Solution structure and allergic cross–reactivity of the wild–type major cherry allergen Pru av 1 and its E45W mutant

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Birch pollinosis is often accompanied by hypersensitivity to fruit as a consequence of the cross–reaction of pollen–allergen specific immunoglobulin E (IgE) antibodies with homologous food proteins. To provide a basis for examining the cross–reactivity on a structural level we used heteronuclear multidimensional nuclear magnetic resonance (NMR) spectroscopy to determine the first high–resolution three–dimensional structure of a pollen–related food allergen, a well–defined structure of the major cherry allergen Pru av 1 in solution. The secondary structure elements and the tertiary fold of Pru av 1 are virtually identical to the major birch pollen allergen Bet v 1. The fact that the P–loop around Glu45, which is known as one of the IgE antibody binding epitopes of Bet v 1, is structurally conserved in Pru av 1 suggests this region to constitute a cross–reactive epitope. This might also explain why Api g 1.0101, the major celery allergen where Glu45 is replaced by lysine, fails to efficiently reduce IgE binding to Pru av 1 in immunoblot inhibition experiments. To verify this hypothesis, we cloned, overexpressed and purified Pru av 1 E45W, whose bulky hydrophobic side–chain instead of the negatively charged carboxyl group at position 45 can be expected to negatively affect the affinity of IgE antibodies binding to the P–loop both in terms of steric and electrostatic interactions. The fact that the NMR spectra of Pru av 1 E45W are virtually superimposable with those of Pru av 1 wt for all but the residues in the immediate vicinity of Trp45 demonstrates that the overall structure of Pru av 1 is not disturbed by the E45W mutation. The reduced capacity of Pru av 1 E45W compared to Pru av 1 wt to inhibit IgE binding to Pru av 1 wt in enzyme allergo–sorbent tests (EAST) therefore has to be attributed to the local structural changes around Trp45, which provides strong evidence that Glu45 is indeed a key residue of one of the cross–reactive IgE binding epitopes of Pru av 1 and thus a promising candidate for mutation in order to develop hypoallergenic allergen variants that can be used as vaccines for a patient–tailored specific immunotherapy with reduced anaphylactic side effects. These findings mark a first step towards a detailed molecular explanation of the observed cross–reactivity patterns of different patients with respect to the individual members of the Bet v 1 family of pollen and food allergens.

Introduction

Up to 70 % of birch pollen allergic patients who suffer from clinical syndromes like rhinitis, asthma, and dermatitis also show hypersensitivity to fresh fruit or vegetables. The allergic reactions after ingestion of foodstuff are predominantly oropharyngeal, for example itching and swelling of lips, tongue and throat, but in rare cases even severe anaphylactic reactions are possible. The symptoms of these type I allergies are caused by an immune response which is triggered when two receptor–bound immunoglobulin E (IgE) antibodies on the surface of a mast cell or basophil are cross–linked by simultaneous binding of an otherwise harmless antigen, the so–called allergen. Pollen–associated food allergies are a consequence of the cross–reaction of pollen–allergen specific IgE antibodies with highly homologous proteins contained in foodstuff. The 17.4 kDa major birch (*Betula verrucosa*) pollen allergen Bet v 1 is responsible for IgE binding in more than 95 % of birch pollen allergic patients. A series of allergens with high sequence identity to Bet v 1 have been reported, pollen allergens from other trees belonging to the *Fagales* order as well as food allergens like Api g 1.0101 from celery (*Apium graveolens*), Mal d 1 from apple (*Malus domestica*), Pru av 1 from cherry (*Prunus avium*), Pyr c 1 from pear (*Pyrus communis*), and Cor a 1.0401 from hazelnut (*Corylus avellana*) (Fig. 1).
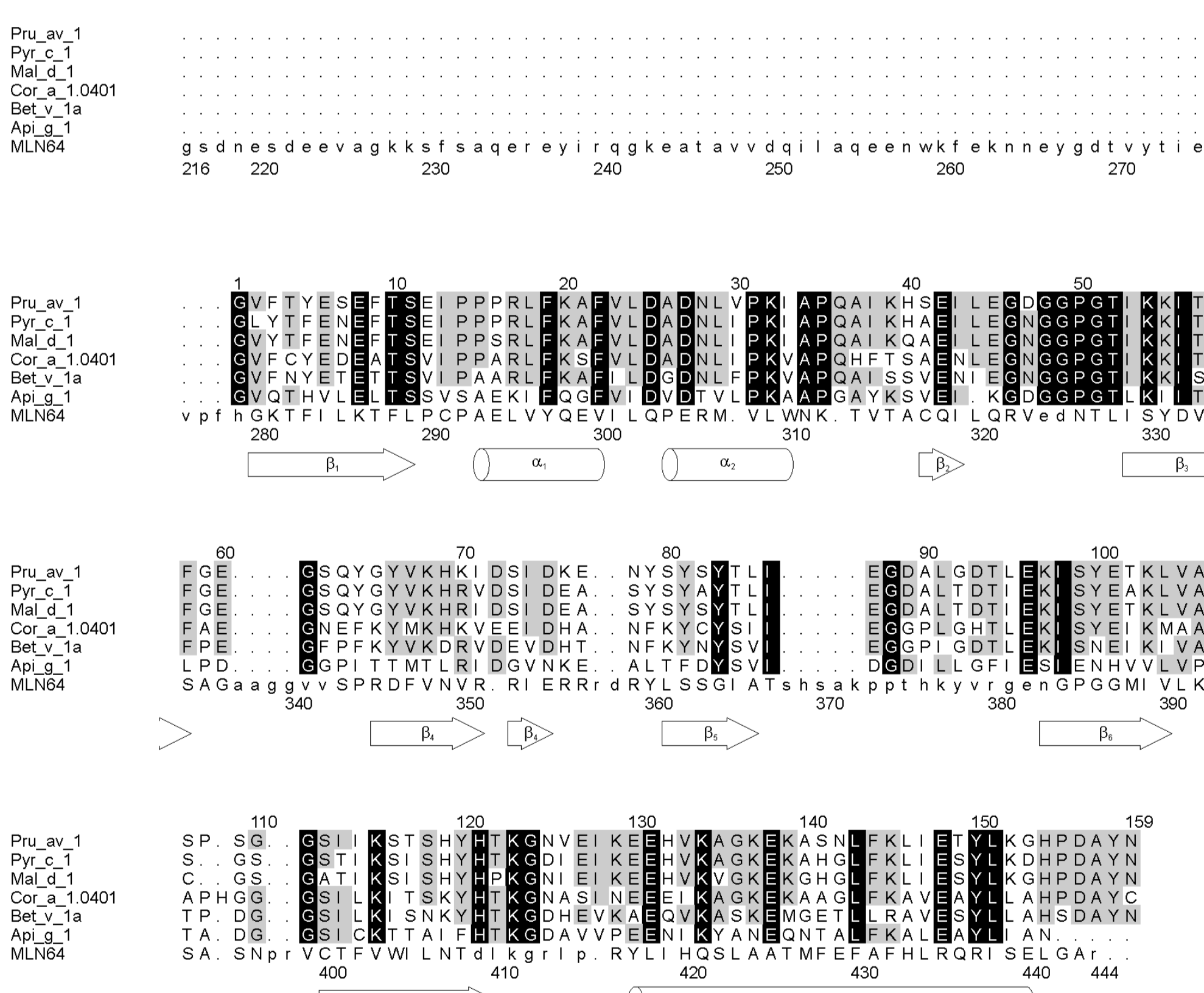
Fig. 1: Structure–based sequence alignment with Pru av 1 of Pyr c 1 (83.5% sequence identity to Pru av 1), Mal d 1 (82.9%), Cor a 1.0401 (64.4%), Bet v 1 isoform a (59.1%), Api g 1.0101 (41.2%), and the START domain of MLN64 (8.5%). The sequence positions above and below the sequences correspond to Pru av 1 and MLN64, respectively. Gaps in the alignment are indicated by dots. Residues conserved in at least 4 of the 6 allergens are highlighted by grey boxes, residues conserved in all 6 allergens by black boxes. The secondary structure elements of Pru av 1 are shown below the alignment. The alignment of the allergens with Pru av 1 is based on homology models created by SWISS–MODEL, the alignment of the START domain of MLN64 with Pru av 1 on a Dali server comparison; the 129 MLN64 residues used for the alignment are printed in uppercase, residues not used for the alignment in lowercase.

Methods and Results

In contrast to Bet v 1[1, 2, 3] as yet no high–resolution three–dimensional structure of any of the corresponding food allergens is available. Since this is a prerequisite for a detailed understanding of the observed immune cross–reactivity, we determined the three–dimensional structure of the major cherry allergen Pru av 1 based on a series of heteronuclear multidimensional NMR experiments performed on uniformly [15]N– and ^{13}C/^{15}N–labeled samples[4, 5]. Pru av 1 shows a well–defined structure in solution (Fig. 2) with average atomic root mean square deviations (RMSDs) from the average structure of 0.60 Å for the backbone and 0.93 Å for all heavy atoms.
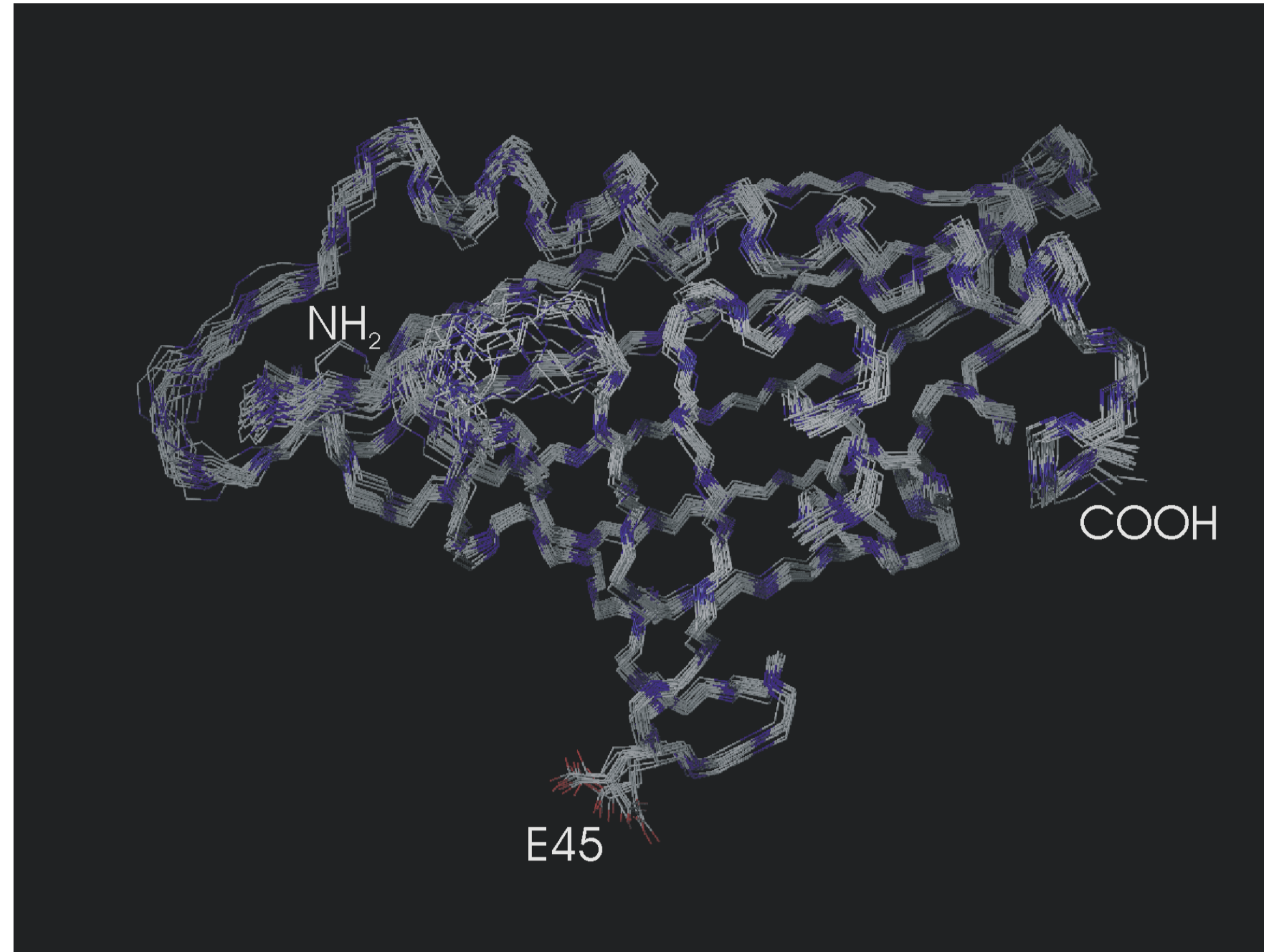
Fig. 2: Backbone overlay of the 22 accepted structures of Pru av 1. C, N, and O atoms are color–coded gray, blue, and red, respectively. The NH₂–terminus on the left–hand side is hidden by the loop from Ile86 to Glu96, the COOH–terminus can be seen on the right–hand side. The structures are in excellent agreement, especially as far as the β–strands are concerned. The side–chain of Glu45 shown at the bottom is clearly solvent–exposed in all structures.
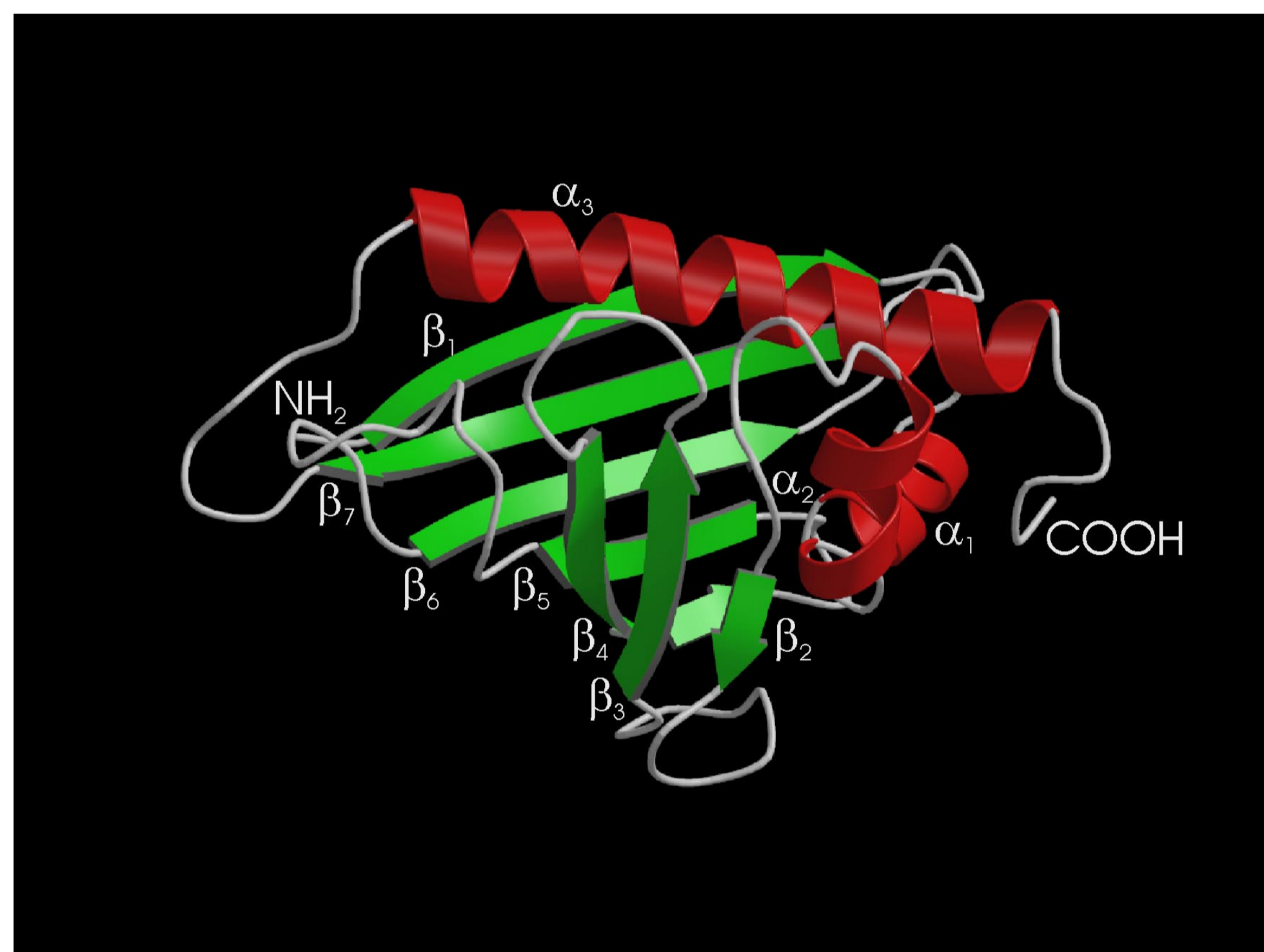
Fig. 3: Schematic representation of the secondary structure elements of Pru av 1. Same view as in Fig. 2. A folded seven–stranded antiparallel β–sheet and two short α–helices arranged in a V–shaped manner wrap around a long COOH–terminal α–helix to form a basket–like structure with the long helix resembling a handle, thus creating a large hydrophobic cavity in the center, which is very unusual for proteins.
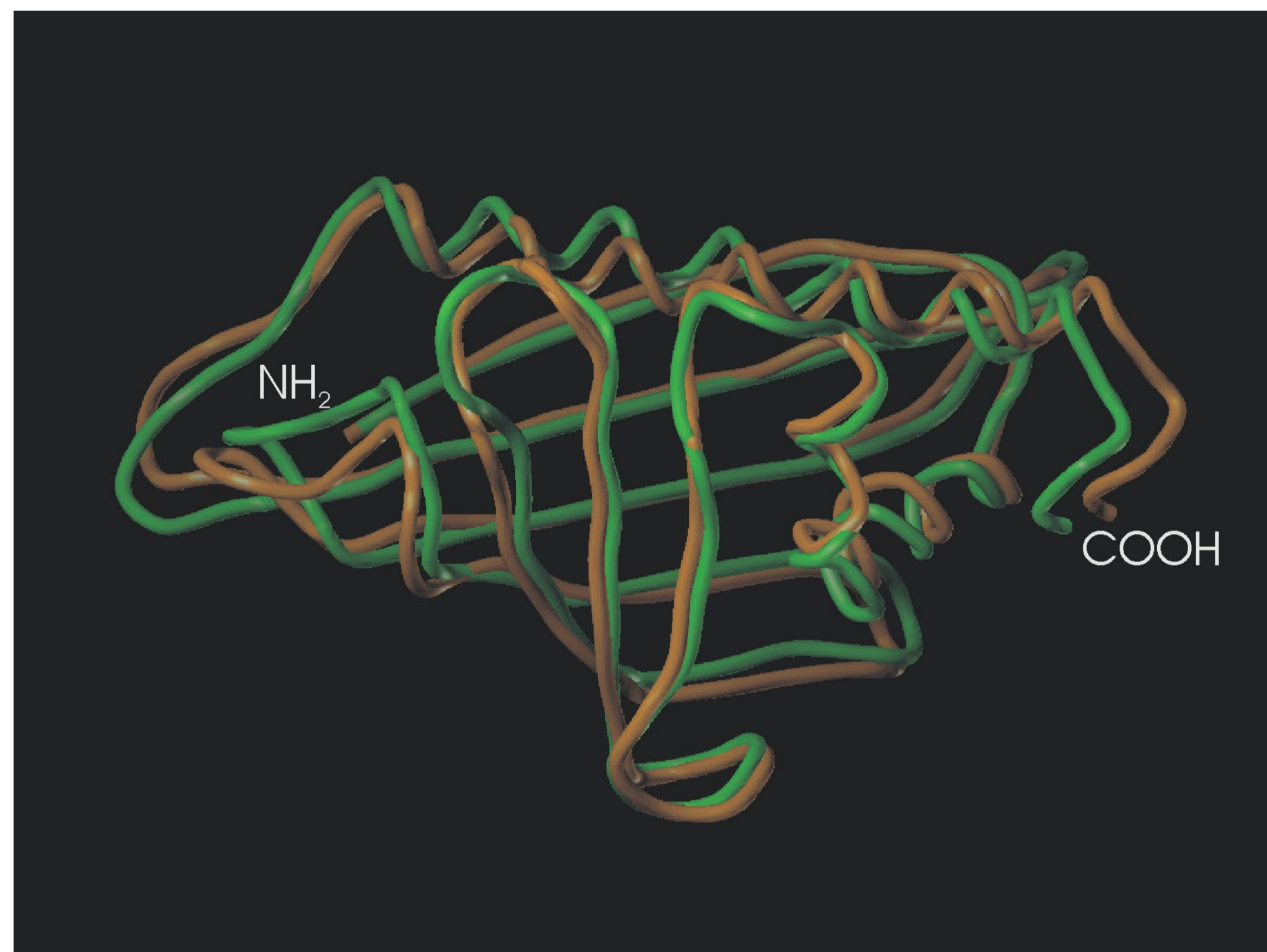
Fig. 4: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of Bet v 1[1] (orange). Same view as in Fig. 2 and 3. The tertiary fold is almost identical.

Together with the considerable sequence identity, the conserved backbone conformation compared to the crystal structure of Bet v 1[1] (Fig. 4) with a backbone atomic RMSD of 1.85 Å leads to a very similar molecular surface, rendering cross–reactive IgE binding epitopes most likely. Preincubation with Bet v 1a indeed inhibits binding of pooled serum IgE to the related food allergens almost completely (Fig. 5), supporting the experience that these food allergies are usually a consequence of cross–reaction after sensitization to birch pollen. For Bet v 1, the P–loop around Glu45 was identified as the binding epitope of a monoclonal murine IgG antibody with high capacity to inhibit binding of serum IgE from allergic patients to Bet v 1[6]. Introduction of four point mutations including E45S indeed reduced the IgE binding capacity by several[sic]fold[3]. In the crystal structure of the complex of Bet v 1 with the IgG Fab fragment the negatively charged side–chain of Glu45 is located in a binding pocket of the antibody with a positive electrostatic potential, where it forms two hydrogen bonds. In addition to Glu45, which is found to be solvent–exposed in all 22 accepted structures of Pru av 1 (Fig. 2), 14 of the remaining 15 residues forming the interface between Bet v 1 and the IgG Fab fragment are conserved or substituted conservatively in Pru av 1 (Fig. 1), which strongly suggests this region to be a cross–reactive IgE binding epitope. This is supported by the significantly decreased binding of serum IgE to the Pru av 1 mutants G46P and ΔT52 observed for some patients[7]. It can also provide an explanation why IgE binding to Pru av 1 is only weakly inhibited by preincubation with Api g 1.0101 (Fig. 6), where the P–loop is shorter by a residue and the negatively charged Glu45 is substituted by a positively charged lysine (Fig. 1).
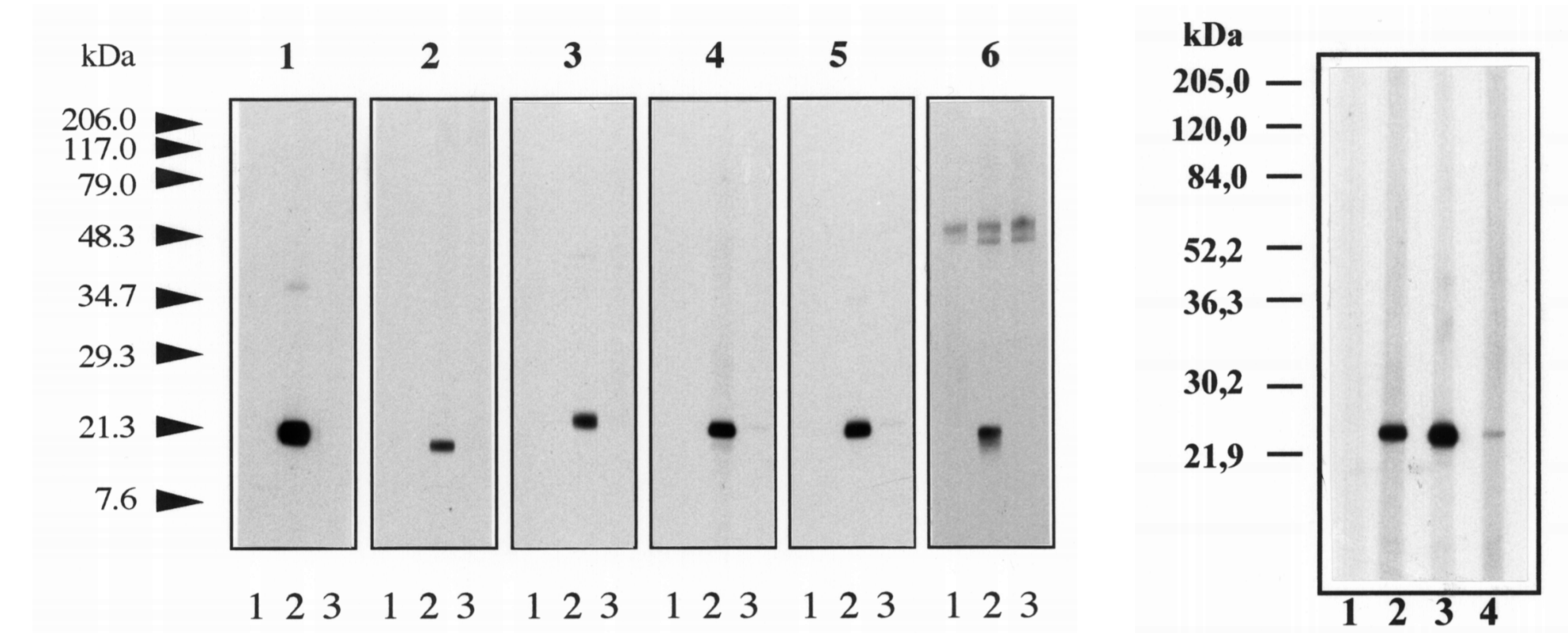
Fig. 5: Immunoblot inhibition of IgE binding to Bet v 1a (1), Api g 1.0101 (2), Mal d 1 (3), Pru av 1 (4), Pyr c 1 (5), and Cor a 1.0401 (6) on the solid phase. A serum pool from birch pollinotic patients with associated food allergy was preincubated with Bet v 1a (3). Serum from a non–allergic subject (1) and samples without inhibitor (2) were used as controls.
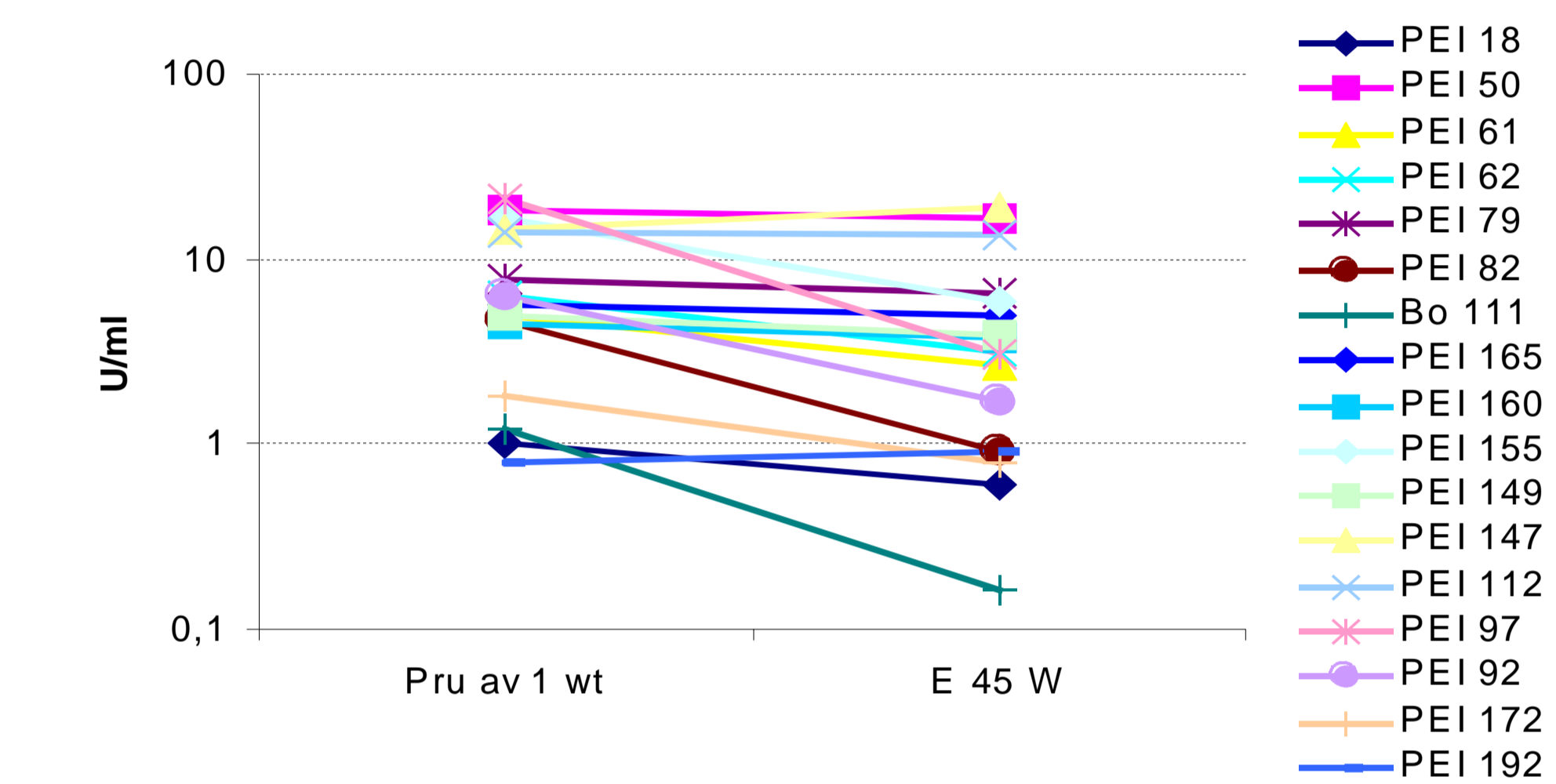
Fig. 6: Immunoblot inhibition of IgE binding to Pru av 1 on the solid phase with Pru av 1 (1) and Api g 1.0101 (2) as inhibitors. A sample without inhibitor (3) and serum from a non–allergic subject (4) were used as controls. Preincubation of the serum pool with Api g 1.0101 only leads to a small reduction of IgE binding to Pru av 1.

Fig. 7: Enzyme Allergosorbent Test (EAST). For determination of the IgE binding capacity of sera from several cherry allergic patients (legend), Pru av 1 wt and E45W were coupled to cyanogen bromide activated filter paper disks at a concentration of 3 μg/ml corresponding to 0.5 μg of recombinant proteins per disk. The EAST was performed with the "Allergopharma Spez. IgE ELISA" (Allergopharma, Germany) according to the manufacturer's instructions. Most of the sera show markedly reduced IgE binding capacity to Pru av 1 E45W compared to Pru av 1 wt. Interestingly, however, there is also one patient (PEI147) for whom the binding capacity increases significantly, although the E45W mutation is completely artificial.
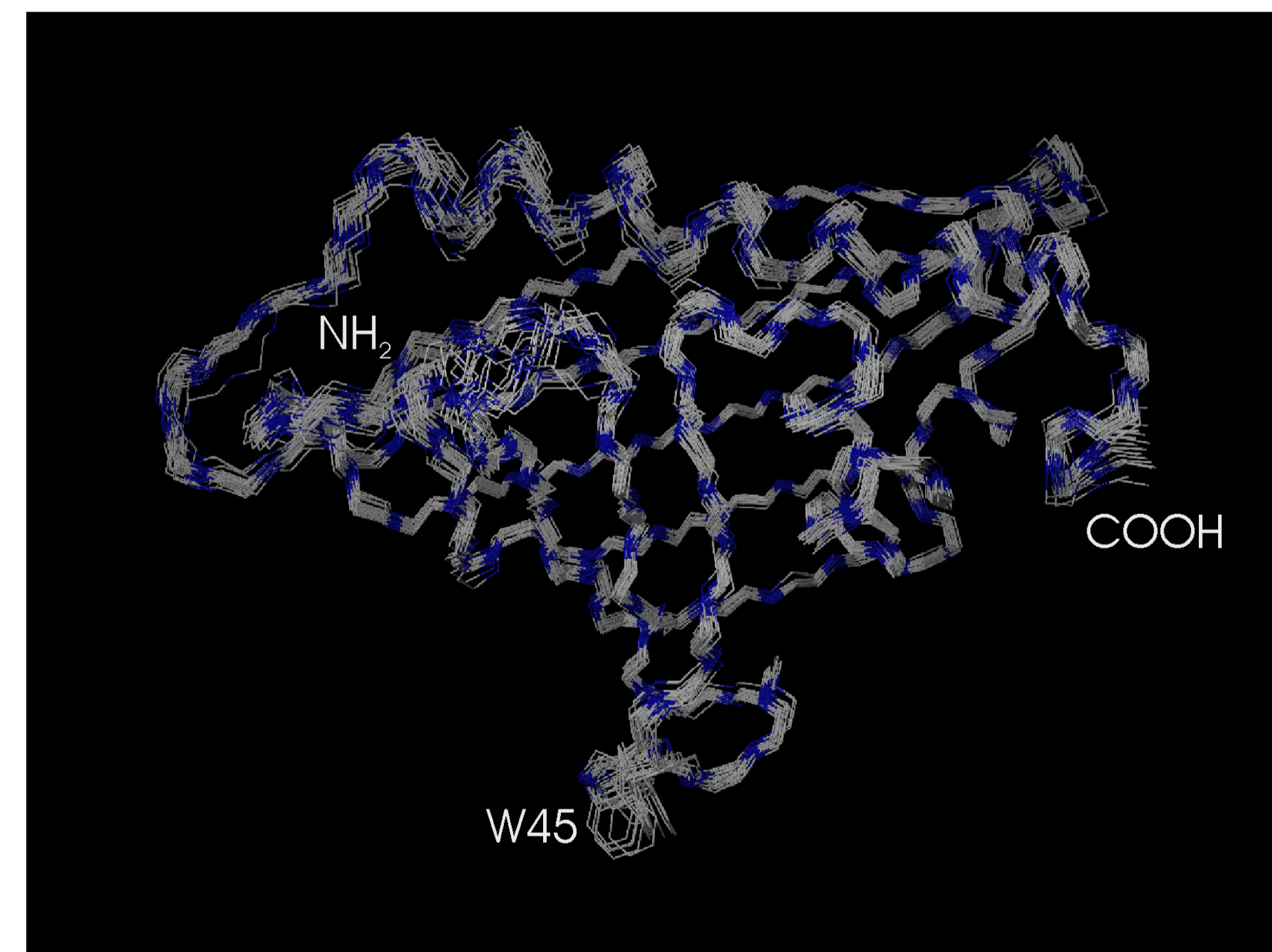
Fig. 8: Backbone overlay of the 22 accepted structures of Pru av 1 E45W. Same view as in Fig. 2, 3 and 4. C and N atoms are color–coded gray and blue, respectively. Even the backbone conformation of the P–loop is identical to that of Pru av 1 wt (Fig. 2), leaving the side–chain of Trp45 shown at the bottom as the only major difference between wild–type and mutant.

To verify this hypothesis, we cloned, overexpressed and purified Pru av 1 E45W, whose bulky hydrophobic side–chain at position 45 can be expected to negatively affect the affinity of IgE antibodies binding to the P–loop both in terms of steric and electrostatic interactions. Since the NMR spectra of a uniformly ^{15}N–labeled sample of Pru av 1 E45W are superimposable with those of Pru av 1 wt for all but seven residues in the P–loop, the three–dimensional structure of Pru av 1 E45W only had to be redetermined locally. Like Pru av 1 wt, Pru av 1 E45W shows a well–defined structure in solution (Fig. 8) with average atomic RMSDs from the average structure of 0.57 Å for the backbone and 0.90 Å for all heavy atoms. The overall structure of Pru av 1 is not disturbed by the E45W mutation, not even the backbone conformation of the P–loop. The reduced IgE binding capacity of the sera of many patients to Pru av 1 E45W compared to Pru av 1 wt (Fig. 7) therefore has to be attributed to the side–chain of Trp45, which provides strong evidence that Glu45 is indeed a key residue of one of the cross–reactive IgE binding epitopes of Pru av 1 and thus a promising candidate for mutation for hypoallergenic allergen variants as vaccines for a patient–tailored specific immunotherapy with reduced anaphylactic side effects.

References

[1] M. Gajhede, P. Osmark, F. M. Poulsen, H. Ipsen, J. N. Larsen, R. J. J. van Neerven, C. Schou, H. Løwenstein and M. D. Spangfort, Nature Struct. Biol. 3, 1040–1045 (1996)

[2] K. Schweimer, H. Sticht, J. Nerkamp, M. Boehm, M. Breitenbach, S. Vieths and P. Rösch, Appl. Magn. Reson. 17, 449–464 (1999)

[3] J. Holm, A. Henriksen, J. N. Larsen, H. Ipsen, M. Gajhede and M. D. Spangfort, Allergy 55, Suppl. 63, 21 (2000)

[4] P. Neudecker, K. Schweimer, J. Nerkamp, M. Boehm, S. Scheurer, S. Vieths, H. Sticht and P. Rösch, J. Biomol. NMR 8, 71–72 (2000)

[5] P. Neudecker, K. Schweimer, J. Nerkamp, S. Scheurer, S. Vieths, H. Sticht and P. Rösch, J. Biol. Chem. 276, 22757–22763 (2001)

[6] O. Mirza, A. Henriksen, H. Ipsen, J. N. Larsen, M. Wissenbach, M. D. Spangfort and M. Gajhede, J. Immunol. 165, 331–338 (2000)

[7] S. Scheurer, D. Y. Son, M. Boehm, F. Karamloo, S. Franke, A. Hoffmann, D. Haustein and S. Vieths, Mol. Immunol. 36, 155–167 (1999)