Handai Nanophotonics, Volume 3
H. Masuhara, S. Kawata and F. Tokunaga (Editors)

Chapter 11

General importance of anomalous diffusion in biological inhomogeneous systems

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1. INTRODUCTION

From the viewpoint of material science, all biological systems from primitive to higher ones are complex compositions of various inhomogeneous substances, for example, polymers, gels, membranes, solutions, and other so-called soft materials. Life is the total activity of the construction, one aspect of which is expressed as material transports within this inhomogeneous space followed by some chemical reactions, including molecular association induced by weak intermolecular interactions. Transporting substance involves gases such as oxygen and carbon dioxide, liquids such as bloods, nourishing matters, hormones, and various signaling molecules which exist both inside and outside of cells. Activity of life, or "live or dead" in other straightforward words, can be distinguished by the conditions of material transports among various organisms in many practical cases. Moreover, organisms with diseases may causes some troubles or difficulties in material transports and therefore their observation can be used as diagnosis of various biological systems. Different from other superficial observations checking the structure or morphology of the biological organs, this new approach through material transports is more closely related to the real life activities.

Material transports in media are quantitatively characterized by diffusion coefficients. Diffusion coefficients can be measured by various methods including several spectroscopic methods, such as fluorescence correlation spectroscopy (FCS) [1-4]. However, diffusion coefficients within inhomogeneous space are not constant showing complex behavior called "Anomalous diffusion (AD)"[5]. AD is defined as the transport phenomenon where the mean-square displacement (MSD) of diffusing particle is not in

proportion to the time progression. Normal diffusion (ND) coefficient cannot be defined any more in such cases.

In this chapter, we discuss the importance of AD in biological systems realizing various bio-activity in life.

2. GENERAL DESCRIPTION OF ANOMALOUS DIFFUSION

2.1. General review about the description of normal diffusion

Diffusion coefficient, which is widely accepted in many fields of science and technology, was first found in Fick's second law [6, 7]. Fick had no microscopic imagination based on molecules but seemed to have derived this concept from thermal conduction theory. Diffusion coefficient namely started as the coefficient in diffusion equation which provides phenomenological explanations about material transports.

Now a day, however, widely accepted definition of diffusion coefficient by physical scientists is given by the relationship with the MSD of diffusing particles which is a statistical quantity obtainable by some experimental techniques [1,8]. This is originally predicted by Albert Einstein for the diffusion induced by Brownian motion [8]. As shown in Fig.1, MSD is often given by a function of time t as $<r(t)^2>$ In normal diffusion (ND), MSD increases in proportion to t and diffusion coefficient D is defined as

$$< r(t)^2 >= 2dDt \qquad (1)$$

where d is the dimension of diffusion, or in other words, the freedom of diffusion. For 3-dimentional isotropic (Euclid) diffusion (1) becomes

$$< r(t)^2 >= 6Dt \qquad (2)$$

t must be sufficiently long to reach a stationery stage where we can rule out the effect of the initial inhomogeneity and local fluctuations but this criteria is fulfilled in normal experimental observation such as in spectroscopy or in diffraction methods.

Diffusion equation describing ND is generally successful in the explanation of the diffusions in ordinal but ideal liquids where the proportional relationship between MSD and t is valid over surprisingly wide time region, from picoseconds to, probably days or years. This success implies the fact that any material transports in normal liquids are induced and driven by microscopic Brownian motions of molecular scale, i.e. the spatial scale is smaller than 0.01 nm and the time scale is smaller then picoseconds. Einstein expressed this concept providing very simple equation called Einstein's equation as

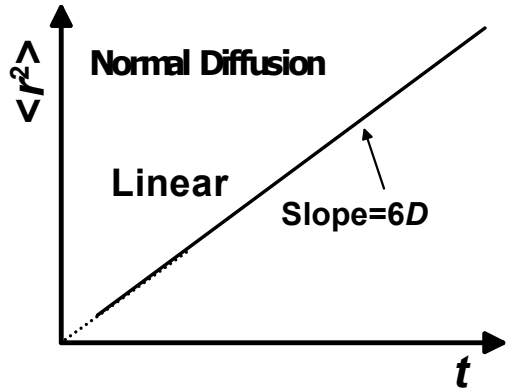

Fig. 1. The definition of diffusion coefficient derived from the time dependent function of mean square displacement (MSD), $<r(t)^2>$. See eq. (1).

$$D = \frac{\beta kT}{m} \tag{3}$$

at temperature T using a parameter β, where m and k stand for the mass of diffusing particle and Boltzmann factor, respectively [8]. Microscopic Brownian motion can be introduced to the diffusion equation by adding some stochastic terms such as random forces. One well-known stochastic differential equation of this kind is Langevin equation [9, 10] where the nature of Brownian motion appears in the time-correlation function of a stochastic random force $F(t)$ as

$$\ddot{x} + \beta\dot{x} = \frac{F(t)}{m} \tag{4}$$

in a style of the equation of motion. The nature of $F(t)$ can be analyzed using its auto-correlation function as

$$R(\tau) =< F(t)F(t+\tau) > \tag{5}$$

When the $F(t)$ is a pure random force (Brownian motion in homogeneous media), $R(t)$ takes a form expressing "white noise" with a delta function as

$$R(\tau) = R_0\delta(\tau) \tag{6}$$

In the diffusion process in homogenous media, the time integrals of $F(t)$ are supposed to show a Gauss distribution and the process belongs to Wiener process. However, this is not a unique solution for general diffusing transports and others are conceivable such as Levy-Flight motion [11, 12]. As far as we

treat homogenous systems classically as ND, we have almost no opportunity to encounter non-Wiener process and no necessity to use these extensions. However it can be revealed in the treatment of AD occurring in inhomogeneous space as simply discussed in the later sections of this article.

2.2. Extention of diffusion coefficient applicable to inhomogeneous systems

The majority of substances existing in this actual world, especially in the biological community, belong to inhomogeneous systems. The diffusion processes in such real substances are affected not only by spatial structures of diffusing spaces but also by some potential forces all of which have some typical scales, from nanometers to meters. One example of spatial structure is the meshwork structure in gels, glasses, or polymer solutions [1, 13-16]. One example of potential forces is the electrostatic interactions in colloidal solutions [17]. As the result, the physical properties concerning material transports (including D) may have some correlations to (or dependence on) the spatial scale [1]. Similarly, the structure of diffusing space also changes gradually along the progress of the time by some fluctuations and the above-mentioned physical properties may also have some correlations to the time scale. Accordingly, the static property in eq. (2) is changed to depend on the time and space scales in inhomogeneous systems as

$$< r(t)^2 >= 6D(t)t \tag{7}$$

or

$$< r(t)^2 >= 6D(L)t \tag{8}$$

where L is a typical length of diffusion traveling (we refer "diffusion length") and one can choose the square root of MSD for this parameter as

$$L = \sqrt{< r(t)^2 >} \tag{9}$$

It should be noted that eq. (2) can be used as the inter-conversion equation between t and L as

$$L = \sqrt{6D(t)t} \text{ or } L = \sqrt{6D(L)t} \tag{10}$$

regarding $D(t)$ and $D(L)$ as simple proportional factors for t [18, 19]. In the later sections of this article, we borrow this inter-conversion in the analyses of our observation of D since any experimental results have there typical t or L in their sampling as far as some spectroscopic or diffraction methods are employed. We

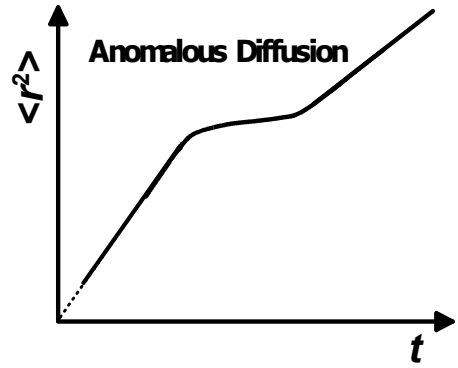

Fig. 2. An example of the lineshape of MSD for anomalous diffusion (AD). $\langle r(t)^2 \rangle$ becomes a variable function of t. (Compare with Fig.1)

call this kind of experiment as "single point measurement of D" and mark the observed results of D as D_{obs} to distinguish them. Even for inhomogeneous diffusions, we allow to use the following equation to obtain rough but sufficiently correct estimation for both t and L in treating a "single" experimental result.

$$L = \sqrt{6D_{obs}t} \tag{11}$$

In recent years, the term of "Anomalous diffusion" is widely used, the definition of which is the diffusion process where MSD is not in proportion to t as indicated in Fig.2. When plotted against linear t, MSD of normal diffusion is a straight line started from the origin (Fig. 1). For AD, however, the lineshape of observable (but actual observation is not easy) MSD is generally a free function of t as shown in Fig. 2. There is one question how we can define the time dependent diffusion coefficient $D(t)$ from the curve in Fig. 2. Another question is what is the relationship between $D(t)$ and experimentally obtained D_{obs}.

3. RELATIONSHIP OF THE OBSERVED DIFFUSION COEFFICIENT (D_{OBS}) WITH THE SAMPLING FUNCTIONS OF EACH EXPERIMENTAL METHOD

In AD, the measurement of diffusion coefficients (we refer this as diffusiometry in this chapter) cannot be straightforwardly connected with the real MSD function. Observation of D is performed under the experimental regulation in the scales of the time and space. If we choose one molecule, the value of D is approximately constant, however, the time scale can be changed by orders depending on the experimental scales which covers, for example, 10^{-9} – 10^{-0} m.

Since most of the experimental techniques have their own setups with a typical spatial scale, one experiment never covers this full time scale of MSD plot in Fig.2.

Another problem is the statistical sampling always accompanied by diffusiometry. When the real displacement ($r_i(t)^2$) of each single particle (i) can be monitored, the definition of MSD is expressed as

$$< r(t)^2 >= \lim_{N\to\infty} \frac{1}{N} \sum_{i=1}^{N} r_i(t)^2 \tag{12}$$

For diffusion of the particles smaller than the optical resolution limit such as ordinary molecules, we have no way to observe the real displacement $r_i(t)$. Instead of this, we choose an event which reflects the particle displacement and measure the period (sometimes statistical) in which the event undergoes. This is one-way derivation and we can never reproduce $r_i(t)$ from MSD.

Here we examine several examples of diffusiometry with spectroscopic techniques.

In FCS [1-4], the event in the view is decrease or increase of the molecules caught in the confocal volume (CV). The fluctuation (i.e. its correlation time) of the fluorescence intensity reflects the period of the stay for single molecule in the CV. In this case, the spatial condition (the size and shape of CV) is defined at first, the event (molecular motion intersecting the CV border) is defined next, and then, a time-dependent behavior (autocorrelation function) is monitored as the experimental result. A fitting function is used for the autocorrelation function to obtain the mean stay period (τ_R) inside CV. Typical size of CV in FCS is hundreds nm in horizontal diameter and typical τ_R is longer than μs.

For PFG-NMR method [18,19], as another example, the spatial resolution realized by magnetic field gradient is defined first, the escape of molecule from the area is used as the event, and a time-dependent behavior (signal decay versus the duration of two pulses) is obtained. Typical size of field gradient resolution is μm and typical decay constant is longer than ms.

FRAP (fluorescence recovery after photobleaching) has also connect the spatial information observed by microscopes with a time-dependent behavior (fluorescence recovery) induced by the diffusion of dye molecules. When a laser scanning microscope is used, the typical size of diffusing space is larger than μm and time scale is longer than ms.

All these experimental methods were developed and designed only for ND. When ND is expected for the system to be investigated, the effect of the shape of sampling function on the result is negligible since the true D value is only one at any t or L range. In most cases in ND, simple averaged values are used to obtain D.

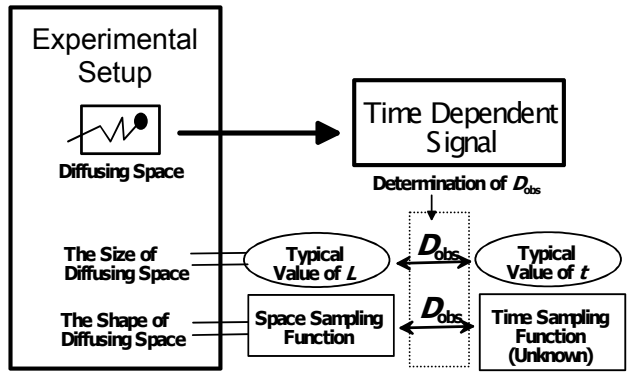

Fig. 3. A schematic diagram describing the effect of the experimental regulation of diffusiometry. The time sampling function is indirectly controlled by the shape and the size of diffusing space.

In Fig.3 we schematically indicate this situation. All these methods have their own space where the particles diffuse and time-dependent signals from the particles are recorded and accumulated. After appropriate analysis, D_{obs} is determined. Each method has its own size of diffusing space and L takes typical values depending on this size. Then this typical L is connected with typical t by

the value of D_{obs}. Therefore the typical value of t is indirectly regulated by the size of the diffusing space. In the same way, the shape and the size of the diffusing space indirectly controls the statistical distribution of the data in the time region through the space sampling function. This distribution, which is precisely unknown, can be regarded as the time sampling function for MSD. Obtained values of D_{obs} are the reflection of this time sampling function and sometimes deviate from the value of $D(t)$.

In AD, where the lineshape of MSD is not linear as shown in Fig.2, there is no guarantee that we can reproduce the true lineshape of $D(t)$ in connecting number of data points of D_{obs} obtained by various diffusiometries. Nevertheless, we dared to have done this approach in the series of our previous works [2-4, 18,19] which seems rather informative and useful in spite of its lack of precise background.

4. DIFFERENTIATION OF MSD AND ANOMALOUS DIFFUSION COEFFICIENT

In the last section we aware that the sampling method in the experiment is essential to discuss the relationship between D_{obs} and $D(t)$. In this section, we provide another remark on the differentiation of MSD in AD. Differentiation can be regarded as a limit ($\Delta t \rightarrow 0$) style of the sampling function in the time region.

For ND, eq.(2) is permanently correct and

$$\frac{d < r(t)^2 >}{dt} = 6D \tag{13}$$

is obtained. For AD, however,

$$\frac{d < r(t)^2 >}{dt} = 6D(t) + 6D'(t) \tag{14}$$

is obtained from eq. (7) and

$$\frac{d < r(t)^2 >}{dt} = 6D(t) \tag{15}$$

is incorrect because the second term in eq. (14) is neglected. In Fig 4, we show the difference between $d<r(t)^2>/dt$ and $6D(t)$ on the MSD curve. In real diffusiometry, for which the sampling function is ambiguous, both cases are possible that obtained D_{obs} is close to $d<r(t)^2>/dt$ or $6D(t)$.

5. EXAMPLES OF DIFFUSIOMETRY FOR INHOMOGENEOUS SYSTEM WITH ANOMALOUS DIFFUSION

In resent years, we performed diffusiometry on aqueous solutions of hyaluronic acid (HA) using several kinds of spectroscopic methods.[2-4, 18, 19] We collected the several numbers of data on single measurement (D_{obs}) and aligned

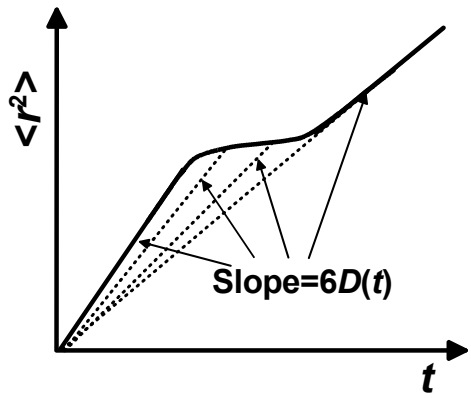

Fig. 4. The $6D(t)$ in the MSD curve of AD is the slope of linear line connecting the origin and $<r(t)^2>$ data points. The $d<r(t)^2>/dt$ is not equal to $6D(t)$. (See eq . (14))

them against the diffusion distance L or the diffusion time t. We could obtain a smooth line in each systems as shown in Fig. 5 and suppose that the resultant lines indicate rough profiles of $D(t)$ or $D(L)$.

The details of experiments were already presented in elsewhere. We used three kinds of diffusiometries for cytchrome c: Photochemical bimolecular reaction (PCBR), SVC-FCS, and PFG-NMR. A smaller molecule Alexa 488 showed a nice AD curve in SVC-FCS results. The value of t was determined as follows: (1) For PCBR, the time constant of the exponential decay of the excited state (fluorescence lifetime) was used. (2) For SVC-FCS, the average stay period in CV (τ_R) was used. (3) For PFG-NMR, the duration between two field

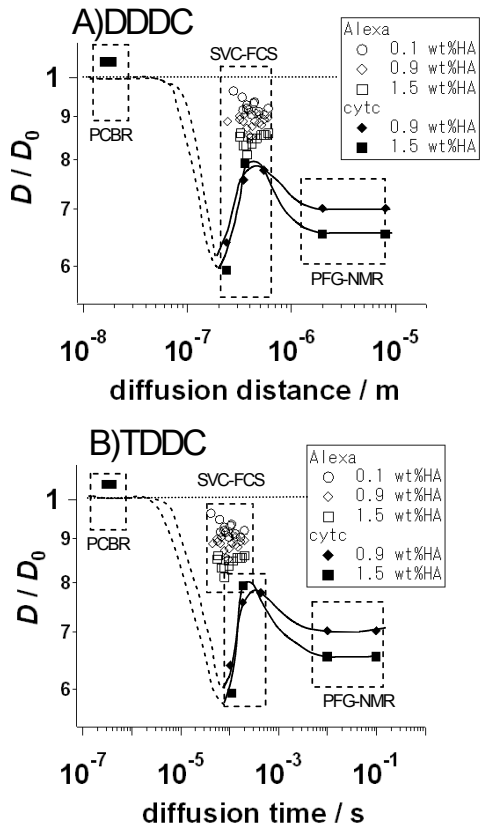

Fig. 5. A) The distance dependence of diffusion coefficient (DDDC) and B) the time dependence of diffusion coefficient (TDDC) plots for Dobs obtained in the aqueous solution of hyaluronan (HA). Diffusing molecules are Alexa 488 and cytchrome c. Use diffusiometries are PCBR (photochemical biomolecular reaction), SVC-FCS (sampling volume controlled fluorescence correlation spectroscopy), and PFG-NMR (pulsed field gradient nuclear magnetic resonance). (Reproduced from Ref. 4)

gradient pulses were used. We obtained corresponding L value for each data point by eq. (11). Both the distance dependence of diffusion coefficient (DDDC) plot and the time dependence of diffusion coefficient (TDDC) plot are shown in Fig. 5. These data points are D_{obs} which involve small deviation from true $D(L)$ or $D(t)$ because of the varieties of the sampling functions for each D_{obs} point.

Since the HA solutions are believed to form meshwork structures, it is reasonable that the profile of $D(L)$ becomes a step function as shown in Fig.6. For HA solutions used in our experiment, the typical mesh size (μ) was 5 - 30nm which was one order larger than the ordinary gel solutions. In the short distance limit (Plateau I: $L \rightarrow 0$), the line was flat and D_{obs} was constant and close to the value in water (buffer solution) without HA. In the long distance limit (Plateau II: $L >\!\!> \mu$), the line was flat again because the mesh size was sufficiently small to be regarded as homogenous media in this large scale of L. The interactions between the diffusing molecules and the HA mesh act as if friction in the continuous media. Different from plateau I, the position of plateau II depends on the mesh size, i.e. the concentration of HA. The HA concentration (C_{HA}) dependence of D_{obs} in plateau II exhibits an exponential curve as

$$D_{obs} = D_0 \exp(-\xi C_{HA}{}^{0.5}) \tag{16}$$

as suggested by Ogston et al [2-4, 21] for general gel solutions.

Between these two plateaus a step was found around which the value of D_{obs} dramatically changes. SVC-FCS method, which we developed recently, is a powerful technique to resolve the lineshape of curvature around the step. The position of the step in the spatial scale was 1-2 order larger than μ and also depends on the size of diffusing particles. The step shifts to lower t and L for larger molecules and realizes the size selection of the molecule like molecular sieving.

The mechanism of decrease in D_{obs} is explained as gradual decrease of the volume of reachable space within diffusing period along which the molecules are occasionally inhibited from their free motions encountering the polymer chains. The term “Ant in the labyrinth” [13, 22, 23] is used to describe this situation. Since the area where the value of D_{obs} changes is restricted in very narrow region of TDDC or DDDC plots, we call this region “local anomalous diffusion area” [4] as shown in Fig. 6. Appearance of local AD area is an importance character of gel-like fluid containing meshwork space.

Since meshwork structures are generally found in almost all biological systems, anomalous diffusion, especially the local anomalous diffusion, should be essential to regulate the biological activity through the material transports. In next section we discuss a typical example of extracellular matrix.

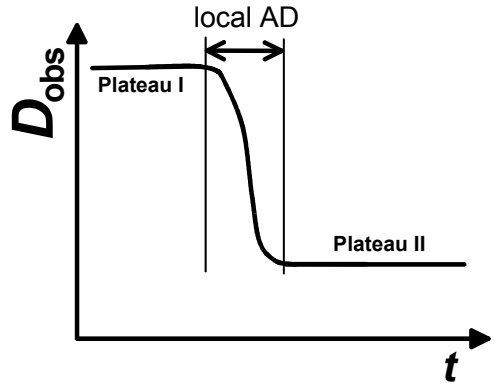

Fig. 6. A typical TDDC curve observed for inhomogeneous solutions containing mesh structure.

6. GENERAL IMPORTANCE OF ANOMALOUS DIFFUSION IN MATERIAL TRANSPORTS IN EXTRACELLULAR MATRICES (ECM)

Extracellular matrix (ECM) [24, 25] is the generalized name of variety of organs in animals other than cells. About a half volume of animal body is constructed by ECM. ECM is generated from cells, surrounds cells, interacts with cells, and is occasionally decomposed by cells. Sometimes cell itself migrates through the ECM space as seen in tumor cell migration.

Until recently, the role of ECM had been thought to be limited to ones of less importance in bio-activity such as constructing the body, holding moisture, etc.. However, recent studies reveal the significance of ECMs which communicate with cells continuously in their life activities. One example is the role of cell adhesion molecules (CAMs). The most famous CAM, cadherins act as connectors in cell-cell adhesion [26]. There are another group of CAMs which interact with ECMs such as integrins [27] (interacting with collagens) and CD44 [28] (interacting with HA). They act as the anchors of the cell contacting with ECM. Cells select cell-cell adhesion or cell-ECM adhesion in their activity and CAMs and ECMs are believed to play crucial roles in development and tumor migrations.

In Fig. 7, a cartoon of typical ECM (assuming ECM in cartilage) is indicated. The ECM is composed of largely of collagen fibrils which forms a stiff mesh structure. Rather small amount of glycoproteins, HA, and proteoglycans exist to fill up the large space between collagens. These glycomaterials form soft meshwork of hydrated gels and also hinder the materials that diffuse in ECM. Our previous results indicate that even a small amount (0.1 wt%) of HA affects the diffusion modes significantly. Therefore, material transports in ECM should be AD including local AD.

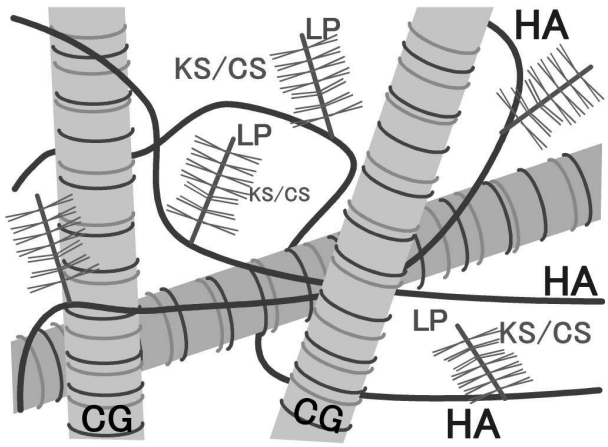

Fig. 7. A cartoon showing the structure of a typical ECM (assuming ECM in cartilage) composed of collagen (CG), hyaluronan (HA), link protein (LP), chondroitin sulfate (CS), keratan sulphate (KS). The LP+CS/KS are called aggrecan.

Now we scope on the cell surface activity in contact with ECM simply comparing two situations with and without ECM as shown in Fig. 8. The cell in the lower position secrets some materials including signaling molecules from P and Q. In free diffusion without ECM, materials secreted from P disperse into open space. Very small number of molecules can reach A, B and C and the majority will be lost in outer space. However, in the existence of ECM, the materials from P are not lost but held in ECM forming a gradient of concentration. The probability to reach B and C is still low but the selectivity to be conveyed to A is improved. In the same way, the material secreted from Q is easily accepted by the adjacent cell in the existence of ECM. The ECM seems to control the yield of chemical reactions by AD especially for the cell-cell communications.

This strategy to improve the yield of chemical reactions is adequately designed. The rate of bimolecular reaction between R and S is naively expressed as

$$v = 4\pi(D_R + D_S)[\mathrm{R}][\mathrm{S}] \tag{17}$$

If S is an acceptor fixed on the cell surface, (17) is simplified to be

$$v = 4\pi D_R[\mathrm{R}]S_0 \tag{18}$$

where S_0 is a constant. To increase the magnitude of v, we have two possibilities to increase [R] or to increase D_R. In biological system, however, the first

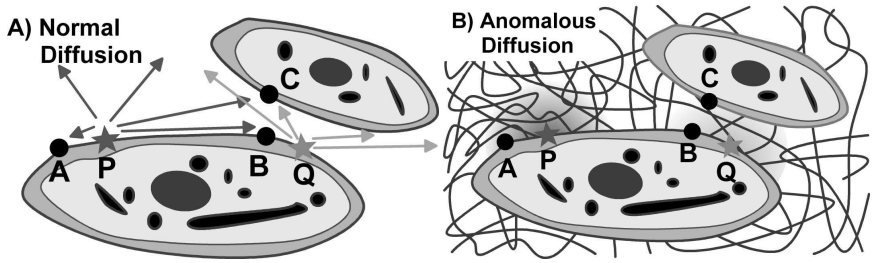

Fig. 8. Two cartoons comparing the cell with and without ECM. Some materials are secreted from P and Q to be accepted at A, B or C. A) Normal diffusion without ECM. B) Anomalous diffusion with ECM.

strategy to increase [R] is inadequate because the excess R is generated and the whole biological system needs to find a way to clean up the existing R which large amount of R and in sweeping up the excess R. Instead of this, the second strategy to increase (or control) D_R is the most rational one in sustaining the life activity.

7. CONCLUSIONS

In this chapter, we described the theoretical background for describing AD, the experimental techniques to resolve AD in spectroscopy together with their results, and one example of ECM showing important roles of AD. Needless to say, AD also occurs in intracellular material transports as observed by FCS and molecular migrations in membranes. Although we have no space to discuss all of them, the general importance of AD in total activity of biological system is apparent.

ACKNOWLEDGEMENTS

This research is partly supported by The Cosmetology Research Foundation, and the Grant-In-Aid for Scientific Research (Kakenhi) Grant No. 17034067 in Priority Area “Molecular Nano Dynamics” and Grant No. 17300166 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

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