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Crystallizing new approaches for antimicrobial drug discovery

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ABSTRACT

Over the past decade, the sequences of microbial genomes have accumulated, changing the strategies for the discovery of novel anti-infective agents. Targets have become plentiful, yet new antimicrobial agents have been slow to emerge from this effort. In part, this reflects the long discovery and development times needed to bring new drugs to market. In addition, bottlenecks have been revealed in the antimicrobial drug discovery process at the steps of identifying good leads, and optimizing those leads into drug candidates. The fruit of structural genomics may provide opportunities to overcome these bottlenecks and fill the antimicrobial pipeline, by using the tools of structure guided drug discovery (SGDD).

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1. Review

1.1. Introduction

A pipeline of new antibiotics is needed to control infectious disease in both the developed and developing world and to sustain an important revenue source for the pharmaceutical industry [1,2].

In the mid-1990's, genomics provided the hope that a wealth of new targets would create a pipeline of new antibiotic compounds entering clinical trials. New targets were identified and genomics provided opportunities to develop both protein-based and cell-based target-specific screens [3]. Despite high throughput screening campaigns that tested hundreds of thousands to millions of compounds from diverse corporate libraries, many of these screens proved fruitless, not yielding compounds that had target-specific inhibition [4]. In other cases, inhibitors were pursued, but efforts to create drug-like molecules from the initial hits proved difficult [5]. A few programs did progress, but met challenges in obtaining a clinically relevant breadth of spectrum that is needed in antimicrobial therapeutics [6,7].

In the late 1990's, structural biologists organized a worldwide initiative to solve 10,000 high resolution protein structures, which was thought to be sufficient that all other proteins could be homology modeled from the information [8]. The worldwide structural genomics initiatives have funded projects in North America, Europe, and Asia. In addition, a substantial amount of money has been expended in private structural genomics initiatives. In the US, the Protein Structure Initiative (PSI) funded 9 structural genomics centers in 2000, with a collective annual budget of approximately US$ 65MM; a second round of funding began in 2005, funding 10 centers [9].

Aiming to rapidly solve the structures of large numbers of diverse proteins and place the structures into the public domain (in the Protein Data Bank (PDB) see Table 1), the PSI and other structural genomics efforts are having an unexpected and substantial impact on the search for new antibiotics. By providing the high resolution structures of

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key bacterial protein targets, structural genomics has enabled structure guided antimicrobial discovery programs.

1.2. *Structural genomics of prokaryotic proteins*

With the aim to solve 10,000 protein structures, and competition for the second round of funding dependent on results, the nine groups in the initial funding round of the PSI sought to solve as many protein structures as possible [9]. Through early structural genomic efforts it became clear that solving the structures of prokaryotic proteins was much easier than solving structures of eukaryotic proteins (see Fig. 1). In nearly every step of the structural genomics pipeline, prokaryotic proteins have advantages [10,11]. Cloning is fundamentally easier with prokaryotic ORFs, because of clear start and stop sites and the lack of introns. Protein expression systems are highly optimized and efficient in bacteria—a wide range of vectors exists for altered expression levels and for making fusions on either the N-terminus or C-terminus of a protein. Bacterial proteins are not generally subject to post-translational modifications, such as glycosylation, and the expressed proteins may have increased propensity to fold into native conformations. Furthermore, expression in bacterial hosts is both cost effective and provides methods for production of large quantities of proteins [12]. In addition, the prokaryotic kingdom is huge. The sequencing of new prokaryotic genomes still reveals ''new'' proteins that do not have primary sequence homologs in previously sequenced genomes, providing a wide variety of proteins for future structural determinations [13].

Over the past 5 years more than 2400 structures have been solved by the combined structural genomics efforts, largely through X-ray crystallographic techniques that have resulted in high resolution structures [14]. Progress at each of the structural genomics centers can be monitored at their web sites or through the TargetDB web site, which collects all of this information into a single searchable database (http://targetdb.pdb.org/; and see Table 2).

Although many of the proteins solved in the structural genomics effort are from thermophiles, many are also from pathogenic bacterial species. Fig. 1 shows data taken from TargetDB summarizing the number of protein structures deposited by researchers in the Protein Data Bank from select

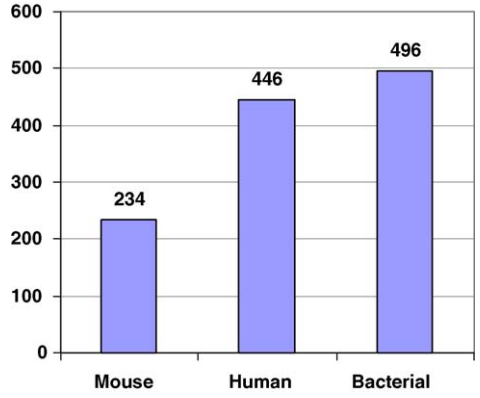


Fig. 1 – Protein structures solved in TargetDB. The TargetDB database was searched for proteins entered into the PDB from various species. The *Y*-axis shows the number of proteins entered into the PDB from mouse, human or bacterial pathogens. Human and mouse proteins were identified by TargetDB searches for "sapiens" and "musculus", respectively. Bacterial pathogen proteins were identified by summing the results from separate organism searches for bacillus, coli, staphylococcus, mycobacterium, pseudomonas, enterococcus, haemophilus, streptococcus, and salmonella. Searching was performed on September 16, 2005.

eubacterial pathogens. From these bacteria, over 4200 ORFs have been cloned, resulting in the public deposition of nearly 500 high resolution structural models of bacterial proteins. Proteins from many other bacterial species, including *Mycobacterium* spp., as well as many thermophilic bacteria have also received focused attention by structural researchers.

Bacterial proteins that will provide targets for most new antibiotics are categorized as ''essential proteins'', because the loss of their function causes cell death (bactericidal effect), or prevents growth (bacteriostatic effect). Many methods have been used to identify the essential proteins in different bacterial species [5]. In general, a few hundred essential genes are identified in a species by these methods [15].

Table 1 – Useful websites

Database	Description	Website
Binding MOAD	''Mother Of All Databases'' provides information on protein-ligand binding	http://www.bindingmoad.org/
PDB	The worldwide Protein Data Bank providing protein structure information from the RCSB PDB (USA); the MSD-EBI (Europe); and PDBj (Japan)	http://www.wwpdb.org/
PDBbind	1762 Protein–ligand complexes. Allows chemical-based similarity or substructure searching to find co-complexes. Academic/governmental users have access to free license. Industrial users license with a moderate subscription fee	http://www.pdbbind.org/
PEC database	Searchable database of information on *E. coli* genes and proteins, including information on essentiality, curated from a variety of sources	http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp
PEDANT	Bioinformatic analysis of completed genomes	http://pedant.gsf.de/
TargetDB	Searchable database of the Protein Structure Initiative that organizes current status of all proteins in the structural genomics pipeline	http://targetdb.pdb.org/

Table 2 – Structural genomics in bacterial pathogens

	Total number genome ORFs[a]	Number of proteins with >1 trans-membrane segment[b]	Number cloned[b]	Number of purified proteins[b]	Number of protein crystals[b]	Diffraction quality crystals[b]	Number of solved structures in PDB[b]
Escherichia coli	4289	776	792	516	204	88	86
Pseudomonas aeruginosa	5565	875	379	140	34	34	36
Haemophilus influenzae	1709	259	281	89	35	12	7
Staphylococcus aureus	2360	510	169	69	38	16	14
Streptococcus pneumoniae	2043	357	289	53	32	10	8
Entercoccus faecalis	3113	ND	310	88	27	15	11
Mycobacterium tuberculosis	3924	506	1853	452	216	136	70
Helicobacter pylori	1491	217	137	37	14	3	3
Total	24494	3500	4210	1444	600	314	235

[a] Information from PEDANT web site (http://pedant.gsf.de).
[b] Information gathered from the TargetDB web site (http://www.targetdb.pdb.org) between August 11–22, 2005, using the built-in query tools, and requesting information on each of the species listed, using both genus and species in the search request. Note that the search terms in this table and Fig. 1 were different, leading to different total.

Assessment of the protein structures solved in the structural genomics effort, along with other structures in the PDB shows high resolution structures of a large percentage of essential proteins have been solved from at least one clinically important species (MBS, unpublished). Of the 250 essential genes of *Escherichia coli* in the PEC database, 179 have a PDB code listed in the PEDANT database, indicating that a protein structure in the PDB is of sufficient primary sequence homology that the *E. coli* protein structure could be successfully homology modeled (see Table 3). Many of these proteins are in the ribosome, and structures of these ribosomal proteins have been solved independently and in the context of solving the structure of the ribosome [16,17]. In order to appreciate the number of new targets that have high resolution structures, the ribosomal proteins were eliminated from this list, resulting 138 non-ribosomal essential proteins of *E. coli* in which a high resolution protein structure is publicly available (see Table 4 for some examples). Of these, 82 structures are of *E. coli* proteins rather than orthologs from other species. Thus, these structures could provide excellent entry points for structure guided antimicrobial discovery efforts.

Table 3 – Structures of essential proteins of *E. coli*[a]

Gene class	Total proteins in each class[b]	Number of proteins or homologs in PDB (% of proteins in each class with a structure)[c]
Essential	250	179 (71.6)
Non-essential	3253	1614 (49.6)
Unknown	906	330 (36.4)
Total	4413	2123 (48.1)

[a] This information was generated by linking information from the PEC database (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp) with information from PEDANT (http://pedant.gsf.de/), using the "GI number" as the link between information in PEC and information in PEDANT. While not perfect, this method captured 2123 of the 2150 proteins of *E. coli* having a PDB code in the PEDANT database.
[b] PEC database (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp).
[c] PEDANT database (http://pedant.gsf.de/).

The structural genomics initiatives are solving large numbers of structures of bacterial proteins, in their quest to solve "non-redundant" protein structures. However, the structural genomics initiatives are not likely to provide the structures of orthologous proteins from clinically important bacterial species, which may be important for structure guided antibiotic discovery efforts. Fortunately, orthologous protein structures can be successfully modeled using a sufficiently homologous protein [18]. Comparisons of predicted protein structural models with independently derived experimental protein structures, have shown that excellent models can be created if the homologous protein and the target protein, share at least 30% amino acid identity [19]. This is a level of identity met by most bacterial orthologs. A cursory examination of the essential proteins of *E. coli* from the PEC database (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp) shows that nearly all of the proteins from *E. coli*, a Gram-negative bacterium, have more than 30% amino acid identity with the orthologous protein from *Staphylococcus aureus*, a Gram positive. Thus, for most bacterial orthologs, the experimentally derived protein structure from one species will be sufficient to create structural models of proteins from other clinically important species.

The increasing numbers of protein structures resulting from structural genomics are fostering the development of additional databases to store the information and make it useful to a variety of researchers (see Table 1) [20]. Among the newer and very relevant databases are MOAD and PDBbind, which house biochemical ligand affinity information for co-structures that are found in the PDB [20–22].

1.3. *The impact of high resolution structures on antimicrobial drug discovery*

Possessing the high resolution structure of a target can provide substantial advantages in several phases of the drug discovery process. The high resolution structures in and of themselves

Table 4 – Novel target-directed antibacterial agents

Pathway	Target	PDB	References to protein structure and SGDD efforts on the target	Programs on the target without SGDD
Cell surface, membrane, peptidoglycan, cell wall	LpxC	1XXE	[31,32]	
	MurA	1UAE	[41,44]	
	MurB	1MBB	[14]	[52]
	YjeE	1FL9	[53,54]	
	FtsZ	1RQ7		[55]
	Signal peptidase	1T7D		[56]
Fatty acid biosynthesis	Acc (acetyl-coA carboxylase)		[57]	[58,59]
	FabF	1OX0	[60,61]	
	FabH	1MZS	[33,37,62]	
	FabI	1LXC		[63]
	YacE (coaE)	1N3B	[57]	
Nucleic acid management	TopoIV (ParC, ParE)	1S16, 1ZVU	[64]	
	Gyrase	1AB4, 1EI1		[65–69]
	RNA polymerase	1IW7	[70]	[71]
	MvaS HMG coA sythase	1TVZ	[29,72]	
Regulation	YycF	1NXO	[73]	
Translation	PheRS	*1EIY*		[74,75]
	Met tRNA synthetase	1PG2		[76]
	Peptidyl deformylase	2AIA	[77]	[78,79]

Several recent reports of new inhibitors of bacterial essential proteins have relied on high resolution protein structures to guide the drug discovery efforts. Several other projects have not been structure guided efforts, sometimes because the structure came out after the work identifying the inhibitors. The PDB code for the protein is listed in the third column; all protein structures are from clinically relevant species, except those that are italicized.

are not a panacea; drug discovery remains hard, and certain aspects of the process are not currently aided by possession of a high resolution structure of the target. Nonetheless, efficiencies can be realized at many points in the drug discovery process when using the structure of a protein target to guide efforts. This is still a very young and active field of research, in which the tools for deriving advantage from the structures are still emerging, and in which there is still substantial room for improvement of the techniques that are being used [23–25]. Summarized below are examples from antimicrobial drug discovery efforts that are using structure guided drug discovery (SGDD) to supplement and expand the tools available to solve the challenges of efficient lead optimization in the discovery of novel classes of antibacterials (see Table 4 for examples).

Tools are emerging to prioritize targets based on the predicted ''druggability'' of their binding pockets. A recent study correlated the characteristics of protein binding pockets with the frequency of experimentally identified binding ligands from NMR-based screening. This analysis led to an algorithm that predicted the ''druggability'' of a binding pocket, based on characteristics that can be derived from a high resolution protein structure [26]. The frequency of NMR-based screening hits from 23 diverse proteins and analysis of the corresponding ligand binding sites was used to create the model, which bins protein binding sites as ''non-druggable'', ''moderately druggable'' or ''highly druggable''. The model was subsequently able to correctly characterize an additional 33 of 35 proteins that have known high affinity ligands, and went on to predict the characteristics of a similar number of proteins in which no ligands have yet been identified. Additional methods are likely to emerge from the increasing numbers of protein structures and co-structures available, and the wealth of high throughput screening information that has been gathered over the past decade. The information databases linking structure and ligand binding information will also aid this effort. Such algorithms will allow future researchers to focus efforts on proteins that are more likely to yield high affinity ligands for drug discovery. Furthermore, the large numbers of essential bacterial proteins with high resolution structures provide an excellent testing ground for such methods.

The availability of a high resolution protein structure at the beginning of a drug discovery project allows in silico screening methods to supplement experimental high throughput screening. In silico screening uses high resolution protein structure information to computationally test and identify small molecules more likely to bind the protein. Typically, millions of compounds can be screened in silico, far surpassing the ability to assess compound diversity by experimental methods [24]. Subsequent experimental testing of compounds identified from a virtual screen can employ more labor-intensive or expensive assays than would be undertaken by a typical experimental high throughput primary screen. To identify inhibitors of the bacterial FabH enzyme, researchers at Quorex Pharmaceuticals undertook a virtual screen of three million commercially available compounds. Filters were applied to eliminate reactive and other undesirable compounds, as well as those that did not satisfy Lipinski guidelines [27]. From these, 2500 compounds were tested experimentally for inhibition of the *Enterococcus faecalis* and *Haemophilus influenzae* FabH enzymes using radioactive-based screens, resulting in 27 hits with inhibition at concentrations less than 10 μM [28]. The experimental screening of such a laborious

assay would likely have been prohibitive without the enrichment of active compounds that resulted from the virtual screening process.

Novel ideas for initial leads can also arise directly from the locations and orientations of fortuitously bound substrates in the high resolution protein structures. Substrates, cofactors and molecules present in crystallization buffers are often found in binding pockets, providing insights into the binding pocket location and the mode of ligand binding. The high resolution structure of the *S. aureus* HMG-CoA synthetase, MvaS had a substrate molecule occupying the tunnel of the protein. This co-structure allowed the characterization of important residues for substrate binding and catalysis, which is facilitating the structure-based design of alternative novel antibiotics for Gram-positive cocci [29]. Likewise, the crystal structure of *Aquifex aeolicus* UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylase, LpxC, had a fatty acid molecule bound fortuitously in a hydrophobic tunnel of the protein [30]. This structure provided both a hypothesis about the binding of previously identified inhibitors of this enzyme, and ideas for designing novel inhibitors [30]. The subsequent solution of structures of the LpxC protein–inhibitor complexes confirmed much of the binding mode, and provided additional information for designing new inhibitors [31,32].

In the drug discovery process, once initial chemical matter is identified, lead optimization can begin. This can be a slow, frustrating and costly phase of the drug discovery process. Without direct visualization of protein–ligand binding complexes, lead optimization progresses largely through a trial and error process to define the structure activity relationship (SAR). Modifications of existing compounds are designed, synthetic routes identified, compounds created and the biological activities determined. From these studies, regions of the compound that are important for activity are inferred. In general, this process results in systematic searches of a relatively small region of compound space and conservative modifications of the initial molecule. The determination of the SAR may require the design and synthesis of hundreds of molecules, costing substantial time and expense that may not be directly aimed at identifying a drug candidate.

More adventurous changes to the starting molecules can be imagined and tested in silico, when a target structure is available. Though predictions are not always correct, the in silico design and testing achieves success rates that are sufficiently high to warrant pursuit of new moieties that would have been unlikely to attract interest without the support of the in silico modeling. Such changes may allow more tractable chemical starting points [33], additional drug-like properties [34], or increased potency [7,28,34]. Even using lower resolution structures can provide new insights and ideas for improving potency or overcoming resistance, as the 3–4 Å structures of the 30S and 50S ribosomal subunits are demonstrating [35,36].

The high resolution structure of a target–inhibitor complex provides direct visualization of the interactions between the small molecule and the protein, and allows rapid hypothesis-generation about the primary contributors of the molecule to binding. To a great extent, solving the structure of a protein–inhibitor complex eliminates the need for trial and error SAR, and can focus energies on designing molecules with additional potency or improved drug-like properties. Though not always straightforward, the design of new molecules using in silico methods is comparatively efficient and can result in substantial improvements in potency. Starting with a 1.6 uM inhibitor of the *E. faecalis* FabH enzyme, Quorex researchers designed new molecules with 400-fold better inhibition of the enzyme [28]. Likewise, starting with 2.4 uM inhibitors of the fatty acid biosynthetic enzyme FabI and using structure guided drug discovery, GSK researchers designed new molecules with 500-fold increased potency [6,7,34]. The initial molecules lacked antimicrobial activity, while the resulting molecules had potent antimicrobial activities, with MICs of 0.06 ug/ml against *S. aureus* strains. Thus, the ability to efficiently improve potency using structure guided methods opens opportunities to explore molecules lacking antimicrobial activity, which might otherwise have been abandoned early in the hit prioritization stage.

Lead optimization requires designing in features other than potency; possessing the structure of the target, and of inhibitor–target complexes, can provide a great advantage in assuring that a large percentage of the molecules that undergo synthesis in this effort are active. In the best of situations, the specific ortholog and inhibitor co-structures are available to guide detailed design and testing of hypotheses. However, even in cases where only homology models are available, structural information can provide substantial benefit. Indole analog inhibitors of the fatty acid biosynthetic enzyme, FabH, encoding the beta-ketoacyl carrier protein synthase III enzyme, were improved using just a homology model and in silico docking [33]. Starting with a relatively insoluble hit from high throughput screening, a docking model of inhibitor-protein binding was made from a homology model of the *Streptococcus pneumoniae* FabH enzyme target. Using this information, new inhibitors were designed based on a high throughput screening hit. More soluble compounds were designed, allowing successful co-crystallization of the *S. pneumoniae* FabH–inhibitor complex. The solved co-structure provided additional insights into the details of inhibitor binding, allowed the rational design of more potent inhibitors, and provided understanding of differences in the inhibitor binding to the *E. coli* and *S. pneumoniae* FabH proteins. In the in silico design of novel FabH benzoylaminobenzoic acid inhibitors by Quorex researchers, molecules became more drug-like, replacing a likely microsome-labile sulfonamide group with a phenoxy that allowed additional interaction with the protein [28].

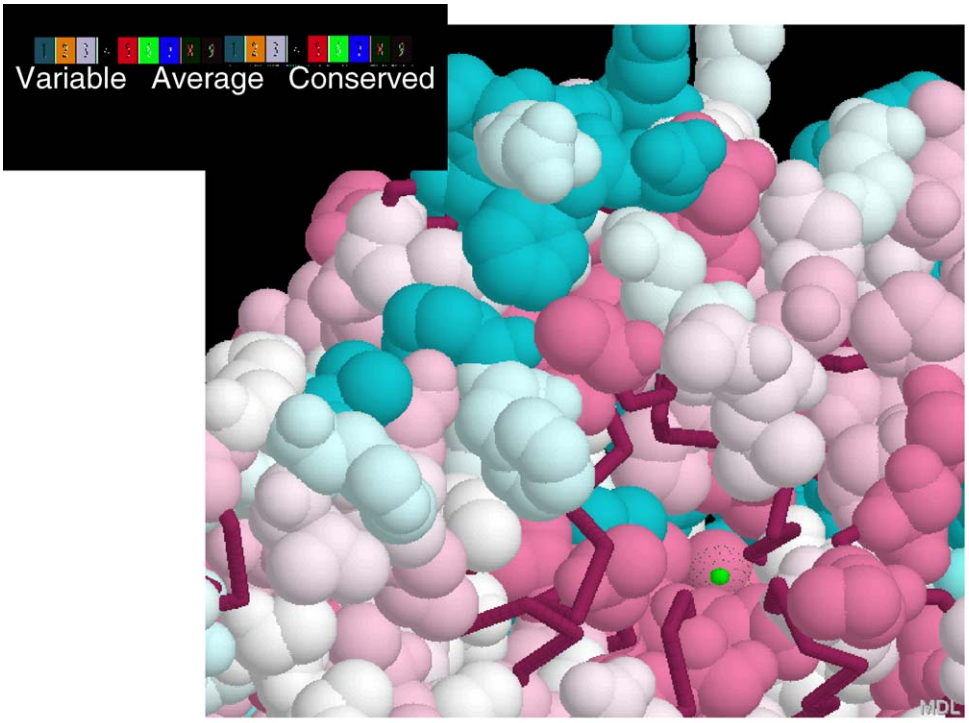
1.4. *Challenges remain*

To obtain a clinically useful antimicrobial spectrum, a small molecule must bind well and inhibit the function of the orthologs from different species. It may require special approaches to obtain molecules with high affinity binding to a wide range of related protein targets. Experience has shown that the relative conservation of active site residues in the primary target sequence does not prevent difficulties in achieving broad spectrum coverage by an inhibitor. Fig. 2 shows the variability found among related

species in the essential fatty acid biosynthetic enzyme FabB, which has a relatively typical conservation of amino acids in the protein binding pocket. Though substantial amino acid conservation exists, small differences in the binding pocket can have substantial and sometimes unexpected effects on the potency of antibiotic agents. The *E. coli* and *S. pneumoniae* FabH enzymes share 80% of their active site residues, yet 10–50-fold differences in *E. coli* and *S. pneumonia* potency was observed for a new class of inhibitors [33,37]. Solving the structure of the orthologous enzymes was required to provide the additional detail needed to understand the source of some of the potency differences [37,38].

Methods are emerging to face the challenges of binding a small molecule to a range of related protein targets. Researchers faced with the need to inhibit rapidly evolving viral protein targets have explored approaches that may have value in antibacterial drug discovery. In one approach, molecules are designed purposely with conformational flexibility, allowing them to bind effectively to a variety of HIV protease pockets that have been altered by resistance mutations [39,40]. This "adaptive inhibitor" approach encourages the design of less constrained molecules, somewhat contrary to current medicinal chemistry practice, in which highly constrained molecules are generally favored in the search for molecules that maximize potency.

Subtle conformational flexibilities of both the small molecules and protein targets limit the correct prediction of the molecular details of the target–inhibitor interactions. Several groups have undertaken high throughput screening to identify inhibitors of UDP-*N*-acetylglucosamine enolpyruvyl transferase, the product of the *murA* gene. Both high resolution solution and crystal structures of the MurA protein have been solved, including the co-structures of MurA with substrate and substrate analogs [41–44]. Through these detailed studies, the N-terminal domain of MurA was shown to undergo substantial conformational change upon binding substrate. Prediction of binding modes for proteins such as MurA can pose huge challenges, and experimental surprises can be revealed when the co-structures of the target with novel inhibitors are solved. The inhibitor T6361 binds to MurA by a novel mode, in which the normal conformational change induced by substrate binding is blocked [44]. The co-structure of the MurA–T6361 complex was solved, and shows an N-terminal structure with substantial differences from the MurA protein bound to the normal substrate (N-terminal r.m.s.d. of the two proteins = 2.1 Å). This example demonstrates the challenges of predicting correct binding, even



FabB (*3-oxoacyl- synthase)* from *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus pyogenes*

(Structure 1ox0 from *S. pneumoniae; Consurf*)

Fig. 2 – FabB from Gram-positive pathogens. The FabB protein structure of *Streptococcus pneumoniae* (1OX0) was used to display the amino acid conservation in the binding pockets of the Gram-positive species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Streptococcus pyogenes*. Identical amino acids are shown in the dark stick figures, while all of the differing amino acids are shown by space filling models, and color-coded for their conservation. Thus, despite substantial conservation of amino acids, the challenges are substantial in identifying small molecules that will bind with high affinity to the target protein in all of these species.

when knowledge of the motions of the protein are known, and reinforces the need to solve co-complex structures during the lead optimization process. The case of MurA is not unusual, as many proteins show substantial motions upon binding substrates, creating challenges for both virtual screening, and determining the correct binding mode of an inhibitor. Several methods to overcome these challenges are emerging, though not yet fully satisfactory. Among the methods is an approach that uses ensembles of experimentally determined structures for the in silico studies [45].

Additional barriers to efficient drug discovery appear when a valuable lead molecule with potency, efficacy, and appropriate pharmacokinetics is found to be toxic. Significant time can be spent in attempting to separate the potency from the toxic effects through medicinal chemistry. Structural guided drug discovery methods now allow researchers to step beyond traditional bounds to experiment with new lead scaffolds. As additional structures accumulate, and as virtual screening methods become more powerful there may be additional opportunities to create methods for virtual ''counter-screening'', to identify potential toxic compounds earlier in the discovery process.

These additional tools of structure guided drug discovery will allow more efficient design of potent, drug-like, target-directed inhibitors. However, history has shown that all biochemical approaches to antimicrobial drug discovery have an additional challenge, which is the ability of the potent target inhibitor to access the target [46]. Over the past decade, the importance of bacterial efflux pumps in both intrinsic and acquired resistance has been recognized [47]. Efflux and entry through the chemically complex bacterial surface layers remain key challenges in designing new antibiotic molecules. Recent structures of Gram-negative efflux pumps [48] and novel efflux pump inhibitors [49–51] may start to provide solutions, yet this challenge will remain until we fully understand and can design around the barriers to entry and efflux of small molecules in bacteria [28].

2. Summary

The global structural genomics initiatives are having unanticipated and very positive consequences on the search for new classes of antibacterial agents. High resolution structures of many essential protein targets are now available from at least one clinically relevant bacterium. Current and future antimicrobial drug discovery researchers have the opportunity to employ the additional tools of structure guided drug discovery in the search for new drugs to treat drug resistant bacterial infections. Hopefully, these new tools of structure guided drug discovery will break through the current barriers to antimicrobial drug discovery, as the most promising targets are prioritized, experimental screens are supplemented by virtual screens, and lead optimization can proceed in more fruitful directions to yield drug-like molecules with exceptional potency. With the public availability of substantial numbers of bacterial target structures, and the importance and recognized need for the discovery of new antibacterials, there is no better proving ground for the value of structure guided drug discovery.

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