s8a.m3.p1 **Thiostrepton**. M.P. Shaw, C.S. Bond, M.S. Alphey, W.N. Hunter, *Dept. of Biochemistry, University of Dundee, WTB/MSI Complex, Dow St., DUNDEE, DD1 5EH.*
Keywords: Thiostrepton, antibiotic, translation.

Thiostrepton is an antibiotic that is active against gram positive bacteria[1] and *Plasmodium falciparum*[2]. The molecule was first isolated from *Streptomyces azureus* and a partial crystal structure was elucidated during the late 60's[3]. Thiostrepton inhibits the translocation step of elongation during translation by binding to the large subunit 23S ribosomal RNA (23SrRNA).

Thiostrepton (M.W. 1664.8) has been crystallized in two tetragonal crystal forms. Form 1 crystallized in space group $P4_32_12$ with dimensions a=b=26.62Å and c=27.46Å. Using data to 1.1Å, the structure was solved from the anomalous signal of the sulphur atoms in the molecule. The model underwent least-squares, anisotropic, partially restrained refinement (R=10.7, R_{free}=13.7). Intramolecular interactions of the improved structure were considered. Form 2 crystallized in space group $P4_22_12$ with dimensions a=b=29.63Å and c=26.74Å. Data were collected to 1.05Å. Using the solved thiostrepton structure and a published structure of the protein:RNA complex[4], we can speculate as to how thiostrepton binds 23SrRNA.

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s8a.m4.p1 **Portrait of a membrane protein in action: bacterio-rhodopsin pumping protons**. K. Edman[1], P. Nollert[2], A. Royant[3,4], H. Belrhali[4], E. Pebay-Peyroula[3], T. Ursby[3,4], J. Hajdu[1], R. Neutze[1] & E.M. Landau[2], *[1]Department of Biochemistry, Uppsala University, Biomedical Centre, Box 576, S-751 23 Uppsala, Sweden, Biozentrum, [2]University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland, [3]Institut de Biologie Structurale, UMR 5075 CEA-CNRS-UJF, 41 rue Jules Horowitz, F-38027 Grenoble Cedex 1, France, [4]European Synchrotron Radiation Facility, 6 rue Jules Horowitz, BP 220, F-38043 Grenoble cedex, France.*
Keywords: membrane protein, intermediate state trapping, bioenergetics.

Bacteriorhodopsin (bR) is the simplest known photon-driven proton pump and, as such, is the simplest protein involved in energy conversion. A chromophore, the retinal, is covalently bound through a Schiff Base linkage in the inner core of the protein. Absorption of a photon causes the isomerisation of the retinal, from the all-*trans* to the 13-*cis* configuration. During its photocycle bR passes through a series of spectral intermediates. With each completed photocycle, a proton has been pumped out of the cell.

Using natural two-dimensional bR crystals, low-resolution diffraction studies on intermediate states have led to a structural consensus. No significant change was observed in the case of the K, L & early M states. Significant changes (tilt of the 6^{th} and 7^{th} helices) were assigned to later stages in the photocycle. A novel crystallisation method for membrane proteins was developed using the lipidic cubic phases[1]. bR crystals thus obtained allowed the quest for high-resolution structures of the ground[2,3] state and the intermediate states of the photocycle.

We elucidate the structure of the K state at 2.1 Å resolution[4]. At 110 K, constant illumination by a green diode laser leads to an equilibrium between the ground state and the K_{LT} state, because there is not enough thermal energy to traverse the barrier associated with the formation of a later state. Diffraction data gave reproducible imaging of the small structural changes associated with the transition bR–>K_{LT}. The key water molecule held between the positively-charged Schiff Base nitrogen and the negatively-charged primary acceptor, Asp85, is displaced, preparing an electrostatic environment for efficient proton transfer. The structural rearrangements of residues in the immediate vicinity of the chromophore weaken the stabilising hydrogen-bond network of the 7^{th} helix. This also places strain on a bulky residue of the 6^{th} helix, possibly announcing the movements of these helices later in the photocycle.

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