Dechema Praxis Forum:
Enzymes for industrial applications
8-Nov-2016

EnginZyme

EnginZyme

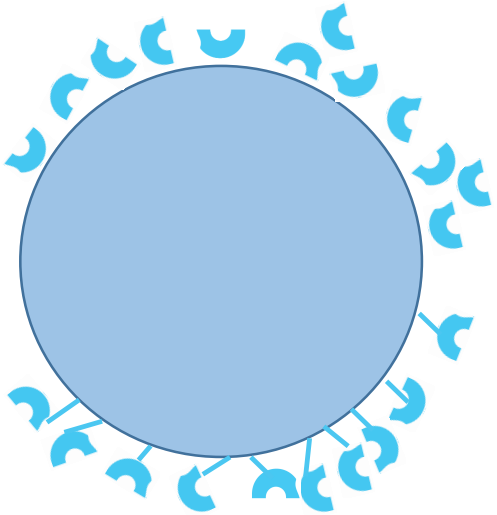
Making biocatalysis your first choice

Outline

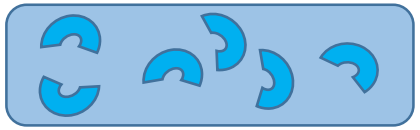
- Common methods for enzyme reuse
- EziG enzyme immobilisation technology
- Examples of EziG applications

Typical methods for enzyme reuse in biocatalysis

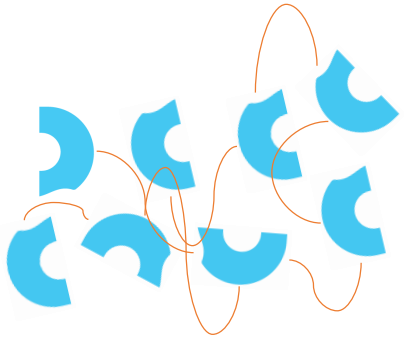
Bind to carrier



Encapsulation



Cross linking



Why are not more processes performed with immobilised enzymes?

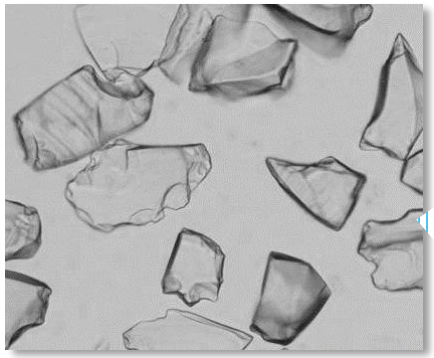
"The usual loss of activity commonly observed in the immobilisation process could be one reason for this observation.

On the other hand, another explanation could be the lack of a generally applicable, and simple to use, method for immobilisation."

- Prof. John Woodley, DTU

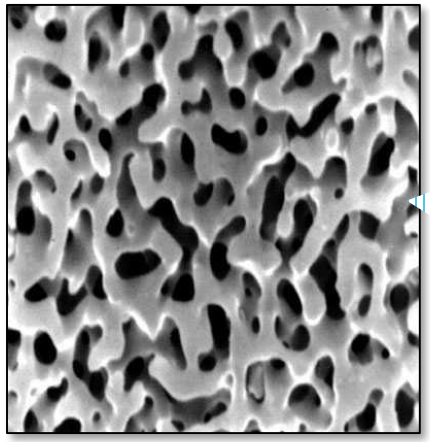
New strategy for enzyme reuse

EziG™ Enzyme Immobilisation



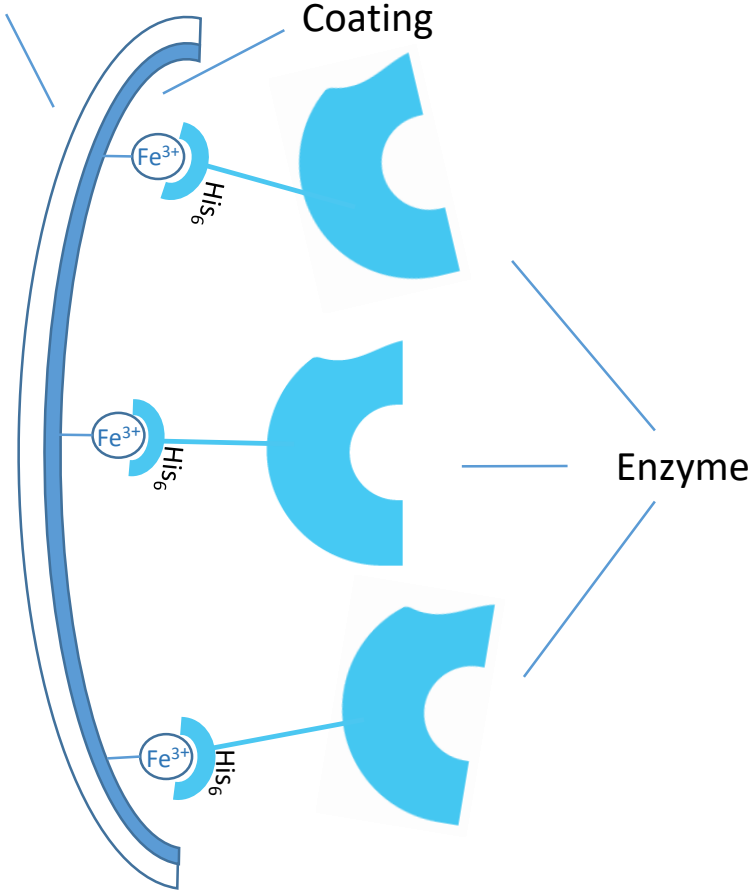
Controlled pore glass (CPG)

- Inert
- Robust
- Non-swelling
- Interconnecting pore structure, excellent fluid properties



Organic polymer coating

- Favourable microenvironment
- Customizable



His-tag binding

- Enrichment/purification
- Non-destructive binding
- Co-immobilization

EziG Products

Varying degree of surface hydrophobicity to suit your enzyme

EziG Opal — **hydrophilic surface**

Pure silica surface, no polymer coating

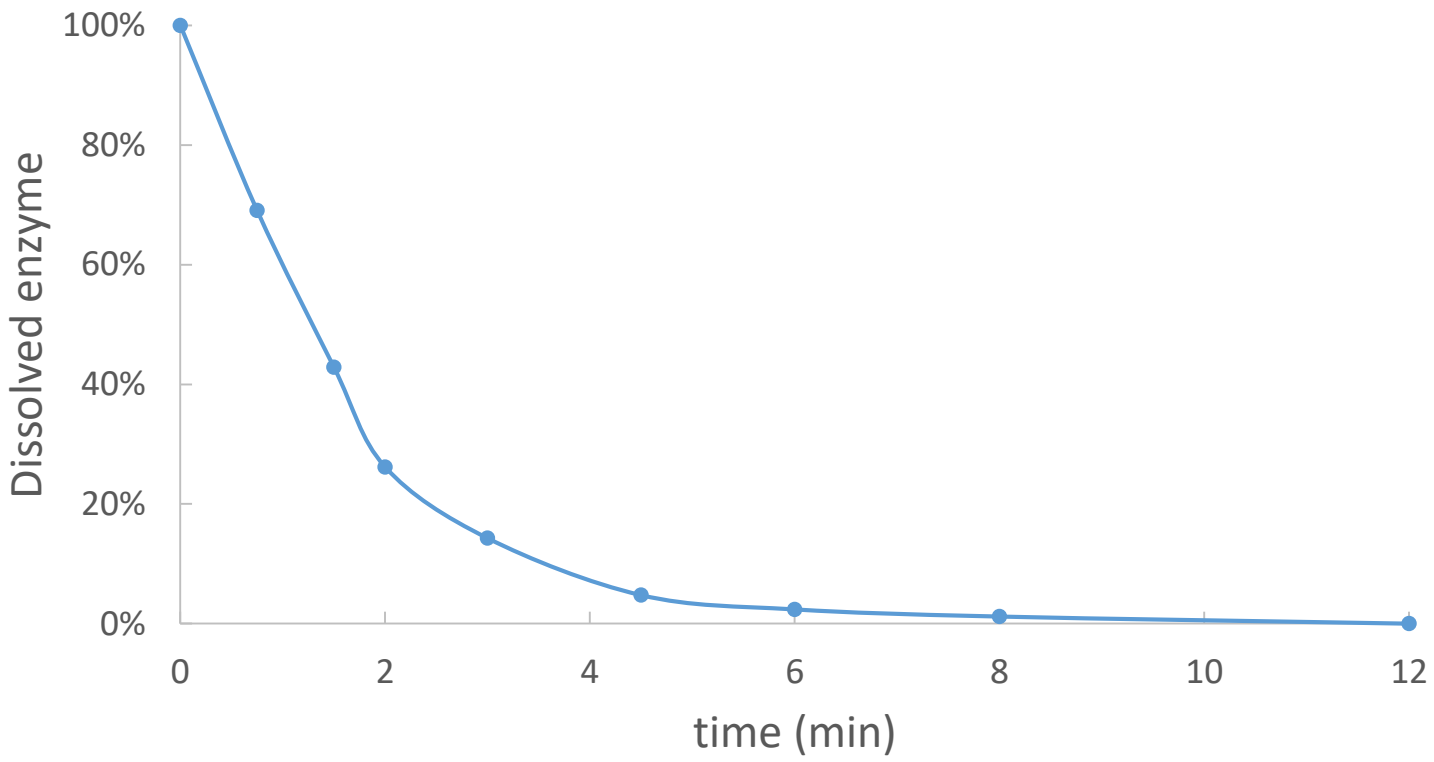
EziG Coral — **hydrophobic surface**

Poly(vinylbenzylchloride) coating

EziG Amber — **semi-hydrophilic surface**

Co-polymer (polystyrene derivative)

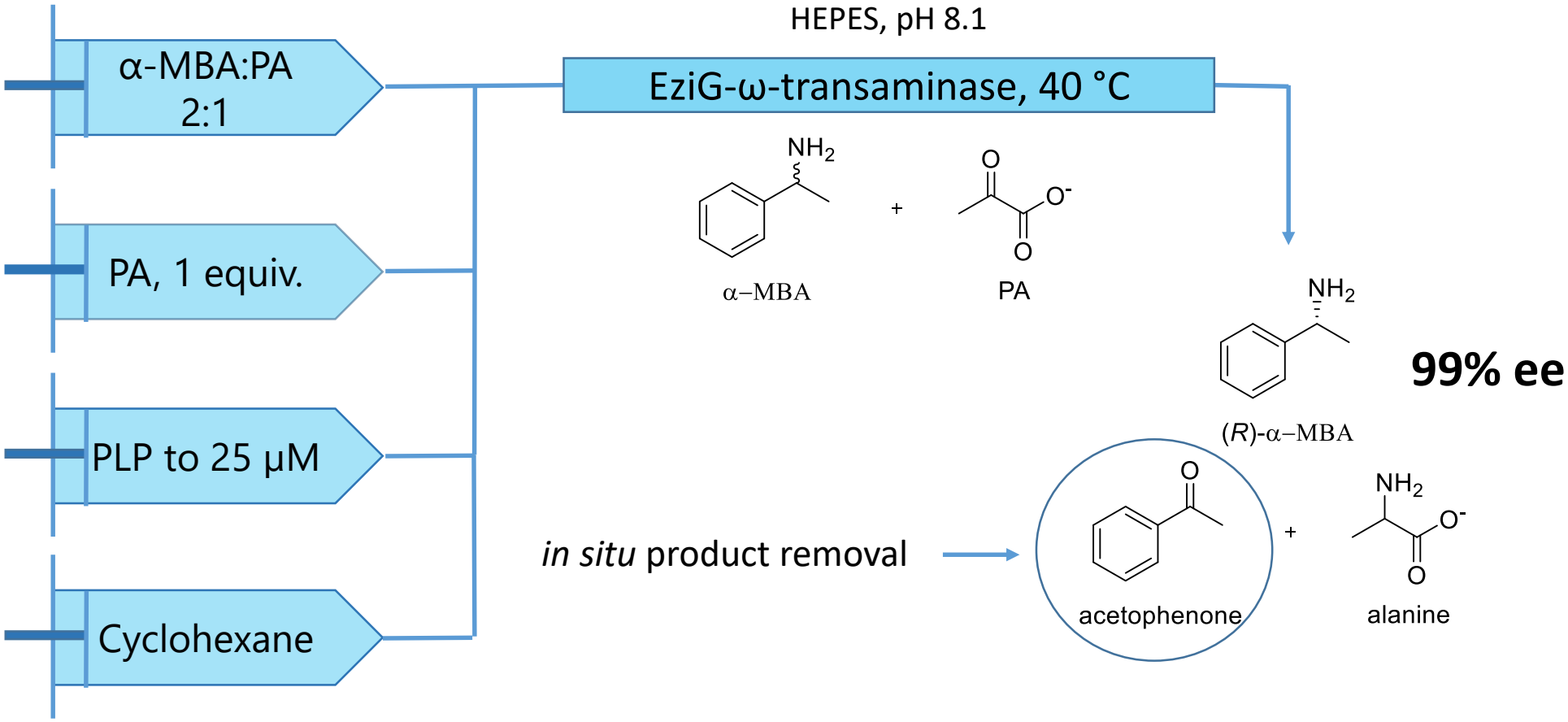
Immobilize your enzyme directly in the reactor



Binding of transaminase from cell free extract (*E. coli*), 100 mL in SpinChem reactor

Continuous Kinetic Resolution

Chromobacterium violaceum ω-transaminase



Multi-phase cascade reaction

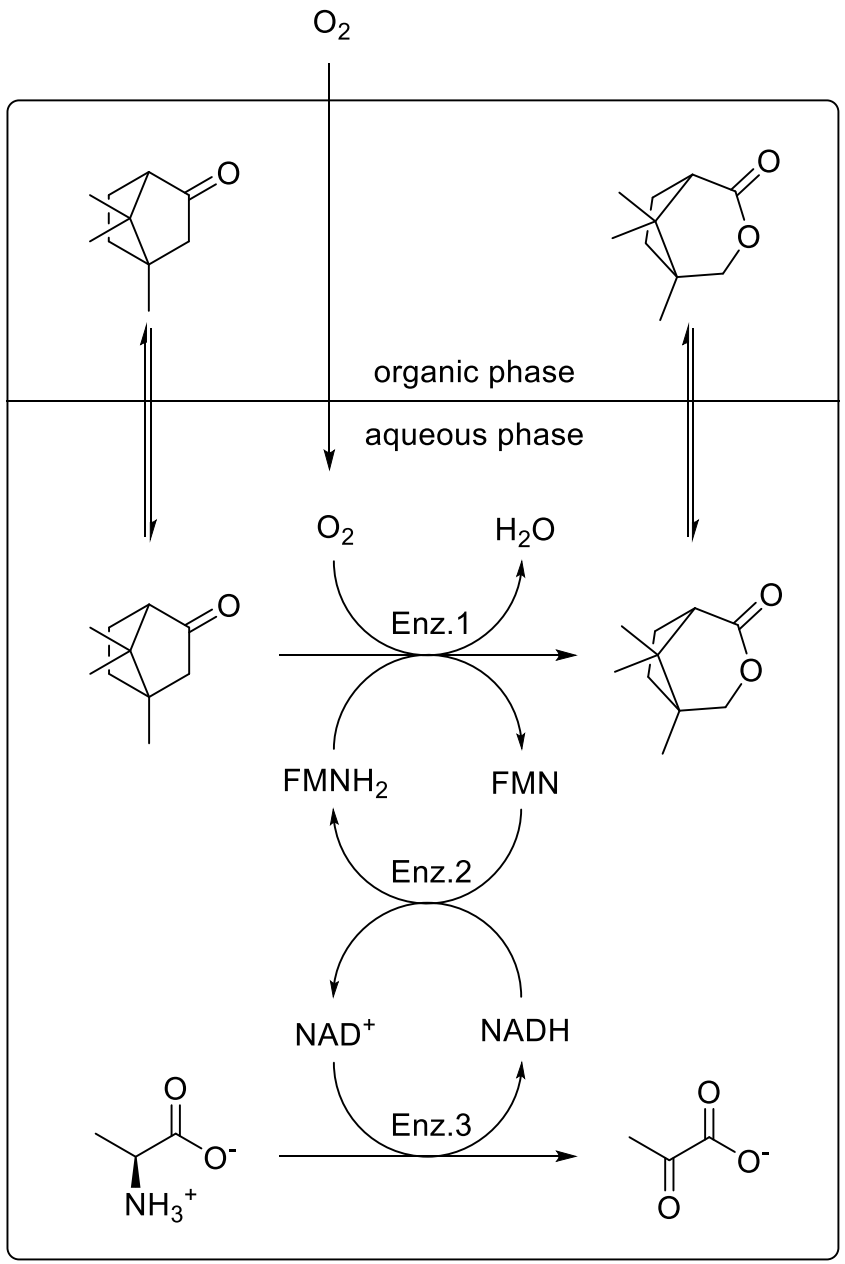
Non-immobilized:

<5% conversion

EziG-immobilized:

56% conversion

Enz.1: Baeyer-Villiger monooxygenase, without $FMNH_2$-regeneration activity
Enz.2: flavin reductase
Enz.3: alanine dehydrogenase



Immobilized *Candida antarctica* lipase B (CalB)
Work in Progress

	Tributyrin hydrolysis (TBU/g)	Stability (%) Remaining activity after 7 days in toluene reaction mixture
EnginLipe™ (EziG-CalB)	19 000	32
Novozym 435 (CalB on acrylic beads)	2 500	22

CalB expressed in *E. coli* – Work in progress

- 300 mg/L active enzyme in shake flask (bioreactor not yet tested)
- Control of secreted vs intracellular expression

- Loaded on EziG from CFE
- EziG-bound CalB not leaching in aqueous conditions (24 h)
- Stable in organic solvent (Toluene, MTBE, MeCN)

In collaboration with

VECTRON BIOSOLUTIONS

How can you save money by immobilizing your enzyme on an expensive material?

- Don’t waste enzyme
- Enrichment – bind from crude extract or culture medium
- Less carrier material needed

- Result: low cost per unit of activity

– Biocatalytic processes – Reusable enzymes –

ALMAC

Partnering to Advance Human Health

EnginZyme

Intermediate Supply

Cost effective processes using immobilised enzymes

Bulk enzyme immobilisation

Immobilised Enzyme Supply

PoC studies and pilot supply

Ready-to-use immobilised selectAZymes™

Lab to Production Scale Bioprocessing

EnginZyme

Immobilisation PoC

Pilot Immobilisation studies

Bulk Immobilised enzyme supply

Scale-up

- Raw material sourcing
- Quality
- Product isolation

Almac Process Dev

- Project Siting
- Almac site
- Partner Selection
- Customer site

Successful PoC

- Use test of Demo batch sample

Enzyme Screening

Enzyme build and selection

Enzyme Evolution

Bioprocess Scale-up

ALMAC

Partnering to Advance Human Health

Summary

- EziG: a general immobilization matrix designed for biocatalysis
  - Batch
  - Flow
  - Cascades
- CalB expressed in *E. coli*
- Ready-to-use immobilized enzymes and process development in collaboration with Almac

EnginZyme

Making biocatalysis your first choice

Leaching

EziG type	Leached enzyme after 24 h at pH 7.0 (%)
EziG Opal	3.4
EziG Coral	n/d
EziG Amber	n/d

EziG type	Leached metal ions after 72 h at pH 7.0 (%)
EziG Opal	1.5
EziG Coral	0.36
EziG Amber	0.61