A model of TLR4 signaling and tolerance using a qualitative, particle–event-based method: Introduction of spatially configured stochastic reaction chambers (SCSRC)

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**ABSTRACT**

Introduction: There have been great advances in the examination and characterization of intracellular signaling and synthetic pathways. However, these pathways are generally represented using static diagrams when in reality they exist with considerable dynamic complexity. In addition to the expansion of existing mathematical pathway representation tools (many utilizing systems biology markup language format), there is a growing recognition that spatially explicit modeling methods may be necessary to capture essential aspects of intracellular dynamics. This paper introduces spatially configured stochastic reaction chambers (SCSRC), an agent-based modeling (ABM) framework that incorporates an abstracted molecular ‘event’ rule system with a spatially explicit representation of the relationship between signaling and synthetic compounds. Presented herein is an example of the SCSRC as applied to Toll-like receptor (TLR) 4 signaling and the inflammatory response.

Methods: The underlying rationale for the architecture of the SCSRC is described. A SCSRC model of TLR-4 signaling was developed after a review of the literature regarding TLR-4 signaling and downstream synthetic events. The TLR-4 SCSRC was implemented in the free-ware software platform, Netlogo. A series of in silico experiments were performed to evaluate the response of the TLR-4 SCSRC with respect to response to simulated administration of lipopolysaccharide (LPS). The pro-inflammatory response was represented by simulated secretion of tumor necrosis factor (TNF). Subsequent in silico experiments examined the response of the TLR-4 SCSRC in terms of a simulated preconditioning effect represented as tolerance of pro-inflammatory signaling to a second dose of LPS.

Results: The SCSRC produces simulated dynamics of TLR-4 signaling in response to LPS stimulation that are qualitatively similar to that reported in the literature. The expression of various components of the signaling cascade demonstrated stochastic noise, consistent with molecular expression data reported in the literature. There is a dose dependent pro-inflammatory response observed with increasing initial doses of LPS, and there was also a dose dependent response with respect to preconditioning effect and the establishment of tolerance. Both of these dynamics are consistent with published responses to LPS.

Conclusions: The particle-based, spatially oriented SCSRC model of TLR-4 signaling captures the essential dynamics of the TLR-4 signal transduction cascade, including stochastic signal behavior, dose dependent response, negative feedback control, and preconditioning effect. This is accomplished even given a high degree of molecular event abstraction. The component detail of the SCSRC may allow for sequential parsing of various preconditioning effects, something not possible without computational modeling and simulation, and may give insight into the expected consequences and responses resulting from manipulation of one or many of these modulating factors. The SCSRC is admirably a work in evolution, and future work will sequentially incorporate additional regulatory mechanisms, both intracellular and paracrine/auto-crine, and improved mapping between the spatial chamber configuration and molecular event rules, and experimentally define biochemical reaction rate constants. However, the SCSRC has promise as a highly modular and flexible modeling method that is suited to the dynamic knowledge representation of intracellular processes.

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1. Introduction

Biological systems are complex and dynamic, and present significant challenges to attempts to understand and manipulate them. The traditional scientific method of reductionism has been
successful in terms of identifying the underlying mechanisms and components of biological systems. However, the process of integrating that knowledge to reconstruct system-level behavior has not progressed to the same degree [1,2]. This deficit is most evident in attempts to characterize and manipulate highly interconnected systems involving multiple positive and negative feedback control loops. These types of processes are primarily involved in homeostasis and maintenance of bodily integrity. The challenge of understanding these systems is seen in the ongoing, and often frustrating, search for effective therapies for ‘system-dysfunction’ conditions such as cancer, sepsis, autoimmune disorders, HIV and transplant rejection [2,3].

In addition to the difficulty associated with formally characterizing the dynamics of complex interconnected systems, biological systems present additional challenges associated with their multi-scale, multi-hierarchical structure [4–9]. This organizational structure renders generative mechanisms ‘opaque’ to observations obtained at a particular phenomenological level. Due to the robust nature of biological systems, successive hierarchies of organization ‘hide’ the underlying details while presenting apparently straightforward observable states. From an analytical standpoint, this does not present a significant problem in accepting a representation at a particular level of resolution as long as (1) there is a relatively linear relationship between perturbations to the system and transitions from one state to another and (2) attempts to manipulate the system do not require making assumptions regarding causality below the resolution of the representation of the system.

Unfortunately, these caveats come into play in the current environment of drug discovery and development for the ‘system-dysfunction’ diseases noted above. Drug candidates are being developed based upon data obtained at finer and finer degrees of mechanistic resolution, without the means to address the fact that this analytical movement places greater and greater demands on the ability to characterize the dynamics of the systems being manipulated. Explicitly representing dynamics at various levels of resolution is important in order to develop an integrative modeling system to address the multi-hierarchical nature of biological systems. An agent-based approach has been proposed to developing this modular, multi-scale architecture [8,9]. As a component of that architecture, the current paper will focus on the representation of intracellular events, the level of resolution that is currently the target of a great deal of biomedical and pharmaceutical research.

I propose that while it is important to be able to explicitly represent multiple levels of biological processes, to a great degree this can be sufficiently accomplished with qualitative representation. Other authors have noted the utility of qualitative modeling of biochemical networks [10–12], and listed below are some justifications for this approach.

(1) The robust nature of biological systems suggests that, in general for most intracellular events, minute changes in intracellular details are not likely to be significant to the overall dynamics of the system. The regions of the system that do emerge as critical points of control will be those that maintain their ‘switching’ capacity across a qualitative range of states. This has been labeled the ‘bowtie’ structure of biological systems [13].

(2) ‘Complete’ knowledge of the state and dynamic control of a system is impossible. Despite significant advances in structural molecular biology and identification of common motifs associated with protein–protein interactions, there will always be limits to the capacity to measure and quantify intracellular processes. This is true not only from a pragmatic methodological standpoint, but also from an epistemological standpoint given the necessity of dealing with the intrinsic issues of ‘incompleteness’ of knowledge. Therefore, some degree of qualitative representation of these processes will always be present in the formation of conceptual mechanistic models of signal transduction.

(3) Current hypotheses/conceptual models/thought experiments exist in qualitative form. These conceptual models represent the ‘knowledge’ extracted from and communicated in the biomedical literature, and define the basis for further experiments and investigation. Currently, however, conceptual models are represented very generally (if they are even represented at all) in diagrams or flow charts. These forms of depiction are ‘static’, in so much that they do not show the consequences of the actions they purport to represent. As a result, despite their utility, the verification or checking of conceptual models represented in this fashion is only possible using indirect methods of additional experiment, observation and assumption.

Being able to ‘bring these diagrams to life’ in a dynamic fashion can be beneficial in the verification of conceptual models, such that the consequences of represented actions and interactions can be seen and evaluated. This involves the construction of simulations, or, as has more recently been labeled, performing ‘executable biology’ [14].

All modeling requires some degree of abstraction. The more traditional methods of mathematical modeling have involved descriptions based on differential equations, usually ordinary differential equations. This method has been successful in modeling kinetic processes in well-mixed systems, classically manifested as biochemical reactions. However, in addition to fluxes of compounds biological systems have spatial structure and configuration. Furthermore, at a basic level, biochemical processes involve discrete events between molecule-particles. These are the events, when scaled up to molar concentrations using statistical mechanics that underlie kinetic rate equations. One approach to modeling these reactions is at a molecular-structure level of resolution, as can be seen in large-scale physical system models (such as protein folding models). However, if the goal is to characterize these processes in the context of their influence on cellular behavior it is possible to accept a level of abstraction that eliminates the details of the molecule–molecule interaction by labeling them generically as ‘interaction events.’ When viewed from the standpoint of intracellular signaling, the validity of this approach becomes immediately evident: the ‘reality’ of the molecule-to-molecule interaction, with respect to participants, conditions and outcomes, is not in dispute. After all, these aspects are abstracted in rate equations. Rather, the emphasis is shifted to representing the physical actions involved and the factors that affect the interaction event. Accomplishing this requires two realizations:

(1) Molecules do not have ‘volition’ to direct their movement to find their reaction partner. Specified reactions can only occur if reactant molecules can come into contact with each other, and this must rely on motion and movement that is ultimately Brownian, and therefore stochastic.

(2) Cells are not ‘bags of molecules,’ i.e. cells are not well mixed systems. Because molecules have no ‘volition’ spatial and environmental conditions within the cell must somehow direct signaling pathways by increasing the likelihood that participants in steps of a signal cascade will actually contact each other. Incorporating a spatial component to the characterization of signaling pathways, such as relating enzymes to the internal cytoskeletal architecture or simulating the effects of molecular crowding (that suggest that biochemical rate constants are dependent upon intracellular context [15]) can accommodate this.

Therefore, perhaps a modeling system can utilize abstraction at the level of the signaling event, without details as to what happens at a molecular structural level during the event. Interaction rates can be qualitatively scaled, as similar classes of interactions act within the same general timeframe, and the emphasis on physical proximity renders fine parsing of these rates unnecessary. Rather,
the focus is on characterizing conditions that lead to interaction events: molecular movement across space, likelihood of interaction events occurring and the ordering of signaling enzymes. This leads to a ‘particle’ view of signal transduction, where interactions within a reaction cascade follow a spatial architecture that is defined by the sequence of the signaling pathway. Particles are used to represent signaling events, and viewing the trajectory of the particle through the various reaction spaces can simulate transduction through a signal cascade. This modeling architecture is termed spatially configured stochastic reaction chambers (SCSRC).

1.1. Spatially configured stochastic reaction chambers (SCSRC)

The SCSRC is an agent-based model (ABM). Agent-based modeling is an object-oriented, discrete-event, rule-based, stochastic modeling method in which the components of a system are represented by computational objects (agents) that interact in a virtual environment, often representing a spatial architecture [16–18]. ABM is a more general description of a model type than the methods defined by the adjectives listed above: it extends object-oriented and rule-based models by utilizing parallelism to implement multiple instances of objects, objects that use class distinctions to specify different rules for agent behavior. Agent rules are executed in a discrete-event fashion, and often incorporate computations involving random number generators to introduce stochasticity into the behaviors of the individual agents. System behavior arises from the aggregated actions of the agents in the form of patterns that define system state [18]. ABMs are related to cellular automata, but extend that modeling method by incorporating multiple classes of agents and motion among the agents.

In the SCSRC each simulation space represents a single cell. The space is a grid of 2-dimensional squares. The agent level is at the ‘particle’ level, where each agent represents an abstracted molecule within the signal transduction cascade. The space is subdivided into a series of smaller rectangular compartments. The design features of the SCSRC are derived from the two central premises listed above: (1) molecules do not behave volitionally, and (2) spatial and structural factors influence the sequence of molecular interactions and signal transduction. The movement rules for agent-molecules in the SCSRC follow a Brownian random walk while they are simulating the molecular events of signal transduction. The subdivided compartments are reaction spaces that represent the close proximity of spatially located signaling enzymes. This proximity simulates either the arrangement of enzymes along cytoskeletal elements or the effects of molecular crowding in the cytoplasm. The apoposed borders of the reaction spaces represent sequential enzymes of the signal cascade. The entry of a molecule-agent into the reaction space through one of these ‘enzyme’ borders is intended to simulate the chemical reaction event catalyzed by the enzyme, producing the next signal molecule and introducing it into the next reaction space. The molecule-agent moves in a random fashion (i.e. it changes its direction within a random 360° arc). When it eventually encounters the opposite border of the reaction space representing the next enzyme in the signaling pathway the molecule-agent is transformed as it passes through that border into the next reaction chamber. As mentioned above, the specifics of the chemical reactions are abstracted into a state transition for the molecule-agent. Primarily, the state transition is merely a change in the labeling of that molecule-agent (to keep track of the signal as it propagates), but occasionally it results in altering the way it interacts with subsequent enzyme-borders.

For instance, inhibitory activity is simulated by agent-border interactions that lead to the affected areas of the enzyme-border being unable to execute the signaling state transition for subsequent encounters with upstream molecule-agents. The number of molecule-agents of a particular type maps to the strength of a signal. The spatial configuration of the chambers of the SCSRC is defined by the sequence of a signaling/synthetic pathway. Specific qualitative types of enzymatic reactions, such as signal amplification, inhibition or activation, can be specified in the encounter rules between the agent-molecules and the enzyme-borders. Currently, there is no accounting for differential reaction rate constants in either the size/shape of the reaction chamber, the movement rate of the molecule-agents, or in the state transition rules when a molecule-agent collides with an enzyme-border. Also, there is no accounting for the possibility of the ‘reverse reaction’ (i.e. passage of the signal-molecule agent backwards through a prior enzyme-border). The justification for this abstraction is that, in general, these enzymatic reactions are heavily favored in the forward direction, though this need not be the case. For purposes of introducing the SCSRC the initial demonstration model will be intentionally ‘ sloppy’ with respect to biochemical kinetics at the expense of component detail, and will demonstrate that this approach is sufficient in capturing key aspects of signal transduction dynamics.

The following sections will describe the implementation and behavior of a SCSRC of Toll-like receptor-4 (TLR-4) signal transduction and its downstream synthetic events (henceforth referred to as the ‘TLR-4 SCSRC’). The simulations performed with the TLR-4 SCSRC will demonstrate how the central behavioral characteristic of pre-conditioning and tolerance can be produced using this relatively abstract modeling method.

1.2. Reference system: Toll-like receptors and inflammation

The body’s response to infection is initiated by the recognition of bacterial cell wall products via a series of Toll-like receptors (TLR), primarily on inflammatory cells [19,20]. Activation of these receptors initiates signaling events that lead to a pro-inflammatory response designed to kill and contain an infectious insult, and also a compensatory anti-inflammatory response intended to attenuate the degree of the pro-inflammatory response. This pattern of response and counter-response is observed at multiple levels of resolution: at systemic levels of mediators and cellular populations, within specific localized tissue beds, and within cells themselves. The balancing behavior of these processes is reflected in the mechanisms of control of TLR signaling, including the phenomenon of tolerance or preconditioning. It is well recognized that the response of cells to inflammatory stimuli is affected by the past history of exposure to lower levels of that stimulus [21–24]. The preconditioning effect results in a dampening of the cellular response to repeated exposure to inflammatory stimulus, and is a manifestation of the same negative feedback processes intended to attenuate the initial pro-inflammatory response. The importance of the tolerance effect becomes more evident in light of increasing evidence of the responsiveness of TLR signaling to endogenous mediators indicating tissue damage, leading to the ‘danger signal’ hypothesis of acute inflammation [22,25,26]. Given this understanding, the inflammatory system can be viewed as being under continued assault as even ‘normal’ existence inevitably leads to minimal, but recurrent tissue damage. In such a situation, tight modulation and tolerance is of vital importance in order to keep the homeostatic inflammatory response appropriately proportional.

The TLR-4 pathway is one of the most intensively studied inflammatory signaling pathways [19,20,27]. While it is acknowledged that negative feedback control of the cellular inflammatory response also involves autocrine and paracrine effects of anti-inflammatory cytokines, such as Interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), and soluble TLR-4 receptor antagonists, this paper focuses on the intracellular regulation of TLR-4 signaling in response to stimulus to the bacterial product lipopolysaccharide (LPS). The rationale for this focus is that under-
standing and characterizing the properties of the intracellular control points provides a guide to the state changes of the constitutive actors (cells), which in turn determine, via their aggregated behavior, the observed phenomenon at higher levels of biological organization. Understanding the dynamics of these control points identifies targets for potential interventions, and modeling these control points provides a method of assessing the potential effects of these interventions.

2. Methods

2.1. Informational basis of the TLR-4 SCSRC

The informational sources for the components and mechanisms for the TLR-4 SCSRC were derived from a series of review articles on the modulation of TLR-4 signaling, as well as some more focused research articles to provide mechanistic insight into selected components [20,21,23,27–34]. The TLR-4 signaling pathway is, as would be expected given its central importance, an extensive and far-reaching system that interacts with multiple synthetic and metabolic pathways, and the intent of this exercise is not to produce a comprehensive model of all aspects of this system (for this description, see Ref. [27]). Rather, the TLR-4 SCSRC is intended to simulate the intracellular feedback effects of a particular module of the TLR-4 signaling pathway and its contribution to preconditioning. As with all modeling endeavors, it is important to delineate the specific assumptions involved in developing a model. The following guidelines were used to decide which components to include, where in the pathway to focus the modeling effort, and the degree of detail to be represented at each point.

(1) All aspects of a pathway control loop needed to be represented in a mechanistic fashion. A control loop is identified as leading from a stimulus to a feedback component, and then back to a control point in the stimulus propagation path. Functionally, this requires modeling signaling events from stimulus to a production of the inhibitory compound (usually implying gene activation and protein synthesis) and the transport of the inhibitory compound back to the signaling cascade. Since the production of the negative feedback mediator, often a protein, needs to be accounted for, both transcriptional and translational events have to be represented in some form.

(2) Following guideline #1 requires that there must be at least some information on the causal mechanisms and actors involved in the generation of inhibitory compounds in order for their inclusion. In fact, it is not sufficient for the literature to state that ‘increased expression of the gene for Inhibitor X lead to down regulation of TLR-4 signaling.’ This type of statement does not give any insight into how endogenous Inhibitor X was produced, or what up regulates gene-producing-X in vivo. Furthermore, a statement such as ‘LPS activation led to increased levels of Inhibitor X’ is also insufficient. There must be at least some mention of the intermediate steps involved in the production of Inhibitor X in order for Inhibitor X to be included. Thus, this restriction precludes the inclusion of such competitive inhibitors such as a shortened version of myeloid differentiation factor 88 (MyD88), MyD88s, and IL-1-associated kinase (IRAK)-M (an inactive isoform of IRAK-1 and IRAK-4) [31].

(3) Very complicated molecular events that can be reduced to simpler state/relationship transitions do not need to be explicitly modeled. This is done to reduce overall model complexity, and is utilized when evidence in the literature suggests that a complex, but relatively well-characterized molecular process leads to a relatively straightforward event. For instance, the interactions associated with tumor necrosis factor-receptor-associated factor 6 (TRAF-6) involve complicated ubiquitination and de-ubiquitination processes, not only with respect to its activation but also in terms of interactions with its putative inhibitor, the zinc ring-finger protein, A20 [30]. However, the end result of these events is that TRAF6 is activated via upstream LPS/TLR-4 receptor complexes, and A20, when present, inactivates TRAF6. Therefore, these interactions were modeled as such.

The determination of the signal cascade components to be included in the TLR-4 SCSRC was guided by the ability to fulfill these criteria as closely as possible for each potential component. It should be emphasized that these guidelines are subjective in nature, and their development and implementation was the sole responsibility of the author. However, to hearken back to the one of the initial goals of this modeling exercise, the dynamic representation of knowledge and verification of conceptual models, this subjective process also represents the current primary method by which researchers develop the knowledge frameworks underpinning their investigations. Recognizing the fact that all modeling (be it conceptual, computational or laboratory-based) is to some degree subjective emphasizes the need to be as explicit as possible with respect to communicating the underlying assumptions of the model. With this in mind, the following modeling decisions were made:

(1) The emphasis would be on intracellular feedback loops. Therefore, soluble TLR-4 receptor antagonists and paracrine/autocrine effects of cytokines such as IL-10 and TGF-β were not considered.

(2) A20 and inhibitor kappa-B proteins (IκB) are the primary intracellular negative feedback compounds whose mechanisms of production, modulation and points of effect could be extracted from the literature [28–31]. It should be noted, however, that information regarding the half-lives of these molecules and their degradation rates is significantly lacking. For purposes of their inclusion in the TLR-4 SCSRC, IκB is consumed only via its binding to NF-κB and A20 is consumed only via its binding to TRAF6. It is acknowledged that these modeling assumptions have potential consequences on the behavior of the TLR-4 SCSRC, particularly with respect to the reproduction of preconditioning and tolerance, and is therefore a recognized source of potential error in the TLR-4 SCSRC. However, it is also an example of how computational modeling of signal transduction can serve as a roadmap for future laboratory investigations, pointing to such gaps in knowledge that may have significant consequences in attempts to develop a mechanistic characterization of the dynamic behavior of the pathway.

(3) The NF-κB (NF-κB) activation pathway via TGF-β-activated kinase 1 (TAK1) provides the common link between upstream TLR-4 signaling and the production of A20, IκB and the putative pro-inflammatory output, tumor necrosis factor (TNF).

(4) While accepting that TAK1 activation also leads to signal transduction in the ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase) and p38 MAPK (mitogen-activated protein kinase) pathways with the subsequent activation of the nuclear transcription factor AP-1 (activator protein 1), also acknowledged to contribute to pro-inflammatory signaling, the decision was made not to incorporate these pathways for the lack of clearly identified intracellular negative feedback mediators generated by this pathway [27]. This is not to say that this mechanism does not exist, only that a causal mechanistic relationship between AP-1 and other intracellular modulators of TLR-4 signaling could not be established by the author’s review of the literature, and therefore precluded their incorporation into this model. In fact, the lack of explicit information regarding the potential relationship between these signaling cascades and the production of other intracellular mediators such as Toll-interacting proteins (Tollip) and suppressors of cytokine signaling (SOCS) might spur additional research in these areas.
The resulting model can be seen in a screenshot of the TLR-4 SCSRC in Fig. 1. A description of the architecture of the TLR-4 SCSRC is in the following section.

2.2. Architecture and properties of the TLR-4 SCSRC

The TLR-4 SCSRC was developed and implemented using the free-ware ABM modeling toolkit Netlogo [35]. The version of Netlogo used is 4.0.2, and the entire TLR-4 SCSRC is available for download at (http://bionetgen.org/SCAI-wiki/index.php/Gary_An#27.27.27Gary_An.27.27.27s_Page.27.27.27). Dimensions of the TLR-4 SCSRC are that of a 91 × 121 2-dimensional grid. Each time-step-iteration (‘ticks’) of the model represents 4 s of simulated time. Therefore, 15 ‘ticks’ represent 1 min of simulation time. Signal propagation is initiated at the top of the simulation space just external to the simulated cell membrane housing the TLR-4 receptors, and progresses ‘downward’ towards the barrier representing ‘DNA.’ Subsequent simulated mRNA transcription and protein synthesis are transmitted ‘upwards’ back towards the cell membrane. In general, molecule-agent collisions with enzyme-borders result in a state transition of the type of molecule-agent as it passes from one compartment to another. Reaction compartments constrain the random movement of specified molecule-agents based on the nature of the relationship between the signaling enzymes. For instance, signal propagation enzymes are assumed to be in close proximity and therefore have a smaller reaction space. On the other hand, the effect of inhibitory molecules (IκB and A20) are presumed to be less tightly coupled to their target enzymes, and therefore their reaction spaces are larger. The following regions and transition rules merit more detailed discussion.

(1) The transduction of the LPS binding signal via the activated trans-membrane TLR-4 receptor complex has two immediate intracellular binding partners that lead to parallel signaling pathways. The first, and likely dominant, pathway is initiated by binding between the intracellular domain of the TLR-4 receptor complex and the MyD88-adaptor-like (MAL, also known as TIRAP) protein, leading to the MyD88 signaling pathway [27,29]. However, blockade of MyD88 does not result in complete attenuation of the inflammatory response, and an alternative pathway acting through the TRIF-related adaptor molecule (TRAM, also known as TICAM-2) has been identified [31,34]. The TRAM–TRIF (TIR-domain-containing adaptor-inducing interferon-beta, also known as TICAM-1) pathway is a point of cross talk between the TLR-4 pathway and the intracellular TLR-3 pathway, but also propagates LPS signals to TRAF6, the common downstream mediator for both the TRAM–TRIF pathway and the MyD88–IRAK pathways. In this model, this parallel pathway structure is represented by dividing the compartment barrier below ‘TLR4-receptor complex’ reaction space into two types, one representing MAL and the other representing TRAM. Subsequent compartments that represent concurrent signaling levels of the parallel pathways are similarly divided. However, the respective enzyme-borders do not transmit molecule agents from the contra-parallel pathway, as there is no evidence of cross talk at these levels, and merely reflect the molecule-agents back into the reaction space when collisions occur. It should also be noted that the TRAM–TRIF–RIP1 pathway has one less ‘step’ than the MAL–MyD88–IRAK-4–IRAK-1 pathway. In order to maintain consistency between the reaction chambers, this is modeled by having the RIP1 signal ‘jump’ to and be constrained by the IRAK-1 border. While currently there is no real consequence for a particle passing down one pathway or the other, the representation of the parallel pathways would allow for future work in which it would be possible to separately measure the contribution of each pathway to signal propagation, allow for matching/validation/calibration of detailed molecular data should it become available, and also allow the simulation of specific pathway/enzyme inhibition/knock-out experiments should those be performed. Give these future considerations it is a useful means of representing bifurcation points in pathway signaling.

(2) The next area that deserves closer focus is at the NF-κB border. The activation of NF-κB actually results from a dis-inhibition of baseline inactive NF-κB resulting from degradation of the bound inhibitor IκB [19]. Therefore, in the resting cell, the ‘native’ population consists of inactive NF-κB bound to IκB. Transmitted signals through the TLR-4 pathway to TAK1 and then on to IκB protein kinases (IκC) lead to the degradation of IκB and subsequent formation of the active NF-κB heterodimer. However, NF-κB increases
production of IkB, leading to reconstitution of the inactive form of NF-κB, resulting in a negative feedback mechanism by which NF-κB regulates its own activity [28]. This process is the first control loop in the regulation of TLR-4 signaling that is modeled in the TLR-4 SCSR. It should also be noted that the activation of NF-κB and the increased transcription of the three DNA sequences represented in the model (IkB, A20, and TNF) represent the primary points of signal amplification in this model (see Fig. 2, letters C and D).

(3) The next area to be examined is the effect of A20 inhibition of TRAF6 activity [21,28,32,33]. As mentioned above, the dynamics of TRAF6 regulation are quite complex, involving ubiquitination and de-ubiquitination of the molecule for both its activation and de-activation, a process that is modulated by A20, which also undergoes ubiquitination [30]. These interactions have been abstracted into an inhibition effect on the TRAF6 border resulting from collision effects with A20 molecule-agents. Points of collision result in those segments of the TRAF6 border being unable to process upstream signals from either RP1 or IRAK-1. The inhibition, however, is not irreversible, as TRAF-6 is not degraded but recirculated by A20. Therefore, the inhibitory effect reverses after a time-frame extracted from the literature, set at ~50 min [28,30].

Secreted levels of TNF represent the pro-inflammatory output of the simulated cell. TNF-agents are produced using the same abstracted transcription/translation process as is used for IkB and A20. Once formed, these molecule-agents are given a straight trajectory to beyond the cell membrane, a process representing the transport mechanisms of secretion. Once they are ‘extracellular’, they are counted and subsequently degrade at a fixed rate [28].

It should be noted again that despite the rather extensive detail with respect to the included components of the signaling cascade, there is an extremely high level of abstraction regarding the biochemical kinetics of the molecular events. As a result, the sizes of the reaction chambers, movement rates for the signal-agents and state transition rules at the enzyme-borders are all the same in terms of reflecting the rates of the reactions. This is predicated upon the modeling abstraction that at the molecular level, the movement rates of molecules are essentially the same, and that in terms of signal transduction, the rates of these reactions are qualitatively similar. Part of the goal of the TLR-4 SCSR is to demonstrate the relative insensitivity of a signal transduction cascade to the details of these rate constants, suggesting that the robust nature of these pathways may be based to a great degree on their component-structure.

3. Simulations and results

Three sets of experiments were done with the model following its construction: (1) observation of the trajectory of a single signaling agent as it progressed from compartment to compartment, (2) demonstration of dose dependent dynamics of the simulated inflammatory response to various levels of administration of LPS, and (3) demonstration of the phenomenon of tolerance resulting from preconditioning doses of LPS.

The results of experiment #1 can be seen in Fig. 2. In this run the signal-molecule agent traces its path as it moves from one compartment to another. Note especially the amplification of the signal at the point of NF-κB activation and at the subsequent simulated transcription events. The random walk trajectory of the signal-molecule agent captures the stochastic effects associated with molecular movement and subsequent effect on the timing of signaling events and internal variance in the distribution effect of the signal.

The results of experiment #2 can be seen in the panels of Fig. 3a–c. Ten runs were performed for each initial condition. There is a clear dose dependent pattern of TNF-secretion seen between doses at LPS-initial = 10, 100, and 1000, a well recognized behavior previously published in the literature [36,37]. Note that the response curves are not exactly the same for each run within each dose group, demonstrating the stochastic effects of the Brownian movement rules seen in Experiment 1.

Experiment #3, the simulation of tolerance, used an initial preconditioning dose of LPS of 10, 100 or 1000. Then, subsequent to this episode, for each preconditioning LPS dose a second dose of LPS = 1000 was administered at 18 h of simulated time. There were 10 runs for each set of conditions, and Fig. 4a–c show the LPS re-dose response curves for preconditioning LPS doses 10, 100, and 1000, respectively. The pattern of response with respect to re-chal-
The dose-dependent preconditioning effect of LPS suggests a dose-dependent preconditioning effect. This is generally consistent with the pattern of preconditioning behavior that has been previously reported in the literature [23,26].

These results demonstrate that, even with a high degree of event abstraction, the particle-based, spatially oriented TLR-4 SCSRC captures the essential dynamics of the signal transduction cascade, including dose-dependent response, negative feedback control, and preconditioning effect. It should be noted that the TLR-4 SCSRC does not reproduce one observed behavior associated with preconditioning, that of 'priming,' in which a very low prior exposure to LPS actually increases the responsiveness of the cell to subsequent LPS stimulus. However, the molecular mechanism of this behavior is unclear, perhaps this may be due to increased synthesis/expression of forward feedback components, including TLR-4 cell surface receptors, however in any case the behavior of the TLR-4 SCSRC suggests that at least it requires additional molecular component detail that is not currently incorporated. The ratio-

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Fig. 3. (a–c) TNF secretion response to initial LPS dose at 10, 100, and 1000. These panels demonstrated the dose-dependent nature of TNF secretion in response to increasing doses of LPS: (a) for initial LPS = 10, (b) for initial LPS = 100, (c) for initial LPS = 1000. The trajectories for 10 runs for each condition are represented in each panel. Note the difference in the plotted trajectories, demonstrating the ability of the SCSRC to reproduce the stochastic nature of signaling dynamics.
The rationale for not including these mechanisms was presented in the Methods section. In fact, this deficit of the TLR-4 SCSRC further emphasizes the need to account for these multiple pathways when planning potential interventions. Future work will sequentially incorporate additional regulatory mechanisms, both intracellular and paracrine/autocrine, into the SCSRC. Additional future experiments have already been alluded to: knock-out versions of various pathway components to identify those ‘essential’ steps in the signaling cascade, improved calibration of reaction chamber size/enzyme-transition rules to real-world reaction rate constants, and integrated investigation into missing mechanistic knowledge that would refine the existing model. Through these investigations and subsequent parsing of various preconditioning effects, something not possible without computational modeling and simulation, insight may be gained into the expected consequences and responses resulting from manipulation of one or many of these.

Fig. 4. (a–c) Effect of preconditioning LPS doses at 10, 100, and 1000 on secondary exposure of LPS = 1000. These panels demonstrate the dose-dependent nature of the effect of preconditioning with respect to TNF response to a second administration of LPS = 1000 at 18 h of simulated time: (a) for initial LPS = 10, (b) for initial LPS = 100, (c) for initial LPS = 1000. In each of these panels, the second peak of TNF secretion represents the preconditioning effect, and these values should be compared to the TNF responses at corresponding initial doses of LPS = 1000 seen in Fig. 3c.
modulating factors, and provide the basis for an ‘engineering approach’ to the development of therapeutic modalities [2].

4. Discussion

One of the defining characteristics of biological systems is that they exist in a state of dynamic equilibrium. They maintain relatively stable structures and configurations despite an internal milieu that is in a constant state of flux, and interactions with an environment that is varied and unpredictable. This ‘stability of form’ occurs at many levels of biological organization; biomedical research focuses on characterizing the processes involved in the transitions from states of health to states of disease. Of these processes, inflammation has emerged as a central, ubiquitous process in diseases ranging from sepsis, cancer, autoimmune disorders, transplant immunology, aging, wound healing and diabetes. The central role of inflammation should not be surprising; it is the key process in the defense of the organism as it interfaces with an often-hostile environment, and in the repair of the organism as its components are damaged or ‘wear out.’ Recognition of the role of inflammation has extended beyond the traditional emphasis on responding to injury and infection towards a view of inflammation as a ‘early response’ monitoring system aimed at identifying and responding to threats to the organism as a whole, the ‘danger signal’ paradigm of examining inflammation [25]. Central to this mechanism is the role of Toll-like receptors, and in particular TLR-4. The control and attenuation of the response to LPS has been well documented, and represents one of the most studied examples of tolerance and preconditioning in biomedical research. The justification of looking at cellular mechanisms of tolerance as they affect the pathogenesis of disease is that total body behavior is generated from the behavior of cellular populations, which in turn represent the output of molecular interactions.

When viewed from a mathematical standpoint, the trans-scalar manifestations of tolerance and preconditioning should not be surprising. As dynamical systems that maintain a non-thermodynamic equilibrium ‘stability’ of form, biological objects can be characterized by differential equations defining an attracting section of its phase space that corresponds to observable behavior. To a certain degree, ‘tolerance’ can be viewed as a phenomenological representation of underlying attractor dynamics, and this recognition may lead to deep understanding and the discovery of an essential underlying abstraction.

The challenge in biomedical research, however, is to go beyond the quest for this type of understanding with a more prosaic goal of developing means of intervention and manipulation. In order to utilize the insights derived from mathematical characterization, it is important to also recognize that biological systems acquire their complexity, to a great degree, through their structure. Their modular, multi-hierarchical organization is itself an example of combinatorial complexity from one scalar level to another. Therefore, another way to approach the search for ‘simplicity’ of explanation is to characterize biological processes through the identification of ‘atomic’ events, and then organize these events in approximations of biological structure. The SCSC is an example of this approach by incorporating process/event simplicity with a means of representing structural complexity. The simplicity of the particle-agent rules means that a great deal of a certain type of detail is rendered unnecessary. For instance, the behavior of the SCSC suggests that qualitative scaling of reaction rates may sufficiently represent system dynamics if implemented in a model architecture that captures the structural configuration of the signaling system.

No one modeling method will optimally suit every investigational situation, and the SCSC is no different. The SCSC is clearly a ‘work in progress.’ There is spatial arrangement put forth in this model may be only one of many that are able to reproduce the dynamics of TLR-4 signaling. There is no accounting for different reaction rates, or in differential amplification aside from that incorporated at the NF-κB and DNA translation level. There are resolved gaps in the component detail associated with parallel pathways and additional feedback loops. However, the SCSC may offer some additional benefits to more traditional equation based models and other computational models such as Petri Nets [38] and cellular automata [39,40], by virtue of its simplistic-but-realistic particle-view of molecular interactions and ability to incorporate structural component detail in the face of incomplete knowledge. Specifically, the SCSC lends itself to potential implementation on parallel processing computer environments (such as graphical processing units) that are suited to running megascale ABMs [41]. It is hoped that ongoing development of the SCSC, particularly as a method of ‘dynamic knowledge representation,’ will provide a useful adjunct in the growing arena of mathematical and computational investigation of biomedical systems.

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References