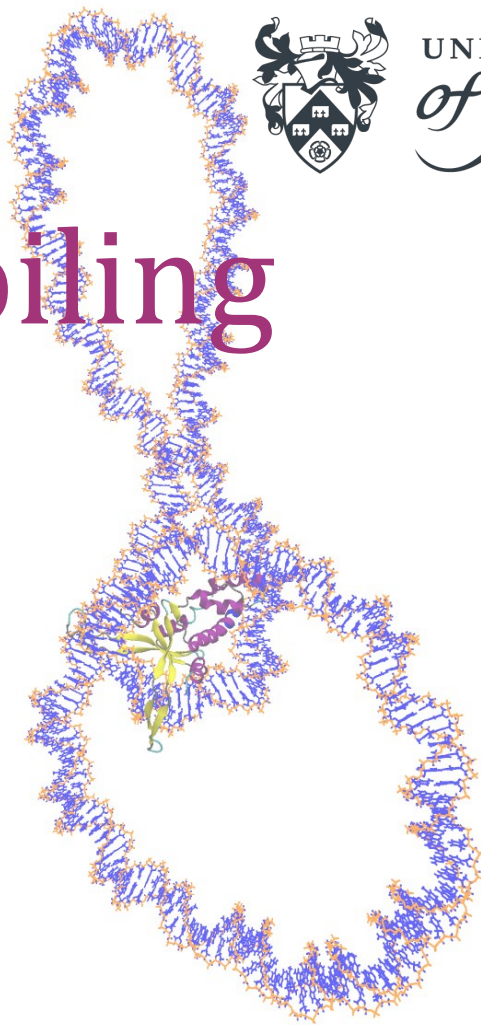




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Simulating supercoiling in DNA minicircles

George Watson





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Introduction

Supercoiling

- Supercoiling is the addition of extra twists to a length of DNA
- Relaxed B-DNA has ~ 10.5 bp/turn

Adding or removing twists induces strain, which causes the DNA to contort into different shapes

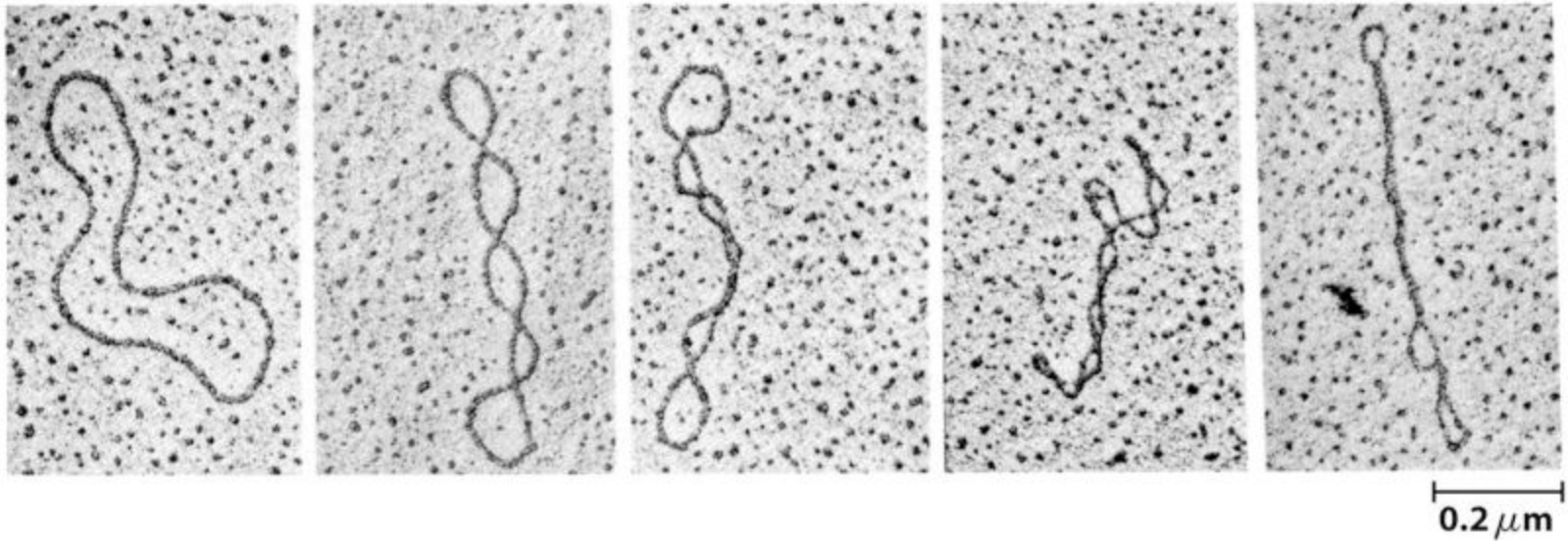
- Supercoiling has many biological functions, including gene regulation, and prokaryotic & eukaryotic genomes are persistently supercoiled



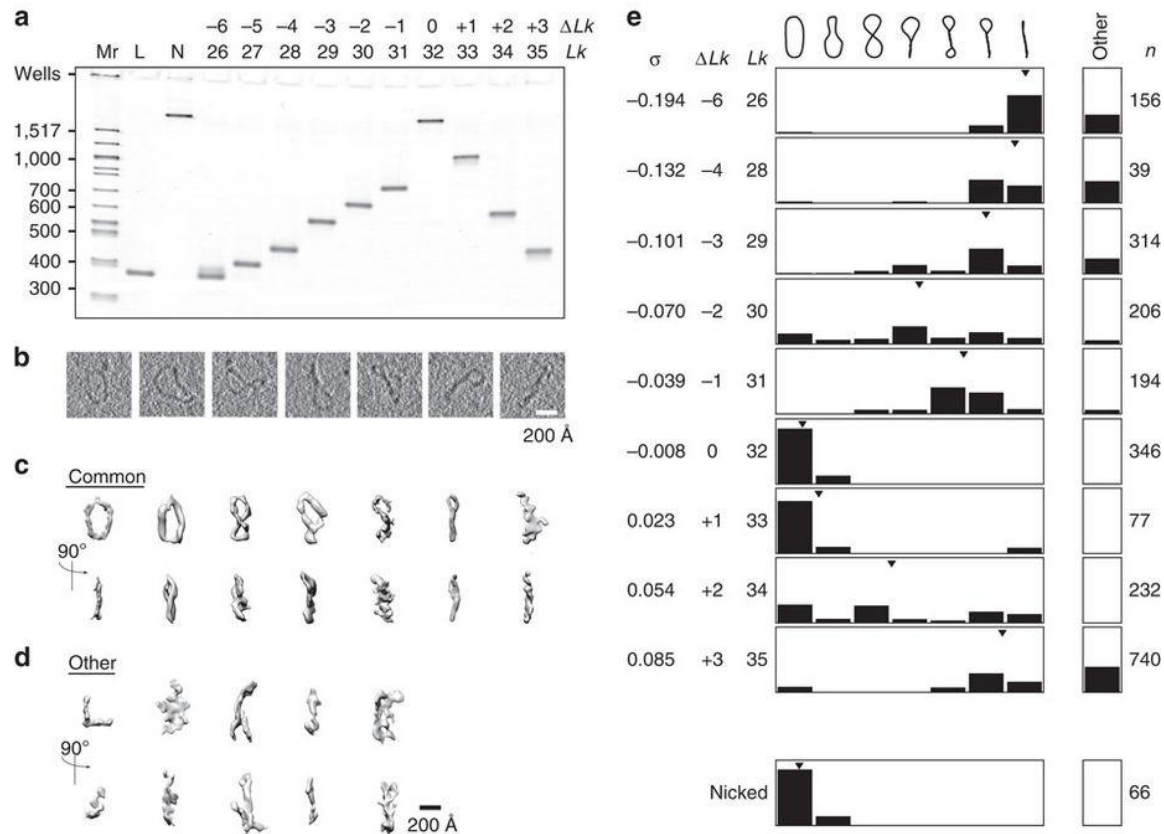
Supercoiling II: Twist & Writhe

- Supercoiling is characterised by
 - **Twist**, Tw , the number of times the two strands coil around one another (typically $N / 10.5$); often given in degrees per base pair (relaxed = $34.3^\circ / \text{bp}$)
 - **Writhe**, Wr , the number of times the helix axis coils around itself
- The **linking number**, $Lk = Tw + Wr$, is usually constant, but twist and writhe can vary smoothly
- The linking number of relaxed B-DNA is denoted Lk_0

$$\Delta Lk = Lk - Lk_0$$



Kornberg 1980 *DNA replication* (San Francisco: W. H. Freeman) 29



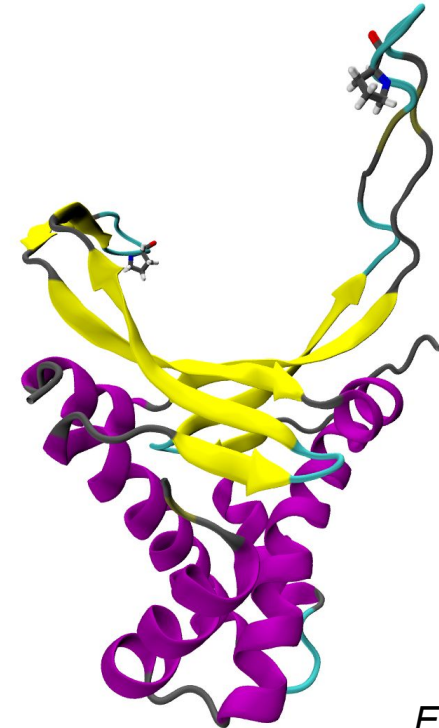
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Irobalieva *et al.* 2015 *Nat. Commun.* **6** 8440 [CC-BY]



DNA-bending proteins: IHF & HU

- Histone-like nucleoid-associated proteins present in virtually all known prokaryotes
- Bend DNA (up to 160°) by binding; intercalating prolines disrupt stacking, causing kinks
- IHF binds specifically to a known sequence; HU binds nonspecifically but prefers damaged DNA





Method



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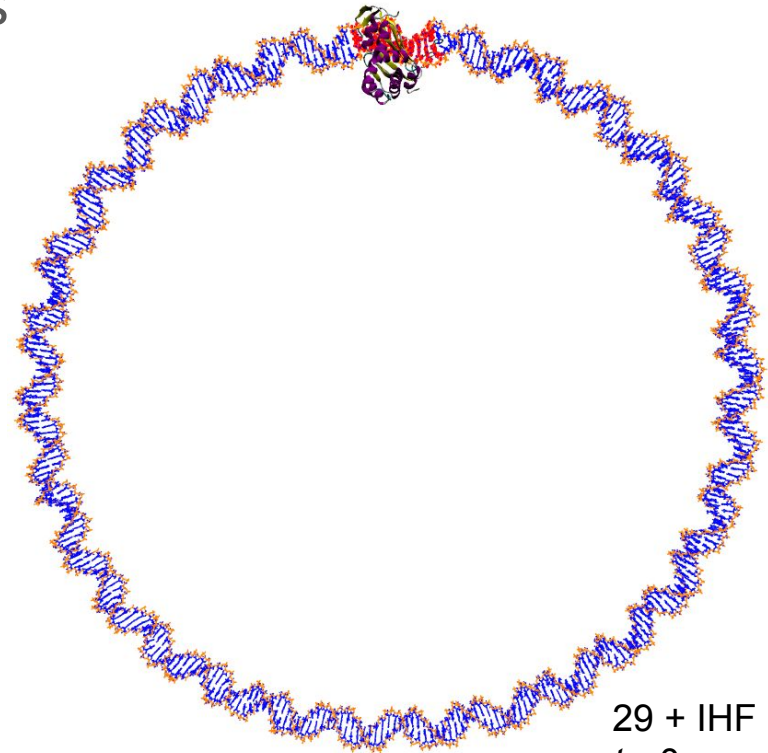
Molecular Dynamics

- Simulation technique that can provide **atomistic insight**
- Construct a **potential** based on the properties of each atom;
integrate to find the force on each atom at every time step
- Powerful, but very **computationally expensive!**
- Simulating water atomistically would be prohibitively slow, so
treat solvent implicitly using **generalised Born** model
- Many potentials and packages (**AMBER**, GROMACS, CHARMM...)



Method

- 336 bp minicircles with 29–34 turns
($Lk_0 = 32 \Rightarrow -3 \leq \Delta Lk \leq +2$)
- Insert IHF binding site (11 bp) & minimise with/without protein (*E. coli*)
- Simulate in implicit solvent for 20 ns using AMBER (pmemd.CUDA)
 - Temperature: 300 K
 - Salt concentration: 0.8 mol/l
 - Potential: ff14SB + parmbsc1



29 + IHF
 $t = 0$



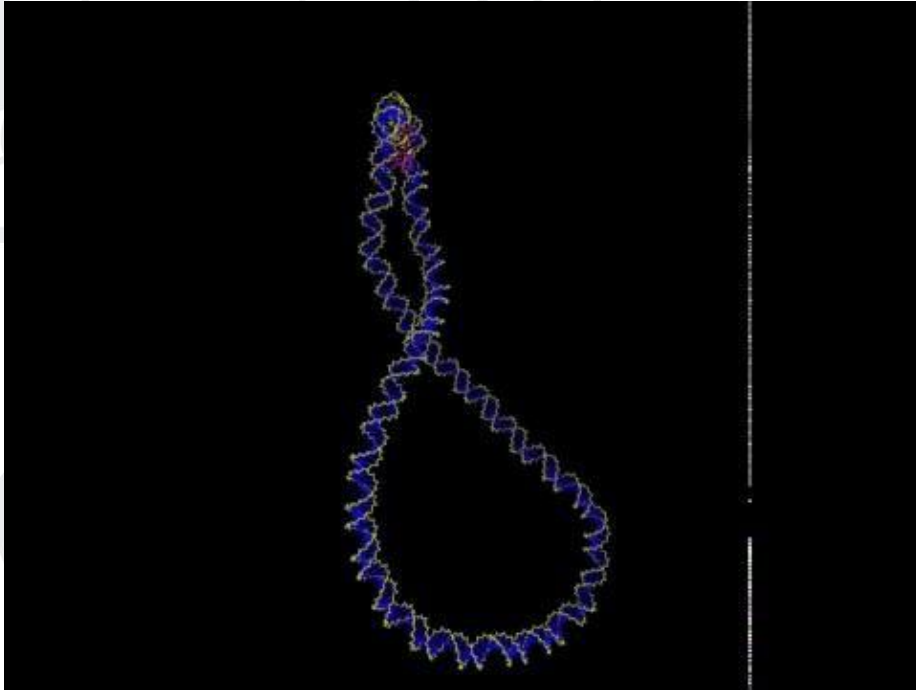
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Results

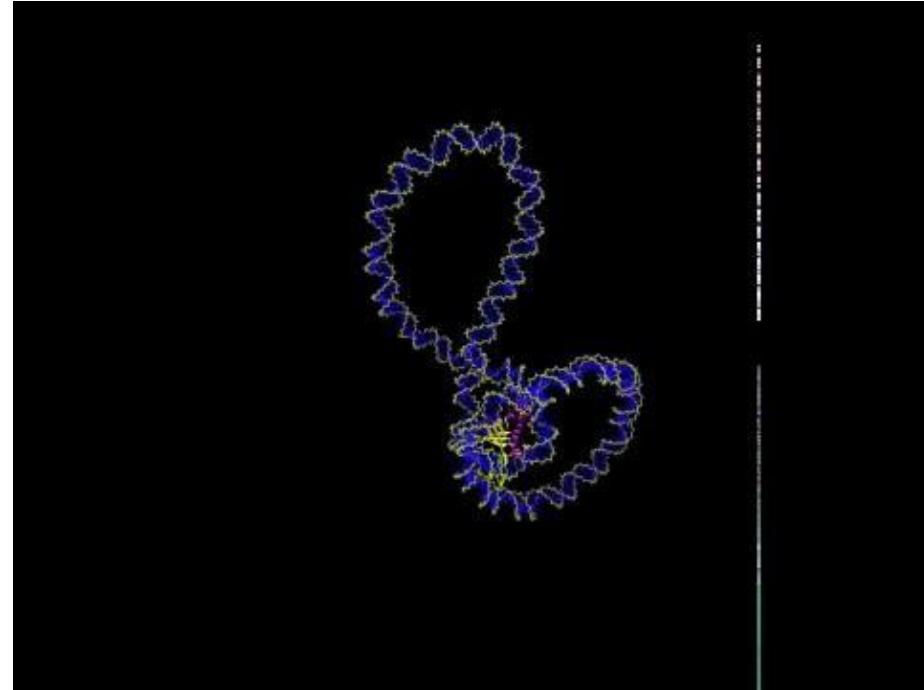
336bp minicircles + IHF



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$$\Delta Lk = -2$$



$$\Delta Lk = -3$$

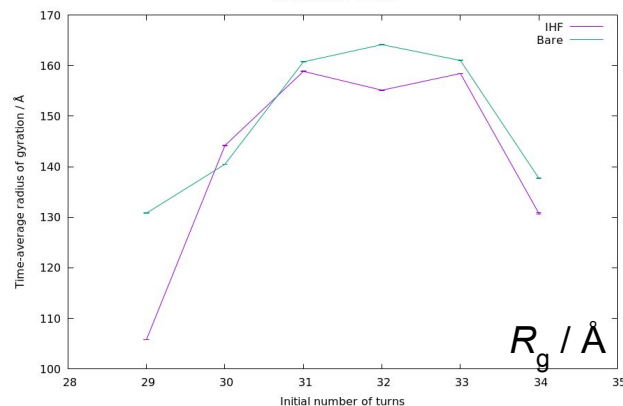
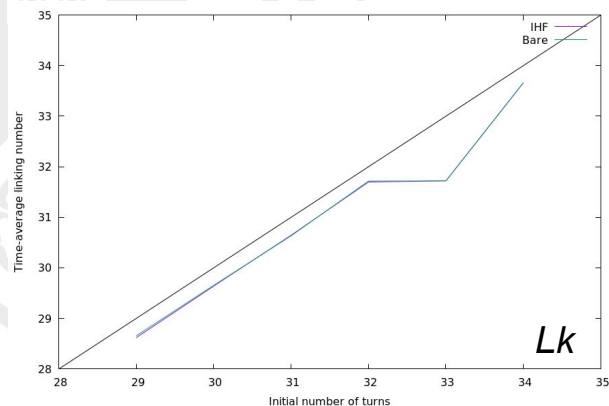
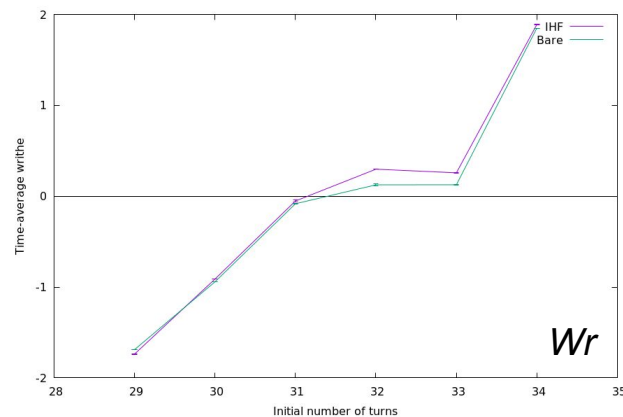
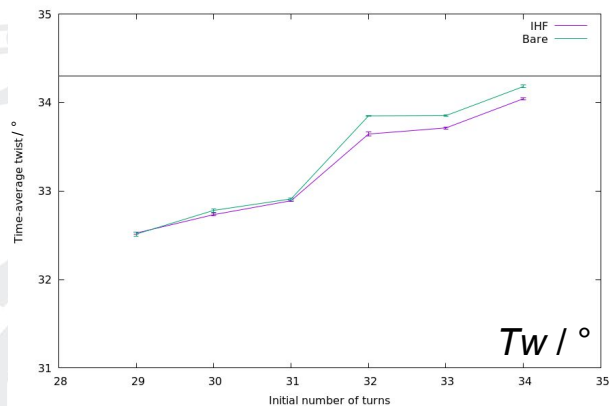
Results

- Supercoiling can **condense DNA & bring distal sites closer together**; this effect appears to be **enhanced by bound IHF**, especially in highly supercoiled systems
- IHF is always positioned at the **apex of the plectoneme**
- IHF can **bridge distal sites** in supercoiled DNA, forming **closed loops** — linked to stability of biofilms?
- The amino acids that form these bridges are **not present in HU-** — are they conserved in other (non-*E. coli*) IHFs?

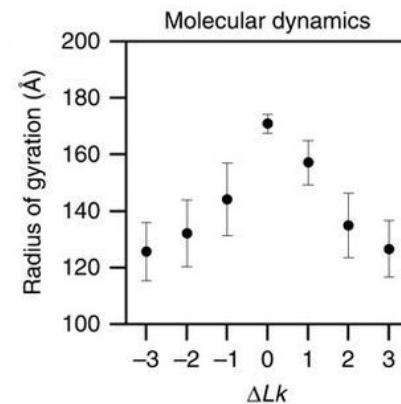
Twist, Writhe, Radius of gyration



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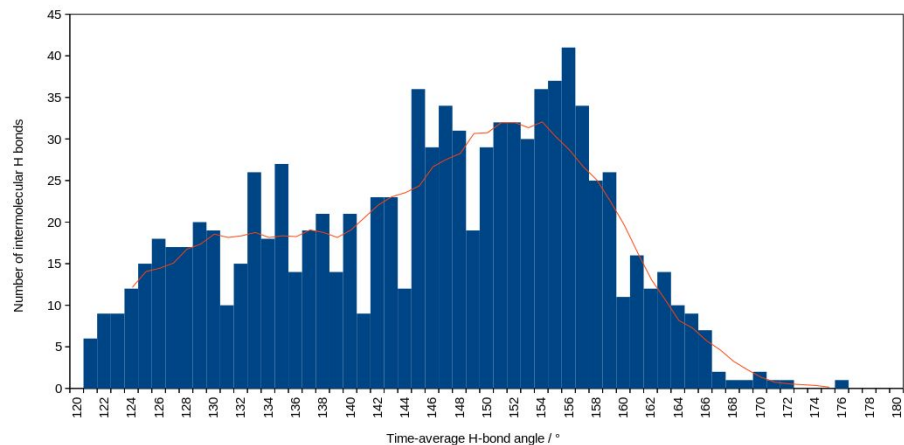
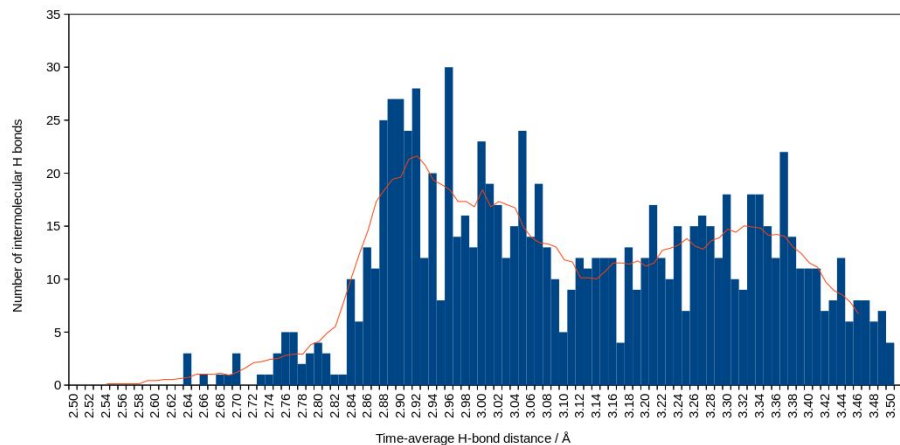
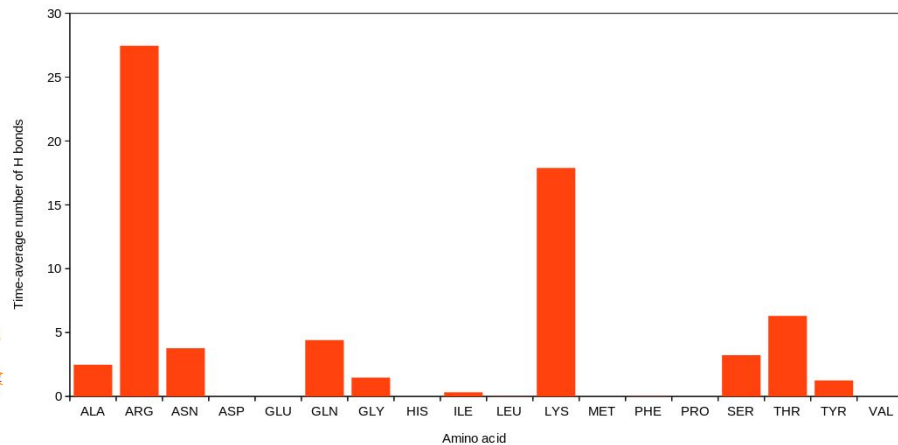
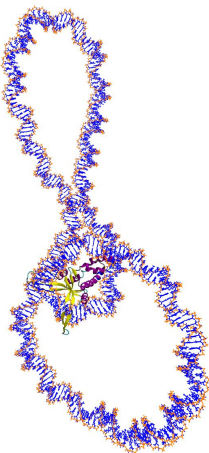


Irobalieva *et al.* 2015 *Nat. Commun.* **6** 8440 [CC-BY]



Hydrogen bonds

Data for 29 + IHF



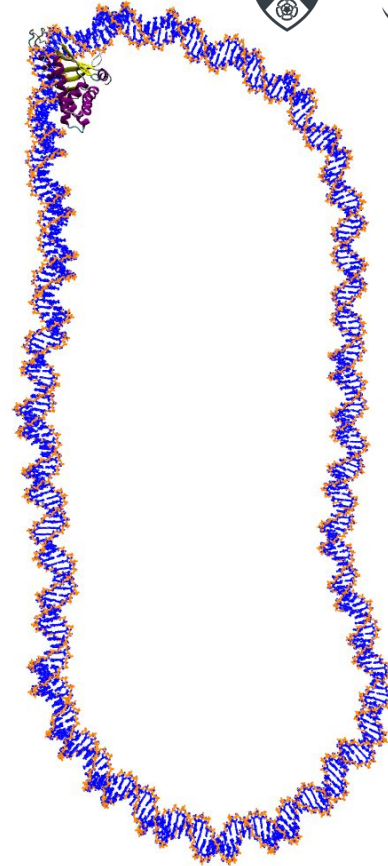


The DNA–IHF interaction is
asymmetric

One side of the protein always
binds first & more strongly

Is this due to the protein structure
or the sequence of this DNA?

IHF is a heterodimer, so
asymmetry is possible

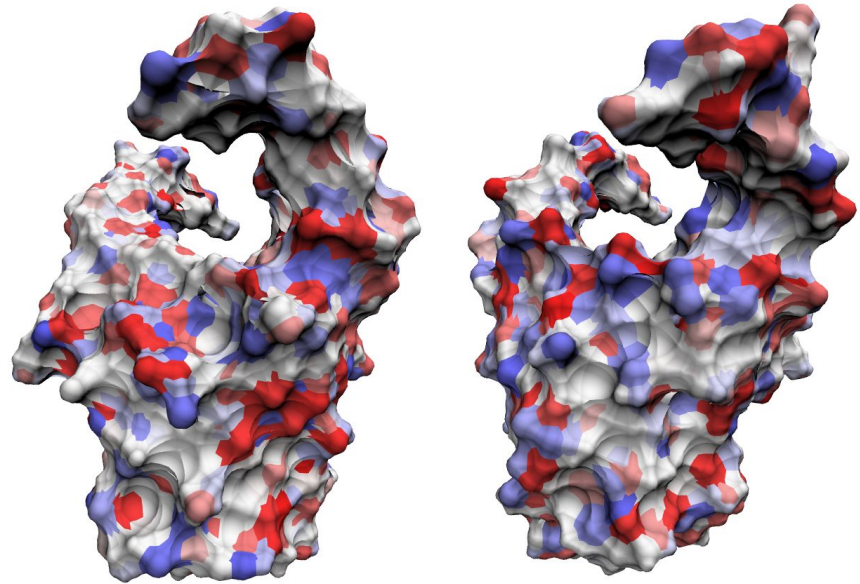
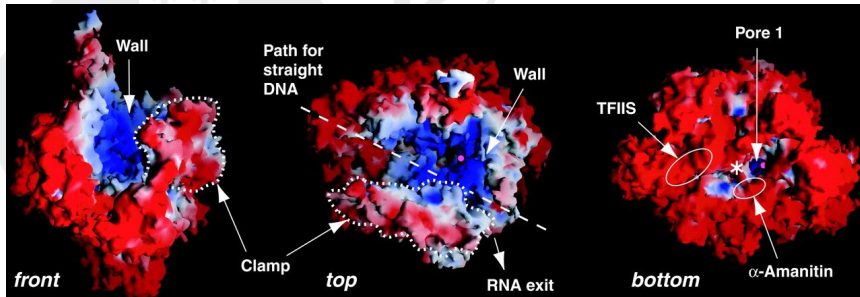


33 + IHF
 $t = 20$ ns



IHF surface charge

- Surface charge distribution is patchy
- Contrast with, e.g., RNA polymerase



Cramer, Bushnell, Kornberg 2001 *Science* **292** 1863–76
(Used under licence)



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Discussion

The Future

- Replicas (*running...*)
- HU (requires crystal structure)
- H bonds; effect of supercoiling on interaction
- Formation of protein bridges between distal sites; dependence on supercoiling?
- Link to experimental work (Seb) — go bigger?

Acknowledgements

- *Supervision* — Agnes Noy, Mark Leake
- *Complementary experiments (soon)* — Sébastien Guilbaud



Further Reading

- **Supercoiling:**
 - Irobalieva *et al.* 2015 *Nat. Commun.* **6** 8440
 - Noy, Sutthibutpong, Harris 2016 *Biophys. Rev.* **8** 145–55
 - Noy, Maxwell, Harris 2017 *Biophys. J.* **112** 523–31
- **DNABII proteins (IHF, HU):**
 - Swinger, Rice 2004 *Curr. Opin. Struct. Biol.* **14** 28–35
- **Software:**
 - **AMBER/AmberTools**: <http://ambermd.org/>
 - **Curves+/Canal**: Lavery *et al.* 2009 *Nucleic Acids Res.* **37** 5917–29
 - **WrLINE**: Sutthibutpong, Harris, Noy 2015 *J. Chem. Theory Comput.* **11** 2768–75