Random loops model can explain the appearance of Topologically Associating Domains (TADs)

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Introduction

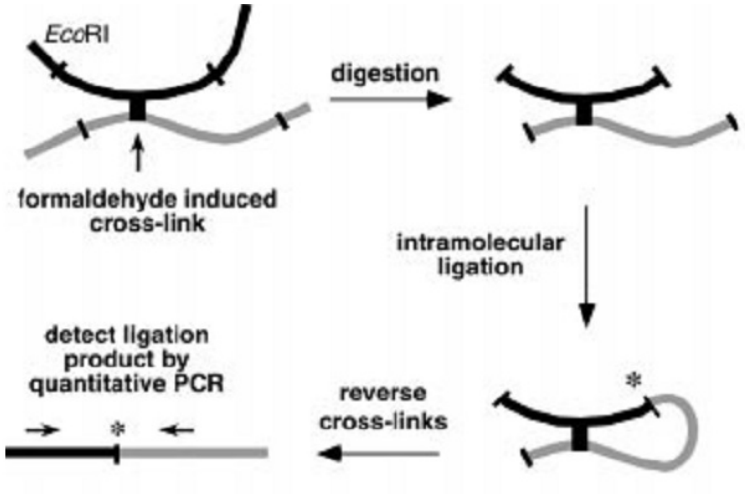
1. The spatio-temporal organization of a chromosome has significant implication to cellular activities, such as gene expression and regulation.
2. However, the spatial organization of the chromatin is not entirely known.
3. It is known that packing of the chromatin allows for long range gene regulation.
4. Furthermore, DNA looping has shown to be a mechanism for long range gene regulation.
5. Here we show that using random polymer looping model and fitting it to the experimental chromatin looping data we can explain the appearance of conserved structures in chromatin called TADs.
6. Using this model, we will be able to say 'something' about the mean structure.

Agenda

Chromosome Conformation Capture Experiments

A set of methods to simultaneously record millions of looping events occurring within the genome (specific or unspecific).
The general steps are:

1. Intact nuclei are extracted from millions of cells;
2. Formaldehyde induces protein-DNA and protein-protein cross-links;
3. restriction enzymes digest the cross-linked DNA;
4. cross-linked DNA is purified, diluted and ligated;
5. cross-links are reversed;
6. PCR to amplify ligation junctions;
7. the histogram of segments' encounter is produced.



The experimental data

- Two replicate of the CC experiments were conducted by Nora et. al 2012.
- Cells taken from undifferentiated mouse embryonic stem cells.
- We focus on a 920,432 bp subset of the data, around the X inactivation center of the X chromosome.
- The region harbors the Xist enhancer and Tisx promoter.
- We have the segments' encounter frequencies from the two experimental replicates.
- The subset of the data contains two Topologically Associating Domains (TADs).

Topologically Associating Domains (TADs)

Conserved structures showing chromosome interactions on the Mb scale, with higher intra than inter-region interactions It is believed that the TAD forms a 'regulatory unit' for gene expression, as can be seen by the correlation of gene expression located on the same TAD. However, it is not yet clear what is its role in a single cell.

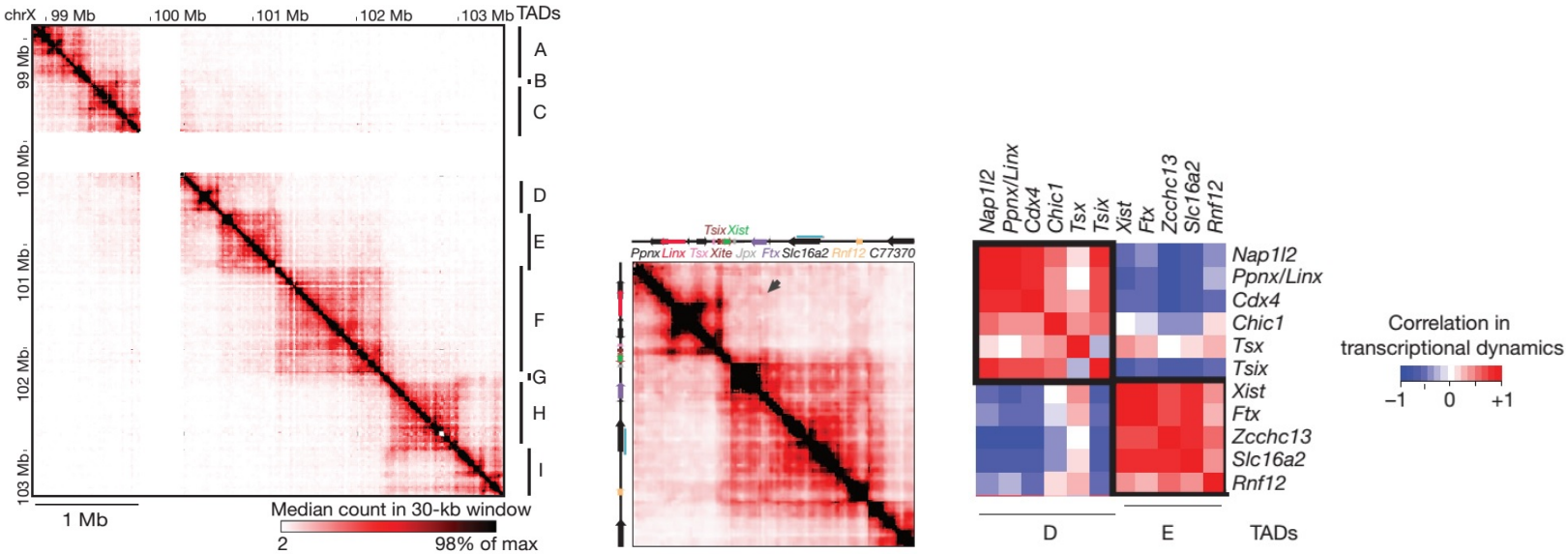
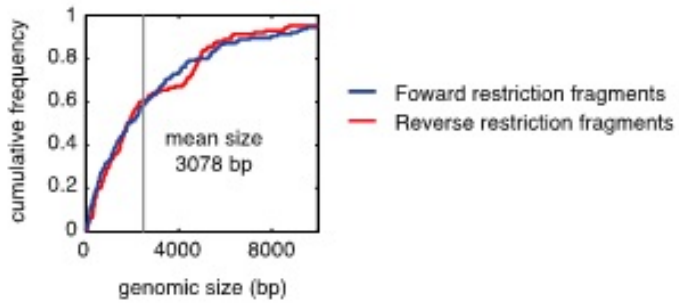


Figure: The 4.5 Mb region (left), enlargement of TAD D and E (center). Displayed median count in a 30kb window every 6kb, gene expression correlation (right)

From restriction segments to beads

- To coarse-grain the data, we choose a bead-size of 3000 bp, corresponding to the mean segment length resulted from the digestion of EcoRIII enzyme.



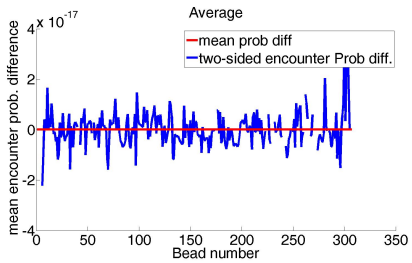
- The genomic section was evenly partitioned. Each bead receives a start and end index according to the segment it covers.
- for example:

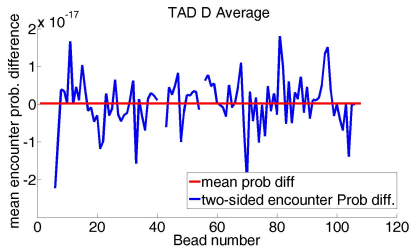
bp range	start ind	end ind
500-3500	1	2
4000-4500	2	2
5000-12001	2	4

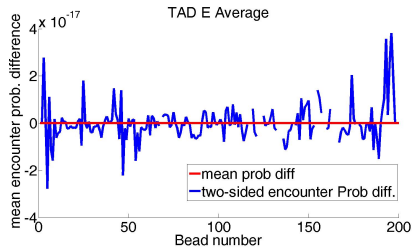
Bead encounter frequecy

TAD D and E

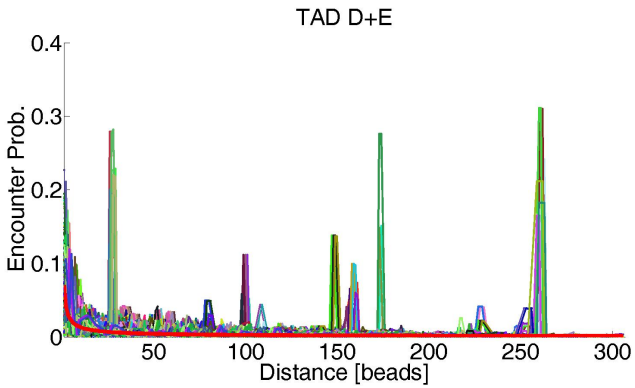
- We work with the average of the two experimental replicates.
- A total length of 920,432 bp - resulting in 307 beads (TAD D 107 beads, TAD E 200 beads).
- We calculate the 'one-sided' encounter probability vs. distance (bead units) for each bead.
- The mean encounter probability's difference, shows that the data is left-right symmetric.

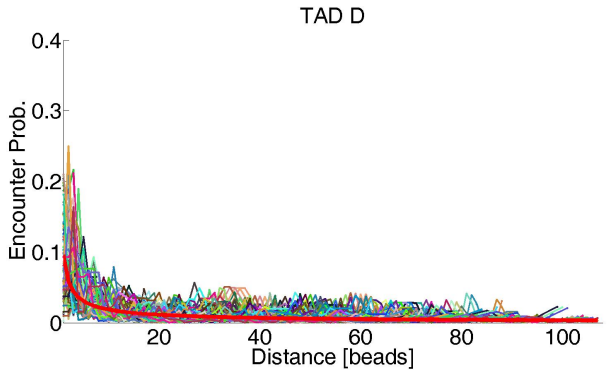


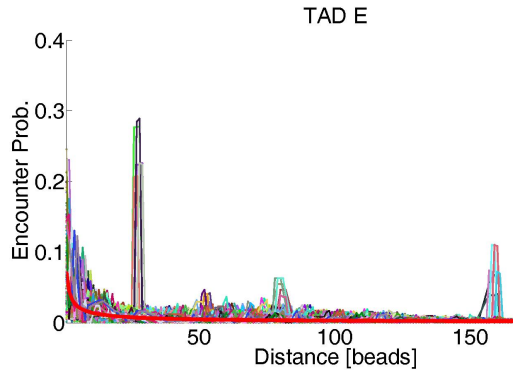




- TAD E has several strong specific interactions. TAD D has almost no specific interactions. Strong inter-TAD specific interactions







Peaks of the encounter data

- About half of the peaks in the encounter data result from specific interactions between TADs.
- The other half comes from specific internal interactions of TAD E.
- To get an impression, a manual marking of the peaks shows:

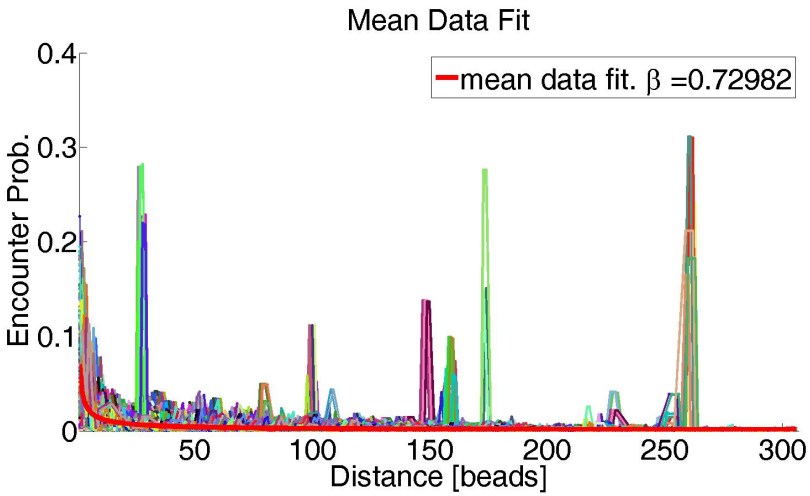
Bead numbers	Encountered beads	TAD
23-26	280-290	$D \leftrightarrow E$
49-53	148-155	$D \leftrightarrow E$
56-59	80-90	$D \leftrightarrow D$
115-117	165-170	$E \leftrightarrow E$
161-162	187 190	$E \leftrightarrow E$
182-184	260-264	$E \leftrightarrow E$
185-186	253-255	$E \leftrightarrow E$
234-236	184-189	$E \leftrightarrow E$
234-236	4-11	$E \leftrightarrow D$
243	88	$E \leftrightarrow D$
264	89-90	$E \leftrightarrow D$
274-277	113-120	$E \leftrightarrow E$

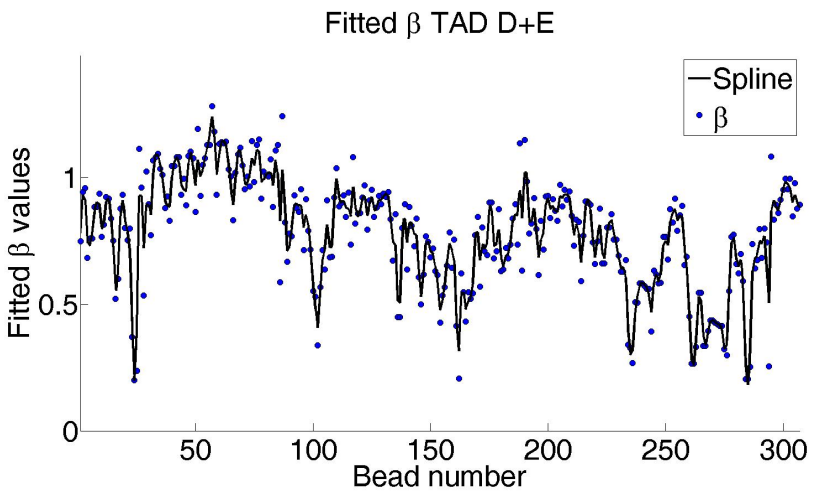
The enconter probability

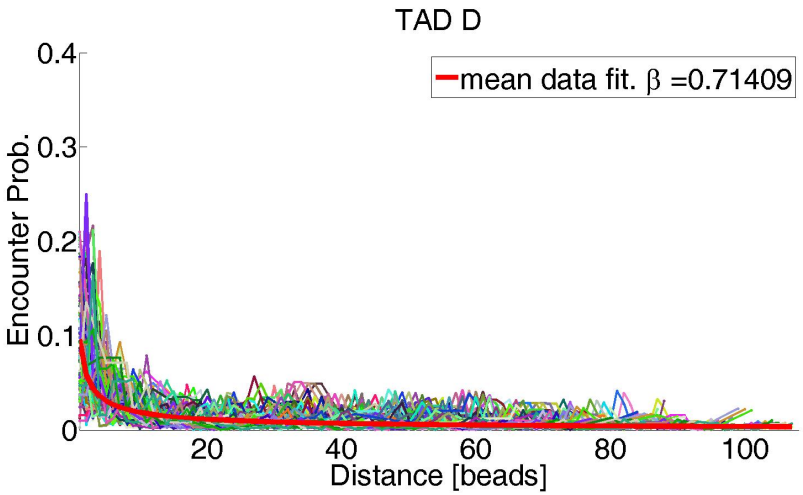
For the case of TAD D, TAD E, and the two together, we estimate the bead encounter probability, p, and fit it with a function of the form

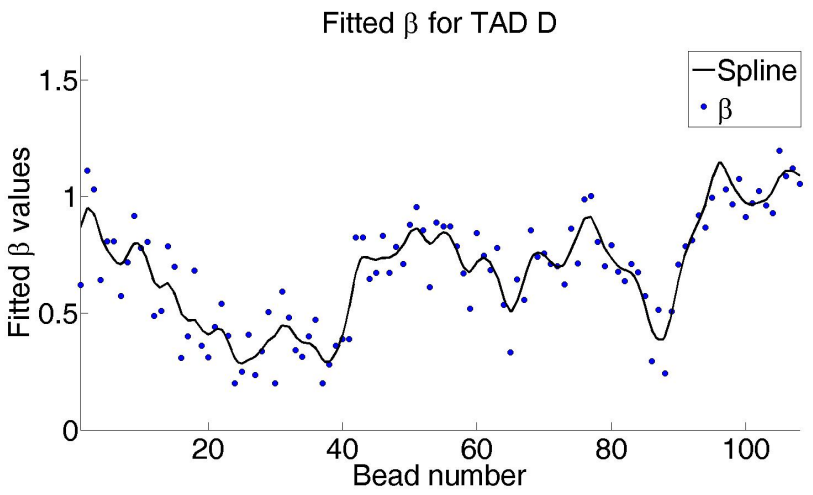
$$p_n(d) = \alpha d^{-\beta}$$

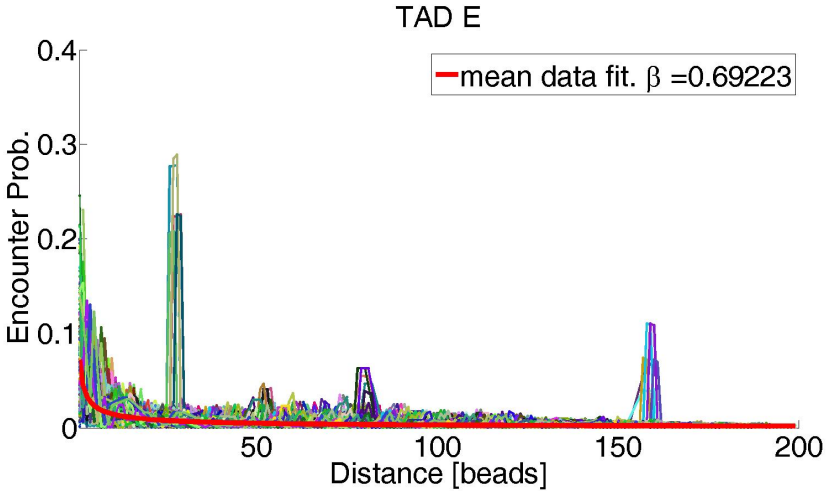
where, d is the distance in [bead] units, n is the bead number, $\alpha = \frac{1}{\sum_{j=1}^{d_{max}} j^{-\beta}}$ and β is a parameter to be estimated. We report the values of β for each bead in each case.

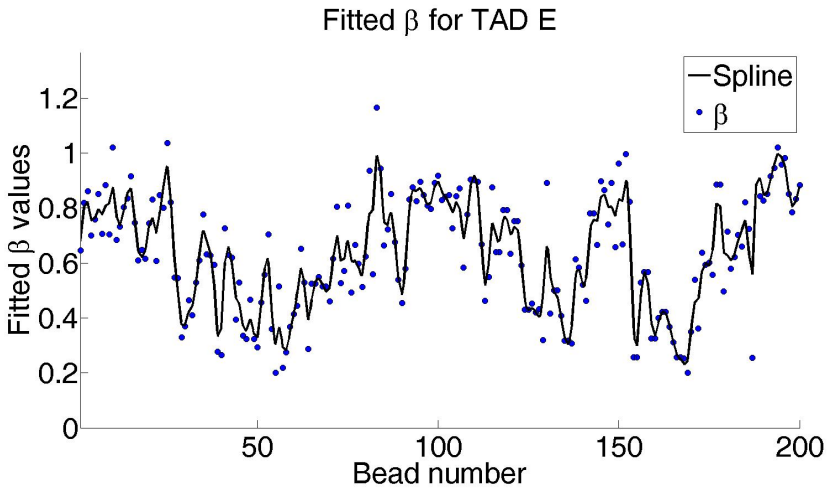





TAD D
mean data fit. β =0.71409
Encounter Prob.
Distance [beads]


Fitted β for TAD D
Spline
β
Fitted β values
Bead number


TAD E
mean data fit. β =0.69223
Encounter Prob.
Distance [beads]


Fitted β for TAD E
Spline
β
Fitted β values
Bead number

Theoretical model

The Rouse model

We start with the classical and most simple model, the Rouse chain.

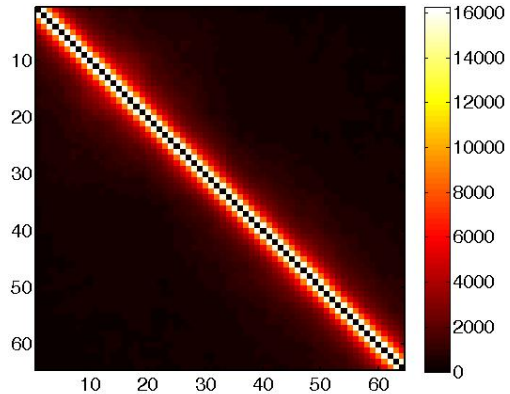
- A Rouse chain describes polymer dynamics as a stochastic motion of a collection of microscopic "beads" connected by harmonic springs
- the 3D motion of bead n in the chain of N beads

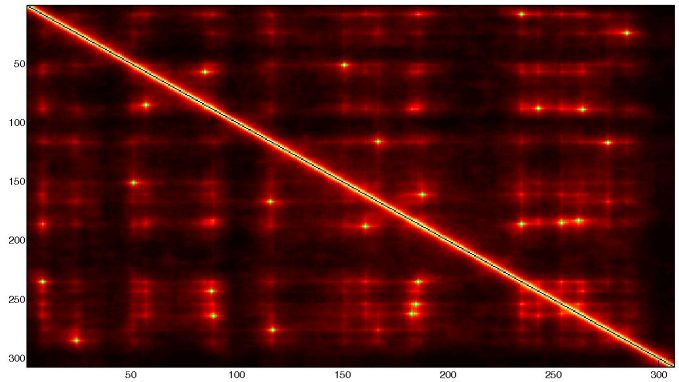
$$\frac{dR_n}{dt} = -\frac{3D}{b^2}(2R_n(t) - R_{n+1}(t) - R_{n-1}(t)) + f_n(t)$$

- R_n- the position of bead n
  b- the standard deviation of the distance between adjacent beads
  D- the diffusion constant
  f_n- white Gaussian noise
- From the theory, $Pr(\|R_n - R_m\| < \epsilon) \sim |n-m|^{-1.5}$

Simulation with simple rouse chain

- A simple Rouse model cannot reproduce the TAD, as expected.
- Placing loops corresponding to the peaks of the encounter data does not reproduce the TADs.





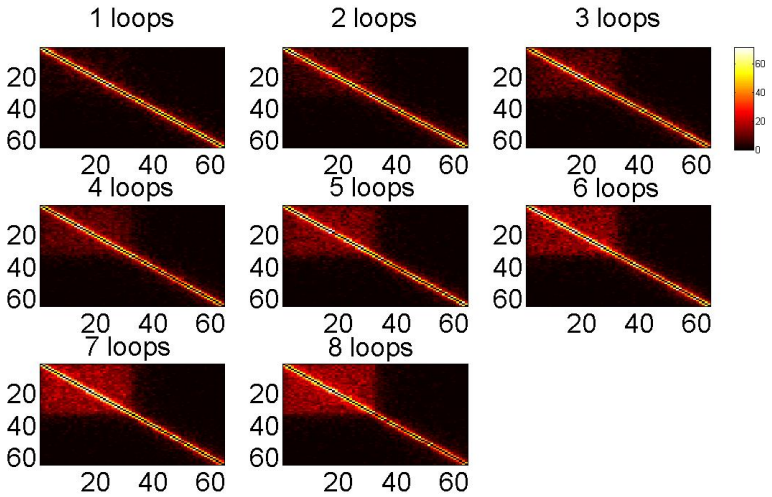
Random Fixed Loop Model

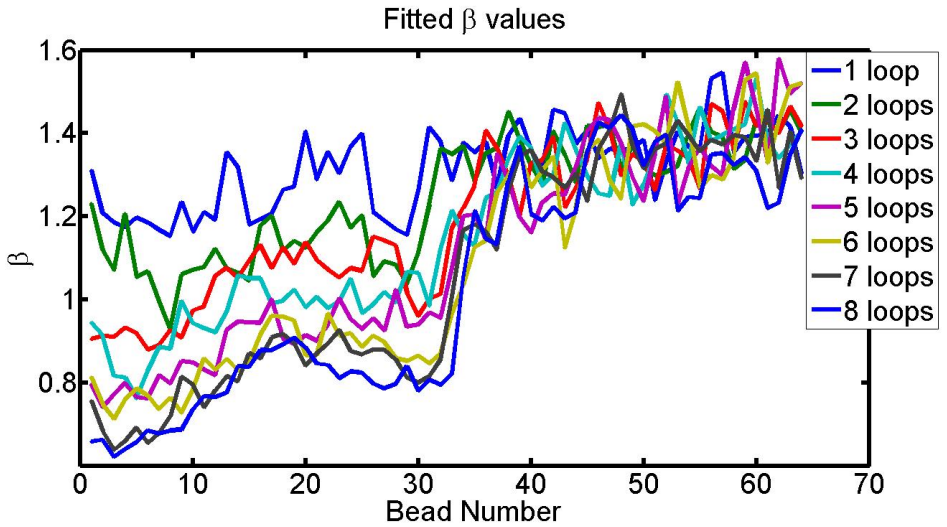
- The enhancer-transcription factor elements' encounter motivates the simulation of a chain with randomly placed loops.
- These encounters might not be as frequent as 'stable' loops in the chromatin and therefore not shown significantly in the encounter maps.
- Simulate a chain of 64 beads, having a random loop in a bounded region.
- Increasing number of loops at random positions.

Random loops in a bounded region

One TAD

We examine the behavior of the encounter probability when we restrict the loops to be in one region of the polymer, increasing the number of loops from 1 to 8.

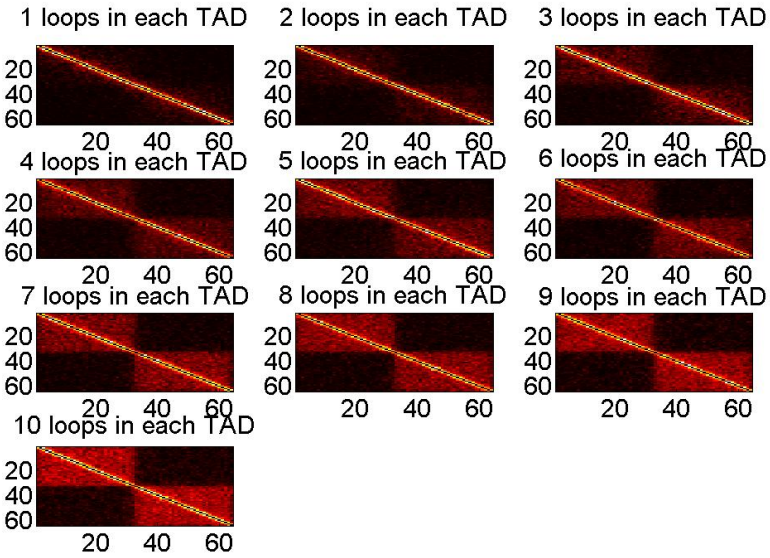


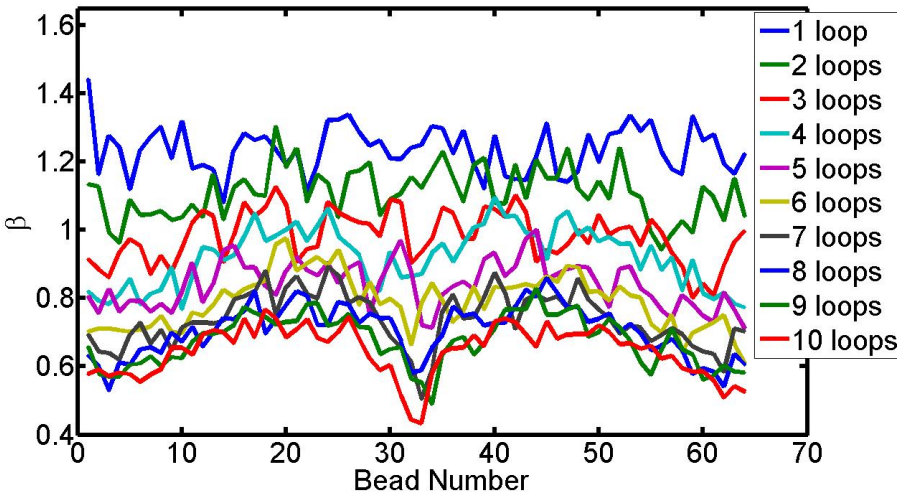


Random loops in a bounded region

Two TADs

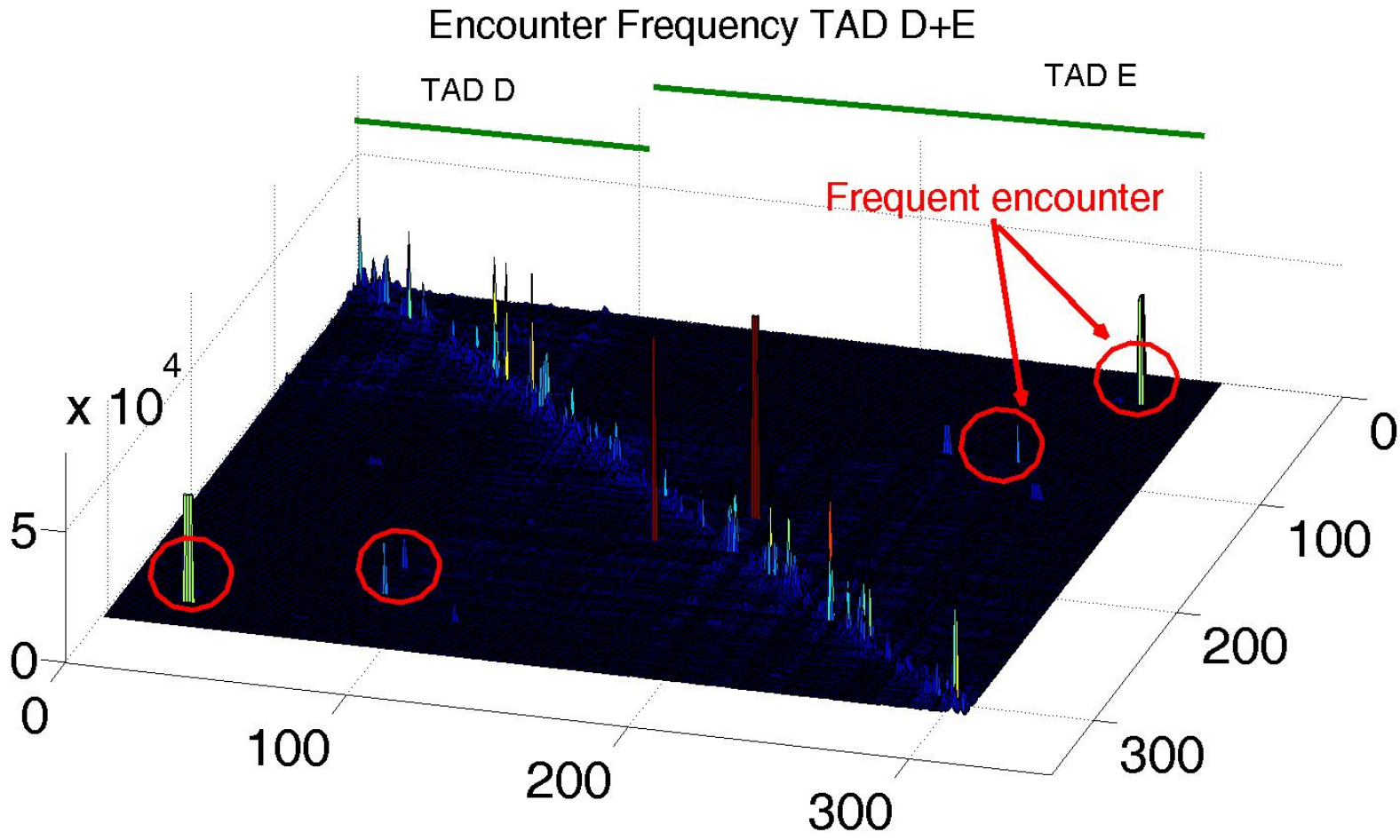
The same can be done with two bounded regions, where the number of loops is increased from 1 to 10 in a 64 beads chain.





Peaks at the edges of TADs

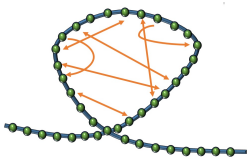
At the edges of TADs there are significant peaks of the encounter frequencies. The encounter data of Nora et al. was smoothed using a median filter. Therefore, those peaks where not clearly shown. Such peaks might indicate a stable loop in the structure of the genomic region and should be taken into account.

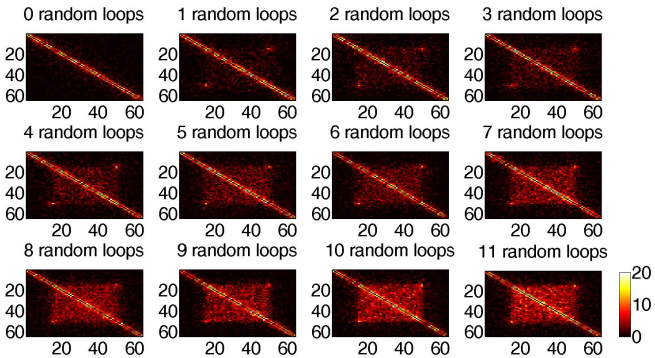


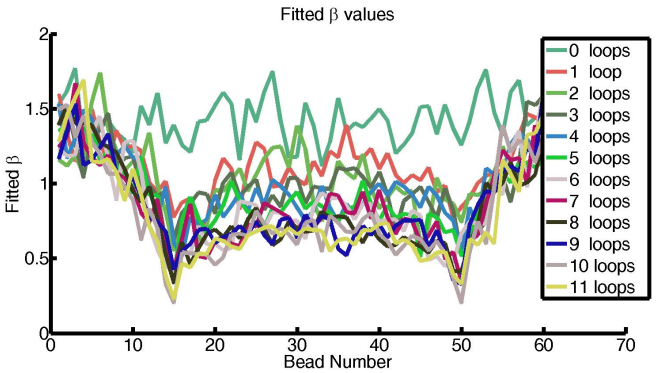
Stable loop with random loops within

one TAD with 'tails'

1. Following the peaks observation, we form a mix of 'stable' big loop with internal random loops.
2. In a 64 beads chain, beads 15 and 50 were connected.
3. We iteratively add 11 loops within the stable loop.
4. We can start seeing the emergence of β curve pattern as in the experimental data.

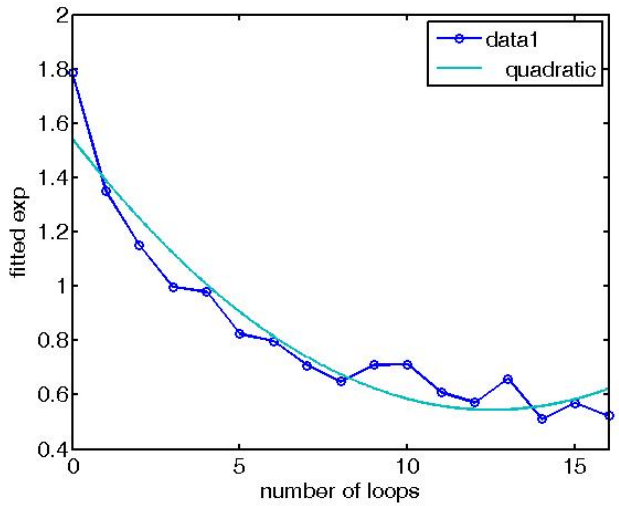






β as a function of the number of loops

1. Through simulation we observe a quadratic relationship between the mean fitted β value and the number of random loops.
2. This enables us to say something about the mean structure.
3. The mean number of loops in a given genomic region can be extracted from experimental data.



Literature supprt for random enhancer promoter interactions

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8. Erez Lieberman-Aiden, Nynke L van Berkum, Louise Williams, Maxim Imakaev, Tobias Ragoczy, Agnes Telling, Ido Amit, Bryan R Lajoie, Peter J Sabo, Michael O Dorschner, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. science, 326(5950):289293, 2009.
9. Erez Lieberman-Aiden, Nynke L van Berkum, Louise Williams, Maxim Imakaev, Tobias Ragoczy, Agnes Telling, Ido Amit, Bryan R Lajoie, Peter J Sabo, Michael O Dorschner, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. science, 326(5950):289293, 2009.

etc..

Summary

- We sowed a plausible and simple model to explain the appearance of the conserved TAD domains.
- A relationship between the mean number of loops and the encounter probability was shown by simulations.
- The parameter used for our analysis, namely β, can easily calculated by experimentalists.

Work in progress

1. Calculation of the mean first encounter time between beads in the Rouse ring
2. Showing analytically the relationship between number of loops and decrease in β
3. Put in more rigorous form the relationship between the pattern of *beta* and the mean observed chromatin structure.
4. Calculating the equilibrium distribution of the random loop model
5. Simulating a model with random dynamic loops, in which loops can form and dissociate.
6. Experiment with more realistic polymer models.