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Food Allergen Epitope Mapping

Changqi Liu*,† and Shridhar K. Sathe*,‡

†School of Exercise and Nutritional Sciences, College of Health and Human Services, San Diego State University, 308 ENS Building, 5500 Campanile Drive, San Diego, California 92182-7251, United States

‡Department of Nutrition, Food & Exercise Sciences, College of Human Sciences, Florida State University, 402 SAN, 120 Convocation Way, Tallahassee, Florida 32306-1493, United States

ABSTRACT: With the increased global awareness and rise in food allergies, a multifold interest in food allergens is evident. The presence of undeclared food allergens results in expensive food recalls and increased risks of anaphylaxis for the sensitive individuals. Regardless of the allergenic food, the immunogen needs to be identified and detected before making any efforts to inactivate/eliminate it. In type I food allergies, protein immunogen cross-links immunoglobulin E, leading to basophil/mast cell degranulation, resulting in the symptoms that range from mild irritation to anaphylaxis. A portion/part of the protein, known as the epitope, can interact with either antibodies to elicit allergic reactions or T-cell receptors to initiate allergic sensitization. Antibody-recognized epitopes can be either a linear sequence of amino acids (linear epitope) or a three-dimensional motif (conformational epitope), while T-cell-receptor-recognized epitopes are exclusively linear peptides. Identifying and characterizing human-allergy-relevant epitopes are important for allergy diagnosis/prognosis, immunotherapy, and developing food processing methods that can reduce/eliminate immunogencity/immunoreactivity of the allergen.

KEYWORDS: *food, protein, allergen, epitope mapping, linear, conformational*

INTRODUCTION

Food choice by humans is governed by hunger and appetite, food availability, affordability, accessibility, and acceptability, nutrient needs, cultural and religious preferences, and food properties, including sensory characteristics. In addition, adverse reactions to food and other food safety issues also play a role. Among the many possible adverse reactions, food allergy appears to be of recent concern. The estimation of food allergy prevalence is difficult and has been suggested to be in the range from 3−6 to <10 and >10% in some regions.[1−3] According to the National Institute of Allergy and Infectious Diseases (NIAID) expert panel,[4] food allergy is defined as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food". The adverse reaction to a food by the affected human involves complex chemistry. The factors that contribute to this complexity includes intrinsic (e.g., genetics, physiology, and biochemistry) and extrinsic (e.g., environment, interaction of food with non-food components, and food processing) parameters. There are four types (I−IV) of food allergies. Type I food allergy is characterized by the immunoglobulin E (IgE)-mediated response to the allergenic food (food component); type II and III are those that involve the immunoglobulin G (IgG)-mediated response; and type IV is attributed to food-antigen-specific T-cell response.[5] Type I food allergy is attributed to food proteins that cross-link IgE on the surface of mast and/or basophil cells because this IgE cross-linking leads to cell degranulation and subsequent release of mediators, resulting in the observed symptoms.[6] Food-protein-induced adverse reactions may not always be the type I food allergy because food-protein-induced enterocolitis, a non-IgE cell-mediated food allergy, is known and of concern.[7] Food-induced allergic reactions may range from mild (e.g., skin irritation) to severe (e.g., anaphylaxis). The mechanism of food allergy remains unresolved. Immunotherapy treatments are one way to address the needs of certain individuals,[8] although the rate of successful outcome is unclear. A lack of cure for food allergy therefore remains a challenge. Consequently, the best defense for a sensitive individual is to avoid the exposure to the offending food. Methods to unequivocally detect and quantify the presence of the offending food are essential.

Immune Epitope Database (IEDB). IEDB (www.iedb.org) is a good resource for epitope information. It catalogs experimentally mapped B-cell and T-cell epitopes. As of February 2, 2018, IEDB has incorporated 431 059 peptidic epitopes and 2576 non-peptidic epitopes from 19 065 references for a variety of immune diseases.[9] As summarized in Table 1, much less allergen epitopes have been characterized in comparison to epitopes involved in infectious diseases or autoimmunity, and the linear epitope data dwarf the conformational epitope data, regardless of the type of disease. It should be noted that the database covers only 89% of the mapped allergen epitopes as a result of the manual data curation process.[10] For example, the linear epitopes of pecan 2S albumin (Car i 1)[11] and the conformational epitopes of cashew 11S legumin (Ana o 2)[12−15] mapped by our research group have not been curated into IEDB.

Epitope. An epitope is a part of an antigen that is recognized by an antibody or antigen receptor (e.g., T-cell receptor). Antibodies and T-cell receptors recognize epitopes in completely different manners. Antibodies can recognize almost any chemical structure on a native or denatured/degraded antigen, while T-cell receptors can only recognize an

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Table 1. Number of Mapped Immune Epitopes[9]

	autoimmune disease	infectious disease	allergy	transplant disease	no disease
linear epitope	33505	19058	8386	249	9569
conformational epitope	108	450	75	18	38
non-peptidic epitope	245	249	317	10	159
total	33858	19757	8778	277	9766

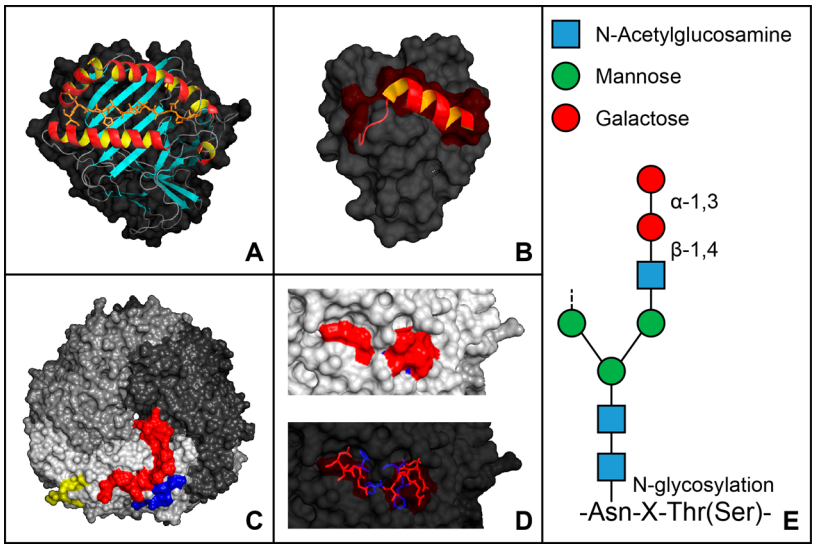

Figure 1. Structure of select food allergen epitopes. (A) Ovalbumin (Gal d 2) synthetic linear epitope amino acids 323−330 (orange) presented in a MHC II molecule (PDB number 1IAO).[101] (B) Major IgE linear epitope (amino acids 95−109) of Atlantic cod parvalbumin (Gad m 1).[98] (C) Amandin (Pru du 6, PDB number 3FZ3) IgG conformational epitope comprised of three discontinuous peptides: amino acids 21−45 (red), 320−328 (yellow), and 460−465 (blue).[27] (D) Partially buried peanut Ara h 3 (PDB number 3C3V) IgE linear epitope (amino acids 260−274). The exposed amino acid residues are in red color, while the buried residues are in blue color.[93] (E) IgE-binding galactose-α1,3-galactose epitope (red) on bovine thyroglobulin.[68]

antigen-derived peptide presented in the major histocompatibility complex (MHC) molecule of an antigen-presenting cell (e.g., dendritic cell).[6]

T-Cell Epitope. Activation of type 2 $CD4^+$ T-cells (T_H2) by allergen-derived peptides is crucial for the development of food allergy. T_H2 cells are distinguished by the production of interleukin (IL)-4, IL-5, and IL-13. These cytokines are not only pivotal in driving the antibody class switch to IgE (IL-4 and IL-13) but also important in the recruitment and activation of mast cells, basophils (IL-4), and eosinophils (IL-5).[6] T_H2 cell epitopes are linear peptides, typically 12−26 amino acids long, because the antigens must be processed into peptidic fragments by endosomal or lysosomal proteases prior to MHC binding and presentation to T-cell receptors[16] (Figure 1). Bindings of a T-cell epitope to both the MHC II molecule and the T-cell receptor are important for the T_H2 cell activation. The core sequence of a T_H2 cell epitope that binds to the MHC II molecule is nine amino acids long with anchor residues at positions 1, 4, 6, and 9.[16] The T-cell receptor interacts with both the MHC-bound epitope and the exposed areas of the MHC α-helices from the top of the MHC II molecule cleft.[17] Examples of mapped T-cell epitopes of select food allergens are presented in Table 2.

B-Cell Epitope. A B-cell epitope is a small region of an antigen recognized by the variable regions of an antibody. The antigen-binding site or paratope of an antibody is located at the tip of each arm of the antibody. Each paratope consists of six hypervariable loops, forming a surface structure that is complementary to the structure of an epitope. The paratope interacts with its corresponding epitope through non-covalent forces. This interaction is highly specific and is the molecular basis of allergen cross-linking of IgEs on the surface of a mast cell or basophil and the subsequent degranulation of the granulocyte.[6] B-cell epitopes of food allergens are mostly peptidic, although carbohydrate epitopes have also been reported.[18] A peptidic epitope can be either a linear peptide or a three-dimensional protein structure comprised of discontinuous peptides/amino acids.

B-Cell Peptidic Epitopes. B-cell linear epitopes are typically 8−15 amino acids long.[19] Although linear epitopes as short as 5 amino acids have been reported, peptides with ≥8 amino acids are required for high avidity bindings with IgE.[20] Linear epitopes of food allergens do not seem to be made of particular groups of amino acids. We analyzed the amino acid distribution in 185 epitopes of peanut 7S vicilin (Ara h 1) and 204 epitopes of bovine α_{S1}-casein (Bos d 9) archived in IEDB. The relative frequency of each amino acid in the epitopes is very similar to the relative frequency observed in the whole allergens (Figure 2). This is in agreement with Ayuso et al., who did not find any difference in amino acid distribution in the IgE-binding regions and the whole molecule of shrimp tropomyosin (Pen a 1).[21] Amino acid sequences of linear epitopes exhibit a high degree of heterogeneity. To date, no common structure of linear epitopes has been identified, with a few exceptions. Sequence analysis of IgE-binding epitopes of white shrimp tropomyosin (Lit v 1) demonstrated a common motif LEX_1X_2L or LEX_1X_2N, where X_1 can be either D, E, N, or K and X_2 can be D or E.[22] Several linear IgE

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Table 2. T-Cell Epitopes of Major Food Allergens

allergen		number of epitopes[9]	epitope example
IUIS	common name		
		Peanut	
Ara h 1	7S vicilin	125	SQLERANLRPCEQ and ANLRPCEQHLM, two distinct yet overlapping T-cell epitopes on Ara h 2[40]
Ara h 2	2S albumin	68	
		Tree Nuts	
Ana o 1	7S vicilin	4	KVKDDELRVIRPSRSQSERG, a T-cell epitope of Ana o 2 that is cross-reactive to pistachio and hazelnut[87]
Ana o 2	11S legumin	6	
Jug r 1	2S albumin	1	
Jug r 2	7S vicilin	18	
Cor a 8	lipid transfer protein	26	
Cor a 9	11S legumin	3	
Cor a 11	7S vicilin	1	
		Soybean	
Gly m 4	Bet v 1-like protein	1	ALFKAIEAYLLAHPD, a T-cell epitope of Gly m 4 cross-reactive with other Bet v 1-related food allergens[88]
		Egg	
Gal d 1	ovomucoid	29	cocktail of Gal d 2 T-cell epitopes, AMVYLGAKDSTRTQI, SWVESQTNGIIRNVL, and AAHAEINEAGREVVG, demonstrated potential in immunotherapy[89]
Gal d 2	ovalbumin	14	
		Milk	
Bos d 4	α-lactalbumin	8	EPMIGVNQELAYFYPELFRQFYQL, an immunodominant T-cell epitope of Bos d 9[90]
Bos d 5	β-lactoglobulin	30	
Bos d 6	bovine serum albumin	7	
Bos d 9	α_{S1}-casein	39	
		Crustacean Shellfish	
Pen m 1	tropomyosin	13	ELRVVGNNLKSLEVS and AYKEQIKTLTNKLKA, two Pen a 1 T-cell epitopes that bind to more than one MHC II allele[91]
Pen m 2	arginine kinase	10	
Pen a 1	tropomyosin	28	
Met e 1	tropomyosin	6	
		Fish Parasite	
Ani s 1	serine protease inhibitor	26	LGKSCDDQFCPE, a T-cell epitope of Ani s 1 that is recognized by eight patients[92]

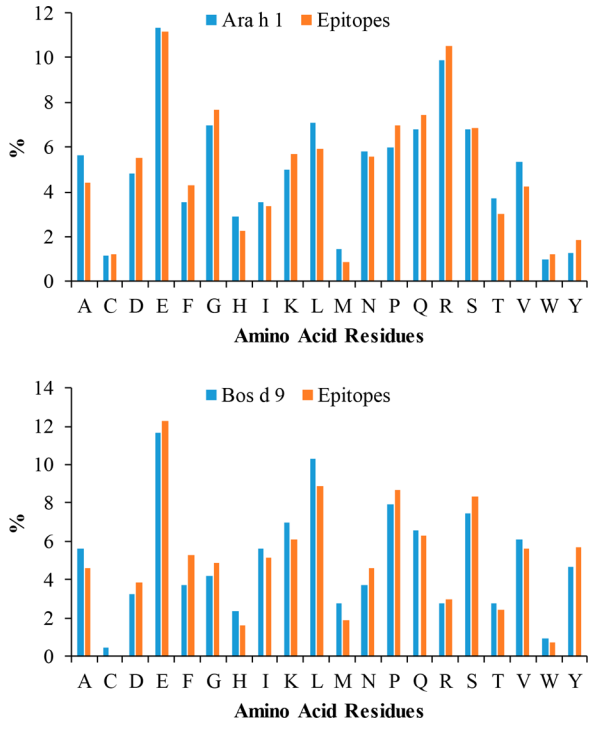

Figure 2. Amino acid distribution in epitopes and whole molecules of Ara h 1 and Bos d 9.

epitopes of cod parvalbumin (Gad c 1) displayed a shared structure comprised of two repetitive sequences DEDK and DELK interspaced by six unrelated amino acids. The tetrapeptides at both termini of the eptiopes were critical for IgE binding, while the spacer was independent of the immunoreactivity.[23] Nevertheless, such "common structures" seem to be unique features of these allergens. Examples of B-cell linear epitopes are shown in Table 3.

B-cell conformational epitopes are comprised of amino acid residues/peptides that are discontinuous in sequence but are brought into spatial proximity by protein folding. It is estimated that the majority of B-cell epitopes are conformational based on the fact that 90% of antibodies raised against an antigen do not react with the peptidic fragments of the antigen.[24] The prevalence of conformational epitopes can also be demonstrated by the contact area of the antigen−antibody complex. On the basis of crystallographic data, an antigen surface of approximately 700−900 Å^2 is recognized by an antibody.[24] The percentage of continuous patches on a protein surface decreases with an increased surface area. Less than 10% of antigen surfaces at 800 Å^2 contain only amino acid residues from a continuous peptide.[25] Despite the predominance of conformational epitopes, mapped structures are scarce to summarize the characteristics of the conformational determinants (Table 4). It has been suggested that conformational epitopes are less important than linear epitopes in food allergy, except for causing oral allergy syndrome, because food proteins are usually subjected to heat denaturation and gastrointestinal digestion, which destabilize the conformational epitopes.[26]

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Table 3. B-Cell Linear Epitopes of Major Food Allergens

allergen		number of epitopes[9]	epitope example
IUIS	common name		
			Peanut
Ara h 1	7S vicilin	185	GNIFSGFTPEFLAQA, an IgE-recognized linear epitope of Ara h 3 that is partially buried (Figure 1D); the buried amino acid residues are in bold[93]
Ara h 2	2S albumin	96	
Ara h 3	11S legumin	109	
Ara h 6	2S albumin	8	
Ara h 7	2S albumin	2	
Ara h 11	14 kDa oleosin	2	
			Tree Nuts
Pru du 6	11S legumin	18	Jug r 4, VFSGFDADFLADAFN; Cor a 9, FSGFDAEFLADAFNV; IgE linear epitopes of Jug r 4 and Cor a 9 with almost identical amino acid sequence are responsible for cross-reactivity of the allergens[94]
Jug n 2	7S vicilin	1	
Ber e 1	2S albumin	7	
Ana o 1	7S vicilin	11	
Ana o 2	11S legumin	22	
Ana o 3	2S albumin	16	
Jug r 1	2S albumin	6	
Jug r 2	7S vicilin	6	
Jug r 4	11S legumin	22	
Cor a 1	Bet v 1-like protein	1	
Cor a 9	11S legumin	25	
Car i 4	11S legumin	19	
			Soybean
Gly m 1	hydrophobic seed protein	2	GSVLSGFSKHFL and GSNILSGFAPEF, two IgE linear epitopes of soy glycinin are overlapped with epitopes of other plant 11S legumins[95]
Gly m 3	profilin	1	
Gly m 4	Bet v 1-like protein	13	
Gly m 5	β-conglycinin	15	
Gly m 6	glycinin	43	
Gly m 8	2S albumin	8	
			Wheat
Tri a 14	lipid transfer protein	10	KSCCRSTLGRNCYNLCRARGAQKLCAGVCR, a B-cell linear epitope of Tri a 37 that is recognized by both patient IgE and rabbit IgG[48]
Tri a 37	α-purothionin	3	
			Egg
Gal d 1	ovomucoid	173	FNPVCGTDGVTYDN, a linear epitope of Gal d 1 recognized by 32% of serum IgE of 50 patients[96]
Gal d 2	ovalbumin	14	
Gal d 5	serum albumin	1	
			Milk
Bos d 4	α-lactalbumin	68	cow and buffalo, NENLLRFFVAPFPEVFGKEK; goat, NENLLRFVVAPFPEVFRKEN; cross-reactive IgE linear epitopes on α_{S1}-casein (Bos d 9) of cow, water buffalo, and goat[97]
Bos d 5	β-lactoglobulin	174	
Bos d 6	bovine serum albumin	10	
Bos d 9	α_{S1}-casein	204	
Bos d 10	α_{S2}-casein	67	
Bos d 11	β-casein	126	
Bos d 12	κ-casein	125	
			Crustacean Shellfish
Pen m 1	tropomyosin	10	MGGDLGQVFRRLTSA, a linear IgE epitope of Lit v 2 recognized more frequently by children than by adults[22]
Pen a 1	tropomyosin	51	
Met e 1	tropomyosin	1	
Lit v 2	arginine kinase	39	
			Fish and Fish Parasite
Gad m 1	β-parvalbumin	45	DGKIGVDEFGAMIKA, a major epitope of Gad m 1 recognized by serum IgE of 13 patients (Figure 1B); this epitope is located in the Ca^{2+}-binding EF domain of parvalbumin[98]
Sal s 1	β-parvalbumin	29	
Ani s 1	serine protease inhibitor	23	
Ani s 5	SXP/RAL-2 family protein	12	

However, we found a conformational epitope on amandin (Pru du 6) that overlaps with IgE-binding epitopes and is stable against thermal processing and *in vitro* digestion in the presence of food matrices.[27−30] Moreover, predominant IgE recognition of conformational epitopes and minor involvement of linear epitopes have been reported for Ara h 2,[31,32] Ara h 6,[32] and Pen a 1.[31,33] These observations suggest that re-evaluation of the implication of conformational epitopes in food allergy is important and needs further investigation.

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Table 4. B-Cell Conformational Epitopes of Major Food Allergens

allergen			
IUIS	common name	number of epitopes[9]	epitope example
			Tree Nuts
Pru du 6	11S legumin	1	amino acids 21−45 (ARQSQLSPQNQCQLNQLQAREPDNR), 320−328 (NRNQIIRVR), and 460−465 (DQEVQQ) (Figure 1C), a conformational IgG epitope that is overlapped with IgE epitopes[27] and stable against thermal processing[29] and digestion[28]
Ana o 2	11S legumin	1[12−14]	
Ber e 1	2S albumin	1[52]	
			Fruits
Cuc m 2	profilin	1	$S_2W_3A_5Y_6D_9H_{10}T_{111}P_{112}G_{113}Q_{114}N_{116}M_{117}R_{121}L_{122}$, a conformational IgE epitope of Cuc m 2 that is responsible for cross-reactivity with timothy grass and birch pollen profilins[99]
Pru p 3	lipid transfer protein	3[53,100]	
			Wheat
Tri a 14	lipid transfer protein	1	Tri a 14, $\mathbf{H_{35}N_{38}R_{39}S_{40}S_{42}D_{43}\ G_{74}V_{75}L_{77}P_{78}Y_{79}}T_{80}$; Pru p 3, $\mathbf{H_{35}N_{38}R_{39}}T_{40}\ \mathbf{D_{43}}R_{44}\mathbf{G_{74}V_{75}L_{77}P_{78}Y_{79}}$; conformational IgE epitopes of Tri a 14 and Pru p 3 exhibit a similar structure[100]
			Egg
Gal d 4	lysozyme	16[55−64]	conformational IgG epitope of Gal d 4;[55] light-chain-binding residues, $D_{18}L_{25}I_{124}L_{129}$; heavy-chain-binding residues, $R_{21}Y_{23}S_{24}N_{27}K_{116}G_{117}T_{118}D_{119}V_{120}$; and light- and heavy-chain-binding residues, $N_{19}G_{22}Q_{121}$
			Milk
Bos d 5	β-lactoglobulin	1	$W_{19}Y_{20}V_{43}E_{44}E_{45}K_{47}L_{57}Q_{59}C_{66}Q_{68}P_{126}E_{127}T_{154}E_{157}$, a conformational IgE epitope of Bos d 5 consists six short fragments covering a flat area on the surface[65]
			Fish
Gad m 1	β-parvalbumin	1	$L_7N_8D_{11}I_{12}L_{16}F_{30}F_{31}K_{33}V_{34}A_{37}S_{40}F_48E_{49}I_{50}I_{51}D_{54}K_{55}E_{61}E_{63}R_{76}L_{78}E_{82}T_{83}E_{102}K_{108}$, a conformational IgE epitope of Gad m 1 overlaps with Baltic cod (Gad c 1) and carp parvalbumin (Cyp c 1) epitopes[66]
Cyp c 1	β-parvalbumin	3[54]	

Table 5. B-Cell Carbohydrate Epitopes of Select Food Allergens

carbohydrate epitope	food	allergen example
α-1,3-fucose	plant foods (e.g., tomato and celery)	tomato β-fructofuranosidase (Sola l 2) and celery flavin adenine dinucleotide-containing oxidase (Api g 5)[34]
β-1,2-xylose	plant foods (e.g., peanut, hazelnut, tomato, and celery)	peanut (Ara h 1), hazelnut 7S vicilin (Cor a 11),[18] tomato β-fructofuranosidase (Sola l 2), and celery flavin adenine dinucleotide-containing oxidase (Api g 5)[34]
galactose-α-1,3-galactose (α-Gal)	red meat (e.g., beef, lamb, and pork)	α-Gal-containing glycoproteins (e.g., beef laminin γ-1, collagen α-1 chain, triosephosphate isomerase, carbonic anhydrase 3, lactate dehydrogenase A, creatine kinase M-type, aspartate aminotransferase, β-enolase, and α-enolase)[36]
galactose-β-1,4-(galactose-β-1,4-galactose-β-1,6)-glucose	prebiotics	galacto-oligosaccharide[37]

Table 6. Commonly Used Epitope Mapping Methods

type of epitope	epitope preparation/preselection method	epitope identification method
T-cell epitopes	overlapping peptide synthesis,[38−41] *in silico* epitope prediction,[39] and *in vitro* MHC-binding assay[39]	T-cell proliferation (^{3}H-labeled thymidine incorporation,[39,40] carboxyfluorescein succinimidyl ester dilution assay,[41] and intracellular cytokine staining[41]) and tetramer-guided epitope mapping[38]
B-cell linear epitopes	overlapping peptide synthesis,[44,46−49] enzymatic digestion,[42,43] epitope prediction,[45] chimeric protein,[46] point mutation,[47] and chemical modification[44]	ELISA,[42−46] dot blot,[42,44−48] Western blot,[44,46] peptide microarray,[49] and hydrogen/deuterium exchange[14]
B-cell conformational epitopes	chimeric molecule production,[12,13,27,52] mutagenesis,[12,27] and phage display[53,54]	ELISA,[13,27,53,54,99,100] dot blot,[12,13,27] protein microarray,[52] hydrogen/deuterium exchange,[14,15,27] X-ray crystallography,[55−65] and nuclear magnetic resonance[66]
B-cell carbohydrate epitopes	deglycosylation,[36,68,69] protease treatment,[68] glycopeptide synthesis,[70] and neoglycoprotein production[69]	ELISA,[68−70] Western blot,[36,68−70] basophil histamine release assay,[69,70] basophil activation test,[68] immunoCAP,[36,68] mass spectrometry,[67−70] and nuclear magnetic resonance[67]

B-Cell Carbohydrate Epitopes. Antibody recognition of non-peptidic structures, such as glycans, has been documented. Two non-mammalian carbohydrate epitopes, α-1,3-fucose and β-1,2-xylose, are known to induce IgE-mediated cross-reactivity in plant and invertebrate foods as a result of their ubiquitous occurrence.[18,34] It was traditionally thought that carbohydrate epitopes have little clinical significance.[35] However, galactose-α-1,3-galactose, a non-primate carbohydrate epitope in red meat, has been shown to cause delayed anaphylaxis.[36] Galacto-oligosaccharide is another carbohydrate allergen responsible for anaphylactic reactions to milk formula product supplemented with this oligosaccharide as prebiotics. The branched structure, galactose-β-1,4-(galactose-β-1,4-galactose-β-1,6)-glucose, is likely the epitope of the allergen.[37] A few examples of carbohydrate epitopes are listed in Table 5.

T-Cell Epitope Mapping. Because the T-cell epitopes are typically linear, synthetic overlapping peptides are used for epitope mapping (Table 6). The overlapping peptides can span the investigated protein over the length or the targeted portion of it. For large protein molecules (e.g., Ara h 1), such investigations become impractical often as a result of the need of large volumes of blood of the patients. The frequency of allergen-specific $CD4^+$ T-cells in circulation is low. It was reported that the average frequency of Ara h 1-specific T-cells

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in blood of peanut-allergic patients was 9 cells per million.[38] Preselection of peptides can be performed by *in silico* MHC II epitope prediction and/or *in vitro* MHC-binding assay to reduce the sample size.[39] The peptides are incubated with peripheral blood mononuclear cells isolated from blood of allergic patients. The antigen-presenting cells take up the peptides and present them to $CD4^+$ T-cells. Recognition of the peptide in the complex of the MHC II molecule by the T-cell receptor leads to proliferation and clone expansion of the peptide-specific T-cell.[39] T-cell proliferation can be quantified by a variety of methods, such as ^{3}H-labled thymidine incorporation,[39,40] carboxyfluorescein succinimidyl ester dilution assay,[41] and intracellular cytokine staining.[41] Alternately, synthetic peptides can be loaded onto soluble MHC II monomer-assembled tetramers that are labeled with fluorochromes. The peptide−tetramer complexes bind with T-cell receptors and stain the allergen-specific $CD4^+$ T-cells. The tetramer-guided epitope mapping allows for identification of MHC restriction and isolation of antigen-specific T-cell clones in the mapping process.[38]

B-Cell Linear Epitope Mapping. Similar to the T-cell epitope mapping, overlapping synthetic peptides are most commonly used for B-cell linear epitope mapping (Table 6), although peptides generated by enzymatic digestion[42−44] or cyanogen bromide fragmentation[44] have also been used. The peptides often span the length of the protein, but fewer peptides can be selectively synthesized on the basis of epitope prediction.[45] In addition, chimeric protein fragments have been used to narrow the antibody-binding region prior to synthesis of overlapping peptides.[46] Peptide mutation (e.g., single-site alanine substitution)[47] and chemical modification (e.g., acetylation and carboxymethylation)[44] are sometimes used to pinpoint the core amino acid residues of the epitope. Epitopes recognized by IgG produced in animals (polyclonal[48] or monoclonal[44]) and patient serum IgE[42,43,45−48] are identified by immunoassays, such as enzyme-linked immunosorbent assay (ELISA),[42−46] dot blot,[42,44−48] and western blot.[44,46] Recently, the peptide microarray has been used as a high-throughput method for large-scale epitope mapping of food allergens.[49] In this assay, overlapping synthetic peptides are printed on the microarray slides. It requires only a small volume of diluted patient sera and allows for concurrent epitope mapping of other immunoglobulin subclasses.[49] In addition, amide backbone hydrogen/deuterium exchange monitored by mass spectrometry can be used for B-cell linear and conformational epitope mapping.[14]

B-Cell Conformational Epitope Mapping. In comparison to B-cell linear epitopes, much less information is available for B-cell conformational epitopes as a result of technical challenges (Table 6). Characterization of the conformational epitopes requires sophisticated techniques, such as chimeric molecule production, large-scale mutagenesis, phage display, hydrogen/deuterium exchange, X-ray crystallography, or nuclear magnetic resonance (NMR).[27,50,51]

Chimeric proteins are produced by replacing a region of a protein with the corresponding region of another structurally related protein. The expressed chimeric proteins are subsequently tested in immunoassays to identify the antibody-binding region(s). Ber e 1 × SFA-8,[52] Ana o 2 × Gly m 6,[12,13] and Pru du 6 × Gly m 6[27] chimeras have been applied in conformational epitope mapping of Brazil nut 2S albumin and cashew and almond 11S legumins.

Mutagenesis is a relatively simple technique for conformational epitope mapping. An amino acid at a given position of the antigen is replaced with another amino acid. Loss of antibody binding after mutagenesis indicates the association of the modified residue with the epitope.[51] Mutagenesis can be laborious as a result of the requirement for a large amount of mutants.[50,51] However, it is very useful for fine mapping of the epitope when the antibody-binding region is narrowed by other techniques (e.g., chimeric molecule production). Alanine point mutation has been used for conformational epitope mapping of Ana o 2[12] and Pru du 6.[27]

The phage display technique uses bacteriophages to generate random peptides on the phage surface. The peptides recognized by an antibody are called mimotopes, which resemble the antigenic epitope. An algorithm is then used to map the putative epitope based on the characteristics of the mimotopes.[50,51] Phage display has been used for conformational epitope mapping of Pru p 3[53] and Cyp c 1.[54]

Recently, hydrogen/deuterium exchange has been used to map the conformational epitopes of Ana o 2[14,15] and Pru du 6.[27] This technique maps protein surfaces based on solvent accessibility. The antigen or antigen−antibody complex is incubated in D_2O, followed by quench and proteolysis. The hydrolysates are analyzed by liquid chromatography−mass spectroscopy. The peptides displaying significant deuterium uptake differences between free antigen and the antigen−antibody complex are the antibody-binding regions.[14,15,27]

Resolution of the X-ray crystal structure of an antigen−antibody complex is an ideal method for conformational epitope mapping. However, this technology is not readily applicable to many antigen/antibody and is time- and resource-intensive.[50,51] Hen egg white lysozyme (Gal d 4) is the most extensively studied antigen using X-ray crystallography. Gal d 4 epitopes recognized by murine monoclonal antibodies D1.3,[55] HyHEL-5,[56] HyHEL-10,[57] D44.1,[58] HyHEL-63,[59] HyHEL-8,[60] HyHEL-26,[60] and F10.6.6,[61] camel antibodies D2-L29, D2-L19, cAb-Lys-2, cAb-Lys-3, D3-L11, and D2-L24,[62] and shark antibodies HEL-5A7[63] and PBLA8[64] have been resolved. In addition, the molecular interactions between an IgE antibody and bovine β-lactoglobulin (Bos d 5) have been revealed[65] (Figure 3).

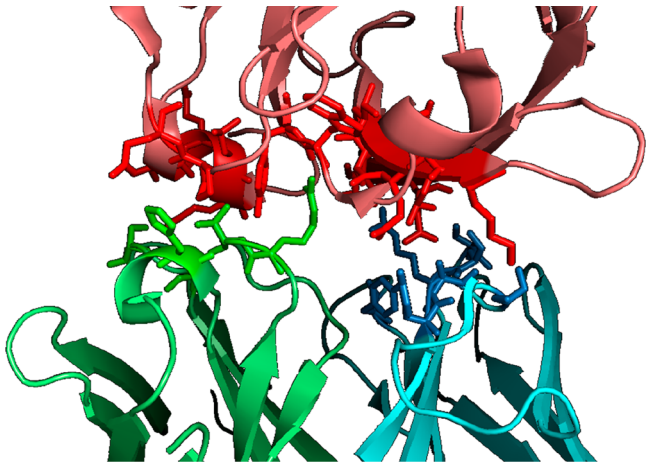

Figure 3. Binding of an IgE to the β-lactoglobulin (Bos d 5) epitope (PDB number 2R56). The β-lactoglobulin molecule and IgE heavy chain and light chain are shown in red, green, and cyan colors, respectively. The epitope and paratope residues are shown in sticks.

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NMR has been used for epitope mapping. This technique is limited by the molecular size of the antigens. Target antigens have to be less than 25 kDa to obtain high-quality *de novo* structures. House dust mite allergens Blo t 5, Der f 2, and Der p 2 have been mapped by NMR. Recently, a major IgE-binding epitope of cod parvalbumin (Gad m 1) was mapped by transversal- and longitudinal-relaxation optimized NMR spectroscopy.[66]

B-Cell Carbohydrate Epitope Mapping. Glycan structures of various foods have been characterized by mass spectrometry and NMR.[67−70] Antibody recognitions of carbohydrates are often determined by loss of immunoreactivity of periodate-oxidized or chemically/enzymatically deglycosylated antigens.[18,34,36,67−69] However, such harsh treatments could also destroy peptidic epitopes.[34] Alternately, unglycosylated protein variants are used to investigate the antibody−antigen interaction.[18] Moreover, protease treatment and glycopeptide synthesis have been employed to demonstrate that the antibody is directed to carbohydrate instead of protein.[18,68,70] Inhibition assay using defined glycans or neoglycoproteins (e.g., glycosylated bovine serum albumin) as inhibitors is another strategy to identify the carbohydrate epitopes.[18,36,69]

Epitope Prediction. With the help of the machine learning technique and currently available epitope databases, various bioinformatic methods have been developed to predict allergen epitopes *in silico*. Such prediction tools are easy, fast, and inexpensive to perform and can complement traditional techniques in epitope mapping and assessment of immunoreactivity and cross-reactivity of allergens.[71]

Several algorithms have been developed for T-cell epitope prediction that are mainly based on MHC II binding. The prediction performance for MHC II binding is much poorer than that of MHC I as a result of the (1) open binding groove of the MHC II molecule that can accommodate peptides of variable lengths and (2) limited data of MHC II binding for training the prediction methods.[72] Moreover, T-cell epitope prediction that is solely based on MHC binding is less accurate because peptide binding to MHC is necessary but insufficient for T-cell recognition. A combination of prediction methods based on proteasomal processing, transporter associated with antigen processing, and MHC binding has resulted in a better predictive performance for $CD8^+$ T-cell epitopes than any single prediction tool.[71] However, prediction tools based on the MHC II processing pathway are not currently available for $CD4^+$ T-cell epitopes. In addition to MHC-binding prediction, IEDB provides an epitope prediction tool based on $CD4^+$ T-cell immunogenicity.[9] A few T-cell epitope prediction tools are listed in Table 7. As a result of the lack of a rich training set of mapped T-cell epitopes, the predictive utility of such tools is limited. Much more epitope mapping work is needed to increase the predictive capability.

B-cell epitope prediction is dependent upon either amino acid sequence, three-dimensional (3D) protein structure, or mimotope information on the target allergen.[73] Amino acid sequence homology has been applied for linear epitope prediction. However, proteins with a high sequence identity may exhibit different immunoreactivity as a result of protein folding.[71] Another linear epitope prediction approach is based on amino acid propensity scales, such as hydrophilicity and chain flexibility. Recently, machine learning algorithms have been used to generalize epitope information and predict new epitopes. A maximum accuracy of 66% has been achieved for linear epitope prediction.[73] Prediction of a B-cell conformational epitope is a more challenging task than a linear epitope prediction as a result of its complexity, high computational cost, and low availability of protein 3D structure and conformational epitope information. Existing conformational epitope prediction tools are generally based on small data sets and exhibit low accuracy.[71] Mimotope-based conformational epitope mapping can be considered as a unique prediction method. It incorporates phage display data and maps the identified mimotopes back to the target antigen to predict the

Table 7. List of Epitope Prediction Tools

method	link	prediction for
T-Cell Epitope Prediction Tools[9,71,72]		
HLA-DR4pred	http://www.imtech.res.in/raghava/hladr4pred/	MHC II epitopes
Imm_Score	http://tools.iedb.org/imm_score/	$CD4^+$ T-cell epitopes
NetMHCII	http://www.cbs.dtu.dk/services/NetMHCII/	MHC II epitopes
NetMHCIIpan	http://www.cbs.dtu.dk/services/NetMHCIIpan/	MHC II epitopes
MHC II	http://tools.iedb.org/mhcii/	MHC II epitopes
MHC2Pred	http://crdd.osdd.net/raghava/mhc2pred/	MHC II epitopes
MHCPred	http://www.ddg-pharmfac.net/mhcpred/MHCPred	MHC I and II epitopes
ProPred	http://www.imtech.res.in/raghava/propred/	MHC II epitopes
RANKPEP	http://imed.med.ucm.es/Tools/rankpep.html	MHC I and II epitopes
SVMHC	http://abi.inf.uni-tuebingen.de/Services/SVMHC	MHC I and II epitopes
SYFPEITHI	http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm	MHC I and II epitopes
TepiTool	http://tools.iedb.org/tepitool/	MHC I and II epitopes
B-Cell Epitope Prediction Tools[9,71,73]		
ABCPred	http://www.imtech.res.in/raghava/abcpred/	linear epitopes
AntiJen	http://www.jenner.ac.uk/AntiJen/	linear epitopes
Bcell	http://tools.iedb.org/bcell/	linear epitopes
Bcepred	http://www.imtech.res.in/raghava/bcepred/	linear epitopes
BepiPred	http://www.cbs.dtu.dk/services/BepiPred/	linear epitopes
CEP	http://196.1.114.49/cgi-bin/cep.pl	conformational epitopes
DiscoTope	http://www.cbs.dtu.dk/services/DiscoTope/	conformational epitopes
ElliPro	http://tools.iedb.org/ellipro/	linear and conformational epitopes
EPCES	http://sysbio.unl.edu/EPCES/	conformational epitopes
EpiSearch	http://curie.utmb.edu/episearch.html	conformational epitopes
Epitopia	http://epitopia.tau.ac.il/	linear and conformational epitopes
EPMeta	http://sysbio.unl.edu/EPMeta/	conformational epitopes
EPSVR	http://sysbio.unl.edu/EPSVR/	conformational epitopes
MIMOX	http://immunet.cn/mimox	conformational epitopes
PEPITO/BEPro	http://pepito.proteomics.ics.uci.edu/	conformational epitopes
Pepitope	http://pepitope.tau.ac.il	linear and conformational epitopes

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possible epitope region.[71,73] A list of B-cell epitope prediction tools are shown in Table 7.

Application of Allergen Epitope Information. Allergen epitope mapping is important for not only understanding the mechanism of food allergy pathogenesis and prediction of potential allergens but also developing effective processing methods that can mitigate food allergenicity. Genetic engineering technology and a myriad of food processing methods have been applied for the reduction or elimination of allergen reactivity.[74,75] Thermal processing and protein/peptide hydrolysis, enzymatic as well as chemical, may or may not result in sufficient destruction of the targeted epitope on an allergen. In the case of the latter, the epitope exhibiting such stability is of concern because it may survive food processing and *in vivo* digestion. For these reasons, understanding the 3D structure of an allergen is useful because it may assist designing suitable conditions and/or structural modifications to reduce/eliminate the immunogenicity or immunoreactivity of the allergen of interest. Every peptide, regardless of its length, and every amino acid occupy finite 3D space. The amino acid side chains, either inherently or through their interactions with the neighboring molecules further add to the complexity of the 3D structure and its potential importance in understanding physicochemical behavior and immunoreactivity of the targeted allergen/epitope. In processed foods, one may anticipate considerable destruction of conformational epitopes with a consequent reduction/elimination of the immunoreactivity. For example, it has been demonstrated that boiling, glycation, and frying downregulate the allergenicity of Ara h 2 in Balb/c mice by reducing the core epitope binding capacity.[76] To assess the effectiveness of the processing methods, oral challenges,[77] animal studies,[76] or a substantial amount of patient IgEs[78] are often required. Animal antibodies recognizing clinically relevant allergenic epitopes have been developed and used as surrogates for such tests.[29,30,79] When the effect of processing on allergen immunoreactivity is assessed using *in vitro* assays, one has to consider that the reduction/elimination of the signal may at least be partly attributed to the loss of protein solubility caused by thermal aggregation and/or the matrix effect (e.g., insoluble protein−polyphenol complex formation).[29] The insoluble allergens may still be active and capable of eliciting clinical symptoms.

Identification of IgE epitopes may also provide information on the clinical history of patients and contribute to food allergy diagnosis and prognosis.[26] Although the double-blind placebo-controlled food challenge remains the gold standard for food allergy diagnosis, it is not always practical to perform the oral challenges as a result of the time requirement and potential risk of severe reactions. Development of diagnostic methods without the need for an oral food challenge is therefore warranted. IgE reactivity of informative epitopes has exhibited great potential in allergy diagnosis with better diagnostic efficiency than the use of whole allergen.[33] A correlation has been found between linear IgE epitope diversity and severity and persistence of egg, milk, peanut, and wheat allergies. Several milk allergen epitopes have been used as biomarkers to predict if a patient can outgrow milk allergy.[26]

Both B-cell and T-cell epitopes have been used in designing immunotherapy for food allergy. B-cell-epitope-based immunotherapies aim to modify IgE-binding epitopes. For example, thermally processed milk and egg with IgE-binding conformational epitopes destroyed markedly accelerated the development of tolerance to such foods in oral immunotherapy.[80,81] Engineered recombinant Ara h 1, Ara h 2, and Ara h 3 with major IgE-binding epitopes modified by amino acid substitution have shown a persistent protective effect in a murine model.[82] However, these engineered allergen vaccines were not successful in desensitizing peanut allergic patients and caused severe allergic reactions in 20% of the patients.[83] T-cell epitopes, on the other hand, are safer alternates with negligible IgE binding and inflammatory cell stimulatory capacities.[84] They can shift the T_H1/T_H2 balance toward T_H1 response and induce immune regulation by regulatory T-cells. Oral immunotherapies using T-cell epitopes have been reported to ameliorate allergic responses in murine models of egg and shrimp allergies.[85,86]

In conclusion, an epitope is defined as the accessible part of an antigen that interacts with an antibody or a T-cell receptor. Knowledge of the allergen epitope characteristics is pivotal for understanding the pathogenesis of food allergy and developing detection, diagnostic, and treatment methods. Mapping of the allergen epitopes have been pursued through strategies and techniques, such as overlapping peptide synthesis, chimeric molecule production, large-scale mutagenesis, phage display, immunoassay, mass spectrometry, X-ray crystallography, and/or NMR. Algorithms have been developed for *in silico* epitope prediction. B-cell conformational epitopes have important implications in food allergy but receive much less attention compared to linear epitopes and, therefore, warrant further investigations. Available information suggests significant efforts are needed to improve our understanding of the role of the food protein structure and allergen immunoreactivity to effectively address the food safety.

AUTHOR INFORMATION

Corresponding Authors

*Telephone: 619-594-7753. E-mail: changqi.liu@sdsu.edu.
*Telephone: 850-644-5837. E-mail: ssathe@fsu.edu.

ORCID

Shridhar K. Sathe: 0000-0001-8339-2473

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Notes

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ABBREVIATIONS USED

ELISA, enzyme-linked immunosorbent assay; IEDB, Immune Epitope Database; IgG, immunoglobulin G; IgE, immunoglobulin E; IL, interleukin; MHC, major histocompatibility complex; NIAID, National Institute of Allergy and Infectious Diseases; NMR, nuclear magnetic resonance

REFERENCES

(1) Sicherer, S. H. Epidemiology of food allergy. *J. Allergy Clin. Immunol.* **2011**, *127*, 594−602.

(2) Javed, B.; Padfield, P.; Sperrin, M.; Simpson, A.; Mills, E. C. A protocol for a systematic review to identify allergenic tree nuts and the

DOI: 10.1021/acs.jafc.8b01967
J. Agric. Food Chem. 2018, 66, 7238−7248

molecules responsible for their allergenic properties. *Food Chem. Toxicol.* **2017**, *106*, 411−416.
(3) Sicherer, S. H.; Sampson, H. A. Food Allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention and management. *J. Allergy Clin. Immunol.* **2018**, *141*, 41−58.
(4) Boyce, J. A.; Assa'ad, A.; Burks, A. W.; Jones, S. M.; Sampson, H. A.; Wood, R. A.; Plaut, M.; Cooper, S. F.; Fenton, M. J.; Arshad, S. H. Guidelines for the diagnosis and management of food allergy in the United States: Summary of the NIAID-sponsored expert panel report. *J. Am. Acad. Dermatol.* **2011**, *64*, 175−192.
(5) Valenta, R.; Hochwallner, H.; Linhart, B.; Pahr, S. Food allergies: The basics. *Gastroenterology* **2015**, *148*, 1120−1131.e4.
(6) Murphy, K.; Weaver, C. *Janeway's Immunobiology*, 9th ed.; Garland Science: New York, 2017.
(7) Nowak-Węgrzyn, A.; Chehade, M.; Groetch, M. E.; Spergel, J. M.; Wood, R. A.; Allen, K.; Atkins, D.; Bahna, S.; Barad, A. V.; Berin, C. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary—Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. *J. Allergy Clin. Immunol.* **2017**, *139*, 1111−1126.e4.
(8) Wai, C. Y. Y.; Leung, N. Y. H.; Leung, P. S. C.; Chu, K. H. Immunotherapy of food allergy: A comprehensive review. *Clin. Rev. Allergy Immunol.* **2017**, 1−19.
(9) Immune Epitope Database (IEDB). http://www.iedb.org/ (accessed Feb 2, 2018).
(10) Vita, R.; Zarebski, L.; Greenbaum, J. A.; Emami, H.; Hoof, I.; Salimi, N.; Damle, R.; Sette, A.; Peters, B. The immune epitope database 2.0. *Nucleic Acids Res.* **2010**, *38*, D854−D862.
(11) Sharma, G. M.; Irsigler, A.; Dhanarajan, P.; Ayuso, R.; Bardina, L.; Sampson, H. A.; Roux, K. H.; Sathe, S. K. Cloning and characterization of 2S albumin, Car i 1, a major allergen in pecan. *J. Agric. Food Chem.* **2011**, *59*, 4130−4139.
(12) Xia, L.; Willison, L. N.; Porter, L.; Robotham, J. M.; Teuber, S. S.; Sathe, S. K.; Roux, K. H. Mapping of a conformational epitope on the cashew allergen Ana o 2: A discontinuous large subunit epitope dependent upon homologous or heterologous small subunit association. *Mol. Immunol.* **2010**, *47*, 1808−1816.
(13) Robotham, J. M.; Xia, L.; Willison, L. N.; Teuber, S. S.; Sathe, S. K.; Roux, K. H. Characterization of a cashew allergen, 11S globulin (Ana o 2), conformational epitope. *Mol. Immunol.* **2010**, *47*, 1830−1838.
(14) Zhang, Q.; Willison, L. N.; Tripathi, P.; Sathe, S. K.; Roux, K. H.; Emmett, M. R.; Blakney, G. T.; Zhang, H.-M.; Marshall, A. G. Epitope mapping of a 95 kDa antigen in complex with antibody by solution-phase amide backbone hydrogen/deuterium exchange monitored by Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* **2011**, *83*, 7129−7136.
(15) Zhang, Q.; Noble, K. A.; Mao, Y.; Young, N. L.; Sathe, S. K.; Roux, K. H.; Marshall, A. G. Rapid screening for potential epitopes reactive with a polycolonal antibody by solution-phase H/D exchange monitored by FT-ICR mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2013**, *24*, 1016−1025.
(16) Malherbe, L. T-cell epitope mapping. *Ann. Allergy, Asthma, Immunol.* **2009**, *103*, 76−79.
(17) Marrack, P.; Scott-Browne, J. P.; Dai, S.; Gapin, L.; Kappler, J. W. Evolutionarily conserved amino acids that control TCR-MHC interaction. *Annu. Rev. Immunol.* **2008**, *26*, 171−203.
(18) Aalberse, R. C. Structural biology of allergens. *J. Allergy Clin. Immunol.* **2000**, *106*, 228−238.
(19) Hileman, R. E.; Silvanovich, A.; Goodman, R. E.; Rice, E. A.; Holleschak, G.; Astwood, J. D.; Hefle, S. L. Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. *Int. Arch. Allergy Immunol.* **2002**, *128*, 280−291.
(20) Ayuso, R.; Lehrer, S.; Reese, G. Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). *Int. Arch. Allergy Immunol.* **2002**, *127*, 27−37.
(21) Ayuso, R.; Sánchez-Garcia, S.; Lin, J.; Fu, Z.; Ibáñez, M. D.; Carrillo, T.; Blanco, C.; Goldis, M.; Bardina, L.; Sastre, J.; Sampson, H. A. Greater epitope recognition of shrimp allergens by children than by adults suggests that shrimp sensitization decreases with age. *J. Allergy Clin. Immunol.* **2010**, *125*, 1286−1293.e3.
(22) Elsayed, S.; Apold, J.; Holen, E.; Vik, H.; Florvaag, E.; Dybendal, T. The structural requirements of epitopes with IgE binding capacity demonstrated by three major allergens from fish, egg and tree pollen. *Scand. J. Clin. Lab. Invest.* **1991**, *51*, 17−31.
(23) Van Regenmortel, M. H. Mapping epitope structure and activity: From one-dimensional prediction to four-dimensional description of antigenic specificity. *Methods* **1996**, *9*, 465−472.
(24) Barlow, D.; Edwards, M.; Thornton, J. Continuous and discontinuous protein antigenic determinants. *Nature* **1986**, *322*, 747.
(25) Lin, J.; Sampson, H. A. The role of immunoglobulin E-binding epitopes in the characterization of food allergy. *Curr. Opin. Allergy Clin. Immunol.* **2009**, *9*, 357−363.
(26) Willison, L. N.; Zhang, Q.; Su, M.; Teuber, S. S.; Sathe, S. K.; Roux, K. H. Conformational epitope mapping of Pru du 6, a major allergen from almond nut. *Mol. Immunol.* **2013**, *55*, 253−263.
(27) Mandalari, G.; Rigby, N. M.; Bisignano, C.; Lo Curto, R. B.; Mulholland, F.; Su, M.; Venkatachalam, M.; Robotham, J. M.; Willison, L. N.; Lapsley, K.; Roux, K. H.; Sathe, S. K. Effect of food matrix and processing on release of almond protein during simulated digestion. *LWT—Food Sci. Technol.* **2014**, *59*, 439−447.
(28) Su, M.; Liu, C.; Roux, K. H.; Gradziel, T. M.; Sathe, S. K. Effects of processing and storage on almond (*Prunus dulcis* L.) amandin immunoreactivity. *Food Res. Int.* **2017**, *100*, 87−95.
(29) Chhabra, G. S.; Liu, C.; Su, M.; Venkatachalam, M.; Roux, K. H.; Sathe, S. K. Effects of the Maillard reaction on the immunoreactivity of amandin in food matrices. *J. Food Sci.* **2017**, *82*, 2495−2503.
(30) Albrecht, M.; Kühne, Y.; Ballmer-Weber, B. K.; Becker, W.-M.; Holzhauser, T.; Lauer, I.; Reuter, A.; Randow, S.; Falk, S.; Wangorsch, A.; Lidholm, J.; Reese, G.; Vieths, S. Relevance of IgE binding to short peptides for the allergenic activity of food allergens. *J. Allergy Clin. Immunol.* **2009**, *124*, 328−336.e6.
(31) Otsu, K.; Guo, R.; Dreskin, S. Epitope analysis of Ara h 2 and Ara h 6: Characteristic patterns of IgE-binding fingerprints among individuals with similar clinical histories. *Clin. Exp. Allergy* **2015**, *45*, 471−484.
(32) Ayuso, R.; Sánchez-Garcia, S.; Pascal, M.; Lin, J.; Grishina, G.; Fu, Z.; Ibáñez, M.; Sastre, J.; Sampson, H. Is epitope recognition of shrimp allergens useful to predict clinical reactivity? *Clin. Exp. Allergy* **2012**, *42*, 293−304.
(33) Fötisch, K.; Vieths, S. N- and O-linked oligosaccharides of allergenic glycoproteins. *Glycoconjugate J.* **2001**, *18*, 373−390.
(34) Altmann, F. The role of protein glycosylation in allergy. *Int. Arch. Allergy Immunol.* **2007**, *142*, 99−115.
(35) van Ree, R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. *Int. Arch. Allergy Immunol.* **2002**, *129*, 189−197.
(36) Apostolovic, D.; Tran, T.; Hamsten, C.; Starkhammar, M.; Cirkovic Velickovic, T.; van Hage, M. Immunoproteomics of processed beef proteins reveal novel galactose-α-1,3-galactose-containing allergens. *Allergy* **2014**, *69*, 1308−1315.
(37) Kaneko, K.; Watanabe, Y.; Kimura, K.; Matsumoto, K.; Mizobuchi, T.; Onoue, M. Development of hypoallergenic galacto-oligosaccharides on the basis of allergen analysis. *Biosci., Biotechnol., Biochem.* **2014**, *78*, 100−108.
(38) DeLong, J. H.; Simpson, K. H.; Wambre, E.; James, E. A.; Robinson, D.; Kwok, W. W. Ara h 1−reactive T cells in individuals with peanut allergy. *J. Allergy Clin. Immunol.* **2011**, *127*, 1211−1218.e3.
(39) Ramesh, M.; Yuenyongviwat, A.; Konstantinou, G. N.; Lieberman, J.; Pascal, M.; Masilamani, M.; Sampson, H. A. Peanut T-cell epitope discovery: Ara h 1. *J. Allergy Clin. Immunol.* **2016**, *137*, 1764−1771.e4.
(40) Prickett, S. R.; Voskamp, A. L.; Dacumos-Hill, A.; Symons, K.; Rolland, J. M.; O'hehir, R. E. Ara h 2 peptides containing dominant

DOI: 10.1021/acs.jafc.8b01967
J. Agric. Food Chem. 2018, 66, 7238−7248

CD4+ T-cell epitopes: Candidates for a peanut allergy therapeutic. *J. Allergy Clin. Immunol.* **2011**, *127*, 608−615.e5.
(41) Turcanu, V.; Maleki, S. J.; Lack, G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J. Clin. Invest.* **2003**, *111*, 1065−1072.
(42) Shanti, K.; Martin, B. M.; Nagpal, S.; Metcalfe, D. D.; Rao, P. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *J. Immunol.* **1993**, *151*, 5354−5363.
(43) Selo, I.; Clement, G.; Bernard, H.; Chatel, J.; Creminon, C.; Peltre, G.; Wal, J. Allergy to bovine β-lactoglobulin: Specificity of human IgE to tryptic peptides. *Clin. Exp. Allergy* **1999**, *29*, 1055−1063.
(44) Song, C. Y.; Chen, W. L.; Yang, M. C.; Huang, J. P.; Mao, S. J. Epitope mapping of a monoclonal antibody specific to bovine dry milk involvement of residues 66−76 of strand d in thermal denatured β-lactoglobulin. *J. Biol. Chem.* **2005**, *280*, 3574−3582.
(45) Maleki, S. J.; Teuber, S. S.; Cheng, H.; Chen, D.; Comstock, S. S.; Ruan, S.; Schein, C. H. Computationally predicted IgE epitopes of walnut allergens contribute to cross-reactivity with peanuts. *Allergy* **2011**, *66*, 1522−1529.
(46) Beardslee, T. A.; Zeece, M. G.; Sarath, G.; Markwell, J. P. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int. Arch. Allergy Immunol.* **2000**, *123*, 299−307.
(47) Robotham, J. M.; Teuber, S. S.; Sathe, S. K.; Roux, K. H. Linear IgE epitope mapping of the English walnut (*Juglans regia*) major food allergen, Jug r 1. *J. Allergy Clin. Immunol.* **2002**, *109*, 143−149.
(48) Pahr, S.; Selb, R.; Weber, M.; Focke-Tejkl, M.; Hofer, G.; Dordić, A.; Keller, W.; Papadopoulos, N. G.; Giavi, S.; Mäkelä, M.; Pelkonen, A.; Niederberger, V.; Vrtala, S.; Valenta, R. Biochemical, biophysical and IgE-epitope characterization of the wheat food allergen, Tri a 37. *PLoS One* **2014**, *9*, e111483.
(49) Lin, J.; Bardina, L.; Shreffler, W. G.; Andreae, D. A.; Ge, Y.; Wang, J.; Bruni, F. M.; Fu, Z.; Han, Y.; Sampson, H. A. Development of a novel peptide microarray for large-scale epitope mapping of food allergens. *J. Allergy Clin. Immunol.* **2009**, *124*, 315−322.e3.
(50) Pomés, A. Relevant B cell epitopes in allergic disease. *Int. Arch. Allergy Immunol.* **2010**, *152*, 1−11.
(51) Gershoni, J. M.; Roitburd-Berman, A.; Siman-Tov, D. D.; Tarnovitski Freund, N.; Weiss, Y. Epitope mapping. *BioDrugs* **2007**, *21*, 145−156.
(52) Alcocer, M. J.; Murtagh, G. J.; Wilson, P. B.; Progias, P.; Lin, J.; Archer, D. B. The major human structural IgE epitope of the Brazil nut allergen Ber e 1: A chimaeric and protein microarray approach. *J. Mol. Biol.* **2004**, *343*, 759−769.
(53) Pacios, L. F.; Tordesillas, L.; Cuesta-Herranz, J.; Compes, E.; Sánchez-Monge, R.; Palacín, A.; Salcedo, G.; Díaz-Perales, A. Mimotope mapping as a complementary strategy to define allergen IgE-epitopes: Peach Pru p 3 allergen as a model. *Mol. Immunol.* **2008**, *45*, 2269−2276.
(54) Untersmayr, E.; Szalai, K.; Riemer, A. B.; Hemmer, W.; Swoboda, I.; Hantusch, B.; Schöll, I.; Spitzauer, S.; Scheiner, O.; Jarisch, R.; Boltz-Nitulescu, G.; Jensen-Jarolim, E. Mimotopes identify conformational epitopes on parvalbumin, the major fish allergen. *Mol. Immunol.* **2006**, *43*, 1454−1461.
(55) Amit, A.; Mariuzza, R.; Phillips, S.; Poljak, R. Three-dimensional structure of an antigen-antibody complex at 2.8 A resolution. *Science* **1986**, *233*, 747−753.
(56) Sheriff, S.; Silverton, E. W.; Padlan, E. A.; Cohen, G. H.; Smith-Gill, S. J.; Finzel, B. C.; Davies, D. R. Three-dimensional structure of an antibody-antigen complex. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 8075−8079.
(57) Padlan, E. A.; Silverton, E. W.; Sheriff, S.; Cohen, G. H.; Smith-Gill, S. J.; Davies, D. R. Structure of an antibody-antigen complex: Crystal structure of the HyHEL-10 Fab-lysozyme complex. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 5938−5942.
(58) Braden, B. C.; Souchon, H.; Eiselé, J.-L.; Bentley, G. A.; Bhat, T. N.; Navaza, J.; Poljak, R. J. Three-dimensional structures of the free and the antigen-complexed Fab from monoclonal anti-lysozyme antibody D44. 1. *J. Mol. Biol.* **1994**, *243*, 767−781.
(59) Li, Y.; Li, H.; Smith-Gill, S. J.; Mariuzza, R. A. Three-dimensional structures of the free and antigen-bound Fab from monoclonal antilysozyme antibody HyHEL-63. *Biochemistry* **2000**, *39*, 6296−6309.
(60) Li, Y.; Li, H.; Yang, F.; Smith-Gill, S. J.; Mariuzza, R. A. X-ray snapshots of the maturation of an antibody response to a protein antigen. *Nat. Struct. Mol. Biol.* **2003**, *10*, 482.
(61) Cauerhff, A.; Goldbaum, F. A.; Braden, B. C. Structural mechanism for affinity maturation of an anti-lysozyme antibody. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 3539−3544.
(62) De Genst, E.; Silence, K.; Decanniere, K.; Conrath, K.; Loris, R.; Kinne, J.; Muyldermans, S.; Wyns, L. Molecular basis for the preferential cleft recognition by dromedary heavy-chain antibodies. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 4586−4591.
(63) Stanfield, R. L.; Dooley, H.; Flajnik, M. F.; Wilson, I. A. Crystal structure of a shark single-domain antibody V region in complex with lysozyme. *Science* **2004**, *305*, 1770−1773.
(64) Stanfield, R. L.; Dooley, H.; Verdino, P.; Flajnik, M. F.; Wilson, I. A. Maturation of shark single-domain (IgNAR) antibodies: Evidence for induced-fit binding. *J. Mol. Biol.* **2007**, *367*, 358−372.
(65) Niemi, M.; Jylhä, S.; Laukkanen, M.-L.; Söderlund, H.; Mäkinen-Kiljunen, S.; Kallio, J. M.; Hakulinen, N.; Haahtela, T.; Takkinen, K.; Rouvinen, J. Molecular interactions between a recombinant IgE antibody and the β-lactoglobulin allergen. *Structure* **2007**, *15*, 1413−1421.
(66) Bublin, M.; Kostadinova, M.; Fuchs, J. E.; Ackerbauer, D.; Moraes, A. H.; Almeida, F. C.; Lengger, N.; Hafner, C.; Ebner, C.; Radauer, C.; Liedl, K. R.; Valente, A. P.; Breiteneder, H. A cross-reactive human single-chain antibody for detection of major fish allergens, parvalbumins, and identification of a major IgE-binding epitope. *PLoS One* **2015**, *10*, e0142625.
(67) Wilson, I. B.; Zeleny, R.; Kolarich, D.; Staudacher, E.; Stroop, C. J.; Kamerling, J. P.; Altmann, F. Analysis of Asn-linked glycans from vegetable foodstuffs: Widespread occurrence of Lewis a, core α1, 3-linked fucose and xylose substitutions. *Glycobiology* **2001**, *11*, 261−274.
(68) Apostolovic, D.; Krstic, M.; Mihailovic, J.; Starkhammar, M.; Cirkovic Velickovic, T.; Hamsten, C.; van Hage, M. Peptidomics of an in vitro digested α-Gal carrying protein revealed IgE-reactive peptides. *Sci. Rep.* **2017**, *7*, 5201.
(69) Bublin, M.; Radauer, C.; Wilson, I. B.; Kraft, D.; Scheiner, O.; Breiteneder, H.; Hoffmann-Sommergruber, K. Cross-reactive N-glycans of Api g 5, a high molecular weight glycoprotein allergen from celery, are required for immunoglobulin E binding and activation of effector cells from allergic patients. *FASEB J.* **2003**, *17*, 1697−1699.
(70) Foetisch, K.; Westphal, S.; Lauer, I.; Retzek, M.; Altmann, F.; Kolarich, D.; Scheurer, S.; Vieths, S. Biological activity of IgE specific for cross-reactive carbohydrate determinants. *J. Allergy Clin. Immunol.* **2003**, *111*, 889−896.
(71) Fu, Z.; Lin, J. An overview of bioinformatics tools and resources in allergy. In *Food Allergens. Methods in Molecular Biology*; Lin, J., Alcocer, M., Eds.; Humana Press: New York, 2017; Vol. *1592*, pp 223−245, DOI: 10.1007/978-1-4939-6925-8_18.
(72) Nielsen, M.; Lund, O.; Buus, S.; Lundegaard, C. MHC class II epitope predictive algorithms. *Immunology* **2010**, *130*, 319−328.
(73) Greenbaum, J. A.; Andersen, P. H.; Blythe, M.; Bui, H. H.; Cachau, R. E.; Crowe, J.; Davies, M.; Kolaskar, A.; Lund, O.; Morrison, S.; Mumey, B.; Ofran, Y.; Pellequer, J.-L.; Pinilla, C.; Ponomarenko, J. V.; Raghava, G. P. S.; van Regenmortel, M. H. V.; Roggen, E. L.; Sette, A.; Schlessinger, A.; Sollner, J.; Zand, M.; Peters, B. Towards a consensus on datasets and evaluation metrics for developing B-cell epitope prediction tools. *J. Mol. Recognit.* **2007**, *20*, 75−82.
(74) Sathe, S. K.; Teuber, S. S.; Roux, K. H. Effects of food processing on the stability of food allergens. *Biotechnol. Adv.* **2005**, *23*, 423−429.

DOI: 10.1021/acs.jafc.8b01967
J. Agric. Food Chem. 2018, 66, 7238−7248

(75) Paschke, A. Aspects of food processing and its effect on allergen structure. *Mol. Nutr. Food Res.* **2009**, *53*, 959−962.
(76) Zhang, W.; Zhu, Q.; Zhang, T.; Cai, Q.; Chen, Q. Thermal processing effects on peanut allergen Ara h 2 allergenicity in mice and its antigenic epitope structure. *Food Chem.* **2016**, *212*, 657−662.
(77) Lemon-Mulé, H.; Sampson, H. A.; Sicherer, S. H.; Shreffler, W. G.; Noone, S.; Nowak-Wegrzyn, A. Immunologic changes in children with egg allergy ingesting extensively heated egg. *J. Allergy Clin. Immunol.* **2008**, *122*, 977−983.e1.
(78) Cai, Q.; Zhang, W.-j.; Zhu, Q.-q.; Chen, Q. Influence of heat treatment on the structure and core IgE-binding epitopes of rAra h 2.02. *Food Chem.* **2016**, *202*, 404−408.
(79) Zhao, L.; Zhao, L.; Zhang, B.; Robotham, J. M.; Roux, K. H.; Tang, H. Identification of a common Ara h 3 epitope recognized by both the capture and the detection monoclonal antibodies in an ELISA detection kit. *PLoS One* **2017**, *12*, e0182935.
(80) Kim, J. S.; Nowak-Węgrzyn, A.; Sicherer, S. H.; Noone, S.; Moshier, E. L.; Sampson, H. A. Dietary baked milk accelerates the resolution of cow's milk allergy in children. *J. Allergy Clin. Immunol.* **2011**, *128*, 125−131.e2.
(81) Leonard, S. A.; Sampson, H. A.; Sicherer, S. H.; Noone, S.; Moshier, E. L.; Godbold, J.; Nowak-Węgrzyn, A. Dietary baked egg accelerates resolution of egg allergy in children. *J. Allergy Clin. Immunol.* **2012**, *130*, 473−480.e1.
(82) Li, X.-M.; Srivastava, K.; Grishin, A.; Huang, C.-K.; Schofield, B.; Burks, W.; Sampson, H. A. Persistent protective effect of heat-killed Escherichia coli producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. *J. Allergy Clin. Immunol.* **2003**, *112*, 159−167.
(83) Wood, R. A.; Sicherer, S. H.; Burks, A. W.; Grishin, A.; Henning, A. K.; Lindblad, R.; Stablein, D.; Sampson, H. A. A phase 1 study of heat/phenol-killed, E. coli-encapsulated, recombinant modified peanut proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) for the treatment of peanut allergy. *Allergy* **2013**, *68*, 803−808.
(84) Prickett, S.; Rolland, J.; O'hehir, R. Immunoregulatory T cell epitope peptides: The new frontier in allergy therapy. *Clin. Exp. Allergy* **2015**, *45*, 1015−1026.
(85) Rupa, P.; Mine, Y. Oral immunotherapy with immunodominant T-cell epitope peptides alleviates allergic reactions in a Balb/c mouse model of egg allergy. *Allergy* **2012**, *67*, 74−82.
(86) Wai, C.; Leung, N.; Leung, P.; Chu, K. T cell epitope immunotherapy ameliorates allergic responses in a murine model of shrimp allergy. *Clin. Exp. Allergy* **2016**, *46*, 491−503.
(87) Archila, L. D.; Chow, I. T.; McGinty, J. W.; Renand, A.; Jeong, D.; Robinson, D.; Farrington, M. L.; Kwok, W. W. Ana o 1 and Ana o 2 cashew allergens share cross-reactive CD4+ T cell epitopes with other tree nuts. *Clin. Exp. Allergy* **2016**, *46*, 871−883.
(88) Jahn-Schmid, B.; Radakovics, A.; Lüttkopf, D.; Scheurer, S.; Vieths, S.; Ebner, C.; Bohle, B. Bet v. 1142−156 is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1−related food allergens. *J. Allergy Clin. Immunol.* **2005**, *116*, 213−219.
(89) Yang, M.; Yang, C.; Mine, Y. Multiple T cell epitope peptides suppress allergic responses in an egg allergy mouse model by the elicitation of forkhead box transcription factor 3- and transforming growth factor-β-associated mechanisms. *Clin. Exp. Allergy* **2010**, *40*, 668−678.
(90) Ruiter, B.; Tregoat, V.; M'rabet, L.; Garssen, J.; Bruijnzeel-Koomen, C.; Knol, E.; Hoffen, E. Characterization of T cell epitopes in α_{s1}-casein in cow's milk allergic, atopic and non-atopic children. *Clin. Exp. Allergy* **2006**, *36*, 303−310.
(91) Ravkov, E. V.; Pavlov, I. Y.; Martins, T. B.; Gleich, G. J.; Wagner, L. A.; Hill, H. R.; Delgado, J. C. Identification and validation of shrimp-tropomyosin specific CD4 T cell epitopes. *Hum. Immunol.* **2013**, *74*, 1542−1549.
(92) Garcia Alonso, M.; Caballero, M.; Umpierrez, A.; Lluch-Bernal, M.; Knaute, T.; Rodríguez-Pérez, R. Relationships between T cell and IgE/IgG4 epitopes of the *Anisakis simplex* major allergen Ani s 1. *Clin. Exp. Allergy* **2015**, *45*, 994−1005.
(93) Jin, T.; Guo, F.; Chen, Y.-w.; Howard, A.; Zhang, Y.-Z. Crystal structure of Ara h 3, a major allergen in peanut. *Mol. Immunol.* **2009**, *46*, 1796−1804.
(94) Robotham, J. M.; Hoffman, G. G.; Teuber, S. S.; Beyer, K.; Sampson, H. A.; Sathe, S. K.; Roux, K. H. Linear IgE-epitope mapping and comparative structural homology modeling of hazelnut and English walnut 11S globulins. *Mol. Immunol.* **2009**, *46*, 2975−2984.
(95) Saeed, H.; Gagnon, C.; Cober, E.; Gleddie, S. Using patient serum to epitope map soybean glycinins reveals common epitopes shared with many legumes and tree nuts. *Mol. Immunol.* **2016**, *70*, 125−133.
(96) Martínez-Botas, J.; Cerecedo, I.; Zamora, J.; Vlaicu, C.; Dieguez, M. C.; Gómez-Coronado, D.; De Dios, V.; Terrados, S.; De La Hoz, B. Mapping of the IgE and IgG4 sequential epitopes of ovomucoid with a peptide microarray immunoassay. *Int. Arch. Allergy Immunol.* **2013**, *161*, 11−20.
(97) Lisson, M.; Novak, N.; Erhardt, G. Immunoglobulin E epitope mapping by microarray immunoassay reveals differences in immune response to genetic variants of caseins from different ruminant species. *J. Dairy Sci.* **2014**, *97*, 1939−1954.
(98) Perez-Gordo, M.; Pastor-Vargas, C.; Lin, J.; Bardina, L.; Cases, B.; Ibáñez, M. D.; Vivanco, F.; Cuesta-Herranz, J.; Sampson, H. A. Epitope mapping of the major allergen from Atlantic cod in Spanish population reveals different IgE-binding patterns. *Mol. Nutr. Food Res.* **2013**, *57*, 1283−1290.
(99) Tordesillas, L.; Pacios, L.; Palacin, A.; Cuesta-Herranz, J.; Madero, M.; Díaz-Perales, A. Characterization of IgE epitopes of Cuc m 2, the major melon allergen, and their role in cross-reactivity with pollen profilins. *Clin. Exp. Allergy* **2010**, *40*, 174−181.
(100) Tordesillas, L.; Pacios, L. F.; Palacin, A.; Quirce, S.; Armentia, A.; Barber, D.; Salcedo, G.; Diaz-Perales, A. Molecular basis of allergen cross-reactivity: Non-specific lipid transfer proteins from wheat flour and peach fruit as models. *Mol. Immunol.* **2009**, *47*, 534−540.
(101) Scott, C.; Peterson, P.; Teyton, L.; Wilson, I. Crystal structures of two I-Ad−peptide complexes reveal that high affinity can be achieved without large anchor residues. *Immunity* **1998**, *8*, 319−329.

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J. Agric. Food Chem. 2018, 66, 7238−7248