

Supplementary data for the article:

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## Supplementary Information

### **Title: Conformational stability of digestion-resistant peptides of peanut conglutins reveals the molecular basis of their allergenicity**

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**Table S1. Masses of identified digestion-resistant peptides from conglutin isoforms**

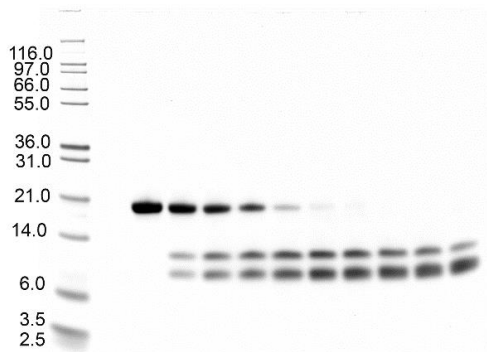
DRP	Sequence mass	pI	Modifications					Calculated mass	Experimental mass
			H <sub>2</sub> O <sup>a)</sup>	Disulfide bond	Alkyl group	Proline hydroxylation	Sequence conflict		
<b>Ara h 2.02 non-reduced</b>									
a	17993.69	5.36	+18	-8	0	+48	0	18051.69	18051.33
b	17559.24	5.35	+18	-8	0	+48	0	17617.24	17617.14
c	16659.28	5.34	+18	-8	0	+32	0	16701.28	16700.72
d	16224.82	5.33	+18	-8	0	+32	0	16266.82	16266.55
e	16068.64	5.1	+18	-8	0	+32	0	16110.64	16110.44
f	3541.67	4.17	0	0	0	+48	0	3589.67	3589.57
g	3030.04	3.79	0	0	0	+32	0	3062.04	3061.28
h	4364.46	4.01	0	0	0	+48	0	4412.46	4411.90
<b>Ara h 2.02 reduced and alkylated</b>									
i	9401.39	5.2	0	0	+342	0	0	9743.39	9743.58
i	9401.39	5.2	0	-2	+285	0	0	9684.39	9684.58
i	9401.39	5.2	0	0	+228	0	0	9629.39	9629.53
j	8966.94	5.18	0	0	+342	0	0	9308.94	9308.38
j	8966.94	5.18	0	0	+285	0	0	9251.94	9252.88
j	8966.94	5.18	0	0	+228	0	0	9194.94	9194.35
j	8966.94	5.18	0	-2	+171	0	0	9135.94	9135.31
k	8610.31	5.64	0	0	+114	+48	0	8772.31	8772.16
k	8610.31	5.64	0	0	+57	+48	0	8715.31	8714.16
l	8454.13	5.05	0	0	+114	+48	0	8616.13	8615.10
m	7275.9	5.61	0	0	+114	+32	0	7421.90	7421.57
<b>Ara h 2.01 non-reduced</b>									
a	16579.23	5.67	0	-8	0	+32	+92	16695.23	16695.72
b	16423.04	5.33	+18	-8	0	+32	+92	16557.04	16555.50
b	16423.04	5.33	0	-8	0	+32	+14	16461.04	16462.54
c	16416.06	5.67	+18	-8	0	+32	0	16458.06	16456.54
d	16259.87	5.1	+18	-8	0	+32	+58	16359.87	16359.54
e	16103.68	5.1	+18	-8	0	+32	+58	16203.68	16202.52
f	1971.03	3.59	0	0	0	+32	0	2003.03	2002.83
<b>Ara h 2.01 reduced and alkylated</b>									
g	9238.21	5.73	0	0	+171	0	0	9409.21	9409.40
g	9238.21	5.73	0	0	+114	0	0	9352.21	9352.31
g	9238.21	5.73	0	0	+57	0	0	9295.21	9295.29
g	9238.21	5.73	0	0	0	0	0	9238.21	9238.31
h	7195.86	7.02	0	0	+171	+32	+14	7412.86	7413.49
i	7039.67	5.59	0	0	+171	+32	+14	7256.67	7257.43
j	2127.21	4.17	0	0	0	+32	0	2159.21	2158.94
<b>Ara h 6 non-reduced</b>									
a	14116.86	4.83	+18	-10	0	0	0	14124.86	14125.29

b	13903.62	4.68	+18	-10	0	0	0	13911.62	13911.17
c	13772.49	4.68	+18	-10	0	0	0	13780.49	13780.12
d	13559.25	4.55	+18	-10	0	0	0	13567.25	13567.02
<b>Ara h 6 reduced and alkylated</b>									
e	9148.37	4.78	0	-2	285	0	0	9431.37	9432.23
e	9148.37	4.78	0	-2	228	0	0	9374.37	9374.24
e	9148.37	4.78	0	-2	171	0	0	9317.17	9317.22
e	9148.37	4.78	0	-2	114	0	0	9260.37	9260.19
e	9148.37	4.78	0	-2	57	0	0	9203.37	9203.17
f	5330.88	5.56	0	0	171	0	0	5501.88	5501.62
f	5330.88	4.9	0	0	114	0	0	5444.88	5444.59
g	5117.64	4.9	0	0	171	0	0	5288.64	5288.50
g	5117.64	4.9	0	0	114	0	0	5231.64	5231.47
h	4986.51	4.9	0	0	228	0	0	5214.51	5214.47
h	4986.51	4.9	0	0	171	0	0	5157.51	5157.45
h	4986.51	4.9	0	0	114	0	0	5100.51	5100.43
i	4773.27	4.55	0	0	171	0	0	4944.27	4943.47
i	4773.27	4.55	0	0	114	0	0	4887.27	4886.29
i	4773.27	4.55	0	0	57	0	0	4830.27	4829.27

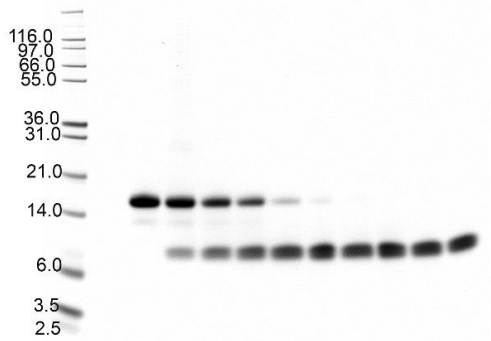
a) Internal peptide bond hydrolysis

**Supplementary Figure 1. Time course of trypsin digestion of conglutin isoforms as analysed by SDS-PAGE.**

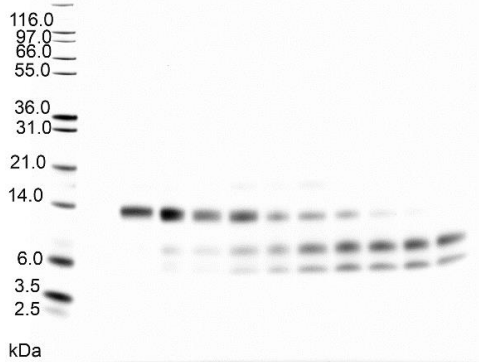
**a.** M 0' 5' 10' 15' 30' 45' 60' 90' 120' 180'



**b.**

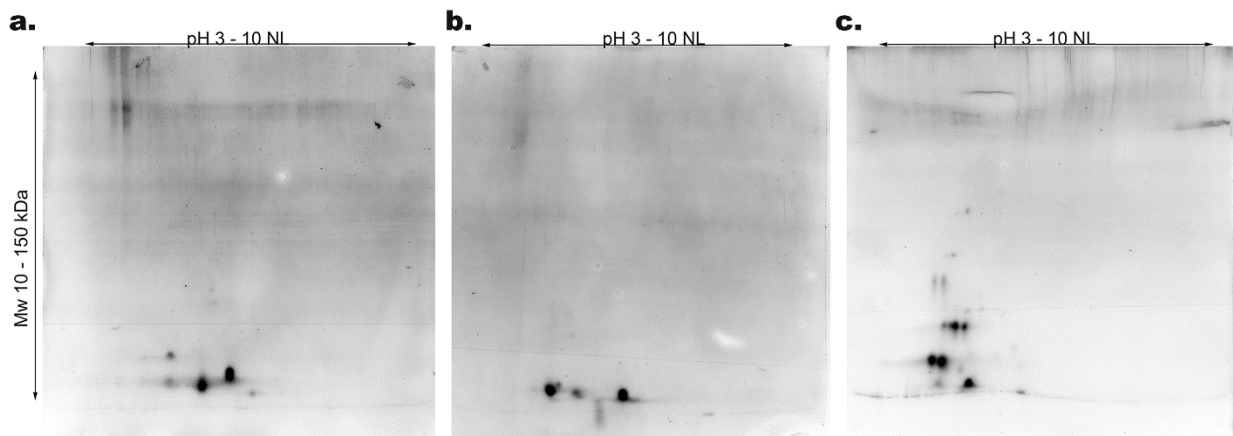


**c.**



Incubation times (in minutes) are indicated at the top of each pane. Panel a: Ara h 2.02; Panel b: Ara h 2.01; Panel c: Ara h 6. M: Molecular markers. For all panels reducing conditions were applied.

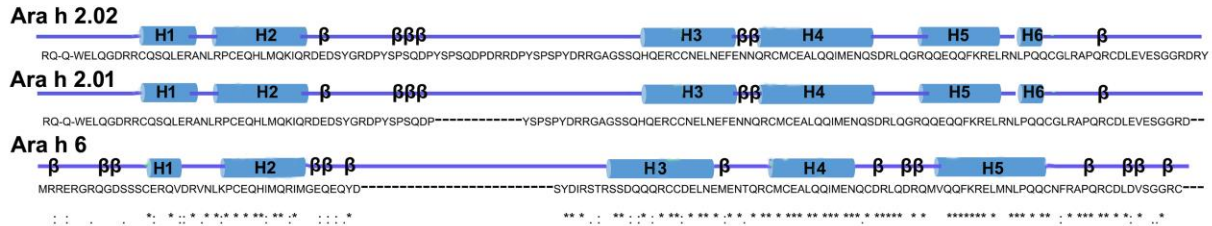
**Supplementary Figure 2. 2-DE analysis of DRPs from conglutin isoforms**



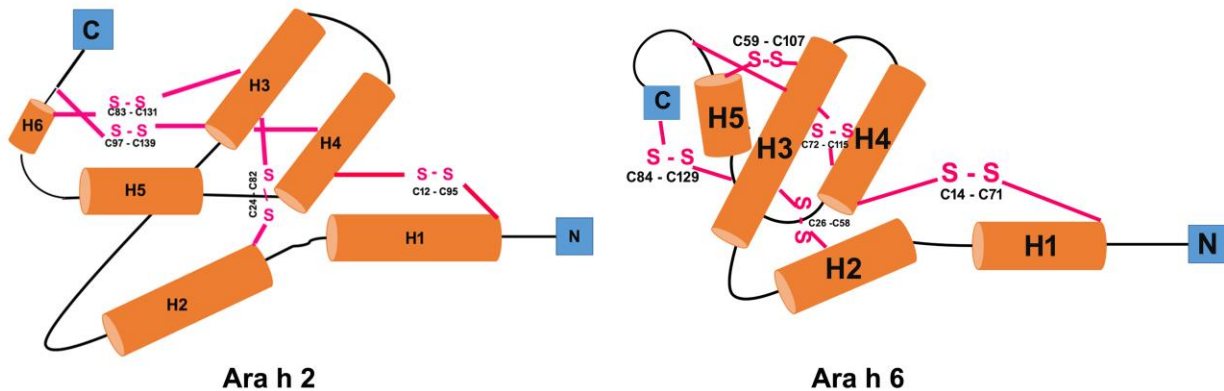
Panel a: DRPs of Ara h 2.02; Panel b: DRPs of Ara h 2.01; Panel c: DRPs of Ara h 6

**Supplementary Figure 3. Sequence alignment and 2D topology diagrams of conglutin isoforms**

**a.**



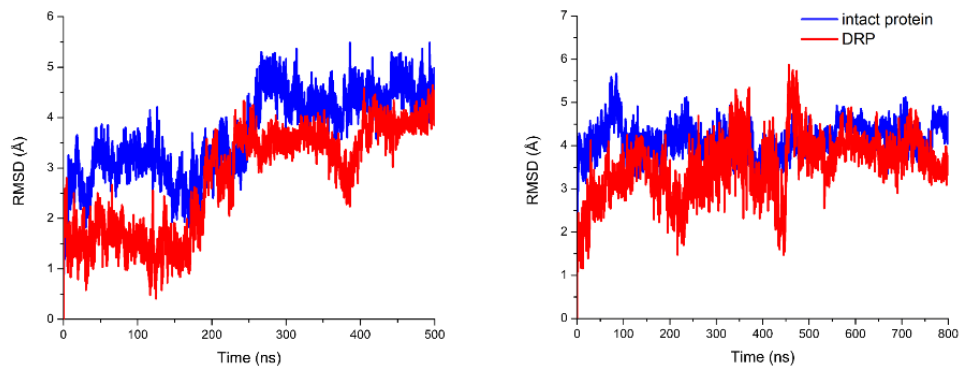
**b.**



**Panel a** – sequence alignment of conglutin isoforms with assigned secondary structures. Symbols underneath the alignment indicate: positions which have a single, fully conserved residue (\*), conservation between groups of strongly similar properties (:), and weakly similar properties (.) Blue cylinder represent helix, and  $\beta$  strands and turns based on PDB sum for PDB codes: 3OB4 (Ara h 2) and 1w2q (Ara h 6).

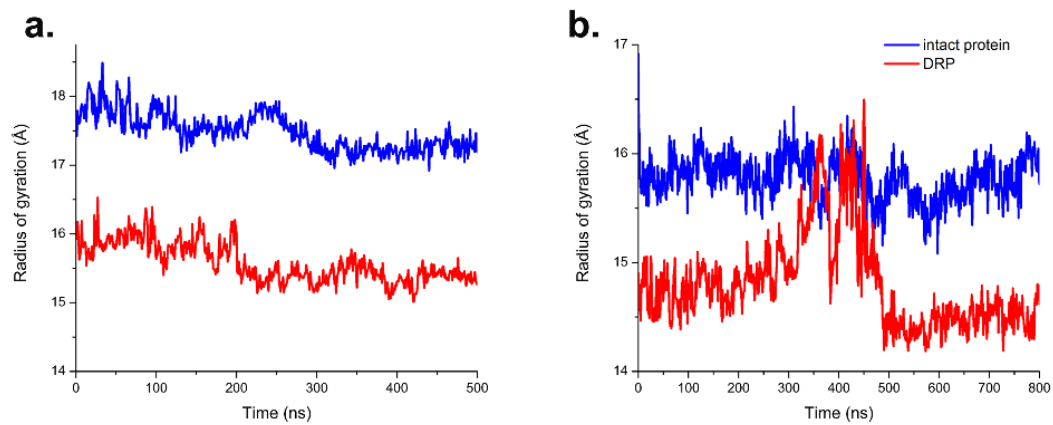
**Panel b** - topology diagram of Ara h 2.02 and Ara h 6 with assigned disulphide bridges. Orange cylinder represent helix; s-s connection for specific cysteine residue into the disulphide bridge; N in blue square represent N terminus of protein; C in blue square represent C terminus of protein.

### Supplementary Figure 4. RMSD variation of conglutin isoforms with DRP



Panel a: Ara h 2.02; Panel b: Ara h 6.

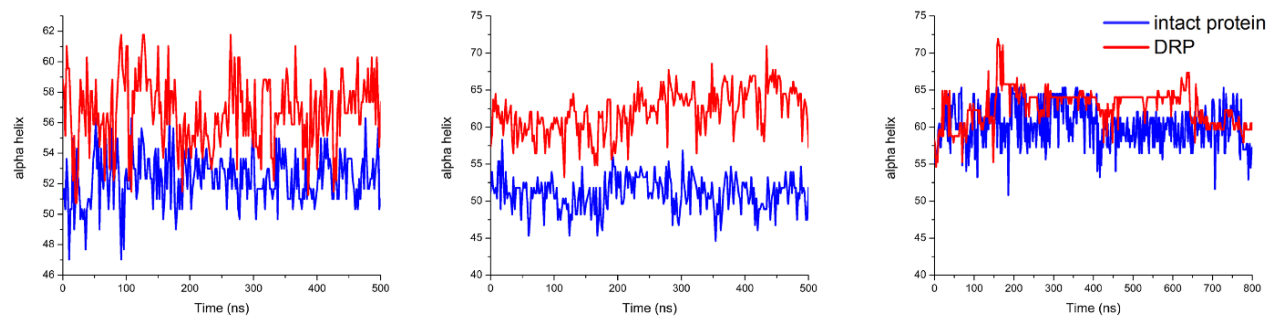
### Supplementary Figure 5. RG variation of conglutin isoforms with DRP



Panel a: Ara h 2.02; Panel b: Ara h 6.



**Supplementary Figure 6. Alpha helix in DRPs calculated according to STRIDE algorithm.**



alpha helix in intact conglutins (blue) and DRPs red). Panel a: Ara h 2.02. Panel b: Ara h 2.01.

Panel c: Ara h 6.