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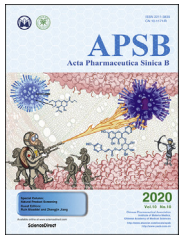

REVIEW

Recent advances in screening active components from natural products based on bioaffinity techniques

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Abstract Natural products have provided numerous lead compounds for drug discovery. However, the traditional analytical methods cannot detect most of these active components, especially at their usual low concentrations, from complex natural products. Herein, we reviewed the recent technological advances (2015−2019) related to the separation and screening bioactive components from natural resources, especially the emerging screening methods based on the bioaffinity techniques, including biological chromatography, affinity electrophoresis, affinity mass spectroscopy, and the latest magnetic and optical methods. These screening methods are uniquely advanced compared to other traditional methods, and they can fish out the active components from complex natural products because of the affinity between target and components, without tedious separation works. Therefore, these new tools can reduce the time

Abbreviations: AAs, amaryllidaceous alkaloids; ABCA1, ATP-binding cassette transporter A1; ACE, affinity capillary electrophoresis; APTES, 3-aminopropyl-triethoxysilane; ASMS, affinity selection mass spectrometry; ChE, cholesterol efflux; CMC, Cell membrane chromatography; CMMCNTs, Cell membrane magnetic carbon nanotube; CMSP, Cell membrane stationary phase; CNT, carbon nanotubes; EGFR, epidermal growth factor receptor; Fe_3O_4−NH_2, aminated magnetic nanoparticles; FP, fluorescence polarization; HCS, high content screen; HTS, high throughout screen; HUVEC, human umbilical vein endothelial cells; IMER, immobilized enzyme microreactor; MAO-B, monoamine oxidases B; MNP, immobilized on nanoparticles; MPTS, 3-mercaptopropyl-trimethoxysilane; MS, mass spectrometry; MSPE, magnetic solid-phase extraction; PD, Parkinson's disease; PMG, physcion-8-*O*-*β*-D-monoglucoside; RGD, arginine-glycine-aspartic acid; SPR, surface plasmon resonance; STAT3, signal transducer and activator of transcription 3; TCMs, traditional Chinese medicines; Topo I, topoisomerase I; TYR, tyrosinase; TYR-MNPs, tyrosinase-immobilized magnetic nanoparticles; UF, affinity ultrafiltration; XOD, xanthine oxidase; α_{1A}-AR, α_{1A}-adrenergic receptor.

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and cost of the drug discovery process and accelerate the development of more effective and better-targeted therapeutic agents.

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1. Introduction

Numerous phytoconstituents from natural resources have been reported to induce pharmacodynamic responses in the body in an additive or synergistic manner[1]. More than 70% of 1562 newly approved drugs from natural origins were introduced between 1981 and 2014[2]. However, the complex matrices of natural products usually contain therapeutically active components at low concentrations. The sample preparation processes of the traditional analytical methods are unable to detect these active components especially at their usual low concentrations. Many investigations adopted the function-based approaches including the extraction and isolation of these therapeutically active components, followed by extensive pharmacological assays[3−6]. The bio-guided assays are selected based on ethnopharmacological approaches[7], simple isolated enzyme assays[8] where the mixture is tested against a particular target[9], and phenotypic assays in which the growth, viability, or function of a cell or a tissue is monitored[10,11]. And, cellular systems are studied in more sophisticated approaches which are often labor-intensive, expensive, and inefficient[12]. However, very low concentrations of the active components could be difficult to detect through conventional analytical and separation techniques. Therefore, researchers designed more efficient experimental assays and the screening technologies to identify the valued components[13].

The term "dereplication" was introduced by Beutler et al.[14] in 1990 as "a process of quickly identifying known chemotypes". Their goal was to evaluate the activity of a range of natural products extracts with simple receptor binding assay and to rapidly identify compounds responsible for this activity without investing time in traditional bioassay-guided fractionation or full structure elucidation procedures[15]. To alleviate the dereplication holdup (a major bottleneck in natural products discovery), scientists have been conducting their research efforts to add tools to their "bag of tricks" aiming to achieve faster, more accurate and efficient ways to accelerate the drug discovery process[16].

Affinity-based screening approach, an extremely convenient and efficient method for separating the potential ligands from a complex mixture, depends up on the principle of macromolecular target-ligand binding. Unlike function-based approaches, the affinity-based screening approach does not require separating every single component of a complex mixture, the mixture can be analyzed as a whole. This approach focuses specifically on the bonded target-ligands, setting aside the unbonded components. Because many macromolecules, such as DNA, proteins, enzymes and receptors, have been demonstrated as the pharmaceutical targets[17−20], the bioaffinity-guided screening or separation method undoubtedly accelerates the discovery process and reduces the research cost[21−23]. These affinity-based screening assays can investigate multiple interacting pairs involved in biological systems, including antigen−antibody, receptor−ligand, enzyme−inhibitor/activator, and protein−protein interactions[24]. These methods include affinity chromatography[25−27], biological chromatography[28,29], ligand fishing[30], affinity electrophoresis[31,32], magnetic separation screening[33,34], spectrum-based methods including fluorescence polarization[35], and surface plasmon resonance (SPR)[36], etc. The principles and applications of these methods have been discussed in this review.

2. On-line screening based on chromatography or electrophoresis

Here, an on-line screening method refers to those which can integrate screening, separation, identification or activity assay procedures in a single automatic instrument. Undoubtedly, chromatographic, electrophoresis and mass spectroscopic methods can meet this requirement. Guo et al.[37] have reviewed the applications of bio-affinity chromatography for screening active compounds from natural products. Various biomolecules or cells have been immobilized onto the surface of supports for screening the target compounds[37,38]. Herein, we have explained some of the latest applications (during 2015−2019).

2.1. Cell membrane chromatography(CMC)−HPLC−mass spectrometry (MS)/MS

CMC was first proposed by He et al.[39,40] in 1996, which has been proven to be an effective method for screening active components interacting with specific receptors in natural products such as traditional Chinese medicines (TCMs). This method utilizes the integrated cell membrane to study the interaction between ligands and transmembrane receptors. Cell membrane stationary phase (CMSP) is prepared by immobilizing cell membranes containing specific receptors on silica carriers and packing them into a steel column[39,41]. When crude extracts pass through the CMC column, the ligands with high affinity can be retained on the column, while nonspecific analytes are eluted quickly. CMC, when combined with multidimensional chromatography or HPLC−MS, can better screen, separate and identify the active components. Fig. 1[29] shows the diagrams of the instrument equipment and the principle of a 10-port switching valve. In Fig. 1A, the CMC part and HPLC−MS/MS are connected through a 10-port switching valve and designated as the 1st and the 2nd dimensions, respectively. CMC screens the active components, and the HPLC−MS/MS identifies them. In the valve position (Fig. 1B), A was the analysis status, and B was the enrichment of retention fractions status. Table 1[29,42−55] shows some applications of various CMC combined with LC−MS/MS system, which have been utilized for screening active compounds from TCMs in the last five years.

Table 1 shows that the CMC method has been used for screening many active components with diverse biological functions. Among these applications, HEK 293 cells with various high expression receptors were the most favorable screening tools. In the future, many more suitable cells or enzymes will be applied as screening tools and the stability and lifetime of the CMC column

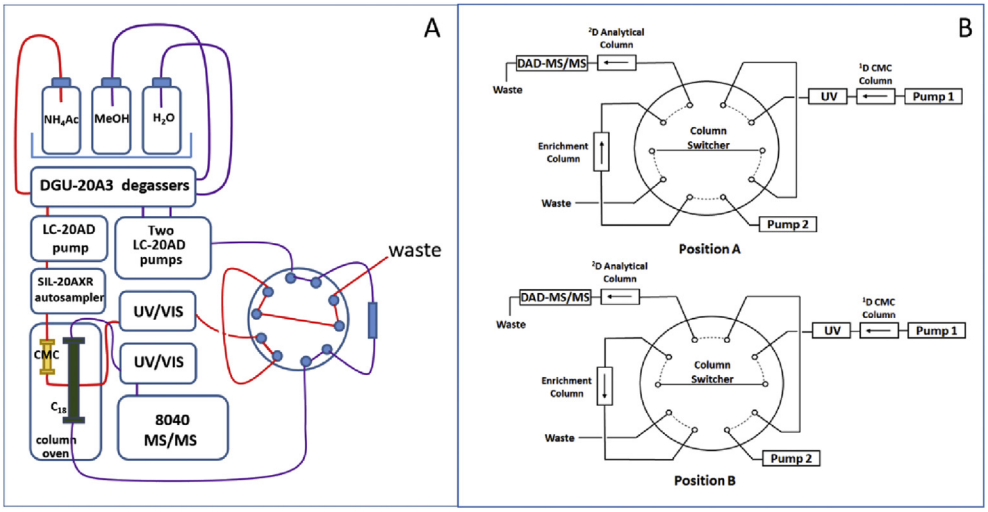

Figure 1 The diagrams of the instrument (A) and the principle of the 10-port switching valve (B). Reproduced with permission from Ref. 45 Copyright © 2017 The Royal Society of Chemistry.

will be improved. At the early stage of CMC, the membrane was immobilized on the surface of a silica-based adsorbent. In recent years, Ding et al.[56] improved the stability of the CMC column using APTES or MPTS to modify silica. Another simple CMC method called the "relative standard method" was developed to determine K_D values[57]. The K_D value of a positive drug with a known target receptor was determined by frontal affinity chromatography (FAC). Other ligands' K_D was calculated by comparing the ratio of retention time between the standard drug and other ligands on the CMC column. A stepwise frontal affinity chromatography model for drug and protein interaction was established, in which the K_D values could be determined within 30 min[58]. Therefore, there are many challenges needed be taken in the development of CMC methods.

2.2. *Cellular membrane affinity chromatography (CMAC)*

A transmembrane protein (GLUT1 transporter) was firstly immobilized by Yang et al.[59] in 1995, which demonstrated that transmembrane protein-based stationary phases could be used for studying ligand−protein interactions. Based on this work, Ruin Moaddel and Irving W. Wainer developed the CMAC method in 1998. In their work, the cellular membranes obtained from an HEK293 cell line expressing the $\alpha 3\beta 4$ nicotinic acetylcholine receptor (nAChR) were entrapped in lipid monolayers and used to study the binding of ligand to the nAChR using frontal affinity chromatography[60]. Therefore, the CMAC technique utilizes the immobilization of a target transmembrane protein onto a stationary phase. The target protein is isolated by homogenizing and solubilizing a source (*e.g.*, cell line) followed by immobilization on either the artificial membrane-phosphatidyl choline (IAM-PC) stationary phase or the surface of an open tubular capillary during a dialysis step. In recent years, they developed mitochondrial membrane affinity chromatography (MMAC) by immobilizing mitochondrial membrane fragments onto the artificial membrane stationary phase[61]. For the detailed information of CMAC, a reference[62] is recommended for readers.

2.3. *Comparison between CMC and CMAC*

For both CMC and CMAC methods, transmembrane proteins are collected from tissues, native cell lines and transfected cell lines; these methods can be used for the initial screening of bioactive components from natural products and the investigation of the affinity interaction between drugs and target receptors; both CMC and CMAC can determine the dissociation constant (K_D) through the frontal affinity chromatography (FAC), nonlinear chromatography (NLC), and zonal elution chromatography.

For CMC, the whole-cell membrane was used to prepare the stationary phase, which can retain the original structure and activity of the transmembrane proteins. While in the CMAC method, transmembrane proteins were isolated by homogenizing and solubilizing cell lines followed by immobilization on the IAM-PC stationary phase. This process requires many reagents, such as protease inhibitors and detergents, to maintain the activity of the transmembrane proteins. This column is commercially available at a high price. Therefore, CMC is easier preparation and cheaper compared to CMAC.

2.4. *Online solid-phase extraction (SPE)−HPLC−MS/MS*

SPE is a widely used sample preparation technique. The analytes are immobilized in a solid sorbent during the sampling process and then recovered by elution. The on-line SPE−LC method is realized through a switching valve in HPLC−MS/MS; this process is faster, high throughput, and automatic. For enhancing the selectivity of the absorbents and analytes, Bagnati et al.[63] firstly prepared an immunoaffinity column (50 mm × 2 mm i. d.) with a commercial immunoaffinity gel (containing antibodies against dexamethasone) for online extraction and purification of dexamethasone and betamethasone in bovine urine.

Recently, an enzyme-based SPE mode has been reported for screening enzyme inhibitors from natural products. Wang et al.[64] have developed a monolith-based AChE-IMER for screening AChEIs from the 70% ethanol extracts of *Corydalis yanhusuo*. As

Table 1 Applications of cell membrane chromatography (CMC)−LC/MS system in screening the active components from the traditional Chinese medicines (TCMs).

No.	CMSP	Natural product	Target component	Biological function	Ref.
1	H9c2 rat cardiac myoblasts	Red yeast rice	New yellow pigment, monacolin K, methylester of monacolin K acid, ethyl ester of monacolin K, dihydromonacolin K, methylester of dihydromonacolin K acid, dehydromonacolin K	Myocardial protective effect	42
2	Rat basophilic leukemia 2H3 cell	*Carthamus tinctorius*	Hydroxysafflor yellow A	Anti-allergic effect	43
3	Platelet	Radix Salviae Miltiorrhiae	Rosmarinic acid, lithospermic acid, salvianolic acid B, two isomers of salvianolic acid B, salvianolic acid C, salvianolic acid D and salvianolic acid H/I	Anti-platelet activity	44
4	Murine macrophage cell	Rheum Officinale, *Angelica dahurica,* and Radix Bupleuri	Dehydrocostus lactone, scopoletin, phellopterin	Anti-inflammatory effect	45
5	Epidermal growth factor receptor	*Marsdenia tenacissima*	Tenacissoside G, tenacissoside H, tenacissoside I	Anti-tumor effect	46
6	Mas-related G protein-coupled receptor X2 cell membrane	Radix Saposhnikoviae	Prim-*O*-glucosylcimifugin, cimifugin, 4′-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol	Anti-allergic effect	47
7	EGFR & FGFR4 HEK293 cell membrane	Radix Salviae Miltiorrhiae	Salvianolic acid C, tanshinone I, tanshinone IIA, cryptotanshinone	Anti-tumor effect	48
8	Rat cardiac muscle	Fructus Schisandrae Chinensis	Deoxyschizandrin, schisantherin A	Myocardial ischemia protecting	49
9	CHO−S/β_1AR	Coptis Chinensis, Rhizoma Corydlis Decumbentis	Coptisine, jatrorrhizine	Myocardial ischemia protecting	50
10	HEK293/EGFR	Radix Scutellariae	Wogonin	Anti-tumor effect	51
11	HEK293/EGFR	Rhizoma Belamcandae	Irisflorentin	Anti-tumor effect	52
12	HEK293/α_{1A} AR	*Peucedanum praeruptorum* Dunn	Paeruptorin A, paeruptorin B, paeruptorin C	α_{1A} Adrenoceptor agonists	53
13	HepG2	Radix Scutellariae	Baicalein, wogonin, chrysin, qroxylin A, neobaicalein, rivularin	Anti-hepatoma effect	54
14	Rat uterus	*Leonurus artemisia*	Genkwanin	Uterine contractions promotion	55
15	MCF-7	Cortex Magnolia Officinalis	Magnolol, honokiol	Anti-tumor effect	29

shown in Fig. 2[20], AChE-ICERs and control-ICERs were prepared through immobilizing AChE onto the surface of a poly (glycidyl methacrylate-*co*-ethylene dimethacrylate) [poly (GMA-*co*-EDMA)] monolithic support and installed in parallel as SPE columns to establish a comparative online ligand fishing platform for rapidly separating and identifying AChE ligands in *C. yanhusuo* extracts. Eight compounds with AChE binding affinity were detected and identified, and their AChE inhibitory activities were further verified through *in vitro* enzymatic inhibition assay. Peng et al.[65] immobilized xanthine oxidase (XOD) on amino-functionalized silica using GA as a crosslinker, and then packing into a stainless-steel column. The resulting SPE column was coupled to an HPLC−DAD−MS/MS system for the online screening of XOD inhibitors from the water extract of *Lonicera macranthoides*. Six compounds, including 3-caffeoylquinic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, were fished out. These methods can be used for screening enzyme inhibitors, and the instruments are similar to the CMC−HPLC−MS/MS system. Screening, separation and identification can be performed online.

2.5. *Affinity capillary electrophoresis (ACE)*

ACE was firstly proposed by Chu et al.[66]. As an effective, rapid, and microscale separation technology, close to physiological conditions, ACE plays a crucial role in studying macromolecular interactions[67]. In the ACE method, enzymes are the most suitable biomolecules for screening[68,69]. The schematic diagram of the in-capillary enzyme assay process and the capillary electropherogram of the reaction mixture is shown in Fig. 3A. Due to reusability of the enzyme, the immobilized capillary enzyme reactor (Fig. 3B) would be helpful for screening inhibitors in a complex mixture using CE[31]. According to the different enzyme incubation processes, the ACE screening method has two modes, off-line and on-line[70]. In the off-line screening mode, the enzymatic reaction is completed out of capillary and the reaction product is analyzed by electrochromatographic system[32,71,72]. In the on-line mode, the steps including reaction, separation, and detection are carried out in capillary.

For screening active components from natural products based on CE, immobilized enzyme microreactor (IMER) is the favored technique[73]. In IMER, the enzyme is immobilized in the capillary

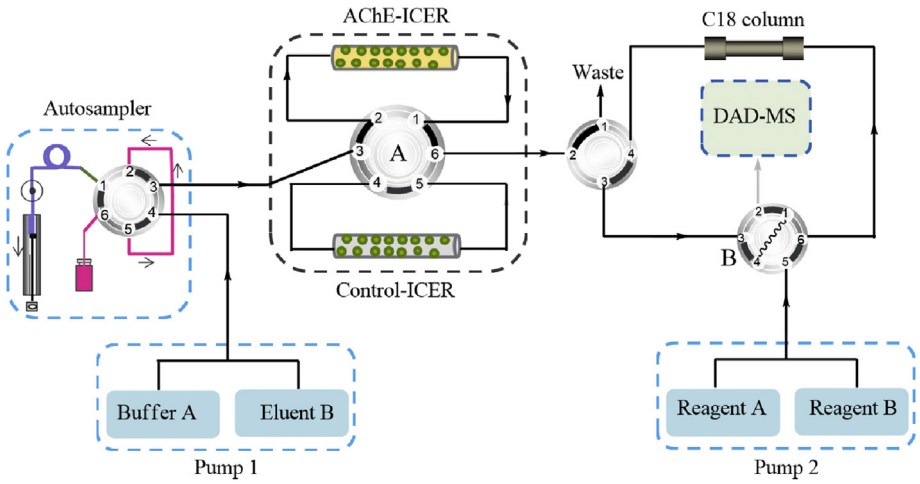

Figure 2 Schematic diagram of the online ligand fishing and identification platform. Reprinted with permission from Ref. 20. Copyright © 2018 Elsevier.

to fabricate the enzymatic reaction. The immobilization of enzyme renders high enzyme activity, relatively good stability and reusability[74]. Additionally, the adsorption of the enzyme can be prevented in the capillaries, which will help improve the efficiency of separation. However, the IMER is not applicable in the enzymatic reaction system, because the buffer for incubation is different from the buffer for separation. Cheng and Chen[75,76] fabricated IMERs for screening tyrosinase inhibitors and trypsin inhibitors from TCMs. They also demonstrated the molecular interaction between enzymes and inhibitors using molecular docking. Zhao et al.[77] established a CE method combined with the pressure mediated microanalysis and electrophoretically mediated microanalysis to study enzyme kinetics and inhibition kinetics of α-glucosidase. The convenient, low-cost and effective in-capillary enzymatic assay could screen the α-glucosidase inhibitors from 9 natural flavonoids. The monolithic column was considered as support with high-efficiency for enzyme immobilization. Zhang et al.[78] fabricated a pepsin−IMER for enzymatic kinetics analysis and inhibitor screening by covalently immobilizing pepsin in a polymer monolithic column. The prepared capillary could screen the pepsin inhibitors from 9 natural products. Some researchers developed IMER combing with mass spectrometry for screening inhibitors. This model demonstrated its applicability and validity using tacrine, galanthamine and the alkaloid uleine as the reference inhibitors for butyrylcholinesterase[79].

3. Off-line screening based on new materials and mass spectrometry methods

3.1. *Affinity selection-mass spectrometry screening (ASMS)*

ASMS was proposed firstly to detect the binding of small sub-sets of ligands to a protein[80]. In the direct ASMS mode, protein−ligand complex is directly detected by MS. The active component is identified through calculation of the difference mass value between the complex and the protein. The disadvantage of this approach is that the ionization process may fully or partially distort the protein−ligand complexes; appropriate MS parameters for each type of complex are challenging and time-consuming[81]. In addition, the mass of the protein−ligand complex is not substantially different from that of the protein itself, even when a binding event is detected, the exact mass of the ligand is difficult to calculate. In another ASMS mode, the protein−ligand complex is isolated, and the ligands are dissociated and identified. Sometimes, the ligands bind weakly and nonspecifically to the protein, and they also can be detected as protein binders in the ASMS assay. This method suffers from a higher incidence of false-positive hits. To solve this problem, Desaire's group[82] developed a method for identifying the tightest-binding lead compounds for target proteins with no false positive identifications. In their approach, the protein and the ligands are incubated together, and

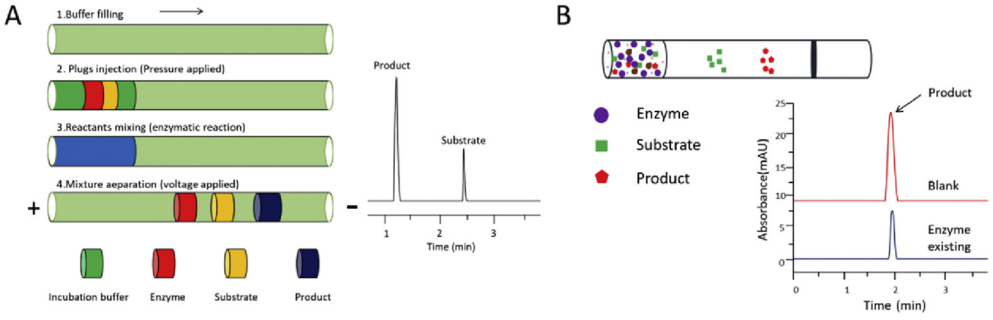

Figure 3 Scheme for screening enzyme inhibitor with in-capillary enzymatic assay (A) and immobilized enzyme microreactor (B).

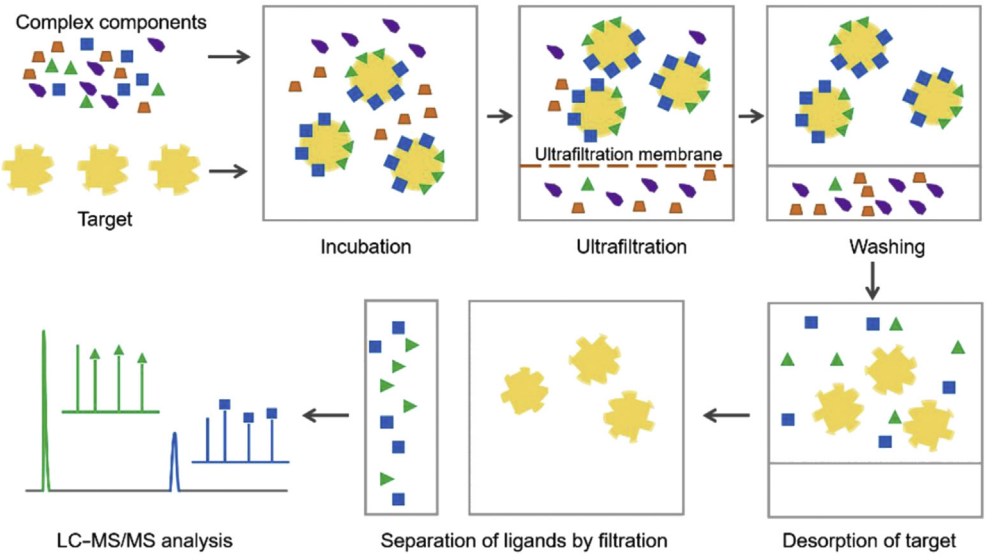

Figure 4 Schematic diagram of the affinity ultrafiltration−HPLC−MS method for screening bioactive components.

the non-binders are separated for detection. They compared the mass spectrum of non-binders before and after incubating with the target.

3.2. *Affinity ultrafiltration (UF)−HPLC−MS/MS*

UF was firstly employed by Luong in 1998 to purify trypsin[83]. Choi et al.[84] firstly developed the ultrafiltration LC−MS method to screen the inhibitors of quinone reductase-2 from the extracts of the marine sediment bacteria and *Humulus lupulus* L. According to the principle (Fig. 4), the ligand−receptor complexes could be retained using an appropriate membrane for ultrafiltration, while impurities would pass through. Secondly, the ligands were released from the complexes using certain solvents. Thirdly, the ligands were identified and quantified by HPLC−MS/MS[85]. This analytical process can maintain the natural conformation of the protein targets, and accurately reflect the physiological conditions for the interaction between the protein targets and small-molecule drugs. Protein targets can be reused in this approach, which is particularly important for some expensive or rare targets.

Many research groups have used affinity UF−HPLC−MS to study the interactions between the chemical components from natural products and biological target proteins[11,86−91]. Chen et al.[92] investigated the interactions between amaryllidaceous alkaloids (AAs) from *Lycoris radiata* and Topo I using affinity UF combined with HPLC−ESI/MS. The results show that 11 AAs specifically bound to Topo I, and hippeastrine displayed the highest enrichment factor. In another study, eight potential components were isolated from *Rhamnus davurica* using affinity UF−HPLC−MS[89]. α-Glycosidase was investigated as a key target for treating type 2 diabetes[93]. Chen et al.[90] developed affinity UF−HPLC−ESI-MS method to screen α-glucosidase inhibitors from *Gymnema sylvestre* extracts. A total of 9 components were considered as potential α-glucosidase inhibitors. Unfortunately, the ultrafiltration technique required repeated manual washing procedures to remove the unbound components[94]. Some components with weak interactions can also be removed. Therefore, this method is inappropriate for investigating the dissociation constant between bioactive components and targets.

3.3. *Magnetic material-based methods*

Compared to the traditional methods, magnetic materials are more useful for screening natural products. Based on the particle size, magnetic materials can be divided into magnetic beads, magnetic nanoparticles, and other magnetic materials[94].

Magnetic particles were firstly prepared by Guesdon and Avrameas in 1977[95]. Later various magnetic beads were fabricated by modifying the surface of the particles with isocyanate-, epoxy- and vinyl groups. The functional groups couple the spacer arms with amino, hydroxy or carboxylic end groups. Strongly hydrophilic substances from both natural and synthetic origin could be attached to the surface (Fig. 5). Magnetic beads can easily and flexibly handle any volume (either large or micro liter scale) of biological samples without repeated pipetting and centrifuging. Many applications with magnetic beads have been used in bioassays (such as antibody or DNA). Moaddel et al.[96] firstly developed magnetic beads immobilized with HSA or SIRT6 protein for ligand fishing in chemical therapeutics and medicinal plant extracts[97]. Many studies have demonstrated that magnetic bead-based approaches can quickly separate and identify active components from complex mixtures, and the integration with liquid chromatography and mass spectrometry helps elucidate the structures of these components accurately[20,98−100]. Most of the investigations focused on the screening of different enzyme inhibitors including lipase[101], transglycosylase[102], α-glucosidase[103,104], acetylcholinesterase[20,105,106], xanthine oxidase[98], angiotensin converting enzyme[107], and α-amylase[108] inhibitors. For example, neuraminidase was immobilized onto the surface of amine-terminated magnetic beads to screen neuraminidase inhibitors. Based on the enzymatic activity, 12 compounds were screened from different herbal secondary metabolites[109]. Despite various advantages of magnetic beads, as described above, few challenges, including the effective desorption of the ligands from the target, are yet to be solved[94].

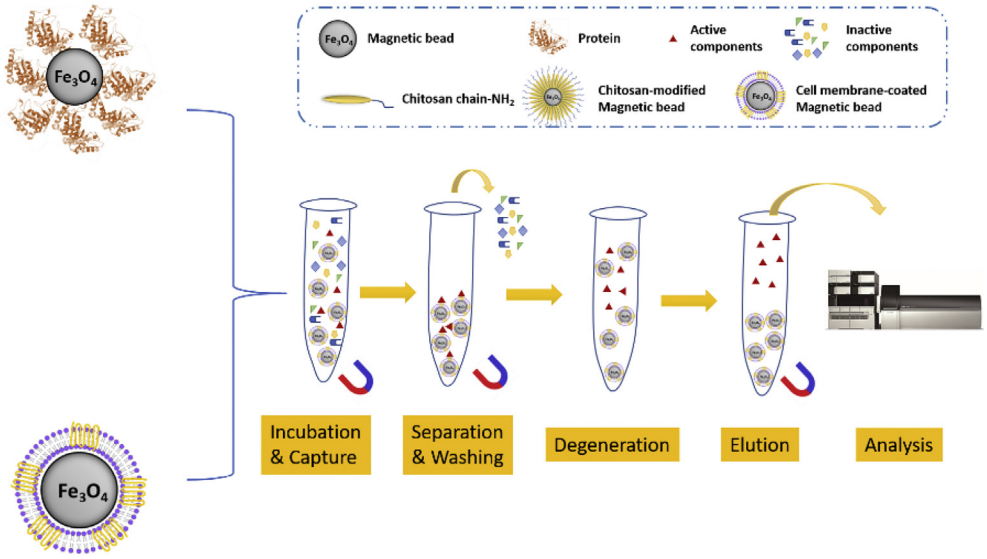

Figure 5 Workflow of the screening method using magnetic particles/nanoparticles.

In recent years, magnetic solid-phase extraction (MSPE) based on enzyme immobilized on nanoparticles (MNP) has become increasing popular owing to their large surface area, and easiness for surface modification and magnetic separation. Many macromolecules, such as α-glucosidase[110], pancreatic lipase[111], folic acid[112], BSA[113], and aptamers[114], have been immobilized on the surface of MNP and used to screen active components from natural resources.

Parkinson's disease (PD), also known as tremor paralysis, is a common degenerative disease of the central nervous system, which causes severe disability in elderly persons. Monoamine oxidases B (MAO-B) inhibitor is a widely recognized target for anti-PD drugs. Some researchers immobilized MAO-B onto MNP to screen inhibitors from *C. fraxini* and *P. granatum*[115]. Tyrosinase (TYR) catalyzes two crucial reactions in the melanogenesis, namely oxidation of monophenols to diphenols, and diphenols to *o*-quinone that ultimately transforms to melanin[116]. TYR is involved in the pathogenesis of Parkinson's disease and skin diseases[117]. Therefore, tyrosinase inhibitors have received tremendous attention[118]. Liu et al.[119] immobilized TYR on the surface of aminated magnetic nanoparticles (Fe_3O_4—NH_2). The immobilized TYR showed enhanced pH and temperature endurances reusability, storage stability and higher catalytic activity compared to free TYR. Immobilized TYR was applied for screening inhibitors

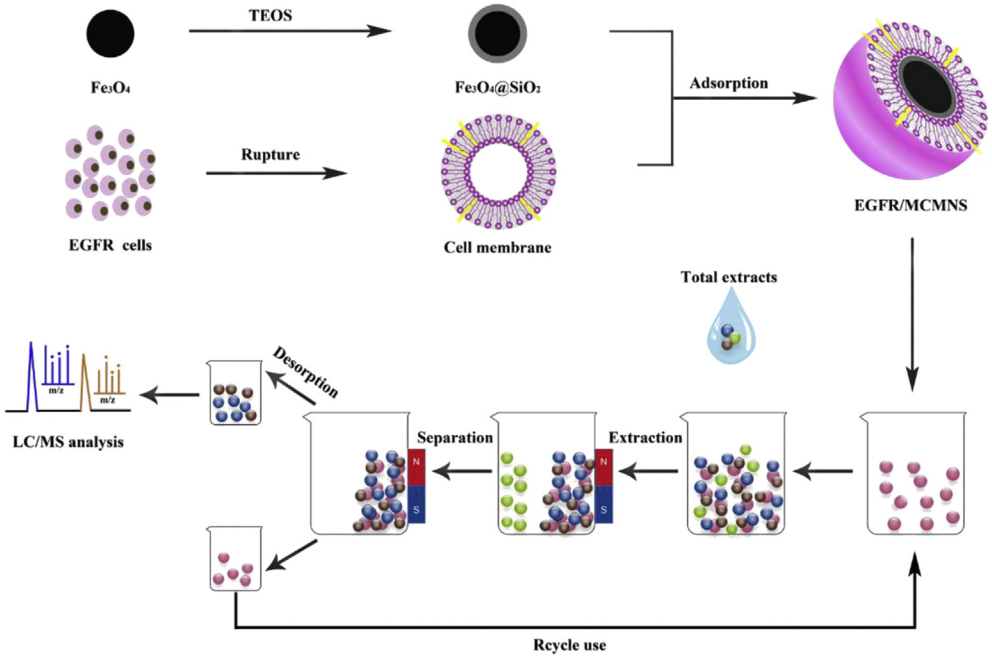

Figure 6 Schematic illustration for high expression of EGFR HEK293 cell membrane coated magnetic nanoparticles for extracting bioactive compounds. Reproduced by the permission of Ref. 119 Copyright © 2019 The Royal Society of Chemistry.

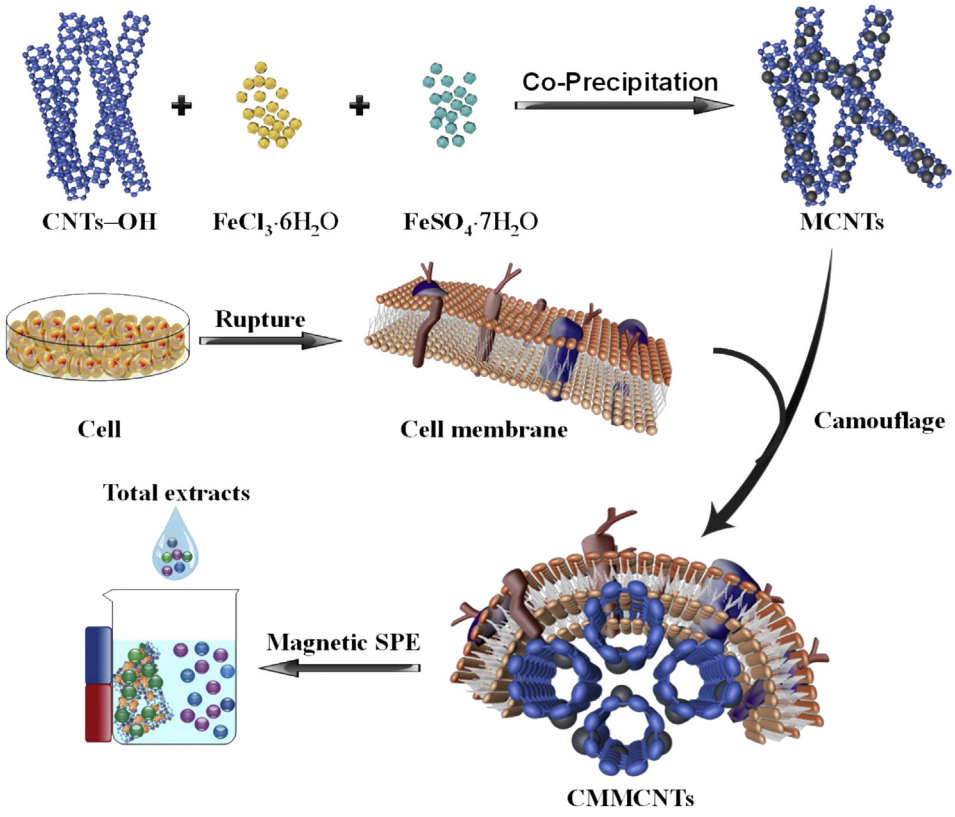

Figure 7 The schematic illustration of preparation and application of CMMCNTs. Reprinted with permission from Ref. 34 Copyright © 2018 Elsevier.

from 11 TCMs. Chen et al.[33] developed a microplate assay integrating TYR-immobilized magnetic nanoparticles (TYR-MNPs) and a homemade magnetic microplate for high-throughput screening compounds, which could interact with the active sites of the enzyme, or copper chelators and bind more strongly than TYR to copper ions, thus distinguishing them from antioxidants or TYR substrates. Integration with a homemade magnetic microplate enables high-throughput inhibitor screening.

Cell membrane cloaked nanoparticles have exhibited great potential drug discovery. In our group, Hu et al.[120] developed a novel method by immobilizing epidermal growth factor receptor (EGFR) HEK 293 cell membrane on MNP. Fig. 6[120] shows that Fe_3O_4 particles have been synthesized as a magnetic core. After coating with a SiO_2 shell, Fe_3O_4 nanoparticle surfaces were covered with polar silanol groups (Si−OH), which helped in the easy absorption of the cell membrane. An optimized amount of the cell membrane with the special self-fusion characteristics could fully cloak the Fe_3O_4@SiO_2. Using this material, benzoylmesaconine and hypaconitine were identified in Radix Aconiti[34]. Based on this method, they also developed a novel dual functionalization carbon nanotubes (CNT) modified with magnetic nanoparticles and high α_{1A}-adrenergic receptor (α_{1A}-AR) expression HEK 293 cell membrane. Fig. 7[34] shows that the MCNTs nanomaterials have been prepared through the electrostatic interaction between MNPs and the surface of CNTs−OH. α_{1A}-AR highly expressed cell membrane was camouflaged on magnetic CNTs. This functionalized cell membrane magnetic carbon nanotube (CMMCNTs) was employed as a drug discovery platform to screen potential α_{1A}-AR antagonists from Radix Aconiti[34]. Two bioactive compounds, namely lappaconitine and benzoylmesaconine were screened out using this method.

3.4. *Surface plasmon resonance (SPR)*

SPR spectroscopy is used for detecting biomolecular binding interactions. In SPR, one molecular partner is immobilized on a metallic film. Then light excites the surface plasmons on the metal; when the ligand binds to the immobilized molecule, a detectable change in the surface plasmon signal can be observed[36]. In the pharmaceutical analysis, scientists can monitor almost any type of molecular interaction in different types of biological molecules (including serum proteins, oligomers, antibodies, and enzymes) using SPR biosensors. Zhang et al.[121] developed an on-line SPR−HPLC−MS/MS method for analyzing human serum albumin binders from Radix Astragali. This application was not widely used due to the incompatibility of the pressure of the SPR and HPLC system.

In most cases, natural products were screened by high throughout methods or molecular docking. Then, the binding affinity of the specific compounds to be the biomolecules was using SPR[122−124]. Cao et al.[125] immobilized TNF-R1 on the SPR sensor surface and *R. officinale* was screened out from four TCMs. Combining with UPLC−QTOF/MS, the bioactive compound was identified as physcion-8-*O*-*β*-D-monoglucoside (PMG). Wang et al.[126] used a novel interactomics approach, Nematic Protein Organisation Technique (NPOT), for identifying ATP-binding cassette transporter A1 (ABCA1), a key membrane transporter contributing to cholesterol efflux (ChE), as a direct binding target of evodiamine. The binding of evodiamine to ABCA1 was confirmed by SPR experiments. Chen et al.[127] developed a SPR biosensor-based active ingredients recognition system (Fig. 8[127]) and applied to screen the signal transducer and activator of

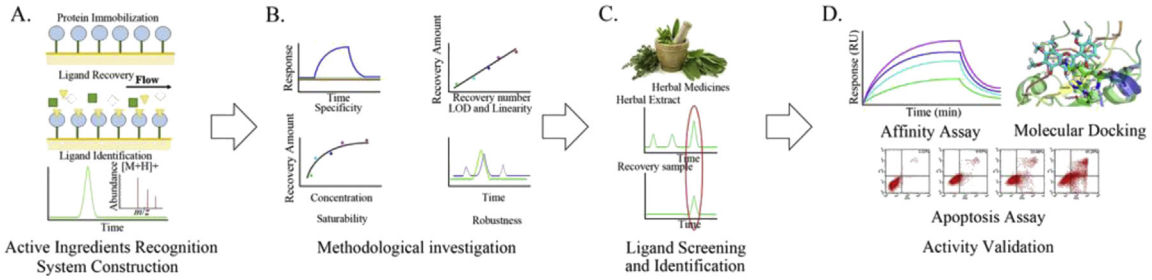

Figure 8 Workflow of SPR−AIRS (A), methodological investigation (B), application in herbal extracts (C), and validation of results (D). Reprinted with permission from Ref. 126 Copyright © 2018 American Chemical Society.

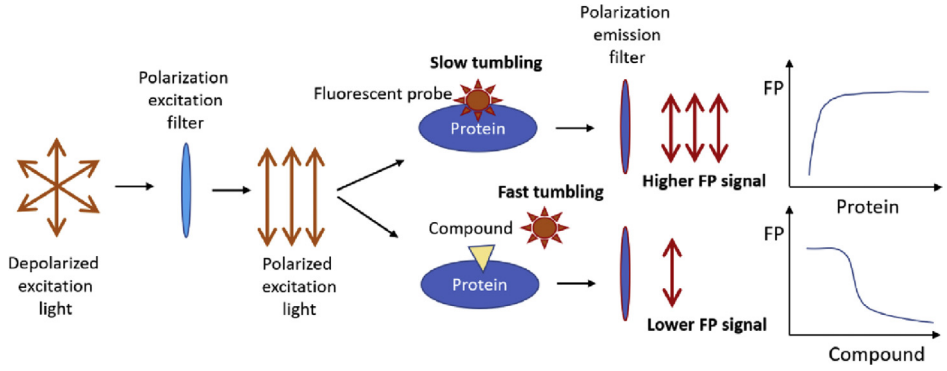

Figure 9 The basic principle of the fluorescence polarization.

transcription 3 (STAT3) ligands from medicinal herbs. Nine candidate compounds were fished out.

In drug discovery, SPR-based screening methods are the most promising tools for high throughput screening, since they can monitor the interactions of low-molecular-weight compounds even at low concentrations. SPR can be coupled with other emerging technologies, and novel sensing materials for developing sustainable *in vitro* methods and improving drug candidates.

3.5. *Fluorescence polarization assay (FP)*

FP is an intrinsically powerful technique for the rapid and homogeneous analysis of molecular interactions in biological/chemical systems. The basic principle of FP is shown in Fig. 9. A fluorescent probe is excited with polarized light, and emission intensity is measured as FP. An unbound fluorescent inhibitor (red) shows a fast tumbling, resulting in a higher depolarized emission signal. The fluorescent probe binds to a larger protein, resulting in a slow tumbling and a higher polarized emission signal[128]. This technique has been successfully used in monitoring enzyme kinetics, protein−protein interactions, DNA diagnostics, biological interactions and high throughput screening in drug discovery.

Cornish's group[129] modified this method and constructed a broadly applicable high-throughput screening for small-molecule targets. The assay requires a target receptor and a reporter molecule, which can couple the target molecule with a fluorophore. The target molecules compete with the reporter for binding to the common receptor. Thus, the target concentration is inversely proportional to the ratio of bound to unbound reporter molecules, which can be measured by FP. They used the receptor FKBP12 and reporter molecule FK506-fluorescein to construct FP assay for screening FK506 (tacrolimus) from *Streptomyces tsukubaensisin* in 384-well, round, black-bottom plates. Berg's group[130] used the STAT5a FP−based binding assay for HTS of chemical libraries comprising a total of 3289 natural products and/or known bioactive substances. NF023 and NF449 display improved selectivity for the SH2 domains of STATa/b and NF449 was the most active candidate among the STATa/b SH2 domain inhibitors. Dysregulation of MLL1 catalytic function is relevant to mixed-lineage leukemia and targeting WDR5−MLL1 interaction could be a promising therapeutic strategy for leukemia harboring MLL1 fusion proteins. Ye et al.[131] discovered several small-molecule inhibitors with potent inhibitory activities *in vitro* against WDR5−MLL1 interaction through FP-based high throughput screening.

3.6. *Other materials*

In recent years, cell-based detection methods have attracted wide attention in drug development. By 2D tissue or 3D cultural on a hydrogel-based chip[132], hollow fibers[133−139], and cell-based assays have been used for screening natural products. For example, Li et al.[140] proposed a cell-based SPE model for screening potential bioactive compounds from *Ligusticum chuanxiong*. They functionalized the poly(oligo (ethylene glycol) methacrylate)-modified amino microspheres using an arginine-glycine-aspartic acid (RGD) peptide. Human umbilical vein endothelial cells (HUVEC) were immobilized on the surface of modified microspheres through the high affinity of RGD and integrin on HUVEC.

The column exhibited good stability after 14 days of intensive sampling. Three bioactive compounds were identified, and two of which were 3-butyl hexahydroisobenzofuran-1(3*H*)-1 and tetramethylpyrazine. For some reason, these methods are not widely used now.

4. Conclusions and perspective

Overall, the screening methods based on affinity techniques successfully employed for revealing the healing mechanism of natural products, finding the active components from the complex natural products, and facilitating drug discovery. However, many challenges should be solved in the future: 1) In affinity techniques, non-specific absorption will lead to false-positive results, so non-specific absorption should be reduced in those techniques. 2) Normally, the healing effect of a natural product depends upon its multi-targets, so multi-targets screening methods should be developed. 3) Combinative technologies, such as combing affinity screening, LC−MS/MS and NMR can greatly improve the screening and identification process of unknown active components. 4) Biosensors, such as SPR or SPR combined with an imaging system, should be developed for readily achieving high-throughput real-time label-free biosensing in two-dimensional (2D) microarrays and parallelly monitoring multiple molecular interactions.

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Author contributions

Sicen Wang and Xiaofang Hou designed the study. Xiaofang Hou drafted the manuscript. Meng Sun, Tao Bao, Xiaoyu Xie and Fen, Wei were major contributors in reviewing the manuscript. Based on the contributions, Xiaofang Hou was listed as the first author while Sicen Wang was the correspondence. All authors read and approved the final manuscript.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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