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The Energy Landscape of Hyperstable LacI-DNA Loops

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The Escherichia coli LacI protein represses transcription of the lac operon by blocking access to the promoter through binding at a promoter-proximal DNA operator. The affinity of tetrameric LacI (and therefore the repression efficiency) is enhanced by simultaneous binding to an auxiliary operator, forming a DNA loop. Hyperstable LacI-DNA loops were previously shown to be formed on DNA constructs that include a sequence-directed bend flanked by operators. Biochemical experiments showed that two such constructs (9C14 and 11C12) with different helical phasing between the operators and the DNA bend form different DNA loop shapes. The geometry and topology of the loops and the relevance of alternative conformations suggested by probable flexible linkers in LacI remain unclear. Bulk and single molecule fluorescence resonance energy transfer (SM-FRET, with D. English) experiments on a dual fluorophore-labeled 9C14-LacI loop demonstrate that it adopts a single, stable, rigid closed-form loop conformation. Here, we characterize the LacI-9C14 loop by SM-FRET as a function of inducer isopropyl-β,D-thiogalactoside (IPTG) concentration. Energy transfer measurements reveal partial but incomplete destabilization of loop formation by IPTG. Surprisingly, there is no change in the energy transfer efficiency of the remaining looped population. Models for the regulation of the lac operon often assume complete disruption of LacI-operator complexes upon inducer binding to LacI. Our work shows that even at saturating IPTG there is still a significant population of LacI-DNA complexes in a looped state, in accord with previous in vivo experiments that show incomplete induction (with J. Maher). Finally, we will report progress on characterizing the "energy landscape" for DNA looping upon systematic variation of the DNA linkers between the operators and the bending locus. Rod mechanics simulations (with N. Perkins) provide testable predictions on loop stability, topology, and FRET.