

1 Identification of NLR-associated amyloid signaling motifs in filamentous bacteria

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19 **Abstract**

20
21 NLRs (Nod-like receptors) are intracellular receptors regulating immunity, symbiosis, non-self
22 recognition and programmed cell death in animals, plants and fungi. Several fungal NLRs employ
23 amyloid signaling motifs to activate downstream cell-death inducing proteins. Herein, we identify in
24 Archaea and Bacteria, short sequence motifs that occur in the same genomic context as fungal
25 amyloid signaling motifs. We identify 10 families of bacterial amyloid signaling sequences (we term
26 BASS), one of which (BASS3) is related to mammalian RHIM and fungal PP amyloid motifs. We find
27 that BASS motifs occur specifically in bacteria forming multicellular structures (mainly in
28 *Actinobacteria* and *Cyanobacteria*). We analyze experimentally a subset of these motifs and find that
29 they behave as prion forming domains when expressed in a fungal model. All tested bacterial motifs
30 also formed fibrils *in vitro*. We analyze by solid-state NMR and X-ray diffraction, the amyloid state of
31 a protein from *Streptomyces coelicolor* bearing the most common BASS1 motif and find that it forms
32 highly ordered non-polymorphic amyloid fibrils. This work expands the paradigm of amyloid signaling
33 to prokaryotes and underlies its relation to multicellularity.

34 Introduction

35 NLRs are intracellular receptors controlling innate immunity and host-symbiont interactions,
36 both in plants and animals (Jones et al. 2016; Mermigka et al. 2019). NLR proteins have a typical
37 tripartite architecture with an N-terminal effector domain, a central (NACHT or NB-ARC) nucleotide
38 binding and oligomerization domain and a C-terminal leucine-rich repeat (LRR) domain. Filamentous
39 fungi also display large and diverse repertoires of up to several hundreds NLR-related genes per
40 genome (Dyrka et al. 2014). These fungal NLR homologs however display WD40, ANK or TPR repeats
41 as ligand recognition domains instead of LRRs found in most plant and animals NLRs (Dyrka et al.
42 2014; Urbach and Ausubel 2017). Fungal NLRs were found to control programmed cell death
43 associated with non-self recognition in several fungal species (Chevanne et al. 2009; Choi et al. 2012;
44 Daskalov et al. 2015b; Espagne et al. 2002; Heller et al. 2018; Saupe et al. 1995). These proteins are
45 considered the fungal counterparts of plant and animal NLRs (Dyrka et al. 2014, Paoletti and Saupe
46 2009, Uehling et al. 2017). Remarkably some fungal NLRs employ an amyloid signaling mechanism to
47 engage cell death inducing effector proteins (Loquet and Saupe 2017). These NLRs display a short 20-
48 25 amino acid long N-terminal amyloid forming motifs upstream of the NACHT (or NB-ARC) domain
49 while their cognate effector protein displays a similar motif C-terminally. The amyloid fold of the
50 activated NLR receptor serves as a structural template to convert the homologous region in the
51 effector protein to a similar amyloid fold (Cai et al. 2014; Daskalov et al. 2015b; Daskalov et al. 2012).
52 This signaling mechanism based on the prion principle (self-propagation of protein polymers) is also
53 operating in several immune signaling cascades in mammals, albeit with a different underlying
54 structural basis (Cai et al. 2016). In fungi, NLR and their cognate regulated effector proteins are
55 typically encoded by adjacent genes and thus form functional gene pairs or clusters (Daskalov et al.
56 2015a; Daskalov et al. 2016; Daskalov et al. 2012). Several classes of fungal amyloid signaling motifs
57 have been described. HRAM motifs (for HET-s Related Amyloid Motifs) (Pfam PF11558) were
58 originally identified in the [Het-s] prion of *Podospora anserina* and have been subject to in-depth
59 functional and structural characterization (Daskalov et al. 2015a; Daskalov et al. 2015b; Wasmer et
60 al. 2008; Wasmer et al. 2010). HRAMs form a β -solenoid amyloid fold comprising 21 amino acid long
61 pseudo-repeats, with one repeat copy on the NLR and two copies on the effector protein. Another
62 family of signaling amyloids is defined by the sigma motif, involved in the propagation of the σ
63 cytoplasmic infectious element of *Nectria haematococca* (Daskalov et al. 2012; Graziani et al. 2004)
64 (Pfam PF17046). Finally, the PP-motif (for pseudo-palindrome) was described in *Chaetomium*
65 *globosum* and displays a sequence similarity with the mammalian RHIM amyloid sequence
66 scaffolding the RIP1K/RIP3K necrosome (Daskalov et al. 2016; Li et al. 2012; Sun et al. 2002). RHIM
67 and RHIM-related motifs were also identified in viruses and *Drosophila* (Kleino et al. 2017; Pham et
68 al. 2019). The similarity between the metazoan RHIM and fungal PP-motifs raised the possibility of an
69 ancient evolutionary origin of this mechanism of cell death-inducing amyloid signaling (Daskalov et
70 al. 2016; Kajava et al. 2014).

71 Several types of downstream effector protein domains activated by NLR-mediated amyloid
72 signaling were described (Daskalov et al. 2012; Dyrka et al. 2014) but the most common are part of a
73 family of membrane targeting proteins including the HeLo, HeLL (Helo-like) and SesA domains (Pfam
74 PF14479, PF17111 and PF17707 respectively). The HeLo domain forms a α -helical bundle with an N-
75 terminal hydrophobic helix and functions as a membrane targeting pore-forming domain (Greenwald
76 et al. 2010; Seuring et al. 2012). The fungal HeLo/Helo-like/SesA domain family shows homology
77 with the N-terminal helical cell death execution domain of the MLKL protein controlling mammalian

78 necroptosis and the RPW8 and Rx-N CC-domains regulating plant immune cell death (Daskalov et al.
79 2016; Murphy et al. 2013). The homology between the membrane-targeting domains suggests a
80 common evolutionary origin for these defense-related programmed cell death processes in plants,
81 animals and fungi. The plant Rx_N and the HET-S HeLo domain share a common mechanism of
82 membrane targeting based on the membrane insertion of a hydrophobic N-terminal α -helix (Seuring
83 et al. 2012; Wang et al. 2019). Other amyloid-controlled effector domains in fungi are predicted to
84 carry enzymatic activity, in particular the SesB α/β hydrolase domain and the PNP-UDP
85 phosphorylase domain (Daskalov et al. 2016; Daskalov et al. 2012). Noteworthy is the observation
86 that fungal NLRs are found in two distinct domain architectures, either as two-component gene
87 clusters involving amyloid signaling or more frequently in an “all-in-one” architecture with the
88 effector/NOD/repeat domains encoded as single polypeptide (Daskalov et al. 2012; Dyrka et al.
89 2014).

90 Several bacterial proteins have received a RHIM amyloid motif annotation in the Pfam or
91 InterPro databases (Rebsamen et al. 2009; Sun et al. 2002). We have analyzed the corresponding
92 protein sequences and found that the region annotated as RHIM occurs in a similar genomic context
93 as fungal amyloid signaling motifs. The motif occurs at the N-terminus of a NLR-like protein and at
94 the C-terminus of a putative effector protein encoded by an adjacent gene. Here, we systematically
95 explore in a genome mining approach the occurrence of putative amyloid signaling sequences in
96 bacterial and archaeal genomes and identify ten families of bacterial amyloid signaling sequences
97 (named here BASS1 to 10). The family designated as BASS3 corresponds to the RHIM-like sequence.
98 We find these motifs specifically in multicellular bacteria in particular in *Actinobacteria* and
99 *Cyanobacteria*. We show for motifs of the BASS1 and BASS3 family, prion formation in the *Podospora*
100 *anserina* fungal model and fibril formation *in vitro*. We use solid-state NMR and X-ray diffraction to
101 structurally characterize the BASS1 motif from *Streptomyces coelicolor* and find that it assembles into
102 highly ordered non-polymorphic amyloids as previously described for fungal amyloid signaling motifs.
103 We propose that NLR-associated BASS motifs are analogous to the amyloid prion motifs identified in
104 fungi and that this signaling mechanism is shared by filamentous fungi and filamentous bacteria.

105

106

107 **Results**

108 **RHIM-like motifs in Bacteria**

109 In the Pfam database, the majority of the sequences with a RHIM annotation are from
110 Metazoan or Metazoan viruses (El-Gebali et al. 2018; Kajava et al. 2014; Kleino et al. 2017; Li et al.
111 2012; Pham et al. 2019). A few hits (24/286) however occur in Bacteria. We examined these bacterial
112 RHIM-annotated proteins and found that ten of them are homologous proteins with the RHIM
113 annotation occurring C-terminally, downstream of \sim 100 amino acid long predicted α -helical domain
114 that we termed Bell (for bacterial domain analogous to Hell, based on shared features with the
115 fungal HeLo-related domains, see below). In the actinobacterium strain *Saccharothrix* sp. ALI-22-I,
116 the gene adjacent to the gene encoding the Bell/RHIM protein (ONI86675.1) encodes a protein with
117 NLR architecture (NB-ARC domain and TPR repeats) (ONI86674.1). Remarkably, the N-terminal region
118 of this NB-ARC/TPR protein shows sequence homology to the C-terminal RHIM-annotated region of

119 the Bell-domain protein (Fig. 1A and B). The ONI86675.1/ ONI86674.1 bacterial gene pair from
120 *Saccharothrix* therefore displays the same features as effector/NLR gene pairs described in
121 filamentous fungi (Daskalov et al. 2015b; Daskalov et al. 2012). This similarity prompted us to analyze
122 further Bell domain proteins in bacterial genomes.

123

124 **Bell-domain occurs in multicellular Bacteria and Archaea**

125 The predicted globular Bell domain of the ONI86675.1 protein from *Saccharothrix* was used
126 as query in HMMER searches to recover homologous proteins. We found that the Bell-domain occurs
127 mainly in short ~120-140 amino acid long proteins and in more rare instances as N-terminal domain
128 of proteins with a NLR domain architecture (as previously described for HeLo, Helo-like and SesA
129 domains) (Dyrka et al. 2014). Figure 1C gives an alignment of Bell domain proteins from
130 phylogenetically diverse prokaryotes including a sequence from an Archaea (*Methanothrix*
131 *soehngeni*). The N-terminal region of the domain was predicted to correspond to an hydrophobic α -
132 helix and a HMM-signature of the domain shows frequent occurrence of a negatively charged residue
133 in position 2 or 3, a feature that is common to fungal HeLo, HeLL domains, mammalian MLKL and
134 plant RPW8 and Rx_N domains (Fig.1D, E and F) (Adachi et al. 2019; Daskalov et al. 2016). Using
135 contact prediction maps based on evolutionary co-variance, the Bell domain of *Saccharothrix* sp. ALI-
136 22-I ONI86675.1 was modelled as a five-helix bundle (Fig.1 F). Secondary structure and fold
137 prediction for the different proteins presented in the alignment in Fig. 1C resulted in five-helix
138 bundles for all proteins although in some cases with different topologies (Fig. S1).

139 We analyzed the phylogenetic distribution of the Bell domain in prokaryotic genomes using
140 the genome-based phylogeny developed by Parks et al. which has been shown to be more accurate
141 than the NCBI taxonomy (Parks et al. 2018). The domain shows a heterogeneous phylogenetic
142 distribution (Table 1, Table2, Table S1). It is most frequent in the *Cyanobacteriota*, *Actinobacteriota*
143 and *Chloroflexota* but very rare or absent in other bacterial phyla. Within these phyla, the domain is
144 specifically present in genera containing multicellular species (described in Bergey's Manual of
145 Systematic Bacteriology) (Table S1). In the *Actinobacteria* class, the domain is frequent in families
146 encompassing filamentous species (*Streptomycetaceae*, *Micronosporaceae*, *Pseudonocardiaceae*,
147 *Streptosporangiaceae*, *Frankiaceae* and *Corynebacteriaceae*) but absent in unicellulars such as the
148 *Bifidobacteriaceae* and *Propionibacteriaceae* (Table 2, Table S1). The same is true at higher
149 phylogenetic resolution, within the *Corynebacteriaceae* family for instance, the domain is highly
150 represented only in the *Nocardia* and *Dietzia* genera encompassing filamentous species (Table S1). In
151 the *Cyanobacteriota*, the domain is highly represented in the *Nostocaceae* family and present in
152 multicellular genera like *Nostoc*, *Calothrix*, *Microcystis* or *Fischerella* but absent in the *Cyanobiaceae*
153 family encompassing unicellular genera like *Prochlorococcus* and *Synechococcus* (Table 2, Table S1)
154 (Shih et al. 2013). In the *Chloroflexota*, the domain is specific to the *Chloroflexus* genus encompassing
155 filamentous species like *Chloroflexus auranticus* and *Chloroflexus aggregans*. Similarly, in Archaea,
156 the Bell domain is found exclusively in the *Methanosarcinales* order, in particular in the genus
157 *Methanosarcina* comprising multicellular species forming cell aggregates like *Methanosarcina*
158 *acetivorans* (Table S1).

159 The phylogenetic distribution of the Bell domain is mirrored by the distribution of NLR-like
160 architecture proteins (here a NB-ARC or NACHT domain associated with TPR or WD repeats) (Table 1,

161 Table S1). The NLR-like domain architecture is frequent in phylogenetic groups in which the Bell
162 domain is represented (for instance in *Actinobacteriota* and *Cyanobacteriota*) but rare or absent in
163 groups lacking Bell domains (for instance *Proteobacteria* or *Firmicutes*) (Table 1). The same is true at
164 higher phylogenetic resolution (Table 2, Table S1). For instance, in *Cyanobacteriota*, the NLR-like
165 architecture is not found in *Cyanobiaceae* but is prevalent in *Nostocaceae*. Similarly, in Archaea, in
166 *Methanosarcina*, the NLR-like architecture is abundant but rare or absent in other genera. The
167 phylogenetic distributions of NLR-like architectures and Bell domain do not fully overlap though, as
168 for instance the Bell domain is not found in the phylum *Myxococcota* where NLR-like architectures
169 are present. Conversely, the genus *Janthinobacterium* contains many Bell domains but lacks NLR-like
170 architecture proteins (Table S1). Specific occurrence in *Cyanobacteria* and *Actinobacteria* of NLR-type
171 architectures (NB-ARC/NACHT with TPR/WD repeats) was reported previously (Asplund-Samuelsson
172 et al. 2012; Koonin and Aravind 2002; Urbach and Ausubel 2017).

173 Overall, the Bell domain was found in roughly 1% of the analyzed bacterial genomes (1237 of
174 113 324) and 10% of the archaeal genomes (94/1183). The domain appears to be typical of Bacteria
175 and Archaea with a multicellular organization either in filamentous or aggregate forming species. In
176 addition, the phylogenetic distribution of the Bell domain proteins is largely mirrored by the
177 phylogenetic distribution of proteins with NLR-like architectures.

178

179 **Prediction of amyloid signaling sequence motifs in bacterial genomes**

180 Amyloid signaling motifs in fungi occur in adjacent gene pairs (Daskalov et al. 2012). Based on
181 the observed Bell-RHIM/NLR-like gene pairs (Fig. 1A), we hypothesized that an amyloid signaling
182 mechanism akin to the one described in filamentous fungi might also operate in some bacterial
183 species. To identify potential amyloid signaling motifs in bacteria we extended a genome mining
184 strategy that we have previously developed to identify amyloid signaling motifs in fungi (Daskalov et
185 al. 2012, 2016). We screened for sequence motifs common to C-termini of Bell-domain proteins and
186 N-termini of NLR-like proteins, which are encoded by adjacent (or closely linked) genes. Specifically,
187 we generated a data set of 1816 non-redundant C-termini of prokaryotic Bell-domain proteins,
188 searched for conserved gapless motifs using MEME and identified 117 motifs. For each of the
189 conserved motifs, we created a profile HMM (pHMM) signature generalizing the motif as described
190 in detail in the Methods section. Next, we identified all NB-ARC and NACHT domain proteins encoded
191 by genes in the genomic vicinity (within 5 kbp) of the genes encoding our set of Bell-domain proteins.
192 Then, we analyzed N-termini of of these NLR-like proteins for occurrences of the pHMM-signatures
193 of Bell-associated C-terminal motifs. We found that half of the motifs identified at least one gene pair
194 encoding a Bell-domain protein and a NACHT/NB-ARC protein. To be more stringent in our analyses,
195 we focused further investigations only on motifs that identified at least 5 non-redundant gene pairs
196 which reduced our set to 29 motifs (comprising up to 75 non-redundant gene pairs per motif) (Table
197 S2). The motifs were clustered based on the overlap between the matched gene pairs (Fig. S2). When
198 at least half of the gene pairs identified for a given motif were also matched by another motif, the
199 two motifs were considered related and were grouped in the same motif cluster. The procedure led
200 to identification of nine clusters (some of which comprised in fact a single motif) (Fig. S2). In each
201 cluster, we selected a representative motif on the basis of the number of underlying sequences (Fig.
202 2). The motifs families defined by each cluster were termed BASS families (for bacterial amyloid

203 signaling sequence) (Fig. 2, Fig. S2). As expected, we recovered the already identified RHIM-related
204 motif represented by 19 gene pairs (BASS3). We also carried out an inverse screen in which we
205 looked for conserved gapless motifs in N-terminal sequences of the NB-ARC/NACHT protein set,
206 generated pHMM signatures for the extracted motifs, and searched for their occurrences in C-
207 termini of the Bell proteins. We retained 12 motifs identifying at least five non-redundant gene pairs
208 and clustered these motifs within the previously defined clusters. While eleven grouped to one of the
209 nine previously identified families (Fig. S2), one novel motif did not, and is thus defining an additional
210 family, we term BASS10.

211 We complemented the search with an alternative procedure consisting of the local pairwise
212 alignment of adjacent Bell C-termini and NLR N-termini. Highly conserved pairs of a length of at least
213 15 amino acids were found in 283 gene pairs including 44 pairs not matched previously with the
214 BASS1 to 10 motifs. In this number were two pairs from archaeal Methanosarcinales species (Table
215 S2).

216 We analyzed the distance and the relative orientation between the Bell encoding gene and
217 the adjacent NLR-encoding gene in our set of 346 matching gene pairs (Table S2). Genes were
218 collinear in 91.3% of the cases. In most collinear gene pairs, the distance between the genes was very
219 small, in 57% of the cases the distance between the two ORFs was 10 bp or less and in 42% of the
220 cases the two ORFs overlapped. In one extreme case, in *Spirosome oryzae*, the Bell domain encoding
221 gene and the NLR-encoding gene overlapped by 57 bp. Gene overlaps are not uncommon in
222 prokaryotes, roughly 20% of the collinear gene pairs overlap, (Fukuda et al. 2003). It is likely that the
223 Bell and NLR-encoding genes pairs with short intergenic distances or gene overlaps are transcribed as
224 polycistronic messenger RNAs reflecting the possible functional link and/or co-regulation between
225 the genes in the pair.

226

227 Paired BASS motifs occur in multicellular species

228 Mirroring the distribution of Bell-domains and NLRs, the vast majority of BASS-motifs pairs
229 occur in *Actinobacteria* and *Cyanobacteria* (Table S1) with the filamentous genus *Streptomyces*
230 dominating the data set with 105 pairs (Table S2). Some motifs appear specific to *Actinobacteria*
231 (BASS 1, 2) others to *Cyanobacteria* (BASS 7, 8, 9, 10), others occur in both classes (BASS 3, 4, 5, 6),
232 (Table S2). All actinobacterial hits occur in filamentous species with the possible exception of
233 *Arthrobacter sp. Rue61a*. No direct information on the growth morphology for this specific strain
234 could be recovered but the corresponding genus is described as unicellular. The hits in *Cyanobacteria*
235 correspond essentially to multicellular filamentous species (*Nostoc*, *Pseudoanabaena*, *Calothrix*,
236 *Aulosira*, *Tolothrix*, *Leptolyngbya* genera). Some species with hits (*Microcystis aeruginosa* and
237 *Crocospaera watsonii*) are described as unicellular (non-filamentous) *Cyanobacteria* but are also
238 found in multicellular aggregates. One hit was found in *Cyanothece sp. PCC 7424* which is a
239 unicellular strain reported to form large amounts of mucilage, as well as in *Chamaesiphon minutus*
240 PCC 6605 a unicellular species forming epiphytic colonies on aquatic plants. *Cyanothece*, *Microcystis*,
241 *Crocospaera* and *Chamaesiphon* are however distinct from the main group of unicellular
242 *Cyanobacteria* (*Prochlorococcus*, *Synechococcus*) in terms of phylogenetic position and abundance of
243 secondary metabolism clusters and homologs of *AmiC* involved in multicellular growth (Shih et al.
244 2013). The remaining gene pairs occur in *Archaea*, *Bacteroidetes* and *Chloroflexi*, in multicellular

245 species, which are either filamentous (like the Chloroflexi *Ktedonobacter racemifer*) or existing as
246 cellular aggregates (like the *Archaea Methanosarcinales* species). We conclude that matching BASS-
247 motif pairs occur with a wide phylogenetic distribution but in the vast majority of cases in
248 multicellular strains and genera.

249

250 **Sequence analysis of BASS motifs**

251 BASS motifs were typically around 25 amino acid in length (Fig. 2). All motifs have a predicted
252 propensity for β -sheet formation (of note is the fact that this criterion was not used for their
253 identification). The sequence logos of the motifs show conservation of G, N and Q residues as well as
254 charged residues and conserved patterns of hydrophobic residues. In comparison to the total of
255 Uniprot entries, the motifs are enriched for Q, N, G, V and I and depleted in K, P and L (Table S3).
256 Kajava and co-workers have reported a similar bias in amino acid composition of β -helical proteins
257 with enrichment in N, V and G and depletion in P and L (Baxa et al. 2006). Fungal amyloid motifs of
258 the HRAM, sigma and PP family share a similar bias in composition, residue conservation and length
259 (Daskalov et al. 2012) (Table S3).

260 Algorithms predicting amyloid propensity derived from the analysis of pathological amyloids
261 generally perform poorly when run on fungal functional amyloid motifs (Ahmed and Kajava 2013). In
262 contrast, the ArchCandy program predicting propensity to form β -arch structures shows good
263 performance for pathological amyloids but also in the case of fungal functional amyloid motifs
264 (Ahmed et al. 2014). We thus evaluated the amyloid propensity of BASS motif containing proteins
265 with ArchCandy and found that their amyloid propensity scores were comparable to those calculated
266 for a selection of validated fungal amyloid motifs and human RHIM-motifs (Fig. S3) (BASS1 and BASS3
267 scored below the recommended stringent score threshold (0.575) but this was also the case for an
268 HRAM5 fungal prion motif (Daskalov et al. 2015a)). In each case, the high scoring region matched the
269 position of the identified BASS motif. Based on this analysis, the identified bacterial motifs are
270 predicted to have amyloid forming propensity (note again that they were not selected on this
271 criterion).

272 The fungal HRAM motifs exemplified by the HET-s prion-forming domain present a two-fold
273 pseudorepeat of the motif in the effector protein (Daskalov et al. 2015b; Ritter et al. 2005). The two
274 pseudorepeats are separated by a variable flexible glycine and proline -rich loop of 6-15 amino acids
275 in length (Daskalov et al. 2015a; Wasmer et al. 2008). We find that some BASS motifs also occur as
276 pseudorepeats. In proteins pairs of different *Streptomyces* species, BASS1 and 6 motifs are present in
277 the Bell-domain protein as two (or three) pseudorepeats separated by a variable proline-rich region
278 and as a single repeat in the corresponding NLR (Fig. S4). These motifs thus are similar in this respect
279 to fungal HRAM motifs.

280 Fungal amyloid signaling motifs can be associated to other effector domains not related to
281 the HeLo-family (HeLo, Helo-like, sesA) but equally encoded by NLR/effector gene pairs (Daskalov et
282 al. 2016; Daskalov et al. 2012; Dyrka et al. 2014). These domains include α/β -hydrolase and PNP-UDP
283 phosphorylase domains. We wondered therefore whether BASS motifs could also be associated to
284 domains distinct from Bell. Thus, we analyzed the occurrence of identified BASS motifs in gene pairs
285 encoding a NLR and a second protein, for which the motif is found in the C-terminal region next to a

286 domain distinct from Bell. At least four such situations were identified with BASS motifs associated to
287 the following domains : a α/β -hydrolase domain, the TIR2 domain, the CHAT metacaspase domain
288 and a guanylate cyclase domain (Fig. S5). All identified examples were found in multicellular species.
289 Bacterial genomes contain genes in which the same effector domains are found in an “all-in-one”
290 association, where the effector domain is directly associated to the NOD and repeat domains of the
291 NLR-like protein, as describe previously for fungal NLRs (Daskalov et al. 2012). While some of these
292 domains have rather general functions, it is of interest to note that all these domains (α/β -hydrolase,
293 TIR2, CHAT and guanylate cyclase) are to various extends documented to be involved in immune and
294 programmed cell death pathways (Daskalov et al. 2016; Freihat et al. 2019; Koonin and Aravind 2002;
295 Nimma et al. 2017; O'Neill and Bowie 2007; Xue et al. 2012).

296

297 **BASS1 and BASS3 from selected bacterial species behave as prion-forming domains when** 298 **expressed in *Podospora***

299 NLR-associated fungal amyloid motifs were initially identified as prion-forming domains (Daskalov et
300 al. 2012). In order to determine if the BASS motifs could behave similarly, we expressed selected
301 BASS motifs in *P. anserina*. used to analyze prion propagation of both homologous and heterologous
302 amyloid signaling motifs (Benkemoun et al. 2011; Daskalov et al. 2015b; Daskalov et al. 2016). It is a
303 valuable alternate model to yeast which was also extensively used to document prion properties of
304 heterologous sequences. In *P. anserina* prion propagation and transmission are easy to monitor
305 because of the syncytial structure of the mycelium and because strains spontaneously fuse and mix
306 their cytoplasmic content when confronted (Benkemoun et al. 2006). We chose a motif
307 corresponding to the most populated family (BASS 1) from the model species *Streptomyces coelicolor*
308 A3(2) and three phylogenetically diverse BASS3 (RHIM-like motifs) from *Actinobacteria* species
309 *Streptomyces atratus* and *Nocardia fusca* and from the *Cyanobacteria* species *Nostoc punctiforme*
310 (Fig.2, Fig.S6, Table 3). The three selected BASS 3 sequences were diverse and share <50% identity
311 between species (Fig.S6). We expressed both the Bell-associated BASS and the corresponding NLR-
312 associated BASS (except for *N. fusca* proteins for which only the Bell-associated sequence was
313 studied) (Table 3). The different BASS motifs were fused with either GFP (in N-terminus) or RFP (in C-
314 terminus) and expressed under a strong constitutive promotor in *P. anserina*. All fusion proteins
315 showed bistability, subsisting initially in a diffuse cytoplasmic state that could convert over time to
316 discrete cytoplasmic dots as previously described for fungal prion-forming domains (Table 4, Fig.3)
317 (Balguerie et al. 2003; Benkemoun et al. 2011; Coustou-Linares et al. 2001; Daskalov et al. 2015b;
318 Daskalov et al. 2016). By analogy with fungal prions, we denote the diffuse state [b*] and the
319 aggregated state [b]. Transition from the diffuse [b*] to the aggregated [b] state could occur either
320 spontaneously or be induced by cytoplasmic contact with a strain expressing the fusion protein in [b]
321 state (Fig.4). We conclude that all tested BASS motifs direct infectious propagation of the aggregated
322 state and thus behave as prion-forming domains in this fungal model.

323

324 **NLR-side BASS induce prion conversion of Bell-side BASS**

325 If BASS motifs are analogous to fungal amyloid signaling motifs, it is expected that the NLR-side and
326 Bell-side BASS co-aggregate and that an aggregated NLR-side BASS is able to convert the matching

327 Bell-side BASS to the prion state (Daskalov et al. 2015b). To test whether BASS motif pairs could co-
328 aggregate, we co-expressed each motif pair in the same fungal strain. We found that all tested pairs
329 co-localize in dots although in some cases the co-localization was not complete (Fig. 5, Fig. S7). Then,
330 we tested whether the NLR-side BASS in the [b] prion state could induce prion aggregation of the
331 corresponding Bell-side BASS. Strains expressing RFP-fused Bell-side motifs in the non-prion [b*]
332 state were confronted with strains expressing the corresponding GFP-fused NLR-side motifs in the
333 [b] prion state. In all tested cases, we found that the NLR-side BASS efficiently converts the Bell-side
334 BASS to the prion state (Fig. 6). The level of amino acid identity within cross-seeding BASS motif pairs
335 is in the range of 43-56% (Fig. 2, Fig. S6) and comparable to the level of identity leading to prion
336 cross-seeding of fungal amyloid signaling motifs (Benkemoun et al. 2011; Daskalov et al. 2015b;
337 Daskalov et al. 2016). When expressed in this fungal model, tested BASS are functionally analogous
338 to fungal amyloid signal motifs in the sense that the NLR-side motif is able to interact with the Bell-
339 side motif and to induce its prion aggregation.

340

341 **BASS1 and BASS3 form fibrils *in vitro***

342 To verify the prediction that the identified bacterial motifs correspond to amyloid forming
343 sequences, we produced the selected Bell-side BASS1 motif (*Streptomyces coelicolor* A3(2)) and the
344 three BASS3 motifs (*Streptomyces atratus*, *Nocardia fusca* and *Nostoc punctiforme*) in recombinant
345 form and analyzed their ability to form fibrils *in vitro*. The proteins encompassing the motifs were
346 expressed as inclusion bodies, purified under denaturing conditions in 8M urea. Upon dilution of the
347 denaturant, all constructs led to the formation of fibrillar aggregates, either as dispersed fibrils or as
348 laterally associated large bundles resembling those formed by HET-s(218-289) (Balguerie et al. 2003;
349 Sabate et al. 2007). Fibril width was in the range of 5-7 nm comparable to the previously identified
350 fungal signaling motifs (Fig. 6), (Daskalov et al. 2015b; Daskalov et al. 2016; Sabate et al. 2007). We
351 conclude that the selected BASS motifs spontaneously assemble into fibrils *in vitro*.

352 For the *Streptomyces coelicolor* A3(2) BASS1 motif (CAB66307.1, residue 38 to 139),
353 belonging to the most commonly occurring BASS family, we further analyzed structural properties of
354 the *in vitro* fibrils (Fig. 7A). We employed X-ray fiber diffraction to examine the presence of a cross- β
355 architecture in the sample. We observed an intense ring at 4.7 Å, and a weak ring at 10 Å (Fig. 7B),
356 this pattern being characteristic for a cross- β structure corresponding to the inter-strand and inter-
357 sheet spacing respectively, typically observed in amyloid fibrils (Sunde et al. 1997). Next, we
358 produced a BASS1 sample isotopically and uniformly ^{13}C labeled to carry out solid-state NMR
359 analysis. Cross-polarization ^{13}C experiment (Fig. 7C) revealed a well-resolved spectrum, implying a
360 high structural order at the local level (Loquet et al. 2018a). In line with the X-ray diffraction analysis,
361 solid-state NMR ^{13}C chemical shifts of BASS1 indicate a protein conformation rich in β -sheet
362 secondary structure, illustrated with a high field effect of the carbonyl region indicative of β -sheet
363 structure (Wang and Jardetzky 2002). Taken together, these analyses show that this BASS-motif
364 forms β -sheet-rich amyloid fibrils, with NMR features highly comparable to amyloid fibrils of the
365 fungal HET-s(218-289) (Ritter et al. 2005; Siemer et al. 2005).

366

367 **Discussion**

368 Amyloids have initially been identified in the context of human protein-deposition diseases
369 and correspond to protein aggregates with a cross- β structure (Riek and Eisenberg 2016). The
370 nucleated-polymerization process governing their assembly allows some amyloids to propagate their
371 conformational state as prions (Colby and Prusiner 2011; Wickner et al. 2016). The amyloid fold also
372 plays a variety of functional roles (Loquet et al. 2018b; Otzen and Riek 2019). In particular, functional
373 amyloids have been found to be involved in signal transduction cascades controlling programmed cell
374 death processes. In mammals, the RHIM motif controls assembly of the RIPK1/RIPK3 complex in the
375 necroptosis pathway (Li et al. 2012; Mompean et al. 2018). In fungi, amyloid motifs control a signal
376 transduction mechanism based on transmission of an amyloid fold from an NLR protein to
377 downstream cell-death execution proteins (Daskalov et al. 2015b; Daskalov et al. 2016). In the
378 prokaryotic reign, functional amyloids have been found to be involved in biofilm formation,
379 development and virulence (Erskine et al. 2018; Rouse et al. 2018; Van Gerven et al. 2018). In
380 addition, prion formation has been reported in bacterial model systems (Giraldo et al. 2011;
381 Shahnawaz et al. 2017; Wang et al. 2017). We show here that phylogenetically diverse bacterial
382 lineages including *Actinobacteria* and *Cyanobacteria* display fungal-like amyloid signaling motifs and
383 thus suggest that NLR-associated amyloid signaling is also present in these multicellular bacterial
384 lineages.

385

386 **Diversity of amyloid signaling motifs in Bacteria**

387 Several families of amyloid signaling motifs were described in fungi and remarkably, PP, one
388 such family appears to be related to the animal RHIM amyloid motifs (Daskalov et al. 2016; Li et al.
389 2012). We now find that a motif similar to RHIM/PP and occurring in the same domain architectures
390 as the fungal PP-motif also exists in Bacteria. Although, the motif consensus is different for RHIM, PP
391 and BASS3 motifs, all share the central G- ϕ -Q- ϕ -G signature (Fig. S6). This signature corresponds to
392 the central core of the RHIM amyloid structure with the tight interdigitation of two such motifs in
393 one β -strand layer (Mompean et al. 2018). The extension of the presence of this amyloid motif to
394 prokaryotic lineages supports the hypothesis of long-term conservation of this motif for amyloid
395 signaling purposes from bacteria, to metazoan and fungi. However, due to the moderate sequence
396 similarity between the motifs the possibility of a convergent evolution towards the amyloid signaling
397 function cannot be ruled out.

398 We have also identified nine other bacterial amyloid motif families. This finding suggests an
399 extensive diversification of this type of signaling domains in prokaryotes. In fungi, amyloid signaling
400 motifs were also found to be diverse with so far three main families described (HRAM, PP and sigma)
401 (Daskalov et al. 2015a; Daskalov et al. 2012). Diversity of such motifs in bacterial lineages appears
402 even greater than in fungi, which might be expected considering the larger phylogenetic breadth of
403 the bacterial lineages compared to fungi (Hug et al. 2016). Diversity of bacterial motifs almost
404 certainly exceed the 10 families described herein, considering that we restricted the analysis to
405 motifs recovering at least 5 non-redundant matching gene pairs. Except for the RHIM/PP-motifs
406 there is no obvious sequence homology between bacterial and fungal motifs, which is not surprising
407 considering that some of the BASS motifs appear specific for a given bacterial phylum. In spite of the
408 lack of direct sequence homology, bacterial and fungal motifs have common features, they show a
409 similar length (typical 20-25 amino acids) and amino acid composition biases, apparently typical of

410 proteins forming β -arch structures (Baxa et al. 2006). In addition, some bacterial motifs appear as
411 double (or triple) pseudo-repeats as described in the case of fungal HRAMs (Daskalov et al. 2015a;
412 Ritter et al. 2005).

413

414 **BASS form prions**

415 When expressed in *Podospora anserina*, selected BASS motifs behaved as prion-forming domains and
416 thus were functionally analogous to previously characterized fungal prion signaling motifs. In other
417 words, they are capable, *in vivo*, in this heterologous setting, to form aggregates and to propagate
418 this aggregation state as prions. In addition, we find that the NLR-side and Bell-side of matching
419 motifs are able to co-aggregate and that, NLR-side motifs convert Bell-side motifs to the prion state.
420 These results are consistent with the proposition that the motifs functionally interact and that the
421 NLR-side motif serves as a template for the transconformation of the Bell-side motif as shown for the
422 fungal prion signaling motifs (Daskalov et al. 2015b). We find that the same motifs form fibrils *in*
423 *vitro*. In case of the BASS1 motif of *Streptomyces coelicolor*, X-ray diffraction and solid-state NMR
424 analyses indicate the formation of a highly-ordered cross- β structure. Comparable observations have
425 been made using the same biophysical techniques for analogous fungal prion-forming domains such
426 as HET-s(218-289) (Ritter et al. 2005; Siemer et al. 2005; Wan and Stubbs 2014), NWD2(1-30)
427 (Daskalov et al. 2015b) or PP (Daskalov et al. 2016). It thus appears that for the BASS motifs that
428 were tested experimentally, we confirm the identified sequences are amyloid prions, again making it
429 plausible that the other motifs also represent analogous prion amyloids.

430

431 **NLRs, Bell domains and amyloid signaling in multicellular bacteria**

432 Proteins with NLR domain architectures control various biotic interactions in plants, animals
433 and fungi (Jones et al. 2016; Uehling et al. 2017). Throughout, we have used the designation NLR to
434 specify proteins displaying a domain architecture associating a NB-ARC or NACHT-type NOD domain
435 (nucleotide binding and oligomerization domain) and ANK, TPR, WD or LRR super-structure forming
436 repeats (SSFR). While some authors reserve the NLR designation to plant and animal NBS-LRR
437 proteins, we adhere to an extended assertion of the term also including NB-SSFR proteins found in
438 fungi and prokaryotes. It has been reported previously that bacterial genomes (in particular in
439 *Actinobacteria* and *Cyanobacteria*) display genes encoding proteins with a NBS-SSFR architecture
440 (Asplund-Samuelsson et al. 2012; Koonin and Aravind 2002; Urbach and Ausubel 2017). In fungi, both
441 NACHT/WD and NB-ARC/TPR proteins have been shown to control non-self recognition and
442 programmed cell death in different species (Chevanne et al. 2009; Choi et al. 2012; Espagne et al.
443 2002; Heller et al. 2018; Saupe et al. 1995). Urbach and Ausubel have shown that these NACHT/WD
444 and NB-ARC/TPR architectures are ancestral to the NBS/LRR architecture, which represent a more
445 recent acquisition (that occurred independently in plant and animal lineages).

446 In our survey of over 100 000 prokaryotic genomes, we find again that NLR architecture
447 proteins are characteristic of bacterial lineages encompassing genera and species with a multicellular
448 organization (filamentous or multicellular aggregates forming species). These are found in
449 filamentous *Actinobacteria*, *Cyanobacteria* and *Chloroflexi* and also in aggregate-forming Archaea.

450 Characteristic of those bacterial lineages that display NLRs is also the presence of the Bell domain.
451 Like fungal NLRs, prokaryotic NLRs typically have NB-ARC/TPR or NACHT/WD architectures and do
452 not display LRR repeats (Dyrka et al. 2014; Koonin and Aravind 2000; Urbach and Ausubel 2017).
453 Another communality between fungal and bacterial NLRs is the presence in both lineages of NLR with
454 WD and TPR-repeats showing high levels of internal conservation (with repeat units showing 80-90%
455 sequence identity, a situation in stark contrast with the bulk of the WD and TPR-repeats in proteins),
456 (Dyrka et al. 2014), (Hu et al. 2017; Marold et al. 2015). In fungi, this internal conservation is related
457 to a mechanism of rapid diversification of repeat domain binding specificity (Paoletti et al. 2007).
458 These NLR-repeats are under positive Darwinian selection and diversify rapidly by a mechanism of
459 repeat-unit reshuffling representing both the cause and consequence of their high internal
460 conservation (Chevanne et al. 2010; Dyrka et al. 2014; Paoletti et al. 2007). Analogous WD and TPR
461 repeats in bacterial NLRs might similarly be under a specific evolutionary regimen allowing rapid
462 diversification. The possibility of an ancestral role of NLR-like proteins in programmed cell death and
463 host defense in multicellular bacteria has been raised before (Koonin and Aravind 2002). Together
464 with the communalities mentioned above, the mechanistic similarity between fungal NLR/amyloid
465 motif/HeLo and bacterial NLR/amyloid motif/Bell domain associations we report, now makes it
466 indeed plausible to envision that the bacterial gene pairs equally function in host defense and
467 programmed cell death. In this hypothesis, the Bell domain might correspond to a programmed cell-
468 death execution domain. In support of this view is the sequence similarity between the signature
469 consensus sequences in the N-terminal α -helical region of Bell, fungal HeLo, metazoan MLKL and
470 plant CC-domains (Fig. 1). Functional studies of the Bell-domain and its possible function in cell death
471 execution are now required to explore this hypothesis. Of note is also the fact that other protein
472 domains related to immune functions and programmed cell death in animals and plants (such as the
473 TIR and caspase-like CHAT domain) can also be found associated to amyloid signaling motifs and that
474 the same domains are frequently found as N-terminal domain of NLRs in multicellular bacteria (Table
475 S1).

476 Remarkably, in fungi NLRs are restricted to filamentous genera (both in Ascomycota and
477 Basidiomycota) and are not found in yeast species (Dyrka et al. 2014). This situation is mirrored in the
478 present study by the fact that NLRs in general as well as the matching NLR/Bell gene pairs are found
479 in *Actinobacteria* and *Cyanobacteria* species with a multicellular morphology but appear to be absent
480 or rare in phylogenetically related unicellular classes. Although, PCD pathways also exist in unicellular
481 species those continue to represent a paradox (Durand et al. 2016; Koonin and Krupovic 2019).
482 Defense-related PCD is generally considered an attribute of multicellular organisms in which altruistic
483 cell death can be advantageous for the survival of the multicellular organism (Iranzo et al. 2014). Our
484 results suggest that filamentous fungi and bacteria have in common the use of NLR-associated
485 amyloid signaling processes. We propose that this shared molecular mechanistic feature between
486 filamentous fungi and filamentous *Actino* and *Cyanobacteria* stems from their common cellular
487 organization and life style. These fungi and bacteria are phylogenetically distant but morphologically
488 alike. In a similar way, abundance and complexity of secondary metabolism clusters involved in
489 allelopathic interactions appears to be a specific feature of filamentous fungi and bacteria compared
490 to related unicellular genera (Keller 2019; Shih et al. 2013; van der Meij et al. 2017). Presence of NLRs
491 and amyloid signaling might represent a common genome hallmark of diverse multicellular microbes.
492 As multicellular organisms, microbes from both reigns could have to cope with parasites and
493 pathogens and thus might rely on altruistic cell suicide for defense, as an immune-related

494 programmed cell death mechanism akin to those operating in multicellular plant and animals
495 lineages. We propose based on these genomic and functional analogies that filamentous fungi and
496 filamentous bacteria share in common the use of NLR-associated amyloid motifs in the control of
497 immune-related programmed cell death. The implication of this hypothesis is that the use of NLRs for
498 immune-related functions might be ancestral and shared universally between multicellular Archaea,
499 Bacteria, fungi, plants and animals. The mechanistic resemblance we report between prokaryotic and
500 eukaryotic NLRs, invites to reconsider the evolutionary trajectory of this protein family, which might
501 have a very ancient history in the control of biotic interactions.

502

503 **Methods**

504 **Homology searches.** The N-terminal 102 amino acid-long fragment of protein ONI86675.1
505 (AOA1V2QF20_9PSEU) (Yeager et al. 2017) was used as the Bell domain query in Jackhmmmer (Eddy
506 2011; Potter et al. 2018) (20 iterations, 2133 hits) and Psi-blast (Altschul et al. 1997; Madeira et al.
507 2019), (5 iterations, 502 hits) homology searches in UniProtKB (Consortium 2018) and NCBI nr
508 databases (Coordinators 2018)(respectively), as of March 2019. In order to extend the coverage, the
509 searches were re-run with the lowest scoring above-the-threshold Jackhmmmer hits in *Archaea*
510 (OPX82726.1 / AOA1V4VC50_9EURY),(Nobu et al. 2017), *Chloroflexi* (ACL23627.1 / B8G4Z4_CHLAD)
511 and *Proteobacteria* (MBN58330.1 / AOA2E7JCT6_9GAMM) (Tully et al. 2018), resulting in 2383, 2553
512 and 939 hits, respectively. In all cases, standard website parameters were used. Combined results
513 consisted of 2797 non-redundant sequences including 2354 sequences shorter than 200 amino acids.
514 The length threshold was chosen to filter out Bell homologues forming “all-in-one” NLR
515 architectures.

516 **Neighboring NLRs identification.** All sequence identifiers in the set were mapped to NCBI accessions
517 using UniProt mapping tool. Accessions of identical sequences in the NCBI nr database were added
518 using the Blast database command tool (Camacho et al. 2009). This resulted in set of 2810 protein
519 accessions from genome-wide studies, restricted to GenBank and RefSeq NP and YP series
520 accessions. An in-house Python (version 3.5) script (aided by requests (Reitz, K., n.d.) and xmltodict
521 (Blech, n.d.) packages) was used to query NCBI Entrez E-utils in order to fetch almost 23k proteins
522 coded by genes within the +/-5000 bp neighborhood of the genes encoding these Bell homologues.
523 Among the proteins coded by the Bell-neighboring genes, 730 were matched with Pfam (El-Gebali et
524 al. 2018), NACHT (PF05729) (Koonin and Aravind 2000) or NB-ARC (PF00931) (van der Biezen and
525 Jones 1998) profiles. This included 467 sequences (426 unique) where the match bordered a
526 relatively short N-terminus of 10 to 150 amino acids in length (396 unique extensions), which is a
527 typical feature of fungal NLR proteins containing amyloid signaling motifs.

528 **Motif extraction.** The C-terminal boundaries of the Bell domains were delimited according to the
529 final Profile HMM (pHMM) (Eddy 2008) of the original Jackhmmmer search using the hmmlalign tool of
530 the standalone HMMER distribution (version 3.2.1) (Eddy 2011). The C-terminal extensions longer
531 than 10 amino acids were extracted. The redundancy of the set was reduced to 90% using cdhit (Fu
532 et al. 2012; Li and Godzik 2006), (version 4.6, standard parameters) resulting in 1814 unique
533 sequences. The common motifs in the C-termini were extracted using MEME (Bailey et al. 2009;
534 Bailey and Elkan 1994) (version 5.0.2, standalone), allowing for motifs of any length from 6 to 30 and
535 possibly repeated in sequence (--anr option), while requiring that each motif was found in at least 10

536 instances. The search yielded 66 motifs (prefixed later “all”) above the standard E-value threshold of
537 0.1. Aiming at motifs restricted to taxonomic branches, additional MEME searches (requiring at least
538 5 motif instances only) were performed for taxonomic subsets of UniProtKB sequences including:
539 *Cyanobacteria* (found 27 motifs in 359 sequences, prefixed later “cya”), combined *Proteobacteria*
540 and *Chloroflexi* (5 in 69, “pch”), and *Streptomyces* (19 in 608, “str”). Finally, the MEME search with
541 the same parameters was performed in N-termini of the NLR-containing genomic neighbors of Bell
542 domains (39 motifs found in 338 sequences, “nlr”).

543 **Motif profile generation.** For each motif, a pHMM was trained using hmmbuild from the HMMER
544 package on instances reported by MEME. Then, the pHMMs were used to re-search the Bell C-
545 terminal domains and NLR N-terminal domains, respectively, using hmmsearch with sequence and
546 domain E-values set to 0.01 and heuristic filters turned off for sensitivity (--max option). The resulting
547 hits were extended each side by 5 amino acids, and then re-aligned to the pHMMs and trimmed of
548 unaligned residues using hmalign (--trim option). Eventually, the resulting alignments were used to
549 train final pHMMs for the motifs again using hmmbuild with standard options. The entire procedure
550 aimed at generalizing the motifs, especially gapped, which could be truncated or over-specialized by
551 MEME.

552 **Motif pairs identification.** Motif pairs were identified whenever hits from the same profile HMM
553 were found in Bell C-termini and their corresponding NLR(s) N-termini, using hmmsearch with
554 sequence and domain E-value set to 0.01 and maximum sensitivity (--max option). There were 1157
555 such pairs (904 using Bell-side motifs and 253 using NLR-side motifs) in 315 (293 and 246,
556 respectively) sequence pairs (there was a considerable overlap between motifs, see below). Only
557 motifs with at least 5 unique pair hits (in terms of sequence) were considered. The criterion was met
558 by 29 Bell-side motifs and 12 NLR-side motifs with 1087 hits in 295 sequence pairs.

559 **Motif clustering.** Motifs were grouped based on overlapping matches (hits in the same sequence
560 pairs). Motifs were joined if at least half of pairs hit by one motif profile HMM were also matched by
561 the other. The procedure yielded ten motif classes termed BASS1 to 10. For each class, the member
562 motif matching most sequences was used as the class representative.

563 **Motif characterization.** For each member motif in classes BASS1-10, the sequence regions matched
564 with its pHMM were extended each side by 5 amino acids and pairwise locally aligned using the
565 Waterman-Eggert method (Huang and Miller 1991; Waterman and Eggert 1987) implemented in
566 EMBOSS (matcher tool, version 6.6.0.0) (Rice et al. 2000). The standard parameters were used
567 including the BLOSUM62 matrix (Henikoff and Henikoff 1992) and gap opening/extending penalties
568 14/4. For each motif class representative, the sequence fragments from the resulting pairwise
569 alignments were combined into multiple sequence alignments using Clustal Omega (Sievers and
570 Higgins 2018; Sievers et al. 2011)(version 1.2.4, standalone) with the --dealign and --auto switches.
571 The resulting MSAs were trimmed manually and profiles were generated using weblogo3 (Crooks et
572 al. 2004) (Weblogo software repository webpage, 2019).

573 In addition, for each motif class and sequence, the pairwise alignments were used to obtain the
574 combined longest fragment matching any member motif. The dealigned fragments were then
575 combined into MSA using Clustal Omega with --full and --full-iter options, curated when necessary
576 (BASS4) and trimmed manually. Amino acid composition of such generated MSAs was then
577 calculated using the quantiprot package (Konopka et al. 2017), after removing redundant sequences.

578 The per-class and overall (unweighted) composition was then compared with amino acid
579 composition of non-redundant sequence sets of fungal functional amyloid motifs including Het-s
580 Related Amyloid Motif (HRAM) (Daskalov et al. 2015a), Pfam NACHT_sigma (PF17106) and Ses_B
581 (PF17046, aka PP-motif) (Daskalov et al. 2015a; Dyrka et al. 2014), beta-solenoid repeat regions
582 extracted from the Protein Data Bank (Berman et al. 2000) according to RepeatsDB (only reviewed
583 entries, as of May 2019), experimentally verified amyloid fragments from AmyLoad (Wozniak and
584 Kotulska 2015) (as of March 2017), and intrinsically disordered protein regions from DisProt
585 (Piovesan et al. 2017) (as of July 2019, only unambiguous entries). Current SwissProt amino acid
586 statistics were used as a reference.

587 **Highly internally conserved pairs search.** Local pairwise alignments of C-termini of Bell domains and
588 their neighboring NLR(s) N-termini were performed using the matcher tool with the standard
589 parameters. For each pair ten best alternatives were filtered for minimum alignment length of 15
590 amino acid and minimum score of 40. The procedure yielded in 292 hits (including 234 unique) in
591 283 sequence pairs. The number included 44 sequence pairs not matched with the BASS1-10 motifs,
592 comprising 25 sequence pairs not matched with pHMM of any motif.

593 **Phylogenetic distribution.** Protein accession lists of genome assemblies listed in the Genome
594 Taxonomy Database (GTDB) (Parks et al. 2018) metadata sheets for Bacteria (113,324 items) and
595 Archaea (1183) were downloaded from NCBI ftp (<ftp.ncbi.nlm.nih.gov/genomes/all/>; as of April
596 2019). The accesions were matched with the ABC_tran (PF00005) (Rosteck et al. 1991), NACHT, NB-
597 ARC, Beta_propeller (CL0186) (Murzin 1992), TPR (CL0020) (Lamb et al. 1995), CHAT (PF12770)
598 (Aravind and Koonin 2002), PNP_UDP_1 (PF01048) (Mushegian and Koonin 1994) and TIR (PF01582)
599 (Bonnert et al. 1997) Pfam profiles hit lists downloaded from Pfam ftp
600 (ftp.ebi.ac.uk/pub/databases/Pfam/current_release/; as of February 2019). In addition, distributions
601 of previously found Bell domain homologues and motif pairs involving their C-termini in GTDB-listed
602 genomes were recored. For each, the total number of hits and the number of genomes with hits is
603 provided. The sequence accession redundancy was resolved with the NCBI Blast nr database (as of
604 March 2019).

605 **Strains and plasmids.** To avoid interference with endogenous prions, the *P. anserina* Δ het-s
606 (Δ Pa_3_620) Δ hellf (Δ Pa_3_9900) Δ Pahellp (Δ Pa_5_8070) strain was used as recipient strain for the
607 expression of molecular fusions of BASS motifs and the GFP (green fluorescent protein) or RFP (red
608 fluorescent protein). These fusions were expressed either from a plasmid based on the pGEM-T
609 backbone (Promega) named pOP plasmid (Daskalov et al. 2016), containing RFP, or from a derivative
610 of the pAN52.1 GFP vector (Balguerie et al. 2004), named pGB6-GFP plasmid containing GFP. In both
611 cases the molecular fusions were under the control of the constitutive *P. anserina* gpd
612 (glyceraldehyde-3-phosphate dehydrogenase) promoter. The Δ Pahellp Δ het-s Δ hellf strain was
613 transformed as described (Bergès and Barreau 1989) with one or two fusion constructs along with a
614 vector carrying a phleomycin-resistance gene ble, pPaBle (using a 10:1 molar ratio). Phleomycin-
615 resistant transformants were selected, grown for 30 h at 26°C and screened for the expression of the
616 transgenes using a fluorescence microscope. Fragments (protein position indicated in brackets) of
617 the following genes (accessions from GenBank or RefSeq) were amplified using specific primers:
618 CAB66307.1 (110-139), CAB66306.1 (1-34), WP_037701008.1 (70-124), WP_037701012.1 (1-37),
619 ACC79696.1 (94-126), ACC79697.1 (1-38), WP_063130184.1 (74-128). The PCR products were cloned
620 upstream of the RFP coding sequence in the pOP plasmid using *PacI/BglII* restriction enzymes or

621 downstream of the GFP in the pAN52.1 plasmid using *NotI/BamHI* restriction enzymes. For
622 heterologous expression in *E. coli*, the following fragments were amplified using specific primers and
623 cloned in pET24a (Novagen) using the *NdeI/XhoI* restrictions sites: CAB66307.1 (110-139) or (38-139),
624 WP_037701008.1 (97-124), ACC79696.1 (94-126), WP_063130184.1 (100-128).

625

626 Since the sequences selected for the BASS3 motif were not included neither in the GenBank nor
627 RefSeq NP or YP series at the time of the genome mining, they were not processed for identifying
628 neighboring NLRs, and hence are not covered in the motif pair list in Table S2. Nevertheless, the
629 BASS3 motifs can be found in these sequences with the motif pHMMs at default identification
630 threshold (E-value of 0.01), except for ACC79697.1 being slightly below (E-value of 0.1).

631

632 **Prion propagation.** The [b] phenotype (acquisition of the [b] prion) was defined in *Podospora* strains
633 expressing fluorescent fusion proteins as the absence ([b*]) or the presence ([b]) of fluorescent dot-
634 like aggregates. To monitor the propagation of the [b] prion, prion free strains were subcultured in
635 presence of [b] prion donor strain and after 72h (contact between tested and donor strains was
636 established after 24h of subculture) the initially prion free tested strain was subcultured on fresh
637 medium and monitored for the presence of aggregates using fluorescence microscopy.

638

639

640 **Protein preparation and fibril formation.** 6his-tagged proteins were expressed from pET24a
641 constructs in *E. coli* BL21-CodonPlus®-RP competent cells as insoluble proteins and purified under
642 denaturing conditions using its terminal 6 histidine tag as previously described (Dos Reis *et al.* 2002).
643 Briefly, cells were grown at 37°C in DYT medium to 0.6 OD₆₀₀ and expression was induced with 1 mM
644 isopropyl β-D-1-thiogalactopyranoside. After, 4 h, cells were harvested by centrifugation, frozen at -
645 80°C sonicated on ice in a lysis buffer (Tris 50 mM, 150 mM NaCl, pH 8) and centrifuged for 20 min at
646 20,000 g to remove *E. coli* contaminants. The pellet was washed in the same buffer and resuspended
647 in denaturing buffer (6M guanidinium HCl, 150 mM NaCl, and 100 mM Tris-HCl, pH 8) until complete
648 solubilization. The lysate was incubated with Talon Resin (CLONTECH) for 1 h at 20°C, and the resin
649 was extensively washed with 8 M urea, 150 mM NaCl, and 100 mM Tris-HCl, pH 8. The protein was
650 eluted from the resin in the same buffer containing 200 mM imidazole. The proteins were pure as
651 judged by sodium-dodecyl-sulfate polyacrylamide-gel electrophoreses (SDS-PAGE) followed by
652 Coomassie-Blue staining and yield was in the range of ~2-4 mg of protein per liter of culture. To
653 eliminate urea, elution buffer was replaced by overnight dialysis at 4°C against Milli-Q water. Fibrills
654 formation resulted spontaneously from dialysis process followed by sample storage in H₂O or in
655 ammonium acetate buffer 100 mM pH 4.5 at 4°C for 7 to 30 days.

656

657 **Light Microscopy.** *P. anserina* hyphae were inoculated on solid medium and cultivated for 24 to 48 h
658 at 26°C. The medium was then cut out, placed on a glass slide and examined with a Leica DMRXA
659 microscope equipped with a Micromax CCD (Princeton Instruments) controlled by the Metamorph
660 5.06 software (Roper Scientific). The microscope was fitted with a Leica PL APO 100X immersion lens.

661

662 **Transmission Electron Microscopy.** For fibrils observations, negative staining was performed:
663 aggregated proteins were adsorbed onto Formvar-coated copper grids (400 mesh) and allowed to
664 dry for 15 min in air, grids were then negatively stained 1 min with 10 μL of freshly prepared 2%
665 uranyl acetate in water, dried with filter paper, and examined with a Hitachi H7650 transmission

666 electron microscope (Hitachi, Krefeld, Germany) at an accelerating voltage of 120 kV. TEM was
667 performed at the Pôle Imagerie Électronique of the Bordeaux Imaging Center using a Gatan USC1000
668 2k x 2k camera.

669
670 **Solid-state NMR of BASS1 fibrils.** The solid-state NMR spectrum of BASS 1 was recorded at 800 MHz
671 on a Bruker Biospin magnet using a triple resonance 3.2 mm probe at a spinning frequency of 11 kHz.
672 64 scans were used using a recycle delay of 3 sec and an acquisition time of 17 ms.

673
674 **X-ray diffraction of BASS1 fibrils.** Fiber diffraction pattern was measured at 4°C on an Excillum
675 MetalJet X-ray generator at the galium wavelength ($K\alpha$, $\lambda = 1.34 \text{ \AA}$). The source was equipped with
676 Xenocs Fox3D optics and a Dectris Eiger 1M detector on a 2θ arm of a STOE stadivari goniometer.
677 The detector has been turned by 90° to have the blind region vertical to hide as much as possible the
678 shadow of the beamstop. The viscous concentrated hydrated sample was mounted in a MicroLoop™
679 from Mitegen on a goniometer head under the cold nitrogen flow. The diffraction pattern
680 corresponds to a 360° rotation along the phi axis with an exposure time of 180 sec.
681

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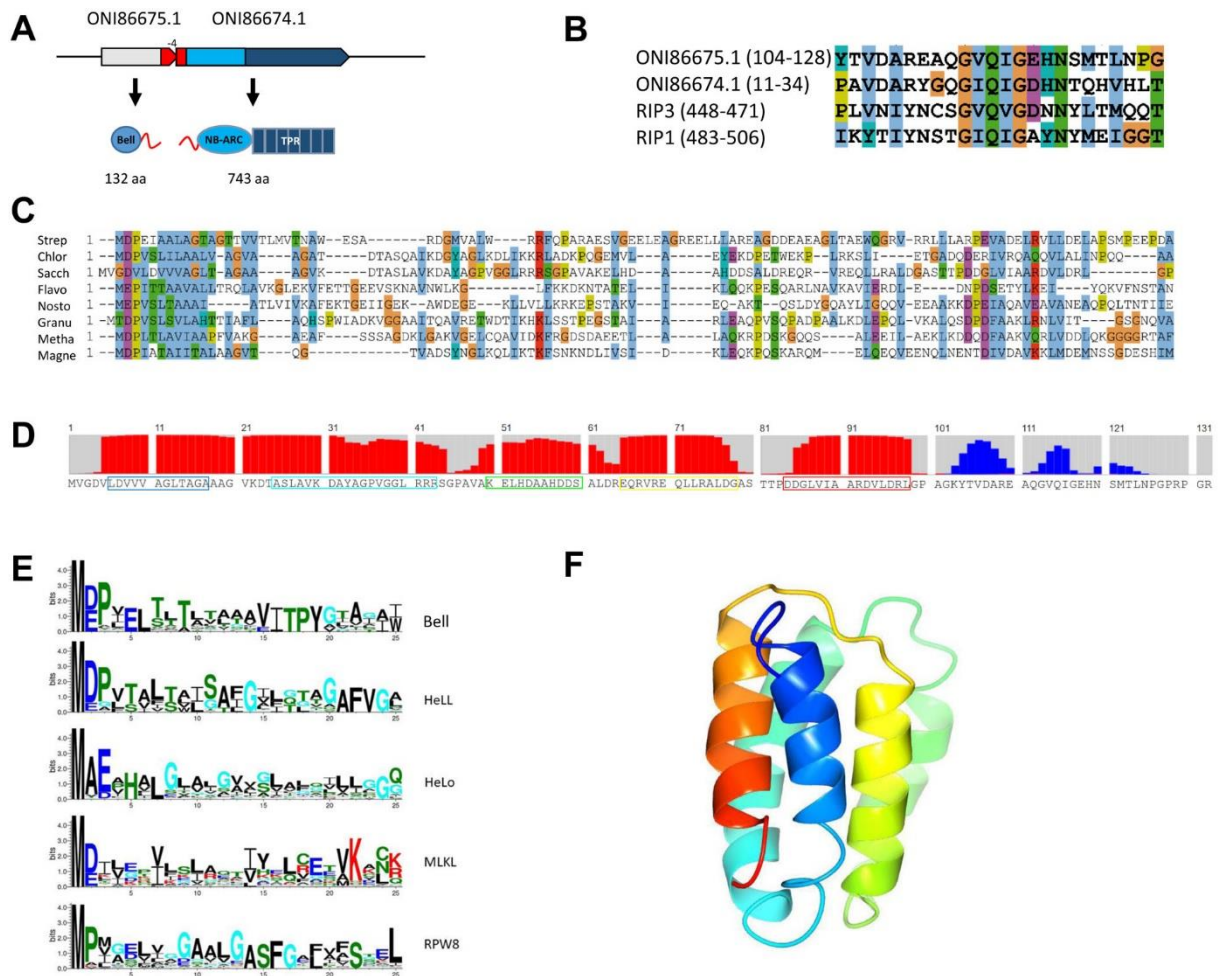


Figure 1. RHIM-like motifs in bacteria are associated to Bell domains. **A.** Genome and domain architecture of the ONI86675.1 and ONI86674.1 gene pair from the actinobacterium strain *Saccharothrix* sp. ALI-22-I. The relative orientation and the overlap between the two ORF are given (the two ORF overlap by 4 bp) as well as the size and domain architecture of the corresponding proteins, respectively for a Bell-domain protein with a C-terminal RHIM-like motif and a NLR-related protein with a NB-ARC and TPR repeat domain and an N-terminal RHIM-like motif. The RHIM-like motifs are represented in red in the ORF diagram and the protein cartoon. **B.** Alignment of the RHIM-like motifs of the proteins encoded by the ONI86675.1 and ONI86674.1 gene pair and the RHIM-motif of the human RIP1 and RIP3 kinases. **C.** Alignment of Bell-domains from various prokaryotes (Strep, Q9RDG0 from *Streptomyces coelicolor* A3(2); Chlor, HBY96210.1 from *Chloroflexi bacterium*; Sacch, ONI86675.1 from *Saccharothrix* sp. ALI-22-I; Flavo, SDZ50707.1 from *Flavobacterium aquidurens*; Nosto, RCJ33357.1 from *Nostoc punctiforme* NIES-2108; Granu, ROP69996.1 from *Granulicella* sp. GAS466; Metha, AEB69174.1 from *Methanoxithrix soehngenii* (strain ATCC 5969); Magne, ETR68090.1 from *Candidatus Magnetoglobus multicellularis* str. Araruama). **D.** Secondary structure prediction for ONI86675.1 from *Saccharothrix* sp. ALI-22-I, red bars represent α -helical propensity, blue bar β -sheet propensity. Boxing corresponds to the α -helices predicted in the homology model given in G. **E.** Consensus sequence of the 25 N-terminal residues of the Bell-domain and other predicted or known cell death execution domains in fungi, plants and mammals. The consensus sequence was generated with Weblogo from a HHMER alignment using the following sequences as queries: ONI86675.1 (Bell), *C. globosum* HELLP (Hell), *P. anserina*, HET-S (HeLo), mouse MLKL (MLKL) and *Arabidopsis thaliana* RPW8.1 (RPW8). **F.** Homology model of the Bell-domain of ONI86675.1 from *Saccharothrix* sp. ALI-22-I based on a contact map (model generated by RAPTOR-X contact).

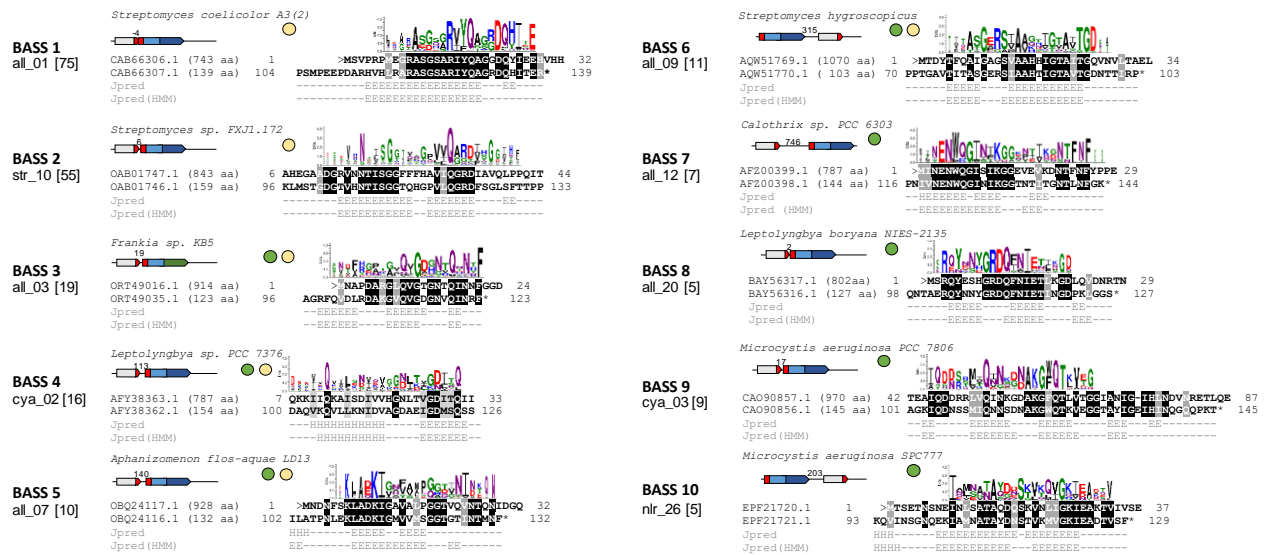


Figure 2. Ten bacterial amyloid signaling sequences motifs (BASS). For each of the ten identified motifs, a representative gene pair is given. A consensus sequence for the motif is given. The consensus was generated using Weblogo from the alignment of all motifs pairs bearing the corresponding motif. For each gene pair chosen as illustrative example, the species name, the genome architecture, the gene number and protein size are given as well as an alignment of the Bell-domain and NLR-associate motifs. The number given above the gene diagram is the distance between the Bell-domain encoding and NLR encoding ORF, negative number represent gene overlaps. The sequences encoding the BASS motif is represented in red, the Bell-domain in grey, NB-ARC domain is light blue, TPR repeats in dark blue and WD repeats in green. Under the alignment the secondary structure prediction for the individual sequences of the Bell-domain motif (Jpred) or for a HMM-alignment of the sequence (Jpred (HMM)) are given (E for extended, H for helical). The number of motif pairs identified with the different motifs is given in parentheses. The dots symbolize the phylogenetic distribution of the motif (green, cyanobacteria, beige, actinobacteria).

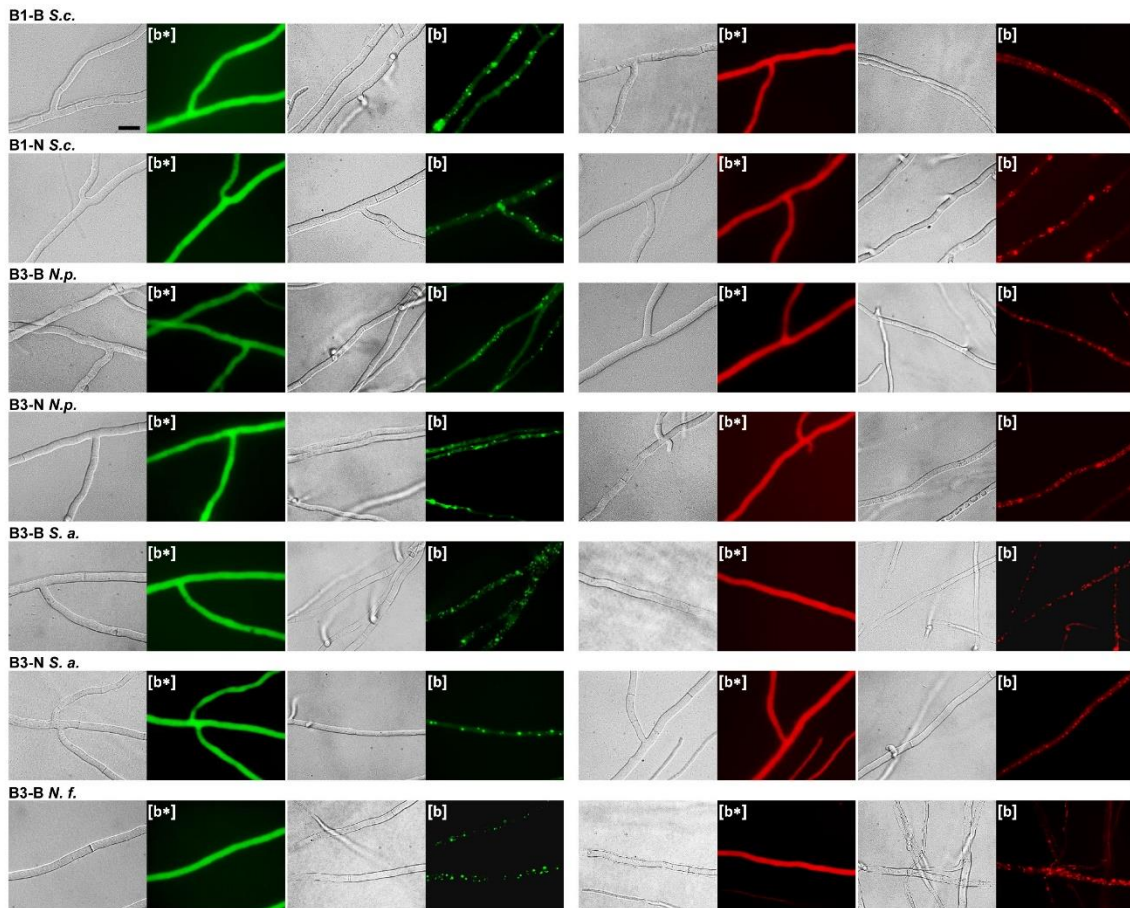


Figure 3. BASS-motif prion a formation in *Podospora anserina*. Micrographs of *P. anserina* strains expressing molecular fusions of BASS1 and BASS3 motifs with GFP (in N-terminus) or RFP (in C-terminus) as indicated above each micrograph (Scale bar 5 μ m). Transformants displayed an initial diffuse fluorescence noted [b*] phenotype (left side of the panels) and acquired dot like fluorescent aggregates, [b] phenotype, after contact with strains already spontaneously expressing the infectious aggregated [b] state (right side of the panels).

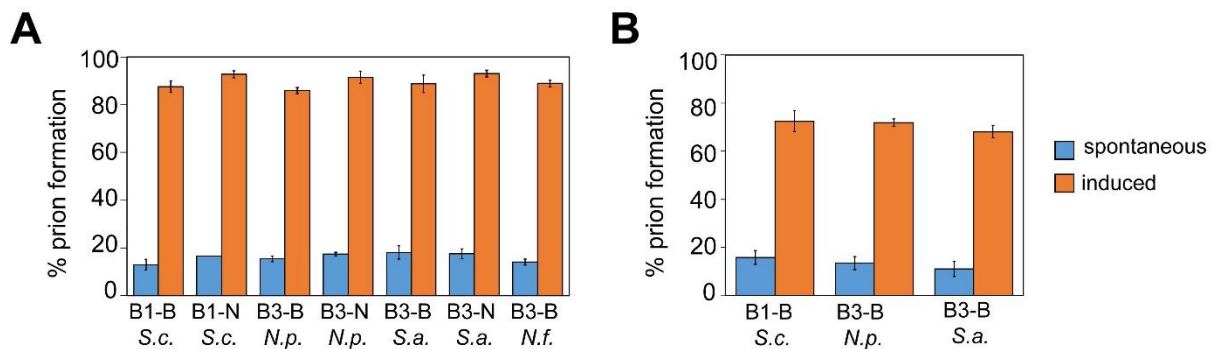


Figure 4. BASS motifs propagate as prions in *Podospora anserina* and NLR-side motifs induce prion formation of Bell-side motifs. A. Histogram representing the percentage of [b*] strains expressing the given BASS motifs (fused to RFP in C-terminus) converted to prion-infected [b] phenotype after contact either with non-transfected prion-free control strain (spontaneous, in blue), or with prion [b] strains expressing the same motif in the aggregated state (induced, in orange). Percentages are expressed as the mean value \pm standard deviation for three independent experiments using six subcultures of four different transformants and corresponding to the analysis of \sim 70 subcultures for each BASS motif. In each case, the BASS motifs are fused to RFP. **B.** Histogram representing the percentage of [b*] strains expressing the given BASS motif (fused to RFP in C-terminus) as indicated converted to prion-infected [b] phenotype by contact either with non-transfected prion-free control strains (spontaneous, in blue), or with prion [b] strains expressing the corresponding NLR-side motif fused to GFP (in N-terminus) in the aggregated states (induced, in orange). Percentages are expressed as the mean value \pm standard deviation for experiments using six to twelve subcultures of six to ten different transformants and correspond to the analysis of 80 to 100 subcultures for each BASS motif.

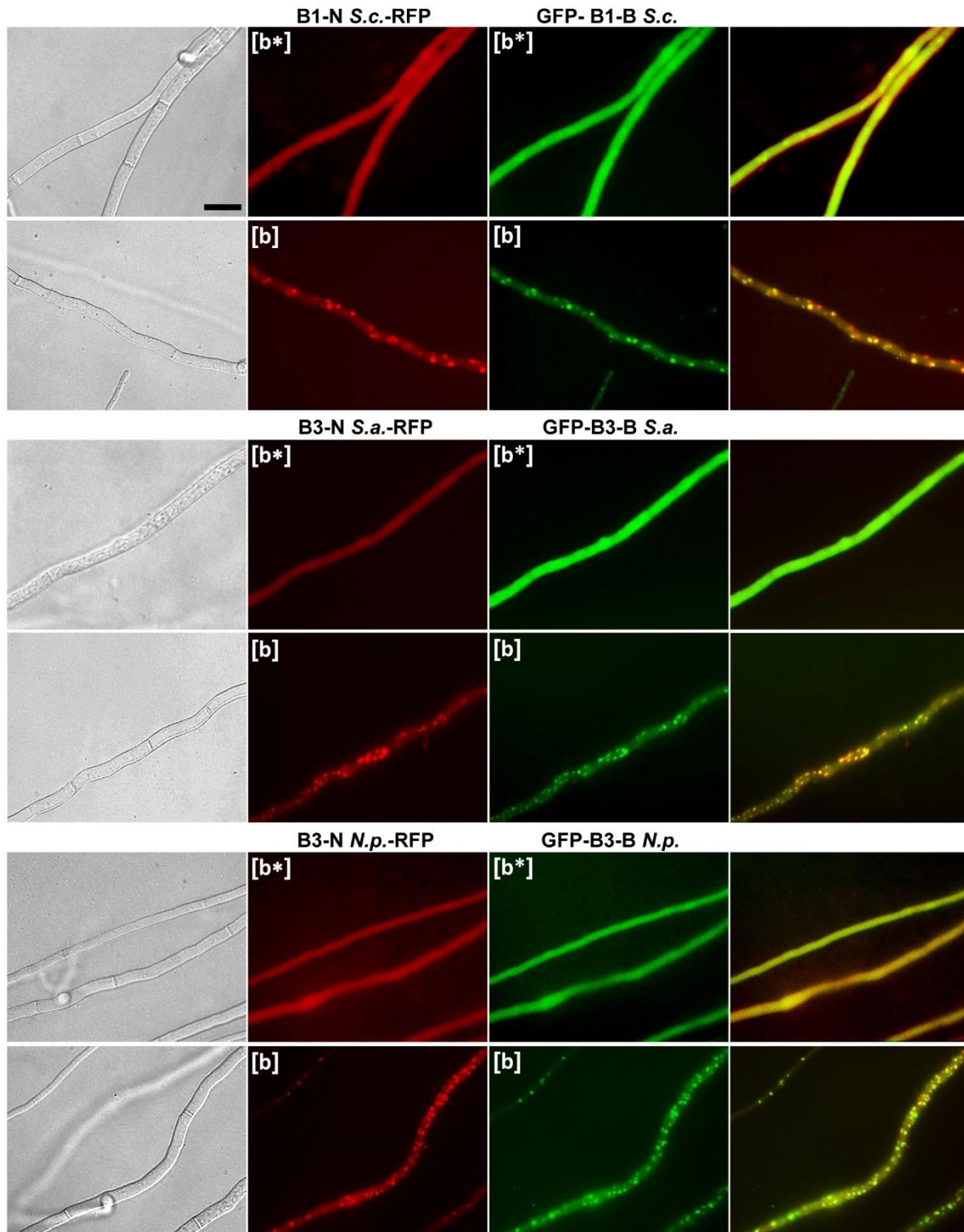


Figure 5. Bell and NLR-side BASS motif co-aggregation in *Podospora anserina*. Micrographs of *Podospora anserina* strains co-expressing Bell-side BASS motifs fused to GFP (in N-terminus) as indicated and the corresponding NLR-side motif fused to GFP (in C-terminus), (Scale bar 5 μm). Panel are from left to right, bright field, RFP, GFP and overlay.

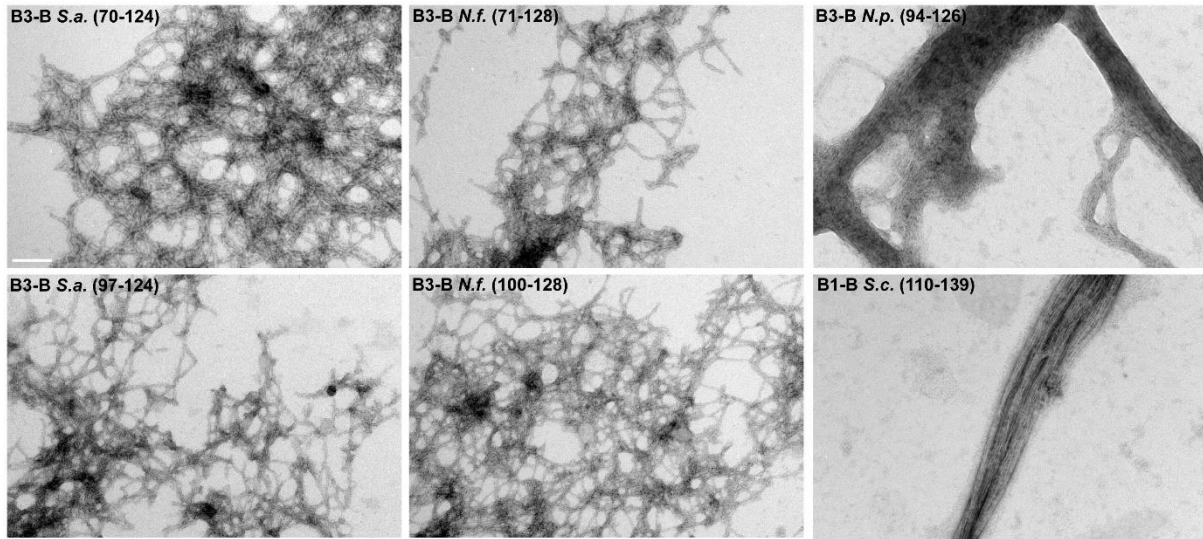


Figure 6. Electron microscopy of BASS-motif fibrils. Electron micrographs of fibrils formed by selected BASS motifs as indicate (scale bar, 100nm).

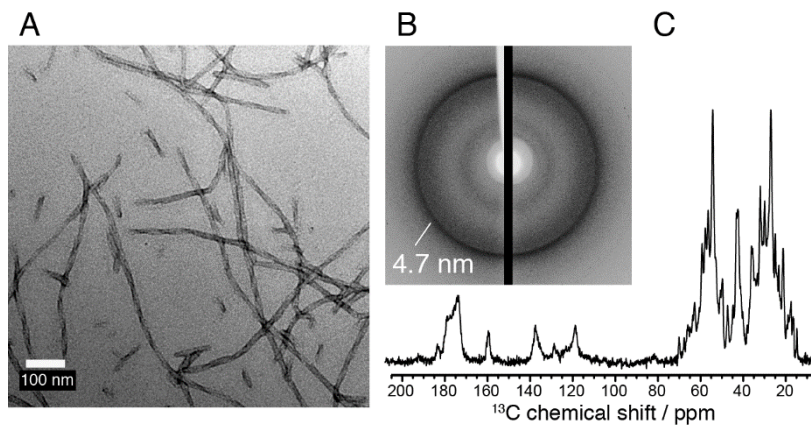


Figure 7: Structural analysis of the *S. coelicolor* BASS 1 motif. **A.** Negatively-stained electron micrograph of BASS 1 fibrils (B1-B S.c.(38-139), CAB66307.1), scale bar is 2 μm . **B.** X-ray diffraction pattern of BASS 1 fibrils, reflection at 4.7 \AA is highlighted, corresponding to the inter-strand spacing. **C.** ^{13}C solid-state NMR spectrum of BASS 1 fibrils.

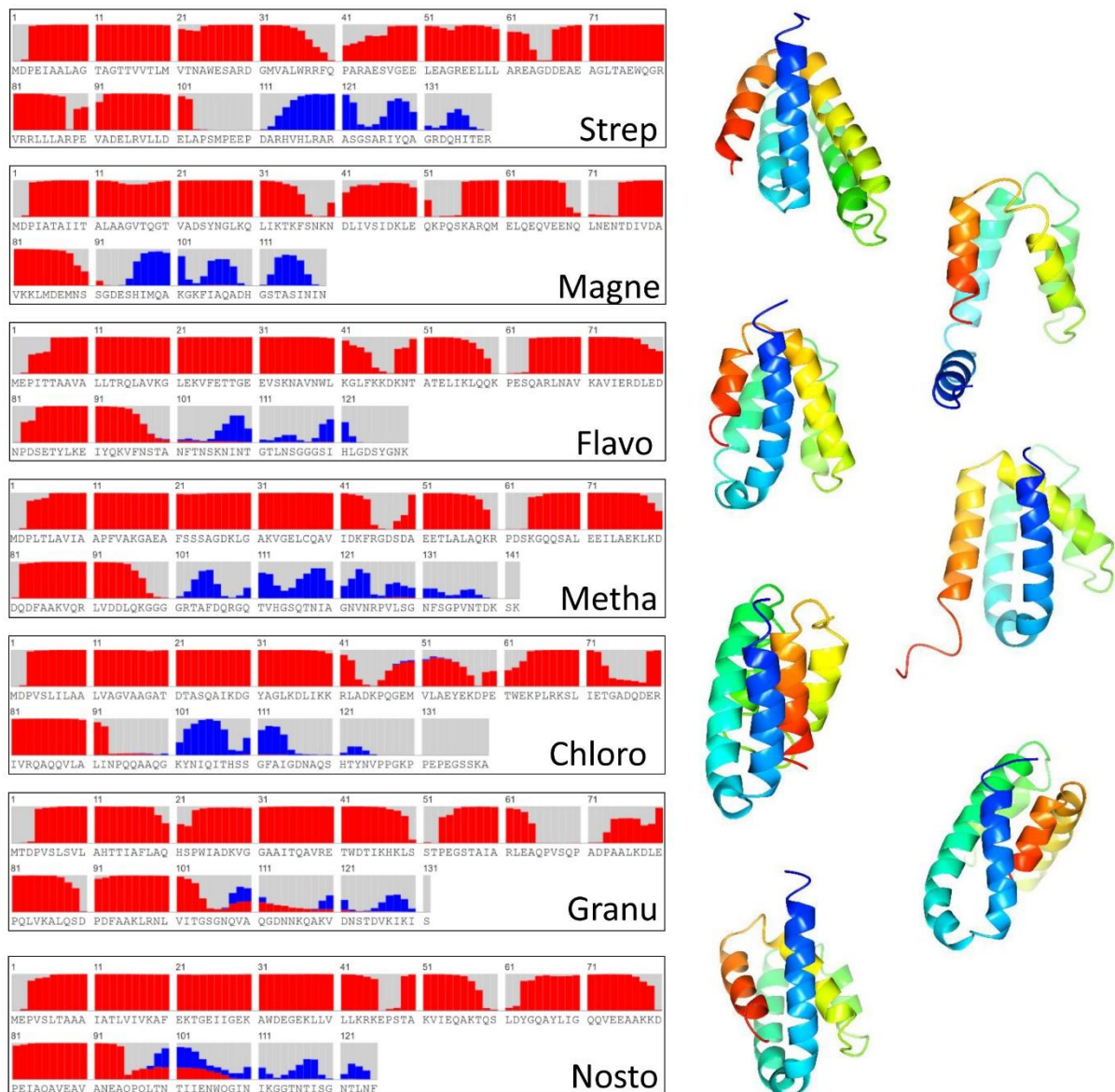


Figure S1. Homology models of Bell-domain proteins. Secondary structure prediction and homology models are given for a set of phylogenetically diverse Bell-domain proteins from prokaryotes ((Strep, Q9RDG0 from *Streptomyces coelicolor* A3(2) ; Chlor, HBY96210.1 from *Chloroflexi bacterium* ; Sacch, ONI86675.1 from *Saccharothrix sp. ALI-22-I* ; Flavo, SDZ50707.1 from *Flavobacterium aquidurens* ; Nosto, RCJ33357.1 from *Nostoc punctiforme NIES-2108* ; Granu, ROP69996.1 from *Granulicella sp. GAS466* ; Metha, AEB69174.1 from *Methanotherx soehngeni* (strain ATCC 5969) ; Magne, ETR68090.1 from *Candidatus Magnetoglobus multicellularis str. Araruama*). Secondary structure prediction and homology models are as given by RAPTOR-X contact. Red bars represent α -helical propensity, blue bar β -sheet propensity. Secondary structure prediction are given for the full-length protein, the homology model for the Bell-domain region only. Note that secondary structure prediction often merged helix 1 and 2 which in some cases are modelled as a continuous helix.

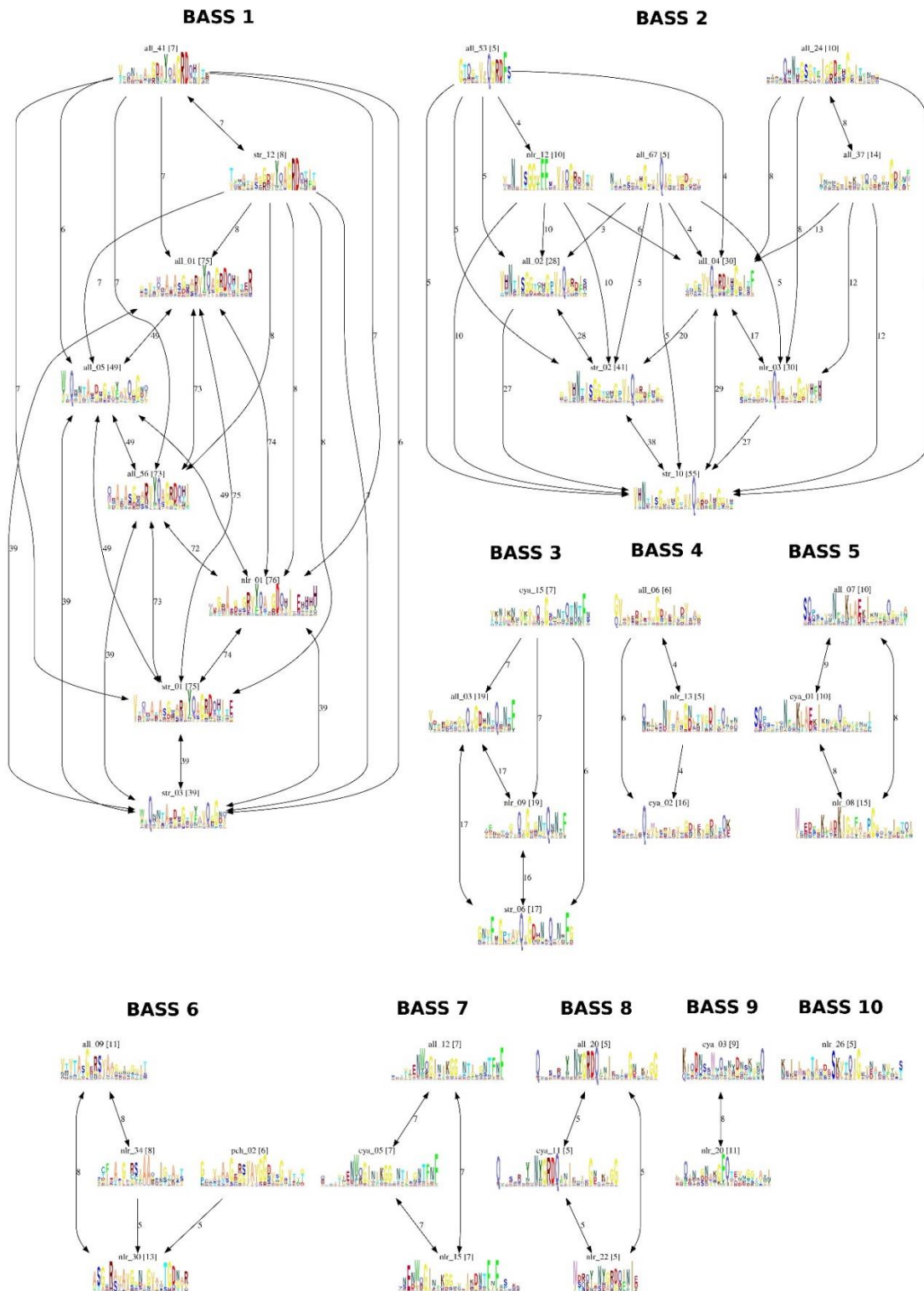


Figure S2. Clustering of motifs. Clustering of motifs. 29 motifs matching at least 5 non-redundant pairs of NLRs and Bell-domain proteins were clustered based on overlapping matches. Motifs (presented as profile HMM logos) were joined if at least half of the pairs hit by one motif profile HMM were also matched by the other. The number in brackets represents the number of pairs for each profile, the number on the arrow joining the motifs indicates the number of common matching underlying sequences. The grouping was preserved in an alternative clustering scheme where motifs sharing at least 40% of their underlying sequences were grouped together.

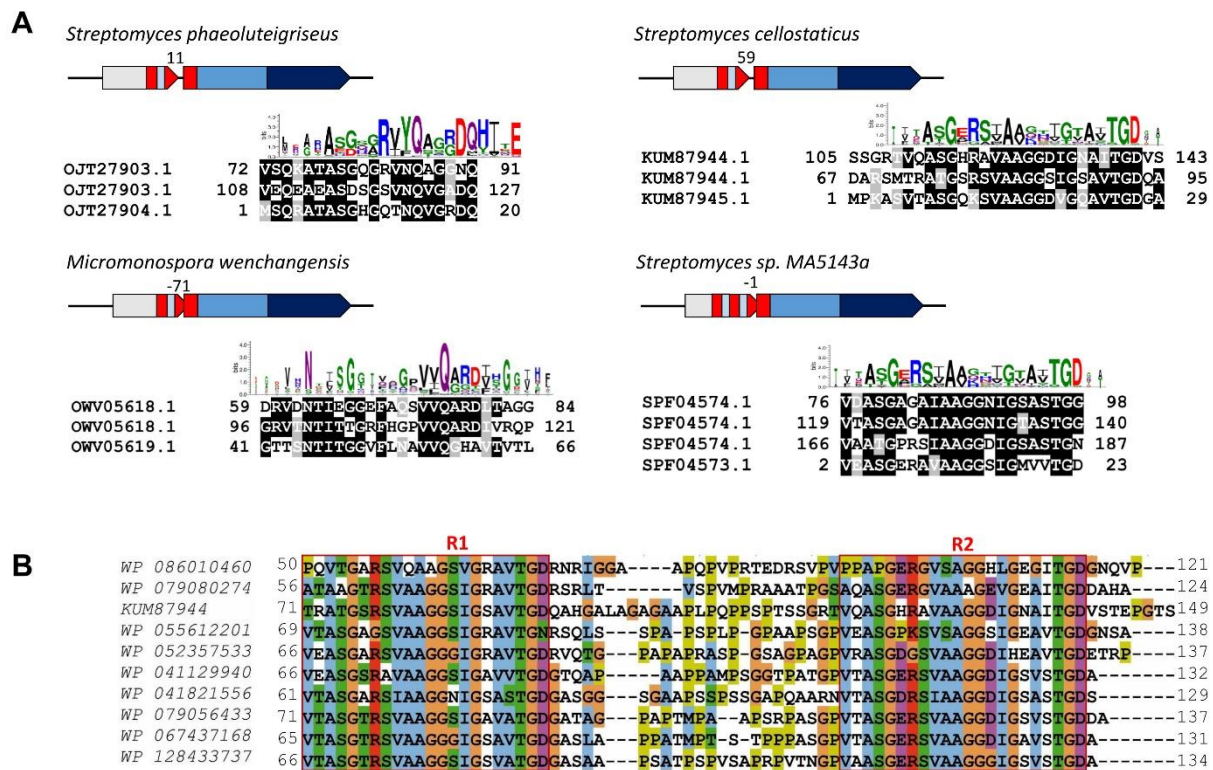
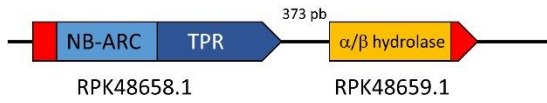


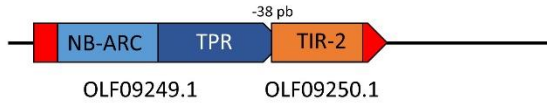
Figure S4. Double and triple BASS motifs. A. Species of origin, gene identification and gene architecture of selected pairs of gene encoding a Bell domain and a NLR and sharing an amyloid signaling motif are given together with an alignment of the two (or three) motifs found associated to the Bell domain with the motif found associated to the NLR. The consensus signature sequence of the motif is given above the alignment. The sequences encoding the BASS motifs are represented in red, the Bell-domain in grey, NB-ARC domain is light blue, TPR repeats in dark blue. **B.** Alignment of 10 orthologs of KUM87944.1 protein from *Streptomyces cellostaticus* given in A, showing the two repeats of the motif and the variable proline and glycine-rich region between the R1 and R2 repeats.

Streptomyces sp. ADI92-24



RPK48658.1 1 M T T N E I D D G I F N A V I Q G H T T V Q L 26
 RPK48659.1 237 R I V I N T F S G C R A V G P V I Q G G T H V S F 262

Actinophytocola xanthii



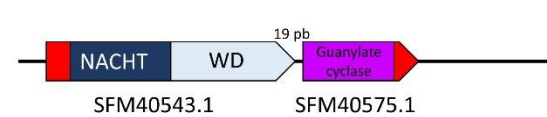
OLF09249.1 1 M V D P A D L R G R A R D D R V Y Q A I R D H V H E 30
 OLF09250.1 161 E R G A V T I N C E A R D N R V Y Q A I R D H L R E 190

Nostoc commune NIES-4072



GBG17166.1 1 M T E D N P K N H T E F Q G E V K G V O V G D H N I I Y N V E Y 31
 GBG17167.1 307 K E S V P S K T I N V V F Q G E V K G S Q V G D H N K Q E N K E A 338

Saccharopolyspora antimicrobica



SFM40543.1 14 G R A T Y S V D A H S A T G V Q G G N T Q I V Y S Y R I G W 47
 SFM40575.1 259 S D Q K Y V V A A D H A Q G V Q G G N T Q H V D F R R P E 292

Figure S5. Other BASS-associated effector domains. Examples of gene pairs sharing an amyloid signaling motif in which the effector domain is not a Bell domain. In each case, the species of origin, gene identification and gene architecture of the selected pairs is given together with an alignment of the motifs associated to the effector domain and associated to the NLR. The sequences encoding the BASS motif are represented in red.

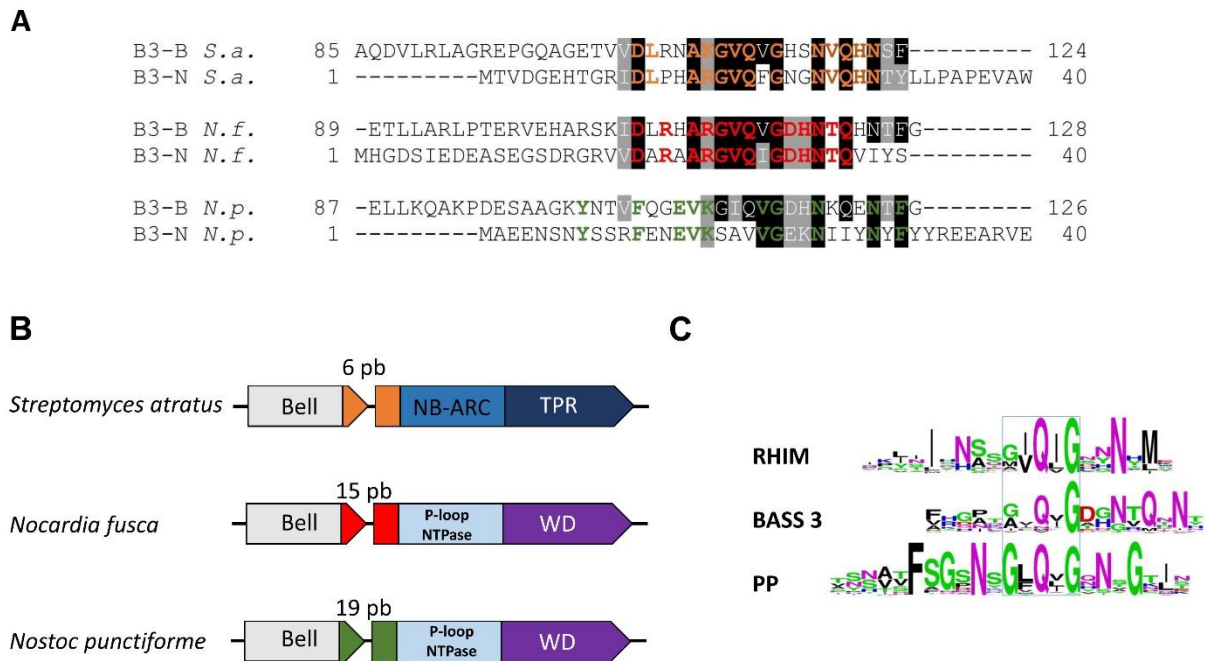


Figure S6. BASS3 motif sequences selected for *in vivo* and *in vitro* expression studies. **A.** Alignment of the BASS3 motif of the selected proteins, the grey and black boxing given residues similar or identical respectively in at least 4 of the 6 sequences. The colored residues highlight identical residues within a gene pair, that is, identical in the Bell (B) and NLR-side (N) motif. Accession number of the sequences are given Table 3. **B.** Species of origin and genome architecture are given for the three selected the Bell-domain and NLR pairs. The number given above the gene diagram is the distance between the Bell-domain encoding and NLR encoding ORF. **C.** Comparison of the consensus sequences of the metazoan RHIM, bacterial BASS 3 and fungal PP-motifs. Logos were generated using all RHIM Pfam entries (PF17721) from Metazoans, all identified BASS 3 pairs motifs (Table S2) and all Pfam entries for PP (PF17046).

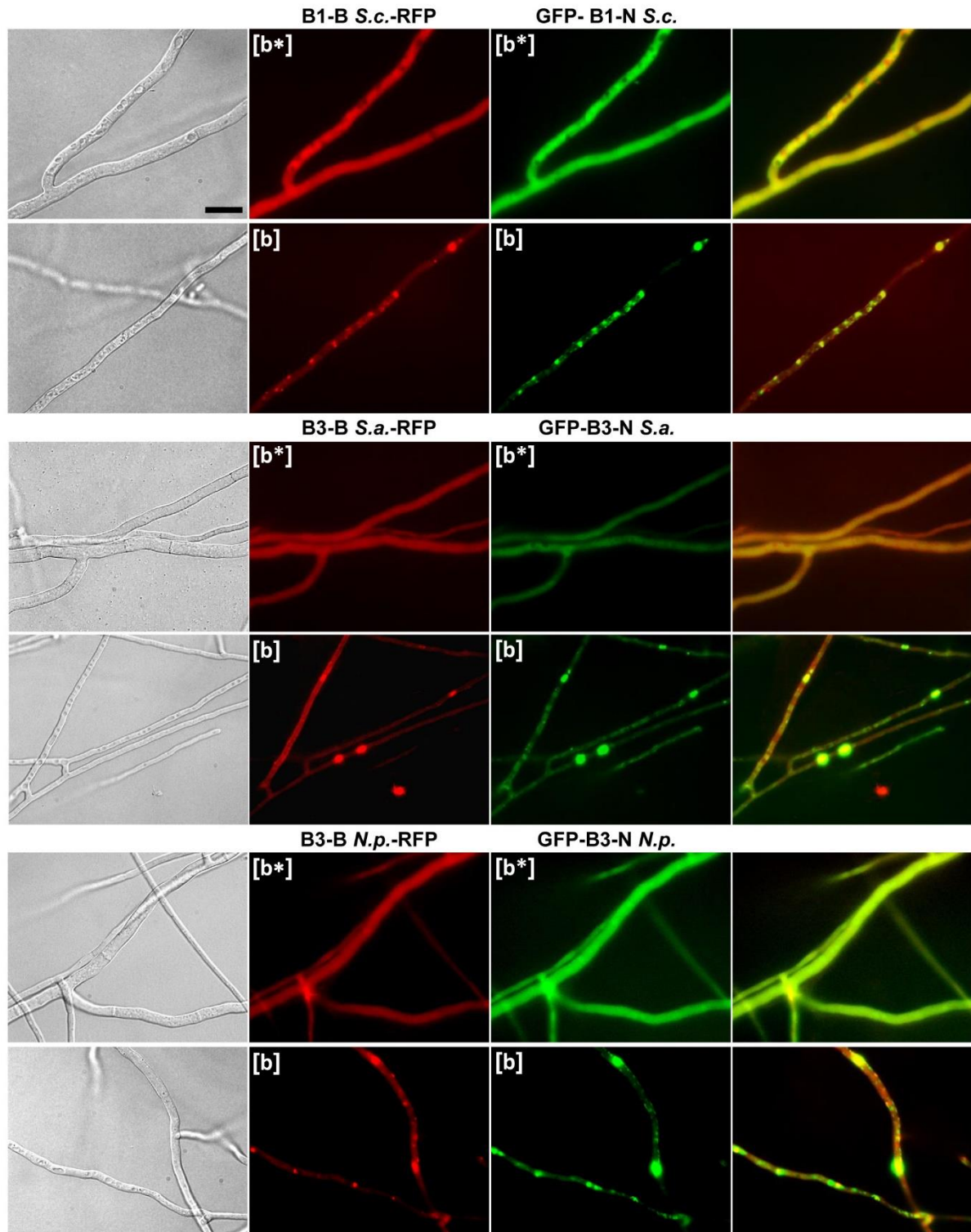


Figure S7. Bell and NLR-side BASS motif co-aggregation in *Podospora anserina*. Micrographs of *Podospora anserina* strains co-expressing Bell-side BASS motifs fused to RFP (in C-terminus) as indicated and the corresponding NLR-side motif fused to GFP (in N-terminus), (Scale bar 5 μ m). Panel are from left to right, bright field, RFP, GFP and overlay.

Table 1. Phylogenetic distribution of Bell domains and NLRs in Bacteria

phylum	#genomes	NBARC-TPR	NACHT-WD	Bell
d__Bacteria;p__Acidobacteriota	91	9	0	2
d__Bacteria;p__Actinobacteriota	13236	2533	284	930
d__Bacteria;p__Bacteroidota	2858	38	8	30
d__Bacteria;p__Campylobacterota	3368	3	0	0
d__Bacteria;p__Chloroflexota	273	48	9	12
d__Bacteria;p__Cyanobacteriota	513	223	152	173
d__Bacteria;p__Deinococcota	81	6	0	0
d__Bacteria;p__Desulfobacterota	267	6	1	3
d__Bacteria;p__Firmicutes	32747	386	2	0
d__Bacteria;p__Firmicutes_A	3076	34	0	0
d__Bacteria;p__Firmicutes_B	130	3	0	0
d__Bacteria;p__Firmicutes_C	182	1	0	0
d__Bacteria;p__Fusobacteriota	195	0	0	0
d__Bacteria;p__Myxococcota	91	20	18	0
d__Bacteria;p__Omnitrophota	88	0	0	0
d__Bacteria;p__Patescibacteria	1772	10	0	0
d__Bacteria;p__Planctomycetota	178	4	0	0
d__Bacteria;p__Proteobacteria	51737	146	26	83
d__Bacteria;p__Spirochaetota	808	4	0	0
d__Bacteria;p__Verrucomicrobiota	582	8	0	0

Table 2. Phylogenetic distribution of Bell-domains and NLRs in *Actinobacteria* and *Cyanobacteria*

family	#genomes	NBARC-TPR	NACHT-WD	Bell
c__Actinobacteria	12979	2523	282	927
o__Actinomycetales;f__Actinomycetaceae	201	22	6	0
o__Actinomycetales;f__Bifidobacteriaceae	558	0	0	0
o__Actinomycetales;f__Brevibacteriaceae	45	2	0	0
o__Actinomycetales;f__Cellulomonadaceae	75	15	0	1
o__Actinomycetales;f__Demequinaceae	32	0	0	0
o__Actinomycetales;f__Dermabacteraceae	26	0	0	0
o__Actinomycetales;f__Dermatophilaceae	79	25	2	2
o__Actinomycetales;f__Microbacteriaceae	415	32	0	4
o__Actinomycetales;f__Micrococcaceae	308	14	8	5
o__Corynebacteriales;f__Corynebacteriaceae	9174	789	20	185
o__Corynebacteriales;f__Geodermatophilaceae	40	13	0	2
o__Corynebacteriales;f__Micromonosporaceae	202	202	36	53
o__Corynebacteriales;f__Pseudonocardiaceae	198	169	20	91
o__Frankiales;f__Frankiaceae	40	38	20	9
o__Jiangellales;f__Jiangellaceae	7	7	0	0
o__Nanopelagiales;f__Nanopelagicaceae	31	0	0	0
o__Propionibacteriales;f__Nocardoidaceae	66	24	0	0
o__Propionibacteriales;f__Propionibacteriaceae	287	3	0	0
o__Streptomycetales;f__Streptomycetaceae	1049	1047	130	540
o__Streptosporangiales;f__Streptosporangiaceae	100	99	37	33
c__Cyanobacteria	467	223	152	173
o__Cyanobacteriales;f__Coleofasciculaceae	7	7	6	6
o__Cyanobacteriales;f__Cyanobacteriaceae	21	2	1	3
o__Cyanobacteriales;f__Microcystaceae	54	39	6	37
o__Cyanobacteriales;f__Nostocaceae	127	99	84	86
o__Cyanobacteriales;f__Phormidiaceae	32	26	11	9
o__Eurycoccales;f__Leptococcaceae	8	0	0	0
o__Leptolyngbyales;f__Leptolyngbyaceae	10	9	4	9
o__Phormidemiales;f__Phormidemiaceae	9	8	7	4
o__Pseudanabaenales;f__Pseudanabaenaceae	8	3	3	2
o__Synechococcales_A;f__Cyanobiaceae	133	0	1	0

Table 3. Sequences selected for functional studies

Motif/Domain/Strain	Accession number and coordinates	Code name
BASS1/Bell/ <i>Streptomyces coelicolor</i> A3(2)	CAB66307.1 (110-139)	B1-B <i>S.c.</i>
BASS1/NLR/ <i>Streptomyces coelicolor</i> A3(2)	CAB66306.1 (1-34)	B1-N <i>S.c.</i>
BASS3/Bell/ <i>Streptomyces atratus</i>	WP_037701008.1 (70-124)	B3-B <i>S.a.</i>
BASS3/NLR/ <i>Streptomyces atratus</i>	WP_037701012.1 (1-37)	B3-N <i>S.a.</i>
BASS3/Bell/ <i>Nostoc punctiforme</i> PCC 73102	ACC79696.1 (94-126)	B3-B <i>N.p.</i>
BASS3/NLR/ <i>Nostoc punctiforme</i> PCC 73102	ACC79697.1 (1-38)	B3-N <i>N.p.</i>
BASS3/Bell/ <i>Nocardia fusca</i>	WP_063130184.1 (74-128)	B3-B <i>N.f.</i>

Table 4. Spontaneous prion formation rates of selected bacterial motifs

transgene	days after transfection											
	5 days			11 days			19 days			26 days		
	[b*] n	[b] n	%	[b*] n	[b] n	%	[b*] n	[b] n	%	[b*] n	[b] n	%
B1-B <i>S.c.</i> (RFP)	30	19	38.8	7	30	81.1	3	36	92.3	3	37	92.5
B1-B <i>S.c.</i> (GFP)	31	5	13.9	11	14	56.0	8	17	68.0	0	18	100.0
B1-N <i>S.c.</i> (RFP)	32	21	39.6	9	29	76.3	8	33	80.5	3	34	91.9
B1-N <i>S.c.</i> (GFP)	15	17	53.1	7	19	73.1	5	26	83.9	0	29	100.0
B3-B <i>N.p.</i> (RFP)	16	1	5.9	5	3	37.5	4	4	50.0	3	10	76.9
B3-B <i>N.p.</i> (GFP)	33	2	5.7	12	10	45.5	8	14	63.6	1	17	94.4
B3-N <i>N.p.</i> (RFP)	30	7	18.9	6	11	64.7	6	16	72.7	3	17	85.0
B3-N <i>N.p.</i> (GFP)	38	8	17.4	20	8	28.6	8	12	60.0	0	15	100.0
B3-B <i>S.a.</i> (RFP)	33	4	10.8	14	16	53.3	8	22	73.3	3	25	89.3
B3-B <i>S.a.</i> (GFP)	5	171	97.2	1	175	99.4	nd	nd	nd	0	83	100.0
B3-N <i>S.a.</i> (RFP)	28	12	30.0	8	16	66.7	7	17	70.8	3	17	85.5
B3-N <i>S.a.</i> (GFP)	16	33	67.3	7	35	83.3	6	36	85.7	1	38	97.4
B3-B <i>N.f.</i> (RFP)	44	8	15.4	13	9	40.9	8	17	68.0	1	11	91.7
B3-B <i>N.f.</i> (GFP)	6	92	93.9	0	98	100.0	nd	nd	nd	nd	nd	nd

nd, not determined.