Physical Nature of the Bacterial Cytoplasm

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***Short Abstract* — It is crucial to understand the physical nature of the cytoplasm since it defines dynamics of intracellular processes and thus cellular physiology. We use single-particle tracking of protein filaments, plasmids, storage granules and foreign probes in *Caulobacter crescentus* and *Escherichia coli* to explore cytoplasm dynamics in bacteria at mesoscale (with probe size>>protein size). Quantitative analysis reveals that the dynamics of the cytoplasm closely resembles dynamics of glassy materials and depends on particle size and metabolic state of the cell. The glassy behavior of the cytoplasm impacts all processes that involve large cellular components and thus has major biological implications.**

***Keywords* — Intracellular dynamics, glassy dynamics, cytoplasmic probe, single-particle tracking, non-equilibrium properties, dormancy, macromolecular crowding.**

I. Abstract

THE bacterial cytoplasm is an aqueous environment that is extremely crowded, highly polydisperse in size of constituents and far from thermodynamic equilibrium. How these features affect cytoplasmic dynamics are poorly understood. However, this understanding is paramount as the physical nature of the cytoplasm underlies dynamics of all intracellular processes and thus defines cellular physiology.

Both normal and anomalous diffusive motions have been reported for cytoplasmic components [1-4], and a unifying picture has yet to emerge. We used single-particle tracking of protein filaments, plasmids, storage granules and foreign particles of different sizes in *Caulobacter crescentus* and *Escherichia coli* to study the physical properties of the cytoplasm as a function of particle size. We showed that the bacterial cytoplasm displays properties characteristic of glass-forming liquids close to the glass transition [5]. The dynamics of the cytoplasm changes from liquid-like for smaller particles (protein-size scale) to solid-like for bigger components (>> protein size). As a consequence, the motion of cytoplasmic components becomes disproportionally constrained with increasing size. These results provide an explanation for the previous seemingly conflicting reports of both anomalous and diffusive motion.

Remarkably, cellular activity fluidizes the cytoplasm, allowing larger components to escape their local environment and explore larger regions of the cytoplasm. Consequently, cytoplasmic fluidity and dynamics dramatically change as cells shift between metabolically active and dormant states in response to fluctuating environments.

Our findings fundamentally alter how we view the bacterial cytoplasm. Currently, the bacterial cytoplasm is typically considered as a simple viscous fluid. Although it may be valid at a protein size scale, our data suggest that larger components (ca. ≥ 30nm) such as plasmids, protein filaments, storage granules and multitude of other large components populating cytoplasm (e.g., polyribosomes, chromosomes, phages, microcompartments, enzymatic megacomplexes, etc.) will be impacted by the glassy behavior of the cytoplasm and the metabolic state of the cell. These non-equilibrium physical properties have to be taken into account during development of quantitative models of intracellular processes.

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