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Crystals of BcrR, a Membrane-Bound Bacitracin Sensor and DNA-binding Protein. Sue Cutfield[a], Jonathan Gauntlett[b], Gregory Cook[b]. [a]*Biochemistry Department,* [b]*Microbiology and Immunology Department, University of Otago, New Zealand.*
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Bacitracin is a polypeptide antibiotic active against gram-positive bacteria and commonly used in the poultry industry where resistance is a big problem. BcrR has been identified as a novel regulatory protein of high level bacitracin resistance encoded by the *bcrABD* operon in *Enterococcus faecalis*[1]. The N-terminal domain of BcrR has similarity to the helix-turn-helix motif of DNA-binding proteins, and topological modelling predicts that the C-terminal domain contains four transmembrane-helices. These data have led to the hypothesis that BcrR functions as both a membrane-bound sensor and transducer of bacitracin availability to regulate *bcrABD* expression. BcrR, with a C-terminal His-tag, was over-expressed in *E. coli* membranes, extracted with DDM and purified by Ni^{2+}-affinity and size-exclusion chromatography. Purified protein was characterised and reconstituted in liposomes to study its binding to *bcrABD* promoter DNA using electrophoretic mobility shift assays, with and without the presence of bacitracin[2]. To further characterise the protein crystallisation trials were undertaken, again with and without bacitracin. Promising, low-resolution (9A) crystals of BcrR have been obtained and work is in progress to improve these.

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Keywords: membrane protein crystals; bacitracin sensor; DNA-binding protein

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Structure of Membrane-bound Cytochrome *c* Nitrite Reductase NrfHA Complex. Margarida Archer[a], M. Luisa Rodrigues[a], Tânia F. Oliveira[a], Inês A. C. Pereira[a]. [a]*Instituto de Tecnologia Química e Biológica, ITQB-UNL, Oeiras, Portugal.*
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Oxidation of membrane bound quinol molecules is a central step in the respiratory electron transport chains used by biological cells to generate ATP by oxidative phosphorylation. We have determined the X-ray structure of cytochrome *c* quinol dehydrogenase NrfH from *Desulfovibrio vulgaris*, which forms a stable complex with its electron partner, the cytochrome *c* nitrite reductase NrfA to 2.3 Å resolution [1]. One NrfH molecule interacts with one NrfA dimer in an asymmetrical manner, forming a large membrane-bound complex with an overall $\alpha_4\beta_2$ quaternary arrangement. The menaquinol-interacting NrfH heme is pentacoordinated, bound by a methionine from the CXXCHXM sequence, with an aspartate residue occupying the distal position. The NrfH heme 1 that transfers electrons to NrfA has a lysine residue from the closest NrfA molecule as distal ligand. The crystal structure of NrfHA complex bound to inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) at 2.8 Å allowed a detailed characterization of the menaquinol binding-site, close to heme 1 that includes several conserved and essential residues [2]. The menaquinol binding cavity is largely polar and has a wide opening to the protein surface.

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Keywords: nitrite reductase; quinol dehydrogenase; membrane protein complex

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Crystallographic Structure of Xanthorhodopsin, the Light-driven Proton Pump with a Dual Chromophore. Hartmut Luecke. *Departments of Biochemistry, Biophysics and Computer Sciene, University of California, Irvine, USA.*
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Homologous to bacteriorhodopsin and even more to proteorhodopsin, xanthorhodopsin is a light-driven proton pump that, in addition to retinal, contains a noncovalently bound carotenoid with a function of a light-harvesting antenna. We determined the structure of this eubacterial membrane protein–carotenoid complex by X-ray diffraction, to 1.9 Å resolution. Although it contains 7 transmembrane helices like bacteriorhodopsin and archaerhodopsin, the structure of xanthorhodopsin is considerably different from the two archaeal proteins. The crystallographic model for this rhodopsin introduces structural motifs for proton transfer during the reaction cycle, particularly for proton release, that are dramatically different from those in other retinal-based transmembrane pumps. Further, it contains a histidine–aspartate complex for regulating the pKa of the primary proton acceptor not present in archaeal pumps but apparently conserved in eubacterial pumps. In addition to aiding elucidation of a more general proton transfer mechanism for light-driven energy transducers, the structure defines also the geometry of the carotenoid and the retinal. The close approach of the two polyenes at their ring ends explains why the efficiency of the excited-state energy transfer is as high as ~45%, and the 46° angle between them suggests that the chromophore location is a compromise between optimal capture of light of all polarization angles and excited-state energy transfer.

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