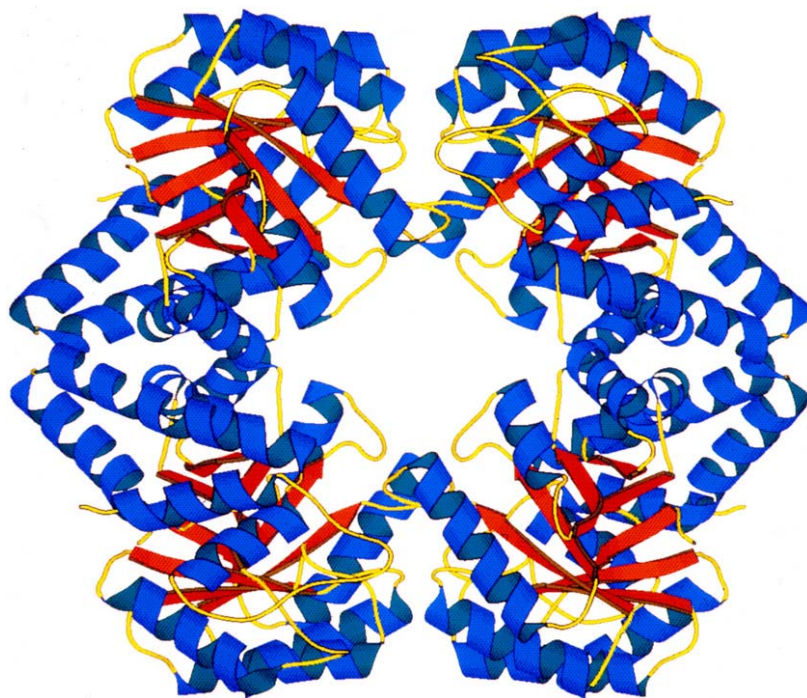


**Three Dimensional Fourier Synthesis of  
*N*-acetylneuraminate Lyase from  
*Escherichia Coli***

by

Tina Izard



A thesis submitted for the degree of Doctor of Philosophy in  
The University of Melbourne, School of Physics  
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## Preface

This thesis reports the research work carried out by the author under the co-supervision of Peter Colman, Biomolecular Research Institute, and Zwi Barnea, School of Physics, The University of Melbourne.

Some of the work described here appeared in the following publications which are enclosed as appendices:

Michael C. Lawrence, Tina Izard, Marc Beuchat, Robert J. Blagrove & Peter M. Colman (1994).

“The structure of phaseolin at 2.2 Å resolution: implications for a common vicilin/legumin structure and the genetic engineering of seed storage proteins.”

*J. Mol. Biol.* **238**, 748 – 776.

Tina Izard, Michael C. Lawrence, Robyn L. Malby, Glenn G. Lilley & Peter M. Colman (1994).

“The Three-Dimensional Structure of *N*-acetylneuraminase Lyase from *Escherichia coli*.”

*Structure* **2**, 361 – 369.

## Abstract

*N*-acetylneuraminase lyase (Neu5Ac lyase, EC 4.1.3.3) from *Escherichia coli* catalyses reversibly the cleavage of *N*-acetylneuraminic acid (sialic acid) to form pyruvate and *N*-acetyl-D-mannosamine. Neu5Ac lyase also catalyses the reverse aldol condensation and has been used in that way to synthesise sialic acid, and some of its derivatives, from pyruvate and *N*-acetyl-D-mannosamine. The enzyme plays a key role in the regulation of the sialic acid metabolism in *Escherichia coli* by controlling levels of intracellular sialic acid. Biosynthetically produced sialic acid and exogenous sialic acid which accumulates inside the cell are both regulated by the Neu5Ac lyase. The enzyme has been found in pathogenic as well as non-pathogenic bacteria, and in mammalian tissues. In bacteria, the enzyme is induced by high levels of the substrate, sialic acid. Sodium borotetrahydride inactivates the enzyme in the presence of either sialic acid or pyruvate, suggesting that a Schiff base is formed during the lyase reaction. Neu5Ac lyase has high sequence identity over its entire length to *Escherichia coli* dihydrodipicolinate synthase (DHDPS), which catalyses the condensation of pyruvate and aspartate  $\beta$ -semialdehyde via a ping pong mechanism in which pyruvate forms a Schiff base with a lysine residue. This lysine is conserved in Neu5Ac lyase and by analogy very likely the active site lysine.

The crystals were shown to belong to the trigonal space group  $P3_221$  with cell parameters  $a = b = 122.8 \text{ \AA}$ ,  $c = 198.8 \text{ \AA}$ ,  $\alpha = \beta = 90^\circ$  and  $\gamma = 120^\circ$

and one tetramer in the asymmetric unit. The Neu5Ac lyase gene encodes a polypeptide of 297 amino acids with a molecular weight of 32 kDa.

The crystal structure of Neu5Ac lyase has been determined by single isomorphous replacement with anomalous scattering and phase extension with non-crystallographic symmetry averaging. The protein protomer contains a central  $\alpha/\beta$  barrel which was first found for triose phosphate isomerase (TIM) followed by three *C*-terminal helices. Crystallographic refinement has been carried out to 2.2 Å resolution. The atomic model has an *R*-factor of 0.206 and root mean square deviations from ideal bond length and angles of 0.015 Å and 2.09° respectively. The current model comprises 291 residues and one sulphate group per protomer as well as sixty two water molecules associated with the tetramer.

The active site of the enzyme has tentatively been identified as a pocket at the *C*-terminal end of the eight-stranded  $\beta$  barrel. Lys-165 lies within this pocket and is probably the reactive residue which forms a Schiff base intermediate with substrate. Sequence similarity of Neu5Ac lyase with DHDPS and MosA over its entire length suggests that these latter enzymes share a similar structure to Neu5Ac lyase.

## Declaration

This thesis is less than 100 000 words in length.

Tina Izard

Tina Izard

## Acknowledgements

I feel deeply indebted to my supervisor, Peter Colman, for giving me this unique opportunity of carrying out this biologically and crystallographically exciting project in his well-equipped laboratory. It was an honour to work under the guidance of an outstanding scientist and the knowledge and confidence I gained during these years is immense. Peter, I am most grateful for your never-ending interest in the progress of this project, for teaching me the theory behind X-ray protein crystallography techniques new to me and for generously providing me with the opportunities of travelling to the Photon Factory in Japan for data collection, and to Erice, Italy, and Atlanta, U.S.A, to present our results at scientific meetings.

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Mark von Itzstein's synthesis of a substrate analogue as a potential deriva-

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with love to all those scientists, who give their advise and wisdom and all the hours of their time to their pupils, the future scientists;

who brilliantly develop the science they created for a better tomorrow for every living creature in the universe and provide mankind with knowledge and the means of treating dreadful diseases;

who are committed to education and science free from discrimination against race, gender, sexual preference, religious and political beliefs, disability, social and economical circumstances;

who do provide the world with all their knowledge in a non-competitive way;

who always stay students inside themselves, haunted by dreams and curiosity and are un-corrupted by fame or fortune

## Auf der Galerie

Wenn irgendeine hinfällige, lungensüchtige Kunstreiterin in der Manege auf schwankendem Pferd vor einem unermüdlichen Publikum vom peitschenschwingendem erbarmungslosen Chef monatelang ohne Unterbrechung im Kreise rundum getrieben würde, auf dem Pferde schwirrend, Küsse werfend, in der Taille sich wiegend, und wenn dieses Spiel unter dem nichtaussetzenden Brausen des Orchesters und der Ventilatoren in die immerfort weiter sich öffnende graue Zukunft sich fortsetzte, begleitet vom vergehenden und neu anschwellenden Beifallsklatschen der Hände, die eigentlich Dampfhämmer sind – vielleicht eilte dann ein junger Galeriebesucher die lange Treppe durch alle Ränge hinab, stürzte in die Manege, rief das: Halt! durch die Fanfaren des immer sich anpassenden Orchesters.

Da es aber nicht so ist; eine schöne Dame, weiß und rot, hereinfliegt, zwischen den Vorhängen, welche die stolzen Livrierten vor ihr öffnen; der Direktor, hingebungsvoll ihre Augen suchend, in Tierhaltung ihr entgegenatmet; vorsorglich sie auf den Apfelschimmel hebt, als wäre sie seine über alles geliebte Enkelin, die sich auf gefährliche Fahrt begibt; sich nicht entschließen kann, das Peitschenzeichen zu geben; schließss—lich in Selbstüberwindung es knallend gibt; neben dem Pferde mit offenem Munde einherläuft; die Sprünge der Reiterin scharfen Blickes verfolgt; ihre Kunstfertigkeit kaum begreifen kann; mit englischen Ausrufen zu warnen versucht; die reifenhaltenden Reitknechte wütend zu peinlichster Achtsamkeit ermahnt; vor dem großen Salto mortale das Ochester mit aufgehobenen Händen beschwört, es möge schweigen; schließlich die Kleine vom zitternden Pferd hebt, auf beide Backen küßt und keine Huldigung des Publikums für genügend erachtet; während sie selbst, von ihm gestützt, hoch auf den Fußspitzen, vom Satub umweht, mit ausgebreiteten Armen, zurückgelehntem Köpfchen ihr Glück mit dem ganzem Zirkus teilen will – da dies so ist, legt der Galeriebesucher das Gesicht auf die Brüstung und, im Schlußmarsch wie in einem schweren Traum versinkend, weint er, ohne es zu wissen.

Franz Kafka (1883 – 1924)

Ah! how harshly the youth of the student passes,  
While all around her, with passions ever fresh,  
Other youths search eagerly for easy pleasures!  
And yet in solitude  
She lives, obscure and blessed,  
For in her cell she finds the ardour  
That makes her heart immense.

But the blessed time is effaced.  
She must leave the land of Science  
To go out and struggle for her bread  
On the grey roads of life.  
Often and often then, her weary spirit  
Returns beneath the roof  
To the corner ever dear to her heart  
While silent labour dwelled  
And where a world of memory has rested.

Marie Skłodowska Curie (1867 – 1934)