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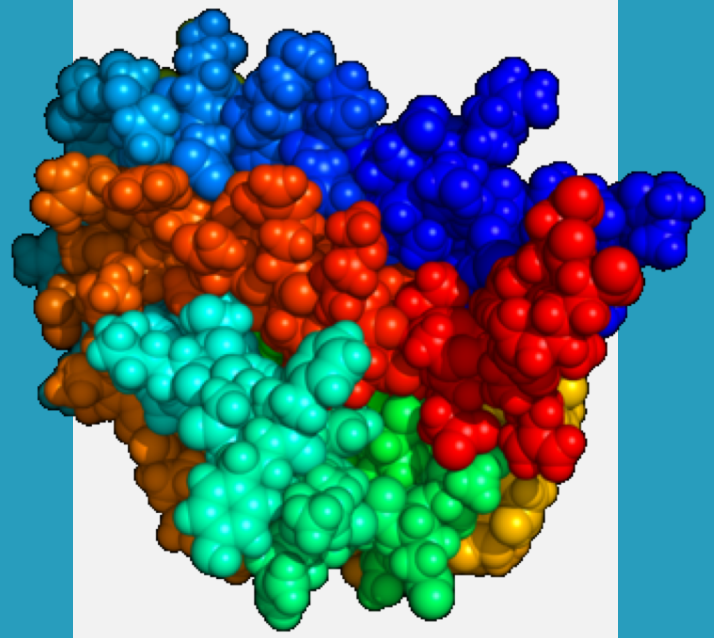
Future of biocatalysis – enzymatic reactions in continuous flow



EnginZyme – Unlocking the potential of enzyme chemistry

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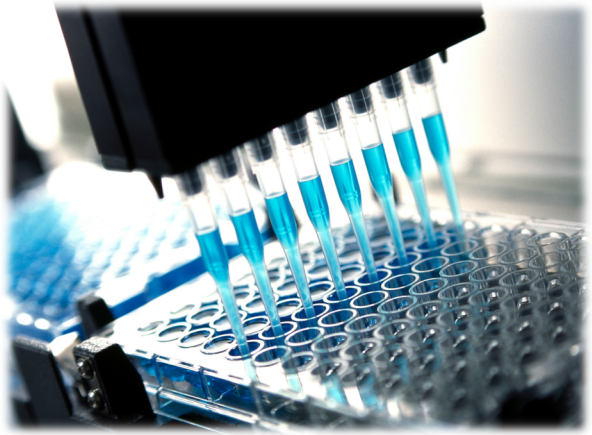
Through efficient enzyme utilization in both biocatalysis and cell-free synthetic biology applications



EnginZyme's immobilisation platform converts enzymes into cost-effective biocatalyst for multiple industries

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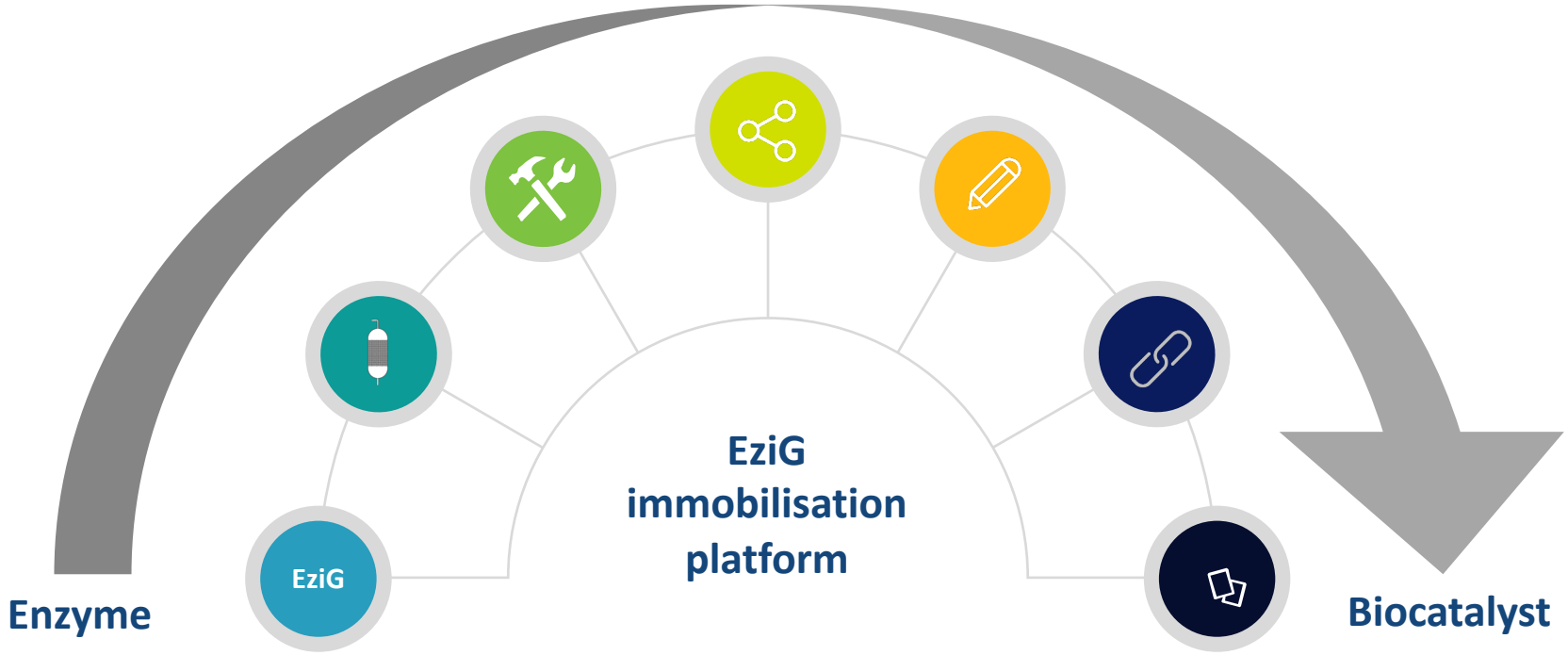

Pharma F&F Chemicals Food Agro

The future needs green chemistry

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Current chemical industry is dominated by efficient, low cost processes which generate excessive waste and rely on petroleum oil as raw material

Chemical industry operations are highly efficient
Continuous flow in fixed bed reactors is *very* common

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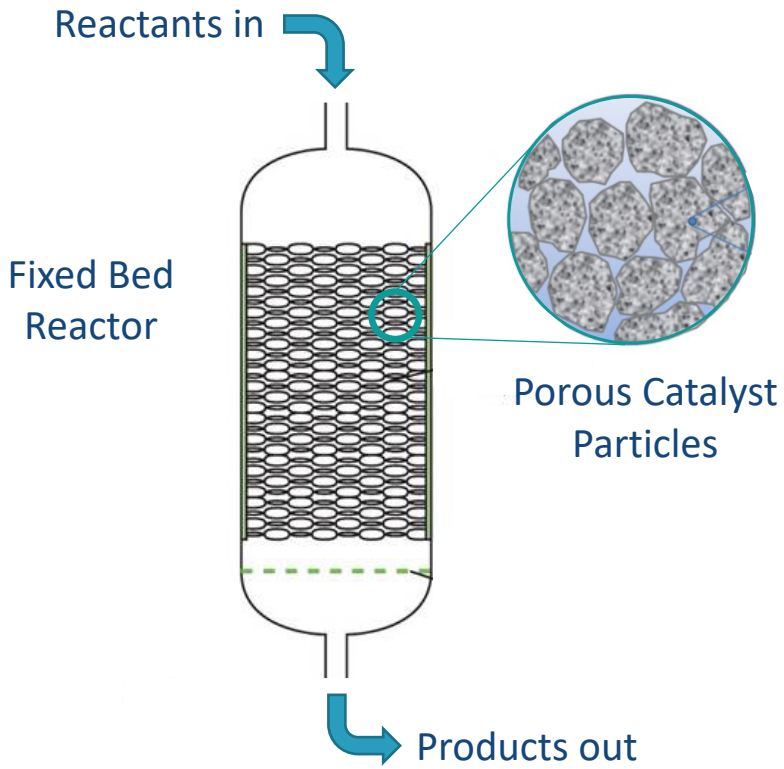

- High throughput and efficiency
- Catalyst fixed in place
- Reagents flow through continuously
- No catalyst separation
- High reactant concentrations
- Simpler downstream separations

Continuous flow reactors offer reduced capital and operating costs

Everything in nature is produced by enzymes performing reactions inside cells

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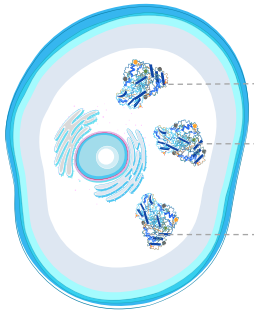
The cell, the basic unit of life



Enzymes, nature's catalysts, highly selective and efficient, performing life sustaining chemistry.

Every living organism

Harnessing enzyme's capabilities forms the basis for biocatalysis and synthetic biology

Enzymes have the capability to significantly reduce costs and CO_2 footprint of current chemical production

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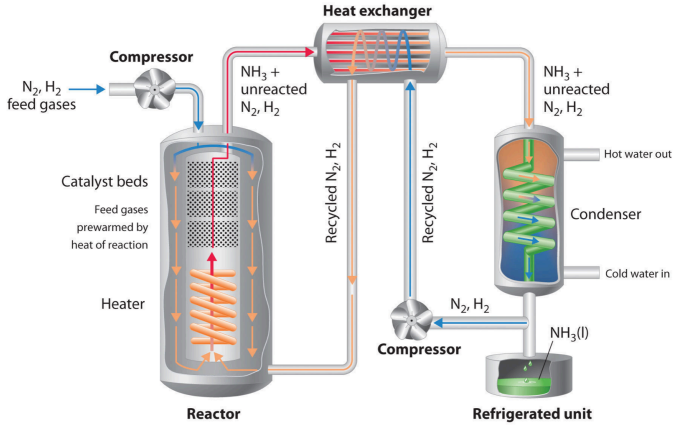
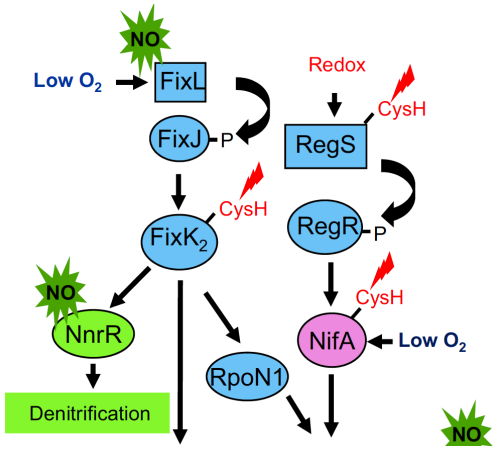


Example:
Nitrogen Fixation



Chemical Process:

- 200 Atmospheres; 500°C
- High GHG contribution
- High energy consumption

Enzymatic Process:

- 1 Atmosphere
- Room Temperature
- Low GHG contribution

Taking advantage of this mild and selective chemistry will be essential to reduce carbon footprint of the chemical industry and our reliance on petroleum oil

The challenges facing biocatalysis have limited utilisation of enzymes

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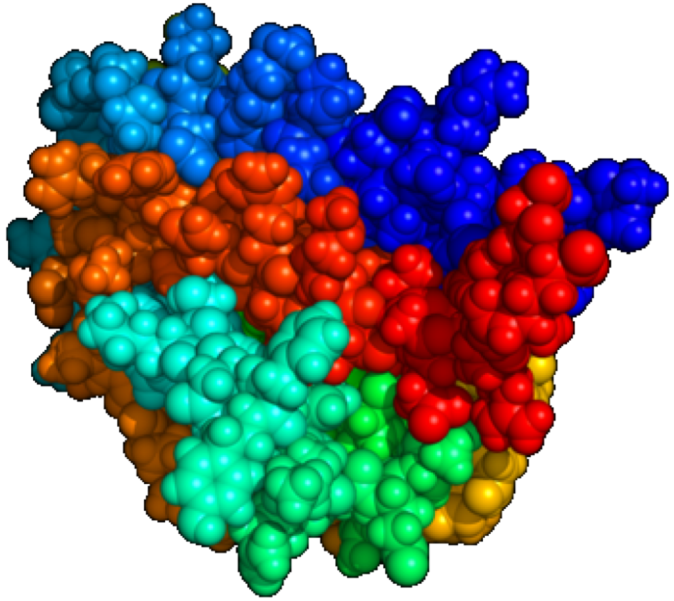

- High enzyme costs
- Long development times
- Difficult to operate in continuous flow
- Typically function in water environments only
- Difficult to scale for commodity applications
- Enzyme stability

New technology solutions are required to truly unlock the potential of enzymes

EnginZyme's EziG™ technology makes enzymes more efficient and easier to use

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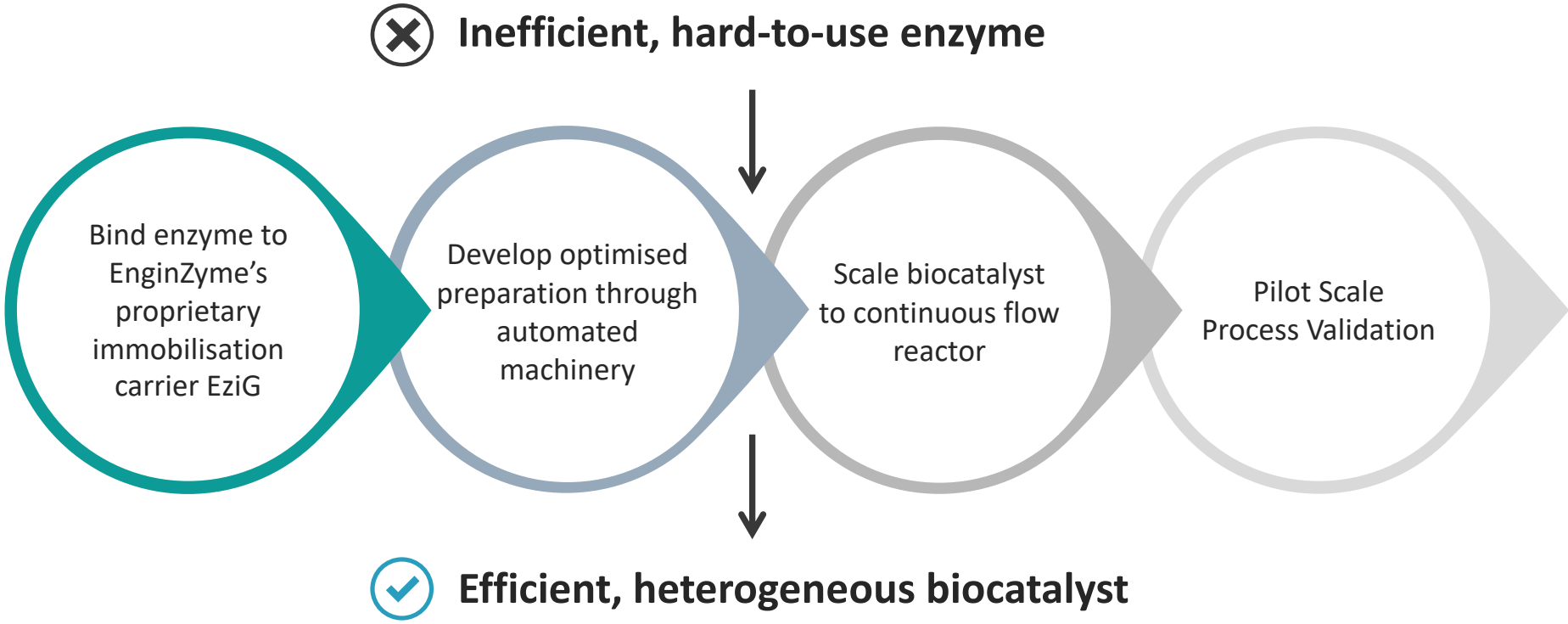
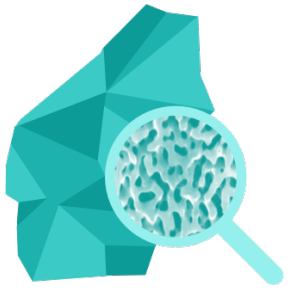


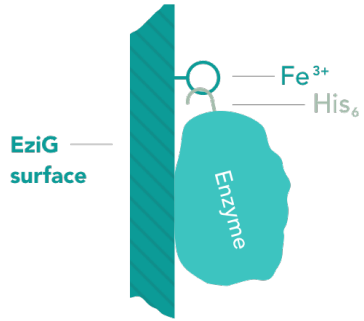
Heterogeneous biocatalysts for continuous flow fixed bed reactors

Stable, affordable, heterogeneous enzyme catalysts

EnginZyme



Controlled Porosity Glass



Chelating Fe(III) binding of His_6-enzyme

Up to 99% activity retention
Up to 30% enzyme loading

- Porous support for high enzyme loading and excellent flow properties
- Strong enzyme bond
- Non-swelling material
- Easy to handle

Universal immobilisation • Minimal activity loss • Repeated enzyme use

Active enzymes in any solvent • Simple to operate in continuous flow

EnginZyme technology utilises enzymes more effectively

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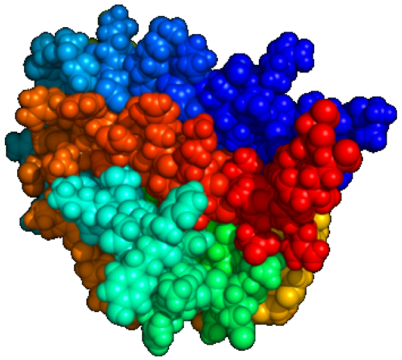
Enzyme Immobilisation



~10% activity
~ 5% loading

Traditional Immobilisation

- Low enzyme loadings
- Poor activity retention
- Large amount of support required
- Lack of general method
- Difficult to scale

Up to 99% activity
Up to 30% loading

With EziG Technology

- High enzyme loadings
- Up to 99% activity retention
- General method for fast development
- Simple to operate in flow
- Readily scalable

Universal immobilisation • Minimal activity loss • Repeated enzyme use

Active enzymes in any solvent • Simple to operate in continuous flow

EziG technology has been applied across numerous enzyme types

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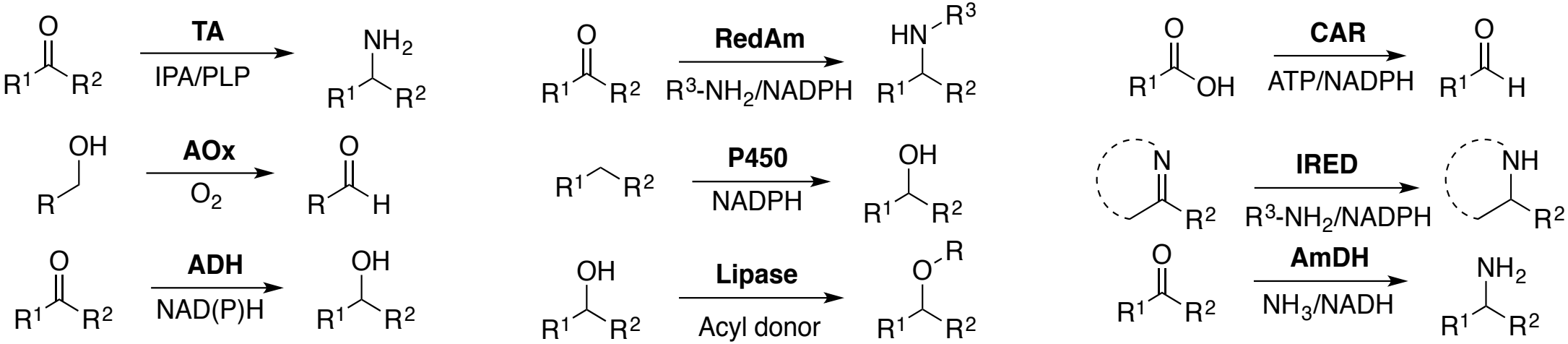

Collaboration with Merck and Manchester Institute of Biotechnology:

Nicholas Turner *et al.*, A generic platform for the immobilisation of engineered biocatalysts , Tetrahedron 75 (2019) 327-334

EziG in continuous flow provides high throughput and efficiency

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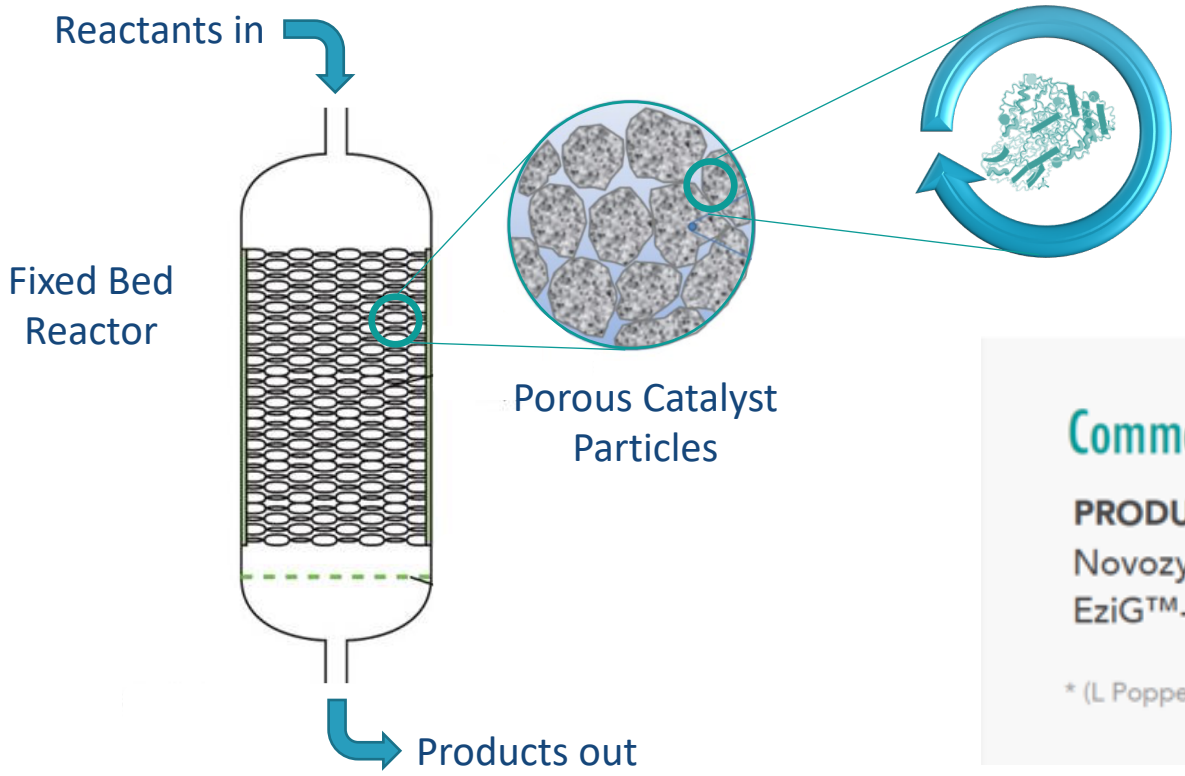


Commonly used immobilized lipases are outperformed

PRODUCTIVITY

Novozym 435*: **~10 µmol/min/g**

EziG™-lipase: **>1000 µmol/min/g**

* (L Poppe, *et. al. Tetrahedron: Asymmetry* 2008, 19, 237-246)

Universal immobilisation • Minimal activity loss • Repeated enzyme use

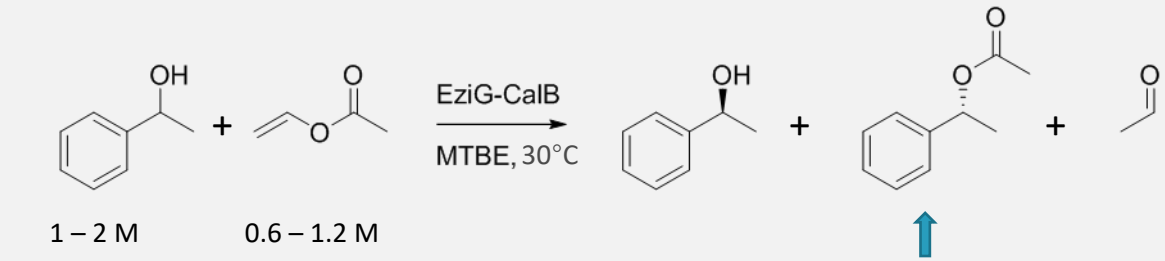
Active enzymes in any solvent • Simple to operate in continuous flow

Translation of EziG results from batch to flow

EnginZyme

Kinetic Resolution of R/S 1-Phenylethanol via Selective Acetylation

Reaction Concentrations and Conditions



- Catalyst is CalB lipase immobilised on EziG support
- Target conversion range 20 - 50% (50% of racemic mixture)
- Reaction conducted with orbital shaking at 1500 rpm

Batch results

Reaction Time:	30 mins
Immobilised Catalyst Loading:	0.3 wt.%
Enzyme loading:	15 wt.%
Conversion:	20%
(R)-α-Methylbenzylacetate ee:	> 99%

Simple and effective immobilisation of CalB Lipase demonstrated with batch reaction

Batch productivity of EziG-CalB lipase is replicated in fixed bed

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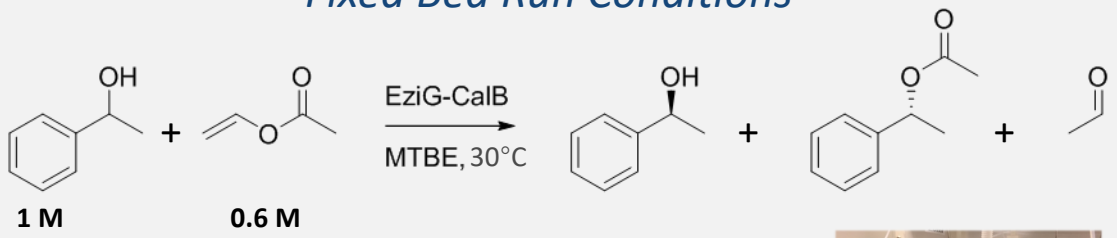
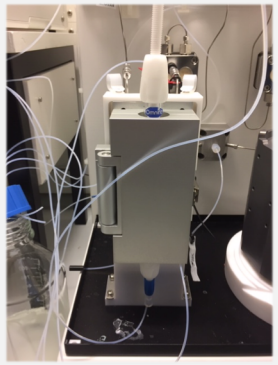
Kinetic Resolution of R/S 1-Phenylethanol via Selective Acetylation

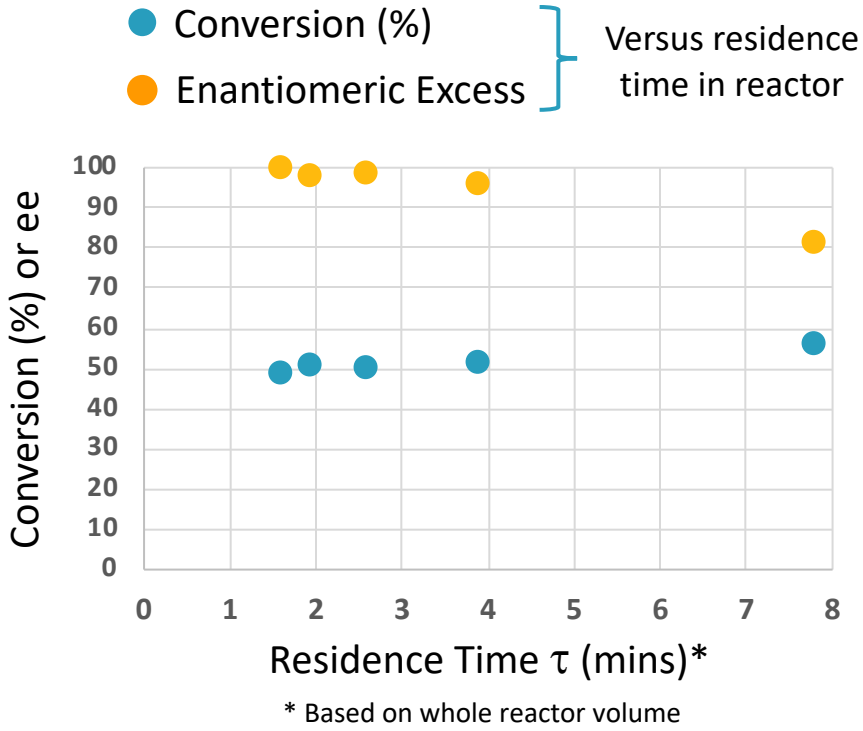
Fixed Bed Run Conditions



1 M **0.6 M**

Reactor Volume: 1.6 ml
Liquid Flow Rate: 0.2 – 1 ml/min
Immobilised Catalyst Charge: 340 mg
Enzyme loading: 20 wt.%





* Based on whole reactor volume

Batch productivity of EziG-CalB Lipase replicated in fixed bed • Target conversion in under 2 minutes

Laboratory scale fixed bed reactor on-stream stability demonstrated

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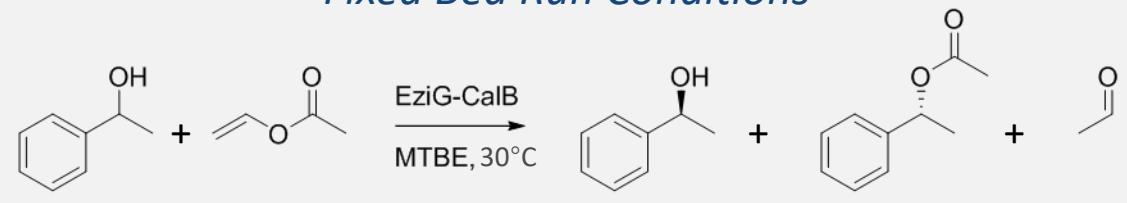
Kinetic Resolution of R/S 1-Phenylethanol via Selective Acetylation

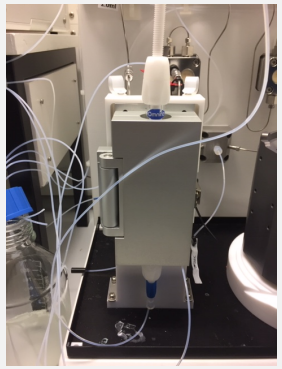
Fixed Bed Run Conditions

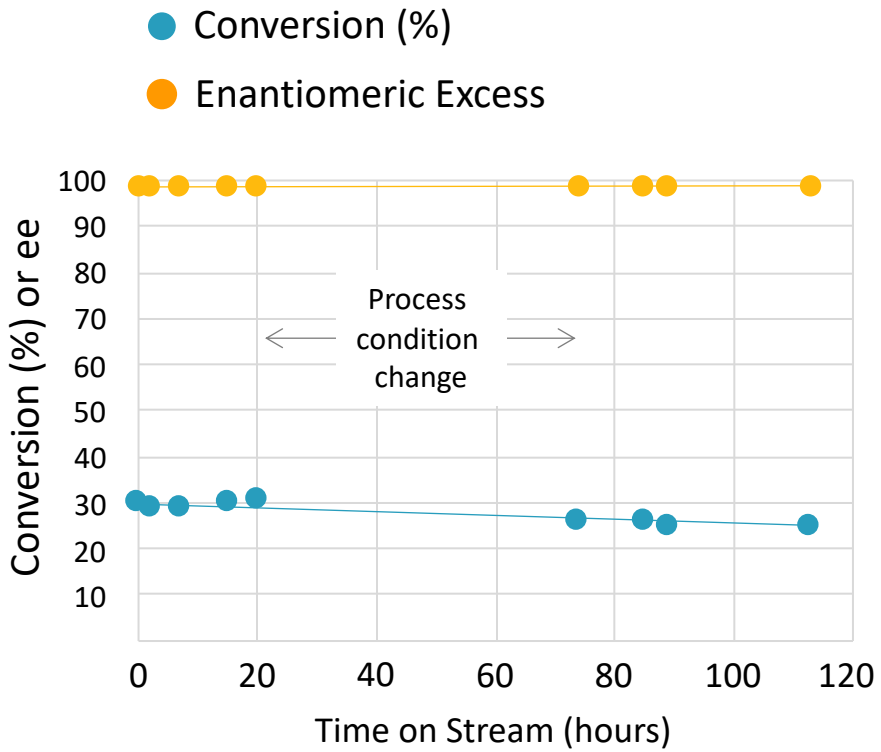


2 M
30 wt.%

1.2 M
12 wt.%

Reactor Volume: 1.6 ml
Liquid Flow Rate: 0.2 – 1 ml/min
Immobilised Catalyst Charge: 300 mg
Enzyme loading: 20 wt.%





Ready for pilot scale validation

From laboratory scale to pilot scale reactor

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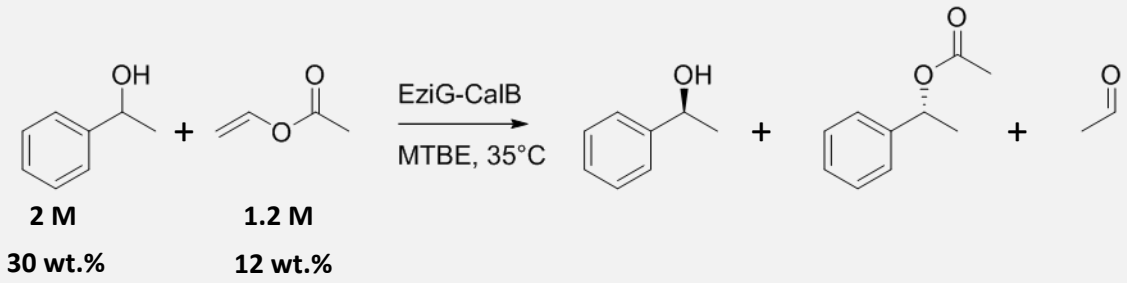
Fixed Bed Run Conditions



2 M **1.2 M**

30 wt.% **12 wt.%**

	Lab Scale		**Pilot Scale**
Reactor Volume:	milliliter scale	→	liter scale
Catalyst Charge:	milligram scale		kilogram scale

EziG-CalB lipase ready for pilot scale operation • Catalyst prepared at kilogram scales

• Pilot campaign this summer

EnginZyme's automated biocatalyst development platform

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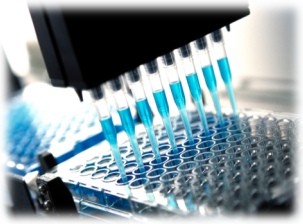
Enzyme Screening & Production

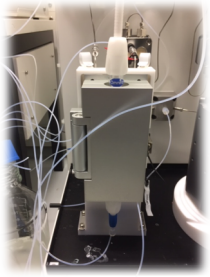
Automated Immobilisation & Reaction Screening

Biocatalyst Design

Flow Development

EziG™ - biocatalyst ready for piloting and scale-up





Simultaneous process development

From an enzyme to a validated continuous process

The future of biocatalysis -
Multi-step cascade reactions using co-immobilised enzymes

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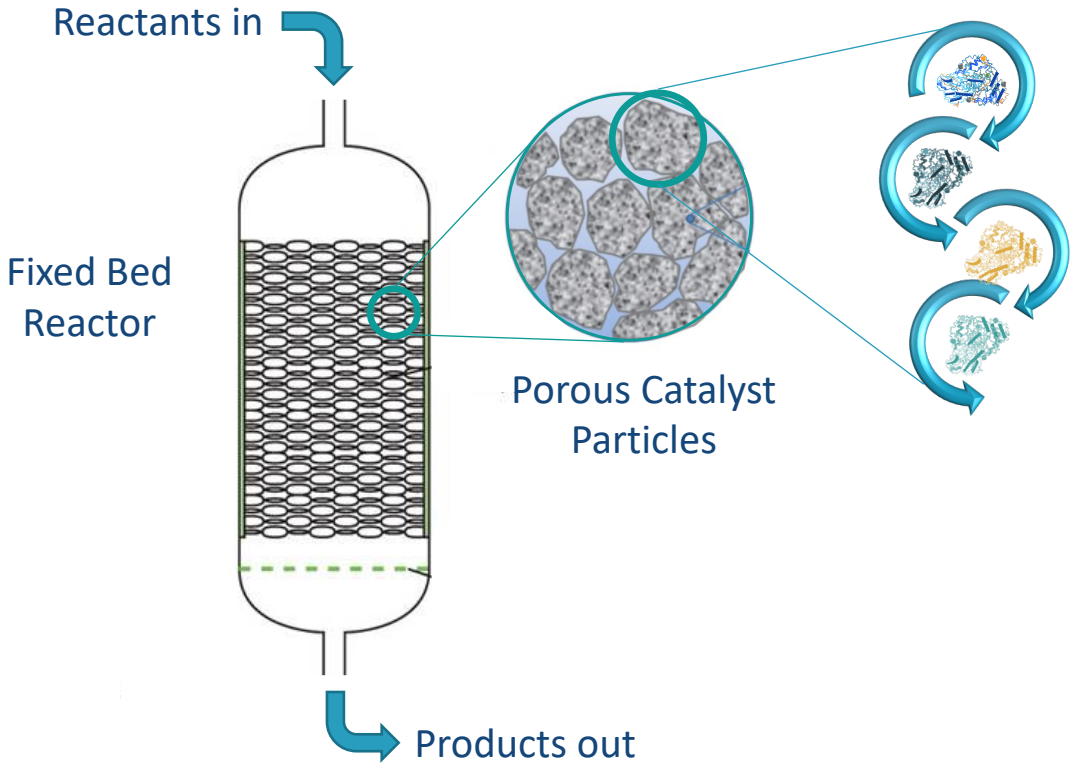


- Multiple enzymes in single reactor
- Enzymes co-immobilised on EziG support
- Mimicking an intracellular process
- Higher substrate concentrations
- Higher yields
- Lower CAPEX and OPEX

Harnessing the power of intracellular biology

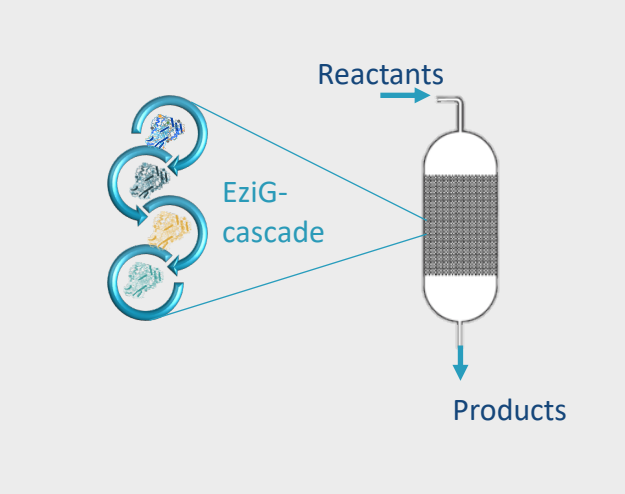
Cell-free synthetic biology is the next generation bioproduction

EnginZyme

20th Century - 2019
Controlled fermentation

- Low product concentrations
- Raw material losses to cell growth
- Long batch reaction sequence
- Large fermentation reactors
- Expensive downstream processing
- High equipment count and high capital cost

2019 and beyond
In vitro cascades in flow



- High yields and high product concentrations
- Simpler downstream processing
- Lower equipment count and lower capital cost

Cascade processes can harness the power of intracellular biology more efficiently

EziG immobilisation platform enables cell-free synthetic biology

EnginZyme

Automated immobilisation
Rapid, multiplexed development of immobilised catalyst.

Automated development
Reduced development time from concept to piloting.

Biocatalyst design
Biocatalyst scaled and validated in flow.

Ability to operate in flow
Employ EziG-enzymes in continuous flow reactors.

Combine multiple enzymatic reaction steps
Co-immobilisation of enzymes onto the EziG support.

EziG technology
Universal, effective immobilisation technology.

Intelligent design for co-factors
Driven by economics : Process or/and chemical solution for co-factor regeneration and recycle.

Pharma F&F Chemicals Food Agro

Biocatalysis in flow opens the door to endless applications

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Sustainable production of existing products

- F&F compounds
- Biochemicals
- Sweeteners
- And many, many more...

Completely novel products

- Artificial spider silk
- Bendable screens
- Biodegradable plastic
- And many, many more...

Scalable solution to climate change

Using CO_2 as feedstock enables carbon-negative production

Harnessing the power of intracellular biology • Cascade processes under development at EnginZyme

Thank You

vince@enginzyme.com