Modeling T-cell antigen discrimination

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1 Introduction

The machinery that the adaptive immune system uses to achieve the cellular equilibrium in front of external aggressions is incredibly efficient. The activation of the T-lymphocyte is found to be remarkably quick, sensitive and very discriminatory in response to peptide-major histocompatibility complex (pMHC) ligand engagement of antigen receptors, the T-cell receptors (TCRs). A few foreign pMHCs on the surface of an antigen trigger a signal response within seconds. Models developed to explain this phenomenon traditionally fail to explain the combination of the absolute distinction between closely related TCR ligands, together with the preservation of the features of the T-cell responses.

Two groups of models have been used to account for these sophisticated discrimination capacity of T-cells. The first turns around the idea that agonist pMHCs induce a specific conformational change in the TCR complex, and the second one is based on a kinetic threshold depending on the lifetime of the pMHC-TCR complex. The first one is discarded by experimental observations, since it has not been observed to be the major conformational changes, although the main flaw is that actually, the potency of pMHCs in activation on T-cells is modulated during intrathymic differentiation. Thus, T-cells can respond differently to the same pMHCs, and this challenges the conformational changes' scheme because responses to a given ligand differ in immature and mature cells in spite of presenting the antigen-receptor structure. More successful models are those based on kinetic proofreading schemes, and they allow modest biophysical differences in the pMHC-TCR interaction to obtain fine functional discrimination.

In this work I will present this standard scheme, first proposed to explain T-cell specificity, the so-called kinetic proofreading (KP), so we are going to observe that it is not sufficient to understand the speed and sensitivity of ligand-induced signaling. Following this line of criticism, we are going to see a modified model that is based in the competition between a digital positive feedback based on extracellular signal-regulated kinase (ERK) and an analog negative feedback involving SH2 domain-containing tyrosine phosphatase (SHP-1). This goes in accordance with the unexpected highly amplified and digital nature of ERK activation in T-cells, a fact that can not be explained solely by KP schemes.

2 Kinetic proofreading scheme

Analogously with the KP, which is essential for describing the protein and DNA synthesis, that early model proposed for T-cell receptor signal transduction is based on the downstream signaling arising from initial variations in pMHC-TCR interaction. McKeithan explain in [1] the successive phosphorylations of the initial complexes, that introduces a lag time between the initial binding and the final activation of the lymphocyte, if the antigen is present. So if the initial complex (pMHC-TCR) dissociates easily, the signal steps do not complete and it prevents the mechanism to trigger functional responses in the case that the pMHC does not present an antigen.

2.1 The model

Initial complexes C_0 formed from the TCR complex and peptide x-MHC undergo N phosphorylations, with constant rate k_p, until the active complex C_N is created. In the same way, at each step the complex dissociates with rate k_{-1}. The last complex triggers the major part of the signals, but earlier complexes may generate early signals.

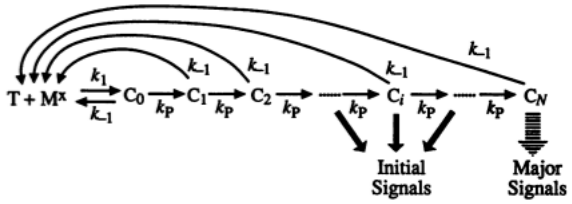


Figure 1: General proofreading scheme.

So we can write down the equations for each complex in this process

$$\frac{d}{dt}[T+M^x] = k_{-1}([C_0]+[C_1]+...+[C_N]) - k_1[T+M^x]$$

$$\frac{d}{dt}[C_0] = k_1[T+M^x] - (k_{-1}+k_p)[C_0]$$

$$\frac{d}{dt}[C_1] = k_p[C_0] - (k_{-1}+k_p)[C_1] \tag{1}$$

$$\frac{d}{dt}[C_2] = k_p[C_1] - (k_{-1}+k_p)[C_2]$$

$$...$$

$$\frac{d}{dt}[C_N] = k_p[C_{N-1}] - k_{-1}[C_N]$$

In this model we assume that the most important signaling molecules are generated by the fully phosphoryled complex, C_N. Let $\alpha \equiv k_p/(k_p+k_{-1})$ be the likelihood that a given modification step will occur before the complex dissociates. Thus, for $i < N$, at steady state we have that $[C_i] = [C_{i-1}]\alpha = C_0\alpha^i$. So $[C_N] = [C_0]k_p\alpha^{N-1}/k_{-1}$ and

$$[C_{total}] = [C_0]\left(\alpha^{N-1}\frac{k_p}{k_{-1}} + \sum_{i=0}^{N-1}\alpha^i\right) = [C_0]\left(1+\frac{k_p}{k_{-1}}\right)$$

Thus, the quantity we are interested in is

$$\frac{C_N}{[C_{total}]} = \alpha^N \tag{2}$$

By this way, we can control the activation by modifying the dissociation rate, k_{-1}, so we can achieve a large-scale signaling event and trigger the activation. What makes the difference is that antigens must bind to the TCR with a larger timescale than the non-agonist ones. We can see that in equation [1], a difference of 10-fold in specific and non-specific peptides ($k_{-1} = 0.1k_p$ in front of $k_{-1} = 10k_p$) generates a 10000-fold difference in activation in the case of $N = 4$ (the fraction of activated complexes are 0.68 versus 6.8 10^{-5}, respectively).

The complete numerical simulation is presented in Figure 2. I ran simulations for the case of $N = 6$ and I show the evolution of all complexes. In the case where the dissociation constant is small, the peptide binds with a large timescale to the TCR and the phosphorylations are more efficient, we can observe in Figure 3 that a large fraction of the complex end up being active (C_5), while there is roughly not activation in the other case.

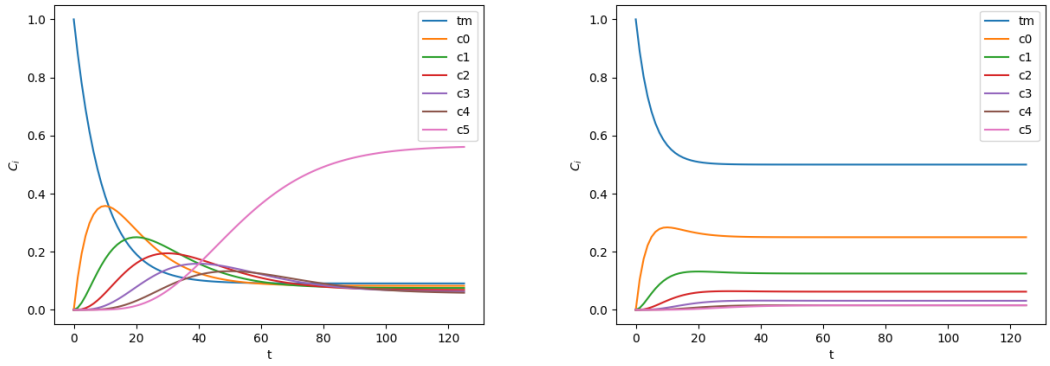


Figure 2: Dynamics of all the complexes in the case of $k_{-1} = 0.1k_p$ (left) and $k_{-1} = k_p$ (right).

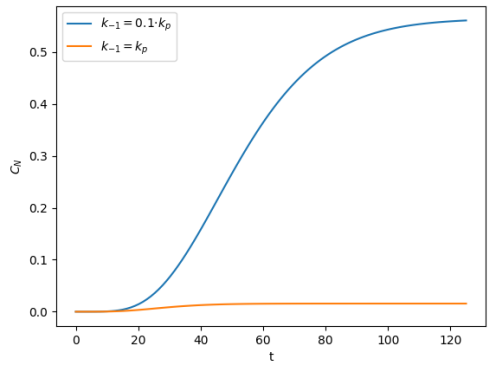


Figure 3: Zoom showing just the active complex comparing both cases of the previous figure.

In Figure 4 I show the age distribution of the complexes so we can observe that there is a peak in the creation of active complexes in the middle of the process, so it would represent the lag time since we begin to see the majors effects in activation of the T-cell.

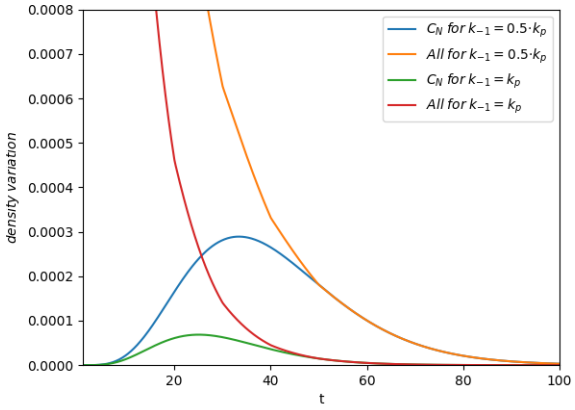


Figure 4: Age distributions, where we can appreciate the rhythm of creation of the active complex in front of the dissociation of all other complexes.

This model allows to increase unlimitedly the selectivity, but in cases where the affinity difference is small, it would require an unacceptable low level of activation from specific stimuli. The problem can be alleviated if the dissociation constant of the active form (k_{-N}) is less than the dissociation constants for the other stages of the complex. By this way it can accumulate active complex even if the initial complex has a small likelihood of passing through N phosphorylations before dissociating.

In order to realize the flaws this model presents, let us take another point of view. Now I run simulations for 3 minutes in a two-step process, setting a characteristic lifetime of 18 s (being thus the rate of dissociation $k_{-1} = 1/18$) for the binding of the agonist pMHC with the TCR, and 3 s ($k_{-1} = 1/3$) for the non-agonist one. The proofreading timescale is 12 s. We begin by putting just one molecule of pMHC, then we increase the number of it so we detect where the threshold is, i.e when we get more than one fully phosphoryled complex after 3 minutes. We can observe the window of discrimination, which is the horizontal distance between the points the agonist and non-agonist pass the threshold, respectively. If we set the mechanism to have 6 phosphorylation steps, we can observe that the non-agonist peptide does not reach the threshold in the same range as before, so the discrimination window is higher. Nevertheless, the number of pMHC needed in order to trigger a minimal response is also higher than in the case with less steps, and it is more than needed in real immunitary systems as well, as observed experimentally.

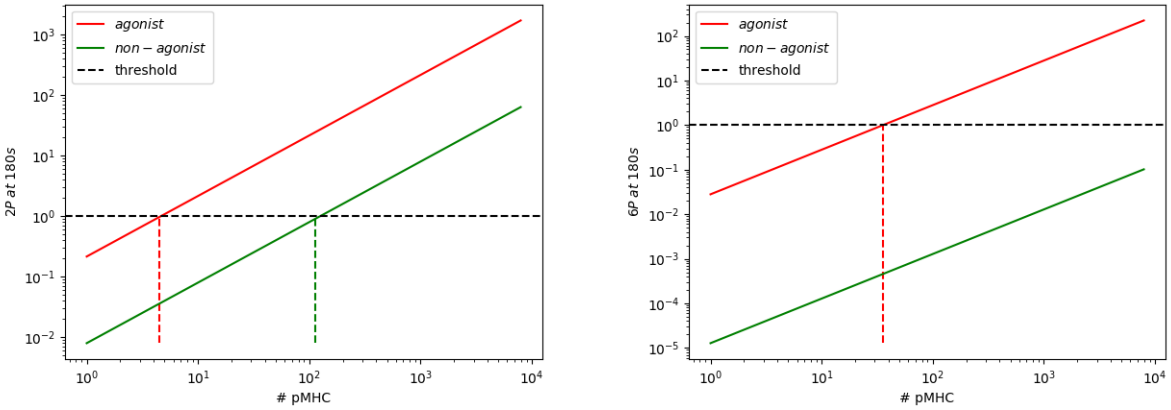


Figure 5: Active complexes formed after 3 min simulations, for the case of sytems with 2 and 6 phosphorylation steps (left and right, respectively).

Now I test the response of this simple biochemical model for proofreading timescales between 0.3 and 30 seconds, observing when we have lymphocyte activation for both agonist and non-agonist (white region), no activation for anyone (black region), and activation just for the agonist, which is our interest case (gray region). In Figure 6 it is compared the case of 2 phosphorylation steps (left), with the case of 6 steps (right). We can observe that even in the case of the 6-step scheme, the gray zone does not span the range of number of pMHC that is observed in the experiments (it is 3 over 4 parts required), not to mention the other flaws remarked earlier, when we saw the increase of sensibility induces an increase of the minimal response, together with the slower response associated.

So we have seen that although this is an interesting model that explains some basic features of the activation of the immune system machinery, specificity is achieved at the expense of speed and sensitivity. There is another caveat of the kinetic proofreading scheme highlighted in the protocol S1 of [2]. The signaling response is macroscopic, including in short timescales. Even with small numbers of pMHC ligands, the response in, for example, calcium ions entering the cytoplasm is shown to be robust. So to translate sparse signals in this robust, macroscopic molecular responses, there needs to be signal amplifications. This model does not achieve the level of amplifications required, so it seems obvious that some additional intracellular amplification is necessary.

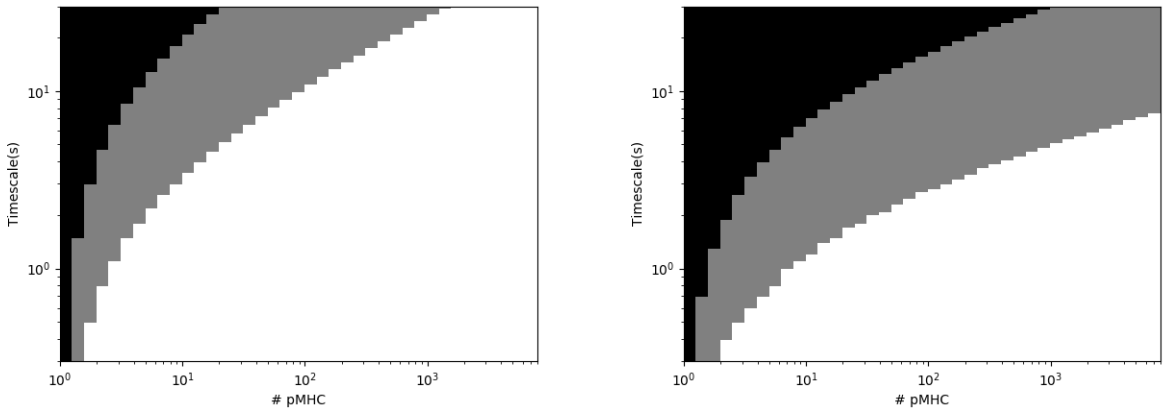


Figure 6: Scanning of the response (black: no response, gray: correct response, white: indistinct response) for different proofreading timescale values, i.e. $1/k_p$. Case of 2 steps (left) and 6 steps (right).

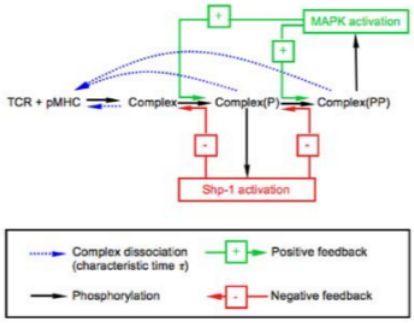
3 Feedback control of digital ERK responses

Thus, the aim of an improved model should be to develop a detailed scheme of early TCR signaling that accounts for all the considerations specified before, into the T-cell's response to antigen's presence. The model I review and test is constructed around a KP scheme, but modified with the inclusion of two competing feedback loops, one positive and another negative. They documented in experiments an unexpected property in the signaling, which is a digital extracellular signal-regulated kinase (ERK) response involving high level of input amplification. Accordingly, their model show that the activation of this ERK amplifier can be prevented while retaining sensitivity to low number of agonist ligands.

3.1 The model

It incorporates a negative-feedback process that acts as some form of noise filtration. The SH2 tyrosine phosphatase (SHP-1) has been observed to play such role in TCR signaling, and it has been used in models to limit responses to high levels of weakly binding ligands. We must have a positive-feedback loop involving ERK-1, so to protect TCRs from the inhibitory effects of the previous one. Beggining shortly after TCR engagement occurs, the phosphatase SHP-1 is tyrosine phosphorylated by active Lck (Src family kinase). The resulting pSHP-1 binds stably to Lck-containing TCR complexes via interaction with the kinase's SH2 domain. Thus, this SHP-1 becomes enzymatically activated upon further tyrosine phosphorylation, leading to dephosphorylation of the Lck.

In the paper they justify all processes and build up all components in great detail, because they compare all the mechanism and simulations with laboratory control experiments. They chose to examine the ERK-phosphorylation response of OT-1 CD8+ T-cells upon activation with peptide-pulsed APCs. They extract all the parameters from experimental tests. Nevertheless, we can schematically represent the model as in the next figure (up). We can observe the intricate full model below.



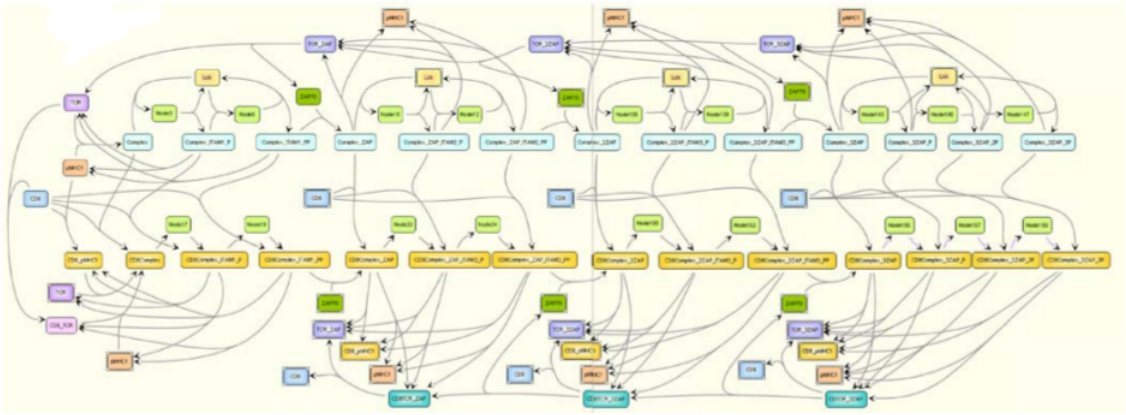


Figure 7: Sketch of the model (up) and full model (down).

I download the particular model they use from here. This is an XML file that is opened with a very useful software, JDesigner, that consists of a graphical modeling environment for biochemical reaction networks. It allows drawing the network on screen and selecting the appropriate kinetic laws from a wide selection of inbuilt rate laws or defining new user defined rate laws. Just opening the XML file allows us to visualize the network and do the simulations easily.

Although it is very interesting to fiddle with this program they use in their paper, I rather present my own results from a simplified model. Grasping the basic aspects of the model and implementing it in the simple KP scheme, I compare it with the one presented earlier. Following the scheme of the previous figure (up), I build a 6-step KP, as the one used before, but now introducing the Shp-1 and MAPK activation. I build it up by allowing the first five complexes to activate together the negative-feedback loop, and then the last complex to enhance the phosphorylation of the same five complexes. So I had to control just the parameters for the activation-deactivation of Shp-1 and MAPK. These has to be smaller than the parameters of the phosphorylation and dephosphorylation, which are still the cornerstone of the model. I also consider that the MAPK activation is bigger than the Shp-1 one. Introducing these in the model enriches it widely, and we can show that the simple KP model presented earlier is enhanced.

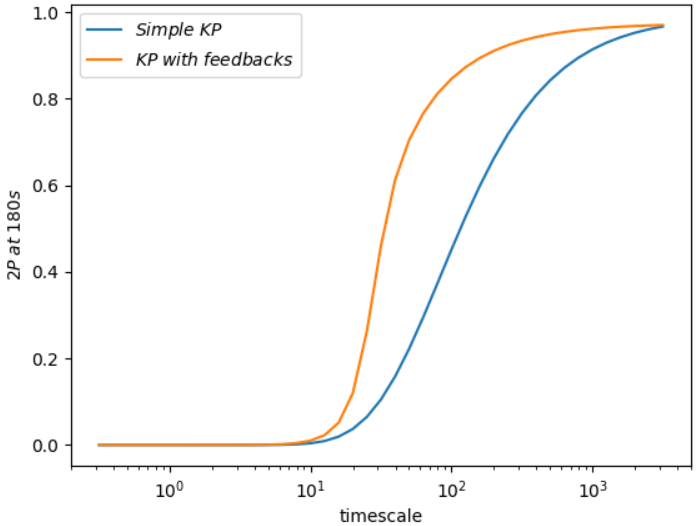


Figure 8: Fully phosphorylations (active complexes C_6) after a 180 s simulation, for increasing timescale for the dissociation of the complexes, i.e $1/k_{-1}$. Comparison of the first and second model.

In Figure 8, we observe that the frontier of antigen and self-peptide discrimination is more recognizable in the model with feedback control. Thus, less dissociation time is needed to mark antigens to be detected by the system. We can compare the new scheme by also obtaining the same results presented in the previous section, now for this modified model. In Figure 9 we see that the window of discrimination is higher than in the simple KP model, in my simulations I get that it is one order of magnitude higher. Nevertheless, the most important difference is that this enhanced discrimination range doesn't come with the effect of losing sensitivity as in the case of the first model, since the threshold for the antigen discrimination is just 3 pMHCs in this case, while in the simple model we needed almost 40 pMHCs to trigger activation, using the same parameters (Figure 5, right).

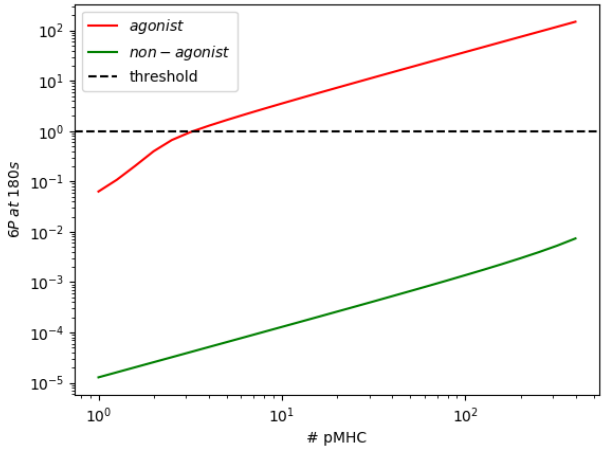


Figure 9: Active complexes formed after 3 min simulations, for the case of 6 phosphorylation steps with positive and negative feedback loops.

Comparing Figure 6 with Figure 10, now the gray zone spans a wide range of pMHC, its surface is pretty much bigger and the no-response area has a sharper frontier than in the simple model, so we can appreciate that we have highly improved the results.

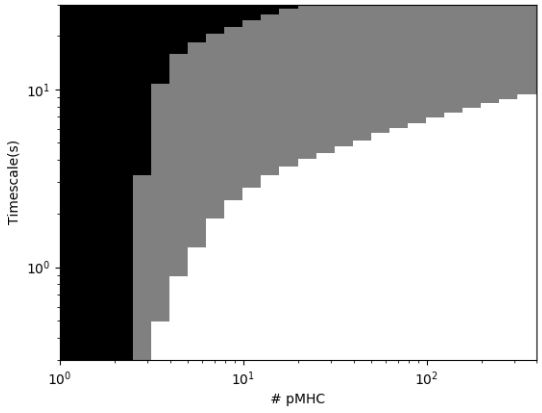


Figure 10: Scanning of the response (black: no response, gray: correct response, white: indistinct response) for different proofreading timescale values, i.e. $1/k_p$, for the KP scheme with 6 steps including the two feedback loops mentioned.

4 Conclusion

So far we have reviewed the cornerstone model for the machinery that involves the immune response, so the question was how T-cells can trigger a rapid, sensitive and highly discriminatory response. The kinetic proofreading scheme allows us to depict qualitatively the process, as it has been presented in the first section of this work. Nevertheless, it has some flaws that suggest that the machinery somehow is far more complicated than a simple KP scheme can explain. One important improvement involves adding two feedback-loop processes, one positive and the other negative. Although following the lines presented in [2], I proved that adding the loops in the most simple way can indeed make the model more efficient and discriminatory. In [2] they present a full biochemical model, and they document additional processes as the ERK response is found to be digital. I also navigated through the model using the program JDesigner, but only my own simulations are presented here in the figures.

On the whole, I feel I have achieved a nice understanding of the basic features of the immune system that we can model, and I realized that it is a highly sophisticated machinery that nature has brought to us through it most perfect tool, evolution, and even now we are still struggling to fully understand and model it in detail.

References

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