Chromatin loops as modulators of enhancer-promoter interactions in their vicinity

Boryana Doyle[1,2,3], Geoffrey Fudenberg[4], Maxim Imakaev[3], and Leonid A. Mirny[3,4,5]

[1] Program for Research in Mathematics, Engineering and Science for High School Students, PRIMES, Massachusetts Institute of Technology
[2] MIT Undergraduate Research Opportunities Program (UROP)
[3] Department of Physics, Massachusetts Institute of Technology
[4] Graduate Program in Biophysics, Harvard University
[5] Institute for Medical Engineering and Science, Massachusetts Institute of Technology

*Corresponding Author: Leonid A. Mirny, E25-526, MIT, 77 Mass Ave. Cambridge MA 02139, 617-452-4862, email: leonid@mit.edu

Abstract

The classic model of eukaryotic gene expression requires direct spatial contact between a distal enhancer and a proximal promoter. However, recent chromosome conformation capture studies (e.g. Hi-C) show that enhancer and promoters are embedded in a complex network of cell-type specific looping interactions. Here we investigate whether, and to what extent, looping interactions between elements in the vicinity of an enhancer-promoter pair can influence the frequency of enhancer-promoter contacts. Our polymer simulations show that a chromatin loop formed by elements flanking either an enhancer or a promoter suppresses enhancer-promoter interactions, working as a topological insulator. A loop formed by elements located in the region between an enhancer and a promoter, on the contrary, facilitates their interactions. We find that these two consequences of chromatin loops have different genomic extents, with facilitation being a local effect and insulation persisting over a large range of genomic distances. Overall, our results show that looping interactions which do not directly involve an enhancer-promoter contact can nevertheless significantly modulate their interactions. This illustrates the intricate effects that local chromatin organization can have on gene expression.

Introduction

Distal enhancer elements in higher eukaryotes are essential for regulating gene expression (1-4). In conjunction with transcription factor binding, the classic model for enhancer function involves the direct spatial interaction between enhancers and their target promoters (Figure **1A**) (1-4). Recent studies have started to reveal the complexity of the enhancer-promoter interaction network, where each enhancer can influence multiple promoters, and each promoter may be influenced by multiple enhancers (5-8). In addition, gene expression and enhancer-promoter interactions occur within the higher-order three-dimensional chromatin organization, which is characterized by an intricate network of interactions at multiple scales. For example, below 1Mb, chromatin is organized into continuous 500-900kb regions of enriched contact frequency called topologically associated domains (TADs), which are largely cell-type independent (9,10). Within TADs, additional cell-type specific looping interactions are formed (6,11,12). These observations raise an important question; namely, how are enhancer-promoter contacts affected by looping interactions in their genomic neighborhood?

Two models for how proximal looping interactions can modulate enhancer-promoter contact have been proposed: the decoy model and the topological model (experiments (13-15), reviewed in (16-19)). The decoy model suggests that insulating elements directly interact with the enhancer, sequestering it from the promoter, and thereby hinder enhancer-promoter interactions. The topological model proposes that two regulatory elements in the vicinity of the enhancer and the promoter can interact with each other to form a chromatin loop; this, in turn, affects enhancer-promoter contacts. Evidence supporting the topological model include experiments in Drosophila using Su(Hw) insulating elements at the 10kb-scale (Figure **1B**, **1C**) (20).

Other studies provide additional examples of, and further complications for, the topological model as a regulatory mechanism (Figure **1D**) (21-23). In a recent study, Kyrchanova et al. (21) observed that a single Drosophila *gypsy* element between an enhancer and a promoter did not change their interactions. However, placing an additional *gypsy* element in the vicinity changed enhancer-promoter interactions depending on *gypsy* position and orientation; the authors explain these observations by *gypsy-gypsy* looping interactions. The regulatory effects of loops may also be relevant at larger genomic distances; in mice, a regulatory element with multiple larger (55kb and 25kb) loops was suggested to control multiple enhancer-promoter contacts at the H19 locus (22). Finally, as proposed for enhancers, loops between insulating elements were suggested to modulate the activity of silencing elements (23).

Due to the complexity observed across experiments, it remains unclear whether, and to what extent, the topological model can mediate enhancer-promoter contacts in eukaryotes. Polymer simulations provide an ideal testing ground to investigate the effects of a loop on enhancer-promoter interactions; many loci can be probed simultaneously at high resolution and more complicated looping arrangements can be systematically addressed. Previously, Mukhopadhyay et al. (24) used polymer simulations to demonstrate that the topological model of insulation applies to an unconfined system of two fused chromatin rings; namely, two loci within the same ring interact more frequently than loci in different rings. We significantly extend this study by asking whether forming loops may affect interactions at scales exceeding the loop size, e.g. interactions of a loop with the rest of the chromosome or between loci in the vicinity of the loop.

Here we use polymer models of chromatin to study how interaction between regulatory elements that form 15-60kb loops in the vicinity of an enhancer-promoter pair can influence direct enhancer-promoter interactions.
For generality, we model chromatin by a long homogeneous flexible fiber with only a few additional interactions between specific elements, as described below. Synthesizing results from the literature, we focused our simulations on two important arrangements of the loop-forming elements relative to an enhancer-promoter pair: (1) an enhancer is flanked by loop-forming elements, while a promoter is beyond the loop (Figure **1E**); and (2) both loop-forming elements are located in the genomic region between an enhancer and a promoter (Figure **1F**). We find that loops can significantly insulate or facilitate the frequency of enhancer-promoter interactions, depending on the loop location relative to the enhancer-promoter pair. These effects are robust for a variety of situations and parameters, including: enhancer-promoter genomic distance, stiffness of the chromatin fiber, size of looping elements, topological constraints on the chromatin fiber (topoisomerase II activity), chromatin densities, and number of looping elements.

Materials and Methods

Model

Using equilibrium simulations of a confined polymer chain, we study how chromatin loops affect enhancer-promoter contact frequency in their vicinity. In most simulations, except for the smallest 10 monomer loop (see Supplemental Figure **S2** caption), we model chromatin as a semi-flexible polymer fiber that consists of 15nm monomers, each representing three nucleosomes or 500bp, with a persistence length of 3 monomers (Figure **1G**)

(25). By considering substantial variations of these parameters, we find that polymers with different flexibilities produce quantitatively similar results (Supplementary Figure **S1**). Excluded volume interactions are modeled by a repulsive potential (see SI Methods). Unless otherwise noted, we allow occasional chromatin fiber crossing to account for topoisomerase II (topo-II) activity by setting a finite energy cost for two monomers to occupy the same volume (i.e. using a truncated repulsive potential, see SI Methods). To account for the dense arrangement of chromatin within the nucleus, we confine the chromatin fiber to impose a 2% density-by-volume; we later vary volume density from 1% to 20% (see Results). For each set of conditions and interactions, we performed Langevin dynamics simulations (Movie **M1**) and sampled conformations from the resulting equilibrium ensemble; these conformations were subsequently analyzed to compute contact frequencies (see below, and SI Methods).

To investigate the effects of a chromatin loop on a larger region of chromatin, we model a loop by forming an irreversible bond between a pair of monomers (Figure **1E**, **1F**, see SI Methods). We considered loops of sizes L=2.5kb, 15kb, 30kb, and 60kb, in a proportionally larger genomic region of size 33*L, i.e. 1Mb for 30kb loop (see Discussion, Supplementary Figure **S2**). Our polymer model contains no additional sequence-specific details, and thus generally addresses how enhancer-promoter interactions are altered in the vicinity of a loop. The model remains agnostic to the chromatin organization at larger genomic scales, assuming that the simulated region is contained within a single chromatin domain (26).

Analysis of simulations

To obtain the contact frequency between loci in our polymer simulations, we first generate an equilibrium ensemble of conformations for each set of parameters using Langevin Dynamics simulations (Figure **2A**, Supplemental Table **T1** for parameter values). From these conformations we compute the pairwise contact frequency between all regions of the chromatin fiber, which we display as a heatmap (Figure **2B**). We note that this heatmap contains information about all possible arrangements of an enhancer and a promoter relative to the loop. Our simulated heatmap is characterized by two features: (i) a decay of contact frequency as a function of increasing genomic distance, and (ii) an off-diagonal interaction between the loop bases. The first feature follows from the polymer connectivity of the simulated chromatin fiber. The second feature alters the typical decline in the contact frequency and is of primary interest in this study.

We define the "contact frequency ratio" as the contact frequency in a model with a loop divided by the contact frequency for an otherwise equivalent

model without a loop, where all other parameters are kept the same; insulation occurs if this ratio is less than 1, whereas facilitation occurs if this ratio is greater than 1. Unless noted otherwise, we report contact frequency ratios for a 30kb loop and a 50kb enhancer-promoter genomic distance.

Results

We used the simulated heatmaps of pairwise contact frequency to determine the effect of a loop on enhancer-promoter contact frequency for two important arrangements reported in the literature (see Figure **1**). The first arrangement involves a chromatin loop formed by elements flanking an enhancer, such that the enhancer is located within the chromatin loop and a promoter is outside of the loop (Figure **2C**). In this case, one of the loop-forming elements is located between an enhancer and a promoter. Simulations show that forming such a loop leads to about a 50% reduction in enhancer-promoter contacts, serving as an insulator (contact frequency ratio of 0.64, Figure **2D**). Below we refer to this arrangement as insulation.

The second arrangement found in the literature and studied here constitutes a chromatin loop located in the genomic region between the enhancer and promoter, i.e. both loop-forming elements are located between the enhancer and promoter (Figure **2C**). Formation of such a loop facilitates enhancer-promoter interactions by increasing their contact frequency by more than 4-fold (contact frequency ratio of 4.15, Figure **2D**). In addition, as the heatmap shows, a looping interaction can increase contact frequency when both the enhancer and promoter are located within the loop, i.e. where the two loop-forming elements flank the enhancer-promoter pair.

Next we varied enhancer-promoter genomic distance and studied whether this changes the strength of loop-induced insulation or facilitation. Interestingly, the two effects behave differently; while facilitation diminishes with distance, insulation appears to be independent of enhancer-promoter genomic distance (Figure **3A**). This shows that facilitation is a local phenomenon, while insulation is a global effect.

For the insulation arrangement, we also varied the relative position of the enhancer within the loop and found that the magnitude of insulation depends on its relative location. Insulation is weaker when the enhancer is placed in the middle of the loop, and strengthens as the enhancer approaches the base of the loop (0.75 to 0.49 contact frequency ratio, Figure **3B**). We note that an extreme case of topological-model insulation is in fact similar to decoy-model insulation, which occurs when the enhancer is placed at the

base of the loop. In this scenario, we observe stronger insulation because the enhancer is permanently interacting with the other loop base, sterically hindering interactions between the enhancer and all other loci.

We additionally investigated how properties of the chromatin fiber influence loop-induced insulation and facilitation. By simulating chromatin fibers with different persistence lengths, we found that this parameter does not significantly affect insulation or facilitation (Supplementary Figure **S1**). This is consistent with the fact that both phenomena are observed at distances much larger than the persistence length, and thus do not emerge solely due to fiber stiffness.

Next, we studied the effect of topological constraints, as they have been suggested to play an important role in chromosome organization (27-29). To investigate this, we performed simulations both with and without allowing the chromatin fiber to cross, which may respectively correspond to cells with and without active topo-II. We found that insulation and facilitation are present irrespective of the absence or presence of topological constraints (Figure **3C**). We note that topological constraints discussed here are distinct from other topological effects such as supercoiling of the chromatin fiber (30) which may be relevant for bacterial chromosome organization (31).

Since these changes of the chromatin fiber did not alter insulation and facilitation, we performed simulations of a phantom polymer chain, which lacks excluded volume (Supplemental Figure **S3**). Remarkably, elimination of excluded volume interactions completely abolishes the insulation effect. In contrast, the degree of facilitation remains largely unaffected by the elimination of excluded volume interactions (contact frequency ratio reduced from 4.15 to 3.20). This result suggests that facilitation mainly arises due to an effective shortening of the enhancer-promoter spacer by the loop. Additional facilitation is observed in simulations with excluded volume, since the chromatin fiber before and after the loop emerges in the same direction, away from the bulky loop. Taken together, these simulations show that excluded volume interactions of a chromatin fiber are essential for insulation, but only moderately impact facilitation.

We further considered how other biological aspects of chromatin organization affect insulation and facilitation. In particular, active and inactive chromatin environments are known to have lower and higher densities, respectively. We performed simulations at densities ranging from low (1%) volume density to high (20%) density (Figure **3D**). We found that while both insulation and facilitation remain qualitatively present, they are both quantitatively stronger at lower density. For comparison, we observe

contact frequency ratios of 0.6 and 5.1 at the lowest density vs. 0.8 and 2.3 at the highest density, for insulation and facilitation respectively.

As an extension of the one-loop model discussed above, we performed simulations in which two consecutive loops are formed. We observed qualitatively similar insulation and facilitation in the two-loop cases (Supplementary Figure **S4**). Furthermore, we note that the average contact frequency between loci within one loop is higher than the average contact frequency between loci from different loops. In this sense, the two loops are insulated from each other as well as from the rest of the fiber. This is consistent with results for an isolated system of two fused rings (24).

Discussion

Using a polymer model of chromatin, we found that a single loop in the vicinity of an enhancer-promoter pair can either insulate or facilitate their interactions. These effects have a considerable magnitude, with about 2-fold insulation and 3-5 fold facilitation; these are comparable to experimentally observed changes in gene expression.

The modulation of enhancer-promoter contacts by chromatin loops is often referred to as the topological model (16,17,19). Studies which consider the topological model often assume a particular mechanism whereby the loop alters enhancer-promoter interactions. Specific mechanisms include: sliding along DNA (32), lamina attachments (19,33), and inter-nucleosome interactions (17). Our simulations show that looping can modulate enhancer-promoter interactions due to polymer effects, independent of the mechanisms above.

The phenomena we observe are very general and follow directly from the polymer connectivity and excluded volume interactions of the chromatin fiber. For example, a loop in the region between an enhancer-promoter pair brings them together and makes them more likely to come into contact. In our model, looping interactions do not necessarily directly prevent or form enhancer-promoter contacts; instead, they steer the conformational ensemble of chromatin toward or away from conformations where an enhancer and a promoter are in contact. This mechanism of action is analogous to classical allosteric regulation in proteins (34), and particularly to disordered proteins, where binding of an allosteric substrate changes the protein conformation, which in turn alters the structure of a distant active site (35).

Our general polymer model of a chromatin loop is qualitatively robust to a variety of situations and the details of the chromatin fiber. This includes: enhancer-promoter genomic distance, stiffness of the chromatin fiber, size of looping elements, topological constraints on the chromatin fiber (topoisomerase II activity), chromatin densities, and number of looping elements. Moreover, our observations of insulation and facilitation do not depend on particular mechanisms of non-specific interactions between monomers, such as dynamic nucleosome switching and bond saturation used in a previous computational study (24).

In specific biological systems, however, the detailed structure and flexibility of the chromatin fiber may become relevant. For example, a chromatin loop with a size approaching several persistence lengths can become very rigid. Consequentially, its effects may depend on the molecular details of the insulating elements, including their orientation as observed in a recent study (21). However, loss/unwrapping of nucleosomes (36) can make the fiber more flexible, either uniformly, or through the formation of kinks (29), allowing small loops to behave similarly to larger loops (Supplementary Figure **S2D**).

Collectively, experiments have observed complex patterns of enhancer-promoter behavior, which depend on the local arrangement of regulatory elements. For example, one insulating element can interact directly with an enhancer and sequester it (decoy insulation), whereas two elements, both between an enhancer and promoter, can cancel each other out by forming a loop (15,16,18,19). Our model predicts that this loop formation would not only cancel out the decoy insulation, but could also topologically facilitate enhancer-promoter contacts. On the contrary, if the second insulator was placed on the other side of the enhancer, our model predicts that decoy insulation would change to topological insulation. Together, these suggest a complex interplay between decoy and topological models of insulation. We further note that decoy insulation can be seen as an extreme case of topological model insulation (Figure **3B**).

An important aspect of the in-vivo networks of local looping interactions is that they may be both dynamic over the course of the cell cycle and different between cells. Our results for insulation and facilitation by fixed loops, where the bases of the loop are always connected, remain relevant for dynamic loops while they are present. Roughly speaking, the effect on insulation or facilitation for a given loop is proportional to its frequency of occurrence in a cell.

Given the complexity of the local looping network, it is likely that there are multiple dynamic loops in the vicinity of the enhancer and promoter. While

we study the permanent single and double loop systems, our results provide intuition even to these more complicated systems. For instance, the global nature of insulation implies it can hinder interactions between a group of enhancer(s) and any number of promoters. Conversely, facilitation is local and thus specific to the regions that directly flank the loop. In conjunction with emerging biological data, future simulations will provide additional insight into the consequences of chromatin's polymeric nature for modulating enhancer-promoter interactions

Supplementary Data Statement

SI Methods
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Table T1
Supplementary Movie M1

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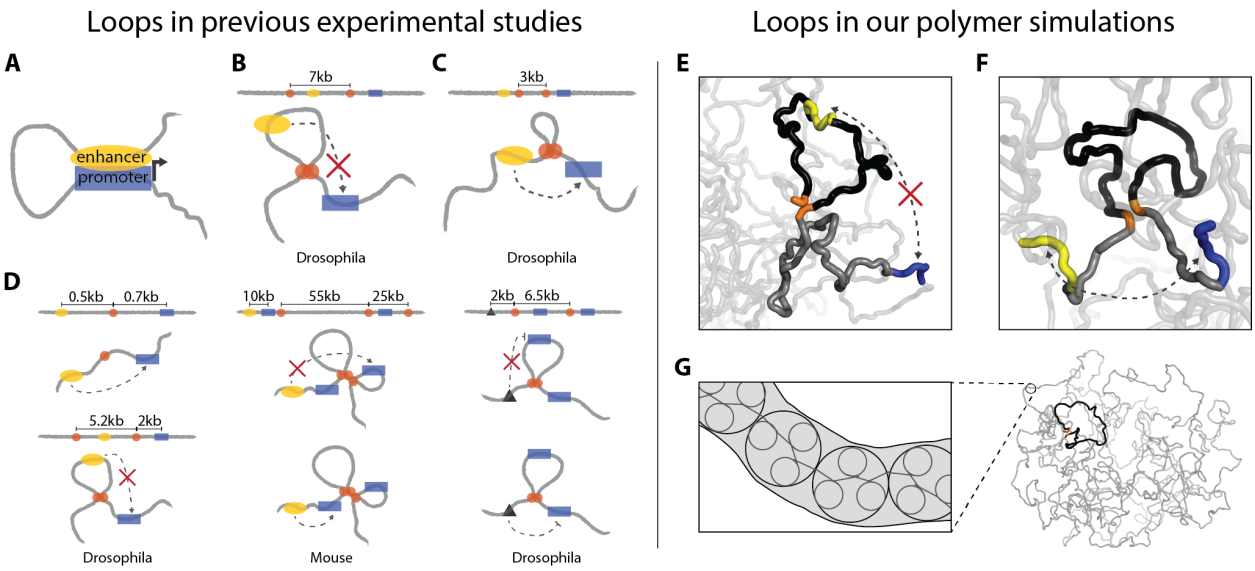

Figure 1. Enhancer-promoter pair in the context of other interactions. *Experimental Studies*, (**A**) Illustration of an enhancer (yellow) spatially interacting with a promoter (blue) along a chromatin fiber. This coloring convention continues throughout the paper. (**B**) A recent study in Drosophila suggested a 7kb chromatin loop formed between Su(Hw) insulators (orange) could decrease enhancer-promoter interactions, indicated by an X (red) (20). (**C**) Conversely, a 3kb chromatin loop in the region between enhancer and promoter was proposed to increase enhancer-promoter interactions. (**D**) Five arrangements for proposed looping interactions from three studies, left to right, Kyrchanova et al. (21), Kurukuti et al. (22), and Comet et al. (23). (*left*) a single Drosophila *gypsy* element between an enhancer and a promoter did not change their interactions (*top*), however an additional *gypsy* element upstream of the enhancer decreases enhancer-promoter interactions (*bottom*), (21). (*center*) at the H19 locus in mice, a regulatory element with multiple larger loops was suggested to control multiple enhancer-promoter contacts; enhancer activity is repressed for the promoter within the loop (*top*), but remains for the promoter before the loop (*bottom*) (22). (*right*) chromatin loops may also modulate spatial interactions between silencing elements (e.g. PRE, black triangles) and their target promoters (23). The promoter within the loop is not silenced (*top)*, whereas the promoter beyond the loop is silenced (*bottom*). *Polymer Simulations*, (**F**) Arrangement 1: polymer conformation where an enhancer is within a chromatin loop and a promoter is beyond the loop. (**G**) Arrangement 2: polymer conformation where an enhancer is before the loop and a promoter is after the loop. (**H**) (*left*) zoom-in on our polymer model of chromatin. The three large circles represent one monomer each; each monomer consists of three nucleosomes (small circles) or 500bp. (*right*) full view of a sample polymer conformation showing a 30kb chromatin loop (black) with highlighted loop-bases (orange) within a 1Mb region.

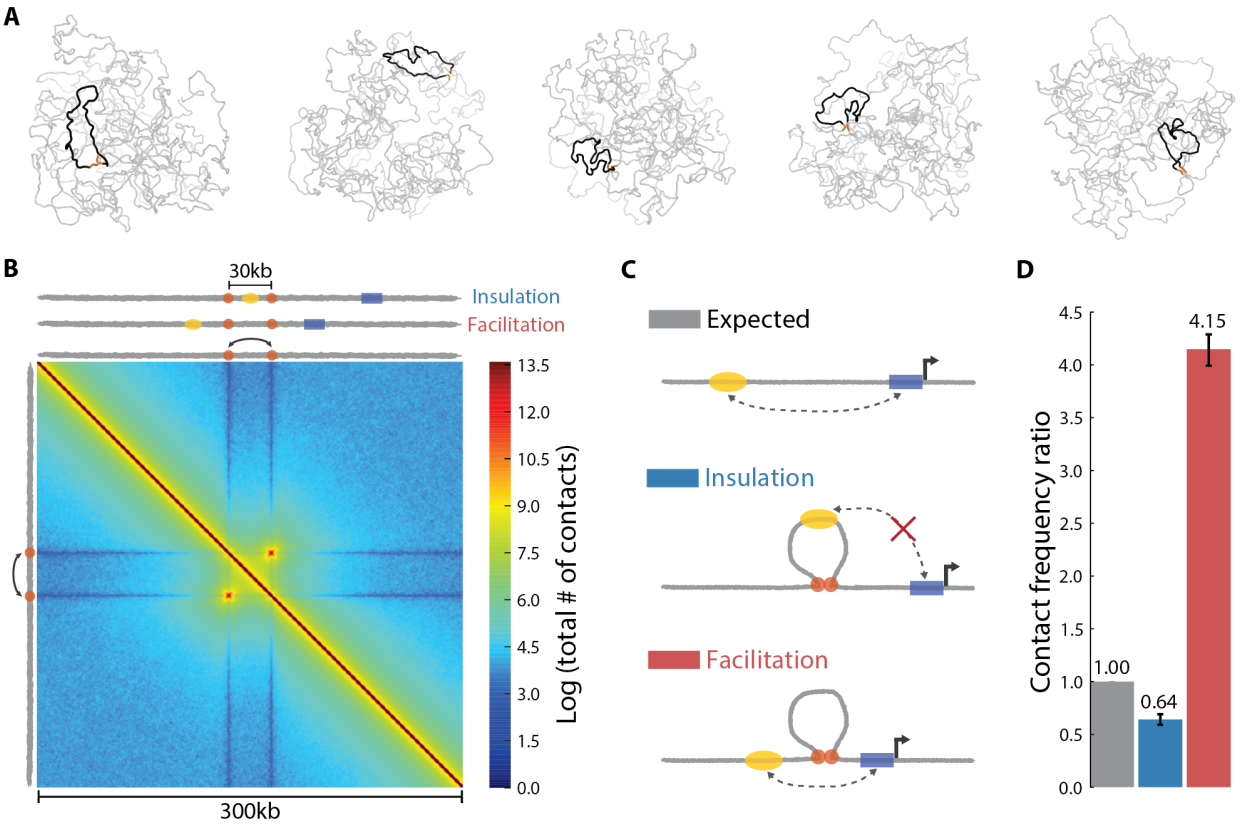

Figure 2. Effect of a chromatin loop on the frequency of enhancer-promoter interactions. (**A**) Four sample conformations from polymer simulations with a 30kb permanent loop (black) formed between two bases (orange) in a 1Mb region of fiber. (**B**) Average heatmap (300kb by 300kb) for polymer simulations of the permanent, one-loop system, with a 30kb loop (aggregated over 800,000 simulated conformations). Top and left edges show positions of the enhancer (yellow), promoter (blue), and loop bases (orange) for insulation and facilitation arrangements. (**C**) Schematics of enhancer-promoter arrangements. (*top*) chromatin fiber without a fixed loop and with enhancer-promoter genomic distance of 50kb, as used to calculate expected (no-loop) contact frequencies (Materials and Methods). (*middle*) arrangement where insulation is observed, represented by the red "X". (*bottom*) arrangement where facilitation is observed. (**D**) Contact frequency ratios (Materials and Methods) for insulation and facilitation arrangements with a 30kb loop and 50kb enhancer-promoter genomic distance.

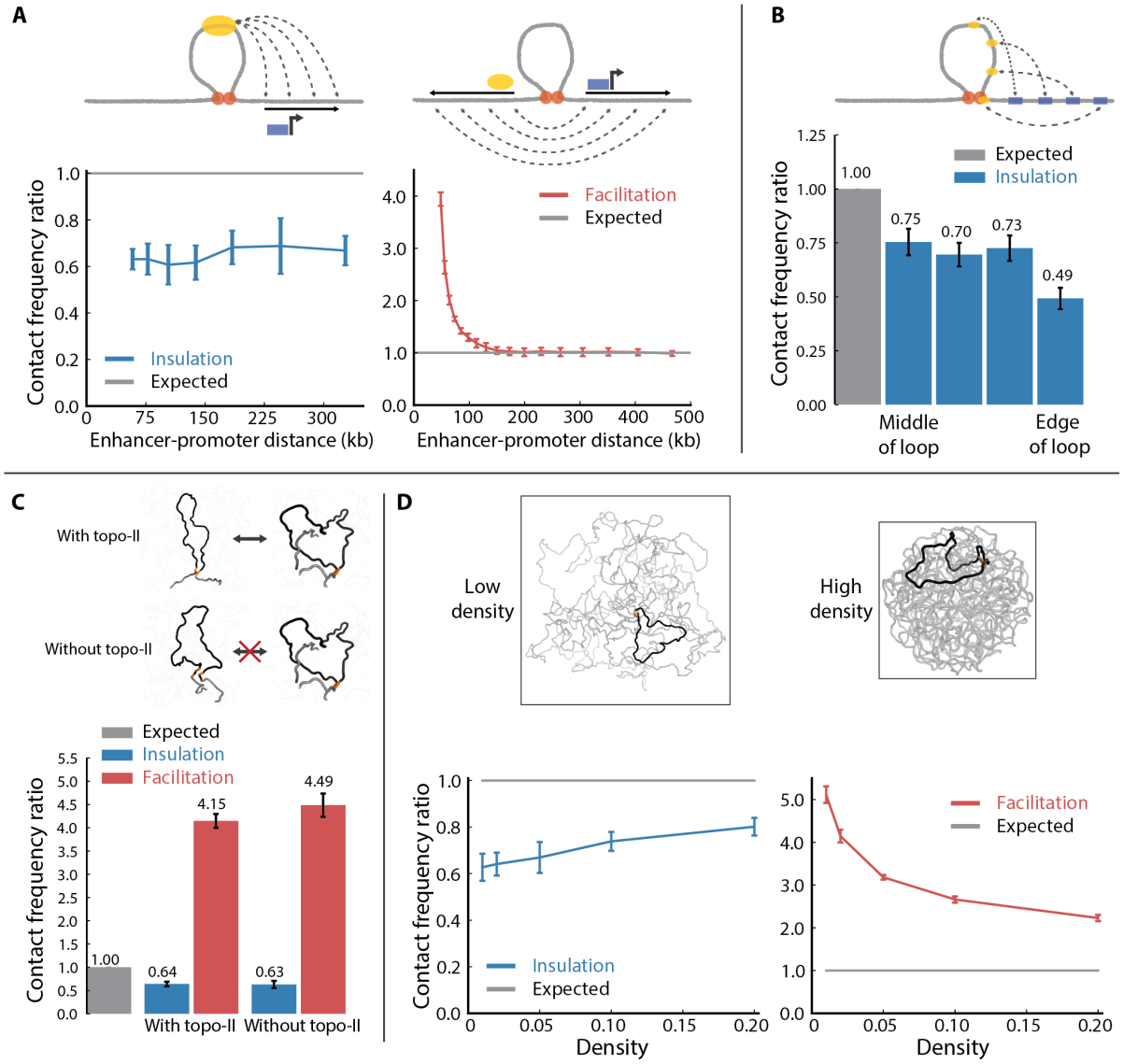

Figure 3. Effect of chromatin organization and density on loop-mediated interactions (**A**) Insulation (left) and facilitation (right) as a function of enhancer-promoter genomic distance. For insulation, enhancer position remains fixed. For facilitation, an enhancer-promoter pair is positioned symmetrically around the loop at each genomic distance. (**B**) Insulation for different positions of the enhancer within the loop with a constant genomic distance of 50kb. (**C**) Effect of topological constraints on insulation and facilitation. With topo-II, there are no topological constraints and a conformation without chromatin threaded through the loop can convert to a conformation with chromatin threaded through the loop. Without topo-II (with topological constraints), chromatin fibers cannot cross and the two conformations cannot interconvert. Bar plot shows the contact frequency ratio for an enhancer-promoter genomic distance of 50kb. (**D**) Effect of density on insulation and facilitation; bar plots show results for 50kb enhancer-promoter genomic distance.