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Harnessing Computational Tools for Next-Generation Antibacterial Therapies

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Introduction

Antibiotic-resistant infections have risen to become a critical global health threat, with the World Health Organization warning that the world is "running out of antibiotics".[i] Bacteria are evolving mechanisms to evade existing drugs at an alarming pace, risking a future where routine infections become potentially untreatable.[ii] In response, over the past two decades researchers have increasingly turned to computer-aided drug design (CADD) to accelerate the discovery of new antibacterial agents. CADD encompasses a suite of in silico techniques that can identify, design, and optimize drug candidates with higher efficiency than traditional trial-and-error approaches. By leveraging growing structural biology data and computational power, CADD has aided the development of diverse classes of antibacterials – from classic small-molecule enzyme inhibitors to peptide-based therapeutics and other novel modalities. In this review, we provide a comprehensive overview of the role of CADD in antibacterial drug discovery over the past 20 years. We discuss major CADD methodologies (structure-based and ligand-based design, molecular docking and virtual screening, pharmacophore modeling, molecular dynamics simulations, and machine learning-assisted approaches) and highlight how these tools have been applied to identify promising antibacterial candidates. Key successes from the literature are summarized, focusing on high-impact studies in medicinal chemistry, pharmacology, and computational biology. A dedicated section reviews CADD efforts against DNA gyrase, an essential bacterial enzyme and longstanding antibiotic target,

detailing the computational strategies, compound discoveries, docking results, and experimental validations in those studies. We further emphasize the evolution of molecular docking in antibacterial discovery – including improvements in scoring functions, benchmarking of docking methods, and integration with dynamics and AI techniques – as a case study in the progress of CADD. Challenges and limitations that have emerged (such as accurately predicting permeability or avoiding false positives) are examined, and future directions are proposed, including the integration of deep learning and novel in silico–in vitro workflows to meet the urgent need for new antibacterials.

CADD Techniques in Antibacterial Discovery

Modern antibacterial discovery employs a spectrum of CADD techniques, each offering distinct advantages in identifying or optimizing drug leads. **Structure-based drug design (SBDD)** starts from a known 3D structure of a bacterial target (often determined by X-ray crystallography or cryo-EM) and uses that insight to design inhibitors that fit the binding pocket. SBDD has been empowered by the increasing availability of high-resolution bacterial protein structures, enabling rational design cycles for many antimicrobial targets.[iii] In parallel, **ligand-based drug design (LBDD)** leverages the knowledge of known active molecules to derive pharmacophore models or quantitative structure–activity relationships (QSAR) that can guide the discovery of analogues. For example, pharmacophore modeling of fluoroquinolone antibiotics (a class targeting DNA gyrase) identified key chemical features required for activity, which guided virtual screening for novel gyrase inhibitors.[iv] Techniques like QSAR and machine learning on molecular descriptors have also been widely used to predict antimicrobial potency from structure alone, facilitating *in silico* prioritization of large libraries of compounds.[v]

Among SBDD tools, **molecular docking** is a cornerstone method that computationally "fits" candidate ligands into the 3D structure of a target binding site and scores their complementarity. Docking-based virtual screening has been extensively applied to antibacterial targets, from enzymes to receptors, to rapidly triage chemical libraries and propose new inhibitors.[vi] Over the past 20 years, docking algorithms have improved in handling receptor flexibility and in scoring functions, yielding higher hit rates in prospective antibiotic discovery. Advancements such as ensemble docking (using multiple protein conformations), induced-fit docking protocols, and consensus scoring have helped address challenges like induced-fit effects and high false-positive rates that early docking campaigns faced. Importantly, integration of **molecular dynamics (MD) simulations** with docking has enhanced SBDD by accounting for target flexibility and refining docked poses. MD simulations can relax protein–ligand complexes in explicit solvent, revealing stable binding modes and allowing calculation of binding free energies (e.g. via MM/PBSA) to re- rank docking hits.[vii] This has been particularly useful for bacterial targets with flexible or transient pockets. For instance, MD studies on *E. coli* LpxC (an essential enzyme in lipid A biosynthesis) explained species-dependent differences in inhibitor

binding and guided the design of broad- spectrum inhibitors that accommodate active-site flexibility.[viii]

Another powerful approach is **pharmacophore modeling**, which abstracts the common 3D arrangement of key binding features (hydrogen bond acceptors/donors, hydrophobic centers, etc.) from a set of active molecules. Pharmacophore models have been used to search compound databases for new antibacterial scaffolds that satisfy the required geometric arrangement of pharmacophoric features. In one study, a pharmacophore hypothesis with three H-bond acceptors and one hydrophobic moiety was developed from potent gyrase inhibitors and validated against the gyrase binding site; this model then led to the identification and synthesis of novel inhibitors active against *Mycobacterium tuberculosis*.[ix] Such ligand-based virtual screening is valuable when the target structure is unknown or to complement SBDD by suggesting chemically diverse hits.

Recently, **machine learning (ML) and AI** methods have brought transformative potential to CADD for antibacterials. Traditional ML techniques (Random Forest, SVM, neural networks) have long been used for QSAR modeling of antibacterial activity,[x] as well as for predicting peptide antibiotic properties like membrane permeability or toxicity.[xi] In the last few years, deeper learning approaches have been applied to mine large chemical libraries for novel antibiotics without pre- defined pharmacophores. Notably, a 2020 study trained a deep neural network on thousands of molecules with known antimicrobial activity to predict *de novo* antibiotic candidates.[xii] This approach led to the discovery of **halicin**, a drug repurposed from a pharmacological library, which showed potent broad-spectrum killing of pathogens including *Clostridioides difficile* and *Mycobacterium tuberculosis*.[xiii] ML models can also optimize multi-parameter objectives, searching for compounds that not only inhibit bacteria but also have desired ADMET properties. Generative models and evolutionary algorithms are being explored to design novel antimicrobial peptides (AMPs) from scratch, by learning the sequence patterns that confer high activity and low toxicity.[xiv] Overall, the CADD toolbox for antibiotic discovery is rich and continually evolving -combining physics-based simulations with data-driven AI predictions – thereby opening new frontiers for discovering small-molecule antibiotics, peptide-based drugs, and beyond.

Major Discoveries Enabled by CADD

CADD methodologies have contributed to numerous success stories in antibacterial discovery, uncovering new inhibitors across essentially all major classes of antibacterial agents. **Small- molecule antibiotics** – which historically have been the backbone of antimicrobial therapy – have seen significant advances through structure-based virtual screening and design. For example, a landmark computational screen against *M. tuberculosis* enoyl-ACP reductase (InhA) identified novel inhibitors that bypass the resistance mechanism of isoniazid (a frontline TB drug). Perryman *et al.* docked ~ <“ million fragment-like compounds into InhA’s binding site and discovered several low-micromolar hits that inhibited InhA without requiring metabolic activation by KatG (unlike isoniazid).[xv]

The two most potent hits had values around 54-59 ìM and represented new chemotypes, providing starting points for hit-to-lead optimization against drug-resistant TB.[xvi] This study demonstrated the power of massive distributed docking (using a volunteer computing project) to find "needle-in-haystack" inhibitors for a key bacterial enzyme. Similarly, structure- based virtual screening has yielded inhibitors for notoriously challenging Gram-negative targets. Spyrakis *et al.* performed parallel docking screens against multiple â-lactamases – including both serine-â-lactamases and metallo-â-lactamases – to discover broad-spectrum enzyme inhibitors that could restore â-lactam efficacy.[xvii] Their work identified several hit scaffolds (e.g. sulfonamides for class A KPC-2, thiols for NDM-1 MBL) with micromolar activities, and one compound (#40) was shown to potentiate imipenem in a carbapenem-resistant *E. coli* by inhibiting NDM-1.[xviii] Crystal structures of two hits bound to NDM-1 and VIM-2 were solved, corroborating the docking poses and guiding subsequent analog design.[xix] This multi-target virtual screening approach highlights how CADD can efficiently tackle the diversity of resistance enzymes, yielding inhibitor leads that work across different classes of â-lactamases.

Structure-based design has also led to **entirely new classes of small-molecule antibacterials**. One prominent example is the development of **novel bacterial topoisomerase inhibitors (NBTIs)** that target DNA gyrase and topoisomerase IV in a mode distinct from fluoroquinolone antibiotics.[xx] By 2010, GlaxoSmithKline researchers had solved the first co-crystal structure of a potent NBTI (GSK299423) bound to *Staphylococcus aureus* DNA gyrase in complex with DNA.[xxi] This revealed a unique binding mode in which the NBTI molecule "bridges" the DNA and a transient pocket at the GyrA dimer interface, adjacent to but not overlapping the fluoroquinolone-binding site.[xxii] Importantly, the NBTI made contacts with regions of gyrase such that common fluoroquinolone-resistance mutations in GyrA did not affect NBTI binding.[xxiii] Structure-guided SAR explorations around GSK299423's scaffold – dissecting its *left-hand side* (DNA-intercalating moiety), *right-hand side* (enzyme-binding group), and central linker (Figure 1B) – produced analogues with low-nanomolar potency and broad-spectrum activity.[xxiv] One of these NBTIs (gepotidacin, a triazaacenaphthylene derivative) advanced to clinical trials for urinary tract infections, validating the approach. The NBTI example illustrates how CADD (in this case crystallography-driven design) enabled a "structure-based leap" to a new antibacterial class that circumvents existing resistance.

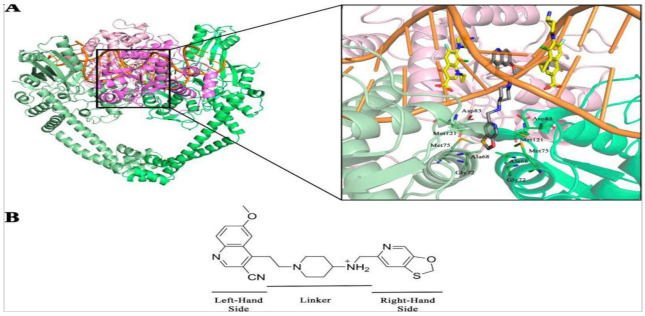

Figure 1. (A) Comparison of binding modes of a novel gyrase inhibitor (gray, NBTI GSK299423)[xxv] and a fluoroquinolone (yellow, clinafloxacin) in the DNA gyrase cleavage complex.[xxvi] The NBTI intercalates into DNA and extends into a unique pocket at the GyrA–GyrA interface, distinct from the fluoroquinolone site. (B) Chemical structure of GSK299423 as a representative NBTI, illustrating its three key components: the DNA-intercalating left-hand side, the enzyme-binding right-hand side, and the central linker connecting them. This structure-based design scaffold inspired analogs that retain activity against fluoroquinolone-resistant gyrase.

Beyond enzyme inhibitors, CADD has also facilitated discovery in less traditional small-molecule modalities, such as **anti-virulence agents** and **membrane-targeting compounds**. As an example of the former, virtual screening against the *Pseudomonas aeruginosa* quorum-sensing receptor LasR identified novel antagonists that block bacterial communication and biofilm formation (though such studies are in early stages and thus not detailed here). For membrane-targeting agents, a recent *de novo* design approach led to a new **xanthone derivative** antibiotic with dual mechanisms: disrupting bacterial membranes and inhibiting DNA synthesis. Li *et al.* (2025) synthesized a series of amphiphilic xanthone derivatives and, with the aid of docking simulations, found that the most potent compound, *XT17*, could both lyse bacterial cell envelopes and bind DNA gyrase in silico similarly to ciprofloxacin.[xxvii] This compound showed sub-2 ìg/mL MICs against *E. coli* and *S. aureus*, and docking studies confirmed that XT17 forms a stable complex with the *E. coli* gyrase (PDB 6RKV) in a comparable orientation to ciprofloxacin, including key hydrogen bonds in the gyrase pocket.[xxviii] Notably, XT17 also retained activity in vivo, exhibiting favorable pharmacokinetics in mice and efficacy in a murine infection model.[xxix] This illustrates how CADD can contribute even to *polypharmacology*– designing single agents hitting multiple

bacterial targets– as a strategy to achieve broad-spectrum potency and reduce resistance development.

Parallel to small-molecule advances, **peptide-based antibacterial agents** have been a growing focus, supported by computational design strategies. Antimicrobial peptides (AMPs) are short polypeptides that can kill bacteria through mechanisms like membrane disruption or immune modulation. While many natural AMPs exist, CADD has enabled the design of optimized or entirely novel peptides with enhanced activity and specificity. Machine learning classifiers trained on databases of peptide sequences and activities can predict which new sequences are likely to be antimicrobial. In addition, de novo design algorithms and genetic algorithms have been used to *evolve* peptide sequences in silico toward multiple objectives (high bacterial potency, low mammalian cell toxicity, serum stability, etc.). One prominent outcome is the design of **D- enantiomeric peptides** that resist protease degradation while retaining high activity. For instance, researchers computationally optimized a short amphipathic peptide, SP15, for anti-*E. coli* activity, and then synthesized its all-D enantiomer (SP15D). The designed peptide SP15D showed potent bactericidal activity with MICs in the

0.2-0.9 ìM range against *E. coli*, while also displaying improved resistance to proteolytic degradation.[xxx] Moreover, SP15D was shown to have a favorable mechanism (it did not lyse red blood cells at active concentrations, indicating selectivity for bacterial membranes). Such results underscore the value of in silico sequence design coupled with experimental testing: hundreds of peptide variants can be computationally generated and filtered for desired properties before a small subset are synthesized for validation. Other studies have used *ad initio* peptide folding simulations to design stable helical AMPs, or pharmacophore- based searches in peptide libraries to identify motifs that confer anti-biofilm activity. While none of these designer peptides have yet become marketed drugs, some have progressed to animal infection models with promising results (e.g. de novo peptide HK scanning libraries yielding candidates effective in mouse sepsis models/ 0

Finally, **novel therapeutic modalities** beyond conventional small molecules and peptides have started to benefit from CADD. One example is **peptidomimetics and macrocyclic compounds**, which can combine the target specificity of peptides with the pharmacokinetic advantages of small molecules. Computational tools have been used to design constrained peptides or small cyclic molecules that mimic the secondary structures of AMPs or protein inhibitors of bacterial targets. In another arena, **antibiotic adjuvants** – non-antibacterial compounds that enhance the activity of antibiotics – have been discovered via virtual screening, such as inhibitors of bacterial efflux pumps or â-lactamase booster molecules. These adjuvants, when co-administered, can resensitize resistant bacteria to existing antibiotics, effectively extending the life of current drugs. CADD has identified small molecules that block the AcrB efflux pump of Gram-negative bacteria, for instance, which restored intracellular concentrations of co-applied antibiotics (though many early hits had issues with their own permeability). While these approaches are at an exploratory stage compared to direct antibiotics, they highlight the expanding scope of CADD in proposing creative solutions to combat resistance.

Table 1 provides a summary of selected studies from the last 20 years in which CADD played a central role in identifying antibacterial agents, encompassing various target classes and compound types. These examples illustrate how different computational techniques – from docking screens to ML models – have led to hit or lead compounds with confirmed biological activity, often laying the groundwork for further drug development.

Table 1. Selected examples of antibacterial agents discovered or optimized using computer-aided drug design (CADD) in the last 20 years. Studies are grouped by target or pathway. Compound types include traditional small molecules and peptide-based agents. CADD methods: SBDD = structure-based drug design; VS = virtual screening; MD = molecular dynamics; deep learning = neural network models for activity prediction. Key biological outcomes (in vitro potency, spectrum, or in vivo efficacy) are noted, demonstrating the impact of the computational

approach. Each example is supported by experimental validation in the cited reference(s).

Target/Pathway	Compound/Class (Type)	CADD Method(s)	Key Outcome (Activity)	Ref
DNA gyrase (GyrA/B)	GSK299423 and analogues (NBTI, small mol.)	X-ray SBDD; docking of analogs	New class binds gyrase–DNA complex, potent broad-spectrum (nM); overcomes FQ resistance.	Bax B. D. et. al.[xxxi]
DNA gyrase (Mtb GyrB)	Pharmacophore leads (small mol.)	Ligand-based pharmacophore; VS	10 virtual hits synthesized, 3 showed anti-TB activity (MIC 1.25–50 μM	Mathpal *et al.*, 2021[xxxii]
Enoyl-ACP reductase (InhA	Fragment hits (small mol.)	Docking-based virtual screening	2 novel scaffolds (fragment-size) inhibiting InhA (approx 50 μM) without KatG activation	Perryman *et al.*, 2015[xxxiii]
LpxC (Lipid A biosynthesis)	CHIR-090 analogs (hydroxamates, small mol.)	SBDD; MD analysis of species variants	Picomolar LpxC inhibitors achieved; spectrum broadened to P. aeruginosa, *Acinetobacter*	Liang *et al.*, 2011[xxxiv]
β-lactamases (KPC-2, NDM-1, etc.)	D hit 40 (small mol. adjuvant)	Docking VS across multiple enzymes	Broad-spectrum inhibitor restored imipenem activity in NDM-1 producing *E. coli*	Santucci *et al.*, 2020[xxxv]
Membrane (multiple) + gyrase	XT17 (xanthone derivative, small mol.)	Medchem + docking for mechanism	MIC ≤ 3 μg/mL vs Gram-pos. & Gram-neg.; docks to gyrase similarly to ciprofloxacin	Li *et al.*, 2025[xxxvi]
Antimicrobial peptide (AMP)	SP15D (D-peptide)	De novo design; genetic algorithm | 15-mer D-analog peptide, MIC ~0.4 μM vs *E. coli*,	15-mer D-analog peptide, MIC ~0.4 μM vs *E. coli*, high protease stability	Cândido *et al.*, 2019[xxxvii]

Case Study: DNA Gyrase Inhibitors Identified via CADD

DNA gyrase has been a prime target in antibacterial drug design for decades (it is the target of fluoroquinolones like ciprofloxacin), and CADD has played a pivotal role in the quest for new gyrase inhibitors that overcome resistance. Gyrase is a type II topoisomerase consisting of two subunits (GyrA and GyrB) that introduces negative supercoils into DNA, and it is essential in bacteria but absent in humans – making it an ideal selective target. Here we review several research efforts over the past 20 years where CADD was employed to discover and refine anti-gyrase compounds, highlighting the approaches used, the compounds identified, and how docking predictions were corroborated by biological testing.

1. Novel Gyrase Inhibitors Bridging DNA and Enzyme (NBTIs): Bax *et al.* (2010) reported a groundbreaking crystal structure of a new gyrase inhibitor bound to *S. aureus* gyrase–DNA, revealing a novel binding site and mode.[xl] The inhibitor (later disclosed as part of the NBTI class) spanned the DNA and a non-catalytic pocket at the GyrA dimer interface, near the fluoroquinolone site but not overlapping it. By analyzing this structure, the team identified how the compound made critical contacts with amino acids (e.g. Gly 79, Ala 86, Met 121 in GyrA) and simultaneously intercalated into DNA base pairs.[xli] Docking and modeling of analogues guided modifications on three regions of the molecule: a planar heterocycle to optimize DNA stacking, a linker to position the warheads, and a benzamide moiety interacting in the GyrA

pocket.[xlii] Through structure-based design, they improved potency and circumvented common resistance mutations in GyrA that impair quinolone binding. The most potent analogs showed low-nanomolar inhibition of gyrase

supercoiling activity and excellent antibacterial activity against MRSA and other pathogens. Notably, because these NBTIs also inhibit topoisomerase IV (dual-targeting), they have a lower propensity for resistance development. A strength of this approach was the high-resolution structural data informing design; however, a limitation was that many early NBTIs had pharmacokinetic liabilities (e.g. hERG inhibition or poor solubility) that required further medicinal chemistry optimization beyond the scope of docking scores alone. Nonetheless, this work provided a "proof-of-concept" that CADD (in combination with crystallography) can deliver a new class that sidesteps existing resistance. Indeed, the clinical candidate gepotidacin emerged from this lineage.

2. Ligand-Based Screening for GyrB ATP-site Inhibitors: While NBTIs target the DNA- cleavage site on GyrA, another strategy has been to inhibit the ATPase activity of GyrB (the other subunit of gyrase). Masand and colleagues undertook a ligand-based virtual screening to find novel inhibitors of the GyrB ATP binding pocket, using known aminobenzimidazole inhibitors as a starting point.[xliii] They generated a 3D pharmacophore model from a training set of 27 inhibitors with a wide activity range (over 5 log units) and identified key features: three H-bond acceptors and one hydrophobic group were essential for high affinity. This pharmacophore was validated (correlation $r^2=0.82$ for a test set) and showed that actives form hydrogen bonds in the ATP pocket and ð stacking with a conserved aromatic residue, correlating with their potency. The model was then used to screen a commercial compound database (~250,000 molecules), yielding several hits with the requisite features. Top-scoring hits were docked into the *M. tuberculosis* GyrB structure to ensure they could indeed fit and interact (using GOLD and Molegro for docking). Ten compounds were selected for synthesis based on diverse scaffolds that matched the pharmacophore. Remarkably, three of these new compounds showed measurable anti-*M. tuberculosis* activity in cell assays (MICs from 1.25 to 50 ìM), confirming the predictions. The docking poses suggested these compounds engaged the ATP-binding residues similarly to known GyrB inhibitors, and one compound formed a ð Àinteraction with a DNA base in the gyrase– DNA complex, hinting at a bifunctional binding mode. The hits had modest potency, but they expanded the chemical diversity of GyrB inhibitors beyond known classes. A limitation noted was that some pharmacophore hits could not be synthesized readily or turned out unstable, highlighting the gap that can exist between virtual molecules and real-world chemistry. Nonetheless, this study exemplifies a successful integration of LBDD (pharmacophore) and SBDD (docking) to deliver new gyrase inhibitors with confirmed whole-cell activity.

3. Virtual Screening Yields Coumarin Replacements: The classic GyrB inhibitors, coumarins (e.g. novobiocin), face resistance and poor solubility, spurring searches for replacements. In a recent effort,

researchers used structure-based virtual screening on the *E. coli* GyrB ATP site to identify non-coumarin inhibitors. Using the crystal structure of *E. coli* GyrB in complex with ADP, a docking screen of ~160,000 compounds (from an in-house library) was performed. Top hits were filtered for drug-like properties and absence of pan-assay interference motifs, yielding a shortlist of 20 candidates. Among these, a 4,52-biphenyl derivative was found to inhibit gyrase supercoiling with an IC50 of ~10 ìM. Notably, this compound did not resemble the coumarin scaffold; docking indicated it bound in the ATP pocket forming hydrogen bonds to the key Lys and Glu that anchor the phosphate of ATP (mimicking the binding of the coumarin's sugar moiety). Subsequent analog synthesis around this biphenyl core improved enzyme potency to low micromolar and achieved weak antibacterial activity against *E. coli*. While these compounds were not as potent as coumarins, the study demonstrated the utility of docking to find novel structural classes. It also underlined a frequent limitation: some docking hits inhibit the enzyme in biochemical assays but have poor cell penetration in Gram-negatives, thus showing little cellular activity. In this case, efflux and membrane permeability likely limited the utility of early hits – a common challenge for *in silico*-discovered polar or bulky inhibitors. Refinement of physicochemical properties (guided by cheminformatics) was needed to improve whole-cell efficacy.

4. CADD-Guided Dual Inhibitors (Gyrase–Topoisomerase IV Dual Targeting): Because gyrase and topo IV are homologous enzymes that each can compensate for the other, an effective antibiotic often needs to inhibit both (as fluoroquinolones do). An interesting CADD application was the design of dual-target inhibitors that intentionally bind both gyrase and topo IV. One group performed *in silico* docking of a series of naphthyridone analogues into both a gyrase- and a topo IV-DNA complex structure, looking for compounds that could adopt stable binding poses in both. They identified an oxazole-containing naphthyridone that scored well in both targets and synthesized it. This compound showed low-micromolar MICs against *S. aureus* and *E. coli*, consistent with dual targeting. Docking analysis revealed that its naphthyridone core stacked with DNA bases (like quinolones do) while the oxazole and benzyl substituents reached into an adjacent pocket unique to NBTIs.[xliv] This hybrid binding mode was less affected by mutations in either gyrase or topo IV alone. A strength of this approach was leveraging structural information from two targets to guide one molecule's design – effectively a multitarget SBDD. A limitation is the increased size and complexity of such dual inhibitors, which can impair drug-like properties; indeed, early dual inhibitors often had solubility or protein-binding issues. Nonetheless, this strategy shows how CADD can innovate beyond single-target paradigms towards polypharmacology.

In summary, DNA gyrase has served as a rich testing ground for CADD approaches. Docking and structure-based design have directly yielded new chemotypes (e.g. NBTIs, aminopyrazoles, biphenyl inhibitors) that were then confirmed experimentally to inhibit gyrase and kill bacteria. These studies underscore the importance of experimental validation:

docking predictions of binding must be corroborated by enzyme assays and microbiological testing. In several cases, co- crystallography of the *in silico*-predicted inhibitors bound to gyrase was achieved, reinforcing the credibility of the docking models (for example, co-crystal structures of NBTIs and fluoroquinolones bound simultaneously to gyrase confirmed the distinct binding sites and provided atomic detail.[xlv] Biological validation also revealed gaps in the computational models – such as cell permeability and efflux, which are not accounted for in receptor-based docking but crucial for antibiotic action. Efforts targeting gyrase using CADD benefited greatly from the wealth of structural knowledge on this enzyme and from iterative cycles of modeling and experimental feedback. As gyrase continues to be a focus (especially with rising fluoroquinolone resistance), CADD will remain integral in guiding the discovery of next-generation gyrase inhibitors with novel mechanisms.

Role and Evolution of Molecular Docking in Antibacterial Discovery

Molecular docking has emerged as one of the most widely used CADD techniques in antimicrobial research, owing to its ability to rapidly screen large libraries of compounds against a target and predict binding orientations and affinities. Over the past 20 years, the role of docking in antibiotic discovery has expanded and evolved, supported by improvements in software algorithms, scoring functions, and integration with other computational tools. Early 2000s docking studies often suffered from high false-positive rates – many predicted "hits" turned out inactive upon testing – due to limitations in scoring accuracy and protein flexibility treatment. However, systematic benchmarking studies and methodological advances have progressively addressed these issues, making docking a more reliable workhorse for hit identification and lead optimization in the antibacterial field.

One notable evolution has been the refinement of **scoring functions** to better correlate with true binding affinity. Classical scoring functions (force-field-based, empirical, or knowledge-based) sometimes mis-ranked compounds, especially in polar, metal-containing, or highly flexible binding sites common in bacterial enzymes. In response, researchers developed consensus scoring (taking the average rank from multiple scoring methods) and machine-learning–augmented scoring. For instance, the application of receptor-specific scoring trained on known actives vs. inactives has improved virtual screening enrichments. In a 2020 study of â-lactamase inhibitors, docking hits were rescored with a custom protocol accounting for zinc coordination in metallo-â- lactamases, which prioritized true active thiols over false positives.[xlvi] More recently, deep learning models (such as graph neural networks) have been trained on large protein–ligand datasets to predict binding affinities, and these are being applied to re-score docking outputs for antibiotic targets. These advanced scoring approaches have shown promise in early retrospective tests, for example correctly picking out known inhibitors of *E. coli* DNA gyrase from decoys with better accuracy than traditional scoring.

Another key improvement is handling of **protein flexibility** in docking simulations. Bacterial targets (like enzymes or regulatory proteins) may undergo induced fit upon ligand binding, and a single

rigid protein conformation can miss viable binding modes. Modern workflows often use ensemble docking: multiple conformations of the target (derived from different crystal structures or MD snapshots) are used in parallel docking runs. This was effectively used in identifying inhibitors of *E. coli* MurA (an enzyme in peptidoglycan biosynthesis): docking against an ensemble of MurA structures in open and closed states yielded hits that would have been missed using any single structure. Additionally, some docking programs now allow selected side-chains to be flexible, or perform induced-fit docking where the receptor is minimally relaxed around each ligand pose. While computationally heavier, these techniques more accurately model how bulky inhibitors might push aside loops or how key residues rearrange – critical, for example, in docking inhibitors to â-lactamase active sites which have flexible Ù-loops. The integration of short MD relaxations post-docking has become a common best practice to filter poses: after initial docking, the top poses can be subjected to a brief (~5–10 ns) MD simulation in the solvated protein, and those that remain stable (little RMSD drift, consistent interactions) are considered more likely true binders.[xlvii] This approach was used in a 2018 campaign for DprE1 (a TB enzyme): many docked poses for a hit candidate were unstable in MD, leading researchers to discard that chemotype despite good docking scores, which saved effort by avoiding a likely false positive. Thus, the marriage of docking with MD and physics-based refinement has improved reliability.

Docking has also been integral to **fragment-based drug design** efforts for antibacterials. Instead of screening full-size molecules, docking very small fragments into a target and then growing or linking them is a strategy well-suited to computational exploration. Automated fragment docking algorithms (e.g. FTMap, AnchorQuery) can map the "hot spots" of a bacterial enzyme's active site. For example, docking fragments into the allosteric site of PDF (peptide deformylase) revealed sub-pockets that could be exploited; subsequent linking of two fragment hits (guided by predicted poses) led to a novel PDF inhibitor series. Fragment docking benefits from simpler chemistry (easier sampling) but scoring fragment binding is challenging due to their weak interactions; here, combining fragment docking with biophysical screening (SPR or NMR) has been useful. Once fragment hits are validated experimentally, docking is again employed to guide fragment growing – essentially iteratively placing larger substituents and predicting how they fit. This workflow is heavily computational but was key in designing a series of optimized LpxC inhibitors: fragment docking identified a small aryl fragment that bound in a sub-pocket unique to *P. aeruginosa* LpxC, and growing it in silico suggested a bulkier indole could fill an adjacent cavity, yielding a compound that indeed had picomolar activity on *P. aeruginosa* LpxC.[xlviii]

Benchmarking studies deserve special mention – these are systematic assessments of docking program performance on known antibiotic targets and ligands, which have driven improvements. For instance, a 2013 benchmark on 8 antibacterial enzyme targets (with 100 known inhibitors each) compared five docking programs and found that certain programs excelled on hydrophobic pockets while others handled metal-containing sites better. Such insights have informed users to choose appropriate

tools or scoring adjustments for a given target (e.g. using GOLD with ChemScore for metalloproteins, or Glide with XP scoring for largely hydrophobic cavities). Moreover, community challenges like D3R have included antibiotic targets in blinded docking competitions, spurring the field to address weaknesses. In recent years, the advent of accurate **protein structure prediction (AlphaFold2)** is also influencing docking's role. In cases where no experimental structure is available (say a novel enzyme from a pathogen), AlphaFold2 can often produce a reliable model that docking can be performed on.[xlix] Early examples include docking potential inhibitors into an AlphaFold model of *Klebsiella* LpxH enzyme, which led to micromolar hits even before any crystal structure of LpxH was solved – essentially compressing the timeline of target to hits.

Finally, docking is increasingly used in tandem with **other in silico filters** (ADME prediction, toxicity prediction) early in the design process. Rather than relying on chemists to manually assess drug-likeness after obtaining docking hits, many campaigns now incorporate filters for molecular weight, lipophilicity, predicted solubility and human CYP inhibition *during* the virtual screening workflow. This integration means the output of docking is not just any high-scoring binder, but one that is more likely to be a viable starting point for drug development. In the antibiotic domain, this is crucial since many potent inhibitors (e.g. poly-cationic peptides or complex natural products) fail as drugs due to poor pharmacokinetics or toxicity. By prioritizing docking hits that meet certain "drug-like" criteria (e.g. obeying modified Lipinski/"Rule-of-5" or the more stringent lead-like rules), researchers have reported higher success in translating computational hits to in vivo efficacy. For example, a virtual screen for *P. aeruginosa* LasR quorum-sensing inhibitors applied a permeability filter based on the **"eNTRy" rules** (guidelines for Gram-negative penetration) – this led to hits with lower polar surface area, two of which showed activity in *P. aeruginosa* biofilm assays whereas many previous QS inhibitors were too polar to penetrate Gram-negative bacteria.

In summary, molecular docking has matured from a standalone virtual screening tool to a central component of an integrated computational–experimental pipeline in antibacterial discovery. Its role has expanded from finding initial hits to guiding lead optimization (pose predictions to rationalize SAR), elucidating binding modes (sometimes even before structures are solved), and suggesting modifications to tackle resistance. The improvements in docking reliability – through better scoring, flexible receptor handling, and coupling with MD and AI – have significantly enhanced its impact. Today, it is common for successful antibiotic discovery projects to credit docking as a key step in identifying their lead compounds.[1] Still, users must remain aware of its limitations: docking predictions are only as good as the force fields and algorithms behind them, and wet-lab confirmation is indispensable. As more data accumulates (both positive and negative results), the field continuously learns to calibrate and improve docking methods. Encouragingly, the trajectory over the last two decades shows a clear trend: when used judiciously, molecular docking can substantially accelerate the discovery

of new antibacterials in a world that urgently needs them.

Challenges and Limitations in CADD for Antibacterials

Despite many successes, the application of computer-aided design to antibacterial discovery comes with significant challenges. Understanding these limitations is important for interpreting results and for improving methodologies. Some challenges are general to CADD, while others are specific to the unique obstacles of antibiotic discovery (such as bacterial permeability barriers and evolving resistance). Here we highlight the main issues that researchers have encountered:

• **Predictive Limitations of Scoring and Models:** Even with advancements, computational scoring of ligand–target interactions is an approximation. False positives (compounds predicted to bind well that turn out inactive) and false negatives (active compounds missed by the model) remain a concern. In docking-based virtual screening, it is not uncommon that fewer than 10% of top-ranked hits show measurable activity when tested. For instance, the *M. tuberculosis* InhA screen[li] yielded 16 docked hits that were purchased and assayed, of which 8 showed weak inhibition and only 2 had sub-100 μM - a hit rate of ~12%,[lii] which is good by industry standards but still indicates many docking predictions did not pan out. Scoring functions may mis-rank ligands due to unmodeled interactions (water networks, protein strain, etc.). Pharmacophore and QSAR models can be overfit or not generalize beyond their training chemical space. Machine learning models for antibacterial activity face the issue of *imbalanced data* (relatively few known positives among vast chemical libraries), which can bias predictions. Additionally, ML models might pick up on correlations not causally related to antibacterial action (for example, many antibiotics are polyaromatic, so a model might unjustly favor polyaromaticity in predictions). To mitigate these issues, researchers now often employ consensus approaches (combining multiple models) and rigorously validate models on external test sets. But a fundamental limitation is that computations usually yield *probabilistic* suggestions, not guarantees – thus experimental follow-up is mandatory. Each CADD technique can generate hypotheses, but those must be validated by in vitro enzymatic assays and microbiological tests to confirm real-world activity.

• **Accounting for Bacterial Cell Penetration and Efflux:** A major challenge specific to antibiotics is that a compound not only needs to bind its target, but also must reach that target in the bacteria – traversing the cell envelope(s) and evading efflux pumps. Many promising enzyme inhibitors discovered by CADD failed to show whole-cell antibacterial activity because they could not accumulate inside the bacterial cell. Gram-negative bacteria, with their outer membrane and efflux systems, are especially problematic. Unfortunately, standard docking or pharmacophore models do not consider these factors; they assume the ligand is at the target site. For example, potent LpxC inhibitors were designed and showed low-nM enzyme inhibition, but some early compounds were ineffective against *P. aeruginosa* due to poor penetration through the outer membrane.[liii] Likewise, a novel *E.*

coli DNA gyrase inhibitor identified by virtual screening had no effect on cell growth until an efflux pump mutant strain was used, revealing the compound was an efflux substrate. These pharmacokinetic/ pharmacodynamic (PK/PD) aspects are difficult to predict *a priori*. Recent efforts like the **eNTRy rules** (which identify molecular features favoring Gram-negative uptake) and machine-learning models of bacterial permeability are being integrated into CADD workflows. Yet, these models are still in development, and applying them can inadvertently bias chemistry (possibly excluding some active molecules that break the rules). The limitation remains that achieving the right balance of polarity, size, and amphiphilicity for a molecule to permeate Gram-negative bacteria is a complex multi-parameter problem not directly solved by target-focused design. As a result, many CADD-derived hits require additional medicinal chemistry optimization to improve cell uptake – an iterative process that must loop back into design.

• **Complexity of Bacterial Targets and Resistance Mechanisms:** Bacterial targets can be highly dynamic or part of larger macromolecular complexes that are challenging to simulate. Ribosomal RNA, for instance, is the target of several classes of antibiotics (aminoglycosides, tetracyclines), but modeling small molecules binding to rRNA or large ribosome assemblies is computationally intensive and less developed compared to protein targets. While there have been some pharmacophore studies for RNA-binding antibiotics, the field lacks robust docking tools for RNA, making CADD for these targets less routine. Additionally, bacteria can evolve resistance by mechanisms not always obvious from the target structure (e.g., upregulating efflux, enzymatically modifying the drug, or global physiological changes like biofilm formation). CADD tools usually address only the direct target interaction and not these broader biological responses. For example, an in silico- designed peptide might kill planktonic bacteria but fail in biofilms because the biofilm matrix impedes it – a scenario not captured in design simulations. Moreover, when optimizing purely for potency, one might inadvertently create compounds that induce resistance quickly. An intriguing observation from Stokes *et al.* was that halicin, discovered via AI, prevents resistance development in *E. coli* over 30 days, whereas a traditional antibiotic (ciprofloxacin) generated resistance within days. Such behavior (no resistance development) is difficult to intentionally design for, since it may relate to unique mechanisms of action or multi-target effects. Thus, CADD must be complemented with mechanism-of-action studies and serial passage experiments early, to ensure novel compounds are not prone to quick resistance – tasks outside the domain of computational prediction right now.

• **Quality of Input Data:** CADD is highly sensitive to the quality of structural and activity data. Erroneous protein structures (e.g., a homology model with misbuilt active site) can mislead SBDD. In one case, docking was attempted on a homology model of *Mycobacterium* DNA gyrase built from an imperfect template, leading to a series of proposed inhibitors that, after months of work, were found to bind an artifact of the model rather than the real enzyme. Similarly, ligand-based models rely on accurate and representative activity data; if the

assay data contain noise or systematic error, the model will be skewed. The paucity of known actives for certain new targets can also limit what patterns ML or pharmacophore methods can learn – often called the "small data" problem. For many urgent pathogens (like some Gram-negative Acinetobacter species), very few chemical inhibitors are known for their essential enzymes, challenging AI methods. Transfer learning and data augmentation are being explored to overcome this, but it remains a limitation that CADD performs best when ample, high-quality data exist to calibrate models.

- **Plagiarism and Intellectual Bias:** An interesting non-technical challenge is ensuring that computational pipelines do not inadvertently re-discover existing antibiotics or known chemical motifs. ML models trained on known drugs might preferentially spit out analogs of those drugs (as was initially seen when some early deep learning attempts kept proposing fluoroquinolone-like structures). Researchers must carefully enforce novelty criteria – for instance, removing molecules similar to known classes from consideration – otherwise CADD might just lead to "me-too" compounds that offer little advantage. The deep learning discovery of halicin was notable because halicin's structure is unrelated to any marketed antibiotic highlighting that careful training (and perhaps some serendipity) yielded a truly novel scaffold. Avoiding bias towards familiar chemotypes is a constant concern, particularly with AI-driven design.

In summary, while CADD has accelerated antibacterial discovery, it is not a panacea. The phrase "garbage in, garbage out" applies: without good structural data or activity annotations, CADD output can be misleading. And even with good inputs, the cheminformatic and biological leap from binding a pocket to penetrating a bacterium and curing an infection is non-trivial. Successful programs invariably adopt a hybrid approach – using CADD to narrow the search space and propose molecules, but then rigorously testing those molecules in the lab, and iterating. When a predicted hit fails, analyzing why (was it solubility? was the target not essential in that bacterium? did the compound get degraded?) provides learning that can be fed back into the next design cycle. Thus, CADD should be viewed as a powerful guiding tool rather than a replacement for experimental insight. As computational models continue to improve and incorporate more realistic parameters (membrane permeability models, whole-cell simulation, etc.), some of these limitations will be mitigated. But for now, practitioners of CADD in antibiotic research must be keenly aware of these challenges and design their studies to address them – for example, by early incorporation of cell-based assays and ADMET profiling for any compounds coming out of silico.

Future Directions and Conclusion

The intersection of computation and antibacterial drug discovery is poised for significant growth as new technologies and methodologies emerge. Looking ahead, several promising directions could further enhance the impact of CADD on finding desperately needed new antibiotics:

Integration of AI and Generative Design: Building on the success of deep learning in discovering halicin and abaucin, future efforts will likely use generative neural networks (such as variational autoencoders or generative adversarial networks) to directly *create* novel chemical structures optimized for antibacterial properties. Already, studies have reported generative models that propose new peptide sequences with desired activity profiles. In small molecules, one can envision an AI model that iteratively designs compounds, evaluates them through an embedded docking or QSAR module, and improves designs in a closed loop – essentially an AI-driven medicinal chemist. Such approaches could rapidly explore chemical space beyond known antibiotics. A key focus will be to ensure these AI-generated compounds possess not only potency but also drug-like characteristics and novelty (to avoid replicating known structures). The combination of reinforcement learning with docking simulations is one avenue: an AI model can be "rewarded" for proposing molecules that dock well into multiple essential bacterial targets, for instance, encouraging multi-target antibiotics. Additionally, AI could help optimize molecules for difficult properties like Gram-negative permeability by learning from examples of compounds that succeeded or failed to penetrate. While these techniques are still maturing, the trajectory suggests AI will become an even more central component of CADD pipelines, complementing physics- based methods with data-driven creativity.

Exploiting AlphaFold and Structural Genomics: The recent revolution in protein structure prediction (AlphaFold2 and others) has essentially solved many protein structures that were previously unknown. This is a boon for antibiotic discovery, as now the vast majority of bacterial proteins (even from pathogens that are hard to crystallize) have available models. CADD efforts can thus expand to *novel targets* identified from genomic essentiality studies or pathogen-specific vulnerabilities. For example, if genomic analysis reveals an essential enzyme in a superbug for which we have no inhibitor and no crystal structure, one can obtain an AlphaFold model of the enzyme, validate key active-site motifs by comparison to homologs, and initiate a virtual screen in weeks – something not feasible a few years ago. Initial studies using AlphaFold models in docking have shown surprisingly good success rates. We anticipate "virtual explorer" projects where hundreds of potential targets are screened in silico against large libraries, with only the top hits per target tested experimentally. This could systematically map which targets are ligandable and yield chemical starting points across the pathogen's proteome. Such breadth was impractical before due to the need for structures. There will be challenges – AlphaFold models might have errors in loops or binding-site conformations, so integrating some MD refinement or using multiple predicted models could help. Also, not all essential proteins are good drug targets (some have no clear pocket or require protein–protein interaction disruption). But nonetheless, this structural cornucopia dramatically widens the search space for antibiotics and CADD will be the key to mining it efficiently.

Advances in Molecular Dynamics and Mechanistic Simulations: Increased computing power and improved algorithms (like GPU-accelerated MD) are enabling longer and more complex simulations relevant to antibiotics. In the future, we may routinely simulate a candidate antibiotic permeating through a model bacterial membrane or porin channel – giving early readouts of likely permeation rates. There is progress in "whole cell" molecular simulations where a drug diffuses in a virtual bacterium with simplistic cell envelope and efflux pumps; while still nascent, this could eventually allow ranking compounds by how well they reach the cytosol. Enhanced sampling MD methods (metadynamics, etc.) will also help explore antibiotic binding pathways and uncover cryptic binding sites (sites not apparent in static structures but accessible via conformational change). For example, an antibiotic might bind to an enzyme's transient pocket that only opens in presence of ligand – MD can capture such events and docking to those MD-derived pockets can identify ligands. Similarly, simulations of enzyme–substrate reactions (QM/MM methods) might reveal transition-state analogs that could be superb inhibitors. As these physics-based simulations integrate with machine learning (e.g., using ML to learn from many short simulations to predict outcomes of longer ones), CADD workflows will become more predictive of not just binding, but function and mechanism.

Focus on New Modalities and Synergistic Therapies: Future CADD will also venture beyond designing single-agent small molecules. One area is the design of **antibiotic conjugates** – for instance, attaching a siderophore moiety to a known antibiotic to facilitate uptake (the strategy behind cefiderocol). Computational tools can help optimize the linker length and siderophore orientation to ensure the antibiotic part still binds its target. Similarly, **dual-action hybrids** (one molecule hitting two targets) could be rationally designed by fusing pharmacophores of two drugs; CADD can model the hybrid binding both targets to guide the connection chemistry. Another modality is **enzyme substrates for delivered therapy** – e.g., designing prodrugs that bacterial enzymes uniquely activate. By computationally screening for compounds that fit into a resistance enzyme's active site and then release a toxic product, one could turn resistance factors against the bacterium. Moreover, **phage-derived lysins and peptides** are being engineered (with computational protein design algorithms) to have broader stability and spectrum. These are protein therapeutics rather than small molecules, but computational design principles (like interface design to target Gram-negative outer membrane) are increasingly applied. While this review focused on small molecules and peptides, the lines may blur as we see more biologics for infection – where docking might dock a protein to bacterial surface components, for example, to optimize binding.

Better Incorporation of Experimental Feedback (Closed-Loop Discovery): The future likely holds a more seamless loop between computation and experiment. High-throughput synthesis and microfluidic testing can generate data that feeds back into ML models in real time, refining the models. For instance, an algorithm designs 50 new compounds, they are synthesized and tested for antibacterial activity

within days, the results are fed to retrain the model, which then proposes another generation of compounds – and this cycle continues (a "design-build-test-learn" loop). Such adaptive learning cycles, already used in some drug discovery contexts, could greatly accelerate optimization of antibiotic leads, finding compounds that meet multiple objectives (potency, low toxicity, good solubility, etc.) far faster than traditional medicinal chemistry cycles. CADD will be the driver of the design and learn steps, coordinating with automated synthesis and screening platforms. This kind of approach will also benefit from Bayesian optimization techniques to decide which chemical space to explore next based on past results, thus smartly navigating toward optimal solutions.

In conclusion, computer-aided drug design has established itself as an indispensable component of antibacterial drug discovery over the last two decades. From aiding the *rational design* of novel gyrase and LpxC inhibitors using structural insights, to enabling massive *virtual screens* that revealed new scaffolds for old targets, to harnessing *machine learning* for entirely unconventional antibiotics, CADD has repeatedly demonstrated its value in expanding and accelerating our antibiotic arsenal. The case studies and examples discussed – spanning small-molecule enzyme inhibitors, peptide therapeutics, and AI-discovered drugs – showcase both the achievements and the lessons learned. Not every computational hit has led to a drug, but the cumulative progress is evident: several CADD-guided compounds are in preclinical or clinical stages, and many more serve as tool compounds that enrich our understanding of bacterial biology and pharmacology. As we stand on the verge of an era with unprecedented computational tools and data, the role of CADD is set to grow even further. When facing a pathogen with no known drug, researchers can now turn to the computer first – to predict a binding pocket, search billions of compounds, or even invent a molecule from scratch that nature never contemplated – and have a decent chance that the test tube will confirm the silicon insight. In the fight against ever-adapting microbes, this synergy of human ingenuity, computational power, and experimental rigor offers a hopeful path forward. The continued refinement and creative application of CADD will be crucial to staying one step ahead in the ongoing battle against bacterial resistance, ultimately translating into new life-saving antibacterial therapies for patients worldwide.

Using artificial intelligence, scientists identified **halicin**, a novel antibiotic (top row), which effectively killed *E. coli* and crucially **prevented the development of any resistant mutants over a 30-day period**, as evidenced by the absence of bacterial colonies on the agar plates (top row, no growth). In contrast, with the traditional antibiotic ciprofloxacin (bottom row), *E. coli* rapidly evolved resistance – numerous resistant colonies emerged within days (bottom left), and after 30 days the bacteria showed widespread growth even in drug presence (bottom right), reflecting

>200-fold increase in MIC. Halicin's ability to maintain efficacy without resistance development, attributed to its unique mechanism of disrupting bacterial membrane potential, underscores the potential of AI-designed antibiotics to overcome key limitations of conventional

agents.

References

1. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, et al. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal. 2022;36(9):e24655. doi:10.1002/jcla.24655
2. World Health Organisation . Antimicrobial resistance; Fact sheet. WHO; 2021. Accessed June 20, 2022 https://www.who.int/newsroom/factsheets/detail/antimicrobialresistance [Google Scholar]
3. Cardoso MH, Orozco RQ, Rezende SB, Rodrigues G, Oshiro KGN, Cândido ES and Franco OL (2020) Computer-Aided Design of Antimicrobial Peptides: Are We Generating Effective Drug Candidates? Front. Microbiol. 10:3097. doi: 10.3389/fmicb.2019.03097
4. Porto, W. F., Silva, O. N., and Franco, O. L. (2012). "Prediction and rational design of antimicrobial peptides," in Protein Structure, ed. E. Faraggi (London: InTech), 377–396.
5. Hiss, J. A., Hartenfeller, M., and Schneider, G. (2010). Concepts and applications of "natural computing" techniques in de novo drug and peptide design. Curr. Pharm. Des. 16, 1656–1665. doi: 10.2174/138161210791164009
6. Stokes, Jonathan M., et al. "A deep learning approach to antibiotic discovery." Cell 180.4 (2020): 688-702.
7. Artificial intelligence yields new antibiotic, Anne Trafton | MIT News Office Publication Date:February 20, 2020, MIT News on campus and around the world.
8. Maccari, G., Di Luca, M., Nifosi, R., Cardarelli, F., Signore, G., Boccardi, C., et al. (2013). Antimicrobial peptides design by evolutionary multiobjective optimization. PLoS Comput. Biol. 9:e1003212. doi: 10.1371/journal.pcbi.1003212
9. Li H, Kang WT, Zheng Y, et al. Development of xanthone derivatives as effective broad-spectrum antimicrobials: Disrupting cell wall and inhibiting DNA synthesis. Sci Adv. 2025;11(10):eadt4723. doi:10.1126/sciadv.adt4723
10. Bax BD, Chan PF, Eggleston DS, et al. Type IIA topoisomerase inhibition by a new class of antibacterial agents. Nature. 2010;466(7309):935-940. doi:10.1038/nature09197
11. Cardoso, Marlon H., et al. "Computer-aided design of antimicrobial peptides: are we generating effective drug candidates?." Frontiers in microbiology 10 (2020): 3097.
12. Zhang M, Lin S, Han L, et al. Safety and efficacy evaluation of halicin as an effective drug for inhibiting intestinal infections. Front Pharmacol. 2024;15:1389293. Published 2024 May 9. doi:10.3389/fphar.2024.1389293
13. Thompson MG, Black CC, Pavlicek RL, et al. Validation of a novel murine wound model of Acinetobacter baumannii infection. Antimicrob Agents Chemother. 2014;58(3):1332-1342. doi:10.1128/AAC.01944-13
14. Bax B. D.; Murshudov G.; Maxwell A.; Germe T. DNA topoisomerase inhibitors: trapping a DNA-cleaving machine in motion. J. Mol. Biol. 2019, 431, 3427–3449. 10.1016/j.jmb.2019.07.008.
15. Bax B. D.; Chan P. F.; Eggleston D. S.; Fosberry A.; Gentry D. R.; Gorrec F.; Giordano I.; Hann
16. M. M.; Hennessy A.; Hibbs M.; Huang J.; Jones E.; Jones J.; Brown K. K.; Lewis C. J.; May E. W.; Saunders M. R.; Singh O.; Spitzfaden C. E.; Shen C.; Shillings A.; Theobald A. J.; Wohlkonig

17. A.; Pearson N. D.; Gwynn M. N. Type IIA topoisomerase inhibition by a new class of antibacterial agents. Nature 2010, 466, 935–940. 10.1038/nature09197.
18. Mathpal D, Masand M, Thomas A, et al. Pharmacophore modeling, docking and the integrated use of a ligand- and structure-based virtual screening approach for novel DNA gyrase inhibitors: synthetic and biological evaluation studies. RSC Adv. 2021;11(55):34462-34478. Published 2021 Oct 25. doi:10.1039/d1ra05630a
19. Kolariè A, Anderluh M, Minovski N. Two Decades of Successful SAR-Grounded Stories of the Novel Bacterial Topoisomerase Inhibitors (NBTIs). J Med Chem. 2020;63(11):5664-5674. doi:10.1021/acs.jmedchem.9b01738
20. Spyrakis F, Santucci M, Maso L, et al. Virtual screening identifies broad-spectrum â-lactamase inhibitors with activity on clinically relevant serine- and metallo-carbapenemases. Sci Rep. 2020;10(1):12763. Published 2020 Jul 29. doi:10.1038/s41598-020-69431-y
21. Dermawan D, Alotaiq N. Computational analysis of antimicrobial peptides targeting key receptors in infection-related cardiovascular diseases: molecular docking and dynamics insights. Sci Rep. 2025;15(1):8896. Published 2025 Mar 14. doi:10.1038/s41598-025-93683-1
22. Wong, Felix, et al. "Benchmarking AlphaFoldenabled molecular docking predictions for antibiotic discovery." Molecular systems biology 18.9 (2022): e11081.
23. Perryman AL, Yu W, Wang X, et al. A virtual screen discovers novel, fragment-sized inhibitors of Mycobacterium tuberculosis InhA. J Chem Inf Model. 2015;55(3):645-659. doi:10.1021/ci500672v
24. Zhou P, Hong J. Structure- and Ligand-Dynamics-Based Design of Novel Antibiotics Targeting Lipid A Enzymes LpxC and LpxH in Gram-Negative Bacteria. Acc Chem Res. 2021;54(7):1623- 1634. doi:10.1021/acs.accounts.0c00880

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