Modeling DNA looping

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1 Main Text

The fraction of time spent in the looped configuration is controlled by several competing effects. For example, suppose that a repressor tetramer is bound to the stronger operator. Shortening the interoperator spacing reduces the volume over which the other operator wanders relative to the second binding site on the repressor, increases the apparent local "concentration" of free operator in the neighborhood of that binding site, and hence enhances looping. But decreasing the interoperator spacing also has the opposite effect of discouraging looping, due to the larger elastic energy cost of forming a shorter loop. Moreover, a shorter overall DNA construct increases the entropic force exerted by bead–wall avoidance, again discouraging looping [1]. To see what our measurement of this looping equilibrium tells us, we therefore needed to calculate in some detail the expected local concentration of operator (the "looping J factor") based on a particular mathematical model of DNA elasticity. We chose a harmonic-elasticity model (a generalization of the traditional wormlike chain model), to see if it could adequately explain our results, or if, on the contrary, some non-harmonic model (for example the one proposed in [6, 7]) might be indicated.

To perform the required calculation, we modified the Gaussian sampling method previously used in [1, 2, 3] (see supplement). Our code generated many simulated DNA chains, applied steric constraints [1], and reported what fraction of accepted chain/bead configurations had the two operator sites separated by 7 nm, the distance between operator centers as seen in PDB structure 1LBG [4]. The standard elastic model as an isotropic rod is inadequate for the description of DNA loops only a few helical repeats in length (see for instance [3]), so we modified the elasticity to account for bend anisotropy and bend–roll coupling. We did not account for sequence dependence, however, so we can only make comparisons to our experimental results with random-sequence DNA. We adjusted the overall magnitude of our DNA elasticity matrix to yield a value of overall persistence length $\xi = 45$ nm appropriate for our experiment's buffer conditions [5]. The chain generation accounted for bead–wall, bead–chain, and wall–chain avoidance, but not chain–chain; nor did we consider any interactions involving the repressor tetramer other than binding.

The result of the simulation was that the looping J factor for short loops was less than 0.015 times as great for the constructs with interoperator spacing around 100 bp as for those with spacing around 300 bp; this ratio was about a hundred times smaller than the experimentally determined ratio of 1.7 at optimum helical phasing. The discrepancy between these results indicates that the hypotheses of harmonic elasticity, plus a rigid V-shaped protein coupler, cannot explain the experimental results.[1] One possible explanation for this discrep-

[1] Our choice of persistence $\xi = 45$ nm was conservative; assuming a large ξ in the simulation

ancy, for which other support has been growing, is the alternate hypothesis of DNA elastic breakdown at high curvature [6, 7].

2 Supplement

Our mathematical model built on our previous work [1, 2], which showed that a Gaussian-sampling simulation could accurately model the experimentally observed relation between DNA tether length and TPM bead motion by including an effective entropic stretching force from bead–wall repulsion. This technique is essentially a Monte Carlo evaluation of the partition function of a chain; instead of a Metropolis implementation, we simply generated many discretized chains using Gaussian distributions for each link's bending and twisting angles, then discarded any such chains that violated the global steric constraints. For the present work, we modified our previous code to monitor the distance between operator centers in the generated chains.

We wished to assess the ability of harmonic elasticity theory (linear elasticity) to explain our experimental results. Because we wished to study tight loops, where it's not adequate to average the elasticity over a helical repeat, we introduced a more detailed elastic model than the usual isotropic-rod model, following a simplified version of the approach of [3]. (Several authors have calculated looping J factors in the isotropic-rod model; see for example [9] and references therein.) We idealized the DNA to be homogeneous, i.e. we neglected sequence effects; also, we did not allow strains in the shift variables (shift, slide, and rise). Thus we needed four elastic constants: three diagonals representing roll, tilt, and twist stiffnesses, and a cross-term for roll–twist coupling. (The remaining entries in the symmetric elastic matrix correspond to couplings forbidden by symmetries, namely tilt–roll and tilt–twist [8].) We inverted the averaged covariance matrix for dimer steps in protein-DNA complexes (`http://rutchem.rutgers.edu/~olson/cov_matrix.html`), observed that the "forbidden" entries were indeed much smaller than the others, then set them exactly to zero. We then multiplied by an overall factor chosen to yield the persistence length 45 nm [5]. This procedure resulted in the elastic deformation free energy per basepair as $\frac{1}{2} k_{\mathrm{B}} T \Delta\theta^t F \Delta\theta$, where $\Delta\theta$ is a vector containing the tilt, roll, and twist angles for a basepair step and

$$F = \begin{bmatrix} 0.0601 & 0 & 0 \\ 0 & 0.0334 & 0.0116 \\ 0 & 0.0116 & 0.0335 \end{bmatrix} \tag{1}$$

(Each entry has the units degrees^{-2}.)

We then generated sequences of random rotation generators, each one close to the zero matrix, in a Gaussian distribution determined by equipartition with the above energy function. Each generator was then exponentiated to give a rotation matrix close to the identity. (To speed evaluation, we actually defined

results in an even greater ratio between short- and long-loop J factors.

an equivalent model to Eq. 1 discretized not at the single-basepair level, but in segments of length $\ell = 10.5\,\mathrm{bp}/5$.) Each such sequence yielded a sequence of segment orientations by successive matrix multiplications; the resulting orientation in turn yielded a chain by following each successive 3-axis a distance ℓ. After a chain was generated, it was checked for steric clashes, and if it survived this check the 3-space distance between operator centers was found.

We now wish to evaluate the concentration of the weaker operator near the free binding site of LacR tetramer bound to the stronger operator (the "looping J factor"). To do this we drew a set of nested spherical shells around the stronger operator and found the fraction of time the weaker operator spent in various shells. The fraction of time spent in the shell at distance 7 nm, divided by this shell's volume, yielded the required concentration. This procedure does not account for the additional requirement that each operator have a specified orientation relative to the LacR tetramer (a similar requirement applies to cyclization [3]). But implementing this condition can only reduce further the predicted J factor for short loops relative to long ones, and our goal in the main text was simply to establish a lower bound on this ratio.

References

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