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Structural insights into a new homodimeric self-activated GTPase family

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Abstract:

The human XAB1/MBDin GTPase and its close homologues form one of the ten phylogenetically distinct families of the SIMIBI (after signal recognition particle, MinD and BioD) class of phosphate-binding loop NTPases. The genomic context and the partners identified for the archaeal and eukaryotic homologues indicate that they are involved in genome maintenance—DNA repair or replication. The crystal structure of PAB0955 from Pyrococcus abyssi shows that, unlike other SIMIBI class G proteins, these highly conserved GTPases are homodimeric, regardless of the presence of nucleotides. The nucleotide-binding site of PAB0955 is rather rigid and its conformation is closest to that of the activated SRP G domain. One insertion to the G domain bears a strictly conserved GPN motif, which is part of the catalytic site of the other monomer and stabilizes the phosphate ion formed. Owing to this unique functional feature, we propose to call this family as GPN-loop GTPase.

INTRODUCTION

Due to the crucial cellular roles of GTPases, tremendous efforts have been dedicated in the past two decades for understanding their structures and function (Bourne *et al.*, 1991; Vetter and Wittinghofer, 2001). Among the P-loop GTPases superfamily, 28 families have been phylogenetically defined (Leipe *et al.*, 2002). The best known includes on one hand, molecular switches such as the Ras-like small G proteins, heterotrimeric G proteins, elongation factor G domains (TRAFAC class), and on the other, Signal Recognition Particle (SRP), its receptor (SR) and HypB that adopt a quite distinct topology (SIMIBI class).

A human GTPase named XAB1/MBD*in* has been shown to interact with XPA, a protein involved in nucleotide excision repair (Nitta *et al.*, 2000), and with MBD2, a component of the MeCP1 large protein complex that represses transcription of densely methylated genes (Lembo *et al.*, 2003). XAB1/MBD*in* was shown to counteract the inhibitory effect of MBD2 at methylated promoters. Furthermore, deletion of the gene coding for its closest homologue in *Saccharomyces cerevisiae* (*Yjr072c*) is lethal (Giaever *et al.*, 2002) indicating that these eukaryotic GTPases are involved in a vital cellular mechanism. Archaeal homologues have been identified. The amino acid sequences of PAB0955 from *Pyrococcus abyssi* and human XAB1/MBDin share strong identities: 27 % (see supplementary Fig S1 online). The *pab0955* gene is located between genes coding for the replicative helicase MCM and the cell division protein MinD. In several distinct archaea, DNA replication-related genes are located in the close neighborhood of the *pab0955* homologous gene (see supplementary Fig S2 online). Such observations strengthen the idea that these GTPases are indeed involved in vital mechanisms at the DNA level, possibly related to DNA replication/repair and conserved from Archaea to Human.

To gain insight into this new GTPase family, we solved the crystal structure of PAB0955 both alone and in complex with different nucleotides. It provides us with different snapshots along the hydrolysis pathway and shows that PAB0955 is the archetype for a new family of homodimeric GTPases called the GPN-loop GTPase family.

RESULTS

The structure of PAB0955 in complex with different nucleotides

The crystal structures of the nucleotide-free form of PAB0955 as well as 6 complexes with different nucleotides were determined (Table 1). Two complexes co-crystallized with guanosine-diphosphate (GDP) contain either just GDP alone (PAB0955-GDP) or GDP and a Mg^{2+} ion (PAB0955-MgGDP). One structure was obtained with guanosine-diphosphate-monothiophosphate (GTPγS) and three other were co-crystallized with guanosine-triphosphate (GTP). The latters contain either GDP and one phosphate ion in the vicinity of the nucleotide (crystals grown at 277K, PAB0955-PiGDP) or GDP and one Mg^{2+} ion (crystals

grown at 293K, PAB0955-GDP^{GTP293}). The presence of GDP in these crystals implies catalytic activity of the protein in either the crystallization drops or the crystals. A last one, obtained with crystals grown in presence of both ethylenediaminetetraacetic acid (EDTA) and GTP actually contains GTP (PAB0955-GTP^{EDTA}).

Overall PAB0955 structure description

PAB0955 is a 248 residues α/β monodomain protein which adopts a canonical "Rossmann fold" shared by all the phosphate-binding loop (P-loop)-containing nucleotide-triphosphate hydrolases. The ^{7}GTAGSGKT^{14} motif located close to the N-terminus of the polypeptide chain constitutes the P-loop that binds the nucleotide phosphate groups. The core of the protein is made of a central six-stranded parallel β-sheet surrounded by 6 α-helices. Two insertions are grafted to this core (residues 41-80 and 183-217) and form two protrusions to the globular shape of the monomer (Fig 1). The first insertion (I1) is reminiscent of the Insertion Box Domain identified for SRP G domains (Freymann *et al.*, 1997). The second insertion (I2) has no equivalent in other GTPases and has the less conserved amino acid sequence among the PAB0955 homologues. The protein is dimeric both in solution (Gras *et al.*, 2005) and in all the crystal forms. The homodimer is either crystallographic, as observed in the $\boldsymbol{P}3_221$ and the $\boldsymbol{P}2_12_12$ crystal forms, or non-crystallographic, as observed in the $\boldsymbol{P}2_1$ crystal form. The accessible surface buried at the dimer's interface is 4420 Å^2 and the two insertions contribute 39% to this interface. 64% of the interface is contributed by hydrophobic residues. The two nucleotide binding sites contained in the PAB0955 dimer are close to the dimer's interface but not connected with each other. The PAB0955 structure in either its apo form or in complex with different nucleotides is well conserved. The root mean square difference (rmsd) calculated for the 154 residues making the core of one monomer ranges from 0.16 to 0.58 Å. Loops involved in the nucleotide binding site (the P-loop, Lys40 next to the G2 motif and the ^{64}Y**GPN**GA^{69} loop) exhibit moderate structural differences with maximum Cα displacement of 1.08 Å, 1.98 Å and 1.16 Å respectively. The I2 region is by far the most flexible with an rmsd of about 2.8 Å for the three conformations observed in PAB0955-MgGDP (two independent molecules) and PAB0955-PiGDP structures. For the structures derived from crystals belonging to the $\boldsymbol{P}3_221$ space group, this region has sparse electron density. An extensive search of the Protein Data Bank led to the identification of only fiveteen GTPases having a fully parallel β-sheet. They include tubulin, FtsZ, and three members of the SIMIBI class: HypB, SRP and SR G domains. A search using DALI confirmed that the *Thermus aquaticus* Ffh fragment of SRP (Freymann *et al.*, 1997) is the GTPase structurally most similar to PAB0955 (Ffh: rmsd of 1.94 Å for 136 Cα, HypB: rmsd of 1.98 Å for 126 Cα) and emphasise the belonging of PAB0955 to the SIMIBI class. However, the dimerization mode observed for PAB0955 drastically differs from the one observed for the SRP/SR complex (Egea *et al.*, 2004) or HypB (Gasper *et al.*, 2006). When

the Ffh SRP G domain and HypB A monomer are superposed on PAB0955 A monomer, the orientation of FtsY SR G domain and HypB B monomer relatively to PAB0955 B monomer differ by a rotation of 66 and 173 degrees respectively. The relative position of the two nucleotide binding sites also differs, as examplified by the distance between the two β-phosphate atoms: 22.0 Å for PAB0955, 17.5 Å for HypB, 9.1 Å for Ffh/FtsY.

Nucleotide-binding mode

The overall nucleotide-binding mode is very well conserved in the six PAB0955 complexes. Figure 2a shows the five consensus motifs in PAB0955, G1-5, involved in GTP binding of GTPases (Bourne *et al.*, 1991). The G2 motif is Asp36 at the C-ter end of the β2-strand and is highly conserved in SIMIBI family proteins (Asp135 in *T. aquaticus* Ffh, Asp69 in HypB). It stabilizes the Mg^{2+} ion through a water-mediated hydrogen bond. The G3 motif (^{101}DTPGQ^{105} in PAB0955) is structurally rather well conserved in all PAB0955 structures since it participates to the dimer's interface. The presence of a proline within this motif probably enhances the rigidity of this loop, whereas in Ras-like small G proteins, this G3 motif (switch II) is quite flexible (Vetter and Wittinghofer, 2001). The conformation of the G3 motif observed in PAB0955 also differs from that of SRP Ffh and HypB. The G4 (^{165}NKVD^{168}) and G5 (^{223}SAK^{225}) motifs specifically stabilize the guanine and the ribose groups as shown in figure 2a. PAB0955 G4 and G5 motifs are very similar to those of HypB (^{167}NKID^{170} and ^{199}SLK^{201}), both in sequence and in structure. In SRP Ffh G domain, the G5 motif differs significantly on both aspects. Surprisingly, the position of γ-monothiophosphate in PAB0955-GTPγS differs by 3.26 Å from that of γ-phosphate in PAB0955-GTP^{EDTA} (Fig 2b) and is close to that of the co-ordinated Mg^{2+} ion observed in PAB0955-MgGDP structure, thus preventing Mg^{2+} binding. Consequently, the conformation of the GTPγS bound to PAB0955 is unlikely the one adopted by GTP prior to hydrolysis, in contrast to other GTPases to which it binds with a conformation very similar to that of GTP and other analogues (Ihara *et al.*, 1998).

GTPase activity of PAB0955

Specific nucleotide hydrolysis in presence of the protein was detected *in vitro* by estimation of the GDP:GTP (or ADP:ATP) ratio. As the protein originates from a hyperthermophilic organism, kinetics were carried out at 80°C. A weak intrinsic GTPase activity (0.012 μmole of GTP hydrolyzed in GDP per min and per mg of protein) was detected while no ATPase activity could be noticed. This activity is consistent with the one observed for the PAB0955 human orthologue XAB1/MBD*in* (Nitta *et al.*, 2000). As already observed for some GTPases such as Era (Sood *et al.*, 1994), PAB0955 is able to phosphorylate itself *in vitro* at 80°C only in presence of [γ^{32}P]-GTP and Mg^{2+} ions (see supplementary information online).

Identification of protein partners related to DNA metabolism.

Since the partners identified for human XAB1/MBD*in* have no homologues in *P. abyssi*, a search for PAB0955 protein partners was undertaken. Pull-down assays with cellular extracts of *P. abyssi* and screening by surface plasmonic resonance led to the identification of three interacting partners: DNA topoisomerase VI (subunit B), DNA primase DnaG and RF-C (small subunit). The equilibrium dissociation constant of about 0.5 nM could be obtained only with the former protein (see supplementary information online).

DISCUSSION

The new GPN-loop GTPase family.

At least 64 sequences homologous to PAB0955 were identified by PSI-BLAST analysis. Sequence alignment of PAB0955 homologues suggests that their topology should be very similar to that of PAB0955, with a core adopting a Rossmann fold and two insertions made of 40 to 43 residues and 35 to 52 residues, respectively. The stable dimeric form observed for PAB0955 is very likely to be conserved in these homologues. Among the 45 residues contributing to the dimer's interface, 20% are strictly conserved and 27% are homologous (supplementary Fig s1 online).

Remarkably, a strictly conserved **GPN** motif in the I1 region is located at the dimer interface, close to the nucleotide-binding site of the other monomer (Fig 2a). In the PAB0955-GTP^{EDTA} structure, the $N^{\delta 2}$ atom of Asn67 (mol B) is 5.9 Å away from the closest GTP γ-phosphate oxygen atom bound to monomer A. In the PAB0955-PiGDP structure, it forms a hydrogen bond with the phosphate ion (mol A) (Fig 3). This Asn67 seems to play the same role to that attributed to Gln61 of the G3 motif in Ras (Prive *et al.*, 1992), which is thought to stabilize the phosphate intermediate. The G3 motif of PAB0955 also contains a Gln105, but it is about 7 Å away from both the γ-phosphate group in PAB0955-GTP^{EDTA} and the phosphate ion in PAB0955-PiGDP. The presence of a proline in the G3 motif and its involvment in the dimer interface prevent PAB0955 Gln105 to get closer to the nucleotide and to fulfill the role of Gln61 in Ras. However, since the amine group of Gln105 (mol A) hydrogen bonds to the amine group of Asn67 (mol B), it may indirectly play a limited role in the catalysis. Remarkably, HypB Asn124, the equivalent of Ras Gln61, points away from the nucleotide and can clearly not hold the same role. HypB His154 (mol B), hypothezised to activate the water molecule that can act as a nucleophile (Gasper *et al.*, 2006), is strickingly close to Asn67 (mol B) in PAB0955. The strict conservation of the GPN motif throughout the whole family further supports an essential catalytic role for Asn67.

Another consensus sequence, (V/I/L)**N**(L/M)**D**(T/P) (^{33}V<u>N</u>L<u>D</u>T^{37} in PAB0955), constitutes the G2 motif of GPN-loop GTPases. Val33 and Leu35 form with Ile48 and Ile100 a hydrophobic cluster while Asn34 blocks the main chain conformation of Asp36 through two hydrogen bonds between the Asn34 amine group and the NH and C=O of Asp36. This very

stable motif forces the conserved Asp36 side chain to point towards the Mg^{2+} ion. In summary, presence of the **G**X_2**G**X**GK**(T/S), (V/I/L)**N**(L/M)**D**, **GPN**, **DXPGQ** and (N/S/T)**K**X**D** motifs constitutes the signature of a GPN-loop GTPase.

An activated conformation for the nucleotide binding site

As illustrated by the comparison of Ffh-GDP (inactivated state) and Ffh-GMPpcp in the Ffh-GMPpcp-FtsY complex (activated state), SRP G domains undergo conformational changes for their G2 and G3 motifs upon activation (Egea *et al.*, 2004). In PAB0955, the conformation of the G2 motif and the position of Asp36 are conserved and much closer to that of Asp135 in the Ffh/FtsY complex (Fig 4a). Moreover, the relative position of the γ-phosphate group and the G1 and G3 motifs in both PAB0955-GTP^{EDTA} and Ffh-GMPpcp structures (Fig 4b) are very similar. Therefore, PAB0955 is structurally significantly closer to the activated conformation of Ffh.

The G domain superposition of PAB0955 dimer with those of Ras-RasGAP (Scheffzek *et al.*, 1997) and SRP-SR (Egea *et al.*, 2004) complexes shows that no steric clash prevent a putative partner to bring an "arginine finger" such as GP120 RasGAP Arg789 or SRP Ffh Arg138 to further activate PAB0955. No positively charged residue is seen in the vicinity of the phosphate groups thanks to PAB0955 dimerization contrasting with HypB where Lys 153 (mol B) hydrogen bonds the γ-phosphate(Gasper *et al.*, 2006). Compared to small GTPases and SRP, the higher GTPase activity of PAB0955 may therefore originate from the fact that Asn67 is already stabilized in its optimal conformation. The hydrogen bond between Gln105 (molA) and Asn67 (mol B) may also enhance the ability of Asn67 to stabilize the negatively charged γ-phosphate in the transition state. The dimeric state of PAB0955 partially mimics Ras-RasGAP interaction by stabilizing the G2 and G3 motifs and bringing the GPN motif close to the nucleotide. To our knowledge, PAB0955 is the first example of a structurally characterized GTPase dimeric in either apo or holo states. This observation implies that the PAB0955 GTPase activity should be controlled *in vivo* by a yet unknown mechanism.

Snapshots of the hydrolysis pathway.

The set of PAB0955 structures provides us with snapshots of the GTP hydrolysis pathway. The PAB0955-GTP^{EDTA} structure is a plausible view of the GTP position prior to hydrolysis. The absence of Mg^{2+} ion, intentionally removed with EDTA to block hydrolysis, should not impact the γ-phosphate position since the nucleotide is sterically constrained by the G3 motif. The PAB0955-PiGDP stands for the next step. The GTP present in the crystallization solution has been hydrolyzed and a free phosphate ion is located 5.2 Å away from the position of the GTP γ-phosphate group observed in the PAB0955-GTP^{EDTA} structure. It is stabilized by 5 hydrogen bonds involving Gly38 and Lys40 (mol A) as well as Asn67 and Gly68 (mol B) GPN loop (Fig 3). It thus confirms that this pocket at the dimer's interface helps to stabilize

the phosphate ion just after GTP hydrolysis. Following the leave of the γ-phosphate group, a 1.1 Å positional shift of the α and β phosphate groups deeper into the nucleotide-binding site towards the G3 loop is observed. The PAB0955-GDP^{GTP293} structure may represent the next snapshot, with GTP fully hydrolyzed and no phosphate ion observed. The GDP molecule is shifted back and superposes well to the GTP molecule in PAB0955-GTP^{EDTA}, possibly illustrating one step towards the release of the nucleotide, likely driven by the mutual electrostatic repulsion of GDP and Pi. These snapshots illustrate how the nucleotide adapts to the rather rigid PAB0955 structure along the hydrolysis pathway, pointing out a marked difference with Ffh, in which the G3 motif is more flexible.

Albeit structurally very well conserved, eukaryal and archaeal GPN-loop GTPases might be involved in different biological functions, since they seem to have different partners. It seems that along the emergence of the eukaryotic branch, and its further evolution, different partners were loss and new ones recruited. As PAB0955 is a good structural template for their eukaryal homologues, it will help in the near future to elucidate how XAB1/MBD*in* functions. Altogether, our results allowed to structurally and mechanistically characterize this new dimeric GTPase family. The fact that this GTPase is dimeric regardless of the presence of a bound nucleotide and that rather small structural changes are observed upon nucleotide binding suggest a yet unobserved mode action, differing from the dimerization–dependant switch mechanism described for the closest member of the SIMIBI family such as SRP/SR and HypB. Therefore, unveiling its precise cellular role in eukaryotes remains an exciting challenge.

METHODS

GTP hydrolysis. Recombinant PAB0955 was obtained as previously described (Gras *et al.*, 2005). The GTPase activity was assessed in triplicate by incubating at 80°C various amounts of purified PAB0955 (0.2-1.0 μM) with 200 μM GTP in 50 mM EPPS buffered at pH 7.0 containing 400 mM potassium glutamate and 5 mM $MgCl_2$ (see supplementary information online).

Crystallization. Native crystals either nucleotide free or co-crystallized with GDP and GTPγS were obtained as described previously (Gras *et al.*, 2005). Derivative crystals and native PAB0955 co-crystallized with GTP or GDP and $NaWO_4$ were obtained as described in supplementary information online.

Crystallographic data collection. Prior to being flash-cooled in liquid nitrogen, crystals were soaked in a cryoprotecting solution containing mother liquor with PEG4000 concentration increased to 30-35%(w/v). Three crystallographic data sets were collected on two DTPA-BMA-Gd soaked crystals at three different wavelengths on the beam-line BM-30A of the European Synchrotron Radiation Facilities (ESRF) using a MarCCD detector. Another data

set was collected on a DTPA-BMA-Gd co-crystallized crystal on the beam-line ID14-eh4 of the ESRF using an ADSC Q4 CCD detector (supplementary Table S1 online). The data collection of the native PAB0955, PAB0955-GTPγS and PAB0955-GDP crystals were previously described (Gras *et al.*, 2005). For the PAB0955-GDP^{GTP293} and PAB0955-PiGDP crystals, crystallographic data were collected on the beam-line BM-30A of the ESRF. For the PAB0955-GTP^{EDTA} and PAB0955-MgGDP crystals, crystallographic data were collected on the beam-line ID14-eh1 of the ESRF using an ADSC Q4 CCD detector (Table 1).

Structure determination and refinement. The nucleotide free PAB0955 structure was solved using the SIRAS/MAD techniques, with crystallographic data collected with the native and the Gd derivative crystals as described in supplementary information online. All the PAB0955-nucleotide complex structures were solved by molecular replacement with AMoRe (Navaza, 1994) using the apo-form of PAB0955 as initial model. The refinement protocol used for all structures included several cycles of refinement with REFMAC (CCP4, 1994) followed by manual model rebuilding with O (Jones *et al.*, 1991), until no interpretable electron density could be identified in the residual map. Final refinement statistics and pdb entry codes are summarized in Table 1.

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Supplementary information is available at EMBO reports online.

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Legends to figures

Fig 1: a) View of the PAB0955-GDP dimer. Monomer A and B are depicted in light pink and light green respectively. GDP molecules are shown with sticks colored according to atom types (light blue for carbons, blue for nitrogens, red for oxygens, green for phosphorus). The G1, G2, G3, G4 and G5 motifs (A monomer) are depicted in yellow, orange, blue, green and cyan respectively. The two insertions I1 and I2 are depicted in fully saturated and partially saturated colors respectively. b) Topology of PAB0955. G1 to G5 boxes are shown with the same color scheme.

Fig 2: a) Detailed view of the nucleotide binding site of PAB0955-MgGDP. G1 to G5 boxes are shown with the color scheme used in Fig 1. Hydrogen bonds are represented with dotted lines. The Mg^{2+} ion is depicted in cyan and water molecules are shown in red. b) Close up view of the superposition of PAB0955-GTP^{EDTA} (GTP with pink carbon atoms) and PAB0955-GTPγS structures (GTPγS with blue carbon atoms). Superposition has been

performed on the 154 residues forming the Rossmann fold core of the protein.

Fig 3: View of the PAB0955 residues stabilizing the phosphate ion in the PAB0955-PiGDP structure. Hydrogen bonds are represented with dotted lines. G1 to G5 boxes are shown with the colour scheme used in Fig 1, the GPN motif is depicted in purple

Fig 4: a) Close-up view of the nucleotide binding site for the superposed PAB0955-GTP^{EDTA}, PAB0955-MgGDP, Ffh-GDP (1ng1) and Ffh-GMPpcp when bound to FtsY (1rj9) structures, depicted in orange, purple, cyan and green respectively. b) Close-up view of the nucleotide binding site for PAB0955-GTP^{EDTA}, depicted in orange, and Ffh in Ffh-FtsY complex, depicted in green. The concerted shift of the γ-phosphate group and the G1 and G3 motifs is highlighted the three arrows.

Table 1: Crystallographic statistics. (Values in parenthesis are for the highest resolution shell.)

	Native apo	GDP	GTPγS	GTP^{EDTA}	PiGDP	GDP^{GTP293}	MgGDP
Data collection							
Wavelength (Å)	0.933	1.542	0.979	0.934	0.979	0.979	0.934
Space group	$\boldsymbol{P}3_221$	$\boldsymbol{P}2_1$	$\boldsymbol{P}3_221$	$\boldsymbol{P}3_221$	$\boldsymbol{P}2_12_12$	$\boldsymbol{P}3_221$	$\boldsymbol{P}2_1$
Unit-cell parameters (Å,°)	a=b=60.3, c=117.1	a=59.1, b=84.9, c=60.2, β=95	a=b=60.2, c=115.9	a=b=60.71, c=116.8	a=58.82, b=84, c=53.16	a=b=60.8, c =117.2	a=59.47, b=85.21, c=60.57, β=94.61
Resolution (Å)	50-2.15 (2.23-2.15)	50-2.30 (2.38-2.30)	50-2.08 (2.15-2.08)	20-2.40 (2.46-2.40)	100-2.80 (2.90-2.80)	100-2.40 (2.45-2.40)	50-1.75 (1.80-1.75)
No. Observations	140472 (10187)	88157 (8488)	83727 (5701)	117734 (8425)	50000 (4710)	93234 (5405)	282790 (18469)
No. unique reflections	13998 (1345)	26182 (2560)	12892 (1245)	10088 (696)	6729 (618)	9976 (574)	59865 (5075)
$\langle I \rangle / \langle \sigma(I) \rangle$	8.2 (2.2)	5.2 (2.1)	6.6 (2.3)	20.7 (7)	15.19 (4.6)	19.55 (5.76)	12.47 (5.91)
Completeness (%)	100 (100)	99.9 (100)	87.6 (94)	98.5 (98.2)	97.8 (93.8)	99 (98.8)	98.6 (93.5)
R_{merge} (%)	5.7 (34.8)	6.8 (36.1)	6.7 (28.4)	7.5 (40)	9.3 (45.5)	6.7 (40.4)	9.8 (37.2)
Refinement							
Resolution (Å)	15-2.15 (2.23-2.15)	15-2.30 (2.38-2.30)	15-2.08 (2.15-2.08)	15-2.40 (2.46-2.40)	15-2.80 (2.90-2.80)	15-2.40 (2.45-2.40)	15-1.75 (1.80-1.75)
No. reflections	12996	23201	11234	8981	6017	8930	53677
free set	*685*	*2607*	*1224*	*994*	*663*	*981*	*6086*
Asymetric unit content	monomer	dimer	monomer	monomer	monomer	monomer	dimer
R_{work}	0.232	0.186	0.208	0.236	0.225	0.211	0.195
R_{free}	0.299	0.265	0.295	0.325	0.346	0.324	0.247
R_{cryst}	0.235	0.190	0.209	0.242	0.232	0.218	0.196
Average B-factor ($Å^2$)	53.6	34.9	45.4	48.9	46.8	50.1	33.8
rms deviations							
Bonds (Å)	0.010	0.011	0.009	0.009	0.008	0.009	0.008
Angles (°)	1.246	1.373	1.297	1.192	1.175	1.185	1.215
PDB entry code	1YR6	1YRA	1YR7	1YR8	1YR9	2OXR	1YRB

Figure 1

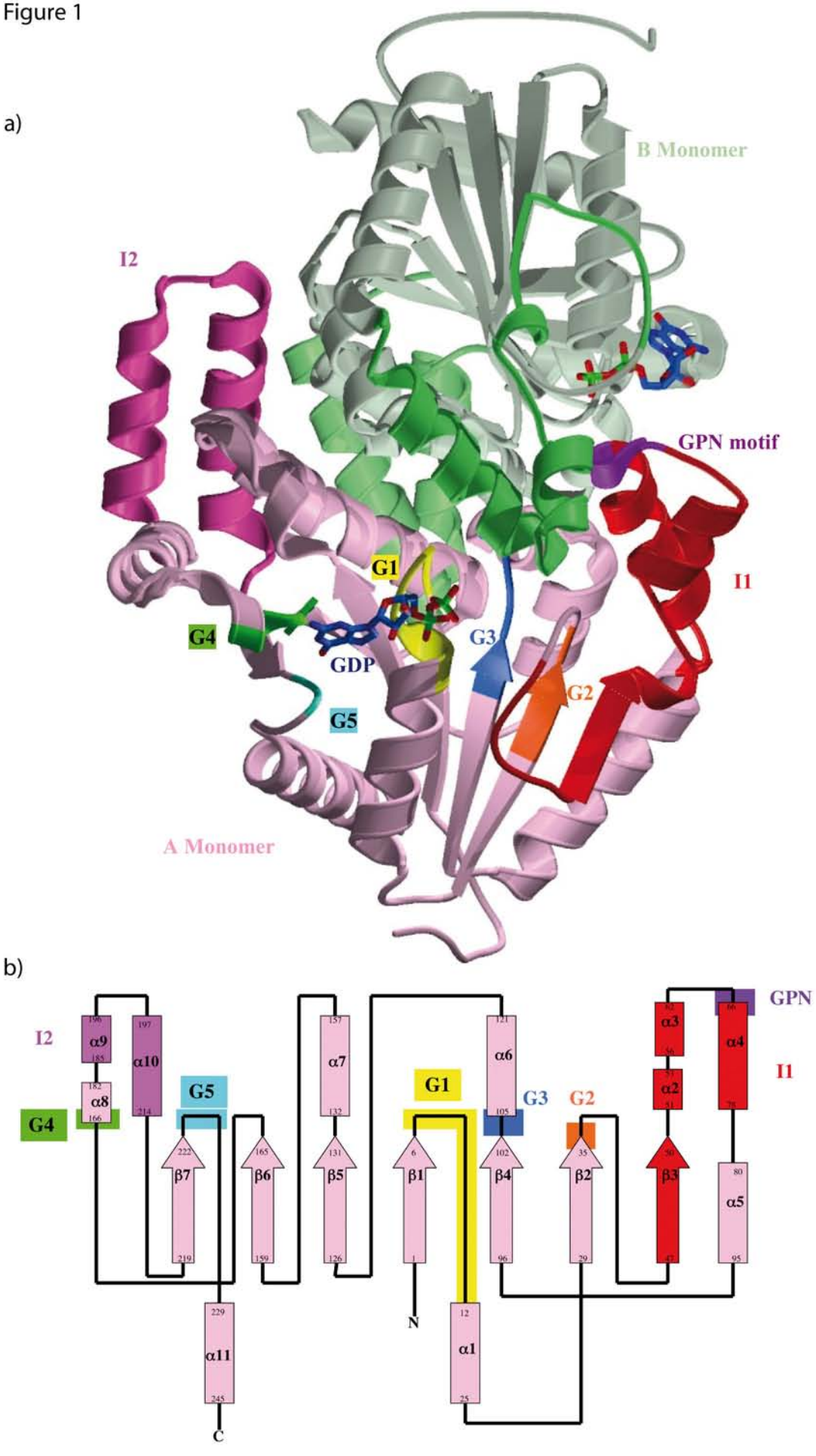

a)
B Monomer
I2
GPN motif
I1
G1
G4
G3
GDP
G2
G5
A Monomer
b)
I2
α9
α10
α8
G4
G5
α7
β7
β6
β5
α11
C
G1
α6
G3
G2
β1
β4
β2
N
α1
α3
α2
β3
GPN
α4
I1
α5

Figure 2

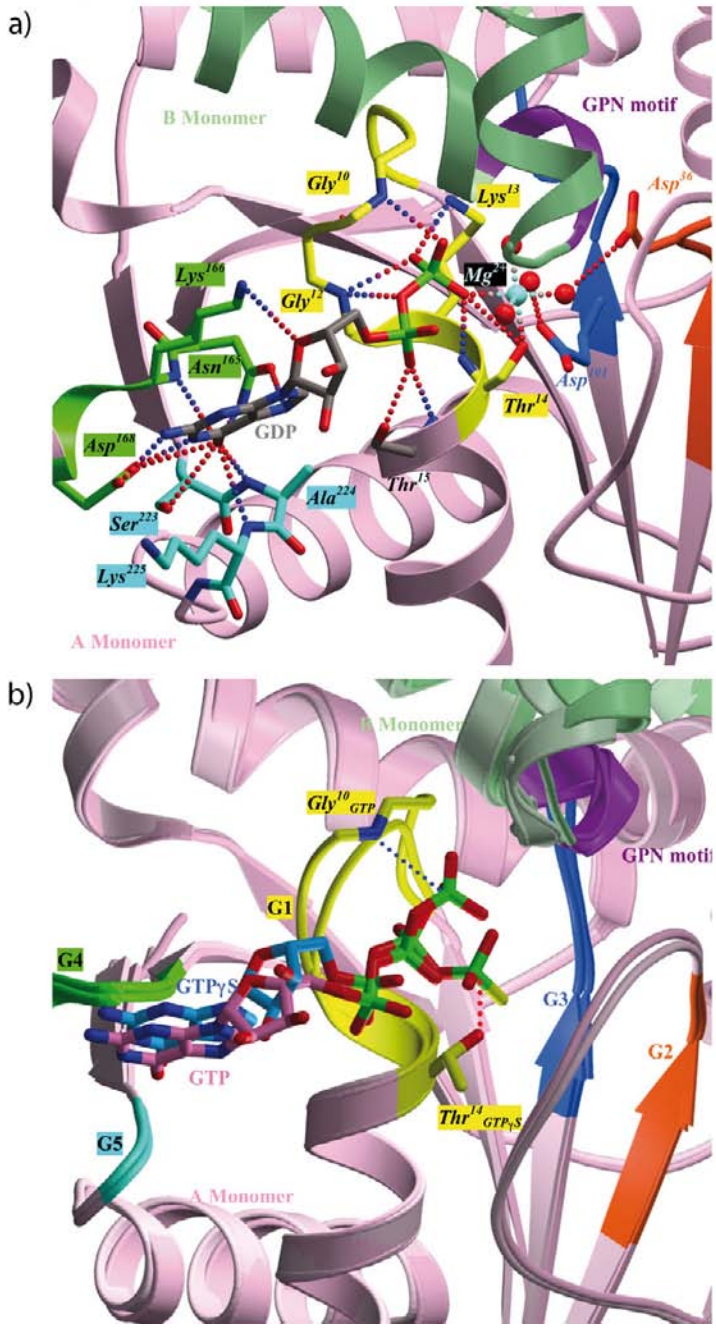

a)
B Monomer
GPN motif
Gly10
Lys13
Asp38
Mg2+
Lys166
Gly12
Asn165
Asp101
Thr14
GDP
Asp168
Thr15
Ala224
Ser223
Lys225
A Monomer
b)
B Monomer
Gly10 GTP
GPN motif
G1
G4
GTPγS
G3
G2
GTP
Thr14 GTPγS
G5
A Monomer

Figure 3

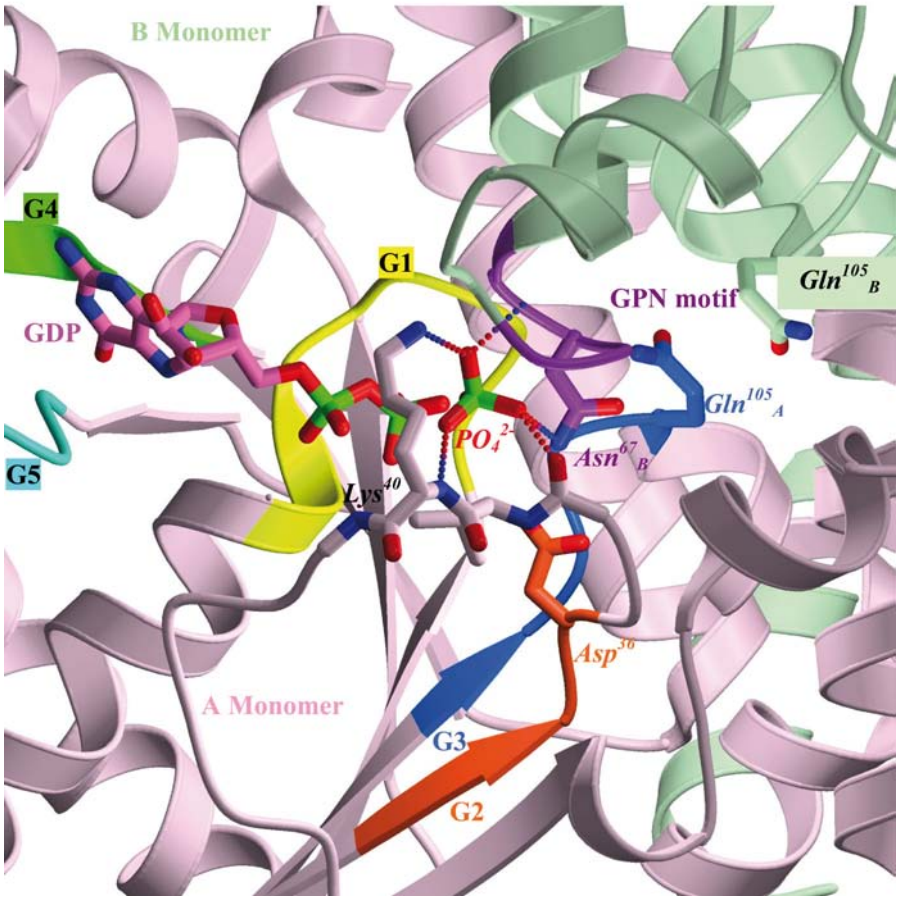
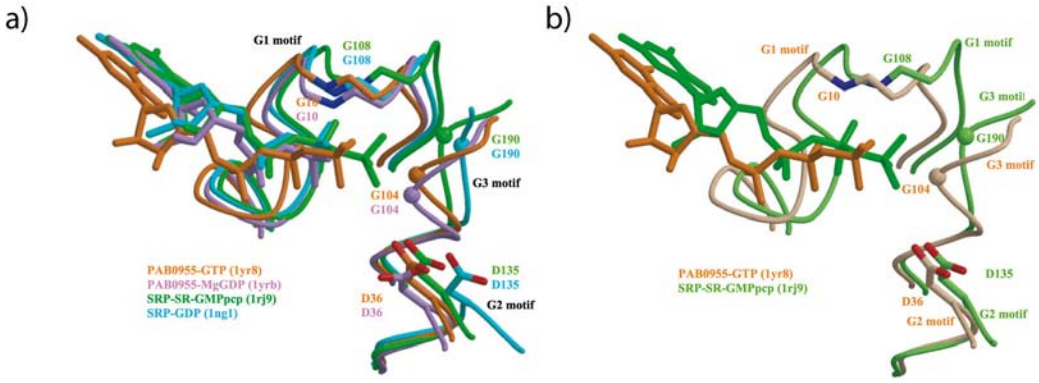

B Monomer
G4
G1
GPN motif
$Gln^{105}{}_{B}$
GDP
$Gln^{105}{}_{A}$
PO_4^{2-}
$Asn^{67}{}_{B}$
G5
Lys^{40}
Asp^{36}
A Monomer
G3
G2

Figure 4


a)
G1 motif
G108
G108
G10
G10
G190
G190
G3 motif
G104
G104
D135
D135
G2 motif
D36
D36
PAB0955-GTP (1yr8)
PAB0955-MgGDP (1yrb)
SRP-SR-GMPpcp (1rj9)
SRP-GDP (1ng1)
b)
G1 motif
G108
G1 motif
G10
G3 motif
G190
G3 motif
G104
D135
D36
G2 motif
G2 motif
PAB0955-GTP (1yr8)
SRP-SR-GMPpcp (1rj9)