

The background of the slide features a close-up, artistic photograph of green leaves, possibly from a plant like a lily or iris, with a soft, blurred effect. The leaves are arranged in a fan-like pattern, creating a sense of depth and natural growth. The colors range from vibrant green to a muted teal, providing a clean and modern aesthetic.

SEQENS

OUR SCIENCE FOR YOUR FUTURE

Tailored-made enzymes for industrial applications:

**Screening from diversity
to enzyme engineering**

Pathway to industrialisation

Which enzymatic biocatalyst solution to convert human made substrate?

- How and where to pick the right enzyme?
- Optimization of R&D conditions
 - Improve the biocatalyst
 - Optimize biocatalytic conditions
- Scale-up process
- Industrialization

FOCUS

Get the first hit!

Apply large range of technologies and know-how to drive the project to industrialization

Integration along the process help to success

Data mining to explore the diversity – get a hit!

Private collection of microorganisms from extreme environments

4,800 Natural strains (Nagoya compliant)

More than 3000 prokaryotic (Meta)genomes

Highly diverse sequences, up to 20 million of available genes

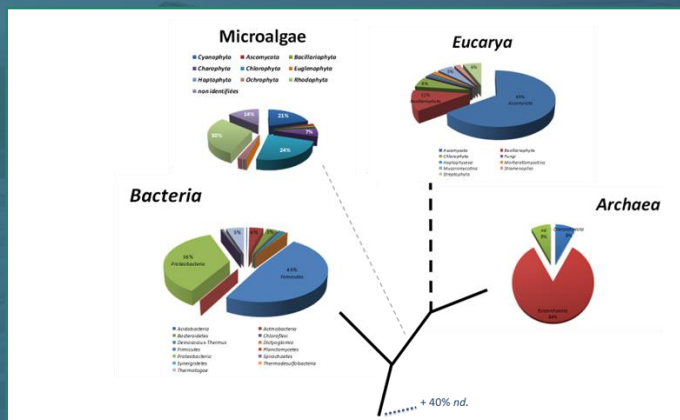
Very high potential for new enzymes discovery

Potential for IP generation

Private genome data mining platform

- Automated genomes annotation and analysis platform
- Genome analysis & exploration
- Metabolism study
- Blast and pattern searches for new enzymes
- Comparative tools to study diversity

High diversity



Worldwide origin

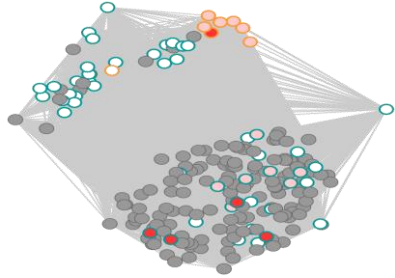


Natural ecosystems

- Deep hydrothermal springs
- Hypersaline anoxic basins, Salt marshes
- Thermal springs
- Volcanic environments, Cold environments
- Oil field
- Industrial & agricultural wastes, soils...



Identification of active & patentable enzymes



Low activity
 Average activity
 Highly active

8 enzymes identified by the customer: starting point

- Dozens of sequences identified in Protéus exclusive collection
- 50 sequences selected for evaluation
- 10 enzymes have shown similar or higher activity, including new patentable sequences

Customer enzyme

Protéus selection:
more efficient
patentable

	Substrate conversion	Identity with client patented sequence
Customer enzyme	~50%	100%
Protéus selection	~80%	99%
Protéus selection	~70%	95%
Protéus selection	~80%	~50%

Substrate defined by customer: starting point

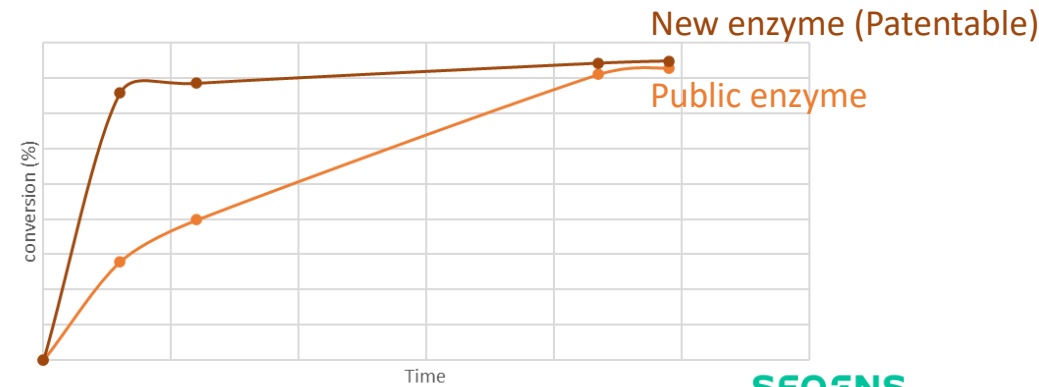
- Compared literature data with well described targets to Protéus collection
- 40 sequences selected for evaluation
- 6 enzymes from our collection have demonstrated specific activity – none from public databases

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

	Enz1	Enz2	Enz3	Enz4	Enz5	Enz6
Enz1	ID	47%	45%	45%	45%	45%
Enz2	47%	ID	67%	66%	68%	77%
Enz3	45%	67%	ID	80%	83%	73%
Enz4	45%	66%	79%	ID	80%	73%
Enz5	45%	68%	83%	80%	ID	73%
Enz6	45%	77%	73%	73%	73%	ID

Seeking for specific phosphorylases

- Compared literature data with well described targets to Protéus collection
- 22 sequences selected for evaluation - 7 enzymes demonstrated specific activity (3 from public databases & 4 from our collection)
- IP issues with publicly available enzymes while Protéus exclusive enzyme offer more patentability options





Strong know-how in Biocatalysis

More than 20 years experience in enzyme optimization & biocatalysis assay development

Custom Biocatalysis process development

Oxido-reductases

- ADH
- ERED
- IRED
- HSDH
- Hydratases
- Amine dehydrogenases
- BVMO
- Cyp450
- Ene-Imine reductases
- Laccases

Hydrolases

- Dehalogenases
- Epoxide hydrolases
- Lipases
- Nitrilases
- Proteases
- Phytases

Transferases

- Threonine aldolase
- Sulfotransferases
- Transaminases

Biomass treatment enzymes

- Amylases
- β -glucosidases
- Cellulases
- Endoglucanases
- Xylanases

Cofactor recycling enzymes

- NADP/H oxydase
- Formate dehydrogenase
- Glucose dehydrogenase
- Lactate dehydrogenase
- L-alanine dehydrogenase

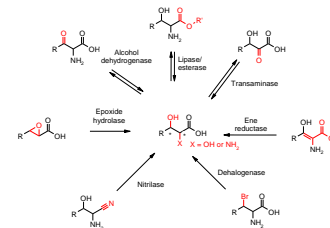
Class of enzymes

600 Enzymes ready for screening

From our discovery work

Tailored kits available

- **Enrichment** of our offer with **additional enzymes** in existing families and new families
- Mixed enzyme families in a plate
- **Customized** amounts on request



Strong know-how

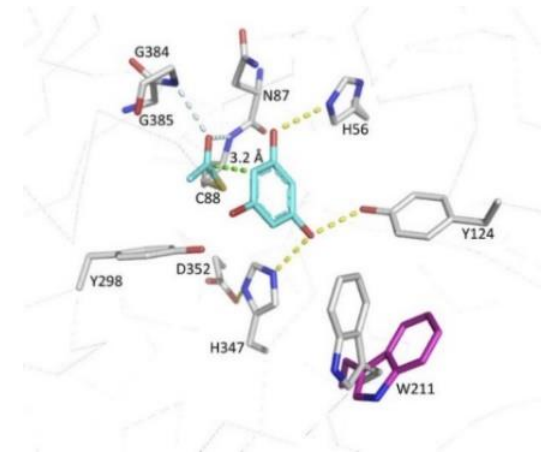
Biocatalysis development on **all classes** of enzymes

Data mining tools for discovery of new enzymes

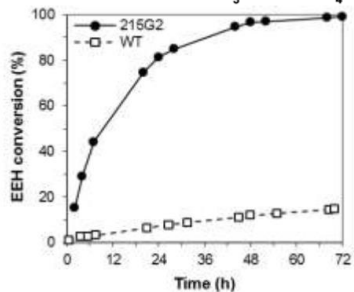
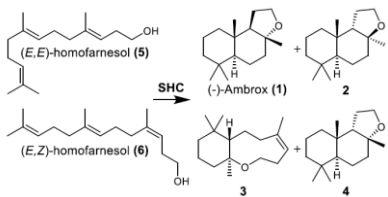
Catalog

R&D optimization – get the conditions right!

- Challenge of reaction conditions
- Selection of the best candidates for further optimizations
- **Evaluate interest for computational modeling**
- Directed evolution – smarter libraries
- **Optimization of the reaction conditions**
(DoE on Automated platform)



Evolution & DoE



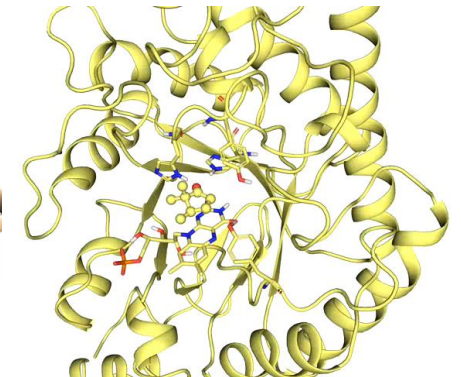
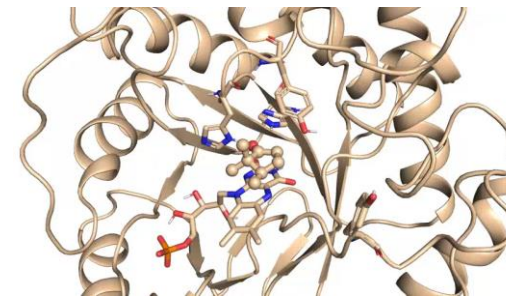
- Conversion obtained with 125g/L of substrate with the best variant
- 1x directed evolution
- DoE x10 improvement

Smart Evolution

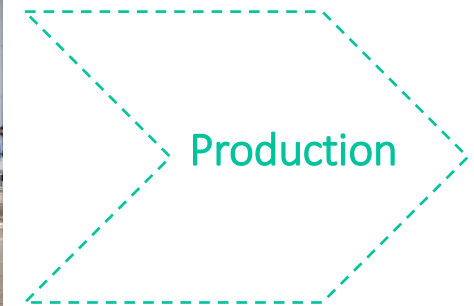
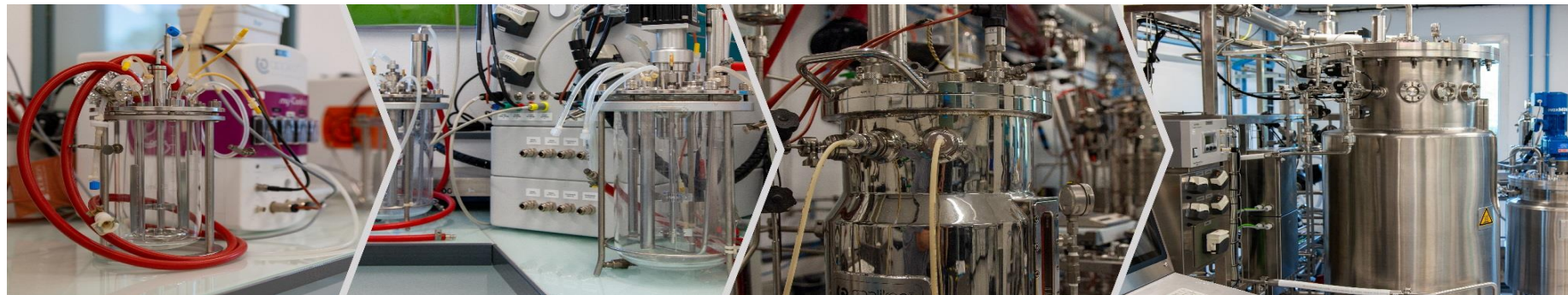
Improved enz1

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

Dynamic Modelling



Scale-up process



Lab-scale (5 – 200 mL) Benchtop bioreactors 1L

- Design of Experiments (DoE)
- Feed stock (Glucose, Glycerol...)
- Fermentation parameters optimization
- 6x 1L Applikon bioreactors

Benchtop bioreactor 3L

- 2x 3L Applikon bioreactors
- 2x 40L Applikon bioreactors
- 300L Global Process Concept (GPC)
- Biomass separation by centrifugation
- Downstream process: cell disruption, membrane separation,...

Pilot plant (40L – 300L)

- Production of a process book
- Biocatalyst process:
At Protéus or transfer to external site
- Biocatalyst reaction:
Integration within SEQENS plants or to customer site

Scale-up examples

Pilot plant (40L – 300L)

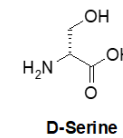
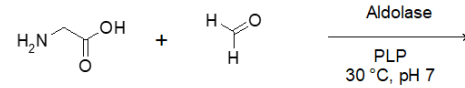
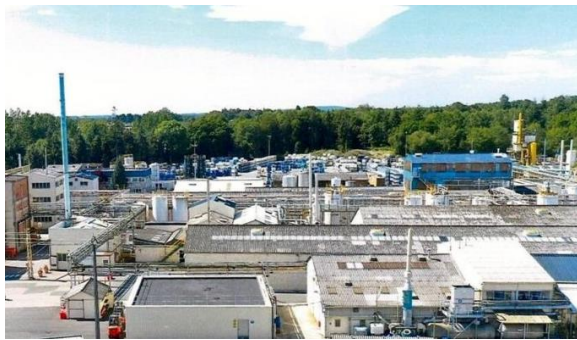
- **Seqenzym® LM**
- Biocatalyst loading 0.2%
- Enzyme cost/kg product is below <5%
- High specificity ee >99.9%
- High productivity 200g/L
- High level of reproducibility

Production (300L – 15m³)

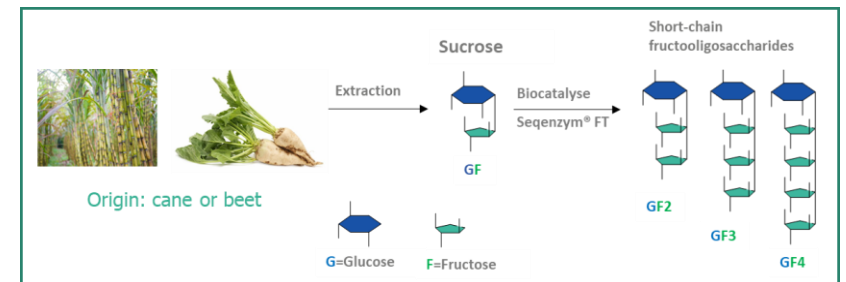
- **Seqenzym® AL**
- High specificity ee >99.9%
- High substrate concentration >30%
- Biocatalyst production up to 15m³
- Enzyme cost/kg product is below <5%
- Enzyme residues in final product are below detection limit

Production (300L – >25m³)

- **Seqenzym® FT**
- Food grade natural enzyme
- Specific activity led to specific composition
- Highly reproducible process



Building block for the synthesis of several APIs



What to remember

There is no universal systematic approaches, and several technologies will be necessary

HT screening is a good start but we still need HT analytical methods !

Computational modelling brought new insight in the way enzymes work but we need to feed them with more data for more accuracy

Industrialization will not compromise between cost and activity. We want both and the development of better solutions for immobilization will help greatly.

For further detail meet
Clement Dince our *Business
Development Manager*



And don't miss his
FLASH CORPORATE
PRESENTATION
tomorrow

SEQENS