Atomistic simulations unveil the influence of DNA topology on IHF–DNA interaction

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Introduction

IHF (fig. 1) is a histone-like nucleoid-associated DNA-bending protein present in all known prokaryotes.

IHF is known to be vital to the stability of biofilms and aggregates at the crossing points of the extracellular DNA lattice [1].

It also mediates DNA topology and supercoiling, which plays an important role in gene regulation.

DNA minicircles (hundreds to thousands of bp) are of special interest: prokaryotic genomes & artificial vectors are circular, and fixed ends are useful to study topology.

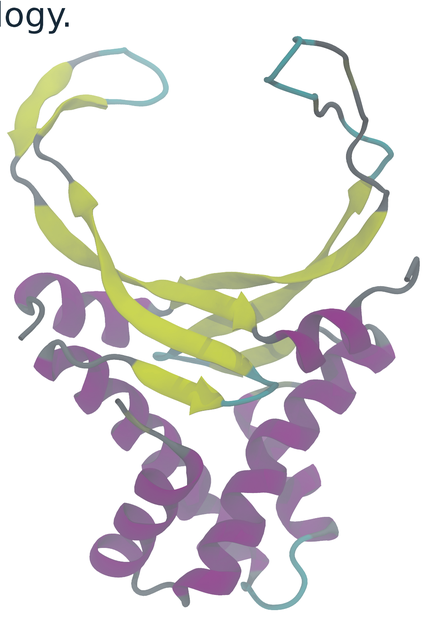

Figure 1.
IHF (*pictured*) is a homodimer with an alpha-helix "body" and two "arms" that bind to DNA.

The end of each arm features a proline that intercalates between base pairs of bound DNA.

DNA topology

Two DNA strands coil around one another to form the double helix; the number of coils is the linking number, Lk.

The twist, Tw, is the number of turns the strands make around the helix axis. This cannot deviate too far from its relaxed value.

So too much ΔLk causes the helix axis to coil around itself; the number of coils is the writhe, Wr, of the system. (figs. 2 & 3)

Topological constraints mean $Lk = Tw + Wr$ at all times.

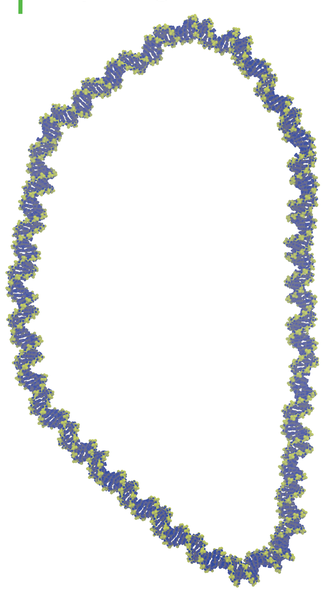

Figure 2.
A 336 bp DNA minicircle with $\Delta Lk = 0$ is relaxed and roughly circular...

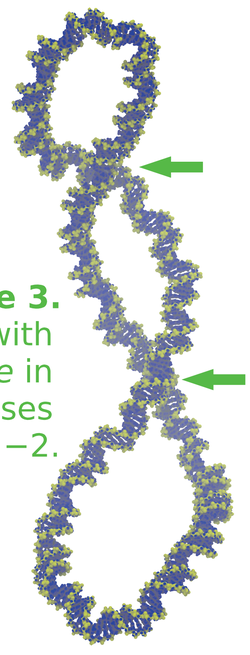

Figure 3.
... while a similar minicircle with $\Delta Lk = -3$ forms a *plectoneme* in which the helix axis crosses itself twice, so $|Wr| = -2$.

Molecular dynamics

Molecular dynamics simulation gives atomistic insight into dynamic behaviour.

Atoms & their positions are defined, then a potential is evaluated at every step to integrate the system in time.

This work used AMBER with the ff14SB + parmbsc1 potentials.

Implicit solvent (Generalised Born) speeds up simulations by removing solvent viscosity.

[1] Gustave J E *et al.* 2013 *J. Cyst. Fibros.* **12** 384–9

IHF bridges DNA minicircles

IHF is always positioned at the apex of a plectoneme (fig. 4).

IHF can bridge supercoiled DNA by forming stable additional contacts, significantly compacting the minicircle (fig. 5).

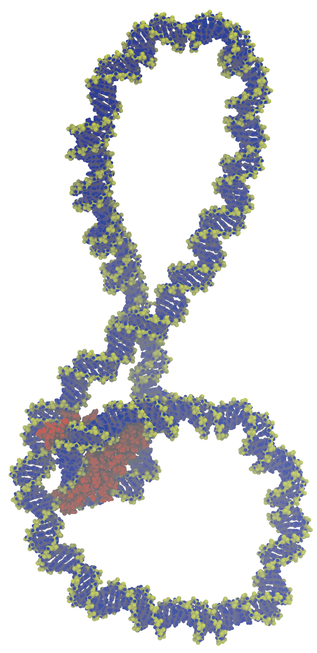

Figure 5.
IHF (red) can form a bridge between distal sites of a writhed minicircle (here, $\Delta Lk = -3$).

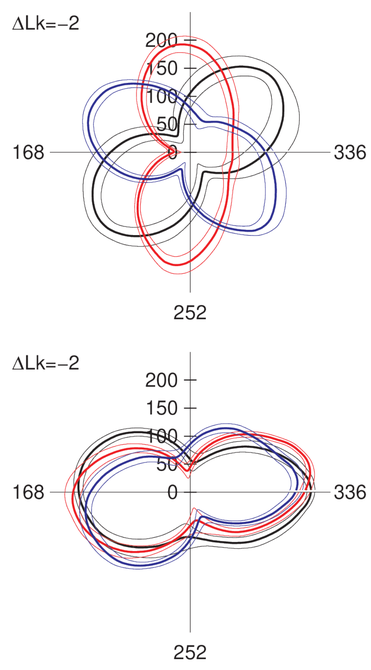

Figure 4.
In the absence of IHF (top), the plectoneme is randomly positioned.

In the presence of IHF (bottom), the plectoneme always has IHF at its apex.

The additional contact appears to be nonspecific, occurring at various points on both the protein and the DNA.

Bridges are remarkably stable and dominated by hydrogen bonding with the DNA backbone.

IHF exhibits two binding modes

IHF is observed to exhbit two binding modes (fig. 6), which depend on the topology of the bound DNA.

IHF binds AT-rich sequences strongly, but binding to non-AT-rich sequences is variable.

Highly supercoiled DNA exhibits both longer and shorter binding sites than torsionally relaxed DNA.

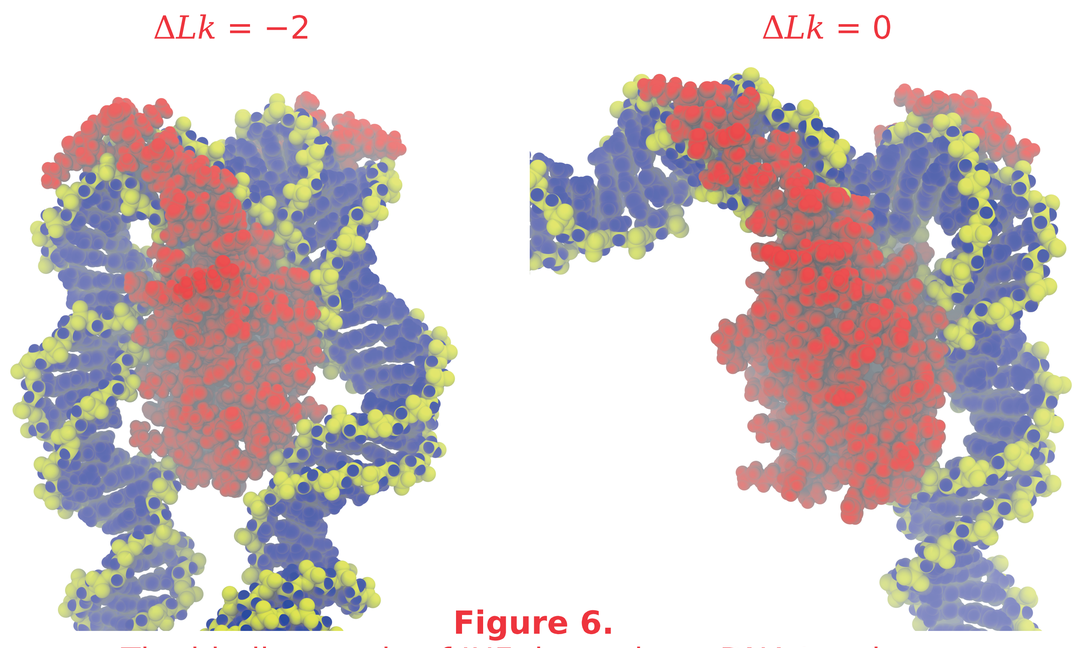

Figure 6.
The binding mode of IHF depends on DNA topology. DNA may wrap around the protein symmetrically (left) or bind only on the AT-rich side (right)

Discussion

Additional protein bridges formed by IHF may explain its importance to the stability of biofilms by stabilising crossing points in extracellular DNA.

IHF bridges also divide DNA into topological domains, and could regulate gene expression.

The dependence of IHF binding on DNA topology adds to this complex regulatory network.

Further work will involve studying interactions between multiple proteins bound to distal sites, and complementary single-molecule experiments using AFM & magneto-optical tweezers.

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