*Supporting Information*

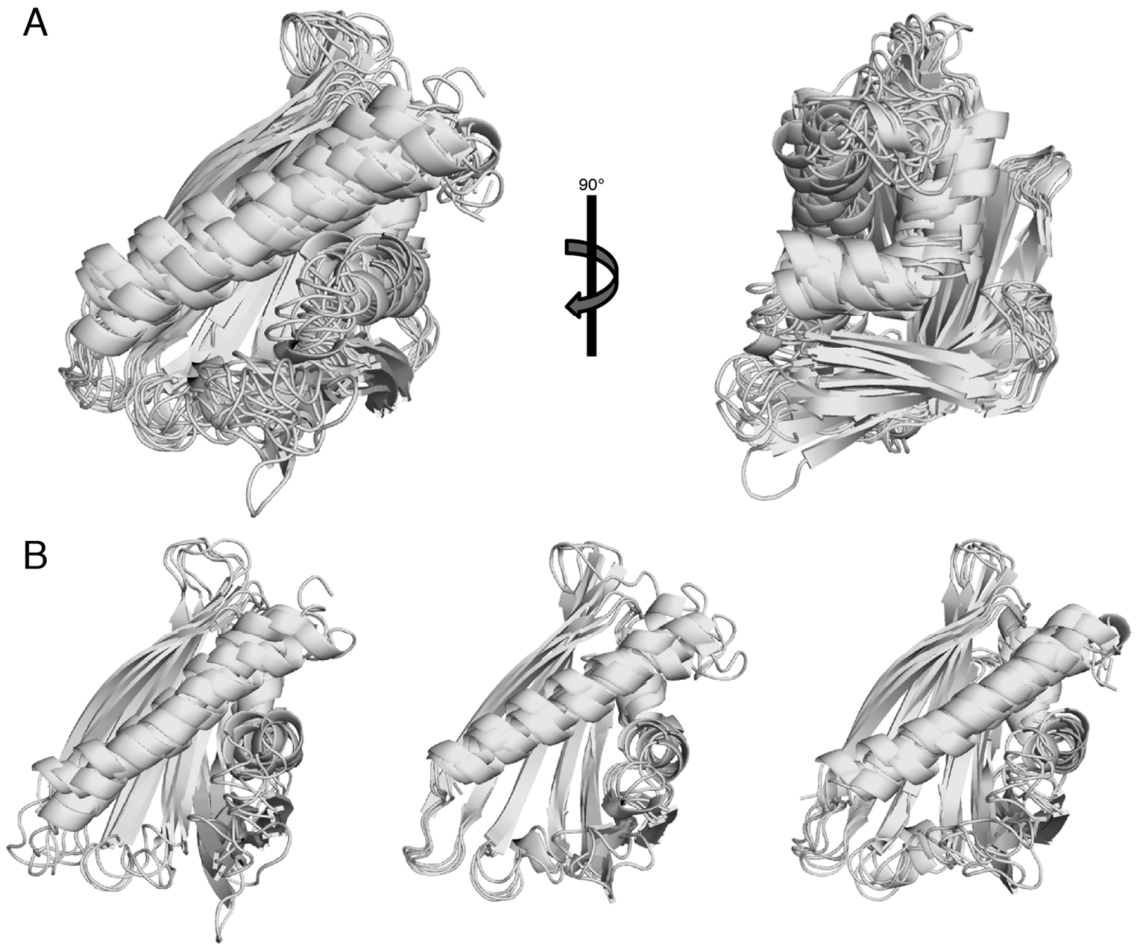
# The structural flexibility of PR-10 food allergens

**Sebastian Führer, Jana Unterhauser, Ricarda Zeindl, Reiner Eidelpes, Monica L. Fernández-Quintero, Klaus R. Liedl and Martin Tollinger**

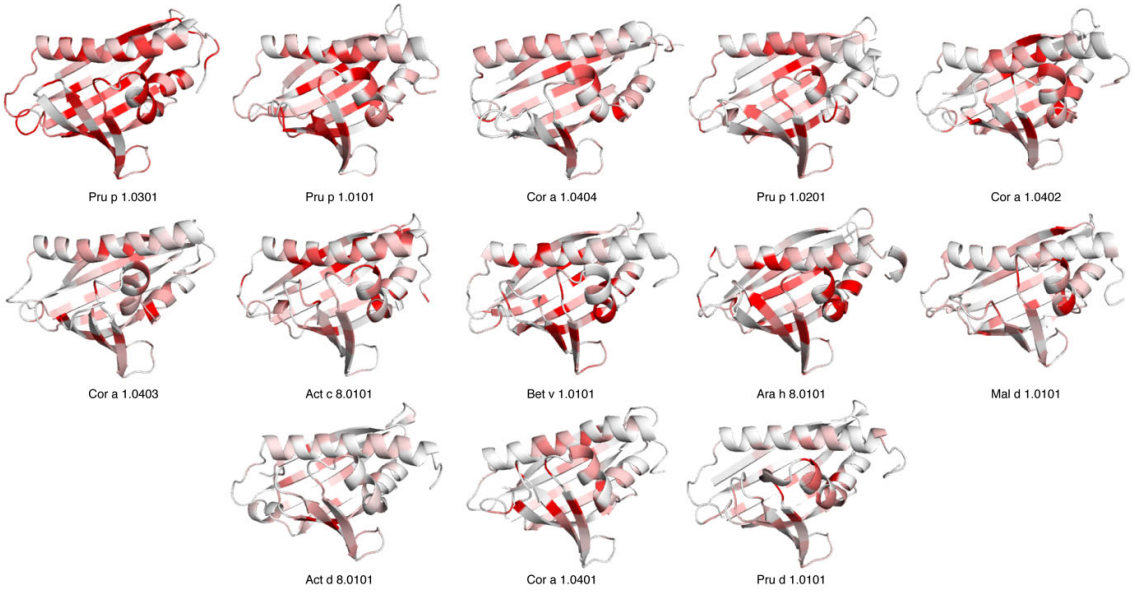
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Bet v 1.0101 GVFNYETETT SVIPAARLFK AFILDGDNLF PKVAPQAISS VENIEGNGGP GTIKKISFPE GFPFKYVKDR VDEVDHTNFK 80
Mal d 1.0101 GVYTFENEFT SEIPPSRLFK AFVLDADNLI PKIAPQAIKQ AEILEGNGGP GTIKKITFGE GSQYGYVKHR IDSIDEASYS 80
Act c 8.0101 GVVTYDMEIP SKVPPVKLYK AFILDGDTLV PKVLPHAIKC VKILEGDGCA GTIKEVTFGE GSHHKCVKQR VDAIDKDNLT 80
Act d 8.0101 GAITYDMEIP SSISAEKMFK AFVLDGDTII PKALPHAITG VQTLEGDGGV GTIKLTTFGE GSVHKSVKHR IDGLDKENFT 80
Pru d 1.0101 GVFTYESEFT SEIPPPRLFK AFVLDADNLV PKIAPQAIKH SEILEGDGGP GTIKKITFGE GSQYGYVKHK IDSIDKENHS 80
Pru p 1.0101 GVFTYESEFT SEIPPPRLFK AFVLDADNLV PKIAPQAIKH SEILEGDGGP GTIKKITFGE GSQYGYVKHK IDSIDKENHS 80
Pru p 1.0201 GVFTYSDEST SVIPPPRLFK ALVLEADTLI PKIAPQSVKS AEIVEGDGGV GTIKKISFGE GSHYSYVKHR IDGLDKDNFV 80
Pru p 1.0301 GVFTYETEFT SVIPPEKLFK AFILDADNLI PKIAPTAVKD TEILEGDGGV GTIKKVTFGE GSQYGYVKHR IDGIDKDNLT 80
Ara h 8.0101 GVFTFEDEIT STVPPAKLYN AM-KDADSIT PKIID-DVKS VEIVEGNGGP GTIKKLTIVE DGETKFILHK VESIDEANYA 78
Cor a 1.0401 GVFCYEDEAT SVIPPARLFK SFVLDADNLI PKVAPQHFTS AENLEGNGGP GTIKKITFAE GNEFKYMKHK VEEIDHANFK 80
Cor a 1.0402 GVFSYEDEAT SVIPPARLFK SFVLDADNLI PKVAPQHFTG AENLEGNGGP GTIKKITFAE GSEFKYMKHK VEEIDHANFK 80
Cor a 1.0403 GVFCYEDEAT SVIPPARLFK SFVLDADNLI PKVAPQHFTG AENLEGNGGP GTIKKITFAE GSEFKYMKHK VEEIDHANFK 80
Cor a 1.0404 GVFSYEDEAT SVIPPARLFK SFVLDADNLI PKVAPQHFTS AENLEGNGGP GTIKKITFAE GNEFKYMKHK VEEIDHANFK 80
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Bet v 1.0101 YNYSVIEGGP IGDTLEKISN EIKIVATPD- GGSILKISNK YHTKGDHEVK AEQVKASKEM GETLLRAVES YLLAHSDAYN 159
Mal d 1.0101 YSYTLIEGDA LTDTIEKISY ETKLVACGS- -GSTIKSISH YHTKGNIEIK EEHVKVGKEK AHGLFKLIES YLKDHPDAYN 158
Act c 8.0101 YSYTIIEGDV LAEKFESISY HIKIVACPD- GGSICKNRSI YTTKGDCKVS EEEIKLGKEK AAEIFKALEA YLLANPDYC- 158
Act d 8.0101 YSYSIIEGGA L-DVFESISY HIKIVATPD- GGCICKNRSI YTPKCDAQVS EEEIKAGKER ASGIFKKVEA YLLANPDC-- 156
Pru d 1.0101 YSYTLIEGDA LGDNLEKISY ETKLVASPS- GGSIIKSISH YHTKGDVEIK EEHVKAGKEK ASNLFKLIET YLKGHPDAYN 159
Pru p 1.0101 YSYTLIEGDA LGDNLEKISY ETKLVASPS- GGSIIKSTSH YHTKGDVEIK EEHVKAGKEK ASNLFKLIET YLKGHPDAYN 159
Pru p 1.0201 YNYTLVEGDA LSDKVEKITY EIKLVASAD- GGSIIKSTSN YHTKGDVEIK EEDVKAGKEK ATGLFKLIEN YLVANPDAYN 159
Pru p 1.0301 YSYTLIEGDA LSDVIEKIVY DIKLVASPN- GGSIVKTISH YHTKGDVEIK EEQVKAGKEK AAGLFKLVEG YLLANPDAYN 159
Ara h 8.0101 YNYSVVGGVA LPPTAEKITF ETKLVEGPN- GGSIGKLTLK YHTKGDAKPD EEELKKGKAK GEGLFRAIEG YVLANPTQY- 156
Cor a 1.0401 YCYSIIEGGP LGHTLEKISY EIKMAAAPHG GGSILKITSK YHTKGNASIN EEEIKAGKEK AAGLFKAVEA YLLAHPDAYC 160
Cor a 1.0402 YCYSIIEGGP LGHTLEKISY EIKMAAAPHG GGSILKITSK YHTKGNASIS EEEIKAGKEK AAGLFKAVEA YLLAHPDTYC 160
Cor a 1.0403 YCYSIIEGGP LGHTLEKISY EIKMAAAPHG GGSILKITSK YHTKGNASIS EEEIKAGKEK AAGLFKAVEA YLLAHPDTYC 160
Cor a 1.0404 YCYSIIEGGP LGHTLEKIPY EIKMAAAPHG GGSILKITSK YHTKGNASIN EEEIKAGKEK AAGLFKAVEA YLLAHPDAYC 160
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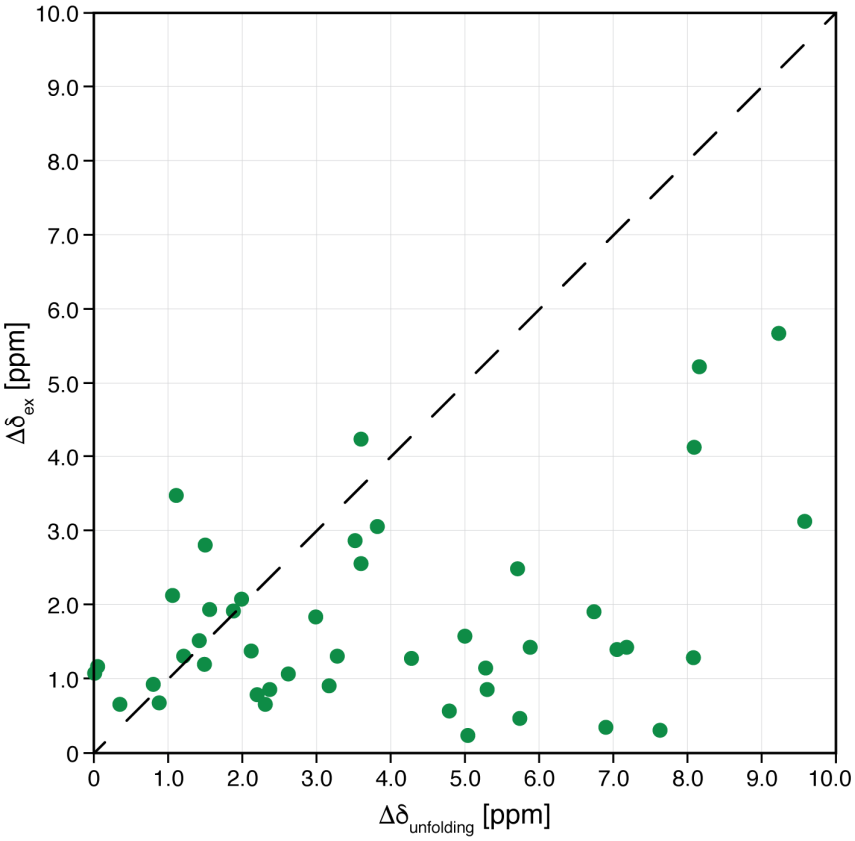
**Supporting Figure S1.** Sequence alignment of PR-10 allergens. Identical amino acid residues are labeled with asterisks, conserved residues with colons and semiconserved residues with dots. Positions of amino acid residues that are not present in a specific allergen are indicated by a dash.




**Supporting Figure S2.** Structural comparison of the thirteen PR-10 allergens in this study. (**A**) Overlay of all structures. (**B**) Overlay of Cor a 1.0101, Cor a 1.0402, Cor a 1.0403, Cor a 1.0404 (left) compared to Pru p 1.0101, Pru p 1.0201, Pru p 1.0301, Pru d 1.0101 (middle) and Bet v 1.0101, Mal d 1.0101, Ara h 8.0101, Act c 8.0101, Act d 8.0101 (right).




**Supporting Figure S3.** Structural flexibility of the thirteen PR-10 allergens. Relaxation dispersion amplitudes ($\Delta R_{2,eff}$ values) exceeding 1 $s^{-1}$ at 700 MHz are plotted on the protein backbones, using a color gradient from rigid (white) to more flexible (red). The same color gradient as in Figure 3 was used for all proteins.




**Supporting Figure S4.** Comparison of $^{15}N$ chemical shift differences, $\Delta\delta_{ex}$, obtained from the backbone amide $^{15}N$ relaxation dispersion data of Ara h 8.0101 with $^{15}N$ secondary chemical shifts, $\Delta\delta_{unfolding}$, as expected for complete unfolding of the protein backbone. Values of $\Delta\delta_{unfolding}$ were calculated as the difference between observed $^{15}N$ chemical shifts and predicted $^{15}N$ chemical shifts for a random coil (Tamiola K, Acar B, Mulder FAA, *J Am Chem Soc* 2010 (132), 18000-18003. doi: 10.1021/ja105656t). The dashed, drawn with a slope of 1.0, line indicates the $^{15}N$ chemical shift differences, $\Delta\delta_{ex}$, that are expected for complete unfolding of the backbone to a random coil.

| Helices | | Sheet | |
|---|---|---|---|
| Ala16 | 0.30 ± 0.16 | Lys37 | 1.51 ± 0.34 |
| Lys 17 | 0.46 ± 0.06 | Ser38 | 5.22 ± 0.51 |
| Asn20 | 0.85 ± 0.07 | Val39 | 2.80 ± 0.25 |
| Ala21 | 1.83 ± 0.14 | Ile41 | 1.30 ± 0.23 |
| Met22 | 0.78 ± 0.06 | Glu43 | 1.42 ± 0.27 |
| Lys23 | 1.39 ± 0.11 | Gly47 | 2.55 ± 0.29 |
| Asp24 | 0.92 ± 0.07 | Thr50 | 0.23 ± 0.10 |
| Ala25 | 1.06 ± 0.07 | Lys53 | 1.16 ± 0.25 |
| Ser27 | 1.57 ± 0.13 | Thr55 | 1.42 ± 0.14 |
| Lys31 | 2.48 ± 0.21 | Ile56 | 2.07 ± 0.22 |
| Ile33 | 0.65 ± 0.07 | Leu66 | 3.47 ± 0.24 |
| Asp123 | 0.90 ± 0.14 | His67 | 3.05 ± 0.16 |
| Glu128 | 1.37 ± 0.10 | Asp73 | 1.93 ± 0.17 |
| Gly134 | 1.19 ± 0.08 | Glu74 | 1.14 ± 0.12 |
| Lys137 | 1.07 ± 0.08 | Val84 | 3.12 ± 1.08 |
| Gly138 | 0.65 ± 0.19 | Val87 | 1.90 ± 0.26 |
| Glu139 | 0.85 ± 0.12 | Ala88 | 5.67 ± 0.81 |
| Ile145 | 0.67 ± 0.08 | Leu89 | 1.30 ± 0.17 |
| Glu146 | 0.56 ± 0.07 | Thr97 | 4.12 ± 0.90 |
| Tyr156 | 0.25 ± 0.16 | Phe98 | 4.23 ± 1.04 |
| | | Glu99 | 1.91 ± 0.16 |
| | | Thr100 | 1.27 ± 0.22 |
| | | Lys101 | 2.86 ± 0.30 |
| | | Leu102 | 2.21 ± 0.18 |
| | | Thr115 | 0.34 ± 0.18 |
| | | Tyr118 | 1.28 ± 0.36 |

**Supporting Table S1.** Residue-specific $^{15}$N chemical shift differences, $\Delta\delta_{ex}$, between major and minor states, obtained from sub-global fits of the backbone amide $^{15}$N relaxation dispersion data for amino acid residues in the three α-helices and loop L2 (left) and in the β-sheet and all other loops (right). Data are shown for Ara h 8.0101. For amino acid residues highlighted in blue and orange the experimental relaxation dispersion data are shown in Figure 4.