Kinetic selectivity modulation of bioactive peptide appearance in the course of enzymatic proteolysis in microreactor

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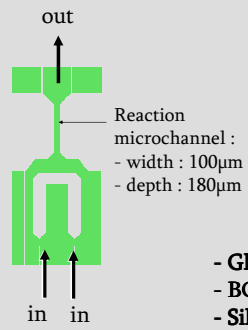
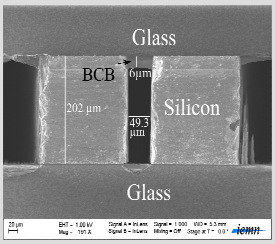
Context and purpose

Microtechnologies development has led to the design of new tools for biology and chemistry. With microfluidic systems, configurations for fluid handling are changed and offer new experimental ways for enzyme engineering. In this context, we propose here to investigate the microfluidic flow regime in biocatalysis. Continuous-flow microfluidic reaction approach provides a good tool to evaluate the impact of reactor miniaturization on enzyme kinetics and proteolytic reaction selectivity. We show here the influence of a strong laminar flow on enzyme activity as a new way for kinetics and selectivity modulating: liquid-liquid parallel laminar flows in microchannel cause kinetic selectivity modification of proteolytic enzyme reaction involving haemoglobin and pepsin.

Reactor microfabrication

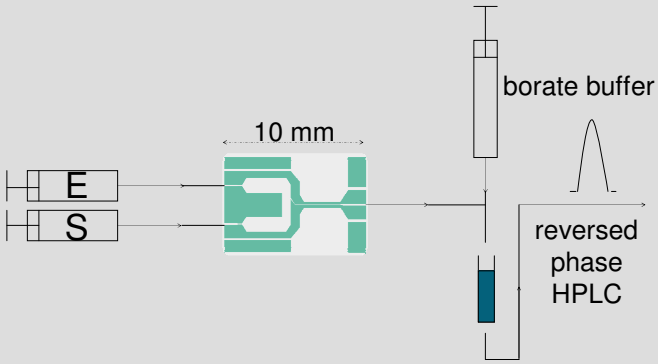
Silicon microchannels



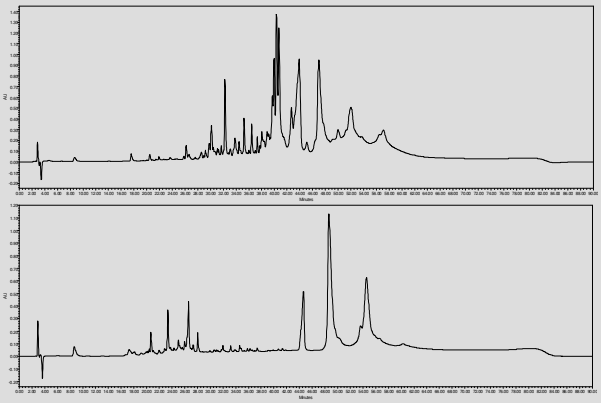


- Glass/silicon/glass technological process
- BCB bonding by thermocompression
- Silicon etching by DRIE

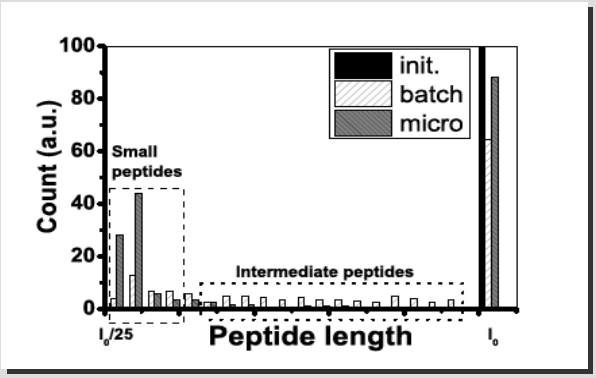
Experimental procedure



Results : kinetic selectivity analysis of haemoglobin hydrolysis by pepsin



RP-HPLC chromatograms for batch and microfluidic experiments after 30 secondes reaction time (1µL.min^{-1})

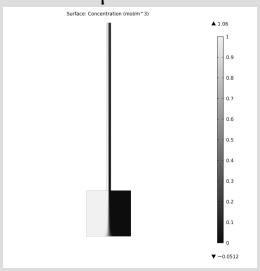


Microfluidic and batch solution contents computed for 30 seconds reaction time by Scilab

We show here that the diffusion based kinetic modulation of haemoglobin peptic hydrolysis induce an alterated peptides kinetic appearance for a portion of the initial substrate population. Peptides appearance modulated kinetics is explained by hydrodynamic simulations and a stochastic algorithm based on Michaelis- Menten approximations.

Diffusion profiles

for 1µL.min^{-1}



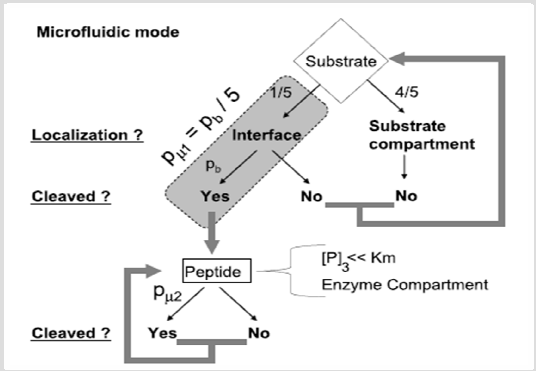
$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \xrightarrow{k_{cat}} E + P$$

Evaluation of probabilities

$$p_b.dt = \frac{d[P]_b}{[S]_b} = \frac{k_{cat}[E]_{0b}}{[S]_b + K_m}dt$$

$$p_{\mu 2}.dt = \frac{k_{cat}[E]_{03}}{K_m + [P]_3}dt \sim \frac{k_{cat}[E]_{03}}{K_m}dt = 2.\frac{k_{cat}[E]_{0b}}{K_m}dt$$

Algorithm used to compute Hb hydrolysis in microfluidic mode



Conclusion

We developed a new approach for kinetics and selectivity modulation leading to a better control of reaction kinetics in microreactors.

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