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Advances in epitope mapping technologies for food protein allergens: a review

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24 **Abstract**

25 *Background:* The incidence of food allergy has been increasing in recent years, but the
26 prevention, diagnosis, and treatment of food allergy remain challenges in the world. Food
27 allergen epitope mapping technology can help us identify the epitope sequence and structure,
28 which is conducive to the development of food allergy prevention, diagnosis, and treatment
29 methods. Moreover, the information about allergic epitopes also plays a positive role in the
30 development of new hypoallergenic or non-allergenic food products in the industry.

31 *Scope and approach:* This article systematically reviews state-of-art research progress and
32 development trend of food epitope mapping technology based on the advantages and
33 disadvantages as well as the potentials of each method. The future development direction and
34 trend of food protein allergen epitope mapping technology were also discussed, with a view to
35 providing a reference for the identification and characterization of food allergens.

36 *Key findings and conclusions:* Compared with traditional technologies including peptide
37 scanning technique, novel technologies have the advantages of high specificity, high
38 sensitivity, high accuracy, and high-throughput. Although some progress has been made in the
39 identification and characterization of food protein allergen epitopes, a large number of
40 epitopes have not yet been identified so far. Some work is still needed for invention of new
41 epitope mapping technology, establishment of systematic and comprehensive databases, and
42 development of allergic omics in the future.

43

44 **Keywords:** Food allergy, Protein, Allergic epitope, Identification technology, Epitope
45 mapping

46

47 **Abbreviation comparison list**

Abbreviation	Full name
APC	Antigen-presenting cell
MHC	Major histocompatibility complex
ELISA	Enzyme linked immunosorbent assay
IgE	Immunoglobulin E
WHO	World Health Organization
IUIS	International Union of Immunological Societies
NCBI	National Center for Biotechnology Information
SDAP	Structural Database of Allergenic Proteins
OAS	Oral allergy syndrome
SYS	Systemic symptoms
NMR	Nuclear magnetic resonance
SPR	Surface plasmon resonance
MS	Mass spectrometry
MALDI-TOF-MS	Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry

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1. Introduction

Food allergy refers to the adverse health effects caused by the immune response for susceptible individuals who are repeatedly exposed to a food allergen (Ho, Wong, & Chang, 2012; Sicherer & Sampson, 2018). As the incidence increases, food allergy has been a global public health issue (Lin & Alcocer, 2017), and nearly 5% of adults and 8% of children all over the world in 2013 were affected (Sicherer & Sampson, 2014), and the recorded incidence of food allergy rose to approximately 10% in 2017 (Sicherer & Sampson, 2018). So far, 370 food allergens from 125 species have been designated by the World Health Organization (WHO) and International Union of Immunological Societies (IUIS) allergen nomenclature subcommittee, and over 50% of species belong to eggs, milk, tree nuts, wheat, crustacean, beans, fish and peanuts (<http://www.allergen.org>, accessed 21 July 2020), which can cause adverse allergic reactions and symptoms, such as asthma, skin irritation, gastrointestinal tract discomfort, and even death. However, no routine and effective immunotherapy for food allergy has been established so far, then avoiding exposure to allergens will be the best precaution for allergic individuals to prevent allergic reactions (Ahsan, Rao, Gruppuso, Ramratnam, & Salomon, 2015; Liu & Krishna, 2018).

Most food allergens are proteins, and during the digestion, the especial amino acids named epitopes on the allergens would be exposed in the intestinal tract, and subsequently, enter the mucosal immune system, bind the immune cells, such as antigen-presenting cells (APCs) and B cell, to trigger a series of immune processes, such as the generation of IgE antibodies and the release of inflammation mediators, then IgE-mediated type I allergic response will occur when the body is exposed to the same food allergen for the second time (Tordesillas, Berin, & Sampson, 2017; Liu & Krishna, 2018; Wai, Leung, Leung, & Chu, 2017). Since there is no definite mechanism of food allergy, the determination of the epitope structure can provide the possibility, and it is also important for establishing effective methods for the prevention, diagnosis, and treatment of food allergy. To be specific, cross-reactivity of potential allergens can be computationally evaluated by comparing the sequence similarity of peptide fragments to the known IgE-binding epitopes (Nugraha, et al., 2019), which is promising to identify more unknown allergenic proteins and help allergic patients prevent

food allergies; besides, epitopes can also be used as biomarkers to increase the specificity of food allergen diagnosis; moreover, immunotherapy can be designed on the basis of the information of epitopes to restore the immune tolerance of allergic patients (Wai, Leung, Leung, & Chu, 2017). Furthermore, during the production of industrialized food, different processing procedures will result in changes in physicochemical properties and structure of food proteins and thereby leading to the alterations of allergenicity in food raw materials. It has been reported that the conformational allergenic epitopes and allergenicity would be changed with the destruction of the structure and conformation of food proteins after the physical processing methods treatments, such as heat treatment, high pressure, radiation, and ultrasound, while linear epitopes might be degraded into shorter fragments by microbial fermentation and enzymatic hydrolysis, and which might lead to the reduction of allergenicity (Rahaman, Vasiljevic, & Ramchandran, 2016). Thus, appropriate food processing methods and reasonable processing parameters will be to the benefit of hypoallergenic food manufacturers in the food industry based on the protein epitope changes.

Therefore, the epitope mapping of food allergens has been popular in recent years. According to the published literatures, epitope mapping methods are mainly divided into four types as follows: 1) methods relying on binding peptides to serum, including peptide scanning technique, phage display technique, peptide microarray, and so on; 2) the binding site information obtained by direct analysis on the structure of the antigen and antibody complex, including X-ray diffraction analysis and nuclear magnetic resonance (NMR); 3) computer-based prediction methods, such as bioinformatics; 4). biomacromolecule interaction analysis for the specific antigen-antibody binding, such as surface plasmon resonance (SPR). In order to provide theoretical reference for the identification and characterization of food allergen epitopes as well as the diagnosis and treatment of allergic diseases, this paper reviews the advantages and disadvantages of traditional and recent methods for the food allergen epitope identification, as well as raise our perspectives on the future development for the epitope identification methods.

2. Brief introduction of epitope

Epitope, also known as the allergic determinant, is the special chemical group on the

surface of an allergen that can specifically bind to the antibody or antigen receptors, such as T cell receptors (Liu & Krishna, 2018). Epitope usually consists of 8-26 amino acids (Liu & Krishna, 2018), and it can be divided into linear epitope and conformation epitope. The linear epitope is composed of continuous amino acids and is recognized by its primary structure, while the conformational epitope consists of spatially adjacent, discontinuous amino acid residues and is related to the three-dimensional structure of proteins.

In adaptive immune, epitopes can be divided into T-cell epitope and B-cell epitope, which can be recognized by the receptor on the surface of T cells or B cells, respectively, and will be involved in the humoral and cellular immunity regulations (Raza, et al., 2019). To be specific, B-cell epitopes are mostly conformational epitopes, while all T-cell epitopes are linear epitopes. T-cell epitopes can be located anywhere in an allergen (Dall'Antonia, Pavkov-Keller, Zangger, & Keller, 2014). It should be noted that T-cell epitopes buried within an allergen may be exposed and then recognized by antibodies when the allergen is denatured. For the conformational B-cell epitope, the three-dimensional structure would be the basis for the specific binding with antibody, therefore, the denaturation of the allergen would result in the connection failure between antigens and antibodies. Moreover, B-cell epitope and T-cell epitope are different in structure, sensitization mechanism, immunotherapeutic use, etc.

2.1 T-cell epitope

T-cell epitopes are short fragments of allergens which consist of 12-20 contiguous amino acids (Ahmad, Eweida, & El-Sayed, 2016). Due to the lack of secondary or tertiary protein structure, T-cell epitopes cannot be cross-linked to IgE antibodies which bind to mast cell receptors (Bohle, 2006). As some T cell peptide epitopes cannot be destroyed by the intracellular proteases, the small peptide fragments might combine with major histocompatibility complex (MHC) II molecules, and the formation of T-cell-epitope-MHC II molecular complex will be expressed on the surface of APCs and then will be recognized by T cell receptor on Th2 cells to produce interleukins, such as IL-4, IL-5, and IL-13 (Fig. 1). Then it is possible that T-cell epitopes could be used to induce immune tolerance. In addition, T-cell epitopes can also regulate immunity by inhibiting Th2 cells and inducing regulatory T cells. These properties make T-cell epitopes a safe and effective immunological modulator which is

suitable to apply to oral immunotherapy. For example, T-cell-epitope-based oral immunotherapy has been reported to be used in the palliative care for shrimp tropomyosin and hen egg ovalbumin allergies in mice by acquiring corresponding immunodominant T cell peptides, respectively (Rupa & Mine, 2012; Wai, Leung, Leung, & Chu, 2016).

2.2 B-cell epitope

B-cell epitopes of most food allergens are polypeptides, which can be linear polypeptides or discontinuous polypeptides with a certain three-dimensional structure. Compared with T-cell epitopes, B-cell epitopes do not need APCs' procession and presentation and can be directly recognized by B cell surface receptors (Fig. 1). In addition, B-cell epitopes on food allergens can specifically link to the variable region of antibodies by non-covalent bonding (Liu & Krishna, 2018). Moreover, B-cell epitopes can be used in the design of immunotherapy for food allergy, such as the genetic engineering strategy based on the modification of B-cell epitopes. For example, in the immunotherapy for peanut allergens, four point-mutations were introduced in B-cell epitopes of major peanut allergen Ara h 3 by amino acid directed mutation, then the binding affinity to patient's IgE antibodies was reduced because of the destruction of B-cell epitope, but the ability to stimulating the proliferation and activation of T cells by T-cell epitopes still retained (Rabjohn, et al., 2002).

The identification and mapping of epitopes is the first step to explore the food allergy mechanism and develop new hypoallergenic or non-allergenic food products during processing. In order to identify the amino acid sequence and structure of epitopes, a few technologies have been developed, such as enzymatic hydrolysis, peptide scanning technique, phage display technique, mass spectrometry (MS), NMR, X-ray diffraction analysis, SPR, peptide microarray, and bioinformatics. As these technologies are applying to food allergen, more and more food allergen epitopes have been mapped, and we have a more thorough and comprehensive understanding of food allergen epitopes.

3. Traditional identification strategies & limitations

3.1 Enzymatic-hydrolysis-based assay

Various proteases can digest food allergens into a lot of small peptides, and the

sensitization of each peptide is tested by serum binding assays to determine immunodominant epitopes of food allergens. Pepsin, trypsin, and chymotrypsin are the commonly used proteases. Although the method is simple to operate, the proteolytic sites are random and the specificity is low. In recent years, only a few studies on the mapping of food antigen epitope were reported to use the enzymatic hydrolysis method alone, and most researches have been combined the enzymatic hydrolysis technology with advanced technologies, such as MS, to study the anti-digestive epitope of food allergens. For example, Di Stasio et al. (2017) obtained digestive peanut peptide fragments were obtained in the process of simulating pepsin and trypsin digestion *in vitro*, and dot blot technique was exploited to study the IgE binding ability of peptide fragments, and then digestion resistant epitopes of peanut were characterized by high-resolution liquid secondary MS.

In addition, the sensitization mechanism of conformational epitopes of food allergens can be also studied by enzymatic hydrolysis. Since the three-dimensional structure of food allergens can be destroyed after the digestion of conformational epitopes in the gastrointestinal tract, the digestive stability of conformational epitopes produced by enzymatic hydrolysis has been investigated. For example, Apostolovic et al. (2016) obtained peanut lectin resistant peptides by using the pepsin digestion to peanut lectin in an acidic environment, and confirming that the binding of peanut lectin to IgE mainly depended on its conformational epitopes by the investigation of the secondary structure and IgE binding ability of natural peanut lectin and digestion-resistant peptides, respectively, via circular dichroism spectroscopy and immunoblotting methods.

3.2 Peptide scanning technique

Peptide scanning technology is one of the most commonly used methods for identifying food allergen epitopes. Several overlapping peptides synthesized by fluoromethoxycarbonyl (Fmoc) solid phase synthetic peptide method will be determined, and the binding affinity of these peptides to antibodies was analyzed by the usage of testes such as enzyme-linked immunosorbent assay(ELISA), western blot, and dot blot. The overlapping peptides of allergens can usually represent the entire allergen amino acid sequence with each peptide length of 10-15 amino acids, and piperidine will be added to remove the Fmoc protecting

group to make the nascent peptide active after coupling and acetylation (Wołczański & Lisowski, 2018). Finally, the cellulose membranes containing synthetic peptides will be used to explore epitopes by serum immunoglobulin detection.

Zhang, Li, Xiao, Nowak-Węgrzyn, and Zhou (2019) obtained amino acid sequence of prawn tropomyosin allergen Exo m 1 by the recombinant protein sequencing, and 46 small peptides with 15 amino acids in length and offset 6 amino acids were synthesized for the IgE epitope peptides detection by using the dot blot inhibition experiment, thereby 5 epitopes of Exo m 1 were identified by this strategy. In order to characterize the epitopes of egg ovomucoid, Mine and Zhang (2002) synthesized 35 overlapping small peptides and overlapped 1 amino acid on the membrane, and 8 ovomucoid IgE epitopes were finally identified by the SPOTs assay to detect the binding to serum.

In addition, peptide scanning technology can also be used for cross-reactivity between different allergens. In order to study the cross-reactivity between peanut lectin PNA and other legume allergens, Rougé, Culerrier, Granier, Rancé, and Barre (2010) synthesized all peptide fragments of peanut lectin PNA, lentil allergens LcA and pea allergen PsA with 15 amino acids in length and overlapped 5 amino acids, then ELISA test was employed to locate the binding area to patient serum IgE with peanut allergen, and amino acid sequence similarity in the IgE binding region of lentil and pea epitopes was detected to explain the cross-reactivity between these allergens.

Although peptide scanning technology can effectively identify some unique, immunodominant epitopes of food allergens, this technology has a few limitations. First, the maximum length of overlapping synthetic peptides might be only 15 amino acids (Steckelbroeck, Ballmer-Weber, & Vieths, 2008), which may be too short compared to an allergen with dozens of overlapping peptides. Second, the process of synthesizing peptide is tedious, and the workload is large and error-prone, besides, the experiment requires a large amount of serum. Last but not least, when different researchers study the same allergen, different lengths of overlapping peptides and offset of the overlapping area may lead to different immunodominant epitopes (Steckelbroeck, Ballmer-Weber, & Vieths, 2008).

Peptide scanning technique has been used for a long time to map linear epitopes of food

allergens, but it cannot be applied to identify conformational epitopes. Since over 90% B-cell epitopes are conformational (Barlow, Edwards, & Thornton, 1986; Pacios, et al., 2011), the peptide scanning technique cannot meet the demand of identifying all epitopes of food allergens. With the development of molecular biology and immunology, methods of epitope mapping are becoming increasingly diverse, and recent methods with high-throughput and high efficiency can overcome the shortcomings of traditional methods to some extent.

4. Recent technologies for the food allergen epitope identification

4.1 Phage display technique

Phage display technology was first proposed by American scientist George P Smith (Smith, 1985) in 1985, and the basic principle is to insert a foreign gene on the structural gene of the bacteriophage virus to identify food allergen epitopes, then the antigen and antibody polypeptide or protein encoded by the foreign gene will be displayed as a fusion protein on the surface of the bacteriophages. Since a variety of foreign genes are randomly inserted into a group of phages, these phage particles will constitute a huge peptide library as the peptides displayed on the surface of bacteriophage still have biological functions and spatial structure, and the specific interaction between the antigen and antibody displayed by phages can be used to screen the epitopes of allergens (Rowley, O'Connor, & Wijeyewickrema, 2004) (Fig. 2).

So far, M13 filamentous bacteriophage, as well as T4, T7, P4, and lambda bacteriophage has been applied to phage display technology (Lenka, Mangesh, & Borszekova, 2016). Among them, M13 filamentous bacteriophage and T7 bacteriophage are commonly applied in phage display technology and are suitable for both peptide and protein displaying (Chen & Dreskin, 2017). The difference between them is that M13 filamentous bacteriophage causes bacterial cells not to lyse when infected with *E. coli*, while bacterial cells can be destroyed by T7 bacteriophage (Chen & Dreskin, 2017). In terms of food allergen epitopes mapping, M13 is the most commonly used bacteriophage. Since the length of the linear epitope was generally believed as 5-7 amino acids previously, a 6-peptide library was applied in the first-generation phage display peptide library. Currently, the commonly used phage peptide libraries for food allergen epitope mapping include 7-peptide libraries, 9-peptide libraries,

10-peptide libraries, and 12-peptide libraries because longer peptide may enhance the interaction affinity and increase the ability to detect important conformational epitopes that may be of lower affinity (Chen & Dreskin, 2017). In addition to phages, bacteria and yeasts can also be used for peptide and protein surface display. Bacterial surface display technology is mainly applied to display some hydrolytic enzymes and proteins with simple structures, and it is difficult to display complex proteins with folded structures such as food allergens. Yeast display technology can be used to identify epitopes of some bacteria (Van Blarcom, et al., 2018; Najjar, Khare, & Varadarajan, 2018), and rarely used in the identification of food allergen epitopes. Thus, phage-based display technology is still the preferred one for identifying food allergens epitopes. In recent years, researchers have developed computer simulation epitope mapping tools based on phage peptide library information, such as FINDMAP, SiteLight, 3DEX, Mapitope, and MIMOX (Huang, Gutteridge, Honda, & Kanehisa, 2006), so epitope mapping will be more rapid and convenient.

There are a few recent researches reported on food allergen epitope mapping using phage display. Taking *Procambarus clarkii* allergen MLC1 as an example, Yang et al. (2020) used three approaches to identify the IgE-binding epitopes of MLC1: i) screening 12-peptide phage display library with purified rabbit anti-MLC1 polyclonal antibody by three rounds of affinity enrichment biopanning, which showed that 9 out of 16 positive phages were preliminarily identified as MLC1 mimotopes, ii) employing the LocaPep program to further identify 5 conformational epitopes located at 3 structure conserved regions of MLC1, indicating that MLC1 of crayfish had the potential for cross-reactivity with other related species, iii) using western blot to confirm the cross-reactivity of crayfish with related species, such as North Sea shrimp, white Pacific shrimp, and so on. Peach allergens, Pru p3, is a lipid transport protein and the main allergen of peaches. Pacios et al. (2008) purified two different Pru p 3-specific IgEs from the serum from patients with OAS (Oral allergy syndrome) and SYS (Systemic symptoms) and employed them to screen the 12-peptide phage display library. After 3 rounds of biopanning, 9 positive phages were selected with IgEs from patients with OAS and 12 positive phages were chosen by using IgEs from patients with SYS, and then two conformational epitopes of Pru p 3 were mapped by DNA sequencing and peptide sequence

alignment. Bøgh et al. (2012) used the 7-peptide phage display library to identify five conformational epitopes of peanut allergen Ara h1.

In the previous study for β -lactoglobulin epitopes of milk allergen, Williams, Badley, Davis, Puijk, and Meloen (1998) compared the peptide scanning method with the phage random 9-peptide library. The former identified 9 linear epitopes and the latter identified 4 epitopes, suggesting that there were differences in the epitopes identified by these two methods, and phage display technology might miss some epitopes recognized by the peptide scanning method. However, compared with peptide scanning technology, phage display technology has the advantages of small workload, technical simplicity (Chen & Dreskin, 2017), large peptide library, high screening efficiency, high-throughput (Wu, Liu, Lu, & Wu, 2016), and low cost (Pande, Szewczyk, & Grover, 2010). Moreover, phage display technology has the ability to identify key amino acids on allergens which are critical for antibody binding (Rowley, O'Connor, & Wijeyewickrema, 2004). In addition, phage display technology can recognize both linear epitopes and conformational epitopes. Finally, it is not necessary to know the structure of peptides before using this technology, and it can be used to recognize the epitopes of multiple antigens (Rowley, O'Connor, & Wijeyewickrema, 2004). In general, phage gene-specific peptide libraries and random peptide libraries are powerful technical means for identifying epitopes.

4.2 Mass-spectrometry-based strategy

Mass spectrometry (MS) is one of the most important technologies for studying proteomics on allergen. Modern mass spectrometers are mainly composed of ion sources, mass analyzers, and detectors. The principle of MS is that the ionized sample molecules lose electrons and become fragmented ions or particles of small molecules. After accelerating in the electric field and entering into the mass analyzer, fragmented ions or particles with different mass-to-charge ratios (m/z) will be separated. The ion current entering the detector is converted into electrical signals of different intensities. Then, the computer converts the mass-to-charge ratio data of the analyzer and the electrical signal data of the detector into a mass spectrum. In addition, the mass spectrometer requires a device to introduce the sample directly or through a separation device, such as gas chromatography, liquid chromatography,

and capillary electrophoresis. Moreover, the single-stage mass spectrometer can only be used to simply measure the molecular mass of the peptide, while the tandem mass spectrometer can be used to infer the detailed structural characteristics of the peptide, such as amino acid sequence, connection site, and the type of posttranslational modifications (Domon & Aebersold, 2006).

Among the MS techniques, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization mass spectrometry are the most widely used for epitope mapping (Hager-Braun & Tomer, 2005). When identifying epitopes, MS needs to be combined with other technologies, such as enzymatic hydrolysis and hydrogen-deuterium exchange (Ahmad, Eweida, & Sheweita, 2016). The experimental approach for the epitope extraction and excision process designed for MS analysis is shown in Fig. 3.

Jankovicova et al. (2008) designed a microfluidic device to extract food allergen epitopes and used MALDI-TOF-MS to characterize the peptide epitopes, which has successfully identified the allergenic epitopes of egg allergen ovalbumin and can be a new strategy for the identification and verification of epitopes. In order to characterize the epitope of the almond protein allergen Pru du 6, Willison et al. (2013) used Fourier transform ion cyclotron resonance MS to monitor hydrogen/deuterium exchange of Pru du 6-antibody complexes and uncomplexed allergens, respectively, and accordingly, several epitope candidate peptides that had differences in deuterium absorption between complexed and uncomplexed allergens were identified, and then conformational epitopes of Pru du 6 were further positioned in three discrete peptide chains by analyzing chimeric molecules substituted Pru du 6 fragments with homologous fragments from soybean allergens Gly m 6 in immunoassays.

In recent years, MS has become a powerful tool for epitope mapping due to its high sensitivity, high accuracy, high-throughput, and short analysis time (Lu, DeFelippis, & Huang, 2009). In addition, the advantage of MS is that it can clearly identify allergens sequences, quantitatively analyze many different allergen proteins at one time with high analysis reliability. However, it is worth noting that food contains a lot of fats, sugars, and other

substances that interfere with MS analysis, so allergens should be purified before applied to MS. Besides, MS requires expensive instruments and skilled operators (Marzano et al., 2020), which is hard to be employed in all laboratories.

4.3 Nuclear-magnetic-resonance-based strategy

To date, nuclear magnetic resonance (NMR) technology has been widely used in analyzing the structure of proteins. When a protein sample is placed in a strong magnetic field, its nucleus will resonate with the applied electromagnetic wave and absorb electromagnetic radiation at the same time. The absorption frequency or resonance position can be detected as chemical shifts which provide information about molecular covalent and non-covalent structures. Besides, splitting peaks generated by the magnetic coupling between adjacent nuclei or covalently bound nuclei can also be used to infer the covalent geometry of the molecule (Wishart, 2005). In addition, NMR experiments provide direct information on binding sites and the structure of protein-protein interactions. Generally, NMR is combined with isotope labeling, hydrogen/deuterium exchange, or saturation transfer experiments to obtain the information on the antigen-antibody binding sites (Dall'Antonia, Pavkov-Keller, Zangger, & Keller, 2014). For example, in order to study the molecular basis of cross-reactivity of fish allergen parvalbumins and identify their IgE-binding epitopes, Bublin et al. (2015) used three approaches: i) applying phage library technology to display the cross-reactive single-chain variable fragment (scFv-gco9) antibodies which can bind with all three fish allergens, cod (Gad m 1), carp (Cyp c 1) and rainbow trout (Onc m 1) parvalbumins, ii) labeling Gad m 1 and scFv-gco9 with a ^{15}N - ^{13}C isotope, iii) using ^1H / ^{15}N heteronuclear single quantum correlation NMR technology to locate the binding site of scFv-gco9 and Gad m 1, and finally, it was found that the conserved amino acid residues in the three fish allergens parvalbumins participated in the cross-reactivity. As another example, Neudecker et al. (2001) applied NMR techniques to study the structure of the purified cherry allergen Pru av 1, and input structural information into molecular dynamics simulation software to obtain the high-resolution three-dimensional structure of Pru av 1. By comparing the sequence similarity and molecular structure of Pru av 1 with birch pollen allergen Bet v 1 in detail and using immunohibition tests, the cross-allergenic epitopes of Pru av 1 and Bet v 1 were

successfully identified, so as to provide a theoretical basis for the detection of cross-reactivity of cherry allergen and birch pollen allergen.

As a tool for studying the molecular structure of compounds, NMR technology can provide rich structural information of proteins, and its frequency resolution is high. However, NMR technology also has some disadvantages. First of all, NMR technology has low sensitivity. Second, NMR is only suitable for continuous small molecular weight (< 30 kDa) proteins with high purity and good water solubility (Wüthrich, 1991). On the contrast, macromolecular proteins with low solubility in water tend to aggregate into complexes or precipitates, which interfere with the collection of NMR spectra (Kustanovich & Zvi, 1996). In addition, this technical instrument is expensive and needs skilled experimenters to operate. Moreover, NMR results sometimes were found to contradict the corresponding X-ray results (Ahmad, Eweida, & Sheweita, 2016).

4.4 X-ray diffraction analysis

X-ray is an electromagnetic wave with a wavelength of about 0.1 nanometer, and its length is equivalent to the interatomic distance of a compound (Iwamoto, 2019). When striking an atom, the X-rays are scattered by the electrons in the atom. Since scattered waves are emitted by atoms at different positions, the interference of the constructive or destructive waves occurs along different directions (Ameh, 2019), thereby generating strong X-ray diffraction in certain directions. Then the position of the diffraction line will reflect the shape and size of the object, and the intensity of the diffraction line will be related to the position and the atomic number of the atom (Ameh, 2019), thereby, the structure of molecules or atoms and their changes over time can be analyzed by X-rays.

Antigen-antibody complex co-crystallization is one of the earliest techniques used for epitope mapping (Ahmad, Eweida, & Sheweita, 2016). Antigen-antibody complex molecules can form stable complexes in the form of crystals. Then, the atomic structure of the complex could be analyzed by X-ray diffraction, and the antigen and antibody binding sites would be obtained by resolving the amino acid electron density in three-dimensional coordinates (Braden & Poljak, 1995). Recently, X-ray crystallography data has shown that the known 15-22 amino-acid-long epitope had a higher affinity to antibody complementarity-determining

regions, while the 7-12 aa long residues known as B cell epitopes might bind to antibody paratope with a very low affinity (Rahman, Chowdhury, Sachse, & Kaltenboeck, 2016; Parvizpour, Pourseif, Razmara, Rafi, & Omid, 2020).

Niemi et al. (2007) used X-ray diffraction technology to analyze the co-crystal structure and the binding sites of the complex formed by bovine milk allergen β -lactoglobulin and IgE antibody variable region Fab, which indicated that the epitope of β -lactoglobulin was composed of six different short amino acid fragments of the polypeptide chain and covered the flat area of the allergen surface, and all six complementarity determining regions of the Fab fragment were involved in antigen binding. The use of variable region Fab fragments to form antigen-antibody complexes not only improves the crystallinity of the allergen but also provides accurate information on the binding site (Bailey, et al., 2017; Ereño-Orbea, et al., 2018).

In conclusion, X-ray diffraction technology can explore the three-dimensional structure of the antigen-antibody complex obtained by co-crystallization, provide detailed information on the antigen-antibody binding site, study conformational epitopes, and detect continuous linear epitopes. However, this technology requires expensive equipment and has particularly high requirements on allergen samples, and a large number of highly purified antibodies-allergen complexes and structures of antibodies must be completely identified (Ahmad, Eweida, & Sheweita, 2016). In addition, this technique requires that the antigen-antibody complex must be crystallizable, even though it could not guarantee to obtain good diffraction crystals (Dall'Antonia, Pavkov-Keller, Zangger, & Keller, 2014). Therefore, this technique is not suitable for most antigens. In recent years, the resolution of the electron microscope has been improved, and X-ray crystallography can be combined with an electron microscope to study the structure of the antigen-antibody complex. This new combined technology can obtain the 'phase' information which is omitted from X-ray crystallography technologies (Jackson, McCoy, Terwilliger, Read, & Wiedenheft, 2015), which can promote the use of X-ray diffraction technology in allergen epitope mapping to some extent.

4.5 Surface-plasmon-resonance-based strategy

Surface plasmon resonance (SPR) is an optical physics phenomenon. When the incident

light enters the interface of two materials with opposite dielectric constants (such as glass and metal) at a critical angle, the light wave interacts with the metal and causes the metal to resonate (Fig. 4a). When the biological macromolecules fixed on the metal surface are combined with other substances, the mass of metal surface increases, and the refractive index changes, which causes the resonance angle to change. By detecting the degree of resonance angle change, the reaction between biological macromolecules can be observed (Fig. 4b, c).

SPR is a good method for analyzing the interaction between proteins and proteins with low-affinity, as well as for real-time monitoring of the reaction between food allergens and antibodies (Ahmad, Eweida, & Sheweita, 2016; Mariani & Minunni, 2014; Šípová & Homola, 2013; Zhou, et al., 2019). SPR biosensors have been currently used to detect the existence of allergens in many food products, such as milk products, peanut products, seafood, and eggs (Zhou, et al., 2019). In addition, SPR experiments have been tried the potentials to identify food allergen epitopes because they can detect the degree of binding affinity between antigen and antibody. For example, in order to characterize the cross-reactive epitopes of peanut lectin and other legume lectins, Rougé, Culerrier, Granier, Rancé, and Barre (2010) used immunoblotting, SPR sensor, and ELISA to evaluate the strength of the interaction between peanut lectin and other legume lectins. Curciarello et al. (2014) used peptide scanning, bioinformatics, and SPR to identify the cross-reactive epitopes of milk and soybean allergens. Shen et al. (2018) developed a new SPR sensor and imaging system for two peptide epitopes of peanut allergen Ara h2, which showed changes of the surface plasmon signal intensity when the fixed Ara h2 combined with the added IgE at different concentrations, and thus, the sensitization risk of two epitopes could be assessed by observing the changing trend, i.e., the binding affinity of these two epitopes of peanut allergen.

To sum up, SPR technology can qualitatively and quantitatively study antigen-antibody interactions (Iraola, Gallego, Taulés, & Carnés, 2013; Zhou, et al., 2019), with advantages including high sensitivity, high selectivity (Zhou, et al., 2019), and real-time detection of reactions (Säfssten, 2009). Besides, this technology does not need to label samples. Different from the traditional SPR equipment that is bulky and expensive and is not suitable for primary laboratories, the newly developed, surface plasmon imaging biosensor technology combined

with local SPR and transmission SPR can provide an accurate, fast, and low-cost method to study allergens (Zhou, et al., 2019).

4.6 Peptide microarray

In recent years, with the widespread use of DNA microarray technology and the rapid development of proteomics, protein microarray technology has played an increasingly important role in the field of natural science. Protein microarray technology has been developed to possess high-throughput, high selectivity, high sensitivity (Qi, Wang, & Tao, 2019), and has the ability to quantitatively detect thousands of samples at the same time. Therefore, it has been used in drug target screening (Zhou, Yang, Li, & Zhu, 2018), psychoneuroimmunology (Ayling, et al., 2017), autoantibody detection (Atak, et al., 2016), the characterization of protein-nucleic acid interactions (Moore, Ajala, & Zhu, 2016), environmental/food safety analysis (Hu, Xie, Qian, Blackshaw, & Zhu, 2011), and so on. However, protein microarray has a few drawbacks to perform. For instance, when the protein is combined on the chip, it needs to maintain its folded state and structural integrity. Then, peptide microarray can make up for the deficiency of protein microarray method and may be a good substitute for protein microarray (Villiers, et al., 2010). There have been some cases that used peptide microarray technology to identify epitopes, due to its simple probe structure, high stability, and easy preparation (Villiers, et al., 2010). The preparation of peptide microarray includes three main steps, namely the construction of solid chip, probe immobilization, and detection of molecular interaction. A peptide microarray model and the schematic of peptide microarray construction to be used in food allergenic sequences identification are shown in Fig. 5.

Vereda et al. (2010) synthesized 135 peptide fragments corresponding to the main sequence of lentil allergen Len c1 and tested the serum binding ability of these peptide fragments by means of microarray immunoassay, which showed that different peptides had different serum binding affinity, and thus, the four IgE-binding epitopes of lentil allergens were characterized and these epitopes are located in the C-terminal of the protein. As another example, in order to develop a peptide microarray-based method for large-scale epitope mapping of food allergens, Lin et al. (2009) synthesized overlapping peptides (20-mers, 3

offset) covering the primary sequences of five milk allergens, i.e. as1-casein, as2-casein, β -casein, κ -casein, and β -lactoglobulin and evaluated their IgE binding ability, and thus, the peptide microarray-based method successfully identified epitopes of these five milk allergens that were basically in agreement with epitopes identified by SPOT membrane method.

Peptide microarray technology has advantages including high-throughput, good specificity, and high sensitivity, and thus it can detect thousands of target peptides at the same time using a small amount of diluted serum so that it greatly improves work efficiency and provides a reliable statistical method for analysis and determination of epitopes (Lin, et al., 2009). However, there are still some deficiencies in this technique, for example, it can only be used to detect linear epitopes, but not conformational epitopes (Hecker, et al., 2012). Besides, this method needs many synthesized peptide fragments, which is costly and tedious (Wang, Wang, Liu, & Fu, 2019).

4.7 Bioinformatics-based assay

With the development of computer technology and bioinformatics, researchers have developed new types of software to predict antigen epitopes and to study the distribution and structure of epitopes by constructing a three-dimensional allergen model. Since the 1980s, a lot of allergen proteins have been identified and characterized so that enough allergen data can be collected to establish various allergen databases with different types and functions (Radauer & Breiteneder, 2019). For example, the WHO/IUIS Allergen Nomenclature database can provide systematic and comprehensive nomenclatures of allergen proteins. The National Center for Biotechnology Information (NCBI) database collects many protein sequences of allergen, and the Structural Database of Allergenic Proteins (SDAP) can provide data such as allergen sequence, epitope, structure, and homology model (Radauer & Breiteneder, 2019). Table 1 lists the common online servers, software, algorithms, and other tools that predict allergen continuous and discontinuous epitopes.

In addition to predicting epitopes of allergens, bioinformatics can also be used to predict cross-reactivity between similar allergens through known epitopes information as typically higher sequence and structural identity can be observed in the cross-reactive allergenic proteins (Nugraha, et al., 2019). Based on this principle, Ivanciuc et al. (2009) developed a

computational strategy named the property distance (PD) value to predict cross-reactivity in the SDAP database (<http://fermi.utmb.edu/SDAP/>, accessed 19 October 2020). Users of SDAP can apply PD search to compare the physicochemical properties of short peptides to those of available IgE epitopes in the SDAP database and deduce potential cross-reacting allergens. In order to assess the cross-reactivity between distantly related allergens, Negi and Braun (2017) developed a structural bioinformatics tool, Cross-React (<http://curie.utmb.edu/Cross-React.html>, accessed 19 October 2020) by comparing the structural similarity of query allergen with the known allergens and the surface exposed residues between query and predicted epitope.

Bioinformatics is a low-cost, high-throughput method to predict the potential epitopes of allergens. The prediction of discontinuous B-cell epitopes mainly depends on the three-dimensional structure of the antigen, while the prediction of continuous B-cell epitopes and T-cell epitopes is mainly based on the amino acid properties, such as hydrophilicity, charge, exposed surface area, and protein secondary structure (Soria-Guerra, Nieto-Gomez, Govea-Alonso, & Rosales-Mendoza, 2015). For example, Zheng, Lin, Pawar, Li, and Li (2011) used DNASTar, Bioinformatics Predicted Antigenic Peptides website, and BepiPred 1.0 server to predict linear epitopes of shrimp allergen myosin. Results showed that DNASTar predicted 9 epitopes, Bioinformatics Predicted Antigenic Peptides website predicted 7 epitopes, and BepiPred 1.0 server predicted 11 epitopes. Ten potential epitope regions were determined by comparing the results obtained by these three tools, and solid phase synthetic peptide method and spot inhibition experiments were used to verify the prediction results of the software, and thus, eight tropomyosin linear epitopes were identified.

Molecular dynamics simulation is a powerful bioinformatics tool for allergenic proteins and epitopes analysis. It can be used to evaluate the binding affinity of epitopes to receptors, including T-cell epitopes to alleles and B-cell epitopes to antibodies, through the molecular docking. Commonly used molecular docking tools include Molecular Operating Environment (Chemical Computing Group Inc, Canada), Discovery Studio (Accelrys Co., Ltd., US), ClusPro server (<https://cluspro.org/login.php>, accessed 19 October 2020), and ZDOCK server (<http://zdock.umassmed.edu/>, accessed 19 October 2020). In addition, molecular dynamics

simulation can also help us analyze the influence of food processing methods on the structural changes of allergenic proteins and epitopes. In order to explore effects of non-thermal processing methods on the allergenicity of Gly m 4 soybean allergen, Vanga, Wang, Singh, and Raghavan (2019) employed molecular dynamics simulation to assess alterations of three linear and conformational epitopes under temperature and pressure deviations, which showed that a high pressure (6 kbar) combined with a low temperature (300 K) could alter the surface properties and conformation of Gly m 4.

Mutation of an amino acid on the epitope of the antigen may lead to an increase or decrease in the binding capacity of the epitope and immunoglobulin (Tan et al., 2012). Bioinformatics can be combined with mutagenesis techniques to study key amino acids on epitopes that have a significant effect on immunoglobulin binding. Mutagenesis techniques can be divided into the shotgun mutagenesis, the site-directed masking, and the site-directed mutation (Ahmad, Eweida, & Sheweita, 2016). In essence, shotgun mutagenesis is a large-scale mutagenesis technique based on the shotgun cloning method (Davidson & Doranz, 2014). What's more, site-directed masking refers to the design of cysteine amino acid mutations based on the three-dimensional structure of protein antigens. Specifically, antibody binding sites on the antigen surface are replaced by cysteine, so as to shield epitopes and prevent the binding of antibodies to epitopes (Ivanenkov, Crawford, Toyama, Sévigny, & Kirley, 2010). Although the shotgun mutagenesis method and the site-directed masking method have some applications in virus epitope mapping, there have been few literature reports on this technology in the mapping of food allergen epitopes so far. Furthermore, site-directed mutation techniques can be divided into alanine scanning mutation and random mutation. Alanine scanning mutation means that each residue in a specific peptide sequence is replaced by alanine in turn. If the peptide contains alanine, glycine will be used to replace the alanine in the original sequence (Ahmad, Eweida, & Sheweita, 2016). Moreover, by comparing the serum binding of mutant peptides with usual peptides, the key amino acids which have a significant effect on immunoglobulin binding can be obtained. For example, Shin et al. (1998) mutated each amino acid of the 23 linear epitopes of Ara h1 with alanines and then tested the IgE binding ability of the mutant polypeptide, and thus, each mutation of

an amino acid in all epitopes caused the loss of IgE binding capacity and the hydrophobic amino acid residues at the epitope center are the most critical for the binding of IgE and epitope. Finally, bioinformatics software was used to construct the Ara h1 three-dimensional model and identify the key amino acids on the molecular model surface. In order to map the epitopes of oyster allergen crag1, Fang et al. (2019) used immunoinformatics tools to predict potential epitopes, then applied ELISA to identify immunodominant epitopes, and employed amino acid site-directed mutagenesis to determine key amino acids on the epitope of oyster allergen crag1, which has successfully confirmed that the changes of amino acids would significantly reduce the affinity of IgE antibodies, but IgE binding capacity was not completely lost, and hydrophilic amino acid residues played the most important role in IgE binding. These results would help to better understand the molecular mechanism of the interaction of food allergens with specific IgEs.

Computer-based results often have gaps with actual results, so this method needs experiments to verify the results. However, the bioinformatics-based method has the advantage of low cost and high-speed data analysis. In addition, it can screen potential allergen epitopes from a large amount of data in the shortest time. Bioinformatics technology can provide a three-dimensional model of the allergen structure which is helpful to visualize the distribution of linear epitopes and conformational epitopes on the protein surface. Furthermore, bioinformatics can be used to verify the results of traditional strategies, thereby ensuring the reliability of conclusions (Wang, Wang, Liu, & Fu, 2019). In recent years, bioinformatics technology has made great progress. With the deepening of research and the accumulation of relevant data, it has become possible to use allergen epitopes or regions to predict new allergens (Wang, Wang, Liu, & Fu, 2019). In addition, the bioinformatics tool can be used as an effective means to assess the potential allergenicity of unknown allergens (Ivanciuc, et al., 2009).

5. Food allergen epitope identification method comparison

The characteristics of the major technologies for allergen epitope identification have been summarized in Table 2 in the current paper. Briefly, peptide scanning technology is the most commonly used technique for mapping epitopes. Combining this technique with serum

binding assays can effectively identify immunodominant epitopes. However, this technique is cumbersome, requires a large amount of serum and the workload is large. Moreover, compared with peptide scanning technology, peptide microarray technology has the advantage of high-throughput, so it can detect thousands of samples at one time using less serum, but it still needs a large number of peptide fragments to be synthesized. Phage display technology can be used to recognize both linear and conformational epitopes with easy operation, large peptide libraries, and high screening efficiency. However, phage display technology may omit some epitopes which can be recognized by peptide scanning. NMR technology and X-ray diffraction technology can provide detailed information on the binding sites of antigens and antibodies, and they are the most direct and effective technologies for mapping antigen epitopes. However, highly purified protein samples, professional skills, and advanced devices are required for these two technologies. Therefore, NMR technology and X-ray diffraction technology are not suitable for primary laboratories. The computer-based bioinformatics method takes the shortest time and has the fastest analysis speed, but it is also the most unreliable method that requires further experiments to verify. MS is one of the most important technologies for studying allergic omics with the advantages of high accuracy and high sensitivity but its technical equipment is expensive and costly.

6. Game-changing strategies for the food allergen epitope identification in close future

In the past few decades, some progress has been made in the identification and characterization of protein allergens (Ciardiello, et al., 2013). However, the number of identified food allergens is far less than the actual allergens, and no complete allergome of any food allergen has been fully identified still now (Ciardiello, et al., 2013; Wang, Wang, Liu, & Fu, 2019). In addition, due to the adjustment of dietary structure and the development of genetically modified food, some new food allergens may appear, and the identification of food allergens will be a new challenge in the food industry.

Although the characterization and identification of food protein allergens and their epitopes are huge tasks but currently, no standardized scheme is proposed. With the continuous development of biotechnology and allergy omics, the low-throughput, long-time, and high-cost traditional epitope mapping technology can no longer meet the requirements.

As a result, discovering advanced epitope mapping technology will be an urgent need with high-throughput, low economic cost, high response sensitivity, high accuracy, and short analysis time. Improving existing technologies and combining existing methods to develop new strategies is an important research direction (Wang, Wang, Liu, & Fu, 2019). For instance, Xu et al. (2017) used a truncated glutathione S-transferase (EC 2.5.1.18) as a carrier for B-cell epitope mapping, and proposed a simpler and more economical method for peptide synthesis than traditional methods, so as to provide a new way for epitope identification. Moreover, multidisciplinary assistance will be involved expected to break the current bottleneck to develop new epitope mapping technology. For instance, spectroscopy is a powerful tool for studying the structure of proteins. Circular dichroism spectroscopy and Fourier transform infrared spectroscopy can be used to resolve the secondary structure of proteins. The second-order ultraviolet spectrum can be used to explore the tertiary structure of proteins, and NMR technology can directly resolve binding sites of antigen and antibody. Based on the benefit from them, researchers can further develop new spectroscopy techniques to study the structure of antigen-antibody complexes, as well as combine spectroscopy with physical methods to invent new immunosensor technologies.

As for the new techniques used to study protein-protein interactions, a few methods, such as yeast two-hybrid, co-immunoprecipitation, and fluorescence resonance energy transfer, can be used. These three methods have not been applied to food allergen epitopes mapping, but are expected to be realized in the future. The basic principle of yeast two-hybrid technology is that the genes of two different proteins can be cloned into DNA binding domain and transcription activation domain of yeast transcription factor GAL4, respectively; and when a protein-protein complex is formed between these two proteins, these two domains will approach to each other and activate the transcription of the reporter gene (Izumi, 2001). The reporter gene can be used to determine whether the two proteins interact with each other. Co-immunoprecipitation is a technique for separating antigens by specifically binding the antigens to antibodies that are previously attached to a solid matrix. Protein A-Sepharose beads, protein G-Sepharose beads, and magnetic beads are commonly used as solid-phase matrices. The source of antigen for immunoprecipitation can be cells or tissues present *in vivo*

and proteins translated *in vitro*, or protein components or polyclonal, monoclonal antibodies separated by other biochemical techniques, such as gel filtration or density gradient precipitation (Bonifacino & Dell'Angelica, 1998). Fluorescence resonance energy transfer technology is based on a special physical phenomenon. When two fluorescent proteins are close enough, the energy of the donor protein can be transferred to the acceptor protein through dipole-dipole interaction. The energy transfer between fluorescent proteins can be determined according to the fluorescence decay characteristics of the donor molecule or the enhancement time of the acceptor fluorescence (Hong, Samson, & Song, 2019). Yeast two-hybrid, co-immunoprecipitation, and fluorescence resonance energy transfer methods have been widely used for protein and protein interactions. Antigens and antibodies are essentially proteins, so these methods can also be used to study the combination of antigens and antibodies in theory. Furthermore, SPR technology has been applied to the field of epitope identification because it can effectively analyze the interaction between low-affinity proteins and proteins, but it is expensive and complicated to operate and is not suitable for primary laboratories. Therefore, yeast two-hybrid, co-immunoprecipitation, and fluorescence resonance energy transfer may be good substitutes for SPR.

6. Conclusion

In recent years, the incidence of food allergies has been rising. Although great progress has been made in understanding food allergens, there is still a lack of effective desensitization methods. It needs to establish effective methods for the prevention, diagnosis, and treatment of food protein allergies. In this context, antibody-binding epitopes are considered to be biomarkers that reflect the persistence and severity of food allergies. Epitope mapping not only helps to understand the pathogenesis but also has to predict potential allergens and develop effective food processing methods to reduce food allergy or make vaccines. Since there has been no standardized scheme for epitopes mapping of food allergens so far, advanced epitope mapping methods that have the advantage of high-throughput, low economic cost, high sensitivity, high accuracy, and short analysis time should be invented. Future research directions for the identification of food allergen epitopes include: 1) improving the existing technologies by increasing analysis speed and reducing the cost on the

premise of ensuring the accuracy of the experimental results; 2) effectively combining multiple technologies to analyze allergen epitopes to achieve complementary advantages; 3) multidisciplinary assistance to develop new epitope mapping technology with high-throughput, low cost, high sensitivity, high accuracy, and high efficiency; 4) establishing a systematic and comprehensive database of food allergen epitopes, so as to facilitate query and analysis of relevant data; 5) drawing on the knowledge of proteomics and screening allergen epitopes based on comprehensively screening of the entire proteome to continuously develop and improve allergic omics.

Conflicts of interest

The authors declare no conflict of interest.

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Figure Captions

Fig. 1. Immune mechanisms contributing to food allergy. The food allergen is digested into small peptides and epitopes are exposed in the gastrointestinal tract. When the body is first exposed to an allergen, APCs identify T-cell epitopes and present epitopes to Th2 cells which can release cytokines, such as IL-4 and IL-13. These cytokines can activate B cells. At the same time, B cells can identify B-cell epitopes and further developed into plasma cells that release immunoglobulin E (IgE). Then, IgE antibodies bind the receptor on the basophil or mast cell. When the body is exposed to the same allergen for the second time, basophil and mast cells are activated by the cross-linking of two receptor-bound IgE antibodies to the antigen. Activated cells release lots of mediators that promote inflammation.

Fig. 2. Principle of phage surface displaying allergic peptides. The genes of the allergen of interest are introduced into phages that can express genes as peptides. The phages containing mimotope peptides are captured by the specific binding of antigen to antibody. Non-binding phages are discarded, while bound phages are eluted and amplified in *E.coil*. To increase the specificity of binding, the biopanning is repeated three to four times. After the last round of biopanning, phage clones containing mimotope peptides are isolated and identified by serum binding assays, such as ELISA and immunoblotting. The sequence of reactive peptides are obtained by inducing from the DNA sequence corresponding to the coding region of the foreign insert.

Fig. 3. Two different methods for epitopes extraction and the identification of mimotope peptides. (A) Various proteases hydrolyze food allergens into peptides, and then these peptides are passed over immobilized antibodies. Epitopes extracted by enzymatic hydrolysis can bind antibodies. Unbound peptides are washed off, while bound peptides are analyzed by MS. (B) Food allergens that bind to immobilized antibodies are digested by some different proteases. Peptides containing epitopes still bind antibodies. Free peptides are washed off, while bound peptides are analyzed by MS.

Fig. 4. (A) Mechanisms for the detection of interaction between allergens and antibodies by SPR. (B) Profile of the reflection intensity and SPR. The letter *a* represents the first binding of antigen and antibody. The letter *b* represents the second binding of antigen and antibody. (C)

SPR angle sensorgram of antigen-antibody interaction.

Fig. 5. (A) A peptide microarray model. (B) The schematic of peptide microarray construction and food allergenic sequences identification. All peptide samples are printed on glass slides. In the following incubation step, diluted human serum containing IgE are applied to the array. Peptides containing food allergen epitopes can bind to IgE. To make the binding visualization, antihuman IgE which is covalently conjugated with fluorescent markers is used to incubate with the array and bind to IgE. The image of experimental results can be obtained by the sample scanning, followed by the peptide sequence analysis.

1068 **Table 1** List of web available tools for continuous and discontinuous epitopes prediction

Types	Names	Websites	Predictive principles
Continuous/ linear B-cell epitope	AAPPred	http://www.bioinf.ru/aappred/	Amino acid pair antigenicity scale, SVM classifier
	ABCpred	https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.html	Recurrent ANNs, fixed length epitope patterns
	BcePred	https://webs.iiitd.edu.in/raghava/bcepred/bcepred_submission.html	Physicochemical properties of epitope residues
	BCPREDS	http://ailab.ist.psu.edu/bcpred/predict.html	SVM classifiers with string kernels
	Bepipred	http://www.cbs.dtu.dk/services/BepiPred	Hidden Markov model, propensity scale method
	COBEpro	http://scratch.proteomics.ics.uci.edu	SVM, epitopic propensity score, secondary structure, solvent accessibility information
	IEDB	http://tools.immuneepitope.org/bcell/	A collection of tools based on various methods
	LBtope	https://webs.iiitd.edu.in/raghava/lbtope/	Large datasets of epitopes, k-Nearest-Neighbor classification algorithm, SVM
	SVMTriP	http://sysbio.unl.edu/SVMTriP/	Tri-peptide similarity, propensity scores, SVM
Discontinuous /conformational B-cell epitope	SEPPA 3.0	http://www.badd-cao.net/seppa3/index.html	Micro-environmental features of glycosylation triangles and glycosylation-related amino acid, neighboring antigenicity
	SEPPA 2.0	http://www.badd-cao.net/seppa2/index.php	3D structure of antigen, subcellular localization of antigen, residue propensity, etc
	DiscoTope	http://www.cbs.dtu.dk/services/DiscoTope/	Epitope propensity amino acid score, surface accessibility
	EPSVR	http://sysbio.unl.edu/EPSVR/	Support vector regression, meta-analysis
	BEPro (PEPITO)	http://pepito.proteomics.ics.uci.edu/	3D structure of antigen, half-sphere exposure values
	EPCES	http://sysbio.unl.edu/services/EPCES/	Antigenic structure and surface characteristics
Both continuous and discontinuous B-cell epitope	Epitopia	http://epitopia.tau.ac.il/	Naïve Bayes classifier, antigen sequence or 3D structure
	ElliPro	http://tools.immuneepitope.org/tools/ElliPro/iedb_input	Prominent index, 3D structure of antigen
T-cell epitope	MHCPred 2.0	http://www.ddg-pharmfac.net/mhcpred/MHCPred/	Additive method

NetCTL 1.2	http://www.cbs.dtu.dk/services/NetCTL	ANN regression
NetMHCII 2.3	http://www.cbs.dtu.dk/services/NetMHCII/	ANNs
NetMHCIpan 4.0	http://www.cbs.dtu.dk/services/NetMHCIpan/	ANNs
ProPred	https://webs.iitd.edu.in/raghava/propred/	Quantitative matrix
RANKPEP	http://imed.med.ucm.es/Tools/rankpep.html	Position-specific score matrix
SYFPEITHI	http://www.syfpeithi.de/bin/mhcserver.dll/epitopeprediction.htm	Scoring system based on position of residue in the epitopes

1069 Note: SVM= Support vector machine, ANN= Artificial neural network

1070

1071 **Table 2** Main features of epitope mapping techniques for food allergen (pros and cons)

	Ease of operation	Cost-effective	Analysis speed	Sensitivity	Specificity	Throughput	Accuracy	Recognize conformational epitopes (if can)
Peptide scanning	++	++	+	+	++	+	++	-
Phage display	++	++	++	+	++	++	++	Yes
MS	+	+	++	+++	+++	+++	+++	Yes
NMR	+	+	++	++	++	++	+++	Yes
X-ray diffraction	+	+	++	++	++	++	+++	Yes
SPR	+	++	++	+++	+++	++	++	Yes
Peptide microarray	+	++	++	+++	+++	+++	++	-
Bioinformatics	+++	+++	+++	+	+	+++	+	Yes

1072 Note: +: low; ++: medium; +++: high

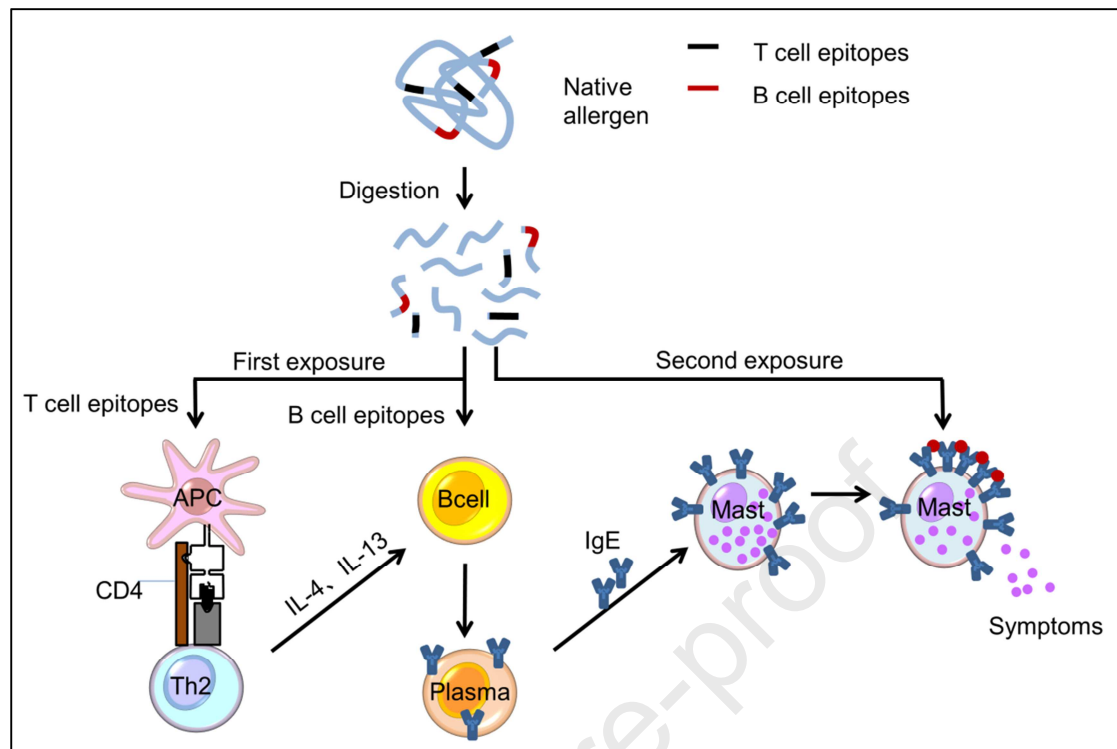


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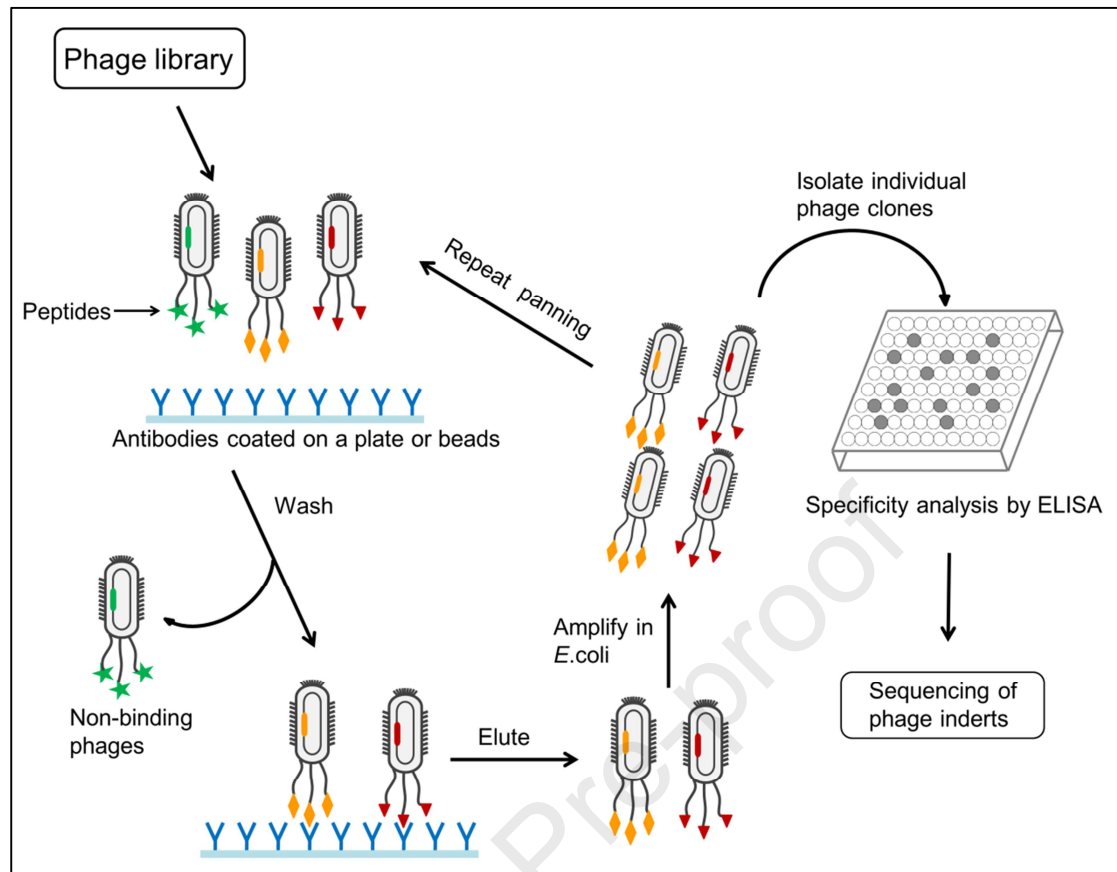


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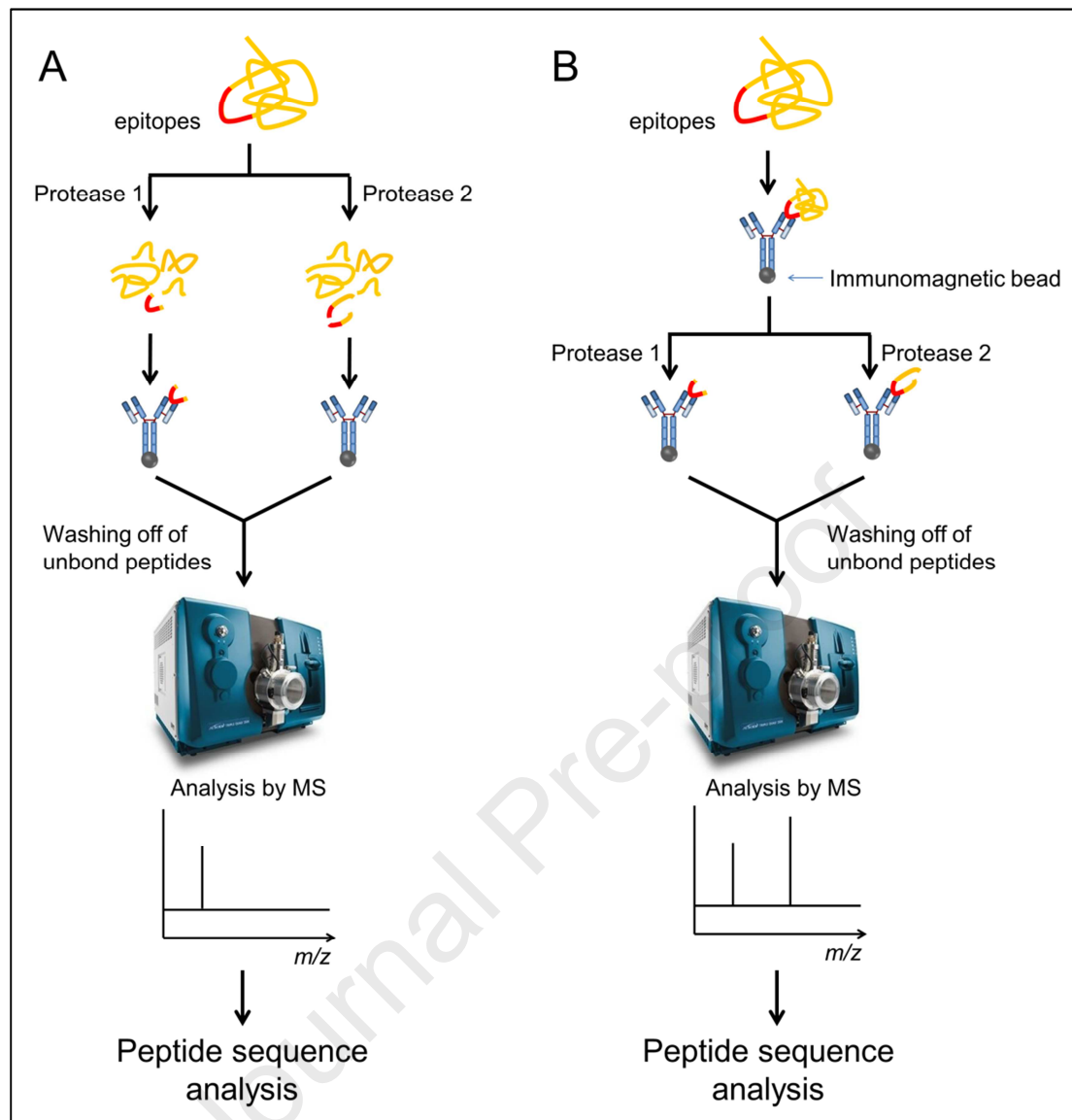


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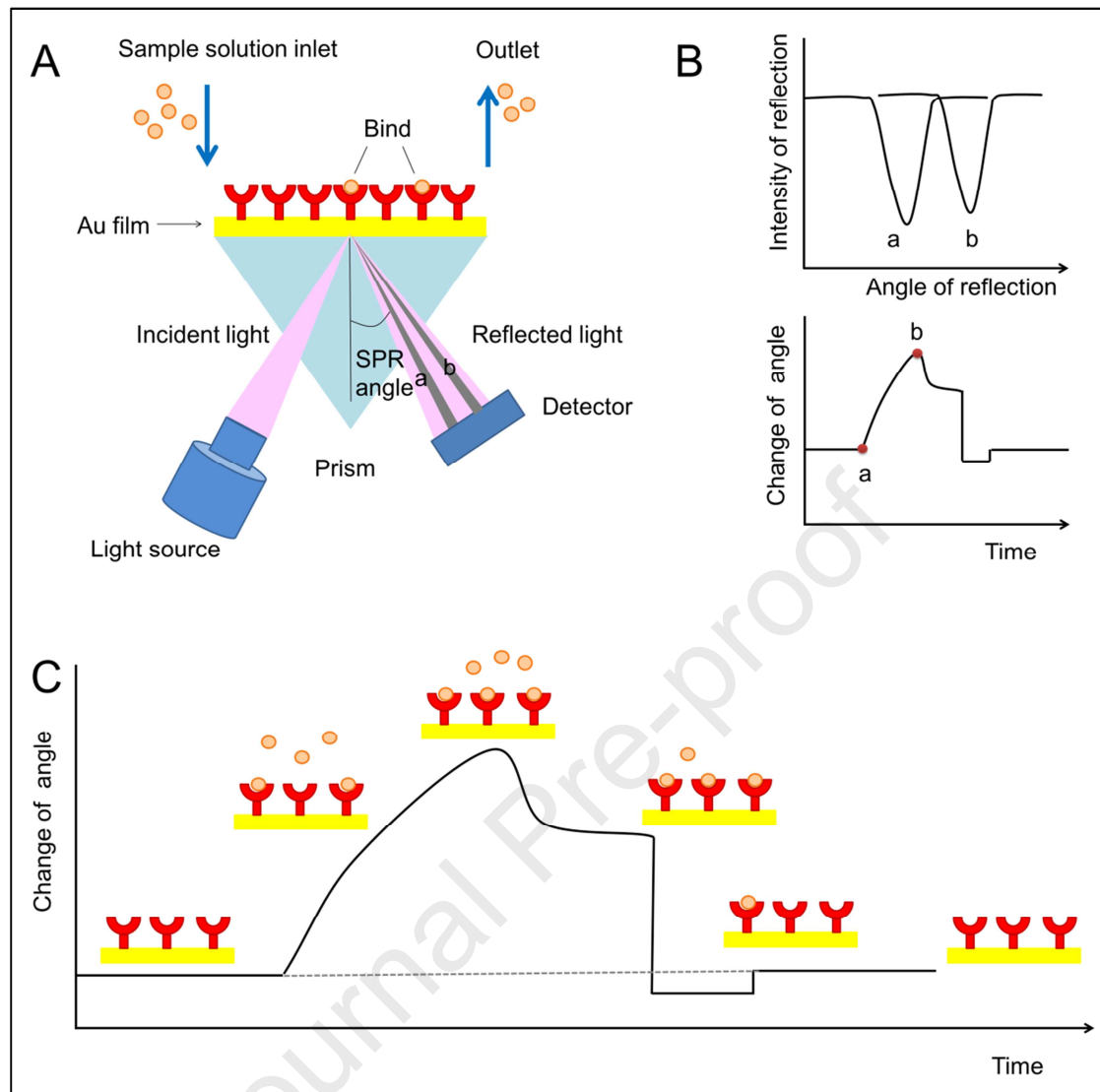


Fig. 4. (A) Mechanisms for the detection of interaction between allergens and antibodies by SPR. (B) Profile of the reflection intensity and SPR angle. The letter *a* represents the first binding of antigen and antibody. The letter *b* represents the second binding of antigen and antibody. (C) SPR angle sensorgram of antigen-antibody interaction.

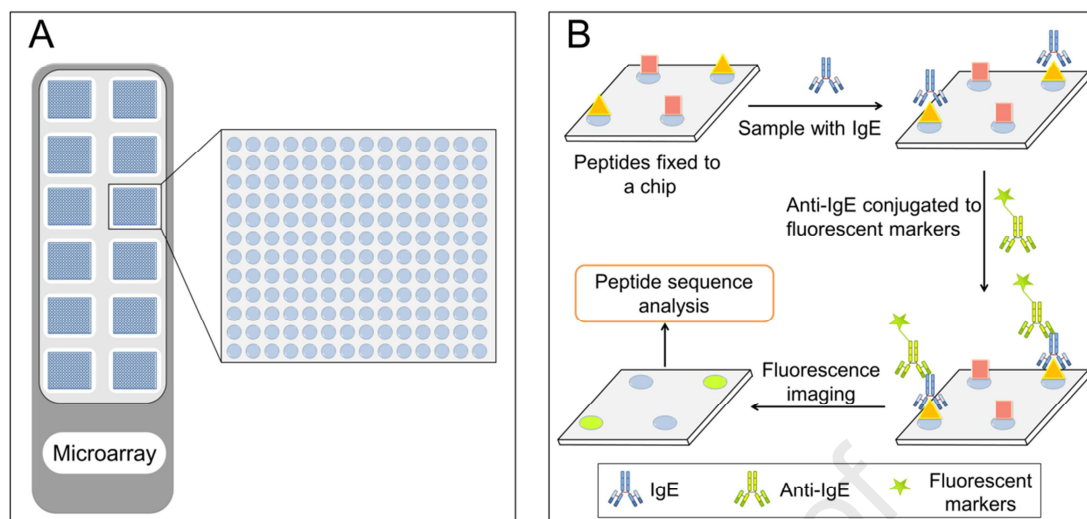


Fig. 5. (A) A peptide microarray model. (B) The schematic of peptide microarray construction and food allergenic sequences identification. All peptide samples are printed on glass slides. In the following incubation step, diluted human serum containing IgE are applied to the array. Peptides containing food allergen epitopes can bind to IgE. To make the binding visualization, antihuman IgE which is covalently conjugated with fluorescent markers is used to incubate with the array and bind to IgE. The image of experimental results can be obtained by the sample scanning, followed by the peptide sequence analysis.

Highlights

- △ Specificity between IgE antibodies and epitopes is indicator of food allergy.
- △ Recent advances in techniques have enabled efficient prediction of epitopes.
- △ Current and development of food epitope mapping technologies were discussed.
- △ Epitope identification mechanism would be better understood.
- △ Diagnosis/prognosis of food protein allergies would be more effectively.