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Unraveling the mechanism of peptidoglycan amidation by the bifunctional enzyme complex GatD/MurT: A comparative structural approach

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ABSTRACT

The bacterial cell wall provides structural integrity to the cell and protects the cell from internal pressure and the external environment. During the course of the twelve-year funding period of the Collaborative Research Center 766, our work has focused on conducting structure-function studies of enzymes that modify (synthesize or cleave) cell wall components of a range of bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Nostoc punctiforme*. Several of our structures represent promising targets for interference. In this review, we highlight a recent structure-function analysis of an enzyme complex that is responsible for the amidation of Lipid II, a peptidoglycan precursor, in *S. aureus*.

1. Introduction

Ever since the discovery of penicillin in 1928 (Fleming, 1929) the bacterial cell wall and its assembly machinery have been a target for antibiotics (Schneider and Sahl, 2010). Now, nearly a century later, many bacteria have developed resistance mechanisms against a wide range of commonly used antimicrobial agents (Nikolaidis et al., 2014). Infections with multi-resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) can often cause severe disease and are extremely hard to treat (Foster, 2004). The situation is particularly critical in hospital environments, where spreading of such pathogens is facilitated by a high availability of susceptible hosts, for instance immunocompromised patients (CDC, 2013; Wang et al., 2017), leading to opportunistic infections. Hence, MRSA and other resistant bacteria are the cause of an increasing number of annual deaths and constitute an immense financial and logistical burden on healthcare worldwide (Chandy et al., 2014). In order to counteract this ever-rising threat, novel strategies to combat resistant bacteria are required.

The major structural component of the bacterial cell wall is the highly crosslinked peptidoglycan heteropolymer (Vollmer et al., 2008). It is composed of polysaccharide chains of alternating beta-1-4 linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) crosslinked by short, branched peptides that vary in composition depending on the species (Schleifer and Kandler, 1972).

Much of our initial work in the Collaborative Research Center 766 has focused on structure-function studies of enzymes that modify the bacterial cell wall. We were able to define the structures and substrate specificities of several autolysins (Büttner et al., 2016, 2014; Lützner et al., 2009; Zoll et al., 2012, 2010), which are needed by bacteria to lyse cell wall structures during cell division (see also review by Büttner et al., 2015). Additional studies have centered on enzymes that glycosylate teichoic acid structures in *staphylococci*, and that represent promising targets for interference (Gerlach et al., 2018; Koç et al., 2015). In recent years, we have become interested in a modification of a peptidoglycan precursor that involves amidation of peptide stem residues.

Peptidoglycan assembly is a multistep anabolic pathway starting with soluble cytosolic precursors and ending with the final heteropolymer network in the periplasmic space. Briefly, the ligases MurC–F (reviewed in Smith, 2006) catalyze the stepwise addition of the peptide stem to the UDP-activated MurNAc moiety. The resulting MurNAc-pentapeptide is then transferred to its membrane anchor undecaprenyl phosphate (bactoprenol) by MraY under UMP release (Chung et al., 2013; van Heijenoort, 2007). This first membrane-bound intermediate is termed Lipid I. Next, the second sugar, GlcNAc, is added by the transglycosylase MurG, yielding Lipid II (van Heijenoort, 2007). In some organisms, the amino acid at position 3 of the peptide stem (L-Lys or meso-diaminopimelic acid (mDAP) in most cases) is further modified by the addition of a short linker peptide, for instance Gly_5 as in *S. aureus* (Schleifer and Kandler, 1972). The final, modified Lipid II is then transferred to the periplasm, where transpeptidase and transglycosylase activities of penicillin binding proteins (PBPs) catalyze the polymerization and crosslinking into the final peptidoglycan layer (Sauvage

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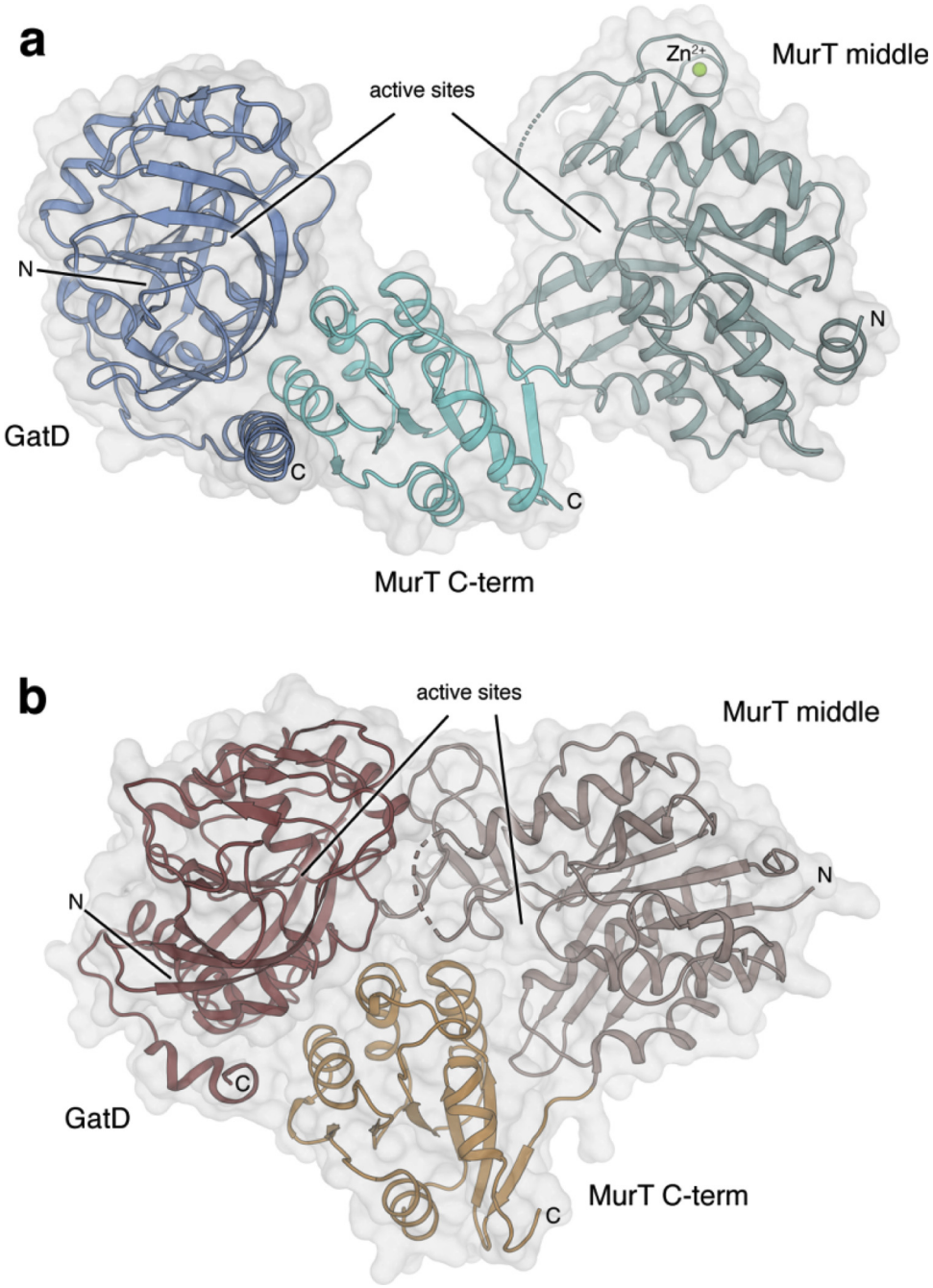

Fig. 1. Ribbon representations of the crystal structures of GatD/MurT. (a) GatD/MurT of *Staphylococcus aureus* displays an open, crescent-shaped conformation that likely represents the state adopted by the enzyme prior to substrate engagement (Nöldeke et al., 2018). (b) The homologous GatD/MurT complex of *Streptococcus pneumoniae* adopts a closed conformation (Morlot et al., 2018).

et al., 2008).

Peptidoglycan of many Gram positive bacteria, including *Staphylococci*, *Streptococci*, and even the distant genus of *Mycobacteria* show a wide variety of modifications in order to enhance its protective efficacy against antimicrobial agents such as lysozyme. The most frequent modifications are sugar moiety N-deacetylation or C_6-O-acetylation (Moynihan et al., 2014; Rajagopal and Walker, 2017), and the amidation of free carboxyl groups in the peptide. The most frequent site of amidation is the α carboxylate of D-glutamate at position 2 in the peptide stem. In peptidoglycan types containing mDAP at position 3, its ε-carboxylate can also be amidated (Schleifer and Kandler, 1972). Additional amidation sites exist, but they are restricted to very particular and rare peptidoglycan variations such as D-aspartate in the peptide crossbridge present in some *Lactococci* (Veiga et al., 2009). All of these different amidation variants appear to contribute to resistance to lysozyme and other autolysins (Figueiredo et al., 2014; Levefaudes et al., 2015; Veiga et al., 2009). While the existence of peptide amidation has been known since the 1960s (Siewert and Strominger, 1968), only a few of the enzymes performing these modifications have been described to date, including LtsA and AsnB1 for mDAP amidation (Bernard et al., 2011; Levefaudes et al., 2015) and AsnH for D-aspartate amidation (Veiga et al., 2009). The most common amidation is carried out by GatD/MurT, which was identified only in 2012 (Figueiredo et al., 2012; Münch et al., 2012). GatD/MurT acts on the membrane-bound peptidoglycan precursor Lipid II and catalyzes the amidation of the second amino acid in the peptide stem from D-glutamic acid to D-isoglutamine.

The ATP-dependent reaction requires glutamine as a nitrogen donor. Interestingly, lack of peptidoglycan amidation as a result of GatD/MurT depletion drastically decreased methicillin tolerance in the context of MRSA (Figueiredo et al., 2014) and even proved lethal in the equally pathogenic *Streptococcus pneumoniae* (Liu et al., 2017; Zapun et al., 2013). These drastic depletion phenotypes have placed GatD/MurT in the spotlight as a potential drug target.

In order to understand the enzymatic mechanism and substrate specificity requirements, and to guide the design of effective inhibitor molecules, high-resolution structural information about the active complex is required. Recently, crystal structures of the GatD/MurT complexes of *Streptococcus pneumoniae* and *Staphylococcus aureus* have been published (Morlot et al., 2018; Nöldeke et al., 2018). This mini-review focuses on the comparison between the two structures and aims to ask specific questions raised by observed similarities or differences in order to help direct future research at them.

2. Structural information on GatD and MurT

Stem peptide amidation by GatD/MurT is known to be crucial for efficient peptidoglycan biosynthesis in a number of Gram positive bacteria, and its essentiality has been inferred for various members of the *Bacilli* family as well as a number of more loosely related *Actinobacteria* (DeJesus et al., 2017; Figueiredo et al., 2012; Münch et al., 2012; Zapun et al., 2013). A homologous operon was found even in the cyanobacterium *Nostoc punctiforme* (Münch et al., 2012). However, mechanistic insights into the enzyme's mode of action are still very limited. Detailed structural information is available only for the complexes from two organisms of the *Bacilli* class, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The structure of isolated, unliganded GatD (Leisico et al., 2018) and two subsequently determined structures of GatD/MurT complexes (Morlot et al., 2018; Nöldeke et al., 2018) have established the fold of the enzymes and identified residues important for ligand binding and catalysis. However, none of the crystal structures contains the full set of substrates (glutamine, ATP, and the peptide stem of Lipid I/II), and the two GatD/MurT complexes also exhibit interesting, profound differences in overall structure and conformation, in the assignment of catalytic residues, and in a small domain that harbors a zinc ion in one case. Therefore, reviewing and comparing the essential structural features of the GatD/MurT complexes provides insights into how they might function in Lipid II amidation.

3. Overall structure of GatD/MurT

In both organisms the two enzymes assemble into a heterodimer, with GatD contacting the C-terminal domain of MurT (Fig. 1). GatD exhibits a mixed alpha/beta hydrolase fold (Ollis et al., 1992) typical for class I glutamine amidotransferases (GATases) (Massière and Badet-Denisot, 1998) and the GatD active site, centered around the catalytic cysteine, faces towards MurT. MurT is composed of two domains, namely a Mur ligase catalytic or middle domain (Mur middle) and a Mur ligase C-terminal domain (Mur C-term). Like in other Mur ligases, MurT contains the canonical ATP binding pocket and catalytic residues required for ATPase activity (reviewed in (Smith, 2006)). Other Mur ligases have been described to use their C-terminal domain to bind part of their respective substrates (Ruane et al., 2013; Smith, 2006), hence it could be inferred that MurT likely employs residues from both domains to coordinate the substrate peptide. However, detailed experimental evidence for the mode of substrate binding is still not available.

While the two available crystal structures of GatD/MurT from *S. aureus* and *S. pneumoniae* adopt largely different conformations overall, the single domains are similar and superimpose very well (Table 1). Hence, a detailed analysis of the similarities and differences between the two structures will help to identify yet undiscovered important features and residues as well as potential organism-specific differences.

Table 1
Root mean square deviations (R.M.S.D.s) of superimpositions of *S. aureus* and *S. pneumoniae* GatD/MurT single domains.

	R.M.S.D. [Å]	aligned residues
GatD	2.14	224
MurT middle	3.21	232
MurT C-term	1.90	128

Such information will paint a more complete picture of the mode of action of this enzyme complex, thus providing a solid foundation for future research aimed at unravelling the reaction mechanism and targeting the active site.

4. Conformational dynamics

The canonical Mur ligases MurC, MurD, MurE, and MurF, which catalyze the formation of the peptidoglycan peptide stem in the cytosolic steps of biosynthesis, have been extensively characterized and crystal structures are available for representatives of all four enzymes (Bertrand et al., 1999; Cha et al., 2014; Mol et al., 2003; Ruane et al., 2013). The middle and C-terminal domains of the Mur ligases are connected *via* a short flexible linker. Mur ligases C, D and F, for which both unliganded and substrate-bound structures are available, display a large degree in domain movement upon ligand binding (reviewed in (Smith, 2006). The strong structural similarities in this region between MurT and the other Mur ligases suggest that such a high degree of mobility would indeed be possible. In support of this hypothesis, the conformations observed in the crystal structures of GatD/MurT from *S. aureus* (*Sa*GatD/MurT) and *S. pneumoniae* (*Sp*GatD/MurT) differ substantially in terms of domain orientation. While *Sp*GatD/MurT adopts a closed conformation similar to that reported for many ligand-bound structures of other Mur ligases, the *S. aureus* structure exhibits a much more open, crescent-shaped conformation that is closer to the apo forms of the aforementioned enzymes. Small angle X-ray scattering experiments performed on *Sa*GatD/MurT show that a similarly open conformation of the enzyme also exists in solution, and indicate that the complex has a considerable amount of flexibility among its domains (Nöldeke et al., 2018).

Based on these observations, it is highly likely that the different conformations adopted by *S. aureus* and *S. pneumoniae* GatD/MurT in their respective crystal structures are not indicative of radical cross-species differences but rather represent two snapshots of a range of possible conformations that can be adopted by both complexes during catalysis. The open conformation observed for *Sa*GatD/MurT likely represents the structure of the complex prior to substrate engagement. As the catalytically active sites of GatD and MurT are separated by about 40 Å in the open conformation, any movement that decreases this distance would seem to be required for catalysis.

Although the evidence available to date clearly points towards a domain rearrangement being a critical step to achieve an active, catalysis-competent complex, the detailed mechanics of catalysis remain elusive. Even the reduced distance between active sites in the closed conformation of around 20 Å is too large to be bridged by nascent ammonia generated in GatD without significant effusive loss. Such a loss would not only hamper MurT catalysis, but if enough ammonia were to escape this could even prove toxic to the cell. Hence, additional domain movements may be required for full and efficient activity.

Is an as of yet undetected ammonia channel formed upon ligand binding to successfully perform the amidation reaction? Does catalysis involve substrate relocation after phosphorylation by MurT to the site of ammonia generation in GatD? High-resolution data on substrate peptide recognition are required in order to answer these questions and to paint a complete picture of GatD/MurT action.

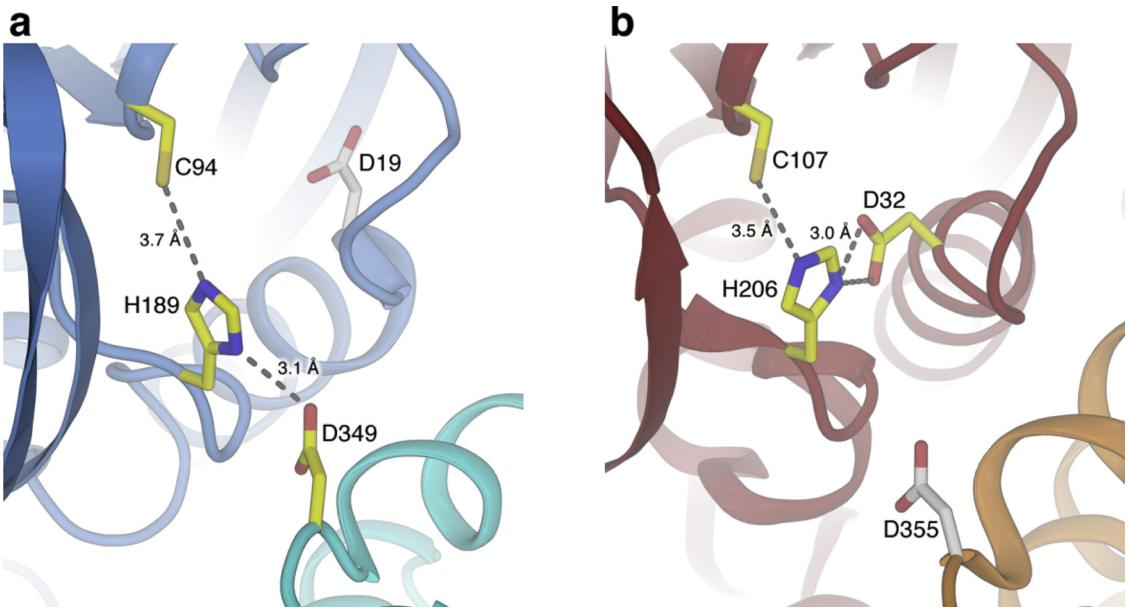

Fig. 2. Comparison of the putative catalytic triads of the GatD/MurT complexes in *S. aureus* and *S. pneumoniae*. (a) Cartoon and stick representation of the intermolecular catalytic triad of *Sa*GatD/MurT. D349 is contributed to the triad by MurT across the dimer interface. (b) The intramolecular triad of *Sp*GatD in the same orientation as in (a). White sticks represent the aspartates homologous to the third triad residue in the respective opposite species. (Morlot et al., 2018; Nöldeke et al., 2018).

5. GatD catalytic triad

Class I or triad type GATases contain a canonical Cys-His-Glu catalytic triad as a means to generate a nucleophile strong enough to initiate glutamine hydrolysis to glutamate (Massière and Badet-Denisot, 1998; Mouilleron and Golinelli-Pimpaneau, 2007). The central nucleophilic cysteine is located at a conserved turn from a β-strand to an α-helix, termed the nucleophile elbow (Ollis et al., 1992). The histidine and glutamate that complete the triad are located on a separate strand, and they are usually separated by a proline. The catalytic triad residues are located nearly in a straight line in order to allow for concerted proton transfer.

While the cysteine and histidine residues are conserved in GatD, the glutamate as the final proton sink is missing. Two alternative aspartic acidic side chains have been proposed to substitute for it in that function, namely *Sa*MurT-D349 (*Sp*MurT-D355) and *Sp*GatD-D32 (*Sa*GatD-D19) (Fig. 2). In the first case, observed in the *S. aureus* enzyme, the aspartic acid is contributed by MurT across the heterodimer interface (Fig. 2a). The intermolecular triad generated in this manner adopts a linear conformation, which would be amenable to concerted proton transfer. In the second case, as found in the structure of *S. pneumoniae* GatD/MurT, the proposed triad is intramolecular, with the aspartic acid being contributed from a flanking helix in GatD (Fig. 2b). While its interatomic distances are smaller than those observed in *Sa*GatD/MurT, the angle of approximately 90° formed by the triad is unusual for such motifs and probably requires slight repositioning of the sidechains involved in order to function in a proton relay reaction.

Intriguingly, both aspartates are conserved in all confirmed and inferred instances of GatD/MurT (Morlot et al., 2018; Nöldeke et al., 2018). However, their relative positions differ largely between the staphylococcal and streptococcal structures. Residue D19 of *Sa*GatD (Fig. 2a) is turned away from the triad site by a twist of helix α2 (secondary structure nomenclature according to (Nöldeke et al., 2018) when compared to *Sp*GatD, thus precluding it from taking the same place and role as *Sp*GatD-D32. On the other hand, *Sp*GatD/MurT cannot form a linear triad with *Sp*MurT-D355, as it is separated from either of the two histidine nitrogens by over 5 Å. It however still plays an important role in *Sp*GatD/MurT function, possibly in complex stability, as its mutation severely impaired activity *in vitro* and even proved lethal *in vivo* (Morlot et al., 2018).

The putative catalytic triads represent a fascinating example of how two different organisms apparently found separate ways to the same end, using exactly the same available tools in terms of conserved residues. It remains to be investigated to which extent the triad conformation may be influenced by the overall conformation of the complex. Thus, a different arrangement of *Sp*GatD/MurT may bring the residues for the intermolecular triad into closer proximity, while a movement in the *S. aureus* complex may lead to a repositioning of *Sa*GatD-D19. Hence, the GatD catalytic triad should remain a focus of research. Structures of the complex from other species may help to fully understand the roles played by the proposed triad residues.

6. The MurT middle domain insertion

One unexpected feature that was revealed by the recent structural studies conducted on the GatD/MurT enzyme complexes from *S. aureus* and *S. pneumoniae* is an insertion of roughly 50 residues between the strands βm6 and βm9 in the middle domain of MurT. This insertion, which to date has not been observed in any other known Mur ligases, bears a strong resemblance to a type of Zinc Finger (ZnF) termed RanBP Zinc ribbon (Gamsjaeger et al., 2007). ZnF motifs exist in various shapes but share the common feature of a Zn^{2+} ion coordinated by four cysteine or histidine sidechains contributed by different portions of the motif, thus conferring stability to the motif (Laity et al., 2001). Most canonical ZnFs are involved in nucleic acid interactions, but several examples of such motifs mediating protein-protein contacts have been reported (Gamsjaeger et al., 2007; Malgieri et al., 2015).

The Zinc ribbon insertion observed in GatD-MurT is well conserved in *Bacilli* (Fig. 3c) and adopts very similar structures in *Sa*MurT and *Sp*MurT. It is composed of two adjacent turns of the protein backbone that contain two cysteines each with one or two linking residues in between. These turns, termed "knuckles", form the beginning and end of a two-stranded antiparallel β-sheet. The cysteines from the knuckles face one another to coordinate a zinc ion that further stabilizes the fold in *Sa*MurT. In the crystal structure of *Sp*MurT, an array of disulfides was modelled instead of the zinc ion. Biochemical analyses however

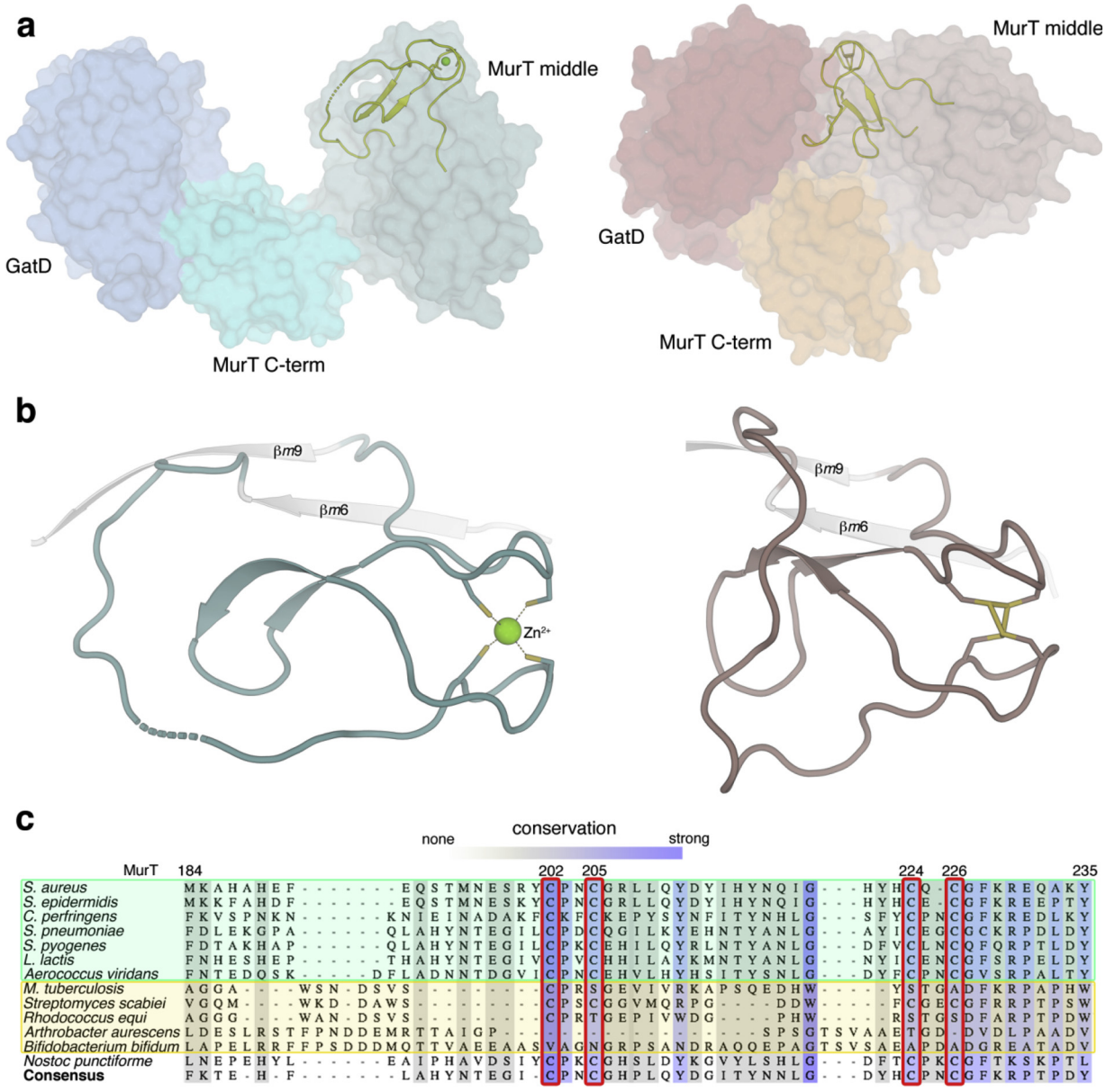

Fig. 3. The MurT insertion: a Zinc Finger. (a) Side by side comparison of the *S. aureus* (left) and *S. pneumoniae* (right) crystal structures (Morlot et al., 2018; Nöldeke et al., 2018). The ZnF is shown as ribbon. (b) Detailed topology of the ZnF highlighting the conserved conformation of the central two-stranded β-sheet and the cys-containing knuckle. The large leading loop adopts a largely different conformation due to the contact with GatD, as seen in (a). (c) Multiple sequence alignment of the insert region of MurT from different species. *Bacilli* (top, green box) share a relatively similar sequence with completely conserved knuckle cysteines (red). In contrast, *Actinobacteria* (yellow box) show a higher interspecies variability in the region.

indicated that the cysteines are initially reduced and the physiological presence of the coordinated metal ion was inferred (Morlot et al., 2018). The segment is located on the side of the MurT middle domain (Fig. 3a), behind the ATP binding site and putative substrate peptide binding region. It forms an extensive contact surface between *Sp*MurT and *Sp*GatD, resulting in a compression of the loop connecting MurT strand βm6 and the first Cys-knuckle (Fig. 3b). In contrast, the same loop is more relaxed and partially flexible in the open form of *Sa*MurT, which has no direct interactions between its middle domain and *Sa*-GatD.

Amino acid sequence comparison of known and putative instances of MurT from different species reveal that conservation of the ZnF is much lower than that of several functionally relevant features. While it probably exists in topologically similar forms across *Bacilli* based on the locations of the cysteines, actinobacterial sequences differ from this pattern (Fig. 3c). *Streptomycetes* (represented here by *S. scabiei*) have a severely shortened insertion while still retaining the characteristic cysteines. However, it cannot be predicted whether this is sufficient to form the characteristic fold. In contrast, most other *Actinobacteria* with GatD/MurT homologues lack the cysteines altogether, and therefore the insertion is unlikely to adopt a zinC–Finger fold.

Together with the lack of detailed knowledge about the precise function of the MurT insertion, this observation raises questions about functional diversity. Is the MurT insertion involved in GatD/MurT activity? If so, have different species evolved different mechanisms to achieve the same goal while relying more or less heavily on a particular sequence, in this case the ZnF, or is catalytic activity completely uncoupled from the nature of the insertion?

RanBP-type Zn ribbons have been associated with protein-protein interactions in the past, such as the complex between Npl4 and Ubiquitin (Alam et al., 2004). The location and conformation of the bacillal ZnF leaves it accessible for an analogous interaction. It is thus

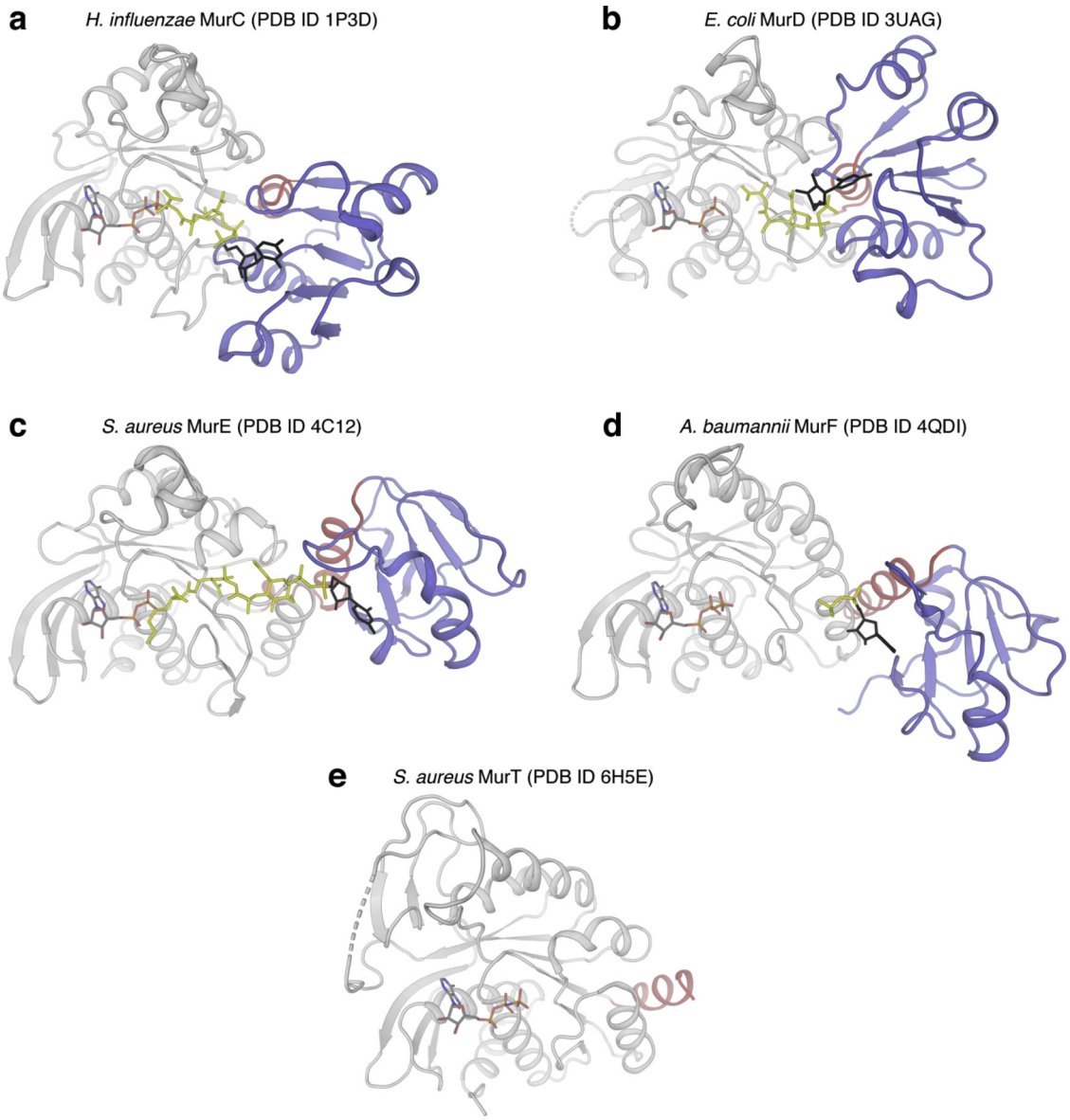

Fig. 4. The N-termini of different Mur ligases. A side by side comparison of the N-terminal and middle domains of MurC-D (Bertrand et al., 1999; Cha et al., 2014; Mol et al., 2003; Ruane et al., 2013) and MurT (Nöldeke et al., 2018) (Panels (a–d) and (e), respectively), aligned for their middle domains, highlight the differences in N-terminus orientation. The N-terminal domain (slate) coordinates the uridine moiety of the substrate (black sticks) thus positioning the substrate peptides of different lengths with respect to the invariable position of ATP in the middle domain (grey cartoon and sticks). The orientation of the N-terminal domain is partially guided by the orientation of a linking helix homologous to MurT helix βm1 (red).

conceivable that the ZnF may be involved in a scaffolding interaction with other components of the membrane-bound phase of peptidoglycan biosynthesis. This could for instance help to bring GatD/MurT into close proximity of its substrate.

7. The short MurT N-terminus

S. aureus GatD/MurT amidates peptidoglycan precursors exclusively in the membrane-bound steps of the biosynthetic pathway (Figueiredo et al., 2012) but shows no preference for either Lipid I, Lipid II or Lipid II - Gly_5 *in vitro* (Münch et al., 2012). The sole difference between the last soluble precursor, UDP-MurNAc-pentapeptide, and Lipid I lies in the exchange of a uridine moiety for a bactoprenol membrane anchor. As the peptide and MurNAc segments are unaltered, the preference of GatD/MurT for this substrate form has to be associated with the membrane anchor. However, neither GatD nor MurT contain exposed hydrophobic segments that could mediate membrane insertion and thus recognition of the isoprenoid tail of the substrate. The N-terminus of MurT, however, has not been resolved in either of the two crystal structures available to date. The unresolved portion spans 30–35 amino acids and contains a high portion of positively charged side chains, totaling 9 and 6 net positive charges for *S. aureus* and *S. pneumoniae*, respectively.

In contrast, the canonical Mur ligases C–F have an additional N-terminal domain of 90–100 amino acids in length. Typically, the N-terminal domains of these Mur ligases adopt a canonical Rossmann fold (Smith, 2006) and coordinate the uridine moiety of the UDP-activated MurNAc-peptide precursor (Fig. 4). Interestingly, the contacts between the Mur middle domain and the growing peptide stem are not particularly extensive. The orientation of the peptide with respect to the unvarying position of ATP in the middle domain is largely controlled by the orientation of the N-terminal domain and, consequently, of the UDP moiety (Smith, 2006). This positioning appears to be mediated at least in part by the orientation of a hinge helix between the N-terminal and

middle domains, homologous to helix βm1 in MurT.

The different nature of the substrate and lack of a comparable N-terminal domain call for a different solution for substrate orientation in MurT. As the orientation of the substrate is not chiefly mediated by the middle and C-terminal domains in other Mur ligases, it can be speculated that MurT may harness external geometrical restraints in order to contact, recognize, and position its substrate peptide for catalysis. The high net positive charge of the unresolved MurT N-terminus suggests such an approach, as it could interact with the negative charges of the phospholipids in the plasma membrane, thus bringing GatD/MurT into the proximity of its substrate. Indeed, Münch et al. showed that *S. aureus* membrane but not cytosolic extracts contain the enzymatic activity required for Lipid II amidation *in vitro* in an ATP and glutamine-dependent fashion, strongly suggesting GatD/MurT to be associated with the plasma membrane (Münch et al., 2012). MurT helix βm1 points in a direction that could orient the enzyme with its active sites facing the membrane. Additionally, cationic terminal stretches have been associated in the past with peripheral membrane association (Whited and Johs, 2015). In such a hypothetical model, spatial sequestration and orientation relative to the membrane rather than direct interactions with the substrate would provide specificity. This hypothesis could provide an explanation for the observed exclusivity in GatD/MurT targeting peptidoglycan precursors at the lipidic stage of biosynthesis.

These hypotheses need to be thoroughly investigated. No experimental evidence is available to date on the role of the MurT N-terminus or the enzyme's subcellular localization.

8. Conclusion and applications

GatD/MurT is highly relevant to worldwide healthcare as a potential drug target to combat infections by multi-resistant bacterial pathogens. By comparing the available GatD/MurT crystal structures of the enzymes of *S. aureus* and *S. pneumoniae,* insights into their mode of action beyond observations on only one protein can be derived. Since the two structures differ in critical ways, the comparison identifies a number of questions that need to be addressed through follow-up studies in order to establish GatD/MurT as a viable platform for the development of structure-guided inhibitors with medical application.

Differences in GatD/MurT across species, such as the MurT middle domain insertion, need to be functionally characterized in order to evaluate their potential as targets for the design of narrow spectrum antimicrobials. Such compounds with a precisely defined target range are more difficult to use due to the necessity to identify the pathogen prior to treatment, but they offer the advantage of a lower impact on beneficial commensal bacteria such as *E. coli*. The observed similar features of the two enzymes, on the other hand, could serve as viable targets for the design of antibiotics with a broader range of action against a wide range of pathogenic bacteria such as *S. aureus*, *S. pneumoniae*, and *M. tuberculosis* while still maintaining its specificity for organisms that express GatD/MurT.

Finally, differences in the crystal structures that cannot be definitively attributed to the difference in species help to paint a detailed picture of the still only rudimentarily understood mechanism of action of GatD/MurT. In this instance, the differences in conformation provide evidence for a major domain rearrangement as an essential step for catalytic activity. Clearly, additional structural and functional studies directed at the mode of substrate engagement by GatD/MurT are needed.

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