Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Data

Supramolecular Propensity of Suckerin Proteins is Driven by β–sheets and Aromatic Interactions as Revealed by Solution NMR

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Experimental Methods

The derived NMR structure was subjected to atomistic molecular dynamics (MD) simulations. The ff99SB force field as implemented in the program AMBER12[1,2] was used. Hydrogen atoms were added using the *Xleap* module of AMBER12[2] to prepare the systems for MD simulations. Each system was neutralized by adding sufficient number of Na^+ and Cl^- counter ions. Each system was then solvated in an octahedral box with TIP3P[3] water molecules that extended 10 Å from any protein atom. The short range non-bonded van der Waals interactions were truncated at 9 Å, while the long range electrostatics were approximated by the particle mesh Ewald[4] method. The covalent bonds involving hydrogen atoms were constrained using SHAKE[5]. The *Sander* module[2] was used for minimization with 250 steps of steepest decent algorithm, followed by 8000 steps of conjugate gradient algorithm. Initially, protein atoms, solvent water molecules, and counter ions were relaxed. This was followed by unrestrained energy minimization to remove any steric clashes. The systems were subject to over 250 ps of heating from 50 to 300 K with weak restraints on the heavy atoms, followed by gradual reduction of the restraints over the next 250 ps, until the restraints were reduced to 0. For next 2 ns the system was equilibrated at 300 K under 1 atm constant pressure. After equilibration production runs of 100 ns with 2 fs time step were carried out and the atomic coordinates were saved every 10 ps. Analyses of the simulation trajectories were performed using the *ptraj* module in Amber. The simulated trajectories were viewed in VMD[6] and figures were generated using PyMol[7].

Supplementary Figures

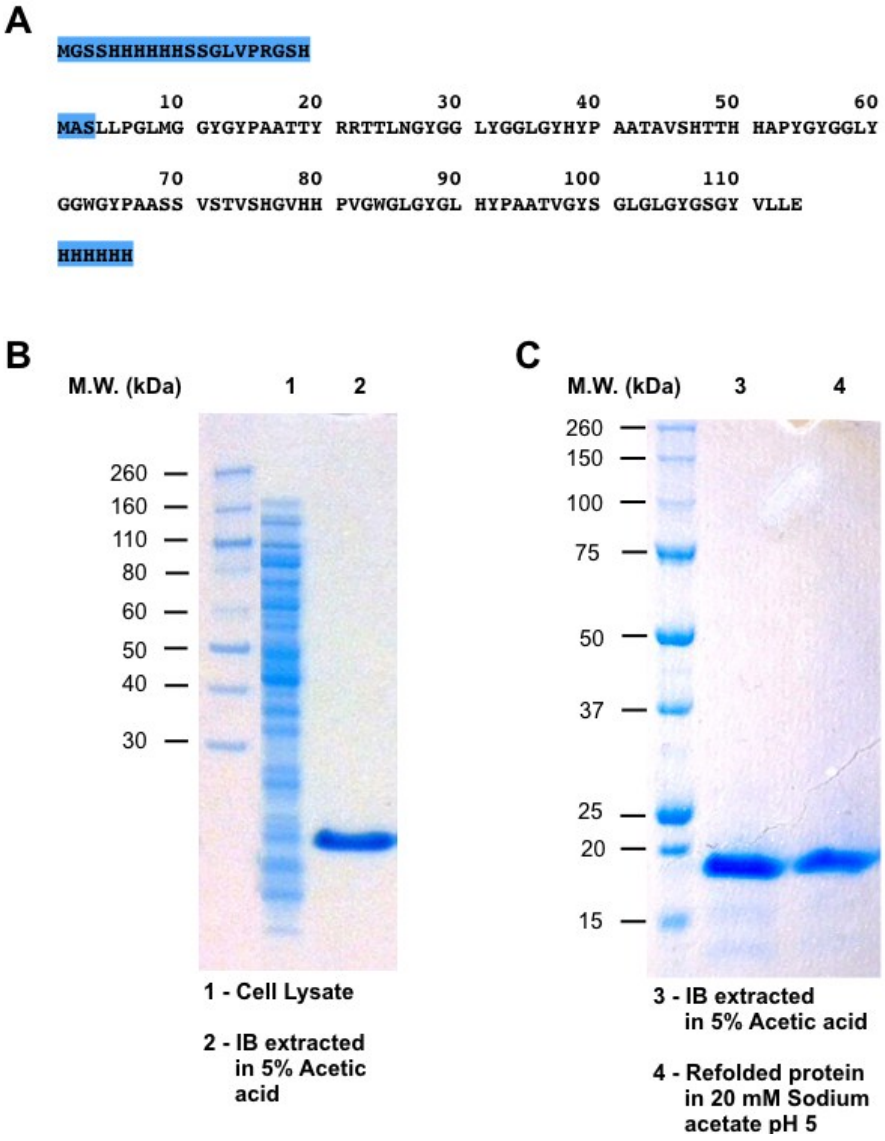

Figure S1. (A) Full length sequence of *SLS-1-mono* (tag residues are highlighted in blue). (B-C) SDS-PAGE analysis of *SLS-1-mono*. (B) Total cell lysate (lane 1) and inclusion body (IB) extracted in 5% acetic acid (lane 2). **(C**) IB extracted in 5% acetic acid. Lane 3: concentrated fraction subsequently used for refolding. Lane 4: refolded and concentrated fraction in 20 mM sodium acetate pH 5 subsequently used for purification by Size Exclusion Chromatography (SEC), shown in Fig. 2B of the main text.

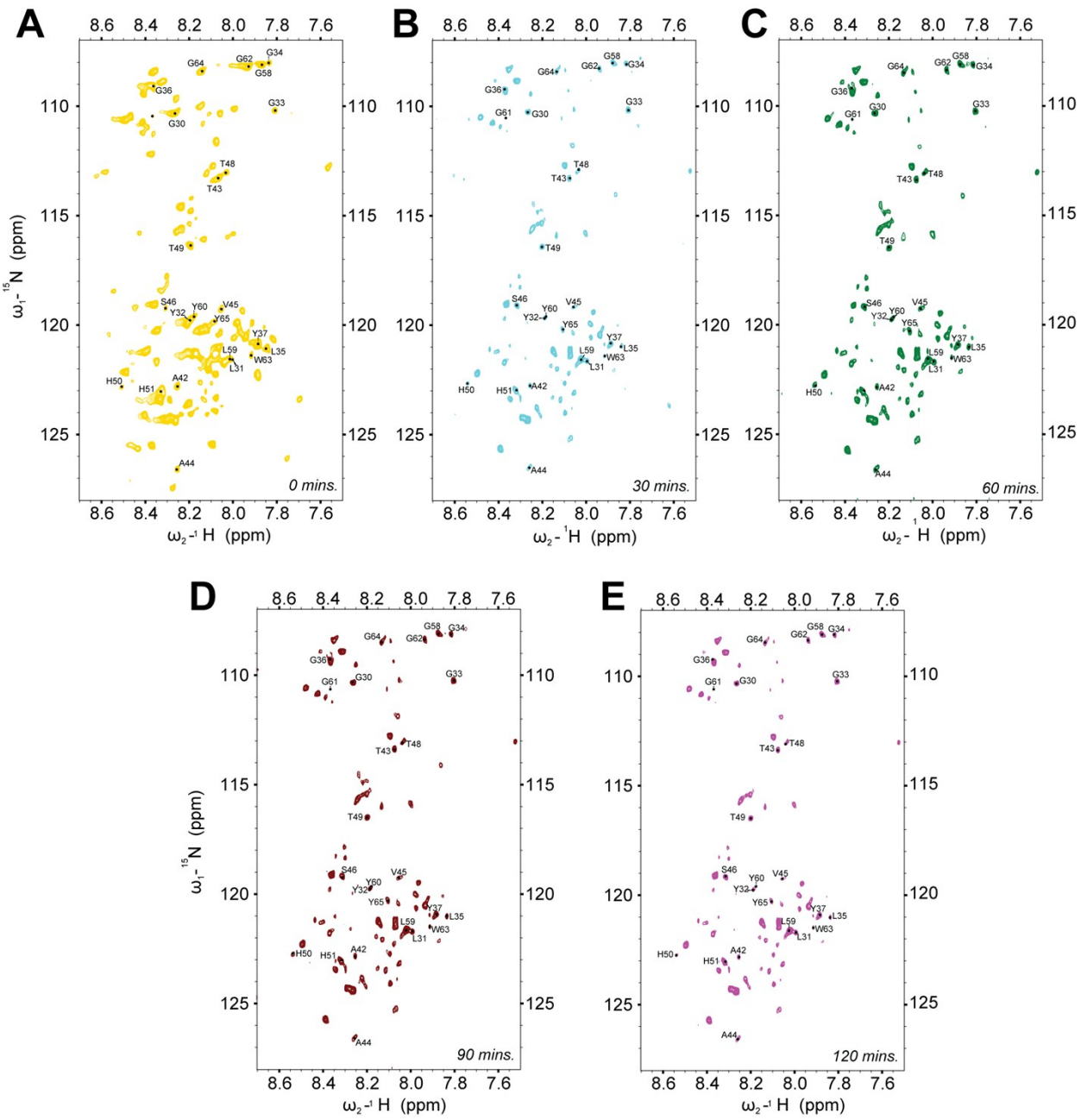

Figure S2. H/D exchange of *SLS-1-mono* in deuterated 20 mM sodium acetate, pH 5.0 recorded at 298 K and at various time points. **(A)** 0 minute; **(B)** 30 minutes; **(C)** 60 minutes; **(D)** 90 minutes; **(E)** 120 minutes.

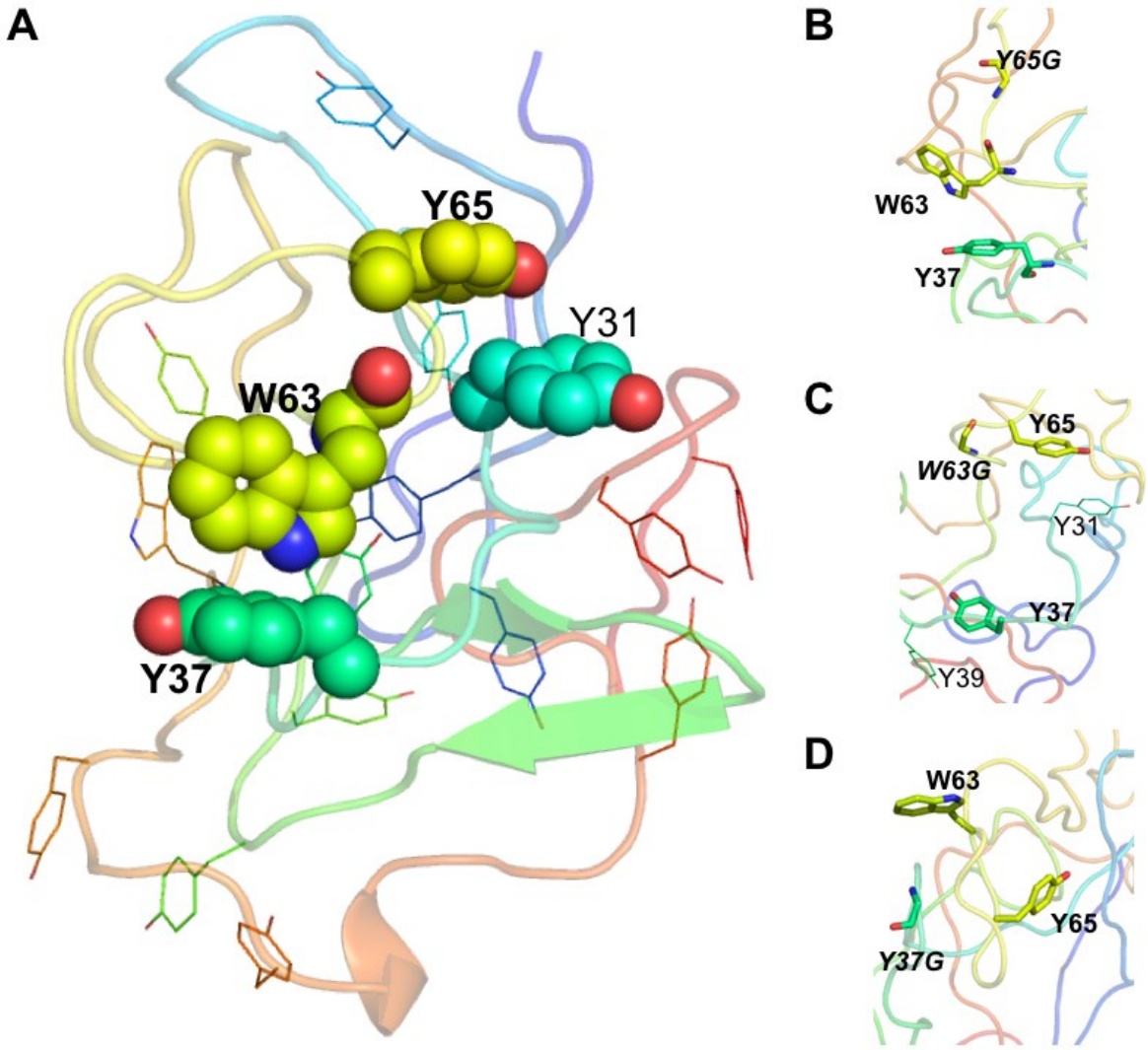

Figure S3. Aromatic interactions observed in *SLS-1-mono* from MD trajectories. (**A**) Snapshot of wild type structure with preserved π-π interaction between Y37 and W63 (spheres) and a newly formed π-π interaction between Y65 and Y31 (spheres representation)**.** All the other Tyr and Trp residues are represented as lines. **(B)** Snapshot from trajectory with **Y65G** mutation, indicating no interaction of G65 but with preserved aromatic interactions between Y37 and W63 (stick representation). (**C**) Snapshot from

trajectory with **W63G** mutation, indicating loss in interaction with Y37 (residues shown as sticks). Y39 and Y31 (line representation) are observed to be spatially accessible for interactions with Y37 and Y65 (stick representation), respectively. (**D**) Snapshot from trajectory with **Y37G** mutation, indicating loss in π-π interaction with W63 (stick representation).

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