

Diffusive Dynamics of biological macromolecules in the cytoplasm of live bacteria

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Background/Relevance

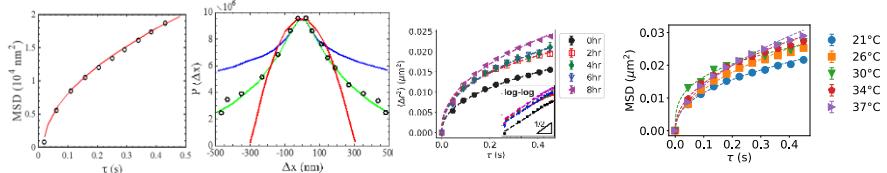
- Quantitative studies on the dynamics of proteins and plasmids inside live bacteria provide new insights to understanding the fundamental processes of life, as well as the properties of the bacterial cytoplasm, and the interactions between the probes and their environments.

Innovation

- Super-resolution fluorescence microscopy in combination with single particle tracking (termed as sptPALM) allows to track individual molecules in live bacteria and cells even if the density of the molecules are very high (>1000 copies per bacterium).

Key Results

- MSD shows that the motion of H-NS proteins in live E. coli is sub-diffusive with a non-Gaussian behavior, and the dynamics of H-NS proteins is cell length and cell age dependent.
- Comparing the MSDs before and after treatment with Ag⁺ and Mg²⁺ shows that Ag⁺ and Mg²⁺ fluidize the bacterial cytoplasm.
- The diffusion coefficients of H-NS proteins and fluorescent dyes in live and dead bacteria is a temperature-dependent.



Approach

- Track the HNS protein in different environmental conditions:
 - Grow k12-E.coli bacteria with fluorescent H-NS proteins in M9 media overnight at 37°C in a shaking incubator.
 - The next day, the culture is diluted to OD=0.05
 - When the OD reached 0.3-0.4, 10 μl of the bacterial culture is added on small square agarose gel pad, incubated at room temperature for mounting on the pad. The remaining culture is treated with Ag⁺, Mg²⁺, pH changes, and temperature shocks.
 - The mounted bacteria samples are imaged using sptPALM, followed by quantitative analysis.

Conclusions

- The displacement of H-NS proteins in the untreated bacterial cells showed a non-Gaussian behavior with sub-diffusive behavior.
- The diffusion coefficients of H-NS proteins is cell length dependent
- Treating the bacterial cells with Ag⁺/Mg²⁺ from 2-8 hr caused faster diffusion of H-NS proteins.
- Raising the temperatures from room temperature to 37 °C caused faster diffusive dynamics of H-NS proteins in live bacteria and slower diffusive dynamics in dead bacteria.

Future work

- Analyze data, prepare manuscript, and write Ph.D. dissertation.
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