Improving Sample Preparation in Cryo-EM

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Cryo Electron Microscopy (Cryo-EM) is a method used to determine the structure of macromolecules. Cryo-EM is used for complexes that are 250kDa or larger and now it is possible to analyze a protein structure at better than 2.3 angstroms resolution. A higher concentration of viruses and proteins is needed within a specific area to rapidly grab the series of images needed to reconstruct their 3D structure using Cryo-EM. The goal of this project is to create a nonlinear voltage gradient, which can control macromolecular structures, like viruses and proteins. Fluorescent microbeads were used instead of viruses and proteins. A sine wave (2 volts peak to peak) induces a dipole moment, which can move the microbeads in a specific pattern. Three test grids were created, each with different amounts of overlap between their opposing finger-like electrodes. Soon after the nonlinear voltage gradient was applied, the microbeads became oriented in diffuse lines, varying in concentration depending on the electrode array. Efficiency was determined via width of the line. A one-way ANOVA [$F(2,27)= 279.39$ $p < 0.05$] showed statistical differences between the grids. A Tukey HSD showed that grid 3 (500 µm) outperformed grid 2 (2 mm) and grid 1 (no overlap). The experiment was repeated on etched PC boards, but the distance between the electrodes was tested. A one-way ANOVA [$F(2,27)= 325.68$ $p < 0.05$] showed statistical differences between the grids. A Tukey HSD showed that grid A (1 mm) outperformed grid B (3 mm) and grid C (5 mm).