

Enzyme Immobilization as an Enabler for Biocatalysis in Flow

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ChemSpec

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EnginZyme

Outline

- General enzyme immobilization: EziG™
- EziG-enzymes in flow
 - Lipase (chiral alcohol and ester)
 - Transaminase (chiral amine)



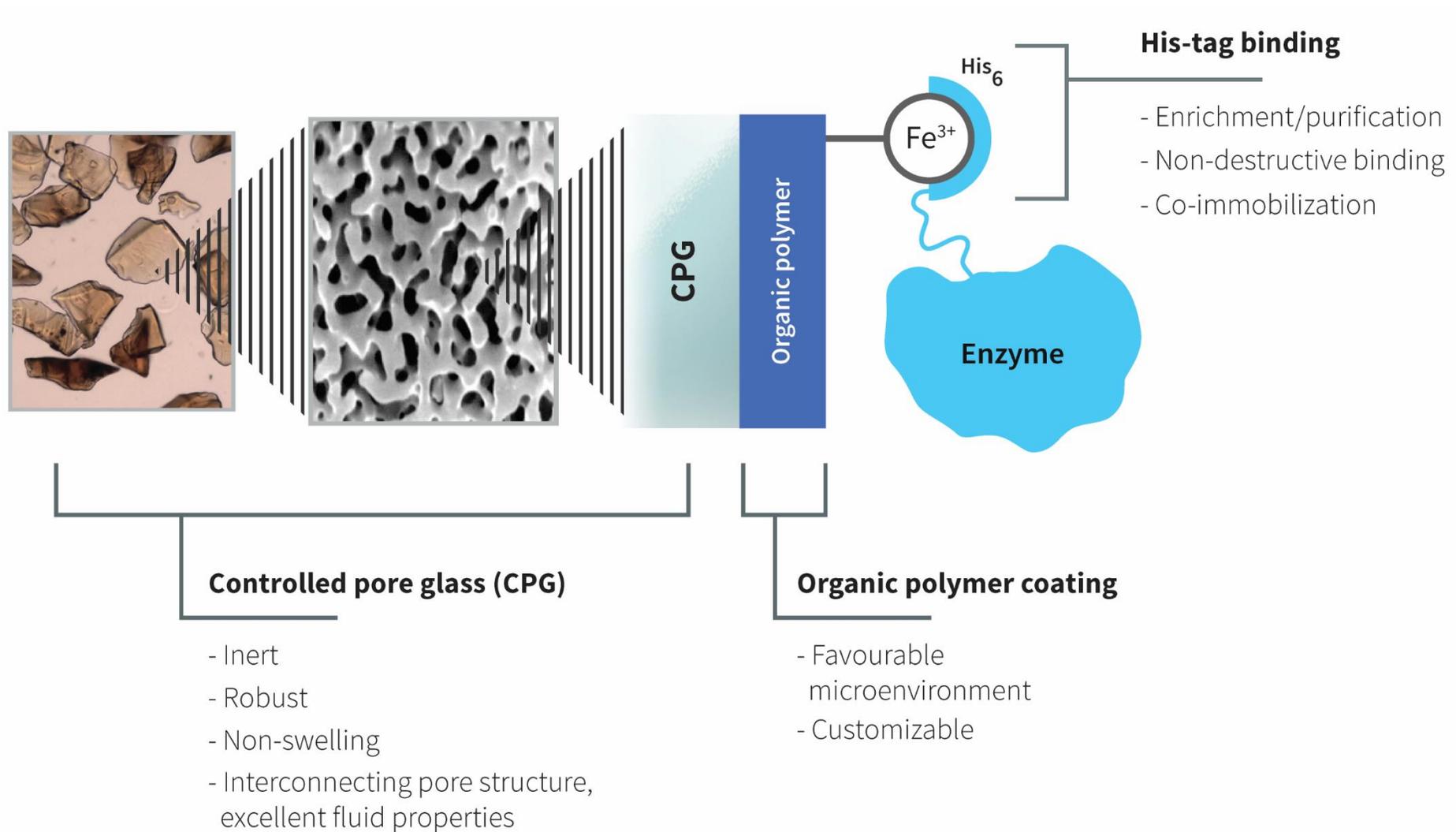
Biocatalysis

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 Immobilization.”

- Prof. John Woodley, Technical Univ. Denmark (DTU)

EziG™ - general enzyme immobilization



EziG Products

Varying degree of surface hydrophobicity to suit your enzyme



EziG Opal

hydrophilic surface

Silica surface, no polymer coating

EziG Coral

hydrophobic surface

Poly(vinylbenzylchloride) coating

EziG Amber

semi-hydrophilic surface

Co-polymer (polystyrene derivative)

Successful EziG-enzymes

- Lipases (several)
- Transaminases (several)
- KRED (several)
- NADPH and NADH regeneration enzymes (GDH, FDH)
- Cutinase
- Protease
- Laccase
- Esterase
- P450
- Baeyer-Villiger monooxygenase
- ...and more

Immobilized *Candida antarctica* lipase B (CalB)

Work in Progress

	Ester hydrolysis, Ethyl methoxyacetate ($\mu\text{mol}/\text{min}/\text{g}$)
EnginLipe™ (EziG-CalB)	13 500
Novozym 435 (CalB on acrylic beads)	1 400

1: Enzyme loading

Apply enzyme solution
(crude extract)
Flush with solvent to
remove water, if needed
10-30%w/w active
enzyme loading

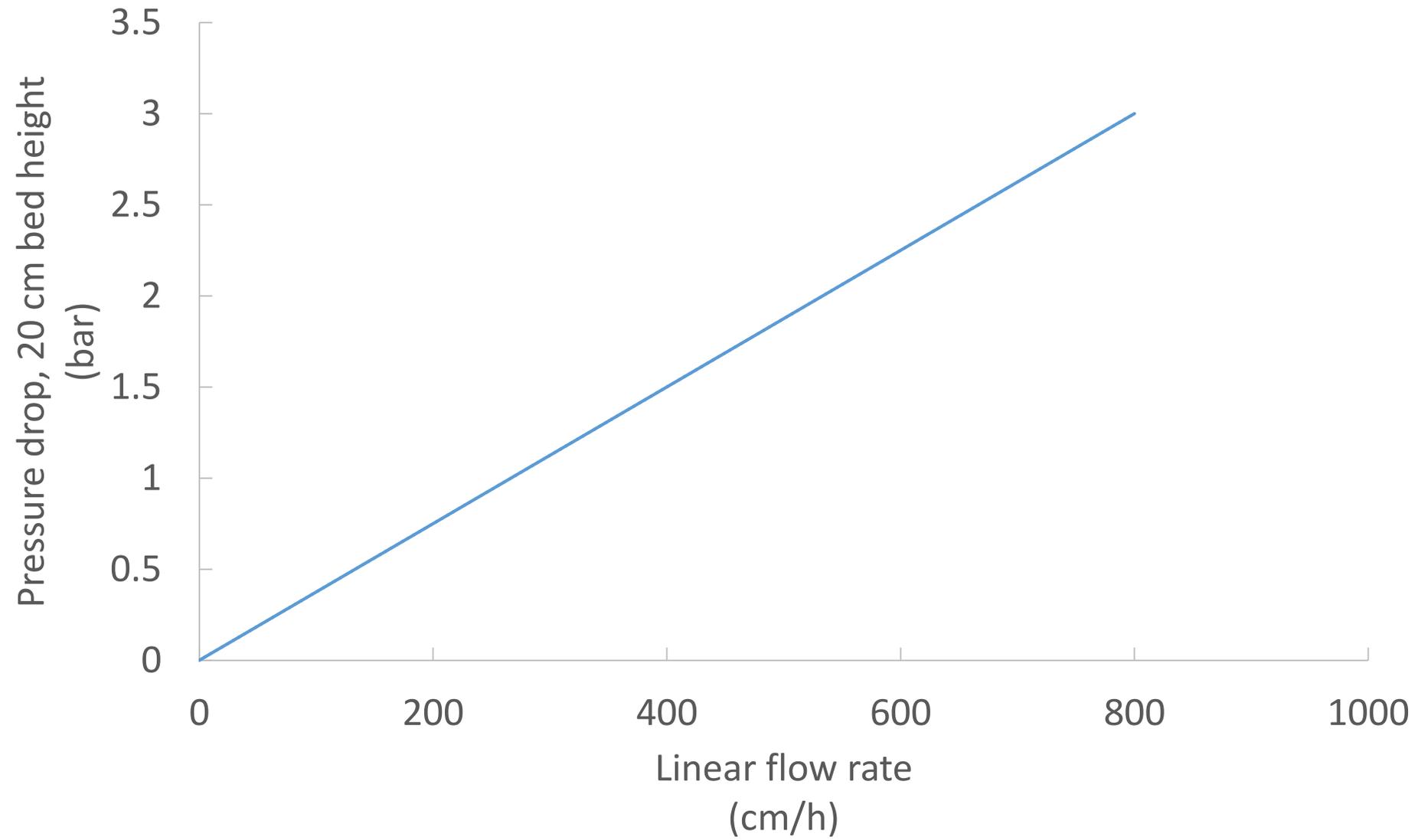
2: Biocatalysis

Apply reaction mixture
Two phase systems
workable

3: Reload enzyme

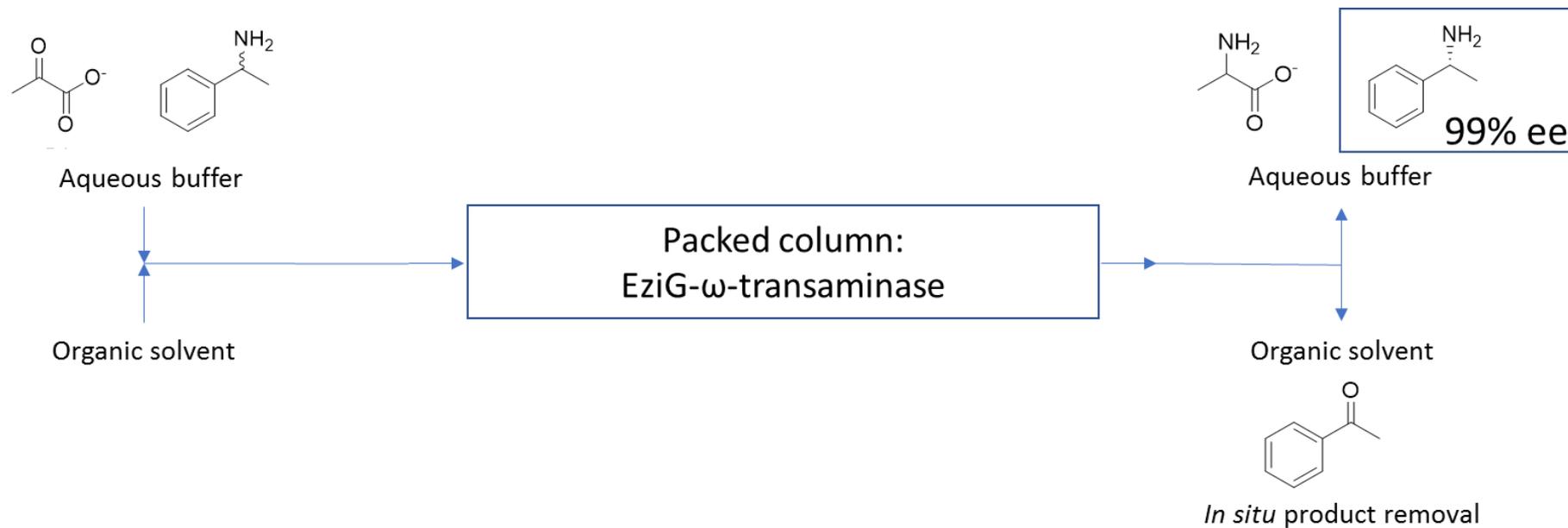
Purge deactivated
enzyme and add fresh

EziG in packed column



Flow chemistry with EziG-transaminase

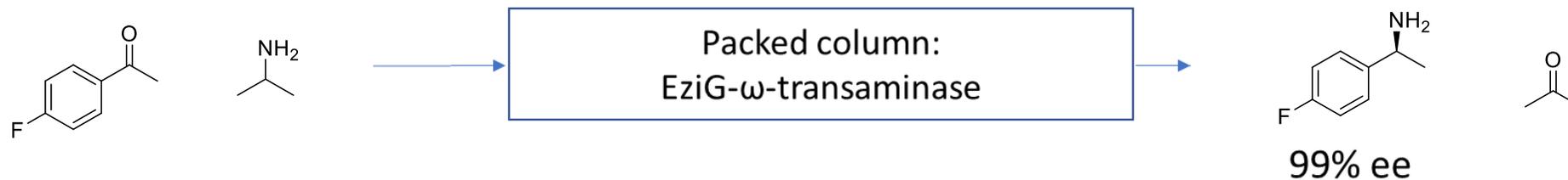
Kinetic resolution proof-of-concept



Less than 5% activity loss over 2 weeks
25%w/w enzyme in column

Flow chemistry with EziG-transaminase

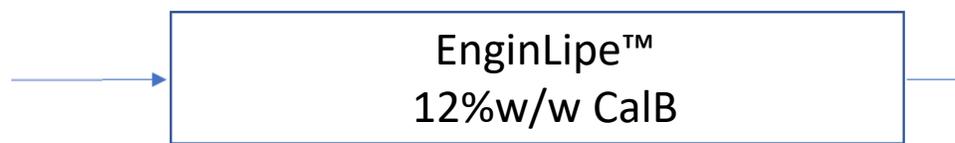
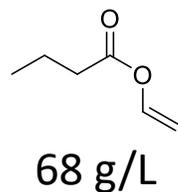
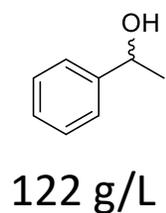
Stereoselective synthesis proof-of-concept



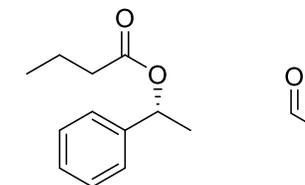
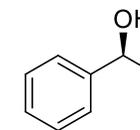
Less than 5% activity loss over 2 weeks
20%w/w enzyme in column

Flow chemistry with EziG-lipase

Candida antarctica lipase B (CalB) proof-of-concept



MTBE 35 °C



>99% conversion

>1000 $\mu\text{mol}/\text{min}/\text{g}$ (>10 000 $\mu\text{mol}/\text{min}/\text{g}$ enzyme)

Novozym 435: ~ 10 $\mu\text{mol}/\text{min}/\text{g}$

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

Thank you!

Dr. Peter Hendil-Forsell



Dr. Alexey Volkov

