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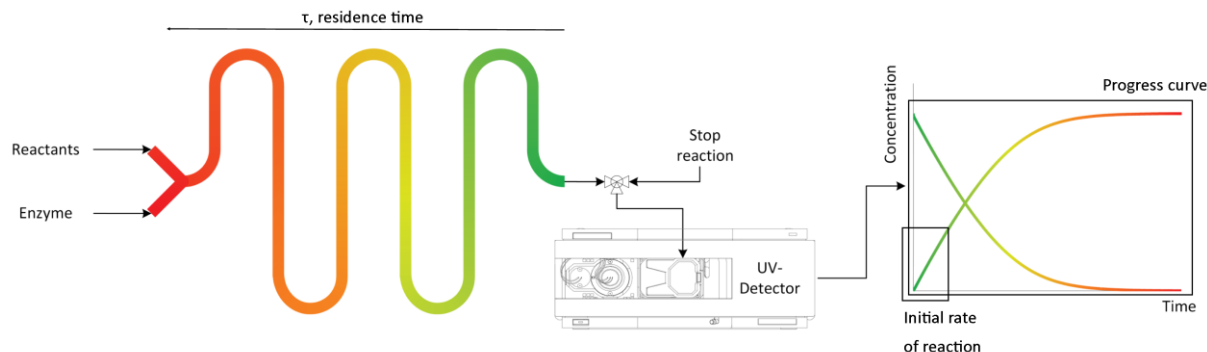
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Enzyme Characterization in Microreactors by UV-Vis Spectroscopy

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In protein engineering mutants are often selected solely on the basis of activity [1], simplifying the analysis and enabling high throughput screening. At a later stage of development, several mutants show comparable performance and this basis for selection becomes indistinct. The basis for selection can at this point be improved by characterization of the enzyme performance where also inhibition and toxicity effects are taken into account. Enzyme characterization is here defined as the effect on initial rate of reaction with respect to pH, enzyme, substrate, co-substrate, product and co-product concentration [2]. From this investigation, it will be possible to determine whether the enzyme meets the criteria for process requirements or not. The development of the process will determine the requirements and this can also reach a state of maturity that resolves obstacles, lowers criteria and paves the way for implementation. As an example ω -transaminase is here investigated, which facilitates the exchange of an amine- and keto-group stereoselectively. The characterization will be carried out in a microreactor [3], this size is currently the only concept that can facilitate this thorough analysis, as the enzyme resource is scarce at this point of development. In the case where the reaction operates with UV active components, UV can be used to detect compounds with high sensitivity supplemented by multivariate data analysis. The spectra are here decorrelated and regressed to yield concentrations of individual compounds. HPLC systems are built for handling small quantities of liquids and the UV detectors for these proves to be fitting excellent. Enzyme characterization is therefore carried out by a combination of a microreactor with a diode array detector from an HPLC system.



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