High-Throughput Crystallographic Screening Method and Device for Membrane Proteins Using Electric Fields

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Membrane proteins comprise approximately 26% of the human proteome. These include receptors involved in cell-cell signaling, pores, channels, and pumps. Over 40% of the current targets of drugs are membrane proteins, but to date only around 155 *unique* human membrane protein structures have been determined. *There are thousands of potential drug targets to be discovered.* This is an extraordinary incentive to obtain high-resolution 3D structural data of membrane proteins. It is well known that membrane proteins must be solubilized prior to crystallization, a procedure that comes with the potential for destabilization. Furthermore, standard crystallization methods include performing large screenings over many different parameters, including sample concentration, pH, precipitants, and temperature, oftentimes yielding negative results. There is a *critical need* for a reliable method of crystallizing membrane proteins. Reproducibility is of particular importance in crystallography methods. The ability to consistently produce high-quality membrane protein crystals opens up the possibility of utilizing different ligands during the crystallization process in order to probe their effects on the conformation of the proteins. All membrane proteins are embedded in a membrane that includes polar lipids and differential ionic concentrations on either side, which results in an electric field across them. Thus, it is fair to hypothesize that the structural conformations of localized domains in membrane proteins are voltage-dependent. This principle is utilized in the high-throughput crystallographic screening device for membrane proteins that we are designing, prototyping, and testing. The device applies a physiological resting membrane potential to a protein-detergent complex in a lipid matrix (RMP@LMx device, US Patent No. 10,155,221, Notice of Allowance - UPR-18280 15/997,728, and provisional patent 15/996,946). It is also capable of applying voltage ramps and other waveforms. This device will be used to conduct high-throughput screening of membrane protein crystals. The overall objective of this project is the design, fabrication, and testing of an instrument that performs high-throughput membrane protein crystallization trials. This may be further applied to many biologically important membrane receptor systems and channels.