



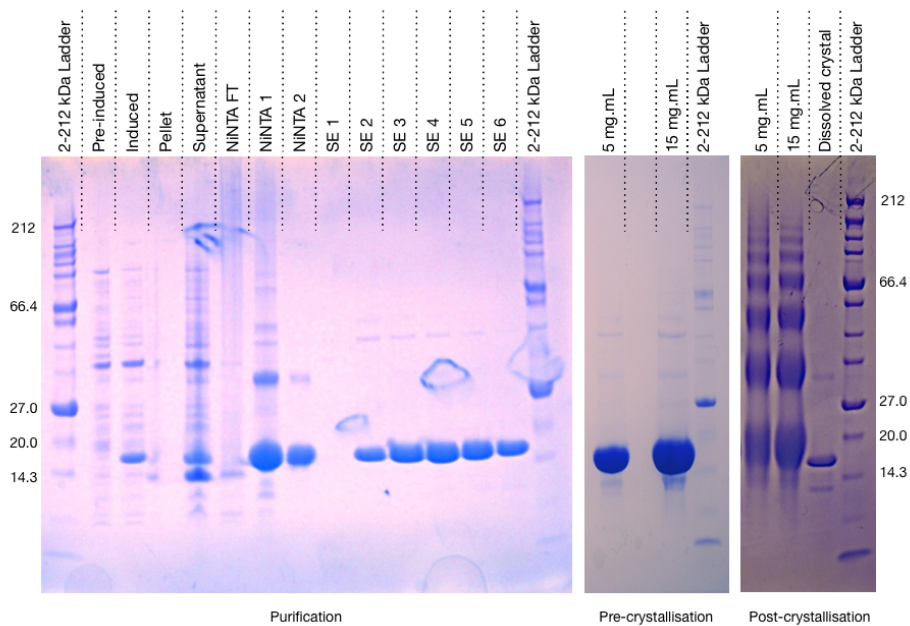
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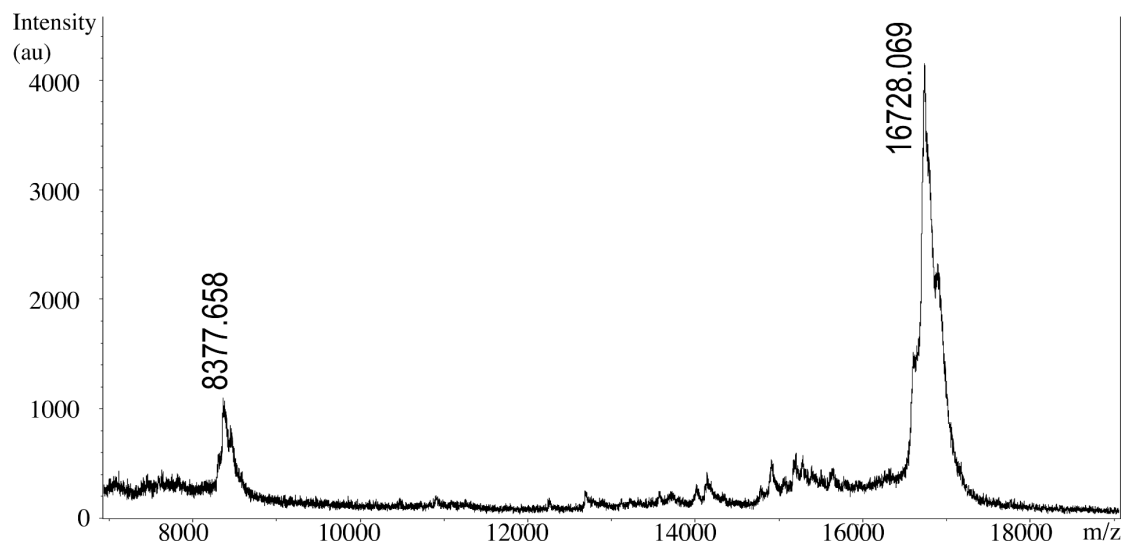
Supporting information for article:

The design and structural characterization of a synthetic pentatricopeptide repeat protein

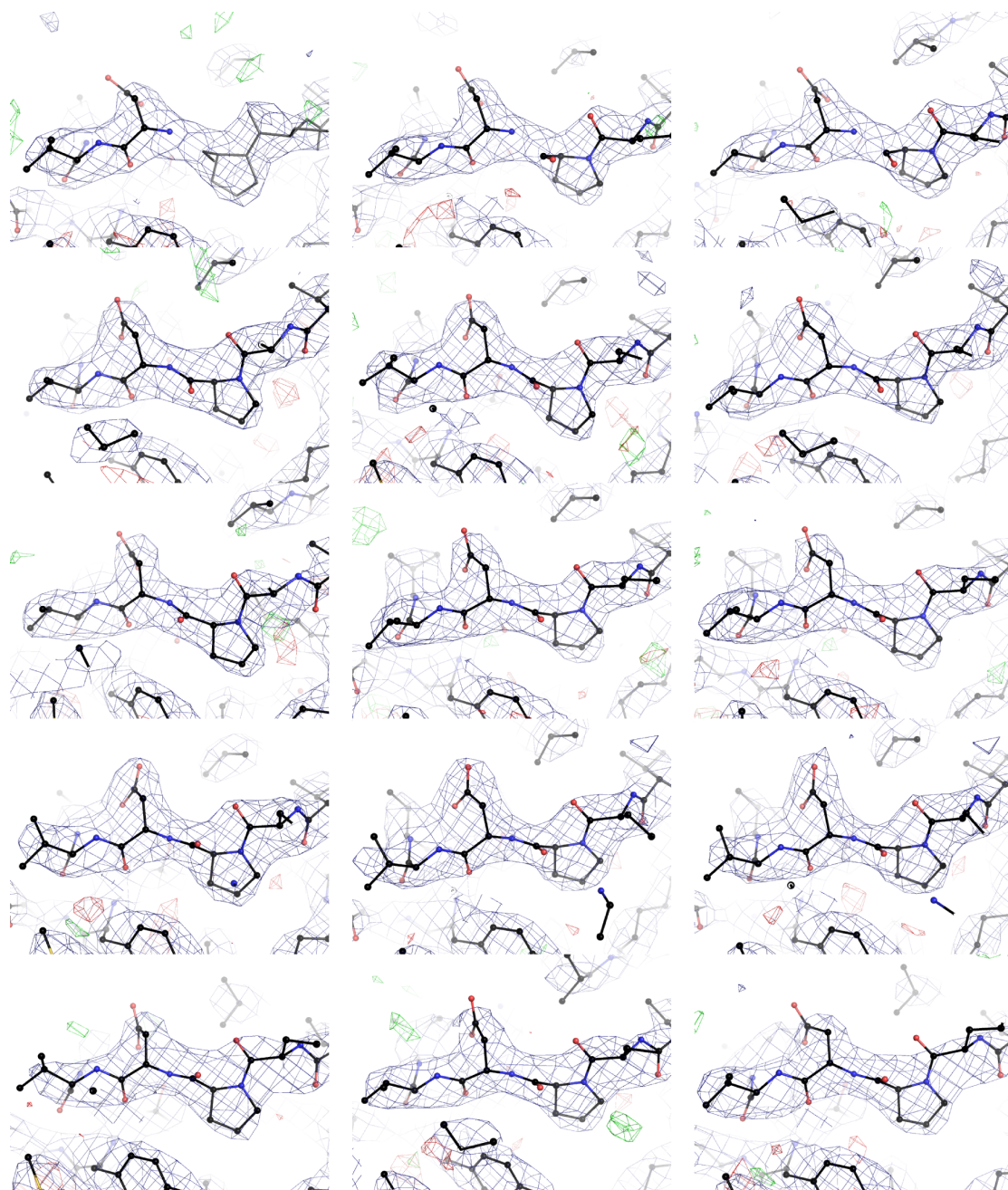
Benjamin S. Gully, Kunal R. Shah, Mihwa Lee, Kate Shearston, Nicole M. Smith, Agata Sadowska, Amanda J. Blythe, Kalia Bernath-Levin, Will A. Stanley, Ian D. Small and Charles S. Bond



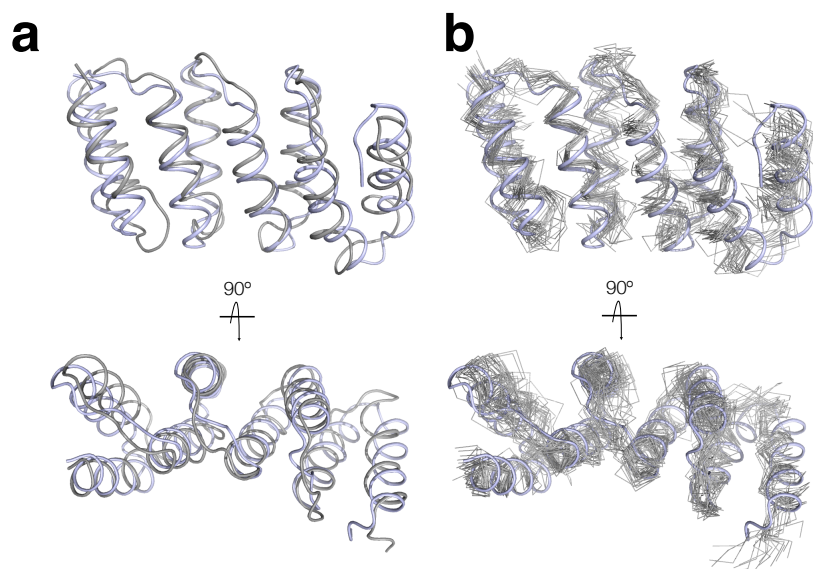
Supplementary Figure S1. Denaturing reducing polyacrylamide gel electrophoresis for the expression, purification and storage of the 16.7 kDa *synthPPR3.5* protein. (Left) Samples taken throughout purification including nickel affinity (NiNTA) and size exclusion chromatography (SE). (Middle) Concentrated samples immediately prior to crystallisation and (Right) after two weeks incubation, including a sample containing five dissolved protein crystals. No proteolytic degradation is visible, but SDS/DTT-persistent laddering of the protein is observed. SDS-PAGE (NuPage®Novex 4-12%, Bis-Tris gel, Invitrogen) stained with Coomassie brilliant blue loaded with 10 μ L per well.



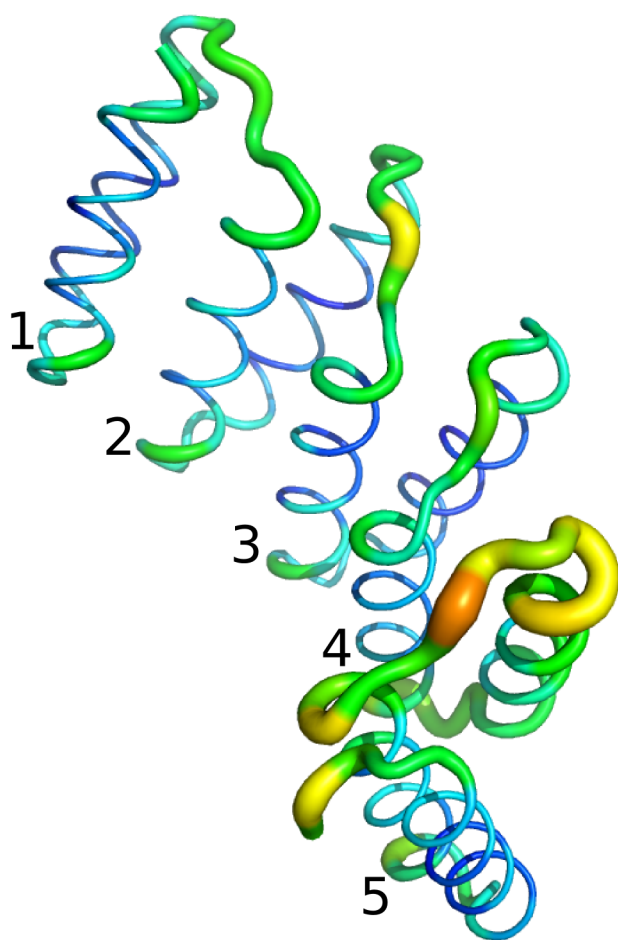
Supplementary Figure S2. MALDI-TOF analysis of a sample harvested from a six-month old crystal tray. The vast majority of the protein is full-length (observed: 16728 Da; theoretical: 16681 Da; $\delta=+49$ Da) with some evidence of minor degradation products around 15kDa.



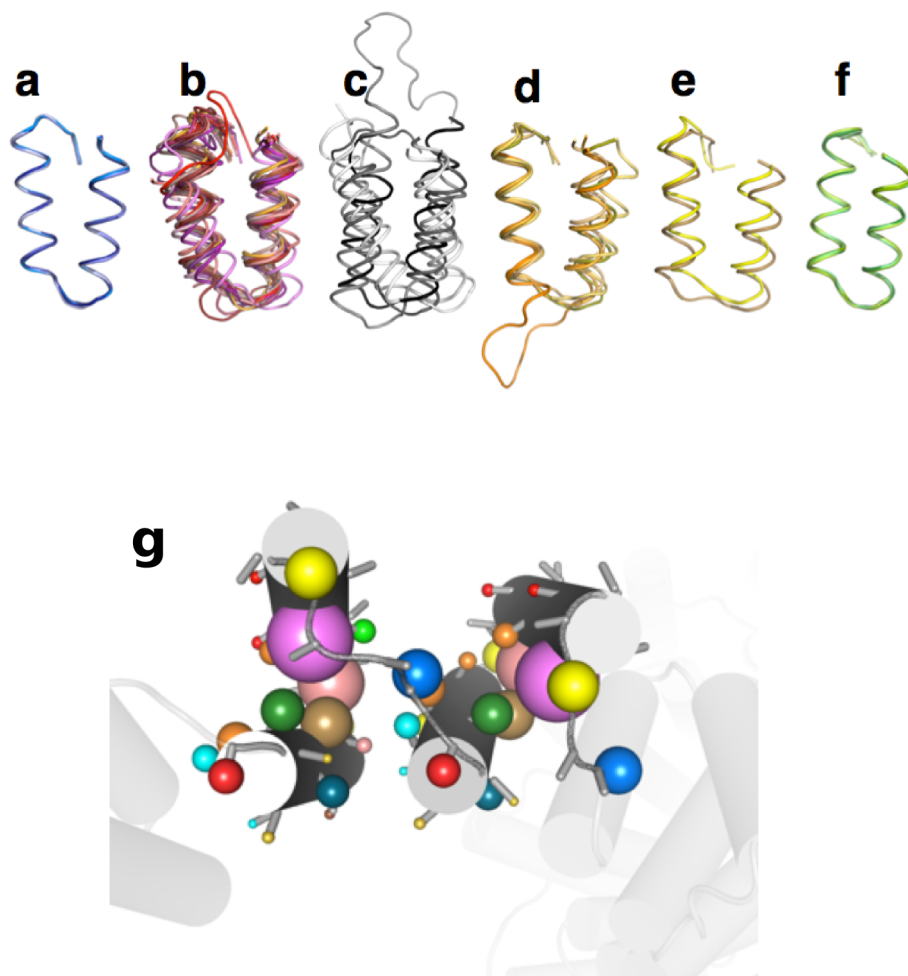
Supplementary Figure S3. Electron density for the synthPPR3.5 structure refined against data with a tripled l -axis, for comparison with Figure 4. Snapshots of structure and electron density ($2m|F_o|-D|F_c|$, α_c (1.2σ , blue) and $m|F_o|-D|F_c|$, α_c (-3.0σ , red; $+3.0\sigma$, green)) at the fifteen junctions between adjacent PPR motifs. Electron density is continuous throughout.



Supplementary Figure S4. Comparison of the predicted PPR structure with the *synthPPR3.5* crystal structure. (a) Superposition of the crystal structure with the most similar model. (b) Superposition of 18 models on the crystal structure. (All-atom RMSDs for between 729 and 876 atoms are, 2.6, 3.0, 2.5, 2.5, 4.2, 3.0, 2.8, 2.7, 2.9, 2.8, 2.7, 2.8, 3.1, 3.2, 2.5, 2.6, 3.5 and 2.8 Å). Models were generated from 20 rounds of modelling two of which did not complete.



Supplementary Figure S5. A cartoon putty representation of the asymmetric unit of *synthPPR3.5*, coloured according to B-factor (blue, minimum; red, maximum). B-factors are higher in the loops than in the helices.



Supplementary Figure S6. (a) Superposition of five PPR motifs of *synthPPR*; RMSDs of 0.239, 0.142, 0.219 and 0.284 Å respectively relative to *synthPPR* motif 1. (b) Superposition of 18 PPR motifs of PPR10 (4M59) with RMSD of 0.847, 0.646, 2.398, 1.334, 3.923, 1.259, 0.740, 2.694, 1.441, 0.824, 2.024, 0.922, 1.568, 2.499, 1.401, 2.368 and 1.928 Å respectively to motif 1 from PPR10. (c) Superposition of the five PPR motifs of THA8 (4N2Q) with RMSDs 6.335, 4.290, 3.836 and 6.518 Å relative to motif 1. (d) Superposition of the five PPR motifs of PRORP1 (4G23) with RMSD of 1.315, 1.575, 1.500 and 2.047 Å respectively to motif 1 from PRORP1. (e) Superposition of two PPR motifs from mtRNAP (3SPA) with RMSD of 3.087 Å. (f) Superposition of four TPR motifs of *synthTPR* (2FO7) with RMSD of 0.333, 0.370 and 0.333 Å respectively to motif 1. (g) A model of two adjacent motifs of *synthPPR3.5* with sidechains represented as spheres with radius proportional to the information content, coloured in the same manner as Figure 1(a).

Supplementary Figure S7. Analysis of superhelical parameters, expressed as intra-motif and inter-motif angles, for known PPR structures; (Top) *synth*PPR, mtRNAP (PDB 3SPA), PRORP1 (4G23), and THA8 (4N2Q) as well as a consensus TPR structure (2FO7). (Bottom) Similar characterisation for PPR10 without (4M57) and with (4M59) RNA bound. Intra-PPR angles calculated with Pymol; \pm represents standard deviation. Helix A shown in light blue and helix B shown in dark blue. Red denotes missing residues and yellow shows residues defined as loop rather than helix.