

What do scaffold proteins really do?

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Abstract

The role of scaffold proteins within signal transduction pathways is an area of current active investigation by biochemists. Much of their effort is presently directed towards identifying which of the many proteins implicated in such pathways, play this role. Moreover the full biological significance of the role is yet to be fully understood. It seems that generic models of the dynamic properties of scaffold proteins and their influence on the signalling can be helpful in interpreting experimental data and developing understanding. We have been developing some PEPA models of the role of scaffold proteins. In this paper we report the results of our preliminary experiments.

1 Introduction

In this paper we present some preliminary work on using PEPA to model scaffold proteins, and investigating their role within the dynamic behaviour of signal transduction pathways within cells. We do not look at any particular pathway, but rather develop generic models which seek to illustrate how the scaffold proteins interact with other proteins in the pathway, and the impact that this has on the signalling dynamics.

This work is just in its infancy and we present here two simple models. The first represents only the formation of the complex on the scaffold and how this is affected by the concentrations of the scaffold and the reagents, and by the ratio between the binding and release rates. This model is analysed using both Markovian and continuous state space semantics. The second model considers the competitive formation of protein complexes in the cytosol, assuming that once proteins have bound in the cytosol they are no longer able to engage with the scaffold. This model is analysed only using the continuous state space semantics. For both models careful consideration of the kinetics is needed, as will be explained.

The rest of the paper is structured as follows. In Section 2 we present a brief introduction to scaffold proteins and their role in cell signalling. Section 3 presents the first PEPA model, considering the binding of reagents to scaffold, and the numerical experiments upon it. Section 4 presents the elaborated model which takes into account the formation of complexes in the cytosol. Finally, in Section 5 we summarise our results and discuss some directions for future work.

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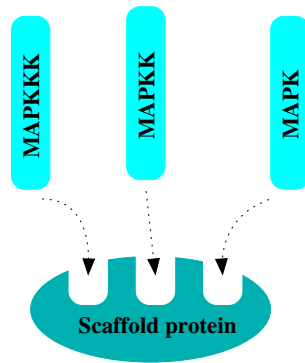


Figure 1: Schematic view of a scaffold protein

2 Scaffold proteins

In signal transduction pathways within a cell, a series of biochemical reactions serve to pass a message from the cell membrane to the nucleus. The message is prompted by the arrival of a signalling molecule at the membrane of the cell, and the result of the signal can be a profound effect on the behaviour of the cell, for example triggering cell replication, differentiation or apoptosis. The *message* is passed as peaks of concentration of proteins along the pathway and sophisticated structures in the pathway can mean that the message is amplified or attenuated as it passes from membrane to nucleus.

Considerable wet lab effort is being applying to identifying the proteins which constitute different signalling pathways. This experimental work has identified some proteins which appear to play an organisational rather than a signalling role. These proteins, termed *scaffold proteins*, serve as additional infrastructure within the cell rather than actually carrying the message themselves [1]. The exact purpose of this infrastructure is not yet clear although it is possible that by increasing local concentrations the scaffolds enhance the message passing capability of the pathway [2]. Certainly, it is known that the scaffold will bind to a number of reagents which constitute successive steps in the pathway forming a complex in which the interacting reagents are in close proximity (see Figure 1).

There have been some previous studies which looked at how the relative concentrations of reagents, and the binding and release rates, affect the formation of protein complexes in general [3] and scaffold complexes in particular [4]. At this early stage of our work we seek to recreate their results as much as anything, although the ultimate aim is to go beyond what has been done before.

Ferrell's paper [2] proposes three implications of scaffold proteins:

1. too little scaffold leads to low signalling;
2. too much scaffold leads to low signalling;
3. intermediate levels generate high signalling.

As a validation for our initial models we have addressed the question of how well we can recreate this behaviour? Moreover, we additionally consider the extent to which rates affect the outcomes.

$$\begin{aligned}
Scaffold_{000} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{100} + (mapkk_{in}, k).Scaffold_{010} \\
&\quad + (mapkkk_{in}, k).Scaffold_{001} \\
Scaffold_{100} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{000} + (mapkk_{in}, k).Scaffold_{110} \\
&\quad + (mapkkk_{in}, k).Scaffold_{101} \\
Scaffold_{010} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{110} + (mapkk_{out}, l).Scaffold_{000} \\
&\quad + (mapkkk_{in}, k).Scaffold_{011} \\
Scaffold_{001} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{101} + (mapkk_{in}, k).Scaffold_{011} \\
&\quad + (mapkkk_{out}, l).Scaffold_{000} \\
Scaffold_{110} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{010} + (mapkk_{out}, l).Scaffold_{100} \\
&\quad + (mapkkk_{in}, k).Scaffold_{111} \\
Scaffold_{101} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{001} + (mapkk_{in}, k).Scaffold_{111} \\
&\quad + (mapkkk_{out}, l).Scaffold_{100} \\
Scaffold_{011} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{111} + (mapkk_{out}, l).Scaffold_{001} \\
&\quad + (mapkkk_{out}, l).Scaffold_{010} \\
Scaffold_{111} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{011} + (mapkk_{out}, l).Scaffold_{101} \\
&\quad + (mapkkk_{out}, l).Scaffold_{110} \\
\\
MapK_{free} &\stackrel{def}{=} (mapk_{in}, \top).MapK_{bound} \\
MapK_{bound} &\stackrel{def}{=} (mapk_{out}, \top).MapK_{free} \\
MapKK_{free} &\stackrel{def}{=} (mapkk_{in}, \top).MapKK_{bound} \\
MapKK_{bound} &\stackrel{def}{=} (mapkk_{out}, \top).MapKK_{free} \\
MapKKK_{free} &\stackrel{def}{=} (mapkkk_{in}, \top).MapKKK_{bound} \\
MapKKK_{bound} &\stackrel{def}{=} (mapkkk_{out}, \top).MapKKK_{free} \\
\\
Scaffold_{000} &\boxtimes_{\mathcal{K}} (MapK_{free} \parallel MapKK_{free} \parallel MapKKK_{free})
\end{aligned}$$

$$\text{where } \mathcal{K} = \{mapk_{in}, mapk_{out}, mapkk_{in}, mapkk_{out}, mapkkk_{in}, mapkkk_{out}\}$$

Figure 2: Simple scaffold model to explore the dynamics of scaffolds and substrates

3 Simple model

Our initial model is shown in Figure 2. In previous work [5] we have developed PEPA models in two distinct style: *reagent-centric* and *pathway-centric*. In the reagent-centric style we focussed on the reagents in the pathway and assumed discrete levels of concentration. There is one set of component definitions for each reagent, each definition corresponding to one of the discrete levels of concentration. The component definition prescribes the actions (reactions) which can increase or decrease the concentration, therefore taking the reagent component to another state (level). In the extreme case this reduces to two local states for the reagent corresponding to *high* and *low* concentration. In the pathway-centric style we focussed on subpathways, or cycles of behaviour within the model. The distinct components of the model correspond to those reagents which have initial concentrations, and the derivatives of the components are the different states (e.g. phosphorylations, complex formations etc.) that the reagents may find themselves in.

$$\begin{aligned}
MapK_{free} &\stackrel{def}{=} (mapk_{in}, m_1).MapK_{bound} \\
MapK_{bound} &\stackrel{def}{=} (mapk_{out}, m_2).MapK_{free} \\
MapKK_{free} &\stackrel{def}{=} (mapkk_{in}, m_3).MapKK_{bound} \\
MapKK_{bound} &\stackrel{def}{=} (mapkk_{out}, m_4).MapKK_{free} \\
MapKKK_{free} &\stackrel{def}{=} (mapkkk_{in}, m_5).MapKKK_{bound} \\
MapKKK_{bound} &\stackrel{def}{=} (mapkkk_{out}, m_6).MapKKK_{free}
\end{aligned}$$

Figure 3: Kinase components for Markovian model

<i>parameter</i>	<i>value</i>
k	1000
l	1000
m_1	1.0
m_2	10.0
m_3	1.0
m_4	10.0
m_5	1.0
m_6	10.0

Table 1: Parameter values for Markovian experiments

For this model where we are interested in the structural rather than the signalling/concentration aspects of the system the reagent-centric style does not seem appropriate. Instead we adopt something closer to the pathway-centric style in which we focus on the possible state that the various components of the system may find themselves. The slight complication of this is that the ODE semantics has generally been applied to the models developed in the reagent-centric style [6].

The model we consider has a three position scaffold and three reagents, which are kinases, which may bind to it. Throughout these experiments we assume that signalling proceeds whenever the three reagents are bound, either in the scaffold (the only possibility here) or in the cytosol.

3.1 Markovian experiments

When conducting Markovian analysis the structure of the model was as above but the passive rates of the kinases ($MapK$, $MapKK$, $MapKKK$) were replaced as shown below in Figure 3

Different configurations of the model were considered. For example, when there are three instances of the scaffold and three instances of each kinase and the release rate is faster than the binding rate then the configuration of the model is:

$$\begin{aligned}
&(Scaffold_{000} \parallel Scaffold_{000} \parallel Scaffold_{000}) \boxtimes_L \\
&(MapK \parallel MapKK \parallel MapKKK \parallel MapK \parallel MapKK \\
&\parallel MapKKK \parallel MapK \parallel MapKK \parallel MapKKK) \\
&\text{where } L = \{mapk_{in}, mapk_{out}, mapkk_{in}, mapkk_{out}, mapkkk_{in}, mapkkk_{out}\}
\end{aligned}$$

and the rates are as shown in the Table 1.

For a model with unlimited amounts of kinase we modify the corresponding part of the model as follows:

$$\begin{aligned} \text{MapK} &\stackrel{\text{def}}{=} (\text{mapk}_{in}, m_1). \text{MapK} + (\text{mapk}_{out}, m_2). \text{MapK} \\ \text{MapKK} &\stackrel{\text{def}}{=} (\text{mapkk}_{in}, m_1). \text{MapKK} + (\text{mapkk}_{out}, m_2). \text{MapKK} \\ \text{MapKKK} &\stackrel{\text{def}}{=} (\text{mapkkk}_{in}, m_1). \text{MapKKK} + (\text{mapkkk}_{out}, m_2). \text{MapKKK} \end{aligned}$$

and the model configuration becomes:

$$(\text{Scaffold}_{000} \parallel \text{Scaffold}_{000} \parallel \text{Scaffold}_{000}) \boxtimes_L (\text{MapK} \parallel \text{MapKK} \parallel \text{MapKKK})$$

3.1.1 Experiment set 1: binding rate == release rate

When the rates are equivalent, consider the likelihood (expressed as probability) of being in a state, in steady state, with at least a certain percentage of scaffolds full.

<i>ratio scaffold:kinase</i>	<i>%100 full</i>	<i>%66 full</i>	<i>%33 full</i>
3:3	.0019	.125	.015
3:2	0	.04	10^{-4}
3:unlimited	.015	.037	.125

Greatest signalling occurs when all scaffolds are full. Clearly when there is too little scaffold, the likelihood of full scaffolds is very low. When the kinases are unbounded, the likelihood of *all full* scaffolds is an order of magnitude higher than when scaffold and kinase are equivalent, but the relative likelihood of *at least 2/3 full* scaffold is smaller, about a third.

3.1.2 Experiment set 2: binding rate > release rate

When the binding rates are an order of magnitude higher than the release rates, then the scaffolds are more likely to be full in the model with equivalent amounts of scaffold and kinase.

<i>ratio scaffold:kinase</i>	<i>%100 full</i>	<i>%66 full</i>	<i>%33 full</i>
3:3	.42	.75	.0075
3:2	0	.22	.02
3:unlimited	.052	.125	.32

So, we can conclude that we see the expected behaviour. But this is only a rough impression, since the rates can only be relative and do not precisely implement known kinetics. The PEPA notation has been useful for formulating the model and the workbench for initial exploration, we now turn our attention to interpretation via the continuous state space semantics, allowing us to conduct experiments with larger populations of molecules.

3.2 Dizzy experiments

In order to analyse the models with larger populations of molecules, we used the tool written by Stephen Gilmore to translate a PEPA model into the format required for input to the Dizzy tool.

This is relatively straightforward but we found that the resulting `.dizzy` file needed some manipulation after the translation in order to get the rates of the synchronisation actions correct. This was because at different parts of the model we found that we wanted different kinetics in order to capture the correct rate of interaction between components. The tool written by Stephen was intended for computer science rather than systems biology case studies, and consequently uses the *min*-kinetics by default.

For the binding of kinase to scaffold we found that we needed mass action kinetics and so the Dizzy file was edited to reflect this, inserting the product of the relevant concentrations, as the rate constant multiplier for such actions. However for the release of a kinase from the scaffold, the *min*-kinetics was correct, as although they are represented apparently as separate entities in the PEPA model, the filled scaffold and the bound kinase are really a single entity.

A number of experiments were carried out on this simple model to study the effect of varying the relative concentrations of scaffold and kinases, and the ratio of the binding and release rates. The results are shown in Tables 2 and 3 respectively.

In Table 2 we can see the effect outlined by Ferrell [2]. As we increase the number of scaffold proteins, keeping the number of kinases the same, we see the number of filled scaffolds increasing but then decreasing again. Recall that signalling is deemed to occur in filled scaffolds so we would expect a similar pattern for the signalling in the cell. The peak is reached when the number of scaffolds and kinases is balanced. This effect is sometimes termed *combinatorial inhibition* [4].

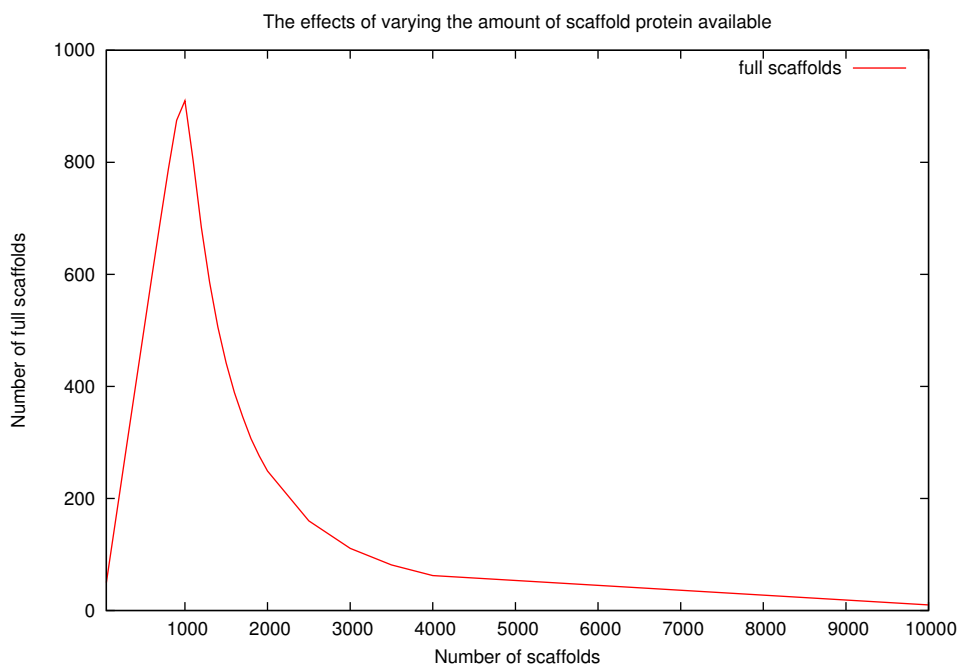
In Table 3 we see the effect of changing the ratio between the binding rate and the release rate. We assume in the current experiments that all the kinases have the same binding rate and the same release rate. Here we see that as the ratio increases, so that binding is more likely than release, so the number of filled scaffolds increases.

4 Enhanced model

In this section we consider a model which also captures the possibility of a complex forming in the cytosol. This free-floating complex formation is now in competition with the formation of a complex on the scaffold. This model is shown in Figure 4. The scaffold aspect of the model remains unchanged but the MAPK reagents now have additional activities, reflecting their ability to bind to each other, as well as binding to the scaffold.

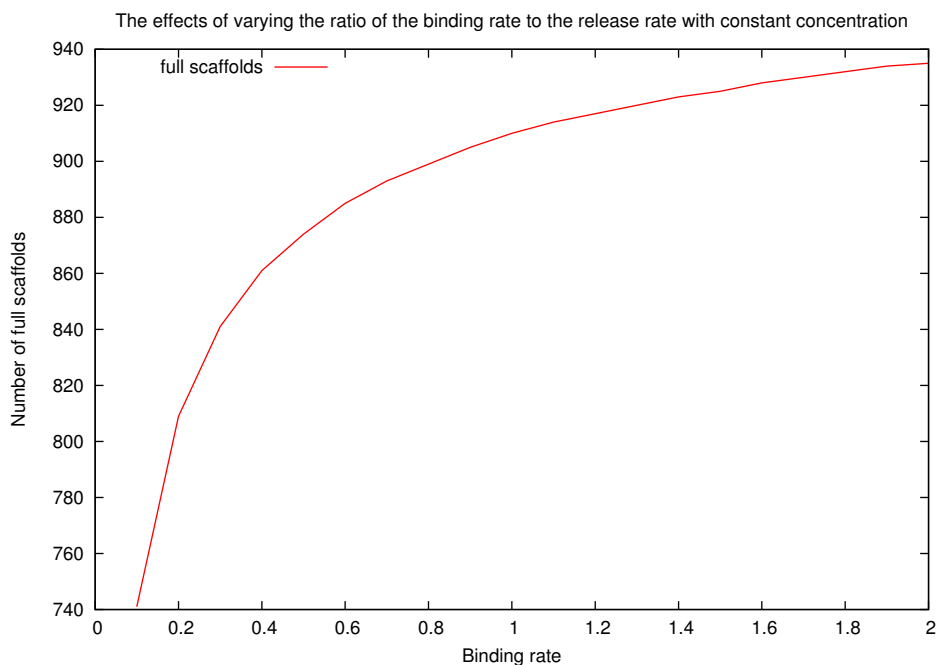
4.1 Dizzy experiments

Again we carried out experiments to investigate the impact of changing the concentration of the scaffold, and the ratio of the binding rate to the release rate (not shown here). We also now considered the impact of changing the concentration of the kinases.



<i>No. of scaffolds</i>	<i>No. of reagents</i>	<i>Binding and Release rate</i>	No. of full scaffolds
50	1000	1.0	49.8
100	1000	1.0	99.7
200	1000	1.0	199
300	1000	1.0	299
400	1000	1.0	398
500	1000	1.0	497
600	1000	1.0	596
700	1000	1.0	693
800	1000	1.0	788
900	1000	1.0	875
1000	1000	1.0	910
1100	1000	1.0	804
1200	1000	1.0	684
1300	1000	1.0	586
1400	1000	1.0	506
1500	1000	1.0	442
1600	1000	1.0	389
1700	1000	1.0	346
1800	1000	1.0	307
1900	1000	1.0	276
2000	1000	1.0	249
2500	1000	1.0	160
3000	1000	1.0	111
3500	1000	1.0	81.5
4000	1000	1.0	62.4
10000	1000	1.0	10.0

Table 2: The effects of varying the amount of scaffold protein available ($k = l = 1.0$)

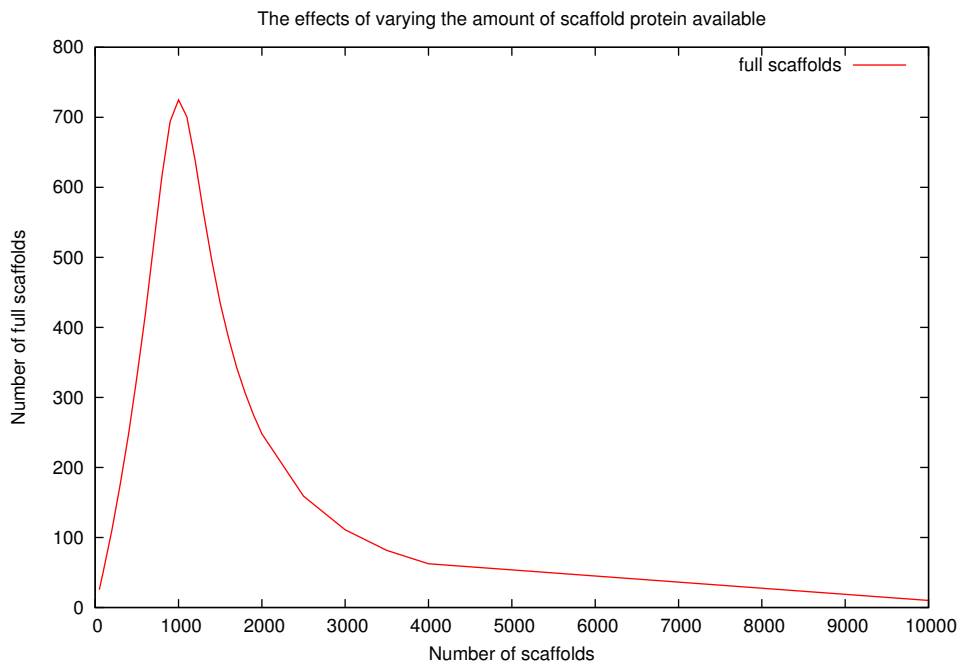


<i>No. of scaffolds</i>	<i>No. of reagents</i>	<i>Binding rate</i>	<i>Release rate</i>	No. of full scaffolds
1000	1000	0.10	1.0	741
1000	1000	0.20	1.0	809
1000	1000	0.30	1.0	841
1000	1000	0.40	1.0	861
1000	1000	0.50	1.0	874
1000	1000	0.60	1.0	885
1000	1000	0.70	1.0	893
1000	1000	0.80	1.0	899
1000	1000	0.90	1.0	905
1000	1000	1.0	1.0	910
1000	1000	1.10	1.0	914
1000	1000	1.20	1.0	917
1000	1000	1.30	1.0	920
1000	1000	1.40	1.0	923
1000	1000	1.50	1.0	925
1000	1000	1.60	1.0	928
1000	1000	1.70	1.0	930
1000	1000	1.80	1.0	932
1000	1000	1.90	1.0	934
1000	1000	2.0	1.0	935

Table 3: The effects of varying the ratio of the binding rate to the release rate with constant concentration levels $[Scaffold] = 1000$

$$\begin{aligned}
Scaffold_{000} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{100} + (mapkk_{in}, k).Scaffold_{010} \\
&\quad + (mapkkk_{in}, k).Scaffold_{001} \\
Scaffold_{100} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{000} + (mapkk_{in}, k).Scaffold_{110} \\
&\quad + (mapkkk_{in}, k).Scaffold_{101} \\
Scaffold_{010} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{110} + (mapkk_{out}, l).Scaffold_{000} \\
&\quad + (mapkkk_{in}, k).Scaffold_{011} \\
Scaffold_{001} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{101} + (mapkk_{in}, k).Scaffold_{011} \\
&\quad + (mapkkk_{out}, l).Scaffold_{000} \\
Scaffold_{110} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{010} + (mapkk_{out}, l).Scaffold_{100} \\
&\quad + (mapkkk_{in}, k).Scaffold_{111} \\
Scaffold_{101} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{001} + (mapkk_{in}, k).Scaffold_{111} \\
&\quad + (mapkkk_{out}, l).Scaffold_{100} \\
Scaffold_{011} &\stackrel{def}{=} (mapk_{in}, k).Scaffold_{111} + (mapkk_{out}, l).Scaffold_{001} \\
&\quad + (mapkkk_{out}, l).Scaffold_{010} \\
Scaffold_{111} &\stackrel{def}{=} (mapk_{out}, l).Scaffold_{011} + (mapkk_{out}, l).Scaffold_{101} \\
&\quad + (mapkkk_{out}, l).Scaffold_{110} \\
\\
MapK_{free} &\stackrel{def}{=} (mapk_{in}, \top).MapK_{sbound} \\
&\quad + (mapk_{bind}, k).MapK_{bound} \\
MapK_{sbound} &\stackrel{def}{=} (mapk_{out}, \top).MapK_{free} \\
MapK_{bound} &\stackrel{def}{=} (mapk_{unbind}, l).MapK_{free} \\
MapKK_{free} &\stackrel{def}{=} (mapkk_{in}, \top).MapKK_{sbound} \\
&\quad + (mapkk_{bind}, \top).MapKK_{kbound} \\
&\quad + (mapkk_{bind}, k).MapKK_{bound} \\
MapKK_{kbound} &\stackrel{def}{=} (mapk_{unbind}, \top).MapKK_{free} \\
MapKK_{bound} &\stackrel{def}{=} (mapkk_{unbind}, l).MapKK_{free} \\
MapKK_{sbound} &\stackrel{def}{=} (mapkk_{out}, \top).MapKK_{free} \\
MapKKK_{free} &\stackrel{def}{=} (mapkkk_{in}, \top).MapKKK_{bound} \\
&\quad + (mapkk_{bind}, \top).MapKKK_{kbound} \\
MapKKK_{bound} &\stackrel{def}{=} (mapkkk_{out}, \top).MapKKK_{free} \\
MapKKK_{kbound} &\stackrel{def}{=} (mapkk_{unbind}, \top).MapKKK_{free} \\
\\
Scaffold_{000} &\stackrel{\mathcal{L}}{\boxtimes} (MapK_{free} \stackrel{\mathcal{M}}{\boxtimes} MapKK_{free} \stackrel{\mathcal{M}}{\boxtimes} MapKKK_{free}) \\
\text{where } \mathcal{L} &= \{mapk_{in}, mapk_{out}, mapkk_{in}, mapkk_{out}, mapkkk_{in}, mapkkk_{out}\} \\
\text{and } \mathcal{M} &= \{mapk_{bind}, mapk_{unbind}\}
\end{aligned}$$

Figure 4: More complex scaffold model to explore the dynamics of scaffolds and substrates including the binding between free-floating *MapK*, *MapKK* and *MapKKK* elements



<i>No. of scaffolds</i>	<i>No. of reagents</i>	<i>Binding and Release rate</i>	No. of full scaffolds
50	1000	1.0	25.5
100	1000	1.0	52.3
200	1000	1.0	110
300	1000	1.0	175
400	1000	1.0	247
500	1000	1.0	328
600	1000	1.0	417
700	1000	1.0	516
800	1000	1.0	615
900	1000	1.0	694
1000	1000	1.0	725
1100	1000	1.0	701
1200	1000	1.0	638
1300	1000	1.0	564
1400	1000	1.0	495
1500	1000	1.0	435
1600	1000	1.0	385
1700	1000	1.0	342
1800	1000	1.0	306
1900	1000	1.0	275
2000	1000	1.0	248
2500	1000	1.0	159
3000	1000	1.0	111
3500	1000	1.0	81.5
4000	1000	1.0	62.4
10000	1000	1.0	10.0

Table 4: The effects of varying the amount of scaffold protein available ($k = l = 1.0$, $r = s = 1.0$)

As can be seen in Table 4 when the concentration of scaffold is varied we again see the characteristic shape of combinatorial inhibition. Note that now the peak is somewhat lower than in the previous experiment. This is because some of the kinases are binding in cytosol and are not available to enter the scaffold.

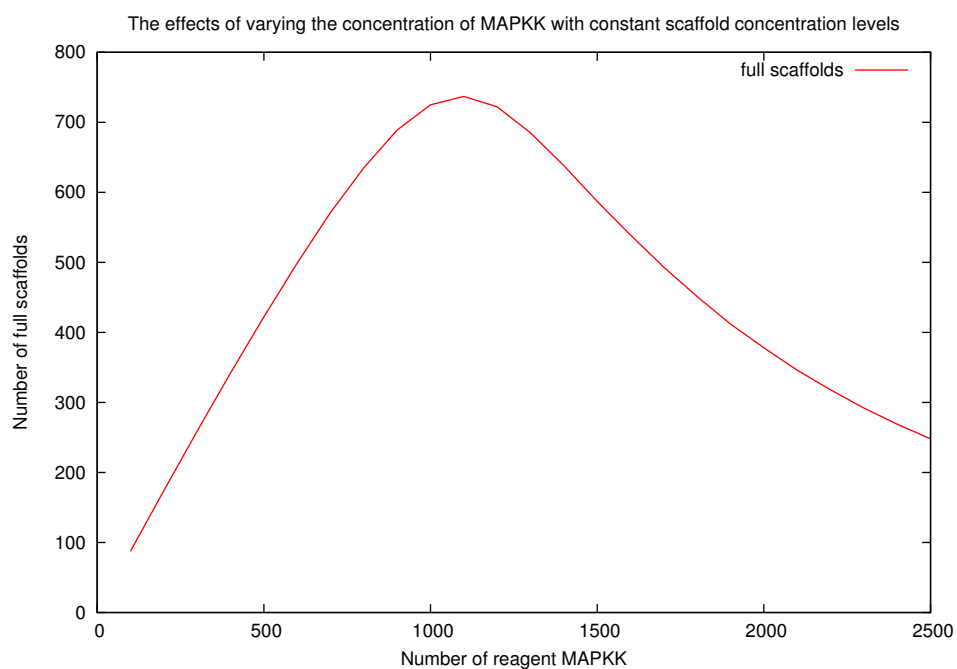
Table 5 shows the results of varying the concentration of one of the kinases, whilst keeping the concentration of scaffold protein and other kinases constant at 1000. As the concentration of *MapKK* increases, we see an increase in the number of filled scaffolds, but then a decrease again. This can be explained as the ability to form a scaffold with a full complement of kinases clearly depends on the availability of the kinases. However, as the concentration of *MapKK* increases, the ability to form complexes in the cytosol also increases (note that *MapKK* plays the central role in such complexes). Thus the number of full scaffolds declines as the amount of *MapKK* continues to increase. Interestingly the peak value for full scaffolds occurs when there is slightly more of *MapKK* than the other reagents (1200).

5 Conclusions and further work

So far our experiments have only been preliminary and to some extent they replicate those already reported in the literature[3, 4]. We have focussed on the formation of the scaffold complex. In future work we plan to look beyond the scaffold complex to its role within the signalling pathway, and investigate the implications of the ability of the scaffold to increase the throughput of the pathway.

References

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<i>No. of scaffolds, MAPK and MAPKKK</i>	<i>No. of reagent MAPKK</i>	<i>Binding and Release rate</i>	No. of full scaffolds
1000	100	1.0	87.7
1000	200	1.0	174
1000	300	1.0	259
1000	400	1.0	342
1000	500	1.0	422
1000	600	1.0	499
1000	700	1.0	571
1000	800	1.0	635
1000	900	1.0	689
1000	1000	1.0	725
1000	1100	1.0	737
1000	1200	1.0	722
1000	1300	1.0	685
1000	1400	1.0	638
1000	1500	1.0	587
1000	1600	1.0	539
1000	1700	1.0	493
1000	1800	1.0	451
1000	1900	1.0	412
1000	2000	1.0	378
1000	2100	1.0	346
1000	2200	1.0	318
1000	2300	1.0	292
1000	2400	1.0	269
1000	2500	1.0	248

Table 5: The effects of varying the concentration of MAPKK with constant scaffold concentration levels [Scaffold] = 1000