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quenched-smFISH: Counting small RNA in Pathogenic Bacteria DOUGLAS SHEPHERD, Department of Physics, University of Colorado Denver, Denver, CO 80217, NAN LI, SOFIYA MICHEVA-VITEVA, Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87544, BRIAN MUNSKY, Department of Chemical and Biological Engineering, Colorado State University, Fort Collins CO 80523, ELIZABETH HONG-GELLER, Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87544, JAMES WERNER, Center for Integrated Nanotechnologies, Los Alamos National Laboratory, Los Alamos, NM 87544 — Here, we present a modification to single-molecule fluorescence in situ hybridization, quenched smFISH (q-smFISH), that enables quantitative detection and analysis of small RNA (sRNA) expressed in bacteria. We show that short nucleic acid targets can be detected when the background of unbound singly dye-labeled DNA oligomers is reduced through hybridization with a set of complementary DNA oligomers labeled with a fluorescence quencher. Exploiting an automated, multicolor wide-field microscope and GPU-accelerated data analysis package, we analyzed the statistics of sRNA expression in thousands of individual Yersinia pseudotuberculosis and Yersinia pestis bacteria before and during a simulated infection. Before infection, we find only a small fraction of either bacteria express the small RNAs YSR35 or YSP8. The copy numbers of these RNA are increased during simulated infection, suggesting a role in pathogenesis. The ability to directly quantify expression level changes of sRNA in single cells as a function of external stimuli provides key information on the role of sRNA in bacterial regulatory networks.

Douglas Shepherd
Department of Physics, University of Colorado Denver, Denver, CO

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