Abstract Submitted
for the MAR12 Meeting of
The American Physical Society

FTIR and RRS Study of the Archaerhodopsin-3 Optogenetic Neural Silencer and Transmembrane Voltage Sensor ERICA SAINT CLAIR, JOHN OGREN, SERGEY MAMAEV, Boston University, Department of Physics, JOEL KRALJ, Harvard University, Chemistry and Chemical Biology, KENNETH ROTHSCHILD, Boston University, Department of Physics — Archaerhodopsin-3 (AR3) is a light driven proton pump from *H Sodemense* with a 74% sequence homology to the more extensively studied bacteriorhodopsin (BR) from *H Salinarum.* Recent studies show that the wild type (WT) AR3 functions as a high-performance, genetically targetable optical silencer of neuronal activity and the mutant D95N functions as a transmembrane fluorescence voltage sensor. In order to understand the molecular similarities and differences between AR3 and BR, we compared light-activated structural changes using resonance Raman spectroscopy (RRS) and Fourier transform infrared (FTIR) difference spectroscopy.RRS pH titration and H/D exchange of WT AR3 showed that the retinylidene chromophore structure and Schiff base hydrogen bond strengths are almost identical to BR. RRS of the mutant D95N revealed a mixture of an N-like and O-like species at a pH greater than 7, unlike WT AR3. Low-temperature and rapid-scan time-resolved FTIR difference spectroscopy of WT AR3 revealed conformational changes during formation of the K, M and N intermediates similar but not identical to BR. Positive/negative bands in the region above 3600 cm^{-1}, which have been assigned to changes in weakly hydrogen bonded internal water molecules, differed substantially between AR3 and BR. These results indicate molecular differences between the AR3 and BR proton pumps which may underlie the ability of AR3 to function as a neurophotonic switch and sensor.

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Date submitted: 11 Nov 2011 Electronic form version 1.4