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Formation of self-assembly aggregates in traditional Chinese medicine decoctions and their application in cancer treatments

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Traditional Chinese Medicine (TCM) formulas, based on the principles of Chinese medicine, have a long history and are widely applied in the treatment of diseases. Compared to single-component drugs, TCM formulas demonstrate superior therapeutic efficacy and fewer side effects owing to their synergistic effects and mechanisms of detoxification and efficacy enhancement. However, various drawbacks, such as the uncertainty of functional targets and molecular mechanisms, poor solubility of components, and low bioavailability, have limited the global promotion and application of TCM formulas. To overcome these limitations, self-assembled aggregate (SA) nanotechnology has emerged as a promising solution. SA nanotechnology significantly enhances the bioavailability and anti-tumor efficacy of TCM by improving its absorption, distribution, and precise targeting capabilities, thereby providing an innovative solution for the modernization and internationalization of TCM. This review delves into the nature and common interactions of SAs based on the latest research developments. The structural characteristics of SAs in TCM formulas, paired-herb decoctions, and single-herb decoctions are analyzed and their self-assembly mechanisms are systematically elucidated. In addition, this article elaborates on the advantages of SAs in cancer treatment, particularly in enhancing the bioavailability and targeting capabilities. Furthermore, this review aims to provide new perspectives for the study of TCM compatibility and its clinical applications, thereby driving the innovative development of nanomaterials in this field. On addressing the technological challenges, SAs are expected to further promote the global application and recognition of TCM in the healthcare sector.

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1 Introduction

Traditional Chinese Medicine (TCM), which uses valuable resources from the nature, has been widely used across Asia and surrounding regions, and it plays a crucial role in improving human health.[1,2] In recent years, owing to its clinical efficacy, TCM has gradually gained acceptance within modern medicine.[3] TCM decoctions represent a concentrated embodiment of the holistic view and syndrome differentiation principles of TCM.[4] Their efficacy is based on the "Monarch–Minister–Assistant–Guide" (Jun–Chen–Zuo–Shi in Chinese) theory, where the "Monarch herb" directly targets the root cause of the disease, the "Minister herb" enhances the effect, the "Assistant herb" reduces the toxicity, and the "Guide herb" directs the formula to the precise target. This formulation strategy not only considers efficacy but also emphasizes on the synergy and safety among the herbs. Nevertheless, the uncertainty regarding the functional targets and molecular mechanisms of TCM formulas, along with issues such as poor solubility and bioavailability of certain components, has limited their widespread global application. To overcome these limitations, the introduction of self-assembled aggregate nanotechnology in TCM has become a crucial approach for improving drug absorption and distribution. Self-assembled aggregate nanotechnology in TCM not only enhances the solubility of its components but also increases their targeting capability within the body, thereby improving the therapeutic efficacy, especially in the context of anti-tumor treatments. The self-assembly of TCM compounds not only enhances anti-cancer activity but also uncovers specific interactions between different components, providing critical insights into the optimization of drug combinations.[5]

Although modern medicine has attempted to address the challenges of inconvenient administration and low utilization of herbal materials by converting decoctions into new dosage forms, such as granules and extracts, these methods tend to overemphasize single components, thereby reducing the therapeutic efficacy. In addition, certain excipients, such as the hydrogen peroxide residues in Tween 80 and lysophospholipids in lecithin, pose safety concerns; it is reported that the synthetic surfactants induces gastrointestinal irritation.[6,7] In contrast,

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TCM formulas leverage the synergistic effects of multiple herbs, eliminating the need for excipients or complex equipment, thereby avoiding these safety issues. In recent years, research has revealed that TCM formulas undergo complex physicochemical changes after decoction, particularly the widespread presence of SAs in aqueous decoctions, a phenomenon that is quite intriguing.[8] These SAs include spontaneously formed monomolecular aggregates, metabolite intermediate aggregates, and complex structures that they form, such as gels, fibers, micelles, and nanoparticles.[9–11] These structures exhibit various biological activities such as hypoglycemic, antioxidant, anti-inflammatory, toxicity-reducing, and antibacterial effects. They can also serve as natural carriers for drug delivery, significantly enhancing the bioavailability and therapeutic efficacy of medications.[12–14] For example, research has shown that through self-assembly techniques, the lipophilic components of herbs can be emulsified during heating, forming stable SAs such as precipitates, colloidal particles, and suspensions.[15,16] The potential of these SAs in TCM-based anti-tumor therapies is particularly noteworthy. The formation of SAs is closely related to the pharmacological efficacy of TCM formulas. For instance, after decoction, Baihu decoction, a formula mentioned in "Treatise on Cold Pathogenic Diseases", has also been found to contain SAs that significantly impact biological activity.[8,17] The stability of medicinal components can be effectively improved, toxicity reduced, and therapeutic efficacy enhanced by these SA structures, thereby making the application prospects of TCM formulas in the field of anti-tumor therapy even broader. In addition, supramolecular chemistry, pioneered by Jean-Marie Lehn, the 1973 Nobel Laureate in Chemistry, and further explored by subsequent researchers, has gradually unveiled the macroscopic behavior of SAs.[2,18] Supramolecular chemistry explores self-assembly processes driven by non-covalent bonding forces, leading to the formation of SAs.[18] Supramolecular chemistry methods are widely applied in TCM research, helping to uncover the complex molecular mechanisms of TCM formulas.[19] These methods help explain their unique pharmacological mechanisms at the microscopic level, opening new avenues for their modernization and application. Hou[19] *et al.* also suggested that multi-component complexes formed spontaneously through self-assembly have the greatest potential to enhance the therapeutic efficacy, as free drugs often lack the full bioactivity of complete TCM formulations. Specifically, interactions between the various components within the complex play a crucial role in determining therapeutic effects. For instance, the processes of thermal reflux and water decoction inadvertently create optimal conditions for the formation of these natural complexes, simultaneously reducing the toxicity of individual components and enhancing the water solubility of bioactive compounds.[5]

As research on SAs progresses, scientists have identified that they are widely present in nature and biological systems, including DNA double helices, cell membranes, and even in fish soup.[20,21] SAs can form naturally from food components under heat induction. In fish soup, spherical SAs primarily consist of triglycerides, which contain carbohydrates and are coated with phospholipids.[22] They exhibit antioxidant, antibacterial, and hepatoprotective effects.[22–24] The formation of these SAs in decoctions suggests that similar structural transformations may occur in the active components of TCM during the decoction process. This provides a new perspective for the further exploration of the molecular mechanisms of TCM formulas. Integrating the self-assembly technology with the anti-tumor properties of TCM formulas offers innovative tools for modern medical applications while establishing a robust foundation for their global advancement. Therefore, building upon previous research, this paper delves into the nature of SAs, their common interactions, and the structural characteristics found in TCM decoctions, including complex formulas, paired-herb decoctions, and single-herb decoctions. It systematically elucidates their mechanisms of action from the perspective of self-assembly involving both identical and different TCM components. Additionally, this paper provides a detailed explanation of the applications and advantages of SAs in cancer treatment, particularly their impact on enhancing bioavailability and targeting capabilities. The aim is to integrate existing research findings and offer a reference for further studies on SAs in TCM decoctions.

2 Essence of SAs and their common forces

Supramolecular self-assembly is the process whereby molecules spontaneously form multi-molecular aggregates ranging from nanometer to micrometer scale, with specific macroscopic and microscopic structures, through weak intermolecular interactions *via* non-covalent bonds.[18,25,26] It is widely believed that non-covalent forces between molecules, such as hydrogen bonding and π–π stacking, are the main drivers of molecular self-assembly.[27,28] These forces lead to the formation of multi-molecular aggregates with various microstructures, such as nanoparticles, gels and so on.[13,25,29] Once SAs are formed, their components retain their original properties while exhibiting unique overall functions through interactions.[30] The arrangement of molecules in self-assembly was determined by non-covalent forces, where hydrogen bonds initially aggregate molecules into dimers, which then are stacked axially or helically. Further aggregation is achieved through π–π stacking and van der Waals forces, ultimately forming higher-order structures and one-dimensional nanostructures. These fragile and reversible non-covalent forces allow SAs to respond specifically to external stimuli, making them widely studied in fields such as organ regeneration, repair, drug delivery, and cell culture.[28,31,32] TCM, the concept of holistic treatment is regarded as essential. The efficacy of TCM is often attributed to the synergistic effects of multiple components rather than a single component. The formation of SAs illustrates this synergy, with key benefits such as improved bioavailability, enhanced stability, optimized drug release, and synergistic enhancement.[16,33] The self-assembled structures formed by the interactions of multiple components in TCM decoctions can be seen as a way to explain the significant efficacy of TCM formulas in treating complex diseases. This holistic approach not only aids

modern scientific understanding of TCM but also provides theoretical support for its modernization and internationalization. Research has shown that the components in TCM decoctions are able to spontaneously assemble into SAs under the influence of various non-covalent forces. These SAs are tightly and orderly structured, possessing unique biophysical and biochemical properties, as illustrated in Table 1 and Fig. 1, 2.

2.1 Hydrogen bonding forces

Hydrogen bonds are intermolecular interactions that occur between a hydrogen atom and an electronegative atom (such as oxygen, nitrogen, or fluorine). It is characterized by its directional nature and moderate strength, playing a crucial role in various chemical and biological systems. It plays a critical role in supramolecular self-assembly. As non-covalent interactions, they enable molecules to spontaneously aggregate into specific structures, drive molecular binding, and offer high selectivity in the assembly process. For example, the double-helix structure of DNA relies on hydrogen bonds for stability, while in nanomaterials, hydrogen bonds can be utilized to design self-assembled functional materials.[20,34] To date, SA structures have been identified in numerous decoctions.[8,16] Berberine (BBR) is a commonly used medication for treating bacterial diarrhea. It also plays a role in reducing the toxicity of other harmful drugs and enhancing their safety. Huang[13] *et al.* in their study on the SAs formed during the co-decoction of *Coptis chinensis* Franch. and *Cinnamomum cassia* (L.) J. Presl found that the active components berberine (BBR) and cinnamic acid (CA) could spontaneously self-assemble into CA–BBR NPs with a size of 65.99 ± 0.43 nm (Fig. 1A). Spectroscopy and XRD analyses revealed that the butterfly-shaped 1D structural units of the CA–BBR NPs spontaneously form, driven by hydrogen bonding and π–π stacking. These 1D units continuously accumulate to construct a layered 3D spatial configuration. The study further validated the exceptional antibacterial activity and biofilm removal capability of CA–BBR NPs against multidrug-resistant *Staphylococcus aureus* through *in vitro* experiments. Additionally, *in vivo* tests using cell-based assays and a zebrafish model confirmed their low toxicity and excellent biocompatibility, highlighting the potential for developing novel antibacterial nanomedicines. During the study of the compatibility between *Coptis chinensis* Franch. and *Rheum tanguticum* Maxim. ex Balf. Lin.[35] *et al.* discovered that when *Coptis chinensis* Franch. and *Rheum tanguticum* Maxim. ex Balf. are decocted together, they spontaneously undergo an exothermic reaction, resulting in the formation of SA Ber–Rhe NPs. Under the influence of hydrogen bonding, rhein (Rhe) forms a layered framework, within which BBR is embedded through electrostatic and π–π stacking interactions, resulting in a stable three-dimensional structure[14] (Fig. 1B). Pharmacological experiments have revealed that untreated bacteria maintain smooth surfaces with intact nucleoid centers. The structure of Rhe contains multiple alternating carbonyl, phenolic hydroxyl, and carboxyl groups, which can form hydrogen bonds with the carboxyl and amide groups at the termini of peptidoglycans on the bacterial cell membrane. Scientists have inferred that when bacteria are co-incubated with Ber-Rhe NPs, a significant number of these nanoparticles adhere to the bacterial surface. The high concentration of BBR and Rhe disrupts the cell membrane ion channel proteins, causing membrane contraction, fusion, and cytoplasm leakage, ultimately leading to bacterial lysis and death. Importantly, the experiments have shown that the synergistic antibacterial action of BBR and Rhe enhances the efficacy of the nanoparticles compared to the free drugs. Furthermore, Zheng[27] *et al.* studied Rhe to explore its application in slow-release hydrogels for the treatment of neuroinflammation. The research revealed that Rhe can self-assemble into a hydrogel through π–π stacking and hydrogen bond interactions. Under conditions of pH 8.0–9.4 and heat, some Rhe is deprotonated to form sodium rheinate. Through π–π stacking and hydrogen bonding, sodium rheinate and Rhe monomers aggregate in a J-type manner to form J-type dimers. Due to electrostatic repulsion between carboxylate ions, the molecules tend to align in an antiparallel fashion, with Rhe molecules joining the aggregate in a left-handed helical arrangement, leading to the formation of left-handed nanofibers. These fibers are further cross-linked to form a three-dimensional network structure. Notably, the three-dimensional network structure of the Rhe hydrogel demonstrates superior sustained release properties, thixotropy, and stability compared to free drugs, showing significant efficacy in the prevention of neuritis. Research suggests that ursolic acid (UA) molecules, pentacyclic triterpenoids derived from TCM such as *Prunella vulgaris* L. and *Hedyotis diffusa* Willd., can self-assemble with shiitake mushroom polysaccharides under the influence of hydrogen bonds and van der Waals forces.[29]

2.2 Hydrophobic interactions

Hydrophobic interactions refer to the tendency of hydrophobic groups to aggregate in water to avoid contact with water molecules. This phenomenon is widely observed in the self-assembly of TCM, aiding in the formation of stable structures, enhancing efficacy and bioavailability, and offering potential values in nanomedicine delivery applications. In many studies involving self-assembly processes, hydrophobic interactions serve as the primary driving forces, while other forces, such as electrostatic interactions and hydrogen bonds, balance these effects, collectively contributing to the formation of stable structures.[36,37] Furthermore, Li[37] *et al.* found that under the influence of electrostatic and hydrophobic interactions, BBR can spontaneously form SAs with flavonoid glycosides—namely, baicalin (BA) and wogonoside (WOG)—derived from *Scutellariae radix* in aqueous solutions (Fig. 1C). The self-assembly of two-component systems with similar molecular skeletons can result in the formation of distinct nanomorphologies. BA–BBR, due to the proximity of its hydrophobic flavonoid sites, is formed into one-dimensional composite units, which then are self-assembled into three-dimensional BA–BBR NPs of approximately 100 nm through hydrophobic interactions. The structure of the BA–BBR NPs is arranged with hydrophilic parts facing outward and hydrophobic parts inward (Fig. 1D). The WOG–BBR unit is a structural unit with hydrophobic ends and

Table 1 Existing studies on SAs in TCM decoction[a]

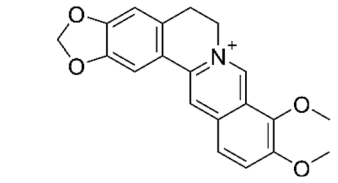
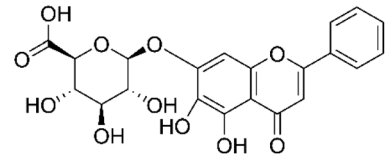
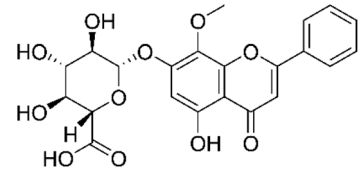
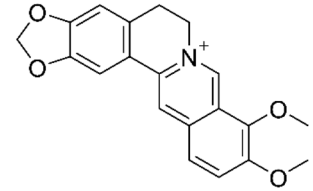
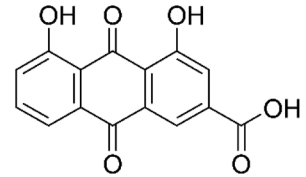
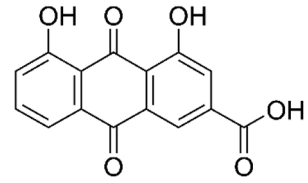
Primary source	Main components of self-assembly	Driving forces	Morphologies	Therapeutic action	Structure	Ref.
Coptis chinensis Franch. and *Scutellaria baicalensis* Georgi co-decoction	Berberine	Hydrophobic interactions and electrostatic interactions	Nano particles or nano fibers	Antibacterial effect		37
	Baicalin					
	Wogonoside					
Coptis chinensis Franch. and *Rheum tanguticum* Maxim co-decoction	Berberine	Hydrogen bond, π–π stacking, electrostatic interactions	Nano particles	Antibacterial effect		27
	Rhein					
Rheum tanguticum Maxim decoction	Rhein	Hydrogen bond, π–π stacking (pH: 8.0–9.4)	Nanofibers	Neuro inflammatory prevention activity		14

Table 1 (*Contd.*)

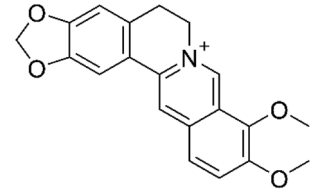
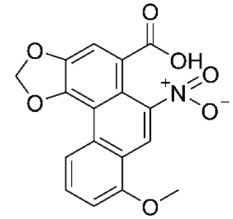
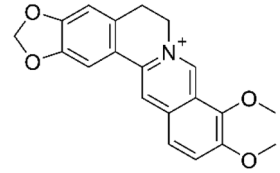
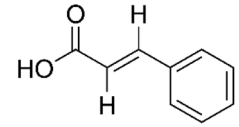
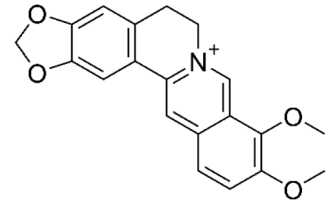
Primary source	Main components of self-assembly	Driving forces	Morphologies	Therapeutic action	Structure	Ref.
Coptis chinensis Franch. and *Aristolochia debilis* Sieb. et Zucc. co-decoction	Berberine	π–π stacking, electrostatic interactions	Cross-linked network structure	↓Acute nephrotoxicity of aristolochic acid		41
	Aristolochic acid					
Coptis chinensis Franch. and *Cinnamomum cassia* (L.) J. Presl co-decoction	Berberine	Hydrogen bond and π–π stacking	Nano particles	Bacteriostatic activity		13
	Cinnamic acid					
Huanglian Jiedu decoction	Berberine	electrostatic interactions	Spherical-like microparticles (precipitate)	Neuroprotective activity; regulate glucose uptake; antibacterial effect		72 and 73
	Baicalin					

Table 1 (*Contd.*)

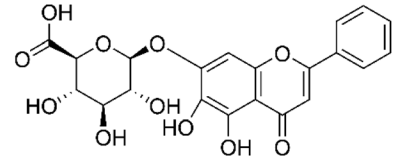
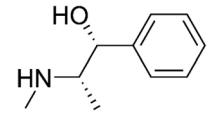
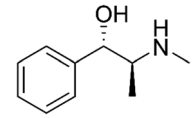
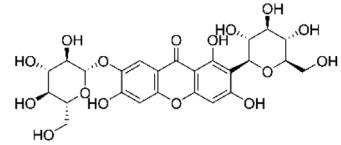
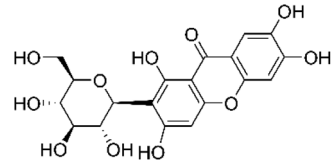
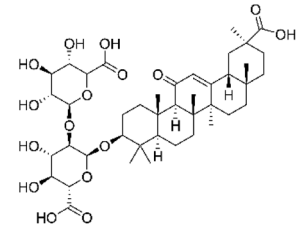
Primary source	Main components of self-assembly	Driving forces	Morphologies	Therapeutic action	Structure	Ref.
Maxing Shigan decoction	Ephedrine Pseudoephedrine	Hydrogen bond, van der Waals forces and electrostatic interactions	Nano particles	Anti-influenza-virus activity	  	84 and 85
Baihu decoction	Polysaccharides; Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}	Coordination interactions	Nano particles	Antipyretic effect; ↓inflammatory factors levels		17, 90 and 91
	Neomangiferin					
	Mangiferin					
	Glycyrrhizic acid					

Table 1 (*Contd.*)

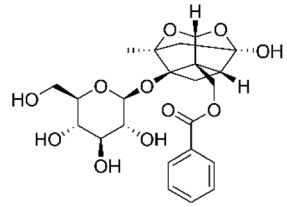
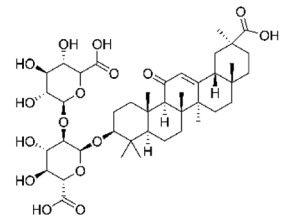
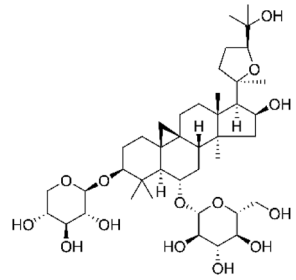
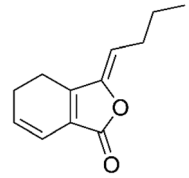
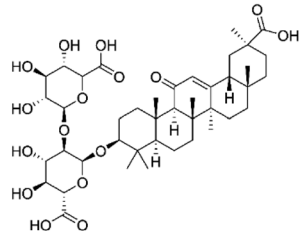
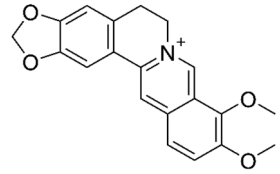
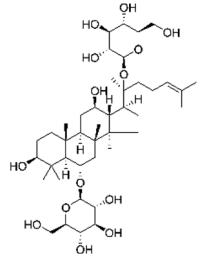
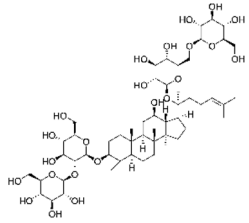
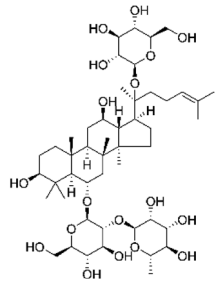
Primary source	Main components of self-assembly	Driving forces	Morphologies	Therapeutic action	Structure	Ref.
Paeonia lactiflora Pall. and *Glycyrrhiza uralensis* Fisch. co-decoction	Paeoniflorin	Hydrophilic interactions and hydrophobic interactions	Micelles	↑Gastrointestinal absorption; ↑anti-inflammatory and immunomodulatory effects		100 and 101
	Glycyrrhizic acid					
Astragalus membranaceus (Fisch.) Bunge and *Angelica sinensis* (Oliv.) Diels co-decoction	Astragaloside IV	Hydrophobic interactions	Nano particles	Anti-myocardial fibrosis		105
	Z-ligustilide					
Glycyrrhiza uralensis Fisch.–*Coptis chinensis* Franch. co-decoction	Glycyrrhizic acid	Hydrophobic interactions and electrostatic interactions	Nano particles	Antibacterial effect		14 and 112
	Berberine					

Table 1 (*Contd.*)

Primary source	Main components of self-assembly	Driving forces	Morphologies	Therapeutic action	Structure	Ref.
						
	Rg1					
Dushen decoction	Rb1	Alkyl–alkyl interactions and hydrogen bonds	Nano micelles	Anti-tumor activity		117 and 118
	Re					
Coptis chinensis Franch. decoction	*Coptis chinensis* Franch. polysaccharide Berberine	Hydrogen bond	Nano particles	Antibacterial activity		59
Angelica sinensis (Oliv.) Diels	*Angelica sinensis* (Oliv.) Diels protein	Hydrophobic interactions	Spherical particles	Free radical scavenging effect		128

[a] ↓: Reduced; ↑: improved.

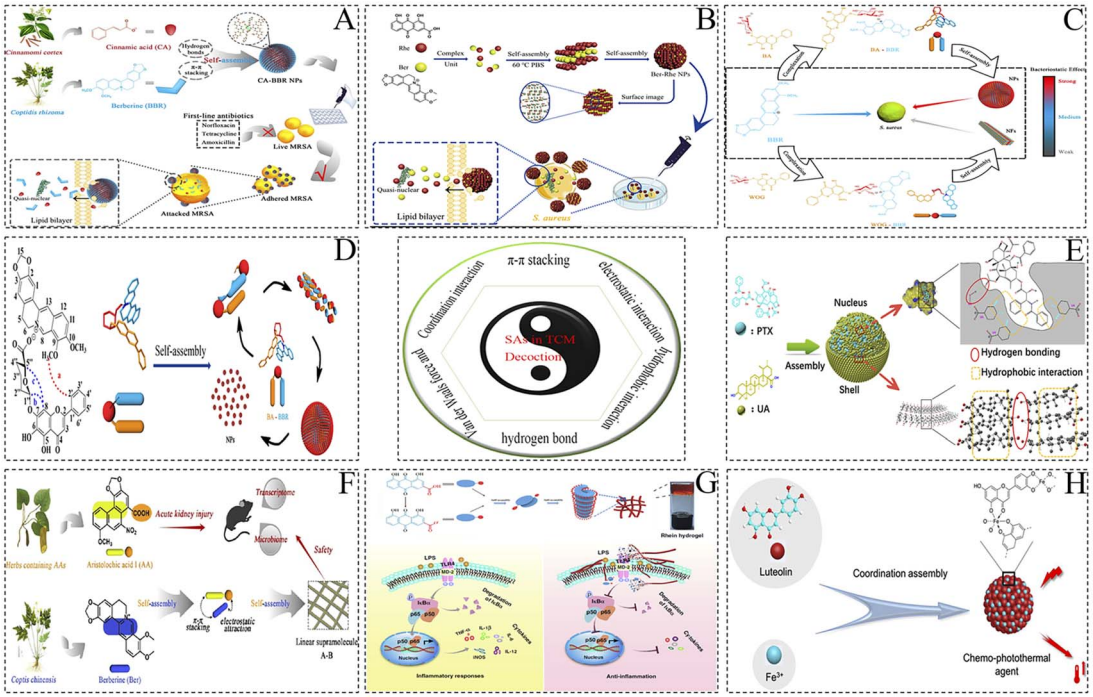


Fig. 1 Different self-assembly forces of SAs in decoction. (A) CA-BBR NPs formed by hydrogen bonding and π–π stacking as the main self-assembly forces. Copyright © 2019 the American Chemical Society; (B) Ber-Rhe NPs formed by π–π stacking and hydrogen bond interactions as the main self-assembly forces. Copyright © 2020 Elsevier B.V.; (C) WOG-BBR NFs and (D) BA-BBR NPs formed through hydrophobic interactions as the main self-assembly forces. Copyright © 2019 the American Chemical Society. (E) UA-PTX NPs formed by hydrophobic interactions as the main self-assembly forces. Copyright © 2020 the American Chemical Society; (F) A–B formed by electrostatic and π–π stacking interactions as the main self-assembly forces. Copyright © 2021 the American Chemical Society; (G) Rhe hydrogel formed by π–π interactions as the main self-assembly forces. Copyright © The Author(s) 2019; (H) Chemo-photothermal agent formed by coordination interactions as the main self-assembly forces. Copyright © 2020 Elsevier B.V. adapted with permission from ref. 13, 14, 27, 37, 38, 41 and 45.

hydrophilic center, capable of forming an I-shaped hydrophobic planar structure. Due to its lack of hydrophobicity, the basic unit is unable to form nanoparticles but can spontaneously assemble in aqueous solutions under strong hydrophobic interactions, resulting in nanofibers with a diameter of 50–100 nm and a length of about 10 μm. More importantly, compared to BBR, the self-assembled BA–BBR NPs exhibit stronger antibacterial effects and biofilm clearance capabilities against *Staphylococcus aureus*, whereas the efficacy of the self-assembled WOG–BBR NFs is less than that of BBR. More importantly, *in vitro* experiments demonstrated that NPs significantly outperformed free BBR in antibacterial activity and biofilm removal, whereas NFs exhibited weaker antibacterial effects. *In vivo* zebrafish models and cytotoxicity tests further revealed that both NPs and NFs possess excellent biocompatibility and low toxicity. This natural small-molecule self-assembly strategy based on NPs and NFs offers significant potential for the development of highly effective and low-toxicity nanoscale antibacterial drugs, particularly in the field of self-delivering antibacterial therapies. Wang[38] *et al.* in their exploration of the drug delivery process using natural active small molecules and their potent synergistic anti-tumor effects discovered that the phenyl rings between UA molecules and paclitaxel (PTX) exhibit strong hydrophobic interactions. These interactions encourage the formation of stable nanocomposites through the coupling of two methyl groups in UA with two phenyl rings connected by amide bonds in PTX, thereby enhancing the drug loading capacity (Fig. 1E). Experiments have demonstrated that the composite material of UA–PTX NPs can prolong the plasma half-life, prevent rapid drug leakage, and preserve the anti-tumor and hepatic protective effects of UA.

2.3 π–π stacking

π–π stacking interactions are important spatial interactions between aromatic compounds, involving weak interactions between electron-rich and electron-poor aromatic rings. This mechanism is equally important as hydrogen bonding in non-covalent interactions, though its underlying principles are more complex.[39] Alkaloids and flavonoids contain aromatic rings, which readily undergo π–π stacking interactions, leading to self-assembly with themselves or other aromatic-ring-containing molecules. These interactions have become some of the primary mechanisms for their assembly.[40] This process has a significant impact on the molecular structure, stability, and functionality. Aristolochic acid (AA), a principal component of TCM such as *Asarum heterotropoides* F. Schmidt and *Aristolochia contorta* Bunge. However, studies have shown that it possesses nephrotoxicity, which can lead to serious health issues such as AA nephropathy and cancer.[41,42] In recent years, scholars have revealed the detoxification mechanism of BBR combined with AA through multicomponent self-assembly

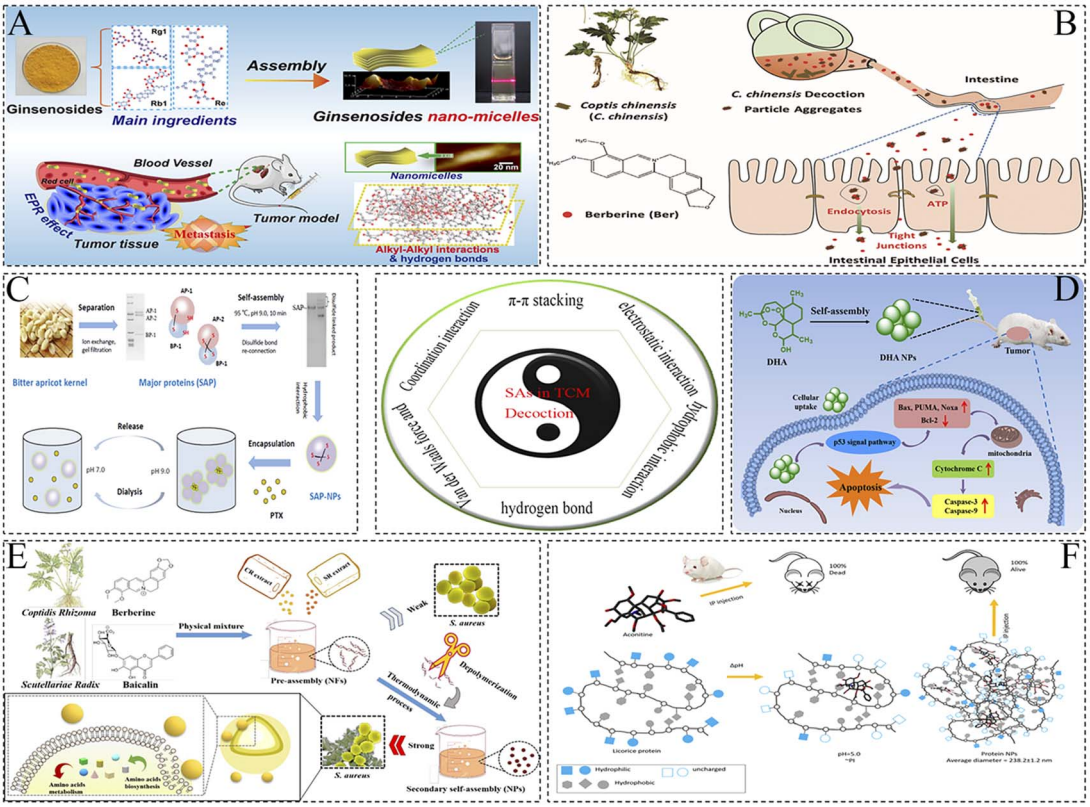


Fig. 2 Different self-assembly forces of SAs in decoction. (A) Ginsenosides nano-micelles formed by hydrogen bonding forces as the main self-assembly forces. Copyright © 2022 Elsevier B.V.; (B) *C. chinensis* decoction particle aggregates formed by hydrogen bonding forces as the main self-assembly forces. Copyright © The Royal Society of Chemistry 2020; (C) PTX-ASP-NPs formed by hydrophobic interactions as the main self-assembly forces. Copyright © 2020 Elsevier B.V.; (D) DHA NPs formed by hydrogen bonding and hydrophobic interactions as the main self-assembly forces. Copyright © 2023, The Royal Society of Chemistry; (E) BBR-BA NPs formed by electrostatic forces as the main self-assembly forces. Copyright © 2022, BMC; (F) GP-AC NPs formed by hydrophobic interactions as the main self-assembly forces. © 2015 Ke *et al.*; adapted with permission from ref. 59, 118, 127, 139, 141 and 144.

studies.[41] Research indicates that under electrostatic and π–π stacking interactions, BBR and AA can self-assemble into linear heterogeneous SAs (A–B), featuring hydrophobic groups on the exterior and hydrophilic groups on the interior. In *in vitro* experiments with HK-2 cells, as well as in zebrafish embryo and C57BL/6 mouse models, the mechanism of the linear heterogeneous SA (A–B) structure and its ability to inhibit the formation of toxic metabolites were investigated. The results indicated that this structure significantly mitigates acute kidney injury induced by AA, while having no significant detrimental effects on gut microbiota homeostasis. These findings provide scientific support for the detoxification potential of TCM combinations (Fig. 1F). Cheng[43] *et al.* co-assembled betulinic acid with Chlorin e6 (Ce6) through hydrogen bonding to form amphiphilic prodrug molecules. These molecules, *via* π–π stacking, interact with the photosensitizer Ce6, resulting in the formation of a hollow shell network structure for photochemotherapy nanoparticles. The nanoparticles exhibit good biocompatibility and low toxicity, and they can synergistically enhance anti-tumor effects when combined with anti-PD-L1 antibodies. Betulonic acid, a derivative of betulinic acid, also co-assembles with Ce6 *via* π–π stacking and hydrophobic interactions. This process enhances its water solubility and boosts its chemical/photodynamic anti-tumor effects.[44] This study developed a photosensitive nanoplatform regulated by natural small molecules, which significantly enhanced the efficiency of type I photochemical reactions and the synergistic anti-tumor effects. *In vitro* experiments confirmed its high efficiency in generating ROS and its potent tumor cell-killing effects. *In vivo* experiments demonstrated excellent tumor inhibition rates and good biosafety, providing new insights for the application of photochemical therapy in hypoxic tumor environments. In addition, quinone compounds typically possess planar structures, where aromatic rings form parallel π-electron systems. This structural feature facilitates intermolecular self-assembly through π–π interactions.[27] Such interactions can enhance intermolecular binding forces, promote the formation of ordered aggregates, and endow quinone compounds with significant potential for applications in materials science and drug delivery. Studies have shown that under mildly alkaline conditions with a pH range of 8.0 to 9.4, Rhe can self-assemble into a hydrogel based on a nanofiber network. This hydrogel facilitates the slow release of Rhe, thereby enhancing its anti-inflammatory effects.[27] Ultraviolet spectroscopy, circular dichroism, X-ray diffraction analysis, and theoretical calculations indicate that Rhe molecules undergo

deprotonation under mild alkaline conditions, forming Rhe sodium salts. These monomers and sodium salts interact through π–π stacking to form dimers. Due to electrostatic repulsion between carboxylate ions, the dimers arrange in opposite orientations and further assemble into higher-order aggregates, eventually crosslinking into a three-dimensional (3D) network structure. *In vitro* experiments confirm that this hydrogel demonstrates effective drug release control, low toxicity, anti-inflammatory activity, and efficient cellular uptake, as shown in Fig. 1G.

2.4 Coordination

In addition, some common forces for self-assembly also include hydrophobic interactions and coordination.[45,46] Coordination interactions are significant intermolecular forces, often stronger than hydrogen bonds and comparable to weaker covalent bonds, and they can be adjusted by environmental conditions. Research indicates that various natural flavonoid constituents of TCM can self-assemble into nanoparticles *via* coordination with metal ions, such as iron (Fe^{3+}), forming nanoparticles like those created by luteolin found in *Chrysanthemum morifolium* Ramat. and *Lonicera japonica* Thunb., which possess photothermal properties[45] (Fig. 1H). *In vitro* experiments have demonstrated that these nanoparticles exhibit significantly enhanced solubility under physiological conditions and show an efficient photothermal effect under 808 nm laser irradiation, with a photothermal conversion efficiency of 26.0%. Additionally, their combined chemotherapy and photothermal therapy exert a notable inhibitory effect on U87MG cells, reducing the IC_{50} value from 30.8 μg mL^{-1} in standalone chemotherapy to 6.6 μg mL^{-1}. *In vivo* experiments further confirmed that the nanoparticles exhibit excellent tumor suppression effects in animal models, with the combined photothermal therapy group showing significantly better efficacy than single therapy approaches. The coordination self-assembly strategy effectively modulates the structure and properties of natural flavonoids, enabling their application as chemotherapeutic agents or photothermal agents. In addition, BA, an active component of *Scutellaria baicalensis* Georgi, exhibits anti-inflammatory and antibacterial properties. The functional groups within its molecule, including phenolic hydroxyl, hydroxyl, carbonyl, and carboxyl groups, can interact with metal ions (such as Al^{3+}) *via* hydrogen bonding and coordination bonding, promoting the self-assembly into nanoparticles. Based on this, Jia[47] *et al.* developed an oral insulin delivery system. Their research revealed that this system could protect insulin in the acidic environment of the stomach and release it into the alkaline environment of the intestine, thereby prolonging its action time. Additionally, BA enhances insulin absorption *via* a paracellular pathway, reduces blood glucose levels, and co-assembles with berberine to improve its solubility.

2.5 Electrostatic forces

In the self-assembly process of TCM, electrostatic interactions serve as crucial non-covalent forces, enabling molecules to form stable structures through charge attraction or repulsion.[48] Charged TCM molecules or their derivatives facilitate self-assembly *via* electrostatic interactions, enhancing the stability and biocompatibility of nanomaterials, thereby optimizing drug delivery and release performance. Electrostatic interactions, combined with other non-covalent forces such as hydrogen bonding and π–π stacking, further improve the efficiency of self-assembly and the therapeutic performance of TCM components. Alkaloids, as nitrogen-containing heterocyclic compounds, can self-assemble with negatively charged groups (such as carboxyl groups) *via* electrostatic interactions, thereby constructing stable drug delivery systems. Research has shown that UA can self-assemble into nanoparticles with a diameter of approximately 150 nm, driven by electrostatic and hydrophobic interactions. *In vitro* studies have demonstrated that these nanoparticles exhibit stronger anti-proliferative activity compared to free UA and significantly enhance the activation of CD^{4+} T cells, indicating their potential for immunotherapy.[49] In addition, UA self-assemblies have been employed for the delivery of various drugs including doxorubicin, PTX, and aspirin.[50–52] Wang[53] *et al.* reported that inulin and gelatin co-assemble to form core nanoparticles, which are subsequently stabilized into core–shell nanogels (CSNGs) through chemical crosslinking. The study encapsulated the antimicrobial peptide Cath30 into CSNGs *via* electrostatic interactions for the treatment of aerobic vaginitis. Under the action of gelatinase secreted by pathogenic bacteria, the nanogels gradually released Cath30 and inulin, achieving a dual therapeutic effect.

Many active components of TCM possess self-assembly capabilities, forming stable nanostructures *via* non-covalent interactions such as hydrogen bonding, π–π stacking, hydrophobic interactions, and coordination. These interaction forces have distinct characteristics and play different roles in the self-assembly process, but each also has its limitations. Many active components in TCM can self-assemble due to their unique structures. However, their ordered structures are influenced by external environmental factors such as pH and protein concentration, which can lead to variations in drug efficacy. Hydrogen bonding and coordination interactions are relatively strong; however, they are highly sensitive to environmental factors such as solvents. π–π stacking and van der Waals forces are weaker; nonetheless, they facilitate the formation of ordered structures. Hydrophobic interactions are notably significant in aqueous solutions; electrostatic forces, meanwhile, are easily influenced by medium conditions. Hydrophilic interactions play a critical role in stabilizing polar systems. In future research, establishing standardized characterization and quantification methods for self-assembly will be crucial. Such advancements will enhance research consistency and provide deeper insights into the pivotal roles of these forces in the self-assembly of TCM. The self-assembled nanoparticles within the *Isatis indigotica* Fortune decoction system exhibit reversible responses to changes in pH and temperature.[54] During the decoction process, the protein components of *Glycyrrhiza uralensis* Fisch. spontaneously self-assemble into protein nanoparticles, which not only promote liver cell proliferation but also enhance the solubility and

bioavailability of insoluble astragaloside IV through encapsulation.[55] However, the particle size distribution of *Glycyrrhiza uralensis* Fisch. protein self-assembled nanoparticles is influenced by heating temperature and pH changes. Notably, the critical impact of different decoction conditions and environmental factors on the structure and function of the aggregates must be recognized, as these are key considerations in the development of SAs.

3 Study on the structure of SAs in TCM decoctions

TCM decoctions are complex dispersed systems in which the active components form SAs *via* interactions such as hydrogen bonding and π–π stacking. This process increases the solubility of poorly soluble components, encapsulates toxic substances to reduce toxicity, and enhances the stability, absorption, bioavailability, and efficacy of the medication.[34,56–59] The structure of SAs in TCM decoctions tends to aggregate and form "precipitates" due to their high surface free energy, as observed in the decoction of Liu Jun Zi Tang.[60] TCM holds that the precipitate should be consumed along with the decoction to ensure its therapeutic effects. Studies dating back to the 1980s revealed that decoctions remain in a suspended state after boiling, and removing the precipitate significantly diminishes their efficacy.[16] Subsequent research has revealed that these SAs are typically composed of proteins, polysaccharides, and surfactants, often forming nanoscale particles. By encapsulating or adsorbing active components, they significantly enhance drug stability, absorption, and bioavailability, serving as a critical material basis for the therapeutic efficacy of TCM.[61] However, during the pharmaceutical process, the precipitate is often filtered out and discarded along with the drugs in an attempt to obtain a "clear liquid". This practice leads to the loss of certain water-soluble and lipid-soluble active components, ultimately reducing the therapeutic efficacy of the decoction.[62] Experiments indicate that co-decoction is a primary condition for the formation of self-assembly aggregates (SAs). For instance, during the co-decoction of Sanhuang Xiexin Tang and Hong Jing Tian decoction, active components from different herbs undergo thorough interactions, resulting in the formation of more stable SAs with smaller particle sizes. Compared to the simple combination of individual decoctions, these co-decocted formulations demonstrate significantly improved antipyretic, analgesic, and anti-inflammatory effects.[63,64] In conclusion, the formation of SAs is a key material basis for the therapeutic effects of decoctions. The investigation of this phenomenon can further uncover the compatibility principles of TCM and provide scientific guidance for optimizing clinical formulations. This approach helps enhance drug efficacy consistency, reduce the waste of active ingredients, and foster the development and application of modern TCM.

3.1 Research on the structure of SAs in prescription decoctions

3.1.1 Huanglian Jiedu decoction (HJD). HJD was first documented in Volume 2 of Ge Hong's "The Handbook of Prescriptions for Emergencies", in "The Thirteenth Prescription for Treating Typhoid Fever with Qi and Temperature". It is noted for its ability to clear away the fire of the sanjiao and heat toxins.[65] As a classic formula for clearing heat and detoxifying, HJD consists of *Coptis chinensis* Franch., *Scutellaria baicalensis* Georgi, *Phellodendron amurense* Rupr. and *Gardenia jasminoides* J. Ellis. It is known for its diverse pharmacological effects including antibacterial, anti-inflammatory, antioxidant, anti-tumor, and immunomodulatory properties.[66,67] HJD has been reported to effectively treat various diseases including diabetes, stroke, and dementia.[68,69] In TCM, "self-precipitation" describes the interactions of chemical components in a compound formula during decoction or solvent extraction, leading to the formation of precipitates in solutions under the guidance of TCM theory.[70] HJD contains glycosides (such as BA) and alkaloids (such as BBR), which undergo up to 7.13% self-precipitation during the water decoction process.[71,72] Through HPLC analysis, it was found that 81.28% of the self-precipitates in HJD consist of acidic and basic compounds, with BA and BBR having the highest proportions, accounting for 42.12% and 31.17%, respectively. Together, these two compounds comprise 73.29% of the total content. Research has shown that the acidic carboxyl group of BA forms a precipitate with alkaloids such as BBR through acid–base complexation.[73] From a molecular thermodynamics perspective, the formation mechanism of HJD self-precipitation was explored, revealing that the combination of BA and BBR produces the most significant self-precipitation. This process is found to be an enthalpy-driven chemical reaction involving non-covalent bonding.[72,74] Furthermore, Liu[75] *et al.* further confirmed that when the concentrations of BBR and BA reach a critical point, a dynamic equilibrium precipitation reaction can occur. Lin[76] *et al.* utilized HPLC and UHPLC-Q-Orbitrap HRMS, confirming that the precipitates contain high concentrations of BA and BBR, with the particles appearing as spherical-like microparticles around 600 nm in size. The study suggested that the precipitates in HJD are formed through a self-assembly process. Chen[68] *et al.* analyzed the components of the supernatant and precipitates in HJD using UHPLC-Q-Orbitrap HRMS, and the results indicated that the concentrations of BA and BBR in the precipitates were significantly higher than that in the supernatant, further confirming their self-assembly characteristics. Wang[72] *et al.* synthesized a BBR–BA complex using a simulated precipitation method and characterized the spontaneous precipitate aggregates, formed *via* electrostatic attraction in a ratio of 1/1, by UV and NMR spectroscopy. It has been reported that HJD self-precipitates exhibit significant anti-inflammatory, antibacterial, and neuroprotective effects. *In vitro* experiments demonstrated that the "self-precipitates" in HJD provide protection against damage in $CoCl_2$-induced NGF–$PC1_2$.[73] It is speculated that the efficacy of "self-precipitation" (acid–base complexes) in HJD may exceed that of the supernatant (monomeric components). Ke[77] *et al.* have demonstrated that the BA, BBR hydrochloride, and their complexes in the self-precipitates of HJD exhibit significant inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*, with the complex's antibacterial effect being weaker than that of BBR hydrochloride alone. It is speculated that the interactions

enhance the activity of BA while reducing the efficacy of BBR, resulting in an antibacterial effect that falls between the two components.

In summary, the self-precipitates of HJD, formed through acid–base complexation and self-assembly, exhibit significant antibacterial, anti-inflammatory, and neuroprotective pharmacological effects. The self-precipitation mechanism enhances drug stability and improves the overall efficacy, showing particular potential in the treatment of complex diseases. However, the differences in activity due to component interactions and the complex formation mechanisms require further research and optimization. Furthermore, extensive clinical validation is essential to support its broader application.

3.1.2 Maxing Shigan decoction (MSD). MSD, a well-known TCMF, is documented as a therapeutic remedy in "the Treatise on Cold Pathogenic Diseases". It is composed of *Ephedra sinica* Stapf, *Prunus acacia* Crantz ex Poir., *Glycyrrhiza uralensis* Fisch., and $CaSO_4 \cdot 2H_2O$,[78,79] which exhibit significant antibacterial, antiviral, antipyretic, and anti-inflammatory pharmacological effects.[80–82] The pharmacological basis of TCM involves both physical and chemical properties, with its mechanism linked not only to chemical components but also to the form and phase state in which these components exist, the latter forming the physical foundation.[83] During the decoction process, these components assemble into nano-colloidal particles ranging from 50 to 150 nm, with key active ingredients such as amygdalin and ephedrine, primarily appearing in nanoparticle form.[84] Reverse-phase chromatography revealed that the contents of ephedrine and pseudoephedrine in the SAs of MSD decoction were 99.7% and 95.5%, respectively, which were significantly higher than those in the dispersed portion of the true solution.[85] It is speculated that amphiphilic molecules such as ephedrine and pseudoephedrine adsorb onto SAs through hydrogen bonding, van der Waals forces, and electrostatic interactions. Additionally, research has shown that the formation of nanoparticles and their antibacterial activity are closely related to their phase structure.[86] *In vitro* pharmacological experiments confirmed that the antiviral activity of MSD against influenza virus is closely related to the SAs present in its decoction.[85] Researchers used the MTT assay to compare the *in vitro* antiviral activity of MSD before and after filtration through a 0.45 μm cellulose acetate membrane. The results indicated that the activity was significantly reduced after filtration. Dynamic light scattering and electron microscopy analyses indicated that filtration removed a large number of large aggregates, reducing the average particle size by approximately 70 nm, with most aggregates larger than 500 nm being retained. It is speculated that the reduction in the antiviral activity of the MSD decoction may be due to the removal of a significant portion of the SAs, as these retained nanoparticle aggregates or the monomer compounds adhered to them may possess anti-influenza A virus activity.

In conclusion, the SAs in MSD decoctions exhibit antiviral properties, which hold significant implications for the study of TCM decoctions. Pharmacological studies have indicated that active components in TCM decoctions may exist in the form of SAs, suggesting that research on TCM efficacy should broaden its perspective to consider aggregates typically viewed as "insoluble".[85] Additionally, nano-scale aggregates may possess physiological and pharmacological activities, thus experimental designs should be adjusted accordingly. *In vivo* activity experiments should take into account the impact of aggregates on the delivery, absorption, and action of active components. *In vitro* cell experiments must evaluate the effects of ultrafiltration and filtration on sample activity, as demonstrated in the aforementioned study on MSD antiviral activity, where 0.45 μm membrane filtration may have influenced the results by removing aggregates.

3.1.3 Baihu decoction (BD). BD, a traditional natural Chinese medicine formula, originates from the "the Treatise on Cold Pathogenic Diseases" written by Zhang Zhongjing during the Eastern Han Dynasty in China. The composition of BD is simplified, containing only four TCMs: $CaSO_4 \cdot 2H_2O$, *Anemarrhena asphodeloides* Bge., *Glycyrrhiza uralensis* Fisch., and *Oryza sativa* subsp. japonica Kato.[87,88] Modern research has shown that BD exhibits antipyretic, anti-inflammatory, and immune-boosting properties.[87] Recently, scholars have employed high-speed centrifugation and dialysis techniques to separate the different phases of BD (true solution, nano, and precipitate phases) and employed high-performance liquid chromatography (HPLC) to analyze the content of key components in each phase.[89] They found that the active ingredients in the nano phase such as neomangiferin, mangiferin, glycyrrhizic acid, and glycyrrhetinic acid were significantly higher than those in the other phases. The nano phase notably enhanced the solubility of these components. Based on this, it is conjectured that the SAs within the nano phase of BD play a significant role in enhancing the solubility of these critical components. To further investigate the formation mechanism of nano SAs in BD, scholars measured parameters such as particle size, salinity, conductivity, and surface tension, combined with TEM and fingerprint analysis.[17] The results revealed that the combination of the four medicinal ingredients in BD is crucial to the formation of the nano phase. The polysaccharides and proteins in *Oryza sativa* subsp. japonica Kato form porous nanoparticle cores that encapsulate chemical components, while the flavonoids and alkaloids in *Anemarrhena asphodeloides* Bge. self-assemble into nanoparticles *via* acid–base neutralization.[90] The inorganic ions in $CaSO4 \cdot 2H_2O$ (such as Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, and Zn^{2+}) stabilize the nano phase by forming a double electric layer. Fe^{2+} and Fe^{3+} act as high-energy aggregation centers, adsorbing insoluble components such as mangiferin and neomangiferin, thus enhancing their solubility. Additionally, ions such as Ca^{2+}, Mg^{2+}, and Zn^{2+} regulate the zeta potential, while glycyrrhizic acid and glycyrrhetinic acid from *Glycyrrhiza uralensis* Fisch., serve as surfactants, further enhancing the stability of the nanostructures.[17,91] *In vivo* pharmacological studies have demonstrated that BD nanoscale SAs are more effective than the true solution fraction in alleviating fever and reducing inflammatory cytokine levels. This superiority is due to their higher cellular uptake efficiency and precise targeting of tissues such as the lungs and brain. These properties not only validate their potential as antipyretic agents but also suggest their applicability in the clinical treatment of

febrile diseases. Further clinical trials are needed to confirm their efficacy and safety.[91,92] Further research revealed that the nano phase of BD significantly alleviates lung tissue damage in a yeast-induced rat fever model, and inhibits the expression of inflammation-related proteins such as IL-1β, TRPV4, NF-κB, and TNF-α.[17,93]

In summary, the nano-phase of BD significantly enhances drug solubility and efficacy, particularly excelling in antipyretic and anti-inflammatory effects, offering innovative approaches for the modernization of TCM. However, despite its clear advantages in improving solubility and bioavailability, further research is needed to explore its long-term safety and the metabolic mechanisms of nanoparticles in the body. Additionally, challenges remain in achieving standardization and quality control during production. A more comprehensive evaluation is required to ensure its safety and feasibility before clinical application.

3.2 Research on the structure of SAs in medicinal-pair decoction

3.2.1 *Paeonia lactiflora* Pall.–*Glycyrrhiza uralensis* Fisch. co-decoction. The *Paeonia lactiflora* Pall.–*Glycyrrhiza uralensis* Fisch. co-Decoction, originating from Zhang Zhongjing's Treatise on Febrile Disease, consists of a 1 : 1 combination of *Paeonia lactiflora* Pall and *Glycyrrhiza uralensis* Fisch. and demonstrates significant clinical efficacy.[94,95] The "monarch" herb, *Paeonia lactiflora* Pall., is rich in active compounds such as paeoniflorin, which exhibits anti-inflammatory, immunomodulatory, and analgesic effects. However, due to its high polarity, these compounds have poor absorption in the gastrointestinal tract when taken orally.[96–98] Research found that the amphiphilic compounds in *Glycyrrhiza uralensis* Fisch. such as glycyrrhizic acid and liquiritin, self-assemble into nanoparticles during the water decoction process. These nanoparticles significantly enhance the absorption efficiency of the active components in *Paeonia lactiflora* Pall.[99,100] Shen[101] *et al.* prepared self-assembling nanoparticles based on paeoniflorin and glycyrrhizic acid using an ultrasonic dispersion method, with a particle size of approximately 200 nm. This nanoparticle system effectively encapsulated paeoniflorin, significantly enhancing its oral bioavailability and controlling its release rate. *In vivo* pharmacokinetic studies showed that compared to paeoniflorin solution, the encapsulated nanoparticles increased paeoniflorin's C_{max} by 2.17 times and AU C_{0-t} by 3.64 times, indicating a significant improvement in both absorption rate and efficacy.[101]

In summary, the self-assembled nanoparticles in the *Paeonia lactiflora* Pall.–*Glycyrrhiza uralensis* Fisch. co-decoction significantly enhance the absorption and efficacy of *Paeonia lactiflora* Pall., components, showing great clinical potential by improving intestinal absorption and strengthening anti-inflammatory and immunomodulatory effects, thereby providing better protection for the body. However, challenges such as the complexity of preparation, long-term safety concerns, and potential risks arising from component interactions hinder its widespread application. Thus, while the technological innovations show promise, further research and optimization are necessary for broader clinical adoption.

3.2.2 *Astragalus membranaceus* (Fisch.) Bunge–*Angelica sinensis* (Oliv.) Diel co-decoction. *Astragalus membranaceus* (Fisch.) Bunge–*Angelica sinensis* (Oliv.) Diel co-decoction, first proposed by Dongyuan Li in the "Theories of Differentiation on Endogenous", is a classic prescription prepared from *Astragalus membranaceus* (Fisch.) Bunge and *Angelica sinensis* (Oliv.) Diels in a ratio of 5/1. It is known for its effects on enriching qi and replenishing blood, and it has been widely used in clinical practice.[102–104] The Monarch herb *Astragalus membranaceus* (Fisch.) Bunge tonifies the Qi of the spleen and lungs, promotes the generation of Qi and blood, and has detoxifying and wound-healing properties. The minister herb *Angelica sinensis* (Oliv.) Diels is an excellent blood tonic, so much so that physicians traditionally say "in ten formulas, nine contain *Angelica sinensis* (Oliv.) Diels". When combined, *Astragalus membranaceus* (Fisch.) Bunge and *Angelica sinensis* (Oliv.) Diels work synergistically to invigorate blood circulation and replenish blood, with the combination strengthening Qi and promoting blood production, effectively alleviating various symptoms. However, the precise therapeutic mechanism remains unclear. Recent studies indicate that decocting *Astragalus membranaceus* (Fisch.) Bunge and *Angelica sinensis* (Oliv.) Diels together can result in the dynamic self-assembly of irregularly shaped, negatively charged spherical SAs. UHPLC-HR-MS analysis revealed that these SAs contain 43 chemical components.[105] Stable and highly water-soluble AA-NPs were successfully isolated using ultrafiltration centrifugation, with eight key components effectively loaded into the particles, exhibiting sustained release properties. Notably, the release rates of Z-ligustilide (LIG) and astragaloside IV (AIV) were below 50% within 24 hours, suggesting hydrophobic interactions between the hydrophobic region of LIG and the tetracyclic backbone of AIV, leading to the formation of AA-NPs in a molar ratio of 2/1. Both *in vivo* and *in vitro* experiments confirmed that AA-NPs effectively treat myocardial fibrosis by restoring cardiac function, reducing collagen deposition, and inhibiting endothelial–mesenchymal transition, thereby demonstrating therapeutic potential.[105]

In conclusion, the SAs of *Astragalus membranaceus* (Fisch.) Bunge and *Angelica sinensis* (Oliv.) Diels offer a potential new mechanism for treating myocardial fibrosis, along with the advantage of sustained drug release. However, the underlying mechanism remains unclear, clinical research is limited, and the preparation process is complex. Despite its innovation and therapeutic potential, further research and optimization are necessary to enable broader clinical applications.

3.2.3 *Glycyrrhiza uralensis* Fisch.–*Coptis chinensis* Franch. co-decoction. *Glycyrrhiza uralensis* Fisch.–*Coptis chinensis* Franch., a classic pair in TCM, are recorded in the "Jin Gui Yao Lue" and are widely applied. *Glycyrrhiza uralensis* Fisch., known for its long history of medicinal use in China, possesses various pharmacological effects including heat-clearing and detoxification, relieving pain, and harmonizing the properties of other herbs.[2,106] Due to its broad application in TCM, it is often referred to as the "national elder" and known as "ten formulas,

nine containing licorice," frequently paired with *Coptis chinensis* Franch. and other heat-clearing, detoxifying herbs to enhance the efficacy. *Coptis chinensis* Franch., first documented in "Shennong Bencao Jing" during the Eastern Han dynasty, is a highly valued herb but may cause "bitter cold damaging the stomach" symptoms.[107,108] *Glycyrrhiza uralensis* Fisch. can mitigate the cold and bitter properties of *Coptis chinensis* Franch., and when decocted together, the heat-clearing and detoxifying effects are enhanced, with a notable self-precipitation phenomenon drawing attention from researchers.[109] Li *et al.* found that when *Glycyrrhiza uralensis* Fisch. and *Coptis chinensis* Franch. are decocted together, self-assembling nanoparticles of approximately 179 nm in size are formed, whereas single decoctions appear clear and transparent.[109] SEM observations confirmed that this self-precipitation only occurs when the two herbs are combined. It is speculated that this precipitation is probably due to the formation of self-assembling nanoparticles during the co-decoction of *Glycyrrhiza uralensis* Fisch. and *Coptis chinensis* Franch. Deng[110] *et al.* detected through HPLC that the precipitates formed from this combination mainly contain glycyrrhizic acid and BBR. Additionally, these precipitates slow down the release of BBR; compared to the supernatant, the AU C_{0-12} value of berberine in the portal vein was reduced by 44.1% in the precipitate.[111] The precipitates formed from the co-decoction of *Glycyrrhiza uralensis* Fisch. and *Coptis chinensis* Franch. not only ensure the therapeutic effect of berberine but also reduce the cold-induced irritation of *Coptis chinensis* Franch. on the spleen and stomach. Studies have shown that during co-decoction, *Glycyrrhiza uralensis* Fisch. protein and BBR self-assemble into nanoparticles driven by electrostatic and hydrophobic interactions, with significantly stronger antibacterial activity than either component alone or in a mechanical mixture.[112] This enhanced effect is due to the amino acid residues on the surface of the *Glycyrrhiza uralensis* Fisch. protein, which adsorb *Staphylococcus aureus*, allowing the self-assembled nanoparticles to attach to the bacterial surface and disrupt its structural integrity. Additionally, the nanoparticles release BBR continuously, increasing the local concentration of BBR around the bacteria, enhancing uptake, and thus producing a strong antibacterial effect.[14]

In conclusion, the self-assembled precipitates formed during the co-decoction of *Glycyrrhiza uralensis* Fisch. and *Coptis chinensis* Franch. are key components responsible for their pharmacological effects and demonstrate significant antibacterial efficacy. However, current research remains largely at the experimental stage, with limited clinical validation. Moreover, the preparation process is complex, and although the potential for application is promising, further research and extensive clinical trials are necessary.

3.3 Research on the structure of SAs in single-herb decoctions

3.3.1 Dushen Decoction. Dushen Decoction, originating from the Yuan Dynasty's "Shi Yao Shen Shu", is a traditional preparation made by decocting ginseng alone in water.[113] *Panax ginseng* C. A. Mey., a well-known medicinal herb, is widely used for treating various diseases and as a nutritional supplement, with its efficacy first recorded in "Traditional Chinese medicine Monographs".[114] Ginsenosides, particularly ginsenoside Rg1, are the key active components in *Panax ginseng* C. A. Mey. Due to its amphiphilic structure, Rg1 can self-assemble into micelles through hydrophilic–hydrophobic interactions.[115] Under these interactions, the hydrophobic regions of Rg1 molecules cluster together to form a hydrophobic core, which is then coated by a hydrophilic shell, resulting in the formation of Rg1 micelles. These polymeric micelles are nano-sized colloids formed by amphiphilic block copolymers in water. They can encapsulate hydrophilic or hydrophobic drugs, protecting them from degradation, enhancing the solubility of poorly soluble drugs, improving drug stability and targeting, and extending circulation time in the bloodstream.[116] Furthermore, polymeric micelles can further optimize antitumor efficacy by adjusting the tissue distribution and pharmacokinetic properties of drugs. Li[117] *et al.* developed a doxorubicin (Dox) nanomedicine based on ginsenoside Rg1 by employing self-assembly technology to load Dox onto an Rg1 nanocarrier, constructing Dox@Rg1 NPs. This approach aimed to reduce cardiotoxicity while enhancing the antitumor activity. *In vitro* experiments demonstrated that this nanomedicine significantly inhibited tumor cell proliferation and reduced ROS levels, showcasing its dual functions of antitumor activity and cardioprotection. Further *in vivo* studies confirmed that the Dox-Rg1 nanomedicine exhibited excellent tumor suppression in a mouse tumor model while effectively mitigating doxorubicin-induced cardiotoxicity by reducing serum cardiac enzyme levels and improving myocardial tissue morphology. Compared to free ginsenosides, Rg1 self-assembled micelles showed higher drug-loading capacity and better biosafety, with superior efficacy and stability in cancer metastasis treatment, particularly in lung cancer metastasis[118] (Fig. 2A).

In conclusion, after decoction, the ginsenosides in Dushen decoction self-assemble into micelles, encapsulating drugs and precisely targeting tumor cells, thereby enhancing their antitumor effects.

4 Mechanism of SA formation in TCM decoctions

4.1 SAs of TCM with the same components

4.1.1 Polysaccharides. Polysaccharides are complex carbohydrates formed by the polymerization of multiple monosaccharides. They are abundant in nature and exhibit pharmacological effects such as antitumor and antioxidant properties.[119–123] The carbohydrate backbone of polysaccharides is rich in hydrophilic groups, allowing them to act as both hydrogen bond donors and acceptors, interacting with other molecules to form nanoparticles. Under specific conditions, polysaccharides spontaneously aggregate through non-covalent bonds to form SAs, such as the cyclic structures of oligosaccharides and the helical structures of polysaccharides, which facilitate the embedding of small-molecule drugs.[124,125] By

introducing hydrophobic aromatic groups or alkyl chains, the hydrophilic–hydrophobic balance of polysaccharides can be regulated, further enhancing their self-assembly capabilities. For example, Wu[59] *et al.* investigated the absorption of BBR and *Coptis chinensis* Franch. polysaccharide nanoparticles from Huanglian decoction in the intestines. They found that these nanoparticles can modulate tight junctions in intestinal epithelial cells, promote paracellular transport of BBR, and enhance its active transport and endocytosis, improving intracellular transport and significantly increasing the absorption efficiency of BBR. This effect is probably due to the hydroxyl-rich and negatively charged surface of the polysaccharides, which form hydrogen bonds with solvent molecules and carry a negative charge, enhancing the transport capacity of poorly soluble drugs (Fig. 2B). Additionally, an acidic branched polysaccharide, VBCP-3-a, extracted from vinegar-processed *Bupleurum chinense* DC. can self-assemble into micelles in water. Through hydrogen bonding and hydrophobic forces, it encapsulates water-insoluble components. It has been shown to enhance the solubility of compounds such as rhein and baicalin better than polysorbate 80 and significantly increases their plasma concentration.[126] Iitsuka[100] *et al.* observed a novel type of polysaccharide nanoparticle in TCM decoctions *via* ultracentrifugation and electron microscopy. These nanoparticles enter macrophages *via* phagocytosis and upregulate IL-6, thereby exerting immunostimulatory effects.

In conclusion, polysaccharide-based SADs show significant potential in drug delivery, particularly for improving the solubility and absorption of poorly soluble drugs. However, despite these promising findings, challenges remain in their practical application. First, the self-assembly mechanisms and stability of polysaccharide-based nanostructures may be influenced by varying conditions such as pH, temperature, and ion concentration, which could limit their effectiveness in complex physiological environments. Second, most current research is still at the laboratory stage, with a lack of systematic *in vivo* and clinical data to validate their safety and efficacy. Therefore, future research should focus on exploring the long-term biocompatibility and pharmacological stability of polysaccharide-based SADs to ensure their practical value in drug delivery systems.

4.1.2 Proteins. Proteins can self-assemble into highly water-soluble nanoparticles through specific interactions such as electrostatic attraction, van der Waals forces, hydrophobic interactions, and hydrogen bonding. In these structures, hydrophilic amino acid residues are located on the exterior, while hydrophobic residues are buried inside. Plant proteins from TCM, such as *Glycyrrhiza uralensis* Fisch. protein and *Armeniacae semen* amarum protein (ASP), have gained widespread attention due to their excellent biocompatibility and self-assembly properties.[55,127] For example, *Angelica sinensis* (Oliv.) Diels protein (ASRP) self-assembles into nanoparticles under specific conditions (pH 8.0, heated at 100 °C for 15 minutes), with this process accompanied by reduced ANS fluorescence, decreased α-helices, and increased β-sheets. These nanoparticles can encapsulate poorly soluble drugs such as ferulic acid (FA), demonstrating excellent antioxidant capacity and high drug-loading ability, with an encapsulation efficiency of approximately 30% and a drug loading capacity of 65%.[128] This indicates that proteins can unfold under certain conditions, exposing hydrophobic groups and assembling into nanostructures that enhance drug delivery capabilities. Additionally, it has been found that ASP in decoctions can self-assemble into ASP-NPs.[127] ASP-NPs can be loaded with the anticancer drug PTX to form PTX–ASP-NPs (Fig. 2C). Compared to free PTX, PTX–ASP-NPs demonstrated greater effectiveness against tumors. Another example is freshwater clam soup, a traditional remedy for liver disease, where nanoparticles isolated from the decoction contain proteins, polysaccharides, lipids, and phytosterols. These nanoparticles exhibited cholesterol uptake inhibition and were highly effective in treating non-alcoholic fatty liver disease.[23,24] Furthermore, studies revealed that glycoproteins BLGP1 and BLGP2, produced *via* glycosylation in the Banlangen decoction, can promote normal cell proliferation while inhibiting the growth of cancer cells and macrophages at the same concentration.[54]

In conclusion, TCM-derived protein-based SAs show significant potential in drug delivery, especially in encapsulating poorly soluble drugs and synergistically enhancing their therapeutic effects. Their excellent biocompatibility offers a new approach for developing drug carriers. However, despite the promising results from experimental studies, there are still challenges in their practical application. First, more *in vivo* and clinical studies are needed to understand the mechanisms of protein self-assembly, nanoparticle stability, and the long-term safety of these drug carriers. Additionally, how to maintain the stability and efficacy of protein nanoparticles in complex physiological environments requires further exploration.

4.1.3 Terpenoids. Terpenoids, composed of isoprene units, are natural plant chemicals that are abundant and widely utilized in nature.[129,130] Recent studies have shown that many small-molecule terpenoids can self-assemble into ordered SAs through non-covalent interactions such as hydrogen bonding, π–π stacking, and van der Waals forces.[131–133] These compounds possess unique chiral centers, rigid structures, and multiple modification sites, including carboxyl, hydroxyl, and carbon–carbon double bonds, which give them excellent self-assembly capabilities.[19,134] One example is glycyrrhizic acid, a triterpenoid derived from *Glycyrrhiza uralensis* Fisch. Due to its structure, which includes two hydrophilic glucuronic acid molecules and one hydrophobic glycyrrhetinic acid molecule, glycyrrhizic acid exhibits both hydrophilic and hydrophobic regions, allowing it to self-assemble in water to form fibrous aggregates with hydrophobic cavities, and even network-like hydrogels at high concentrations.[135] Glycyrrhizic acid SAs can encapsulate poorly soluble drugs such as baicalin, enhancing their solubility and stability.[136] *In vitro* experiments demonstrated that the release rate of baicalin in pH 6.8 and 8.3 buffers after 6 hours was 18.20% and 47.96%, respectively, and the release rate can be controlled by adjusting the pH. Selyutina[137] *et al.* utilized glycyrrhizic acid micelles to improve the oral absorption of the water-soluble monoterpenoid glycoside paeoniflorin. Compared to free paeoniflorin, the micelle-loaded form enhanced drug release and intestinal permeability. Additionally, glycyrrhetinic acid, due to its self-assembly properties in

water, can effectively combine with other drugs, significantly improving the solubility of poorly soluble compounds.[137] Yang[138] *et al.* applied glycyrrhizic acid micelles to deliver PTX *in vivo*, achieving an increase in oral bioavailability of nearly sixfold compared to free PTX. Recent studies have pointed out that dihydroartemisinin (DHA), a derivative of artemisinin derived from *Artemisia annua*, not only exhibits antimalarial activity but also combats various cancers.[139] Notably, under the influence of hydrogen bonding and hydrophobic interactions, DHA can self-assemble into spherical, carrier-free DHA NPs with a diameter of 93 nm, significantly enhancing its anti-cancer efficacy (Fig. 2D). *In vitro* and *in vivo* experiments have shown that DHA NPs exhibit good stability, high encapsulation efficiency, and strong release response, with significantly greater anti-cancer efficacy than that of free DHA.

In conclusion, although terpenoids, particularly triterpenoids, exhibit promising self-assembly properties for drug delivery, challenges remain in their clinical application. Although self-assembly systems such as glycyrrhizic acid have made progress in improving drug solubility, their stability, large-scale production, and cost-effectiveness still require further investigation. Additionally, while DHA nanoparticles exhibit significant anti-cancer effects, their mechanisms, safety, and performance in humans have not yet been fully validated, limiting their clinical translation.

4.2 SAs of TCM with different components

4.2.1 Alkaloids and flavonoids for forming SAs. Flavonoid components are widely present in TCM, primarily as acidic or neutral glycosides. During the decoction process, acidic flavonoids often undergo acid–base neutralization reactions with alkaloids, leading to the formation of SAs, a common phenomenon in TCM decoctions. When herbs rich in alkaloids are co-decocted with herbs containing acidic flavonoids, acid–base neutralization occurs. For instance, in HJD, BA (an acidic glycoside) and BBR form a precipitate during the decoction process, accounting for about 2.63% of the total decoction.[140] Studies have shown that this precipitate primarily consists of one-dimensional composite units formed *via* electrostatic interactions between BA and BBR. These units then self-assemble into three-dimensional BA–BBR NPs under hydrophobic interactions, with the hydrophilic parts facing outward and hydrophobic parts inward.[37] This self-assembled structure not only exhibits excellent biocompatibility but also shows superior antibacterial activity to BBR, particularly in disrupting bacterial biofilms. Furthermore, *in vitro* hemolysis, cytotoxicity, and *in vivo* zebrafish toxicity experiments have all confirmed its safety. In a recent study, Huang *et al.* have used BBR and BA, the main components of *Coptis chinensis* Franch.–*Scutellaria baicalensis* Georgi herb pair, to reveal the differences and mechanisms of self-assembly between physical mixtures and co-decoctions.[141] They discovered that the self-assembly of the *Coptis chinensis* Franch.–*Scutellaria baicalensis* Georgi herb pair and the phytochemicals berberine and baicalin in their physical mixture forms both resulted in NFs, while only the co-decoction formed self-assembled NPs (Fig. 2E). Under heat-driven conditions, BBR–BA NPs exhibited greater structural regularity and enhanced antibacterial activity, and the NFs could further be converted into BBR–BA NPs.

In summary, nanoparticles formed by the self-assembly of flavonoids and alkaloids hold great potential for drug development and delivery, especially in improving drug solubility, stability, and controlled release. Additionally, these self-assembled nanostructures demonstrate good biocompatibility and enhanced antibacterial activity. However, current research remains focused primarily on laboratory simulations and *in vitro* studies, lacking systematic *in vivo* validation and clinical research. Future efforts should be directed toward investigating their stability and efficacy under complex physiological conditions to ensure their safety and effectiveness in clinical applications.

4.2.2 Alkaloids and proteins for forming SAs. Research suggests that in clinical practice, the combined use of *Glycyrrhiza uralensis* Fisch. and *Aconitum kusnezoffii* Reichb. can effectively eliminate the toxicity of *Aconitum kusnezoffii* Reichb. and enhance the treatment efficacy.[142] The self-assembly nanotechnology of TCM provides a new method for studying this phenomenon. *Aconitum kusnezoffii* Reichb. and *Glycyrrhiza uralensis* Fisch., when combined, are recognized as a classic drug pairing that reduces toxicity and enhances therapeutic effectiveness. Recent studies have pointed out that in addition to the acid–base neutralization reaction between the tertiary amine N in alkaloids and the carboxyl group in glycyrrhizic acid, *Glycyrrhiza uralensis* Fisch. protein is also a key element in revealing the mechanism behind its toxicity reduction and efficacy enhancement.[143,144] Ke[144] *et al.* identified that a stable 31 kDa protein (GP) can be extracted from *Glycyrrhiza uralensis* Fisch. At pH 5, GP can form self-assembled GP–AC NPs with aconitine (AC), with a particle diameter of over 200 nm and an encapsulation efficiency of 28.2%. Acute toxicity experiments in mice demonstrated that the toxicity of aconitine was reduced after encapsulation by GP (Fig. 2F). This study demonstrates that encapsulating toxic alkaloids within nanoparticles made from TCM proteins can reduce their toxicity. It is hypothesized that other toxic alkaloids present in Chinese herbal decoctions could similarly interact with the amphiphilic compounds found in *Glycyrrhiza uralensis* Fisch., potentially yielding similar effects.

In conclusion, encapsulating toxic alkaloids in herbal protein-based self-assembling nanoparticles is a key mechanism for toxicity reduction and efficacy enhancement, as evidenced in the combination of *Glycyrrhiza uralensis* Fisch. and *Aconitum kusnezoffii* Reichb. This self-assembly strategy not only reduces toxicity but also enhances the therapeutic effects of the drugs. However, current research is mainly focused on animal models, and there is a lack of human clinical data. Future studies should further explore the biocompatibility of these nanoparticles and assess their safety and efficacy in humans to ensure their widespread clinical application.

5 Application and advantages of SAs in cancers treatment with TCM

5.1 Applications of SAs in TCM for antitumor therapy

Malignant tumors remain a primary cause of premature mortality worldwide. Although significant progress has been

Table 2 Applications of SAs in the field of cancer treatment[a]

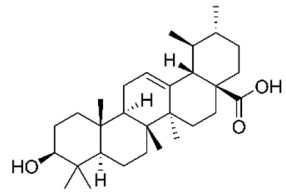
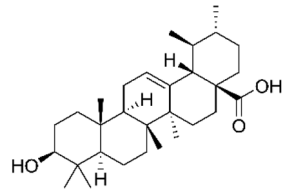
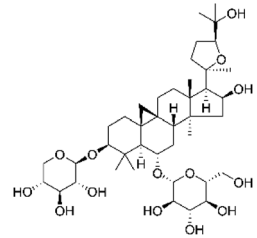
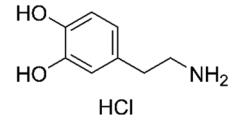
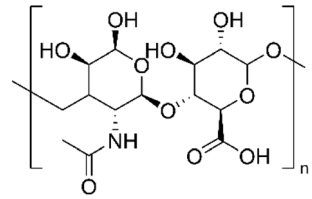
Cancer type	SAs preparation	Drug composition	Effects	Drug structure	Ref.
Lung cancer	UA NPs	UA (ursolic acid)	↑Immunostimulatory activity of TNF-α, IL-6 and IFN-β, ↑activation of CD^{4+} T cells, ↑cells apoptosis; ↓expression of COX-2/VEGFR2/VEGFA, ↓the activity of STAT-3		49
		UA (ursolic acid)	↓Tumor proliferation and metastasis; ↑tumor targeting ability 3		
Lung cancer	UA/(AS-IV) @PDA–HA NPs	AS-IV (astragaloside IV)			160
		DA (dopamine hydrochloride)			
		HA (hyaluronic acid)			

Table 2 (*Contd.*)

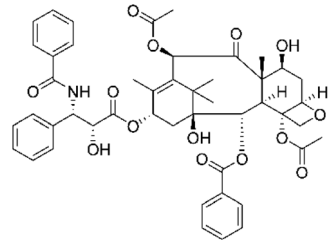
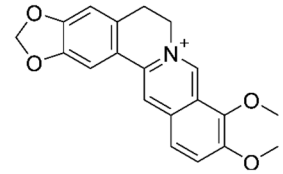
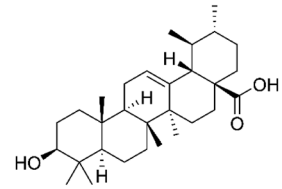
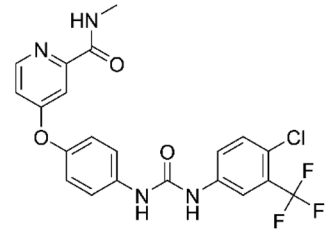
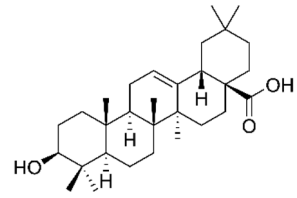
Cancer type	SAs preparation	Drug composition	Effects	Drug structure	Ref.
Lung cancer	PTX-ss-BBR NPs	PTX (paclitaxel)	↑Level of ROS in cancer cells, ↑block the cell cycle and cells apoptosis; ↓mitochondria membrane potential		164
		BBR (berberine)			
Liver cancer	US NPs	UA (ursolic acid)	↓Proliferation of HepG2 cells, SMMC7721 cells and H22 cells, ↓the migration of HepG2 cells and SMMC7721 cells, ↓membrane potential of mitochondrial; ↑cells apoptosis		161
		Sora (sorafenib)			
Liver cancer	OA–GA–PTX NPs	OA (oleanolic acid)	↑Antitumor activity; ↑drug releasing capacity and cellular uptake, ↑SOD, GSH; ↓ALT, AST, LDH, CK, MDA, ↓liver damage caused by PTX		52
		GA (glycyrrhetinic acid)			

Table 2 (*Contd.*)

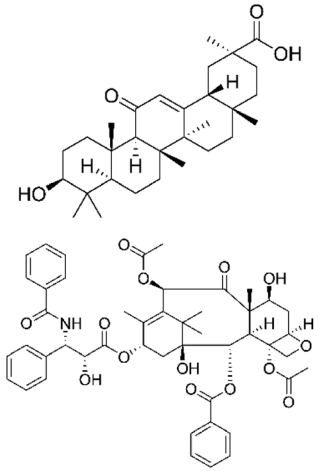
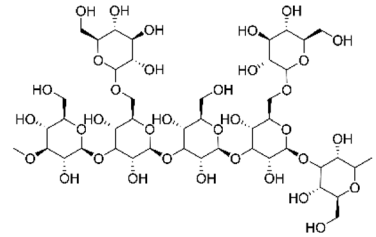
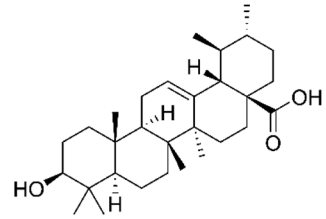
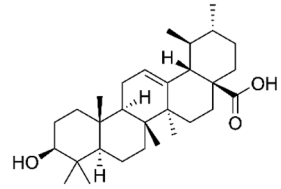
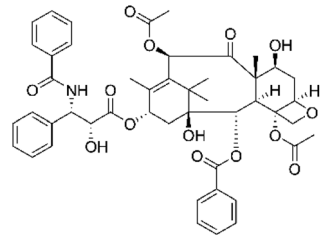
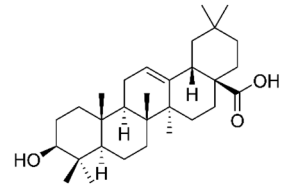
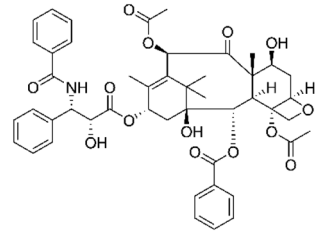
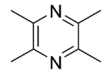
Cancer type	SAs preparation	Drug composition	Effects	Drug structure	Ref.
		PTX (paclitaxel)			
		LNT (lentinan)	↓Tumor growth and metastasis; ↑antitumor immunity; ↑drug anticancer and bioavailability performance		
Colorectal cancer	LNT–UA NPs				29
		UA (ursolic acid)			
Breast cancer	UA–PTX NPs	UA (ursolic acid)	↑Encapsulation efficiency and drug-loading, ↑block the cell cycle and cells apoptosis; ↓proliferation of breast tumor		38
		PTX (paclitaxel)			

Table 2 (*Contd.*)

Cancer type	SAs preparation	Drug composition	Effects	Drug structure	Ref.
Breast cancer	OC	Ce6 (chlorin e6)	Improved solubility, efficacy and safety of the OA		165
		OA (oleanolic acid)			
Ovarian cancer	PTX-ss-TMP NPs	PTX (paclitaxel)	↑Block the cell cycle and cell apoptosis, ↑cytotoxicity, ↑cellular uptake and rapid intracellular drug release; ↓the expression levels of p-VEGFR2/VEGFR2, p-AKT/AKT, p-mTOR/mTOR, and p-p38/p38, ↓the VEGFR2-AKT/mTOR/p38 signaling axis		167
		TMP (tetramethylpyrazine)			

[a] ↓: Reduced or inhibited; ↑: improved.

made in treatment, the incidence of new cancer cases and the proportion of cancer-related deaths are reported to be increasing, particularly in China and other regions.[145–147] The pathogenesis of tumors is complex and influenced by multiple risk factors, and effective treatment options for advanced-stage tumors remain lacking. Current treatments primarily rely on surgical resection combined with chemotherapy and/or radiotherapy. However, challenges such as the side effects of chemotherapy and the development of drug resistance persist.[148–152] In this context, plant-based TCM formulations are increasingly recognized as potential alternative cancer treatments, owing to their synergistic therapeutic effects, minimal side effects, and lower likelihood of inducing drug resistance.[153,154] SAs, formed through non-covalent interactions such as hydrogen bonding, π–π stacking, and hydrophobic interactions, create nanostructures that demonstrate significant potential in TCM for antitumor therapy. Natural small molecules found in TCM, such as UA, flavonoids, and terpenoids, are capable of self-assembling into stable nanoparticles. This self-assembly enhances drug solubility, bioavailability, and targeting specificity while simultaneously reducing side effects and strengthening antitumor activity.[5] TCM can also enhance the activity of immune cells, such as NK cells and T cells, thereby further improving the antitumor efficacy.[155] In recent years, combination therapy strategies involving active components of TCM and chemotherapeutic drugs have attracted increasing attention, particularly for their remarkable efficacy in reversing tumor drug resistance, enhancing therapeutic outcomes, and reducing side effects.[156] The application of SAs in different cancers is summarized in Table 2. For example, UA, a natural compound derived from herbs such as *Mespilus japonica* Thunb., *Prunella vulgaris* L., and *Hedyotis diffusa* Willd., has been shown to exhibit significant anticancer effects against various cancers, including NSCLC, colorectal cancer, breast cancer, liver cancer, and colorectal carcinoma.[29,157–160] UA exerts its anticancer effects through various mechanisms, including the induction of apoptosis and the inhibition of cell proliferation and migration. Furthermore, its self-assembly into nanoparticles significantly enhances both its stability and therapeutic efficacy. Fan[49] *et al.* found that UA self-assembles into carrier-free UA NPs *via* hydrophobic interactions and hydrogen bonding between molecules (Fig. 3). In a lung cancer model, these UA NPs showed stronger anticancer effects than free UA, inhibiting the proliferation of A549 human lung adenocarcinoma cells, inducing apoptosis, reducing COX-2/VEGFR2/VEGFA expression, and enhancing immune-stimulatory activities of TNF-α, IL-6, and IFN-β. UA also combines with astragaloside IV (AS-IV) to form multifunctional nanoparticles, UA/(AS-IV)@PDA–HA, which fuse chemotherapy, PTT, and immunotherapy to further enhance the antitumor effect (Fig. 4). This combination is especially effective in treating NSCLC, significantly increasing cytotoxicity and inhibiting NSCLC metastasis while optimizing AS-IV's immune response to suppress NSCLC growth and metastasis.[160] Additionally, UA combined with Sora forms US NPs, which protect liver cells, exhibiting excellent particle size, dispersibility, and stability.

Fig. 3 Schematic of the preparation of UA NPs with a carrier-free, self-delivery system and its anticancer mechanism, Copyright © 2018 the American Chemical Society, adapted with permission from ref. 49.

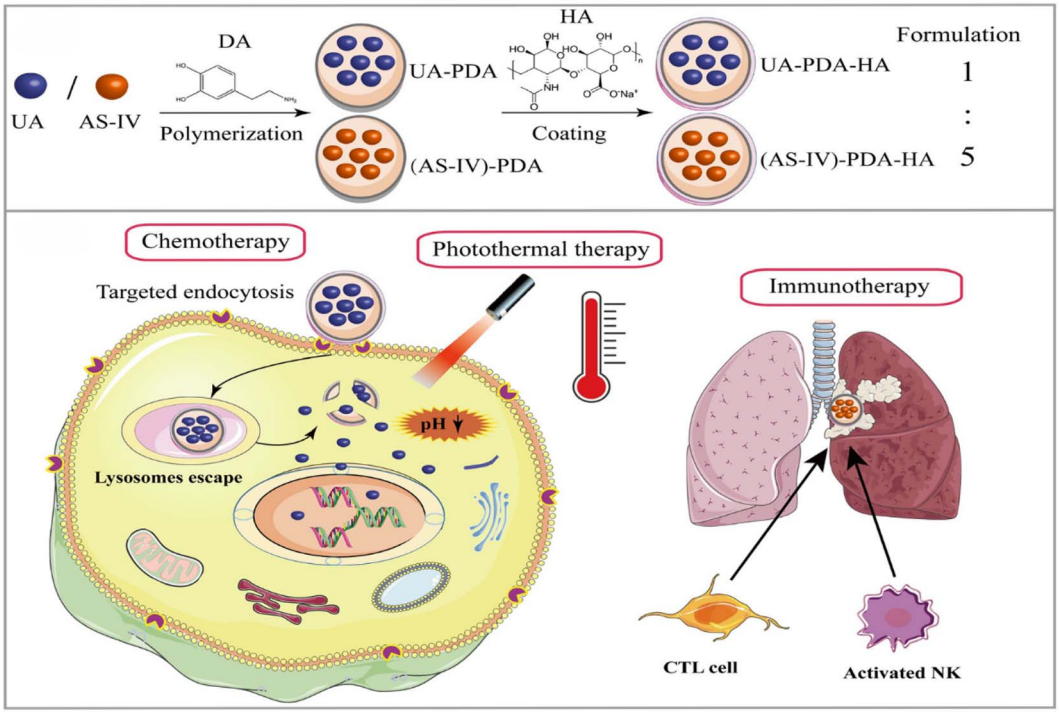


Fig. 4 Schematic of the synthesis of the core–shell UA/(AS-IV)@PDA–HA nanomedicine, which combined chemo-, immuno-, and photothermal-therapy in inhibiting the growth and metastasis of lung cancer, Copyright © The Royal Society of Chemistry 2023, adapted with permission from ref. 160.

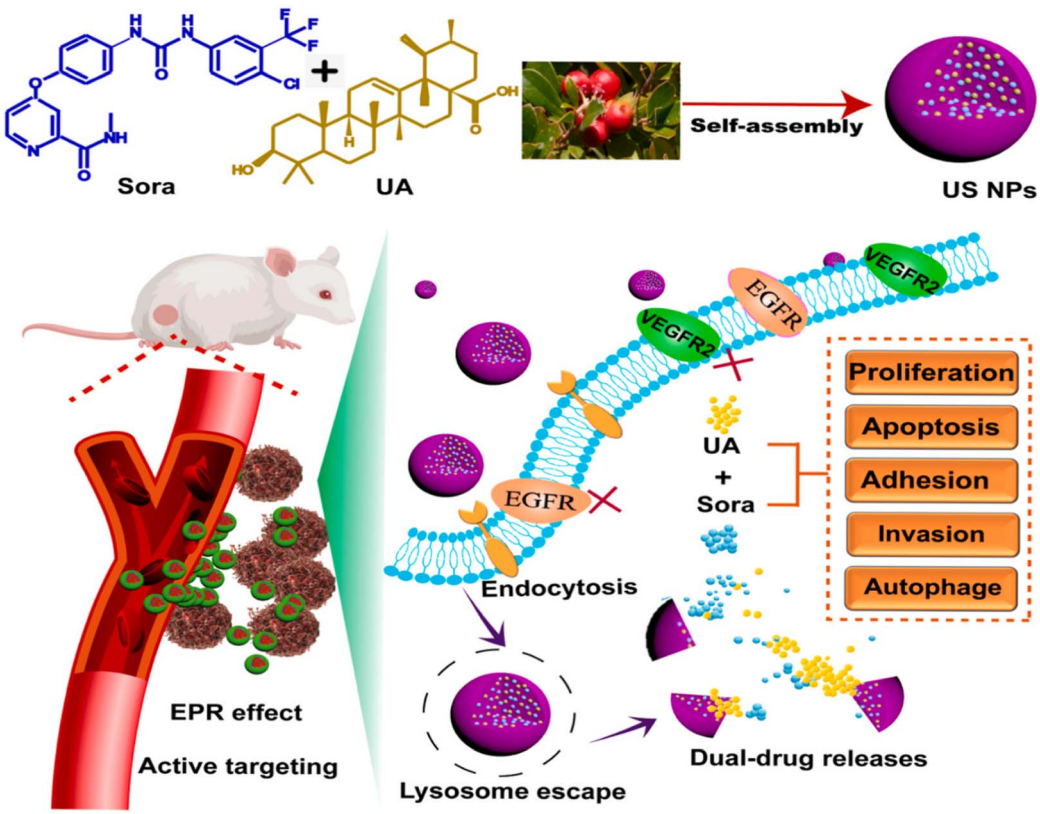


Fig. 5 Schematic of the preparation and antitumor effect of US NPs, Copyright © 2023 Published by Elsevier B.V, adapted with permission from ref. 161.

These nanoparticles synergistically inhibit the proliferation of multiple liver cancer cell lines, suppress migration, and reduce colony formation[161] (Fig. 5).

5.2 Combined applications of SAs with chemotherapy, photothermal therapy (PTT), and immunotherapy

5.2.1 Combined application of SAs with chemotherapy. Combined chemotherapy achieves synergistic anticancer effects by targeting multiple signaling pathways involved in tumor growth through the use of two or more drugs.[156,162,163] Active components in TCM are capable of simultaneously acting on multiple targets, resulting in synergistic therapeutic effects. The use of self-assembly nanoparticle strategies further improves the solubility, stability, and targeted release efficiency of these compounds, thereby enhancing their antitumor activity while mitigating the adverse effects associated with chemotherapy. The combination of SAs with chemotherapeutic agents has been shown to produce remarkable synergistic and potentiating effects in cancer treatment. Additionally, PTX, a chemotherapy drug, effectively inhibits cell division and is commonly used to treat breast, ovarian, and lung cancers. Studies have shown that UA and PTX can self-assemble into UA–PTX NPs, which significantly enhance their ability to inhibit breast cancer cell proliferation, induce apoptosis, and cause cell cycle arrest.[38] *In vivo*, these nanoparticles exhibit excellent targeting and antitumor effects while reducing PTX-induced liver damage, achieving a synergistic anticancer effect. PTX can also self-assemble with BBR to form PTX-ss-BBR NPs, which target mitochondria, induce ROS production in A549 cells, and cause cell cycle arrest, thus inhibiting tumor growth. These nanoparticles also have glutathione responsiveness, showing potential in treating bacteria-induced lung cancer.[164] Glycyrrhetinic acid (GA) and oleanolic acid (OA) have also been found to possess antitumor activity, though OA has poor water solubility and weak bioactivity. Research indicates that the self-assembled OA–GA NPs not only exhibit strong stability and high drug-loading capacity but also possess sustained-release functionality (Fig. 6). When combined with PTX, they enhance the anticancer effect and reduce liver toxicity.[52]

5.2.2 Combined application of SAs with PTT. The combined use of self-assembled nanoparticles with PTT can significantly enhance the antitumor efficacy. Additionally, Chlorin e6 (Ce6) is a photosensitizer used in cancer treatment, exhibiting both photodynamic and sono-photodynamic therapy (SPDT) effects. Research indicates that Ce6 can self-assemble with OA to form a carrier-free nanoparticle drug, OC, which significantly enhances the efficacy of combined chemotherapy and SPDT (Fig. 7).[165] After intravenous injection, OC accumulates at the tumor site and enters cancer cells through the enhanced permeability and EPR effect. Under light and ultrasound stimulation, OC synergistically enhances chemotherapy and SPDT by generating ROS, reducing the mitochondrial membrane potential and inducing apoptosis. OA and Ce6 self-assemble under the driving forces of electrostatic interactions, π–π stacking, and hydrophobic effects, forming OC NPs in aqueous solutions with good monodispersity and photostability. Compared to free drugs, OC NPs demonstrate significantly enhanced solubility, efficacy, and safety, exhibiting particularly strong toxicity against cancer cells, thus showcasing immense potential for improving cancer treatment. The PDA nanomaterial-mediated PTT also plays a crucial role in inhibiting tumor growth. UA/(AS-IV)@PDA–HA not only eliminates primary tumors but also inhibits distant metastasis in NSCLC, demonstrating great potential as an anti-metastasis drug for NSCLC. In addition, Lee[166] *et al.* pioneered the development of an AIE-active theranostic platform using the natural compound

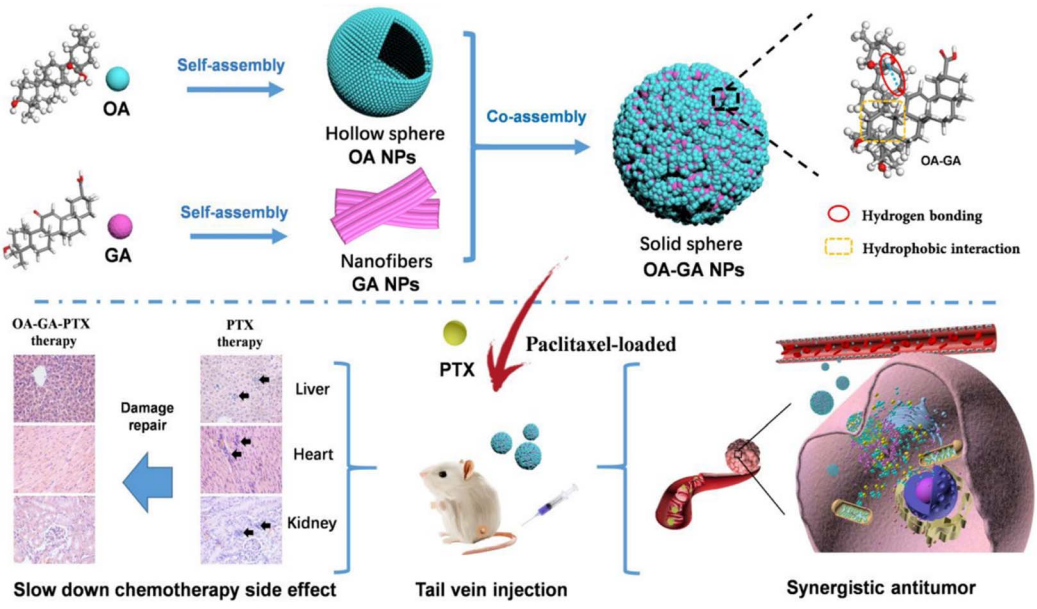


Fig. 6 Schematic of the preparation of OA–GA NPs with a high drug loading and self-delivery system, and its antitumor mechanism, Copyright © the American Chemical Society, adapted with permission from ref. 52.

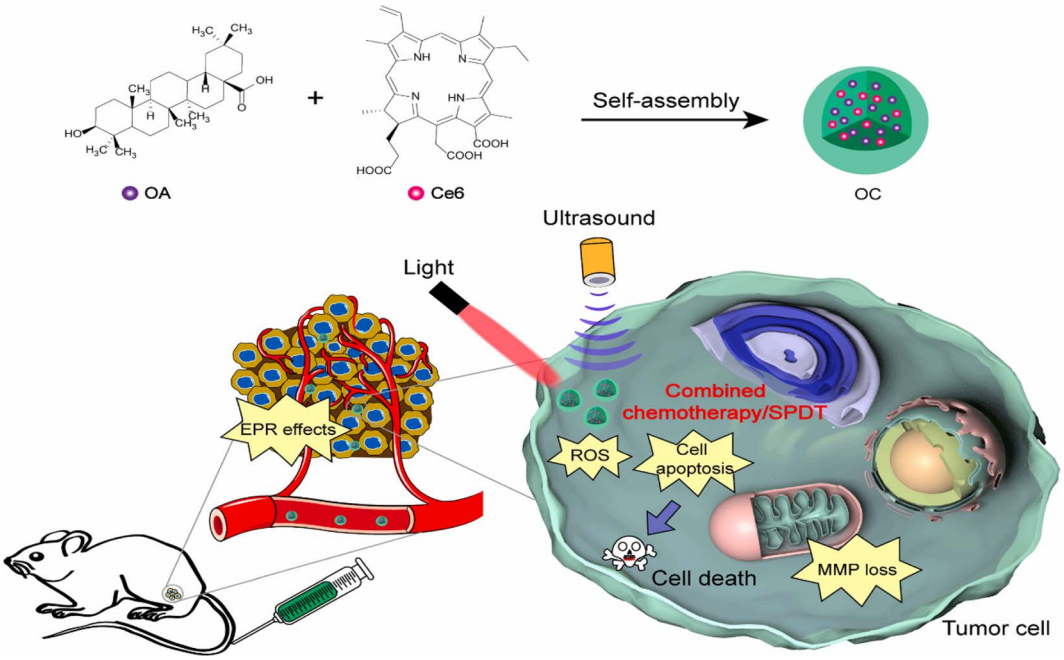


Fig. 7 Schematic of the preparation procedures of self-assembled nanoparticles and their application for synergistic chemo-/sono-photodynamic anti-tumor therapy. Copyright © 2021, Elsevier, adapted with permission from ref. 165.

BBR and demonstrated its potential through both *in vitro* and *in vivo* experiments. BBR has been shown to exhibit high selectivity at the cellular level, labeling mitochondria in cancer cells and migrating to the nucleus under light irradiation. This process, accompanied by the generation of ROS, significantly enhances the efficiency of PDT. *In vivo* studies have demonstrated that, upon light exposure, BBR effectively eliminates Gram-positive bacterial infections and accelerates wound healing. With its low toxicity and high therapeutic efficacy, BBR offers a promising new direction for the application of natural AIE materials in integrated diagnosis and treatment.

5.2.3 Combined application of SAs with immunotherapy. The application of TCM SAs in immunotherapy has also attracted widespread attention. For example, the LNT–UA

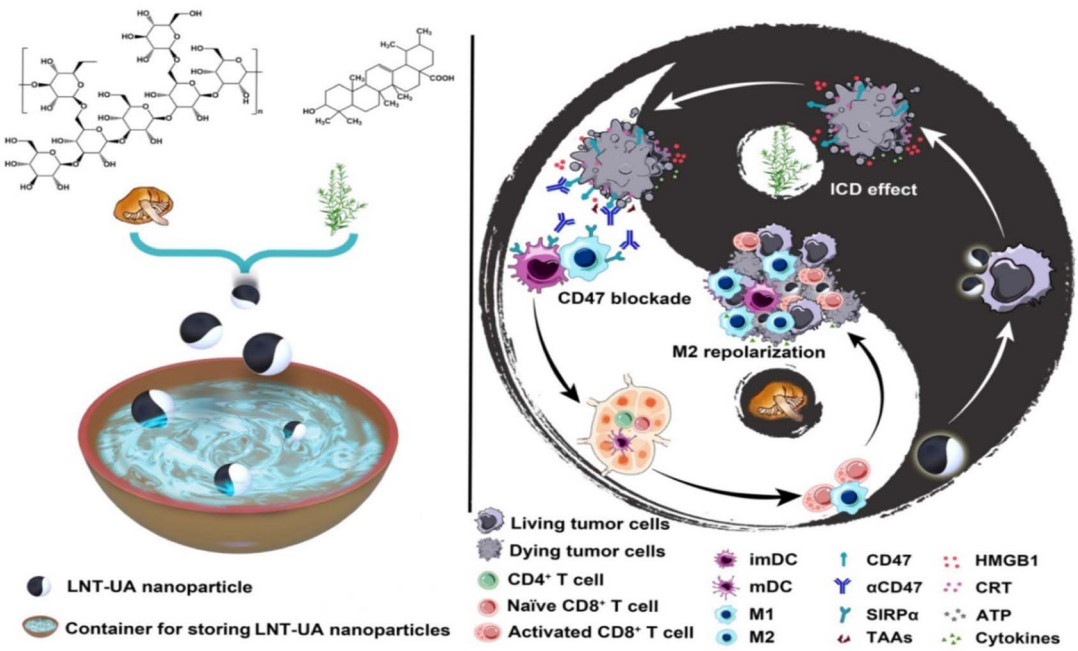


Fig. 8 Schematic of LNT–UA preparation and its combination with αCD47 for CRC immunotherapy *via* modulating the tumor immunosuppressive microenvironment, Copyright © 2022 Ivyspring International Publisher, adapted with permission from ref. 29.

nanodrug, self-assembled from UA and lentinan, a polysaccharide extracted from *Lentinus edodes* (Berk.) sing, significantly enhances antitumor immunity through synergistic effects, effectively inhibiting the growth and metastasis of colorectal cancer[29] (Fig. 8). Zou[167] *et al.* discovered that PTX, when self-assembled with tetramethylpyrazine (TMP), forms

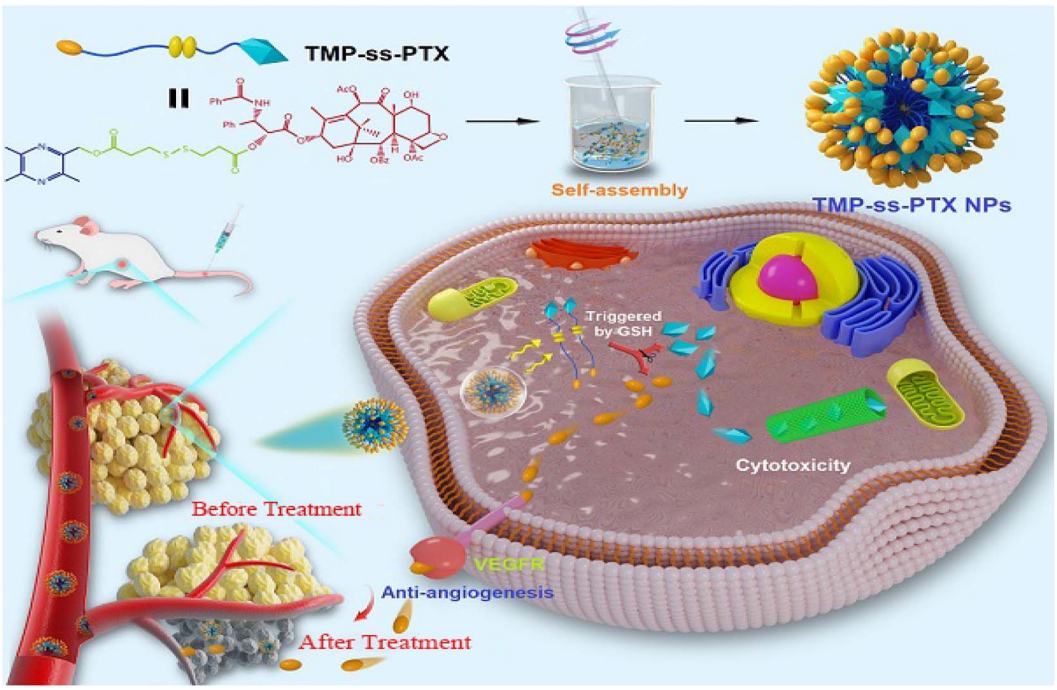


Fig. 9 Schematic of the preparation of PTX-ss-TMP NPs with a redox-responsive carrier-free nanosystem and its mechanism for suppressing ovarian carcinoma growth, Copyright © 2021 Ivyspring International Publisher, adapted with permission from ref. 167.

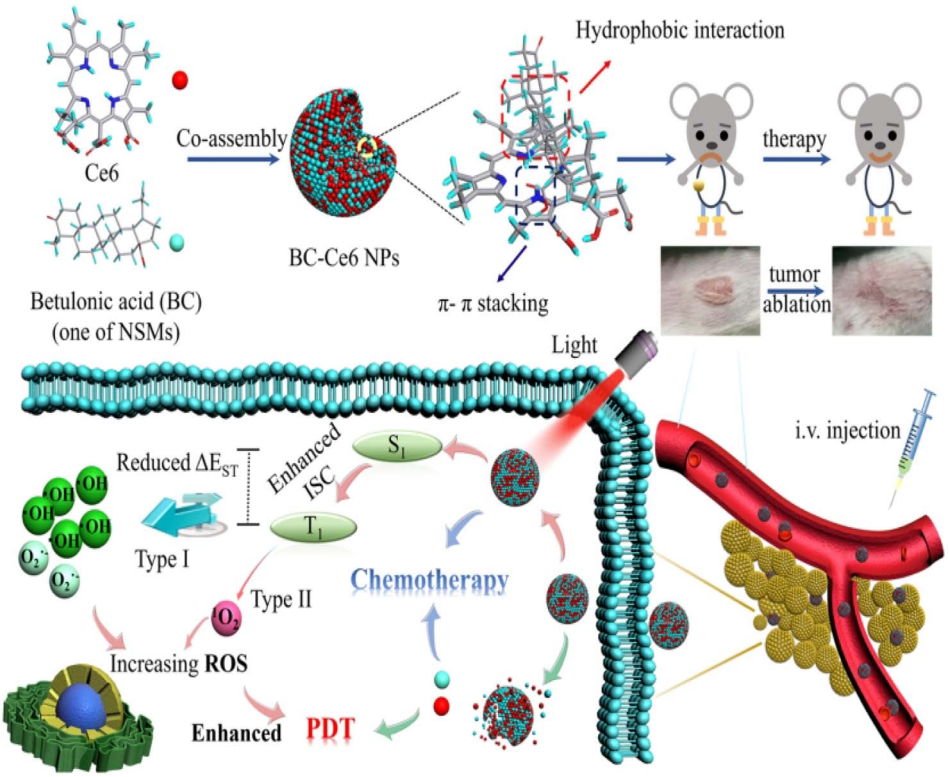


Fig. 10 Schematic of a representative small molecule, betulonic acid (BC)-meditated co-assembled synergistic antitumor BC–Ce6 NP for significantly enhanced chemo-photodynamic combination. Copyright © American Chemical Society, adapted with permission from ref. 44.

PTX-ss-TMP NPs, which show synergistic efficacy against ovarian cancer (Fig. 9). These nanoparticles effectively inhibit cancer cell proliferation, angiogenesis, and tumor progression, displaying higher cytotoxicity and apoptosis induction compared to PTX alone. In addition, Cheng[43] *et al.* designed and developed a carrier-free self-assembled nanoprodrug, COS–BA/Ce6 NPs, for combined photodynamic-chemotherapy-immunotherapy. This nanoprodrug integrates the natural small-molecule BA, COS, and the photosensitizer Ce6. COS–BA/Ce6 NPs form nanostructures through self-assembly, exhibiting pH responsiveness, low toxicity, and excellent biodegradability. *In vitro* experiments showed that this nanodrug effectively killed tumor cells by enhancing the generation of singlet oxygen ($^1O_2$) and improving the efficacy of chemotherapy. *In vivo* studies revealed that, when combined with anti-PD-L1 immunotherapy, it successfully activated systemic antitumor immune responses and efficiently inhibited both primary and metastatic tumors. Its intelligent release mechanism ensures precise drug delivery within the tumor microenvironment while reducing toxicity to normal tissues (Fig. 10). This study provides a novel strategy and a preclinical foundation for the application of highly efficient and low-toxicity nano-immunostimulants in cancer therapy.

In conclusion, by utilizing nanoparticles to enhance permeability and modify active targeting functions, SAs can effectively address the challenges of poor solubility and low bioavailability in antitumor active compounds, enabling more precise targeting of tumor cells. These self-assembly systems not only enhance the antitumor effects of active components but also synergize with other chemotherapy drugs, improving therapeutic outcomes while reducing toxicity and side effects. The self-assembly of active components from TCM with chemical drugs into nanomedicines offers an innovative strategy for cancer treatment, surpassing the efficacy of single drugs or simple combination therapies. These nanomedicines not only significantly enhance drug stability and targeting capabilities but also provide a foundation for constructing multifunctional diagnostic and therapeutic platforms, demonstrating broad potential applications in cancer diagnosis and treatment.[168] Additionally, through modification, nanomedicines can be equipped with photothermal or magnetic functionalities, enabling integrated tumor diagnosis and treatment. With their efficiency, versatility, and safety, SAs hold great promise for future applications in tumor diagnosis and therapy.

6 Conclusion

TCM decoctions, a vital component of TCM, are rooted in the holistic perspective of compound formulations, reflecting the scientific principles of the "Monarch–Minister–Assistant–Guide" theory. In recent years, the SA behavior of secondary metabolites, such as flavonoids and saponins, in decoctions has received widespread attention, offering molecular-level explanations for the pharmacological mechanisms of TCM. Studies have found that SAs are driven by non-covalent interactions such as hydrogen bonding, hydrophobic interactions, and π–π stacking to form stable supramolecular structures. These structures not only enhance the solubility, stability, and bioavailability of active components but also prolong the duration of therapeutic effects, providing a scientific basis for the "synergistic enhancement" observed in TCM compound formulations. For example, the synergistic self-assembly of glycyrrhizic acid and BA significantly improves solubility, while the self-assembled system of BBR and Rhe enhances antibacterial activity by increasing the drug concentration.[14,136] These findings support the traditional wisdom of "appropriate formulation" in TCM, providing a new scientific perspective for modern TCM research.

Nevertheless, current research on SAs in TCM is still in its early stages. Many aspects, such as their formation mechanisms, structure–function relationships, and *in vivo* pharmacodynamics, remain unresolved. The complexity of components in decoctions further complicates the identification of key driving forces and the dynamic structural changes of SAs. Moreover, certain self-assembled products may lead to potential side effects due to inadequate release control or extended half-life, raising the need for more rigorous safety evaluations. Future research, integrating TCM theories with modern scientific approaches, can be developed in the following directions: (1) Deepening the molecular study of the "Monarch–Minister–Assistant–Guide" theory in TCM: using modern supramolecular chemistry techniques to analyze the formation mechanisms of SAs in decoction formulations. This approach aims to uncover the roles and interactions of different medicinal components in the self-assembly process, providing quantitative evidence to support the scientific rationale behind TCM formulations. (2) Establishing a multiscale research framework for TCM decoctions: leveraging molecular dynamics simulations and omics technologies to systematically analyze the behavior of SAs from the microscopic level (molecular assembly behavior) to the macroscopic level (overall pharmacological effects). This approach aims to build a comprehensive bridge between "TCM efficacy" and "molecular structure". (3) Enhancing the stability and targeting efficiency of SAs: using technologies such as liposomes, polyethylene glycol (PEG) modification, and biomaterial coatings to improve the stability and biocompatibility of SAs. Additionally, aligning with the TCM principle of "treatment based on syndrome differentiation", targeted self-assembled nanomedicines can be developed for specific diseases.[169] (4) Innovating TCM formulations and clinical translation: building on the traditional advantage of TCM decoctions being "convenient for consumption", developing novel liquid or nanoformulations based on SAs to enhance the quality and stability of these preparations, and enabling the modernization and broader application of traditional medicines. (5) Scientific explanation for the internationalization of TCM: using SAs as a focal point to elucidate the molecular mechanisms by which TCM decoctions enhance drug activity, reduce toxicity, and improve efficacy. This approach aims to advance the internationalization of TCM and provide unique solutions for the global health industry. (6) New explorations in intelligent and precision medicine: integrating advanced intelligent nanotechnology to develop multifunctional self-assembled drugs that meet the precision treatment needs of

TCM principles such as "different treatments for the same disease" and "similar treatments for different diseases". This approach further highlights the unique value of TCM in the management of complex diseases.

In conclusion, research on SAs in TCM decoctions has, on a theoretical level, deepened the scientific understanding of the core principles behind TCM formulations and, on a practical level, advanced the modernization and internationalization of TCM. By integrating supramolecular chemistry with TCM theories, studying the formation mechanisms and pharmacological effects of SAs in decoctions not only optimizes the modern clinical application of TCM but also offers promising scientific prospects for the development of novel TCM formulations and contributions to global health governance.

Data availability

No new data were used during the preparation of this review.

Author contributions

Chunqiu Fang and Zhi Pan conceived and designed the research. Chunqiu Fang wrote the paper. Yinghang Wang created figures and tables. Zhi Pan revised the manuscript.

Conflicts of interest

There are no conflicts to declare.

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References

1 T.-L. Lin, C.-C. Lu, W.-F. Lai, T.-S. Wu, J.-J. Lu, Y.-M. Chen, C.-M. Tzeng, H.-T. Liu, H. Wei and H.-C. Lai, *Protein Cell*, 2020, **12**, 394–410.
2 Z. Wang, W. Li, J. Lu, Z. Yuan, W. Pi, Y. Zhang, H. Lei, W. Jing and P. Wang, *J. Ethnopharmacol.*, 2023, 300115704.
3 H.-H. Zhu, D.-P. Wu, X. Du, X. Zhang, L. Liu, J. Ma, Z.-H. Shao, H.-Y. Ren, J.-D. Hu, K.-L. Xu, J.-W. Wang, Y.-P. Song, M.-Y. Fang, J. Li, X.-Y. Yan and X.-J. Huang, *Lancet Oncol.*, 2018, **19**, 871–879.
4 X. Luan, L.-J. Zhang, X.-Q. Li, K. Rahman, H. Zhang, H.-Z. Chen and W.-D. Zhang, *J. Ethnopharmacol.*, 2020, **254**, 112687.
5 Q. Li, Y. Lianghao, G. Shijie, W. Zhiyi, T. Yuanting, C. Cong, Z. Chun-Qin and F. Xianjun, *Biomater. Sci.*, 2024, **12**, 1662–1692.
6 T. Du, R. Sun, S. Du, S. Gao, M. Hu, Y. Zhang, J. Chen and G. Yang, *J. Chromatogr. B*, 2019, **1128**, 121767.
7 W. Jia, J. Liu, R. Hu, A. Hu, W. Tang, L. Li and J. Li, *Front. Pharmacol*, 2020, **11**, 382.
8 Y. Zhuang, J. Yan, W. Zhu, L. Chen, D. Liang and X. Xu, *J. Ethnopharmacol.*, 2008, **117**, 378–384.
9 G. M. Whitesides and B. Grzybowski, *Science*, 2002, **295**, 2418–2421.
10 B. Rybtchinsk, *ACS Nano*, 2011, **5**, 6791–6818.
11 M. e. Malík, J. r. Velechovský and P. Tlustõs, *Fitoterapia*, 2021, **151**, 104845.
12 J. Hu, Z. Wu, J. Yan, W. Pang, D. Liang and X. Xu, *J. Ethnopharmacol.*, 2009, **123**, 267–274.
13 X. Huang, P. Wang, T. Li, X. Tian, W. Guo, B. Xu, G. Huang, D. Cai, F. Zhou, H. Zhang and H. Lei, *ACS Appl. Mater. Interfaces*, 2020, **12**, 227–237.
14 X. Tian, P. Wang, T. Li, X. Huang, W. Guo, Y. Yang, M. Yan, H. Zhang, D. Cai, X. Jia, F. Li, B. Xu, T. Ma, C. Yan and H. Lei, *Acta Pharm. Sin. B*, 2020, **10**, 1784–1795.
15 A. H. Elcock, *Curr. Opin. Struct. Biol.*, 2010, **20**, 196–206.
16 Y. Gao, Y. Dong, Q. Guo, H. Wang, M. Feng, Z. Yan and D. Bai, *Molecules*, 2022, **27**, 3268.
17 Y. Ping, Y. Li, S. Lü, Y. Sun, W. Zhang, J. Wu, T. Liu and Y. Li, *Biomed. Pharmacother.*, 2020, **124**, 109826.
18 F. Huang and E. V. Anslyn, *Chem. Rev.*, 2015, **115**, 6999–7000.
19 Y. Hou, L. Zou, Q. Li, M. Chen, H. Ruan, Z. Sun, X. Xu, J. Yang and G. Ma, *Mater. Today Bio*, 2022, **15**, 100327.
20 E. Mahon, T. Aastrup and M. Barboiu, Dynamic Nanoplatforms in Biosensor and Membrane Constitutional Systems, in *Constitutional Dynamic Chemistry*, 2011, pp. 139–163.
21 X. Dou, N. Mehwish, C. Zhao, J. Liu, C. Xing and C. Feng, *Acc. Chem. Res.*, 2020, **53**, 852–862.
22 X. Qian, X. Fan, H. Su, J. Zhang, N. Tao, J. Zhong, X. Wang and B. Han, *LWT*, 2019, **111**, 69–76.
23 Z. Yu, G. Gao, H. Wang, L. Ke, J. Zhou, P. Rao, T. Chen, Z. Peng, J. Zou and S. Luo, *Int. J. Biol. Macromol.*, 2020, **151**, 781–786.
24 G. Gao, H. Wang, J. Zhou, P. Rao, L. Ke, J. J. Lin, B. S. Pan, Y. Zhang and Q. Wang, *J. Agric. Food Chem.*, 2021, **69**, 1610–1618.
25 Q. Zhao, X. Luan, M. Zheng, X.-H. Tian, J. Zhao, W.-D. Zhang and B.-L. Ma, *Pharmaceutics*, 2020, **12**, 128.
26 L. Qiao, H. Yang, S. Gao, L. Li, X. Fu and Q. Wei, *J. Mater. Chem. B*, 2022, **10**, 1908–1922.
27 J. Zheng, R. Fan, H. Wu, H. Yao, Y. Yan, J. Liu, L. Ran, Z. Sun, L. Yi, L. Dang, P. Gan, P. Zheng, T. Yang, Y. Zhang, T. Tang and Y. Wang, *Nat. Commun.*, 2019, **10**, 1604.
28 L. Cai, S. Liu, J. Guo and Y.-G. Jia, *Acta Biomater.*, 2020, **113**, 84–100.
29 Q. Mao, J. Min, R. Zeng, H. Liu, H. Li, C. Zhang, A. Zheng, J. Lin, X. Liu and M. Wu, *Theranostics*, 2022, **12**, 6088–6105.
30 E. Mattia and S. Otto, *Nat. Nanotechnol.*, 2015, **10**, 111–119.
31 L. Li, R. Sun, R. Zheng and Y. Huang, *Mater. Des.*, 2021, **205**, 109759.
32 J. Xu, G. Qi, W. Wang and X. S. Sun, *NPJ Sci. Food*, 2021, **5**, 14.

33 L. Li, R. Zheng and R. Sun, *Pharmacol. Res.*, 2022, **4**, 100158.
34 J. Huang, Y. Zhu, H. Xiao, J. Liu, S. Li, Q. Zheng, J. Tang and X. Meng, *Chin. Med.*, 2023, **18**, 66.
35 X. Lin, X. Huang, X. Tian, Z. Yuan, J. Lu, X. Nie, P. Wang, H. Lei and P. Wang, *ACS Omega*, 2022, **7**, 43510–43521.
36 C. M. A. Leenders, M. B. Baker, I. A. B. Pijpers, R. P. M. Lafleur, L. Albertazzi, A. R. A. Palmans and E. W. Meijer, *Soft Matter*, 2016, **12**, 2887–2893.
37 T. Li, P. Wang, W. Guo, X. Huang, X. Tian, G. Wu, B. Xu, F. Li, C. Yan, X.-J. Liang and H. Lei, *ACS Nano*, 2019, **13**, 6770–6781.
38 J. Wang, H. Zhao, K. Zhi and X. Yang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 6827–6839.
39 D. Yang, S. Gao, Y. Fang, X. Lin, X. Jin, X. Wang, L. Ke and K. Shi, *Nanomedicine*, 2018, **13**, 3159–3177.
40 N. Han, X. Huang, X. Tian, T. Li, X. Liu, W. Li, S. Huo, Q. Wu, Y. Gu, Z. Dai, B. Xu, P. Wang and H. Lei, *Curr. Drug Delivery*, 2021, **18**, 914–921.
41 P. Wang, W. Guo, G. Huang, J. Zhen, Y. Li, T. Li, L. Zhao, K. Yuan, X. Tian, X. Huang, Y. Feng, H. Lei and A. Xu, *ACS Appl. Mater. Interfaces*, 2021, **13**, 32729–32742.
42 S. Das, S. Thakur, M. Korenjak, V. S. Sidorenko, F. F.-L. Chung and J. Zavadil, *Nat. Rev. Cancer*, 2022, **22**, 576–591.
43 J. Cheng, H. Zhao, B. Li, H. Zhang, Q. Zhao, S. Fu, Y. Han, W. Lu, J. Shi and X. Yang, *Acta Pharm. Sin. B*, 2023, **13**, 879–896.
44 J. Cheng, H. Zhao, J. Wang, Y. Han and X. Yang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 43488–43500.
45 Y. Liu, L. Zhao, G. Shen, R. Chang, Y. Zhang and X. Yan, *Colloids Surf., A*, 2020, **598**, 124805.
46 S. Garde, *Nat. Commun.*, 2015, **517**, 277–279.
47 X. Jia, Z. Yuan, Y. Yang, X. Huang, N. Han, X. Liu, X. Lin, T. Ma, B. Xu, P. Wang and H. Lei, *J. Nanobiotechnol.*, 2022, **20**, 116.
48 S. Song, Q. Zheng, A. Song and J. Hao, *Langmuir*, 2011, **28**, 219–226.
49 L. Fan, B. Zhang, A. Xu, Z. Shen, Y. Guo, R. Zhao, H. Yao and J.-W. Shao, *Mol. Pharm.*, 2018, **15**, 2466–2478.
50 K. Jiang, L. Han, Y. Guo, G. Zheng, L. Fan, Z. Shen, R. Zhao and J. Shao, *J. Mater. Chem. B*, 2017, **5**, 9121–9129.
51 C. Li, J. Lin, P. Wu, R. Zhao, J. Zou, M. Zhou, L. Jia and J. Shao, *Bioconjugate Chem.*, 2018, **29**, 3495–3502.
52 J. Wang, H. Zhao, W. Qiao, J. Cheng, Y. Han and X. Yang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 42537–42550.
53 X. Wang, Y. Wang, M. Tang, X. Wang, W. Xue, X. Zhang, Y. Wang, W.-H. Lee, Y. Wang, T.-Y. Sun, Y. Gao and L.-L. Li, *Adv. Healthcare Mater.*, 2023, **12**, 2202432.
54 J. Zhou, J. Liu, D. Lin, G. Gao, H. Wang, J. Guo, P. Rao and L. Ke, *J. Tradit. Complementary Med.*, 2017, **7**, 178–187.
55 J. Zhou, J. Zhang, G. Gao, H. Wang, X. He, T. Chen, L. Ke, P. Rao and Q. Wang, *J. Agric. Food Chem.*, 2019, **67**, 9354–9361.
56 K. Matsuoka, R. Miyajima, Y. Ishida, S. Karasawa and T. Yoshimura, *Colloids Surf., A*, 2016, **500**, 112–117.
57 D. Lin, Q. Du, H. Wang, G. Gao, J. Zhou, L. Ke, T. Chen, C. Shaw and P. Rao, *BioMed Res. Int.*, 2017, **2017**, 1–8.
58 Q. Weng, X. Cai, F. Zhang and S. Wang, *Food Chem.*, 2019, **274**, 796–802.
59 J. Wu, Y. Yang, X. Yuan, H. Xu, Q. Chen, Q. Zhang, Z. Hou, F. Jiao and D. Yin, *Food Funct.*, 2020, **11**, 10480–10492.
60 E. Shang, X. Ma, X. Yu, S. Sun and G. Shen, *J. PharmTech Res.*, 2021, **40**, 216–220.
61 M. Wan, L. Liu, H. Wu, C. Lu, Y. Fan and H. Huang, *J. Chin. Mater. Med.*, 2011, **34**, 455–458.
62 L.-M. Qin, Z. Zheng, F.-L. Niu, R.-Y. Dong, Y.-F. Yam, F.-H. Lang and G.-P. Fan, *Chin. J. Exp. Tradit. Med. Formulae*, 2000, **6**, 3–5.
63 Z.-J. Chen, J. Wang, S. Gan, J. Li, X. Wang, Y.-F. Cheng and W. Li, *Pharm. Clin. Chin. Mater. Med.*, 2018, **9**, 23–27.
64 X. Li, Z. Liang, J. Du, Z. Wang, S. Mei, Z. Li, Y. Zhao, D. Zhao, Y. Ma, J. Ye, J. Xu, Y. Zhao, J. Chang, Y. Qin, L. Yu, C. Wang and C. Jiang, *Sci. China Life Sci.*, 2019, **62**, 333–348.
65 X.-J. Zhang, Y.-X. Deng, Q.-Z. Shi, M.-Y. He, B. Chen and X.-M. Qiu, *Phytomedicine*, 2014, **21**, 615–623.
66 X. Li, H. Tang, Q. Tang and W. Chen, *Front. Cell Dev. Biol.*, 2021, **9**, 638366.
67 R. Tian, X. Liu, Y. Xiao, L. Jing, H. Tao, L. Yang and X. Meng, *J. Ethnopharmacol.*, 2024, **323**, 117686.
68 M. Chen, P. Wang, T. Li, L. Li, J. Li, H. Bai, H. Lei and Q. Ma, *J. Pharm. Biomed. Anal.*, 2021, **195**, 113820.
69 J.-Y. Zheng, X.-X. Li, W.-Y. Lin, S. Su, H.-C. Wu, R.-D. Hu, H.-F. Pan, J.-H. Ye, Y.-F. Cai and S.-J. Zhang, *J. Ethnopharmacol.*, 2023, **315**, 116658.
70 Y. Chen, Q. Li, X. Yang, W. Wu, Z. Jin and J. Liaoning, *Univ. China Med.*, 2023, **25**(07), 214–220.
71 L. Pan, J. Fu, H. Zhu and L. Guo, *China J. Chin. Mater. Med.*, 2010, **35**, 40–43.
72 H. Wang, T. Li, H. Xiang, X. Zhang, K. Fang, G. Wu, M. Yan, N. Xue, M. Chen, T. Xie, Y. Zhang, P. Wang and H. Lei, *Molecules*, 2017, **22**, 1456.
73 K. Fang, G.-R. Wu, H. Wang, Z. Rui, X.-Y. Zhang, N.-N. Xue, M. Chen, W.-B. Guo, F.-H. Chu, B. Xu, P.-L. Wang and H.-M. Lei, *Chin. Tradit. Herb. Drugs*, 2017, **48**, 3714–3719.
74 T. Li, H. Wang, H. Zhang, X.-H. Tian, Q.-H. Chen, K. Fang, G.-R. Wu, B. Xu, F.-H. Chu, P.-L. Wang and H.-M. Lei, *Chin. Tradit. Herb. Drugs*, 2017, **48**, 3505–3510.
75 D.-M. Liu, S.-M. Liu, J.-X. Zu, F. Lu, L. Jing, W. He and Z.-H. Huang, *Chin. Tradit. Pat. Med.*, 2012, **34**, 74–78.
76 L.-F. Lin, G.-S. Chen, H. Ll and H.-J. Yang, *China J. Chin. Mater. Med.*, 2023, **48**, 5790–5797.
77 X. Ke, L. Zhang, L. Xin, D. Zhang, L. Han and C. Chuan, *Chin. Tradit. Pat. Med.*, 2020, **42**, 2192–2195.
78 X. An, C. Shi, Y. Han, X. Li, L. Dong, Y. Li, H. Chen, Y. Wang, J. Li, G. Liu, F. Lian, R. Ma and X. Tong, *Front. Pharmacol*, 2023, **14**, 1279519.
79 Y. Xu, L. Bao, S. Cao, B. Pang, J. Zhang, Y. Zhang, M. Chen, Y. Wang, Q. Sun, R. Zhao, S. Guo, J. Sun and X. Cui, *J. Ethnopharmacol.*, 2024, **320**, 117424.
80 L.-Q. Ma, C.-S. Pan, N. Yang, Y.-Y. Liu, L. Yan, K. Sun, X.-H. Wei, K. He, M.-M. Xiao, J.-Y. Fan and J.-Y. Han, *Microcirculation*, 2014, **21**, 649–663.

81 Q. Li, C. Bai, R. Yang, W. Xing, X. Pang, S. Wu, S. Liu, J. Chen, T. Liu and X. Gu, *Front. Pharmacol*, 2020, **11**, 581691.
82 T. Guo, Y. Guo, Q. Liu, Y. Xu, L. Wei, Z. Wang, S. Chen, C. Wang, Y. Tian, J. Cui, Y. Wang, Y. Wang and L. Sun, *J. Ethnopharmacol.*, 2021, **275**, 114133.
83 H.-Z. Qiao, L.-Q. Di, Q.-N. Ping and L.-H. Hu, *China J. Chin. Mater. Med.*, 2021, **46**, 2443–2448.
84 C. He and Y. Pu, *Chin. J. Exp. Tradit. Med. Formulae*, 2022, **28**(15), 259–266.
85 Q. Du, Y. Huang, H.-Q. Wang, G.-Z. Gao, J.-W. Zhou, L.-J. Ke and P.-F. Rao, *China J. Tradit. Chin. Med. Pharm.*, 2014, **29**, 3746–3750.
86 Y.-X. Zhu, W. Chen, Z.-Z. Wang, H.-Z. Qiao and L.-Q. Di, *Acta Pharmacol. Sin.*, 2021, **56**, 2112–2118.
87 X. Wang, H. Long, M. Chen, Z. Zhou, Q. Wu, S. Xu, G. Li and Z. Lu, *Front. Physiol.*, 2022, **13**, 1023453.
88 T. Lu, L. Li, Y. Li and X. Li, *J. Poult. Sci.*, 2023, **60**, 2023012.
89 S.-W. Lv, Y.-Q. Wu, Y.-P. Li, Y.-H. Wang, Z.-X. Yang, R. Wang, Q.-X. Guan and Y.-J. Li, *Chin. J. Exp. Tradit. Med. Formulae*, 2020, **26**, 154–160.
90 J.-W. Hu, G.-X. Jia, Y.-Q. Dong, Q. Lu, S.-Y. Tian, S.-S. Yang and Y.-B. Li, *Chin. Tradit. Herb. Drugs*, 2022, **53**, 7307–7316.
91 S. Lü, H. Su, S. Sun, Y. Guo, T. Liu, Y. Ping and Y. Li, *Sci. Rep.*, 2018, **8**, 12209.
92 S.-W. Lv, Y.-Q. Wu, Y.-P. Li, S. Sun, D.-Y. Yang, Y.-Y. Guo, H. Su and Y.-J. Li, *J. Int. Pharm. Res.*, 2020, **47**, 870–875.
93 J. Wu, *Preliminary Study on the Antipyretic Effect and Mechanism of Bai-hu Decoction and its Nanometer Phase*, Heilongjiang University of Chinese Medicine, 2018.
94 R. He, S. Wang, S. Yang, R. Liu, N. Nan, X. Lu, M. Gong and J. Li, *J. Ethnopharmacol.*, 2023, **309**, 116300.
95 R. He, Y. Xu, J. Peng, T. Ma, J. Li and M. Gong, *J. Nat. Med.*, 2016, **71**, 198–207.
96 Z. Q. Liu, Z. H. Jiang, L. Liu and M. Hu, *Pharm. Res.*, 2006, **23**, 2768–2780.
97 C. Wang, J. Yuan, L. L. Zhang and W. Wei, *Xenobiotica*, 2016, **46**, 1142–1150.
98 B. Yan, M. Shen, J. Fang, D. Wei and L. Qin, *J. Pharm. Biomed. Anal.*, 2018, **160**, 276–288.
99 P. Gan, M. Zhong, X. Huang, M. Sun, Y. Wang, Y. Xiao, C. Zeng, Q. Yuan, Z. Liu and H. Zhou, *Planta Med.*, 2011, **78**, 237–243.
100 H. Iitsuka, K. Koizumi, A. Inujima, M. Suzaki, Y. Mizuno, Y. Takeshita, T. Eto, Y. Otsuka, R. Shimada, M. Liu, K. Ikeda, M. Nakano, R. Suzuki, K. Maruyama, Y. Zhou, H. Sakurai and N. Shibahara, *Biochem. Biophys. Res.*, 2018, **16**, 62–68.
101 C. Shen, B. Shen, J. Zhu, J. Wang, H. Yuan and X. Li, *Drug Dev. Ind. Pharm.*, 2020, **47**, 207–214.
102 K. K. L. Kwan, T. T. X. Dong and K. W. K. Tsim, *Phytomedicine*, 2021, **88**, 153605.
103 G.-s. Chai, J. Gong, J.-j. Wu, R.-k. Ma, J. Zhu, D.-d. Jia, Y.-q. Zhang, X.-r. Zhai, H.-x. Sun, Y. j. Nie, P. Zhao, Y.-l. Xu and H. t. Yu, *J. Ethnopharmacol.*, 2023, **313**, 116554.
104 Y. Gao, Y. Zhang, W. Liu, N. Zhang, Q. Gao, J. Shangguan, N. Li, Y. Zhao and Y. Jia, *Pharm. Biol.*, 2023, **61**, 710–721.
105 P. Liang, T. Bi, Y. Zhou, Y. Ma, X. Liu, W. Ren, S. Yang and P. Luo, *ACS Appl. Mater. Interfaces*, 2023, **15**, 47939–47954.
106 C. Zhang, N. Li, G.-S. Zhong, L.-L. Xiu, H.-Y. Liu, S.-H. Chen, F. Chen, M. Li, W.-Y. Liao and Y.-N. Ren, *Chin. Tradit. Herb. Drugs*, 2021, **52**, 6425–6430.
107 Y. Yang, C. T. Vong, S. Zeng, C. Gao, Z. Chen, C. Fu, S. Wang, L. Zou, A. Wang and Y. Wang, *J. Ethnopharmacol.*, 2021, **268**, 113573.
108 B. Chen, L. Zhang, D. Lu, J. Yan, Z. Sun and J. Hunan, *Univ. China Med.*, 2021, **41**, 224–229.
109 W. Li, Z.-J. Wang, X.-Y. Lin, X.-J. Liu, N.-N. Han, W.-M. Pi, Z.-H. Yuan, H.-M. Lei and P.-L. Wang, *Acta Pharmacol. Sin.*, 2022, **57**, 1901–1908.
110 J. Deng, *Chemical Changes of Berberine-Containing Traditional Chinese Medicine and Glycyrrhiza Uralensis Fisch during Decocting – Study on Precipitation Reaction Mechanism of Berberine and Glycyrrhizic Acid*, Hunan University of Chinese Medicine, 2007.
111 R. Wang, *Mechanisms underlying the pharmacokinetic influence of Glycyrrhizae Radix et Rhizoma on berberine in Coptidis Rhizoma*, Shanghai University of Traditional Chinese Medicine, 2019.
112 W. Li, Z.-J. Wang, X.-J. Liu, N.-N. Han, T. Li, H.-M. Lei and P.-L. Wang, *Acta Pharmacol. Sin.*, 2021, **56**, 2119–2126.
113 S.-J. Chu, Y.-N. Dong, Q. Zhang, L.-J. Xle, J.-H. Ll, F. F. Xu, J. Yu, H.-L. Feng, Z.-Q. Co and Z.-H. Lou, *Ginseng Res.*, 2022, **34**, 34–39.
114 D. T.-K. Yun, *Lancet Oncol.*, 2001, **2**, 49–55.
115 J. Xiong, J. Guo, L. Huang, B. Meng and Q. Ping, *Int. J. Pharm.*, 2008, **360**, 191–196.
116 M. Li, L. Lin, Y. Wang, J. Shi, C. Wu and C. Zhu, *Chin. Tradit. Herb. Drugs*, 2024, **55**, 688–696.
117 C. Li, *Doxorubicin Nanomedicine Based on Ginsenoside Rg1 with Alleviated Cardiotoxicity and Enhanced Antitumor Activity*, Tianjin University of Technology, 2022.
118 X.-R. Tan, C. Li, K.-K. Feng, J.-Q. Le, J.-W. Shen and J.-W. Shao, *J. Ind. Eng. Chem.*, 2022, **116**, 303–309.
119 F. Chen, G. Huang, Z. Yang and Y. Hou, *Int. J. Biol. Macromol.*, 2019, **138**, 673–680.
120 T. Khan, A. Date, H. Chawda and K. Patel, *Carbohydr. Polym.*, 2019, **210**, 412–428.
121 X. Mei, W. Yang, G. Huang and H. Huang, *Int. J. Biol. Macromol.*, 2020, **142**, 232–236.
122 N. S. Chandel, *Cold Spring Harbor Perspect. Biol.*, 2021, **13**, a040568.
123 M. Guo, S. Shao, D. Wang, D. Zhao and M. Wang, *Food Funct.*, 2021, **12**, 494–518.
124 X.-F. Yan, Y. Yang and D.-K. Yin, *Chin. J. Biochem. Pharm.*, 2014, **34**, 175–178.
125 C. Cao, C. K. Wong, R. P. Kuchel, S. D. Luca, J. M. Hook, C. J. Garvey, S. Smith, J. Ho and M. H. Stenzel, *Nat. Commun.*, 2019, **10**, 582.
126 Y. Zhao, P. Wan, J. Wang, P. Li, Q. Hu and R. Zhao, *Carbohydr. Polym.*, 2020, **229**, 115473.
127 D. Lin, W. Lin, G. Gao, J. Zhou, T. Chen, L. Ke, P. Rao and Q. Wang, *Int. J. Biol. Macromol.*, 2020, **159**, 850–858.

128 X. Li, *Self-assembly Effect of Angelica Sinensis Protein Andits Application*, Fuzhou University, 2018.
129 L. Wang, B. Yang, X.-P. Lin, X.-F. Zhou and Y. Liu, *Nat. Prod. Rep.*, 2013, **30**, 455–473.
130 Y. Zhuo, M. Li, Q. Jiang, H. Ke, Q. Liang, L.-F. Zeng and J. Fang, *Front. Endocrinol.*, 2022, **13**, 901545.
131 P. B. G. Bag and D. R. Majumdar, *Chem. Rec.*, 2017, **17**, 841–873.
132 K. Zhi, H. Zhao, X. Yang, H. Zhang, J. Wang, J. Wang and J. M. Regenstein, *Nanoscale*, 2018, **10**, 3639–3643.
133 X. Yang, C. Ma, Z. Chen, J. Liu, F. Liu, R. Xie, H. Zhao, G. Deng, A. T. Chen, N. Gong, L. Yao, P. Zuo, K. Zhi, J. Wang, X. Gao, J. Wang, L. Fan and J. Zhou, *Nano Res.*, 2019, **12**, 2468–2476.
134 X. Li, S. Lee and J. Yoon, *Chem. Soc. Rev.*, 2018, **47**, 1174–1188.
135 D. A. Saha, D. J. Adamcik, D. S. Bolisetty, S. Handschin and P. D. R. Mezzenga, *Angew Chem. Int. Ed. Engl.*, 2015, **54**, 5408–5412.
136 G. You, T. Feng, G. Zhang, M. Chen, F. Liu, L. Sun, M. Wang and X. Ren, *Int. J. Pharm.*, 2021, **601**, 120546.
137 O. Y. Selyutina and N. E. Polyakov, *Int. J. Pharm.*, 2019, **559**, 271–279.
138 F.-H. Yang, Q. Zhang, Q.-Y. Liang, S.-Q. Wang, B.-X. Zhao, Y.-T. Wang, Y. Cai and G.-F. Li, *Molecules*, 2015, **20**, 4337–4356.
139 Y. Li, W. Zhang, N. Shi, W. Li, J. Bi, X. Feng, N. Shi, W. Zhu and Z. Xie, *Biomater. Sci.*, 2023, **11**, 2478–2485.
140 C. Zhang, R. Zhao, W. Yan, H. Wang, M. Jia, N. Zhu, Y. Zhu, Y. Zhang, P. Wang and H. Lei, *Molecules*, 2016, **21**, 1094.
141 X. Huang, X. Liu, X. Lin, Z. Yuan, Y. Zhang, Z. Wang, W. Pi, H. Zhao, H. Lei and P. Wang, *J. Nanobiotechnol.*, 2022, **20**, 1662–1692.
142 B. Zhou, J. Zhang, S. Wu, Q. Zhuo, W. Gao, J. Hao and S. Man, *J. Ethnopharmacol.*, 2015, **169**, 1–7.
143 J.-M. Zhang, C.-M. Fu, Y.-X. He, J.-R. Lu, F. Gao, J.-S. Wang and W. Liao, *Chin. Tradit. Herb. Drugs*, 2013, **44**, 165–169.
144 L.-J. Ke, G.-Z. Gao, Y. Shen, J.-W. Zhou and P.-F. Rao, *Nanoscale Res. Lett.*, 2015, **10**, 449.
145 R. Pirlog, P. Chiroi, I. Rusu, A. M. Jurj, L. Budisan, C. Pop-Bica, C. Braicu, D. Crisan, J.-C. Sabourin and I. Berindan-Neagoe, *Int. J. Mol. Sci.*, 2022, **23**, 5346.
146 F. Bray, M. Laversanne, H. Sung, J. Ferlay and R. L. Siegel, *Ca - Cancer J. Clin.*, 2024, **74**, 229–263.
147 B. Han, R. Zheng, H. Zeng, S. Wang, K. Sun, R. Chen, L. Li, W. Wei and J. He, *J. Natl. Cancer Cent.*, 2024, **4**, 47–53.
148 A. Sancar and R. N. V. Gelde, *Science*, 2021, **371**, eabb0738.
149 B. Li, H. Shao, L. Gao, H. Li, H. Sheng and L. Zhu, *Drug Deliv.*, 2022, **29**, 2130–2161.
150 A. J. K. Phillips, A. J. Lawther and A. K. Walker, *Brain Behav. Immun.*, 2022, **100**, 172–173.
151 J. Kim, S. Lee, Y. Kim, M. Choi, I. Lee, E. Kim, C. G. Yoon, K. Pu, H. Kang and J. S. Kim, *Nat. Rev. Mater.*, 2023, **8**, 710–725.
152 Y. Hu, J. Zou, Q. Wang, Y. Chen, H. Wang and J. Li, *Eur. J. Pharm. Biopharm.*, 2024, **196**, 114184.
153 A. Mandal, R. Bisht, I. D. Rupenthal and A. K. Mitra, *J. Contr. Release*, 2017, **248**, 96–116.
154 J. Sun, Y. Wei, J. Wang, M. Hou and L. Su, *Front. Pharmacol*, 2024, **15**, 1377592.
155 Y. Liu, S. Yang, K. Wang, J. Lu, X. Bao, R. Wang, Y. Qiu, T. Wang and H. Yu, *Cell Prolif.*, 2020, **53**, e12894.
156 Q. Gao, J. Feng, W. Liu, C. Wen, Y. Wu, Q. Liao, L. Zou, X. Sui, T. Xie, J. Zhang and Y. Hu, *Adv. Drug Deliv. Rev.*, 2022, **188**, 114445.
157 R. Yin, T. Li, J. X. Tian, P. Xi and R. H. Liu, *Crit. Rev. Food Sci. Nutr.*, 2017, **58**, 568–574.
158 G.-H. Kim, S.-Y. Kan, H. Kang, S. Lee, H. M. Ko, J. H. Kim and J.-H. Lim, *Int. J. Mol. Sci.*, 2019, **20**, 4767.
159 J.-L. Zheng, S.-S. Wang, K.-P. Shen, L. Chen, X. Peng, J.-F. Chen, H.-M. An and B. Hu, *BMC Complementary Med. Ther.*, 2021, **21**, 52.
160 F. Xu, M. Li, Z. Que, M. Su, W. Yao, Y. Zhang, B. Luo, Y. Li, Z. Zhang and J. Tian, *J. Mater. Chem. B*, 2023, **11**, 3453–3472.
161 L.-W. Tong, J.-Q. Le, X.-H. Song, C.-L. Li, S.-J. Yu, Y.-Q. Lin, Y.-F. Tu and J.-W. Shao, *Colloids Surf. B Biointerfaces*, 2024, **234**, 113724.
162 J. Chen, Z.-y. Ding, S. Li, S. Liu, C. Xiao, Z. Li, B.-x. Zhang, X.-p. Chen and X. Yang, *Theranostics*, 2021, **11**, 1345–1363.
163 A. E. Pomeroy, E. V. Schmidt, P. K. Sorger and A. C. Palmer, *Trends Cancer*, 2022, **8**, 915–929.
164 Y. Cheng and Y. Ji, *J. Contr. Release*, 2020, **318**, 38–49.
165 Y. Zheng, Z. Li, Y. Yang, H. Shi, H. Chen and Y. Gao, *Phytomedicine*, 2021, **93**, 153788.
166 M. M. S. Lee, L. Zheng, B. Yu, W. Xu, R. T. K. Kwok, J. W. Y. Lam, F. Xu, D. Wang and B. Z. Tang, *Mater. Chem. Front.*, 2019, **3**, 1454–1461.
167 L. Zou, X. Liu, J. Li, W. Li, L. Zhang, C. Fu, J. Zhang and Z. Gu, *Theranostics*, 2021, **11**, 4171–4186.
168 M. M. S. Lee, E. Y. Yu, J. H. C. Chau, J. W. Y. Lam, R. T. K. Kwok, D. Wang and B. Z. Tang, *Biomaterials*, 2022, **288**, 121712.
169 J. Liu, *Mechanistic Insight into the Interaction of Gastrointestinal Mucus with Oral Administration of Nanopolymeric Carriers*, Qingdao university, 2018.