Unravelling the Hidden Choreography of Energy Conversion: Subcellular Dynamics and Distribution of Oxidative Phosphorylation Complexes in Bacterial Cells

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Biological cell membranes are complex environments displaying dynamic lipid and protein nanoclustering with implications for key processes. Among the latter, energy conversion in most living organisms relies on the activity of proton transporters associated with the membrane and connected by electron shuttles, quinones, collectively describing the oxidative phosphorylation process (OXPHOS). The speed of this enzymatic reaction is limited on diffusional heterogeneity properties of the proteins. Thus, understanding how a specific molecule gets to the right place, at the right moment, in the right cascade of any biological events poses a notable challenge. Importantly, the hypothesis that congregation of OXPHOS complexes empowers energy conversion and consequently maximize connectivity with the quinone pool requires further experimental validations.

Despite substantial knowledge of the enzymatic reactions involved in OXPHOS in prokaryotes, there is a stark lack of understanding regarding the relationship between the distribution and diffusion of OXPHOS components in bacterial membranes and the efficiency of the process. Therefore, the objective of our work is to provide a comprehensive picture of the organization and dynamics of all components of OXPHOS at the single-cell level.

Investigating the subcellular localization and dynamics of nanometric and oligomeric OXPHOS complexes is challenging due to the micrometer size of bacterial cells. To break this limit, we employed super-resolution microscopy to decipher the behavior of individual molecules. To this end, the methods based on direct stochastical optical reconstruction microscopy (dSTORM) or fluorescence correlation spectroscopy (FCS) demanded the introduction of suitable fluorescent probes and genetically fused protein of interest to HaloTag enzymes. Leveraging the superior brightness of Promega fluorescent ligands, such as cell-permeant TMR® or Sir-647®, we achieved dual-color molecule tracking with sufficient spatial resolution to visualize, quantify, and resolve protein proximities and oligomerization states. The unique photophysics properties of the two ligands showcase a nanoscale visualization at lateral resolutions well below 20 nm.

Overall, this project aims to record unprecedented biologically relevant parameters of freely diffusing or confined complexes over multiple time intervals and resolve dynamic molecular heterogeneity on different spatial scales of observations, ranging from few tens of nanometer to a full cellular image.