

Fig. 7. Structure-based sequence alignment of *TcPRACA* with the protein of unknown function Lr31 from *Brucella mellitensis* (Protein Data Bank ID code 1TM0) and the diaminopimelate epimerase DapF from *Haemophilus influenzae* (Protein Data Bank ID code 1BWZ). Secondary structural elements are depicted above the sequences. Light-shadowed (conserved) and dark-shadowed (strictly conserved) boxes depict residue conservation. A double-line beneath the sequences indicates structurally equivalent regions. The catalytic cysteines conserved in *TcPRACA* and DapF are marked with stars. Although the structural (rms deviation, 1.7 Å) and sequence (29% identity) relatedness between *TcPRACA* and Lr31 is significant, the Lr31 residues equivalent to the catalytic Cys residues are Ser-92 and Thr-255, thus making unlikely a function of amino acid racemase for this enzyme.

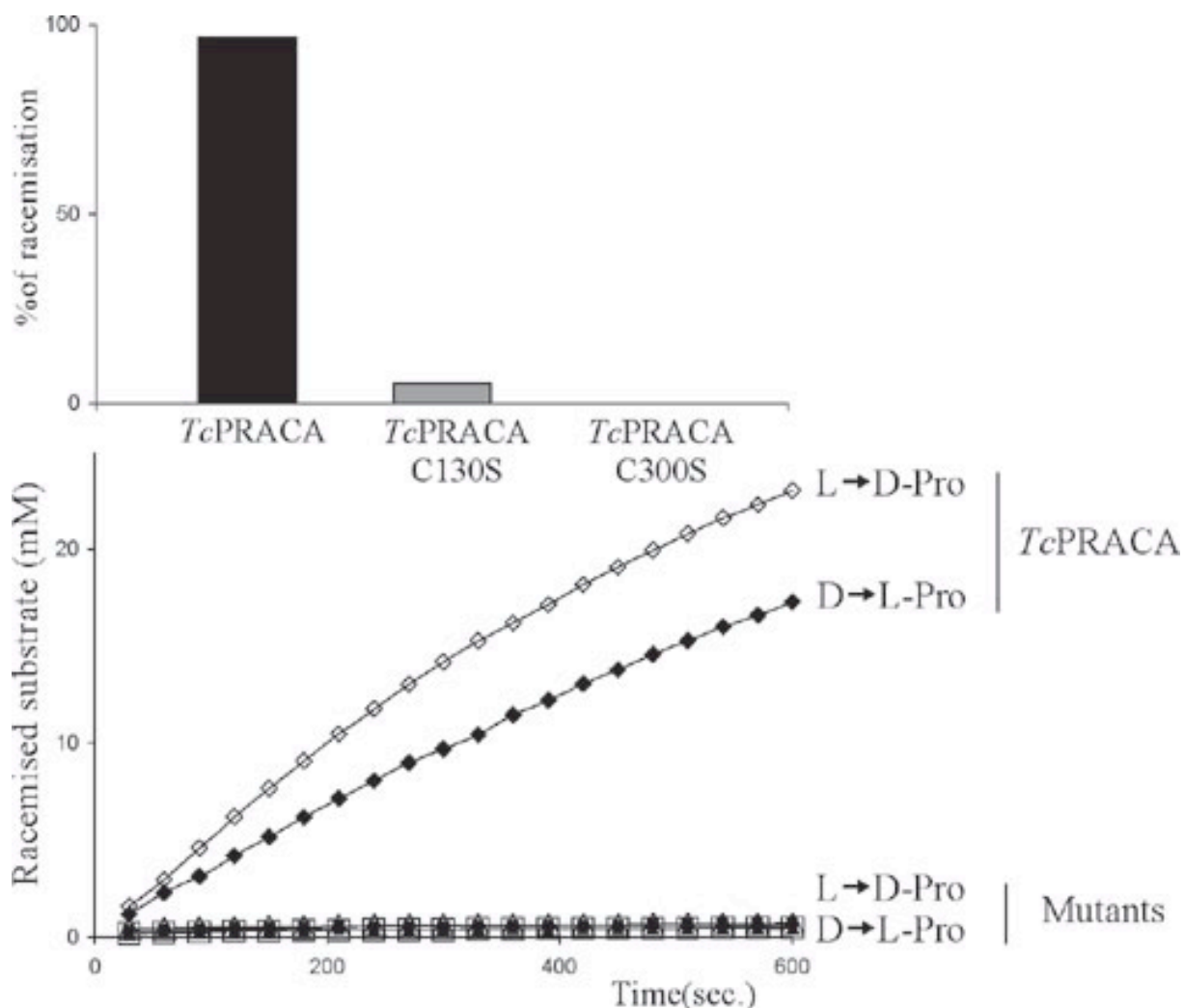


Fig. 8. Mutagenesis of key cysteine residues of *TcPRACA* drastically affects proline racemase activity. (A) The racemization activities of *TcPRACAC130S* and *TcPRACAC300S* are given as percentages of the wild-type activity obtained with the substrate L-proline (40 mM) and 0.25 μ M of enzyme in 0.2 M sodium acetate, pH 6, after 30 min incubation at 37°C in a 500- μ l volume. The reactions were stopped by incubating for 10 min at 80°C; then 1 ml of water was added, and the optical rotation was measured in a polarimeter 241 MC (Perkin–Elmer) at 365 nm (mercury lamp), in a cell with 10-cm path length. (B) Kinetics of racemization. Measurements were performed every 30 s during 1 h after incubation of 40 mM L-proline (open symbols) or D-proline (closed symbols) with 0.25 μ M wild-type *TcPRACA* (diamonds), *TcPRACAC300S* (squares), or *TcPRACAC130S* (triangles). All reagents were purchased from Sigma.

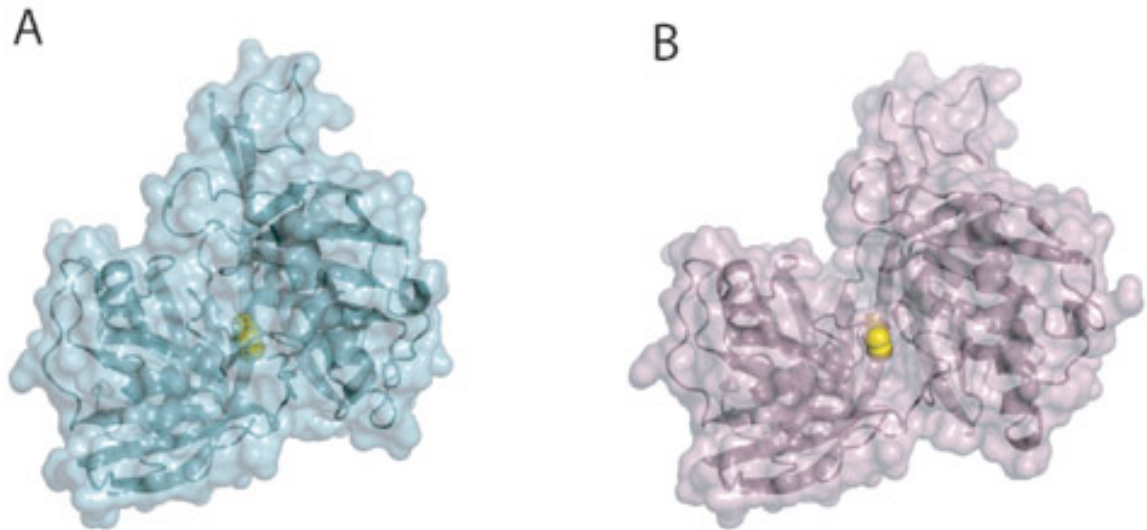


Fig. 9. Inhibitor binding promotes a structural rearrangement of *TcPRACA*. (A) Closed structure of the *TcPRACA* monomer in the presence of pyrrole-2-carboxylic acid (PYC), with the substrate-binding cavity completely buried within the monomer. (B) In the absence of PYC, the monomer displays a more relaxed, open structure with the active site accessible from the bulk solvent (the inhibitor is shown here for visualization purposes).

Table 2. ELISPOT assays

Three Balb/cJ male mice (Janvier, France) per group were injected i.p., respectively, with 50 μg of wild-type *TcPRACA*, *TcPRACAC130S*, or *TcPRACAC300S* mutant proteins and compared with control non injected mice. After 4 days, spleen cells were recovered in RPMI medium 1640/5% FCS, and numbers of immunoglobulin-producing cells were determined by ELISPOT for each mouse individually. Microplates (96-well) were coated overnight with 7.5 $\mu\text{g}/\text{ml}$ goat anti-mouse heavy- and light-chain-purified immunoglobulins. Plates were then saturated with PBS/1% gelatin and further incubated with serial dilutions of spenic cells for 16 hours, at 37°C under 5% CO₂. Cells were lysed with water, and the plates were then incubated with goat anti-mouse IgM or goat anti-mouse IgG coupled with biotin. After 6 h of incubation at 37°C, plates were washed and further incubated for 1 h at 37°C with streptavidin coupled to alkaline phosphatase. Reactions were revealed by using 5-bromo-4-chloro-3-indoyl phosphate substrate solution during 4 h, and the spots were counted. All antibodies were from Southern Biotechnology Associates (Birmingham, AL). I/N, infected/naïve ratios.

Table 2.

Treatment	Mouse #	Total spleen cells x 10 ⁶	Mean \pm SD	I/N	IgM-B cells /10 ⁶ cells	Mean \pm SD	I/N	IgG-B cells /10 ⁶ cells	Mean \pm SD	I/N	Total Ig-B cells /10 ⁶ cells	Mean \pm SD	I/N
none	1	1,68			2240			20,0			6390		
	2	1,34			2240			22,0			8626		
	3	1,39			2720			20,0			9585		
			1,47 $\pm 0,18$			2400 ± 277			20,7 $\pm 1,2$			8200 ± 1639	
<i>TcPRACA</i> -WT	1	1,54			4000			44,0			10256		
	2	1,06			5760			44,0			14102		
	3	0,96			7200			36,0			14743		
			1,19 $\pm 0,31$	0,81		5653 ± 1302	2,36		41,3 $\pm 4,6$	2,00		13034 ± 2426	1,56
<i>TcPRACA</i> -C130S	1	1,15			6709			72,0			13461		
	2	1,68			5431			92,0			10256		
	3	1,39			6070			72,0			12820		
			1,41 $\pm 0,27$	0,96		6067 ± 639	2,53		78,7 $\pm 8,0$	3,80		12179 ± 1695	1,50
<i>TcPRACA</i> -C300S	1	1,20			5112			60,0			15384		
	2	1,63			6709			68,0			11538		
	3	1,06			5431			72,0			10897		
			1,30 $\pm 0,30$	0,88		5751 ± 845	2,40		66,7 $\pm 6,1$	3,22		12606 ± 2426	1,54

Supporting Info : PNAS site at :

http://www.pnas.org/cgi/content/full/0509010103/DC1?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&fulltext=buschiazzo&searchid=1139249637841_6278&FIRSTINDEX=0&journalcode=pnas