



HAL
open science

^1H , ^{13}C and ^{15}N chemical shift assignments of the ZnR and GYF cytoplasmic domains of the GltJ protein from *Myxococcus xanthus*

Bouchra Attia, Bastien Serrano, Olivier Bornet, Françoise Guerlesquin, Laetitia My, Jean-Philippe Castaing, Tâm Mignot, Latifa Elantak

► To cite this version:

Bouchra Attia, Bastien Serrano, Olivier Bornet, Françoise Guerlesquin, Laetitia My, et al.. ^1H , ^{13}C and ^{15}N chemical shift assignments of the ZnR and GYF cytoplasmic domains of the GltJ protein from *Myxococcus xanthus*. *Biomolecular NMR Assignments*, 2022, 16 (2), pp.219-223. 10.1007/s12104-022-10083-6 . hal-03872154

HAL Id: hal-03872154

<https://hal.science/hal-03872154v1>

Submitted on 28 Nov 2022

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.

¹H, ¹³C and ¹⁵N chemical shift assignments of the ZnR and GYF cytoplasmic domains of the GltJ protein from *Myxococcus xanthus*

Bouchra Attia¹, Bastien Serrano¹, Olivier Bornet², Françoise Guerlesquin¹, Laetitia My³, Jean-Philippe Castaing³, Tâm Mignot³ and Latifa Elantak^{1*}

¹Laboratoire d'Ingénierie des Systèmes Macromoléculaires, Aix-Marseille Université, Centre National de la Recherche Scientifique (CNRS), UMR7255, 13009 Marseille, France

²Institut de Microbiologie de la Méditerranée, Centre National de la Recherche Scientifique (CNRS), FR3479, 13009 Marseille, France

³Laboratoire de chimie bactérienne, Aix-Marseille Université, Centre National de la Recherche Scientifique (CNRS), UMR7283, 13009 Marseille, France

*Corresponding author: Latifa Elantak, elantak@imm.cnrs.fr

Statements and Déclarations

Competing interests : The authors declare that they have no conflict of interest.

Funding : This work was funded by the Centre de la recherche scientifique (CNRS and Aix-Marseille University).

Availability of data : The chemical shift values have been deposited in the BioMagResBank (<http://www.bmrwisc.edu/>) under the accession numbers 51096 and 51104.

Abstract

Bacterial cell motility is essential for a range of physiological phenomena such as nutrient sensing, predation, biofilm formation and pathogenesis. One of the most intriguing motilities is bacterial gliding, which is defined as the ability of some bacteria to move across surfaces without an external appendage. In *Myxococcus xanthus*, gliding motility depends on the assembly of focal adhesion complexes (FAC) which include the Glt multiprotein complex and allow directional movement of individual cells (A-motility). Within the Glt multiprotein complex, GltJ is one of the key proteins involved in FAC assembly. In this work we report complete backbone and side chain ^1H , ^{13}C and ^{15}N chemical shifts of the two cytoplasmic domains of GltJ, GltJ-ZnR (BMRB No. 51104) and GltJ-GYF (BMRB No. 51096). These data provide the first step toward the first high resolution structures of protein domains from the Glt machinery and the atomic level characterization of GltJ cytoplasmic activity during FAC assembly.

Introduction

Cell migration is an essential behavior of all living cells with directional motility. This fundamental survival capacity is tightly regulated and mediates a plethora of fundamental biological and pathological processes (Stuelten et al. 2018). This is true for all complex organisms, but also for unicellular organisms such as bacteria. Bacterial motility is essential for carrying out nutrient detection (Porter et al. 2011), biofilm formation (Guttenplan and Kearns 2013) and interaction with their host during infection (Ribet and Cossart 2015). To do so, bacteria have evolved a wide range of sophisticated macromolecular machines resulting either in extracellular appendages such as flagella and surface pili, or in focal adhesion complexes (FACs) mediating the so-called gliding motility (also known as A-motility) (Wadhwa and Berg 2021). A-motility have been extensively studied in the model organism *Myxococcus xanthus* (Mignot et al. 2007; Islam and Mignot 2015; Faure et al. 2016; Mignot and Nöllmann 2017). In this bacterium, FACs assembly allows single cells to move at the colony periphery, exploring their environment for food. A-motility is controlled by cytoplasmic proteins, MglA and AglZ, coupled to the bacterial cytoskeleton MreB and a protein machinery named Agl-Glt which contains fourteen proteins (AglQRS and GltA to GltK) predicted to localize in all layers of the cell envelope (Islam and Mignot 2015). This protein complex assembles at the leading pole of the cell and moves in the direction of the lagging cell pole, attaching to the substrate thus powering the directional movement of the cell (Schumacher and Søggaard-Andersen 2017) (Faure et al. 2016). While in-depth knowledge is available at the single cell and genetic levels, there is no structural data or exploration of protein interactions in the Agl-Glt system that would provide a molecular and structural understanding of the assembly and function of Glt proteins in this motility system.

A previous study has shown that GltJ deletion strongly affects AglZ, AglQ and GltI localization, indicating that GltJ plays a central role in FAC assembly (Faure et al. 2016). Indeed, GltJ is an inner membrane protein containing a N-terminal cytoplasmic region, a transmembrane segment and a C-terminal periplasmic region. The N-terminal cytoplasmic region includes two predicted structural domains: a zinc-ribbon (GltJ-ZnR¹⁻⁴²) and a glycine-tyrosine-phenylalanine (GltJ-GYF¹³⁸⁻²²²) domain. The zinc ribbon family is characterized by the presence of two pairs of cysteines in a CXXC-X₁₈₋₂₆-CXXC motif that can chelate a zinc ion (Fig. 1a). Proteins containing zinc ribbon domains are predicted to bind nucleotides/nucleic acids but also proteins or small molecules (Klug 2010). In *Caulobacter crescentus*, a similar ZnR domain has been shown to regulate protein recruitment at the cell pole (Bergé et al. 2016). Thus, the GltJ-ZnR is a strong candidate for intermolecular interactions within the Agl-Glt machinery or with cytoplasmic effectors. The GYF domains are found in most eukaryotic species (Kofler and Freund 2006; Heinze et al. 2007) and have never been characterized in prokaryotes. In eukaryotes, they act as small adaptor domains recognizing proline-rich sequences with the core motif PPG ϕ (ϕ = hydrophobic or aromatic residue) (Kofler et al. 2005; Gu et al. 2005). GYF signature allowing specific protein partner recognition is contained in the conserved sequence W-X-Y-X₆₋₁₁-GPF-X₄-M-X₂-W-X₃-GYF. In *M. xanthus* GltJ-GYF sequence, only 6 residues of the GYF signature are conserved (Fig. 1a) suggesting a new distinct mode of interaction with ligands as compared to eukaryotic GYF domains. In order to investigate the network of interactions and the structural basis that underpins the cytoplasmic biological activity of GltJ, we undertook the complete NMR resonance assignment of the ZnR and GYF domains of this protein. To our knowledge, this is the first NMR study of protein domains from the Glt multiprotein complex.

Methods and experiments

Expression and purification of GltJ-ZnR and GltJ-GYF. The gene sequence corresponding to residues 1-42 of the GltJ protein (ZnR domain) from *M. xanthus* was inserted into a pET28a plasmid vector for expression of a C-terminal His-tagged domain. GYF gene sequence corresponding to residues 138-222 of GltJ was inserted into a modified pET28a plasmid for expression as a N-terminal His6-tagged lipoyl fusion protein. Both constructs were transformed into *Escherichia coli* strain BL21(DE3) for protein overexpression and purification. The ¹⁵N/¹³C-labeled domains were expressed in one liter of M9 medium supplemented with 1 g of ¹⁵N-labeled NH₄Cl and 2 g of ¹³C-labeled glucose. The cells were grown at 37 °C with shaking until OD_{600nm} = 0.7 and protein overexpression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) followed by overnight incubation at 25°C. For GltJ-ZnR, 0.1mM ZnCl₂ was added at the time of induction. Cells were harvested by centrifugation at 4000 rpm for 30 min at 4 °C. The cell pellets were resuspended in buffer A (20 mM Tris-HCl pH 8, 500 mM NaCl, 20 mM imidazole) supplemented with cOmplete™ EDTA-free protease inhibitor cocktail (Roche). The cell suspension was lysed using high pressure homogenizer (Avestin Emulsiflex C5). The cell lysate was then separated by ultracentrifugation at 90,000×g for 30 min at 4 °C. Cell lysates were filtered using a 0.22 μM syringe filter and passed through a HisTrap™ HP column (GE HealthCare) equilibrated with 10 column volumes of buffer A. Then, the column was washed with 10 column volumes of buffer A and eluted in buffer A supplemented with 500 mM imidazole. Afterwards, GltJ-ZnR was further purified by size exclusion chromatography using a Superdex-75 column (GE Healthcare Life Sciences). The His6-tagged-lipoyl-GYF fusion protein was cleaved using TEV protease at 4 °C overnight. The excess TEV protease and His-lipoyl tag were removed using a HisTrap™ HP column, the GltJ-GYF sample was further purified using a Superdex-75 column. NMR experiments were performed on samples containing 450 μM and 1 mM protein, for GltJ-

ZnR and GltJ-GYF, respectively, dissolved in 550 μ L of buffer (10 mM NaPO₄, 5% D₂O), pH 7.

NMR spectroscopy. All NMR experiments were performed at 298 K and 303 K for GltJ-ZnR and GltJ-GYF, respectively, on a Bruker Avance III 600 MHz spectrometer equipped with a cryogenically cooled triple resonance (¹H/¹⁵N/¹³C) 5 mm probe. The backbone assignments were carried out by recording 3D HNCO, HN(CA)CO, HN(CO)CA, HNCA, CBCA(CO)NH and HNCACB experiments. Additionally, a two-dimensional ¹H-¹³C-HSQC and three-dimensional (H)CCH-TOCSY, ¹H-¹⁵N-TOCSY-HSQC, ¹H-¹⁵N-NOESY-HSQC and ¹H-¹³C-NOESY-HSQC experiments were used for side-chain assignments. Aromatic side-chains of phenylalanines and tyrosines were assigned with aromatic ¹H-¹³C-NOESY-HSQC experiments. The data were processed using Topspin3.1 (Bruker) and analyzed with CCPNMR (Vranken et al. 2005).

Extent of assignments and data deposition

Fig. 1b presents the ¹H-¹⁵N HSQC spectra of GltJ-ZnR and GltJ-GYF to illustrate representative backbone resonance assignments. Chemical shift assignments ¹H, ¹⁵N, and ¹³C have been deposited in Biological Magnetic Resonance Bank (BMRB, <http://www.bmrwisc.edu>) under IDs 51104 and 51096 for GltJ-ZnR and GltJ-GYF, respectively. For GltJ-ZnR domain, we assigned 99% of the backbone nuclei (C ^{α} , C', H ^{α} , H^N, and N), and 84% of ¹³C and 88% of ¹H of the ¹³CH_n side chain moieties. Only the C' and H ^{α} , of the initiator methionine were not obtained. C ^{α} and C ^{β} chemical shift values for GltJ-ZnR cysteines are consistent with Zn-ligated cysteine residues (Kornhaber et al. 2006). For GltJ-GYF domain, we assigned 99% of the backbone nuclei (C ^{α} , C', H ^{α} , H^N, and N), and 89% of ¹³C and 91% of ¹H of the ¹³CH_n side chain moieties. Only the H^N, and N of Arg186 were not obtained.

The secondary structures were predicted based on H^N , N, C', C^α and C^β backbone chemical shifts from each GltJ domain using TALOS+ webserver (Shen et al. 2009). Fig. 2a shows TALOS-derived helix/strand probability for GltJ-ZnR, which consist of four β -strands and two short helical regions. The location of the four cysteines flanks two loops where the zinc ion can be accommodated, which is a characteristic of the zinc ribbon family (Krishna et al. 2003). Secondary structure predictions for GltJ-GYF (Fig. 2b) indicate the presence of five β -strands and two α -helices. The global structural organization is similar to that described in the GYF domains previously characterized in humans (Freund et al. 2002) and plant (PDB ID: 1WH2), confirming the classification of this domain in this protein family despite the absence of the full GYF sequence signature. Comparison to GYF human domain shows the presence of an extended helix and an additional β -strand in the GltJ-GYF C-terminal region (Fig. 2b). These slight differences may be in part due to the shorter length of the human protein construct (Freund et al. 2002). These results suggest that there is a different recognition code for GltJ-GYF towards its ligands within a conserved structural fold. The NMR chemical shifts reported here set the ground for further structural investigations and interaction studies of the GltJ cytoplasmic domains with potential partners within the A-motility machinery, and characterization of their role in FAC assembly.

Acknowledgement

This work was funded by the Centre national de la recherche scientifique (CNRS) and Aix-Marseille University.

References

- Bergé M, Campagne S, Mignolet J, et al (2016) Modularity and determinants of a (bi-)polarization control system from free-living and obligate intracellular bacteria. *eLife* 5:e20640. <https://doi.org/10.7554/eLife.20640>
- Faure LM, Fiche J-B, Espinosa L, et al (2016) The mechanism of force transmission at bacterial focal adhesion complexes. *Nature* 539:530–535. <https://doi.org/10.1038/nature20121>

- Freund C, Kühne R, Yang H, et al (2002) Dynamic interaction of CD2 with the GYF and the SH3 domain of compartmentalized effector molecules. *EMBO J* 21:5985–5995. <https://doi.org/10.1093/emboj/cdf602>
- Gu W, Kofler M, Antes I, et al (2005) Alternative Binding Modes of Proline-Rich Peptides Binding to the GYF Domain †. *Biochemistry* 44:6404–6415. <https://doi.org/10.1021/bi0479914>
- Guttenplan SB, Kearns DB (2013) Regulation of flagellar motility during biofilm formation. *FEMS Microbiol Rev* 37:849–871. <https://doi.org/10.1111/1574-6976.12018>
- Heinze M, Kofler M, Freund C (2007) Investigating the functional role of CD2BP2 in T cells. *International Immunology* 19:1313–1318. <https://doi.org/10.1093/intimm/dxm100>
- Islam ST, Mignot T (2015) The mysterious nature of bacterial surface (gliding) motility: A focal adhesion-based mechanism in *Myxococcus xanthus*. *Seminars in Cell & Developmental Biology* 46:143–154. <https://doi.org/10.1016/j.semcdb.2015.10.033>
- Klug A (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu Rev Biochem* 79:213–231. <https://doi.org/10.1146/annurev-biochem-010909-095056>
- Kofler M, Motzny K, Freund C (2005) GYF Domain Proteomics Reveals Interaction Sites in Known and Novel Target Proteins. *Mol Cell Proteomics* 4:1797–1811. <https://doi.org/10.1074/mcp.M500129-MCP200>
- Kofler MM, Freund C (2006) The GYF domain. *FEBS Journal* 273:245–256. <https://doi.org/10.1111/j.1742-4658.2005.05078.x>
- Kornhaber GJ, Snyder D, Moseley HNB, Montelione GT (2006) Identification of Zinc-ligated Cysteine Residues Based on $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ Chemical Shift Data. *J Biomol NMR* 34:259–269. <https://doi.org/10.1007/s10858-006-0027-5>
- Krishna SS, Majumdar I, Grishin NV (2003) Structural classification of zinc fingers: SURVEY AND SUMMARY. *Nucleic Acids Research* 31:532–550. <https://doi.org/10.1093/nar/gkg161>
- Mignot T, Nöllmann M (2017) New insights into the function of a versatile class of membrane molecular motors from studies of *Myxococcus xanthus* surface (gliding) motility. *Microb Cell* 4:98–100. <https://doi.org/10.15698/mic2017.03.563>
- Mignot T, Shaevitz JW, Hartzell PL, Zusman DR (2007) Evidence that focal adhesion complexes power bacterial gliding motility. *Science* 315:853–856. <https://doi.org/10.1126/science.1137223>
- Porter SL, Wadhams GH, Armitage JP (2011) Signal processing in complex chemotaxis pathways. *Nat Rev Microbiol* 9:153–165. <https://doi.org/10.1038/nrmicro2505>
- Ribet D, Cossart P (2015) How bacterial pathogens colonize their hosts and invade deeper tissues. *Microbes Infect* 17:173–183. <https://doi.org/10.1016/j.micinf.2015.01.004>
- Schumacher D, Søgaaard-Andersen L (2017) Regulation of Cell Polarity in Motility and Cell Division in *Myxococcus xanthus*. *Annu Rev Microbiol* 71:61–78. <https://doi.org/10.1146/annurev-micro-102215-095415>
- Shen Y, Delaglio F, Cornilescu G, Bax A (2009) TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. *J Biomol NMR* 44:213–223. <https://doi.org/10.1007/s10858-009-9333-z>
- Stuelten CH, Parent CA, Montell DJ (2018) Cell motility in cancer invasion and metastasis: insights from simple model organisms. *Nat Rev Cancer* 18:296–312. <https://doi.org/10.1038/nrc.2018.15>
- Vranken WF, Boucher W, Stevens TJ, et al (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins* 59:687–696. <https://doi.org/10.1002/prot.20449>

Figure caption

Fig. 1 a Upper panel, GltJ-ZnR sequence with conserved cysteines chelating the zinc ion highlighted. Lower panel, multiple sequence alignment of GYF domains (GltJ-GYF from *Myxococcus xanthus*, CD2BP2-GYF from *Homo sapiens*, SMY2-GYF from *Saccharomyces cerevisiae*, GYF domain of a hypothetical protein from *Arabidopsis Thaliana*). Residues from the GYF consensus sequence are highlighted in red and boxed. Conserved corresponding residues are colored purple in the GltJ-GYF sequence. Numbering is according to *M. xanthus* GltJ-GYF sequence. **b** 600 MHz ^1H - ^{15}N -HSQC of GltJ-ZnR (left panel, 450 μM), and GltJ-GYF (right panel, 1mM) in 10 mM sodium phosphate (pH 7), supplemented with 5% D_2O at 298K and 303K for GltJ-ZnR and GltJ-GYF, respectively. Assigned residues are indicated by single letter codes and horizontal lines indicate asparagine and glutamine NH_2 side-chain resonances. Resonance assignments are available online at the BMRB repository (accession number for GltJ-ZnR is 51104 and 50096 for GltJ-GYF)

Fig. 2 Secondary structure of **a** GltJ-ZnR and **b** GltJ-GYF as predicted by TALOS+, based on H^{N} , N , C' , C^{α} , and C^{β} chemical shifts. Secondary structure probabilities (red: α -helices; blue: β -strands) are plotted versus residue numbers. At the bottom of each plot, the blue arrows represent the predicted β -strands and red rectangles the helices. Location of characteristic residues are indicated, cysteines for GltJ-ZnR and GYF consensus sequence for GYF domains. For GltJ-GYF the β -strands and α -helices distribution is aligned with the secondary structure topology of GYF domains from *H. sapiens* (PDB 1GYF) (Freund et al. 2002) and *A. Thaliana* (1WH2).