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Using Surface Entropy Reduction to Improve the Crystallization Properties of a Recombinant Antibody Fragment RNA Crystallization Chaperone

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Abstract : Phage displaying synthetic Fab libraries have been used to obtain Fabs that bind to specific RNA targets with high affinity and specificity. These Fabs have been demonstrated to facilitate RNA crystallization. However, the antibody framework used in the construction of these phage display libraries contains numerous bulky, flexible, and charged residues, which facilitate solubility and hinder aggregation. These residues can interfere with crystallization due to the entropic cost associated with burying them within crystal contacts. To systematically reduce the surface entropy of the Fabs and improve their crystallization properties, a protein engineering strategy termed surface entropy reduction (SER) is being applied to the Fab framework. In this approach, high entropy residues are mutated to smaller ones such as alanine or serine. Focusing initially on Fab BL3-6, which binds an RNA AAACA pentaloop with 20nM affinity, the SER P server (http://services.mbi.ucla.edu/SER/) was used and analysis was performed on existing RNA-Fab BL3-6 co-crystal structures. From this analysis twelve surface entropy reduced mutants were designed. These SER mutants were expressed and are now being measured for their crystallization and diffraction performance with various RNA targets. So far, one mutant has generated 3.02 angstrom diffraction with the yjdF riboswitch RNA. Ultimately, the most productive mutations will be combined into a new Fab framework to be used in a optimized phage displayed Fab library.

Keywords : antibody fragment, crystallography, RNA, surface entropy reduction
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