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

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# Introduction

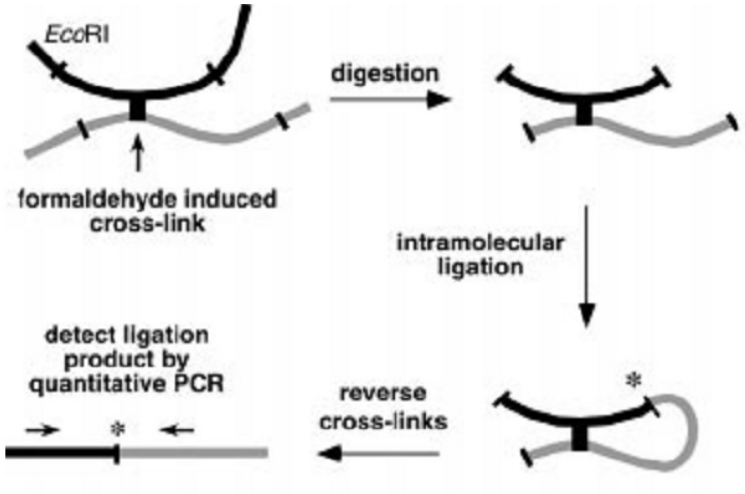
1. The spatio-temporal organization of the chromatin has significant implication of cellular activities, such as gene expression and regulation.
2. however, the spatial organization of the chromatin is not entirely known.
3. DNA looping has shown to be a mechanism for long range gene regulation.
4. here 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.

# 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. histogram of segment encounter is produced



# The experimental data

- Two replicate of the CC experiments were conducted by Nora et. al 2012.
- we focus on a 920,432 bp subset of the data, around the X inactivation center of the X chromosome in mouse embryonic stem cells.
- the region harbors the Xist enhancer and Tisx promoter.
- we have the segments' encounter frequencies from the two experimental replicates.

# Topologically Associating Domains (TADs)

Conserved structures of chromosome interactions on the Mb scale, with higher inter than intra-segment interactions It is believed that the TAD forms a 'regulatory unit' for regulating gene expression, as can be seen by the correlation of gene expression located on the same TAD

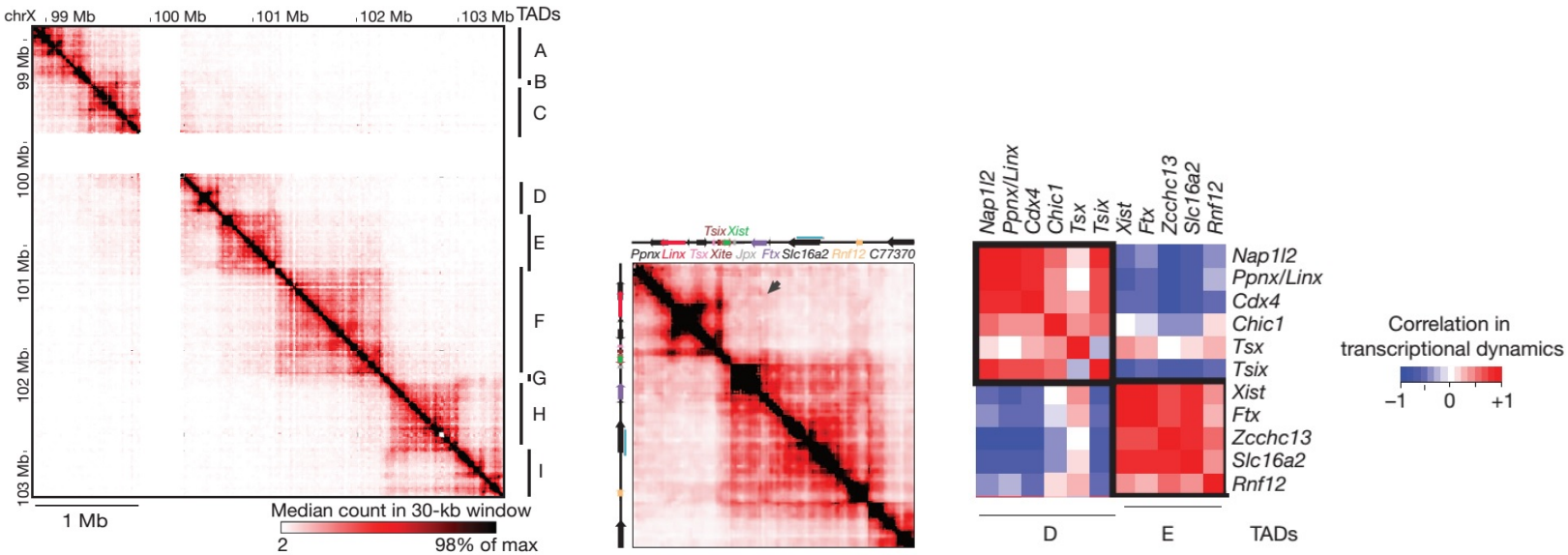
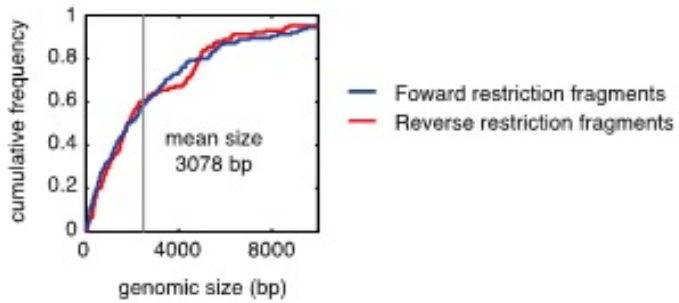



Figure: The 4.5 Mb region (left), enlargement of TAD D and E (center). Displayed median count in a 30kb window every 6kb, gene expression correlation (right)

# 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.



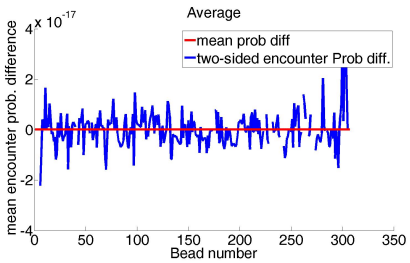

- the genomic section was evenly partitioned by 3000 bp beads. Each segment receives a start and end index according to the beads it covers.
- for example,

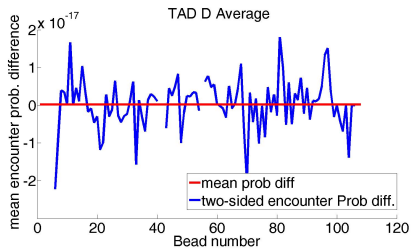
| bp range | start ind | end ind |
|---|---|---|
| 500-3500 | 1 | 2 |
| 4000-4500 | 2 | 2 |
| 5000-12001 | 2 | 4 |

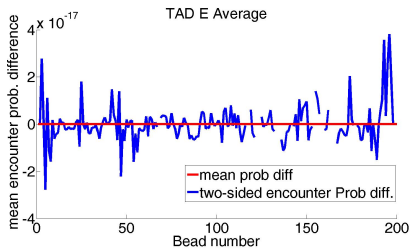
# Bead encounter frequecy

## TAD D and E

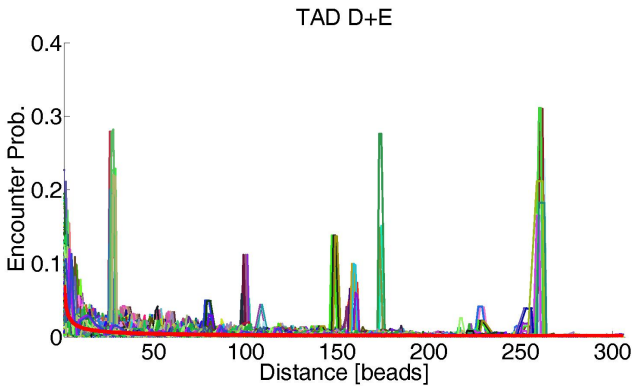
- We work with the average of the two experimental replicates
- a total length of 920,432 bp - resulting in 307 beads (TAD D 107 beads, TAD E 200 beads)
- We calculate the 'one-sided' encounter probability vs. distance (bead units) for each bead
- the mean encounter probability difference, shows that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

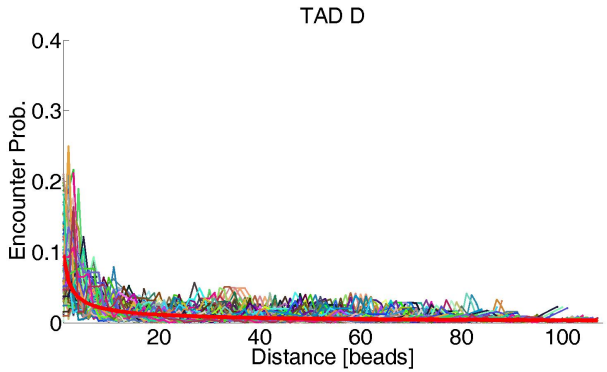


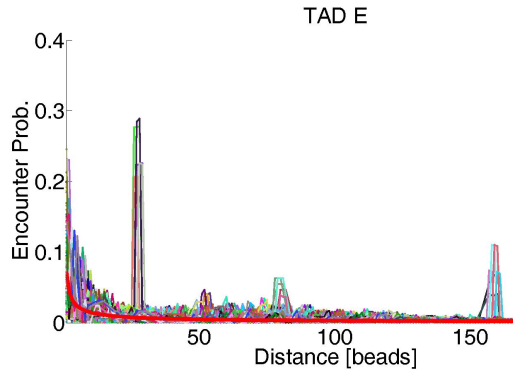




- TAD E has several strong specific interactions. TAD D has almost no specific interactions. Strong inter-TAD specific interactions



# 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

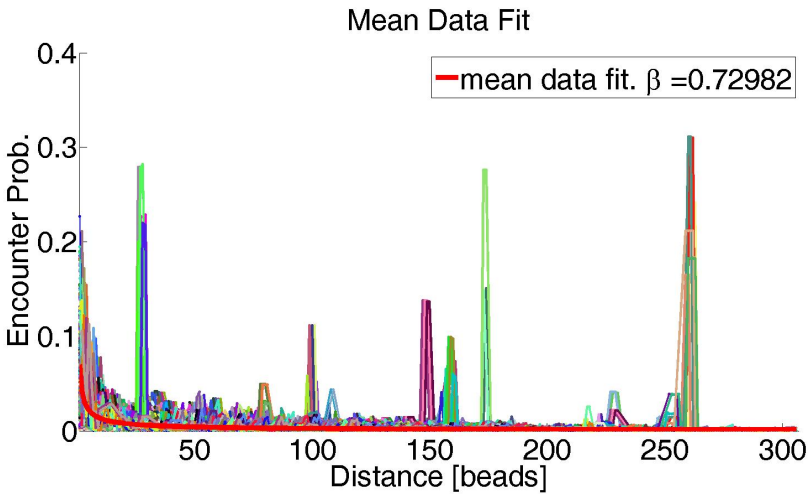
| Bead numbers | Encountered beads | TAD |
|---|---|---|
| 23-26 | 280-290 | $D \leftrightarrow E$ |
| 49-53 | 148-155 | $D \leftrightarrow E$ |
| 56-59 | 80-90 | $D \leftrightarrow D$ |
| 115-117 | 165-170 | $E \leftrightarrow E$ |
| 161-162 | 187 190 | $E \leftrightarrow E$ |
| 182-184 | 260-264 | $E \leftrightarrow E$ |
| 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 | $E \leftrightarrow E$ |

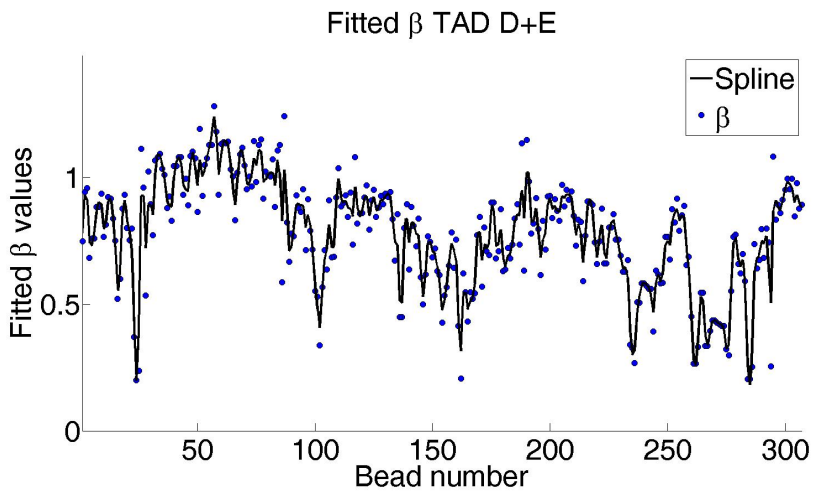
# The enconter probability

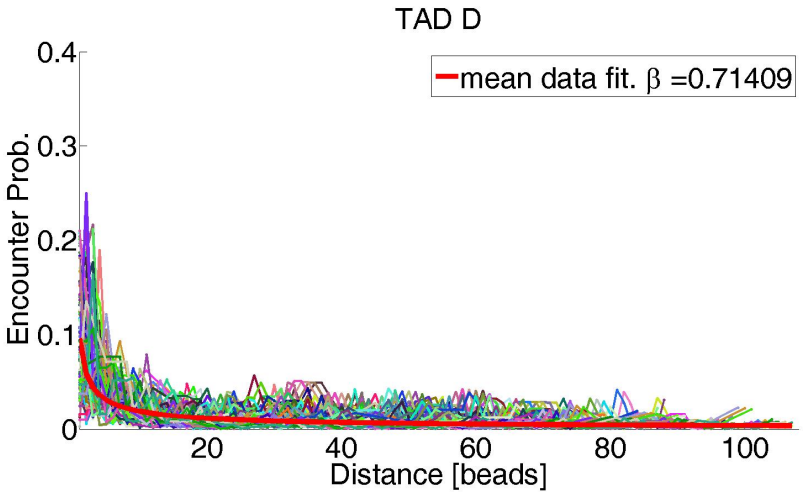
For the case of TAD D, TAD E, and the two together, we estimate the bead encounter probability, $p$, and fit it with a function of the form

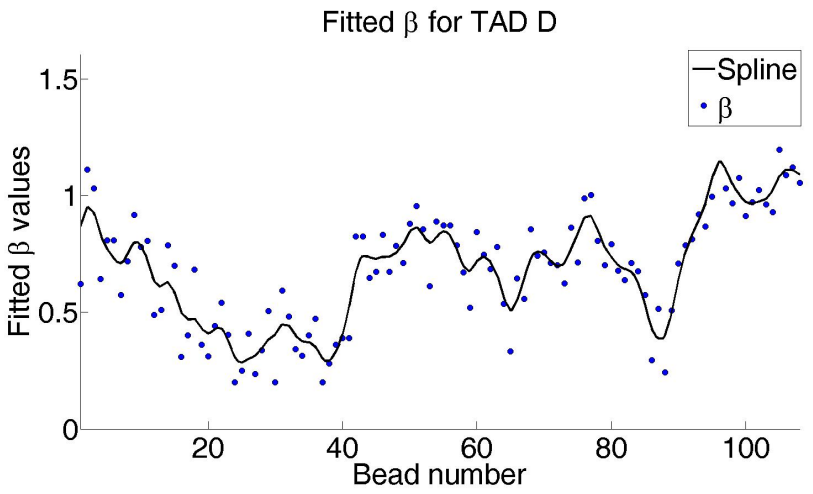
$$p_n(d) = \alpha d^{-\beta}$$

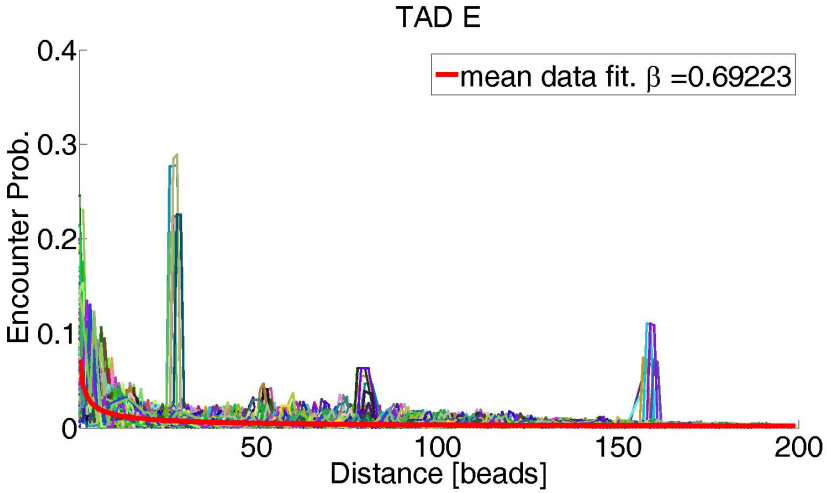
where, $d$ is the distance in bead units, $\alpha = \frac{1}{\sum_{j=1}^{d_{max}} j^{-\beta}}$ and $\beta$ is a parameter to be estimated. We report the values of $\beta$ for each bead in each case

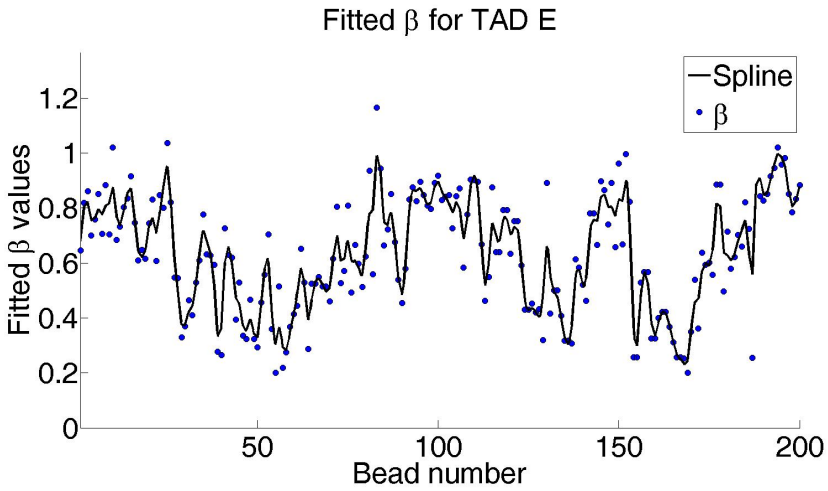



TAD D
mean data fit. β =0.71409
Encounter Prob.
Distance [beads]


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.

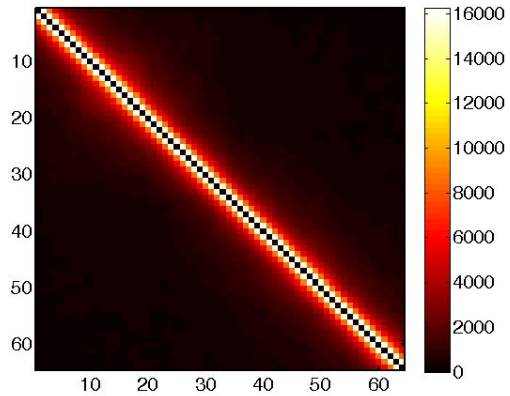
- A Rouse chain describes polymer dynamics as a stochastic motion of a collection of microscopic "beads" connected by harmonic springs
- the 3D motion of bead $n$ in the chain of $N$ beads

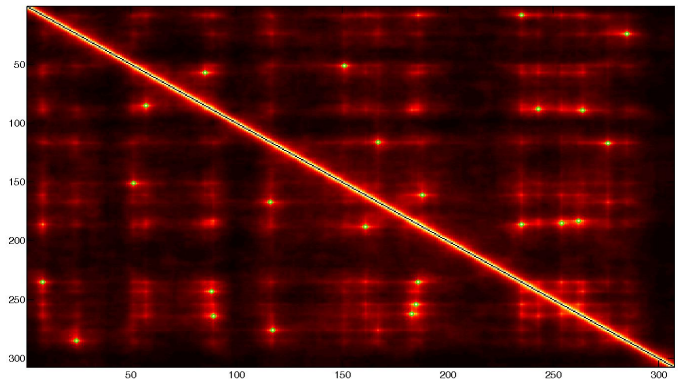
$$\frac{dR_n}{dt} = -\frac{3D}{b^2}(2R_n(t) - R_{n+1}(t) - R_{n-1}(t)) + f_n(t)$$

- $R_n$- 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.



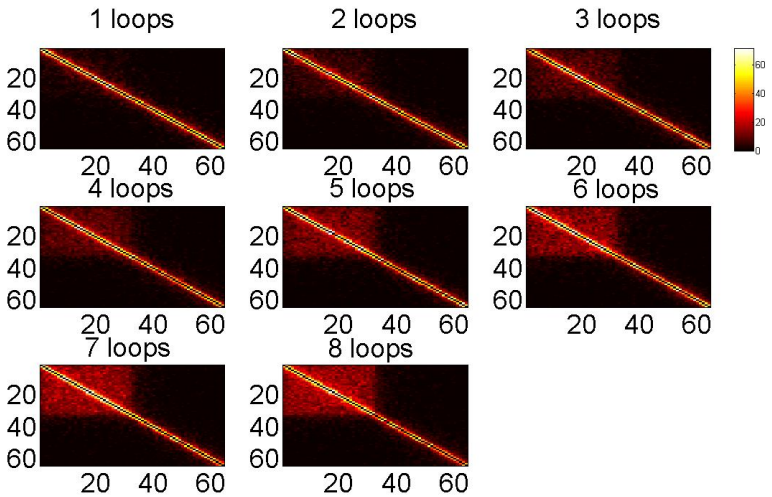
# Random Fixed Loop Model

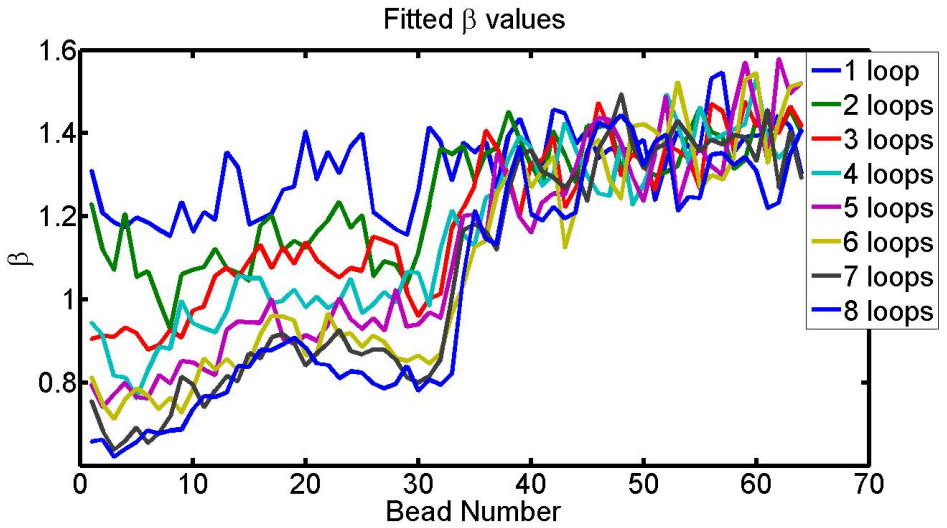
## One TAD

- 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 genomic region
- increasing number of loops at random position is simulated

# Random loops in a bounded region

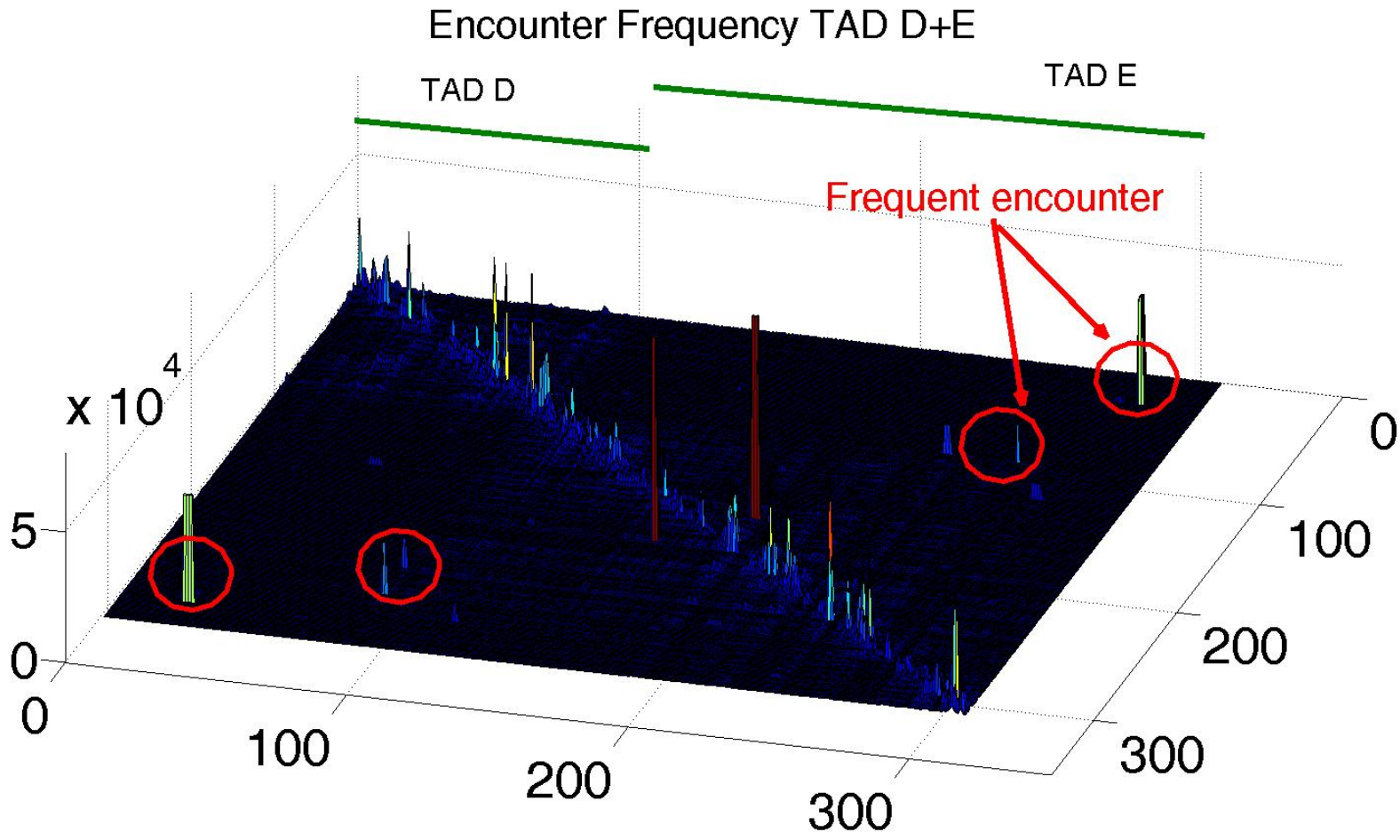
We examine the behavior of the encounter probability when we restrict the loops to be in one region of the polymer, increasing the number of loops from 1 to 8.



# peaks at the edges of the 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 shown clearly. Such peaks might indicate a stable loop in the structure of the genomic region and should be taken into account.



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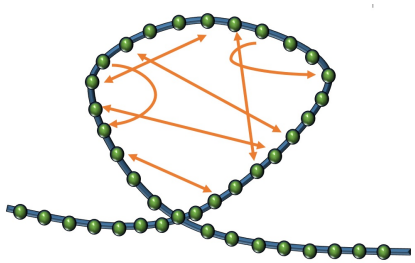

# Future perspective

- A model with variable encounter distance
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