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Crystallization of N-terminal Strep-tagged Fusion Lipase from Thermostable Bacillus sp. Strain 42. Mahiran Basri, Raja Noor Zaliha, Raja Abd Rahman, Tengku Haziyamin Tengku Abid. Hamid, Abu Bakar Salleh. *Department of Chemistry, Faculty of Science. 1Department of Microbiology; 1Department of Biochemistry; 1Department of Biotechnology and Biomolecular Sciences, University Putra Malaysia 43400 Serdang, Selangor, Malaysia.

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A novel thermoalkaliphilic lipase producer Geobacillus zalihae strain T1 was isolated from palm oil mill effluent in Malaysia [1]. The mature T1 lipase was overexpressed in Escherichia coli harboring pGEX/TIS recombinant plasmids with lipase activity of 42 U/ml [2]. The enzyme can be crystallized up to 60°C [3]. Optimization process revealed that a balance of hydrophobic interaction, packing rate, and some flexibility was needed to obtain a good crystal solved at 1.5Å [4]. Point mutation D311E (inter-loops networking) created an additional one salt-bridge and two hydrogen bonds as compared to K344R (intra-loops networking) with additional two salt-bridges and one hydrogen bond. Denatured protein analysis revealed that mutation D311E gave higher Tm (70.59 °C) as compared to K344R (68.54 °C) and native T1 lipase (68.52 °C). The mutant D311E was able to form preliminary crystal interface with formulation 9, 13 and 21 of Crystal Screen 2 with 2 M NaCl, 30% PEG-MME-2000 and 2 M NaCl as precipitants, respectively.

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The paper introduces a new concept of “protein surface shielding agents” into protein crystallization. This concept follows from “analytical crystallography”, i.e. analysis of processes leading to formation of crystals and the analysis of intermolecular contacts observed in the crystalline state “crystal contact areas” (CCA) [1]. It shows that large molecules have many various modes of mutual adhesion but only some of these “adhesion modes” (AM) are suitable for compact stacking of macromolecules into the crystal lattice. It shows also that different adhesion modes lead to crystals of different diffraction quality, and to different space groups, and some adhesion modes are