Fusion to the TELSAM Protein Polymer Dramatically Improves the Speed of Target Protein Crystallization by Stabilizing Weak Crystal Contacts

James Moody[1], Supeshala Sarath Nawarathnage[2], Sara Soleimani[3], Moriah Longhurst[4], Braydan Bezzant[5], Diana Ramirez[6], Parag Gajjar[7], Derick Bunn[8], Cameron Stewart[9], Tobin Smith[10], Maria Pedroza Romo[11], Seth Brown[12], Nathan Towne[13]

[1]No affiliation given [2]No affiliation given, [3]Brigham Young University, [4]Brigham Young University, [5]Brigham Young University, [6]California State University, Chico, [7]Brigham Young University, [8]Brigham Young University, [9]Brigham Young University, [10]Brigham Young University, [11]Brigham Young University, [12]Brigham Young University

[13]Brigham Young University

jdmoody@chem.byu.edu

We investigate the requirements for TELSAM protein polymers to reliably form well-diffracting crystals when fused to target proteins. We tested various numbers of target proteins fused per turn of the TELSAM helical polymer, both semi-rigid and flexible TELSAM–target connections, and a variety of target proteins. The time to crystal appearance versus the target protein alone was quantified, as was the diffraction quality of the resulting crystals. We determined in multiple cases that fusion to TELSAM accelerates the rate of crystal formation by as much as 27-fold versus the target protein alone. While crystals can be obtained by fusing either 2, 3, or 6 copies of the target protein per turn of the 6-fold helical TELSAM polymer, fusions of 2 or 3 copies frequently result in datasets that are difficult to phase, while fusion of 6 copies invariably gives datasets that can be readily phased and refined. We determined that adjacent polymers need not make direct contacts to form well-diffracting crystals of TELSAM–target protein fusions, being separated by at least 40 Å in some cases. Flexible linkers between TELSAM polymers and target proteins do not appear to impair crystal quality, as the target proteins are able to find low-energy binding modes against the polymers. In some cases, TELSAM is seen to stabilize extremely weak inter-target protein crystal contacts, likely through an immense avidity effect. In cases where the target protein's structure is not known, the TELSAM polymer is able to solve the X-ray phases, allowing the target protein to be built de novo into the electron density. Additionally, fusion to TELSAM is able to rapidly grow crystals at protein concentrations as low as 1 mg/mL, opening the door to crystallizing marginally soluble proteins. We conclude that TELSAM is a powerful crystallization chaperone meriting further investigation.