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"From one seed a whole handful": homologous proteins as seeds in crystallisation

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Protein structures have significantly impacted and aided drug discovery efforts. However, it is not enough to know the structure of a protein; it must be the right structure. Small alteration in sequence can lead to different conformations and oligomerization states, cause changes which lead to different active site architecture and also which modify function. Protein crystallization is an essential prerequisite for the determination of protein structures by X-ray crystallography. We have obtained encouraging initial results for a hitherto unexplored crystallization method with the enzyme arylamine N-acetyltransferase from M. tuberculosis (TBNAT). Despite prolonged and varied trials to crystallize TBNAT, an important anti-tubercular drug target, no crystals were obtained. In an alternative approach, cross-seeding of TBNAT protein with micro-crystalline seeds from a homologous NAT from M. marinum (74 % sequence identity (SID)) surprisingly resulted in a single 20 micron sized TBNAT crystal that diffracted to 2.1 Å and allowed for TBNAT structure determination (Abuhammad et al., 2013). To our knowledge, cross-seeding crystallisation using homologous proteins has only been previously successful in cases with more than 85% SID. In this study, we have explored the effect of low sequence homology on cross seeding using β-lactamases with SID as low as 30%. Despite the low SIDs, the results show cross seeding leads to an increase in hits obtained, the identification of new crystallization conditions, shortening of crystallization time and an improvement in the quality of the crystals obtained.

[1] *Abuhammad A, Lowe ED, McDonough MA, et al. (2013). Structure of arylamine N-acetyltransferase from Mycobacterium tuberculosis determined by cross-seeding with the homologous protein from M. marinum: triumph over adversity. Acta Crystallogr D Biol Crystall*

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