The propensity of the bacterial rodlin protein RdlB to form amyloid fibrils determines its function in *Streptomyces coelicolor*

Wen Yang1,†, Joost Willemse2, Elizabeth B. Sawyer1,2,‡, Fei Lou1,3, Weibin Gong1, Hong Zhang1,3, Sally L. Gras4, Dennis Claessen2 & Sarah Perrett1,3

*Streptomyces* bacteria form reproductive aerial hyphae that are covered with a pattern of pairwise aligned fibrils called rodlets. The presence of the rodlet layer requires two homologous rodlin proteins, RdlA and RdlB, and the functional amyloid chaplin proteins, ChpA-H. In contrast to the redundancy shared among the eight chaplins, both RdlA and RdlB are indispensable for the establishment of this rodlet structure. By using a comprehensive biophysical approach combined with *in vivo* characterization we found that RdlB, but not RdlA, readily assembles into amyloid fibrils. The marked difference in amyloid propensity between these highly similar proteins could be largely attributed to a difference in amino acid sequence at just three sites. Further, an engineered RdlA protein in which these three key amino acids were replaced with the corresponding residues from RdlB could compensate for loss of RdlB and restore formation of the surface-exposed amyloid layer in bacteria. Our data reveal that RdlB is a new functional amyloid and provide a biophysical basis for the functional differences between the two rodlin proteins. This study enhances our understanding of how rodlin proteins contribute to formation of an outer fibrillar layer during spore morphogenesis in streptomycetes.

A variety of microorganisms can establish a complex, yet highly ordered extracellular matrix in response to various environmental cues. This matrix serves multiple purposes for microorganisms and can alter the surface properties of the enveloped structure1, facilitate spore dispersal into the air2, mediate cell attachment to surfaces3 and in some cases help pathogens to invade their host4. The specific composition of this matrix varies from species to species, but these materials often share a highly ordered, fibrous protein component known as amyloid fibrils5. Amyloids possess a characteristic cross-β structure, which gives the fibrils both physical strength and chemical stability6. Amyloid formation has traditionally been associated with a range of neurodegenerative diseases in humans, yet an increasing number of studies indicate that amyloid proteins often functionally contribute to the physiological well-being of organisms7. Within the diverse list of functional amyloids identified over the last decade or so, a significant number have been reported in bacteria, including the well-studied *Escherichia coli* curli fibrils and the TasA amyloids in *Bacillus* biofilms5,8,9. Functional amyloids are also formed by streptomycetes. In *Streptomyces* the aerial mycelium is covered with an amyloid surface layer, called the rodlet layer, which appears as a network of pair-wise aligned nanorods10. Two kinds of secreted proteins, named rodlins and chaplins, are essential for the formation of this rodlet layer. A series of genetic and ultrastructural studies led to a model in which the chaplin proteins serve as the functional amyloid component11–13. Chaplins are secreted by growing aerial hyphae to form a tightly-packed fibrillar layer rendering the surface of aerial hyphae and spores hydrophobic, which is required for efficient aerial growth11–16. Rodlins were found to be less crucial for aerial

---

1National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, 100101, China. 2Molecular Biotechnology, Institute of Biology, Leiden University, 2333 BE Leiden, the Netherlands. 3University of the Chinese Academy of Sciences, Beijing, 100049, China. 4Department of Chemical and Biomolecular Engineering and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, 3010 VIC, Australia. 5Present address: Leiden University, 2333 BE Leiden, the Netherlands. 6Present address: London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK. Correspondence and requests for materials should be addressed to S.L.G. (email: sgras@unimelb.edu.au) or D.C. (email: d.claessen@biology.leidenuniv.nl) or S.P. (email: sarah.perrett@cantab.net)
hyphae formation, and were suggested to align chaplin fibrils into the higher order rodlet structure\textsuperscript{12,17}. Notably, both of the rodlin proteins (RdlA and RdlB) are indispensable for formation of rodlets in the wild-type strain\textsuperscript{11,16}. Despite their high degree of sequence similarity, the \textit{in vivo} data strongly imply that RdlA and RdlB may each have a unique functional contribution to the structural integrity of the rodlet layer, which cannot be compensated for by the other rodlin protein.

In contrast to the chaplin proteins, the biochemical characterization of rodlins has so far been limited. This is largely due to the fact that the trifluoroacetic acid (TFA) extraction procedure used to isolate rodlins directly from the insoluble cell wall components of \textit{S. coelicolor} results in a mixture of the two rodlin proteins in a completely denatured state\textsuperscript{12}. The large size of rodlins compared to the chaplins also makes the proteins less amenable to peptide synthesis. In this study, we have thoroughly investigated the biophysical properties of the RdlA and RdlB proteins expressed recombinantly in \textit{E. coli}. Using a variety of biophysical methods we discovered that RdlB, but not RdlA, readily self-assembles into amyloid fibrils that closely resemble the fibrils observed on the cell surface of aerial structures. The difference in the propensity of the two rodlins to assemble into amyloid fibrils is attributed to small differences in the N-terminal part of the two rodlin proteins. The introduction of three amino acid changes in the N-terminus of RdlA was not only sufficient to impart the ability to form amyloid fibrils \textit{in vitro}, but also restored the formation of the rodlet layer \textit{in vivo} in a strain lacking both RdlA and RdlB. These data show RdlB to be a new functional amyloid, and provide a biophysical basis for the functional differences between the two rodlin proteins.

Results

RdlA and RdlB are highly similar. The rodlin proteins RdlA and RdlB of \textit{S. coelicolor} consist of 131 and 133 amino acids, respectively. Lalign analysis\textsuperscript{18} of the mature proteins indicates that the rodlins are 69.6% identical and 93.1% similar (Fig. 1A). The two rodlins also share highly similar hydrophobicity profiles based on the Kyte & Doolittle scale\textsuperscript{19} (Fig. 1B). Notably, RdlA possesses significantly more polar or charged residues near its N-terminus compared to RdlB. As a result, RdlB has a lower net charge and a greater hydrophobic score than RdlA.

TANGO was used to predict the secondary structure of RdlA and RdlB\textsuperscript{20,21}. TANGO calculation suggests that the regions between residues 51 and 57 in both RdlA (VAVINLV) and RdlB (VSVIGLV) have a high propensity for $\beta$-sheet aggregation. The algorithms AGGREGSCAN\textsuperscript{22} and FoldAmyloid\textsuperscript{23} which predict aggregation-prone regions showed agreement with the results obtained using TANGO.

Figure 1. (A) The ESPript output of the mature rodlin sequences aligned by ClustalW. Conserved residues have a black background: 71 out of 105 of residues in RdlB are identical to RdlA. (B) The hydropathy patterns of RdlA (black) and RdlB (red) appear to be highly similar.
Common features of RdlA and RdlB in solution. When rodlin are purified from the cell walls of *Streptomyces*, the trifluoroacetic acid extraction process causes denaturation of the proteins, hampering studies of rodlin structure and function. In order to characterize the rodlin proteins, both RdlA and RdlB were produced recombinantly in *E. coli* without their N-terminal signal peptide. The molecular mass values of the purified rodlin, 10605 Da for RdlA and 10799 Da for RdlB, were confirmed by MALDI-TOF (Fig. S1). Both RdlA and RdlB were shown to be intrinsically disordered by far-UV circular dichroism (CD) (Fig. 2A) and ¹H-¹⁵N HSQC NMR spectra (Fig. S2).

**Figure 2.** RdlA and RdlB have a different propensity to assemble into amyloid-like fibrils. (A) Vortexing induces RdlB to adopt β-sheet rich secondary structure (red solid line) from random coil (red dash line), while RdlA (black solid line) retains the random coil conformation observed before vortexing (black dash line). The protein concentration used was 4 μM in 100 mM sodium phosphate buffer (pH 7.0) measured in a 1 mm cuvette. (B, C) Increase in ThT fluorescence intensity by RdlA (B) and RdlB (C) at different concentrations. Readings were taken every 3 min for over 15 hours in a microplate reader while shaking at 700 rpm. The data shown are the average of at least 4 replicates and the error bars represent the standard error of the mean. (D) No well-defined structure was found in negatively-stained electron micrographs of RdlA (40 μM) after samples were incubated for 24 hours. (E) Negatively stained electron micrograph of mature RdlB fibrils (40 μM) incubated for 24 hours. (F) Negatively-stained electron micrograph of RdlB fibrils after 24 hours proteinase K digestion; the thin, needle-like fibrils tend to align into bundles. Scale bars represent 100 nm in images (D–F).
We then analysed the surface activity of the two proteins by interfacial tension (IFT) measurements. RdlA and RdlB at a concentration of 100 μg/ml lowered the surface tension by 13 mN/m and 18 mN/m, respectively. In comparison, the chaplin peptide, ChpH was more surface active and was capable of altering the interfacial tension from 72 mN/m to 46 mN/m, in agreement with a previous study. These results indicate that the surfactant properties of the rodlins are less potent than those of the chaplin peptides.

**RdlB forms amyloid-like fibrils in vitro.** The bioinformatics analysis reveals little difference between RdlA and RdlB, and both rodlins were found to be intrinsically disordered in solution. However, when CD spectra were recorded after 10 min of vortexing, a process known to increase the interfacial surface area and promote mixing that can induce fibril formation, a distinct difference was observed between the two rodlins. RdlB readily adopted a β-sheet-rich secondary structure, as indicated by the emergence of a strong negative peak near 220 nm (Fig. 2A). In contrast, the CD spectrum of RdlA barely changed after vortexing.

The amyloid binding dye, Thioflavin T (ThT) was used to monitor fibril formation kinetics. No detectible increase in ThT fluorescence was observed for RdlA indicating little polymerization during prolonged incubation with agitation (Fig. 2B). In contrast, the kinetic curves of the polymerization of RdlB showed a significant increase in ThT fluorescence, suggesting the formation of amyloid-like fibrils (Fig. 2C). Within the range of concentrations tested, the kinetics of RdlB fibril formation was concentration-dependent and a short lag time of ~1 hour was observed. This is consistent with the CD results described above showing a conformational change in RdlB upon vortexing. The lag time is interesting as chaplin peptides assemble rapidly without a detectable lag phase, although both systems are sensitive to agitation.

To assess the morphology of the aggregates formed, transmission electron microscopy (TEM) was used. Unlike samples of RdlA in which only a few irregular aggregates were observed (Fig. 2D), abundant fibrils were observed for RdlB (Fig. 2E). Notably, the RdlB fibrils strongly resemble the fibrillar structures observed after isolation of the rodlet layer with mechanical disruption. The typical fibrils formed by RdlB are highly uniform and of significant length, often exceeding 1 μm. Some very well aligned fibrils could be observed in a parallel pattern. Proteinase K digestion was used to investigate the stability of the RdlB fibrils. The bundles of thin, needle-like structures observed using TEM showed that the polymerization products of RdlB were proteinase K resistant (Fig. 2F), which implies that they have an amyloidial nature with little globular protein structure displayed on the fibril surface, consistent with structural data obtained by CD. Taken together, these data show that RdlB, but not RdlA, is capable of assembling into amyloid-like fibrils.

**X-ray diffraction demonstrates that RdlB fibrils have a cross-β structure.** The X-ray diffraction pattern of the partially aligned RdlB fibrils displays the features typical of a cross-β structure (Fig. 3). The sharp peak at a spacing of 4.7 ± 0.02 Å indicates the inter-strand distance within β-sheets, while the broad peak centered at 10.2 ± 0.1 Å represents the inter-sheet spacing within the fibril cross-section, confirming that the fibrils formed by RdlB are amyloid fibrils. The reflection at 30.9 ± 1.4 Å in the equatorial axis may arise from the arrangement of multiple protofilaments within the RdlB fibrils. This reflection could also indicate the length of a single β-strand consisting of residues involved in the fibril core. Given that the average distance between residues within a polypeptide chain is 3.8 Å, this observation suggests there might be a 9–10 residue β-aggregation-prone region in the RdlB sequence which directly constitutes the fibril core. It is plausible that the β-strand incorporates only part of the RdlB sequence, with some residues extending from the fibril core remaining disordered and flexible to a certain degree. Fibril stalks formed by chaplins also gave an axial inter-strand reflection at ~4.7 Å, similar to the X-ray diffraction pattern of RdlB fibrils, but lack equatorial reflections at 30.9 Å, suggesting that the alignment of RdlB fibrils is more ordered than in Chp fibrils.
RdlB has an amyloid-forming region near the N-terminus. Given the high similarity between the sequences of the two rodlin proteins, the difference in aggregation properties of these two proteins was surprising. The N-terminal 50 or so residues is the region of greatest difference between RdlA and RdlB (Fig. 1A), and residues 51–57 in both proteins was predicted to be prone to aggregation. Taking this into account, we first constructed a series of complementary and overlapping fragments of RdlB to narrow down the region(s) of RdlB responsible for amyloid assembly. We found that the N-terminal fragments residues 1–42 (Fig. 4A) and residues 1–58 (not shown) readily formed aggregates, whereas the complementary C-terminal fragments residues 43–105 (Fig. 4A) and residues 59–105 (not shown) did not, narrowing the amyloid fibril forming region to the N-terminal 42 residues. We then further screened truncations in RdlB by deleting regions located near the N-terminus and tested the aggregation propensity of the resulting proteins. Whilst ThT binding can vary between fibrils, this assay together with EM observation provides a good assessment of whether aggregation has occurred. We found that deleting the region between residues 17 and 42 (Δ17-42RdlB) prevented RdlB from assembling into amyloid fibrils. There was little detectable increase in fluorescence of the dye ThT, even after prolonged incubation of Δ17-42RdlB (Fig. 4A). In contrast, two mutants with smaller deletions within this same region, Δ17-29RdlB and Δ31-42RdlB, were still able to assemble into amyloid fibrils (Fig. S3). The mutant Δ51–57RdlB also formed fibrils (Fig. S3), confirming that residues 51–52, which are predicted to be aggregation prone, do not in fact contribute to aggregation of RdlB. Taken together, these results identify the region between residues 17–42 as critical for initiating the polymerization of RdlB.

To further dissect the region important for amyloid fibril formation, short synthetic peptides based on the sequence of the N-terminal region of RdlB were screened for their aggregation capability. The peptide RdlB11–27 (NGNGASQYFGNSMTTGN) and the peptide RdlB28–42 (MSPQMALIQGSFNKP) both showed a significant propensity for amyloid aggregation in a ThT fluorescence assay (Fig. 4B). The RdlB11–27 fibrils observed by TEM appeared to associate into tangled networks with some fibrils displaying a slight twist (Fig. 4C). Meanwhile, fibrils
formed by the peptide RdlB28–42, displayed a greater tendency to align in parallel clumps (Fig. 4D). The ThT results and EM examination suggest that the two peptides both contain residues that directly contribute to the amyloid formation propensity in full length RdlB, although the properties of the resulting fibrils differ. In contrast, the first 19 residues of RdlB (IGDDSGPVSANGNGASQYF) was not able to assemble into amyloid fibrils suggesting these residues are less important to fibril formation (Fig. 4B). Therefore, a 26 residue amyloid determining region was defined as residues 17–42 in the N-terminal of RdlB (Fig. 5A).

The propensity to form amyloid fibrils can be swapped between RdlA and RdlB. We then compared the sequences of the two rodlins within this 26 residue amyloid region at the residue level. Although the overall hydropathy plots of the N-terminal regions of the two rodlins are similar (Fig. 1B), RdlA possesses more charged residues compared to RdlB (Fig. 5A). In particular, RdlA has glutamate or aspartate residues at positions 16, 27 and 36, and these long charged side chains are generally considered to disfavor β-sheet stacking. In RdlB, the residues at the corresponding sites are serine, glutamine or asparagine, which are considered more favourable for β-sheet formation. We therefore constructed a RdlA mutant, named RdlA*, which contains the three site mutations E16Q, D27N and E36Q, corresponding to the differences between the wild-type RdlA and RdlB sequences. In parallel, we created the corresponding RdlB variant protein, RdlB*, in which the three putative amyloid-favoring residues were replaced by glutamate or aspartate residues, as found in the RdlA sequence. However, as these three changes in RdlB* did not completely remove amyloid forming ability in vitro (Fig. S4), we introduced an additional three charged residues into RdlB*, yielding RdlB− (Fig. 5).

The amyloid propensity was strongly diminished in RdlB−, and was comparable to that of RdlA, suggesting there is little accumulation of amyloid-like structure (Fig. 5B). Conversely, the RdlA* mutant gained the propensity to form amyloid-like structures, as deduced from the significant increase in ThT fluorescence. Even though the self-assembly of RdlA* revealed an extended lag time and a longer exponential phase compared to RdlB, TEM analysis confirmed the formation and presence of abundant fibrillar structures (Fig. 5C); the observed fibrils of RdlA* were relatively short and tended to clump together to form a large tangled structure, possibly reflecting the relatively high protein concentration used to obtain sufficient material for EM analysis. Taken together, these data highlight these three residues as crucial determinants for the different propensity of RdlA and RdlB to assemble into amyloid fibrils, namely residues at positions 16, 27 and 36.

The amyloid propensity of rodlins correlates with the presence of rodllets. Previous work indicates that both RdlA and RdlB are essential for rodllet assembly. Given that RdlB exhibits strong fibril forming capability in vitro, we created Streptomyces strains that express variants of RdlA or RdlB with altered amyloid-forming propensity. The rodllet layer, which is described as a sheet of ordered rods in a mosaic pattern, is typically observed on the outer surface of aerial mycelia and spore chains in S. coelicolor (Fig. 6A). In the ∆rdlB strain, the surface of the spores appeared smooth without a detectable fibrous structure, even though it is suggested to be covered with chaplin proteins in a fibrillar form (Fig. 6B). In agreement with earlier work, we first confirmed that the rodllet layer was restored in the ∆rdlB strain after reintroduction of the wild-type rdldB gene (Fig. 6C). In contrast, the introduction to the ∆rdlB strain of plasmid pIJ8630-Δ17-42rdlB, which expresses the RdlB mutant lacking amyloid fibril forming capability, failed to restore the formation of rodllets in vivo (Fig. 6D). This confirms that this 26 residue amyloid region in RdlB is crucial for formation of the rodllet layer. Also, no rodllets were detected on the
Figure 6. The propensity of rodlins to form amyloid fibrils correlates with the presence of rodlets. The outer surface of spores of the wild-type strain (A) is characterized by the rodlet layer. In contrast, no rodlets are detected on the surface of spores of the $\Delta rdLB$ strain (B). Introduction of the wild-type $rdLB$ gene (contained on plasmid pIJ8630-rdLB) in the $\Delta rdLB$ strain restored rodlet formation (C). No complementation is observed when pIJ8630-$\Delta 17-42rdLB$ (D) or pIJ8630-rdLB$^{−}$ (E) are introduced in the $\Delta rdLB$ mutant strain. (F) Rodlets are also formed by the introduction of plasmid pIJ8630-rdLA$^{*2}$ in the $\Delta rdLAB$ mutant. (G) Spore chains of the *S. coelicolor* wild-type strain as observed with scanning electron microscopy without platinum plasma coating. A transparent sheath-like structure is visible surrounding the separating spores. (H) Proposed model for the developmental transition of *S. coelicolor* aerial hyphae into chains of spores, which are enveloped by two amyloidal layers containing assembled chaplins (blue/purple) and rodlins (orange/green). Note that the rodlins are part of the outermost layer. The scale bar represents 100 nm (A–F) or 1 $\mu$m (G).
spore surface when the ΔrdlB strain was complemented with pIJ8630-rdlB—, which leads to the production of the RdlB— mutant protein with a diminished propensity to form amyloid fibrils (Fig. 6E). The secretion and correct localization of the RdlB— mutant along with RdlA were confirmed by MALDI-TOF mass spectrometry of spores collected from the complemented strain (Fig. S5A)32.

To see if the amyloid-promoting mutations in RdlA* were also functional in vivo, we introduced rdlA* in the ΔrdlAB strain that lacks the rodlet pattern (Fig. S6A). Given our previous observation that the absence of either rodlin gene affects the expression level of the remaining copy12, two copies of rdlA* were introduced to ensure sufficient production of the mutant RdlA* protein to potentially compensate for both RdlA and RdlB, and intriguingly, rodlets were evident on the spore surface of the complemented mutant (Fig. 6F). The rodlet pattern, in terms of both the coverage and the paired alignment of the fibrous structure, observed on the RdlA* expressing strain (Fig. 6F) closely resembled the rodlet layer of the wild-type strain (Fig. 6A), or the ΔrdlAB mutant complemented with the pIJ8630-rdlArdlB plasmid that restores both RdlA and RdlB (Fig. S6B). MALDI-TOF mass spectrometry of spores of the complemented strain indicated that RdlA* was correctly localized on the spore surface with RdlB absent in this strain (Fig. S5B). Taken together, these data show that the ability of RdlB or RdlA* to form amyloid fibrils plays an instrumental role in rodlet formation on the cell surface. Further, the rodlins may contribute to formation of the thin sheath-like structure that has been previously reported to encapsulate the spores9,40, and which we also detected using scanning electron microscopy (SEM) imaging without applying the typical plasma coating (Fig. 6G).

Discussion

Functional amyloids have been recognized in a wide range of prokaryotic and eukaryotic organisms. In streptomycetes, functional amyloids formed by the chaplin proteins confer surface hydrophobicity to aerial structures, which may facilitate spore dispersal. Here, we demonstrate that the rodlin protein RdlB, also assembles into amyloid fibrils, while RdlA shows no fibril-forming capability. The difference in amyloid propensity of the rodlin proteins arises from a few amino acid differences within the N-terminal regions of these two proteins. Notably, three amino acid changes were sufficient to change the non-assembling RdlA into a functional amyloid. Our data provide new insight into the regulation of amyloid assembly, and the organization of the surface layers enveloping aerial hyphae in streptomycetes.

RdlB forms functional amyloid fibrils. We purified rodlin proteins produced recombinantly in E. coli, and found them to be natively disordered. Such intrinsically disordered proteins have significant structural plasticity, allowing them to adopt different tertiary structures, depending on the environment and specific ligands available for binding33. RdlB consists primarily of random-coil structure in its native soluble state, similar to many other amyloidogenic polypeptides, yet it can readily form β-sheet structure and polymerize into amyloid fibrils. Amyloid aggregation is often initiated by a relatively small region of the polypeptide chain, referred to as an aggregation nucleus31. For RdlB, a 26 residue region located in the N-terminus was identified as the crucial amyloid-forming region (residues 17–42). Interestingly, this region was not detected by the β-sheet or amyloid formation propensity prediction algorithms TANGO, AGGRESCAN or FoldAmyloid, which identified only a 7 residue region (SVSIGL) corresponding to residues 51–57 of RdlB as highly prone to aggregation. Those algorithms tend to give weight to hydrophobic residues when locating aggregation-prone clusters in protein sequences so this difference is not unexpected nor without precedent. The 7 residue region identified by the algorithms was initially of interest because it contains the motif SVIGL, which is also present in the amyloid-determining region of CphF14. However, without these residues RdlB (A51-57RdlB) was still able to form fibrils efficiently (Fig. S3) and the C-terminal fragment of RdlB (i.e. 43-105RdlB) is incapable of forming fibrils. Further, the same region, with similar sequence, is also predicted to be aggregation prone in RdlA, but RdlA is unable to form fibrils. Thus we conclude that the aggregation-prone SVSIGL sequence does not provide the crucial driving force for RdlB amyloid formation, although this region may favor the association of RdlB monomers by hydrophobic stacking.

Two sub-regions within the 26 residue amyloidogenic region identified in RdlB, residues 17–29 (QYFGNSMTTGNMS) and residues 31–42 (QMALIQGSFNKP), were individually able to promote the fibril formation of the RdlB protein (Fig. S3). We propose that these two sub-regions can individually form β-strands and together adopt a β-hairpin structure, serving as the structural template for polymerization of full length RdlB. In comparison with RdlA, the lower density of charged residues was the main distinguishing feature of this region. This is supported by the observation that the RdlB variant RdlB—, in which six polar residues in this region were replaced by charged residues, no longer assembles into amyloid fibrils in vitro and in vivo. Conversely, the ability to form amyloid fibrils is conferred to RdlA*, a variant of RdlA in which three charged residues that strongly disfavor amyloid aggregation were replaced with the hydrogen bond favoring residues present in RdlB. Interestingly, with its newly acquired amyloid formation propensity, RdlA* was able to rescue the rodlet morphology in the ΔrdlAB mutant (Fig. 6F), which wild-type RdlA is unable to do13. Taken together, we have demonstrated a correlation between amyloid-forming propensity and the presence of a fibrous rodlet structure on mature spores, and identify RdlB as a functional amyloid involved in extracellular coating in S. coelicolor.

The role of RdlA in rodlet layer assembly remains elusive. Our in vitro and in vivo data clearly demonstrate that RdlB forms amyloid fibrils that are required for formation of the rodlet layer in the wild-type bacterium. However, the in vivo function of RdlB also requires RdlA. This is concluded from the fact that the rodlet layer is absent in the ΔrdlA strain, whether or not it is supplemented with an additional copy of the rdlB gene12. In other words, with a high amyloid-forming propensity, the protein RdlB itself cannot form the rodlet layer. Given that RdlA and RdlB are both abundantly present on the cell surface11, and that only RdlB appears to form amyloid fibrils, the question remains: how does RdlA contribute to rodlet formation? One classic example of the coordination between homologous proteins in extracellular functional amyloid assembly is the E. coli...
curli system, where CsgB functions as a nucleator for CsgA. However, such cooperation does not seem to apply to RdLA and RdLB, as no seeding effect was observed when RdLB fibrils seeds were added into a RdLA protein solution (Fig. S7A). Furthermore, the presence of RdLA did not interfere with the tendency of RdLB to form amyloid fibrils (Fig. S7B). However, due to the unknown dynamic stoichiometry of the two homologous rodlin proteins during aerial growth, it is hard to exclude the possibility that RdLA may be indispensable for achieving the appropriate assembly speed of RdLB fibrils. It is plausible that RdLA serves as a ‘regulator’ in vivo by interfering within the expression pathway to avoid the possible cytotoxicity or incorrect localization of amyloid oligomers due to the premature self-aggregation of RdLB, as an autoregulatory system is necessary for precise functional protein assembly. However, titration of RdLA and RdLB had no effect on the position of the NMR peaks (data not shown), which indicates that the two proteins do not interact directly in solution. We therefore assume that the coordination between the two rodlins in vivo may require participation of additional components present in the extracellular matrix. Indeed, as a smooth sheath-like outer layer could be observed for the strain lacking rodlins, it is suggested that other yet unidentified components contribute to the rodlet layer in addition to rodlins in their fibrillar form (see below).

Developing aerial structures are enveloped by two amyloid layers. Aerial growth in *S. coelicolor* is initiated by the accumulation of sufficient amounts of chaplins, in particular the small chaplins ChpE and ChpH, protein assembly. However, titration of RdLA and RdLB had no effect on the position of the NMR peaks (data not shown), which indicates that the two proteins do not interact directly in solution. We therefore assume that the coordination between the two rodlins in vivo may require participation of additional components present in the extracellular matrix. Indeed, as a smooth sheath-like outer layer could be observed for the strain lacking rodlins, it is suggested that other yet unidentified components contribute to the rodlet layer in addition to rodlins in their fibrillar form (see below).

**Materials and Methods**

**Strains and growth conditions.** *Streptomyces coelicolor* strains used in this work are listed in Table S1. All *S. coelicolor* strains were cultured at 30 °C on solid MS agar medium. *E. coli* DH5α was used for plasmid construction, while *E. coli* ET12567/pUZ8002 was used for conjugation as described.

**Plasmids.** Plasmids used for the *in vivo* complementation are listed in Table S1. All oligonucleotides used for constructing mutational variants of rodlins listing in Table S2 were obtained from Sangon Biotechnology (Shanghai, China). The mutants of *rdLB* were constructed by site-directed mutagenesis of an EcoRV-flanked 1.4 kb Plasmids. The biophysical characterization of the rodlin proteins from *S. coelicolor* reveals an unexpected divergence in the properties of the two highly homologous proteins. As we have shown here for the first time, RdLB forms amyloid fibrils that are essential for the structural integrity of the rodlet layer in *S. coelicolor*. This represents a significant development in terms of guiding further investigation of the assembly of the rodlet layer in *Streptomyces*. Besides the identification of a new functional amyloid, our results also indicate that *S. coelicolor*, as it recruits multiple amyloids for its extracellular matrix assembly, could be an important model for further study of how functional amyloids are exploited in organisms under intricate regulatory control. In addition, our results are also of importance in understanding the mechanism of amyloid fibril aggregation at the residue level and could provide valuable insight into the differences between pathogenic and functional amyloids.
fragment cloned in pBluescript II KS +, encompassing the putative promoter region, the coding sequencing of wild-type rdLB and a 748 bp sequence 3’ of the stop codon of rdLB. The rdLA* mutant was acquired through site-directed mutagenesis of a 1.5 kb EcoRV-flanked fragment in pBluescript II KS +, containing the putative promoter and coding sequence of rdLA, as well as 843 bp sequence 3’ of the stop codon of rdLA. The sequences of all mutations were confirmed by bi-directional sequencing. In all plasmids containing the mutant rdLA or rdLB genes, a 2.2 kb BamHI fragment encompassing the hygromycin resistance gene from plasmid pHP45Ωhyg was inserted. The resulting plasmids were cut with Kpnl/XbaI to excise fragments carrying the mutant rdLA or rdLB genes together with the hygromycin resistance gene, and which were subsequently ligated into pJ8630 cut with the same enzymes.

**Bioinformatics.** All input for bioinformatics analysis was according to the previously published sequences of the mature rodlin proteins (accession numbers: RdlA (AJ315950) and RdlB (AJ315951)). The rodlin sequences were characterized without the N-terminal signal peptide (residues 1–28) based on the reported cleavage sites. The Lalign server was employed to produce sequence alignments and identity calculations of rodlin with the default scoring matrix in which the open gap penalty and the extended gap penalty were −14 and −4, respectively. The hydrophobicity scores for rodlin were calculated by ProtScale via the ExPASy proteomics server. TANGO was used for predicting the secondary structure and the β aggregation propensity. The parameters for aggregation propensity calculation were 298 K, pH 7.0 and an ionic strength of 0.225 M (which is the ionic strength of a 100 mM sodium phosphate buffer at pH 7.0). The aggregation-prone regions were also predicted using the programs AGGRESCAN and FoldAmyloid.

**Protein Preparation.** Rodlins and mutant variants thereof were recombinantly expressed in *E. coli* without their 28 residue signal sequence. The rdLA and rdLB genes were obtained by PCR from genomic DNA of *Streptomyces coelicolor* and confirmed by DNA sequencing. Mutants of RdlB were generated using the primers listed in Table S2. Rodlins and mutational variants were cloned into the pET-28a expression vector. The recombinant rodlin proteins containing a His-tagged SMT3 protein followed by a ULP1 cleavage site at the N-terminus. Note that the recombinant rodlin has an extra serine residue at the N-terminus after cleavage of the fusion tag. Proteins were expressed in *E. coli* BL21 (DE3) cultured at 37 °C in 2YT medium supplemented with 200 μg/ml kanamycin till the OD600 of the medium reached 0.7. Protein production was induced with 0.7 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 18 °C for 12 hours. Cells were harvested at 4800 × g at 4 °C for 30 min by centrifugation. Cell pellets were resuspended in 100 mM sodium phosphate buffer, pH 7.0. After cell lysis and centrifugation, the lysate was purified by Ni-column (Chelating Sepharose Fast Flow). Fractions containing the target proteins underwent ULP1 digestion followed by a second Ni2+-affinity purification. After the SMT3 protein was removed, the rodlin was concentrated and loaded onto the Superdex G75 column (GE Healthcare) and purified by gel filtration chromatography, except truncated mutant 1-42RdlB which precipitated during the ULP1 digestion due to its strong tendency to rapidly form from fibrils. The buffer for gel filtration and final protein storage was 100 mM sodium phosphate buffer, pH 7.0. The purity of proteins was checked by SDS-PAGE, and the concentrations were determined by BCA assay. Proteins were stored at −80 °C in sodium phosphate buffer directly after flash-freezing.

**Peptide Preparation.** Synthetic peptides corresponding to the N-terminus sequence of RdlB were purchased from Sangon Biotechnology (Shanghai, China). The purity (>95%) and composition of the peptides was confirmed by high performance liquid chromatography along with electrospray mass spectrometry.

**Interfacial Tension.** The surface activity of both RdlA and RdlB were analysed by pendant drop tensiometry on a contact angle system (Contact Angle System OCA, DataphysicsCo., Germany). The rodlin solution was prepared in 10 mM sodium phosphate buffer pH 7.0 at a concentration of 100 μg/ml. As a control, ChpH was dissolved in the same buffer at the same concentration as RdlA and RdlB. For all measurements, the volumes of the droplets were between 18 μl and 21 μl. All measurements were performed at ambient temperature.

**NMR Spectroscopy.** NMR experiments were performed at 298 K on an Agilent DD2 600 MHz NMR spectrometer. NMR samples contained 400 μM 15N-labeled rodlin protein in 100 mM sodium phosphate buffer pH 7.0 with 5 mM CHAPS, 4 mM DL-Dithiothreitol, 0.5 mM EDTA and 10% (v/v) D2O. All data were processed with NMRPipe and analyzed with NMRView.

**Circular Dichroism Spectroscopy.** Far-UV CD spectra of rodlin solutions were acquired on a Chirascan plus CD spectrometer (Applied Photophysics Ltd., UK). Rodlins were diluted with 100 mM sodium phosphate buffer pH 7.0 to give a final concentration of 4 μM. The spectra were recorded between 195 nm and 280 nm at 25 °C in a 1 mm path length cuvette. The scan rate was 20 nm/min with steps of 0.5 nm. The spectra were generated based on 3 scans per sample and corrected by subtracting the reference spectrum of the sodium phosphate buffer. To determine the secondary structure changes of RdlA and RdlB induced by contact with the water-air interface, agitation was introduced by vortexing vigorously for 10 min. After vortexing, samples were immediately analysed as described above.

**Amyloid Fibril Formation.** The kinetics of amyloid fibril formation of RdlA, RdlB and the mutant variants thereof were monitored using Thioflavin T (Sigma-Aldrich). A 150 μl protein sample in 100 mM sodium phosphate buffer pH 7.0 was mixed with ThT (20 μM) in 96 well plates with an optical flat bottom (Coning, NY, USA) which were subsequently covered with PlateMax film (Coning, NY, USA) to prevent evaporation. The fluorescence was measured using a microplate reader (FLUOstar Omega, BMG, Germany) at 25 °C, with orbital shaking
at 700 rpm between readings. Samples were excited at 450 nm, after which the emission was measured at 480 nm. For each sample at least 4 replicates were measured.

**Proteinase K Digestion.** Proteinase K (Ameresco) was added to RdLB fibrils (80 μM) after 2 days incubation in 100 mM sodium phosphate buffer pH 7.0 to reach a working concentration of 0.5 mg/ml. CaCl₂ was added to give a final concentration of 10 mM. Digestion was carried out at room temperature overnight. Samples were then quickly frozen using liquid nitrogen and stored at −80 °C until further use.

**Transmission Electron Microscopy.** For TEM examination, samples of RdLA, RdLB and mutants thereof were prepared at desired concentrations after being filtered through a 0.22 μm filter. Samples were incubated overnight at room temperature with gentle rotation to allow fibril formation. TEM specimens were prepared by adding 8 μl of fibril solution to glow discharged carbon-coated copper grids for 1 min. The grids were then washed with distilled water and negatively stained with 2% uranyl acetate for 20 s followed by another wash. Grids were dried with filter paper wicking off excessive solution between each washing step. Samples were imaged using a Tecnai G2TF30 microscope (FEI Company, Eindhoven, The Netherlands) operating at 100 kV.

**X-ray fibril diffraction.** RdLB fibrils were prepared in 10 mM sodium phosphate buffer pH 7.0 at a peptide concentration of 100 μM and left overnight at room temperature on a rocking platform shaker set at low speed (Ratek Instruments, VIC, Australia). Fibrils were pelleted by centrifugation at 1500 × g for 10 min and then suspended in distilled water. Buffer salts were removed by repeating the centrifugation and resuspension steps three times. The pelleted RdLB fibrils were then resuspended in a small volume of distilled water at a final concentration of 2 mM. The dry stalk samples were prepared by suspending aliquots of fibril-containing solutions (8–10 μl) between two wax-filled capillary ends and allowed to dry in air at room temperature, as previously described 50.

X-ray diffraction images were collected at the Australian Synchrotron on the macromolecular crystallography beamline (MX2) at a wavelength of 0.954 Å 51. The sample to detector distance was 300 mm and the exposure time was 5 s. The 2-D diffraction images were converted into jpeg files using the program ADXV 52. The diffraction patterns were radially integrated in both axial and equatorial directions on either side of the reflection within a 50° sector in the azimuthal direction using the analysis program Clearer 53. The positions of the reflections and associated errors were automatically calculated by Clearer based on the positions of the maximal intensity on either side of the diffraction pattern. The average radial intensity was plotted against reciprocal spacing (1/d Å⁻¹) for both the axial and equatorial direction.

**Scanning Electron Microscopy.** Cryo-SEM was performed on a JEOL JSM-6700F. Six day old colonies of *S. coelicolor* were cut from solid MS agar medium and transferred onto the surface of an aluminum stub and frozen in nitrogen slush. Samples were subsequently transferred to the cryo-stage of the microscope. After the surface frost was sublimated (10 min at −90 °C), samples were coated with platinum plasma for 3–5 minutes at 7 mA and viewed at 3.0 kV.

**References**

1. Wessels, J. G. Hydrophobins: proteins that change the nature of the fungal surface. Adv. Microb. Physiol. 38, 1–45 (1997).
2. Beever, R. E. & Dempsey, G. P. Function of rodlets on the surface of fungal spores. Nature 272, 608–610 (1978).
3. Wösten, H., Schuren, F. & Wessels, J. Interfacial self-assembly of a hydrophobin into an amphiphilic protein membrane mediates fungal attachment to hydrophobic surfaces. *The EMBO Journal* 13, 5848 (1994).
4. Aimananda, V. et al. Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* 460, 1117–1121 (2009).
5. Larsen, P. et al. Amyloid adhesins are abundant in natural biofilms. *Environ. Microbiol.* 9, 3077–3090 (2007).
6. Smith, J. F., Knowles, T. P., Dobson, C. M., MacPhee, C. E. & Welland, M. E. Characterization of the nanoscale properties of individual amyloid fibrils. *Proc. Natl. Acad. Sci.* 103, 15806–15811 (2006).
7. Fowler, D. M., Koulav, A. V., Balch, W. E. & Kelly, J. W. Functional amyloid – from bacteria to humans. *Trends Biochem. Sci.* 32, 217–224 (2007).
8. Jorid, P. B. et al. Widespread abundance of functional bacterial amyloid in mycolata and other gram-positive bacteria. *Appl. Environ. Microbiol.* 75, 4101–4110 (2009).
9. Gebbink, M. F., Claessen, D., Bouma, B., Dijkhuizen, L. & Wösten, H. A. B. Amyloids—a functional coat for microorganisms. *Nat. Rev. Microbiol.* 3, 333–341 (2005).
10. Wildermuth, H., Wehrli, E. & Horne, R. W. The surface structure of spores and aerial mycelium in *Streptomyces coelicolor*. *J. Ultrastruct. Res.* 35, 168–180 (1971).
11. Claessen, D. et al. Two novel homologous proteins of *Streptomyces coelicolor* and *Streptomyces lividans* are involved in the formation of the rodlet layer and mediate attachment to a hydrophobic surface. *Mol. Microbiol.* 44, 1483–1492 (2002).
12. Claessen, D. et al. The formation of the rodlet layer is the result of the interplay between rodlins and chaplins. *Mol. Microbiol.* 53, 433–443 (2004).
13. Elliot, M. A. et al. The chaplins: a family of hydrophobic cell surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. *Genes Dev.* 17, 1727–1740 (2003).
14. Bokhove, M. et al. Chaplins of *Streptomyces coelicolor* self-assemble into two distinct functional amyloids. *J. Struct. Biol.* 184, 301–309 (2013).
15. Petrus, M. L. & Claessen, D. Pivotal roles for Streptomyces cell surface polymers in morphological differentiation, attachment and mycelial architecture. *Antonie Van Leeuwenhoek* 106, 127–139 (2014).
16. Claessen, D. et al. A novel class of secreted hydrophobic proteins is involved in aerial hyphae formation in *Streptomyces coelicolor* by forming amyloid-like fibrils. *Genes Dev.* 17, 1714–1726 (2003).
17. Claessen, D., de Jong, W., Dijkhuizen, L. & Wösten, H. A. B. Regulation of *Streptomyces* development: reach for the sky? *Trends Microbiol.* 14, 313–319 (2006).
18. Myers, E. W. & Miller, W. Optimal alignments in linear space. *Comput. Appl. Biosci.* 4, 11–17 (1988).
19. Röse, J. & Doolittle, R. F. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157, 105–132 (1982).
20. Fernández-Escamilla, A.-M., Rousseau, F., Schymkowitz, J. & Serrano, L. Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. *Nat. Biotechnol.* 22, 1302–1306 (2004).
21. Linding, R., Schymkowitz, J., Rousseau, F., Diella, F. & Serrano, L. A comparative study of the relationship between protein structure and 3-aggregation in globular and intrinsically disordered proteins. *J. Mol. Biol.* 342, 345–353 (2004).
Conchillo-Solé, O. et al. AGGRESCAN: a server for the prediction and evaluation of “hot spot” of aggregation in poly peptides. *BMC Bioinformatics* **8**, 65 (2007).

Garbuzynskiy, S. O., Lobanov, M. Y. & Galitskaya, O. V. FoldAmyloid: a method of prediction of amyloidogenic regions from protein sequence. *Bioinformatics* **26**, 326–332 (2010).

Sawyer, E. B., Claessen, D., Haas, M., Hurgobin, B. & Gras, S. L. The assembly of individual chaplin peptides from *Streptomyces coelicolor* into functional amyloid fibrils. *PLoS One* **6**, e18839 (2011).

Neumann, M. et al. Misfolded proteinase K–resistant hyperphosphorylated α-synuclein in aged transgenic mice with locomotor deterioration and in human α-synucleinopathies. *J. Clin. Invest.* **110**, 1429–1439 (2002).

Makin, O. S., Sikorski, P. & Serpell, L. C. Diffraction to study protein and peptide assemblies. *Curr. Opin. Chem. Biol.* **10**, 417–422 (2006).

Wong, T. Q., Binger, K. L., Howlett, G. J. & Griffin, M. D. Identification of an amyloid fibril forming peptide comprising residues 46–59 of apolipoprotein AI. *FEBS Lett.* **586**, 1754–1758 (2012).

Gras, S. L. et al. Functionalised amyloid fibrils for cell adhesion. *Biomaterials* **29**, 1553–1562 (2008).

Blake, C. & Serpell, L. Synchrotron X-ray studies suggest that the core of the transthyretin amyloid fibril is a continuous β-sheet helix. *Structure* **4**, 989–998 (1996).

Wang, W. & Hecht, M. H. Rationally designed mutations convert de novo amyloid-like fibrils into monomeric β-sheet proteins. *Proc. Natl. Acad. Sci.* **99**, 2760–2765 (2002).

Michelitsch, M. D. & Weissman, J. S. A census of glutamine/asparagine-rich regions: implications for their conserved function and the prediction of novel prions. *Proc. Natl. Acad. Sci.* **97**, 11910–11915 (2000).

de Jong, W., Vigenboom, E., Dijkhuizen, L., Wösten, H. A. B. & Claessen, D. SapB and the rodlins are required for development of *Streptomyces coelicolor* in high osmolarity media. *FEBS Microbiol. Lett.* **239**, 154–159 (2012).

Uversky, V. N., Oldfield, C. J. & Dunker, A. K. Intrinsically disordered proteins in human diseases: introducing the D2 concept. *Annu. Rev. Biophys.* **37**, 215–246 (2008).

Capstick, D. S., Jomaa, A., Hanke, C., Ortega, J. & Elliott, M. A. Dual amyloid domains promote differential functioning of the chaplin proteins during *Streptomyces* aerial morphogenesis. *Proc. Natl. Acad. Sci.* **108**, 9821–9826 (2011).

Chapman, M. R. et al. Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295**, 851–855 (2002).

Shu, Q. et al. The E. coli CsgA nucleator of curli assembly to β-sheet oligomers that alter the CsgA fibrillization mechanism. *Proc. Natl. Acad. Sci.* **109**, 6502–6507 (2012).

Greenwald, J. & Riek, R. Biology of amyloid: structure, function, and regulation. *Structure* **18**, 1244–1260 (2010).

de Jong, W., Wösten, H. A. B., Dijkhuizen, L. & Claessen, D. Attachment of *Streptomyces coelicolor* is mediated by amyloid fibrillae that are anchored to the cell surface via cellulose. *Mol. Microbiol.* **73**, 1128–1140 (2009).

Glaeser, A. M. & Hopwood, D. A. The fine structure of *Streptomyces coelicolor* (S. coelicolor): III. The walls of the mycelium and spores. *J. Biophys. Biochem. Cytol.* **10**, 505–516 (1961).

Williams, S., Bradshaw, R., Costerton, J. & Forge, A. Fine structure of the spore sheath of some *Streptomyces* species. *Microbiology* **72**, 249–258 (1972).

van Wezel, G. P. et al. ssgA is essential for sporulation of *Streptomyces coelicolor* A3 (2) and affects hyphal development by stimulating septum formation. *J. Bacteriol.* **182**, 5653–5662 (2000).

Kieser, T., Bibb, M., Buttner, M., Chater, K. & Hopwood, D. *The *Streptomyces* coelicolor* A3(2) and affects hyphal development by stimulating septum formation. *J. Bacteriol.* **182**, 5653–5662 (2000).

Kiess, T., Bibb, M., Buttner, M., Chater, K. & Hopwood, D. Practical *Streptomyces* genetics. (The John Innes Foundation, 2000).

Hanahan, D. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* **166**, 557–580 (1983).

Prentki, P. & Krisch, H. M. *In vitro* insertional mutagenesis with a selectable DNA fragment. *Gene* **29**, 303–313 (1984).

Gasteiger, E. et al. *The proteomics protocols handbook* (ed. Walker, J. M.) 571–607 (Springer, 2005).

Lorenzen, A. & Kennedy, S. W. A fluorescence-based protein assay for use with a microplate reader. *Anal. Biochem.* **214**, 346–348 (1993).

Delaglio, F. et al. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* **6**, 277–293 (1995).

Johnson, B. A. & Blevis, R. A. NMR View: A computer program for the visualization and analysis of NMR data. *J. Biomol. NMR* **4**, 603–614 (1994).

LeVine, H. [18] Quantification of β-sheet amyloid fibril structures with thioflavin T *Methods Enzymol.* **309**, 274–284 (1999).

Gras, S. L. & Squires, A. M. *Protein folding, misfolding, and disease* (eds Hill, A. F., Barnham, K. J., Bottomley, S. P. & Cappai, R.) 147–163 (Springer, 2011).

McPhillips, T. M. et al. Blu-Ice and the Distributed Control System: software for data acquisition and instrument control at macromolecular crystallography beamlines. *J. Synchrotron Radiat.* **9**, 401–409 (2002).

Arvai, A. ADXV - a program to display X-ray diffraction images http://www.scripps.edu/~arvai/adxv.html (2009).

Sumner Makin, O., Sikorski, P. & Serpell, L. C. CLEARER: a new tool for the analysis of X-ray fibre diffraction patterns and diffraction simulation from atomic structural models. *J. Appl. Crystallogr.* **40**, 966–972 (2007).

Acknowledgements

The X-ray diffraction data was collected on the macromolecular crystallography beamline (MX2) at the Australian Synchrotron, Victoria, Australia. The authors wish to acknowledge the MX2 beamline staff for their support. This work was supported by a Major International Collaborative Grant from the National Natural Science Foundation of China [311110003914] and by the Chinese Ministry of Science 973 Program [2013CB910700, 2012CB911000] to S.P.; and by the Netherlands Organization for Scientific Research (NWO) via a VIDI grant (12957) to D.C. SG is supported by The ARC Dairy Innovation Hub (IH121000005).

Author Contributions

W.Y., E.B.S., W.B.G., H.Z., S.L.G., D.C. and S.P. designed the research; S.L.G., D.C. and S.P. supervised the research; W.Y., J.W., E.B.S. and F.L. performed the experiments; all authors analyzed the data; W.Y., S.L.G. D.C. and S.P. wrote the paper; all authors revised the paper and approved the final version.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Yang, W. et al. The propensity of the bacterial rodlin protein RdlB to form amyloid fibrils determines its function in *Streptomyces coelicolor*. *Sci. Rep.* **7**, 42867; doi: 10.1038/srep42867 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
