MS06 P11

Heating enables reproducible crystallization of amyloidogenic transthyretin. Anders Karlsson, A. Elisabeth Sauer-Eriksson, *Umeå Center for Molecular Pathogenesis, Umeå University, Sweden*
E-mail: anders.karlsson@ucmp.umu.se

Keywords: Crystallization, Protein heating, Transthyretin

The use of high temperature incubation in the purification of heat-stable proteins is a well established technique. Recently, heating of proteins was also described as a final polishing step to remove less stabile forms of apparently pure protein, thereby improving the diffraction properties of crystals [1]. In the present study, we show an example where high temperature incubation of a highly aggregation prone mutant removed heterogenic forms of the protein and allowed us to reproducibly produce well diffracting crystals.

The triple substitution mutant of the plasma transport protein transthyretin (TTR) G53S/E54D/L55S rapidly forms amyloid-like aggregates in solution. Despite being very heterogenic and aggregation prone in solution, one batch of the protein was successfully crystallized in our lab [2]. The solved structure (PDB ID 1G1O), revealed significant structural changes, suggested to be of direct relevance for the mutant's propensity for aggregation. Despite extensive attempts, reproducing these crystals proved impossible, thus preventing further structural and biochemical studies of the crystallizable form of the mutant. After optimization of the protein expression and purification protocols, the homogeneity was increased and crystals were obtained. These were however small, fragile and non-diffracting.

By prolonged incubation of the protein at a high temperature, less heat-stable forms of the mutant were precipitated, leaving stabile tetrameric TTR in solution. No major improvement of the resolution was detected. However, by being able to reproducibly isolate and crystallize a stabile species of the *in vitro* amyloidogenic transthyretin mutant G53S/E54D/L55S, further biochemical and structural characterization of this aggregation prone mutant is now possible.

[1] Pusey, M. L., Liu, Z. J., Tempel,W., Praissman, J., Lin, D.,Wang, B.-C., Gavira, J. A., Ng, J. D. (2005). Prog. Biophys. Mol. Biol. 88, 359–386.
[2] Eneqvist T., Andersson K., Olofsson A., Lundgren E., Sauer-Eriksson A.E. (2005). Mol Cell. 2000 Nov;6(5):1207-18.