

# Plant Defense Elicitation by the Hydrophobin Cerato-Ulmin and Correlation with Its Structural Features

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## TABLE AND FIGURES OF SUPPLEMENTARY MATERIAL

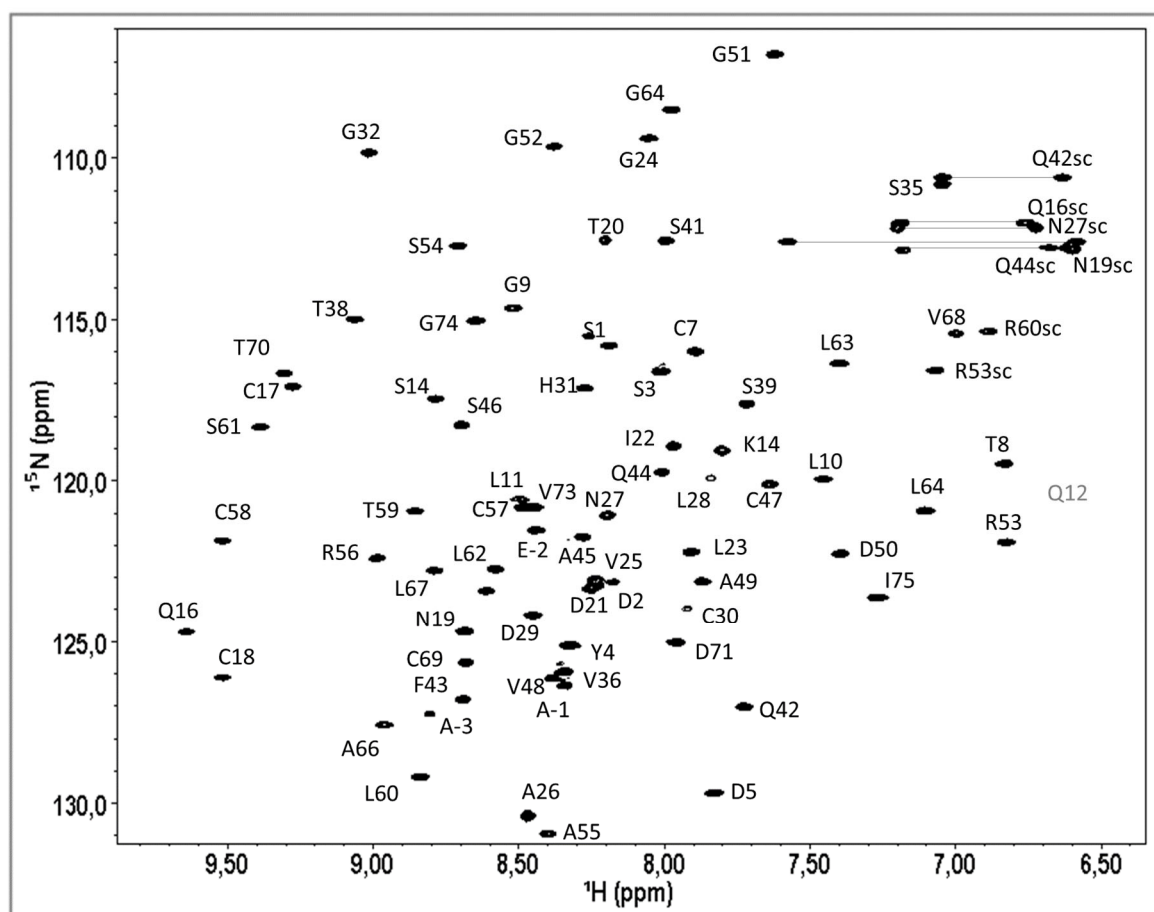
Table S1. Experimental RDCs ( $D_{\text{HN}}^{\text{obs}}$  column) versus calculated RDCs ( $D_{\text{HN}}^{\text{calc}}$  column). The third column reports  $D_{\text{HN}}^{\text{obs}} - D_{\text{HN}}^{\text{calc}}$  for each residue considered in the analysis. According to equation (1) the Q factor is estimated to be 0.551.

	$D_{\text{HN}}^{\text{obs}}$	$D_{\text{HN}}^{\text{calc}}$	$\Delta D_{\text{HN}}$			$D_{\text{HN}}^{\text{obs}}$	$D_{\text{HN}}^{\text{calc}}$	$\Delta D_{\text{HN}}$
SER5	0.7	0.4673	0.2327		SER45	1	0.6743	0.3257
ASP6	1.9	1.612	0.288		GLN46	0	-0.03775	0.03775
SER7	1	0.4702	0.5298		PHE47	0	-0.5417	0.5417
TYR8	0.5	0.9436	-0.4436		GLN48	1	0.4778	0.5222
ASP9	0.5	-0.277	0.777		ALA49	1	0.711	0.289
CYS11	1	0.937	0.063		SER50	0.5	0.2077	0.2923
THR12	1	0.351	0.649		CYS51	0.5	-0.1234	0.6234
GLY13	0	-0.5811	0.5811		VAL52	0.5	0.1268	0.3732
LEU14	1.6	0.9994	0.6006		ALA53	1.1	0.8006	0.2994
GLN16	0.6	0.7041	-0.1041		ASP54	0	0.0349	-0.0349
LYS17	0	0.6011	-0.6011		GLY55	0	0.0432	-0.0432
SER18	1.6	1.3131	0.2869		GLY56	1	0.7525	0.2475
GLN20	0	0.7793	-0.7793		ARG57	0.5	-0.1629	0.6629
CYS21	0.5	-0.2256	0.7256		SER58	1.1	0.2855	0.8145
CYS22	0.5	-0.0279	0.5279		ALA59	0.6	-0.1592	0.7592
ASN23	0.5	-0.1248	0.6248		ARG60	0.5	0.3498	0.1502
THR24	0.5	0.7175	-0.2175		CYS62	0	0.7537	-0.7537
ASP25	1.6	1.7177	-0.1177		THR63	0	0.579	-0.579
ILE26	0.5	0.1553	0.3447		LEU64	1	1.0377	-0.0377
LEU27	1.1	0.3804	0.7196		SER65	0.6	1.1189	-0.5189
GLY28	0	-0.6621	0.6621		LEU66	0.5	0.0774	0.4226
ALA30	0.5	0.4035	0.0965		LEU67	0	0.3076	-0.3076
ASN31	1	1.1342	-0.1342		GLY68	0.3	0.1413	0.1587
LEU32	2	1.1632	0.8368		LEU69	0.5	1.0275	-0.5275
ASP33	1.5	1.4575	0.0425		ALA70	0	0.2758	-0.2758
CYS34	1.6	1.4112	0.1888		LEU71	0.5	0.3528	0.1472



<b>HIS35</b>	1.6	0.7185	0.8815		<b>VAL72</b>	1.5	1.1362	0.3638
<b>GLY36</b>	0.5	-0.0364	0.5364		<b>CYS73</b>	0	0.341	-0.341
<b>SER39</b>	0.5	0.1124	0.3876		<b>THR74</b>	0	0.244	-0.244
<b>VAL40</b>	1	0.5153	0.4847		<b>ASP75</b>	0.5	0.8241	-0.3241
<b>THR42</b>	0.6	0.5301	0.0699		<b>GLY78</b>	0.6	0.332	0.268
<b>SER43</b>	0.5	0.0762	0.4238		<b>ILE79</b>	0.5	0.0899	0.4101

**Figure S1.**  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectrum of U- $^{15}\text{N}$ -rCU in 30% ACN- $d_3$ :70% 50 mM phosphate buffer pH 5.8. at 15 °C. Negative numbers refer to the residues of the non-native additional EAEA N-terminal sequence



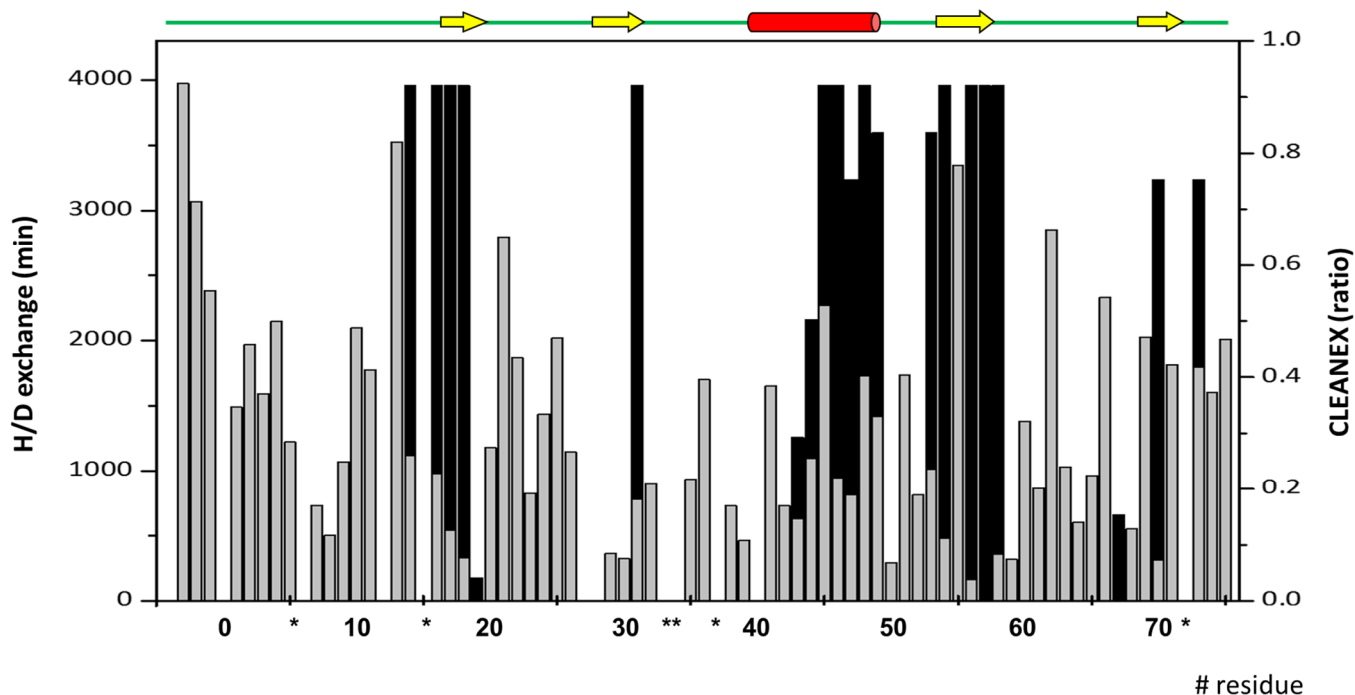


**Figure S2. Solvent accessibility data: H/D exchange and CLEANEX-PM.**

Amide protons with low H/D exchange rates, that remain visible after more than 4000 min in the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum upon  $\text{D}_2\text{O}$  resuspension (black bars) and CLEANEX-PM data, reported as the ratio of the CLEANEX-PM/HSQC peak intensities (grey bars).

A cartoon of the topology of the CU model is reported in the upper part of the figure.

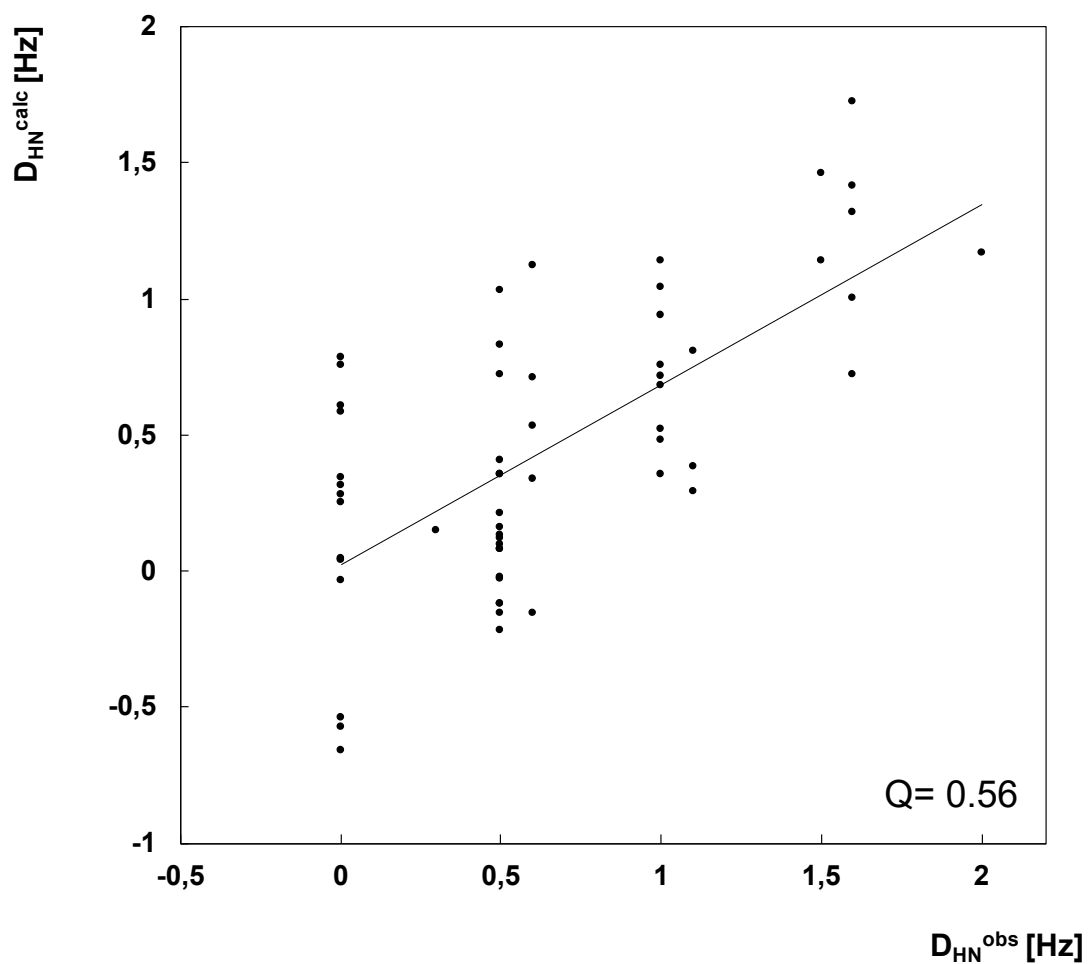
The secondary structure elements are coincident with regions protected from solvent exchange, with lower H/D exchange rates and amide low water-NOE correlations. Prolines (missing signals) are marked with an asterisk (\*).





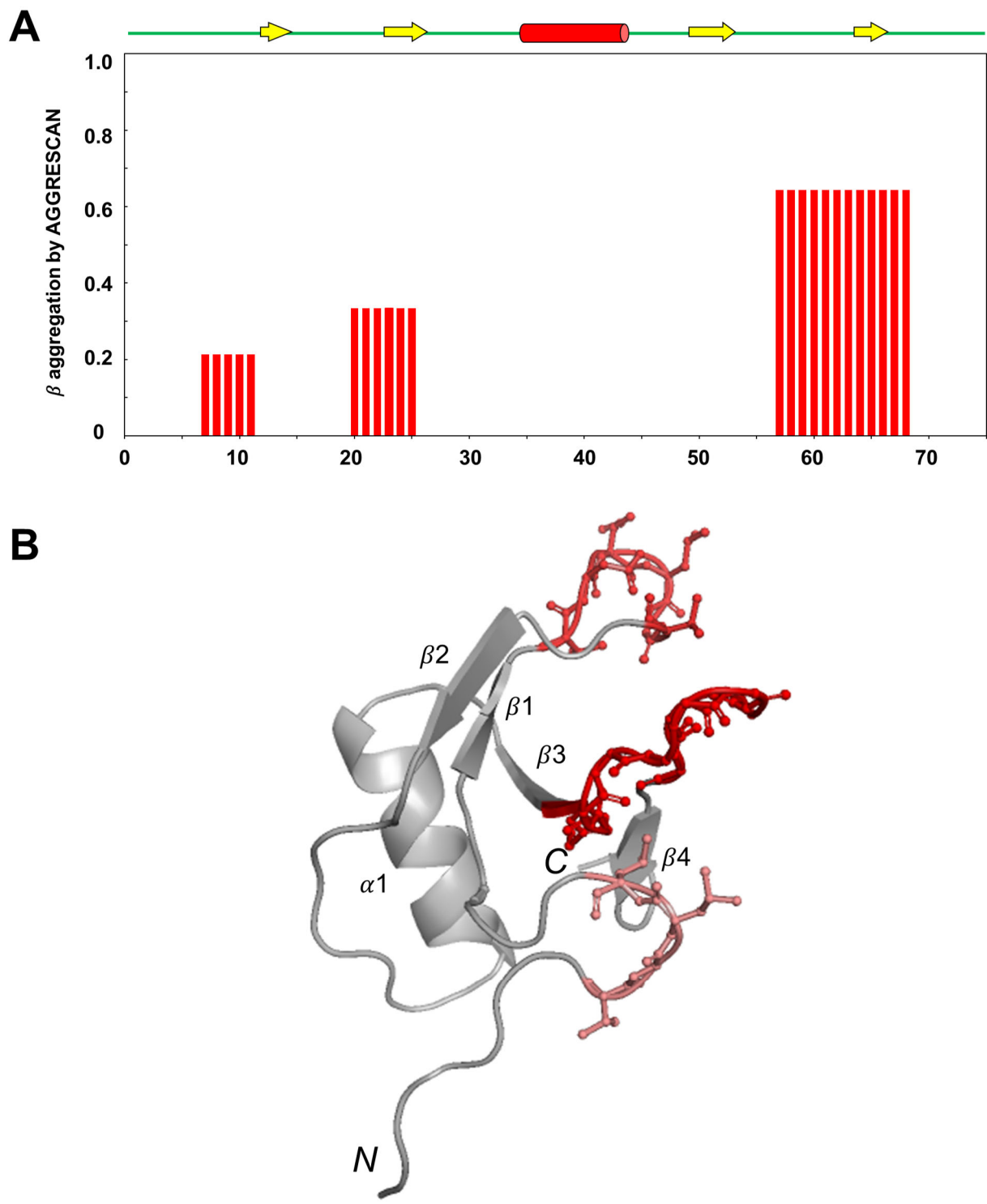
**Figure S3. Experimental RDCs ( $D_{\text{HN}}^{\text{obs}}$ ) versus calculated RDCs ( $D_{\text{HN}}^{\text{calc}}$ ).**

Experimental validation of CU's model. The correlation plot shows the degree of agreement between experimentally measured RDCs and those calculated from the calculated homology model. Q factor is estimated to be 0.56.





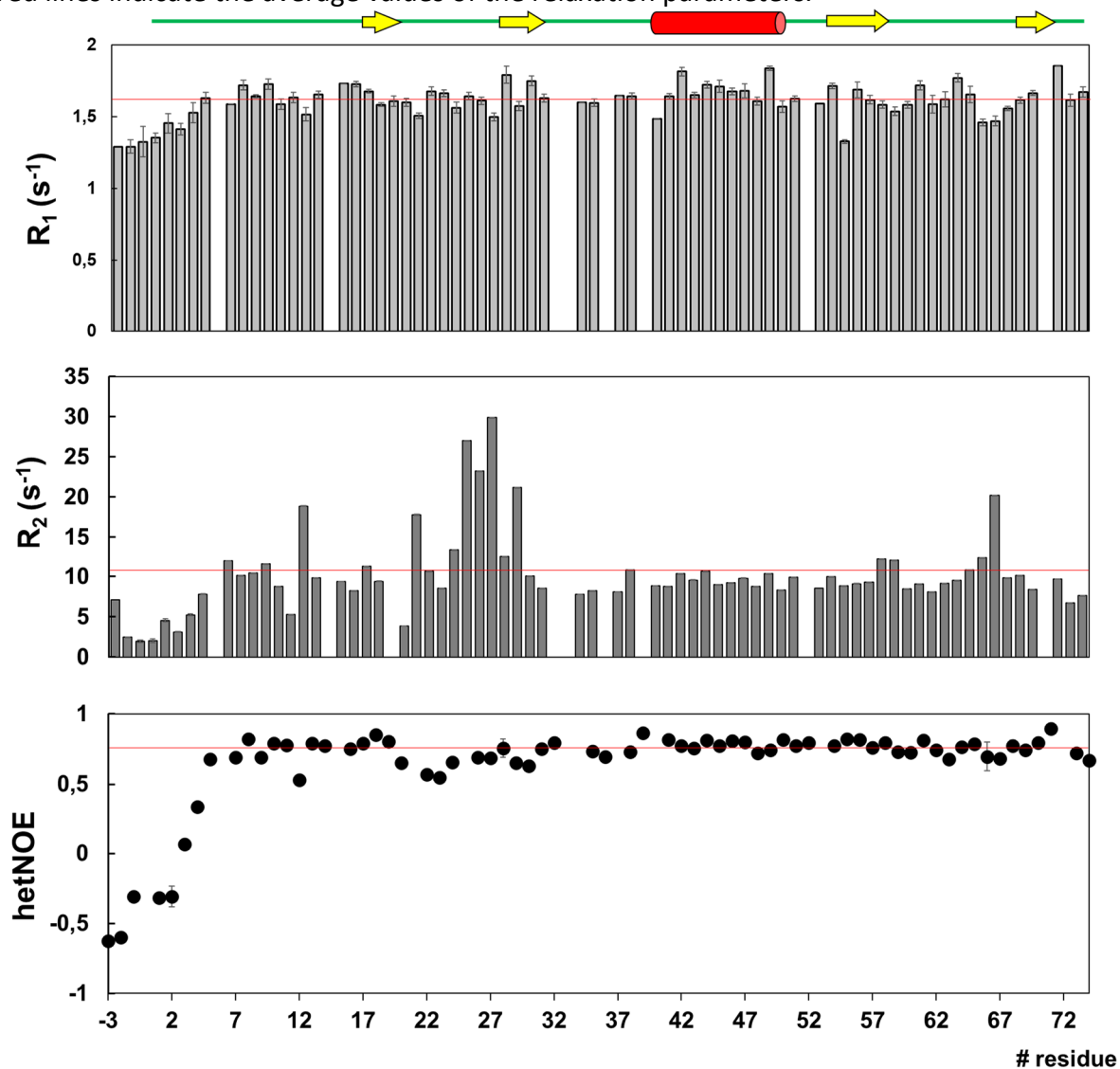
**Figure S4. CU residues with aggregation tendency.** The CU aggregation profile was analyzed using the AGGRESCAN server. The probability of aggregation propensity is plotted against the protein sequence (A) and mapped onto the 3D CU model (B). A cartoon of the topology of the CU model is reported in the upper part of the figure.





**Figure S5.**  $^{15}\text{N}$ -NMR dynamics experiments.

$R_1$ ,  $R_2$ , and hetNOE of rCU 700  $\mu\text{M}$  in 30%  $\text{ACN-}d_3$ :70% 50 mM phosphate buffer pH 5.8. at 15  $^\circ\text{C}$ . red lines indicate the average values of the relaxation parameters.

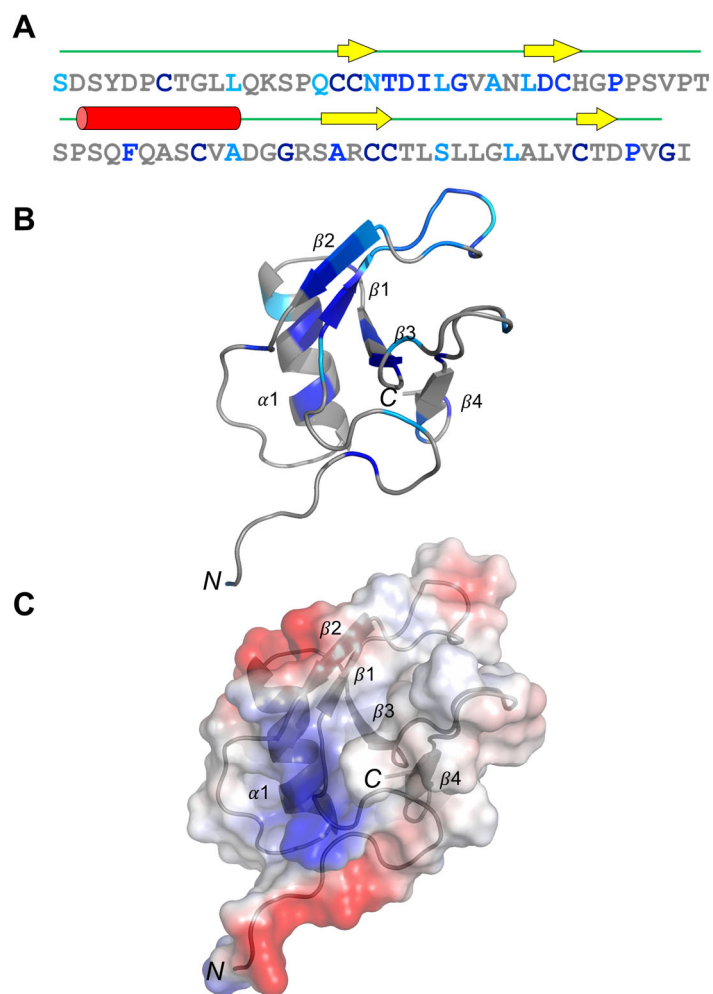




**Figure S6. Conserved residues and hydrophobic patches.**

Conserved residues amongst class-II hydrophobins calculated with the ConSurf server. Conserved residues are coloured on the CU sequence (A) and on the 3D model (B) (dark blue for highly conserved amino acids and progressively lighter colours for medium and low conserved).

C) Surface electrostatic potential in the CU model calculated with Pymol. showing the hydrophobic character of the  $\beta 1\beta 2$ - and  $\beta 3\beta 4$ -loops.





**Figure S7. Chemical shift titration experiments.**

Section of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of rCU in phosphate buffer. showing chemical shift perturbations upon addition of increasing amounts of  $\text{ACN-}d_3$ .

