

2.3 Å X-ray Structure of the Heme-Bound GAF Domain of Sensory Histidine Kinase DosT of *Mycobacterium tuberculosis*

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SUPPLEMENTAL FIGURES

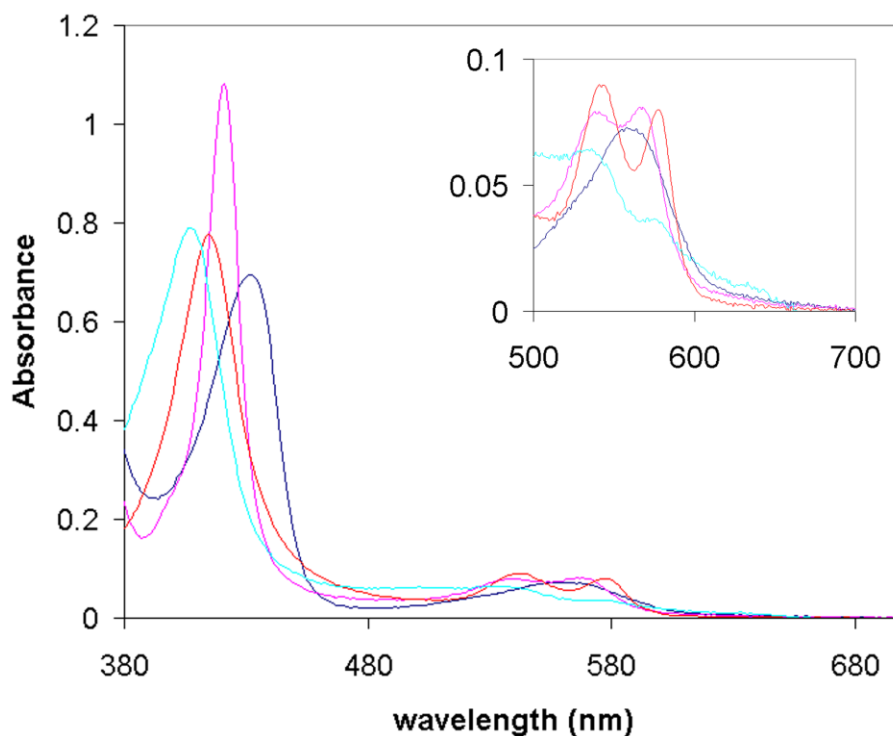


Figure S1. UV-visible spectral characterization of GAF_{DosT}. Spectra of the ferric GAF_{DosT}, *cyan*; ferrous GAF_{DosT}, *dark blue*; ferrous GAF_{DosT}-CO complex, *magenta*; and ferrous GAF_{DosT}-O₂ complex, *red*, are shown. The insert shows an enlargement of the visible region of the spectra.

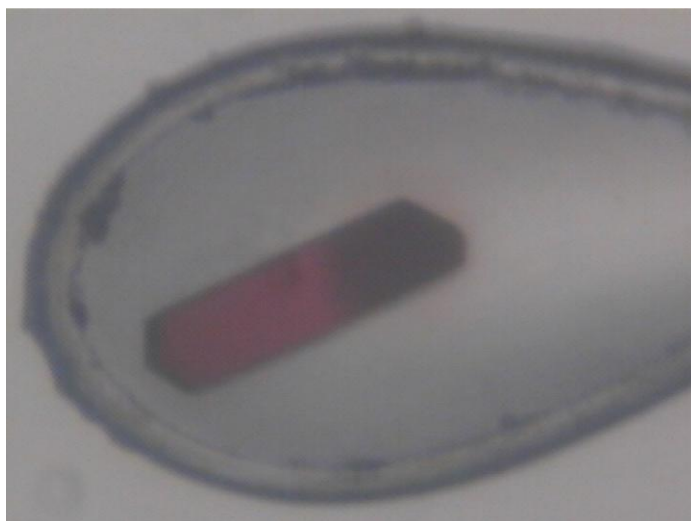


Figure S2. GAF_{DosT} x-ray induced photoreduction. A snapshot of the GAF_{DosT} crystal after collection of the two-wavelength anomalous diffraction data set is shown. The irradiated part of the crystal on the right is of a much darker red color.

Fig. S3 (page1)

DosT_M. tuberculosis (O53473)

DosT_M. tuberculosis (O53473)MTHPDRANVPNGSPPIRETLQLRLRELLLELVQDRIEQIVEGRDRLDGLDAI LAITS
M. smegmatis (ABK70610)MGGVNGHHPRRDDQRTPLRDTLSQLRLRELLSEVQDRVEQIVEGRDRLDGLVEAMLVVTS
M. vanbaalenii (ABM12229)MRPLRDTLSQLRLRELLNEVQDRVEQIVEGRDRLDGLJAMLVVTS
M. gilvum (ABP46316)MHDGAVADADDHRRDARGPVRTLSQLRLRELLTEVQDRVEQIVIRGDRDLGLVEAMLAITS
DosS_M. ulcerans (ABL04774) MTDEPHPDAGPSLGVPSGDGLRLRLTLQLRLRELLLEVQDRVEQIVEGRDRLDGLVEAMLVVTS
DosS_M. bovis (BCG_CAL73144)MTTGGVLDENDGAAMRPLRHTLSQLRLRELLLEVQDRVEQIVEGRDRLDGLVEAMLVVTA
DosS_M. tuberculosis (NP217648)MTTGGVLDENDGAAMRPLRHTLSQLRLRELLLEVQDRVEQIVEGRDRLDGLVEAMLVVTA
N. farcinica (BAD57741)MLGMEGRSSRPGSNDPPIETLAQSRRLRELLAEVQDRIAEIVNVDRQMDRLIEAMLVVTA
Rhodococcus_RHA1 (ABG91885)MTRQPDAGDRGPLLGSLSQLRLRELLAEVQDRIEQIVDA RDHV DGLIEAMLAITS
T. fusca (AAZ57079)MADDTPNGERGVSFVPMRLDELLSELQVRINAVMLVDRVRSLLJAVVSI GS
S. coelicolor (CAB53427)MENAEETREARVRLQLRLDELLLEELQARLDAAGTRDRVRSLLJAVVSVGR
S. tropica (ABP54044)MTPSPQHHPATPSLGLTPLSRVHLDELLQEMLDLVSQVVASRRLLELJAVVGI ST
Nocardioide JS614 (ABL80469)MIDPGAAQTGATVSEHDGALSASTQA LLDAAVAIIS

DosT_M. tuberculosis (O53473)

DosT_M. tuberculosis (O53473) G L K I D A T L R A I V H T A A E L V D A R Y G A L G V R G Y D H R ... L V E F V Y E G I D E E T R H L I G S L P F G R G V L G A L
M. smegmatis (ABK70610) G L E L D V I L R T I V H T A I E L V D A R Y G A L G V R G E D H H ... L I E F T Y E G I T P E V R E I G P L P G R G V L G V L
M. vanbaalenii (ABM12229) G L E L D E L T I V R T A I D L V D A Q Y G A L G V R G H D H E ... L V E F T Y Q G I D E Q S K E L I G H L P G R G V L G V L
M. gilvum (ABP46316) G L E L D E L T I V R T A I D L V D A Q Y G A L G V R G H D Q E ... L V E F T Y E G I D E P M R E I G H L P G R G V L G V L
DosS_M. ulcerans (ABL04774) G L E L D A T L R T I V H S A T N L V D A R Y G A L E V H D R D K R ... L V Q F V Y E G I D E T V A R I G H L P G L G I I G H L
DosS_M. bovis (BCG_CAL73144) G L D L E A T L R A I V H S A T S L V D A R Y G A M E V H D R Q H R ... V L H F V Y E G I D E T V R R I G H L P K G L G V I G L L
DosS_M. tuberculosis (NP217648) G L D L E A T L R A I V H S A T S L V D A R Y G A M E V H D R Q H R ... V L H F V Y E G I D E T V R R I G H L P K G L G V I G L L
N. farcinica (BAD57741) G L D L D N T L R S I V H T A I E L V D A K Y G A L G V R E S D R D S K E L A E F V Y E G I D D R T R V L I G D L P R G H G V L G L L
Rhodococcus_RHA1 (ABG91885) G L D L D N I L T I V H A A I L V D A R Y G A L G V L G P G G G ... L S E F V F E G I D E E T R E I G D L P R G R G V I G L L
T. fusca (AAZ57079) G L D L E S T L R R I V S T A M D L V D A R Y G A M G V I G P G G E ... L E Q F T P L G M S D E I A A V E H W P G R G L I G L L
S. coelicolor (CAB53427) B I D L E Q A I R S I V E A A A A L V D A E Y A A L G V I G P D G K R ... L S A F H T V G V D A Q Q I A R I G P Y P G H G I I G L L
S. tropica (ABP54044) D L D L R S T L Q R I V E A A S E L A G A R Y G A L G V L G R D . R T . L H D F V T H G I D D E Q R A K I G E L P H G R G V I G L L
Nocardioide JS614 (ABL80469) D L D L R A V L T R I V E A A S R T E G A E Y G A L G V V G S G S L ... L V E F V T T G I D D T R R L I G D P P H G H G I I G L L

Surface crevice

DosT_M. tuberculosis (O53473)

DosT_M. tuberculosis (O53473) T E E P K P T I R L D D I S R H P A S V G F P L P P M R T F L G V P V R I R D E V F G N L L T E K A D Q P F S D D D E V L V Q A
M. smegmatis (ABK70610) I D D P K P I R L D D I R L P A S V G F P P N P P M R T F L G V P V R I R D K V F G N L L T E K T N Q P F S E D D E V L V Q A
M. vanbaalenii (ABM12229) I D D P K P I R L D R I S Q H P A S V G F P P N P P M R T F L G V P V R I R D E V F G N L L T E K A D Q P F S E D D E V L V Q A
M. gilvum (ABP46316) I N D P K P I R L E D I S R H A S V G F P P N P P M R T F L G V P V R I R D E V F G N L L T E K S D G Q P F S E D D E V L V Q A
DosS_M. ulcerans (ABL04774) I D E P K P I R L E D I S Q H P A S I G F P P S H P P M R T F L G V P V R V R D V S I G T L L T D K T N G Q P F S D D D E V L V Q A
DosS_M. bovis (BCG_CAL73144) I E D P K P I R L D D V S A H P A S I G F P P Y P P M R T F L G V P V R V R D E S F G T L L T D K T N G Q P F S D D D E V L V Q A
DosS_M. tuberculosis (NP217648) I E D P K P I R L D D V S A H P A S I G F P P Y P P M R T F L G V P V R V R D E S F G T L L T D K T N G Q P F S D D D E V L V Q A
N. farcinica (BAD57741) I T D P R P I R L A D M S A H P A S V G F P D H P P M R T F L G V P V Q V G G R V F G N L L T E K A G S G H F T E D D E V V V Q A
Rhodococcus_RHA1 (ABG91885) I T N P K P V R L D D L S H S A S V G F P A B P P M K T F L G V P I Q I R D E V F G N L L T E K A N G S Q P F T E D D E V L I R A
T. fusca (AAZ57079) I K E P K T I R L S C I S E H P S A G F P P G P P M R T F L G V P I R V R N E V F G N L L T E K R N N A V F D E E D E A I V T A
S. coelicolor (CAB53427) I R H P E P I R L A K I L S D H P A S V G F P A H P P M N T F L G V P I R V R D E V F G N L L T E K R G G Q Q F D E D D E S V I A T
S. tropica (ABP54044) I R D P R P V R M P D I T Q H P A S V G F P P N P P M R S F L G V P V R I R D Q V F G N L L A E A K R D A A E F T E D D E E I V V A
Nocardioide JS614 (ABL80469) I R D P E P I R L D D L T Q S S A V G F P P H P P M R S F L G V P V R I R G T V F G N L L T E K S G G A F T A E D D Q L L V E S

Proximal heme-binding motif

DosT_M. tuberculosis (O53473)

DosT_M. tuberculosis (O53473) L A A A A G I A V D N A R L F E E S R T R E A W I E A T R I G T Q M I A G A D F A M V F R L I A E A L T L M A G A A T L V A V P L
M. smegmatis (ABK70610) L A A A A G I A V D N A R L Y E E S Q A R Q A W I A A T R I G T Q L L S G T D F A T V F R L V A A E A L T L T G A D G T L V A V P A
M. vanbaalenii (ABM12229) L A A A A G I A I D N A R L Y Q S K T R R S W I E A T R I G T E M L S G A D E S K V F R L V A D Q S R L S G A Q S T L V A V P A
M. gilvum (ABP46316) L A A A A G I A I D N A R L Y A Q S Q A R Q A W I E A T R I G T E L L S G I D F A G V F R R V A D R A Q L S G A A T L V A V P D
DosS_M. ulcerans (ABL04774) L A A A A G I A I A N A R L Y E Q A R Q A W I G A T R I A T E L L S G T E F A S V F R L V A E A L K L T S A D A L V A I P V
DosS_M. bovis (BCG_CAL73144) L A A A A G I A V A N A R L Y Q Q A K A R Q S W I E A T R I A T E L L S G T E F A T V F R L V A A E A L K L T A D A L V A V P V
DosS_M. tuberculosis (NP217648) L A A A A G I A V A N A R L Y Q Q A K A R Q S W I E A T R I A T E L L S G T E F A T V F R L V A A E A L K L T A D A L V A V P V
N. farcinica (BAD57741) L A A A A G I A I A N A R L Y E E S R I R Q A W I E A T Q V A T R L L A G G E T S E V F T V I T E R A L A L T G S A C A F L A I P E
Rhodococcus_RHA1 (ABG91885) L A A A A G I A I E N A R L Y E E S R T R Q A W I E A T R E I A T E L L A G S E T A E V L Q V I A D D A L T A L T G A D S A F L A V P D
T. fusca (AAZ57079) L A T A A G I A I D N A R L Y E E T R R R E T W L D A S D E I T T R L L S G T A P R E V L H L V A K K A R M A D A D L V A I A T P D
S. coelicolor (CAB53427) L A V A A G V A I D N A R L Y E E S R L R E R L Q V N A B I T H T L S G A D Q G G V L P L A E R A R E I T G S A L S V A T P V
S. tropica (ABP54044) L A A A A G V A I E N A R L Y E L A H R R E R W L A A A A E I T T L L G E V H R T D A L S L V A R R A R E V A E A E L A L V L L Y D
Nocardioide JS614 (ABL80469) L A S T A G F V I D N A R A Y G L S E R R R O H L E A S A E L T E M L Q P P I E L G R A L S Q V A R S A R S M S G A V A S V L R L D

DosT_M. tuberculosis (O53473)

DosT_M. tuberculosis (O53473) D D E A P A C E V D D I V I V E V A G E I S P A V K Q M T V A V S G T S I G G V F H D R T P ... R R F D R . L D I A . V D G P V E
M. smegmatis (ABK70610) D P D A S A A E . . E L V I V E V A G A V P A P E A S A I P V Q D N A I G Q A F R D R A P ... R R L D V . L D G P G L
M. vanbaalenii (ABM12229) D L D E L D G R V D E L V V V A T A G D . G P G V S L S A I P T H G S A I G E V F S T K T P V F R D T . L E L E . P . R H L A
M. gilvum (ABP46316) D P D L P V G E V A E L T V A A S A G E . T T A A A H D P I P V A G T A I G D A F V H R T P G M T D R . A D I G . I . G A T V
DosS_M. ulcerans (ABL04774) D E D V P A S E V A Q L L V I E T V G N A V A A E G C T I A V A G T A L A E V L L D S A P R Q V D K . I A V E D V D E L G D
DosS_M. bovis (BCG_CAL73144) D E D M P A A D V G E L L V I E T V G S A V A S I V G R T I P V A G A V L R E V F V N G I P R R V D R . V D E L G L D E I A D
DosS_M. tuberculosis (NP217648) D E D M P A A D V G E L L V I E T V G S A V A S I V G R T I P V A G A V L R E V F V N G I P R R V D R . V D E L G L D E I A D
N. farcinica (BAD57741) D P D V P P E D L T E L V V V A A G A D A D . I T G T R L P M D E T H T G V A F R D R P L A V D T . A D A P S F A T S L E
Rhodococcus_RHA1 (ABG91885) D P D I P S D E V T E L V V I A S A G T V S D R I I G R T I P V D K S T S G E A F R E R T P L R V T A L A F D P G F E T G T R
T. fusca (AAZ57079) R D E E L V V R I A D G P D A S V V R G R S T P V E G S L T G R A F R G A D P I I T . E L A R E A S T P T L L H G L D
S. coelicolor (CAB53427) S G T D T L A V E I A D G H E A D A W R G V L P V E G T L I G Q A F V Q R A P V H S A D E C R D S R V S A G P F E G
S. tropica (ABP54044) P G A D T F T V D V V D G A G E Q A A L V G T V L P A A D T S F G A A V I H G R H D R V A D L A Y A A P W P A I F D
Nocardioide JS614 (ABL80469) N G T P V H T T A V D E A D A D R V G G A L E L I G D E V N F R G D V S P V G R T I G G L E

Fig. S3 (page2)

DosT M.tuberculosis (053473)

DosT_M.tuberculosis (O53473)
M.smegmatis (ABK70610)
M.vanbaalenii (AEM12229)
M.gilvum (ABP46316)
DosS_M.ulcerans (ABL04774)
DosS_M.bovis (BCG_CAL73144)
DosS_M.tuberculosis (NP217648)
N.farcinica (BAD57741)
Rhodococcus_RHA1 (ABG91885)
T.fusca (AAZ57079)
S.coelicolor (CAB53427)
S.tropica (ABP54044)
Nocardioideis JS614 (ABL80469)

320 330 340 350 360 370 380

PGP ALVPLRLADTVAGVLVALRSADQPPFSDKQLDMMMAAFADQAALAWRLATFQRQMRSEVILTDR
GCP ALVPLRLRADTVAGVLVAVQSGGARPFTELEDDMMATGFADQAALAVQLASSQRMRSELILADR
PGP ALVPLRLRADTVAGVLVAVREFGAPPFNAELDMMMAAFVDQAALAWQLATFQRQLRELVLDR
.GP ALLPLRLRADPAVGVLLVLRSVGAPQFPRSEDMLMAAFADQAALAVQLASTQHRQLRELGLTDR
MGP ALVPLRLRADTVAGGVVLLHPSSESIVTEELDMMMAAFADQAALALQLATFSQRMRREL DVVTR
AGP ALLPLRLRARGTVAGVVVVLSCQGGPFAFTELEDDMMMAAFADQAALAWQLATSQRMRREL DVLTDR
AGP ALLPLRLRARGTVAGVVVVLSCQGGPFAFTELEDDMMMAAFADQAALAWQLATSQRMRREL DVLTDR
YGP VLTPLRLRAGNAVGLLTMRALGMPVDAAGQAMMSAFADQAALALQMAATQRRMRELDVVSER
FGPALADPLRAAQSGTVGLVTLRHVDALPFTDQLALMSGFADQAALAVQLANSQRMRRELDVLSDR
IGPVVLVPPGASRRGVLLTGRKAGRLPFPFMTVQMLHAFAGHAAVLELAELARRHAEKRLIVLEDR
LGP GVAVPIGSSAEARGVLLVRQSGGQEFSEETEPPLLVFAQAQAVAMELAERRADAEQIALLEDRE
TGP AVLSPLATADTLHGVLLVAFEGDHHDTSEDIALLGSEFAADQAALAMERARQAEERELVLEDR
... ALLVPLRLTHLVGSLVLLVFCAGHTPPPAFERALLASFAADQAALADRAOQIEDREELAVTSDR

Dost M.tuberculosis (053473)

DosT_M.tuberculosis (O53473)
M.smegmatis (ABK70610)
M.vanbaalenii (AEM12229)
M.gilvum (ABP46316)
DosS_M.ulcerans (ABL04774)
DosS_M.bovis (BCG_CAL73144)
DosS_M.tuberculosis (NP217648)
N.farcinica (BAD57741)
Rhodococcus_RHA1 (ABG91885)
T.fusca (AAZ57079)
S.coelicolor (CAB53427)
S.tropica (ABF54044)
Nocardioideis JS614 (ABL80469)

390			400			410			420			430			440			450																																															
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	V	G	L	T	L	Q	G	A	P	R	A	V	P	A	V	R	E	S	T	Y	S	S	I	D	D	L	Q	E	I	Q	E	I	R	S	A	I	F	D	L	H	A	G	P	S	R	T				
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	V	G	L	T	L	Q	G	T	I	P	R	A	S	A	E	V	Q	Q	R	I	A	D	C	V	D	D	L	Q	Q	V	I	Q	E	I	R	T	A	I	F	D	L	H	T	P	A	G	G	T		
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	V	G	L	T	L	Q	G	T	I	P	R	A	S	A	E	V	Q	Q	R	I	A	D	C	V	D	D	L	Q	Q	V	I	Q	E	I	R	T	A	I	F	D	L	H	G	H	G	G	S	N		
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	V	G	L	T	L	Q	G	S	I	A	R	A	S	E	V	Q	Q	R	I	A	D	C	V	D	D	L	Q	S	V	I	Q	E	I	R	T	A	I	F	D	L	H	G	S	S	A	T				
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	I	G	L	A	L	Q	G	S	V	P	R	T	R	E	P	K	V	Q	Q	R	L	S	D	V	D	D	L	Q	Q	V	I	Q	E	I	R	T	I	F	D	L	H	G	S	S	Q	V	T			
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	I	G	L	A	L	Q	G	A	V	P	H	E	R	N	P	E	V	Q	Q	R	L	S	D	V	D	D	L	Q	Q	V	I	Q	E	I	R	T	T	I	F	D	L	H	G	A	S	Q	G	I	T	
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	I	G	L	A	L	Q	G	A	V	P	H	E	R	N	P	E	V	Q	Q	R	L	S	D	V	D	D	L	Q	Q	V	I	Q	E	I	R	T	T	I	F	D	L	H	G	A	S	Q	G	I	T	
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	V	G	L	T	L	Q	G	T	V	S	R	A	R	A	P	E	I	K	T	R	L	M	D	T	V	N	D	V	Q	S	V	I	Q	E	I	R	H	S	I	F	D	H	S	S	S	A	E	A	E	A
D	R	I	A	D	L	H	D	H	V	I	Q	R	L	F	A	V	G	L	T	L	Q	G	S	T	L	Q	R	A	P	I	V	K	Q	R	L	Q	S	I	D	D	L	H	D	I	V	R	D	I	R	T	A	I	F	D	L	H	G	S	S	G	G	M	T		
D	R	I	A	K	D	L	H	D	V	I	Q	R	L	F	A	I	A	M	S	L	M	S	V	R	R	E	D	D	T	V	A	Q	R	I	Q	A	V	D	D	L	H	D	T	I	Q	R	S	T	I	F	A	L	H	G	N	T	E	G	G	F					
D	R	I	A	D	L	H	D	L	A	I	Q	R	L	F	A	T	G	M	T	L	Q	S	A	G	R	R	V	Q	D	P	M	A	S	E	R	I	L	R	A	V	D	D	L	D	E	T	I	K	I	R	S	T	I	F	G	L	R	S	R	D	D	A	V		
D	R	I	A	D	L	H	D	V	I	Q	R	L	F	A	T	G	L	Q	S	A	P	M	S	T	R	E	L	A	N	R	I	N	A	V	D	D	L	D	A	T	I	K	D	I	R	T	I	F	E	R	T	I	F	E	R	T	...	P	M	T					
E	R	I	A</																																																														

Autophosphorylation site

DosT M. tuberculosis (053473)

DosT_M.tuberculosis (O53473)
M.smegmatis (ABK70610)
M.vanbaalenii (AEM12229)
M.gilvum (ABP46316)
DosS_M.ulcerans (ABL04774)
DosS_M.bovis (BCG_CAL73144)
DosS_M.tuberculosis (NP217648)
N.farcinica (BAD57741)
Rhodococcus_RHA1 (ABG91885)
T.fusca (AAZ57079)
S.coelicolor (CAB53427)
S.tropica (ABP54044)
Nocardioideis JS614 (ABL80469)

460 470 480 490 500 510
 G. LRHRIDKVIDLAIPALHTVQYTGPLSVVDTVLANEAEAVLRBAVSNVVRHASTSLTNVS
 R. LRQRIDEAIAQFS.GAGLHTTSCFVGPLSVVDAELDEAEAVLRBAVSNVVRHAGNELTVRK
 R. LRQRIDEAIAQFT.SADVHTVQFVGPLSVVDPALSDAEAVVRBAVSNVVRHSGATLTDVSVT
 R. LRQRIDAAVAAFPE.SGVRTSMQCAGPLSVVDAGLDEAEAVVRBAVSNVVRHAEASTVAVTVT
 R. LRQRIDAAVAAFG.SGLRRTVQFVGPLSVVDNVLDAEAEAVVRBAVSNVVRHAAATLTLTVRK
 R. LRQRIDAAVAQFAD.SGLRRTVQFVGPLSVVDSALADQAEAVVRBAVSNVVRHAKASTLTLTVRK
 R. LRQRIDAAVAQFAD.SGLRRTVQFVGPLSVVDSALADQAEAVVRBAVSNVVRHAKASTLTLTVRK
 PALRKRLHVAIAEMTEETDLRTSRLSGPVVLAPAMFDVEAVLRBAVSNVVRHAKASSLVRILT
 Q.FRRRLHIEIVAEITADSGRLRTVHMSGPLSVIDAALDEAEAVLRBAVSNVVRHAGATVTVSIS
 AWRRLRIDVVGAAATESLGFSPVRLDGPISAVPDSIAEEVLAVLRBAVSNVVRHARASQDITVQV
 PGLRSRAVRAVGEAAPLLGFAPSVRMEGLDTHVPARTADEVMAVLTESLTNVARHARADRADVVLE
 AQLRSRTRAEVDAAESLGFAPRLALAGPDSAVSDLEPDLTVLVRBAVSNVVRHARADRAVAVTVQ
 PSRLRLRLQRLAREYAPLLGFDPTVTITVGGVDVAVPTVEVRDQLLVLRBAVSNVVRHAAAHAEIELA

Dost *M. tuberculosis* (053473)

DosT_M.tuberculosis (O53473)
M.smeagmati (ABK70610)
M.vanbaalenii (AEM12229)
M.gilvum (ABP46316)
DosS_M.ulcerans (ABL04774)
DosS_M.bovis (BCG_CAL73144)
DosS_M.tuberculosis (NP217648)
N.farcinica (BAD57741)
Rhodococcus_RHA1 (ABG91885)
T.fusca (AAZ57079)
S.coelicolor (CAB53427)
S.tropica (ABP54044)
Nocardioideis JS614 (ABL80469)

[illegible]

DosT M.tuberculosis (053473)

DosT_M.tuberculosis (O53473)
M.smegmatis (ABK70610)
M.vanbaalenii (AEM12229)
M.gilvum (ABP46316)
DosS_M.ulcerans (ABL04774)
DosS_M.bovis (BCG_CAL73144)
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Nocardioideis JS614 (ABL80469)

Figure S3. Heme-binding and ligand-binding motifs. Multiple sequence alignments between putative two-component sensory protein-histidine kinases from different organisms are shown. Accession numbers of proteins in Swiss-Prot/TrEMBL (<http://us.expasy.org/sprot>) or NCBI (<http://www.ncbi.nlm.nih.gov>) databases are given next to the name of the host organism (*M. tuberculosis*, *Mycobacterium tuberculosis*; *M. smegmatis*, *Mycobacterium smegmatis*; *M. vanbaalenii*, *Mycobacterium vanbaalenii*; *M. gilvum*, *Mycobacterium gilvum*, *M. ulcerans*, *Mycobacterium ulcerans*; *M. bovis*, *Mycobacterium bovis*; *N. farcinica*, *Nocardia farcinica*; *T. fusca*, *Thermobifida fusca*; *S. coelicolor*, *Streptomyces coelicolor*, *S. tropica*, *Salinispora tropica*). Secondary structure annotation and residue numbering is according to GAF_{DosT} of *M. tuberculosis*. The α -helices are represented by *spirals* and β -strands by *arrows*. Alignments were performed using the MAP algorithm as implemented in the BCM Search Launcher (<http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html>). Residues constituting the proximal heme binding motif as deduced from the crystal structure are marked with *blue triangles*. Residues constituting the distal ligand-binding pocket are marked with *green squares*. Residues making up the surface crevice are marked with *pink triangles*. Invariant H147 and Y169 are highlighted in *cyan*. Highly conserved H392 implicated as the site of autophosphorylation is highlighted in *green*. The image was prepared by using program ESPript (<http://esprpt.ibcp.fr/ESPript/ESPript/>).