Randomly cross-linked polymer models

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Polymer models are used to describe chromatin, which can be folded at different spatial scales by binding molecules. By folding, chromatin generates loops of various sizes. We present here a randomly cross-linked (RCL) polymer model, where monomer pairs are connected randomly. We obtain asymptotic formulas for the steady-state variance, encounter probability, the radius of gyration, instantaneous displacement and the mean first encounter time between any two monomers. The analytical results are confirmed by Brownian simulations. Finally, the present results can be used to extract the minimum number of cross-links in a chromatin region from conformation capture data.

DNA in the nucleus is constantly remodeled by regulatory factors and compacted genomic regions form transient and stable loops [1][2]. Looping frequencies, but does not contain neither directly nor any transient genomic encounter times. To reconstruct chromatin at a given scale and explore its transient properties, polymer models are used as a coarse-grained representation. The Rouse model [7], characterized by nearest neighbors interactions, predicts an encounter probability (EP) that decays with $|m-n|^{-3/2}$ between monomer $m$ and $n$, but cannot account for long-range interactions, observed inside TADs of the CC data [1][8]. Other polymer models include attractive and repulsive forces between monomers [9][10] to account for long-range interactions and have been used to probe the heterogeneous steady-state organization of the chromatin, characterized by binding molecules [11].

We study here a randomly cross-linked (RCL) polymer model used in [8] to describe the ensemble of chromatin conformation, where random cross-links are formed by binding molecules [1]. The space of RCL polymer configurations was so far explored only numerically [12][16], but no analytical formulas have been derived for studying the steady-state or transient properties and thus explore the large parameter space. We derive here novel analytical formulas for the encounter probability (EP), variance, and the radius of gyration of the RCL polymer, that we use to study the polymer dynamics. The present model can be used to determine from CC empirical EP, the minimal number of cross-links, inaccessible from CC experiments. We further study the mean first encounter time between any two monomers, which plays a key role in gene regulation [17]. Most of the asymptotic derivations are confirmed by Brownian simulations.

The RCL polymer model. We start with a linear polymer in dimension $d(d = 3)$ consisting of $N$ monomers with positions $R = [r_1, r_2, ..., r_N]^T$, connected sequentially by harmonic springs [1] and we added connectors between random non-nearest neighboring (NN) monomer pairs (Fig. 1A). The potential energy of the RCL polymer is the sum of the spring potential of linear backbone with positions $R$ and $\zeta$ is the diffusion constant, $b$ the standard-deviation of the connector between connected monomers, $k_B$ is the Boltzmann’s constant and $T$ the temperature. The ensemble $G$ is composed of $N_c$ randomly chosen indices $m, n$ among the non-NN monomers. We define the connectivity fraction $0 \leq \xi \leq 1$, as the fraction of connector numbers $N_L = \frac{(N-1)(N-2)}{2}$,

$N_c(\xi) = [\xi N_L].$ (2)

For each polymer realization, we choose $N_c$ pairs from the possible $N_L$ NN monomers. The dynamics of the resulting polymer monomers (vector $R$) is driven by Brownian motion and the field of force due to the potential energy leading to the stochastic description

$$\frac{dR}{dt} = -\frac{d}{b^2} D (M + B(\xi)) R + \sqrt{2D}\zeta \frac{d\omega}{dt},$$

where $D = \frac{k_B T}{\zeta}$ is the diffusion constant, $\zeta$ is the friction coefficient, $\omega$ are independent white noise with mean 0 and variance 1, $M$ is the $N \times N$ Rouse matrix [7]

$$M_{m,n} = \begin{cases} -\sum_{j \neq m} M_{m,j}, & m = n; \\ -1, & |m-n| = 1; \\ 0, & \text{otherwise}. \end{cases}$$ (4)
For a given \( \xi \), the square symmetric matrix \( B(\xi) \) with random connectivity is defined by
\[
B_{mn}(\xi) = \begin{cases} 
-1, & |m - n| > 1, \text{ and connected;} \\
-\sum_{j,n \neq j}^{N} B_{mj}(\xi), & m = n; \\
0, & \text{otherwise.}
\end{cases}
\]

To derive the steady-state properties of an ensemble of RCL polymers, we adopt a mean-field model where we replace the matrix \( B(\xi) \) in Eq. 3 by its average \( \langle B(\xi) \rangle \) (averaging over all configurations of non NN connected monomer pairs). We thus construct \( \langle B(\xi) \rangle \), using the probability density of the monomer connectivity. For a fixed number of connector \( N_c \), the probability that monomer \( m \) has \( k \leq (N - 2) \) non-NN connections is obtained by choosing \( k \) position in row \( m \) of the matrix \( B(\xi) \) (excluding the super- and sub- and the diagonal), and the remaining \( N_c - k \) connections in any row or column \( n \neq m \):
\[
Pr_m(k) = \begin{cases} 
\frac{C_{N_c}^k C_{N_c - k}^1}{C_{N_c}^N}, & 1 < m < N; \\
\frac{C_{N_c}^k C_{N_c - k}^1}{C_{N_c}^N}, & m = 1, N,
\end{cases}
\]
where the binomial coefficient is \( C_i^j = \frac{i!}{(i-j)!j!} \). This probability is the hyper-geometric distribution for the number of connections for monomer \( m \). The mean number of connectors for each monomer is therefore
\[
\beta_m(\xi) = \begin{cases} 
-\xi, & |m - n| > 1; \\
\beta_m(\xi), & m = n; \\
0, & \text{otherwise},
\end{cases}
\]
Using the mean values in 6, we obtain the expression for the matrix \( \langle B(\xi) \rangle \), with entries
\[
\langle B_{mn}(\xi) \rangle = \begin{cases} 
-\xi, & |m - n| > 1; \\
\beta_m(\xi), & m = n; \\
0, & \text{otherwise},
\end{cases}
\]
which can be decomposed as the sum
\[
\langle B(\xi) \rangle = \xi (N I_d - M - 1_N),
\]
where \( I_d \) is the \( N \times N \) identity matrix, and \( 1_N \) is a \( N \times N \) matrix of ones. To study the mean properties of the RCL polymer, we study the stochastic process 3 using the average matrix \( \langle B(\xi) \rangle \).

**Eigenvalues of the RCL polymer.** To study the steady-state properties of \( \langle B(\xi) \rangle \) we diagonalize the averaged connectivity matrix \( M + \langle B(\xi) \rangle \). Using Rouse normal coordinates \( U = [u_0, u_1, \ldots u_{N-1}] \) [7], defined as
\[
U = VR,
\]
where
\[
V = (\alpha_p^n) = \begin{cases} 
\sqrt{\frac{1}{N}}, & p = 0; \\
\sqrt{\frac{2}{N}} \cos \left( \frac{(n - \frac{1}{2}) \pi}{N} \right), & \text{otherwise}
\end{cases}
\]
is the Rouse orthonormal basis 7, which diagonalizes \( M \):
\[
VMV^T = \Lambda = \text{diag}(\lambda_0, \lambda_1, \ldots, \lambda_{N-1}),
\]
where
\[
\lambda_p = 4 \sin^2 \left( \frac{p \pi}{2N} \right), \quad p = 0, \ldots, N-1,
\]
are the eigenvalues of the Rouse matrix. We obtain from 3 the mean-field equations
\[
\frac{dU}{dt} = -\frac{1}{6} D \left[ \Lambda + V \langle B(\xi) \rangle V^T \right] U + \sqrt{2D} \frac{d\eta}{dt},
\]
where \( \eta = V \omega \) are independent white noises with mean 0 and variance 1. From 8, the matrix \( \langle B(\xi) \rangle \) commutes with \( M \) and therefore is diagonalizable using the same orthonormal basis \( V \):
\[
V \langle B(\xi) \rangle V^T = \text{diag}(\gamma_0(\xi), \ldots, \gamma_{N-1}(\xi)).
\]
Using 8 and 14, we obtain the eigenvalues
\[
\gamma_p(\xi) = \begin{cases} 
0, & p = 0; \\
\xi (N - \lambda_p), & 1 \leq p \leq N-1.
\end{cases}
\]
Finally, the eigenvalues of system \[ \mathbf{RCL} \] are the sum of eigenvalues of the Rouse matrix \( \mathbf{M} \) and \( \langle B(\xi) \rangle \):

\[
\chi_p(\xi) = \gamma_p(\xi) + \lambda_p = N\xi + 4(1 - \xi) \sin^2 \left( \frac{p\pi}{2N} \right). \tag{16}
\]

The system \[ \mathbf{RCL} \] is decoupled and consists of an ensemble of \( N \)-independent equations. For \( \xi = 0 \), we recover the Rouse polymer \[ \mathbf{R} \], whereas for \( \xi = 1 \), we obtain a fully connected polymer, for which all eigenvalues equal to \( N \) except for the first vanishing one. Using 16, the potential energy of the RCL polymer is written in the form

\[
\phi_\xi(U) = \frac{\kappa}{2} \sum_{p=1}^{N-1} \chi_p(\xi) u_p^2. \tag{17}
\]

The statistics of the RCL system (relation 3), can be recovered from 13 in the diagonalized form (expression 17), by scaling \( \xi \) with the ratio of mean number of random connectors to the mean of total number of connectors:

\[
\xi_* = \frac{N_c}{N + N_c}. \tag{18}
\]

We plotted in Fig.1B the eigenvalues 16 for RCL polymers, for \( N = 50 \) monomers, and \( N_c = 5, 25 \) and 50 added random connectors.

**Encounter probability (EP) between monomers of the RCL polymer.** The RCL polymer belongs to the class of generalized Gaussian chain models \[ \mathbf{G} \], for which the EP between any two monomers \( m \) and \( n \) at equilibrium is given by

\[
P_{m,n}(\xi) = \left( \frac{d}{2\pi \sigma_{m,n}^2(\xi)} \right)^\frac{1}{2}. \tag{19}
\]

To compute expression 19 we estimate the variance \( \sigma_{m,n}^2(\xi) \) in normal coordinates (Eq. 9):

\[
\sigma_{m,n}^2(\xi) = \langle (r_{m,n})^2 \rangle = \sum_{p=1}^{N-1} \left( \sigma_p^m - \sigma_p^n \right)^2 \langle u_p^2(\xi) \rangle. \tag{20}
\]

From the Ornstein-Uhlenbeck equations 13 19, we obtain the time-dependent variance of the normal coordinates

\[
\langle u_p^2(\xi) \rangle = \frac{b^2}{\chi_p(\xi)} \left( 1 - \exp \left( - \frac{2D\chi_p(\xi)}{b^2} \right) \right). \tag{21}
\]

We define the hierarchy of relaxation times \( \tau_0 \geq \tau_1(\xi) \geq \ldots \tau_{N-1}(\xi) \), with

\[
\tau_p(\xi) = \frac{b^2}{2D\chi_p(\xi)}, \tag{21}
\]

where the slowest time \( \tau_0(\xi) \) corresponds to the diffusion of the center of mass. At steady-state,

\[
\langle u_p^2(\xi) \rangle = \frac{b^2}{2(1 - \xi) \left( y(N,\xi) - \cos \left( \frac{p\pi}{N} \right) \right)} \tag{22}
\]

where

\[
y(N,\xi) = 1 + \frac{N\xi}{2(1 - \xi)}. \tag{23}
\]

Replacing 10 and 22 into 20 we get

\[
\sigma_{m,n}^2(\xi) = \frac{b^2}{N(1 - \xi)} \sum_{p=0}^{N-1} \left( \cos \left( \frac{p(m - n)\pi}{N} \right) - \cos \left( \frac{(p + n - 1)m\pi}{N} \right) \right)^2 y(N,\xi) - \cos \left( \frac{p\pi}{N} \right) \right]. \tag{24}
\]

For \( N \gg 1 \), the sum \[ 24 \] is computed in the complex plane using the contour of the unit disk parameterized by \( z = e^{ix} \)

\[
\sigma_{m,n}^2(\xi) = \frac{-b^2}{4\pi i(1 - \xi)} \oint_{|z|=1} \left( z - \zeta(0,N,\xi) \right) \left( z - \zeta(1,N,\xi) \right) \left( z^{2(m+n)+1} \right) \tag{25}
\]

where

\[
\zeta_0(N,\xi) = y(N,\xi) + \sqrt{y^2(N,\xi) - 1}, \quad \zeta_1(N,\xi) = y(N,\xi) - \sqrt{y^2(N,\xi) - 1}. \tag{26}
\]

When \( \zeta_0(0,0) = 1 \), we recover the variance \( \sigma_{m,n}^2(0) = b^2 \left[ m - n + 1 \right] \) of the Rouse chain (\( N_c = 0 \)). The integrand in \[ 24 \] is symmetric in \( m \) and \( n \) and has a pole of order \( 2(m+n)+1 \) at \( z = 0 \) and simple poles at \( z = \zeta_0(N,\xi) \), \( z = \zeta_1(N,\xi) \). Because \( y(N,\xi) \geq 1 \), we have \( \zeta_0(N,\xi) \geq 1 \), which is outside of the unit disk \( |z| = 1 \), and \( \zeta_1(N,\xi) \leq 1 \), for all \( N, \xi \geq 0 \). The pole \( \zeta_0(N,\xi) \) is not inside the disk and does not contribute in the calculation of the residues of \[ 24 \]. For \( \xi < 1 \), we obtain an exact expression for the variance

\[
\sigma_{m,n}^2(\xi) \approx \frac{b^2}{N\xi} \left( 1 - \exp(-|m-n|\sqrt{N\xi}) \right). \tag{26}
\]

Using 25 and 19 we obtain a novel expression for the steady-state encounter probability \( P_{m,n}(\xi) \) between any two monomers. We compare the EP obtained from Brownian simulations of RCL polymer for \( N = 20, 50 \) with the analytical formula 19 for \( N_c = 25 \) connectors (Fig. 2a), which shows a very good agreement.

**Mean square radius of gyration (MSRG) of the RCL polymer.** The MSRG \( \langle R_g^2(\xi) \rangle \) characterizes the size of the RCL polymer and can be computed from the variance 26 as

\[
\langle R_g^2(\xi) \rangle = \frac{1}{N^2} \sum_{m=1}^{N} \sum_{n=1}^{m} \sigma_{m,n}^2(\xi). \tag{27}
\]

Using 25 and 27 with the notations \( \zeta_0 = \zeta_0(N,\xi), \quad \zeta_1 = \zeta_1(N,\xi) \), we obtain

\[
\langle R_g^2(\xi) \rangle = \frac{b^2}{N^2(1 - \xi)} \left[ \frac{(1 + 2\zeta_0)N(1 + N)}{2\zeta_0} \right] \tag{28}
\]

\[
+ N(2(1 + \zeta_0)^2 - \zeta_0^2) \frac{\zeta_0(1 - \frac{1}{G_0})}{(1 - \zeta_0^2)^2} + \frac{2(1 + \zeta_0)(1 - \frac{1}{G_0})}{(1 - \zeta_0^2)} \right].
\]
Mean First Encounter Time (MFET) $\langle \tau^e(\xi) \rangle$ between monomers of the RCL polymer. We compute here the mean time for two monomers of the RCL polymer to enter for the first time in a ball of radius $\epsilon > 0$, at which they can possibly interact to form a chemical bond (Fig. 3(a)). The MFET for both the Rouse and beta polymer were computed (see [20]) from the first eigenvalue $\lambda_0^e$ of the Fokker-Planck operator associated to the stochastic equation [19] so that

$$\langle \tau^e(\xi) \rangle \approx \frac{1}{D\lambda_0^e(\xi)}. \quad (35)$$

The first order approximation in $\epsilon$ is given by [20]

$$\lambda_0^e(\xi) = \frac{4\pi \epsilon}{\int_{C-P} e^{-\phi_\xi(U)} dU} \frac{e^{-\phi_\xi(U)}}{|\hat{\Omega}(\xi)|} + O(\epsilon^2), \quad (36)$$

where $\phi_\xi(U)$ is the diagonalized potential [17] $|\hat{\Omega}(\xi)|$ is the integral over the entire RCL configuration space

$$\hat{\Omega}(\xi) = \int e^{-\phi_\xi(U)} dU = \left( \frac{2\pi N^{-1}}{\prod_{p=1}^{N-1} \kappa \chi_p(\xi)} \right)^{\frac{1}{2}}. \quad (37)$$

The integral over $C - P$ in [36] is computed over the space of closed RCL polymer ensemble, with fixed connector between monomers $m$ and $n$ and additional $N_c(\epsilon)$ random connectors. A direct computation gives

$$\int_{C-P} e^{-\phi_\xi(U)} dU = (2\pi)^{\frac{N-1}{2}} \left( \frac{\kappa b^2 \prod_{p=1}^{N-1} \kappa \chi_p(\xi)}{\sigma_{m,n}^2(\xi)} \right)^{\frac{1}{2}}. \quad (38)$$

Using relations [37] and [38] in [35] we obtain the MFET between any two monomers $m$ and $n$ of the RCL polymer for a given connectivity fraction $\xi$ in dimension $d = 3$:

$$\langle \tau^e_{m,n}(\xi) \rangle \approx \frac{1}{4\pi D \epsilon} \left( \frac{2\pi \sigma_{m,n}^2(\xi)}{\kappa b^2} \right)^{\frac{1}{2}}, \quad (39)$$

Using [26] into [39] we obtain the approximation

$$\langle \tau^e_{m,n}(\xi) \rangle \approx \frac{b^2 (1 - \exp(-lm + n\sqrt{N})) d/2}{4\sqrt{N} \pi D \epsilon (\kappa b^2) d/2} + O(N\xi),$$

For $N\xi \gg 1$, the MSD behaves like

$$\langle r^2_m(t) \rangle \approx \frac{db\sqrt{dDt}}{2\pi(1 - \xi)}. \quad (33)$$

We conclude that the homogeneous behavior of MSD for the RCL polymer model gives an anomalous exponent $\alpha = 0.5$, similar to the Rouse model. Finally, for long time scales ($t \gg \tau_1(\xi)$), (slow diffusion of the polymer’s center of mass), the error function in [31] is almost constant and therefore

$$\langle r_m(t)^2 \rangle = 2dD_{cm} t + \frac{db^2}{2\pi N\xi(1 - \xi)}. \quad (34)$$
FIG. 3. Transient properties of the RCL polymer (a) Two monomers $m$ (red) and $n$ (purple) of the RCL polymer meet when they enter a sphere of radius $\epsilon$. Random connectors (dashed arrows) are added to a linear Rouse backbone (b) Stochastic simulations (marked by a dot) MFET between monomer 1 and monomers 2-20 of RCL polymers with $N = 20$ (blue), 50 (yellow) and 100 (green) monomers, with $N_c = 25$ random connectors, are in good agreement with the analytical formula (Eq. 39) dashed). Parameters: $\epsilon = b/10, D = 1, b = \sqrt{3}, \Delta t = 0.01s$, the RCL system is $3$ (we used Eq. 39 with $\xi^*$, Eq. 18).

Applications of the RCL polymer model. We derived here several analytical formula for the steady-state variance, encounter probability, the radius of gyration, mean-square displacement and the mean first encounter time of the RCL polymer model. These formula can be used to extract parameters from chromatin conformation in CC experiments [11,12]. In particular, using formula [19] it is possible to fit the empirical encounter probability obtained from experimental data to extract the connectivity fraction $\xi$. This parameter has a direct interpretation and represents the mean number of cross-links, that can be mediated by CTCF molecules present in a genomic region. The parameter $\xi$ depend on the coarse-grained scale (see [8]). The extracted parameter $\xi$ can then be used to estimate the radius of gyration (Eq. 28) of any region of interest. This radius characterizes the size of the genomic region, at least relative to other genomic segments, hence providing insightful information about the local organization of the chromatin in the cell nucleus.

To demonstrate out methodology, we coarse-grained the 5C data reported in [1] of male neuronal progenitors NPC-E14 cells, replicate 1, TAD H, containing 679 kbp, at a scale of 3kbp, resulting in $N = 226$ monomers. We fit the EP (Eq. 19) to 5C data of each of the 226 monomers and obtain the average connectivity $\xi = 0.0022$, corresponding $N_c = 56$ added connectors. We use the persistence length of $b = 0.05 \mu m$. Substituting $N = 226, b = 0.05, \xi = 0.0022$ in Eq. 28 we compute the radius of gyration to be 43 nm for TAD H. Thus, the 679 kbp TAD H is compacted in a sphere of volume $3.4 \times 10^5 \text{ nm}^3$ (2 bp per $\text{nm}^3$).

The structural information extracted from the static CC maps using the RCL polymer model was recently used to interpret the dynamics of single particle trajectories (SPT) [21,22]. By fitting the MSD (Eq. 31) to SPT data and by extracting the degree of connectivity $\xi$, we interpret the mean deviation of the loci dynamic from pure diffusion as the confined dynamics of the loci in a cross-links genomic environment [8,21,25]. We provided a direct formula to extract $\xi$, so that the simulations of [23] can now be bypassed. Finally, once the connectivity fraction is extracted, the mean first encounter time between any two monomers can be computed using formula [39]. Encounter times are key for understanding processes, such as mammalian X chromosome inactivation [1] or non-homologous-end joining after DNA double-strand break [20,23].

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