

What do scaffold proteins really do?

Jane Hillston.
LFCS, University of Edinburgh

30th June 2006

Joint work with Muffy Calder, University of Glasgow

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Scaffold proteins

Simple model

Markovian analysis
ODE analysis

Enhanced model

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Motivation

BioSPA: do we need it?

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And if we do, what should it look like?

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In seeking to answer these questions we have been undertaking a number of modelling studies of signal transduction pathways with PEPA to assess what features a new modelling language might need.

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Scaffold proteins seem to be important in signal transduction pathways but have largely been ignored by the systems biology community at the moment

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Scaffold proteins seem to be important in signal transduction pathways but have largely been ignored by the systems biology community at the moment

Thus we sought to develop some generic models of scaffold proteins partly for their own intrinsic interest but also as another type of component to test the expressiveness of PEPA.

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The story so far....

- ▶ Currently we have only considered **two simple models**.

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The story so far....

- ▶ Currently we have only considered two simple models.
- ▶ Both are developed in the **pathway-centric** rather than the reagent-centric style.

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The story so far....

- ▶ Currently we have only considered two simple models.
- ▶ Both are developed in the pathway-centric rather than the reagent-centric style.
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- ▶ Currently we have only considered two simple models.
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- ▶ There is an issue about the kinetics of the models (capturing **mass action** kinetics).

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- ▶ Interesting to note that a **mix of kinetics** is required within the model.

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- ▶ We have applied both Markovian and ODE analysis.
- ▶ There is an issue about the kinetics of the models (capturing mass action kinetics).
- ▶ Interesting to note that a mix of kinetics is required within the model.
- ▶ Otherwise the modelling features offered by PEPA seem adequate.

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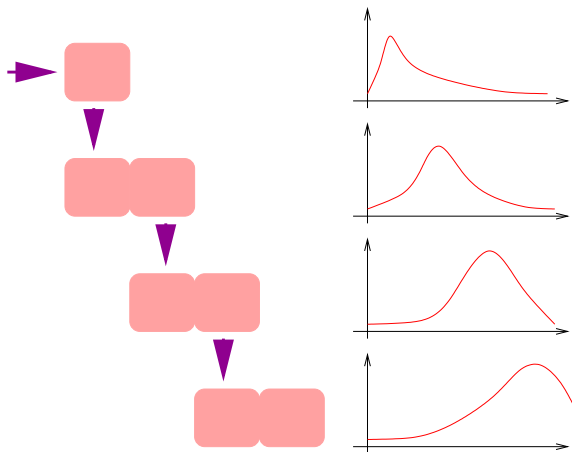
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Signal transduction pathways

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A series of biochemical reactions serve to pass a message from the cell membrane to the nucleus.

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Scaffold proteins: what are they?

- ▶ Wet lab experiments seek to find all the proteins which constitute different signalling pathways.

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Scaffold proteins: what are they?

- ▶ Wet lab experiments seek to find all the proteins which constitute different signalling pathways.
- ▶ Some proteins seem to be involved in a **organisational** rather than signalling role.

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Scaffold proteins: what are they?

- ▶ Wet lab experiments seek to find all the proteins which constitute different signalling pathways.
- ▶ Some proteins seem to be involved in a organisational rather than signalling role.
- ▶ These are termed **scaffold proteins**.

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- ▶ Wet lab experiments seek to find all the proteins which constitute different signalling pathways.
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- ▶ These are termed scaffold proteins.
- ▶ They seem to serve as **additional infrastructure** for the pathway, rather than carrying the message themselves.

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- ▶ Some proteins seem to be involved in a organisational rather than signalling role.
- ▶ These are termed scaffold proteins.
- ▶ They seem to serve as additional infrastructure for the pathway, rather than carrying the message themselves.
- ▶ They have been found to **form complexes** with reagents which themselves constitute consecutive steps in the pathway.

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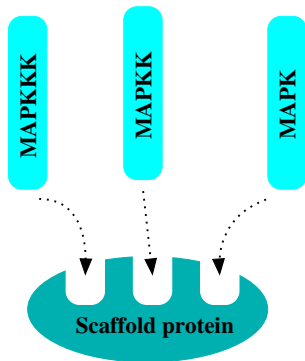
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Schematic view of a scaffold protein



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Scaffold proteins: what is their impact?

Ferrell's paper [Ferrell00] proposes three implications of scaffold proteins:

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Scaffold proteins: what is their impact?

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1. too little scaffold leads to low signalling;

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

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Previous studies [Bray & Lay, PNAS 97], [Levchenko et al, PNAS 00] investigated how the relative concentrations, and binding and release rates, affect the formation of protein/scaffold complexes.

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Previous studies [Bray & Lay, PNAS 97], [Levchenko et al, PNAS 00] investigated how the relative concentrations, and binding and release rates, affect the formation of protein/scaffold complexes.

We seek to recreate those results in the first instance to validate our approach.

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Scaffold component

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$$\begin{aligned} \text{Scaffold}_{000} &\stackrel{\text{def}}{=} (\text{mapk}_{in}, k). \text{Scaffold}_{100} + (\text{mapkk}_{in}, k). \text{Scaffold}_{010} \\ &+ (\text{mapkkk}_{in}, k). \text{Scaffold}_{001} \end{aligned}$$

$$\begin{aligned} \text{Scaffold}_{100} &\stackrel{\text{def}}{=} (\text{mapk}_{out}, l). \text{Scaffold}_{000} + (\text{mapkk}_{in}, k). \text{Scaffold}_{110} \\ &+ (\text{mapkkk}_{in}, k). \text{Scaffold}_{101} \end{aligned}$$

$$\begin{aligned} \text{Scaffold}_{010} &\stackrel{\text{def}}{=} (\text{mapk}_{in}, k). \text{Scaffold}_{110} + (\text{mapkk}_{out}, l). \text{Scaffold}_{000} \\ &+ (\text{mapkkk}_{in}, k). \text{Scaffold}_{011} \end{aligned}$$

$$\begin{aligned} \text{Scaffold}_{001} &\stackrel{\text{def}}{=} (\text{mapk}_{in}, k). \text{Scaffold}_{101} + (\text{mapkk}_{in}, k). \text{Scaffold}_{011} \\ &+ (\text{mapkkk}_{out}, l). \text{Scaffold}_{000} \end{aligned}$$

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$$\text{MapK}_{\text{free}} \stackrel{\text{def}}{=} (\text{mapk}_{\text{in}}, \infty). \text{MapK}_{\text{bound}}$$

$$\text{MapK}_{\text{bound}} \stackrel{\text{def}}{=} (\text{mapk}_{\text{out}}, \infty). \text{MapK}_{\text{free}}$$

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$$\text{Scaffold}_{000} \boxtimes_{\mathcal{K}} (\text{MapK}_{\text{free}} \parallel \text{MapKK}_{\text{free}} \parallel \text{MapKKK}_{\text{free}})$$

where

$$\mathcal{K} = \{\text{mapk}_{\text{in}}, \text{mapk}_{\text{out}}, \text{mapkk}_{\text{in}}, \text{mapkk}_{\text{out}}, \text{mapkkk}_{\text{in}}, \text{mapkkk}_{\text{out}}\}$$



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Markovian analysis

We first considered a Markovian analysis — explicitly building the **discrete state space** and solving it for the **steady state** probability distribution.

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The size of system considered was necessarily limited but we considered to distinct cases:

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1. Explicit numbers of instances of scaffold and kinase proteins;

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2. An explicit number of instances of the scaffold protein and unlimited numbers of the kinases.

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1. Explicit numbers of instances of scaffold and kinase proteins;
2. An explicit number of instances of the scaffold protein and unlimited numbers of the kinases.

In each case we considered the influence of the ratio between the binding rate and the release rate.

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Three instances of the each kinase and scaffold

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where

$$L = \{ \text{mapk}_{in}, \text{mapk}_{out}, \text{mapkk}_{in}, \text{mapkk}_{out}, \text{mapkkk}_{in}, \text{mapkkk}_{out} \}$$

Unlimited amounts of kinase

What do scaffold proteins really do?

Jane Hillston. LFCS,
University of Edinburgh.

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

$$\text{MapK} \stackrel{\text{def}}{=} (\text{mapk}_{in}, m_1). \text{MapK} + (\text{mapk}_{out}, m_2). \text{MapK}$$

$$\text{MapKK} \stackrel{\text{def}}{=} (\text{mapkk}_{in}, m_1). \text{MapKK} + (\text{mapkk}_{out}, m_2). \text{MapKK}$$

$$\text{MapKKK} \stackrel{\text{def}}{=} (\text{mapkkk}_{in}, m_1). \text{MapKKK} + (\text{mapkkk}_{out}, m_2). \text{MapKKK}$$

and the model configuration becomes:

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

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Experiment set 1: binding rate == release rate

What do scaffold proteins really do?

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When the rates are equivalent, we consider the steady state probability of being in a state with at least a certain percentage of scaffolds full.

<i>scaffold:kinase</i>	<i>%100 full</i>	<i>%66 full</i>	<i>%33 full</i>	<i>throughput</i>
3:3	.0019	.125	.015	0.8
3:2	0	.04	10^{-4}	0.24
3:unlimited	.015	.037	.125	0.64

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Experiment set 2: binding rate $>$ release rate

What do scaffold proteins really do?

Jane Hillston. LFCS,
University of Edinburgh.

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>scaffold:kinase</i>	<i>%100 full</i>	<i>%66 full</i>	<i>%33 full</i>	<i>throughput</i>
3:3	.42	.75	.0075	5.78
3:2	0	.22	.02	1.39
3:unlimited	.052	.125	.32	1.87

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Markovian conclusions

- ▶ These models are necessarily small because we represent the state space explicitly.
- ▶ However, the current models do not reach the limit of what can be analysed using the Markovian approach.
- ▶ Nevertheless, even these simple models show some of the trends which we are looking for.
- ▶ Experiments with larger populations are more easily conducted in the continuous state space setting

What do scaffold proteins really do?

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Using the continuous state space semantics

- ▶ Previous work deriving ODE from signal transduction models had been based on the **reagent-centric** modelling style.
- ▶ Here we are using something closer to the pathway-centric style.
- ▶ However we were able to use the Dizzy translator developed by Stephen for computer modelling case studies.
- ▶ This uses the bounded capacity kinetics by default (min) so some manipulation of the resulting .dizzy file was needed to get the kinetics correct for all actions.

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Kinetic issues

- ▶ The binding of kinase to scaffold needed mass action kinetics and so the Dizzy file was edited to reflect this.
- ▶ However, for the release of a kinase from the scaffold, the *min*-kinetics was correct.
- ▶ Although they are represented as separate entities in the PEPA model, the filled scaffold and the bound kinase are really a single entity.

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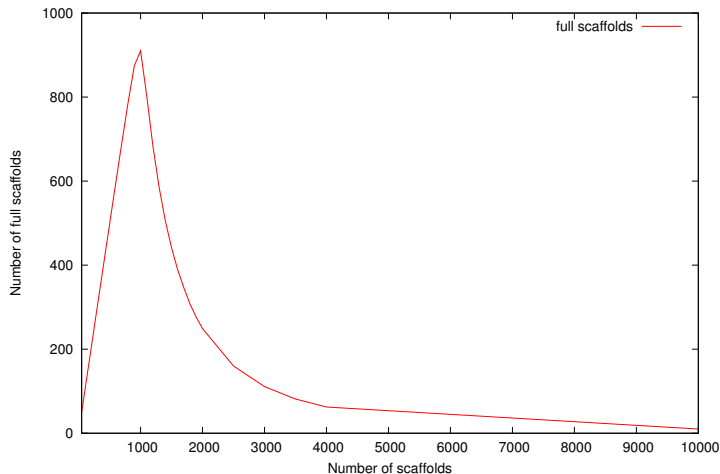


Varying the scaffold concentration

What do scaffold proteins really do?

Jane Hillston. LFCS,
University of Edinburgh.

The effects of varying the amount of scaffold protein available



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Varying the scaffold concentration

What do scaffold proteins really do?

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The graph shows the **combinatorial inhibition** effect outlined by Ferrell.

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.

The peak is reached when the number of scaffolds and kinases is balanced.

Recall that signalling is deemed to occur in filled scaffolds so we would expect a similar pattern for the signalling in the cell.

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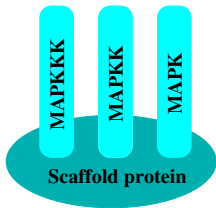
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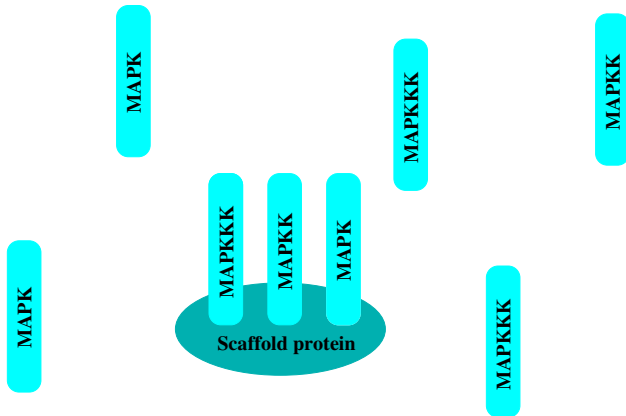
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Balanced amounts results in full scaffolds: **high signalling**

Combinatorial inhibition



Too little scaffold leaves kinases unbound: **low signalling**

What do scaffold proteins really do?

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University of Edinburgh.

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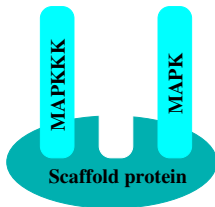
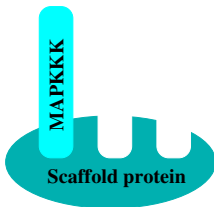
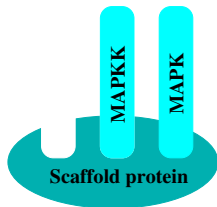
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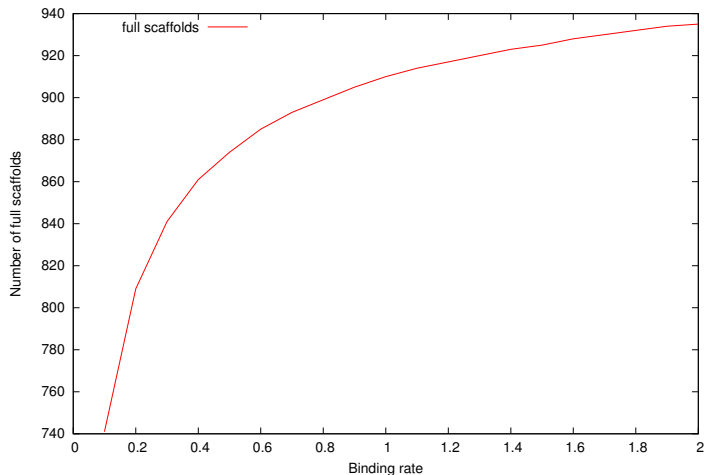
Too much scaffold leads to unfilled scaffolds: **low signalling**

Varying binding rate : release rate ratio

What do scaffold proteins really do?

Jane Hillston. LFCS,
University of Edinburgh.

The effects of varying the ratio of the binding rate to the release rate with constant concentration



Scaffolds are more likely to fill if the binding rate is greater than the release rate.

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Modified kinases to include binding in cytosol

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$$\begin{aligned} \text{MapK}_{\text{free}} &\stackrel{\text{def}}{=} (\text{mapk}_{\text{in}}, \infty). \text{MapK}_{\text{sbound}} \\ &+ (\text{mapk}_{\text{bind}}, k). \text{MapK}_{\text{bound}} \\ \text{MapK}_{\text{sbound}} &\stackrel{\text{def}}{=} (\text{mapk}_{\text{out}}, \infty). \text{MapK}_{\text{free}} \\ \text{MapK}_{\text{bound}} &\stackrel{\text{def}}{=} (\text{mapk}_{\text{unbind}}, l). \text{MapK}_{\text{free}} \\ \text{MapKK}_{\text{free}} &\stackrel{\text{def}}{=} (\text{mapkk}_{\text{in}}, \infty). \text{MapKK}_{\text{sbound}} \\ &+ (\text{mapkk}_{\text{bind}}, \infty). \text{MapKK}_{\text{kbound}} \\ &+ (\text{mapkk}_{\text{bind}}, k). \text{MapKK}_{\text{bound}} \\ \text{MapKK}_{\text{kbound}} &\stackrel{\text{def}}{=} (\text{mapk}_{\text{unbind}}, \infty). \text{MapKK}_{\text{free}} \\ \text{MapKK}_{\text{bound}} &\stackrel{\text{def}}{=} (\text{mapkk}_{\text{unbind}}, l). \text{MapKK}_{\text{free}} \\ \text{MapKK}_{\text{sbound}} &\stackrel{\text{def}}{=} (\text{mapkk}_{\text{out}}, \infty). \text{MapKK}_{\text{free}} \\ \text{MapKKK}_{\text{free}} &\stackrel{\text{def}}{=} (\text{mapkkk}_{\text{in}}, \infty). \text{MapKKK}_{\text{bound}} \\ &+ (\text{mapkk}_{\text{bind}}, \infty). \text{MapKKK}_{\text{kbound}} \\ \text{MapKKK}_{\text{bound}} &\stackrel{\text{def}}{=} (\text{mapkkk}_{\text{out}}, \infty). \text{MapKKK}_{\text{free}} \\ \text{MapKKK}_{\text{kbound}} &\stackrel{\text{def}}{=} (\text{mapkk}_{\text{unbind}}, \infty). \text{MapKKK}_{\text{free}} \end{aligned}$$



Modified kinases to include binding in cytosol

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$$\text{MapK}_{\text{sbound}} \stackrel{\text{def}}{=} (\text{mapk}_{\text{out}}, \infty). \text{MapK}_{\text{free}}$$

$$\text{MapK}_{\text{bound}} \stackrel{\text{def}}{=} (\text{mapk}_{\text{unbind}}, l). \text{MapK}_{\text{free}}$$

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$$\text{MapKK}_{\text{kbound}} \stackrel{\text{def}}{=} (\text{mapk}_{\text{unbind}}, \infty). \text{MapKK}_{\text{free}}$$

$$\text{MapKK}_{\text{bound}} \stackrel{\text{def}}{=} (\text{mapkk}_{\text{unbind}}, l). \text{MapKK}_{\text{free}}$$

$$\text{MapKK}_{\text{sbound}} \stackrel{\text{def}}{=} (\text{mapkk}_{\text{out}}, \infty). \text{MapKK}_{\text{free}}$$

$$\begin{aligned} \text{MapKKK}_{\text{free}} &\stackrel{\text{def}}{=} (\text{mapkkk}_{\text{in}}, \infty). \text{MapKKK}_{\text{bound}} \\ &+ (\text{mapkk}_{\text{bind}}, \infty). \text{MapKKK}_{\text{kbound}} \end{aligned}$$

$$\text{MapKKK}_{\text{bound}} \stackrel{\text{def}}{=} (\text{mapkkk}_{\text{out}}, \infty). \text{MapKKK}_{\text{free}}$$

$$\text{MapKKK}_{\text{kbound}} \stackrel{\text{def}}{=} (\text{mapkk}_{\text{unbind}}, \infty). \text{MapKKK}_{\text{free}}$$

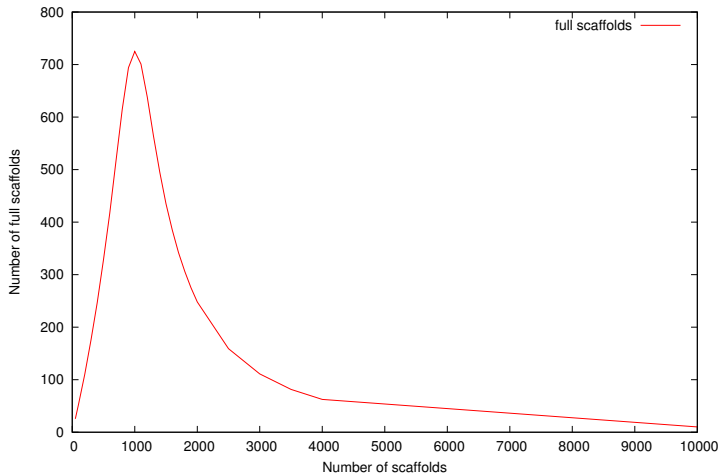


Varying scaffold protein concentration

What do scaffold proteins really do?

Jane Hillston. LFCS,
University of Edinburgh.

The effects of varying the amount of scaffold protein available



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Varying scaffold protein concentration

What do scaffold proteins really do?

Jane Hillston. LFCS,
University of Edinburgh.

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.

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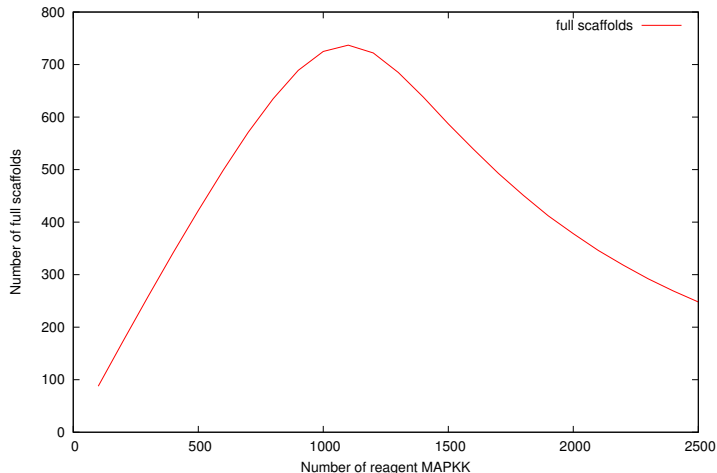


Varying MAPKK kinase concentration

What do scaffold proteins really do?

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University of Edinburgh.

The effects of varying the concentration of MAPKK with constant scaffold concentration levels



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Varying MAPKK kinase concentration

As the concentration of *MapKK* increases, we see an increase in the number of filled scaffolds, but then a decrease again.

Clearly 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. 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).

What do scaffold proteins really do?

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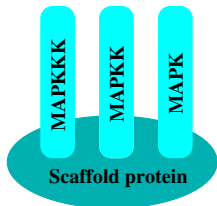
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Combinatorial inhibition — again

What do scaffold proteins really do?

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Balanced amounts results in full scaffolds: **high signalling**

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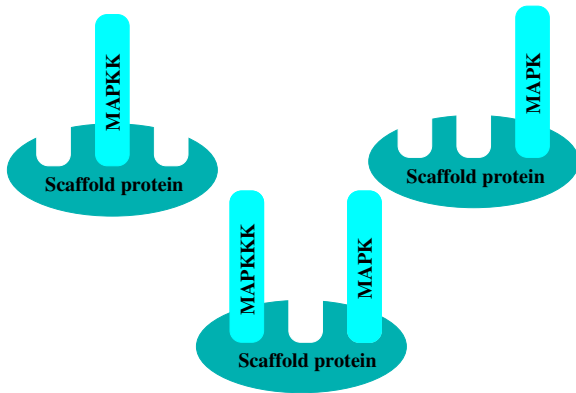
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Too little kinase leads to unfilled scaffolds: **low signalling**

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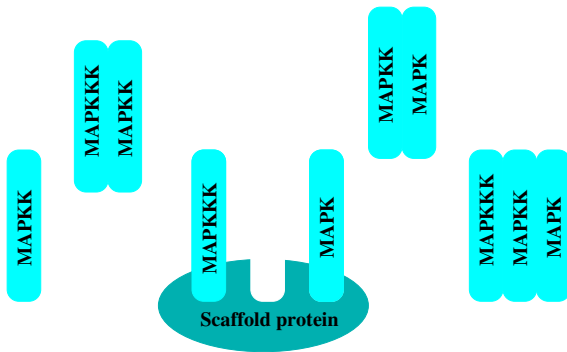
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Combinatorial inhibition — again



Too much kinase binds in the cytosol: **low signalling**

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Ongoing work

- ▶ There are clearly more experiments to do with the enhanced model.
 - ▶ We have yet to consider a Markovian analysis of this model;
 - ▶ More work is needed to here to devise ways to elicit the interesting informaton from the Markovian analysis;
 - ▶ In the continuous state space setting there is more to investigate about the interplay between the different rates associated with the different bindings and unbindings in the system.
- ▶ We have not built a reagent-centric model of this system but it could be interesting to do so.
- ▶ We would also like to conduct some model checking on a model of the system.

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Conclusions

- ▶ Initial indications are that the PEPA model is able to reproduce the phenomena uncovered by earlier, much more complicated models.
- ▶ The current models make clear the case for the use of both the mass action kinetics and the bounded capacity kinetics in any future language intended for signal transduction modelling.

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