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Concurrency in leukocyte vascular recognition: developing the tools for a predictive computer model

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Leukocyte recruitment has a crucial role in inflammation and immunity. An interplay between adhesion molecules and pro-adhesive agonists generates a complex molecular network controlling tissue-specific and inflammation-dependent leukocyte vascular recognition. Recent findings highlight the importance of quantitative parameters in controlling the specificity of leukocyte vascular recognition. Introduction of quantitative parameters demonstrates the non-linear behavior of the process and suggests the necessity for a revision of the traditional model. We propose a formalization of the original multi-step model of leukocyte vascular recognition by introducing the notion of concurrency that explains how the quantitative variation of pro-adhesive parameters might control the specificity and the sensitivity of this process. Moreover, we discuss how concurrency, by integrating quantitative parameters, constitutes a central concept for the implementation of a predictive computer modeling of leukocyte vascular recognition.

Leukocyte recruitment is a highly controlled process that is crucial in inflammation and immunity. Leukocyte recruitment is initiated by vascular recognition of cells flowing in blood [1]. Tissue-specific leukocyte vascular recognition and extravasation from blood into tissues relies on the functional interplay between adhesion molecules and chemoattractants [2,3]. Numerous molecular components of this system have been identified and their mode of action deeply investigated at the molecular level.

Leukocyte vascular recognition is commonly thought to involve three major steps, each mediated by a distinct protein family. Selectins control initial tethering and rolling of free-flowing white blood cells on carbohydrate moieties present on endothelia [4]. The slow motion of rolling leukocytes then facilitates sensing of chemoattractants exposed on the endothelial surfaces. Subsequently, chemoattractants rapidly deliver intracellular signals through seven transmembrane domain G protein-coupled receptors (GPCRs) which, in turn, promote leukocyte firm adherence and transendothelial migration by upregulation of integrin adhesiveness to Ig-like family endothelial ligands [5].

The discovery of selectins, integrins, chemoattractants and their receptors, endowed with leukocyte- as well as tissue-specific expression patterns, fostered the development of the concept of tissue-specific area codes for leukocyte trafficking [2,6]. In such a model, selectins, chemoattractants and integrins are proposed to act together, generating a great combinatorial diversity depending on the type of selectin–carbohydrate, chemoattractant–receptor and integrin–Ig ligand pairs involved in leukocyte–endothelium interactions. The combinatorial logic of the multi-step model illuminates how, by combining a relatively small set of address signals, it is possible to generate a variety of leukocyte 'area codes' for different tissues. It was therefore postulated that each leukocyte subtype is equipped with a specific combination of receptors, enabling it to enter those tissues that display the appropriate counter-receptors. This mechanism would generate an unambiguous tissue-specific molecular code. Thus, the multi-step paradigm not only models the leukocyte extravasation process but also provides a conceptual framework for the exquisite specificity of leukocyte vascular recognition.

However, despite the increased knowledge gathered in the last decade, the bewildering complexity and redundancy of the system still defeats our ability to describe lymphocyte vascular recognition at a systemic level. Accumulating evidence shows an unexpectedly high degree of promiscuity and redundancy in ligand–receptor interactions [7,8]. Most leukocytes possess largely overlapping patterns of receptors and a multiple overlapping series of chemokines are often found expressed on endothelial cells from distinct tissues. These findings have blurred the ability of the original multi-step model to predict how a specific combination of pro-adhesive molecules might control selective leukocyte vascular

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recognition. Importantly, the regulatory significance of quantitative variations of pro-adhesive parameters is poorly emphasized by the model.

Towards a concurrent model for leukocyte vascular recognition

It might now be useful to analyze the combinatorial logic of the original leukocyte vascular recognition model. In this model, leukocyte vascular recognition is controlled by a molecular regulatory circuit that brings a circulating cell from a free-flowing state (state A) to a rolling state (state B), followed by an activated state (state C) and a final arrested state (state D). We can consider this as a serial process in which transitions are triggered by a sequence of non-overlapping molecular signals: signal 1 (rolling by selectins) for A|B transition state, signal 2 (activation by chemoattractant receptors) for B|C transition state, signal 3 (adhesion by integrins) for C|D transition state, (Figure 1). But is this model adherent to experimental observations?

It is important to note that states B, C and D are transitional and reversible parts of the system that are attained when signals are of sufficient strength to overcome intrinsic threshold values. For instance, rolling (state B) occurs only until selectins or integrins are engaged and is characterized by weak biochemical interactions. Furthermore, integrin activation by chemoattractants is a rapid but transient phenomenon [5]. This rapid reversibility of each of the states of the system imposes the need for a simultaneous, rather than a serial, delivery of multiple signals. Indeed, because every step depends on the previous one, termination of a step before initiation of the next one would stop the entire process. Furthermore, integrins can mediate both rolling and arrest of leukocytes [9]. Thus, in certain circumstances, the same molecule can provide signal 1 or signal 3 to promote the A|B as well as the C|D transition. Moreover, recent observations have indicated that chemokine receptor signaling is capable of triggering rolling or immediate arrest of leukocytes under conditions of flow [10]. Thus, signal 2 can promote the B|C or the A|C transition, in addition to the A|B transition. Altogether, these observations indicate the presence, during leukocyte vascular recognition, of overlapping regulatory mechanisms and dynamic interactions, whose persistency is necessary to drive the execution of the entire process.

This close inspection of molecular mechanisms indicates that leukocyte vascular recognition depends on a continuum of overlapping transitional states. In this context, vascular recognition should be modeled as a 'concurrent process'. Indeed, concurrency implies overlapping events. The concept of 'concurrency' is well known in computer science, where computation of contemporary events often occurs (see later). Thus, in a concurrent model of leukocyte vascular recognition, the final state of the system is achieved only if the three input signals are delivered simultaneously. So, in our model, the state transition A|B is triggered by signal 1; B|C by signals $1 + 2$; and C|D by summation of signals $1 + 2 + 3$ (Figure 2).

These aspects of molecular cooperativity can be formalized in a Boolean AND gate with three input signals that need to be delivered simultaneously to trigger the final output response (vascular recognition). Thus, leukocyte vascular recognition is properly described by the combinatorial logic of a concurrent model. Would a concurrent model be adequate to predict the functional outcome of the leukocyte vascular recognition process?

Concurrency and the importance of quantitative parameters in leukocyte vascular recognition

In a 'strictly' serial model of leukocyte vascular recognition, each transition state is triggered when a threshold value of a particular receptor–ligand interaction is reached, independently of the strength of the other type of signals. This model is still combinatorial at a qualitative level but fails to convey information regarding the amount of signals delivered at each step. In fact, each transition of

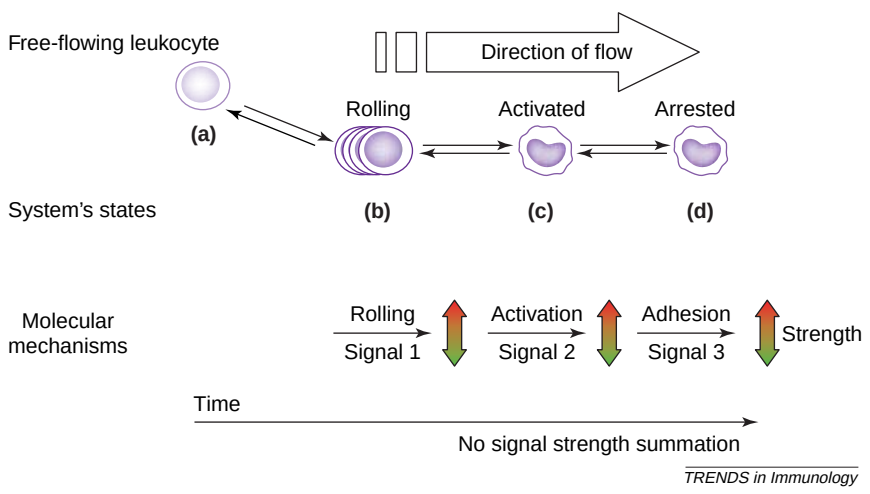

Figure 1. The serial model for leukocyte extravasation. The distinct states into which a free-flowing leukocyte must transit before arrest on endothelial surfaces can occur are depicted. The system's states are indicated in parentheses: **(a)** free-flowing leukocyte, **(b)** rolling leukocyte and **(c)** arrested leukocyte. In a serial model, the molecular signals are delivered in a temporally ordered 'serial' sequence. The figure illustrates the concept that in a serial model there is no summation of signal strength in time. In this model, the strength or quantity of each of the molecular signals dictating each of the transition cannot be fully integrated. This model enables 'qualitative summation' and combinatorial usage of information but because each process proceeds independently of the other no quantitative integration of signal strength is permitted.

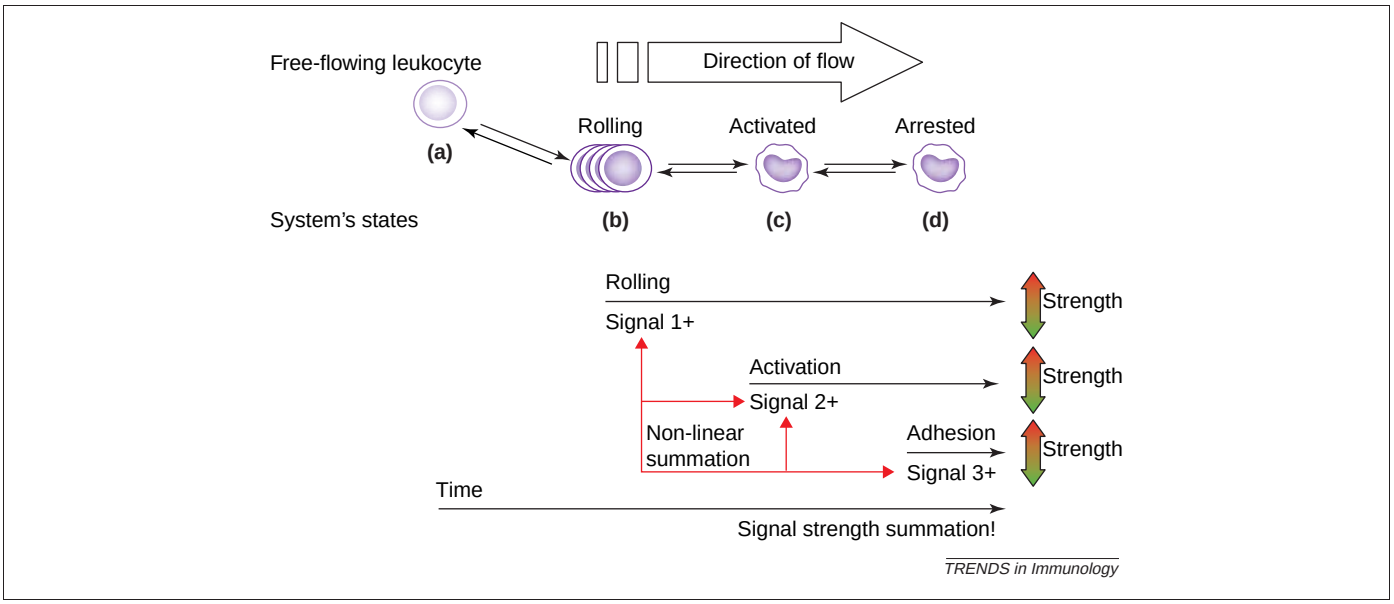

Figure 2. The concurrent model for leukocyte extravasation. The distinct states into which a free-flowing leukocyte must transit before arrest on endothelial surfaces can occur are depicted. The system's states are indicated in parentheses: **(a)** free-flowing leukocyte, **(b)** rolling leukocyte and **(c)** arrested leukocyte. In a concurrent model, the molecular signals overlap temporally and physically and are applied contemporarily. The figure illustrates the concept that in a concurrency based model there is summation of signal strength in time. In this model, each concurrent process influences the response of the others and the strength of each of the molecular signals dictating each of the transitions can be added up and integrated. Red arrows connecting distinct signals indicate the ability of these concurrent processes to influence the response of each other. These influences are ultimately responsible for complex non-linear behavior of the system.

state occurs, or fails to, depending on whether or not a defined threshold value of the signal is achieved. There is no summation of strength value between distinct signals; summation is 'qualitative' but not 'quantitative'. In such a model, 'quantitative' information about signal strength, below or above the threshold values required for each transition of state, is not computed by the system and becomes irrelevant. For instance, the well known variability in the expression level of integrins, Ig-like ligands and chemokine receptors, which are dependent on leukocyte subtype, cell activation state and regional microvasculature, is scarcely emphasized in a serial model.

However, interpretation of leukocyte vascular recognition as a concurrent process enables quantitative variation of pro-adhesive parameters to be combined along the different steps of the cascade. As an example, in a serial model, the expression level of chemokine receptors on leukocytes (one factor controlling the strength of the activation signal) dictates whether or not arrest of rolling cells is induced independently of the expression level of integrins, selectins and their ligands. By contrast, in a concurrent model, quantitative variation of different parameters can be combined over time. The effectiveness of a discrete expression level of a given chemokine receptor in triggering leukocyte arrest will be amplified by the strength of other concurrent signals, for example, the expression level of integrin ligands or the amount of presented chemokine. Such a system would be able to store and combine both qualitative as well as quantitative information. Thus, quantitative differences in signal strength become much more relevant in a concurrent versus a serial model.

Another crucial aspect of concurrency deserves elaboration. Concurrent processes can exert reciprocal influences on the response amplitude of each other. This means that the strength of one process can be affected by the strength of other concurrent processes. This feature is responsible for generating the non-linear behavior of a system of concurrent processes. To better illustrate this concept, let us see how it applies to leukocyte vascular recognition. In our model, rolling, activation and adhesion are three concurrent processes organized in a small network that constitutes the system of leukocyte vascular recognition (Figure 2). The initial process of leukocyte rolling enables sensing of chemoattractants presented on the endothelium. Intuitively, it is conceivable that the slower the rolling, the higher is the probability for leukocytes to perceive the appropriate chemokine signal, and subsequently adhere. Hence, the rolling velocity (the strength of the output response for the rolling process), which is dependent on the density and type of adhesion molecules engaged between leukocytes and endothelium, could modify the response curve of the concurrent processes of cell activation and cell arrest. Taken together, these observations elucidate how quantitative summation of the output values for concurrent processes can be non-linear and ultimately result in large variations of the final output of the system triggered by even small changes in the parameters of each concurrent process.

These features of a concurrent multi-step model could explain the high noise-filtering capacity, non-linear behavior and great flexibility of the process of leukocyte vascular recognition allowing for its ability to perform digital-like responses. Importantly, this provides a framework to interpret how quantitative differences in chemokine receptor engagement can lead to qualitative diversification in lymphocyte recruitment. As an example of this phenomenon, we have recently documented that quantitative differences in the expression of chemokine receptors in functionally distinct subsets of T helper cells

can lead to qualitative differences in dynamic adhesion under conditions of physiologic flow [11]. In this experimental setting, the specificity of lymphocyte subset-integrin-mediated adhesion depends on the integration of multiple quantitative parameters, including the expression level of the chemokine receptor and the amount and affinity of the immobilized chemokine. Thus, a concurrent model provides a way to understand how selective recruitment of specific cell subsets could be achieved on the basis of quantitative differences in chemokine potency and/or amount (Figure 3).

In addition, the introduction of a concurrent model could help to illuminate the regulatory role of the dynamic expression of pro-adhesive molecules, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), by the inflamed endothelium. Finally, concurrency could help to explain why vascular recognition triggered by chemokine receptors under flow is a much more threshold-sensitive phenomenon compared to chemotaxis [12,13] (see later). Thus, the proposed concurrent model enables the potential investigation of the physiologic relevance of quantitative variations in pro-adhesive parameters.

Concurrency and chemokine receptor redundancy

The chemokine system bears a high degree of redundancy and promiscuity, which is proposed to increase the robustness of the system, thus enabling tolerance to attacks by pathogens [7]. In this system, several chemokine receptors are simultaneously expressed on the surface of any given leukocyte subtype and are likely to be engaged by multiple chemokines presented, at the same time, on the inflamed endothelium [14,15]. Because chemokine receptors trigger intracellular signals, leading to integrin activation and chemotaxis, the ability of rolling leukocytes to arrest and extravasate will be influenced by the summation of signals delivered by the multiple chemokine receptors engaged on the surface of the leukocyte. In a concurrent model, the strength of a single activation signal will be weighted by the cell and integrated with the strength of the other signals. Thus, the concurrency of multiple signals would enable the cell to overcome the activation threshold. Overall, concurrency highlights a new biological meaning for redundancy and robustness in the chemokine network and suggests how the integration of multiple signals might increase the flexibility of leukocyte vascular recognition. It is perhaps interesting to highlight at least one example of redundancy and unexplained complexity in chemokine receptor biology that could find a novel interpretation based on our model. It has been suggested that a subset of skin-homing T cells uses at least two chemokine receptors, CCR4 and CCR10, to transmigrate in the inflamed skin [16–18]. In one study, blocking of CCR10 sufficed in inhibiting inflammatory cell recruitment [19], whereas in another, simultaneous ablation of CCR4 and CCR10 were required [20]. These conflicting observations could potentially find an interpretation based on concurrency of poorly observed quantitative differences in pro-adhesive factors (particularly the amount of chemokines presented on the endothelium) induced under distinct experimental conditions.

Concurrency and the role of speed

Several quantitative parameters are likely to influence integrin activation by chemokines, including the number of chemokine receptors expressed on the leukocyte surface, the affinity constants for ligand–receptor interactions and the density of chemokines presented by the endothelium [11]. The effectiveness of all these factors in controlling integrin activation is, in turn, influenced by hemodynamic parameters, such as the velocity of the blood flow. The velocity of blood flow determines the speed of flowing or rolling leukocytes and this imposes a temporal restraint for their activation and arrest. Indeed, a fast flowing or rolling cell has less time to interact with chemokines or with adhesion molecules expressed by the endothelium. Thus, cell movement tends to reduce the efficiency of integrin activation and this should be computed in a

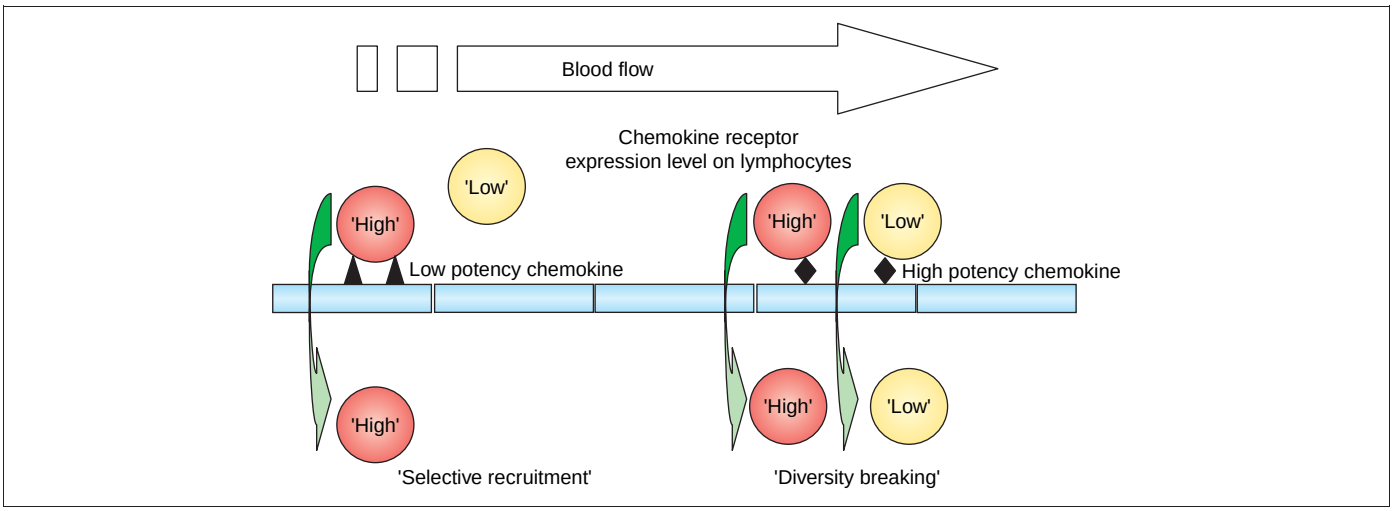

Figure 3. A model based on concurrency in vascular recognition, explaining how quantitative variations in chemokine potency and receptor level might be integrated to control the specificity of lymphocyte subset recruitment. In our model, endothelial display of a low potency chemokine will result in selective vascular recognition by a lymphocyte subset expressing high levels of receptor, thus achieving a high degree of specificity. By contrast, endothelial display of a high potency chemokine will promote vascular recognition by lymphocyte subsets expressing both high as well as low levels of receptor, potentially breaking the diversity of lymphocyte subset vascular recognition. Reproduced with permission from Ref. [11]. (Copyright 2002. The American Association of Immunologists, Inc.)

realistic model. In a concurrent process, quantitative variations of blood-flow shear stress in distinct anatomical districts can be computed and, thus, become relevant to establish adjustable thresholds for leukocyte extravasation [21,22]. Interestingly, recent data lend support to a crucial role for blood-flow shear stress in the regulation of lymphocyte transmigration in inflammation. Lymphocyte–endothelial adhesion and extravasation have been found to associate with the appearance of focal structural dilations of microvessels in inflamed skin that lead to a marked reduction in wall shear stress [23]. Thus, quantification of hemodynamic properties of the microvasculature becomes as important as quantification of molecular parameters involved in the process.

Concurrency in leukocyte vascular recognition and computer modeling

Is it conceivable to develop a computational method that is able to integrate experimental quantitative data, leading to reliable prediction of leukocyte behavior in the microvasculature? To respond to this question, it might now be useful to introduce some concepts and recent advances in computer science.

In computer science, concurrent systems are defined as a group of co-existing computational processes that can communicate with each other in a synchronous or asynchronous way [24,25]. Computer science proposed abstract formal languages, initially developed for the specification of concurrent computational processes, which were found particularly suitable to describe interactions between molecules in biological networks. Significant progresses in the mathematical theory of concurrent computation have been recently made in the context of the so-called π-calculus [25–27]. The π-calculus has been proposed as appropriate to model a system of interacting molecules in a network of concurrent biochemical reactions [28]. Moreover, a significant improvement of the π-calculus was introduced [29,30] by assigning different rates to the involved biochemical reactions (BioSpi, http://www.wisdom.weizmann.ac.il/~aviv/). Lecca *et al.* have recently been able to implement a computer simulation of the process of leukocyte vascular recognition in the context of the BioSpi model by taking into account quantitative parameters and leading to some predictive outcomes [31] (http://www-smi.stanford.edu/projects/helix/psb04/; www-smi.stanford.edu/projects/helix/psb04/lecca.pdf). Importantly, by computing pro-adhesive parameters typical of inflamed brain microvessels, a tissue environment crucial to the pathogenesis of multiple sclerosis, the model is able to calculate the number of encephalitogenic lymphocytes recruited to the brain microcirculation as a function of molecule expression density and hemodynamic parameters of the microvasculature, in agreement with experimental observations [32–33] (http://portal.acm.org/citation.cfm?doid = 967900.967944; http://arc.cs.odu.edu:8080/dp9/getrecord/oai_dc/UNITN.Eprints/oai:UNITN.Eprints:484). These recent developments exemplify the importance of a model able to compute quantitative parameters, and suggest the concrete possibility in the future of developing accurate mathematical models of leukocyte vascular recognition that can consider cell- and microvasculature-specific quantitative parameters, thus leading to organ- and, perhaps, disease-specific computer simulations and predictions.

Conclusions

Leukocyte vascular recognition is an example of a complex dynamic system displaying non-linear behavior. The original multi-step model of leukocyte extravasation elucidated how selective recruitment of leukocyte subtypes can be achieved in different tissues and organs by qualitatively combining distinct sets of molecules, however, it paid scarce attention to the importance of quantitative parameters in the regulation of selective leukocyte vascular recognition. Moreover, investigation of the molecular networks controlling cell migration has revealed a high level of redundancy, promiscuity and complexity, not envisioned in the original formulation of the model. A systematic analysis of leukocyte vascular recognition enabled us to propose a novel interpretation based on concurrency that emphasizes the crucial importance of concurrency of distinct molecular signals and hemodynamic parameters in controlling the specificity of leukocyte vascular recognition. This model implies the existence of qualitative, as well as quantitative, area codes controlling leukocyte vascular recognition in different physiopathological situations. Notably, in the context of the emerging field of systems biology, in which massive cell biology scale-up experimentation and mathematics combine to develop predictive computer modeling in life sciences, representation of leukocyte vascular recognition as a concurrent computational process might open completely new scenarios. Indeed, because concurrency can take into due account the importance of quantitative variations in pro-adhesive parameters, a computer model based on concurrency might help to deeply analyze the intricate nature of leukocyte vascular recognition and increase our ability to predict, and thus pharmacologically manipulate, the evolution of this complex process in health and disease.

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