



HAL
open science

Domain motions of glucosamine-6P synthase: Comparison of the anisotropic displacements in the crystals and the catalytic hinge-bending rotation

Stéphane Mouilleron, Béatrice Golinelli-Pimpaneau

► **To cite this version:**

Stéphane Mouilleron, Béatrice Golinelli-Pimpaneau. Domain motions of glucosamine-6P synthase: Comparison of the anisotropic displacements in the crystals and the catalytic hinge-bending rotation. *Protein Science*, 2007, 16, pp.485 - 493. 10.1110/ps.062598107 . hal-03281514

HAL Id: hal-03281514

<https://hal.science/hal-03281514>

Submitted on 9 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Domain motions of glucosamine-6P synthase: comparison of the anisotropic displacements in the crystals and the catalytic hinge-bending rotation.

Stéphane Mouilleron^{1,2,3}, Béatrice Golinelli-Pimpaneau¹

¹Laboratoire d'Enzymologie et Biochimie structurales, Bâtiment 34 et ²Institut de Chimie des Substances Naturelles, UPR 2301, CNRS, 1 avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France, ³Present address: Structural Biology Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom

Corresponding author: Béatrice Golinelli-Pimpaneau, tel: 33 1 69 82 42 35, fax: 33 1 69 82 31 29, e-mail: beatrice.golinelli@lebs.cnrs-gif.fr

Abbreviations: GlmS, glucosamine-6-phosphate synthase; Fru6P, D-fructose-6-phosphate; Glc6P, D-glucose-6-phosphate; DON, 6-diazo-5-oxo-L-nor-leucine; TLS, translation-libration-screw; NCS, non crystallographic symmetry; rmsd, root mean square deviation.

Keywords: dynamic domains; hinge-bending domain motion, domain movement; translation-libration-screw refinement, anisotropic temperature factor.

Running title: domain motions of glucosamine-6-P synthase

Abstract

Glucosamine-6-phosphate synthase channels ammonia over 18 Å from glutamine at the glutaminase site to fructose-6-P at the synthase site. We have modeled the anisotropic displacements of the glutaminase and synthase domains from the two crystallized states, the enzyme in complex with fructose-6-P or in complex with glucose-6P and a glutamine affinity analog, using TLS (rigid-body motion in terms of translation, libration and screw motions) refinement implemented in *REFMAC*. The domains displacements in the crystal lattices are compared to the movement of the glutaminase domain relative to the synthase domain that occurs during the catalytic cycle upon glutamine binding, which was visualized by comparing the two structures. This movement was analyzed by the program *DYNDOM* as a 22.8° rotation around an effective hinge axis running approximately parallel to helix 300-317 of the synthase domain, the glutaminase loop that covers the glutaminase site upon glutamine binding acting as the mechanical hinge.

Introduction

Glucosamine-6-phosphate synthase (GlmS) catalyzes the synthesis of glucosamine-6P from D-fructose-6P (Fru6P) and L-glutamine (Gln) and uses a channel to transfer ammonia from its glutaminase to its synthase active site. We have recently reported the crystal structure determination of two consecutive states of GlmS from *Escherichia coli*, the complex with Fru6P at 2.05 Å resolution and the complex with glucose-6P (Glc6P) and the glutamine affinity analogue 6-diazo-5-oxo-L-nor-leucine (DON) at 2.35 Å resolution (Mouilleron et al., 2006). The protein consists of two globular domains, the glutaminase domain (residues 1-239) and the synthase domain (residues 249-608), separated by a flexible linker region. Conformational flexibility of several loops of the glutaminase and synthase domains plays a key role in catalysis. In particular, the C-terminal loop is thought to close the synthase site when the first substrate Fru6P binds and, when the second substrate glutamine binds, the glutamine or Q-loop (residues 73 to 81) has been shown to shield the glutaminase site concomitantly with domain motion. Indeed, DON binding to the sugar-bound form of the enzyme induces a rotation of the

glutaminase domain relative to the synthase domain in order to maintain the dimer interface of the enzyme (Mouilleron et al., 2006). The indole group of Trp74 functions as a molecular gate, opening and closing up the ammonia channel.

The GlmS·Fru6P crystal unit consists of three copies of the protein (monomers A to C) together with three fructose-6P molecules. The model of this complex was refined with isotropic B factors to an R factor of 24.4 % and an R_{free} of 28.5 % at 2.05 Å resolution (Mouilleron et al. 2006). The average isotropic temperature factors were significantly different for each domain of the three non crystallographic symmetry (NCS)-related molecules. In addition, the monomer C glutaminase domain with the highest B factors had a weak and ill-defined electron density, indicating static disorder. The relatively high R factors and large differences in B factors for the different molecules could be a consequence of unmodeled anisotropic displacements. Therefore, as individual atomic anisotropic refinement is not feasible with data collected at 2.05 Å resolution, we describe here the use of translation-libration-screw (TLS) parameterization that uses collective variables to describe the translation, libration (torsional vibration) and screw-rotation displacements of pseudo-rigid bodies to model anisotropic atomic displacement (Winn et al. 2001) (Table 1A).

By providing detailed information about atomic displacements, anisotropic refinement of X-ray crystallography data is an experimental method that can elucidate the dynamic structure of proteins (Artymiuk et al. 1979; Painter and Merritt, 2006). Therefore, we also pursued the previously reported refinement of the other crystallized state of whole GlmS, Glms in complex with Glucose-6-P (Glc6P) and DON (Mouilleron et al. 2006), by performing TLS refinement (Table 1A). The TLS analysis of the two complexes allows to get insight into the intrinsic motions of two consecutive functional states of GlmS.

Finally, a systematic analysis of the structural change occurring between the two X-ray structures of whole GlmS was done with the program *DYNDOM* (Hayward and Berendsen 1998). This allows to delineate the dynamical domains and visualize the conformational change occurring upon DON binding in term of domain movements. The domains displacements observed in the crystals are then compared to the domains motion during the catalytic cycle in order to know if it is related to the biological activity of the protein.

Results and discussion

Crystal packing analysis

The crystallographic asymmetric unit of the GlmS·Fru6P structure contains three monomers A, B and C. Monomers A and B are related by a 176.0° rotation and a 0.769 \AA translation and monomers B and C by a 178.5° rotation and a 0.296 \AA translation. The enzyme is functional as a dimer; monomers B and C constitute one dimer while monomer A forms a tightly packed dimer with its counterpart in the neighboring asymmetric unit (Fig. 1A). The synthase active site is located at the dimer interface formed by two synthase domains and the two corresponding glutaminase domains are located on opposite sides of the synthase dimer. The structural differences between the different monomers are minor. The overall rmsd for $C\alpha$ atoms is 0.55 \AA between monomers A and B and 0.61 \AA between monomers A and C.

Although displacements are generally limited in crystals due to packing constraints, the GlmS·Fru6P crystal has a high solvent content (60.9 %) and high static disorder of the monomer C glutaminase domain is indicated by the high isotropic temperature factors (Table 1B) and the ill-defined electron density (Figure S1). The disorder of this domain, which is directly related to its weak participation in crystal contacts (Fig. 1A, Table 1B), did not allow to trace 36% of its side chains.

The asymmetric unit of the GlmS·Glc6P·DON crystal contains one dimer (Fig. 1B). The two monomers are related by a 179.8° rotation and a 0.067 \AA translation with an overall rmsd for $C\alpha$ atoms of 0.35 \AA . The GlmS·Glc6P·DON crystal has a lower solvent content (52.9 %) and is involved in more crystallographic contacts than the GlmS·Fru6P crystal (Table 1B). As the glutaminase domain of monomer B is less constrained by the crystal packing than the glutaminase domain of monomer A (Table 1B), its internal motion reflects better the mobility of the glutaminase domain in solution.

In general, the glutaminase domains of all monomers of both structures are involved in less crystal-packing interactions than the synthase domains that are involved in the dimer interface (Table 1B) and appear therefore to be more flexible, allowing large conformational changes

during the catalytic cycle.

TLS refinement

The TLS refinement allows to improve the fit of the model to the observed data by accounting for the anisotropy of the data. In addition, it accounts for differences in displacement parameters between NCS-related molecules observed in the isotropic refinement. The TLS refinement implemented in *REFMAC* evaluates three separate contributions to the total atomic displacement parameter: the overall anisotropy of the crystal, the translations and librations of pseudo-rigid bodies within the asymmetric unit of the crystal and the local displacements of individual atoms. All atoms within a TLS group are assumed to constitute a rigid body and the displacements about the rigid-body degrees of freedom for this group are refined to optimize the agreement between the model and the measured intensity data. Atomic displacements are represented by 20 refinement parameters for each TLS group instead of one or six parameters per atom, for isotropic or individual anisotropic refinement, respectively. An important component of the TLS model of anisotropic displacements is the choice of rigid groups. The whole asymmetric unit, each protein monomer or domain are usually treated as TLS groups. TLS refinement of crystallographic data has been used to investigate domain displacements in a few cases (Ramirez et al. 2002; Yousef et al. 2002; Papiz et al. 2003; Wilson and Brunger 2003; Chaudhry et al. 2004; Schultz-Heienbrok et al. 2004; Akif et al. 2005; Newstead et al. 2005; Painter and Merritt, 2006), sometimes together with other complementary approaches.

The relatively high crystallographic factors after isotropic refinement (Table 1C), the different average isotropic *B* factors for the three monomers (Table 1B) and the loose packing of the crystal led us to investigate three TLS models for the GlmS·Fru6P structure (Table 1C). In the first model, only one TLS group including the three protein monomers and the three fructose-6P molecules was considered. In the second model, each of the three monomers in the asymmetric unit including its fructose-6P ligand was treated individually as a TLS group. In the third model, two TLS groups for each monomer were considered, one for the glutaminase domain and one for the synthase domain with fructose-6P. Solvent molecules were not included in any TLS group. The models have been refined with or without NCS restraints (Table 1B). Although all

three TLS models give a drop in R and in R_{free} the inclusion of one TLS group for each monomer has the most significant effect with a decrease of R and R_{free} respectively of 3.3 % and 3.5 %. A further 0.3 % decrease in the R factor is observed when each domain is treated as an independent TLS group and this TLS model is further considered here. The GlmS·Glc6P·DON crystal has a much less pronounced anisotropic behavior than the GlmS·Fru6P crystal. The R factors are similar whether TLS groups for the asymmetric unit, for each monomer or for each domain are considered (data not shown). The TLS refinement results in a drop for R and R_{free} respectively of 1.9 % and 1.1 % (Table 1C). For both structures, while applying NCS restraints in the isotropic refinement has a negative effect on both R and R_{free} factors, when TLS parameters are included, the R and R_{free} factors have the same values whether NCS restraints are imposed or not.

The B factor can be decomposed into two components, B_{TLS} accounting for the rigid body displacements of the TLS groups and B_{res} , corresponding to the individually refined atomic residual B factors. The relative contributions of each component to the B factor (Figure 2) indicate that the TLS parameters account for most of the large-scale variations of the total B factors. Therefore, after inclusion of TLS parameters, the residual B factors for the different protein monomers that describe the static disorder in the crystal are very similar to each other and the application of NCS restraints is reasonable (Table 1C).

Analysis of domain displacements in the crystal

The full TLS refinement yields atomic coordinates, translation, libration, and screw tensors for each TLS group chosen and residual individual B factors for each atom. The TLS parameters for the refinement in which each domain is considered as a TLS group describe the displacements of the domains in the crystal, giving a rough measure of the degree of order (Yousef et al. 2002). The eigenvalues of the libration tensor represent the magnitude of rotational displacement around three perpendicular axes. A significantly different magnitude of the eigenvalues around the three axes indicates an anisotropic motion. Yet, direct viewing of the axes representing the libration motion does not lead to any simple physical interpretation.

The libration tensor results of the TLS refinements where the NCS restraints have been released

(Fig. 3B and 4) indicate a particularly large and anisotropic libration motion of monomer C glutaminase domain of the GlmS·Fru6P complex, which is indeed the least ordered part of the structure, as judged by the quality of the electron density (Fig. S1) (mean-square displacement of $8.1^{\circ 2}$). The directions of libration axes of the different domains are generally different (Fig. 4), indicating individual displacements of these domains. However, it is interesting to note that the axes of libration for the synthase domains of monomers B and C of the GlmS·Fru6P crystal have similar directions (Fig. 4A), implying that these two domains move together as one rigid-body. It is the same for the synthase domains of monomers A and B of the GlmS·Glc6P·DON crystal (Fig. 4B). This is in agreement with the corresponding pairs of monomers forming compact dimers through the synthase domains (Fig. 1).

An estimate of the mean-square amplitude of intramolecular translation of of the GlmS glutaminase and synthase domains is respectively 0.2 and 0.3 Å² (Scheringer 1972). For the synthase domain of monomer B and both domains of monomer C of the GlmS·Fru6P crystal and the glutaminase domain of monomer B of the GlmS·Glc6P·DON crystal, the mean-square translational displacements are much higher than these values (Fig. 3A), which indicates a rigid-body translational motion of these domains.

Analysis of the domain movement occurring upon glutamine analog binding to sugar-bound glucosamine-6-P synthase.

Insight into protein domain motion most often comes from analyzing crystal structures of open and closed conformations. Examining multiple crystal structures of a protein in different conformations has shown that domain motions can be classified into two extreme types, shear and hinge (Gerstein et al. 1994; Wriggers and Schulten 1997). In hinge-type motion, movement is perpendicular to the interdomain surface, whereas it is parallel in the shear type.

No analysis of the domain movements was made in our previous paper reporting the GlmS·Fru6P and GlmS·Glu6P·DON structures determination (Mouilleron et al. 2006). In order to further investigate the dynamics of *E.coli* GlmS, we analyze here the conformational change of GlmS occurring upon glutamine analog binding in terms of domain movements by comparing the two structures with *DYNDOM* (Hayward and Berendsen 1998; Hayward 1999) (Table 2,

Fig. 5A and B). The program *DYNDOM*, based on the idea that dynamic domains can be identified by their differing rotational properties, delineates the quasi-rigid domains and identifies the interdomain screw axes and the residues involved in the interdomain motion.

GlmS was divided into two dynamic domains, which correspond closely to the structural domains: the moving domain, corresponding to the glutaminase domain, is composed of residues 3 to 236 and the fixed domain, corresponding to the synthase domain, of residues 244 to 606. When either monomer of the GlmS·Glc6P·DON was superposed on monomer A of the GlmS·Fru6P structure, the Q-loop delineated by *DYNDOM* as residues 73 to 82 (belonging to the glutaminase domain) was included in the fixed domain. Therefore, the shielding of the glutaminase site upon DON binding that was analyzed as the closure of the Q-loop on the glutaminase site (Mouilleron et al. 2006) can also be viewed as a rocking motion of the glutaminase domain on the Q-loop, which does not move (Fig. 5A). The bending regions (defined as the regions of the backbone where a transition is seen between the rotational properties of the two dynamic domains) were defined as residues 235 to 248, the linker region. The mechanical hinge, where the hinge axis and the rotational transition coincide, involves several residues of the Q-loop (73 to 75 and 80 to 82) that play a crucial role in catalysis. In particular, Arg73 serves as an anchor to the carboxylate group of glutamine and Trp74 acts as the gate of the channel. The location of the interdomain screw axis within 5.5 Å of the bending residues identifies an effective hinge motion with an angle of rotation of 22.8° ($\pm 1.7^\circ$) (Table 2). The hinge axis runs approximately parallel to helix 300-317 of the synthase domain (Fig. 5B) and is located 3.3 Å from C α of Asp29 (participating in the dimer interface) and 2.1 Å from C α of Trp74. The translation component of the screw operation describing the domain movement is 1.07 Å (± 0.09 Å) so that the movement is essentially a pure domain rotation.

The mean-square libration is 8.1 $^\circ$ ² and 2.9 $^\circ$ ² for the least constrained glutaminase domains of the GlmS·Fru6P and GlmS·Glc6P·DON structures, respectively, while the hinge motion that accompanies the conformational change upon DON binding is 22.8 $^\circ$, indicating that the scales of these two types of motions are different. The angle between the inter-domain hinge rotation axis and the closest predominant axis of libration of the glutaminase domain of the least constrained monomer (C) of the GlmS·Fru6P complex is 40.5 $^\circ$ (Fig. 5B and C). Yet, the

libration motion of the GlmS·Glc6P·DON complex is very different and of smaller magnitude than that of the GlmS·Fru6P complex (Fig. 5D). In fact, in the GlmS·Glc6P·DON complex that mimicks the conformation of the enzyme with two bound-substrates, the two active sites are linked by the ammonia channel and no major domain movement is expected at this stage of the reaction while in the GlmS·Fru6P complex, a conformational change of the enzyme that shields the glutamine binding site is observed upon glutamine binding. Therefore, the libration motion of the GlmS·Fru6P complex, which is of high magnitude and not observed in the following state of the enzyme, seems to be functionally significant and may be related to the conformational change that occurs upon glutamine binding.

Conclusion

The isotropic refinement of the crystal structure of GlmS in complex with fructose-6P at 2.05 Å resolution yielded relative high crystallographic factors. In addition, the three non crystallographic symmetry-related molecules in the asymmetric unit showed significantly different overall displacement parameters. Therefore, the GlmS·Fru6P structure represents a typical example where anisotropic refinement can improve the fit of the model to the diffraction data by taking into account anisotropic rigid body displacements. As TLS refinement allows to take out experimental information about the dynamical properties of macromolecules from X-ray data, it was also performed for the GlmS·Glc6P·DON complex, which represents the following step in the reaction. This allows to understand the basis of the transitions from one state to the other.

The refinement of TLS parameters implemented in the macromolecular refinement program *REFMAC* (Winn et al. 2001) gave best results when one TLS group for each domain was used. Removing large-scale domain displacements while making residual *B* factors more meaningful results in a large reduction in *R* and R_{free} factors. For the GlmS·Fru6P structure, there was a spectacular improvement in *R* and R_{free} values respectively of 3.4 % and 3.8 %. TLS refinement of the displacement parameters of both structures indicates that the two domains are dynamically distinct, with the glutaminase domain possessing significantly more flexibility than the synthase domain. One glutaminase domain of the GlmS·Fru6P structure, for which weak crystallographic

contacts and low electronic density was observed, presented a markedly anisotropic behavior for the libration motion. Since this domain is involved in few crystal contacts, the internal dynamics of this domain probably reflect its mobility in solution.

To avoid wasteful escape of ammonia, glutamine amidotransferases shield their active sites against the surrounding water environment. This involves the closure of one domain onto the other upon substrate binding as well as loop closure to trap the substrates. The comparison of the anisotropic domain displacements observed in the crystals and the domain motion during the catalytic cycle suggest that the intramolecular mobility properties of the sugar-bound enzyme contribute to facilitate the structural change that is observed upon glutamine binding and have therefore a biological significance.

Although TLS tensors do not describe normal modes of vibration, they approximate the effects of a collective motion of these modes as the first three low-frequency normal modes describe correlated domain movements. To further explore the conformational dynamics of *E. coli* GlmS, this work will be complemented by a normal mode analysis (Floquet et al, in preparation).

Acknowledgements

Financial support from ICSN to SM is gratefully acknowledged. We are grateful to Joël Janin for most valuable discussions and advice throughout this work. We thank Marie-Ange Badet-Denisot and Bernard Badet for initiating this project, and Thierry Prangé for reading the manuscript.

Materials and methods

Protein preparation and data collection

The protein preparation, crystallization and structure determination has been reported previously (Mouilleron et al. 2006). The diffraction data sets were collected at 100K on beamlines ID14-EH1 or ID14-EH2 at the European Synchrotron Radiation Facility in Grenoble for the GlmS·Fru6P crystal and GlmS·Glu6P·DON crystal, respectively. The coordinates have been deposited in the Protein Data Bank (PDB code 2BPL and 2J6H).

TLS refinement

All TLS refinements were performed in *REFMAC5* (Murshudov et al. 1999; Winn et al. 2001) using data between 15-2.05 Å or 15-2.35 Å resolution for the GlmS·Fru6P and GlmS·Glu6P·DON crystals respectively. When used, NCS restraints are applied with medium restraints for main-monomer atoms and loose restraints for side-monomer atoms. As the best results are usually observed when all atomic *B* factors are fixed at a constant value before refining the overall scale factor and TLS parameters (Winn et al. 2001), this procedure was adopted here. All TLS refinements used the same starting coordinates with all isotropic *B* factors set to 35 Å². TLS refinements were carried out for 12 cycles of conjugate-gradient least-squares refinement against all measured data, with a matrix weighting term of 0.2. A bulk-solvent model was employed and an overall anisotropic scale factor was applied. After the TLS refinement was complete, an additional 15 cycles of restrained conjugate-gradient least-squares of both coordinates and isotropic *B* factors was performed. The same 5% of the data were used to calculate R_{free} through the *CNS* and *REFMAC5* portions of the refinement. The CCP4 program *TLSANL* (Howlin et al. 1993) was used to determine the principal axes of the TLS tensors relative to an orthogonal frame with the centre of reaction as origin as well as the magnitudes along these axes. The final model of the GlmS·Fru6P structure includes 64% and 92% of the side chains for the monomer C glutaminase and synthase domains, respectively. The number of contacts between the different domains have been calculated with *NCONT* from CCP4i (version 5).

Domain rotation analysis

The protein dynamical domains, hinge axes and amino acids involved in the hinge bending motion upon DON binding were determined with the CCP4 program *DYNDOM* (Hayward and Berendsen 1998) based on the comparison of the GlmS·Fru6P and GlmS·Glc6P·DON structures. A sliding window of five residues was used in the analysis. The minimum value for the ratio of interdomain to intradomain displacement was set to 1.0 and the minimum domain size was 60 residues.

References

- Akif, M., Suhre, K., Verma, C., and Mande, S.C. 2005. Conformational flexibility of Mycobacterium tuberculosis thioredoxin reductase: crystal structure and normal-mode analysis. *Acta Crystallogr D Biol Crystallogr* **61**: 1603-1611.
- Artymiuk, P.J., Blake, C.C., Grace, D.E., Oatley, S.J., Phillips, D.C., and Sternberg, M.J. 1979. Crystallographic studies of the dynamic properties of lysozyme. *Nature* **280**: 563-568.
- Chaudhry, C., Horwich, A.L., Brunger, A.T., and Adams, P.D. 2004. Exploring the structural dynamics of the E.coli chaperonin GroEL using translation-libration-screw crystallographic refinement of intermediate states. *J Mol Biol* **342**: 229-245.
- Gerstein, M., Lesk, A.M., and Chothia, C. 1994. Structural mechanisms for domain movements in proteins. *Biochemistry* **33**: 6739-6749.
- Hayward, S. 1999. Structural principles governing domain motions in proteins. *Proteins* **36**: 425-435.
- Hayward, S., and Berendsen, H.J. 1998. Systematic analysis of domain motions in proteins from conformational change: new results on citrate synthase and T4 lysozyme. *Proteins* **30**: 144-154.
- Howlin, B., Butler, S.A., Moss, D.S., Harris, G.W., and Driessen, H.P.C. 1993. TLSANL: TLS parameter-analysis program for segmented anisotropic refinement of macromolecular structures. *J. Appl. Cryst.* **26**: 622-624.
- Mouilleron, S., Badet-Denisot, M.-A., and Golinelli-Pimpaneau, B. 2006. Glutamine binding opens the ammonia channel and activates glucosamine-6P synthase. *J. Biol. Chem.* **281**: 4404-4412.
- Murshudov, G.N., Vagin, A.A., Lebedev, A., Wilson, K.S., and Dodson, E.J. 1999. Efficient anisotropic refinement of macromolecular structures using FFT. *Acta Crystallogr. D* **55**: 247-255.
- Newstead, S.L., Watson, J.N., Bennet, A.J., and Taylor, G. 2005. Galactose recognition by the carbohydrate-binding module of a bacterial sialidase. *Acta Crystallogr D Biol Crystallogr* **61**: 1483-1491.
- Painter, J., and Merritt, E. A. 2006. Optimal description of a protein structure in terms of multiple groups undergoing TLS motion. *Acta Crystallogr D Biol Crystallogr* **62**: 439-450.

Papiz, M.Z., Prince, S.M., Howard, T., Cogdell, R.J., and Isaacs, N.W. 2003. The structure and thermal motion of the B800-850 LH2 complex from *Rps.acidophila* at 2.0Å resolution and 100K: new structural features and functionally relevant motions. *J Mol Biol* **326**: 1523-1538.

Ramirez, U.D., Minasov, G., Focia, P.J., Stroud, R.M., Walter, P., Kuhn, P., and Freymann, D.M. 2002. Structural basis for mobility in the 1.1 Å crystal structure of the NG domain of *Thermus aquaticus* Ffh. *J Mol Biol* **320**: 783-799.

Scheringer, C. 1972. On the contributions of the internal modes of molecules to the Debye-Waller factors. *Acta Cryst.* **A28**: 516-522.

Schultz-Heienbrok, R., Maier, T., and Sträter, N. 2004. Trapping a 96 degrees domain rotation in two distinct conformations by engineered disulfide bridges. *Protein Sci* **13**: 1811-1822.

Wilson, M.A., and Brunger, A.T. 2003. Domain flexibility in the 1.75 Å resolution structure of Pb²⁺-calmodulin. *Acta Crystallogr D Biol Crystallogr* **59**: 1782-1792.

Winn, M.D., Isupov, M.N., and Murshudov, G.N. 2001. Use of TLS parameters to model anisotropic displacements in macromolecular refinement. *Acta Crystallogr. D* **57**: 122-133.

Wriggers, W., and Schulten, K. 1997. Protein domain movements: detection of rigid domains and visualization of hinges in comparisons of atomic coordinates. *Proteins* **29**: 1-14.

Yousef, M.S., Fabiola, F., Gattis, J.L., Somasundaram, T., and Chapman, M.S. 2002. Refinement of the arginine kinase transition-state analogue complex at 1.2 Å resolution: mechanistic insights. *Acta Crystallogr D Biol Crystallogr* **58**: 2009-2017.

Table 1:**A Data collection and refinement statistics**

	GlmS·Fru6P	GlmS·Glc6P·DON
Space group	C2	P212121
Cell dimensions		
a, b, c (Å)	132.2, 109.7, 176.3	83.3, 91.23, 184.99
α , β , γ (°)	90, 97.1, 90	90, 90, 90
Resolution Å	30.00 - 2.05 (2.09 - 2.05)	30.00 - 2.35 (2.39 - 2.35)
R_{sym}	0.083 (0.72)	0.097 (0.446)
$I/\sigma I$	16.75 (2.00)	9.55 (2.09)
Completeness (%)	98.6 (97.2)	96.0 (83.0)
Redundancy	5.45 (4.62)	3.11 (2.09)
Anisotropic refinement statistics		
Resolution Å	15.00 - 2.05	15-2.35
(Outer resolution shell)	2.06-2.05	2.41-2.35
R _{free} %	28.83 (41.08)	23.7 (34.4)
R %	26.23 (38.47)	18.7 (30.0)
No. reflections	145921	54035
No. atoms		
Protein	14037	
Ligand	3	4
Water	405	383
R.m.s. deviations		
Bond lengths (Å)	0.021	0.022
Bond angles (°)	1.93	1.807

B

Correlation between the isotropic *B* factors and the number of crystallographic contacts.

	Isotropic <i>B</i> Factor (Å ²)	Number of crystallographic contacts/residues
GlmS·Fru6P		
Chain A		
Glutaminase	38.7	0.68
Synthase	30.2	1.38
Chain B		
Glutaminase	36.1	0.72
Synthase	53.4	1.06
Chain C		
Glutaminase	71.9	0.10
Synthase	53.7	1.05
GlmS·Glc6P·DON		
Chain A		
Glutaminase	29.5	0.94
Synthase	36.2	1.69
Chain B		
Glutaminase	58.1	0.43
Synthase	41.1	1.63

¹The number of crystallographic contacts of one domain is defined by the number of atoms belonging to another domain (either of the same molecule or of a symmetry-related molecule) that are located at a distance less than 4 Å.

C: Isotropic and anisotropic refinement of the GlmS·Fru6P and GlmS·Glc6P·DON structures.

	TLS group	NCS	R factor	R _{free}
GlmS·Fru6P				
	no	no	0.244	0.285
	no	yes	0.254	0.297
	1 asymmetric unit	no	0.238	0.280
	1 asymmetric unit	yes	0.239	0.281
	3 monomers	no	0.211	0.250
	3 monomers	yes	0.211	0.251
	6 domains	no	0.210	0.247
	6 domains	yes	0.210	0.247
GlmS·Glc6P·DON				
	no	no	0.206	0.248
	no	yes	0.227	0.261
	4 domains	no	0.187	0.237
	4 domains	yes	0.187	0.239

Table 2: Dynamical domains and hinge bending residues determined by the program *DYNDOM* in CCP4 for the superposition of the different monomers of the GlmS·Fru6P (PDB code 2BPL) and GlmS·Glc6P·DON (PDB code 2J6H) structures.

Superposition (Monomer)	Domain 1	Domain 2	Bending residues	Rotation (°)	Translation along axis (Å)	% Closure motion (°) ¹
2BPL (A) 2J6H (A)	74-81; 244-606	3-73; 82-243	73-82; 235-248	21.4	1.0	31.1
2BPL (A) 2J6H (B)	73-81; 238-606	3-72; 82-237	72-75; 77-82; 235-248	21.1	1.0	33.4
2BPL (B) 2J6H (A)	244-606	3-243	235-248	22.9	0.9	23.4
2BPL (B) 2J6H (B)	237-239 244-606	3-236; 240-243	235-248	22.5	0.9	21.8
2BPL (C) 2J6H (A)	244-606	3-236	235-248	24.0	1.1	34.1
2BPL (C) 2J6H (B)	237-606	3-236	235-248	23.5	1.0	33.7

¹ The unit vector of the rotation vector corresponding to the hinge axis can be decomposed into components parallel or perpendicular to the line joining the centers of mass of the two domains, respectively g_t and g_c , with $g_t^2 + g_c^2 = 1$. The degree of the closure motion is defined as $g_c^2 \times 100$ %.

Figure legends

Figure 1: A Crystallographic contacts of the three molecules of the GlmS·Fru6P crystal. The synthase domain is colored in purple, dark green and dark blue and the glutaminase domain in pink, light green and light blue for monomers A, B or C, respectively. The asymmetric unit contains one dimer (constituted of monomers B and C) and one monomer (A) that forms a dimer with its counterpart (A#) in the neighboring asymmetric unit.

B Crystallographic contacts of the two molecules of the GlmS·Glc6P·DON crystal. The asymmetric unit contains one dimer, constituted of monomers A and B. The synthase domain is colored in dark green and dark blue and the glutaminase domain in light green and light blue for monomers A and B, respectively.

Figure 2: Comparison of the displacement parameters before and after the anisotropic refinement. A GlmS·Fru6P crystal. B GlmS·Glc6P·DON crystal. Residues are numbered 1 to 608 for monomer A, 609 to 1216 for monomer B, 1217 to 1824 for monomer C. B_{TLS} , the contribution from the TLS motion, B_{res} , the residual B factors after applying the TLS model, and their sum B are shown. The isotropic B factors, B_{iso} , are also indicated. For each residue, the B factors are merged over the main-monomer atoms.

Figure 3: Mean displacements given as the TLS tensor eigenvalues when each domain is treated as a rigid group in the TLS refinements.

Left: GlmS·Fru6P structure, right: GlmS·Glc6P·DON structure

A Eigenvalues of the translation tensor.

B Eigenvalues of the libration tensor

C Eigenvalues of the screw tensor

The eigenvalues are shown as a cumulative stack bar. The axis with the lowest eigenvalue is at the bottom and the one with the highest at the top. The S tensor was made symmetric by referring it to a coordinate system whose origin is at the center of reaction for the rigid group.

Figure 4: Stereoview of the TLS-derived libration axes when each domain is considered as a TLS group.

A GlmS·Fru6P

B GlmS·Glc6P·DON

The domains are colored as in Fig. 1. The length of each axis is proportional to the magnitude of libration along the axis. The sugar is represented in ball-and-stick. This figure was drawn with *MOLSCRIPT*.

Figure 5: Comparison of the domain movements during the catalytic cycle and in the crystals.

A Hinge-bending domain rotation upon glutamine binding.

The dynamical domains as determined by the superimposition of monomers A of the GlmS·Fru6P (in yellow) and GlmS·Glc6P·DON structures with *DYNDOM* (Hayward and Berendsen 1998). In the GlmS·Glc6P·DON structure, the fixed domain is shown in blue, the moving domain in green, the linker and Q-loops in black.

B Location of the hinge axis of the rotation upon glutamine binding.

The dynamical domains of GlmS shown in the open GlmS·Fru6P conformation are colored according to the absolute rotation of each segment except helix 300-317 almost parallel to the rotation axis, which is colored black. The rmsd of $0.88 \text{ \AA} (\pm 0.17 \text{ \AA})$ and $0.68 \text{ \AA} (\pm 0.35 \text{ \AA})$, between the two structures when the moving and fixed domains, respectively, are superimposed indicate that the two domains rotate essentially as rigid domains with little perturbation of the domain structures. The interdomain rotation axis is shown in red, with the arrow indicating direction of the rotation of the glutaminase domain by the right hand rule (Hayward and Berendsen 1998). This figure was drawn with *RASMOL*.

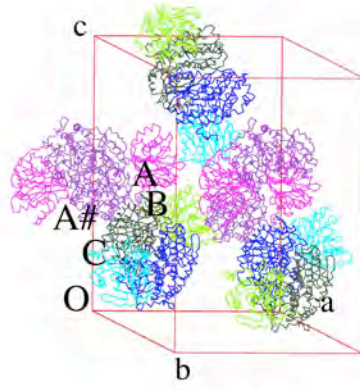
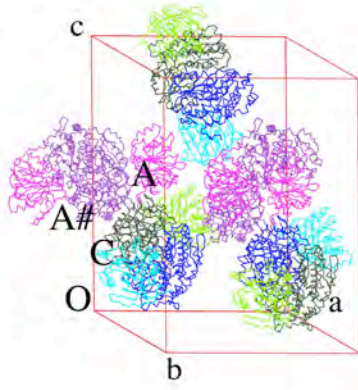
C Stereoview of the TLS-derived libration axes for monomer C of the GlmS·Fru6P structure.

The monomer is oriented as in (A). The glutaminase and synthase domains are colored cyan and blue, respectively.

D Stereoview of the TLS-derived libration axes for monomer B of the GlmS·Glc6P·DON structure.

Figure 1

A



B

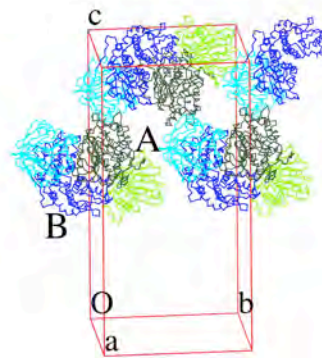
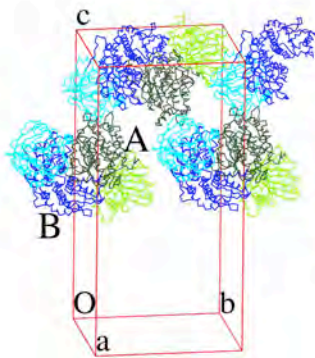
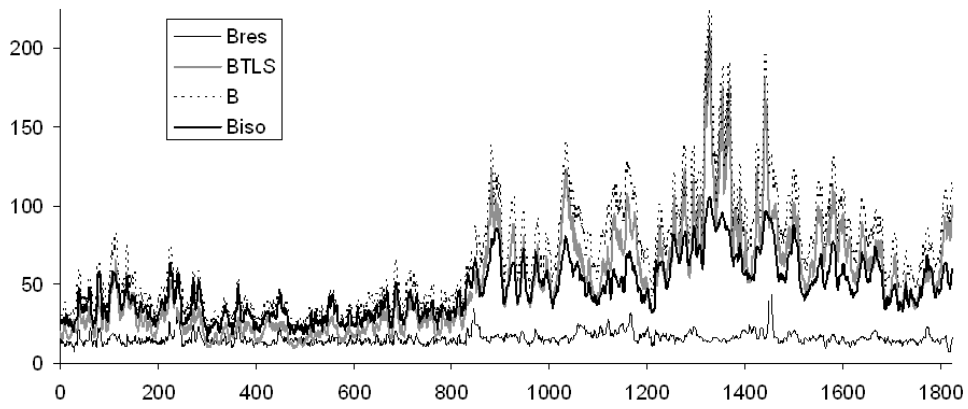


Figure 2

A



B

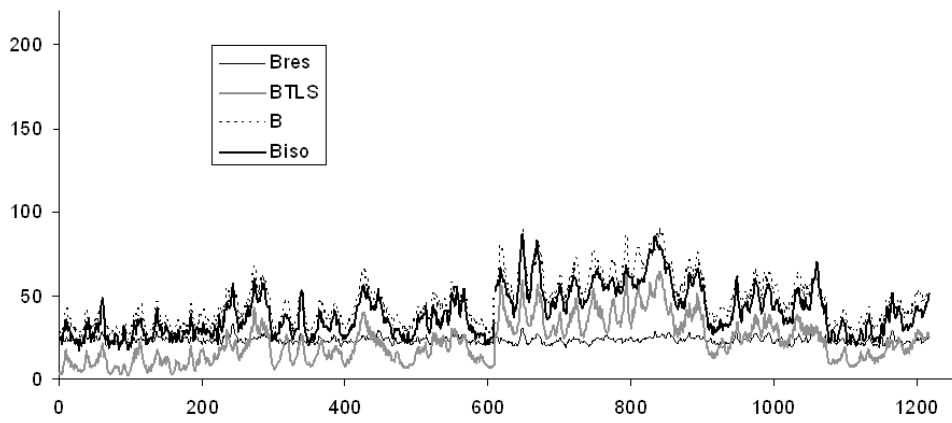
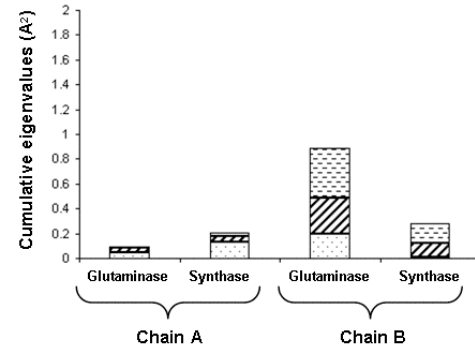
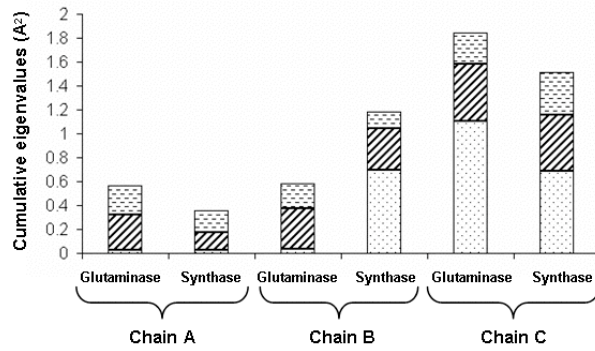


Figure 3

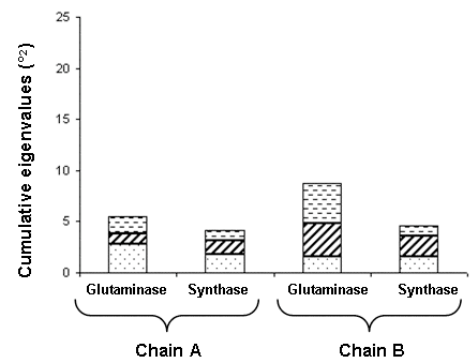
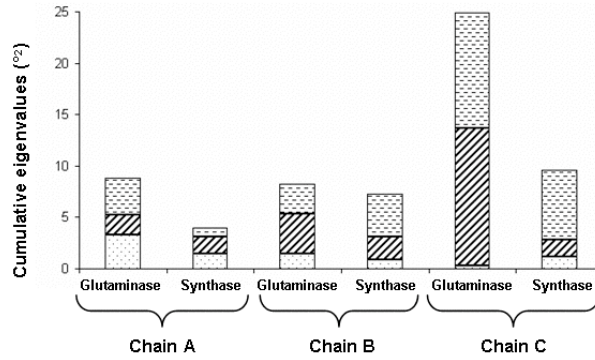
GlmS-Fru6P

GlmS-Glc6P·DON

A



B



C

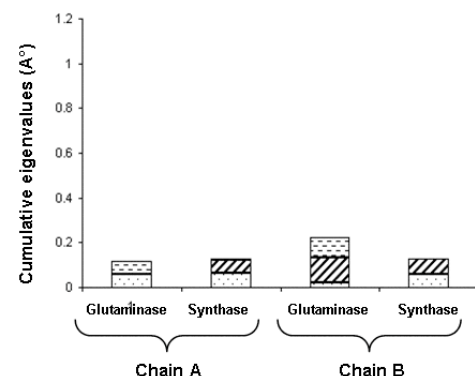
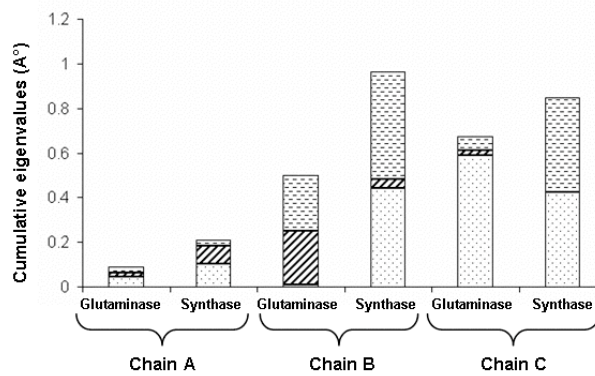


Figure 4

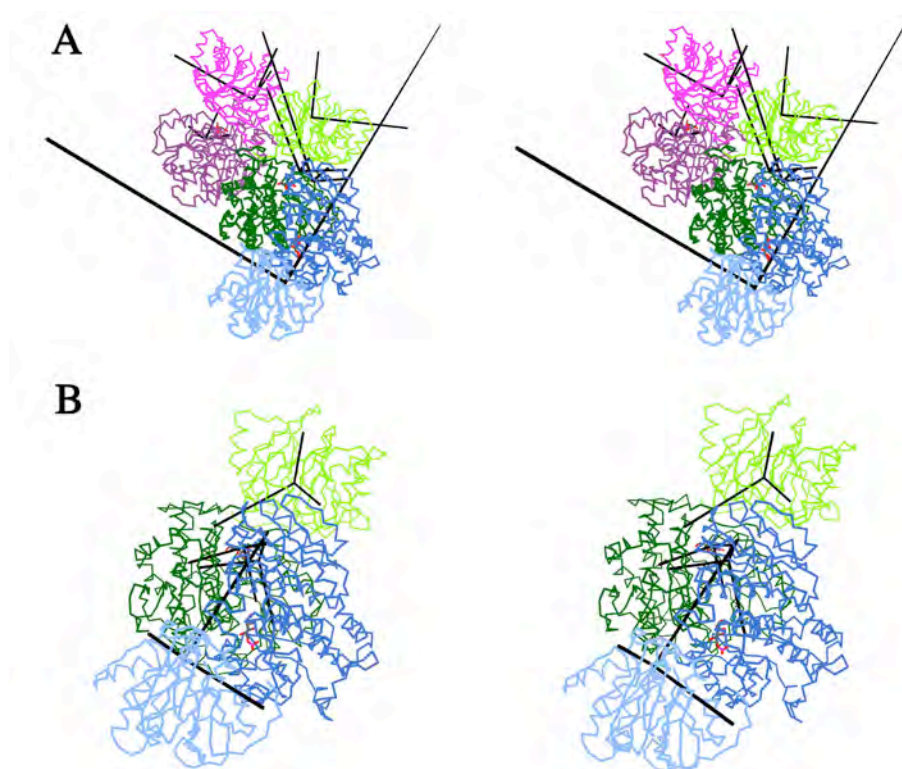


Figure 5

