SEQENS

OUR SCIENCE FOR YOUR FUTURE

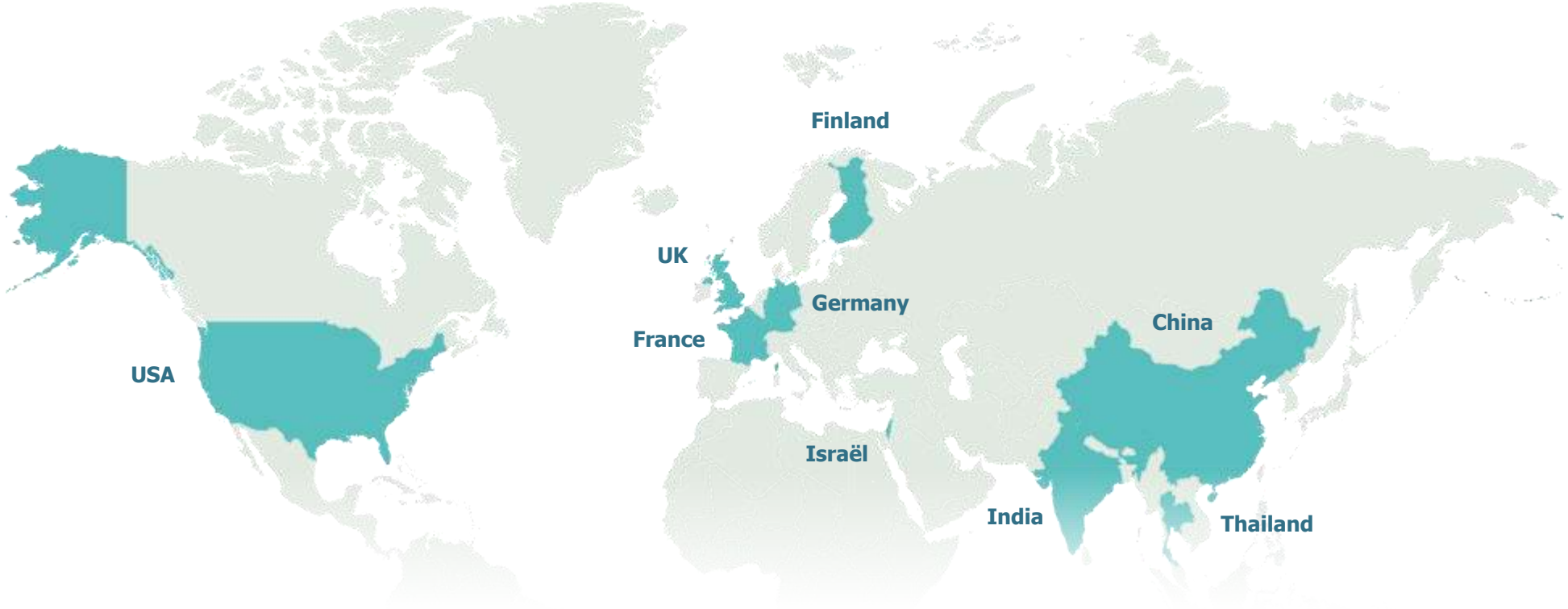
The power of Biocatalysis in the development of Active Pharmaceutical Ingredients

December, 5th 2024

Juliette Martin, PhD
SEQENS
Scientific Communication Manager

Seqens at a glance

SEQENS is an integrated global leader
in health, personal care and specialty ingredients



>€1,1BN
Revenue

c.3,300
Employees

1,500+
Clients

16
Manufacturing Sites

09
Countries

State of the art science platform

09 R&D
Centers

>300
Research scientists & experts (130 PhDs)

>12,000 SQM
Lab surface

LABS
Up to 50L & 7 Pilot Plants

SEQENS

SEQENS Biotechnologies

Comprehensive global R&D platform with unique scientific skills

Complementary biotechnology solutions

Protéus by SEQENS

Equipment
1,000 sqm
1 kilo-lab
3 L, 40 l, 300 L fermentation
DSP capabilities
Robotic platform HTS

Product categories
Pharma solutions
Cosmetics
Food & feed
Chemicals

Expertise
Biocatalysis services
Enzyme screening
Biocatalyst devpt. & optimization
Biocatalysis scale-up
Multi-tons manufacturing

Alganelle

Equipment
100 sqm
Lab-scale production
3 L, 20 L & 30 L Photobioreactors
DSP capabilities

Product categories
Pharma solutions
Cosmetics
Biomedicals
Biomaterials

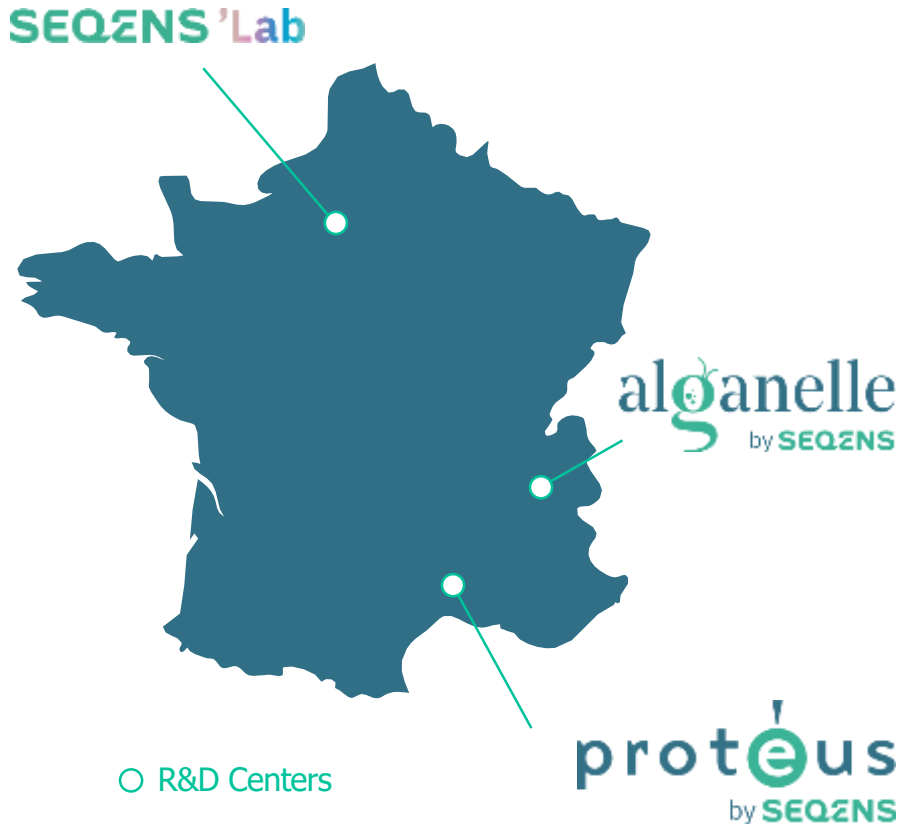
Expertise
Synthetic biology
Metabolic engineering
Microorganism engineering
(microalgae, bacteria, yeast)
Recombinant production
(metabolites, bioactive
peptides & proteins)

SEQENS'Lab

Equipment
5,000 sqm
3 kilo-lab suites
3 cGMP pilot plants
(incl. 1 flowchem pilot)
Robotic platform HTE

Product categories
Pharma solutions
Specialty ingredients

Expertise
Flow chemistry
Sloid-state design
High potency API devpt.
Analytical excellence
Quality by design



Towards more Sustainable Manufacturing Process

In chemical industry, some key challenges include:

- develop more **sustainable** processes for chemical manufacturing (pharma, fine chemicals, active ingredients, etc…) within **greener synthetic steps** and **process intensification**.

- more pressure to **accelerate** the development stages for reaching **competitive** processes.


Improve yield

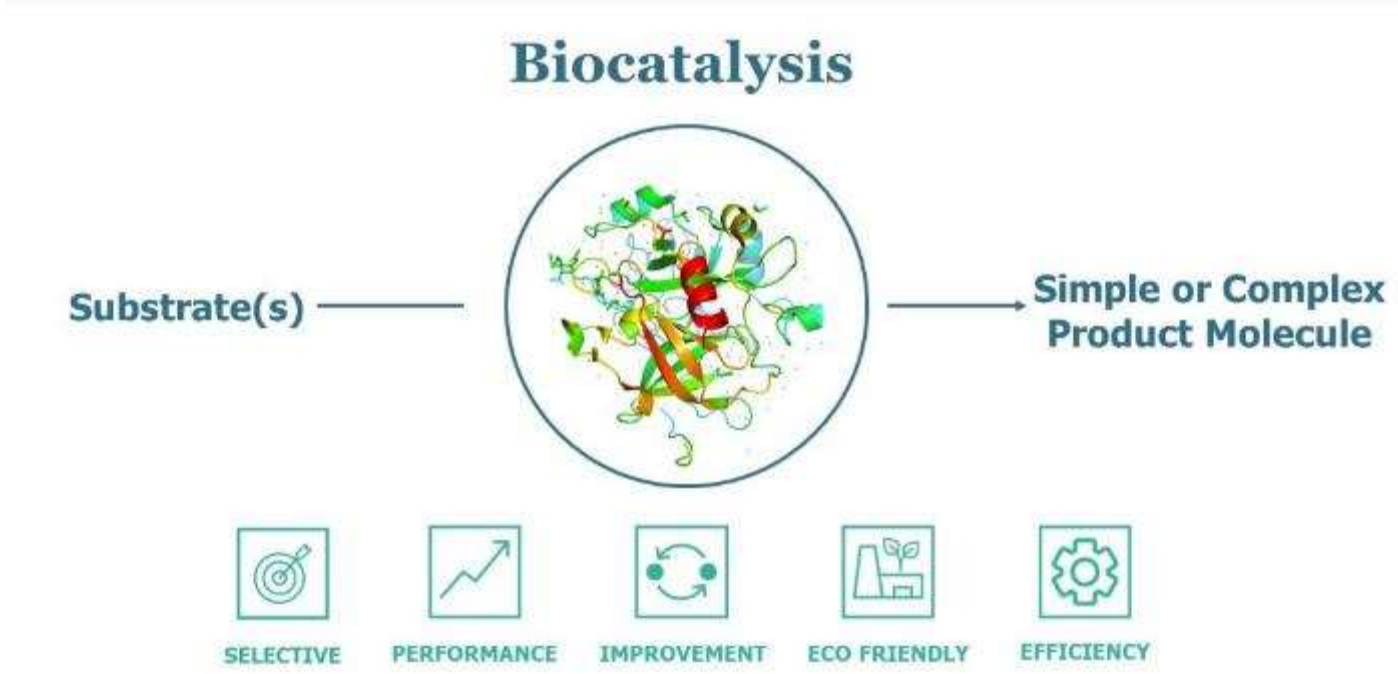

Improve selectivity → higher purity

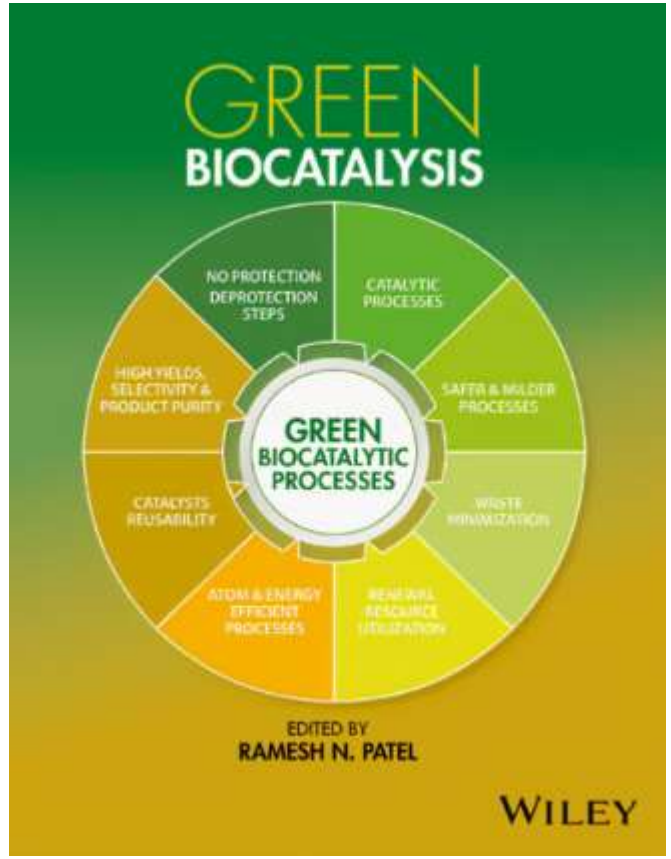

Shorter cycle time


Access to wider reaction types

SEQENS

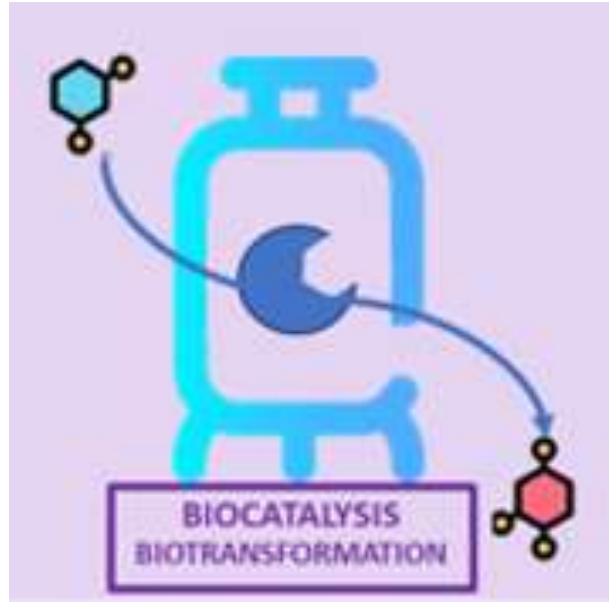
Biocatalysis means greener chemistry





Biocatalysis in Organic Chemistry

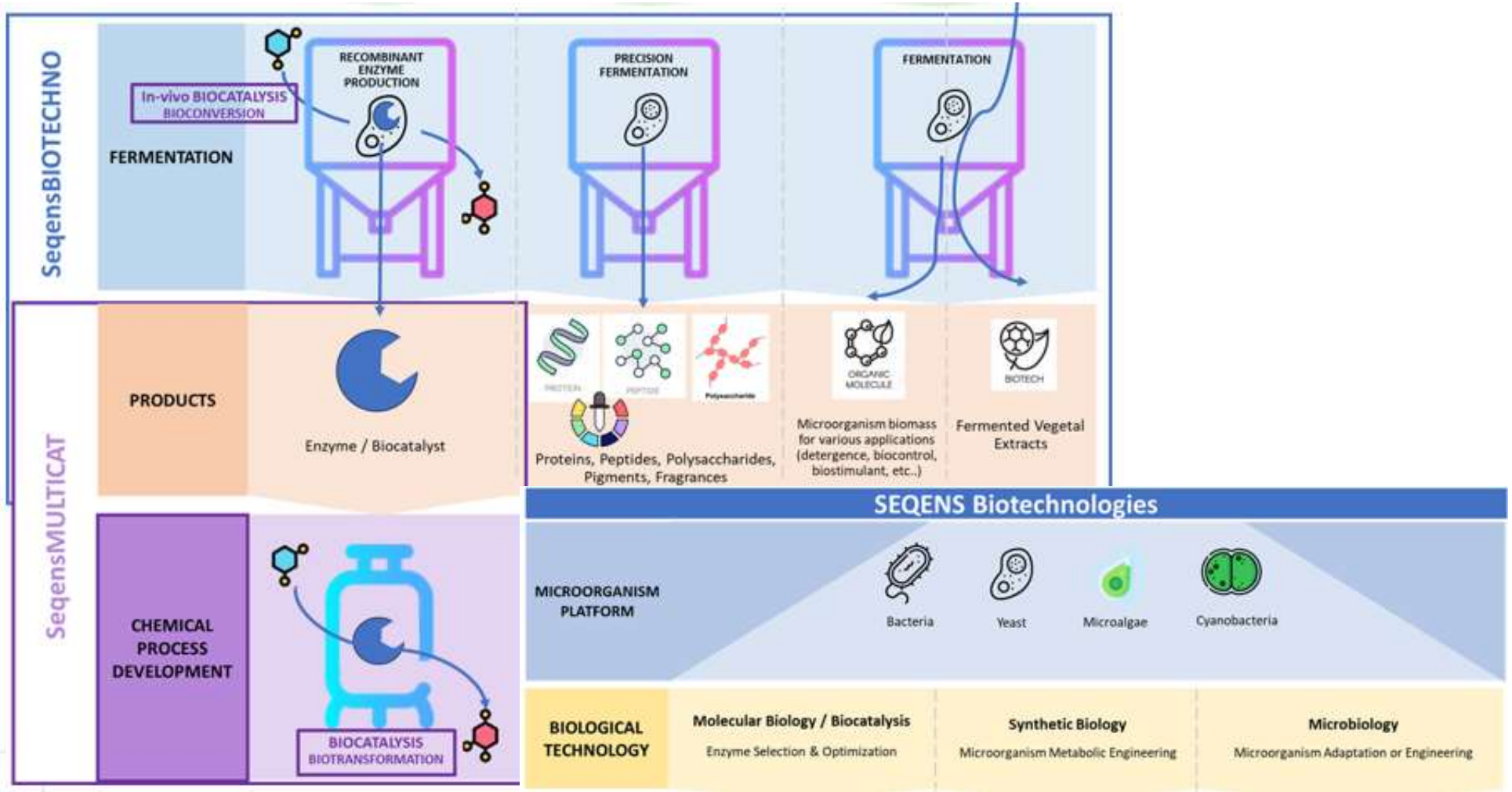
- **Enzymes benefits for organic chemistry**
  - Green chemistry principles
  - Operate in mild conditions
  - Biodegradable and non toxic
  - Enantio-, regio-, chemoselectivity



- The term **biocatalyst** is rather imprecise, as it is employed, for example, for:
- a **wild-type organism**,
- a **single** enzyme,
- a **crude** enzyme preparation,
- or an enzyme (preparation) **immobilized** on a carrier

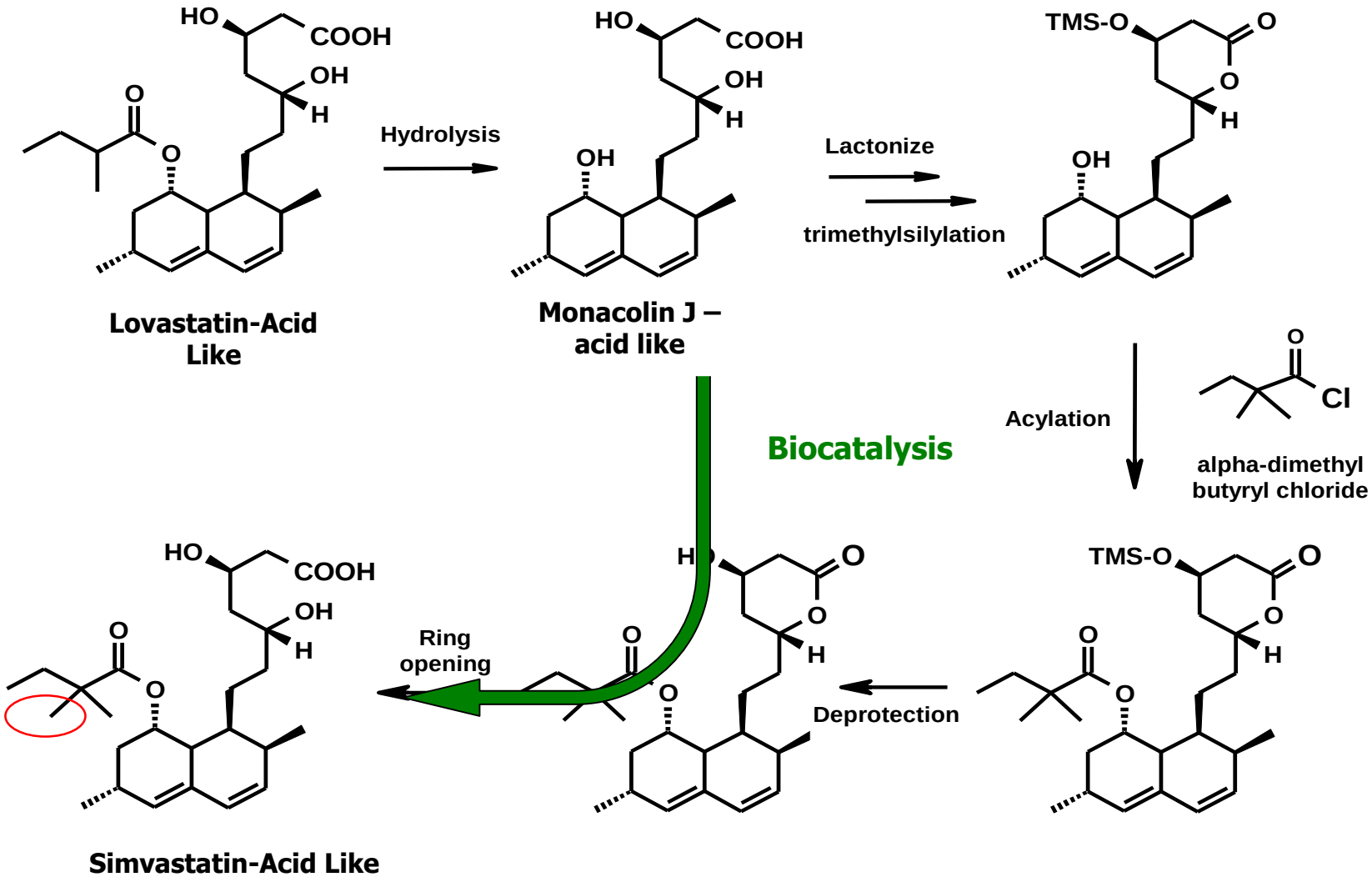
- The preparation of the catalyst requires, in general, the machinery of the protein synthesis of microorganisms via fermentation.
- The production of enzymes will take, in general, between few hours up to a few days, in one step process (pretty quick compared to multistep syntheses of some nonnatural ligands).

Using living machineries



Case Studies

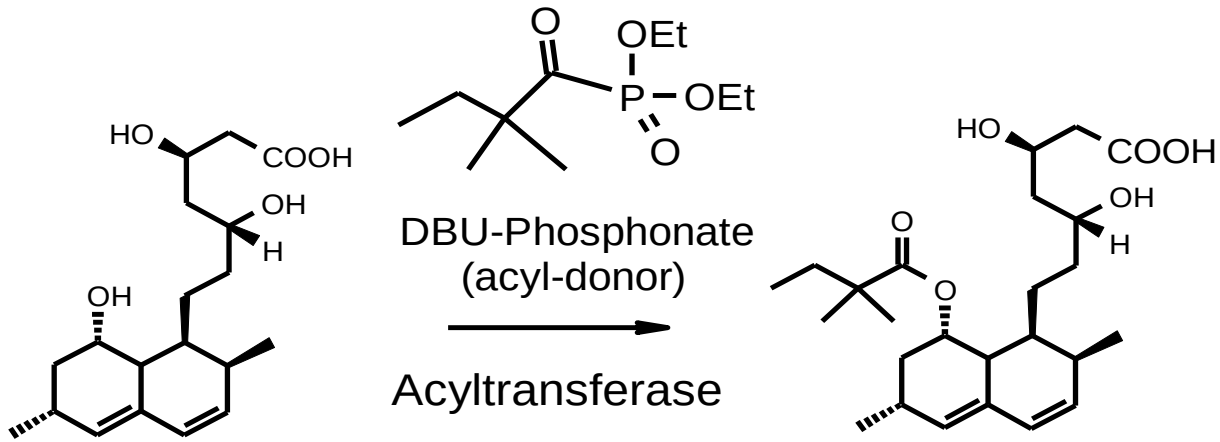
The Power of Biocatalysis – Case study 1



Aspergillus terreus

- ✓ **Reduce chemical steps:** protection/deprotection, waste, purification steps
- ✓ **Enzyme specificity** avoid **quality deviation** (manufacturing)
- ✓ **Cost reduction**

The Power of Biocatalysis – Case study 1



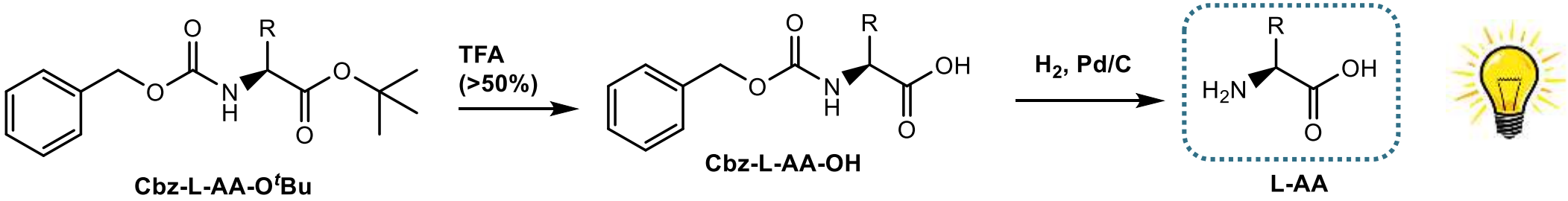
Monacolin J acid **Simvastatin acid**

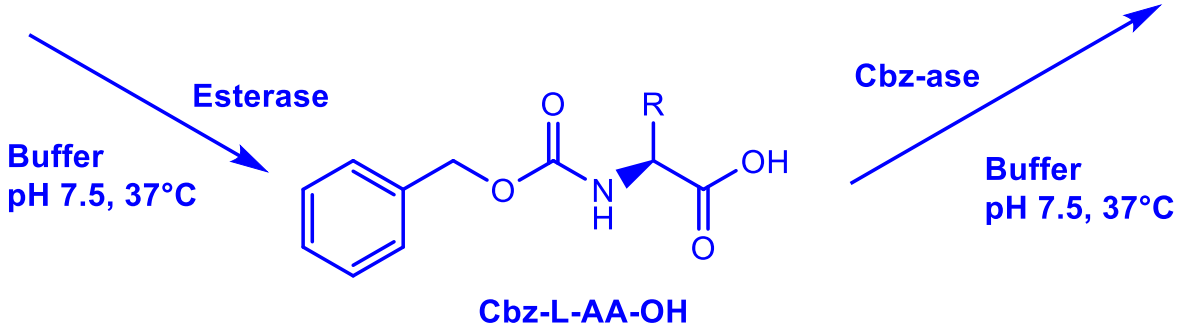
Within multicatalysis expertise, our chemists have designed a new acyl donor suitable with enzyme process operability

Benefits of Acyl-phosphonates (Technology patented by SEQENS)

- Low cost acyl donors, easy to produce,
- Acyl transferases accomodate acyl-phosphonates donors,
- Non reactive leaving groups.

Developing leaving groups synthesis with enzymes





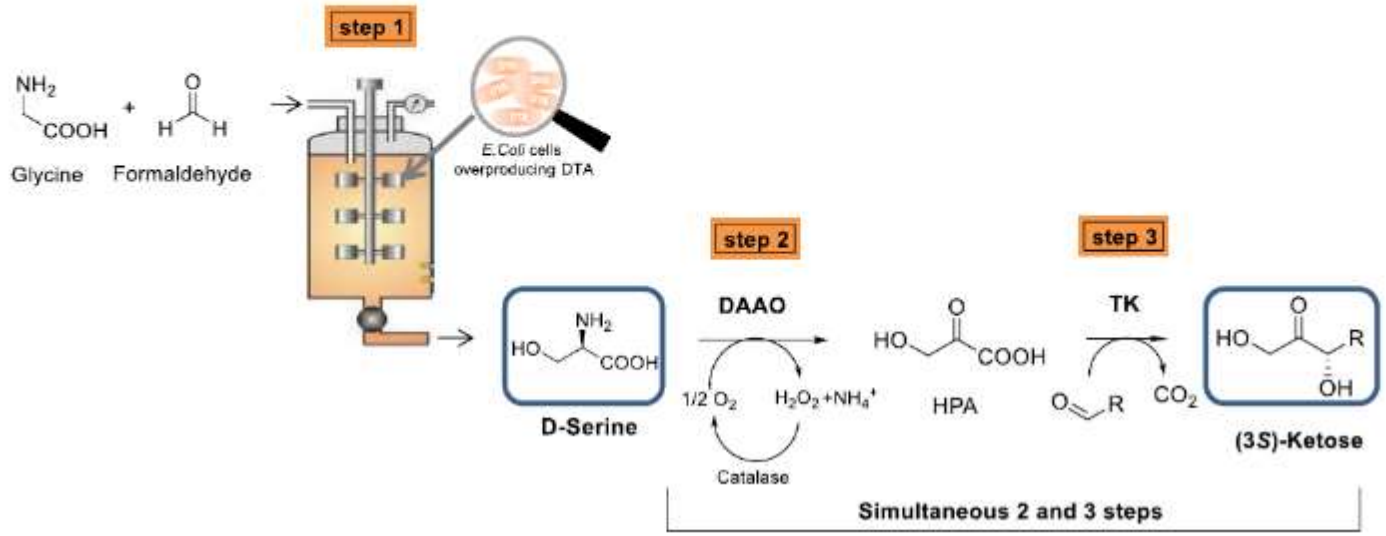
Campopiano et al. Faraday Discuss., 2024,252, 174-187

Opportunity to investigate new protecting groups that are typically known in biocatalysis

D-serine as a key building block: enzymatic cascades

D-Serine as a Key Building Block: Enzymatic Process Development and Smart Applications within the Cascade Enzymatic Concept

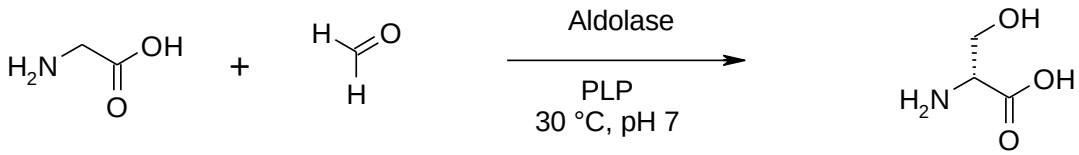
Nazim Ocal, Mélanie L'enfant, Franck Charmantray, Loredano Pollegioni, Juliette Martin,* Pascal Auffray, Jérôme Collin, and Laurence Hecquet*



Org. Process Res. Dev. 2020, 24, 5, 769–775

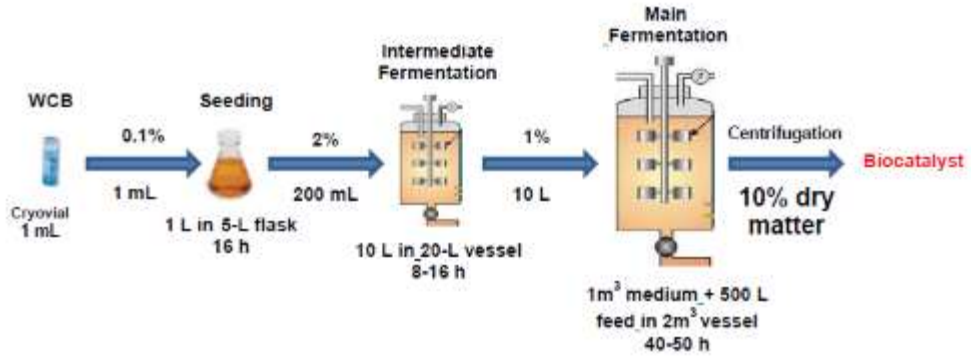
Multi-ton production using biocatalysis at Seqens: D-Serine

Seqenzym® AL



Glycine **D-Serine**

Parameters	Process Values
pH	7 ± 0,5
$MgCl_2$	0.003 eq
PLP	0.05% w/w
Base	KOH 35.7%
Temperature	30°C
Conversion rate of both substrates	~90%



Biocatalysis developed as an alternative to conventional chromatographic separation process

High specificity: ee > 99,9%

High substrate concentration > 30%

Enzyme fermentation at 15 m3-scale

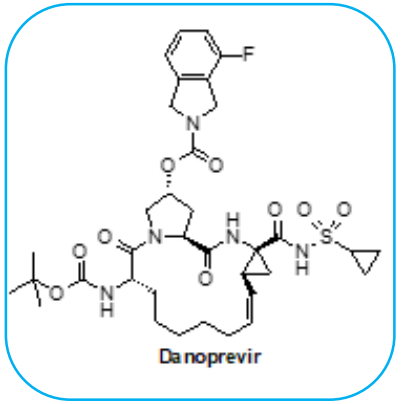
Enzyme impact on production cost is below 6%

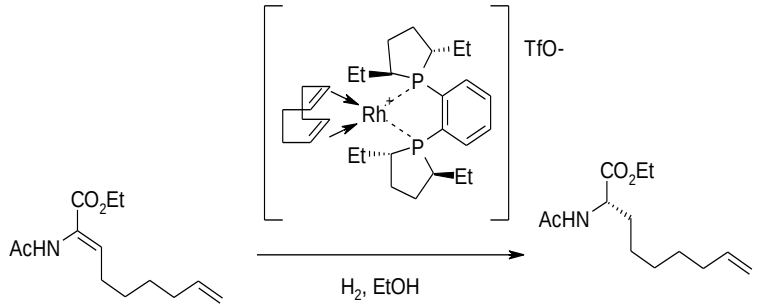
Enzyme residues in final product are under detection limit

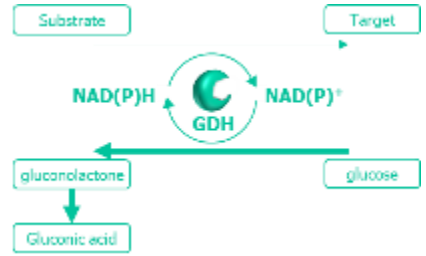
Seqens Biotechnologies

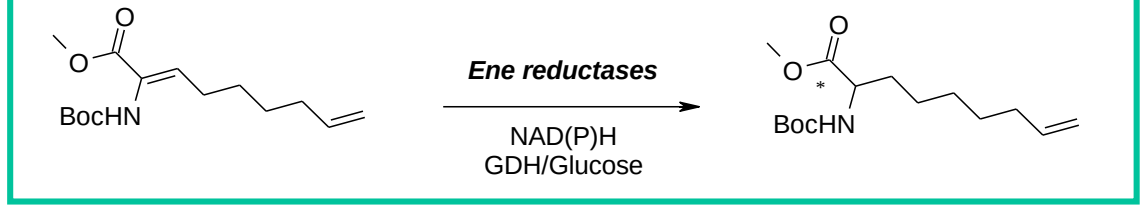
Enzymes for Precious Metal Replacement

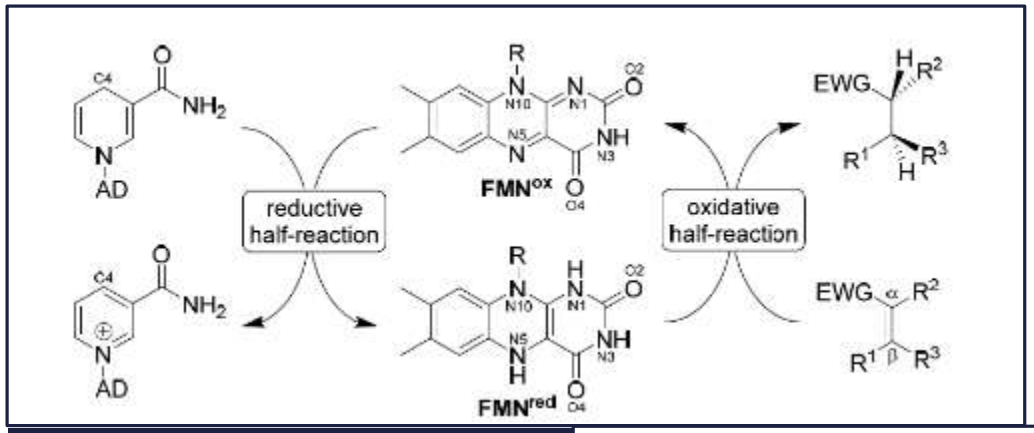
ENE-REDUCTASE (ERED) for precious transition metal replacement



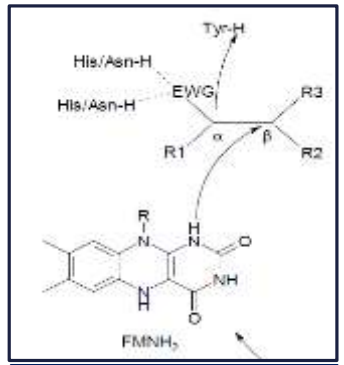








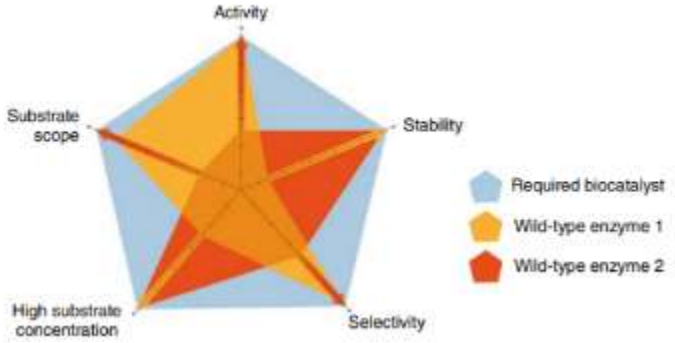
EWG: electron withdrawing groups | Aldehydes, ketones >>> carboxylic acids, nitriles, esters

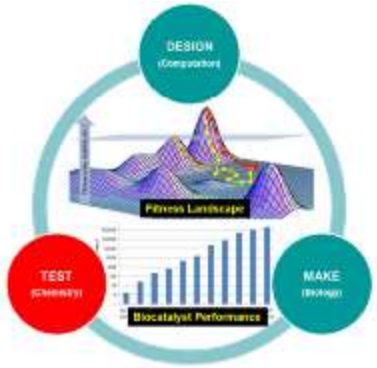


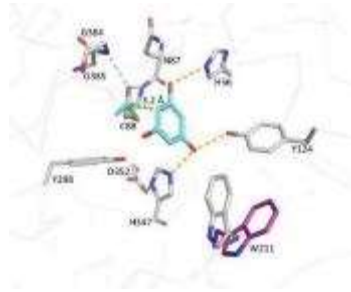
Mechanism

- ERED catalyse asymmetric CC double bond reduction
  - ⇒ **Allow introduction of asymmetric center(s) on the targeted molecules**
- Cofactors: FMN & NAD(P)H
  - ⇒ **Require NAD(P)H recycling**
    - Glucose dehydrogenase (GDH) with glucose can be utilized
    - Alternatives: ADH with isopropanol or FDH with formate

Industrial enzymes : reaching specifications (technico-economics)





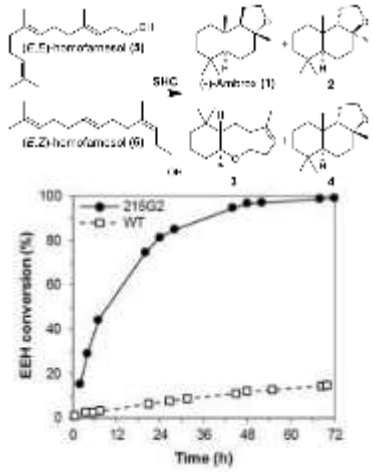


Truppo, ACS Med. Chem. Lett., 2017.

Evolution & DoE

Dynamic Modelling

Smart evolution



- Conversion obtained with 125g/L of substrate with the best variant
- 1x directed evolution
- DoE x10 improvement

	Substrate conversion
Improved enz1	~98%
Enz1	~10%
Enz2	~46%
Enz3	~67%
Enz4	~20%
Enz5	~27%
Enz6	~97%

SEQENS

Pioneer in enzyme optimization

Tailored enzymes, development & scale-up of biocatalytic processes from lab to commercial scale

BIOMASS TREATMENT ENZYMES

Amylases	6
β-glucosidases	5
Cellulases	5
Xylanases	6

COFACTOR RECYCLING ENZYMES

NAD(P)H oxidases	2
Formate dehydrogenases	2
Glucose dehydrogenase	1
L-lactate dehydrogenase	1
L-alanine dehydrogenase	1

HYDROLASES

Dehalogenases	4
Epoxide hydrolases	11
Lipases	263
Nitrilases	14
Proteases	3
Phytases	2

LYASES

Hydratases	15

OXIDOREDUCTASES

Alcohol dehydrogenases	69
Amine dehydrogenases	18
Bayer-Villiger MonoOxygenases	10
Cytochromes P450	4
Ene-reductases	129
Hydroxy Steroid Dehydrogenases	13
Imine reductases	41
Laccases	21

TRANSFERASES

Threonine aldolases	4
Sulfotransferases	3
Transaminases	10

AUTOMATION HTS & HTE PLATFORMS

PROTEIN & STRAIN DESIGN

- Enzyme modeling
- Smart library design
- Machine learning
- Directed & Adaptative Evolution

DATA MINING

- 650+ SEQENZYM enzyme collection
- 5000+ SEQENBIOTICS strain collection
- In silico selection

Created in **1998** and within **SEQENS** since 2017

20 Research scientists & experts

SEQENS

At Seqens, we foster Innovation & Collaborations

Worldwide leader in the development and the production of pharmaceutical active ingredients, intermediates and specialty ingredients

Science & Technology Platform:

- **SeqensUP** (Bioavailability, early-formulation…)
- **SeqensMULTICAT** (Multi-catalysis)
- **SeqensANALYTICS** (Expertise & High tech equipments)
- **SeqensBIOTECHNO** (natural, bio-products)
- **SeqensFAST** (FLOW)
- **SeqensPOLYMERS** (polymer synthesis)

Digitalization Platform:

Advanced Tools = Kinetic Modelling, Statistical Design, Digital Catalysis, PAT

HTE = High Thoughput Experimentation Platform →

← **HTS = High Throughput Screening Platform**

INNOVATION

SEQENS

OUR SCIENCE FOR YOUR FUTURE

Thank you for your attention !

juliette.martin@seqens.com

WWW.SEQENS.COM