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Target Identification Using Affinity Selection Mass Spectrometry and a Protein Library of 17,000 Proteins, Including Membrane Proteins

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Overview

- Phenotypic screening has led to the discovery of numerous bioactive compounds, but identifying their molecular targets remains a challenge
- The absence of target identification for the compound hinders both compound optimization and the development of targeted therapies
- We developed an innovative Affinity Selection Mass Spectrometry (ASMS) method that measures interactions of the compound and 17,000 different proteins.
- The method, which uses proteins in their native states, including membrane protein, enables highly accurate target identification and accelerates the drug discovery process

Introduction

- Phenotypic screening is a powerful approach for discovering bioactive compounds with therapeutic potential
- However, the inability to identify molecular targets often hinders compound optimization and clinical translation
- Our novel ASMS technology is designed for efficient and precise target identification
- Unlike traditional methods, ASMS does not require protein solubilization or immobilization, preserving the native structure and interactions of proteins
- This approach facilitates the elucidation of mechanisms of action for compounds identified in phenotypic screens, accelerating drug development

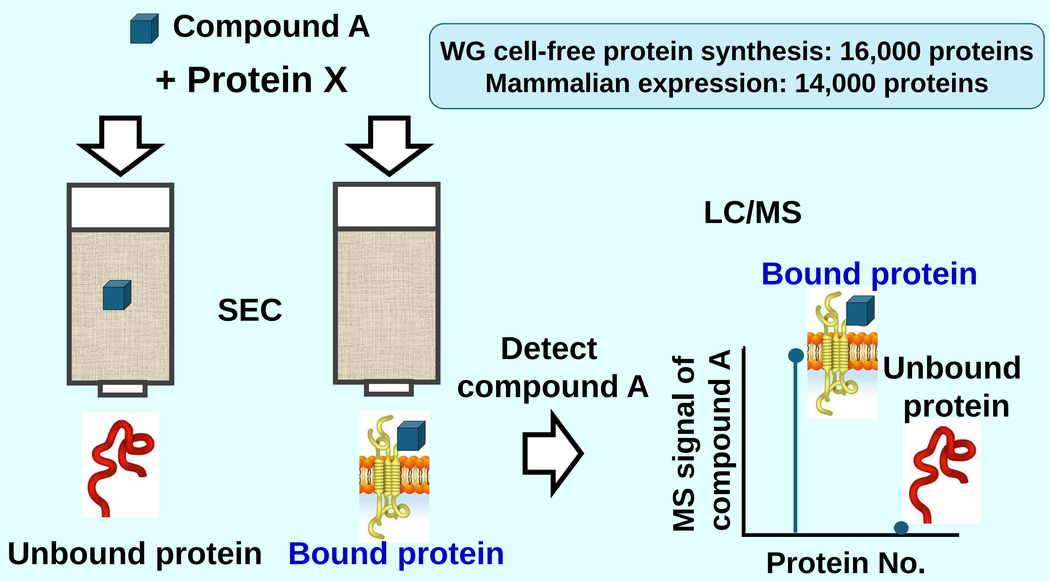
Methods & Results

1. ASMS Technology:

- Using size exclusion chromatography (SEC) and LC/MS, the target protein of compound A is identified based on the MS signal of compound A binding (1)

2. Comprehensive Protein Library:

- Includes approximately 17,000 proteins, covering diverse classes such as soluble and membrane proteins

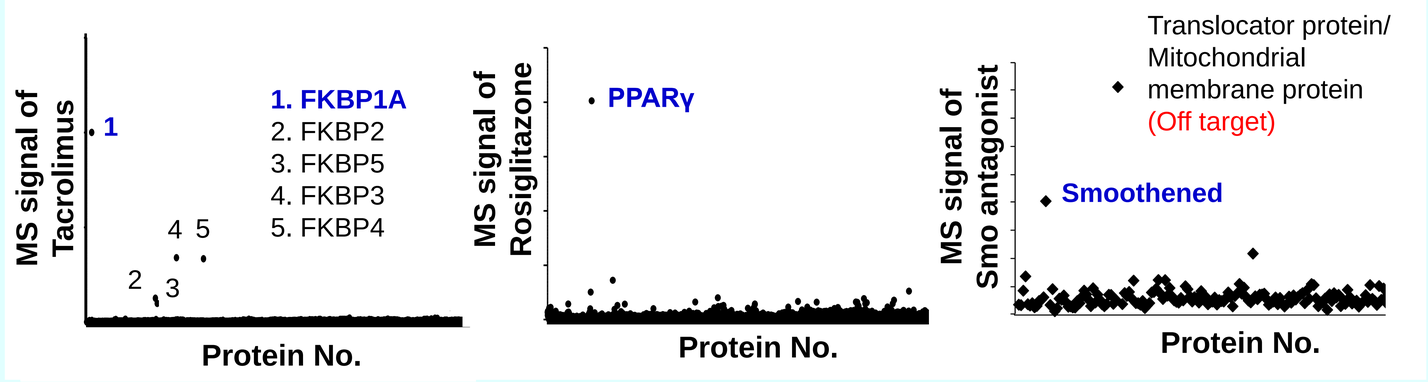


3. Validation Studies:

We evaluated the robustness and accuracy of ASMS by testing it with well-characterized bioactive compounds:

- **Tacrolimus** (Immunosuppressant): Identified FKBP1A (FKBP12) as its molecular target
- **Rosiglitazone** (Antidiabetic): Confirmed binding to PPARγ
- **Smoothened (Smo) antagonist**: Successfully identified the Smoothened receptor (membrane protein) as the target

For each compound, ASMS accurately identified their known molecular targets, demonstrating the reliability of this method



(WG cell-free protein synthesis protein library)

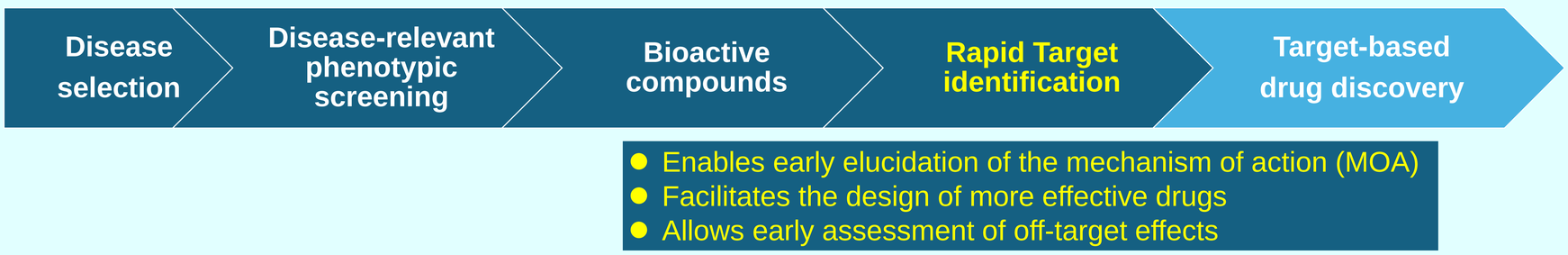
(Mammalian expression protein library)

Conclusion

Our ASMS technology represents a significant advancement in target identification for phenotypic screening.
By preserving native protein-compound interactions, this method provides:

- Highly accurate molecular target identification, including for challenging membrane proteins
- Reduced artifacts through the elimination of protein immobilization
- A streamlined approach from phenotypic screens to drug development

These innovations support the efficient discovery and optimization of bioactive compounds, accelerating the development of targeted therapies



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Conflict of Interest Disclosure: The authors declare no competing financial interest.

Reference
(1) McMillan E.A., Ryu M.J., Diep C.H., Mendiratta S., Clemenceau J.R., Vaden R.M., Kim J.H., Motoyaji T., Covington K.R., Peyton M., et al. Chemistry-first approach for nomination of personalized treatment in lung cancer. Cell. 2018;173:864–878.e29