

Towards a comprehensive strategy for target identification of small molecule drug candidates using chemical proteomics and orthogonal approaches

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Target identification and the elucidation of mechanism of action (MoA) for bioactive small molecules are key steps in drug discovery, in particular in the context of phenotypic approaches to drug discovery. In phenotypic screening, compound libraries are used to screen cell-based or *in vivo* assays to identify compounds that modulate cellular models of disease. These strategies have seen a resurgence in recent years as an alternative to target-based strategies since they offer several advantages, e.g.: The potential to identify novel and unexpected druggable proteins and mechanisms in a given disease context, pre-selection for compounds with proven cellular activity and the ability to identify compounds that elicit their phenotype via activity on multiple targets. In order to enable further progression of primary compound hits into drug development, target identification and MoA elucidation are typically required and various strategies have evolved to address these aspects:

Affinity-based approaches aim to describe the protein interactome of drug candidates that constitutes the full spectrum of potential efficacy and off-targets. Quantitative chemical proteomics has emerged as a powerful affinity-based approach that is directly applicable to disease-relevant cell lines and tissues. Standard lysate-based, non-covalent affinity chromatography approaches based on affinity probes that have been validated to retain cell activity have been highly successful for certain target classes, including kinases and other enzyme families and in particular soluble members. However, they have a significantly lower success rate for important target classes such as ion channels and G-protein coupled receptors that require the intact cellular environment for compound-binding competence. In these cases, covalent strategies such as photocrosslinking-based experiments using live cell treatment have proven to be useful but require careful experimental design and optimization. On the other hand, several approaches have been introduced recently that conceptually circumvent the requirement for identification and synthesis of specific affinity probes and thus can be suitable alternatives where generation of affinity probes is challenging. These include random compound display using photocrosslinker-based affinity matrices as well as the Cellular Thermal Shift Assay (CETSA).

Our efforts towards a comprehensive chemical proteomics strategy combining the various covalent and non-covalent approaches will be discussed in the context of a multipronged strategy for de-novo target deconvolution that also includes functional genetic strategies, cellular profiling and in-silico approaches that often provide crucial orthogonal information.