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Introduction

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Over the last two decades, biophysics has reemerged as a core discipline in drug discovery. Many may argue that biophysical methods never truly left discovery, but all will note the renewed present importance and central role of such methods. This reemergence is driven by three primary forces: the birth of fragment-based drug discovery schemes, the recognition of and desire to mitigate artifacts in traditional biochemical screening, and a desire to accelerate the transition from first-in-class to best-in-class molecules by focusing on hit and lead kinetics. Each of these strategies or goals requires various information-rich biophysical methods to experimentally execute. This text aims to summarize some of the key methods emerging from these three broad enterprises. First, though, it will map the contours of these three drivers of biophysics' reemergence and link them to the chapters that follow.

Fragment-based drug discovery and fragment-based lead discovery are slightly different names for the same discovery approach: using a library of relatively small compounds to probe the surface of a target protein for binding sites. Fragment-based discovery approaches are animated by the information theory-based idea that relatively simple, small compounds sample chemical space more effectively than larger, more complex molecules [1, 2]. In practice, this approach drives one to develop low complexity screening libraries [3, 4]; consequently, the binding interactions with target proteins are generally very weak. Weak interactions require sensitive methods to unambiguously detect the binding event [5]. In simple bimolecular binding, the concentration of the complex is driven by the concentration of the ligand; this drives many scientists to screen their fragment libraries at relatively high concentrations. Effective screening methods must both be able to detect relatively weak interactions in the context of relatively high compound concentrations; several biophysical methods are well suited for this demanding screening campaign [6]. Various NMR approaches have been successfully applied to identify and characterize weak small molecule–protein interactions [7]. This text explores both traditional protein-detected NMR [8] approaches in Chapters 9 and 10

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and nontraditional NMR [9, 10] approaches in Chapter 8. Both approaches have merit and are usefully applicable in partially overlapping circumstances. Surface plasmon resonance (SPR) [11, 12] and microscale thermophoresis (MST) [13] have also been successfully deployed in fragment screening campaigns to detect weak interactions. Chapters 5 and 6 explore applications of MST and SPR beyond fragment-based discovery, respectively.

A second force driving the reemergence of biophysical methods in drug discovery has been the desire to identify and eliminate high-throughput screening hits that operate through uninteresting nuisance mechanisms. Brian Schoichet recognized and characterized some commonly observed nuisance phenomena; many of these nuisance mechanism enzymatic assay hits had weak micromolar activities and showed either a flat or highly irregular SAR [14]. Schoichet's team determined that the aberrant behavior in biochemical screening assays was driven by poor solubility resulting in compound aggregate formation. These compound aggregates, present in extremely low concentration, serve as protein sinks, adsorbing most of the target protein, yielding what appeared to be detectable but weak inhibition [15]. His team demonstrated that many of these aggregation-based inhibitors could be culled from screening hits by comparing activity in an assay with no or very low detergent to a high detergent assay condition. Compounds that lose activity in the high detergent assay were likely to be uninteresting nuisance hits.

Several biophysical methods complement the differential detergent biochemical assay [16]. In the biochemical assay approach, the presence of aggregates is inferred, whereas in the biophysical approaches, the aggregates are directly detected. SPR is uniquely suited such direct detection of nuisance behavior in a buffer matched to the original biochemical screening buffer [17]. Aggregated compounds generate complex binding responses that are not simple 1 : 1 interactions but rather reflect the partitioning of the aggregated compound between the free buffer and the protein captured on the sensor chip. Aggregated compounds also show complex binding to the sensor surface with no target protein captured, providing a simple, parallel means to detect nonideal interactions in real time during library screening. Hit validation workflows now commonly employ SPR, mass spectrometry, and other biophysical methods to remove nuisance mechanism hits [18].

A third trend driving the reemergence of biophysics in drug discovery is the desire to optimize kinetic or thermodynamic properties with an aim to rapidly progress from a first-in-class compound to a best-in-class compound. When comparing a first-in-class compound to a best-in-class compound, the best-in-class molecule generally has high selectivity for the pharmacologic target and consequently a lengthy residence time with that target [19]. Detailed understanding of compound binding kinetics [20] and inhibitory mechanism leads to better candidates with properties more like an ideal best-in-class compound [21]. SPR allows real-time analysis of binding kinetics [22]; streamlined experimental approaches allow rapid compound sorting based on kinetic parameters [23]. Combining thermodynamic data with affinity and kinetic data further characterizes the intermolecular interactions, enabling detailed SAR and further compound optimization [24]. This idea is explored and different methods applied inform interaction quality in Chapters 2, 4, 7, and 11.

The text concludes with a case study in Chapter 14 that joins many of the methods and concepts discussed in earlier chapters. The Pfizer research team used a combination of traditional biochemical analysis, focused structural information derived from NMR,

SPR kinetics, and NMR dynamics to optimize a *Staphylococcus aureus* DHFR inhibitor. Data from no one method assured success; it was the conjunction of data from the several biophysical techniques that enabled their focused, hypothesis-driven prospective library design that ultimately yielded novel, nonacid cell-active inhibitors. Importantly, the dynamics and kinetic data incorporated common resistance mutations, informing the library design and ultimately the candidate compounds. This discovery case study exemplifies the fully integrated discovery approach where data-rich biophysical techniques continually inform discovery. This approach enables research teams to target transient protein conformations, protein–protein interaction surfaces, or complex enzyme targets—all examples of targets that have met will have little success with traditional high-throughput enzymatic screening [25].

This text is a survey of contemporary biophysical methods in drug discovery. Biophysical methods report on intermolecular interactions directly with rich detail; these methods naturally complement traditional high-throughput screening [26, 27], particularly when attacking irregular, nonenzymatic [28, 29], or membrane protein [30, 31] targets.

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