Revue Roumaine de Chimie, **2008**, *53*(5), 415–419

COMPUTATIONAL STUDY OF DIFFUSION IN CELLULAR TWO-DIMENSIONAL CROWDED MEDIA MODELED AS MIXTURES OF MOBILE AND IMMOBILE OBSTACLES

Adriana ISVORAN,[a*] Eudald VILASECA,[b,c] Laura UNIPAN,[d] Josep-Lluis GARCÉS[e] and Francesc MAS[b,c]

[a]Department of Chemistry, Faculty of Chemistry Biology Geography, University of the West Timişoara, Pestallozi 16, 300115 Timişoara, Roumania
[b] Theoretical Chemistry Research Centre (CeRQT) of Scientific Park of Barcelona (PCB), Barcelona, Spain
[c]Physical Chemistry Department, Barcelona University (UB), Barcelona, Spain
[d]Department of Agriculture, Faculty of Agriculture, University of Agricultural Sciences of Banat, Calea Aradului 119, 300645 Timişoara, Roumania
[e]Chemistry Department, Lleida University (UdL), Lleida, Spain

Received August 30, 2007

The aim of this paper is to reveal how diffusion in cellular obstructed media is affected by obstacles' mobility using a Monte Carlo simulation algorithm. Macromolecular crowding is a characteristic of cellular media and it has strong effects on many physical and chemical phenomena, diffusion being one of the most affected. We model small particles diffusion processes in cell membranes using two-dimensional lattices with obstacles having different sizes and different degrees of mobility. There are two types of obstacles: the first ones mimic membrane proteins, are bigger and immobile and the second ones mimic membrane lipids, are smaller and have different degrees of mobility. Our simulations show that in all obstructed lattices diffusion is anomalous for short times and becomes normal for long times. They also reveal that for increasing mobility of obstacles diffusion is less anomalous and the crossover time from one regime to another decreases with increasing mobility of obstacles. These results reflect that membrane fluidity strongly facilitates small particles diffusion processes and underline the applicability of these kind of computational studies for analysing molecular diffusion in cellular media.

INTRODUCTION

It is well known that environment properties always affect molecular mobility and this is also true for intracellular diffusion, which has been described as hindered. The main physical reason for this behaviour seems to be molecular crowding. If it is associated with binding interactions with immobile or mobile molecules, the effects are more important. As diffusion is implied in many cellular mechanisms, in order to understand the dynamics of the cell or of its different compartments it is important to understand the diffusion of different types of molecules in crowded environment. Diffusion properties in random, disordered, crowded media are subject to considerable theoretical and experimental work.[1-25] For cellular media there are two separate directions: one of them concerns diffusion in membranes considered as two-dimensional (2D) media and the other concerns diffusion in cytoplasm, organelles and nucleus considered as three-dimensional media (3D). Theoretical studies made in the last twenty years suggested that in crowded media diffusion is anomalous for short times and normal for long times for both 2D and 3D media.[1-17] Taking into account that there are numerous single-particle events in living cells, we have also simulated single-particle diffusion in both 2D and 3D media with obstacles. We use two manners for obstacles distribution: random distribution and uniform distribution with different free spaces between two parallel obstacles.[1] We have found that randomly distributed obstacles are much more efficient barriers for small particle diffusion than uniformly distributed ones and that a higher free space between the parallel obstacles facilitates the diffusion process.[1] There are also quite recent experimental studies revealing that theoretical predictions are correct. [18-25]

* Corresponding author: aisvoran@cbg.uvt.ro

Even if there is a big amount of theoretical and experimental studies concerning diffusion in cellular media, this process is not completely understood and described. **A**nomalous diffusion seems to be caused by multiple mechanisms whose effects are complex. Within this paper we extend the algorithm that we have used in previous simulations[1-4] for more realistic situations within cell membrane and we perform a computational analysis of small particles diffusion (water molecules, ions, gas molecules, small therapeutic agents, small metabolites) in 2D crowded media. We are interested to reveal the effect of obstacles mobility on anomalous diffusion. In order to simulate realistic situations within living cells, we consider two kinds of obstacles: ones bigger and immobile to mimic proteins in cell membrane and the others smaller and having different degrees of mobility to mimic lipids in membranes having different fluidity. We consider proteins as immobile because their mobility is clearly lower than that of lipids.

RESULTS

In Figure 1 we show the plots of mean square displacement of tracers versus time and in Figure 2 we show the plot $log(\langle r^2 \rangle/t)$ versus $log(t)$ for diffusion of tracers in 2D lattices with obstacles having different degrees of relative mobility (M) in comparison to diffusion of tracers in lattices without obstacles, which mimic homogenous media.

From Figure 1 we notice that increasing obstacles' mobility means a less anomalous diffusion, the slopes of the lines corresponding to higher mobility being higher. For tracers diffusion in unobstructed lattice the slope of the line is 1 and diffusion is normal. If we put the plot from Figure 1 in double logarithmical scale, the slopes equal anomalous diffusion exponents, their values being presented in table 1. These values increase with increasing the obstacles mobility and it also underlines that increased mobility means less anomalous diffusion.

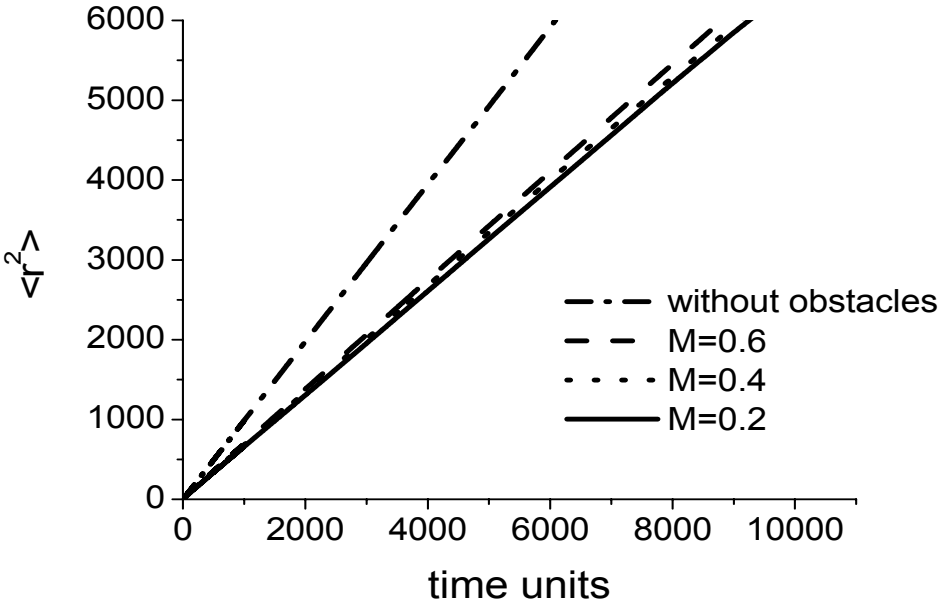

Fig. 1 – The of mean square displacement versus time for tracers diffusion ([T]=0.01) in 2D media with immobile quasi-octagonal obstacles 7x7 ($[O]_{im}$=0.111) and mobile quasi-octagonal obstacles 5x5 ($[O]_{m}$=0.168) with different degrees of mobility: M=0.2 for solid line, M=0.4 for dotted line, M=0.6 for dashed line. The dot-dashed line corresponds to diffusion in unobstructed lattice.

Table 1

The anomalous diffusion exponents and crossover time values for tracer diffusion in obstructed lattices with obstacles having different degrees of mobility in comparison to tracer diffusion in unobstructed lattice

Obstacles mobility M	Obstacles relative mobility	Anomalous diffusion exponent, α	Crossover time (time steps)
0.2	0.175	0.9746 ± 0.0002	1000 ± 10
0.4	0.350	0.9766 ± 0.0001	660 ± 10
0.6	0.520	0.9803 ± 0.0001	494 ± 10
Unobstructed lattice	Unobstructed lattice	1.0002 ± 0.0001	-

According to equation (3), the ratio $<r^2>/t$ represents diffusion coefficient and Figure 2 shows its time dependence for obstructed diffusion. For unobstructed diffusion it is constant (dash-dotted line in figure 3). The irregularities at the end of these curves are due to statistical noise.

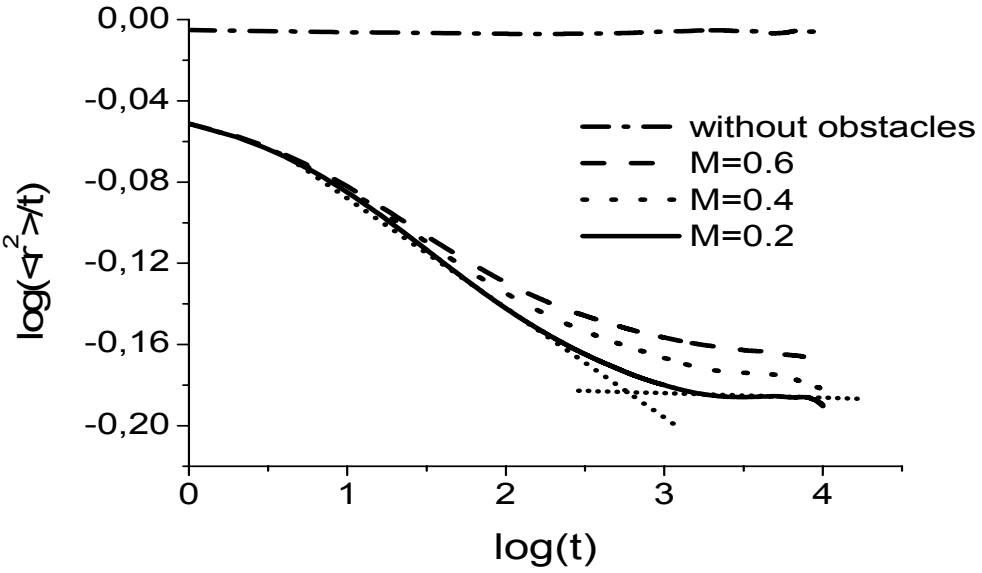

Fig. 2 – The plot log($<r^2>/t$) versus log(t) for tracers diffusion ([T]=0.01) in 2D media with immobile quasi-octagonal obstacles 7x7 ($[O]_{im}$=0.111) and mobile obstacles ($[O]_m$=0.168) with different degrees of mobility: M=0.2 for solid line, M=0.4 for dotted line, M=0.6 for dashed line. The dot-dashed line corresponds to diffusion in unobstructed lattice and short-dotted lines show the manner to determine the crossover time from anomalous diffusion to normal one.

When diffusion is obstructed, we notice that there is a crossover from anomalous diffusion at short times to normal diffusion at long times. The crossover time from one regime to another could be determined as the intersection of linear fitting lines for the regions of the curve corresponding to the two regimes[8], as illustrated in Figure 2. The crossover time decreases as the obstacles' mobility increases, see Table 1. Also, the diffusion coefficient decrease is more accentuated for smaller mobility of obstacles.

DISCUSSION

The results presented here suggest anomalous diffusion of small particles in a 2D media modeled as a mixture of mobile and immobile obstacles. We compare the results obtained here with those obtained for small particles diffusion in 2D crowded media where all the obstacles are immobile, tracers' concentration being the same.[2,3] For tracers diffusion in a 300x300 2D lattice with immobile obstacles 5x5 with truncated corners in concentration 0.008 the anomalous diffusion exponent is 0.9300 ± 0.0001[3] and for tracers diffusion in a 300x300 lattice with immobile obstacles 7x7 with truncated corners in concentration 0.003 the anomalous diffusion exponent is 0.9320 ± 0.0001.[3] In this study, for a higher total concentration of mobile and immobile obstacles (0.011) and the lowest relative mobility of small obstacles (0.175) the anomalous diffusion exponent is higher, 0.9746 ± 0.0002 (see Table 1). For the other higher relative mobilities, anomalous diffusion coefficients are even higher (see also Table 1).

In case of the same concentration of tracers ([T]=0.01), their diffusion in a 300x300 lattice with obstacles 5x5 with truncated corners in concentration 0.006 and having the same mobility as in this study (0.2, 0.4 and 0.6 respectively) the anomalous diffusion coefficients are[2]: 0.9860±0.0002 for M=0.2, 0.9881±0.0002 for M=0.4 and 0.9921±0.0001 for M= 0.6. All these coefficients are higher than those presented in this study, but there all the obstacles are mobile and their density is smaller.

All these results strongly favor the idea that obstacles mobility favours diffusion of small particles and they underline the importance of cell membrane fluidity for cellular transport phenomena.

Other simulation data reveal that diffusion process in 2D cellular media is strongly dependent on obstacles concentration, geometry and size,[2-4, 8, 14] on tracer concentrations[3, 12] and on the manner the obstacles are distributed within the lattice.[1] For

single-particle diffusion in 2D obstructed lattices with obstacles distributed randomly respective uniformly we have noticed that a random distribution of obstacles is a much more efficient barrier against diffusion than their uniform distribution.[1] Also, the diffusion process is facilitated when there are larger free spaces between the parallel obstacles.[1] These simulation results also underline the importance of membrane structural organization for small particle diffusion through it. Diffusion process is also affected by aggregation and structuring processes.[11, 15]

METHODOLOGY

i) Anomalous diffusion

In most real systems disorder may exist over a finite range of distances and in this range diffusion is not classical, but anomalous.[5] At longer distances the effect of disorder disappears due to statistical effects which cancel each other and diffusion becomes normal. For normal diffusion the mean-square displacement of the diffusing particle, $< r^2 >$, is proportional to time, *t*, being dependent on the topological dimensionality, *d*, of the medium

$$< r^2 > = (2d)Dt \qquad (1)$$

where D is diffusion constant.[5]

In anomalous diffusion the mean-square displacement is non-linear with time, i.e.

$$< r^2 > \sim t^{\alpha} \qquad (2)$$

where α is called anomalous diffusion exponent[6]. If $\alpha < 1$ we talk about sub-diffusion (and it is the case for diffusion in crowded media) and if $\alpha > 1$ we talk about super-diffusion[6]. In case of sub-diffusion we may define a normalised, time dependent diffusion coefficient[7]

$$D(t) \sim < r^2 > / t \sim t^{\alpha - 1} \qquad (3)$$

When $\alpha = 1$ D becomes a constant and diffusion process is normal.

ii) Computational study

In order to illustrate anomalous diffusion in crowded media we perform simulations using a Monte Carlo algorithm that was described elsewhere.[1-3] This algorithm was improved in this study in order to allow taking into account of two kinds of obstacles, ones smaller and mobile with different degrees of mobility and the other bigger and immobile. The degree of mobility is modeled within the algorithm by considering the movement of small obstacles with different probabilities: 0.2, 0.4 and 0.6 respectively. The algorithm generates a random number and compares it with the imposed probability. If the randomly generated number is smaller or equal to the probability the obstacle has the possibility to move and if it is greater than this probability the obstacle does not move. We have used 300x300 square lattices with boundary conditions. We have chosen this size of the lattice for two reasons: we have noticed a dependence of simulation data on lattice sizes and that a higher lattice gives more accurate data[4] and we also have taken into account the computational time which strongly increases for a bigger lattice.

So we have decided to make a compromise between lattice size and computational time and to use a lattice which is big enough and requiring reasonable computational time.

Every tracer occupies a single site in the lattice, tracers' concentration being [T]=0.01. Each small obstacle occupies a square of 5×5 sites with truncated corners (it is quasi-octagonal occupying 21 sites) and each big obstacle occupies a square of 7×7 sites with truncated corners (37 sites).

As the obstacles occupy many sites, we must distinguish between their concentration and the density of occupied sites by obstacles. Density of occupied sites means the percent of individual occupied sites by obstacles in the lattice. For immobile obstacles, their concentration is 0.003 and the density of sites occupied by them is $[O]_{im}$=0.111 being under percolation threshold, $[O]_{p.t.}$=0.4. For mobile obstacles, their concentration is 0.008 and the density of sites occupied by them is $[O]_m$=0.168. In case of tracers, as every tracer occupies a site in the lattice, their concentration is equal to their density.

Smaller obstacles are mobile having different degrees of mobility (M): 0.2, 0.4 and 0.6 respectively. We must underline that these values illustrate the frequency that we propose for the displacement of a small obstacle. We consider repulsive interactions between particles (every site in the lattice can be occupied by a single particle at the same time) and it decreases the real mobility of the obstacles. We register the number of movements for every tracer and also for every

obstacle and we may calculate after each simulation the real mobility for both tracers and obstacles. Then we calculate a relative mobility of mobile obstacles in comparison to tracers. We used 10000 time steps in each simulation and we performed 20 independent runs, the mean-square displacement for diffusing particle being averaged for these independent runs. The Monte Carlo routines were implemented using in-house Fortran 77 programs.

CONCLUSIONS

Our simulation data reveal anomalous diffusion of small particles in 2D crowded media for short times and normal diffusion for long times. There is a crossover time between the two regimes that decreases as the mobility of obstacles increases because the degree of mixing within the lattice increases with increasing mobility and this fact facilitates the diffusion process. This observation is very important for biological media where some diffusion processes are very rapid. Also, as obstacles' mobility increases, diffusion is less anomalous the reason being the same – a better mixing within the system. These results are in good agreement with other simulation data which reveal that in 2D crowded media diffusion is anomalous for short times and normal for long times.[1-4, 8-15] Our considerations are quite different from the other simulations presented in the literature because we analyze diffusion of small particles in a two-dimensional crowded media modeled as a mixture of mobile obstacles (that mimic membrane lipids) and immobile obstacles (that mimic membrane proteins). In order to reveal more realistic situations within the cell, the immobile obstacles have higher sizes than mobile ones. Also, we take into account different degrees of mobility for mobile obstacles in order to analyze small particle diffusion through membranes in different states, from gel to fluid state. There is in the literature another simulation study of diffusion in mixture of mobile and immobile obstacles concerning lateral diffusion of lipids in cell membrane.[10] Within this study all the particles (tracers and obstacles) have the same sizes. Our approach is different, we consider small tracers and obstacles having different sizes, but the results are qualitatively similar. Our results are also in good qualitative agreement with experimental data showing hindered diffusion of proteins, [17-19] nanoparticles[20-24] and water[25] in 2D crowded media.

In our opinion, another important conclusion which results from this paper is that theoretical investigations and modelling of cellular media must take into account the fact that cell organization is dynamic, accounting for motions and rearrangements at different space and time scales. They also must consider the interplay between diffusive motions and, if possible, between them and other various biochemical and biophysical events.

REFERENCES

1. A. Isvoran, E. Vilaseca., L. Unipan,. J. L. Garces and F. Mas**,** *Rom. J. Biophys*., **2007,** *17*, 21-32.
2. A. Isvoran, E. Vilaseca, L. Unipan, J. L. Garces and F. Mas, *Proceedings of the 6th Balkan Physics Union Conference*, *AIP*, **2006**, *889*, 469-471.
3. A. Isvoran and L. Unipan, "Living cell – a fractal structure and crowded medium", West University of Timişoara, **2006,** p. 70 – 85.
4. A. Isvoran, E. Vilaseca, L. Unipan, J. L. Garces and F. Mas, *Ann.West Univ. Tim., Series Chemistry*, **2007**, in press.
5. D. Ben-Avraham and S. Havlin, "Diffusion and reactions in fractals and disordered systems", Cambridge University Press, 2000, p. 59-68.
6. A. Bunde and S. Havlin, "Fractal and disordered systems", Springer, Berlin, 1990**,** p. 25-34.
7. T. G. Dewey, "Fractals in molecular biophysics", Oxford University Press, New York, 1997, p. 120 -128.
8. M. J. Saxton, *Biophys. J.,* **1987**, *54,* 989-997.
9. M. J. Saxton, *Biophys. J*., **1989**, *56*, 615-626.
10. M. J. Saxton., *Biophys. J.,* **1990,** *58,* 1303-1306.
11. M. J. Saxton, *Biophys. J*., **1992,** 61, 119-129.
12. M. J. Saxton, *Biophys. J.,* **1993,** *64,* 1053-1062.
13. M. J. Saxton, *Biophys. J.,* **1993**, *64*, 1766-1780.
14. M. J. Saxton, *Biophys. J*., **1994**, *64,* 394-401.
15. M. J. Saxton, *Biophys. J*., **1997**, *72,* 1744-1756.
16. M. J. Saxton, *Biophys. J*., **2001,** *81*, 2226-2232.
17. D. S. Banks and C. Frandin, *Biophys. J*., **2005,** *89,* 2960-2971.
18. M. Vachsmuth, T. Weidemann, G. Muller, M.W. Hoffmann-Rohrer, T.A. Knoch, W. Walder and L. J. Langovski, *Biophys*. J., **2003,** *84*, 3353-3363.
19. N. Fatin-Rouge, K. Starkev and J. Buffle, *Biophys. J.,* **2004,** *86,* 2710-2719.
20. H. P. Kao, J. R. Abney and A. S. Verkmann, *J. Cell Biol*., **1993,** *120,* 175-184.
21. A. Masuda, K. Ushida and T. Okamoto, *Biophys. J.,* **2005,** *88,* 3584-3591.
22. H. Sanabria, Y. Kubota and M. N. Waxham, *Biophys. J*., **2007,** *92,* 313-319.
23. R. P. Kulkarni, D. D. Wu, M. E. Davis and S. E. Fraser, *Proc. Nat. Am. Sci.*, **2005,** *102,* 7523-7531.
24. M. Weiss, M. Elsner, F. Kartberg and T. Nilsson, *Biophys. J*., **2004,** *87,* 1-7.
25. M. Kopf, C. Korinth, O. Haferkamp and T. F. Nonnenmacher, *Biophys. J.,* **1996,** *70,* 2950-2957.