Profiling Protein Interactions with Label-Free Quantification Proteomics in Combination with Affinity Purification by micro-Affinity Column

Guizhen Liu, Jingwu Kang*

State Key Laboratory of Bioorganic and Natural Products Chemistry, Center for Excellence in Molecular Synthesis, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China

Gold statement

- Affinity purification with sepharose resins and magnetic particles is not convenient for profiling protein-protein interactions
- Affinity purification with Spin-tip or capillary monolithic affinity columns is more easily to manipulate automatically
- The new affinity purification approach improved the quality of Label-Free Quantification Proteomics analysis

Introduction

Protein-protein interactions (PPIs) play an essential role in conducting various fundamental cellular processes [1]. Deciphering the PPIs is thus a foundation work for understanding protein functions and uncovering novel therapeutic targets [2]. Historically, co-immunopreciplitation (co-IP) and yeast two-hybrid are standard tools used for this purpose5. However, in the last two decades, affinity purification coupled with mass spectrometry (AP-MS) has emerged as a powerful tool for unbiased identification of PPIs on the proteomic scale [3].

Body

In this communication, We report an AP-MS method for profiling protein-protein interactions [4]. The AP was implemented with monolithic micro immobilized metal ion affinity chromatography columns (m-IMAC) which were prepared by photoinitiated polymerization in the tip of a pipette (Spin-tip columns) or a fused silica capillary. The recombinant His_6-tagged protein (bait protein) was reversibly immobilized on the affinity column through chelating group the nitrilotriacetic acid (NTA)-Ni^{2+}. The bait protein and its interacting partners can be easily eluted from the affinity matrix. The pulled-down cellular proteins were then analyzed with the label-free quantitative proteomics. We used this method for probing the interactome concerning the GOLD (Golgi dynamics) domain of the autophagy associated adaptor protein FYCO1. Totally 96 proteins including 7 literature reported FYCO1-associating proteins were identified. Among them CCZ1 and MON1A were further biochemically validated, and the direct interaction between FYCO1 GOLD domain with CCZ1 were confirmed by co-immunoprecipitation experiments. This method is further applied to profile the interactome of anti-apoptotic protein Bcl-XL. Beside 7 known interacting proteins, two new interacting proteins were identified.

Conclusion

It is demonstrated that the affinity purification with m-IMAC columns in combination of Label-free quantification proteomics provide high confident and robust approaches for profiling of protein interactions.

References

[1] I. Bonetta, Nature 468(2010) 851-854.
[2] A. A.Ivanov, F. R. Khuri, H. Fu, Trends Pharmacol Sci 34 (2013) 393-400.
[3] X. Li, W. Wang, J. Chen, Proteomics 15 (2015) 188-202.
[4] Liu GZ, Kang JW et al. Anal. Chem. 92(2020) 3913-3922.