Fluid Flow, Motility and Cell Shape Set the Found. for Biofilm Architecture of *Caulobacter crescentus*

**T. Rossy**¹, C. D. Nadell², A. Persat¹;
¹Ecole Polytechnique Fédérale de Lausanne, Lausanne, SWITZERLAND, ²Dartmouth College, Hanover, NH.

In the wild, biofilms comprise multiple strains or species. The spatial arrangement of these communities influences the nature of their interactions through the expression of social phenotypes, which itself feeds back onto spatial organization. However, little is known about how environmental factors affect this organization. The morphology of biofilms is altered as they experience forces arising from fluid flow, but whether these also impact biofilm mixing is unknown. Here, we demonstrate that biofilms of the freshwater bacterium *Caulobacter crescentus* form distinct patterns of surface colonization depending on hydrodynamic conditions. Surface-associated, stalked *C. crescentus* divide asymmetrically into a stalked cell staying attached and a flagellated swarmer cell that either immediately attaches to the surface, or explores the fluidic environment. Therefore, the fate of planktonic daughter cells is affected by both flow and swimming motility. Based on this, we sought to answer whether and how hydrodynamic forces affected *C. crescentus* biofilm architecture and spatial lineage structure. To address this, we performed high-resolution visualizations of *C. crescentus* biofilms grown in microfluidic chambers under controlled flow. First, we observed a clear decrease of surface colonization with increasing flow velocity. In weak flows, the surface was densely and uniformly covered, whereas it was sparsely colonized by large colonies in strong flows. Second, we probed the effect of flow intensity on spatial lineage structure by using two *C. crescentus* strains expressing distinct fluorescent proteins and measured lineage segregation during surface colonization. Surprisingly, segregation increased with flow velocity, in contrary to the assumption that flow induces lineage mixing. Finally, to describe these colonization patterns, we developed a theoretical model based on the balance between advective transport and diffusion of swarmer cells. Planktonic *C. crescentus* indeed experiences diffusive transport from random swimming trajectories and advective directional transport from fluid flow. As flow intensity increases, the residence time of planktonic cells in the channel decreases. This reduces their chances to spread across the channel and to encounter the surface through swimming. Consistent with our model, in weak flows, a non-motile mutant recapitulated the wild-type biofilm structure obtained in strong flow. We thus demonstrate that hydrodynamic forces strongly impact not only biofilm architecture, but also lineage distribution in multi-strain biofilms. By modulating lineage mixing, flow is expected to influence the social interactions between biofilm-dwelling bacteria. This indicates that social interactions are not only dictated by biological factors, but also by mechanical conditions imposed onto the community.