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Immobilized enzyme reactors as a cost-effective solution to enhance (bio)assay throughput

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Low-volume immobilized enzyme reactors (IMERs) are a cost-effective way to increase bioassay throughput. Over the years, different materials have been suggested as support for IMER preparation. However, short bed, high-performance monolithic columns such as CIMac™ and CIMic™ Analytical columns are among the best supports for IMER preparation, especially when integration into a downstream separative system is required and large molecules need to be analyzed. In recent years, the therapeutic field has seen remarkable progress and has moved towards larger and more complex drug structures. In this context, monolith columns with flow-through micrometer pores represent an advantageous option due to their large pore size, low limitations to mass transfer, high stability, and low backpressure.
This presentation focuses on our recent advances in the IMER field using short-bed convective interaction media (CIM™) columns, with a specific focus on IMERs that are functionalized with enzymes used in omic fields. Specifically, the presentation will primarily focus on trypsin-IMERs used for on-line protein digestion and subsequent MS-based peptide analysis, and PNGase F-IMERs for N-glycan release from glycoproteins. Both enzymes are commonly used in solution for large (glyco)protein characterization as well as within biotherapeutics quality control. The definition of the glycosylation profile is a critical aspect of biological and biotechnological drug production because it can affect drug distribution, stability, localization, and ultimately activity. On the other hand, glycoanalysis is also a challenging analytical field. To overcome the limitations of in-solution assays, tailored IMERs have been developed and optimized to achieve the best stability and performance under the best operational conditions. Moreover, short digestion/deglycosylation time (within minutes) has significantly reduced the time required for (glyco)protein processing. Lastly, insertion into specific analytical LC-MS platforms has allowed for performing in-line enzymatic reactions followed by the direct analysis of the released products.