a ten-compound, shape-diverse mixture, the structure of the target protein is automatically redetermined by molecular replacement using a reference target structure predefined in the SGX LIMS. Once this step is complete, the structure is partially refined and a difference Fourier synthesis is calculated to reveal any superficial electron density features that cannot be explained by either the structure of the protein target or surrounding water molecules. For each unexplained electron density feature, an attempt is made to automatically identify the fragment within the mixture that best corresponds to the shape of the electron density feature.

Once the automated processing/fragment identification is complete, "snapshots" of each difference electron density feature, with accompanying ligand atomic stick-figure interpretation, can be accessed via the SGX LIMS. Visual inspection of these images represents the first point at which manual intervention by a protein crystallographer is required. Expert inspection of the electron density images facilitates prioritization of the three-dimensional map viewing process and assessment of the results of automated fragment fitting. This combination of proprietary and public domain software tools provides an efficient process for analyzing the results of SGX core fragment library x-ray screening.

In addition to providing the all important "direct look" at the fragment binding to the target, x-ray screening has proven remarkably sensitive. Fragments with measurable IC_{50} values up to 50 mM have been detected, and in some cases we have not even been able to measure the binding affinity of a fragment hit. The sensitivity of the x-ray screening approach obtains from the very high local protein concentration within a typical crystal (~0.1 M). The other advantage of x-ray screening comes, paradoxically, from the limitation of the crystallographic method itself. Visualization of fragment hits via difference Fourier syntheses depends critically on the fraction of individual protein molecules comprising the crystal to which ligand is bound (i.e., fractional occupancy) and on how well the fragment is anchored to its binding site on the surface of the protein. Average occupancy levels must exceed ~30% for a ligand to be detectable by x-ray screening. The ligand must also bind to the target with a single, well-defined set of intermolecular interactions. If a fragment binds to multiple subsites within an enzyme active site, the resulting electron density feature(s) will be weak and blurry and will, therefore, be scored as an uninterpretable negative. Thus, fragment hits detected by x-ray screening exhibit both high fractional occupancy and well-defined anchoring to the surface of the target protein.

Complementary biophysical screening

As a complement to crystallographic screening, SGX frequently conducts both biochemical and surface plasmon resonance (SPR) screening of the ~1,500-compound core fragment library. Biochemical screening is performed using a Beckman BioMek FX liquid-handling system equipped with a Sagian rail. Our core fragment library can be screened one compound at a time via the appropriate biochemical assay in less than a day to complement results from crystallographic screening. It is often challenging to use biochemical assays to characterize weakly binding ligands, because of the problem of spectral interference. We screen the SGX core fragment library at 500 μM ligand concentration, using biochemical assays formatted to minimize spectral interference, while maximizing throughput. IC_{50} values are determined for all biochemical hits (defined as >~50% inhibition). Spectral interference is not a shortcoming of SPR screening, which is performed with a Biacore T-100 instrument using either 96- or 384-well compound array formats. Our core fragment library can be screened one compound at a time in a week to complement results from both crystallographic screening and biochemical assays. Data from biochemical/SPR screening are automatically imported into the SGX LIMS for comparison with the results of x-ray screening.

Comparison of x-ray, biochemical, and SPR screening

Combining x-ray screening with biochemical assays and SRP studies typically reveals compound hits common to all three approaches plus hits limited to two of three methods and hits peculiar to a single method. Follow-up x-ray crystallographic studies of individual fragment soaks or cocrystallization are used as the final arbiter of the utility of hits coming independently from biochemical assays and/or SPR screens. X-ray validated biochemical/SPR hits are retained for further evaluation. Biochemical/SPR hits that cannot be confirmed by crystallography are abandoned. Although rare, because the hit rate is ~1–5%, x-ray screening sometimes fails to detect a fragment hit because of masking by the presence of a more potent compound in the same shape-diverse fragment screening mixture. Biochemical assays and/or SPR screening of single fragments can overcome this shortcoming. The following section discusses criteria used to select fragment hits for structure-guided optimization.

Structure/activity relationship optimization

As discussed above, a typical crystallographic screen yields approximately fifteen to seventy-five hits per target with binding affinities (IC_{50}) ranging from low micromolar to low millimolar levels. A fragment hit is useful only if it can be elaborated through efficient synthesis in directions that rapidly lead to dramatic improvements in activity. Computational prediction of which fragments represent the best candidates for optimization is not feasible because of the huge number of possible analogs that can be generated from each fragment and the computational time required for predicting binding free energies. Instead, we select four to five of the most promising fragments to optimize in parallel. Our experience has shown that careful selection and prioritization of fragment hits typically provides two to