Chemical Proteomics versus Leishmaniasis

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In this issue of *Chemistry & Biology*, Wright et al. (2015) describe an elegant approach to evaluating substrates and the drug target potential of *Leishmania donovani* N-myristoyltransferase (NMT) using a technically simple and straightforward chemical proteomics approach.

The identification and validation of novel drug targets is often the first step in current drug discovery (Overington et al., 2006). Due to the high costs and risks as well as long development times, potential drug targets are stringently evaluated before a new drug discovery campaign is launched. Economic aspects such as a favorable intellectual property situation or promising marketing opportunities aside, these evaluations naturally also involve the "suitability" of the drug target, such as an assessment of its disease relevance or druggability (Gashaw et al., 2011). Obviously, such investigations frequently require time, labor, and cost-intensive molecular, genetic, and pharmacological studies.

These issues weigh heavily in the development of drugs against neglected tropical diseases, a family of biologically diverse disorders found in poorly developed tropical or subtropical regions. Despite an irrefutable unmet medical need, weak marketing perspectives and low academic funding rates severely restrict the discovery of novel chemotherapeutics against these fatal diseases. Neglected tropical disease drug development is not solely hampered by economic aspects, however, but also by a lack of suitable approaches to overcome these disorders (Nwaka and Hudson, 2006). In fact, for many neglected tropical diseases, target identification as the starting point for drug discovery has been and still is a tedious and challenging task, because molecular manipulations of the causing pathogens, disease models for target assessment, or even a basic understanding of the underlying disease mechanisms are still limited.

These challenges call for alternative complementary approaches. In the last years, chemical proteomics, an approach in which small-molecule probes are used to covalently tag proteins in a function-dependent manner, has emerged into just such a complementary technology for drug-target discovery (Jeffery and Bogyo, 2003; Cravatt et al., 2008). A major advantage of chemical proteomics is that once the overall workflow has been established, the methodology can be adapted relatively simply and rapidly to different biological systems. This advantage of chemical proteomics has been exploited by Wright et al., who used an in-house established approach for elucidating the global impact of N-myristoyltransferase (NMT)-dependent protein myristoylation (Wright et al., 2015). In their approach, a combination of an alkyne-tagged myristic acid analog and chemical knockdown experiments is used to identify NMT substrates and to quantify their relative abundance in diverse biological systems and processes. These studies led, for example, to the elucidation of plasmodial NMT as a potential antimalarial drug target (Wright et al., 2014) or enabled the mapping of myristoylation patterns in cancer cells (Thinon et al., 2014).

Wright et al. now adapted this approach to investigate the relevance of *Leishmania donovani* NMT for parasital protein myristoylation (Wright et al., 2015). *L. donovani* is the causing pathogen of visceral leishmaniasis (VL), the most fatal manifestation of leishmaniasis, which results in a systemic infection causing organ swelling, fever, and anemia up to organ failure and death. Current treatment options are limited and alternative chemotherapeutic approaches are thus highly desirable. Although previous molecular and bioinformatics studies suggested that NMT plays an important role for proper myristoylation and survival of *L. donovani*, a deeper understanding of its overall contribution to proteome myristoylation and thus drug-target potential so far has not been reached. Wright et al. (2015) therefore incubated *L. donovani* promastigotes or amastigotes with the alkyne myristic acid analog, resulting in an alkyne myristate-modified lipidome (Figure 1). Subsequent affinity capture of these proteins via copper-catalyzed cycloaddition (CuAAC) and quantitative mass spectrometry permitted a quantitative and global view on *L. donovani* myristoylation. This approach led to a "high confidence" identification of, overall, 49 myristoylated proteins (including known myristoylated substrates such as ARL1 or SMP1) in the amastigote and 113 myristoylated proteins in promastigotes. These are many more myristoylated proteins than predicted by bioinformatics estimations, indicating that protein myristoylation in *L. donovani* is much more important than previously anticipated.

The analysis further revealed that, unexpectedly, 59% of the myristate-modified proteins in amastigotes and even 78% in promastigotes did not contain an N-terminal glycine residue. This motif is, however, supposed to be an essential motif for NMT-dependent myristoylation, indicating that besides NMT, further enzymes may contribute to protein myristoylation. To better assess the individual contribution of NMT, the authors combined their metabolic labeling in a second step with a chemical knockdown approach. To this end, they preincubated *L. donovani* amastigotes and promastigotes with two previously characterized, biochemically equipotent NMT inhibitors that, however, displayed significantly

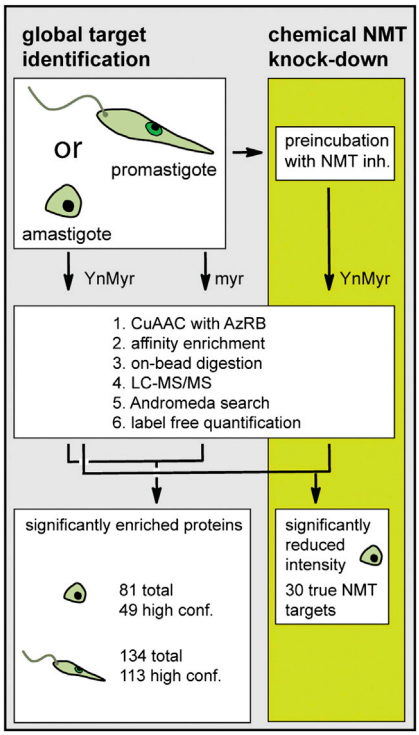

Figure 1. Workflow of the Global Target Identification and Chemical Knockdown Approach

Two complementary experiments were performed to assess parasital NMT substrates. For the first approach, amastigotes and promastigotes were grown in the presence of YnMyr (alkyne myristic acid analog) or myr (myristic acid). Subsequently, labeled proteins were decorated with AzRB (using CuAAC), affinity purified on NeutrAvidin, and on-bead digested with trypsin. After LC-MS/MS the obtained spectra were searched with MaxQuant using the Andromeda search engine and label free quantification turned on. Comparison of the different data set resulted in a total of 49 high-confidence (81 total), YnMyr-modified proteins in amastigotes and 113 high-confidence (134 total) proteins in promastigotes. For the second approach, amastigotes were preincubated for 1 hr with or without the NMT inhibitors and then labeled for 12 hr with YnMyr. After work up and mass spectrometry (same as for first approach), the four data sets were statistically compared, resulting in the identification of 30 proteins whose intensity was significantly reduced in the presence of the NMT inhibitors. These proteins were classified true NMT targets/substrates.

different inhibitory activities on a cellular level. Preincubation of *L. donovani* cultures with both inhibitors led to an efficient reduction of parasital myristoylation in a dose-dependent manner that correlated well with the experimentally determined EC_{50} values, thus confirming target engagement of the compounds in living parasites. Chemical knockdown in amastigotes in combination with quantitative proteomics, however, did not lead to a uniform reduction in protein myristoylation. Instead, only a subset of 30 proteins (in a high-confidence interval) was less myristoylated; interestingly, all of the proteins carried an N-terminal glycine residue, indicating that these 30 proteins are direct NMT substrates. A subsequent GO term analysis of these 30 proteins then revealed that 50% of identified proteins are of unknown function, whereas the other half are involved in highly diverse biological processes such as signal transduction, transport, or degradation. This indicates that a small-molecule-mediated NMT inhibition may have extensive implications on *L. donovani* biology.

Although additional experiments are required to further validate NMT as a leishmaniasis drug target, e.g., to exclude potential off-target effects of the NMT inhibitors that might contribute to the observed antiparasital effect, the present study elegantly demonstrates the potential of chemical proteomics in conjunction with quantitative mass spectrometry to rapidly catalog posttranslationally modified proteins and to evaluate potential drug targets in various biological systems, using one common approach. This study might therefore serve as a blueprint for similar studies on other enzymes and/or posttranslational modifications that surely will be addressed in the future.

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