Trends in
Parasitology

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Spotlight

Finding optimal drug target sites in parasite pathogens

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Benns *et al*. have recently combined a chemoproteomic profiling method with a CRISPR-based gene-editing method to identify chemically targetable residues essential for fitness in the parasite *Toxoplasma gondii*. The result is a strategy that enables rapid discovery of new drug targets to combat *T. gondii* and other related parasites.

Parasites are organisms that have evolved to efficiently survive in close connection with a host. They maintain a host–guest symbiosis through evolutionary adaptation to host-derived pressures. As a result, parasites are particularly adept at finding mechanisms to evade therapeutic drug pressures. By identifying highly essential targets or multiple targets that enable combination therapies, it is possible to reduce the chances of resistance generation. As a result, several omics-based methods have been used to identify and prioritize as many new classes of drug targets as possible. However, most of these methods suffer from an inability to globally consider key factors such as essentiality and 'drugability' of potential targets. Thus, target selection largely remains biased by the availability of functional information, and many optimal targets are overlooked.

Benns *et al.* address these limitations by developing a combination approach using activity-based protein profiling (ABPP) platform to identify chemically reactive 'hotspots' and a clustered regularly interspaced short palindromic repeats (CRISPR)-based oligo recombineering (CORe) platform to identify which of the hotspots are the most functionally important to the parasite [1]. This enables target selection based on both overall potential to be targeted with a small-molecule drug and the likelihood that modifying that site will have detrimental consequences to the parasite. The result is an unbiased, functionally validated list of binding sites with high potential value for therapeutic development.

The technique of ABPP utilizes chemically reactive activity-based probes (ABPs) that form covalent bonds with target proteins [2]. The majority of ABPs target nucleophilic active-site residues of functional enzymes such as proteases and hydrolases [3,4]. This enables global profiling of members of a related enzyme family in complex proteomic samples. By mapping changes in enzyme activity, for example, over the life cycle of a parasite, it is possible to identify proteins that play essential roles in essential biological processes, thus making them optimal drug targets [5].

To broaden the scope of ABPP, more generally reactive probes have been developed that chemically modify specific amino acids based only on their intrinsic reactivity [6–8]. The first example of this approach characterized the reactivity of all cysteine residues in a proteome using a chemical probe containing an iodoacetamide (IA)-reactive functional group [9]. This created a list of thousands of cysteine residues in hundreds of proteins with a wide range of overall chemical reactivities. Further analysis found that cysteine residues with high reactivity generally tend to be involved in important functions such as mediating redox reactions, performing catalysis, or facilitating cofactor binding. However, the analysis of the full dataset was limited by the fact that many proteins have poorly defined or unknown functions.

Benns *et al.* use ABPP to initially characterize all the reactive cysteine residues in the proteome of the human parasite pathogen *T. gondii*. This provided a list of over a thousand chemically reactive cysteines in hundreds of proteins that could be grouped into categories of low, medium, and high reactivity (Figure 1A). The set of 130 highly reactive cysteine residues identified in 97 proteins was further refined using a phenotypic scoring approach based on predicted essentiality of each gene as defined by a genome-wide knockout screen. This resulted in a final set of 75 cysteine residues in 56 proteins as potential therapeutic targets. However, because this set included proteins with known functional cysteine residues, proteins with established function but no known role for cysteine, and hypothetical proteins of no known function, it was impossible to assign priorities to the list.

To complement the ABPP data, Benns *et al.* developed a technology (CORe) to rapidly assign functional importance to each of the reactive cysteine residues (Figure 1B). This method uses a CRISPR-Cas9-based homology-directed repair (HDR) strategy to replace the codon for each cysteine residue with a stop codon or codon for a point mutation. The resulting parasites with edited genomes are then used for fitness competition studies to assess which mutations are detrimental to the parasite. The main premise is that mutations that negatively impact fitness are functionally essential residues and therefore ideal target sites for small-molecule drugs.

Perhaps one of the most impressive aspects of CORe technology is its overall speed. In this study, it was possible to assess fitness effects of five mutations for each of the 75 cysteine residues within 1 month. This dataset provided some striking findings, including the fact that mutation of ~83% of the cysteine sites showed no impact on fitness and that

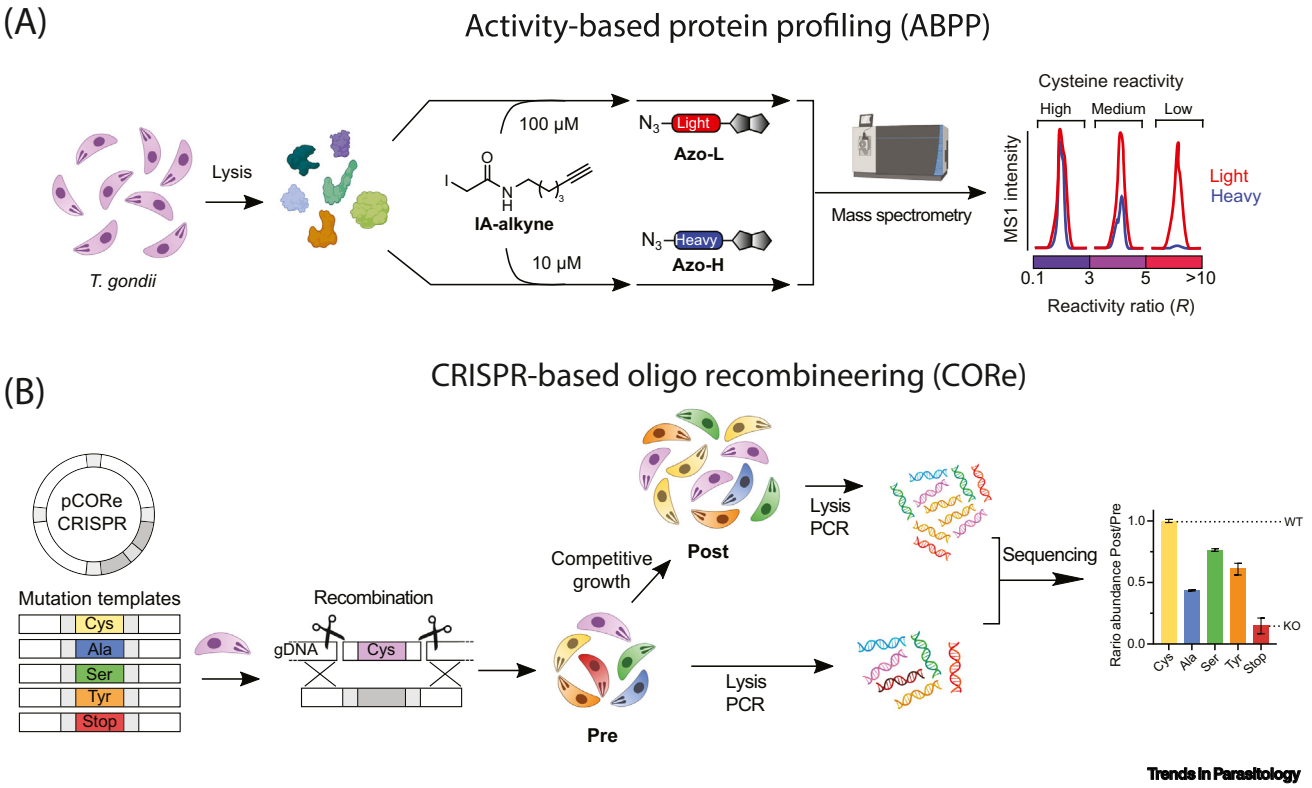

Figure 1. Schematic of the workflow for activity-based protein profiling (ABPP) and clustered regularly interspaced short palindromic repeats (CRISPR)-based oligo recombineering (CORe) methods for prioritization of drug targeting sites in *Toxoplasma gondii*. (A) ABPP using the cysteine reactive iodoacetamide (IA-Alkyne) tag. Cysteines are labeled at low (10 μM) and high (100 μM) concentrations of the probe and then analyzed by mass spectrometry to determine the overall reactivity of each residue [1]. (B) The CORe platform enables fitness testing of mutants of specific cysteine residues. Pools of CRISPR-edited parasites are grown in competition, and relative levels of specific mutants are measured by sequencing. A drop in the levels of any mutant indicates a negative impact on overall fitness [1]. Part of the figure was created with BioRender.com.

there was no direct correlation between levels of cysteine reactivity and impact on fitness. Furthermore, the distribution of conservation scores for residues that were fitness-conferring was bimodal, suggesting that selecting target residues based on conservation across species is not an effective strategy for prioritization.

Ultimately, the ABPP–CORe approach created a list of specific cysteine residues in *T. gondii* that are fitness-conferring. As a final filter of this data, the authors performed analysis of the functional annotations of the corresponding proteins and found an enrichment in genes associated with protein translation. Functional validation of cysteine residues on the 80S ribosome and the 60S ribosomal protein RPL4 confirmed that multiple residues are important for function and that mutation of those residues results in loss of fitness. Importantly, many of these essential cysteine residues on translation-associated proteins were conserved in both *T. gondii* and the human malaria parasite *Plasmodium falciparum* but not in humans. Consistent with this finding, the cysteine-reactive IA electrophile blocked *in vitro* translation of proteins in *P. falciparum* but not in human extracts. Finally, screening of a library of cysteine-reactive molecules identified a lead compound with specific inhibition of *P. falciparum* protein translation.

In summary, the work by Benns *et al.* highlights the importance of using highly parallel and rapid methods to directly assess the functional importance of specific targetable residues on proteins. While it is tempting to directly use 'omics' methods to pick drug targets, these approaches are generally biased by the availability of functional information. When omics methods can be combined with global functional screens, as demonstrated by Benns *et al.*, the result is a treasure trove of potential new drug targets for important human pathogens.

Acknowledgments

M.B. is supported by grants from the National Institutes of Health (R01EB026332, R01 EB026285, R33AI127581, R01EB028628, R01DK130293). The author thanks Dr Matthew A. Child for providing images used in the figure.

Declaration of interests

The author declares no competing interests.

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https://doi.org/10.1016/j.pt.2022.12.003

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