Computational Approaches for Antibacterial Drug Discovery

Prachi Srivastava and Neha Srivastava

Abstract

With the emerging problem of antibacterial drug resistance and resurgence of disease, there is immediate need for the development of new and effective therapeutic interventions to combat pathogens. Traditional methods of drug discovery are very expensive and time consuming, and carry high error rates. Computational approaches, on the other hand, predict drug targets and therapeutic agents with fewer side effects (i.e., minimal disease resurgence) and reduce the time and cost for discovery. Thus, the computational approaches have become a crucial part of drug development, as they streamline processing and testing in a cost-effective manner. This chapter highlights the significance and progress of computational approaches in antibacterial drug discovery.

1 Introduction

Bacterial diseases, such as tuberculosis, pneumonia, cholera, diphtheria, meningitis, tetanus, Lyme disease, gonorrhea, and syphilis, are a leading global health threat and represent a major cause of morbidity and mortality. The disease pathologies are

P. Srivastava (✉)
AMITY Institute of Biotechnology, AMITY University, Lucknow, Uttar Pradesh, India
e-mail: psrivastava@amity.edu

N. Srivastava
AMITY Institute of Biotechnology, AMITY University, Lucknow, Uttar Pradesh, India

Dr. A.P.J. Abdul Kalam Technical University, Lucknow, Uttar Pradesh, India

largely caused by the production of toxins or by an aggressive immune response to bacterial antigens. Although improved awareness about sanitation, advancements in vaccination, and the discovery of antibiotics have greatly minimized the impact of these diseases, many hurdles yet remain. The emergence of drug-resistant bacterial strains and the subsequent resurgence of diseases such as tuberculosis have reestablished infection as a prominent global threat.

Despite the rising problem of drug resistance, no effective alternative therapeutics has emerged to protect against these deadly bacterial infections. According to the World Health Organization (WHO), approximately 700,000 people die every year due to drug-resistant infections worldwide (de Kraker et al. 2016), with those numbers estimated to reach ten million by 2050. Currently available approaches to drug discovery are very expensive and time consuming, and thus there is a great need to develop new and advanced perspectives to overcome this problem. Advancements in computational methods are currently playing a key role in scientific research, not only in the discovery of novel drug targets but in regard to many other fields, such as genomics, proteomics, metabolomics, transcriptomics, systems biology, and molecular phylogenetics. These methods allow scientists to link disease symptoms to particular mutations, epigenetic modifications, and various other genetic and environmental alterations, thereby identifying potential drug targets and novel therapeutics with fewer side effects and potential for drug resistance (i.e., minimizing long-term adverse effects on human health while improving cost- and time-effectiveness). Thus, the current chapter focuses on the significant role and real-world applicability of computational approaches with respect to antibacterial drug discovery.

2   Genomic Approaches to Drug Discovery

Genomics approaches are regularly used for the detailed study of an organism's genetic code and are applied in the fields of DNA sequencing and recombinant DNA technology, and in understating the assembly, annotation, and interpretation of the structure and function of a genome (Mishra and Srivastava 2017). The rise of the genomics era has played a significant role in vaccine and drug development from sequence-based approaches and has provided a novel path to the investigation of underlying disease mechanisms. Genomic study is well suited for the identification of potential drug targets and the design of novel therapeutics and vaccines, as well as the prediction of their side effects on human health, and represents a promising approach to combatting drug resistance and disease resurgence.

Identification of potential antibacterial drug targets based on genome sequences is a major challenge, as the number of genes with unknown biological function is still high. The emergence of bioinformatics has played a crucial role in the identification of homologs of known genes by comparative genomic analysis of new sequences with biochemically characterized sequences of proteins/enzymes. The complete genome sequencing of bacteria provides insight into new information about the disease, its pathogenesis, resurgence, and drug resistance. Thus, genomics

is used to predict potential antibacterial drug targets for disease. After the completion of the first whole genome bacterial sequence of *Haemophilus influenza* in 1995, computational approaches have been vital in providing significant information about pathogens, pathogenesis, and antibiotic resistance. To date, thousands of bacterial genomes have been sequenced and many more are presently ongoing. The genome sequence of a particular bacterial pathogen can contain thousands of genes, providing a massive collection of potential antigens and drug targets. Thus, genomics can be used to identify potential drug candidates faster and more accurately than conventional methods. Genomic approaches are not only used in target-based screening studies but also in whole genome expression profiles to study the cellular response to therapeutic manipulation of an antimicrobial drug target. Additionally, genomics can be used in screening large numbers of herbal compounds possessing antibacterial activity and can identify novel structural classes of antibiotics. Thus, the genomics era has had a significant influence on vaccine and therapeutic development.

2.1   Reverse Vaccinology

Reverse vaccinology is defined as a genome-based approach to vaccine development. It uses computation to design novel vaccines by taking information present in the genome without the need to grow specific microorganisms in the laboratory. The first vaccine developed by reverse vaccinology was the meningococcal B (MenB) vaccine for the prevention of Neisseria meningitides (R. Rappuoli et al. 2012). In this study, the complete genome of MenB was sequenced and computationally analyzed. The selected in silico vaccine candidate was then expressed in *Escherichia coli* to allow for in vitro testing. This work represented a significant contribution to the field of vaccine development. Since then, the approach has been successfully applied in the development of vaccines against various organisms, including *Bacillus anthracis*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Chlamydia pneumoniae*, *Porphyromonas gingivalis*, *Edwardsiella tarda*, and *Mycobacterium tuberculosis* (Motin and Torres 2009). The approach is also being used in the development of protein-based vaccines against antibiotic-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*, and to interpret transcriptomic and proteomic data in order to generate a short list of candidate antigens that can be used for in vitro analysis, thereby reducing the cost and time of downstream processing (Del Tordello et al. 2017). Thus, reverse vaccinology represents a revolutionary new vaccine development strategy that greatly improves upon conventional methods.

2.2   Next-Generation Sequencing

Present approaches for the identification of human pathogen are not sufficient to provide complete information related to disease pathogenesis. Next-generation sequencing (NGS) identifies the complete genome of an organism in a single

sequence. The results interpreted from these data provide, in detail, the underlying mechanisms of disease virulence and resistance, provides information on disease outbreak, and is being used to monitor the current and historic emergence of drug resistance in bacteria and other microbes. NGS technologies are used in various medical microbiology laboratories for the identification and characterization of causal pathogens, rapid identification of bacteria using the 16S–23S rRNA region, and in taxonomic and metagenomic approaches to the study of infectious disease. It is specifically used in the study of evolution and dynamics of drug resistance in bacterial pathogens (ECDC 2016). Various NGS software are available for analysis, including CLC Genomic Workbench (Qiagen) (Powell 2018), SPAdes (Lapidus et al. 2014), and Velvet (Zerbino 2010) for genome assembly analysis, multi-locus sequencing typing (Belén et al. 2009) (MLST) approaches, and conserved core genome (Ghanem et al. 2018) (cgMLST), or whole genome (wgMLST), for investigating genetic relationships (Chen et al. 2017). Many others, such as SeqSphere (Ridom) (Kohl 2014) and BioNumerics (Applied Maths, Biomérieux) (Hunter et al. 2005), or online tools, such as EnteroBase and BIGSdb (Bacterial Isolate Genome Sequence Database) (Jolley and Maiden 2010), are also available.

3 Proteomic Approaches to Drug Discovery

Proteomics is a science dealing with the global analysis of cellular proteins (Osman et al. 2009). It is defined as a complete set of proteins that are produced by an organism under certain conditions (Wasinger et al. 1995). In recent years, proteomics has become a powerful tool for the analysis of complex biochemical mechanisms, and identification of new protein structure, function, and protein–protein interactions. In addition, investigating protein profiles in response to antibiotic sensitivity and drug resistance can significantly contribute to the development of therapeutics for disease recurrence. Furthermore, with the emergence of bioinformatics it has become easier to retrieve information about specific genomes and proteomes for in-depth analysis. In short, proteomics has a diverse range of applications, including drug target identification and validation, efficacy and toxicity testing, and the investigation of drug mechanisms and activities.

3.1 Molecular Modeling

Protein 3D structure is an important perspective for structure-based drug design, as protein structure is vital to ligand binding. With the rapid emergence of homology modeling, it has fast become the first choice for protein 3D structure prediction (Srivastava and Tiwari 2017). Homology modeling techniques for structure prediction are based on sequence similarity to a homologous structure. It is an easy, reliable, low-cost, and less-time-consuming method than conventional means. The 3D structures of proteins provide valuable information about the underlying mechanisms and functions of the molecules, which plays a significant role in drug

discovery. Presently, over 137,000 experimental protein structures are available in the Protein Data Bank (PDB) (19).

Homology modeling involves several steps, including template identification, multiple sequence alignment, and model building based on the 3D structure of a template, as well as model refinement, optimization, and validation. Various studies have been conducted that reveal the significance of homology modeling in drug discovery. In one such study, the 3D structure of the N315 stp1 protein produced by a clinical strain of *Staphylococcus aureus* was predicted using homology modeling through modeler software (Jain et al. 2014). The predicted structure was then validated through PROCHECK, ERRAT, VERIFY-3D, and ProSA tools. Further, the structure was refined through GROMACS software to obtain a more stable and refined configuration. Thus, the predicted 3D structure of *Staphylococcus aureus* N315 stp1 provided valuable information about structure–activity relationships and interactions with the protein. Another study found that drug resistance in *Mycobacterium tuberculosis* is due to a multidrug efflux mechanism of the Mycobacterium multidrug resistant (MMR) protein (Malkhed et al. 2013), which belongs to small multidrug-resistant family of proteins (SMR). Thus, considering the MMR protein as a novel target, an in silico tertiary structure of protein was designed and constructed in order to identify a novel drug molecule for drug resistance in *Mycobacterium tuberculosis*. Another study on a virulent strain of CGSP 14 *Streptococcus pneumoniae* (Karavadi et al. 2014a, b) revealed that the genome codes for 2206 proteins, among which the polysaccharide polymerase protein (B2ILP9) and capsular polysaccharide biosynthesis protein (B2ILP4) act as efficient drug targets and were modeled through homology in order to discover the role of the proteins in the disease pathway of pneumonia. Hence, homology modeling plays a significant role in providing information about protein structure and function in less time, and in a cost-effective manner than conventional means, representing a boon for drug design and development. Various tools for molecular modeling are available, such as Modeller, I-TASSER, LOMETS, SWISS-MODEL, and Gromacs (Frantisek et al. 2007; Zhou et al. 2015; Chou 2004).

3.2 Virtual Screening and Molecular Docking

Virtual screening is defined as the process of screening the molecules from a library of chemical compounds based on their scoring and binding affinity to the specific target (Chen 1977). It is performed for the screening of most potential drug candidates based on their chemical properties, the Lipinski rule of five (Lipinski et al. 2001), their ADMET (Cheng et al. 2013) properties (absorption, distribution, metabolism, excretion, and toxicity), their interaction with the specific target, and their binding affinity as determined by a molecular docking study.

These methods have been used to screen a host of potential therapeutics. For example, in a study involving abnormal prion protein (PrP^{Sc}), which is responsible for the pathogenesis of prion diseases, structure-based drug discovery approaches were used to filter out the most promising compound with anti-prion effects,

showing high binding score and spatial interaction with the target, and remarkably reducing prion disease pathogenesis (Ishibashi et al. 2016). In another study, proteins identified from highly virulent strains of *Streptococcus pneumonia* (HUNGARY19A-6, D39, TIGR4, G54, CGSP14, TCH8431-19A) were modeled and validated (Nastasa 2018). Structure-based virtual screening was then performed to identify novel drug molecules against the proteins, and a docking study was utilized to analyze the protein–ligand interactions and binding affinity. Peptide deformylase protein (PDF), which is essential to the pathogenesis of several bacterial diseases, is used as an attractive target to identify novel antibacterial drug molecules based on binding affinity with hydrazine derivatives using virtual screening and molecular docking study (Karavadi et al. 2014a, b). Bacterial topoisomerase (khursheed 2013), a key target in antibacterial and anticancer drug discovery, was used to discover potential bacterial topoisomerase I inhibitors and structural motif using in silico screening. Various online and offline tools and software, such as *Pharmer, Catalyst, PharmaGist, Blaster, Anchor Query, Ligandscout, Autodock, Swiss-Dock,* and *GOLD*, are available for study and analysis (Sandhaus et al. 2018; Peter 2010; Kujawski et al. 2012; Jones and Rowland 2013; Parrott et al. 2014; Dubey et al. 2011).

3.3   Molecular Dynamic Simulation

Molecular dynamic (MD) simulation plays a very significant role in the drug discovery process. Through MD simulation, one can track the rapid processes of biological systems that can occur in less than a millisecond. It is used to study the physical movement of all the macromolecules, proteins, nucleic acids, atoms, and carbohydrates of biological significance (Cumming et al. 2013). Calculating the free binding energy of ligand–protein and protein–protein interactions is an important feature of the simulations.

Structure-based virtual screening with MD simulation and free energy calculation was used to study the activity of anon–peptide compound against falcipain 1 and 2 (FP-1 and FP-2), *Plasmodium*-produced proteins that catalyze hemoglobin degradation, and their analogs (D W. Borhani 2012). A South African natural compound, 5PGA, and five further potential compounds were identified as having inhibitory activity against FP-1, FP-2, and their analogs. In another study utilizing MD simulation of two *E. coli*–produced Resistance-Nodulation-Division (RND) transporters, AcrB and AcrD, which play a major role in multidrug resistance, researchers connected various specificity patterns of the two transporters to their physicochemical and topographical properties based on calculation of multifunctional recognition sites on the molecular surface (Musyoka et al. 2016). Another molecular docking and MD simulation study was performed on meropenem and imipenem, two antibiotics having different binding affinity for the efflux pump in *P. aeruginosa* and AcrB structures, revealed a greater susceptibility of meropenem over imipenem to the binding site of AcrB, and in-depth analysis identified a key residue involved in the binding interaction (Ramaswamy et al. 2017). These

examples illustrate how MD simulation is a very beneficial tool for structure-based drug design. Various MD simulation software is available, such as *Amber, CHARMm, Gromacs, NAMD,* and *Schrodinger's Desmond* (Bajic et al. 2016; Salomon-Ferrer et al. 2013; Brooks 2009; Phillips et al. 2005).

3.4 Toxicity Prediction

Determining the safety and toxicity of chemical compounds represents a crucial step in the drug discovery process. In silico toxicology prediction is a computational method used to visualize, analyze, simulate, and predict the toxicity of a chemical (David E. Shaw 2006). The aim of toxicity testing is to identify the effects of harmful chemicals on both the patient and the environment. Various parameters are involved in toxicity testing, such as the rate, frequency and dosages of exposure, ADMET (absorption, distribution, metabolism, excretion/elimination, and toxicity) properties, as well as other biological and chemical properties. In silico toxicology testing using computational approaches minimizes the use of animals while also reducing the cost and time requirements. Along with this, it also improves the safety and assessment of the chemicals. Various tools and software are available for computational toxicity testing, including *OSIRIS Property Explorer, ALOGPS, ADMET Prediction, Molinspiration,* and *TOPKAT* (Parthasarathi and Dhawan 2018; Dearden 2003; Tetko and Bruneau 2004; Ekins et al. 2017; Nadeem et al. 2015).

4 Antibacterial/Antimicrobial Databases

Biological databases are the most important feature of bioinformatics. They contain vast libraries of biological information, including genes, proteins, metabolic pathways, microarray data, next-generation sequencing data, and much more. Many antibacterial/antimicrobial databases are freely available that provide valuable information about the sequences, structures, and signatures of genes and proteins. This information plays a vital role in the drug discovery process in terms of gene/protein and screening of novel synthetic/herbal therapeutic compounds. Selections of important and useful antibacterial/microbial databases are listed in Table 11.1.

5 Future Perspectives

The emergence of drug-resistant bacteria is a major threat facing the world today, and there is a great need to improve the methods by which we investigate antibacterial drug-resistance mechanisms and to discover new therapeutic interventions to combat the issue. Traditional approaches to antibacterial drug discovery are very time consuming and expensive, and thus, the emerging field of computational approaches allows researchers to combine biological and chemical parameters in order to streamline the drug discovery pipeline in a time- and cost-effective manner.

Table 11.1 List of antibacterial/antimicrobial databases

Name of Database	Description	Website Address
Antimicrobial peptide database (APD)	Database of antimicrobial and peptides	*http://aps.unmc.edu/AP/*
Antimicrobial drug database (AMDD)	Includes a detailed repository of antibacterial and antifungal compounds, along with their basic information and properties	*http://www.amddatabase.info*
Antimicrobial compounds database	Provides information about antimicrobial compounds	*http://www.dsf.unica.it/~gmalloci/abdb/*
Collection of antimicrobial peptides (CAMP$_{R3}$)	Database of antimicrobial peptide family-based studies, including information related to sequence, structure, and signatures of peptides	*http://www.camp3.bicnirrh.res.in/*
The antimicrobial index	Contains information on microorganisms and antimicrobial agents	*http://antibiotics.toku-e.com/*
Data repository of antimicrobial peptides (DRAMP)	Includes information regarding sequences, structures, antimicrobials, physiochemical data, patents, references, etc.	*http://dramp.cpu-bioinfor.org*
PhytAMP database	Contains information about taxonomic, microbiological, and physicochemical data on small cysteine-rich antimicrobial peptides (AMPs)	*http://phytamp.hammamilab.org/*
Antibiotic resistance genes database (ARDB)	Provides information on antibiotic resistance	*https://ardb.cbcb.umd.edu/*
MIC database	Contains 2D structures of synthesized compounds/antibiotics, IUPAC names, smiles, MIC values, etc.	*www.trimslabs.com/mic/index.htm*
Antibacterial biocide and metal resistance genes database (BacMet)	Includes antibacterial biocide and metal resistance genes	*http://bacmet.biomedicine.gu.se/*
A database linking antimicrobial peptides (LAMP)	Provides a useful resource and tools for AMP studies.	*http://biotechlab.fudan.edu.cn/database/lamp*
MilkAMP database	Provides information on antimicrobial dairy peptides, including microbiological and physicochemical data	*http://milkampdb.org/home.php*
Antimicrobial consumption database (ESAC-net)	Provides data on antimicrobial consumption based on community and the hospital sector	*https://ecdc.europa.eu/en/antimicrobial-consumption/*

(continued)

Table 11.1 (continued)

Name of Database	Description	Website Address
The comprehensive antibiotic resistance database (CARD)	Provides information about resistance genes, their products, and associated phenotypes	*https://card.mcmaster.ca/*
Antimicrobial combinations database	Provides information on antimicrobial combination therapies	*http://sing.ei.uvigo.es/ antimicrobialCombination/*

Combining computational methods with wet laboratory experiments provides efficient ways to understand the entire mechanism of drug resistance, as well as pathogen virulence and progression. Computational approaches play a significant role in predicting the functions, properties, and activities of antimicrobial agents and their interactions with therapeutic targets throughout the drug discovery process. Bioinformatics provides advanced tools and software that allow microbiologists to analyze and interpret high throughput experimental data in a cost-effective manner, while the use of various databases, tools, and software by R&D laboratories makes practical application that much easier to realize. It is foreseen that the continued synergy of experimental data with computational approaches will lead to a new age of antibacterial drug discovery.

References

Bajic et al. (2016, March/April) *In silico toxicology: Computational methods for the prediction of chemical toxicity* (Vol. 6). WIREs Computational Molecular Science published by Wiley.

Belén, A., Pavón, I., & Maiden, M. C. J. (2009). Multilocus sequence typing. *Methods in Molecular Biology, 551*, 129–140.

Borhani, D. W. (2012). The future of molecular dynamics simulations in drug discovery. *Journal of Computer-Aided Molecular Design, 26*, 15–26.

Brooks, B.R. (2009, July 30). CHARMM: The biomolecular simulation program. *Journal of Computational Chemistry* 30(10): 1545–1614.

Chen, N. Y. (1977). The biological functions of low-frequency phonons. *Scientia Sinica, 20*, 447–457.

Yi Chen et al. (2017). Whole genome and core genome multilocus sequence typing and single nucleotide 2 polymorphism analyses of Listeria monocytogenes associated with an outbreak linked to 3 cheese, United States, 2013. *Applied and Environmental Microbiology*.

Cheng, F., Li, W., Liu, G., & Tang, Y. (2013). In silico ADMET prediction: Recent advances, current challenges and future trends. *Current Topics in Medicinal Chemistry., 13*(11), 1273.

Chou, K. C. (2004). Review: Structural bioinformatics and its impact to biomedical science. *Current Medicinal Chemistry, 11*, 2105–2134.

Cumming, J. G., Davis, A. M., Muresan, S., Haeberlein, M., & Chen, H. (2013). Chemical predictive modelling to improve compound quality. *Nature Reviews Drug Discovery, 12*, 948–962.

David E. Shaw (2006). Scalable algorithms for molecular dynamics simulations on commodity clusters. *Proceedings of the ACM/IEEE Conference on Supercomputing (SC06)*, Tampa, Florida, November 11–17, 2006.

de Kraker, M. E. A., Stewardson, A. J., & Harbarth, S. (2016, November). Will 10 million people die a year due to antimicrobial resistance by 2050? *PLoS Medicine* 13(11): e1002184.

Dearden, J. C. (2003). In silico prediction of drug toxicity. *Journal of Computer-Aided Molecular Design, 17*(4), 119–127.

Del Tordello, E., Rappuoli, R., & Delany, I. (2017) *Reverse vaccinology. Human vaccines.* Academic press.

Dubey, R. D., Chandraker, G., Sahu, P. K., Paroha, S., Sahu, D. K., Verma, S., Daharwal, S. J., & Reddy, S. L. N. P. (2011). Computer aided drug design: A review. *Research Journal of Engineering and Technology, 2*(3), 104–108.

ECDC. (2016). Expert Opinion on Whole Genome Sequencing for Public Health Surveillance.

Ekins, S., Spektor, A. C., Clark, A. M., Dole, K., & Bunin, B. A. (2017). Collaborative drug discovery for More Medicines for Tuberculosis (MM4TB). *Drug Discovery Today, 22*(3), 555–565.

Frantisek, F., Christopher, G. J., & Smytha, W. F. (2007). A simple, fast hybrid pattern-matching algorithm. *Journal of Discrete Algorithms, 5*(4), 682–695.

Ghanem, M., Wang, L., Zhang, Y., Edwards, S., Lu, A., Ley, D., & El-Gazzar, M. (2018). Core genome multilocus sequence typing: A standardized approach for molecular typing of mycoplasma gallisepticum. *Journal of Clinical Microbiology, 56*(1), e01145–e01117.

Hunter, S. B., Vauterin, P., Lambert-Fair, M. A., Van Duyne, M. S., Kubota, K., Graves, L., Wrigley, D., Barrett, T., & Ribot, E. (2005). Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: Converting the national databases to the new size standard. *Journal of Clinical Microbiology, 43*(3), 1045–1050. https://doi.org/10.1128/JCM.43.3.1045-1050.2005. http://www.applied-maths.com/bionumerics.

Ishibashi, et al. (2016). Structure-based drug discovery for prion disease using a novel binding simulation. *EBioMedicine, 9*, 238–249.

Jain et al. (2014). *Homology modeling and molecular dynamics simulations of a protein serine/threonine phosphatase stp1 in Staphylococcus aureus N315: a potential drug target* (pp. 592–599). Received 20 Dec 2013, Accepted 03 Mar 2014, Published online: 15 Apr 2014.

Jolley, K. A., & Maiden, M. C. (2010). BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics, 11*, 595.

Jones, H., & Rowland, Y. K. (2013). Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT: Pharmacometrics & Systems Pharmacology, 2*(8), e63.

Karavadi et al. (2014a) A novel approach of inter strain docking in accelerating the process of lead identification in pneumonia. *Journal of Chemical and Pharmaceutical Sciences* ISSN: 0974-2115.

Karavadi, et al. (2014b). Homology modeling of polymerase and cps biosynthesis proteins in cgsp14 strain of streptococcus pneumonia and its ligand identification: An insilico approach. *I. Asian Journal of Pharmaceutical and Clinical Research, 7*(Suppl 2), 162–165.

Khursheed, A. (2013). *In silico development of broad spectrum antibacterial by targeting peptide deformylase*. ethesis.nitrkl.ac.in/5132/.

Kohl, et al. (2014). Whole-genome-based *Mycobacterium tuberculosis* surveillance: A standardized, portable, and expandable approach. *Journal of Clinical Microbiology, 52*(7), 2479–2486.

Kujawski, J., Bernard, M. K., Janusz, A., & Weronika, K. (2012). Prediction of log P: Alogps application in medicinal chemistry education. *Journal of Chemical Education, 89*(1), 64–67.

Lapidus, A., Antipov, D., Bankevich, A., Gurevich, A., Korobeynikov, A., Nurk, S., Prjibelski, A., Safonova, Y., Vasilinetc, I., & Pevzner, P. A. (2014) New Frontiers of Genome Assembly with SPAdes 3.0. (poster).

Lipinski, C. A, Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001, March). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 46(1–3): 3–26. https://doi.org/10.1016/S0169-409X(00)00129-0.

Malkhed et al**.** (2013) *Identification of novel leads applying in silico studies for Mycobacterium multidrug resistant (MMR) protein* (pp. 1889–1906). Received 01 Jan 2013, Accepted 04 Sep 2013, Published online: 14 Oct 2013.

Mishra and Srivastava. (2017). Review on computational approaches for identification of new targets and compounds for fighting against Filariasis. *The Open Bioactive Compounds Journal* 5.

Motin, V. L., & Torres, A. G.. (2009). *Molecular approaches to bacterial vaccines* (pp. 63–76). Vaccines, 2009, Academic press.

Musyoka et al. (2016). Structure based docking and molecular dynamic studies of *Plasmodial* cysteine proteases against a South African natural compound and its analogs. *Scientific Reports* 6: 23690.

Nadeem, et al. (2015). Synthesis, spectral characterization and in vitro antibacterial evaluation and Petra/Osiris/Molinspiration analyses of new Palladium (II) iodide complexes with thioamides. *Alexandria Journal of Medicine, 52*, 279–228.

Nastasa, et al. (2018). Antibacterial Evaluation and Virtual Screening of New Thiazolyl-Triazole Schiff Bases as Potential DNA-Gyrase Inhibitors. *International Journal of Molecular Sciences, 19*, 222.

Osman, K. M., Ali, M. M., Radwan, M. I., Kim, H. K. & Han, J. (2009, July 21). Comparative Proteomic Analysis on Salmonella Gallinarum and Salmonella Enteritidis Exploring Proteins That May Incorporate Host Adaptation in Poultry. *Journal of Proteomics* 72(5): 815–821. ISSN 1876-7737.

Parrott, N., Hainzl, D., Scheubel, E., Krimmer, S., Boetsch, C., Guerini, E., & Martin-Facklam, M. (2014). Physiologically based absorption modelling to predict the impact of drug properties on pharmacokinetics of bitopertin. *AAPS Journal, 16*(5), 1077–1084.

Parthasarathi, R., & Dhawan, A.. (2018). *In Silico Approaches for Predictive Toxicology. In Vitro Toxicology* (pp. 91–109).

Peter, E. (2010). Molecular structure input on the web. *Journal of Cheminformatics, 2*, 1.

Phillips, et al. (2005). Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry, 26*, 1781–1802.

Powell, M. (2018, April 23). ECCMID18: QIAGEN announces European launch of platform for syndromic insights, QIAstat-Dx. *Infectious Diseases Hub*. Retrieved 28 June 2018. https://www.qiagenbioinformatics.com/products/clc-genomics-workbench/

Ramaswamy, et al. (2017). Molecular rationale behind the differential substrate specificity of RND transporters AcrB and AcrD. *Scientific Reports volume, 7*.

Rappuoli, R. et al. (2012, October). Developing vaccines in the era of genomics: a decade of reverse vaccinology. *Clinical Microbiology and Infection* 18 (Supplement 5).

Salomon-Ferrer, R., Case, D. A., & Walker, R. C. (2013). An overview of the Amber biomolecular simulation package. *WIREs Comput. Mol. Sci., 3*, 198–210.

Sandhaus, et al. (2018). Discovery of novel bacterial topoisomerase I inhibitors by use of in silico docking and in vitro assays. *Scientific Reports volume, 8*, 1437.

Srivastava and Tiwari. (2017). Critical Role of Computer Simulations in Drug Discovery and Development. *Current Topics in Medicinal Chemistry, 17*.

Tetko, I. V., & Bruneau, P. (2004). Application of ALOGPS to predict 1-octanol/water distribution coefficients, logP, and logD, of AstraZeneca in-house database. *J Pharm Sci*.

The Protein Data Bank and the challenge of structural genomics. (2000). Nature. *Structural Biology, 7*(11), 957–959. https://doi.org/10.1038/80734. http://www.rcsb.org/.

Wasinger, V. C., Cordwell, S. J., Cerpa-Poljak, A., Yan, J. X., Gooley, A. A., Wilkins, M. R., Duncan, M. W., Harris, R., Williams, K. L. & Humphery-Smith, I. (1995, July). Progress with Gene-Product Mapping of the Mollicutes: Mycoplasma Genitalium. *Electrophoresis* 16(7): 1090–1094. ISSN 0173-0835.

Zerbino, D. R. (2010). Using the Velvetde novo Assembler for Short-Read Sequencing Technologies. In Andreas D. Baxevanis (ed.), *Using the Velvet de novo assembler for short-read sequencing technologies*. pp. Unit 11.5. https://doi.org/10.1002/0471250953.bi1105s31. ISBN 0471250953.

Zhou, G. P., Huang, R. B., & Troy, F. A. (2015). 3D structural conformation and functional domains of poly sialyltransferase st8sia iv required for polysialylation of neural cell adhesion molecules. *PPL, 22*, 137–148.