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**The inducer maltotriose binds in the central cavity of the tetratricopeptide-like sensor domain of MaIT, a bacterial STAND transcription factor**

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1 **The inducer maltotriose binds in the central cavity of the**  
2 **tetratricopeptide-like sensor domain of MalT, a bacterial STAND**  
3 **transcription factor**

4

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10 Running title: Maltotriose-binding site of MalT sensor domain

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19

20 # Abbreviations: STAND, Signal Transduction ATPases with Numerous Domains; NOD, nucleotide

21 Oligomerization Domain; SUPR, Superhelical Repeats; AAA+ ATPases, ATPases associated with

22 various cellular activities; APAF1, apoptosis activating factor 1; NLRP, NOD, Leucine rich repeat and

23 Pyrin domain containing; TPR, tetratricopeptide repeats; DT3, sensor domain of MalT; rmsd, root

24 mean square deviation; Ni-NTA, nickel-nitrilotriacetic acid.

25

26

## 1 **Summary**

2 Signal transduction ATPases with numerous domains (STAND) are sophisticated proteins that  
3 integrate several signals and respond by building multimeric platforms allowing signaling in various  
4 processes: apoptosis, innate immunity, bacterial metabolism. They comprise a conserved nucleotide  
5 oligomerization domain (NOD), which functions as a binary switch that oscillates between the OFF  
6 (ADP-bound) and the ON (ATP-bound) conformation, and non conserved sensor and effector  
7 domains. Transition from the OFF form to the ON form strictly depends on the binding of an inducer to  
8 the sensor domain. The interaction of the inducer with this domain was studied in MalT, a model  
9 STAND protein. MalT sensor domain has a SUPR (superhelical repeats) fold resembling a cylinder  
10 with a central cavity. The cavity was subjected to an alanine-scanning approach, and the effects of the  
11 alanine substitutions on inducer binding and transcription activation were analyzed. This work  
12 unambiguously showed that the inducer maltotriose binds inside the cavity, and a patch on the inner  
13 surface was proposed to be the primary maltotriose binding-site. Furthermore, limited proteolysis  
14 suggested that maltotriose binding changes the conformation of the sensor domain.

## 1 Introduction

2 AAA+ ATPases constitute a large class of P-loop ATPases functioning as multimers and involved  
3 in extremely diverse cellular activities. Signal Transduction ATPases with Numerous Domains  
4 (STAND) are atypical AAA+ (Erzberger *et al.*, 2006) involved in signal transduction (Danot *et al.*, 2009;  
5 Leipe *et al.*, 2004) that are able to assemble into large multimeric scaffolds upon integrating several  
6 positive and negative signals. These platforms bring together target molecules of various kinds or carry  
7 out enzymatic reactions, which triggers downstream signaling. Signaling by STAND proteins pertains to  
8 various cellular functions: for example apoptosis (like the proapoptotic factor APAF1), innate and  
9 adaptive immunity (the numerous NLRP and plant R proteins, NOD1 and NOD2, CIITA) in eukaryotes.  
10 In prokaryotes, STAND have also multiple roles: primary or secondary metabolism, like transcription  
11 activators MalT (regulator of the *Escherichia coli* maltose regulon, Boos and Shuman, 1998) and AfsR  
12 (a global regulator controlling antibiotic production in *Streptomyces coelicolor*, Tanaka *et al.*, 2007) but  
13 also envelope integrity and probably survival in activated macrophages, like *Mycobacterium*  
14 *tuberculosis* serine/threonine kinase PknK (Kumar *et al.*, 2009 and references therein).

15 STAND proteins share a multidomain architecture (Fig. 1A) comprising a NOD (Nucleotide-  
16 binding Oligomerization Domain) conserved module, followed by a non-conserved sensor domain  
17 sometimes separated from it by a short spacer called the arm. This architecture is completed by one or  
18 several effector domains, which lie at either end of the NOD-sensor polypeptide. The NOD module is  
19 the centerpiece of the mechanism of action of STAND proteins. The NOD consists of the conserved  
20 core of AAA+ ATPases, with Walker boxes A and B, followed by a winged helix domain specific to the  
21 STAND. The presence of this domain enables the NOD module to act as a binary switch that oscillates  
22 between two conformations: an ADP-bound long-lived OFF form (inactive) and an ATP-bound ON form  
23 prone to multimerize (Bao *et al.*, 2007; Marquetet and Richet, 2007; Riedl *et al.*, 2005; Tameling *et al.*,  
24 2006), which leads to the multimeric platform that is the active species.

25 Signaling by STAND proteins relies on the control of this NOD binary switch by an inducer, i.e. a  
26 molecule that binds and activates the protein (Bao *et al.*, 2007; Marquetet and Richet, 2007; Schreiber  
27 and Richet, 1999). However, no direct interaction between the inducer and the NOD was observed in  
28 any STAND protein, whereas at least in two cases, those of the rice R protein Pi-ta (Jia *et al.*, 2000)  
29 and the MalT transcription activator (Danot, 2001), the inducer bound the sensor domain, hence its  
30 name. Yet, the precise mechanism of action of the inducer on the NOD switch is unknown. Deletion of  
31 the sensor domain in STAND proteins had opposite results depending on the protein. In certain

1 instances it caused protein activation in the absence of inducer (Ade *et al.*, 2007; Hsu *et al.*, 2008; Hu  
2 *et al.*, 1998; Tanabe *et al.*, 2004), leading to the notion that the apo-sensor domain maintains the NOD  
3 in the OFF position till inducer binding to the sensor relieves this inhibition. In other cases deletion of  
4 the sensor abolished the activity, which could sometimes be restored by co-producing the sensor  
5 domain in trans (Moffett *et al.*, 2002), suggesting that this domain can also play a positive role.

6 Sensor domains are not conserved throughout the STAND family. They belong almost exclusively  
7 to four classes of superstructure-forming domains made of repeated motifs: leucine-rich repeats,  
8 WD40 repeats, tetratricopeptide repeats (TPR), and superhelical repeats (SUPR), a variation of the  
9 TPR. Bacterial proteins of the STAND family have a sensor domain either of the TPR or of the SUPR  
10 kind, the latter being specific of the MalT/PknK clade. Even though numerous WD40, leucine-rich  
11 repeats and TPR domains have been crystallized in other contexts, the only STAND sensor domain  
12 whose three-dimensional structure is known to date is that of MalT (abbreviated as DT3, Steegborn *et al.*,  
13 2001). Like TPR, SUPR are made of a two  $\alpha$ -helix antiparallel bundle motif, but SUPR helices are  
14 longer and the motif is more degenerated. In both cases, the succession of the  $\alpha$ -helices forms a right-  
15 handed solenoid with adjacent bundles parallelly stacking against each other (Fig. 1B, inset), whose  
16 envelope has the shape of a superhelical slab. In the MalT SUPR structure the superhelical slab winds  
17 upon itself to form a hollow cylinder, due to the head-to-tail stacking of helix bundles one superhelical  
18 turn (i.e. 5 bundles) apart. The cylinder is closed at one end by a C-terminal three-helix subdomain. It  
19 has an axial opening (Fig. 1C, left panel) at the other end of the cylinder (near the N-terminus) and a  
20 second, lateral opening between the contact point between bundles 1 and 6 and that between bundles  
21 2 and 7 (Fig. 1C, right panel).

22 Whereas inducer binding is a key event in STAND signaling, no inducer binding site of a STAND  
23 protein has been characterized yet. To study the molecular basis of inducer-sensor domain interaction  
24 on the model of the MalT transcription activator, an alanine-scanning mutagenesis approach was  
25 undertaken. Functional analyses revealed the precise location of the binding site for the inducer  
26 maltotriose and suggests that this binding changes the conformation of the sensor domain.

## 28 Results

### 29 Strategy

30 Statistics bearing on ligand-binding proteins with a known three-dimensional structure show that  
31 the ligand binding site is harbored by the largest cavity on the surface of the protein in about 70% of

1 the cases (Nayal and Honig, 2006). A candidate for inducer binding in the MalT sensor domain is  
2 therefore the large central cavity (3530 Å<sup>3</sup> as calculated using the CASTp software (molecular surface  
3 calculation (Dundas *et al.*, 2006)). The Van der Waals volume of a maltotriose molecule is only 420 Å<sup>3</sup>  
4 (calculated as described (Zhao *et al.*, 2003)). There are therefore numerous ways in which maltotriose  
5 could be accommodated in this cavity. To determine whether maltotriose binds in this pocket, and to  
6 accurately locate its binding site, residues that line the cavity (identified by CASTp (Table S2)) were  
7 individually replaced by alanines. Glycine and proline residues were excluded from this analysis. Since  
8 MalT proteins from other bacteria are expected to bind the same inducer (maltotriose) in the same way  
9 as *E. coli* MalT, the analysis was also restricted to residues conserved in a set of 6 MalT sequences\*.  
10 According to these rules, 29 codons (Fig. 1B) of the part of the *malT* gene encoding DT3 borne by an  
11 M13 vector were individually changed to alanine codons by site-directed mutagenesis, thus creating a  
12 homogeneous library of sensor domain mutations.  
13

#### 14 *A majority of the mutations affect MalT activity in vivo*

15 The pop8083 strain bears a *malP'*-*lacZYA* fusion at the *malPQ* locus, which directs the synthesis  
16 of a hybrid MalP'-LacZ active β-galactosidase under the control of the *malPp* MalT-dependent  
17 promoter. The *malK-malM* region of this strain is replaced by a zeocin resistance cassette: in the  
18 absence of the MalK protein, which is known to inhibit MalT activity, MalT-dependent transcription  
19 becomes constitutive, and this constitutivity is at least partially due to the presence of endogenous  
20 maltotriose in the cytoplasm. All the mutations were transferred to the chromosomal *malT* gene of  
21 pop8083, strains were grown at 25°C and their β-galactosidase activity was assayed.

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\* The MalT sequences used were those from gammaproteobacteria *Aggregatibacter actinomycetemcomitans*, *Actinobacillus pleuropneumoniae*, *Photobacterium luminescens*, *Vibrio cholerae*, *Vibrio vulnificus*, *Yersinia pseudotuberculosis*. They are all annotated as MalT proteins by the NCBI (<http://www.ncbi.nlm.nih.gov/>) and the corresponding genes map immediately next to orthologs of *malP* and *malQ*, as in the *E. coli* chromosome. In addition, the proteins of *V. cholerae* and *V. vulnificus* (those with the lowest percentage of identity with *E. coli* MalT) have been purified and showed maltotriose dependent multimerization like *E. coli* MalT (Danot, unpublished results).

1 A majority of the mutations had a deleterious effect on MalT activity (Fig. 2), with those belonging  
2 to the N-terminal part of the domain (i.e. close to the openings of the cavity) being in general more  
3 detrimental. Only 5 mutations out of 29 did not decrease the activity of the *malPp* promoter. Among  
4 these mutations, *D647A*, *Q690A* and *N693A* did not alter MalT activity, while *R729A* and *R692A*  
5 increased it. Three substitutions (*W581A*, *R696A* and *I769A*) caused complete abolition of MalT  
6 activity. A western blot of soluble extracts prepared from cultures of all the pop8083 derivatives grown  
7 in the conditions of the  $\beta$ -galactosidase assays (Fig. 2, bottom panel) shows that the amount of soluble  
8 MalT was not significantly affected by the mutations: after quantification, all strains appeared to  
9 produce the MalT variants at a level within 20% (close to the experimental error) of the wt except for  
10 *W529A* (70%), *D647A* (150%), *W648A* (125%), *R696A* (55%), *I769A* (60%). In conclusion, a majority  
11 of the residues that line the large central cavity of the MalT sensor domain and that are conserved in  
12 MalT orthologs seem to play a role in the activity of MalT. These observations are compatible with a  
13 role of these residues in the binding of maltotriose and suggest that the inducer binding-site is indeed  
14 contained by the large central cavity of the sensor domain.

15

#### 16 *Purification of the variants of the isolated sensor domain.*

17 The  $\beta$ -galactosidase assays described above evaluate the influence of the substituted residues  
18 on the global signal transduction process carried out by MalT. To determine which residues of the  
19 sensor domain play a role in the physical interaction with maltotriose, the mutations were transferred in  
20 the context of the isolated sensor domain. The mutations were introduced in the gene encoding DT3  
21 present in a pET28b(+) derived expression plasmid, pOM164, harbored by the BL21(DE3) $\Delta$ *malT220*  
22 strain. In this expression system, wild-type DT3 and all variants could be produced in a soluble form  
23 except *W581A*, *R692A*, *R696A* and *I769A*. Proteins were purified by a one-step Ni-NTA  
24 chromatography. *R692A*, but not the three other insoluble variants, could be rescued by replacing  
25 IPTG induction by autoinduction (Studier, 2005). Moreover, to obtain *R692A* at sufficient concentration  
26 and purity, it was purified as a His<sub>6</sub>-tagged version named hR692A, along with a control His<sub>6</sub>-tagged  
27 version of DT3, hDT3. Finally, a total of 26 variants of the MalT sensor domain could be purified.

28 Noteworthy, the three mutations *W581A*, *R696A* and *I769A* that completely abolish  $\beta$ -  
29 galactosidase activity in pop8083 also render the isolated DT3 completely insoluble. This behavior



1 suggests that the effect of these mutations is due to a global destabilization of the structure. Note that  
2 *R696A* and *I769A* decrease the amount of soluble MalT *in vivo* even at low protein levels, as shown by  
3 the Western blot on Fig. 2, and that W581 is the most buried tryptophan residue of the whole domain.  
4 Interestingly, these mutations all affect residues involved in the interaction between the SUPR repeats  
5 and the three helix C-terminal subdomain: W581 (bundle 4) interacts with S765 (C-terminal  
6 subdomain), I769 (C-terminal subdomain) with M662 (bundle 6), and R696 (bundle 7) with E770 (C-  
7 terminal subdomain). Interactions between the SUPR fold and the C-terminal subdomain, which could  
8 play the role of the solvation or capping helix in TPR structures (Main *et al.*, 2003), might therefore be  
9 important for the overall stability of the sensor domain.

10

#### 11 *Effect of the mutations on maltotriose affinity: a fluorescence study*

12 The MalT sensor domain comprises 11 tryptophan residues, which means that its fluorescence  
13 emission spectrum is highly composite. It has been observed that maltotriose binding exalts the overall  
14 intrinsic fluorescence of DT3 and shifts the maximum of the emission spectrum towards shorter  
15 wavelengths (Danot, 2001). To examine the effects of the substitutions on maltotriose binding to the  
16 sensor domain, fluorescence spectra of all purified variants were recorded in the presence and  
17 absence of maltotriose (Fig. 3A). In general, the shape of the spectra of the purified variants without  
18 maltotriose is nearly identical to that of the wt protein, suggesting that the 26 substitutions tested do not  
19 change the protein three-dimensional structure significantly. Only two mutations, D726A and W648A,  
20 had a significant effect on the global position of the spectrum without maltotriose: they shifted it  
21 towards short wavelengths (Table 1). A straightforward explanation in the case of W648A is that W648  
22 has a maximum fluorescence emission in the vicinity of 350 nm (consistent with the fact that it is  
23 exposed to the solvent) and therefore contributes more to the right part of the overall fluorescence  
24 spectrum.

25 The maltotriose-induced changes in the fluorescence emission spectrum were differentially  
26 affected by the substitutions (Fig. 3A, Table 4). Out of 26 substitutions, 10 (group 1: E496A, H499A,  
27 W529A, Q533A, E536A, R574A, H645A, N684A, F686A, D726A) completely abolished the  
28 maltotriose-induced fluorescence increase, suggesting that the corresponding residues play an  
29 important role in maltotriose binding. Spectra of other variants like N693A or Q690A responded to  
30 maltotriose in the same way as the wt, whereas others had intermediate spectral changes. To quantify  
31 the differences between the variants, maltotriose binding curves were obtained by recording the  
32 fluorescence increase at a given emission wavelength as a function of maltotriose concentration (see

1 Fig. 3C for examples of binding curves and Fig. S2 for the totality of the binding curves). Dissociation  
2 constants derived from these experiments are reported (Table 2). 3 substitutions decreased the affinity  
3 of the sensor domain for maltotriose by a factor of more than 10 (group 2: L504A, Q688A, L723A), 2  
4 had a 5- or 6-fold effect (group 3: Q457A and H685A), and the 11 others (group 4: N461A, I537A,  
5 E570A, F571A, L600A, D647A, W648A, Q690A, hR692A, N693A, R729A) had less than a 3-fold  
6 effect. The large number of residues of the cavity whose substitution impacts maltotriose binding  
7 corroborates the *in vivo* study and strongly suggests that the cavity of the MalT sensor domain  
8 contains the maltotriose-binding site. To be more specific, residues of group 1 are most probably  
9 involved in maltotriose binding, while residues of group 2 and 3 might play accessory roles, and  
10 residues of group 4 do not participate importantly in the binding of maltotriose to DT3.

11

### 12 *The primary maltotriose-binding site*

13 Fig. 4A shows a surface representation of DT3 split in two halves to show the wall of the cavity. All  
14 of the 26 altered residues are colored according to the group which they belong to. The right half  
15 contains fewer residues that are conserved in MalT orthologs (and that were therefore tested). All  
16 residues of group 1 and 2 but H645 and R574 form a contiguous patch. This patch extends between  
17 the two openings of the cavity but does not reach towards the bottom of the pocket. It is entirely  
18 contained in the left moiety of the cavity (as defined from Fig. 4A) and more precisely in a quadrilateral  
19 whose summits are F686, E496, W529 and D726. It is contributed for by two pairs of helix bundles that  
20 are one superhelical turn apart: bundles 2 and 7, bundles 3 and 8. The largest distance between any  
21 two aminoacids of this patch, 16.5 Å (between D726 and W529) is slightly superior to the length of a  
22 maltotriose molecule (~13 Å).

23 In order to determine how maltotriose could interact with the putative binding-site built up by group  
24 1 residues, automated docking of maltotriose to the wall of the cavity was performed using AutoDock  
25 4.0 (Goodsell and Olson, 1990; Morris *et al.*, 1996; Morris *et al.*, 1998). The 450 docked conformations  
26 obtained were grouped in clusters (sets of similar conformations) according to their mutual rmsd  
27 values with a 3.0 Å tolerance. A large number of clusters was found (27 containing more than 4  
28 conformations), possibly reflecting the relatively high number of torsions of the ligand and the large  
29 protein surface tested. The lowest energy cluster (Table 3) had only 3 members and the 4 largest  
30 clusters fell within 2.5 kcal/mol (the standard error of the force field (Huey *et al.*, 2007)) of the lowest  
31 energy. Since the real conformation of the ligand can be approximated by the lowest energy  
32 conformation but also by a low-energy conformation from one of the most populated clusters (Bikadi

1 and Hazai, 2009), maltotriose conformations within 2.5 kcal/mol from the lowest energy and belonging  
2 to the 5 clusters mentioned above were studied further. To determine which one of these  
3 conformations is most likely to correspond to the real position of the maltotriose molecule bound to  
4 DT3, their average distance to group 1 residues was measured as described in Material and Methods.  
5 Conformations of cluster 7 scored best according to this test. The best scoring conformation of cluster  
6 7 (average distance to group 1 residues : 3.3 Å) is shown in Fig. 4A and 4C. The docked molecule,  
7 which lies roughly parallel to helix 3, with its reducing end directed towards the bottom of the pocket, is  
8 closer than 3.7 Å from 7 group 1 residues: E496, H499, W529, Q533, E536, F686 and D726, and  
9 several protein-ligand hydrogen bonds might be formed in this conformation (Fig. 4C). Moreover, it  
10 displays a good surface complementarity with the inner surface of the cavity (see Fig. 4A) and the  
11 central glucosyl unit is in a position to stack against F686, although imperfectly. For all these reasons,  
12 this theoretical conformation seems to be a reasonable working model of the bound maltotriose  
13 molecule. The 7 group 1 residues mentioned above belong to the contiguous patch described at the  
14 beginning of this chapter, which will be tentatively called the primary maltotriose binding site  
15 hereinafter.

16 Yet, H645, N684 and R574 (group 1) and Q457, H685, L504, L723 (groups 2 and 3) are more  
17 distant from the docked maltotriose. Three of them might play an indirect role by positioning important  
18 residues: N684 and H685 lie ~3 Å away from F686, and Q457 is hydrogen bonded to E496. As for  
19 L504 (bundle 2), L723 (turn between bundle 7 and 8, facing L504) and H645, they are probably  
20 important for the head-to-tail stacking of bundles 2 and 7 (H645A renders the R519-Q520 peptide bond  
21 sensitive to trypsin (not shown), suggesting a "breathing" of bundles 1 and 2 with respect to the rest of  
22 the domain).

23 Interaction of the last group 1 residue, R574, with maltotriose in the conformation of Fig.4C  
24 requires a long distance conformational change which is supported by the following observation.  
25 W648A, D648A and E570 variants have reduced maltotriose-induced blue-shifts (Table 1): the W648A  
26 substitution grossly mimics the absence of maltotriose, while D647A and E570A mimic its presence.  
27 An appealing hypothesis is that in the wt, maltotriose binding causes the two charged residues D647  
28 and E570 to come closer to W648 and quench its fluorescence, which requires a long distance  
29 conformational change.

30

31 *Maltotriose probably causes a global conformational change in the MalT sensor domain*

32 To test whether maltotriose binding exerts a long-range effect on the conformation of the MalT

1 sensor domain, limited proteolysis of DT3 by trypsin was performed in the presence and absence of  
2 maltotriose. The presence of 200 mM maltotriose drastically protected the domain from trypsin attack  
3 (Fig. 5). Control experiments showed that protease sensitivity of a control protein was barely affected  
4 by 200 mM maltotriose, as was trypsin sensitivity of DT3 by 200 mM glucose. In the crystal used to  
5 solve the structure of the MalT sensor domain, the primary maltotriose binding site proposed here is  
6 not accessible to the trypsin catalytic site because it is contained by the central cavity. Therefore,  
7 maltotriose binding protects the sensor domain from trypsin proteolysis, not by directly shielding  
8 peptide bonds of this binding site, but rather by changing the conformation of more exposed peptide  
9 bonds from a distance. This strongly suggests that maltotriose elicits a long-range conformational  
10 change of DT3.

11

## 12 Discussion

### 13 *The maltotriose binding site of MalT*

14 The first conclusion of this work is that maltotriose binds in the large inner cavity of the sensor  
15 domain of MalT. The primary maltotriose binding site defined by the mutational analysis extends  
16 between the two main openings of the cavity and comprise residues from two pairs of head-to-tail  
17 stacked helix bundles: 2 and 7 on one hand, 3 and 8 on the other hand. Interestingly, the binding-site  
18 lies in the upper moiety of the pocket. This is unexpected because a glycerol molecule was present in  
19 the bottom of the pocket in the crystals used to solve the structure of the MalT sensor domain, leading  
20 to the notion that maltotriose might bind in that region (Steegborn *et al.*, 2001). On the contrary,  
21 substitution of two arginines (R692 and R729) close to the glycerol in the crystal had virtually no effect  
22 on the affinity, and even increased transcription activation *in vivo*. One possible explanation for the  
23 latter observation is that these arginine residues are part of the binding site of a small molecule which  
24 would inhibit either maltotriose binding or subsequent signaling.

25

26

### 27 *The primary maltotriose binding site shows classical features of carbohydrate binding sites.*

28 Carbohydrate-binding sites have several characteristics that reflect the particular and  
29 amphiphilic nature of their ligands. Their most outstanding feature is the high frequency of two kinds of  
30 residues with different properties: aromatic residues that either stack against the pyranoside ring or  
31 establish Van der Waals contacts, and residues with polar planar groups that are suited to form

1 bidentate hydrogen bonds, as commonly observed in protein carbohydrate complexes (Quiocho,  
2 1989). In that respect, the proposed maltotriose binding site is fairly satisfactory, since all group 1  
3 residues but one (F686) belong to an aminoacid type that is more frequent in the sugar-binding sites of  
4 the Procarb40 database (Malik and Ahmad, 2007) than in the whole protein sequences of the same  
5 database. Furthermore, the repartition of the different residues in the proposed binding site makes  
6 sense: F686 lies in a central position, while residues with planar polar groups at the periphery (E496,  
7 Q533, E536 H499, Q688, D726) are well positioned for an interaction with the equatorial hydroxyl  
8 groups of the glycosyl units; finally, at the expense of a local conformational change, W529 could stack  
9 against the hydrophobic side (A-face) of the glycosyl moiety at the non-reducing end of maltotriose.  
10 The fact that each one the two aromatic residues of the primary maltotriose-binding site is close to an  
11 opening of the pocket suggests that they might also be involved in the translocation of maltotriose  
12 through these openings to its final binding site. Indeed, stacking interactions are often involved in  
13 guided diffusion of polysaccharides (Divne *et al.*, 1998; Schirmer *et al.*, 1995). However, the proposed  
14 model (Fig. 4 A, C) is not necessarily exact in all its details: protein-carbohydrate complex structures  
15 generally show a denser network of hydrogen bonds (Quiocho, 1989). It must also be recalled that the  
16 alanine-scanning was restricted to residues conserved in a sample of MaIT orthologs, so that a non-  
17 conserved secondary binding determinant could have been missed.

18 Finally, the shape of the primary maltotriose binding site proposed here provides a nice  
19 explanation for the specificity of MaIT for maltotriose (Raibaud and Richet, 1987, see also Dippel *et al.*,  
20 2005). Maltodextrins longer than 3 glucosyl units are too long to fit in, as shown by the poor effect of  
21 maltotetraose on the DT3 fluorescence spectrum (Fig. 3B). On the other hand, the binding site  
22 provides interacting partners all along the maltotriose molecule, which would explain the preference for  
23 maltotriose over maltose (Fig. 3B and Table 2). Interestingly, the specificity for maltotriose over  
24 maltose seems stronger in the whole protein (Raibaud and Richet, 1987) than in the isolated sensor  
25 domain. It is therefore likely that, in addition to its lower affinity for the sensor domain, maltose is also  
26 less efficient in triggering the conformational changes that ensue.

27

### 28 *Comparison with other tetratricopeptide domains*

29 From the beginning, TPR domains, the closest structural relatives of the MaIT SUPR domain,  
30 have been recognized as protein-protein interaction domains. Typically, binding of their partner protein  
31 has little if any effect on their conformation (Grove *et al.*, 2008). However, several TPR proteins have  
32 recently been shown to diverge from this simple model. In the case of protein phosphatase 5, the

1 ligand has been proposed to act allosterically on the TPR domain. Namely, binding of its Hsp90 C-  
2 terminal peptide ligand slightly changes the conformation of the protein phosphatase 5 TPR domain in  
3 a way that probably alters its inhibitory interaction with the catalytic domain (Cliff *et al.*, 2006). In  
4 another system, recognition of the peroxisomal targeting signal PTS1 by the PEX5 peroxisomal  
5 receptor was also shown to involve a long distance conformational change of the TPR domain of PEX5  
6 (Stanley *et al.*, 2006). Interestingly, the PEX5 TPR domain has the shape of a flattened cylinder with a  
7 central channel in which the PTS1 peptide binds, resulting in constriction of the channel due to a  
8 relative movement of the C-terminal helix bundles with respect to the N-terminal ones.

9 In the case of MalT, several observations point towards a maltotriose-induced conformational  
10 change of the SUPR domain. Assuming that maltotriose first binds to the primary maltotriose binding-  
11 site presented here, the involvement of R574 in the binding of the inducer could suggest a contraction  
12 of the structure analogous to that observed in PEX5, which would shrink the cavity and enable this  
13 residue to interact with maltotriose.

14

#### 15 *Maltotriose binding and signal transduction*

16 As said above, the  $\beta$ -galactosidase activities presented here are the outcome of a signal  
17 transduction process, whereas the fluorimetry experiments probe only one step of the signaling:  
18 maltotriose binding by the isolated sensor domain. Nevertheless, the two kinds of experiments give a  
19 relatively good correlation (Table 4). For instance, all 13 mutations causing more than a 10-fold  
20 reduction in maltotriose affinity for the sensor domain decrease the  $\beta$ -galactosidase activity of pop8083  
21 by at least 3-fold and 5 out of the 11 mutations causing less than a 3-fold effect on maltotriose affinity  
22 for the sensor domain confer a  $\beta$ -galactosidase activity superior or equal to that of the wt allele.  
23 However the correlation is broken for the 6 remaining mutations that only mildly affect maltotriose  
24 affinity. *I537A* gives a relatively low  $\beta$ -galactosidase level (46%), for which there is no obvious  
25 explanation. As to the remaining substitutions which barely alter the affinity of the sensor domain for  
26 maltotriose but confer a very low (12-25%)  $\beta$ -galactosidase activity (N461A, E570A, F571A, L600A,  
27 W648A), they are involved, not in maltotriose binding but in another step of the signal transduction  
28 process. The fact that they cluster around an opening of the cavity (Fig. 4B) further suggests that this  
29 step consists in establishing or destroying a contact between these residues and another part of the  
30 protein (for instance the NOD-arm region). How such an interaction between the sensor and the NOD-  
31 arm region enables maltotriose to regulate the NOD switch and whether a similar scheme also applies

1 to other STAND proteins with sensor domains of the SUPR and tetratricopeptide family remains to be  
2 determined.

3

## 4 **Experimental procedures**

### 5 *Strains and plasmids*

6 pop8080 is derived from pop3125 (MC4100 ( $\Phi$ *malPQ-lacZY*), Débarbouillé *et al.*, 1978) as  
7 follows: an F factor has been introduced and the joint of the *malPQ-lacZY* fusion has been replaced by  
8 that of the *malP'-lacZ'* translational fusion of phage M13malP3 (Danot and Raibaud, 1993), using  
9 transfer from M13 onto the chromosome as described by Danot and Raibaud (1993). pop8081 differs  
10 from pop8080 by a deletion encompassing the entire *malT* gene which was transferred from  
11 M13mp11::*malT* $\Delta$ *malT220* (Schreiber *et al.*, 2000) onto the chromosome. pop8083 and pop8084 were  
12 built from pop8080 and pop8081, respectively, by replacing the *malK-lamB-malM* operon by a cassette  
13 conferring zeocin resistance (Richet, unpublished results).

14 M13malT9 was obtained by cloning the *malT* HpaI-Acc65I fragment of pOM180 (Richet *et al.*,  
15 2005) that encompasses the *malT* fragment encoding the sensor domain (DT3), into the XbaI site of  
16 M13mp11 DNA after filling in all cohesive ends, the '*malT*' and '*lac*' ORFs being divergent.  
17 M13malT9 $\Delta$ *lac* is derived from M13malT9 by a 420 bp deletion starting immediately downstream of the  
18 EcoRI site of the polylinker and removing the whole '*lac*' sequence.

19 All the mutations except one (Table S1) were introduced in M13malT9 by mutagenesis by the  
20 method of Kunkel (1987). To facilitate transfer to the chromosome in the case of *W648A*, *Q690A*,  
21 *N693A* and *R729A*, the BssHII-BssHII fragment of the M13malT9 derivatives carrying these mutations  
22 were cloned between the BssHII sites of M13malT9 $\Delta$ *lac*. All the *malT* sequences in these phages were  
23 checked. Mutations were then transferred from M13malT9 or M13malT9 $\Delta$ *lac* onto the chromosomal  
24 *malT* gene of pop8080 as described (Danot and Raibaud, 1993). The success of the transfer was  
25 verified by amplifying the DT3 encoding fragment and digesting the amplicon by the relevant restriction  
26 enzyme (see Table S1) or by sequencing it in the case of *R729A*. Finally, all the pop8080 derivatives  
27 were converted into pop8083 derivatives (i.e. *malK lamB MalM*) by P1 transduction using a P1 lysate  
28 of pop8083 followed by zeocin selection.

29 pOM164 and derivatives were constructed as follows: pET28b(+) (Novagen) was first rid of its  
30 unique BssHII site by amplifying with Pwo DNA polymerase (Roche) the whole plasmid DNA with  
31 oligonucleotides ET1 (creating a silent mutation in the *lacI* BssHII site;

1 5'-IGCCATTACCGAGTCCGGGCT) and ET2 (5'-CGCATTGCGCCCAGCGCCA). The NcoI-HindIII  
2 fragment of pOM152 that encodes DT3 was inserted into the same sites of this pET28b(+) derivative,  
3 to give pOM164. pOM164H is derived from pOM164 by inserting the HTAG1-HTAG2 double stranded  
4 oligonucleotide (Danot, 2001) in the NcoI site. The *malT* mutations were transferred into pOM164 by  
5 cloning the small BssHII-BssHII fragment of the mutagenized M13malT9 into the BssHII sites of  
6 pOM164, except for mutations *N693A* and *L723A*, which create a new BssHII site: in this case, the  
7 fragment of M13malT9 that encodes DT3 was PCR amplified using oligonucleotides DT3G and DT3D  
8 (Danot, 2001), digested by NcoI and HindIII and cloned between the same sites of pOM164.  
9 pOM164HR692A was constructed by cloning the small BssHII-BssHII fragment of M13malT9R692A  
10 into the BssHII sites of pOM164H as a receiver.

11

### 12 *β-galactosidase assay*

13 Cells were grown in M9 medium (Sambrook *et al.*, 1989) supplemented with 0.4% sorbitol.  
14 Cultures were inoculated with 100 μl overnight precultures and grown at 37°C, or inoculated with 1-10  
15 μl of a day preculture and grown overnight at 25°C. When they reached an  $A_{600}$  of 0.5-0.8,  
16 β-galactosidase was assayed as described (Miller, 1972), using chloroform and 0.0019% SDS to  
17 permeabilize the cells and using DTT (0.5 mM final concentration) instead of 2-mercaptoethanol.

18

### 19 *Western blots*

20 Cells were grown at 25°C as for the β-galactosidase assays. For each strain, cells (the equivalent  
21 of 5 ml at  $A_{600} = 0.5$ ) were collected and resuspended in 500 μl of 50 mM Tris-HCl (pH7.7), 10%  
22 sucrose, 0.3M KCl, 1 mM ATP. Cells were disrupted by sonication, and centrifuged in a labtop  
23 centrifuge (Eppendorf) at 13000 rpm for 60 min. Soluble extracts (5 μl) were loaded on an SDS-PAGE  
24 gel containing 9.3% acrylamide and 0.12% bisacrylamide. Proteins were transferred on a Hybond™ C  
25 membrane, probed with anti-MalT antibodies and a horse-radish peroxidase coupled secondary  
26 antibody. As a control, membranes were stripped and reprobed with an anti-sigma<sup>70</sup> antibody.  
27 Membranes were treated with the ECL+ reagents (GE Healthcare, Fairfield, CT) and scanned on a  
28 Storm 840 (GE Healthcare).

29

### 30 *Protein purification*

31 BL21(DE3) Δ*malT220* carrying pOM164 or derivatives thereof were grown at 37°C in 200 ml LB



1 medium + 50 µg/ml kanamycine to  $A_{600} = 2$  and induced with 0.1 mM IPTG. Growth was continued at  
 2 24°C and cells were harvested 5 h later, washed and resuspended in 5 ml TSK buffer (50 mM Tris-HCl  
 3 (pH 7.7), 10% sucrose, 0.5M KCl). In the case of hDT3 (His-tagged DT3), hR692A, E570A, N693A and  
 4 D726A, 200 ml ZYP5052 medium (Studier, 2005) without trace metals and with bactotryptone instead  
 5 of N-Z amine + 100 µg/ml kanamycine were inoculated at  $A_{600} = 0.01$  with BL21(DE3)  $\Delta malT220$   
 6 carrying pOM164H, pOM164HR692A, pOM164E570A, N693A and D726A respectively. Bacteria were  
 7 grown under strong agitation (200 rpm), at 16°C for 48h (hDT3, hR692A) or at 22°C for 22-28h  
 8 (N693A, D726A, E570A) washed and resuspended in 12 ml TSK buffer.

9 Cells were disrupted with a French press at 16000 psi after addition of 20 µg/ml DNaseI and  
 10 centrifuged in a Ti75 rotor at 40000 rpm for 30 min. 2 ml (3 ml for L504A, 4 ml for N461A, Q533A  
 11 E570A and D726A, 5 ml for E496A, 14 ml for hR692A) of the supernatant were loaded on a 1 ml Ni-  
 12 NTA (Qiagen) column. The column was washed with TSK buffer, TSK buffer + 5 mM imidazole and  
 13 eluted in TSK buffer + 20 mM imidazole, except for hR692A and hDT3, for which the columns were  
 14 washed with TSK buffer, TSK buffer + 40 mM imidazole and eluted with TSK + 400 mM imidazole.  
 15 Proteins were recovered at concentrations (determined by the method of Lowry (1951)) between 5 and  
 16 15 mg/ml except for hDT3 (30 mg/ml) and their purity was over 80% (as estimated by quantification of  
 17 Coomassie Blue stained gels loaded with different protein amounts) except for E496A and hR692A  
 18 (75%).

19

## 20 Fluorimetry

21 Fluorescence measurements were performed on a PTI (Lawrenceville, NJ, USA) QuantaMaster™  
 22 (or PTI QuantaMaster QM4CW for the experiments of Fig. 3B) spectrofluorometer at 20°C on 1 ml  
 23 samples containing 1 µM protein in a 50 mM Tris-HCl (pH 7.7), 0.1 M KCl degassed buffer under  
 24 continuous stirring. The excitation wavelength was 284 nm, excitation and emission bandwidths were 1  
 25 and 4 nm, respectively. Fluorescence emission spectra were recorded between 300 and 450 nm in the  
 26 absence and in the presence of the indicated maltotriose concentration. The spectral center of gravity  
 27 was calculated as:

$$28 \frac{\sum_{\lambda=300}^{420} \lambda F(\lambda)}{\sum_{\lambda=300}^{420} F(\lambda)}$$

29 with  $\lambda$  designating the wavelength in nm and  $F(\lambda)$  the fluorescence intensity in arbitrary units at

1 that wavelength. Maltotriose binding studies were carried out by adding increasing concentrations of  
2 maltotriose to the sample without exceeding a 5% dilution and by recording fluorescence emission  
3 once per second at the indicated wavelength (between 316 and 338 nm) during ~100 s after each  
4 maltotriose addition. Each point is the average of 50 scans, generally those between 46 s and 95 s. In  
5 a control experiment using DT3, two spectra were recorded instead of the 100 scans after each  
6 maltotriose addition. The  $K_D$  which were calculated for each wavelength between 315 and 350 nm  
7 were all within 10% of 3.3 mM (not shown), suggesting that the wavelength used does not affect  
8 importantly the calculation of the dissociation constant.

9 The overall shape of the DT3 fluorescence spectrum repeatedly obtained in this work with the PTI  
10 QuantaMaster was different from that of Danot (2001) (Fig. 3A). The new spectrum showed a "flat top",  
11 with fluorescence emission intensity only slowly increasing between 342 and 348 nm, where the  
12 maximum is attained. This spectrum was reproducibly obtained for several years with different DT3  
13 preparations, and it undergoes similar changes upon maltotriose addition as that of Danot (2001) (a 12  
14 % increase in the maximum fluorescence and a blue-shift of  $1.7 \pm 0.32$  nm of the spectral center of  
15 gravity, with a new  $K_D$  measurement of 3.5 mM). A control experiment was carried out on a Perkin  
16 Elmer LS 50B spectrofluorometer: the Perkin Elmer spectrum obtained had the same overall shape as  
17 the new spectrum (with a shift to longer wavelengths, which might reflect differences in the calibration  
18 and in the optics of the two spectrofluorometers), and the effect of maltotriose was similar (Fig. S1;  
19 shift in the spectral center of gravity: 1.7 nm). Finally, the shape of the spectrum obtained with the  
20 recently purchased PTI QuantaMaster QM4CW (Fig. 3B,  $K_D$  measured for maltotriose, 2.7 mM,  
21 increase in maximum fluorescence 12 %, shift in the spectral center of gravity: 1.6 nm), was much  
22 closer to that of Danot (2001), suggesting that ageing of components of the PTI Quantamaster was the  
23 cause of the difference observed between Fig. 3A and Danot (2001).

24

### 25 *Automated docking*

26 Automated docking was performed using Autodock 4.0 (Goodsell and Olson, 1990; Morris *et al.*,  
27 1998; Morris *et al.*, 2009). Energy grids of 63x87x68 points centered on the (-25.76; 55.38; -42.963)  
28 point with a spacing of 0.375 Å were calculated using the 1hz4 structure of the Protein Data Bank.  
29 Grids contained all atoms of residues from group 1-3. Preparation of the ligand and protein files was  
30 performed using the AutodockTools 1.4.5 package. Nine runs of 50 dockings of the maltotriose  
31 molecule (idealized coordinates from the MSDchem database (<http://www.ebi.ac.uk/msd-srv/msdchem>)) were performed using the Lamarckian genetic algorithm with default parameters

1 except for the population size (300) and number of energy evaluations ( $10^8$ ). The average distance  
2 from a ligand pose to group 1 residues was measured as the mean over all ligand atoms of the  
3 minimum over all group 1 residue atoms of the distances between the ligand atoms and group 1  
4 residue atoms.

5  
6 *Limited proteolysis and microsequencing* Eight  $\mu\text{g}$  DT3 or GST-'T (Vidal-Ingigliardi *et al.*, 1993) were  
7 preincubated for 5 min at 25°C in 26  $\mu\text{l}$  of 62 mM Tris-HCl (pH7.7), 0.38 M KCl, 0.8% sucrose (0.9%  
8 glycerol for GST-'T) and 0, 200 mM maltotriose or 200 mM glucose. Reactions were initiated by  
9 addition of 4  $\mu\text{l}$  trypsin at different concentrations and stopped after 30 min by adding 10  $\mu\text{l}$  AEBSF (4-  
10 (2-aminoethyl) benzenesulfonyl fluoride hydrochloride) at 16 mM and 40  $\mu\text{l}$  of sample buffer (0.125 M  
11 Tris-HCl pH (6.8), 4% SDS, 20% glycerol, 0.01% bromophenol blue). Ten  $\mu\text{l}$  were loaded on a 12%  
12 (37.5:1 acrylamide-bisacrylamide) SDS-PAGE. The gel (0.8 mm thickness) was stained using Sypro®  
13 Ruby (BioRad, Hercules, CA) (0.1 ml/cm<sup>2</sup>) according to the manufacturer specifications and scanned  
14 with a STORM 840 (GE Healthcare).

15 For N-terminal microsequencing of the H645A tryptic fragment, 12  $\mu\text{g}$  H645A were digested as  
16 above without maltotriose in 20  $\mu\text{l}$  at 35  $\mu\text{g/ml}$  trypsin. Digested products were transferred to a  
17 Hybond™ P membrane (GE Healthcare) and microsequenced at the Microsequencing Platform of the  
18 Pasteur Institute on a Procise® sequencer (Applied Biosystems, Carlsbad, CA).

19

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27

28

## 1 TABLES

MaT variant	Spectral center of gravity (nm)	
	- maltotriose	+ maltotriose
DT3	349.9 ± 0.17	348.2 ± 0.15
E570A	349.7 ± 0.18	349.1 ± 0.07
D647A	349.6 ± 0.21	349.2 ± 0.12
W648A	348.8 ± 0.09	348.1 ± 0.16
D726A	348.6 ± 0.28	348.3 ± 0.27

2 **Table 1:** Effect of particular substitutions on the maltotriose induced blue-shift. Spectral centers of  
3 gravities were measured between 300 and 420 nm for particular mutants as indicated in  
4 Experimental Procedures. Each value is the mean of at least 4 independent experiments.

1

MalT variant	$K_D$ (mM)
DT3	$3.5 \pm 0.6$ (325)
hDT3	$4.6 \pm 0.6$ (325)
Q457A	$22 \pm 7$ (317, 321)
N461A	$5.1 \pm 0.3$ (321)
L504A	$46 \pm 19.5$ (316)
I537A	$4.6 \pm 0.5$ (321, 325)
E570A	$4.4 \pm 0.5$ (321, 334)
F571A	$5.7 \pm 0.8$ (328, 334)
L600A	$8.3 \pm 0.5$ (321)
D647A	$9.2 \pm 0.8$ (321, 332)
W648A	$6.0 \pm 0.6$ (321)
H685A	$20 \pm 4$ (321)
Q688	$83 \pm 48$ (316)
Q690A	$2.7 \pm 0.3$ (325, 332)
hR692A	$13 \pm 2$ (321)
N693A	$6.8 \pm 0.3$ (323, 329)
L723A	$45 \pm 18$ (321)
R729A	$5.4 \pm 1.3$ (321, 338)

2

3 **Table 2:** Dissociation constants of MalT variants for maltotriose measured by fluorimetry.

4 Each value is the average of two independent experiments, except for DT3 (four experiments).

5 Number in parentheses give the emission wavelength(s) used for each experiment. The dissociation

6 constant of DT3 and maltose measured in the same conditions was  $28.6 \pm 8.8$  mM.

1

cluster n°	energy (kcal/mol)	population	distances to group1 average (max-min) in Å
1	-13.6	3	3.87 (3.69-4.17)
3	-12.3	18	4.25 (3.79-4.38)
5	-12.1	24	4.04 (4.01-5.52)
7	-12.0	18	3.52 (3.34-3.74)
15	-11.6	20	4.10 (4.31-4.47)

2 **Table 3:** Summary of the automated dockings. Data concerning the lowest energy cluster and the 4  
3 most populated clusters are presented. The last column gives the average, maximum and minimum  
4 over all conformations of the cluster with an energy <-11.1 kcal/mol of the average distance  
5 between the ligand and group 1 residues (as defined in Experimental procedures).

1

MalT variant	numbering of PDB file	$K_D/K_D(\text{wt})$	$\beta$ -galactosidase activity (% of wt)
Q457A	20	6.3	18
N461A	24	1.5	12
E496A	59	–	11
H499A	62	–	20
L504A	67	13.2	24
W529A	92	–	6
Q533A	96	–	14
E536A	99	–	34
I537A	100	1.3	46
E570A	133	1.3	15
F571A	134	1.6	18
R574A	137	–	9
W581A	144	N.D.	0
L600A	163	2.4	18
H645A	208	–	16
D647A	210	2.6	101
W648A	211	1.7	25
N684A	247	–	26
H685A	248	5.6	47
F686A	249	–	33
Q688A	251	23.7	14
Q690A	253	0.8	116
R692A	255	2.9 <sup>a</sup>	206
N693A	256	2.0	122
R696A	259	N.D.	1
L723A	286	12.8	26
D726A	289	–	11
R729A	292	1.5	245
I769A	332	N.D.	1

2 **Table 4:** Summary of the results. The fluorescence data of the purified proteins and the  $\beta$ -  
3 galactosidase activities of the pop8084 strains producing the different MalT variants are presented as  
4 ratios to the data obtained with the wt. – means that the  $K_D$  was too high to be measured. N.D.  
5 indicates that the protein could not be purified.

1 <sup>a</sup> Ratio between the  $K_D$  of hR692 and that of hDT3.

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1

2 **Figure legends**

3

4 **Fig. 1.** (A) Representation of the MalT primary sequence with indication of the boundaries of the  
5 domains. AAA+: conserved core of AAA+ ATPases. The MalT effector domain is a DNA-binding  
6 domain. (B) Schematic representation of the MalT sensor domain. The aminoacid sequence is shown  
7 with boxes representing the helices arranged in bundles as in the three-dimensional structure.  
8 Numbering starts with the first residue of the MalT protein, with arrowheads every ten residues.  
9 Residues substituted in this work are circled. Inset: three dimensional representation from 1hz4 entry  
10 of the Protein Data Bank (<http://www.rcsb.org>) with cylindrical helices, numbered from the beginning of  
11 the domain. Bundle 1 is made of helices 1 and 2, and so on. Even and odd helices are colored in light  
12 grey and black, respectively, to show the two-layer nature of the superhelix. The three helices that are  
13 not numbered compose the C-terminal subdomain. (C) Surface representation of the MalT sensor  
14 domain with residues of the mouths of the central cavity (as defined by CASTp) represented in blue  
15 (axial mouth) and cyan (lateral mouth). Left: view along the axis of the cavity. Center: front view, as in  
16 the inset of Fig.1B. Right: left view. Rotation angles and axes (going from the left to the right panel) are  
17 indicated.

18

19 **Fig. 2.** Effect of the mutations on transcription activation by MalT *in vivo*.  $\beta$ -galactosidase activities of  
20 cultures of pop8083 and its mutated derivatives grown at 25°C were calculated as the mean of at least  
21 three independent experiments. A background of 52 Miller units was subtracted from all the activities.  
22 Error bars correspond to one standard deviation. Bottom panel: western blot of soluble extracts of the  
23 same strains grown in the conditions of the  $\beta$ -galactosidase assays, revealed by anti-MalT and anti-  
24 sigma<sup>70</sup> antibodies as indicated. The three right hand panels are croppings of the scan of one  
25 membrane while the left hand panel is a scan of another membrane.

26

27 **Fig. 3.** Fluorescence spectra and binding curves. (A) Fluorescence emission spectra recorded at 20°C  
28 after excitation at 284 nm without (blue) and with maltotriose (red) are shown, reassembled according  
29 to the group they belong to (see text). The group is indicated in the upper left corner of the panel.  
30 Spectra without maltotriose have been normalized to have the same maximal value as the wt, and for  
31 each variant the spectrum with maltotriose was normalized by the same factor. Each pair of spectra is

1 vertically shifted from the precedent pair for clarity. A.U. : fluorescence arbitrary units. The buffer  
 2 spectrum of the wt experiment is drawn in black. Maltotriose concentrations used for the spectra with  
 3 maltotriose were: Q690A, R729A: 15 mM; E570A, F571A, W648A: 20mM; wt, N461A, I537A, L600A,  
 4 D647A, H685A: 25 mM; N693A, hR692A, L723A, 30 mM; Q457A, L504A : 40 mM; E496A, H499A,  
 5 W529A, Q533A, E536A, R574A, N684A, H645A, F686A, Q688A, D726A: 50 mM. Note that saturation  
 6 by maltotriose could not always be obtained. Percentages of saturation calculated from the binding  
 7 curve fits are the following: wt: 86%; Q457A: 61%; N461A: 81%; L504A: 65%; I537A: 81%; E570A:  
 8 80%; F571A: 78%; L600A: 74%; D647A: 68%; W648A: 74%; H685A: 55%; Q688A: 38%; Q690A: 82%;  
 9 hR692A: 69%; N693A: 81%; L723A: 38%; R729A: 74%. (B) Fluorescence emission spectra of wt DT3  
 10 (20°C, excitation at 284 nm) in the presence of 25 mM maltotriose, maltose or maltotetraose. The  
 11 difference in the wt spectra between Fig.3A and 3B is due to the use of two different fluorometers (see  
 12 Experimental Procedures). (C) Examples of binding curves. See Experimental Procedures and Fig. S2  
 13 for details.

14  
 15 **Fig. 4.** Structure-function relationship in DT3. (A) Surface representation of the MalT sensor domain  
 16 with a docked maltotriose molecule (see text) split in two to reveal the inner wall of the pocket. The left  
 17 moiety (same viewing axis as in Fig. 1C (center panel)) contains most of bundles 1, 2, 3, 6, 7 and 8  
 18 while the right comprises most of bundles 4 and 5 and the 3-helix subdomain. Color code: group 1  
 19 residues: red; group 2, orange; group 3, yellow; group 4, green; maltotriose, cyan.. The axial opening is  
 20 indicated by a vertical arrow, the lateral one by a horizontal arrow. (B) Top view. The rotation indicated  
 21 transforms the viewing axis of (A, left panel) in that of (B). Residues whose alanine substitution does  
 22 not affect the  $K_D$  but decreases the  $\beta$ -galactosidase activity of pop8083 are indicated. Same color code  
 23 as in (A). (C) Stereo view of a sticks representation of the primary maltotriose binding-site with docked  
 24 maltotriose in the conformation shown in (A). All group 1-3 residues are shown, with hypothetical  
 25 hydrogen bonds in green dashed lines. R692 is also represented even though it belongs to group 4  
 26 because it is predicted to donate a hydrogen bond to the maltotriose in this conformation. Backbone,  
 27 R692 and maltotriose atoms are colored according to their nature (C: grey (backbone, R692) or cyan  
 28 (maltotriose), O, red, H, white). Side-chains are colored according to the color-code of (A).

29  
 30 **Fig. 5.** Limited proteolysis of the MalT sensor domain in the presence and absence of maltotriose or  
 31 glucose. + indicates the presence of 200 mM of maltotriose or glucose. Lanes 0-14, DT3; trypsin  
 32 concentrations in  $\mu\text{g/ml}$ : 0 (lane 0), 2.1 (lane 1), 4.2 (lanes 2, 6), 8.3 (lanes 3, 7), 17 (lanes 4, 8), 33

1 (lanes 5, 9, 12 and 13), 67 (lane 10 and 14). Lanes 15-17, GST-<sup>-</sup>T; lane 15, undigested; lanes 16,17,  
2 1.9 µg/ml trypsin. Samples in lanes 15-17 have been run on the same gel.

3

#### 4 **References**

5

6 Ade, J., DeYoung, B.J., Golstein, C., and Innes, R.W. (2007) Indirect activation of a plant nucleotide  
7 binding site-leucine-rich repeat protein by a bacterial protease. *Proc. Natl. Acad. Sci. U S A*  
8 **104**: 2531-2536.

9 Bao, Q., Lu, W., Rabinowitz, J.D., and Shi, Y. (2007) Calcium blocks formation of apoptosome by  
10 preventing nucleotide exchange in APAF-1. *Mol. Cell* **25**: 181-192.

11 Bikadi, Z., and Hazai, E. (2009) Application of the PM6 semi-empirical method to modeling proteins  
12 enhances docking accuracy of AutoDock. *J. Cheminform.* **1**: 15.

13 Boos, W., and Shuman, H. (1998) Maltose/maltodextrin system of *Escherichia coli*: transport,  
14 metabolism, and regulation. *Microbiol. Mol. Biol. Rev.* **62**: 204-229.

15 Cliff, M.J., Harris, R., Barford, D., Ladbury, J.E., and Williams, M.A. (2006) Conformational diversity in  
16 the TPR domain-mediated interaction of protein phosphatase 5 with Hsp90. *Structure* **14**: 415-  
17 426.

18 Danot, O., and Raibaud, O. (1993) On the puzzling arrangement of the asymmetric MalT-binding sites  
19 in the MalT-dependent promoters. *Proc. Natl. Acad. Sci. USA* **90**: 10999-11003.

20 Danot, O. (2001) A complex signaling module governs the activity of MalT, the prototype of an  
21 emerging transactivator family. *Proc. Natl. Acad. Sci. USA* **98**: 435-440.

22 Danot, O., Marquenet, E., Vidal-Ingigliardi, D., and Richet, E. (2009) Wheel of life, wheel of death: a  
23 mechanistic insight into signaling by STAND proteins. *Structure* **17**: 172-182.

24 Débarbouillé, M., Shuman, H.A., Silhavy, T.J., and Schwartz, M. (1978) Dominant constitutive  
25 mutations in *malT*, the positive regulator gene of the maltose regulon in *Escherichia coli*. *J.*  
26 *Mol. Biol.* **124**: 359-371.

27 Dippel, R., Bergmiller, T., Boehm, A., and Boos, W. (2005) The maltodextrin system of *Escherichia*  
28 *coli*: glycogen-derived endogenous induction and osmoregulation. *J. Bacteriol.* **187**: 8332-  
29 8339.

- 1 Divne, C., Stahlberg, J., Teeri, T.T., and Jones, T.A. (1998) High-resolution crystal structures reveal  
2 how a cellulose chain is bound in the 50 Å long tunnel of cellobiohydrolase I from *Trichoderma*  
3 *reesei*. *J. Mol. Biol.* **275**: 309-325.
- 4 Dundas, J., Ouyang, Z., Tseng, J., Binkowski, A., Turpaz, Y., and Liang, J. (2006) CASTp: computed  
5 atlas of surface topography of proteins with structural and topographical mapping of  
6 functionally annotated residues. *Nucleic Acids Res.* **34**: W116-118.
- 7 Erzberger, J.P., Mott, M.L., and Berger, J.M. (2006) Structural basis for ATP-dependent DnaA  
8 assembly and replication-origin remodeling. *Nat. Struct. Mol. Biol.* **13**: 676-683.
- 9 Goodsell, D.S., and Olson, A.J. (1990) Automated docking of substrates to proteins by simulated  
10 annealing. *Proteins* **8**: 195-202.
- 11 Grove, T.Z., Cortajarena, A.L., and Regan, L. (2008) Ligand binding by repeat proteins: natural and  
12 designed. *Curr. Opin. Struct. Biol.* **18**: 507-515.
- 13 Hsu, J.L., Peng, H.L., and Chang, H.Y. (2008) The ATP-binding motif in AcoK is required for regulation  
14 of acetoin catabolism in *Klebsiella pneumoniae* CG43. *Biochem. Biophys. Res. Commun.* **376**:  
15 121-127.
- 16 Hu, Y., Ding, L., Spencer, D.M., and Nuñez, G. (1998) WD-40 repeat region regulates APAF-1 self-  
17 association and procaspase-9 activation. *J. Biol. Chem.* **273**: 33489-33494.
- 18 Huey, R., Morris, G.M., Olson, A.J., and Goodsell, D.S. (2007) A semiempirical free energy force field  
19 with charge-based desolvation. *J. Comput. Chem.* **28**: 1145-1152.
- 20 Jia, Y., McAdams, S.A., Bryan, G.T., Hershey, H.P., and Valent, B. (2000) Direct interaction of  
21 resistance gene and avirulence gene products confers rice blast resistance. *EMBO J.* **19**:  
22 4004-4014.
- 23 Kumar, P., Kumar, D., Parikh, A., Rananaware, D., Gupta, M., Singh, Y., and Nandicoori, V.K. (2009)  
24 The *Mycobacterium tuberculosis* protein kinase K modulates activation of transcription from  
25 the promoter of mycobacterial monooxygenase operon through phosphorylation of the  
26 transcriptional regulator VirS. *J. Biol. Chem.* **284**: 11090-11099.
- 27 Kunkel, T.A., Roberts, J.D., and Zakour, R.A. (1987) Rapid and efficient site-specific mutagenesis  
28 without phenotypic selection. *Methods in Enzymology* **154**: 367-382.
- 29 Leipe, D.D., Koonin, E.V., and Aravind, L. (2004) STAND, a class of P-loop NTPases including animal

- 1 and plant regulators of programmed cell death: multiple, complex domain architectures,  
2 unusual phyletic patterns, and evolution by horizontal gene transfer. *J. Mol. Biol.* **343**: 1-28.
- 3 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the  
4 Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- 5 Main, E.R., Xiong, Y., Cocco, M.J., D'Andrea, L., and Regan, L. (2003) Design of stable alpha-helical  
6 arrays from an idealized TPR motif. *Structure* **11**: 497-508.
- 7 Malik, A., and Ahmad, S. (2007) Sequence and structural features of carbohydrate binding in proteins  
8 and assessment of predictability using a neural network. *BMC Struct. Biol.* **7**: 1.
- 9 Marquenet, E., and Richet, E. (2007) How integration of positive and negative regulatory signals by a  
10 STAND signaling protein depends on ATP hydrolysis. *Mol. Cell* **28**: 187-199.
- 11 Miller, J.H. (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring  
12 Harbor, NY, USA.
- 13 Moffett, P., Farnham, G., Peart, J., and Baulcombe, D.C. (2002) Interaction between domains of a  
14 plant NBS-LRR protein in disease resistance-related cell death. *EMBO J.* **21**: 4511-4519.
- 15 Morris, G.M., Goodsell, D.S., Huey, R., and Olson, A.J. (1996) Distributed automated docking of  
16 flexible ligands to proteins: parallel applications of AutoDock 2.4. *J. Comput. Aided Mol. Des.*  
17 **10**: 293-304.
- 18 Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., and Olson, A.J. (1998)  
19 Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy  
20 function. *J. Comput. Chem.* **19**: 1639-1662.
- 21 Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., and Olson, A.J.  
22 (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility.  
23 *J. Comput. Chem.*
- 24 Nayal, M., and Honig, B. (2006) On the nature of cavities on protein surfaces: application to the  
25 identification of drug-binding sites. *Proteins* **63**: 892-906.
- 26 Quioco, F.A. (1989) Protein-carbohydrate interactions: basic molecular features. *Pure & Appl. Chem.*  
27 **61**: 1293-1306.
- 28 Raibaud, O., and Richet, E. (1987) Maltotriose is the inducer of the maltose regulon of Escherichia coli.  
29 *J. Bacteriol.* **169**: 3059-3061.

- 1 Richet, E., Joly, N., and Danot, O. (2005) Two domains of MalT, the activator of the *Escherichia coli*  
2 maltose regulon, bear determinants essential for anti-activation by MalK. *J. Mol. Biol.* **347**: 1-  
3 10.
- 4 Riedl, S.J., Li, W., Chao, Y., Schwarzenbacher, R., and Shi, Y. (2005) Structure of the apoptotic  
5 protease-activating factor 1 bound to ADP. *Nature* **434**: 926-933.
- 6 Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular cloning*. Cold Spring Harbor, N.Y.: Cold  
7 Spring Harbor Laboratory press.
- 8 Schirmer, T., Keller, T.A., Wang, Y.F., and Rosenbusch, J.P. (1995) Structural basis for sugar  
9 translocation through maltoporin channels at 3.1 Å resolution. *Science* **267**: 512-514.
- 10 Schreiber, V., and Richet, E. (1999) Self-association of the *Escherichia coli* transcription activator MalT  
11 in the presence of maltotriose and ATP. *J. Biol. Chem.* **274**: 33220-33226.
- 12 Schreiber, V., Steegborn, C., Clausen, T., Boos, W., and Richet, E. (2000) A new mechanism for the  
13 control of a prokaryotic transcriptional regulator: antagonistic binding of positive and negative  
14 effectors. *Mol. Microbiol.* **35**: 765-776.
- 15 Stanley, W.A., Filipp, F.V., Kursula, P., Schuller, N., Erdmann, R., Schliebs, W., Sattler, M., and  
16 Wilmanns, M. (2006) Recognition of a functional peroxisome type 1 target by the dynamic  
17 import receptor Pex5p. *Mol. Cell* **24**: 653-663.
- 18 Steegborn, C., Danot, O., Huber, R., and Clausen, T. (2001) Crystal structure of transcription factor  
19 MalT domain III: a novel helix repeat fold implicated in regulated oligomerization. *Structure* **9**:  
20 1051-1060.
- 21 Studier, F.W. (2005) Protein production by auto-induction in high density shaking cultures. *Protein*  
22 *Expr. Purif.* **41**: 207-234.
- 23 Tameling, W.I., Vossen, J.H., Albrecht, M., Lengauer, T., Berden, J.A., Haring, M.A., Cornelissen, B.J.,  
24 and Takken, F.L. (2006) Mutations in the NB-ARC domain of I-2 that impair ATP hydrolysis  
25 cause autoactivation. *Plant Physiol.* **140**: 1233-1245.
- 26 Tanabe, T., Chamailard, M., Ogura, Y., Zhu, L., Qiu, S., Masumoto, J., Ghosh, P., Moran, A.,  
27 Predergast, M.M., Tromp, G., Williams, C.J., Inohara, N., and Nuñez, G. (2004) Regulatory  
28 regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J.* **23**:  
29 1587-1597.

- 1 Tanaka, A., Takano, Y., Ohnishi, Y., and Horinouchi, S. (2007) AfsR recruits RNA polymerase to the  
2 *afsS* promoter: a model for transcriptional activation by SARPs. *J Mol Biol* **369**: 322-333.
- 3 Vidal-Ingigliardi, D., Richet, E., Danot, O., and Raibaud, O. (1993) A small C-terminal region of the  
4 *Escherichia coli* MalT protein contains the DNA-binding domain. *J. Biol. Chem.* **268**: 24527-  
5 24530.
- 6 Zhao, Y.H., Abraham, M.H., and Zissimos, A.M. (2003) Fast calculation of van der Waals volume as a  
7 sum of atomic and bond contributions and its application to drug compounds. *J. Org. Chem.*  
8 **68**: 7368-7373.

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