London, London, UK. [b]*Molecular Genetics Group, Molecular and Metabolic Signalling Centre, Division of Basic Medical Sciences, St. George's, University of London, London, UK.* [c]*Diamond Light Source, Didcot, Oxford, UK.*
E-mail: mark.sanderson@kcl.ac.uk, lfisher@sgul.ac.uk, ivan.laponogov@kcl.ac.uk

Topoisomerase IV belongs to the type II class of DNA topoisomerases, which are responsible for changing and stabilizing DNA supercoiling and are also involved in chromosome segregation in prokaryotes. Topo IIs are essential enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones. They change DNA topology by forming a transient covalent cleavage complex with a gate-DNA (G-segment) duplex and transporting the second duplex (T-segment) though a double-stranded break in the formed protein-DNA gate. Although the biological importance of these enzymes is well known, cleavage complex formation and reversal is not fully understood for any type II topoisomerase. In order to further our understanding of the topo II action, we have solved the crystal structures representing sequential states in the formation and reversal of a DNA cleavage complex by topoisomerase IV from
S. pneumoniae. A 3.1 Å resolution structure of the complex captured by a novel antibacterial dione represents a drug-arrested form of the cleavage intermediate and reveals two drug molecules intercalated at a symmetrically cleaved B-form DNA gate and stabilized by drug-specific protein contacts. Similar protein/DNA/drug complex formation was observed for the 2.9 Å resolution structure of topo IV/DNA/levofloxacin solved by us and representing the first high-resolution quinolone-stabilized cleavage complex. Subsequent dione release allowed us to obtain drug-free cleaved and resealed DNA complexes in which the DNA gate, in contrast to the previous state, adopts an unusual A/B-form helical conformation. It also revealed an important reposition of a Mg^{2+} ion towards scissile phosphodiester group allowing its coordination and promoting reversible cleavage by active-site tyrosines. These are the first structures solved for putative reaction intermediates of a type II topoisomerase. They indicate how a type II enzyme reseals DNA during its normal reaction cycle as well as how the complex is stabilized by different antibacterial drugs, which is important for the development of new topoisomerase-targeting therapeutics.

Keywords: Topoisomerases, breakage-reunion, Protein-DNA complexes

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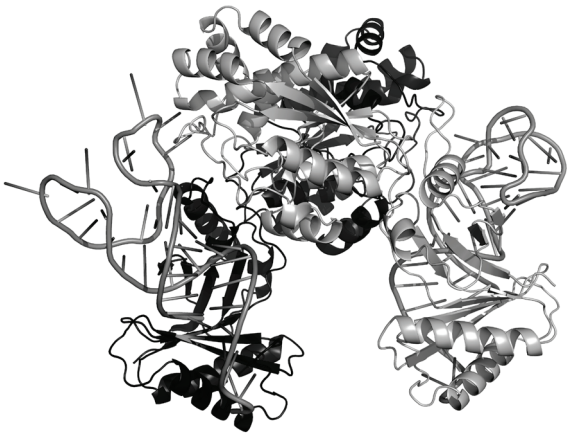
Crystal structure of a homodimeric 4-thiouridine synthetase - RNA complex. Piotr Neumann[a], Kristina Lakomek[a], Peter-Thomas Naumann[b], Achim Dickmanns[a], Charles T. Lauhon[b], Ralf Ficner[a], [a]*Abteilung für Molekulare Strukturbiologie, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany.* [b]*School of Pharmacy, Univeristy of Wisconsin, Madison, Wisconsin 53705, USA.*
E-mail: pneuman2@uni-goettingen.de

The maturation of all types of RNA in all domains of life includes the posttranscriptional modification of nucleosides. A wide variety of rare nucleosides has been identified, among them there are 16 different thio-nucleotides including the 4-thiouridine, the 2-thiouridine and several alkylated derivatives of 2-thiouridine. Modified nucleotides have been reported to influence vastly different cellular processes. Thiouridines are prerequisites to a correct and efficient translation process and contribute to the structural stability of the tRNA molecule. The modified nucleoside 4-thiouridine (s^4U) is ubiquitously located at position eight (U8) of eubacterial and archaeal tRNAs in the loop region between the acceptor and the D stem. s^4U does not only stabilize the fold of the tRNA, but also plays a central role in bacterial UV protection acting as a sensor for near-UV radiation. U8 is post-transcriptionally modified by a set of enzymes including the 4-thiouridine synthetase ThiI.
Here we report the crystal structure of ThiI from *T. maritima* in complex with a truncated substrate tRNA. The structure demonstrates that ThiI functions only as homo-dimer, since the tRNA acceptor stem including the 3'-recognition element ACCA is bound by the N-terminal ferredoxin-like and THUMP domains of one monomer thereby correctly positioning U8 close to the active site in the pyrophosphatase domain of the other monomer. The structure also indicates that full-length substrate tRNA has to adopt a non-canonical conformation upon binding to ThiI.



Keywords: protein-RNA complexes, RNA-binding proteins, RNA structure

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Combined biophysical techniques used to derive a model for alpha crustacyanin John R Helliwell[a], Ming-chuan Wang[b], Natasha Rhys[b], Clair Baldock[b] and J Günter Grossmann[c,*], [a] *School of Chemistry, University of Manchester M13 9PL, UK,* [b]*Faculty of Life Sciences, University of Manchester M13 9PL, UK,* [c] *STFC Daresbury Laboratory, Warrington WA4 4AD, UK*
** Current address: School of Biological Sciences, University of Liverpool, UK*
E-mail: john.helliwell@manchester.ac.uk

Alpha crustacyanin is a carotenoprotein responsible for the blue-black colouration of lobster shell, perhaps beneficial for camouflage. It is a multi-macromolecular complex of 320kDa, of eight beta crustacyanin dimers and sixteen astaxanthins as the chromophores. Within each beta crustacyanin are two astaxanthins. The chromophore-protein interaction in the beta crustacyanin subunits explains the likely molecular tuning parameters for the first 2/3rds of the 150nm wavelength shift that occurs when a lobster is cooked, thereby turning it red. X-ray crystal structure analysis of the alpha crustacyanin has so far not proved possible and so this study has aimed to create a model of alpha crustacyanin with lower resolution techniques. Such a model can aid in understanding the overall ultrastructure, and specifically the additional wavelength shift seen in the complex but also with possible applications within the colouration industries We have predominantly used negative-stain electron microscopy. A model has been produced via the beta crustacyanin protein crystal structure PDB code 1GKA [1] docked into the EM map of ~30Å resolution, the map being created by analysing 10,021 particle images. The derived EM model has an open conformation and lacks any evident symmetry. The open conformation is consistent with the crystal packing of the beta crustacyanin with its very open layout due to its ~85% crystal solvent content. Further cross checks have been made with small-angle X-ray scattering (SAXS), analytical ultracentrifugation (AUC) and other biophysical approaches to corroborate as much as possible this EM model for alpha crustacyanin. The beta crustacyanin positions have incorporated interfaces analysed by the PISA web server [http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html], as being energetically stable, with the docking procedure incorporating both automated approaches, as well as a by-eye fit i.e. to take into account EM density not considered by the EM software used. The calculated SAXS for the EM derived model is consistent, but not in perfect agreement, with the SAXS data [2]. Separate SAXS data modelling has been made both via ab initio and rigid body modelling methods. Though in some cases the pseudo-helical looping is seen, as in the microscopy envelope, there are too many variables present to produce a conclusive model. The sedimentation velocity AUC indicates a single species with the expected molecular mass. But the sedimentation equilibrium AUC gives evidence of numerous species, implying that dissociation perhaps occurs both randomly and easily. With such an EM model there is always a risk of error such as from the staining and conformational heterogeneity producing artefacts in the single particle images. The development of this study will ideally involve cryo-electron microscopy or fresh crystals; even a crystal diffraction resolution of ~10 Å [2], and harnessing the EM model at 30 Å resolution reported here, would be a further next step forward in ultrastructure detail.

[1] M. Cianci, P.J. Rizkallah, A. Olczak, J. Raftery, N.E. Chayen, P.F. Zagalsky and J.R. Helliwell (2002) PNAS USA 99, 9795-9800.
[2] N. Chayen, M. Cianci, J.G. Grossmann, J. Habash, J.R. Helliwell, G.A. Nneji, J. Raftery, P.J. Rizkallah & P.F. Zagalsky Acta Cryst 2003, D59, 2072-2082.

Keywords: Electron Microscopy, SAXS, crustacyanin