ONI Application note

Bacteria research

Exploring adaptive responses in nitrogen starved *E. coli* using the Nanoimager

Based on publication by: McQuail, J., Switzer, A., Burchell, L., & Wigneshweraraj, S. (2020). The RNA-binding protein Hfq assembles into foci-like structures in nitrogen starved *Escherichia coli*. The Journal of Biological Chemistry, 295(35), 12355–12367.

Challenge

Bacteria are often starved of essential nutrients and have consequently evolved complex strategies that enable them to withstand prolonged periods without vital resources. To maximize chances of survival, bacteria spend the majority of their time in a state of little to no growth. However, the pathways initiating and regulating these metabolic adaptation mechanisms are poorly understood, in part due to the limited technology required to accurately study them[1].

Conventional imaging techniques cannot clearly distinguish and trace individual molecules that are highly expressed within a bacterial cell. This is due to the diffraction of light that limits the ability to attain spatial information of particles smaller than 250 nm, making it challenging to examine how bacteria respond to nutrient (e.g. nitrogen) starvation.

To examine the intracellular behavior of individual molecules of the RNA binding protein Hfq, known to regulate gene expression during stress[2], McQuail *et al*. (2020) turned to single-molecule localization microscopy using the Nanoimager to overcome the diffraction limit of light[3]. By combining photoactivated localization microscopy (PALM) with single-particle tracking (SPT), they were able to activate subsets of Hfq molecules to assess their dynamic behavior in live nitrogen starved bacteria.

Results

In order to visualize Hfq, the protein was tagged with a PA-mCherry. This enabled McQuail *et al*. to track individual molecules of Hfq in real-time and calculate their diffusion coefficients using the dedicated Nanoimager software, in both nitrogen starved bacteria and non-starved controls. Hfq molecules in long-term (24-hours) nitrogen

Summary

The Nanoimager provides a multiplex solution to explore the microscopic details of bacterial adaptation mechanisms.

Using single-molecule localization we can:

- Visualize single molecules involved in the regulation of adaptive processes in bacteria
- Understand the dynamic behavior of RNA binding proteins
- Explore protein-protein interactions and aggregation
- Elucidate and analyze protein trafficking networks in real-time using integrated tools

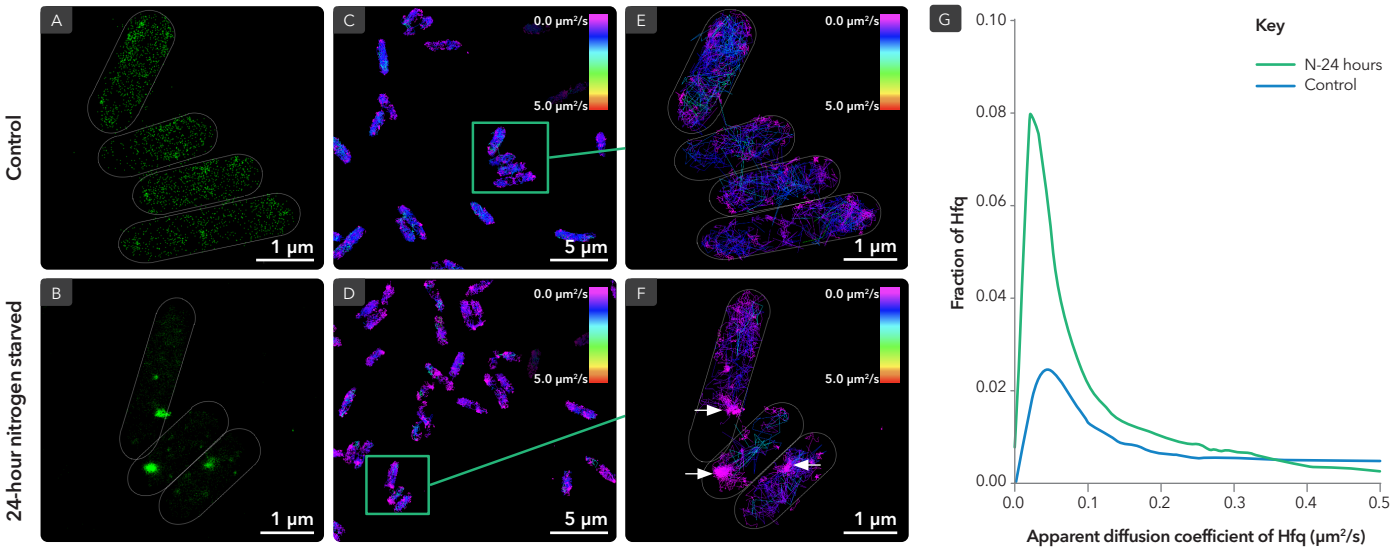

Figure 1 | Localizations from PALM-SPT imaging of the RNA binding protein Hfq (tagged to PA-mCherry protein, green) in control cells (A) or following 24-hours nitrogen starvation (B). Hfq molecules were less motile in the nitrogen starved bacteria compared to controls, displaying confined regions of Hfq, SPT tracks are colored by their diffusion coefficient. (C-D). Zoomed in view of tracks, foci indicated with white arrow (E-F). Histogram comparing the diffusion coefficients between the controls (blue) and nitrogen starved bacteria (green) for the sampled populations (G).

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starved bacteria exhibited less motility compared to controls (Figure 1). This was due to the reduced movement of Hfq molecules within confined spaces presenting as clusters, with one significantly larger cluster or 'foci' per bacterium.

To further examine the Hfq foci at 24-hours post-starvation, HDBSCAN (density-based spatial cluster analysis) was performed on the PALM-SPT localization data. The analysis identified that the larger Hfq foci were present in approximately 90% of the nitrogen starved bacteria sampled and rarely observed in controls (Figure 2). By combining advanced microscopy techniques with streamlined analysis tools, the Nanoimager can rapidly visualize and quantify individual clusters of molecules. This facilitates novel experimental approaches to further explore the formation of the confined localizations of Hfq, not observed previously in *E.coli*.

Figure 2 | HDBSCAN cluster analysis. Cluster analysis was performed on the PALM-SPT localization data (A-B), to identify the large Hfq foci in the 24-hour nitrogen starved bacteria. Clusters were filtered by area, density and the number of localizations for the nitrogen starved bacteria (D) with the same filters then applied to the control sample for analysis (C). Histogram displaying average area of clusters in nitrogen starved bacteria, with a mean of 0.015 µm² (E).

Solution with the Nanoimager

The Nanoimager provides a complete solution for understanding bacteria. Using powerful techniques such as PALM-SPT, enables novel research into transcriptional cascades, diffusion pathways, as well as protein-protein interactions in a fast and accessible manner. The ability to use sophisticated analysis tools to further characterize behavior provides valuable insight into the role of adaptation mechanisms. ONI is focused on advancing the scientific tools that empower researchers to continue exploring the microscopic details of bacteria.

For more information on single-molecule localization microscopy and PALM-SPT in bacteria research visit www.oni.bio/pathogens

References

1. Switzer, A., Brown, D. R. & Wigneshweraraj, S. (2018). New insights into the adaptive transcriptional response to nitrogen starvation in *Escherichia coli*. Biochemical Society Transactions 46(6), 1721-1728.
2. Brown, D. R., Barton, G., Pan, Z., Buck, M., & Wigneshweraraj, S. (2014). Nitrogen stress response and stringent response are coupled in *Escherichia coli*. Nature Communications, 5, 4115.
3. McQuail, J., Switzer, A., Burchell, L., & Wigneshweraraj, S. (2020). The RNA-binding protein Hfq assembles into foci-like structures in nitrogen starved *Escherichia coli*. The Journal of Biological Chemistry, 295(35), 12355–12367.

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