Microparticle assisted precipitation screening method for robust drug target identification

Jiawen Lyu[1,2], Chengfei Ruan[1,2], Xiaolei Zhang[1,2], Yan Wang[1,2], Kejia Li[1,2], Mingliang Ye[1,2,*]

[1] *CAS Key Laboratory of Separation Sciences for Analytical Chemistry, National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), 116023, Dalian, China;*
[2] *University of Chinese Academy of Sciences, 100049, Beijing, China*

Gold statement
- A novel method to separate protein precipitate for drug target identification
- Streamlined proteomic sample preparation using microparticles
- Compatible with minute samples and scalable to automation

Introduction
While thermal proteome profiling (TPP) shines in the field of target screening by analyzing the soluble fraction of the proteome samples treated with high temperature [1], the counterpart, insoluble precipitate has been overlooked for a long time. Inspired by a Protein Aggregation Capture (PAC) method [2], we propose a novel method, termed Microparticle Assisted Precipitation Screening (MAPS), for drug target identification. This method exploits the principle that drug bound proteins are more resistant to thermal unfolding, thus less aggregate on the surface of microparticles. The whole sample preparation is processed on the surface of microparticles including wash, alkylation, and digestion.

Body
Theoretically, the ligand induced thermal stabilization of target protein can be revealed by profiling either the supernatant or the precipitate. However, most of the reported methods still adopt the supernatant for drug target deconvolution due to the inconvenience in the study of precipitate. MAPS method is compatible with minute amount of initial proteins. We evaluated the MAPS method with several drug-target systems of different levels of promiscuity with the initial protein amount of only 20 µg in each sample. The main targets of several well-studied drugs, including Methotrexate, Raltitrexed, Cyclosporin A, and SHP099, were successfully identified. The known target HSP90 family, and an off-target PRDX family were also identified for Geldanamycin. Then, the method was applied to pan-kinase inhibitor staurosporine and detected 32 protein kinases from 40 candidates within a cellular proteome comprising over 6000 proteins. With the same initial protein amounts, MAPS outperformed classic TPP by identifying more target kinases with higher specificity.

Conclusion
MAPS is an unbiased robust method for drug target screening, filling the vacancy of stability-based target screening using precipitate. It was demonstrated that MAPS is compatible with minute amount of sample and is scalable to automation attributed to the bead-based workflow.

References
[1] Savitski, M. M., *Science* **346** (2014), 1255784.
[2] Batth, T. S., *Molecular & Cellular Proteomics* **18** (2019), 1027-1035.