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LETTER

Affinity Selection Mass Spectrometry (AS-MS) as a Tool for Prospecting Target Ligands

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Affinity selection mass spectrometry (AS-MS) has been shown to be a powerful tool for identifying bioactive molecules in synthetic and/or natural libraries. The selection provided by the formation of the target-ligand complex allows the identification of hits irrespective of their functional effect. Moreover, it precludes the use of label, since the binders are identified by their exact mass.[1] The binders are determined by an affinity or index ratio calculated through control assays.[2–4] The target protein can be used in solution or immobilized in a solid support (Figure 1). Both approaches have pros and cons.[5,6]

Unlike most conventional high-throughput screening assays, AS-MS has fewer or no limitations when it comes to target selection. It is important, however, to understand the implications of choosing membrane proteins as targets. Membrane proteins correspond to 42% of all drug targets listed in DrugBank. Moreover, they are likely to be selected as protein targets due to their participation in many disease pathways, acting as ion channels, molecular transporters, solute carriers, receptors, and anchors.[7] One of the bottlenecks in working with membrane proteins comes from the need to use a detergent for solubilization, folding, and structure maintenance. Detergents are usually used above the critical micelle concentration, which can lead to empty micelles and thus to false positive results, caused by nonspecific interactions with the detergent micelles.[8] Interference in the ionization of the binders also needs to be examined.[9]

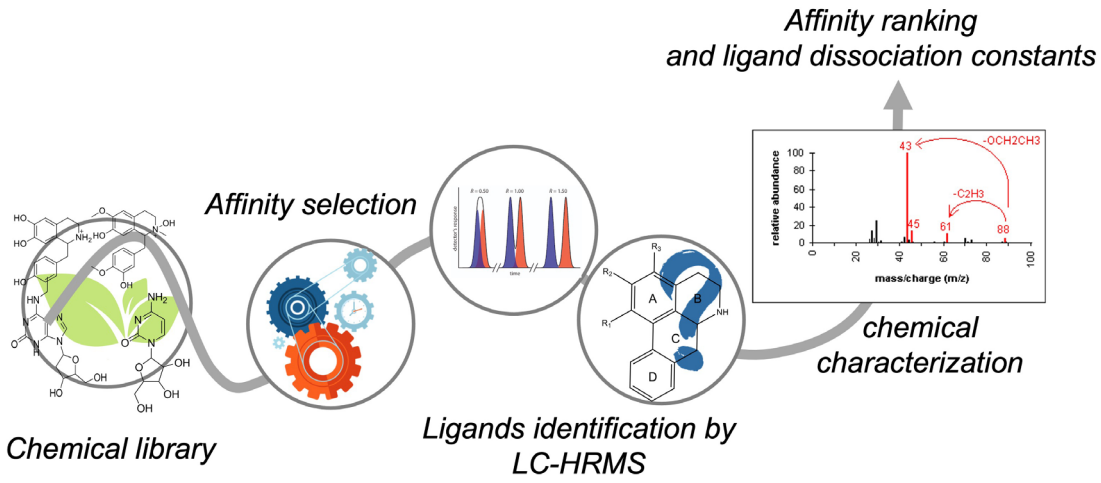

Figure 1. Schematic AS-MS for ligand screening in a chemical library.

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The AS-MS technologies mainly used with protein targets in solution are size exclusion chromatography (SEC) and pulsed ultrafiltration (PUF). These approaches are used to separate the protein–ligand complexes from unbound ligands. The collected protein–ligand complexes are then denatured by a variety of experimental conditions and the ligands are thus analyzed, usually by liquid chromatography high-resolution mass spectrometry (LC-HRMS).[1,6]

Two commercial settled technologies are used for SEC: SpeedScreen (Novartis) and the Automated Ligand Identification System (ALIS) (Merck & Co.).[1] SpeedScreen uses a micro-plate for the target-ligand complex formation and SEC for the separation step,[9] while ALIS relies on continuous-flow chromatography for the isolation and dissociation of the complex.[10] PUF is carried out either with a solvent pump or a centrifuge to push the protein–ligand complex through the membrane. The pore size and chemical composition of the membranes are important parameters to consider, to minimize adsorption of the protein and/or small molecules.[1,5]

AS-MS based on a solid supported target (Figure 2) has been explored with a diversity of applications and a variety of supports.[11] The workflow comprises the same four main steps used in the solution-based assay: load, wash, ligand extraction or desorption, and LC-MS analysis.

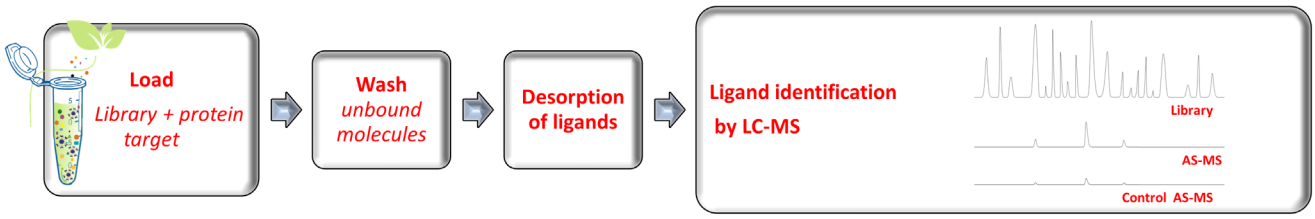

Figure 2. Workflow of AS-MS using a solid supported target.

Among the diversity of solid supports, magnetic beads have been the most used, probably because they provide large specific surfaces and can be separated rapidly and consistently by the application of external magnetic forces. The beads are composed of a magnetic material, such as iron, cobalt, nickel, and metal oxides, and a chemical moiety, which is used for target immobilization.[12] The beads' size and chemical composition are important parameters for their use in AS-MS.[13]

When screening synthetic libraries, one main advantage is that they are formed by known molecules, and the structural characterization of the identified ligands is thus made by the correlation of the exact mass, isotopic pattern, and LC retention time of each ligand present in the collection.[1] There are numerous synthetic library suppliers that provide multiple formats (96, 384, 1536 well plates), pooled compounds, and concentrations. In using a synthetic library, it is possible to design the configuration that is most suitable for the purpose. Ideally, the target should be present in molar excess relative to the pool of compounds. This avoids missing binders with lower affinity.[1] Another important aspect of synthetic libraries is that the chemical space can be covered in a more controlled fashion. Moreover, most of the compounds in these libraries have physicochemical properties that are associated with acceptable aqueous solubility and intestinal permeability, which comprise the first steps in oral bioavailability.[14] Consequently, when confirmed as binders, they have a better chance of progressing.

It is acknowledged that synthetic libraries should have a diversity of chemical scaffolds, while having synthetic availability. These requirements have recently been met by a "synthetic methodology-based natural product-like library", which enabled the identification of small molecules that cause the disruption of GIT1/β-Pix interactions.[15] Nonetheless, it has not yet been used in AS-MS.

Finally, the best benefit one can have with a synthetic library in AS-MS is the capability of validating the ligands as singlets, as the suppliers also provide any compound individually from the library.

The molecular and stereochemical complexities encoded in natural product libraries play a significant role in the drug discovery and development processes,[15,16] but hold a huge challenge in their use, and demands innovative assays method, in which AS-MS has been explored.

As an example, an AS-MS hemp screen[17] was carried out using a recombinant SARS-CoV-2 spike protein S1 subunit (~72 kDa) containing an N-terminal His-tag immobilized on Ni^{2+}-nitrilotriacetic acid-derivatized magnetic microbeads. Based on the dereplication of the ligands, and using cannabinoid standards for the equilibrium dialysis experiments, cannabigerolic acid (CBGA), tetrahydrocannabinolic acid (THCA-A), and cannabidiolic acid (CBDA) were identified as the ligands with the highest affinities. To this end, CBGA and CBDA blocked the infection of human epithelial cells by a pseudovirus expressing the spike protein.

The use of HRMS, software for data processing and curation, fragmentation experiments, spectral libraries, and molecular networks are all necessary for these collections, represented by the natural product extracts, to infer the molecular structures of the identified ligands.[2,4] It is an arduous and not always successful step in structural elucidation.[2,3] Anyhow, the clear identification of isobaric binders, either in a very compressed synthetic library or in natural products, is always a problem that requires additional deconvolution experiments.

It is important to stress that suitable biochemical and/or cellular assays are required to furnish further biological information, so as to characterize the identified ligands. Zonal or frontal bioaffinity chromatography can be used to this end. The AS-MS platform also serves to characterize target-ligand interaction mechanisms and can be used either to identify molecular targets or in phenotyping experiments. We expect to see more intensive use of AS-MS as a means of identifying low concentration binders from natural product extracts and the use of more diverse scaffold synthetic libraries.

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Fernando G. Almeida is Senior Scientist at OMass Therapeutics in Oxford, UK. He was a pioneer in setting up the affinity-based ligand screening platform at the company and has been successful in applying the AS-MS screening platform across different targets/projects. With more than 10 years of experience in LC-MS, it is worth mentioning his trajectory at the Research Facility (BioMass – CEFAP), Institute of Biomedical Sciences, University of São Paulo establishing the proteomics platform, followed by a post-doctoral fellowship at the Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, working with protein interaction and cross-linking proteomics.