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EDITORIAL OVERVIEW

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Although the development of new drugs can in no case ever be considered easy, the investment of enormous intellectual and financial resources over many decades has brought us to a point where high quality drug candidates for certain classes of targets can be routinely generated. However, major unmet medical needs continue to exist across a wide range of diseases, driving the drug discovery enterprise to solve increasingly complex problems and to confront ever more difficult targets. This evolution requires, among other things, an increasingly sophisticated understanding of how drugs work, a willingness to go after non-traditional target classes, the use of an expanded range of drug compound types and modalities, and improved methods for tracking drug distribution *in vivo*. Particular challenges include how to assess target druggability, and how to tackle target classes previously considered undruggable. We additionally need to continually improve our understanding of drug mechanism of action, to enable the efficient discovery and development of drugs with better attributes, and to improve dosing and safety profiles. In putting together this Special Issue on Next Generation Therapeutics, we have solicited articles that describe recent progress and future prospects covering many of the above needs.

Virtual screening – the computational assessment of a collection of compound structures to identify those most likely to bind at a particular site on a target – remains a mainstay of lead identification, especially for conventionally druggable protein targets such as the nucleotide binding sites of kinases. However, the application of computational docking to unusual or particularly challenging targets, such as protein-protein interaction interfaces and others discussed in this issue, has been less successful. Many different approaches to docking and pose selection are now available involving, for example, different scoring functions, or different ways of accounting for flexibility on the part of the receptor. Wingert and Camacho

discuss recent advances in both the software and strategies used for computer-aided drug design. They compare the predictive utility of various approaches across a range of target classes, concluding that the choice of which method is best is contextual, and depends on target type, and on what other information is available about the structure of the target and its known ligands. Often the choice of software and scoring function is less important than proper matching of strategy to target, and selection of which experimental target structure is most appropriate to use.

There is increased awareness that drug-target kinetics must be considered in the selection and optimization of drug candidates to improve the success rate of new drug discovery. However, our understanding of the molecular factors that control the lifetime of the drug-target complex is still in its infancy. The article by Lu *et al.* discusses the current state of knowledge about the mechanisms that govern drug-target residence time, illustrated by discussion of examples from several target classes, including kinases and G protein-coupled receptors (GPCRs). Their article highlights current approaches to unravelling the drug binding reaction coordinate, including structure-kinetics relationships and molecular dynamics simulations, with a view to developing the ability to tailor the residence time of a drug candidate to its particular application.

A class of drug targets that has received much attention in recent years, but which still remains highly challenging, is that of protein-protein interaction (PPI) interfaces. As a field, we are still in the early stages of developing reliable approaches to the discovery of potent, selective and bioavailable small molecule PPI inhibitors. Ran and Gestwicki review the 66 small PPI inhibitors that have been reported during the past three years, with a view to learning which approaches are working and which are not. They discuss how PPI interface area correlates with inhibitor binding affinity, and with whether success was found using conventional small molecule compounds versus peptides. They extract lessons concerning how considerations such as the availability of an experimental X-ray structure of the target, or whether the target undergoes a conformational change, affect prospects of finding an inhibitor. Finally, the authors present an overview of what these recently published examples reveal concerning how to think about the “druggability” of a PPI target, and discuss the outlook for inhibiting that subset of PPI targets that is truly intractable to conventional small molecule inhibitors.

Another class of targets that is considered to be highly challenging includes proteins involved in protein folding disorders, such as those associated with serious neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's, Amyotrophic Lateral Sclerosis (ALS), and others. Scannevin reviews current efforts toward developing therapies for protein misfolding disorders, focusing on drugs that have entered human clinical trials. He considers these therapies not in terms of which disease indication(s) they address, or the identify of their molecular target, but instead in terms of the different strategies they employ. He identifies four classes of approach: inhibiting production of the parent protein, inhibiting the aggregation of the misfolded protein, removing the toxic aggregated forms of the misfolded protein, and mitigating the toxicity of the misfolded proteins. Comparing the clinical candidates in this way allows useful

comparisons to be made between drugs that address different diseases and target different proteins, but work in a similar manner.

Structure-based drug design involves the development of molecules that bind to defined pockets or sites on the target, resulting in a desired pharmacological outcome. However, in many cases suitable binding pockets cannot be readily identified in unliganded X-ray structures, hindering the development of antagonists and inhibitors using rational design and approaches such as *in silico* docking. Cryptic sites are binding sites that are not found in the unbound protein but, as a result of conformational change, become apparent when a ligand is bound. Targeting cryptic sites is a topic of high recent interest as a strategy for inhibiting targets for which the main active site is poorly druggable. Vajda *et al.* point to ambiguity in the literature concerning how a cryptic site should be defined, and review recently published computational strategies for identifying cryptic sites. They review evidence showing that a substantial fraction of the putative cryptic sites identified by Molecular Dynamics (MD) simulations appear unlikely to be druggable, and discuss how the reliable detection of pharmacologically relevant cryptic sites can be improved by supplementing MD with computational hot spot mapping, or by employing machine learning approaches.

Another approach to drug discovery against poorly druggable targets that has received substantial recent attention involves the use of compound types that violate conventional guidelines for “druglikeness”, as embodied for example in Lipinski’s Rule-of-Five or Veber’s Rules. These so-called “beyond Rule-of-Five” (bRo5) compounds, many of which have molecular weights well above 500 Da., can be better suited to bind to poorly druggable sites. However, at least since the concept of “druglikeness” was developed in the 1990s, such compounds have been strongly disfavored, primarily due to the increased difficulty in achieving good oral bioavailability and other desirable pharmaceutical properties. Poongavanam, Doak and Kihlberg review instances of approved oral drugs with molecular weight >500 Da, and discuss our rapidly growing understanding of how oral absorption and cell permeability can be achieved with such bRo5 compounds. They also provide an overview of current guidelines for the design and optimization of compounds that lie in bRo5 space.

Yet another emerging approach to drug discovery against challenging targets is the use of Targeted Covalent Inhibitors (TCI). TCIs are compounds that bind with moderate affinity to the target site, but in so doing place a reactive “warhead” in a position that promotes rapid covalent reaction with a specific amino acid side-chain on the protein. Formation of a covalent adduct with the target promotes high inhibitor occupancy of even a poorly druggable site, can provide a long drug-target residence time if desired, and can confer enhanced selectivity over related proteins if the covalently-targeted residue is poorly conserved across the protein family. To-date, efforts to develop TCIs have focused almost exclusively on labeling a cysteine residue on the target of interest, limiting the approach to the subset of proteins that have an exposed cysteine adjacent to a functionally relevant binding site. Mukherjee and Grimster review recent progress towards expanding the scope of TCIs to achieve efficient and selective covalent labeling of other amino acid types, including lysine, tyrosine, serine, threonine, and others. They survey recent publications in which residues other than cysteine have been targeted by covalent inhibitors, and discuss the

chemistry of the reactive “warheads” that have been used to covalently label each residue type.

An orthogonal approach to antagonizing the activity of highly challenging targets is to promote the degradation of the protein target in the cell using Proteolysis Targeting Chimeras (PROTACS). PROTACS are bifunctional molecules that bind to the target protein and recruit E3 ubiquitin ligases into the complex, thereby causing the target to become ubiquitinated and then proteolytically degraded by the proteasome. However, the properties required for a given PROTACS agent to be most effective are still being worked out. Philips and Fisher provide an overview of the fundamental principles involved in the design and application of these heterobivalent degradation promoting molecules. In particular, the authors describe how the catalytic action of PROTACS can be described using kinetic models developed for enzymes, and point out that the concentration dependence of their activity will be bell-shaped due to the “prozone” or “hook” effect. These models provide a foundation for the rational optimization of such degraders.

Therapeutic antibodies have a long and successful history as drugs, working to inhibit extracellular protein targets, or to kill disease-causing cells through antibody-mediated cytotoxicity or the targeted delivery of cytotoxic drugs. In their article, Lee *et al.* focus on a third therapeutic mechanism for the application of therapeutic antibodies to cancer, involving the use of antibodies to stimulate the anti-cancer immune system. Checkpoint inhibitor antibodies function by restarting the cancer-immunity cycle in order to target tumor cells that have evaded the immune system. The authors discuss therapies such as ipilimumab, an anti-CTLA-4 antibody, as well as antibodies that target the programmed death-ligand 1 (PD-L1) and programmed death-1 (PD-1) pathway that have reduced immune-related adverse effects.

Antibodies do not always possess ideal properties for the therapeutic goal at hand. Consequently, there has been great interest in exploring other protein scaffolds as platforms for drug discovery. These non-antibody protein scaffolds are frequently highly engineered, both to achieve binding affinity and specificity toward the target, and to optimize other properties. Wuo and Arora review different the classes of engineered protein scaffolds relevant for drug discovery, and compare the geometries and physicochemical features of their target binding interfaces. They then discuss how these proteins can be used as starting points for the development of conformationally-defined synthetic compounds that mimic key secondary structure elements of the binding epitope, thereby preserving key binding features in the context of a much smaller molecule.

The choice of target is crucial to the prospects for successful therapeutic intervention in a given disease. The reason why certain diseases continue to be major problems in human health, despite years of research into their causes and treatments, can be not only due to a technical inability to identify a molecule that will effectively modulate the activity of a key target protein. In some cases, the challenge has instead been to identify the appropriate point(s) for pharmacological intervention. A particularly important example is tuberculosis (TB), caused by infection with *Mycobacterium tuberculosis* (*Mtb*), which remains the leading cause of death worldwide. In this issue, Bonds and Sampson review recent literature

suggesting that one way to treat *Mtb* infection is to target the organism's acquisition and metabolism of host derived lipids such as cholesterol. They propose an indirect approach to drug development against TB, by targeting *Mtb* cholesterol metabolism through dysregulation of its pathways for nutrient utilization.

In addition to advances in our understanding of drug-target binding and the implementation of new drug modalities, such as those described above, developing new and better drugs additionally requires improved approaches for tracking how a given drug distributes within the cell or within and between tissues. This is a pressing need, for example, in the development of antibiotics to treat infections caused by Gram negative bacteria, which is hindered by both the impermeability of the bacterial cell envelope and the presence of pumps that cause rapid efflux of drug molecules. Six *et al.* compare and contrast current approaches to quantifying the accumulation of small molecules in bacteria. Label free methods based on mass spectrometry are now starting to offer the possibility of assessing the subcellular localization of compounds in bacteria. When implemented in high throughput, these approaches will allow structure-accumulation studies to be performed on compound libraries to reveal the factors that govern bacterial cell penetration and efflux.

In addition to penetration into bacteria, drugs also have to reach the site of infection at therapeutically relevant concentrations. Prideaux *et al.* describe the use of mass spectrometry imaging (MSI) to quantify drug distribution in tissue. The authors compare and contrast several approaches, including an emerging technique in which laser capture microdissection (LCM) is coupled with LC/MS/MS to provide high spatial resolution and accurate compound quantification. The power of LCM-LC/MS/MS is exemplified by a study in which this approach was used to quantify the distribution of the tuberculosis drug bedaquiline in tissue from a mouse model of *Mtb* infection.

As the above articles make clear, this is an exciting time to be involved in drug discovery research. Significant advances in our understanding of fundamental aspects of drug design, innovative new drug modalities, and major developments in analytical methods and other areas of pharmaceutical science, appear with astounding frequency. It is an impossible task to summarize all of the recent major advances in a single journal issue, and we regret that there were many significant developments that we were unable to include. However, we hope that the selection of articles assembled herein provides a set of timely and authoritative updates on several important areas of rapid recent and current progress, that are likely to have a major impact on the next generation of therapeutics and beyond.