SERS-based detection methods for screening of genetically modified bacterial strains

The importance of metabolic engineering has been growing over the last decades, establishing the use of genetically modified microbial strains for overproduction of metabolites at industrial scale as an innovative, convenient and biosustainable method. Nowadays, application areas of microbial factories vary largely, including industrial production of valuable compounds for biofuels, polymer synthesis and food, cosmetic and pharmaceutical industry. The improvement of computational and biochemical tools has revolutionized the synthesis of novel modified microbial strains, opening up new possibilities for rapid genome modification and high-throughput development of large-size microbial libraries. However, there is still a need for fast, high-throughput and real-time screening techniques, in order to speed up the testing of newly produced strains.

In the frame of this PhD project, surface enhanced Raman scattering (SERS) has been identified as a fast and molecule-specific detection technique, increasingly applied to sensing in life sciences. Also due to its great potential for miniaturization and automation, SERS could represent a possible solution for specific, robust and high-throughput sensing in metabolic engineering.

As the main goal of this Ph.D. project, we explored the potential of SERS for quantitative and reproducible screening of genetically modified E. coli strains, based on the amount of specific secondary metabolites found in supernatant. However, due to the intrinsic sensitivity of SERS, and due to the matrix complexity of real supernatant samples, a pre-treatment step was needed to exclude salts and other unwanted compounds from detection. Liquid-liquid extraction (LLE) and supported liquid membrane (SLM) extraction were combined with SERS, enabling a robust and quantitative discrimination between different E. coli strains, validated with high-performance liquid chromatography (HPLC). Centrifugal microfluidics, based on the actuation of microfluidic discs by simply controlling a spinning motor, represents an appealing alternative to traditional microfluidics, placing special emphasis on parallelization, short time-to-response and ease of use of the developed devices. We developed a solvent-resistant lab-on-disc (LoD) device, integrating filtration, LLE and SERS-based sensing; besides achieving fast pre-treatment and sensing of supernatant samples on disc, the use of large-scale fabrication techniques (injection molding and ultrasonic welding) enabled the production of tens of microfluidic modules within two working days, demonstrating the scalability of the developed device.