A BIOSPI MODEL OF LYMPHOCYTE-ENDOTHELIAL INTERACTIONS IN INFLAMED BRAIN VENULES

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This paper presents a stochastic model of the lymphocyte recruitment in inflammed brain microvessels. The framework used is based on stochastic process algebras for mobile systems. The automatic tool used in the simulation is the BioSpi. We compare our approach with classical hydrodinamical specifications.

1 Introduction

Lymphocytes roll along the walls of vessels to survay the endothelial surface for chemotactic signals, which stimulate the lymphocyte to stop rolling and migrate through the endothelium and its supporting basement membrane. Lymphocyte adhesion to the endothelial wall is mediated by binding between cell surface receptors and complementary ligands expressed by the endothelium. The dynamic of adhesion is regulated by the bond association and dissociation rates: different values of these rates give rise to different dynamical behaviors of the cell adhesion.

The most common approach to the simulation of rolling process of lymphocyte is based on hydrodynamical models of the particle motion under normal or stressed flow [1,16,18]. At a macroscopic scale, the process is generally modeled with the typical equations of mass continuity, momentum transport and interfacial dynamic. At a microscopic scale, the cell rolling is simulated as a sequence of elastic jumps on the endothelial surface, that result from sequential breaking and formation of molecular bonds between ligands and receptors [16,6,9]. This kind of model is able to simulate the time-evolution of bond density.

A major challenge for a mechanical approach is to treat the disparate scales between the cell (typically of the order of micrometers) and the bonds (of the order of nanometers). In fact, rolling involves either dynamical interaction between cell and surrounding fluid or microscopic elastic deformations of the bonds with the substrate cells. Moreover recent studies have revealed

that the process leading to lymphocyte extravasation is a sequence of dynamical states (contact with endothelium, rolling and firm adhesion), mediated by partially overlaped interactions of different adhesion molecules and activation factors. The classical mechanical models are inefficent tools to describe the concurrency of the molecular interactions; also if they treat the physical system at the scale of intermolecular bonds with appreciable detail, they are not able to reproduce the sensitivity to the small pertubations in the reagent concentrations or in reaction rates typical of microscopic stochastic systems governed by complex and concurrent contributions of many different molecular reactions. The probabilistic nature of a biological system at the molecular scale requires new languages able to describe and predict the fluctuations in the population levels. We rely on a stochastic extension [21,22] of the π-calculus [17], a calculus of mobile processes based on the notion of naming. The basic idea of this biochemical stochastic π-calculus is to model a system as a set of concurrent processes selected according to a suitable probability distribution in order to quantitatively accommodate the rates and the times at which the reactions occur.

We use this framework to model and simulate the molecular mechanism involved in encephalitogenic lymphocyte recruitment in inflammed brain microvessels.

Our development can also be interpreted as a comparison between the most common modeling method based on hydrodynamical and mechanical studies and π-calculus representation, in order to point out the ability of this new tool to perform a stochastic simulation of chemical interactions that is higly sensitive to small perturbations. We also present data obtained from BioSpi simulations.

2 Molecular mechanism of autoreactive lymphocyte recruitment in brain venules

A critical event in the pathogenesis of multiple sclerosis, an autoimmune disease of the central nervous system, is the migration of the lymphocytes from the brain vessels into the brain parenchima. The extravasation of lymphocytes is mediated by highly specialized groups of cell adhesion molecules and activation factors. The process leading to lymphocytes migration, illustrated in Fig. 1 , is divided into four main kinetic phases: 1) initial contact with the endothelial membrane (tethering) and rolling along the vessel wall; 2) activation of a G-protein, induced by a chemokine exposed by the inflamed endothelium and subsequent activation of integrins 3) firm arrest and 4) crossing of the endothelium (diapedesis). For this study, we have used a model of

early inflammation in which brain venules express E- and P-selectin, ICAM-1 and VCAM-1 [20]. The leukocyte is represented by encephalitogenic $CD4^+$ T lymphocytes specific for PLP139-151, cells that are able to induce experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis.

Tethering and rolling steps are mediated by binding between cell surface receptors and complementary ligands expressed on the surface of the endothelium. The principal adhesion molecules involved in these phases are the selectins: the P-selectin glyco-protein ligand-1 (PSGL-1) on the autoreactive lymphocytes and the E- and P-selectin on the endothelial cells. The action of integrins is partially overlaped to the action of selectins/mucins: α_4 integrins and LFA-1 are also involved in the rolling phase, but they have a less relevant role.

Chemokines have been shown to trigger rapid integrin-dependent lymphocyte adhesion in vivo through a receptor coupled with G_i proteins. Integrin-dependent firm arrest in brain microcirculation is blocked by pertussis toxin (PTX), a molecule able to ADP ribosylate G_i proteins and block their function. Thus, as previously shown in studies on naïve lymphocytes homing to Peyer's patches and lymph nodes, encephalitogenic lymphocytes also require an in situ activation by an adhesion-triggering agonist which exerts its effect via Gi-coupled surface receptor.

The firm adhesion/arrest is mediated by lymphocyte integrins and their ligands from the immunoglobulin superfamily expressed by the endothelium. The main adhesion molecules involved in cell arrest is integrin LFA-1 on lymphocyte and its counterligand ICAM-1 on the endothelium. The action of α_4 integrins is partially overlaped to the action of LFA-1: α_4 integrins are involved in the arrest but they have a less relevant role [20].

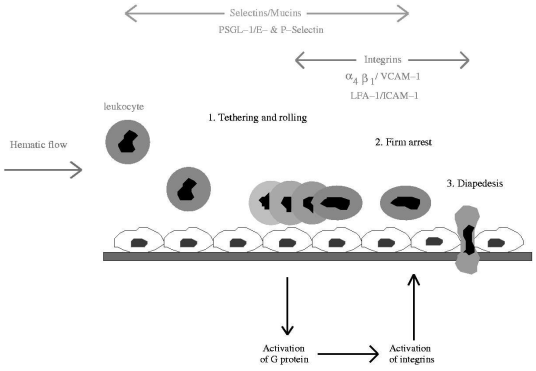

Figure 1. The process leading to lymphocyte extravasation is a finely regulated sequence of steps controlled by both adhesion molecules and activating factors.

3 Kinetics models of cell adhesion

In this section we firstly describe the micro-scale model of cell adhesion proposed by Dembo et al. [6], that computes the time-evolution of the of the bonds density between ligands and receptors during the phase of rolling. Secondly, we briefly report the recent results of the computational method called *Adhesive Dynamics* developed by Chang et al. [3] and based on the Bell model [1], that expresses the dissociation rate as a function of the total force applied on the lymphocyte, simulates the adhesion of a cell to a surface under flow. Here the relationship between ligand/receptor functional properties and the dynamics of adhesion are expressed in state diagrams, drawing the variation of the lymphocyte centroid position in time.

We have considered here these two models, because they describe the two main aspects of the cell motion: the molecular interaction at molecular bond scale and the dynamics of the motion at the lymphocyte scale, to compare the two kinds of results with the π-calculus simulations.

Dembo adhesion model. Rolling is a state of dynamic equilibrium in which there is rapid breaking of bonds at the trailing edge of the lymphocyte-endothelium contact zone, matched by rapid formation of new bonds at the leading edge. The process of lymphocyte rolling and adhesion under blood flow involves the balance of the forces arising from hydrodinamic effect including shear and normal stresses and the number and strength of the molecular bonds [7,12,23,24,25].

The kinetic reaction model proposed by Dembo et al.[6] simulates the rolling lymphocyte as a viscous newtonian fluid enclose in a pre-stressed elastic membrane and the adhesion bonds formed between the rolling cell and its substrate are simulated as elastic springs perpendicular to substrate. The parameters considered by this model are: N_l (lingands density) = N_r (receptors density) = 400 μm^2, k_{on}^0 (equilibrium association rate) = 84s^{-1}, k_{off}^0 (equilibrium dissociation rate) = 1s^{-1}, σ (equilibrium spring constant) = 5 dyne/cm, σ_{ts} (transient bond elastic constant) = 4.5 dyne/cm, K_BT (thermal energy) = 3.8 $\times$ 10^{-7} ergs and λ (equilibrium bond length) = 20 nm. They are used to compute the bond density N_b assuming the adhesion bond force $F_b = N_b\sigma(L-\lambda)$ [16,18]. The hyperbolic analytic solution for the time-evolution of bond density N_b is given by $N_b(t) = -\frac{1}{84t} + 400$ and it is plotted in Fig. 2.

Bell model and adhesive dynamics. The physicochemical properties that give rise to the various dynamic states of cell adhesion are mainly the rates of reaction. In particular the bond dissociation rate and its dependence on the resultant of the applied forces play an important role in rolling process. Bell [1] proposed that the net dissociation rate k_{off} of a bond under an applied

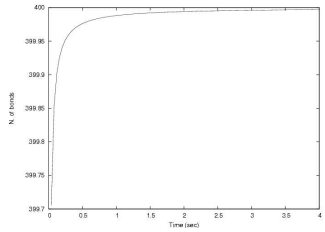

Figure 2. Time-evolution of bonds desity.

external force f can be modeled as $k_{off} = k_{off}^{(u)} \exp\left(\frac{sf}{K_B T}\right)$ where $k_{off}^{(u)}$ is the unstressed dissociation rate and $K_B T$ is the thermal energy; s is a parameter with units of length that relates the reactivity of the molecule to the distance to the transition state in the intramolecular potential of mean force for single bonds [1,8]. The Bell model paramenter $k_{off}^{(u)}$ and s are functional properties of the molecules.

Using the equation above to model the force dependence of dissociation, Chang et. al performed Adhesive Dynamics computer simulations to obtain the states diagrams of the lymphocyte motion. In the Adhesive Dynamic method [3,13,14], the simulation begins with a freely moving cell (modeled as a sphere with receptors distributed at random over its surface and kinetic parameters [3]). The cell is allowed to reach a steady translational velocity in absence of specific interactions, after which receptor-mediated binding is initiated. The involved adhesion molecules and the uniformely reactive substrate react with association rate k_{on} and dissociation rate k_{off}. During each time step, bond formation and breakage are simulated by Monte Carlo methods, in which random numbers are compared with the probabilities for binding and unbinding to determine whether a bond will form or break in the time interval [3]. The dynamic of motion involves the elastic bond force, given by the Hooke's law, colloidal force [3] and the force imparted to the cell by the fluid shear. The motion of the lymphocyte is obtained from the mobility matrix for a sphere near a plane in viscous fluid. The new positions of free receptors and tethers at $t + dt$ are updated from their positions at t, using the translational and angular velocity of the cell. The process is repeated until the cell travels 0.1 cm, or 10s of simulated time has elapsed. The adhesive dynamics simulation parameters are: R (cell radius) = 5 μm, λ (equilibrium bond length) = 20 nm, σ (spring constant) = 100 dyne/cm, μ (viscosity) = 0.01 $g\ cm^{-1}\ s^{-1}$, T (temperature) = 310 K and γ_w (wall shear rate) = 100s^{-1}.

From different values of rates constants in the Bell model (see caption of Fig.3) different motion state diagrams emerge [16]. *Tethering*, in which

lymphocytes move at a translational velocity $v < 0.5v_h$ (where v_h is the hydrodinamical velocity of the blood flow) but exhibit no durable arrest is shown in Fig. 3 (upper left). *Rolling* for which cells travel at $v < 0.5v_h$, but experience durable arrests, is shown in Fig. 3 (upper right). Finally in *firm adhesion*, shown in Fig. 3(lower), cells bind to the endothelium and remain motionless.

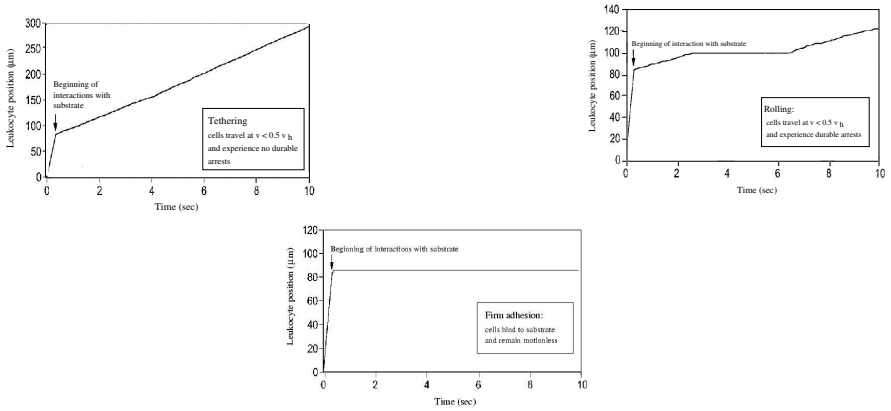

Figure 3. Representative trajectory of lymphocyte tethering at a mean velocity v equal to one half of the hydrodinamic velocity v_h. The parameters are the following: $\gamma = 0.001$ nm, $k_{on} = 84s^{-1}$, $k_{off}^{(u)} = 1s^{-1}$ (upper left). Representative trajectory of rolling motion of lymphocyte, with a mean velocity $v < 0.5v_h$, that experience durable arrests (upper right). Representative trajectory of lymphocyte for firm adhesion. The parameters are the following: $\gamma = 0.001nm$, $k_{on} = 84s^{-1}$, $k_{off}^{(u)} = 20s^{-1}$ (lower).

4 The BioSpi model implementation and results

We first recall the syntax and the intuitive semantics of the stochastic π-calculus [22]. We then describe our specification of the lymphocyte recruitment process, and eventually we discuss the simulation results.

Biomolecular processes are carried out by networks of interacting protein molecules, each composed of several distinct independent structural parts, called *domains*. The interaction between proteins causes biochemical modification of domains (e.g. covalent changes). These modifications affect the potential of the modified protein to interact with other proteins. Since protein interactions directly affect cell function, these modifications are the main mech-

anism underlying many cellular functions, making the stochastic π-calculus particularly suited for their modeling as mobile communicating systems. The syntax of the calculus follows

$$P ::= \mathbf{0} \mid X \mid (\pi, r).P \mid (\nu x)P \mid [x = y]P \mid P|P \mid P + P \mid A(y_1, \ldots, y_n)$$

where π may be either $x(y)$ for *input*, or $\overline{x}y$ for *output* (where x is the *subject* and y is the *object*) or τ_i for *silent* moves. The parameter r corresponds to the basal rate of a biochemical reaction and it is an exponential distribution associated to the channel occurring in π. The order of *precedence* among the operators is the order (from left to right) listed above.

Processes model molecules and domains. Global channel names and co-names represent complementary domains and newly declared private channels define complexes and cellular compartments. Communication and channel transmission model chemical interaction and subsequent modifications. The actual rate of a reaction between two proteins is determined according to a constant *basal rate* empirically-determined and the concentrations or quantities of the reactants . Two different reactant molecules, P and Q, are involved, and the reaction rate is given by $Brate \times |P| \times |Q|$, where $Brate$ is the reaction's basal rate, and $|P|$ and $|Q|$ are the concentrations of P and Q in the chemical solution computed via the two auxiliary functions, In_x, Out_x that inductively count the number of receive and send operations on a channel x enabled in a process.

The semantics of the calculus thereby defines the dynamic behaviour of the modeled system driven by a *race condition*, yielding a probabilistic model of computation. All the activities enabled in a state compete and the fastest one succeeds. The continuity of exponential distributions ensures that the probability that two activities end simultaneously is zero.

The reduction semantics of the biochemical stochastic π-calculus is

$$(\ldots + (\overline{x}\langle z\rangle, r).Q)|((x(y), r).P + \ldots) \xrightarrow{x, r_b \cdot 1 \cdot 1} Q|P\{z/y\}$$

$$\frac{P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} P'}{P|Q \xrightarrow{x, r_b \cdot r_0' \cdot r_1'} P'|Q}, \begin{cases} r_0' = r_0 + In_x(Q) \\ r_1' = r_1 + Out_x(Q) \end{cases}$$

$$\frac{P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} P'}{(\nu\, x)P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} (\nu\, x)P'} \quad \frac{Q \equiv P, P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} P', P' \equiv Q'}{Q \xrightarrow{x, r_b \cdot r_0 \cdot r_1} Q'}$$

A reaction is implemented by the three parameters r_b, r_0 and r_1, where r_b represents the basal rate, and r_0 and r_1 denote the quantities of interacting molecules, and are computed compositionally by In_x and Out_x.

4.1 Specification

The system of interacting adhesion molecules that regulate the lymphocytes recruitment on endothelial surface illustrated in Fig. 1 has been implemented in the biochemical stochastic π-calculus. The system is composed by eight concurrent processes, corresponding to the eight species of adhesion molecules, that regulate the cell rolling and arrest: PSGL1, PSELECTIN, CHEMOKIN, CHEMOREC, ALPHA4, VCAM1, LFA1 and ICAM1. The code implements the four phases of the lymphocyte recruitment: the interaction between PSGL1 and PSELECTIN, the ALPHA4 and LFA1 activation by chemokines and the firm arrest mainly caused by the interaction between the active form of LFA1, LFA1_ACTIVE, and ICAM1 and in part also due to the interaction of the active form of ALPHA4, ALPHA4_ACTIVE, with VCAM1. Its specification is

We simulated the role and the contribution of the different interactions as bi-molecular binding processes occuring at different rates. The selectins interaction PSGL1/PSELECTIN plays a crucial role in guaranting an efficient rolling, therefore the channels rates for the communication in the binding process between PSGL1 and PSLECTIN have been calculated from the deterministic rates of the Bell model, that reproduce the tethering and rolling motion. Analogously, for the ALPHA4_ACTIVE/VCAM1 interaction, that contributes to rolling and, in part, also to cell arrest, the channels rate have been calculated from the Bell model rates that recreate the rolling motion. The interaction LFA1_ACTIVE/ICAM1 is the main responsible of firm arrest of the cell on the endothelium and thus the rates of communication between LFA1_ACTIVE and ICAM1_ACTIVE have been calculated from those reproducing the firm adhesion in Bell model simulations.

The activation of ALPHA4 and LFA1 integrins by the chemokines is implemented in two steps: firstly a chemokine CHEMOKIN binds to its receptors CHEMOREC and changes to a "bound" state CEHMOKIN_BOUND. Then the complex CHEMOKIN_BOUND sends two names *sign1 and* sign2 on the channels *act_alpha and* act_lfa, on which the processes ALPHA4 and LFA1 are ready to receive them as inputs. After ALPHA4 and LFA1 have received the signals from CHEMOKIN_BOUND, they change to the active form ALPHA4_ACTIVE and LFA1_ACTIVE.

The whole process of lymphocyte recruitment occur in a space of $V = 1.96 \times 10^5 \mu\text{m}^3$, corresponding to a volume of a vessel of 25μm of radius and 100μm of length, and in a simulated time of 15s. In the considered volume V, the number of mulecules is of the order of 10^6. In our simulations the values

$SYSTEM ::= PSGL1|PSELECTIN|CHEMOKIN|CHEMOREC|ALPHA4$
$|VCAM1|LFA1|ICAM1$

$PSGL1 ::= (\nu\ backbone)BINDING\_PSITE1$
$BINDING\_PSITE ::= (\overline{bind}\langle backbone\rangle, RA).PSGL1\_BOUND(backbone)$
$PSGL1\_BOUND(bb) ::= (\overline{bb}, RD_0).PSGL1$
$PSELECTIN ::=$
$\quad (bind(cross\_backbone), RA).PSELECTIN\_BOUND(cross\_backbone)$
$PSELECTIN\_BOUND(cbb) ::= (\overline{cbb}, RD_0).PSELECTIN$

$CHEMOKIN ::= (\nu\ chemobb)BINDING\_CSITE$
$BINDING\_CSITE ::= (\overline{lig}\langle chemobb\rangle, RA\_C).CHEMOCHIN\_BOUND(chemobb)$
$CHEMOCHIN\_BOUND(chemobb) ::= ACT1|ACT2|ACT3(cbb)$
$ACT1 ::= (\overline{alpha\_act}\langle sign1\rangle, A).ACT1$
$ACT2 ::= (\overline{lfa\_act}\langle sign2\rangle, A).ACT2$
$ACT3(chb) ::= (\overline{chb}, RD\_C).CHEMOKIN$
$CHEMOREC ::=$
$\quad (lig(cross\_chemobb), RA\_C).CHEMOREC\_BOUND(cross\_chemobb)$
$CHEMOREC\_BOUND(ccr) ::= (ccr, A).CHEMOREC$
$ALPHA4 ::= (alpha\_act(act\_a), A).ALPHA4\_ACTIVE$
$LFA1 ::= (lfa\_act(act\_l), A).LFA1\_ACTIVE$

$ALPHA4\_ACTIVE ::= (\nu\ backbone2)BINDING\_ASITE$
$BINDING\_ASITE ::= (\overline{bind2}\langle backbone2\rangle, RA).ALPHA4\_BOUND(backbone2)$
$ALPHA4\_BOUND(bb2) ::= (\overline{bb2}, RD_1).ALPHA4$
$VCAM1 ::= (bind2(cross\_backbone2), RA).VCAM1\_BOUND(cross\_backbone2)$
$VCAM1\_BOUND(cbb2) ::= (\overline{cbb2}, RD_1).VCAM1$

$LFA1\_ACTIVE ::= (\nu\ backbone3)BINDING\_SITE3$
$BINDING\_SITE3 ::= (\overline{bind3}\langle backbone3\rangle, RA).LFA1\_BOUND(backbone3)$
$LFA1\_BOUND(bb3) ::= (\overline{bb3}, RD_2).LFA1\_BOUND$
$ICAM1 ::= (bind3(cross\_backbone3), RA).ICAM1\_BOUND(cross\_backbone3)$
$ICAM1\_BOUND(cbb3) ::= (\overline{cbb3}, RD_2).ICAM1\_BOUND$

$RA = 6.500 \quad RA\_C = RD_0 = 0.051 \quad RD_1 = 5.100$
$RD_2 = 1.000 \quad RD\_C = 3.800 \quad A = infinite$

$Radius\ of\ vessel = 25\ micromenters \quad Length\ of\ vessel = 100\ micromenters$
$Volume\ of\ vessel = 1.96 \times 10^5\ cubic\ micrometers \quad Radius\ of\ lymphocyte = 5\mu m$

of the volume and of the molecules number have been proportionally re-scaled by this factor, to make the code computationally faster.

The stochastic reaction rates for bimolecular binding/unbinding reaction are inversely proportial to the volume of space in that the reactions occur [10], in particular for the stochastic association rate we have that $RA = k_{on}/V$ and for the stochastic dissociation rate we have $RD = 2k_{off}/V$, where the k_i's are the deterministic rates.

The output of simulation is the time-evolution of number of bonds (shown in Fig. 4) assuming the following densities expressed in μm^{-2}: PSGL-1 [19] and P-SELECTIN 5600, ALPHA4 [4] and VCAM-1 85, CHEMOREC and CHEMOKINES 15000, LFA-1 [11] and ICAM-1 5500. The characterization of the steps and the adhesion molecules implicated in lymphocyte recruit-

ment in brain venules was performed by using intravital microscopy, a potent technique allowing the visualization and analysis of the adhesive interactions directly through the skull in live animal

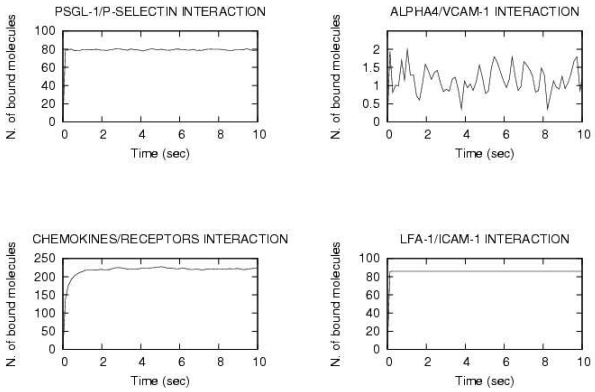

Figure 4. BioSpi simulation of 4-phases model of lymphocyte recruitment.

The BioSpi simulations reproduce the hyperbolic behavior predicted by the Dembo model. However unlike Dembo model, the BioSpi model is more sensitive to the variations of the dissociation constant rate $k_{off}^{(u)}$.

Moreover the plots in Fig. 4 show the relevant roles played by PSGL-1/P-Selectin and LFA-1/ICAM-1 interactions. The curve describing the time-evolution of the bonds number of LFA-1/ICAM-1 interaction presents an approximately linear steep increasing (with an angular coefficient of the order of 10^3) followed by a clearly constant behavior: this curve represents the firm adhesion of lymphocyte and it is comparable with the state diagram of the Bell model of Fig. 3. In fact, the firm arrest is reached when the number of bonds become stably constant in the time or, analogously, when the position of cell centroid does not change anymore. On the contrary, the plots representing PSGL-1/P-SELECTIN and ALPHA4/VCAM-1 interactions present, after a steep increasing with about the same slope of that of LFA-1/ICAM-1 binding, an oscillating behavior respect to the equilibrium positions given by the $y = 80$ and $y = 1$, respectively. This behavior represents the sequential bonds breaking and formation in the selectins and integrins binding during the rolling (see Fig. 3 for comparison).

The results obtained in this work assert that the formal description provided by BioSpi model represents in a concise and expressive way the basic physics governing the process of lymphocyte recruitment.

More generally, physics describes either microscopic or macroscopic interactions between bodies by means of the concept of *force*, that expresses the action of the field generated by a particle (or a set of particle) on the other bodies of the system. BioSpi representation hits this remarks, that is just the central paradigma of the physical description of the nature and summarizes it in the new concepts of *communications exchange* or (*names passing*). Moreover, the rates of communication in stochastic π-calculus include all the dynamic of the system, because they contain the quantitative information about the intensity of the forces transmitted between the particles. Finally, the main advantage of the BioSpi model is that thte π-calculus permits to better investigate dynamics, molecular and biochemical details. It has a solid theoretical basis and linguistical structure, unlike other apporaches [5].

5 Conclusion

The usage of new languages such as stochastic π calculus to describe and simulate the migration of autorective lymphocytes in the target organ will help us better understand the complex dynamics of lymphocyte recruitment during autoimmune inflammation in live animal. Furthermore, our approach may represent an important step toward future predictive studies on lymphocyte behavior in inflamed brain venules. The stochastic calculus may, thus, open new perspectives for the simulation of key phenomena in the pathogenesis of autoimmune diseases, implicating not only better knowledge, but also better future control of the autoimmune attack.

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