

Solution structure determination and ligand interaction studies of the major cherry allergen Pru av 1 by NMR spectroscopy

Philipp Neudecker¹, Kristian Schweimer¹, Jörg Nerkamp¹, Katrin Lehmann¹,
Stephan Scheurer², Stefan Vieths², Heinrich Sticht¹ and Paul Rösch¹

¹ Lehrstuhl für Struktur und Chemie der Biopolymere, Universität Bayreuth, Bayreuth, Germany

² Abteilung Allergologie, Paul-Ehrlich-Institut, Langen, Germany

Phone: +49-921-553869; Fax: +49-921-553544; E-mail: philipp.neudecker@uni-bayreuth.de

Birch pollenosis 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, rendering the existence of cross-reactive IgE binding epitopes most likely. In particular, the fact that the P-loop around Glu45, which is known as one of the IgE binding epitopes of Bet v 1, is structurally conserved in Pru av 1 suggests this region to constitute a cross-reactive epitope. The large hydrophobic cavity expected to be important for the still unknown physiological function of Bet v 1 is also conserved in Pru av 1. NMR spectroscopy provides first experimental evidence that Pru av 1 indeed interacts with phytosteroids, and molecular modeling shows that the hydrophobic cavity is large enough to accommodate two such molecules.

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).

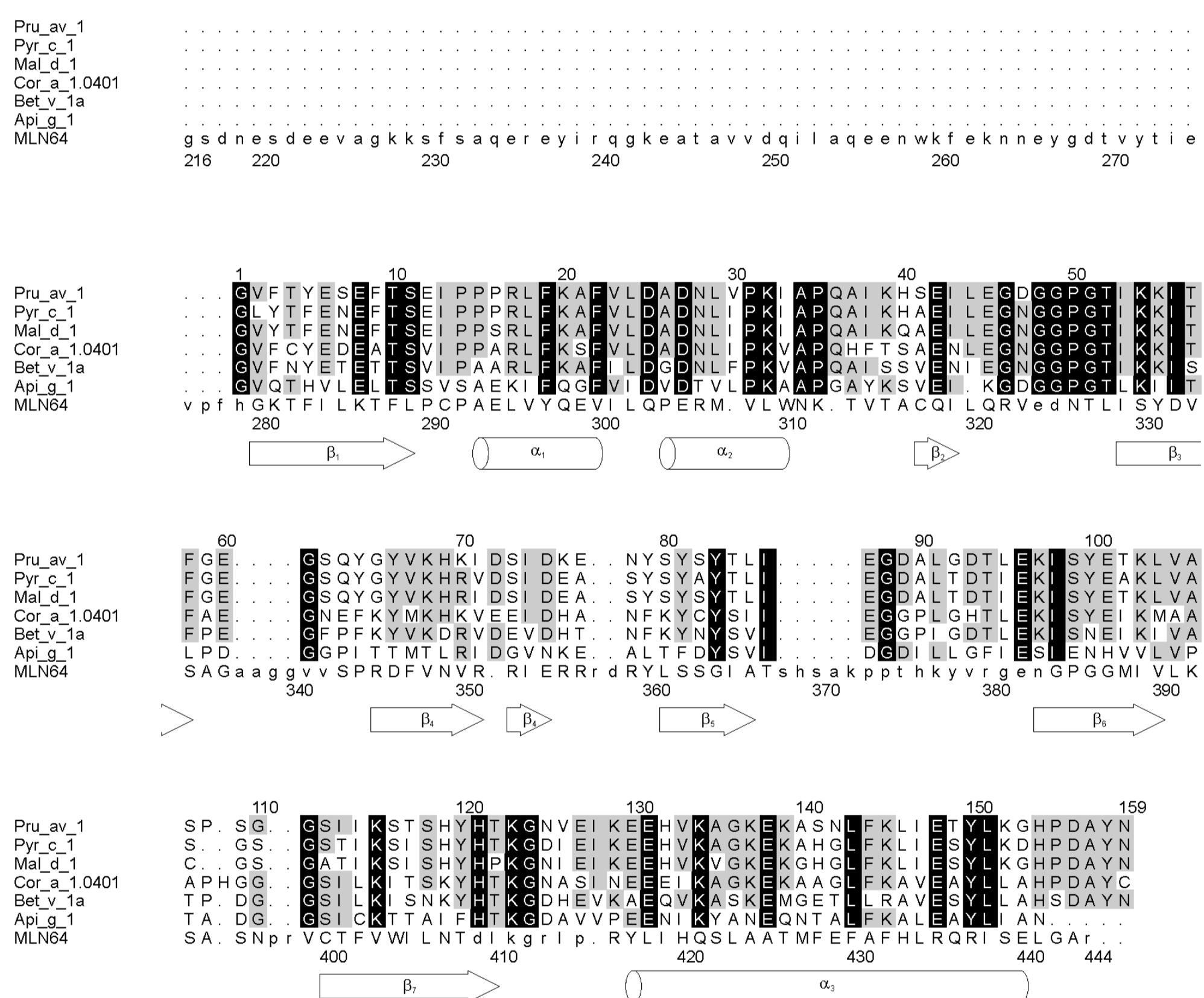


Fig. 1: Structure-based sequence alignment with Pru av 1 of Pyr c 1 (83.5% sequence identity to Pru av 1), Mal d 1 (82.9%), Cor a 1.0401 (64.4%), Bet v 1 isoform a (59.1%), Api g 1.0101 (41.2%), and the START domain of MLN64 (8.5%). The sequence positions above and below the sequences correspond to Pru 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} no high-resolution three-dimensional structure 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 17.5 kDa major cherry allergen Pru av 1 in solution based on 2438 experimental restraints derived from a series of mostly heteronuclear multidimensional NMR experiments performed on uniformly ¹⁵N- and ¹³C/¹⁵N-labeled samples^{3,4}. 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. A schematic representation is shown in Fig. 3. A backbone overlay with the crystal structure of Bet v 1¹ (Fig. 4) confirms that the secondary structure elements and the tertiary fold are virtually identical (backbone atomic RMSD 1.85 Å). Together with the high sequence identity, the conserved backbone conformation leads to a very similar molecular surface as far as shape and charge distribution are concerned, rendering the existence of cross-reactive IgE binding epitopes most likely. The physiological function of all these allergens is still unknown. They show high sequence similarity to pathogenesis-related proteins, but most of them are expressed constitutively. The striking structural homology with the recently determined crystal structure of the START domain of human MLN64⁵ (Fig. 5) in spite of low sequence identity (Fig. 1) suggests phytosteroids as putative ligands for Bet v 1 and Pru av 1, which is supported by first NMR spectroscopic data (Fig. 6 and 7).

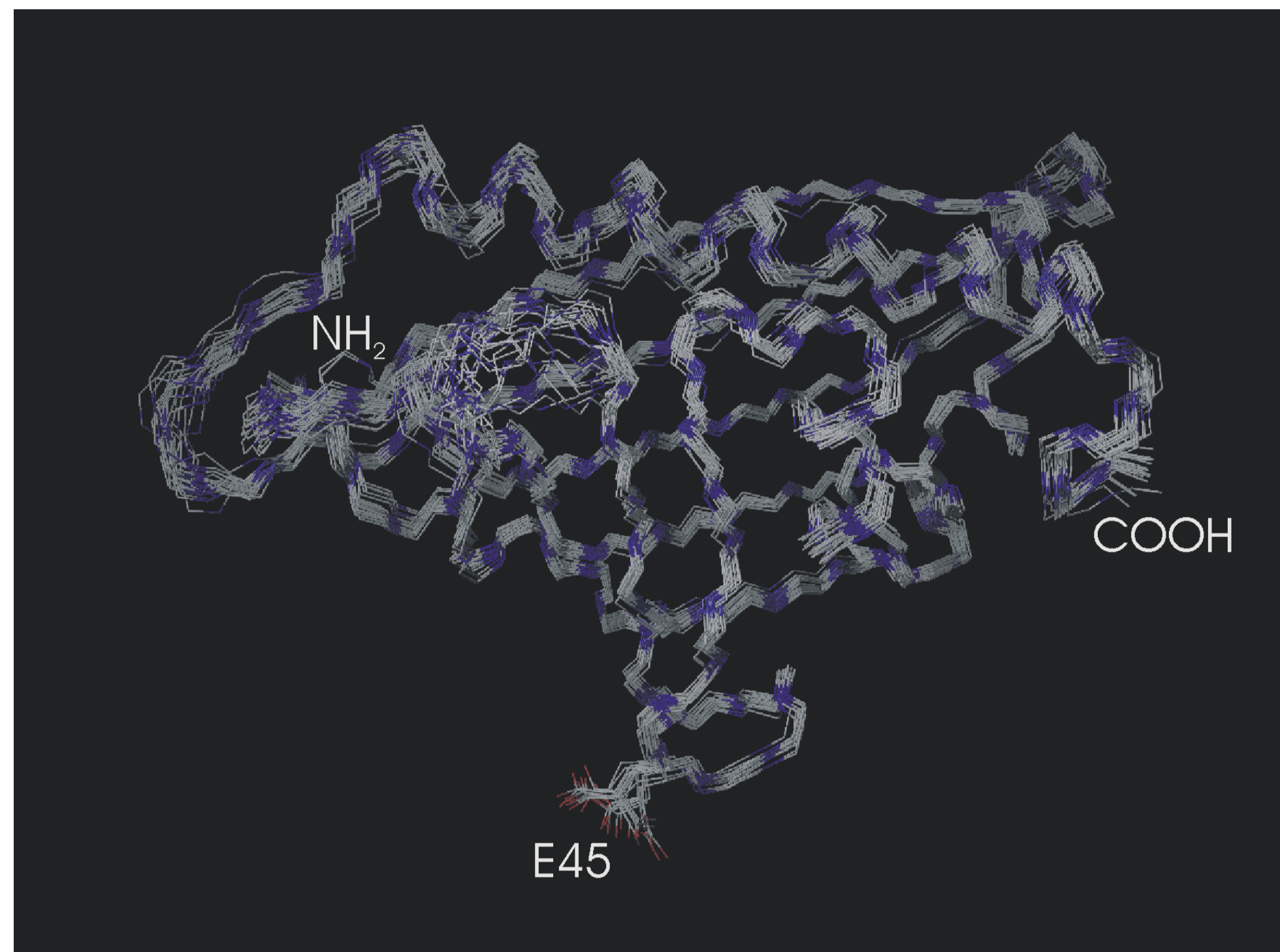


Fig. 2: Backbone overlay of the 22 accepted structures of Pru av 1. C, N, and O atoms are color-coded gray, blue, and red, respectively. The NH₂-terminus on the left-hand side is hidden by the loop from Ile86 to Glu96, the COOH-terminus can be seen on the right-hand side. The structures are in excellent agreement, especially as far as the β -strands are concerned. The side-chain of Glu45 shown at the bottom, which is known as a key residue of one of the IgE binding epitopes of Bet v 1⁶, is clearly solvent-exposed in all structures.

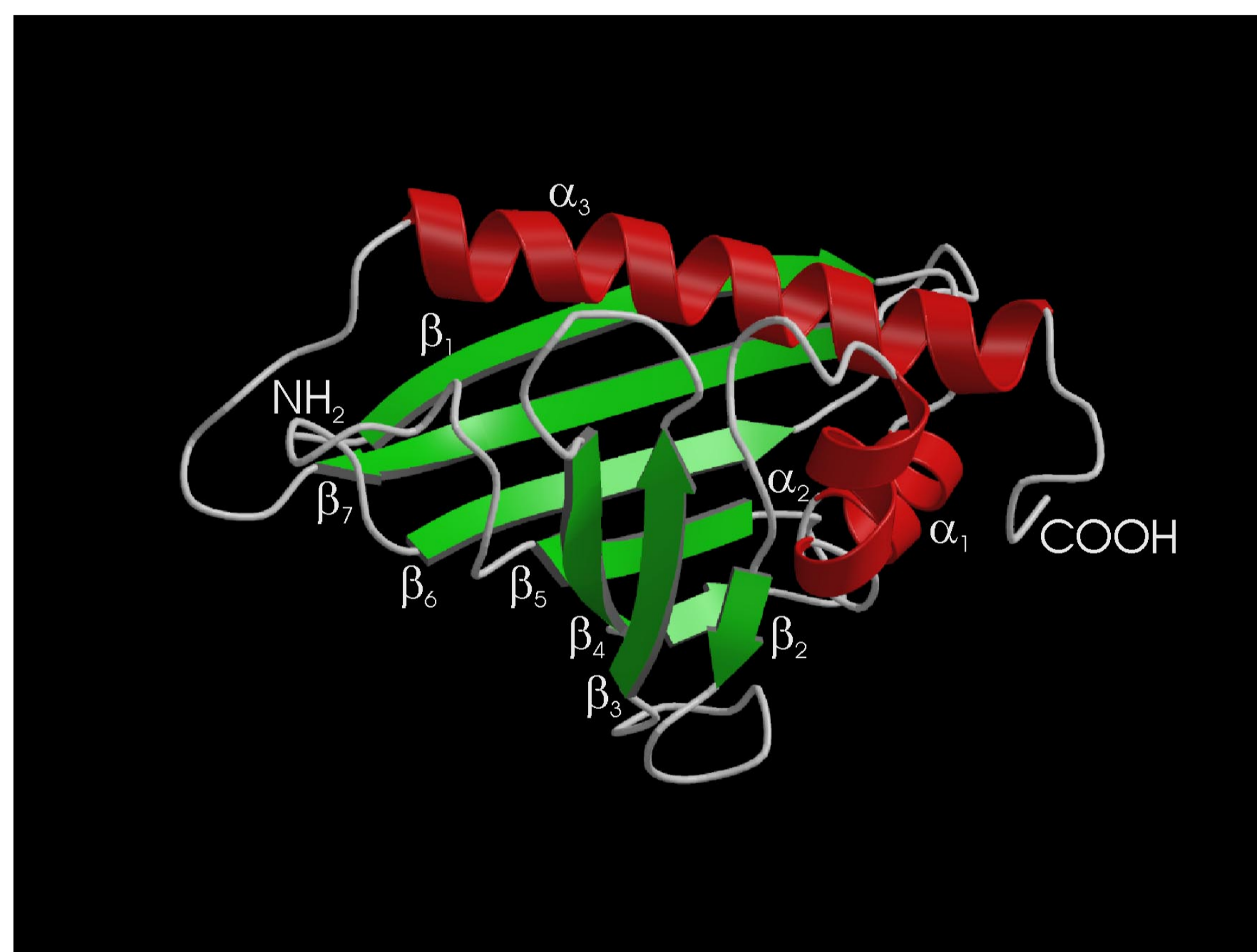


Fig. 3: Schematic representation of the secondary structure elements of Pru av 1. Same view as in Fig. 2. A folded seven-stranded antiparallel β -sheet and two short α -helices arranged in a V-shaped manner wrap around a long COOH-terminal α -helix to form a basket-like structure with the long helix resembling a handle, thus creating a large hydrophobic cavity in the center, which is very unusual for proteins.

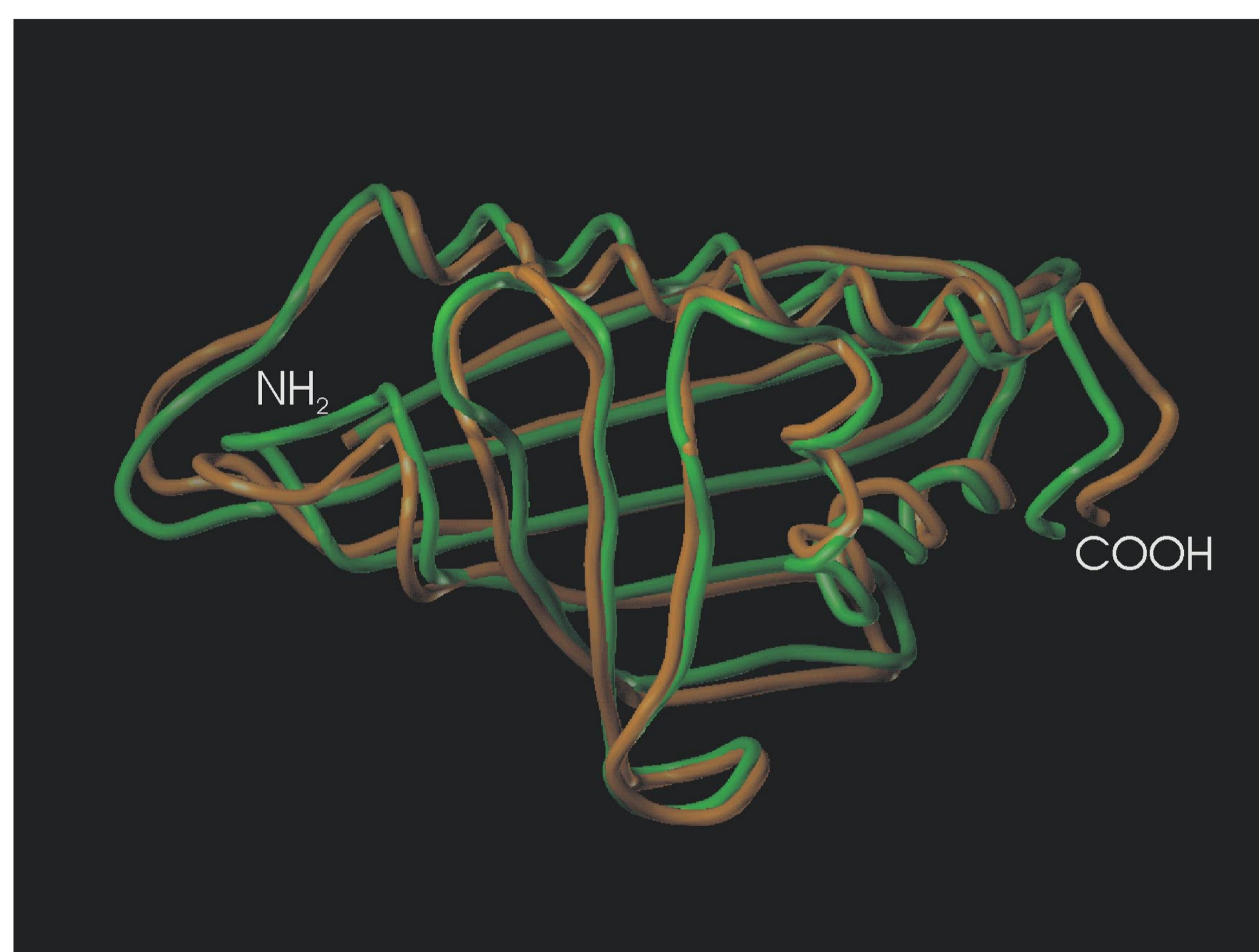


Fig. 4: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of Bet v 1¹ (orange). Same view as in Fig. 2 and 3. The tertiary fold is almost identical.

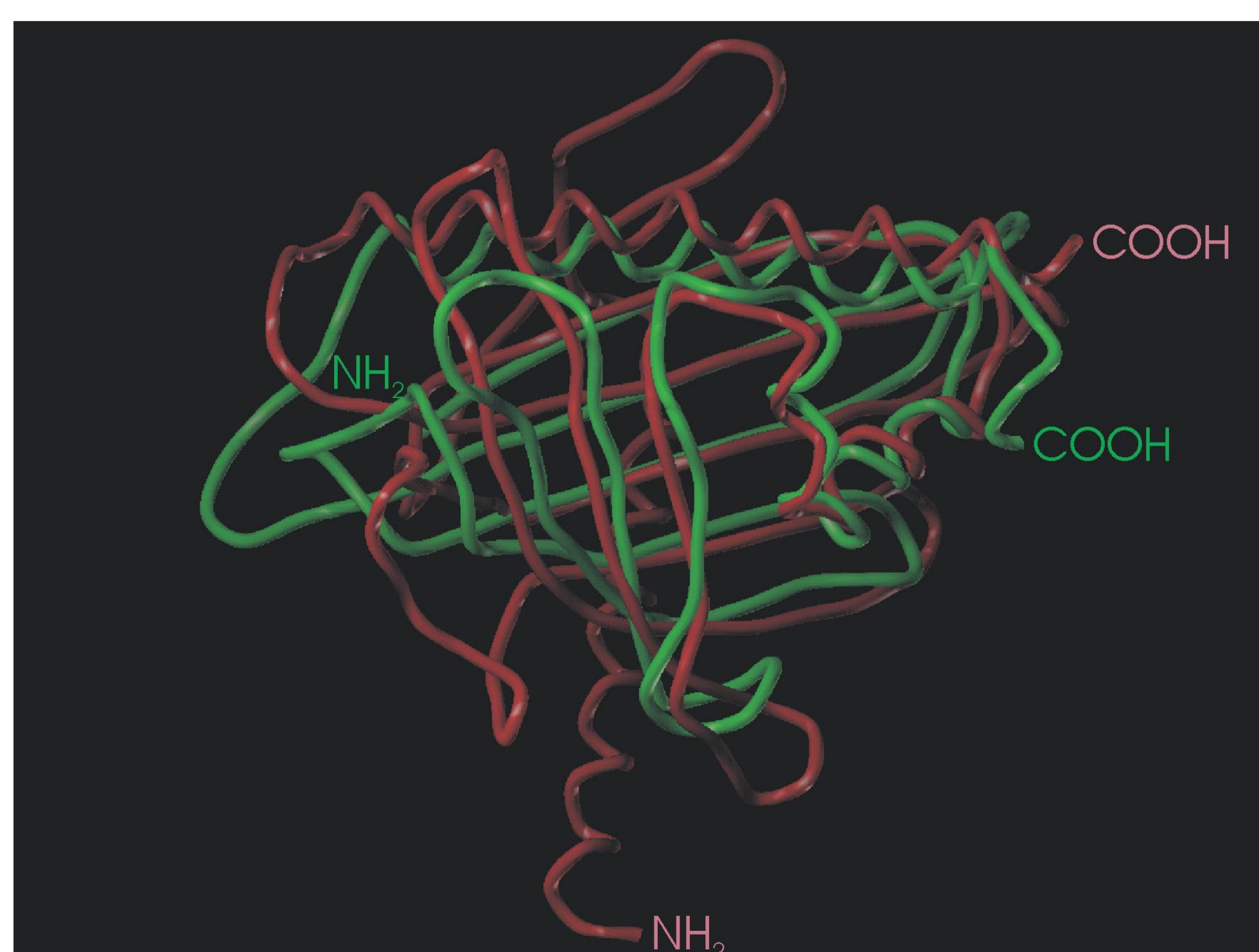


Fig. 5: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of the START domain of human MLN64⁵ (red), a protein that is associated with cholesterol transport. Same view as in Fig. 2, 3 and 4. The backbone atomic RMSD over the 129 residues used for the alignment is only 2.89 Å, even though the sequence identity over these 129 residues is only 8.5 % (Fig. 1).

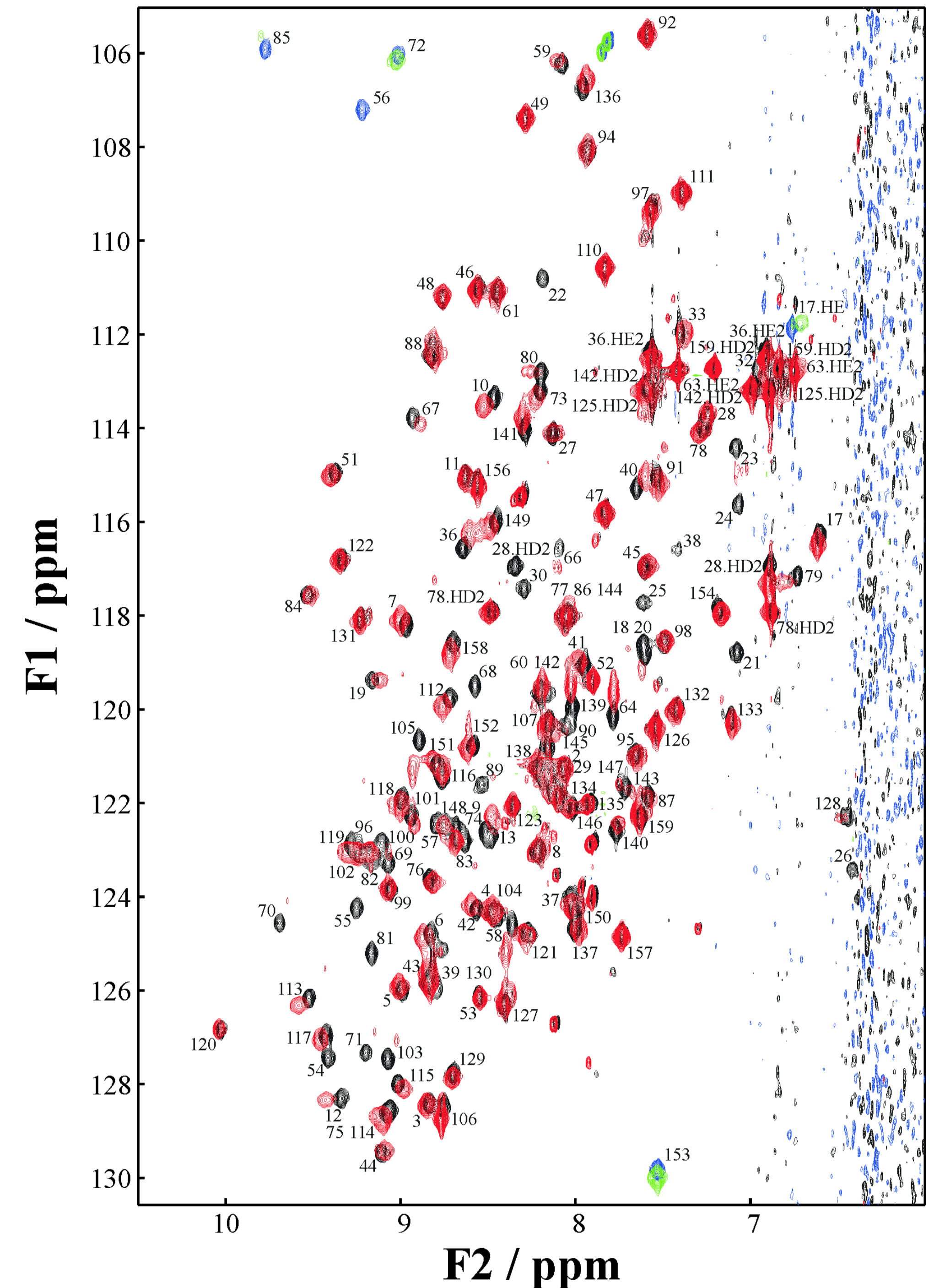


Fig. 6: Overlay of the [¹H,¹⁵N] HSQC spectra of uniformly ¹⁵N-labeled Pru av 1 with (positive signals in red, negative signals in green) and without (positive signals in black, negative signals in blue) homocastasterone in H₂O/DMSO-d₆ (9:1). Amide proton resonances are labeled according to their residue numbers. Negative resonances are aliased in the indirect ¹⁵N dimension F1.

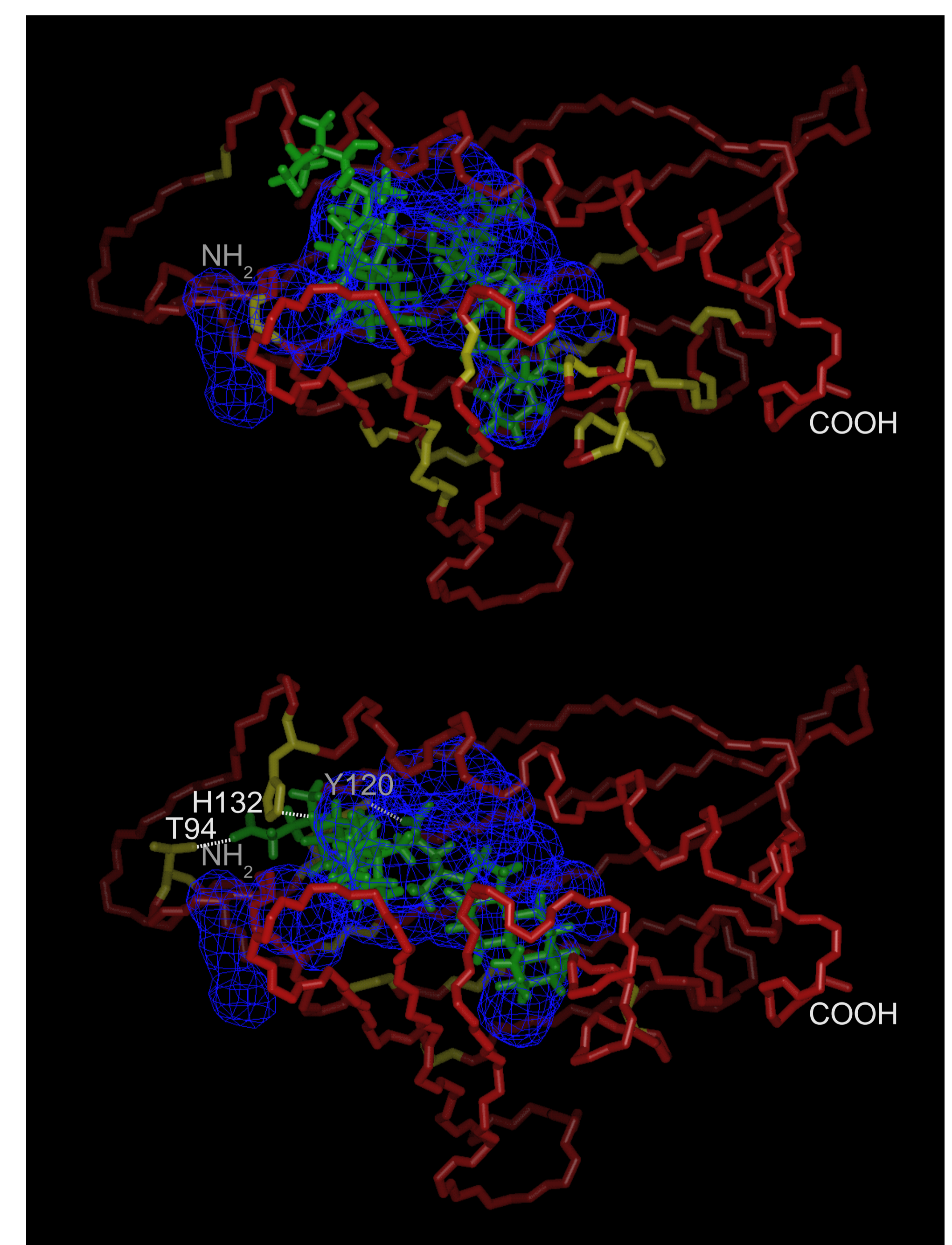


Fig. 7: Visualization of the hydrophobic cavity. Similar view as in Fig. 2, 3, 4 and 5. The backbone of the solution structure of Pru av 1 is shown in red, the cavity is indicated by blue lines. The location of two castasterone molecules modeled into the cavity (top) is shown in green, residues whose amide proton resonances disappeared from the [¹H,¹⁵N] HSQC (Fig. 6) upon presence of homocastasterone due to intermediate exchange processes are colored yellow. The location of two deoxycholate ligands in a recently determined crystal structure of Bet v 1 (PDB access code 1FM4; bottom) is shown in green, residues with resonances disappearing from the ¹H spectrum upon titration with deoxycholate (data not shown) and the side-chains of Thr94, Tyr120 and His132 probably involved in hydrogen bonds are colored yellow.

References

- M. Gajhede, P. Osmark, F. M. Poulsen, H. Ipsen, J. N. Larsen, R. J. J. van Neerven, C. Schou, H. Löwenstein and M. D. Spangfort, *Nature Struct. Biol.* **3**, 1040–1045 (1996)
- K. Schweimer, H. Sticht, J. Nerkamp, M. Boehm, M. Breitenbach, S. Vieths and P. Rösch, *Appl. Magn. Reson.* **17**, 449–464 (1999)
- P. Neudecker, K. Schweimer, J. Nerkamp, M. Boehm, S. Scheurer, S. Vieths, H. Sticht and P. Rösch, *J. Biomol. NMR* **8**, 71–72 (2000)
- P. Neudecker, K. Schweimer, J. Nerkamp, S. Scheurer, S. Vieths, H. Sticht and P. Rösch, *J. Biol. Chem.* **276**, 22757–22763 (2001)
- Y. Tsujishita and J. H. Hurley, *Nature Struct. Biol.* **7**, 408–414 (2000)
- O. Mirza, A. Henriksen, H. Ipsen, J. N. Larsen, M. Wissenbach, M. D. Spangfort and M. Gajhede, *J. Immunol.* **165**, 331–338 (2000)