

## MS41-02 | FOUR FOR THE PRICE OF ONE: CROSS-SEEDING TO OBTAIN CRYSTALS OF ANCESTRAL ELONGATION FACTOR TUS

Majumdar, Soneya (Department of Cell and Molecular Biology, Uppsala University, Uppsala, SWE); Bergfors, Terese (Department of Cell and Molecular Biology, Uppsala University, Uppsala, SWE); Sanyal, Suparna (Department of Cell and Molecular Biology, Uppsala University, Uppsala, SWE)

Elongation factor Tu (EF-Tu) is an essential translation factor which brings aminoacyl tRNAs to the ribosome. The strong correlation between the thermal stability of EF-Tu and the optimal growth temperature of the host makes EF-Tu an attractive candidate for studying protein evolution. Gaucher et al. [1] have reconstructed several sequences of EF-Tu, which represent the ancient nodes of the bacterial evolutionary tree.

In this study we crystallised, and subsequently determined, high-resolution structures of four of these nodal EF-Tus—EF-Tu 170, 184, 262, and 317. They have high sequence identity with each other (84-92%), and  $T_m$  values ranging from 39.1 to 66.7°C. Crystals of EF-Tu 262 which were obtained first, diffracted to 2Å and were used to cross-seed the other three homologs by matrix microseeding [2]. Hits generated in this round of matrix microseeding were then used for further cross-seeding experiments of the proteins with each other in various permutations. Intriguingly, the best crystals were obtained by cross-seeding the nodes that had more similar biophysical characteristics, rather than the highest sequence identity.

Despite the many successes reported with cross-seeding [2], it is still unclear why it works for some homologs and not others. We suggest that the biophysical properties of the homologs may be a more important consideration than their sequence identity when selecting an appropriate cross-seed.

[1] Gaucher EA, Govindarajan S & Ganesh OK (2003) *Nature* 451, 704–707.

[2] D'Arcy A, Bergfors T, Cowan-Jacob SW & Marsh M (2014) *Acta Crystallogr. F Struct. Biol. Commun.* 70(Pt 9): 1117–1126.