Affinity selection-mass spectrometry towards membrane protein drug discovery

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Introduction

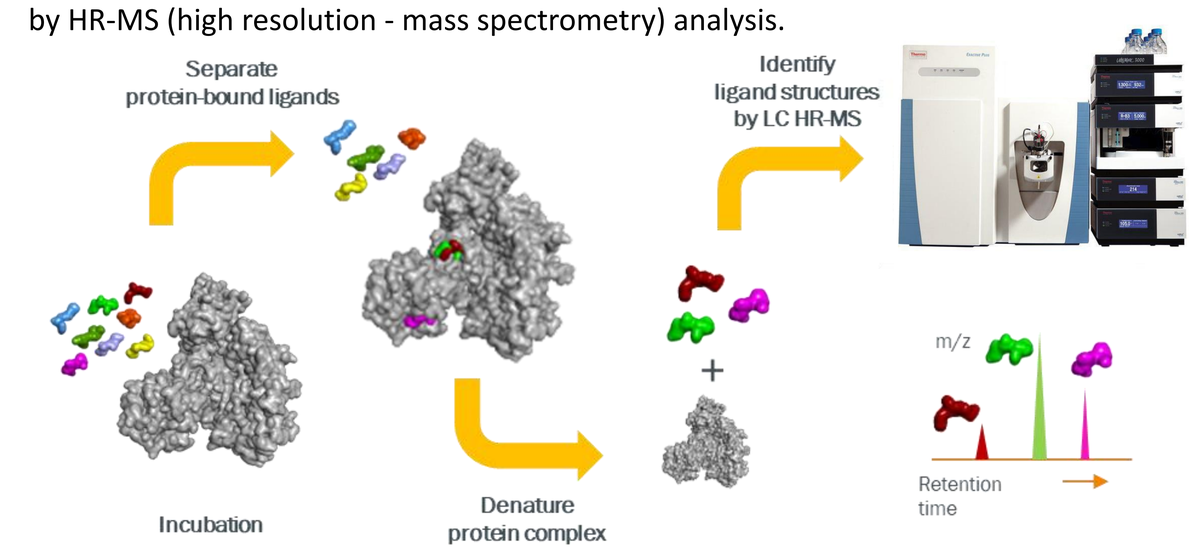
In recent years, affinity screening has gained momentum as an innovative and alternative method to HTS, used for hit generation in drug discovery. This screening technology is becoming especially attractive due to the emergence of new modalities such as PROTACs/Molecular Glues[1], resulting in a quest for selective binders versus functional hits.

The emergence of **Affinity Selection - Mass Spectrometry (AS-MS)**[3] enables the screening of large collections being assayed as defined compound mixtures, each compound encoded by its exact mass. The historical development of this approach and the latest breakthroughs is presented with a particular focus on membrane proteins.

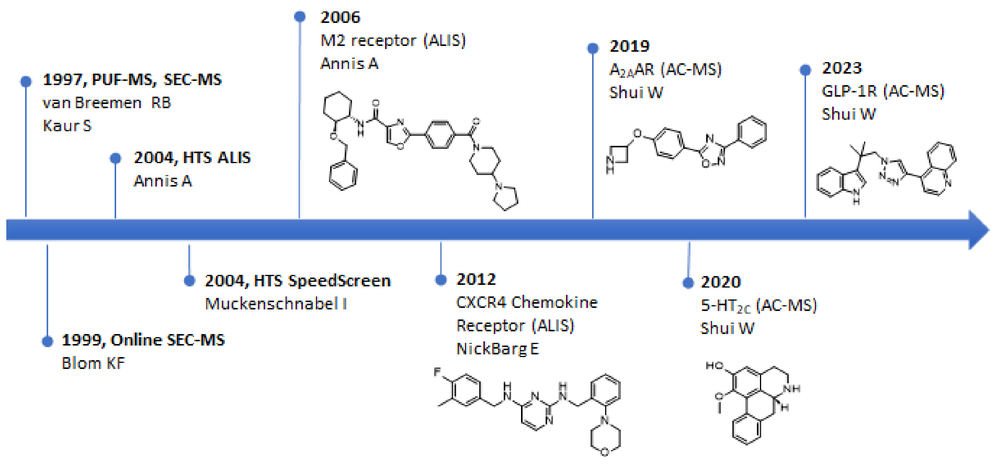
Methods

1. AS-MS Principle

The biological target of interest is incubated with the pre-defined compound mixtures; target-binder complexes are then separated by size exclusion media from unbound compounds. After separation from the target, the identities of the binders are revealed by HR-MS (high resolution - mass spectrometry) analysis.



2. Towards membrane protein screening

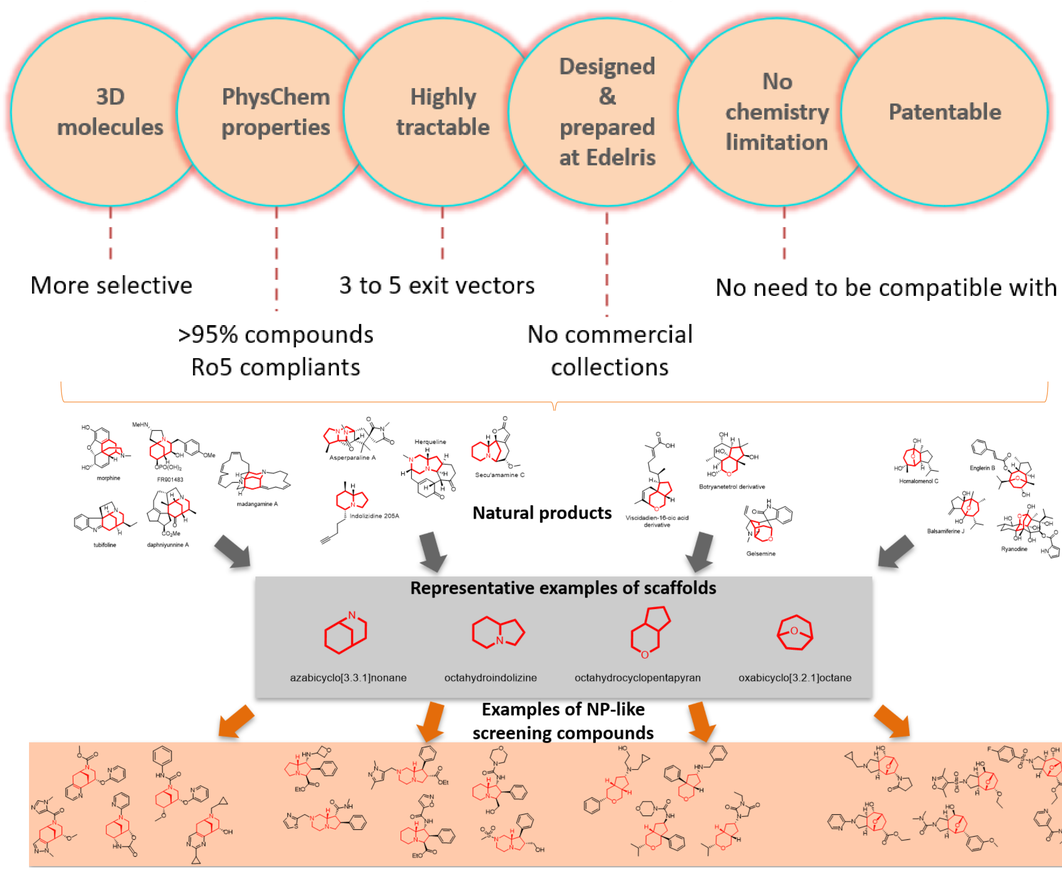


3. Edelris AS-MS platform

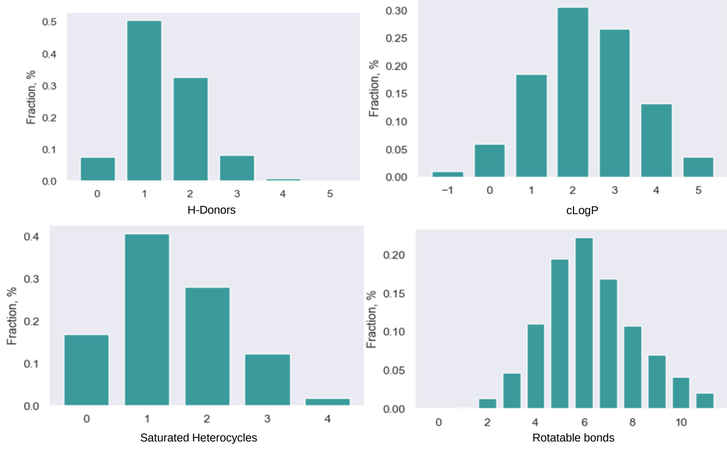
✓ *Our process*

Target qualification & Pilot screening → Full Screening **2M compounds** from Edelris Keymical Space™ → Hit confirmation & Compounds shipment

✓ *Our unique collection: a 2M compounds diverse library*



4. Edelris library: optimized Phys-Chem properties

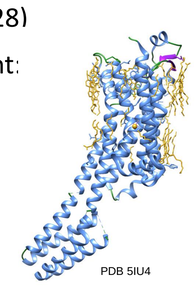


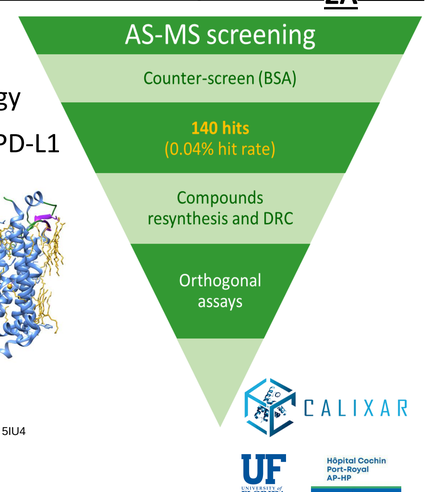
Results on therapeutic target: A2A Adenosine Receptor (A_{2A}AR)

Towards new allosteric modulators of A_{2A}AR

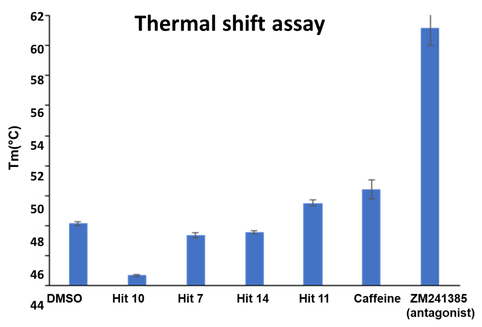
- Therapeutic targets: Parkinson's disease and immuno-oncology
- Clinical studies on solid tumors: combination with anti-PD-1/PD-L1 treatment (EOS-100850, CPI-444, AZD-4635, AB-928)
- High level of adenosine in tumor microenvironment: high potency needed for orthosteric inhibitors

Strong interest to identify allosteric antagonists

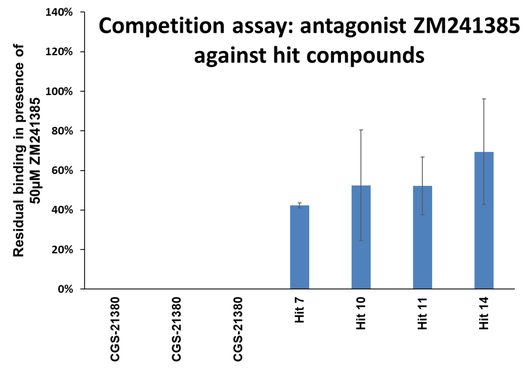




- **Orthogonal assay**



- **Competition assay**

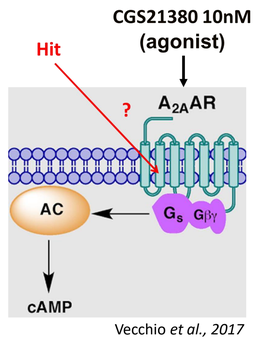


Orthosteric radioligand competition assay
[^{3}H]ZM241385

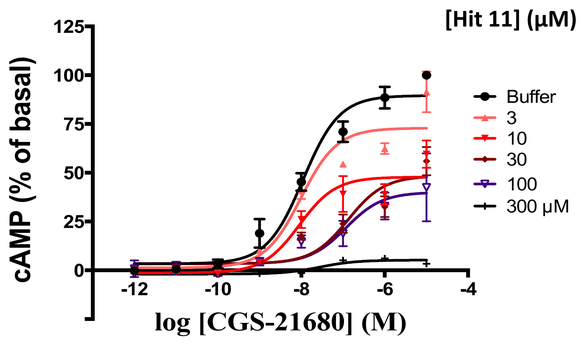
Compound	Ki (µM)
Hit 7	65.6
Hit 10	>1000
Hit 11	>1000
Hit 14	348

- **Cellular assay and antagonist effect**

Stimulation of Adenylate cyclase cAMP production on A_{2A}AR-expressing HEK293 cells



Vecchio *et al., 2017*



Conclusions

AS-MS leads the way to fast identification of new ligands for GPCR targets including active forms of ion channels. *In silico* molecular modeling is underway to identify the orthosteric binding site.

[1] M. Toure, CM.Crews,. *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 1966-1973
[2] R. M. Franzini, L.H. Yuen, *ChemBioChem,* **2017**, *18*, 829-836
[3] Prudent, R., Annis, D.A., Dandliker, P.J., Ortholand, J-Y., Roche, D. *Nat Rev Chem* **2021**, *5*, 62–71