

Experiences with Fluorescence Methods in Ultra-High Throughput Screening and Drug Discovery Using the Analyst

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Exelixis has established an ultra-large compound library of >2 million compounds. The compound library encompasses both structural diversity and depth as represented by the number of molecular scaffolds and the number of analogs present of each scaffold, respectively. Evaluation of the library against discovery targets requires the use of high-throughput screening formats that are efficient in terms of speed and cost. Homogeneous optical methods are well suited to meet those requirements; therefore fluorescence intensity, fluorescence polarization and luminescence have been employed in assay development for HTS at Exelixis. Two multipurpose plate reader systems are available to support HTS, including the Analyst (Molecular Devices). For example, several protein kinases were screened using the STX fluorescence polarization assay (Molecular Devices), in which the sensitivity of the Analyst made it the preferred platform. Examples of HTS results obtained with the STX assay will be provided, including a comparison to a radiometric ³³P-phosphate transfer assay for the determination of compound potency. A disadvantage of the STX assay is its sensitivity to interference from intrinsic compound fluorescence, resulting in false positives. Another example application of the Analyst is a series of high-throughput assays of drug metabolizing cytochrome P450s. These fluorogenic assays employ coumarin- or fluorescein-labeled substrates of various cytochrome isoforms to detect inhibition. Representative dose response (IC₅₀) curves will be provided.