



BIOLOGICAL  
CRYSTALLOGRAPHY

**Volume 71 (2015)**

**Supporting information for article:**

**Structure of the novel monomeric glyoxalase I from *Zea mays***

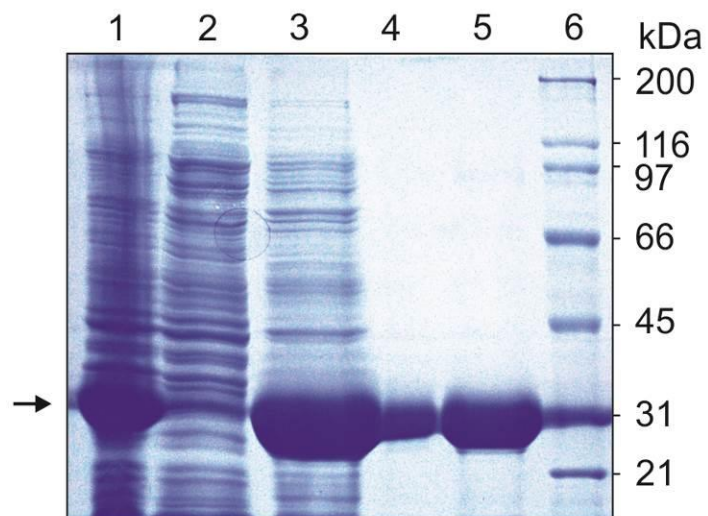
**Gino L. Turra, Romina B. Agostini, Carolina M. Fauguel, Daniel A. Presello,  
Carlos S. Andreo, Javier M. González and Valeria A. Campos-Bermudez**

**Table S1** Supplementary Table S1

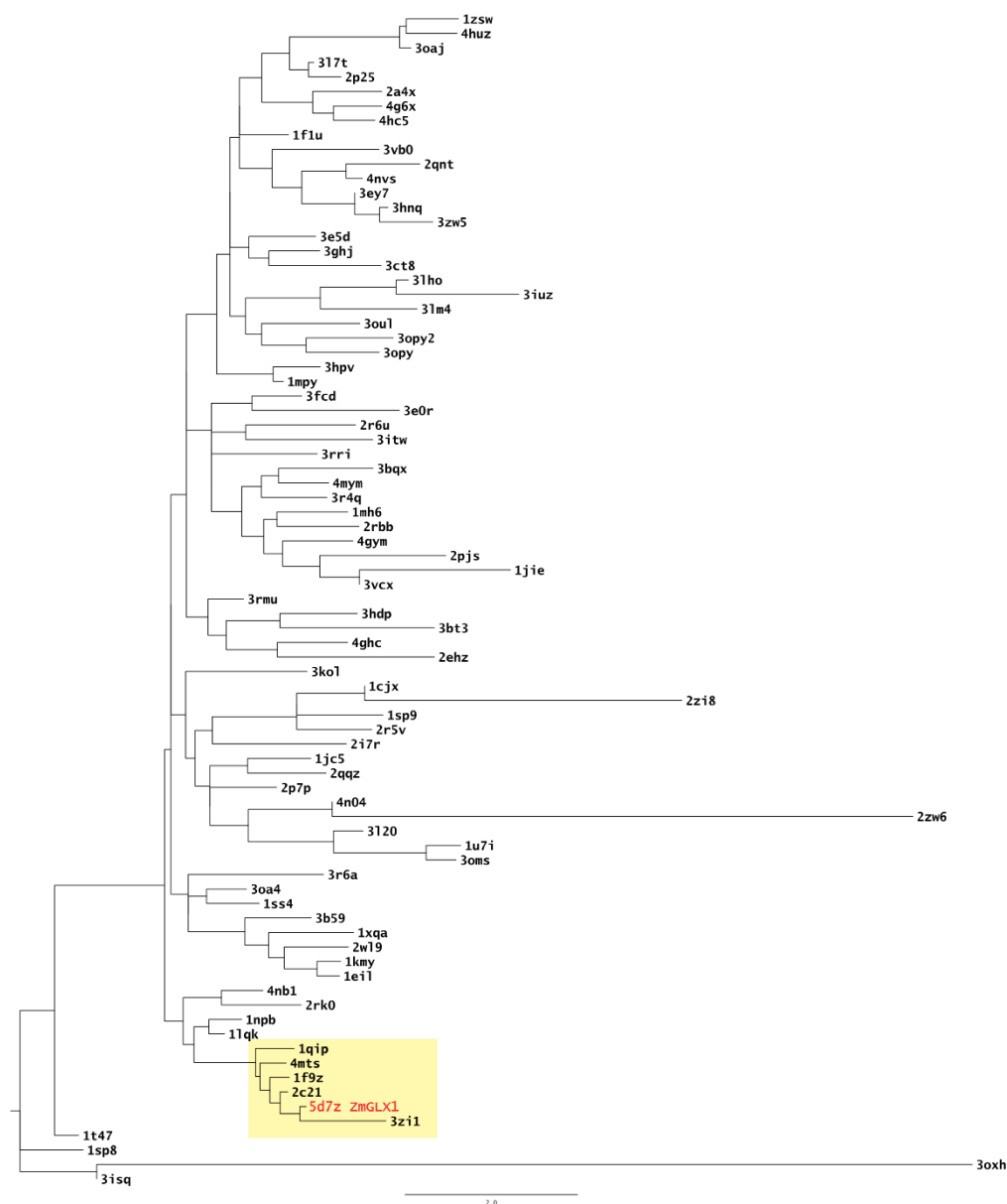
| Primer name                | Sequence 5'-->3'           | Notes  |
|----------------------------|----------------------------|--|
| ZmGLX1 Fw                  | TACCATGGCTATGGCAACCGGTAG   | Primer set used to amplify ZmGlx1 and cloning to a non cleavable C-terminus His-Tag  |
| ZmGLX1 Rv                  | GAGCTCTCAGTGGAGTTCCTTGAG   |  |
| <i>His6-less</i> ZmGLX1 Fw | ATGCTAGCATGGCAACCGGTAGTG   | Primer set used to amplify ZmGlx1 and cloning to a cleavable N-terminus His-Tag This primer was also used for E144Q sequence generation in the overlap extension PCR |
| <i>His6-less</i> ZmGLX1 Rv | AACTCGAGTCAGTGGAGTTCCTTGAG |  |
| <i>His6-less</i> E144Q Fw  | TACATGTTTCAGCTTATCCAGAGGG  | Inner primers used to the overlap extension PCR  |
| <i>His6-less</i> E144Q Rv  | CCCTCTGGATAAGCTGAAACATGTA  |  |

**Table S2** Supplementary Table S2.

| ZmGLX1's Proteins             | Induction conditions                                  | Notes   |
|-------------------------------|---|---|
| <i>Trace metals</i> ZmGLX1    | Autoinduction medium with trace metal supplementation | The cloning strategy allowed the expression of C-terminal fusion to the His-Tag present in pET 28b                                |
| <i>Ni-only</i> ZmGLX1         | Autoinduction medium with 1 mM Ni(II) added           | The cloning strategy allowed the expression of C-terminal fusion to the His-Tag present in pET 28b                                |
| <i>His6-less</i> ZmGLX1       | Autoinduction medium with 1 mM Ni(II) added           | The cloning strategy allowed the expression of N-terminal fusion to the His-Tag provided by pET 28b which was eventually cleaved. |
| <i>His6-less</i> E114Q-ZmGLX1 | Autoinduction medium with 1 mM Ni(II) added           | The cloning strategy allowed the expression of N-terminal fusion to the His-Tag provided by pET 28b which was eventually cleaved. |



**Figure S1** SDS-PAGE of the fractions obtained in each IMAC purification step. Lane 1: crude cell extract; lane 2: sample flow-through; lane 3: wash; lane 4 and 5: elution; lane 6: molecular weight standard (Thermo fisher). The pure protein with a mass of 32 kDa was obtained and indicated with an arrow.

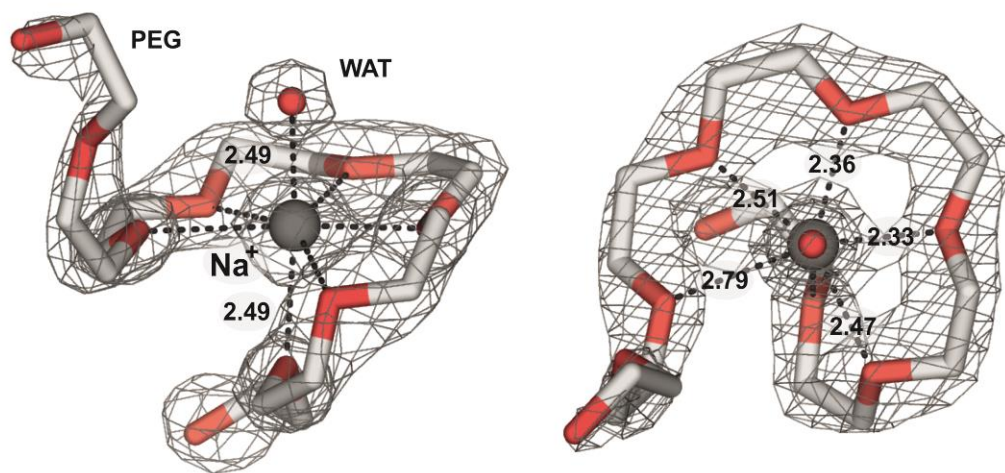


**Figure S2** Phylogenetic tree calculated with MEGA 6, using the structure-guided compact sequence alignment of the Dali Lite Server output, based on the PDB90 database. Taxa are indicated as the corresponding PDB identifiers (see the Table below for details in alphabetical order). The *yellow* box highlights the clade with the closest structural homologs of ZmGLX1 (*red*). Note that the closest homolog is the human protein of unknown function GloD4 (PDB 3zi1), followed by the glyoxalases I from *L. major* (trypanothione-dependent) (PDB 2c21), *E. coli* (PDB 1f9z), *P. aeruginosa* (PDB 4mts), and *H. sapiens* (PDB 1qip).

| PDB  | UniProt | Protein names  | Gene names                            | Organism   |
|------|---------|--|---------------------------------------|--|
| 1CJX | P80064  | 4-hydroxyphenylpyruvate dioxygenase (4HPPD) (HPD) (HPPDase) (EC 1.13.11.27)  | hpd                                   | <i>Pseudomonas</i> sp. (strain P.J. 874)   |
| 1EIL | P17297  | Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39) (2,3-dihydroxybiphenyl dioxygenase) (DHBD) (23OHBP oxygenase)  | bphC                                  | <i>Pseudomonas</i> sp. (strain KKS102)   |
| 1F1U | Q44048  | 3,4-dihydroxyphenylacetate 2,3-dioxygenase   | mndD                                  | <i>Arthrobacter globiformis</i>  |
| 1F9Z | P0AC81  | Lactoylglutathione lyase (EC 4.4.1.5) (Aldoketomutase) (Glyoxalase I) (Glx I) (Ketone-aldehyde mutase) (Methylglyoxalase) (S-D-lactoylglutathione methylglyoxal lyase) | gloA b1651 JW1643                     | <i>Escherichia coli</i> (strain K12)   |
| 1JC5 | Q8VQN0  | Methylmalonyl CoA epimerase (EC 5.1.99.1) (Methylmalonyl-CoA epimerase) (EC 5.1.99.1)  |                                       | <i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i>  |
| 1JIE | Q53793  | Bleomycin/pleomycin binding protein  | ble                                   | <i>Streptomyces verticillus</i>  |
| 1KMY | P47228  | Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39) (2,3-dihydroxybiphenyl dioxygenase) (DHBD) (23OHBP oxygenase)  | bphC Bxeno_C1125<br>Bxe_C1191         | <i>Burkholderia xenovorans</i> (strain LB400)  |
| 1LQK | Q914K6  | Glutathione transferase FosA (EC 2.5.1.18) (Fosfomycin resistance protein)   | fosA PA1129                           | <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1 / IC / PRS 101 / LMG 12228)                            |
| 1MH6 | P13081  | Bleomycin resistance protein (BRP) (CL990 resistance protein)  | ble                                   | <i>Klebsiella pneumoniae</i>   |
| 1MPY | P06622  | Metapyrocatechase (MPC) (EC 1.13.11.2) (CatO2ase) (Catechol 2,3-dioxygenase)   | xylE                                  | <i>Pseudomonas putida</i> ( <i>Arthrobacter siderocapsulatus</i> )   |
| 1NPB | Q56415  | Glutathione transferase FosA (EC 2.5.1.18) (Fosfomycin resistance protein)   | fosA fos                              | <i>Serratia marcescens</i>   |
| 1QIP | Q04760  | Lactoylglutathione lyase (EC 4.4.1.5) (Aldoketomutase) (Glyoxalase I) (Glx I) (Ketone-aldehyde mutase) (Methylglyoxalase) (S-D-lactoylglutathione methylglyoxal lyase) | GLO1                                  | <i>Homo sapiens</i> (Human)  |
| 1SP9 | P93836  | 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) (4-hydroxyphenylpyruvic acid oxidase) (4HPPD) (HPD) (HPPDase)  | HPD PDS1<br>At1g06570 F12K11.9        | <i>Arabidopsis thaliana</i> (Mouse-ear cress)  |
| 1SS4 | Q81F54  | Glyoxalase family protein  | BC_1747                               | <i>Bacillus cereus</i> (strain ATCC 14579 / DSM 31 / JCM 2152 / NBRC 15305 / NCIMB 9373 / NRRL B-3711)         |
| 1T47 | Q53586  | 4-hydroxyphenylpyruvate dioxygenase (4HPPD) (HPD) (HPPDase) (EC 1.13.11.27)  | hpd SAV_5149                          | <i>Streptomyces avermitilis</i> (strain ATCC 31267 / DSM 46492 / JCM 5070 / NCIMB 12804 / NRRL 8165 / MA-4680) |
| 1U7I | Q913Y6  | Uncharacterized protein  | PA1358                                | <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1 / IC / PRS 101 / LMG 12228)                            |
| 1XQA | Q81A18  | Uncharacterized protein  | BC_3580                               | <i>Bacillus cereus</i> (strain ATCC 14579 / DSM 31 / JCM 2152 / NBRC 15305 / NCIMB 9373 / NRRL B-3711)         |
| 1ZSW | Q81H03  | Glyoxalase family protein  | BC_1024                               | <i>Bacillus cereus</i> (strain ATCC 14579 / DSM 31 / JCM 2152 / NBRC 15305 / NCIMB 9373 / NRRL B-3711)         |
| 2A4X | O05205  | Mitomycin-binding protein (Mrd)  | mrd                                   | <i>Streptomyces lavendulae</i>   |
| 2C21 | Q68RJ8  | Trypanothione-dependent glyoxalase I (EC 4.4.1.-)  | GLO1<br>LMJF_35_3010                  | <i>Leishmania major</i>  |
| 2EHZ | P0A108  | 1,2-dihydroxynaphthalene dioxygenase (1,2-DHN dioxygenase) (DHND0) (EC 1.13.11.56) (1,2-dihydroxynaphthalene oxygenase)  | doxG                                  | <i>Pseudomonas</i> sp. (strain C18)  |
| 2P25 | Q832L2  | Glyoxylase family protein  | EF_2214                               | <i>Enterococcus faecalis</i> (strain ATCC 700802 / V583)   |
| 2P7P | Q8Y612  | Fosfomycin resistance protein FosX   | fosX lmo1702                          | <i>Listeria monocytogenes</i> serovar 1/2a (strain ATCC BAA-679 / EGD-e)                                       |
| 2PJS | A9CIG0  | Uncharacterized protein  | Atu1953                               | <i>Agrobacterium fabrum</i> (strain C58 / ATCC 33970) ( <i>Agrobacterium tumefaciens</i> (strain C58))         |
| 2QNT | A9CII6  | Uncharacterized protein  | Atu1872                               | <i>Agrobacterium fabrum</i> (strain C58 / ATCC 33970) ( <i>Agrobacterium tumefaciens</i> (strain C58))         |
| 2QQZ | Q81Y56  | Glyoxalase-like domain protein (Glyoxalase/Bleomycin Resistance protein) (Putative glyoxalase family protein)  | GBAA_3701<br>BASH2_02231<br>BF27_3975 | <i>Bacillus anthracis</i>  |
| 2R5V | O52791  | 4-hydroxymandelate synthase (HMS) (HmaS) (EC 1.13.11.46) (4-hydroxyphenylpyruvate dioxygenase II)  |                                       | <i>Amycolatopsis orientalis</i> ( <i>Nocardia orientalis</i> )   |
| 2R6U | Q0S814  | Uncharacterized protein  | RHA1_ro04536                          | <i>Rhodococcus jostii</i> (strain RHA1)  |
| 2RBB | B2TEV0  | Glyoxalase/bleomycin resistance protein/dioxygenase  | Bphyt_4242                            | <i>Burkholderia phytofirmans</i> (strain DSM 17436 / PsJN)   |
| 2RK0 | A8LBF3  | Glyoxalase/bleomycin resistance protein/dioxygenase  | Franean1_1561                         | <i>Frankia</i> sp. (strain EAN1pec)  |
| 2WL9 | Q6REQ5  | Catechol 2,3-dioxygenase   | akbC                                  | <i>Rhodococcus</i> sp. DK17  |
| 2ZI8 | P9WNW7  | Iron-dependent extradiol dioxygenase (EC 1.13.11.-)  | hsaC bphC Rv3568c                     | <i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)  |
| 2ZW6 | Q53796  | Bleomycin acetyltransferase  |                                       | <i>Streptomyces verticillus</i>  |

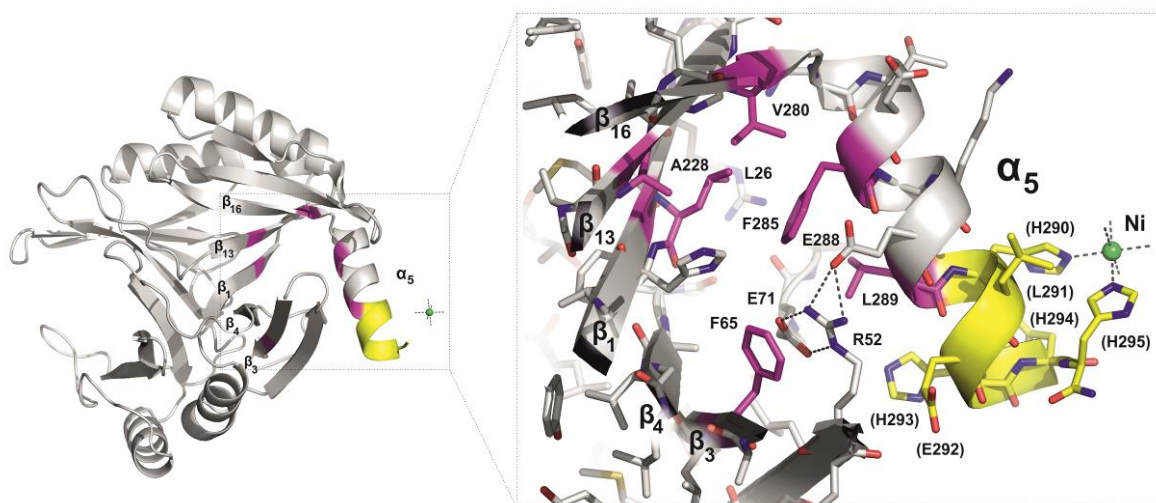
|             |        |   |                                   |  |
|-------------|--------|---|-----------------------------------|--|
| <b>3B59</b> | Q2GAG3 | Glyoxalase/bleomycin resistance protein/dioxygenase   | Saro_0713                         | <i>Novosphingobium aromaticivorans</i> (strain DSM 12444 / F199)   |
| <b>3BQX</b> | Q0G7J9 | Uncharacterized protein   | FP2506_05986                      | <i>Fulvamarina pelagi</i> HTCC2506   |
| <b>3BT3</b> | A9KJQ6 | Transcriptional regulator, AraC family  | Cphy_0674                         | <i>Clostridium phytofermentans</i> (strain ATCC 700394 / DSM 18823 / ISDg) ( <i>Lachnoclostridium phytofermentans</i> )  |
| <b>3CT8</b> | Q9KAX6 | BH2160 protein  | BH2160                            | <i>Bacillus halodurans</i> (strain ATCC BAA-125 / DSM 18197 / FERM 7344 / JCM 9153 / C-125)  |
| <b>3E0R</b> | Q9X4I9 | C3-degrading proteinase (CppA protein)  | cppA<br>ERS019718_00184           | <i>Streptococcus pneumoniae</i>  |
| <b>3EY7</b> | Q9K3D3 | Biphenyl-2,3-diol 1,2-dioxygenase III-related protein   | VC_A0328<br>VC_A0341<br>VC_A0463  | <i>Vibrio cholerae</i> serotype O1 (strain ATCC 39315 / El Tor Inaba N16961)   |
| <b>3FCD</b> | Q93AH5 | Orf125EGC139  |                                   | uncultured bacterium   |
| <b>3GHJ</b> | B0BGV9 | Putative integron gene cassette protein   | ORF1                              | uncultured bacterium   |
| <b>3HDP</b> | Q97H22 | Lactoylglutathione lyase, YQJC B.subtilis ortholog  | CA_C2192                          | <i>Clostridium acetobutylicum</i> (strain ATCC 824 / DSM 792 / JCM 1419 / LMG 5710 / VKM B-1787)   |
| <b>3HNQ</b> | Q8ZM36 | Virulence protein STM3117   | STM3117                           | <i>Salmonella typhimurium</i> (strain LT2 / SGSC1412 / ATCC 700720)  |
| <b>3HPV</b> | Q7WYF5 | Catechol 2,3-dioxygenase (Catechol 2,3-dioxygenase, LapB)   | lapB<br>PSAKL28_05860             | <i>Pseudomonas alkylphenolia</i>   |
| <b>3ISQ</b> | P32754 | 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) (4-hydroxyphenylpyruvic acid oxidase) (4HPPD) (HPD) (HPPDase)   | HPD PPD                           | <i>Homo sapiens</i> (Human)  |
| <b>3ITW</b> | Q333U2 | Putative uncharacterized protein tioX   | tioX                              | <i>Micromonospora</i> sp. ML1  |
| <b>3IUZ</b> | Q46PQ5 | Uncharacterized protein   | Reut_B5534                        | <i>Cupriavidus pinatubonensis</i> (strain JMP 134 / LMG 1197) ( <i>Ralstonia eutropha</i> (strain JMP 134))  |
| <b>3KOL</b> | B2IY64 | Glyoxalase/bleomycin resistance protein/dioxygenase   | Npun_R6391                        | <i>Nostoc punctiforme</i> (strain ATCC 29133 / PCC 73102)  |
| <b>3L7T</b> | Q8DU42 | Uncharacterized protein   | SMU_1112c                         | <i>Streptococcus mutans</i> serotype c (strain ATCC 700610 / UA159)  |
| <b>3LHO</b> | Q07XY2 | Uncharacterized protein   | Sfri_3296                         | <i>Shewanella frigidimarina</i> (strain NCIMB 400)   |
| <b>3LM4</b> | Q0S9X1 | Catechol 2,3-dioxygenase (EC 1.13.11.2)   | RHA1_ro03865                      | <i>Rhodococcus jostii</i> (strain RHA1)  |
| <b>3OA4</b> | Q9KCV1 | BH1468 protein  | BH1468                            | <i>Bacillus halodurans</i> (strain ATCC BAA-125 / DSM 18197 / FERM 7344 / JCM 9153 / C-125)  |
| <b>3OAJ</b> | P96693 | Putative ring-cleaving dioxygenase MhqO (EC 1.13.11.-)  | mhqO ydfO<br>BSU05490             | <i>Bacillus subtilis</i> (strain 168)  |
| <b>3OMS</b> | Q81DD6 | PhnB protein  | BC_2431                           | <i>Bacillus cereus</i> (strain ATCC 14579 / DSM 31 / JCM 2152 / NBRC 15305 / NCIMB 9373 / NRRL B-3711)   |
| <b>3OPY</b> | A7MAS3 | ATP-dependent 6-phosphofructokinase subunit gamma (ATP-dependent 6-phosphofructokinase) (ATP-PFK) (Phosphofructokinase 3) (Phosphohexokinase)                     | PFK3                              | <i>Komagataella pastoris</i> (Yeast) ( <i>Pichia pastoris</i> )  |
| <b>3OPY</b> | Q8NJU8 | ATP-dependent 6-phosphofructokinase subunit alpha (EC 2.7.1.11) (ATP-dependent 6-phosphofructokinase 1) (ATP-PFK 1) (Phosphofructokinase 1) (Phosphohexokinase 1) | PFK1                              | <i>Komagataella pastoris</i> (Yeast) ( <i>Pichia pastoris</i> )  |
| <b>3OPY</b> | Q8TGA0 | ATP-dependent 6-phosphofructokinase subunit beta (EC 2.7.1.11) (ATP-dependent 6-phosphofructokinase 2) (ATP-PFK 2) (Phosphofructokinase 2) (Phosphohexokinase 2)  | PFK2                              | <i>Komagataella pastoris</i> (Yeast) ( <i>Pichia pastoris</i> )  |
| <b>3OUL</b> | E3SET7 | Toxoflavin degrading enzyme   | tflA                              | <i>Paenibacillus polymyxa</i> ( <i>Bacillus polymyxa</i> )   |
| <b>3OXH</b> | P9WIR3 | 27 kDa antigen Cfp30B   | cfp30B TB27.3<br>Rv0577 MTV039.15 | <i>Mycobacterium tuberculosis</i> (strain ATCC 25618 / H37Rv)  |
| <b>3R4Q</b> | Q7CXA0 | Lactoylglutathione lyase  | Atu2344                           | <i>Agrobacterium fabrum</i> (strain C58 / ATCC 33970) ( <i>Agrobacterium tumefaciens</i> (strain C58))   |
| <b>3R6A</b> | Q8PS64 | Conserved protein (EC 4.4.1.5)  | MM_3218                           | <i>Methanosarcina mazei</i> (strain ATCC BAA-159 / DSM 3647 / Goe1 / Go1 / JCM 11833 / OCM 88) ( <i>Methanosarcina frisia</i> )  |
| <b>3RMU</b> | Q96PE7 | Methylmalonyl-CoA epimerase, mitochondrial (EC 5.1.99.1) (DL-methylmalonyl-CoA racemase)  | MCEE                              | <i>Homo sapiens</i> (Human)  |
| <b>3RRI</b> | C8WSF5 | Glyoxalase/bleomycin resistance protein/dioxygenase   | Aaci_2434                         | <i>Alicyclobacillus acidocaldarius</i> subsp. <i>acidocaldarius</i> (strain ATCC 27009 / DSM 446 / JCM 5260 / NBRC 15652 / NCIMB 11725 / NRRL B-14509 / 104-1A) ( <i>Bacillus acidocaldarius</i> ) |
| <b>3VB0</b> | A5VGW5 | Glyoxalase/bleomycin resistance protein/dioxygenase   | Swit_4902                         | <i>Sphingomonas wittichii</i> (strain RW1 / DSM 6014 / JCM 10273)  |
| <b>3VCX</b> | Q6NBC6 | Glyoxalase/Bleomycin resistance protein/dioxygenase domain  | RPA0902                           | <i>Rhodospseudomonas palustris</i> (strain ATCC BAA-98 / CGA009)   |
| <b>3ZII</b> | Q9HC38 | Glyoxalase domain-containing protein 4  | GLOD4 C17orf25<br>CGI-150 My027   | <i>Homo sapiens</i> (Human)  |
| <b>3ZWS</b> | A6NK44 | Glyoxalase domain-containing protein 5  | GLOD5                             | <i>Homo sapiens</i> (Human)  |

|             |        |  |                             |   |
|-------------|--------|--|-----------------------------|---|
| <b>4G6X</b> | C7PY34 | Glyoxalase/bleomycin resistance protein/dioxygenase                        | Caci_4633                   | <i>Catenulispora acidiphila</i> (strain DSM 44928 / NRRL B-24433 / NBRC 102108 / JCM 14897) |
| <b>4GHC</b> | Q45135 | Homoprotocatechuate 2,3-dioxygenase  |                             | <i>Brevibacterium fuscum</i>  |
| <b>4GYM</b> | D3F7P7 | Glyoxalase/bleomycin resistance protein/dioxygenase                        | Cwoe_2487                   | <i>Conexibacter woesei</i> (strain DSM 14684 / JCM 11494 / NBRC 100937 / ID131577)          |
| <b>4HC5</b> | D1C967 | Glyoxalase/bleomycin resistance protein/dioxygenase                        | Sthe_2956                   | <i>Sphaerobacter thermophilus</i> (strain DSM 20745 / S 6022)                               |
| <b>4HUZ</b> | Q9ZBB0 | 2,6-dichloro-p-hydroquinone 1,2-dioxygenase (EC 1.13.11.-)                 | pcpA                        | <i>Sphingobium chlorophenolicum</i>   |
| <b>4MTS</b> | Q9I5L8 | Lactoylglutathione lyase   | gloA2 PA0710                | <i>Pseudomonas aeruginosa</i> (strain ATCC 15692 / PAO1 / IC / PRS 101 / LMG 12228)         |
| <b>4MYM</b> | A1SPF2 | Glyoxalase/bleomycin resistance protein/dioxygenase                        | Noca_4190                   | <i>Nocardioides</i> sp. (strain BAA-499 / JS614)  |
| <b>4N04</b> | C7Q893 | Glyoxalase/bleomycin resistance protein/dioxygenase                        | Caci_7252                   | <i>Catenulispora acidiphila</i> (strain DSM 44928 / NRRL B-24433 / NBRC 102108 / JCM 14897) |
| <b>4NB1</b> | P60864 | Metallothiol transferase FosB (EC 2.5.1.-) (Fosfomycin resistance protein) | fosB SA2124                 | <i>Staphylococcus aureus</i> (strain N315)  |
| <b>4NVS</b> | Q18CP6 | Putative enzyme, glyoxalase family   | CD630_01590                 | <i>Peptoclostridium difficile</i> (strain 630) ( <i>Clostridium difficile</i> )             |
| <b>5D7Z</b> | C0PK05 | Lactoylglutathione lyase (EC 4.4.1.5) (Glyoxalase I)                       | Zm.67052<br>ZEAMMB73_635823 | <i>Zea mays</i> (Maize)   |



**Figure S3** Structure of the PEG-Na<sup>+</sup> crown ether complex found in ZmGLX1 active site. Wireframe surface indicates the electron density contoured at 1  $\sigma$ , and numbers indicate distances in Å.





**Figure S4** Amino acid residues involved in hydrophobic interactions (*magenta*) and electrostatic interactions (*dotted lines*) stabilizing the alpha-helical segment  $\alpha_5$  bound to the site B concavity ( $\beta$ -sheets 1-4 and 13-16). Note that the helical segment belonging to the hexa-histidine tag (amino acid residues labeled between parentheses, *yellow*), does not significantly contribute amino acid sidechains for this interaction.