Supporting Information

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SI Text

Model Construction. $EAS_{\Delta 15}$ is chosen for model construction due to its reduced complexity. This mutant is identical to full length EAS in terms of the morphology and physiochemical properties of the rodlets that it forms[22].

A rodlet segment consisting of six $EAS_{\Delta 15}$ monomers was modeled using HADDOCK2.1 [26,27] starting with the lowest energy structure of the $EAS_{\Delta 15}$ monomer (PDB ID code 2K6A) in the first round of docking. A total of six rounds of docking were performed. In rounds 2–6, the middle molecule in the lowest energy structure from the previous round was used to create six identical starting structures. During each round of HADDOCK runs, residues A1–T2 and Q20-$G44_{\Delta 15}$ (the remaining 10 residues in the Cys3–Cys4 region in $EAS_{\Delta 15}$) and K62–N79 (residues in the Cys7–Cys8 region) were defined as flexible residues. F72 was defined as "active" and the neighboring residues S71–I75 as "passive" according to the definition of ambiguous interaction restraints in HADDOCK. Standard parameters were used, except that the numbers of molecular dynamics steps were increased to 500 for rigid body high temperature annealing, 1,000 for first rigid body cooling stage, 8,000 for second cooling stage, and 8,000 for third cooling stage in rounds 1–5. These numbers were doubled again in the last round. In all rounds, 200 rigid body docking calculations were carried out in iteration 0 with the 50 lowest energy structures subjected to refinement in iteration 1. No symmetry restraints were used. The five conformers with the lowest total energy (E_{tot}) were analyzed.

A number of single runs were also carried out with HADDOCK-evolved structures from round 4 to test various hydrogen-bonding arrangements. We note the length of the strand in the spine model is limited by the length of the Cys7–Cys8 region and its attachment to the β-barrel core. A longer β-spine than about 6–7 residues appear to clash with the β-barrel, although the length of the strand in the spine must be long enough to provide stability to the rodlets and must at least cover the F72–I75 region. For the antiparallel model, we found that more than one hydrogen-bonding pattern is sterically acceptable (e.g., offsetting the hydrogen-bonding register by one residue or changing the length of the β-strand in the spine from 4 to 6 can be accommodated). The final models (Model A and B; see below) that are presented contain the hydrogen bond set in the β-spine which yields the lowest total energy structure.

The following restraints were used in the final calculations as presented in the paper:

1. Hydrogen bond restraints in antiparallel (Models A and B) or parallel (Model C) format between neighboring monomers over residues S71–I75 and D64–T68. All hydrogen bond restraints are included as unambiguous distance restraints with HN…O distance set to 1.8 to 2.3 Å and N…O distance set to 2.8 to 3.3 Å between hydrogen bonded pairs.
2. Dihedral angle restraints over S71–I75 and D64–T68 ($\phi = -120 \pm 30°$ and $\phi = 115 \pm 30°$ for parallel β-sheet or $\phi = -140 \pm 30°$ and $\phi = 135 \pm 30°$ for antiparallel β-sheet).
3. Vector angle restraints prevent the β-sheet twisting over the length of the rodlet segment (using the CA atoms between the first and last residue in each strand as reference points).
4. Asparagine ladder restraints between N67 sidechains in neighboring monomers and an intramolecular restraint between CB of N67 and CB of F72 to keep Asn inside the β-sheet spine (Model A only).

A summary of the results from HADDOCK calculations for models A, B, and C are shown in Table S2.

Thermal Stability Monitored by Circular Dichroism Spectropolarimetry. Lyophilized $EAS_{\Delta 15}$ and $EAS_{\Delta 15}$-F72G as prepared above were resuspended in 300 μL of 10 mM sodium phosphate (pH 7) to a concentration of approximately 25 μg/mL according to absorbance at 280 nm. All CD data were acquired on a Jasco J-720 or J-815 spectropolarimeter using a 1 mm quartz cuvette. The spectra shown are the average of three successive scans in continuous mode (20 nm/min) with a step size of 0.5 nm, a 1 s response time, and a 1 nm bandwidth. For temperature melts, the ellipticity (mdeg) was monitored at 198 nm for $EAS_{\Delta 15}$ and $EAS_{\Delta 15}$-F72G as the temperature was increased from 20 °C to 85 °C in increments of 0.1 °C. Samples were incubated for 10 sec at each 0.1 °C interval to allow for equilibration.

Fourier Transform Infrared Spectroscopy. Soluble $EAS_{\Delta 15}$ and rodlets samples were prepared in water at a protein concentration of 50 μg/mL. Rodlet formation was induced by a 30-min agitation at 25 °C using a vortex mixer. FTIR spectra were collected in absorbance mode at 2 cm^{-1} resolution using a Varian Scimitar 800 FTIR spectrometer. The system was constantly purged with a stream of dried N_2 gas. All spectra were first zeroed with the absorbance value at 1,800 cm^{-1}. To account for the slight differences in residual moisture content in the samples, a variable weight factor was assigned to the zeroed blank spectrum (air with holder) before spectra subtraction was carried out. The weight factor was adjusted until sharp spikes in the spectra were matched out.

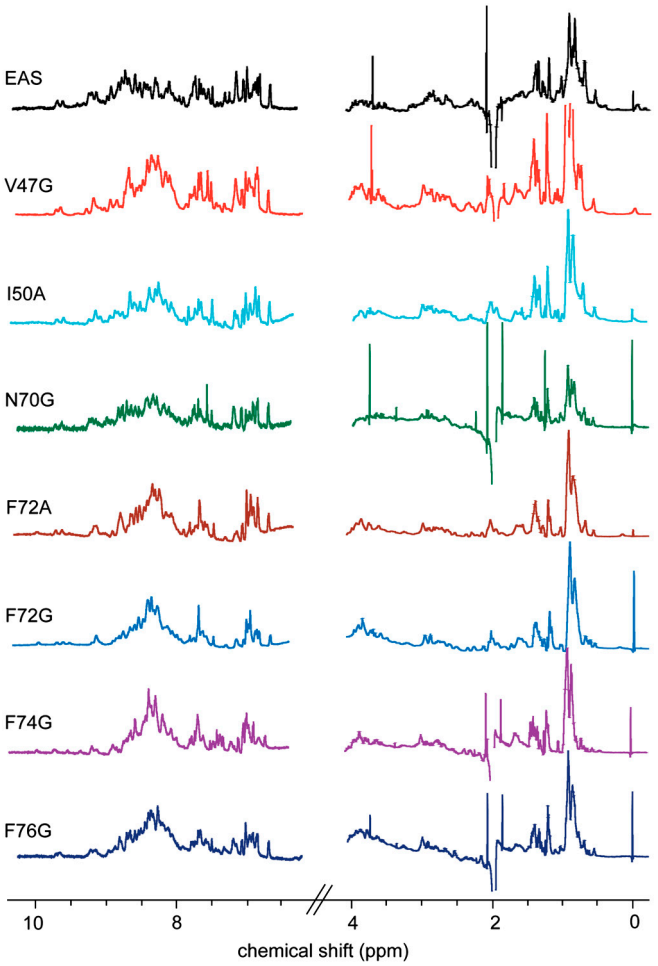

Fig. S1. The ^{1}H one-dimensional NMR spectra of wild-type EAS and selected mutants indicate that these proteins are folded, with a structure similar to that of the parent protein.

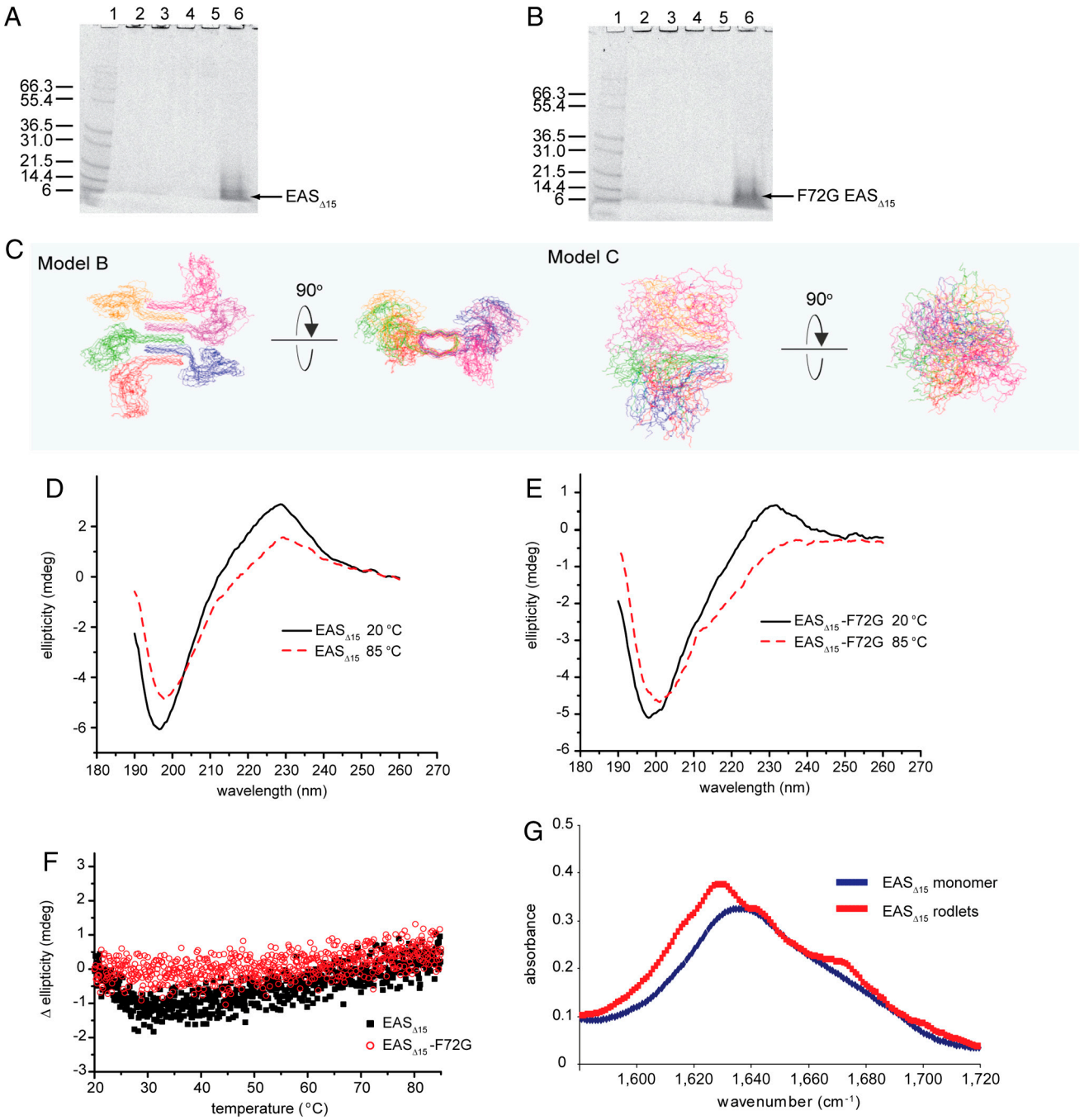

Fig. S2. (*A*) EAS$_{\Delta15}$ and (*B*) F72G EAS$_{\Delta15}$ rodlet samples were resuspended in MQW (lane 2) or 10% SDS with boiling (lane 3), 70% ethanol (lane 4), 8 M urea (lane 5), or neat TFA (lane 6). Lane 1 contains protein MW standards in both gels. (*C*) Rodlet models B and C. Structures of EAS$_{\Delta15}$ hexamer calculated from six rounds of HADDOCK starting with six copies of the monomeric EAS$_{\Delta15}$ structure in the first round. Overlay of 5 lowest energy structures from the last round of docking are shown for Model B (antiparallel β-spine and no Asn restraints) and Model C (parallel β-spine). The parallel model has numerous steric clashes and the minimized structures do not converge to a single cluster. (*D* and *E*) Far-UV circular dichroism spectra of EAS$_{\Delta15}$ and EAS$_{\Delta15}$-F72G recorded at 20 °C and 85 °C. (*F*) Plot showing the change in ellipticity (mdeg) at 198 nm for EAS$_{\Delta15}$ and EAS$_{\Delta15}$-F72G as measured by circular dichroism spectropolarimetry over the temperature range of 20 °C to 85 °C. (*G*) FTIR spectra recorded over 1,580 cm^{-1} to 1,720 cm^{-1} from lyophilised EAS$_{\Delta15}$ monomer (blue) and rodlets (red) at 25 °C.

Table S1. NMR and refinement statistics for $EAS_{\Delta 15}$-F72G

	Protein
NMR distance and dihedral constraints	
Distance constraints	
Total NOE	1,400
Intraresidue	635
Interresidue	
Sequential ($\|i - j\| = 1$)	314
Medium-range ($\|i - j\| < 4$)	75
Long-range ($\|i - j\| > 5$)	266
Intermolecular	0
Hydrogen bonds	0
Total dihedral angle restraints	25
ϕ	25
ψ	0
Structure statistics	
Violations (mean and s.d.)	
Distance constraints >0.5 Å	0
Dihedral angle constraints >5 Å	0
Deviations from idealized geometry	
Bond lengths (Å)	0.0034 ± 0.0001
Bond angles (°)	0.428 ± 0.019
Impropers (°)	1.58 ± 0.11
Average pairwise rmsd* (Å)	
Heavy	1.06 ± 0.13
Backbone	0.73 ± 0.10

*Pairwise rmsd was calculated among 20 refined structures over the backbone atoms of residues T2-Q20, L43-D63, and I74-C81.

Table S2. Summary of results from HADDOCK calculations for models A, B, and C

	Model A	Model B	Model C
β-sheet geometry	antiparallel	antiparallel	parallel
β-spine residues (for which hydrogen bond restraints are applied)	SFLII, VTNTG	SFLII, VTNTG	SFLII, DVTNT
Asn ladder restraints	Yes	No	No
Average total energy (lowest 5 energy structures)	−586.1 ± 8.6	−531.0 ± 2.5	−205.5 ± 13.3
No. of distance violations over 1 Å	0	1	93
No. of angle violations over 10°	0	0	17
rmsd (over backbone atoms of residues in β-spine)	0.5 Å	0.7 Å	3.2 Å