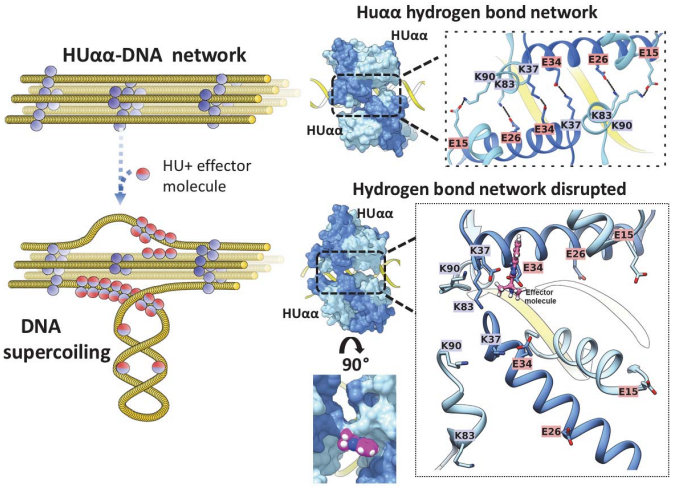
Regulating bacterial gene expression with small molecules by altering DNA supercoiling

Soumya G. Remesh[1], Subhash Verma[2], Zhong Qian[2], Sankar Adhya[2], Michal Hammel[1]
[1]Molecular Biophysics and Integrated Biology, Lawrence Berkeley National Laboratory, Berkeley, CA - 94720
[2] National Cancer Institute, National Institutes of Health, 37 Convent Drive, BLDG 37 RM 5138
Bethesda MD USA
sgremesh@lbl.gov.

Bacterial chromosome (nucleoid) organization is dependent on conserved nucleoid associated proteins (NAPs), mainly the histone-like protein HU involving interactions spanning the nanoscale and mesoscale to direct DNA supercoiling. By means of macromolecular crystallography, small angle X-ray scattering (SAXS) and soft X-ray tomography (SXT) we showed that DNA dependent HU networking controls DNA supercoiling to maintain the nucleoid structure. Moreover, we characterized the molecular switch of gain-of-function HU mutations that altered DNA supercoiling and reprograms *Escherichia coli* to an invasive form. The fact that DNA supercoiling also controls expression of virulence factors in many pathogens makes HU an attractive target for novel antibacterial agents. We showed that the HU networking can be disrupted by small molecules and revealed new molecular mechanism to regulate gene expression. Using a virtual screening of a small molecule fragment library, we identified a bioactive molecule that targets the HU-HU networking interface. Our structural characterization of the molecular mechanism of this prototypic small molecule has validated the HU networking as a bona-fide target for regulating bacterial gene expression. Using our integrated structural biology approach the mechanistic insights we provide underpins a rational strategy for the discovery of new antimicrobial agents that re-program gene expression in the pathogenic state.



HU multimerization and HU-DNA network directs bacterial DNA supercoiling. We show that addition of a prototypic effector molecule disrupts critical hydrogen bond network altering DNA supercoiling.

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5. Characterization of porin and ompR mutants of a virulent strain of *Salmonella typhimurium*: ompR mutants are attenuated in vivo. Infect Immun. 1989;57(7):2136-40