Serial Crystallography-Optimized Sample Preparation With MYELIn: Novel Homogeneous Protein Microcrystal Yield Density Control via Sequential Pulsed Electric Fields and Ultrasonic Irradiation

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G protein-coupled receptors (GPCRs) are targeted by 30-40% of FDA-approved drugs, yet structure determination remains hindered by cryogenic conditions of conventional methods such as macromolecular crystallography and cryo-EM that introduce non-physiological conformations. Serial crystallography (SX) allows room-temperature experiments but lacks a universal sample preparation method that allows the tuning of size and density of the microcrystal population. This study developed a sequential optimization setup through pulsed electric fields (PEF1, PEF2) and ultrasonic waves (US) by targeting three distinct windows of the two-step nucleation pathway—during LLPS formation, nuclei homogenization shortly after nucleation, and crystal growth. Phase-field simulations (Cahn-Hilliard/Allen-Cahn) identified 2 Hz and 0.05 amplitude as optimal for PEFs. Proof-of-principle and proof-of-concept experiments used lysozyme (HEWL) as model protein in 8-channel indium tin oxide (ITO) reaction cells for PEFs and a plate transducer with deionized water as medium for US, with the fields applied in the sequence of PEF1–US–PEF2, commensurate with the windows. Simulations confirmed PEF1 increases nucleation sites while PEF2 favors growth. Experimentally, with respect to control, PEF1 created more crystals below 25 microns. US produced a <10-micron population. PEF2 generated larger microcrystals up to ~90 microns. The sequential optimization concentrated the population in the SX-optimal 30-50 micron range with a tighter distribution than controls. This presents a proof-of-concept for a tunable, from-scratch microcrystal preparation for SX, where PEF1 controls quantity, US homogeneity, and PEF2 size, allowing preparation tailored to the idiosyncratic requirements of certain structural experiments.

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