Supplementary Material to the article

Functional identification of a *Streptomyces lividans* FKBP-like protein involved in the folding of overproduced secreted proteins.

Vicente RL¹, Marín S¹, Valverde JR², Palomino C¹, Mellado RP¹, Gullón S¹*

¹Departamento de Biotecnología Microbiana. Centro Nacional de Biotecnología (CNB-CSIC). Darwin 3. 28049 Madrid. Spain.
²Scientific Computing Service, Centro Nacional de Biotecnología (CNB-CSIC). Darwin 3.

28049 Madrid. Spain.

**Corresponding author: Sonia Gullón Blanco Centro Nacional de Biotecnología (CSIC) c/ Darwin 3. 28049 Madrid. Spain

> Phone: +34915854523 Fax: +34915854506 E-mail: sgullon@cnb.csic.es





Figure S1. **Alpha-amylase and agarase models.** (A) Left: model of mature alphaamylase with Pro350 residue in cis conformation. Right, model of immature amylase with P350 in trans conformation. The alpha-amylase Pro350 residue has been coloured in red and indicated by a red arrow. The active site is indicated by a green arrow. The cis-isomerisation of the Pro residue favours the enzyme to acquire its active conformation. (B) Left: model of mature agarase with P127 and P183 in cis conformation. Right: model of immature agarase with P127 and P183 in trans conformation. Prolines P127 and P183 are shown in red and indicated by red arrows, the active site has been coloured cyan, and the allosteric site in magenta. The models were produced using I- TASSER (alpha-amylase) or Raptor-X (agarase) and UCSF chimera, and optimized with the Amber force field.





ADLEWEQYPVPAAPGGNRSWQLLPSHSDDFNYTGKPQTFRGRWLDQHKDG WSGPANSLYSARHSWVADGNLIVEGRRAPDGRVYCGYVTSRTPVEYPLYT EVLMRVSGLKLSSNFWLLSRDDVNEIDVIECYGNESLHGKHMNTAYHIFQR NPFTELARSQKGYFADGSYGYNGETGQVFGDGAGQPLLRNGFHRYGVHWISA TEFDFYFNGRLVRRLNRSNDLRDPRSRFFDQPMHLILNTESHQWRVDRGIEP TDAELADPSINNIYYRWVRTYQAV

Figure S3. Agarase sequence coverage obtained by nano LC-MS/MS Triple TOF analysis. Band corresponding to the protein with a faster mobility when Sli-FKBP was overproduced respect to the wild type was sliced out of the gel and subject to trypsin digestion and analysed by nano LC-MS/MS Triple Tof. Sequences from the mature agarase in **bold** were identified in the experiment. Yellow letters indicate catalytic residues.



Figure S4. Predicted models of agarase.

Top row, left to right: A, mature agarase with both P127 and P183 in *cis* conformation. B, agarase with P127 in *trans* and P183 in *cis* conformation. C, agarase with both P127 and P183 in *trans* conformation. All the structures are shown using the same orientation to show the conservation of the active site in the P127*trans*, P183*cis* conformation. In the lower row we have superposed agarose bound to the active site (green) and the allosteric processive site (red) to illustrate the suggested disruption of the allosteric site in both *trans* conformations (B, C) and of the active site in the double *trans* conformer (C).