dependent activation that rapidly reverses in the dark. Thus, the new re- 
shifted MAO further validates that the two-component system tolerates vari-
ation of the PTL and provides tunable control of channel photoactivation 
properties.

1922-Pos Board B652
Site-Specific Tagging of Channelrhodopsins with Genetically-Encoded Azido Groups
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Channelrhodopsins (ChRs) are light-gated cation channels widely used in op- 
togenetics because they can trigger depolarization of membrane potential 
upon illumination. In order to investigate the mechanism of channel opening, 
we used amber stop codon suppression to introduce the unnatural amino acid 
(uaa) p-azido-phenylalanine (azF) into expressed ChRs with high efficiency. 
Based on the recent crystal structure of a ChR-hybrid, amino acid residues in 
vicinity of regions that might be involved in the channel gating process were 
chosen as targets for replacement with azF. AzF-containing mutants were pu-
rified from mammalian cells in satisfactory yields with expression levels of 
up to ~35% compared with wild-type receptor, which matches earlier expe-
rience with CCR5 and bovine rhodopsin. We also developed a simple proce-
dure to reconstitute ChR azF mutants into POPC-bilayer-membranes for 
future spectroscopy studies. The site-specific azF tag provides a useful 
FT-IR (Fourier Transform Infrared) spectroscopy probe because of its small 
size and its unique vibrational signature, which is well separated from 
intrinsic protein backbone signals. FT-IR difference spectroscopy in combi-
nation with uaa-mutagenesis can be used to track changes in the electrostatic 
environment of the azido probe and reveal local structural movements 
without impairing significantly the native protein architecture. In addition 
to direct interrogation of azF tags, exploiting the chemical property of the 
azido group as bio-orthogonal coupling site could allow specific functionali-
ization of ChR. ChR azF mutants were reacted with fluorophore adducts using 
strain-promoted azide-alkyne cycloaddition chemistry. Coupling efficiency at 
multiple sites was determined by in-gel fluorescence scanning and UV-Vis 
spectroscopy. The current work describes for the first time the successful 
introduction of uaa into ChR variants, demonstrating a robust and powerful 
technology to investigate function and mechanism of this important class of 
photoreceptors.

1923-Pos Board B653
Encoding the Light-Sensitivity of Channelrhodopsin-2
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Microbial rhodopsins are routinely used as light-controlled switches in 
neurobiology. Their versatile applicability relies on the simplicity of the op-
togenetic approach. Light-sensitivity is promoted to the host cell encoded in 
genetic information. However, the signal output is limited by the expression 
threshold of about 180mV which is associated with an increase in the base-
line intensity of Pado (Δlmax). This ChR has properties advantageous for light modulated neuronal 
control including a red-shifted λmax and slow light inactivation compared to 
ChR2 (Hou, S. et al. (2012) Photochem Photobiol 88, 119-128). Near-

1924-Pos Board B654
Resonance Raman and Low Temperature FTIR Characterization of the Red Shifted Channelrhodopsin 1 from Chlamydomonas Austriaca
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Channelrhodopsins (ChRs) control phototaxis in green algae and function as 
light-gated cation channels when expressed in animal cells. Because ChRs 
can be functionally expressed in neuronal membranes, this distinct family of 
microbial rhodopsins have rapidly become an important tool in neuroscience. 
While the light-activated molecular changes occurring in channelrhodopsin-2 
from Chlamydomonas reinhardtii (ChR2) have been extensively studied, lit-
tle is known about such changes in the diverse groups of other ChRs including 
the major class of channelrhodopsin-1 (ChR1). Here, we have characterized the 
structure and molecular changes in ChR1 from Chlamydomonas austriaca 
(CaChR1). This ChR has properties advantageous for light modulated neuronal 
control including a red-shifted λmax and slow light inactivation compared to 
ChR2 (Hou, S. et al. (2012) Photochem Photobiol 88, 119-128). Near-

1925-Pos Board B655
Pado, a Novel Fluorescent Voltage-Sensing Protein, Identified by a Highly 
Conserved Motif in the S2 Trans-Membrane Segment
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A highly conserved sequence motif in the S2 trans-membrane segment of the voltage-sensing domain was used to search and identify novel voltage-
sensing proteins. This motif pulled down in silico proteins with homology to 
voltage-gated calcium, potassium, sodium and proton channels, as well as 
voltage-sensing phosphatases in addition to several proteins of unknown 
function. To test for voltage-sensitive optical responses, we fused the puta-
tive voltage-sensing domain from 8 novel proteins to the fluorescent protein 
super ecliptic pHlorin A227D. The cytosolic amino terminus of the Ciona 
voltage sensitive phosphatase was used to improve plasma membrane expres-
sion. The voltage-gated proton channel from liver fluke, which we call Pado 
(ΔpH), gave a large optical signal (>10%) in response to a 200mV depolar-
ization. Decreasing the extracellular pH resulted in a reduced current and 
caused a substantial decrease in the optical signal upon membrane depolar-
ization. Decreasing the extracellular pH resulted in a reduced current and 
also reduced the optical signal upon membrane depolarization. These results 
suggest that the voltage-gated current is due to the voltage-gated proton channel and that the corresponding change in including pH affects the fluores-
cent intensity of Pado (ΔpH). This search criterion is capable of identifying 
novel voltage-gated proteins that can be used to generate voltage-sensing 
probes.

1926-Pos Board B656
Light-Regulated GABA_A Receptors: An Optogenetic Toolset for Studying Neural Inhibition
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GABA_A receptors (GABA_ARs) are the major mediators of inhibitory neuro-
transmission in the brain. The diverse composition of the GABA_AR pen-
tamers, which determines receptor localization and biophysical properties, 
leads to the heterogeneity and complexity of GABAergic transmission. To