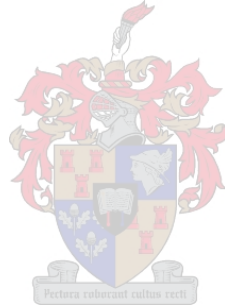


Comparison of progress curve analysis and  
initial rate kinetics for the estimation of  
identifiable enzyme kinetic parameters: a case  
study using two glycolytic enzymes from  
*Sulfolobus solfataricus*.

by

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of Science at Stellenbosch University*

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# Declaration

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# List of Abbreviations / Nomenclature

1,3-BPG	1,3-Bisphosphoglycerate
3-PG	3-Phosphoglycerate
DHAP	Dihydroxyacetone phosphate
DTT	Dithiothreitol
ED	Entner-Doudoroff pathway
EMP	Embden-Meyerhof-Parnas pathway
ENO	Enolase
F6P	Fructose 6-phosphate
FBPA	Fructose 1,6-bisphosphate aldolase
PBPase	Fructose 1,6-bisphosphate phosphatase
G6P	Glucose 6-phosphate
G6PDH	Glucose 6-phosphate dehydrogenase
GAP	Glyceraldehyde 3-phosphate
GAPN	Non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase
GAPOR	Ferredoxin-dependent glyceraldehyde 3-phosphate oxidoreductase
G1P	Glucose 1-phosphate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
IPTG	Isopropyl thio- $\beta$ -D-galactopyranoside
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	reduced Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear Magnetic Resonance spectroscopy
PGAM	Phosphoglycerate mutase
PGI	Phosphoglucose isomerase
PGK	Phosphoglycerate kinase
PK	Pyruvate kinase
TEP	Triethyl phosphate
TPI	Triosephosphate isomerase
TRIS	Tris(hydroxymethyl)aminomethane



# Summary

Obtaining accurate parameter value estimations is imperative to studying enzyme kinetics. It is also important that these parameter values be identifiable. The non-identifiability of parameter estimations is usually indicative of parameter correlation, but may also be caused by other factors. These factors include the quality and quantity of the data used for parameter characterisation, the method of data analysis used as well as the model or rate equation to which the data is fitted. The non-identifiability of parameter estimations can either be structural or practical non-identifiability. Structural non-identifiability is caused by the model structure and is an indication of parameter correlations while practical non-identifiability is an indication of qualitatively or quantitatively insufficient data. The choice in kinetic assay and the type of data that will be collected is often dictated by the ligands, the enzyme itself or the availability of equipment. However, in the absence of these limitations, either progress curve or initial rate analysis may be selected. There are advantages and limitations to using either of these methods. The aim of this study is to try and determine if either of these two methods returns a greater number of identifiable parameter estimations for specific enzyme kinetic attributes.

Experimental progress curve and initial rate data are collected for two different enzymes, GAPN and PGI, of *Sulfolobus solfataricus*. The GAPN and PGI enzymes represent different enzyme kinetic characteristics. Parameter value estimations are then obtained from the data fitting and an identifiability analysis for all possible combinations of the parameters are completed. When considering all the combinations of parameter fits it is possible to see trends in the combinations that return identifiable or non-identifiable parameters. With the identifiability analysis approach used in this study it is possible to determine if the non-identifiability of a parameter is structural or practical. Thereafter it is possible to speculate if the non-identifiability of the parameter estimations are due to parameter correlation or other factors and which method is superior for the analysis of certain enzyme kinetic attributes.

# Opsomming

Die verkryging van akkurate parameterwaarde skattings is noodsaaklik vir die studie van ensiem kinetika. Dit is ook belangrik dat hierdie parameterwaardes identifiseerbaar is. Die nie-identifiseerbaarheid van parameterwaardes is gewoonlik 'n aanduiding van parameter korrelasie, maar kan ook veroorsaak word deur ander faktore. Hierdie faktore sluit in die kwaliteit en kwantiteit van die data wat gebruik is vir parameter karakterisering, die data analise metode wat gebruik is asook die model of vergelyking waarop die datapassing gedoen is. Die nie-identifiseerbaarheid van parameterwaardes kan of strukturele of praktiese nie-identifiseerbaarheid wees. Strukturele nie-identifiseerbaarheid word veroorsaak deur die modelstruktuur en is 'n aanduiding van parameter korrelasie terwyl praktiese nie-identifiseerbaarheid 'n aanduiding is van kwalitatiewe of kwantitatiewe onvoldoende data. Die keuse in kinetiese toets en die tipe data wat ingesamel sal word, word dikwels bepaal deur die ligande, die ensiem self of die beskikbaarheid van toerusting. Egter, in die afwesigheid van hierdie beperkinge, is dit moontlik om van of vorderingskurwe of inisiële snelheid analise gebruik te maak. Daar is voordele en beperkings met die gebruik van enige een van hierdie twee metodes. Die doel van hierdie studie is om te probeer bepaal of enige van hierdie twee metodes meer identifiseerbare parameterwaardes lewer vir spesifieke ensiem kinetika eienskappe.

Eksperimentele vorderingskurwe en inisiële snelheid data vir twee verskillende ensieme, GAPN en PGI, van *Sulfolobus solfataricus* is verkry. Die GAPN en PGI ensieme verteenwoordig verskillende ensiem kinetika eienskappe. Parameterwaardes is verkry deur middel van die datapassing en 'n identifiseerbaarheids-analise vir alle moontlike kombinasies van die parameters is daarna voltooi. Met die inagneming van alle kombinasies van parameter passings is dit moontlik om tendense te sien in die kombinasies wat identifiseerbare en nie-identifiseerbare parameters lewer. Die identifiseerbaarheids-analise benadering wat in hierdie studie gebruik is maak dit moontlik om te bepaal of die nie-identifiseerbaarheid van 'n parameter struktureel of prakties is. Dit is dan moontlik om te spekuleer of die nie-identifiseerbaarheid van die parameterwaardes is as gevolg van parameter korrelasie of ander faktore en watter metode beter is vir die analise van spesifieke ensiem kinetika eienskappe.

# Chapter 1

## Introduction

Accurate parameter estimation and determination of parameter identifiability are imperative to any enzyme kinetic study. The methods used to determine parameter values are crucial to this process [52]. There are two major experimental methods used for acquiring data for parameter value estimation, namely initial rate estimation of steady state kinetics and progress curve analysis. Initial rate estimation has been the preferred method for decades [21]. The negligible inhibitory effect of product formation on initial enzyme kinetics and simple experimental procedures are advantageous features of initial rate analysis [12, 32]. This traditional method allows for simple data analysis which was crucial prior to the development of strong computational tools [32]. The main advantage of the initial rate approach is that specific perturbations can be made for each parameter in determining the enzyme kinetics [12]. The surge in technological development in recent decades has facilitated the analysis of large quantities of complex data [12, 13, 21]. Thus progress curve analysis has become a feasible alternative to initial rate analysis for the task of parameter estimation [13]. More information is obtained from progress curve data than initial rate data per enzyme kinetic assay. It is expected that progress curve analysis will require fewer full time courses for the estimation of the same set of parameters, while initial rate analysis will require the use of multiple partial enzyme assays. These partial assays only include the initial data points and possibly useful data collected beyond this point are intentionally discarded [12, 39].

When fitting experimental data, a problem that one is often faced with is the non-identifiability of parameter values [52]. As models are becoming increasingly more complex, completing identifiability analyses on parameters have become a pivotal part of parameter characterisation [61]. Depending on the type of data, and which method of data analysis is used, parameter values can be difficult to identify when fitting for multiple parameter values [21, 52]. It is useful to distinguish between structural and practical non-identifiable parameter value estimations to improve experimental design and model reduction. Structurally non-identifiable parameters occur when the model dynamics cannot be constrained by the measurable experimental data, regardless of the quantity or quality of the data. Practically non-identifiable parameters occur when too few datasets,

or datasets of poor quality are used for parameter estimations [52]. Methods for investigating the identifiability of parameters include the Power Series Expansion method, the Exhaustive Modelling method and the differential algebraic method. The identifiability analysis of these methods are based purely on the priori analysis of the kinetic model [40, 51, 63, 65]. Other methods that include the effect that the data might have on the identifiability of parameter estimations make use of the Hessian and the Covariance matrices [19]. Another method which also includes the effect of data on the identifiability of parameters is the profile likelihood method. This empirical method utilises the profile likelihood of mathematical models to provide a visual representation of parameter non-identifiability [52]. With this method one is also able to determine both practical or structural non-identifiability of a model [52].

As the method of parameter value estimation can influence the identifiability of the parameter value [52], choosing between either initial rate or progress curve analysis is by no means unimportant. The aim of this study is to analyse progress curve and initial rate data, and to investigate differences in identifiability of the parameter value estimations. Furthermore, we speculate as to whether it is possible to identify generic enzyme kinetic attributes for which either initial rate or progress curve analysis is the superior method.

Two glycolytic enzymes of the thermophilic Archaea bacteria *S. solfataricus* are studied which describe different types of enzyme kinetics. The first is the irreversible, bi-bi enzyme kinetics of the non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase (GAPN) enzyme, with allosteric activation and substrate inhibition, and the second is the reversible, uni-uni enzyme kinetics of the phosphoglucose isomerase (PGI) enzyme.

- The first objective of this study is to complete experimental enzyme kinetic assays to collect progress curve and initial rate data for both GAPN and PGI.
- The second objective is to complete the fitting of the progress curve and initial rate data to characterise the kinetic parameters for both enzymes.
- The third objective is to conduct a comparative identifiability analysis of the parameter estimations of the initial rate and progress curve data.

The outline of this thesis is as follows: Chapter 2 starts with an introduction to the two methods of data analysis. Thereafter different data fitting algorithms are discussed as well as the methods used for identifiability analysis. Chapter 3 includes a more in depth description of the two enzymes chosen for this study and covers the experimental methods for collecting progress curve and initial rate data for both enzymes. In Chapter 4 the results of the identifiability analysis of the GAPN and PGI parameter estimations are shown. Chapter 5 includes a general discussion of the results while Chapter 6 includes the conclusions drawn from this study.

## Chapter 2

# Overview of analytical methods

### 2.1 Initial rate estimation vs. Progress curve analysis

In 1902 Henri postulated that the rate of an enzymatic reaction is proportional to the concentration of the enzyme-substrate complex, but failed to prove his hypothesis experimentally [26, 32]. Later Michaelis and Menten were successful in experimentally proving Henri's original hypothesis and the Michaelis-Menten equation was derived. Although they are known for their initial rate experiments, Michaelis and Menten also completed the first global analysis of progress curve data. With a rigorous, yet no doubt tedious, analysis of several progress curves they were able to derive a value for the constant  $\frac{V_{max}}{K_m}$  of the invertase enzyme by hand. Furthermore they were also the firsts to study the effects of competitive product inhibition on enzyme kinetics. Their product inhibition study included the derivation of competitive product inhibition equations and the characterisation of dissociation constants for each of the products [32, 47]. Henri, and Michaelis and Menten completed much of the groundwork for the enzyme kinetic analysis that we do today.

For years initial rate analysis has been the most used method for parameter estimation [32]. Without the help of computational tools, the use of linear transformations of data and linear regression was initially more feasible. For instance the parameter values for the maximal rate ( $V_{max}$ ) and half-saturation constant ( $K_m$ ) can easily be determined with Lineweaver-Burk plots, with the reciprocal plotting of enzyme kinetic rate against substrate concentration [12, 21, 32]. With this method many of the primary kinetic values can be estimated, even for rate kinetics where an allosteric modifier is present [32].

It is relatively easy to obtain many different perturbations by running multiple assays with varying concentrations of all the applicable ligands [29]. This process can be laborious and time consuming, depending on the choice of assay equipment and the complexity of the enzyme. For example, the use of a 96-well microplate reader as opposed to a spectrophotometer, reading a single 1 mL cuvette, will be significantly faster. Also,

## 2.1. Initial rate estimation vs. Progress curve analysis

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studying the kinetics of a system with less substrates, products, and effectors will require less perturbations than that of a more complicated system with numerous substrates, products, and effectors.

The use of initial rate estimations is advantageous for several reasons; the substrate concentration is identical to that of the initial substrate concentration during the course of an assay, the amount of product formed has negligible inhibitory effects on the enzyme, and there is no risk of the enzyme to have been partially inactivated [12]. Initial rate estimation is simple, both in terms of experimental procedure and data analysis, making it the preferred method in some cases [12, 32].

The use of progress curve analysis as parameter estimation method, which entails the fitting of data to an integrated rate equation, has increased in recent years [13, 21]. Parameter estimation from time course data was challenging in the past due to lack of computational resources. It has only recently become possible to successfully analyse large quantities of progress curve data [12, 13, 21]. The main setback was the need to integrate often complex differential rate equations as one of the steps of progress curve data fitting [13, 49]. In 1988 the program AGIRE, a nonlinear regression program that converts a differential equation into its integrated form, and fits progress curve data to the integrated rate equation was created [13]. The theoretical framework upon which the development of this program is based, was done by Boeker [6, 7]. This program was a major advance in the use of progress curve data as it was user-friendly, eliminating the tedious step of deriving an integrated rate equation. Although this program was limited to reversible or physiologically irreversible uni-uni, bi-uni, uni-bi or bi-bi enzyme kinetics, it simplified the use of progress curve data analysis and made it more accessible [13].

During progress curve analysis the entire time course is used as opposed to only the first few data points used for initial rate estimation [12, 49]. Each progress curve assay renders far more data than an initial rate assay, allowing for the enzyme kinetics to be characterised from fewer experimental assays [12, 16, 49].

The advantages of progress curve analysis also include the fact that the progress curve data describes the enzyme's dependence on both substrate and product concentrations as the enzyme reaction runs to equilibrium [12, 21]. The type of data analysis that will be used influences the experimental design [32]. In the absence of allosteric product inhibition, a single assay run to completion, providing a full progress curve, may be just as sufficient for parameter estimation as an array of initial rate assays with varying substrate concentrations of which only the initial section of data is used [32]. Nonetheless, progress curves are often not sufficient for parameter identifiability due to correlations between parameters [32, 52].

There are also several other disadvantages to using progress curve data. It is often difficult to study single parameters or specific subsets of the enzyme kinetics as progress curve data describe the complete enzymatic rate equation [12]. As simple as it is to

parameterise the rate kinetics of a certain enzyme in the absence of allosteric product inhibition with progress curve analysis, so difficult can it be to do so in the presence thereof. More time courses may be needed to characterise parameter values when any product has an allosteric inhibitory effect on the enzyme [12].

The efficiency of either progress curve or initial rate analysis is also defined by the experimental design. In a comparative analysis of the initial rate and progress curve data of the triosephosphate isomerase (TPI) enzyme of *Saccharomyces cerevisiae*, it was found that the progress curve data analysis returned less accurate parameter estimations than the initial rate data analysis. The progress curve data were obtained by allowing the initial rate assays to run longer. However, the net forward reactions were not sufficiently inhibited by the reverse reactions. As such the progress curves, containing data of the TPI kinetics in the forward direction, did not contain enough information about the kinetics in the reverse direction and *vice versa*. With the experimental design being completely biased towards the initial rate kinetics, this method of data analysis returned the most accurate parameter values estimations [46]. When comparing the efficiency of the two methods, the experimental design should not be of such a nature that either of the two methods are favoured.

## 2.2 Data fitting

The linear regression of initial rate data, such as Lineweaver-Burk plots, was originally used for parameter value estimation and is still used today [12, 32]. There are two key disadvantages to using linear regression; experimental errors are not weighted equally [12, 32] and linear transformation of the rate equations of complex enzyme kinetics may not be possible [12]. These issues can be avoided by using nonlinear regression [12]. There are two types of methods to consider when using nonlinear regression; search and gradient methods. In search methods the sum of the squared residuals is calculated as the parameter values are varied randomly within a feasible range of solutions until a local minimum is reached [12, 54]. These methods include the pure random search method, several adaptive search methods and the pure localisation search method. The pure random search method generates a list of evenly distributed points within the feasible range of solutions and returns the optimal solution among the points [50, 54]. The adaptive search methods are similar to the pure random search method, however the methods are forced to improve the fit with each iteration over the points in the feasible solution [50, 67]. The genetic algorithm is an example of such an algorithm, the search technique is improved after each iteration thereby mimicking the effect of natural selection [20]. The pure localisation method adapts the feasible solution range according to the results of the previous iteration [50, 54]. The random search methods are robust in the sense that they are used in cases where other optimisation methods, that use specific mathematical



structures, fail. Therefore these methods are often used as an initial approach to new problems whereafter other, more specialised, optimisation methods are implemented [54].

In gradient methods the slope of the sum of the squared residuals at a certain point is an indication of the change in the parameter value estimations. As such it is used to locate the approximate minimum point. This process is repeated until the best predicted minimum has been identified [12]. The gradient methods include the Gauss-Newton method, the gradient descent method, the Levenberg-Marquardt method and the Nelder-Mead method. The gradient descent or steepest descent method refits for the parameters in the negative direction of the objective function's slope. The step size is proportional to the gradient; the step size is smaller if the slope is steep as not to skip over the minimum and the step size is larger if the slope is flatter as to reach the minimum faster [43]. This method works well with simple objective functions and for cases where many parameters need to be estimated [43]. The gradient descent method is however very slow once it nears the minimum [42]. This Gauss-Newton method is deterministic and may not be able to escape local minima [24]. It is however fast once it nears the minimum, albeit local or global [42, 43], but this is dependent on accurate initial predictions of the parameter values [42]. The Levenberg-Marquardt method is a combination of the gradient descent and Gauss-Newton methods [43]. When iterating over areas of the feasible solution range with a less steep slope, the gradient descent method is used while the Gauss-Newton method is used to iterate over areas with a steeper slope [42, 43]. The Nelder-Mead algorithm works on the basis of reducing the size of a simplex to find the coordinates of a minimum in a solution space. A simplex is an  $N$  dimensional triangle representing the estimates of  $N$  parameters. With each iteration the largest vertex of the simplex is replaced by a smaller vertex until the minimum is reached [44]. The use of gradient methods have been preferred as they are generally faster than search methods and provide additional information regarding the standard errors of the estimated parameter values [12]. These standard errors only take into account the variance of each parameter [12] and does not rigorously account for parameter correlation. The covariance or correlation of the parameters are addressed in the next section.

The standard deviation of experimental data and how the data is weighted is also an important part of the data fitting process [32]. The sum of the squared differences returned by the objective function can be weighted to an overall standard deviation [32] or each squared difference can be weighted individually [12, 32].

## 2.3 Parameter identifiability

Experimental data may not be adequate for the parameterisation of a sizeable model, resulting in non-identifiable parameters [52]. When using mathematical models to describe enzyme kinetic networks, it is necessary to determine if the data used for model



construction correctly characterise the parameter values. The quantity and quality of experimental data can greatly influence the outcome of parameter estimations [21, 52]. The identifiability of parameters depends on both the data and the model selected for fitting. Using a complex model along with data of which the inherent information content does not constrain all parameters will result in non-identifiable parameter estimations [32]. Structurally non-identifiable parameters occur when the model dynamics are too complex to be described by the measurable experimental data, or when there are correlations between parameters. The maximum likelihood of the parameter estimations is not altered by changes in the parameter estimations, thus the confidence intervals of these parameters are infinite. Practical non-identifiable parameters occur when qualitatively or quantitatively inadequate data are used for parameter estimations. These parameters are bounded in both directions, but not sufficiently to achieve a desired degree of confidence [52].

The identifiability of a parameter is defined by the fact that the parameter vector is a unique solution of the system of ordinary differential equations that describe the model [51] and that the confidence intervals of the parameter estimations are finite [52].

There are various methods for the validation of parameter identifiability. One of these methods is the Power Series Expansion method. The identifiability of the parameters of a model is determined by analysing the power series expansion of the ordinary differential equations that describe the model, evaluated at time point zero. This method is based on the assumption that the model derivatives can be presented in the form of a power series expansion. Thus this method cannot be used for the identifiability analysis of more complex systems. As the identifiability analysis is based purely on the model equations and does not take into consideration the quality of experimental data, this method is only sufficient for determining structural non-identifiability [51]. Another method is the Exhaustive Modelling method. For this method the set of ordinary differential equations describing the model kinetics are presented in matrix form. The identifiability analysis of the parameter estimations are conducted by means of similarity transformations of the matrix. This method also does not take experimental data into account and therefore is only sufficient for determining the structural non-identifiability of parameters [63, 65]. The use of differential algebra, especially Ritt's algorithm, can also be used for the identifiability analysis of parameter value estimations. However, these methods also only consider the model structure and not the influence of experimental data on the parameter identifiability [40]. Other methods, that do take the influence of experimental data on identifiability into account, consider the curvature of the likelihood. The use of the Hessian and Covariance matrices is one such a method [19]. For a model, described by a system of ordinary differential equations, let  $\theta$  be the vector of parameters and  $\chi^2(\theta)$  the sum of the squared residuals returned by the optimisation of the objective function. Then  $m$  is the number of data points  $(t_i, y_i)$ ,  $y(t_i)$  is the measured data and  $\hat{y}(t_i, \theta)$  is the

fit.  $w_i$  is the error in the experimental data point  $(t_i, y_i)$ . These experimental errors form the diagonal weighting matrix  $W$  where  $W_{ii} = \frac{1}{w_i^2}$ . The  $\chi^2(\theta)$  values can be expressed as:

$$\chi^2(\theta) = \sum_{i=1}^m \left[ \frac{y(t_i) - \hat{y}(t_i, \theta)}{w_i} \right]^2$$

The Jacobian matrix is a matrix of the first-order partial derivatives of the objective function that is indicative of the objective function's sensitivity to changes in  $\theta$ :

$$J = \frac{d\hat{y}}{d\theta}$$

The Hessian matrix is a matrix of second-order partial derivatives of the objective function:

$$H = J^T J$$

The Covariance matrix is the inverse of the Hessian matrix weighted to the error in the experimental data:

$$V_\theta = [HW]^{-1}$$

The standard errors of the parameters are the diagonal elements of the square root of the Covariance matrix:

$$\sigma_\theta = \sqrt{V_\theta}$$

The confidence intervals of the parameter estimations are then derived from these standard errors [52].

Another method for the validation of parameter identifiability is the profile likelihood approach. This approach entails the exploration of the parameter space in each direction after the initial parameter estimation has been completed. The profile likelihood is calculated for each of the parameters ( $\theta$ ) by re-optimising the sum of the squared residuals scaled to the standard deviation ( $\chi^2$ ), for variations in the parameter estimate ( $\hat{\theta}_i$ ). While fixing the value of  $\hat{\theta}_i$  within a specified solution space, the re-optimised values for  $\chi^2$  will indicate whether the rest of the parameters are able to compensate for the change in  $\hat{\theta}_i$ . If not, the parameter is identifiable, given that the profile likelihood can also be bounded with confidence intervals. Although no assumptions are made in determining the identifiability of the parameter values, the profile likelihoods are created empirically [32, 52].

The profile likelihood can also be used to determine structural and practical non-identifiable parameters as well as the confidence intervals of parameter estimations [52]. The advantages of this is that the type of non-identifiability of parameter estimations can be detected [52], the confidence intervals can be determined, it allows for the visual representation of parameter identifiability, and it can be used for experimental design and model reduction [52]. The identifiability of individual parameters can also be determined,

allowing one to consider only the biologically relevant parameters [52]. This method is used for the analysis of parameter identifiability in this study.

The parameter value estimations can only be considered identifiable if all the parameters fitted for are identifiable. The profile likelihood plots show the change in the  $\chi^2$  value when conducting an identifiability analysis on a parameter. With normally distributed observational noise the minimisation of the  $\chi^2$  yields a maximum likelihood estimate. If a parameter is identifiable, the other parameters fitted for cannot compensate for change in this parameter value resulting in an increase in the  $\chi^2$  value. This creates a curve with the identifiable parameter estimation located at the minimum, see Figure 2.1 (a). Likelihood-based confidence intervals are determined by a threshold in the likelihood [45, 52]. If the curve is steep around the minimum, the confidence intervals are narrower. The smaller the confidence interval the more probable the parameter estimation [52]. We selected a solution space in the range of  $\frac{1}{10}x$  to  $10x$  the estimated parameter value. If the parameter is not identifiable within this range we consider the estimated parameter value to be non-identifiable. We also distinguish between practical and structural non-identifiability by examining the shape of the profile likelihood plots. A flat profile likelihood plot with an infinite confidence interval represents structural non-identifiability, see Figure 2.1 (b). A profile likelihood plot that is curved, with an optimal solution for the parameter estimation located at the minimum, but is not constrained sufficiently by the confidence interval, represents practical non-identifiability [52], see see Figure 2.1 (c).

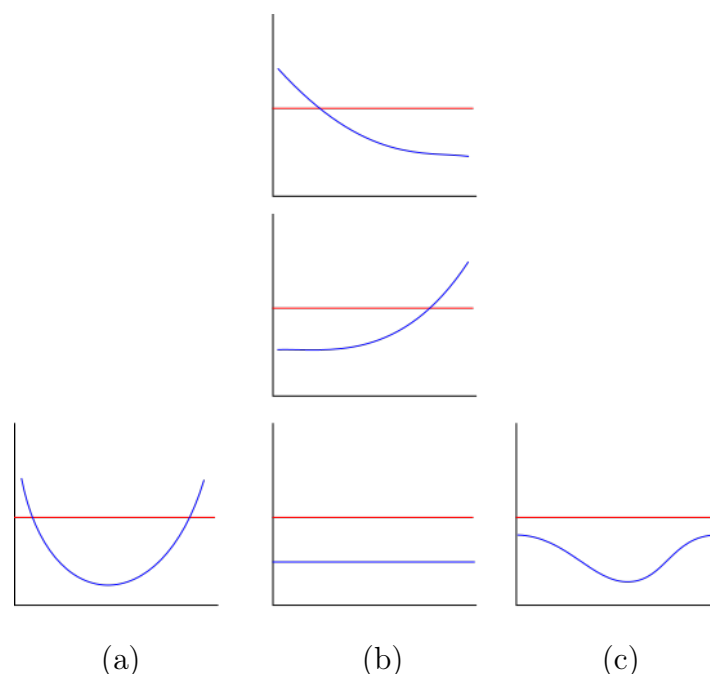


Figure 2.1: Examples of the general shape of the profile likelihood plots for identifiable (a), structurally non-identifiable (b), and practically non-identifiable (c) parameters. The profile likelihood is shown in blue along with the threshold in the likelihood determined by the likelihood-based confidence interval shown in red.

# Chapter 3

## Overview of experimental methods

### 3.1 Selecting enzymes

As mentioned in Chapter 1, GAPN and PGI collectively represent a relatively wide range of enzyme kinetic characteristics. The irreversible, bi-bi enzyme kinetics with allosteric activation and substrate inhibition, as well as reversible, uni-uni enzyme kinetics of GAPN and PGI respectively, represent many glycolytic enzyme kinetics.

The metabolism of archaea is known for having unusual, modified pathways containing novel enzymes that replace the typical eukaryotic and bacterial enzymes [9, 18, 31, 35, 56, 59]. *S. solfataricus* and other thermophilic organisms have evolved to survive under extreme temperatures of between 60°C and 80°C [34, 35]. These high temperatures however can threaten the thermal stability of some of the pathway intermediates and as a result a carbon loss is observed. The stability of the larger molecular structures of *S. solfataricus*, like proteins, membranes, DNA and RNA, under extreme temperatures, has been thoroughly analysed. However, it is only recently that the thermal stability of the smaller molecular structures, such as pathway intermediates, have been analysed [34].

#### PGI

The basic pathways, such as the Entner-Doudoroff (ED) and Embden-Meyerhof-Parnas (EMP) pathways, of glycolysis are slightly modified in archaea compared to that of bacteria or eukaryotes [9, 35]. Some of the enzymes of the pathways differ from the classic ED or EMP enzymes or are new. PGI is such an example, however, the PGI that has been identified in *S. solfataricus* is of the classic form [31, 35, 56, 59]. PGI enables the reversible conversion of glucose 6-phosphate (G6P) to fructose 6-phosphate (F6P) via the EMP pathway [9, 31, 55], and is essential for both glycolysis and gluconeogenesis [23, 31, 59].

## GAPN

Ferredoxin-dependent glyceraldehyde 3-phosphate oxidoreductase (GAPOR), fructose 1,6-bisphosphate aldolase/phosphatase (FBPA/ase) and GAPN are also examples of thermophiles' adaption to life at high temperatures [9, 31, 48, 55, 59, 64]. In *S. solfataricus* glucose is converted to pyruvate via the modified ED pathway, either via the non-phosphorylative or semi-phosphorylative branch [3, 9, 18, 31, 35, 36, 68]. The regulation of these two branches is still unclear [1, 2]. In the semi-phosphorylative branch the enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK) facilitate gluconeogenesis via the EMP pathway [3, 9, 18, 35, 55, 59, 68]. Glycolysis operates via the ED pathway and is facilitated by GAPN which irreversibly converts glyceraldehyde 3-phosphate (GAP) to 3-phosphoglycerate (3-PG), while  $\text{NADP}^+$  is converted to NADPH [9, 18, 59]. The GAPN enzyme is allosterically activated by glucose 1-phosphate (G1P), a glycogen metabolism intermediate. It is also the only enzyme in the pathway subject to allosteric regulation and the only regulation point of the ED pathway [3, 9, 35].

Normally, in mesophilic organisms, the conversion of GAP to 3-PG happens via the GAPDH and PGK enzymes with 1,3-bisphosphoglycerate (1,3-BPG) as intermediate. 1,3-BPG is however very thermolabile and in the majority of thermophiles the conversion of GAP to 3-PG is done via GAPN, thereby bypassing 1,3-BPG [9, 18]. Triose phosphates of the central carbohydrate metabolism, like GAP, dihydroxyacetone phosphate (DHAP) and 1,3-BPG, are also not stable at extreme temperatures [34]. The thermolability of substrates and product need to be taken into consideration when conducting the enzyme kinetic assays.

3.1. Selecting enzymes

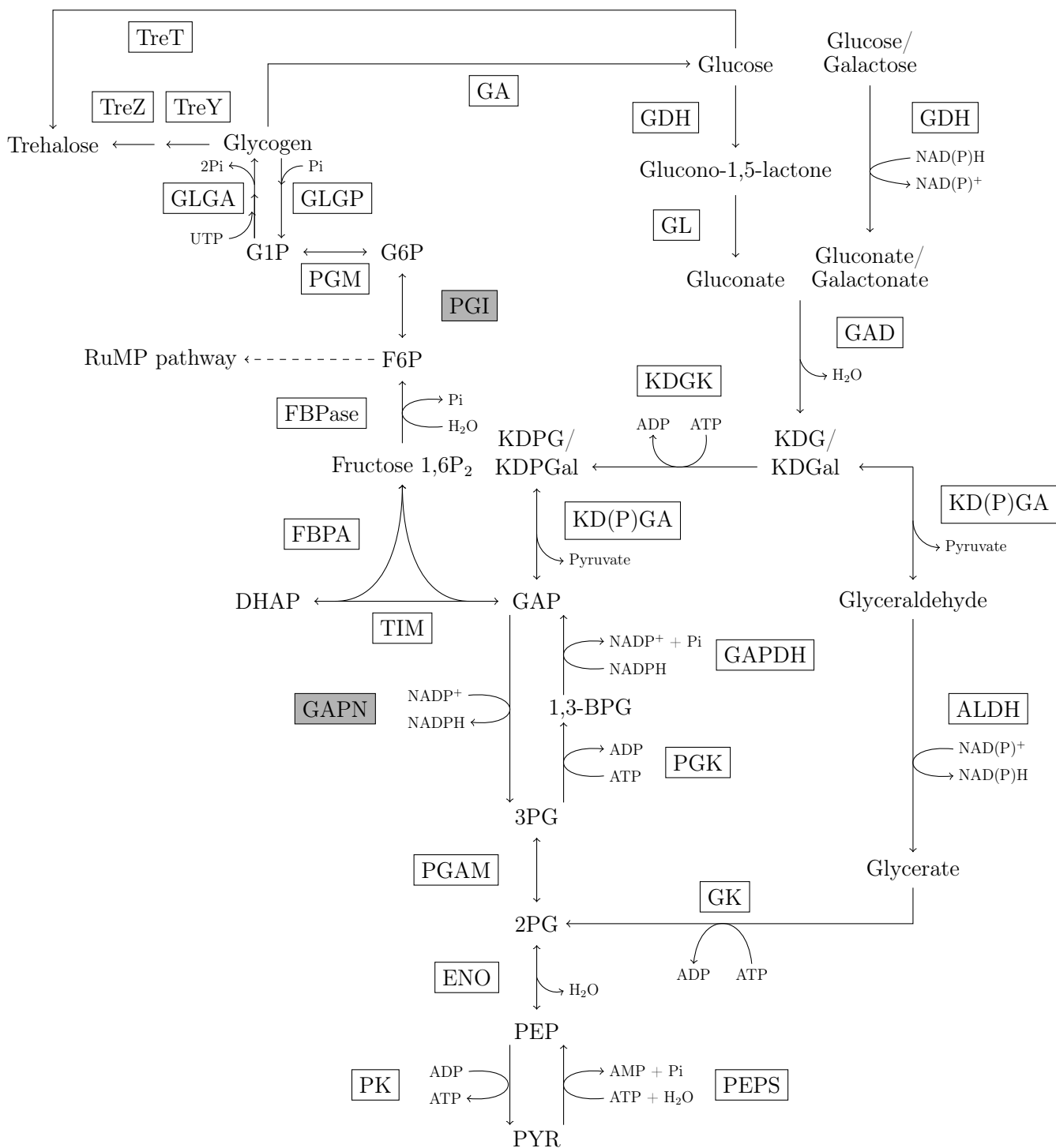


Figure 3.1: The central carbohydrate metabolism of *S. solfataricus*. Enzymes: ALDH, aldehyde dehydrogenase; ENO, enolase; FBPA, fructose-1,6-bisphosphate aldolase; FB-Pase, fructose-1,6-bisphosphatase; GA, glucan-1,4- $\alpha$  glucosidase; GAD, gluconate dehydratase; GDH, glucose dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAPN, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; GK, glycerate kinase; GL, gluconolactonase; GLGA, glycogen synthase; GLGP, glycogen phosphorylase; KD(P)GA, 2-keto-3-deoxy-(6-phospho)-gluconate aldolase; KDGK, 2-keto-3-deoxygluconate kinase; PEPS, phosphoenolpyruvate synthetase; PGAM, phosphoglycerate mutase; PGI, phosphoglucose isomerase; PGK, phosphoglycerate kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; TIM, triose phosphate isomerase; TreT, trehalose glycosyltransferring synthase; TreY, malto-oligosyltrehalose synthase; TreZ, malto-oligosyltrehalose trehalohydrolase. Intermediates: 1,3-BPG, 1,3-biphosphoglycerate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; DHAP, dihydroxyacetonephosphate; F6P, fructose 6-phosphate; Fructose 1,6P<sub>2</sub>, fructose 1,6-biphosphate; G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; KD(P)G, 2-keto-3-deoxy-(6-phosphate)-gluconate; KD(P)Gal, 2-keto-3-deoxy-(6-phosphate)-galactonate; PEP, phosphoenolpyruvate; PYR, pyruvate.

## 3.2 Experimental and *in silico* datasets

The use of an experimental dataset was sufficient for the identifiability analysis of PGI enzyme kinetics. However, for the identifiability analysis of the GAPN enzyme kinetics, the use of an experimental dataset alone was not sufficient. The model structure was not the only contributing factor to the non-identifiability of the parameters. Experimental noise and temperature lag, which lead to practical non-identifiability were also present in the data. Unpublished parameter values determined by Dr. Kouril (personal communication) were used to conduct the identifiability analysis. Our experimental results differed from those of Dr. Kouril in terms of enzyme saturating assay concentrations. Due to these discrepancies between our experimental results and the unpublished results of Dr. Kouril, and the lack of literature values for the GAPN parameters, the use of a supplementary *in silico* analysis of the GAPN parameter identifiability was a viable option. Although these discrepancies in the experimental results should be addressed at some point, the aim of this study is to conduct a comparative analysis of the progress curve and initial rate methods. Thus, finding an explanation for the difference in the experimental results fell outside the scope of this study. These results are discussed in more detail in [Chapter 4](#). [Figure 3.2](#) shows an outline of the different datasets, both experimental and *in silico* generated, used throughout this study:

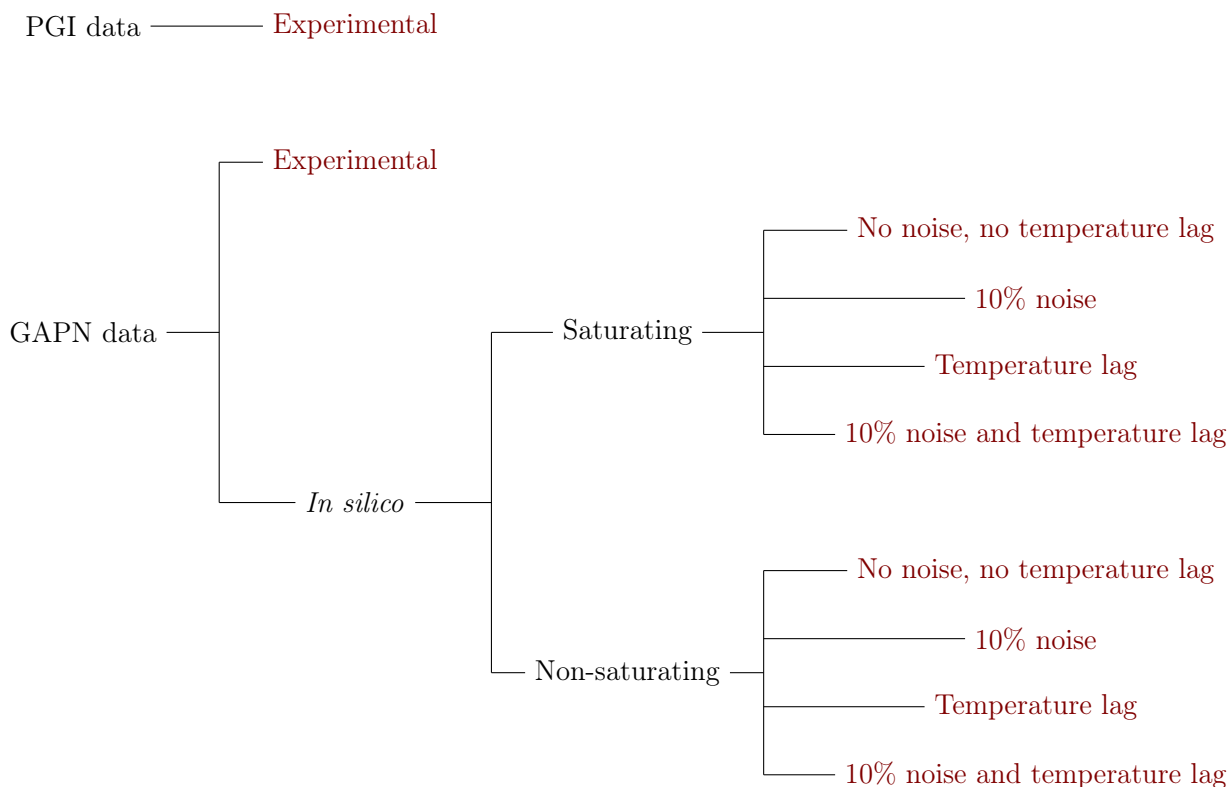


Figure 3.2: Outline of experimental and *in silico* generated datasets used in this study.

### 3.3 Experimental methods for GAPN

#### Experimental design and methodology

Micro-organisms such as *Escherichia coli* and *S. cerevisiae* as well as *S. solfataricus* itself can be used for the over expression of archaeal genes [27, 38, 41, 57]. Although *S. solfataricus* grows heterotrophically on an array of different carbohydrates, making it fairly easy to culture under certain conditions [35, 38, 68], this process can be costly [27]. Making use of mesophilic hosts that facilitate the heterologous expression of genes is easier and more cost effective than culturing *S. solfataricus* itself and extracting the required enzymes. Enzyme extraction and purification from mesophilic expression systems are completed with simple heat precipitation [27, 57, 68]. For the study of the proteins FBPA/ase, TPI, GAPDH, and PGK, these proteins were expressed in *E. coli* and extracted by heat treatment for 20 minutes at 80°C. The enzyme extraction was followed by anion exchange and gel filtration chromatography [34]. In another study, this technique, with slight changes in the duration and temperature of the heat precipitation step, was also used for the determination of the kinetic parameters of GAPN. Half-saturating concentration of NADP<sup>+</sup> (0.1 mM), and 0.01 mM of G1P was used to study the effect of an allosteric activator [18].



For this study the transformed *E. coli* strain Rosetta(DE3) was cultivated in LB broth at 37°C with 34 mg·L<sup>-1</sup> chloramphenicol and 50 mg·L<sup>-1</sup> kanamycin. To induce overexpression 1 mM isopropyl thio-β-D-galactopyranoside (IPTG) was added to the culture, once it had reached an optical density (600 nm) of 0.6, and incubated for 3-4 hours [17, 34, 68]. Thereafter cells were harvested by centrifugation for 20 min at 6 000 rpm (4°C) and resuspended in 20 mL TRIS/HCl buffer (100 mM, pH 7, room temperature). The suspension was then divided into 1.5 mL aliquots in Eppendorf tubes and centrifuged for 5 min at 13 000 rpm whereafter the supernatant was discarded [14]. Pellets were stored at -80°C. The frozen pellets were resuspended in 1 mL TRIS/HCl buffer (100 mM, pH 7, room temperature) supplemented with 10 mM dithiothreitol (DTT) [17]. The cell contents were extracted by glass-bead extraction (1 g, ≤ 106 μm diameter) for 6 min and centrifugation (13 000 rpm, 45 min, room temperature) [5, 14]. The supernatant was removed and subjected to heat precipitation for 20 min at 80°C. The enzyme extracts were then isolated from the supernatant by a final centrifugation step (13 000 rpm, 30 min, room temperature) to remove denatured proteins [34]. The protein concentrations were determined by means of Bradford assays [8].

The GAPN catabolic activity was measured in a mixture containing 100 mM TRIS/HCl buffer (pH 7), 0.983 μg GAPN and varying concentrations of either GAP (0.03-0.5 mM, 1.0 mM NADP<sup>+</sup>) or NADP<sup>+</sup> (0.001-0.5 mM, 0.1 mM GAP). The GAPN activity with varying concentrations of NADPH (0.005-0.1 mM, 0.1 mM GAP, 1.0 mM NADP<sup>+</sup>) and varying concentrations of GAP in the presence of 1.0 mM G1P (0.01-0.5 mM GAP, 1.0 mM NADP<sup>+</sup>) was measured in a mixture containing 100 mM TRIS/HCl buffer (pH 7) and 0.775 μg GAPN. The GAPN activity with varying concentrations of the allosteric modifier G1P (0.0005-0.1 mM, 0.1 mM GAP, 1.0 mM NADP<sup>+</sup>) was measured in 100 mM TRIS/HCl buffer (pH 7) and 1.458 μg GAPN. The buffer and enzyme mixture was preheated to 70°C before the substrates, products and cofactors were added to the mixture to initiate the assays. All NADPH formation data were collected at 70° with a Rayleigh UV-1800 UV/VIS Spectrophotometer Peltier Temperature Control System at 340 nm. The data collected were used for both progress curve and initial rate analysis.

### Thermal degradation

The thermal degradation constant for GAP was previously determined to be 0.056 min<sup>-1</sup> with a half-life time of 12.4 minutes at 70°C [34]. G1P is not subject to thermal degradation [11]. NADP<sup>+</sup> is stable at 70°C, NADPH however is sensitive to temperatures above 40°C when in solution [28, 37].

For this study the thermal degradation constants for GAP and NADPH were experimentally determined at 70°C. The GAP data were collected with NMR spectroscopy and the NADPH data were collected with a Rayleigh UV-1800 UV/VIS Spectrophotometer. The thermal degradation constants were determined to be 0.084 min<sup>-1</sup> and 0.147 min<sup>-1</sup> for GAP and NADPH respectively.

### Evaporation of assay solution

The influence of evaporation of the assay samples during the preheating step and the assay itself was tested. A cuvette containing 1 mL distilled H<sub>2</sub>O was sealed during the preheating process and weighed before preheating for 15 minutes. Thereafter the unsealed cuvette was placed in the heated spectrophotometer for a further 15 minutes and weighed again. The weight loss during the complete experimental process was less than 2% and as a result will have little influence on the absorbance data collected.

### GAPN rate equation

The model and rate equation (3.1) used by Dr. Kouril were used for data fitting for the case study on GAPN (personal communication).

$$v_{gapn} = \frac{V_m \cdot \left( \frac{GAP \cdot NADP}{K_{mgap} \cdot K_{mnadp}} \right) \left( 1 + \frac{G1P}{\alpha \cdot K_{mg1p}} \right)}{\left( 1 + \frac{GAP}{K_{igap}} \right) \left( 1 + \frac{G1P}{K_{mg1p}} + \frac{GAP}{K_{mgap}} \cdot \left( 1 + \frac{G1P}{\alpha \cdot K_{mg1p}} \right) \right) \left( 1 + \frac{NADP}{K_{mnadp}} + \frac{NADPH}{K_{mnadph}} \right)} \quad (3.1)$$

### Temperature lag

As mentioned above, GAP and NADPH are subject to thermal degradation and were therefore kept on ice before being added to the preheated enzyme and buffer mixture during the experimental procedures.

The activity of the GAPN enzyme changes with fluctuations in temperature. Thermophilic enzyme activity is very low at temperatures of between 20°C and 30°C while enzyme activity is very high at temperatures that exceed the organism's optimal growth temperature, which is often even higher than 100°C [10]. The addition of cold ligands to the enzyme mixture resulted in a decrease in temperature which need to be corrected for. We assume that the kinetics of the GAPN enzyme are similar to most biological reactions in terms of its temperature coefficient ( $Q_{10}$ ) being equal to two. For each 10°C increase or decrease in temperature (T) the kinetic rate (R) will increase or decrease by a factor of two [53, 58].

$$Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}} \quad (3.2)$$

We use the  $R_2$  constant as a scaling factor when determining the initial rates and during progress curve analysis. To do so the starting temperatures of the assays need to be calculated. As all the products, substrates and cofactors used in the assays were suspended in buffer (100 mM TRIS/HCl, pH 7), the specific temperature of all the solutions are the same. Thus the Richmann equation can be used to calculate the starting

temperatures of the assays, where the starting assay temperature ( $T$ ) is calculated by using the volumes ( $m_{1,2}$ ) and temperatures ( $T_{1,2}$ ) of the substrates, products, buffer, and enzyme [25].

$$T = \frac{(m_1 T_1 + m_2 T_2)}{(m_1 + m_2)} \quad (3.3)$$

## Data analysis

The full time course data collected were analysed in the open-source programming language, Python. Dr. Eicher provided example scripts for the fitting of the experimental initial rate and progress curve data, as well as for the identifiability studies. These scripts were then adapted for the analysis of the supplementary *in silico* generated data.

The most linear section of the first minute of each progress curve was used as initial rate data. The fitting of the initial rate data to the rate equation (3.1) was completed by using the `scipy.optimize.leastsq` module [33]. The initial decrease in temperature was corrected for with equation 3.2 for each initial rate.

The full time courses were fitted to the rate equation (3.1) with the `scipy.optimize.leastsq` module for the progress curve data analysis [33]. As the data were collected in triplicate, the averages of the time courses were fitted, weighted to the variance. The Levenberg-Marquardt method was originally considered for this study, but this method terminated the optimisation prematurely, thus the Nelder-Mead method was our method of choice.

The method described in Chapter 2 was used for the identifiability analysis of the parameter value estimations [52]. The `scipy.integrate.odeint` module was used in the identifiability analysis script. Computations were performed using the University of Stellenbosch's Rhasatsha HPC: <http://www.sun.ac.za/hpc> as well as a personal Macbook Pro (2.5 GHz Intel Core i5 processor with 4 GB RAM). However, due to backlog on the Stellenbosch cluster the identifiability analysis for some datasets were completed on the laptop only; these analyses took about a week per dataset to complete. To interpolate the sampled profile likelihood points spline interpolation was used [16]. All figures were created with Matplotlib [30].

## 3.4 Experimental methods for PGI

### Experimental design and methodology

Similar to GAPN, the PGI enzyme of *S. solfataricus* can easily be expressed in mesophilic hosts and extracted by a heat precipitation process. The heat precipitation can be completed at 90°C for 20 minutes [60], although it has also been completed successfully at

80°C for 20 minutes [34]. Both of these heat precipitation methods were followed by a centrifugation step.

In a study conducted on the PGI enzymes of several organisms, including thermophilic archaea, the PGI anabolic activity was studied at different temperatures of between 37°C and 80°C, depending on the organism. Glucose 6-phosphate dehydrogenase (G6PDH) from hyperthermophilic *Thermotoga maritima* was used as auxiliary enzyme for linked enzyme assays. Auxiliary enzymes were also used in linked enzyme assays to study the PGI activity in the catabolic direction [22].

For this study transformed *E. coli* strain (Bl21 RIL) was cultivated in LB broth at 37°C with 50 mg·L<sup>-1</sup> kanamycin antibiotic. The rest of the culturing and enzyme extraction processes are the same as for the GAPN enzyme as described above.

### Initial rate data

To collect data in the catabolic direction requires the use of multiple auxiliary enzymes that are stable at 70°C. As these enzymes were not available to us, data could only be collected in the anabolic direction. The initial rate data in the anabolic direction could be collected with NMR spectroscopy, however the relaxation time between the free induction decays are long and much of the initial rate data is lost. Thus the data could only be collected in the anabolic direction with the Rayleigh UV-1800 UV/VIS spectrophotometer. This required the use of a linked enzyme kinetic assay. G6PDH from *T. maritima* is used as linking enzyme which removes G6P from the system by converting it to Glucono- $\delta$ -lactone 6-phosphate, and NADP<sup>+</sup> to NADPH. The assay mixture contained 100 mM TRIS/HCl buffer (pH 7), 4.464  $\mu$ g PGI, 20  $\mu$ g G6PDH, 1.0 mM NADP<sup>+</sup>, and varying concentrations of F6P (0.005 -10 mM) [60, 66, 69]. The enzymes and buffer were preheated to 70°C before substrates and cofactors were added to initiate the assays.

### Progress curve data

PGI progress curve data in both the anabolic and catabolic directions could be collected with NMR spectroscopy as the relaxation time between free induction decays do not affect the quality of the time course data. The data were collected on a Varian 600 MHz spectrometer at 70°C, the major advantage being that both G6P and F6P data could be collected for each assay. Five sets of G6P and F6P data were collected from the following assays: 20 mM G6P and 0 mM F6P, 10 mM G6P and 0 mM F6P, 5 mM of both G6P and F6P, 0 mM G6P and 20 mM F6P, and 0 mM G6P and 10 mM F6P. In addition each assay solution contained 4.464  $\mu$ g PGI enzyme extract, 50 mM triethyl phosphate (TEP), which is used as a standard, 100  $\mu$ L D<sub>2</sub>O, and 10 mM MgCl<sub>2</sub>, made up to a final volume of 1 mL with 50 mM TRIS buffer (pH 7, room temperature) [16].

## Thermal degradation

Neither G6P, F6P, nor the PGI enzyme itself are subject to significant thermal degradation [66, 69].

## PGI rate equation

The reversible Michaelis-Menten equation is used for the fitting of the PGI kinetic data:

$$v_{pgi} = \frac{\frac{V_f \cdot G6P}{K_{mG6P}} - \frac{V_r \cdot F6P}{K_{mF6P}}}{1 + \frac{G6P}{K_{mG6P}} + \frac{F6P}{K_{mF6P}}} \quad (3.4)$$

The JWS Online Model Database contains many examples of models that include PGI, all of which use the reversible Michaelis-Menten equation and Haldane relationship to characterise the PGI kinetics:

$$v_{pgi} = \frac{V_f \cdot \left( G6P - \frac{F6P}{K_{eq}} \right)}{K_{mG6P} \cdot \left( 1 + \frac{F6P}{K_{mF6P}} + \frac{G6P}{K_{mG6P}} \right)} \quad (3.5)$$

Equation 3.5 should describe the full time course data collected with NMR spectroscopy and is used for the fitting of the PGI progress curve data. With the use of a linked enzyme assay for the collection of the PGI initial rate data, all G6P is converted to Glucono- $\delta$ -lactone 6-phosphate by G6PDH. As there is no accumulation of G6P the reversible equation 3.4 reduces to equation 3.6:

$$v_{pgi} = \frac{V_m \cdot F6P}{K_{mF6P} + F6P} \quad (3.6)$$

## Data analysis

The NMR time course data were processed with NMRPy, a Python based open-source software suite [15]. Dr Eicher provided custom NMRPy scripts adapted for this project and performed the processing of the raw NMR data. The fitting of the experimental data to the rate equation (3.5) was completed with the Nelder-Mead least squares algorithm by using the `scipy.optimize.leastsq` module [33], see Figure 4.19.

Only one time course of data were collected per assay with NMR spectroscopy. It is not possible to replicate progress curve experiments with NMR spectroscopy exactly as

the time points of each data point will be different. With no duplicates or triplicates of the data the sum squared residuals could not be weighted to the variance in the data. Spline interpolation, specifically the `scipy.interpolate.UnivariateSpline` module, was used for determining the standard error of the data [15, 33]. This however only represents the noise of the NMR spectrometer and not experimental error as well. The `scipy.integrate.odeint` module was used. Spline interpolation was used to interpolate the sampled profile likelihood points [16].

The initial rate data were processed in a custom Python script adapted from an example script provided by Dr. Eicher. Initial rate fitting was done with spline interpolation, the module `scipy.interpolate.UnivariateSpline` was used [33].

The fitting of the initial rate data to the rate equation (3.6) was completed with the Nelder-Mead least squares algorithm from the `lmfit.minimize` module, see Figure 4.17. The initial rate data were collected in duplicate, thus the averages of the initial rate were fitted to the rate equation, weighted to the variance.

Thereafter the identifiability analysis was completed and rendered with spline interpolation [30, 52]. All figures were created with Matplotlib [30].

# Chapter 4

## Results

### 4.1 GAPN results

#### Identifiability analysis of parameter value estimation from experimental data

Enzyme kinetic experiments with saturation curves for substrates, products and allosteric regulator were performed. Figure 4.1 shows the averaged experimental initial rate data, collected with a UV/VIS spectrophotometer. Plot (a) of Figure 4.1 shows the effect that the allosteric regulator, G1P, had on the the GAPN enzyme.

NADPH absorbs light at a wavelength of 340 nm and is the only GAPN ligand which can be observed with the use of light spectroscopy. Thus the other ligand concentrations are inferred from the changes in NADPH concentration. 33 time courses of progress curve data were collected. In Figure 4.2 two plots of progress curve data are shown. Both of the assays had starting concentrations of 0.5 mM GAP and 1.0 mM NADP<sup>+</sup>. Plot (a) shows the assay with the addition of 0.1 mM G1P and plot (b) shows the assay without any G1P.

When fitting progress curve data, parameters are often correlated; not fixing any of the parameter values can result in extreme correlation between parameters [32]. To avoid adding more degrees of freedom to the model, and thus introducing more correlations, the thermal degradation constants were fixed to the experimentally determined values before fitting the data for the remaining parameters. The degradation constants are chemical parameters and not enzyme parameters and can therefore be excluded from the fit.

## 4.1. GAPN results

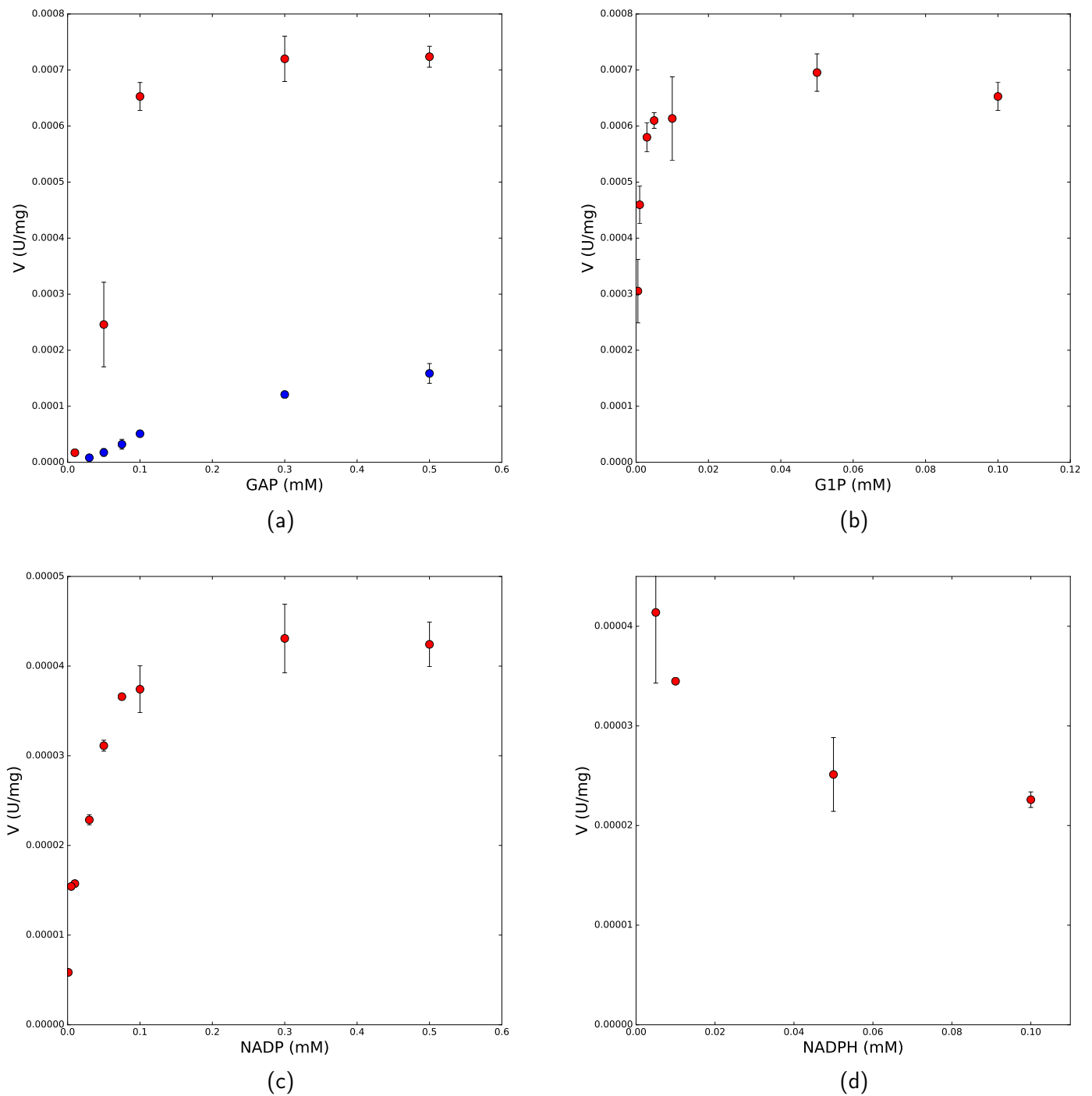


Figure 4.1: Initial rate plots of the experimental data. (a) Varying concentrations of GAP without G1P, shown in blue, and with 0.1 mM G1P, shown in red. (b) Varying concentrations of G1P. (c) Varying concentrations of NADP<sup>+</sup>. (d) Varying concentrations of NADPH. The error bars are an indication of the variance in the experimental data.



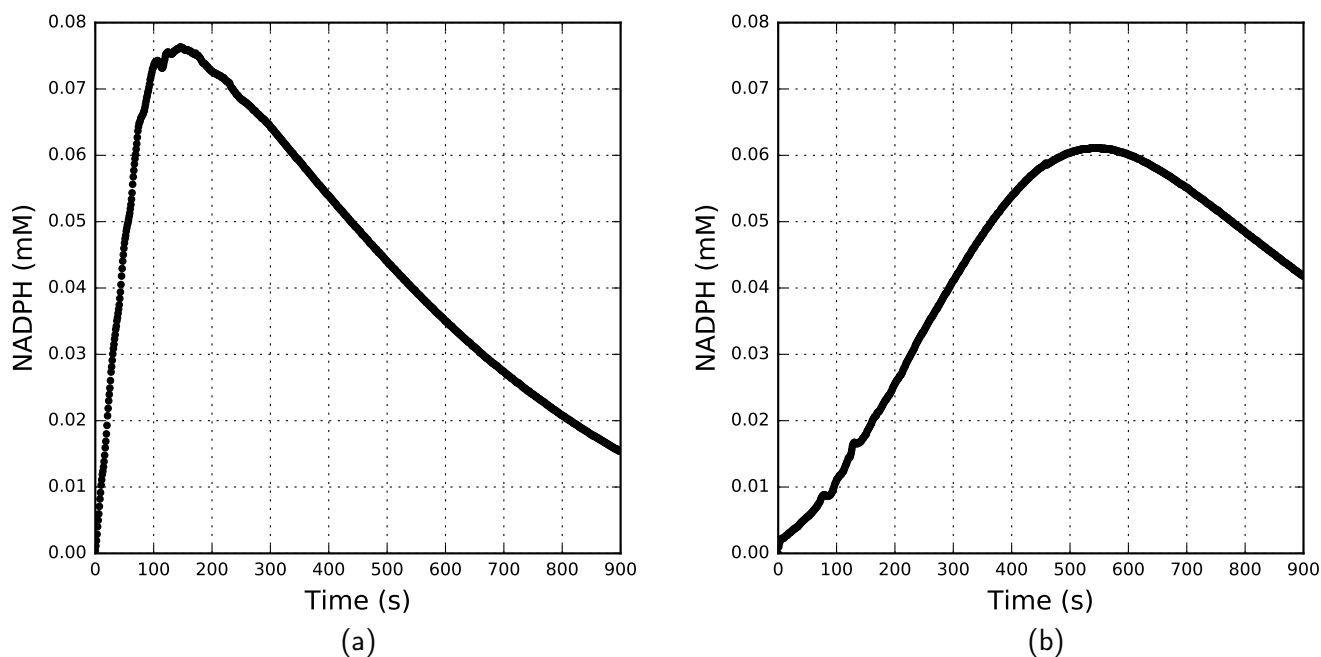


Figure 4.2: Plots of two of the 33 time courses of experimental data. (a) 0.5 mM GAP and 1.0 mM NADP<sup>+</sup> with 0.1 mM G1P. (b) 0.5 mM GAP and 1.0 mM NADP<sup>+</sup> without any G1P present.

If realistic parameter values can be estimated, the next question is how accurate the parameter estimations are. To determine if using the full time course data as opposed to only the initial few points improves the identifiability of parameter value estimations, we started by fitting for all seven parameters simultaneously. Thereafter one parameter was fixed to its value that has been experimentally determined by Dr. Kouril while the remaining six parameters were fitted for (personal communication). These results obtained by Dr. Kouril are yet to be published. By fixing one of the parameter values to its experimentally determined value, the correlation between the parameter estimations may be decreased. This process was continued until only one parameter was fitted for while the remaining six parameters were fixed to their experimentally determined values. All possible combinations of parameters were considered. As mentioned previously there is quite a difference between our experimental results and those of Dr. Kouril. As such the parameter value set describing our experimental data will differ from the unpublished parameter set of Dr. Kouril. Using these unpublished parameter values to conduct the identifiability analysis of our experimentally determined parameter values may influence the outcome thereof. However, as there are no literature values available for the parameters of GAPN, using these unpublished values was the most promising option. The results of this identifiability analysis is given in the next paragraph. A more thorough, *in silico* analysis of parameter identifiability is introduced at the end of this section.

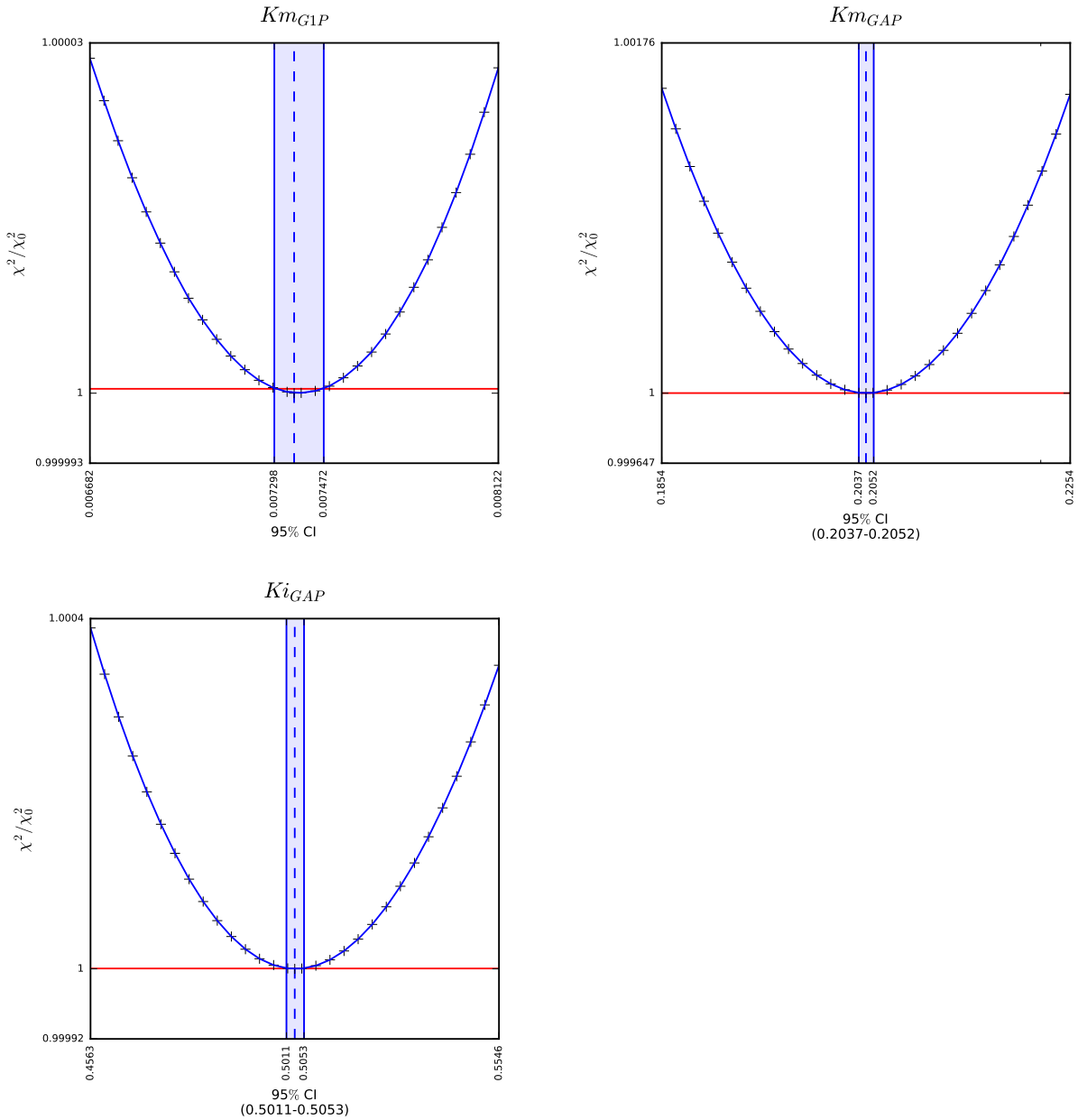


Figure 4.3: Profile likelihood plots of one of the 127 combinations of parameters fitted for with progress curve data.  $Km_{GAP}$ ,  $Km_{G1P}$  and  $Ki_{GAP}$  are fitted for while the values of  $V_m$ ,  $K_{mNADP}$ ,  $K_{mNADPH}$  and  $\alpha$  are fixed. The 95% confidence intervals are depicted by the two solid blue lines parallel to the estimated parameter values depicted by the dashed lines. The solid red lines are the thresholds in the  $\chi^2$  values that indicate the 95% confidence intervals.

In each line of Tables 1 to 72 the blank spaces indicate the parameter that was fixed while the other parameters were fitted for. The 'x' symbols indicate that the particular parameter estimation was not identifiable. The value and the accompanying range displayed in brackets are the identifiable parameter estimation and confidence interval respectively. Figures 4.4, 4.9, 4.10, 4.11, 4.12, 4.13, 4.14, 4.15 and 4.16 indicate whether

all the fitted parameters for each possible combination were identifiable or not.

When fitting for all seven parameters, then combinations of six parameters, etc. until only one parameter is fitted for, there are a total of 127 possible parameter combinations. Each of the parameters are fitted for 64 of these combinations and are fixed for the remaining 63 combinations.

For the progress curve analysis of the experimental GAPN data only 15 out of a possible 127 combinations returned all the parameters fitted for as identifiable, see Figure 4.4. Out of these 15 combinations, the highest number of identifiable parameters were four combinations of three parameters.  $K_{mGAP}$  was present in these four combinations, as well as in the majority of the other completely identifiable combinations. Figure 4.3 shows the profile likelihood plots of one of these combinations where all three parameters fitted for were identifiable.

When considering all the combinations, including those where not all the parameters fitted for were identifiable,  $K_{mGAP}$  and  $\alpha$  were most frequently identifiable.  $K_{mGAP}$  was identifiable for 46 and  $\alpha$  was identifiable for 44 out of a possible 64 fits. There was no noticeable correlation between either  $K_{mGAP}$  or  $\alpha$  and any of the other parameters.  $V_m$  was identifiable for 17 of the combinations it was fitted for and was only identifiable if either  $K_{mGAP}$  was fixed or the pair of  $K_{mNADP}$  and  $K_{mNADPH}$  were fixed.  $K_{mNADP}$  was only identifiable for 11 fits;  $V_m$  was fixed for nine of these fits. It is difficult to deduce from the results if  $K_{mNADP}$  and any of the other parameters are correlated.  $K_{mNADPH}$  was identifiable for 21 of the fits with  $K_{mNADP}$  having been fixed for 19.  $K_{mG1P}$  was identifiable for 14 out of the possible 64 combinations it was fitted for, but in each instance the value for  $\alpha$  was fixed.  $K_{iGAP}$  was only identifiable for three fits with  $\alpha$  having been fixed for all three fits, see Tables 1 to 7.

None of the combinations fitted for with the initial rate analysis returned any identifiable parameter estimations (see Figure 4.4).

The results of the identifiability analysis suggest that progress curve analysis is the superior method, although only by a slight margin as there were few combinations of parameter fits that returned identifiable parameter estimations. As mentioned previously the non-identifiability of parameters can be due to practical or structural non-identifiability [52]. The majority of the profile likelihood plots rendered by the identifiability analysis of the initial rate data indicate that the parameters are structurally non-identifiable. The results of the identifiability analysis of the progress curve data suggested that some of the parameters were structurally non-identifiable, but some were also practically non-identifiable. The practical non-identifiability of the parameters may be due to experimental error and the initial temperature lag in the data or possible non-saturating concentrations of ligands used for the assays.

It is however possible that the discrepancies between the parameter set describing our experimental data and the unpublished parameter set used for the identifiability analysis contributed to the non-identifiability of the parameters. Although it seems that our experimental assay ranges do include enzyme saturating concentrations (see Figure 4.1), these concentrations do not correspond to those determined by Dr. Kouril (personal communication). For the assays with varying concentrations of GAP, both with and without G1P, enzyme saturating concentrations are much lower than that determined by Dr. Kouril. The specific activity of the enzyme is also much lower than the specific activity determined by Dr. Kouril. Enzyme saturation similar to that seen in Dr. Kouril's results is seen in our experimental results, with concentrations more than 10 times lower. The two main differences between the experimental assays completed for this study and that of Dr. Kouril is the use of DL-GAP and recombinant GAPN enzyme as opposed to pure D-GAP and pure GAPN enzyme.

The effects of enzyme saturating and non-saturating ligand concentrations, initial temperature lag and experimental error were not investigated experimentally, but with the use of *in silico* generated data. The next question to consider is which of these conditions contribute the most to the non-identifiability of parameter estimations.



## Identifiability analysis of parameter value estimation from *in silico* data

In the following sections all combinations of the above mentioned factors are investigated. The datasets were generated *in silico* with equation 3.1 and unpublished parameter values determined by Dr. Kouril (personal communication) with the MATHEMATICA programming language. The data analysis and identifiability analysis were the same as for the experimental data. The datasets are as follows:

Enzyme saturating data	Non-saturating data
Without noise or temperature lag	Without noise or temperature lag
With 10% noise and without temperature lag	With 10% noise and without temperature lag
Without noise and with temperature lag	Without noise and with temperature lag
With 10% noise and temperature lag	With 10% noise and temperature lag

The data consists of 10, 10, 9, 6 and 10 time courses for varying concentrations of GAP (without G1P), G1P, NADP<sup>+</sup>, NADPH, and GAP (with G1P) respectively. The full time courses were used for progress curve analysis and the initial linear part of each time course was used to calculate the initial rate data.

For the enzyme saturating conditions the GAP concentrations were varied between 0.5-250.0 mM with 1.0 mM NADP<sup>+</sup> with and without 0.1 mM G1P. G1P was varied between 0.0001-2.0 mM with 0.1 mM GAP and 1.0 mM NADP<sup>+</sup>. NADP<sup>+</sup> was varied between 0.001-5.0 mM with 0.1 mM GAP. NADPH was varied between 0.0001-2.0 mM with 5.0 mM GAP and 1.0 mM NADP<sup>+</sup>.

For the non-saturating conditions the GAP concentrations were varied between 0.001-1.0 mM with 1.0 mM NADP<sup>+</sup>. GAP was varied between 0.001-1.0 with 1.0 mM NADP<sup>+</sup> and 0.1 mM G1P. G1P was varied between 0.005-1.0  $\mu$ M with 0.1 mM GAP and 1.0 mM NADP<sup>+</sup>. NADP<sup>+</sup> was varied between 0.001-0.5 mM with 0.1 mM GAP. NADPH was varied between 0.0005-0.05 mM with 0.1 mM GAP and 1.0 mM NADP<sup>+</sup>. These concentration ranges were selected such that neither the progress curve analysis method nor the initial rate analysis methods was favoured [46].

For the datasets that include the effect of temperature lag, equations 3.2 and 3.3 were used to incorporate a decrease of 10°C in the starting temperature of the time courses. For the datasets that include the effect of noise, up to 10% noise were added randomly to each data point of the generated time courses. Spline interpolation, specifically the `scipy.interpolate.UnivariateSpline` module, was used for determining the variance

of the *in silico* datasets with the added 10% noise. The variance in the noiseless *in silico* data is determined by floating point error. Figure 4.5 shows one of the *in silico* generated time courses with the effect of 10% noise. Figure 4.6 shows the same time course, but with the effects of both temperature lag and 10% noise. The starting concentrations for this time course were: 1.0 mM GAP, 1.0 mM NADP<sup>+</sup>, 0 mM NADPH and 0 mM G1P.

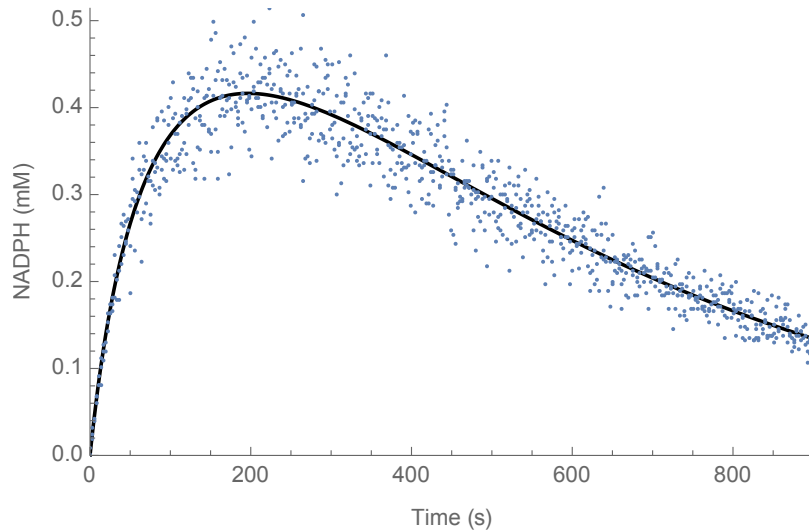


Figure 4.5: An example of one time course of *in silico* generated GAPN data (solid line) with the added effect of 10% noise (blue data points). Starting concentrations are: 1.0 mM GAP, 1.0 mM NADP<sup>+</sup>, 0 mM NADPH and 0 mM G1P.

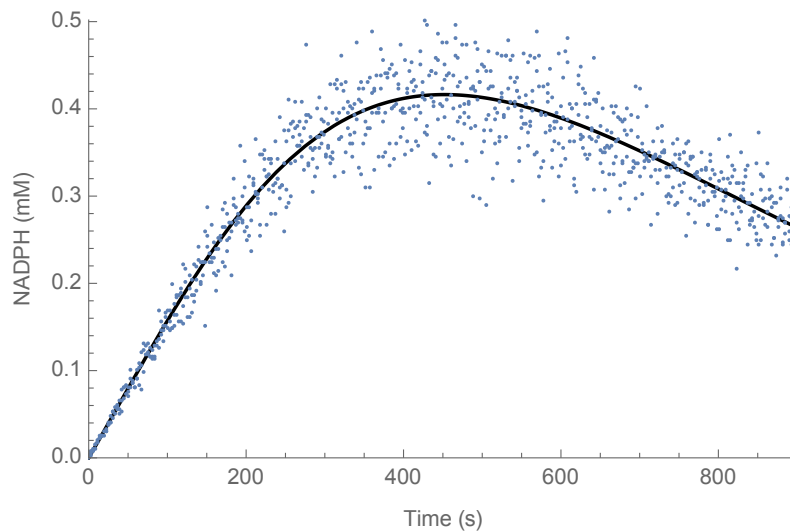


Figure 4.6: An example of one time course of *in silico* generated GAPN data (solid line) with the added effect of 10% noise and temperature lag (blue data points). Starting concentrations are: 1.0 mM GAP, 1.0 mM NADP<sup>+</sup>, 0 mM NADPH and 0 mM G1P.

### ***In silico* data: parameter identifiability with SATURATING data, NO NOISE and NO TEMPERATURE LAG**

In this section the identifiability of parameters with the use of enzyme saturating *in silico* generated data, without the effect of experimental noise or initial temperature lag is studied.

Figure 4.8 shows the saturating *in silico* generated initial rate data. Plot (a) of Figure 4.8 shows the effect that the allosteric regulator, G1P, had on the the GAPN enzyme. Figure 4.7 shows two plots of the saturating *in silico* generated progress curve data, 46 time courses were generated. The starting concentrations for both simulated time courses were 0.5 mM GAP and 1.0 mM NADP<sup>+</sup>. Plot (a) shows the assay with the addition of 0.1 mM G1P and plot (b) shows the assay without any G1P.

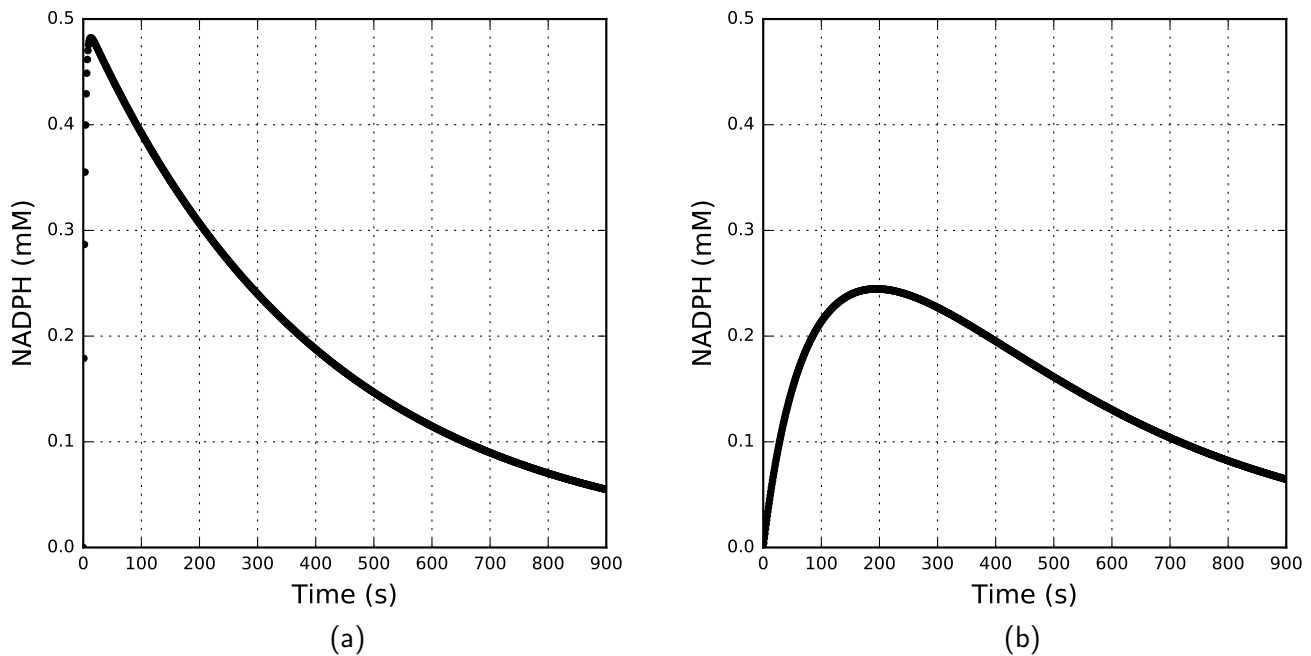


Figure 4.7: Plots of two of the 46 time courses of saturating *in silico* generated data. (a) 0.5 mM GAP and 1.0 mM NADP<sup>+</sup> with 0.1 mM G1P. (b) 0.5 mM GAP and 1.0 mM NADP<sup>+</sup> without any G1P present.



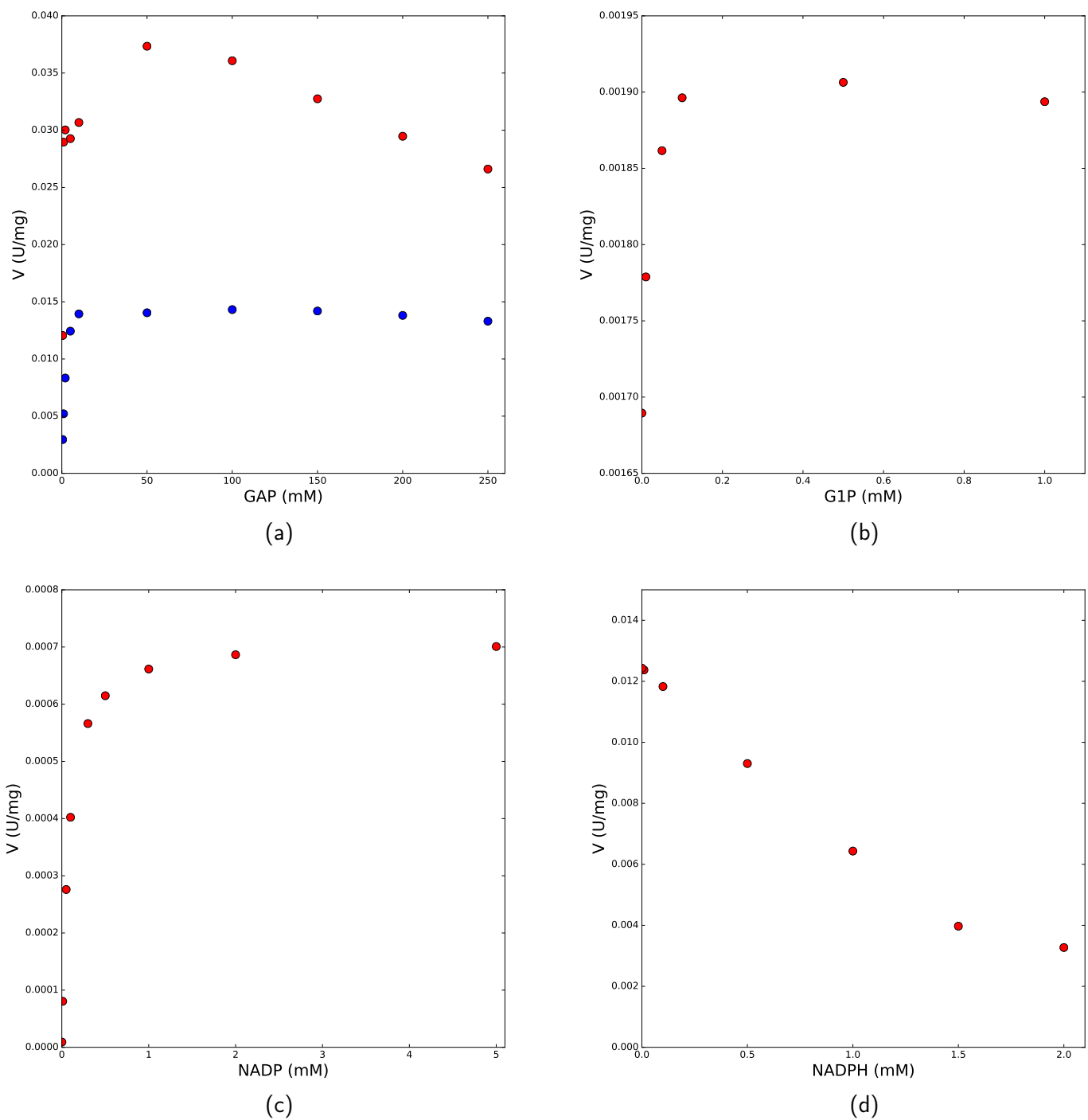


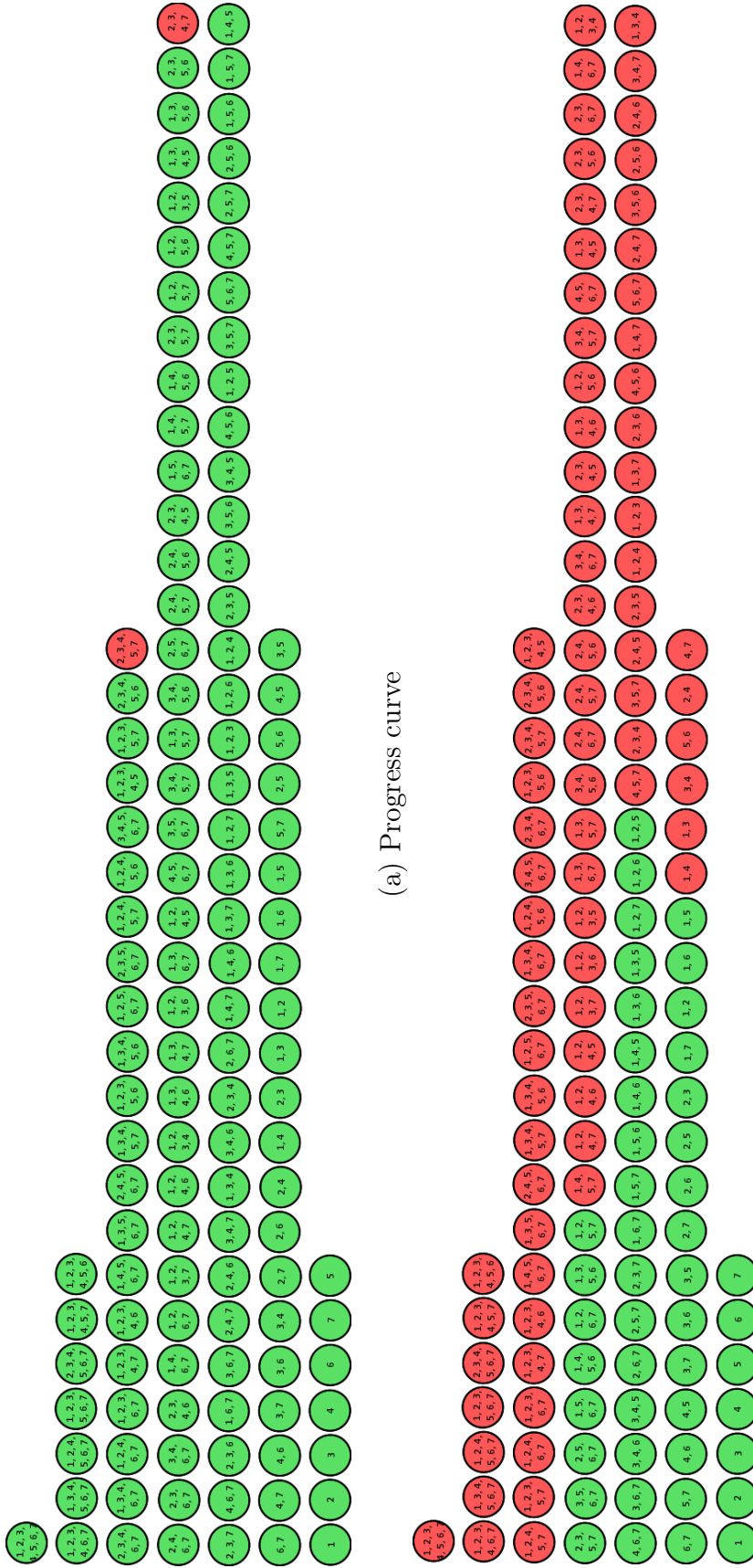
Figure 4.8: Initial rate plots of the saturating *in silico* generated data. (a) Varying concentrations of GAP without G1P, shown in blue, and with 0.1 mM G1P.

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For the progress curve analysis all the parameters fitted for were identifiable for 125 out of a possible 127 combinations, see Figure 4.9. The case where  $V_m$  and  $\alpha$  were fixed while the other parameters were fitted for was one of the cases where not all the parameters fitted for were identifiable,  $K_{mNADP}$  and  $K_{mNADPH}$  were not identifiable, see Table 12. The other case was where  $V_m$ ,  $\alpha$  and  $K_{mG1P}$  were fixed while the other parameters were fitted for,  $K_{mNADP}$  and  $K_{mNADPH}$  again were not identifiable, see Table 14. There does not seem to be any correlation between the parameters.

For the initial rate analysis all the parameters fitted for were identifiable for 47 out of a possible 127 combinations, see Figure 4.9. Out of these combinations the highest number of identifiable parameters were combinations of four parameters.

When considering the combinations of parameter fits where not all parameters fitted for were identifiable,  $V_m$ ,  $K_{mGAP}$ ,  $K_{mG1P}$ ,  $\alpha$  and  $K_{iGAP}$  were identifiable for 49, 52, 41, 45 and 54 out of a possible 64 fits respectively. These parameters do not seem to be correlated with any of the other parameters.  $K_{mNADP}$  was identifiable for 25 of the fits and was only identifiable if either  $V_m$  or  $K_{mGAP}$  was fixed. If  $V_m$  and  $K_{mGAP}$  were also fitted for along with  $K_{mNADPH}$ ,  $K_{mNADP}$  was not identifiable.  $K_{mNADPH}$  was identifiable for 12 of the fits. Similar to  $K_{mNADP}$ ,  $K_{mNADPH}$  was only identifiable if either  $V_m$  or  $K_{mGAP}$  was identifiable and was not identifiable if  $V_m$  and  $K_{mGAP}$  were also fitted for, see Tables 9, 11, 13, 15, 17, 19 and 21.

Identifiable parameter estimations with enzyme saturating *in silico* generated data for GAPN

(a) Progress curve

(b) Initial rate

Figure 4.9: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with saturating *in silico* generated data. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mG1P}$  (5),  $\alpha$  (6),  $K_{iGAP}$  (7).

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### ***In silico* data: parameter identifiability with SATURATING data, 10% NOISE and NO TEMPERATURE LAG**

In this section the identifiability of parameters with the use of enzyme saturating *in silico* generated data, with the effect of 10% of experimental noise and without the effect of initial temperature lag is studied.

None of the combinations fitted for with the initial rate analysis returned any identifiable parameter estimations (see Figure 4.10).

For the progress curve analysis all the parameters fitted for were identifiable for 107 out of a possible 127 combinations, see Figure 4.10. The highest number of parameters that were identifiable are six, while one parameter has been fixed. There are two cases where all six parameters were identifiable: where  $K_{mNADP}$  and  $K_{mNADPH}$  has been fixed respectively.  $K_{mNADP}$  and  $K_{mNADPH}$  were the only two parameters that contribute to the non-identifiability of this dataset. All the other parameters were identifiable in all instances.

When considering the combinations of parameter fits where not all parameters fitted for were identifiable,  $K_{mNADP}$  and  $K_{mNADPH}$  were identifiable for 45 and 46 out of a possible 64 fits respectively.  $K_{mNADP}$  was identifiable in all cases where  $K_{mNADPH}$  was fixed and vice versa.  $K_{mNADP}$  and  $K_{mNADPH}$  were not identifiable for various combinations where either  $V_m$  or  $K_{mGAP}$  were fixed along with  $K_{mG1P}$ ,  $\alpha$  or  $K_{iGAP}$ .  $K_{mNADP}$  and  $K_{mNADPH}$  were identifiable if both  $V_m$  and  $K_{mGAP}$  were fixed, see Tables 22 - 28.

Identifiable parameter estimations with enzyme saturating *in silico* generated data with 10% noise for GAPN

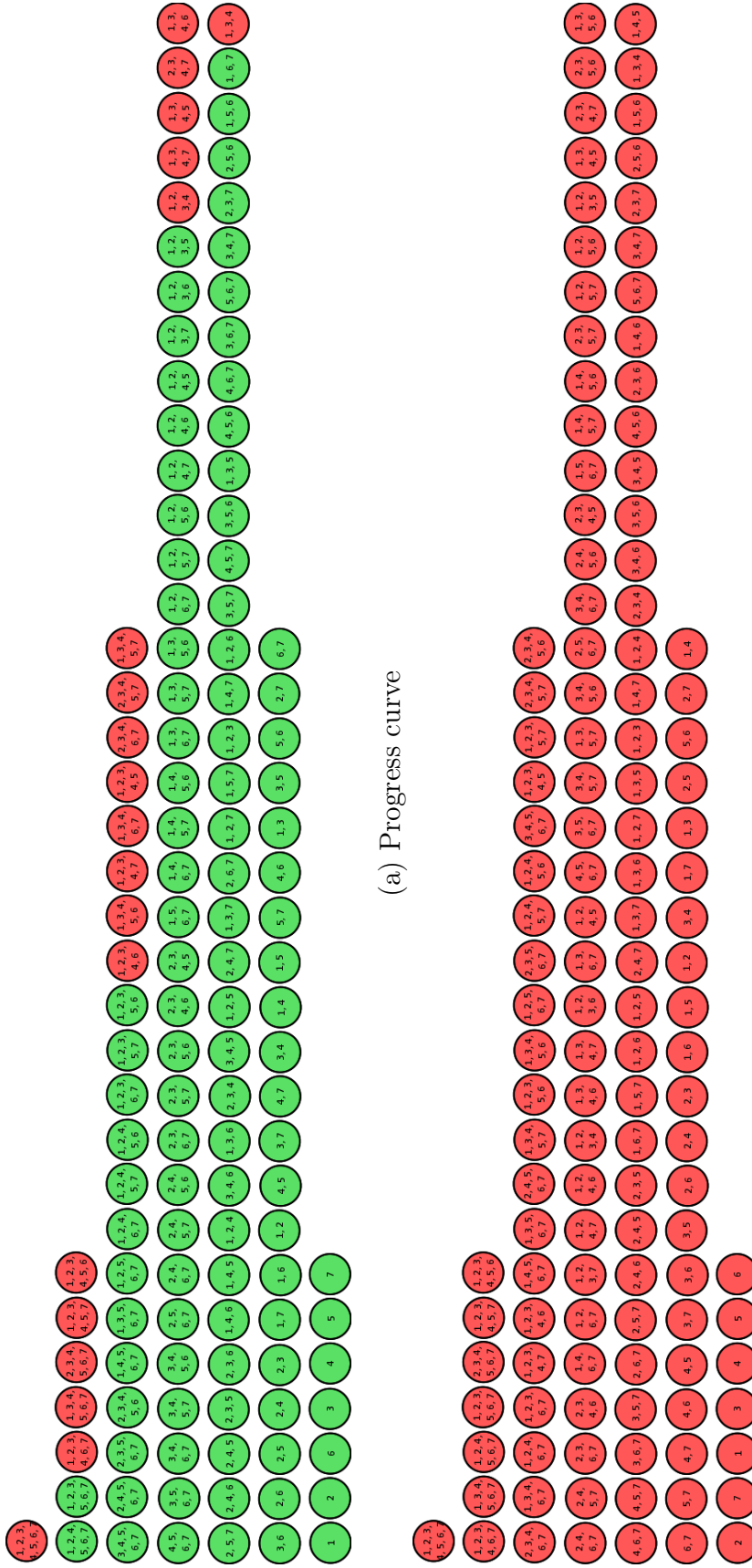


Figure 4.10: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with saturating *in silico* generated data with 10% noise. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mGIP}$  (5),  $\alpha$  (6),  $K_{iGAP}$  (7).

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### ***In silico* data: parameter identifiability with SATURATING data, TEMPERATURE LAG and NO NOISE**

In this section the identifiability of parameters with the use of enzyme saturating *in silico* generated data, without the effect of experimental noise and with the effect of initial temperature lag is studied.

With progress curve analysis there were 41 out of a total of 127 combinations where all the parameters fitted for were identifiable. Five was the highest number of identifiable parameters. The combination of  $V_m$ ,  $K_{mGAP}$ ,  $K_{mG1P}$ ,  $\alpha$  and  $K_{iGAP}$  was the only case where all five parameters fitted for were identifiable, see Figure 4.11.

For the other combinations, where some parameters were identifiable and some not,  $V_m$ ,  $K_{mGAP}$ ,  $K_{mG1P}$ ,  $\alpha$  and  $K_{iGAP}$  were identifiable for 23, 55, 40, 42, 39 out of a total of 64 fits respectively. These parameters do not seem to be correlated.  $K_{mNADP}$  and  $K_{mNADPH}$  were identifiable for 16 fits. For all of these combinations  $K_{mNADP}$  was only identifiable if both  $V_m$  and  $K_{mNADPH}$  were fixed and similarly  $K_{mNADPH}$  was only identifiable if both  $V_m$  and  $K_{mNADP}$  were fixed, see Tables 29 - 35.

Only one parameter fit with initial rate data returned identifiable parameter estimations. It is the case where only  $V_m$  was fitted for while the other six parameters were fixed (see Figure 4.11 and Table 36).

Identifiable parameter estimations with enzyme saturating *in silico* generated data with temperature lag for GAPN

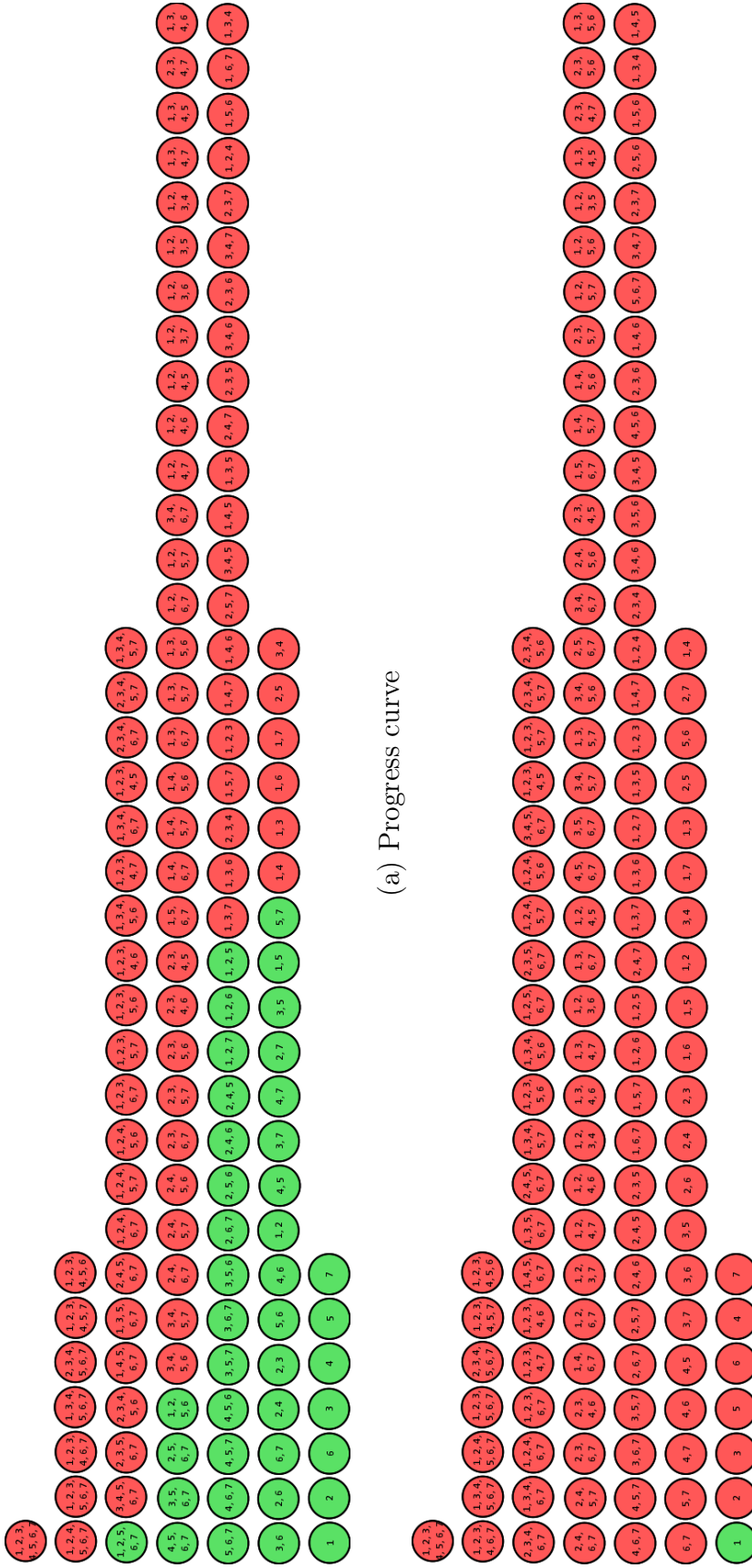


Figure 4.11: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with saturating *in silico* generated data with initial temperature lag. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mGIP}$  (5),  $\alpha$  (6),  $K_{iGAP}$  (7).

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### ***In silico* data: parameter identifiability with SATURATING data, TEMPERATURE LAG and 10% NOISE**

In this section the identifiability of parameters with the use of enzyme saturating *in silico* generated data, with the effect of 10% of experimental noise and initial temperature lag is studied.

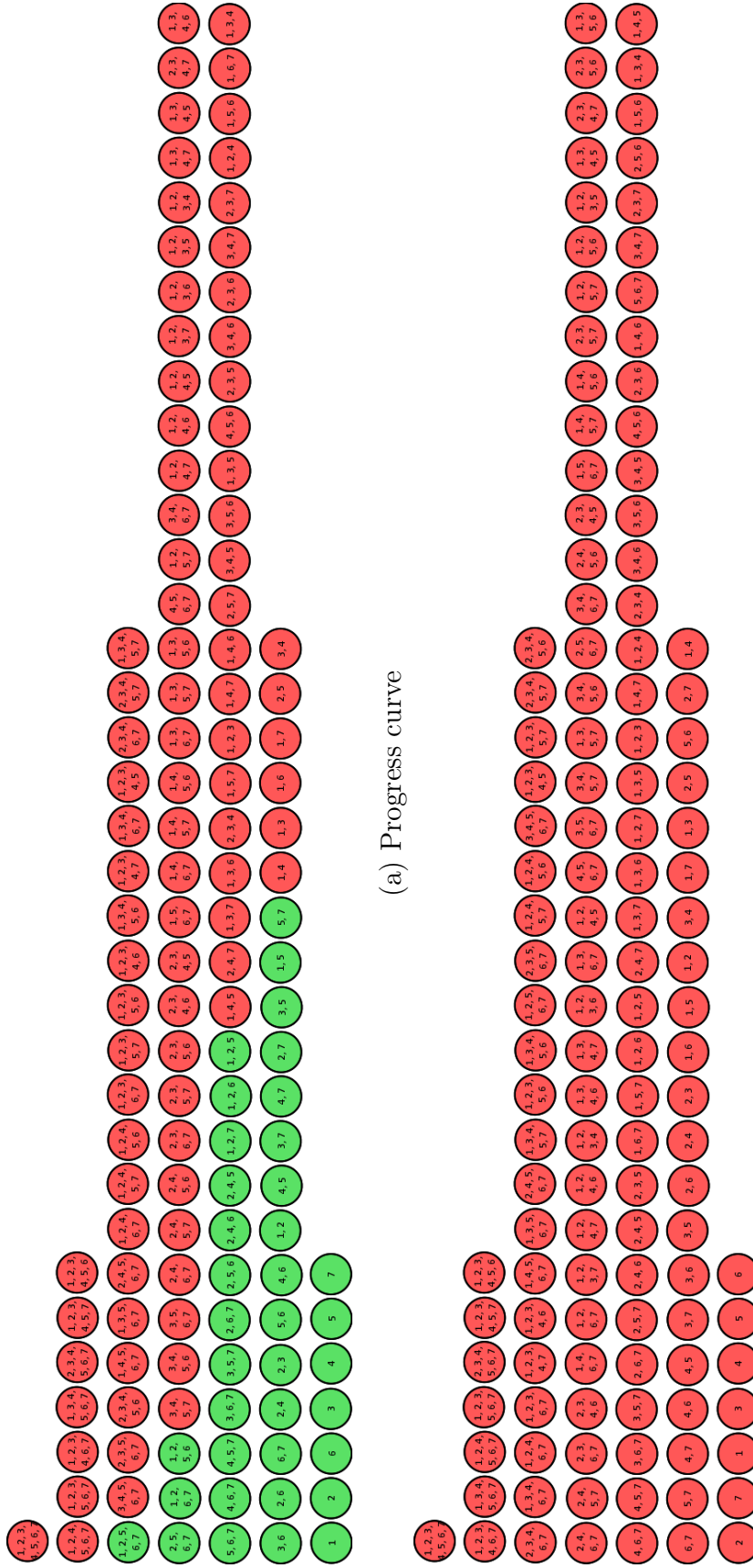
With progress curve analysis there were 38 out of a total of 127 combinations where all the parameters fitted for were identifiable. Five was the highest number of identifiable parameters, however there is only one combination of five parameters that was completely identifiable. It is the case where  $K_{mNADP}$  and  $K_{mNADPH}$  was fixed while the other five parameters were fitted for, (see Figure 4.12).

For the other combinations, where some parameters were identifiable and some not,  $V_m$ ,  $K_{mGAP}$ ,  $K_{mG1P}$ ,  $\alpha$  and  $K_{iGAP}$  were identifiable for 18, 60, 39, 36, 37 out of a total of 64 fits respectively. These parameters do not seem to be correlated.  $K_{mNADP}$  and  $K_{mNADPH}$  were identifiable for 14 and 16 fits respectively. For all of these combinations  $K_{mNADP}$  was only identifiable if both  $V_m$  and  $K_{mNADPH}$  were fixed and similarly  $K_{mNADPH}$  was only identifiable if both  $V_m$  and  $K_{mNADP}$  were fixed, see Tables 37 - 42.

None of the combinations fitted for with the initial rate analysis returned any identifiable parameter estimations (see Figure 4.12).



### Identifiable parameter estimations with enzyme saturating *in silico* generated data with 10% noise and temperature lag for GAPN



(b) Initial rate

Figure 4.12: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with saturating *in silico* generated data with 10% noise and initial temperature lag. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mGIP}$  (5),  $\alpha$  (6),  $K_{iGAP}$  (7).

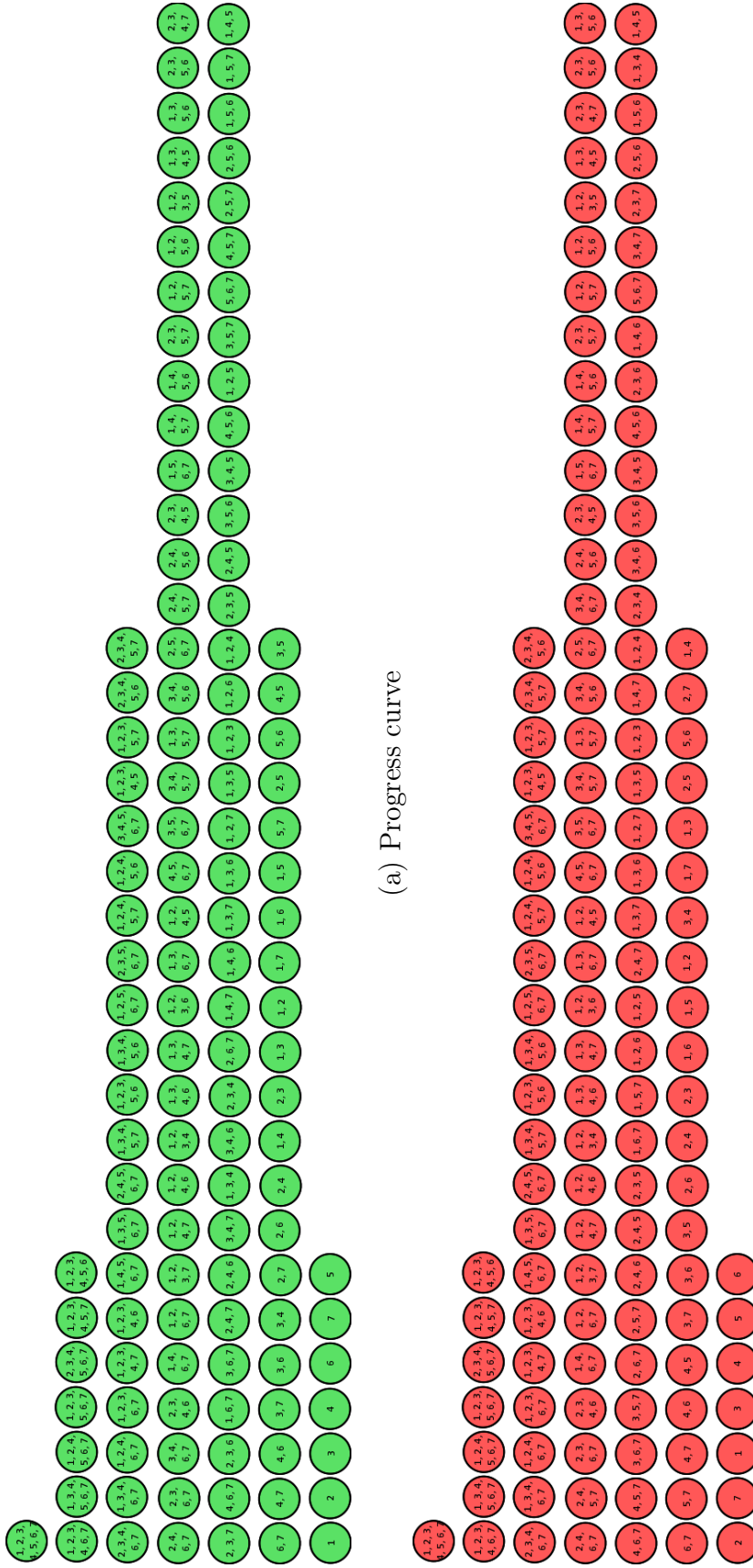
### ***In silico* data: parameter identifiability with NON-SATURATING data, NO NOISE and NO TEMPERATURE LAG**

In this section the identifiability of parameters with the use of non-saturating *in silico* generated data, without the effect of experimental noise or initial temperature lag is studied.

The parameter estimations of all 127 possible combinations were identifiable when fitted for with progress curve analysis, see Figure 4.13 and Tables 43, 44, 45, 47, 49, 50 and 51.

Although there are a few parameters that were identifiable with the analysis of the initial rate data, none of the combinations of parameters fitted for returned all the parameters as identifiable, see Figure 4.13. For the fitting of combinations of five parameters,  $V_m$  was the only parameter that was identifiable, but only for two combinations. The one is with  $K_{mGAP}$  and  $K_{mNADPH}$  fixed and the other is with  $K_{mGAP}$  and  $K_{mG1P}$  fixed while the other parameters were fitted for, see Table 46. With the fitting of combinations of four parameters  $V_m$  and  $\alpha$  were identifiable for three and four fits respectively, see Table 48.

Identifiable parameter estimations with non-saturating *in silico* generated data for GAPN



(a) Progress curve

(b) Initial rate

Figure 4.13: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with non-saturating *in silico* generated data. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mGIP}$  (5),  $\alpha$  (6),  $K_iGAP$  (7).

***In silico* data: parameter identifiability with NON-SATURATING data, 10% NOISE and NO TEMPERATURE LAG**

In this section the identifiability of parameters with the use of non-saturating *in silico* generated data, with the effect of 10% experimental noise and without the effect of initial temperature lag is studied.

All 127 possible combinations of parameters were identifiable when fitted for with progress curve analysis while none of the parameters fitted for with initial rate analysis were identifiable, see Figure 4.14 and Tables 52 - 58.



### ***In silico* data: parameter identifiability with NON-SATURATING data, with TEMPERATURE LAG and NO NOISE**

In this section the identifiability of parameters with the use of non-saturating *in silico* generated data, without the effect of experimental noise and with the effect of initial temperature lag is studied.

With progress curve analysis there were 107 out of a possible 127 combinations where all of the parameters fitted for were identifiable. All seven parameters were identifiable when fitted for, see Figure 4.15.

For the other combinations, where some parameters were identifiable and some not,  $V_m$ ,  $K_{mGAP}$  and  $K_{iGAP}$  were identifiable for all 64 of the fits.  $K_{mNADP}$ ,  $K_{mNADPH}$ ,  $K_{mG1P}$  and  $\alpha$  were identifiable for 52, 47, 61 and 62 of the fits respectively. For all of the combinations of fits where  $K_{mNADP}$  and  $K_{mNADPH}$  were not identifiable, either  $\alpha$  or  $K_{iGAP}$  was fixed, see Tables 59 - 65.

None of the initial rate data fits returned any identifiable parameters, see Figure 4.15.

Identifiable parameter estimations with non-saturating *in silico* generated data with temperature lag for GAPN

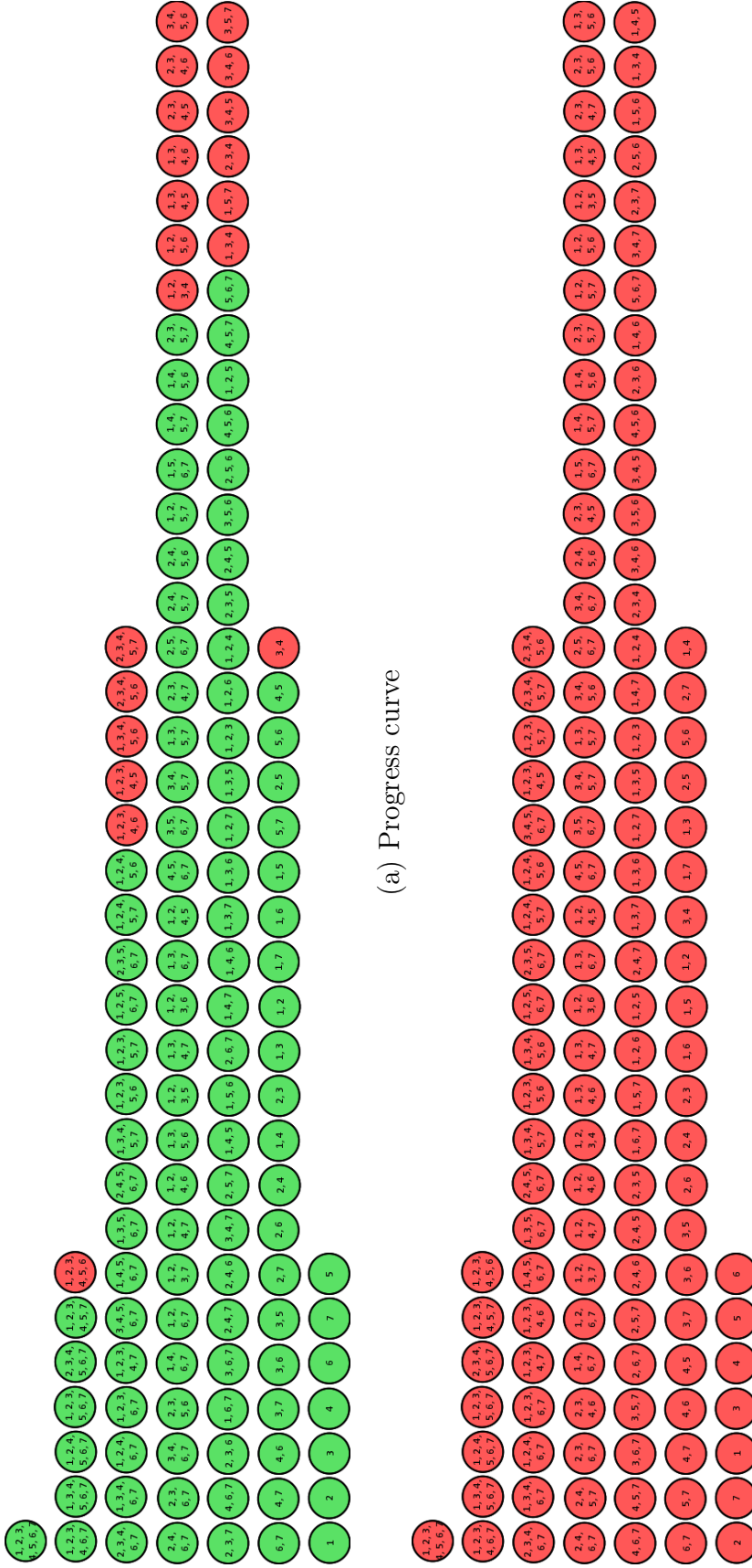


Figure 4.15: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with non-saturating *in silico* generated data with initial temperature lag. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mG1P}$  (5),  $\alpha$  (6),  $K_{iGAP}$  (7).

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### ***In silico* data: parameter identifiability with NON-SATURATING data, TEMPERATURE LAG and 10% NOISE**

In this section the identifiability of parameters with the use of non-saturating *in silico* generated data, with the effect of 10% experimental noise and the effect of initial temperature lag is studied.

With progress curve analysis there were 111 out of a possible 127 combinations where all of the parameters fitted for were identifiable. All seven parameters were identifiable when fitted for, see Figure 4.16.

For the other combinations, where some parameters are identifiable and some not,  $V_m$ ,  $K_{mGAP}$  and  $K_{iGAP}$  were identifiable for all 64 of the fits.  $K_{mNADP}$ ,  $K_{mNADPH}$ ,  $K_{mG1P}$  and  $\alpha$  were identifiable for 56, 54, 56 and 60 of the fits respectively. For all of the combinations of fits where  $K_{mNADP}$  was not identifiable, either  $V_m$  and  $K_{iGAP}$  or  $K_{mGAP}$  and  $K_{iGAP}$  were fixed. For all of the combinations of fits where  $K_{mNADPH}$  was not identifiable, either  $\alpha$  or  $K_{iGAP}$  was fixed. Either  $\alpha$  or  $K_{iGAP}$  was fixed when  $K_{mG1P}$  was not identifiable and for the combinations where  $\alpha$  was not identifiable  $K_{iGAP}$  was fixed, see Tables 66 - 72.

None of the initial rate data fits returned any identifiable parameters, see Figure 4.16.

The results of the identifiability analysis of the GAPN experimental data and GAPN *in silico* generated data are summarised in Chapter 5.



Identifiable parameter estimations with non-saturating *in silico* generated data with 10% noise and temperature lag for GAPN

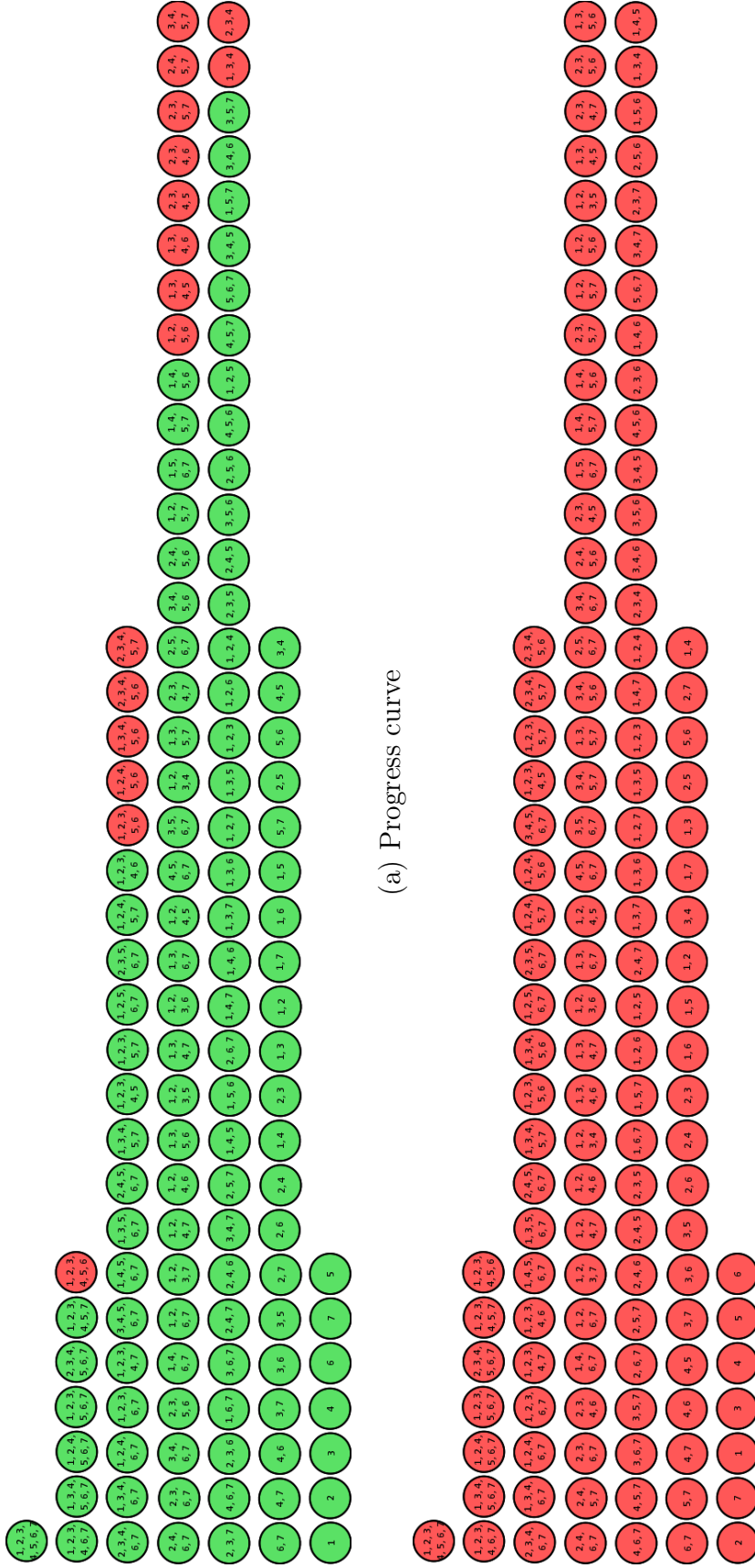


Figure 4.16: Graphical representation of the combination of fits where all the parameters fitted for are identifiable (green nodes) and where one or more of the parameters fitted for are not identifiable (red nodes) with non-saturating *in silico* generated data with 10% noise and initial temperature lag. The numbers indicate which of the parameters are fitted for:  $V_m$  (1),  $K_{mGAP}$  (2),  $K_{mNADP}$  (3),  $K_{mNADPH}$  (4),  $K_{mGIP}$  (5),  $\alpha$  (6),  $K_{iGAP}$  (7).

## 4.2 PGI results

### Initial rate data fitting and identifiability analysis

Anabolic initial rate data, where F6P is converted to G6P, were collected for PGI. Figure 4.17 shows the averaged initial rate data, collected with a UV/VIS spectrophotometer, and the estimated fit to equation 3.6. With only two parameters the identifiability analysis of the initial rate PGI data is much simpler than that of the GAPN initial rate data. When fitting for both of the parameters,  $V_m$  and  $K_{mF6P}$ , parameter value estimations of 2.474 U/mg and 0.04609 mM were returned respectively. The parameter values of both the parameters were identifiable, see Figure 4.18. The parameter value estimations and their respective 95% confidence intervals can be seen in Table 4.1.

$V_m$	$K_{mF6P}$
2.474 (1.869-3.079)	0.04609 (0.04144-0.05073)

Table 4.1: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for both parameters with anabolic initial rate data.

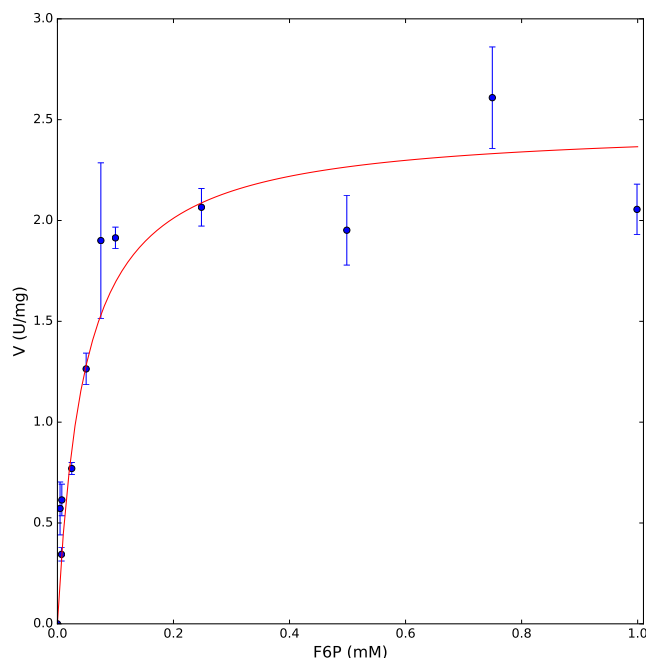


Figure 4.17: The initial rates of the PGI enzyme with varied concentrations of F6P (blue data points with error bars) and the fit to the rate equation 3.6 (solid red line). The error bars are an indication of the variance in the experimental data.

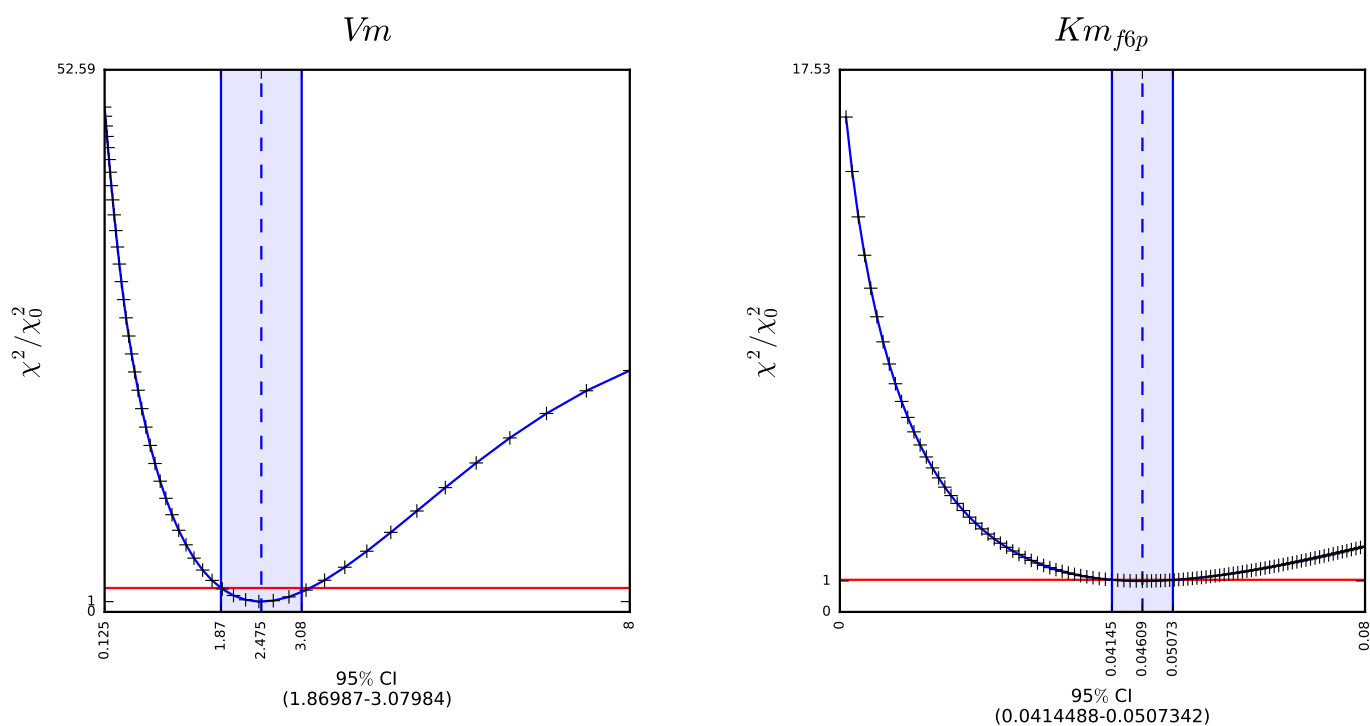


Figure 4.18: Profile likelihood plots of  $V_m$  and  $K_{m_{F6P}}$  with the fitting of initial rate data. The 95% confidence intervals are depicted by the two solid blue lines parallel to the estimated parameter values depicted by the dashed lines. The solid red lines are the thresholds in the  $\chi^2$  values that indicate the 95% confidence intervals.

### Progress curve data fitting and identifiability analysis

Five time courses of PGI data were collected with NMR spectroscopy for progress curve analysis. Four of the five sets of time course data, that have been fitted to equation 3.5 can be seen in Figure 4.19. As we did for the identifiability analysis of the GAPN data, we started the identifiability analysis of the PGI progress curve data by fitting for all four parameters of equation 3.5 simultaneously. Thereafter one parameter was fixed while the remaining three parameters were fitted for. Parameter value estimations for *S. solfataricus* PGI were not found in literature, therefore unpublished values for these were used (personal communication with Prof. Snoep). This process was continued until only one parameter was fitted for while the remaining three parameters were fixed to the unpublished parameter values. The following unpublished values have been used for the fitting of all possible combinations of the four parameters:  $V_f$ : 23.58,  $K_{mG6P}$ : 0.2000 and  $K_{mF6P}$ : 0.4400. The literature value of 0.3300 for  $K_{eq}$  was used [62].

When fitting for all four parameters,  $V_f$ ,  $K_{eq}$ ,  $K_{mG6P}$  and  $K_{mF6P}$ , only the parameter value estimation for  $K_{eq}$  was identifiable (see Table 1). The combination of  $V_f$ ,  $K_{eq}$  and  $K_{mG6P}$  returned the highest number of identifiable estimated values when fitted for, see Figures 4.20 (a) and 4.21. For the other combinations of three parameters fitted for,  $K_{mF6P}$  was not identifiable. Complete parameter value identifiability was only obtained when  $K_{mF6P}$  was not fitted for (see Table 2). For the fitting of two parameters, there are two cases that returned non-identifiable parameter value estimations: the fitting of  $K_{mG6P}$  and  $K_{mF6P}$ , while  $V_f$  and  $K_{eq}$  were fitted for and *vice versa*. For the four other cases, that returned identifiable parameter estimations, one parameter of both of the above mentioned pairs were fixed while the other was fitted for (see Table 3). Fitting for only one parameter returned identifiable parameter estimations for all four parameters as is shown in Table 4.

The parameter value for  $K_{mF6P}$ , obtained from the initial rate data analysis shown in the previous section, was also used for the parameter value identifiability analysis of the progress curve data. The value of 0.04609 for  $K_{mF6P}$  was used in conjunction with the unpublished values for  $V_f$  (23.58) and  $K_{G6P}$  (0.2000), and the literature value of 0.3300 for  $K_{eq}$  [62]. The use of the experimentally determined value for  $K_{mF6P}$  did not alter the outcome of the identifiability analysis significantly. The combination of  $V_f$ ,  $K_{eq}$  and  $K_{mG6P}$  again returned the highest number of identifiable estimated values when fitted for, see Figures 4.20 (b) and 4.22. The identifiability of the parameter values for all possible combinations were the same, save for slight variations in the parameter values themselves, (see Tables 5 - 8) and that identifiable parameter estimations for  $V_f$  and  $K_{eq}$  could be determined while  $K_{mF6P}$  and  $K_{mG6P}$  were fixed. This combination of parameters were not identifiable with the use of the unpublished value for  $K_{mF6P}$  (see

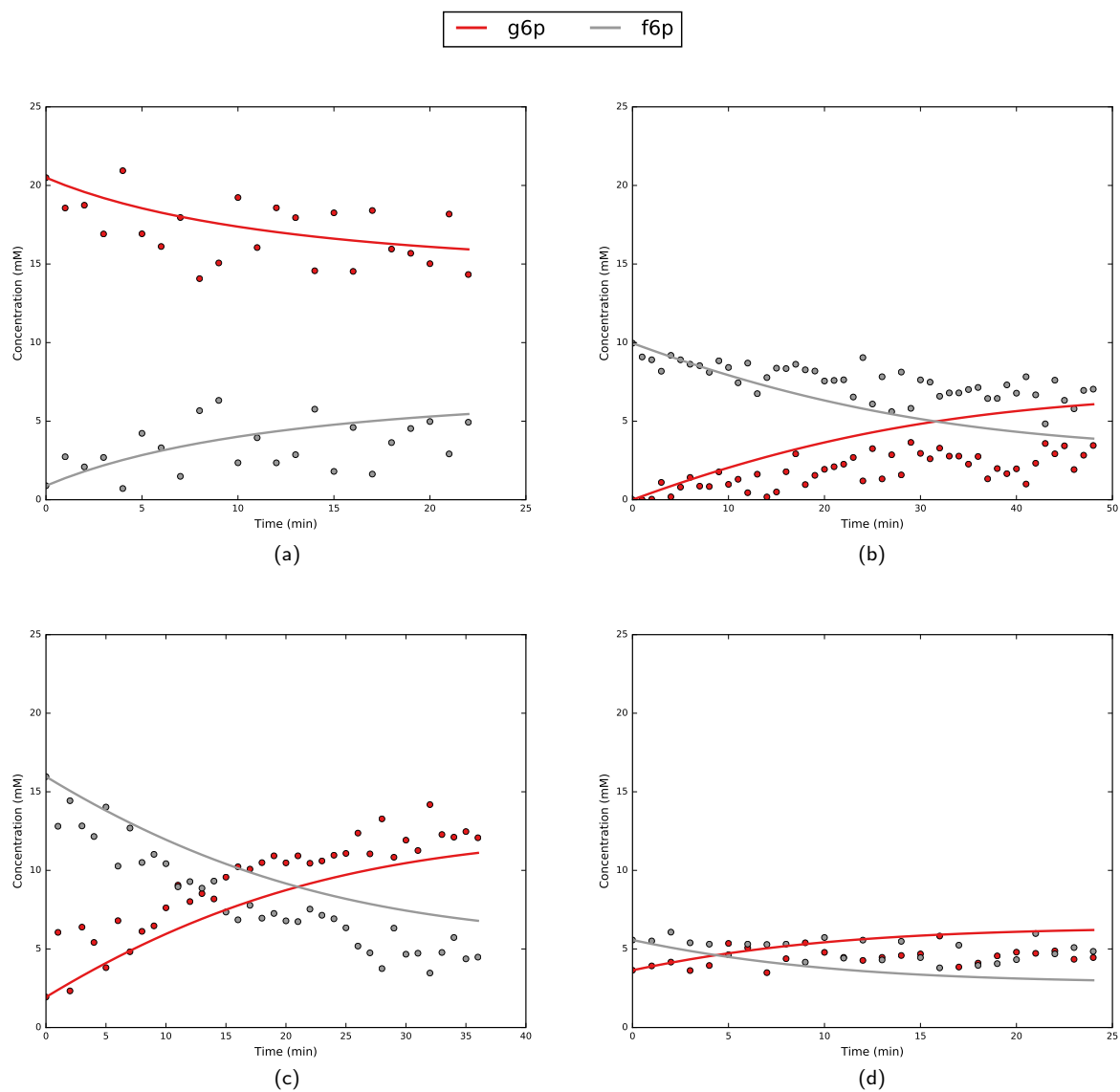


Figure 4.19: The progress curve data collected with NMR spectroscopy (dots) and the fit to the rate equation 3.5 (lines) for the initial concentrations of 20 mM G6P and 0 mM F6P (a), 10 mM F6P and 0 mM G6P (b), 20 mM F6P and 0 mM G6P (c), and 5 mM F6P and 5 mM G6P (d).

Figure 4.20 (b) and Table 7).

The results of the identifiability analysis of the PGI data are summarised in Chapter 5.

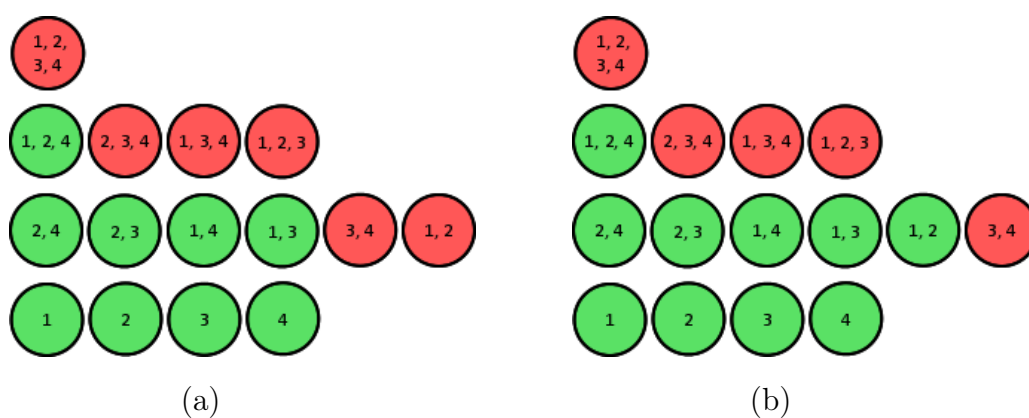


Figure 4.20: Graphical representation of the combination of fits that return identifiable parameter estimations with progress curve data.  $V_f$  (1),  $K_{eq}$  (2),  $K_{mF6P}$  (3),  $K_{mG6P}$  (4). The green nodes represent cases where all the parameters fitted for are identifiable. The red nodes represent cases where one or more of the parameters fitted for are not identifiable. The parameter set used for the identifiability analysis shown by (a) are the unpublished values for  $V_f$ ,  $K_{mF6P}$  and  $K_{mG6P}$  and the literature value for  $K_{eq}$ . The parameter set used for the identifiability analysis shown by (b) are the unpublished values for  $V_f$  and  $K_{mG6P}$ , the literature value for  $K_{eq}$  and the value for  $K_{mF6P}$  determined experimentally in this study from initial rate data.

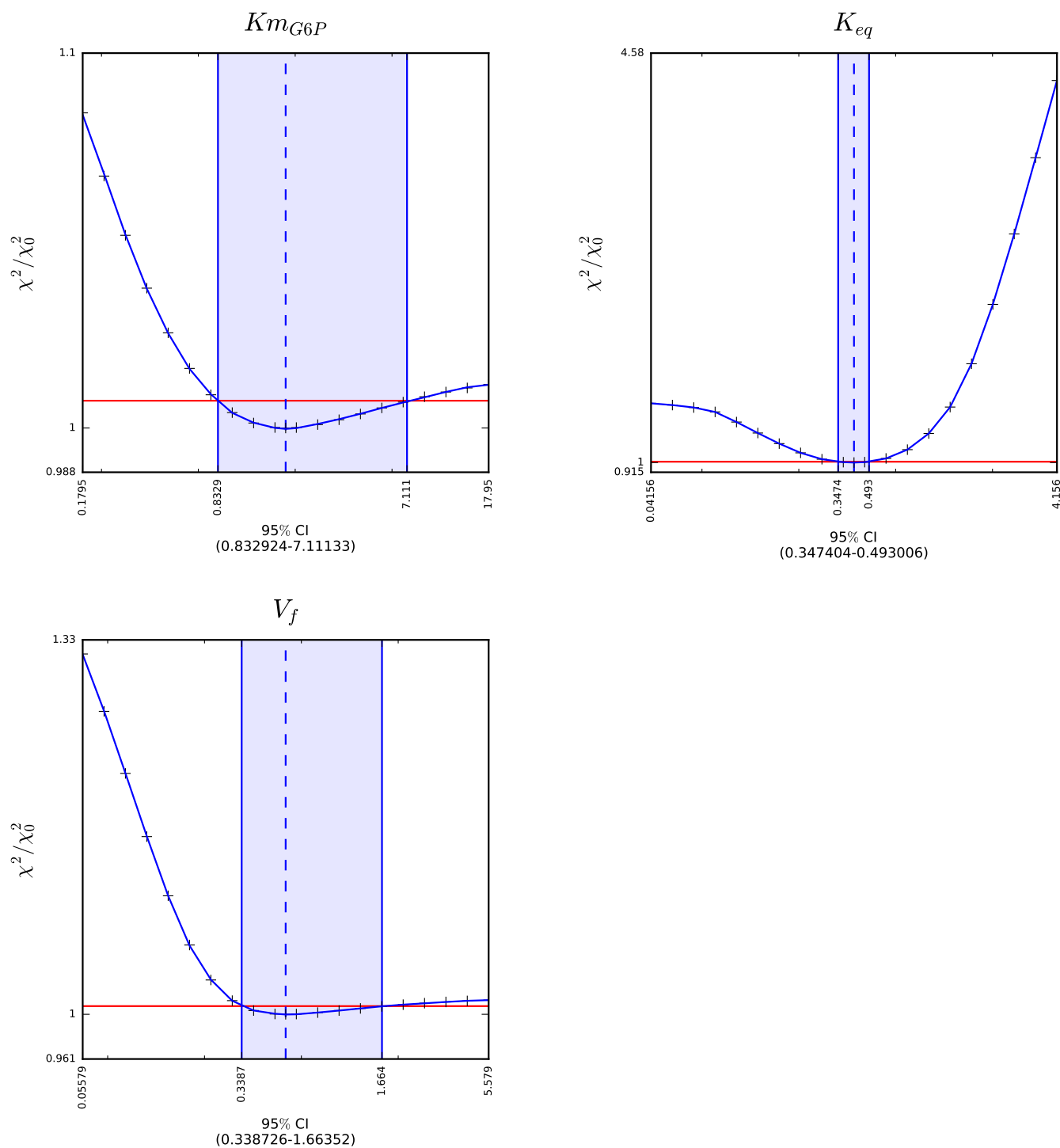


Figure 4.21: Profile likelihood plots of  $V_f$ ,  $K_{eq}$  and  $K_{mG6P}$  with the fitting of progress curve data, while the value of  $K_{mF6P}$  is fixed to the unpublished value of 0.4400. The 95% confidence intervals are depicted by the two solid blue lines parallel to the estimated parameter values depicted by the dashed lines. The solid red lines are the thresholds in the  $\chi^2$  values that indicate the 95% confidence intervals.

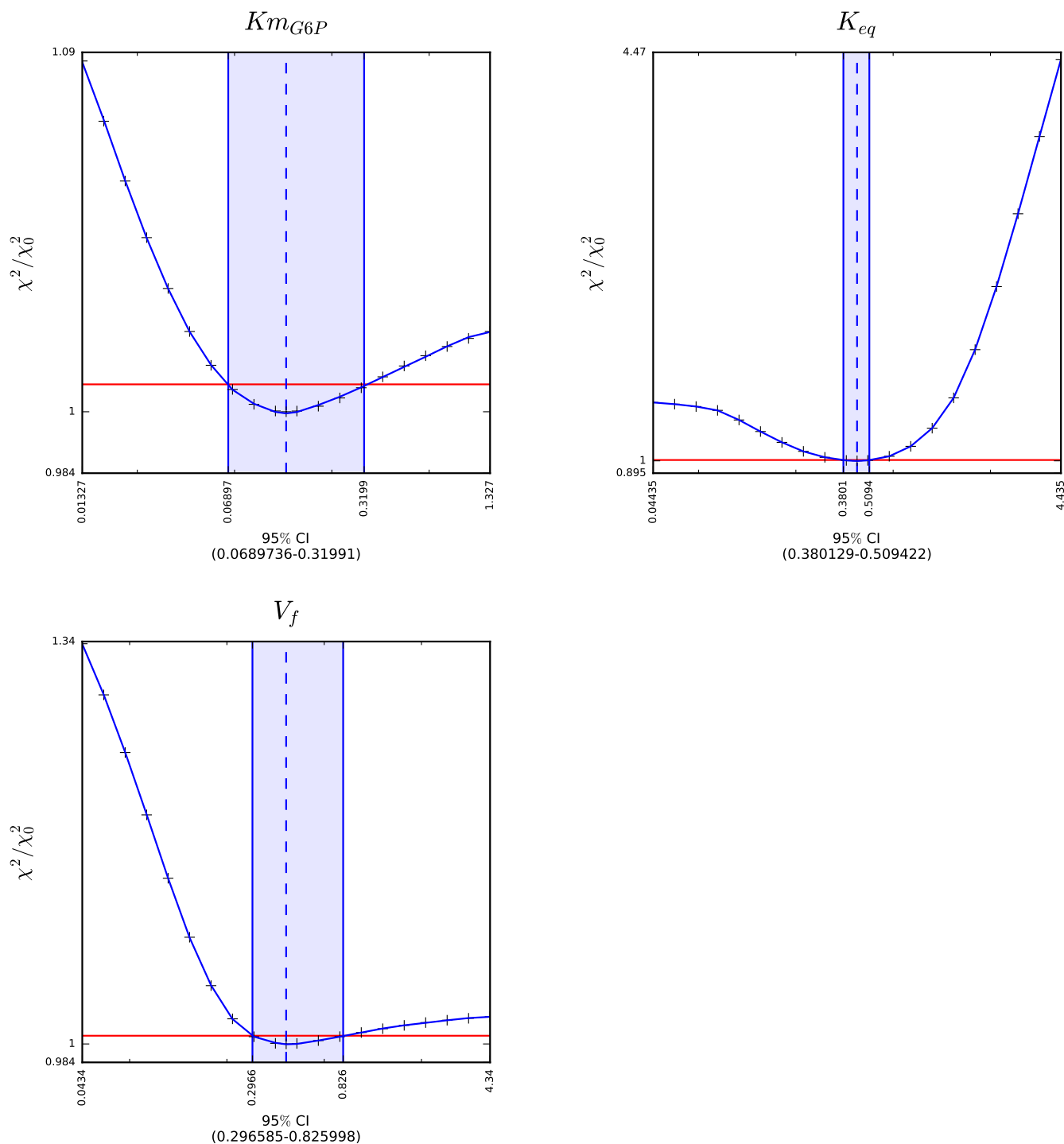


Figure 4.22: Profile likelihood plots of  $V_f$ ,  $K_{eq}$  and  $K_{mG6P}$  with the fitting of progress curve data, while the value of  $K_{mF6P}$  is fixed to the value of 0.04609 determined experimentally with the analysis of initial rate data. The 95% confidence intervals are depicted by the two solid blue lines parallel to the estimated parameter values depicted by the dashed lines. The solid red lines are the thresholds in the  $\chi^2$  values that indicate the 95% confidence intervals.



# Chapter 5

## Discussion

Identifiability analysis of both experimental and *in silico* generated GAPN data and experimental PGI data were completed. The profile likelihood plots of the parameters were examined to determine if the non-identifiability of certain parameter values are of a structural or practical nature.

### GAPN

For a comprehensive analysis of the identifiability of the GAPN parameters a supplementary *in silico* analysis was required due to discrepancies in our experimentally determined parameter values and previously determined, unpublished parameter values. The identifiability analysis of the GAPN experimental data showed very few completely identifiable parameter combinations for the progress curve data and none at all for the initial rate data, see Figure 5.1 (i). Figure 5.1 presents graphs of the number of completely identifiable parameter combinations in contrast with the number of combinations that include non-identifiable parameter estimations for experimental and *in silico* generated GAPN data.

When considering the enzyme saturating datasets (Figure 5.1 (ii) - (v)), the non-identifiability of parameter estimations returned by the progress curve analysis are quite evenly distributed between structural and practical non-identifiability. The practical non-identifiability, in which the data is lacking in either quality or quantity to accurately describe the GAPN kinetics, can largely be attributed to temperature lag. The initial temperature lag effect reduces the quality of the data more than the effect of the 10% noise. The structural non-identifiability of the progress curve data parameter estimations can be attributed to the possible parameter correlations. The non-identifiability of  $K_{mNADP}$  and  $K_{mNADPH}$  contribute most to the overall non-identifiability of the parameter fits. For nearly all of the fits where  $K_{mNADP}$  and  $K_{mNADPH}$  are identifiable, the value for  $V_m$  is fixed. This non-identifiability of  $K_{mNADP}$  and  $K_{mNADPH}$  if  $V_m$  is also fitted for is an indication of possible parameter correlation.

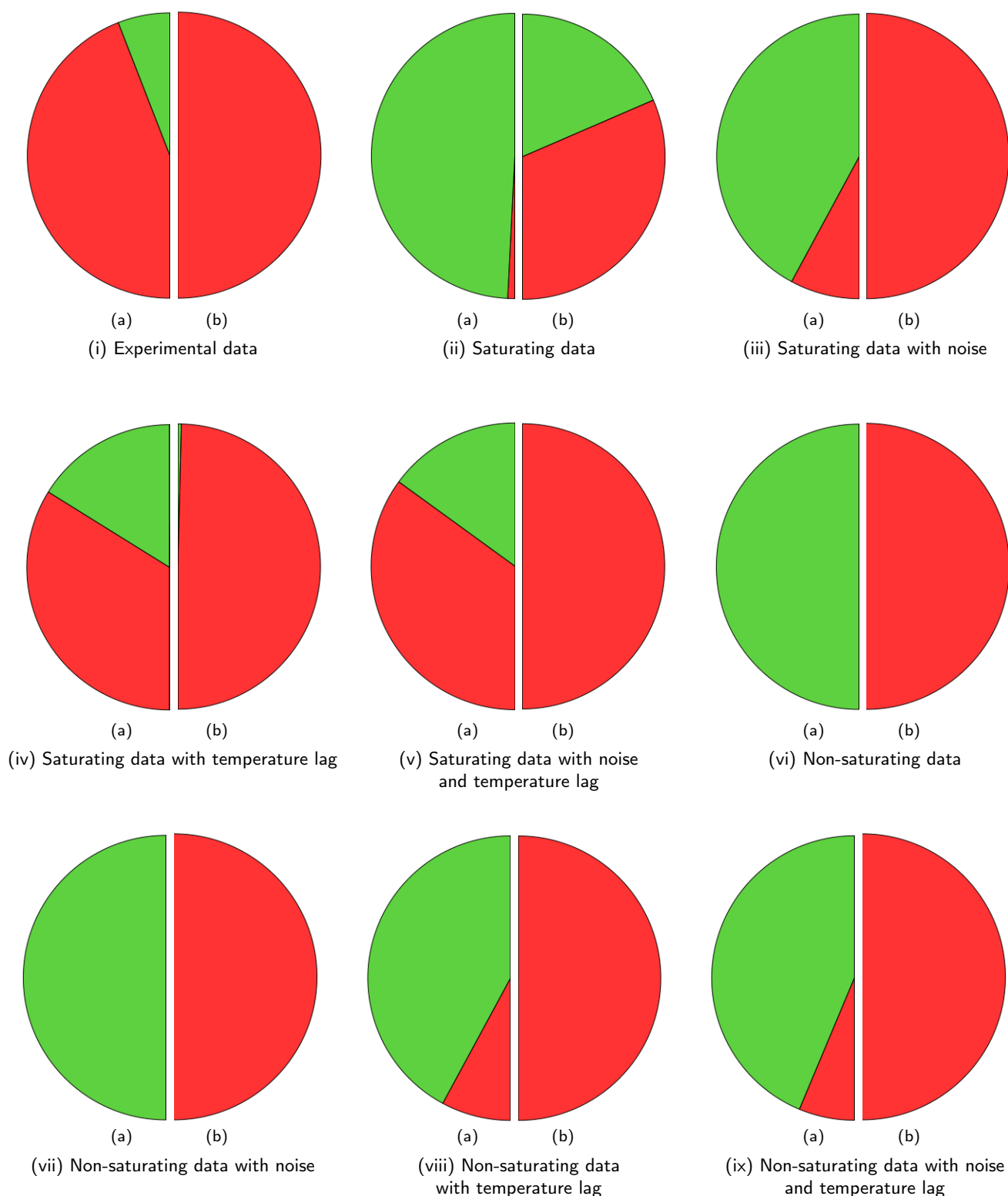


Figure 5.1: Visual representation of the identifiability analysis of the progress curve (a) and initial rate (b) experimental and *in silico* generated GAPN data. The green areas of the graphs represent the number of fits where all the parameters fitted for are identifiable while the red areas represent the number of fits where one or more of the parameters fitted for are not identifiable.

In the majority of the fits where either  $K_{mNADP}$  or  $K_{mNADPH}$  have been fixed while the other parameter is fitted for, and *vice versa*, the one parameter is able to compensate for the change in the other parameter. This means that for most of the fits  $K_{mNADP}$  is identifiable if  $K_{mNADPH}$  is fixed and  $K_{mNADPH}$  is identifiable if  $K_{mNADP}$  is fixed. This parameter correlation is not seen in the initial rate data as the structural non-identifiability is not due to parameter correlation, but due to the GAPN model being too complex to be characterised by the initial rate type data.

The non-identifiability of parameter estimations returned by the initial rate analysis is mostly structural non-identifiability. This explains why the majority of the datasets are not able to return identifiable parameter estimations with initial rate analysis (see Figures 5.1 (ii) - (ix)). The initial rate data cannot adequately constrain the model dynamics. Some of the non-identifiability is also of practical nature, as such the set of enzyme saturating data, without any initial temperature lag or noise, is the only dataset able to return identifiable parameter estimations with initial rate analysis, albeit only a few combinations (see Figure 5.1 (ii)). The temperature lag, noise, and non-saturating concentration ranges result in poor quality datasets which are inadequate for the characterisation of identifiable parameter values as the model is underdetermined by the data (see Figure 5.1 (iii) - (ix)). This is an example of structurally identifiable parameters that are practically non-identifiable due to the use of insufficient data [52].

As was seen with the analysis of the initial rate and progress curve data of *S. cerevisiae* TPI, mentioned in Chapter 2, the progress curve analysis method is robust when given imperfect datasets. The TPI progress curve data were incomplete time courses, having not yet reached equilibrium, and the experimental design favoured initial rate analysis. Some accurate parameter estimations were still obtained with progress curve analysis [46]. Similarly the experimental data used in our GAPN case study were used for both progress curve and initial rate analysis. However, the experimental design was such that neither of the two methods were disadvantaged. The ligand concentration ranges chosen were enzyme saturating concentrations and the assays were run to completion, allowing for equilibrium to be reached.

Interestingly, there are some datasets where the fitting of all seven parameters returned all seven parameters as identifiable, but for various fits, where some of the parameters were fixed, not all of the parameters were identifiable. It is unexpected that parameter non-identifiability is seen when some parameters have been fixed in a datasets where all of the parameters are identifiable when all parameters are fitted for. This phenomenon can be attributed to the discrepancies between our experimentally determined parameter values and the parameter values previously determined by Dr. Kouril (personal communication). The difference between these parameter values might be such that fixing some

parameters to the previously determined values causes the system to no longer be able to find identifiable parameter values. However, when the system is not constrained it is able to find identifiable parameter estimations for all seven parameters.

## PGI

Unlike for the GAPN enzyme, the experimental PGI data were sufficient for the completion of the identifiability analysis and therefore a supplementary *in silico* analysis was not necessary.

When considering the PGI data analysis, no non-identifiability occurred with the identifiability analysis of the initial rate PGI data. The non-identifiability of the PGI parameter combinations with the fitting of progress curve data are structural non-identifiability. This suggests some correlation between the four parameters. The results of the identifiability analysis of all possible combinations of these four parameters suggests correlation between  $K_{mF6P}$  and  $K_{mG6P}$ . Identifiable parameter estimations for both of these parameters cannot be obtained if both are fitted for. Due to the non-identifiability being structural and not practical, the contribution of other factors, such as noise or initial temperature lag, towards the non-identifiability is irrelevant. Parameters that are structurally identifiable are not necessarily practically identifiable [52].

Given the results of the identifiability analysis of this case study, there is a clear difference between the identifiability of parameter value estimations with the use of progress curve and initial rate analysis. There are seven GAPN parameters as opposed to only two PGI parameters that were fitted for with the anabolic PGI initial rate data and four PGI parameters fitted for with progress curve data. When disregarding the differences in enzyme kinetics and the fact that this is an isolated case study of only two examples of enzyme kinetics, it would seem that the degrees of freedom, represented by the number of parameters that are to be characterised by a dataset, contribute to the identifiability or non-identifiability of the parameters. If it were possible to extend this case study to more generic conditions, our results would imply that either progress curve or initial rate analysis is sufficient for the characterisation of identifiable parameter estimations when a small number of parameters are involved. However, with an increase in the number of parameters that need to be characterised it would seem that progress curve analysis is more robust in terms of returning identifiable parameter estimations.

As this case study only includes two examples of enzyme kinetics, a uni-uni system and a bi-bi system, the results may be more generalisable if more examples of enzyme kinetics are included in the comparative analysis.

In the light of the amount of information given to each method, specifically the number of data points, determining the superiority of either method is trivial as we favour

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progress curve analysis. In this case study we consider the following: given a certain number of incubations, does it make sense to let the initial rate assays run to completion in order to conduct progress curve analysis instead. This however raises the issue of the type of equipment used. If the use of NMR spectroscopy is the only viable option, the comparison of these two methods is rendered futile. Conducting initial rate analysis on NMR data is hardly ever considered. However, with the use of light spectroscopy an assay run can either be terminated once initial rate data have been collected or the assay can be allowed to run to completion. If an incubation is allowed to reach equilibrium, no additional experimental effort is required, only more time, depending on how long it takes the enzyme(s) to reach equilibrium. However this approach returns much more data per enzyme assay. To the question whether it is unnecessary to collect the additional data or if it greatly improves the parameter fitting and identifiability results, we could reply that for complex rate equations it would appear to be beneficial, while the added advantage for simple kinetic equations is less clear.

The model and rate equation used for data fitting can influence the identifiability of parameter estimations [52]. The use of increasingly complex rate equations can lead to the overparameterisation of systems. The use of model reduction methods can facilitate the increase in the number of identifiable parameters [4, 61]. The different model reduction methods can be categorised into three groups. The first group of methods are lumping methods which reduce models by creating single, new parameters for groups of parameters. The second group of methods are sensitivity analysis methods which identifies parameters that are less crucial for the description of model dynamics and these parameters are excluded. The third group of methods are time-scale methods which are used if some parts of the model dynamics function much faster or slower than the rest [61]. These methods aim to improve parameter identifiability by altering the model and rate equation. This project however focuses only on the importance of the improvement of data quality to increase parameter identifiability.

To further investigate the difference between the two methods, another type of comparison to consider is to provide each of the two methods with the exact same number of data points before completing the parameter fitting and identifiability analysis. A distinct number of data points can be selected for the comparative study, say 100 points for example. Initial rate data can then be collected in the form of 10 initial rate assays consisting of 10 data points each and 5 time courses of 20 data points each can be collected for progress curve analysis. By following this approach, the comparative study of the progress curve and initial rate data analysis methods are far less biased towards progress curve analysis.

# Chapter 6

## Conclusion

The aim of this study was to investigate the difference in identifiability of parameter estimations from initial rate and progress curve data analysis. Subsequently we aimed to determine what the contributing factors are to the difference in the identifiability analysis. A comparative identifiability analysis of GAPN parameter estimations with initial rate and progress curve analysis of experimental and different *in silico* generated datasets was completed. Identifiability analysis of experimental PGI initial rate and progress curve data was also completed. Determining identifiable parameter estimations for the bi-bi, irreversible enzyme kinetics of the GAPN enzyme, with allosteric activation and product inhibition, proved a greater challenge, as expected than for the reversible, uni-uni enzyme kinetics of the PGI enzyme. When considering the type of enzyme kinetics of GAPN, with seven parameters present, overall the progress curve analysis method is more robust than the initial rate analysis method in terms of returning identifiable parameter value estimations with the use of imperfect data. When considering the type of enzyme kinetics of PGI, with fewer parameters than GAPN, both the initial rate and progress curve analysis methods return identifiable parameter estimations.

Our analysis is based on the preferential treatment of progress curves as we provided this method with considerably more data. However, allowing the initial rate assays to run to equilibrium and processing the data as progress curve data instead proved more beneficial for enzyme kinetics with more degrees of freedom. It is not possible to give a conclusive answer to which of the two methods is superior for the analysis of a specific type of enzyme kinetic mechanism. Much will be dependent on the equipment that is available for the assay, e.g. NMR versus spectrophotometer; or the assay itself, i.e. directly linked to NADPH or only assessable via multiple linked reactions. However, our data do show that for more complicated enzyme kinetic mechanisms it is beneficial for the identifiability of enzyme kinetic parameters to prolong the period over which the experimental data are captured and to analyse the data with a progress curve analysis.

# Appendix A

## GAPN parameter value estimations and confidence intervals

### Experimental data

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
3.052222 (3.051-21.49)	1.092604 (1.092-8.427)	x	x	x	0.268213 (0.2681-0.2862)	x

Table 1: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of the experimental data.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	x	x	x	x	x	x
145.718047 (80.81-159.5)		x	x	x	0.331816 (0.2931-0.3319)	x
x	x		0.000602 (0.0005-0.0006)	x	0.287975 (0.2879-0.3164)	x
x	x	x		x	x	x
x	x	x	x		0.240256 (0.1876-0.2403)	x
x	0.300785 (0.1931-0.6929)	x	0.000017 (1.61-1.72x10 <sup>-5</sup> )	0.009521 (0.0094-0.0098)		x
x	x	x	x	x	x	

Table 2: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of the experimental data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		x	x	x	0.000553 (0.0005-0.0006)	x
	x		x	x	x	x
	x	x		x	x	x
	0.183356 (0.1765-0.1834)	0.000019 (1.88-1.90x10 <sup>-5</sup> )	x		0.058169 (0.0578-0.0596)	x
	0.181539 (0.1584-0.1816)	x	x	0.004622 (0.0036-0.0047)		x
	x	x	x	x	x	
x			x	x	x	x
x		x		x	x	x
x		x	x		0.294370 (0.2943-0.7111)	x
x		x	x	x		2.3313x10 <sup>5</sup> (2.33-2.61x10 <sup>5</sup> )
184.712351 (184.7-207.5)		x	x	x	0.324048 (0.2166-0.4906)	
0.488707 (0.1757-0.4887)	0.349705 (0.1642-0.4069)			x	0.349757 (0.3487-0.3506)	x
x	0.801447 (0.2628-0.8015)		0.000410 (0.0004-0.0005)		0.243037 (0.1910-0.2431)	x
x	0.301081 (0.2026-0.6580)		0.000417 (0.0004-0.0005)	0.009621 (0.0094-0.0097)		x
x	x		x	x	x	
x	x	x			0.293080 (0.2751-0.2932)	x
x	0.192303 (0.1598-0.1923)	x		0.007187 (0.0070-0.0073)		x
x	x	x		x	x	
x	0.300992 (0.2030-0.6597)	x	x			x
x	x	x	x		0.254621 (0.1349-0.4407)	
x	0.301251 (0.3011-0.3130)	x	x	0.009354 (0.0047-0.0219)		

Table 3: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of the experimental data.



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
			0.003475 (0.0034-0.0035)	x	0.000507 (0.0005-0.0006)	x
		x		x	x	x
		x	x		0.000060 (5.89-6.06x10 <sup>-5</sup> )	x
		x	x	x		x
		x	x	x	0.000551 (0.0005-0.0006)	
	x			x	x	x
	0.170997 (0.1705-0.1714)		0.003661 (0.0036-0.0037)		0.056574 (0.0557-0.0573)	x
	0.167462 (0.1669-0.1679)		0.003048 (0.0030-0.0031)	0.003727 (0.0036-0.0038)		x
	0.202924 (0.2023-0.2034)		0.006126 (0.0060-0.0062)	x	0.201934 (0.2007-0.2029)	
	0.161854 (0.1615-0.1622)	0.260235 (0.2158-0.2603)			0.180566 (0.1630-0.1807)	x
	0.137328 (0.1299-0.1374)	0.439088 (0.4390-0.4391)		0.001681 (0.0015-0.0017)		x
	0.217867 (0.1746-0.2179)	0.149436 (0.1267-0.1495)		x	0.273580 (0.2727-0.2745)	
	0.179057 (0.1606-0.1791)	x	x			x
	0.172372 (0.1723-0.1922)	x	x		x	
	0.178482 (0.1784-0.1933)	x	x	x		
x				x	x	x
345.230976 (345.2-388.7)			0.000374 (0.0003-0.0004)		0.380696 (0.3726-0.3808)	x
x			x	0.000039 (3.43-4.14x10 <sup>-5</sup> )		x
204.443314 (204.4-229.7)			0.000446 (0.0004-0.0005)	x	0.324111 (0.2007-0.6035)	
x		x			0.000213 (0.0002-0.0003)	x
x		x		x		x
x		x		x	x	
x		x	x			x
x		39.535502 (9.528-44.55)	0.000893 (0.0002-0.0009)		0.365969 (0.2254-0.8124)	
8.551x10 <sup>8</sup> (2.13-9.67x10 <sup>8</sup> )		3834.7068 (3823-4311)	x	x		
x	0.231077 (0.2147-0.2311)				0.361708 (0.3548-0.3620)	x

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.246590 (0.2465-0.2578)	0.130144 (0.1238-0.1302)			x		x
0.498540 (0.4508-0.6143)	0.356241 (0.3543-0.3576)			x	0.352599 (0.3511-0.3534)	
x	0.301229 (0.2033-0.6578)		0.000432 (0.0004-0.0005)			x
x	x		0.000410 (0.0004-0.0005)		0.248353 (0.1947-0.2484)	
x	0.302026 (0.2031-0.6601)		x	0.009689 (0.0096-0.0097)		
x	0.192572 (0.1588-0.1926)	x				x
x	x	x			x	
x	0.193162 (0.1592-0.1932)	x		0.007298 (0.0071-0.0074)		
x	x	x	x			

Table 4: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of the experimental data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				x	x	x
			0.002435 (0.0024-0.0025)		0.000052 (5.16-5.31x10 <sup>-5</sup> )	x
			x	x		x
			0.003503 (0.0034-0.0035)	x	0.000505 (5.01-5.08x10 <sup>-4</sup> )	
		x			x	x
		x		x		x
		0.239685 (0.2385-0.2410)		x	0.000847 (0.0008-0.0009)	
		x	x			x
		x	x		0.000059 (5.85-6.01x10 <sup>-5</sup> )	
		x	x	x		
	x				x	x
	0.204418 (0.2040-0.2048)			0.007367 (0.0072-0.0075)		0.503065 (0.5011-0.5049)
	0.263007 (0.2630-0.2859)			x	0.310522 (0.3104-0.3162)	
	0.167058 (0.1534-0.1671)		0.003059 (0.0011-0.0031)			x
	0.170606 (0.1658-0.1707)		0.003706 (0.0012-0.0037)		0.056793 (0.0556-0.0571)	
	0.167077 (0.1537-0.1671)		0.003079 (0.0011-0.0031)	0.003720 (0.0036-0.0038)		
	0.137054 (0.1301-0.1371)	0.435119 (0.4351-0.4517)				x
	x	x			x	
	0.137352 (0.1301-0.1374)	0.434089 (0.4334-0.4349)		0.001675 (0.0016-0.0017)		
	0.178146 (0.1607-0.1782)	x	x			
0.244841 (0.2448-0.2666)					0.000051 (5.04-5.20x10 <sup>-5</sup> )	x
x				x		x
45.958667 (45.95-46.38)				x	0.510373 (0.5010-0.5104)	
x			x			x
x			0.000201 (0.0001-0.0002)		0.375477 (0.2228-0.8264)	
x			x	x		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
x		x				x
x		x			0.000215 (2.11-2.16x10 <sup>-4</sup> )	
x		x		x		
x		x	x			
0.248312 (0.2483-0.2601)	0.130175 (0.1238-0.1302)					x
x	0.235847 (0.2178-0.2359)				0.368028 (0.3610-0.3683)	
0.248078 (0.2480-0.2584)	0.130455 (0.1243-0.1305)			x		
x	0.301896 (0.2034-0.6620)		0.000426 (0.0004-0.0005)			
x	0.192918 (0.1591-0.1930)	x				

Table 5: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of the experimental data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.000264 (0.0002-0.0003)	x
				x		x
				x	0.001608 (0.0016-0.0017)	
			x			x
			x		0.000052 (5.13-5.25x10 <sup>-5</sup> )	
			x	x		
		x				x
		0.476257 (0.4722-0.4764)			0.000048 (4.73-4.85x10 <sup>-5</sup> )	
		x		x		
		x	x			
	0.205012 (0.1956-0.2051)					x
	0.226300 (0.2142-0.2263)				0.355014 (0.3305-0.3551)	
	0.281527 (0.2815-0.2816)			0.009984 (0.0098-0.0100)		
	0.166656 (0.1532-0.1667)		0.003090 (0.0011-0.0031)			
	0.137117 (0.1302-0.1372)	0.429955 (0.4296-0.4314)				
3.125203 (3.122-3.413)						0.113949 (0.0839-0.2120)
0.246269 (0.2462-0.2682)					0.000052 (5.11-5.25x10 <sup>-5</sup> )	
x				x		
x			x			
x		x				
0.249858 (0.2498-0.2506)	0.130527 (0.1302-0.1307)					

Table 6: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of the experimental data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						x
					0.000683 (0.0006-0.0007)	
				x		
			x			
	0.286014 (0.2856-0.2863)	x				
1.329697 (1.328-1.330)						

Table 7: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for one parameter using progress curve analysis of the experimental data.

## GAPN parameter value estimations and confidence intervals

Enzyme saturating *in silico* generated data

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.919038 (0.9190-1.001)	81.328101 (74.70-81.33)	0.117850 (0.1081-0.1179)	0.066237 (0.0607-0.0663)	0.007238 (0.0066-0.0073)	0.007647 (0.0070-0.0077)	16.741743 (16.74-18.23)

Table 8: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of enzyme saturating *in silico* generated data.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
x	x	x	x	0.008686 (0.0008-0.0009)	x	358.018 (358.0-369.9)

Table 9: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using initial rate analysis of enzyme saturating *in silico* generated data.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	55.714764 (50.83-55.72)	0.004574 (0.0041-0.0046)	0.002981 (0.0027-0.0030)	0.011721 (0.0117-0.0127)	0.004326 (0.0039-0.0044)	24.900482 (24.90-27.39)
0.557201 (0.5572-0.6100)		0.093086 (0.0853-0.0931)	0.050676 (0.0464-0.0507)	0.015247 (0.0152-0.0165)	0.003745 (0.0034-0.0038)	26.855624 (26.85-29.23)
0.865623 (0.7953-0.8657)	80.796370 (74.21-80.80)		0.040427 (0.0371-0.0405)	0.007164 (0.0065-0.0072)	0.007648 (0.0070-0.0077)	16.782672 (16.78-18.27)
0.870169 (0.7994-0.8702)	80.767297 (74.18-80.77)	0.072122 (0.0721-0.0785)		0.007190 (0.0066-0.0072)	0.007647 (0.0070-0.0077)	16.786467 (16.78-18.28)
0.923515 (0.9235-1.006)	81.835391 (75.17-81.84)	0.117048 (0.1074-0.1171)	0.065969 (0.0605-0.0660)		0.008131 (0.0074-0.0082)	16.642332 (16.64-18.12)
0.953105 (0.9531-1.038)	83.927531 (77.08-83.93)	0.117504 (0.1078-0.1175)	0.066106 (0.0606-0.0661)	0.005343 (0.0049-0.0054)		16.194609 (16.19-17.63)
0.624336 (0.6243-0.6772)	57.412964 (57.41-62.03)	0.113015 (0.1045-0.1131)	0.062391 (0.0576-0.0624)	0.012058 (0.0120-0.0130)	0.004524 (0.0041-0.0046)	

Table 10: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	x	x	x	x	x	16.769092 (16.28-16.77)
x		x	x	x	0.022822 (0.0228-0.0262)	84.020428 (81.58-84.02)
0.088533 (0.0885-0.0886)	378.371083 (367.2-378.4)		x	0.008902 (0.0089-0.0091)	x	362.504295 (351.5-362.6)
x	x	x		x	x	x
x	x	x	x		0.003172 (0.0030-0.0032)	352.350668 (352.3-368.4)
x	x	x	x	x		x
0.247296 (0.2472-0.5544)	153.308279 (153.3-436.6)	x	x	x	x	

Table 11: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using initial rate analysis of enzyme saturating *in silico* generated data.



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		0.044623 (0.0409-0.0447)	0.026254 (0.0240-0.0263)	0.014733 (0.0147-0.0160)	0.003710 (0.0034-0.0037)	27.019284 (27.01-29.42)
	52.448879 (48.11-52.45)		0.043089 (0.0395-0.0431)	0.013586 (0.0135-0.0147)	0.003746 (0.0034-0.0038)	26.827166 (26.82-29.24)
	52.901933 (49.53-52.91)	0.066443 (0.0664-0.0723)		0.013271 (0.0121-0.0133)	0.003803 (0.0034-0.0038)	26.606515 (26.60-29.00)
	56.032913 (51.01-56.04)	0.002623 (0.0023-0.0027)	0.001722 (0.0015-0.0018)		0.005331 (0.0048-0.0054)	24.772909 (24.77-27.27)
	56.181144 (51.42-56.19)	x	x	0.004100 (0.0037-0.0041)		24.658380 (24.65-26.99)
	55.547473 (51.01-55.55)	0.006258 (0.0057-0.0063)	0.004063 (0.0037-0.0041)	0.0011819 (0.0118-0.0128)	0.004300 (0.0039-0.0043)	
0.542649 (0.5426-0.5916)			0.038369 (0.0352-0.0384)	0.015086 (0.0138-0.0151)	0.003764 (0.0034-0.0038)	26.797542 (26.79-29.17)
0.547405 (0.5474-0.5921)		0.076105 (0.0761-0.0822)		0.015133 (0.0151-0.0163)	0.003761 (0.0034-0.0038)	26.810574 (26.81-28.96)
0.555003 (0.5550-0.6023)		0.090446 (0.0836-0.0904)	0.049477 (0.0457-0.0495)		0.004996 (0.0046-0.0050)	26.842771 (26.84-29.00)
0.561101 (0.5611-0.6089)		0.085642 (0.0792-0.0857)	0.046331 (0.0428-0.0464)	0.004521 (0.0041-0.0046)		26.570722 (26.57-28.70)
0.568176 (0.5681-0.6168)		0.077120 (0.0713-0.0772)	0.041552 (0.0384-0.0416)	0.015046 (0.0150-0.0162)	0.003959 (0.0036-0.0040)	
0.853623 (0.7903-0.8537)	81.583367 (75.51-81.59)			0.006925 (0.0064-0.0070)	0.007674 (0.0070-0.0077)	16.699049 (16.69-18.04)
0.870590 (0.8642-0.8964)	81.298384 (77.77-80.67)		0.040507 (0.0389-0.0404)		0.008121 (0.0074-0.0077)	16.684019 (16.80-17.43)
0.897860 (0.8978-0.9697)	83.367044 (77.16-83.37)		0.040454 (0.0374-0.0405)	0.005295 (0.0049-0.0053)		16.237378 (16.23-17.54)
0.592877 (0.5928-0.6411)	57.188654 (57.18-61.78)		0.039562 (0.0366-0.0396)	0.011924 (0.0119-0.0128)	0.004537 (0.0041-0.0046)	
0.875031 (0.8101-0.8751)	81.273842 (75.23-81.28)	0.071964 (0.0719-0.0778)			0.008127 (0.0075-0.0082)	16.686843 (16.68-18.03)
0.902575 (0.9025-0.9748)	83.342921 (77.14-83.35)	0.072068 (0.0720-0.0778)		0.005311 (0.0049-0.0054)		16.239711 (16.23-17.54)
0.597186 (0.5971-0.6458)	57.193083 (57.19-61.78)	0.073771 (0.0737-0.0797)		0.011967 (0.0110-0.0120)	0.004535 (0.0041-0.0046)	
0.952547 (0.9525-1.029)	83.906610 (77.66-83.91)	0.117402 (0.1086-0.1174)	0.066070 (0.0611-0.0661)			16.200027 (16.20-17.50)
0.623247 (0.6232-0.6760)	57.555299 (57.55-62.18)	0.111360 (0.1030-0.1114)	0.061774 (0.0571-0.0618)		0.005542 (0.0051-0.0056)	
0.629530 (0.6295-0.6828)	57.798449 (57.79-62.44)	0.109865 (0.1016-0.1099)	0.060497 (0.0559-0.0605)	0.004449 (0.0041-0.0045)		

Table 12: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		4.721323 (4.721-4.857)	x	0.009034 (0.0090-0.0093)	0.024143 (0.0234-0.0242)	83.760240 (83.75-86.21)
	270.544555 (270.5-278.4)		x	x	x	17.770703 (17.27-17.78)
	277.196950 (277.1-286.6)	x		x	x	16.569107 (16.56-17.18)
	279.250782 (279.2-287.2)	x	x		0.0968912 (0.0941-0.0969)	16.748634 (16.28-16.75)
	x	x	x	x		x
	272.671628 (272.6-280.9)	0.387160 (0.3734-0.3871)	x	x	x	
0.096013 (0.0959-0.0987)			x	0.009084 (0.0090-0.0093)	0.024111 (0.0234-0.0242)	83.817041 (83.81-86.23)
0.089804 (0.0898-0.0923)		x		0.009093 (0.0088-0.0091)	0.024107 (0.0234-0.0242)	83.834218 (83.81-86.24)
0.089887 (0.0898-0.0924)		x	x		0.025104 (0.0251-0.0258)	83.696083 (83.68-86.09)
0.079776 (0.0797-0.0820)		x	x	0.111573 (0.1084-0.1116)		105.371065 (105.3-108.3)
x		x	x	0.007343 (0.0073-0.0076)	x	
x	x			x	x	x
x	x		x		x	360.390235 (349.9-360.4)
0.107384 (0.1043-0.1074)	x		x	0.000179 (0.0001-0.0002)		x
0.397653 (0.3864-0.3976)	273.598064 (265.8-273.6)		x	x	x	
x	x	x			x	x
0.100444 (0.0976-0.1005)	225.112709 (218.8-225.2)	x		0.000178 (0.0001-0.0002)		155.304729 (155.3-159.7)
0.371730 (0.3613-0.3717)	273.454692 (265.8-273.4)	x		x	0.080167 (0.0779-0.0802)	
0.101385 (0.0985-0.1014)	217.688636 (211.6-217.7)	x	x			149.199008 (149.1-153.4)
0.371564 (0.3611-0.3716)	273.205686 (265.5-273.2)	x	x		0.076492 (0.0743-0.0765)	
x	x	x	x	x		

Table 13: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using initial rate analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.041184 (0.0411-0.0448)	0.014753 (0.0135-0.0148)	0.003601 (0.0033-0.0036)	27.384209 (27.38-29.82)
		0.070245 (0.0644-0.0703)		0.014729 (0.0147-0.0160)	0.003601 (0.0033-0.0036)	27.378149 (27.37-29.82)
		0.044575 (0.0408-0.0446)	0.026253 (0.0240-0.0263)		0.004925 (0.0045-0.0050)	27.001713 (27.00-29.49)
		0.035140 (0.0322-0.0352)	0.020699 (0.0189-0.0207)	0.004330 (0.0039-0.0044)		26.768306 (26.76-29.23)
		0.020004 (0.0183-0.0200)	0.011915 (0.0109-0.0119)	0.014359 (0.0143-0.0156)	0.003923 (0.0035-0.0040)	
	51.646832 (47.42-51.65)			0.014198 (0.0141-0.0154)	0.003657 (0.0033-0.0037)	27.186202 (27.18-29.62)
	52.667186 (48.32-52.67)		0.043379 (0.0398-0.04434)		0.004870 (0.0044-0.0049)	26.745273 (26.74-29.15)
	52.797672 (48.44-52.80)		0.043788 (0.0402-0.0438)	0.003998 (0.0036-0.0040)		26.615476 (26.61-29.01)
	54.333119 (49.90-54.34)		0.046238 (0.0462-0.0503)	0.012264 (0.0122-0.0133)	0.003964 (0.0036-0.0040)	
	53.167100 (48.77-53.16)	0.065931 (0.0659-0.0717)			0.004910 (0.0045-0.0050)	26.501171 (26.50-28.89)
	53.417019 (49.00-53.42)	0.065123 (0.0651-0.0709)		0.003974 (0.0036-0.0040)		26.317093 (26.31-28.69)
	54.782484 (50.31-54.79)	0.062336 (0.0572-0.0624)		0.012020 (0.0120-0.0130)	0.004031 (0.0036-0.0041)	
	56.253768 (51.61-56.26)	x	x			24.599244 (24.59-26.81)
	55.685217 (51.14-55.69)	0.005966 (0.0054-0.0060)	0.003886 (0.0035-0.0039)		0.005296 (0.0048-0.0053)	
	55.781355 (51.21-55.79)	0.001233 (0.0011-0.0013)	0.000805 (0.0007-0.0008)	0.004132 (0.0037-0.0042)		
0.511644 (0.5116-0.5574)				0.014431 (0.0144-0.0157)	0.003659 (0.0033-0.0037)	27.112372 (27.11-29.52)
0.542043 (0.5420-0.5910)			0.038466 (0.0353-0.0385)		0.005008 (0.0045-0.0050)	26.791732 (26.79-29.16)
0.550861 (0.5508-0.6006)			0.037884 (0.0347-0.0379)	0.004513 (0.0041-0.0046)		26.532470 (26.53-28.88)
0.562811 (0.5628-0.6140)			0.037463 (0.0344-0.0375)	0.014988 (0.0149-0.0163)	0.003966 (0.0036-0.0040)	
0.546620 (0.5466-0.5961)		0.075889 (0.0758-0.0826)			0.005005 (0.0045-0.0050)	26.804971 (26.80-29.18)
0.556100 (0.5561-0.6064)		0.077105 (0.0771-0.0839)		0.004516 (0.0041-0.0046)		26.549266 (26.54-28.90)
0.568728 (0.5678-0.6205)		0.078009 (0.0780-0.0849)		0.015055 (0.0150-0.0163)	0.003959 (0.0036-0.0040)	
0.561822 (0.5618-0.6152)		0.085897 (0.0788-0.0859)	0.046402 (0.0425-0.0464)			26.558966 (26.55-28.91)
0.566093 (0.5660-0.6202)		0.075006 (0.0688-0.0750)	0.040597 (0.0372-0.0406)		0.005222 (0.0047-0.0053)	
0.570805 (0.5708-0.6254)		0.073062 (0.0670-0.0730)	0.039171 (0.0359-0.0392)	0.004717 (0.0043-0.0048)		
0.858702 (0.7889-0.8587)	82.010551 (75.32-82.01)				0.008099 (0.0074-0.0081)	16.613054 (16.61-18.09)

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.885405 (0.8134-0.8854)	84.144346 (77.28-84.15)			0.005153 (0.0047-0.0052)		16.162512 (16.16-17.60)
0.575096 (0.5750-0.62766)	57.653894 (57.65-62.78)			0.011400 (0.0113-0.0124)	0.004522 (0.0041-0.0046)	
0.897696 (0.8311-0.8977)	83.362943 (77.16-83.37)		0.040459 (0.0374-0.0405)			16.239197 (16.23-17.54)
0.592969 (0.5929-0.6412)	57.338145 (57.33-61.94)		0.039695 (0.0367-0.0397)		0.005545 (0.0051-0.0056)	
0.599986 (0.5999-0.6488)	57.587089 (57.58-62.21)		0.039353 (0.0364-0.0394)	0.004424 (0.0040-0.0045)		
0.902243 (0.8353-0.9023)	83.326948 (77.12-83.33)	0.072056 (0.0720-0.0778)				16.243944 (16.24-17.55)
0.597034 (0.5970-0.6456)	57.341345 (57.34-61.94)	0.073497 (0.0734-0.0794)			0.005548 (0.0051-0.0056)	
0.604537 (0.6045-0.6538)	57.595150 (57.59-62.22)	0.074166 (0.0741-0.0801)		0.004434 (0.0041-0.0045)		
0.630283 (0.302-0.6836)	57.782416 (57.78-62.43)	0.110223 (0.1019-0.1102)	0.060588 (0.0560-0.0606)			

Table 14: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.035110 (0.0341-0.0352)	0.008876 (0.0083-0.0089)	x	5.153891 (5.009-5.154)
		4.805816 (4.671-4.805)		0.009132 (0.0088-0.0092)	0.023499 (0.0228-0.0235)	86.035422 (86.03-88.50)
		4.718770 (4.586-4.719)	x		0.025101 (0.0250-0.0258)	83.724393 (81.38-83.73)
		x	x	x		x
		2.147714 (2.147-2.209)	2.127804 (2.126-2.189)	0.007289 (0.0070-0.0073)	x	
	265.102918 (265.0-272.7)			$7.761 \times 10^{-6}$ ( $7.54-7.97 \times 10^{-6}$ )	0.099493 (0.0967-0.0995)	17.617419 (17.12-17.62)
	270.528286 (270.5-278.3)		x		0.093680 (0.0910-0.0937)	17.755248 (17.25-17.76)
	290.181425 (290.1-299.6)		x	1.028540 (0.9937-1.029)		18.588412 (18.58-19.14)
	398.666104 (398.6-410.1)		x	x	0.090563 (0.0880-0.0906)	
	279.250707 (279.2-287.2)	x			0.096891 (0.0941-0.0968)	16.748633 (16.28-16.75)
	251.356732 (244.3-251.4)	4.076770 (3.962-4.077)		0.000138 (0.0001-0.0002)		163.116037 (163.1-167.8)
	438.474249 (425.9-438.5)	x		x	x	
	216.846819 (216.8-223.5)	4.079565 (3.960-4.080)	x			149.129056 (149.1-153.4)
	273.216397 (265.5-273.3)	0.383310 (0.3725-0.3834)	x		0.076492 (0.0743-0.0765)	
	295.706471 (287.4-295.8)	0.333439 (0.3333-0.3430)	x	0.820349 (0.7973-0.8204)		
0.098125 (0.0980-0.1008)				0.009034 (0.0087-0.0091)	0.025030 (0.0243-0.0251)	80.721211 (80.67-83.03)
0.096091 (0.0960-0.0988)			x		0.025107 (0.0243-0.0258)	83.693403 (83.68-86.09)
0.085282 (0.0852-0.0877)			x	0.111591 (0.1084-0.1116)		105.369678 (105.3-108.3)
0.174834 (0.1699-0.1749)			0.200105 (0.1998-0.2058)	0.000214 (0.0002-0.0003)	0.077443 (0.0649-0.0775)	
0.089887 (0.0898-0.0924)		x			0.025104 (0.0251-0.0258)	83.696079 (83.69-86.09)
0.079776 (0.0797-0.0820)		x		0.111573 (0.1084-0.1116)		105.371064 (105.3-108.3)
0.165524 (0.1608-0.1656)		0.013653 (0.0132-0.0137)		0.008017 (0.0065-0.0081)	0.071582 (0.0715-0.0738)	
x		0.001815 (0.0017-0.0022)	x			153.418652 (153.4-157.8)
x		x	x		0.073443 (0.0713-0.0735)	
x		x	x	1.069575 (1.069-1.100)		
0.087101 (0.0871-0.0872)	408.435313 (397.0-408.5)				0.002798 (0.0027-0.0028)	405.825624 (405.7-417.5)

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.107772 (0.1047-0.1078)	237.778455 (231.1-237.8)			0.000157 (0.0001-0.0002)		159.250878 (159.2-163.8)
0.400538 (0.3893-0.4006)	282.094661 (274.2-282.1)			x	0.078889 (0.0766-0.0789)	
0.108381 (0.1053-0.1084)	217.691347 (211.6-217.7)		x			149.202128 (149.2-153.4)
0.397201 (0.3861-0.3973)	273.200710 (265.5-273.3)		x		0.076491 (0.0743-0.0765)	
0.412026 (0.4005-0.4121)	295.643711 (287.3-295.8)		x	0.820125 (0.8199-0.8436)		
0.101385 (0.0985-0.1014)	217.689465 (211.6-217.7)	x				149.199051 (149.1-153.4)
0.371564 (0.3611-0.3716)	273.205639 (265.5-273.3)	x			0.076492 (0.0743-0.0765)	
x	x	x		x		
0.334246 (0.3248-0.3343)	1525.0730 (1482-1526)	x	x			

Table 15: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using initial rate analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.014347 (0.0142-0.0144)	0.003705 (0.0036-0.0037)	26.918389 (26.91-27.05)
			0.041228 (0.0412-0.0449)		0.004805 (0.0044-0.0048)	27.367171 (27.36-29.81)
			0.041475 (0.0414-0.0451)	0.004153 (0.0038-0.0042)		27.271373 (27.27-29.70)
			0.042825 (0.0428-0.0466)	0.014224 (0.0142-0.0154)	0.003738 (0.0034-0.0038)	
		0.070171 (0.0644-0.0702)			0.004805 (0.0044-0.0048)	27.360333 (27.36-29.80)
		0.069742 (0.0640-0.0698)		0.004150 (0.0038-0.0042)		27.247905 (27.24-29.68)
		0.067639 (0.0621-0.0677)		0.014209 (0.0130-0.0142)	0.003752 (0.0034-0.0038)	
		0.034518 (0.0316-0.0346)	0.020327 (0.0186-0.0204)			26.757848 (26.75-29.14)
		0.020139 (0.0184-0.0202)	0.012006 (0.0110-0.0120)		0.005137 (0.0047-0.0052)	
		0.014743 (0.0135-0.0148)	0.008784 (0.0080-0.0088)	0.004478 (0.0041-0.0045)		
	51.654522 (47.43-51.65)				0.004815 (0.0044-0.0049)	27.196813 (27.19-29.63)
	51.490683 (47.28-51.49)			0.004085 (0.0037-0.0041)		27.194740 (27.19-29.63)
	50.884434 (46.72-50.89)			0.014693 (0.0146-0.0159)	0.003607 (0.0033-0.0036)	
	52.734257 (48.38-52.74)		0.043722 (0.0401-0.0438)			26.632373 (26.63-29.03)
	54.457700 (50.01-54.46)		0.046393 (0.0463-0.0505)		0.004982 (0.0045-0.0050)	
	54.446100 (50.00-54.45)		0.046586 (0.0465-0.0507)	0.003893 (0.0035-0.0039)		
	53.350001 (48.94-53.35)	0.065220 (0.0652-0.0710)				26.335147 (26.33-28.71)
	54.907727 (50.43-54.91)	0.062141 (0.0570-0.0622)			0.005029 (0.0046-0.0051)	
	54.943600 (50.46-54.95)	0.061829 (0.0567-0.0619)		0.003893 (0.0035-0.0039)		
	55.744801 (51.18-55.75)	0.000869 (0.0008-0.0010)	0.000567 (0.0005-0.0006)			
0.511931 (0.5119-0.5577)					0.004851 (0.0044-0.0049)	27.097652 (27.09-29.50)
0.515144 (0.5151-0.5612)				0.004185 (0.0038-0.0042)		26.880559 (26.88-29.26)
0.524728 (0.5247-0.5715)				0.014098 (0.0140-0.0153)	0.003867 (0.0035-0.0039)	
0.551383 (0.5513-0.6012)			0.037841 (0.0347-0.0379)			26.520426 (26.52-28.87)
0.562136 (0.5621-0.6132)			0.037558 (0.0344-0.0376)		0.005222 (0.0047-0.0053)	
0.568137 (0.5681-0.6198)			0.037130 (0.0340-0.0372)	0.004713 (0.0043-0.0048)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.556727 (0.5567-0.6071)		0.077206 (0.0772-0.0840)				26.536812 (26.53-28.89)
0.567794 (0.5677-0.6194)		0.077775 (0.0777-0.0847)			0.005220 (0.0047-0.0053)	
0.574362 (0.5743-0.6266)		0.078709 (0.0787-0.0857)		0.004719 (0.0043-0.0048)		
0.571190 (0.5711-0.6202)		0.073213 (0.0677-0.0733)	0.039219 (0.0362-0.0393)			
0.885452 (0.8197-0.8855)	84.147204 (77.89-84.15)					16.161535 (16.16-17.46)
0.576134 (0.5761-0.6228)	57.772550 (57.77-62.42)				0.005479 (0.0050-0.0055)	
0.580454 (0.5804-0.6275)	58.100642 (58.10-62.77)			0.004222 (0.0039-0.0043)		
0.600468 (0.6004-0.6493)	57.570358 (57.57-62.19)		0.039297 (0.0363-0.0393)			
0.605095 (0.6050-0.6544)	57.578045 (57.57-62.20)	0.074279 (0.0742-0.0802)				

Table 16: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of enzyme saturating *in silico* generated data.



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.008658 (0.0084-0.0087)	x	5.098004 (4.955-5.098)
			0.035084 (0.0341-0.0351)		0.210856 (0.2108-0.2169)	5.155439 (5.011-5.156)
			x	x		x
			$3.5540 \times 10^{-6}$ ( $3.45-3.55 \times 10^{-6}$ )	x	0.711283 (0.7112-0.7317)	
		4.799646 (4.665-4.800)			0.024488 (0.0244-0.0251)	85.870660 (85.85-88.33)
		x		x		x
		1.402222 (1.402-1.442)		x	x	
		x	x			x
		2.147228 (2.147-2.208)	2.126461 (2.124-2.187)		0.073423 (0.0713-0.0735)	
		2.067996 (2.067-2.127)	1.913448 (1.912-1.968)	1.069951 (1.040-1.070)		
	264.820300 (264.8-272.4)				0.095078 (0.0924-0.0951)	17.606382 (17.11-17.61)
	292.000681 (291.9-300.3)			1.018278 (0.9898-1.019)		18.634088 (18.11-18.64)
	409.151521 (409.1-420.9)			x	0.088242 (0.0857-0.0883)	
	2695.9842 (2695-2773)		x			20.653467 (20.07-20.66)
	398.587429 (398.5-410.0)		x		0.086506 (0.0840-0.0866)	
	404.056507 (404.0-415.7)		x	0.940187 (0.9139-0.9402)		
	244.764745 (237.9-244.8)	4.022644 (3.910-4.023)				156.866503 (156.8-161.3)
	437.537616 (437.5-450.1)	x			0.090113 (0.0875-0.0902)	
	442.527761 (442.5-455.2)	x		0.988796 (0.9611-0.9888)		
	1525.1040 (1482-1526)	0.537779 (0.5227-0.5379)	x			
0.098116 (0.0981-0.1009)					0.026053 (0.0260-0.0268)	80.569239 (78.32-80.76)
0.085806 (0.0858-0.0882)				0.114412 (0.1112-0.1145)		104.140065 (104.0-107.1)
0.178679 (0.1736-0.1787)				0.007131 (0.0071-0.0073)	0.076200 (0.0740-0.0762)	
0.071098 (0.0691-0.0711)			x			153.408275 (153.4-157.8)
0.174596 (0.1697-0.1746)			0.201121 (0.2010-0.2069)		0.073432 (0.0713-0.0735)	
0.179105 (0.1740-0.1791)			0.183211 (0.1829-0.1884)	1.070109 (1.040-1.070)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.066509 (0.0665-0.0666)		x				153.408826 (153.3-157.8)
0.165555 (0.1609-0.1656)		0.013658 (0.0136-0.0140)			0.073422 (0.0734-0.0755)	
0.170061 (0.1652-0.1701)		0.015019 (0.0145-0.0151)		1.070124 (1.040-1.070)		
x		x	x			
0.108856 (0.1058-0.1089)	230.685574 (224.2-230.7)					153.054085 (153.0-157.4)
0.400364 (0.3891-0.4004)	281.831035 (273.9-281.9)				0.075274 (0.0731-0.0753)	
0.415219 (0.4036-0.4153)	306.116676 (297.5-306.2)			0.799491 (0.7771-0.7995)		
0.357312 (0.3472-0.3573)	1525.1071 (1482-1526)		x			
0.334246 (0.3248-0.3343)	1525.0729 (1482-1526)	x				

Table 17: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using initial rate analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.004889 (0.0044-0.0049)	26.927141 (26.92-29.34)
				0.004170 (0.0038-0.0042)		26.972905 (26.97-29.39)
				0.014716 (0.0135-0.0148)	0.003615 (0.0033-0.0037)	
			0.041481 (0.0414-0.0451)			27.268996 (27.26-29.70)
			0.042859 (0.0428-0.0466)		0.004926 (0.0045-0.0050)	
			0.043043 (0.0430-0.0468)	0.004192 (0.0038-0.0042)		
		0.069730 (0.064-0.0698)				27.244904 (27.24-29.67)
		0.067591 (0.0620-0.0676)			0.004934 (0.0045-0.0050)	
		0.067302 (0.0617-0.0673)		0.004202 (0.0038-0.0042)		
		0.014368 (0.0143-0.0156)	0.008559 (0.0085-0.0093)			
	51.475807 (47.27-51.48)					27.191056 (27.19-29.62)
	50.887951 (46.72-50.89)				0.004803 (0.0040-0.0048)	
	50.727944 (46.57-50.73)			0.004152 (0.0038-0.0042)		
	54.392534 (49.95-54.40)		0.046540 (0.0465-0.0506)			
	54.891311 (50.41-54.90)	0.061885 (0.0568-0.0619)				
0.515296 (0.5152-0.5614)						26.867763 (26.86-29.25)
0.524910 (0.5249-0.5717)					0.005055 (0.0046-0.0051)	
0.526806 (0.5268-0.5738)				0.004340 (0.0039-0.0044)		
0.556446 (0.5684-0.6201)			0.037103 (0.0340-0.0371)			
0.574744 (0.5747-0.6270)		0.078782 (0.0787-0.0858)				
0.580504 (0.5805-0.6325)	58.093653 (58.09-63.26)					

Table 18: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.209223 (0.2092-0.2152)	5.099417 (4.956-5.100)
				4.520799 (4.520-4.650)		5.877769 (5.713-5.878)
				0.211276 (0.2053-0.2173)	x	
			x			0.008474 (0.0082-0.0085)
			$3.554 \times 10^{-6}$ (3.45-3.55 $\times 10^{-6}$ )		0.700995 (0.7009-0.7211)	
			$3.554 \times 10^{-6}$ (3.45-3.55 $\times 10^{-6}$ )	27.552663 (27.54-28.34)		
		6.865666 (6.865-7.063)				159.167830 (159.1-163.7)
		2.065583 (2.065-2.124)			0.078098 (0.0759-0.0781)	
		1.977250 (1.977-2.034)		1.163621 (1.131-1.163)		
		4.685065 (4.685-4.820)	x			
	2729.4056 (2729-2807)					20.777824 (20.19-20.78)
	409.063344 (409.0-420.8)				0.084281 (0.0819-0.083)	
	415.323422 (415.3-427.2)			0.908654 (0.8832-0.9087)		
	3225.3730 (3225-3318)		x			
	1696.2182 (1648-1697)	0.472526 (0.4592-0.4726)				
0.070890 (0.0689-0.0709)						154.347887 (154.3-158.7)
0.178712 (0.1736-0.1788)					0.077558 (0.0753-0.0776)	
0.183605 (0.1784-0.1836)				1.147135 (1.115-1.148)		
0.096652 (0.0939-0.0967)			x			
0.090414 (0.0878-0.0905)		x				
0.364372 (0.3541-0.3644)	1605.0577 (1560-1606)					

Table 19: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using initial rate analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						26.973793 (26.97-29.39)
					0.004813 (0.0044-0.0048)	
				0.004149 (0.0038-0.0042)		
			0.043051 (0.0430-0.0468)			
		0.067288 (0.0617-0.0673)				
	50.715104 (46.56-50.72)					
0.526878 (0.5268-0.5273)						

Table 20: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using progress curve analysis of enzyme saturating *in silico* generated data.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						0.008370 (0.0083-0.0086)
					0.700955 (0.7008-0.7211)	
				27.552721 (27.54-28.34)		
			$3.6135 \times 10^{-6}$ ( $3.47-3.62 \times 10^{-6}$ )			
		4.727272 (4.7272-4.8636)				
	3247.5908 (3247-3341)					
0.096782 (0.0940-0.0968)						

Table 21: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using initial rate analysis of enzyme saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

Saturating *in silico* generated data with 10% noise

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.488736 (0.3494-0.7323)	49.193986 (35.11-70.09)	0.039649 (0.0070-0.1190)	x	0.006208 (0.0020-0.0193)	0.008272 (0.0045-0.0124)	25.905412 (18.40-37.00)

Table 22: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	50.923375 (34.10-52.36)	x	x	0.005954 (0.0020-0.0169)	0.008668 (0.0051-0.0126)	24.945560 (24.09-42.63)
0.504930 (0.4898-1.107)		x	x	0.005930 (0.0020-0.0165)	0.008624 (0.0052-0.0125)	25.066871 (20.45-35.23)
0.507330 (0.3627-0.7318)	49.372225 (35.27-70.16)		0.039422 (0.0280-0.0490)	0.006267 (0.0021-0.0184)	0.008249 (0.0046-0.0122)	25.880260 (18.41-36.87)
0.509415 (0.3640-0.7352)	49.430894 (35.31-70.24)	0.073560 (0.0575-0.1060)		0.006255 (0.0021-0.0183)	0.008249 (0.0046-0.0122)	25.870182 (18.40-36.86)
0.490990 (0.3512-0.7330)	49.456004 (35.36-70.33)	x	x		0.008596 (0.0053-0.0125)	25.760484 (18.32-36.63)
0.501350 (0.3592-0.7503)	50.389600 (36.01-71.19)	x	x	0.005162 (0.0019-0.0115)		25.222183 (18.00-35.87)
0.504647 (0.4903-1.058)	50.674148 (44.20-71.27)	x	x	0.005948 (0.0020-0.0167)	0.008625 (0.0051-0.0125)	

Table 23: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		0.049966 (0.0109-0.0875)	0.028852 (0.0060-0.0523)	0.005992 (0.0023-0.0150)	0.008634 (0.0057-0.0117)	25.021512 (21.99-31.85)
	49.924285 (34.86-51.43)		0.039356 (0.0280-0.0503)	0.006173 (0.0022-0.0164)	0.008382 (0.0051-0.0121)	25.560096 (24.61-38.18)
	49.799278 (34.76-51.56)	0.073664 (0.0553-0.1046)		0.006197 (0.0022-0.0165)	0.008338 (0.0050-0.0121)	25.655644 (24.57-38.49)
	51.009242 (34.26-52.27)	x	x		0.008898 (0.0057-0.0127)	24.903193 (24.16-42.21)
	51.201676 (34.63-52.49)	x	x	0.005224 (0.0021-0.0110)		24.788912 (24.05-41.27)
	50.815302 (36.48-62.32)	0.049714 (0.0126-0.0814)	0.028735 (0.0072-0.0482)	0.005981 (0.0021-0.0157)	0.008639 (0.0052-0.0125)	
0.521799 (0.5020-0.7836)			0.039486 (0.0280-0.0478)	0.006021 (0.0022-0.0158)	0.008563 (0.0052-0.0124)	25.134543 (20.50-35.26)
0.523228 (0.5001-0.7920)		0.073437 (0.0595-0.1056)		0.006017 (0.0022-0.0158)	0.008550 (0.0052-0.0123)	25.256172 (20.64-35.29)
0.504610 (0.4913-1.102)		x	x		0.008835 (21.03-35.21)	25.065651
0.505165 (0.4920-1.107)		x	0.023237 (0.0011-0.0606)	0.005178 (0.0021-0.0109)		25.033253 (21.04-35.16)
0.505243 (0.4957-1.2547)		x	0.023562 (0.0034-0.0603)	0.005933 (0.0021-0.0155)	0.008635 (0.0053-0.0125)	
0.492913 (0.3533-0.7089)	49.942343 (35.71-70.77)			0.005912 (0.0021-0.0159)	0.008300 (0.0048-0.0122)	25.777508 (18.36-36.63)
0.509980 (0.3652-0.7337)	49.651290 (35.53-70.43)		0.039470 (0.0280-0.0479)		0.008592 (0.0054-0.0124)	25.727923 (18.33-36.49)
0.521011 (0.3732-0.7442)	50.605176 (36.19-71.33)		0.039443 (0.0280-0.0479)	0.005197 (0.0021-0.0110)		25.181208 (17.99-35.73)
0.523297 (0.5031-0.7793)	50.815913 (44.23-71.33)		0.039447 (0.0280-0.0478)	0.006011 (0.0022-0.0158)	0.008591 (0.0052-0.0124)	
0.511989 (0.3665-0.7369)	49.708613 (35.57-70.50)	0.073462 (0.0594-0.1056)			0.008589 (0.0054-0.0124)	25.718611 (18.32-36.48)
0.523189 (0.3746-0.7479)	50.669816 (36.24-71.41)	0.073523 (0.0593-0.1057)		0.005187 (0.0021-0.0110)		25.169245 (17.98-35.72)
0.525238 (0.5023-0.7906)	50.858533 (44.50-71.41)	0.073515 (0.0594-0.1057)		0.006006 (0.0022-0.0158)	0.008588 (0.0052-0.0124)	
0.501464 (0.3596-0.7466)	50.399114 (36.05-71.04)	x	x			25.217409 (18.02-35.79)
0.504472 (0.4919-1.137)	50.694943 (45.09-71.29)	x	x		0.008839 (0.0057-0.0126)	
0.505075 (0.4926-1.143)	50.731357 (44.95-71.31)	x	x	0.005183 (0.0021-0.1097)		

Table 24: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.040268 (0.0394-0.0573)	0.005953 (0.0024-0.0145)	0.008497 (0.0053-0.0122)	25.287753 (24.29-36.08)
		0.071791 (0.00511-0.0731)		0.005941 (0.0024-0.0144)	0.008486 (0.0053-0.0122)	25.307286 (24.21-36.05)
		0.050075 (0.0129-0.0821)	0.028923 (0.0073-0.0487)		0.008859 (0.0058-0.0126)	25.020138 (23.90-35.70)
		0.048841 (0.0120-0.0805)	0.028213 (0.0067-0.0477)	0.005235 (0.0023-0.0105)		24.989449 (23.88-35.66)
		0.049948 (0.0157-0.0788)	0.028842 (0.0139-0.0468)	0.005991 (0.0024-0.0146)	0.008634 (0.0054-0.0124)	
	51.920464 (37.10-52.50)			0.005596 (0.0022-0.0134)	0.008761 (0.0055-0.0125)	24.700716 (24.19-35.64)
	49.979030 (34.94-51.27)		0.039425 (0.0281-0.0491)		0.008658 (0.0057-0.0123)	25.537759 (24.72-38.05)
	50.040299 (35.02-51.35)		0.039534 (0.0282-0.0496)	0.005154 (0.0023-0.0103)		25.499991 (24.68-37.93)
	50.532391 (36.19-53.69)		0.040260 (0.0374-0.0570)	0.005983 (0.0024-0.0146)	0.008511 (0.0053-0.0122)	
	49.868975 (34.85-51.37)	0.073510 (0.0576-0.1042)			0.008621 (0.0056-0.0122)	25.626040 (24.70-38.32)
	49.963297 (34.96-51.49)	0.073240 (0.0566-0.1036)		0.005132 (0.0023-0.0102)		25.570021 (24.64-38.15)
	50.595799 (36.28-55.20)	0.071680 (0.0511-0.0759)		0.005949 (0.0024-0.0145)	0.008511 (0.0053-0.0122)	
	51.201344 (34.64-52.30)	x	x			24.789600 (24.16-41.20)
	50.841852 (36.51-60.39)	0.049733 (0.0149-0.0795)	0.028767 (0.0085-0.0469)		0.008865 (0.0058-0.0126)	
	50.86638 (36.53-60.43)	0.048814 (0.0143-0.0783)	0.028246 (0.0081-0.0463)	0.005219 (0.0023-0.0105)		
0.501412 (0.4972-0.7206)				0.005775 (0.0023-0.0139)	0.008487 (0.0053-0.0122)	25.335988 (22.85-35.66)
0.521576 (0.5046-0.7821)			0.039516 (0.0281-0.0467)		0.008794 (0.0058-0.0125)	25.135386 (21.17-35.24)
0.522606 (0.5059-0.7849)			0.039451 (0.0280-0.0465)	0.005201 (0.0023-0.0104)		25.100981 (21.23-35.20)
0.523051 (0.5125-0.8097)			0.039427 (0.0280-0.0461)	0.006025 (0.0024-0.0147)	0.008587 (0.0053-0.0123)	
0.522996 (0.5031-0.7901)		0.073382 (0.0614-0.1053)			0.008779 (0.0057-0.0125)	25.157478 (21.28-35.28)
0.524074 (0.5044-0.7931)		0.073504 (0.0616-0.1056)		0.005190 (0.0023-0.0104)		25.122793 (21.34-35.24)
0.524803 (0.5115-0.8162)		0.073577 (0.0621-0.1057)		0.006025 (0.0024-0.0147)	0.008574 (0.0053-0.0123)	
0.505205 (0.4941-1.107)		x	x			25.031601 (21.70-35.15)
0.504903 (0.4968-1.249)		x	x		0.008844 (0.0058-0.0125)	
0.505252 (0.4972-1.253)		x	x	0.005185 (0.0023-0.0104)		
0.494912 (0.3552-0.7102)	50.132298 (35.91-70.92)				0.008537 (0.0055-0.0122)	25.671688 (18.31-36.30)



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.506000 (0.3631-0.7204)	51.154093 (36.62-71.94)			0.004949 (0.0022-0.0099)		25.099163 (17.95-35.53)
0.506734 (0.4978-0.7333)	51.225569 (47.85-72.24)			0.005701 (0.0023-0.0137)	0.008604 (0.0054-0.0123)	
0.521001 (0.3735-0.7415)	50.605612 (36.23-71.17)		0.039444 (0.0280-0.0466)			25.181392 (18.01-35.65)
0.523255 (0.5059-0.7780)	50.839709 (45.26-71.34)		0.039477 (0.0281-0.0467)		0.008823 (0.0058-0.0125)	
0.524073 (0.5070-0.7804)	50.879048 (45.02-71.36)		0.039444 (0.0280-0.0467)	0.005213 (0.0023-0.0104)		
0.523164 (0.3749-0.7451)	50.668903 (36.27-71.25)	0.073518 (0.0614-0.1055)				25.169362 (18.01-35.64)
0.525110 (0.5054-0.7890)	50.883388 (45.47-71.42)	0.073443 (0.0613-0.1054)			0.008817 (0.0058-0.0125)	
0.525984 (0.5065-0.7915)	50.923748 (45.26-71.44)	0.073509 (0.0614-0.1056)		0.005202 (0.0023-0.0104)		
0.505088 (0.4945-1.140)	50.732175 (45.80-71.29)	x	x			

Table 25: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
				0.005658 (0.0026-0.0126)	0.008926 (0.0057-0.0126)	24.385126 (24.05-35.35)
			0.040275 (0.0395-0.0573)		0.008713 (0.0058-0.0123)	25.285084 (24.46-36.06)
			0.040316 (0.0396-0.0573)	0.005079 (0.0025-0.0095)		25.269080 (24.45-36.04)
			0.040459 (0.0400-0.0577)	0.005930 (0.0027-0.0133)	0.008531 (0.0054-0.0121)	
		0.071781 (0.0511-0.0729)			0.008699 (0.0058-0.0123)	25.305140 (24.40-36.03)
		0.071705 (0.0511-0.0728)		0.005063 (0.0025-0.0095)		25.286234 (24.38-36.00)
		0.071435 (0.0509-0.0721)		0.005915 (0.0027-0.0132)	0.008527 (0.0054-0.0121)	
		0.048875 (0.0149-0.0781)	0.028233 (0.0085-0.0461)			24.991315 (24.07-35.63)
		0.050073 (0.0180-0.0771)	0.028921 (0.0103-0.0456)		0.008860 (0.0059-0.0125)	
		0.049287 (0.0175-0.0761)	0.028466 (0.0100-0.0450)	0.005231 (0.0026-0.0099)		
	51.921618 (37.10-52.40)				0.008876 (0.0059-0.0125)	24.701671 (24.28-35.63)
	51.905373 (37.09-52.38)			0.004989 (0.0025-0.0094)		24.702172 (24.28-35.63)
	52.045119 (37.14-52.42)			0.005573 (0.0025-0.0123)	0.008768 (0.0056-0.0124)	
	50.039250 (35.03-51.11)		0.039534 (0.0282-0.0478)			25.499938 (24.83-37.89)
	50.560726 (36.22-53.12)		0.040291 (0.0380-0.0570)		0.008734 (0.0058-0.0123)	
	50.577360 (36.23-53.18)		0.040335 (0.0380-0.0571)	0.005111 (0.0025-0.0096)		
	49.957752 (34.97-51.21)	0.073249 (0.0595-0.1035)				25.572432 (24.80-38.10)
	50.620448 (36.31-54.36)	0.071635 (0.0511-0.0751)			0.008728 (0.0058-0.0123)	
	50.643996 (36.32-54.45)	0.071551 (0.0510-0.0749)		0.005085 (0.0025-0.0096)		
	50.868370 (36.53-58.54)	0.048837 (0.0167-0.0762)	0.028260 (0.0096-0.0449)			
0.501443 (0.4980-0.7206)					0.008655 (0.0057-0.0122)	25.334597 (23.29-35.64)
0.501944 (0.4985-0.7217)				0.004932 (0.0024-0.0093)		25.301793 (23.29-35.60)
0.503446 (0.5010-0.7145)				0.005770 (0.0026-0.0128)	0.008540 (0.0054-0.0121)	
0.522602 (0.5090-0.7838)			0.039451 (0.0281-0.0453)			25.101301 (21.98-35.19)
0.522819 (0.5141-0.8081)			0.039459 (0.0281-0.0449)		0.008817 (0.0058-0.0124)	
0.523506 (0.5149-0.8102)			0.039407 (0.0280-0.0449)	0.005212 (0.0026-0.0099)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.524080 (0.5080-0.7918)		0.073506 (0.0637-0.1054)				25.123082 (22.06-35.22)
0.524556 (0.05136-0.8143)		0.073520 (0.0641-0.1054)			0.008807 (0.0058-0.0124)	
0.525255 (0.5144-0.8164)		0.073607 (0.0642-0.1055)		0.005208 (0.0026-0.0098)		
0.505296 (0.4987-1.251)		x	x			
0.506336 (0.3637-0.7185)	51.182240 (36.67-71.81)					25.081131 (17.96-35.42)
0.506833 (0.4995-0.7333)	51.237649 (48.47-72.23)				0.008748 (0.0058-0.0123)	
0.507465 (0.5001-0.7336)	51.289713 (48.45-72.27)			0.004956 (0.0024-0.0093)		
0.524082 (0.5100-0.7791)	50.880059 (46.09-71.34)		0.039444 (0.0281-0.0454)			
0.525977 (0.5100-0.7900)	50.924092 (46.29-71.42)	0.073507 (0.0635-0.1054)				

Table 26: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.009063 (0.0061-0.0127)	24.385305 (24.13-35.34)
				0.005178 (0.0028-0.0091)		24.389098 (24.13-35.35)
				0.005622 (0.0029-0.0114)	0.008985 (0.0059-0.0126)	
			0.040318 (0.0397-0.0573)			25.267694 (24.64-36.02)
			0.040467 (0.0401-0.0577)		0.008744 (0.0059-0.0122)	
			0.040493 (0.0401-0.0577)	0.005088 (0.0028-0.0090)		
		0.071705 (0.0511-0.0725)				25.286043 (24.59-35.98)
		0.071428 (0.0509-0.0719)			0.008728 (0.0005-0.0122)	
		0.071382 (0.0508-0.0718)		0.005074 (0.0028-0.0089)		
		0.049253 (0.0210-0.0740)	0.028445 (0.0120-0.0436)			
	51.902917 (37.09-52.26)					24.701363 (24.38-35.62)
	52.045752 (37.14-52.33)				0.008877 (0.0060-0.0124)	
	52.030033 (37.13-52.31)			0.004972 (0.0027-0.0088)		
	50.574528 (36.23-52.54)		0.040332 (0.0386-0.0570)			
	50.642180 (36.33-53.51)	0.071551 (0.0510-0.0741)				
0.501979 (0.4994-0.7213)						25.299547 (23.76-35.58)
0.503468 (0.5016-0.7145)					0.008705 (0.0059-0.0122)	
0.503744 (0.5019-0.7151)				0.004964 (0.0027-0.0087)		
0.523484 (0.5169-0.8091)			0.039409 (0.0281-0.0436)			
0.525267 (0.5169-0.8150)		0.073610 (0.0664-0.1053)				
0.507474 (0.5018-0.7333)	51.287884 (49.11-72.24)					

Table 27: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						24.388709 (24.22-35.34)
					0.009112 (0.0062-0.0126)	
				0.005184 (0.0031-0.0084)		
			0.040502 (0.0403-0.0577)			
		0.071366 (0.0508-0.0716)				
	52.027793 (37.13-52.21)					
0.503775 (0.5026-0.7148)						

Table 28: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using progress curve analysis of enzyme saturating *in silico* generated data with the effect of 10% noise.

## GAPN parameter value estimations and confidence intervals

Saturating *in silico* generated data with initial temperature lag

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
x	4.870481 (4.859-4.889)	x	x	x	x	x

Table 29: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	4.220570 (4.213-4.233)	x	x	x	x	x
x		x	x	x	x	24.008684 (23.98-24.08)
119.945358 (87.05-120.3)	4.274273 (4.267-4.292)		x	x	x	x
x	4801.7136 (4794-4853)	x		239.131911 (239.0-248.5)	x	4.085700 (4.074-4.103)
x	4.871506 (4.862-4.889)	x	x		0.107979 (0.1073-0.1090)	x
x	x	x	x	x		x
x	44.499416 (44.47-44.61)	x	x	x	x	

Table 30: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		x	x	0.002201 (0.0006-0.0100)	x	42.862053 (42.83-43.00)
	3.969780 (3.964-3.980)		0.000288 (0.0002-0.0003)	x	x	x
	4.006978 (4.001-4.015)	8.539669 (8.537-8.542)		x	x	x
	3.987153 (3.981-3.998)	0.000444 (0.0004-0.0037)	x		0.109732 (0.1091-0.1107)	x
	4.152061 (4.145-4.163)	x	x	1.472487 (1.456-1.490)		x
	28.558463 (28.54-28.59)	x	x	x	x	
x			x	x	x	23.981684 (23.96-24.05)
x		x		x	x	25.428727 (25.41-25.49)
x		x	x		0.183165 (0.1827-0.1839)	24.447001 (24.42-24.51)
314.956954 (292.2-315.0)		x	x	2.450878 (2.443-2.473)		23.958762 (23.94-24.02)
x		x	x	2.138005 (2.138-2.141)	0.010337 (0.0098-0.0105)	
0.403269 (0.4030-0.4052)	373.459232 (373.2-375.3)			0.007518 (0.0051-0.0213)	0.000419 (0.0002-0.0005)	700.318123 (696.3-778.6)
x	4.261425 (4.255-4.276)		x		0.109639 (0.1090-0.1105)	x
x	4.250193 (4.244-4.266)		x	1.293529 (1.284-1.311)		x
x	44.342612 (44.31-44.44)		x	x	x	
x	x	x			0.552571 (0.5523-0.5530)	1.209335 (1.206-1.213)
x	x	x		13.187959 (13.17-13.29)		x
x	x	x		x	x	
x	3.951901 (3.946-3.962)	x	x			x
169.151482 (160.9-170.7)	44.262695 (44.23-44.36)	x	x		0.186368 (0.1860-0.1870)	
x	44.329710 (44.30-44.42)	x	x	2.416563 (2.409-2.436)		

Table 31: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
			0.001586 (0.0015-0.0016)	0.001936 (0.0005-0.0058)	0.104400 (0.0894-0.1056)	44.258099 (44.23-44.38)
		1.495866 (1.495-1.496)		0.000410 (0.0001-0.0010)	0.095503 (0.0884-0.0958)	43.051807 (43.03-43.16)
		x	x		0.104596 (0.1043-0.1049)	42.857821 (42.83-42.98)
		x	x	1.193401 (1.190-1.200)		42.714442 (42.69-42.83)
		x	x	0.002132 (0.0006-0.0128)	0.131907 (0.1316-0.1323)	
	473.652107 (473.6-473.8)			0.003631 (0.0028-0.0069)	0.000798 (0.0007-0.0008)	481.781008 (480.7-502.4)
	3.945884 (3.941-3.953)		0.000288 (0.0002-0.0003)		0.109247 (0.1087-0.1100)	x
	3.964976 (3.960-3.973)		0.000288 (0.0002-0.0003)	1.290251 (1.281-1.306)		x
	27.945197 (27.93-27.97)		0.001456 (0.0014-0.0015)	x	x	
	4.004653 (4.000-4.011)	8.547773 (8.546-8.549)			0.101179 (0.1006-0.1019)	x
	4.005108 (4.000-4.012)	8.539484 (8.538-8.541)		1.206344 (1.198-1.220)		x
	30.477156 (30.46-30.50)	1.602708 (1.602-1.603)		x	x	
	3.697185 (3.692-3.704)	0.000601 (0.0005-0.0035)	x			x
	28.550230 (28.53-28.57)	x	x		0.156575 (0.1563-0.1570)	
	28.526640 (28.51-28.55)	x	x	1.958306 (1.953-1.970)		
0.088337 (0.0883-0.0884)				x	x	x
1183.5932 (1183-1184)			x		0.189222 (0.1888-0.1899)	23.912575 (23.89-23.97)
1280.2758 (1280-1281)			x	2.445728 (2.439-2.466)		23.903816 (23.88-23.96)
x			x	x	x	
x		x			0.150653 (0.1503-0.1512)	25.449010 (25.43-25.50)
x		x		1.865928 (1.860-1.879)		25.338124 (25.32-25.39)
x		x		x	x	
x		x	x			24.987721 (24.96-25.06)
x		x	x		0.185454 (0.1851-0.1860)	
x		x	x	2.380596 (2.374-2.398)		
18.036456 (17.97-18.32)	x				0.004389 (0.0043-0.0044)	1.877322 (1.871-1.889)



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.490384 (0.4901-0.4930)	429.753596 (429.4-432.1)			x		463.558974 (462.1-485.8)
1.508203 (1.507-1.513)	1210.5804 (1210-1214)			0.001264 (0.0009-0.0023)	0.002133 (0.0020-0.0022)	
x	3.936707 (3.931-3.945)		x			x
909.824675 (710.5-911.2)	44.588133 (44.56-44.67)		x		0.186207 (0.1858-0.1868)	
802.317568 (648.3-803.8)	44.204444 (44.18-44.29)		x	2.395307 (2.389-2.412)		
x	6483.0201 (6473-6551)	x				3.610818 (3.603-3.623)
x	x	x			0.153873 (0.1535-0.1544)	
x	1831.243471 (1829-1840)	x		0.000511 (0.0003-0.0008)		
x	51.214494 (51.18-51.40)	x	x			

Table 32: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.004925 (0.0039-0.0080)	0.006138 (0.0057-0.0063)	60.961950 (60.94-61.09)
			0.001586 (0.0015-0.0016)		0.101405 (0.1012-0.1017)	44.254123 (44.23-44.36)
			0.001590 (0.0015-0.0016)	1.149964 (1.147-1.155)		44.100869 (44.08-44.20)
			0.002016 (0.0020-0.0021)	0.001839 (0.0005-0.0057)	0.129443 (0.1294-0.1297)	
		1.494225 (1.494-1.495)			0.091397 (0.0912-0.0916)	42.986893 (42.97-43.08)
		1.488347 (1.488-1.489)		1.018152 (1.015-1.023)		42.756808 (42.74-42.85)
		1.202097 (1.202-1.203)		0.000426 (0.0002-0.0011)	0.114845 (0.0976-0.1151)	
		x	x			45.926415 (45.90-46.06)
		x	x		0.128552 (0.1283-0.1288)	
		x	x	1.530620 (1.527-1.537)		
	473.656992 (473.6-473.7)				0.000755 (0.0007-0.0008)	481.948309 (481.0-498.9)
	450.150757 (450.1-450.2)			x		437.446328 (436.7-450.3)
	449.982501 (449.9-450.1)			0.006637 (0.0053-0.0129)	0.000499 (0.0004-0.0005)	
	3.673355 (3.669-3.679)		0.000274 (0.0002-0.0003)			x
	27.961907 (27.95-27.98)		0.001456 (0.0014-0.0015)		0.155330 (0.1551-0.1557)	
	27.938150 (27.92-27.96)		0.001456 (0.0014-0.0015)	1.939887 (1.936-1.950)		
	3.722436 (3.718-3.728)	8.987022 (8.984-8.988)				x
	x	x			x	
	x	x		x		
	30.672629 (30.66-30.70)	x	x			
0.080329 (0.0803-0.0804)					x	x
0.079354 (0.0793-0.0794)				0.000126 (3.07-23.0x10 <sup>-5</sup> )		x
0.076886 (0.0768-0.0769)				x	x	
x			x			24.857242 (24.84-24.91)
x			x		0.185289 (0.1850-0.1858)	
x			x	2.405551 (2.400-2.419)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
x		x				23.974806 (23.96-24.02)
x		x			0.151868 (0.1516-0.1523)	
x		x		1.879714 (1.875-1.890)		
x		x	x			
0.492193 (0.4919-0.4943)	429.965956 (429.7-431.9)					458.729270 (457.6-476.6)
1.503572 (1.503-1.508)	1207.1014 (1206-1210)				0.001962 (0.0019-0.0020)	
1.762706 (1.762-1.769)	1322.4285 (1321-1327)			0.000093 (93.0-180x10 <sup>-5</sup> )		
x	52.387549 (52.36-52.54)		x			
x	x	x				

Table 33: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.006071 (0.0058-0.0062)	60.959510 (60.94-61.06)
				0.002649 (0.0023-0.0034)		61.079238 (61.06-61.18)
				0.007173 (0.0060-0.0111)	0.004393 (0.0040-0.0045)	
			0.001503 (0.0015-0.0016)			47.655526 (47.63-47.77)
			0.002016 (0.0020-0.0021)		0.125780 (0.1256-0.1260)	
			0.002018 (0.0020-0.0021)	1.491445 (1.489-1.496)		
		1.439861 (1.439-1.440)				41.543595 (41.53-41.62)
		1.201558 (1.201-1.202)			0.110143 (0.1099-0.1103)	
		1.199775 (1.199-1.200)		1.273484 (1.271-1.278)		
		x	x			
	448.733169 (448.7-448.8)					434.793207 (434.2-444.5)
	449.963609 (449.9-450.0)				0.000528 (0.0005-0.0006)	
	412.815129 (412.7-412.9)			x		
	29.943050 (29.93-29.96)		0.001480 (0.0014-0.0015)			
	35.373242 (35.36-35.39)	1.450084 (1.449-1.451)				
0.088621 (0.0886-0.0887)						x
0.076887 (0.0768-0.0769)					x	
0.076307 (0.0763-0.0764)				0.000117 (3.57-19.5x10 <sup>-5</sup> )		
x			x			
x		x				
1.777134 (1.776-1.782)	1326.8404 (1326-1330)					

Table 34: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						61.087576 (61.07-61.15)
					0.004755 (0.0046-0.0048)	
				0.002469 (0.0022-0.0029)		
			0.002057 (0.0020-0.0021)			
		1.144314 (1.144-1.145)				
	410.784939 (410.7-410.8)					
0.076343 (0.0763-0.0764)						

Table 35: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using progress curve analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						x
					x	
				x		
			x			
		x				
	x					
0.206678 (0.2066-0.2633)						

Table 36: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using initial rate analysis of enzyme saturating *in silico* generated data with the effect of initial temperature lag.

### Saturating *in silico* generated data with 10% noise and temperature lag

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	4.881036 (2.923-4.898)	x	x	x	x	x
x		x	x	x	x	22.936964 (17.83-32.05)
x	4.078438 (2.909-4.347)		x	x	x	x
x	4.117131 (4.110-4.127)	x		x	x	x
x	4.411119 (4.403-4.428)	x	x		0.113235 (0.1125-0.1144)	x
x	4.301735 (4.294-4.318)	x	x	1.350107 (1.340-1.370)		x
x	42.873751 (42.84-42.97)	x	x	x	0.002647 (0.0025-0.0031)	

Table 37: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using initial rate analysis of enzyme saturating *in silico* generated data with the effect of 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		x	x	0.002289 (0.0006-0.0097)	x	41.283497 (39.57-58.58)
	3.775963 (2.690-3.966)		0.000284 (0.0002-0.0004)	x	x	x
	3.828566 (2.727-4.008)	8.681681 (6.142-8.758)		x	x	x
	3.744931 (2.682-4.235)	x	x		0.114132 (0.0809-0.1369)	x
	5.952340 (2.143-5.931)	x	x	1.730653 (1.022-1.758)		x
	27.690475 (19.89-30.32)	x	x	x	x	
x			x	x	x	22.984317 (16.53-32.04)
x		x		x	x	24.890852 (21.04-34.11)
x		x	x		0.203937 (0.1459-0.2380)	22.931372 (17.38-32.01)
x		x	x	2.714727 (1.944-3.780)		22.935202 (18.11-32.03)
x		x	x	x	x	
0.401758 (0.3788-0.5717)	373.450493 (345.3-529.0)			0.008241 (0.0035-0.0276)	0.000440 (0.0001-0.0006)	777.121939 (558.4-1266)
x	4.043717 (2.887-4.306)		x		0.114418 (0.0812-0.1384)	x
x	4.082982 (2.911-4.305)		x	1.355564 (0.9632-1.883)		x
x	43.010361 (38.81-60.18)		x	x	x	
x	4.112980 (2.932-4.320)	x			0.104811 (0.0743-0.1254)	x
x	4.115260 (2.934-4.322)	x		1.252895 (0.8900-1.735)		x
x	45.517839 (43.47-64.11)	x		x	x	
x	3.712698 (2.653-4.024)	x	x			x
x	46.441077 (42.41-46.53)	x	x		0.195899 (0.1955-0.1966)	
62.033405 (60.91-62.04)	43.303143 (43.28-43.39)	x	x	2.598398 (2.597-2.617)		

Table 38: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using initial rate analysis of enzyme saturating *in silico* generated data with the effect of 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.001608 (0.0011-0.0017)	0.002029 (0.0006-0.0071)	x	42.632673 (41.15-60.52)
		1.483504 (1.463-2.086)		0.000432 (0.0001-0.0011)	x	41.668795 (40.26-59.12)
		x	x		0.111949 (0.0800-0.1267)	41.278676 (39.81-58.56)
		x	x	1.292765 (0.9254-1.666)		41.149691 (39.68-58.37)
		x	x	0.002256 (0.0006-0.0106)	x	
	475.567555 (472.9-671.0)			0.004029 (0.0021-0.0097)	0.000814 (0.0005-0.0011)	520.529170 (449.5-810.2)
	3.752958 (2.674-3.918)		0.000283 (0.0002-0.0003)		0.114042 (0.0810-0.1362)	x
	3.772545 (2.688-3.937)		0.000284 (0.0002-0.0004)	1.358234 (0.9665-1.888)		x
	27.129465 (19.48-29.05)		0.001427 (0.0013-0.0019)	x	x	
	3.820981 (2.723-3.980)	8.691979 (6.215-8.758)			0.105105 (0.0745-0.1232)	x
	3.822604 (2.724-3.981)	8.683221 (6.145-8.748)		1.262244 (0.8972-1.688)		x
	29.602752 (21.27-32.83)	1.638218 (1.176-1.686)		x	x	
	4.772881 (2.457-4.783)	x	x			x
	27.707584 (19.90-29.88)	x	x		0.164617 (0.1177-0.1824)	
	27.687026 (19.88-29.84)	x	x	2.080962 (1.492-2.895)		
0.087596 (0.0628-0.0882)				x	x	x
x			x		0.204449 (0.1462-0.2283)	22.897849 (19.68-32.00)
x			x	2.705635 (1.941-3.774)		22.926045 (18.99-32.01)
x			x	x	x	
x		x			0.163589 (0.1168-0.1824)	24.465118 (21.89-34.23)
x		x		2.062039 (1.477-2.866)		24.334652 (22.21-34.08)
x		x		x	x	
x		x	x			24.286325 (18.54-33.91)
x		x	x		0.195860 (0.1955-0.1965)	
x		x	x	2.559384 (2.553-2.578)		
0.403334 (0.4031-0.4050)	374.911893 (374.72-376.5)				0.000504 (0.0004-0.0006)	771.493332 (767.2-854.3)



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.482832 (0.4825-0.4854)	425.930483 (425.6-428.3)			x		511.570023 (509.8-540.4)
1.505268 (1.504-1.510)	1218.1405 (1217-1222)			0.001418 (0.0010-0.0027)	0.002106 (0.0020-0.0022)	
x	3.724927 (3.720-3.733)		x			x
707.425270 (587.7-709.0)	42.985024 (42.96-43.06)		x		0.197604 (0.1972-0.1982)	
660.885739 (556.8-662.5)	43.169990 (43.15-43.25)		x	2.605995 (2.600-2.624)		
x	6594.1340 (6584-6664)	x				3.519935 (3.513-3.532)
x	45.785180 (45.76-45.87)	x			0.164442 (0.1641-0.1649)	
x	1826.0567 (1824-1835)	x		0.000581 (0.0003-0.0009)		
x	48.117068 (48.08-48.26)	x	x			

Table 39: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using initial rate analysis of enzyme saturating *in silico* generated data with the effect of 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.005245 (0.0029-0.0104)	0.006278 (0.0041-0.0088)	59.773316 (58.74-85.76)
			0.001608 (0.0011-0.0017)		0.108465 (0.0776-0.1202)	42.628532 (41.40-60.50)
			0.001612 (0.0011-0.0017)	1.244502 (0.8912-1.530)		42.487529 (41.27-60.30)
			0.002012 (0.0014-0.0021)	x	x	
		1.481907 (1.465-2.084)			0.097912 (0.0699-0.1074)	41.606630 (40.45-59.01)
		1.476225 (1.459-2.076)		1.102808 (0.7887-1.299)		41.390181 (40.24-58.70)
		1.208312 (1.191-1.685)		0.000454 (0.0001-0.0012)	x	
		x	x			44.653808 (43.15-63.51)
		x	x		0.135715 (0.0970-0.1464)	
		x	x	1.631855 (1.169-2.001)		
	475.585991 (473.4-671.0)				0.000787 (0.0005-0.0010)	520.709026 (461.6-805.8)
	453.036045 (453.0-453.1)			x		471.605898 (470.7-487.4)
	453.065663 (453.0-453.2)			0.007357 (0.0056-0.0156)	0.000512 (0.0004-0.0006)	
	3.481587 (3.477-3.487)		0.000269 (0.0002-0.0003)			x
	27.138715 (27.13-27.16)		0.001427 (0.0014-0.0015)		0.163246 (0.1630-0.1636)	
	27.116955 (27.10-27.13)		0.001427 (0.0014-0.0015)	2.060303 (2.056-2.071)		
	3.540877 (3.537-3.546)	9.140429 (9.138-9.142)				x
	x	x			x	
	x	x		x		
	29.148722 (29.13-29.17)	x	x			
0.080281 (0.0802-0.0803)					x	x
x				0.000136 (3.67-25.3x10 <sup>-5</sup> )		x
0.075527 (0.0755-0.0756)				x	x	
x			x			24.244738 (24.23-24.30)
x			x		0.195665 (0.1953-0.1961)	
x			x	2.557540 (2.552-2.572)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
x		x				23.483553 (23.47-23.53)
x		x			0.161858 (0.1615-0.1623)	
x		x		2.027536 (2.023-2.038)		
x		x	x			
0.484534 (0.4843-0.4867)	426.137707 (425.9-428.1)					506.463071 (505.0-529.6)
1.501242 (1.500-1.505)	1215.0811 (1214-1218)				0.001951 (0.0019-0.0020)	
1.755342 (1.754-1.761)	1329.6923 (1329-1334)			0.000099 (1.32-19.3x10 <sup>-5</sup> )		
x	48.330895 (48.30-48.45)		x			
x	x	x				

Table 40: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using initial rate analysis of enzyme saturating *in silico* generated data with the effect of 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.006282 (0.0043-0.0087)	59.772701 (58.98-85.74)
				0.002857 (0.0017-0.0047)		59.884574 (59.09-85.91)
				0.007547 (0.0044-0.0145)	0.004565 (0.0029-0.0064)	
			0.001512 (0.0010-0.0016)			46.322295 (45.14-65.90)
			0.002011 (0.0014-0.0021)		0.132789 (0.0949-0.1408)	
			0.002013 (0.0014-0.0021)	1.589443 (1.139-1.854)		
		1.442687 (1.430-2.035)				40.736393 (39.78-57.81)
		1.207838 (1.195-1.684)			0.117353 (0.0837-0.1238)	
		1.206014 (1.193-1.681)		1.369621 (0.9796-1.533)		
		x	x			
	451.659561 (449.9-635.2)					468.485332 (434.4-718.0)
	453.056603 (451.6-645.6)				0.000549 (0.0003-0.0007)	
	416.815726 (415.5-591.2)			x		
	28.475600 (20.46-30.31)		0.001423 (0.0013-0.0019)			
	33.666185 (24.20-37.05)	1.513058 (1.085-1.537)				
0.087841 (0.0630-0.0882)						x
0.075508 (0.0751-0.1057)					x	
0.074862 (0.0744-0.1047)				0.000123 (3.23-25.0x10 <sup>-5</sup> )		
x			x			
x		x				
1.768808 (1.743-2.578)	1333.6354 (1309-1927)					

Table 41: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using initial rate analysis of enzyme saturating *in silico* generated data with the effect of 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						59.891830 (59.38-85.89)
					0.004956 (0.0034-0.0065)	
				0.002650 (0.0017-0.0041)		
			0.002039 (0.0014-0.0021)			
		1.157316 (1.148-1.614)				
	414.825153 (414.0-588.4)					
0.074892 (0.0746-0.1047)						

Table 42: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using initial rate analysis of enzyme saturating *in silico* generated data with the effect of 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

Non-saturating *in silico* generated data

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.514298 (0.5103-0.5258)	50.891413 (50.46-113.8)	0.069913 (0.0545-0.0873)	0.042291 (0.0330-0.0520)	0.005224 (0.0049-0.0081)	0.009435 (0.0084-0.0097)	25.765679 (25.55-41.62)

Table 43: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of non-saturating *in silico* generated data.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	50.861422 (50.61-50.89)	0.069911 (0.0561-0.0851)	0.042287 (0.0340-0.0508)	0.005226 (0.0050-0.0062)	0.009431 (0.0091-0.0096)	25.777877 (25.66-33.87)
0.522916 (0.5227-0.5802)		0.073508 (0.0434-0.1095)	0.045042 (0.0267-0.0653)	0.005700 (0.0053-0.0082)	0.009029 (0.0085-0.0094)	21.417665 (21.22-33.26)
0.512143 (0.5036-1.805)	49.960542 (49.09-176.2)		0.042336 (0.0421-0.0432)	0.005765 (0.0051-0.0131)	0.008920 (0.0068-0.0094)	21.692502 (21.42-41.83)
0.512182 (0.5033-1.802)	49.966166 (49.10-176.0)	0.068542 (0.0682-0.0705)		0.005773 (0.0052-0.0131)	0.008916 (0.0068-0.0094)	21.739267 (21.47-41.71)
0.515885 (0.5128-0.6213)	51.050284 (50.75-51.77)	0.069931 (0.0561-0.0855)	0.042306 (0.0339-0.0509)		0.009463 (0.0093-0.0096)	25.721038 (25.53-36.85)
0.518088 (0.5154-0.5265)	51.265058 (51.00-63.84)	0.069940 (0.0561-0.0855)	0.042314 (0.0340-0.0509)	0.005185 (0.0050-0.0057)		25.647297 (25.51-34.14)
0.527949 (0.5252-0.8309)	52.171062 (51.83-82.73)	0.070341 (0.0567-0.0857)	0.042626 (0.0344-0.0509)	0.005129 (0.0049-0.0069)	0.009619 (0.0090-0.0098)	

Table 44: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of non-saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		0.070852 (0.0635-0.0741)	0.042796 (0.0382-0.0462)	0.005239 (0.0051-0.0061)	0.009425 (0.0092-0.0095)	25.703858 (25.62-32.25)
	50.913909 (50.86-50.93)		0.041750 (0.0416-0.0421)	0.005222 (0.0050-0.0061)	0.009435 (0.0092-0.0095)	25.792780 (25.69-32.84)
	50.886528 (50.82-50.91)	0.069434 (0.0693-0.0700)		0.005225 (0.0050-0.0061)	0.009432 (0.0092-0.0095)	25.791088 (25.69-32.71)
	50.871851 (50.69-50.90)	0.069860 (0.0578-0.0829)	0.042237 (0.0350-0.0496)		0.009448 (0.0093-0.0095)	25.774028 (25.67-32.87)
	50.872851 (50.70-50.90)	0.069776 (0.0577-0.0826)	0.042237 (0.0349-0.0495)	0.005182 (0.0050-0.0056)		25.727246 (25.63-32.50)
	50.751660 (50.55-50.77)	0.070386 (0.0585-0.0837)	0.042737 (0.0356-0.0500)	0.005220 (0.0050-0.0061)	0.009471 (0.0092-0.0096)	
0.512456 (0.5124-0.5154)			0.041750 (0.0416-0.0421)	0.005234 (0.0051-0.0061)	0.009416 (0.0092-0.0095)	25.834511 (25.72-33.32)
0.512726 (0.5126-0.5166)		0.069435 (0.0693-0.0702)		0.005235 (0.0051-0.0061)	0.009416 (0.0092-0.0095)	25.826075 (25.71-33.12)
0.512915 (0.5128-0.5346)		0.069910 (0.0577-0.0865)	0.042300 (0.0350-0.0499)		0.009440 (0.0093-0.0095)	25.795493 (25.67-33.47)
0.513137 (0.5130-0.5347)		0.070021 (0.0579-0.0837)	0.042373 (0.0351-0.0499)	0.005184 (0.0050-0.0056)		25.720305 (25.61-32.82)
0.514446 (0.5144-0.5348)		0.070748 (0.0591-0.0839)	0.042923 (0.0359-0.0839)	0.005223 (0.0050-0.0060)	0.009471 (0.0092-0.0096)	
0.513740 (0.5112-1.014)	50.989767 (50.65-100.6)			0.005190 (0.0050-0.0074)	0.009464 (0.0087-0.0096)	25.575392 (25.43-36.25)
0.515384 (0.5128-0.7895)	51.053159 (50.79-78.05)		0.041755 (0.0416-0.0421)		0.009460 (0.0093-0.0096)	25.752793 (25.59-35.33)
0.517747 (0.5155-0.6289)	51.285300 (51.05-62.33)		0.041758 (0.0416-0.0421)	0.005184 (0.0050-0.0056)		25.674046 (25.55-32.99)
0.528115 (0.5258-0.7921)	52.263685 (51.97-78.79)		0.041838 (0.0417-0.0421)	0.005122 (0.0049-0.0066)	0.009628 (0.0091-0.0097)	
0.516093 (0.5134-0.7908)	51.096855 (50.84-78.12)	0.069423 (0.0693-0.0701)			0.009465 (0.0093-0.0096)	25.728173 (25.57-35.16)
0.517946 (0.5157-0.6291)	51.278235 (51.05-62.31)	0.069420 (0.0693-0.0702)		0.005185 (0.0050-0.0056)		25.666362 (25.55-32.85)
0.528265 (0.5258-0.7900)	52.255922 (51.97-78.52)	0.069295 (0.0691-0.0700)		0.005124 (0.0049-0.0066)	0.009627 (0.0091-0.0097)	
0.518241 (0.5160-0.6289)	51.273588 (51.04-62.30)	0.069947 (0.0578-0.0836)	0.042312 (0.0350-0.0499)			25.636443 (25.52-32.96)
0.524815 (0.5228-0.7099)	51.832095 (51.60-70.35)	0.070450 (0.0586-0.0838)	0.042687 (0.0355-0.0499)		0.009553 (0.0094-0.0096)	
0.522111 (0.5203-0.6262)	51.560837 (51.34-62.03)	0.070506 (0.0586-0.0838)	0.042742 (0.0356-0.0499)	0.005204 (0.0051-0.0056)		

Table 45: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of non-saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		X	X	X	X	X
	X		X	X	X	X
	X	X		X	X	X
	X	X	X		X	X
	X	X	X	X		X
	X	X	X	X	X	
X			X	X	X	X
0.444751 (0.0020-84.33)		X		X	X	X
0.559551 (0.2597-41.81)		X	X		X	X
X		X	X	X		X
X		X	X	X	X	
X	X			X	X	X
X	X		X		X	X
X	X		X	X		X
X	X		X	X	X	
X	X	X			X	X
X	X	X		X		X
X	X	X		X	X	
X	X	X	X			X
X	X	X	X		X	
X	X	X	X	X		

Table 46: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using initial rate analysis of non-saturating *in silico* generated data.



## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.041600 (0.0415-0.0418)	0.005252 (0.0051-0.0059)	0.009430 (0.0092-0.0095)	25.625645 (25.56-31.08)
		0.069647 (0.0695-0.0697)		0.005250 (0.0051-0.0059)	0.009424 (0.0092-0.0095)	25.690617 (25.62-31.30)
		0.070910 (0.0647-0.0736)	0.042840 (0.0389-0.0457)		0.009451 (0.0093-0.0095)	25.690350 (25.62-31.22)
		0.070811 (0.0645-0.0735)	0.042798 (0.0389-0.0457)	0.005193 (0.0051-0.0055)		25.641305 (25.57-30.87)
		0.070298 (0.0641-0.0727)	0.042690 (0.0388-0.0456)	0.005220 (0.0051-0.0059)	0.009473 (0.0093-0.0095)	
	51.015547 (51.00-51.02)			0.005188 (0.0050-0.0059)	0.009467 (0.0093-0.0096)	25.568265 (25.49-30.20)
	50.919655 (50.88-50.93)		0.041760 (0.0417-0.0420)		0.009450 (0.0093-0.0095)	25.790170 (25.70-31.81)
	50.917431 (50.87-50.93)		0.041778 (0.0417-0.0420)	0.005181 (0.0051-0.0055)		25.744500 (25.66-31.47)
	50.827795 (50.78-50.84)		0.041921 (0.0418-0.0421)	0.005214 (0.0051-0.0059)	0.009479 (0.0093-0.0096)	
	50.894621 (50.84-50.91)	0.069413 (0.0693-0.0699)			0.009448 (0.0093-0.0095)	25.787642 (25.70-31.70)
	50.893562 (50.84-50.91)	0.069382 (0.0692-0.0698)		0.005182 (0.0051-0.0055)		25.728791 (25.65-31.35)
	50.811551 (50.76-50.82)	0.069155 (0.0690-0.0696)		0.005217 (0.0051-0.0060)	0.009477 (0.0093-0.0096)	
	50.865216 (50.72-50.88)	0.067930 (0.0594-0.0808)	0.042242 (0.0360-0.0485)			25.716837 (25.63-31.43)
	50.759535 (50.61-50.77)	0.070345 (0.0602-0.0814)	0.042722 (0.0366-0.0489)		0.009484 (0.0094-0.0095)	
	50.759737 (50.62-50.77)	0.070310 (0.0602-0.0812)	0.042700 (0.0366-0.0487)	0.005201 (0.0051-0.0055)		
0.511433 (0.5114-0.5122)				0.005207 (0.0051-0.0059)	0.009435 (0.0092-0.0095)	25.635118 (25.55-30.91)
0.512398 (0.5123-0.5148)			0.041761 (0.0416-0.0420)		0.009437 (0.0093-0.0095)	25.828675 (25.73-32.21)
0.512554 (0.5125-0.5150)			0.041771 (0.0417-0.0420)	0.005182 (0.0051-0.0055)		25.753222 (25.66-31.68)
0.513507 (0.5134-0.5159)			0.041899 (0.0418-0.0421)	0.005223 (0.0051-0.0059)	0.009468 (0.0093-0.0095)	
0.512652 (0.5126-0.5161)		0.069413 (0.0693-0.0700)			0.009439 (0.0093-0.0095)	25.820632 (25.72-32.05)
0.512815 (0.5127-0.5161)		0.069401 (0.0693-0.0700)		0.005184 (0.0051-0.0055)		25.745421 (25.65-31.54)
0.513753 (0.5137-0.5170)		0.069210 (0.0691-0.0698)		0.005220 (0.0051-0.0059)	0.009474 (0.0093-0.0095)	
0.513215 (0.5132-0.5316)		0.070044 (0.0596-0.0817)	0.042381 (0.0361-0.0488)			25.709523 (25.61-31.68)
0.514403 (0.5143-0.5318)		0.070752 (0.0607-0.0820)	0.042933 (0.0369-0.0489)		0.009487 (0.0094-0.0095)	
0.514464 (0.5144-0.5319)		0.070758 (0.0607-0.0821)	0.042929 (0.0369-0.0489)	0.005205 (0.0051-0.0056)		
0.513053 (0.5114-0.7437)	50.921462 (50.70-73.74)				0.009452 (0.0093-0.0095)	25.593492 (25.47-32.96)

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.515886 (0.5143-0.6090)	51.201194 (51.00-60.53)			0.005169 (0.0050-0.0055)		25.512755 (25.42-30.97)
0.525094 (0.5238-0.7332)	52.048978 (51.81-73.22)			0.005119 (0.0049-0.0064)	0.009610 (0.0092-0.0097)	
0.517810 (0.5159-0.6126)	51.285446 (51.09-60.73)		0.041754 (0.0416-0.0420)			25.663637 (25.56-31.82)
0.524620 (0.5229-0.6827)	51.892112 (51.69-67.65)		0.041834 (0.0417-0.0421)		0.009556 (0.0094-0.0096)	
0.521786 (0.5203-0.6108)	51.611077 (51.43-60.54)		0.041858 (0.0417-0.0421)	0.005203 (0.0052-0.0056)		
0.518049 (0.5161-0.6129)	51.281773 (51.08-60.71)	0.069429 (0.0693-0.0700)				25.656820 (25.56-31.69)
0.524902 (0.5232-0.6816)	51.896531 (51.70-67.49)	0.069304 (0.0692-0.0699)			0.009557 (0.0094-0.0096)	
0.521993 (0.5204-0.6107)	51.609434 (51.43-60.48)	0.069265 (0.0691-0.0698)		0.005204 (0.0051-0.0056)		
0.522089 (0.5205-0.6112)	51.560145 (51.38-60.53)	0.070504 (0.0603-0.0819)	0.042743 (0.0366-0.0488)			

Table 47: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of non-saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
			X	X	X	X
		X		X	X	X
		X	X		X	X
		X	X	X		X
		X	X	X	X	
	X			X	X	X
	X		X		0.099809 (0.0099-1.094)	X
	X		X	X		X
	X		X	X	X	
	X	X			0.100177 (0.0016-10.77)	X
	X	X		X		X
	X	X		X	X	
	X	X	X			X
	X	X	X		X	
	X	X	X	X		
0.452872 (0.0307-15.07)				X	X	X
0.568461 (0.0326-63.89)			X		0.105542 (0.1055-5.253)	X
X			X	X		X
X			X	X	X	
0.560849 (0.5608-97.36)	X				0.105453 (0.1054-5.439)	X
X	X			X		X
X	X			X	X	
X	X		X			X
X	X		X		X	
X	X		X	X		
X	X	X				X

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
x	x	x			x	
x	x	x		x		
x	x	x	x			
x		x			x	x
x		x		x		x
x		x		x	x	
x		x	x			x
x		x	x		x	
x		x	x	x		

Table 48: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using initial rate analysis of non-saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.005213 (0.0051-0.0058)	0.009504 (0.0093-0.0096)	24.759974 (24.75-25.47)
			0.041610 (0.0416-0.0417)		0.009463 (0.0094-0.0095)	25.602266 (25.55-30.00)
			0.041626 (0.0416-0.0418)	0.005207 (0.0051-0.0055)		25.568358 (25.52-29.76)
			0.041827 (0.0418-0.0419)	0.005227 (0.0051-0.0058)	0.009475 (0.0093-0.0095)	
		0.069633 (0.0695-0.0697)			0.009457 (0.0094-0.0095)	25.671271 (25.61-30.22)
		0.069603 (0.0695-0.0697)		0.005202 (0.0051-0.0055)		25.627919 (25.57-29.93)
		0.069259 (0.0692-0.0693)		0.005226 (0.0051-0.0058)	0.009476 (0.0093-0.0095)	
		0.070791 (0.0657-0.0729)	0.042785 (0.0396-0.0451)			25.639579 (25.58-29.93)
		0.070349 (0.0653-0.0723)	0.042725 (0.0395-0.0451)		0.009483 (0.0094-0.0095)	
		0.070281 (0.0652-0.0722)	0.042681 (0.0394-0.0451)	0.005201 (0.0051-0.0055)		
	51.014497 (51.00-51.02)				0.009460 (0.0094-0.0095)	25.564693 (25.50-29.35)
	51.011697 (50.99-51.01)			0.005168 (0.0051-0.0054)		25.556453 (25.49-29.31)
	50.876942 (50.87-50.88)			0.005202 (0.0051-0.0057)	0.009486 (0.0093-0.0095)	
	50.909970 (50.87-50.92)		0.041772 (0.0417-0.0420)			25.731786 (25.66-30.38)
	50.831665 (50.80-50.84)		0.041927 (0.0418-0.0421)		0.009488 (0.0094-0.0095)	
	50.830712 (50.79-50.84)		0.041927 (0.0418-0.0421)	0.005200 (0.0051-0.0055)		
	50.885969 (50.84-50.90)	0.069392 (0.0693-0.0697)				25.728270 (25.66-30.28)
	50.816397 (50.77-50.83)	0.069144 (0.0690-0.0694)			0.009488 (0.0094-0.0095)	
	50.815578 (50.77-50.83)	0.069143 (0.0690-0.0694)		0.005202 (0.0051-0.0055)		
	50.760226 (50.64-50.77)	0.070309 (0.0620-0.0793)	0.042701 (0.0377-0.0477)			
0.511430 (0.5114-0.5120)					0.009440 (0.0093-0.0095)	25.636993 (25.56-29.98)
0.511576 (0.5115-0.5122)				0.005168 (0.0051-0.0054)		25.586455 (25.52-29.69)
0.512932 (0.5129-0.5130)				0.005208 (0.0051-0.0057)	0.009477 (0.0093-0.0095)	
0.512622 (0.5125-0.5146)			0.041765 (0.0417-0.0420)			25.741837 (25.67-30.54)
0.513513 (0.5134-0.5154)			0.041899 (0.0418-0.0421)		0.009484 (0.0094-0.0095)	
0.513513 (0.5134-0.5155)			0.041899 (0.0418-0.0421)	0.005204 (0.0051-0.0055)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.512890 (0.5128-0.5155)		0.069411 (0.0693-0.0699)				25.734734 (25.66-30.42)
0.513689 (0.5136-0.5162)		0.069196 (0.0691-0.0696)			0.009486 (0.0094-0.0095)	
0.513705 (0.5136-0.5163)		0.069195 (0.0691-0.0696)		0.005203 (0.0051-0.0055)		
0.514452 (0.5144-0.5287)		0.070776 (0.0625-0.0801)	0.042943 (0.0379-0.0478)			
0.515997 (0.5147-0.5925)	51.204196 (51.04-58.88)					25.483982 (25.41-29.79)
0.521459 (0.5205-0.6346)	51.664250 (51.51-63.16)				0.009535 (0.0094-0.0096)	
0.519905 (0.5190-0.5883)	51.502385 (51.35-58.49)			0.005190 (0.0051-0.0054)		
0.521799 (0.5205-0.5951)	51.612903 (51.46-58.96)		0.041859 (0.0418-0.0420)			
0.522020 (0.5207-0.5950)	51.613430 (51.46-58.92)	0.069263 (0.0691-0.0697)				

Table 49: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of non-saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.009513 (0.0094-0.0095)	24.760168 (24.75-25.31)
				0.005215 (0.0051-0.0054)		24.759820 (24.75-25.31)
				0.005212 (0.0051-0.0056)	0.009509 (0.0094-0.0096)	
			0.041627 (0.0416-0.0417)			25.567810 (25.53-28.78)
			0.041825 (0.0418-0.0419)		0.009487 (0.0094-0.0095)	
			0.041830 (0.0418-0.0419)	0.005208 (0.0051-0.0054)		
		0.069604 (0.0695-0.0697)				25.628898 (25.59-28.92)
		0.069270 (0.0692-0.0693)			0.009493 (0.0094-0.0095)	
		0.069259 (0.0692-0.0693)		0.005212 (0.0051-0.0054)		
		0.070308 (0.0665-0.0717)	0.042700 (0.0402-0.0445)			
	51.003125 (50.99-51.01)					25.528880 (25.48-28.28)
	50.876995 (50.87-50.88)				0.009487 (0.0094-0.0095)	
	50.876554 (50.87-50.88)			0.005193 (0.0051-0.0054)		
	50.830798 (50.80-50.84)		0.041927 (0.0419-0.0420)			
	50.815877 (50.78-50.82)	0.069143 (0.0690-0.0693)				
0.511661 (0.5116-0.5121)						25.556605 (25.50-28.56)
0.512927 (0.5129-0.5130)					0.009485 (0.0094-0.0095)	
0.512913 (0.5129-0.5130)				0.005197 (0.0051-0.0054)		
0.513534 (0.5135-0.5150)			0.041898 (0.0418-0.0420)			
0.513699 (0.5136-0.5156)		0.069192 (0.0691-0.0695)				
0.519874 (0.5192-0.5723)	51.498670 (51.39-56.85)					

Table 50: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of non-saturating *in silico* generated data.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						24.759853 (24.75-25.11)
					0.009515 (0.0094-0.0096)	
				0.005216 (0.0051-0.0053)		
			0.041823 (0.0418-0.0419)			
		0.069261 (0.0692-0.0693)				
	50.876056 (50.87-50.88)					
0.512917 (0.5129-0.5130)						

Table 51: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using progress curve analysis of non-saturating *in silico* generated data.



## GAPN parameter value estimations and confidence intervals

Non-saturating *in silico* generated data with 10% noise

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.514265 (0.5064-0.5891)	49.914320 (48.98-171.1)	0.073526 (0.0403-0.1137)	0.045044 (0.0245-0.0676)	0.005770 (0.0051-0.0139)	0.008926 (0.0066-0.0095)	21.588978 (21.27-44.19)

Table 52: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	49.889187 (49.34-49.96)	0.073510 (0.0435-0.1086)	0.045034 (0.0269-0.0648)	0.005772 (0.0053-0.0085)	0.008923 (0.0083-0.0093)	21.594584 (21.42-32.77)
0.512977 (0.5129-0.5282)		0.069910 (0.0561-0.0855)	0.042286 (0.0339-0.0509)	0.005234 (0.0050-0.0062)	0.009418 (0.0091-0.0096)	25.805874 (25.67-34.58)
0.513773 (0.5102-1.080)	50.891299 (50.50-107.2)		0.041750 (0.0416-0.0421)	0.005224 (0.0050-0.0078)	0.009433 (0.0085-0.0096)	25.798734 (25.61-39.78)
0.514027 (0.5102-1.082)	50.889705 (50.50-107.2)	0.069433 (0.0693-0.0703)		0.005224 (0.0050-0.0078)	0.009433 (0.0085-0.0096)	25.789665 (25.60-39.55)
0.551832 (0.5430-0.8229)	53.638170 (52.87-133.3)	0.073409 (0.0434-0.1093)	0.045114 (0.0267-0.0654)		0.009527 (0.0092-0.0099)	20.858106 (20.61-35.21)
0.551394 (0.5430-0.8229)	53.509997 (52.87-133.3)	0.072664 (0.0434-0.1093)	0.045202 (0.0267-0.0654)	0.005396 (0.0092-0.0099)		20.803781 (20.61-35.21)
0.450384 (0.4464-0.4644)	44.025674 (43.53-86.50)	0.071301 (0.0414-0.1059)	0.043250 (0.0254-0.0618)	0.006423 (0.0058-0.0126)	0.007979 (0.0066-0.0084)	

Table 53: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		0.065589 (0.0492-0.0729)	0.040690 (0.0301-0.0484)	0.05644 (0.0053-0.0078)	0.008972 (0.0085-0.0093)	22.061877 (21.94-31.65)
	50.142212 (50.03-50.19)		0.042337 (0.0421-0.0431)	0.005750 (0.0054-0.0081)	0.008943 (0.0084-0.0093)	21.653643 (21.50-31.46)
	50.143495 (50.00-50.21)	0.068541 (0.0683-0.0700)		0.005757 (0.0054-0.0082)	0.008937 (0.0084-0.0092)	21.700402 (21.55-31.39)
	50.080478 (49.69-50.14)	0.072458 (0.0463-0.1019)	0.044652 (0.0286-0.0617)		0.009256 (0.0090-0.0095)	21.558442 (21.41-31.31)
	49.979252 (49.63-50.04)	0.072323 (0.0462-0.1012)	0.044577 (0.0285-0.0614)	0.005364 (0.0051-0.0064)		21.297693 (21.15-30.34)
	50.464807 (49.98-50.51)	0.071050 (0.0445-0.1018)	0.042650 (0.0268-0.0590)	0.005804 (0.0054-0.0083)	0.008724 (0.0082-0.0090)	
0.520284 (0.5201-0.5270)			0.042343 (0.0421-0.0431)	0.005699 (0.0053-0.0079)	0.009018 (0.0085-0.0093)	21.530594 (21.36-31.75)
0.520256 (0.5201-0.5291)		0.068527 (0.0683-0.0702)		0.005707 (0.0053-0.0079)	0.009012 (0.0085-0.0093)	21.575889 (21.41-31.61)
0.522089 (0.5219-0.5723)		0.073439 (0.0470-0.1050)	0.045180 (0.0290-0.0630)		0.009317 (0.0091-0.0096)	21.353374 (21.18-31.62)
0.523876 (0.5237-0.5742)		0.074110 (0.0478-0.1058)	0.045529 (0.0294-0.0633)	0.005388 (0.0051-0.0065)		21.077639 (20.92-30.33)
0.514817 (0.5147-0.5592)		0.068980 (0.0429-0.0980)	0.041577 (0.0260-0.0568)	0.005763 (0.0054-0.0080)	0.008754 (0.0083-0.0090)	
0.511866 (0.5060-1.633)	49.801588 (49.05-158.8)			0.005818 (0.0053-0.0122)	0.008877 (0.0071-0.0093)	21.921614 (21.69-38.12)
0.549306 (0.5422-1.320)	53.655958 (52.99-121.5)		0.042467 (0.0422-0.0432)		0.009516 (0.0092-0.0098)	20.958211 (20.74-33.44)
0.549435 (0.5437-0.8569)	53.598935 (53.02-82.75)		0.042408 (0.0420-0.0430)	0.005388 (0.0051-0.0065)		20.896543 (20.72-30.33)
0.450383 (0.4471-0.8208)	44.135515 (43.71-81.09)		0.041896 (0.0417-0.0426)	0.006408 (0.0059-0.0118)	0.007993 (0.0068-0.0084)	
0.549330 (0.5420-1.321)	53.674211 (53.01-121.5)	0.068323 (0.0681-0.0700)			0.009515 (0.0092-0.0098)	21.005123 (20.79-33.38)
0.549738 (0.5438-0.8576)	53.637640 (53.06-82.80)	0.068423 (0.0681-0.0701)		0.005391 (0.0051-0.0065)		20.936185 (20.77-30.26)
0.450697 (0.4472-0.8183)	44.145520 (43.72-80.77)	0.069216 (0.0690-0.0708)		0.006410 (0.0059-0.0118)	0.007994 (0.0068-0.0084)	
0.549916 (0.5440-0.8543)	53.458410 (52.88-73.16)	0.073395 (0.0470-0.1048)	0.045108 (0.0290-0.0629)			20.899275 (20.73-30.37)
0.486997 (0.4831-0.8222)	48.000493 (47.56-70.48)	0.069656 (0.0434-0.0992)	0.042323 (0.0265-0.0583)		0.008896 (0.0086-0.0091)	
0.515389 (0.5112-0.7560)	50.803401 (50.32-75.00)	0.069306 (0.0429-0.0987)	0.041826 (0.0260-0.0575)	0.005241 (0.0050-0.0063)		

Table 54: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.042951 (0.0429-0.0434)	0.005620 (0.0053-0.0074)	0.008964 (0.0085-0.0092)	22.182251 (22.09-30.33)
		0.067526 (0.0673-0.0675)		0.005627 (0.0053-0.0074)	0.008972 (0.0086-0.0092)	22.082391 (21.98-30.31)
		0.066180 (0.0523-0.0723)	0.041162 (0.0322-0.0476)		0.009237 (0.0090-0.0094)	21.946889 (21.85-29.94)
		0.065316 (0.0513-0.0715)	0.040700 (0.0316-0.0473)	0.005286 (0.0051-0.0061)		21.736211 (21.64-29.13)
		0.068191 (0.0545-0.0737)	0.041175 (0.0324-0.0477)	0.005754 (0.0054-0.0076)	0.008754 (0.0083-0.0090)	
	50.008699 (49.97-50.02)			0.005802 (0.0055-0.0077)	0.008901 (0.0085-0.0092)	21.876931 (21.76-28.66)
	50.269909 (50.18-50.31)		0.042563 (0.0424-0.0432)		0.009262 (0.0091-0.0095)	16.831144 (21.47-29.91)
	50.164604 (50.07-50.21)		0.042571 (0.0424-0.0432)	0.005359 (0.0051-0.0063)		21.345059 (21.22-29.02)
	50.576324 (50.48-50.60)		0.041457 (0.0413-0.0420)	0.005793 (0.0055-0.0078)	0.008735 (0.0083-0.0090)	
	50.298842 (50.19-50.35)	0.068151 (0.0679-0.0692)			0.009261 (0.0091-0.0095)	21.653228 (21.52-29.86)
	50.193147 (50.08-50.25)	0.068137 (0.0679-0.0692)		0.005361 (0.0051-0.0063)		21.388881 (21.26-28.97)
	50.517733 (50.40-50.55)	0.069951 (0.0697-0.0709)		0.005801 (0.0055-0.0079)	0.008729 (0.0083-0.0090)	
	50.048898 (49.73-50.10)	0.072156 (0.0498-0.0968)	0.044529 (0.0308-0.0590)			21.363850 (21.24-29.06)
	50.679062 (50.34-50.72)	0.069913 (0.0473-0.0950)	0.042222 (0.0287-0.0560)		0.009068 (0.0089-0.0092)	
	50.678980 (50.35-50.72)	0.069059 (0.0465-0.0933)	0.041697 (0.0282-0.0550)	0.005239 (0.0050-0.0061)		
0.521694 (0.5216-0.5236)				0.005740 (0.0054-0.0076)	0.008992 (0.0086-0.0093)	21.727108 (21.59-29.27)
0.519499 (0.5194-0.5249)			0.042511 (0.0423-0.0431)		0.009307 (0.0091-0.0095)	21.465072 (21.32-30.10)
0.520902 (0.5208-0.5266)			0.042467 (0.0423-0.0431)	0.005378 (0.0051-0.0063)		21.190076 (21.06-29.00)
0.514796 (0.5147-0.5204)			0.041593 (0.0414-0.0421)	0.005762 (0.0054-0.0077)	0.008753 (0.0083-0.0090)	
0.519316 (0.5192-0.5265)		0.068252 (0.0680-0.0696)			0.009304 (0.0091-0.0095)	21.517005 (21.37-30.01)
0.520819 (0.5207-0.5283)		0.068340 (0.0681-0.0698)		0.005381 (0.0051-0.0063)		21.241376 (21.11-28.93)
0.515129 (0.5150-0.5225)		0.069687 (0.0695-0.0710)		0.005762 (0.0054-0.0077)	0.008750 (0.0083-0.0090)	
0.522998 (0.5228-0.5657)		0.073846 (0.0513-0.1008)	0.045434 (0.0316-0.0606)			21.168422 (21.03-29.04)
0.513671 (0.5135-0.5516)		0.068803 (0.0465-0.0936)	0.041646 (0.0283-0.0547)		0.009067 (0.0089-0.0092)	
0.514873 (0.5148-0.5530)		0.069145 (0.0468-0.0940)	0.041738 (0.0284-0.0548)	0.005242 (0.0050-0.0061)		
0.553718 (0.5487-1.220)	53.891075 (53.32-112.5)				0.009527 (0.0092-0.0098)	21.178356 (21.00-31.24)

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.552679 (0.5484-0.8156)	53.739701 (53.24-78.73)			0.005415 (0.0052-0.0064)		21.086113 (20.94-28.43)
0.448892 (0.4471-0.7426)	44.035084 (43.68-73.69)			0.006406 (0.0060-0.0110)	0.007982 (0.0070-0.0083)	
0.548071 (0.5432-0.8086)	53.540257 (53.05-78.21)		0.042470 (0.0423-0.0431)			20.985102 (20.84-29.01)
0.486812 (0.4837-0.7728)	48.015732 (47.64-76.49)		0.041937 (0.0417-0.0425)		0.008897 (0.0087-0.0091)	
0.515827 (0.5124-0.7217)	50.864400 (50.45-71.45)		0.041645 (0.0415-0.0422)	0.005240 (0.0050-0.0061)		
0.548294 (0.5433-0.8091)	53.577423 (53.09-78.25)	0.068320 (0.0681-0.0697)				21.028010 (20.88-28.96)
0.486933 (0.4837-0.7705)	48.018807 (47.65-76.19)	0.069116 (0.0689-0.0705)			0.008897 (0.0087-0.0091)	
0.515849 (0.5123-0.7206)	50.838649 (50.43-71.27)	0.069595 (0.0694-0.0710)		0.005240 (0.0050-0.0061)		
0.515665 (0.5121-0.6683)	50.855943 (50.44-71.59)	0.069096 (0.0465-0.0943)	0.041722 (0.0282-0.0551)			

Table 55: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
				0.005719 (0.0055-0.0072)	0.008800 (0.0085-0.0090)	23.886084 (23.86-25.35)
			0.043032 (0.0430-0.0434)		0.009218 (0.0090-0.0094)	22.052140 (21.97-28.53)
			0.043158 (0.0431-0.0435)	0.005256 (0.0051-0.0059)		21.854563 (21.78-27.89)
			0.041708 (0.0417-0.0418)	0.005745 (0.0055-0.0073)	0.008753 (0.0084-0.0089)	
		0.067420 (0.0673-0.0675)			0.009231 (0.0091-0.0094)	21.964368 (21.88-28.55)
		0.067228 (0.0671-0.0673)		0.005271 (0.0051-0.0059)		21.753895 (21.68-27.85)
		0.069432 (0.0694-0.0695)		0.005748 (0.0055-0.0073)	0.008753 (0.0084-0.0089)	
		0.065550 (0.0542-0.0704)	0.040851 (0.0335-0.0461)			21.749813 (21.67-27.83)
		0.069127 (0.0581-0.0735)	0.041813 (0.0347-0.0471)		0.009067 (0.0089-0.0092)	
		0.068294 (0.0571-0.0728)	0.041300 (0.0341-0.0467)	0.005232 (0.0050-0.0059)		
	50.054520 (50.02-50.06)				0.009238 (0.0091-0.0094)	21.994974 (21.90-27.63)
	49.931272 (49.90-49.94)			0.005939 (0.0052-0.0061)		21.694460 (21.60-27.07)
	50.919014 (50.91-50.93)			0.005698 (0.0054-0.0072)	0.008785 (0.0084-0.0090)	
	50.223753 (50.15-50.26)		0.042619 (0.0425-0.0431)			21.410470 (21.31-27.70)
	50.728839 (50.65-50.75)		0.041686 (0.0416-0.0421)		0.009070 (0.0089-0.0092)	
	50.682630 (50.61-50.70)		0.041662 (0.0415-0.0421)	0.005239 (0.0050-0.0059)		
	50.257137 (50.16-50.30)	0.068059 (0.0679-0.0689)				21.457575 (21.36-27.66)
	50.696890 (50.61-50.73)	0.069541 (0.0694-0.0703)			0.009068 (0.0089-0.0092)	
	50.654998 (50.56-50.68)	0.069568 (0.0694-0.0703)		0.005239 (0.0050-0.0059)		
	50.692908 (50.42-50.72)	0.069025 (0.0505-0.0890)	0.041692 (0.0306-0.0526)			
0.521580 (0.5215-0.5231)					0.009301 (0.0091-0.0095)	21.760427 (21.65-27.99)
0.522897 (0.5228-0.5244)				0.005408 (0.0052-0.0062)		21.431732 (21.32-27.12)
0.512333 (0.5123-0.5126)				0.005712 (0.0054-0.0072)	0.008762 (0.0084-0.0089)	
0.520218 (0.5201-0.5247)			0.042521 (0.0424-0.0430)			21.272952 (21.16-27.66)
0.513807 (0.5137-0.5182)			0.041758 (0.0416-0.0422)		0.009065 (0.0089-0.0092)	
0.514819 (0.5147-0.5194)			0.041646 (0.0415-0.0421)	0.005240 (0.0050-0.0059)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.520041 (0.5199-0.5260)		0.068241 (0.0680-0.0694)				21.327458 (21.22-27.61)
0.513950 (0.5138-0.5197)		0.069398 (0.0692-0.0704)			0.009067 (0.0089-0.0092)	
0.515037 (0.5149-0.5211)		0.069579 (0.0694-0.0706)		0.005240 (0.0050-0.0059)		
0.514749 (0.5146-0.5460)		0.069095 (0.0507-0.0895)	0.041721 (0.0307-0.0525)			
0.551557 (0.5480-0.7663)	53.684656 (53.28-74.03)					21.223604 (21.11-27.15)
0.485789 (0.4841-0.6934)	47.944497 (47.65-68.98)				0.008890 (0.0087-0.0090)	
0.511051 (0.5091-0.6675)	50.582856 (50.26-66.55)			0.005209 (0.0050-0.0059)		
0.515668 (0.5129-0.6850)	50.862006 (50.52-67.81)		0.041664 (0.0415-0.0421)			
0.515789 (0.5129-0.6843)	50.845816 (50.51-67.67)	0.069569 (0.0694-0.0706)				

Table 56: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.009098 (0.0090-0.0092)	23.889942 (23.87-25.01)
				0.005231 (0.0051-0.0057)		23.898598 (23.88-25.02)
				0.005715 (0.0055-0.0069)	0.008813 (0.0085-0.0089)	
			0.043154 (0.0431-0.0434)			21.861021 (21.80-26.49)
			0.041728 (0.0417-0.0418)		0.009075 (0.0089-0.0092)	
			0.041771 (0.0417-0.0418)	0.005225 (0.0051-0.0057)		
		0.067239 (0.0671-0.0673)				21.766042 (21.70-26.43)
		0.069408 (0.0693-0.0695)			0.009071 (0.0089-0.0092)	
		0.069340 (0.0693-0.0694)		0.005225 (0.0051-0.0057)		
		0.068379 (0.0599-0.0717)	0.041357 (0.0359-0.0453)			
	49.979948 (49.95-49.99)					21.813839 (21.74-25.83)
	50.925400 (50.91-50.93)				0.009069 (0.0089-0.0092)	
	50.896107 (50.89-50.90)			0.005206 (0.0050-0.0057)		
	50.695357 (50.64-50.71)		0.041679 (0.0416-0.0420)			
	50.667552 (50.60-50.69)	0.069543 (0.0694-0.0701)				
0.522362 (0.5223-0.5234)						21.564922 (21.48-25.81)
0.512367 (0.5123-0.5126)					0.009063 (0.0089-0.0092)	
0.512733 (0.5127-0.5129)				0.005206 (0.0050-0.0057)		
0.514652 (0.5146-0.5180)			0.041671 (0.0415-0.0420)			
0.514945 (0.5149-0.5193)		0.069562 (0.0694-0.0703)				
0.511223 (0.5097-0.6311)	50.602081 (50.35-62.83)					

Table 57: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						23.898726 (23.89-24.62)
					0.009105 (0.0090-0.0092)	
				0.005232 (0.0051-0.0055)		
			0.041775 (0.0417-0.0418)			
		0.069339 (0.0693-0.0694)				
	50.896267 (50.67-50.90)					
0.512709 (0.5126-0.5128)						

Table 58: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using progress curve analysis of non-saturating *in silico* generated data with 10% noise.



## GAPN parameter value estimations and confidence intervals

Non-saturating *in silico* generated data with temperature lag

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.237671 (0.2365-0.2575)	29.883970 (29.60-46.12)	0.064300 (0.0579-0.0754)	0.122969 (0.1101-0.1500)	0.003596 (0.0035-0.0043)	0.019281 (0.0186-0.0198)	3.555017 (3.541-3.974)

Table 59: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	61.729574 (49.34-49.96)	0.070747 (0.0435-0.1086)	0.139156 (0.0269-0.0648)	0.002723 (0.0053-0.0085)	0.026601 (0.0083-0.0093)	2.916152 (21.42-32.77)
0.400556 (0.5129-0.5282)		0.062800 (0.0561-0.0855)	0.122772 (0.0339-0.0509)	0.002773 (0.0050-0.0062)	0.024145 (0.0091-0.0096)	3.230851 (25.67-34.58)
0.240723 (0.2398-0.2479)	29.553832 (29.35-44.10)		0.132049 (0.1319-0.1336)	0.003675 (0.0036-0.0043)	0.019592 (0.0190-0.0199)	3.447015 (3.428-3.678)
0.216540 (0.2158-0.3118)	32.022416 (31.80-46.06)	0.022687 (0.0226-0.0228)		0.003227 (0.0031-0.0038)	0.017212 (0.00166-0.0175)	4.403808 (4.376-4.833)
0.201871 (0.2013-0.2088)	23.967696 (23.86-29.52)	0.071327 (0.0669-0.0815)	0.133558 (0.1222-0.1607)		0.016768 (0.0165-0.0173)	3.474646 (3.445-3.813)
0.140208 (0.1400-0.1454)	19.225681 (19.14-20.36)	0.052923 (0.0469-0.0615)	0.097846 (0.0867-0.1173)	0.006773 (0.0066-0.0076)		4.766914 (4.730-5.288)
0.138383 (0.1382-0.1389)	37.065559 (36.94-45.08)	0.004703 (0.0035-0.0087)	x	0.002340 (0.0022-0.0028)	0.008741 (0.0084-0.0088)	

Table 60: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		0.107659 (0.1046-0.1117)	0.212664 (0.2061-0.2318)	0.003337 (0.0032-0.0035)	0.029342 (0.0292-0.0294)	2.331612 (2.331-2.359)
	62.096761 (61.95-62.14)		0.135619 (0.1355-0.1370)	0.002704 (0.0026-0.0029)	0.026541 (0.0264-0.0267)	2.933904 (2.926-3.078)
	74.983661 (74.80-75.02)	0.022175 (0.0221-0.0222)		0.002274 (0.0022-0.0024)	0.024157 (0.0240-0.0243)	3.687601 (3.675-3.954)
	51.216686 (50.90-51.37)	0.100232 (0.0952-0.1110)	0.194092 (0.1839-0.2278)		0.027147 (0.0270-0.0274)	2.347858 (2.342-2.435)
	129.774996 (116.4-129.7)	x	x	0.002482 (0.0024-0.0028)		12.834647 (12.58-19.63)
	152.502639 (152.2-152.6)	x	x	0.001091 (0.0010-0.0011)	0.014760 (0.0147-0.0148)	
0.409768 (0.4097-0.4146)			0.135478 (0.1353-0.1368)	0.002812 (0.0027-0.0030)	0.024551 (0.0244-0.0248)	3.140072 (3.128-3.325)
0.337644 (0.3376-0.3419)		0.022225 (0.0221-0.0223)		0.002505 (0.0024-0.0026)	0.021049 (0.0209-0.0213)	4.106761 (4.088-4.469)
0.472016 (0.4718-0.4887)		0.084914 (0.0798-0.0949)	0.163126 (0.1528-0.1928)		0.025244 (0.0250-0.0255)	2.584923 (2.572-2.756)
0.221088 (0.2210-0.2242)		0.011742 (0.0052-0.0152)	0.023831 (0.0100-0.0314)	0.002856 (0.0028-0.0030)		11.083137 (11.00-13.15)
0.182736 (0.1827-0.1830)		x	x	0.001766 (0.0017-0.0018)	0.010680 (0.0106-0.0107)	
0.170788 (0.1706-0.1766)	10.352742 (10.32-10.99)			0.037877 (0.0363-0.0730)	0.006917 (0.0057-0.0074)	3.147456 (3.136-3.234)
0.200373 (0.2000-0.2408)	23.964074 (23.89-28.53)		0.128995 (0.1288-0.1302)		0.016634 (0.0164-0.0169)	3.506217 (3.489-3.706)
0.141819 (0.1417-0.1459)	18.356939 (18.30-19.06)		0.128218 (0.1281-0.1295)	0.007352 (0.0072-0.0079)		4.468941 (4.446-4.723)
0.128520 (0.1284-0.1457)	31.535300 (31.46-36.05)		0.153158 (0.1530-0.1549)	0.002837 (0.0027-0.0034)	0.007624 (0.0073-0.0077)	
0.171819 (0.1715-0.1991)	24.262035 (24.19-27.86)	0.023397 (0.0233-0.0234)			0.013724 (0.0135-0.0140)	4.413424 (4.388-4.769)
0.138457 (0.1383-0.1431)	21.486841 (21.42-22.50)	0.023041 (0.0230-0.0231)		0.005655 (0.0055-0.0061)		5.577429 (5.551-6.059)
0.135265 (0.1351-0.1579)	35.587558 (35.49-41.91)	0.019610 (0.0195-0.0197)		0.002435 (0.0023-0.0029)	0.008474 (0.0081-0.0085)	
0.139894 (0.1397-0.1432)	21.066979 (21.00-21.85)	0.043174 (0.0388-0.0483)	0.081113 (0.0728-0.0934)			5.447316 (5.432-5.685)
0.104631 (0.1045-0.1050)	26.530443 (26.49-27.55)	0.006734 (0.0013-0.0099)	x		0.005119 (0.0050-0.0052)	
0.147971 (0.1478-0.1555)	40.026673 (39.94-42.21)	x	x	0.002099 (0.0020-0.0021)		

Table 61: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.128761 (0.1286-0.1297)	0.003463 (0.0034-0.0036)	0.030166 (0.0300-0.0302)	2.225333 (2.225-2.232)
		0.024664 (0.0246-0.0247)		0.003663 (0.0036-0.0038)	0.030834 (0.0307-0.0309)	2.135947 (2.135-2.142)
		0.102271 (0.0997-0.1054)	0.198213 (0.1928-0.2124)		0.027306 (0.0272-0.0273)	2.325881 (2.325-2.348)
		0.157790 (0.1545-0.1637)	0.291474 (0.2846-0.3217)	0.014834 (0.0146-0.0177)		2.604431 (2.604-2.645)
		1.537804 (1.537-1.538)	x	x	x	
	31.175403 (31.09-31.19)			0.10172 (0.0100-0.0117)	0.024712 (0.0243-0.0249)	2.217431 (2.212-2.261)
	55.419961 (55.32-55.49)		0.129992 (0.1299-0.1310)		0.026092 (0.0260-0.0262)	2.513154 (2.508-2.576)
	92.264992 (91.73-92.28)		0.137133 (0.1370-0.1386)	0.004329 (0.0042-0.0048)		6.115565 (5.951-6.843)
	141.981566 (141.9-142.0)		0.166349 (0.1162-0.1680)	0.001114 (0.0011-0.0012)	0.014632 (0.0145-0.0147)	
	63.256032 (63.14-63.32)	0.023579 (0.0235-0.0236)			0.024108 (0.0240-0.0242)	2.879945 (2.874-2.968)
	120.355528 (119.6-120.4)	0.020600 (0.0205-0.0207)		0.002802 (0.0027-0.0030)		10.441151 (10.33-13.22)
	150.252128 (150.1-150.3)	0.018368 (0.0183-0.0184)		0.001088 (0.0010-0.0011)	0.014749 (0.0147-0.0148)	
	97.872602 (97.23-97.89)	0.026538 (0.0222-0.0305)	0.050229 (0.0420-0.0589)			6.380932 (6.355-6.734)
	147.010993 (146.7-147.1)	x	x		0.010262 (0.0102-0.0103)	
	151.734511 (151.5-151.8)	x	x	0.001874 (0.0018-0.0019)		
0.761426 (0.7613-0.7697)				0.007919 (0.0078-0.0088)	0.026567 (0.0262-0.0268)	2.384542 (2.377-2.441)
0.450233 (0.4501-0.4543)			0.130719 (0.1306-0.1317)		0.024274 (0.0241-0.0244)	2.722603 (2.715-2.829)
0.262852 (0.2628-0.2684)			0.144247 (0.1441-0.1456)	0.003597 (0.0035-0.0038)		7.868081 (7.802-8.563)
0.195329 (0.1953-0.1956)			0.159659 (0.1595-0.1612)	0.001769 (0.0017-0.0018)	0.010718 (0.0106-0.0108)	
0.383855 (0.3838-0.3875)		0.023340 (0.0233-0.0234)			0.020959 (0.0208-0.0211)	3.328972 (3.318-3.510)
0.227547 (0.2275-0.2317)		0.020509 (0.0204-0.0206)		0.002969 (0.0029-0.0031)		10.344072 (10.28-11.70)
0.185150 (0.1851-0.1854)		0.019092 (0.0190-0.0191)		0.001754 (0.0017-0.0018)	0.010700 (0.0106-0.0108)	
0.257478 (0.2574-0.2602)		0.020238 (0.0160-0.0231)	0.039099 (0.0308-0.0456)			7.458028 (7.440-7.834)
0.185462 (0.1854-0.1857)		x	x		0.007117 (0.0070-0.0072)	
0.182645 (0.1826-0.1829)		x	x	0.002021 (0.0020-0.0021)		
0.243760 (0.2435-0.2619)	15.777914 (15.72-16.96)				0.019323 (0.0192-0.0195)	2.940161 (2.931-3.011)

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.183308 (0.1832-0.1887)	11.027764 (11.00-11.27)			0.026941 (0.0266-0.0295)		3.012007 (3.003-3.069)
0.110745 (0.1107-0.1136)	14.459324 (14.44-14.88)			0.101602 (0.0864-7.101)	x	
0.141997 (0.1419-0.1454)	20.051103 (20.01-20.63)		0.132078 (0.1319-0.1331)			5.101155 (5.092-5.251)
0.107016 (0.1069-0.1100)	25.267116 (25.23-26.04)		0.147893 (0.1477-0.1492)		0.005049 (0.0050-0.0051)	
0.149101 (0.1489-0.1551)	37.451968 (37.38-39.08)		0.156019 (0.1559-0.1575)	0.002137 (0.0021-0.0022)		
0.138668 (0.1385-0.1423)	22.028091 (21.98-22.72)	0.022857 (0.0228-0.0229)				5.797461 (5.786-6.002)
0.104696 (0.1046-0.1078)	26.183438 (26.15-27.02)	0.020425 (0.0204-0.0205)			0.005089 (0.0050-0.0051)	
0.147664 (0.1475-0.1540)	39.353508 (39.28-41.15)	0.019434 (0.0194-0.0195)		0.002098 (0.0020-0.0021)		
0.136131 (0.1360-0.1414)	35.553053 (35.49-37.02)	x	x			

Table 62: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.005926 (0.0058-0.0062)	0.017568 (0.0174-0.0176)	4.835495 (4.835-4.840)
			0.127562 (0.1275-0.1283)		0.028157 (0.0281-0.0282)	2.233418 (2.233-2.389)
			0.113893 (0.1138-0.1146)	0.020458 (0.0202-0.0313)		2.339007 (2.338-2.345)
			0.022147 (0.0221-0.0222)	0.009904 (0.0098-0.0105)	0.010001 (0.0099-0.0100)	
		0.024819 (0.0247-0.0249)			0.029094 (0.0290-0.0291)	2.142250 (2.142-2.147)
		0.027577 (0.0275-0.0276)		x		2.257173 (2.257-2.263)
		0.126690 (0.1266-0.1267)		0.009106 (0.0090-0.0096)	0.009977 (0.0099-0.0100)	
		0.238293 (0.2352-0.2423)	0.488503 (0.4805-0.5422)			2.873341 (2.873-2.910)
		1.520899 (1.520-1.521)	x		0.007790 (0.0077-0.0078)	
		1.522502 (1.522-1.523)	x	0.002456 (0.0024-0.0025)		
	32.780751 (32.71-32.79)				0.026865 (0.0268-0.0270)	2.374774 (2.370-2.412)
	25.672996 (25.62-25.71)			0.199728 (0.1949-0.2028)		1.731969 (1.729-1.771)
	82.514059 (82.51-82.52)			0.003582 (0.0035-0.0037)	0.011976 (0.0119-0.0120)	
	87.453849 (87.35-87.46)		0.134381 (0.1343-0.1353)			5.492597 (5.482-5.673)
	136.861446 (136.8-136.9)		0.145690 (0.1456-0.1467)		0.010161 (0.0101-0.0102)	
	141.300590 (141.2-141.2)		0.160539 (0.1604-0.1618)	0.001893 (0.0018-0.0019)		
	99.079873 (98.96-99.09)	0.022286 (0.0222-0.0223)				6.503304 (6.490-6.768)
	144.613351 (144.5-144.7)	0.020602 (0.0205-0.0207)			0.010230 (0.0102-0.0103)	
	149.467874 (149.4-149.5)	0.018947 (0.0189-0.0190)		0.001867 (0.0018-0.0019)		
	147.255590 (147.0-147.3)	x	x			
0.743220 (0.7431-0.7496)					0.027757 (0.0276-0.0279)	2.477393 (2.471-2.525)
0.572116 (0.5720-0.5820)				x		3.887745 (3.841-3.981)
0.325025 (0.3250-0.3251)				0.004696 (0.0046-0.0049)	0.009749 (0.0097-0.0098)	
0.284991 (0.2849-0.2860)			0.139183 (0.1391-0.1401)			6.500112 (6.489-6.709)
0.198353 (0.1983-0.1985)			0.149944 (0.1498-0.1510)		0.007156 (0.0071-0.0072)	
0.195261 (0.1952-0.1954)			0.159245 (0.1591-0.1605)	0.002019 (0.0020-0.0021)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
0.258290 (0.2582-0.2592)		0.021691 (0.0216-0.0217)				7.418466 (7.405-7.701)
0.188107 (0.1881-0.1883)		0.020144 (0.0201-0.0202)			0.007105 (0.0070-0.0072)	
0.185103 (0.1851-0.1853)		0.019149 (0.0191-0.0192)		0.002013 (0.0020-0.0021)		
0.187348 (0.1873-0.1883)		x	x			
0.178417 (0.1783-0.1811)	13.423453 (13.39-13.68)					4.128956 (4.121-4.202)
0.148428 (0.1483-0.1520)	20.843875 (20.82-21.38)				0.006649 (0.0066-0.0067)	
0.166017 (0.1659-0.1698)	23.696594 (23.66-24.30)			0.005375 (0.0053-0.0055)		
0.137123 (0.1370-0.1412)	33.064313 (33.01-34.12)		0.143279 (0.1431-0.1443)			
0.135591 (0.1355-0.1398)	34.651821 (34.60-35.81)	0.020949 (0.0209-0.0210)				

Table 63: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.018061 (0.0180-0.0181)	4.835460 (4.835-4.838)
				0.014215 (0.0141-0.0147)		4.826453 (4.826-4.829)
				0.005532 (0.0054-0.0057)	0.019026 (0.0189-0.0191)	
			0.112662 (0.1126-0.1131)			2.349689 (2.349-2.354)
			0.022203 (0.0222-0.0223)		0.011912 (0.0118-0.0120)	
			0.022126 (0.0221-0.0222)	0.010586 (0.0105-0.0108)		
		0.028103 (0.0280-0.0282)				2.279564 (2.279-2.284)
		0.126464 (0.1264-0.1265)			0.011634 (0.0116-0.0117)	
		0.126793 (0.1267-0.1268)		0.009695 (0.0096-0.0099)		
		x	x			
	47.461519 (47.40-47.47)					4.246112 (4.239-4.315)
	82.448057 (82.44-82.45)				0.010918 (0.0109-0.0110)	
	82.689681 (82.68-82.69)			0.004734 (0.0047-0.0048)		
	137.073234 (137.0-137.1)		0.146017 (0.1459-0.1468)			
	144.861064 (144.8-144.9)	0.020558 (0.0205-0.0206)				
0.478555 (0.4785-0.4800)						5.658091 (5.651-5.763)
0.325018 (0.3250-0.3251)					0.009442 (0.0094-0.0095)	
0.324923 (0.3249-0.3250)				0.004844 (0.0048-0.0049)		
0.200330 (0.2003-0.2005)			0.146557 (0.1464-0.1473)			
0.190135 (0.1901-0.1903)		0.020574 (0.0205-0.0206)				
0.166482 (0.1664-0.1694)	23.780911 (23.75-24.24)					

Table 64: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						4.82456 (4.824-4.826)
					0.019271 (0.0192-0.0193)	
				0.015022 (0.0149-0.0154)		
			0.022090 (0.0220-0.0221)			
		0.127061 (0.1270-0.1271)				
	82.639080 (82.63-82.64)					
0.325074 (0.3250-0.3251)						

Table 65: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameter using progress curve analysis of non-saturating *in silico* generated data with initial temperature lag.



## Non-saturating *in silico* generated data with 10% noise and temperature lag

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.116797 (0.1164-0.1173)	16.631592 (16.52-25.60)	0.046286 (0.0397-0.0583)	0.104657 (0.0802-0.1468)	0.003347 (0.0031-0.0053)	0.019307 (0.0173-0.0198)	4.236011 (4.231-4.406)

Table 66: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all seven parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
	71.564223 (71.31-71.63)	0.050805 (0.0414-0.0619)	0.123384 (0.0993-0.1678)	0.001679 (0.0016-0.0019)	0.036482 (0.0362-0.0367)	2.910770 (2.908-2.946)
0.361258 (0.3611-0.3666)		0.047904 (0.0388-0.0588)	0.114797 (0.0920-0.1570)	0.001797 (0.0017-0.0020)	0.033993 (0.0337-0.0342)	3.071130 (3.067-3.113)
0.119987 (0.1196-0.1915)	16.631670 (16.57-26.46)		0.161768 (0.1614-0.1668)	0.003340 (0.0031-0.0050)	0.019526 (0.0177-0.0200)	4.180175 (4.172-4.336)
0.112042 (0.1117-0.1733)	16.470406 (16.41-25.41)	0.019603 (0.0195-0.0197)		0.003413 (0.0032-0.0052)	0.018751 (0.0170-0.0192)	4.346114 (4.338-4.511)
0.097790 (0.0976-0.1105)	13.909214 (13.87-16.80)	0.046098 (0.0367-0.0568)	0.102038 (0.0805-0.1383)		0.014511 (0.0142-0.0150)	4.625688 (4.617-4.763)
0.082196 (0.0821-0.0849)	11.774746 (11.75-12.26)	0.045097 (0.0357-0.0556)	0.098235 (0.0769-0.1327)	0.007219 (0.0070-0.0081)		5.169862 (5.163-5.300)
0.039622 (0.0396-0.0401)	6.920125 (6.912-7.016)	0.016423 (0.0077-0.0257)	0.028106 (0.0122-0.0464)	x	x	

Table 67: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for six parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
		0.335294 (0.3307-0.3372)	1.417169 (1.377-2.104)	0.01988 (0.0019-0.0022)	0.037776 (0.0375-0.0380)	2.544333 (2.544-2.568)
	70.035578 (69.99-70.08)		0.173017 (0.1727-0.1777)	0.001688 (0.0016-0.0019)	0.036516 (0.0363-0.0374)	2.890338 (2.888-2.920)
	74.403074 (74.35-74.46)	0.018525 (0.0184-0.0186)		0.001664 (0.0016-0.0018)	0.036177 (0.0359-0.0363)	2.964090 (2.962-2.995)
	69.598298 (69.31-69.66)	0.053385 (0.0453-0.0639)	0.124473 (0.1046-0.1646)		0.031816 (0.0316-0.0320)	2.840744 (2.838-2.870)
	72.175737 (71.56-72.24)	0.056914 (0.0480-0.0709)	x	x		3.171212 (3.168-3.209)
	122.820986 (122.7-122.9)	x	x	0.001292 (0.0012-0.0014)	0.019621 (0.0195-0.0197)	
0.370525 (0.3704-0.3715)			0.171607 (0.1713-0.1762)	0.001803 (0.0017-0.0020)	0.034217 (0.0340-0.0344)	3.036412 (3.033-3.072)
0.348628 (0.3485-0.3496)		0.018664 (0.0186-0.0187)		0.001799 (0.0017-0.0020)	0.033529 (0.0333-0.0337)	3.137845 (3.135-3.175)
0.369175 (0.3691-0.3758)		0.049429 (0.0413-0.0592)	0.114010 (0.0945-0.1501)		0.029509 (0.0293-0.0297)	3.010773 (3.007-3.047)
0.339540 (0.3394-0.3439)		0.036785 (0.0282-0.0448)	0.076945 (0.0585-0.1008)	0.008493 (0.0083-0.0102)		3.542111 (3.538-3.591)
0.214869 (0.2148-0.2155)		x	x	0.001648 (0.0016-0.0018)	0.016219 (0.0160-0.0163)	
0.105318 (0.1052-0.1155)	10.814577 (10.79-11.86)			0.042090 (0.0367-0.6298)	0.002812 (0.0002-0.0038)	8.365457 (8.340-8.790)
0.100290 (0.1001-0.1191)	13.885897 (13.85-16.46)		0.158255 (0.1580-0.1620)		0.014673 (0.0144-0.0151)	4.566950 (4.557-4.683)
0.083887 (0.0837-0.0868)	11.684968 (11.66-12.10)		0.155715 (0.1554-0.1594)	0.007297 (0.0071-0.0080)		5.119068 (5.107-5.230)
0.040974 (0.0409-0.0415)	6.733252 (6.726-6.805)		0.123811 (0.1236-0.1260)	x	x	
0.094975 (0.0948-0.1120)	13.935993 (13.90-16.40)	0.019949 (0.0198-0.0200)			0.014273 (0.0140-0.0147)	4.720462 (4.711-4.843)
0.080573 (0.0804-0.0834)	11.884044 (11.86-12.31)	0.020194 (0.0201-0.0203)		0.007142 (0.0070-0.0079)		5.246297 (5.234-5.362)
0.039720 (0.0397-0.0402)	6.868248 (6.861-6.942)	0.024314 (0.0242-0.0244)		x	x	
0.081337 (0.0812-0.0841)	11.790563 (11.73-12.21)	0.043824 (0.0355-0.0529)	0.096052 (0.0772-0.1259)			5.268997 (5.261-5.385)
0.041403 (0.0413-0.0421)	7.420118 (7.412-7.541)	0.018011 (0.0060-0.0247)	0.031361 (0.0104-0.0450)		0.005047 (0.0049-0.0051)	
0.042262 (0.0422-0.0428)	7.535759 (7.527-7.668)	0.018046 (0.0058-0.0248)	0.030866 (0.0099-0.0442)	0.004502 (0.0044-0.0048)		

Table 68: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for five parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_mGAP$	$K_mNADP$	$K_mNADPH$	$K_mG1P$	$\alpha$	$K_iGAP$
			0.133698 (0.1335-0.1360)	0.002685 (0.0026-0.0030)	0.042943 (0.0425-0.0431)	1.962460 (1.962-1.973)
		0.025139 (0.0250-0.0252)		0.002915 (0.0028-0.0033)	0.042962 (0.0425-0.0431)	1.927388 (1.927-1.939)
		0.312826 (0.3089-0.3145)	1.150007 (1.124-1.506)		0.033489 (0.0333-0.0336)	2.510937 (2.510-2.531)
		0.382927 (0.3618-0.3846)	x	x		2.841279 (2.840-2.867)
		1.215787 (1.212-1.216)	1.901790 (1.882-2.463)	0.001903 (0.0018-0.0020)	0.016158 (0.0160-0.0162)	
	52.329998 (52.30-52.34)			0.004634 (0.0045-0.0053)	0.023933 (0.0236-0.0241)	4.433712 (4.430-4.499)
	68.338325 (68.30-68.38)		0.165119 (0.1648-0.1687)		0.031883 (0.0317-0.0320)	2.824913 (2.823-2.849)
	71.138372 (71.10-71.18)		0.146777 (0.1465-0.1496)	x		3.155388 (3.153-3.187)
	113.630252 (113.5-113.7)		0.086492 (0.0863-0.0874)	0.001334 (0.0013-0.0014)	0.019424 (0.0193-0.0195)	
	72.478023 (72.44-72.53)	0.0192299 (0.0192-0.0194)			0.031540 (0.0314-0.0317)	2.893422 (2.891-2.919)
	75.372996 (75.33-75.42)	0.021304 (0.0212-0.0214)		x		3.238212 (3.235-3.271)
	119.145650 (119.0-119.2)	0.032909 (0.0328-0.0331)		0.001289 (0.0012-0.0014)	0.019602 (0.0195-0.0197)	
	74.155800 (73.62-74.21)	0.053441 (0.0458-0.0648)	0.112897 (0.0961-0.1480)			3.280986 (3.278-3.314)
	118.448681 (118.3-118.5)	x	x		0.015141 (0.0150-0.0152)	
	120.453058 (120.3-120.5)	x	x	0.003296 (0.0032-0.0035)		
0.492915 (0.4928-0.4941)				0.004640 (0.0045-0.0053)	0.023415 (0.0231-0.0236)	4.563533 (4.558-4.641)
0.377921 (0.3778-0.3788)			0.164440 (0.1642-0.1680)		0.029726 (0.0295-0.0299)	2.979372 (2.977-3.009)
0.353014 (0.3529-0.3553)			0.151089 (0.1508-0.1541)	0.008691 (0.0085-0.0103)		3.477963 (3.474-3.517)
0.231263 (0.2312-0.2319)			0.093805 (0.0936-0.0948)	0.001667 (0.0016-0.0018)	0.016232 (0.0161-0.0163)	
0.356018 (0.3555-0.3569)		0.019368 (0.0193-0.0194)			0.029068 (0.0289-0.0292)	3.076392 (3.074-3.107)
0.333010 (0.3329-0.3339)		0.020692 (0.0206-0.0208)		0.008396 (0.0082-0.0098)		3.586527 (3.582-3.628)
0.220536 (0.2205-0.2212)		0.030503 (0.0304-0.0306)		0.001631 (0.0016-0.0017)	0.016275 (0.0161-0.0163)	
0.332986 (0.3329-0.3380)		0.034979 (0.0276-0.0417)	0.073745 (0.0577-0.0936)			3.644918 (3.642-3.687)
0.219620 (0.2196-0.2202)		x	x		0.012155 (0.0120-0.0122)	
0.215765 (0.2157-0.2163)		x	x	0.003162 (0.0031-0.0033)		
0.142396 (0.1422-0.1565)	14.713286 (14.68-16.16)				0.012345 (0.0121-0.0126)	6.867187 (6.852-7.114)

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.130905 (0.1307-0.1355)	13.376278 (13.35-13.86)			0.011744 (0.0115-0.0129)		7.063687 (7.049-7.278)
0.071472 (0.0714-0.0721)	8.489042 (8.481-8.645)			x	x	
0.083086 (0.0830-0.0855)	11.696146 (11.68-12.05)		0.157063 (0.1568-0.1602)			5.216031 (5.210-5.312)
0.042772 (0.0427-0.0432)	7.228567 (7.222-7.326)		0.125921 (0.1257-0.1278)		0.005053 (0.0049-0.0051)	
0.043561 (0.0435-0.0441)	7.315989 (7.309-7.423)		0.123447 (0.1232-0.1253)	0.004585 (0.0045-0.0049)		
0.079850 (0.0797-0.0822)	11.899361 (11.88-12.26)	0.020045 (0.0199-0.0201)				5.341077 (5.335-5.442)
0.041482 (0.0414-0.0419)	7.385892 (7.379-7.487)	0.023912 (0.0238-0.0240)			0.005042 (0.0049-0.0051)	
0.042364 (0.0423-0.0429)	7.500646 (7.493-7.611)	0.024349 (0.0242-0.0244)		0.004507 (0.0044-0.0048)		
0.042167 (0.0421-0.0427)	7.483408 (7.476-7.595)	0.018297 (0.0078-0.0241)	0.031136 (0.0133-0.0425)			

Table 69: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for four parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
				0.004792 (0.0047-0.0054)	0.024505 (0.0242-0.0246)	4.182529 (4.182-4.190)
			0.131852 (0.1317-0.1337)		0.039464 (0.0393-0.0396)	1.972457 (1.972-1.981)
			0.131149 (0.1310-0.1330)	0.338949 (0.3344-0.3415)		1.977069 (1.977-1.986)
			0.021008 (0.0210-0.0211)	0.008389 (0.0082-0.0096)	0.013351 (0.0131-0.0134)	
		0.025380 (0.0253-0.0254)			0.039887 (0.0397-0.0400)	1.936061 (1.935-1.945)
		0.025386 (0.0253-0.0254)		0.342699 (0.3382-0.3453)		1.936771 (1.936-1.947)
		0.132997 (0.1329-0.1330)		0.007581 (0.0074-0.0086)	0.013414 (0.0132-0.0135)	
		0.422064 (0.4190-0.4233)	1.683774 (1.656-2.276)			2.951943 (2.951-2.974)
		1.157178 (1.154-1.157)	1.485938 (1.475-1.727)		0.012654 (0.0125-0.0127)	
		1.199323 (1.196-1.200)	1.698411 (1.684-2.045)	0.003662 (0.0036-0.0038)		
	52.248930 (52.22-52.26)				0.023489 (0.0234-0.0236)	4.419950 (4.417-4.472)
	53.524045 (53.50-53.53)			0.018539 (0.0180-0.0260)		4.624378 (4.621-4.684)
	83.028248 (83.02-83.03)			0.002817 (0.0027-0.0030)	0.016564 (0.0164-0.0166)	
	72.795844 (72.76-72.83)		0.148956 (0.1487-0.1513)			3.260293 (3.258-3.287)
	109.579411 (109.5-109.6)		0.078803 (0.0787-0.0794)		0.015011 (0.0149-0.0151)	
	111.358110 (111.2-111.4)		0.080446 (0.0803-0.0811)	0.003362 (0.0033-0.0035)		
	77.103384 (77.06-77.14)	0.0201026 (0.0209-0.0211)				3.343403 (3.341-3.371)
	114.512703 (114.4-114.6)	0.036015 (0.0359-0.0362)			0.015087 (0.0150-0.0151)	
	116.483478 (116.4-116.5)	0.035300 (0.0352-0.0354)		0.003287 (0.0032-0.0034)		
	118.834384 (118.8-118.9)	x	x			
0.493491 (0.4934-0.4944)					0.022990 (0.0228-0.0231)	4.551858 (4.548-4.615)
0.470119 (0.4700-0.4711)				0.015739 (0.0154-0.0200)		5.049579 (5.044-5.127)
0.320961 (0.3209-0.3210)				0.003484 (0.0034-0.0038)	0.014184 (0.0140-0.0142)	
0.346695 (0.3466-0.3473)			0.152878 (0.1527-0.1554)			3.578831 (3.576-3.612)
0.236403 (0.2363-0.2369)			0.087913 (0.0878-0.0886)		0.012208 (0.0121-0.0123)	
0.232230 (0.2322-0.2327)			0.091089 (0.0909-0.0919)	0.003200 (0.0031-0.0033)		

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
0.327281 (0.3272-0.3279)		0.020478 (0.0204-0.0205)				3.685160 (3.682-3.719)
0.225830 (0.2258-0.2264)		0.032438 (0.0323-0.0325)			0.012165 (0.0121-0.0122)	
0.221626 (0.2216-0.2222)		0.031366 (0.0313-0.0315)		0.003138 (0.0030-0.0033)		
0.218427 (0.2184-0.2189)		x	x			
0.128307 (0.1282-0.1318)	13.386061 (13.36-13.76)					7.476860 (7.468-7.671)
0.078997 (0.0789-0.0801)	9.748204 (9.730-9.915)				0.006415 (0.0063-0.0065)	
0.079805 (0.0797-0.0809)	9.807197 (9.797-9.972)			0.007588 (0.0074-0.0080)		
0.043464 (0.0434-0.0439)	7.272051 (7.266-7.358)		0.122872 (0.1227-0.1244)			
0.042249 (0.0422-0.0426)	7.446416 (7.441-7.536)	0.024467 (0.0244-0.0245)				

Table 70: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
					0.024156 (0.0240-0.0242)	4.182319 (4.182-4.188)
				0.023226 (0.0228-0.0320)		4.172858 (4.172-4.178)
				0.004480 (0.0044-0.0049)	0.026536 (0.0263-0.0266)	
			0.110834 (0.1107-0.1118)			2.114182 (2.114-2.122)
			0.021045 (0.0210-0.0211)		0.014926 (0.0148-0.0150)	
			0.020874 (0.0208-0.0209)	0.013557 (0.0133-0.0145)		
		0.030004 (0.0299-0.0300)				2.109051 (2.108-2.118)
		0.132864 (0.1328-0.1329)			0.014650 (0.0146-0.0147)	
		0.133792 (0.1337-0.1338)		0.012183 (0.0210-0.0130)		
		1.173893 (1.171-1.174)	1.546162 (1.537-1.747)			
	55.572924 (55.55-55.58)					4.989349 (4.986-5.040)
	82.926041 (82.92-82.93)				0.014626 (0.0145-0.0147)	
	83.387005 (83.38-83.39)			0.005667 (0.0056-0.0059)		
	109.953887 (109.9-110.0)		0.078228 (0.0781-0.0787)			
	114.850088 (114.7-114.9)	0.036296 (0.0362-0.0364)				
0.454350 (0.4543-0.4549)						5.458223 (5.454-5.524)
0.321022 (0.3210-0.3211)					0.012842 (0.0127-0.0129)	
0.319659 (0.3196-0.3197)				0.005666 (0.0056-0.0059)		
0.235094 (0.2350-0.2355)			0.088421 (0.0883-0.0890)			
0.224503 (0.2244-0.2249)		0.032243 (0.0322-0.0323)				
0.080724 (0.0807-0.0816)	9.985758 (9.978-10.11)					

Table 71: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.

## GAPN parameter value estimations and confidence intervals

$V_m$	$K_{mGAP}$	$K_{mNADP}$	$K_{mNADPH}$	$K_{mG1P}$	$\alpha$	$K_{iGAP}$
						4.171128 (4.171-4.174)
					0.025874 (0.0258-0.0259)	
				0.026538 (0.0262-0.0509)		
			0.020819 (0.0208-0.0209)			
		0.134154 (0.1341-0.1342)				
	83.428272 (83.42-83.43)					
0.319481 (0.3194-0.3195)						

Table 72: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for only one parameters using progress curve analysis of non-saturating *in silico* generated data with 10% noise and initial temperature lag.



## Appendix B

### PGI parameter value estimations and confidence intervals

#### Identifiability analysis of fits with unpublished parameter values

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
x	0.404636 (0.3534-0.4531)	x	x

Table 1: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all four parameters using progress curve analysis of NMR progress curve data.

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
	0.404952 (0.3581-0.4491)	x	1972.9669 (1695-2312)
x		x	x
0.557933 (0.3387-1.663)	0.415604 (0.3474-0.4930)		1.795292 (0.8329-7.111)
x	0.727948 (0.6935-0.7547)	x	

Table 2: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of NMR progress curve data.

## PGI parameter value estimations and confidence intervals

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
		x	2514.1077 (2352-2763)
	0.344780 (0.3070-0.3855)		109.206851 (95.17-125.5)
	0.346170 (0.3167-0.3773)	0.000770 (0.000671-0.000878)	
1.374375 (0.5980-11.53)			6.476989 (2.732-55.94)
0.799635 (0.4427-2.485)		0.022930 (0.006943-0.0447)	
x	0.727948 (0.6969-0.7503)		

Table 3: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of NMR progress curve data.

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
			115.305774 (110.5-121.7)
		0.000726 (0.000696-0.000765)	
	0.728781 (0.6977-0.7512)		
0.089043 (0.08318-0.09440)			

Table 4: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for one parameter using progress curve analysis of NMR progress curve data.

## Identifiability analysis of fits with unpublished parameter values and our experimentally determined value for $K_m F6P$

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
x	0.727948 (0.6906-0.7584)	x	x

Table 5: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for all four parameters using progress curve analysis of NMR progress curve data.

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
	0.404952 (0.3581-0.4491)	x	1972.9669 (1703-2307)
x		x	x
0.433930 (0.2965-0.8259)	0.443520 (0.3801-0.5094)		0.132635 (0.06897-0.3199)
x	0.727948 (0.6935-0.7547)	x	

Table 6: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for three parameters using progress curve analysis of NMR progress curve data.

$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
		x	2514.1071 (2286-2698)
	0.345959 (0.3163-0.3772)		11.912527 (10.38-13.69)
	0.346185 (0.3167-0.3773)	0.000770 (0.000671-0.000878)	
0.908070 (0.4762-3.913)			0.459218 (0.2297-2.083)
0.799665 (0.4427-2.485)		0.022929 (0.006942-0.04471)	
0.565832 (0.5037-0.6362)	0.414979 (0.3775-0.4523)		

Table 7: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for two parameters using progress curve analysis of NMR progress curve data.

PGI parameter value estimations and confidence intervals

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$V_f$	$K_{eq}$	$K_{mF6P}$	$K_{mG6P}$
			12.636215 (12.12-13.33)
		0.000726 (0.000696-0.000765)	
	0.739296 (0.7151-0.7727)		
0.425964 (0.4072-0.4509)			

Table 8: Parameter value estimations and confidence intervals (indicated in brackets) when fitting for one parameter using progress curve analysis of NMR progress curve data.

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