

Supplementary Materials

The Common Bean Small Heat Shock Protein Nodulin 22 from *Phaseolus vulgaris* L. Assembles into Functional High-Molecular-Weight Oligomers

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- Figure S1. *PvNod22* Sequence alignment.

- Figure S2. ACS-*PvNod22* homology dimmer model.

- Figure S3. AlphaFold model.

- Figure S4. Glutaraldehyde crosslinking of *PvNod22*.

- Figure S5. Correlation curves of *PvNod22* at different temperature and concentrations.

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PvNod22      DADVVEEDPDCFRFVAETEG-IG-DVRAHTIEIHPGVTKIVVRDGGSVELS-----LDQLELDMWRFLPESTRPELASAVFVDGELIVTVPKGHGEED 157
Hsp T. thalictroides DADVLEENPECFRFVVTE-DVGHDVQTQTMEIYPGVTKIVLRGNVLELD-----LDELELDLWRFLPSTKPELASAVYVDGELIVTVPKDMNMDE 146
Hsp A. chinensis      DADVSVETADCFRFVVDITDDGIGGDVRAHTVEIYPGVTKIVVRGNDVVDLS-----VDELELDLWRFLPEATVPERASAVYSEGELIVTVPKDANFDV 147
Hsp P. frutescens     DADVSIDETPESIRFTAATD-DISGDVRADAVEIYPGVTKIVIRGNVVDLS-----GTEFELDLWRFLPSTRPPELVSSAYEDGELIVTVPKGAATED 144
Hsp S. asiatica       DADVSIDETPGSLRFTAATD-DIGGGVWADAI EHPGVTKIVVRGGGVADLS-----GTELELDLWRFLPASTRPELASAAYDDGELIVTVPKGAATTE 145
Hsp C. melo          DADVSIVENSNCFRFVAETDG-VSEEFRAHTVQIHPGVTKIVVRQIGDLEST-----LDELEIDMWRFLPETTLPELATAVFDGGLIVTVPKREDAA-- 148
Hsp S. tora          DADVSVEESPDCFRFVAETDG-IG-DVQAHTVEIHPGVTKIVVRESGGSVELL-----LDELELDLWRFLPESTRPELASAVFVDGELIVTVPKGEEELG 148
Hsp M. truncatula    DADVAVEEAADCFRFVAETEG-IG-EVRAHTVEIHPGVTKIVVRDGSLELS-----LDQLELDMWRFLPDBTLPELASAVFVDGELIVTVPKGHEVQN 148
Nod22 C. melo       DADVLVEENTDCFRFIAVTDGNISDGVRAHAVEIHPGVTKIVVRENESLEMA-----IDELELDMWRFLPETTRPELASAAAFVDGELIVTVPKGNDEEN 148
Hsp G. australes     DADVAVEESPDCFKFVAETDGRIGDVRAHTVEIHPGVTKIVIRSNLSLVGFG-----LLDLELDMWRFLPETTRPELASAVYEDGELIVTVPKGGEVEN 152
Hsp T. cacao         DADVAVEESPDCFKFVAETDGRIGDVRAHTVEIHPGVTKIVIRSNLSLVDFS-----LLDLELDMWRFLPETTRPELASAVYEDGELIVTVPKGGEVEN 224
PBP S. suchowensis   DADVSVEENPDCFRFVAETDDDDIGDV-RAHTVEIHPGVTKIVIRPNGYFDLS-----TLDDFELDMWRFLPESTRPELASAVLAHAGELIVTVPKGEE--G 141
PPT S. suchowensis  DAEVSVEENPDCFRFVAETDNNIGEV-RAHTVEIYPGVTKIVIRPNGYLELS-----PLADLELDMWRFLPETTRPELASAVLADGELIVTVPKGEEVEE 201
Hsp P. sativum       --RVDWKETPEAHVFKADLPGLKKEEVK---VEVEDDRVL-QISGERSVEKEDKNDEWHRVERSSGKFLRRFLPENAKMDKVKA SMENGVLTVTVPK----- 95
Hsp T. aestivum      NARMDWKETPEAHVFKADLPGVKKEEVK---VEVEDGNVL-VVSGERTKEKEDKNDEWHRVERSSGKFVRRFLLEDAKVVEVKAGLENGVLTVTVPKAEVKKP 142
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Figure S1. *PvNod22* Sequence alignment. Sequence alignment of ACS-*PvNod22* with selected homologous protein obtained from BLAST protein analysis. Conserved residues with *PvNod22* are colored in red. The asterisk indicate fully conserved residue, colon indicates conservation between groups of strongly similar properties and period show conservation between groups of weakly similar properties. PBP: Protein binding protein, PPT: Pre protein translocase

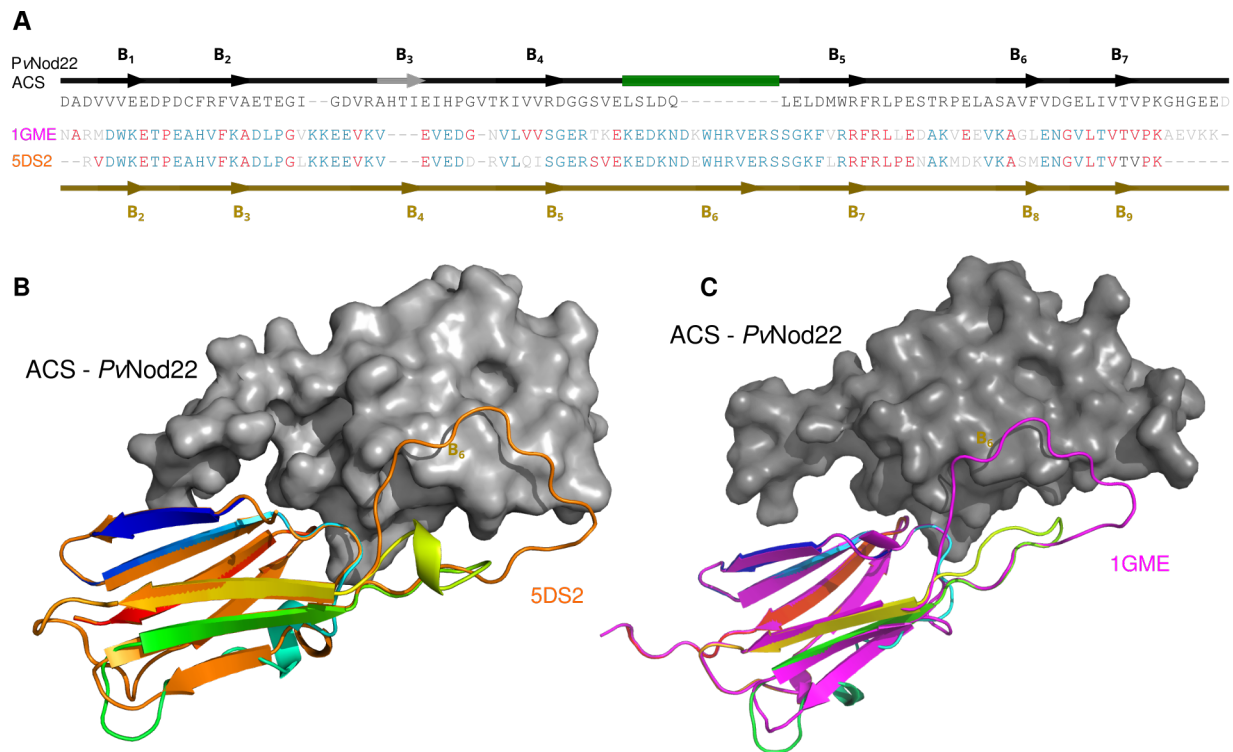


Figure S2. ACS-*PvNod22* homology dimer model. A) Sequence alignment of ACS-*PvNod22* with *Triticum aestivum* sHsp16.9 (PDB access number 1GME) and *Pisum sativum* sHsp18.1 (PDB access number 5DS2). Conserved residues with *PvNod22* are colored in red. Conserved residues between *TasHsp16.9* and *PssHsp18.1* are colored in blue. B) ACD dimer homology model using the crystallographic structure of *TasHsp16.9* (PDB code 5DS1) as template. The seven β -sheets predicted are shown multicolored. Template is shown in orange. C) ACD dimer homology model using the crystallographic structure of *PssHsp18.1* (PDB access number 5DS2) as template. The seven β -sheets predicted are shown multicolored. Template is shown in magenta.

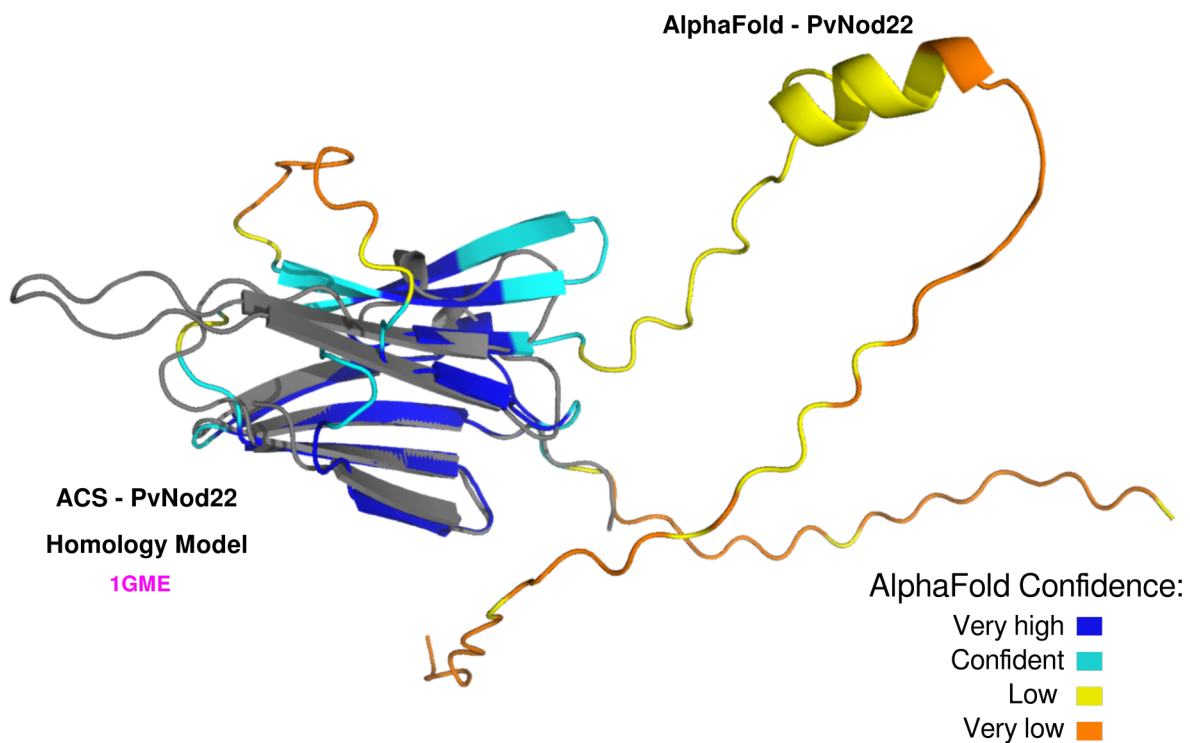


Figure S3. Superposition of ACS-*PvNod22* AlphaFold model and the homology model. Superposition of the ACS homology model using the crystallographic structure of *TasHsp16.9* (PDB code 5DS1) and the AlphaFold model downloads from the EMBO database. The Alphamodel include the N and C domains. Alphamodel color coded according to model confidence, Very high (pLDDT > 90) dark blue; Confident (90 > pLDDT > 70) blue; Low (70 > pLDDT > 50) yellow; Very low (pLDDT < 50) orange. The RMSD calculated between the confident regions of AlphaFold model and the homology model is 0.49 Å.

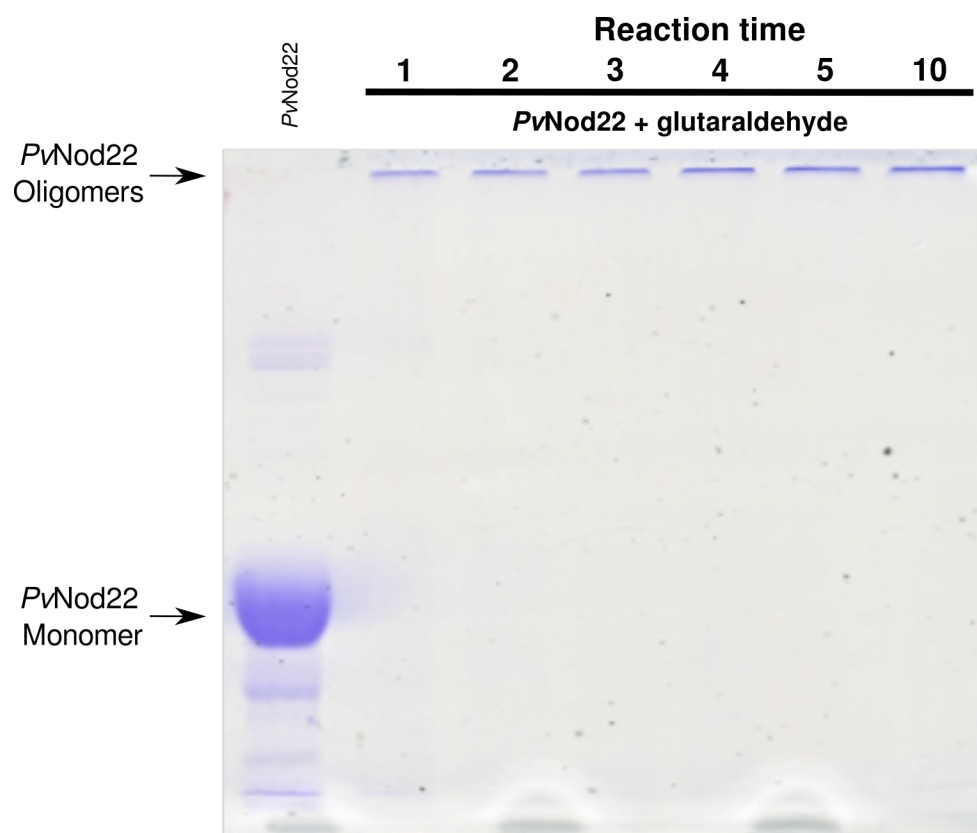


Figure S4. Glutaraldehyde crosslinking of *PvNod22* for the identification of quaternary protein structure. SDS-PAGE, 6% Laemmli of *PvNod22* recombinant protein (1 mg/mL) treated with glutaraldehyde 0.05% (v/v) for limited times (min). Lane C. Purified recombinant *PvNod22* untreated with glutaraldehyde. Lanes 1-5 and 10, min after glutaraldehyde treatment. The figure show that the proteins form high molecular weight complex are immediately formed after glutaraldehyde addition.

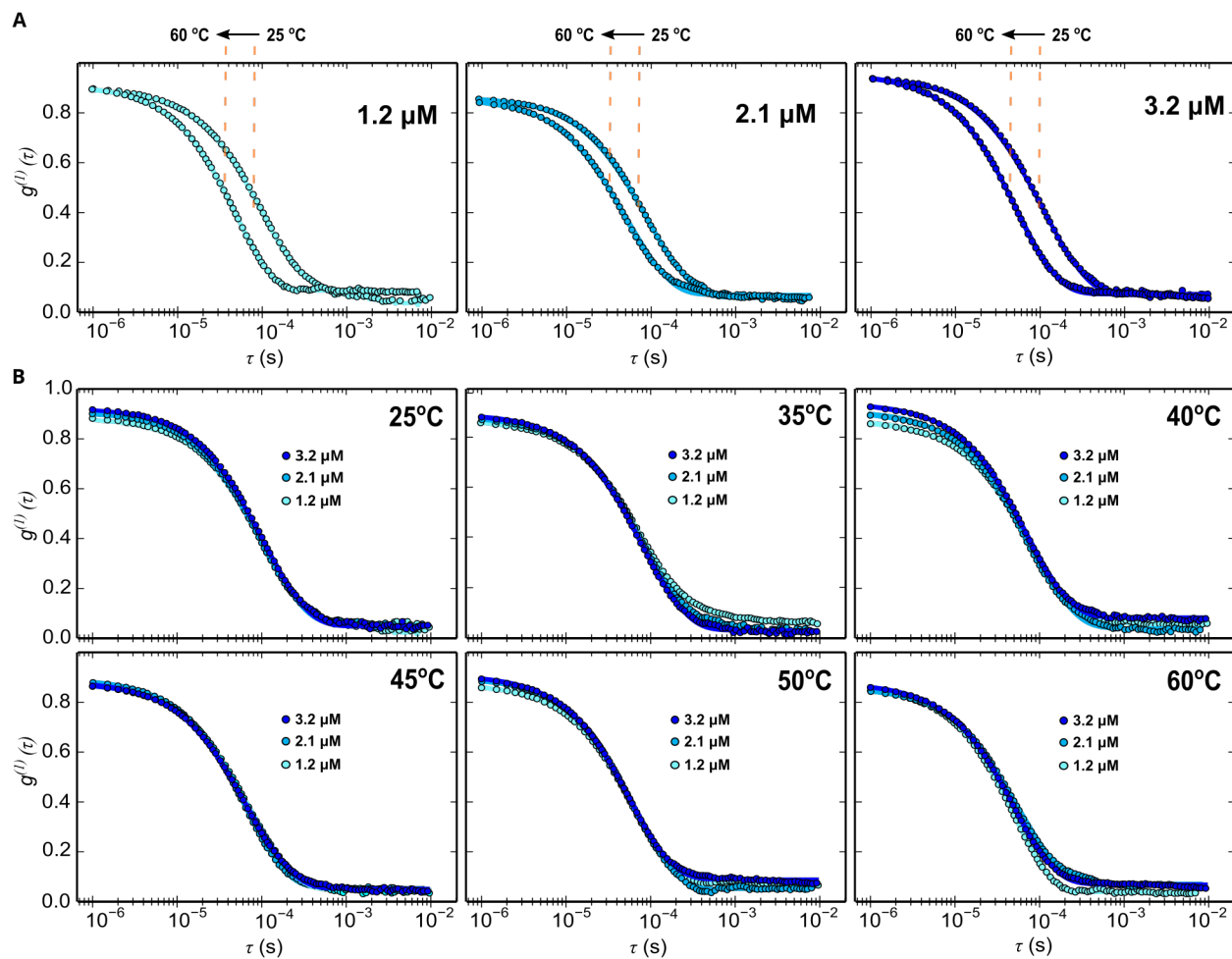


Figure S5. Correlation curves of *PvNod22* at different temperature and concentrations. A) Correlation curves at 1.3, 2.1 and 3.2 μM of *PvNod22* at different temperatures. Discontinuous lines show the movement from left to right observed with the temperature increased from 25 to 60 $^{\circ}\text{C}$. B) Correlation curves at 25, 35, 40, 45, 50 and 60 $^{\circ}\text{C}$ at different concentration. Light scattering measurements were carried out using a Malvern Zetasizer Nano ZSP at 633 nm with a scatter angle of 173 $^{\circ}$. *PvNod22* samples were previously diluted in TBS. The figure show that the concentrations of *PvNod22* do not have relevant effect on the molecular size of the oligomers.