



Short Communication

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# New Promises of Chemical Proteomics for Drug Development



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**Abbreviations:** TPP: Thermal Proteome Profiling; FITeXP: Functional Identification of Target by Expression Proteomics;

## Short Communication

The road to development of a new drug often begins with the idea of its target protein, which could result from evidence emerging from different research sources, including academia, clinics and industry. However, taking a single compound to the drug market involves a very high cost, often over a billion of US dollars, and requires on average a time commitment between ten and fifteen years. The entire process is very complicated and, even in the lucky cases when the final development steps are reached, it sometimes collapses because of a failure related to the drug target, which is its starting point. Indeed, one of the major reasons of drug failures in clinical trials is the lack of efficacy due to low specificity of the drug towards the desired target. Sometimes, when the promising candidate compound is found in a large-scale phenotypic screening, the target is not even known a priori. Thus both identification and validation of targets are fundamental processes, and major bottlenecks, of modern drug development. A good example is the anti-cancer drugs, which probably represent the biggest portion of the entire drug development sector. There, a significant number of perspective compounds as well as many approved drugs are still poorly characterized in terms of their targets and mechanisms of action. Some of these drugs have been discovered in hypotheses-driven studies, and their thorough validation by unbiased methods is still missing. The lack of certainty that the observed efficacy resides exceptionally within the hypothesized molecular action is also one of the reasons why approved drugs are now increasingly being explored for different uses than those envisioned during their development. The need of having unbiased target-related approaches in drug

development welcomed the application of omics-based methods to drug target identification and characterization. Despite the fast growing volume of post-genomic data and the impressive advancements in genomics and transcript omics in terms of throughput and coverage, mass spectrometry (MS)-based proteomics remains the most valuable player in the elucidation and validation of drug targets, due to its unique capability to provide unbiased characterization of the cellular proteins that are effectively targeted by the drugs. Proteomics methods rapidly evolve beyond the old paradigm that limited their focus to protein identification, quantification, as well as PTM profiling. Newly emerging methods probe a vast array of protein characteristics, including thermal stability, rates of expression and degradation, co-regulation, protein-protein, protein-RNA and protein-molecule interactions, etc. One of the fastest growing proteomics areas is Chemical proteomics that aims to characterize the interactions with the cellular proteome of small molecules (e.g., drug candidates), including their targets and off-targets. Chemical proteomics integrates several disciplines and methodological approaches, combining synthetic organic chemistry, cell biology, biochemistry, immunochemistry and MS-based proteomics. Earlier approaches used activity- or affinity-based probes, where the drug was covalently attached to a linker and a tag, as to be captured by affinity beads or isolated by affinity chromatography. Although these approaches were successful in specific cases, they required sophisticated chemical design, and more importantly, demanded chemical modifications not to cause significant changes in drug interaction with its targets and not to alter the mechanism of action. The latter turned out to be significant limitations in many cases, as even a small chemical

variation is capable of changing the drug efficacy by orders of magnitude. Thus the development of Chemical proteomics over the last few years is focused on unbiased approaches that do not involve chemical modification of the drug molecule. Here we single out two complementary approaches that emerge as pillars of modern Chemical proteomics: thermal proteome profiling (TPP) and functional identification of target by expression proteomics (FITeXP). Both approaches can provide unbiased proteome-wide identification of primary and secondary drug targets in cells, lysate (TPP) as well as in tissues. Both approaches have limitations; however, their combined application provides unprecedented insight into the drug interaction with its targets.

Thermal Proteome Profiling (TPP) is a modern, MS-based and proteome-wide version of a well-known method widely applied on a single protein level [1]. The method takes advantage of the biophysical principle of ligand-induced thermal stabilization of proteins. TPP is an proteome-wide extension of the proof-of-principle study on cellular thermal shift assay (CETSA [1]) for monitoring interactions between target proteins and drugs directly in cells [2]. Like most molecules, some proteins tend to become more soluble with increasing temperature, which is a basis of both chicken soup cooking and of the harsh protein-solubilization methods involving boiling in detergent solution. However, many proteins possess peculiar tertiary structure with hydrophobic core and hydrophilic surface. Thermal unfolding of such structures results in their lower solubility, like in boiled chicken egg. These “chicken-egg” proteins are the subjects of interest in CETSA and TPP, while the “chicken soup” proteins, as well as normally insoluble proteins, escape attention of these methods.

In CETSA and TPP, the sample-cell lysate, intact cells or tissues-is heated to different temperatures, from the innocent 37 °C to the scorching 70 °C, and incubated at these conditions for several minutes. The proteins that have denatured and precipitated during this treatment are then removed by thorough centrifugation, while the soluble fraction gets digested and analyzed by LC-MS. Comparison of the protein abundances at different temperatures produces “melting curves” for thousands of proteins. The experiment is performed with and without the drug (incubation time with the drug is typically 2h), and the proteins are then sorted by the shifts in their melting curves. The most shifted proteins typically relate to the drug targets. To validate these findings and increase the specificity of drug target identification, a second experiment is performed where a single temperature point is chosen, and the variable parameter is the drug concentration: the true targets show engagement at lower concentrations than secondary targets. Typically, the experiments on cells and tissues yield, besides primary targets, also a multitude of secondary targets due to protein-protein interactions in complexes. Then, to filter out these secondary phenomena, TPP analysis is performed on cellular lysate, which usually provides data with a higher contrast. TPP was enabled

by many technological advances in the MS field and, particular, by the possibility to reliably perform multiplexing of up to 10 samples by using isotopically labeled tags (iTRAQ, TMT, etc.). The limitations of TPP, besides the above mentioned, involves the cases when the protein thermal stability changes only marginally upon interaction with a drug on the other hand, the information coded in the shape of the melting curve is not yet fully harvested, and represents an untapped potential for further method development in TPP.

Functional identification of target by expression proteomics (FITeXP) is a completely new approach for effective identification of drug target candidates, which seems to possess fewer limitations and higher specificity than TPP, but which doesn't provide direct information on protein engaging the drug. The original work provided both a proof-of-principle study as well as extensive validation using multiple cancer cell lines and several anti-cancer drug compounds [3]. In FITeXP, the drug is applied to cells for 48 h at a compound concentration inducing death in about half of the cancer cell population, after which the proteome is extracted and analyzed in comparison with cells incubated with a vehicle for the same time interval. The proteins significantly up- or down-regulated in treatment, especially the proteins showing larger regulation in response to a particular drug, are the target candidates for that drug. FITeXP is largely based on the empirical observation that target proteins are exceptionally strongly regulated in late apoptosis regardless the cell type, provided the cells are sensitive to the drug. However, the concrete molecular mechanisms behind that observation are yet to be fully investigated. FITeXP, similarly to TPP, produces a multitude of potential drug target candidate, only the top ones of which are primary targets. Unlike TPP, there is no option in FITeXP to resort to cell lysate analysis to differentiate the primary targets from the secondary ones. However, FITeXP is more specific and true targets are usually closer to the top of the list than in TPP. Besides, FITeXP holds a promise (not yet realized) to explore the concentration dimension, by observing how protein abundance changes after a given incubation time when the drug concentration increases. Also, larger list of protein candidates (50-100 molecules) in FITeXP, being mapped on protein networks, provide molecular mechanism of drug action. Finally, FITeXP can be performed not only on the surviving cells ( $\approx 50\%$  of the total cell population, still attached to the flask bottom), but also on already dying (floating) cells, which could provide complementary target information, as well as reveal the details of surviving mechanisms. The latter features are missing in TPP because of the short incubation time.

Summarizing, TPP and FITeXP are two completely complementary and probably the most advanced Chemical proteomics tools for identifying the targets and molecular mechanisms of action of small-molecule compounds in an unbiased way, with no need of chemical modification. The much deeper knowledge of drug targets and underlying mechanisms

of actions provided by these methods could empower drug development, shorten the development time, lessen the burden of cost and facilitate drug development towards personalized cancer treatment. In both TPP and FITeXP, bioinformatics treatment of large quantitative proteomics information is a very important element of analysis. These two methods are a clear indication of the future direction in drug development.

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