MONTE CARLO SIMULATION OF SINGLE-PARTICLE DIFFUSION IN TWO-DIMENSIONAL AND THREE-DIMENSIONAL CROWDED MEDIA

ADRIANA ISVORAN[*], *E. VILASECA*[**, ***], *LAURA UNIPAN*[****], *J.L. GARCÉS*[*****], *F. MAS*[**, ***]

*Department of Chemistry, University of the West Timişoara, 16, Pestallozi, 300115 Timişoara, Romania, e-mail: aisvoran@yahoo.com
**Theoretical Chemistry Research Centre (CeRQT) of Scientific Park of Barcelona (PCB), Barcelona, Spain
***Physical Chemistry Department, Barcelona University (UB), Barcelona, Spain
****Department of Agriculture, University of Agricultural Sciences of Banat, 119, Calea Aradului, 300645 Timişoara, Romania
*****Chemistry Department, Lleida University (UdL), Lleida, Spain

Abstract. This paper focuses on Monte Carlo simulations of single-particle diffusion in two-dimensional (2D) and three-dimensional (3D) media with obstacles distributed randomly and, respective, uniformly. The simulation data show anomalous diffusion for short times and normal diffusion for long times and they suggest that the uniform distribution of obstacles facilitates diffusion in comparison to their random distribution. The simulation results also reveal that, for the same dimensionality of the media, anomalous diffusion increases, as the average space between obstacles decreases and it is always much less anomalous in 3D crowded media than in 2D ones.

Key words: diffusion, crowded media, Monte Carlo simulations.

INTRODUCTION

In many early studies the cytoplasm of mammalian cell was considered as an aqueous solution in which the different types of molecules were dissolved and each type could perform free diffusion in this environment. More recent studies have taken into account that the interior of the cell contained a high total concentration of macromolecules and that the cytoplasm was structured on many length scales. Such media were called crowded [20] and it was demonstrated both experimentally and theoretically that the macromolecular crowding had considerable consequences on physical and chemical processes, especially on the diffusion. The experimental studies have shown that diffusional mobility of many types of particles in the cytoplasm was strongly reduced by comparison with their mobility in aqueous solutions [1, 2, 18, 19]. Computer simulations have also shown that the diffusion

Received: October 2006;
in final form: November 2006.

ROMANIAN J. BIOPHYS., Vol. 17, No. 1, P. 21–32, BUCHAREST, 2007

process was affected by characteristics of the environment being anomalous in obstructed media: the diffusion coefficient has been reduced by increasing concentration of obstacles until complete immobilization occurred beyond the percolation threshold value for concentration [16], it was dependent on the ratio of tracers mobility versus obstacles mobility [9] and it was also dependent on sizes and mobility of both tracers and obstacles [10–16].

Even if the amount of experimental data concerning the diffusion process in crowded media constantly increases, there are still aspects that cannot be understood and explained in detail. This understanding could be improved by computer simulations results and it would be beneficial for many interdisciplinary fields such as protein diffusion in concentrated solution or pharmaceutical researches concerning drug delivery systems and mechanisms.

In this study we perform Monte Carlo simulations of single-particle diffusion on two-dimensional (2D) and three-dimensional (3D) lattices with obstacles in order to obtain more detailed information about the diffusion process in crowded media.

THEORETICAL BACKGROUND: ANOMALOUS DIFFUSION

In free diffusion the mean-square displacement of the diffusing particle, $< r^2 >$, follows the well-known Einstein-Smoluchowski relation, being proportional to time, *t*, and depending on the topological dimensionality, *d*, of the medium

$$< r^2 > \; = \; (2d)Dt \tag{1}$$

In this equation, *D* is the well-known diffusion coefficient in the limit of infinitely diluted solutions (Brownian motion) being dependent on the diffusing particle's hydrodynamic radius and on the viscosity of the medium [14].

In anomalous diffusion the mean-square displacement is proportional to a non-integer power of time

$$< r^2 > \sim t^{\alpha} \tag{2}$$

where $0 < \alpha < 1$ and the exponent α is called anomalous diffusion exponent [3]. In case of anomalous diffusion we may define a normalised, time dependent diffusion coefficient

$$D(t) \propto \frac{< r^2 >}{t} \sim t^{\alpha-1} \tag{3}$$

and if we assume that equation (3) holds for all $t > 0$ we obtain

$$D(t) = \Gamma t^{\alpha-1} \tag{4}$$

with Γ being a constant [17]. When $\alpha = 1$ we obtain $D = \Gamma$; it is constant and the diffusion process is normal.

METHODOLOGY: SIMULATION ALGORITHM

We have built Fortran 77 programs to implement the Monte Carlo routines. The simulation algorithm for single particle diffusion in a crowded medium is organized as follows:

i) In all simulations the diffusion medium is a lattice distribution of position sites. For 2D simulations we use square lattices and for 3D simulations we use cubic lattices, both with periodic boundary conditions. The lattice sizes are specified for every considered situation.

ii) In the lattice we put a single-particle as tracer and a distribution of obstacles to mimic a crowded medium. In 2D simulations each obstacle is a 5×5 site square with truncated corners occupying 21 sites or a 7×7 site square with truncated corners occupying 37 sites. In 3D simulations each obstacle is a $5\times5\times5$ cube with truncated corners occupying 81 sites or a $7\times7\times7$ cube with truncated corners occupying 179 sites. Tracer occupies 1 single site in the lattice.

iii) We use different modalities to distribute the obstacles within the lattice: randomly and uniformly. In the uniform case the obstacles form a square (2D) or a cubic (3D) regular lattice with a constant separation between every pair of nearest-neighbours. To make the comparison of the results reliable, the calculations with random distribution of obstacles and those with uniform distribution should have a similar density of occupied sites, $[O]_{\text{sites}}$. Thus the space between two nearest-neighbour obstacles in the uniform distribution and the size of the simulation lattices must be defined for each case according to the obstacle density chosen. In Figure 1 we illustrate a small region of a 2D lattice where a single tracer (T) is present and two obstacles (o), each occupying a square of 5×5 sites with truncated corners. In this case the obstacles are distributed uniformly with 2 spaces between the parallel obstacles. The situations considered in this study are presented in Table 1.a for 2D media and in Table 1.b for 3D media.

		o	o	o					o	o	o		
	o	o	o	o	o			o	o	o	o	o	
	o	o	o	o	o			o	o	o	o	o	
	o	o	o	o	o			o	o	o	o	o	
		o	o	o					o	o	o		
		o	o	o					o	o	o		
	o	o	o	o	o	T		o	o	o	o	o	
	o	o	o	o	o			o	o	o	o	o	
	o	o	o	o	o			o	o	o	o	o	
		o	o	o					o	o	o		

Fig. 1. A region of a 2D lattice (10×10) illustrating a tracer particle and four obstacles occupying 5×5 sites with truncated corners. The obstacles are distributed uniformly with 2 spaces between two parallel obstacles.

Table 1.a

Obstacle sizes, density of sites occupied by obstacles, and lattice sizes considered in the simulations of single-particle diffusion in 2D media. In each case the type of obstacle distribution (random or uniform, indicating the separation between two nearest-neighbours) is also given

Obstacle size	$[O]_{sites}$	Distribution	Lattice size
5×5	0.58	random	300×300
5×5	0.58	uniform with 1 space	300×300
5×5	0.43	random	301×301
5×5	0.43	uniform with 2 spaces	301×301
5×5	0.33	random	304×304
5×5	0.33	uniform with 3 spaces	304×304
5×5	0.26	random	306×306
5×5	0.26	uniform with 4 spaces	306×306
7×7	0.37	random	300×300
7×7	0.37	uniform with 3 spaces	300×300
7×7	0.31	random	308×308
7×7	0.31	uniform with 4 spaces	308×308

iv) There is a hard sphere repulsive interaction between tracer and obstacles and between obstacles themselves, so every site in the lattice cannot be occupied by two particles at the same time.

Table 1.b

Obstacle sizes, density of sites occupied by obstacles, and lattice sizes considered in the simulations of single-particle diffusion in 3D media. In each case the type of obstacle distribution (random or uniform, indicating the separation between two nearest-neighbours) is also given

Obstacle size	$[O]_{sites}$	Distribution	Lattice size
5×5×5	0.38	random	102×102 ×102
5×5×5	0.38	uniform with 1 space	102×102 ×102
5×5×5	0.24	random	105×105×105
5×5×5	0.24	uniform with 2 spaces	105×105×105
7×7×7	0.35	random	104×104×104
7×7×7	0.35	uniform with 1 space	104×104×104
7×7×7	0.25	random	99×99×99
7×7×7	0.25	uniform with 2 spaces	99×99×99

v) A random number is used to choose one of the nearest-neighbour sites for the single-particle to move. For 2D simulations we consider four nearest-neighbours and for 3D simulations we consider six nearest-neighbours. The particle moves only if the selected position is empty.

vi) We use 100000 time steps in each simulation and we performed 1000 independent runs. The mean-square displacement for diffusing particle is averaged for these 1000 independent runs.

RESULTS

In both two-dimensional and three dimensional media diffusion is anomalous for short times and normal for long times. In order to illustrate the passage from anomalous diffusion to normal diffusion, in Figure 2 we plot the log ($<r^2>/t$) *versus* log (t) curves for single particle diffusion in a 2D medium having an obstacle density of $[O]_{sites}$ = 0.33. The curves for the random and uniform distribution are compared. In both cases the obstacles have a squared size of 5×5 sites with truncated corners. In this figure we also illustrate the manner to determine the crossover time, t^*, from one regime to another: we perform the linear fit of the region describing anomalous diffusion (descending curve) and the linear fit of the region describing normal diffusion. The time corresponding to their intersection gives us the crossover time.

In Figure 3 we show the plot for single particle diffusion in a 3D medium having an obstacle density of $[O]_{sites}$ = 0.38. The curves for the random and uniform distribution are compared. In both cases the obstacles have a cubic size of 5×5×5 sites with truncated corners. The irregularities at the end of curves in Figures 2 and 3 are due to statistical noise.

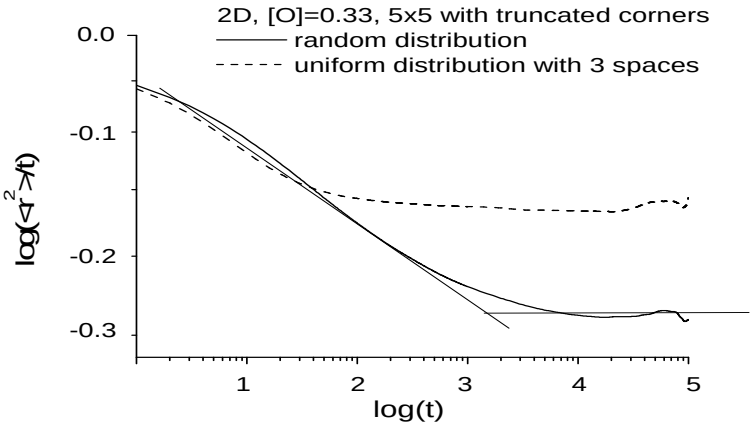

Fig. 2. The plot log($<r^2>/t$) versus log(t) for single particle diffusion in 2D media with quasi-octagonal obstacles 5×5 ($[O]_{sites}$ = 0.33) distributed randomly (continuous line) and uniformly (dashed line).

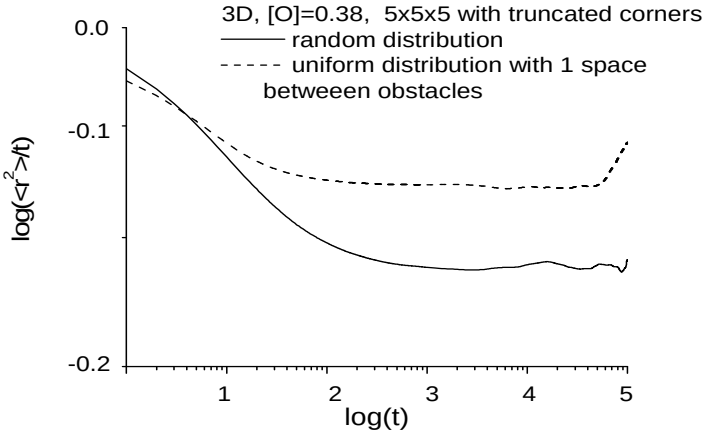

Fig. 3. The plot $\log(<r^2>/t)$ versus $\log(t)$ for single particle diffusion in 3D media with quasi-octagonal obstacles 5×5×5 ($[O]_{sites}$ = 0.38) distributed randomly (continuous line) and uniformly (dashed line).

In Figure 4 we plot the $\log(<r^2>/t)$ versus $\log(t)$ for single particle diffusion in 2D media with obstacles having a squared size of 7×7 sites with truncated corners, with a density of $[O]_{sites}$ = 0.37 and distributed randomly, respectively the same density of obstacles uniformly distributed. In Figure 5 we show a similar plot for a 3D medium having an obstacle density of $[O]_{sites}$ = 0.35. The curves for the random and uniform distribution are compared. In both cases the obstacles have a cubic size of 7×7×7 sites with truncated corners. The irregularities at the end of curves in Figures 4 and 5 are also due to statistical noise.

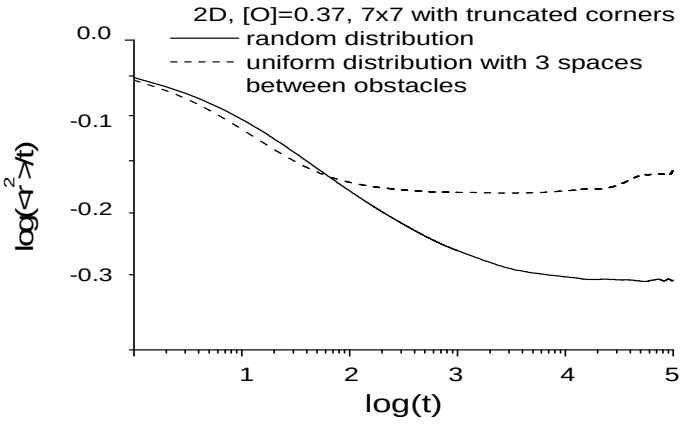

Fig. 4. The plot $\log(<r^2>/t)$ versus $\log(t)$ for single particle diffusion in 2D media with quasi-octagonal obstacles 7×7 ($[O]_{sites}$ = 0.37) distributed randomly (continuous line) and uniformly (dashed line).

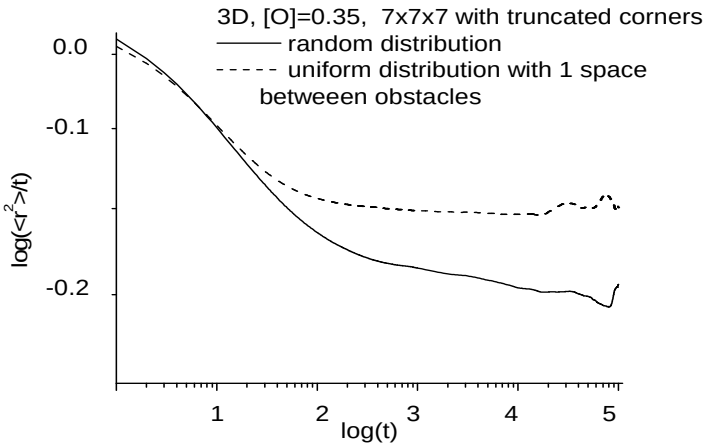

Fig. 5. The plot $\log(\langle r^2\rangle/t)$ versus $\log(t)$ for single particle diffusion in 3D media with quasi-octagonal obstacles 7×7×7 ($[O]_{sites}$ = 0.35) distributed randomly (continuous line) and uniformly (dashed line).

From all these figures we notice the crossover from anomalous diffusion regime to normal one. The crossover time strongly depends on the manner the obstacles are distributed, being considerably higher for random distribution than for uniform one. It also depends on the obstacle densities and sizes and on the topological dimension of the media. The crossover times for all the investigated cases are presented in Table 2. The transition from anomalous diffusion at short times to normal one for long times has been noticed in all previously published results for tracer's diffusion in 2D obstructed media [6, 9–15].

We notice that the two lines in every figure have different slopes, the decreasing in time of the apparent diffusion coefficient being stronger for random distribution of obstacles. It means that for the same obstacle density the abnormality of diffusion is higher for the random distribution than for the uniform one.

For a 2D square lattice with punctual obstacles the percolation threshold is $[O]_{sites}$ = 0.407 [9]. In case of obstacles with higher sizes the concept of percolation limit must be different because for the same density of occupied sites the obstacles form compact regions and the percolation limit must be superior of the value corresponding to punctual obstacles. It is also true for the uniform distribution of obstacles, because in this situation we cannot define a percolation limit. This is illustrated in our paper for single-particle diffusion in 2D lattices with quasi-octagonal obstacles 5×5 and obstacles density $[O]_{sites}$ = 0.43, respectively $[O]_{sites}$ = 0.58. Both these concentrations are upper the percolation limit value corresponding to square lattices with punctual obstacles, but only for the second value in the case of random distribution of obstacles we notice that the mean square displacement has a limiting value (data not shown). Also, for this last situation we

do not notice the crossover from anomalous diffusion to normal one, the diffusion process is anomalous for all the simulation time, see Figure 6.

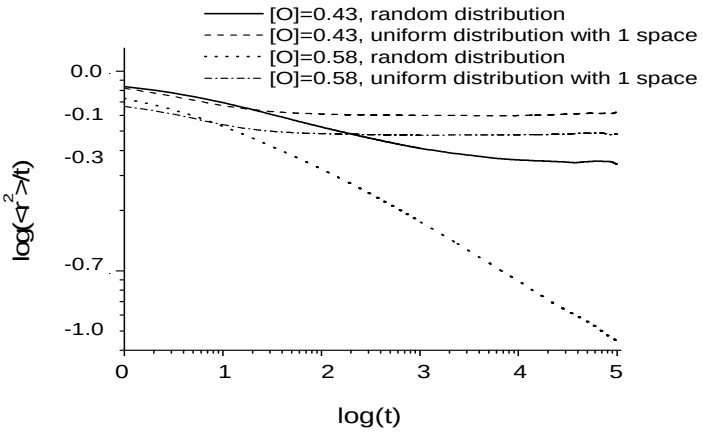

Fig. 6. The plot $\log(\langle r^2\rangle/t)$ versus $\log(t)$ for single particle diffusion in 2D media with quasi-octagonal obstacles 5×5 with density $[O]_{sites} = 0.43$ distributed randomly (continuous line) and uniformly with 1 space between two parallel obstacles (dashed line), respectively with density $[O]_{sites} = 0.58$ distributed randomly (dotted line) and uniformly with 1 space between two parallel obstacles (dash-dotted line).

In Table 2 we also show the values of anomalous diffusion exponents for all investigated cases.

Table 2

The anomalous diffusion exponents, α, and crossover times, t^*, for single-particle diffusion in 2D and 3D crowded media

$d = 2$				$d = 3$			
Obstacles sizes and distribution	$[O]_{sites}$	α	t^* (time units)	Obstacles sizes and distribution	$[O]_{sites}$	α	t^* (time units)
5×5 random	0.58	0.711±0.003	–	–	–	–	–
5×5 uniform	0.58	0.958±0.001	10000	–	–	–	–
5×5 random	0.43	0.901±0.002	2000	–	–	–	–
5×5 uniform	0.43	0.963±0.004	800	–	–	–	–
5×5 random	0.33	0.946±0.004	1000	5×5×5 random	0.38	0.984±0.003	400
5×5 uniform	0.33	0.988±0.003	300	5×5×5 uniform	0.38	0.994±0.001	100

Table 2 (continued)

5×5 random	0.26	0.963±0.004	800	5×5×5 random	0.24	0.990±0.002	300
5×5 uniform	0.26	0.991±0.001	200	5×5×5 uniform	0.24	0.996±0.001	100
7×7 random	0.37	0.937±0.005	2000	5×5×5 random	0.35	0.982±0.003	400
7×7 uniform	0.37	0.985±0.005	200	5×5×5 uniform	0.35	0.991±0.002	100
7×7 random	0.31	0.953±0.004	2000	5×5×5 random	0.25	0.987±0.002	300
7×7 uniform	0.31	0.986±0.004	100	5×5×5 uniform	0.25	0.996±0.002	100

The anomalous diffusion exponent is determined from the slope of mean-square displacements versus time in double logarithmical scale for times corresponding to anomalous diffusion. These exponents are always higher for uniform distribution than for random distribution, which means that the uniform distribution of obstacles facilitates diffusion in comparison with their random distribution, see also Figure 7. These values strongly depend on obstacles density, as it increases diffusion, which is much more anomalous and the system behaviour tends to the trapped diffusion case.

In Table 2 it can also be seen that for an almost similar obstacle density, the anomalous diffusion is stronger for smaller size obstacles (5×5 or 5×5×5 with truncated corners) than for the greater ones (7×7 or 7×7×7 with truncated corners). In the second case the sites occupied by the obstacles are more grouped forming a smaller number of particles and leaving greater spaces free for diffusion.

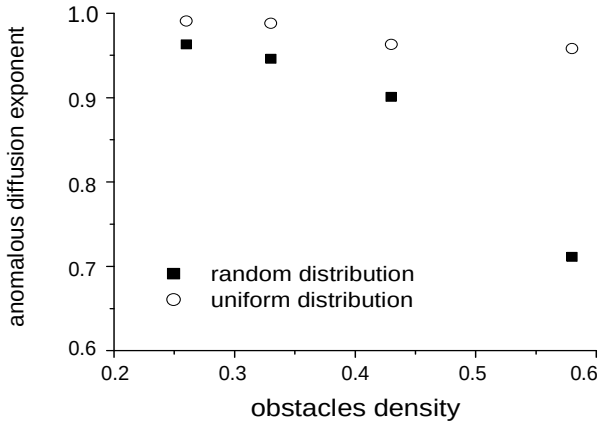

Fig. 7. Anomalous diffusion exponent values for different densities of 5×5 quasi-octagonal obstacles distributed randomly (■), respectively uniformly with different spaces between two parallel obstacles (○).

For 3D media the anomalous diffusion exponents are always higher than for 2D media. The explanation is that the degree of mixing of obstacles in 3D media is always better than in 2D ones.

DISCUSSIONS AND CONCLUSIONS

Transport and binding of molecules to specific sites are necessary for the assembly and function of ordered supramolecular structures in living cells. Mobility of molecular species within the complex environment of mammalian cell is known to deviate notably from mobility in dilute solutions. There are also single molecular events inside living cells (e.g., signal transduction and gene transcription) and detailed knowledge about their transport could improve our understanding of basic cellular processes as well as improving our knowledge on the intracellular transport of therapeutic agents. The technique of single molecule tracking can be used to probe the dynamics of intracellular macromolecules, but the trajectories of single-particles obtained from these experiments are not truly understood [5, 16].

In order to better understand the diffusion process for small molecules in living cells we have performed simulations concerning single-particle diffusion in 2D and 3D crowded media. In our approach we have considered only spatially obstructed motion without other types of interactions such hydrodynamics, binding or reactions. Our approach is based on the experimental results obtained by Kao and co-workers [7] and Wachsmuth and co-workers [18] which have shown that probe collisions with intracellular components was determined to be the principal diffusive barrier that slowed the translational diffusion of small solutes.

Our simulation data reveal anomalous diffusion for small times and normal diffusion for long times in both 2D and 3D obstructed media. These results are in good agreement with other simulation data [6, 9–15] and also with experimental data. Wachsmuth and co-workers [18] have measured the effective diffusion constants and transport velocities of polyplexes in the cytoplasm to understand how they are intracellularly transported. For short intervals, the motion of the polyplex was highly correlated and it had a pronounced memory effect. For longer intervals, the memory effect was lost, and the motions could be described as a pure random walk. Similar findings concerning two temporal regimes of intracellular motion have also been reported for μm beads moving in the cytoplasm and chromosome motion in the nucleus [8].

The crossover time from one regime to another depends on the following factors: the manner to distribute the obstacles, obstacle density, obstacle size and the topological dimension of the media. The crossover time decreases with decreasing density of obstacles and increasing sizes of obstacles. This behaviour is strongly correlated to that of an anomalous diffusion exponent, which increases

with decreasing density and increasing sizes of obstacles. As the density of obstructed sites in the lattice is smaller, the diffusion is easier to occur, its degree of abnormality being smaller, and also the crossover time is smaller. The same behaviour of particles diffusion in 2D crowded media was also noticed in other simulations [6, 10, 11] and we have shown here the same qualitative behaviour for 3D media.

For the same density of occupied sites but obstacles with bigger sizes, the mean distance between two obstacles is higher and also the mean displacement of diffusing particle between two consecutive obstacles is higher. This is true for both 2D and 3D media and it illustrates that small obstacles are more efficient barriers for diffusion than bigger ones. This result was also reported for other simulations of diffusion in 2D crowded media [6, 10, 11].

For the same dimensionality of the media and the same density of occupied sites in the lattice, the crossover time is always shorter for uniform distribution of obstacles than for their random distribution. The anomalous diffusion exponent also increases for uniform distribution of obstacles in comparison to their random distribution. These observations allow us to conclude that the uniform distribution of the obstacles facilitates single-particle diffusion in comparison to their random distribution. This observation could be related with structural organization of living cells. In case of uniformly distributed obstacles there are “channels” in the lattice where all the vacancies are connected by a continuous path. When tracer enters in such a channel, its diffusion is obstructed only by the “walls” and the diffusing distance is higher than in case of randomly distributed obstacles. As the channel is larger, the area of connected vacancies is higher and the diffusion is easier to occur. For the diffusion process in 2D obstructed media we consider the transport of small particles through membranes and for 3D media we consider the diffusion process through microtubules in the cytoplasm. Membranes contain channels that facilitate small particle diffusion and the diameters of channels are very important for membrane permeability for any type of particle. As the diameter of the channel is larger, there are fewer collisions with its walls and the diffusion rate is higher, the same considerations being also applicable to diffusion through microtubules.

Our simulation data also show much less anomalous diffusion in 3D crowded media than in 2D ones for all the investigated cases. This result is in good agreement with theoretical predictions, which show, even for homogeneous environment, a recurrent diffusion (the probability for diffusing particle to revisit one of its old positions is very high) for media with small topological dimensions ($d \leq 2$) and non-recurrent diffusion (the probability for diffusing particle to revisit one of its old positions is very small) for 3D media [4]. It is also related to the degree of mixing in the system. 2D media never assure a perfect mixing, but 3D media always assure a higher degree of mixing than 2D ones.

Acknowledgements. We acknowledge the financial support from: CNCSIS 551 Romania, Spanish Ministry of Education and Science (Projects: BQU2003-09698, UNBA05-33-001) and "Direcció General de Recerca" of the "Generalitat de Catalunya" (Projects: 2005-SGR-00616, 2005-PEIR-0051/69).

REFERENCES

1. ARRIO-DUPONT, M., G. FOUCAULT, M. VACHER, P.F. DEVAUX, S. CRIBIER, Translational diffusion of globular proteins in the cytoplasm of cultured muscle cells, *Biophys. J.*, 2000, **78**, 901–907.
2. BANKS, D.S., C. FRANDIN, Anomalous diffusion of proteins due to molecular crowding, *Biophys. J.*, 2005, **89**, 2960–2971.
3. BEN-AVRAHAM, D., S. HAVLIN, *Diffusion and Reactions in Fractals and Disordered Systems*, Cambridge University Press, 2000, pp. 59–74.
4. DEWEY, T.G., *Fractals in Molecular Biophysics*, Oxford University Press, New York, 1997, pp. 120–124.
5. GOULIAN, M., S.M. SIMON, Tracking Single Proteins within Cells, *Biophys J.*, 2000, **79**, 2188–2198.
6. ISVORAN, A., E. VILASECA, J.L. GARCÉS, L. UNIPAN, F. MAS, Simulation of diffusion in two-dimensional crowded media, Proceedings of the 6th Balkan Physics Union Conference, AIP, 2006, in press.
7. KAO, H.P., J.R. ABNEY, A.S. VERKMAN, Determinants of the translational mobility of a small solute in cell cytoplasm, *J. Cell Biol.*, 1993, **120**, 175–184.
8. KULKARNI, R.P., D.D. WU, M.E. DAVIS, S.E. FRASER, Quantitating intracellular transport of polyplexes by spatio-temporal image correlation spectroscopy, *PNAS*, 2005, **102**, 7523–7528.
9. SAXTON, M.J., Lateral diffusion in an archipelago. The effect of mobile obstacles, *Biophys. J.*, 1987, **52**, 989–997.
10. SAXTON, M.J., Lateral diffusion in an archipelago. Distance dependence of the diffusion coefficient, *Biophys. J.*, 1989, **56**, 615–622.
11. SAXTON, M.J., Lateral diffusion in a mixture of mobile and immobile particles. A Monte Carlo study, *Biophys. J.*, 1990, **58**, 1303–1306.
12. SAXTON, M.J., Lateral diffusion and aggregation. A Monte Carlo study, *Biophys. J.*, 1992, **61**, 119–128.
13. SAXTON, M.J., Lateral diffusion in an archipelago. Dependence on tracer size, *Biophys. J.*, 1993, **64**, 1053–1062.
14. SAXTON, M.J., Lateral diffusion in an archipelago. Single-particle diffusion, *Biophys. J.*, 1993, **64**, 1766–1780.
15. SAXTON, M.J., Anomalous diffusion due to obstacles. A Monte Carlo study, *Biophys. J.*, 1994, **66**, 394–401.
16. SAXTON, M.J., Single particle tracking: The distribution of the diffusion coefficients, *Biophys. J.*, 1997, **72**, 1744–1753.
17. SAXTON, M.J., Anomalous subdiffusion in fluorescence photobleaching recovery. A Monte Carlo study, *Biophys. J.*, 2001, **81**, 2226–2240.
18. WACHSMUTH, M., T. WEIDEMANN, G. MULLER, M.W. HOFFMANN-ROHRER T.A. KNOCH, W. WALDEK, J. LANGOVSKI, Analyzing intracellular binding and diffusion with continuous fluorescence photobleaching, *Biophys. J.*, 2003, 84, 3353–3363.
19. WEISS, M., M. ELSNER, F. KARTBERG, T. NILSSON, Anomalous subdiffusion is a measure of cytoplasmic crowding in living cells, *Biophys. J.*, 2004, **87**, 3518–3524.
20. ZIMMERMAN, S.B., A.P. MINTON, Macromolecular crowding: biochemical, biophysical and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.*, 1993, **22**, 27–65.