Visualising the diversity of membrane potential changes in cyanobacteria during photosynthesis, exoelectrogenesis and nitrogen fixation

Many phototrophic microorganisms, algae and plants export energetic electron carriers out of the cell, carrying electrons derived from photosynthesis, in a process termed exoelectrogenesis. Exoelectrogenesis remains one of the least well understood electron transport pathways within photosynthesis, with both its biochemical mechanism and its function within cellular life unknown [1]. An increased understanding of exoelectrogenesis would aid the development bioelectrochemical systems the production of solar electricity and fuels, and illuminate the nature of a significant energetic ‘leak’ in the photosynthetic apparatus. Typically, approaches to studying this phenomenon rely on bulk methods, which reduces the activity of a diverse, complex community of cells to single-output measurements.

Here we investigate the function that exoelectrogenesis plays within the cyanobacterial biofilm, and relate this to the understanding of electron flux in phototrophs more generally. To do so, we apply the fluorescent reporter ThT to biofilms of the model cyanobacterium *Synechocystis* to visualise and quantify membrane potential during electron export. By coupling this technique with electrochemistry, we both probe and control the redox chemistry at the interface between cells and coverslip. Further, we aim to study the spatial heterogeneity of photosynthetic and exoelectrogenic activity across cyanobacterial biofilms. To do so we study the multicellular community at the single-cell level, analysing distinct sub-populations within the biofilm of varying photosynthetic activities.

Finally, we apply this tool to the diazotroph *Trichodesmium*, to monitor photosynthesis in real-time at single-cell resolution along the trichome, to shed light on the mechanism of segregation of N_2 fixation and photosynthesis in *Trichodesmium* [2].

[1] Wroe, E. I., Egan, R. M., Willyam, S. J., Shang, L., & Zhang, J. Z. *Current Opinion in Electrochemistry*, **2024** 46, 101535.

[2] Hania, A.; Lopez-Adams, R.; Prasil, O.; Eichner, M. *Photosynthetica* **2023**, *61* (1), 58–72. doi.org/10.32615/ps.2023.007.