Directed Evolution of *Bacillus subtilis* Lipase A Towards Thermostability: Role of Aggregation

Augustyniak W (1), Brzezinska AA (1), Wienk H (2), Boelens R (2), Reetz MT (1)
(1) Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany
(2) Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands

Lipase A from *Bacillus subtilis* is one of the best known lipases and a good target for examination of concepts in biocatalysis. Recently, B-FIT driven iterative saturation mutagenesis yielded mutants that retain their activity upon heating and cooling down [1,2]. The availability of these mutants opens up the opportunity of studying the evolutionary process using biophysical tools.

Three mutants and wild-type lipase were prepared and characterized using thermal inactivation profiles, circular dichroism, fluorescence, activity assays and NMR spectroscopy. The results suggest that the mutants retain their activity upon heating and cooling due to prevention of aggregation. Wild-type Lipase A does not show this effect; it unfolds and precipitates upon heating and does not re-fold whilst cooling down. Interestingly, the mutants are thermodynamically slightly less stable than the wild-type enzyme.

The wild-type and the best mutant were selected for comparative NMR studies. Structure determination, dynamics, H/D exchange and temperature coefficients are reported for the mutant. Currently, similar studies of wild-type Lipase A are underway.

References:
[1] M. T. Reetz, J. D. Carballeira, A. Vogel: Iterative saturation mutagenesis on the basis of B factors as a strategy for increasing protein thermostability, *Angew. Chem. Int. Ed. Engl.*, **2006**, *45*, 7745-7751.
[2] M. T. Reetz, J. D. Carballeira: Iterative saturation mutagenesis (ISM) for rapid directed evolution of functional enzymes, *Nature Protocols*, **2007**, *2*, 891-903.