سمینار هفتگي ماده چگال نرم

عنوان سمینار

Super-Resolved Microscopy as a Tool to Unveil Intracellular Structure in Biological Systems

ارائه دهنده

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چکیده

We have employed a combination of super-resolution microscopy techniques to explore the spatial redistribution of the heat-stable nucleoid structuring (H-NS) protein within E. coli in response to high osmolarity. H-NS is an abundant nucleoid association protein in gram-negative bacteria and plays a dual role as a global gene regulator and nucleoid organizer. By imaging the spatial organization of H-NS, we hoped to gain insight into its complicated role in osmoadaptation. While we saw no effect on the spatial organization of H-NS in osmotically stressed, exponentially growing cells, we observed a profound rearrangement of H-NS proteins in osmotically stressed, stationary phase cells. In this case, H-NS was dynamically translocated to the cell periphery, over the course of 5-10 minutes, and appears to be excluded from a tightly condensed chromosome. While the mechanism remains elusive, we were able to connect this response to the overall superhelicity of the bacterial chromosome. By inhibiting DNA gyrase in exponential phase, we were able to observe a very similar reorganization of H-NS, and chromosomal collapse, that we previously observed only in stationary phase. This behavior implies that the superhelicity of the chromosome plays a role in regulating osmoadaptation. To perform these studies, owing to the small size of bacteria and the diffraction limit of light, we established an optical setup on which we can conduct one- or two-color PALM or dSTORM in 3-dimensions. These single-molecule localization microscopy (SMLM) methods can achieve a resolution of roughly 20nm. In addition, we developed image-processing tools to reconstruct accurate SMLM images and studied the photo-physics of various photo-switchable fluorophores to confirm their suitability for high-resolution SMLM. Further applications of these methods and developments are discussed herein.

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