HU multimerization shift controls nucleoid compaction

Michal Hammel,¹* Dhar Amlanjyoti,² Francis E. Reyes,¹ Jian-Hua Chen,³ Rochelle Parpana,¹ Henry Y. H. Tang,¹ Carolyn A. Larabell,¹,3 John A. Tainer,¹,4* Sankar Adhya²

Molecular mechanisms controlling functional bacterial chromosome (nucleoid) compaction and organization are surprisingly enigmatic but partly depend on conserved, histone-like proteins HUαα and HUββ and their interactions that span the nanoscale and mesoscale from protein-DNA complexes to the bacterial chromosome and nucleoid structure. We determined the crystal structures of these chromosome-associated proteins in complex with native duplex DNA. Distinct DNA binding modes of HUαα and HUββ elucidate fundamental features of bacterial chromosome packing that regulate gene transcription. By combining crystal structures with solution x-ray scattering results, we determined architectures of HU-DNA nucleoproteins in solution under near-physiological conditions. These macromolecular conformations and interactions result in contraction at the cellular level based on in vivo imaging of native unlabeled nucleoid by soft x-ray tomography upon HUβ and ectopic HUα38 expression. Structural characterization of charge-altered HUαα-DNA complexes reveals an HU molecular switch that is suitable for condensing nucleoid and reprogramming noninvasive Escherichia coli into an invasive form. Collective findings suggest that shifts between networking and cooperative and noncooperative DNA-dependent HU multimerization control DNA compaction and supercoiling independently of cellular topoisomerase activity. By integrating x-ray crystal structures, x-ray scattering, mutational tests, and x-ray imaging that span from protein-DNA complexes to the bacterial chromosome and nucleoid structure, we show that defined dynamic HU interaction networks can promote nucleoid reorganization and transcriptional regulation as efficient general microbial mechanisms to help synchronize genetic responses to cell cycle, changing environments, and pathogenesis.

INTRODUCTION

Bacterial and archaeal chromosomes exist in a microscopically visible, variably condensed nucleoid entity analogous to eukaryotic chromatin (1, 2). Similar to histones in eukaryotes, nucleoid-associated proteins (NAPs) are essential for bacterial nucleoid condensation and resulting expression patterns (3, 4). HU, a ubiquitous bacterial histone-like protein, is among the most conserved and abundant NAPs in eubacteria (5). In Escherichia coli, HU forms a dimer by HUα self-association (HUαα) or by HUα-HUβ (HUαβ) interactions. HUα is mostly expressed during the early exponential growth phase and HUβ is expressed only during the stationary phase (6), supporting the importance of distinct HUαα/DNA and HUαβ/DNA packaging of the nucleoid in regulating expression patterns during growth and stasis. HU dimers show high affinity to specialized or damaged DNA structures [for example, kinked or cruciform DNA (7, 8)], but it is as a largely sequence-independent DNA binding protein that HU has key architectural roles in DNA nucleoid compaction and in constraining negative supercoils in DNA (9), but this has remained structurally undefined. DNA supercoiling modulates gene transcription in a promoter-specific way (10) and influences gene expression in many pathogens (11). Moreover, nucleoid organization into local topological domains contributes to its overall compactness (2, 9) and to transcriptional regulation by enigmatic mechanisms that are directly connected. Global transcription profiles change when bacterial cells transition from the lag phase to the exponential growth phase (12–14), and HU regulates these changes. Yet, despite notable efforts to unravel the molecular mechanisms of HU interactions with DNA that affect the nucleoid state, structures with undamaged, nonspecific DNA remain unknown and the molecular connections between HU-DNA interactions and nucleoid architecture that regulate gene expression remain mysterious (2).

A noninvasive E. coli strain can be switched to an invasive form solely by mutational changes in HU (15, 16). Mutations in HUα residues E38K and V42L (termed HUα38) transform the E. coli nucleoid and reorganize the transcriptional program to activate mammalian cell invasion–associated factors, such as the curly fiber encoding gene products (15, 16); thus, HU interactions with DNA can affect global transcriptional regulation and key processes, such as pathogenesis.

HUαα homodimers are mostly present during the lag and exponential growth phase, whereas HUαβ heterodimers are mostly present during the early and late stationary phases. The exchange of HUαα with HUβ influences the corresponding gene expression pattern reviewed by Dorman (2)]. HU binds at high affinities (in the nanomolar range) to specific DNA structures that are of limited occurrence and that have specific gene regulatory or recombination functions. However, HU molecules (~50,000 molecules per cell) far outnumber such specific binding sites; thus, DNA structure and sequence-independent binding of HU likely predominate its functions in bacterial DNA condensation.

To help elucidate the fundamental and mechanistic components of bacterial chromosome structure (packing) and function (transcription), we first determined the crystal structures of the complexes of HUαα, HUββ, and HUα38β with a nonspecific DNA duplex with random sequences (Fig. 1). To determine the functional relationships of these HU-DNA structures to DNA networks and nucleoid architecture, we characterized the overall organization of HU’s nucleoprotein complexes...
in solution by small-angle x-ray scattering (SAXS) (17). We found that HUαα, HUαβ, and HUα38α38 organize DNA differently, in ways suitable for bacteria to use dynamic transformations of HU nucleoprotein networks to control DNA supercoiling and to thereby modify transcriptional programming. To test whether these changes in DNA binding by HU can affect the nucleoid state at the cellular level, we imaged unlabeled DNA in vivo by soft x-ray tomography (SXT). Comparisons of bacterial cells with HUα and HUα38 expression show that the specific HUα38 mutations that affect DNA binding and pathogenesis have a marked impact on nucleoid structure at the cellular level.

RESULTS AND DISCUSSION

Distinct HU-DNA interfaces reveal plasticity in nucleoid packing

Crystallization of HU with native duplex DNA has eluded researchers for over a decade. We therefore used SAXS (17, 18) to optimize nonspecific DNA interactions with HU in solution and efficiently approach effective crystallizations of solution interactions with a suitable order for structure determination (see Materials and Methods). We thereby crystallized and determined structures of HUαα, HUαβ, and HUα38α38 in complex with 19– or 20–base pair (bp) DNA at resolutions of 2.7 to 3.5 Å (Fig. 1, fig. S1, and table S1). Structures of HU-DNA complexes were solved by molecular replacement with the structure of HUαα (19) or HUαβ (20) (table S1). All of these structures show linear DNA conformation across HU’s α-helical “body” (Fig. 1, A to C, and fig. S1) rather than bent DNA located between extended β-ribbon “arms” as seen for structurally specific DNA interactions with two appropriately placed extra-helical bases (21) (Fig. 1, A to C, and movie S1). The asymmetric unit contains one dimer and one DNA strand that is paired to a symmetry-related strand with the out-of-register duplex positions, such that backbones superimpose but base identity differs. This “virtual sliding” of DNA along HU means that complexes with either 19– or 20-bp DNA show identical continuous DNA electron density in the unit cell (fig. S1). All HU dimers adopt similar secondary structures forming two HU faces. Crystallographic symmetry reveals an assembly in which each HU face bound to different DNA duplexes (Fig. 1E and fig. S2). All HU

**Fig. 1.** HU-DNA crystal structures, functional interactions, and interfaces. (A to C) Crystal structures of HUαα-DNA, HUαβ-DNA, or HUα38α38-DNA for four asymmetric units across continuous DNA are colored as indicated. V45 inserts into the DNA minor groove as shown for interfaces 1 and 2. The E38K mutation site in the HUα38α38-DNA structure is highlighted (green). (D) Two asymmetric units of the *Anabaena* HUαα crystal structure bound to a distorted DNA substrate. The positions of DNA, extra bases, residue P63, and V45 are colored as indicated. (E) Superimposition of HUαα-DNA and HUαβ-DNA interfaces. The protein portion was superimposed to highlight different DNA paths.
dimers bind DNA through locking a DNA phosphate between the G\textsuperscript{46} and K\textsuperscript{83} peptide amides and inserting V\textsuperscript{45}s into the minor groove (phosphate lock) (Fig. 1E and fig. S2). The importance of the G\textsuperscript{46} and G\textsuperscript{82}–K\textsuperscript{83} backbone in the locking mechanism explains their strict conservation among prokaryotes (fig. S3). In contrast, the previously described intercalation of P\textsuperscript{36} on the extended arms with the DNA minor groove may be a lock-and-key mechanism for specific limited DNA sites (22) (Fig. 1D). The interactions defined here seem universal for the sequence-independent DNA binding acting in nucleoid compaction. Unlike HU interaction with structurally specific aberrant DNA (Fig. 1D), interaction with native B-form DNA does not stabilize the extended arms, evidently distinguishing specific aberrant DNA complexes from nucleoid compaction complexes. The weak electron density of the extended arms at the contact regions is consistent with limited DNA interactions, allowing functionally important flexibility for sequence-independent DNA networking (fig. S1).

HU interactions with native DNA trigger HU multimerization, which promotes straightening of the DNA axis, and not bending, as seen for individual HU dimers on structurally aberrant DNA (Fig. 1 and fig. S1). Two HU\textalpha\textalpha~ interact across the DNA in opposite orientations, primarily through a hydrogen bond network (fig. S1). Alternating HU\textalpha\textalpha–HU\textalpha\textalpha~ couples lie alongside the DNA (in the 20-bp distance) oriented oppositely to the first couple (Fig. 1A). These HU\textalpha\textalpha–HU\textalpha~ couples interact with two distinct DNA-protein interfaces, with ~30° difference in the tilt of the HU\textalpha~–HU\textalpha~ couple relative to the main DNA axis (fig. S2A). Although V\textsuperscript{45}s inserts into the DNA minor groove at both sites, variability in the interfaces suggests that V\textsuperscript{45}s acts as the fulcrum of a ball-and-socket joint, permitting tilt and twist of HU\textalpha~ on DNA (Fig. 1E and fig. S2B). V\textsuperscript{45}s is replaced by arginines in HU family members that are DNA-specific binders [R\textsuperscript{58} of Hbb and R\textsuperscript{46} of IHF (integration host factor) \beta chain] to stabilize DNA with a specific TTA sequence (23, 24) (fig. S2D). Arginines insert into the DNA minor groove and explain higher binding affinities (7) compared to the sequence-independent flexible valine ball-and-socket joint.

The \alpha and \beta chains share 69% sequence similarity (fig. S3A), forming two HU\textbeta~ faces (\alpha\beta and \beta\alpha) (Fig. 1, B and E) and two HU\textalpha\textbeta~ pairs bind across the DNA to form an HU\textalpha~–HU\textbeta~ couple. However, the couple at the other side of the DNA is in close contact with the first couple facing the same direction (Fig. 1B). Alternating HU\textalpha~–HU\textbeta~ couples adopt distinct protein-DNA interfaces resembling interface 1 or 2 in the HU\textalpha~ homodimer (Fig. 1E and figs. S2B and S4). Interface 1 is formed by \alpha\beta and interface 2 is formed by the \beta\alpha protein face. In contrast, mutant HU\textalpha~38x38 adopts a substantially different arrangement along the DNA with two HU\textalpha~38x38 couples lying along the same side of the DNA within close proximity (Fig. 1C). Although only one face of HU\textalpha~38x38 supports formation of continuous DNA through interface 2, weak electron density for noncontinuous DNA is visible at the opposite face (fig. S1C). This marked rearrangement of HU mutant along DNA, without perturbing the DNA-protein interface, suggests the plasticity of HU multimerization along native DNA.

Parallel DNA networking dominates nucleoid packing in growth phase

By combining multiple HU\textalpha~–DNA asymmetric units perpendicular to the DNA, we visualized lateral protein multimerization (Fig. 2A). Alternating lateral multimers form a DNA network that can grow in both lateral and medial directions (Fig. 2B and movie S2). To test this networking in solution, we measured SAXS on mixtures of HU\textalpha~ with 20- and 80-bp DNA duplexes at varied protein/DNA ratios. SAXS profiles show strong diffraction peaks, confirming the formation of periodic nucleoprotein networks in solution (Fig. 2B and fig. S5). Diffraction at d = 50, 57, 78, and 2 × 78 Å spacings corresponds to the distances between the DNAs. SAXS curves of HU\textalpha~–20-bp DNA complexes show sharper peaks due to a more ordered assembly, with peaks matching the first-order diffractions of the crystal structure. Diffraction peaks for low HU\textalpha~/DNA ratios (fig. S5) suggest HU’s capability to network DNA, with several HU\textalpha~ mimicking concentration in cells. Mutations of the HU\textalpha~–HU\textalpha~ interface (K38E, V42L, and E34K) completely diminished diffraction peaks, suggesting a critical role of HU\textalpha~–HU\textalpha~ coupling in DNA networking (Fig. 2C). Mutation of the HU\textalpha~–DNA interface (G82E and V45E) does not disrupt the DNA network (Fig. 2C and fig. S5). However, introducing the G82E and K83E double mutation at the phosphate lock leads to a disruption of the network (Fig. 2C), most likely due to weakened DNA affinity (Fig. 2D). The P63E mutation on the extended arms diminished diffraction peaks at d = ~78 Å and d = ~2 × 78 Å, which suggests disruption of medial multimerization, although the d = ~57 Å peak indicates persistence of lateral multimerization (Fig. 2C). The extended and disordered character of the arms is suitable for allowing multiple interactions with neighboring DNAs or HU\textalpha~s that were disrupted by P63E mutation. This observation uncovers an independent role for HU arms or body in forming dynamic networks. Such dynamic DNA networking mediated by HU may explain efficient nucleoid compaction in rapidly growing bacteria. Moreover, overproduction of HU\textalpha~ or HU\textbeta~ does not increase nucleoid condensation (9). Thus, collective data support a model for nucleoid condensation specific to the early bacterial lag and exponential growth phase in which DNA is organized into a network maintained by sparsely bound HU\textalpha~. Protein-free gaps may allow the efficient exchange of various DNA metabolic proteins and other NAPs required for exponential growth phase–specific condensation of the bacterial chromosome (fig. S9) (9).

HU\textalpha~ and HU\textbeta~ interplay allows dynamic changes in nucleoid architecture

By combining multiple HU\textalpha~–DNA asymmetric units along the continuous DNA, we modeled an assembly that fully covers double-stranded DNA (dsDNA). Although this assembly bridges the centrally located dsDNA to two flanking DNAs, the lateral HU\textbeta~–HU\textalpha~ multimerization for DNA networking is not maintained (Fig. 3A). The \beta chain substitutions of K90N, E15D, and E12A, which participate in the hydrogen bonds maintaining lateral multimerization of HU\textalpha~s, disrupt multimerization in HU\textbeta~ (Fig. 3B). This destabilization of the lateral multimerization permits the intercalation of arms from oppositely oriented HUs, allowing for tight condensation of HU\textbeta~ to surround DNA (Fig. 3A and movie S3).

To test this disruption of lateral multimerization in solution, we measured SAXS on the HU\textbeta~ with 80-bp DNA at various ratios. SAXS data show formation of HU\textbeta~–DNA filaments in solution with a length of ~250 Å (Fig. 3C and fig. S6). Missing diffraction peaks in the curves support the absence of the DNA network. Atomistic models of HU\textbeta~/80-bp DNA filaments match the 250 Å length defined by the P(r) function (Fig. 3C) and fit experimental SAXS curves well (Fig. 3C and fig. S6A). The fitting of SAXS curves and SAXS–defined volumes (25) shows two binding modes where HU\textbeta~ fully covers DNA at high HU\textbeta~/DNA ratios or bridges two DNAs at low ratios (Fig. 3C and fig. S6A). The two binding modes described support and extend previous
biophysical (26, 27) and imaging experiments (28). Our structures show variability in the HU-DNA interfaces (Fig. 1E) that allows DNA binding in the HU saddle at low-salt concentration (29, 30) or binding of short DNAs strands (31) where cooperative HU multimerization could not persist. To further test implications of our crystal structures, we collected SAXS data on the 1× HU/DNA complex. Models derived from crystal structures give an excellent match with the SAXS, whereas the model with the DNA bound to the HU saddle does not (Fig. 3C and fig. S6D). HU can transform the HU-DNA network into filaments, as shown by diminished diffraction peaks upon adding HU into the preformed DNA network (fig. S7A). In considering the combined structural implications for a distinct role of HU in DNA condensation, we posit that HU subunits are a foil for HU action.

A charge blocking assembly switch synchronizes activation of virulence genes
To examine how altered HU multimerization affects the transcription profile, we determined the structure of the DNA-bound HU mutant (HU38). Similar to wild type, HU interacts with DNA through interfaces 1 and 2, although only interface 1 supports continuous DNA (Fig. 4A and fig. S1C). Weak electron densities of noncontinuous DNA visible at interface 2 suggest an inability of HU to network DNAs (fig. S1C). The surface-exposed K38

Fig. 2. HU-DNA network interactions and assembly. (A) Two orthogonal views of HU-DNA assembly built by combining multiple crystal asymmetric units along two lateral and one medial DNA. Location of the phosphate lock is highlighted with V45 residues. (B) Two orthogonal views of the HU-DNA nucleoprotein network displayed in molecular surface. The bottom panel shows experimental SAXS of the 16× HU/80-bp DNA complex, 8× HU/20-bp DNA, and free HU with the diffraction peaks at the spacings corresponding to the distances between DNAs as indicated. The red line indicates theoretical SAXS calculated from the two parallel DNAs matching observed SAXS oscillations. SAXS curve of the HU-DNA complex shows sharper peaks due to a more ordered assembly with the peaks matching the reflections (hkl: 001, 200, 110) of the crystal structure related to the highlighted distances. SAXS of free HU matches the theoretical profile (blue) of an oligomeric mixture as indicated. (C) SAXS for 18× HU, 13× HU, 16× HU, and 5× HU with 80-bp DNA shows the diffraction peaks indicating the DNA networks. Note that the HU mutant is missing the first diffractions at d ~ 78 and d ~ 2 × 78 Å, suggesting disruption of the DNA network. SAXS for 10× HU and 18× HU with 80-bp DNA shows the absence of peaks indicating filament-like assemblies. WT, wild type. (D) Electrophoretic mobility shift assay (EMSA) gel for each mutation at homodimer/80-bp DNA ratios of 30:1, 10:1, 5:1, and 2:1 at a DNA concentration of 0.0027 mM.
mutation creates a positive charge that alters the original electrostatic surface acting in $\text{HU}^{\alpha}\text{-HU}^{\alpha}$ coupling (Fig. 4B). New coupling through $Q^5\cdot D^8$ hydrogen bonding is formed (Fig. 4B). Disruption of wild-type coupling allows an arm-to-tail multimerization of $\text{HU}^{\alpha}$ along the DNA (Fig. 4A). Two HU chains in forward and backward directions along the DNA create a grooved U-shaped channel holding DNA (Fig. 4A and movie S4). Eight HU$^{\alpha}$s form one superhelical turn, allowing alignment of 80-bp DNA located at the base of the grooved DNA binding surface (Fig. 4C).
Fig. 4. HUα38β38-DNA interactions and assembly. (A) Two orthogonal views of HUα38β38 assembly along DNA. Alternating HUα38β38s are shown for six asymmetric units of the HUα38β38–19-bp DNA crystal structure. V35 residues and the K38E mutation are highlighted. (B) Arrangements of HUα38β38–HUα38β38 and HUαα-HUαα coupling across DNA are shown as electrostatic surfaces. The right panel shows changes in the electrostatic surface between HUαα and the HU38α38 mutant. Close-up views highlight the network of hydrogen bonds required for coupling of HUα38β38–HUα38β38. Mutations E38K and V42L are highlighted in green. (C) Experimental SAXS curves for 16× HUα38β38/DNA, 8× HU38α38/DNA, and 4× HU38α38/DNA ratios and for free HUα38β38 matching the theoretical profiles of atomistic models shown in the right panel (see also fig. S8). Multiple asymmetric units along 80-bp DNA are displayed as low-resolution molecular surfaces. P(r) functions (inset) were calculated from the corresponding SAXS curves and normalized based on experimentally determined volumes of assemblies (25) indicated in (D). P(r) functions indicate maximal dimensions between 250 and 290 Å and volumes of assemblies, which are consistent with atomistic models. (D) SAXS-determined cross-sectional Rg’s and volumes show the formation of HUαβ/80-bp DNA complexes that are thicker and bulkier than HU38α38/80-bp DNA complexes. These parameters cannot be determined for crystalline HUαα complexes formed at the >3× HUαβ/80-bp DNA ratio.

dimension of 250 Å or with the volume of particles determined by SAXS but also fit the experimental curves well (Fig. 4C and fig. S8). Smaller cross-sectional radii of gyration and volumes derived from SAXS (Fig. 4D) in relation to HUαβ/80-bp DNA suggest that HUα38β38s do not bridge DNAs but rather spread out on independent DNAs. HU38α38-DNA SAXS parameters, together with derived models, correlate with observed nucleoprotein particles shown by atomic force microscopy (AFM) (32), which are in contrast to bridged HUαβ nucleoprotein particles also seen by AFM (28). However, similar to HUαβ, HUα38β38 transforms the HUαα/80-bp DNA network into filaments, as seen by diminishing diffraction peaks after adding HU38α38 (fig. S7B). The inability of the HU38 mutation to network or bridge DNAs links such mutation to increased DNA superhelicity in vivo (32) (fig. S9).

To test the implications of our in vitro HU-DNA complexes for nucleoid structural changes occurring upon HUβ and ectopic HUα38 expression, we imaged E. coli wild-type MG1655 and mutant HU38 (SK3842) strains with SXT. SXT is a high-resolution imaging method that is applied to fully hydrated, unfixed, and unstained cells and that directly measures carbon and nitrogen concentration in biological samples (33). Orthoslices and three-dimensional (3D) reconstructions of SXT imaging of MG1655 in the early growth phase reveal the expected spreading out of dispersed nucleoid with a lobular structure (Fig. 5A). Local nucleoid condensation in wild type was visible during all stages of cell cycle (from a resting cell to a newly divided cell) (Fig. 5A). Changes in nucleoid condensation are linked to HUβ expression, which supports negative supercoiling (20) (fig. S9). Thus, cellular HU-DNA assembly is a dynamic equilibrium between HUαβ and HUαβ, where HUαβ-DNA patches (3, 34) alternate with an HUαα-HUαα network (fig. S9). This alteration of HU multimerization appears suitable for changing accessibility to factors, such as DNA gyrase or topoisomerase I to maintain DNA supercoiling and provide a mechanism for HUαβ to dictate the global transcription profile. In contrast, the nucleoid in the mutant SK3842 strain was organized into a densely condensed unit in all growth phases (Fig. 5A, bottom). As we showed previously, nucleoid condensation observed in the mutant is a direct effect of HUαβ and not mediated through other nucleoid-condensing proteins, such as H-NS and Dps or higher levels of the mutant HU (15). Overall, nucleoid condensation induced by HUβ and HUα38 expression can be quantified using the nucleoid volume and linear absorption coefficient (LAC) (Fig. 5B). Although the HUβ expression in the late growth phase decreases the nucleoid volume from 3.5 to 1.5 μm³, induced condensation by ectopic
HUα38 expression is strikingly more marked with a nucleoid volume of <1 μm³ in all growth phases (Fig. 5B).

For HU and nucleoid architecture, the molecular functionality key to macroscopic behavior begins to manifest itself at the nanoscale and extends into the mesoscale (or middle area) that uncovers the emergence of collective behavior. Rather than site-specific DNA bending, HUβ expression condenses the chromosome (Fig. 5) and supports negative supercoiling. We find that this occurs via disruption of DNA networks by inducing DNA patches that transform periodic DNA condensation into more superhelical structures. Further, altering HU surface charges shows how HU can play regulatory roles in the acidic stress response in *Helicobacter pylori* and uncovers a mechanism of gene regulation by altering HU. The *E. coli* HUα38 mutant shows a markedly condensed chromosome that is directly linked to positive supercoiling. The structural characterization of HUα38-DNA presented here thus links HU charges to cooperative multimerizations that in turn help control DNA supercoiling.

Using these combined multiscale structures, we define a mechanism whereby the level and type of superhelicity are automatically generated through an altered mode of supercoil restraints rather than requiring changes in cellular topoisomerase activities. These data therefore support a unified, testable model for the relationships of HU structural assemblies and networks with compaction and transcription that explains and extends multiple observations. The shift in HU’s multimerization provides an efficient general mechanism to synchronize the genetic response to external conditions. Furthermore, effector molecules present in pathogenicity-inducing environments may elicit changes in HU-mediated nucleoid condensation, leading to swift and concerted changes in the basal transcription program. These observations make HU interactions an attractive target for controlling not only pathogenesis but also microbial systems in general.

**MATERIALS AND METHODS**

**Protein purification**

HUβ was purified from an expression plasmid (pRLM118) (36). HU-hyperexpressing cells were grown at 30°C in 200 ml of LB medium...
containing ampicillin (100 μg/ml) to an A600 (absorbance at 600 nm) of 1.5. The culture was quickly placed in a water bath at 42°C and was shaken for 30 min. The cells were washed once and suspended in 50 ml of buffer [20 mM Hepes-NaOH (pH 7.5), 60 mM KCl, and 1 mM dithiothreitol (DTT)] and disrupted by passing through a French press three times. After removing the cell debris by centrifugation at 7000g for 10 min, the supernatant was fractionated by ammonium sulfate precipitation at 70 to 90% saturation. The ammonium sulfate precipitation was repeated twice. The fraction rich in Huαβ was loaded on a 10-ml Q-Sepharose column (Pharmacia Biotech) pre-equilibrated with buffer. A flow-through fraction was taken and directly applied on a 5-ml heparin-Sepharose column (Pharmacia Biotech) pre-equilibrated with buffer. After thoroughly washing the column with buffer containing 400 mM KCl, Hu was eluted by a linear gradient with buffer containing buffer. After thoroughly washing the column with buffer containing 0.6 to 0.8 M KCl was taken and stored at 4°C. Eluate at 0.6 to 0.8 M KCl was taken and stored at 4°C. Protein concentration was measured by using the BCA Protein Assay Kit (Pierce) with bovine serum albumin (BSA) as standard.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.

Hammel et al.
SAXS diffraction peaks with our HUα-DNA structure (Fig. 2B), we calculated structure factors from the crystal structure with PHENIX.MODEL (45) and transferred them into the reflection data file with MTZDUMP (46). Atomic coordinates and structure factors for the crystal structures were deposited in the PDB under accession codes 4YEX, 4YEY, 4YFH, 4YF0, 4YFT, and 4YEW.

**SAXS data collection and evaluation**

SAXS data were collected at the ALS beamline 12.3.1 (SIBYLS), Lawrence Berkeley National Laboratory (Berkeley, CA) (47) for HU concentrations of 17 to 200 μM spanning the measured intracellular HU concentrations of 25 to 100 μM (48). The wavelength was set to λ = 1.0 Å and the sample-to-detector distance was set to 1.5 m, resulting in scattering vectors, with q ranging from 0.01 to 0.32 Å⁻¹. The scattering vector is defined as q = 4π sinθ/λ, where 2θ is the scattering angle. All experiments were performed at 20°C, and data were processed as previously described (47). The buffer for SAXS experiments was 50 mM tris-HCl (pH 7.5), 150 mM NaCl, 1 mM DTT, and 1 mM PMSF. To collect SAXS for protein-DNA assemblies, proteins HUα, HUβ, and HUαβ38, respectively, were mixed with DNA directly before the data collection (~5 min) at the protein/DNA ratios, as indicated in Figs. S5, S6, and S8. The SAXS curve of HUα-DNA showed diffraction peaks, indicating the formation of a crystalline phase in solution. These SAXS experiments revealed diffraction peaks defining the d spacing between parallel DNAs. This type of SAXS curve cannot be used to obtain the pair distribution function [P(r)] or radius of gyration (Rg). Observed diffraction peaks in HUα–20-bp DNA samples were identical with the lattice planes calculated for the refined HUα-DNA crystal structure. The theoretical scattering profiles for two parallel DNAs, as indicated in Fig. 2B, were calculated with FoXS (49). To test disruption of the HUα–80-bp DNA network, we titrated HUβ (fig. S7A) or HUαβ38 (fig. S7B) into the preformed HUα–80-bp DNA assembly (HUα/DNA ratio, 17:1; DNA concentration, 0.02 mM). The relative level of networking was quantified by calculating the area under the diffraction peak for the initial HUα–80-bp DNA, HUαβ38–80-bp DNA, HUαβ38–20-bp DNA, and HUαβ38–80-bp DNA data were matched with an ensemble of two different complexes. The match of theoretical profiles to the experimental curve could be improved with larger ensemble sizes; however, an ensemble size of two for HUαβ38–80-bp DNA (Fig. S3C and fig. S6) and one for HUαβ38–80-bp DNA (Fig. 4C and fig. S8) was sufficient to fit the data. Ensemble modeling was also applied to determine the solution state of 1× HUαβ20-bp DNA (Fig. 3C and fig. S6). The various atomistic models of HUαβ20-bp DNA were built by combining multiple positions of one or two 20-bp DNA along with one HUβ as seen in their crystal structure. An alternative model with the 20-bp DNA located between HUαβ extended arms was built by positioning DNA according to previously reported HU crystal structures with specific DNA (21). A FoXS–MES approach was applied to define a minimal ensemble of two, as shown in Fig. 3C and fig. S6D. The oligomeric mixture of free HUα and free HUαβ38 was determined by a FoXS–MES approach. Higher oligomeric states of HUα were built based on the crystal packing reported for the *Bacillus anthracis* HU structure (PDB ID: 3RHI). A BILBOMD approach (56) was used to explore the flexibility of HUαβ extended arms in free state (Fig. 3C). BILBOMD uses molecular dynamics (MD) simulations to explore the conformational space of the extended arms. The MD simulations provide an ensemble of molecular models from which a SAXS curve is calculated and compared to the experimental curve. A genetic algorithm (MES) (56) was used to select and weight two conformers, which best fit the SAXS curve of HUαβ. SAXS data and SAXS-derived models were deposited with the BIOSIS database, and experimental SAXS parameters are reported in table S2.

**Soft x-ray microscopy**

*E. coli* strain MG1655 and HUα38 mutant strain SK3842 were used for SXT. Mutagenesis of HUα38 and integration of the mutant *hupA* gene into the chromosome have been previously described by Kar and Adhya (57). Cells were grown in 5 ml of LB medium at 37°C. Cells were put on ice and promptly cryoimmobilized after reaching an OD₆₀₀ (optical density at 600 nm) of 0.2 for the lag phase, 0.5 for the exponential phase, and >1.0 for the stationary phase. Cells were mounted in thin-walled glass capillary tubes and rapidly cryoimmobilized before being mounted in the cryogenic specimen rotation stage of the XM-2 soft x-ray microscope at the National Center for X-ray Tomography (ALS). Each data set (that is, 90 projection images spanning a range of 180°) was collected using a microscope equipped with a Fresnel zone plate–based objective lens with a resolution of 50 nm (58). The projections for every tilt were recorded using a Peltier-cooled, back-thinned, and direct-illuminated 2048 × 2048 pixel soft x-ray charge-coupled device camera (Roper Scientific iKon-L). Projection images were manually aligned using IMOD software by tracking fiducial markers on adjacent images. The 3D x-ray tomograms were segmented using Chimera (43) and were used to reconstruct volumes, measure voxel values (that is, absorption values in the volume element of the reconstructed data), and calculate LACs. To quantitatively compare the volume and length of a nucleoid for various cells, x-ray tomograms were filtered with a Gaussian filter and segmented at LAC = 0.25 μm⁻¹. Ten cells per growth phase were used to calculate the volume and length of a nucleoid structures. A sub-ensemble of complexes coexisting in solution was selected by a genetic algorithm guided by the fit to the experimental SAXS data using FoXS–MES (Minimal Ensemble Search) (49, 56). The selected complexes were weighted, allowing the selection of the minimal ensemble to avoid overfitting with the larger ensemble. The size of the minimal ensemble was selected based on the level of improvement in the SAXS fit between different ensemble sizes. HUβ/80-bp DNA data were matched with an ensemble of two different complexes. The match of theoretical profiles to the experimental curve could be improved with larger ensemble sizes; however, an ensemble size of two for HUαβ38–80-bp DNA (Fig. 3C and fig. S6) and one for HUαβ38–80-bp DNA (Fig. 4C and fig. S8) was sufficient to fit the data. Ensemble modeling was also applied to determine the solution state of 1× HUαβ/20-bp DNA (Fig. 3C and fig. S6). The various atomistic models of HUαβ/20-bp DNA were built by combining multiple positions of one or two 20-bp DNA along with one HUβ as seen in their crystal structure. An alternative model with the 20-bp DNA located between HUαβ extended arms was built by positioning DNA according to previously reported HU crystal structures with specific DNA (21). A FoXS–MES approach was applied to define a minimal ensemble of two, as shown in Fig. 3C and fig. S6D. The oligomeric mixture of free HUα and free HUαβ38 was determined by a FoXS–MES approach. Higher oligomeric states of HUα were built based on the crystal packing reported for the *Bacillus anthracis* HU structure (PDB ID: 3RHI). A BILBOMD approach (56) was used to explore the flexibility of HUαβ extended arms in free state (Fig. 3C). BILBOMD uses molecular dynamics (MD) simulations to explore the conformational space of the extended arms. The MD simulations provide an ensemble of molecular models from which a SAXS curve is calculated and compared to the experimental curve. A genetic algorithm (MES) (56) was used to select and weight two conformers, which best fit the SAXS curve of HUαβ. SAXS data and SAXS-derived models were deposited with the BIOSIS database, and experimental SAXS parameters are reported in table S2.

**SAXS modeling**

To match SAXS curves of different HUαβ/80-bp DNA and HUαβ38/ 80-bp DNA ratios to various HU-DNA binding modes, we applied ensemble analysis (54, 55). This approach introduces the concept of ensemble fitting of the SAXS data from multicomponent systems. A large pool (more than 20) of various HU/DNA complexes (covering one, two, or three neighboring 80-bp DNAs) was generated based on the crystal
Electrophoretic mobility shift assay

EMSA were performed for HUa and HU mutants (HUa E38K,V42L, HUa E34K, HUa E34K, HUa G32E, and HUa G32E,K35I). EMSAs were run on 11% agarose gels in 1× tris-acetate-EDTA buffer and visualized with ethidium bromide. Eighty–base pair DNA (1.5 μg) (1.5 μl at 1 mg/ml) was combined with HU to yield protein/DNA ratios of 30:1, 10:1, 5:1, and 2:1; diluted with buffer [50 mM tris (pH 7.5) and 150 mM NaCl] to 10 μl; combined with loading dye; and run on a gel (at 200 V for 20 min).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/2/7/e1600650/DC1

REFERENCES AND NOTES


Acknowledgments: We thank the SIBYLS beamline (BL 12.3.1) staff, G. Williams for imaging, J. Holton and M. Kuzdovich for aiding data collection, F. Guo for early-stage HU purification, and S. Kar for providing E. coli strain SK3842. Funding: This work was supported by the NIH Structural Cell Biology of DNA Repair Machines (grant P01 CA02594) and NIH grant MINOS (Macromolecular Insights on Nucleic Acids Optimized by Scattering) GM105404 (to M.H. and J.A.T.). SIBYLS beamline efforts to combine SAXS and crystallography at the ALS were supported in part by the U.S. Department of Energy (DOE) program Integrated Diffraction Analysis Technologies. The National Center for X-ray Tomography was supported by the National Institute of General Medical Sciences of the NIH (grant P41GM103445) and the DOE’s Office of Biological and Environmental Research (grant DE-AC02-05CH11231). This work was also supported in part by the NIH Intramural Research Program and the Center for Cancer Research of the National Cancer Institute. Author contributions: M.H. developed the project with S.A. and performed the structure data collection and analyses with input from J.A.T., D.A., J.-H.C., F.E.R., R.P., and H.Y.H.T. performed some of the experiments. M.H., S.A., C.A.L., and J.A.T. wrote the article with input from all authors. Competing interests: The authors declare that they have no competing interests. Data and materials availability: Crystal structures were deposited in the PDB under accession codes 4YEX, 4YEY, 4YFH, 4YF0, and 4YEW. SAXS data and solution models were deposited in BIOSIS with the BIOSIS IDs HUaa1P, HUab1P, HUab2Y, HUab3Y, HUa38P, and HUa38Y. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.