

Supplementary information

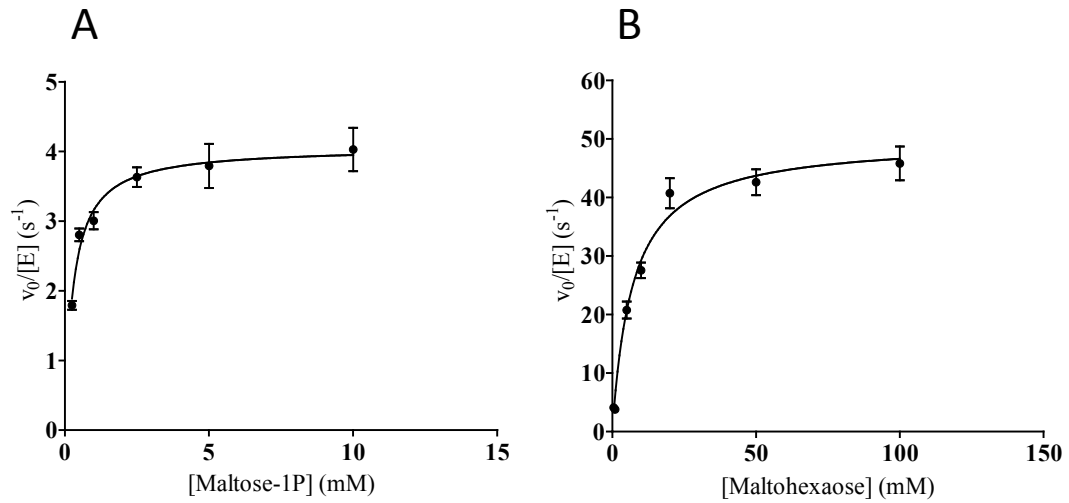
Structure of *Mycobacterium thermoresistibile* GlgE defines novel conformational states that contribute to the catalytic mechanism.

Vitor Mendes^{1,2}, Michal Blaszczyk¹, Ana Maranhã², Nuno Empadinhas², Tom L Blundell^{1*}

1 - Department of Biochemistry, University of Cambridge, Cambridge CB2 1GA, UK

2 – Molecular Mycobacteriology Group, CNC-Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal.

* - Corresponding author: tom@cryst.bioc.cam.ac.uk



Supplementary figure S1: *M. thermoresistibile* GlgE kinetics with maltohexaose and maltose-1P. Maltohexaose concentration was kept at 1mM in A and maltose-1P concentration was kept at 5mM in B.

M. thermoresistibile

N β1 N α1 N β2 N β3

```

1  ...VA GRIV IDDV QP VVSN GRVY PAKAVY GEVVFVVAATV WRECHDAVA
M. thermoresistibile 1  ...MSGRAIGTETIEWWVGRV IDDVAPVVSNGVY PAKAVY GEVVFVVAATV WRECHDAVA
M. tuberculosis 1  ...MTGR IIDDVAPVVSNGVY PAKAVY GEVVFVVAATV WRECHDAVA
Nocardia_farcinica 1  ...MHYTR IIDDVAPVVSNGVY PAKAVY GEVVFVVAATV WRECHDAVA
Streptomyces_coelicolor 1  MPATHHSSATSARPTV VGRIPVLDVRRPVRGR PAKAVY GESFVSAATV WRECHDAVA
Streptomyces_coelicolor

```

Variable loop

N β4 N β5 N β6

```

45  A L L V V R V H G T T Y P D L A D P P P G V P G A D R T A V P I G D V M T P A A P V K P Q R L I P M S P G H T P D V F H
M. thermoresistibile 45  A L L V V R V L G V R Y P H L T D R P R A R V . . . . . L P T H S E P Q Q R V K P L L I P M T S G Q E P F V F H
M. tuberculosis 58  A L L V V R C L G P S Y P Q L S E G P L R R I A A P R E . . . . . K E T S A T R I K P L H I P M Q P G G A L D R F H
M. abscessus 45  A L L A V R G P G A T . . . . . P T R I R M T P D P E P D V F N
Nocardia_farcinica 46  A L L V V L R D P G R . G . . . . . P W T E M R E L A P G T D R M G
Streptomyces_coelicolor 61  A L L V V L R D P G R . G . . . . . P W T E M R E L A P G T D R M G
Streptomyces_coelicolor

```

N β7 N β8 S α1 S α2

```

104  G H F T P D R V G L W T Y R V D G W D P I A S W R H N V T A K L D A G Q G E L N N D L L V G A R L L E R A A T G
M. thermoresistibile 104  G H F T P D R V G L W T Y R V D G W D P I A S W R H N V T A K L D A G Q G E L N N D L L V G A R L L E R A A T G
M. tuberculosis 109  G Q F T P D R V G L W T F R V D G W D P I A T W R H N V E A K L E A G Q G A L A N D L L V G A R L L E R A A T G
M. abscessus 98  A S F V P D R E G L W T F R V D G W D P I A T W R H N V E A K L E A G Q G A L A N D L L V G A R L L E R A A T G
Nocardia_farcinica 74  A T F I P N T P G T W T F R I E G W D P I A T W R H N V E A K L A V G S F A D L A N D L L E L G A R L L E R A A Q A
Streptomyces_coelicolor 90  A T V T A G E T G T W S Y T V E A W G D P V T W R H H A R I K I P A G L D T . . . D L V L E G A R L L E R A A Q A
Streptomyces_coelicolor

```

S α3 S α4 N β9

```

163  V P R E L R E A L L E A A A L R A P G D F F T R A G A A L S A E V S D L L A E Y P L R E F V T R G E Q Y G V W V
M. thermoresistibile 163  V P R E L R E A L L E A A A L R A P G D F F T R A G A A L S A E V S D L L A E Y P L R E F V T R G E Q Y G V W V
M. tuberculosis 168  V P R G L R D P L L A A A A L R T P G D F V T R T A L A L T P E I E E L A D Y P L R D L V T R G E Q F G V W V
M. abscessus 157  V P R K L R R G P L L A S A E A L R T Q D D F G A R F Q H A L S D E V Q V L A E F P L R D L L T R G T Y G I W V
Nocardia_farcinica 133  L P R A K F E K L R A A A A L R S D E Q L P A R V A P A F G A V E A E L R A T P L R D L V T R G P H C V A V
Streptomyces_coelicolor 146  V P G R E D R E L L A A V D A L R D E S R P A A S R I A A A L T P Q V D A V L A R H P L R D L V T S S D E L P L V
Streptomyces_coelicolor

```

A α1 A β1 A α2 A α3 A β2

```

220  D R P . E A R F S S W Y E M F P R S T G G W D A E G R P V H G T F A T A A E A L P R I A R M G F D V V Y L P P H P I G
M. thermoresistibile 220  D R P . E A R F S S W Y E M F P R S T G G W D D D G N P V H G T F A T A A E A L P R I A R M G F D V V Y L P P H P I G
M. tuberculosis 225  D R P . L A R F G A W Y E M F P R S T G G W D D D G N P V H G T F A T A A E A L P R I A R M G F D V V Y L P P H P I G
M. abscessus 214  D R T . R A L Y G S W Y E F F P R S T G G V G P D G R P M H G T L A T T G A D L A R V A R M G F D V V Y L P P H P I G
Nocardia_farcinica 190  E R P . R A L F G S W Y E F F P R S T G G R D A E G R P V H G T F A T A A K E L P R I A R M G F D V V Y L P P H P I G
Streptomyces_coelicolor 205  E R E . R A L Y G A W Y E F F P R S E G T P . . . H T P H G T F R T A A R R L A I A R M G F D V V Y L P P H P I G
Streptomyces_coelicolor

```

Inst1 α1 A α4 A β3

```

279  K V H R . K G R N N S V T A A P G D V G S P W A I G S D E G G H D A V H P Q L G T E D F E F V A S A R D L G L E V A
M. thermoresistibile 279  K V H R . K G R N N S V T A A P G D V G S P W A I G S D E G G H D A V H P Q L G T E D F E F V A S A R D L G L E V A
M. tuberculosis 284  K V H R . K G R N N S P T A A P T D V G S P W A I G S D E G G H D I V H S L G T I D D F D F V S A A R D L G L E V A
M. abscessus 273  E I N R . K G R N N S P I A E P G D V G S P W A I G S A G G C H D T H B E L G T I K D F E F V A T A R K L G L E V A
Nocardia_farcinica 249  E V N R . K G R N N S L V A E P G D V G S P W A I G S K D G G H D A V H P A L G T E A D F E F V A T A A R L G L E V A
Streptomyces_coelicolor 260  T I H R . K G R N N I L S A T G D V G V P W A I G S P E G G H D S L H P A L G T E D F E F V F A G K L G L E V A
Streptomyces_coelicolor

```

B β1 B α1 B loop2 B β2 B β3 B β4 A α5

```

338  L D F A L O C M P D H P W A R B H P E W F T V L P D G T I A M A E N P P K K Y C D I Y P L N F D N D P A G L Y Q E V L
M. thermoresistibile 338  L D F A L O C M P D H P W A R B H P E W F T V L P D G T I A M A E N P P K K Y C D I Y P L N F D N D P A G L Y Q E V L
M. tuberculosis 343  L D F A L O C M P D H P W A R B H R Q W F T E L P D G T I A M A E N P P K K Y C D I Y P L N F D N D P A G L Y D E V L
M. abscessus 332  L D F A L O C M P D H P W V S A H P E W F T E L P D G T I A M A E N P P K K Y C D I Y P L N F D N D P A G L Y T E V L
Nocardia_farcinica 308  L D F A L O C M P D H P W V R B H P E W F T L P D G T I A M A E N P P K K Y C D I Y P V N F D N D P D G L Y A E V L
Streptomyces_coelicolor 319  L D F A L O C S P D H P W H R K H P E W F H R P D G T I A M A E N P P K K Y C D I Y P L A F D A D P G L A T E T V
Streptomyces_coelicolor

```

A α6 A β4 A α7 A α8 A β5 A α9

```

397  R V V R F W T S H G V N I E R V D N P H T K P P N F W A M I T G Q I K N E N P D V L F L E A F T P A R L Y G L A K
M. thermoresistibile 397  R V V R F W T S H G V N I E R V D N P H T K P P N F W A M I T G Q I K N E N P D V L F L E A F T P A R L Y G L A K
M. tuberculosis 402  R V V Q H W V N H C V K F E R V D N P H T K P P N F W A M I T A Q V K T V D P D V L F L E A F T P A R Q Y G L A K
M. abscessus 391  R V V Q H W T A H G V K I E R V D N P H T K P P D F W Q E L T G E V K A A D P D V L F L E A F T P A R L Y G L A R
Nocardia_farcinica 367  R I V R H W T I G L G V K I E R V D N P H T K P A D F W E L H A T V R K D D P D V L F L E A F T P A R L Y G L A R
Streptomyces_coelicolor 378  R I L R H W T M D H G V R I E R V D N P H T K P V A F W E R V H A D I N G T D P D V L F L E A F T P A M M A T L A Q
Streptomyces_coelicolor

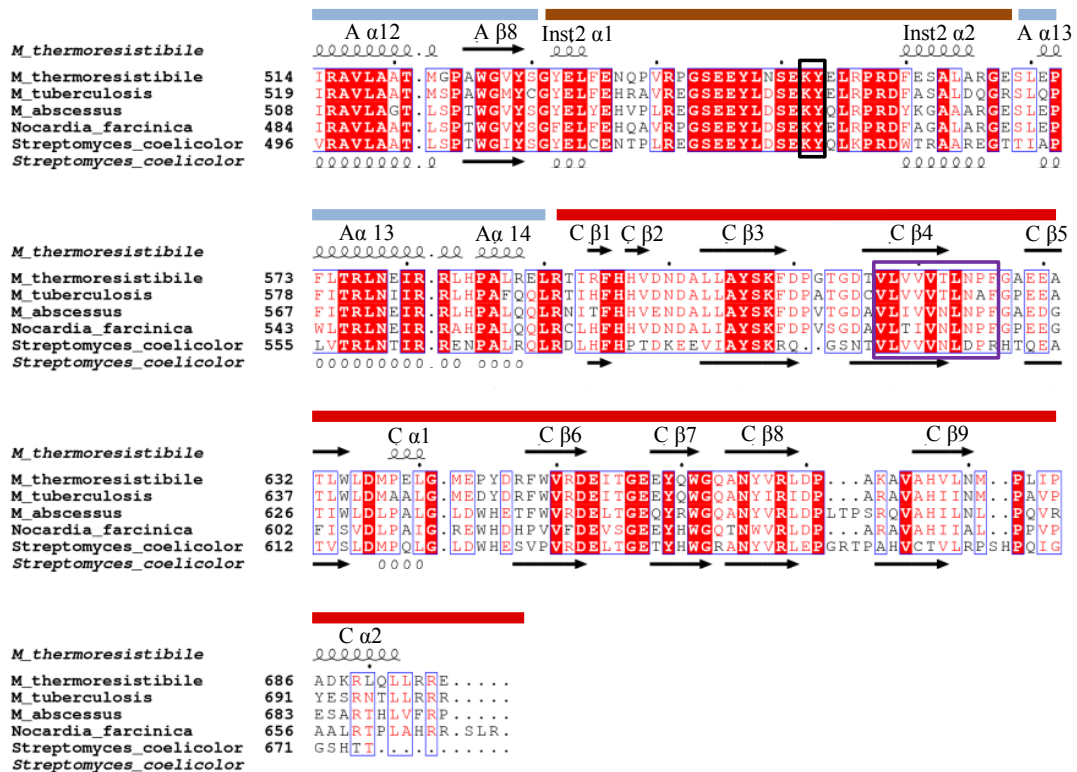
```

A β6 A α10 A α11 A β7 A α12

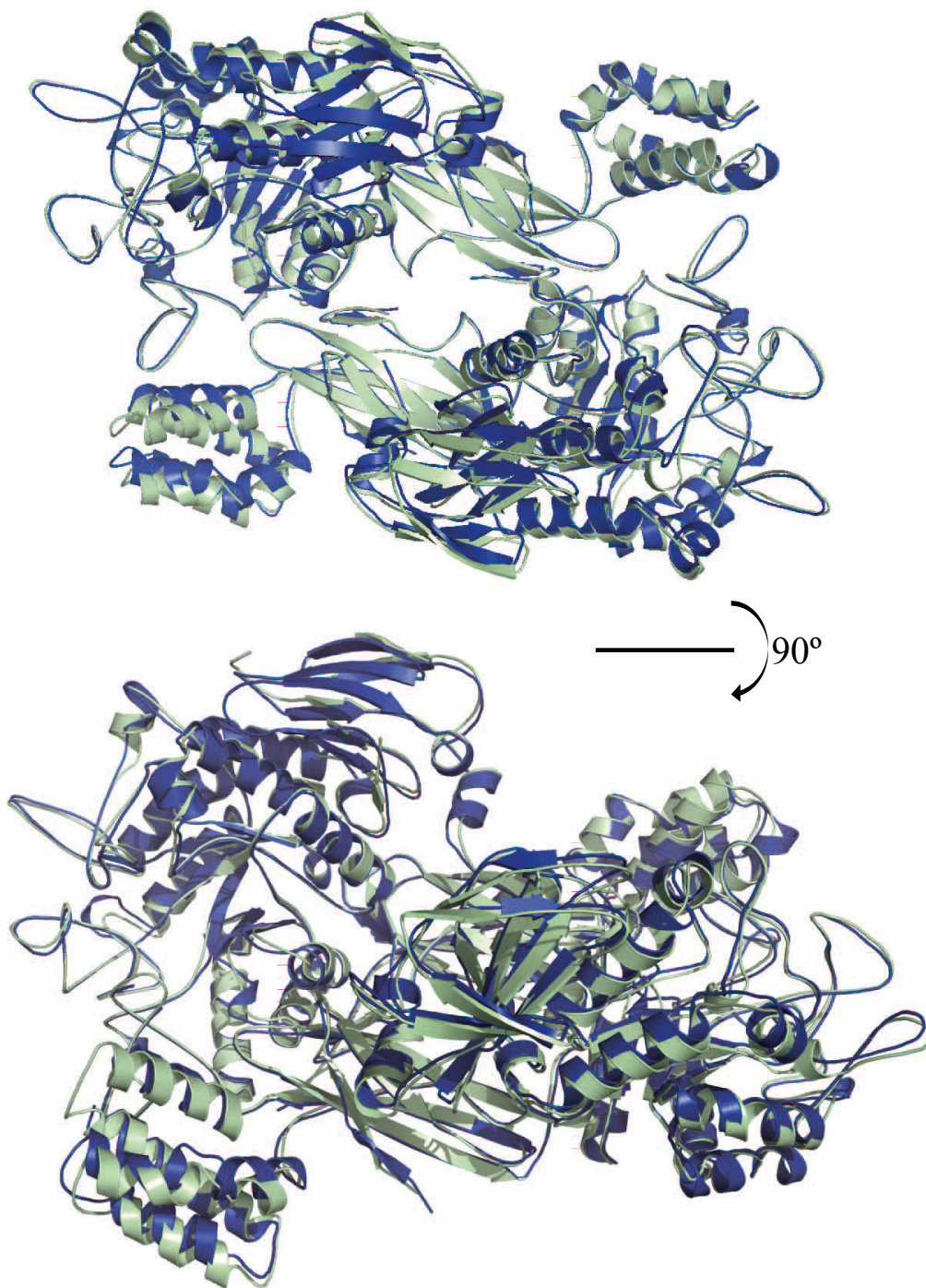
```

456  L G F Q S Y T Y F T W R T S K W E L T E F G Q E I A A K A D I A R P N L F V N T E D I L H S L O H G G F G M F A
M. thermoresistibile 456  L G F Q S Y T Y F T W R T S K W E L T E F G Q E I A A K A D I A R P N L F V N T E D I L H S L O H G G F G M F A
M. tuberculosis 461  L G F Q S Y T Y F T W R T T K W E L T E F G N Q I A E L A D Y R R P N L F V N T E D I L H A V L O H N G G M F A
M. abscessus 450  L G F Q S Y T Y F T W R T A K E I A E F G R E I A A Q A D V A R P N L F V N T E D I L H S L O Y G G G M F A
Nocardia_farcinica 426  R G F Q S Y T Y F T W R V A K W E L T E F G N E L A A K A D E A R P N L F V N T E D I L H S L O H G G F G M F A
Streptomyces_coelicolor 437  I G F Q S Y T Y F T W R N T K S E L T E V L T E L S G E A A S Y M R P N F F A N T E D I L H A V L O H G R P A F E
Streptomyces_coelicolor

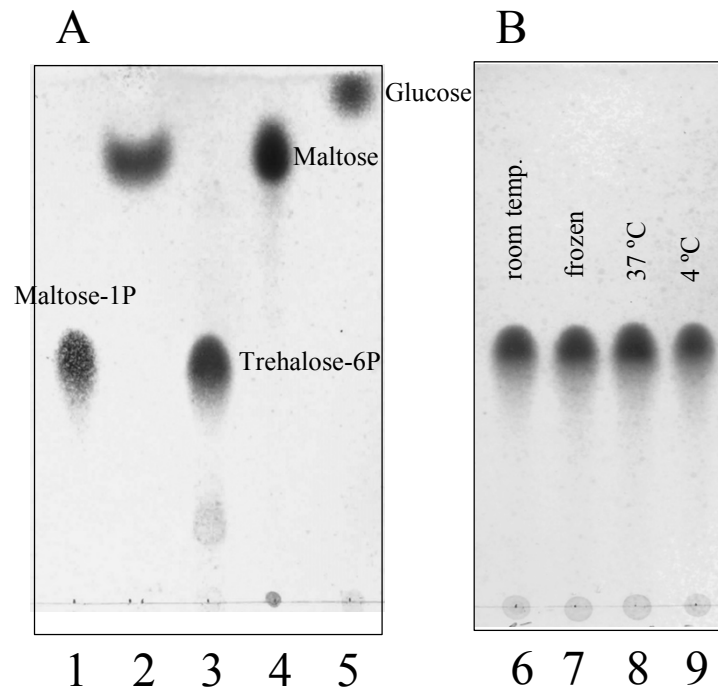
```



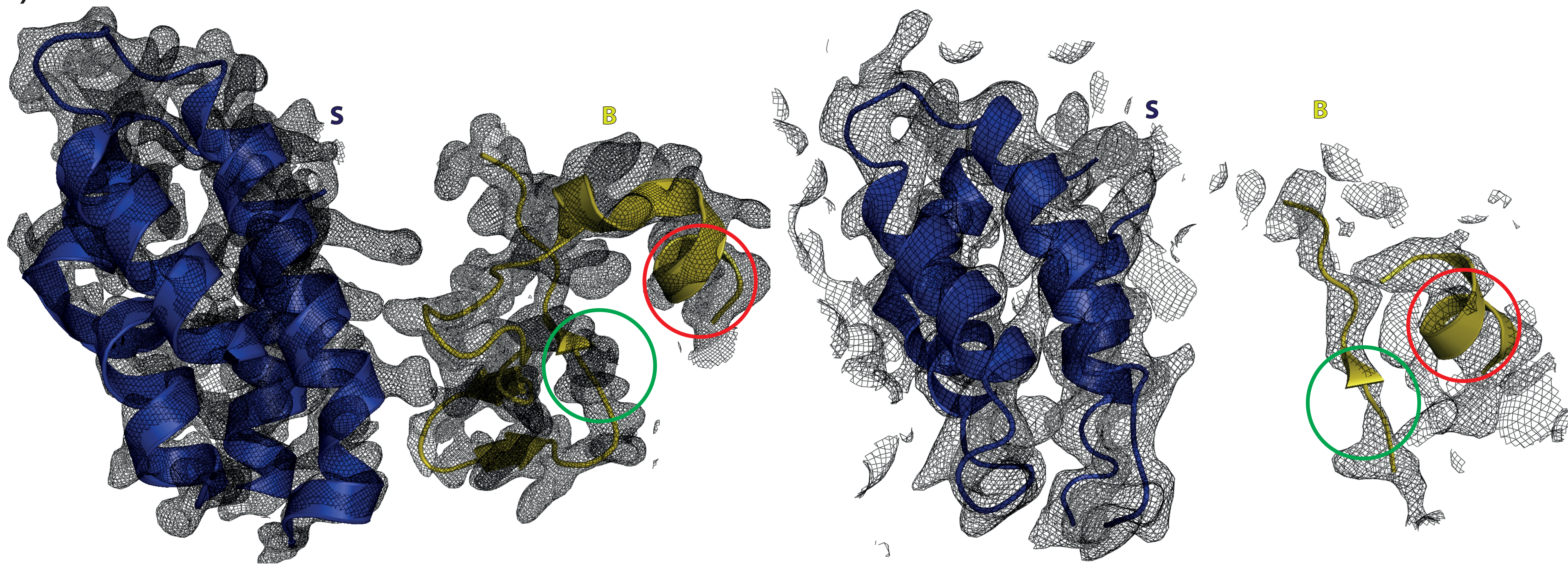
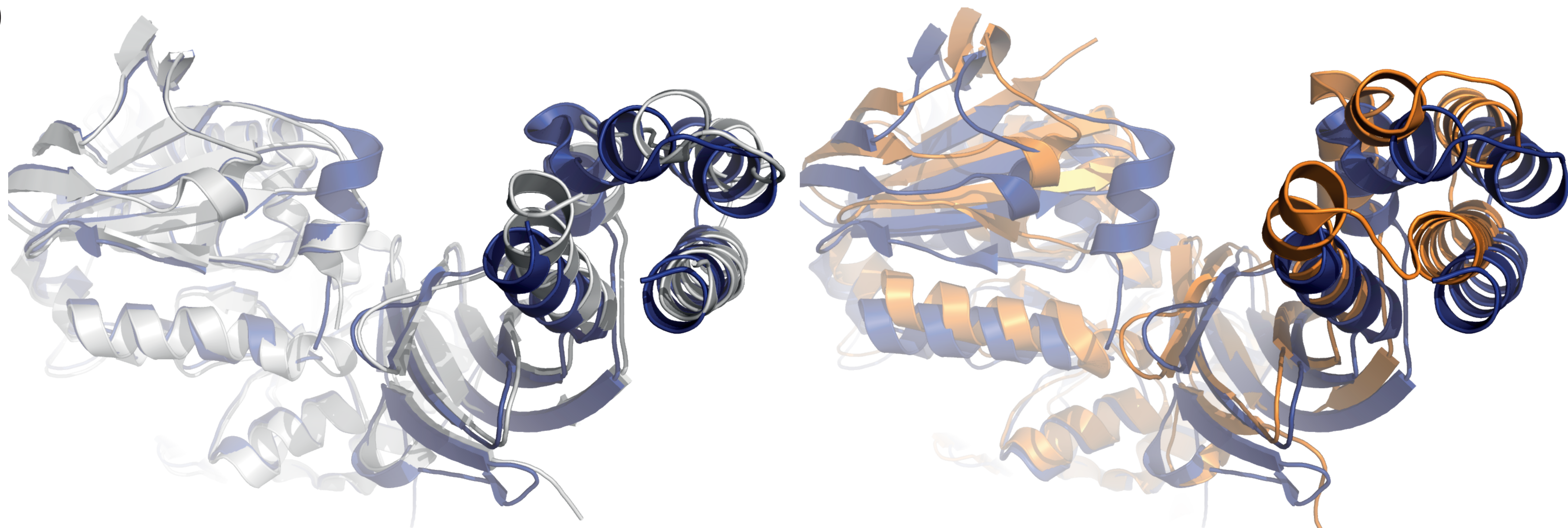
Supplementary Figure S2: Conservation of active-site and phosphorylation-site residues in GlgE orthologues. The multiple sequence alignment was performed with ClustalOmega^{1,2}. Secondary structure features above the sequences correspond to *M. thermoresistibile* GlgE maltose co-crystallization structure (5GCM). The secondary structure features indicated below the sequences correspond to *S. coelicolor* GlgE structure (3ZSS). A horizontal line in different colours represents the several domains: Domain N is represented in brown, Domain S in blue, Domain A in light blue, Domain B in yellow, Insert 1 in cyan, Insert 2 in dark orange and Domain C in red. Black boxes highlight residues that directly interact with maltose-1P. Green Boxes highlight GlgE residues know to be phosphorylated by PknB in *M. tuberculosis*³. Purple boxes highlight residues found to interact with linear α -glucan chains⁴. Red boxes highlight conserved residues. This figure was prepared with ESPript 3⁵.



Supplementary Figure S3: Superposed structures of maltose co-crystallization (5CGM) in blue and apo form (5CJ5) in green.



Supplementary figure S4: (A) TLC analysis of maltose, maltose-1P, glucose and trehalose-6P. Lane 2 corresponds to maltose-1P treated with alkaline phosphatase. (B) TLC analysis of 1 week (6, 7, 9) and 1h (8) maltose-1P solutions.

A)**B)**

Supplementary figure S5: (A) Electron density maps of maltose co-crystallization structure (right panel) and maltose-1P co-crystallization structure (left panel) focusing on the S and B domains of opposing protomers. The represented S domain of the maltose-1P co-crystallization structure belongs to the maltose bound protomer. Red and green circles highlight the same regions between both panels. (B) Superposition of *M. thermoresistibile* GlgE in blue (maltose co-crystallization structure) in blue with *M. tuberculosis* GlgE (4U33) (right panel) and *S. coelicolor* GlgE (3ZT5) (left panel).

References

- 1 Goujon, M. *et al.* A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res* **38**, W695-699, (2010).
- 2 Sievers, F. *et al.* Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular systems biology* **7**, 539, (2011).
- 3 Leiba, J. *et al.* Mycobacterium tuberculosis maltosyltransferase GlgE, a genetically validated antituberculosis target, is negatively regulated by Ser/Thr phosphorylation. *J Biol Chem* **288**, 16546-16556, (2013).
- 4 Lindenberger, J. J., Kumar Veleti, S., Wilson, B. N., Sucheck, S. J. & Ronning, D. R. Crystal structures of Mycobacterium tuberculosis GlgE and complexes with non-covalent inhibitors. *Sci Rep* **5**, 12830, (2015).
- 5 Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res* **42**, W320-324, (2014).