Understanding biochemical processes in the presence of sub-diffusive behavior of biomolecules in solution and living cells

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Abstract

In order to maintain cellular function, biomolecules like protein, DNA, and RNAs have to diffuse to the target spaces within the cell. Changes in the cytosolic microenvironment or in the nucleus during the fulfillment of these cellular processes affect their mobility, folding, and stability thereby impacting the transient or stable interactions with their adjacent neighbors in the organized and dynamic cellular interior. Using classical Brownian motion to elucidate the diffusion behavior of these biomolecules is hard considering their complex nature. The understanding of biomolecular diffusion inside cells still remains elusive due to the lack of a proper model that can be extrapolated to these cases. In this review, we have comprehensively addressed the progresses in this field, laying emphasis on the different aspects of anomalous diffusion in the different biochemical reactions in cell interior. These experiment-based models help to explain the diffusion behavior of biomolecules in the cytosolic and nuclear microenvironment. Moreover, since understanding of biochemical reactions within living cellular system is our main focus, we coupled the experimental observations with the concept of sub-diffusion from in vitro to in vivo condition. We believe that the pairing between the understanding of complex behavior and structure-function paradigm of biological molecules would take us forward by one step in order to solve the puzzle around diseases caused by cellular dysfunction.

Keywords Anomalous diffusion · Macromolecular crowding · Protein stability · Conformational equilibria · Protein–protein interaction · Biological functions · Gene expression

Introduction

The cellular system is complex comprising of harmonious interaction of different organelles to achieve different cellular processes. Cells have several housekeeping biomolecules, which are constitutively expressed inside them, whereas other biomolecules traverse across the cellular membrane based on necessity. Each of these biomolecules have their own characteristic functions and properties which help maintaining cellular homeostasis. Irregularities in interaction patterns between

these biomolecules may give rise to cellular discrepancies which might lead to the manifestation of disease conditions. Progressively, in order to design inhibitors for these diseases, the conceptualization of the fundamentals of the associated biochemical reactions is essential. The cellular functions depend on the availability of active biomolecules at the right reaction sites and time in order to complete biochemical reactions. The effects of the surrounding neighbors facilitate these required molecules to diffuse to the desired site and it helps to obtain these diffusion-limited biochemical reactions. Cell signaling (Bu and Callaway 2011), transport (Fulton 2011), gene expression (Levsky et al. 2002), etc. are the few examples where main cell constituents; multiple proteins interact with DNA, RNA, or with each other in order to carry out cellular functions. Interestingly, advancements in science in the form of CRISPR/Cas9 (Wang et al. 2016b), phase transitions (Shin and Brangwynne 2017), mRNA count to understand gene expression (Chen and Larson 2016), single molecule studies of translation, transcription (Prabhakar et al. 2019), DNA repair (Uphoff et al. 2013), and replication (Tanner and van Oijen 2010) enable us to visualize these cellular events more

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closely from their neighboring backgrounds. However, there still remains a lack of comprehensive understanding regarding the mathematical perspective of interpreting these phenomena. The main reason for this can be attributed to the unavailability of proper mathematical models and limiting the reactions to just in vitro conditions without extrapolation to in vivo conditions.

The specific and non-specific interactions of biomolecules with each other inside a cellular system cannot be explained simply by simple Brownian motion. This is because, their chemical properties, interaction specificity, mostly affect their diffusion in cellular space and thus it governs the association and dissociations. For example, in a cell when RNA and RNA binding proteins interact, they form droplets more dense than the cellular cytosol. This in turn hinders the diffusion of the RNA–protein complex (Lampo et al. 2017) in the cell cytoplasm and successively influences the cellular function. Naturally under this heterogeneous condition, the normal diffusion model given by Einstein (Einstein 2005) and Smoluchowski (Smoluchowski 1907) in the year of 1905 cannot be implemented. Similarly, the non-monotonic diffusion of RNA polymerase over a DNA strand (Jülicher and Bruinsma 1998) is a consequence of specific interactions and the participation of several transcription factors (Liu and Tjian 2018) and are not explainable by simple diffusion. This makes the whole system extremely complex, and far different from the simple motions that have been modeled as observed in solutions. Sometimes, their residence times are so large that the distribution of their diffusion coefficients deviates completely from Gaussian distribution. Due to these shortcomings, over time, several mathematical models were proposed to explain cellular events which cannot be understood from the simple Brownian motions. Some of the models created for understanding biochemical reactions include fraction of Brownian motion (Wyłomańska et al. 2016), fraction of Langevin equation (Wyłomańska et al. 2016), continuous time correlated random walk (CTRW) (Chechkin et al. 2009), Lorentz model (Höfling and Franosch 2013), and Gaussian model of fractal (Shin and Brangwynne 2017); these have been popularly used to track the sub diffusion of the biomolecules within the cell. In this review, our major focus would be to explain the biochemical reactions with their complexities in the light of different aspects of anomalous diffusion. In this process, we will discuss briefly the evolution of our knowledge from simple Brownian diffusion to more complex diffusion processes. The improvisations where fractional Brownian motion contains memory law power with time, CTRW deals with the distribution of waiting time instead of distribution of diffusion time, and Lorentz model allows the concept of molecule diffusion in a disorder medium are embraced to frame the diffusion controlled biochemical reactions within cell. Readers who are interested in more detailed studies of mathematical expression and their derivations could go refer to

these literatures (Höfling and Franosch 2013; Metzler et al. 2014).

To validate these models, experimental evidence is essential; however, the problem with ensemble-averaged experiment is that the noise created by these sub-diffusive natures is merged within the observations. To fish out these noises, different biophysical tools such as NMR (Fan and Gao 2015) and single-molecule fluorescence spectroscopy (Schwille et al. 1999; Seisenberger et al. 2001; Dix et al. 2006) have been used. The spatial and temporal details in terms of fluorescence fluctuation or chemical shift of desired molecules extracting from these instruments' data along with mathematical models maneuver the interaction information during its diffusion through crowded cytosol or nucleus which are not assessable through traditional ensemble-averaged study. Interestingly, sometimes these data appear as a temporal trajectory of a fluorescently labeled molecule in the cell over time in single-particle tracking (SPT) or sometimes as a noise generated from the labeled molecules when it comes in and out from the confocal volume focused in the cell in fluorescence correlation spectroscopy (FCS) or sometimes as the recovery of fluorescence of a fluorescent molecule after it is bleached with more high power of laser in fluorescence recovery after photo bleaching (FRAP) .

Again, focusing on the diffusion-limited biochemical reaction would give an equation like:

Experimental observation of biochemical reaction

$$= f(\text{Diffusion}) \times \text{Other parameters}$$

This implies that the consequence of the diffusion on the biochemical reactions provides more meaningful features regarding the spatiotemporal dynamics of biomolecules. However, deciphering these two phenomena in order to understand their interconnections have been a complicating task to perform. There are three types of biochemical reactions: (i) biomolecular association, (ii) alterations in conformation, and (iii) adsorption equilibria which are affected by these four types of environmental effects (a) non-specific intermolecular interactions, (b) side reactions, (c) partitioning between micro-environments for the different species, and (d) surface interaction as introduced by A.P. Minton (Zimmerman and Minton 1993; Minton 2015; Zhou et al. 2008), one of the pioneers of this field. We have mentioned some examples regarding a few types of biochemical reactions in this review in the context of sub diffusion. Moreover, the dynamic interaction of DNA, RNA, and proteins and the resultant noise generated from it are other avenues of our discussion pertaining to gene expression in brief. A few mathematical models which talk about

these have been discussed as these topics are beyond the scope of this review.

The aim of the review is to explain biochemical reactions behind the different cellular mechanisms using the idea of anomalous diffusion in a crowded environment with the help of different biophysical tools (Fig. 1). To approach the idea thoroughly, the modeling of diffusion behavior or waiting time distribution to trace out the physical parameters, those are encoded with the influence of microenvironment change during the process, is needed. In this review, we start with the basic introduction of diffusion in solution. We have then attempted to elaborate different modes of diffusion to interpret biological functions within the cell. Gradually, the combination of brief mathematical derivations and basic description of the model are recapitulated to show the process of mining the parameters from the observables. In conclusion, this review will discuss the future perspectives of this field and what kind of new biological problem can be addressed with this approach.

Overview of in vitro diffusion

The concept of simple diffusion was established by the Scottish botanist *Robert Brown* when he observed the continuous restless motion of tiny particles from pollen grains under a microscope. The unpredictable movement of colloidal

particles suspended in a simple dilute solvent is referred to as *Brownian motion* (Mörts et al. 2010). Thereafter, the theoretical and mathematical explanation was developed by Einstein and Smoluchowski (Einstein 1956) in terms of probability distribution of diffusion. In suspended colloidal systems, particles experience continuous collision with the solvent molecules. During the collision, a tiny amount of momentum is exchanged and it occurs in the liquid dynamics time regime, i.e., picosecond. In this small time window, if any displacement (S) of suspended particle happens due to the inter-particle collision significantly, then the increment can be measured as:

$$\Delta S = S(t + \Delta t) - S(t) \quad (1)$$

Where t is the random time variable which is identically and independently distributed. Gradually, these kinds of tiny displacements increases and the summation of the total displacement can be understood using central limit theorem which is governed by Gaussian distribution. The probability of finding a suspended particle in “ a ” dimension of surrounded space as a function of time and space is:

$$P(s, t) = \left[\frac{2\pi\delta s^2(t)}{a} \right]^{-\frac{a}{2}} \exp\left(\frac{-s^2 a}{2\delta s^2(t)}\right) \quad (2)$$

In Eq. (2), the variance $\delta s^2(t)$ increases linearly with the number of events in the *propagator* or *van hove self-*

Fig. 1 The schematic representation of how to understand the biochemical reaction by monitoring the diffusive behavior of the molecules in the cellular environment and then through the construction of mathematical models to interpret them. Reprinting permission were taken

correlation function (Akcasu et al. 1970) suggesting a linear increase of the mean square displacement (MSD) $\delta s^2(t) = 2aDt$. Considering the only transport coefficient indicating the Brownian motion as diffusion coefficient D , the propagator can be described as follows:

$$P(s, t) = \frac{1}{(4\pi Dt)^{a/2}} \exp\left(\frac{-s^2}{4Dt}\right) \quad (3)$$

In this prospect, the Fourier transformation of $P(s, t)$ will be advantageous to scattering parameters, which can be implemented as:

$$P(k, t) = \int a^a s e^{-ik \cdot s} P(s, t) \quad (4)$$

The scattering generated from the diffusion of the suspended molecules within the solution can be monitored by spectroscopic methods. Here, we need to consider the transfer of momentum from the molecule of interest to another molecule or a photon and the propagator is calculated as:

$$P(k, t) = \exp(-Dk^2 t) \quad (5)$$

Thus the propagator $P(s, t)$ can be converted to any measurable variables and is the result of an average of possible individual variables of the motion for the particle.

The idea of Brownian motion was again evolved by the introduction of Langevin equation where the dynamics of the probe within the medium gives a unique trajectory which is a consequence of stochastic fluctuation force and viscous force. The equation of motion for the position of the particle is given by:

$$m \frac{d^2 s}{dt^2} = -m\gamma \frac{ds}{dt} + \xi(t) \quad (6)$$

Where $-m\gamma (ds/dt)$ denotes the viscous force and $\xi(t)$ represents the random fluctuation force. Langevin's equation (Langevin 1908) was the first example of a stochastic differential equation with a random term $\xi(t)$ hence having a solution which is a random function. Each solution of the Langevin equation denotes a different random trajectory and using rather simple properties of the fluctuating force $\xi(t)$, measurable results can be derived. Ornstein formulated a modified Langevin equation introducing a notion which is called as random Gaussian white noise. These noise terms, i.e., $\xi(t) = (\xi_1(t), \dots, \xi_d(t))$ have been considered to be independent and random quantities and have been averaged over many independent processes in a short time scale in such a way that the central limit theorem applies. The correlation between noises can be represented as:

$$\langle \xi_i(t_1) \xi_j(t_2) \rangle = 2k_B T / \gamma \delta_{ij} \delta(t_1 - t_2) \quad (7)$$

Again, from Stokes–Einstein derivation (Kholodenko and Douglas 1995), variance σ is derived from $D = \sigma^2/2 = (k_B T / \gamma)$ and one can calculate that as:

$$\langle \xi_i(t_1) \xi_j(t_2) \rangle = 2D \delta_{ij} \delta(t_1 - t_2) \quad (8)$$

The correlation between the noises only exists within the short time range as it disappears with time. Further, the correlation of displacement appears as:

$$\langle \Delta D_i(t) \Delta D_j(t) \rangle = 2Dt \delta_{ij} \quad (9)$$

Hence, if $i=j$, the MSD is as:

$$\delta d^2(t) = 2dDt \quad (10)$$

Apart from the mathematical expression, another breakthrough in this field was achieved when the experimental evidence of trajectory of a colloidal particle using microscope was observed by Perrin et al. (1909).

In vivo diffusion

While most of the biophysical studies continue to reveal important insights into the properties and functions of biological macromolecules, much attention is being directed at clarifying the discrepancy of the observations extrapolated from in vitro to in vivo (Schurgers et al. 2010; Checkley et al. 2015). In a physiological environment, all biomolecules experience specific, regulated interactions with the surrounded intracellular milieu. Proper clarifications are required to explain that. Even if such interactions are only steric in nature, significant alterations in biochemical reaction to maintain cellular function may be caused from that. In this context, *Escherichia coli* cells contain 25% of macromolecules within its cytoplasm and among them 90% are globular proteins and nucleic acids. The concentration of proteins is 20–250 mg/ml, whereas the concentration of DNA and RNAs are 20–50 mg/ml. In comparison to *E. coli*, mammalian cells contain less concentrated biomolecules and have more organized architecture. Thus, the macromolecules occupy 10–35% of cell volume and make this space unavailable to other molecules. A simple diffusion is believed as a solute diffuses through a continuous hydrodynamic fluid (Ritz and Caltagirone 1999). In most biological systems, for example, cell cytoplasm contains heterogeneous composition of solutes (Luby-Phelps 2013) and thus diffusion of individual solute in the cytoplasm violates the hydrodynamic fluid assumption. Furthermore, the interaction of solutes with the cytoskeletal networks or organelle membranes makes the diffusion process more complex. Due to these

conditions, it is not possible to assume that the diffusion of biomolecules inside a cell follows the equations of normal diffusion. However, there have been cases observed where solutes do follow normal diffusion equation (Kühn et al. 2011). Normal diffusion can be explained with the help of central limit theorem. According to this statistical law, the MSD of a molecule in a simple solution is expected to show linear behavior with time, whereas a nonlinear profile of MSD can be considered as a sign of unusual diffusion (Fig. 2a, b). Generally, MSD is proportional to this power law (Banks and Fradin 2005),

$$\delta r^2(t) \propto t^\alpha \quad (11)$$

Where α lies between 0 and 1. Thus the MSD increases slower than the normal diffusion, and is referred to as anomalous diffusion. Equation 11 has nonetheless been used extensively as a semi-empirical description of anomalous diffusion. If $\alpha < 1$, the diffusion is called as anomalous or sub diffusion (Banks and Fradin 2005), and if $\alpha > 1$, the diffusion is called super diffusion (Abad

et al. 2010; Reverey et al. 2015) (Fig. 2a). During the process, simple diffusion is not able to explain the phenomena as the central limit theorem is not applied to it at the desired time scale. There are some interesting trends observed in MSD over time. For example, even if a particle undergoes normal diffusion, the MSDs may not be linear with time for all spatiotemporal variations. There would be two types of relationship that can be observed over time. In the first case, the diffusion of a particle is too slow that it violates the central limit theorem at a time frame of interest but over time normal diffusion conquers (Ellery et al. 2016). In such cases, a crossover can be observed from a short time range to a long time range diffusion. In the second case, the correlations in the increments decay slowly and the extent of sub-diffusion sustains for a longer time. Hence, the central limit theorem is never applied to it in the biological relevant time scale. Thus, the interpretation of MSD that varies linearly or nonlinearly with time depends on the characteristic time over which the measurement is made. In this review, we have discussed only the possibilities and experimental evidences those that are consistent with the observations of the complex and anomalous diffusion.

Fig. 2 **a** The classification of diffusion depending upon their power law with time where for normal diffusion, mean square displacement (MSD) linearly correlates with time ($\alpha = 1$), whereas for sub-diffusion and super diffusion $\alpha < 1$ and $\alpha > 1$ relationship respectively. **b** The consequences of the power law with time on the trajectories of the particles and their MSDs are shown. **c** The schematic representation of diffusion patterns of molecules when they are of different sizes or when they experience different nonspecific interactions in vitro or in vivo. Depending upon the

sizes of the crowders and solute molecules, diffusion varies from anomalous diffusion, Brownian slow and fast to restricted diffusion. **d** A glimpse of time scale of different biochemical reactions and the accessibility of these time scales with help of different fluorescence measurement like life time, anisotropy, etc. are presented. Here, diffusion (Cy) means the diffusion of a molecule in cytoplasm and diffusion (Mb) means the diffusion of a molecules in membrane. Reprinted with permission taken from the respective publishers

Models for the anomalous diffusion

Due to the nonlinear behavior of MSD with time, several methods were used to explain the slowness of a particle in the crowded environment depending upon their characterized motion in the different cellular environment. Some of these methods that have been discussed here are fractional Gaussian motion and fractional Langevin equation motion of Gaussian model where noise comes from the fluctuation force within medium and they are power-correlated with time. Continuous-time random walk where the infinite waiting time is Gaussian distributed instead of mean square displacement and Lorentz model where the model deals with the diffusion of a particle in the disordered medium are the most popular ones. In this context, Metzler et al. (2014) explained several other variety of CRTW model depending upon their different characteristics. Those interested with the improvisation of models in terms of mathematical derivations will find more information about these in these papers (Höfling and Franosch 2013; Metzler et al. 2014; Metzler et al. 2016; Luchko 2012; Sokolov 2012).

Gaussian models

Apart from the simple diffusion in solution, stochastic noises may come from more complex and anomalous diffusions in crowded environment and can be incorporated with the diffusion. They are power-correlated and have been explainable by introduction of a Gaussian propagator.

Fractional Brownian motion

This is one of the popular stochastic models that explains in a simple manner the process of anomalous diffusion in the presence and absence of external fields. This model was introduced by Mandelbrot and Van Ness (2005) as a combination of Brownian motion along with power law memory. Fractional Brownian motion (FBM) is widely applied to different fields of science, engineering, and extensive efforts have been invested to broaden its spectrum of problem solving which pertains to more fields than the two mentioned above (Dieker and Mandjes 2003; Xu and Luo 2018). The special feature of FBM is that it is not differed from self-similar Gaussian process but only with stationary increment. In Langevin's stochastic differential equation, we have seen that the noise is correlated with time and the MSD increases linearly. Considering the noises also follow Gaussian statistics, we can incorporate it into the diffusion propagator ($P(d, t)$) which characterizes the MSD and the transport behavior. Mathematically, this can be presented as:

$$\frac{dx(t)}{dt} = \xi_{fgn}(t) \quad (12)$$

Further, the correlation of noise appears as:

$$\langle \xi_{fgn(i)}(t_1) \xi_{fgn(j)}(t_2) \rangle = d F(|t_1 - t_2|) \delta_{ij} \quad (13)$$

Where $\xi_{fgn}(t)$ is the fractional Gaussian noise and has been considered to follow Gaussian statistics over time but is power-correlated as incorporation of persistent correlation in the noise makes the transport drastically slow compared to the normal diffusion, as is seen in anomalous diffusion, i.e.; $\delta x^2(t) \propto t^\alpha$ with an exponent $0 < \alpha < 1$. In that case, the correlation function can be represented as:

$$\langle \xi_{fgn}(t_1) \xi_{fgn}(t_2) \rangle = \alpha(\alpha-1) K_\alpha |t_1 - t_2|^{\alpha-2} \quad (14)$$

Notably, due to the factor $(\alpha-1)$, the fractional Gaussian noise is positively correlated for the case $1 < \alpha < 2$ and negatively correlated for $0 < \alpha < 1$. The Gaussian diffusion propagator for FBM is given by,

$$P_{FBM}(x, t) = \frac{1}{\sqrt{4\pi K_\alpha t^\alpha}} \cdot \exp\left(-\frac{x^2}{4K_\alpha t^\alpha}\right) \quad (15)$$

We need to remember that FBM is not a Markovian process and thus applying only van Hove correlation function cannot explain the statistical properties completely. Thus with the introduction of multiple function, the position autocorrelation of FBM is:

$$\langle x(t_1)x(t_2) \rangle = K_\alpha (t_1^\alpha + t_2^\alpha - |t_1 - t_2|^\alpha) \quad (16)$$

From Eq. (16), the averaged MSD can be obtained as:

$$\langle x^2(\Delta) \rangle = 2K_\alpha \Delta^\alpha \quad (17)$$

When $t = t_1 = t_2$, it FBM appears ergodic and is equivalent to the MSD shown in Eq. 11.

Fractional Langevin equation motion

There are several application of Fractional Langevin equation (FLE) in studying biological problem. This model is used to study the internal dynamics of proteins (Sande et al. 2014), motion of lipid molecules in membranes (Jeon et al. 2012), and the sub-diffusion of mRNA and chromosome loci (Vitali et al. 2018) in living cells. The FLE deals with the description of particle motion in a viscous-elastic environment and thus can be considered to be a combination of elastic and hydrodynamic interaction models (Talon et al. 2010). As we discussed earlier, Langevin equation explains about how a spherical particle diffuses through a complex medium and its subsequent displacement characterized by velocity $v(t)$.

Langevin equation can explain that behavior by Eq. (6) where the first term is the frictional force experienced from the viscous medium and $\xi_{fbm}(t)$ is the noise from the fractional Brownian motion. In case of a viscous medium, the noise comes from the fluctuation force when the particle tries to diffuse through the medium. As the noise also follows the Gaussian distribution, the correlator of the noise can also be represented by Eq. (7) as discussed earlier. Considering fluctuation-dissipation relation (Kubo 1966) and the convolution integral, fractional Langevin equation can be written as:

$$m \frac{d^2 x(t)}{dt^2} = -\gamma \Gamma(\alpha-1) \frac{d^{2-\alpha} x(t)}{dt^{2-\alpha}} + \eta \xi_{fGn}(t) \quad (18)$$

Here, this equation is obtained on the basis of fluctuation-dissipation relation between Stokes drag of the medium and the fluctuation noise. They are instantaneously coupled with each other and create the underdamped motion which is different from the normal diffusion in solution.

Thus the MSD obtained from the underdamped FLE is,

$$\langle x^2(\Delta) \rangle = \frac{2k_B T \Delta^2}{m} E_{\alpha,3} \left(-\Gamma(\alpha-1) \frac{\gamma}{m} \Delta^\alpha \right) \quad (19)$$

Over several mathematical derivations, the limiting behavior of MSD for the anomalous diffusion can be understood as:

$$\langle x^2(t) \rangle = 2k_B T (\Gamma(\alpha-1) \gamma)^{-1} t^{2-\alpha} \quad (20)$$

In this regime, the motion is normally overdamped sub-diffusion with persistent noise with power component $(2-\alpha)$ for $1 < \alpha < 2$.

There are several other models like scaled Gaussian motion (SGM) (Metzler et al. 2014), transient aging, and heterogeneous anomalies (Berry and Soula 2014) to describe the complex transport behavior of the particles in the cellular environment.

Continuous-time random walks

This model was proposed by Montroll and Weiss (1965) to describe the stochastic process and is currently one of the most popular models to explain anomalous diffusion behavior of molecules in crowded environment. Initially, continuous-time random walk was considered as a one-dimensional walk, where a particle jumps with a waiting time of τ and the step size of x , which have been considered to be random variables. Suppose a particle moves with an increment of Δx_i , the distance covered by the particle $X(t)$ with $N(t)$ steps within the time t can be represented as:

$$X(t) = \sum_{i=1}^{N(t)} \Delta x_i \quad (21)$$

The probability for the process covering the distance $X(t)$ at time t is then given by:

$$P(X, t) = \sum_0^t P_n(x) P(n, t) \quad (22)$$

Where $P_n(x)$ is the probability of covering distance X after n jumps and $P(n, t)$ is the probability of doing n steps after time t . Then a step ahead with the distribution of waiting time $\varphi(t)$ and step size $\sigma(\Delta x)$ and the subsequent Laplace-Fourier transformation of probability of $P(X, t)$ gives:

$$W(k, s) = \frac{1-\varphi(s)}{s} \cdot \frac{1}{1-\varphi(s) \cdot \sigma(k)} \quad (23)$$

Where $\varphi(s)$ and $\sigma(k)$ are the Laplace and Fourier transformation of $\varphi(t)$ and $\sigma(\Delta x)$. During this walk, the particles show well-behaved and separated distribution of waiting time and step size and interestingly after each jump, the waiting times and step size appear to be independent from the previous step and they contribute to this same distribution. The inverse Laplace-Fourier transformation of $W(k, s)$ gives the probability density function of $P(X, t)$ as:

$$P(X, t) = \frac{1}{2\pi} \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} ds [dk e^{st-ikx} W(k, s)] \quad (24)$$

Clearly, $P(X, t)$ depends on the asymptotics of $\varphi(t)$ and $\sigma(\Delta x)$. For normal diffusion, one can expect that waiting time pdf with finite average waiting time τ appears as:

$$\varphi(t) = \frac{1}{\tau} \exp\left(-\frac{t}{\tau}\right) \quad (25)$$

In contrast, for a long-tailed pdf displaying the asymptotic long-time behavior,

$$\varphi(t) \sim \frac{\alpha}{\Gamma(1-\alpha)} \frac{\tau^\alpha}{t^{1+\alpha}} \quad (26)$$

It shows anomalous diffusion in the regime $0 < \alpha < 1$. Several groups are working on the improvisation of CTRW (Le Vot et al. 2017; Hou et al. 2018) with modifications to get more detailed spatial and temporal explanation of the biological events within the cell. In this progress, the ensemble averaged MSD is calculated as,

$$x^2(\Delta) \sim \Delta^\alpha \quad (27)$$

Whereas, time averaged MSD can be represented as,

$$\overline{\langle \delta^2(\Delta) \rangle} \sim \frac{\Delta}{t^{1-\alpha}} \quad (28)$$

Normally, this time averaged MSD is derived from the collection of data from single-molecule fluorescence and

single-particle tracking experiments. This model has helped shed light toward understanding the sub-diffusive behavior of RNA molecules in *E. coli* (Lampo et al. 2017), the diffusion of potassium channels in two-dimensional plasma membrane (Weigel et al. 2011) where the anomalous diffusion is caused due to the transient interaction with the actin cytoskeleton. In biology, CTRW provides enormous opportunities to define the different states of biomolecules where the active state can be considered as walker displacement and the passive or inactive state can be explained by the distribution of waiting time.

The Lévy flights (Chechkin et al. 2008) can be modeled by a CTRW with a finite mean of a long-tailed jump length pdf and a waiting time pdf. The model is assumed to have the walks with finite fixed velocity instead of instantaneous jumps. Thus combined with temporal distribution having finite velocity, the probability $P(X, t)$ is:

$$P(X, t) \sim \frac{1}{t^{\frac{1}{\beta}}} L_{\beta} \left(\frac{x}{t^{\frac{1}{\beta}}} \right) \quad (29)$$

Where L_{β} is a Lévy stable law of index β . Therefore, ordinary Lévy flights with a sub-diffusive scaling with time are expressed as:

$$X(t) \sim t^{\frac{1}{\beta}} \quad (30)$$

The presence of finite velocity of the particles makes this model more advance but at the same time extracting analytical solution from it is a complex matter. As a standard improvement of Levy walk model, it is now not limited to the fixed particle velocity (Zaburdaev et al. 2015) but it applies to varying velocity (Denisov et al. 2012), such as self-similar hierarchical structure.

Lorentz model

CTRW deals with molecularly homogeneous medium but Lorentz model opens a new path to determine the motion of a particle in a heterogeneous medium (Bouchaud and Georges 1990) which includes the anomalous or sub-diffusion in the medium, crossover phenomena, immobilized particles, and long-time tails. Originally, Lorentz (Höfling and Franosch 2013; Höfling et al. 2006) developed a model to evaluate the motion of a ballistic particle placed amidst random and scattered obstacles in order to define the microscopic explanation of Drude's electricity in metal. There are few examples (Saxton 1994; Sung and Yethiraj 2006, 2008) where they have used this model to explain the motion of proteins in a cellular environment.

The sub-diffusive behavior of the molecules is different from each other and their unique motions are comprehended

with the evolution of models over time. Actually, the existence of the anomalous diffusion is observed in all the cellular compartments and at every stage of cellular metabolism, signaling, and gene expression (Ten Wolde and Mugler 2014). In this part, the presence of the anomalous diffusion from the process of transcription in the nucleus to the interaction of the protein with membranes through the transport of these biomolecules in the cytoplasm is discussed.

Bridging between physics and biology

The three main constituents of a cell are protein, DNA, and RNA. The function of the cell depends entirely upon the interactions of these three molecules with each other. For the fulfillment of cellular functions, these molecules interact either strongly or transiently and their dynamic behaviors in cellular processes create noise in our observations (fluorescence, absorbance) in wide range of time scale (Fig. 2d). In order to understand these dynamic interactions which take place within a cell, we need to evaluate the parameters extracted from the noise with precision (Fig. 1). Thus, we need to be more accurate considering all the physical or chemical events associated with these changes in the crowded environment (Fig. 2c). Interestingly, the individual nature of these molecules is different (Valastyan and Weinberg 2011) and as a consequence, folding and dynamics of them will be more complicated when they are in the midst of other diverse molecules which can influence them and the interaction with others. We need to remember that sometimes the self-association of these molecules drives them to do their function and that is reflected in cellular malfunction causing several diseases like cancer (Zhivotovsky and Orrenius 2010), neurodegenerative diseases (Wong and Cuervo 2010), infectious diseases (Aguzzi and Calella 2009), etc. Hence, the integrity of the cell function is built on the fine tuning of cross-talk between its constituents (Engler et al. 2009). The basic need of human health lies on the deciphering of functional and dysfunctional interactions of these biomolecules at their specific locations in the cell. We can hope that the connection between explaining the responsible interactions of biomolecules in cellular context, disease phenotype, extrapolating our knowledge from the mathematical models, and the physics of the particle's motion in colloidal solution may pave more realistic pathway for the improvement of disease diagnosis.

Considering their importance in the human health, biomolecules, mainly nucleic acids, namely DNA and RNA, carry the unique function of keeping the blueprint of genetic code of an organism. Later on, these sequences of nucleotides determine the amino acid sequence of proteins, which are crucial for the life on Earth. There are 20 different naturally occurring amino acids, which play a fundamental role in determining protein structure and function. Proteins are necessary for the

structural integrity of cells as they participate in transporting, moving other biomolecules in and out of cells, and catalyzing majority of chemical reactions that take place in a living organism. In this section, we will elucidate the application of the concepts of complex diffusion, i.e., anomalous diffusion, of proteins required for their folding, stability, and functions while encountering complex interactions and traveling through different cellular environments.

Anomalous diffusion in protein folding, dynamics, and stability

According to the energy landscape theory of protein folding, the folding can be modeled as diffusion along a one-dimensional reaction coordinate. In this diffusion model, the rate of folding is measured by the height of the free energy barrier and a kinetic prefactor. This prefactor depends on the diffusion coefficient along the reaction coordinate. The diffusion coefficient at the top of the barrier contributes only to the rate for proteins with a pronounced free energy barriers separating the folded and unfolded states. Thus, there are several aspects of protein folding that are affected by the diffusion namely (1) folding free energy surface, (2) transition path time, (3) internal friction, and (4) contact formation time from unfolded state to folded state. Principally, they are interconnected and the consequence of the whole is responsible for the folding and stability of a protein. Although the effect of the anomalous diffusion on the folding free energy has not appreciated as it should have been, but nowadays, several groups are working to reveal the connection of this effect in protein folding experimentally (Jennifer Lippincott-Schwartz 2001) as well as by using MD simulation (Satija et al. 2017). In this section, the behavior of the diffusion and their effect on the protein stability have been discussed and thus it includes the diffusion measurement, evaluating the relationship of mean square displacement with time (Fig. 3a). The ergodicity of diffusion over time are observed in many cases (Schwarzl et al. 2017) and will make these events of protein folding more complex (Cote et al. 2012). In the field of protein folding, the motion of the protein over the folding free energy surface describes the folding and dynamics of the protein where each coordinate of the energy landscape consists of the information of the interaction of the backbone and side chains within the protein interior (Onuchic et al. 1997). Under folding conditions, the protein follows a funnel-like energy landscape to reach the most stable native state (Dill and Chan 1997). Sometimes, local interactions include unwanted roughness in the folding free energy surface in the form of non-native conformations. The uneven motion of the protein over its unfolded state is generated from the attractive and repulsive interaction or may come from the unfavorable side chain interaction. Interestingly, the pathway of folding is not only governed by

the initial conformation in folding state but also on the statistical fluctuations on the hierarchy of the energy landscape which makes multitude of pathways accessible. Thus the motion of protein molecule over this multidimensional rough energy surface explains the folding behavior of it and the crossing of the heights of the local barriers. It determines the speed of the motion through the conformational space and describes the time scale of folding to the bottom of the funnel or to the other non-native states. In this context, the notion of “internal friction” (De Sancho et al. 2014) comes from the intra-protein interaction or from the solvent viscosity, and slows down the protein’s movement over the rough folding free energy surface. Hence, the roughness which defines the folding and dynamic behavior of the protein is affected in two different ways: (1) the effect of the surrounding (solvent viscosity and crowding reagents) and (2) intramolecular interaction. In case of intra-protein interaction, the presence of the denaturants eliminate or reduces the height of the rough energy surface, whereas the increase of the temperature increases the roughness of the folding free energy surface due to the predominant contribution of hydrophobic effect. On the other hand, the distribution of the crowding molecules around the protein molecule restrict the motion and entropy of the unfolded state in the folding landscape and thus this induced spatial distribution of the unfolded state finds new pathway to the stable folded state and contributes to the different stability of the protein in the induced environment.

Even though folding of a protein involves reconfiguration of several of the bonds and interactions between a number of atoms, as discussed earlier that energy landscape theory avows that protein folding actually can be understood simply in terms of diffusion (Best and Hummer 2010; Hagen 2010) where the phenomenon can be represented as a system accomplishing stochastic diffusive motion on a free energy surface. Such diffusion-controlled processes are defined by Kramer’s reaction rate theory (Hänggi et al. 1990; Pollak and Talkner 2005). The fact that the conformational dynamics of a protein molecule are very strongly damped by the surrounding solvent, considerable attention has been given to explore the applications of Kramer’s rate theory in order to understand protein dynamics (Hagen 2010). A number of authors have used Kramer’s theory as a tool for probing the diffusional character of protein folding and dynamics (Bilsel and Matthews 2000). However, evidences from experiment and theory indicate that the dynamics of a folding polypeptide chain are diffusion-based under experimental condition (Zwanzig 1997; Sabelko et al. 2002; Naganathan et al. 2006; Hagen 2010). Interestingly, if we consider that the effects of protein and solvent friction are additive, then the Kramer’s equation would appear as:

$$k = \frac{B}{\alpha\zeta_p + (1-\alpha)\zeta_s} \exp\left(-\frac{E_0}{RT}\right) \quad (31)$$

Fig. 3 **a** The application of single-molecule fluorescence spectroscopy in order to understand the solution whether it shows poly or mono-dispersity. In the poly-dispersed solution, it shows power law with the diffusion time in contrast to the mono-dispersed solution. **b** The effect of the excluded volume effect on the diffusion of time of different proteins ranging from smaller protein like lysozyme (~ 10 kDa) to larger protein like anti-goat donkey antibody (150 kDa). As the excluded volume decreases, protein experiences more obstacles to diffuse through and it takes

longer times. **c** Due to that smaller excluded volume, the unfolded state of the bovine serum albumin (BSA) gets destabilized and this protein shows higher stability in gel measured by FCS (black) than the solution measured by circular dichroism (CD, red). In the inset, the distribution of diffusion time against different % of acrylamide are shown. **d** Decrease in the activity of yeast cytochrome C (y-CytC) with the increment of crowding by ficolls and y-CytC showed highest activity at native buffer condition. Reprinted permission were taken from the respective journals

Where E_0 is the average height of the energy barrier separating two protein conformations, B is viscosity- and temperature-independent parameters that depends on the shape of the surrounding surface, R is the gas constant, T is the temperature, ξ_p is the friction constant for the motion in the protein, and ξ_s is the friction constant for the motion in the solvent. They are proportional to the viscosity according to Stokes' law (Zwanzig 2012).

Thus the effect of the surrounding medium in the solution or cellular environment has a dominant effect. It is evident that an increase in viscosity could slow down the diffusion that initiates the conformational changes significantly and therefore influence the stability of the protein in the cellular environment. Then, it would be more relevant to discuss the impact of sub-diffusive nature on the spatial coordinates of the folding landscape of a protein along with its induced entropically changed conformation and how they will change the pathway of folding and the contact formation rate in the

presence of crowded molecules over the higher energy barriers generated from the viscous solvent according to Kramer's equation. Moreover, intra-protein backbone diffusion has been found not only in local motions in shorter time but also in larger conformational change in longer time. The sub-diffusive behavior can be caused for several reasons such as crowded environment, the presence of randomly distributed heterogeneous traps with a wide distributed trapping times (Zumofen et al. 1983; Feig et al. 2017). Sometimes, the chain connectivity of polymer (Semenov and Meyer 2013) causes this kind of actions. The inclusion of internal friction has highlighted the importance of the unfolded backbone motion in this regard. Few groups have been investigating the effects of anomalous diffusion in the internal friction as proteins execute conformational fluctuations in their rough energy landscape and these findings are very insightful to understand the protein dynamics at molecular level. Satija et al. (Satija et al. 2017) demonstrated the existence of anomalous diffusion

while crossing over the energy barrier as a discrepancy was observed between the energy barriers inferred from the experimentally observed transition times and the independently determined energy barriers. With the help of several molecular simulations, they were able to understand that the memory effect was the reason for the discrepancy and hence the dynamics of the protein is sub-diffusive on the one dimension of the reaction coordinate. Fractional Brownian motion (FBM) model that was used for the anomalous diffusion in the presence of a potential mean force was used to extract broader distributions of transition path times computed directly from protein trajectories. To strengthen the understanding, Boyer et al. (2014) used a path-dependent random-walk model with long-range memory to obtain the mean square displacement (MSD) as well as the propagator in the asymptotic limit. The model deals with a random walker, walking at constant rate and occupying a site at earlier time stochastically. Interestingly, in the weakly non-Markovian regime, the transition happens with sub-diffusive nature due to the divergence of the jump to a prior site in time. There are several other experiments that have been performed to extract more information about parameters like diffusion constant over the diffusive barrier (Trimble and Grinstein 2015), rate constant at particular force (Jagannathan and Marqusee 2013), and transition path time for different potentials (Zhang and Chan 2012) using Kramer's theory for diffusive barrier crossing. These provide progressive knowledge toward the understanding of protein folding more closely even at the cellular interior.

Due to the discrepancy between experimentally observed power law diffusion and the simulation assuming normal diffusion, there are few other parameters included in the potentials. Friedel et al. accounting the contribution of tethering effect on the diffusional protein dynamics as well as Lapidus et al. accounting the chain stiffness with a persistence length respectively in the potential modifies the motion of the protein backbone and thus the dynamics very small at shorter time range but deviations were observed after the times corresponding to the diffusion over the length (Lapidus et al. 2002; Friedel et al. 2006).

The protein folding involves the transition between the folded state and the unfolded state and the folding stability is associated with the folding free energy difference between these two states. In the crowded and confined environment, the major effect is exerted by the “excluded volume.” The unfolded state is considered as a more expanded form than the folded state, hence the motion of this state is more adversely affected in the confined space. Das et al. (2018) recently mentioned the presence of anomalous diffusion in the internal friction and memory effect while explaining the dynamics of unfolded protein in confinement. Its variation in different proteins with different length, with the increment of denaturing concentration indicates the dependency of compactness of the protein on internal friction. Confined environment induces

more compact unfolded state of a protein and this gives rise to the exponential increment of reconfiguration time and broadening the spectrum of the relaxation times exhibited by a protein. This kind of behavior restricts the applicability of Rouse and Zimm model (Zwanzig 2004) and rather increases the possibility of sub-diffusive motion due to the long-tailed distribution of relaxation time. Thus, in the confinement and crowding environment, the protein conformation is in different space in the folding landscape coordinate which would favor the folded state and increase folding stability.

To understand the stability in the context of diffusion, the diffusion equation can be written in terms of probability density considering n as a continuous variable:

$$\frac{\partial P(r, n)}{\partial n} = \frac{b^2}{6} \nabla^2 P(r, n) \quad (32)$$

Further, the survival fraction of all the possible conformations are expressed as probability density of a protein with n residues and starts from a fixed position of R_0 provided the dimension of the unfolded protein does not cross the boundary of the excluded volume,

$$S(R_0) = \int d^3R P(R, N; R_0) \quad (33)$$

With the same analogy of Gaussian chain and Brownian trajectory, the survival fraction of a polymer molecule in crowded environment is modeled in the presence of crowders of radius R_m and concentration of C_m as:

$$S(N) = \exp \left(-4\pi \left(\frac{Nb^2}{6} \right) R_m C_m \left[1 + 2R_m \left(\frac{\pi Nb^2}{6} \right)^{-\frac{1}{2}} \right] \right) \quad (34)$$

The statistical weightage of the unfolded state (f_U) in the crowded environment (Zhou 2004) can be represented as:

$$f_U = S(N)(1 - \varnothing) \quad (35)$$

Where $\varnothing$ is the fractional volume ($\varnothing = \frac{4}{3}\pi R_m^3 \cdot C_m$) where the Brownian trajectory can be launched and then the reduction factor is $1 - \varnothing$. From this mathematical expression, it is easily understood that the population (survival fraction) of the unfolded state is dependent on the concentration and thus the diffusion. Again, the folding free energy is like

$$\Delta\Delta G_F = -k_B T \ln \left(\frac{f_U}{f_F} \right) \quad (36)$$

In the presence of crowding, due to the boundary restriction, the population of the unfolded state decreases significantly compared to the folded state and thus protein gets stabilized in the crowded environment or cellular environment. The stabilization of protein in crowded and cellular environment are

also shown experimentally by several groups (Samiotakis et al. 2009; Ebbinghaus and Gruebele 2011; Wang et al. 2012b; Gershenson 2014). Basak and Chattopadhyay (2013, 2014) worked on four sets of protein having molecular weights ranging from 12 to 150 kDa namely lysozyme, intestinal fatty acid binding protein (IFABP), bovine serum albumin (BSA), and anti-donkey goat antibody to understand the diffusive behavior of these proteins in the presence of different excluded volume which is generated by the increment of % of acrylamide concentration (Fig. 3b). Larger proteins were affected more prominently than the smaller protein, which is consistent with our above mathematical explanation. Moreover, by using single-molecule spectroscopy (FCS) and ensemble averaged spectroscopy (CD), they have shown that the stability of BSA protein is more in gel than the solution and the unfavorable interactions in the unfolded state is the reason behind it (Fig. 3c). Additionally, the polymer scaling law (Flory 1953), the dimension of a protein also behaves differently for folded and unfolded states in gel when compared to solution. The dimensional difference between these states introduces new aspect of thoughts about the resolution in detecting these two states. From the same group, Paul et al. (2016) also investigated the effect of crowding agents Ficoll70 and Dextran70 on the native-like state of yeast cytochrome c. It was observed that the crowding medium affects equilibrium between the compact and expanded states, shifting its population toward the compact conformer. As a result, y-cytc loses its peroxidase activity (Fig. 3d). Urea-induced protein stability measurements showed that the compaction destabilizes the protein due to charge repulsions between similar charged clusters in the unfolded state. Furthermore, the increase in the time constant between the compact and expanded state in the cellular milieu indicated the presence of solution micro-viscosity in this crowded environment. The inclusion of interaction between solute and the crowded molecule during their diffusion through it would be more insightful to understand the biochemical reactions which are going on in the cell (Harada et al. 2013).

Anomalous diffusion in contact formation rate or folding rate

Contact formation is more relevant to protein folding as it is associated with the formation of secondary and tertiary structure of the biomolecules. It has been observed that the probability density of the end to end contact formation is maximum at the root mean square (R_{rms}). Crowding makes this separation more closer than the R_{rms} and the diffusion controlled contact formation rate (Zhou 2004) appears as:

$$k_f = 3 \cdot \left(\frac{6}{\pi}\right)^{\frac{1}{2}} D\sigma / R_{\text{rms}}^3 \quad (37)$$

Where D is the apparent diffusion constant of the solute in the medium. To avoid the possibilities of elimination of the unfolded states from the restriction boundary, the survival function consists of more compact unfolded state and the R_{rms} values will be smaller than the solution. However, the crowded environment makes the diffusion of the solute slower and it comes from the interaction of the compact unfolded state with the neighbors. Such observation are observed in several studies (Fierz and Kiefhaber 2007; Guigas and Weiss 2008). Interestingly, the interplay between anomalous diffusion and the reduction of entropy of the unfolded state in the crowded environment optimizes the folding rate and thus the stability.

Anomalous diffusion in protein aggregation of IDPs and globular proteins

Due to the cell's necessity, intrinsically disordered proteins evolve linearly with evolution and hence, eukaryotes contain more IDPs than the prokaryotes. More than 50% of the proteins in the eukaryotic proteome are disordered and they play various roles in maintaining the cell's integrity, cell-cell connection (Babu 2016), etc. The liberty of having a large number of degrees of freedom makes IDPs completely different from the proteins those follow traditional “lock-key” mechanism (Morrison et al. 2006) while they interact with their binding partners. This behavior introduces wide range of molecular recognition depending upon the situation that the cell is facing. Thus, their stability, intactness for being functional, and degradation are tightly regulated within the cell and that is determined by their rotational and translational motions in the cell which can be sometimes explained by sub-diffusive behavior. Failure to perform its functions lead to the accumulation of aggregated IDPs in the cellular milieu and several complications such as neurological disorders (Jucker and Walker 2013), cancer (Levine and Kroemer 2008), etc. may result from it. In this context, diffusion of IDPs are also affected as the unfolded state of the globular protein is affected but not to the same extent and hence shows anomalies in diffusion in solution as well as inside the cell (Pauwels et al. 2017). The details of behaviors shown by IDPs are discussed in several literatures (Wang et al. 2012a; Soranno et al. 2014; Theillet et al. 2014; Pauwels et al. 2017). Here, our main focus is on the effect of anomalous diffusion on the IDP and its binding partner interactions. In order to understand the contribution of anomalous diffusion in the self-assembly of IDPs, a simple model with Brownian motion is needed to start with as the basic knowledge of this behavior is still not well-appreciated.

The work of Smoluchowski (2017) helps to determine the rate constant for the instantaneous association of two spherical particles freely diffusing with a relative diffusion constant of

D (in the absence of a long-range potential) at a contact distance a . This can be represented as:

$$k_a = 4\pi aD \quad (38)$$

Thereafter, several modifications were implemented to improve our knowledge about the influence of diffusion on the protein association (Zhou 1993; Wieczorek and Zielenkiewicz 2008). Interestingly, the above calculations are obtained by assuming that they are in steady state. Under such an assumption, the supply of reactant pairs by diffusion (reaction flux) which becomes the Smoluchowski rate constant (Zhou 2004) at steady state at time t , is:

$$k_a(t) = 4\pi aD \left[1 + \frac{a}{\sqrt{\pi Dt}} \right] \quad (39)$$

Further on, under the influence of a potential $U(r)$, the steady-state binding rate constant is derived by Debye (2011) as:

$$k_a = D \cdot f(U(r)) \quad (40)$$

Thus if the protein–protein interaction is diffusion-limited, then this slowing down of the diffusion will affect the intermolecular interaction. Whereas if the interaction is reaction-limited, then the slowed-down diffusion does not have any effect on this association. In addition, the incorporation of soft nonspecific interactions in the induced reaction potential would make more exciting to interpret the fate of a protein in cell. The combined effects of anomalous diffusion and favorable or non-favorable interaction potential will determine the conformation and stability of a protein and thus aggregation. The presence of anomalous diffusion was observed between two hard sphere by Tokuyama and Oppenheim (Felderhof 1990) as:

$$\frac{D_a}{D} = \frac{1 - \frac{9\phi}{32}}{1 + H(\phi) + \left(\frac{\phi}{0.5718} \right) \left(1 - \frac{\phi}{0.5718} \right)^2} \quad (41)$$

Where $H(\phi)$ is a function of volume fraction and it helps to explain the diffusion of labeled protein like bovine serum albumin in its own concentrated solution (Balbo et al. 2013).

Most importantly, anomalous diffusion has greater influence on effective concentrations and enhanced thermodynamic activities and that result in direct consequence on protein aggregation (Coquel et al. 2013). Here, we outline how it effects protein–protein association with few examples.

Contributions to equilibrium thermodynamics

The minimizing volume occupancy in high concentration of proteins and pronounced anomalous diffusion favor protein–

protein association, because these combined effects enable the system to increase the probability of collision of each proteins in the cellular milieu. For that case, the self-diffusion of the protein along the time scale and the length of the space is somehow responsible for this anomalous diffusion in the cellular environment. Roosen-runge et al. (2011) probed that the measured diffusion coefficient $D(\phi)$ of the bovine serum albumin (BSA) drastically decreased with the increment of the protein volume fraction ϕ explored in the range $7\% \leq \phi \leq 30\%$. With the help of an ellipsoidal protein model and an analytical framework involving colloid diffusion theory, the protein's self-diffusion at biological volume fractions is found to be slowed down to 20% of the dilute limit solely due to the effect of the neighboring molecules. This kind of behavior is seen in a transmembrane protein VSVG ts045 (Malchus and Weiss 2010) while it interacts with endoplasmic reticulum (ER) and the folded and unfolded form, both show anomalous diffusion in fluorescence correlation spectroscopy (FCS). They also demonstrated that the folding sensor of quality control (UTG1) accelerates the oligomerization of this VGVs ts045 protein and also shows anomalies in its diffusion. It is easily understandable that the multimeric complexes have a greater dimension than the monomeric proteins. The success of this simple mapping of the complex diffusion is promising in order to understand of the formation of aggregations compared to the functional one. Interestingly, the varying properties such as molecular weight, size, shape, and electrostatic interactions of the surrounding molecules influence the self-diffusion and thus the stability of the complexes in that environment (McGuffee and Elcock 2010; Basak and Chattopadhyay 2013). Moderate stability were observed in vitro for the formation of heterodimers of *E. coli* polymerase III theta-epsilon subunits (Conte et al. 2012) and the complex of superoxide dismutase and xanthine oxidase (Zhou et al. 2006) in different crowder environments. In contrast, the formation of complex of barnase-barstar (Phillip and Schreiber 2013) and the complex of TEM1 and β -lactamase-BLIP inhibitor in PEG- and dextran-crowded solutions (Phillip et al. 2009) are disfavored and opens a new avenue of rethinking about the correlation between the subdiffusive behavior and the aggregation mechanism. Although the examples of the protein aggregation of IDPs under the influence of anomalous diffusion are limited but it is believed that the intrinsic unfolded features and aggregation propensity of IDPs would implicate complex diffusion in cellular environment and accelerates the aggregation process.

Contributions to kinetics

Statistically, anomalous diffusion generates enormous probabilities for protein–protein interactions when these two proteins are in the close proximity at their binding interfaces. In vivo, the compartmentalization in the viscous surrounding

provides more confinement for these abundant two proteins of interest and increases the probability of them coming into each other's vicinity. Additionally, the steric repulsion also exacerbates the effect of slower translational diffusion. Collectively, these behaviors favor toward intracellular association rates and are oblivious to the dissociation kinetics and vice versa. This difference may help us to understand the discrepancy of the association rates in vitro and in vivo (Phillip and Schreiber 2013). One of the meaningful examples in this direction is that the association rates of the TEM1-BLIP are slower in crowded solutions than in HeLa cells (Phillip et al. 2012).

The anomalous diffusion of protein may play an important role in the action of chaperonins as its power law diffusion has a beneficial effect on the yield of proteins undergoing chaperonin-assisted folding by increasing the chance of a partially folded chain which will be educed for new rounds of accurate folding (Ellis 2003; Spiess et al. 2004).

Anomalous diffusion in gene expression

Gene expression is a molecular process which is regulated in order to utilize the genetic information to produce genetic product. These genetic products would be different proteins or functional RNA. The process includes several steps like transcription (Venters and Pugh 2009), RNA splicing (Wang et al. 2015), translation (Ramakrishnan 2002), post-translational modification (PTM) of proteins (Prabakaran et al. 2012). Interestingly, to fulfill the requirement, several proteins need to move from one region to another side in the cell compartments. The motion of the proteins for gene expression is also governed by the sub-diffusive behavior as these processes happen in the cell nucleus or cytoplasm, a highly dynamics and organized environment where the interactions within the constituents vary from strong to transient interactions (Fig. 4a). The in-depth understanding of gene expression is important as it is not only considered as the central phenomena in development but also a helpful tool for uncovering different diseases such as cancer, neurodegenerative diseases, etc.

As mentioned earlier, gene expression is associated with several preinitiation complex formation involving several domains and proteins to integrate a large number of transient signals within the nucleus. Thus the movement of the proteins, especially transcription factors (TF) (Elf et al. 2007), determines the shape of the nuclear landscapes and elucidates how fast or slow a TF exploring its accessible contact sites somehow regulates the gene expression in cell. In recent times, gene expression is believed to be a stochastic process (Friedman et al. 2006; Raj and van Oudenaarden 2009) in both prokaryotes and eukaryotes and the production of mRNA or protein (in translation) may occur in bursts (Yan et al. 2016). In the prokaryotes, the promoter switches

between “active states” to “inactive states” and thus mRNA synthesis is consistent within the long time series as the promoter follows a simple two-state switching model (Rieckh and Tkačik 2014). Therefore, one can therefore interpret that the process occurs for the sub-diffusion behavior of transcription factors at high concentration of protein. As gene expression is tightly regulated by TFs and their concentration in the cell milieu is in the order of nano-molar, hence the stochastic nature of the gene expression definitely comes from the diffusion of the TFs. Prior to the initiation of transcription, TFs come to bind to the promoter and then dissociate after making several copies of mRNA. When it dissociates from the promoter, there is a high probability of binding of the TFs to the promoter again and these extended rounds of rebinding and then dissociation makes the effective active and inactive state of the promoter last longer thereby producing mRNA in bursts (Corrigan et al. 2016; Yan et al. 2016). In the cellular environment, the sub-diffusive nature of TFs while reaching the desired target site decreases with the association rate of TFs with the corresponding binding sites and it increases with the probability of round rebinding and decreases the dissociation rate. The complexity arises from the diffusion behavior of the TFs, making the gene expression more stochastic in nature. Several biochemical reactions in the cell are affected by this diffusional phenomena where it is believed that it would be “facilitated diffusion” (Esadze and Stivers 2018) with fast target search time or “3D diffusion” in a crowded environment (Dey and Bhattacharjee 2018) or “anomalous diffusion” with recurrent binding and dissociation with template DNA (Zhao et al. 2017) but the proper model and the understanding is still elusive.

Although they are interconnected, the effect of the entropic contribution in the form of exclusion volume effect (Matsuda et al. 2014) and the weak interactions are discussed separately to decipher their part in the sub-diffusion of TFs in the cellular milieu. The participation of weak interactions in the anomalous diffusion is discussed elaborately by Woringer et al. (Jin et al. 2007) where they discussed the term “reduced dimensionality.” The interplay between the dimension of the random walk d_w and that of the available space to diffuse d_f determines the motions of the protein diffusion in the compact and non-compact category. Interestingly, due to the recurrent manner, compact exploration accelerates the reaction rate and decreases the target searching time. The entropic contribution part was discussed by Morelli et al. (2011) and talks about the effect available space on not only the molecular diffusion but also the shift of association-dissociation equilibrium. They showed that as a consequence of the shift of equilibrium of the binding of RNA polymerase (RNAP) and the regulatory proteins, slow diffusion of the latter increase the noise in gene expression, although the mean expression level is unaltered. Thus the effect of several interactions on the anomalous diffusion and on the biochemical reactions are

Fig. 4 **a** Variety of diffusion modes of the biomolecules in the bacterial cell. These diffusions are ranging from the slow diffusion in the macromolecular crowding and increased viscosity to the non-monotonic behavior in the mobility (hopping, jumping, and sliding) of the DNA-binding proteins due to the specific and non-specific interactions. Slow

diffusions are also observed in the oligomer formation and transient confined compartments. **b** Representative correlation functions for the different kinds of motions of the molecules in different media. Reprinting permissions were taken

understood to some extent but most is still ill-defined and is waiting for the advent of new models.

RNA splicing is another aspect of gene expression where in eukaryotes mRNA is modified by removing alternative introns in pre mRNAs and neighboring exons are put together with the help of an RNA–protein catalytic complex known as spliceosome. In this context, it would not be wrong if the contribution of the interplay of the interaction with neighboring molecules and RNA–protein complex to the anomalous diffusion is speculated. This is also evident when Notelaers et al. (2014) showed that alpha3 GlyRs has the tendency to show different types of anomalous diffusion. Thereby strengthening its role in RNA splicing and in determining lateral membrane trafficking. Thus these types of complex formation are also dependent on the mobility of the RNA as well as the participating protein. When the motion of the RNA comes into the discussion, it is well known that most RNA molecules are rapidly exported from the nucleus after their synthesis. The abundance of the mRNA, tRNA, and rRNA in the cytoplasm instantly after their synthesis indicates the

transport efficiency of these molecules within the nucleus. While transporting, the weak interactions may influence the MSD or diffusion of RNAs in this crowded environment (Ghosh et al. 2016) as mentioned in several literatures (Ando and Skolnick 2010; Trovato and Tozzini 2014; Kapanidis et al. 2018). Politz et al. (2002) measured the diffusional motion of labeled endogenous polyadenylated RNAs by *in vivo* hybridization using a poly (T)-oligonucleotide. It has been shown that the diffusional coefficient determined for the RNAs with the help of several labeling and imaging methods suggesting that diffusion is sufficient in order to effectively transport an mRNA from its sites of transcription to the nuclear periphery. In the case of rRNA, the observed diffusion properties were different as the diffusion of RNAs under the influence of frequent, transiently interaction with the pre-ribosomal subunits follow power law rule.

The interior of the nucleus is dynamic as well as crowded, whereas the chromatin is more composed and the mechanism of this organization is still unknown. The 20–30% of nucleus interior space is occupied by the chromatin and all most every

molecule has to weave around the heterogeneous chromatin environment. Interaction of these molecules with the chromatin regulates the nuclear organization and dynamics and the absence of it shows drastic transition of diffusion behavior in the nucleus as it is evident from the work by Bronshtein et al. (2015), where they showed that depletion of lamin A surprisingly amends genome dynamics and a dramatic transition is observed from slow anomalous diffusion to fast and normal diffusion. This shows that anomalous and localized motion has foremost implication on the core biophysical mechanisms. Due to the anomalous diffusion of chromatin, it takes a longer time in the order of hours to diffuse through a chromosomal territory and thus regulate it efficiently. This anomalous diffusion would be caused by the temporal chromatin-chromatin binding facilitated by few proteins. Interestingly, the anomaly of motion of these proteins are also affected depending upon their size and shape because the excluded volume generated by the chromatin applies more restrictions for the large protein and makes it sub-diffusive; however, smaller proteins are mostly unaffected. Furthermore, in the context of considering lower dimension of the motion on the heterogeneous chromatin, the concept of fractional reaction kinetics (Shinkai et al. 2016) is more pertinent to explain the peculiar motion of proteins (Golding and Cox 2006; Guigas and Weiss 2008) during transcription.

Translational mechanism is tightly regulated in a temporal and spatial manner. The localization and movement of mRNA is important to understand the translational behavior and consequently cellular motility. As discussed earlier, during the movement of a product of transcription from the nucleus to the cytoplasm of the cell, mRNA experiences several interactions with its neighbors and thus it shows different form of diffusion. This kind of interactions and hence diffusions have different impact on the (1) rates of the translation (Klump et al. 2013), (2) distribution of ribosomal mRNA (Chou 2003), (3) fraction of active translating mRNAs (Wang et al. 2016a), (4) diffusion models of translation complexes in cells (Cottrell et al. 2012), and (5) ribosome stalling (Dao Duc and Song 2018). With the advancement of methodology, several interesting aspects of translational dynamics of single mRNAs have been discovered, such as (a) the transition between translational and non-translational states of mRNA happens in a longer time scale, indicating mRNA may undergo through several translational shutdowns and re-initiation within their lifetime (Yan et al. 2016); and (b) tracking real-time movements of mRNA–ribosome complexes expressing cytosolic proteins experience three different behaviors: stationary, sub-diffusive, and diffusive (Wang and Chen 2016). Katz et al. (2016) showed that mRNA co-moving with ribosome demonstrates slower diffusion features in the thousands of single-particle trajectories compared to the non-translational mRNAs that are exposed to the translational inhibitors. The mRNA–ribosome complexes display a broad distribution of

mobility, which is barely correlated with the number of ribosomes on the mRNA. The heterogeneity of mobility is likely caused by interaction between the translating complexes and neighboring molecules (Kapanidis et al. 2018).

Several works have been implemented to reveal the complexity of the gene expression and our apologies as only a few of these could be discussed in this review. At the end of this section, gene expression can be explained mathematically as (Vilar and Saiz 2013):

Where TS_i and TS_j are the different transcription states and k_{ij} is the rate of interconversion. k_1 and k_3 are the rate of transcription and translation and k_2 and k_4 are the degradation rate of mRNA and protein. According to the above discussion, it is apparently proven that all these rate constants are the function of the diffusion of the individual biomolecules thus the appearance of anomalous diffusion whether it comes from the weak interactions within the complexes or from the excluded volume effect of the cellular biomolecules ultimately defines the basic biochemical reactions within the cell.

Anomalous diffusion in membrane protein interaction

Till now, it has been conceptualized that the transient interaction between protein and neighboring molecules and the limited space given by the surrounding gives rise of sub-diffusion of the molecules in cell. Membrane protein interaction is also not an exception where cellular membrane also restricts the available space for protein through a tight binding interaction and a compact phospholipid architecture and consequently imparts highly crowded environment with varying concentrations of macromolecules. Therefore, as a result of crowding, constraints of space alter the biochemical reactions. In addition, the diffusive transport is slowed down and it may appear as more anomalous on intermediate length and time for the protein in the presence of crowded membrane and vice versa. Thus the transition of smooth geometrical deformations due to protein crowding or even to topological changes during the formation of a transport vesicle makes in-depth understanding of crowded membrane systems mandatory to gain insights into the physiochemical basis of cellular membrane trafficking.

In my opinion, the literature survey of the effect of individual protein molecules on the diffusion of lipid membrane and the other way around would be helpful if they are discussed in a more segregated way but considering the length of the review, we briefly describe the appearance of power law diffusion in this system in a collective way. During the initial studies, Saffmann and Delbruck (1975) showed that the membrane components perform simple Brownian motion but later on several experiments using FCS (Mueller et al. 2011), FRAP (Favard 2018), and SPT (Jin et al. 2007) proved that there is the presence of anomalous diffusion in membrane protein interaction in a cell (Goiko et al. 2016; Yamamoto et al. 2017) in a certain time and length scale. The motion of a labeled dextran molecule was observed as anomalous diffusion in membrane using FCS by Schwille et al. (1999) as one of the pioneering work in this field (Fig. 4b). Further, Weiss et al. (2003, 2004) also showed sub-diffusion of fluorescently labeled enzyme on the membrane of ER and Golgi bodies in HeLa cell using FCS. The anomalies of diffusion of lipid is dependent on the lipid composition of the membrane and the membrane fluidity and this is also evident by early single-particle tracking experiments that showed different degrees of sub-diffusion for lipids (Seu et al. 2006). The presence of proteins affects the diffusion of the lipid bilayer and shows anomalous diffusion. FCS measurements have revealed that lipid diffusion coefficients in GUVs decreased by 30% for large membrane protein densities (Kyoung and Sheets 2008; Machán and Hof 2010; Ramadurai et al. 2010). In this direction, several simulations works have been done to improvise our understanding about cellular biochemistry (Noguchi and Gompper 2006; Yamamoto et al. 2015; Chavent et al. 2016; Metzler et al. 2016), where stronger effects were measured in CG-MD simulations. It was shown that lipid diffusion was slowed down in membranes crowded with WALP peptides as compared to bilayers without crowded molecules (Domański et al. 2012). In this system, the diffusion behavior of the protein molecules in the presence of membrane will be other phase of the coin. In this study, FRAP measurements revealed that the diffusion of proteins in the plasma membrane of living cells showed sub-diffusive nature as the total protein content of the membrane was increased (Alenghat and Golan 2013; Trimble and Grinstein 2015). The protein diffusion in GUV membranes experiences a twofold reduction in the diffusion constant, i.e., obstructed diffusion in the FCS measurement with the increment of protein concentrations. An anomalous diffusion of proteins in crowded endo membranes in living cells is also featured with similar anomaly exponents in FCS experiments (Guigas and Weiss 2008).

Several new approaches have been done to take a closer look into this phenomena in the form of different experimental (Deich et al. 2004; Hoskins et al. 2011; Fazal et al. 2015) and simulation works (Netz and Dorfmueller 1995; Saenko 2016).

Here, we try to put more emphasis on the experimental observations but there are several other literatures dealing with mainly simulation works (Slater and Yan Wu 1995; Nixon and Slater 1999; Ando and Skolnick 2010; McGuffee and Elcock 2010; Tabatabaei et al. 2011). Our apologies if we missed to mention interesting works of this field due to the length of the paper.

Perspectives

In this review, it has been shown that the biological mechanism in cells are tightly controlled and regulated by the spatial and temporal heterogeneity in the cellular constituents and the modeling of these heterogeneities help to understand the network machinery and the physiological implication of the transport system, e.g., anomalous diffusion. Due to this motion, the generation of noise encoded with variation information of the active and stochastic processes can govern a distinct cellular function and consequently the deconstruction of it can drastically alter the function. Several improvements have been incorporated in the experimental and theoretical levels to broaden our knowledge about cellular mechanism. New experimental methods (especially single-molecule biophysical methods) in studying of gene expression over time, the activity of large number of proteins, and tracking their interactions over long time and theoretical models with considering more complex parameters would be useful to trace out the responsible factors at the level from protein complex–DNA interaction (in cell size and growth) to protein–protein interaction (aggregation and degradation). In this context, few parts are well-appreciated but few which are the main constituents of system biology like transcription termination in eukaryotes, consequence of time delay, protein sliding on DNA, stalling, the dynamics of protein folding and unfolding, etc. are neglected. Realizing the weightage of every event inside cellular compartment and working on it makes the whole scenario complete. As a progressive effort, the increase of complexity in vitro assays and reduction of complexity in vivo assays will be more advantageous as the simplicity can facilitate interference and analysis of complex biological phenomena in defining the contribution of the individual particles in a crowded environment more closely. In a theoretical point of view, an optimization of generic design principles of cell mechanism is needed to be done on the basis of past models so that this universal generic model at least frames the mechanism in a generalized way if it exists. Thus, the future of biology will be built on more qualitative, model-based approach, with a balance between experimentation and modeling to study the biological living systems to the fullest.

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Compliance with ethical standards

Conflict of interest Sujit Basak declares that he has no conflict of interest. Sombuddha Sengupta declares that he has no conflict of interest. Krishnananda Chattopadhyay declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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