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Main allergens in European Hornet (*Vespa crabo* Linnaeus, 1758) and Asian Hornet (*Vespa velutina* Lepetelier, 1836): A Comparative Study of Their Structural Properties †

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Abstract: The yellow-legged Asian hornet, (*Vespa velutina* Lepetelier, 1836), native to Southeast Asia, India, and China, was accidentally introduced into South Korea in 2003 and France in 2004. The spread of this invasive hornet throughout Europe has been very rapid, resulting in frequent stinging incidents. Various studies have been carried out on the nature of the protein components of the *Vespidae* venom. It has been found that the allergens of *Vespa velutina* (VV), the European hornet (VC, *Vespa crabo* Linnaeus, 1758) and the common wasp (*Vespula* spp) have a high similarity in their main proteins.The structures generated by homology-modelling for the antigen 5 (Vesp v 5) from Asian hornet together with the obtained for phospholipase A1 (Vesp c 1) and for the antigen 5 (Vesp c 5) from European hornet were studied using APBS (Adaptive Poisson-Boltzmann Solver), a continuum solvation method. The results obtained, along with other parameters calculated such as: LogP, VLogP, Molecular Lipophilicity Potential, among others, not only allow to check the high degree of similarity among these proteins but also to study the differences that make possible the analysis of their epitopes. The obtained information could be useful in the characterization of Hymenoptera venom allergy (HVA) as well as for specific immunotherapy, since knowledge of the structure of allergens is essential for the precise diagnosis and treatment of HVA.

1. Introduction

The yellow-legged Asian hornet, (*Vespa velutina* Lepetelier, 1836), native to Southeast Asia, India, and China, was accidentally introduced into South Korea in 2003 and France in 2004 [1]. Vespa velutina is one of the most aggressive and fearful Hymenoptera species in China where it is known as killer-wasp because it causes tens of deaths every year [2]. The spread of the invasive hornet throughout Europe has been very rapid, resulting in frequent stinging incidents [3], these stings can cause allergic reactions, being the most common cause of anaphylaxis due to hymenopterans in some European regions [4].

Various studies have been carried out on the nature of the protein components of the *Vespidae* venom. Recently, it has been reported that two relevant allergens in *Vespa velutina* (VV) are Vesp v 5 and glycosylated Vesp v1 with amino acid sequences of 202 (mass: 22,718 Da) and 304 (mass: 33,957 Da), respectively [5]. Antigens 5 are proteins of unknown function in Hymenoptera venoms with high allergenic potency. In addition to Phospholipase A1, antigen 5 represents one of the most important major venom allergens in almost all allergy-relevant Vespoidea species [6].

The invasion of VV in Europe makes the cases of stings in humans more and more frequent, and the tension of the moment means that the victims often do not accurately identify the species of hornet that attacked them, possibly because of the native species: *Vespa crabo* Linnaeus, 1758 (VC) or *Vespula* spp. Hymenoptera venom allergy (HVA) is one of the most common causes of anaphylaxis in adults and is frequently associated with severe anaphylaxis, a prevalence of up to 3.5% is reported in Europe, and causal treatment in the form of venom immunotherapy (VIT) is effective and well tolerated [7]. The similarity existing among Vespa and Vespula components would justify that the treatment of these patients with Vespula-containing preparations is really being effective in protecting these patients, as already shown for Vespa spp.-sensitized patients, that had been protected by immunotherapy with Vespula commercial preparations [8,9].

It has been found that the allergens of VV, VC and Vespula spp. have a high similarity in their main proteins [10]. A recent clinical study [4] opens the possibility that the already known validity of the VIT of Vespula spp. for VC [8] could be extended to VV.

To our knowledge to date, VV and VC antigens 5 have not been modelled in order to compare their three-dimensional structures and properties. So, with the objective of seeing interactions in analogous regions, we are interested in their study. This would constitute data in favour of their similarity in the anaphylactic reactions observed in patients with hornet stings, since not all the scientific community agree with this general therapy claiming for a species-specific one [11]. Here we present the preliminary results of our research on this field.

2. Methods, Results and Discussion

Coordinates for Vesp v 5 were modelled with SWISS-MODEL (a fully automated protein structure homology-modelling server), accessible via the ExPASy web server (https://swissmodel.expasy.org/) with the sequence determined by Monsalve et al. [10]. VC shows two variants of allergen 5, namely Vesp c 5.01 (P35781, [12]) and Vesp c 5.02 (P35782, [13]) [14]. Otherwise, RCSB PDB database [15] was used to retrieve coordinates for Vesp v 5 in Vespula vulgaris, which from now on we will refer to as 1QNX to avoid confusion with those corresponding to Vesp v 5 from VV.

The properties of the allergens were obtained with the VegaZZ software [16], calculating LogP, Lipole and VlogP. LogP is a molecular descriptor assimilated to the lipophilicity of the molecule, lipole is a measure of the lipophilicity distribution and it can be calculated as the sum of local values of log *P* and *Virtual logP*. The *Virtual logP* was obtained by the Molecular Lipophilicity Potential (*MLP*) that is calculated projecting the Broto-Moreau lipophilicity atomic constants on the molecular surface [17].

In patients with ascertained VC systemic reactions, a VC-specific VIT should be more adequate at least from the safety viewpoint. However, where VC inmunotherapy is not commercially available, the *Vespula spp.* VIT can be used with a comparable efficacy [18]. This idea, along with the fact that X-Ray determined coordinates are available for 1QNX [19], led us to the introduction of this allergen in this study.

The SPADE web service performs epitope predictions for allergens and IgE, based on structural data in terms of 3D atom coordinates and the cross-reactivity information [20]. SPADE computation steps include the pair-wise 3D alignment and comparison of reference against all other models, followed by the combination of surface similarity data in a cross-reactivity-weighted fashion. Finally, the amino acid residues with the highest probability to bind IgE are selected and spatially clustered. As standard of 100% cross-reactivity Vesp v 5 (PDB ID: 1QNX) was used.

Once the surface regions and their electrostatic sign have been defined, the search for a similarity in the antibody-allergen interaction can be considered. An additional filtering based on the molecular electrostatic potential (MEP) can be made and it could be checked whether Vesp v 5, Vesp c 5.01 and Vesp c 5.02 have the same type of interaction with IgE.

In each study, the structural location of the epitopes, the amino acid composition of each, and a graphic are depicted. The bar graph shows the individual similarity scores of the amino acid residues resulting from the combined comparisons. Putatively IgE-binding residues were filtered at

a threshold of 70. For every residue, the surface similarity score to its compared homologue is displayed as a bar, where the base line is at the epitope threshold. Residues with lower surface similarity get down-facing bars and are classified as non-IgE-binding. By definition, they cannot be part of a Predicted Epitope Patch (PEP). Among all surface residues with scores above the threshold (up-facing bars), those that are clustered in immediate spatial proximity become assigned to a PEP.

The seminal idea was to use 1QNX as a 100% standard for the cross-reactivity of VC and VV antigens, assigning them also a cross-reactivity of 100% to indicate to the program that it should interpret regions of high 1QNX surface similarity to Vesp c 5.01, Vesp c 5.02 and Vesp v 5 as likely IgE epitopes. For these inputs SPADE software identified 4 epitopes (Figure 1).

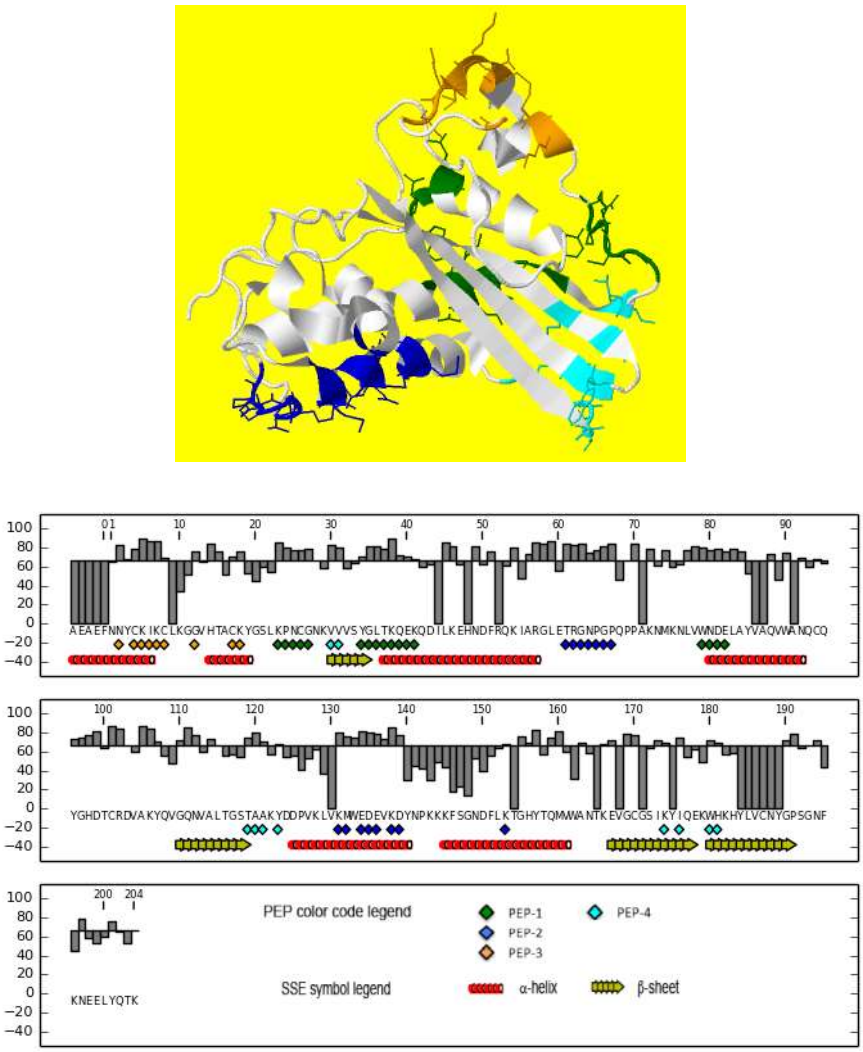


Figure 1. Epitopic regions on 1QNX structure and average similarity of surface residues, as compared between allergens.

- PEP1 with 17 residues: K23, P24, N25, C26, G27, Y34, G35, L36, T37, K38, Q39, E40, K41, W79, N80, D81, E82; area: 1153.2 Å^2, epitope FOM: 0.778, mean accessibility: 33.6%.
- PEP2 with 15 residues: T61, R62, G63, N64, P65, G66, P67, K131, M132, E134, D135, E136, K138, D139, K153. area: 995.5 Å^2, epitope FOM: 0.795, mean accessibility: 33.1%.
- PEP3 with 9 residues: N2, C4, K5, I6, K7, C8, G12, C17, K18; area: 772.0 Å^2, epitope FOM: 0.794, mean accessibility: 40.5%.
- PEP4 with 10 residues: V30, V31, T119, A120, A121, Y123, K174, I176, W180, H181; area: 671.5 Å^2, epitope FOM: 0.741, mean accessibility: 34.3%.

These results indicate that the three allergens studied had four possible epitopes for the IgE antibody. Then, it was studied each of them separately with the *Vespa vulgaris* v5 allergen (1QNX). For this, we followed two strategies, one using 1QNX as reference for 100% reactivity against each of the other antigens individually, and the other one using separately each one of the three antigens as a reference against 1QNX, considering each allergen as target structure and 1QNX as a cross-reactive (100%) allergen. The first approach using 1QNX as the reference is summarized below.

For Vesp c 5.01 allergen, SPADE predicted four PEP (Figure 2):

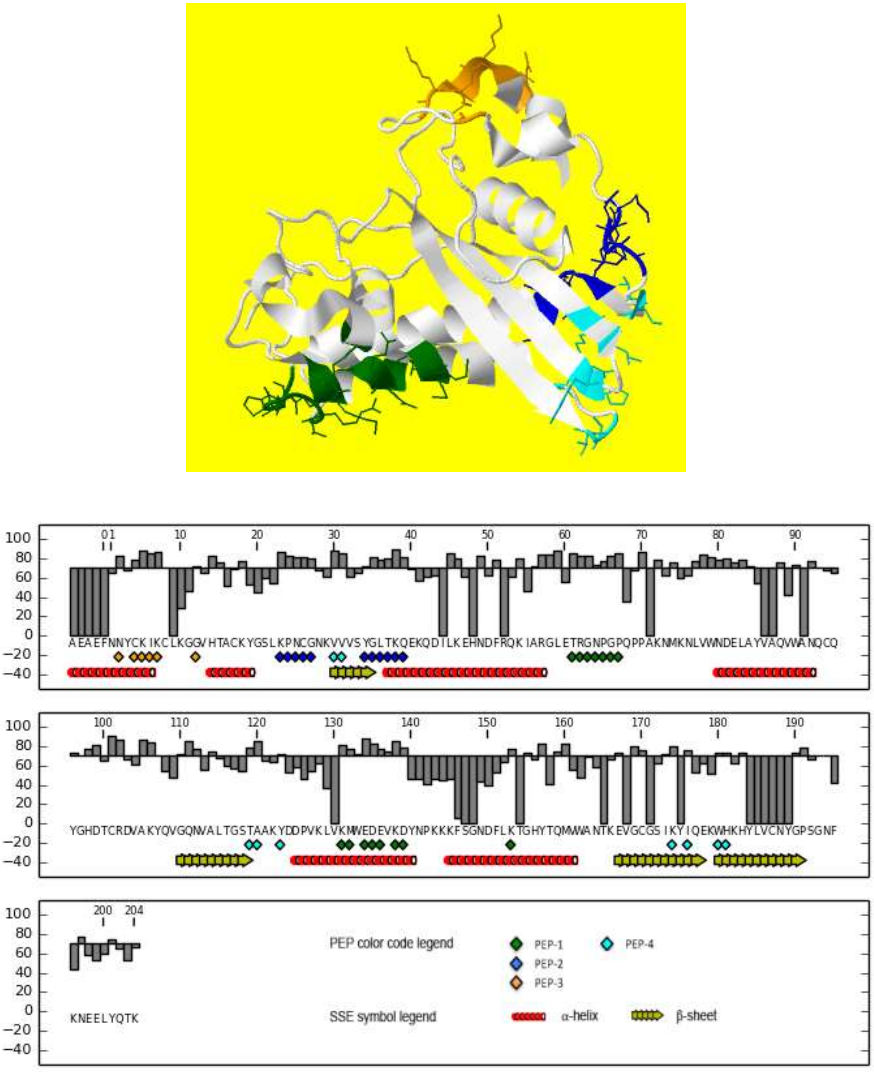


Figure 2. PEP on 1QNX structure and average similarity of surface residues, as compared between allergens.

- PEP1 with 15 residues: T61, R62, G63, N64, P65, G66, P67, K131, M132, E134, D135, E136, K138, D139, K153, area = 995.5 Å^2, epitope FOM: 0.809, mean accessibility: 33.1%
- PEP2 with eleven residues: K23, P24, N25, C26, G27, Y34, G35, L36, T37, K38, Q39, area: 861.1 Å^2, epitope FOM: 0.812, mean accessibility: 39.7%
- PEP3 with 6 residues: N2, C4, K5, I6, K7, G12; area: 637.9 Å^2, epitope FOM: 0.822, mean accessibility: 50.6%
- PEP4 with 9 residues: V30, V31, T119, A120, Y123, K174, I176, W180, H181; area: 598.0 Å^2, epitope FOM: 0.790, mean accessibility: 32.2%.

For Vesp c 5.02 allergen, SPADE only predicted two PEP (Figure 3):

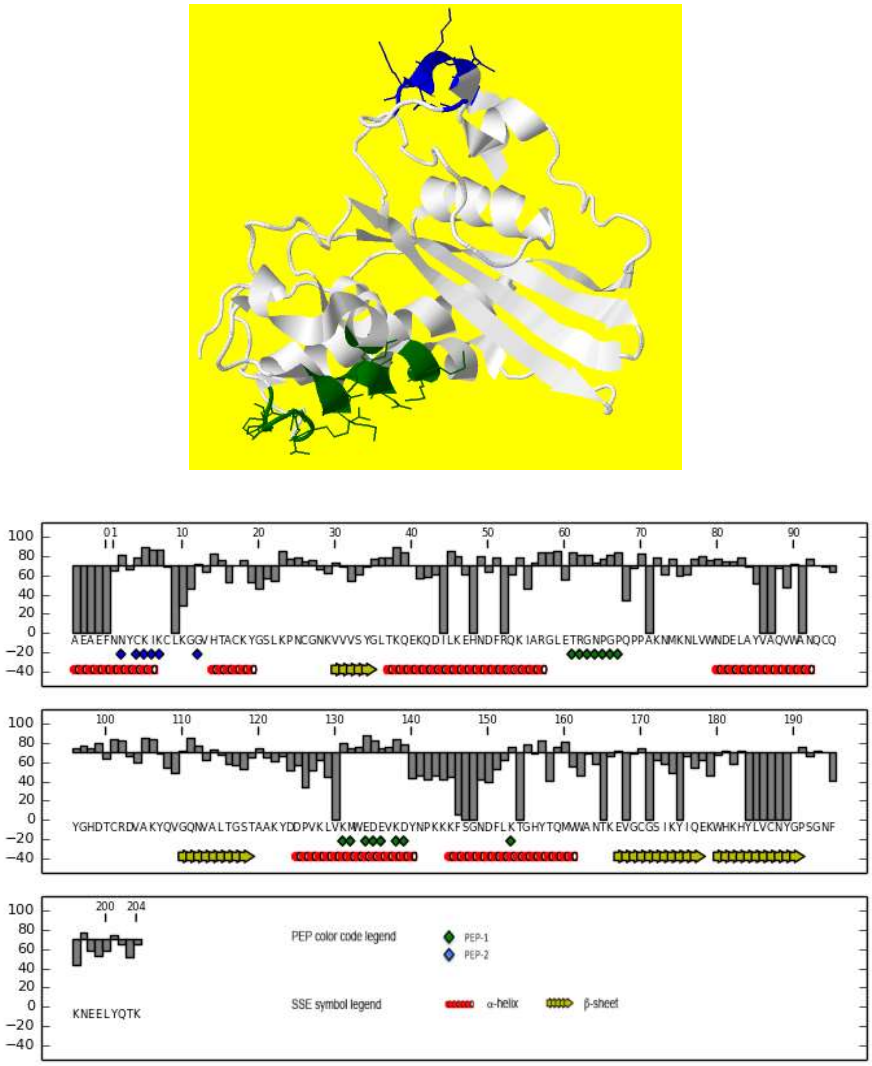


Figure 3. PEP on 1QNX structure and average similarity of surface residues, as compared between allergens.

- PEP1 with fifteen amino acids: T 61, R 62, G 63, N64, P65, G66, P67, K131, M132, E134, D135, E136, K138, D139, K153; area: 995.5 Å^2, epitope FOM: 0.799, mean accessibility: 33.1%.
- PEP2 with six components: N2, C4, K5, I6, K7, G12; area: 637.9 Å^2, epitope FOM: 0.823, mean accessibility: 50.6%.

For Vesp v 5 allergen, SPADE predicted three epitope patches (Figure 4):

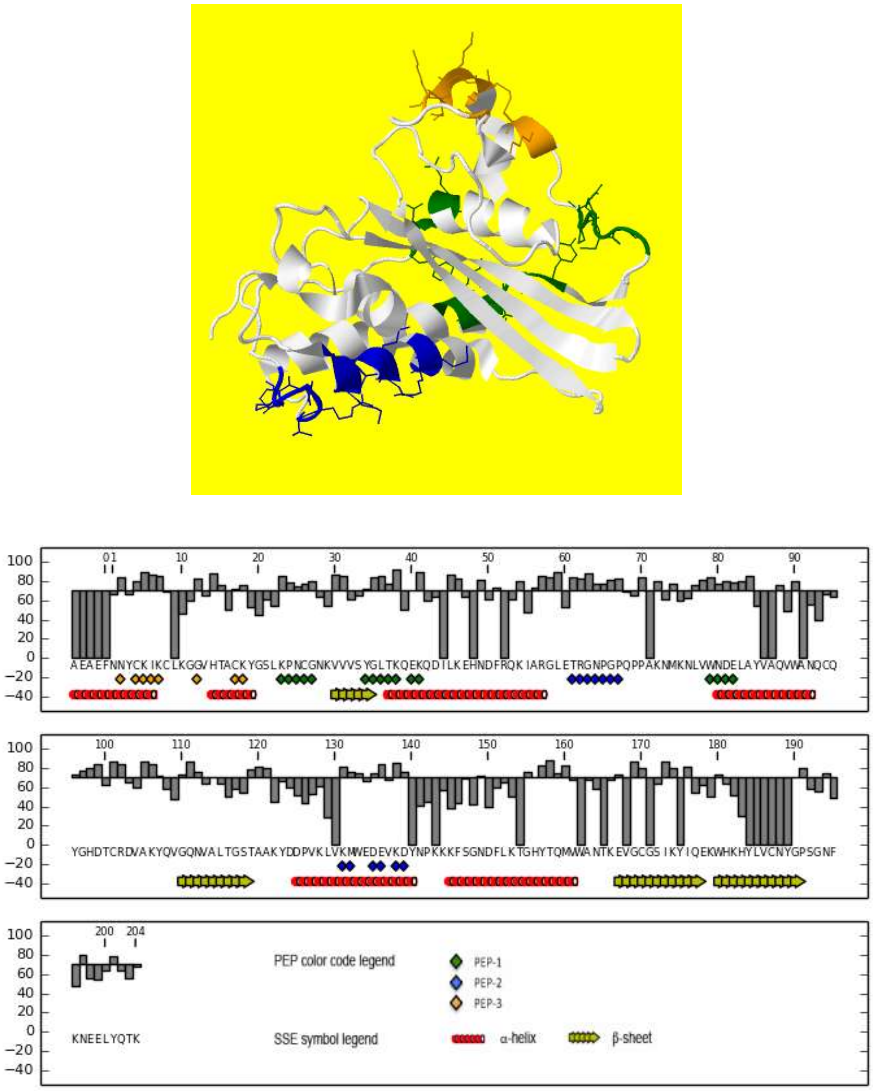


Figure 4. PEP on 1QNX structure and average similarity of surface residues, as compared between allergens.

- PEP1 with 16 residues: K23, P24, N25, C26, G27, Y34, G35, L36, T37, K38, E40, K41, W79, N80, D81, E82; area: 1052.0 Å^2, epitope FOM: 0.805, mean accessibility: 32.8%.
- PEP2 with 13 residues: T61, R62, G63, N64, P65, G66, P67, K131, M132, D135, E136, K138, D139; area: 874.6 Å^2, epitope FOM: 0.807, mean accessibility: 34.5%.
- PEP3 with 8 residues: N2, C4, K5, I6, K7, G12, C17, K18; area: 765.1 Å^2, epitope FOM: 0.818, mean accessibility: 45.1%

As above mentioned, each allergen was also studied as target structure for 1QNX as a cross-reactive (100%) allergen.

SPADE for Vesp c 5.01 only two PEP zones were predicted (Figure 5).

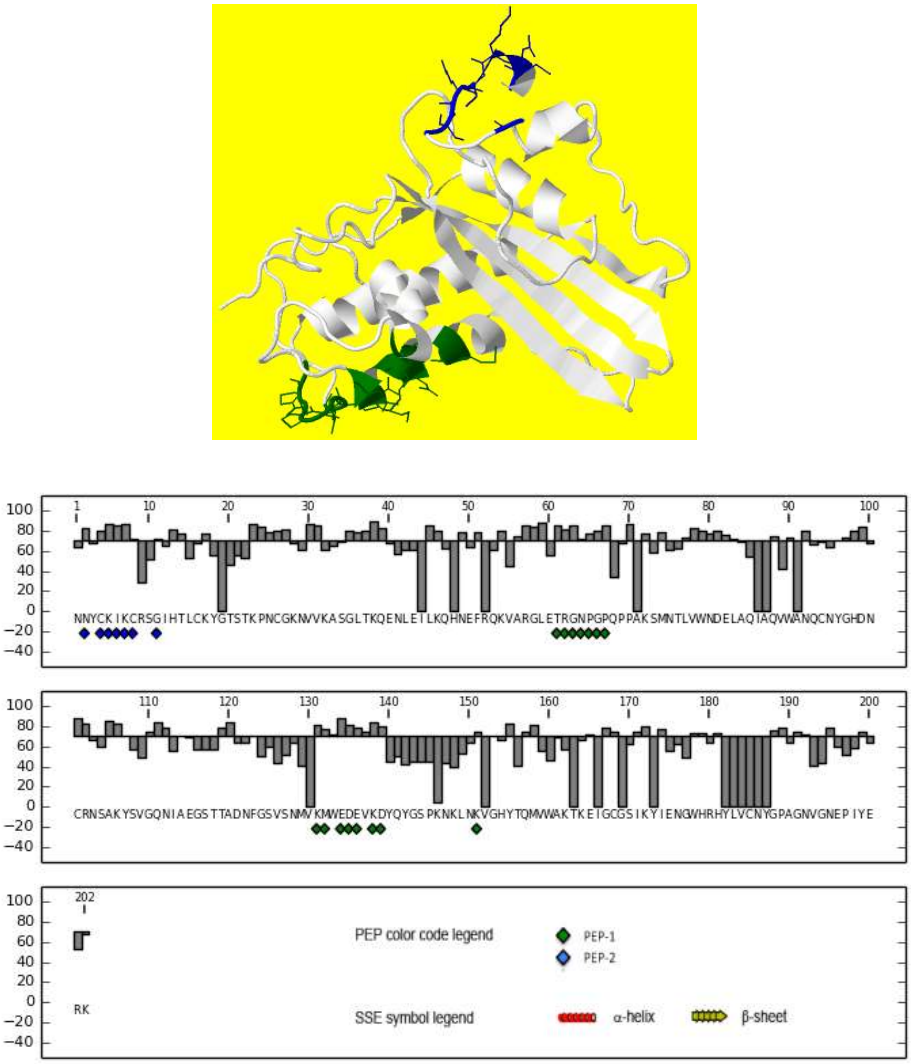


Figure 5. PEP on Vesp c 5.01 structure and average similarity of surface residues, as compared between allergens.

- PEP1 with 15 residues: T61, R62, G63, N64, P65, G66, P67, K131, M132, E134, D135, E136, K138, D139, K151. area: 984.2 Å^2, epitope FOM: 0.807, mean accessibility: 32.6%.
- PEP2 with 7 amino acids: N2, C4, K5, I6, K7, C8, G11; area: 650.7 Å^2, epitope FOM: 0.809, mean accessibility: 43.8%.

SPADE for Vesp c 5.02 only two PEP zones were predicted (Figure 6).

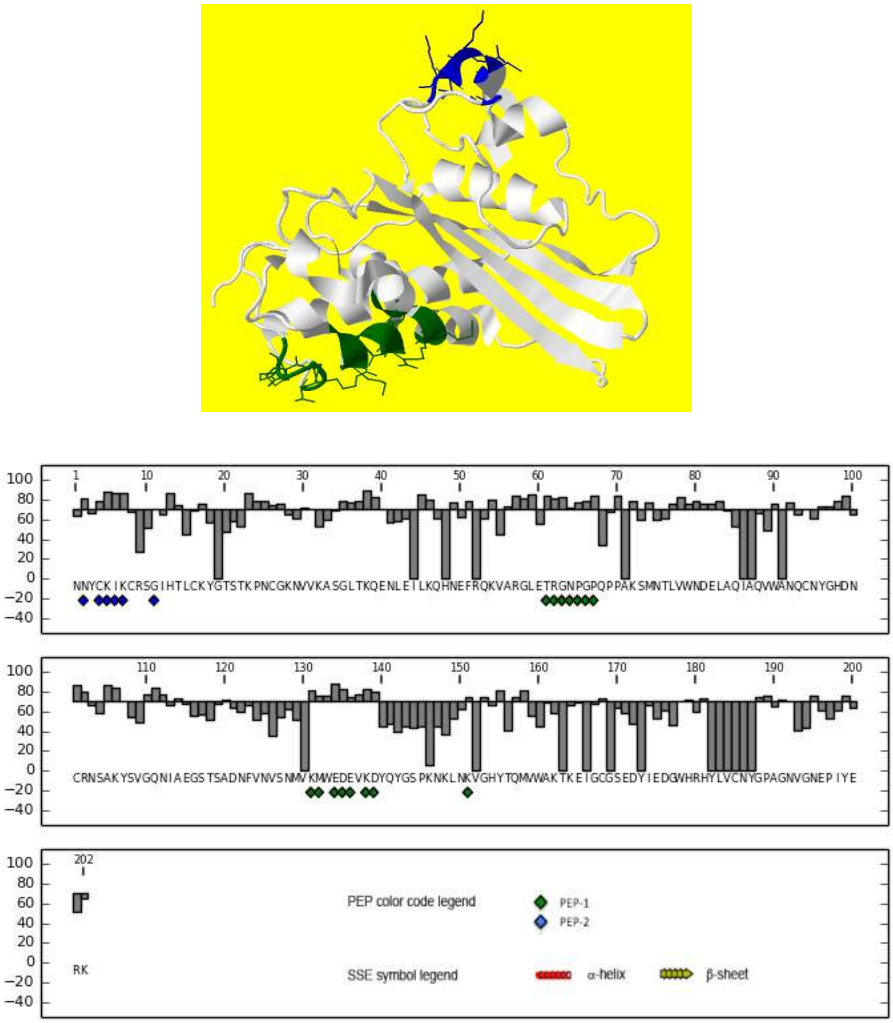


Figure 6. PEP on Vesp c 5.02 structure and average similarity of surface residues, as compared between allergens.

- PEP1 with 15 residues: T61, R62, G63, N64, P65, G66, P67, K131, M132, E134, D135, E136, K138, D139, K151. area: 992.7 Å^2, epitope FOM: 0.799, mean accessibility: 32.8%.
- PEP2 with 6 amino acids: N2, C4, K5, I6, K7, G11; area: 638.2 Å^2, epitope FOM: 0.816, mean accessibility: 49.9%.

SPADE for Ves v 5 three PEP zones were predicted (Figure 7)

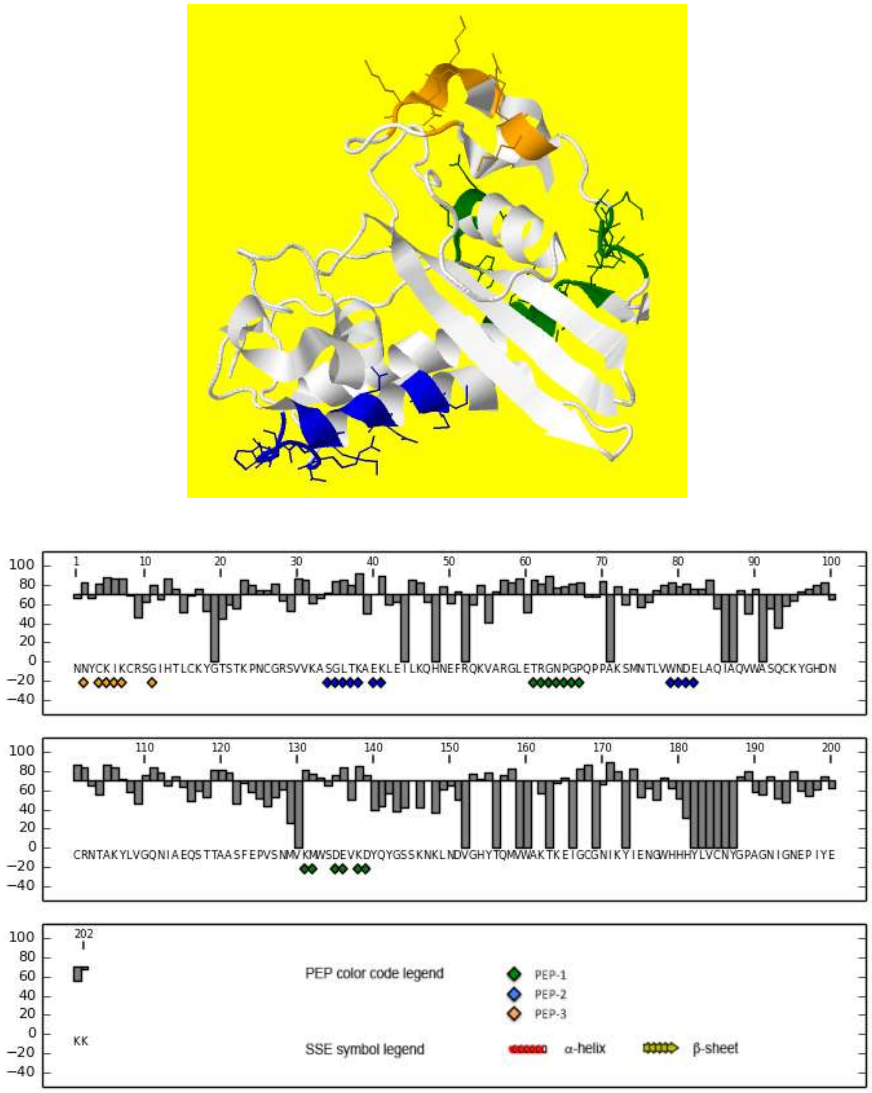


Figure 7. PEP on Vesp c 5.02 structure and average similarity of surface residues, as compared between allergens.

- PEP1 with 13 residues: T61, R62, G63, N64, P65, G66, P67, K131, M132, D135, E136, K138, D139, area: 911.2 Å^2, epitope FOM: 0.812, mean accessibility: 35.9%
- PEP2 with 11 residues: S34, G35, L36, T37, K38, E40, K41, W79, N80, D81, E82; area: 668.0 Å^2, epitope FOM: 0.808, mean accessibility: 29.9%
- PEP3 with 6 aminoacids: N2, C4, K5, I6, K7, G11; area: 626.6 Å^2, epitope FOM: 0.845, mean accessibility: 49.1%

In order to find common sequences in the epitopes of all SPADE outcomes, multiple sequence alignment software Clustal Omega [21] was used, finding only two common amino acid sequences. These two sets of epitopes are represented in Figure 8: PEP-A (blue) N2, C4, K5, I6, K7, G11 and PEP-B (fucsia) T61, R62, G63, N64, P65, G66, P67, K131, M132, D135, E136, K138, D139.

CLUSTAL O(1.2.4) multiple sequence alignment

Table 1. Superposition of 1QNX, v 5, c 5.01 and c 5.02.

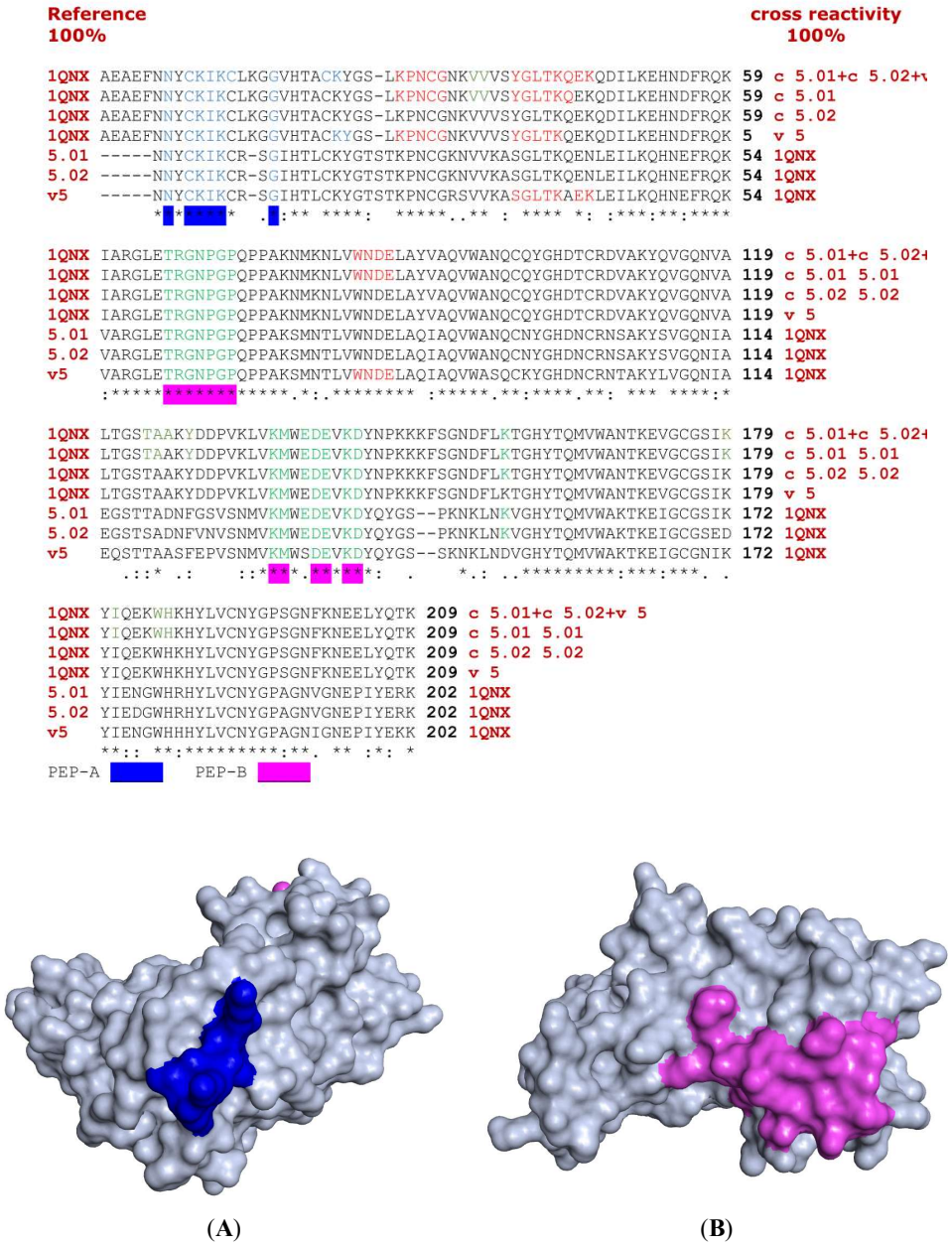


Figure 8. PEP-(**A**) (blue) and PEP-(**B**) (fucsia) areas.

Once the possible epitopes common to the 4 allergens against IgE had been identified, the properties of these compounds were calculated in order to verify the similarity between the allergens. The results for lipophilic properties, calculated with VegaZZ [17], are indicated in Table 2. From these values, it can be inferred that the affinity for the water phase is similar in VV and VC allergens, meanwhile 1QNX is slightly more soluble in water.

Table 2. Values for logP, lipole and virtual logP calculated with VegaZZ.

Descriptor	Vesp v 5	Vesp c 5.01	Vesp c 5.02	1QNX
logP (Crippen)	−125.4042	−126.7743	−122.6103	−140.7915
Lipole (Crippen)	0.4645	0.3314	0.4339	0.6363
logP (Broto)	−317.0903	−323.2975	−323.4015	−336.1422
Lipole (Broto)	0.6049	0.5291	0.5315	1.1018
Virtual logP	−102.4231	−105.4014	−103.6493	−112.9504

Another interesting property when studying the antigen-antibody interaction is the MEP. This is defined as the interaction energy between the charge distribution of a molecule and a unit positive charge, and it is a good tool for assessing the molecules reactivity towards positively or negatively charged reactants. This allows to identify the key structural features to understand the relationships between the structure and the activity of these molecules. Therefore, a high similarity in the MEP zones corresponding to the PEPs in the four allergens can be expected since this would imply a similar binding behaviour.

The calculation of the MEP surface showed values are indicated in Table 3 (negative values in red, positive values in blue). The representation of the MEP is calculated with VegaZZ 3.2.1 [22] by projecting the atomic charges on the molecule surface.

Molecular electrostatic potentials were also calculated using APBS (Adaptive Poisson-Boltzmann Solver), which solves the equations of continuum electrostatics for large biomolecular assemblages (software used PBEQ solver [23]). Table 4 shows the surface electrostatic potentials from +2 kcal/(mol·*e*), in blue, to −2 kcal/(mol·*e*), in red, for the four allergens.

When the MEP values of the common PEP-A and PEP-B are compared, some differences can be detected. This leads us to think that considering the cross-reactivity of the four allergens as 100% could be inadequate, and the values should be confirmed on the basis of more extensive experimental studies, since in the field of hymenopteran allergens there is still some divergence in the published results. Therefore, if experimental data of cross-reactivity are achieved, along with molecular docking studies with the IgE antibody, results more closely adjusted to reality could be obtained. Hence, additional studies are of great interest to improve the effectiveness of VIT.

Table 3. MEP surfaces calculated with VegaZZ.

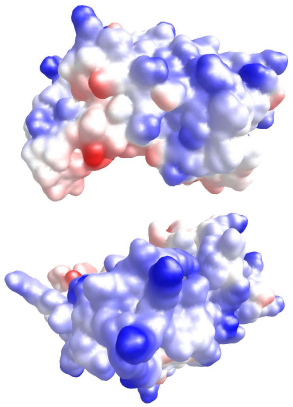
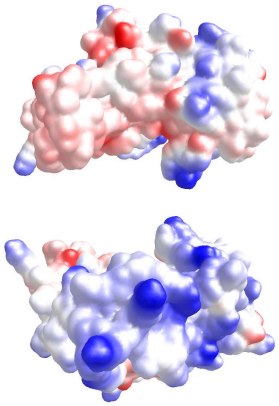
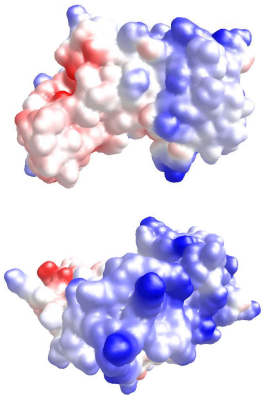
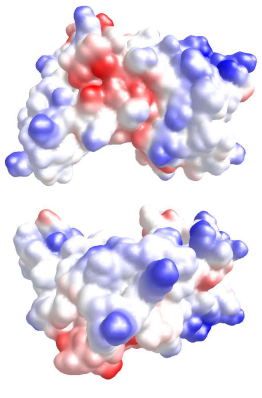
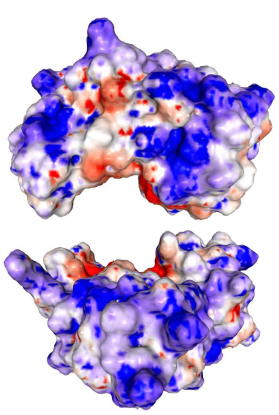
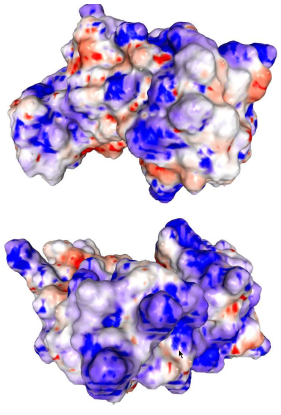
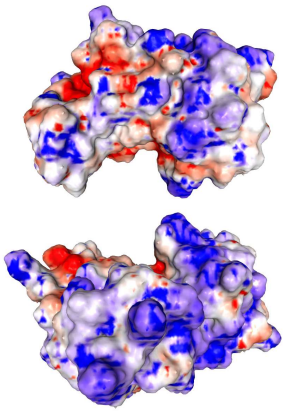
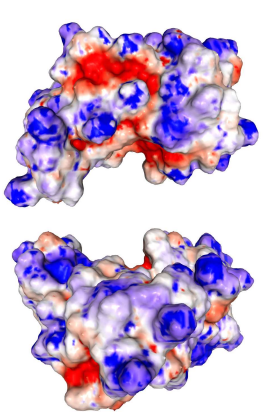
Vel v 5 **MEP Range: −0.2511⇔0.8756**	**Vesp c 5.01** **MEP Range: −0.1112⇔0.8597**	**Vesp c 5.02** **MEP Range:−0.5278⇔0.7412**	**1qnx** **MEP Range: −0.2829⇔0.8787**
			

Table 4. MEP surfaces calculated with PBEQ solver.

Vel v 5	Vesp c 5.01	Vesp c 5.02	1qnx
			

3. Conclusions

The evaluation of the prediction of epitopes in allergens Vesp c 5.01, Vesp c 5.02 and Vesp v 5 with the software tool "Surface comparison-based Prediction of Allergenic Discontinuous Epitopes" (SPADE), specialized in the IgE antibody, led to the identification of two regions common in the different cases studied. The comparison of the areas of the molecular electrostatic potential (MEP) surface for the four allergens allowed to detect slight changes in their values, which could imply somewhat different behaviours between them when interacting with the IgE antibody.

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