

UNIVERSIDADE FEDERAL DO PARANÁ

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MODELAGEM MATEMÁTICA DA ATIVIDADE DE URIDILILTRANSFERASE  
DA PROTEÍNA GlnD DE *Escherichia coli*



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2015

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Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Bioquímica, no Curso de Pós-Graduação em Ciências – Bioquímica, Setor de Ciências Biológicas, Universidade Federal do Paraná.

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## TERMO DE APROVAÇÃO

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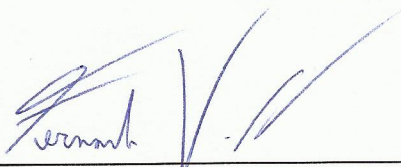
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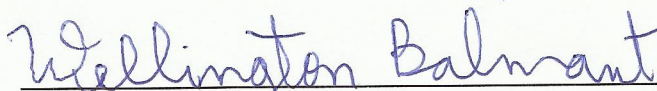
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Curitiba, 25 de junho de 2015.

## RESUMO

UTase é uma enzima regulatória em *Escherichia coli* que realiza a modificação covalente por uridililação das duas proteínas PII encontradas neste organismo, GlnB e GlnK. Além de sofrerem esta modificação covalente, as proteínas PII sofrem modulação alostérica pelos efetores ATP, ADP e 2-OG. O estado de PII, em termos da ligação destes efetores e da uridililação, define a ação de PII frente a seus diversos alvos regulatórios. Modelos matemáticos anteriores para a atividade de UTase não foram consistentes com seu mecanismo, de complexo ternário ordenado, pois não eram capazes de descrever seu perfil de inibição pelos produtos (Jiang *et al.*, 1998). Esta dissertação é dividida em duas partes. Na primeira, é deduzido um conjunto de equações para a atividade de UTase que descrevem corretamente seu perfil de inibição pelos produtos. A limitação deste primeiro modelo, comum aos modelos prévios para UTase, é não descrever como o estado de ligação de PII, em termos de seus efetores, afeta sua uridililação por UTase. Na segunda parte desta dissertação, o primeiro modelo é integrado ao modelo de Rocha *et al.* (2013) para a ligação de efetores alostéricos à PII, resultando no primeiro modelo da atividade de UTase que leva em conta o estado de ligação de PII. Valores para as constantes do modelo foram obtidas da literatura diretamente (Jiang *et al.* 1998) ou estimados ajustando o modelo a dados experimentais extraídos (Jiang and Ninfa, 2011), mas conjunto de valores obtido não é consistente. Apesar disso, a abordagem e o conjunto de equações do modelo integrado fazem uma contribuição válida para a modelagem da uridililação de PII em *E. coli*.

Palavras-chave: modelagem matemática, cinética enzimática, Uridililtransferase, *Escherichia coli*, PII, efetores alostéricos

## ABSTRACT

UTase is a regulatory enzyme in *Escherichia coli* that modifies by uridylylation the two PII proteins found in this organism, GlnB and GlnK. Besides uridylylation, these PII proteins also suffer allosteric modulation by the effectors ATP, ADP and 2-oxoglutarate. The state of PII, in terms of the binding of these effectors as well as uridylylation, defines the action of PII towards its various regulatory targets. Previous mathematical models for the activity of UTase were not consistent with its mechanism, which is an ordered ternary complex mechanism, because they were not capable of describing its product inhibition profile (Jiang *et al.* 1998). This dissertation has two parts. In the first part, a set of equations is deduced for the activity of UTase which correctly describe its product inhibition profile. The limitation of this first model, common to the previous UTase models, is that it does not describe how the ligation state of PII, in terms of its effectors, affects the uridylylation of PII by UTase. In the second part of this dissertation, the first model is integrated with the model number 4 of Rocha *et al.* (2013) for the binding of allosteric effectors to PII, resulting in the first model of UTase activity to take into account the binding of effectors to PII. Values for the constants of the model were obtained from the literature directly (Jiang *et al.*, 1998) or were estimated by adjusting the model to extracted experimental data from Jiang and Ninfa (2011), but the final set of values is not consistent. Despite that, the approach and set of equations of the integrated model make a valid contribution to the modelling of the uridylylation of PII in *E. coli*.

Key-words: mathematical modelling, enzyme kinetics, Uridylyltransferase, *Escherichia coli*, PII, allosteric effectors

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## LISTA DE ABREVIATURAS

ATP – adenosina trifosfato

ADP – adenosina difosfato

2-OG – 2-oxoglutarato

UTase – uridililtransferase

UTP – uridina trifosfato

UMP – uridina monofosfato

GS – glutamina sintetase

GDH – glutamato desidrogenase

GOGAT – glutamato sintase



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## 1 INTRODUÇÃO

As proteínas PII são proteínas transdutoras de sinal presentes em uma grande diversidade de organismos, incluindo bactérias, arqueias e plantas. Elas atuam sobre várias proteínas e enzimas, coordenando o metabolismo de nitrogênio. Esta ação depende de seu estado, em termos do número de efetores alostéricos ligados. Em proteobactérias, como *E. coli*, e em algumas actinobactérias, seu estado também depende de modificação covalente, realizada pela enzima GlnD, também conhecido como UTase/UR, como resposta à concentração celular de glutamina. Esta modificação é uma uridililação em seu *loop T*, uma estrutura flexível que é projetada para fora da estrutura principal das proteínas PII e que tem papel importante na interação com seus alvos regulatórios (Huerger *et al.*, 2012). Em outros organismos, ao invés de uridililação, o *loop T* é modificado por adenililação ou por fosforilação (Hesketh *et al.*, 2002; Strosser *et al.*, 2004; Forchhammer e Tandeau de Marsac, 1994, 1995; Kloft *et al.*, 2005).

Nas proteobactérias, o sistema de controle do qual participam PII e UTase/UR regula a expressão e a atividade da proteína de transporte AmtB e da enzima glutamina sintetase (GS), que participam do transporte de amônia para dentro da célula e a assimilação dela ao metabolismo. Já em bactérias fixadoras de nitrogênio, este sistema atua também na regulação da fixação de nitrogênio. A descoberta recente de que uma das proteínas PII de *E. coli* regula acetil-CoA carboxilase, uma enzima chave da biossíntese de ácidos graxos (Rodrigues, 2014; Gerhardt, 2015), sugere este sistema atua ainda na coordenação dos metabolismos de nitrogênio e carbono.

A modelagem matemática deste sistema tem o potencial de ajudar a compreendê-lo melhor. Ao integrar o conhecimento atual do sistema e traduzi-lo para uma linguagem matemática, um modelo é capaz de fazer simulações e previsões quantitativas do sistema. A comparação destas a resultados experimentais permite testar hipóteses sobre o funcionamento do sistema e apontar novas questões para guiar o trabalho experimental futuro. O organismo ideal para a modelagem deste sistema de controle envolvendo PII e UTase/UR é *E. coli*, por ser um dos organismos nos quais este sistema e a assimilação de amônia que ele controla são mais bem estudados.

A próxima seção abordará primeiramente este sistema de controle e o sistema de assimilação de amônia em *E. coli*. A seção seguinte fará uma revisão dos modelos matemáticos previamente utilizados para descrever e estudar este sistema em *E. coli*.

### 1.1 A assimilação de amônia em *Escherichia coli*

A Figura 1 mostra a via de assimilação de amônia em *E. coli*. Amônia entra na célula de duas maneiras: por difusão passiva através da membrana ou pela ação de AmtB (Kleiner, 1993; Khademi, 2004; Soupene 1998). Uma vez dentro da célula, a amônia é assimilada para glutamato, o principal metabólito distribuidor de nitrogênio celular (Reitzer, 2003), por duas vias. A principal via envolve a glutamina sintetase (GS) (Yuan *et al*, 2009; van Heeswijk, 2013) em conjunto com a glutamato sintase (GOGAT). Esta via é capaz de suprir a demanda celular de nitrogênio em baixas concentrações de amônia, devido à alta afinidade de GS por amônia, com a desvantagem de consumir uma molécula de ATP por molécula de amônia assimilada. A outra via envolve a glutamato desidrogenase (GDH). Como sua afinidade por amônia é menor que a de GS, ela somente é capaz de suprir a demanda de nitrogênio quando a concentração de amônia é alta, porém com a vantagem de menor custo energético para a célula.

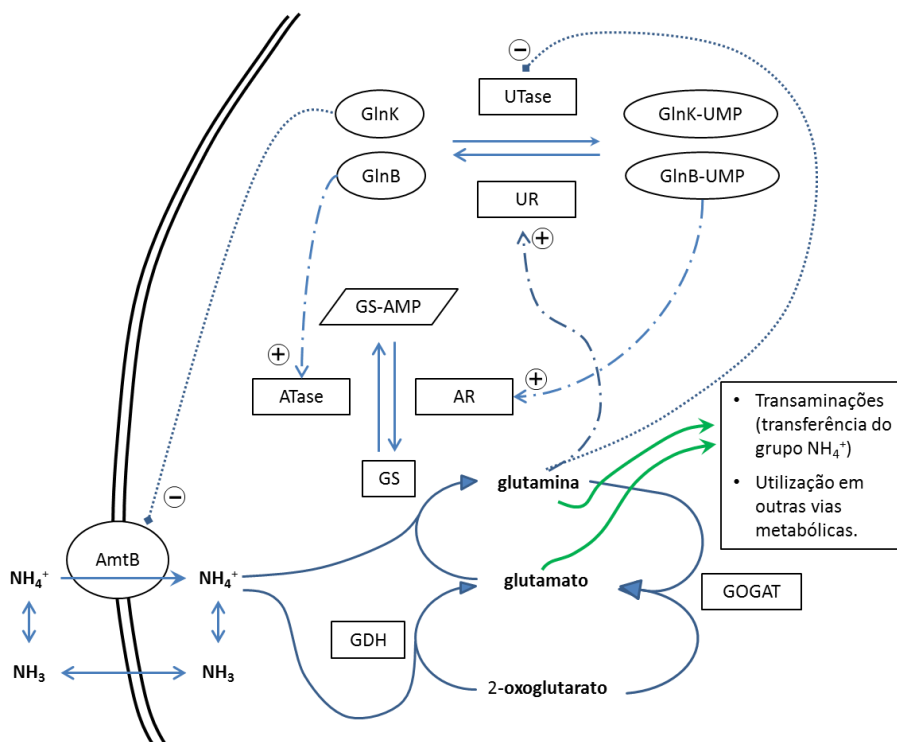


Figura 1 - Esquema da assimilação de amônia em *Escherichia coli*. *GlnB* e *GlnK* são as proteínas PII de *E. coli*. *GlnB-UMP* e *GlnK-UMP* são suas formas uridililadas. *UTase* e *UR* são as duas atividades opostas da enzima bifuncional *UTase/UR*. *ATase* e *AR* são as duas atividades opostas da enzima bifuncional *ATase/AR*. *GS* é glutamina sintetase em sua forma não modificada, ativa. *GS-AMP* é glutamina sintetase em sua forma adenilada, inativa. *AmtB* é a proteína transportadora específica para amônia. *GOGAT* é a enzima glutamato sintase, também chamada glutamato-oxoglutarato-aminotransferase. *GDH* é glutamato desidrogenase. As setas acompanhadas de um círculo com sinal positivo indicam ativação. As setas acompanhadas de um círculo com sinal negativo indicam inibição.

Além de suprir a demanda por glutamato em baixas concentrações de amônia, GS é a enzima responsável por suprir a demanda de glutamina. É a única enzima capaz de produzir este metabólito em *E. coli* (Reitzer, 2003). Como GS apresenta essa diversidade de funções e alto potencial de gasto energético, podendo ser responsável por até 15% do consumo de ATP celular (Reitzer, 2003), a regulação de sua atividade é de suma importância para a célula. Esta regulação acontece em três níveis: expressão gênica, inibição alostérica (retroalimentação negativa por diversos metabólitos) e modificação covalente reversível por adenililação (Reitzer, 2003; van Heeswijk, 2013). A regulação da expressão de GS é controlada pelo sistema de dois componentes NtrB-NtrC, enquanto o estado de adenililação de GS é controlado pela enzima bifuncional adenililtransferase/enzima removedora de adenilil (ATase/AR). Tanto o sistema NtrB-NtrC quanto a enzima ATase/AR estão subordinados regulatoriamente à proteína transdutora de sinal GlnB.

*E. coli* tem duas proteínas PII, GlnB e GlnK, que são homotriméricas. GlnB é expressa constitucionalmente enquanto GlnK é expressa apenas em condições de restrição de nitrogênio. A interação das proteínas PII com seus alvos regulatórios (NtrB e ATase/AR para e AmtB para GlnK) depende de seu estado de uridililação e de ligação aos efetores alostéricos ATP, ADP e 2-oxoglutarato (2-OG). Os sítios alostéricos para os quais ATP e ADP competem localizam-se entre as subunidades de PII. Cada um destes sítios possui um sítio vizinho para a ligação de 2-OG. A uridililação ocorre no *loop* T de cada subunidade. Em baixas concentrações de glutamina, a enzima bifuncional uridililtransferase/removedora de uridilil (UTase/UR) catalisa a adição de um grupamento UMP ao resíduo Tyr-51, localizado no ápice do *loop* T. Em altas concentrações de glutamina, UTase/UR catalisa a reação de hidrólise que remove este grupamento. Desta forma, as proteínas PII integram sinais celulares de energia (razão ATP/ADP), carbono (2-OG) e nitrogênio (glutamina).

## 1.2 Modelos publicados para o sistema de assimilação de amônia

Dentre os trabalhos que contribuem para a modelagem da assimilação de amônia e de seu sistema de controle, há três que buscam modelar o sistema de forma global e integrada. São estes os modelos de Kurata *et al.* (2005), Bruggeman, Boogerd e Westerhoff (2005) e de Ma, Boogerd e Goryanin (2009). Há, também, os modelos que tratam individualmente apenas um dos elementos do sistema. Há dois modelos que descrevem a ligação dos efetores alostéricos ATP, ADP e 2-oxoglutarato a GlnB. Estes são os modelos de Jiang e Ninfa (2007) (para PII ou

específico para GlnB) e de Rocha e colaboradores (2013) (específico para GlnB, pelo menos as constantes). As contribuições e limitações destes cinco modelos serão abordadas a seguir.

### 1.2.1 Modelo de Kurata *et al.* (2005)

O modelo que descreve a ação dinâmica do maior número de proteínas e enzimas que compõem a via de assimilação de amônia é o de Kurata e colaboradores (2005). Esse modelo trata de todas as enzimas e proteínas mostradas na Figura 1, com exceção da proteína transportadora AmtB, e descreve a regulação tanto a nível proteico quanto a nível gênico. Também trata das proteínas NtrB e NtrC, que interagem com GlnB para atuar na regulação gênica da via. Apesar de incluir todos esses elementos, o modelo os trata de maneira muito simplificada. As cinco principais limitações do modelo serão abordadas a seguir.

A primeira limitação do modelo diz respeito às proteínas PII, que foram tratadas como monômeros sem efetores alostéricos. Uma vez que a uridililação de GlnB e suas interações com seus alvos depende do estado de ligação dos efetores alostéricos, ATP, ADP e 2-OG (Jiang e Ninfa, 2007; Rocha *et al.*, 2013), o modelo é incapaz de descrever corretamente o efeito destes efetores na rede metabólica mostrada em Fig. 1.

A segunda limitação do modelo foi o pressuposto de que 2-oxoglutarato se liga a UTase/UR para regular sua atividade, mesmo que já tenha sido demonstrado que isto não ocorre (Kamberov, Atkinson e Ninfa, 1995). Devido a este pressuposto errado, o modelo não foi capaz de descrever o aumento de mais do que cinco vezes na concentração de GS que ocorreu em experimento em que um mutante de *E. coli* com deleção para *glnD* foi transferido de um meio rico em amônia para um meio pobre em amônia (Kurata *et al.*, 2005).

A terceira limitação do modelo foi que GlnK foi tratada como sendo funcionalmente idêntica à GlnB, enquanto há diferenças importantes entre as duas. Há duas diferenças importantes. Primeiro, GlnK-UMP é desuridililada muito mais lentamente que GlnB-UMP. Segundo, GlnB é 40 vezes mais eficaz que GlnK em ativar a ATase: é necessária uma concentração de GlnK de 20  $\mu\text{M}$  para obter a velocidade inicial de adenililação que é obtida com 0,5  $\mu\text{M}$  de GlnB (Jiang e Ninfa, 1999).

A quarta limitação do modelo é que somente PII e PII-UMP são consideradas como efetores alostéricos de ATase/AR, enquanto glutamina favorece a atividade de ATase por

regulação alostérica. Tanto GlnK quanto GlnB têm sinergia com glutamina nesta ativação de ATase (Jiang, Mayo e Ninfa, 2007). No caso de GlnK, não há ativação de ATase *in vitro* na ausência de glutamina (Atkinson e Ninfa, 1999).

A quinta limitação do modelo é que GS é tratada como um monômero, enquanto é um dodecâmero. Da maneira descrita pelo modelo, a adenililação alterna a atividade de GS entre dois estados: ativa ou inativa. Como dodecâmero, com um sítio de adenililação por subunidade, GS pode ter de 0 a 12 grupos adenilil ligados e, mesmo completamente adenililada, possui uma atividade residual. Com esse grau variável de adenililação, a regulação da atividade de GS é muito mais gradual e refinada do que o modelo é capaz de representar.

Como afirmado pelos próprios autores (Kurata *et al.*, 2005), seu modelo não busca representar os mecanismos reais dos fenômenos envolvidos. Sua abordagem é de representar, da maneira simples, todos elementos do sistema para fazer previsões sobre a resposta sistêmica. No entanto, as diversas limitações do modelo colocam dúvida sobre a confiabilidade de suas previsões.

### 1.2.2 Modelo de Bruggeman, Boogerd e Westerhoff (2005)

O modelo de Bruggeman *et al.* (2005) trata de todos os elementos do sistema mostrados na Figura 1, exceto AmtB e GlnK. Apesar de não descrever a expressão gênica e sua regulação (i.e. o sistema NtrB-NtrC), como faz o modelo de Kurata *et al.* (2005), este modelo utiliza equações cinéticas mais complexas, que descrevem os elementos do sistema de maneira mais completa.

Uma limitação do modelo é que ele ignora a competição de ADP pelo sítio de ligação de ATP na proteína GlnB, um fenômeno que só foi confirmado após publicação deste modelo (Conroy *et al.*, 2007; Rocha *et al.*, 2013). Isso afeta também a previsão da ligação de 2-oxoglutarato à GlnB, já que a ligação de 2-oxoglutarato e de ATP à GlnB apresenta sinergia. O modelo não prevê corretamente a ação de GlnB, já que ela depende de estado de ligação de GlnB a seus efetores.

Para descrever atividade de UTase, os autores se embasaram na caracterização feita por Jiang *et al.* (1998), e extraíram as constantes da equação diretamente desse trabalho. Segundo Jiang *et al.* (1998), a UTase segue um mecanismo de complexo ternário ordenado, e a reação é

reversível. No entanto, a equação cinética utilizada por Bruggeman e colaboradores difere da equação clássica para complexo ternário ordenado (Cornish-Bowden, 2012), e não possui diversos dos termos para inibição pelos produtos, o que pode ser uma limitação outra do modelo.

Como não havia resultados publicados para a cinética de adenililação da GS pela ATase, Bruggeman *et al.* (2005) propuseram uma equação cinética. Tomaram como ponto de partida a equação de Michaelis-Menten para reações irreversíveis, e adicionaram termos para a ligação em equilíbrio dos efetores glutamina, PII-G<sub>1</sub> e PII-UMP-G<sub>1</sub> (ou seja, as duas formas de PII ligadas a apenas uma molécula de 2-oxoglutarato) (Bruggeman *et al.*, 2005). A mesma abordagem foi utilizada para descrever a atividade de AR. Com novos dados experimentais apresentados por Jiang, Mayo e Ninfa (2007), a abordagem de Bruggeman e colaboradores não é mais satisfatória.

Para descrever a cinética de GDH, os autores propõem uma equação e fornecem os valores das constantes utilizadas, e apenas citam uma base de dados (Bruggeman *et al.*, 2005). Não fica claro se tanto a equação quanto os valores dos parâmetros foram obtidos desta base de dados ou se a equação foi desenvolvida pelos autores e foram obtidas apenas as constantes da base de dados (NIST Standard Reference Database. O endereço citado pelos autores não fornece nenhuma informação específica para esta enzima, apenas dá acesso à base de dados, que fornece valores de constantes de equilíbrio.

Os autores afirmam que a cinética de GOGAT é descrita por um “mecanismo ter-ter irreversível” com uma etapa de equilíbrio rápido, à qual foi incorporado um efeito inibitório para um metabólito imaginário, criado em seu modelo para simular a demanda de glutamato pela célula. Eles referenciam uma caracterização de GOGAT de Redina e Orme-Johnson (1978). No entanto, não descrevem o desenvolvimento das equações utilizadas para modelar GOGAT.

### 1.2.3 Modelo de Ma, Boogerd e Goryzanin (2009)

Ma *et al.* (2009) estenderam o modelo de Bruggeman *et al.* (2005) para descrever o transporte de amônia para o meio intracelular. O modelo final descreve todos os elementos do sistema de assimilação de amônia em *Escherichia coli* mostrados na Figura 1. Ma *et al.* (2009)



descreveram a difusão através da membrana e o transporte mediado pela proteína AmtB, bem como a regulação desta por GlnK. Em relação às proteínas PII, fizeram duas considerações. A primeira foi de que apenas GlnK, em sua forma não uridililada, bloqueia o transporte por AmtB, enquanto GlnB não interage com AmtB. Esta consideração foi baseada em dados experimentais de Coutts *et al.* (2002) de que GlnB liga-se à AmtB, mas não impede o transporte de amônia. A segunda foi de que GlnK não atua na regulação de ATase/AR. Para esta consideração, se embasaram na informação já citada de que GlnK seria 40 vezes menos eficiente que GlnB como ativadora de ATase (Jiang e Ninfa, 1999).

#### 1.2.4 Modelo de Jiang e Ninfa (2007) para a ligação de efetores a PII

O modelo de Jiang e Ninfa (2007) descreve a ligação dos efetores ATP, ADP e 2-OG à proteína PII. Ele é o primeiro a levar em conta a ligação de ADP à proteína PII. Sua principal limitação é que suas equações são capazes de descrever a ligação de apenas um efetor por vez: a ligação de ATP à GlnB, de ADP à GlnB, ou ainda de 2-oxoglutarato à GlnB-ATP (GlnB já associada a ATP). Isso torna sua aplicação limitada a estes casos, que ocorrem apenas *in vitro*. Ele não é apropriado para descrever o que ocorre *in vivo* porque não é capaz de prever a ligação destes efetores à proteína PII quando os três estão presentes simultaneamente.

#### 1.2.5 Modelo de Rocha *et al.* (2013) para a ligação de efetores a PII

Rocha e colaboradores (2013) modelaram a ligação dos efetores ATP, ADP e 2-OG à proteína PII. Propuseram quatro modelos, que possuem a mesma abordagem porém diferentes considerações sobre as afinidades de ligação dos efetores a PII. Sua modelagem destas interações de GlnB é superior que as de Bruggeman *et al.* (2005) e de Jiang e Ninfa (2007), já que é capaz de descrever os estados de ligação de GlnB quando todos os três efetores alostéricos estão presentes.

Rocha *et al.* (2013) tem como pressuposto que 2-OG somente pode se ligar a uma subunidade de PII que já possua uma molécula de ATP ligada. Portanto, dado um trímero de PII, cada uma de suas três subunidades pode: (a) não ter ligante algum; (b) ter ADP ligado; (c)

ter ATP ligado; ou (d) ter tanto ATP quanto 2-OG ligados. Isso resulta em 20 espécies possíveis de PII em equilíbrio.

Os quatro modelos de Rocha et al. (2013) possuem as mesmas 20 espécies. Os modelos diferenciam-se entre si pelo número de classes de sítios de ligação considerados para ATP, ADP e 2-OG. No modelo 1, considera-se que há 3 classes de sítio de ligação ATP e 1 para ADP, ou seja, a ligação da primeira, segunda e terceira moléculas de ATP a PII ocorrem com afinidades diferentes, enquanto a ligação das três moléculas de ADP ocorre com a mesma afinidade. Nos modelos 2 e 3, considera-se que há 2 classes de sítios tanto para ATP quanto para ADP. Após a ligação da primeira molécula de ATP a afinidade muda, e as afinidades para a segunda e terceira moléculas de ATP são as mesmas. O mesmo ocorre para ADP. A diferença entre estes modelos é que o modelo 2 pressupõe que ATP e ADP não podem se ligar a um mesmo trímero, enquanto o modelo 3 pressupõe que eles podem. Por fim, o modelo 4 considera três classes de sítios tanto para ATP quanto para ADP. Em relação a 2-OG, os quatro modelos consideram que há três classes de sítios.

Rocha e colaboradores (Rocha *et al.*, 2013) obtiveram os valores das constantes de afinidade entre PII e ATP, ADP e 2-OG ao ajustar cada um dos quatro modelos a vários conjuntos de dados experimentais de Jiang e Ninfa (2007). De acordo com sua análise de erros, o modelo 4 foi o que apresentou melhor ajuste, e por este motivo será o modelo utilizado para descrever a ligação dos efetores ATP, ADP e 2-OG à PII na segunda etapa deste trabalho.

### 1.3 Justificativa e Objetivos

Os modelos até agora publicados fazem grande avanço na simulação da assimilação de amônia em *Escherichia coli*, mas apresentam limitações que podem ser superadas para obter um modelo mais realista. O modelo de Kurata *et al.* (2005) é o único que descreve a regulação da expressão dos genes envolvidos e a síntese dos produtos desses genes. Por isso, também o único capaz de descrever o comportamento do sistema em tempos de resposta mais longos. No entanto, trata os mecanismos bioquímicos de maneira demasiado simplificada, e o modo como trata a ação alostérica de 2-oxoglutarato sobre a uridililação é incorreta.

Ma *et al.* (2009) ampliaram o modelo de Bruggeman *et al.* (2005), resultando em um modelo que descreve a ação de todos os componentes do sistema, excetuando-se a expressão

gênica. As atividades enzimáticas são descritas com atenção para seus mecanismos, contribuindo mais que o modelo de Kurata *et al.* (2005), portanto. No entanto, a descrição de PII é inadequada, pois não considera que ADP se liga a PII, competindo pelo mesmo sítio de ligação de ATP. Ainda, algumas das equações cinéticas são inventadas, como a de ATase, AR, e possivelmente GDH e GOGAT, e não determinadas experimentalmente. O modelo de Jiang e Ninfá (2007) melhora a modelagem de PII ao levar em conta a ligação de ADP, além de ATP, e 2-oxoglutarato a PII, mas ainda de forma incompleta. Sua deficiência é resolvida por Rocha e colaboradores (2013), cujo modelo é capaz de prever a ligação de GlnB a seus efetores alostéricos quando os três estão presentes, que é a condição encontrada *in vivo*. Seu modelo utiliza regras para a ligação dos efetores, algumas das quais podem não representar os mecanismos reais, e podem ser melhoradas.

Dentro deste contexto, o objetivo geral desta dissertação de mestrado foi desenvolver um modelo que descreva corretamente a atividade de UTase. Tal modelo contribuirá para a modelagem do sistema de assimilação de amônia em *E. coli*. O trabalho foi feito em duas etapas. Na primeira etapa, o objetivo foi obter um modelo cinético para UTase que fosse capaz de prever seu perfil de inibição pelos produtos (Jiang *et al.*, 1998), sendo, assim, consistente com o mecanismo da enzima. Esta etapa é tratada no primeiro artigo contido nesta dissertação. Na segunda etapa, o objetivo foi integrar este modelo ao modelo número 4 de Rocha *et al.* (2013) para a ligação de efetores a PII para obter um modelo que fosse capaz de descrever a influência do estado de ligação de PII, em termos de seus efetores alostéricos, sobre a atividade de UTase. Esta etapa é tratada no segundo artigo contido nesta dissertação.

## 2 ARTIGO 1: Modelling of the uridylyltransferase activity of *Escherichia coli* UTase/UR revisited

### 2.1 ABSTRACT

The ammonia assimilation pathway in *Escherichia coli* and many other bacteria is a tightly regulated system. The PII proteins are central in this system as signal transducers. Besides allosteric modulation, they also suffer reversible covalent modification by Uridylyltransferase. The resulting ligation and uridilation state of the PII proteins defines their ability to interact with regulatory targets. The UTase product inhibition pattern shows this enzyme follows a ternary complex mechanism. Previous mathematical models of UTase have been unable to describe its product inhibition correctly. This work contributes to the modelling of the ammonia assimilation pathway in *E. coli* with a model for UTase activity which is capable of describing its product inhibition pattern correctly and is therefore consistent with its mechanism.

### 2.2 INTRODUCTION

The ammonia assimilation pathway in *Escherichia coli* and many other bacteria is a tightly regulated system. Besides allosteric regulation of the assimilatory enzymes, there is a complex regulatory cascade involving two bifunctional enzymes, uridylyltransferase/uridylyl-removing enzyme (UTase/UR) and adenylyltransferase/adenylyl-removing enzyme (ATase/AR), and two signal transduction proteins, GlnB and GlnK, which are members of the PII family. GlnB and GlnK integrate multiple cellular signals and, depending on these signals, act upon various target proteins to control the activity and expression of glutamine synthetase (GS) and the activity of the ammonia transporter (AmtB) (Fig. 1). In *E. coli*, GlnB is constitutively expressed, whereas GlnK is only expressed under nitrogen starvation.

Both GlnB and GlnK are homotrimers that contain three identical nucleotide-binding sites, located in the clefts between the subunits of the trimer. Next to each nucleotide-binding site, there is a 2-oxoglutarate (2-OG) binding site. ATP and ADP compete for the nucleotide-binding site, while 2-OG can only bind to its site if an ATP molecule is bound to the adjacent nucleotide-binding site. If 2-OG is bound to a subunit, then the T-loop of that subunit can be uridylylated by the uridylyltransferase activity of UTase/UR, which is favored by low intracellular glutamine concentrations. This UMP group is removed by the uridylyl-removing

activity of UTase/UR, which is favored by high intracellular glutamine concentrations. In this manner, PII proteins integrate intracellular signals of energy status (ATP/ADP), carbon status (2-OG) and nitrogen status (glutamine).

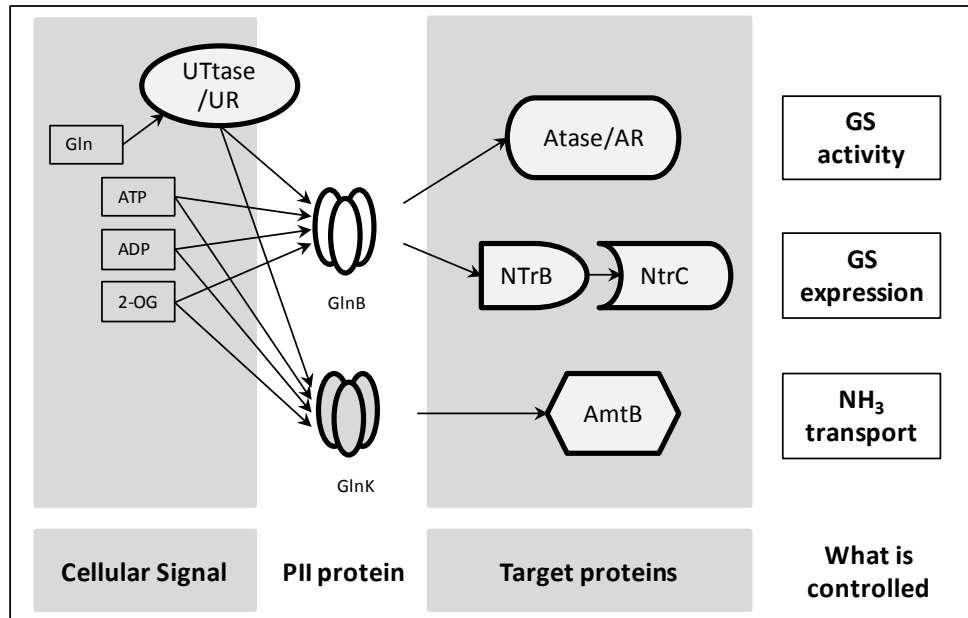


Figure 1 - Overview of the interactions of the PII proteins GlnB and GlnK in *E. coli* and the control they exert over the ammonia assimilation system.

*GlnB* and *GlnK* are directly affected by the cellular levels of ATP, ADP and 2-oxoglutarate (2-OG) and indirectly, through the action of UTase/UR, by the levels of glutamine (Gln). Depending on these signals, *GlnB* acts upon the target proteins ATase/UR to control GS activity and NtrB to control GS expression, while *GlnK* acts upon the AmtB transporter to control ammonia transport.

The interaction of PII proteins with their target molecules depends on their particular states, in terms of the effectors (i.e. ATP, ADP and 2-OG) bound to the trimer and the number of subunits that are uridylylated. For example, only non-uridylylated GlnB can bind allosterically to NtrB, and only non-uridylylated GlnK can bind AmtB (Rodrigues, 2011). When complexed to GlnB, NtrB dephosphorylates NtrC, with this resulting in decreased expression of glutamine synthetase. Likewise, when complexed with GlnK, AmtB is unable to transport ammonia to the inside of the cell.

A mathematical model of the ammonia assimilation system and its regulation would be a useful tool to further our understanding of how this system works. Amongst the few mathematical models previously proposed for this system in *E. coli* (Mutalik *et al.*, 2003; Bruggeman *et al.*, 2005; Kurata *et al.*, 2005; Lodeiro and Melgarejo, 2008; Ma *et al.*, 2009; Kidd and Wingreen, 2010), those of Kurata *et al.* (2005), Bruggeman *et al.* (2005) and Ma *et al.* (2009) are the most complete. In the Kurata model, PII is considered to be a monomer and

ATP, ADP and 2-OG are not effectors of PII, rather 2-OG is a direct effector of UTase (Kurata *et al.*, 2005), even though it has been known since 1995 that 2-OG does not bind to UTase (Kamberov *et al.*, 1995). Since the effectors bound to PII determine how it interacts with its target proteins, the Kurata model does not properly describe these interactions. Further, since PII is a trimer that can suffer three uridylylations, the Kurata model cannot describe the uridylylation kinetics correctly. The model of Bruggeman *et al.* (2005) describes both the binding of effectors to PII trimers and the uridylylation of the subunits in greater detail. It assumes that PII is saturated by ATP and does not consider the competitive binding of ADP. However, it does predict the concentrations of various states of PII with different numbers of bound 2-OG molecules and different numbers of uridylylated subunits. The kinetic equations for uridylylation of PII by UTase take into account product inhibition by uridylylated forms of PII and by pyrophosphate (Table 1), but the denominators of these equations lack several of the terms that would be expected for the ordered ternary-complex mechanism, which this reaction follows (Jiang *et al.* 1998). Ma *et al.* (2009) expanded the model of Bruggeman *et al.* (2005) by including the AmtB transporter and adjusting the values of some of the constants of the model, but the equations regarding PII and UTase/UR were unaltered from Bruggeman *et al.* (2005).

Within mathematical models of the ammonia assimilation system, it is important to describe the binding of effectors to PII and the uridylylation of PII correctly. Recently, our group developed a model for the equilibrium binding of ATP, ADP and 2-OG to PII that recognized twenty states with different combinations of bound effectors (Rocha *et al.*, 2013). In the present paper, we develop a kinetic model for the uridylylation of PII by UTase, based on the ordered ternary complex mechanism. We show that our model makes predictions about product inhibition that are consistent with the experimental results of Jiang *et al.* (1998), whereas the model of Bruggeman *et al.* (2005) does not.

Table 1 – Equations of the model of Bruggeman *et al.* (2005) for the uridylylation of PII by UTase.  
Uridylylation of PII

$$v_1 = \frac{[UT]_T k_{catF} [PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \left( 1 + \frac{[GLN]}{K_{GLN}} \right) \left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PII}} [PIIUMP_1]}{K_{iS_{PII}} K_{i_{PIIUMP_1}}} + \frac{[PIIUMP_1]}{K_{iS_{PII}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_1][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PII}} [PIIUMP_2]}{K_{iS_{PII}} K_{i_{PIIUMP_2}}} + \frac{[PIIUMP_2]}{K_{iS_{PII}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_2][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PII}} [PIIUMP_3]}{K_{iS_{PII}} K_{i_{PIIUMP_3}}} \right) \quad (1a)$$

Uridylylation of PIUMP<sub>1</sub>

$$v_2 = \frac{[UT]_T k_{catF} [PIUMP_1][UTP]}{K_{iS_{PIUMP_1}} K_{M_{UTP}}} \left( 1 + \frac{[GLN]}{K_{GLN}} \right) \left( 1 + \frac{K_{MS_{PIUMP_1}} [UTP]}{K_{iS_{PIUMP_1}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PIUMP_1}}} + \frac{[PII][UTP]}{K_{iS_{PIUMP_1}} K_{M_{UTP}}} + \frac{[PII][UTP][PP_i]}{K_{iS_{PIUMP_1}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PIUMP_1}} [PIIUMP_1]}{K_{iS_{PIUMP_1}} K_{i_{PIIUMP_2}}} + \frac{[PIIUMP_1]}{K_{iS_{PIUMP_1}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PIUMP_1}} K_{M_{UTP}}} + \frac{[PIIUMP_1][UTP][PP_i]}{K_{iS_{PIUMP_1}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PIUMP_2}} [PIIUMP_2]}{K_{iS_{PIUMP_1}} K_{i_{PIIUMP_2}}} + \frac{[PIIUMP_2]}{K_{iS_{PIUMP_1}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PIUMP_1}} K_{M_{UTP}}} + \frac{[PIIUMP_2][UTP][PP_i]}{K_{iS_{PIUMP_1}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PIUMP_3}} [PIIUMP_3]}{K_{iS_{PIUMP_1}} K_{i_{PIIUMP_2}}} \right) \quad (1b)$$

Uridylylation of PIUMP<sub>2</sub>

$$v_3 = \frac{[UT]_T k_{catF} [PIUMP_2][UTP]}{K_{iS_{PIUMP_2}} K_{M_{UTP}}} \left( 1 + \frac{[GLN]}{K_{GLN}} \right) \left( 1 + \frac{K_{MS_{PIUMP_2}} [UTP]}{K_{iS_{PIUMP_2}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PIUMP_2}}} + \frac{[PII][UTP]}{K_{iS_{PIUMP_2}} K_{M_{UTP}}} + \frac{[PII][UTP][PP_i]}{K_{iS_{PIUMP_2}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PIUMP_2}} [PIIUMP_1]}{K_{iS_{PIUMP_2}} K_{i_{PIIUMP_3}}} + \frac{[PIIUMP_1]}{K_{iS_{PIUMP_2}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PIUMP_2}} K_{M_{UTP}}} + \frac{[PIIUMP_1][UTP][PP_i]}{K_{iS_{PIUMP_2}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PIUMP_2}} [PIIUMP_2]}{K_{iS_{PIUMP_2}} K_{i_{PIIUMP_3}}} + \frac{[PIIUMP_2]}{K_{iS_{PIUMP_2}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PIUMP_2}} K_{M_{UTP}}} + \frac{[PIIUMP_2][UTP][PP_i]}{K_{iS_{PIUMP_2}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PIUMP_3}} [PIIUMP_3]}{K_{iS_{PIUMP_2}} K_{i_{PIIUMP_3}}} \right) \quad (1c)$$

In order to facilitate comparisons with our equations, these equations are rewritten using the same nomenclature that we used in our equations

### 2.3 MODEL DEVELOPMENT

GlnB and GlnK are treated generically as PII in this model. UTase is assumed to follow a non-processive mechanism since it is possible to separate PII with none, one, two or three uridylylated subunits after partial uridylylation assays (Atkinson *et al.*, 1994). Application of the ordered ternary complex mechanism to the uridylylation of the three substrates, PII, PIIUMP<sub>1</sub> and PIIUMP<sub>2</sub>, results in the reaction scheme shown in Figure 2.

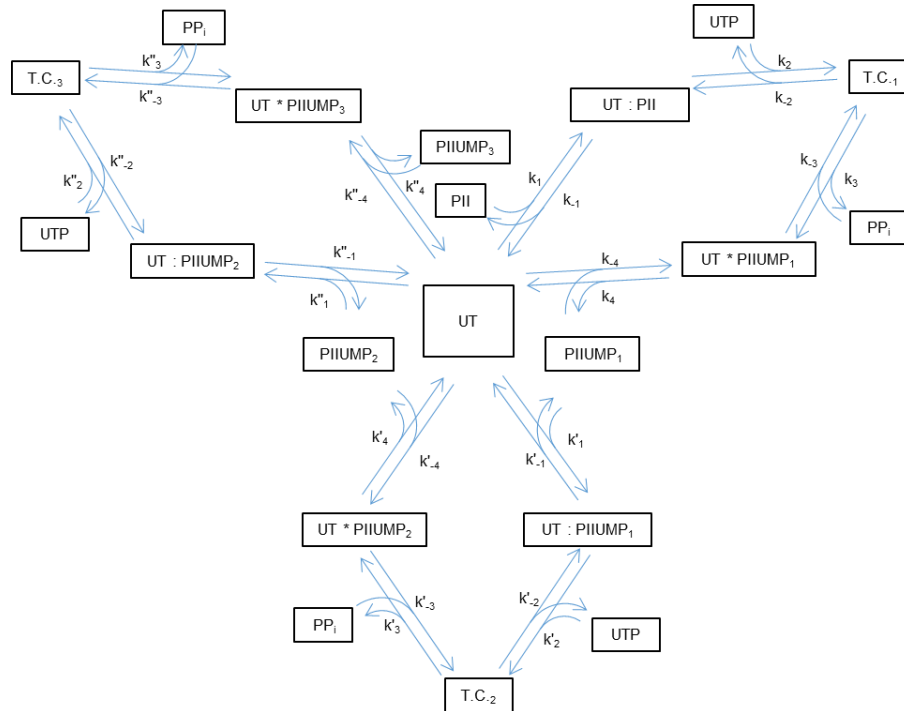


Figure 2 - The ternary complex mechanism proposed for UTase. Whenever a form of PII is complexed to the enzyme through a non-uridylylated subunit, the complex is written with a “:” as in “UT:PIIUMP1”. If the interaction is through a uridylylated subunit, however, the complex is written with an asterisk “\*”, such as in “UT\*PIIUMP1”. The abbreviations T.C.1, T.C.2 and T.C.3 refer to the ternary complexes between the enzyme and the two substrates (or products) of each of the reactions. Since the ternary complex between enzyme and the two substrates cannot be distinguished from the ternary complex between enzyme and the two products until either the substrate UTP or the product PPi dissociates, they are treated as a single enzyme form.

Based on the scheme in Figure 1, kinetic equations were deduced for the three UTase reactions, utilizing the King-Altman method (King and Altman, 1956) as described by Cornish-Bowden (2012). The detailed deduction is available in the Supplementary Material.

The velocity of uridylylation of PII is given by:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} - [UT_{Total}] k_{catR} \frac{[PPi][PIIUMP_1]}{K_{M_{PPi}} K_{iP_{PIIUMP_1}}} \right) F_2 F_3}{\left( 1 + \frac{[GLN]}{K_{GLN}} \right) (F_1 F_2 F_3 + F_2 F_3 D_1 + F_1 F_3 D_2 + F_1 F_2 D_3)} \quad (2a)$$



The velocity of uridylylation of PIIUMP<sub>1</sub> is given by:

$$v_2 = \frac{\left( [UT_{Total}]k_{catF} \frac{[PIIUMP_1][UTP]}{K_{iS_{PIIUMP_1}} K_{M_{UTP}}} - [UT_{Total}]k_{catR} \frac{[PPi][PIIUMP_2]}{K_{M_{PPi}} K_{iP_{PIIUMP_2}}} \right) F_1 F_3}{\left( 1 + \frac{[GLN]}{K_{GLN}} \right) (F_1 F_2 F_3 + F_2 F_3 D_1 + F_1 F_3 D_2 + F_1 F_2 D_3)} \quad (2b)$$

The velocity of uridylylation of PIIUMP<sub>2</sub> is given by:

$$v_3 = \frac{\left( [UT_{Total}]k_{catF} \frac{[PIIUMP_2][UTP]}{K_{iS_{PIIUMP_2}} K_{M_{UTP}}} - [UT_{Total}]k_{catR} \frac{[PPi][PIIUMP_3]}{K_{M_{PPi}} K_{iP_{PIIUMP_3}}} \right) F_1 F_2}{\left( 1 + \frac{[GLN]}{K_{GLN}} \right) (F_1 F_2 F_3 + F_2 F_3 D_1 + F_1 F_3 D_2 + F_1 F_2 D_3)} \quad (2c)$$

All three equations have the same denominator. The factors  $D_1$ ,  $D_2$  and  $D_3$  in this denominator are related to the first, second and third uridylylations, respectively. They are given by:

$$D_1 = \left( \frac{[PII] + [PII][UTP]}{K_{iS_{PII}} K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII] K_{MP_{PIIUMP_1}} [PPi]}{K_{iS_{PII}} K_{iP_{PIIUMP_1}} K_{M_{PPi}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPPi}} + \frac{[PIIUMP_1]}{K_{iP_{PIIUMP_1}}} + \frac{[PIIUMP_1][PPi]}{K_{iP_{PIIUMP_1}} K_{M_{PPi}}} + \frac{[PIIUMP_1][UTP] K_{MS_{PII}}}{K_{iP_{PIIUMP_1}} K_{M_{UTP}} K_{iS_{PII}}} + \frac{[PIIUMP_1][UTP][PPi]}{K_{iP_{PIIUMP_1}} K_{iUTP} K_{M_{PPi}}} \right) \quad (3a)$$

$$D_2 = \left( \frac{[PIIUMP_1]}{K_{iS_{PIIUMP_1}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PIIUMP_1}} K_{M_{UTP}}} + \frac{[PIIUMP_1] K_{MP_{PIIUMP_2}} [PPi]}{K_{iS_{PIIUMP_1}} K_{iP_{PIIUMP_2}} K_{M_{PPi}}} + \frac{[PIIUMP_1][UTP][PPi]}{K_{iS_{PIIUMP_1}} K_{M_{UTP}} K_{iPPi}} + \frac{[PIIUMP_2]}{K_{iP_{PIIUMP_2}}} + \frac{[PIIUMP_2][PPi]}{K_{iP_{PIIUMP_2}} K_{M_{PPi}}} + \frac{[PIIUMP_2][UTP] K_{MS_{PII}}}{K_{iP_{PIIUMP_2}} K_{M_{UTP}} K_{iS_{PII}}} + \frac{[PIIUMP_2][UTP][PPi]}{K_{iP_{PIIUMP_2}} K_{iUTP} K_{M_{PPi}}} \right) \quad (3b)$$

$$D_3 = \left( \frac{[PIIUMP_2]}{K_{iS_{PIIUMP_2}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PIIUMP_2}} K_{M_{UTP}}} + \frac{[PIIUMP_2] K_{MP_{PIIUMP_3}} [PPi]}{K_{iS_{PIIUMP_2}} K_{iP_{PIIUMP_3}} K_{M_{PPi}}} + \frac{[PIIUMP_2][UTP][PPi]}{K_{iS_{PIIUMP_2}} K_{M_{UTP}} K_{iPPi}} + \frac{[PIIUMP_3]}{K_{iP_{PIIUMP_3}}} + \frac{[PIIUMP_3][PPi]}{K_{iP_{PIIUMP_3}} K_{M_{PPi}}} + \frac{[PIIUMP_3][UTP] K_{MS_{PIIUMP}}}{K_{iP_{PIIUMP_3}} K_{M_{UTP}} K_{iS_{PIIUMP}}} + \frac{[PIIUMP_3][UTP][PPi]}{K_{iP_{PIIUMP_3}} K_{iUTP} K_{M_{PPi}}} \right) \quad (3c)$$

Likewise, the expressions  $F_1$ ,  $F_2$  and  $F_3$  in the denominators of Eqs (2a) to (2c) are related to the first, second and third uridylylations (Figure 1), respectively. They are given by:

$$F_1 = \left( \frac{K_{MP_{PIIUMP_1}} [PPi]}{K_{iP_{PIIUMP_1}} K_{iPPi}} + 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right) \quad (4a)$$

$$F_2 = \left( \frac{K_{MP_{PIIUMP_2}}}{K_{iP_{PIIUMP_2}}} \frac{[PPi]}{K_{iPPi}} + 1 + \frac{K_{MS_{PIIUMP_1}}}{K_{iS_{PIIUMP_1}}} \frac{[UTP]}{K_{M_{UTP}}} \right) \quad (4b)$$

$$F_3 = \left( \frac{K_{MP_{PIIUMP_3}}}{K_{iP_{PIIUMP_3}}} \frac{[PPi]}{K_{iPPi}} + 1 + \frac{K_{MS_{PIIUMP_2}}}{K_{iS_{PIIUMP_2}}} \frac{[UTP]}{K_{M_{UTP}}} \right) \quad (4c)$$

$F_1$ ,  $F_2$  and  $F_3$  also appear in pairs in the numerators of Eqs (2a), (2b) and (2c). Within each of these expressions, the constants  $K_{MS}$  and  $K_{iS}$  are related to the form of PII that serves as the substrate in the corresponding uridylylation. Likewise, the constants  $K_{MP}$  and  $K_{iP}$  are related to the form of PII that serves as the product in the corresponding uridylylation.

Since the interaction of UTase with a PII subunit is not affected by the uridylylation state of the other two subunits (Atkinson *et al.*, 1994), it is possible to derive relationships between the constants related to the three substrates (PII, PIIUMP<sub>1</sub> and PIIUMP<sub>2</sub>) and between the constants related to the three products (PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub>). Detailed deductions are given in the supplementary material. The  $K_{MS}$  values for the three substrates are related by:

$$K_{MS_{PIIUMP_1}} = \frac{3}{2} K_{MS_{PII}} \quad (5a)$$

$$K_{MS_{PIIUMP_2}} = 3 K_{MS_{PII}} \quad (5b)$$

The  $K_{iS}$  values for the three substrates are related by:

$$K_{iS_{PIIUMP}} = \frac{3}{2} K_{iS_{PII}} \quad (6a)$$

$$K_{iS_{PIIUMP_2}} = 3 K_{iS_{PII}} \quad (6b)$$

The  $K_{MP}$  values for the three products are related by:

$$K_{MP_{PIIUMP_2}} = \frac{1}{2} K_{MP_{PIIUMP_1}} \quad (7a)$$

$$K_{MP_{PIIUMP_3}} = \frac{1}{3} K_{MP_{PIIUMP_1}} \quad (7b)$$

Finally, the  $K_{iP}$  values for the three products are related by:

$$K_{iP_{PIIUMP_2}} = \frac{1}{2} K_{iP_{PIIUMP_1}} \quad (8a)$$

$$K_{iP_{PIIUMP_3}} = \frac{1}{3} K_{iP_{PIIUMP_1}} \quad (8b)$$

If these identities are substituted into Eqs (4b) and (4c), these equations become identical to Eq. (4a). As a consequence, the expressions  $F_1$ ,  $F_2$  and  $F_3$  cancel out of Eqs. (2a) to (2c) in such a manner as to result in the following simplified velocity equations:

$$v_1 = \left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} - [UT_{Total}] k_{catR} \frac{[PPi][PIIUMP]}{K_{M_{PPi}} K_{iP_{PIIUMP}}} \right) / D^* \quad (9a)$$

$$v_2 = \left( [UT_{Total}] k_{catF} \frac{[PIIUMP][UTP]}{K_{iS_{PIIUMP}} K_{M_{UTP}}} - [UT_{Total}] k_{catR} \frac{[PPi][PIIUMP_2]}{K_{M_{PPi}} K_{iP_{PIIUMP_2}}} \right) / D^* \quad (9b)$$

$$v_3 = \left( [UT_{Total}] k_{catF} \frac{[PIIUMP_2][UTP]}{K_{iS_{PIIUMP_2}} K_{M_{UTP}}} - [UT_{Total}] k_{catR} \frac{[PPi][PIIUMP_3]}{K_{M_{PPi}} K_{iP_{PIIUMP_3}}} \right) / D^* \quad (9c)$$

These equations have the same denominator,  $D^*$ , given by:

$$D^* = \left( 1 + \frac{[GLN]}{K_{GLN}} \right) (D_0^* + D_1^* + D_2^* + D_3^*) \quad (9d)$$

In this denominator, the terms  $D_0^*$  to  $D_3^*$  are given by:

$$D_0^* = 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{K_{MP_{PIIUM_1}} [PPi]}{K_{iP_{PIIUM_1}} K_{iPPi}} \quad (9e)$$

$$D_1^* = \left( \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][PPi] K_{MP_{PIIUM_1}}}{K_{iS_{PII}} K_{M_{PPi}} K_{iP_{PIIUM_1}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPPi}} + \frac{[PIIUMP_1]}{K_{iP_{PIIUM_1}}} + \frac{[PIIUMP_1][PPi]}{K_{iP_{PIIUM_1}} K_{M_{PPi}}} + \frac{[PIIUMP_1][UTP] K_{MS_{PII}}}{K_{iP_{PIIUM_1}} K_{M_{UTP}} K_{iS_{PII}}} + \frac{[PIIUMP_1][UTP][PPi]}{K_{iP_{PIIUM_1}} K_{iUTP} K_{M_{PPi}}} \right) \quad (9f)$$

$$D_2^* = \left( \frac{[PIIUMP_1]}{K_{iS_{PIIUMP_1}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PIIUMP_1}} K_{M_{UTP}}} + \frac{[PIIUMP_1][PPi] K_{MP_{PIIUM_2}}}{K_{iS_{PIIUMP_1}} K_{M_{PPi}} K_{iP_{PIIUM_2}}} + \frac{[PIIUMP_1][UTP][PPi]}{K_{iS_{PIIUMP_1}} K_{M_{UTP}} K_{iPPi}} + \frac{[PIIUMP_2]}{K_{iP_{PIIUM_2}}} + \frac{[PIIUMP_2][PPi]}{K_{iP_{PIIUM_2}} K_{M_{PPi}}} + \frac{[PIIUMP_2][UTP] K_{MS_{PIIUM_1}}}{K_{iP_{PIIUM_2}} K_{M_{UTP}} K_{iS_{PIIUM_1}}} + \frac{[PIIUMP_2][UTP][PPi]}{K_{iP_{PIIUM_2}} K_{iUTP} K_{M_{PPi}}} \right) \quad (9g)$$

$$D_3^* = \left( \begin{aligned} & \frac{[PIUMP_2]}{K_{iS_{PIUMP_2}}} + \frac{[PIUMP_2][UTP]}{K_{iS_{PIUMP_2}} K_{M_{UTP}}} + \frac{[PIUMP_2][PPi] K_{MP_{PIUMP_3}}}{K_{iS_{PIUMP_2}} K_{M_{PPi}} K_{iP_{PIUMP_3}}} + \frac{[PIUMP_2][UTP][PPi]}{K_{iS_{PIUMP_2}} K_{M_{UTP}} K_{iPPi}} + \\ & \frac{[PIUMP_3]}{K_{iP_{PIUMP_3}}} + \frac{[PIUMP_3][PPi]}{K_{iP_{PIUMP_3}} K_{M_{PPi}}} + \frac{[PIUMP_3][UTP] K_{MS_{PIUMP_2}}}{K_{iP_{PIUMP_3}} K_{M_{UTP}} K_{iS_{PIUMP_2}}} + \frac{[PIUMP_3][UTP][PPi]}{K_{iP_{PIUMP_3}} K_{i_{UTP}} K_{M_{PPi}}} \end{aligned} \right) \quad (9h)$$

## 2.4 RESULTS AND DISCUSSION

Product inhibition patterns for UTase were deduced from Eqs. (9a) to (9c). In product inhibition studies, the initial velocity of the reaction is measured in several sets of assays. Each of the three uridylylation reactions catalyzed by UTase has two substrates and two products: in such a case, both substrates are always added, but only one of the products is added in any particular assay set. In their product inhibition studies, Jiang *et al.* (1998) used the substrates of the first uridylylation, namely UTP and non-uridylylated PII, but the form of PII tested as the product was the product of the third uridylylation, PIIUMP<sub>3</sub>, and not the product of the first uridylylation, PIIUMP<sub>1</sub>. Although Jiang *et al.* (1998) did not explain explicitly why they used this experimental strategy, it does avoid the complications that would arise from the use of PIIUMP<sub>1</sub>: this product of the first uridylylation is also a substrate of the second uridylylation. It would have been very difficult to differentiate its inhibitory action as a product from its inhibitory action as a competing substrate. To do this they would have had to measure the rates of both uridylylation reactions individually, despite them occurring simultaneously.

Equations (9a) to (9c) were used to determine the inhibition patterns that would be expected when each of the PII products (PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub>) were added, with this analysis being done for each of the uridylylation cycles. Table 2 shows the results of the analysis for the particular combination used by Jiang *et al.* (1998), namely the addition of PP<sub>i</sub> and PIIUMP<sub>3</sub> as inhibitors of the first uridylylation reaction. This analysis was undertaken with Eq. (9a), of the model developed in the present work, and also with Eq. (1a), of the model of Bruggeman *et al.* (2005) (see Supplementary Material for the detailed analysis).

For the experiment undertaken by Jiang *et al.* (1998), when PP<sub>i</sub> is added as the inhibitor, the equation developed in the current work for the first uridylylation, Eq. (9a), simplifies to

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{K_{MP_{PIIUMP_3}} [PP_i]}{K_{iP_{PIIUMP_3}} K_{iPP_i}} + \frac{[PII][PP_i]}{K_{iS_{PII}} K_{M_{PP_i}}} \frac{K_{MP_{PIIUMP_3}}}{K_{iP_{PIIUMP_3}}} + \frac{[PII][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPP_i}} \right)} \quad (10a)$$

and when PIIUMP<sub>3</sub> is added as the inhibitor it simplifies to

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right.}$$

$$\left. + \frac{[PIIUMP_3]}{K_{iP_{PIIUMP_3}}} + \frac{[PIIUMP_3][UTP]}{K_{iP_{PIIUMP_3}} K_{M_{UTP}}} \frac{K_{MS_{PIIUMP_1}}}{K_{iS_{PIIUMP_1}}} \right) \quad (10b)$$

Likewise, for the experiment undertaken by Jiang *et al.* (1998), when PP<sub>i</sub> is added as the inhibitor, the equation developed by Bruggeman *et al.* (2005) for the first uridylylation, Eq. (1a), simplifies to

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right.}$$

$$\left. + \frac{[PII][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPP_i}} \right) \quad (11a)$$

and when PIIUMP<sub>3</sub> is added as the inhibitor it simplifies to

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right.}$$

$$\left. + \frac{K_{MS_{PII}} [PIIUMP_3]}{K_{iS_{PII}} K_{iP_{PIIUMP_1}}} \right) \quad (11b)$$

The product inhibition pattern predicted by the model developed in the current work exactly matches the experimental product inhibition pattern for UTase obtained by Jiang *et al.* (1998), whereas the model of Bruggeman *et al.* (2005) predicts a quite different product inhibition pattern (Table 2).

Our model is capable of predicting the experimentally observed product inhibition pattern because it contains all the terms in the denominator that are consistent with an ordered ternary-complex mechanism. The model of Bruggeman *et al.* (2005) fails to predict the experimentally observed product inhibition pattern because its denominator lacks several of

these terms, as can be seen by comparing Eqs (10a) and (11a) and also Eqs (10b) and (11b). It is not clear why Bruggeman *et al.* (2005) omitted these terms in their model: although they stated that UTase follows the ordered ternary complex mechanism, they did not describe the development of the model.

Table 2 – Comparison of the product inhibition patterns for UTase predicted by the model developed in the current work and the model of Bruggeman *et al.* (2005) with the experimental pattern reported by Jiang *et al.* (1998)

Product used as inhibitor	Variable Substrate	Fixed Substrate	Inhibition type (non-saturating fixed substrate)	Inhibition type (saturating fixed substrate)
Product inhibition pattern determined experimentally by Jiang <i>et al.</i> (1998)				
PP <sub>i</sub>	PII	UTP	Mixed	Uncompetitive
	UTP	PII	Mixed	Mixed
PIIUMP <sub>3</sub>	PII	UTP	Competitive	Competitive
	UTP	PII	Mixed	No inhibition
Product inhibition pattern predicted by the model developed in the present work				
PP <sub>i</sub>	PII	UTP	Mixed	Uncompetitive
	UTP	PII	Mixed	Mixed
PIIUMP <sub>3</sub>	PII	UTP	Competitive	Competitive
	UTP	PII	Mixed	No inhibition
Product inhibition pattern predicted by the model of Bruggeman <i>et al.</i> (2005)				
PP <sub>i</sub>	PII	UTP	Competitive	Competitive
	UTP	PII	Competitive	Competitive
PIIUMP <sub>3</sub>	PII	UTP	Uncompetitive	No inhibition
	UTP	PII	Uncompetitive	No inhibition

Whereas in our model the denominators for the three reactions are identical, in the model of Bruggeman *et al.* (2005) they are not. This is quite strange: The denominator of the velocity equation for any particular enzyme-catalyzed reaction contains terms that are related to each form of the enzyme within the reaction mixture; the terms are not limited to those related to the particular reaction being considered. Consequently, when the enzyme is catalyzing several different reactions within the reaction mixture, the velocity equations for these different reactions must have the same denominators.

The lack of identity of the denominators of the Bruggeman model (i.e. in Eqs (1a) to (1c)) is due to their substrate and product constants. In both the Bruggeman and our equations, there is a  $K_{iS}$  constant in all terms that contain the concentration of PII, PIIUMP<sub>1</sub> or PIIUMP<sub>2</sub> as substrates. Similarly, there is a  $K_{iP}$  constant in all terms that contain the concentration of PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> or PIIUMP<sub>3</sub> as products. The denominators of Eqs (1a) to (1c) each have the same number of terms. Although the terms in one denominator appear to be equivalent to the terms in the other denominators, they are not identical because the  $K_{iS}$  and  $K_{iP}$  constants in each equation are different. In Eq. (1a),  $K_{iSP_{II}}$  is present in each term that contains the concentration of one of the PII species as a substrate and  $K_{iP_{PIIUMP_1}}$  is present in each term that contains the concentration of one of the PII species as a product. In the corresponding terms,



Eq. 1(b) contains the constants  $K_{iS_{PIIUMP_1}}$  and  $K_{iP_{PIIUMP_2}}$ , while Eq. (1c) contains the constants  $K_{iS_{PIIUMP_2}}$  and  $K_{iP_{PIIUMP_3}}$ . In other words, in each equation of Bruggeman, the  $K_{iS}$  and  $K_{iP}$  constants in the denominator all correspond to the substrate and product, respectively, of the particular uridylylation reaction in question (PII and PIIUMP<sub>1</sub> for the first reaction, PIIUMP<sub>1</sub> and PIIUMP<sub>2</sub> for the second reaction and PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub> for the third reaction).

In our equations, the  $K_{iS}$  and  $K_{iP}$  constants in the denominator of a particular reaction equation correspond to the form of PII that appears in that particular term. In other words, all terms containing the concentration of PII as a substrate have  $K_{iS_{PII}}$ , all terms with the concentration of PIIUMP<sub>1</sub> as a substrate have  $K_{iS_{PIIUMP_1}}$  and all terms with the concentration of PIIUMP<sub>2</sub> as a substrate have  $K_{iS_{PIIUMP_2}}$ . Similarly, all terms with the concentration of PIIUMP<sub>1</sub> as a product have  $K_{iP_{PIIUMP_1}}$ , all terms with the concentration of PIIUMP<sub>2</sub> as a product have  $K_{iP_{PIIUMP_2}}$ , and all terms with the concentration of PIIUMP<sub>3</sub> as a product have  $K_{iP_{PIIUMP_3}}$ . This results in our three equations having exactly the same denominator.

To exemplify the difference between our equations and those of Bruggeman, the denominator of Eq. (1a) has the term “[PII]/ $K_{iS_{PII}}$ ”, while Eq. (2a) has “[PII]/ $K_{iS_{PIIUMP_1}}$ ” and Eq. (1c) has “[PII]/ $K_{iS_{PIIUMP_2}}$ ”. In all three of our equations (i.e. Eqs (9a) to (9c)), the term is “[PII]/ $K_{iS_{PII}}$ ”.

There is a further important difference between our denominators and those of Bruggeman. When the denominators of Bruggeman (Eqs (1a) to (1c)) are compared to the denominator of our equations (Table 3), several terms are identical. However, our denominator contains several terms with the concentration of PP<sub>1</sub> that are missing from the denominators of Eqs (1a) to (1c). Likewise, our denominator has several terms with the concentration of PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> or PIIUMP<sub>3</sub> as products that are missing from the denominators of Eqs (1a) to (1c). There is only one term containing each of the concentrations of PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub> as products in the denominators of Bruggeman *et al.* (2005), and these have no equivalent counterpart in our denominators.

The numerators of the equations of Bruggeman *et al.* (2005) only contain the term for the forward reaction and not the term for the reverse reaction, as they assume uridylylation to be irreversible. This is not unreasonable, since it has been shown that the reverse reaction is very slow compared to the forward reaction (Jiang *et al.*, 1998). However, in the equations deduced in the current work, the reversibility of the reaction is responsible not only for the

presence of the term for the reverse reaction in the numerator but also for many of the terms in the denominator that contribute to prediction of the correct product inhibition pattern.

The terms that are missing from the equations of Bruggeman *et al.* (2005) are the terms that contain either  $K_{MPi}$ ,  $K_{iUTP}$  or  $K_{MP}$  for PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> or PIIUMP<sub>3</sub>. Values for these constants are not given by Jiang *et al.* (1998), which was the source used by Bruggeman *et al.* (2005) for the values for all other constants. It may be that Bruggeman *et al.* (2005) removed the constants for which there were no available numerical values.

Table 3 – Comparison of the terms of the denominators of the equations of Bruggeman *et al.* (2005) and the denominator of our equations.

	Denominator for the equation of Bruggeman <i>et al.</i> (2005) for the first uridylylation reaction	Denominator for the equation of Bruggeman <i>et al.</i> (2005) for the second uridylylation reaction	Denominator for the equation of Bruggeman <i>et al.</i> (2005) for the third uridylylation reaction	Denominator for the uridylylation equations in this work
Terms without the concentration of any form of PII	$1, \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}}$	$1, \frac{K_{MS_{PIIUM_1}} [UTP]}{K_{iS_{PIIUM_1}} K_{M_{UTP}}}$	$1, \frac{K_{MS_{PIIUM_2}} [UTP]}{K_{iS_{PIIUM_2}} K_{M_{UTP}}}$	$1, \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}},$ $\frac{K_{MP_{PIIUM_1}} [PPi]}{K_{iP_{PIIUM_1}} K_{i_{PPi}}}$
Terms with the concentration of PII as a substrate	$\frac{[PII]}{K_{iS_{PII}}}, \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}},$ $\frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PII]}{K_{iS_{PIIUM_1}}}, \frac{[PII][UTP]}{K_{iS_{PIIUM_1}} K_{M_{UTP}}},$ $\frac{[PII][UTP][PPi]}{K_{iS_{PIIUM_1}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PII]}{K_{iS_{PIIUM_2}}}, \frac{[PII][UTP]}{K_{iS_{PIIUM_2}} K_{M_{UTP}}},$ $\frac{[PII][UTP][PPi]}{K_{iS_{PIIUM_2}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PII]}{K_{iS_{PII}}}, \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}},$ $\frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}},$ $\frac{[PII][PPi]}{K_{iS_{PII}} K_{M_{PPi}}}, \frac{K_{MP_{PIIUM_1}}}{K_{iP_{PIIUM_1}}}$
Terms with the concentration of PIIUM <sub>1</sub> as a substrate	$\frac{[PIIUM_1]}{K_{iS_{PII}}},$ $\frac{[PIIUM_1][UTP]}{K_{iS_{PII}} K_{M_{UTP}}},$ $\frac{[PIIUM_1][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PIIUM_1]}{K_{iS_{PIIUM_1}}},$ $\frac{[PIIUM_1][UTP]}{K_{iS_{PIIUM_1}} K_{M_{UTP}}},$ $\frac{[PIIUM_1][UTP][PPi]}{K_{iS_{PIIUM_1}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PIIUM_1]}{K_{iS_{PIIUM_2}}},$ $\frac{[PIIUM_1][UTP]}{K_{iS_{PIIUM_2}} K_{M_{UTP}}},$ $\frac{[PIIUM_1][UTP][PPi]}{K_{iS_{PIIUM_2}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PIIUM_1]}{K_{iS_{PIIUM_1}}},$ $\frac{[PIIUM_1][UTP]}{K_{iS_{PIIUM_1}} K_{M_{UTP}}},$ $\frac{[PIIUM_1][PPi]}{K_{iS_{PIIUM_1}} K_{M_{PPi}}}, \frac{K_{MP_{PIIUM_2}}}{K_{iP_{PIIUM_2}}},$ $\frac{[PIIUM_1][UTP][PPi]}{K_{iS_{PIIUM_1}} K_{M_{UTP}} K_{i_{PPi}}}$
Terms with the concentration of PIIUM <sub>2</sub> as a substrate	$\frac{[PIIUM_2]}{K_{iS_{PII}}},$ $\frac{[PIIUM_2][UTP]}{K_{iS_{PII}} K_{M_{UTP}}},$ $\frac{[PIIUM_2][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PIIUM_2]}{K_{iS_{PIIUM_1}}},$ $\frac{[PIIUM_2][UTP]}{K_{iS_{PIIUM_1}} K_{M_{UTP}}},$ $\frac{[PIIUM_2][UTP][PPi]}{K_{iP_{PIIUM_1}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PIIUM_2]}{K_{iS_{PIIUM_2}}},$ $\frac{[PIIUM_2][UTP]}{K_{iS_{PIIUM_2}} K_{M_{UTP}}},$ $\frac{[PIIUM_2][UTP][PPi]}{K_{iS_{PIIUM_2}} K_{M_{UTP}} K_{i_{PPi}}}$	$\frac{[PIIUM_2]}{K_{iS_{PIIUM_2}}},$ $\frac{[PIIUM_2][UTP]}{K_{iS_{PIIUM_2}} K_{M_{UTP}}},$ $\frac{[PIIUM_2][UTP][PPi]}{K_{iS_{PIIUM_2}} K_{M_{UTP}} K_{i_{PPi}}}$

				$\frac{[PIIUMP_2] [PPi] K_{MP_{PIIUMP_3}}}{K_{iS_{PIIUMP_2}} K_{M_{PPi}} K_{iP_{PIIUMP_3}}},$ $\frac{[PIIUMP_2] [UTP] [PPi]}{K_{iS_{PIIUMP_2}} K_{M_{UTP}} K_{iPPi}}$
Terms with the concentration of PIIUMP <sub>1</sub> as product	$\frac{K_{MS_{PII}} [PIIUMP_1]}{K_{iS_{PII}} K_{iP_{PIIUMR_1}}}$	$\frac{K_{MS_{PIIUMR_1}} [PIIUMP_1]}{K_{iS_{PIIUMR_1}} K_{iP_{PIIUMP_2}}}$	$\frac{K_{MS_{PIIUMP_2}} [PIIUMP_1]}{K_{iS_{PIIUMP_2}} K_{iP_{PIIUMP_3}}}$	$\frac{[PIIUMP_1]}{K_{iP_{PIIUMR_1}}},$ $\frac{[PIIUMP_1] [UTP] K_{MS_{PII}}}{K_{iP_{PIIUMR_1}} K_{M_{UTP}} K_{iS_{PII}}},$ $\frac{[PIIUMP_1] [PPi]}{K_{iP_{PIIUMR_1}} K_{M_{PPi}}},$ $\frac{[PIIUMP_1] [UTP] [PPi]}{K_{iP_{PIIUMR_1}} K_{iUTP} K_{M_{PPi}}}$
Terms with the concentration of PIIUMP <sub>2</sub> as product	$\frac{K_{MS_{PII}} [PIIUMP_2]}{K_{iS_{PII}} K_{iP_{PIIUMR_1}}}$	$\frac{K_{MS_{PIIUMR_1}} [PIIUMP_2]}{K_{iS_{PIIUMR_1}} K_{iP_{PIIUMP_2}}}$	$\frac{K_{MS_{PIIUMP_2}} [PIIUMP_2]}{K_{iS_{PIIUMP_2}} K_{iP_{PIIUMP_3}}}$	$\frac{[PIIUMP_2]}{K_{iP_{PIIUMP_2}}},$ $\frac{[PIIUMP_2] [PPi]}{K_{iP_{PIIUMP_2}} K_{M_{PPi}}},$ $\frac{[PIIUMP_2] [UTP] K_{MS_{PIIUMR_1}}}{K_{iP_{PIIUMP_2}} K_{M_{UTP}} K_{iS_{PIIUMR_1}}},$ $\frac{[PIIUMP_2] [UTP] [PPi]}{K_{iP_{PIIUMP_2}} K_{iUTP} K_{M_{PPi}}}$
Terms with the concentration of PIIUMP <sub>3</sub> as product	$\frac{K_{MS_{PII}} [PIIUMP_3]}{K_{iS_{PII}} K_{iP_{PIIUMR_1}}}$	$\frac{K_{MS_{PIIUMR_1}} [PIIUMP_3]}{K_{iS_{PIIUMR_1}} K_{iP_{PIIUMP_2}}}$	$\frac{K_{MS_{PIIUMP_2}} [PIIUMP_3]}{K_{iS_{PIIUMP_2}} K_{iP_{PIIUMP_3}}}$	$\frac{[PIIUMP_3]}{K_{iP_{PIIUMR_3}}},$ $\frac{[PIIUMP_3] [PPi]}{K_{iP_{PIIUMR_3}} K_{M_{PPi}}},$ $\frac{[PIIUMP_3] [UTP] K_{MS_{PIIUMR_2}}}{K_{iP_{PIIUMR_3}} K_{M_{UTP}} K_{iS_{PIIUMR_2}}},$ $\frac{[PIIUMP_3] [UTP] [PPi]}{K_{iP_{PIIUMR_3}} K_{iUTP} K_{M_{PPi}}}$

Our model has a further advantage over that of Bruggeman *et al.* (2005): by recognizing that the interaction of UTase with a subunit within a trimer is not affected by the uridylylation

state of the other two subunits (Atkinson *et al.*, 1994), we showed that UTase has different apparent values for the  $K_{MS}$ ,  $K_{iS}$ ,  $K_{MP}$  and  $K_{iP}$  constants related to the various PII species as substrates or products, with these apparent values depending on the number of uridylylated subunits (See Eqs. (5) to (8)). In contrast, Bruggeman *et al.* (2005) used the same values of  $K_{MS}$  and  $K_{iS}$  for the three substrates (PII, PIIUMP<sub>1</sub> and PIIUMP<sub>2</sub>) and the same values of  $K_{MP}$  and  $K_{iP}$  for the three products (PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub>). Effectively, the model of Bruggeman *et al.* (2005) assumes that the chance of a productive collision between UTase and a PII trimer, with this trimer acting either as a substrate or as an inhibiting product, is not affected by the spatial orientation of the trimer. As we show in the supplementary material, UTase would have twice as much chance of binding to a non-uridylylated subunit in an encounter with PIIUMP<sub>1</sub> than in an encounter with PIIUMP<sub>2</sub>. Likewise, it would have twice as much chance of binding to a uridylylated subunit in an encounter with PIIUMP<sub>2</sub> than in an encounter with PIIUMP<sub>1</sub>.

Since our model describes the inhibitory effects of the products (PP<sub>i</sub>, PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub>) correctly and also provides correlations for the various  $K_{MS}$ ,  $K_{iS}$ ,  $K_{MP}$  and  $K_{iP}$  constants related to the different species of PII, it will have a superior capability of predicting transitory uridylylation profiles, where the various forms of PII are present in the reaction mixture, when compared with the model of Bruggeman *et al.* (2005). However, at the moment, it is limited to describing systems in which the three 2-OG binding sites of the PII trimer are completely occupied by 2-OG. In order to describe situations in which PII trimers have varying numbers of bound ADP, ATP and 2-OG molecules, the model developed in the current work must be extended to recognize these states explicitly.

## 2.5 SUPPLEMENTARY MATERIAL 1

### 2.5.1 Deduction of the three UTase equations

The King-Altman method requires the drawing of a master pattern. Figure S shows the King and Altman's master pattern for the UTase mechanism from Figure 1.

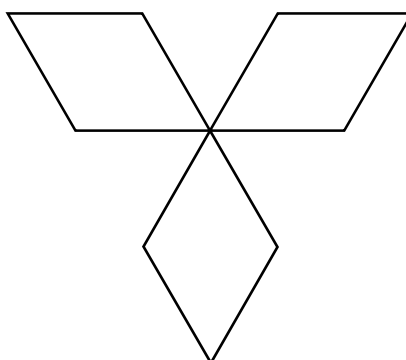


Figure S1 – Master pattern of the King-Altman method for the UTase mechanism.

Each vertex in the master pattern represents a form of the enzyme (compare Figure S1 to Figure 1). There is a line connecting two enzyme forms whenever there are reactions leading to one from the other. In order to obtain an expression for the fraction of one enzyme form relative to the total enzyme, all individual patterns which lead to this enzyme form are drawn.

Each individual pattern must be made up of lines only from the master pattern and the lines in an individual pattern cannot form a closed loop. If a closed loop would be formed, one of the lines in this pattern must be removed. A mathematical expression must be obtained for each of 10 the enzyme forms. The mathematical expression for an enzyme form is obtained from all possible patterns that lead to the enzyme form in question. In order to simplify the procedure the loops of the master pattern can be treated one at a time. An expression for each of the three enzyme forms unique to the first loop, i.e. the upper right loop, can be obtained by considering the first loop individually. Each of these expressions must then be multiplied by an expression relative to the two remaining loops. These “*remaining loop*” expressions are named  $F$ ,  $F'$  and  $F''$  for the first, second and third loops, respectively. These three expressions are all related to the enzyme form that connects the loops to one another, i.e. free UTase. The patterns for each loop that lead to each of these expressions are shown in Figure S. The top, middle and bottom patterns are relative to the first, second and third loop of the master pattern respectively and therefore related to the first, second and third uridylylation reaction, again respectively.

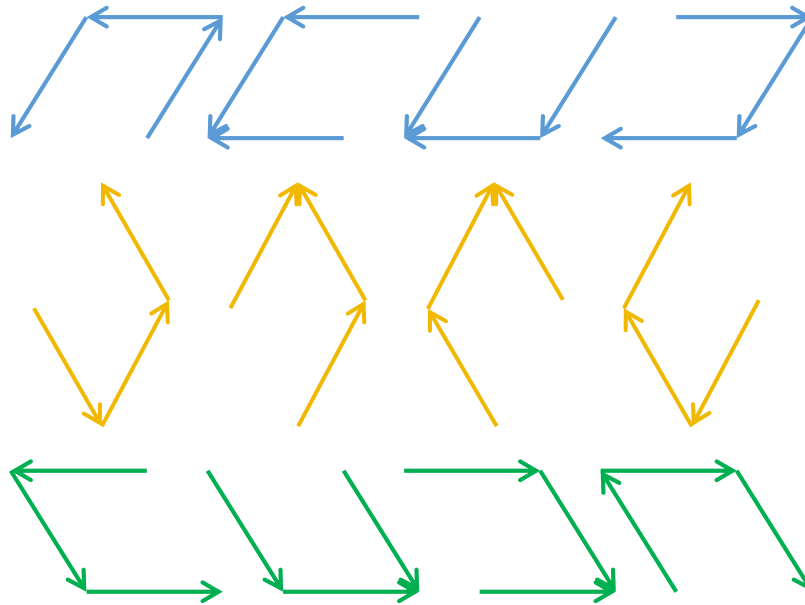


Figure S2 – The patterns used to obtain expressions F (top), F' (middle) and F'' (bottom).

The four top patterns produce the expression

$$F = k_{-1}k_{-2}k_{-3}[PPi] + k_{-1}k_{-2}k_4 + k_{-1}k_3k_4 + k_2k_3k_4[UTP] \quad (S.3)$$

The four middle patterns produce the expression

$$F' = k'_{-1}k'_{-2}k'_{-3}[PPi] + k'_{-1}k'_{-2}k'_4 + k'_{-1}k'_3k'_4 + k'_2k'_3k'_4[UTP] \quad (S.4)$$

Finally, the four bottom patterns produce the expression

$$F'' = k''_{-1}k''_{-2}k''_{-3}[PPi] + k''_{-1}k''_{-2}k''_4 + k''_{-1}k''_3k''_4 + k''_2k''_3k''_4[UTP] \quad (S.5)$$

Eqs (S.3), (S.4) and (S.5) have terms that can be joined, resulting in the simplified equations:

$$F = k_{-1}k_{-2}k_{-3}[PPi] + k_{-1}k_4(k_{-2} + k_3) + k_2k_3k_4[UTP] \quad (S.6)$$

$$F' = k'_{-1}k'_{-2}k'_{-3}[PPi] + k'_{-1}k'_4(k'_{-2} + k'_3) + k'_2k'_3k'_4[UTP] \quad (S.7)$$

$$F'' = k''_{-1}k''_{-2}k''_{-3}[PPi] + k''_{-1}k''_4(k''_{-2} + k''_3) + k''_2k''_3k''_4[UTP] \quad (S.8)$$

By following the set of patterns leading to each enzyme form in the first loop (except free UTase), the expressions below are obtained. Equation (S.9) is relative to UT:PII, the enzyme form complexed to PII. Equation (S.10) is relative to the Ternary Complex 1, the enzyme form complexed to both PII and UTase or to PIIUMP and PPi depending on the catalysis step. This enzyme form will be denominated T.C.<sub>1</sub> from here on. Equation (S.11) is relative to UT\*PIIUMP, the enzyme form complexed only to PIIUMP, interacting specifically through the uridylylated subunit.

$$k_1k_{-2}k_{-3}[PII][PPi] + k_1k_4k_{-2}[PII] + k_1k_4k_3[PII] + k_{-2}k_{-3}k_{-4}[PPi][PIIUMP] \quad (S.9)$$

$$k_1 k_2 k_3 [PII][UTP][PPi] + k_1 k_2 k_4 [PII][UTP] + k_{-1} k_{-3} k_{-4} [PPi][PIIUMP] + k_2 k_{-3} k_{-4} [UTP][PPi][PIIUMP] \quad (S.10)$$

$$k_1 k_2 k_3 [PII][UTP] + k_{-1} k_{-4} (k_{-2} + k_3) [PIIUMP] + k_2 k_3 k_{-4} [UTP][PIIUMP] \quad (S.11)$$

These expressions, once multiplied by  $F'$  and  $F''$  and divided by a denominator  $D$ , to be resolved after all enzyme form expressions are obtained, will give the concentration fraction of the respective enzyme form, that is, the enzyme form's concentration divided by the total enzyme concentration. The equations (S.12), (S.13) and (S.14) are obtained from equations (S.9), (S.10) and (S.11) respectively, by following this procedure. Equations (S.15), (S.16) and (S.17) refer to enzyme forms which are found in the second loop or UTase reaction, UT:PIIUMP, Ternary Complex 2 (T.C.2) and UT\*PIIUMP<sub>2</sub>, respectively. Enzyme form UT:PIIUMP is the complex between UTase and PIIUMP through a non-uridylylated subunit (differing from UT\*PIIUMP), enzyme form T.C.2 is the ternary complex of the second loop and enzyme form UT\*PIIUMP<sub>2</sub> is the complex between enzyme doubly uridylylated PII through a uridylylated subunit. These last three equations, relative to the second loop, are multiplied by  $F$  and  $F''$ , relative to the two other loops. The same logic applies to the next three equations, (S.18), (S.19) and (S.20). They are relative the third loop, and they must be multiplied by  $F$ , relative to the first loop, and  $F'$ , relative to the second loop. Lastly, the expression for the free enzyme is equation (S.21).

$$\frac{[UT : PII]}{[UT]_T} = \frac{\left( k_1 k_2 k_3 [PII][PPi] + k_1 k_4 (k_{-2} + k_3) [PII] \right)}{D} \times F' \times F'' \quad (S.12)$$

$$\frac{[T.C._1]}{[UT]_T} = \frac{\left( k_1 k_2 k_3 [PII][UTP][PPi] + k_1 k_2 k_4 [PII][UTP] + k_{-1} k_{-3} k_{-4} [PPi][PIIUMP] + k_2 k_3 k_{-4} [UTP][PPi][PIIUMP] \right)}{D} \times F' \times F'' \quad (S.13)$$



$$\frac{[UT * PIIUMP_1]}{[UT]_T} = \frac{\left( k_1 k_2 k_3 [PII][UTP] + k_{-1} k_{-4} (k_{-2} + k_3) [PIIUMP_1] \right)}{D} \times F' \times F'' \quad (S.14)$$

$$\frac{[UT : PIIUMP]}{[UT]_T} = \frac{\left( k'_1 k'_{-2} k'_{-3} [PIIUMP][PPi] + k'_1 k'_4 (k'_{-2} + k'_3) [PIIUMP] \right)}{D} \times F \times F'' \quad (S.15)$$

$$\frac{[T.C._2]}{[UT]_T} = \frac{\left( k'_1 k'_2 k'_{-3} [PIIUMP][UTP][PPi] + k'_1 k'_2 k'_4 [PIIUMP][UTP] + k'_{-1} k'_{-3} k'_{-4} [PPi][PIIUMP_2] + k'_2 k'_{-3} k'_{-4} [UTP][PPi][PIIUMP_2] \right)}{D} \times F \times F'' \quad (S.16)$$

$$\frac{[UT * PIIUMP_2]}{[UT]_T} = \frac{\left( k'_1 k'_2 k'_3 [PIIUMP][UTP] + k'_{-1} k'_{-4} (k'_{-2} + k'_3) [PIIUMP_2] \right)}{D} \times F \times F'' \quad (S.17)$$

$$\frac{[UT : PIIUMP_2]}{[UT]_T} = \frac{\left( k''_1 k''_{-2} k''_{-3} [PIIUMP_2][PPi] + k''_1 k''_4 (k''_{-2} + k''_3) [PIIUMP_2] \right)}{D} \times F \times F' \quad (S.18)$$

$$\frac{[T.C._3]}{[UT]_T} = \frac{\left( k''_1 k''_2 k''_{-3} [PIIUMP_2][UTP][PPi] + k''_1 k''_2 k''_4 [PIIUMP_2][UTP] + k''_{-1} k''_{-3} k''_{-4} [PPi][PIIUMP_3] + k''_2 k''_{-3} k''_{-4} [UTP][PPi][PIIUMP_3] \right)}{D} \times F \times F' \quad (S.19)$$

$$\frac{[UT * PIIUMP_3]}{[UT]_T} = \frac{\left( k''_1 k''_2 k''_3 [PIIUMP_2][UTP] + k''_{-1} k''_{-4} (k''_{-2} + k''_3) [PIIUMP_3] \right)}{D} \times F \times F' \quad (S.20)$$

$$\frac{[UT]}{[UT]_T} = \frac{F \times F' \times F''}{D} \quad (S.21)$$

### 2.5.1.1 Change of constants

The constants in the equations for the fraction of each enzyme form are the constants for the fundamental reactions that comprise the proposed mechanism. It is useful to substitute these fundamental constants for constants which are more meaningful to each substrate and product in the complete mechanism. Equations (S.22) to (S.51) are the identities between the fundamental constants and the constants in the final equation.

Constant identities for the first loop

$$K_{MS_{PII}} = \frac{k_3 k_4}{k_1 (k_3 + k_4)} \quad (S.22)$$

$$K_{iS_{PII}} = \frac{k_{-1}}{k_1} \quad (S.23)$$

$$K_{M_{UTP}} = \frac{k_4 (k_{-2} + k_3)}{k_2 (k_3 + k_4)} \quad (S.24)$$

$$K_{i_{UTP}} = \frac{k_{-1} + k_{-2}}{k_2} \quad (S.25)$$

$$K_{M_{PPi}} = \frac{k_{-1} (k_{-2} + k_3)}{k_{-3} (k_{-1} + k_{-2})} \quad (S.26)$$

$$K_{i_{PPi}} = \frac{k_3 + k_4}{k_{-3}} \quad (S.27)$$

$$K_{MP_{PIIUM\text{\textcircled{1}}}}} = \frac{k_{-1} k_{-2}}{k_{-4} (k_{-1} + k_{-2})} \quad (S.28)$$

$$K_{i_{P_{PIIUM\text{\textcircled{1}}}}} = \frac{k_4}{k_{-4}} \quad (S.29)$$

$$k_{cat_F} = \frac{k_3 k_4}{k_3 + k_4} \quad (S.30)$$

$$k_{cat_R} = \frac{k_{-1} k_{-2}}{k_{-1} + k_{-2}} \quad (S.31)$$

Constant identities for the second loop:

$$K_{MS_{PIIUM\text{\textcircled{2}}}}} = \frac{k'_3 k'_4}{k'_1 (k'_3 + k'_4)} \quad (S.32)$$

$$K_{iS_{PIIUM\text{\textcircled{2}}}}} = \frac{k'_{-1}}{k'_1} \quad (S.33)$$

$$K_{M_{UTP}} = \frac{k'_4 (k'_{-2} + k'_3)}{k'_2 (k'_3 + k'_4)} \quad (S.34)$$

$$K_{i_{UTP}} = \frac{k'_{-1} + k'_{-2}}{k'_2} \quad (S.35)$$

$$K_{M_{PPi}} = \frac{k'_{-1} (k'_{-2} + k'_3)}{k'_{-3} (k'_{-1} + k'_{-2})} \quad (S.36)$$

$$K_{i_{PPi}} = \frac{k'_3 + k'_4}{k'_{-3}} \quad (S.37)$$

$$K_{MP_{PIIUM\text{\textcircled{2}}}}} = \frac{k'_{-1} k'_{-2}}{k'_{-4} (k'_{-1} + k'_{-2})} \quad (S.38)$$

$$K_{iP_{PIUMP_2}} = \frac{k'_{-4}}{k'_{-4}} \quad (S.39)$$

$$k_{catF} = \frac{k'_3 k'_4}{k'_3 + k'_4} \quad (S.40)$$

$$k_{catR} = \frac{k'_{-1} k'_{-2}}{k'_{-1} + k'_{-2}} \quad (S.41)$$

Constant identities for the third loop:

$$K_{MS_{PIUMP_2}} = \frac{k''_3 k''_4}{k''_1 (k''_3 + k''_4)} \quad (S.42)$$

$$K_{iS_{PIUMP_2}} = \frac{k''_{-1}}{k''_1} \quad (S.43)$$

$$K_{M_{UTP}} = \frac{k''_4 (k''_{-2} + k''_3)}{k''_2 (k''_3 + k''_4)} \quad (S.44)$$

$$K_{i_{UTP}} = \frac{k''_{-1} + k''_{-2}}{k''_2} \quad (S.45)$$

$$K_{M_{PPi}} = \frac{k''_{-1} (k''_{-2} + k''_3)}{k''_{-3} (k''_{-1} + k''_{-2})} \quad (S.46)$$

$$K_{i_{PPi}} = \frac{k''_3 + k''_4}{k''_{-3}} \quad (S.47)$$

$$K_{MP_{PIUMP_3}} = \frac{k''_{-1} k''_{-2}}{k''_{-4} (k''_{-1} + k''_{-2})} \quad (S.48)$$

$$K_{iP_{PIUMP_3}} = \frac{k''_4}{k''_{-4}} \quad (S.49)$$

$$k_{catF} = \frac{k''_3 k''_4}{k''_3 + k''_4} \quad (S.50)$$

$$k_{catR} = \frac{k''_{-1} k''_{-2}}{k''_{-1} + k''_{-2}} \quad (S.51)$$

The identities (S.22) to (S.51) can be used to perform a change of constants in the equations for the enzyme form fractions. It is more interesting that the enzyme form fraction equations be summed for each loop or reaction. The addition of the equations for the enzyme forms in loop one, (S.12), (S.13) and (S.14), results in equation (S.52). The same procedure is done for the equations of the second loop, (S.15), (S.16) and (S.17), in order to obtain equation (S.53), and for the equations of the third loop, (S.18), (S.19) and (S.20), in order to obtain equation (S.54).

$$\frac{\begin{pmatrix} [UT : PII] + \\ [T.C._1] + \\ [UT * PIIUMP] \end{pmatrix}}{[UT]_T} = \frac{\begin{pmatrix} k_1 k_{-2} k_{-3} [PII][PPi] + k_1 k_4 (k_{-2} + k_3) [PII] \\ + k_{-2} k_{-3} k_{-4} [PPi][PIIUMP] + k_1 k_2 k_4 [PII][UTP] + \\ k_1 k_2 k_{-3} [PII][UTP][PPi] + k_{-1} k_{-3} k_{-4} [PPi][PIIUMP] + \\ k_2 k_{-3} k_{-4} [UTP][PPi][PIIUMP] + k_1 k_2 k_3 [PII][UTP] + \\ k_{-1} k_{-4} (k_{-2} + k_3) [PIIUMP] + k_2 k_3 k_{-4} [UTP][PIIUMP] \end{pmatrix}}{D} \times F' \times F'' \quad (S.52)$$

$$\frac{\begin{pmatrix} [UT : PIIUMP] \\ + [T.C._2] + \\ [UT * PIIUMP_2] \end{pmatrix}}{[UT]_T} = \frac{\begin{pmatrix} k'_1 k'_{-2} k'_{-3} [PIIUMP][PPi] + k'_1 k'_4 (k'_{-2} + k'_3) [PIIUMP] + \\ k'_{-2} k'_{-3} k'_{-4} [PPi][PIIUMP_2] + k'_1 k'_2 k'_4 [PIIUMP][UTP] + \\ k'_1 k'_2 k'_{-3} [PIIUMP][UTP][PPi] + k'_{-1} k'_{-3} k'_{-4} [PPi][PIIUMP_2] + \\ k'_2 k'_{-3} k'_{-4} [UTP][PPi][PIIUMP_2] + k'_1 k'_2 k'_3 [PIIUMP][UTP] + \\ k'_{-1} k'_{-4} (k'_{-2} + k'_3) [PIIUMP_2] + k'_2 k'_3 k'_{-4} [UTP][PIIUMP_2] \end{pmatrix}}{D} \times F \times F'' \quad (S.53)$$

$$\frac{\begin{pmatrix} [UT : PIIUMP_2] \\ + [T.C._3] + \\ [UT * PIIUMP_3] \end{pmatrix}}{[UT]_T} = \frac{\begin{pmatrix} k''_1 k''_{-2} k''_{-3} [PIIUMP_2][PPi] + k''_1 k''_4 (k''_{-2} + k''_3) [PIIUMP_2] \\ + k''_{-2} k''_{-3} k''_{-4} [PPi][PIIUMP_3] + k''_1 k''_2 k''_{-3} [PIIUMP_2][UTP][PPi] + \\ k''_1 k''_2 k''_4 [PIIUMP_2][UTP] + k''_{-1} k''_{-3} k''_{-4} [PPi][PIIUMP_3] + \\ k''_2 k''_{-3} k''_{-4} [UTP][PPi][PIIUMP_3] + k''_2 k''_3 k''_{-4} [UTP][PIIUMP_3] + \\ k''_1 k''_2 k''_3 [PIIUMP_2][UTP] + k''_{-1} k''_{-4} (k''_{-2} + k''_3) [PIIUMP_3] \end{pmatrix}}{D} \times F \times F' \quad (S.54)$$

The terms in these three equations are reorganized so as to have each combination of product and substrate concentrations appear only once. This simplifies equations (S.52), (S.53) and (S.54) to equations (S.55), (S.56) and (S.57), respectively.

$$\frac{\begin{pmatrix} [UT : PII] + \\ [T.C._1] + \\ [UT * PIIUMP] \end{pmatrix}}{[UT]_T} = \frac{\begin{pmatrix} k_1 k_4 (k_{-2} + k_3) [PII] + k_1 k_2 (k_3 + k_4) [PII][UTP] + \\ k_1 k_2 k_{-3} [PII][UTP][PPi] + k_1 k_{-2} k_{-3} [PII][PPi] + \\ k_{-3} k_{-4} (k_{-1} + k_{-2}) [PPi][PIIUMP] + k_2 k_3 k_{-4} [UTP][PIIUMP] + \\ k_2 k_{-3} k_{-4} [UTP][PPi][PIIUMP] + k_{-1} k_{-4} (k_{-2} + k_3) [PIIUMP] \end{pmatrix}}{D} \times F' \times F'' \quad (S.55)$$

$$\frac{\begin{pmatrix} [UT : PIIUMP] \\ + [T.C._2] + \\ [UT * PIIUMP_2] \end{pmatrix}}{[UT]_T} = \frac{\begin{pmatrix} k'_1 k'_4 (k'_{-2} + k'_3) [PIIUMP] + k'_1 k'_2 (k'_3 + k'_4) [PIIUMP][UTP] + \\ k'_1 k'_{-2} k'_{-3} [PIIUMP][PPi] + k'_1 k'_2 k'_{-3} [PIIUMP][UTP][PPi] + \\ k'_2 k'_3 k'_{-4} [UTP][PIIUMP_2] + (k'_{-1} + k'_{-2}) k'_{-3} k'_{-4} [PPi][PIIUMP_2] + \\ k'_2 k'_{-3} k'_{-4} [UTP][PPi][PIIUMP_2] + k'_{-1} k'_{-4} (k'_{-2} + k'_3) [PIIUMP_2] \end{pmatrix}}{D} \times F \times F'' \quad (S.56)$$

$$\frac{\left( \begin{array}{l} [UT : PIIUMP_2] \\ + [T.C_3] + \\ [UT * PIIUMP_3] \end{array} \right)}{[UT]_r} = \frac{\left( \begin{array}{l} k''_1 k''_4 (k''_{-2} + k''_3) [PIIUMP_2] + k''_1 k''_2 (k''_3 + k''_4) [PIIUMP_2] [UTP] + \\ k''_1 k''_{-2} k''_{-3} [PIIUMP_2] [PPi] + k''_1 k''_2 k''_{-3} [PIIUMP_2] [UTP] [PPi] + \\ k''_2 k''_3 k''_{-4} [UTP] [PIIUMP_3] + (k''_{-1} + k''_{-2}) k''_{-3} k''_{-4} [PPi] [PIIUMP_3] + \\ k''_2 k''_{-3} k''_{-4} [UTP] [PPi] [PIIUMP_3] + k''_{-1} k''_{-4} (k''_{-2} + k''_3) [PIIUMP_3] \end{array} \right)}{D} \times F \times F' \quad (S.57)$$

Both the numerators and denominators of Eqs. (S.21), (S.55), (S.56) and (S.57) are divided by:

$$k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3) \quad (S.58)$$

All terms corresponding to the first uridylylation cycle are grouped together, as are the terms corresponding to the second uridylylation cycle and to the third uridylylation cycle. Equation (S.21) results in:

$$\frac{[UT]}{[UT]_r} = \frac{F_1 \times F_2 \times F_3}{D / \left( (k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3)) \right)} \quad (S.59)$$

Equation (S.55) results in:

$$\frac{\left( \begin{array}{l} [UT : PII] + \\ [T.C_1] + \\ [UT * PIIUMP_1] \end{array} \right)}{[UT]_r} = \frac{\left( \begin{array}{l} \frac{k_1 k_4 (k_{-2} + k_3)}{k_{-1} k_4 (k_{-2} + k_3)} [PII] + \frac{k_1 k_2 (k_3 + k_4)}{k_{-1} k_4 (k_{-2} + k_3)} [PII] [UTP] + \\ \frac{k_1 k_2 k_3}{k_{-1} k_4 (k_{-2} + k_3)} [PII] [UTP] [PPi] + \frac{k_1 k_2 k_3}{k_{-1} k_4 (k_{-2} + k_3)} [PII] [PPi] + \\ \frac{k_{-3} k_{-4} (k_{-1} + k_{-2})}{k_{-1} k_4 (k_{-2} + k_3)} [PPi] [PIIUMP_1] + \frac{k_2 k_3 k_{-4}}{k_{-1} k_4 (k_{-2} + k_3)} [UTP] [PIIUMP_1] + \\ \frac{k_2 k_3 k_{-4}}{k_{-1} k_4 (k_{-2} + k_3)} [UTP] [PPi] [PIIUMP_1] + \frac{k_{-1} k_{-4} (k_{-2} + k_3)}{k_{-1} k_4 (k_{-2} + k_3)} [PIIUMP_1] \end{array} \right)}{D / \left( (k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3)) \right)} \times F_2 \times F_3 \quad (S.60)$$

Equation (S.56) results in:

$$\frac{\left( \begin{array}{l} [UT : \text{PIIUMP}_1] \\ + [T.C._2] \\ [UT * \text{PIIUMP}_2] \end{array} \right)}{[UT]_T} = \frac{\left( \begin{array}{l} \frac{k'_1 k'_4 (k'_{-2} + k'_3)}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PIIUMP}_1] + \frac{k'_1 k'_2 (k'_3 + k'_4)}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PIIUMP}_1][UTP] + \\ \frac{k'_1 k'_2 k'_{-3}}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PIIUMP}_1][\text{PPi}] + \frac{k'_1 k'_2 k'_{-3}}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PIIUMP}_1][UTP][\text{PPi}] + \\ \frac{k'_2 k'_3 k'_{-4}}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [UTP][\text{PIIUMP}_2] + \frac{(k'_{-1} + k'_{-2}) k'_{-3} k'_{-4}}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PPi}][\text{PIIUMP}_2] + \\ \frac{k'_2 k'_{-3} k'_{-4}}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [UTP][\text{PPi}][\text{PIIUMP}_2] + \frac{k'_{-1} k'_{-4} (k'_{-2} + k'_3)}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PIIUMP}_2] \end{array} \right) \times F_1 \times F_3}{\frac{D}{(k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3))}} \quad (\text{S.61})$$

Finally, Equation (S.57) results in:

$$\frac{\left( \begin{array}{l} [UT : \text{PIIUMP}_2] \\ + [T.C._3] \\ [UT * \text{PIIUMP}_3] \end{array} \right)}{[UT]_T} = \frac{\left( \begin{array}{l} \frac{k''_1 k''_4 (k''_{-2} + k''_3)}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [\text{PIIUMP}_2] + \frac{k''_1 k''_2 (k''_3 + k''_4)}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [\text{PIIUMP}_2][UTP] + \\ \frac{k''_1 k''_2 k''_{-3}}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [\text{PIIUMP}_2][\text{PPi}] + \frac{k''_1 k''_2 k''_{-3}}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [\text{PIIUMP}_2][UTP][\text{PPi}] + \\ \frac{k''_2 k''_3 k''_{-4}}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [UTP][\text{PIIUMP}_3] + \frac{(k''_{-1} + k''_{-2}) k''_{-3} k''_{-4}}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [\text{PPi}][\text{PIIUMP}_3] + \\ \frac{k''_2 k''_{-3} k''_{-4}}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [UTP][\text{PPi}][\text{PIIUMP}_3] + \frac{k''_{-1} k''_{-4} (k''_{-2} + k''_3)}{k''_{-1} k''_4 (k''_{-2} + k''_3)} [\text{PIIUMP}_3] \end{array} \right) \times F_1 \times F_2}{\frac{D}{(k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3))}} \quad (\text{S.62})$$

such that  $F_1$ ,  $F_2$  and  $F_3$  are given by:

$$F_1 = \frac{F}{k_{-1} k_4 (k_{-2} + k_3)} \quad (\text{S.63})$$

$$F_2 = \frac{F'}{k'_{-1} k'_4 (k'_{-2} + k'_3)} \quad (\text{S.64})$$

$$F_3 = \frac{F''}{k''_{-1} k''_4 (k''_{-2} + k''_3)} \quad (\text{S.65})$$

which in turn can be rewritten as:

$$F_1 = \frac{k_{-1} k_{-2} k_{-3}}{k_{-1} k_4 (k_{-2} + k_3)} [\text{PPi}] + \frac{k_{-1} k_4 (k_{-2} + k_3)}{k_{-1} k_4 (k_{-2} + k_3)} + \frac{k_2 k_3 k_4}{k_{-1} k_4 (k_{-2} + k_3)} [UTP] \quad (\text{S.66})$$

$$F_2 = \frac{k'_{-1} k'_{-2} k'_{-3}}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [\text{PPi}] + \frac{k'_{-1} k'_4 (k'_{-2} + k'_3)}{k'_{-1} k'_4 (k'_{-2} + k'_3)} + \frac{k'_2 k'_3 k'_4}{k'_{-1} k'_4 (k'_{-2} + k'_3)} [UTP] \quad (\text{S.67})$$

$$F_3 = \frac{k''_{-1} k''_{-2} k''_{-3}}{k''_{-1} k''_{-4} (k''_{-2} + k''_{-3})} [PPi] + \frac{k''_{-1} k''_{-4} (k''_{-2} + k''_{-3})}{k''_{-1} k''_{-4} (k''_{-2} + k''_{-3})} + \frac{k''_{-2} k''_{-3} k''_{-4}}{k''_{-1} k''_{-4} (k''_{-2} + k''_{-3})} [UTP] \quad (S.68)$$

The identities of the final constants from Eqs. (S.22) to (S.51) are substituted into Eq. (S.66) to give:

$$F_1 = \frac{K_{MP_{PIIUM_1}}}{K_{IP_{PIIUM_1}} K_{M_{PPi}}} [PPi] + 1 + \frac{K_{MS_{PII}}}{K_{IS_{PII}} K_{M_{UTP}}} [UTP] \quad (S.69)$$

into Eq. (S.67) to give:

$$F_2 = \frac{K_{MP_{PIIUM_2}}}{K_{IP_{PIIUM_2}} K_{M_{PPi}}} [PPi] + 1 + \frac{K_{MS_{PIIUM_1}}}{K_{IS_{PIIUM_1}} K_{M_{UTP}}} [UTP] \quad (S.70)$$

into Eq. (S.68) to give:

$$F_3 = \frac{K_{MP_{PIIUM_3}}}{K_{IP_{PIIUM_3}} K_{M_{PPi}}} [PPi] + 1 + \frac{K_{MS_{PIIUM_2}}}{K_{IS_{PIIUM_2}} K_{M_{UTP}}} [UTP] \quad (S.71)$$

into Eq. (S.60) to give:

$$\frac{\begin{pmatrix} [UT : PII] + \\ [T.C._1] + \\ [UT * PIIUM_1] \end{pmatrix}}{[UT_{Total}]} = \frac{D}{(k_{-1} k_{-4} (k_{-2} + k_{-3}) \times k'_{-1} k'_{-4} (k'_{-2} + k'_{-3}) \times k''_{-1} k''_{-4} (k''_{-2} + k''_{-3}))} \left( \begin{array}{l} \frac{[PII]}{K_{IS_{PII}}} + \frac{[PII][UTP]}{K_{IS_{PII}} K_{M_{UTP}}} + \\ \frac{[PII][UTP][PPi]}{K_{IS_{PII}} K_{M_{UTP}} K_{i_{PPi}}} + \frac{K_{MP_{PIIUM_1}} [PII][PPi]}{K_{i_{PIIUM_1}} K_{IS_{PII}} K_{M_{PPi}}} + \\ \frac{[PPi][PIIUM_1]}{K_{i_{PIIUM_1}} K_{M_{PPi}}} + \frac{K_{MS_{PII}} [UTP][PIIUM_1]}{K_{IS_{PII}} K_{i_{PIIUM_1}} K_{M_{UTP}}} + \\ \frac{[UTP][PPi][PIIUM_1]}{K_{M_{PPi}} K_{i_{UTP}} K_{i_{PIIUM_1}}} + \frac{[PIIUM_1]}{K_{i_{PIIUM_1}}} \end{array} \right) \times F_2 \times F_3 \quad (S.72)$$

into Eq. (S.61) to give:

$$\begin{aligned}
& \left( \begin{array}{l} [UT : PIIUMP_1] \\ + [T.C._2] + \\ [UT * PIIUMP_2] \end{array} \right) = \frac{D}{(k_{-1}k_4(k_{-2}+k_3) \times k'_{-1}k'_4(k'_{-2}+k'_3) \times k''_{-1}k''_4(k''_{-2}+k''_3))} \times F \times F_3 \\
& \left( \begin{array}{l} \frac{[PIIUMP_1]}{K_{iS_{PIIUM\phi_1}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PIIUM\phi_1}}K_{M_{UTP}}} + \\ \frac{[PIIUMP_1][UTP][PPi]}{K_{iS_{PIIUM\phi_1}}K_{M_{UTP}}K_{i_{PPi}}} + \frac{K_{MP_{PIIUM\phi_2}}[PIIUMP_1][PPi]}{K_{iP_{PIIUM\phi_2}}K_{iS_{PIIUM\phi_1}}K_{M_{PPi}}} + \\ \frac{[PPi][PIIUMP_2]}{K_{M_{PPi}}K_{iP_{PIIUM\phi_2}}} + \frac{K_{MS_{PIIUM\phi_1}}[UTP][PIIUMP_2]}{K_{iS_{PIIUM\phi_1}}K_{iP_{PIIUM\phi_2}}K_{M_{UTP}}} + \\ \frac{[UTP][PPi][PIIUMP_2]}{K_{iP_{PIIUM\phi_2}}K_{M_{PPi}}K_{i_{UTP}}} + \frac{[PIIUMP_2]}{K_{iP_{PIIUM\phi_2}}} \end{array} \right) \times F \times F_3 \quad (S.73)
\end{aligned}$$

and into Eq. (S.62) to give:

$$\begin{aligned}
& \left( \begin{array}{l} [UT : PIIUMP_2] \\ + [T.C._3] + \\ [UT * PIIUMP_3] \end{array} \right) = \frac{D}{(k_{-1}k_4(k_{-2}+k_3) \times k'_{-1}k'_4(k'_{-2}+k'_3) \times k''_{-1}k''_4(k''_{-2}+k''_3))} \times F_1 \times F_2 \\
& \left( \begin{array}{l} \frac{[PIIUMP_2]}{K_{iS_{PIIUM\phi_2}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PIIUM\phi_2}}K_{M_{UTP}}} + \\ \frac{K_{MP_{PIIUM\phi_3}}[PIIUMP_2][PPi]}{K_{iP_{PIIUM\phi_3}}K_{iS_{PIIUM\phi_2}}K_{M_{UTP}}} + \frac{[PIIUMP_2][UTP][PPi]}{K_{iS_{PIIUM\phi_2}}K_{M_{UTP}}K_{i_{PPi}}} + \\ \frac{K_{MS_{PIIUM\phi_2}}[UTP][PIIUMP_3]}{K_{iS_{PIIUM\phi_2}}K_{iP_{PIIUM\phi_3}}K_{M_{PPi}}} + \frac{[PPi][PIIUMP_3]}{K_{iP_{PIIUM\phi_3}}K_{M_{PPi}}} + \\ \frac{[UTP][PPi][PIIUMP_3]}{K_{iP_{PIIUM\phi_3}}K_{M_{PPi}}K_{i_{UTP}}} + \frac{[PIIUMP_3]}{K_{iP_{PIIUM\phi_3}}} \end{array} \right) \times F_1 \times F_2 \quad (S.74)
\end{aligned}$$

Equations (S.59), (S.72), (S.73) and (S.74) are added to give:



$$\begin{aligned}
& \left( \begin{array}{l} [UT] + \\ [UT : PII] + \\ [T.C._1] + \\ [UT * PIIUMP_1] + \\ [UT : PIIUMP_1] + \\ [T.C._2] + \\ [UT * PIIUMP_2] + \\ [UT : PIIUMP_2] + \\ [T.C._3] + \\ [UT * PIIUMP_3] \end{array} \right) = \left( \begin{array}{l} \left( \begin{array}{l} \frac{F_1 \times F_2 \times F_3 + [PII] + [PII][UTP]}{K_{iS_{PII}} + K_{iS_{PII}} K_{M_{UTP}}} + \\ \frac{[PII][UTP][PPi] + K_{MP_{PIIUM_1}} [PII][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}} + K_{i_{P_{PIIUM_1}}} K_{iS_{PII}} K_{M_{PPi}}} + \\ \frac{[PPi][PIIUMP_1] + K_{MS_{PII}} [UTP][PIIUMP_1]}{K_{i_{P_{PIIUM_1}}} K_{M_{PPi}} + K_{iS_{PII}} K_{i_{P_{PIIUM_1}}} K_{M_{UTP}}} + \\ \frac{[UTP][PPi][PIIUMP_1] + [PIIUMP_1]}{K_{M_{PPi}} K_{i_{UTP}} K_{i_{P_{PIIUM_1}}} + K_{i_{P_{PIIUM_1}}}} \end{array} \right) \times F_2 \times F_3 + \\ \left( \begin{array}{l} \frac{[PIIUMP_1] + [PIIUMP_1][UTP]}{K_{iS_{PIIUM_1}} + K_{iS_{PIIUM_1}} K_{M_{UTP}}} + \\ \frac{[PIIUMP_1][UTP][PPi] + K_{MP_{PIIUM_2}} [PIIUMP_1][PPi]}{K_{iS_{PIIUM_1}} K_{M_{UTP}} K_{i_{PPi}} + K_{i_{P_{PIIUM_2}}} K_{iS_{PIIUM_1}} K_{M_{PPi}}} + \\ \frac{[PPi][PIIUMP_2] + K_{MS_{PIIUM_1}} [UTP][PIIUMP_2]}{K_{M_{PPi}} K_{i_{P_{PIIUM_2}}} + K_{iS_{PIIUM_1}} K_{i_{P_{PIIUM_2}}} K_{M_{UTP}}} + \\ \frac{[UTP][PPi][PIIUMP_2] + [PIIUMP_2]}{K_{i_{P_{PIIUM_2}}} K_{M_{PPi}} K_{i_{UTP}} + K_{i_{P_{PIIUM_2}}}} \end{array} \right) \times F_1 \times F_3 + \\ \left( \begin{array}{l} \frac{[PIIUMP_2] + [PIIUMP_2][UTP]}{K_{iS_{PIIUM_2}} + K_{iS_{PIIUM_2}} K_{M_{UTP}}} + \\ \frac{K_{MP_{PIIUM_3}} [PIIUMP_2][PPi] + [PIIUMP_2][UTP][PPi]}{K_{i_{P_{PIIUM_3}}} K_{iS_{PIIUM_2}} K_{M_{UTP}} + K_{iS_{PIIUM_2}} K_{M_{UTP}} K_{i_{PPi}}} + \\ \frac{K_{MS_{PIIUM_2}} [UTP][PIIUMP_3] + [PPi][PIIUMP_3]}{K_{iS_{PIIUM_2}} K_{i_{P_{PIIUM_3}}} K_{M_{PPi}} + K_{i_{P_{PIIUM_3}}} K_{M_{PPi}}} + \\ \frac{[UTP][PPi][PIIUMP_3] + [PIIUMP_3]}{K_{i_{P_{PIIUM_3}}} K_{M_{PPi}} K_{i_{UTP}} + K_{i_{P_{PIIUM_3}}}} \end{array} \right) \times F_1 \times F_2 \end{array} \right) \quad (S.75) \\
& \frac{[UT_{Total}]}{D / (k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3))}
\end{aligned}$$

Since:

$$[UT]_T = \left( \begin{array}{l} [UT] + [UT : PII] + [T.C._1] + [UT * PIIUMP_1] + \\ [UT : PIIUMP_1] + [T.C._2] + [UT * PIIUMP_2] + \\ [UT : PIIUMP_2] + [T.C._3] + [UT * PIIUMP_3] \end{array} \right) \quad (S.76)$$

The numerator and denominator on the left side of Eq.(S.75) cancel out. The denominator from the right side can then be “brought over” to the left side:

$$\begin{aligned}
D' = & \left( \begin{aligned} & F_1 \times F_2 \times F_3 + \\ & \left( \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \right. \\ & \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}} + \frac{K_{MP_{PIIUM_1}} [PII][PPi]}{K_{i_{PIIUM_1}} K_{iS_{PII}} K_{M_{PPi}}} + \\ & \frac{[PPi][PIIUM_1]}{K_{i_{PIIUM_1}} K_{M_{PPi}}} + \frac{K_{MS_{PII}} [UTP][PIIUM_1]}{K_{iS_{PII}} K_{i_{PIIUM_1}} K_{M_{UTP}}} + \\ & \left. \frac{[UTP][PPi][PIIUM_1]}{K_{M_{PPi}} K_{i_{UTP}} K_{i_{PIIUM_1}}} + \frac{[PIIUM_1]}{K_{i_{PIIUM_1}}} \right) \times F_2 \times F_3 + \\ & \left( \frac{[PIIUM_1]}{K_{iS_{PIIUM_1}}} + \frac{[PIIUM_1][UTP]}{K_{iS_{PIIUM_1}} K_{M_{UTP}}} + \right. \\ & \frac{[PIIUM_1][UTP][PPi]}{K_{iS_{PIIUM_1}} K_{M_{UTP}} K_{i_{PPi}}} + \frac{K_{MP_{PIIUM_2}} [PIIUM_1][PPi]}{K_{i_{PIIUM_2}} K_{iS_{PIIUM_1}} K_{M_{PPi}}} + \\ & \frac{[PPi][PIIUM_2]}{K_{M_{PPi}} K_{i_{PIIUM_2}}} + \frac{K_{MS_{PIIUM_1}} [UTP][PIIUM_2]}{K_{iS_{PIIUM_1}} K_{i_{PIIUM_2}} K_{M_{UTP}}} + \\ & \left. \frac{[UTP][PPi][PIIUM_2]}{K_{i_{PIIUM_2}} K_{M_{PPi}} K_{i_{UTP}}} + \frac{[PIIUM_2]}{K_{i_{PIIUM_2}}} \right) \times F_1 \times F_3 + \\ & \left( \frac{[PIIUM_2]}{K_{iS_{PIIUM_2}}} + \frac{[PIIUM_2][UTP]}{K_{iS_{PIIUM_2}} K_{M_{UTP}}} + \right. \\ & \frac{K_{MP_{PIIUM_3}} [PIIUM_2][PPi]}{K_{i_{PIIUM_3}} K_{iS_{PIIUM_2}} K_{M_{UTP}}} + \frac{[PIIUM_2][UTP][PPi]}{K_{iS_{PIIUM_2}} K_{M_{UTP}} K_{i_{PPi}}} + \\ & \frac{K_{MS_{PIIUM_2}} [UTP][PIIUM_3]}{K_{iS_{PIIUM_2}} K_{i_{PIIUM_3}} K_{M_{PPi}}} + \frac{[PPi][PIIUM_3]}{K_{i_{PIIUM_3}} K_{M_{PPi}}} + \\ & \left. \frac{[UTP][PPi][PIIUM_3]}{K_{i_{PIIUM_3}} K_{M_{PPi}} K_{i_{UTP}}} + \frac{[PIIUM_3]}{K_{i_{PIIUM_3}}} \right) \times F_1 \times F_2 \end{aligned} \right) \quad (S.77)
\end{aligned}$$

Such that:

$$D' = \frac{D}{k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3)} \quad (S.78)$$

## 2.5.1.2 Numerators

The rate of uridylylation of PII is the rate at which PIIUMP<sub>1</sub> is released from the complex UT\*PIIUMP<sub>1</sub>, given by:

$$v_1 = [UT * PIIUMP_1]k_4 - [UT][PIIUMP_1]k_{-4} \quad (S.79)$$

the rate of uridylylation of PIIUMP<sub>1</sub> is the rate at which PIIUMP<sub>2</sub> is released from the complex UT\*PIIUMP<sub>2</sub>, given by:

$$v_2 = [UT * PIIUMP_2]k'_{-4} - [UT][PIIUMP_2]k'_{-4} \quad (S.80)$$

and the rate of uridylylation of PIIUMP<sub>2</sub> is the rate at which PIIUMP<sub>3</sub> is released from the complex UT\*PIIUMP<sub>3</sub>, given by:

$$v_3 = [UT * PIIUMP_3]k''_{-4} - [UT][PIIUMP_3]k''_{-4} \quad (S.81)$$

Equation (S.14) can be modified to:

$$[UT * PIIUMP_1] = \frac{[UT]_T \left( \frac{k_1 k_2 k_3 [PII][UTP] + k_{-1} k_{-4} (k_{-2} + k_3) [PIIUMP_1]}{+ k_2 k_3 k_{-4} [UTP][PIIUMP_1]} \right) \times F \times F''}{D} \quad (S.82)$$

and Eq. (S.21) can be modified to:

$$[UT] = \frac{[UT]_T F \times F \times F''}{D} \quad (S.83)$$

By substituting Eqs. (S.82) and (S.83) into Eq. (S.79) while substituting  $F$  for Eq. (S.6) we produce:

$$v_1 = \frac{[UT]_T \left( \left( \frac{k_1 k_2 k_3 [PII][UTP] + k_{-1} k_{-4} (k_{-2} + k_3) [PIIUMP_1]}{k_2 k_3 k_{-4} [UTP][PIIUMP_1]} \right) k_4 - \left( \frac{k_{-1} k_{-2} k_{-3} [PPi] + k_{-1} k_{-4} (k_{-2} + k_3)}{k_2 k_3 k_{-4} [UTP]} \right) [PIIUMP_1] k_{-4} \right) \times F \times F''}{D} \quad (S.84)$$

By multiplying out the constants and concentration in evidence we obtain:

$$v_1 = \frac{[UT]_T \left( \frac{k_1 k_2 k_3 k_4 [PII][UTP] + k_{-1} k_{-4} k_4 (k_{-2} + k_3) [PIIUMP_1]}{+ k_2 k_3 k_{-4} k_4 [UTP][PIIUMP_1]} - \frac{k_{-1} k_{-4} k_4 (k_{-2} + k_3) [PIIUMP_1]}{- k_2 k_3 k_{-4} k_{-4} [UTP][PIIUMP_1]} - \frac{k_{-1} k_{-2} k_{-3} k_{-4} [PPi][PIIUMP_1]}{+ k_2 k_3 k_{-4} k_{-4} [UTP][PIIUMP_1]} \right) \times F \times F''}{D} \quad (S.85)$$

There are two pairs of opposite terms which cancel out, to give:

$$v_1 = \frac{[UT]_T (k_1 k_2 k_3 k_4 [PII][UTP] - k_{-1} k_{-2} k_{-3} k_{-4} [PPi][PIIUMP_1]) \times F' \times F''}{D} \quad (S.86)$$

Both numerator and denominator are divided by Eq. (S.58) to give:

$$v_1 = \frac{\left( [UT]_T \left( \frac{k_1 k_2 k_3 k_4}{k_{-1} k_4 (k_{-2} + k_3)} [PII][UTP] - \frac{k_{-1} k_{-2} k_{-3} k_{-4}}{k_{-1} k_4 (k_{-2} + k_3)} [PPi][PIIUMP_1] \right) \right) \times \frac{F'}{k'_{-1} k'_4 (k'_{-2} + k'_3)} \times \frac{F''}{k''_{-1} k''_4 (k''_{-2} + k''_3)}}{D / (k_{-1} k_4 (k_{-2} + k_3) \times k'_{-1} k'_4 (k'_{-2} + k'_3) \times k''_{-1} k''_4 (k''_{-2} + k''_3))} \quad (S.87)$$

Equations (S.64), (S.70) and (S.78) into Eq. (S.87), as well as the identities in Eqs.(S.23), (S.24), (S.26) and (S.29) to give:

$$v_1 = \frac{[UT]_T \left( k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} - k_{catR} \frac{[PPi][PIIUMP_1]}{K_{iP_{PIIUMP_1}} K_{M_{PPi}}} \right) \times F_2 \times F_3}{D'} \quad (S.88)$$

The equivalent procedure applied to Eq. (S.80) will give:

$$v_2 = \frac{[UT]_T \left( k_{catF} \frac{[PIIUMP_1][UTP]}{K_{iS_{PIIUMP_1}} K_{M_{UTP}}} - k_{catR} \frac{[PPi][PIIUMP_2]}{K_{iP_{PIIUMP_2}} K_{M_{PPi}}} \right) \times F_1 \times F_3}{D'} \quad (S.89)$$

and applied to Eq. (S.81) will give:

$$v_3 = \frac{[UT]_T \left( k_{catF} \frac{[PIIUMP_2][UTP]}{K_{iS_{PIIUMP_2}} K_{M_{UTP}}} - k_{catR} \frac{[PPi][PIIUMP_3]}{K_{iP_{PIIUMP_3}} K_{M_{PPi}}} \right) \times F_1 \times F_2}{D'} \quad (S.90)$$

When inhibition by glutamine is considered, with the assumption that it is able to bind to any of the ten forms of the enzyme considered in the mechanism with equal affinity, a term is added to the denominators of these three velocity equations, resulting Eqs. (2a), (2b) and (2c).

### 2.5.2 Correlations between constants for PII forms

The constants used in the final form of the kinetic equations are in fact combinations of the fundamental constants shown in Figure 1. In order to establish correlations between the final constants, correlations between the fundamental constants are established first and then converted into the correlations between the final constants. We assume that the binding between the enzyme and a trimer of PII happens mainly through interactions to the PII subunit whose

T-loop enters the active site. Whatever the effectors bound or the uridylylation status of the two other “secondary” subunits, they are assumed not to affect the binding.

The first step in the mechanism (Figure 1) for the first reaction is the binding of PII to the enzyme. Since the only subunit whose state matters is the “binding subunit”, it is useful to visualize the binding of that subunit alone, while disregarding the “secondary subunits” (Figure S1). These two ways of visualization, of the whole trimer and of only the binding subunit, are also applied to the binding of PIIUMP<sub>1</sub> (Figure S2) and PIIUMP<sub>2</sub> (Figure S3) to UTase, the first steps of the second and third reactions, respectively. The binding always occurs through a non-uridylylated subunit. The proportionalities between the concentration of each of these trimers, when the trimer visualization is used, and the concentration of non-uridylylated subunits, when the subunit visualization is used, are given in Equations (S.91), (S.92) and (S.93).

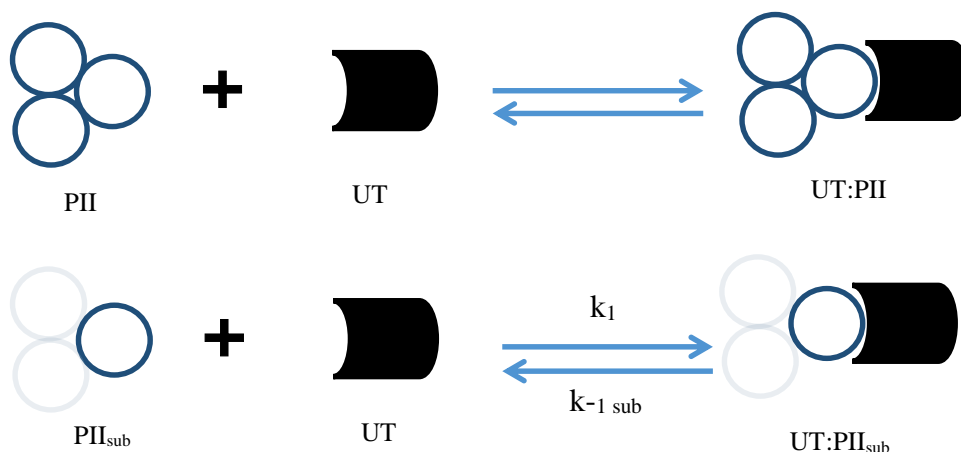


Figure S3 – Binding of PII to UTase, visualization as the trimer binding (above) and as the subunit binding (below).

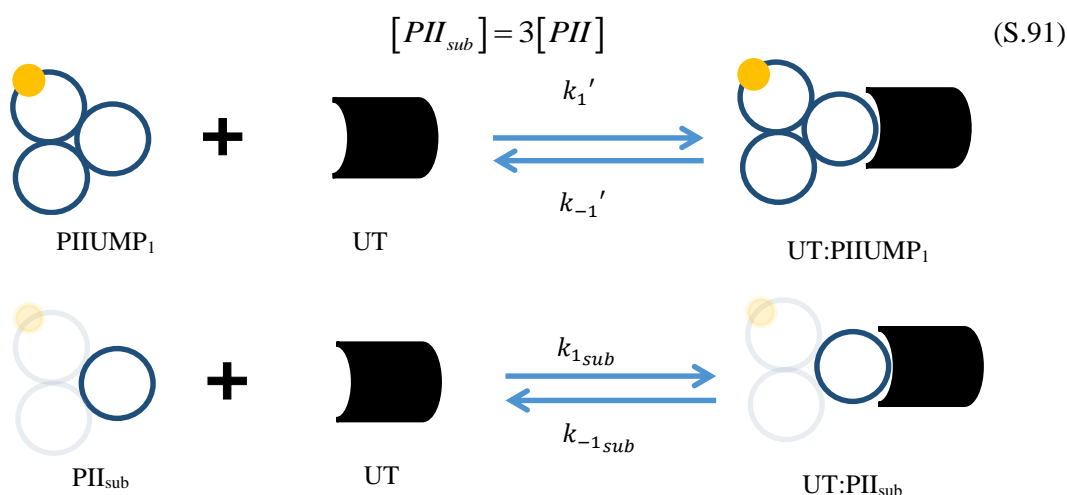


Figure S4 – Binding of PIIUMP<sub>1</sub> to UTase, visualization as the trimer binding (above) and as a non-uridylylated subunit binding (below).

$$[PII_{sub}] = 2[PIIUMP_1] \quad (S.92)$$

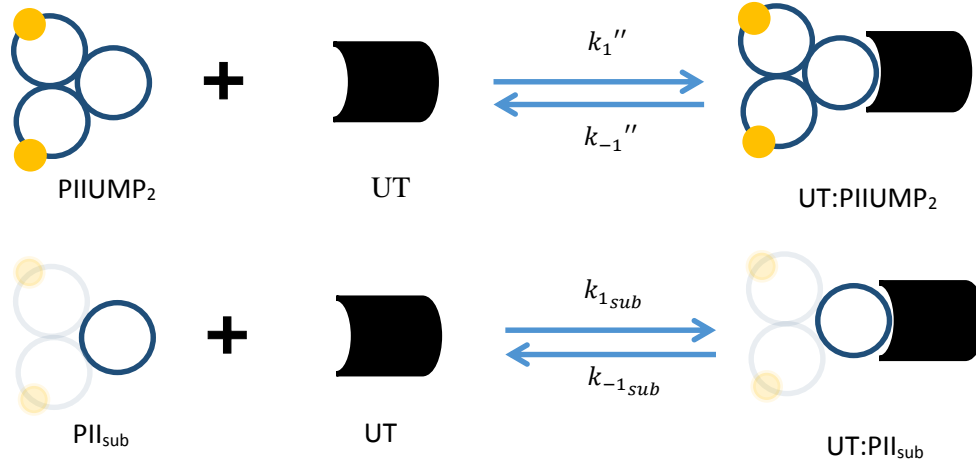


Figure S5 – Binding of PIIUMP<sub>2</sub> to UTase, visualization as the trimer binding (above) and as a non-uridylylated subunit binding (below).

$$[PII_{sub}] = [PIIUMP_2] \quad (S.93)$$

The velocity of the binding of the binding reactions are written, from the perspective of the trimer as well as of the binding subunit, for the first step of the first reaction:

$$V_1 = k_1 [PII][UT] \quad (S.94)$$

$$V_1 = k_{1sub} [PII_{sub}][UT] \quad (S.95)$$

for the first step of the second reaction:

$$V_1' = k_1' [PIIUMP_1][UT] \quad (S.96)$$

$$V_1' = k_{1sub} [PII_{sub}][UT] \quad (S.97)$$

and for the first step of the third reaction:

$$V_1'' = k_1'' [PIIUMP_2][UT] \quad (S.98)$$

$$V_1'' = k_{1sub} [PII_{sub}][UT] \quad (S.99)$$

Since what changes is only the perspective of visualization, the velocity of the binding reaction  $V_1$  is the same despite the perspective, Equations (S.94) and (S.95) can be rewritten as:

$$k_1 [PII][UT] = k_{1sub} [PII_{sub}][UT] \quad (S.100)$$

Equations (S.96) and (S.97) can be rewritten to:

$$k_1' [PIIUMP_1][UT] = k_{1sub} [PII_{sub}][UT] \quad (S.101)$$

and Equations (S.98) and (S.99) can be rewritten to:

$$k_1'' [PIIUMP_2][UT] = k_{1sub} [PII_{sub}][UT] \quad (S.102)$$

Each of the proportions from Equations (S.91), (S.92) and (S.93) is substituted Equations (S.100), (S.101) and (S.102), respectively:

$$k_1 [PII][UT] = k_{1sub} 3 [PII][UT] \quad (S.103)$$

$$k'_1 [PIIUMP_1][UT] = k_{1sub} 2 [PIIUMP_1][UT] \quad (S.104)$$

$$k''_1 [PIIUMP_2][UT] = k_{1sub} [PIIUMP_2][UT] \quad (S.105)$$

Which result in the correlations between the fundamental constants for each trimer and for the non-uridylylated subunit:

$$k_1 = 3k_{1sub} \quad (S.106)$$

$$k'_1 = 2k_{1sub} \quad (S.107)$$

$$k''_1 = k_{1sub} \quad (S.108)$$

The velocity of the dissociation reactions in Figures S1, S2 and S3 are given by:

$$V_{-1} = k_{-1} [UT : PII] \quad (S.109)$$

$$V'_{-1} = k'_{-1} [UT : PIIUMP_1] \quad (S.110)$$

$$V''_{-1} = k''_{-1} [UT : PIIUMP_2] \quad (S.111)$$

Which can be written from the perspective of the binding subunit as well:

$$V_{-1} = k_{-1sub} [UT : PII_{sub}] \quad (S.112)$$

$$V'_{-1} = k'_{-1sub} [UT : PII_{sub}] \quad (S.113)$$

$$V''_{-1} = k''_{-1sub} [UT : PII_{sub}] \quad (S.114)$$

By substituting Equations (S.109), (S.110) and (S.111) into Equations (S.112), (S.113) and (S.114), we obtain:

$$k_{-1} [UT : PII] = k_{-1sub} [UT : PII_{sub}] \quad (S.115)$$

$$k'_{-1} [UT : PIIUMP_1] = k_{-1sub} [UT : PII_{sub}] \quad (S.116)$$

$$k''_{-1} [UT : PIIUMP_2] = k_{-1sub} [UT : PII_{sub}] \quad (S.117)$$

Since the variation in perspective does not change the concentration of the complex, the concentrations cancel out to give:

$$k_{-1} = k_{-1sub} \quad (S.118)$$

$$k'_{-1} = k_{-1sub} \quad (S.119)$$

$$k''_{-1} = k_{-1sub} \quad (S.120)$$

The second step in each of the uridylylation reactions is the binding of UTP to the complex between enzyme either PII, PIIUMP<sub>1</sub> or PIIUMP<sub>2</sub>. In all these three complexes the binding is through a non-uridylylated subunit. From the assumption that the state of the two remaining “secondary subunits” does not affect the interaction of the trimer and the enzyme, all three of these complexes should interact identically with UTP in this second step. Therefore, the fundamental constants for the binding of UTP are equal for the three uridylylation reactions:

$$k_2 = k'_2 = k''_2 \quad (\text{S.121})$$

as are the constants for the reverse reaction of the second step, the dissociation of UTP:

$$k_{-2} = k'_{-2} = k''_{-2} \quad (\text{S.122})$$

The third step in the mechanism is the dissociation of PP<sub>i</sub> from the ternary complex, after catalysis. The interaction between enzyme and either PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub> is now through a uridylylated subunit, and since we assume it is not affected by the two other subunits, the interaction with the dissociating PP<sub>i</sub> molecule is identical in all three complexes. Thus the constants the dissociation of PP<sub>i</sub> are equal for the three uridylylation reactions:

$$k_3 = k'_3 = k''_3 \quad (\text{S.123})$$

as are the constants for the reverse reaction, of binding of PP<sub>i</sub>:

$$k_{-3} = k'_{-3} = k''_{-3} \quad (\text{S.124})$$

The fourth and last step in the mechanism is the dissociation of the product from the enzyme, PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> or PIIUMP<sub>3</sub>, depending on the reaction. The reverse is the binding of these forms of uridylylated PII to the enzyme. The interaction between PII and the enzyme in these cases is through a uridylylated subunit. Similarly to the first step, the analysis of this step through the visualization in the perspective of the trimer and in the perspective of the binding subunit is important for obtaining the correlations and is shown in Figures S4, S5 and S6.



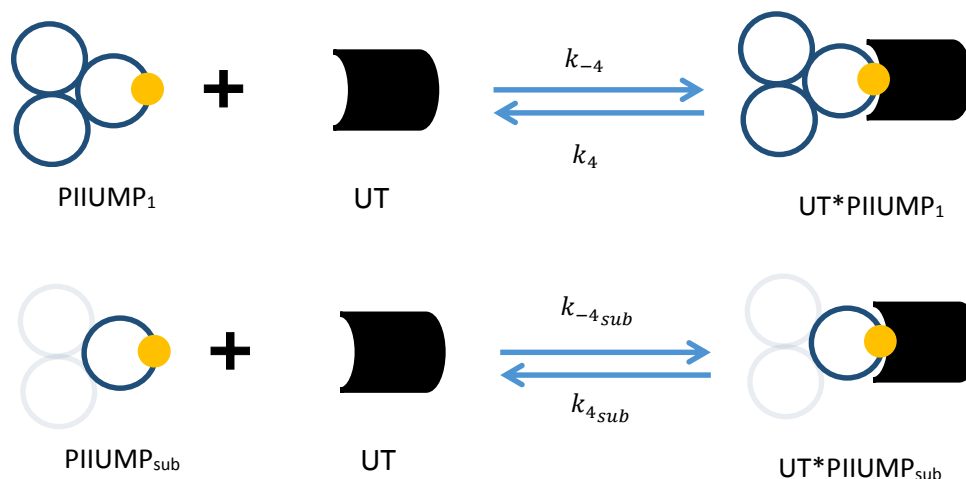


Figure S6 – Binding and dissociation of PIIUMP<sub>1</sub> to UTase, from the perspective of the trimer (above) and of the binding subunit (below).

The proportion between the concentration of the trimer and of the subunit and between the concentration of the complex in the perspective of trimer and of subunit are given in Equations (S.125) to (S.130).

$$[\text{PIIUMP}_1] = [\text{PIIUMP}_{\text{sub}}] \quad (\text{S.125})$$

$$[\text{UT} * \text{PIIUMP}_1] = [\text{UT} * \text{PIIUMP}_{\text{sub}}] \quad (\text{S.126})$$

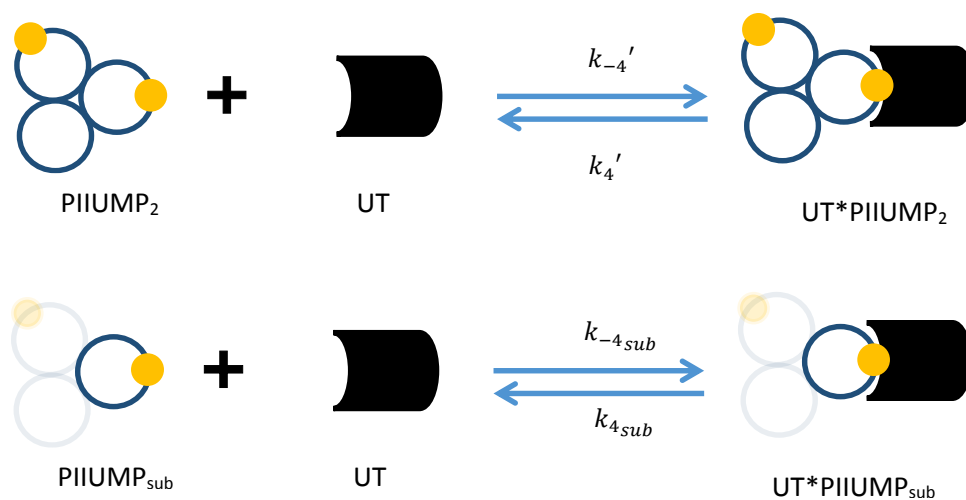


Figure S7 – Binding and dissociation of PIIUMP<sub>2</sub> to UTase, from the perspective of the trimer (above) and of the binding subunit (below).

$$[\text{PIIUMP}_{\text{sub}}] = 2[\text{PIIUMP}_2] \quad (\text{S.127})$$

$$[\text{UT} * \text{PIIUMP}_2] = [\text{UT} * \text{PIIUMP}_{\text{sub}}] \quad (\text{S.128})$$

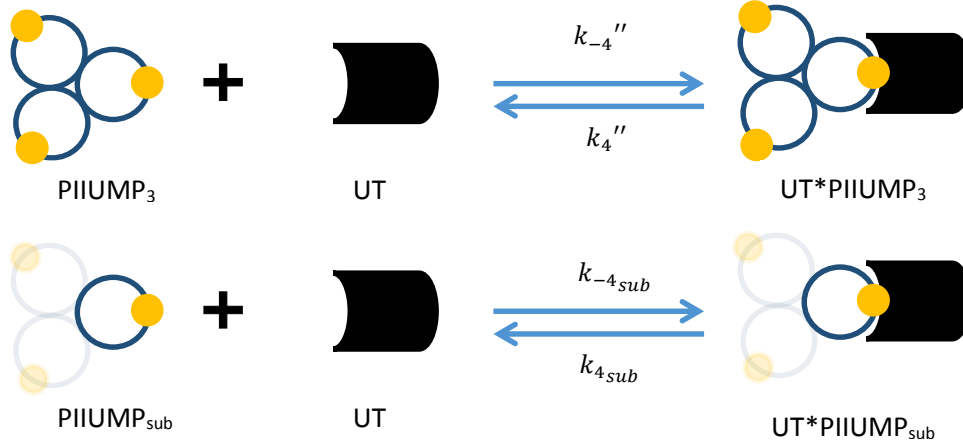


Figure S8 – Binding and dissociation of PIIUMP<sub>3</sub> from UTase, from the perspective of the trimer (above) and of the binding subunit (below).

$$[PIIUMP_{sub}] = 3[PIIUMP_3] \quad (S.129)$$

$$[UT * PIIUMP_3] = [UT * PIIUMP_{sub}] \quad (S.130)$$

The velocity of dissociation of PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub> can be written from the perspective of the trimer as:

$$V_4 = k_4 [UT * PIIUMP_1] \quad (S.131)$$

$$V'_4 = k'_4 [UT * PIIUMP_2] \quad (S.132)$$

$$V''_4 = k''_4 [UT * PIIUMP_3] \quad (S.133)$$

and from the perspective of the subunit as:

$$V_4 = k_{4sub} [UT * PIIUMP_{sub}] \quad (S.134)$$

$$V'_4 = k_{4sub} [UT * PIIUMP_{sub}] \quad (S.135)$$

$$V''_4 = k_{4sub} [UT * PIIUMP_{sub}] \quad (S.136)$$

One of the two equations for each velocity can be substituted into the other to produce:

$$k_4 [UT * PIIUMP_1] = k_{4sub} [UT * PIIUMP_{sub}] \quad (S.137)$$

$$k'_4 [UT * PIIUMP_2] = k_{4sub} [UT * PIIUMP_{sub}] \quad (S.138)$$

$$k''_4 [UT * PIIUMP_3] = k_{4sub} [UT * PIIUMP_{sub}] \quad (S.139)$$

Equations (S.126), (S.128) and (S.130) are substituted into Equations (S.137), (S.138) and (S.139), respectively:

$$k_4 [UT * PIIUMP_1] = k_{4sub} [UT * PIIUMP_1] \quad (S.140)$$

$$k'_4 [UT * PIIUMP_2] = k_{4sub} [UT * PIIUMP_2] \quad (S.141)$$

$$k''_4 [UT * PIIUMP_3] = k_{4sub} [UT * PIIUMP_3] \quad (S.142)$$

The concentrations of the complexes cancel out to give:

$$k_4 = k_{4sub} \quad (S.143)$$

$$k'_4 = k_{4sub} \quad (S.144)$$

$$k''_4 = k_{4sub} \quad (S.145)$$

The velocities of the reverse fourth step in the mechanism, the binding of PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> or PIIUMP<sub>3</sub> to the enzyme through a uridylylated subunit, are given in the perspective of the trimer by:

$$V_{-4} = k_{-4} [UT][PIIUMP_1] \quad (S.146)$$

$$V'_{-4} = k'_{-4} [UT][PIIUMP_2] \quad (S.147)$$

$$V''_{-4} = k''_{-4} [UT][PIIUMP_3] \quad (S.148)$$

and in the perspective of the binding subunit by:

$$V_{-4} = k_{-4sub} [UT][PIIUMP_{sub}] \quad (S.149)$$

$$V'_{-4} = k_{-4sub} [UT][PIIUMP_{sub}] \quad (S.150)$$

$$V''_{-4} = k_{-4sub} [UT][PIIUMP_{sub}] \quad (S.151)$$

The substitution of Eqs. (S.146), (S.147) and (S.148) into Eqs. (S.149), (S.150) and (S.151) result in:

$$k_{-4} [UT][PIIUMP_1] = k_{-4sub} [UT][PIIUMP_{sub}] \quad (S.152)$$

$$k'_{-4} [UT][PIIUMP_2] = k_{-4sub} [UT][PIIUMP_{sub}] \quad (S.153)$$

$$k''_{-4} [UT][PIIUMP_3] = k_{-4sub} [UT][PIIUMP_{sub}] \quad (S.154)$$

Equations (S.125), (S.127) and (S.129) are substituted into Eqs. (S.152), (S.153) and (S.154), respectively, to produce:

$$k_{-4} [UT][PIIUMP_1] = k_{-4sub} [UT][PIIUMP_1] \quad (S.155)$$

$$k'_{-4} [UT][PIIUMP_2] = 2k_{-4sub} [UT][PIIUMP_2] \quad (S.156)$$

$$k''_{-4} [UT][PIIUMP_3] = 3k_{-4sub} [UT][PIIUMP_3] \quad (S.157)$$

The concentrations of enzyme and trimer cancel out to give:

$$k_{-4} = k_{-4sub} \quad (S.158)$$

$$k'_{-4} = 2k_{-4sub} \quad (S.159)$$

$$k''_{-4} = 3k_{-4sub} \quad (S.160)$$

The fundamental constants for the second and third uridylylation reactions can be written in function of the constants for the first reaction. From Eqs. (S.106) to (S.108):

$$k'_1 = \frac{2}{3} k_1 \quad (S.161)$$

$$k''_1 = \frac{1}{3} k_1 \quad (S.162)$$

From Eqs. (S.118) to(S.120):

$$k'_{-1} = k_{-1} \quad (S.163)$$

$$k''_{-1} = k_{-1} \quad (S.164)$$

From Eq. (S.121):

$$k'_{-2} = k_2 \quad (S.165)$$

$$k''_{-2} = k_2 \quad (S.166)$$

From Eq.(S.122):

$$k'_{-2} = k_{-2} \quad (S.167)$$

$$k''_{-2} = k_{-2} \quad (S.168)$$

From Eq.(S.123):

$$k'_3 = k_3 \quad (S.169)$$

$$k''_3 = k_3 \quad (\text{S.170})$$

From Eq.(S.124):

$$k'_{-3} = k_{-3} \quad (\text{S.171})$$

$$k''_{-3} = k_{-3} \quad (\text{S.172})$$

From Eqs. (S.143) to (S.145):

$$k'_4 = k_4 \quad (\text{S.173})$$

$$k''_4 = k_4 \quad (\text{S.174})$$

From Eqs. (S.158) to (S.160):

$$k'_{-4} = 2k_{-4} \quad (\text{S.175})$$

$$k''_{-4} = 3k_{-4} \quad (\text{S.176})$$

The correlations from the fundamental constants from Eqs. (S.161) to (S.176) can be substituted into the identities for the final constants for the second and third reactions from Eqs. (S.32) to (S.51). The constants for UTP and PPI<sub>i</sub>, as well as both the forward and reverse  $k_{cat}$ , are equal for the three uridylylation reactions:

$$K_{M_{UTP}} = \frac{k_4(k_{-2} + k_3)}{k_2(k_3 + k_4)} = \frac{k'_4(k'_{-2} + k'_3)}{k'_2(k'_3 + k'_4)} = \frac{k''_4(k''_{-2} + k''_3)}{k''_2(k''_3 + k''_4)} \quad (\text{S.177})$$

$$K_{I_{UTP}} = \frac{k_{-1} + k_{-2}}{k_2} = \frac{k'_{-1} + k'_{-2}}{k'_2} = \frac{k''_{-1} + k''_{-2}}{k''_2} \quad (\text{S.178})$$

$$K_{M_{PPI}} = \frac{k_{-1}(k_{-2} + k_3)}{k_{-3}(k_{-1} + k_{-2})} = \frac{k'_{-1}(k'_{-2} + k'_3)}{k'_{-3}(k'_{-1} + k'_{-2})} = \frac{k''_{-1}(k''_{-2} + k''_3)}{k''_{-3}(k''_{-1} + k''_{-2})} \quad (\text{S.179})$$

$$K_{I_{PPI}} = \frac{k_3 + k_4}{k_{-3}} = \frac{k'_3 + k'_4}{k'_{-3}} = \frac{k''_3 + k''_4}{k''_{-3}} \quad (\text{S.180})$$

$$k_{cat_F} = \frac{k_3 k_4}{k_3 + k_4} = \frac{k'_3 k'_4}{k'_3 + k'_4} = \frac{k''_3 k''_4}{k''_3 + k''_4} \quad (\text{S.181})$$

$$k_{cat_R} = \frac{k_{-1} k_{-2}}{k_{-1} + k_{-2}} = \frac{k'_{-1} k'_{-2}}{k'_{-1} + k'_{-2}} = \frac{k''_{-1} k''_{-2}}{k''_{-1} + k''_{-2}} \quad (\text{S.182})$$

The constants for the PII substrate and product of the second uridylylation reaction are:

$$K_{MS_{PIUMR_1}} = \frac{k_3 k_4}{\frac{2}{3} k_1 (k_3 + k_4)} \quad (\text{S.183})$$

$$K_{IS_{PIUMR_1}} = \frac{k_{-1}}{\frac{2}{3} k_1} \quad (\text{S.184})$$

$$K_{MP_{PIUMP_2}} = \frac{k_{-1} k_{-2}}{2k_{-4} (k_{-1} + k_{-2})} \quad (\text{S.185})$$

$$K_{IP_{PIUMP_2}} = \frac{k_4}{2k_{-4}} \quad (\text{S.186})$$

The constants for the PII substrate and product of the third uridylylation reaction are:

$$K_{MS_{PIUMP_2}} = \frac{k_3 k_4}{\frac{1}{3} k_1 (k_3 + k_4)} \quad (\text{S.187})$$

$$K_{IS_{PIUMP_2}} = \frac{k_{-1}}{\frac{1}{3} k_1} \quad (\text{S.188})$$

$$K_{MP_{PIUMP_3}} = \frac{k_{-1} k_{-2}}{3k_{-4} (k_{-1} + k_{-2})} \quad (\text{S.189})$$

$$K_{IP_{PIUMP_3}} = \frac{k_{-4}''}{k_{-4}''} \quad (\text{S.190})$$

These give the correlations presented in the main body of the article:

$$K_{MS_{PIUMR_1}} = \frac{3}{2} K_{MS_{PII}} \quad (5a)$$

$$K_{MS_{PIUMP_2}} = 3K_{MS_{PII}} \quad (5b)$$

$$K_{iS_{PIIUMP}} = \frac{3}{2} K_{iS_{PII}} \quad (6a)$$

$$K_{iS_{PIUMP_2}} = 3K_{iS_{PII}} \quad (6b)$$

$$K_{MP_{PIUMP_2}} = \frac{1}{2} K_{MP_{PIUMR_1}} \quad (7a)$$

$$K_{MP_{PIUMP_3}} = \frac{1}{3} K_{MP_{PIUMP_1}} \quad (7b)$$

$$K_{iP_{PIUMP_2}} = \frac{1}{2} K_{iP_{PIUMP_1}} \quad (8a)$$

$$K_{iP_{PIUMP_3}} = \frac{1}{3} K_{iP_{PIUMP_1}} \quad (8b)$$

### 2.5.3 Deduction of product inhibition patterns

In product inhibition studies, various sets of assays are done. In each set, the concentration of one of the substrates is varied (the “variable substrate”) while the concentration of the other is held constant (the “fixed substrate”). In each set of assays, only one of the products is added; the concentration of the other product is zero and all terms that contain it disappear from the equation. After this simplified equation is obtained, the type of product inhibition to be expected in the set of assays can be inferred from the terms that remain the denominator: if the concentration of the added product appears in terms that also contain the concentration of the variable substrate, the inhibition is uncompetitive; if it appears in terms that do not contain the concentration of the variable substrate, the inhibition is competitive; if it appears in both types of terms, the inhibition is mixed; finally, if it does not appear in any term, then there is no inhibition. This procedure is done for each possible combination of variable substrate, fixed substrate and added product; for a reaction involving two substrates and two products, there are eight such combinations. The whole procedure is repeated, but this time assuming that the fixed substrate is present at such a high concentration (denominated “saturating concentration”) that those terms in the denominator that do not contain the concentration of the fixed substrate are negligible and can be cut from the denominator. This may change the type of inhibition that the added product is predicted to cause for the variable substrate. These analyses give a “product inhibition pattern”.

#### 2.5.3.1 Product inhibition patterns predicted by the model of Bruggeman *et al.* (2005)

This section explains the deductions of the product inhibition patterns that are given in Table 2 of the main article for the model of UTase developed by Bruggeman *et al.* (2005).

The substrates of the first uridylylation loop, namely non-uridylylated PII and UTP, are always present in the assays. All these experiments were undertaken without added GLN, such that the term  $(1+[GLN]/K_{GLN}) = 1$ . When  $PP_i$  is added to be tested as an inhibitor,  $PIIUMP_1$ ,  $PIIUMP_2$  and  $PIIUMP_3$  are not present, so any terms with the concentrations of these species, in gray in Eq.(S.191), are null:

$$v_1 = \frac{[UT]_T k_{catF} [PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \left( 1 + \frac{[GLN]}{K_{GLN}} \right) \left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PII}} [PIIUMP_1]}{K_{iS_{PII}} K_{i_{P_{PIIUMR_1}}}} + \frac{[PIIUMP_1]}{K_{iS_{PII}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_1][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PII}} [PIIUMP_2]}{K_{iS_{PII}} K_{i_{P_{PIIUMR_2}}}} + \frac{[PIIUMP_2]}{K_{iS_{PII}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_2][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PP_i}}} + \frac{K_{MS_{PII}} [PIIUMP_3]}{K_{iS_{PII}} K_{i_{P_{PIIUMR_3}}}} \right) \quad (S.191)$$

When these terms are removed, the result is Eq. (10a).

#### 2.5.3.1.1 Product inhibition of the first uridylylation reaction when $PP_i$ is added as the inhibitor

When PII is the variable substrate and UTP is added at a fixed, non-saturating concentration, there are three terms in the denominator containing the added product  $PP_i$ . This is shown in Eq.(S.192), in which  $PP_i$  is in red and PII, whenever in the same term as  $PP_i$ , is shown in blue:



$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{K_{MP_{PIIUM}} [PPi]}{K_{iP_{PIIUM}} K_{iPPi}} + \frac{[PII][PPi]}{K_{iS_{PII}} K_{M_{PPi}}} + \frac{K_{MP_{PIIUM}} [PPi]}{K_{iP_{PIIUM}} K_{iPPi}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPPi}} \right)} \quad (S.192)$$

Of the three terms that contain [PPi], two also contain the concentration of the variable substrate, PII, which means there is uncompetitive inhibition. The other PPi-containing term does not have PII concentration, meaning there is competitive inhibition. Since both types of inhibition are present, there is mixed inhibition.

When PII is the variable substrate and UTP the fixed “saturating” substrate, the UTP concentration is high enough that all terms that do not contain the UTP concentration are negligible and are removed from the equation. The equation without these terms, showing PPi in red and PII in blue, is:

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPPi}} \right)} \quad (S.193)$$

Since the only term containing PPi concentration also contains PII concentration, the predicted inhibition is uncompetitive.

The next two sets of assays have UTP as the variable substrate and PII as the fixed substrate. For the set of assays in which the PII concentration is not “saturating”, the following equation holds:

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{K_{MP_{PIIUMP}} [PPi]}{K_{iP_{PIIUMP}} K_{iPPi}} + \frac{[PII][PPi]}{K_{iS_{PII}} K_{M_{PPi}}} \frac{K_{MP_{PIIUMP}}}{K_{iP_{PIIUMP}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPPi}} \right)} \quad (S.194)$$

There are two terms that contain the PP<sub>i</sub> concentration but that do not contain the UTP concentration, meaning there is competitive inhibition. There is also a term with both PP<sub>i</sub> and UTP concentration, so there is also uncompetitive inhibition. Since both types of inhibition are present, the predicted inhibition is mixed.

For the set of assays in which PII concentration is “saturating”, all terms not containing PII concentration are negligible and are removed from Eq. (S.194), giving:

$$v_1 = \frac{[UT]_T k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}}}{\left( \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][PPi]}{K_{iS_{PII}} K_{M_{PPi}}} \frac{K_{MP_{PIIUMP}}}{K_{iP_{PIIUMP}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPPi}} \right)} \quad (S.195)$$

Of the two terms containing PP<sub>i</sub> concentration in the denominator of this equation, one also has UTP concentration, while the other does not. This corresponds to mixed inhibition, which is unchanged from the situation in which the PII concentration was not “saturating”.

#### 2.5.3.1.2 Product inhibition of the first uridylylation reaction when PIIUMP<sub>3</sub> is added as the inhibitor

The other product that Jiang *et al.* (1998) tested as an inhibitor of the first uridylylation reaction was PIIUMP<sub>3</sub>. The molecular species PP<sub>i</sub>, PIIUMP<sub>1</sub> and PIIUMP<sub>2</sub> were not added to this set of assays. Since their concentrations were null, any terms containing the concentrations of these species are negligible. These concentrations are in gray in Eq. (S.196):

$$v_1 = \frac{[UT]_T k_{cat+} [PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \left( 1 + \frac{[GLN]}{K_{GLN}} \right) \left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPP_i}} + \frac{K_{MS_{PII}} [PIIUMP_1]}{K_{iS_{PII}} K_{iPP_{PIIUM_1}}} + \frac{[PIIUMP_1]}{K_{iS_{PII}}} + \frac{[PIIUMP_1][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_1][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPP_i}} + \frac{K_{MS_{PII}} [PIIUMP_2]}{K_{iS_{PII}} K_{iPP_{PIIUM_2}}} + \frac{[PIIUMP_2]}{K_{iS_{PII}}} + \frac{[PIIUMP_2][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_2][UTP][PP_i]}{K_{iS_{PII}} K_{M_{UTP}} K_{iPP_i}} + \frac{K_{MS_{PII}} [PIIUMP_3]}{K_{iS_{PII}} K_{iPP_{PIIUM_3}}} \right) \quad (S.196)$$

There are two sets of assays in which PII is the variable substrate and UTP the fixed substrate. In the first set of assays, the concentration of UTP is not “saturating”. Equation (S.197) shows the concentration of PIIUMP<sub>3</sub> in red:

$$v_1 = \frac{[UT]_T k_{catF} [PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{K_{MS_{PII}} [PIIUMP_3]}{K_{iS_{PII}} K_{iPP_{PIIUM_3}}} \right) \quad (S.197)$$

Since there is only one term with the concentration of PIIUMP<sub>3</sub> and it does not contain the concentration of the variable substrate, PII, the inhibition is competitive.

In the set of assays in which the concentration of UTP is “saturating”, any terms not containing the concentration of UTP are negligible. After these terms are removed, Eq. (S.198) simplifies to:

$$v_1 = \frac{[UT]_T k_{cat+} [PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \left( \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right) \quad (S.199)$$

Since there are no terms containing the concentration of PIIUMP<sub>3</sub> in the denominator, there is no predicted inhibition.

There are two sets of assays in which UTP is the variable substrate and PII the fixed substrate. The type of inhibition for the set of assays in which the concentration of PII is not “saturating” can be predicted from Eq. (S.197). Since the term containing the concentration of PIIUMP<sub>3</sub>, does not contain the concentration of UTP, the type of inhibition is competitive.

When the concentration of PII is “saturating”, the removal of any terms not containing the concentration of PII results in:

$$v_1 = \frac{[UT_{total}]k_{cat+}[PII][UTP]}{K_{iS_{PII}}K_{M_{UTP}}} \left( \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}}K_{M_{UTP}}} \right) \quad (S.200)$$

Since there are no terms with the concentration of PIIUMP<sub>3</sub>, there is no inhibition.

### 2.5.3.2 Product inhibition patterns predicted by the model developed in the current work

This section explains the deductions of the product inhibition patterns that are given in Table 2 of the main article for the model of UTase developed in the current work. The same procedures of analysis were undertaken as those presented in the previous section for the model of Bruggeman *et al.* (2005).

For the set of assays with PII as the fixed substrate and UTP as the fixed substrate, present at a non-saturating concentration, the equation with the remaining terms is:

$$v_1 = \frac{\left( [UT_{Total}]k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}}K_{M_{UTP}}} \right)}{\left( 1 + \frac{K_{MS_{PII}}[UTP]}{K_{iS_{PII}}K_{M_{UTP}}} + \frac{K_{MP_{PIIUM\text{A}_1}}[PPi]}{K_{iP_{PIIUM\text{A}_1}}K_{i_{PPi}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}}K_{M_{UTP}}} + \frac{[PII][PPi]}{K_{iS_{PII}}K_{M_{PPi}}} + \frac{K_{MP_{PIIUM\text{A}_1}}[PPi]}{K_{iP_{PIIUM\text{A}_1}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}}K_{M_{UTP}}K_{i_{PPi}}} \right)} \quad (S.201)$$

which produces mixed inhibition. For a “saturating” concentration of UTP instead, the equation simplifies to:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( \frac{K_{MS_{PII}} [UTP] [PII]}{K_{iS_{PII}} K_{M_{UTP}} K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}} \right)} \quad (S.202)$$

which produces uncompetitive inhibition.

For  $PP_i$  tested as the inhibitor, UTP as the variable substrate and PII as the fixed substrate at a non-saturating concentration, our equation simplifies to:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{K_{MP_{PIIUM_1}} [PPi]}{K_{i_{PPi}} K_{i_{PPi}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][PPi] K_{MP_{PIIUM_1}}}{K_{iS_{PII}} K_{M_{PPi}} K_{i_{PPi}} K_{i_{PPi}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}} \right)} \quad (S.203)$$

which produces mixed inhibition. If PII is added at a saturating concentration instead, the equation further simplifies to:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][PPi] K_{MP_{PIIUM_1}}}{K_{iS_{PII}} K_{M_{PPi}} K_{i_{PPi}} K_{i_{PPi}}} + \frac{[PII][UTP][PPi]}{K_{iS_{PII}} K_{M_{UTP}} K_{i_{PPi}}} \right)} \quad (S.204)$$

which still produces mixed inhibition.

For  $PIIUM_3$  tested as the inhibitor, PII as the variable substrate and UTP as the fixed substrate at a non-saturating concentration, the equation simplifies to:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUM_3]}{K_{i_{PPi}} K_{i_{PPi}}} + \frac{[PIIUM_3][UTP] K_{MS_{PIIUM_2}}}{K_{i_{PPi}} K_{i_{PPi}} K_{M_{UTP}} K_{iS_{PIIUM_2}}} \right)} \quad (S.205)$$

which produces competitive inhibition. If UTP is added at a saturating concentration instead, the equation further simplifies to:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_3][UTP] K_{MS_{PIIUMP_2}}}{K_{iP_{PIIUMP_3}} K_{M_{UTP}} K_{iS_{PIIUMP_2}}} \right)} \quad (S.206)$$

which still produces competitive inhibition.

For PIIUMP<sub>3</sub> tested as inhibitor, UTP as the variable substrate and PII as the fixed substrate, added at a non-saturating concentration, the simplified equation is:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( 1 + \frac{K_{MS_{PII}} [UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} + \frac{[PIIUMP_3]}{K_{iP_{PIIUMP_3}}} + \frac{[PIIUMP_3][UTP] K_{MS_{PIIUMP_2}}}{K_{iP_{PIIUMP_3}} K_{M_{UTP}} K_{iS_{PIIUMP_2}}} \right)} \quad (S.207)$$

which produces mixed inhibition. If PII is added at a saturating concentration instead, the equation simplifies further to:

$$v_1 = \frac{\left( [UT_{Total}] k_{catF} \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)}{\left( \frac{[PII]}{K_{iS_{PII}}} + \frac{[PII][UTP]}{K_{iS_{PII}} K_{M_{UTP}}} \right)} \quad (S.208)$$

which produces no inhibition.

### 3 ARTIGO 2: A Unified Model of *E. coli* Uridylyltransferase and PII effector binding

#### 3.1 INTRODUCTON

Several mathematical models have been proposed to describe the *Escherichia coli* ammonia assimilation system and its regulation, either entirely or in part (Mutalik *et al.*, 2003; Bruggeman *et al.*, 2005; Kurata *et al.*, 2005; Lodeiro and Melgarejo, 2008; Ma *et al.*, 2009; Kidd and Wingreen, 2010; ARTIGO 1). Up until recently, the model to best describe the activity of uridylyltransferase (UTase) was the one by Bruggeman *et al.* (2005). It has a kinetic equation for each of the three uridylylation reactions catalyzed by UTase and these equations take into account product inhibition by pyrophosphate and by the uridylylated forms of PII. However, they do not correctly predict the product inhibition pattern for UTase (Jiang *et al.* 1998), as shown in our previous paper (Mallmann *et al.* 2015). In that same paper, we have also proposed a set of kinetic equations for UTase that do predict the correct product inhibition pattern. However, neither our model nor the model of Bruggeman *et al.* (2005) take into account that the uridylylation of a PII trimer by UTase is affected by the binding of allosteric effectors to PII.

PII has three sites for the binding of either ATP or ADP, each located in a cleft where two of the subunits interact. Next to each of the ATP/ADP binding sites is located a site for the binding of 2-OG. A 2-OG molecule is only able to bind to its site if there is an ATP molecule bound to the neighboring ATP/ADP site (Fokina *et al.*, 2010), and the binding of the second and third 2-OG molecules to the same PII trimer present strong negative cooperativity (Kamberov *et al.*, 1995; Jiang, Ninfa, 2007). PII can only be uridylylated if ATP and 2-OG are bound to it (Jiang *et al.* 1998). The previous kinetic models for UTase ignore this, and treat all PII as uridylylatable. That is effectively the same as assuming that PII is fully bound with 2-OG. Since 2-OG binds to PII with strong negative cooperativity (the  $K_M$  for the binding of the third 2-OG may be as high as 6000  $\mu\text{M}$ ), PII fully bound with 2-OG will only be possible at the end of the physiological range of 2-OG concentration, which varies from 1 mM to 10 mM (Ref), the previous models for UTase have their application limited to very high 2-OG concentrations. Since PII uridylylation is affected by 2-OG binding, which in turn is affected by ATP/ADP binding, a model for PII uridylylation should take the binding of these small-molecule effectors to PII into account.

Of the models that describe the binding of small-molecule effectors to PII (Jiang, Ninfa, 2007; Bruggeman *et al.*, 2005; Rocha *et al.*, 2013) only that of Rocha *et al.* (2013) describes

the full range of PII ligation states when ATP, ADP and 2-OG are present simultaneously. The Rocha model considers 20 possible states for PII. These states take into account the competitive binding of ATP and ADP, and also the binding of 2-OG to ATP-bound PII subunits only. The Rocha model considers negative cooperativity for the binding of additional ATP molecules per trimer, no cooperativity for the binding of ADP, and strong negative cooperativity for the binding of additional 2-OG molecules per trimer. An interesting assumption of the Rocha model is that the binding of 2-OG, mandatorily to an ATP-bound subunit, traps the bound ATP molecule in its site. Therefore, the 2-OG must dissociate before the ATP in that same subunit is able to. This would provide an explanation that had not been suggested previously for the fact that in the presence of 2-OG there is more ATP bound to PII (Rocha *et al.*, 2013).

In order to obtain a model that is able to correctly describe the uridylylation of PII in various concentrations of ADP, ATP and 2-OG, in this paper the uridylylation model of Mallmann *et al.* (previous work) and the PII effector-binding model of Rocha *et al.* (2013) are joined into a unified model for the PII signal transduction protein and its uridylylation.

### 3.2 MODEL DEVELOPMENT

The model is based on 5 key assumptions. First, the binding of the effectors ADP, ATP and 2-OG to PII subunits follows model 4 of Rocha *et al.* (2013). Second, the uridylylation state of the trimer does not affect the dissociation constants associated with the binding of these effectors. Third, the UTase activity can only uridylylate or deuridylylate a subunit of PII that has a bound 2-OG (Jiang *et al.*, 1998). Fourth, the catalytic constants related to the uridylylation of a subunit are not affected by the uridylylation states of the other subunits (Mallmann *et al.* 2015). Fifth, after the T-loop of a subunit is uridylylated, it is possible for 2-OG to dissociate from that subunit.

Based on these assumptions, Figure 1 shows the various uridylylation reactions that are possible with PII. In this figure, the PII trimers in any particular row have the same number of bound 2-OG molecules. Likewise, the PII trimers in any particular column have the same “uridylylation state” (i.e. the same number of uridylylated subunits). The horizontal arrows represent uridylylation reactions catalyzed by UTase, while vertical bidirectional arrows represent reversible, non-covalent binding of 2-OG. Importantly, each PII trimer shown in



Figure 1 represents what we will refer to as a superstate. Each superstate represents a pool of substates with a common number of uridylylated subunits and also a common number of bound 2-OG molecules (i.e. PII:G<sub>1</sub> is the superstate that represents all substates with no uridylylated subunits and with one 2-OG molecule bound, whereas PIIUMP<sub>2</sub>:G<sub>3</sub> represents all substates with two uridylylated subunits and with three 2-OG molecules bound). Also, the substates comprised in a single superstate have different numbers of bound ATP and ADP molecules and the 2-OG molecules can be bound to different subunits of the trimer (Section 1.1 of the Supplementary Material). Subunits with a bound 2-OG always have a bound ATP, but subunits without a bound 2-OG could have a bound ATP, a bound ADP, or no bound effector. In this manner, each column in Figure 1 represents a combination of many substates in equilibrium. Since uridylylation is assumed not to affect the binding of the effectors, model 4 of Rocha *et al.* (2013) describes the relative concentrations of these substates in each column.

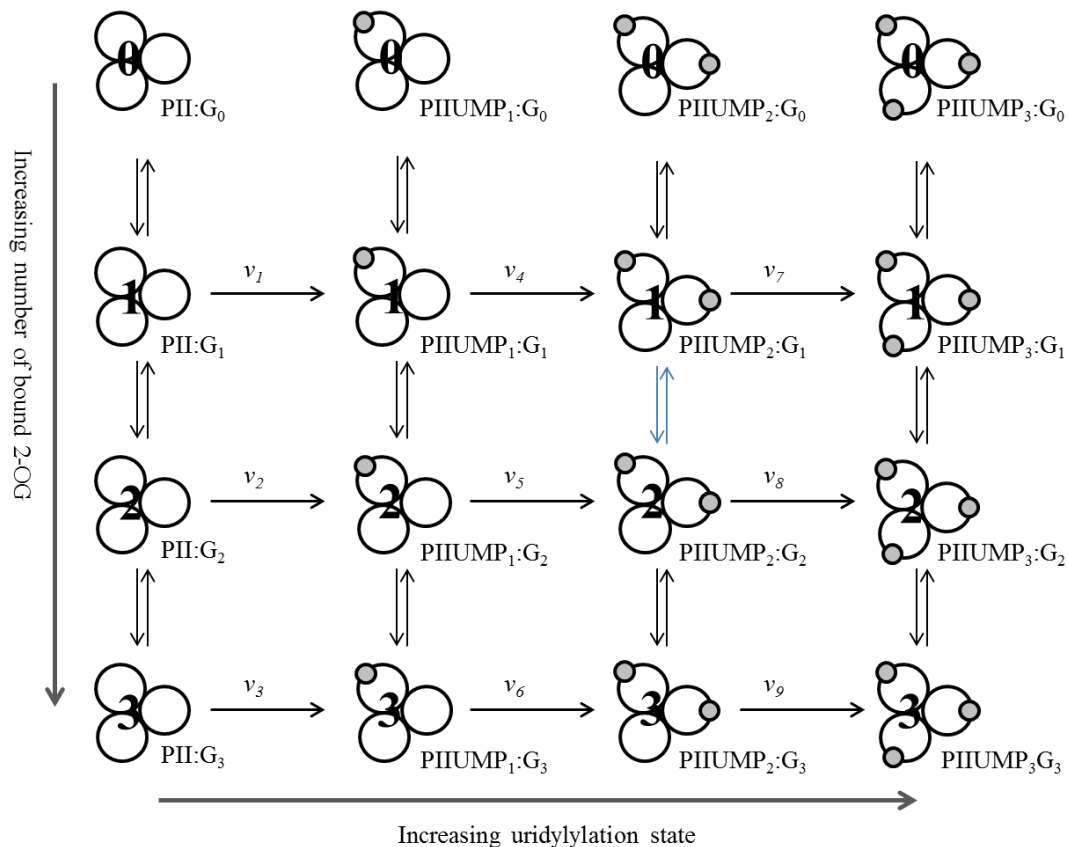


Figure 1 – Scheme for the uridylylation of PII. The vertical arrows show association (down arrows) and dissociation (up arrows) of 2-oxoglutarate and PII. The number of bound 2-OG is indicated by the number in the center of the trimer. Given that the model allows 2-OG to dissociate from a subunit once it is uridylylated, these 2-OG molecules could be bound to any of the subunits. The horizontal arrows show uridylylation of PII. The added UMP groups are shown as small gray circles

The model of Mallmann *et al.* (2015) only described the three reactions in the bottom row of Figure 1, namely the sequence  $\text{PII:G}_3 \rightarrow \text{PIIUMP}_1:\text{G}_3 \rightarrow \text{PIIUMP}_2:\text{G}_3 \rightarrow \text{PIIUMP}_3:\text{G}_3$ . The existence of more uridylylation reactions in the scheme shown in Figure 1 has two consequences. Firstly, since a velocity equation needs to be written for each reaction, the total number of velocity equations must increase from 3 to 9. Secondly, each possible reaction increases the number of terms in the denominator, which is common to all velocity equations. In the numerator,  $k_{catF}$  (for the forward reaction) and  $k_{catR}$  (for the reverse reaction) are assumed to be unaffected by the uridylylation state, so the same symbols are used for all nine reactions.

Application of the King-Altman (King and Altman, 1956; Cornish-Bowden, 2012) approach leads to the following equations for the nine uridylylation reactions:

$$v_1 = \left( [UT]_T k_{catF} \frac{[PII:G_1][UTP]}{K_{iS_{PII:G_1}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_1:G_1]}{K_{M_{PPi}} K_{iP_{PIIUMP_1:G_1}}} \right) / D \quad (1)$$

$$v_2 = \left( [UT]_T k_{catF} \frac{[PII:G_2][UTP]}{K_{iS_{PII:G_2}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_1:G_2]}{K_{M_{PPi}} K_{iP_{PIIUMP_1:G_2}}} \right) / D \quad (2)$$

$$v_3 = \left( [UT]_T k_{catF} \frac{[PII:G_3][UTP]}{K_{iS_{PII:G_3}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_1:G_3]}{K_{M_{PPi}} K_{iP_{PIIUMP_1:G_3}}} \right) / D \quad (3)$$

$$v_4 = \left( [UT]_T k_{catF} \frac{[PIIUMP_1:G_1][UTP]}{K_{iS_{PIIUMP_1:G_1}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_2:G_1]}{K_{M_{PPi}} K_{iP_{PIIUMP_2:G_1}}} \right) / D \quad (4)$$

$$v_5 = \left( [UT]_T k_{catF} \frac{[PIIUMP_1:G_2][UTP]}{K_{iS_{PIIUMP_1:G_2}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_2:G_2]}{K_{M_{PPi}} K_{iP_{PIIUMP_2:G_2}}} \right) / D \quad (5)$$

$$v_6 = \left( [UT]_T k_{catF} \frac{[PIIUMP_1:G_3][UTP]}{K_{iS_{PIIUMP_1:G_3}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_2:G_3]}{K_{M_{PPi}} K_{iP_{PIIUMP_2:G_3}}} \right) / D \quad (6)$$

$$v_7 = \left( [UT]_T k_{catF} \frac{[PIIUMP_2:G_1][UTP]}{K_{iS_{PIIUMP_2:G_1}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_3:G_1]}{K_{M_{PPi}} K_{iP_{PIIUMP_3:G_1}}} \right) / D \quad (7)$$

$$v_8 = \left( [UT]_T k_{catF} \frac{[PIIUMP_2:G_2][UTP]}{K_{iS_{PIIUMP_2:G_2}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_3:G_2]}{K_{M_{PPi}} K_{iP_{PIIUMP_3:G_2}}} \right) / D \quad (8)$$

$$v_9 = \left( [UT]_T k_{catF} \frac{[PIIUMP_2:G_3][UTP]}{K_{iS_{PIIUMP_2:G_3}} K_{M_{UTP}}} - [UT]_T k_{catR} \frac{[PPi][PIIUMP_3:G_3]}{K_{M_{PPi}} K_{iP_{PIIUMP_3:G_3}}} \right) / D \quad (9)$$

The denominator, which is common to all velocity equations, is given by:

$$D = \left( 1 + \frac{[GLN]}{K_{GLN}} \right) (D_0 + D_1 + D_2 + D_3 + D_4 + D_5 + D_6 + D_7 + D_8 + D_9) \quad (10)$$

The terms that make up this denominator are as follows:

$$D_0 = 1 + R \frac{K_{M_{substrate}}}{K_{i_{substrate}}} \frac{[UTP]}{K_{M_{UTP}}} + R \frac{K_{M_{product}}}{K_{i_{product}}} \frac{[PPi]}{K_{i_{PPi}}} \quad (11)$$

$$D_1 = \frac{[PII:G_1]}{K_{iS_{PII:G_1}}} \times S + \frac{[PIIUMP_1:G_1]}{K_{iP_{PIIUMP_1:G_1}}} \times P \quad (12)$$

$$D_2 = \frac{[PII:G_2]}{K_{iS_{PII:G_2}}} \times S + \frac{[PIIUMP_1:G_2]}{K_{iP_{PIIUMP_1:G_2}}} \times P \quad (13)$$

$$D_3 = \frac{[PII:G_3]}{K_{iS_{PII:G_3}}} \times S + \frac{[PIIUMP_1:G_3]}{K_{iP_{PIIUMP_1:G_3}}} \times P \quad (14)$$

$$D_4 = \frac{[PIIUMP_1:G_1]}{K_{iS_{PIIUMP_1:G_1}}} \times S + \frac{[PIIUMP_2:G_1]}{K_{iP_{PIIUMP_2:G_1}}} \times P \quad (15)$$

$$D_5 = \frac{[PIIUMP_1:G_2]}{K_{iS_{PIIUMP_1:G_2}}} \times S + \frac{[PIIUMP_2:G_2]}{K_{iP_{PIIUMP_2:G_2}}} \times P \quad (16)$$

$$D_6 = \frac{[PIIUMP_1:G_3]}{K_{iS_{PIIUMP_1:G_3}}} \times S + \frac{[PIIUMP_2:G_3]}{K_{iP_{PIIUMP_2:G_3}}} \times P \quad (17)$$

$$D_7 = \frac{[PIIUMP_2:G_1]}{K_{iS_{PIIUMP_2:G_1}}} \times S + \frac{[PIIUMP_3:G_1]}{K_{iP_{PIIUMP_3:G_1}}} \times P \quad (18)$$

$$D_8 = \frac{[PIIUMP_2:G_2]}{K_{iS_{PIIUMP_2:G_2}}} \times S + \frac{[PIIUMP_3:G_2]}{K_{iP_{PIIUMP_3:G_2}}} \times P \quad (19)$$

$$D_9 = \frac{[PIIUMP_2:G_3]}{K_{iS_{PIIUMP_2:G_3}}} \times S + \frac{[PIIUMP_3:G_3]}{K_{iP_{PIIUMP_3:G_3}}} \times P \quad (20)$$

The substrate terms (S) and the product terms (P) are as follows:

$$S = 1 + \frac{[UTP]}{K_{M_{UTP}}} + \frac{[PPi]}{K_{M_{PPi}}} R \frac{K_{M_{product}}}{K_{i_{product}}} + \frac{[UTP][PPi]}{K_{M_{UTP}} K_{i_{PPi}}} \quad (21)$$

$$P = 1 + \frac{[UTP]}{K_{M_{UTP}}} R \frac{K_{M_{substrate}}}{K_{i_{substrate}}} + \frac{[PPi]}{K_{M_{PPi}}} + \frac{[UTP][PPi]}{K_{i_{UTP}} K_{M_{PPi}}} \quad (22)$$

The symbol  $R_{K_i^{product}}^{K_M^{product}}$  represents the ratio between the values of  $K_{MP}$  and  $K_{iP}$  for the particular PII state that acts as a product in the particular equation being considered (i.e. from Eq. (11) to Eq. (20)). As can be seen by comparing the  $K_{MP}$  and  $K_{iP}$  in each line of Table 1, this ratio has always the same value, irrespective of which form of PII is being considered as the product. In other words:

$$R_{K_i^{product}}^{K_M^{product}} = \frac{K_{MP_{PIIUM\beta_1G_1}}}{K_{iP_{PIIUM\beta_1G_1}}} = \frac{K_{MP_{PIIUM\beta_1G_2}}}{K_{iP_{PIIUM\beta_1G_2}}} = \dots = \frac{K_{MP_{PIIUM\beta_3G_3}}}{K_{iP_{PIIUM\beta_3G_3}}} \quad (23)$$

The symbol  $R_{K_i^{substrate}}^{K_M^{substrate}}$  the ratio between the values of  $K_{MS}$  and  $K_{iS}$  for the particular PII state that acts as a substrate in the particular equation being considered (i.e. from Eq. (11) to Eq. (20)). As can be seen by comparing the  $K_{MS}$  and  $K_{iS}$  in each line of Table 2, this ratio has the same value, irrespective of which form of PII is being considered as the substrate. In other words:

$$R_{K_i^{substrate}}^{K_M^{substrate}} = \frac{K_{MS_{PII:G_1}}}{K_{iS_{PII:G_1}}} = \frac{K_{MS_{PII:G_2}}}{K_{iS_{PII:G_2}}} = \dots = \frac{K_{MS_{PIIUM\beta_2G_3}}}{K_{iS_{PIIUM\beta_2G_3}}} \quad (24)$$

### 3.2.1 Correlations between values of constants

It is possible to deduce relationships between the apparent dissociation constants that appear in Eqs (1 to 9). In this analysis,  $K_{iS}^*$  and  $K_{MS}^*$  are used as basic, reference constants. They have the value of the kinetic constants for a PII trimer with only one uridylylatable subunit, namely an non-uridylylated subunit with a bound 2-OG). Likewise,  $K_{iP}^*$  and  $K_{MP}^*$ , have the value of the kinetic constants for a PII trimer with only one deuridylylatable subunit (in which the UMP is removed by the reverse reaction of UTase, not by the UR activity), namely a uridylylated subunit with a bound 2-OG.

Since the model not only allows 2-OG to dissociate from a uridylylated subunit, but also allows 2-OG to bind to a subunit with a bound ATP (but a vacant 2-OG binding site), each of the PII superstates at the intersections of Figure 1 has three different metastates that have the same number of uridylylations and bound 2-OG molecules (Figure 2), but differ from each other in where these uridylylations and 2-OG are located spatially (i.e. in which subunits). Therefore, the three metastates represent the three possible spatial distributions of effectors of the corresponding superstate. Since it is assumed that the presence of the uridylylation on the

T-loop does not affect the affinity of that subunit for 2-OG, then in equilibrium, the 2-OG will be evenly distributed between the three subunits. Non-uridylylated subunits with bound 2-OG can act as substrates for UTase (Figure 2).

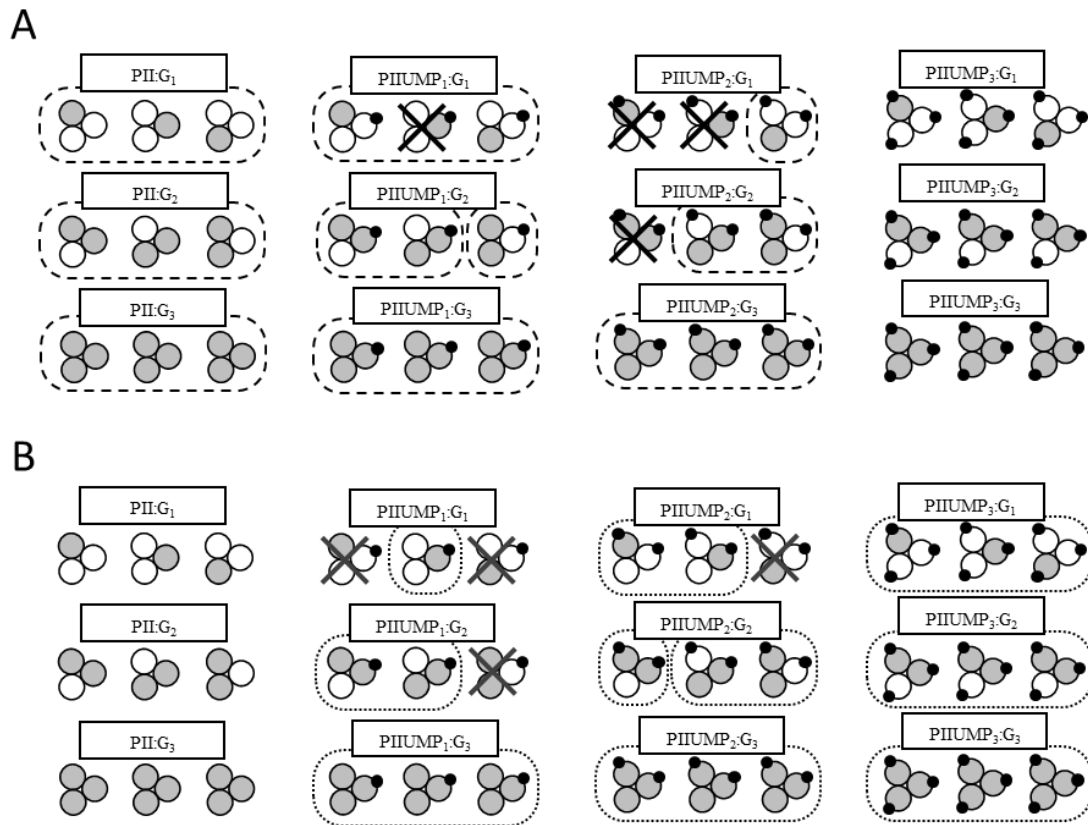


Figure 2 – The three metastates contained in each PII superstate in the bottom three rows of Figure 1. (A) Highlighted as substrates: Metastates with a non-uridylylated subunit that are encircled by a dashed line are uridylylatable and therefore can act as substrates. Metastates with a non-uridylylated subunit but which are marked with a black cross are not uridylylatable and therefore cannot act as substrates. (B) Highlighted as products: Metastates with a uridylylated subunit that encircled by a dotted line are deuridylylatable and can therefore act as products. Metastates with a uridylylated subunit but which are marked with a gray cross are not deuridylylatable, and therefore cannot act as products.

The three metastates of PII:G<sub>1</sub> are identical through rotation, each having one non-uridylylated subunit with a bound 2-OG. The three identical metastates of PII:G<sub>2</sub> all have two non-uridylylated subunits with bound 2-OG. The three identical metastates of PII:G<sub>3</sub> all have three non-uridylylated subunits with a bound 2-OG.

Of the three metastates of PIIUMP<sub>1</sub>:G<sub>1</sub>, only two can act as substrates of UTase; they are identical, each having one non-uridylylated subunit with a bound 2-OG. All three metastates of PIIUMP<sub>1</sub>:G<sub>2</sub> can act as substrates of UTase: two are identical, each having one non-uridylylated subunit with a bound 2-OG, the third has two non-uridylylated subunits each with a bound 2-OG. All three identical metastates of PIIUMP<sub>1</sub>:G<sub>3</sub> have two non-uridylylated subunits with bound 2-OG.

Of the three metastates of PIIUMP<sub>2</sub>:G<sub>1</sub>, only one can act as a substrate of UTase. Of the three metastates of PIIUMP<sub>2</sub>:G<sub>2</sub>, two can act as substrates of UTase. Of the three metastates of PIIUMP<sub>2</sub>:G<sub>3</sub>, three can act as substrates of UTase. In all cases, the metastates that can act as substrates have only one non-uridylylated subunit with a bound 2-OG.

Consideration of these metastates, in combination with the assumption that binding of UTase to the subunit of a trimer is not affected by the uridylylation states and effector binding states of the other two subunits of the trimer, leads to correlations between the different  $K_{iS}$  and  $K_{MS}$  values and between the different  $K_{iP}$  and  $K_{MP}$  values. Details of the analysis used to obtain these correlations are shown in the supplementary material. The resulting apparent values of these constants for each superstate are shown in Tables 1 and 2.

Table 1 – Correlation of values of constants for each PII superstate acting as a substrate

Reaction	PII superstate acting as a substrate	Average number of uridylylatable subunits per trimer	Correlation with $K_{iS}^*$	Correlation with $K_{MS}^*$
1	PII:G <sub>1</sub>	1	$K_{iS_{PII:G_1}} = K_{iS}^*$	$K_{MS_{PII:G_1}} = K_{MS}^*$
2	PII:G <sub>2</sub>	2	$K_{iS_{PII:G_2}} = \frac{1}{2} K_{iS}^*$	$K_{MS_{PII:G_2}} = \frac{1}{2} K_{MS}^*$
3	PII:G <sub>3</sub>	3	$K_{iS_{PII:G_3}} = \frac{1}{3} K_{iS}^*$	$K_{MS_{PII:G_3}} = \frac{1}{3} K_{MS}^*$
4	PIIUMP <sub>1</sub> :G <sub>1</sub>	2/3	$K_{iS_{PIIUMP_1:G_1}} = \frac{3}{2} K_{iS}^*$	$K_{MS_{PIIUMP_1:G_1}} = \frac{3}{2} K_{MS}^*$
5	PIIUMP <sub>1</sub> :G <sub>2</sub>	4/3	$K_{iS_{PIIUMP_1:G_2}} = \frac{3}{4} K_{iS}^*$	$K_{MS_{PIIUMP_1:G_2}} = \frac{3}{4} K_{MS}^*$
6	PIIUMP <sub>1</sub> :G <sub>3</sub>	2	$K_{iS_{PIIUMP_1:G_3}} = \frac{1}{2} K_{iS}^*$	$K_{MS_{PIIUMP_1:G_3}} = \frac{1}{2} K_{MS}^*$
7	PIIUMP <sub>2</sub> :G <sub>1</sub>	1/3	$K_{iS_{PIIUMP_2:G_1}} = 3K_{iS}^*$	$K_{MS_{PIIUMP_2:G_1}} = 3K_{MS}^*$
8	PIIUMP <sub>2</sub> :G <sub>2</sub>	2/3	$K_{iS_{PIIUMP_2:G_2}} = \frac{3}{2} K_{iS}^*$	$K_{MS_{PIIUMP_2:G_2}} = \frac{3}{2} K_{MS}^*$
9	PIIUMP <sub>2</sub> :G <sub>3</sub>	1	$K_{iS_{PIIUMP_2:G_3}} = K_{iS}^*$	$K_{MS_{PIIUMP_2:G_3}} = K_{MS}^*$

Table 2 – Correlation of values of constants for PII superstate acting as a product

Reaction	PII superstate acting as a product	Average number of deuridylylatable subunits per trimer	Correlation with $K_{iP}^*$	Correlation with $K_{MP}^*$
1	PIIUMP <sub>1</sub> :G <sub>1</sub>	1/3	$K_{iP_{PIIUMP_1 G_1}} = 3K_{iP}^*$	$K_{MP_{PIIUMP_1 G_1}} = 3K_{MP}^*$
2	PIIUMP <sub>1</sub> :G <sub>2</sub>	2/3	$K_{iP_{PIIUMP_1 G_2}} = \frac{3}{2}K_{iP}^*$	$K_{MP_{PIIUMP_1 G_2}} = \frac{3}{2}K_{MP}^*$
3	PIIUMP <sub>1</sub> :G <sub>3</sub>	1	$K_{iP_{PIIUMP_1 G_3}} = K_{iP}^*$	$K_{MP_{PIIUMP_1 G_3}} = K_{MP}^*$
4	PIIUMP <sub>2</sub> :G <sub>1</sub>	2/3	$K_{iP_{PIIUMP_2 G_1}} = \frac{3}{2}K_{iP}^*$	$K_{MP_{PIIUMP_2 G_1}} = \frac{3}{2}K_{MP}^*$
5	PIIUMP <sub>2</sub> :G <sub>2</sub>	4/3	$K_{iP_{PIIUMP_2 G_2}} = \frac{3}{4}K_{iP}^*$	$K_{MP_{PIIUMP_2 G_2}} = \frac{3}{4}K_{MP}^*$
6	PIIUMP <sub>2</sub> :G <sub>3</sub>	2	$K_{iP_{PIIUMP_2 G_3}} = \frac{3}{2}K_{iP}^*$	$K_{MP_{PIIUMP_2 G_3}} = \frac{1}{2}K_{MP}^*$
7	PIIUMP <sub>3</sub> :G <sub>1</sub>	1	$K_{iP_{PIIUMP_3 G_1}} = K_{iP}^*$	$K_{MP_{PIIUMP_3 G_1}} = K_{MP}^*$
8	PIIUMP <sub>3</sub> :G <sub>2</sub>	2	$K_{iP_{PIIUMP_3 G_2}} = \frac{1}{2}K_{iP}^*$	$K_{MP_{PIIUMP_3 G_2}} = \frac{1}{2}K_{MP}^*$
9	PIIUMP <sub>3</sub> :G <sub>3</sub>	3	$K_{iP_{PIIUMP_3 G_3}} = \frac{1}{3}K_{iP}^*$	$K_{MP_{PIIUMP_3 G_3}} = \frac{1}{3}K_{MP}^*$

### 3.2.2 Solving the model

The differential equations of the model (Eqs (1) to (9)) are solved using a Runge-Kutta method, more specifically the Dormand-Prince method (Dormand, Prince, 1982), implemented in the software MATLAB, using the “ode45” subroutine. This gives profiles for the concentrations of UTP and PP<sub>i</sub> and of the four different uridylylation states of PII (i.e. PII, PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> and PIIUMP<sub>3</sub>). During the integration, the concentrations of free ATP, ADP and 2-OG are assumed to remain constant and the model of Rocha *et al.* (2013) is used,

with these concentrations, to determine the concentrations of the 20 substates that constitute each uridylylation state (these 20 substates are contained within the four superstates in a particular column of Figure 1). When a superstate appears as a substrate or as a product, its concentration is expressed as the sum of the concentrations of the substates that constitute that particular superstate (i.e. substates with a particular number of uridylylated subunits and a particular number of 2-OG bound subunits). Section 3.5.1 of the supplementary material gives details about which substates constitute each superstate. The added velocities of the first three reactions represent the overall rate of conversion of PII into PIIUMP<sub>1</sub>. Likewise, the added velocities of reactions 4, 5 and 6 represent the overall rate of conversion of PIIUMP<sub>1</sub> into PIIUMP<sub>2</sub> and the added velocities of reactions 7, 8 and 9 represent the overall rate of conversion of PIIUMP<sub>2</sub> into PIIUMP<sub>3</sub>.

### 3.3 RESULTS

The values of some of the parameters of the kinetic model for UTase were obtained directly from Jiang *et al.* (1998) (Table 3). In order to use their values for  $K_M$  and  $K_i$  of PII and for  $K_i$  of PIIUMP<sub>3</sub>, it is necessary to deduce the predominant superstate in which these two PII forms were present in their assays. They used a 2-OG concentration of 33  $\mu\text{M}$ . For this concentration, model 4 of Rocha *et al.* (2013), which has  $K_M$  values of 11  $\mu\text{M}$  and 143  $\mu\text{M}$  for the binding of the first and second 2-OG molecules to PII, respectively, predicts that the predominant form of PII as a substrate is PII:G<sub>1</sub>, namely PII with one uridylylatable subunit. Therefore, the values that Jiang *et al.* (1998) reported for  $K_M$  (3.0  $\mu\text{M}$ ) and  $K_i$  (1.8  $\mu\text{M}$ ) are assumed to be the apparent values of  $K_{MS}^*$  and  $K_{iS}^*$ . Likewise, model 4 of Rocha *et al.* (2013) predicts that the predominant form of PIIUMP<sub>3</sub>, which Jiang *et al.* (1998) tested as a product in their product inhibition studies, was PIIUMP<sub>3</sub>:G<sub>1</sub>, namely PII with one deuridylylatable subunit. Therefore, the value that Jiang *et al.* (1998) reported for the  $K_i$  of PIIUMP<sub>3</sub> (3.5  $\mu\text{M}$ ) is assumed to be the apparent value of  $K_{iP}^*$ . Since the product inhibition studies of Jiat *et al.* (1998) give two possible values for the  $K_i$  of PP<sub>1</sub> (49.6  $\mu\text{M}$  and 113.5  $\mu\text{M}$ ), the value of  $K_{iPP_i}$  was taken as the geometric mean of the two (i.e. they were multiplied and the square root was taken), of 75  $\mu\text{M}$ .



Table 3 – Values of the constants obtained for the model.

Constant	Values obtained from Jiang <i>et al.</i> (1998)	Values obtained by optimization
$K_{iS}^*$	1.8 $\mu\text{M}$	-
$K_{MS_{PII:G_1}}$	3.0 $\mu\text{M}$	-
$K_{M_{UTP}}$	40 $\mu\text{M}$	-
$K_{i_{UTP}}$	-	$1.5 \times 10^5 \mu\text{M}$
$K_{M_{PPI}}$	-	0.82 $\mu\text{M}$
$K_{i_{PPI}}$	75 $\mu\text{M}$	-
$K_{iP}^*$	3.5 $\mu\text{M}$	-
$K_{MP}^*$	-	$3.3 \times 10^{15} \mu\text{M}$
$k_{catF}$	137 $\text{min}^{-1}$	
$k_{catR}$	2 $\text{min}^{-1}$	

The values of the three remaining constants,  $K_{i_{UTP}}$ ,  $K_{M_{PPI}}$  and  $K_{MP}^*$ , were estimated by fitting the model to the experimental uridylylation results from Jiang and Ninfa (2011) (Figure 3) in MATLAB using the “fminsearch” subroutine. Only the values of  $K_{M_{PPI}}$  and  $K_{MP}^*$  were allowed to be varied by the subroutine. Section 3.5.3 of the supplementary material demonstrates that all ten kinetic constants are correlated in such a manner that any one may be expressed as a combination of all the others. The value of  $K_{i_{UTP}}$  was calculated by this correlation:

$$K_{i_{UTP}} = \frac{K_{M_{UTP}} K_{iS_{PII:G_1}} K_{MP_{PIIUM\lambda:G_1}} K_{i_{PPI}} k_{catF}}{K_{i_{PPIUM\lambda:G_1}} K_{MS_{PII:G_1}} K_{M_{PPI}} k_{catR}} \quad (25)$$

Therefore, the values of nine constants are needed to give the value of  $K_{i_{UTP}}$ . The values of four of the constants, namely  $K_{M_{UTP}}$ ,  $K_{i_{PPI}}$ ,  $k_{catF}$  and  $k_{catR}$ , are readily available (second row of Table

3). The values of another three of the constants, namely  $K_{iS_{PII:G1}}$ ,  $K_{MS_{PII:G1}}$  and  $K_{iP_{PIIUMP1:G1}}$ , are calculated from  $K_{MS}^*$ ,  $K_{iS}^*$  and  $K_{iP}^*$  (second row of Table 3) and the correlations from Tables 1 and 2. The values of the two other constants, namely  $K_{MP_{PIIUMP1:G1}}$  and  $K_{MP_{Pi}}$ , change in each iteration of the “fminsearch” subroutine. In each iteration, “fminsearch” “guesses” a value for  $K_{MP_{Pi}}$  and  $K_{MP}^*$ . The value of  $K_{MP_{PIIUMP1:G1}}$  is calculated from  $K_{MP}^*$  using the correlation from Tables 2. The value for  $K_{i_{UTP}}$  is then calculated in each iteration of “fminsearch” using Eq. (25). At the end of the optimization, the values obtained of  $1.5 \times 10^5 \mu\text{M}$  for  $K_{i_{UTP}}$ ,  $0.82 \mu\text{M}$  for  $K_{MP_{Pi}}$  and  $3.3 \times 10^{15} \mu\text{M}$  for  $K_{MP}^*$ .

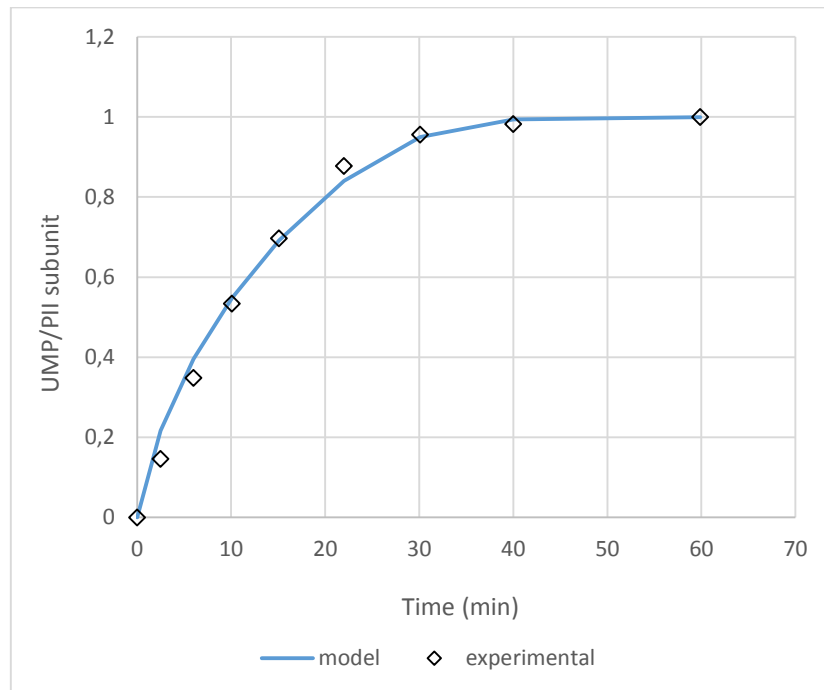


Figure 3 – Fitting of model to data to estimate  $K_{i_{UTP}}$ ,  $K_{MP_{Pi}}$  and  $K_{MP}^*$ . Experimental points were extracted from Jiang and Ninfa (2011)

### 3.4 DISCUSSION

In the current work, the model of Rocha *et al.* (2013) was used describe the binding of ATP, ADP and 2-OG to both non-uridylylated PII and uridylylated PII, using the same dissociation constants in both cases. Rocha *et al.* (2013) obtained these dissociation constants by fitting their model to sets of experimental data for non-uridylylated PII from Jiang and Ninfa (2007). It is not necessarily the case that these dissociation constants remain unaffected by uridylylation, but this is difficult to ascertain, since there is limited experimental data regarding

the binding of effectors to partially and fully uridylylated PII. Jiang *et al.* (1998) present a single (apparent)  $K_D$  value for 2-OG for fully uridylylated PII, however, they did not present the experimental data from which they deduced this value. Since there is negative cooperativity in the binding of the second and third 2-OG molecules to the PII trimer, this  $K_D$  from Jiang *et al.* (1998) is not applicable. The use of the same dissociation constants to describe the binding of the effectors to both non-uridylylated PII and uridylylated PII implicitly assumes that the presence of the UMP moiety on the T-loop does not affect the binding sites for ATP, ADP and 2-OG that are located near the base of the T-loop. This may be reasonable, since the UMP moiety binds to Tyr-51, which is situated near the tip of the T-loop and the T-loop itself is very flexible and points outwards from the main body of the PII trimer. However, there is the possibility that the uridylylated T-loop bends inwards, such that the UMP moiety interacts with some other part of the main body of PII; if this were to happen, uridylylation of the T-loop could affect the dissociation constants of the effectors.

Rocha (2013) suggested that 2-OG might not dissociate from a uridylylated PII subunit, which would result in the scheme for the UTase reactions shown in Figure 4. The superstates  $\text{PIIUMP}_1:\text{G}_0$ ,  $\text{PIIUMP}_2:\text{G}_0$ ,  $\text{PIIUMP}_2:\text{G}_1$ ,  $\text{PIIUMP}_3:\text{G}_0$ ,  $\text{PIIUMP}_3:\text{G}_1$ ,  $\text{PIIUMP}_3:\text{G}_2$  are not possible in this scheme since if 2-OG is necessary for uridylylation of a subunit, these PII states can only be obtained by subsequent dissociation of 2-OG. A model similar to that developed in the current work was derived for this scheme, but it was not possible to fit it to the data of Jiang and Ninfa (2011) using the constants for 2-OG dissociation reported by Rocha *et al.* (2013) (data not shown). It is necessary to have experimental evidence as to whether 2-OG can, in fact, dissociate from a uridylylated subunit. Beyond this, it is essential to have experimental data to determine whether the 2-OG dissociation constants are affected or not by the uridylylation of subunits of the trimer.

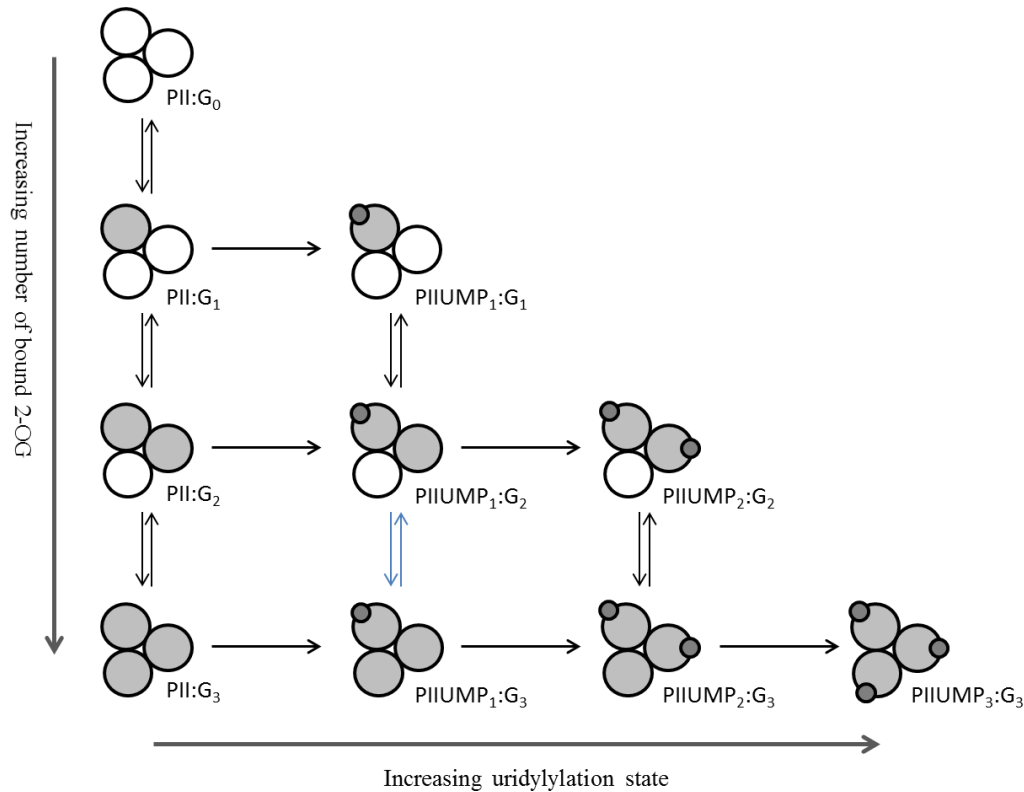


Figure 4 – A scheme for uridylylation of PII in which 2-OG is not allowed to dissociate from a uridylylated subunit. The vertical arrows show association (down arrows) and dissociation (up arrows) of 2-oxoglutarate and PII. The PII subunits bound to 2-oxoglutarate are painted light gray. The horizontal arrows show uridylylation of PII. The added UMP groups are shown as small dark gray circles.

The use of the constants from Jiang *et al.* (1998) presents 3 issues. Firstly, they determined their constants for PII by considering the full PII concentration as the substrate concentration. For this model, the concentration of non-uridylylated PII subunits with a bound 2-OG should be used instead. Secondly, there are two possible values for  $K_{iPPi}$  from the product inhibition assays from Jiang *et al.* (1998), 49.6  $\mu\text{M}$  and 113.5  $\mu\text{M}$ . They are still the same order of magnitude, however, and the geometric average of the two values was used to attenuate the issue. Thirdly, Jiang *et al.* (1998) obtained these values from their product inhibition assays by fitting simple inhibition equations to the assay data. Since in this fitting procedure they used the substrate constants they had previously obtained ( $K_{iPII}$ ,  $K_{MPiI}$  and  $K_{MUTP}$ ), errors in the substrate constants will be cause erros in the value obtained for  $K_{iPPi}$ .

There is a major issue with the values estimated for  $K_{MP}^*$  and  $K_{iUTP}$  in this work: they are several orders of magnitude larger than the other constants, especially  $K_{MP}^*$ , which is thirteen to fourteen orders of magnitude larger. There are two considerations to be made about this. The first is whether these values may be trusted. There are issues with the constants

obtained from Jiang *et al.* (1998), which were pointed out in the previous paragraph. If the assumption that the uridylylation state of PII does not affect the dissociation constants of ATP, ADP and 2-OG is wrong, then the values of these constants that were used, namely those obtained by Rocha *et al.* (2013), are also wrong. Since the dissociation constants from Rocha *et al.* (2013) and the kinetic constants from Jiang *et al.* (1998) were used in the model that was fitted to the uridylylation curve of Jiang and Ninfa (2011), any error in their values would have led to errors in the fitted values. Ideally, the model would be fitted simultaneously to a large amount of experimental data in order to obtain all constants. The second consideration are the implications of this. The denominator of the rate equation contains several terms that contain a form of  $K_{MP}$  in their numerators:  $K_{MP}$  multiplies the pyrophosphate concentration in term  $D_0$ , and it multiplies the pyrophosphate concentration and the respective PII substrate in each of the other “ $D$ -terms”. Since any other terms in the denominator would be negligible in comparison, the denominator would be reduced to  $D^*$ :

$$D^* = \frac{[PPi]}{K_{M_{PPi}}} R^{K_{M_{product}}}_{K_{i_{product}}} \left( \frac{K_{M_{PPi}}}{K_{i_{PPi}}} + \frac{[PII : G_1]}{K_{iS_{PII.G_1}}} + \frac{[PII : G_2]}{K_{iS_{PII.G_2}}} + \frac{[PII : G_3]}{K_{iS_{PII.G_3}}} + \frac{[PIIUMP_1 : G_1]}{K_{iS_{PIIUMP_1.G_1}}} + \frac{[PIIUMP_1 : G_2]}{K_{iS_{PIIUMP_1.G_2}}} + \frac{[PIIUMP_1 : G_3]}{K_{iS_{PIIUMP_1.G_3}}} + \frac{[PIIUMP_2 : G_1]}{K_{iS_{PIIUMP_2.G_1}}} + \frac{[PIIUMP_2 : G_2]}{K_{iS_{PIIUMP_2.G_2}}} + \frac{[PIIUMP_2 : G_3]}{K_{iS_{PIIUMP_2.G_3}}} \right) \quad (26)$$

while the numerators of the rate equations (Eqs (1) to (9)) would remain unchanged.

The product inhibition pattern deduced from this denominator would be significantly different from that reported by Jiang *et al.* (1998). Pyrophosphate would be a mixed inhibitor towards the PII substrates. There would be no product inhibition from the uridylylated forms of PII and no inhibition of any kind towards UTP, i.e. when UTP is the variable substrate in the product inhibition assays. Fully uridylylated PII would not cause any product inhibition, which is inconsistent with the study of Jiang *et al.* (1998), where it was used as one of the products and did, indeed, cause inhibition.

All of these show that the set of values for the constants (Table 5) is inconsistent. In order to obtain an appropriate set of values for these constants, a large set of uridylylation assays would be performed, with varying concentrations of all important species (substrates, products and PII effectors), and the model would be made to fit the data from all assays simultaneously. Another set of uridylylation assays would then be performed, with different conditions than

those used to obtain the constants, and the predictions of the model for these conditions would be compared to the experimental values in order to validate the model. Most assays focus on obtaining the initial velocity of the reaction. Indeed, these assays are very useful in obtaining the values of constants for the substrates. In reversible reactions, the assays to obtain the constants for the products are usually use the same strategy, but measuring the initial velocity of the reverse reaction. Since the velocity of the reverse reaction is so low for UTase (Jiang *et al.*, 1998), this is probably not a feasible strategy. An alternative is to perform uridylylation assays until full uridylylation is achieved. Another is to perform uridylylation assays to obtain the initial velocity when both  $PP_i$  and uridylylated PII are added. Both need to be present because two of the constants in the model, namely  $K_{iUTP}$  and  $K_{iPP_i}$ , only appear in terms where both products are present (Eq. (22)).

The issues with the values of the constants notwithstanding, this model is an important contribution to the modelling of ammonia assimilation in *E. coli*, Since it is the first model to take into account the influence of the binding of ATP, ADP and 2-OG to PII in the activity of UTase.

### 3.5 SUPPLEMENTARY MATERIAL 2

#### 3.5.1 Superstates

Each of the superstates shown in Figure 1 of the main article are actually pools of the various effector-binding states described by Rocha *et al.* (2013). The concentrations of the superstates of non-uridylylated PII are given by:

$$[PII:G_0] = \left( \begin{array}{l} [PII] + [PII:ATP_1] + [PII:ATP_2] + \\ [PII:ATP_3] + [PII:ADP_1] + [PII:ADP_2] + \\ [PII:ADP_3] + [PII:ATP_1:ADP_1] + \\ [PII:ATP_1:ADP_2] + [PII:ATP_2:ADP_1] \end{array} \right) \quad (S2.1)$$

$$[PII:G_1] = \left( \begin{array}{l} [PII:ATP_1:OG_1] + [PII:ATP_2:OG_1] + \\ [PII:ATP_3:OG_1] + [PII:ADP_1:ATP_1:OG_1] + \\ [PII:ADP_1:ATP_2:OG_1] + [PII:ADP_2:ATP_1:OG_1] \end{array} \right) \quad (S2.2)$$

$$[PII:G_2] = [PII:ATP_2:OG_2] + [PII:ATP_3:OG_2] + [PII:ADP_1:ATP_2:OG_2] \quad (S2.3)$$

$$[PII:G_3] = [PII:ATP_3:OG_3] \quad (S2.4)$$

The concentrations of the superstates of mono-uridylylated PII are given by:

$$[PIIUMP_1:G_0] = \left( \begin{array}{l} [PIIUMP_1] + [PIIUMP_1:ATP_1] + \\ [PIIUMP_1:ATP_2] + [PIIUMP_1:ATP_3] + \\ [PIIUMP_1:ADP_1] + [PIIUMP_1:ADP_2] + \\ [PIIUMP_1:ADP_3] + [PIIUMP_1:ATP_1:ADP_1] + \\ [PIIUMP_1:ATP_1:ADP_2] + [PIIUMP_1:ATP_2:ADP_1] \end{array} \right) \quad (S2.5)$$

$$[PIIUMP_1:G_1]=\left(\begin{array}{l} [PIIUMP_1:ATP_1:OG_1]+[PIIUMP_1:ATP_2:OG_1]+ \\ [PIIUMP_1:ATP_3:OG_1]+[PIIUMP_1:ADP_1:ATP_1:OG_1]+ \\ [PIIUMP_1:ADP_1:ATP_2:OG_1]+[PIIUMP_1:ADP_2:ATP_1:OG_1] \end{array}\right) \quad (S2.6)$$

$$[PIIUMP_1:G_2]=\left(\begin{array}{l} [PIIUMP_1:ATP_2:OG_2]+[PIIUMP_1:ATP_3:OG_2] \\ +[PIIUMP_1:ADP_1:ATP_2:OG_2] \end{array}\right) \quad (S2.7)$$

$$[PIIUMP_1:G_3]=[PIIUMP_1:ATP_3:OG_3] \quad (S2.8)$$

The concentrations of the superstates of bi-uridylylated PII are given by:

$$[PIIUMP_2:G_0]=\left(\begin{array}{l} [PIIUMP_2]+[PIIUMP_2:ATP_1]+ \\ [PIIUMP_2:ATP_2]+[PIIUMP_2:ATP_3]+ \\ [PIIUMP_2:ADP_1]+[PIIUMP_2:ADP_2]+ \\ [PIIUMP_2:ADP_3]+[PIIUMP_2:ATP_1:ADP_1]+ \\ [PIIUMP_2:ATP_1:ADP_2]+[PIIUMP_2:ATP_2:ADP_1] \end{array}\right) \quad (S2.9)$$

$$[PIIUMP_2:G_1]=\left(\begin{array}{l} [PIIUMP_2:ATP_1:OG_1]+[PIIUMP_2:ATP_2:OG_1]+ \\ [PIIUMP_2:ATP_3:OG_1]+[PIIUMP_2:ADP_1:ATP_1:OG_1]+ \\ [PIIUMP_2:ADP_1:ATP_2:OG_1]+[PIIUMP_2:ADP_2:ATP_1:OG_1] \end{array}\right) \quad (S2.10)$$

$$[PIIUMP_2:G_2]=\left(\begin{array}{l} [PIIUMP_2:ATP_2:OG_2]+[PIIUMP_2:ATP_3:OG_2] \\ +[PIIUMP_2:ADP_1:ATP_2:OG_2] \end{array}\right) \quad (S2.11)$$

$$[PIIUMP_2:G_3]=[PIIUMP_2:ATP_3:OG_3] \quad (S2.12)$$

Finally, the concentrations of the superstates of tri-uridylylated PII are given by:



$$[PIIUMP_3:G_0]=\left(\begin{array}{l} [PIIUMP_3]+[PIIUMP_3:ATP_1]+ \\ [PIIUMP_3:ATP_2]+[PIIUMP_3:ATP_3]+ \\ [PIIUMP_3:ADP_1]+[PIIUMP_3:ADP_2]+ \\ [PIIUMP_3:ADP_3]+[PIIUMP_3:ATP_1:ADP_1]+ \\ [PIIUMP_3:ATP_1:ADP_2]+[PIIUMP_3:ATP_2:ADP_1] \end{array}\right) \quad (S2.13)$$

$$[PIIUMP_3:G_1]=\left(\begin{array}{l} [PIIUMP_3:ATP_1:OG_1]+[PIIUMP_3:ATP_2:OG_1]+ \\ [PIIUMP_3:ATP_3:OG_1]+[PIIUMP_3:ADP_1:ATP_1:OG_1]+ \\ [PIIUMP_3:ADP_1:ATP_2:OG_1]+[PIIUMP_3:ADP_2:ATP_1:OG_1] \end{array}\right) \quad (S2.14)$$

$$[PIIUMP_3:G_2]=\left(\begin{array}{l} [PIIUMP_3:ATP_2:OG_2]+[PIIUMP_3:ATP_3:OG_2] \\ +[PIIUMP_3:ADP_1:ATP_2:OG_2] \end{array}\right) \quad (S2.15)$$

$$[PIIUMP_3:G_3]=[PIIUMP_3:ATP_3:OG_3] \quad (S2.16)$$

### 3.5.2 Correlations for the values of the $K_{iS}$ and $K_{MS}$ for different PII superstates

The kinetic equations include terms in which the concentration of a particular superstate is divided by the  $K_{iS}$  value for that superstate. This can be written as the sum of similar terms, one for each of the uridylylatable metastates that compose that particular superstate. In general terms, this can be written as:

$$\frac{[PIIUMP_x:G_x]}{K_{iS_{PIIUMP_x:G_x}}}=\frac{[A]}{K_{iS_A}}+\frac{[B]}{K_{iS_B}}+\frac{[C]}{K_{iS_C}} \quad (S2.17)$$

The first case study is undertaken for the superstate  $PIIUMP_1:G_1$  (Figure S1).

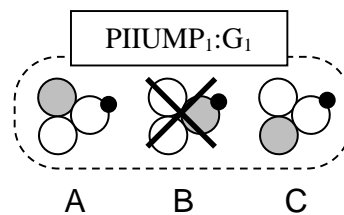


Figure S1 – Metastates A, B and C of the superstate  $PIIUMP_1:G_1$ .

Since “B” in Figure S1 is not uridylylatable, application of Eq. (S2.17) gives:

$$\frac{[PIIUMP_1:G_1]}{K_{iS_{PIIUMP_1:G_1}}} = \frac{[A]}{K_{iS_A}} + \frac{[C]}{K_{iS_C}} \quad (S2.18)$$

As both metastate A and metastate C have only one uridylylatable subunit, their  $K_{iS}$  constants are equal to  $K_{iS}^*$ . Since the concentration of each metastate is a third of the concentration of the superstate:

$$\frac{[PIIUMP_1:G_1]}{K_{iS_{PIIUMP_1:G_1}}} = \frac{1}{3} \frac{[PIIUMP_1:G_1]}{K_{iS}^*} + \frac{1}{3} \frac{[PIIUMP_1:G_1]}{K_{iS}^*} \quad (S2.19)$$

Addition of the two terms on the right-hand side gives:

$$\frac{[PIIUMP_1:G_1]}{K_{iS_{PIIUMP_1:G_1}}} = \frac{2}{3} \frac{[PIIUMP_1:G_1]}{K_{iS}^*} \quad (S2.20)$$

Both the numerator and denominator of the right-hand side are multiplied by 3/2 to give:

$$\frac{[PIIUMP_1:G_1]}{K_{iS_{PIIUMP_1:G_1}}} = \frac{[PIIUMP_1:G_1]}{\frac{3}{2} K_{iS}^*} \quad (S2.21)$$

Since the concentrations terms are the same, this means that:

$$K_{iS_{PIIUMP_1:G_1}} = \frac{3}{2} K_{iS}^* \quad (S2.22)$$

As shown by Mallmann *et al.* (2015), the correlation between the  $K_{iS}$  values for two different PII trimers also holds for their  $K_{MS}$  values. Therefore:

$$K_{MS_{PIIUMP_1:G_1}} = \frac{3}{2} K_{MS}^* \quad (S2.23)$$

The second case study is undertaken for the superstate  $PIIUMP_1:G_2$  (Figure S2);

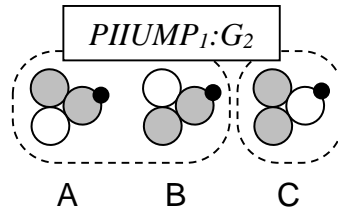


Figure S2 – Metastates A, B and C of the superstate PIIUMP<sub>1</sub>:G<sub>2</sub>.

In this case, metastates A and B have one uridylylatable subunit, while metastate C has two. As shown by Mallmann *et al.* (2015), the  $K_{iS}$  for a PII trimer with two uridylylatable subunits is half of  $K_{iS}^*$ . This means that for PIIUMP<sub>1</sub>:G<sub>2</sub> Equation (S2.17) can be written as:

$$\frac{[PIIUMP_1:G_2]}{K_{iS_{PIIUMP_1:G_2}}} = \frac{[A]}{K_{iS}^*} + \frac{[B]}{K_{iS}^*} + \frac{[C]}{\frac{1}{2}K_{iS}^*} \quad (S2.24)$$

Since the concentration of each metastate is equal to a third of the concentration of the superstate, it is possible to write:

$$\frac{[PIIUMP_1:G_2]}{K_{iS_{PIIUMP_1:G_2}}} = \frac{\frac{1}{3}[PIIUMP_1:G_2]}{K_{iS}^*} + \frac{\frac{1}{3}[PIIUMP_1:G_2]}{K_{iS}^*} + \frac{\frac{1}{3}[PIIUMP_1:G_2]}{\frac{1}{2}K_{iS}^*} \quad (S2.25)$$

The last term on the right hand side is reorganized to give:

$$\frac{[PIIUMP_1:G_2]}{K_{iS_{PIIUMP_1:G_2}}} = \frac{\frac{1}{3}[PIIUMP_1:G_2]}{K_{iS}^*} + \frac{\frac{1}{3}[PIIUMP_1:G_2]}{K_{iS}^*} + \frac{\frac{2}{3}[PIIUMP_1:G_2]}{K_{iS}^*} \quad (S2.26)$$

Adding the terms on the terms on the right-hand side of the equation results in:

$$\frac{[PIIUMP_1:G_2]}{K_{iS_{PIIUMP_1:G_2}}} = \frac{\frac{4}{3}[PIIUMP_1:G_2]}{K_{iS}^*} \quad (S2.27)$$

Both the numerator and denominator of the right-hand term are multiplied by  $\frac{3}{4}$ , giving:

$$\frac{[PIIUMP_1:G_2]}{K_{iS_{PIIUMP_1:G_2}}} = \frac{[PIIUMP_1:G_2]}{\frac{3}{4}K_{iS}^*} \quad (S2.28)$$

Since the concentrations terms are the same, this means that:

$$K_{iS_{PHUMR_1G_2}} = \frac{3}{4} K_{iS}^* \quad (S2.29)$$

Extension of this analysis to the  $K_{iS}$  and  $K_{MS}$  values of the other superstates and to the  $K_{iP}$  and  $K_{MP}$  values of all superstates leads to the correlations shown in Tables 1 and 2 of the main article. The factor that correlates the  $K_{iS}$  value to  $K_{iS}^*$  (and also the  $K_{MS}$  values to  $K_{MS}^*$ ) for a particular superstate is the reciprocal of the average number of uridylylatable subunits per trimer for that superstate. Likewise, the factor that correlates the  $K_{iP}$  value to  $K_{iP}^*$  (and also the  $K_{MP}$  values to  $K_{MP}^*$ ) for a particular superstate is the reciprocal of the average number of deuridylylatable subunits per trimer for that superstate.

### 3.5.3 Proof of Equation (25) of the main paper

All ten constants related to a uridylylation cycle are related in the following manner:

$$K_{MS_{PH}} K_{iP_{PHUMP}} K_{iUTP} K_{M_{PPi}} k_{catR} = K_{MP_{PHUMP}} K_{iS_{PH}} K_{M_{UTP}} K_{i_{PPi}} k_{catR} \quad (S2.30)$$

This can be proven by replacing each of these constants with its identity in terms of the fundamental constants, with these identities being given by Eqs. (S21) to (S30) of the Supplementary Material of Mallmann *et al.* (2015):

$$\left( \frac{k_3 k_4}{k_1 (k_3 + k_4)} \right) \left( \frac{k_4}{k_{-4}} \right) \left( \frac{k_{-1} + k_{-2}}{k_2} \right) \left( \frac{k_{-1} (k_{-2} + k_3)}{k_{-3} (k_{-1} + k_{-2})} \right) \left( \frac{k_{-1} k_{-2}}{k_{-1} + k_{-2}} \right) = \left( \frac{k_{-1} k_{-2}}{k_{-4} (k_{-1} + k_{-2})} \right) \left( \frac{k_{-1}}{k_1} \right) \left( \frac{k_4 (k_{-2} + k_3)}{k_2 (k_3 + k_4)} \right) \left( \frac{k_3 + k_4}{k_{-3}} \right) \left( \frac{k_3 k_4}{k_3 + k_4} \right) \quad (S2.31)$$

All terms cancel out, proving that equation the left-hand side is identical to the right-hand side. This means one of the constants can be obtained if values are available for all the others. In the current work,  $K_{iUTP}$  was isolated:

$$K_{iUTP} = \frac{K_{M_{UTP}} K_{iS_{PH}} K_{MP_{PHUMP}} K_{i_{PPi}} k_{catF}}{K_{iP_{PHUMP}} K_{MS_{PH}} K_{M_{PPi}} k_{catR}} \quad (S2.32)$$

When Eq. (S2.32) is written specifically for the first reaction of Figure 1 of the main paper, which has the superstate  $\text{PII:G}_1$  as the substrate and the superstate  $\text{PIIUMP}_1:\text{G}_1$  as the product, Eq. (25) from the main paper is obtained:

$$K_{i_{UTP}} = \frac{K_{M_{UTP}} K_{i_{S_{\text{PII:G}_1}}} K_{M_{P_{\text{PIIUMP}_1:\text{G}_1}}} K_{i_{\text{PPi}}} k_{catF}}{K_{i_{P_{\text{PIIUMP}_1:\text{G}_1}}} K_{M_{S_{\text{PII:G}_1}}} K_{M_{\text{PPi}}} k_{catR}} \quad (25)$$

## 4 CONSIDERAÇÕES FINAIS

Os objetivos desta dissertação foram:

- (i) Propor um modelo matemático para UTase de *Escherichia coli* capaz de corretamente prever seu perfil de inibição pelos produtos.
- (ii) Propor um modelo matemático para UTase que descreva os efeitos dos efetores alostéricos de PII nas reações de uridililação.

Estes objetivos foram atingidos. As contribuições e limitações dos modelos desenvolvidos nesta tese serão discutidas nesta seção.

O modelo para UTase proposto no primeiro artigo prevê o perfil de inibição pelos produtos adequadamente. Ele é capaz de melhor descrever a atividade de UTase durante a transição entre PII não uridililada e PII completamente uridililada, quando as concentrações dos produtos variam. Sua principal limitação é a de considerar que toda a PII é uridililável. Isto limita sua aplicação aos casos em que PII esteja saturada por 2-OG, situação que pode ser produzida *in vitro* e que apenas acontece *in vivo* no extremo mais alto da faixa de concentração fisiológica de 2-OG.

O modelo proposto no segundo artigo continua sendo capaz de prever o perfil de inibição pelos produtos, mas supera a principal limitação do primeiro modelo. Ele é capaz de descrever a atividade de UTase de acordo com o estado de ligação de PII por seus efetores, em toda a faixa de concentração de ATP, ADP e 2-OG. Sua principal limitação está relacionada aos valores numéricos das constantes cinéticas. Os valores de todas as constantes, exceto  $K_{MP}$ ,  $K_{MPPi}$  e  $K_{iUTP}$  são obtidos da tabela 2 de Jiang *et al.* (1998). Estes valores foram obtidos por Jiang *et al.* em uma única concentração de 2-OG, de 33  $\mu\text{M}$ , então são valores aparentes. No caso de  $K_{iPII}$ ,  $K_{MPII}$  e  $K_{iP}$  especialmente, a adoção dos valores de Jiang *et al.* (1998) é uma aproximação, baseada em que os estados predominantes em seus ensaios foi PII:G<sub>1</sub> e PIIUMP<sub>3</sub>:G<sub>1</sub>. Ainda assim, para esta concentração de 2-OG, haveria quantidades significantes dos estados PII:G<sub>0</sub>, PII:G<sub>2</sub>, PIIUMP<sub>3</sub>:G<sub>0</sub> e PIIUMP<sub>3</sub>:G<sub>2</sub>. Quando os valores das constantes  $K_{MP}$ ,  $K_{MPPi}$  e  $K_{iUTP}$  foram otimizados para descrever dados de um ensaio de uridililação no segundo artigo contido nesta dissertação, os valores obtidos para  $K_{MP}$ ,  $K_{MPPi}$  foram várias ordens de grandeza maiores que os demais, tornando o conjunto de valores das constantes cinéticas do modelo inconsistentes

Além dos dois modelos para UTase em si, a principal contribuição desta dissertação é a abordagem aplicada às constantes cinéticas dos diferentes estados de uridililação e ligação de efetores de PII. Bruggeman havia considerado que  $K_{IS}$  e  $K_{MS}$  teriam valores idênticos para PII, PIIUMP<sub>1</sub> e PIIUMP<sub>2</sub> (Bruggeman *et al.*, 2005). Como elas possuem número diferente de subunidades que são uridililáveis, os valores de  $K_{IS}$  e  $K_{MS}$  não podem ser idênticos. Da mesma forma, Bruggeman havia considerado que  $K_{IP}$  teria valores idênticos para PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> e PIIUMP<sub>3</sub> (Bruggeman *et al.*, 2005), mas como estas espécies não possuem o mesmo número de subunidades que atuam como produto, os valores de  $K_{IP}$  não podem ser idênticos. Nesta dissertação, foi reconhecido que além de terem valores distintos, os valores destas constantes estão correlacionados entre si. A análise feita no Material Suplementar ao primeiro artigo (Seção 2.5) mostrou que os valores das constantes de substratos estão relacionados ao número de subunidades uridililáveis por trímero, sendo inversamente proporcionais, e que os valores das constantes de produtos estão relacionados ao número de subunidades desuridililáveis por trímero, sendo, também, inversamente proporcionais. Esta abordagem se assemelha àquela utilizada por Monod para descrever a atividade de enzimas multiméricas que possuem sítios ativos idênticos em cada subunidade. Como não foram encontradas análises previamente publicadas, esta pode ser a primeira análise de reações enzimáticas com substratos multiméricos possuam com sítios idênticos para modificação covalente em cada subunidade. A abordagem utilizada para o caso de UTase e PII pode ser aproveitada para outras reações enzimáticas deste tipo. Proteínas e enzimas multiméricas que sofrem modificação covalente não são incomuns, como a própria GS, que é um dodecâmero que sofre adenililações em cada uma de suas doze subunidades.

O desenvolvimento destes dois modelos para UTase levantou uma série de questões que podem orientar o trabalho experimental. Caso respondidas, elas podem não apenas levar ao aprimoramento do próprio modelo, mas também ao aprofundamento do entendimento deste sistema. Há três questões principais:

- (i) Como estão relacionadas as constantes cinéticas para trímeros de PII com diferentes estados de ligação a 2-OG e de uridililação?
- (ii) De que maneira o estado de uridililação de PII afeta as constantes de dissociação dos efetores ATP, ADP e 2-OG?
- (iii) A ligação de uma única molécula de 2-OG ligada a um trímero de PII permite que suas três subunidades sejam substratos para UTase, ou cada subunidade precisa ter 2-OG ligado para ser substrato?

À primeira questão foi proposta uma resposta nesta dissertação, de que a comentada no parágrafo anterior. Para responder à questão com evidências experimentais e, desta forma, também confirmar ou refutar a validade da abordagem citada, uma série de ensaios de uridililação precisaria ser realizada. Dentre eles, iniciar com ensaios que tenham como substratos PII, PIIUMP<sub>1</sub> e PIIUMP<sub>2</sub> saturados com 2-OG, podem-se obter valores experimentais para  $K_{iS_{PII:G_3}}$ ,  $K_{MS_{PII:G_3}}$ ,  $K_{iS_{PIIUMP_1:G_3}}$ ,  $K_{MS_{PIIUMP_1:G_3}}$ ,  $K_{iS_{PIIUMP_2:G_3}}$  e  $K_{MS_{PIIUMP_2:G_3}}$  do segundo modelo. Este conjunto de ensaios permitiria verificar a correlação com PII de diferentes estados de uridililação. Outro conjunto de ensaios de interesse utilizaria como substrato PII na presença de diferentes concentrações de 2-OG. Estes ensaios permitiriam confirmar ou refutar as correlações entre  $K_{iS_{PII:G_1}}$ ,  $K_{iS_{PII:G_2}}$  e  $K_{iS_{PII:G_3}}$  e entre  $K_{MS_{PII:G_1}}$ ,  $K_{MS_{PII:G_2}}$  e  $K_{MS_{PII:G_3}}$ , bem como confirmar que as correlações entre  $K_{iS_{PII:G_1}}$  e  $K_{iS_{PII:G_2}}$  por exemplo são as mesmas que entre  $K_{MS_{PII:G_1}}$ ,  $K_{MS_{PII:G_2}}$ .

No modelo, a segunda questão foi tratada com o pressuposto de que o estado de uridililação de PII não afeta as constantes de dissociação dos efetores. Para responder definitivamente esta questão, uma série de ensaios variando as concentrações dos três efetores na presença de PII, PIIUMP<sub>1</sub>, PIIUMP<sub>2</sub> e PIIUMP<sub>3</sub> precisaria ser realizada. Se a determinação utilizar isótopos, como as realizadas por Jiang e Ninfa (2007) para obter os dados usados para determinar as constantes de seu modelo e do modelo de Rocha *et al.* (2013), cada ensaio precisa ser repetido até três vezes, cada uma com um dos efetores marcado.

A terceira questão foi tratada no modelo do segundo artigo com o pressuposto de que cada subunidade precisa ter uma molécula de 2-OG ligada a ela mesma, para que ela possa ser uridililada por UTase, em um trímero individual. A confirmação experimental deste pressuposto for seria um indício de que a ligação dos efetores a PII levam a mudanças conformacionais localizadas à região próxima dos sítios aos quais estes efetores estão ligados. Caso ser verifique experimentalmente o oposto, de que uma única molécula de 2-OG ligada ao trímero de PII possibilita a uridililação das suas três subunidades, ficará evidente que a ligação dos efetores em um sítio é comunicada para o restante da estrutura terciária de PII, alterando sua conformação. Como diversos estados de ligação de PII coexistem em um dado conjunto de concentrações dos efetores (Rocha *et al.*, 2013), a resposta desta questão teria implicações sobre as faixas de concentrações dos efetores necessárias para elicitar um estado com ação regulatória em PII.



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