Once the fragment hits have been validated, the hit-to-lead process begins. It is essential for the success of this process to have an assay that can reliably measure the affinities of very weak binders, is robust enough to tolerate high concentrations of compounds and solvent [i.e., dimethyl sulfoxide (DMSO)], and has sufficient throughput to test tens to hundreds of compounds. Hits are typically ranked on the basis of ligand efficiency and other factors (e.g., synthetic attractiveness, availability of structural information), and analogs are screened to expand the scaffold classes, find more potent compounds, and identify structure/activity relationships. In choosing hits for follow-up, an LE > 0.3 kcal/mol-atom cutoff is sometimes imposed because this is the minimum LE value predicted to produce a 10 nM final inhibitor that conforms to the MW = 500 (rule-of-five) limit.

The most desirable result from the hit-to-lead process is to find multiple scaffolds that bind to the same hot spot on the receptor using a similar pattern of interactions and that offer chemically tractable sites for adding functional groups that can access nearby subsites. Using the bound structures of overlapping hits it is sometimes possible to design hybrid molecules that combine the features of multiple molecules. As fragments are optimized into potent inhibitors, they usually maintain the original binding mode of the fragment hit. Caution is indicated if the hits are found to bind at multiple locations in the active site and/or in a variety of different orientations, without making a consistent set of interactions with the receptor, or if the binding mode changes dramatically during optimization. These observations indicate that the fragments can bind in many different modes that have approximately the same energy, rather than a single mode that is much lower in energy than the rest, and the binding energy landscape of the receptor may not offer a suitable hot spot for placing a molecular anchor.[33] We suspect that proteins exhibiting a high degree of conformational flexibility may be particularly susceptible to this problem, because they are more likely to possess multiple conformational states that have similar energies.

For cases where two or more fragments are found to bind in close proximity, the additivity of binding energies[37,46,47] favors linking them together (e.g., linking two millimolar fragments to make a micromolar binder). Because the linked molecule suffers an entropic penalty on binding of just one rather than two molecules, it is expected to be much more potent than the sum of the fragments. Although the linking approach is conceptually elegant, it is seldom used because of the practical difficulty of connecting two fragments using the limited set of synthetically accessible bond lengths and angles provided by nature, without straining or perturbing the favorable interactions of either fragment or introducing unfavorable entropic or enthalpic effects from the linker. In practice, the actual potency gained by linking fragments is usually significantly less than theory (in one study the gains were, on average, fivefold lower than expected).[48] Also, in many cases the binding site contains only one hot spot consistently targeted by fragments, so a set of independent second site binders is not available. For these reasons, it is far more common for fragments to be optimized by elaborating or building out from scaffolds bound to a single site.

EXPERIMENTAL METHODS: DETECTION OF FRAGMENT BINDING BY NMR

In the early development of NMR fragment-based screening techniques, literature descriptions of new experiments focused on methods of detection rather than the lead generation strategies. As the number of studies grew, and real drug discovery problems were addressed, it became apparent that experimental approaches and fragment follow-up strategies could be combined in many different ways to uniquely address each target and drug design program. For this reason, it is best to consider the physical methods used to detect binding in NMR-based screening separately from the strategies used to elaborate fragment hits into medicinal chemistry leads. In the following sections, the most commonly used experimental techniques are reviewed.

NMR has long been established as a sensitive method for detecting binding of small molecules to macromolecular targets. Methods for detecting ligand binding by NMR can be either target directed or ligand directed. Target-directed methods rely on observing a change in an observable NMR parameter of the target biomolecule that results from its interaction with a ligand. Alternatively, ligand-directed techniques rely on the observation of a change in an NMR parameter of the ligand, which arises as a consequence of its interaction with the target receptor. Each method has advantages and disadvantages, and choosing the optimal approach will depend on a number of factors such as the molecular weight of the macromolecule, solubility and expression yield of the target protein, and, most importantly, the overall requirements of the drug discovery project.

Target-directed methods

Target-directed detection of ligand binding is most often accomplished by observing differences between the chemical shift of one or more resonances of the receptor spectrum in the presence of a mixture of ligands relative to those of a reference spectrum of the receptor in the unliganded state. When differences are observed, additional spectra are then acquired using the individual components of the ligand mixture to deconvolute the mixture spectrum and determine the identity of the binding ligand. In principle, any NMR spectrum can be acquired for this purpose, but the high sensitivity and resolution of two-dimensional ^{15}N-^{1}H correlation spectroscopy using uniformly ^{15}N labeled protein makes it the most frequently chosen method. If sequence-specific resonance assignments are available for the target protein, the amino acid residues at the interaction site of the ligand can be readily identified by comparison with resonances observed to undergo chemical shift perturbations.