Characterization of membrane protein using mass spectrometry-based technologies — affinity selection-mass spectrometry (AS-MS)

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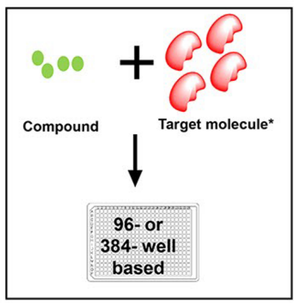
Abstract

Affinity Selection-Mass Spectrometry (AS-MS) is an emerging technology in drug discovery. In practice, its primary focus is on discovering binders for target molecules. As reported publicly, AS-MS is employed for hit finding in high-throughput screening campaigns for various target molecules, ranging from soluble proteins to challenging targets. Recently, it has been applied for binder exploration of functionally challenging target molecules, **such as membrane proteins,** RNA, and proteins of interest (POI) involved in targeted protein degradation (TPD). Even membrane proteins can be utilized in AS-MS technology, along with soluble target molecules. However, **in many cases, proper sample preparation is necessary for membrane proteins**, which may involve solubilization with mild detergents to maintain their proper folding. In this poster presentation, we demonstrate that **AS-MS, using membrane fractions or microsome as protein material, is useful not only for evaluating binding affinity but also for profiling the inhibition mode of membrane proteins and structure determination of membrane protein complex with inhibitor.**

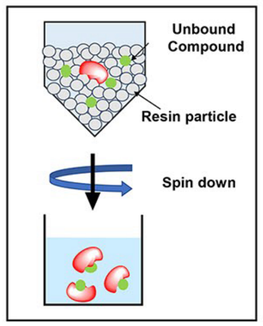
Method

1. Preparation



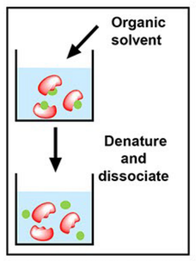
Incubate a compound and a target molecule.
* **Crude membrane proteins, such as membrane fractions and microsome** expressing the target molecules.

2. B/F separation



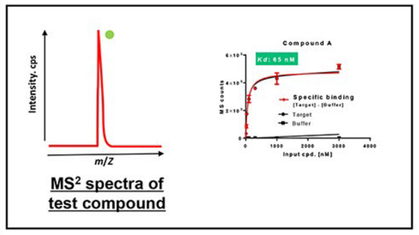
Remove unbound compounds with a size exclusion chromatography using a 384-well plate of which wells were filled with ADDP's custom resin for AS-MS

3. Dissociation



Denature and release bound compound from complex

4. Detection & Analysis



MS^2 spectra of test compound

Measure by **RapidFire-MS/MS**** or LC-MS/MS

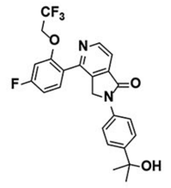
Fig. 1. Workflow of K_D determination by AS-MS with MRM* mode

Appropriate resin and centrifugation conditions for B/F separation can deal with even membrane fractions.

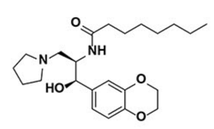
* Multiple reaction monitoring. ** Agilent technologies, Inc.

Case 1
Characterization of membrane protein enzyme and inhibitors: GCS inhibitors[1,2]

Glucosylceramide synthase (GCS) is a membrane protein enzyme which catalyzes glucosylceramide (GlcCer) synthesis from ceramide (Cer) and uridine diphosphate-glucose (UDP-glucose). This enzyme involved in lysosomal storage disorders, such as Gaucher's disease (GD).



T-036 (Takeda)



Eliglustat

Fig. 2 GCS inhibitors used for characterization

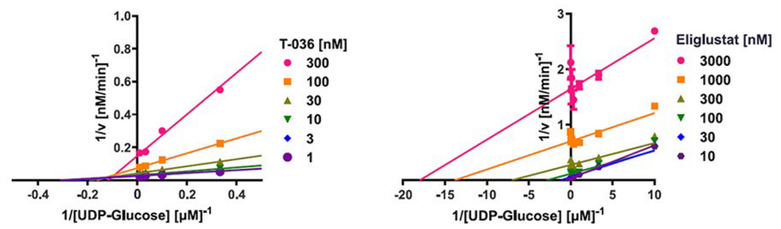


Fig. 3. Kinetic analysis by Lineweaver-Burk plots.
T-036 showed a noncompetitive manner in inhibitory activity of enzyme (left). Meanwhile, eliglustat showed an uncompetitive inhibition manner (right).

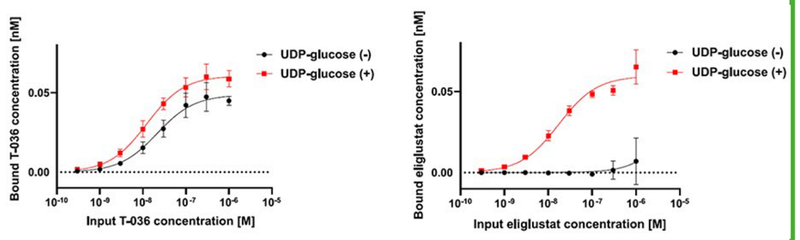


Fig. 4. Binding analysis by AS-MS using membrane fractions.
T-036 bound to the enzyme without affecting UDP-glucose binding as the substrate molecule (left). Meanwhile, eliglustat bound to the enzyme only in the presence of UDP-glucose (right).

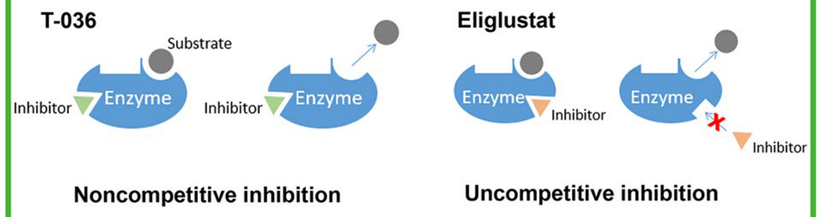
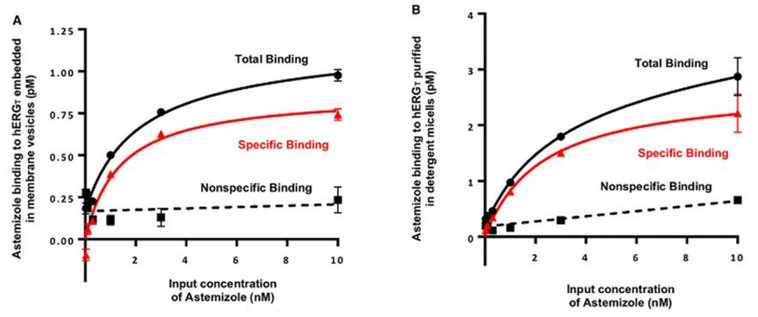


Fig. 5. Schematic figure of binding mechanism for GCS inhibitors.
Combining enzymatic kinetics assay with binding analysis using AS-MS could clearly demonstrate the binding mechanism and the inhibitory modes of GCS inhibitors.

Case 2
Structure determination of hERG/Astemizol complex by Cryo-EM[3,4]

For membrane protein structure determination in complex with compounds, it's important to know the binding affinity of compound **before and after purification of membrane protein.** In addition, K_D values are helpful to set an appropriate compound concentration of cryo-EM sample.



K_D: 1.4 ± 0.29 nM for membrane fractions

K_D: 2.3 ± 0.58 nM for purified hERG

Fig. 6. Binding analysis of astemizole to $hERG_T$* by AS-MS.
K_D values of astemizole were determined for $hERG_T$ embedded in a cell membrane (A) and $hERG_T$ purified in LMNG micelles (B). Specific binding signals (red triangles) were calculated by subtracting nonspecific binding signals (solid square), conducted without $hERG_T$, from the total binding signals (solid circles). The results are shown as the mean ± standard error in triplicate.

* $hERG_T$ was truncated from residues 141-350 and 871-1005, prepared for structure determination.

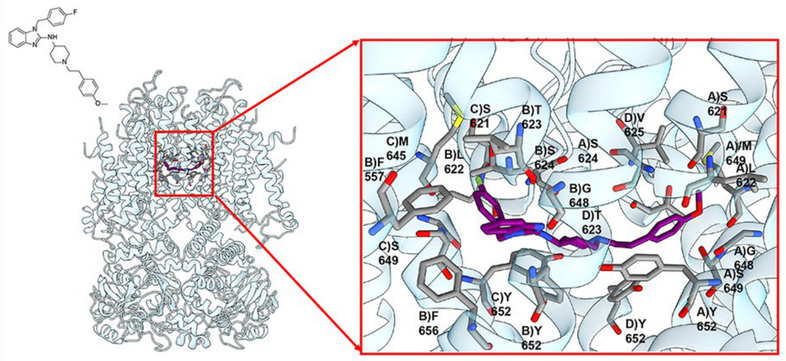


Fig. 7. Complex structure of hERG and astemizole complex.
The cryo-EM structure of hERG in complex with astemizole was determined to **a resolution of 2.7 Å**. The key residues involved in astemizole (purple) binding are shown as grey sticks.

Claudio Catalano *et al.*, COMPPa 2022, NanoImaging Services

References

1. Fujii, Takahiro *et al. Journal of neurochemistry* 159,3 (2021): 543-553.
2. Tanaka, Yuta *et al., Journal of medicinal chemistry.* 65,5 (2022): 4270-4290.
3. Asai, T. *et al. Structure* 29(3), (2021): 203-212
4. Claudio Catalano *et al.*, the 2nd COMPPÅ meeting, held in New York, NY in June 2022.

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