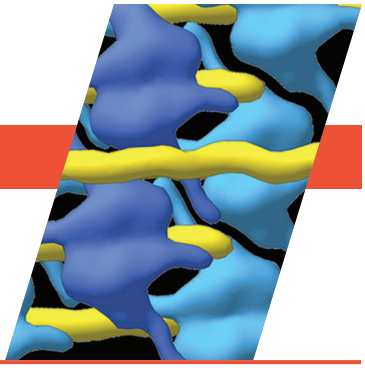
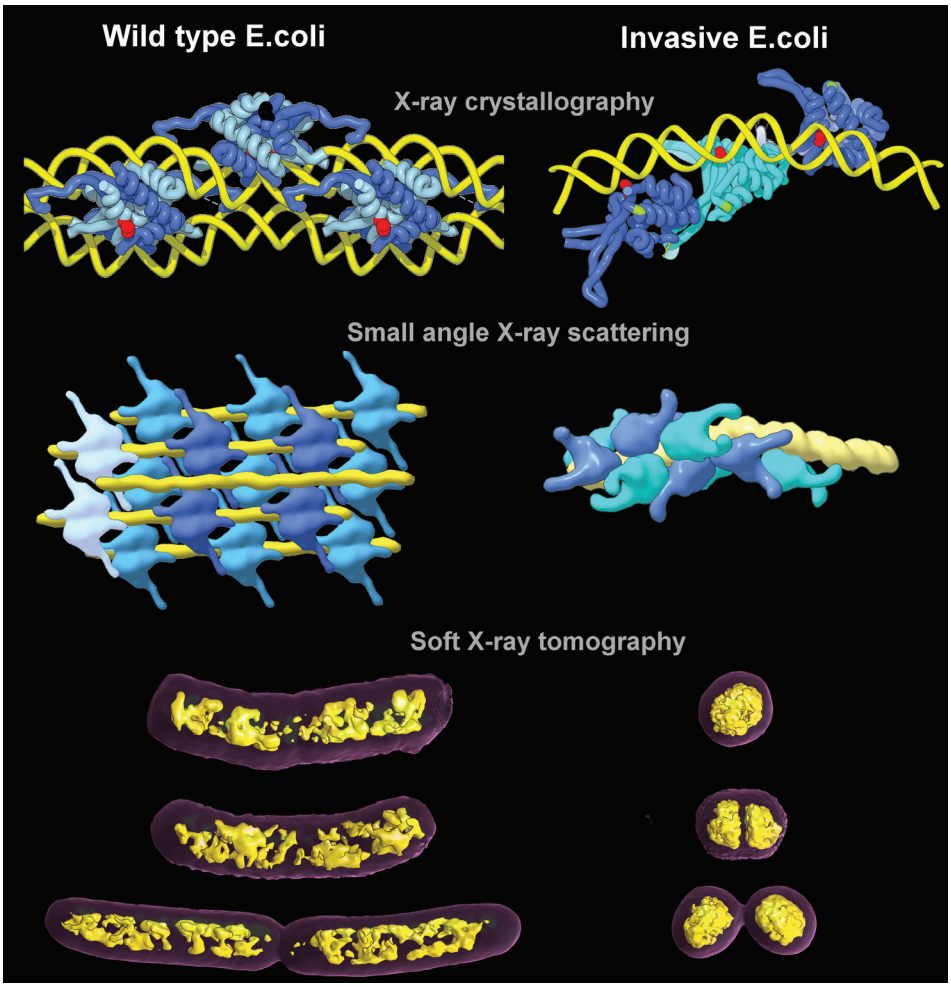


BIOLOGICAL SCIENCES

Molecular Switch Triggers Bacterial Pathogenicity



The top two rows show illustrations of crystals and solution structures of bacterial HU proteins with DNA represented by x-ray crystallography and small-angle x-ray scattering, respectively. DNA strands are yellow and HU proteins are shades of blue. Soft x-ray tomography was used to visualize bacterial chromatin (in yellow) in wild-type and invasive *E. coli* cells, shown in the bottom row. (Credit: Michal Hammel/Berkeley Lab.)

Scientists have revealed for the first time the molecular steps that turn on bacteria's pathogenic genes. Using an array of high-powered x-ray imaging techniques at the ALS, the researchers showed that certain bacterial DNA-binding proteins, denoted HU, are involved in the physical twisting of the genetic strand and that its supercoiling can trigger the expression of genes that make a microbe invasive. The study could open up new avenues in the development of drugs to prevent or treat bacterial infection.

The researchers looked at how long strands of DNA are tightly packed into chromosomes, a necessity if they are to fit into compact spaces. In eukaryotes (organisms whose cells have a nucleus—including humans), the strands of DNA wrap around proteins called histones, like thread on a spool. In contrast, the DNA strands in single-celled prokaryotes (such as bacteria) are compacted into a region inside the cell called a nucleoid, analogous to a nucleus but not enclosed by a membrane. And like histone in eukaryotes, HU in prokaryotes helps organize the DNA into more-compact chromosomes.

However, when the normal twists and turns of DNA compaction turn into

Fighting the Resistance

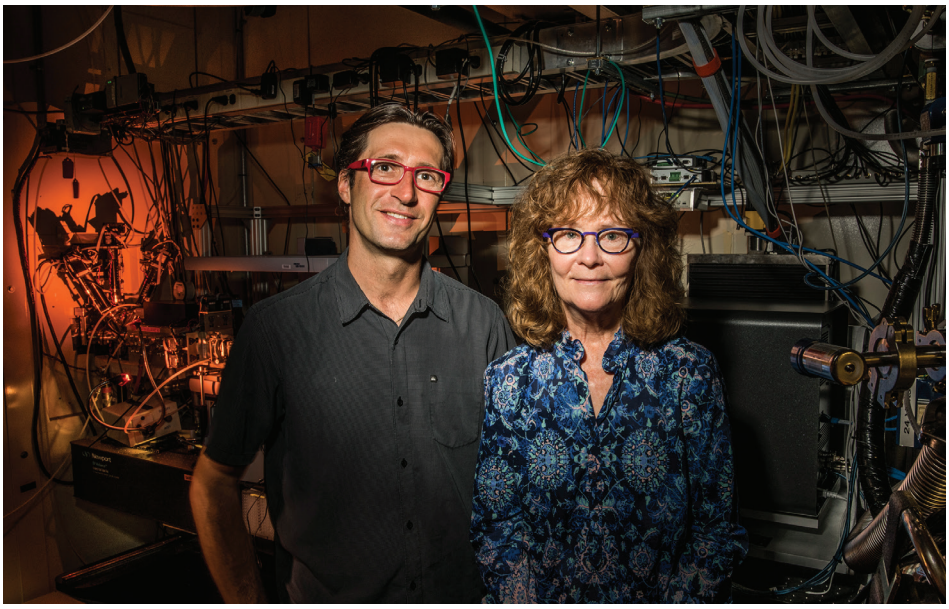
Alexander Fleming, who received a Nobel Prize in 1945 for discovering penicillin, warned of the dangers of its negligent use in his Nobel lecture. "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body." Today, the Centers for Disease Control and Prevention (CDC) conservatively estimates that over two million people are sickened every year in the United States due to antibiotic-resistant infections, with at least 23,000 dying as a result. Because antibiotic resistance is a natural evolutionary process that can be slowed, but not stopped, the development of new antibiotics is a crucial part of the fight. However, the number of new antibiotics developed and approved has decreased over the last 30 years. In this work, Hammel et al. describe a molecular mechanism for switching *E. coli* bacteria from noninvasive to invasive by triggering structural changes in DNA, a discovery that could lead to new classes of antibiotics.

supercoiling (over- or under-winding of the double helix), trouble can begin. It has been known that DNA supercoiling leads to pathogenicity in bacteria, but exactly how the bacterial chromosome is condensed, organized, and ultimately segregated has been a puzzle for over half a century. In this work, the researchers were able to visualize for the first time how this packing is done in *E. coli*, and they also discovered that the way HU proteins pack the chromosomes can trigger gene expression.

Elucidating these molecular mechanisms entailed imaging interactions that span the nanoscale and mesoscale, from HU-DNA molecular complexes to bacterial chromosome and nucleoid structure, using three ALS beamlines. The Structurally Integrated Biology for Life Sciences (SIBYLS) Beamline 12.3.1 combines x-ray crystallography and small-angle x-ray scattering (SAXS) capabilities. The crystallography performed there, as well as at Beamline 8.3.1, provided atomic-level details of how the HU proteins—both wild type (normal) and invasive (pathogenic)—interacted with the bacterial DNA, while SAXS was able to show how the HU proteins assembled and affected the longer strands of DNA in solution.

Then, to get a clear sense of how that twisting and packing manifests at the cellular level, the team took their samples to the National Center for X-Ray Tomography (NCXT) at ALS Beamline 2.1. With x-ray tomography, they were able to see the natural contrast in organic material in as close to a living state as possible, and they obtained quantitative comparisons of how compacted the chromosomes were in normal and pathogenic strains of *E. coli*. The results showed that the genetic material in the pathogenic *E. coli* was so tightly packed that it consumed less than one-half the volume of its non-pathogenic counterpart.

Initially, it had been believed that the enzyme topoisomerase was the primary driver of DNA coiling in bacteria. This study showed that, independent of topoisomerase, changing the assembly of HU proteins was enough to induce changes in DNA coiling at different stages of bacterial growth. What is notable about HU proteins as a trigger for gene expression is that it is quick. This makes sense as a survival mechanism for bacteria, which need to adapt quickly to different environments. The study results also beg the question: If pathogenicity can be switched on, could it also be switched off? The researchers certainly expect to answer that question in future studies. These HU interactions could be an attractive target for drugs that control pathogenesis, not only of bacteria, but of other microbes with comparable genetic structures.



Michal Hammel and Carolyn Larabell at the SIBYLS Beamline (12.3.1). (Credit: Paul Mueller/ Berkeley Lab.)

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