orientation of the M1, M2, and M3 transmembrane segments of the AChR with respect to the pore and each other is very similar to that in the closed-channel structural model developed on the basis of the cryo-EM images or Torpedo's receptor at 4-Å resolution. To the extent that this structural model corresponds to the actual closed-channel conformation, our results indicate that the expansion of the pore that underlies channel opening involves only a limited rearrangement of these three helices. Such a modest change seems optimal to ensure rapid closed-open interconversion rates, and hence, a fast postsynaptic response upon neurotransmitter-binding.

**2905-Symp**
**Modulation of AMPA and kainate receptors by accessory subunits**
**James Howe**.
Yale University, New Haven, CT, USA.

**2906-Symp**
**Mechanisms For Information Processing By NMDA Receptors: Insights From Single-channels**
**Gabriela K. Popescu**.
University at Buffalo (SUNY), Buffalo, NY, USA.
NMDA receptors are glutamate-activated ion channels with key roles in synaptic transmission and information processing in the brain. Their calcium permeability and voltage-dependent magnesium block have been long identified as critical to these roles. In addition, NMDA receptors have exceptionally slow kinetics and are sensitive to a broad array of neuromodulators. We hypothesized that these two latter features allow NMDA receptors to recognize and integrate a variety of extracellular signals.
After rapidly binding glutamate and glycine, NMDA receptors activate slowly by navigating a complex gating pathway marked by peaks and valleys in the energy landscape of their activation reaction. At equilibrium, fully liganded receptors distribute among multiple pre-open, open and desensitized states which can be resolved in single-channel records and characterized kinetically with statistical analyses. With this approach, we measured changes in the gating energy landscape induced by individual allosteric modulators to learn how NMDA receptors process and integrate chemical information presented in their extracellular environment,
We characterized the effects of protons, zinc, partial agonists, and the neurosteroids pregnanolone sulfate and pregnenolone sulfate on the gating reaction of NR1-1a/NR2A receptors. We found that each of these modulators exert specific, signature effects on the gating reaction. These changes can be expressed as unique sets of gating rate constants. The resulting quantitative kinetic models represent valuable tools for further structure-function investigations, as well as for determining how neuromodulators affect the NMDA receptor response to a variety of physiologic and pathologic stimulation patterns.

**2907-Symp**
**Problems In Determining A Mechanisms Of Receptor Activation And Relating It To Structure**
**David Colquhoun**.
UCL, London, United Kingdom.
Our aim is to understand receptors well enough to be able to make predictions about the activity of a new ligand or about the effect of mutations. This has yet to be achieved but it will entail knowing about both structure and function, and about the relation between them.
Receptors on ion channels that mediate synaptic transmission are essentially never at equilibrium. To understand how they function in real life requires a physical mechanism to be postulated and, if verified, the rate constants in that mechanism to be estimated. The mechanism must describe states that have real physical reality (to a sufficient approximation) if the aim of achieving predictive ability is to be attained. Recent advances in single channel analysis have allowed measurement of up to 18 rate constants, far more than can be obtained from any macroscopic analysis, and it has allowed more stringent tests of proposed mechanisms too. At a limited level some predictive ability has been achieved. For example, the synaptic current produced by glycine can be predicted from steady state single channel analysis (Burzomato et al. 2004, J. Neurosci. 24, 10924-10940), but the ability to predict the effects on function of changes in structure of either the ligand or the receptor is still very limited (though this fact is often disguised by exaggerated claims). It is impossible to say what the next major step forward will be. My guess, for what it's worth, is that single molecule fluorescence methods, perhaps combined with improved NMR and molecular dynamics, may improve enough to allow a firm structural interpretation of the postulated intermediate states in channel opening, despite their brief lifetime of 10 microsec or less (Lape et al. 2008, Nature, 454, 722 - 728)

# Minisymposium 4: Electron Transfer and Energy Coupling Reactions in Organelles

**2908-MiniSymp**
**The Reaction Of Cytochrome $aa_3$-600 With Radical Trapping Agents**
**Bruce C. Hill**, Diann Andrews, Graeme B. Mulholland.
Queen's University, Kingston, ON, USA.
Cytochrome $aa_3$-600 or menaquinol oxidase from *Bacillus subtilis* is a member of the heme-copper oxidase family that includes mitochondrial cytochrome *c* oxidase. A distinguishing feature of cytochrome $aa_3$-600 is that it does not oxidize cytochrome *c* and does not contain a $Cu_A$ center, but instead uses menaquinol as reducing substrate to convert $O_2$ to water. A radical signal is observed when cytochrome $aa_3$-600 is frozen during the course of steady-state catalysis. The nature of this radical is not fully characterized and we aim to understand it further by using the radical traps 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 2-methyl-2-nitrosopropane (MNP) and N-*tert*-butyl-phenylnitrone (BPN). TEMPO appears to inhibit menaquinol oxidase's steady state activity, whereas MNP and BPN are without effect. Heme-copper oxidases form a series of intermediates when exposed to $H_2O_2$ that are related to the intermediates formed in the much faster reaction with oxygen. Addition of $H_2O_2$ to oxidized cytochrome $aa_3$-600 leads to formation of the ''P-state'' (606 nm), which is followed by progression to the ''F-state'' (580 nm). The progress of this reaction is halted at the P-state when performed in the presence of TEMPO (50 μM- 5mM). In addition if TEMPO is added at the end of the $H_2O_2$ reaction the F-state is converted back to the P-state. We propose that the inhibition of cytochrome $aa_3$-600 by TEMPO is mediated by its ability to trap the P-state of the enzyme and slow its progress through the catalytic cycle.

**2909-MiniSymp**
**Two Conformations Of The Cytochrome C Oxidase Discriminated By Spectro-electrochemistry Using Seiras**
**Renate L.C. Naumann**.
MPI for Polymer Research, Mainz, Germany.
Electronic wiring of cytochrome c oxidase (CcO) from *R. sphaeroides* to gold surfaces was employed to monitor redox changes through redox centers, $Cu_A$, heme a, heme $a_3$ and $Cu_B$. Electrochemical investigations revealed that under aerobic and reducing conditions the enzyme undergoes a gradual transition into an activated state. It is only in this state that proton pumping and catalytic currents can be observed. The potential of the catalytic current, however, is shifted by 450 mV negative from the standard redox potential of $Cu_A$. In contrast, ''correct'' standard redox potentials of all the centers in the positive potential range can be observed if the enzyme kept under anaerobic and oxidizing conditions. Then no proton pumping does take place. This state is therefore considered as a non-activated state. The transition between the two states is fully reversible. This was also verified by electrochemically-controlled surface-enhanced infrared absorption spectroscopy (SEIRAS) and surface-enhanced resonance Raman spectroscopy (SERRS).
**Activated Non-activted**
Ch. Nowak, Ch. Luening, W. Knoll, R. L. C. Naumann, A two-layer gold surface with improved surface-enhancement for spectro-electrochemistry using SEIRAS, JPC(C) under review

**2910-MiniSymp**
**Rapid Freeze-quench Trapping Of Intermediates In The Reaction Of Cytochrome *c* Oxidase With Hydrogen Peroxide**
**Michelle A. Yu**, Gary J. Gerfen, Syun-Ru Yeh, Denis L. Rousseau.
Albert Einstein College of Medicine, Bronx, NY, USA.
Hydrogen peroxide treatment of Cytochrome *c* Oxidase (C*c*O) at low and high pH is commonly used to form the ''P'' and ''F'' oxygen-intermediates. The structure and function of intermediates formed in the reaction of bovine C*c*O with $H_2O_2$ are studied using a custom rapid freeze-quench device designed to trap fast biochemical intermediates on the 50 to 500 μsec time scale. Oxidized bovine C*c*O was mixed with $H_2O_2$ at pH 6 and pH 8 at room temperature. Reaction intermediates were freeze-quenched at 77 K and examined by optical absorption and EPR spectroscopy. A new carbon-based radical signature arises, which is distinct from the ''broad'' and ''narrow'' species trapped via manual mixing of $H_2O_2$ and bCcO at low and high pH. A comparison between the new radical, the narrow, and the broad species was conducted at X-band (9 GHz) and D-band (130 GHz) EPR. Assignment to oxygen-intermediates was made by low-temperature optical absorption. The reaction mechanism is reassessed based on these results.