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Voltage Dependent Conformational States of the Transmembrane Protein Prestin Measured By FLIM-FRET Techniques

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The transmembrane protein prestin forms an integral part of the mammalian sense of hearing by providing the driving force for the electromotility of the outer hair cell, a specialized cell that resides within the cochlea. This provides the cochlea with an ability to amplify mechanical vibrations, allowing for a high degree of sensitivity and selectivity in auditory transduction. This phenomenon, driven by changes in the transmembrane potential, is thought to be the result of conformational changes in self-associating prestin oligomers. We have previously utilized Forster resonance energy transfer (FRET), by acceptor photobleach methods, to detect the changes in these conformational states in response to controlled voltage stimulus. While these methods reported positive results, the standard deviation of the FRET efficiency was not sufficient for determining nanoscale changes in prestin organization. Here we expand upon this work by utilizing fluorescence lifetime imaging (FLIM) detected by time correlated single photon counting (TCSPC), the most accurate FRET measurement technique available. FLIM techniques measure the characteristic fluorescence decay profiles of reporters; changes in these profiles can be caused by quenching processes such as FRET activity. Human embryonic kidney cells are used to host prestin molecules that are genetically encoded with either teal fluorescent protein (TFP) or the yellow fluorescent protein variant citrine. Individual cells are then voltage-clamped and the intermolecular distance between TFP and citrine is probed via FLIM-FRET methods. This has revealed large changes in detected FRET efficiencies between hyperpolarized cells (32% efficiency) and depolarized cells (13% efficiency), implying significant changes in prestin conformation.