

Dynamics of RNA Polymerase in E. Coli determined by Raster Image Correlation Spectroscopy and Number and Brightness Analysis

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RNA Polymerase (RNAP) is fundamental in all living organisms, as it transcribes DNA into RNA molecules. Visualizing the molecular copy number and dynamics of RNAP in single living cells is difficult using conventional fluorescence microscopy. We use two techniques based on Fluorescence Correlation Spectroscopy (FCS) to determine the molecular copy number and mobility of RNAP in single living cells of Escheri coli. Raster Image Correlation Spectroscopy (RICS) is an image based FCS technique that measures the average molecular diffusion at every point in an image. This is useful in living cells for characterizing molecular diffusivity in addition to DNA, membrane or cytoskeletal binding (Digman et al. 2005; Digman & Gratton 2009). Number and Brightness Analysis (N&B) is an image based FCS technique which measures molecular concentration and molecular brightness at every point in an image. This is useful in living cells for characterizing expression level and oligomerization state (Digman et al. 2008). We found that RNAP is expressed in copy numbers of ~ 1000 molecules per cell and diffuses at approximately 1 $\mu\text{m}^2/\text{s}$. We have established the utility of RICS and N&B for monitoring RNAP in E. coli, and intend to measure RNA-protein interactions between RNAP and the sigma 70 subunit using RNA Mango labelling of sigma 70.

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