Tunable kinetic proofreading in a model with molecular frustration

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Abstract In complex systems, feedback loops can build intricate emergent phenomena, so that a description of the whole system cannot be easily derived from the properties of the individual parts. Here, we propose that intermolecular frustration mechanisms can provide non-trivial feedback loops which can develop non-trivial specificity amplification. We show that this mechanism can be seen as a more general form of a kinetic proofreading (KP) mechanism, with an interesting new property, namely the ability to tune the specificity amplification by changing the reactants concentrations. This contrasts with the classical KP mechanism in which specificity is a function of only the reaction rate constants involved in a chemical pathway. These results are also interesting because they show that a wide class of frustration models exists that share the same underlining KP mechanisms, with even richer properties. These models can find applications in different areas such as evolutionary biology, immunology, and biochemistry.

Keywords Kinetic proofreading · T cell activation · Specificity · Cellular frustration · Molecular frustration · Assortativeness

Introduction

An important issue in biochemistry consists in unveiling mechanisms that increase specificity in highly degenerate reactions. A major breakthrough was achieved by Hopfield (1974) after proposing the kinetic proofreading (KP) mechanism. The KP attempts to explain how DNA replication and protein synthesis occur with very small errors, even though the energies involved in the biochemical recognition processes are very close. The range of possible applications of these ideas was later recognized to be much broader, and opened an active field of research (Hlavacek et al. 2001; Qian 2006; Burroughs and Merwe 2007; Inoue and Kaneko 2010; Owens et al. 2010; Kirkilionis 2010). McKeithan (1995) argued that the KP could also help explaining the high specificity reached in cellular recognition processes during T cell activation (McKeithan 1995). In this case, highly specific cellular interactions could benefit from the same type of underlying mechanism which helped explaining the high specificity achieved in molecular interactions (Chan et al. 2003; George et al. 2005). In a quite different context, one of the authors of this contribution identified a mechanism that highly increased assortativeness in a mating population. Assortativeness is another kind of specific interaction between individuals which favors some matings relatively to others. In the study of Almeida and de Abreu (2003), it was argued that these specifically amplified interactions could explain the emergence of new species in sympathy. Later, it was also proposed that similar ideas could be applied in immunology (de Abreu et al. 2006) in what it was called the cellular frustration framework. However, these later models are substantially different from Hopfield’s and McKeithan’s KP models. In the later case, increased specificity depends only on the specific rate constants associated to each
molecular interaction, whereas in the study of de Abreu et al. (2006) interactions depend non-trivially on the presence of other agents (molecules, cells, or individuals).

Since both types of models can appear to be very different, it is important to show that they both share the same underlying mechanism, namely the existence of a long series of time lasting interactions until a final reaction stage is reached. The purpose of this article is thus to show that the mechanism identified in the study of de Abreu et al. (2006) can be seen as a general form of a KP mechanism. For this purpose, we organize this article as follows. In the next section, we will discuss the main ideas involved in the KP mechanism as discussed by McKeithan. McKeithan’s model is extremely appealing due to its simple mathematical formulation. We will concentrate on those aspects that allow a straightforward comparison between both classes of models. Afterward, we introduce a simple model that captures the main ideas of cellular frustration models and discuss a molecular version of these models. We will show that it is possible to use the formulation introduced by McKeithan within this more general class of models. We will be able to show that in the later models increased specificity can indeed be achieved and, furthermore, that this class of models can exhibit a new property. This is the possibility of tuning specificity amplification by changing reactants concentrations. This result is extremely interesting because the classical KP pathway did not benefit from this type of flexible behavior.

**Classical Kinetic Proofreading**

Consider three types of molecules A, A*, and B. Molecules A and A* can both react with B molecules with very close free energies. The KP mechanism shows that it is possible to produce an almost unlimited larger number of final products from one reaction than the other. The KP mechanism makes two important assumptions. It assumes that the final products are obtained after a long chain of reactions, and also that intermediate reactants can be destabilized toward the initial step in the pathway, as described in Fig. 1. If destabilization rates are bigger for reactions involving one molecule (A or A*) than the other, then a several fold increase in the production of the final products can be achieved in one pathway relatively to the other. This is the essence of KP explanation for the emergence of high specificity arising from almost degenerate reactions.

From a mathematical point of view, the specificity increase can be straightforwardly understood. Following McKeithan (1995), we would write the chemical kinetics equations:

\[
\frac{d[AB_0]}{dt} = k_1 \cdot [A][B] - k_{-1} \cdot [AB_0] - k_p \cdot [AB_0] \quad (1)
\]

\[
\frac{d[AB_i]}{dt} = k_p \cdot [AB_{i-1}] - k_{-1} \cdot [AB_i] - k_p \cdot [AB_i] \quad (2)
\]

\[
\frac{d[AB_N]}{dt} = k_p \cdot [AB_{N-1}] - k_{-1} \cdot [AB_N] \quad (3)
\]

and similarly for the pathway involving the A* molecule. The total number of molecules A and B is assumed constant. As a result, the concentration of free molecules evolves according to \(d[A]/dt = d[B]/dt = -\Sigma d[AB_i]/dt \) (\(i = 0, \ldots, N\)).

It is easy to obtain the following relations for the concentrations at the steady state:

\[
\frac{[AB_N]_{ss}}{[AB_0]_{ss}} = x \quad (4)
\]

\[
\frac{[AB_N]_{ss}}{[AB_0]_{ss}} = x^{N-1} \cdot \frac{k_p}{k_{-1}} \quad (5)
\]

\[
\frac{[AB_i]_{ss}}{[AB_0]_{ss}} = x^i \quad (6)
\]

The total concentration of complexes ([AB\text{total}]i) can be obtained by adding (5) and the summation of all the other complexes concentrations using (6):

\[
[AB\text{total}]_{ss} = [AB_0]_{ss} \cdot \left( x^{N-1} \cdot \frac{k_p}{k_{-1}} + \sum_{i=0}^{N-1} x^i \right)
\]

Finally, it is possible to write for the normalized concentration of final products:

\[
\frac{[AB_N]_{ss}}{[AB\text{total}]_{ss}} = x^N \quad (8)
\]

Identical expressions can be obtained for the pathway involving A* molecules, replacing \(k_{-1}\) by \(k_{-1}^*\), or, correspondingly, \(x\) by \(x^*\).

To quantify the effectiveness of the KP mechanism, we follow (Chan et al. 2003) and define true positive (TP) events when A*B\text{N} complexes are formed. On the other hand, false positive (FP) events occur when AB\text{N} complexes are formed. True negative (TN) and false negative (FN)
events occur every time $AB_0$ or $A^*B_0$ complexes do not end in $AB_N$ or $A^*B_N$ complexes, respectively. The following statistical measures of performance can then be defined:

Specificity = \( \frac{\text{Number of TP}}{\text{Number of TP} + \text{Number of FP}} \)  
Sensitivity = \( \frac{\text{Number of TP}}{\text{Number of TP} + \text{Number of FN}} \)  
Tolerance = \( \frac{\text{Number of TN}}{\text{Number of TN} + \text{Number of FP}} \)

Specificity measures the fraction of final products, $AB_N$, that corresponds to the desired complex. Sensitivity measures the fraction of complexes $A^*B_0$ that produce the desired final product, $A^*B_N$. Finally, tolerance measures the fraction of complexes $AB_0$ that do not form the undesired final product, $AB_N$.

Tolerance was not considered in the study of Chan et al. (2003) because in McKeithan’s model tolerance can be expressed as a function of sensitivity and specificity. However, we include it here because its behavior will be useful to discuss separate phenomena occurring in the molecular frustration model. From (7) and (8), we have:

\[
\text{Sensitivity} = \left( \frac{k_p}{k_p + k_{-1}} \right)^N = x^* \quad (12)
\]
\[
\text{Tolerance} = 1 - \left( \frac{k_p}{k_p + k_{-1}} \right) = 1 - x^N \quad (13)
\]
\[
\text{Specificity} = \frac{x^N}{x^* + x^N} \quad (14)
\]

Since $x < x^* < 1$, it is clear that sensitivity decreases exponentially with the number of reaction steps $N$, while specificity approaches 1. Specificity amplification depends only on the dissociation rate constants, $k_{-1}$ and $k^*_p$, their ratio and absolute values (Table 1). It should be remarked that according to McKeithan’s scheme, specificity is not necessarily close to 1. For small values of $k_{-1}/k^*_p$, specificity can be only slightly higher than 50%. To increase specificity, it is required to increase dissociation rate constants. However, this has a drawback, as it reduces the overall sensitivity (reactivity) of the system (Table 1).

According to McKeithan’s scheme, specificity depends only on chemical constants and cannot be easily changed. In the next section, we will show that molecular frustration also increases specificity, but in that case specificity depends on the reactants concentrations, and hence it can be easily tuned.

### Inter-molecular frustration

An alternative mechanism to increase specificity in systems of complex agents was proposed in the study of Almeida and de Abreu (2003), in the context of a theory for sympatric speciation. Instead of molecules involved in multi-step chained reactions, it was considered a population of individuals performing time lasting mating decisions. This approach shares a common ingredient with the classical KP mechanism, namely the requirement of time demanding intermediate processes preceding the final stage. During these intermediate steps, dissociations occur with different probabilities depending on the already formed associations ($A^*B$ or $AB$ in the example from the previous section). However, now dissociations arise depending on which molecules are interacting. As a result, dissociation rates become frequency dependent: they depend on the concentrations of molecules that can destabilize a given complex.

According to the classical KP approach, intermediate steps could be due to a set of conformational configurations (McKeithan 1995; de Abreu et al. 2006), or other intermediate chemical reactions (Hopfield 1974). The final step in the pathway would be associated with the production of major signals like cellular activation (McKeithan 1995; de Abreu et al. 2006), or the assembly of the final synthesis product (Hopfield 1974). On the contrary, in the case of individuals in a mating population, the time-consuming intermediate states would arise from the time investment required before a decision is made. The major signal produced in the end could be related to reproduction (Almeida and de Abreu 2003).

However, a fundamental difference exists between the two approaches. In the traditional KP mechanism, intermediate steps are destabilized by spontaneous dissociation. This is fixed according to the chemistry of the reactions involved. On the contrary, in the approach taken in the study of Almeida and de Abreu (2003), this would be due to the other individuals decisions that could change a mating stability. This led to the concept of “cellular frustration” (de Abreu et al. 2006): an individual’s decision may frustrate other individual’s previously taken decisions. In this study, we propose that similar ideas may also be applicable to describe interactions between large molecules or complexes. Furthermore, we want to show that the frustration mechanism proposed fits in a more general form of a KP mechanism, which displays new features.

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**Table 1** Specificity for several systems with four states ($N = 3$) in the pathway

| $k_{-1}/k_p$ | 1 | 10 | 0.01 | 0.1 |
|--------------|---|----|------|-----|
| Tolerance    | $\approx 1.0000$ | $\approx 1.0000$ | 0.8750 | 0.8750 |
| Specificity  | $\approx 1.0000$ | 0.9987 | 0.8859 | 0.8574 |
| Sensitivity  | 0.1250 | 0.0008 | 0.9706 | 0.7513 |
The main ideas involved in the “cellular frustration” concept can be easily understood with the example in Fig. 2. Consider three molecules (A, B, and C) interacting according to a ranking of affinities as described in the Table in Fig. 2 (right). For instance, molecules A would bind to molecules B with higher binding energy than if they would bind to molecules C. Similarly, molecules B would bind to molecules C with higher binding energy than to molecules A* and A, respectively, and in decreasing order.

Note that the frustration mechanism discussed here is different from the one discussed in the context of protein folding (Bryngelson et al. 1995). In protein folding, frustration occurs when amino acid sequences produce many conformations that are closely linked local minima in the energy landscape. Frustration is mainly an intra-molecular concept. In our case, frustration emerges from inter-molecular interactions, producing also many unstable configurations. To distinguish the two types of frustration, we refer to the frustration mechanisms discussed in this article as inter-molecular frustration.

Crucially important is that it is also assumed that a molecule can only form a stable binding with one other molecule at a time. Consequently, when two molecules are simultaneously bound to a third, it is assumed that this creates an instability reducing drastically the affinity in the weaker bond. This could arise, for instance, if conformations develop whenever stronger bindings are formed, weakening previous ones. While in the case of mating decisions, this kind of decision dynamics seems to be most natural; in the context of molecular interactions, it requires a bigger complexity in the dynamics of individual molecules. Nevertheless, this is still a reasonable possibility for many systems. And indeed, if one thinks of transmembrane proteins, it is well known that binding extracellular molecules can induce release of intracellular molecules in a far away binding site at a later time (Hlavacek et al. 2001; Burroughs and Merwe 2007).

Molecular frustration could then be a natural outcome in this kind of complex systems. As shown in Fig. 2, molecules A, B, and C would tend to form continuously transient bindings because a third molecule can always destabilize already established pairs. The question we raised in this study is whether the introduction of a fourth molecule, different and yet similar to molecule A, may lead to longer lasting (less frustrated) interactions, so that the final interaction step in the chemical pathway is more easily reached?

**Inter-molecular frustration and increased specificity**

We considered a biochemical model with four molecules (A, A*, B, and C) interacting according to the Table in Fig. 2 (right) and undergoing a sequence of conformational steps before the final AB or A*B complexes are reached. We will assume that molecule A is maximally frustrated (de Abreu and Mostardinha 2009), so that the introduction of a new molecule A* necessarily reduces frustration leading to more stable A*B complexes. This model allows a direct comparison with McKeithan’s model, and highlights how molecular frustration embodies a generalization of a KP mechanism.

In Fig. 3, the full set of reactions is presented. If alone, A and B molecules can react with a reaction rate constant \(k_+\), forming an AB complex. Afterward, they can undergo a sequence of transformations \((AB \rightarrow AB_{k+})\), at a reaction rate constant \(k_{r}^{+}\). During these intermediate configurations, AB complexes can also be destabilized, if free C molecules interact with the B molecule in the complex. In this case, a new BC complex is formed and an A molecule is freed. Afterward, the BC complex can be destabilized if a molecule A interacts with the C molecule in the complex. It should be noted that, according to the table in Fig. 2, an A* molecule can also destabilize an AB complex. AB complexes are more frustrated than A*B and consequently it should be harder that AB complexes reach the final product stage. It is our aim to quantify the specificity increase that is introduced by the fact that A* molecules are necessarily less frustrated than A molecules.

Note that for the sake of mathematical simplicity, we assumed that the \(k_+\) reaction constant incorporates two processes. One concerning the binding of two molecules, and the other the release of formerly bonded molecules on a distant binding site. The later could result from a change...
in the conformational state of that binding site. This simplification allows a more transparent mathematical description and should not influence qualitatively the results provided \( k_+ \gg k_p \), i.e., the time scale involved in binding and change on the conformational structure on distant binding sites is a fast process relatively to the time required to reach final products.

Simple mean field dynamical equations can be derived for the concentrations of the several possible complexes. Equations for the \( A^*B \) complexes are:

\[
\frac{d[A^*B_0]}{dt} = k_+ \cdot \left( \sum_{i=0}^{N} [AB_i] + [B\varnothing] \right) \cdot ([A^*\varnothing] + [A^*C]) \\
- k_p \cdot [A^*B_0] - k_+ \cdot [A^*B_0] \cdot [C\varnothing]
\]

\[ (15) \]

\[
\frac{d[A^*B_i]}{dt} = k_p \cdot [A^*B_{i-1}] - [A^*B_i] \cdot (k_p + k_+ \cdot [C\varnothing])
\]

\[ (16) \]

\[
\frac{d[A^*B_N]}{dt} = k_p \cdot [A^*B_{N-1}] - k_+ \cdot [A^*B_N] \cdot [C\varnothing]
\]

\[ (17) \]

For equations involving the \( AB \) complexes, we got

\[
\frac{d[AB_0]}{dt} = k_+ \cdot [B\varnothing] \cdot ([A\varnothing] + [AC]) - k_p \cdot [AB_0] \\
- k_+ \cdot [AB_0] \cdot ([C\varnothing] + [A^*C] + [A^*\varnothing])
\]

\[ (18) \]

\[
\frac{d[AB_i]}{dt} = k_p \cdot [AB_{i-1}] - [AB_i] \\
\cdot (k_p + k_+ \cdot [C\varnothing] + [A^*C] + [A^*\varnothing])
\]

\[ (19) \]

\[
\frac{d[AB_N]}{dt} = k_p \cdot [AB_{N-1}] - k_+ \cdot [AB_N] \\
\cdot ([C\varnothing] + [A^*C] + [A^*\varnothing])
\]

\[ (20) \]

Similar equations were derived for the other complexes (e.g., \( AC, BC, \) etc.) to simulate the whole system.

The concentration of free \( A \) molecules is obtained using

\[
\frac{d[A\varnothing]}{dt} = -\sum_i d[AB_i]/dt - d[AC]/dt \quad \text{(i = 0, ..., N)}
\]

and similarly for the other free molecules.

In Eqs. 15–20, negative contributions involving the rate constant \( k_+ \) account for dissociations resulting from interactions with other molecules. Contrary to what happened in McKeithan’s model, the rate constant \( k_+ \) is now modulated by the concentration of molecules that can destabilize a complex. For instance, the \( A^*B_N \) complex can be dissociated on interaction with a free \( C \) molecule (Eq. 17), whereas the \( AB_N \) complex can furthermore be destabilized by \( A^*C \) complexes and free \( A^* \) molecules. \( AB_N \) complexes are thus less stable than \( A^*B_N \) complexes. By performing a similar analysis to Eqs. 15–16 and (18–19), we can conclude in general that \( AB \) complexes are more unstable than \( A^*B \) complexes as there are more processes destabilizing the former complexes (Fig. 3).

At the steady state, \( d[AB_i]/dt = d[AB]/dt = 0 \), and we can define constants \( \beta \) similar to McKeithan’s \( \chi \) constants:

\[
\frac{[A^*B_i]_{ss}}{[A^*B_{i-1}]_{ss}} = \frac{k_p}{k_p + k_+ \cdot [C\varnothing]_{ss}} = \beta_i
\]

\[ (21) \]

\[
\frac{[AB_i]_{ss}}{[AB_{i-1}]_{ss}} = \frac{k_p}{k_p + k_+ \cdot ([C\varnothing]_{ss} + [A^*C]_{ss} + [A^*\varnothing]_{ss})} = \beta
\]

\[ (22) \]

A relation can also be obtained relating the concentrations at the initial and the last step in the pathway:

\[
\frac{[A^*B_N]_{ss}}{[A^*B_0]_{ss}} = \frac{k_p}{k_p + k_+ \cdot [C\varnothing]_{ss}} \cdot \beta^{N-1}
\]

\[ (23) \]

Finally, the total concentration of \( A^*B \) complexes in the system is given by
that self-organized molecular interactions (Karsenti 2008) interactions. Consequently, this calls attention to the fact emergent behavior of a fairly simple set of inter-molecular of conformational pathways. Instead it builds upon the KP mechanism that does not require a numerous sequence molecular frustration embodies a more general kind of a First, because it shows that the mechanism of inter-specificity is now a function of reactants steady state mechanisms are present. However, there is an important difference: specificity similar to that predicted by the classical KP concentration of single molecules decreases because it shows that there are mechanisms in which specificity could be tuned to a desired level by an external control, or by a mechanism developed inside a complex biochemical system, for instance, a regulated synthesis of C molecules. Hence, molecular frustration can be a flexible specificity amplification mechanism.

In order to confirm these theoretical predictions, we simulated numerically the reaction rate dynamical equations derived before. In Fig. 4, we show the evolution of the concentration of $AB_i$ and $A^*B_i$ complexes for $i = 0, \ldots, N$, together with $[AO]$ and $[A^*O]$. It is clear that the concentration of single molecules decreases straightforwardly along the time, while the concentration at the steady state of intermediate complexes decreases steadily along the pathway. As a result, while there is a specificity increase, there is simultaneously an overall loss in reactivity, typical of KP mechanisms (Chan et al. 2003; George et al. 2005).

In Fig. 5, we show the dependence of tolerance, specificity, and sensitivity as a function of the initial concentration of C molecules and for pathways with $N = 3$ and $N = 4$. These results are interesting for several reasons. First, because they confirm that specificity varies considerably with the concentration of one of the reactants. In particular, it shows that the introduction of a third molecule influences the specificity dramatically. As a result, specificity becomes a tunable property because it can be increased to a desired level by tuning the third molecule concentration, at the expense of some decrease in sensitivity. These results are also interesting because specificity shows a maximum at a location that does not coincide with the maximal increase in tolerance. Furthermore, sensitivity is still non-negligible when tolerance approaches the maximal value. This puts in evidence that there are two different mechanisms taking place. Specificity amplification uses the same mechanism leading to increased tolerance. Indeed, both require frustration to build the necessary feedback loops. However, if the concentration of C reactants is too large, a jamming transition takes place that again blocks these amplification mechanisms, reducing both sensitivity and specificity. The non-monotonous decay in the sensitivity plot in Fig. 5 indeed shows that these two mechanisms are present.

In Fig. 6, we also show that specificity increases when the molecular reactivity, $k_+$, increases. This agrees with (25) and highlights that specificity is an emergent property in this model. In fact, one could naively think that by increasing the molecular reactivity of all species, specificity could be hindered. On the contrary, specificity increases and the production of some species over others can differ by orders of magnitude. In this figure, the amplification ratio was computed according to:

$$[A^*B_{\text{total}}] = [A^*B_{\text{ss}}] + \left( \beta \left( \frac{k_p}{k_+} \left( C \right)_{\text{ss}} + \sum_{i=0}^{N-1} \beta_s \right) \right)$$

(24)

Expressions are identical to the ones obtained by McKeithan if we establish the following correspondences:

$$k_{i-1} \leftrightarrow k_+ \cdot [C \text{ss}]$$

$$k_{-1} \leftrightarrow k_+ \cdot ([C \text{ss}] + [A^*C]_{\text{ss}} + [A^*O]_{\text{ss}})$$

(25)

Similarly, expressions for the sensitivity, specificity, and tolerance remain identical if we replace $\alpha$ by $\beta$ and $\alpha_s \rightarrow \beta_s$. An important consequence of this analysis is that molecular frustration can also produce an increase in specificity similar to that predicted by the classical KP mechanism. However, there is an important difference: specificity is now a function of reactants steady state concentrations. This is interesting for two main reasons. First, because it shows that the mechanism of inter-molecular frustration embodies a more general kind of a KP mechanism that does not require a numerous sequence of conformational pathways. Instead it builds upon the emergent behavior of a fairly simple set of inter-molecular interactions. Consequently, this calls attention to the fact that self-organized molecular interactions (Karsenti 2008) can achieve specificity amplification similar to what happened in the less flexible KP schemes. Second, because it shows that there are mechanisms in which specificity could be tuned to a desired level by an external control, or by a mechanism developed inside a complex biochemical system, for instance, a regulated synthesis of C molecules. Hence, molecular frustration can be a flexible specificity amplification mechanism.

**Fig. 4** The evolution of the concentrations for unbound molecules A and $A^*$ (AO and $A^*O$) and intermediate complexes $AB_i$, $A^*B_i$ (i = 0, ..., N) in a system with $N = 3, k_A/k_B = 10$. In the initial configuration, there were only unbound molecules with relative concentrations: $[AO]_0/[BO]_0 = [A^*O]_0/[BO]_0 = [CO]_0/[BO]_0 = 0.5$.
Final discussion and conclusions

In this article, we discussed a possible mechanism for specificity amplification based on molecular frustration. Molecular frustration requires that each molecule has at least two different binding sites for different molecules. It also requires that each molecule can only form stable bindings with one single molecule at a time. If it binds to a third molecule, then the weaker bond becomes unstable. This could result from a kind of “induced fit” mechanism, in which after the initial binding conformations follow and destabilize other binding sites, a process resembling long-ranged allosteric regulation (Kenakin and Miller 2010; Gandhi et al. 2008). It is, however, not possible to exclude a “conformation selection” scenario, probably of an extended type (Csermely et al. 2010), in which both conformational selection and adjustments follow each other. In fact, both mechanisms are likely to coexist in many cases (Hammes et al. 2009). In any way, we believe that the specificity amplification phenomenon here discussed, is robust against the particular type of binding events, “induced fit” or “conformational selection”.

Inter-molecular frustration arises if a sequence of bindings and unbindings occurs so that molecules never form stable complexes. In case the set of molecules in the system forms a maximally frustrated set (de Abreu and Mostardinha 2009), we showed that a less frustrated molecule introduced in the system can produce final products at a rate one or twofold higher than products involving other similar molecules in the system. Furthermore, we showed that this specificity amplification depended on the reactants concentrations. This result is interesting because it contrasts with the conventional KP mechanism in which specificity is a sole function of chemical rate constants and consequently it is not as flexible. It would be interesting to consider other biochemical systems displaying similar increased specificity properties, but with different biochemical requirements.

Finally, the results reported in this article also established a clear link between KP mechanisms, well known in biochemistry or theoretical immunology, and assortativeness amplification mechanisms relevant in evolutionary biology (Almeida and de Abreu 2003).
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