ERYTHROCYTES AGGREGATION DUE TO ENTROPIC EFFECTS

Author: David Ledesma Moraga.
*Facultat de Física, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain.**

Advisor: Carles Calero Borrallo
(Dated: June 11, 2017)

Abstract: In this paper we analyze the creation of disks through the depletion interaction, creating a simple model and running some computer simulations using Langevin's dynamics. We demonstrate that this effect is possible in the system described, and that the simulations can be related to the model proposed, linking it with the creation of erythrocytes columns in the blood.

I. INTRODUCTION

In this paper we discuss the aggregation of particles due to entropic effects (also known as depletion interaction), and we will analyze its implications in a particular biological media: blood.

The depletion interaction is an effective attractive entropic force which appears in solutions of large colloidal particles, when they are in the vicinity of other smaller solutes. This force makes a group of colloids interact among themselves in order to create cumulus and columns, also called aggregates [1]. The case of aggregates which have inspired this work, is the one of the erythrocytes in the blood, which is provided in Fig.(1). [2]

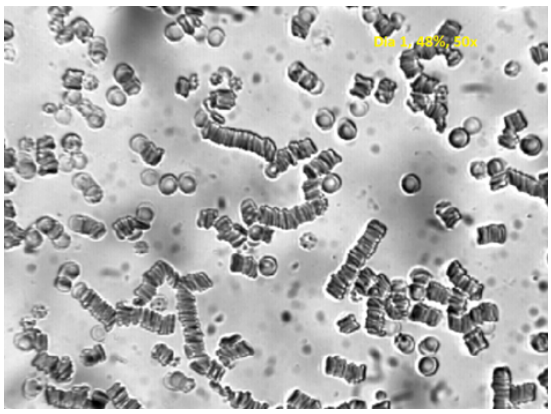

FIG. 1: Picture of erythrocytes aggregates taken in an experiment. Courtesy of: Claudia Trejo-Soto, Hernan Barrio-Zang and Aurora Hernandez-Machado (2016)

In our study, the large colloidal particles will be the erythrocytes, while the fibrinogen polymer present in blood will play the role of the smaller solutes. The erythrocytes are disk-shaped cells which transport oxygen through the blood, and the fibrinogen is a polymer used to create fibrin in the coagulation.

*Electronic address: dledesmo7.alumnes@ub.edu

There are two main basis for this interaction: the maximization of the entropy and the excluded volume between particles. The excluded volume in our case can be explained in the following simplistic way: the particles cannot overlap their volume, therefore, given a cell in a certain position, any other particle won't be able to occupy the same space in the system of the first one. Thats makes that in the surroundings of the first cell, there will be an excluded volume where all the other particles (their position) cannot be found. Consequently, the particles will be able to get in contact with the cell's membrane (as seen in position 1 of Fig.(2)), but they will never penetrate it (position 2). [3]

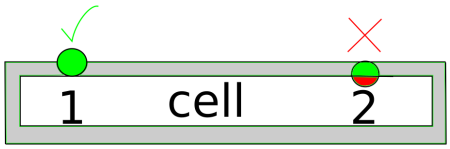

FIG. 2: Picture showing the concept of the excluded volume in a cell, for a polymer's centre of mass. In grey can be seen the cell's surrounding excluded volume there.

Then, in order to reach the equilibrium of an isolated system we need to maximize its entropy, which also means that we will need to have a maximum number of micro-states, as seen in Boltzmann's entropy. In our system, under some conditions, with the formation of aggregates the partial entropy of the cells will decrease, but leaving a greater space for the polymers. Then, the polymers will have a greater number of possible micro-states, and therefore, they will be able to reach a higher entropy leaving a net increase in the system.

To analyze this phenomenon, we have carried out two different works; first of all we have introduced a simple model that tries to characterize the depletion of these two bodies using basic physical concepts; in addition, we have also implemented computer simulations of this system.

II. MINIMAL MODEL

In the model that we are studying, we consider a suspension composed by hards-shell polymers and disks,

where the only interaction that takes part is an effective short ranged entropic force among disks, characterized along the Z-axis direction of them, with a bond energy $-\epsilon_0$.

To have an aggregate of 1 disk, means having only a free disk, whereas having an aggregate of n disks consists on having n disks grouped together through this force, with a total of (n-1) bonds, leaving a total bond energy (n-1)ϵ_0.

To characterize this entropic force we depart from a well known expression for the chemical potential of an aggregate of n-particles [4]

$$\mu_n = \mu^0 + \frac{1}{n}\left[k_B T ln\left(\frac{\phi_n}{n}\right) - \epsilon_0(n-1)\right] \quad (1)$$

This Bond energy is characterized by an osmotic pressure through [1]

$$\epsilon_0 = \Pi(\Psi_P) \times V_{excluded} = (\psi_P K_B T) \times (f R_P D^2) \quad (2)$$

(ψ_P is the concentration of polymers, D is the diameter of the disks, R_P is the radius of gyration of the polymer, and f is a geometric constant).

From (1) and (2), under the hypothesis of reaching the equilibrium, we are able to get to

$$\mu_n = \mu_{n-1} = ... = \mu_1 = \mu^0 + K_B T ln(\Phi_1) \quad (3)$$

And taking $\psi_D = \sum_{n=1}^{\infty} \Phi_n$ where ψ_D is the volume fraction of disks in the system, and then defining $x \equiv \Phi_1 e^{\Psi_P D^2 R_P f}$, we obtain

$$\psi_D = \sum_{n=1}^{\infty} \Phi_n = \frac{x e^{-\Psi_P D^2 R_P f}}{(1-x)^2} \quad (4)$$

From which we can extract

$$x = 1 + \frac{1}{2N^{*2}}\left[1 - \sqrt{1+4N^*}\right] \quad (5)$$

where $N^* \equiv \sqrt{\psi_D \exp(\psi_P D^2 R_P f)}$

Finally, the volume fraction of aggregates composed by n disks is given by

$$\frac{\Phi_n}{n} = x^n e^{-\Psi_P D^2 R_P f} \quad (6)$$

And now we are able to derive quantities that characterize our system as

Probability of obtaining an aggregate of n-disks

$$P(n) = \frac{x^n}{\sum_{n=1}^{\infty} x^n} = (1-x)x^{n-1} \quad (7)$$

Average number of disks per aggregate

$$\langle N \rangle = \sum nP(n) = \frac{2N^{*2}}{\sqrt{1+4N^{*2}} - 1} \quad (8)$$

As the dependence of the system on the different variables is not trivial, in the Fig.(3) we have plotted the dependence of $\langle N \rangle$ with the diameter of the disks at different volume fractions. Finally, in Fig.(5), we have taken a typical value for the erythrocyte diameter $D_{erythrocyte} \approx 7\mu m$, and some values from the simulations as $\psi_P = 0.05$ and $R_P = 0.3\mu m$.

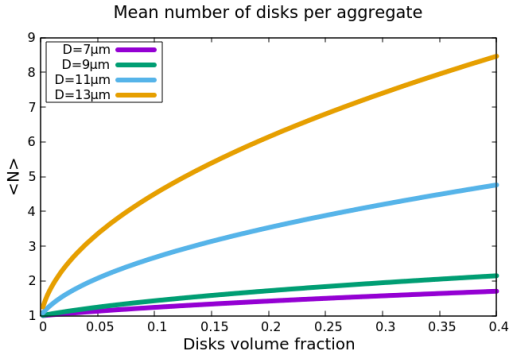

FIG. 3: Average number of disks per aggregate dependency with the disks concentration for different values of the disk's diameter. Values taken: $\psi_P = 0.05$ and $R_P = 0.3\mu m$.

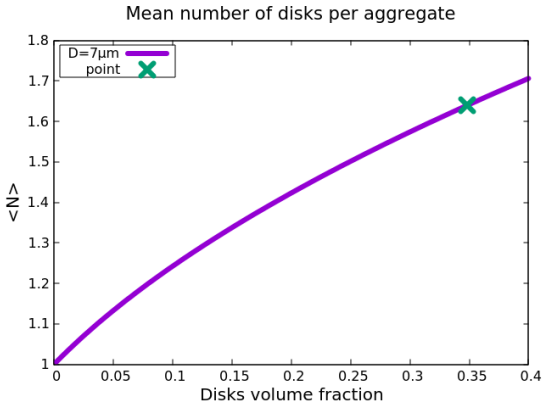

FIG. 4: Dependency of the average number of disk per aggregate with the disks volume fraction. There is a point marked which will be useful for the simulations analysis.

As can be seen, the model predicts the creation of disks' aggregates in the equilibrium. As $\langle N \rangle$ increases with this variables, it means that aggregates of n>1 have been produced, and the strongest dependency has been seen to be with the disk's diameter and volume fraction.

III. COMPUTER SIMULATIONS

A. Procedure

In order to see if this effect is possible and relevant in the system described, we have run some simulations using a general particle simulator which uses the computing capabilities of GPUs, called HOOMD [5], [6]

Our depletant particles are characterized as many little spheres of diameter 1σ stuck together which generates a rigid disk [7], and our solutes are free rigid spheres with a radius of 0.3σ. (σ is the length unit we set for the system simulated.)

The only two forces acting in the system are: a truncated repulsion Lennard-Jones potential for each sphere, which symbolizes the hard-shell of the particles, and a random force, typical of a Brownian particle. The expression for the truncated potential is shown in Eq. (9), and we have used it with $r_{cut} = 2^{1/6}\sigma$ and a shift that moves the V_{min} to 0 in order to make all values V$\geq$ 0. This shift is done to emulate the hard-shell phenomena, as the L-J interactions include an attractive part in the potential, and our excluded volume doesn't. This potential defines the units of length σ and energy ϵ of our simulation.

$$V_{LJ} = \begin{cases} 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] & r < r_{cut} \\ 0 & r \geq r_{cut} \end{cases} \tag{9}$$

The system is confined in a box with periodic boundary conditions, which is a method to simulate very large systems using an smaller cell. It basically means considering that when a particle passes through one side of the unit cell, it re-appears on the opposite side with the same properties.The whole system is also in a thermal bath, which proportionates every particle an energy of $kT = 0.2\epsilon$.

The simulations compute the energies, positions, momentums, orientations and other physical quantities for each particle in the system through solving the Langevin equation

$$m\frac{d^2\vec{x}}{d\tau^2} = -\lambda\frac{d\vec{x}}{d\tau} + \eta(\tau) \tag{10}$$

in each step, where $\eta(\tau)$ is the stochastic force, and τ is the time unit.

The system simulated have been run with 125 disks of $D = 7\sigma$ and 6250 spheres of $R = 0.3\sigma$. The simulations departed from an ordered generated state, which has been stochastically disordered by leaving the system evolve for some time. Then, having the system in a random state, it has been evolved while compressing in order to reach higher densities, and to make the aggregation more evident. In this process we have reached a disk volume fraction of 0.348, and a polymer's concentration of 0.05. Finally, once the compression is finished, we have let the system evolve free, in order to reach the equilibrium.

Lastly, we can visualize the simulations results using VMD [8], [9]: in Fig.(5) we can observe clearly the generation of aggregates in the whole system, where the longest one observed has been of 15 disks, and it can be seen in the middle of the left half part of this figure.

In Fig.(6) we can observe more efficiently the aggregation effect looking only at the bonds, where every bond illustrates an interaction among 2 disks.

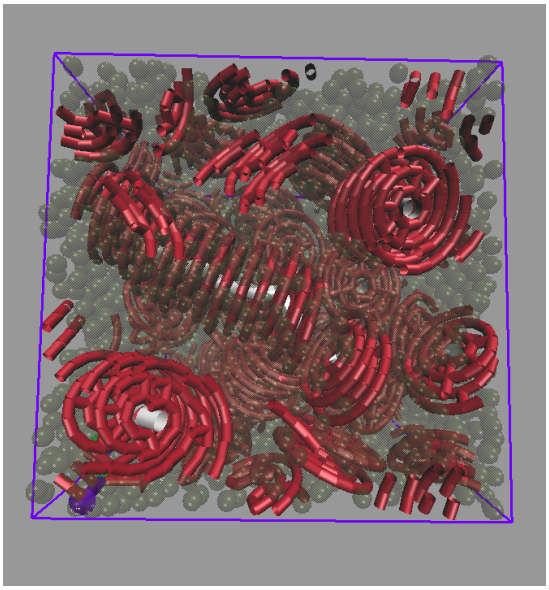

FIG. 5: Snapshot of one simulation that illustrates the whole system simulated. The purple line shows the limits of the simulation box, the ocher semi-transparent spheres are the polymers, and in red are represented the disks. The white cylinders between disks indicates that there is an aggregate between them. Figure generated with VMD [8], [9].

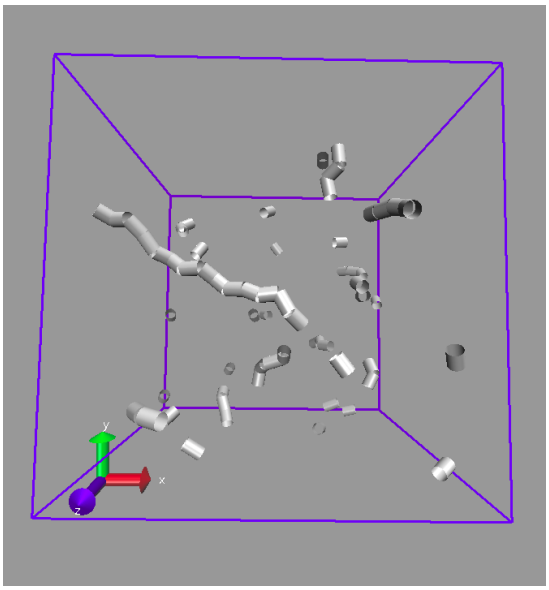

FIG. 6: Snapshot of a simulation illustrating the aggregates bonds. The purple line shows the limits of the simulation box and the white cylinders emulate an aggregate between disks. Figure generated with VMD [8], [9].

B. Analysis and relations with the model

To determine whether the simulation has been running correctly or not, we can examine the consistency between different energies. Because of the interactions tak-

ing part, we can approximate our system to an ideal gas (as the potential only takes the role of excluded volume), which will follow the equipartition Law. Having the same number of degrees of freedom, both rotational and translational kinetic energies will contribute the same value for each particle:

$$\left\langle H_t^{kin} \right\rangle = \left\langle H_r^{kin} \right\rangle = \frac{3}{2} N k_B T \tag{11}$$

In ideal gases, summing over all the particles in the system, we obtain the Virial:

$$-\left\langle \sum_{k=1}^{N} \vec{q_k} \vec{F_k} \right\rangle = 3 N k_B T \tag{12}$$

where $\vec{F_k}$ is the net force on the particle k.

Then, as the only force interacting between particles is the random force imposed through the thermal bath, we should obtain that the total kinetic energy of the simulation is 3N times the energy given to each particle (0.2ϵ). Therefore, with $N = 6375$ particles we should obtain:

$$\left\langle H_t^{kin} \right\rangle = \left\langle H_r^{kin} \right\rangle = \frac{3}{2} N k_B T = 1912.5\epsilon \tag{13}$$

$$-\left\langle \sum_{k=1}^{N} \vec{q_k} \vec{F_k} \right\rangle = 3 N k_B T = 3825\epsilon \tag{14}$$

Analyzing the Fig.(7), we can observe that the system's energy has some little fluctuations. This fact is due to the stochastic forces considered. Although having this noise, if we compare the results of the simulation with the ones of the expressions (13) and (14), we can say with high accuracy that the temperature of the system has been constant during all the simulation, and therefore, that the compression of the system and the simulation have been run correctly.

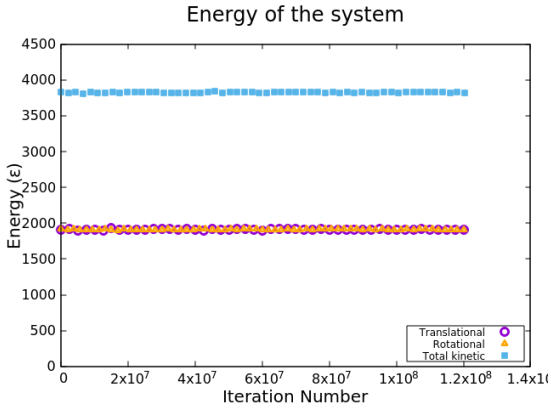

FIG. 7: Translational and rotational kinetic energies of the whole system, and the total kinetic energy.

One important fact that must be taken into account is if we have already reached the equilibrium or not. In Fig.(8) this fact is analyzed looking at the potential energy of the excluded volume interaction. The system, after the compression, tries to minimize it's potential energy in order to reach the equilibrium.As this energy has converged with the evolution time of the system, we can say that we have reached the equilibrium.

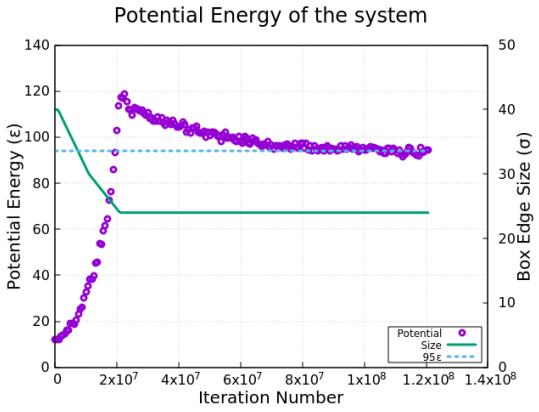

FIG. 8: Evolution of the potential energy along the simulation. On the left hand, there can be seen an increase due to the compression, and on the right hand the free evolution. We also show the evolution of the system's size along the simulation.

In Fig.(9) we have also analyzed the evolution of the system after the compression, computing the mean number of disks per aggregate, related with Eq. (8) of the model.

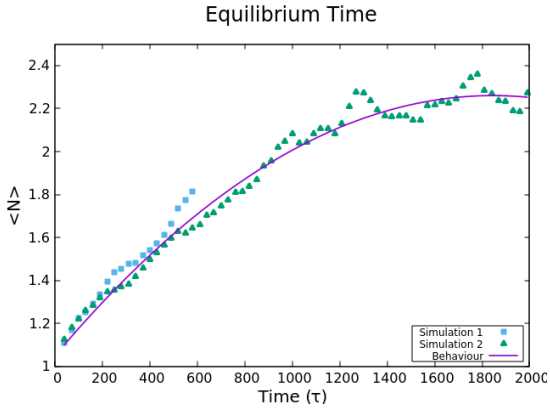

FIG. 9: Dependency of the mean number of disks per aggregate with the evolution time for two simulations. The blue simulation lasted 1990τ, while orange 600τ, where τ is the simulation unit of time. The behaviour line shows an approximation to the system's evolution.

Looking at Fig.(9), we can observe that although having some fluctuations, $\langle N \rangle$ converges to a value of $\langle N \rangle \approx 2.3$. We can compare this result with the one of the point in Fig.(4) given by the model, which is $\langle N \rangle \approx 1.64$. The results are not close enough one to each other, but the most important fact is that both predict a notori-

ous aggregation of disks in the system under the same conditions.

In table 1, there can be seen another relation between the simulations and the model. We can relate the probability of having an aggregate of n-disks predicted by the model in Eq. (7), with the frequency they appear in the simulation. In the simulation we have obtained 31 free disks (n=1), 22 pairs of disks, 3 aggregates of 3 disks, and 4,2 and 1 columns of 4,5 and 15 disks respectively. These numbers, for example, give a frequency of 0.492 for the n=1, whereas the model predicts a probability of 0.610. This fact tells us that our model and the simulations give a very similar distribution of aggregates.

n	p_{model}	simulation aggregates	simulation frequency
1	0.610	31	0.492
2	0.238	22	0.349
3	0.093	3	0.048
4	0.036	4	0.063
5	0.014	2	0.032
15	1.14E-6	1	0.016

TABLE I: Data of the frequencies from the system simulated and from calculus made with the model.

IV. CONCLUSIONS

- First of all, we have demonstrated under certain conditions, that the production of disks aggregates in our system can be explained by the depletion interaction. These concrete conditions are: $\psi_P = 0.05$, $\psi_D = 0.348$, $R_P = 0.3\sigma$, $D = 7\sigma$ and the hypothesis of equilibrium.

- Then, we have also analyzed exhaustively the realization and process of the simulations, which include the thermalization of the system during the whole simulation, the consistency of the energies obtained with the equipartition law, and the system's equilibrium itself. Moreover, we have related the results of the simulations with the ones of the model, giving some similarities in the results like in the Table 1.

- Finally, with all this results, we are able to explain the generation of disks aggregates in our simple system. Therefore, as our system is a simple analogy to the blood, we can conclude that the depletion interaction can also produce erythrocytes aggregates.

Acknowledgments

Special thanks to Carles Calero Borrallo who has offered the opportunity to carry out this research. Also thanks to Claudia Trejo-Soto, Hernan Barrio-Zang and Aurora Hernandez-Machado who have provided some experimental pictures; HOOMD-blue and VMD for providing the tools for the simulations; and finally my parents, who have supported me along all the path.

[1] S.-W. Hu and Y.J. Sheng, *Phase Diagram of Solvophilic Nanodiscs in a Polymer Solution: Depletion Attraction*, J. Phys. Chem. B, **vol. 117**, 4098-4108 (2013)

[2] D.A.F., W.P., B.C., G.G., and G.E.K.,*Predicting human blood viscosity in silico*, PNAS, **vol. 108**, no. 29 (2011)

[3] H.N.W. Lekkerkerker and R. Tuinier, *Colloids and the Depletion Interaction*, Springer Editorial

[4] J. Faraudo, J. S. Andreu, C. Calero and J. Camacho, *Predicting the Self-Assembly of Superparamagnetic Colloids under Magnetic Fields*, Advanced Functional Materials, **vol. 22**, 3837-3858 (2016)

[5] J. A. Anderson, C. D. Lorenz, and A. Travesset. *General purpose molecular dynamics simulations fully implemented on graphics processing units*, Journal of Computational Physics, **vol. 227**, 5342-5359 (2008)

[6] J. Glaser, T. D. Nguyen, J. A. Anderson, P. Liu, F. Spiga, J. A. Millan, D. C. Morse and S. C. Glotzer, *Strong scaling of general-purpose molecular dynamics simulations on GPUs*, Computer Physics Communications, **vol. 192**, 97-107 (2015)

[7] . D. Nguyen, C. L. Phillips, J. A. Anderson and S. C. Glotzer. *Rigid body constraints realized in massively-parallel molecular dynamics on graphics processing units*, Computer Physics Communications, **vol. 182**, 2313-2307 (2011)

[8] W. Humphrey, A. Dalke and K. Schulten, *VMD - Visual Molecular Dynamics*, J. Molec. Graphics, **vol. 14**, 33-38 (1996)

[9] URL: http://www.ks.uiuc.edu/Research/vmd/