Combining C1s STXM with atomic force microscopy (AFM) to study EPS in biofilms

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Extracellular polymeric substances (EPS) are produced by many microorganisms to perform diverse functions such as the initial attachment of cells to solid surfaces, cell-to-cell adhesion, scavenging of nutrients or protection from dessication or toxic substances. EPS are composed of polysaccharides and proteins with minor contributions of nucleic acids and lipids, but the exact composition depends on on the specific bacterial or fungal strain, the growth stage, and the physicochemical conditions of the environment. In addition, the composition of EPS is often studied after separating it from liquid cultures by extraction, a procedure which is prone to several artifacts.

We used carbon spectromicroscopy at the K-edge (STXM) and atomic force microscopy (AFM) to investigate composition and mechanical properties of EPS at high spatial resolution in intact biofilms of *Bacillus subtilis*. STXM allowed to quantify the contribution of proteins, non-aromatic proteins, polysaccharides, and lipids on EPS patches between bacterial cells at a resolution of 30 nm, while evaluation of force-distance curves gave Young`s modulus, deformation and tip-sample adhesion of the same patches.

In comparison to EPS extracted from a liquid culture, the EPS inside the biofilm were depleted in proteins and enriched in lipids. During adsorption of EPS on goethite we observed a preferential adsorption of proteins and lipids. When the biofilm was grown in the presence of goethite, the secreted (non adsorbed) EPS were also enriched in lipids. AFM images revealed that the biofilm-EPS is made up of domains of different mechanical properties. However, this was not reflected in the chemical composition as seen by STXM.

We conclude, that STXM is able to characterize the chemical composition of EPS in biofilms grown under different conditions (e.g., in the absence and presence of goethite) and that AFM my add further detail on the spatially heterogenous surface properties of EPS.