Electronic Supporting Information

Cold-induced aldimine bond cleavage by Tris in *Bacillus subtilis* alanine racemase

Noelia Bernardo-García[a], Pedro A. Sánchez-Murcia[b], Akbar Espaillat[c], Siseth Martínez-Caballero[a], Felipe Cava[c], Juan A. Hermoso*[a], and Federico Gago*[b]

[a] Department of Crystallography and Structural Biology, Institute of Physical Chemistry "Rocasolano", CSIC, 28006 Madrid, Spain
[b] Department of Biomedical Sciences, "Unidad Asociada IQM-CSIC", University of Alcalá, E-28871 Alcalá de Henares, Madrid, Spain
[c] Laboratory for Molecular Infection Medicine, Department of Molecular Biology, Umeå Centre for Microbial Research, Umeå University, 90187 Umeå, Sweden

*Corresponding authors. Email: xjuan@iqfr.csic.es / Email: federico.gago@uah.es

Supplementary information

Following the first round of reviews, fresh protein was produced and new crystals of *Bs*-AlaR were grown by the microbatch method in a medium containing 15% PEG 4000, 0.2 M $MgCl_2$, 0.1 M Tris (TRS) pH = 8.5.

Three X-ray diffraction data sets from these new *Bs*AlaR crystals were collected on beamline bl13-Xaloc at ALBA (Barcelona, Spain), with oscillation ranges of 0.15º for two of them and 0.20º for the other one. These crystals diffracted at 2.3, 2.5 and 2.7 Å resolution, respectively, and in all of them we observed the same active site arrangement and aldimine bond cleavage that we originally described in the main text for *Bs*AlaR–TRS (**Figure S1**).

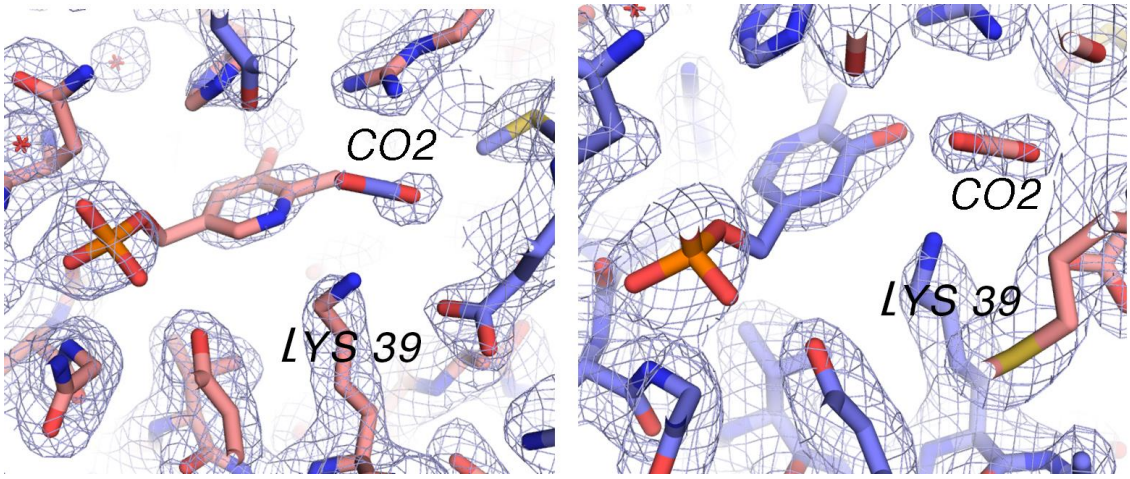

Figure S1. Electron density map ($2F_o$-F_c map contoured at 1σ) of both active sites from the crystal at 2.3 Å resolution. The other datasets at 2.5 and 2.7 Å present the same active site conformation observed in the dataset at 2.2 Å (reported in the main text) and 2.3 Å albeit with less quality details because of the slightly worse resolution.

Table S1. Crystallographic data collection and refinement statistics for crystal #1.

	*Bs*AlaR Xtal 1
Data collection	
Wavelength (Å)	0.980110
Space group	$P4_322$
Unit cell *a, b, c* (Å)	73.10, 73.10, 333.0
Unit cell α,β,γ (º)	90, 90, 90
T (K)	100
X-ray source	Synchrotron
Resolution range (Å)	46.9-(2-30-2.22)
Unique reflections	41560
Completeness (%)	99.9 (100.0)
Redundancy	7.8
R_{merge}	0.2 (1.83)
R_{pim}	0.115 (1.029)
Average $I/\sigma(I)$	8.5 (1.7)
Refinement	
Resolution range (Å)	46.9-2.3
R_{work}/R_{free}	0.19/0.24
Monomers per AU	2

Figure S2. Proposed mechanism for the Tris-assisted 4'-deformylation of PLP in *Bs*AlaR.