Developing new antibiotics: from target identification to mechanism validation

Summary

The Nanoimager provides a multiplex solution for novel antibiotic development. Through its single-molecule capabilities is supports:

- Understanding mechanisms of resistance
- Novel antibiotic target identification
- Antibiotic selection and validation
- In-depth description of the MoA

Challenge

"There is no time to wait. Unless the world acts urgently, antimicrobial resistance will have a disastrous impact within a generation." Although antibiotics were discovered nearly 100 years ago, their overuse led to a growing problem of resistant bacteria impossible to eradicate with available therapies. In 2019 the WHO report showed that multidrug-resistant bacteria are expected to be one of the leading causes of death globally, overtaking cancer, in 30 years.

A thorough understanding of bacterial resistance is hindered by the fact that bacterial populations are sometimes heterogeneous, with resistance arising from a few individuals. Understanding cell-to-cell variations at a single-molecule level provides new insights into sources of resistance, as well as potential novel antibiotic mechanism of action. The Nanoimager effectively addresses these challenges and provides an accessible platform for a robust and fast treatment validation.

Results

The Nanoimager provides single-molecule sensitivity for quantification of *E.coli* cell response to drug treatment. In this example, photoactivatable protein Dendra2 was fused to a transcription factor and expressed at native levels. Antibiotic-treated and control (untreated) cells were fixed and imaged by PALM (Figure 1A). The number of molecules within each sample showed approx. 7-fold increase in the level of the transcription factor production upon antibiotic treatment (Figure 1B). This observation suggests upregulation of target genes in a coordinated response against the antibiotic.

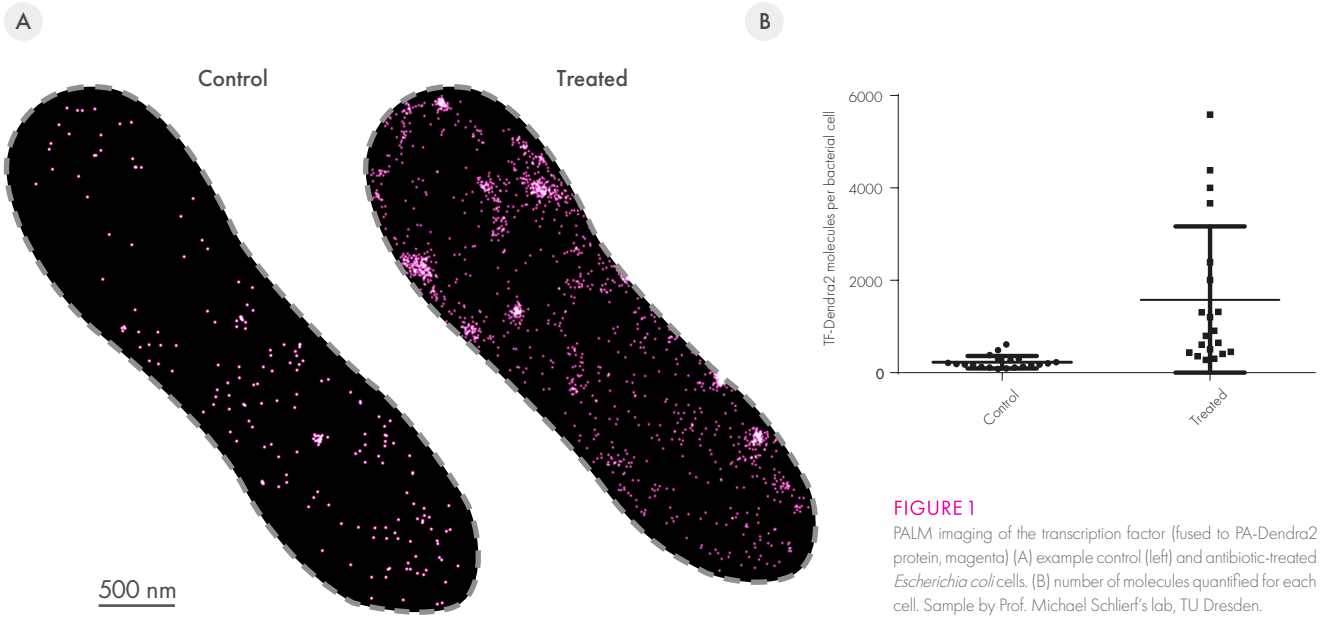

FIGURE 1

PALM imaging of the transcription factor (fused to PA-Dendra2 protein, magenta) (A) example control (left) and antibiotic-treated *Escherichia coli* cells. (B) number of molecules quantified for each cell. Sample by Prof. Michael Schlierf's lab, TU Dresden.

In the second example the *Bacillus subtilis* PBP2 protein, an ubiquitous penicillin target, was fused with HaloTag, stained with JF549 and followed by single-particle tracking (Figure 2A). Analysis of PBP2 spatio-temporal distribution revealed 2 types of molecular behavior: pole-associated molecules exhibiting random motion and mid-cell localised molecules diffusing directionally (Fig.2A, tracks 1&2 and tracks 3&4, respectively). Measurement of diffusion coefficient provides insights into the number of molecules and their characteristic dynamics, as presented in the example chart (Figure 2B). Understanding the activity, interactions and partners of proteins essential for bacterial cell cycle enables researchers to discover and engineer new classes of antibiotics.

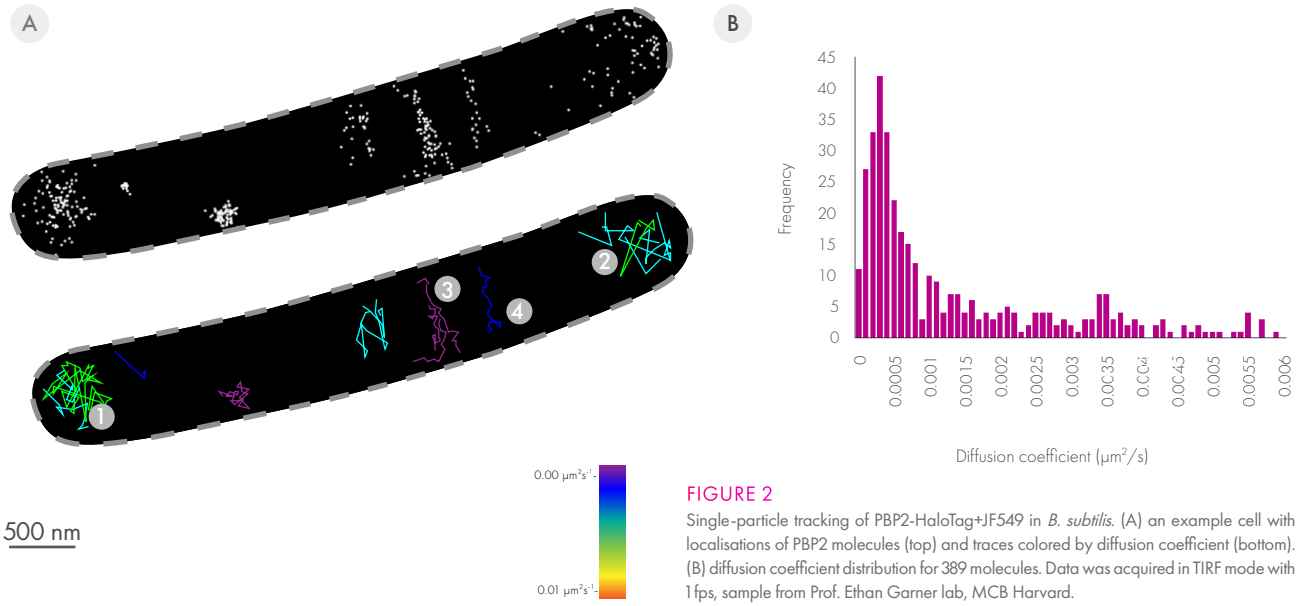

FIGURE 2

Single-particle tracking of PBP2-HaloTag+JF549 in *B. subtilis*. (A) an example cell with localisations of PBP2 molecules (top) and traces colored by diffusion coefficient (bottom). (B) diffusion coefficient distribution for 389 molecules. Data was acquired in TIRF mode with 1 fps, sample from Prof. Ethan Garner lab, MCB Harvard.

Solution With The Nanoimager

The Nanoimager provides a complete solution for understanding bacterial antibiotic resistance mechanisms, robust development and validation of novel therapeutics in a fast and accessible manner.

Compatible with microfluidics and live-cell acquisition, this imaging platform enables detection with high sensitivity of the effects and killing consequences of antibiotics, correlating function with bacterial viability.

For more information on super-resolution bacteria imaging visit **www.oni.bio**

e: hi@oni.bio • t: +44 (0) 1865 951 820
Linacre House, Jordan Hill Road, Oxford, OX2 8TA, UK
ONI Inc. SPACES, 101 Jefferson Drive, Menlo Park, CA, 94025 USA
www.oni.bio

@oniHQ @ONI

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