Anomalous diffusion of tracer particles in polymer globule: possible model of nuclear diffusion

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Motivated by recent observation of subdiffusion in different part of living cell, and in particular in dense nuclear compartments, we model the diffusion of tracer particles in dense polymer globule. For certain values of parameters the subdiffusion is observed. We perform further analysis of the data to discriminate between three possible scenarios: diffusion of fractal, continuous time random walk (CTRW) and fractional Brownian motion (FBM). We get zero fractal dimension, effectively ruling out the possibility of fractal origin of subdiffusion, being unable to discriminate between remaining two possibilities. We discuss remaining two scenarios as well as possible further simulations that could lead to better understanding of the diffusion in a polymer globule and connecting simulations to real experiments.

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I. INTRODUCTION

In a recent decade single particle tracking experiments became available. This gave another tool for investigation of molecular organization of cytoplasm and nucleus in a cell. In particular, many experiments [1–4] observe subdiffusive motion of a tracer particles in cytoplasm as well as in nucleus and nucleoli.

Subdifusion, as a deviation from diffusion law of linear scaling of mean square displacement (MSD) with time can be observed in a variety of different setups. The most frequently discussed scenarios are diffusion in a *fractal environment*, *continuous time random walk* (CTRW) with wide distribution of waiting times and *fractional Brownian motion* (FBM). In particular, diffusion in the nuclear compartments of the living cell, as measured by Bancaud *et. al.* [1] is attributed to the fractal organization of hetero- and euchromatin.

In the present Project we attempt to perform similar experiment *in silico*. We do not try to describe cell environment in a realistic way by modeling all possible cytoplasmic ingredients (e.g. in a spirit of [5]). Instead of this we hope to capture essential features of heterochromatin by a dense polymer chain with steric interactions. We show that motion of tracer particles in such environment leads to subdiffusion for certain parameters of the system. We investigate the probability distribution of MSD, and use so-called maximal excursion method [6]. This gives us more information about origin of subdiffusive behavior, and allows us to exclude fractality from the possible origins of the subdiffusion (more exactly, we get an estimate for fractal dimension to be zero).

The organization of this Project paper is following. We start by discussing basic theory behind diffusion in fractal environment and other scenarios of anomalous diffusion, mostly following along the lines of book [7] and recent paper [6]. After this, we review experiment by Bancaud *et. al.* [1] that serve as a motivation for this Project. Finally, we describe setup used for simulation and interpret our results. The paper is concluded with brief discussion of results and proposing further simulations.

II. THEORY

In this section we briefly describe three possible setups where one encounters subdiffusion. We introduce basic notions relevant for diffusion of fractals [7], and discuss recently proposed way to discriminate between different subdiffusion scenarios [6].

The diffusion process in d dimension is typically characterized by mean square displacement,

$$MSD = \langle r^2(t)\rangle = 2dDt^{\alpha}, \tag{1}$$

where α is diffusion exponent. If $\alpha = 1$, we encounter normal Brownian diffusion, whereas for $\alpha < 1$ the process is referred to as a subdiffusion. Below we describe three different models that all ultimately lead to Eq. (1).

A. Diffusion on fractals

Diffusion in any integer dimension is very simple and well-studied phenomena, with mean square displacement (MSD) scaling proportional to time,

$$\text{MSD} = 2dDt. \tag{2}$$

Naively, one may think of a fractal to be a space with non-integer dimension, and expect Eq. (2) to hold for this case as well. However, in reality, relation (2) is violated due to inherent *non-Markovian* property of diffusion in fractals. For fractal the correlation between displacements at different steps, $\langle \delta \boldsymbol{r}_i \cdot \delta \boldsymbol{r}_j\rangle \neq 0$ is non-zero, as it used to be for diffusion in $\mathbb{R}^d$ space. For diffusion in fractals MSD is given by

$$\text{MSD} = 2dDt^{2/d_w}, \tag{3}$$

where we defined diffusion exponent $d_w \equiv 2/\alpha$. It is $d_w = 2$ for regular diffusion, and $d_w > 2$ for subdiffusion. Diffusion exponent d_w, along with d_f – fractal dimension of the space where diffusion occurs, constitute two main exponent that are relevant for describing diffusion in fractal. Depending on the relation between d_w and d_f,

diffusion can be *compact* ($d_w > d_f$, all sites are visited) or *non-compact* ($d_w < d_f$) [8].

Third exponent that is commonly used is the spectral exponent d_s (fracton dimension). It is not independent and is expressed through diffusion exponent and fractal dimension: $d_s = 2d_f/d_w$. Alternatively, d_s can be defined as a scaling exponent for the *number of distinct sites* visited by a walker,

$$S(t) \sim t^{d_s/2}, \tag{4}$$

which makes obvious its connection to kinetic rates (see [9]). For the incipient percolation cluster Alexander-Orbach conjecture suggests $d_s = 4/3$, that agrees very well with experiments. However, for general fractals no general value for d_s can be established.

Finally, the scaling form of the probability distribution $P(r,t)$ will be relevant for us in what follows. For fractals, one can argue that it has the following form

$$P(r,t) = t^{-\alpha d_f/2} P\left(\frac{r}{t^{\alpha/2}}, 1\right). \tag{5}$$

B. Continuous Time Random Walk and Fractional Brownian Motion

CTRW and FBM are two additional scenarios that could also lead to subdiffusion. CTRW is described as a process of RW with random waiting times. Provided the distribution of the waiting times has the long-tailed form $p(t) \sim 1/t^{1+\alpha}$, $0 < \alpha < 1$, so that mean waiting time diverges, the resulting process becomes subdiffusive with diffusion exponent α.

FBM bases on a different physical mechanism: it accounts for the correlation in a random walk. Namely, the state at time t is influenced by state at time $t' < t$, what is achieved by changing Gaussian white noise into fractional Gaussian white noise.

C. Mean Maximal Excursion Method

Finally, we briefly discuss a recent proposal of Tejedor *et. al.* [6] that, in principle, should allow to discriminate between fractal, CTRW, and FBM mechanisms of subdiffusion. In addition to studying MSD and its moments, Tejedor *et. al.* propose to analyze the distribution of maximal excursion distances. One can define the probability $\Pr(r_{\max} = r_0, t)$ for particle to reach *maximal distance* r_0 during time t and study its moments, e.g.

$$\langle r^2_{\max}\rangle = \int_0^\infty dr_0\, r_0^2 \Pr(r_{\max} = r_0, t) = \mathcal{D} t^{\alpha_{\max}}, \tag{6}$$

$$X_{\max} = \langle r^4_{\max}\rangle / \langle r^2_{\max}\rangle^2, \tag{7}$$

where $\alpha_{\max}$ is defined to be MME exponent. (Note that corresponding quantities without index "max" refer to MSD and its moments.)

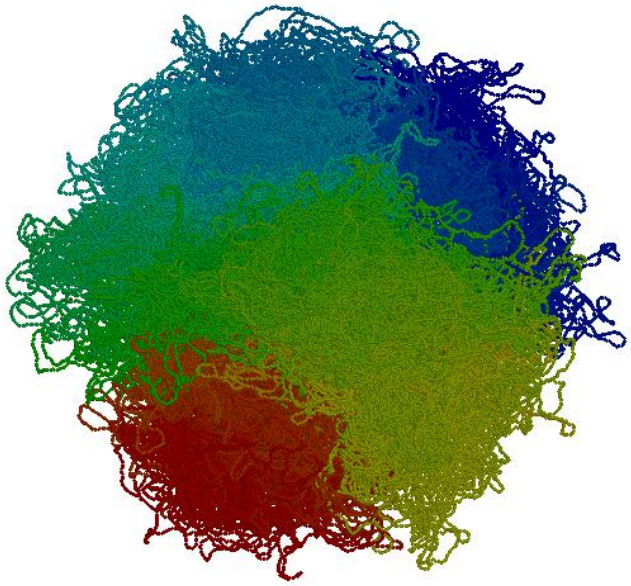

FIG. 1. Initial state of polymer: dense polymer on a three-dimensional torus with a circumference $l = 210$ Å. The opposite faces of the cube have to be identified, so that spreading of polymer means self-penetration. Coloring goes from blue to red along the length of the chain.

Tejedor *et. al.* argue that using values of α, $\alpha_{\max}$, X, and $X_{\max}$, one can discriminate different mechanisms leading to subdiffusion. FBM is characterized by (for $d = 3$)

$$\alpha \neq \alpha_{\max}, \quad X = 5/3, \quad X_{\max} < 1.36. \tag{8}$$

Whereas for fractals

$$\alpha = \alpha_{\max}, \quad X < 5/3, \quad X_{\max} < 1.36, \tag{9}$$

and for CTRW

$$\alpha = \alpha_{\max}, \quad X > 5/3, \quad X_{\max} > 1.36. \tag{10}$$

Finally, fractal subdiffusion differs from CTRW and FBM by the *growing spheres scaling*: from Eq. (5) one can deduce the probability for particle to be inside a sphere of growing radius $r_0 t^{\alpha/2}$ to be

$$\Pr_{\leq}(t) \equiv \Pr(r \leq r_0 t^{\alpha/2}) = A(r_0) t^{\alpha(d-d_f)/2}. \tag{11}$$

Thus, if $\Pr_{\leq}(t)$ is constant in time, this suggests $d_f = d$ that can be interpreted as the absence of fractal structure.

III. DIFFUSION *IN VIVO*: EXPERIMENT

We briefly review the seminal paper by Bancaud *et. al.* [1]. Authors find anomalous diffusion in the living cell and attribute it to the fractal organization of dense

nuclear compartments. The study goes along three independent lines of evidence: Bancaud *et. al.* investigate volume exclusion effects, qualitative change of diffusion, and change in reaction kinetics. Leaving aside volume exclusion and reaction kinetics, we primarily concentrate on diffusion, as the most interesting for us.

First, Bancaud *et. al.* study the diffusion of Green Fluorescent Proteins (GFP) multimers by Fluorescent Correlation Spectroscopy (FCS). This allows one to extract values diffusion exponent $\alpha = 0.79 \pm 0.02$ and $\alpha = 0.75 \pm 0.07$ for euchromatin and heterochromatin correspondingly. For euchromatin, change in diffusion coefficient compared to water $D_{\text{euchr}}/D_{\text{water}} \approx 0.3$ is almost size independent. The absence of dependence of measured quantities on the size of diffusing particles is interpreted as an evidence for fractal diffusion.

Next, tracking of (aggregates of [10]) Quantum Dots (QD) is performed. For euchromatin the subdiffusive motion *at short timescales* is observed with $\alpha = 0.73$. At longer timescales motion changes its character to purely diffusive. The crossover happens at timescale $\Delta t = 20$ ms: for longer times fits within standard diffusion model are said to be accurate. Using Δt authors estimate the upper fractal scale to be $H \sim D\delta t \sim 100$ nm. Given α that is obtained from MSD fitting, fractal dimension is found to be $f = 2.61 \pm 0.15$ from fitting histograms for $p(\Delta r, \Delta t_1)/p(\Delta r, \Delta t_2)$ for $\Delta t_{1,2} < \Delta t$.

In case of heterochromatin, fractal dimension can be obtained from measured fractal exponent (governing kinetic rates), ε that can be related to fracton dimension (see Eq. (4) and Ref. [9]), $\varepsilon = 1 - d_s/2$. Resulting value of fractal dimension is $d_f \approx 2.2 \pm 0.2$. Upper scale of self-similarity, H, can be estimated to be $H \sim 60$ nm by Flory-like argument $H \sim c^{-3/4} \sim N^{3/4}$, using $n_{\text{hetero}}/n_{\text{eu}} \sim 2$ and upper scale of self-similarity for euchromatin.

To summarize, Bancaud *et. al.* conjecture fractal structure of eu- and heterochromatin with upper and lower self similarity limits $H = 100$ nm, $h \sim 3$ nm [11], and estimate fractal dimension and diffusion exponents of these fractals.

IV. DIFFUSION *IN SILICO*: MOLECULAR DYNAMICS SIMULATIONS

In this section, after briefly describing the protocol of simulations, we present the results of MSD fitting as well as of MME fitting and their interpretation.

A. Protocol

The simulation was done using OpenMM package for molecular dynamics simulations on videocards by Simbios group at Stanford.

The polymer consisted of $N_{\text{poly}} = 150,000$ monomers of "radius" $R_{\text{poly}} = 1.5$ Å joined together. Initial configuration of polymer was obtained as a self-avoiding random walk, after this it was contracted by attractive potential, so that it formed fractal globule [12]. After this external potential was relaxed and periodical boundary conditions were imposed in all three dimensions, so that polymer effectively lived on a three-dimensional torus, Fig. 1. This was done in order to avoid boundary-related effects. The final state of the polymer was dense, with polymer taking approximately 1/2 of the total volume of the system. Finally, $N_{\text{tr}} = 27$ tracer particles were added to the systems. The evolution of the system was done, using Langevin dynamics at a room temperature $T = 300$ K and scattering rate $\gamma = 0.4$ ps^{-1}. After every interval Δt ($\Delta t = 1.3$ ps, unless otherwise specified) snapshots of position of all the particles were taken.

Masses of tracers and *polymer particles*, as well as the *radius of tracers* were varied while the diffusion of tracer particles was studied. As a result we obtained a set of data, only some of which will be presented here. In addition, the "test experiment" was performed, when all bonds between polymer particles were relaxed, and we observed a diffusion of tracer in a liquid consisting from (former) polymer particles. In what follows we will refer to our experimental setups using Latin numbers as follows:

- **i** "test experiment": polymer is relaxed, all parameters are the same as in **ii**.
- **ii** big heavy tracer diffusing in light polymer, $R_{\text{tracer}} = 10R_{\text{poly}}$, $M_{\text{tracer}} = 1000$ amu, $M_{\text{poly}} = 100$ amu
- **iii** big light tracer, diffusing in (ultra) heavy polymer, $R_{\text{tracer}} = 10R_{\text{poly}}$, $M_{\text{tracer}} = 100$ amu $M_{\text{poly}} = 10000$ amu,
- **iv** small heavy tracer diffusing in light polymer, $R_{\text{tracer}} = R_{\text{poly}}$, $M_{\text{tracer}} = 1000$ amu, $M_{\text{poly}} = 100$ amu,

and we remind that "radius" of polymer $R_{\text{poly}} = 1.5$ Å, and all radii are defined as a parameters in the repulsive part of Van der Waals potential, $U_{12} \sim [(R_1+R_2)/R_{12}]^{12}$.

B. MSD fitting

Having specified the system, we proceed with scaling of MSD with time. Results for MSD as a function of time are presented as a log-log plot in Fig. 2. From the plot one can see that normal diffusion seems to realize for **i** and **iv** setup, whereas for **ii** and **iii** setups diffusion is slower than normal. This is confirmed by the values of α (see Eq. 1), presented in Table I. We see that our "test experiment" **i** reproduces normal diffusive scaling very well, with α being almost exactly one. Value of α being relatively close to one, has also been obtained for **iv** setup, where heavy and relatively small tracers were diffusing in a polymer. Remaining two cases, **ii** and

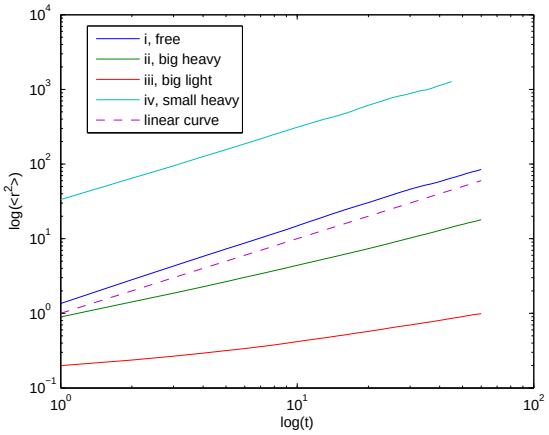

FIG. 2. Double logarithmic plot of MSD as a function of time reveals deviation from normal diffusion for **ii** and **iii** setups. Pink dotted curve represents scaling for normal diffusion, $\langle r^2\rangle \propto t$ (i.e. $\alpha = 1$). Green (**ii**) and red (**iii**) curves clearly have different scaling, moreover α changes over time. The time step here is $10\Delta t$, MSD is plotted for times up to $600\Delta t$.

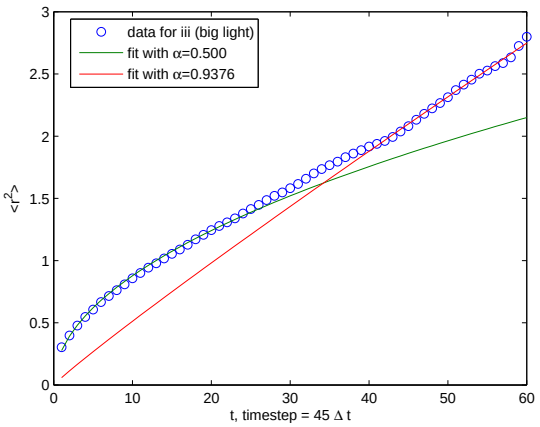

FIG. 3. Plot of of MSD as a function of time for **iii** setup reveals change in scaling exponent α from $\alpha = 0.5$ to $\alpha = 0.94$ for large time. Timestep is $45\Delta t$, MSD is plotted for times up to $2700\Delta t$.

iii show strong deviation from normal diffusive scaling, with scaling exponent growing with time. Fig. 3 displays plot for **iii** case, where one can clearly see that over time interval of $2700\Delta t$, exponent α changes from ≈ 0.5 to ≈ 1. Therefore, we conclude that *on a larger timescales diffusion becomes normal.*

In addition to scaling of MSD we study higher moments of probability distribution $P(r,t)$. We consider the ratio $X = \langle r^4\rangle/\langle r^2\rangle^2$, that is equal to $5/3 \approx 1.666$ for the three-dimensional Brownian motion. Behavior of the ratio $X = \langle r^4\rangle/\langle r^2\rangle^2$ is shown in Fig. 4. Results of fitting are presented in the third column of Table I. Fitting the case **iii** for small and large timescales gives different values, $X = 1.86$ and $X = 1.61$ respectively, giving additional support that diffusion at larger timescales gradually tends to normal.

C. MME fitting

Having studied the MSD scaling and distribution, we turn to mean maximal excursion method [6], described in more details in Sec. II C.

As for MSD, first we find scaling exponent for $\langle r^2_{\max}\rangle$, that appears to be very close to the MSD exponent α (compare columns 2 and 4 in Table I).

Fitting of $X_{\max} = \langle r^4_{\max}\rangle/\langle r^2_{\max}\rangle^2$ (note, that here we used large timescales) gives us values close to theoretical value 1.36 for the normal diffusion, in all cases, except for the **iii** system. In the latter case the value obtained for medium timescale is $X_{\max} = 1.2477$, whereas for maximum available timescales $\alpha_{\max} = 0.5544$ and $X_{\max} = 1.15$.

Finally, we perform growing spheres test (see Sec. II C),

System	α	X	$\alpha_{\max}$	$X_{\max}$	$\Pr_{\leq}$
RW	1.	1.6666	1.	1.36	const
i	0.9999	1.6358	1.0753	1.3540	—
ii	0.7540	1.7408	0.7716	1.3325	const
iii	0.4335	1.8624	0.4682	1.2477	const
iv	0.9562	1.7463	0.9690	1.3429	—

TABLE I. Summary of data analysis of tracers trajectories. α is diffusion exponent, $X = \langle r^4\rangle/\langle r^2\rangle^2$, $\alpha_{\max}$ is defined in Eq. (6), $X_{\max} = \langle r^4_{\max}\rangle/\langle r^2_{\max}\rangle^2$. First row represents theoretical predictions for Brownian random walk in $d = 3$ dimensions. All other numbers are obtained from fitting simulation data.

in order to find out if subdiffusion originates from fractal environment. Plot of the probability $\Pr_{\leq} \equiv \Pr(r \leq r_0 t^{\alpha/2})$ (see Eq. (11)) is shown in Fig. 5. We see that this probability is constant except for the very small times, thus giving us the fractal dimension $d_f \approx 0$. This can be interpreted as *evidence against fractal origin of observed subdiffusion.*

Finally, collected all data into Table I we try to apply criteria Eq. (8)-(10). Since we ruled out the possibility of fractal diffusion, and $\alpha \approx \alpha_{\max}$ one could suggest CTRW as possible scenario. However, the condition for $X_{\max}$ in Eq. (10) does not hold for (mostly interesting) setups **ii** and **iii**. In summary, available data does not allow us to clearly identify the mechanism leading to subdiffusion. Nor do the physical reasoning: one could equally imagine trapping of particle into "holes" inside the polymer with long waiting times (leading to CTRW), or presence of correlation in random walk due to interactions with polymer (FBM), or finally some exotic fractal organiza-

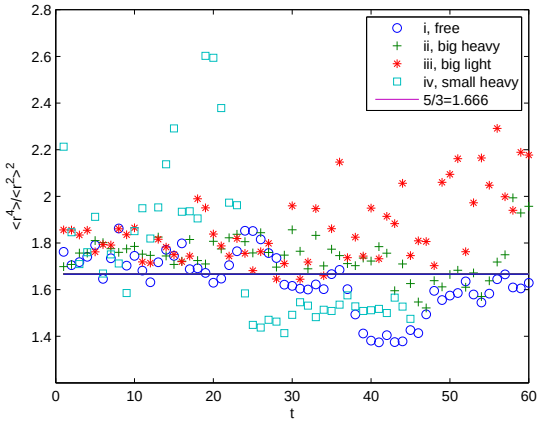

FIG. 4. $X = \langle r^4 \rangle / \langle r^2 \rangle^2$ as a function of time. The blue line indicates theoretical value obtained from Gaussian distribution.

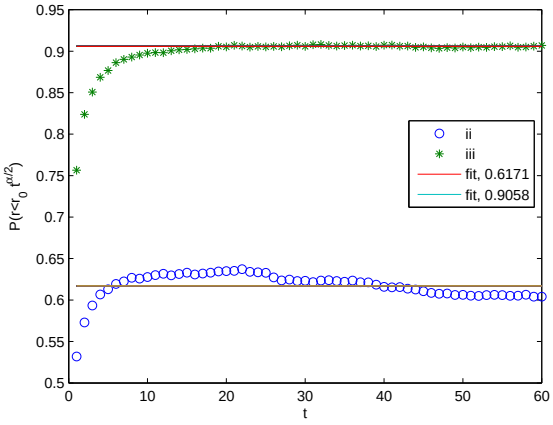

FIG. 5. Constant behavior of the probability of encountering the particle in the growing sphere $\Pr(r \leq r_0 t^{\alpha/2})$ as a function of time rules out the fractal origin of subdiffusion in polymer globule. Plot shows results for two setups: **ii** and **iii**, as well as corresponding fits.

tion of globule with $d_f \approx 0$ leading to fractal diffusion.

V. DISCUSSION

To conclude, motivated by experiment [1], we performed simulations of tracers diffusion in polymer *in silico*. Analyzing the data we indeed discovered the subdiffusion for certain values of parameters. More careful study of particle trajectories ruled out the possibility of diffusion due to fractal structure of available space. However, available data does not give possibility to discriminate between CTRW and FBM scenarios of subdiffusion.

There remains many other parameters in our system that have not been explored in this study. In particular, studies of diffusion for *different polymer densities* (note, that densities much higher than $\sim 50\%$ are not accessible due to divergencies occurring in simulations) could possibly contribute to our understanding of diffusion in a polymer. Also, diffusion in a polymer with *different organization* is of great interest. We expect that equilibrium globule (i.e. polymer that is allowed to cross itself in the process of equilibration [12]) should obstruct diffusion more strongly, and, possibly lead to different behavior. Hopefully exploration of all these possibilities could lead to better understanding of current setup and possibly its connection to the experiments in the nuclear compartments of living cells.

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