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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s8a.m4.p2 **Novel Ion Coordination in Gramicidin, a Membrane Channel.** W.L. Duax, B. Burkhart, *V. Pletnev, N. Li, and W. Pangborn, *Hauptman-Woodward Inst., 73 High St., Buffalo, NY 14203 and *Shemyakin Inst., Moscow, Russia.*
Keywords: ion channel, coordination, ion/π bonding.

The structure determinations of seven ion complexes of gramicidin A provide reliable information on the coordination of K^+, Cs^+, Rb^+ and Tl^+ ions in the channels. All complexes studied thus far crystallize with two gramicidin dimers in the asymmetric unit, antiparallel β-ribbons that coil into right handed β-barrels. At least seven distinctly different sites of ion coordination have been characterized in the fourteen channels. Based upon current data there appears to be between one and two cations per channel distributed over a subset of the seven sites, with partial occupancies ranging from 15 to 90%. There are differences in patterns of ion distribution in the channels. K^+ ions stay closer to the ends of the channel and leave a gap in the channel center. Rb^+, Tl^+, and Cs^+ ions are more uniformly distributed throughout the channel and almost always occupy a position in its exact center. Ion coordination is primarily through the π electron clouds of the peptide carbonyl groups. In the majority of cases, each ion makes three such contacts. The coordination distances between the ions and the carbonyl oxygens range from 2.56 to 3.70°Å. The angles defining the relative positions of the cations and the carbonyl groups (μ_1,M^+...O C) range from 70° to 150° (average 108°). The absolute magnitude of the torsion angles defining the relationship of the ion and the sp^2 plane of the carbonyl groups (μ_2,M^+...O–C_1-N_1) range from 54° to 104° (average 81°). The peptide bonds ω($C\alpha_n$-C_1-N_1- $C\alpha_{n-1}$) of residue involved in ion coordination exhibit significant non-planarity (as much as 18°). ^{13}C NMR chemical shift measurements attributed to Na^+ ion interactions with leucine residues in oriented gramicidin channels in dimyristoyl phosphatidylcholine (DMPC) bilayers correlate with these observations. This coordination geometry differs significantly from that typically observed in ion carbonyl complexes in which the μ_1 angles are near 120° and the ions are more nearly coplanar with the sp^2 plane of the carbonyl group. The observed coordination properties are consistent with the function of gramicidin as a monovalent cation selective membrane channel.