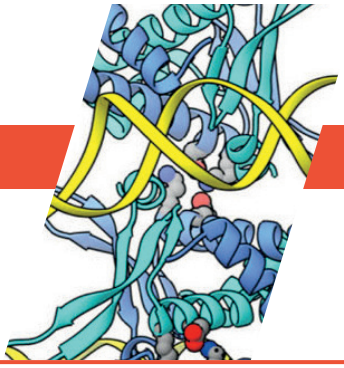
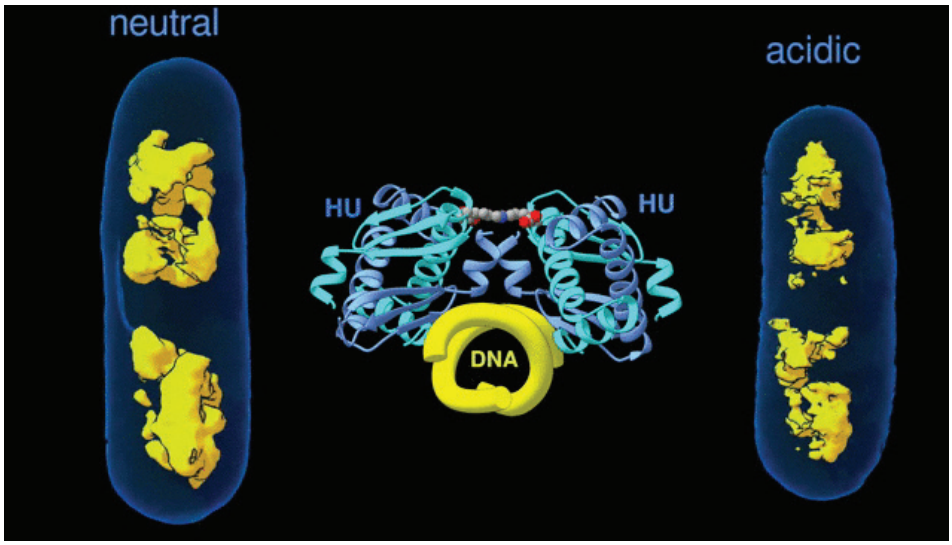
ADVANCED LIGHT SOURCE

BIOLOGICAL SCIENCES

How Proteins Remodel DNA in Bacteria Under Stress





Change in the architecture of the bacterial chromosome during the adaptation to an acidic environment is controlled by the DNA binding protein called HU (shown in shades of blue) and its interaction with DNA (yellow). (Credit: Michal Hammel/Berkeley Lab)

Scientific Achievement

Multiscale, multimodal visualization techniques at the Advanced Light Source (ALS) enabled researchers to clarify how proteins remodel bacterial DNA in response to stressful environments.

Significance and Impact

The discovery could lead to new strategies for controlling microbial behavior and, eventually, new ways to fight bacterial infections.

Bacterial rapid response

When bacteria are put in different environments, such as one that is more acidic or anaerobic, their genes start to adapt remarkably quickly. They're able to do so because the proteins making up their chromosomes can pack and unpack rapidly, regulating the expression of genes that could help them respond to the changing environment. Now, researchers have visualized this process at the molecular level using advanced imaging techniques, an achievement that could eventually enable scientists to develop strategies to control microbial behavior.

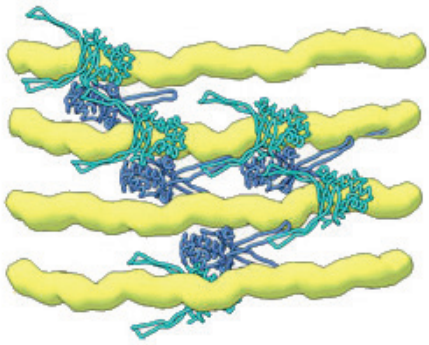
DNA-packing proteins

In bacteria, the proteins responsible for DNA packing are called HU proteins. They are dimers—made up of two identical or nearly identical subunits that, when joined, form a bilaterally symmetrical "body" with two "arms." To perform their packing function, these dimers "hug" the DNA, with arms on either side of a strand. They link up with each other (multimerize) to form arrays that hold the DNA strands parallel to each other. Changes that loosen or tighten this DNA bundling make the genetic information in the DNA more or less accessible to the enzymes responsible for gene expression.

In earlier work, the researchers used the ALS's complementary visualization capabilities to show how HU proteins can trigger pathogenicity in *E. coli*. In this work, the researchers focused on how external environmental factors, such as acidity or salinity, affect HU function.

Visualization in three ways

For a multiscale perspective on DNA packing by HU under various conditions (different pH levels and salt concentrations, different growth phases, and wild type vs mutated), the researchers used three x-ray visualization techniques at the ALS. At the microscale, soft x-ray tomography at Beamline 2.1 enabled characterization of the higher-order organization of the *E. coli* nucleoid (the bacterial analogue of a cell nucleus). At the mesoscale, small-angle x-ray scattering (SAXS) at Beamline 12.3.1



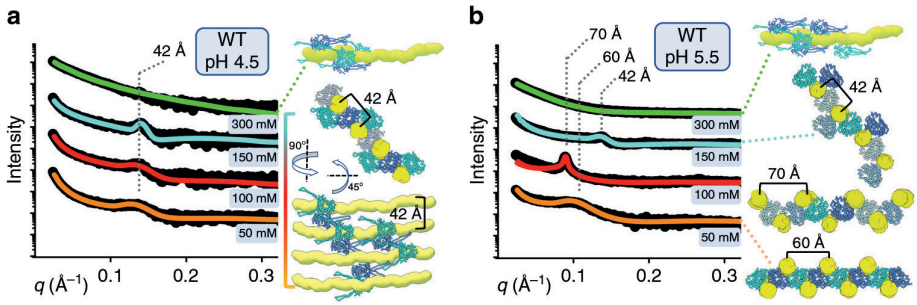
HU dimers (blue) link up to form a network that arranges DNA strands (yellow) into an orderly parallel bundle.

made it possible to determine the overall shapes of HU–DNA complexes in solution. At the nanoscale, protein crystallography at Beamline 8.3.1 allowed identification of the molecular-level mechanisms that mediate DNA bundling.

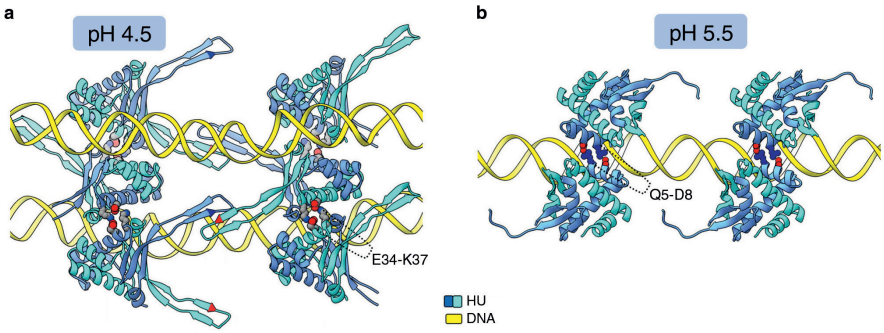
The combination of SAXS and crystallography was crucial to gaining a detailed understanding of the HU response to environmental changes. Ready access to all three beamlines, as well as to experts onsite, greatly facilitated the work. Finally, the researchers also performed gene expression measurements, to correlate expression levels with the tomographic and scattering data.

Surviving the acid test

The tomography results revealed that, under acidic conditions, the bacterial chromatin is less condensed, with more diffuse borders. This "remodeling" of the chromatin was correlated with an increase in gene expression, consistent with the need to respond quickly to survive the stress of an acidic environment. The SAXS and crystallography data showed that HU multimerization is dependent on pH and salt concentrations. These effects mechanistically rely on HU's promiscuity in forming multiple electrostatically driven multimerization interfaces. The next step will be to figure out how to control DNA packing in order to change bacterial behavior, with the ultimate goal of developing new approaches to fighting bacterial infections.



SAXS curves for wild-type (WT) HU–DNA complexes at two values of pH (4.5 and 5.5) and four salt (NaCl) concentrations (50 to 300 mM). Under most of these conditions, DNA strands (yellow) readily self-assemble into bundled structures, facilitated by HU proteins (blue). (a) At pH 4.5, the peak in the 1D SAXS profile corresponds to a DNA separation of 42 Å. (b) At pH 5.5, the peaks correspond to spacings of 70, 60, and 42 Å, depending on salt concentration. At both pH levels, the bundles separate into filament-like structures when the salt concentration reaches 300 mM.



Crystal structures of HU–DNA complexes at pH 4.5 and 5.5, showing details of the molecular-level mechanism that alters the DNA bundling. Red and blue spheres indicate electrostatically charged residues involved in HU multimerization.

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