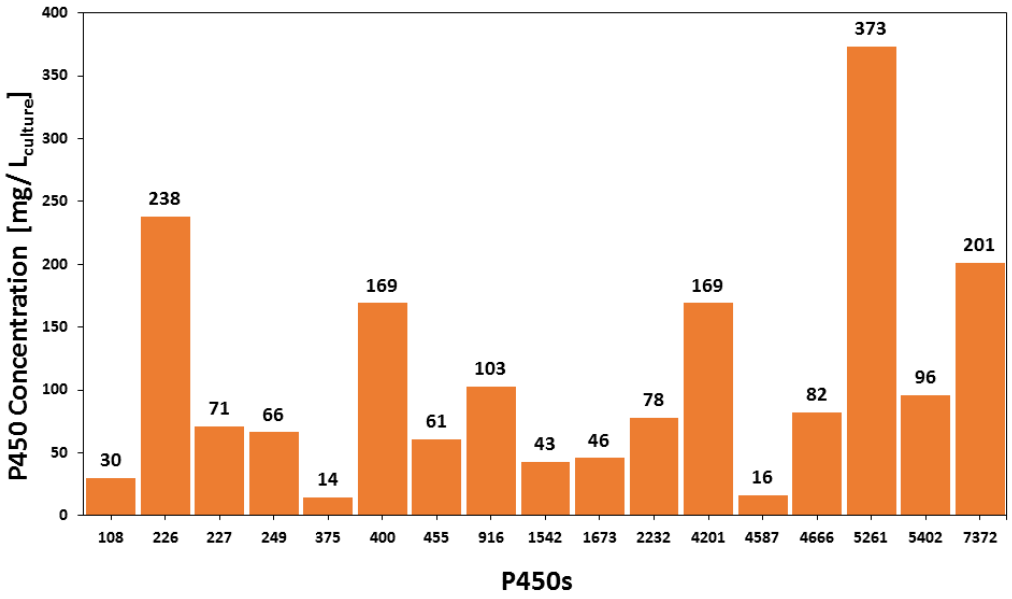
- *open for partners*
- *~ 53,000 P450-sequences*
- *186 structures*
- *annotation of sequences*
- *structural information*
- *description of domains*
- *available publications*
- *authors and their affiliations*
- *sequential patterns*

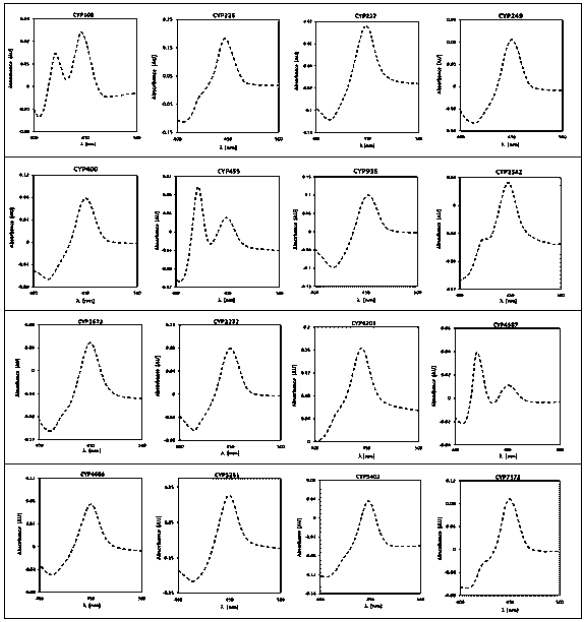
http://193.239.206.14/menu_login.php



Technical overview

- *Recombinant expression of P450s in E. coli*

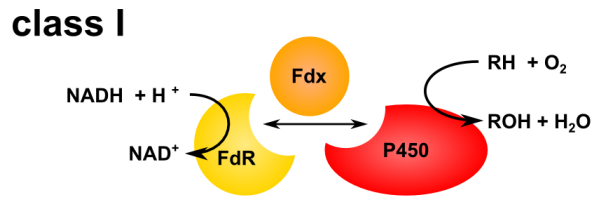


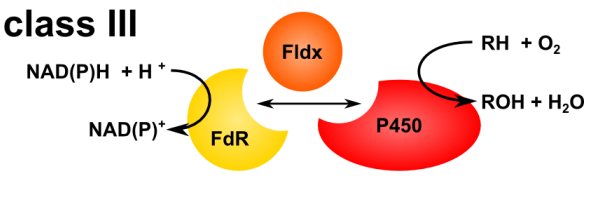


Concentration of recombinant P450s in *E. coli*: 20 - 400 mg per L culture

Technical overview

- *Identification and (co-)expression of appropriate redox partners for P450s*



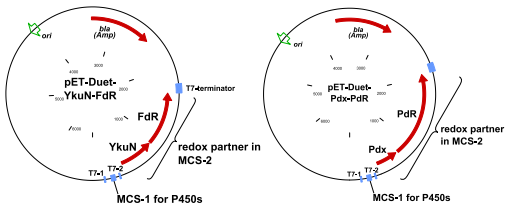


Redox partners

YkuN/FdR **Pdx/PdR**

+

P450 gene of interest



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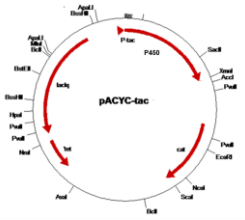
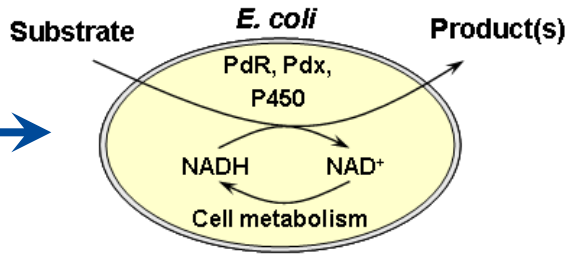
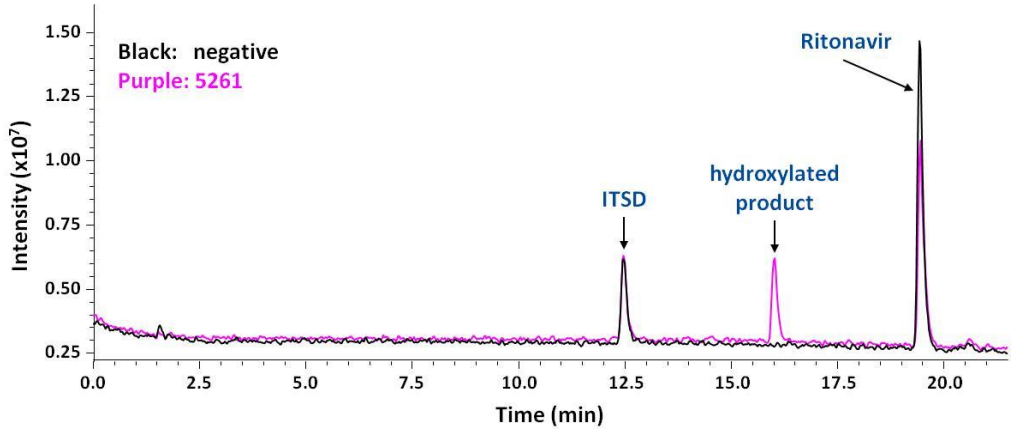


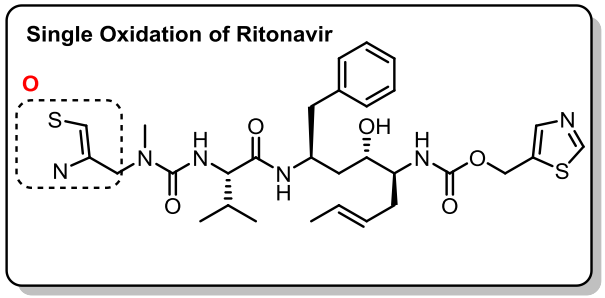
in vitro & in vivo

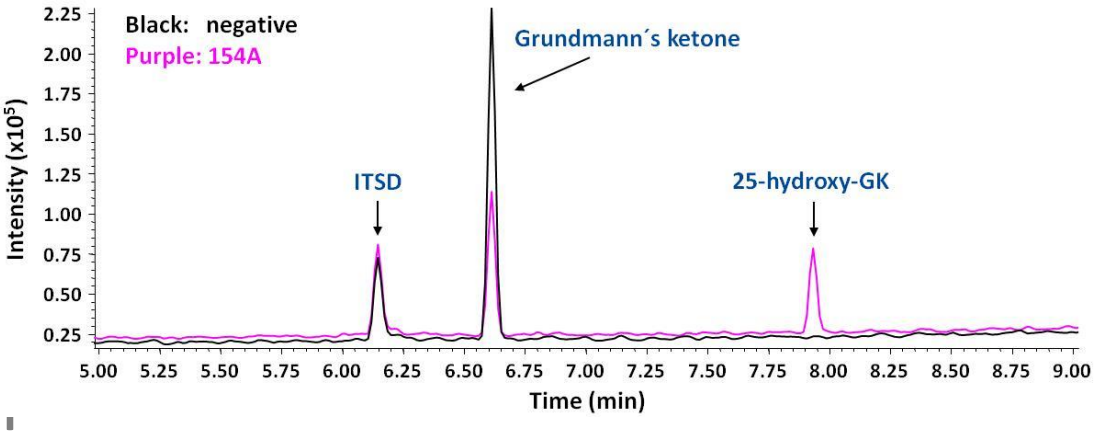


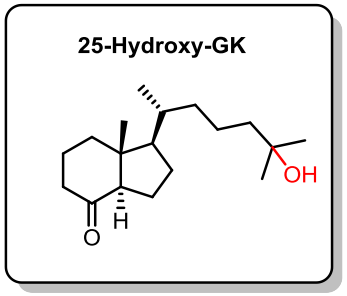
Technical overview

- *Identification of lead P450 candidates (selected examples)*







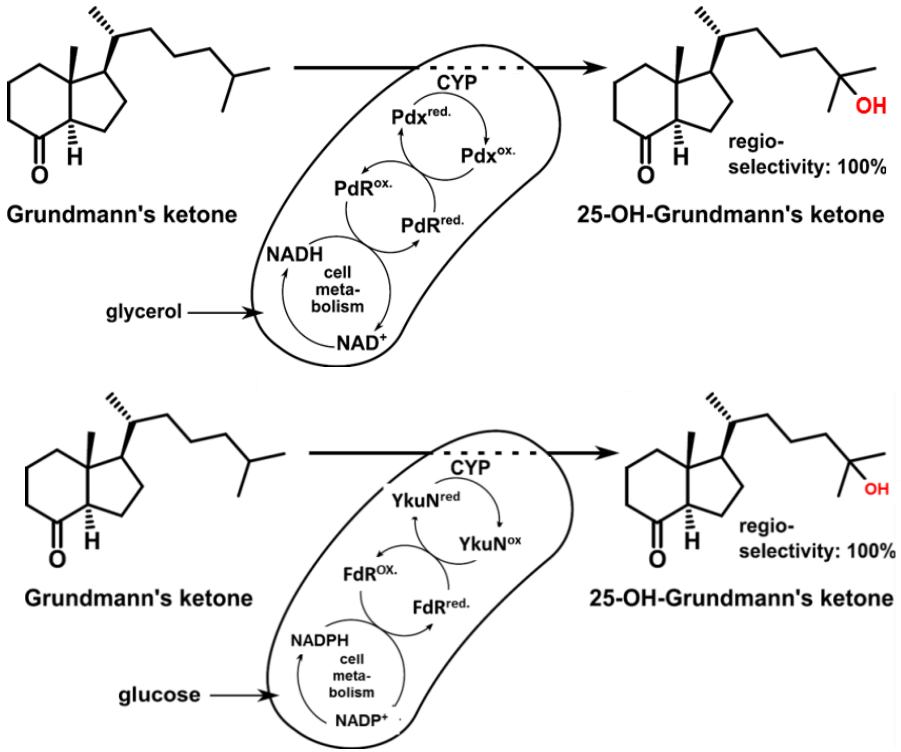


Technical overview

- *Designer biocatalyst with optimized P450 system expression in host systems*

Optimization of:

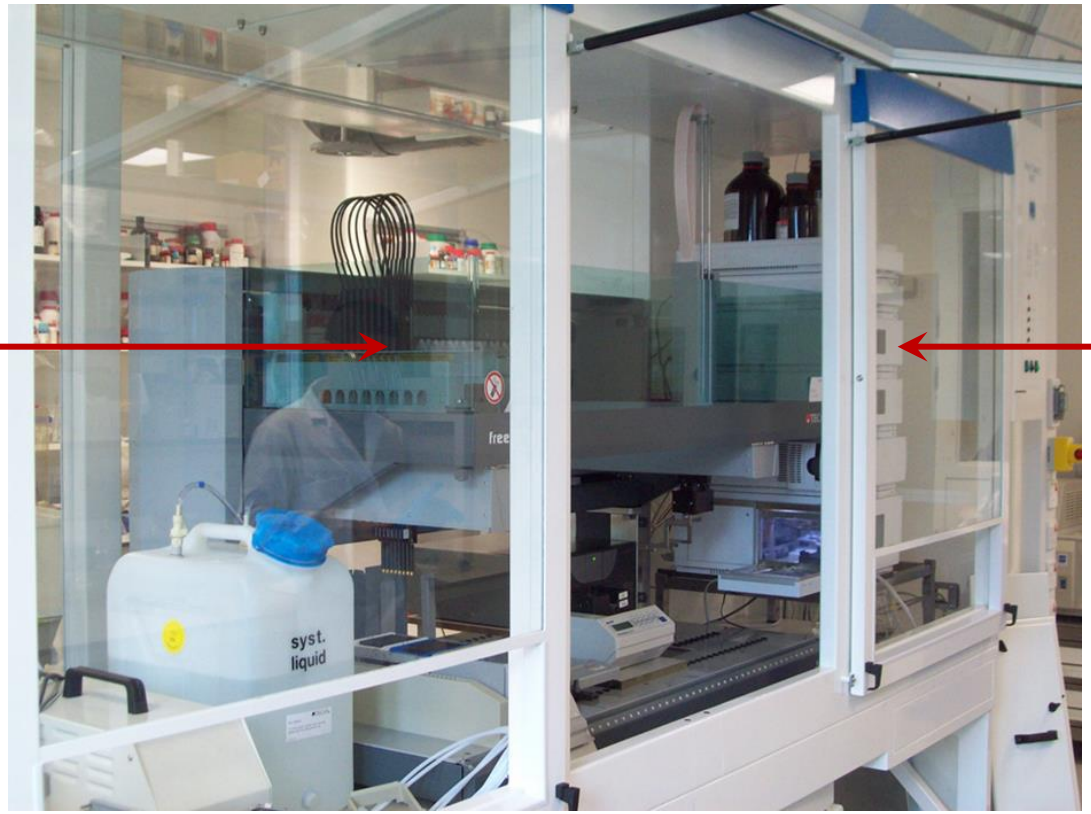
- Cell concentration
- Substrate concentration
- Co-solvents
- Redox partners
- Cofactor availability



Technical overview

- *Creation of automated high throughput methods*

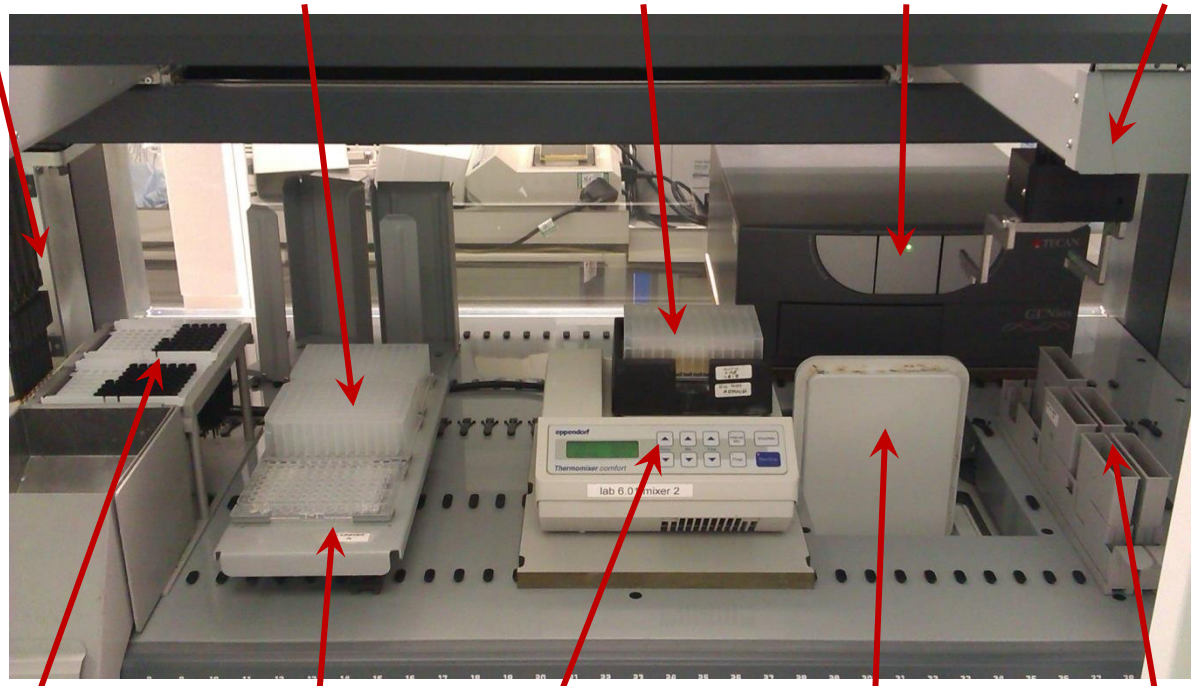
Automated platform



HPLC

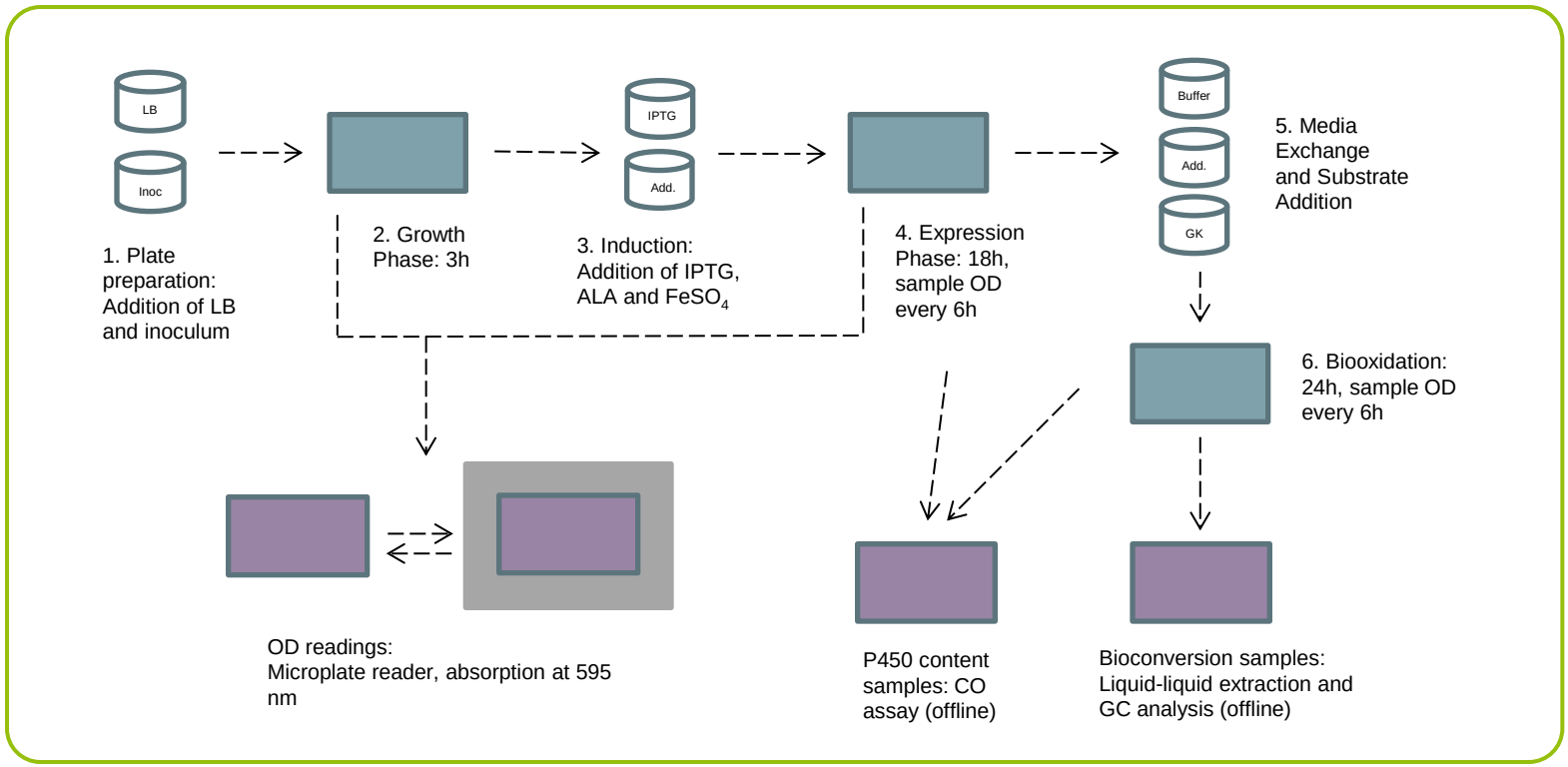
Technical overview

- *Creation of automated high throughput methods*



Technical overview

- *Optimization of P450 biooxidation processes at µl-scale*



Technical overview

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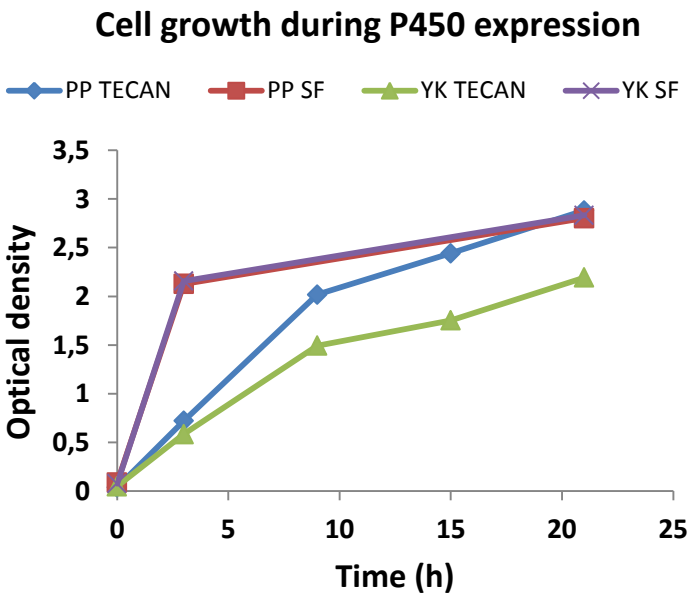

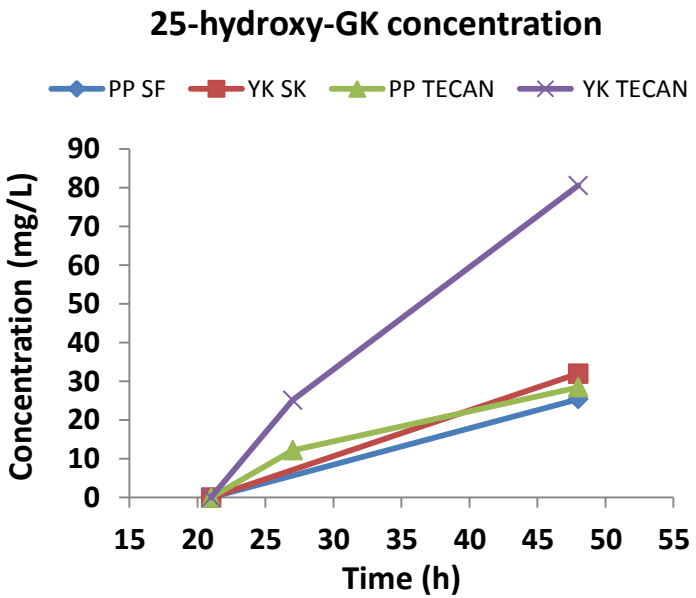

Summary

- *Start of HyPerIn was delayed*
- *Extension of project duration until 03/2017*
- *Work packages in progress:*
  - *Process scale-up and verification*
  - *Pilot scale production of a fine chemical compound*
  - *Industrial evaluation for a pharma compound*

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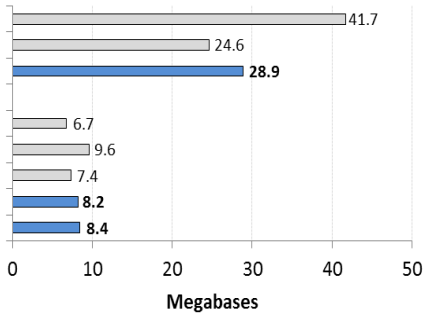
Fraunhofer

Novartis

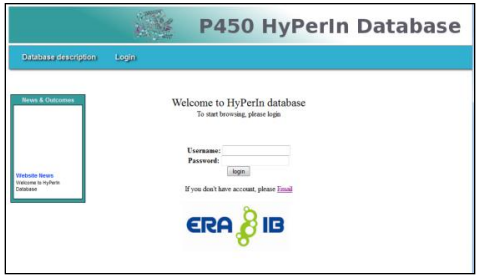
Project outcome

I. A collection of wild-type strains with known P450 activities



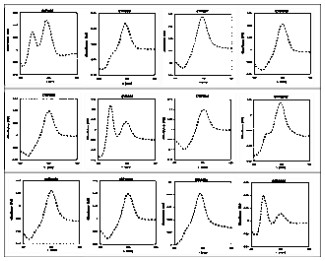
II. Genomes of 8 microbial strains with P450 activities are sequenced and annotated

III. More than 450 new P450-genes identified

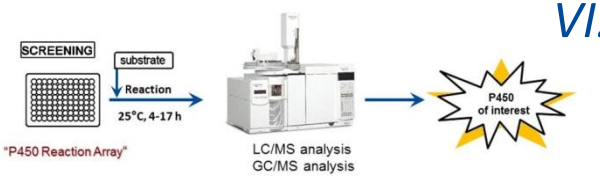


IV. A new P450 database created

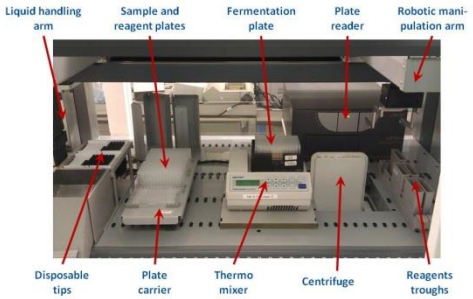
Project outcome



V. A library of novel P450 enzymes expressed in recombinant E. coli

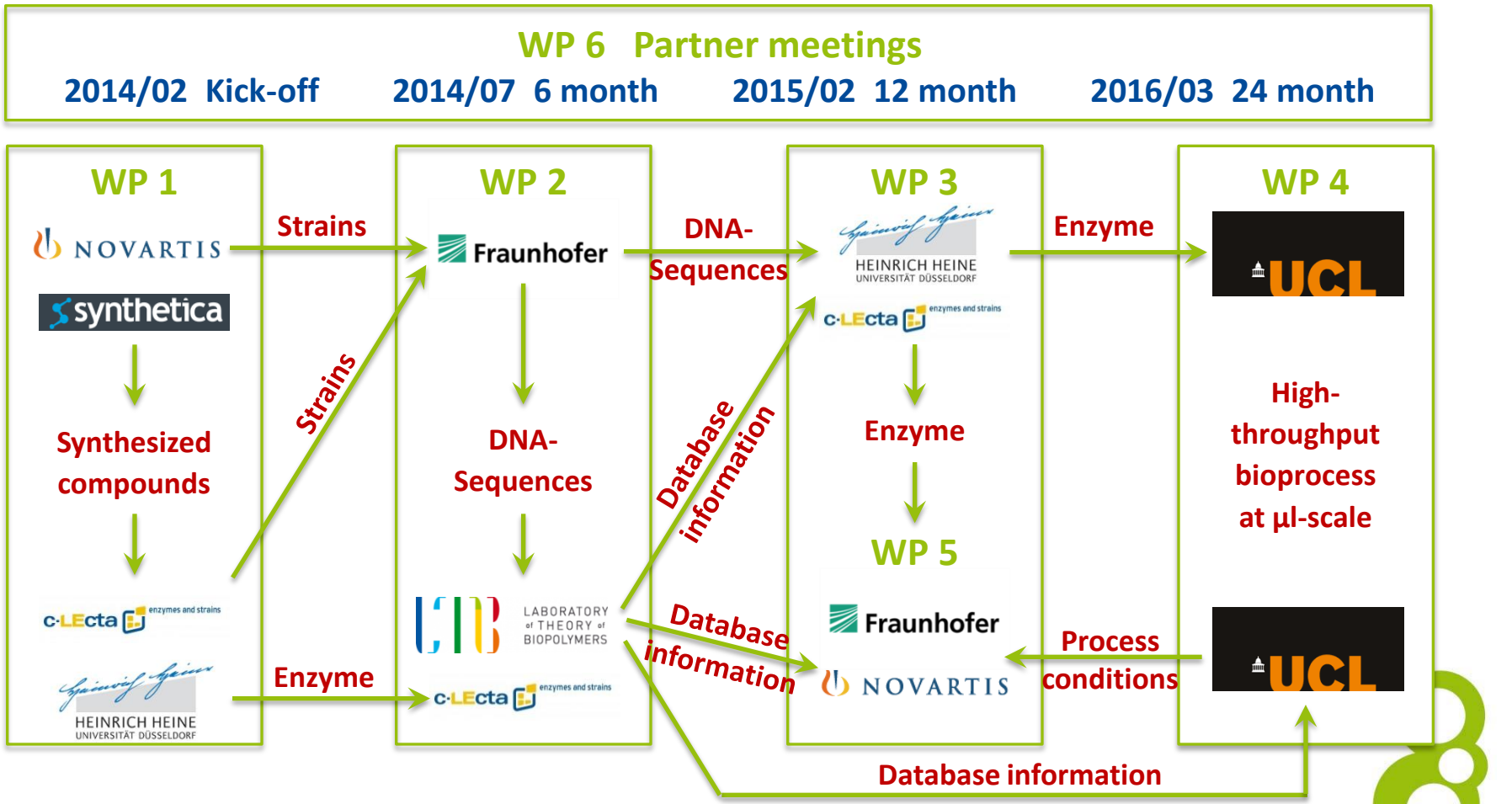


VI. A set of recombinant P450-based whole-cell biocatalysts with activity toward target compounds



VII. A high-throughput platform for P450 biocatalyst evaluation at µl-scale

General Evaluation



Acknowledgements

ERA-IB network

National funding organizations

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