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 IIIs are essential enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones. They change enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones. They change enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones.

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.

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

FA1-MS07-T04

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

The modified nucleoside 4-thiouridine (s4U) is ubiquitously found in many bacteria and archaea and is involved in the regulation of bacterial functions. The modifcation of s4U is catalyzed by the enzyme thiuridine synthetase ThiI, which forms a complex with a truncated substrate tRNA. The crystal structure of this complex was solved and reveals the molecular basis for the formation and reversal of a DNA cleavage complex.

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


Keywords: Electron Microscopy, SAXS, crustacyanin