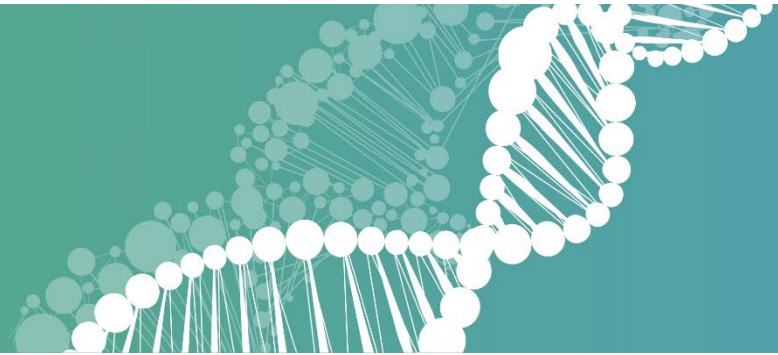




ERA CoBioTech

BIO TECH RESEARCH AND INNOVATION HACK 2021

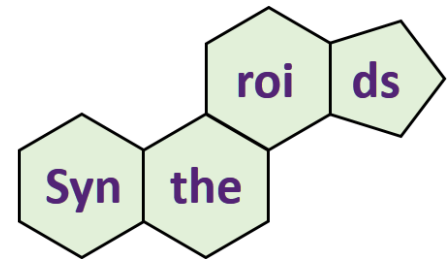
# Final seminar of the cofunded projects of ERA CoBioTech



Project name: Synthetic Biology for Industrial Production of Steroids

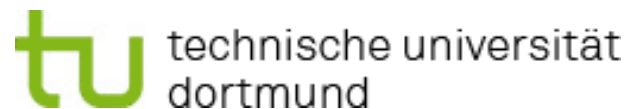
Project acronym: Syntheroids

Name: Alberto Sola-Landa (INBIOTEC)



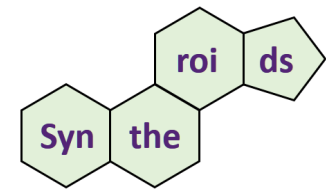
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

28.09.2021



● Total project budget: 2,308,000 €

● Period: 15/05/2018 – 30/11/2021

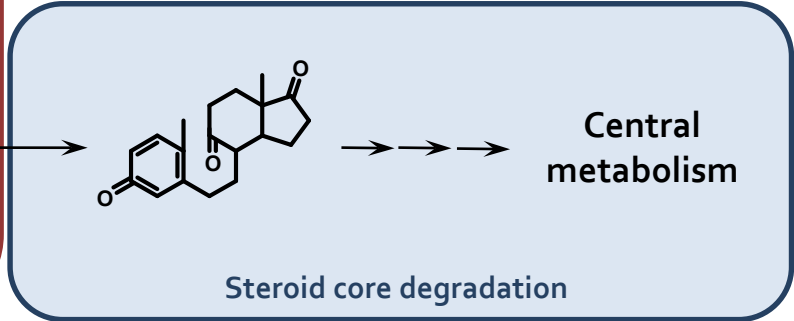
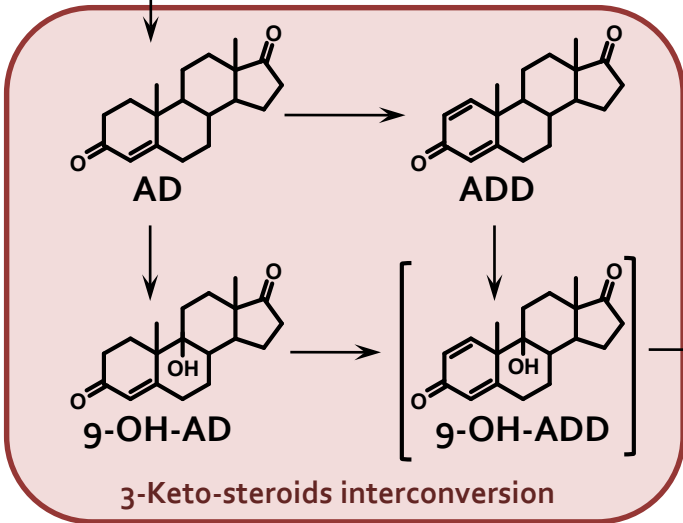
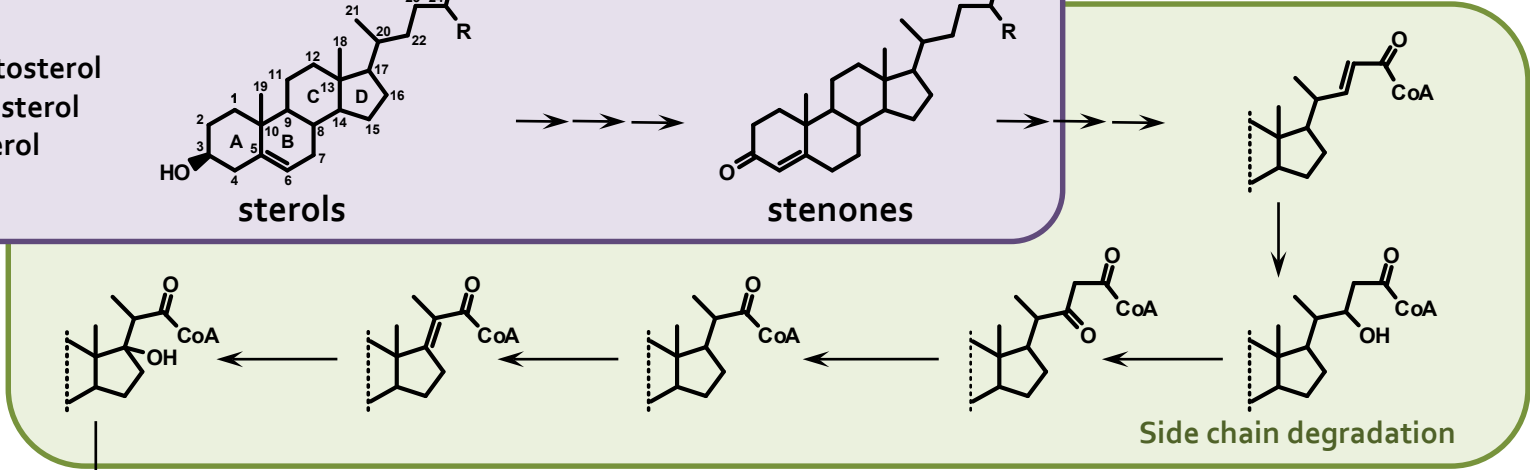
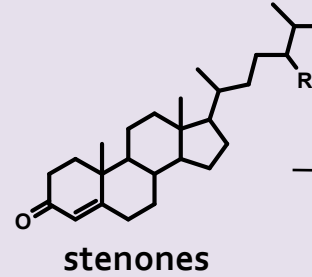
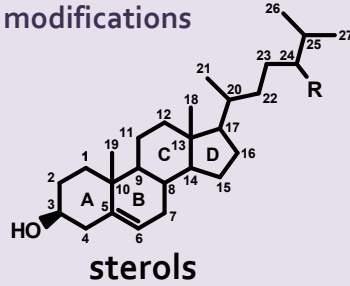


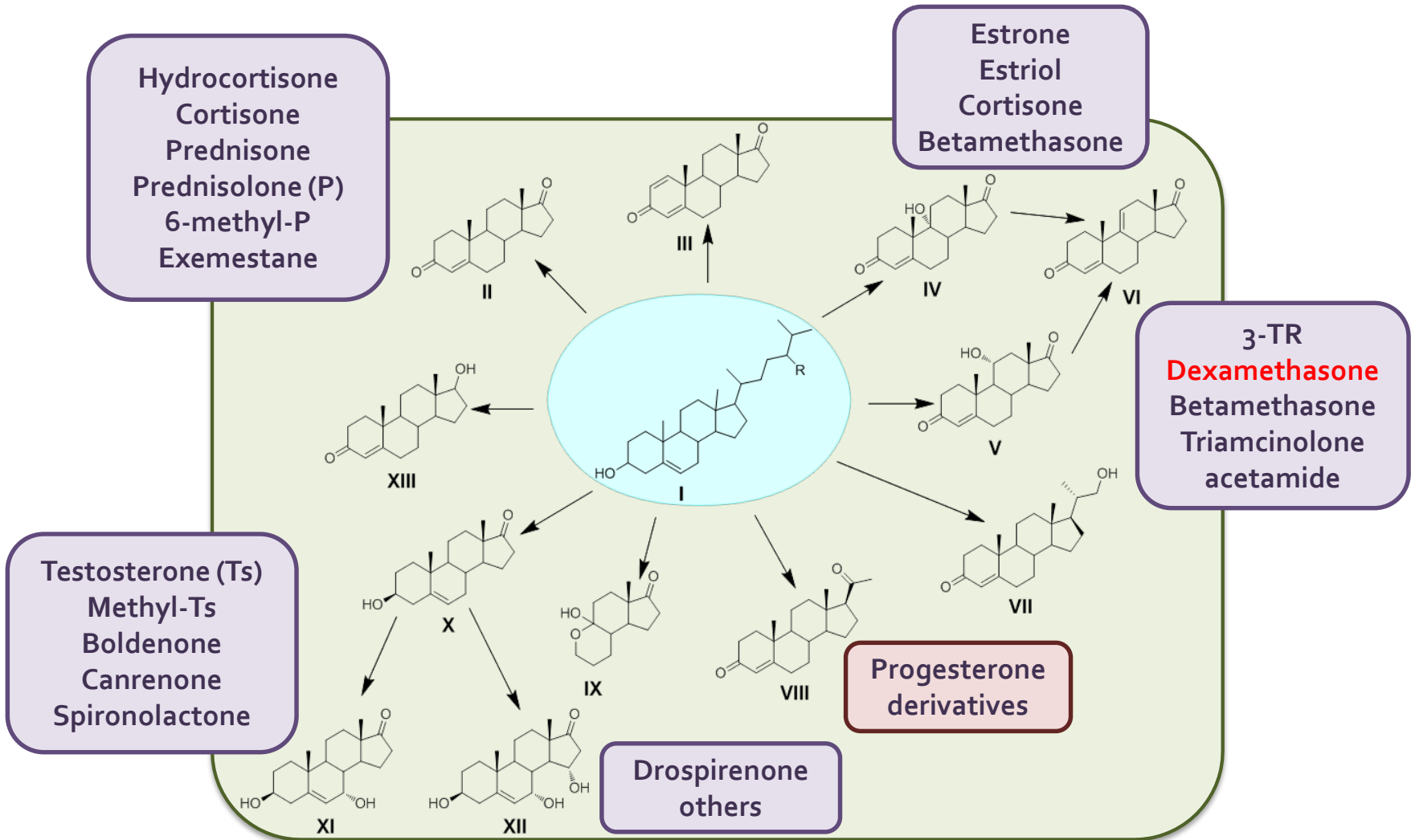
## Steroids

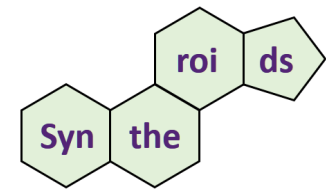
- Second largest class of drugs
- More than 300 clinically approved steroidal compounds
- Clinical use since the late 1940 s
- Multiple applications
  - ✓ Rheumatoid arthritis
  - ✓ Neurodegenerative diseases
  - ✓ Cancer
  - ✓ Inflammatory diseases → SARS-CoV-2 (COVID-19)
  - ✓ Metabolic disorders
  - ✓ Contraception
  - ✓ Hormonal insufficiencies
  - ✓ Others

Initial steroid core A-B rings modifications

R = CH<sub>2</sub>-CH<sub>5</sub>, sitosterol  
 CH<sub>3</sub>, campesterol  
 H, cholesterol

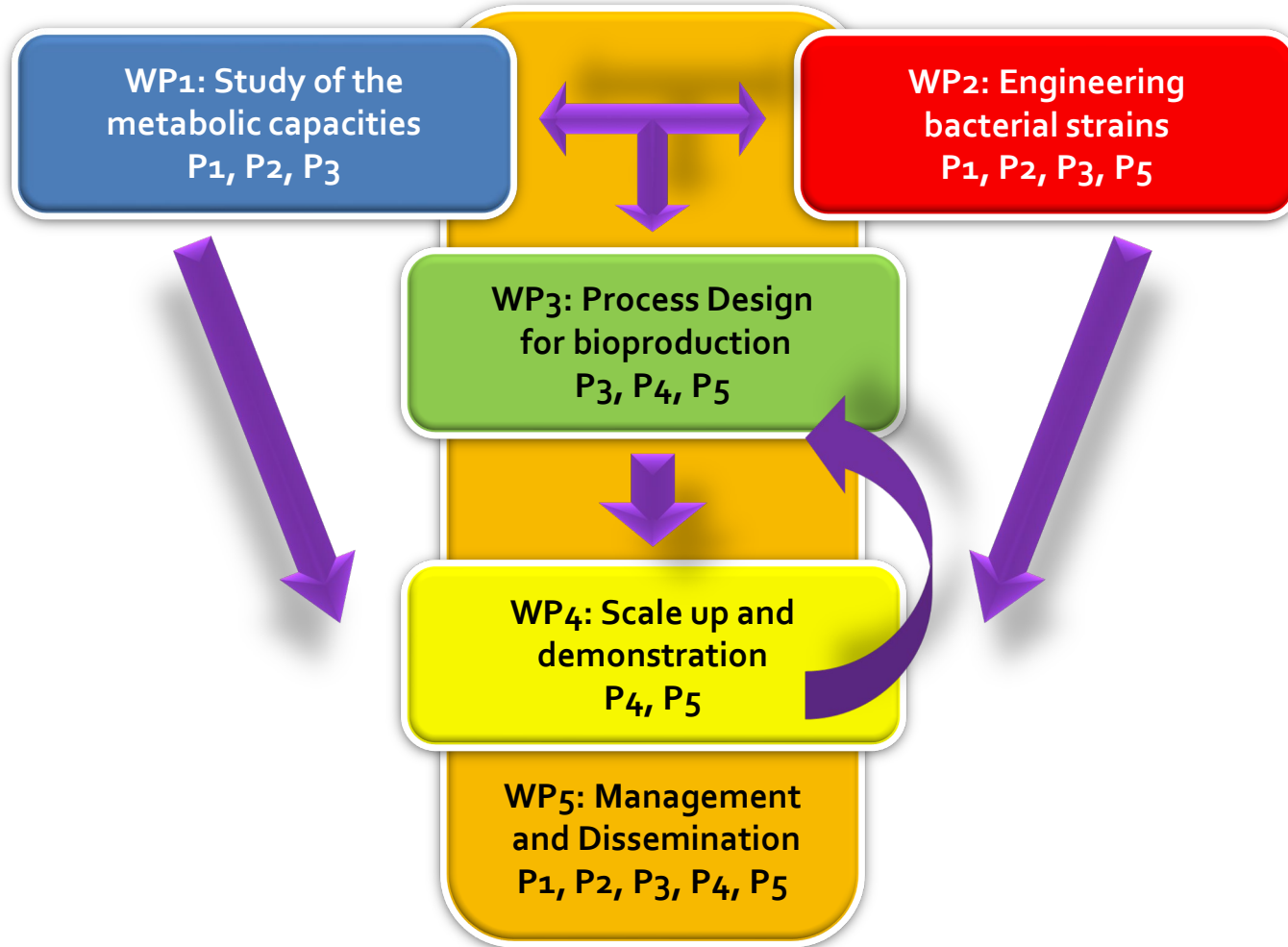




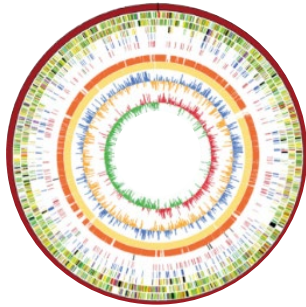


The **central objective** of Syntheroids is **to develop an integrated production process for pharmaceutical steroids** using Synthetic Biology and improved processing technology. Syntheroids project has four **specific objectives**:

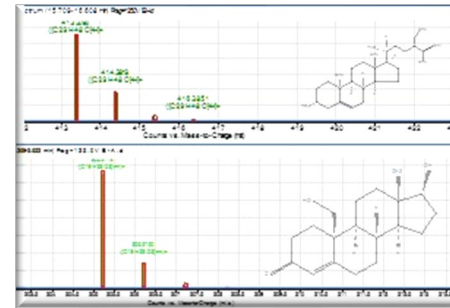
- **Omics data integration** from steroid producing Actinobacteria as a source of Synthetic Biology targets for productive strain evolution.
- **Creating genetically engineered bacterial strains** capable of producing innovative **C22-steroid precursors**.
- **Reduce or eliminate end-product inhibition** by mutagenesis, genetic engineering and process optimization.
- **Integrate up- and downstream processes** for an eco-friendly bioconversion.



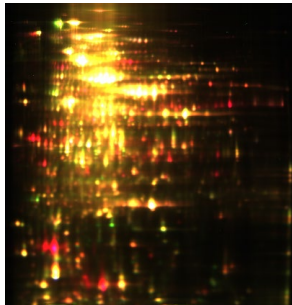
# WP1. Metabolic capacities of steroid-producing strains



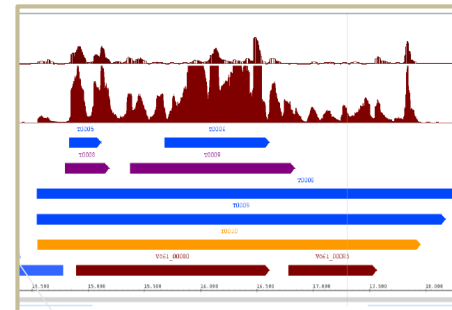
*Genome sequencing*



*Metabolomics*



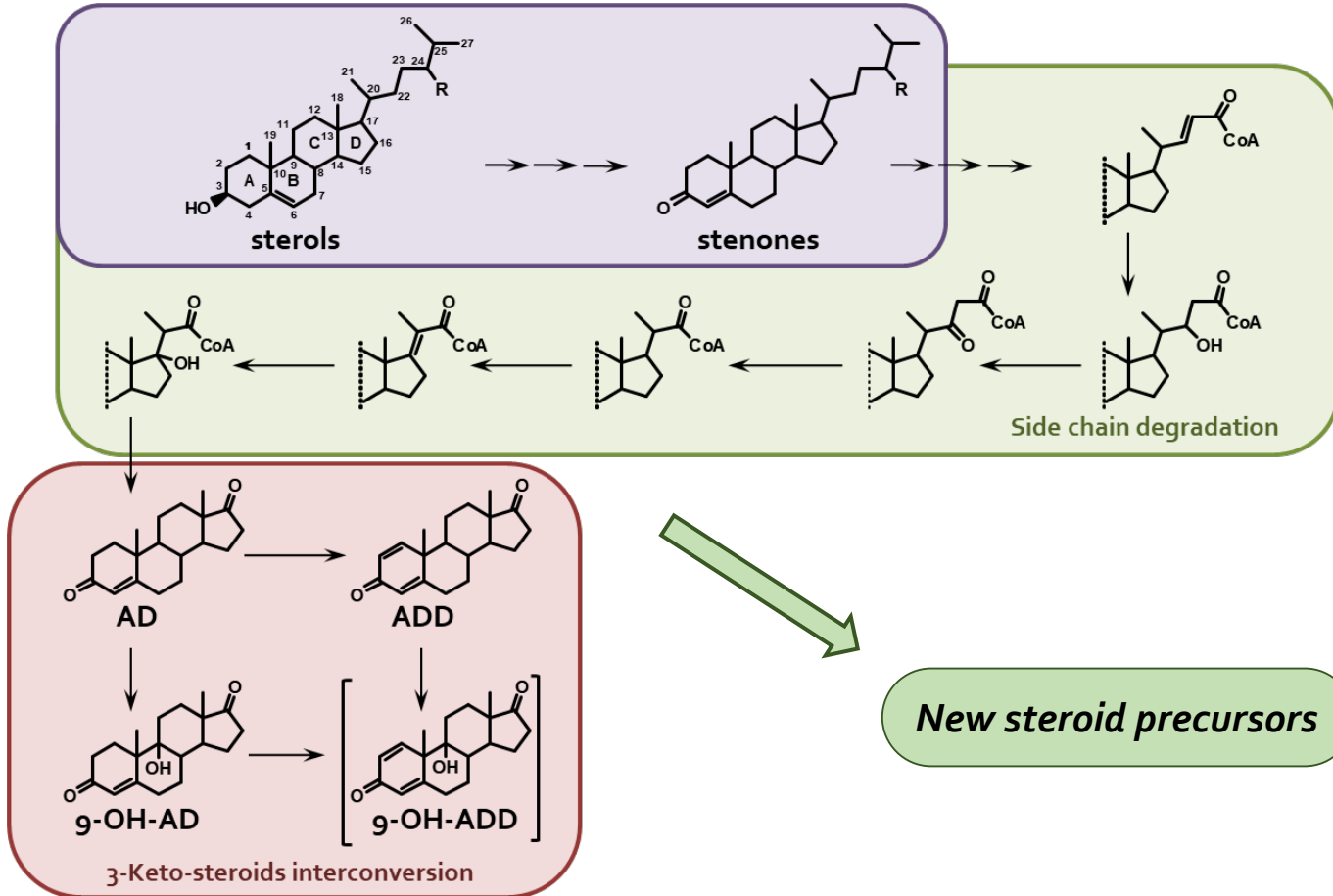
*Proteomics*



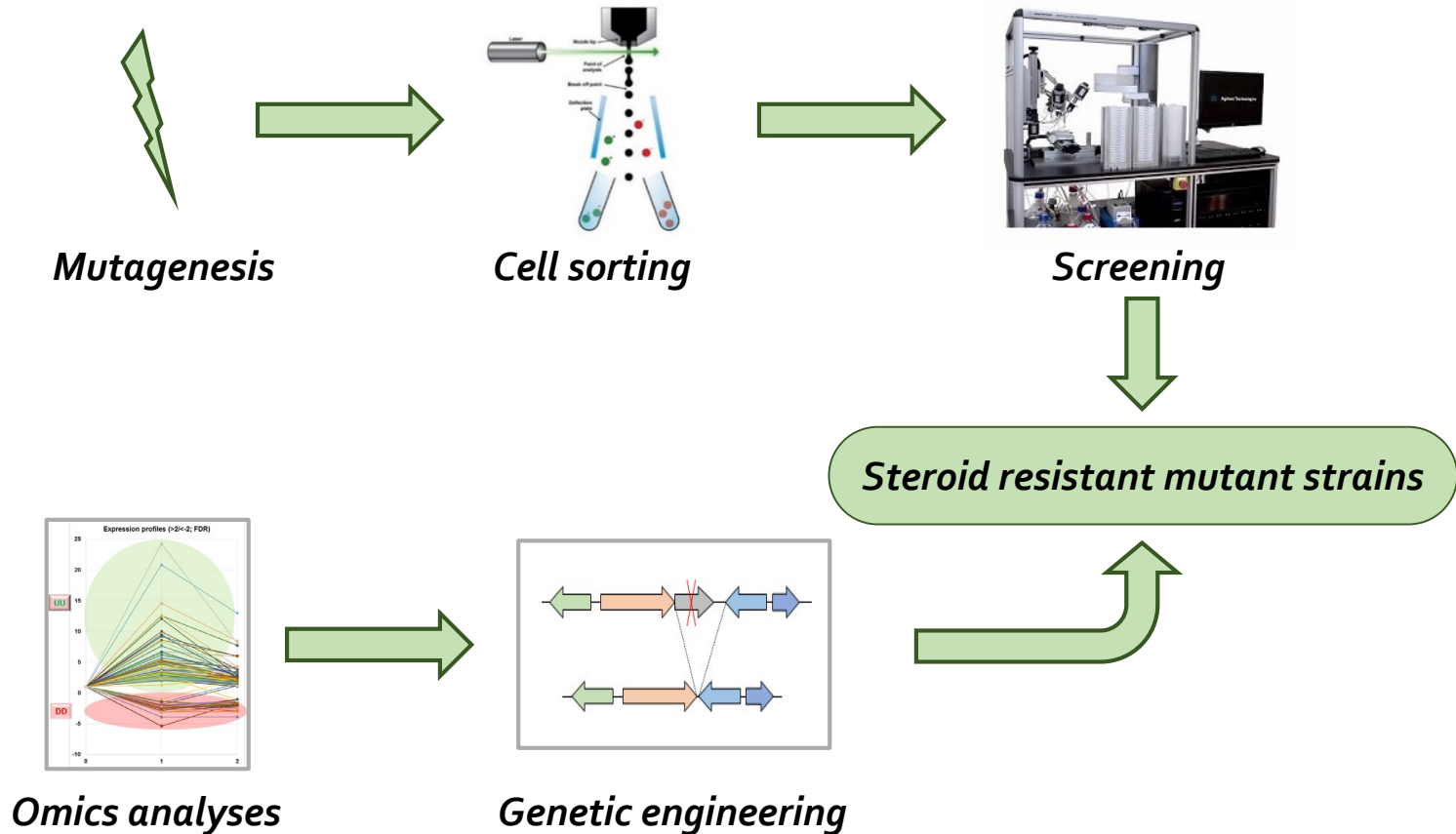
*Transcriptomics*



## WP2. Engineering bacterial strains for steroid production

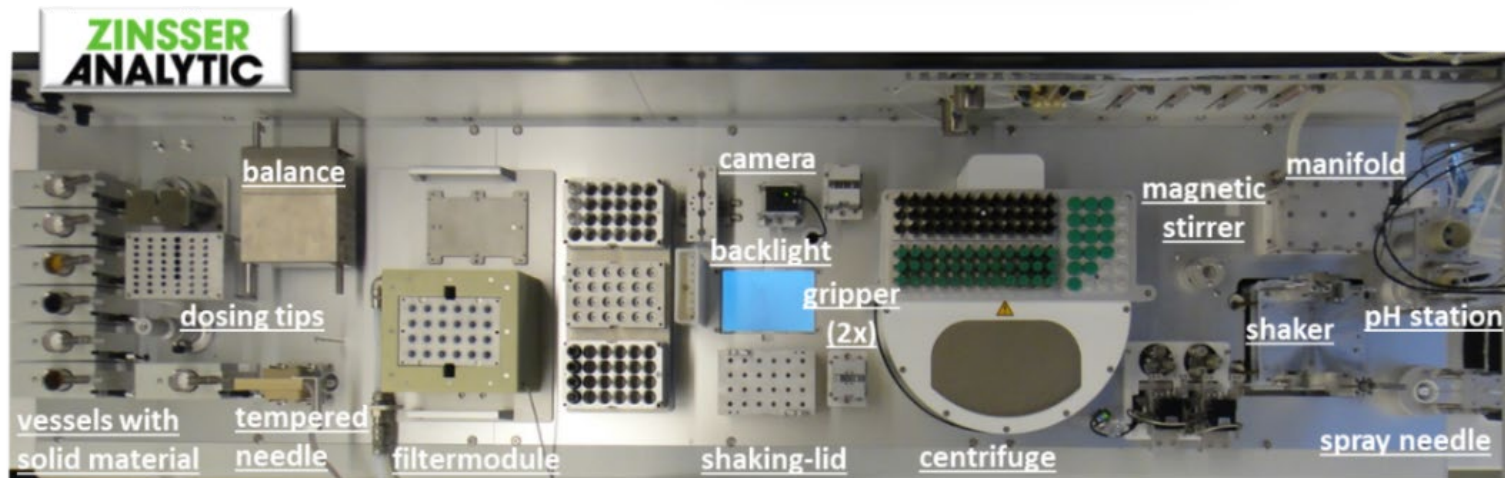
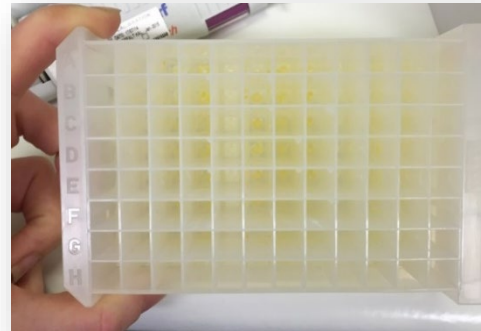


# WP2. Engineering bacterial strains for steroid production



## WP3. Process design for bioproduction of steroids

- Miniaturized cultivations
- Downstream processing



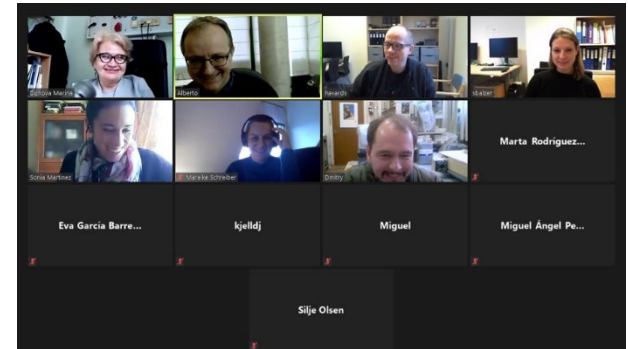
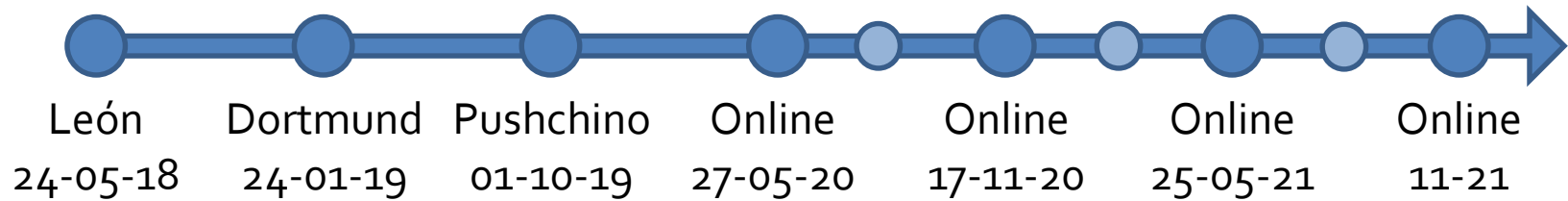
## WP4. Scale up and demonstration





## WP5. Management and Dissemination

- Project management and coordination



## WP5. Management and Dissemination

### ● Data Management



#### Syntheroids - Data Management

Share Room AN SU S AS +11

- 📁 Data Management Plan
- 📁 Deliverables
- 📁 Dissemination
- 📁 Literature-References
- 📁 Meetings
- 📁 Project formalities
- 📁 Protocols and SOPs
- 📁 Strain Information

## WP5. Management and Dissemination

- Dissemination and communication

Priority 1:  
Pharmaceutical  
companies

Industrial  
Fairs



Priority 2:  
Scientific  
community

Publications  
  
Congresses

BMC Biotechnology

Phytochemistry

Steroids



PROCESSNET  
EINE INITIATIVE VON DECHEMA UND VDI-GVC



Priority 3:  
General  
public

@Syntheroids

Mass media



Diario de León

La Nueva Crónica  
*Pura información de León*

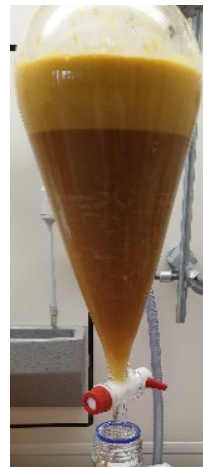
ELMUNDO

# WP5. Management and Dissemination

- Life Cycle Assessment (LCA)



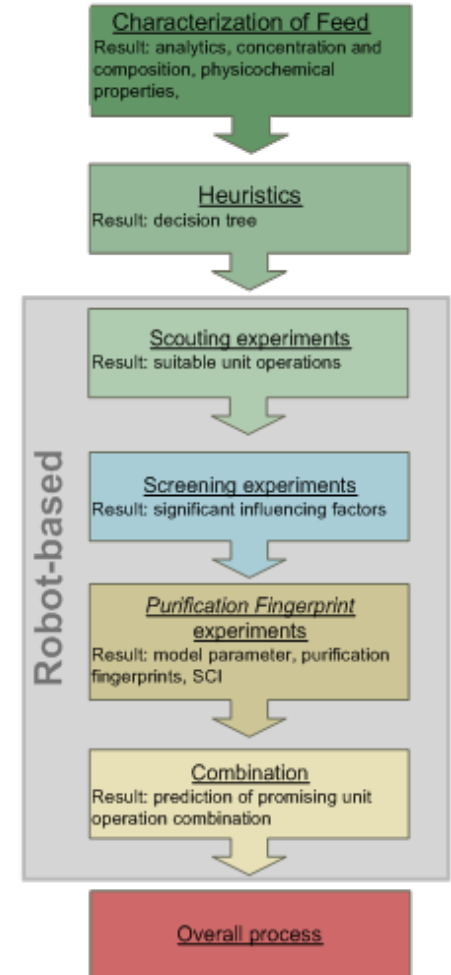
USP →



DSP →



Purified product





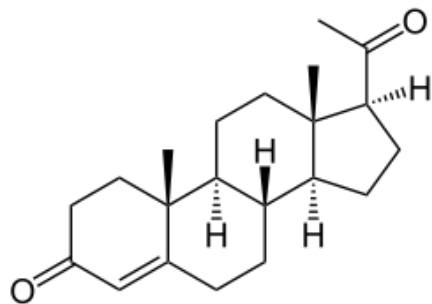
## WP5. Management and Dissemination

- Exploitation

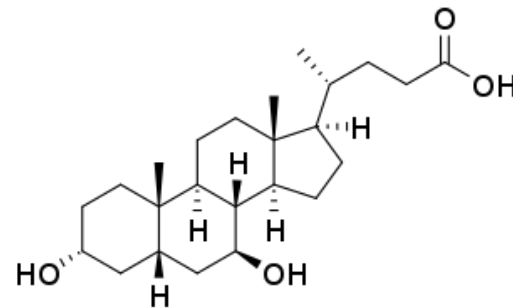


- *Gender/diversity/culture dimension in the project*

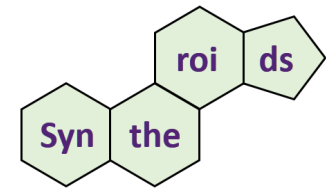
- *Diversity in gender, country of origin and age of people involved in the project*
- *Diversity reflected in the recipients of steroids produced by precursors*



Progesterone



Ursodeoxycholic acid



- **Starting TRL → 3** (*experimental proof of concept*)
- **Proposed final TRL → 6** (*technology demonstrated in industrially relevant environment*)
- **TRL reached → 5** (*technology validated in industrially relevant environment*)
- **Main bottlenecks:** *Strain used was not a real industrial strain*  
*Production of by-products*

## O1. Omics data integration from steroid producing Actinobacteria

Bragin et al. *BMC Biotechnology* (2019) 19:39  
<https://doi.org/10.1186/s12896-019-0533-7>

BMC Biotechnology

**RESEARCH ARTICLE** Open Access

Genome-wide response on phytosterol in 9-hydroxyandrostenedione-producing strain of *Mycobacterium* sp. VKM Ac-1817D

Eugeny Y. Bragin<sup>1,2\*</sup>, Victoria Y. Shtratnikova<sup>4</sup>, Mikhail I. Schelkunov<sup>3,5</sup>, Dmitry V. Dovbnya<sup>1,2</sup> and Marina V. Donova<sup>1,2</sup>




Shtratnikova et al. *BMC Biotechnology* (2021) 21:7  
<https://doi.org/10.1186/s12896-021-00668-9>

BMC Biotechnology

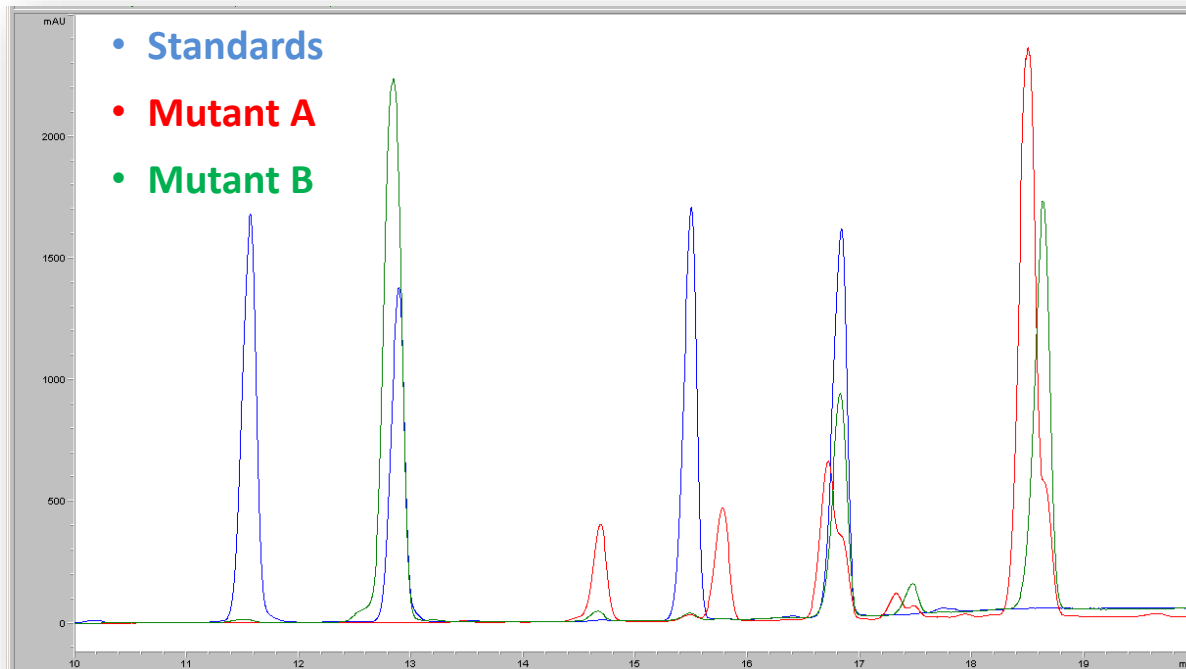
**RESEARCH ARTICLE** Open Access

Different genome-wide transcriptome responses of *Nocardioides simplex* VKM Ac-2033D to phytosterol and cortisone 21-acetate

Victoria Yu Shtratnikova<sup>1\*</sup>, Mikhail I. Schelkunov<sup>2,3</sup>, Victoria V. Fokina<sup>4,5</sup>, Eugeny Y. Bragin<sup>4</sup>, Andrey A. Shutov<sup>4,5</sup> and Marina V. Donova<sup>4,5</sup>

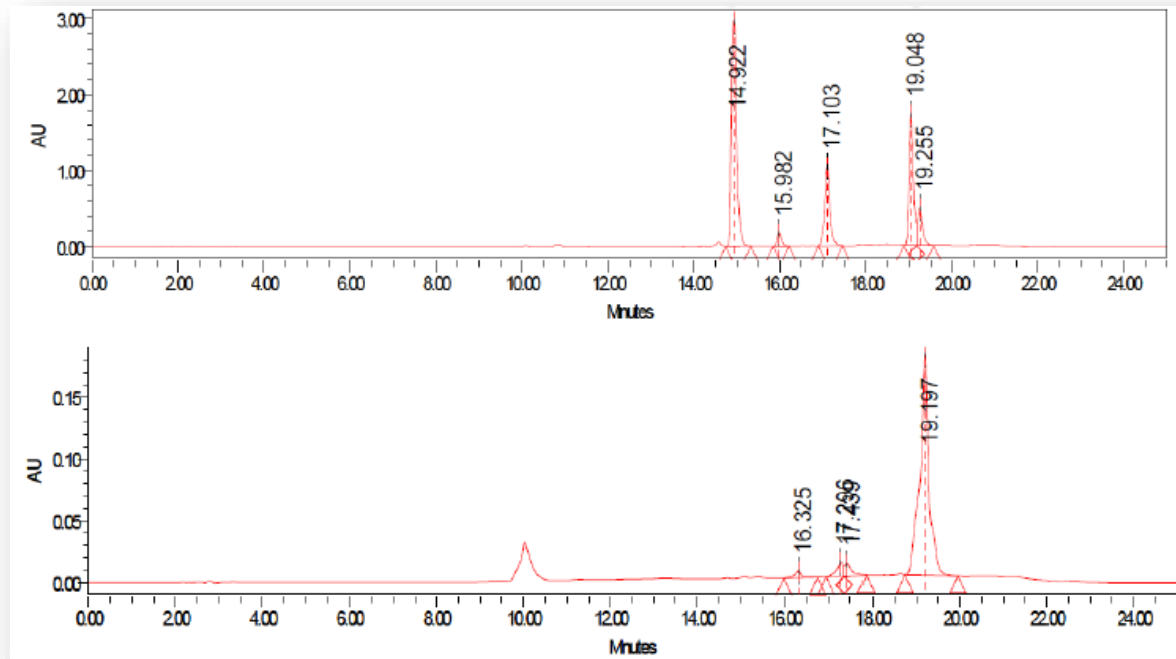


## O2. Creating engineered bacterial strains producing C-22 steroids



- *Enzymes with low substrate specificity*
- *Identification of by-products which can be also used as steroid precursors*

## O2. Creating engineered bacterial strains producing C-22 steroids



- Improved production modifying the composition of culture medium

## O2. Creating engineered bacterial strains producing C-22 steroids

Phytochemistry 169 (2020) 112160



Contents lists available at ScienceDirect

**Phytochemistry**

journal homepage: [www.elsevier.com/locate/phytochem](http://www.elsevier.com/locate/phytochem)

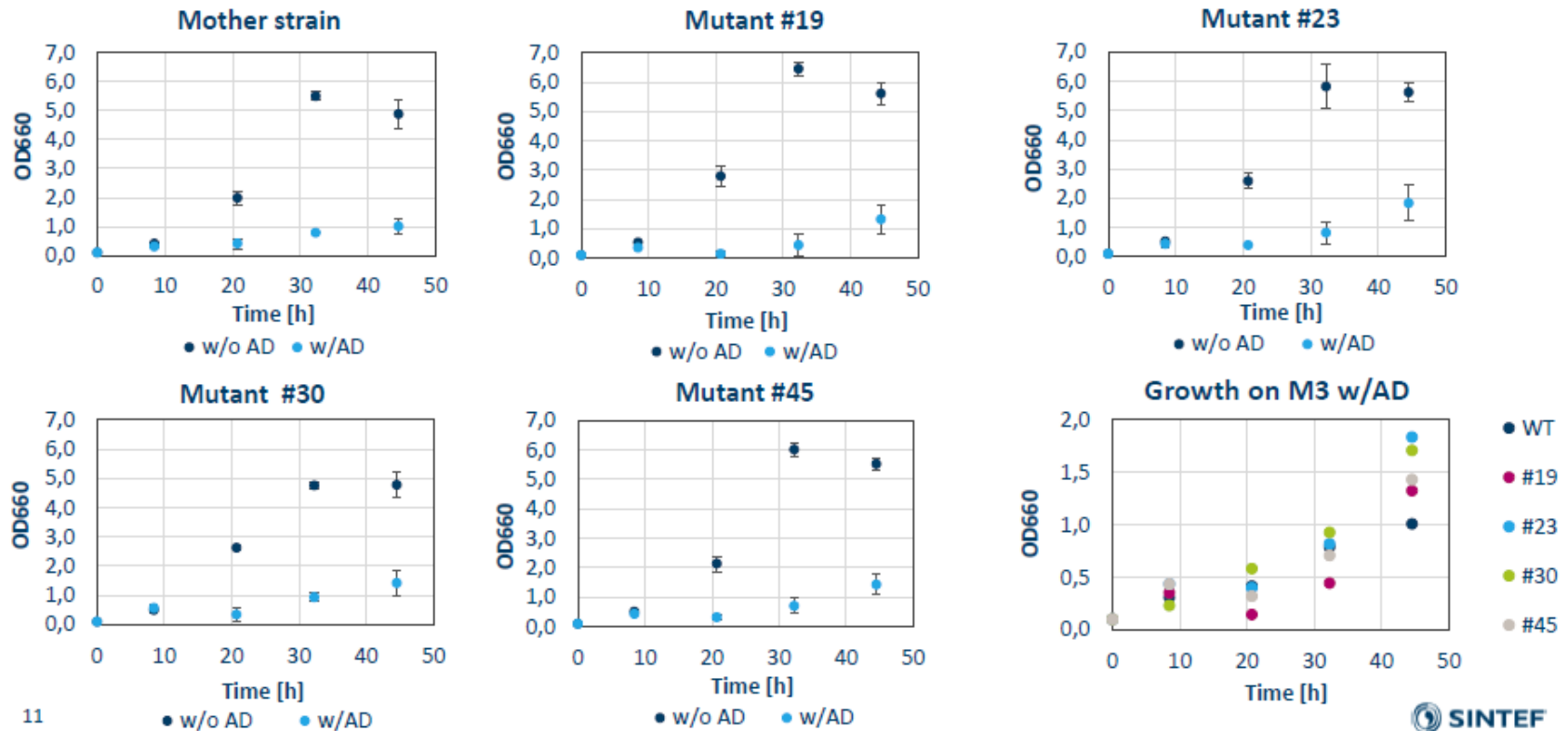
**Biotransformation of androstenedione and androstadienedione by selected *Ascomycota* and *Zygomycota* fungal strains**

Vyacheslav Kollerov<sup>a,b,\*</sup>, Andrei Shutov<sup>a,b</sup>, Alexey Kazantsev<sup>c</sup>, Marina Donova<sup>a,b</sup>

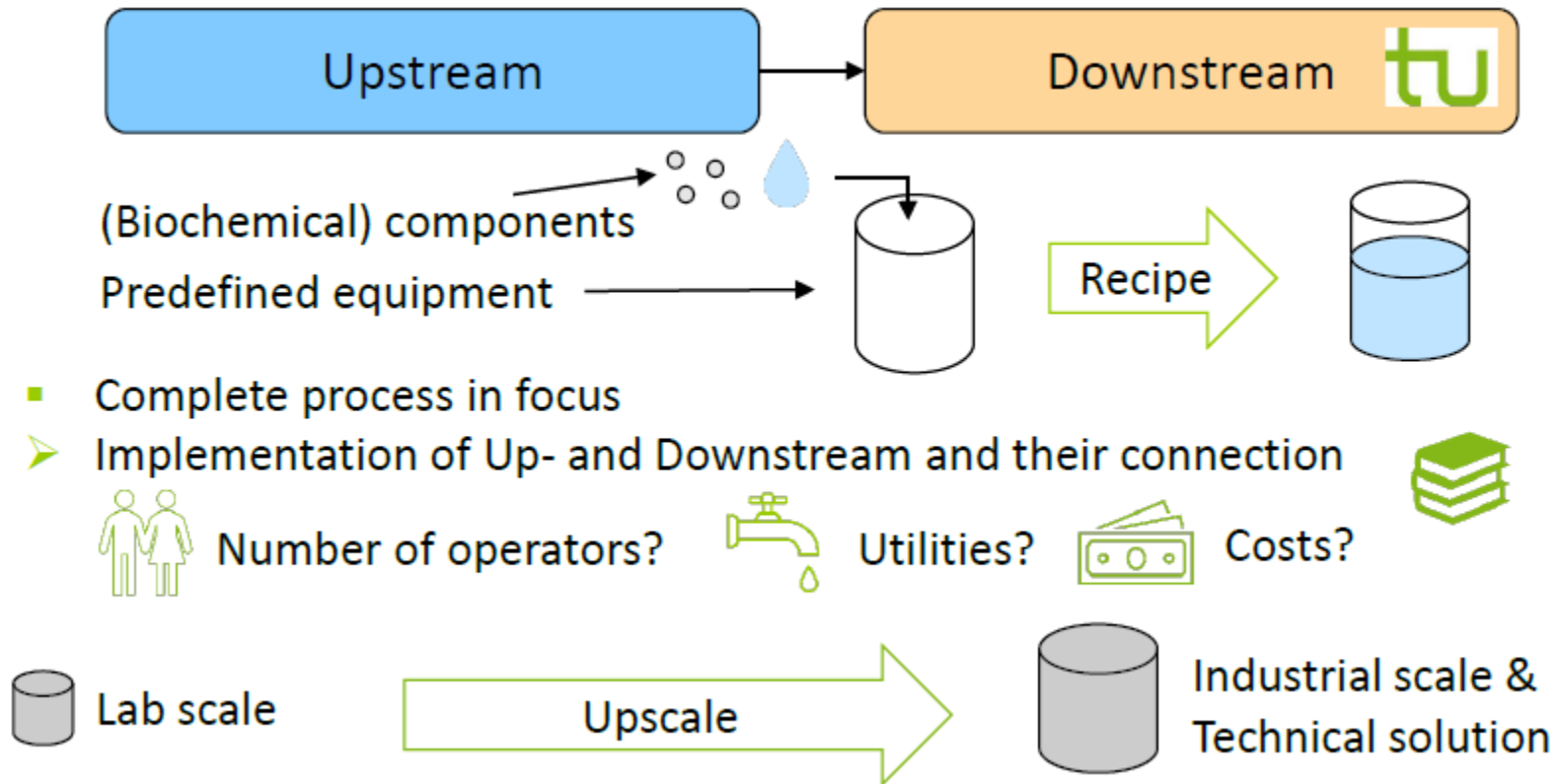

- *Ability to catalyze structural modifications of 3-oxo-androstane steroids*

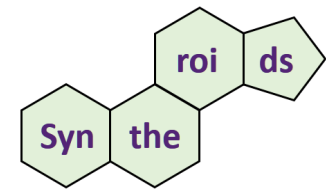
## O3. Reduce or eliminate end-product inhibition





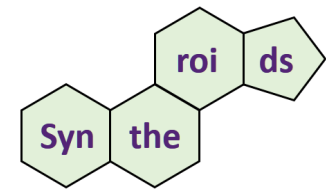
## O4. Integrate up- and downstream processes





## Objectives proposed:

- O1. Omics data integration from steroid producing Actinobacteria
- O2. Creating engineered bacterial strains producing new steroids
  - ✓ C-22 precursors
  - ✓ 1-dehydroanalogs
  - ✓ Hydroxylated androstanes
- O3. Reduce or eliminate end-product inhibition
- O4. Integrate up- and downstream processes



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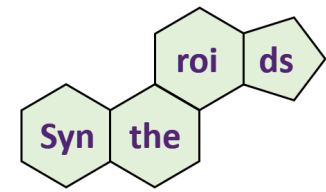
E-mail: [simone.balzer.le@sintef.no](mailto:simone.balzer.le@sintef.no)

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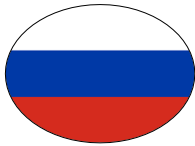
E-mail: [JoseLuis.BarredoFuente@curiaglobal.com](mailto:JoseLuis.BarredoFuente@curiaglobal.com)



● *The National funding Agencies*



*Ministry of Science, Innovation and Universities*



*Ministry of Science and Higher Education*



*The Research Council of Norway*



*Federal Ministry of Education and Research*



*The Centre for the Development of Industrial Technology*