NEAR ATOMIC-RESOLUTION CRYO-EM STUCTURE OF THE *ZYMOMONAS MOBILIS* GH68 LEVANSUCRASE FIBRILS

Yuval Shoham[a], Yiran Yu[b], Shaked Katzelnick[a], Noam Hadad[c], Ravi R. Sonani[d], Rachel Salama[a], Edward H. Egelman[d], Gil Shoham[c], Dganit Danino[a,b]

[a] Department of Biotechnology & Food Engineering, Technion-IIT, Haifa, 32000 Israel
yshoham@technion.ac.il
[b] Biotechnology & Food Engineering Program, GTIIT, Shantou 515063, China
[c] Institute of Chemistry, the Hebrew University of Jerusalem, Jerusalem, 91904, Israel
[d] Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA 22903, USA

Levansucrases (LS) are retaining glycoside hydrolases (GH) that hydrolyze sucrose and release glucose and a high molecular weight β-(2-6)-fructose chain termed levan. Previously, we demonstrated that the GH68 LS from *Zymomonas mobilis* exhibits two active forms depending on the pH. At pH above 7, the enzyme is soluble as either a monomer or a dimer, primarily hydrolyzing sucrose to glucose and fructose and synthesizing levantriose. At pH below 6, the enzyme self-assembles into stable fibrils, predominantly synthesizing high molecular weight levan from sucrose [1]. The transition of the enzyme between the two forms is completely reversible, simply by changing the pH. Attempts to obtain high-resolution crystal structures of both soluble and fibrils forms were unsuccessful since the protein was not amended for crystallization. Recently, utilizing high-resolution Cryo-Electron Microscopy (Cryo-EM), we achieved a near atomic resolution structure of the *Z. mobilis* LS fibrils, at 2.1 Å resolution (Figure 1). The helical parameters are twist -163.328 degrees (left-handed helix) and rise 23.144 Å. The basic unit is a dimer which is stabilized by six salt bridges and twelve hydrogen bonds. Dimer units assemble into helical structure and interact with each other via three hydrophobic interfaces, highest along the 2-start (~523 Å^2), followed by 3-start (~408 Å^2) and 1-start (~228 Å^2) helical interfaces, consistent with our previous observation that fibril formation is driven by hydrophobic interactions [1]. To further understand the pH-dependent behavior, we computed the electrostatic potential of the levansucrase dimeric structure at different pH values using the PDB2PQR web server, which includes the APBS algorithm. At pH above 7, the surface potential was highly negative, while at pH 5.5, it was nearly neutral, explaining the pH-dependent assembly and disassembly of the fibrils.

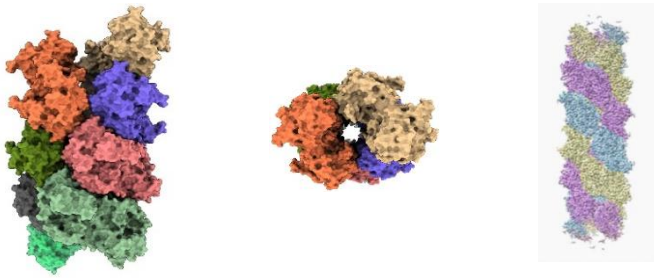

Figure 1. Cryo-EM helical fibril structure of *Z. mobilis* GH68 levansucrase. Each dimer appears in a different color. On the right LS fibril colored as a 3-start helix.

References:
1. D. Goldman, N. Lavid, A. Schwartz, G. Shoham, D. Danino, and Y. Shoham, *J. Biol. Chem*. **2008,** 283, 32209-32217.