

How affinity influences tolerance in an idiotypic network

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Abstract

Idiotypic network models give one possible justification for the appearance of tolerance for a certain category of cells while maintaining immunization for the others. In this paper, we provide new evidence that the manner in which affinity is defined in an idiotypic network model imposes a definite topology on the connectivity of the potential idiotypic network that can emerge. The resulting topology is responsible for very different qualitative behaviour of the network. We show that using a 2D shape-space model with affinity based on complementary regions, a cluster-free topology results that clearly divides the space into distinct zones; if antigens fall into a zone in which there are no available antibodies to bind to, they are tolerated. On the other hand, if they fall into a zone in which there are highly concentrated antibodies available for binding, then they will be eliminated. On the contrary, using a 2D shape space with an affinity function based on cell similarity, a highly clustered topology emerges in which there is no separation of the space into isolated tolerant and non-tolerant zones. Using a bit-string shape space, both similar and complementary affinity measures also result in highly clustered networks. In the networks whose topologies exhibit high clustering, the tolerant and intolerant zones are so intertwined that the networks either reject all antigen or tolerate all antigen. We show that the distribution and topology of the antibody network defined by the complete set of nodes and links—an autonomous feature of the system—therefore selects which antigens are tolerated and which are eliminated.

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1. Introduction

One of the key aspects of the idiotypic network first proposed by Jerne is the manner in which the affinity between clones is defined, this affinity ultimately giving rise to the network itself. Jerne himself specifically addressed this issue in his Nobel lecture in 1984 (Jerne, 1985) in which he compared this affinity with the matching problem between pieces of sentence (for example referring to Chomsky's work on universal grammar). Jerne's suspicion was that the manner in which affinity was defined would determine the properties of the resultant networks of connected clones. The key role played by the definition of the pair-wise affinities between the components of a network has been apparent in many proposed models

which simulate the ontogenesis of the immune network, e.g., De Boer and Perelson (1991), Calenbuhr et al. (1995) and Detours et al. (1996). However, the work of Varela and Coutinho (Varela and Coutinho, 1991; Varela et al., 1988) pushes this concept even further in making a clear link between the affinity and the topology of the resulting network connectivity. Despite the lack of empirical data relating to the connectivity matrix which made it impossible to make any definitive statement on the analytical nature of the topology, it is evident that Varela did not see the connectivity of the network as simply random like in an Erdős graph (Bollobás, 2001), but rather well structured and playing a key role in the functionality of the system. For instance, he discussed the topology of this connectivity as a possible cause or signature of some auto-immune diseases whose treatment was inspired by this new network perspective. He showed, again using very scant data, that people suffering from auto-immune disease could present a less densely connected network than healthy

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ones. This reduction in connectivity could decrease the network effect and thereby provoke homeostatic failure by perturbing the emergent regulatory effect of this network.

The quest to understand the structure and functionality of all types of biological networks continues, but is making great advances with new experimental techniques which facilitate the study of large sets of empirical data, for example protein-interaction networks. Further advancements in our knowledge and understanding are being made by a new generation of physicists enthusiastic about small-world effects and scale-free topology (Barabási and Albert, 1999; Sole et al., 2002). This paper carries on this quest with new and very unexpected findings, first presented in a preliminary form in Hart et al. (2006).

1.1. Background: affinity measures in idiotypic networks

The study of the effects of affinity between cells was facilitated by the notion of shape space introduced by Perelson (1989) as a method for representing biological molecules and therefore capturing affinities between them. This affords the modeller a variety of alphabets and therefore spaces in which to study the interaction of cells. The most common choices are to adopt a bit-string universe or an n -dimensional real (or integer) valued space. In practice, the bit-string model is predominantly used as it offers a rich diversity in the manner in which strings can interact; a number of affinity functions have been proposed which are physiologically plausible based on finding *complementary* matching regions between two strings (Perelson, 1989).

However, a low-dimensional integer representation of cells offers some advantages in that interactions between cells can be visualized and therefore analysed. Although it is perhaps more natural to define affinity between two cells in for example a 2D integer-space in terms of their proximity and therefore *similarity* to each other, affinity in terms of complementarity can also be defined in such a universe. Stewart and Coutinho (2004), propose a model based on a simplified version of shape space in which points on a 2D grid represented a *pair* of perfectly complementary shapes with maximal affinity. Their simple model showed that a self-sustaining network could arise as a result which they propose correspond to *molecular self*. Furthermore, they go on to show that external antigens are *tolerated* by this network, i.e. they are assimilated into the molecular self by the network.

In related work, Bersini (2002) proposed a shape-space model implemented in 2D in which affinity was based upon complementary matching between cells by supposing that a cell exerts a domain of affinity in a zone which is situated in region obtained by reflecting the cell through the centre of symmetry of the space. It was shown that this led to a model in which regions of tolerance and intolerance emerged naturally from the dynamics of the idiotypic network, without need for pre-defining cells as being of a particular type. This model was later explored in greater

depth by Hart in Hart and Ross (2005a, b), and Hart (2005) and later Dilger in Dilger and Strangfield (2006) which confirmed that these zones exist and furthermore showed that the shape of the zones, and therefore the subsequent properties of the network could be controlled by altering the shape of the domain of affinity exerted by a cell.¹

In this paper, we show that contrary to opinion, the definition of affinity imposes a very definite topology on an emerging network, which has subsequent important consequences for the properties that we can expect a network to exhibit. The paper is organized as follows. First, two different network models are introduced, in 2D and in a bit-string universe. We then show how the 2D model with complementary matching gives rise to tolerant and intolerant zones in the shape space. This is then contrasted to the bit-string shape space with an affinity function based on Hamming distance in which tolerance cannot be obtained. We explain the anomalous results we find by first analysing a 2D model with a similarity-based affinity function which can be visualized in a straightforward manner. This indicates that the topology of the network defined by the affinity function plays a key role in the dynamics. Finally, we confirm our hypothesis by directly investigating the dynamics of network growth on a range of potential topologies.

2. Description of network models

In this section, we describe the 2D and bit-string models in which we obtain our results. In as far as possible, the models are equivalent in the manner in which potential cells are introduced, and in the methods by which a cell stimulates (or is stimulated by) other cells. The differences lie only in the representation of cells used, and therefore the manner in which affinity between cells is calculated.

2.1. 2D shape-space model

The following 2D shape-space model was first proposed by Bersini (2002) and subsequently adopted in further work by Hart and Ross (2005a, b), and Hart (2005) in which the effect of the shape of the cell recognition region was explored. The shape space is defined on a 2D integer-grid of dimension X, Y . A cell is specified by a position (x, y) on the grid. The potential network therefore consists of a possible $X \times Y$ cells. Cells can be considered as

¹Note that the usefulness of shape space as a concept for modelling protein interaction has been challenged by Carneiro and Stewart (Carneiro et al. (1996)) who take the view that interactions between proteins (both idiotypic and otherwise) are essentially a relational phenomenon and that therefore the affinity between molecules cannot be predicted from the characteristics of the two isolated molecules. They therefore propose a model in which affinities are generated by a procedure based on random assignment which implies no intrinsic topology in the resulting matrices.

connected nodes on a graph if one cell is *stimulated* by another cell. The manner in which one cell stimulates another depends on the affinity function defined. If affinity is defined as complementary, then two cells *A* and *B* have a non-zero affinity if *B* lies within a circular region of radius *r* centred on the point $(X - x, Y - y)$. On the other hand, if affinity is defined between *similar* cells, then *A* and *B* have a non-zero affinity if *B* lies within a circular region of radius *r* centred on *A* itself. For all cells that lie within a distance *r* of one of the four boundaries of the shape space, then part of their circular recognition region will fall outside of the shape space, (X, Y) . Any region outwith the shape space is simply ignored, i.e., there are no periodic boundary conditions. (The choice of concentration values given for new antibodies and antigens in the algorithm below was determined through empirical investigation. This is discussed in detail in Section 3.1.) Using these definitions, the following algorithm can be used to simulate the growth on an idiotypic network in which there are potential interactions between both antibodies and antibodies, and antibodies and antigens:

1. Generate at random a new antibody (x, y) and add with concentration 10.
2. (Possibly) add a new antigen with coordinates (x_a, y_a) and concentration 1000.
3. Calculate the stimulation S_{Ab} of each antibody.
4. If $L < S_{Ab} < U$, increase the concentration of the antibody by 1, otherwise decrease it by 1.
5. Calculate the stimulation S_{Ag} of each antigen.
6. If $L < S_{Ag}$, decrease the concentration of the antigen A_c such that $A_c \leftarrow A_c - S_{Ag}/(L * f)$, where f is simply a scaling factor.
7. Remove any cells (antibody or antigen) with concentration 0.

Stimulation of antibodies and antigens is calculated according to the equations below, where S_{Ab} represents the total stimulation of an antibody *Ab* and S_{Ag} represents the total stimulation of an antigen *Ag*. The affinity between any two cells represented in a 2D shape space by points *i* and *j* is given by Eq. (1). (Note that the equation holds given that $r > D(i, j')$, otherwise the affinity is zero.) The term $D(i, j')$ represents the Euclidean distance in shape space between the cells, where j' is the complement of point *j*, on which the recognition region of *j* is centred. (In the similarity model, $j = j'$.) The term C_i represents the concentration of cell *i*. r represents the radius of the circular recognition region surrounding each complementary point. The total affinity exerted by a cell represented by a point in shape space *i* is influenced by the concentration of the cell *i*, and is given by Eq. (1). According to this equation, affinity is maximal when $D(i, j')$ is zero, i.e., j' lies exactly at the centre of the complementary region. The affinity decreases as $D(i, j')$ increases, that is, cells lying closer to the centre of the circular recognition region provide more stimulation than those further away.

If $D(i, j') > r$ then *i* exerts no affinity on j' :

$$\begin{aligned} \text{affinity}(i, j) &= C_i(r - D(i, j')) \\ \text{if } D(i, j') \leq r, &= 0 \text{ otherwise} \end{aligned} \quad (1)$$

$$\begin{aligned} S_{Ab} &= \sum_{\text{antibodies } E} \text{affinity}(E, Ab) \\ &+ \sum_{\text{antigens } A} \text{affinity}(A, Ab), \end{aligned} \quad (2)$$

$$S_{Ag} = \sum_{\text{antibodies } E} \text{affinity}(E, Ab). \quad (3)$$

2.2. Bit-string model

Except for the definition of clone identity, the bit-string model closely follows the previous description given for the 2D shape space, maintaining a complementarity-based affinity rule. Instead of a point in a plane, each cell is now identified by a binary bit-string of *N* bits and the affinity a cell *i* exerts on another cell *j* is defined by the following equation:

$$\text{affinity}(i, j) = 100 \cdot C_i \cdot (HD(i, j) - T) / (N_{bits} - T) \quad (4)$$

with C_i being the concentration of the cell *i*, HD the hamming distance between the two bitstrings and T , the affinity threshold, playing an equivalent role of the parameter r in the 2D shape-space model, provided that $(HD(i, j) - T)$ is positive, otherwise the affinity is 0.

Like before, the total affinity (field) received by a cell *i*, S_i , is obtained by summing the affinity for all cells present in the system, given that this affinity can either be positive or null, as in Eqs. (2) and (3). As in the 2D scenario, antibodies can be stimulated by antigens and antibodies, while antigens interact only with antibodies, and not with other antigens.

Keeping the system as similar as possible to the 2D shape-space model, the algorithm is as follows:

1. Generate at random a new antibody cell (bit-string) having an affinity field between L and U , with concentration 75.
2. (Possibly) add a new antigen with concentration 100.
3. Calculate the stimulation S_{Ab} received by each antibody.
4. If $L < S_{Ab} < U$, increase the concentration of the antibody by 1, otherwise decrease it by 1.
5. Calculate the stimulation S_{Ag} received by each antigen accordingly.
6. If $L < S_{Ag}$, decrease the concentration by 1.
7. Remove any cells whose concentration has reached 0.

Regarding the idiotypic network as just a graph, we may say that a cell *A* is connected with a cell *B*, if the hamming distance between *A* and *B* is higher than this threshold T . A high T value imposes a system where an almost perfect complementarity is needed for stimulation, whereas a low

T tolerates very poor complementarity for the network to pop up. Each combination of parameters gives rise to different stabilized networks. The size of the stable network will depend primarily on the threshold level (T) and the size of the window (U and L). Low specificity (low T) leads to potential networks in which the nodes have potentially high connectivity; this high connectivity, however, leads to nodes becoming easily over-stimulated (exceeding the upper limit) which in turn leads to a decrease in their concentration. At very low T , a network never pops up as no cells can be sustained for long enough. The opposite can also happen. When almost perfect complementarity is needed for stimulation (very high T), the average degree of the network will be so low that nodes cannot be stimulated beyond the lower stimulation limit. So, for an idiotypic network to pop up, an optimal individual average stimulation value must be found in the algorithm. A similar phenomenon is observed in the 2D model as the radius r is increased; in fact, the recognition radius of a cell r in the 2D model can be seen as playing a similar role to the threshold value T in the bit-string model in that both parameters regulate the number of potential matches that any cell can make in each model. In 2D, when r is low, the probability of cell finding another cell lying within its recognition zone to provide stimulation is low, and a network is unable to pop-up due to the low average degree. At the other end of the spectrum, when r is large, every cell is stimulated by every other cell; all cells become over-stimulated and are suppressed, preventing the emergence of a self-sustaining network. Therefore, as with the bit-string model, there is a balance to be found between r , and also the concentration at which cells are added (see Eq. (2)).

2.3. A comment on the proposed model

It should be noted that the model proposed above is in fact a continuation of a whole series of idiotypic network models that have been proposed in the past by many authors, e.g. Stewart and Coutinho (2004), Calenbuhr et al. (1995), Detours et al. (1996), and many others such as De Boer and Perelson. All of these models in fact implement a number of common features (although they differ in the exact mechanisms by which these features are implemented):

1. The idea of a shape space: either 2D or binary.
2. The notion that both antibodies and antigens grow or decrease in concentration depending on how they bind to each other, i.e. whether the binding is from antibody to antibody, or antibody–antigen. Each antibody i receives a field of affinity exerted by all others and defined as the sum over the whole existing set of their concentration multiplied by a degree of match between each element of the set and antibody i . The network arises as the result of the connections existing between matching antibodies.
3. If the field received by a cell is too high, this causes a decrease in concentration of the received antibody or

antigen. For the antibody, this decrease is a compromise between the stimulation that makes them proliferate (i.e. they are produced by the B lymphocytes) and their mutual binding that causes them to die. For the antigen, the decrease is due to the effect of the binding only.

4. If the field received is too low, this also causes a decrease in the concentration of the received antibody.
5. In between these two thresholds (too low and too high) the effect of the field for the receiving antibody is an increase in concentration as the B-lymphocyte production exceeds the binding.

The model presented in this paper is the simplest instance of such a scenario. However, it has been shown by the authors quoted above that such a simplification does not modify the relevant phenomenology, and therefore we utilize this simplified model in order to investigate some of the deeper issues concerning the topology that can arise in a connected network.

3. Experimental results

We first perform a simple set of experiments in which the bit-string and 2D models are run for 10,000 iterations in the absence of any antigens. Following this, a set of 50 randomly generated antigens are presented to the network and evolution continued for a further 2000 iterations.

In order to be consistent with work reported previously in Hart (2005), 2D experiments are performed on a grid of size 100×100 , resulting in 10,000 potential cells. The values of the lower limit L and upper limit U are fixed at $L = 100$ and $U = 10,000$. Antibody cells are added to the simulation with concentration 10; antigen cells are added with concentration 1000. In the simulations with bit-strings, we consider strings of length 13, creating a space of 8192 possible cells, a potential repertoire size of similar size to the 2D shape space. The lower limit L and upper limit U take, respectively, values 5000 and 10,000. Unless otherwise stated, the scaling factor f is set to 100 in all experiments.

Initial experiments performed with both network models give surprising results (shown in Figs. 1 and 2) which can be summarized as follows:

- The network obtained via the 2D model is always able to tolerate a subset of antigens.
- The network obtained via the bit-string model is intolerant of all antigens for the majority of the threshold range (when $T > 10$ the network collapses as a result of a lack of connections).

3.1. Exploration of parameter space

Experimentation shows that these results hold over a wide range of parameters for each model. Fig. 1 presents the results of an investigation of the main parameters of the 2D model, in particular the recognition radius r , the

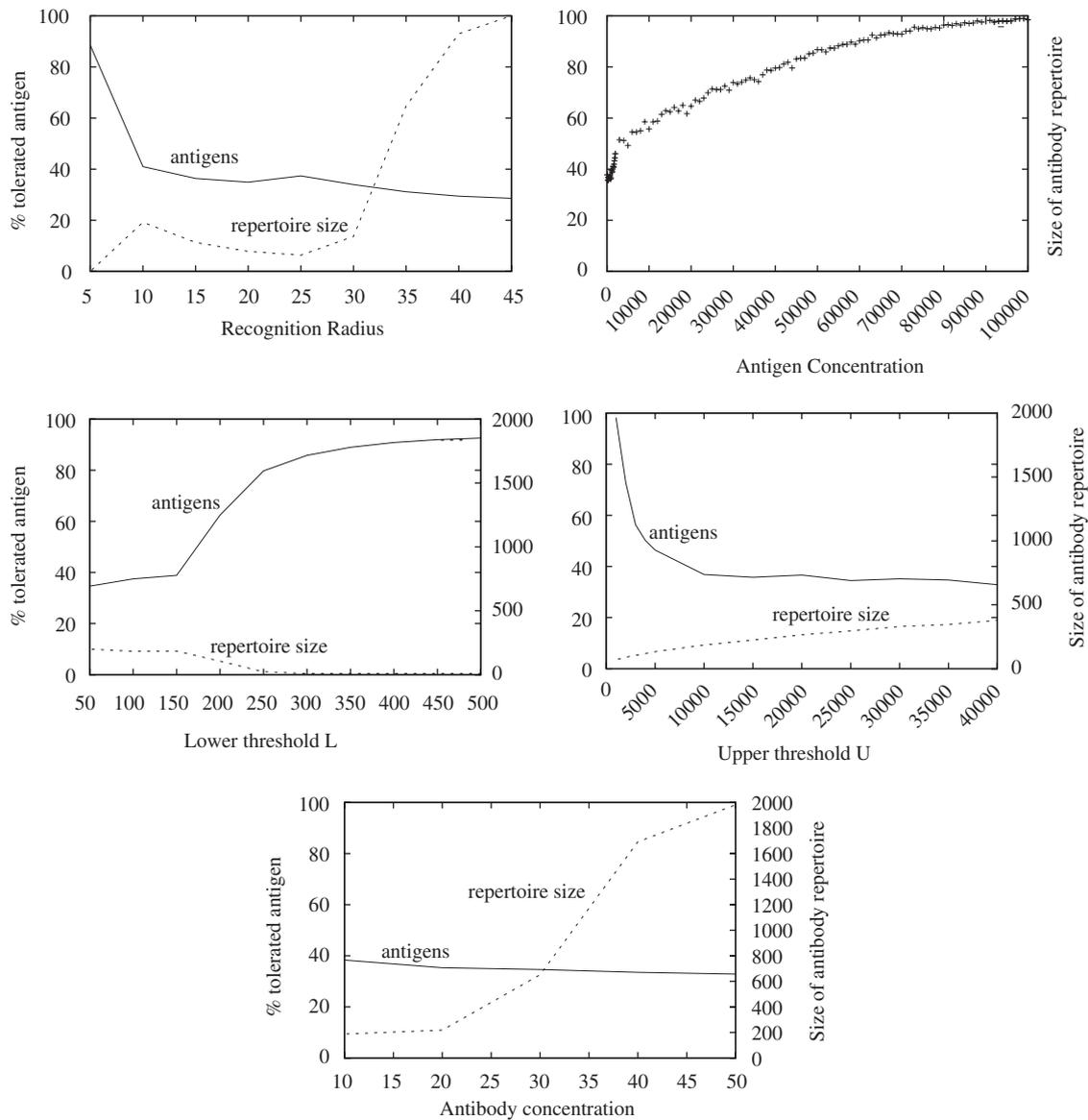


Fig. 1. Figures show exploration of parameter space of 2D model with complementary affinity function. In all experiments shown, the scaling factor f which influence the decrease in antigen concentration is set to 100. Graphs show the % of antigens tolerated by the network following presentation of a set of 50 random antigens to a network evolved in the absence of antigen for 10,000 iterations. The tolerance is plotted as a function of: (top left) radius of recognition region (top right) concentration of added antigens (middle left) value of the lower stimulation threshold L (below which concentration of antibodies is reduced) (middle right) value of upper stimulation threshold U (above which the concentration of antibodies is reduced), (bottom centre) concentration of added antibodies. In each diagram, all parameters other than the one being explored are fixed at $r = 15$, $L = 10$, $U = 10,000$, initial concentration antigen = 100, initial concentration antibody = 10, scaling factor $f = 100$.

concentration of added antigens, A_c , and the lower and upper limits L and U of the stimulation thresholds. The ultimate outcome of any simulation is determined by a complex interplay between these parameters. In essence, in order for a network to emerge, then a balance must be found which allows a number of antibodies to maintain a level of stimulation which lies between the two threshold limits. Insufficient stimulation causes antibodies to be eliminated almost immediately; excess stimulation similarly causes antibodies to be suppressed. In both cases, a network cannot be maintained and therefore any antigens

to which the system is exposed remain in the system indefinitely.

Fig. 1 shows, however, that there is a wide range of parameters over which a stable network can emerge. For example, the network is stable over a wide range of values of r , springing into existence when $r > 10$. Behaviour as a function of antigen concentration is as expected; at high doses, even though a proportion of antigens are recognized by the network, and correspondingly reduce in concentration, they cannot be completely eliminated in the time allowed during the experiment, hence 100% tolerance is

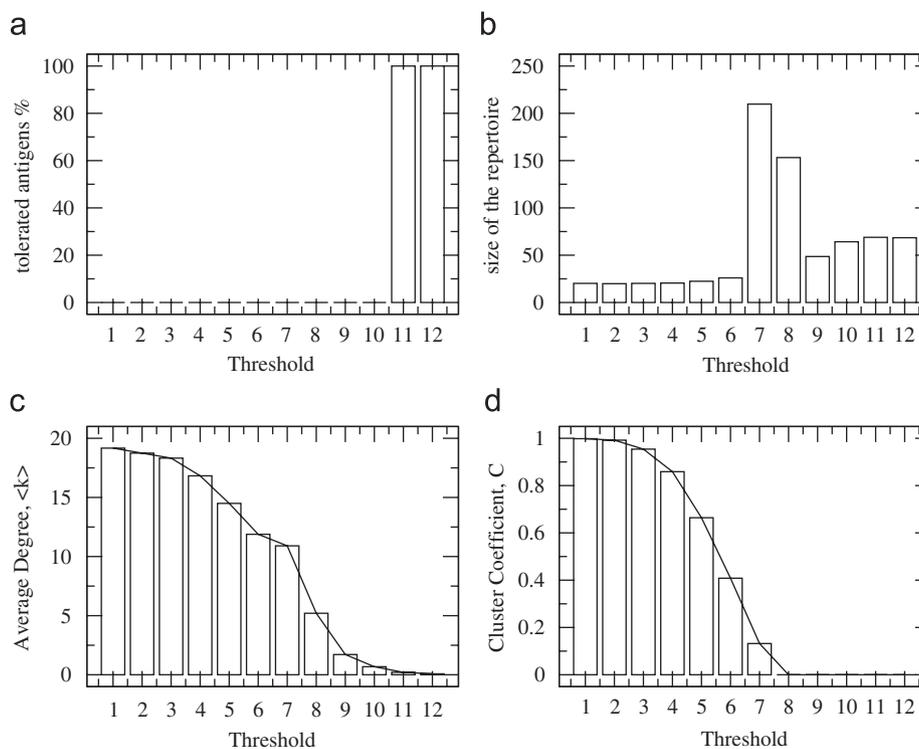


Fig. 2. Bit-string model with complementary affinity function: graphs show % of tolerated antigens as the recognition threshold of cells varies (top left), the size of the repertoire (top right), the average degree of the biggest connected component of the network (bottom left) and the average cluster coefficient of the network (bottom right). The network is intolerant for almost all values of the threshold. When $T > 10$, the network cannot survive and the repertoire is basically constituted by antigens. (initial concentration (Abs) = 20; initial concentration (Ags) = 90; number of iterations: 10^7 ; average over 20 simulations; Upper limit: 1000; lower limit: 500; a random set of antigens is introduced every 10^3 iterations after a transient period of 10^4 iterations).

observed. The lower threshold limit L affects the stimulation level at which antibodies are suppressed. When it rises over 150, an abrupt change in network behaviour is observed, and the repertoire is no longer maintained, again allowing any added antigen to be tolerated. Correspondingly, if the upper threshold U is reduced, a similar transition occurs, and no network emerges. The final diagram in Fig. 1 shows the response of the network as the concentration at which new antibodies are added is increased. Although this has little effect on the emergence of a stable network and the corresponding behaviour of that network to presented antigens, it has a profound influence on the size of the repertoire. In the remainder of this paper, for the 2D model, the following set of parameters is used in the experiments described, unless stated otherwise: ($r = 15$, $f = 100$, $L = 100$, $U = 10,000$, new $Ag_{conc} = 100$, new $Ab_{conc} = 10$). These parameters are chosen as being representative of the range of parameters over which a stable network can emerge. However, as the discussion above shows, the model is relatively robust to changes in these values.

In contrast to this, experimentation with the bit-string model shows very different results. For threshold $T \leq 10$, the network shows zero tolerance to antigens. At greater thresholds, the network cannot survive, and the repertoire is constituted entirely by antigens (see Fig. 2, top left). A wide-ranging exploration of the parameter space of the bit-

string model was performed before coming to this conclusion; experimental results are not given for the full investigation as they show little of interest. The results shown in Fig. 2 are typical of those obtained with all combinations of parameters. Therefore, we conclude that the bit-string model used in conjunction with a complementary affinity function does not produce similar results to the 2D model used with a complementary affinity function. This suggests that different network dynamics emerge due to the intrinsic features of the model, that is the node definitions and binding rules used.

3.2. Further analysis of results

Following a cell's birth, its ultimate outcome, i.e. whether it is tolerized or immunized, is determined by the level of stimulation or field strength it receives from cells currently sustained in the network. Therefore, an analysis of the field at all potential locations in the shape space ought to indicate local tolerant and reactive zones. This is straightforward to visualize in 2D; Fig. 3 shows the potential field received by a hypothetical cell occupying each of the potential sites on the grid given the existing network after 10,000 iterations. This field is calculated according to Eq. (2). Darker shading indicates higher field, and vice versa. Two distinct zones are apparent. The top half of the diagram clearly shows a zone in which all

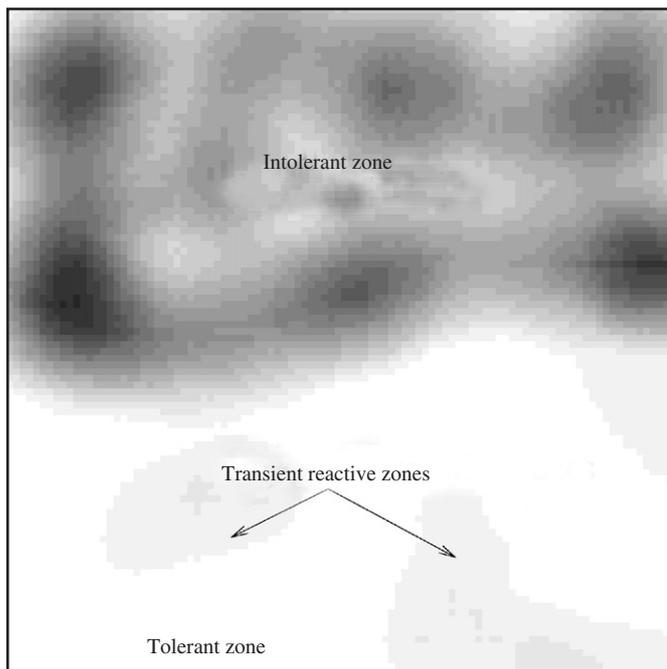


Fig. 3. Field experienced by a cell occupying each potential site of the grid following emergence of the network.

potential sites experience some field; the strength of the field itself varies throughout the zone. In the lower half of the diagram, the majority of sites experience no field at all (antibodies cannot survive in the complementary zone) therefore are tolerant to any cell. Transient reactive regions occur in this region; due to the nature of the algorithm, cells are continuously added to the grid and survive for a minimum of 10 iterations. If these cells occur in the intolerant zone, they temporarily stimulate cells in the lower half—observe, however, that the shading indicates that the reactivity is very low at these sites. Note that the position of the boundary line separating the two zones is entirely an emergent result of the network dynamics, and does not require any pre-labelling of cells as being of a particular type, e.g. to be tolerated or to be rejected.

Although straightforward visual representation cannot be achieved in the bit-string model, we can compare the distribution of field strengths received by a potential node placed at each possible location in the shape space, given the networks that emerge from both models. In the 2D model, the results above imply a distribution in which a large number of the cells receive little or no field, and the remainder receives high field. Figs. 4 and 5 illustrate the result for both shape spaces. The histograms obtained in both models show very similar distributions—in both cases a very broad degree distribution is observed. For the 2D shape space, as expected, a large number of sites receive no field whatsoever. The remainder receive a spread of field-values, indicating their reactivity. The bit-string results are similar—despite the fact that the experimental results suggested that a much more homogeneous field distribu-

tion, with every site receiving approximately the same average field. Thus, the field distributions appear to *both* support the notion that tolerant and non-tolerant regions should be observed in the shape space. One possible explanation is that although these distinct zones do exist in a bit-string space, they are so intertwined that no separation in behaviour can occur. We explore this hypothesis in the next section considering the topology of the networks.

4. The relationship between affinity and topology

An affinity function based on complementarity between cells appears to give rise to networks with very different topological properties. In order to investigate this further, we examine some physical properties of the observed networks in each model. These are given in Table 1.² One main difference between the networks is obvious; the networks obtained in the bit-string universe with a complementarity-based affinity function and threshold $T = 7$ have a cluster coefficient which is one order of magnitude larger than that obtained using the 2D model also with a complementarity-based affinity. In contrast, the bit-string model with $T = 8$ has a cluster coefficient of zero. The cluster coefficient stands for a rough measure of the number of triangles present in the network, providing a normalized value indicating the extent to which direct neighbours of a given node are direct neighbours of each other. Hence, the high value of the cluster coefficient in the bit-string model at $T = 7$ can be explained by the occurrence of the motif shown on the left in Fig. 7 in the topology of the networks, when compared with the 2D model. The fact that this motif can occur at all is an artefact of the affinity function which permits such connections via a series of different matching sequences, over a certain range of thresholds. Fig. 8 illustrates one such sequence for $T = 7$. In contrast, at $T = 8$, no such motif can occur. In fact, it can trivially be shown that for any bitstring of length L , then such triangles can occur given that $T < 2L/3$, where T is the threshold used in Eq. (4)—this is confirmed experimentally by the

²Note that a discussion of topological properties of a network often includes reference to the average path length of the network (APL), but we have omitted this information, as it is in fact impossible to perform a correct analysis of this property for the networks under discussion. This results from the fact that the topological structure of the networks co-evolve with the concentrations of the cells present, and thus the structure is continuously and dynamically changing. Therefore, during any simulation, there will inevitably be a number of time-steps at which the network is not connected anymore, and therefore the APL cannot be calculated. Specifically in the case of the 2D model, the resultant effective immune network is often a collection of disconnected components, which become connected and then disconnected again through topological fluctuations during the evolution of the network. In the bit-string model, a similar situation is observed, particularly following the collapse of unstable, temporary hubs at high values of T . At time-steps when the networks obtained are connected, the networks are observed to have a very small APL due to the large degree heterogeneity of these topologies. (This is particularly true in the bit-string model.)

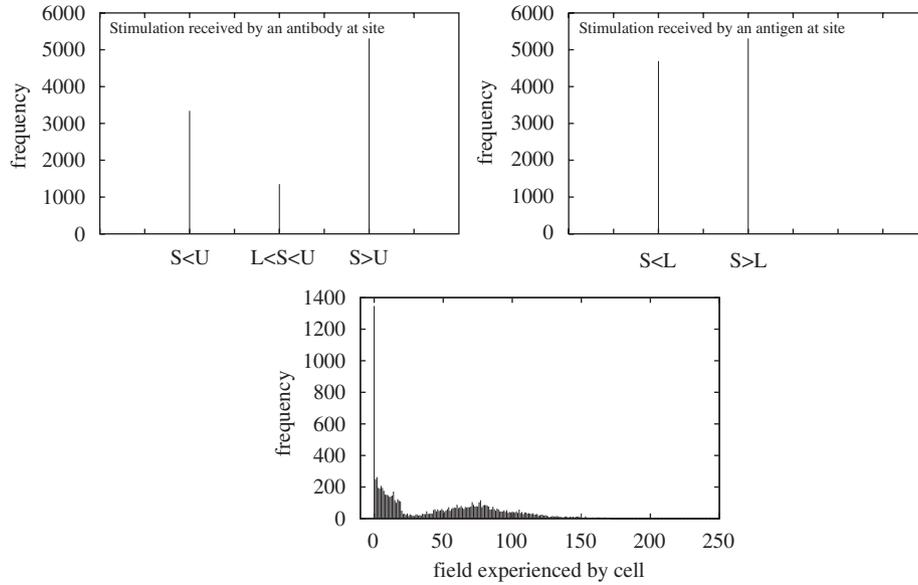


Fig. 4. The diagrams examine the field that would be experienced by a potential cell (antibody or antigen) placed at each of the possible 10,000 sites on the 100×100 grid given an existing evolved network. In these diagrams, S , stimulation, L , lower limit (100) and U , upper limit (10,000). The upper left diagram depicts the number of *antibodies*, which if occupying these sites, would have a total stimulation which (a) lies in the window $L < S < U$ and therefore increase in concentration, and (b) falls outside this window and would decrease in concentration (the field is calculated according to Eq. (2)). The upper right diagram depicts the situation should each potential site be occupied by an *antigen*, and shows the number of antigens which would decrease in concentration ($L < S$), or remain unaltered (field calculated according to Eq. (3)). The lower diagram shows the full distribution of field strengths of cells placed at each potential site (in order to facilitate plotting, the concentration term in Eq. (2) is assumed to be 1 for all cells).

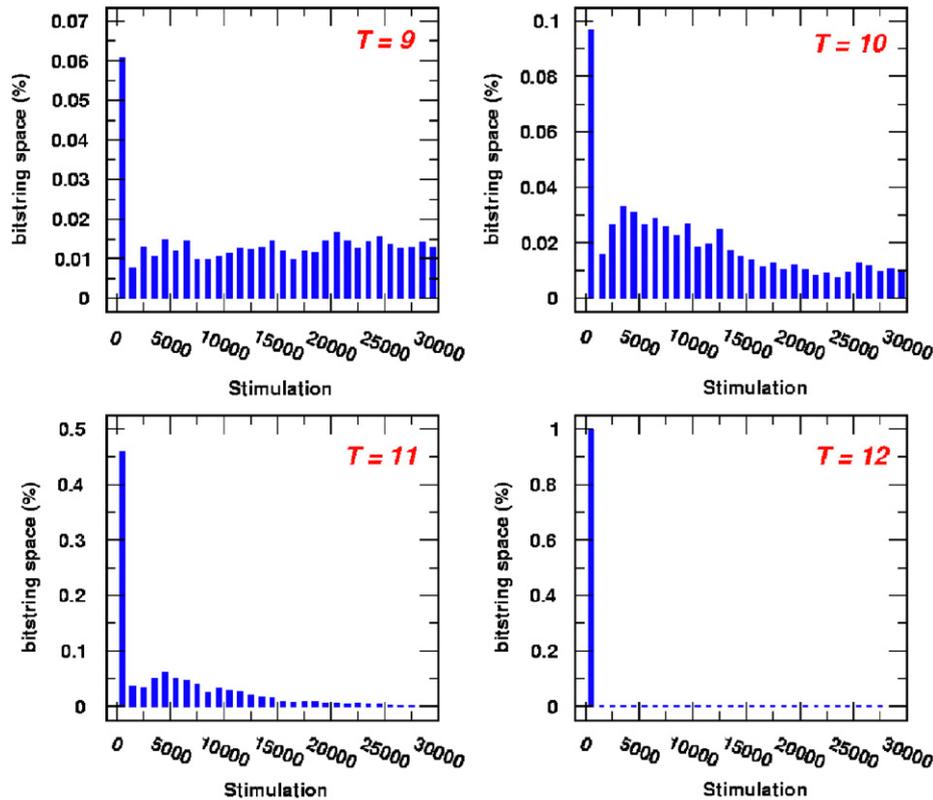


Fig. 5. Field experienced by every potential bit-string in the bit-string space, following emergence of the network, for four different affinity thresholds.

relationship between threshold and cluster coefficient shown in Fig. 2, bottom right, which shows zero cluster coefficient for $T \geq 8$.

The presence of this motif would explain the observed results; any antigen can always find itself with two kinds of responding antibodies closely located in the bit-string

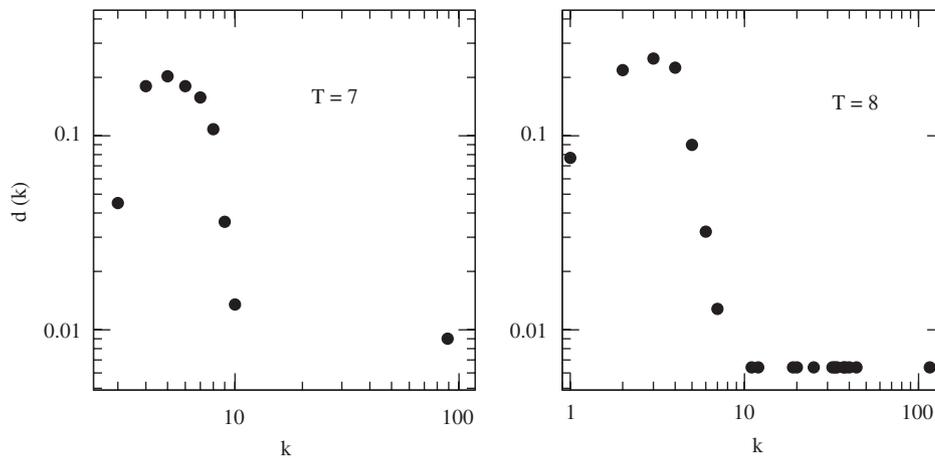


Fig. 6. Final degree distribution $d(k)$ for the bit-string model with $T = 7$ and 8 . $d(k) = N_k/N$ where N_k gives the number of nodes with k connections (degree) and N the total number of nodes. The figure illustrates an important point: at both thresholds, the observed distributions are similar, exhibiting high levels of heterogeneity. However, the internal structure of the networks at $T = 8$ and $T = 8$ is completely different, with the former exhibiting high levels of clustering, while the latter has zero cluster coefficient. The existence of this kind of random network with hubs is very unstable because hubs cannot remain in network for a long time. After appearing they will get over-stimulated and will collapse immediately after.

Table 1
Network properties of shape spaces with varying affinity measures (2D results obtained with $r = 15$ and with $T = 7, 8$ for the bit-string model)

	2D complementary	2D similar	Bit-string model	
			$T = 7$	$T = 8$
Number of nodes	167.8	102.42	209.7	153.2
Maximum degree	29.4	18.17	158.8	113.5
Average degree	4.7	8.52	10.9	5.2
Cluster Coeff.	0.022	0.792	0.13	0.0

This corresponds to a space of size 10,000 in 2D and of 8192 in the bit-string model. The values of $r = 15$ and $T = 7$ are chosen such that the number or potential matching pairs in each model is as close as possible. Results with $T = 8$ are also included to illustrate the difference in the network structure when the threshold level prevents the formation of clusters. Each value results from an averaging over 20 simulations. The *degree* of a node is defined as the number of connections existing between a node and other nodes. The *cluster coefficient* of the network is calculated by averaging over all vertices, the fraction of vertices adjacent to a given vertex that are adjacent to each other. Therefore, its possible values range from 0 to 1, with 1 indicating that all the neighbouring nodes are connected to one another.

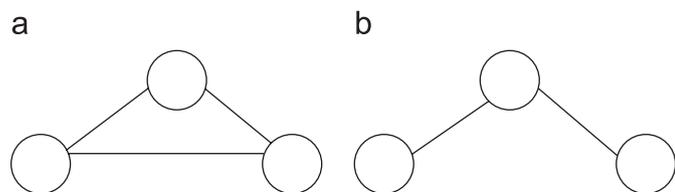


Fig. 7. (a) Motif obtained using similarity-based affinity metric. (b) Motif obtained using complementary-based affinity metric.

space, one in high and the other in low concentration. At the end, the response of the network to any antigen intrusion just depends on the initial concentration of this antigen and therefore no longer on the position of this

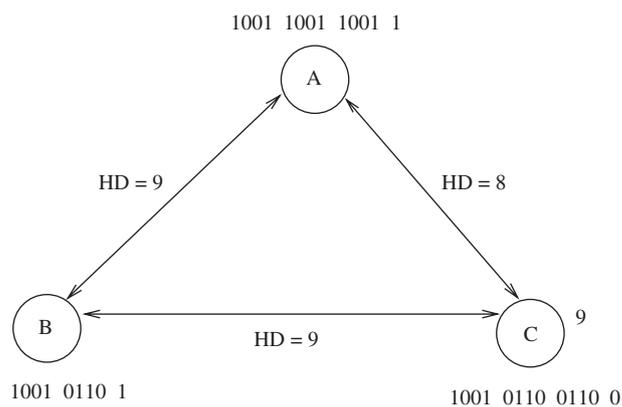


Fig. 8. The figure shows an actual example of a triangular motif that could be obtained in a network where nodes are represented by bit-strings of length 13, with threshold $T = 7$. A non-zero affinity exists between all pairs of cells with $HD > 7$. In this example, A connects with B, as the hamming distance $HD = 9$. Similarly, a connection exists with A and C ($HD = 8$), and also with B and C ($HD = 9$).

antigen, as the space has been uniformly filled up with all types of antibodies.

In contrast, in the network obtained in 2D, there is a high incidence of the motif shown in the right hand side of Fig. 7, creating the conditions for a zero cluster coefficient—if a stimulates b and b stimulates c , then c cannot stimulate a . This automatically creates a bi-partite structure which does not allow any creation of loops among sets of three nodes. It is clear that a network composed predominantly of motifs of this type will necessarily have cluster coefficient close to 0. In fact, the value obtained in the 2D space used is slightly larger than zero (see Table 1) as clustering can occur between cells located very close to the centre of the space, where $(X - x, Y - y)$ is approximately equal to (x, y) . The network topology therefore prevents clusters, but facilitates the emergence of *chains* of cells which are able to separate

the space into distinct regions. Therefore, the location of any antigen in shape space will determine whether it is tolerated or eliminated. Thus, we hypothesize that an affinity function which tolerates the existence of a *triangular* motif as observed in the bit-string universe will result in a topology in which it is impossible to separate distinct zones of high reactivity from those of low reactivity, therefore resulting in a network which reacts identically to any presented antigen. On the other hand, an affinity function which supports the emergence of the “V” shaped motif obtained with the 2D model will result in a topology which clearly separates reactive zones from non-reactive zones. In the following sections, we present some supporting evidence for this argument.

The cluster coefficient is just one indicator of the topology of a network, and appears to provide some justification for the existence of intertwined tolerant and reactive zones as outlined above. Clearly, however, there are other factors which could also result in mixed zones in which it is impossible to separate tolerant from reactive behaviours. This is apparent from Fig. 2 which shows that the bit-string network is still unable to tolerate any antigens when the threshold ≥ 8 , despite the fact that the cluster coefficients of such networks are zero. However, other types of motifs could also occur in these networks which potentially may result in the same end result, i.e. that there is intermingling of zones of high and low field. For example, it is likely that a number of closed loop motifs, of the form $A \leftrightarrow B \leftrightarrow C \leftrightarrow D \leftrightarrow E \leftrightarrow A$ exist which result in the same effect. The main message of this paper remains the same, however; the topology of network impacts its behaviour. The clustering coefficient is one of a number of ways in which topology can be defined, and appears to explain the 2D results and provide partial explanation in the bit-string case.

A further insight into network topology can be gleaned by examining the average degree of the obtained networks. Fig. 6 compares the degree distribution of two bit-string networks at $T = 7$ and 8. Both show a high heterogeneity in terms of degree, and similar distributions, despite the fact the network with $T = 8$ has a completely different internal structure. The existence of nodes with such high degree shows that hubs can appear in the immune-network

models used. However, this topological feature is very unstable—hubs easily appear but disappear promptly due to becoming over-stimulated as result of an excessive number of connections. The structure of such networks is therefore very unstable, even if their capacities to reject antigens are extremely powerful. The fact that the distributions of both networks shown are similar despite the differences apparent in the structure of the networks emphasizes the role of the overall topology of a network rather than individual cell connectivity in determining functionality.

4.1. A similarity-based affinity function in 2D

In order to confirm our hypothesis that the presence of the triangular motif in the network topology prevents the emergence of distinct tolerant and intolerant zones, we perform one further experiment in the 2D space, modifying the affinity function to one which is based in *similarity* between nodes. Thus, we assume a cell A recognizes another cell B if B lies within a circular recognition region *centred on* A . It is trivial to see that such a function supports the emergence of the triangular motif. For example, any three points lying at the corners of an equilateral triangle of side length $< r$ will be connected in this model as points in the 2D space are connected if the Euclidean distance between them is less than the recognition radius r . If the hypothesis is true, we would therefore expect this affinity function to result in a network topology which has high cluster coefficient and is intolerant of antigens, as in the bit-string case.

Similar experiments to those described in Section 3 are performed. Fifty antigens are presented to a evolved network, and the percentage of them tolerated measured, as a function of c , the concentration at which antigens are added, and f , the scaling factor in the antigen concentration-update function introduced in Section 2.1. The results, presented in Fig. 9 show two distinct behaviours. Essentially, two extremes are observed; zero tolerance or 100% tolerance, depending on the balance between concentration, radius and scaling factor, with a switch between the two behaviours over a small concentration range when f is very low. This is in accord with Eq. (2).

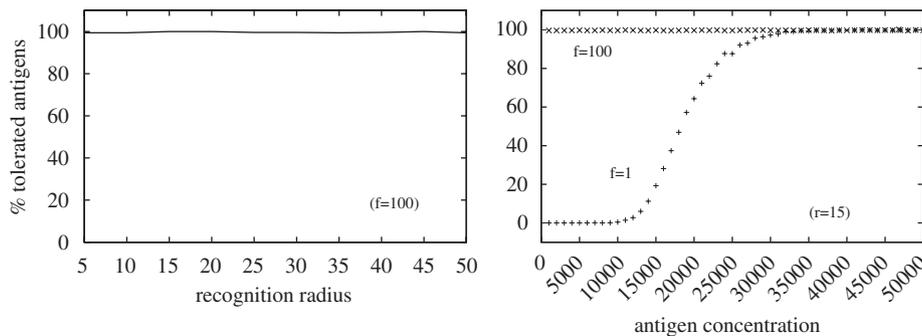


Fig. 9. 2D model with similarity-based affinity function: graphs show % of tolerated antigens as the recognition radius of cells varies (left) and as a function of antigen concentration and scaling factor f (right).

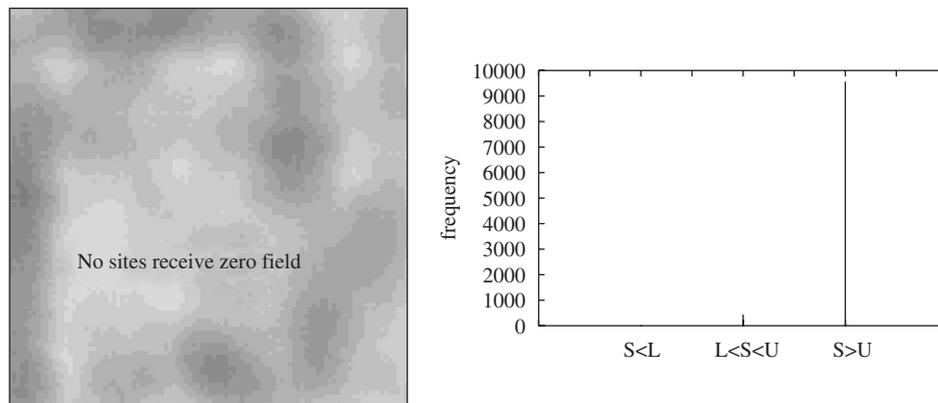


Fig. 10. Examination of field and stimulation received at potential network sites for 2D shape space with similarity-based affinity.

An analysis of the field strength (Fig. 10) at each site corroborates the explanation suggested by bit-string results; the field is relatively homogeneous across the entire network, caused by the intertwining of tolerant and intolerant regions. This averages out the total field received at any site, resulting in the behaviours observed above.

Analysis of the bit-string model with a similarity-based affinity measure is not included in this manuscript, as it follows from the discussion just presented that such an analysis would not contribute any further evidence to our argument for the following reason: as in the 2D model, an affinity function based on similarity would only serve to further enhance the incidence of the triangular motif in the networks (reflected in the value of the cluster coefficient), therefore magnifying the effect already observed with bit-strings and a complementary affinity function. Such a high cluster coefficient simply creates conditions under which antigens are all tolerated or all eliminated, depending on the initial concentration of the antigens.

5. Potential networks

The 2D shape-space network described above defined a potential network of 10^4 cells. This potential network is almost completely homogeneous in that the majority of cells in the network can potentially stimulate exactly the same number of other cells.³ The *average degree* of any cell in the network is influenced by the size of the stimulation zone surrounding the cell, i.e. by r . The *potential degree* of any cell is therefore the maximum number of other cells to which it can potentially connect, governed of course by the area defined by r and is equal for all cells. The results described above have shown that depending on the affinity rule chosen, the *effective* network, i.e. the one that actually emerges, is a homogeneous graph with high or low cluster coefficient. Likewise, the N -bit-string model implies a potential network of 2^N cells. This potential network is

³Note, however, that due to the boundary effects discussed earlier cells lying within a distance r of the boundaries of the space have fewer potential connections due to the fact that part of their stimulation zone will lie outside of the shape space.

also homogeneous as the affinity rule is the same for every cell. In this case, the affinity rule implicitly defines the average path length in the network, and the global affinity threshold T defines the average degree in the network. Therefore, in both cases, in our experiments we have implicitly created a homogeneous potential network containing all possible cells, and have studied the dynamics on this network of all cells with concentration greater than 0. The resulting real immune network is simply a sub-graph of the potential network in which nodes are defined by cells with concentration greater than 0, and links by edges connecting cells with non-zero concentration.⁴ Based on these results, we make a tentative claim that potential networks in which the cluster coefficient does not equal zero cannot sustain a separation between tolerant and non-tolerant regions of the shape space.

Clearly, the 2D and bit-string models presented here with simple affinity functions represent a gross simplification of real interactions between immunological cells. However, let us assume that there exists a plausible representation \mathcal{R} in which cells interact via a realistic affinity function \mathcal{A} . This gives rise to a network N with a particular topology. This facilitates an alternative experimental approach: Assume a potential, homogeneous graph G which defines a potential network. This network implicitly defines a shape space (by defining a certain topology), and affinity function, and even the stimulation area of any cell (by defining the individual potential degree). This allows us to bypass any definitions of these parameters and directly study the effect of network topology on the emergence of tolerant zones within a shape space.

Therefore, we consider graphs in which a cell X stimulates another cell Y if X has concentration different from 0 and if X has a link with Y in the underlying potential network. All connections are assumed to have the same weighting. The stimulation $s_{(X,Y)}$ received by a cell X

⁴This viewpoint corresponds exactly to that observed in Stumpf et al. (2005) by Stumpf et al. who note that real-world protein networks correspond to potential networks, and that experimental results correspond to a sampling of this network.

from Y is zero if X does not have a link with Y in the potential network, and is equal to $\alpha * C_Y$ otherwise, where α is simply a pre-defined constant and C_Y is Y 's concentration. Depending on the total amount of stimulation received by X , its concentration will either increase or decrease, just like in the previous models. If the total stimulation is between a lower and a higher stimulation thresholds its concentration will increase by one, and decrease also by one unit otherwise. The ability of the evolving network to tolerate antigens is measured by exposing the network to a set of 50 randomly generated antigens every 1000 iterations. The antigens are permitted to remain in the network for a period of 1000 iterations. At the end of this period, the number which have non-zero concentration is recorded, and these antigens are considered as tolerated. These remaining antigens are then removed from the network before a new set of 50 antigens is added again.

Therefore, for a potential network of 10^4 nodes, at each time-step:

- Introduce a new antibody cell by randomly choosing an empty node ($C_i = 0$) from the potential network and assign it a concentration $C_i > 0$.
- Calculate the total stimulation of each cell with non-zero concentration, summing the stimulation received from each first neighbour in the potential network.
- Update the concentration of each cell as before, using the usual stimulation window.
- Every 1000 generations, record the number of tolerated antigens. Following this, remove them from the network and then add a new set of 50 random antigens.

5.1. Results in homogeneous potential networks

In this section, we study the topological effects on the emergence of tolerance assuming a homogeneous graph in which all nodes share the same potential degree. We consider potential networks of the same size as before— 10^4 nodes. To tackle the main differences between the studied affinity functions in terms of a potential network approach, we study two kinds of regular graphs: a regular ring of size N and a bi-partite regular network made of two rings of size $N/2$ (see Fig. 11) with an average degree $\langle k \rangle$. The former case corresponds to an affinity function based on the similarity between cells. This creates a potential network where triangles or loops are often present. In the latter case, we create a regular network that can be pictured as two parallel rings of nodes—ring A and B. Nodes belonging to the first ring can only be connected with nodes of ring B and vice versa. For instance, for an average degree of 4, a node i belonging to the ring A will be connected to the nodes at the positions $i - 1$, $i - 2$, $i + 1$ and $i + 2$ of the ring B. In this way, we produce the bi-partite equivalent of a regular network, with exactly the same spatial constraints and average degree, but implicitly defining two groups of interacting

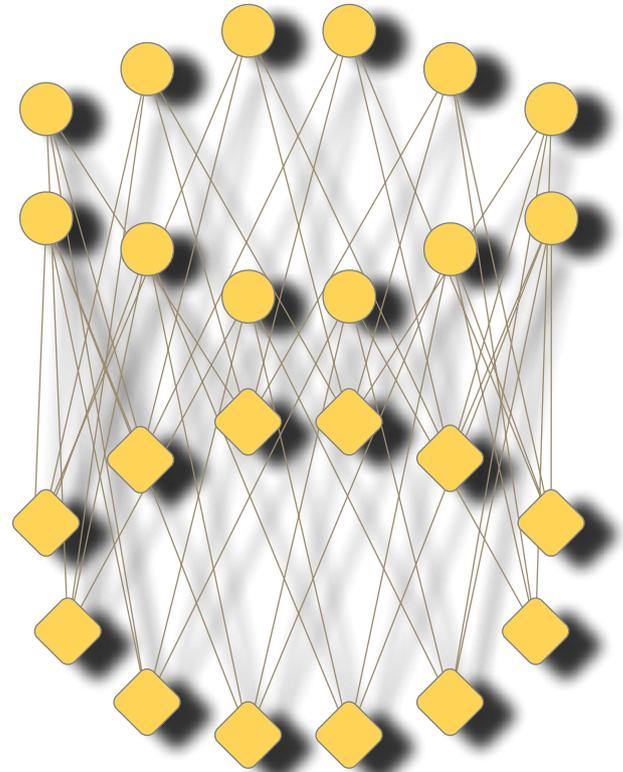


Fig. 11. This scheme illustrates a bi-partite equivalent of a regular graph corresponding to the complementarity affinity rule. In this example the average degree is 4 (see main text). The ring made of circles on the top is the complement of the ring formed of square on the bottom.

cells in a bi-partite fashion. The cluster coefficient will be trivially equal to zero in this case, where in the normal regular network will be given by $(3k - 6)/(4k - 4)$, with k standing for the average degree of the network (Dorogotsev and Mendes, 2003). This bi-partite regular graph corresponds to the complementarity affinity function, which has been shown to give rise to two perfectly distinct areas in terms of tolerating antigens, contrary to the similarity rule (see previous sections). The results for this simplified model are shown in Fig. 12. They can be compared directly to the previous results in 2D in which we plot radius vs % tolerated antigens, as the potential average degree is topologically equivalent to the radius of stimulation. It shows that the existence of a bi-partite topology defining the set of all possible/potential interactions promotes the emergence of high levels of tolerance for most of the values of $\langle k \rangle$. This result corroborates with the previous results obtained with the 2D shape-space model and validates the simplification and abstraction introduced here. This emphasizes our main point, i.e. that the existence of a non-null value for the cluster coefficient emerges acts as an important deterrent for the existence of tolerance zones in the shape space, at least when homogeneous potential networks are considered. Moreover, Fig. 12 shows three regions (regarding $\langle k \rangle$) limited by abrupt transitions. The first one occurs when the

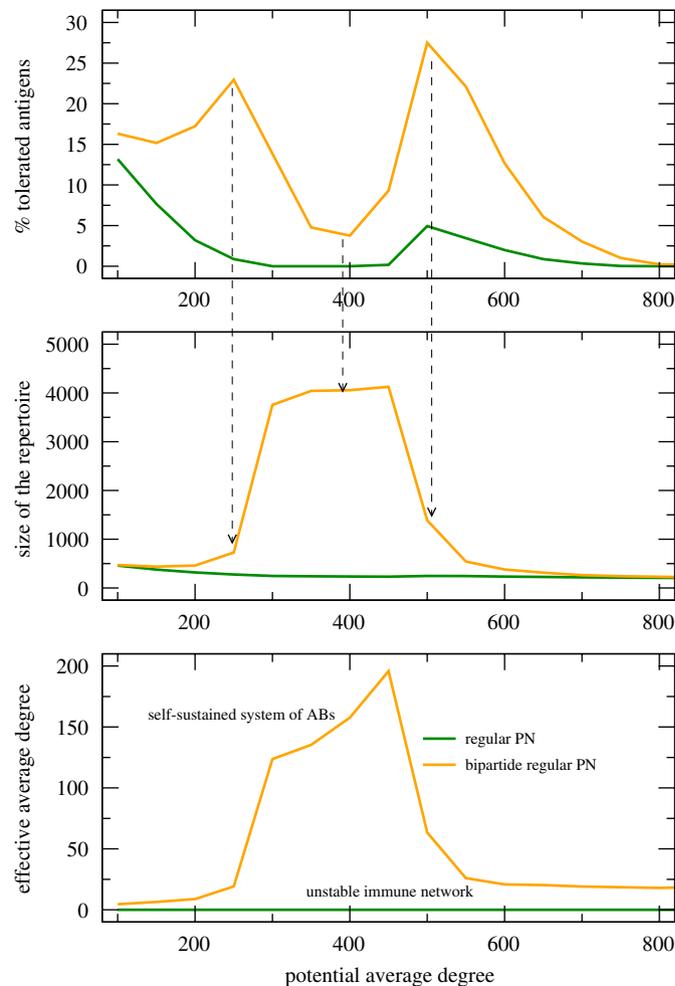


Fig. 12. In the upper panel we show the percentage of tolerated random antigens as a function of the average degree of the underlying regular network for two types of regular networks: a regular ring and bipartite regular ring. Previously we have shown that similarity and complementarity can give a completely different outcome in terms of tolerance probability. Here we study the same phenomena after isolating the topologically characteristics for each affinity definition (see main text for details). These results correspond to an average over 50 runs. In the middle panel we show the dependence of the equilibrium size of the network in the potential average degree. The lower panel represents the relationship between the potential average degree and the effective average degree of the nodes with concentration different from 0. (*initial Ab concentration = initial Ag concentration = 200; upper stimulation limit = 1000 = 2 · lower stimulation limit*).

potential network does not provide enough stimulation to maintain the majority of the antibodies inside the window range (low potential average degree). For a moderate level of potential links, a second type of behaviour emerges. Here the potential network offers the ideal conditions for the emergence of a self-sustained system of antibodies, avoiding both under and over stimulation. In this range the effective size of the network increases together with the effective average degree. Finally, when the potential average degree becomes too high, the majority of nodes are able to become over-stimulated. Under these conditions, the equilibrium size of the network collapses together with the effective average degree.

6. Discussion and future work

We have presented results using two instantiations of a homogeneous network model coupled with various affinity

measures, and obtained with more abstract *potential* networks. Our work, as in the majority of previously published models of idiotypic models, has made the assumption that the affinity function is defined in such a way that every cell type has intrinsically the same number of potential stimulation partners. This disregards a plethora of recent results in the area of complex biochemical networks showing that the majority of the real-world network do not share this homogeneous feature. In fact, it has been extensively shown that most of the biological networks are intrinsically heterogeneous, where some nodes, considering their intrinsic chemical properties, are able to stimulate a large number of cells, contrary to others that stimulates only a few number of cell types. The first ones are naturally *made* to connect to a large number of other cells—they are *natural hubs*. Moreover, it has been shown that most of the biochemical networks can be characterized with broad-scale degree distribution or even

by a scale-free degree distribution, recently popularized by their remarkable robustness properties. Therefore, although our results provide a novel connection between the topological characteristics of a network (resulting from the affinity measure) and its ability to tolerate or immunize antigens, a natural extension to this work would therefore be to consider the emergence of effective networks on heterogeneous potential graphs, and furthermore, potential graphs exhibiting scale-free (or nearly scale-free) degree distributions. Heterogeneity in a network may offer the potential for certain types of antigen (depending on their degree and position in the global interaction network) to become topologically protected against antibodies, which are not able to destroy them, therefore increasing the global level of tolerance. Moreover, heterogeneity effects also play an important role in determining the equilibrium size of the repertoire, which in turn influences the capacity of an idiotypic immune network to tolerate antigens. Therefore, this avenue of research is clearly ripe for further exploration.

In addition, we have alluded to the existence of the affinity \mathcal{A} which has biological plausibility and at the same time results in a network with the topological properties required to create tolerant and immunizing zones in a network. Therefore, in conjunction with continuing studies of abstract potential networks, it may also be fruitful to continue with the search for an affinity measure which gives rise to the identified motifs. The vast amount of biological data now available pertaining to protein–protein interaction networks no doubt holds clues to such measures, though clearly it remains a non-trivial task to make use of this data in this way.

7. Conclusion

We have shown the role played by the affinity function and the corresponding potential network (the network defined by all possible cells and all possible interactions) in defining whether or not it is possible for tolerant and intolerant zones to co-exist in a idiotypic immune network. We showed that affinity functions assuming connections between complementary regions of the shape space promotes the emergence of tolerant areas of the shape space, contrary to configurations based on stimulation of cells with similar properties. We also identified the topological features inherent in both definitions by defining an abstract model to describe a generalized way of defining affinity among cells jumping over an explicit definition of an affinity equation. Under this approach, it has been shown that a null cluster coefficient and the bipartite topology resulting from the manner in which potential networks are organized in the complementarity scenario creates the possibility of two distinct zones being able to co-exist.

Ever since the idea of networks in immunology (essentially with idiotypic networks) was first postulated, the topology of these networks has always raised a great

deal of interest. However, the majority of previous simulations have never addressed the nature of the binding in such detail, particularly in terms of its impact on the final outcome of a simulation, i.e. the structure of the resulting network. We have shown for the first time that the topology of a potential network—defined by the choice of affinity function—influences the effective network that actually emerges, and that the effective network in turn influences one of the network's essential capabilities: to separate zones of tolerance from immunization zones. In addition, the results offer further support to the notion that intrinsic features of biological nodes create non-trivial shape spaces which require careful analysis, in conjunction with the sampling dynamics responsible for the emergence of the effective network. While previous authors have often arbitrarily selected affinity functions, this paper intends to show that this choice is far from neutral. Although we have selected to define binding functions at the extrema of the potential spectrum (i.e. binding occurs if antibodies have symmetrical or equal profile) in order to illustrate our point, many more intermediary functions could have been studied without changing the essential message of this paper. There is now a wealth of literature concerning protein networks and their topology. This paper lends further weight to the growing evidence that the topology of a biological network plays a key role in understanding its behaviour, by presenting experimental evidence showing that this phenomena is indeed true in the case of idiotypic networks.

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