Enhancing Protein Crystallization Screening Results Using Engineered Nucleation Features

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Protein structure determination remains a critical facet in understanding cellular functions and developing treatments for disease, and macromolecular X-ray crystallography remains the benchmark method for determining these structures at atomic resolution. Despite advances in high throughput crystallization workflows, the time- and sample-consuming challenges of crystallization still result in 80% of crystallization screens failing to produce positive hit information, let alone crystals of diffraction quality. DeNovX is developing crystallization platforms that use engineered nucleation features (ENFs) to improve crystallization outcomes while retaining the requisite diffraction quality. Overall, DeNovX has demonstrated 1.1-8.7-fold increases in crystallization hit percentages while reducing time to crystallization by an average of 40% for a group of proteins known to crystallize (e.g., lysozyme, BPT, etc.) and for proteins with less well characterized crystallization behavior. In order to assess crystal quality, synchrotron X-ray diffraction data were collected and structure determinations were conducted for control samples and for crystals formed more rapidly using ENFs, and the resolution and quality metrics are comparable to their relevant benchmark structures in the PDB. In addition, crystallization using ENFs generates an average of 2.5-fold more crystalline material, which is beneficial in emerging techniques like fixed-target serial crystallography that are acutely sample-destructive and can require hundreds of crystals per study.