Chemical Proteomics of Host-Pathogen Interaction

Jingyan Ge[1] and Shao Q. Yao[1,*]
[1]Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
*Correspondence: chmyaosq@nus.edu.sg
http://dx.doi.org/10.1016/j.chembiol.2015.04.002

In less than two decades, activity-based protein profiling (ABPP) has expanded to become the de facto tool for the study of small molecule-protein interactions in a proteomic environment. In this issue, Na et al. (2015) present another ABPP method, which they called reactive probe-based chemical proteomics, to study host-pathogen interaction and subsequently identify the protein PheA as a potential key effector during the pathogen infection process.

Activity-based protein profiling, a concept coined in the late 1990s by Ben Cravatt (Liu et al., 1999), has rapidly grown up in recent years to become a powerful technology for proteome-wide interrogation of small molecule-protein interactions. Initially conceived as a tool for large-scale profiling of enzymes on the basis of their response to different classes of irreversible suicide inhibitors, the laboratories of Cravatt and Bogyo introduced the so-called activity-based probes (ABPs), which typically contain a reactive warhead, a reporter, and a linker (Jeffery and Bogyo, 2003; Evans and Cravatt, 2006). By varying the reactive warhead, many enzyme classes have been successfully profiled with different ABPs. Later, a similar concept named affinity-based protein profiling (A*f*BPP) was introduced to interrogate general enzyme-inhibitor interactions, which in most cases are noncovalent. The corresponding affinity-based probes (A*f*BPs) used in such studies achieved covalent modifications of enzymes with noncovalent small molecule inhibitors under UV irradiation conditions via a preinstalled photo-crosslinker (Saghatelian et al., 2004 and Chan et al., 2004). A*f*BPs thus significantly expanded the coverage of ABPP applications. More recently, aided by the accelerated development of liquid chromatography-tandem mass spectrometry (LC-MS/MS), ABPP has further extended its reach into the realm of in situ drug profiling for proteome-wide identification of potential cellular targets of bioactive compounds (Su et al., 2013). By taking advantage of clickable probes derived from the original bioactive compounds, first the FDA-approved Orlistat (a covalent drug for obesity and diabetes; Yang et al., 2010) and then Dasatinib (a noncovalent anti-cancer drug; Shi et al., 2012) were successfully studied inside live mammalian cells, where genuine drug-target interactions typically take place. As a clickable probe was minimally modified from the original drug in its size and cell permeability, it retained most of the drug's potency and binding to its cellular targets. Increasingly, this in situ drug profiling method, due to its numerous obvious advantages over most other large-scale proteomic profiling strategies, has gained popularity for both on- and off-target identification of drugs and other bioactive compounds (Ziegler et al., 2013).

In the current issue, Jun Seok Lee and coworkers (Na et al., 2015) present an apparently simple but effective approach to study host-pathogen infection (Figure 1). By utilizing readily available, chemically reactive fluorescent dyes, this so-called reactive probe-based chemical proteomic strategy is different from previously developed methods for studies of host-pathogen interaction. The team infected RAW 264.7 macrophages with *Salmonella typhimurium* natively labeled with each of four commonly used fluorescein (Flu)/tetramethylrhodamine (Rho) dyes having either an *N*-hydroxysuccinimide (NHS) or iodoacetamide (IA) reactive group, and they subsequently found that infected macrophages produced highly distinct fluorescence signals located exclusively in the cell nuclei. This points to the presence of possible "hit" bacterial proteins that were labeled by Flu-NHS and involved in the host infection process. Next, to positively identify these bacterial proteins, the authors employed a comparative proteomic strategy by using excessive Biotin-NHS to label/capture only proteins whose labeling profiles were altered in samples prelabeled with Flu-NHS; in doing so, the authors successfully identified 313 highly enriched proteins, 29 of which were likely candidates due to their nuclear localization. The next step was to prove the biological effectiveness of these candidate targets. The authors focused on the top candidate-Chorismate mutase-P/prephenate dehydratase (PheA). First, cell-cycle analysis was carried out, demonstrating that cell-cycle arrest was observed during G1/S phase in PheA-transfected RAW264.7 cells. Next, ChIP analysis was performed to show EGFP-fused PheA bound to a DNA oligomer containing E2F7-binding motif. Further RT-PCR analysis showed similar levels of up/downregulations of E2F7-binding genes in macrophages either infected with *Salmonella* or transiently expressed with PheA-EGFP. Finally, in RAW264.7 cells infected with PheA-EGFP-overexpressing *Salmonella typhimurium*, strong fluorescence signals were detected predominantly around the nucleus of macrophages. All these lines of evidence thus prompted the authors to conclude PheA was likely a key effector during the pathogen infection process. The results are somewhat unexpected but novel enough to warrant future studies on the exact role of PheA plays in host-pathogen interaction.

The use of reactive probe-based chemical proteomics is reminiscent of a previously reported hyperreactive cysteine profiling approach (Weerapana et al., 2010): by combining with fluorescence microscopy, it offers a convenient but effective strategy to study host-pathogen interaction. Looking ahead, there is plenty of room for improvement of this technique; for example, the nonspecific nature of Flu-NHS toward proteins, as well as the

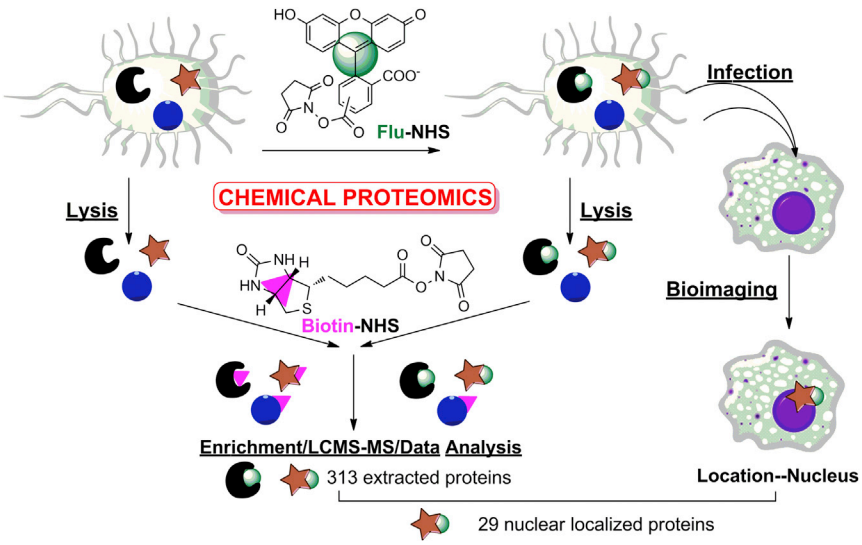

Figure 1. General Workflow of Reactive Probe-Based Chemical Proteomics of Host-Pathogen Interactions

Comparative profiling to identify Flu-NHS labeled proteins, and cellular imaging experiments showing nuclear localized bacterial proteins as the most likely candidates involved in pathogen infection.

size of the fluorophore itself, is potentially problematic, as in many cases, a potential "hit" protein might lose its biological activity upon labeling and thus will not be positively identified. In the present study, the authors managed to identify and validate a candidate hit, but it is likely that many more candidates were missed. One possible improvement might be through the use of reactive probes containing "minimalist" clickable tags in lieu of the fluorophore (e.g., an N_3- or terminal alkyne-containing NHS); this will ensure labeled proteins were minimally perturbed structurally. The current approach lies in simplicity and will add another useful tool in the burgeoning field of activity-based profiling for different biological studies. The authors have so far given us a glimpse of what this strategy can do in a case study of host-pathogen interactions. It might work with other similar types of biological interactions, especially in the field of infectious diseases caused by Ebola virus, malaria, and others, to facilitate the identification of potential biomarkers and therapeutic agents during different stages of infection.

REFERENCES

Chan, E.W.S., Chattopadhaya, S., Panicker, R.C., Huang, X., and Yao, S.Q. (2004). J. Am. Chem. Soc. *126*, 14435–14446.

Evans, M.J., and Cravatt, B.F. (2006). Chem. Rev. *106*, 3279–3301.

Jeffery, D.A., and Bogyo, M. (2003). Curr. Opin. Biotechnol. *14*, 87–95.

Liu, Y., Patricelli, M.P., and Cravatt, B.F. (1999). Proc. Natl. Acad. Sci. USA *96*, 14694–14699.

Na, H.N., Yoo, Y.H., Yoon, C.N., and Lee, J.K. (2015). Chem. Biol. *22*, this issue, 453–459.

Saghatelian, A., Jessani, N., Joseph, A., Humphrey, M., and Cravatt, B.F. (2004). Proc. Natl. Acad. Sci. USA *101*, 10000–10005.

Shi, H., Zhang, C.J., Chen, G.Y.J., and Yao, S.Q. (2012). J. Am. Chem. Soc. *134*, 3001–3014.

Su, Y., Ge, J., Zhu, B., Zheng, Y.G., Zhu, Q., and Yao, S.Q. (2013). Curr. Opin. Chem. Biol. *17*, 768–775.

Weerapana, E., Wang, C., Simon, G.M., Richter, F., Khare, S., Dillon, M.B., Bachovchin, D.A., Mowen, K., Baker, D., and Cravatt, B.F. (2010). Nature *468*, 790–795.

Yang, P.Y., Liu, K., Ngai, M.H., Lear, M.J., Wenk, M.R., and Yao, S.Q. (2010). J. Am. Chem. Soc. *132*, 656–666.

Ziegler, S., Pries, V., Hedberg, C., and Waldmann, H. (2013). Angew. Chem. Int. Ed. Engl. *52*, 2744–2792.