Strategies To find Low-Occupancy Ligands in A Protein-Peptide Crystal

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DNA repair in pathogenic bacteria is a promising area for drug target development to tackle the challenge of antimicrobial resistance. Bacterial DNA repair pathways contain an array of protein-protein and protein-DNA interactions that if inhibited, would prevent DNA repair. In turn, lack of DNA repair may increase the efficacy of current antibiotics or prevent the evolution of resistance against antibiotics that generate DNA damage as a killing mechanism. Therefore, atomic structures of the essential protein-protein and protein-DNA interactions are vital to understand the molecular mechanism of DNA repair and provide a template for structure-based drug design. Using biochemical and biophysical analysis, we have identified a protein-protein interaction that is necessary for repair of a DNA break. We have crystallized a complex of one protein bound to a fluorescently labelled peptide of the second protein. The structure of the first protein has been previously solved and it readily crystallizes, therefore the fluorescent labelling identifies protein crystals that also contain the peptide. However, it is still unclear if the peptide is present with sufficient occupancy to be visible in the experimental electron density maps. We will show the additional optimizations we have made to crystallizing the protein-peptide complex to increase peptide occupancy and strategies for data collection and refinement that will ideally lead to determining the structure of the protein-peptide complex. This structure will help us to better understand how the protein-protein interaction leads to efficient DNA repair, while providing a structural model for inhibitor design.