

Structural and functional insights into the recombinant PR-10 tomato allergen Sola I 4.02

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Note S1. CASTpFold pocket analysis and ligand-proximal residues

CASTpFold analysis of the Sola l 4.02 AlphaFold model identified Pocket 1 as the largest internal cavity. The 49 pocket-lining residues were Phe6, Thr7, His8, Ser10, Leu23, Val24, Phe27, Asp28, Leu35, Asp38, Val39, Ile42, Lys55, Met57, Phe59, Val60, Gly63, Pro64, Ile65, Leu68, His70, Ile72, Ile75, Thr82, Tyr84, Leu86, Val91, Leu96, Ile99, Tyr101, Val103, Phe105, Cys115, Thr117, Thr119, Tyr121, His127, Val129, Glu133, His134, Val136, Gly137, Lys138, Lys140, Ala141, Ile142, Asp143, Leu144, and Phe145.

ALL1_APIGR	MGVQTHVLELTSSVSAEKIFQGFVIDVDTVLPKAAPGAYKSVEI-KGDGGPGTILKIITLP	59
LUPLU	MGVFTFQDEYTSTIAPAKLYKALVTDADIIIPKAV-ETIQSVEIVEGNGGPGTIKKLTFI	59
ARAH8	MGVFTFEDEITSTVPPAKLYNAM-KDADSITPKII-DDVKSVEIVEGNGGPGTIKKLTIV	58
SOLA	MGVNTFTHESTTTIAPTRLFKGLVLDFTSLVPKLLSHDVKSIEIVEGDGGAGSIKQMNFEV	60
FRA1E	MGVYTYENEFTSDIPAPKLFAFVLADNLIPIKAPQAVKCAEILEGDGGPGTIKKITFG	60
BETV1A	MGVFNJETETTSVIPARLFKAFILDGDNLFKVPAPQAISSVENIEGNGGPGTIKKISFP	60
CORA1.0401	MGVFCYEDEATSVIPPARLFKSFVLADNLIPIKVPQHFSTAENLEGNGGPGTIKKITFA	60
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ALL1_APIGR	DGGPITTMTLRIDGVNKEALTFDYVIDGDILLGFIESIENHVVLVPTAD-GGSICKTTA	118
LUPLU	EGGESKYVLHKIEAIDEANLGYNSIVGGVGLPDTIEKISFETKLVEGAN-GGSIGKVTI	118
ARAH8	EDGETKFIHLHKVESIDEANYAYNSVVGVALPPTAEKITFETKLVEGPN-GGSIGKLTL	117
SOLA	EGGPYIKYLKHKIHVIDDKNLVTKYSLIEGDVLGDKLESIAVDVKFEAAGD-GGCVCKTTT	119
FRA1E	EGSHYGYVKHKIHSIDKVNHTYSYSLIEGDALSENIEKIDYETKLVSAPH-GGTIIKTTS	119
BETV1A	EGFPFKYVKDRVDEVDHTNFKYNSVIEGGPIGDTLEKISNEIKIVATPD-GGSILKISN	119
CORA1.0401	EGNEFKYMKHKVEEIDHANFKYCYSIIEGGPLGHTLEKISYEIKMAAAPHGGGSILKITS	120
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ALL1_APIGR	IFHTKGDVAVPEENIKYANEQNTALFKALEAYLIAN-----	154
LUPLU	KIETKGDAPNEEEGKAAKARGDAFFKAIESYLSAHPDYN-	158
ARAH8	KYHTKGDAPDEEELKKGKAKGEGLFRAIEGYVLNPTQY-	157
SOLA	EYHTKGDHVVSEEEHNVGKGKAIDLFAIEAYLLANPSVYA	160
FRA1E	KYHTKGDVEIKEEHVKAGKEKAHLFKLIEGYLKDHPSEYN	160
BETV1A	KYHTKGDHEVKAEQVKASKEMGETLLRAVESYLLAHS DAYN	160
CORA1.0401	KYHTKGNASINEEIKAGKEKAAGLFKAVEAYLLAHPDAYC	161
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Figure S1. Multiple sequence alignment of representative PR-10 proteins. Full-length amino acid sequences of Api g 1 (P49372; displayed as ALL1_APIGR in the alignment), *Lupinus luteus* PR-10 (Q9LLQ2), Ara h 8 (Q6VT83), Sola l 4.02 (K4CWC4), Fra a 1E (Q256S2), Bet v 1a (P15494), and Cor a 1.0401 (Q9SWR4) were aligned using Clustal Omega. Asp28, Lys55, Tyr84, and Ile99 are invariant across all seven sequences analyzed. Asp28, Lys55, and Ile99 are highlighted in red, whereas Tyr84 is highlighted in blue because it is also ligand-proximal to the structurally projected naringenin. Lys138, also highlighted in blue, is ligand-proximal and conserved in six of seven sequences. Residue numbering refers to Sola l 4.02.

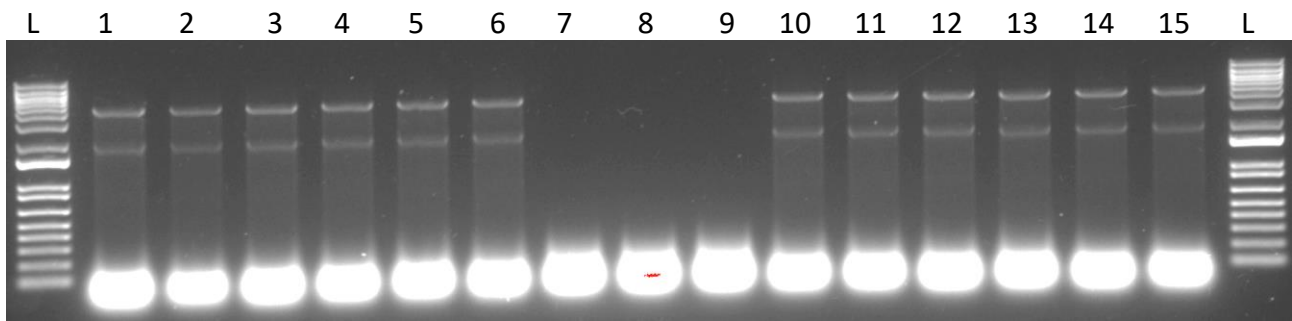


Figure S2. Ribonuclease activity assay of recombinant Sola l 4.02 protein. RNase activity of Sola l 4.02 protein at different pH after 3 hours at 37°C. L: 1 Kb Plus Ladder. Lane 1-3: three different RNAs (1ug each) in pH 5.0 buffer treated with 1ug of protein. Lane 4-6: three different RNAs (1ug each) in pH 7.5 buffer treated with 1ug of protein. Lane 7-9: positive control (RNAs treated with RNase). Lane 10-12: negative control (RNAs untreated at pH 5.0). Lane 12-15: negative control (RNAs untreated at pH 7.5).

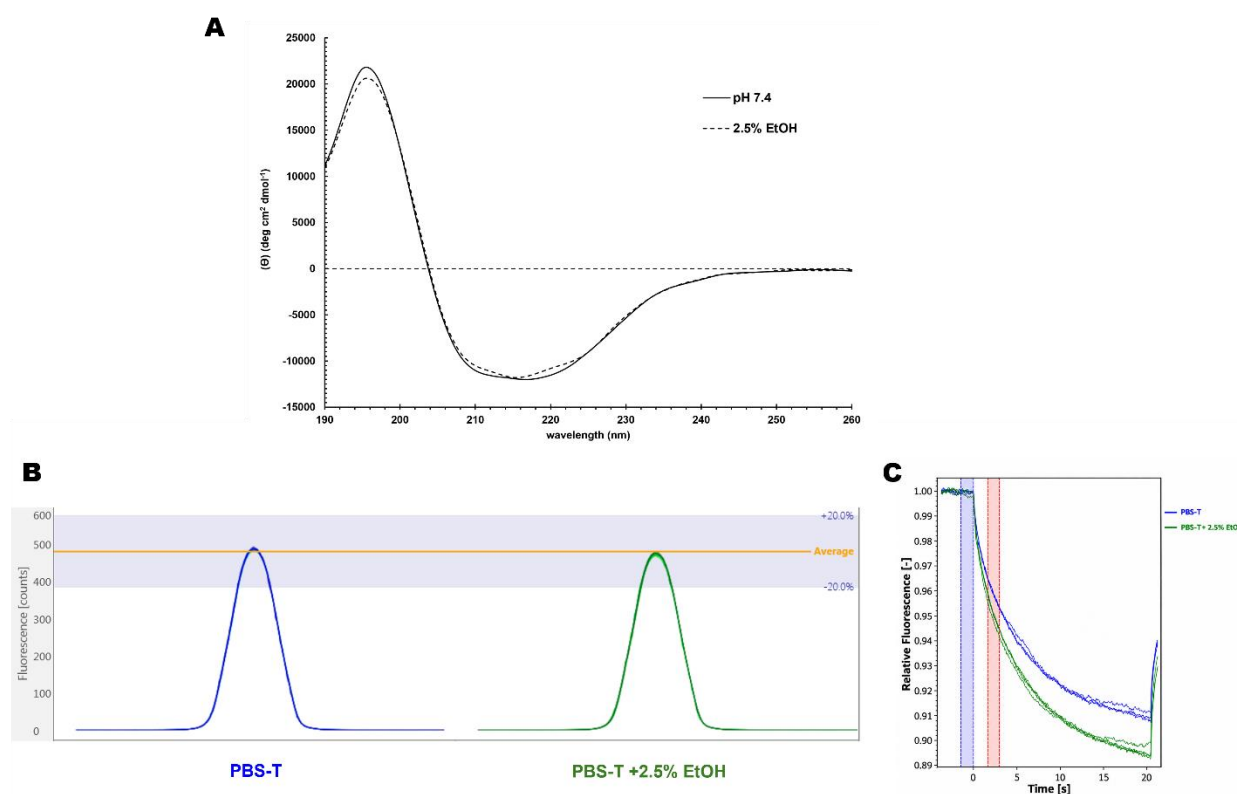


Figure S3. A) Effect of ethanol on the secondary structure of Sola l 4.02. Far-UV CD spectra of Sola l 4.02 recorded at pH 7.4 in the absence (solid line) or presence of 2.5% (v/v) ethanol (dashed line). **B)** Fluorescence profiles of labeled Sola l 4.02 in $1\times$ PBS containing 0.1% Tween-20 (PBS-T, blue) and PBS-T supplemented with 2.5% (v/v) ethanol (PBS-T + 2.5% EtOH, green). **C)** MST thermophoresis profiles of Sola l 4.02 in PBS-T (blue traces) and PBS-T + 2.5% EtOH (green traces). These control assays were performed under the same conditions used for the naringenin-binding MST experiments.

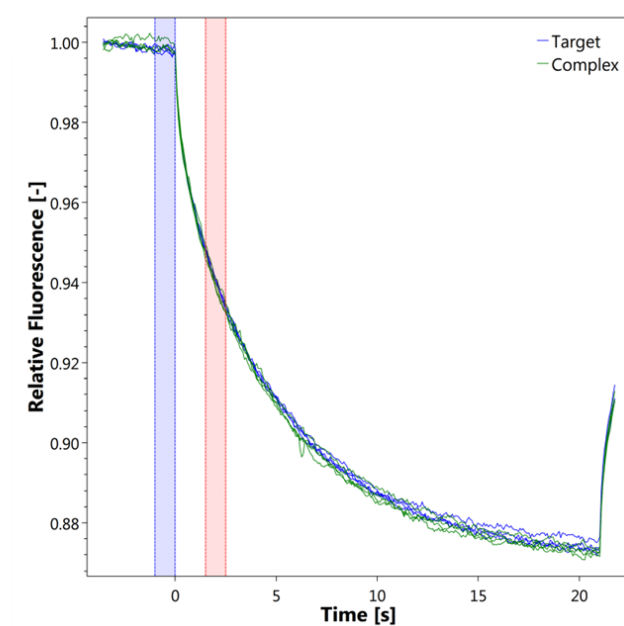


Figure S4. Binding assessment between Sola l 4.02 and Hydroxycinnamic Acid. Thermophoresis of Sola l 4.02 alone and in presence of Hydroxycinnamic Acid at final concentration of 500 μ M.

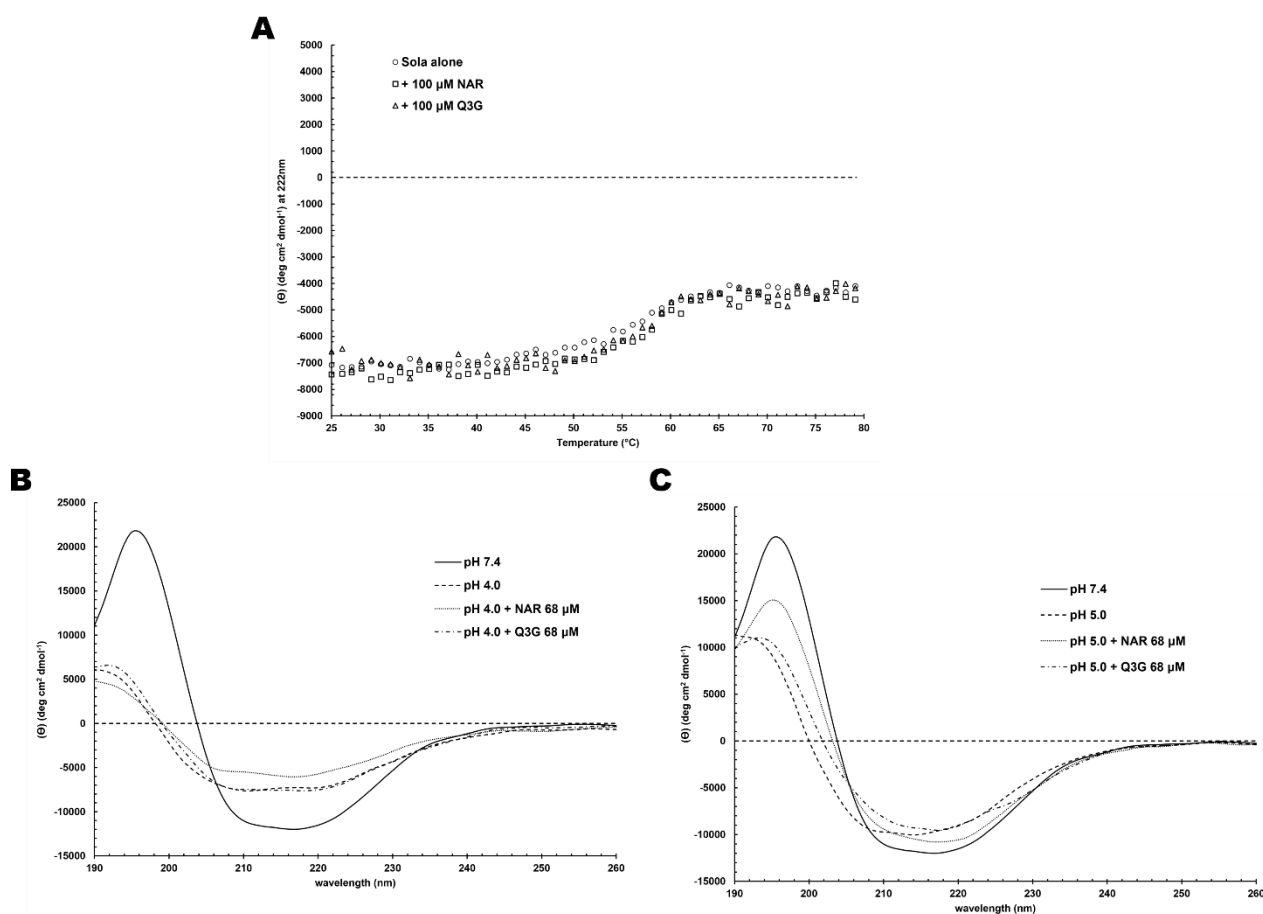


Figure S5. Effect of NAR and Q3G on Sola l 4.02 stability. **A)** Thermal stability of Sola l 4.02 monitored following the CD signal at 222 nm in the absence of ligand and in the presence of 100 μ M Naringenin (NAR) or quercetin-3-O-glucoside (Q3G). **B)** Far-UV CD spectra of Sola l 4.02 recorded at pH 7.4 and pH 4.0 in the absence or presence of 68 μ M NAR or Q3G. **C)** Far-UV CD spectra of Sola l 4.02 recorded at pH 7.4 and pH 5.0 in the absence or presence of 68 μ M NAR or Q3G.