

731-Pos Board B496

Bacterial Type 3 Secretion Systems: High-Throughput 3D Single-Molecule Tracking of Sorting Platform Proteins in Live Cells

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Bacterial secretion systems are large biomolecular assemblies that rely on static and transient interactions between individual molecular subunits. A central example is the Type 3 Secretion System (T3SS) which consists of both the static membrane-embedded needle complex and the much more dynamic cytoplasmic sorting platform. Single-subunit turnover in the sorting platform and the resulting structural heterogeneity have made it challenging to decipher the molecular-level mechanism of Type 3 secretion. Live-cell single-molecule super-resolution microscopy is ideally suited to measure spatial locations and trajectories of individual molecular subunits with nanoscale precision. Extracting meaningful biological results, however, requires characterizing the entire distribution of molecular behaviors, which in turn, necessitates a large number of individual measurements. Here, we apply high-throughput aberration-corrected 3D single-molecule localization microscopy to quantitatively measure the diffusion behaviors of over 100,000 individual T3SS sorting platform proteins. The single-molecule trajectories reveal multiple diffusive populations in the bacterial cytoplasm suggesting the pre-formation of functionally important higher-order molecular complexes. By providing information on the spatiotemporal regulation of protein function in living cells, our results complement recent structural and biochemical findings that the cytoplasmic T3SS sorting platforms contain large pod-like structures and that cytoplasmic C-ring proteins may pre-assemble into oligomeric complexes prior to binding to the T3SS sorting platforms.