three orders of magnitude enhancement in activity during the first round of fragment elaboration.

The primary determinant for choosing a fragment for chemical elaboration is a high-quality, unambiguous crystal structure of the target-fragment complex at better than 2.5Å resolution, which clearly reveals the orientation of the bound ligand and the conformation of the polypeptide chain segments forming the ligand binding site. As discussed earlier, the ligand efficiency of the fragment hit represents an important parameter with which to prioritize among potential starting points for medicinal chemistry elaboration. Biochemical measurements of IC_{50} and/or SPR measurements of binding kinetics (k_{on} and k_{off}) permit estimation of ligand efficiency. In aggregate, fragment hits are prioritized for synthetic elaboration based on the following criteria: location of the fragment binding site, fragment binding mode, structural accessibility of chemical handles for synthesis, a preliminary evaluation of synthetically accessibility, potential novelty in terms of patentability, the conformation of the protein, and ligand efficiency.

Ideally fragment hits will bind to either the active site or a known allosteric site. Fragments that bind at previously unknown sites remote from a lattice packing interface represent opportunities for discovery of novel/selective lead compounds, but such sites require validation through fragment elaboration into more potent compounds with which to perform definitive biochemical or cellular assays. The mode of fragment binding must orient synthetic handles toward pockets or subsites on the surface of the target protein. If the intrinsic synthetic handles of the fragment hit are oriented only toward solvent or are sterically blocked, alternative handles may be found by searching for available fragment analogs or introduced via synthesis of a fragment analog. Synthetic feasibility is assessed by considering the diversity of available reagents that are compatible with the fragment hit and related synthons. Ligand efficiency is assessed by examining the ratio of biochemical activity to the size of the fragment (see above). Novelty is evaluated in terms of both the fragment hit and its binding mode. A familiar fragment can be observed to bind in an unusual way, which can provide novel elaboration opportunities. Observing a common binding mode for similar fragments sometimes provides an initial SAR and gives support, albeit indirectly, for fragment hit selection. Previous experience with the same or a related target and the same or a similar scaffold represented by the fragment hit can also help support the choice of a fragment hit. Fragment biochemical activity is usually less important than the criteria described above, because poorly oriented or ligand-inefficient fragments can be difficult if not impossible to optimize.

A detailed account of how we use computational chemistry tools to plan fragment elaboration chemistry has been published by Blaney et al.[22] Our current methods of predicting binding free energies of partially elaborated fragment hits are used to compare different elaboration routes for the selected fragment hits, to select the best of these routes, and to prioritize analogs for synthesis. This approach is currently too expensive from the computational standpoint to apply to all of the possible virtual libraries for all fragment hits.

Our goals for the first stage of fragment optimization are to improve binding affinity by at least 100-fold for each chemical handle (i.e., $IC_{50} \sim 1-10\,mM \rightarrow \sim 10-100\,\mu M$), to validate the selected fragment by establishing an initial SAR at each available synthetic handle, and to correlate this SAR with observed cocrystal structures and computational predictions of potency. As discussed previously, optimization of fragment hits into nanomolar leads typically requires an increase in binding energy of ~4–9 kcal/mol (three to six orders of magnitude). Without appeal to structural information, this task would be daunting.

With timely access to the right cocrystal structures, weakly binding fragments have been successfully optimized into potent lead compounds. At SGX, access to our proprietary x-ray beamline at the APS provides very rapid turnaround between compound synthesis and cocrystal structure determination. The median time required for x-ray data collection and structure determination is forty-eight hours, with 90% of requested cocrystal structures being delivered to the project team within ninety-six hours. With such rapid turnaround our multidisciplinary design teams (consisting of medicinal chemists, computational chemists, and protein crystallographers) can make decisions regarding the next round of compound synthesis with a full three-dimensional view of the SAR for the evolving lead series.

SGX has had considerable success with the fragment engineering method, wherein optimization involves "growing" each fragment with small focused analog libraries at each synthetic handle, followed by synthesis of multiply elaborated fragments using the better substituents identified for each chemical handle. This approach is stepwise, systematic, and lends itself to maintaining and even improving ligand efficiency. Once fragments have been selected for synthesis, available reagents are assembled to generate various small focused analog libraries. Compounds are prioritized for synthesis using predicted binding free energies for each fragment analog and on the basis of medicinal chemistry SAR considerations. In silico docking of fragments is not part of the SGX fragment-based drug discovery strategy. Experience has shown that much more reliable results can be obtained using experimentally determined structures of protein-fragment complexes as starting points for planning synthetic chemistry.

The resource needs of such an exercise depend on the particulars of the target. SGX metrics for this initial SAR exploration are as follows: duration = 2–8 months (average 4 months), number of fragment analogs synthesized = 10–150 (average 60 analogs), potency gain = 500–10,000 fold (average 3,500 fold). Following experimental validation of the optimization potential of a given fragment hit, further