What is the best target protein density for TELSAM fusion crystallization?

Crystallization is the rate-limiting step in macromolecular X-ray diffraction studies. Usually, crystallization methods are only successful for a fraction of known proteins, and producing a single crystal with high resolution is a time-consuming, expensive, and laborious process. TELSAM is a protein crystallization chaperone which enhances crystallization propensity. We investigated the effects of target protein loading and histidine tag inclusion on crystallization dynamics and crystal quality. We tested displaying 2, 3, or 6 copies of a 9 kDa UBA domain or a 19 kDa vWa domain per turn of the TELSAM polymer. We used a flexible linker between TELSAM and the target proteins. Fusing different numbers of TELSAM subunits dictates the number of target proteins displayed per turn of the TELSAM polymer. In 1TEL there are 6 target proteins per turn while there are 3 and 2 target proteins in 2TEL and 3TEL constructs. To test the effect of the 10x histidine tag on TELSAM fusion crystallization, we made each construct with either a permanent or cleavable His tag. Protein crystals were obtained for all the UBA and vWA constructs. The crystallization time, crystallization propensity, crystal size, crystal shape, crystal lattice architecture, structure refinement statistics, diffraction resolution, fraction of reflections indexed, mosaicity, presence of twinning or broken periodicity, and ease of solving the structure was compared for the obtained crystals. We found that 1TEL-UBA fusions crystalized within 24hrs, and they crystalized better without the His tag while the 2TEL and 3TEL constructs crystalized better with the His tag. The 1TEL- vWA fusion crystalized better with the His tag and resulted in the current highest-resolution (1.2 Å) structure of this protein. Our work reveals 1TEL to be the superior TELSAM variant. The His tag presence has positive or negative effects depending on the display density and the specific target protein. These insights enable increased understanding and utilization of TELSAM fusion crystallography and will speed protein structure studies and open new pathways in drug design.

BY: Prasadika Samarawickrama
September 24, 2024