

## Session: Tools & modelling

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### **Gold optimized nanohybride for a selective labeling and imaging in micro tomography of biofilm grown in porous media**

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Biofilm development in porous media, whether it is in biofilters or in the hyporheic zone, is particularly difficult to visualize. Optical approaches such as confocal laser scanning microscopy cannot generally be used because of the opacity of the porous matrix. In this context, imaging using X-ray computed microtomography has proven successful in resolving the spatial distribution of biofilms within the porous structure. However, this technique requires the use of contrast agents to differentiate between the biofilm and the aqueous phase. Here we develop a novel contrast agent based on gold nanoparticles (AuNP) functionalized with concanavalin A (ConA). The AuNP-ConA attaches to glycoproteins within the biofilm thanks to the lectin functionalization and the AuNP increases X-ray absorption and thus contrast. This presents several advantages over existing contrast agents, including **1-** biocompatibility, **2-** good penetration inside the biofilm, **3-** good affinity between the contrast agent and the biofilm with little attachment onto the porous surfaces, **4-** introduction of nanoparticles at low concentrations in the culture medium. We validated our approach in microfluidic devices by comparing fluorescence microscopy of *Pseudomonas aeruginosa* GFP and commercial AuNP-ConA labeled with rhodamine, thus allowing us to verify colocalization of biofilm and AuNP-ConA. We further proceeded to imaging *Pseudomonas aeruginosa* biofilm grown in a 3D printed porous medium under a constant flow of nutrients using X-ray computed microtomography. For this purpose, we developed a new chemical approach to the production of the AuNP-ConA that allows us to obtain large quantities of contrast agent in a few easy steps at a reasonable cost. Such functionalized gold nanoparticles open new perspectives for imaging bacterial biofilms in a variety of opaque porous structures, with applications ranging from chemical engineering to environmental sciences.