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Original Article

Structural Prediction and Domain Analysis of GyrB in *Helicobacter pylori*

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Abstract

DNA gyrase, classified as type II topoisomerase, is critical for regulating bacterial DNA topology and serves as primary targets for fluoroquinolone antibiotics. Resistance to these antibiotics frequently arises from mutations in the gyrase subunits, particularly GyrA and GyrB. This study addresses the structural characterization of the GyrB subunit of *Helicobacter pylori* DNA gyrase, whose tertiary structure remains experimentally uncharacterized. Utilizing homology-based modeling via the Swiss-Model server, we predicted the 3D structure of GyrB, leveraging sequence data from public databases (NCBI, Pfam, UniProt). Conserved functional domains—HATPase_c (ATPase catalytic domain), DNA_gyrase_B, and Toprim—were identified, with sequence alignments revealing high evolutionary conservation across bacterial species. Structural annotation further localized critical residues within these domains, including catalytic sites (e.g., ATP-binding motifs) and regions associated with fluoroquinolone resistance. Notably, mutations proximal to the Toprim domain, implicated in stabilizing the DNA-enzyme complex, were mapped to residues previously linked to quinolone resistance in other pathogens. These predictions provide the first computational structural framework for *H. pylori* GyrB, elucidating potential mechanisms underlying drug resistance. The model underscores the utility of homology-based approaches in bridging structural knowledge gaps for antimicrobial targets. Our findings establish a foundation for targeted mutagenesis studies and structure-guided drug design to combat fluoroquinolone-resistant *H. pylori* strains, emphasizing the need for experimental validation to corroborate these insights.

Keywords: GyrB, Swiss-Model, *Helicobacter pylori*, fluoroquinolone resistance, homology modeling.

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Introduction

DNA gyrase is a critical bacterial enzyme that introduces negative supercoils into DNA, a process essential for replication, transcription, and recombination. It belongs to the type II topoisomerase family and is unique to bacteria, making it an ideal target for antibiotics. DNA gyrase is composed of two GyrA and two GyrB subunits, forming a heterotetramer (A_2B_2), with each subunit playing a distinct role in the DNA cleavage and re-ligation process. The GyrA subunit mediates DNA breakage and rejoining, while GyrB supplies energy through ATP hydrolysis to drive the reaction forward (1,2).

The process of supercoiling involves a multistep mechanism whereby the enzyme cuts a segment of the double-stranded DNA, passes another segment through the break, and then reseals the cut. This complex mechanism is not only essential for maintaining DNA topology but also helps bacteria manage torsional strain during cellular processes (3). Due to their critical role in bacterial survival, DNA gyrases have long been considered promising targets for antibacterial therapy. Fluoroquinolones, which stabilize the DNA-gyrase complex after DNA cleavage, effectively inhibit this enzyme by preventing the religation step (4).

Among the clinically significant bacteria, *Helicobacter pylori* has received special attention due to its role in gastritis, peptic ulcers, and gastric cancer. Treatment of *H. pylori* infections often includes fluoroquinolones, particularly in second-line therapies when first-line treatment fails. However, growing antimicrobial resistance has become a global concern, with resistance to fluoroquinolones in *H. pylori* reported at varying rates across different countries (5). In Iran, resistance rates to ciprofloxacin and levofloxacin have shown a significant increase, suggesting a need for local surveillance and alternative strategies (6, 7).

Resistance to fluoroquinolones in *H. pylori* is commonly due to point mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *gyrB* genes. These mutations alter the target binding site of the antibiotic, thereby reducing drug efficacy. While *gyrA* mutations are more frequently reported, alterations in *gyrB* may also contribute to resistance, particularly in combination with *gyrA* mutations, leading to higher levels of resistance (7). Despite the recognized importance of *gyrB*, it remains less well studied than *gyrA*.

Given the essential role of DNA gyrase and the increasing clinical significance of resistance, detailed molecular analysis of the *gyrB* gene and its protein product in *H. pylori* is warranted. Computational tools enable the prediction of protein structure and function based on gene sequence data, allowing for in silico exploration of resistance mechanisms and potential drug-binding sites. Such studies can aid in the development of new therapeutic targets or inhibitors that circumvent current resistance pathways (8,9).

In this study, we aim to characterize the *gyrB* gene and protein of *H. pylori* through bioinformatics analysis. By predicting its functional domains and tertiary structure, we hope to provide insights into the enzyme's role in fluoroquinolone resistance and highlight potential avenues for novel drug design. Our findings will contribute to a better understanding of antimicrobial resistance at the molecular level and support ongoing efforts to combat persistent *H. pylori* infections.

Methods

Retrieval of Gene and Protein Sequences

The *gyrB* gene sequence of *Helicobacter pylori* ATCC 26695 was retrieved from the NCBI database (GenBank accession number: AE000511).. The corresponding protein sequence of GyrB (UniProt ID: P55992) was downloaded from the UniProt database for further functional and structural analysis (10,11).

Gene structure and genomic location were confirmed using NCBI’s Genome Data Viewer. Genomic context was visualized to identify neighboring genes and potential functional associations.

Sequence Homology and Comparative Genomics

Nucleotide-level similarity searches were performed using BLASTn (NCBI) to identify homologous sequences in other organisms. Hits with over 50% sequence identity were recorded, with a focus on cross-kingdom homologs to explore evolutionary conservation.

Phylogenetic relationships were inferred by aligning *gyrB* homologs from various species using ClustalW. A distance tree was generated using the Neighbor-Joining method embedded in NCBI's BLASTn Distance Tree tool. Phylogenetic clustering patterns were interpreted to evaluate the evolutionary divergence of *H. pylori* GyrB.

Protein Domain and Functional Analysis

Conserved domains in the GyrB protein were identified using Pfam, UniProt, and Prosite databases. Domains such as HATPase-c, Toprim, and Mg^{2+}-binding motifs were annotated based on sequence alignment and domain-specific profiles. Ligand-binding sites and catalytic residues were further confirmed using PDBsum and literature references.

Template Selection for Homology Modeling

Homology modeling was carried out using both manual and automated approaches via the Swiss-Model web server:

Manual Approach: The protein sequence of *H. pylori* GyrB was submitted to Swiss-Model, and DNA topoisomerase IV subunit B from *Streptococcus pneumoniae* (PDB ID: 4JUO, UniProt ID: Q59961) was selected as the modeling template based on domain similarity, functional relevance, and structure quality.

Automated Approach: The sequence was submitted without a predefined template, allowing Swiss-Model's built-in algorithm to identify optimal matches. The same PDB entry (4JUO) was suggested as the top-ranked model, validating the manual selection.

Model Construction and Evaluation

Structural models were generated by Swiss-Model using both manual and automated template inputs. Two main models were analyzed:

Model 1: 48.83% sequence identity, covering residues 18–595.

Model 2: 27.73% sequence identity, covering residues 223–706.

Model 1 was selected for further analysis due to its higher identity and more complete coverage of functional domains. Structural validation was performed using GMQE and QMEAN scores provided by Swiss-Model. Ligand-binding sites (DG35, Mg^{2+}) were identified through structural annotation and mapped using PyMOL.

Functional Residue Identification

Critical amino acid residues involved in ATP binding, Mg^{2+} coordination, and DNA interaction were identified through UniProt, Pfam, PDBsum, and Prosite analyses. These residues were mapped onto the modeled structure to assess their spatial location and potential involvement in enzymatic function or drug interactions.

Results

The *gyrB* gene of *Helicobacter pylori* spans 2322 base pairs and, in accordance with its prokaryotic origin, lacks introns or exons. It is localized at locus HP_0501, flanked by HP_0500 and HP_0502 on the *H. pylori* genome. Sequence analysis identified three functionally important regions within the gene: a magnesium-binding site, an ATP-binding HATPase-c domain, and a Toprim domain implicated in catalytic DNA cleavage. The exact genomic coordinates, retrieved from NCBI (Accession: AE000511), place the gene between nucleotides 530007 and 527686 on the chromosome (12).

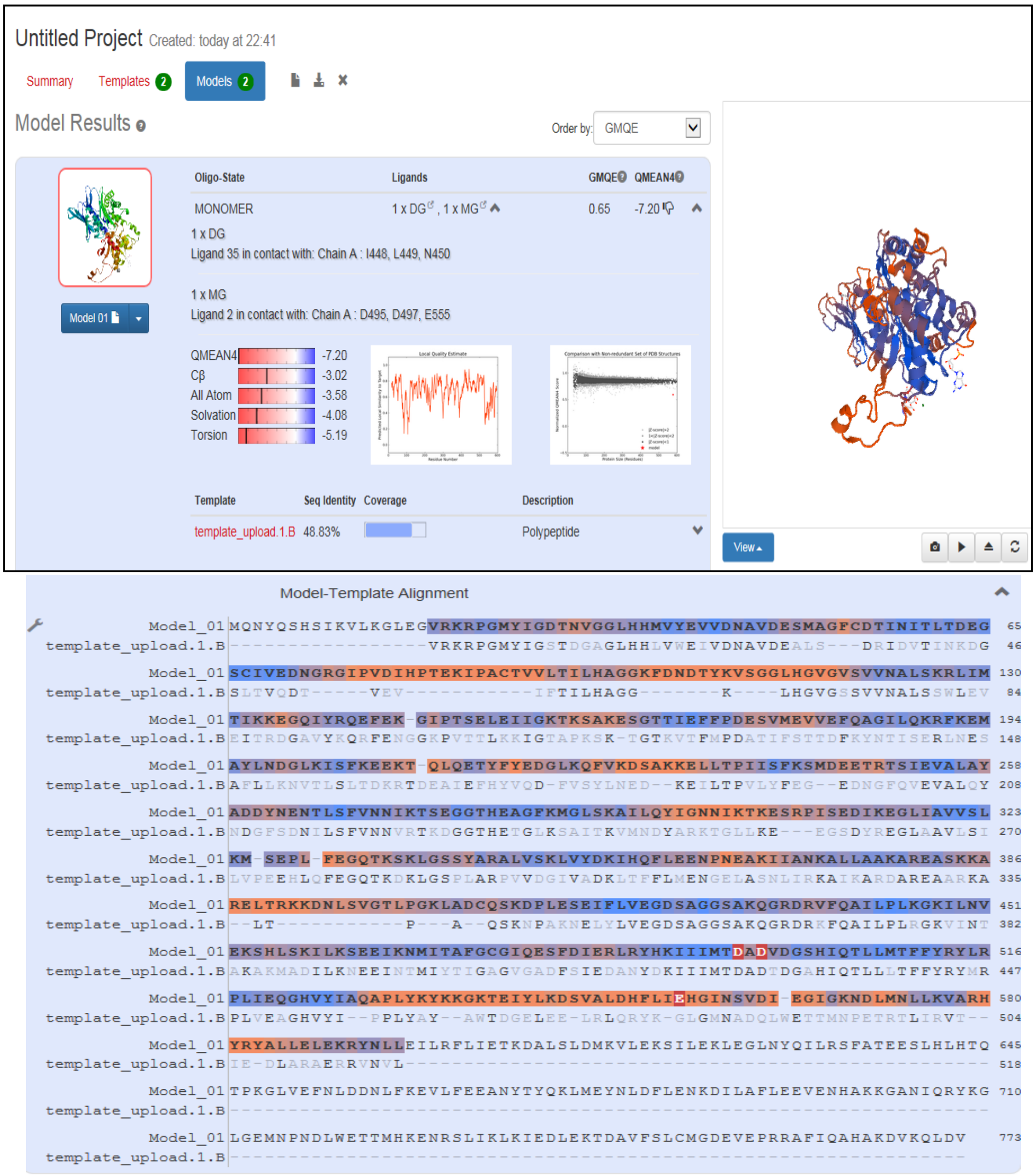
Comparative sequence analysis using BLASTn revealed that *gyrB* shares over 50% identity with homologous sequences in various organisms, including some non-bacterial species. Notably, alignment with *Plasmodium knowlesi* revealed two conserved regions on chromosome 4 with 73% and 83% nucleotide similarity, indicating evolutionary conservation of functional motifs.

At the protein level, the GyrB enzyme of *H. pylori* consists of 773 amino acids with a molecular weight of approximately 87.37 kDa. Domain analysis using UniProt (P55992) identified three conserved domains: HATPase_c, DNA_gyrase_B, and Toprim, which highlight its role in ATP hydrolysis and DNA supercoiling.

Phylogenetic analysis based on sequence alignment showed that *H. pylori* GyrB forms a distinct clade, diverging from its bacterial and eukaryotic homologs. The phylogenetic tree was generated using Distance Tree results in BLASTn and validated with ClustalW, illustrating the unique evolutionary trajectory of this protein.

To investigate the 3D structure of GyrB, homology modeling was conducted using Swiss-Model. BLASTp searches identified DNA topoisomerase IV subunit B from *Streptococcus pneumoniae* (PDB ID: 4JUO; UniProt ID: Q59961) as the most suitable structural template due to its domain similarity, resolution, and structural coverage. Despite the presence of other templates with marginally higher sequence identity, they were excluded due to limited coverage or poor domain overlap.

Two modeling approaches were applied. In the first, a user-defined template (4JUO) was selected, and two structural models were generated. Model 1 (48.83% identity, residues 18–595) was chosen over Model 2 (27.73% identity, residues 223–706) due to superior sequence alignment and domain coverage. The final model preserved all major conserved domains and allowed identification of key ligand-binding regions. Specifically, the DG35 ligand-binding site involved residues Ile448, Leu449, and Asn450, while a magnesium-binding (MG2) site was coordinated by Asp495, Asp497, and Gln555 (Figure 1, 2).



Oligo-State	Ligands	GMQE	QMEAN4
MONOMER	1 x DG, 1 x MG	0.65	-7.20

1 x DG
Ligand 35 in contact with: Chain A : I448, L449, N450

1 x MG
Ligand 2 in contact with: Chain A : D495, D497, E555

Template	Seq Identity	Coverage	Description
template_upload.1.B	48.83%		Polypeptide

```
         Model_01 MQNYQSHSIKVLKGLEGVRKRPGMYIGDTNVGGLHHMVYEVVDNAVDESMAGFCDTINITLTDEG  65
template_upload.1.B -----------------VRKRPGMYIGSTDGAGLHHLVWEIVDNAVDEALS---DRIDVTINKDG  46

         Model_01 SCIVEDNGRGIPVDIHPTEKIPACTVVLTILHAGGKFDNDTYKVSGGLHGVGVSVVNALSKRLIM 130
template_upload.1.B SLTVQDT-----VEV-----------IFTILHAGG-------K----LHGVGSSVVNALSSWLEV  84

         Model_01 TIKKEGQIYRQEFEK-GIPTSELEIIGKTKSAKESGTTIEFFPDESVMEVVEFQAGILQKRFKEM 194
template_upload.1.B EITRDGAVYKQRFENGGKPVTTLKKIGTAPKSK-TGTKVTFMPDATIFSTTDFKYNTISERLNES 148

         Model_01 AYLNDGLKISFKEEKT-QLQETYFYEDGLKQFVKDSAKKELLTPIISFKSMDEETRTSIEVALAY 258
template_upload.1.B AFLLKNVTLSLTDKRTDEAIEFHYVQD-FVSYLNED--KEILTPVLYFEG--EDNGFQVEVALQY 208

         Model_01 ADDYNENTLSFVNNIKTSEGGTHEAGFKMGLSKAILQYIGNNIKTKESRPISEDIKEGLIAVVSL 323
template_upload.1.B NDGFSDNILSFVNNVRTKDGGTHETGLKSAITKVMNDYARKTGLLKE---EGSDYREGLAAVLSI 270

         Model_01 KM-SEPL-FEGQTKSKLGSSYARALVSKLVYDKIHQFLEENPNEAKIIANKALLAAKAREASKKA 386
template_upload.1.B LVPEEHLQFEGQTKDKLGSPLARPVVDGIVADKLTFFLMENGELASNLIRKAIKARDAREAARKA 335

         Model_01 RELTRKKDNLSVGTLPGKLADCQSKDPLESEIFLVEGDSAGGSAKQGRDRVFQAILPLKGKILNV 451
template_upload.1.B --LT-----------P---A--QSKNPAKNELYLVEGDSAGGSAKQGRDRKFQAILPLRGKVINT 382

         Model_01 EKSHLSKILKSEEIKNMITAFGCGIQESFDIERLRYHKIIIMTDADVDGSHIQTLLMTFFYRYLR 516
template_upload.1.B AKAKMADILKNEEINTMIYTIGAGVGADFSIEDANYDKIIIMTDADTDGAHIQTLLLTFFYRYMR 447

         Model_01 PLIEQGHVYIAQAPLYKYKKGKTEIYLKDSVALDHFLIEHGINSVDI-EGIGKNDLMNLLKVARH 580
template_upload.1.B PLVEAGHVYI--PPLYAY--AWTDGELEE-LRLQRYK-GLGMNADQLWETTMNPETRTLIRVT-- 504

         Model_01 YRYALLELEKRYNLLEILRFLIETKDALSLDMKVLEKSILEKLEGLNYQILRSFATEESLHLHTQ 645
template_upload.1.B IE-DLARAERRVNVL-------------------------------------------------- 518

         Model_01 TPKGLVEFNLDDNLFKEVLFEEANYTYQKLMEYNLDFLENKDILAFLEEVENHAKKGANIQRYKG 710
template_upload.1.B -----------------------------------------------------------------

         Model_01 LGEMNPNDLWETTMHKENRSLIKLKIEDLEKTDAVFSLCMGDEVEPRRAFIQAHAKDVKQLDV   773
template_upload.1.B ---------------------------------------------------------------
```

Figure 1: Homology model generated by SWISS-MODEL from a template (up) and Alignment (down).

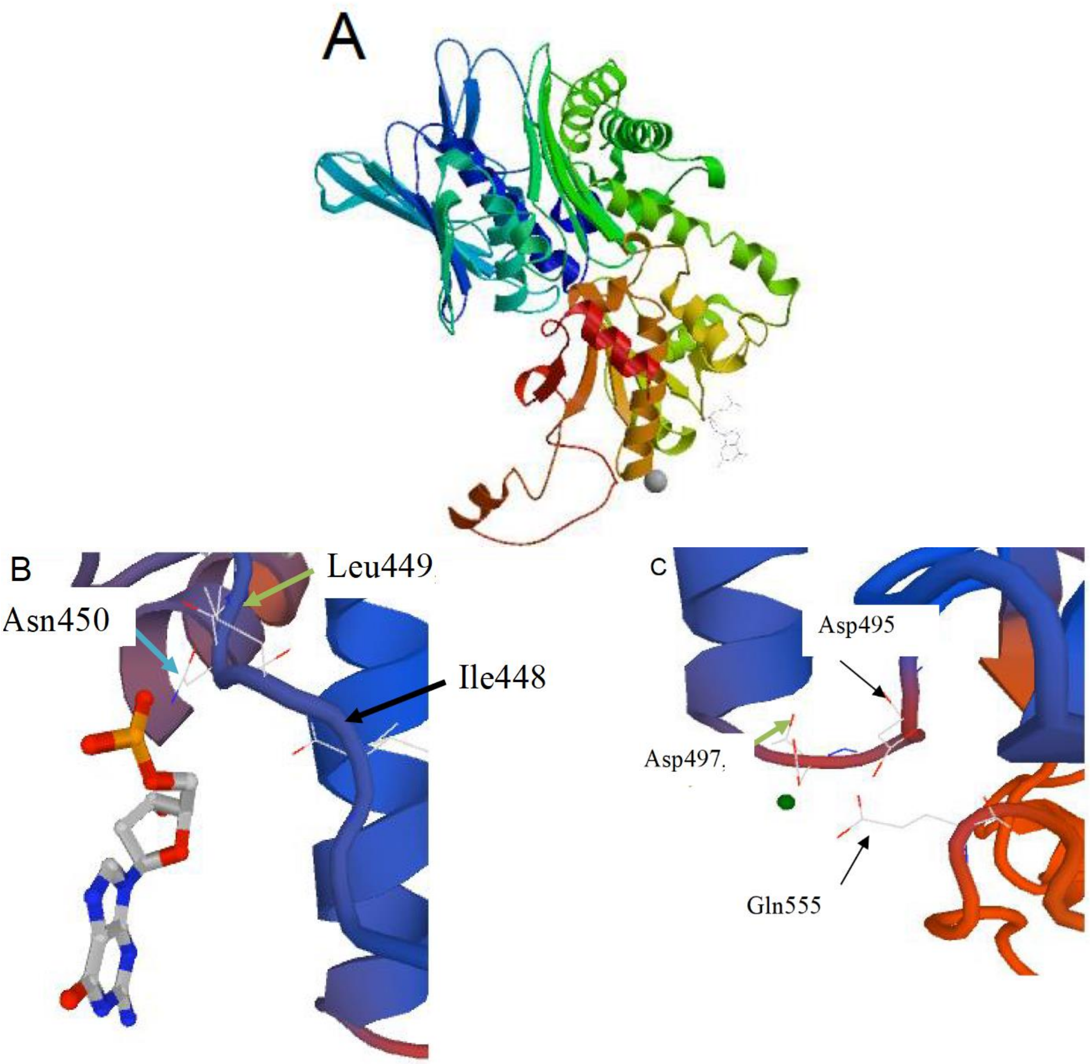


Figure 2. Predicted tertiary structure of *H. pylori* GyrB based on homology modeling using the DNA topoisomerase IV subunit B of *Streptococcus pneumoniae* (PDB ID: 4JUO).The model illustrates the spatial conformation of GyrB, colored from N-terminus (blue) to C-terminus (red) (A). Two ligand-binding sites are identified: Ligand DG35 interacts with residues Ile448, Leu449, and Asn450 (Chain A) (B). Ligand MG2 interacts with Asp495, Asp497, and Glu555 (Chain A) (C). These residues are functionally important for DNA binding and Mg^{2+} coordination. The model supports the presence of conserved domains critical for ATP hydrolysis and DNA cleavage activity.

Homology Modeling Using Swiss-Model (Automated Template Search)

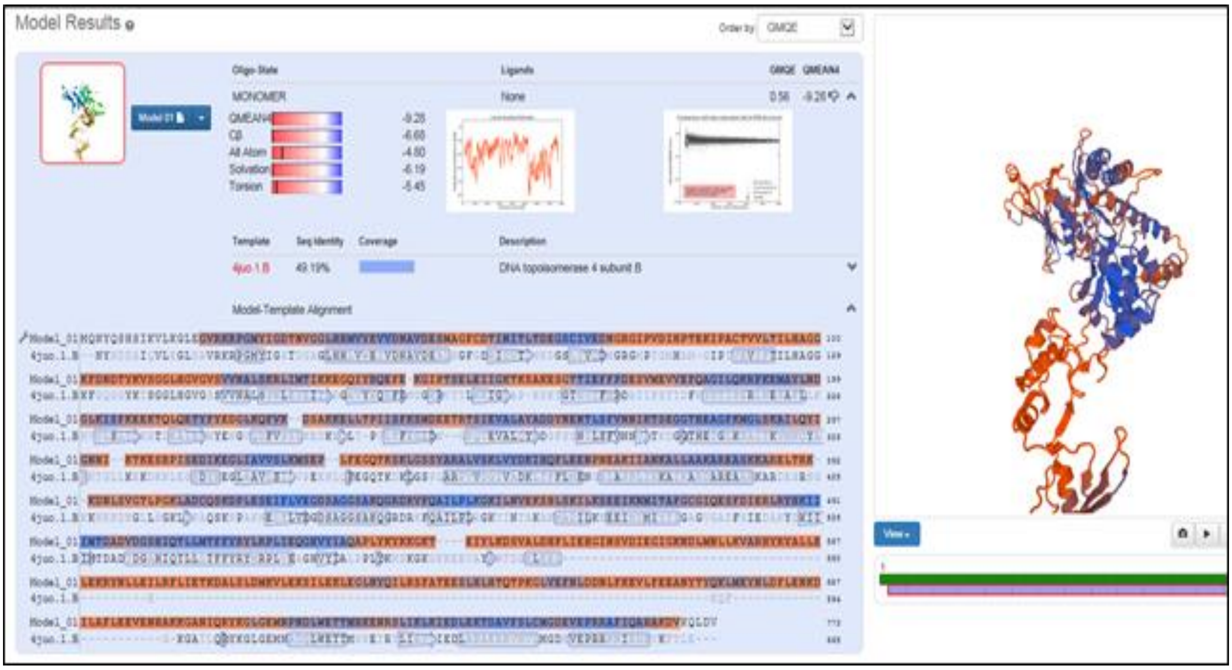
In addition to manual template selection, Swiss-Model's automated template search tool was employed by submitting the *H. pylori* GyrB FASTA sequence without a predefined template. Remarkably, the system's top recommendation was again 4JUO, validating the manual selection (Figures 3). Although the third-ranked model shared structural similarity, it exhibited domain mismatches and was thus excluded.

Functional Domain and Active Site Identification

Functional annotation using UniProt and Pfam databases confirmed the presence of three key domains (Figure 4):

HATPase-c domain, involved in ATP binding and hydrolysis,Toprim domain, which contributes to Mg^{2+}-dependent DNA cleavage and religation, Mg^{2+}-binding site, essential for catalytic activity, particularly involving residues Asp495 and Asp497.

These functional domains are structurally conserved among homologs and are vital for the DNA strand-passage mechanism characteristic of DNA gyrases.



Model Results

Order by: GMQE

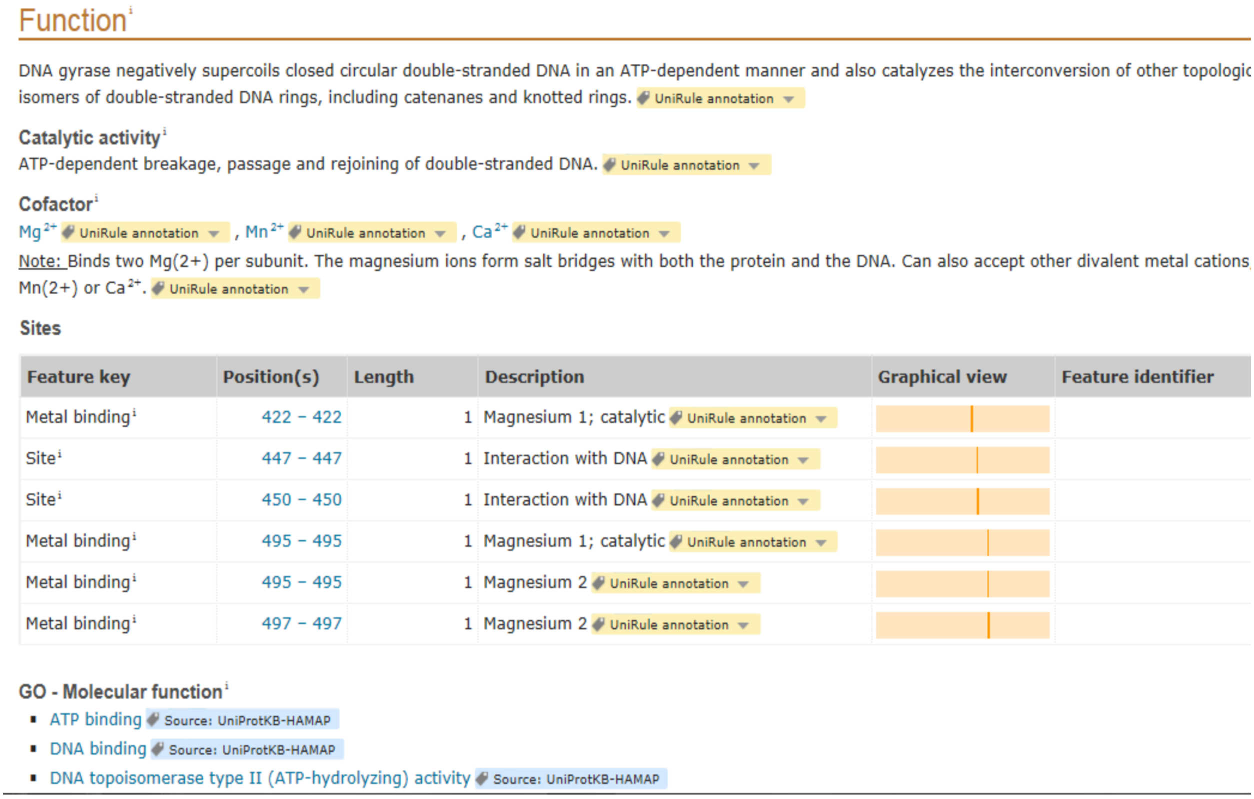
Oligo State	Ligands	GMQE	QMEAN4
MONOMER	None	0.56	-9.26

QMEAN4 -9.26
Cβ -6.68
All Atom -4.80
Solvation -6.19
Torsion -5.45

Template	Seq Identity	Coverage	Description
4juo.1.B	49.19%		DNA topoisomerase 4 subunit B

Model-Template Alignment

Figure 3. Amino acid alignments between gyrase B and 4juo by swiss-model for the proposed spatial structure model of *Helicobacter pylori* gyrase B.



Function

DNA gyrase negatively supercoils closed circular double-stranded DNA in an ATP-dependent manner and also catalyzes the interconversion of other topologic isomers of double-stranded DNA rings, including catenanes and knotted rings. UniRule annotation

Catalytic activity

ATP-dependent breakage, passage and rejoining of double-stranded DNA. UniRule annotation

Cofactor

Mg^{2+} UniRule annotation , Mn^{2+} UniRule annotation , Ca^{2+} UniRule annotation

Note: Binds two Mg(2+) per subunit. The magnesium ions form salt bridges with both the protein and the DNA. Can also accept other divalent metal cations Mn(2+) or Ca^{2+}. UniRule annotation

Sites

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Metal binding	422 – 422	1	Magnesium 1; catalytic UniRule annotation		
Site	447 – 447	1	Interaction with DNA UniRule annotation		
Site	450 – 450	1	Interaction with DNA UniRule annotation		
Metal binding	495 – 495	1	Magnesium 1; catalytic UniRule annotation		
Metal binding	495 – 495	1	Magnesium 2 UniRule annotation		
Metal binding	497 – 497	1	Magnesium 2 UniRule annotation		

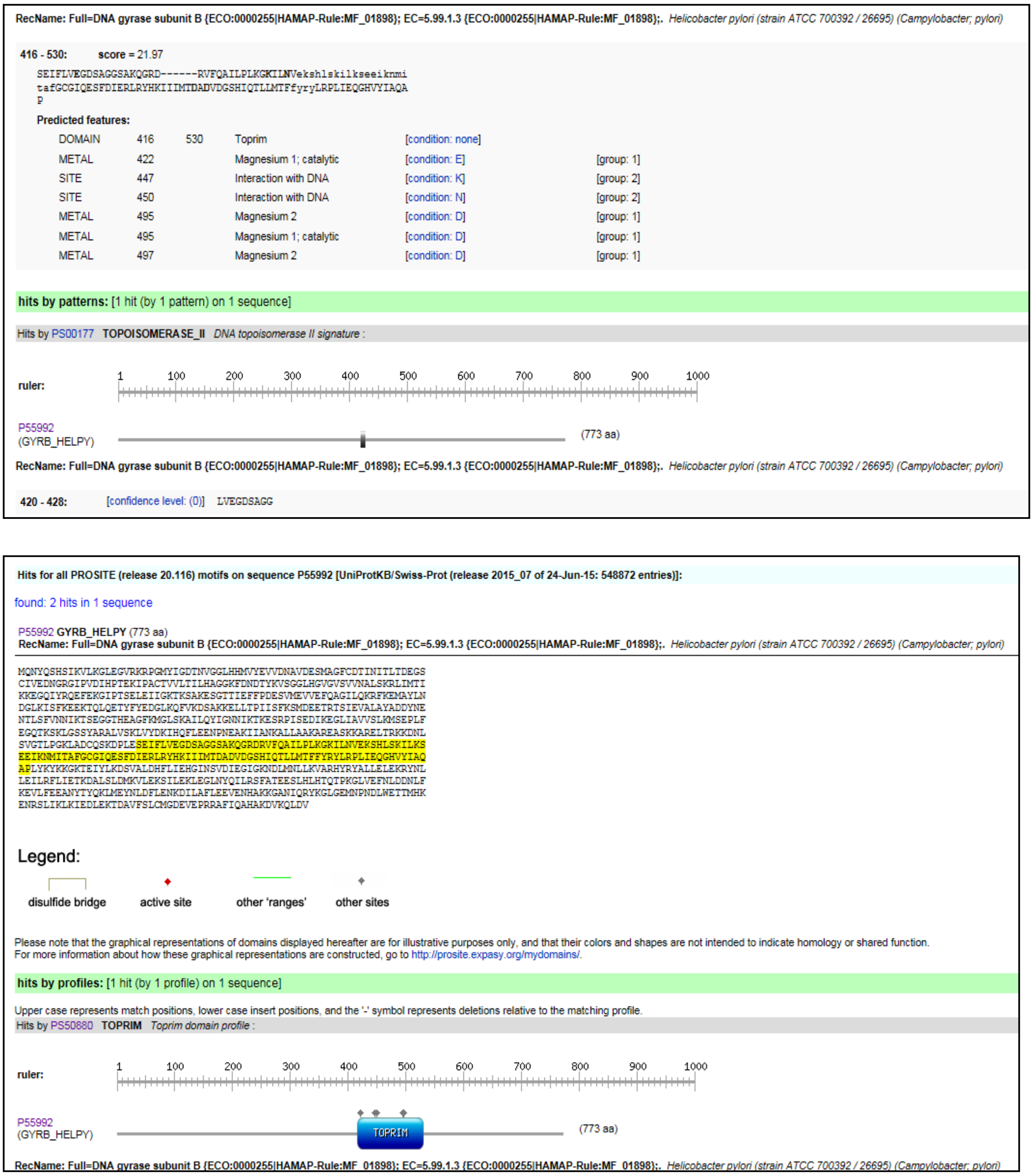
GO - Molecular function

- ATP binding Source: UniProtKB-HAMAP
- DNA binding Source: UniProtKB-HAMAP
- DNA topoisomerase type II (ATP-hydrolyzing) activity Source: UniProtKB-HAMAP

Figure 4: Functional regions of *Helicobacter pylori* gyrase B in UniProt.

Functional Domain Mapping with Pfam and Prosite

Domain mapping using the Pfam database revealed that the Toprim domain spans residues 417–529, a region confirmed through Prosite analysis (Figures 5A, B). This segment likely plays a pivotal role in the structural transition from secondary to tertiary conformations, particularly near the enzyme's active site.



RecName: Full=DNA gyrase subunit B {ECO:0000255|HAMAP-Rule:MF_01898}; EC=5.99.1.3 {ECO:0000255|HAMAP-Rule:MF_01898};. *Helicobacter pylori (strain ATCC 700392 / 26695) (Campylobacter; pylori)*

416 - 530: **score** = 21.97

```
SEIFLVEGDSAGGSAKQGRD------RVFQAILPLKGKILNVekshlskilkseeiknmi
tafGCGIQESFDIERLRYHKIIIMTDADVDGSHIQTLLMTFfyryLRPLIEQGHVYIAQA
P
```

Predicted features:

```
DOMAIN   416   530   Toprim                    [condition: none]
METAL    422         Magnesium 1; catalytic    [condition: E]      [group: 1]
SITE     447         Interaction with DNA      [condition: K]      [group: 2]
SITE     450         Interaction with DNA      [condition: N]      [group: 2]
METAL    495         Magnesium 2               [condition: D]      [group: 1]
METAL    495         Magnesium 1; catalytic    [condition: D]      [group: 1]
METAL    497         Magnesium 2               [condition: D]      [group: 1]
```

hits by patterns: [1 hit (by 1 pattern) on 1 sequence]

Hits by PS00177 **TOPOISOMERASE_II** *DNA topoisomerase II signature* :

P55992
(GYRB_HELPY) (773 aa)

RecName: Full=DNA gyrase subunit B {ECO:0000255|HAMAP-Rule:MF_01898}; EC=5.99.1.3 {ECO:0000255|HAMAP-Rule:MF_01898};. *Helicobacter pylori (strain ATCC 700392 / 26695) (Campylobacter; pylori)*

420 - 428: [confidence level: (0)] LVEGDSAGG

Hits for all PROSITE (release 20.116) motifs on sequence P55992 [UniProtKB/Swiss-Prot (release 2015_07 of 24-Jun-15: 548872 entries)]:

found: 2 hits in 1 sequence

P55992 **GYRB_HELPY** (773 aa)
RecName: Full=DNA gyrase subunit B {ECO:0000255|HAMAP-Rule:MF_01898}; EC=5.99.1.3 {ECO:0000255|HAMAP-Rule:MF_01898};. *Helicobacter pylori (strain ATCC 700392 / 26695) (Campylobacter; pylori)*

```
MQNYQSHSIKVLKGLEGVRKRPGMYIGDTNVGGLHHMVYEVVDNAVDESMAGFCDTINITLTDEGS
CIVEDNGRGIPVDIHPTEKIPACTVVLTILHAGGKFDNDTYKVSGGLHGVGVSVVNALSKRLIMTI
KKEGQIYRQEFEKGIPTSELEIIGKTKSAKESGTTIEFFPDESVMEVVEFQAGILQKRFKEMAYLN
DGLKISFKEEKTQLQETYFYEDGLKQFVKDSAKKELLTPIISFKSMDEETRTSIEVALAYADDYNE
NTLSFVNNIKTSEGGTHEAGFKMGLSKAILQYIGNNIKTKESRPISEDIKEGLIAVVSLKMSEPLF
EGQTKSKLGSSYARALVSKLVYDKIHQFLEENPNEAKIIANKALLAAKAREASKKARELTRKKDNL
SVGTLPGKLADCQSKDPLESEIFLVEGDSAGGSAKQGRDRVFQAILPLKGKILNVEKSHLSKILKS
EEIKNMITAFGCGIQESFDIERLRYHKIIIMTDADVDGSHIQTLLMTFFYRYLRPLIEQGHVYIAQ
APLYKYKKGKTEIYLKDSVALDHFLIEHGINSVDIEGIGKNDLMNLLKVARHYRYALLELEKRYNL
LEILRFLIETKDALSLDMKVLEKSILEKLEGLNYQILRSFATEESLHLHTQTPKGLVEFNLDDNLF
KEVLFEEANYTYQKLMEYNLDFLENKDILAFLEEVENHAKKGANIQRYKGLGEMNPNDLWETTMHK
ENRSLIKLKIEDLEKTDAVFSLCMGDEVEPRRAFIQAHAKDVKQLDV
```

Legend:

Please note that the graphical representations of domains displayed hereafter are for illustrative purposes only, and that their colors and shapes are not intended to indicate homology or shared function.
For more information about how these graphical representations are constructed, go to http://prosite.expasy.org/mydomains/.

hits by profiles: [1 hit (by 1 profile) on 1 sequence]

Upper case represents match positions, lower case insert positions, and the '-' symbol represents deletions relative to the matching profile.
Hits by PS50880 **TOPRIM** *Toprim domain profile* :

P55992
(GYRB_HELPY) (773 aa)

RecName: Full=DNA gyrase subunit B {ECO:0000255|HAMAP-Rule:MF_01898}; EC=5.99.1.3 {ECO:0000255|HAMAP-Rule:MF_01898};. *Helicobacter pylori (strain ATCC 700392 / 26695) (Campylobacter; pylori)*

Figure 5. Prediction of Toprim area in the Prosite database.

Critical Residues in Functional Regions

Integration of data from UniProt, PDBsum, Pfam, and Prosite identified several critical amino acid residues within functional domains and ligand-binding regions:

Toprim / Mg^{2+} binding site: Asp495, Asp497.
ATP-binding / HATPase-c domain: Glu422, Lys447.
Ligand/DNA-binding residues: Asn450, Ile448, Leu449, Glu555.

These residues are integral to enzymatic catalysis, nucleotide binding, and substrate interaction. Their conservation and spatial arrangement further confirm the structural and functional validity of the modeled GyrB structure.

Together, these modeling efforts provide a robust and functionally annotated structure of *H. pylori* GyrB. The structural insights form a strong foundation for downstream applications such as docking studies, inhibitor design, and investigation into antibiotic resistance mechanisms.

Discussion

This study employed a comprehensive bioinformatics approach integrating genomic analysis, phylogenetics, and homology modeling to investigate the structure and function of the GyrB subunit of DNA gyrase in *Helicobacter pylori*. As a crucial enzyme responsible for maintaining DNA topology through ATP-dependent supercoiling and relaxation, GyrB is not only essential for bacterial survival but also a validated target of fluoroquinolone antibiotics. Understanding its molecular architecture provides critical insight into mechanisms of drug resistance and informs therapeutic design.

The gene structure analysis confirmed the prokaryotic nature of *gyrB*, with no introns or exons, and identified three functionally essential domains—HATPase-c, Toprim, and a magnesium-binding site. These regions are integral to the enzyme’s ATP hydrolysis and DNA strand cleavage-religation functions. The preservation of these domains across bacterial species suggests strong evolutionary conservation driven by functional necessity.

Comparative sequence analysis revealed moderate identity with genes from unrelated species, such as *Plasmodium knowlesi*, suggesting that conserved catalytic motifs persist even across distant taxa. However, the limited sequence identity (~73–83%) and alignment confined to short regions reflect evolutionary divergence likely driven by host-specific adaptations or structural constraints unique to *H. pylori*.

One of the central achievements of this work was the successful prediction of the GyrB 3D structure using homology modeling. The DNA topoisomerase IV subunit B from *Streptococcus pneumoniae* (PDB ID: 4JUO) emerged as the most suitable template, based on both manual curation and Swiss-Model’s automated template selection. Despite the presence of templates with slightly higher sequence identity, 4JUO offered superior domain overlap and functional congruence. This dual validation reinforces the robustness of the structural model.

Among the generated models, the one with 48.83% identity and coverage of residues 18–595 was chosen for downstream analysis, as it captured all the key enzymatic domains. This model also revealed conserved ligand-binding sites, including those for magnesium ions (Asp495, Asp497) and potential small-molecule inhibitors (DG35-binding site), which are crucial for catalytic activity and drug targeting.

Importantly, the modeling revealed critical residues involved in ATP binding (E422, K447), DNA interaction (N450, I448, L449), and Mg^{2+} coordination (D495, D497). These residues, located within or adjacent to conserved domains, represent potential hotspots for functional disruption through mutation—an insight particularly relevant in the context of fluoroquinolone resistance. Substitutions at these positions may interfere with drug binding or enzymatic efficiency, potentially conferring resistance or reducing susceptibility to antibiotics.

The repeated identification of 4JUO as the optimal template by both manual and automated approaches further validates its use and highlights the reliability of the modeled structure. Minor structural variations observed in alternative models underscore the precision required in homology modeling, particularly when targeting conserved functional domains for inhibitor design.

In summary, this study provides a detailed structural and functional map of the *H. pylori* GyrB protein, offering a strong foundation for future experimental investigations. The model supports structure-based drug discovery efforts, including: Molecular docking studies with fluoroquinolones and novel inhibitors, Mutagenesis experiments to probe resistance mechanisms, virtual screening and repurposing of therapeutic compounds.

In conclusion, this work contributes to a deeper understanding of DNA gyrase function in *H. pylori* and facilitates the rational development of targeted therapies against antibiotic-resistant gastric infections.

Acknowledgement

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