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Supplemental Information

Structure of the Bacterial Cytoskeleton Protein Bactofilin by NMR Chemical Shifts and Sequence Variation

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Fig S1. Lowest energy structure of the control simulation using only the Profasi energy and chemical shifts.

Table S1. Validation using ssNMR distance measurements. The table reports the distances measured in the final MD refined structure. The distances correspond to the contacts observed by ssNMR. In the cases of ambiguous contacts we show the contacts with the smallest distances.

#	Contact	Distance (Å)
1	G54HN-V70HA	3.0
2	D45HN-S43HA	4.4
3	S84HN-D67HA	2.5
4	G115HN-A99HA	5.5
5	V70HN-A87HA	3.3
6	D116HN-A99HA	3.2
7	S111HN-G109HA1	3.7
8	R93HN-G109HA1	9.3
9	I100CD-I117CD	4.7
10	V90CG1-I100CG2	3.9
11	V90CG1-I100CD	5.1
12	L107CD2-V92CG2	4.1
13	I60CD-S43C	8.2
14	I48CG2-V64C	6.3
15	A44CB-D61CG	5.2
16	V97CG1-E82CD	6.3
17	A89CB-E88CD	7.0
18	L135CD2-D116CG	5.4
19	L135CD1-D116CG	4.4
20	K103CD-E120CD	4.8

Validation by comparison with mutational data

We grouped the mutations according to how they change the chemistry and size of the amino acid, whether or not they affected polymerization, and whether the mutated residue points

towards the hydrophobic core (inwards) or is surface exposed (outwards) in our structure (Table S2). To provide a link between our structure and any effect of the mutations on filament assembly, we made the following simplifying assumptions: (i) The monomer stability is correlated to the change in polymerization, (ii) mutations at outward facing positions are likely not to be destabilizing, (iii) mutations at inward facing positions are likely to be destabilizing when the mutation significantly changes the size and/or hydrophobicity of the amino acid.

In line with these assumptions, we find that mutations at positions that are solvent exposed, independently of whether they involve charged residues (group B) or changing polarity (group D), do not affect filament assembly. At inward pointing positions, we generally find that the substitution of either Val, Leu or Ile to Ala (i.e. changing a medium sized residue to a smaller one, but preserving the apolar nature) has no effect on assembly (group A). In contrast, changing a larger Phe or Met to Ala affects assembly (group C). Here we note an outlier, in that the mutation V75A in the same group also affects assembly, despite Val-to-Ala mutations being tolerated elsewhere (group A). In contrast to the A123S mutation on the surface, which is tolerated (group D), (single or multiple) substitutions of buried apolar residues for the more polar Ser consistently affects filament assembly (group E and F). Thus, despite our simple model for the relationship between structure, mutation and assembly we find that the structure we determined can rationalize all but one of the 27 mutations studied.

Table S2. Comparison of our structure with mutational data. We divided the mutations into six groups depending on the type of mutations that were performed and whether or not the mutated side chain points inwards towards the core or is surface exposed in our structure. The table also indicates whether the mutations were found experimentally to affect filament assembly in the cell.

Group	Mutations	Changed polymerization	Type	Orientation
A	V52A, L58A, I60A, V64A, V68A, L73A, V81A, V85A, V96A, V105A, V113A, L122A	No	Hydrophobic to A	Inwards
B	E77K, E88K, K103E, D116A	No	Charge change	Outwards
C	V75A, M124A, F130A	Yes	Hydrophobic to A	Inwards
D	A123S	No	Hydrophobic to S	Outwards
E	L73S, V75S, L122S, M124S, L73S/V75S	Yes	Hydrophobic to S	Inwards
F	L41S/L42S, L122S/A123S/M124S	Yes	Hydrophobic to S	Inwards and outwards

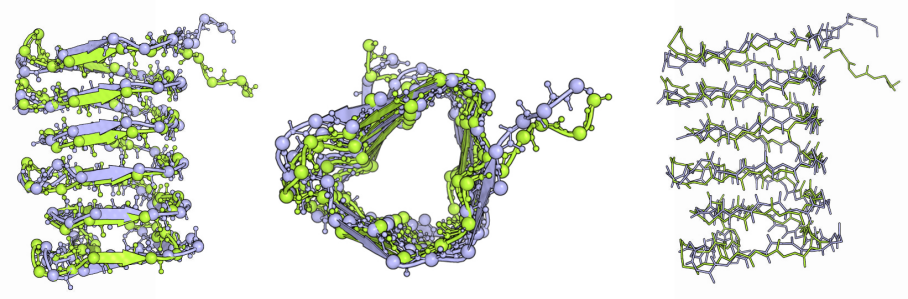

Fig S2. Overlay of our structure (lime) with a recently determined ssNMR structure (light blue).