

Novel hydrophobins Hydph6, Hydph7, and Hydph16 from *Pleurotus ostreatus*: *In silico* characterization reveals Hydph6 chitin affinity and industrial potential

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SUPPLEMENTARY MATERIAL

Signal Peptide Prediction:

Sequence

Prediction: Signal Peptide (Sec/SPI)

Cleavage site between pos. 23 and 24.

Probability 0.924045

Protein type	Other	Signal Peptide (Sec/SPI)	Lipoprotein signal peptide (Sec/SPII)	TAT signal peptide (Tat/SPI)	TAT Lipoprotein signal peptide (Tat/SPII)	Pilin-like signal peptide (Sec/SPIII)
Likelihood	0.0003	0.9991	0.0001	0.0002	0.0001	0.0001

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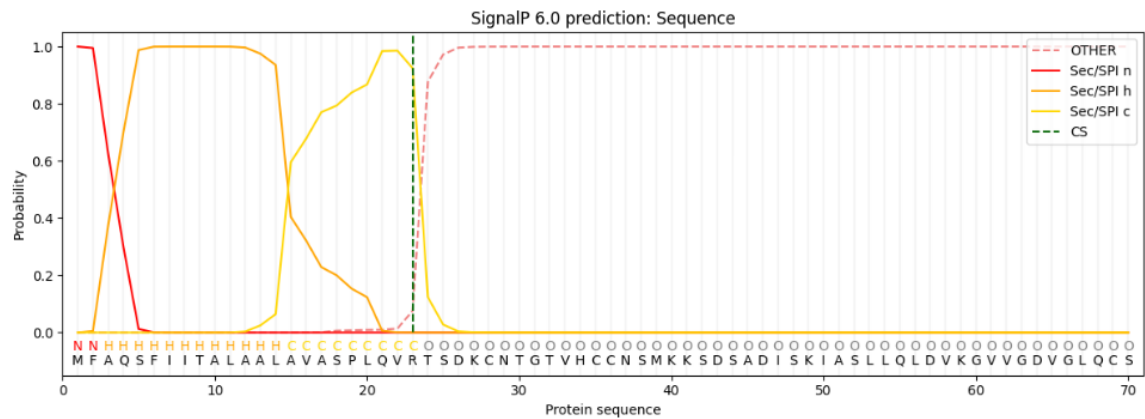


Figure S1: Signal peptide prediction of Hydph7

Prediction: Signal Peptide (Sec/SPI)

Cleavage site between pos. 19 and 20.

Probability 0.978073

Protein type	Other	Signal Peptide (Sec/SPI)	Lipoprotein signal peptide (Sec/SPII)	TAT signal peptide (Tat/SPI)	TAT Lipoprotein signal peptide (Tat/SPII)	Pilin-like signal peptide (Sec/SPIII)
Likelihood	0.0003	0.9991	0.0001	0.0002	0.0001	0.0001

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SignalP 6.0 prediction: Sequence

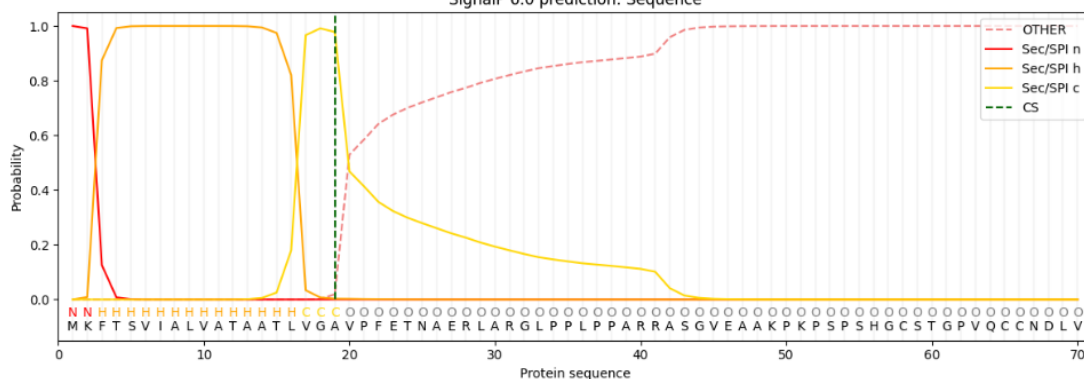


Figure S2: Signal peptide prediction of Hydph16

Sequence

Prediction: Signal Peptide (Sec/SPI)

Cleavage site between pos. 23 and 24.

Probability 0.886458

Protein type	Other	Signal Peptide (Sec/SPI)	Lipoprotein signal peptide (Sec/SPII)	TAT signal peptide (Tat/SPI)	TAT Lipoprotein signal peptide (Tat/SPII)	Pilin-like signal peptide (Sec/SPIII)
Likelihood	0.0003	0.999	0.0001	0.0002	0.0002	0.0001

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SignalP 6.0 prediction: Sequence

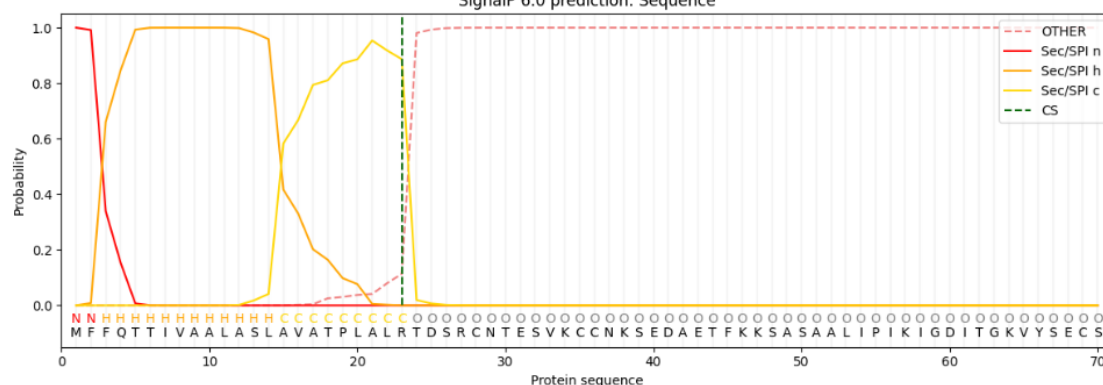
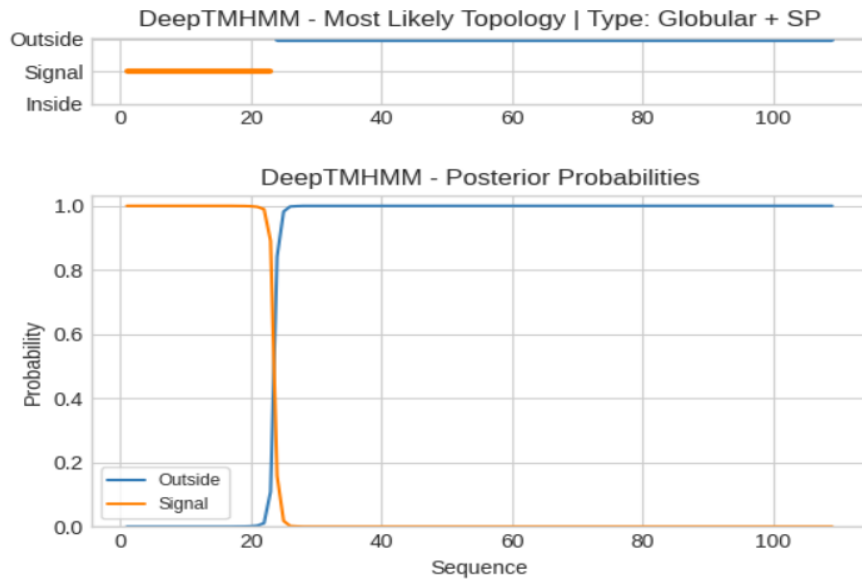


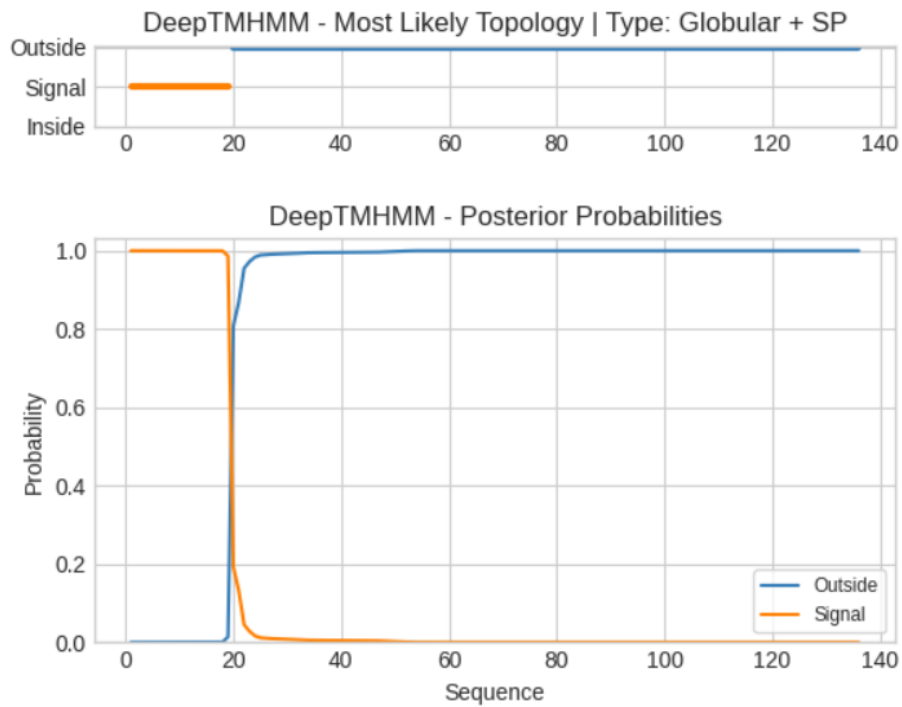
Figure S3: Signal peptide prediction of Vmh3-1

Transmembrane Region Prediction



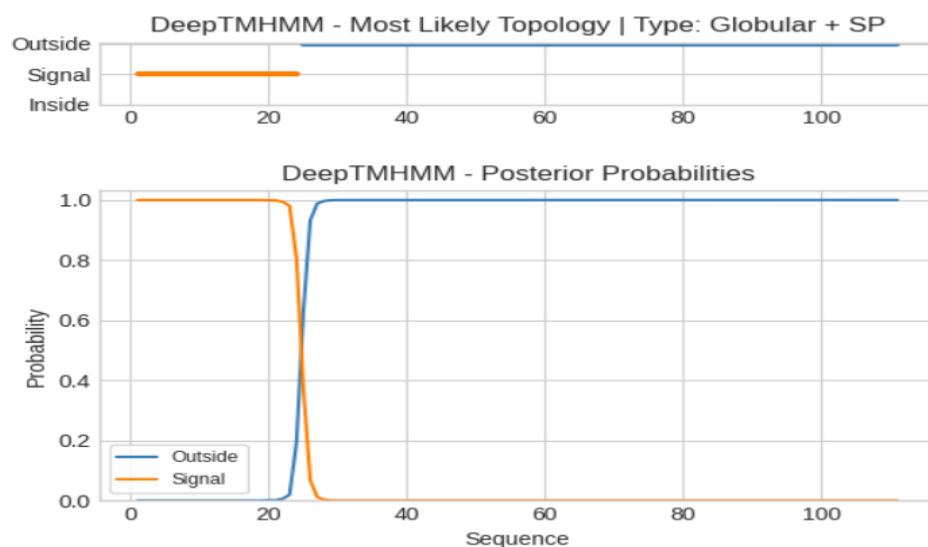
Sequence Length: 109
 # Sequence Number of predicted TMRs: 0
 Sequence signal 1 23
 Sequence outside 24 109

Figure S4: Transmembrane region prediction of Hydph7



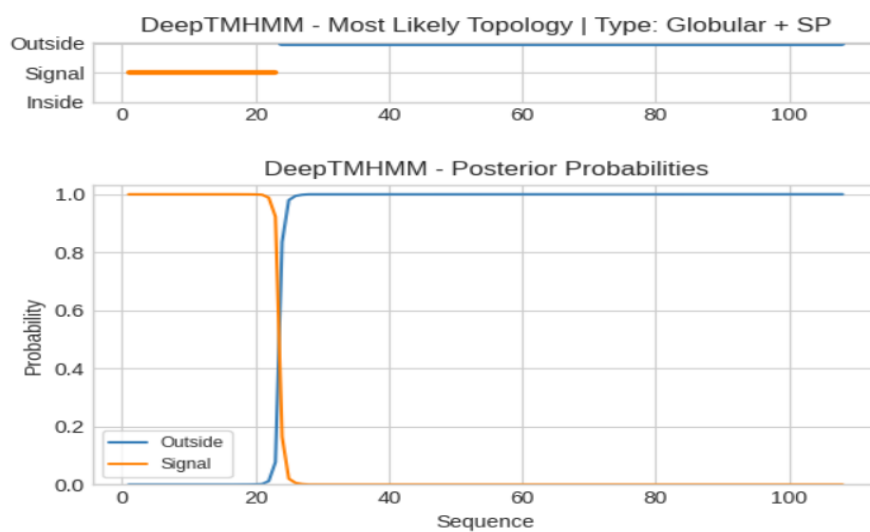
Sequence Length: 136
 # Sequence Number of predicted TMRs: 0
 Sequence signal 1 19
 Sequence outside 20 136

Figure S5: Transmembrane region prediction of Hydph16



```
# Sequence Length: 111
# Sequence Number of predicted TMRs: 0
Sequence signal 1 24
Sequence outside 25 111
```

Figure S6: Transmembrane region prediction of Vmh2



```
# Sequence Length: 108
# Sequence Number of predicted TMRs: 0
Sequence signal 1 23
Sequence outside 24 108
```

Figure S7: Transmembrane region prediction of Vmh3-1

Secondary structure of Hydrophobins:

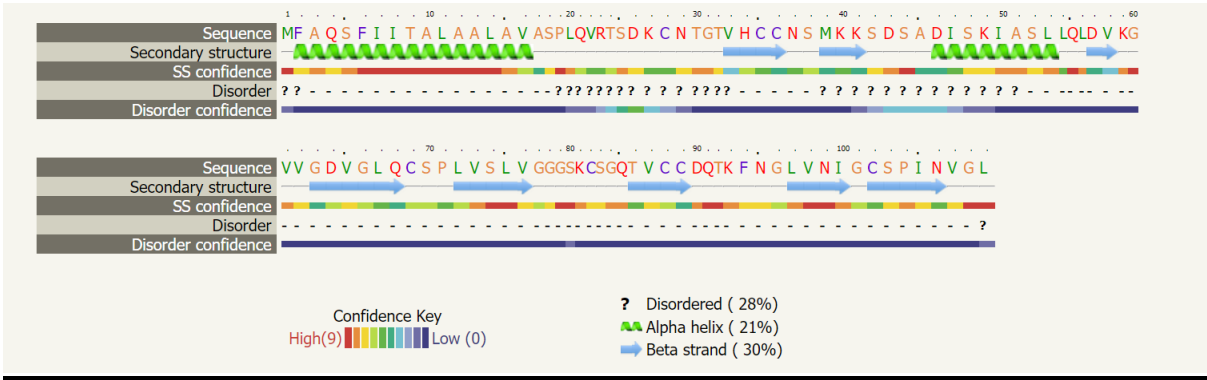


Figure S8: Secondary structure of Hydph7

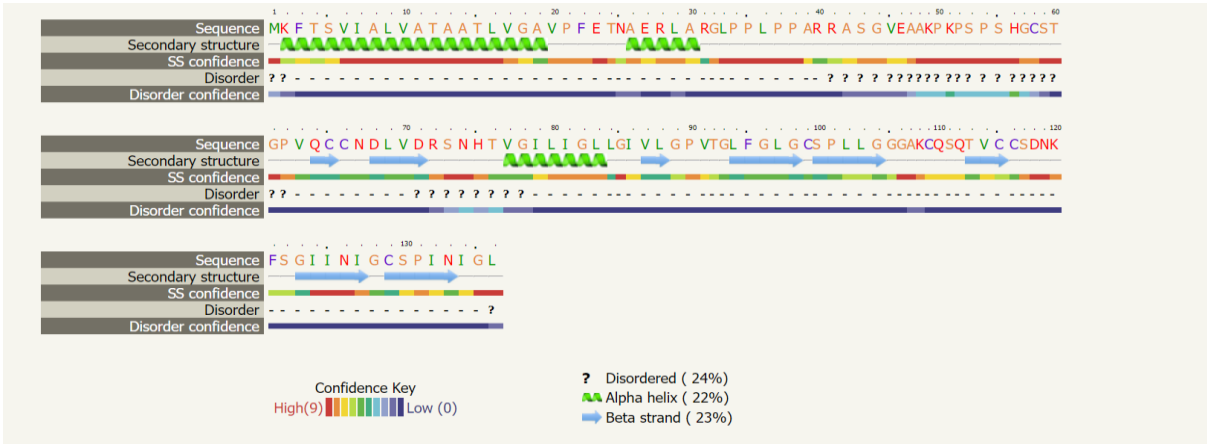


Figure S9: Secondary structure of Hydph16

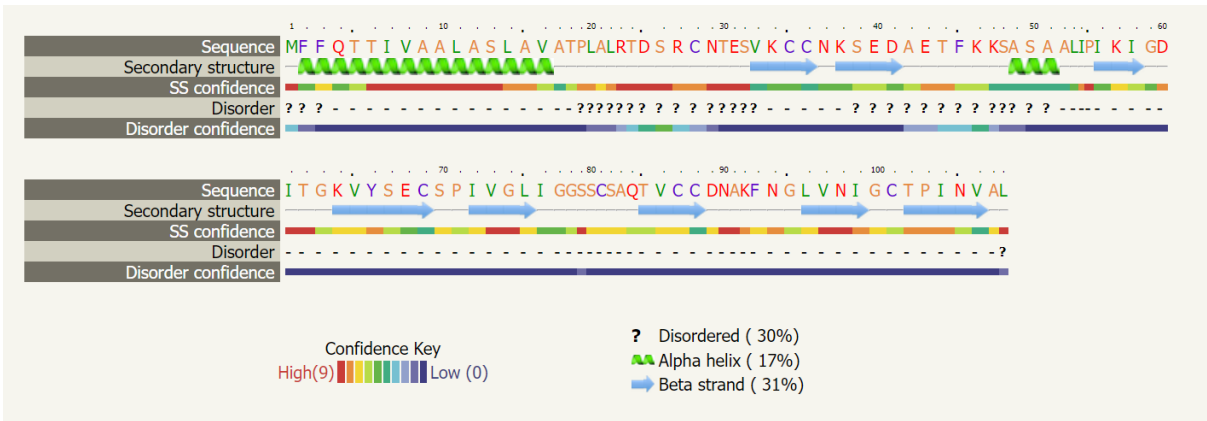



Figure S10: Secondary structure of Vmh3-1



CLUSTAL 2.1 multiple sequence alignment

Pairwise sequence alignment Hydph7 Hydrophobin
with 7s7s

A scatter plot showing the Z-score (Y-axis, ranging from -20 to 10) versus the Number of residues (X-axis, ranging from 0 to 1000). The plot compares data from X-ray (light blue) and NMR (dark blue) experiments. The NMR data points are concentrated at lower residue counts (below 200) and show a wider distribution of Z-scores, including a prominent outlier at approximately (100, -3). The X-ray data points are more spread out across the residue count range (up to 1000) and generally show lower Z-scores, particularly for larger proteins.

Local model quality

Y-axis: Knowledge-based energy (ranging from -3.0 to 3.0)

X-axis: Sequence position (ranging from 1 to 100)

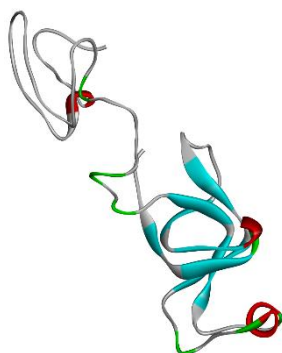
Legend:

- WINDOW SIZE 10 (light green line)
- WINDOW SIZE 40 (dark green line)

The graph shows that the knowledge-based energy generally decreases as the sequence position increases. The window size 40 model (dark green) shows a more stable and generally lower energy profile compared to the window size 10 model (light green), which exhibits significant fluctuations and higher energy values in the early sequence positions.

Hydph16

A.



Predicted 3D structure of Hydph16 Hydrophobin

B.

CLUSTAL 2.1 multiple sequence alignment

```

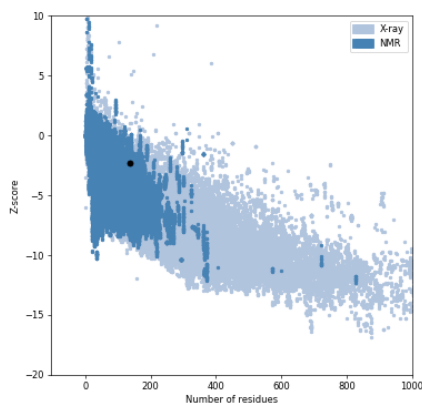
Target      MKFTSVIALVATAATLVGAVPFETNAERLARGLPPLPPARRASGVEAAKPKPSPSHGCS
2nbh      -----KAMADIGSTAVPRDVNGG-----TPPKSCSS
              * *      *** :.*
              :*.:**

Target      GPVQCCNDLVDRSNHTVG--ILIGLLGIVLGPVTGLFGLGCSPLLG---GGAKCQSQTVC
2nbh      GPVYCCNKTEDSKHLDKGTALLGLLNKIGDLKDLVGLNCSPLSVIGVGGNSCSAQTV
              *** **,* * :. * * :*:*:* * :* :*:*:* * :* :* :*:*
              * :. :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*

Target      CSDNKFSGIINIGCSPINIGL
2nbh      CTNTYQHGLVNVGCTPINIGL
              * :. :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*
  
```

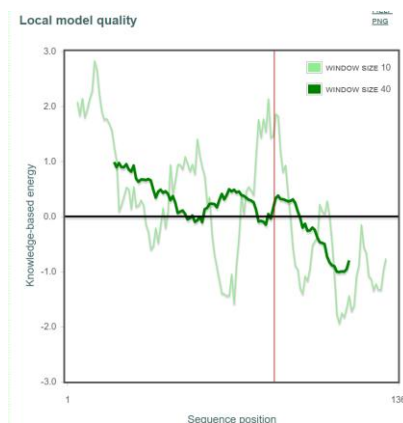
Pairwise sequence alignment Hydph16 Hydrophobin with 2nbh

C.



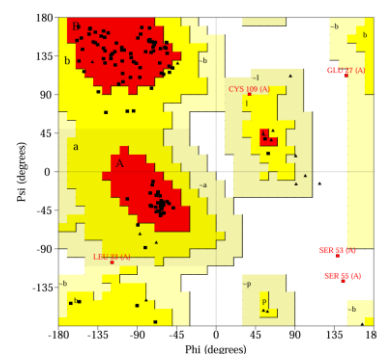
ProSA-web Z- score plot

D.



Local quality estimate

E.



Ramachandran Plot Analysis

Figure S12: Structure analysis and validation of (A.) predicted 3D structure of **Hydph16** Hydrophobin protein by Modeller 9.11. (B.) pairwise sequence alignment with 2nbh using ClustalW, where the conserved amino acid residues are elucidated as (), highly similar residues as (:) and weakly similar residues as (.). (C.) ProSA-Z-score plot shows the overall model quality Z score i.e. -2.28, (D) Local model quality. (E.) Ramachandran plot analysis of 3D structure of Hydph16 Hydrophobin, where the red region defines most favourable area of residues; the yellow region is additionally allowed; and generously allowed residues in the light-yellow region.

Vmh3-1

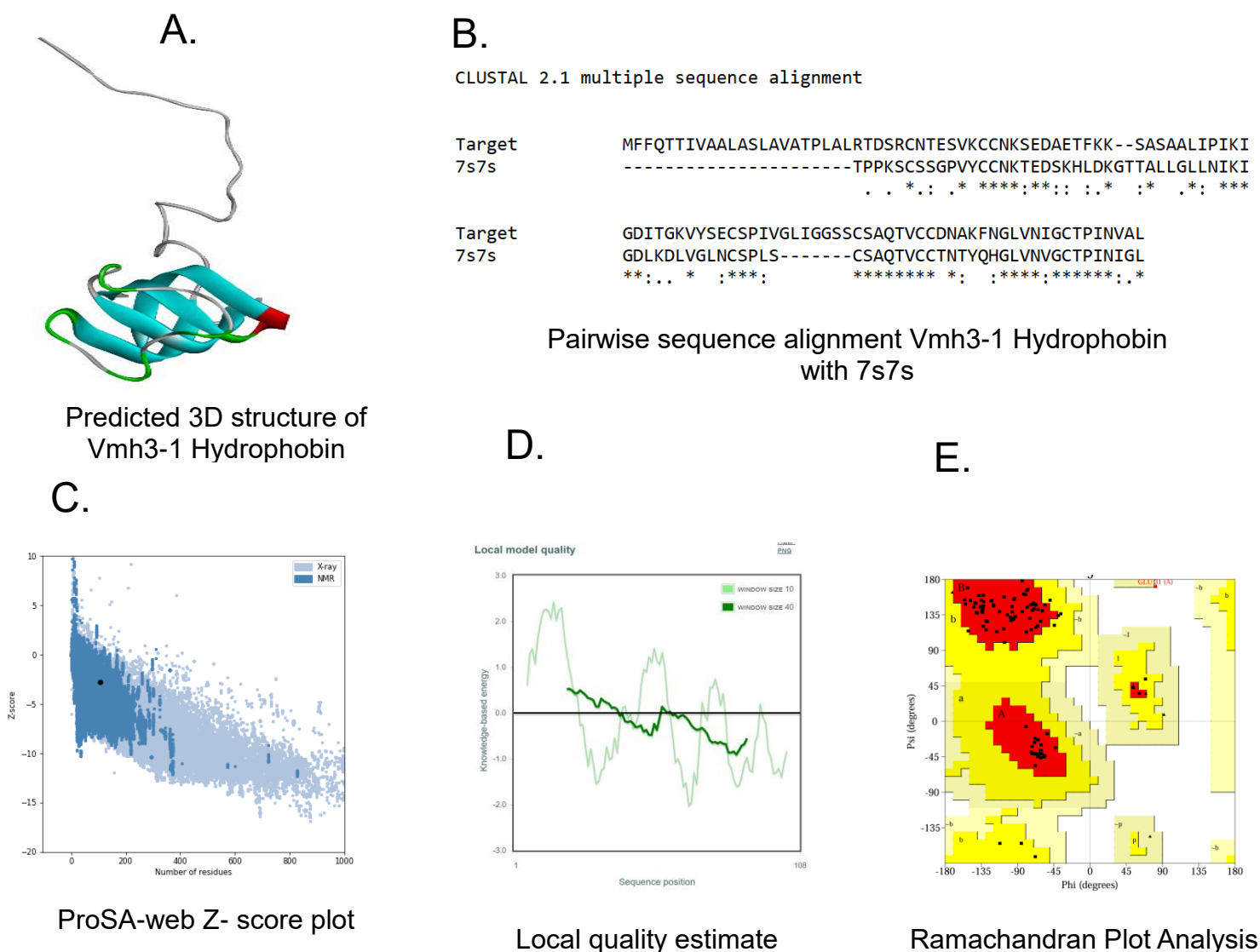


Figure S13: Structure analysis and validation of (A.) predicted 3D structure of **Vmh3-1** protein by Modeller 9.11. (B.) pairwise sequence alignment with 7s7s using ClustalW, where the conserved amino acid residues are elucidated as (), highly similar residues as (:) and weakly similar residues as (.). (C.) ProSA-Z-score plot shows the overall model quality Z score i.e. -2.79, (D) Local model quality. (E.) Ramachandran plot analysis of 3D structure of Vmh3-1, where the red region defines most favourable area of residues; the yellow region is additionally allowed; and generously allowed residues in the light-yellow region.

Protein–Protein Interactions:



Figure S14: Protein-protein interactions of HYDPH 7

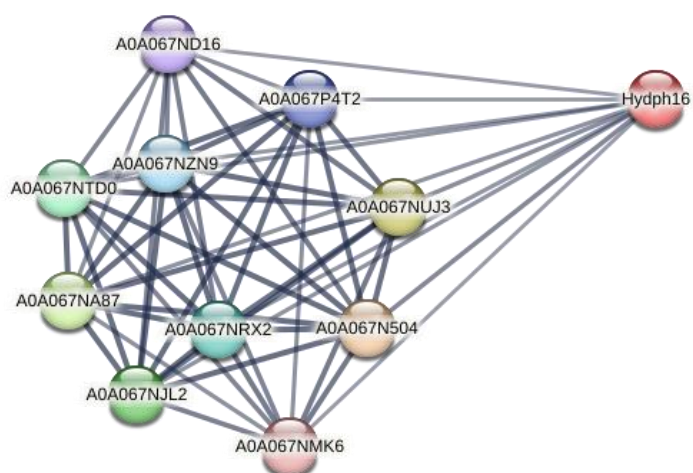


Figure S15: Protein-protein interactions of HYDPH 16

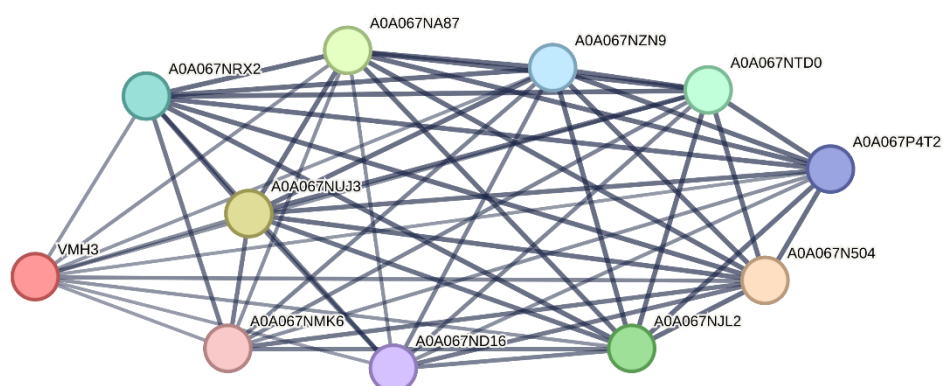
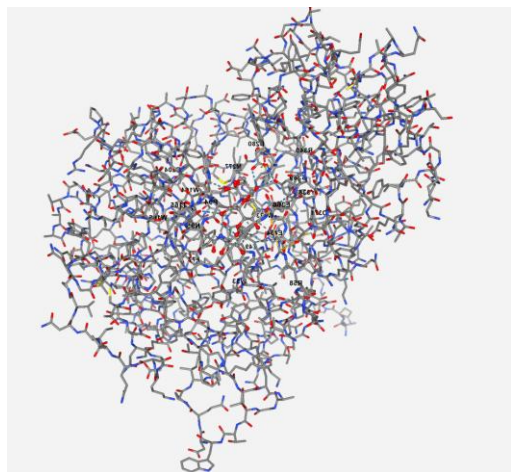
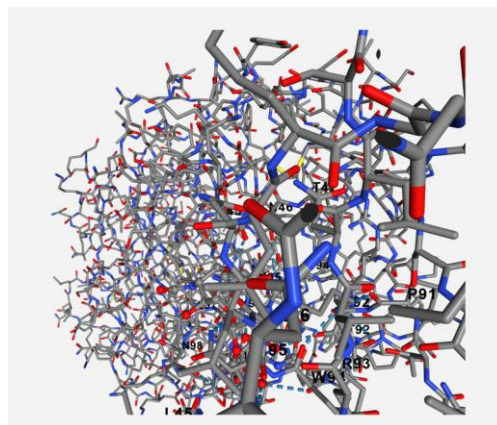


Figure S16: Protein-protein interactions of Vmh3-1



a. Chitinase A-Chitin Interaction



b. Chitin-binding Protein 21 (CBP 21)- Chitin Interaction

Figure S17: Interaction of Chitin with a) Chitinase A b) Chitin-binding Protein 21 (CBP 21) for studying docking of chitin and HPs