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Ultra-Miniaturized Weak Affinity Chromatography Coupled with Mass Spectrometry (nano-WAC-MS) as a Powerful Screening Strategy of Native Membrane Proteins in Fragment Based Drug Discovery: Adenosine Receptor as a Case-Study

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Summary

Fragment-based drug discovery (FBDD) is a structure-guided approach in which the first screening step (against a target protein) aims to identify small binding molecules ("hits") with potential for subsequent evolution into high-affinity ligands ("drug-like" compounds). Due to their inherent small size, these binding molecules (called fragments) have a weak affinity (µM to mM range) for the target. Although several biophysical techniques are available for fragment screening of soluble proteins, none are suitable for native membrane proteins such as G protein-coupled receptors (GPCRs), which are important targets for therapeutic agents. The key criteria for membrane protein fragment screening are low protein consumption (very difficult to produce proteins), unbiased conformational states and speed (low stability proteins).

Here we propose a strategy that combines the preparation of nanodisc-embedded GPCRs and the ultra-miniaturisation of weak affinity chromatography coupled with mass spectrometry.

The preparation of home-made affinity nanocolumns (less than 1 µL in volume) is proposed according to a "generic process" (i.e. applicable to any membrane protein): (i) in-situ synthesis (75 µm i.d. capillary) of a new highly hydrophilic monolith to minimize non-specific interactions [1], (ii) in-situ grafting of streptavidin (resulting in the so-called generic columns), (iii) in-situ capture of the biotinylated nanodiscs containing the receptor. The full characterization of affinity nanocolumns by frontal affinity chromatography is presented in terms of total target density (approximately 100 pmol/µL, i.e. less than 1 µg per column, an unrivalled protein consumption), active target density (approximately 92 pmol/µL) and low non-specific binding. The coupling of the affinity nanocolumns (flow rate 200 nL/min) to a nanospray mass spectrometer was then optimized by addition of a make-up flow (600 nL/min), resulting in a robust coupling.

Finally, the results of a fragment screening against the adenosine receptor AA2AR (50 fragments/injection) are presented and the relative advantages of different types of "control columns" are discussed.

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References

[1] J. Gil, I. Krimm, V. Dugas, C. Demesmay, Preparation of miniaturized hydrophilic affinity monoliths: Towards a reduction of non-specific interactions and an increased target protein density, J. Chromatogr. A 1687 (2023) 463670.