Final Report Summary - PLASMAQUO (Development of plasmonic quorum sensors for understanding bacterial-eukaryotic cell relations)

We aimed at developing a sensing platform to monitor chemical and biological processes within bacterial and cell cultures. In particular, we aimed at the real-time observation of the Quorum Sensing mechanism for communication between bacteria. The critical factor was the fast identification of low concentrations of molecules involved in the communication mechanism. The technique to be used is surface enhanced Raman scattering (SERS) and relies on the generation of high electric fields at the surface of metal nanoparticles through illumination under surface plasmon resonance conditions. Such electric fields can induce huge enhancements of the Raman scattering by target molecules, so that ultradetection is possible.

Because the efficiency of nanoparticles toward electric field enhancement strongly depends on their morphology and composition, we designed and synthesized novel plasmonic nanoparticles, and nanoparticle assemblies of various morphologies and compositions. Detailed optical and morphological characterization confirmed that such nanoparticles are efficient SERS platforms.

Additional enhancement can be obtained through organization of the nanoparticles into 2D and 3D ordered arrays. Tiny gaps are created between neighboring nanoparticles, in which the electric field is further enhanced, thereby improving the SERS activity. Chemical design allowed us to obtain highly ordered arrays for different types of nanoparticles and their collective plasmonic response was optimized, as confirmed through the detection of standard target analytes.

We provided high quality experimental data regarding the application of plasmonics and SERS for sensing diverse analytes, including biomolecules and bacterial quorum sensing-regulated processes. We thus went beyond the state of the art in the fields of colloidal nanoparticle synthesis, colloidal phase behavior and understanding SERS mechanisms for designing highly efficient plasmonic nanoparticles and assemblies. We demonstrated the capacity of the new materials for non-invasive, label-free monitoring of intra-species and inter-species Quorum Sensing in relevant bacterial species, such as P. aeruginosa and C. violaceum, designated as human opportunistic pathogens.

We rationally designed hybrid plasmonic nanostructured substrates composed of microporous materials and gold nanoparticles (AuNPs) as SERS-active platforms for label-free detection and imaging of quorum sensing chemical communication in Pseudomonas aeruginosa bacterial cultures. Different plasmonic substrates allowed label-free detection and visualization of the quorum sensing signal pyocyanin upon secretion from live biofilms and small clusters of bacteria into the underlying SERS sensors in a non-invasive way. Owing to the high enhancement factor of the plasmonically-active substrates we achieved ultrasensitive detection down to 10-15 M. We expanded the application of our SERS approach for simultaneous detection of two quorum sensing microbial metabolites, violacein and pyocyanin, expressed in co-cultures of Chromobacterium violaceum (C. violaceum) and P. aeruginosa on agar-based plasmonic substrates. This approach provides an additional tool for spatio-temporal analysis and detection of inter-species chemical interactions between microbes on agar, a standard support matrix for culturing microbial cells on solid medium.

We demonstrated that our plasmonic platforms can be applied to investigate cellular chemotypes and metabolite exchange processes linked to the development and organization of microbial populations. They can also be implemented toward the screening and identification of SERS-active bioactive metabolites with potential clinical, pharmacological or biotechnological interest produced by microbes in defined synthetic consortia and in mixed species obtained from environmental samples. Understanding microbial chemical communication processes is important to provide fundamental insights into microbial
ecology and necessary knowledge for potential applications in medicine, drug discovery, biotechnology and synthetic biology. The ultrasensitive detection of microbial metabolites through plasmonics opens the possibility for diagnosis of bacterial infections, which will aid in the development of more effective strategies of prevention and control of infectious diseases.

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