DNA Looping in Topologically Constrained Domains

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SUMMARY

- **New techniques are needed in order to study the effects of DNA supercoiling on loop-mediated regulation**
- **The thermodynamics of DNA looping depend periodically on the helical phasing between recognition sites**
- **A long-term goal is to provide enabling technologies for ensemble and single-molecule FRET studies of DNA looping mediated by *lac* repressor *in vitro* and *in vivo***
- **Other applications of the methodology include engineering of DNA substrates for studies of site-specific recombination and other enzyme systems**

INTRODUCTION

Protein-mediated DNA-loop formation is a fundamental mechanistic aspect of many important biological processes, including gene regulation, recombination, and replication.

Single-molecule tethered-particle experiments conducted by Normanno *et al.1* demonstrated a dependence of LacI-mediated looping on supercoiling. Repeated spontaneous transitions between two distinct loop structures were observed by Wang *et al.2*

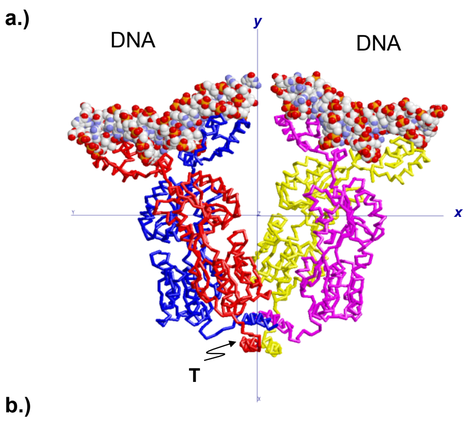

Figure 1. (a.) Crystallographic "v- shaped" LacI tetramer in complex with operator DNA3 **(b.)** "Extended" LacI tetramer visualized by EM 4.

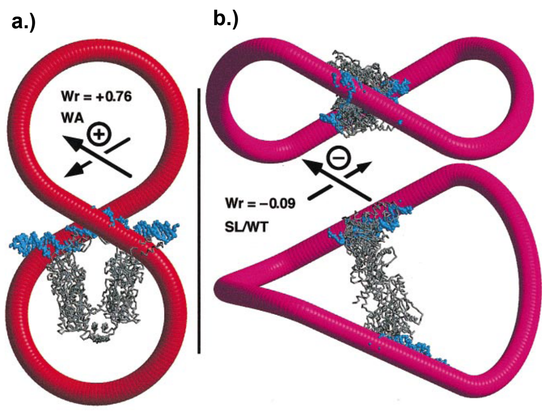

Figure 2.(a.) Schematic model of a DNA circle with a positive writhe crossover enforced by LacI **(b.)** Two views of a more open DNA model bridged by an extended LacI conformation5.

RESULTS

A hallmark of protein-mediated DNA looping is the periodic dependence of loop formation on binding-site separation. This periodicity arises because of DNA's limited torsional flexibility and the requirement for correct torsional alignment of the two protein binding sites (**Figure 2**).

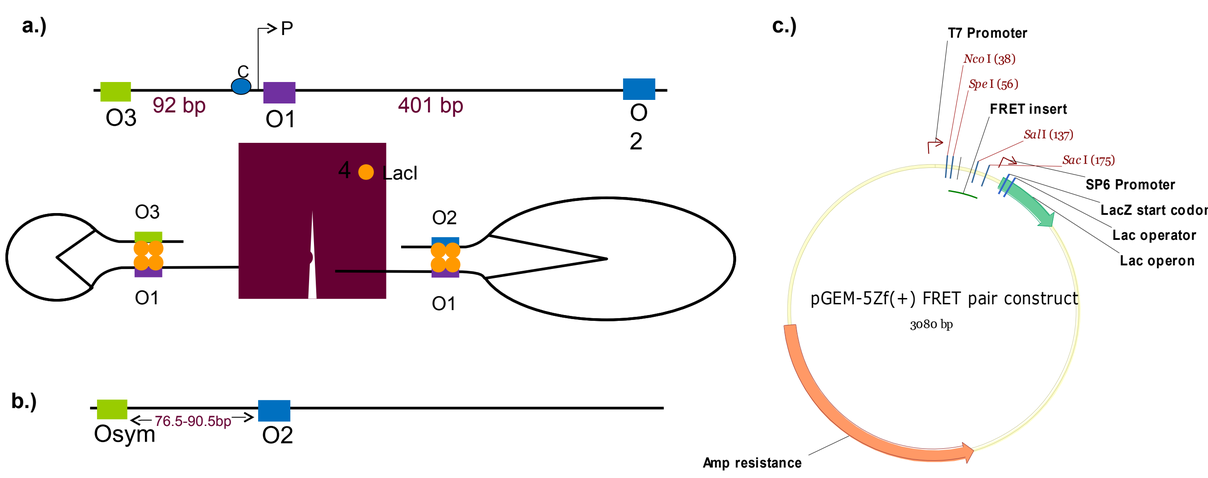

Figure 3. (a.) Wild-type *E. coli lac* operon with cis regulatory elements. The three operators O1, O2 and O3 are positioned at the indicated distances relative to the transcription start site (P). The catabolite activator protein (CAP) binding site is indicated by (C). Looping between the primary operator, O1, and either one of two auxillary operators, O2 or O3, increases the concentration of *lac*I at the promoter, thereby enhancing repression **(b.)** Design of the experimental constructs. The O2 operator is positioned at distances from Osym that vary in 1-bp increments (76 - 90 bp), generating a set of regularly phased operator sequences over about 1.5 turns of DNA duplex **(c.)** Restriction map of pLS(0), a construct in which the O2-Osym distance is 76 bp.

By analyzing the thermodynamics of LacI-mediated looping as a systematic function of the spacing of operator sequences (**Figure 3**), the associated helical-phase dependence of DNA looping can be analyzed to investigate the equilibrium between different loop conformations.

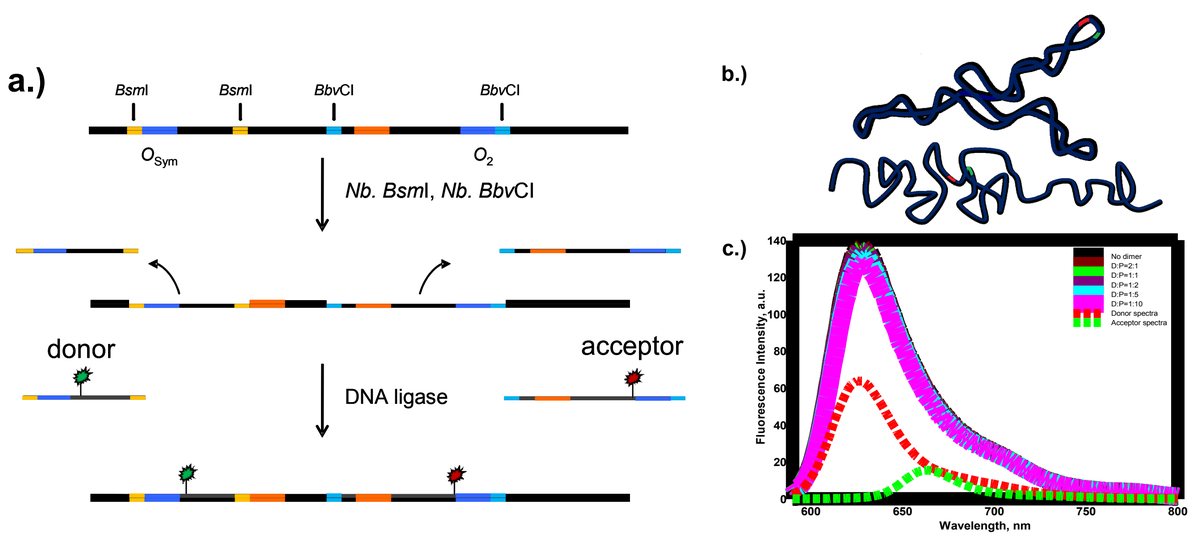

Figure 4. (a.) Method for generating plasmid DNAs labeled with multiple fluorophores. **(b.)** Configurations of fluorophore-labeled supercoiled (sc) and linear DNAs. Locations of donor (green) and acceptor (red) moeities are indicated by the colored chain segments. **(c.)** FRET results for sc pLS(6). Increasing quenching of donor emission is observed with increasing concentrations of LacI protein accompanied by an increase in the acceptor signal.

Fluorescence resonance energy transfer (FRET) is used to quantitate loop formation in LacI titration experiments using supercoiled DNA (**Figure 4**). Donor/acceptor fluorophore pairs (Atto 594 and Atto 647N, *R*0 = 7.5 nm) are conjugated to thymine residues located adjacent to the operator sites.

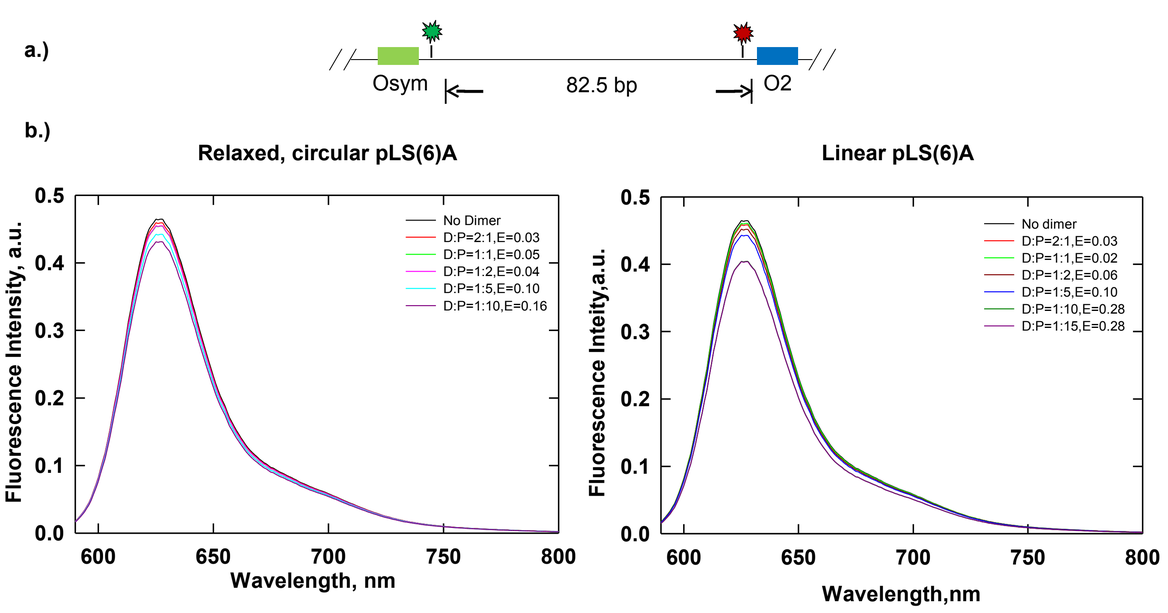

Figure 5. (a.) Location of donor and acceptor fluorophores in Atto594/647N-labeled pLS(6)A. **(b.)** Deconvolved donor-emission spectra of Atto 594/Atto 647N-labeled pLS(6)A (**Figure 4c**).

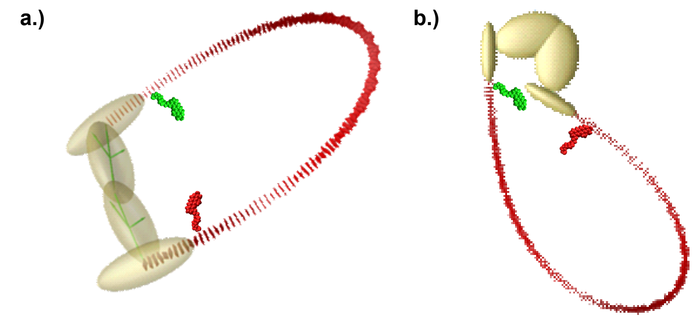

Figure 6. (a.) Loop geometry for the extended form of the LacI tetramer taken from 6. **(b.)** 'LB' lac-repressor loop geometry formed by the V-shaped tetramer. Both models are shown with donor and acceptor

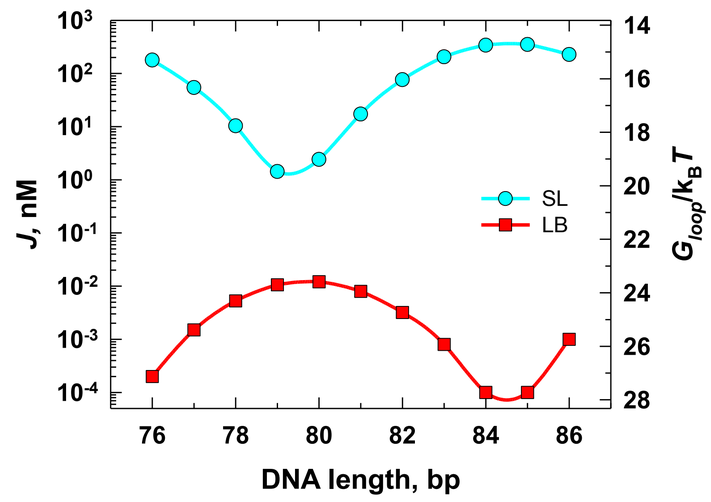

Figure 7. Length dependence of J factors and Δ*G*loop for the extended (SL) and LB LacI-loop conformations computed using the harmonic-approximation method6. The J-factor length dependence shows that the extended LacI conformation is thermodynamically more favorable than v-shaped forms for loops in this size range. Note the difference in J-factor helical phasing between SL and LB loop conformations.

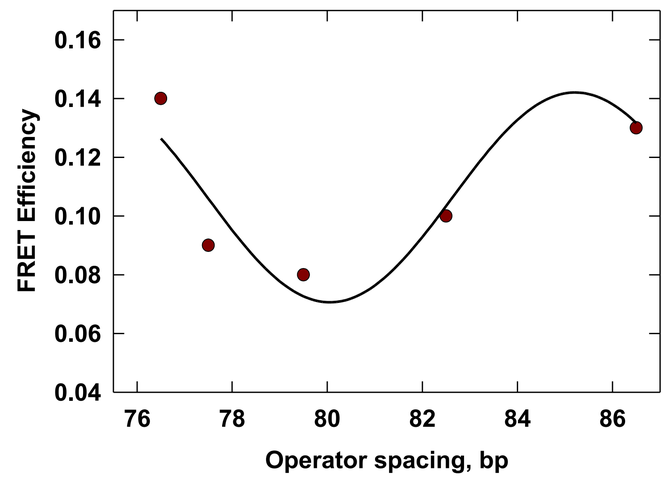

Figure 8. FRET efficiency as a function of operator spacing in relaxed, circular pLS(6)A. The smooth curve is a fit to a sinusoidal dependence and is intended only as a guide to the eye.

CONCLUSIONS

We present bulk (ensemble) FRET results for LacI-mediated DNA loops involving closely-spaced operator sequences in covalently closed plasmids. FRET-efficiency data support a model in which DNA looping is mediated by an extended form of the LacI tetramer. The effect of excess supercoiling on repressor-loop conformation is the subject of current studies.

REFERENCES

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ACKNOWLEGEMENTS

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