Supplementary Files

The odorant-binding proteins of the spider mite *Tetranychus urticae*

Jiao Zhu[1,2], Giovanni Renzone[3], Simona Arena[3], Francesca Romana Dani[4], Harald Paulsen[2], Wolfgang Knoll[1,5], Christian Cambillau[6], Andrea Scaloni[3], Paolo Pelosi[1]

[1] Austrian Institute of Technology GmbH, Biosensor Technologies, Konrad-Lorenz Straße, 24, 3430 Tulln, Austria
[2] Faculty of Biology, Institute of Molecular Physiology, Johannes Gutenberg-Universität, 55099 Mainz, Germany
[3] Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, Napoli, Italy
[4] Department of Biology, University of Firenze, via Madonna del Piano 6, 50019 Sesto Fiorentino, Italy
[5] Department of Physics and Chemistry of Materials, Faculty of Medicine/Dental Medicine, Danube Private University, Krems, Austria
[6] Architecture et Fonction des Macromolécules Biologiques (UMR 6098), CNRS, 13288 Marseille Cedex 09, France

Correspondence to: Paolo Pelosi, Austrian Institute of Technology GmbH, Biosensor Technologies, Konrad-Lorenz Straße, 24, 3430 Tulln, Austria. E.mail: paolo.pelosi@ait.ac.at; ppelosi.obp@gmail.com

Supplementary files

Table S1. Amino acid sequences of ticks and mites OBPs used to construct the tree of Figure 1.

>TurtOBP1 _XP_015786166.1 uncharacterized protein LOC107363453 [Tetranychus urticae]
MKNLQSITILCLIFGFIVSTVYSQPLGSSDSNPDTQDTKEASTSVNDKSSDQEGSSESDEDAPTPVAGPHRPPTINWGKC
PQLEPKEAEKKQKADIIRTCLKSITLPENITQESVEKHRTEVAKCALNAENWFAEDGSYRYDKAESEIKNKKLQPEIEPQ
IVNQHSECRKEAEEQFPSGIAQIQLYQACMDYHISMICGIQIVGTGYSQ
>TurtOBP2 XP_015794342.1 uncharacterized protein LOC107370892 [Tetranychus urticae]
MNYLSLCVFIIFFLTIGFSESARLPRSTSSPATSSSTTTTSSTVNNKKNEENQPSTSSASSSAGSATGGPTINWGRCPQL
EPTNEEKIKKAAVITKCLESTPVPVNITRETVELHREQVAACALRMEGWFNGQGLYRFDKAENEIKAKKLTREVEEKVLS
FHRQCKDEAEEKYPVSSNQLIAQIQLYQACMDYFISEVCGIEVQIAGSEGSSE
>TurtOBP3 XP_015782945.1 uncharacterized protein LOC107360741 [Tetranychus urticae]
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ETVEKHRTEVARCALNAENWFTEDGKYRYDKAESEIKAKKLSQEVEPQILRQHGECRKEAEGQFPSGIAQIQLYQACMDY
HISQICGITIVGPSSPQ
>TurtOBP4 XP_015791022.1 uncharacterized protein LOC107367785 [Tetranychus urticae]
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QVQLYQACMDYHISQICGITIVGASSPQ
>Ssca_KAF7495847 .1 hypothetical protein SSS_793 [Sarcoptes scabiei]
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LEQQQELLDQLAQLQRQTQQLLAAQQSLAAVAAAAAVNGNNNGVSIGTPLDGNSNNDAGSHSPITSGSIQYHRQQNQQQT
HPTTQQLNNPTSNQQSAAIRNDNIDQGSLEQSQSKSNQLNQMINNAQNSQSQSMSGLPQSPQINWGKCPELEPSEQEKLA
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EEKYPTSNSSIIAQIQLYQACMDYFISDVCGIEVNDTDSPQF
>SscaKAF7496567 .1 hypothetical protein SSS_1960 [Sarcoptes scabiei]
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SITPELIIGHQKNVTECALKIENWFDKKGDYKFEKAEKEIKNKKLSSEMQKALVGSHQDCAEVSKTQKKQSKTPMDVVEQ
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>Dpte_XP_027195173 .1 GATA zinc finger domain-containing protein 10-like
[Dermatophagoides pteronyssinus]
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EPSEQEKLAKANVITKCLETTPLPTNITRESVEQHREQIAACALRSEGWFTQQGGYDFSKAEKEIKNKRLQEDLENQVLN
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pteronyssinus]
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KNMTIVEQVQVYQGCMDAHITQNCQIEISAV
>Ldel_RWS22631 .1 hypothetical protein B4U80_07214, partial [Leptotrombidium deliense]
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VCGIDVLRR
>Ldel_RWS28635 .1 hypothetical protein B4U80_06376 [Leptotrombidium deliense]
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IVQHQREVALCALTTEQWFNEQGKYRYEKAENEIKRKGLQPVIQTRIVFHHNKCQTEAKEKQDVIQEVQMYQACMDYYIA
QICGITVTTQ
>Dtin_RWS11187 .1 hypothetical protein B4U79_15176 [Dinothrombium tinctorium]
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NENNRQHHNQPQQPPPPLPPIGPPQISWGQCPQLEPTMEEKIKKAAVITKCLELTPVPTNITRESVELHREQVAACALQM
EGWFNEKGTYRFDKAESEIKNKKLQKDIENRVLTYHKQCKEEAEEKFPISSQNLIAQIQLYQACMDYFISEVCGIEVGPS
APAEF
>Dtin_RWS02013 .1 hypothetical protein B4U79_11820 [Dinothrombium tinctorium]
MWKTVAIVIFFCVLFVDCQQPQEPKINWGKCSKIEPTEQDKQEKAKVIQGCLKQVPLPKNVTRESVAQHQREVAVCALNA
ENWFNEEGKYRYEKAERQIKSKKLEKEIETKILFHHNKCKREAQEKTEIIEEVQLYQACMDYYIAQICEITVN
>Msub_CAD7629358 .1 unnamed protein product [Medioppia subpectinata]
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GIHCQQMFSQQPPQAQAPSQPQPQSQQPPATQGPQINWGKCPQLEPSEKEKMTKAQVITKCLETTPLPQNITRDTVELHR
EQIAACALQSEGWFTANNAYDFGKAETEIKNKQLTKQIEDQVLGYHKQCKEEAEEKFPNTKNTVIAQIQLYQACMDYFIS
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NLTQQSIESHQKAIAECALRAEKWFTKAGNYRFSKAETEIRAKKLQKPIESQLLANHKNCESEAKREFPKTAIIDQIQLY

QACVRHVTRSYGFTAFLTLNIHFQMDFHISNVCEIKVENSK

>Onov_CAD7648203 .1 unnamed protein product [Oppiella nova]
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SDAEFQTPPTLLILYKSRETFAQKTPKMETLLKVSLFLCIVCAFSAFAAPANKKVDKQTATTTGTTPAANATTDKAPTID
WGKCPQLEPKDAEKRAKAAVLETCLARHPIPANLTQETIESHQKAIAECALRTEKWFTAKGNYRYSKAENEIKAKKLPKD
VESALLAHHKNCESEAKREFASKERVIDQIQLYQACMDYHITSACGIQLKDN
>Onov_CAD7650725 .1 unnamed protein product, partial [Oppiella nova]
MSLWLAEHRQERQEFSATRLAIKHKDQTLLATVFTHLLFFDPNQQLPPFFGGQPSGPPQQHFFNPQQFLVQPNQQFNGQP
PQAQPQLAQSQPSQPQLQAQPPAPQGPQINWGKCPQLEPSEKEKSTKAQVITKCLETTPLPPNITRD
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>Vdes_XP_022672530 .1 uncharacterized protein LOC111255133 [Varroa destructor]
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>Vdes_XP_022653281 .1 uncharacterized protein LOC111247024 [Varroa destructor]
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>Vdes_XP_022653293 .1 uncharacterized protein LOC111247029 [Varroa destructor]
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KNHKVDLKKMSMTAASAGSPELKKAYENCPKDDKNTSEDRSIKCLIDHLEVDCKLASK
>Vdes_XP_022666940 .1 uncharacterized protein LOC111252778 [Varroa destructor]
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VEEALPNLSRCMLKVIGWVRANGNMNLTKYMDYLSQAGLDDKTLELTKTAHEKCSQRALSDGSGDYKEQDKIYVECIFRH
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KDHKIDQAKMASTATSITSPELKKAFTECPKDDKNVSEDRTIKCVIDNLETTCKLST
>Gocc_XP_003742126 .1 uncharacterized protein LOC100907366 [Galendromus occidentalis]
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>Gocc_XP_003741537 .1 uncharacterized protein LOC100907454 [Galendromus occidentalis]
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KVMV
>Tmer_OQR73552 .1 hypothetical protein BIW11_09656 [Tropilaelaps mercedesae]
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>Tmer_OQR79252 .1 hypothetical protein BIW11_05867 [Tropilaelaps mercedesae]
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>Tmer_OQR80083 .1 hypothetical protein BIW11_00054, partial [Tropilaelaps mercedesae]
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>Emay_OTF72123 .1 hypothetical protein BLA29_010316, partial [Euroglyphus maynei]
QLPRAPKINWGRCPQLEPTQRDIEAKSSILQECLRQNPPPQSESITQEQIIQREYKYEKAEKEIKNKNLAKEMEKVLLES
HKQCSQIAKDRKQAVKNLSNVEQVQIYQGCMDAHITQNCQIQISAV
>Hfla_AYH64978 .1 immunogenic protein [Haemaphysalis flava]
MVAFKAALFICALALPVYAQNAPAAEPQRPEINWGKCPQLQPSKEERQQKALVIDTCLEKVPLPDVEHANETVIQQHRED
VTTCALHSEGWFNKNGQYRFDRARTEILNKKLAAEVEPQVLAKHDECKKEAEEKFAHQFVAQVQLYQACMDYHISQICGI
QIQALPQAGAAPPAQG
>Hlon_ADK47399 .1 immunogenic protein [Haemaphysalis longicornis]
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VTTCALHSEGWFNKNGQYRFDRARTEILNKKLAAEVEPQVLAKHDECKKEAEEKFAHQFVAQVQLYQACMDYHISQICGI
QIQAGAPAGGVPQQPPQQG
>Hqin_AAX37829 .1 Hq05 [Haemaphysalis qinghaiensis]
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VTTCALKTEGWFNKNGHYRFDRAKTEISNKKLPADVQPQVIAKHDECKKEAETKFPKNFIAQVQLYQACMDHHISQLCGV
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NDTVIQQHRQDVTTCALKTEGWFNSNGQYRFDRARTEILNKKLAKDVEPQVLSKHDECKKEAEEKFAKNFVAQVQLYQAC
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IDLAAMTTRASSVSGKLGQAFTDCPKDDHNRNAAKGVKCIIDRLETDCPVPTGSTAH
>Iper_KAG0425362 .1 hypothetical protein HPB47_027463, partial [Ixodes persulcatus]
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KDDHNRNASKGVKCIIDRLE
>Isca_XP_002405987 .2 uncharacterized protein LOC8033800 [Ixodes scapularis]
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HPQDIEEYLPRLSRCMLKSTGWVRSNGNMNLSKYLQYLSLLGLDDETMNGTREAHKGCIAKAYKEQDYKTQDKVYVECIF
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IQGAGGAGAPAHG
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RTDVDLSRMQQAAQNVTQQELRAAFQECPQDENNKKVGKAVKCLIDHLETSCPVPAGVNRE
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RTDVDLSRMQHLAQNITQPEVRAAFQECAQDENNKKVGKAVKCFIDHLETSCPVPDATKRE
>Rsan_XP_037519110 .1 uncharacterized protein LOC119396094 isoform X1 [Rhipicephalus
sanguineus]
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KNDVNLERMAQLAKDITQQDLKGAFQECPKNDRNKSLQRTVKCVIDHLEKTVPCARWRCWSQGVRR
>Rsan_XP_037519113 .1 uncharacterized protein LOC119396094 isoform X2 [Rhipicephalus
sanguineus]
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IQGGPQAGAPAHG
>Dsil_XP_037578762 .1 uncharacterized protein LOC119461497 [Dermacentor silvarum]
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>Dsil_XP_037581089 .1 uncharacterized protein LOC119464249 [Dermacentor silvarum]
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DIEEYLPRLSRCMLKSTGWVRSNGNMNLSKYLQYLSLLGLDNETMDSTREAHKGCISKAYKETDFKTQDKLYVECIFRHF
TKVCGANFQGAVKEGYIVGITQLSFMPRMGPLEVVDDVDGDDVVETPPGEERVPRPINETAPIEP

Table S3. Ligands tested with the four TurtOBPs

Ligand	OBP1	OBP4	OBP2	OBP3
	Competitive binding		Tryptophan quenching	
1-NPN	v	v	v	v
Coniferyl aldehyde	v	v	v	v
Vanillin	v	v	v	v
β-Ionone	v	v	v	v
Safranal	v	v	v	v
Eugenol	v	v	v	v
Farnesol	v			
(*E*)-β-Farnesene	v	v		v
Methyl jasmonate	v			
Linalool	v	v		
Decanal	v			
(*Z*)-3-Hexenol	v			
Hexanal	v			
Dopamine	v	v		
Decanoic acid	v			
Geranyl acetone	v			v
Z11-Hexadecenal		v		
Farnesol		v		
Oleic acid		v		
8-Pentadecanone		v		
Citral		v		v
Benzyl cyanide			v	v
Methoxyeugenol			v	v
4-Methylcathecol			v	v
p-Isopropylphenol			v	v
α-Methoxycinnamaldehyde			v	
α-Pentylcinnamaldehyde			v	v
Carvacrol			v	v
Methoxyvinylphenol			v	
p-tert-Butylbenzaldehyde			v	v
Thymol			v	
Homovanillic alcohol			v	
Cyclamen aldehyde			v	v
Piperonyl alcohol			v	
Octanoic acid				v
Decanoic acid				v
Dodecanoic acid				v
Methyleugenol				v
β-Caryophyllene	v			v
Geraniol	v			v
Santalol	v			v
β-Pinene	v			v

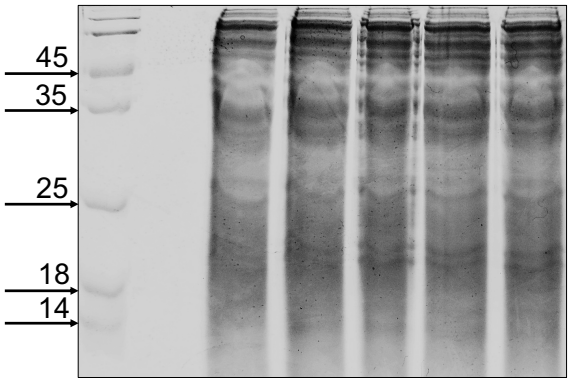

Figure S1. Electrophoretic separation of a crude extract of whole *T. urticae* proteins used for proteomic analysis. The region between apparent molecular mass 12-30 kDa was excised from the five lanes (all loaded with the same sample), pooled and subjected to proteomic analysis as described in the Material and Methods section.

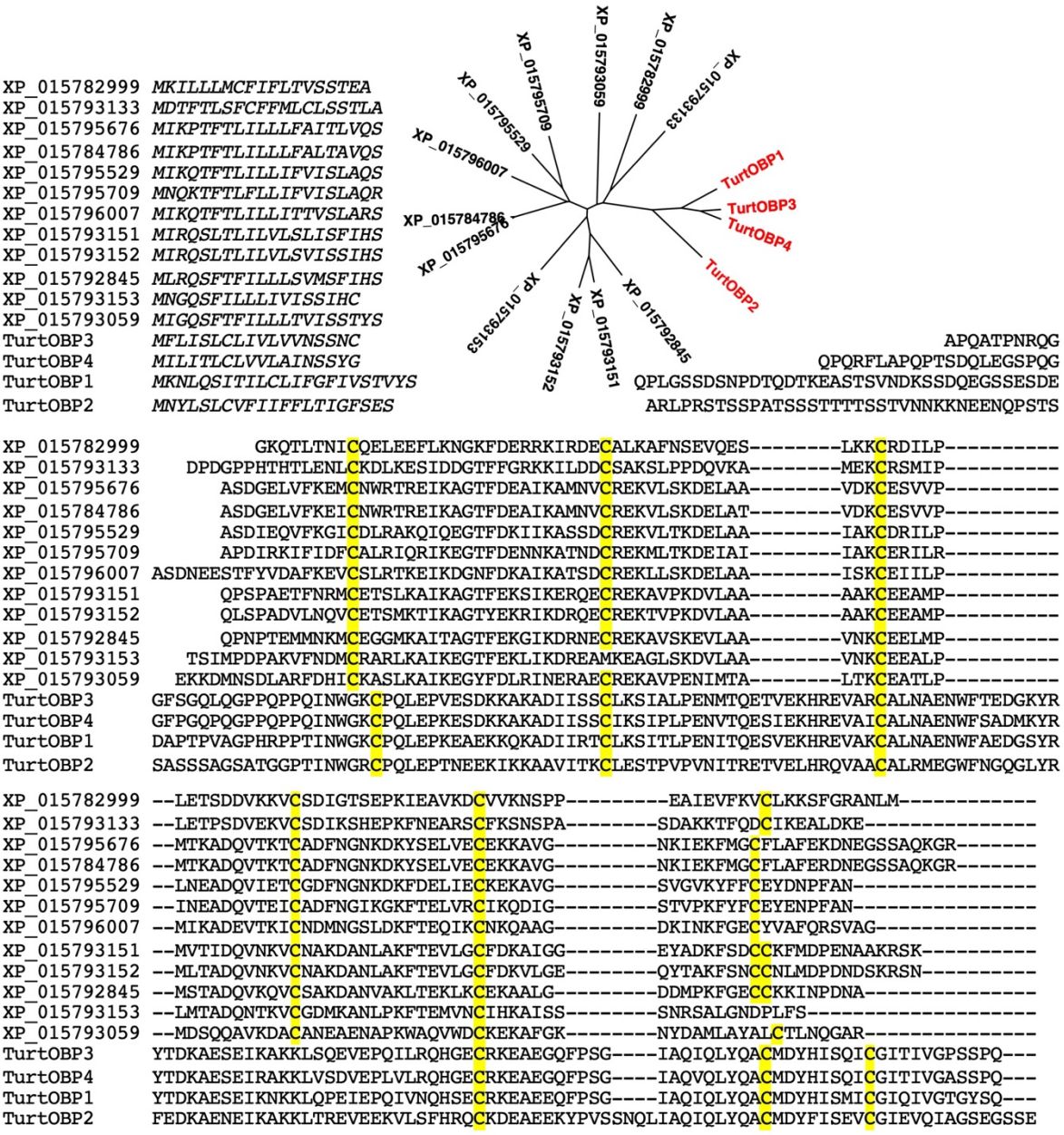

Figure S2. Alignment of four TurtOBPs with 12 sequences of a putative second OBP family of OBPs in *T. urticae*. Identity values between members of the two groups of proteins are around 10-15% at the amino acid level. Such low similarity is still significant, considering also the alignment of most of the cysteine residues.

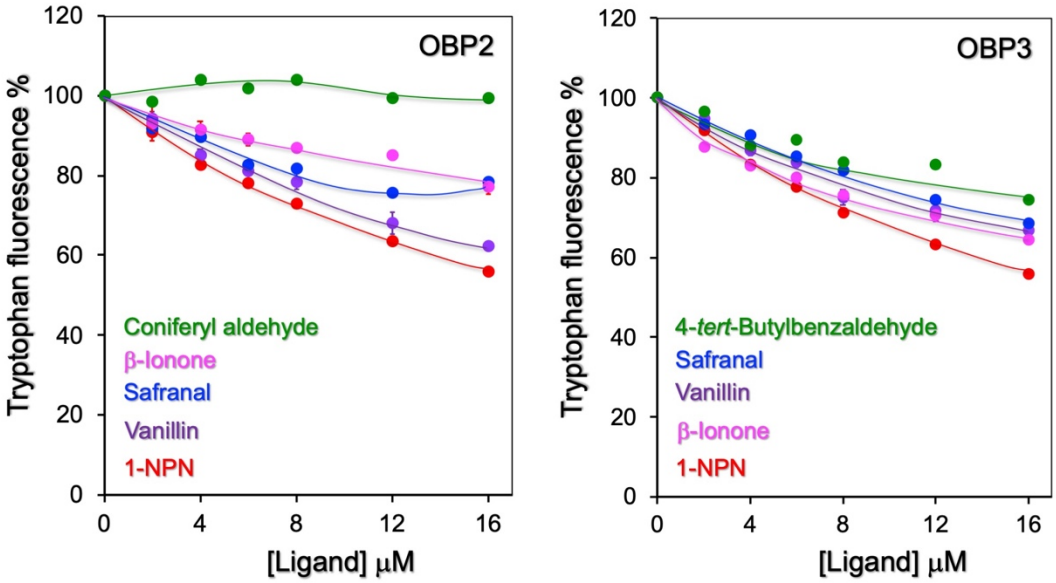

Figure S3. Quenching of the intrinsic tryptophan fluorescence of TurtOBP2 and TurtOBP3 by selected ligands.

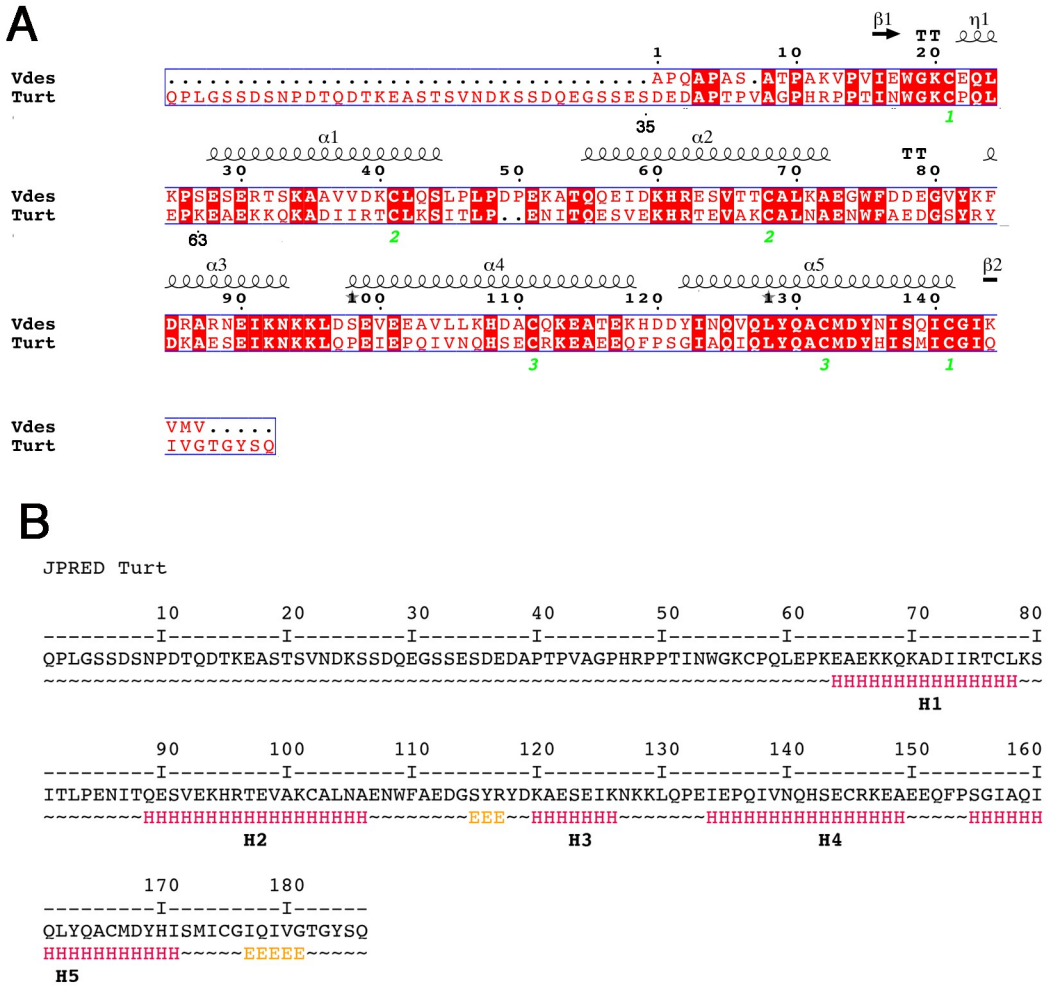

Figure S4. Secondary structure analysis of OBP1 from *T. urticae* and *V. destructor.* A. Sequence alignment of these OBPs was perform with Multalin [43] and EsPrit [44], which allow to superimpose sequence data with secondary structure. B. The secondary structure analysis of TurtOBP1 was also performed with Jpred4 [45] using standard parameters.

A

Summary statistics

Protein Geometry	Poor rotamers	1	0.83%	Goal: <0.3%
	Favored rotamers	117	96.69%	Goal: >98%
	Ramachandran outliers	1	0.74%	Goal: <0.05%
	Ramachandran favored	127	93.38%	Goal: >98%
	Rama distribution Z-score	2.53 ± 0.72		Goal: abs(Z score) < 2
	Cβ deviations >0.25Å	0	0.00%	Goal: 0
	Bad bonds:	0 / 1124	0.00%	Goal: 0%
	Bad angles:	7 / 1517	0.46%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 9	0.00%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	1	0.7%	Goal: <1.0%
	CA Geometry outliers	0	0.00%	Goal: <0.5%
Additional validations	Chiral volume outliers	0/161		

In the two column results, the left column gives the raw count, right column gives the percentage.
Key to table colors and cutoffs here:

B

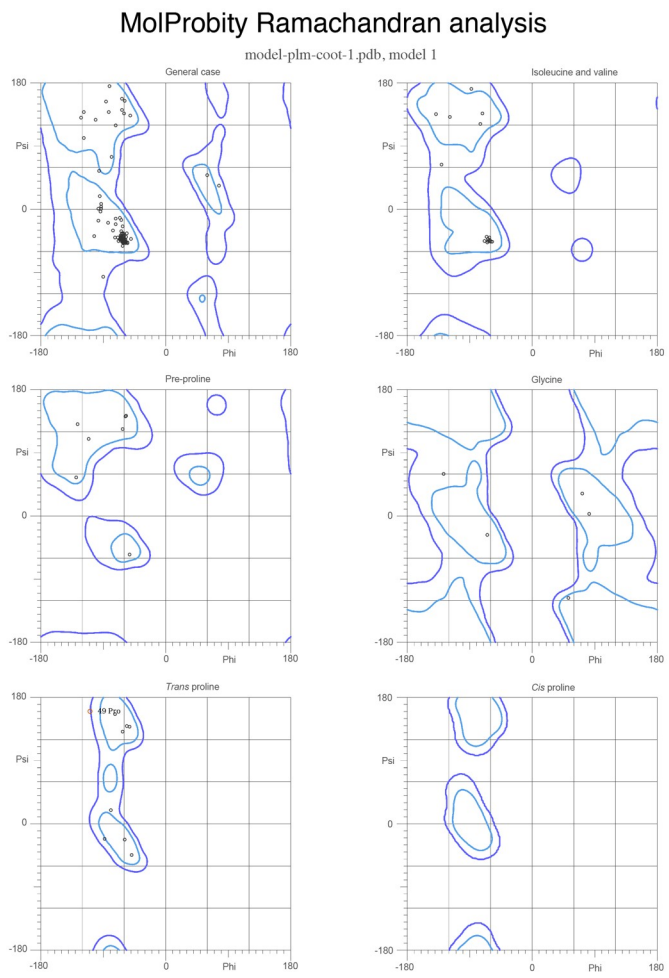

Figure S5.Output files from Molprobity

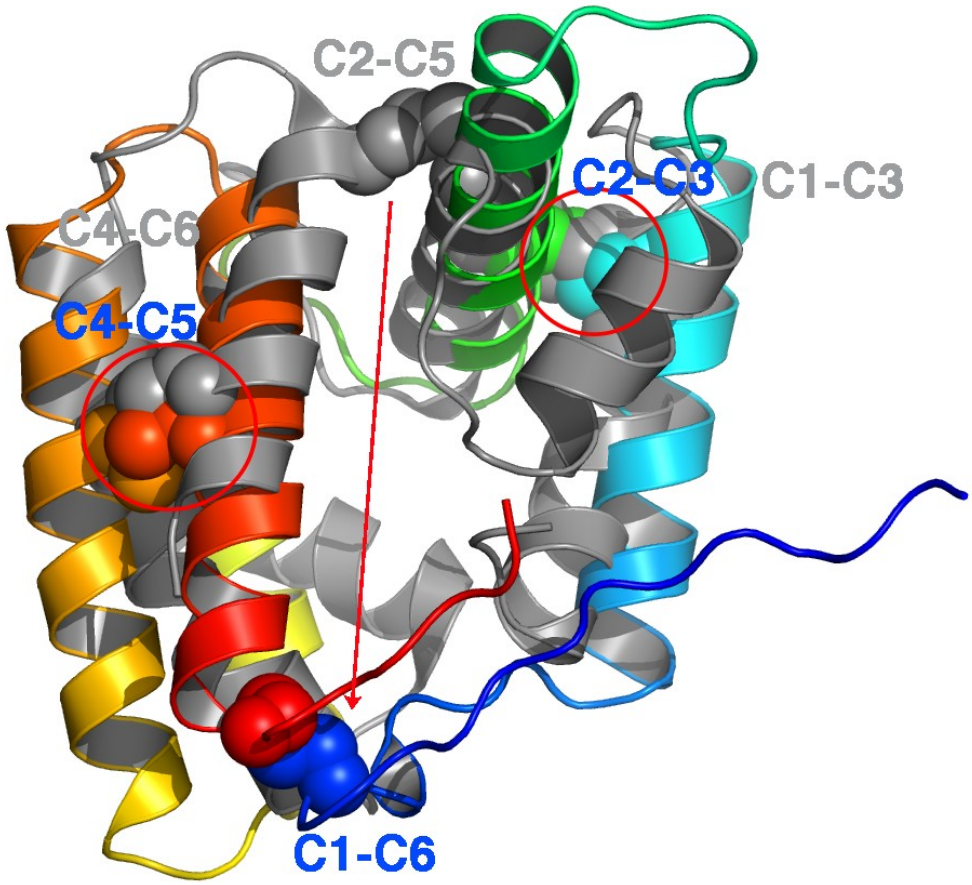

Figure S6. Ribbon view of the structure of TurtOBP1 model superimposed to that of the classical OBP fold of LmadPBP. TurtOBP1 is rainbow colored, LmadPBP is colored grey. The three disulfide bridges of each structure are displayed as atomic spheres. The superimposed disulfide briges are identified by a red circle, while the C2- C5 disulfide of the classical OBP fold is displaced at the opposite end of the molecule in TurtOBP1 (red arrow).