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Supporting information for article:

Cloning, expression, purification, characterization, crystallization and X-ray crystallographic analysis of recombinant Der f 21 (rDer f 21) from *Dermatophagoides farinae*

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Table S1 Constructs and molecular weight of reported recombinant protein Der f 21.

Constructs of Der f 21	Sequence	Number of Residues	Molecular Weight	Vector	References
Full length	**MKPIIPCAIVMAVSVS* ***G*FIVDVDT**EDKWRNAF DHMLMEEFEEKMDQIE HGLLMLSEQYKELEKT KSKELKEQILRELTIAEN YLRGALKFMQQEAKRT DLNMFERYNFETAVSTI EILVKDLAELAKKVKA VKSDD	136 residues (consist of predicted 17 residues signal peptide and 119 residues of mature Der f 21)	Deduced from SDS-PAGE: 14 kDa	pCold-TF	Cui *et al*., 2014
Predicted Mature Der f 21	#**FIVDVDT**EDKWRNAF DHMLMEEFEEKMDQIE HGLLMLSEQYKELEKT KSKELKEQILRELTIAEN YLRGALKFMQQEAKRT DLNMFERYNFETAVSTI EILVKDLAELAKKVKA VKSDD	119 residues (consist of residue 18 to 136 of full length Der f 21)	Calculated: 14.16 kDa	-	Cui *et al*., 2014
rDer f 21	+**MHHHHHHSSGLVPR GS**EDKWRNAFDHMLM EEFEEKMDQIEHGLLM LSEQYKELEKTKSKELK EQILRELTIAENYLRGA LKFMQQEAKRTDLNMF ERYNFETAVSTIEILVK DLAELAKKVKAVKSDD	128 residues (consist of 16 residues from His-tag fusion and 112 residues of Der f 21 (residue 25-136).	Calculated: 15.2 kDa SDS-PAGE: 13 kDa Mass-Spec: 15.2 kDa	pET-M	This study

* The predicted signal peptides is in bolded italic form.

The seven amino acid residues that eliminated in rDer f 21 construct is bolded.

+ The extra His-tag fusion sequence consisted of 16 amino acid residues is in bold.

Protein sequence coverage: 41%

Matched peptides shown in ***bold red***.

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  1 MKFIIFCAIV MAVSVSGFIV DVDTEDKWRN AFDHMLMEEF EEKMDQIEHG
 51 LLMLSEQYKE LEKTKSKELK EQILRELTIA ENYLRGALKF MQQEAKRTDL
101 NMFERYNFET AVSTIEILVK DLAELAKKVK AVKSDD
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Figure S1 Der f 21 matched peptides sequence obtained using the mass spectrometry 5800 MALDI-TOF/TOF MS (Applied Biosystems-SCIEX).

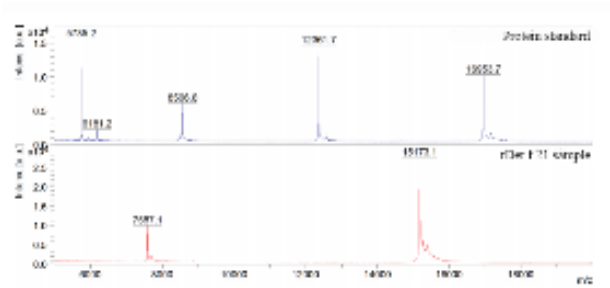

Figure S2 MALDI TOF/TOF mass spectra of rDer f 21 compared with the profile of protein standard in the m/z range of 5 to 20 kDa. Molecular weight values are indicated on the top of each peak.

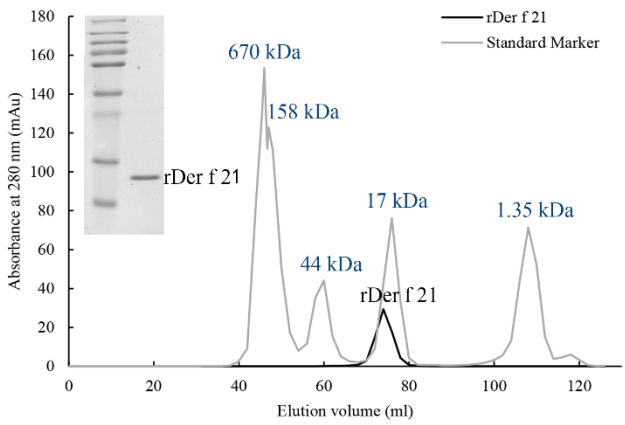

Figure S3 Size-exclusion chromatography result of rDer f 21 using HiLoad 16/600 Superdex 75 pg gel filtration column (GE healthcare, UK). The rDer f 21 protein was eluted as a single peak bigger than 17 kDa of myoglobin. The chromatography peak suggests that the rDer f 21 protein may be monomer or dimer in solution.

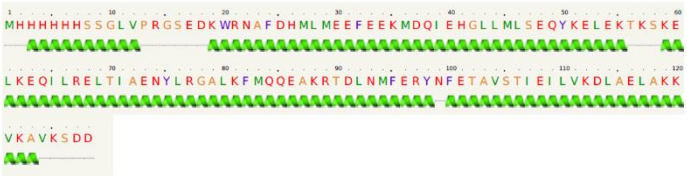

Figure S4 Secondary structure prediction of rDer f 21 determined by PHYRE2 (Kelley & Sternberg, 2009). The rDer f 21 protein structure is predicted to have three long alpha helices and one short alpha helix at the N-terminal fusion-tag region.

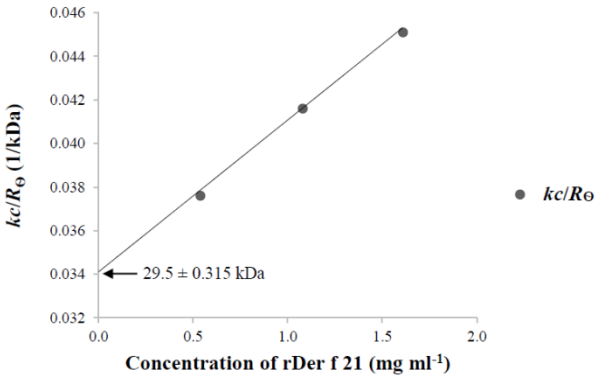

Figure S5 Debye plot generated using static light scattering data of rDer f 21. The second-virial-coefficient value, A_2 of rDer f 21 protein is 0.00350 ± 1.58 X 10^{-4}, and molecular weight of rDer f 21 calculated using $kc/R_\Theta = (1/M + 2A_2c)$ equation is 29.5 kDa.

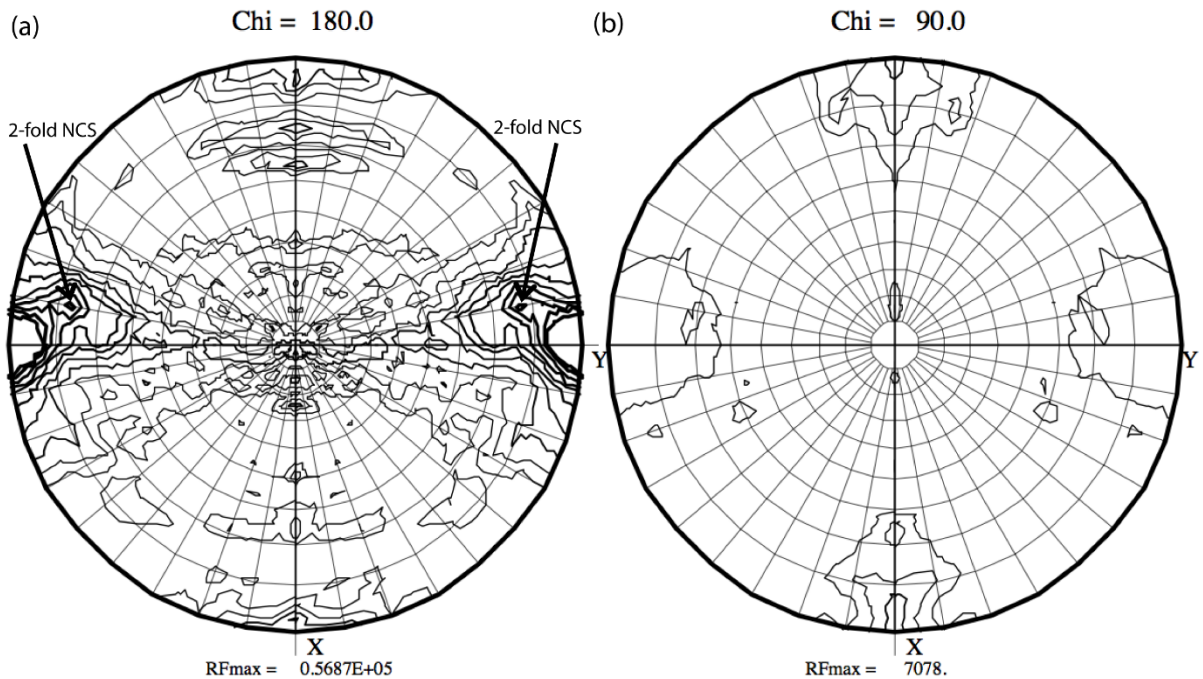

Figure S6 Stereographic projections of the self-rotation function peaks for Der f 21 data was calculated by MOLREP. Sections of (a) κ = 180° and (b) κ = 90° are shown. The arrows indicate the non-crystallographic symmetry (NCS) two-fold axis located nearby the crystallographic axis in the section chi=180°.